Monitoring Flow Streams in Multi-step Transformations

Reaction optimisation conference

Flow Chemistry Lecture

24th of April 2013

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Ian R. Baxendale

‘For 60% of today's chemical reactions the easiest approach is a batch based synthesis.’

French chemist Antoine Lavoisier (the "father of modern chemistry") started the chemical revolution in 1773.

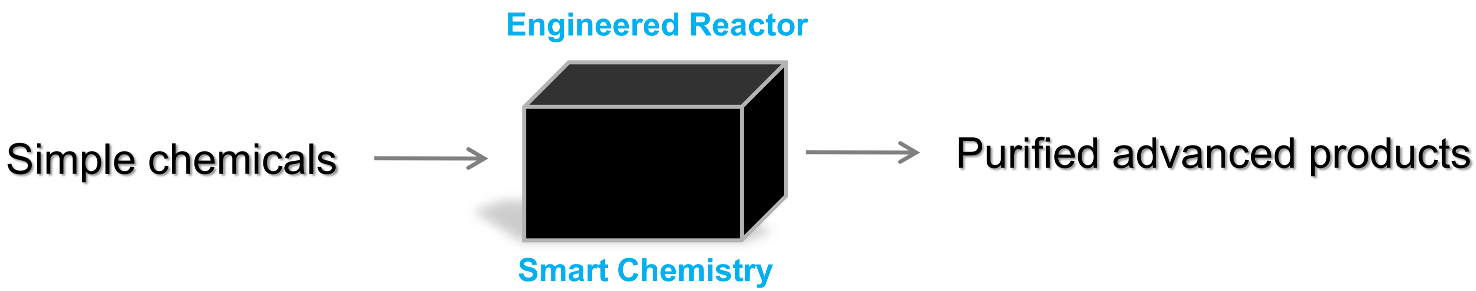
We have now had over 240 years to perfect batch based approaches.

So why adopt flow chemistry as an additional tool?

To overcome some of the current limitations in working practice.

A few example:

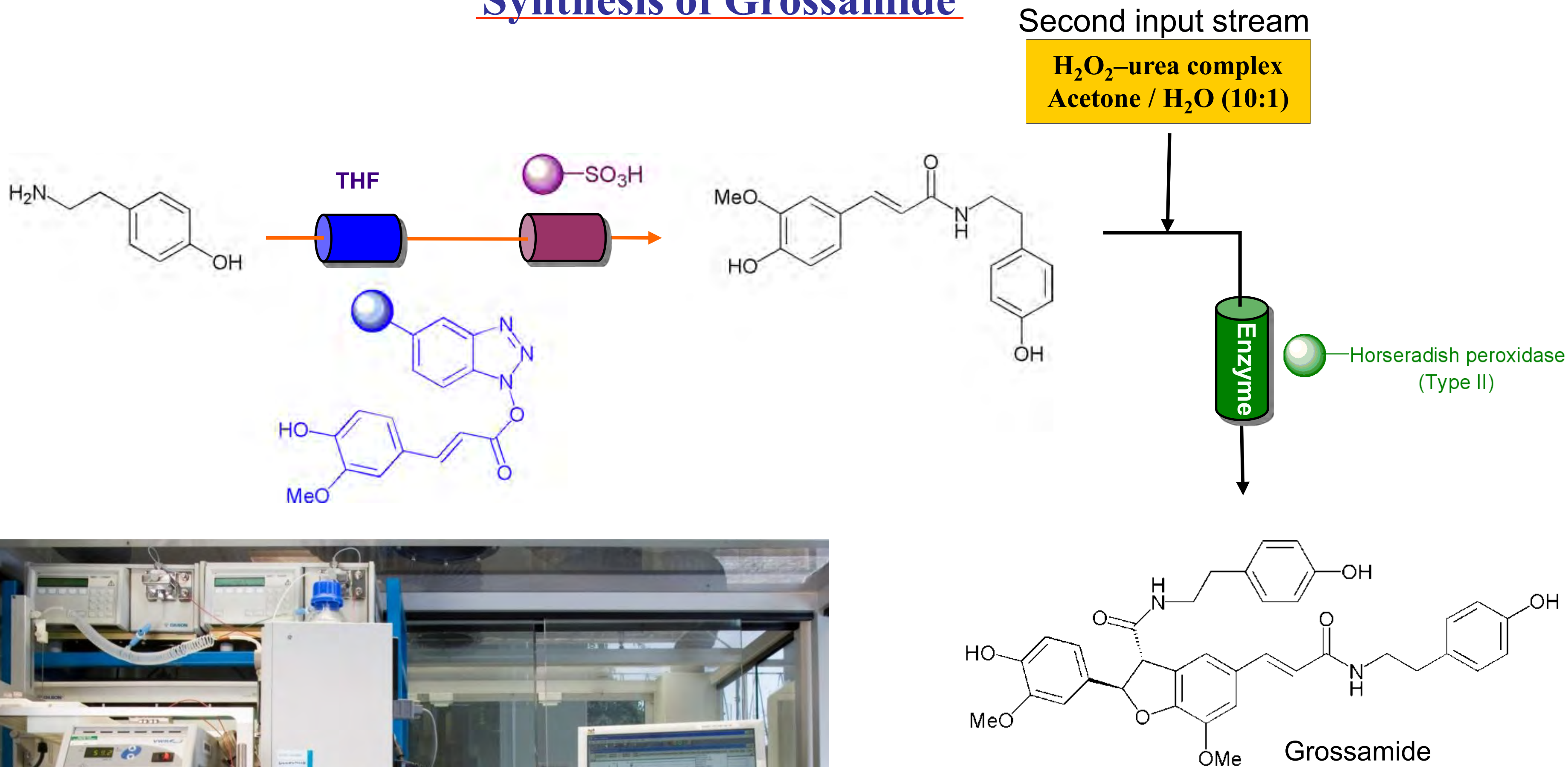
- **Solvent boiling point as upper temperature limit (avoid DMSO/DMF) – the problem of ‘reflux restriction’ (Microwave chemistry/scale-up)**
- **Use of large dilution, e.g. to control heat releases or to simply create a working volume.**
- **The direct scale up of a reaction is not always a linear progression or multiplication of substrates/solvents and parameters.**
- **Achieve controlled reactivity rather than relying upon aggressive or labile reagents – quick and dirty chemistry reliant on chromatography.**
- **In-line monitoring means problems can be rectified early - not all your eggs are in one basket.**



100% efficient, 100% yielding and 100% reproducible!

We still have a ‘little’ bit of work to do.

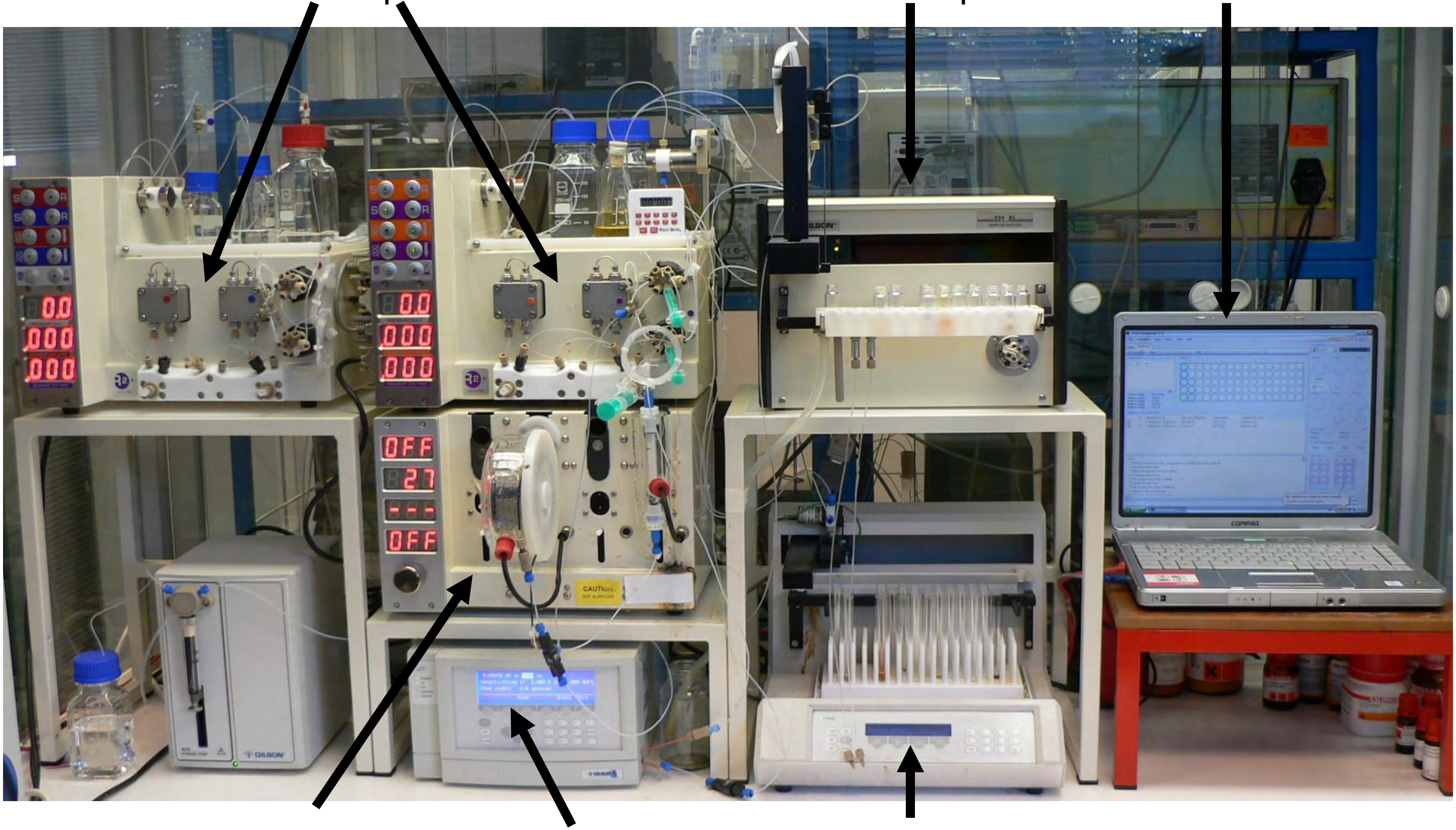
Synthesis of Grossamide



Heater/Cooler module a reactor chip -40-150 °C
Two HPLC pumps 100-10,000 µL/min 35 bar system pressure
2 reagent loading channels from liquid handling unit
UV/diode array detection
Split stream real-time MS analysis for optimisation
In-line reverse phase preparative purification – Mass directed
Fully software controlled via UNIPOINT + DoE software

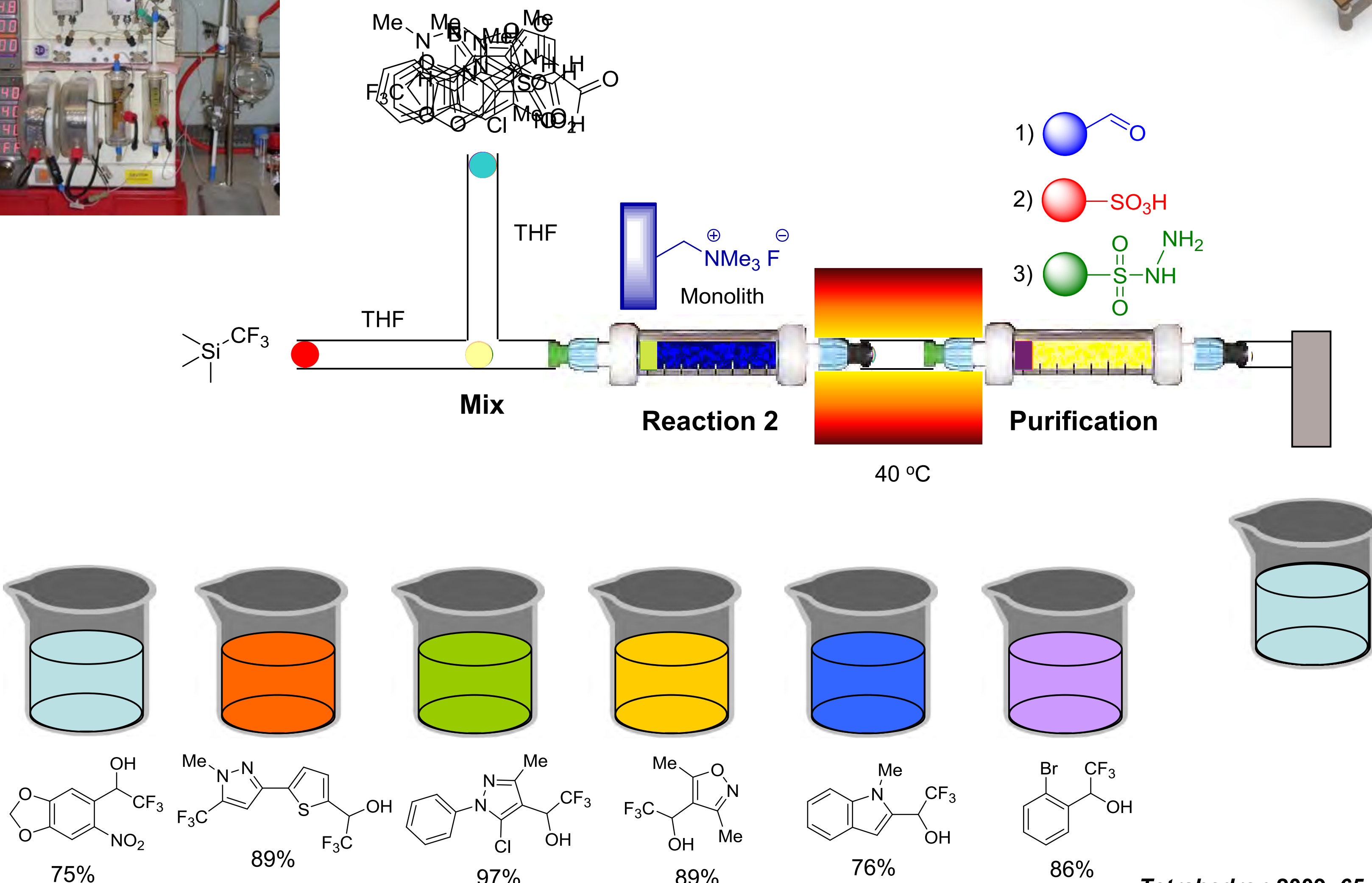
Synlett 2006, 427.

