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Quick Guide: Inclusion Moments

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Green-by-Design: Award-winning Innovations in Biocatalysis

HARSHKUMAR PATEL, PHD
Principal Scientist, Bristol Myers Squibb

MATTHEW WINSTON, PHD
Principal Scientist, Merck

JOHN TUCKER, PHD
Executive Director, Chemical Development, CMC, Neurocrine Biosciences

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for Green Chemistry & Engineering Impact
in the Pharmaceutical Industry

Excellence in R&D and execution of pharmaceutical green chemistry
Compelling environmental, safety and efficiency improvements over existing technologies
Established in 2016

2024 Nominations open Fall 2023: https://www.acsgcipr.org/awards/

Two is better than one!
• Less Hazardous Chemical Synthesis
• Atom Economy
• Catalysis
• Reduction of Solvents and Auxiliaries
• Waste Prevention
Sustainable Manufacturing of BMS-986278 Leveraging an ERED/KRED Biocatalytic Cascade

ACS Webinar
Oct 12th, 2023

Dr. Harshkumar Patel
Harshkumar.patel@bms.com

Bristol-Myers Squibb
Chemical & Process Development (CPD)

2023 Peter J. Dunn Award: Overall Summary

The Power of Biocatalysis

11 steps, 2.8% yield, PMI = 2017

10 steps, 23.4% yield, PMI = 287

100% removal TMSCH₂N₂ & DCM
>80% in $/kg API

86% in waste

3 P's of Sustainability

Planet
People
Portfolio
2023 Peter J. Dunn Award: Overall Summary

Our Approach

CPD Mission: Create **safe**, **economic**, and **sustainable** processes to supply high quality active ingredients for the medicines we deliver to patients.

- **Route Invention**
  - What is optimal sequence of steps to API?
  - Step Count
  - Safety
  - Simplify Supply-chain

- **Process Invention**
  - What are optimal conditions for each step?
  - Yield & purity
  - Simplify process

- **Process Characterization**
  - How can each step be made ‘unbreakable’?
  - Risk Management
  - Mechanism
  - Long-term robustness

**commercial medicine**
Program Background

- BMS-986278 is currently under development for treatment of Idiopathic Pulmonary Fibrosis (IPF) + other ILD (Interstitial Lung Diseases)
  - IPF affects ~200K in US and ~50K new cases per year (worldwide)
  - Increasing incidence, prevalence and severity
  - Most prevalent of the fibrosing lung diseases - doubling in the last decade
  - Avg life expectancy after diagnosis = 3-5 years

Towards a Proposed New Route: Using Modelling & Prediction

PMI correlates to production cost

Step PMI is unique and can be predicted

Overall route PMI can be simulated

Shiny Web App PMI Predictor

https://acsgcipr-predictpmi.shinyapps.io/pmi_calculator/
Background: Enabling Route for 8-30 kg Deliveries

Safety Challenge

Sustainability Challenge

Overall: 11 total steps; Longest Linear Sequence: 7 steps

Chemical & Process Development Strategy for New-Route Development

- Opportunity to use cyclohexyl side chain fragment as limiting reagent (vs 2.5 equiv A)
- Eliminates azide safety risks
- Maintains same API step
- Two reaction sequences possible (optionality)
- Requires POC for proposed end-game AND syntheses of new triazole and trans-hydroxy acid fragments

Predictions:

- ≥50% reduction in overall cost and PMI to API
**Fragment Coupling Decisions**

- No red flags wrt OBN deprotection conditions to afford penultimate intermediate
- No significant differences between either Pd coupling under initial Kumada conditions
- SNAr 1st approach selected since more reactive SNAr system/decreased impurities

**Overall Planned Endgame**
Preparing Fragment ‘D’ for POC of Endgame

This enabled the preparation of initial lab supplies of ‘D’, but was not planned for bulk quantities

Initial Route Scouting to ‘D’:

Biocatalysis (i.e., use of enzymes) was initial focus....

