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Selected case studies in Pharmaceutical Process R&D: from Quality by Design to Sustainable Chemistry.

Mariano Stivanello

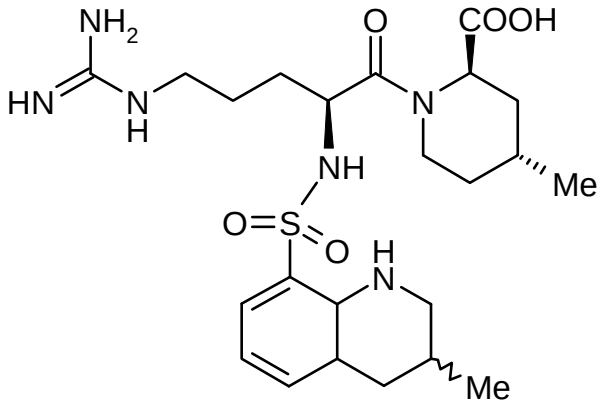
Process R&D Department

THREE SELECTED CASE STUDIES

- **Pharmaceutical Process R&D**: a novel synthetic approach to the key intermediate of Argatroban monohydrate
- **Quality by Design**: application of RAMAN spectroscopy in the optimization of the enantiomeric separation of the key intermediate of the Escitalopram process
- **Green and Sustainable Chemistry** in the Development and Optimization of a novel API process

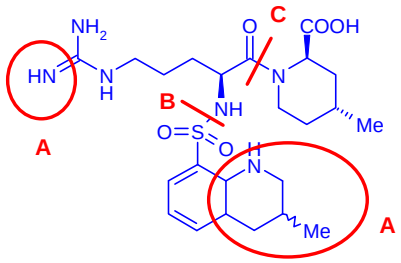
ARGATROBAN MONOHYDRATE (Novastan®)

- Highly selective direct **thrombin inhibitor**
- **Anticoagulant** for the treatment and prophylaxis of thrombosis in patients with *heparin-induced thrombocytopenia (HIT),* a complication of heparin therapy.
- Type 2 HIT particularly is an *immune-mediated disorder* that has life- and limb-threatening thrombotic complications
- Discovered and developed by **Mitsubishi (JP)**
- Approved by FDA in 2000, now Generic API
- Co-marketed by **Glaxo-Smith Kline (GSK)** in all major countries
- Synthetic dipeptide
- Low-volume & high-price API
- **Crystal structure: 2:1 diastereomeric mixture (66/34) monohydrate salt**



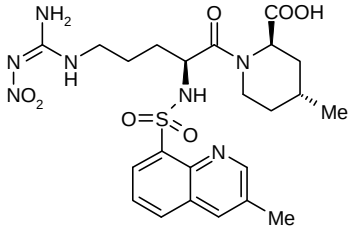
ARGATROBAN MONOHYDRATE

ARGATROBAN retrosynthetic analysis



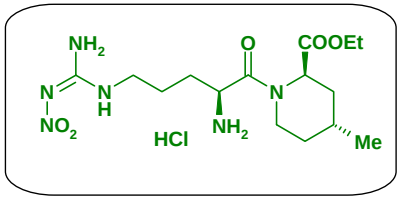
ARGATROBAN MONOHYDRATE

A



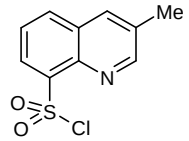
ARGA6

B



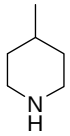
ARGA4

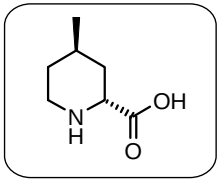
+



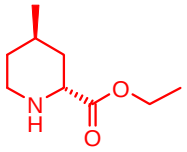
QSC

C



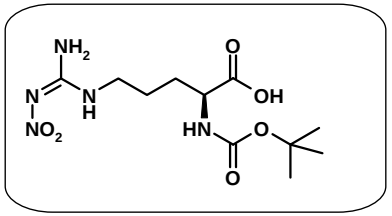


TRANS 4-METHYL-
2-PIPECOLIC ACID



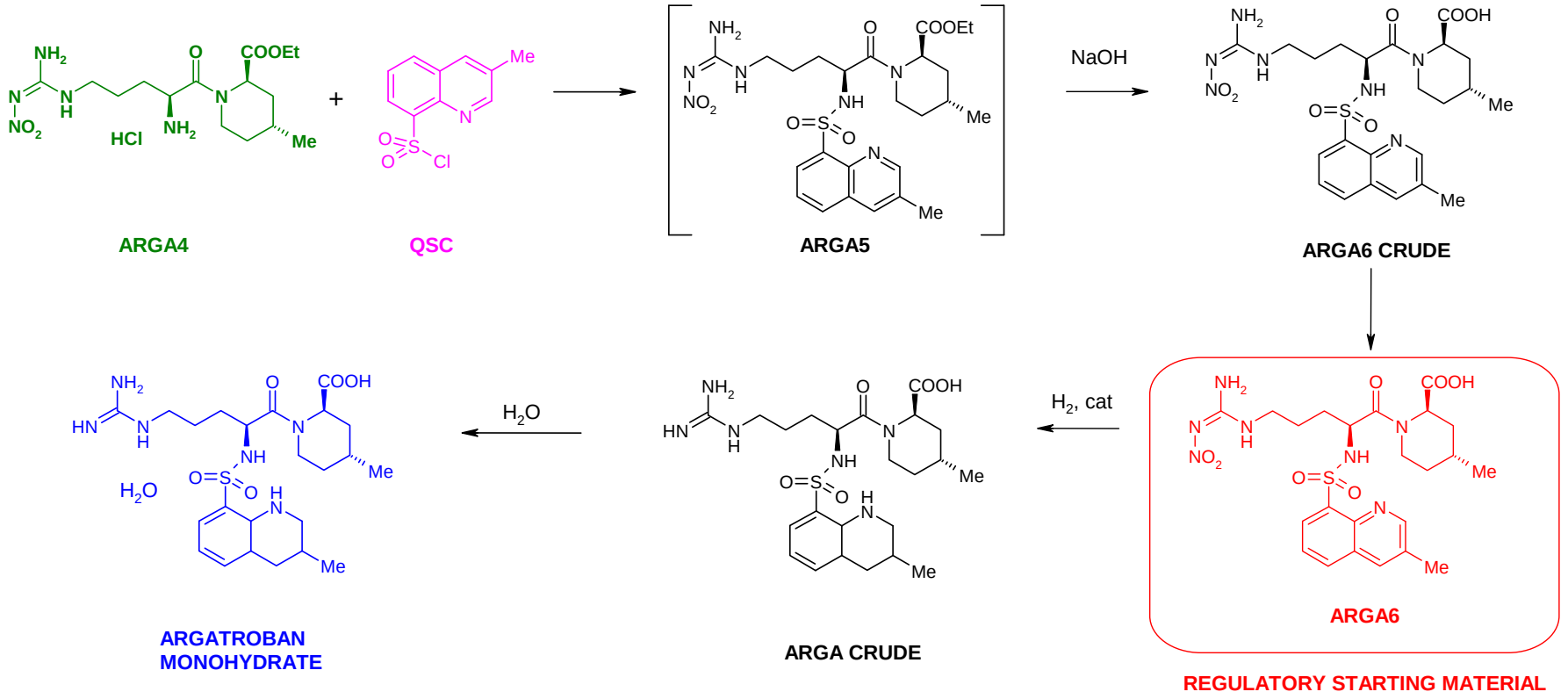
ETHYL (2R,4R)-4-METHYL-2-PIPECOLATE

+



N-Boc-N'-NITRO-L-ARGININE

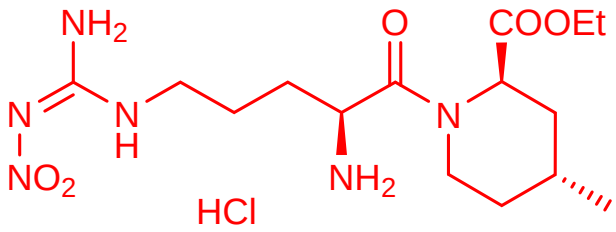
ARGATROBAN: LUPI CURRENT GMP ROUTE



Key intermediate ARGA4

COMMERCIAL ISSUES:

- Single supplier (Far East)
- supply chain risk issue
- Very high cost

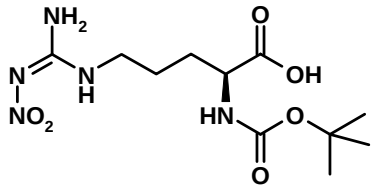


ARGA 4

STRATEGY and TARGETS:

- In house production
- reduced risk
- cost reduction

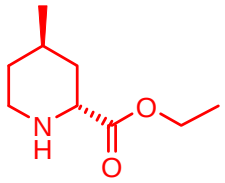
ARGA4 synthesis via enantiopure 4-methyl-2-pipecolic ester



Boc-nitro-arginine

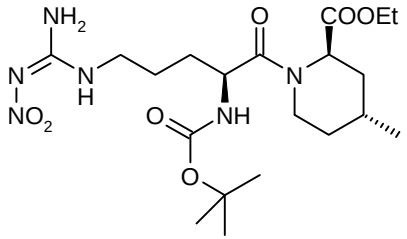
BOC-L-ARG(NO2)-OH

+



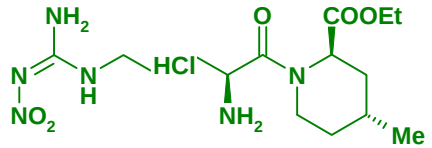
(2R,4R)-4-methyl-2-pipecolic acid ethyl ester

COUPLING



ARGA3

DEPROTECTION

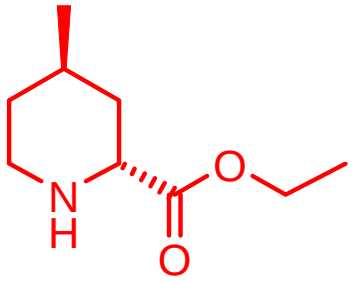


HCl

ARGA4

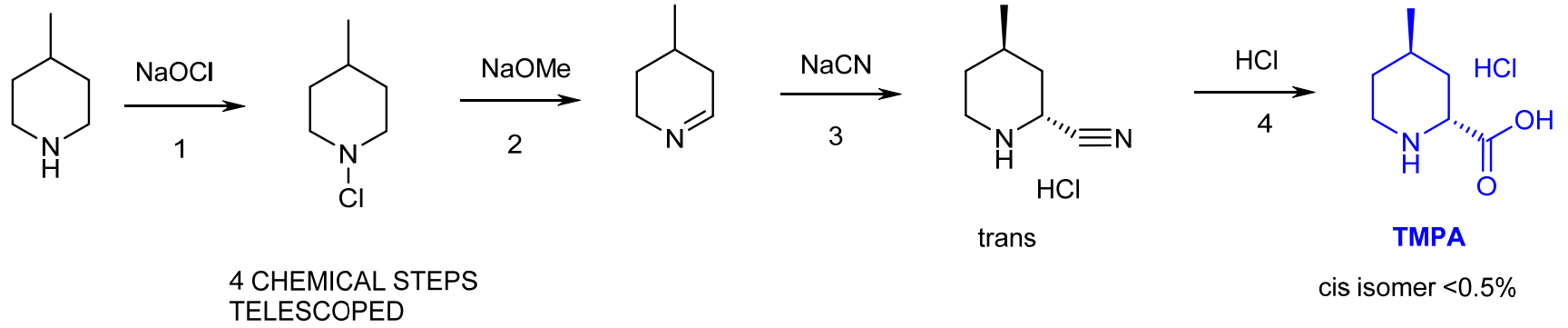
Ethyl (2R,4R)-4-methyl-2-piperidinecarboxylate

- Two stereo centres present: trans and cis configuration (a total of 4 stereoisomers)
- Sourcing of both enantiopure ester and carboxylic acid proved to be difficult (few suppliers, unsustainable high prices)
- In literature the enantiopure ester is obtained by:



  - **Enantioselective syntheses:** multistep, from costly starting materials, not suitable for multi-Kg production
  - **Enantioselective hydrogenation** of 1,2,5,6-tetrahydro-4-methyl-2-pyridinecarboxylic acid derivatives (costly)
  - **Classical resolution** of the corresponding racemic trans ester with *L-tartaric acid* (expired Mitsubishi JP patent)
  - **Enzymatic resolution** of racemic esters with lipase or protease (patented)

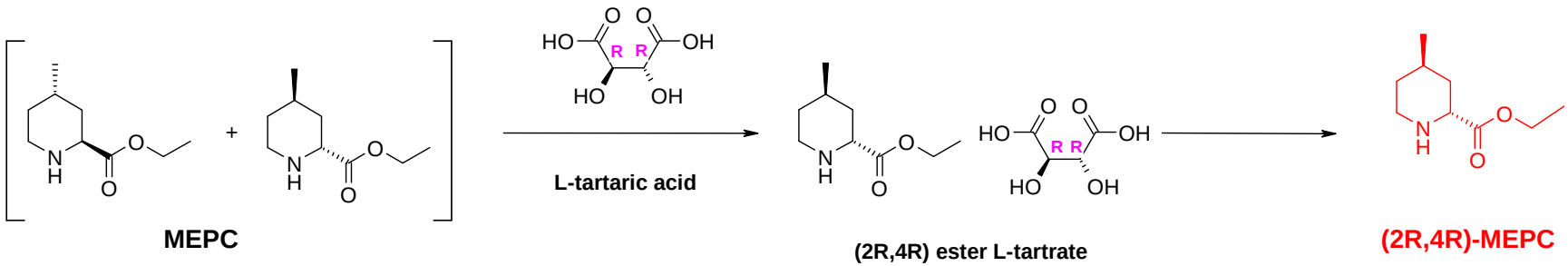
Synthesis of racemic trans-4-methyl-2-pipecolic acid (TMPA) and its ethyl ester



OVERALL YIELD to TMPA: ca. 40% mol

- Original synthesis of racemic trans 4-methyl-2-pipecolic acid/ester is not straightforward
- Few new shorter, more effective routes recently published and/or patented
- **TMPA** intermediate:
  - in-house produced
  - Outsourced (different process)

Ethyl (2R,4R)-4-methyl-2-piperidinecarboxylate resolution process

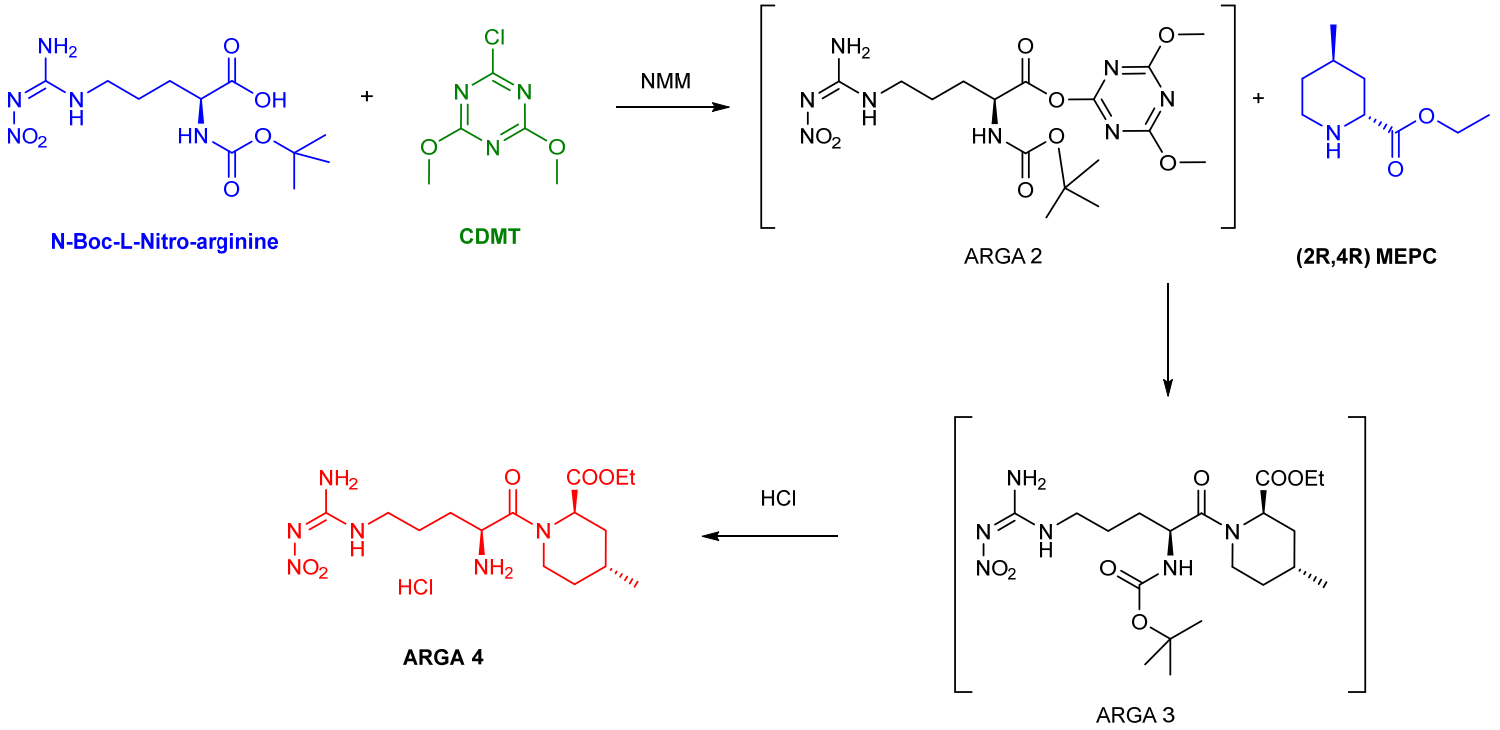


- Original Mitsubishi patent (expired) uses **L-tartaric acid** as resolving agent
- Best exp. conditions afforded the diastereomeric salt in low yield and purity
- Screening of different chiral acids failed !
- Original resolution was optimised in LUPI R&D with the help of the Raman in-line probe.
- Final LuPI lab process:
  - Crude L-tartrate salt was isolated with low enantiomeric purity (ca. **70%** e.e.)
  - Its recrystallization allowed increasing e.e. only up to **90%**

- Overall yield of resolution process remained anyway rather low. (ca. 15% mol, 30% th.)

