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6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

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The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Nathan Collins
UU	UU	UU	UU		19b. TELEPHONE NUMBER 650-859-3889

RPPR Final Report
as of 07-Apr-2020

Agency Code:

Proposal Number: 68394CHDRP **Agreement Number: W911NF-16-C-0051**
INVESTIGATOR(S):

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DUNS Number: 009232752 EIN: 941160950

Report Date: 22-Apr-2020 Date Received: 06-Apr-2020
Final Report for Period Beginning 23-Mar-2016 and Ending 22-Mar-2020
Title: SynFini - An Automated Chemical Synthesis Platform
Begin Performance Period: 23-Mar-2016 **End Performance Period:** 22-Mar-2020
Report Term: 0-Other

Submitted By: Leslie Hokama Email: leslie.hokama@sri.com
Phone: (650) 859-4632

Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 0 **STEM Participants:** 0

Major Goals: To develop an automated chemical synthesis platform from chemical design and reaction conditions to production on a microfluidic-based system with in-line analytics. Specific goals/milestones for the last phase of the contract (Phase 3) are shown below:
Brief summaries are included below:

Software
Complete integration of SynRoute route method translation to automated hardware protocols (achieved goal)
Completed alpha version of Process Builder (achieved goal)
Build the SynChem database architecture and integrate data with SynRoute (achieved goal)
Develop and implement self-optimizing systems, including feedback and control that can be applied to both SynJet and AutoSyn (achieved goal)
Evaluate ability for multi-step optimization using machine learning approaches

Hardware
Develop a more stable, robust pump system and demonstrate more reproducible analytics and product output (achieved goal)
Develop and implement a reduced temperature/cooling reactor on AutoSyn (achieved goal)
Develop approach for convergent synthesis on AutoSyn (achieved goal)
Perform more extensive user beta testing and plan for transitioning of technology to a broader user base (achieved goal)

System Integration
Demonstrate and test an end-to-end integrated system that allows users to seamlessly design and execute the synthesis of target compounds (achieved goal)

Accomplishments: Accomplished under goals are summarized in the PDF document in the Upload section.

Training Opportunities: Nothing to Report

Results Dissemination: Several meetings and conferences have been attended by the project staff to both promote the Make-It/SRI SynFini Chemical Synthesis Platform as well as learn where and what the industry wants or needs and how this system can accelerate the drug or chemical development process.

RPPR Final Report
as of 07-Apr-2020

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Annual patent reports were submitted during the period of performance for this contract. There were no new patents filed during the last phase of this contract.

PARTICIPANTS:

Participant Type: PD/PI
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Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Co PD/PI
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Other Professional
Participant: Leslie Hokama
Person Months Worked: 3.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Staff Scientist (doctoral level)
Participant: Jeremiah Malerich
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

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Person Months Worked: 4.00 **Funding Support:**
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International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Other Professional
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Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Other Professional
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Person Months Worked: 7.00 **Funding Support:**
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International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Other Professional
Participant: Vi-Anh Vu
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

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Participant: Dominique Tartar
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

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Participant: Judy Szeto
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Project Contribution:
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International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Kristina Rucker
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Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Other Professional
Participant: Noeli Paz Soldan Cruz
Person Months Worked: 4.00 **Funding Support:**
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International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Other Professional
Participant: Daniel Matsiev
Person Months Worked: 5.00 **Funding Support:**
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International Travel:
National Academy Member: N
Other Collaborators:

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Participant: Sahana Mallya
Person Months Worked: 5.00 **Funding Support:**
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Participant: Mario Latendresse
Person Months Worked: 6.00 **Funding Support:**
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International Travel:
National Academy Member: N
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Participant Type: Other Professional
Participant: Markus Krummenacker
Person Months Worked: 7.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Other Professional
Participant: Alex Barszap
Person Months Worked: 2.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N

Other Collaborators:

CONFERENCE PAPERS:

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: Chemical & Biological Defense Science & Technology Conference
Date Received: Conference Date: 29-Dec-2017 Date Published: 29-Dec-2017
Conference Location: San Diego, California
Paper Title: AutoSyn: A Versatile, Compact, Automated Chemical Synthesis Device
Authors: Jeremiah P. Malerich, David Stout, Jin-Ping Lim, Vi-Anh Vu, Judy Szeto, Joseph Kozocas, Jason D. Wh
Acknowledged Federal Support: **Y**

Publication Type: Conference Paper or Presentation **Publication Status:** 1-Published
Conference Name: CCP Summit 2018 (Commercializing COntinuous Processing in Pharma)
Date Received: Conference Date: 31-Jan-2018 Date Published: 31-Jan-2018
Conference Location: Boston, MA
Paper Title: Designing Your Synthetic Process for CM at the Discovery Stage
Authors: Nathan Collins
Acknowledged Federal Support: **Y**

PATENTS:

Intellectual Property Type: Patent Date Received:
Patent Title: Modular Systems for Performing Multi-Step Chemical Reactions, and Methods of Using same
Patent Abstract: Disclosed are modular chemical reaction systems and methods of using such chemical reaction
Patent Number: 06557
Patent Country: USA
Application Date: 06-Apr-2018 Application Status: 1
Date Issued:

DARPA Make-It Phase 3 Final and Program Final Report
March 22, 2020

SynFini—An Automated Chemical Synthesis Platform

Contract: W911NF-16-C-0051

PI: Nathan Collins
Co-PI: Peter Madrid
PM: Leslie Hokama

Peter Karp
Mario Latendresse
Jin-Ping Lim
Jeremy Malerich
Jason White

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SynFini Core Value Proposition:

Automated high quality, multistep chemical synthesis, with reaction data capture, analysis and archive, that enables…

- optimal synthetic route discovery
- digital reproducibility, transferability and scalability

…ultimately unlocking innovative design that is focused on **"what to make" rather than "how to make"**

Summary of Phase 1 – 3 Performance Against Contract Milestones and SRI Objectives

Phase 1A Milestones

√ indicates goal achieved

Month 9 Demonstration Milestones:

- ✓ Demonstrate computational (*in silico*) reaction pathway design of all target molecules
- ✓ Demonstrate the lab-based continuous synthesis of three of the target molecules
- ✓ Deliver completed engineering plans for Phase 1 synthesis system
- ✓ Demonstrate partial routes to targets using at least two modules common with the schemes for other targets (in future phases these schemes will be defined by the knowledge-based tools)
- ✓ Deliver a cost model for the Phase 1 system, which includes details on commercially available vs. custom parts, as well as projections on component lifetime and depreciation
- ✓ **Month 9 Challenge:** Demonstrate reaction pathway design and lab-based continuous synthesis of a DARPA-defined molecule. FOX-7.