A) Achieved POC using KRED P2-G03
B) No resolution hits
C) No good hits with commercial enzyme kits
D) No good hits with commercial enzyme kits

...but did not lead to quick POC
2nd-Gen Route to Prepare >100 kg BMS-986278

- 1st time scale-up produced 120 kg API
- Route to ‘D’ not expected to be ‘long-term’

Initial Development & Scale-Up to BMS-986278

Tools Predicted Enzymatic Route to ‘D’
- >50% reduction in PMI
- 20-50% cost reduction

With POC for end-game, can we return to envisioned ERED/KRED cascade, *w/ a focus on enzyme evolution?*
CodeEvolver® Directed Evolution Platform for API Manufacturing

Key performance indicators:
- Target: Substrate loading → >50 g/L
- ERED * KRED loading: → <12 wt% total
- Conversion & selectivity: → >98% @ >99% de/ee

Screening & Early Evolution

• After 2 rds of evolution (IPA recycling system @ pH 6):
  • Obtained POC, but: 100 wt% ERED, 100 wt% KRED and only 5 g/L SM loading
  • Before continuing with evolution, conducted some initial process development. Data supported:
    • pH 7 (vs pH 6) optimal for ERED/KRED cascade
    • Glucose/GDH significantly outperformed IPA recycling system
  • Led to major shift in enzyme evolution conditions....
Results from a Key Partnership in Enzyme Evolution

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Conditions</th>
<th>Conditions after 11 Rounds of Evolution + Process Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>ERED-001 KRED-P2-G03</td>
<td>ERED-310 KRED-457</td>
</tr>
<tr>
<td>Substrate Load</td>
<td>5 g/L</td>
<td>67 g/L</td>
</tr>
<tr>
<td>ERED Load</td>
<td>100 wt%</td>
<td>8 wt%</td>
</tr>
<tr>
<td>KRED Load</td>
<td>100 wt%</td>
<td>3 wt%</td>
</tr>
<tr>
<td>Recycling System</td>
<td>IPA (20%)</td>
<td>Glucose + GDH</td>
</tr>
<tr>
<td>pH</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Temperature</td>
<td>30°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Time</td>
<td>21 hours</td>
<td>12 hours</td>
</tr>
<tr>
<td>Pdt/Int/SM</td>
<td>75/15/10</td>
<td>99/0/0</td>
</tr>
<tr>
<td>Selectivity</td>
<td>&gt;99% ee, &gt;99% de</td>
<td>&gt;99% ee, &gt;99% de</td>
</tr>
</tbody>
</table>

Key performance indicators:
- Substrate loading: target > 50 g/L
- ERED * KRED loading: target < 12 wt% total
- Conversion & selectivity: target > 98% @ > 99% de/ee

Going from Lab to Plant Scale

1st Gen Process:
- SM added as soln over ~ 10 h to soln of ERED/KRED/GDH & NADP+
- As rxn proceeds, gluconic acid forms and pH drops
- pH is continuously monitored and aq NaOH dosed to maintain pH ~ 7
  ➢ Overall, aq NaOH addition curve serves as PAT for conversion

Results:
- No issues on 1.5 kg scale
- Stalling observed on 105 kg scale
Troubleshooting the ERED/KRED Cascade

Results:
• Took slipstreams of stalled 105 kg rxn mixture and added either:
  • KRED only → no significant change
  • ERED only → no significant change
  • GDH-105 only → no significant change
  • NADP+ only → complete conversion
• NADP+ charge alone recovered activity
• Since oxidation of glucose by GDH-105 is very fast → proposed NADPH is predominant species during reaction
• Additional expts supported while NADPH is stable at pH 10-13, it has some stability issues at pH 7 (note: NADP+ is stable for >24 h at pH 7)
• How can we leverage this info to develop a more robust process......

New Protocol & Scale-Up Results

• ….add NADP+ in SM solution (vs enzymes) to minimize build up of NADPH

200 kg batch w/ revised solution prep:
• >98% conv; no kickers needed
• 87% isolated yield
• 100% chiral purity
Comparison of Routes to ‘D’

**Enabling Route to ‘D’**

**New Route to ‘D’**

<table>
<thead>
<tr>
<th>Step Count</th>
<th>Overall Yield</th>
<th>Est Cost Saving</th>
<th>PMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>35%</td>
<td>-</td>
<td>650</td>
</tr>
<tr>
<td>2</td>
<td>73%</td>
<td>&gt;50% savings in $/kg final API</td>
<td>112</td>
</tr>
</tbody>
</table>

PMI: kg of all inputs leading to 1 kg of Compound D

Overall Route Modifications

**1st-Gen Route**

**3rd-Gen Route**

BMS-386278
Optimized Route to BMS-986278

Overall Route Metrics Summary

Social/People

- **100%** elimination of **hazardous** TMSCH<sub>2</sub>N<sub>3</sub> & dichloromethane (DCM)
- eliminates **339 kg TMSCH<sub>2</sub>N<sub>3</sub>** & **>39,000 kg DCM** per **100 kg API delivered**!