- Influenced by purity profile of starting racemic ester ?
  → *see Org. Process Res. Dev. 2014, 18, 709−716*

Synthesis of ARGA4 from N-Boc-L-Nitro-arginine and (2R,4R) MEPC

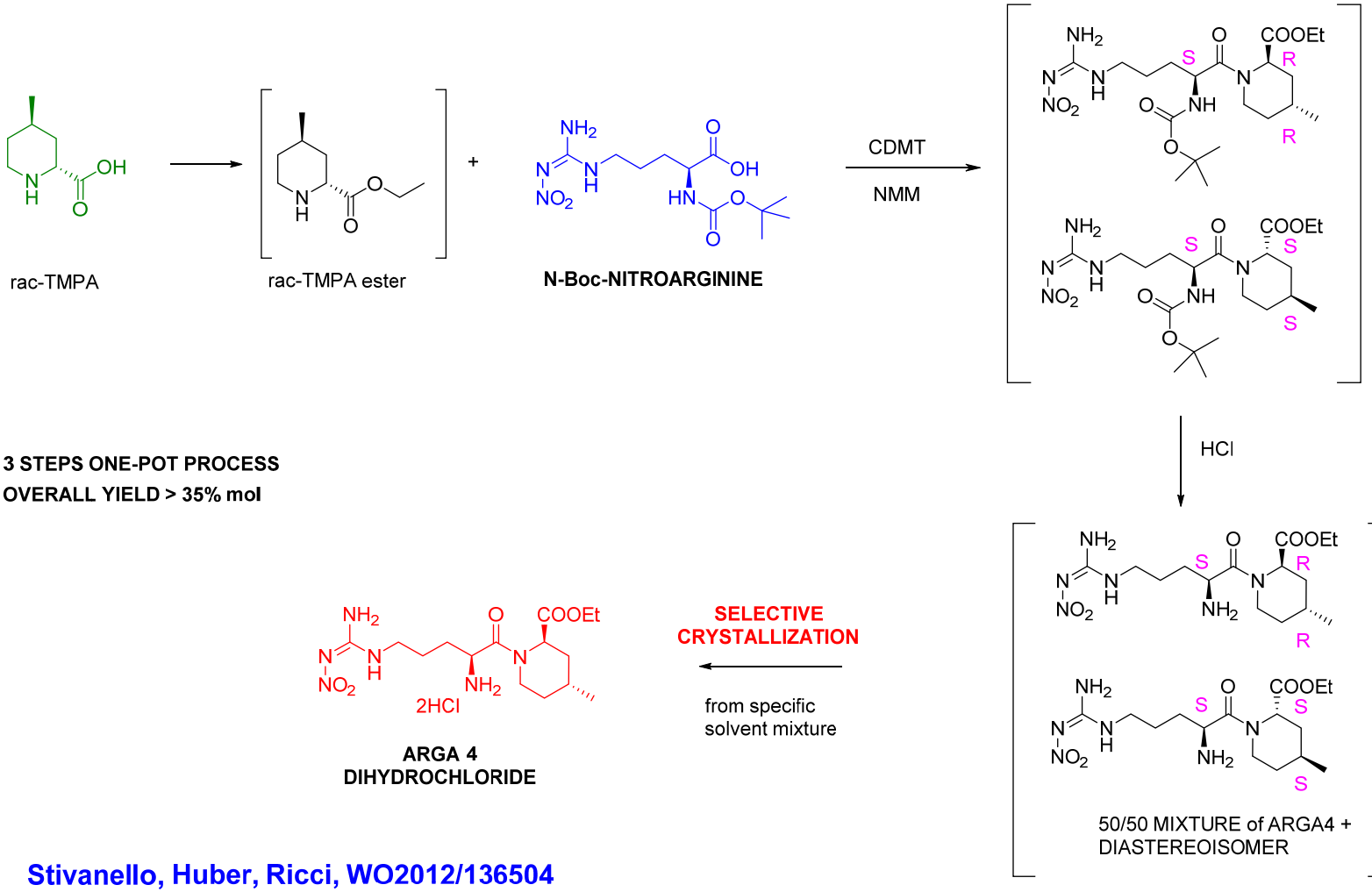


- Very efficient peptide coupling using 2-chloro-4,6-dimethoxy-1,3,5-triazine (inexpensive)
- Higher yield and much cleaner reaction than using other 'old fashioned' and more toxic coupling reagents used in literature (chloroformates, carbodiimides etc).
- Use of (2R,4R) ester afforded a highly pure ARGA4 despite its rather low e.e (90%)

- Even (2R,4R) ester with lower e.e. (70%) afforded ARGA4 intermediate with reasonable purity

- Might be possible to avoid the resolution process?

ARGA4 synthesis: direct route from racemic TMPA



3 STEPS ONE-POT PROCESS
OVERALL YIELD > 35% mol

Stivanello, Huber, Ricci, WO2012/136504

ARGA4 : Highlights of new LuPI approach

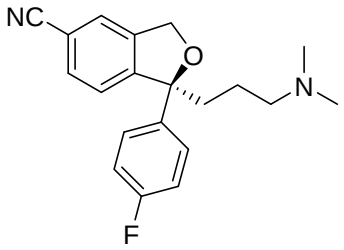
- **Highly diastereo-selective crystallization**:
  - Peptide-like coupling with CDMT + Boc deprotection with HCl allow the isolation of the crude 50:50 mixture of ARGA4 + diastereoisomer with high HPLC purity (>96%)
  - a new crystal form of **ARGA4 dihydrochloride ethanol solvate** is isolated from ethanol or from a specific mixture of **ethanol/ethyl acetate**
  - The solubility difference between the two stereoisomers is so high that the unwanted ARGA4 diastereoisomer is fully removed in the mother liquors
  - ARGA4 is isolated with HPLC purity >99.0% with 0.7% of the unwanted stereoisomer
- **Overall yield** (from racemic TMPA) **doubled** (**39**% mol vs. 18% mol)
- **Patent application WO2012/136504 now granted in major countries:** claiming
  - The whole new process (general and specific conditions)
  - New crystal form of ARGA4 dihydrochloride ethanol solvate

ARGA4 : economical considerations

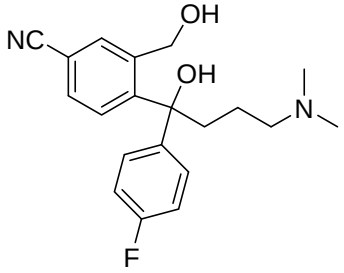
- Direct process **economically more convenient:**
  - Overall yield **doubled**
  - Raw material costs also **30% lower** despite using >2 amount of Boc-nitro arginine
    - Due to costly trans-methyl-pipecolic acid (outsourced or in-house produced) vs. cheaper N-Boc-N'-nitro-L-arginine
- But we have always to consider the **overall industrial cost:**
  - Direct process is more straightforward and shorter (TMPA resolution process with 3 products isolations avoided)
  - Lower **Direct cost** (manpower as hours/Kg of product)
  - Simpler **equipment train** (less reactors and filters used)
  - Reduced **cycle time** and equipment utilization
  - Higher industrial **productivity**
  - Lower **indirect costs** (overheads), e.g. lower costs for QC, waste treatment, etc.