Phase 1 Target Molecules

- ✓ fluconasol
- ✓ ibuprofen
- ✓ nevirapine
- ✓ hydroxychloroquine
- ✓ diphenhydramine
- ✓ diazepam
- ✓ atropine

Phase 1B Milestones

√ indicates goal achieved

Month 18 Demonstration Milestones:

- ✓ Demonstrate the ***continuous synthesis of all Phase 1 target molecules*** in a single, automated system
- ✓ Demonstrate ***switching of the device*** from synthesis of one target to that of another in less than two hours, while maintaining system performance (e.g., scale, purity, etc.)
- ✓ Demonstrate ***synthesis of one target via two alternate pathways*** that were generated by the reaction pathway design software; demonstrate a complete computational/experimental feedback loop
- Demonstrate system scalability by building a continuous reactor for one target molecule (proposer-defined by month nine) capable of 1 MT/year (114 g/h) equivalent - ***GOAL DROPPED BY DIRECTION OF DARPA PROGRAM MANAGER TO FOCUS ON DVELOPMENT OF CORE PLATFORM***

Month 18 Challenge: Demonstrate reaction pathway design and continuous synthesis of three DARPA-defined molecules in an integrated, automated device

- ✓ 6-carboxytetramethylrhodamine
- ✓ (s)-warfarin
- ✓ Tranexamic acid

Progress Against Phase 2 Milestones

√ indicates goal achieved

Contract Objective

- ✓ Demonstrate the continuous synthesis of all Phase 2 target molecules in a single, automated system (tramadol, lamivudine, pregabalin, naproxen, and imatinib) with
  - ✓ ***Optical purity monitored*** continuously
  - ✓ Syntheses should include ***real-time, on-line characterization,*** *separation and purification*
  - ✓ Continuous syntheses of tramadol and imatinib
  - ✓ Continuous, asymmetric synthesis of lamivudine & naproxen **utilize new chemistry** to AutoSyn
  - ✓ Continuous, asymmetric synthesis of pregabalin analog phenibut
- ✓ Develop a computational map of synthetic capability of existing modules that predicts the suite of molecules that can/cannot be synthesized by the current device
- ✓ Assess reaction screening and optimization platform SynJet as method for rapid profiling of synthetic routes. Determine how SynJet may be implemented into chemistry development workflow to materially speed design process
- ✓ Month 34 Challenge: Demonstrate reaction pathway design and continuous synthesis of a DARPA-defined molecule in an integrated, automated device (priority for maximum integration of Base Period modules)

SRI Objectives

- ✓ Complete SynFini components SynRoute, AutoSyn
- ✓ Establish reaction optimization and process analytical technology strategy, technology and systems, and integrate into AutoSyn
- ✓ Integrate all SynFini components into seamless operation and define and demonstrate workflows for automated chemical synthesis
- ✓ Create preliminary SynChem Database to capture optimized reaction protocols for future reaction screening

Progress Against Phase 3 Milestones

√ indicates goal achieved

- ✓ Demonstrate the *continuous synthesis of all Phase 3 target molecules and two unknowns* in a single, automated system
  - ✓ Quinapril, bortezomib, itraconazole completed
  - ▪ Atorvastatin – long reactions times (days) requires a different 'flow' strategy

- ✓ Demonstrate *software that enables the design of modules for a given set of products in real-time* (in silico) for a DARPA-defined molecule
  - ✓ Completed - identified synthetic process for 90% of FDA approved drugs

- ✓ Build a second AutoSyn system and demonstrate efficient process transfer from one system to another
  - ✓ Completed - shown transferability for DARPA target molecules

- ✓ Continuing from ribavirin challenge example, create route planning algorithm for efficient production of compound sets; extend hardware and software to enable synthesis of focused compound sets on AutoSyn.
  - ✓ Completed – testing in progress

SRI Objectives for SynFini Phase 3

√ indicates goal achieved

Software

- ✓ Complete integration of SynRoute route method translation to automated hardware protocols
  - ✓ Completed alpha version of Process Builder
- ✓ Build the SynChem database architecture and integrate data with SynRoute
- ✓ Develop and implement self-optimizing systems, including feedback and control that can be applied to both SynJet and AutoSyn
  - ✓ Evaluate ability for multi-step optimization using machine learning approaches

Hardware

- ✓ Develop a more stable, robust pump system and demonstrate more reproducible analytics and product output
- ✓ Develop and implement a reduced temperature/cooling reactor on AutoSyn
- ✓ Develop approach for convergent synthesis on AutoSyn
- ✓ Perform more extensive user beta testing and plan for transitioning of technology to a broader user base

System Integration

- ✓ Demonstrate and test an end-to-end integrated system that allows users to seamlessly design and execute the synthesis of target compounds

Applications of Make-It Technologies to the Ongoing COVID-19 Outbreak

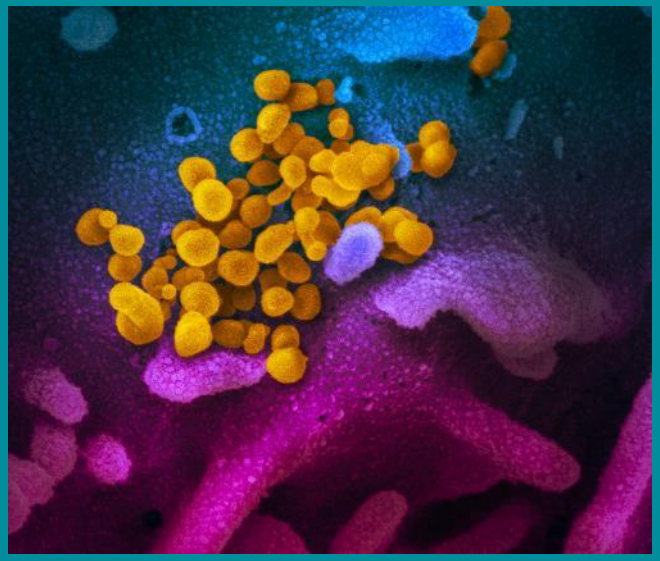
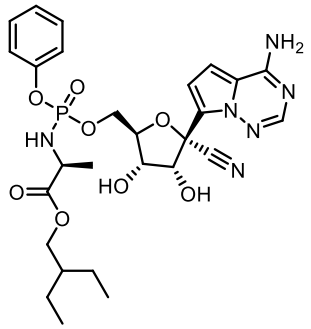