Integrated Flow Chemistry Platform



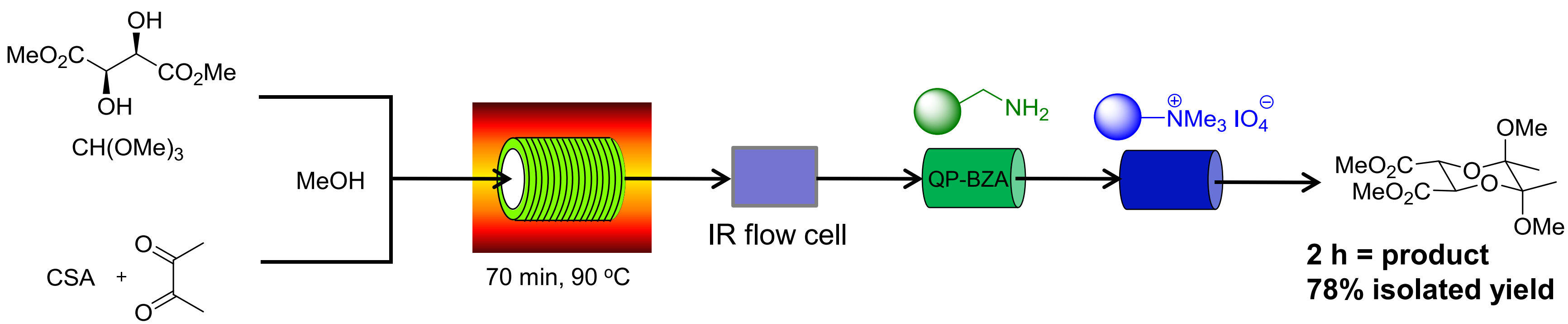
Automated Sequential Reactions

Trifluoromethylation with Ruppert's Reagent

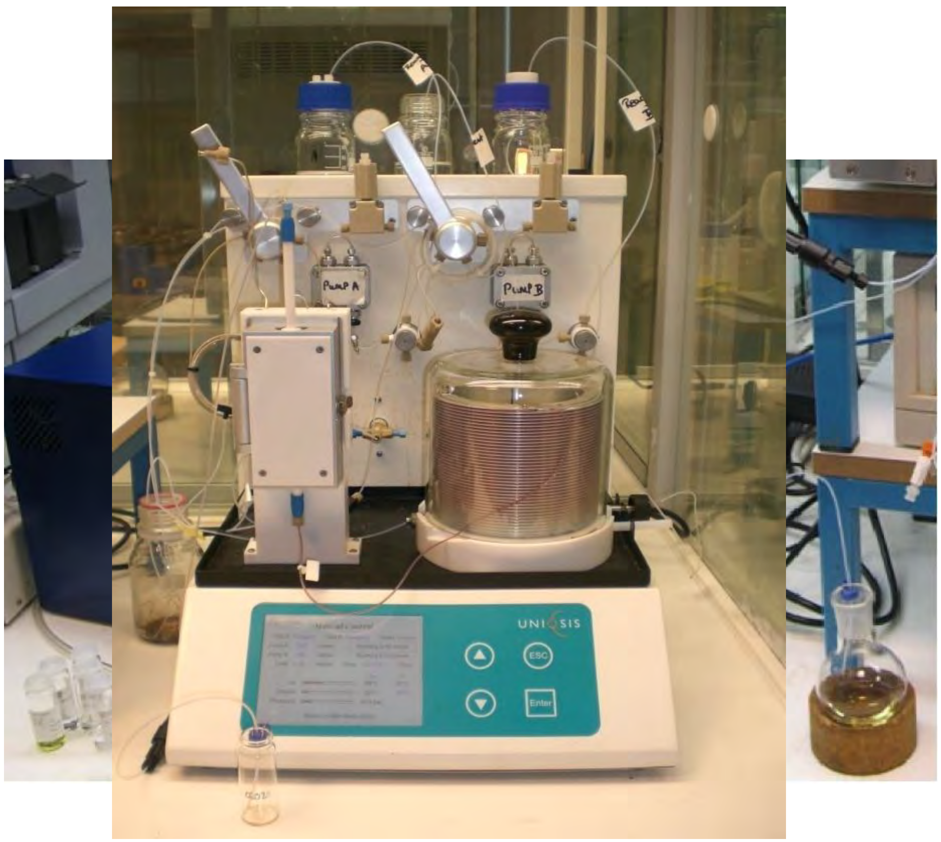


Tetrahedron 2009, *65*, 6611-6625

BDA tartrates –monitoring concentrations

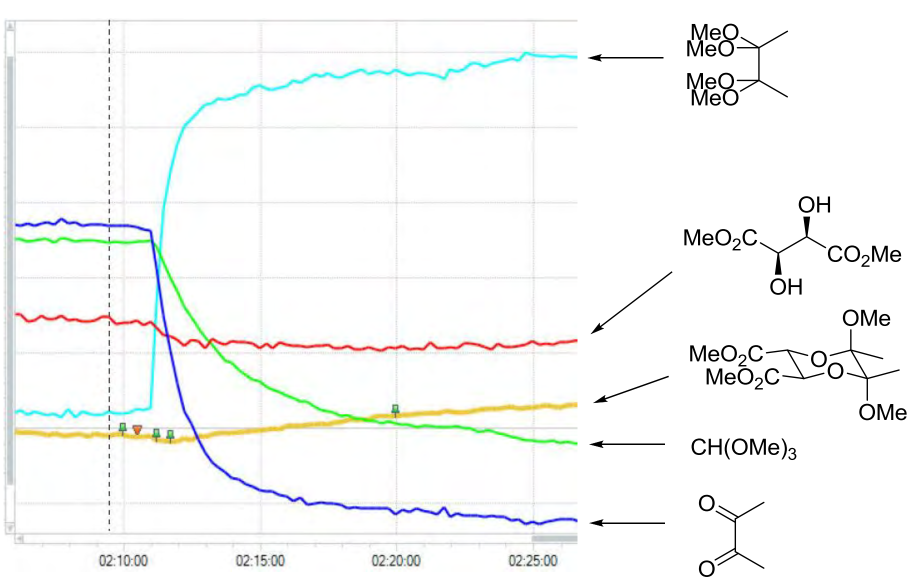


2 h = product
78% isolated yield



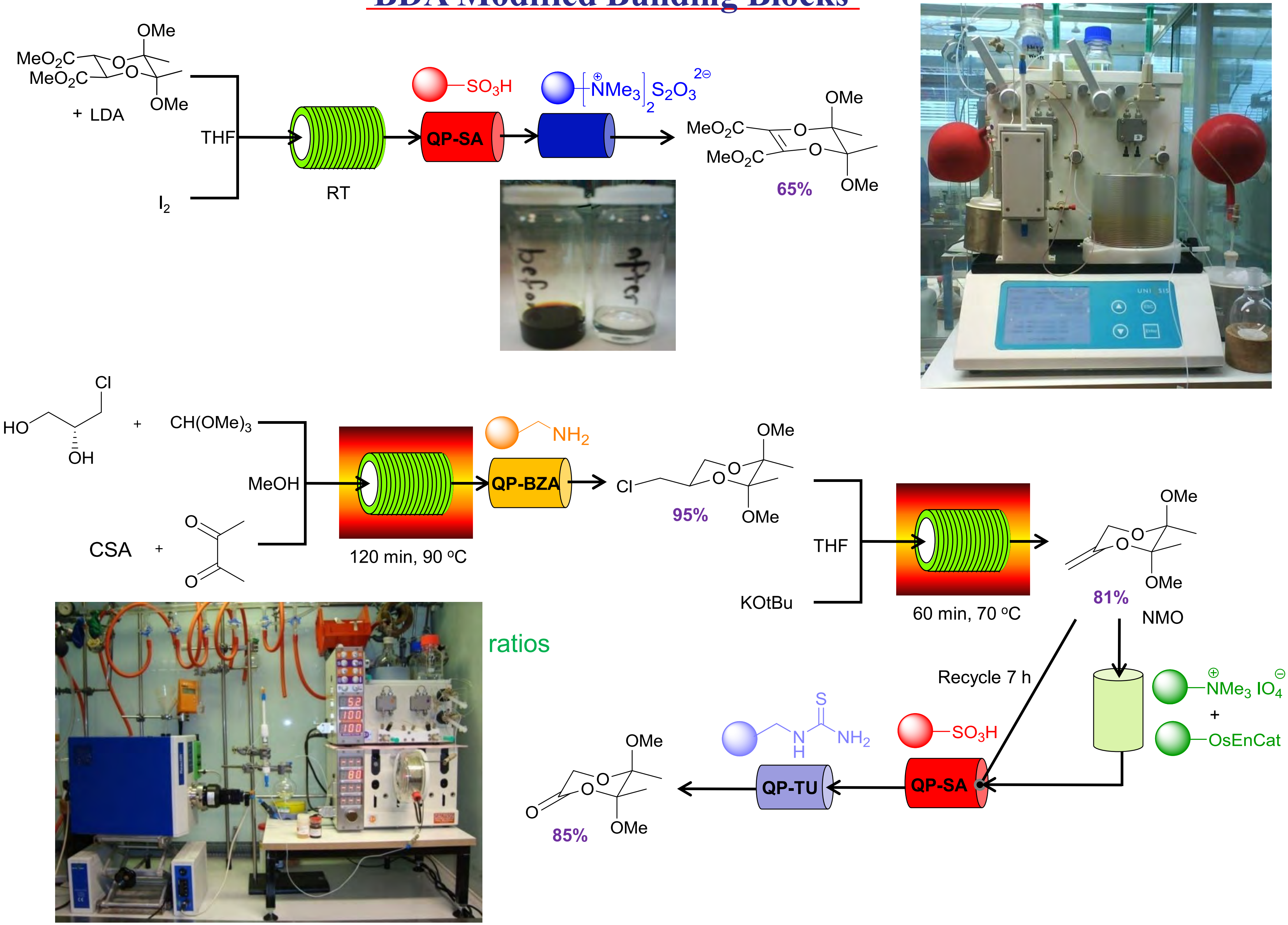
Uniqsis FlowSyn

Batch:14 h reflux, bicarbonate work-up, Et_2O extraction - aqueous washing, vac down, 2 x recyrst. 50-70%

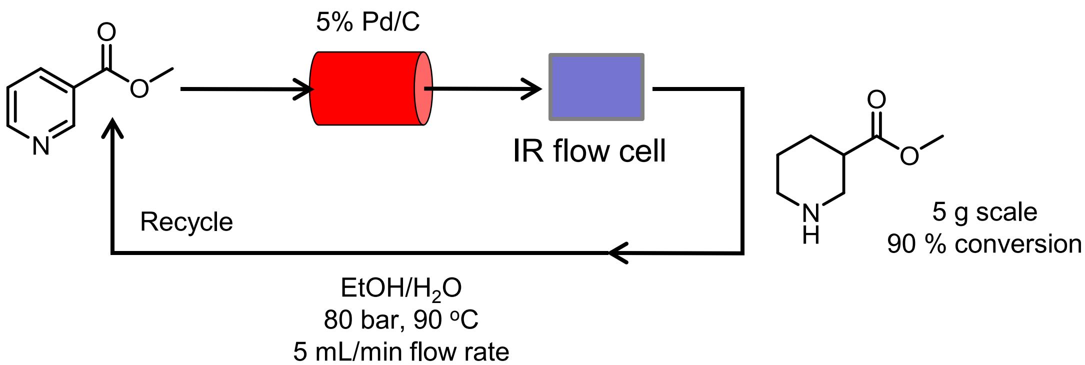


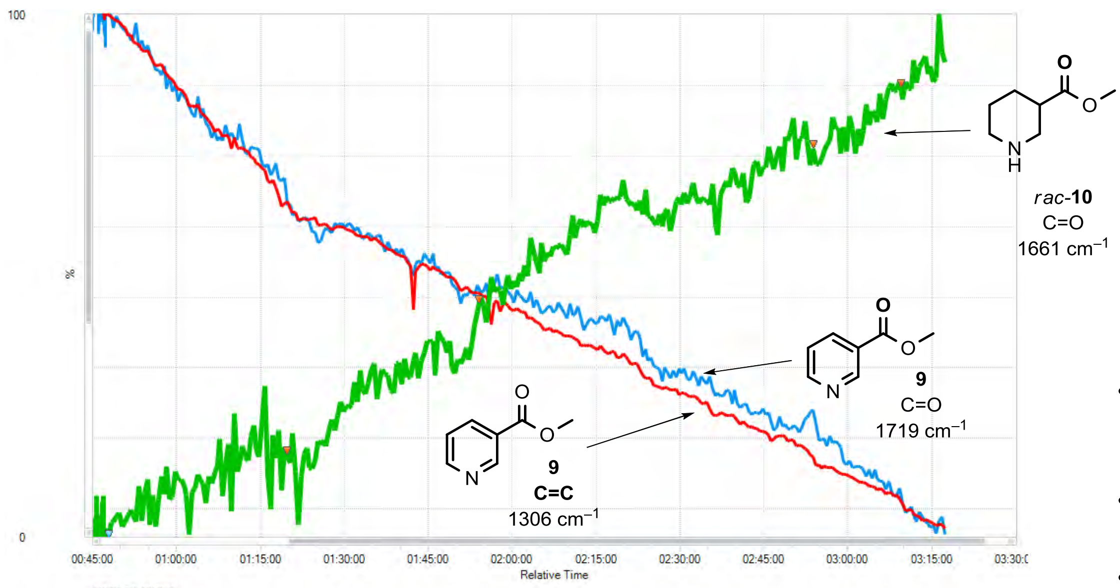
Org. Biomol. Chem. 2009, 4594.

BDA Modified Building Blocks

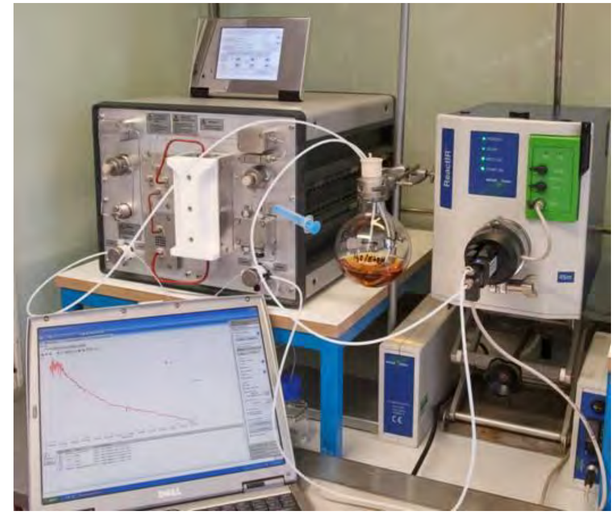


MT React IR™ 45m flow cell



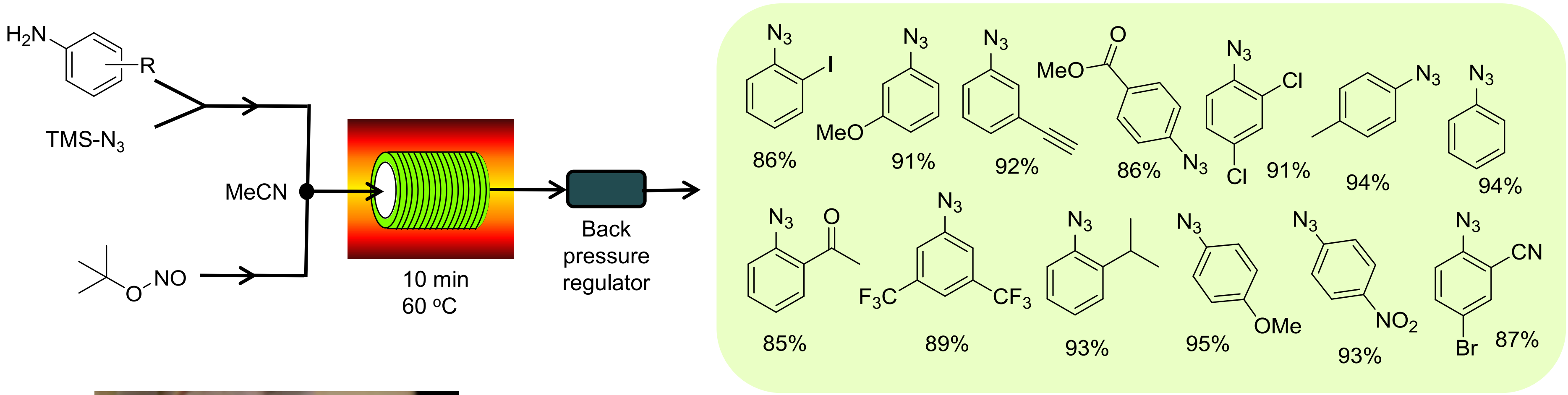


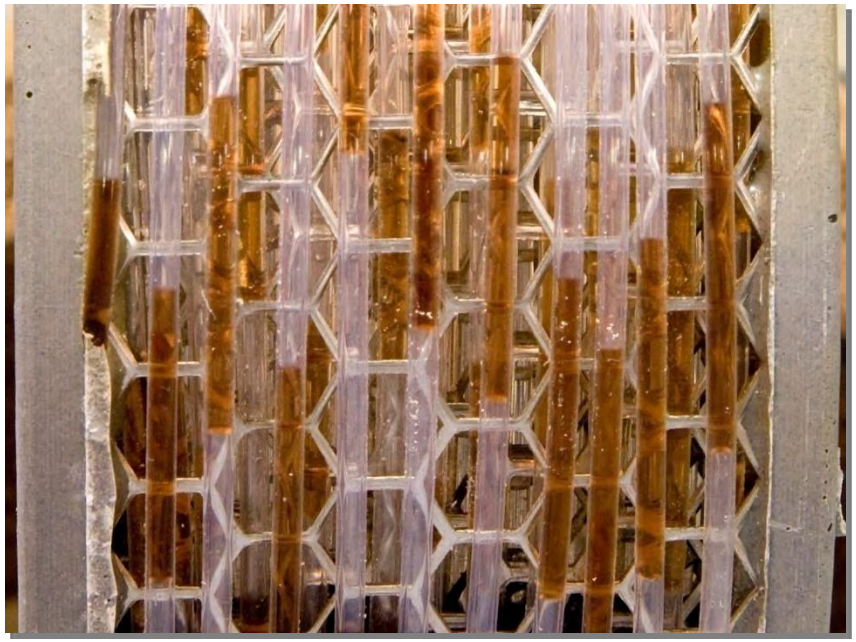
Org. Proc. Res. Dev., 2010, 14, 393

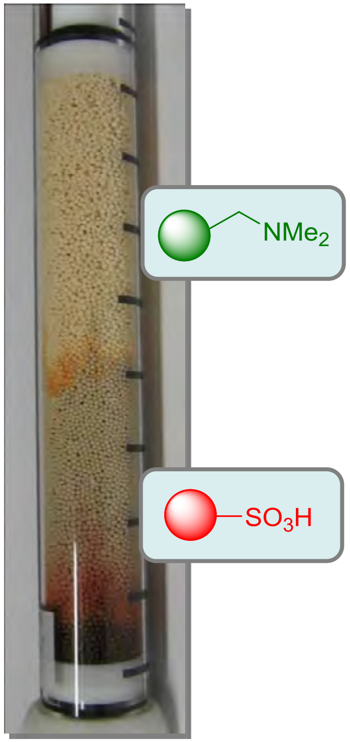


- Body: ReactIR™ 45m, fitted with a Mercury Cadmium Telluride (MCT) detector. Flow cell: Attenuated Total Reflectance (ATR) diamond sensor
- Full infrared spectral region from 650 to 1950 cm^{-1} and from 2250 to 4000 cm^{-1}
- Head can be heated and can stand pressures up to 30 bar
- HPLC connections to flow chemistry equipment
- iC IR 4.0 software for system operation and data analysis