QdB/PAT application in the optimization of a resolution process

- **Escitalopram (Cipralex ®, Lexapro ®)** is a Lundbeck CNS antidepressant

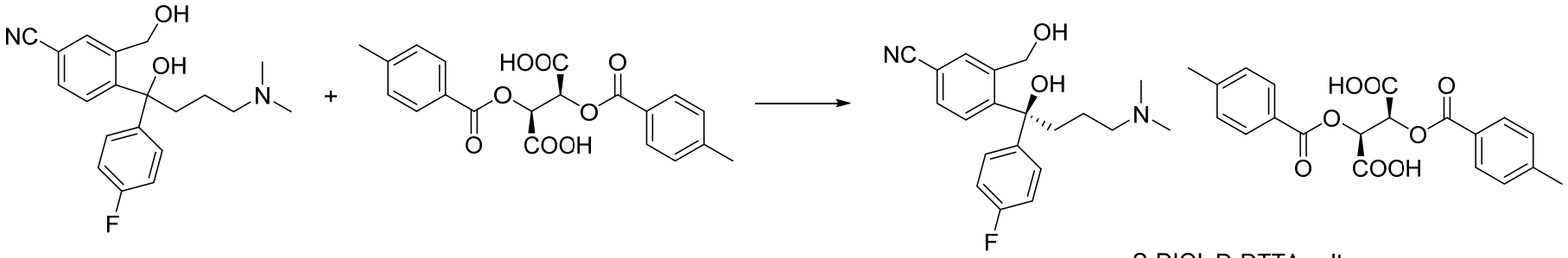


- obtained industrially from the separation of its key racemic-diol intermediate
  - via SMB chromatography
  - via 'classical resolution'



rac-DIOL

The ‘classical resolution’ process or rac-diol



rac-DIOL

D-DTTA
D-(+)-di-p-toluoyl-L-tartaric acid

S-DIOL D-DTTA salt
DIASTEREOMERIC SALT A

Lundbeck Patents: EP0347066B1 (product), US8022232B2 (resolution process)

- Small differences in the **solubilities** of salt A (wanted) and salt B (unwanted)
- Diastereomeric salts A precipitates first, then afterwards also the undesired salts B precipitates.
- Resolution process is therefore **kinetically** and not thermodynamically controlled.
- Isolation of diastereomeric salt A has to be done **prior** to precipitation of B

Goals at LUPI

☺ **Optimization** of crystallization **yield**

☺ Increase **process robustness** by delaying precipitation of unwanted diastereomeric salt B as much as possible in order to obtain salt A with the **highest chiral and chemical purity** possible.

☺ **Industrialization** of the final process (industrial batch size, multi MT/y)

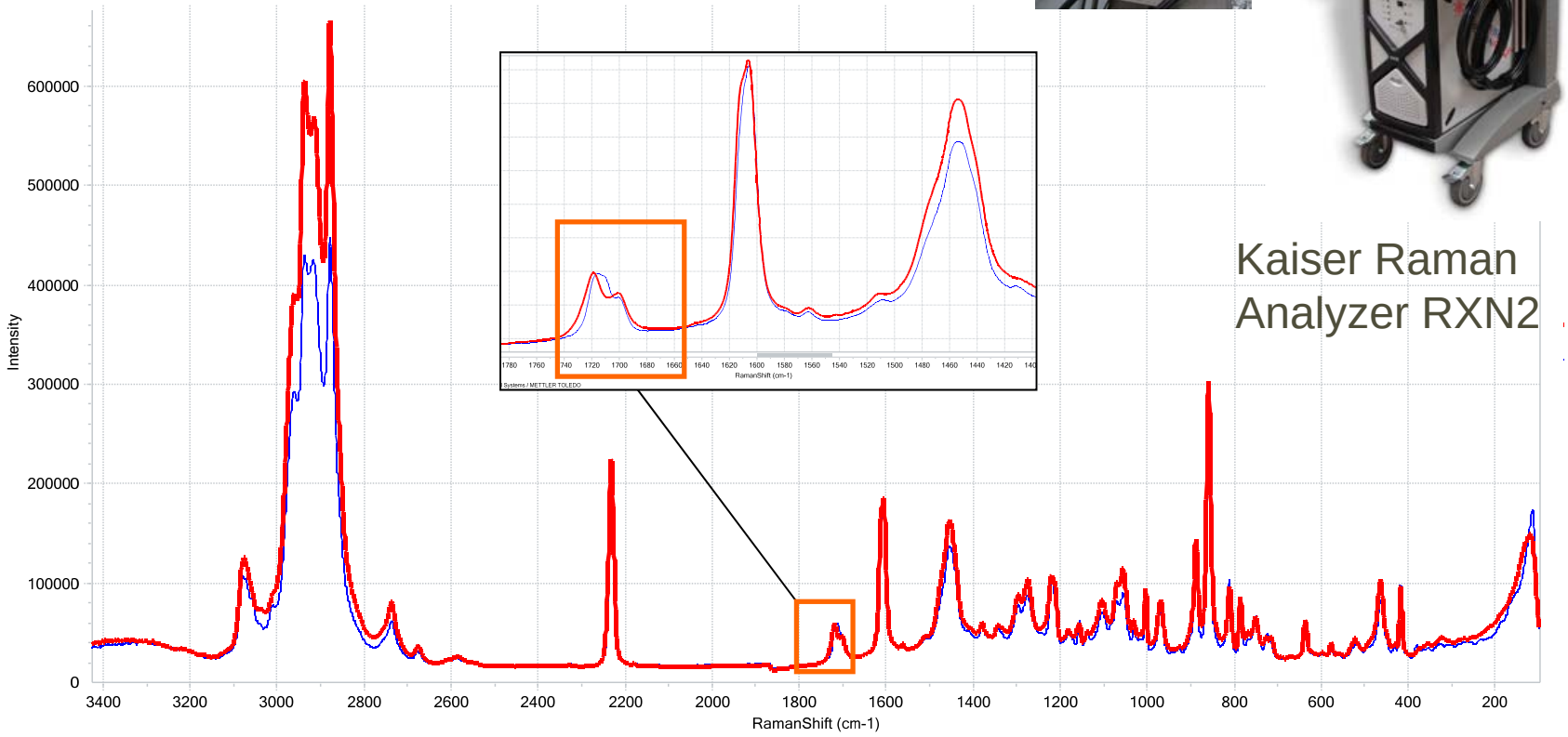
Monitoring of Optical Purity:

- In-process analysis (chiral HPLC)
- Use of **PAT** (Process Analytical Technology) tools

Process Optimisation using Raman in-line probe

Raman spectroscopy proved to be the **only (available) technique** able to distinguish between the two diastereomeric salts in **suspension** of the **resolution mixture.**

Kaiser Raman Analyzer RXN2

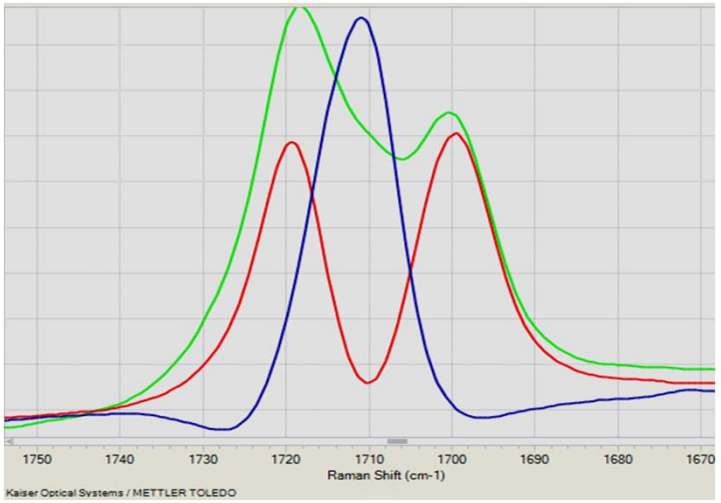


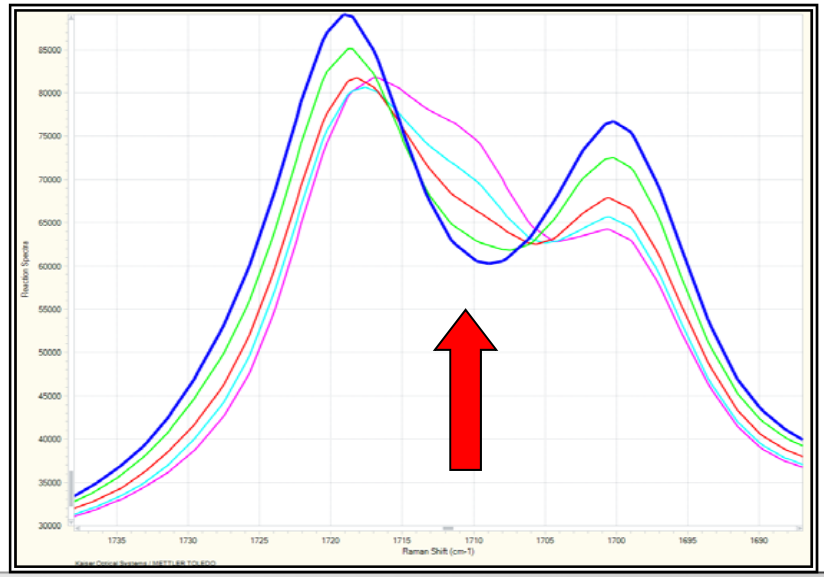
Untreated spectra (sequence of single spectra)

Main problems:

diagnostic peak of unwanted salt B lies **in between** the two peaks of wanted salt A

small changes in the Raman Spectra are **difficult** to follow in the diagnostic region (around 1700 cm-1).