Image Source: U.S. NIH/NIAID Wikipedia

Routes to Experimental COVID-19 Therapies on SynRoute



Remdesivir

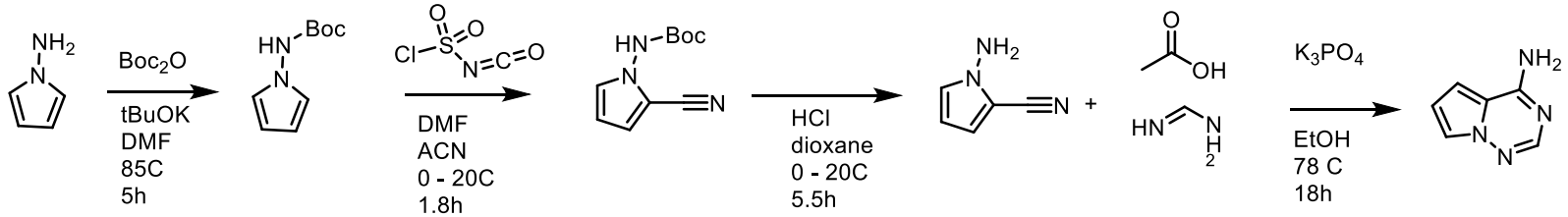
- Gilead nucleoside antiviral in development for treatment of Ebola virus disease (EVD)
- Demonstrated to be effective against SARS-CoV in NHP disease model
- Being tested for treatment of COVID-19 under emergency use authorization

Search for complete synthesis:

Identified 56 reactions that produce this compound, but no complete routes to feedstock

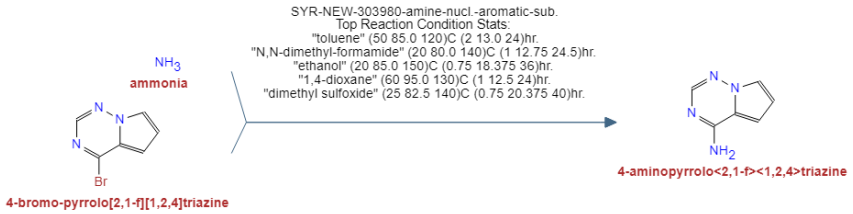
Synthetic routes of key heterocycle intermediate:

Gilead synthesis (4-step from N-amino-pyrrole):



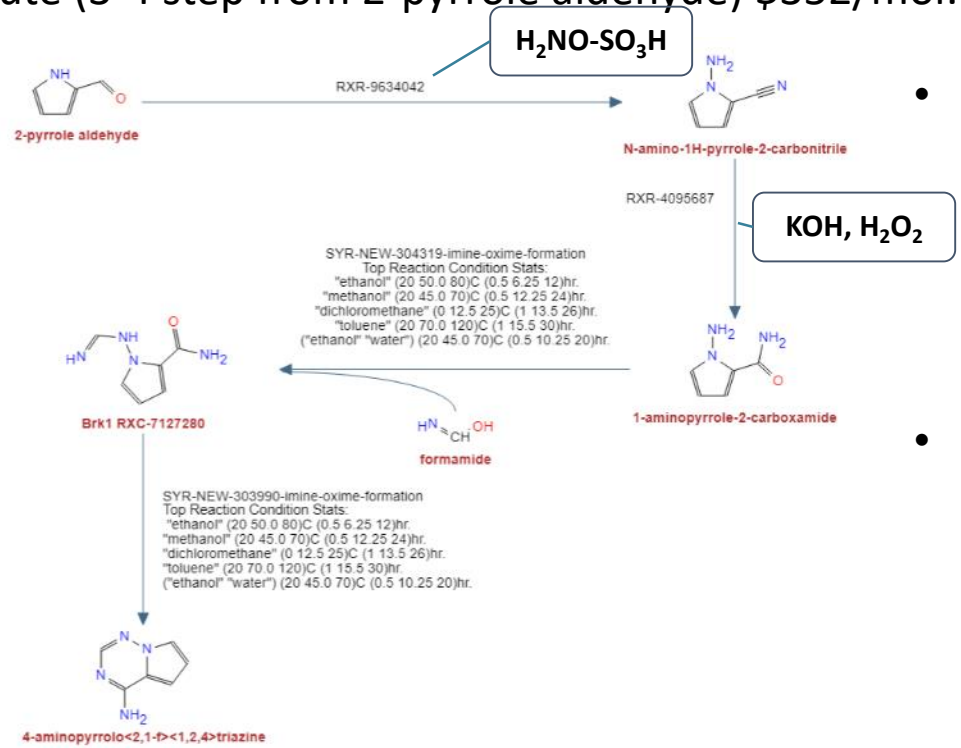
Routes to Key Remdesivir intermediate on SynRoute

SynRoute (1-step from 4-bromopyrrolotriazine) $148/mol:



- **Requires advanced intermediate feedstock**

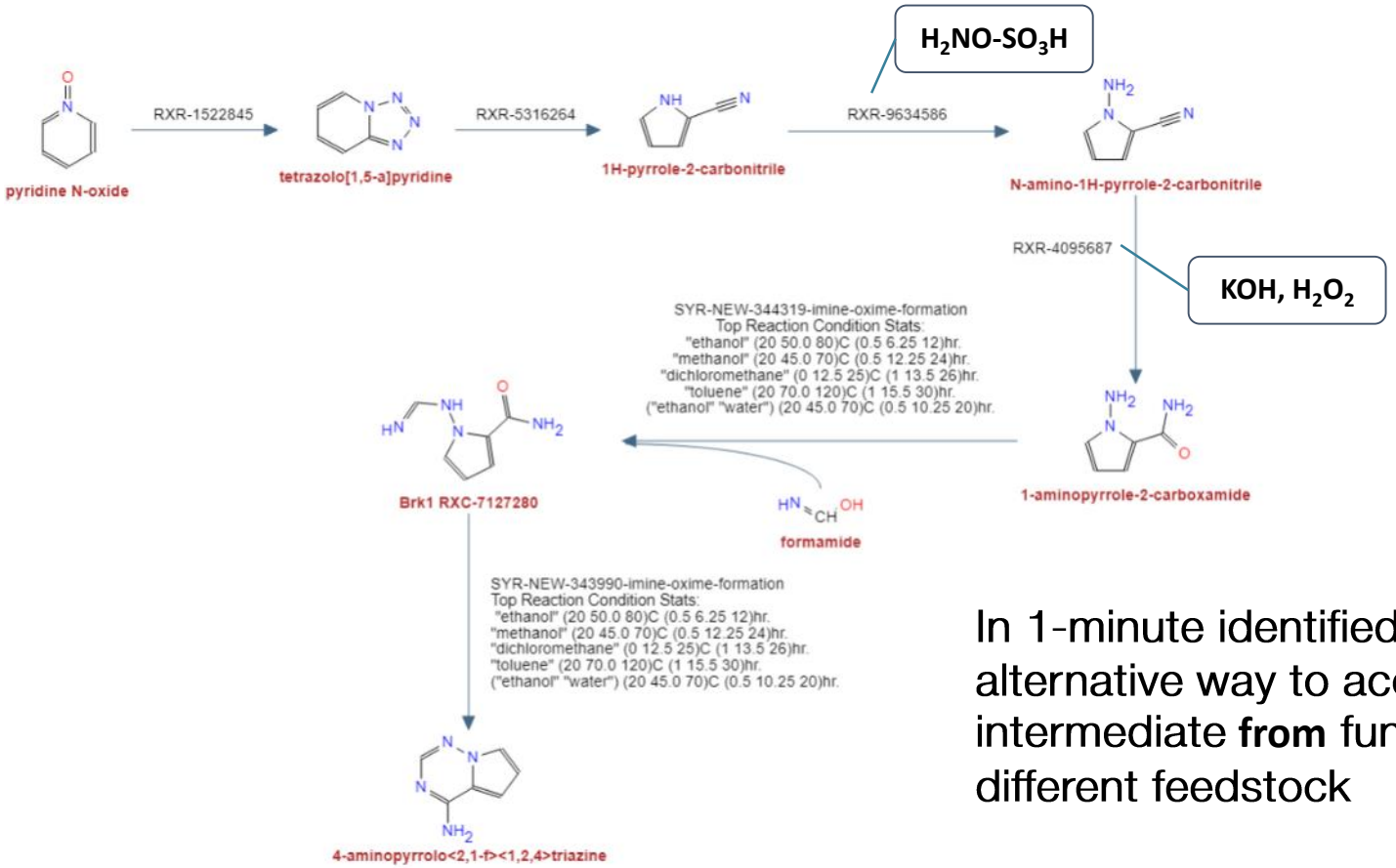
SynRoute (3-4 step from 2-pyrrole aldehyde) $352/mol:



- **The last two MCT-generated steps are effectively the same as the Gilead heterocycle formation done in one-pot – so effective a shorter 3-step route**
- **Route involves different and lower cost feedstocks than used in Gilead patent**

Modeling Unavailability of Key Feedstock

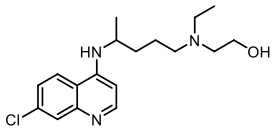
- With key feedstock potentially becoming unavailable, SynRoute can quickly identify alternative synthesis route
- Route search with both: N-amino-pyrrole and 2-pyrrole aldehyde unavailable:



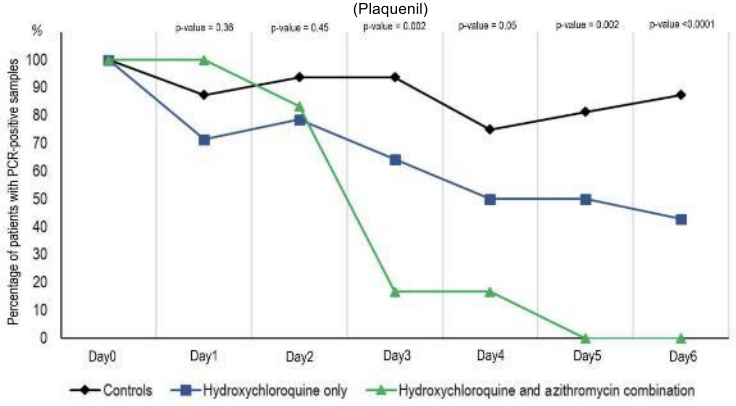
In 1-minute identified an alternative way to access this key intermediate **from** fundamentally different feedstock

Continuous Flow Synthesis of Hydroxychloroquine

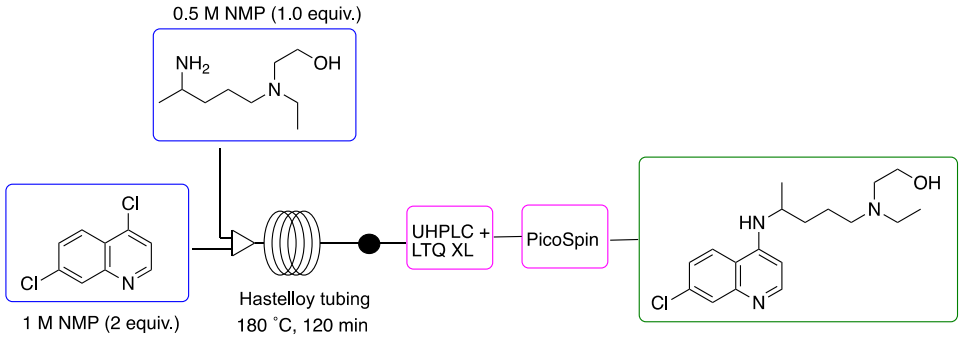
Hydroxychloroquine (Plaquenil, PLQ) tested on a small cohort of 24 patients infected with SARS-CoV-2 shows reduction in viral load.



Hydroxychloroquine (Plaquenil)



Source:
Gautret et al. (2020)
DOI: 10.1016/j.ijantimicag.2020.105949



- 66% conversion by NMR
- Repeating now on updated AutoSyn system with integrated analytics