Azides: Preparation in Flow



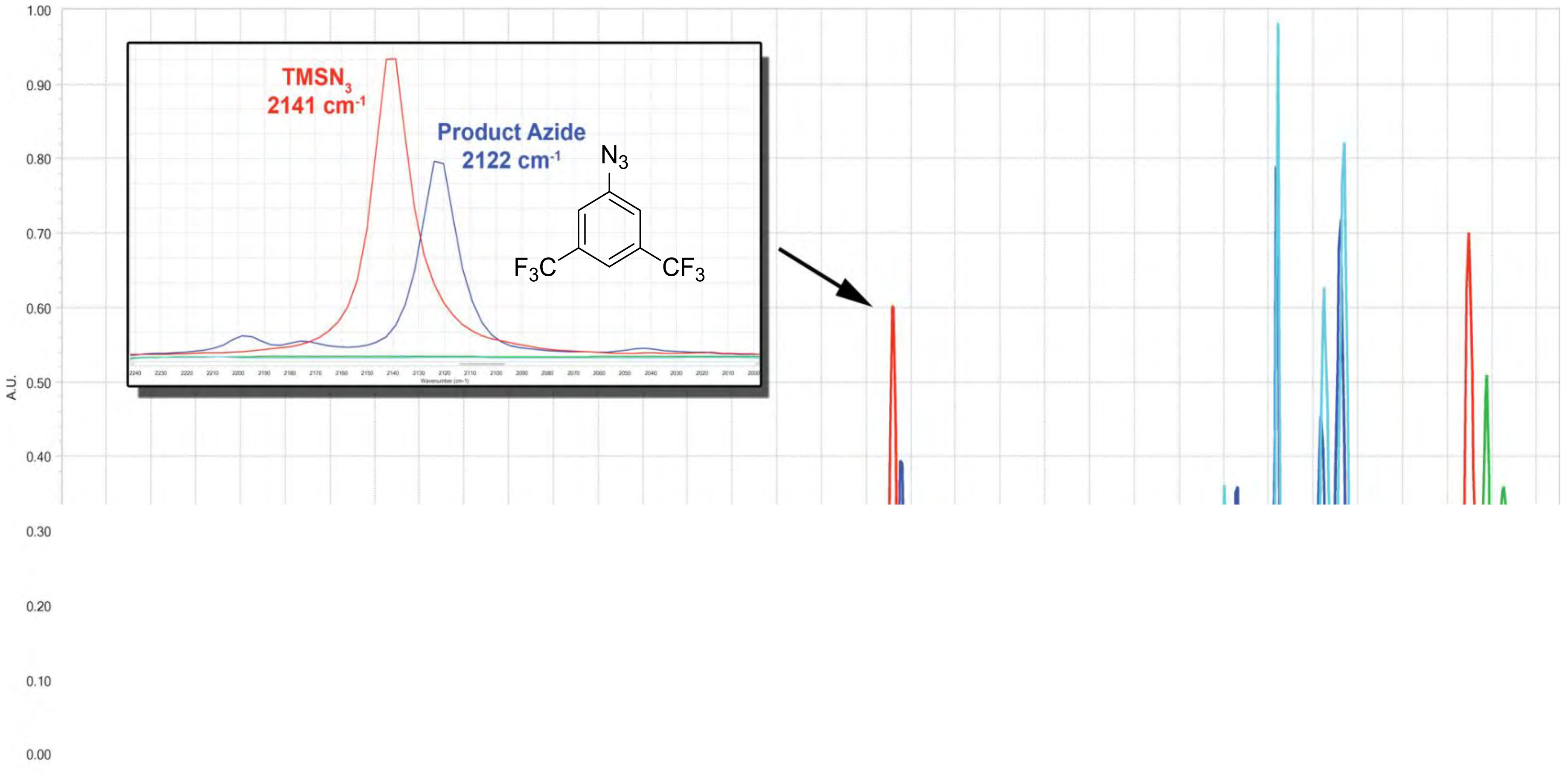


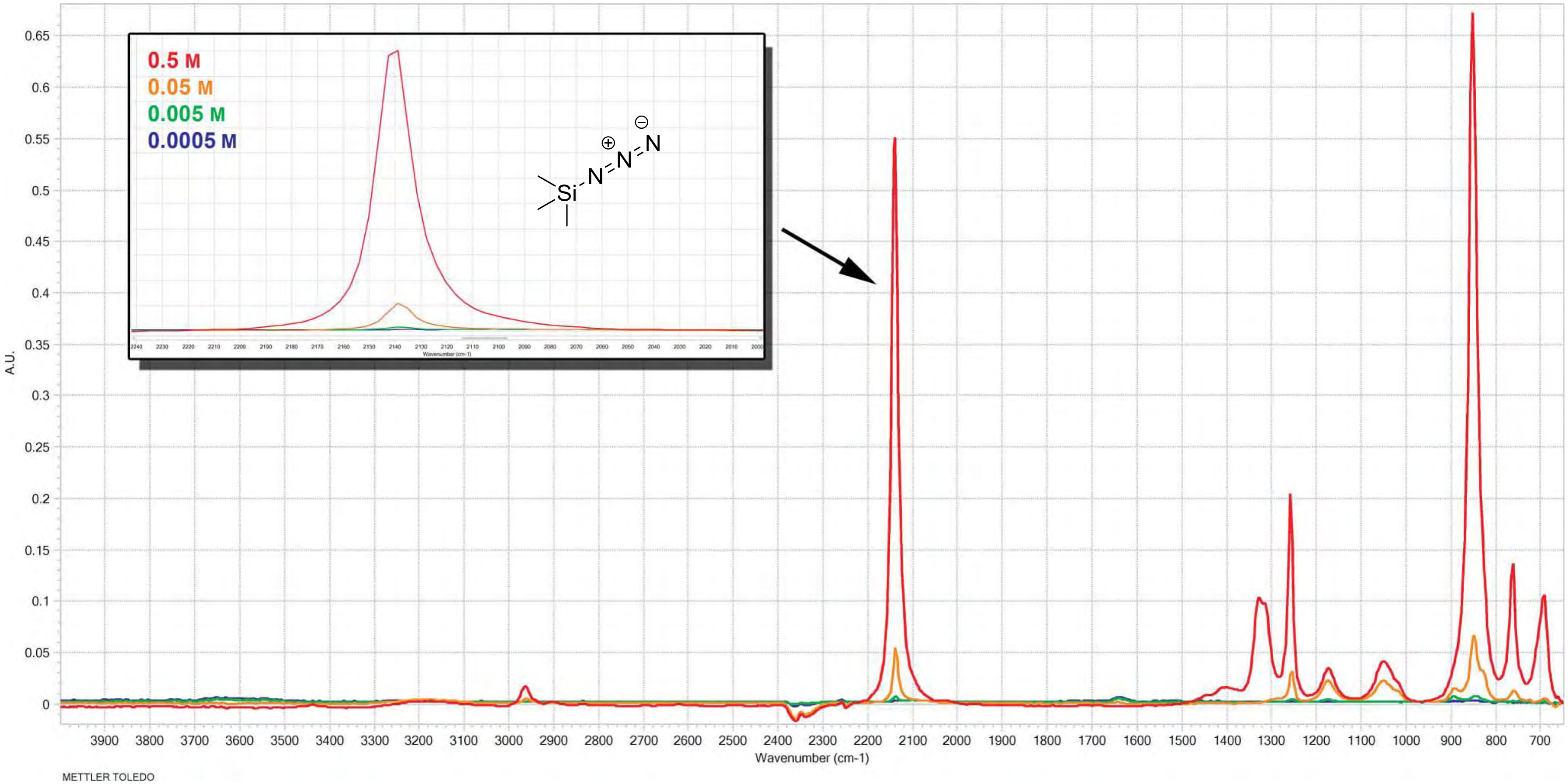


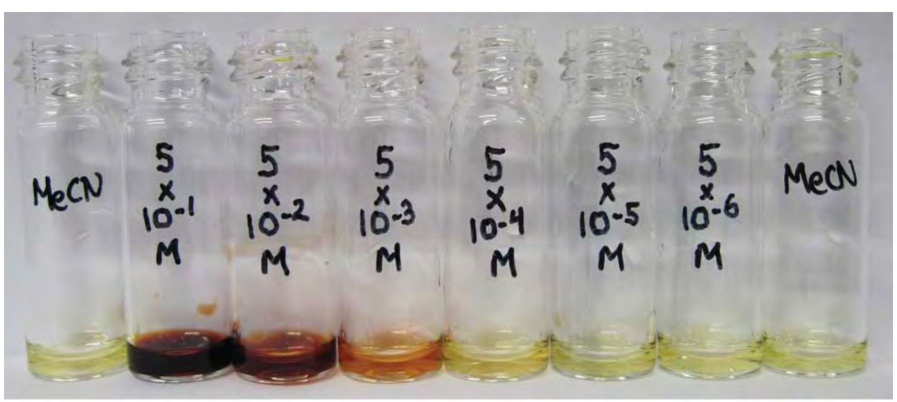
Org. Biomol. Chem. 2011, 9, 1927

In-line monitoring for Azides

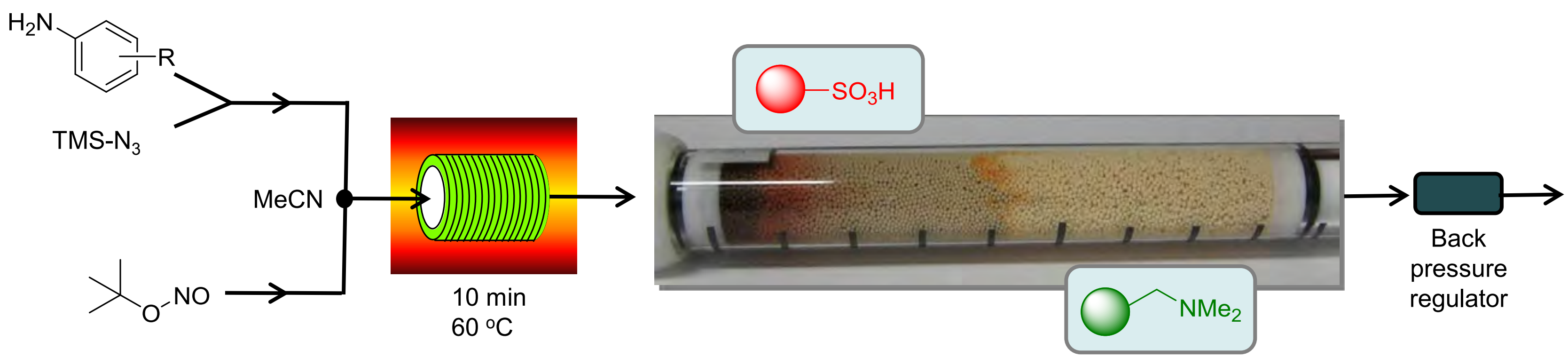
Mettler-Toledo ReactIR flow cell

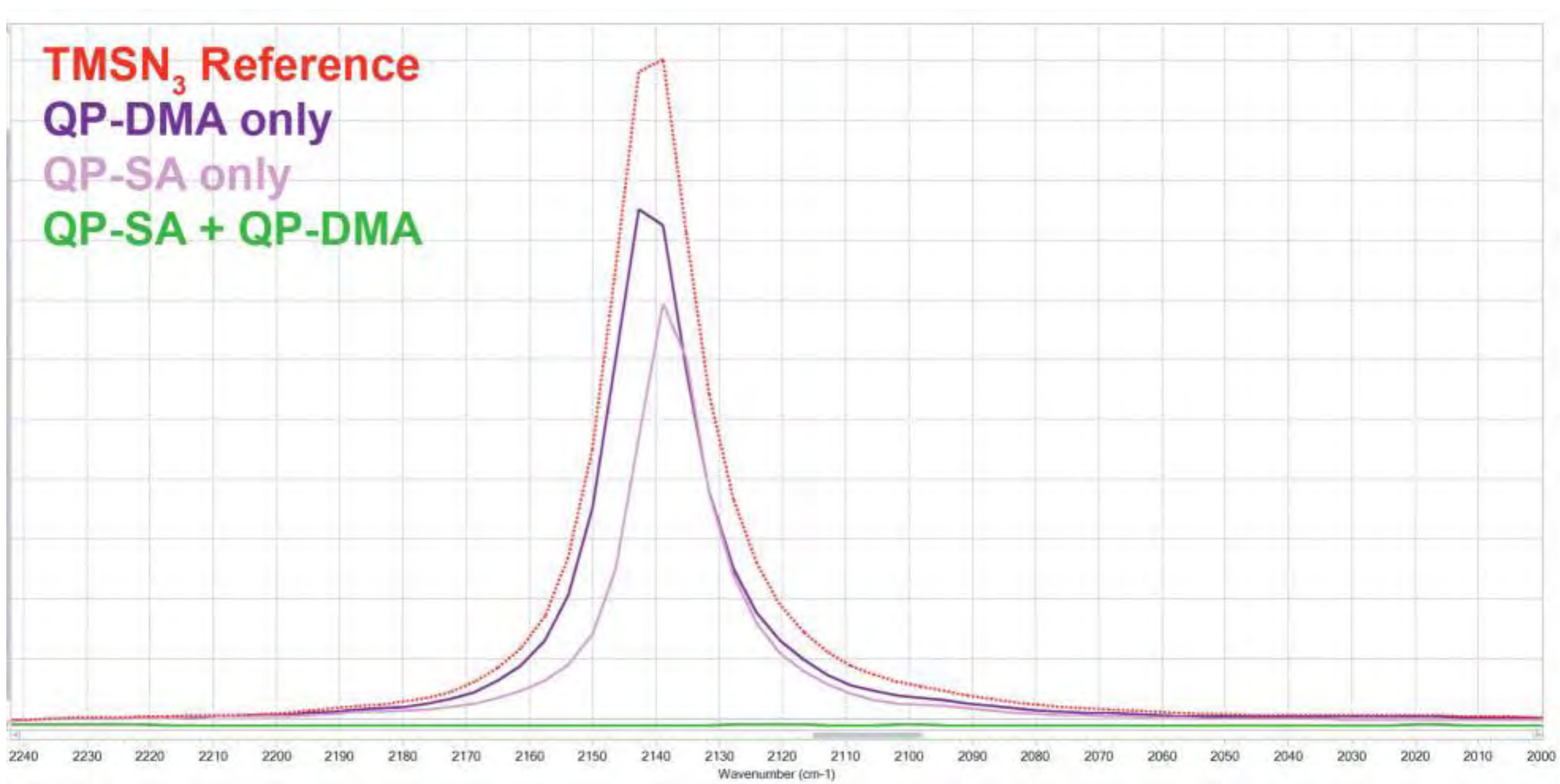




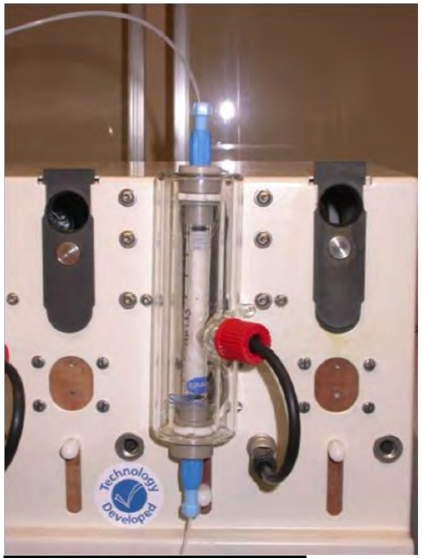


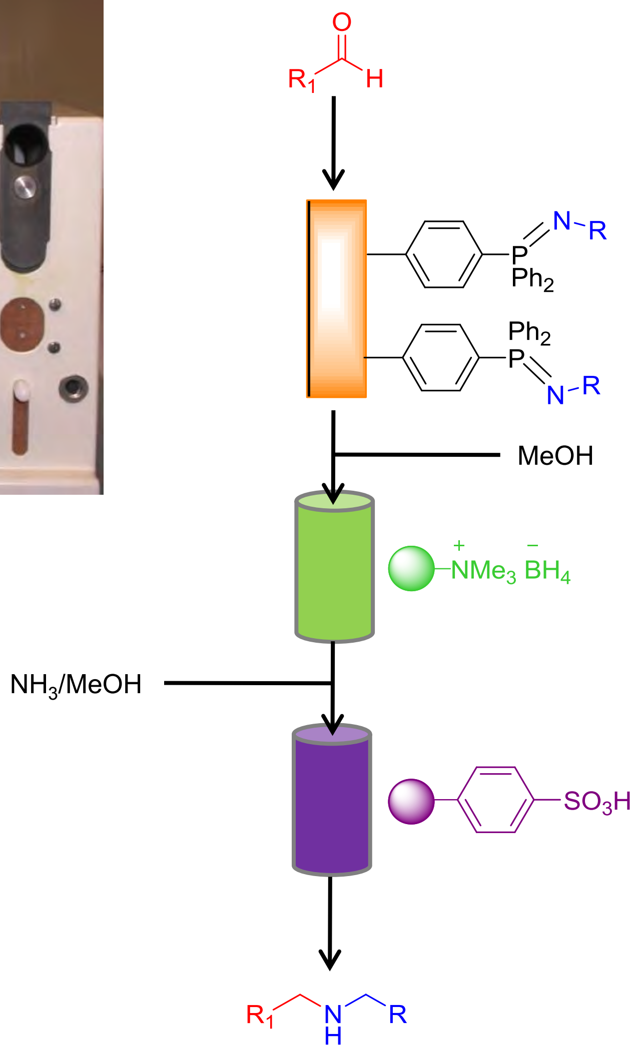
Ferric chloride colourimetric test


H2N
R
TMS-N3
MeCN
NO
O
10 min
60 °C
SO3H
NMe2
Back
pressure
regulator

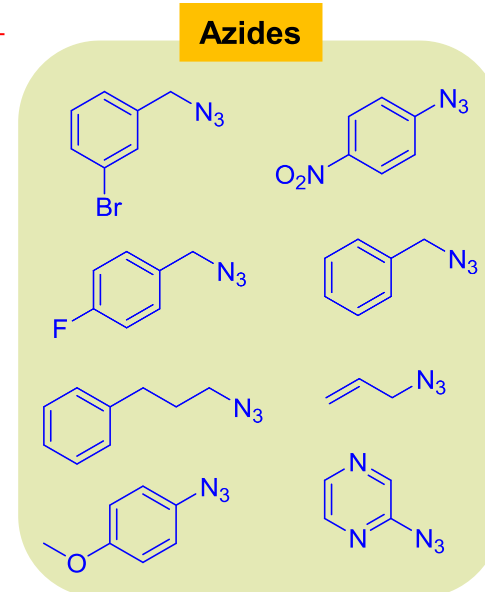

TMSN3 Reference
QP-DMA only
QP-SA only
QP-SA + QP-DMA
2240 2230 2220 2210 2200 2190 2180 2170 2160 2150 2140 2130 2120 2110 2100 2090 2080 2070 2060 2050 2040 2030 2020 2010 2000
Wavenumber (cm-1)