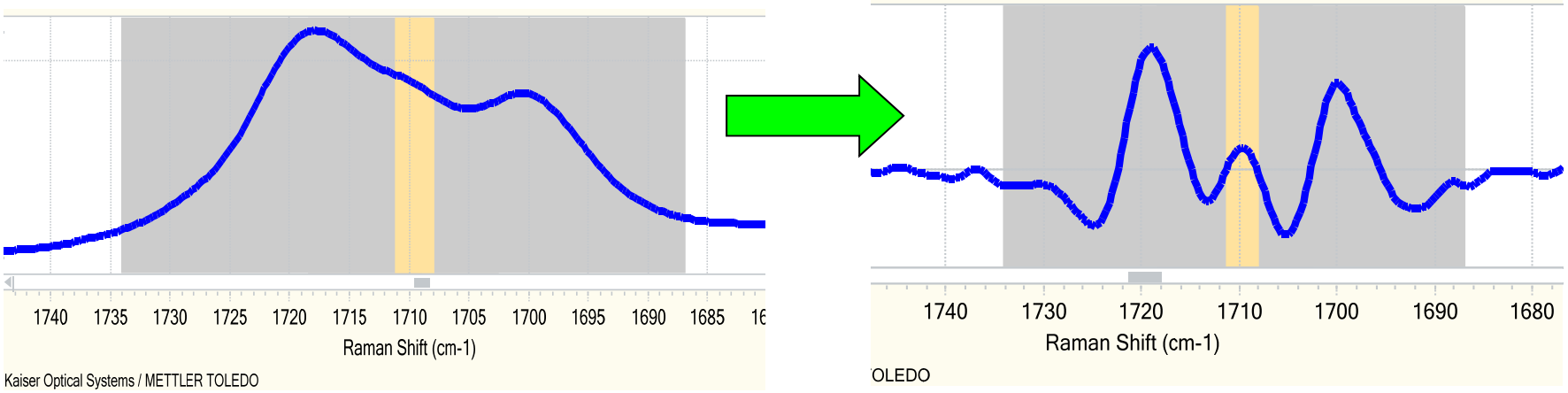




Levels of **B less than 15%** (A%=85%) are difficult/impossible to see.

Treated spectra: application of a second derivative

- RAMAN software (iC Raman) allows to apply a **second derivative** on the entire spectrum.
- This can help to detect levels of **less than 10% B** (A%=90).

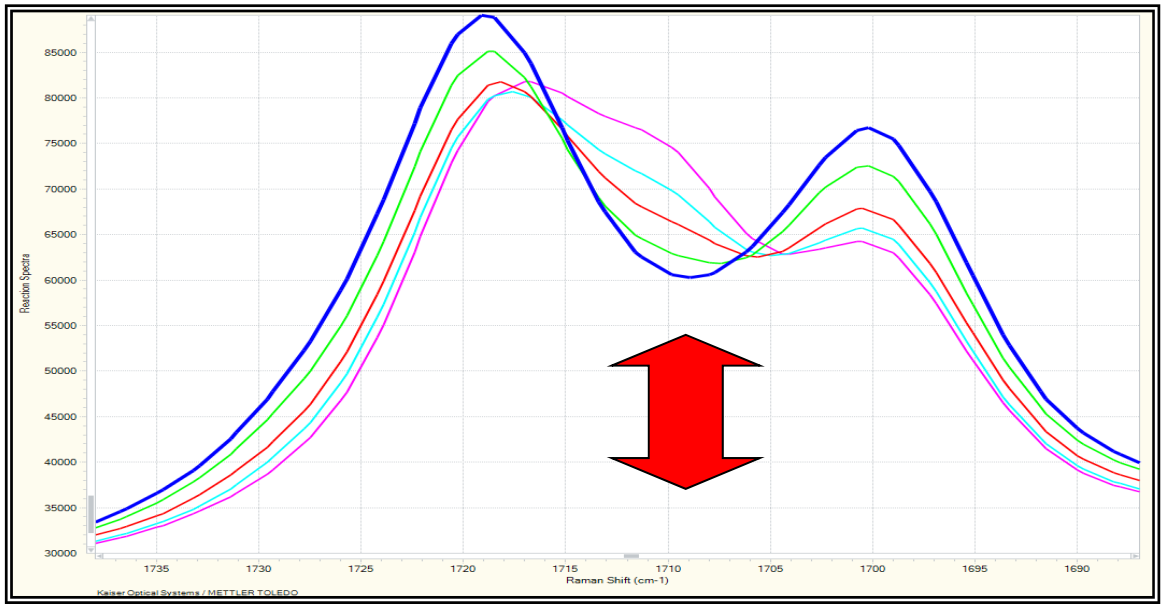


Trend-graph based on optical purity model

A model can be built with the Raman software correlating:

- ☞ **Peak Area Ratios (RAMAN) vs.**
- ☞ **optical purity (HPLC) of isolated in-process samples (composition A vs. B)**
- ☞ **linear correlation with excellent fit (R squared = 0.999)**

Stacked RAMAN spectra



Chiral HPLC

IPC1
97.5 %

IPC2
87.6 %

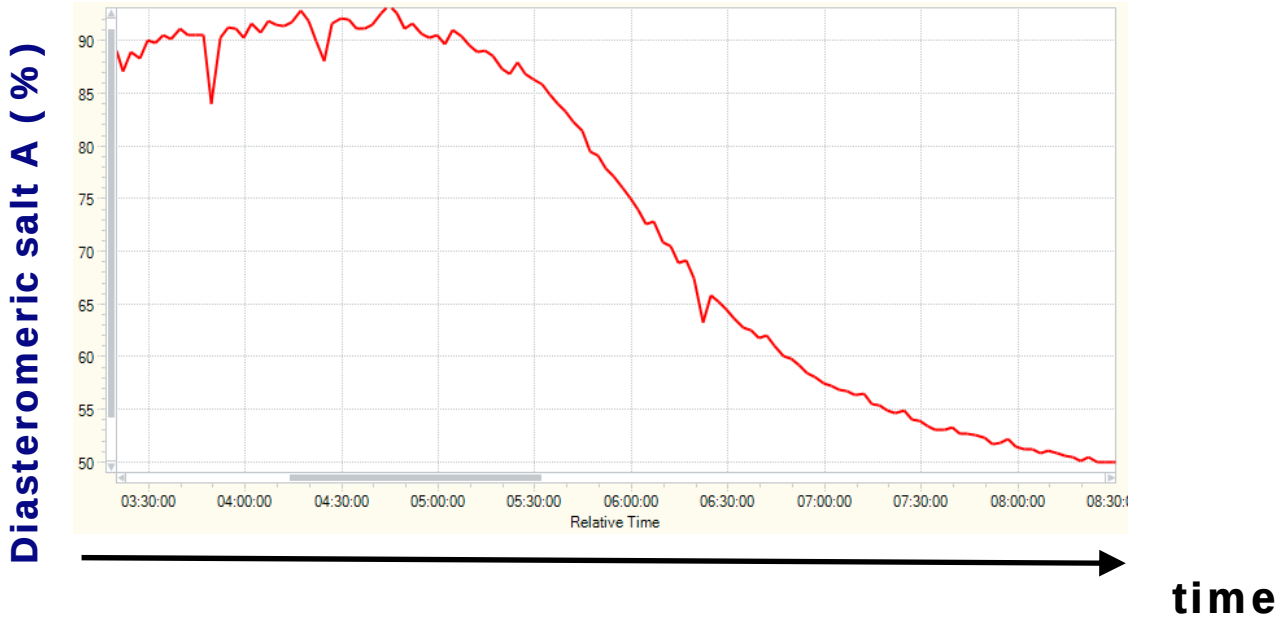
IPC3
74.3 %

IPC4
66.6 %

IPC5
60.0 %

RESOLUTION MONITORING: optical purity

- This model can be applied in a **new resolution experiment**
- the crystallization can thus be followed in **real time** viewing the new trend graph.
- The **optical purity** of the precipitated diastereomeric salts – expressed as % of salt A - is given in **real time**.