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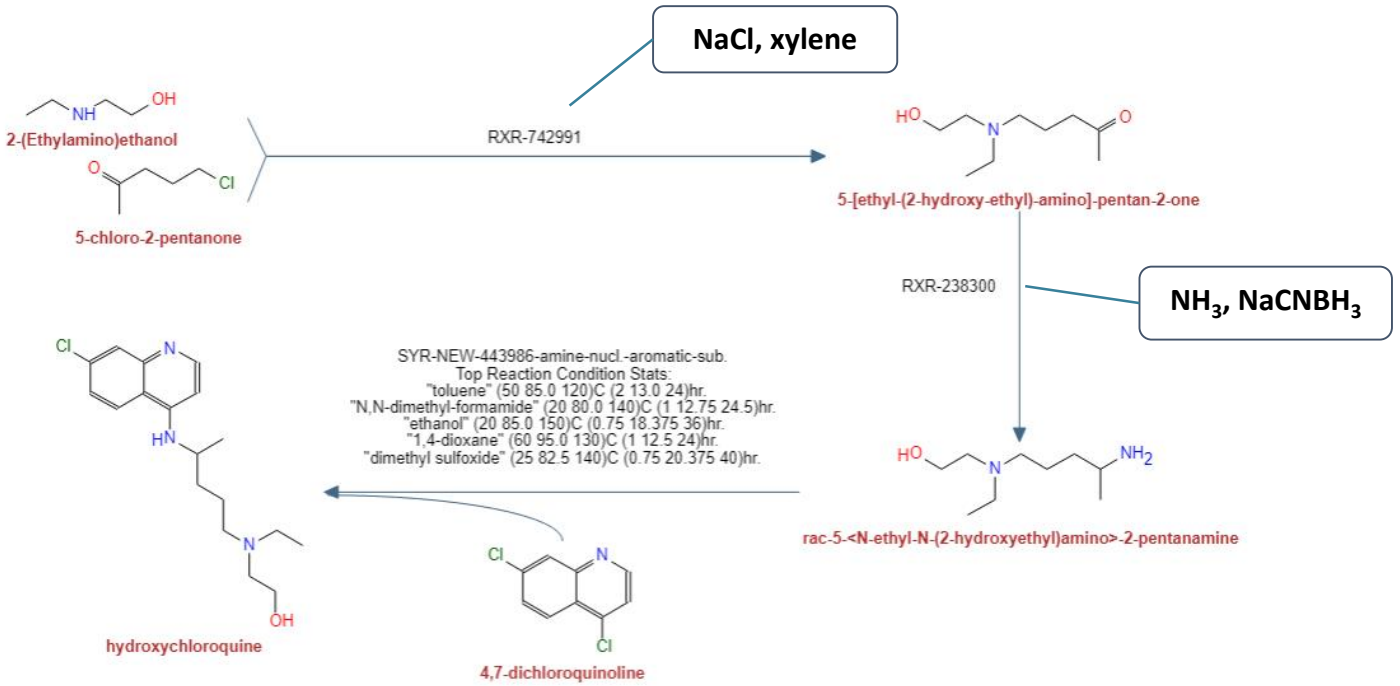
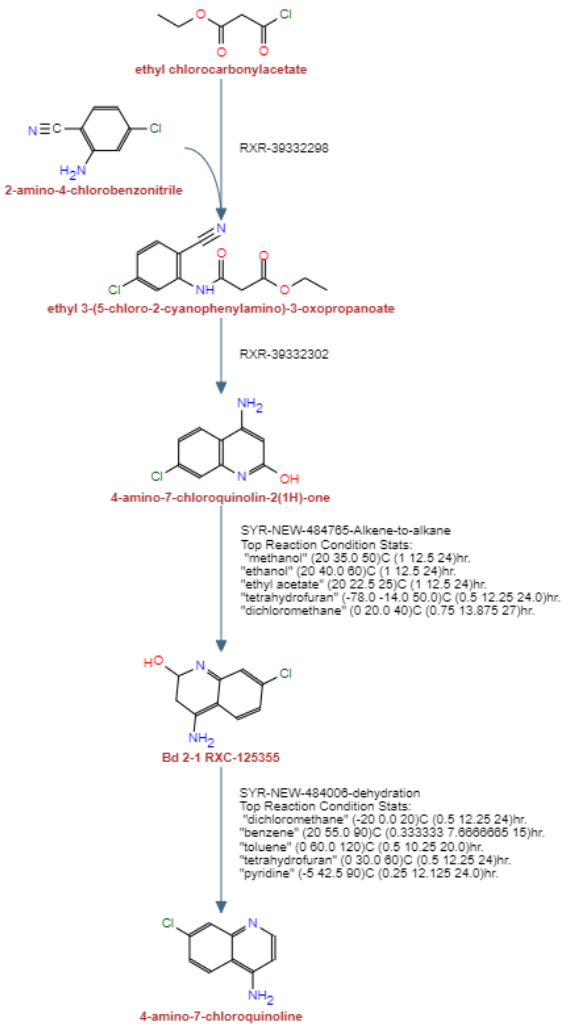
SynRoute Top Route for Hydroxychloroquine

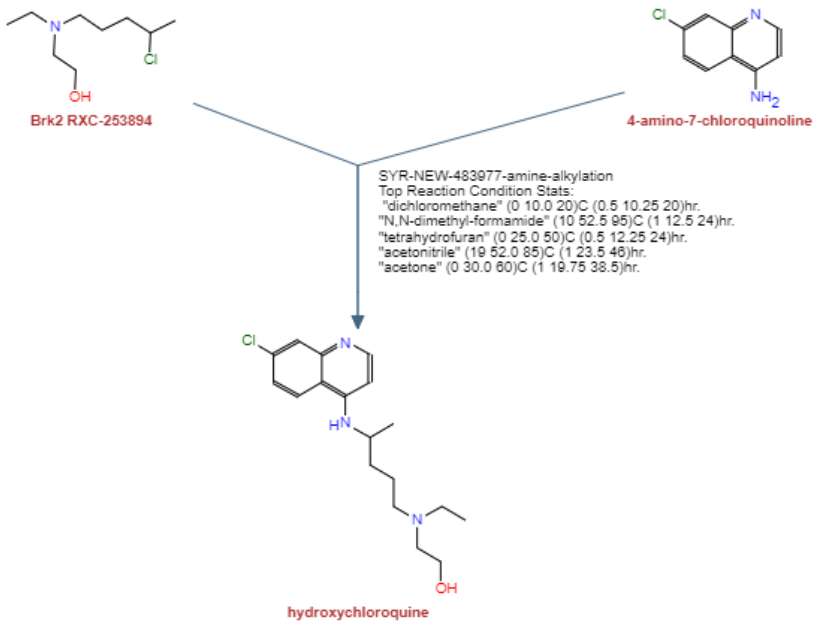


- All of the patented process routes for hydroxychloroquine also use 4,7-dichloroquinoline as a feedstock
  - Patents: CN103724261A, WO2010027150, CA2561987A1, WO2005062723A2, US2546658

If Quinoline Feedstock becomes Unavailable?



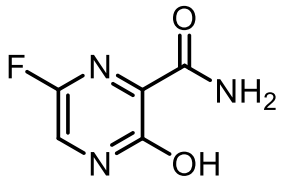
- SynRoute quickly finds an alternative route from very cheap malonate feedstock
- Utilizes modified side chain intermediate to produce the final API



Favipiravir – An RNA-dependent RNA-polymerase Inhibitor

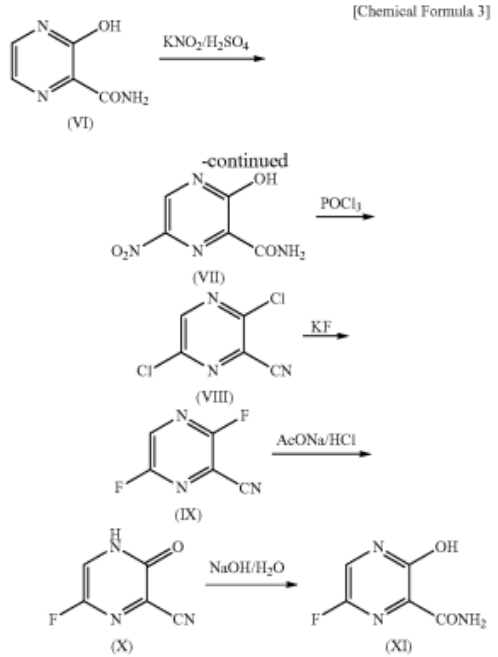
- Favipiravir is a fluorinated nucleoside antiviral drug that was developed in Japan for treatment of influenza
- Viral polymerase enzymes are a broad-spectrum target that have been effectively targeted with nucleoside analogs that selectively inhibit viral enzymes over the human homologs
- Favipiravir has been found to be effective in a small open-label trial in China for treatment of COVID-19
  - Median time to viral clearance – 4 days
  - Improvement in lung pathology in 91% of patients compared to 62% in controls

Source: www.theguardian.com/world/2020/mar/18/japanese-flu-drug-clearly-effective-in-treating-coronavirus-says-china?CMP=share_btn_link



Favipiravir
(Avigan)

Original Patent Route:



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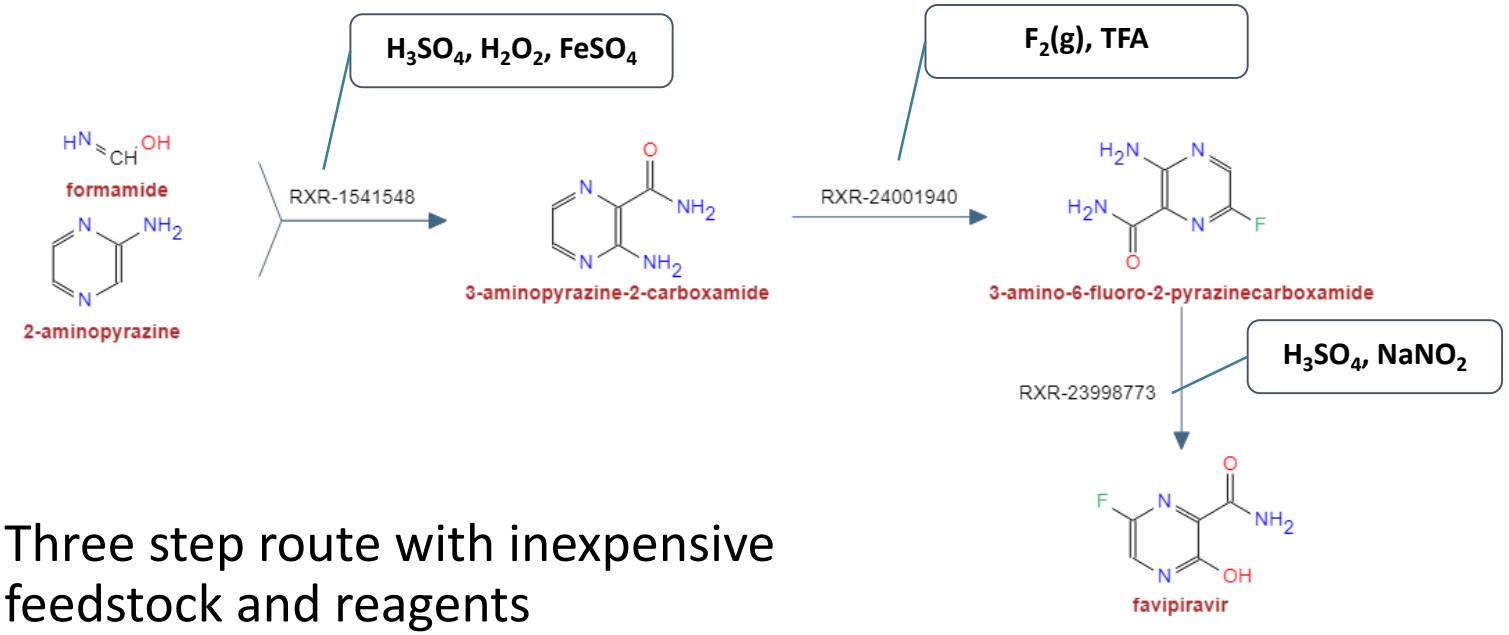
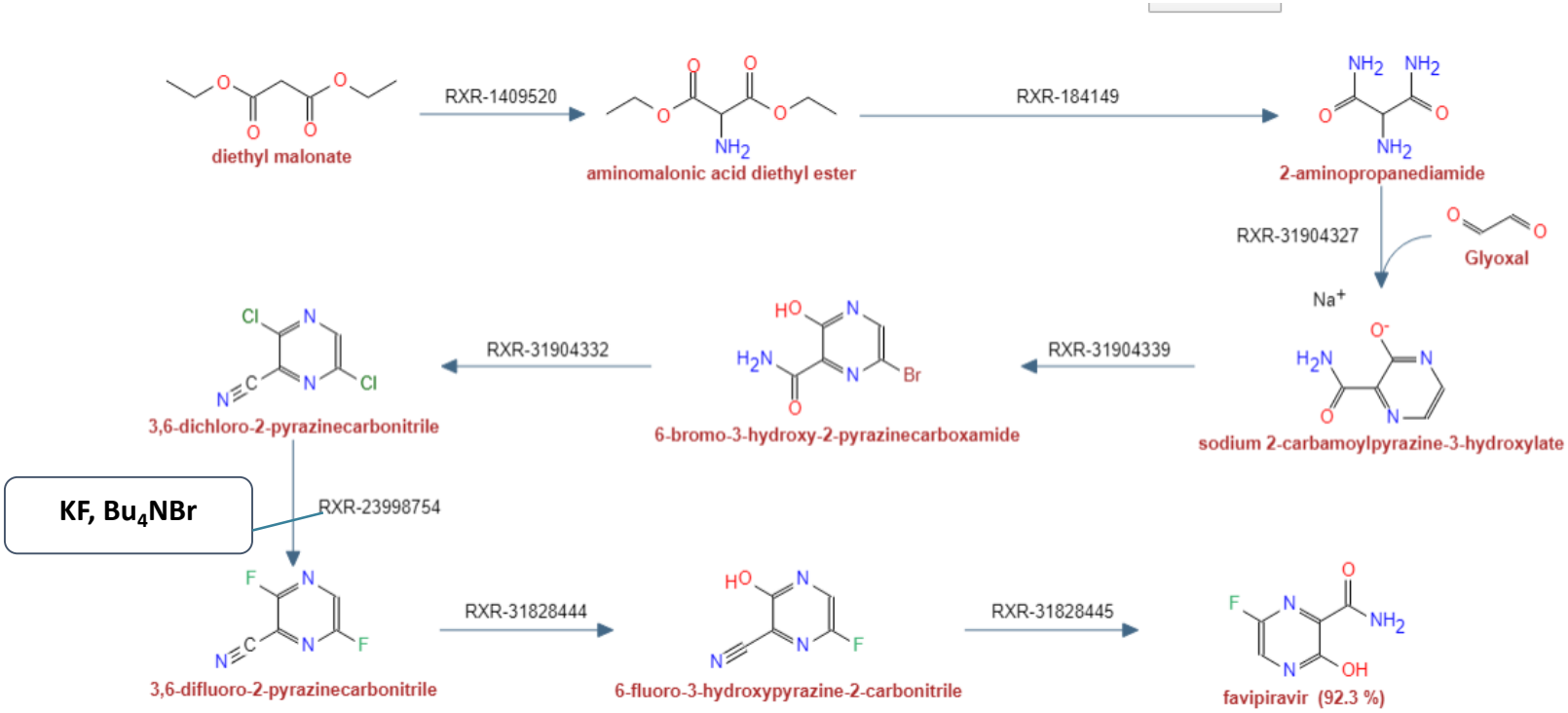
SynRoute Top Route for Favipiravir



- Three step route with inexpensive feedstock and reagents
- Requires electrophilic fluorination that can be challenging to perform on large production scale

If Pyrazine Feedstock becomes Unavailable?