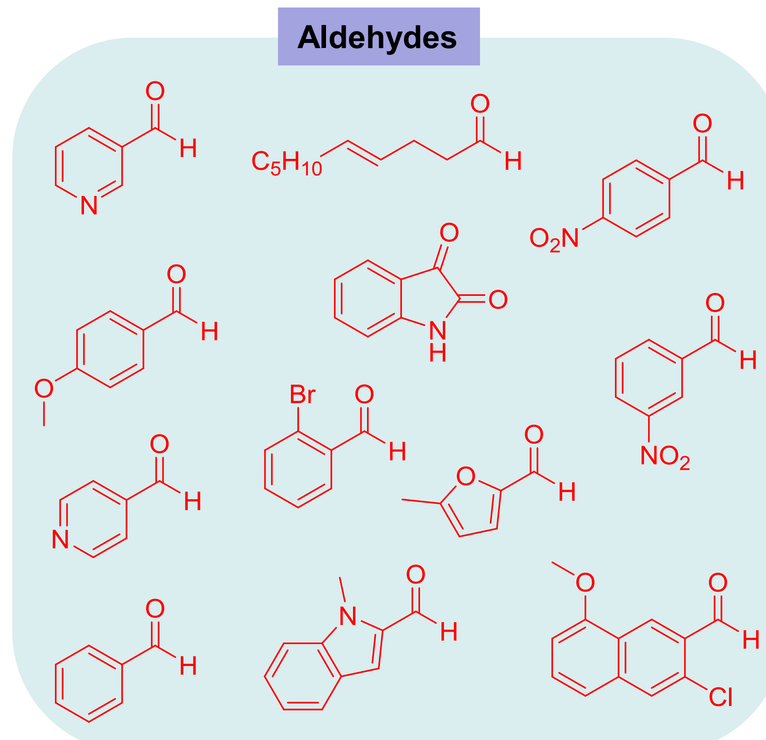
Stauginger Aza Wittig Reaction





65-99% isolated yield, >95% purity

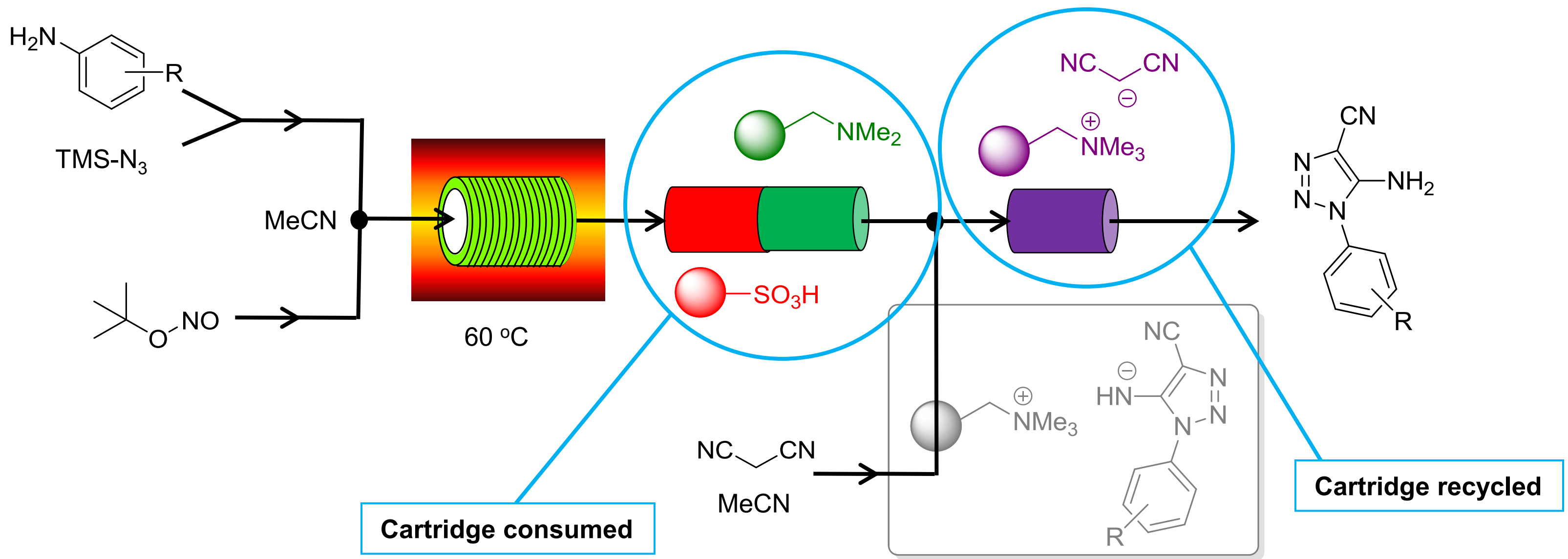


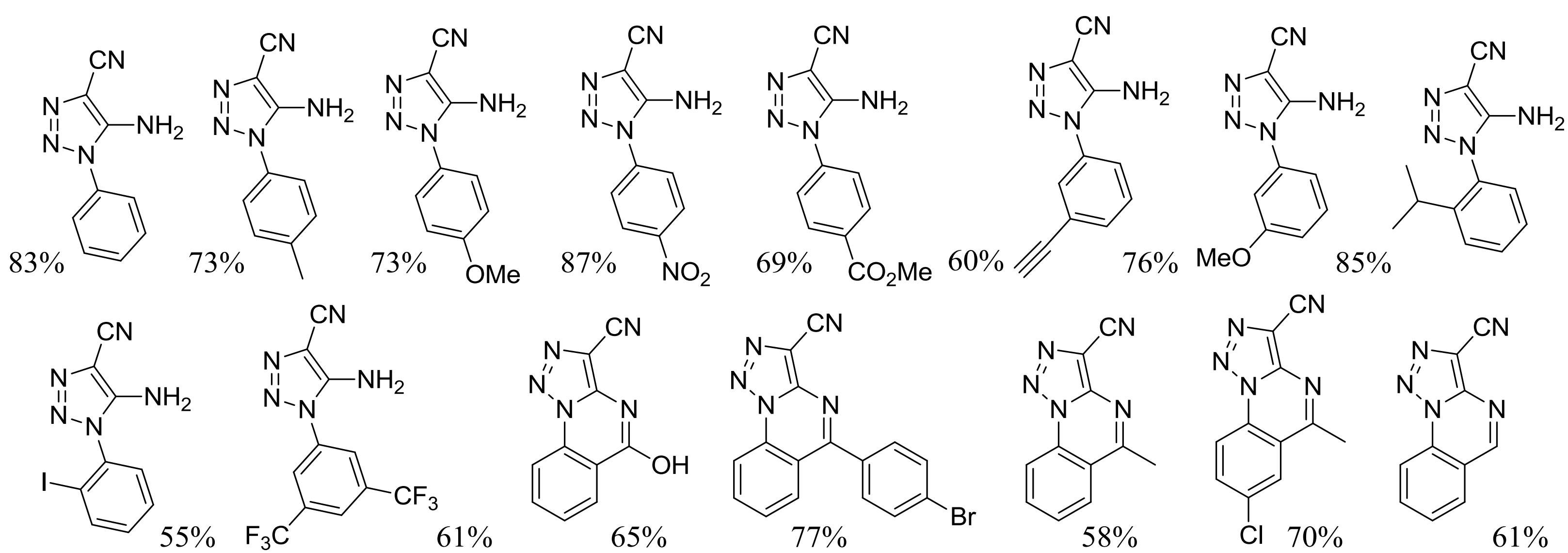


Org. Biomol. Chem. 2011, 9, 1927

Triazole Synthesis in Flow: Automation

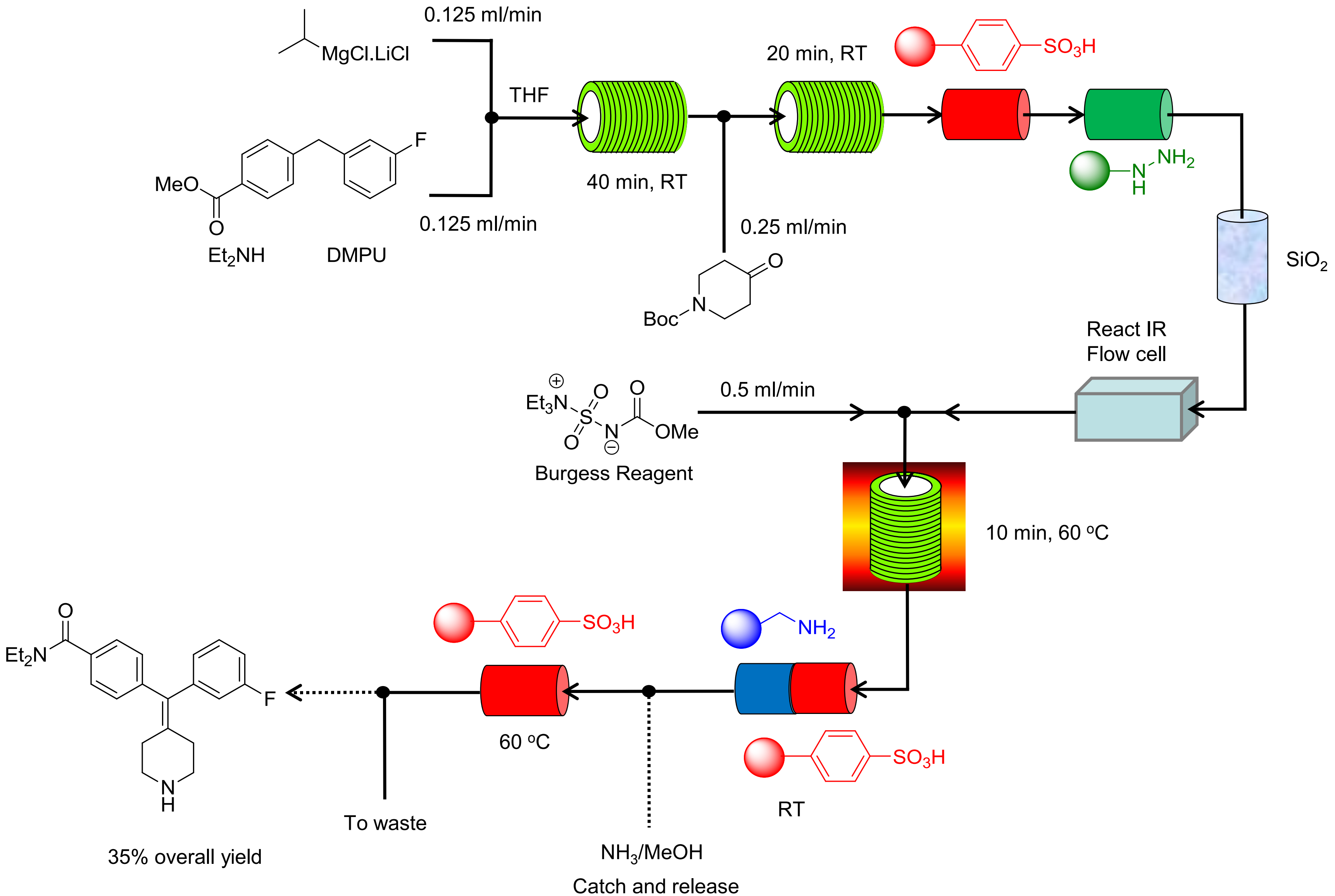
Triazole Synthesis in Flow





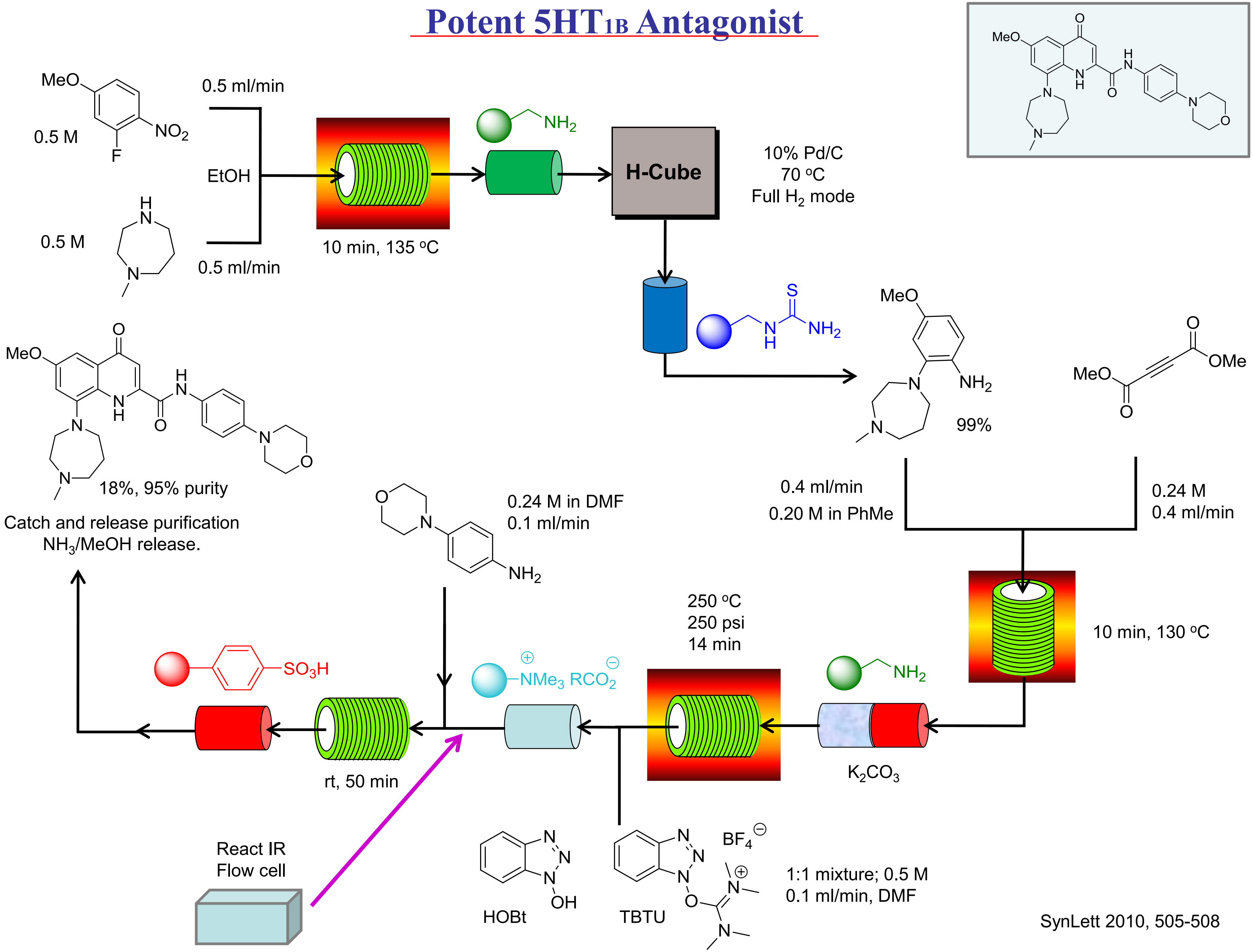
Org. Biomol. Chem. 2011, 9, 1938

δ-Opioid Receptor Agonist



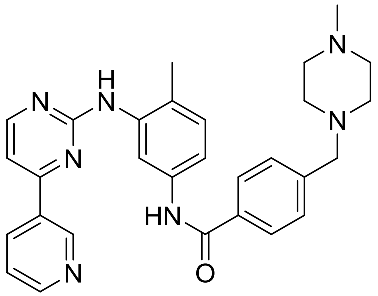
Chem. Eur. J. , 2010, 16, 12342.

Potent 5HT1B Antagonist



SynLett 2010, 505-508

Synthesis of Gleevec

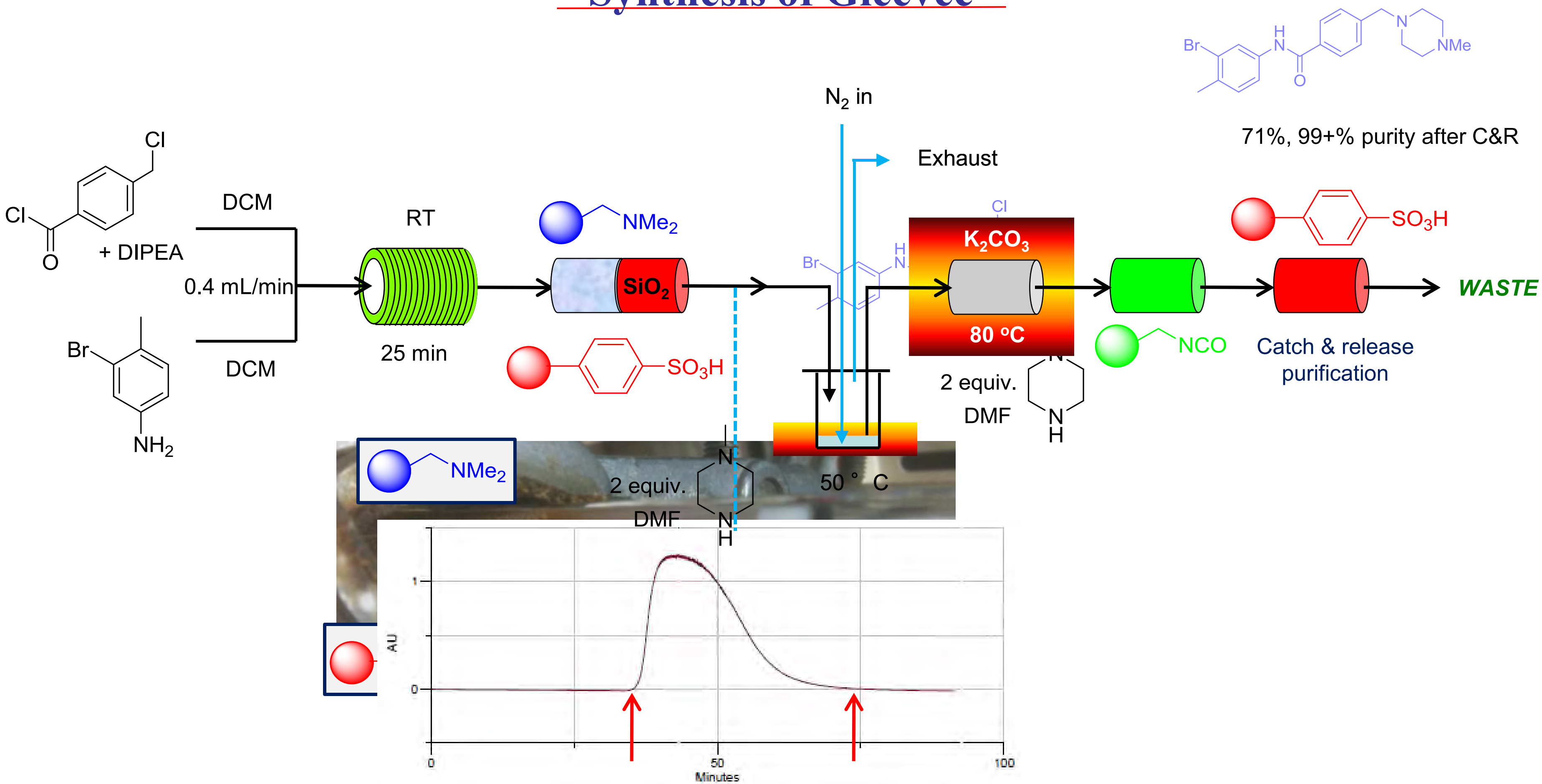


Gleevec

Aim:

- **Devise a flow-based synthesis of Gleevec that reduces the need for manual work-up/purification.**
- **Use the generic reactor to demonstrate library production from commercially available building blocks.**
- **Create a route that allowed for increased points of diversity in analogues construction.**
- **Demonstrate production and in-line screening of drug candidates using a known pharmaceutical.**

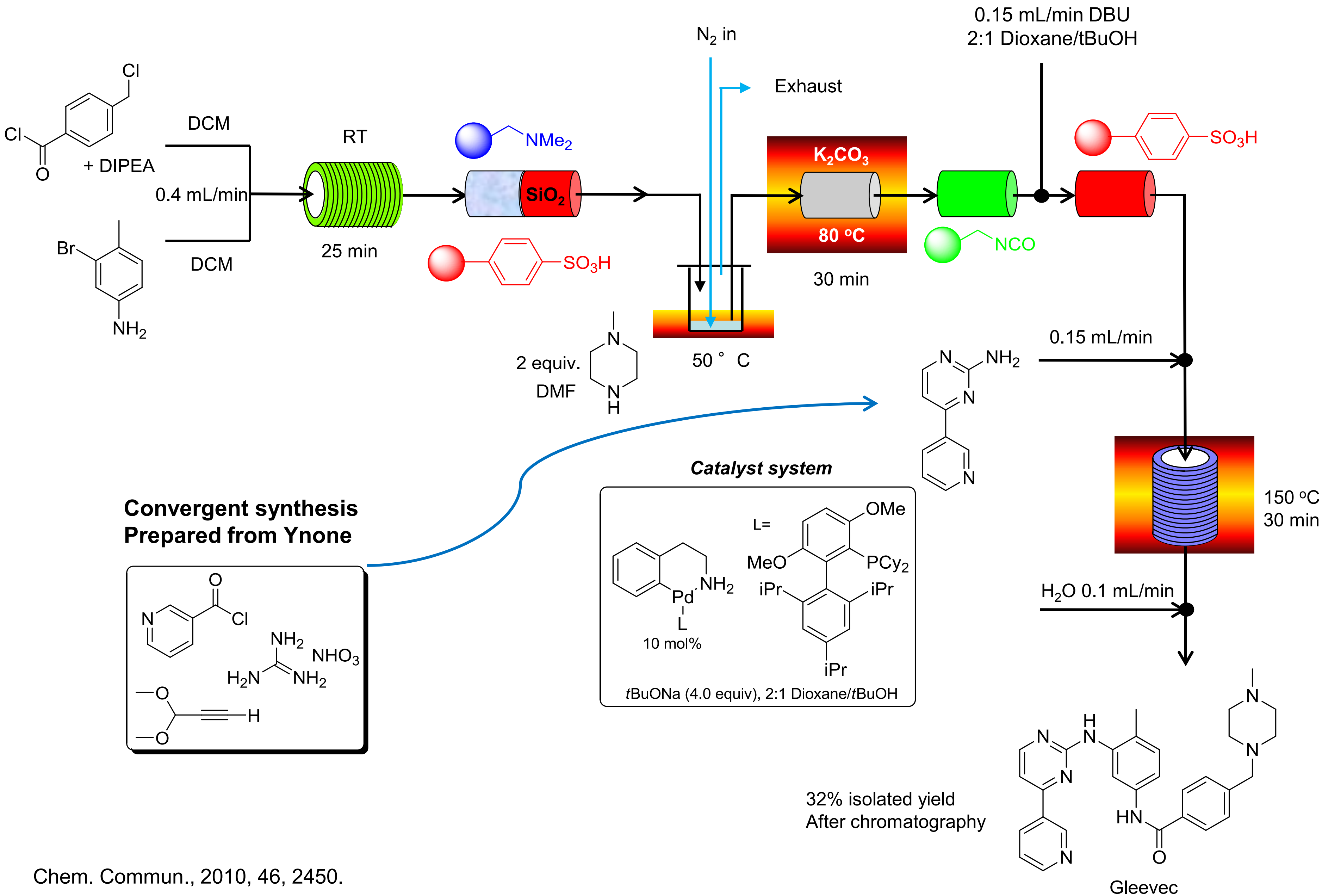
Synthesis of Gleevec



- Use UV triggered fraction collector to collect product into heated vial
- N_2 gas bubbled through the solution removes the DCM
- All product can be collected from the previous step – increased overall yield
- 80% isolated yield

Chem. Commun., 2010, 46, 2450.

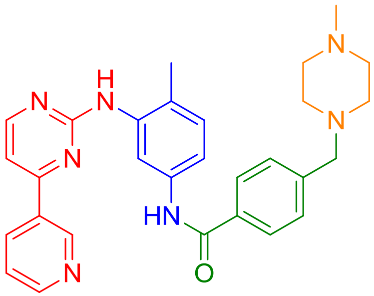
Synthesis of Gleevec



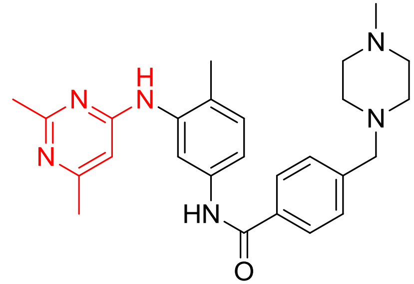
Chem. Commun., 2010, 46, 2450.

Analogues of Gleevec

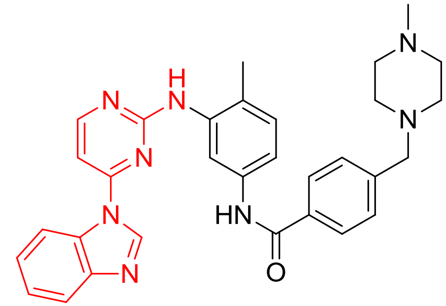
Route provides access to analogues not achievable via process route



Gleevec 32%

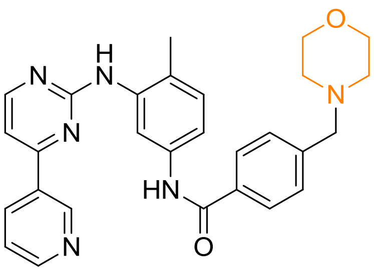


33%

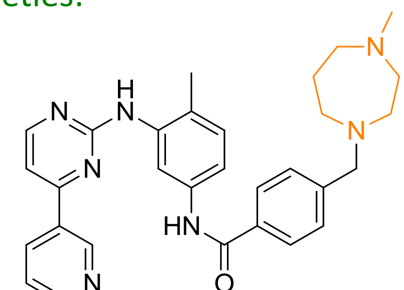


29%

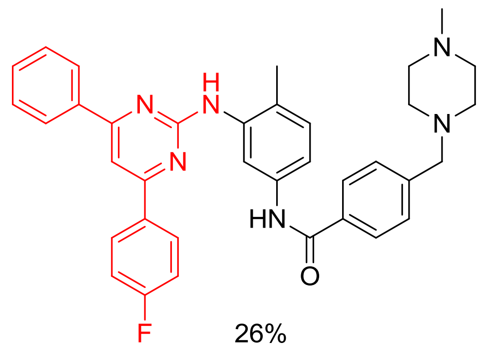
Alternative solubilising moieties:



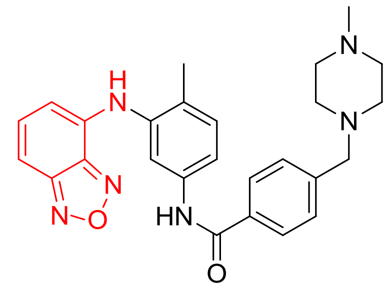
24%



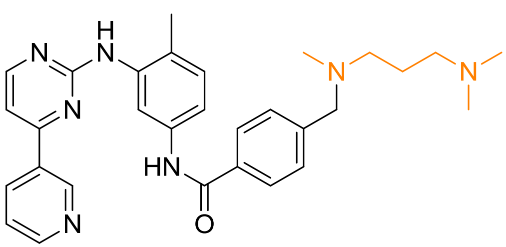
25%



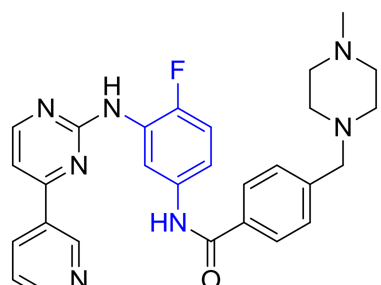
26%



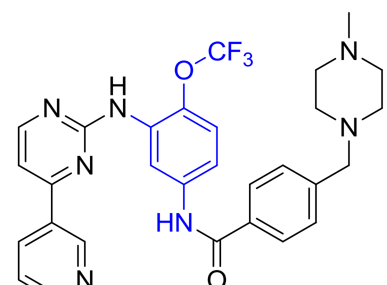
31%



24%



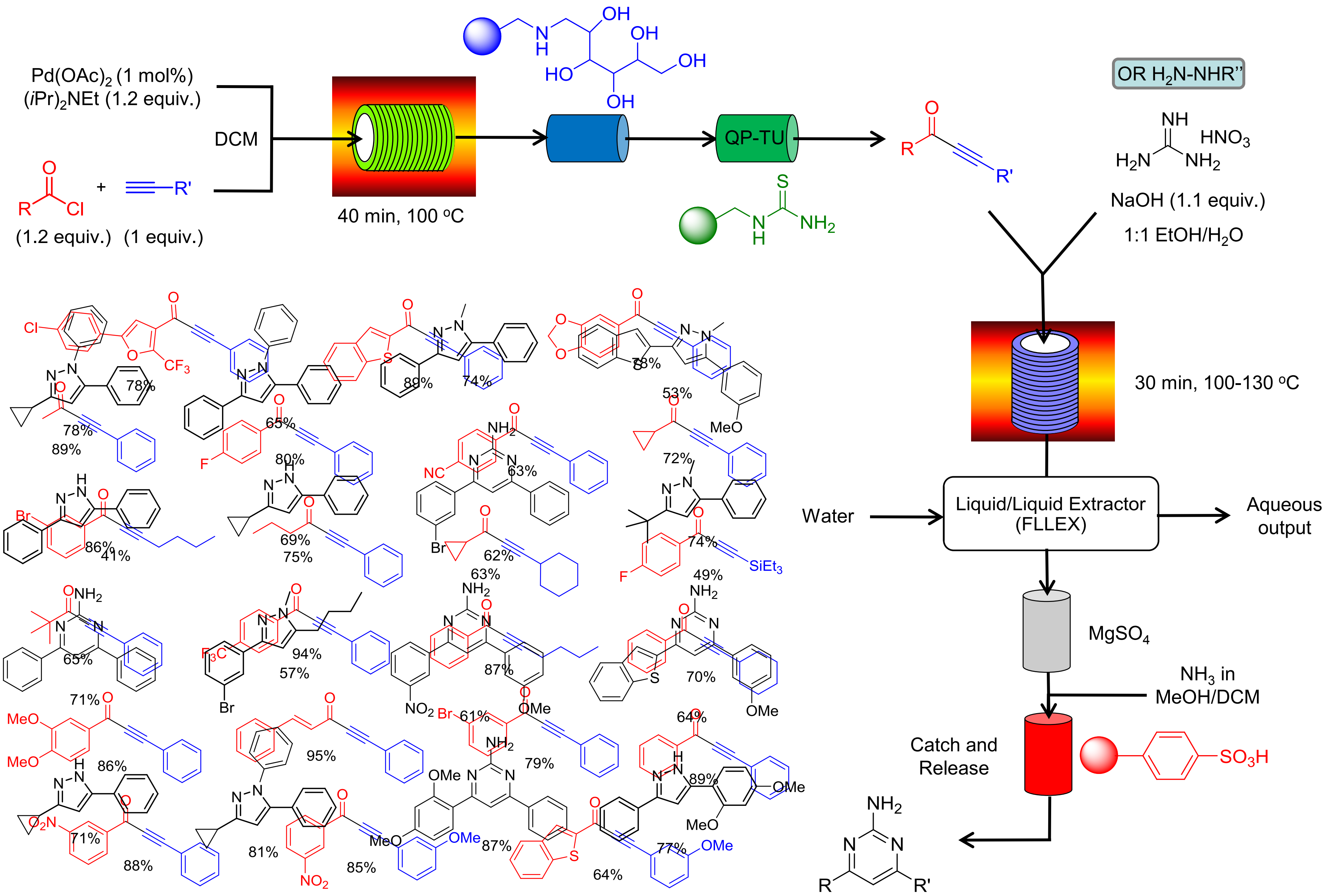
35%



28%

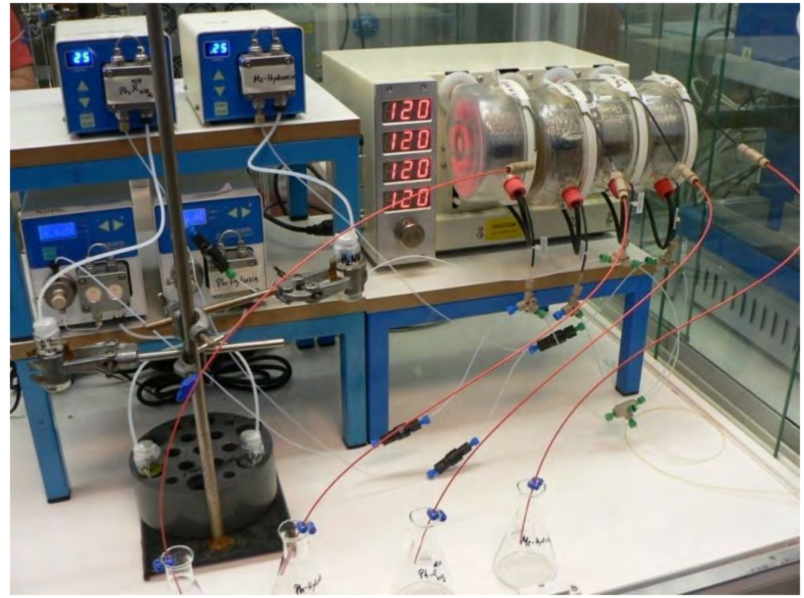
Org. Biomol. Chem., 2013, 11, 1822.