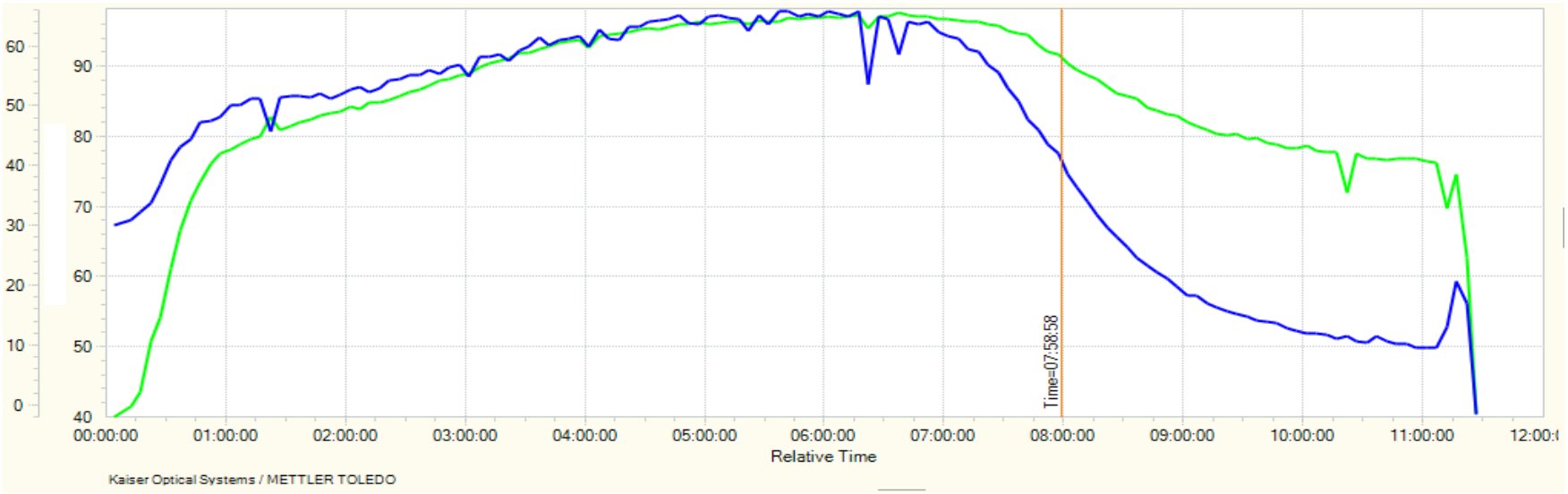
RESOLUTION MONITORING: isolated Yield

- Real time trend graphs can be build also based on the **amount of isolated solid (resolution yield)** of a single diastereomeric salt (e.g. salt A).
- Experimental model based on correlation between **isolated product** (grams) and **ratio** of the two **peak areas**
- **Sensitivity** was checked initially adding small portions of A to the initial suspension corresponding to 1% mol yield increase

we can actually detect well the increase of solid salt A corresponding to 1% mol yield !

Final models for Yield and Optical purity

Yield (grams) and optical purity (% of salt A)



With these two models we can hence monitor the resolution in real time regarding:

- ☺ **Optical purity (salt A%): sensitivity ca. 5%**
- ☺ **Yield: sensitivity 1% mol yield increase**

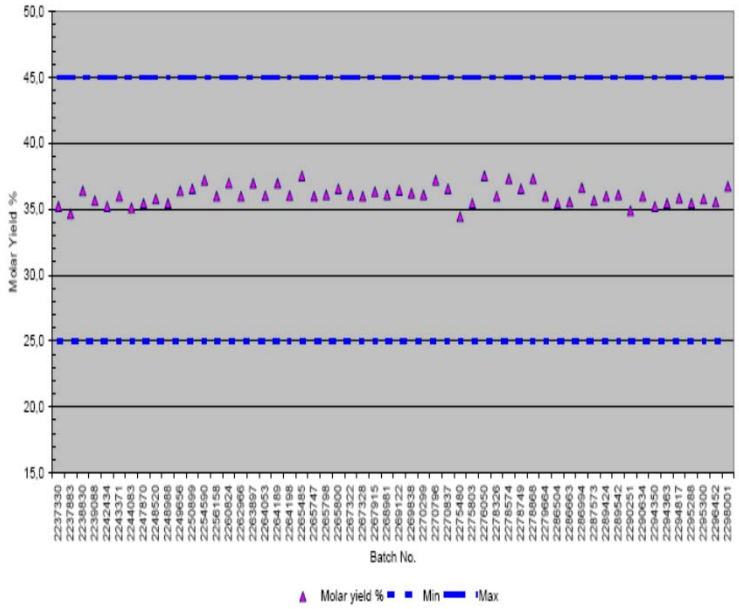
Critical Process Parameters (CPPs) of the Resolution Process

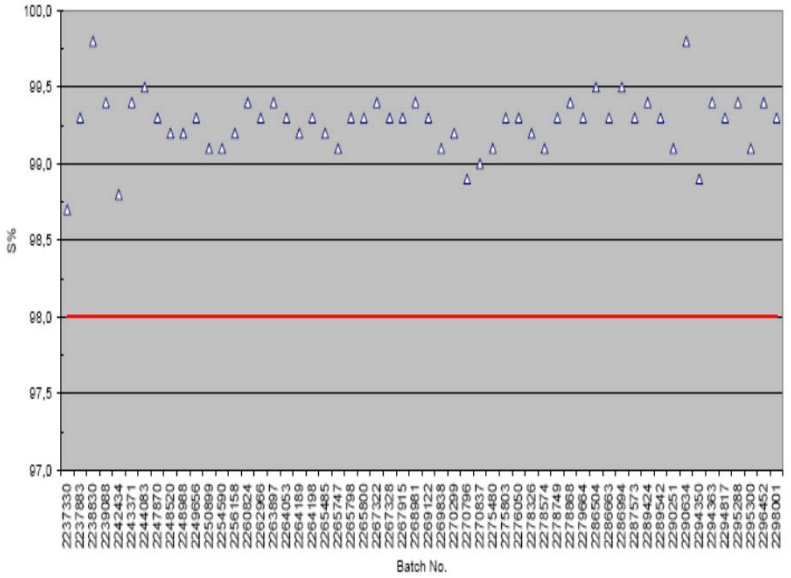
The monitoring with Raman helped Process R&D to increase the process understanding with the assessment of the **Critical Process Parameters (CPPs)** and definition of their **proven acceptable ranges (PAR)**

- **Crystallisation temperature** is critical, since a decrease of **only 2°C** influences the crystallization robustness anticipating the unwanted salt B precipitation.
- Presence of **traces of water and/or organic solvents** delays unwanted salt B precipitation, but do not lead to any yield increase.
- A **higher stirring rate** anticipates the unwanted salt B precipitation.
- Temperature and time of **final 'aging'** influence product yield, as expected. Longer aging time and lower temperatures increases the yield but are detrimental to the optical purity (a **compromise** must be taken).

PROCESS INDUSTRIALIZATION

- Final **fine-tuning** in Production department to adjust the whole process to existing equipment and **filter-drier**
- Process robustness demonstrated by **high reproducibility and consistency** in 4 years of commercial scale production (approx. **60 batch/year**)





Av. Yield = 36 ± 1.5% (72% th. ± 1.5%)