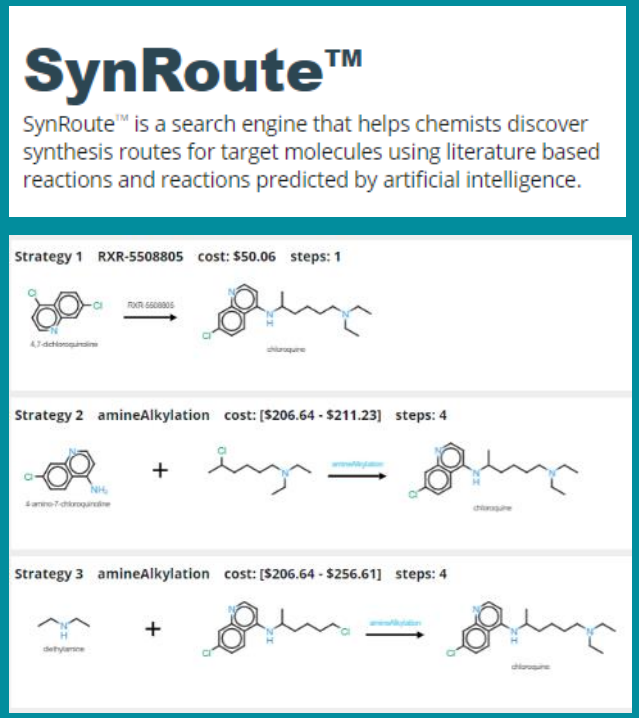
- SynRoute finds a new route utilizing very inexpensive feedstock (diethyl malonate) and no longer requires the electrophilic fluorination reaction



Application of Make-It Technologies to COVID-19 Outbreak

- SRI's SynFini technology developed under the DARPA Make-It program can be applied towards the production of candidate COVID-19 therapeutics
- SynRoute – a rapid synthetic route planning tool can:
  - Find multiple synthetic routes for active pharmaceutical ingredients (APIs) or key intermediates for potential COVID-19 therapeutics
  - Find alternative synthetic routes as feedstock reactants become unavailable
  - Develop synthetic routes for novel compounds in a medicinal chemistry optimization program for COVID-19 therapeutics
- AutoSyn – an automated multi-step flow synthesizer can:
  - Produce gram-scale amounts of COVID-19 APIs or intermediates
  - Rapidly switch between chemical production processes on a single, fixed-configuration instrument

Task 1: SynRoute - Knowledge-Based Route Design, Planning, and Automation



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SynRoute Phase 3 Updates

- Implemented a new search algorithm that allows diversified routes beside the optimal route
- Developed a new algorithm to find routes for synthesizing multiple target compounds by minimizing the number of different reactions
- Developed an approach to add new reaction transformations to MCT ML classifiers from other validated reactions (e.g., from Reaxys reaction set)
- Integrated the US patent database to SynRoute after applying a series of modifications to remove inconsistencies between reactants, solvents, and reagents
- Designed and implemented a completely new user interface to search, display routes, and more *[Outside Make-It contract]*
- Partnership with Elsevier to port SynRoute on their new Entellect platform *[Outside Make-It contract]*

Algorithm for Diversification of Routes

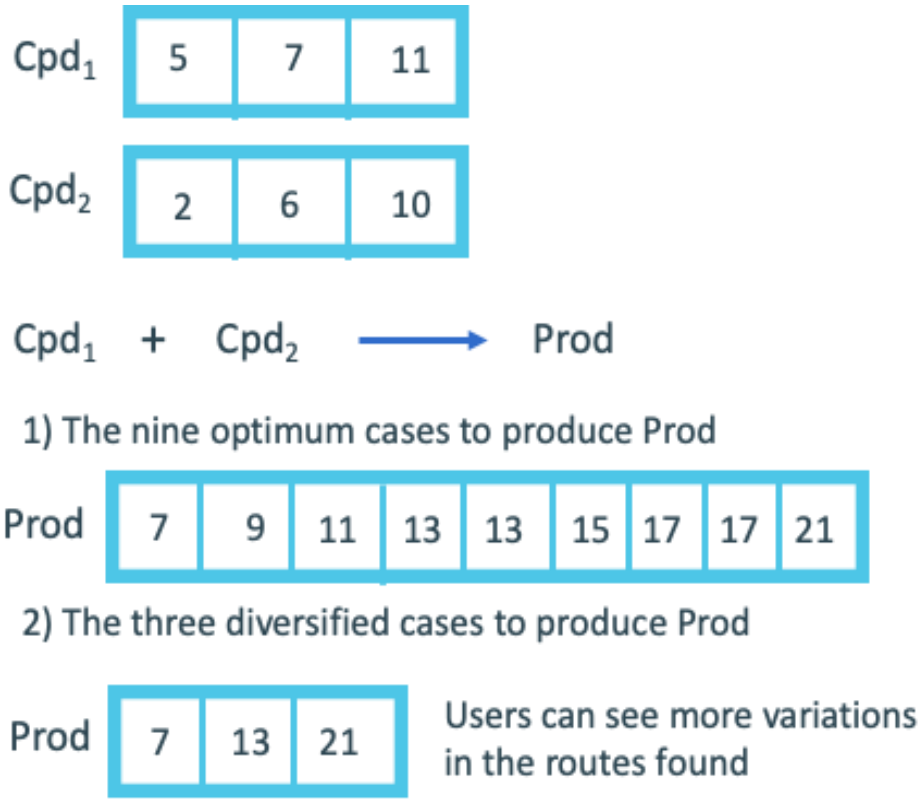
- Suppose a reaction of the form
  $Cpd_1 + Cpd_2 \rightarrow Prod$

where Cpd_1 and Cpd_2 have three different costs coming from sub-routes and that the reaction has a perfect yield.

- Then there are nine combinations of costs that can be assigned to Prod as shown on the right.