Environmental/Planet

- **>8x** yield vs Ph2 route
- **50%** reduction in steps needing aq wkup
- **86%** reduction in overall PMI
- eliminates **6 million Kg** of waste annually at peak!

Economic/Portfolio

- **60%** reduction in $/kg API (100 kg scale)
- **>$3M in cost avoidance** to deliver **1st 100 kg API**
- **>80%** reduction in $/kg API on **>1000 kg scale**....

Embedding ‘Green By Design’ principles as part of our development mindset to deliver enhanced value to our People & Patients
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Bristol Myers Squibb
MK-1454: A Challenging Synthetic Target

Key Synthetic Challenges
- 10 stereocenters, including 2 at phosphorus
- 2 unnatural nucleosides with fluorinated stereocenters
- 13-membered macrocyclic core
- Unsymmetrical 2'5' and 3'5' linkages

MK-1454: A Challenging Synthetic Target

Key Synthetic Challenges
- 10 stereocenters, including 2 at phosphorus
- 2 unnatural nucleosides with fluorinated stereocenters
- 13-membered macrocyclic core
- Unsymmetrical 2'5' and 3'5' linkages
1st Generation Route

8 steps from advanced intermediates
30% overall yield
Chromatography required
PMI ~ 9,000

From Commodity to Active Pharmaceutical Ingredient (API)
Overview of 3’-F-Guanosine (3’-FG) Process


Overview of 2’-F-Adenosine (2’-FA) Process

Organocatalytic Thiophosphorylation


From Commodity to API

What is the most direct route from monomers to MK-1454?
MK-1454 is an analog of natural signaling molecule cGAMP

Sun, L. et al. Science 2013, 786

Taking Cues from Biology: cGAMP Biosynthesis

Sun, L. et al. Science 2013, 786
Taking Cues from Biology: cGAMP Biosynthesis

Sun, L. et al. Science 2013, 786
Drawing Inspiration from Nature to Make MK-1454

Can we engineer cGAS to produce MK-1454?

Discovery of a Promiscuous Wild-Type cGAS

Express and screen animal cGAS homologs for trace activity
Discovery of a Promiscuous Wild-Type cGAS

Express and screen animal cGAS homologs for trace activity

Express in *E. coli* → Lyse cells → Screen for reactivity on authentic substrates

Screening hit! cGAS from bald eagle: MK-1454 ~0.1% + 2 other diastereomers

Directed Evolution of an MK-1454-Producing cGAS

3'F-thio-GTP + 2'F-thio-ATP → cGAS (Round 11) → 35% assay yield single diastereomer
Directed Evolution of an MK-1454-Producing cGAS

**Challenge:** Isolation, handling and stability of thiotriphosphates

Can we produce nucleotide triphosphates in situ? (Biocatalytic cascade!)
Taking Cues from Biology: Nucleotide Triphosphate Synthesis

Protein Engineering Enables Phosphorylation of Unnatural Nucleotides

98% assay yield
single P-diastereomer

95% assay yield
single P-diastereomer
Clicking Together Nucleotides in Telescopied Enzymatic Cascade

$\text{3F-thio-GMP} \quad \text{GK (0.9 wt%)} \quad \text{cGAS (100 wt%)} \quad \text{MK-1454}$

$\text{2F-thio-AMP} \quad \text{AK (0.55 wt%)} \quad \text{AcK (0.75 wt%)} \quad \text{pH 7.4, 10 °C}$

$\text{>90% AY, >99% de}$

$\text{>90% AY, >99% de}$

63% assay yield >99% de

500 g scale

Single-pot enzymatic cascade!