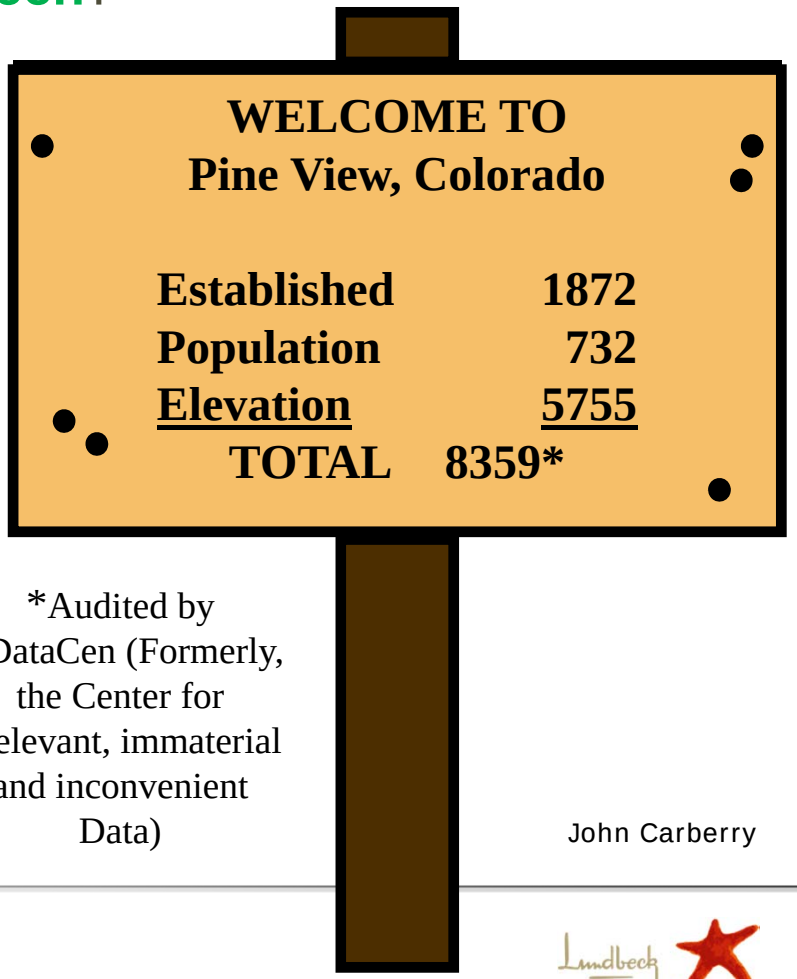
Av. Optical purity 99.3%, lowest 98.3% Limit 98%

Green and Sustainable Chemistry in the Development and Optimization of a novel API process

- How Do you know Your Process is **Green**?

"If you don't keep **score**, you're only practicing"

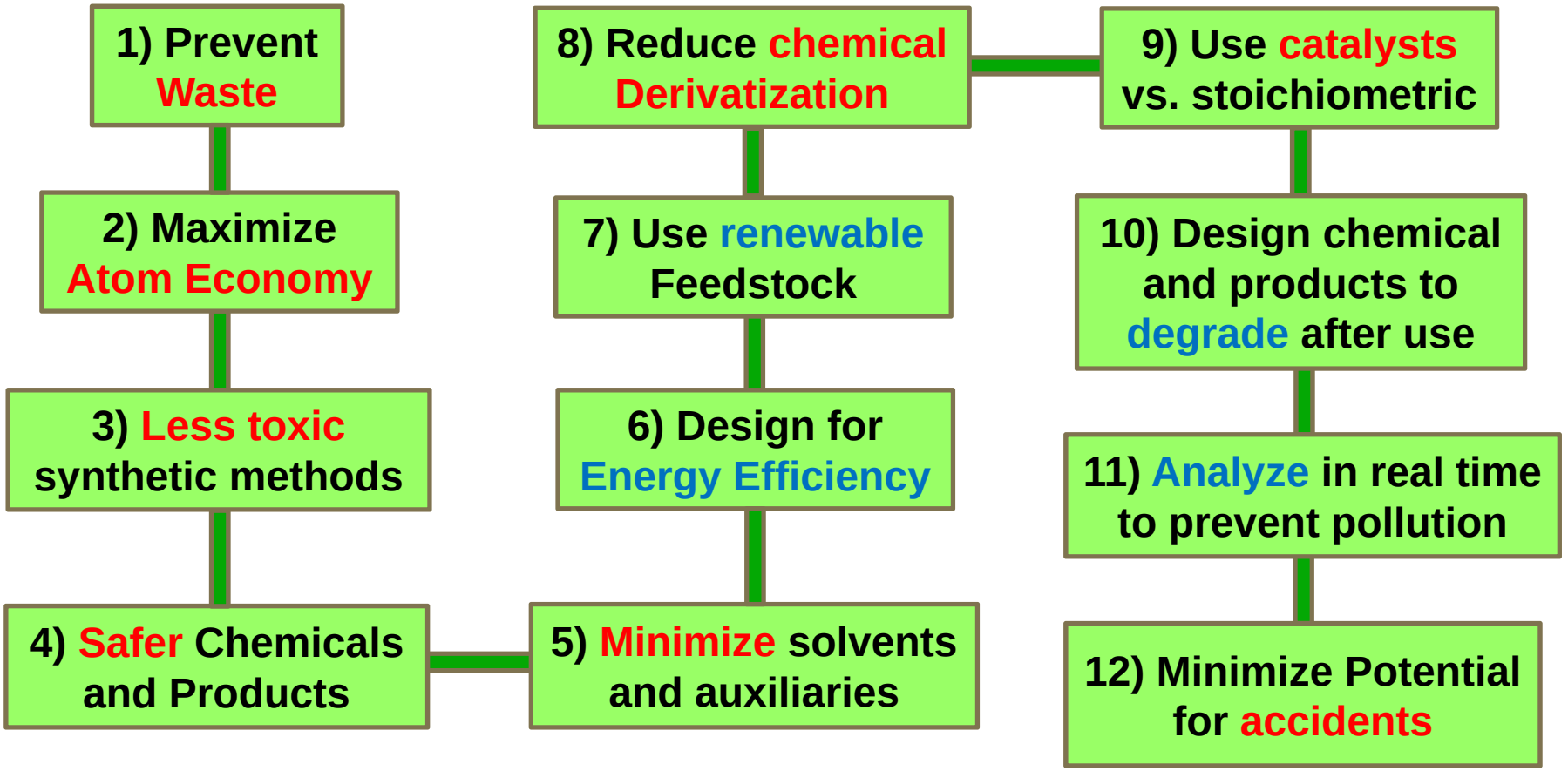
Jan Leschly,
former CEO of Smith-Kline Beecham



*Audited by 3iDataCen (Formerly, the Center for irrelevant, immaterial and inconvenient Data)

John Carberry

The 12 Principles of Green Chemistry



Green Metrics: the E-Factor

E-Factor = Total mass of materials required to produce 1kg product (mass intensity) – 1.

Industry	E-factor	Annual Production (tonnes)	Total Waste tpa	No of transformations	Years of development
Oil Refining	*ca*. 0.1	10^6 - 10^8	10 million	Separations	100+
Bulk Chemicals	<1 to 5	10^4 - 10^6	5 million	1-2	10 – 50
Fine Chemicals	5 to >50	10^2 - 10^4	0.5 million	3-4	4 - 7
Pharmaceuticals	25 to >100	10 – 10^3	0.1 million	6+	3 - 5

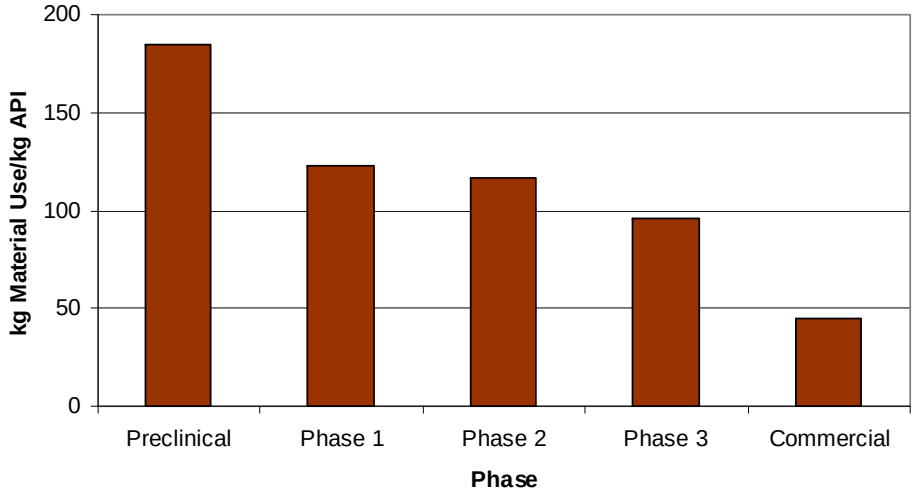
Process Mass Intensity Metric (PMI)

$$\text{Process mass intensity} = \frac{\text{quantity of raw materials input (kg)}}{\text{quantity of bulk API out (kg)}}$$

All Process steps from *commonly available materials* have to be considered

Raw Materials are all materials *including water* that are used in the whole process