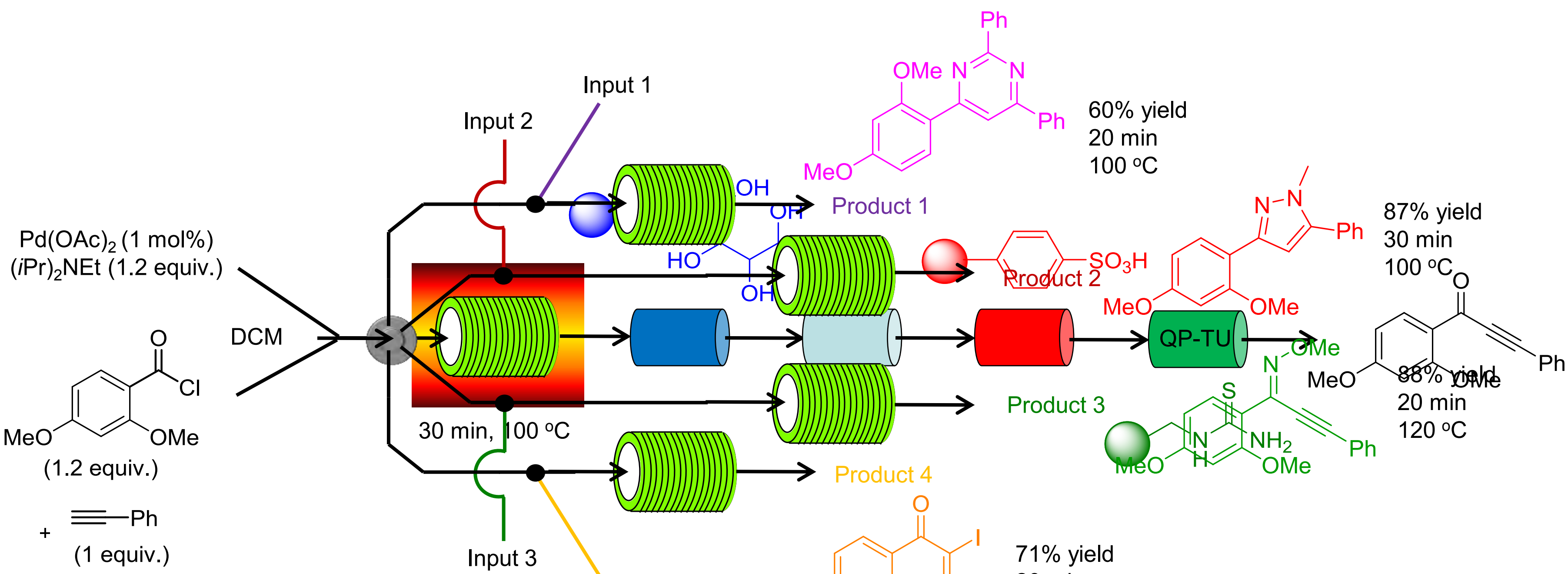
Ynone Synthesis – numbering out



Chem. Eur. J., 2010, 16, 89-94

Stream Splitting in Flow



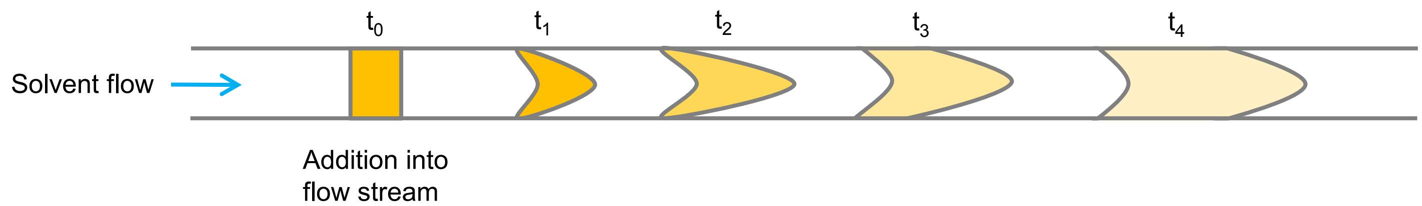


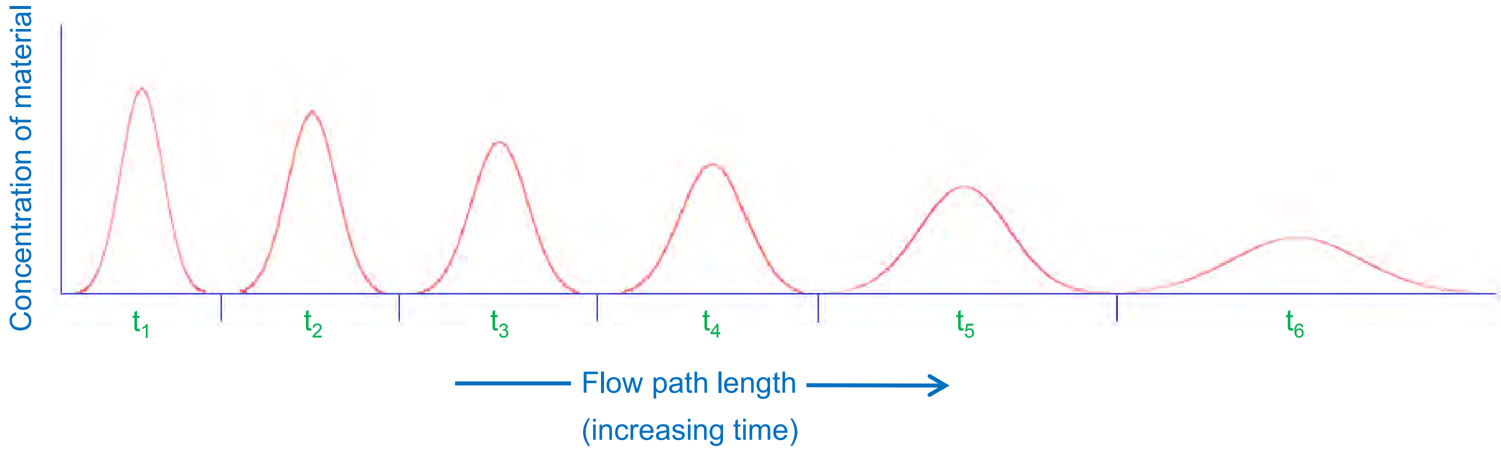
Chem. Eur. J., 2010, 16, 89-94

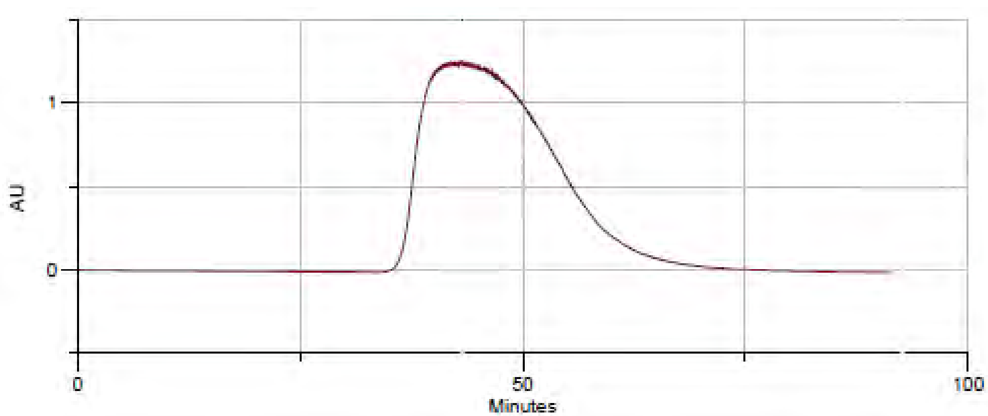
Mixing and Dispersion

Dispersion Model accounts for diffusion

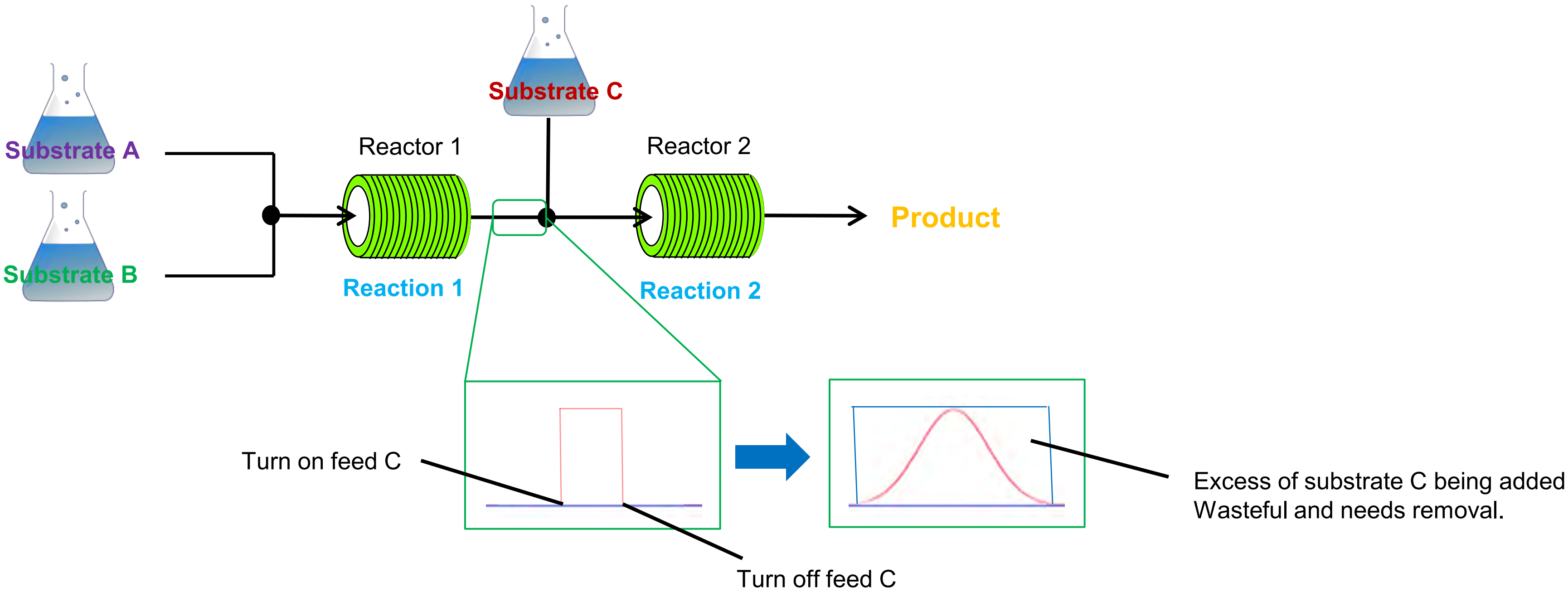
- diffusion molecular
- diffusion convective
  - Aris-Taylor dispersion in laminar flow or turbulent dispersion from eddies

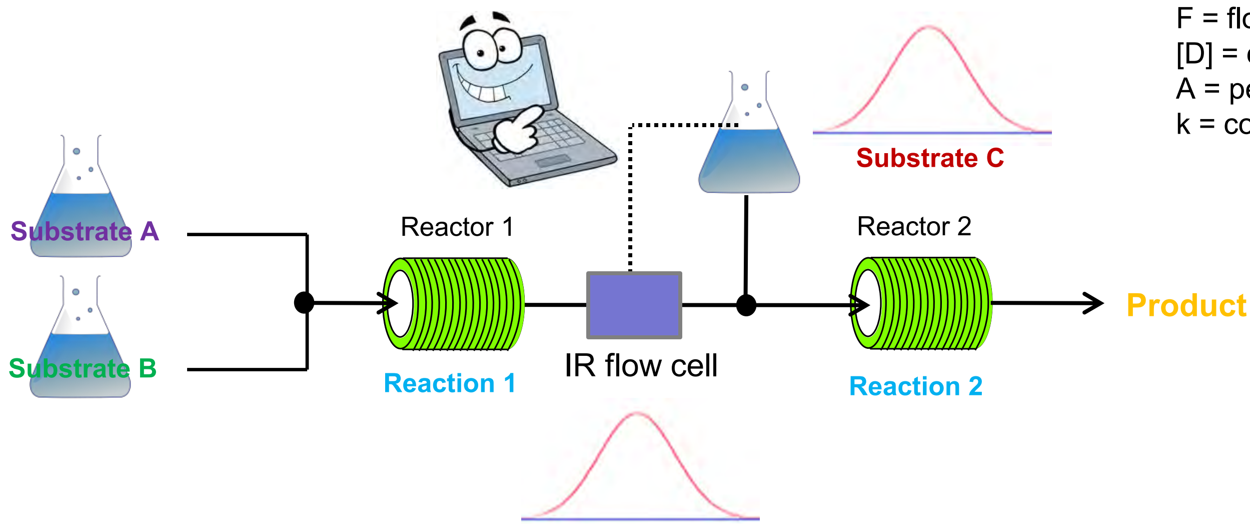






The third stream issue

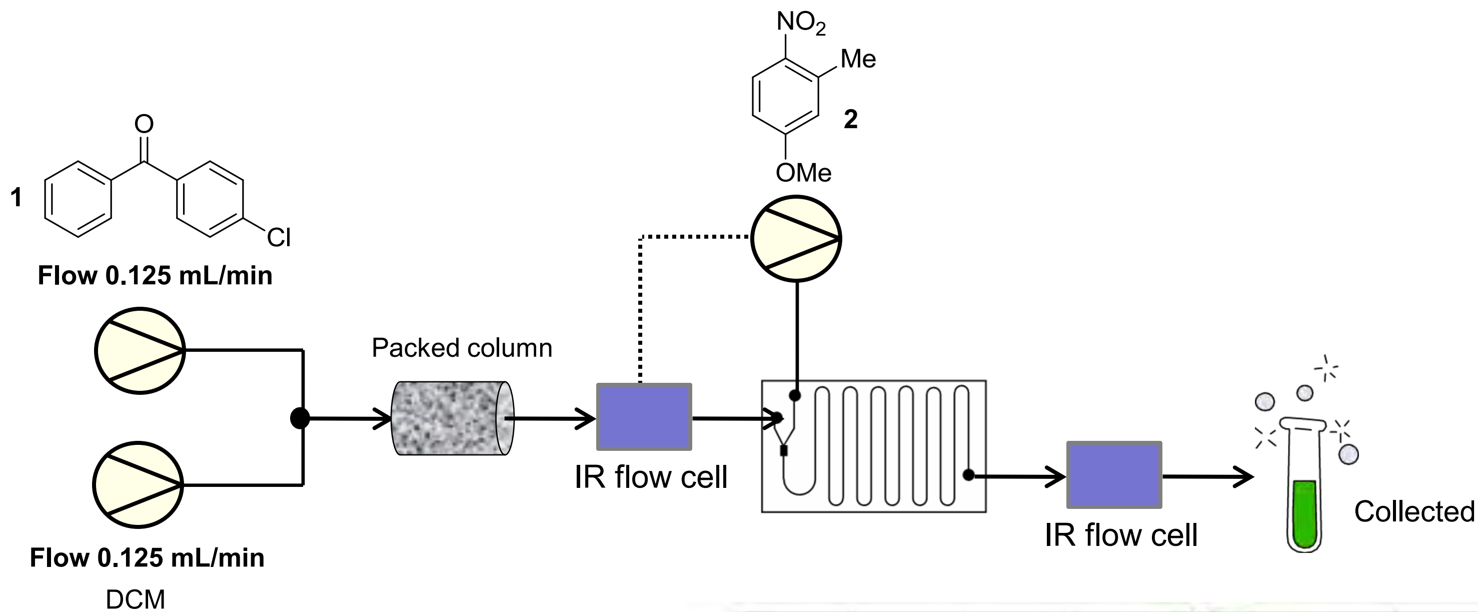


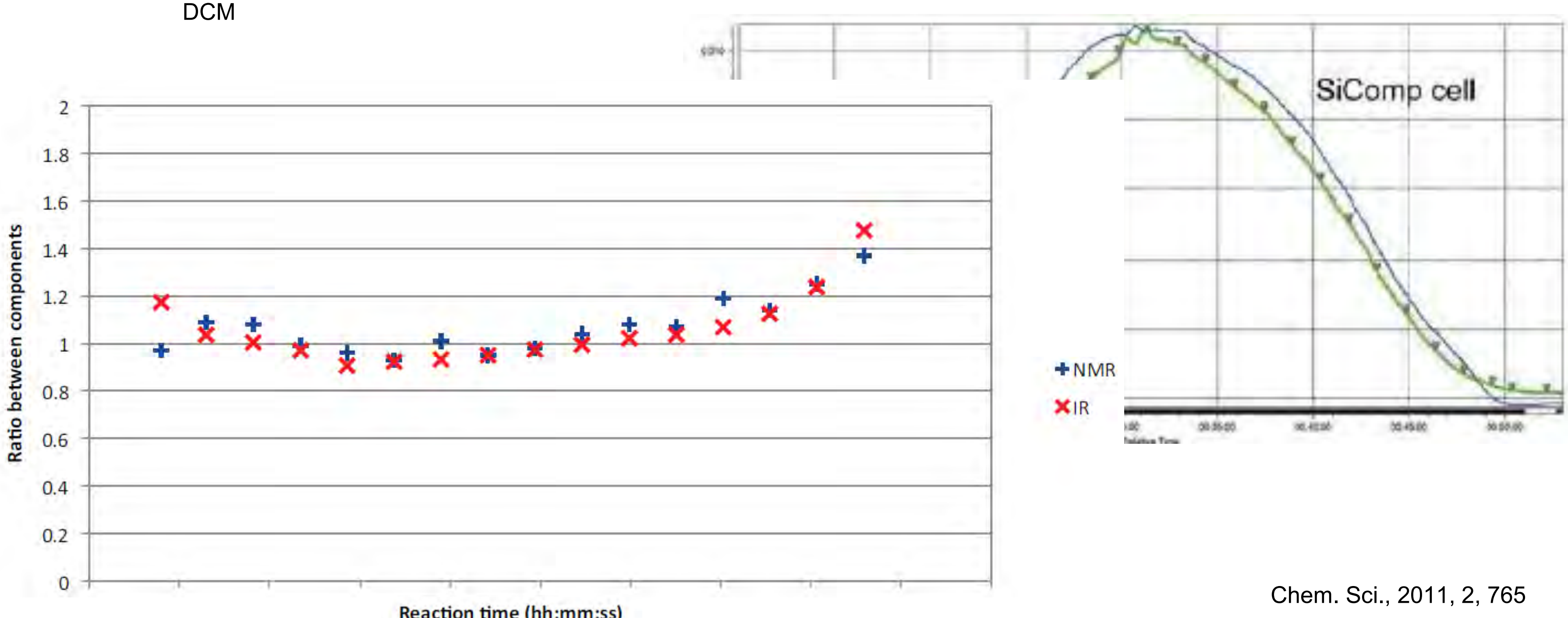


X = stoichiometry between the two components
F = flow rate of output process
[D] = conc. of third stream component
A = peak height value measured by IR
k = conversion factor