Lyophilized Cell Lysates As Standard Biocatalyst Sources

Biocatalyst overexpression in E. coli fermentation

cell lysis

Lyophilization

Biocatalyst

Bacterial host cell protein

Bacterial genetic material

Bacterial endotoxins

Lipids

Added directly to reactions without purification
Post-synthetic Process Chemistry Challenges

MK-1454 is injected into patients
Higher standard for API purity compared to orally administered drugs

*E. coli* lysed cell powder can elicit adverse immune response

**Challenge!** Protein in final product must be undetectable (<20 ppm)

Sequential Crystallizations of MK-1454

**crude MK-1454** in aqueous biocatalytic reaction stream

Solubility \( (\text{H}_2\text{O}) < 1 \text{ mg/mL} \)

**MK-1454**

High residual *E. coli* protein after 2 crystallizations!!

Aqueous tri-sodium salt
Very soluble

\[ \text{filter / isolate} \]
Sequential Crystallizations of MK-1454

Aqueous crystallizations due to limiting solubility properties of MK-1454

Workup and Isolation Challenges

Like MK-1454, major impurities are highly polar, highly soluble in water, and poorly soluble in organics
Poor Extraction of MK-1454 Into Organic Solvents

MK-1454 is insoluble in most organic solvents

Quaternary Ammonium Extractants for MK-1454 Purification

No detectable protein in organic layer
Intercepting an Aqueous Crystallization: Back-Extraction into Water

Middle:

Fresh MX aqueous salt solution

Equation: $M^+X^- = NaCl, NaOAc, (NH_4)_2Cl, (NH_4)_2OAc, CaCl_2, CaOAc, MgCl_2, MgOAc, ...$

Tertiary Amines as pH-Switchable Extractants?

Bottom:

Fresh aqueous NaOH solution
d - activate tertiary ammonium phase-transfer agent

Extraction

NaOH

NaOH

NaOH
**Hypothesis:** Tertiary Amines as pH-Switchable Extractants

[Diagram showing the hypothesis with chemical structures and reaction pathways]

High-Throughput Discovery of Tertiary Ammonium Extractants

Extractant library synthesized by combinatorial acid / base reactions

[Diagram showing the synthesis of extractant library and its application]

pH-Switchable Extractants: Proof-of-Concept Achieved!

Tri-n-octylamine

2-MeTHF

H₂PO₄

aqueous

protein-enriched aqueous

Fresh aqueous NaOH solution

protein-enriched aqueous

tri-n-octylamine remains in organic layer

No protein detected!
 Prep Scale Demonstration of Extraction

Organic phase
(enriched in product)

Extraction into 2-MeTHF

N/(n-oct)₃ + H₃PO₄
2-MeTHF

15 vol% 1-PrOH

Aqueous phase
(cell debris, solids,
and host cell protein)

Back-extraction into water
NaOH (aq.)

Crystallization
HCl (aq.)

48% isolated yield
Non-detectable host
cell protein

Single solid-handling step

Effective concentration of aqueous solution!

Probing Solution-State Extractant Interactions

DOSY NMR (d₈-THF)

\[ \text{Diffusion in } d_8\text{-THF (corrected for viscosity)} \]

\[ \text{Dependence on } [\text{HN}(n\text{-oct})_3]^+ \text{ Concentration} \]

\[ \Delta \delta = 4.65 \text{ ppm} \]
\[ \Delta \delta = 0.11 \text{ ppm} \]
\[ \Delta \delta = 0.05 \text{ ppm} \]
\[ \Delta \delta = 0.00 \text{ ppm} \]
Probing Solution-State Extractant Interactions

MK-1454 likely extracted in reverse micelles
General phenomenon demonstrated with other polar hydrophilic molecules

Reverse Micellization of pH-Switchable Extractants
Summary and Lessons Learned

- **Enzyme engineering** in concert with process development enabled a complex biocatalytic cascade to MK-1454.
- Leveraged **enzyme substrate specificity** and **diastereoselectivity** to prepare a single cyclic dinucleotide diastereomer.
- >10x improvement in **process mass intensity** (~800) over 1st generation non-biocatalytic route.

### Function inspires medicinal chemists

**Biomolecular Analogues**
- Hydrophilic and Polar
- Synthetic Biocatalytic Applications

**Biosynthesis** inspires process chemists

Advances in biocatalysis necessitate novel process chemistry solutions
Overview of MK-1454 Commercial Manufacturing Route
Overview of MK-1454 Commercial Manufacturing Route

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