PMI by Pharmaceutical Development Phase

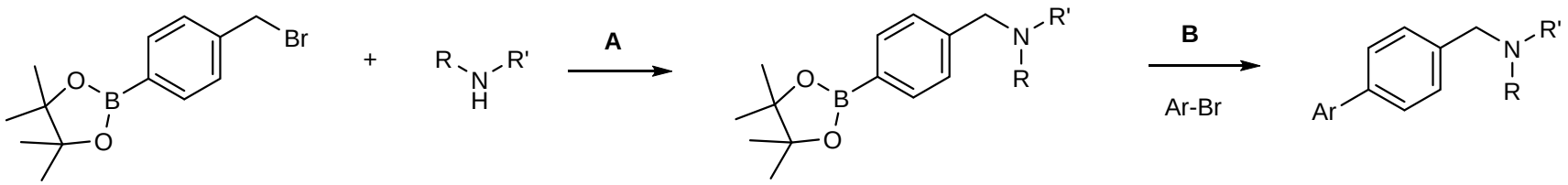


https://www.acs.org/content/acs/en/greenchemistry.html

A recent case study at LUPI

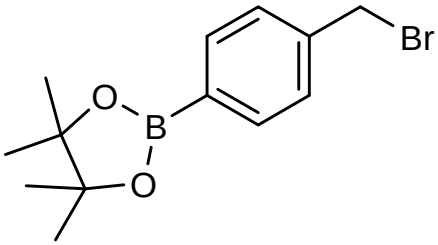
- New API in development phase II-III for treatment of autoimmune diseases

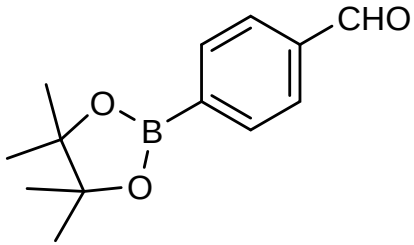
- Original synthesis developed for the first small-scale campaigns to support early clinical trials (Phase I, IIA)



A. Amine alkylation
B. Suzuki coupling

Why developing an alternative route?

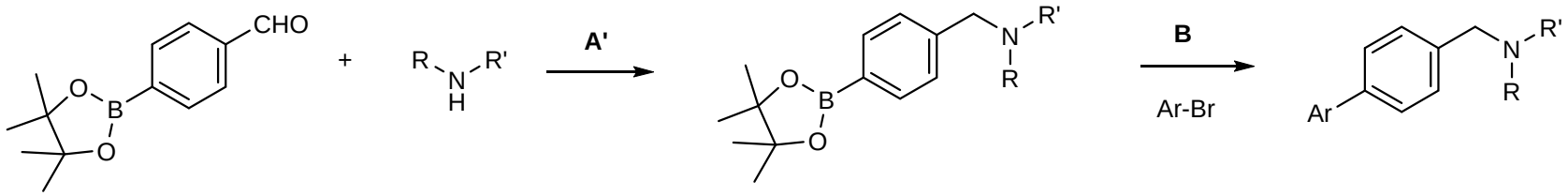




The bromobenzyl boronic ester derivative was found to be **genotoxic** (Ames positive) so the corresponding non-genotoxic **benzaldehyde** derivative was considered a potential substitute

3rd principle: wherever practicable, synthetic methodologies should be designed to use and generate substances that possess **little or no toxicity** to human health and the environment.

New process via reductive amination



- Reductive amination:
  - Via **sodium triacetoxyborohydride** reduction
  - Via **catalytic hydrogenation**

Reductive amination: comparison

	Sodium triacetoxyborohydride (STAB)	Catalytic hydrogen.
Yield	83%	73%
Purity	>99%A	>99%A
Solvent/ volumes	DCM 10vol + NaOH 30% 1vol + MeOH 9 vol	MeOH 8vol
Reductive	STAB 1.2 eq	JM 5% Pt/C 0.1mol %
Reaction time	8 hours to charge STAB portion wise	Hydrogenation time
Temperature	25°C	70°C
Pros	Higher yield	Better atom economy Single solvent Simple work-up
Cons	Difficult charge of STAB, exothermic reaction, water sensitive reagent high MW molecule STAB (211.94) to introduce one hydrogen (1.008)! Chlorinated solvent Longer and complex work-up	Potential safety concerns due to the use of hydrogen Slightly lower yield (but not fully optimized process)
Relative cost of raw materials	1	1
PMI	**20.3**	**9.0**

Reductive amination & Green Principles

1st principle: it is better to prevent waste than to clean up waste after it is formed.

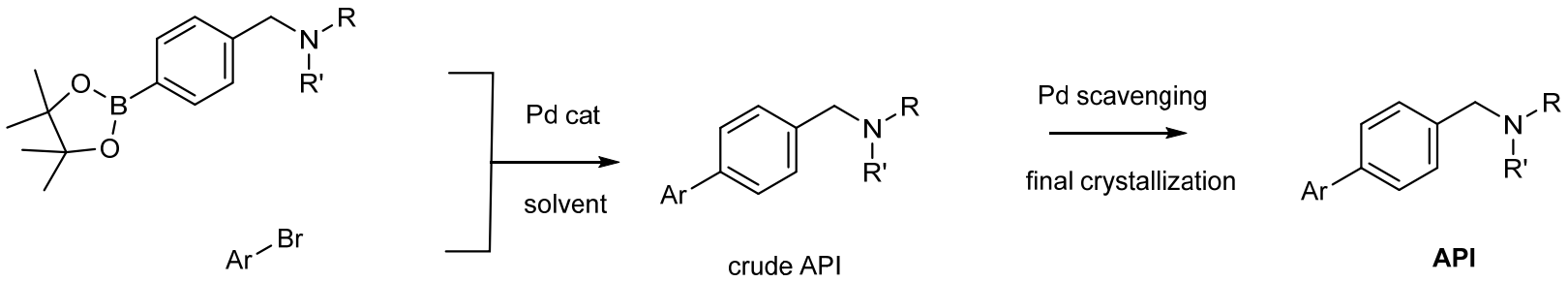
2nd principle: synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product (atom economy).

5th principle: the use of auxiliary substances (e.g. solvents, separating agents) should be made unnecessary wherever possible and innocuous when used.

9th principle: catalytic reagents (as selective as possible) are superior to stoichiometric reagents.

11th principle: analytical methodologies need to be further developed to allow for real-time, in-process monitoring, and control prior to the formation of hazardous substances.

Suzuki coupling & Pd scavenging



- Performed both a solvents and a catalysts screening and then optimized the most promising conditions

- Identified the catalytic couple $Pd(OAc)_2$ / Ph-phospho-adamantane (Ph-PA) far superior to the original catalyst $Pd(dppf)_2Cl_2 \cdot CH_2Cl_2$ and the combination of n-propanol/water superior to methanol alone
  - Higher yield
  - Lower amount of heavy metal
  - More concentrated conditions
  - Much easier Pd/catalyst removal

Suzuki coupling: comparison

	Starting process	Current process
Solvent/volumes	methanol 15vol	n-propanol/water 3/2vol
Catalytic reagent	$Pd(dppf)_2Cl_2 \cdot CH_2Cl_2$ 2.0 mol%	$Pd(OAc)_2$ 0.075mol% Ph-phosphaadamantane 0.15mol%
Reaction time	At least 72 hours	Less than 20 hours
Temperature	Approx. 65°C	87-88°C
Yield	87%	95%
Purity	97.7 %	>99%A
comments		Lower amount of Pd, more concentrated conditions, better yield and purity
Relative cost of raw materials	**2.5**	**1**
PMI	**21.5**	**7.2**

Pd scavenging: comparison

- Pd(OAc)2/Ph-PA complex easier to be scavenged than Pd(dppf)2Cl2.CH2Cl2
- Moreover, the new process also implies a definitely lower Pd loading (1:25)
- Expensive Smopex resins could be effectively substituted by common activated charcoal

	Starting process	Current process
Solvent/volumes	THF/water 75/10 vols	DCM/methanol 15/5 vols
Scavenger	**Smopex 234** 0.1 wt	**activated charcoal** type MV-125 0.05 wt
Residual Pd	< 50 ppm	<20 ppm
Relative cost of raw materials	**50**	**1**
PMI	**78**	**25**

Suzuki coupling/Pd scavenging & Green Principles

Suzuki coupling:

- **1st principle**: it is better to prevent waste than to clean up waste after it is formed.
- **3rd principle**: wherever practicable, synthetic methodologies should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
- **6th principle**: energy requirements should be recognized for their environmental and economic impacts and should be minimized.

Pd scavenging:

- **1st principle**: it is better to prevent waste than to clean up waste after it is formed
- **5th principle**: the use of auxiliary substances (e.g. solvents, separating agents) should be made unnecessary wherever possible and innocuous when used.

Comparison between initial and new process for PMI and cost

	Initial process	New process
Cumulative PMI	**331**	**80**
Cumulative PMI Substrate, Reagents, Solvents	226	44
Cumulative PMI Substrates and Reagents	12	4
Cumulative PMI Solvents	214	40
Cumulative PMI Water	105	36
Relative cost of raw materials	**3.6**	**1**