$$Flow\ rate = \frac{X \cdot F \cdot k}{[D]}$$

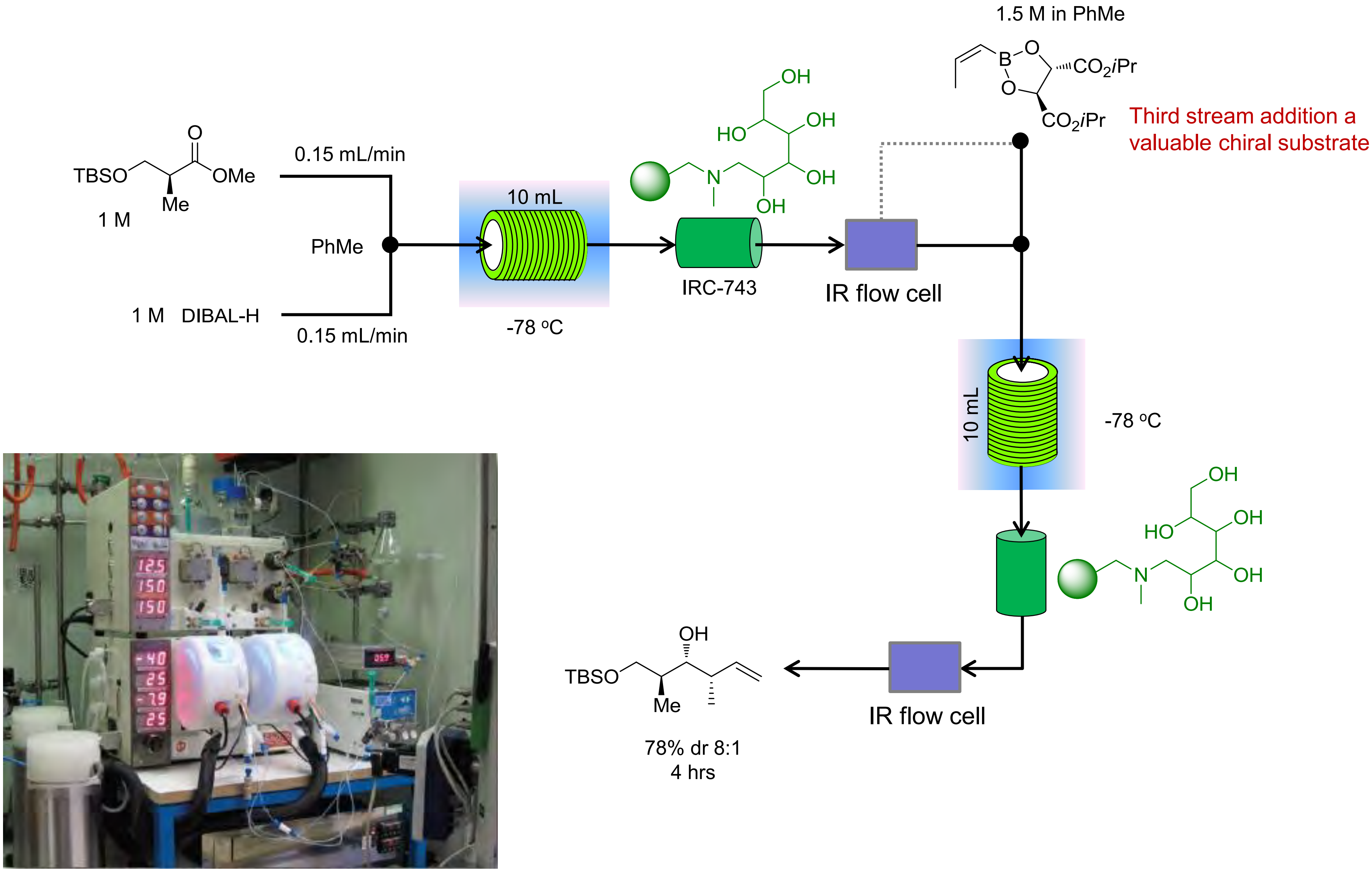
The third stream issue





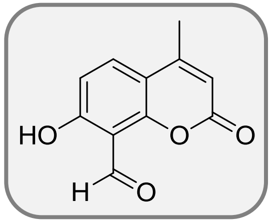
Chem. Sci., 2011, 2, 765
OPRD 2010, 14, 393

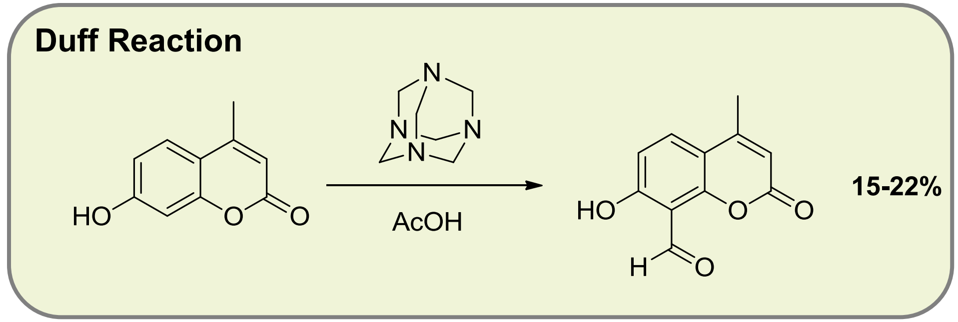
The third stream issue

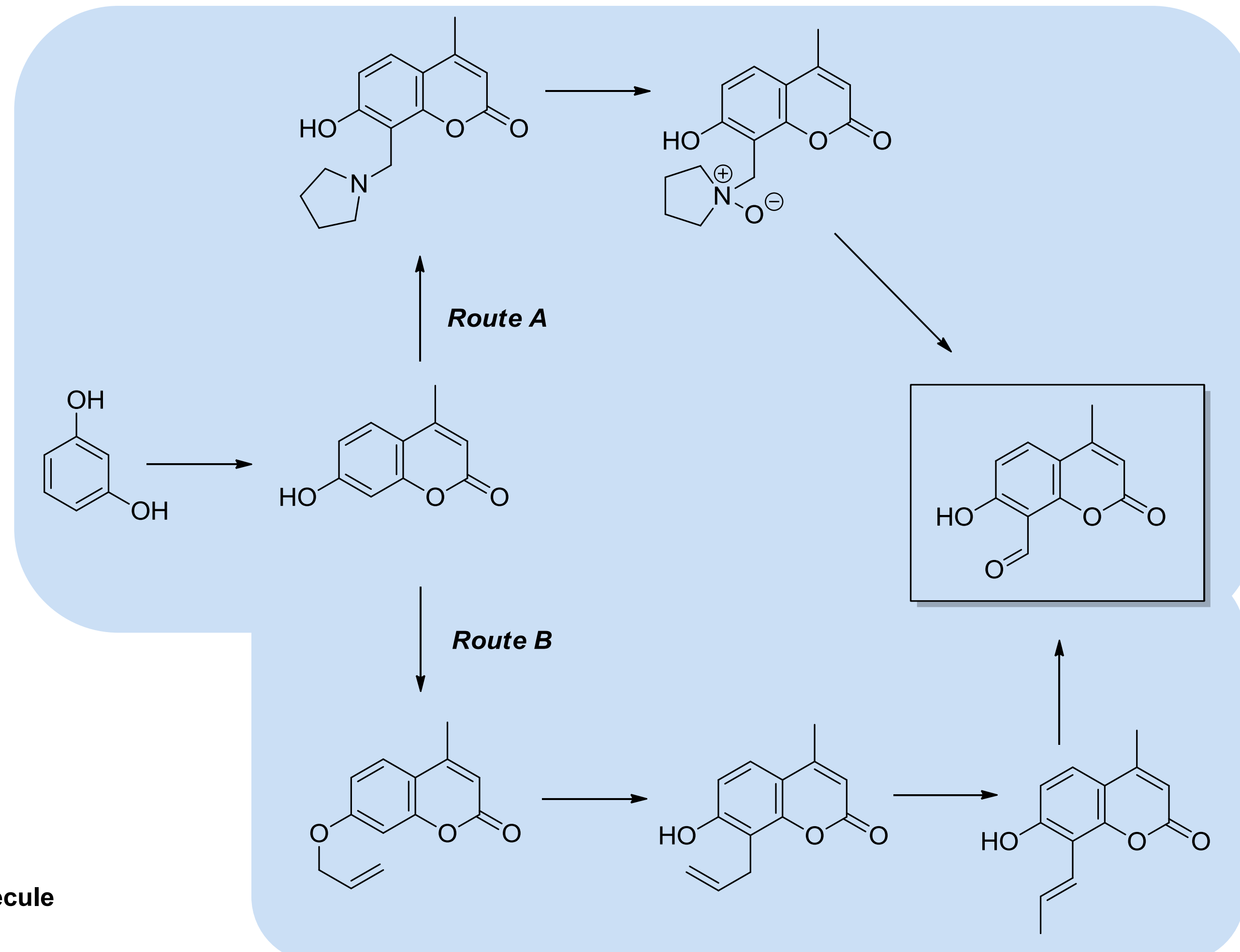


Chem. Euro. J., 2011, 17, 3398
OPRD 2010, 14, 393

Synthesis of an IRE-1 binding probe





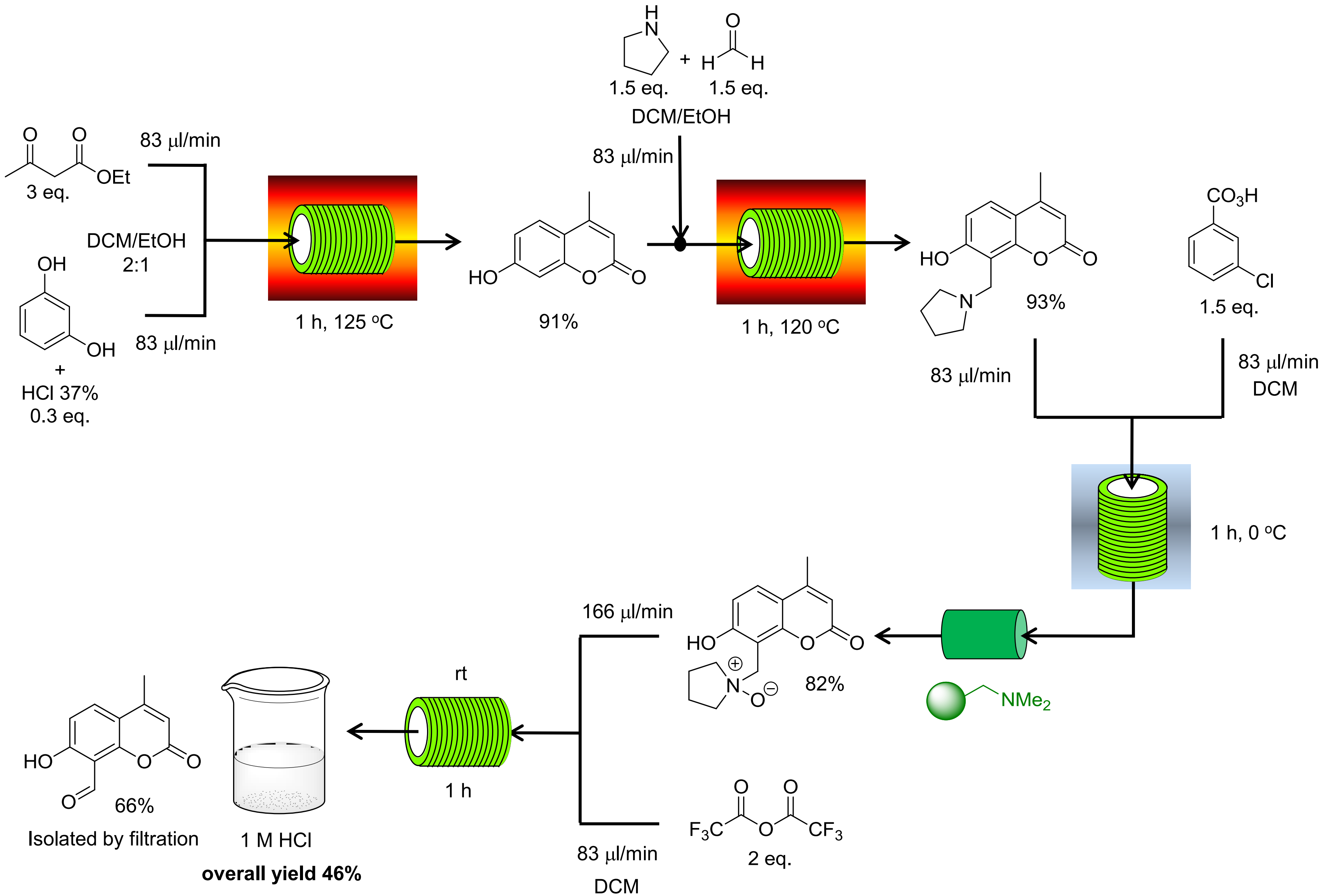


- Cheap starting materials/reagents
- Scaleable via the same route
- Highly reproducible
- Automated synthesis
- Avoid work-up/purification

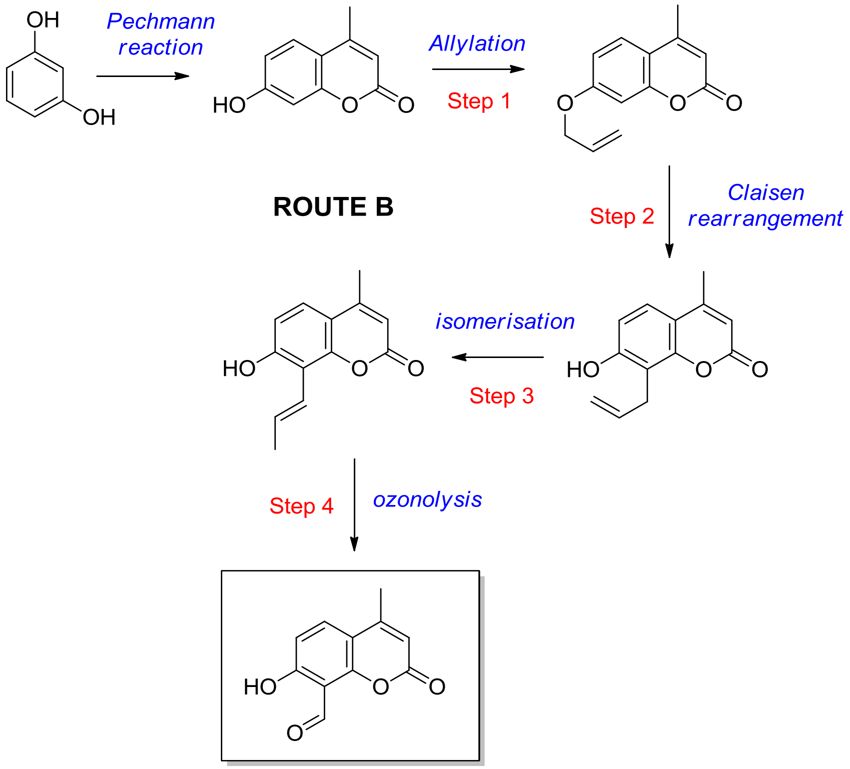
Selective inhibition of unconventional mRNA splicing by an IRE1-binding small molecule

PNAS**,** 2012**,** doi:10.1073/pnas.1115623109

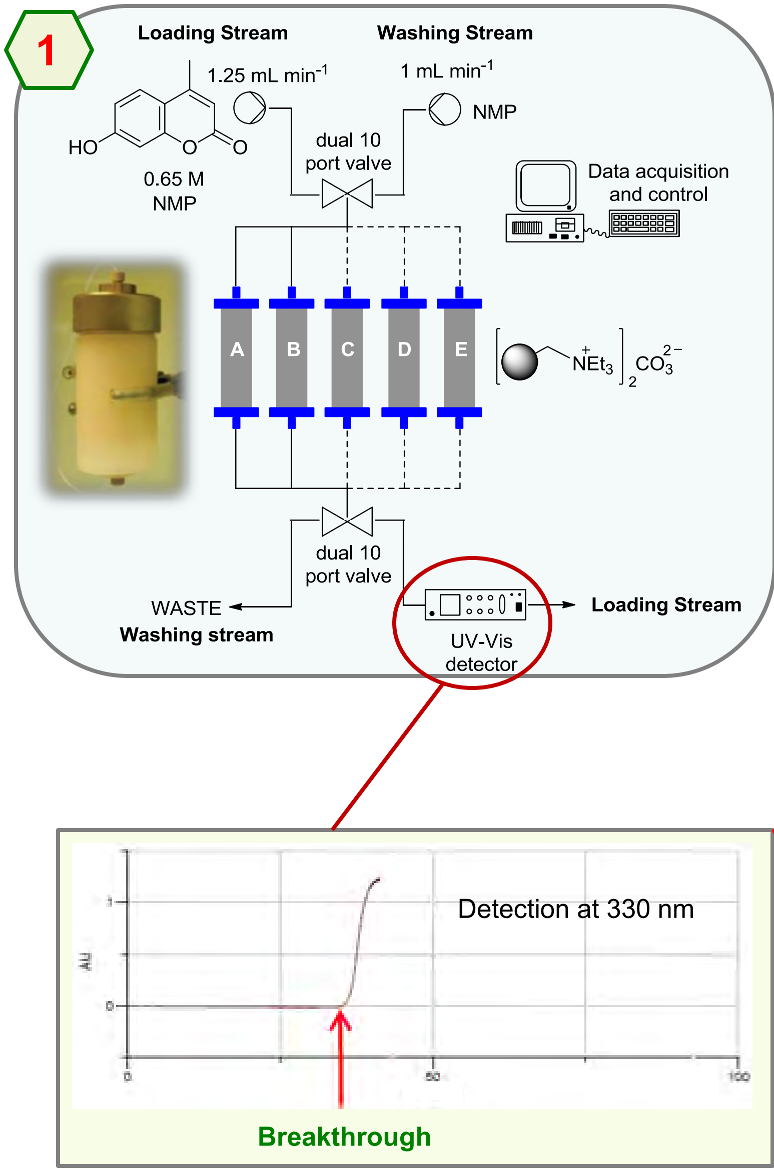
Route A: *N*-oxide Derived Synthesis

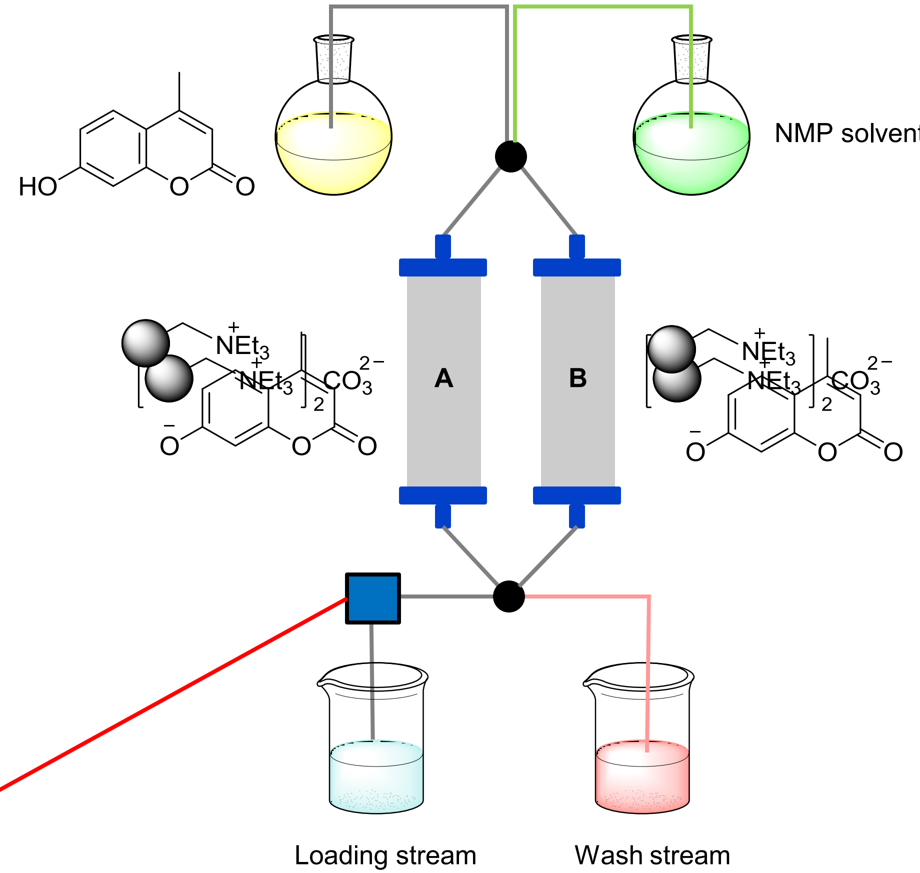


Route B: Claisen Rearrangement



Route B: Claisen Rearrangement

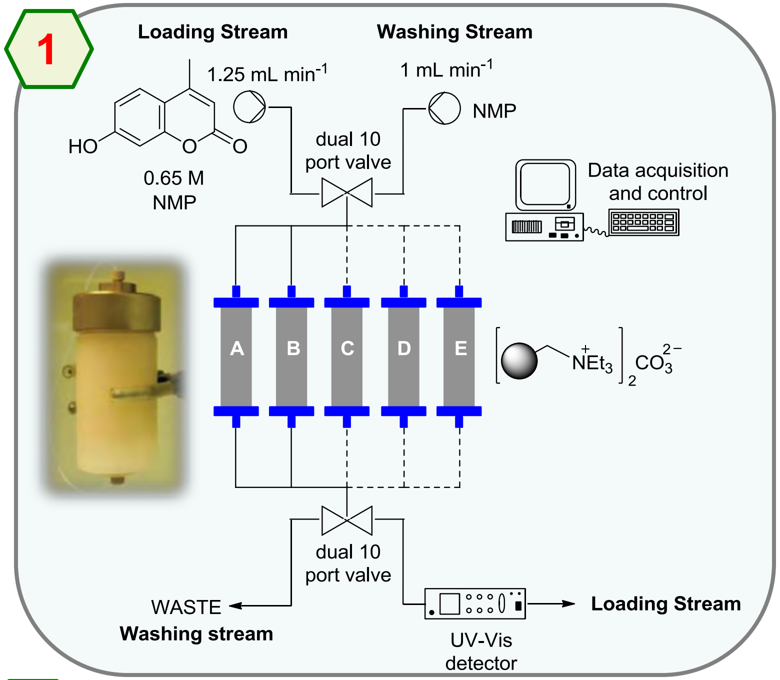


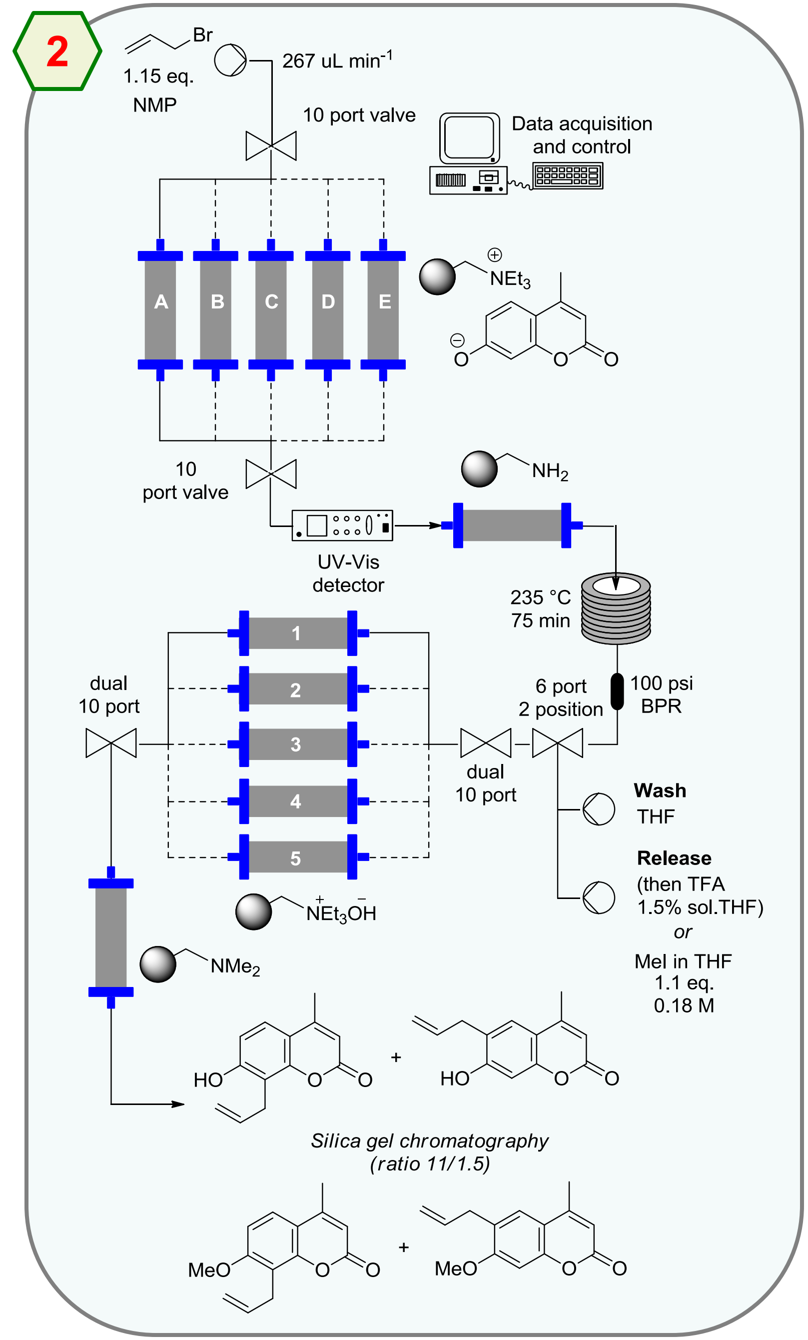


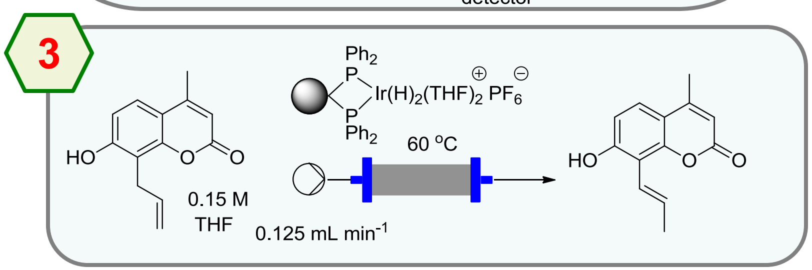
10 cartridges loaded sequentially in a fully automated procedure

~ 2 M of substrate

Route B: Claisen Rearrangement

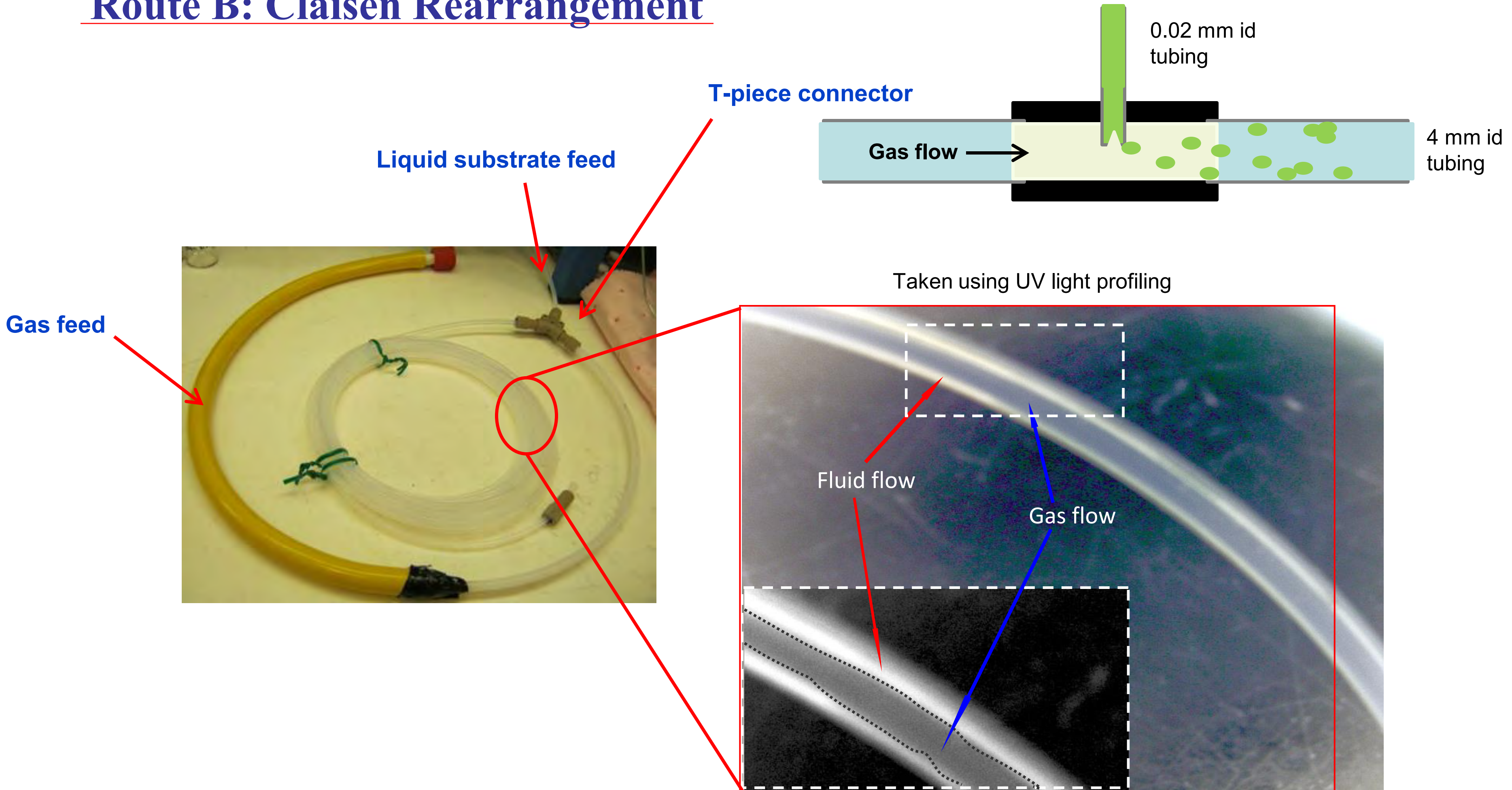


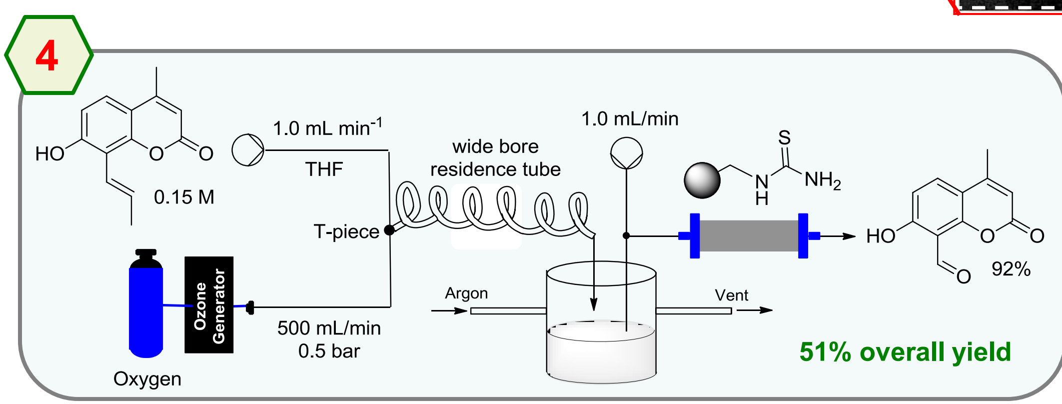




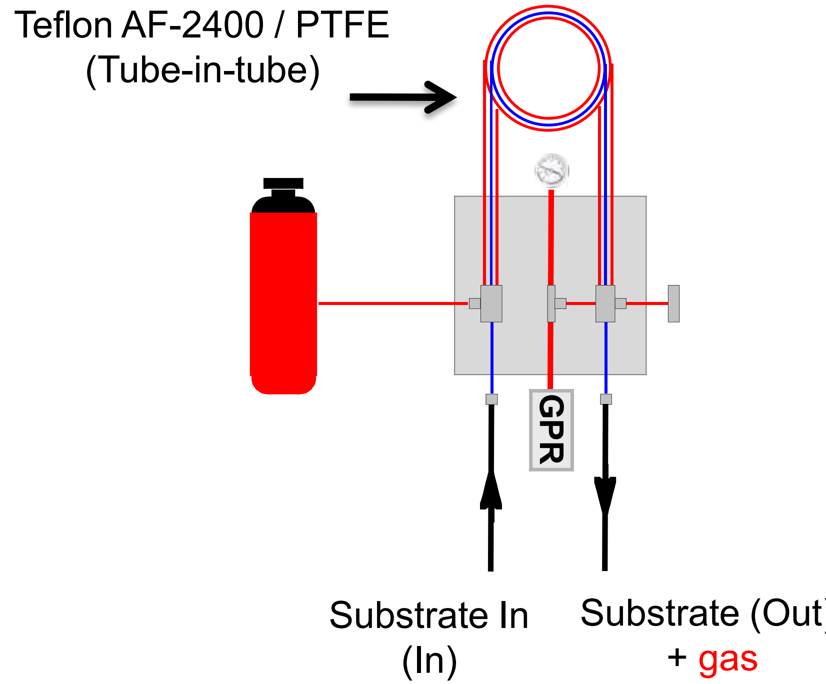
Chem. Euro. J. 2012, 32, 9901-9910

Route B: Claisen Rearrangement

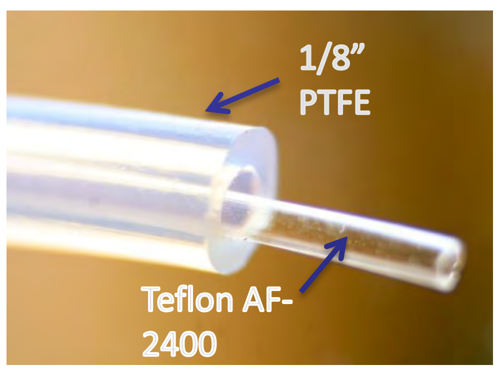


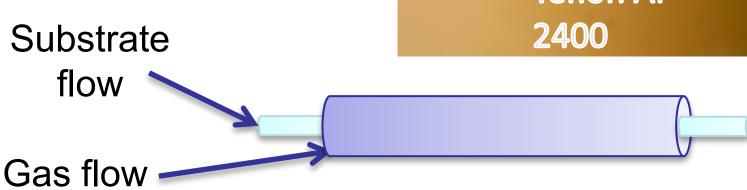


Tube-In-Tube Gas-Liquid Flow Reactor



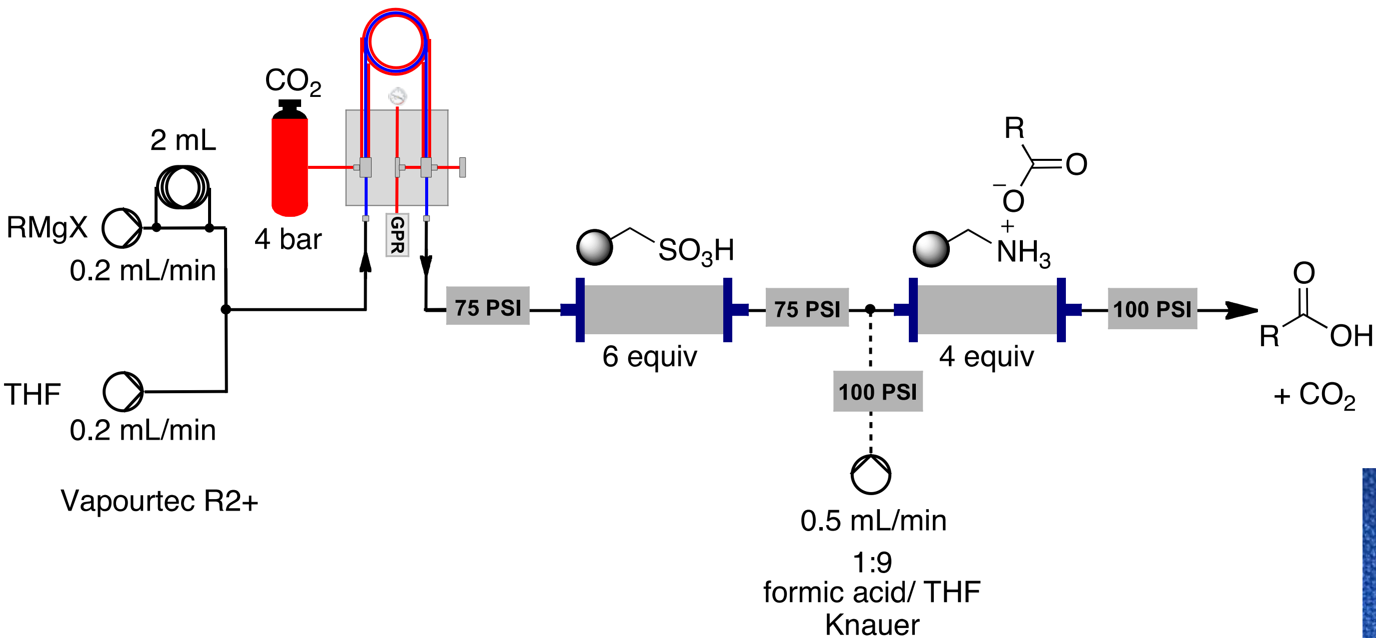
- reactor volume 0.28-0.56 mL (1-2 m Teflon AF-2400)
- gas pressure 10-35 bar
- flow rates 0.1-10 mL/min

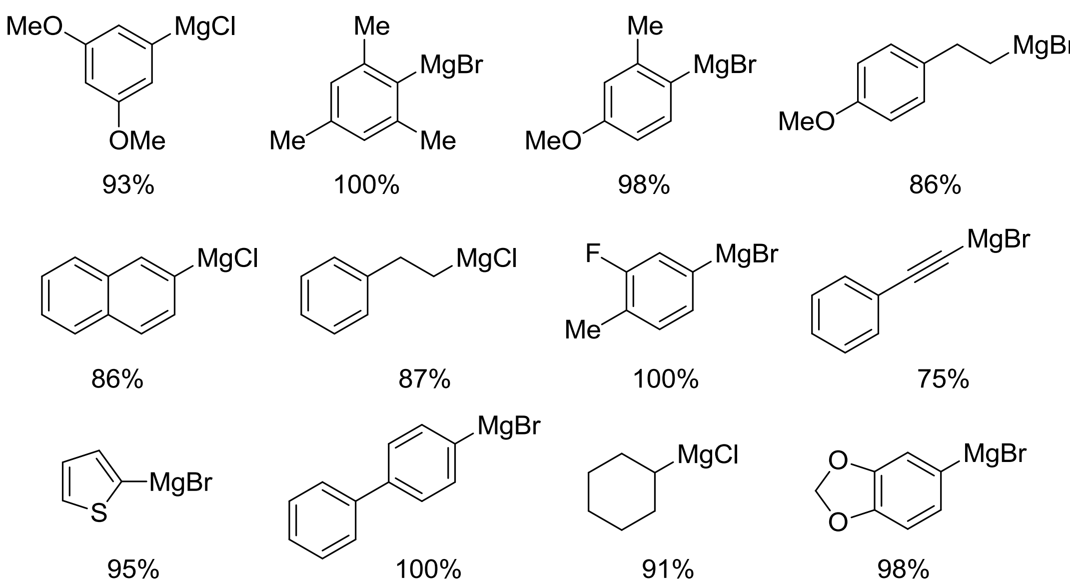




Angew. Chem. Int. Ed., 2011, 49, 1190.

Carbonylations of Grignard Reagents

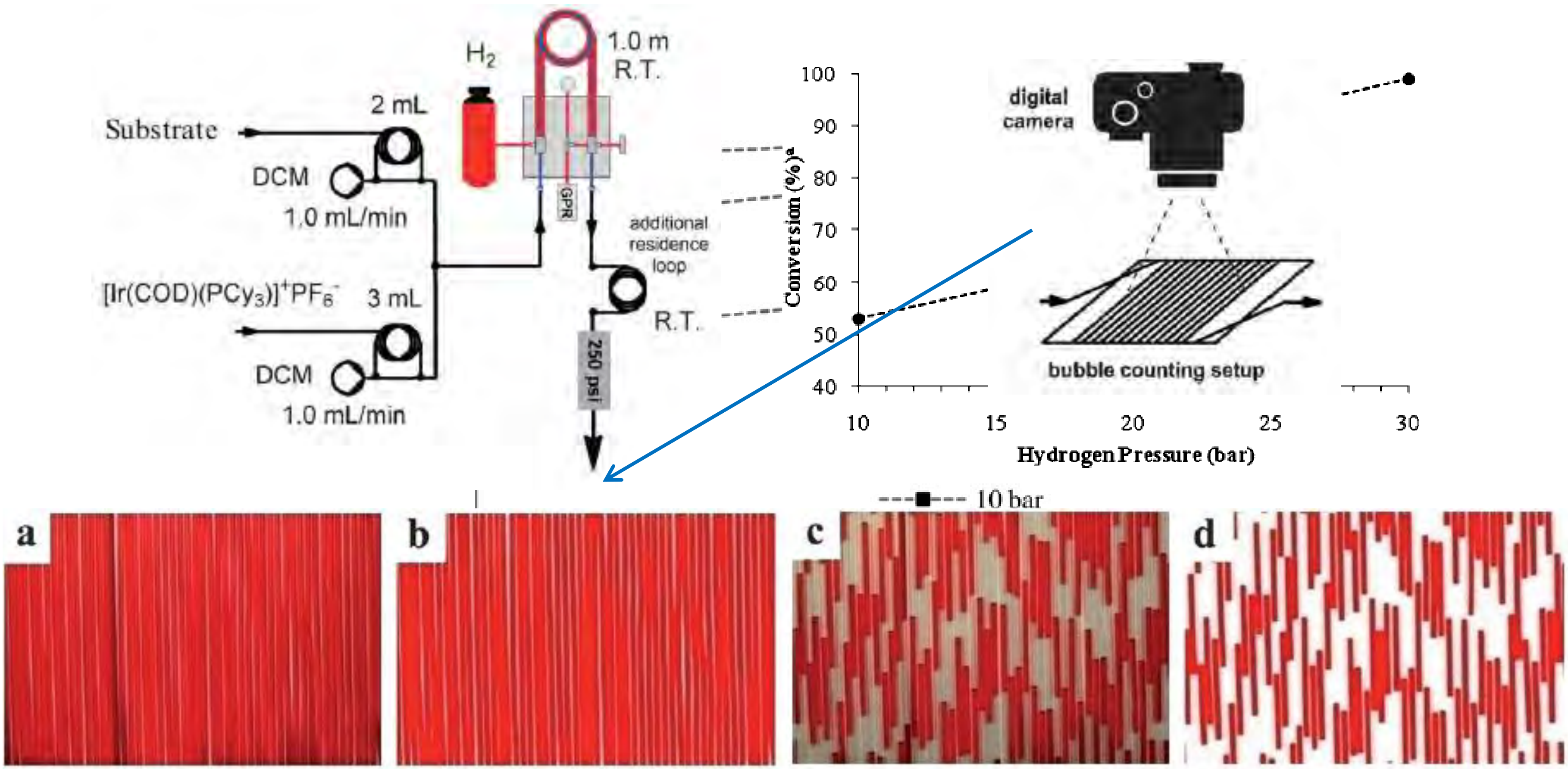




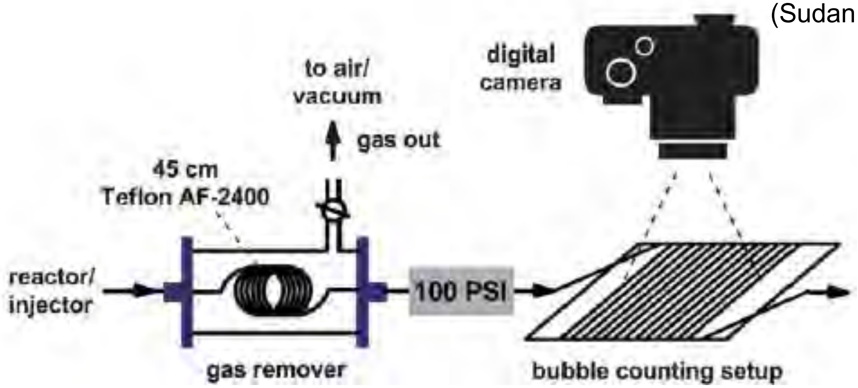
5-8 g resin

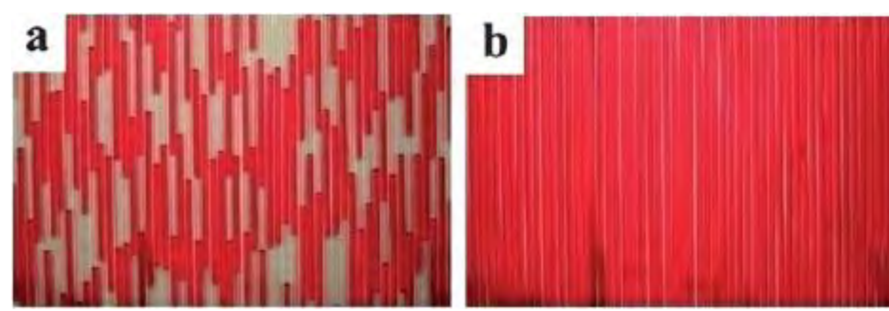
55-65 g of resin

Hydrogenations using a Tube-in-Tube reactor

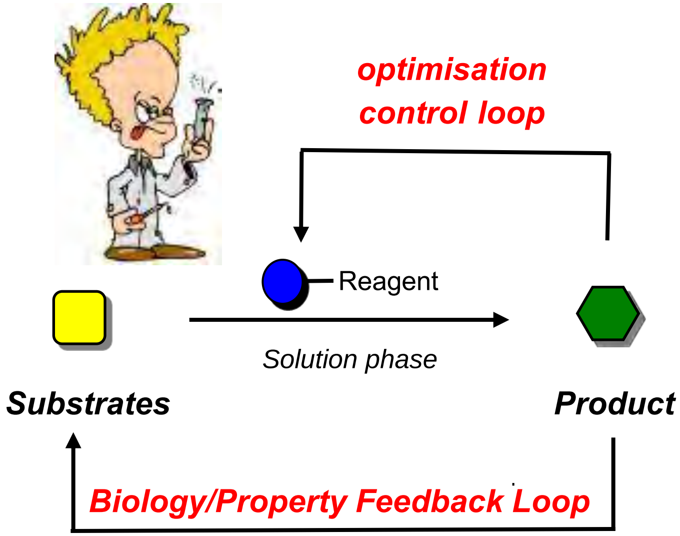


(Sudan red 7B in DCM)



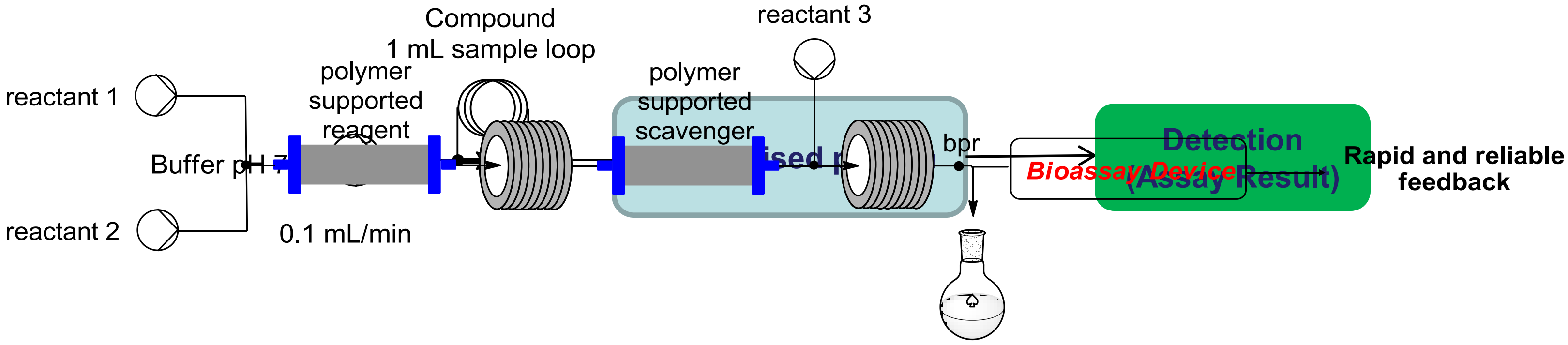


Proof of Concept Design



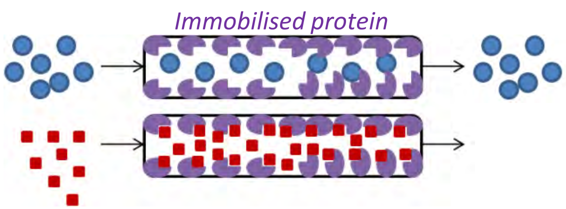
What to make

- SAR
- ADME/Tox
- Chemotype profiles
- Validation



Frontal Affinity Chromatography

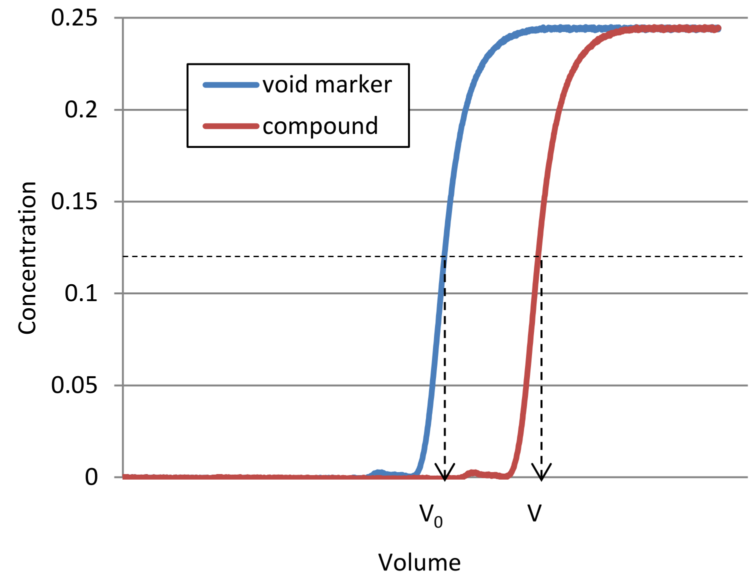
- Continuous infusion of analyte over immobilised target
- Injection of several concentrations gives access to K_d
- Compatible with existing Flow Chemistry platforms
- Method to calculate B_t directly: biocatalyst application

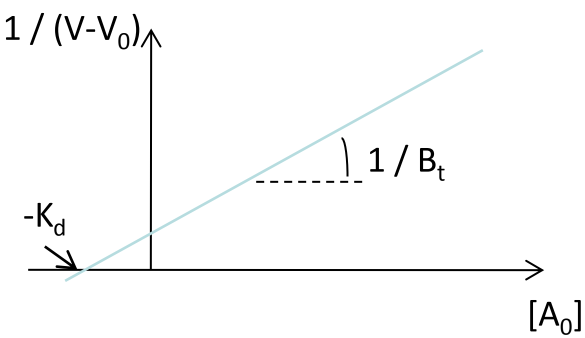


No affinity = No retention (V_0)

Affinity = Retention (V)

($V-V_0$) = Breakthrough volume α affinity



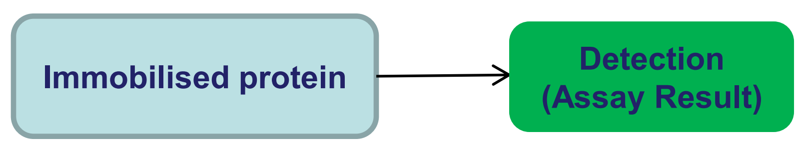


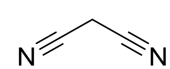
$$1 / (V-V_0) = (1 / B_t) [A_0] + K_d / B_t$$

B_t = amount of immobilised protein

Amount bound give access to K_d using:

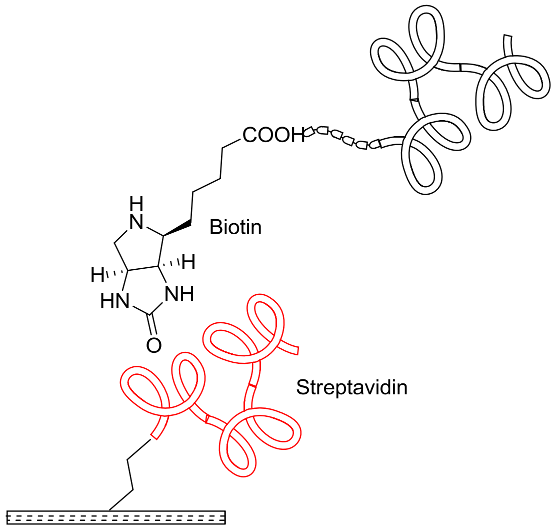
$$[A_0] (V-V_0) = \frac{B_t [A_0]}{[A_0] + K_d}$$





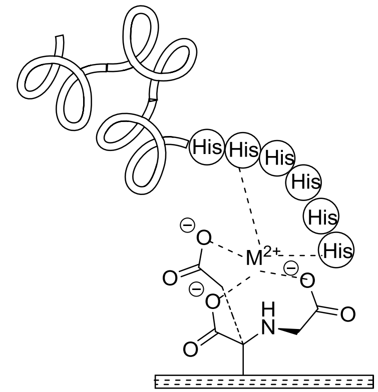
Void marker

Anal. Biochem. **2003**, 1-12



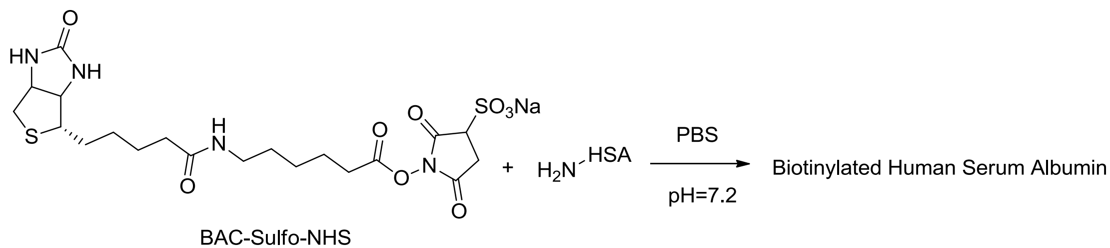
Biotin-streptavidin $k_d=10^{-15}$

- MALDI-TOF was used for characterisation of biotinylated HSA



Hexahistidine tagged protein
Weaker interaction, Cu strongest

- His-tag not strong enough, protein is washed out



Downsizing the column:

2.5-23 µL

15 µL

31 µL

175-353 µL

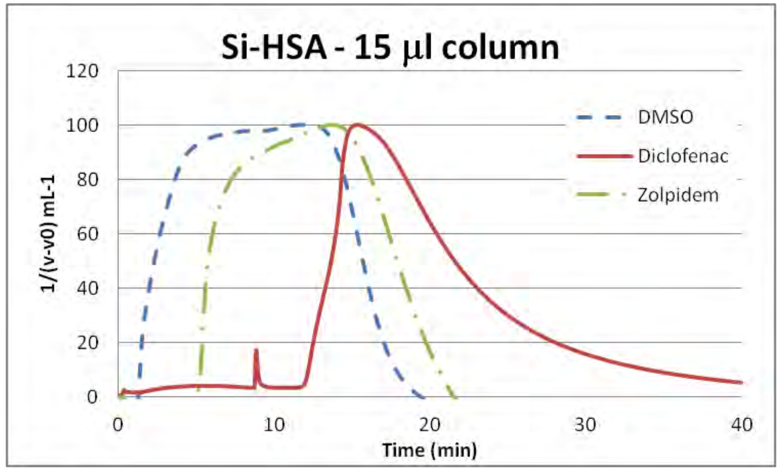
Objective:

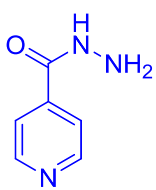
To assess the system using Human Serum Albumin (HSA)

HSA column

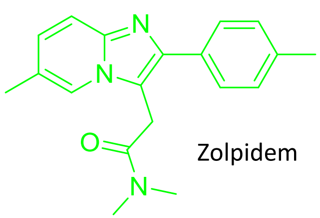
(1 mm x 2 cm)
volume of 15 µl

10 mM sodium phosphate
2.7 mM KCl
137 mM NaCl , pH = 7.4

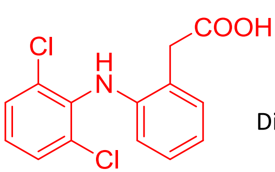




Isoniazid



Zolpidem

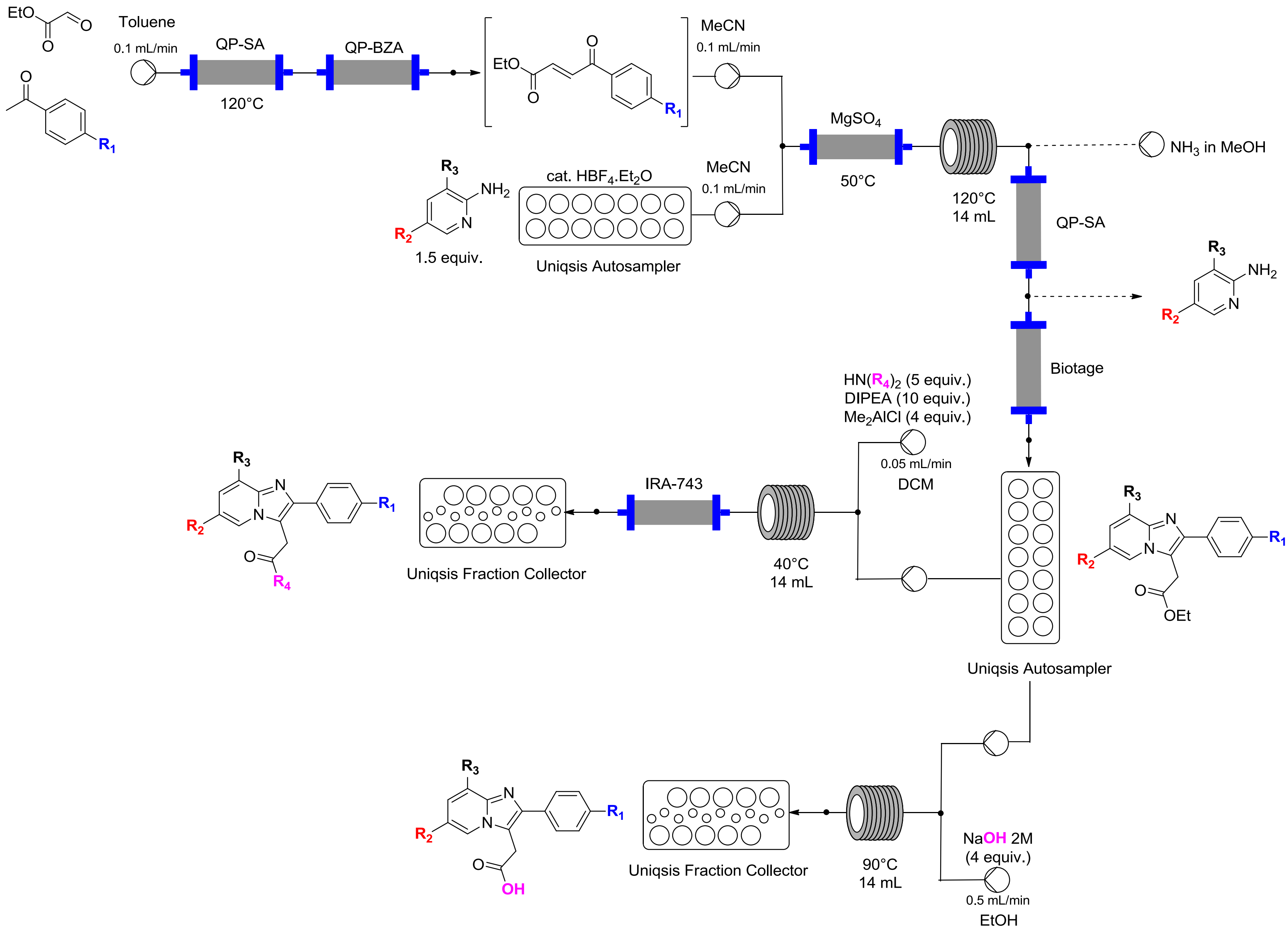


Diclofenac

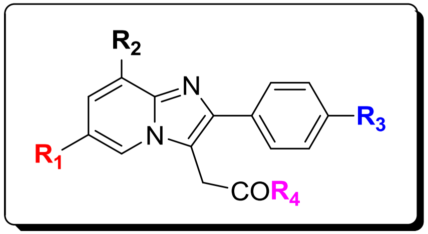
Molecule	Bound in plasma (%)	Rt Volume	Estimated Kd (microM)
DMSO	-	127 µL	- V_o
Isoniazid	0	130 µL	-
Zolpidem	92	414 µl	52
Diclofenac	99.8	838 µl	1.2

- Diclofenac, zolpidem and DMSO were retained with the correct order and reproducibly
- The assay was used to evaluate the binding of a series of $GABA_A$ agonists to HSA

Automated Flow Synthesis of GABAA Ligands

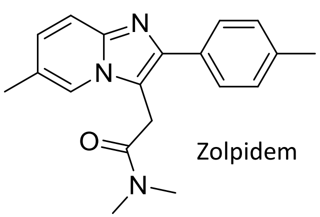


Synthesised GABAA Ligands



	R_1	R_2	R_3	R_4	V (µL) @ 8 µM	K_d (µM)	B_t (nmoles)
DMSO	-	-	-	-	115.9	-	-
1	CH_3	H	CH_3	NMe_2	430.1	60	20.5
2	CH_3	H	CH_3	OH	680.5	25	21.5
3	CH_3	H	CH_3	NPr_2	520.2	42	32.8
4	Cl	H	Cl	NMe_2	456.0	64	24.9
5	Cl	H	Cl	OH	822.5	37	31.2
6	H	H	CH_3	NMe_2	267.5	152	23.5
7	H	H	CH_3	OH	428.9	49	18.0
8	CH_3	H	Cl	NPr_2	552.2	79	36.6
9	CH_3	H	Cl	NMe_2	425.1	78	26.2
10	CH_3	H	Cl	OH	486.4	57	22.9
11	Cl	H	CH_3	NMe_2	332.4	88	20.2
12	Cl	H	CH_3	OH	494.3	40	17.5
13	CH_3	Br	CH_3	OH	203.7	33	14.4
14	CH_3	Br	CH_3	NMe_2	378.4	84	24.1

K_d (µM) 52



Zolpidem

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