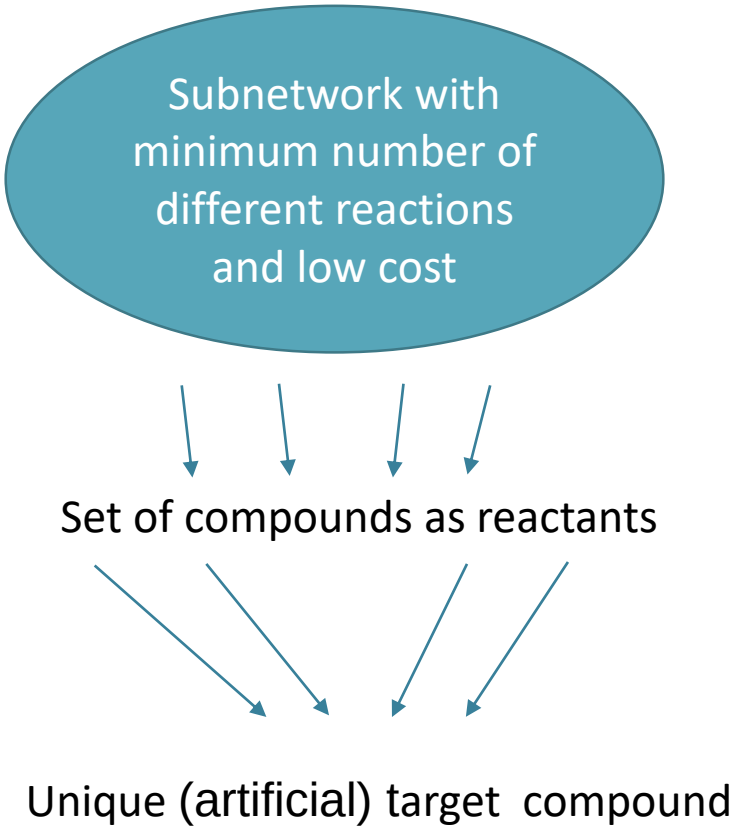
- But for the diversified approach, only three cases are considered, one from each pair of costs (5,2), (7,6), (11,10).

- The result has the optimal, but more variations after the optimal.



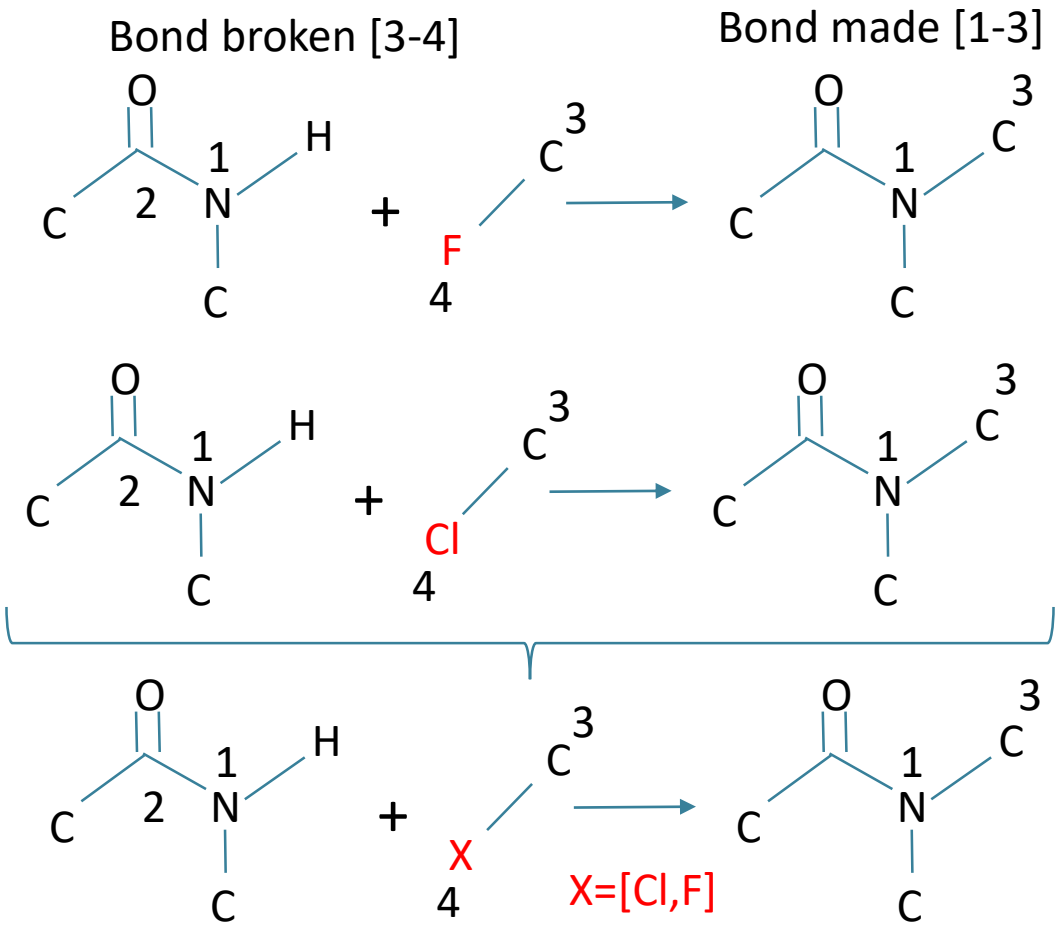
Finding Efficient Routes for Sets of Compounds

- A set of compounds could be produced with a smaller set of total reactions when considered together rather than individually
- For example, two or more compounds may share an intermediate that can be first produced, then used as a common reactant
- SynRoute was extended to find optimal routes based primarily on minimizing the total number of different reactions used



Generating New Reaction Transformations from Literature based Reactions

- SynRoute has 62 MCT reaction transformations
- They do have some limitations (e.g., heterocyclic formation transformations)
- Generating new reaction transformations by programmatically analyzing reactions from literature (e.g., Reaxys)
- Atom mappings are computed for > 100K reactions
- Reaction centers are used to generate basic patterns (SMARTS can be used)
- Similar reaction centers are iteratively merged to form general transformations

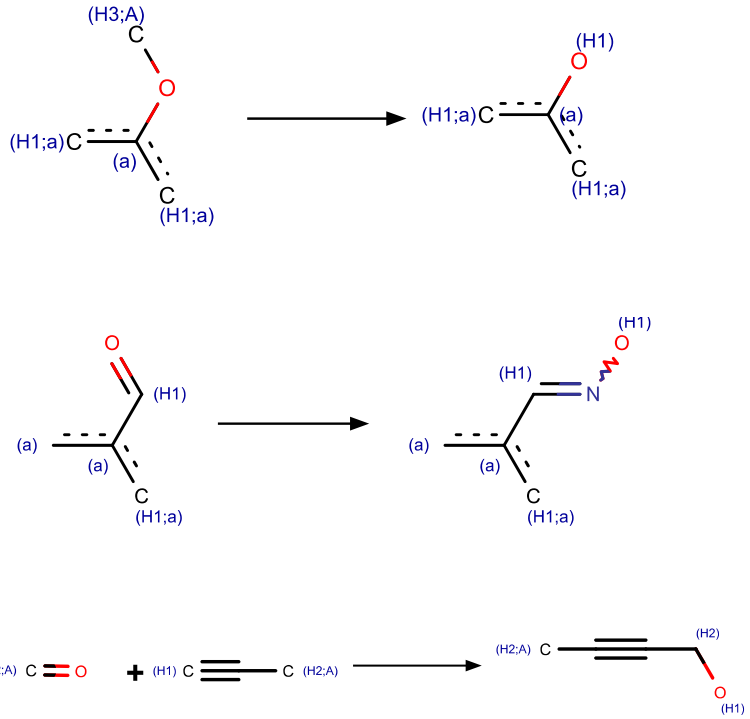


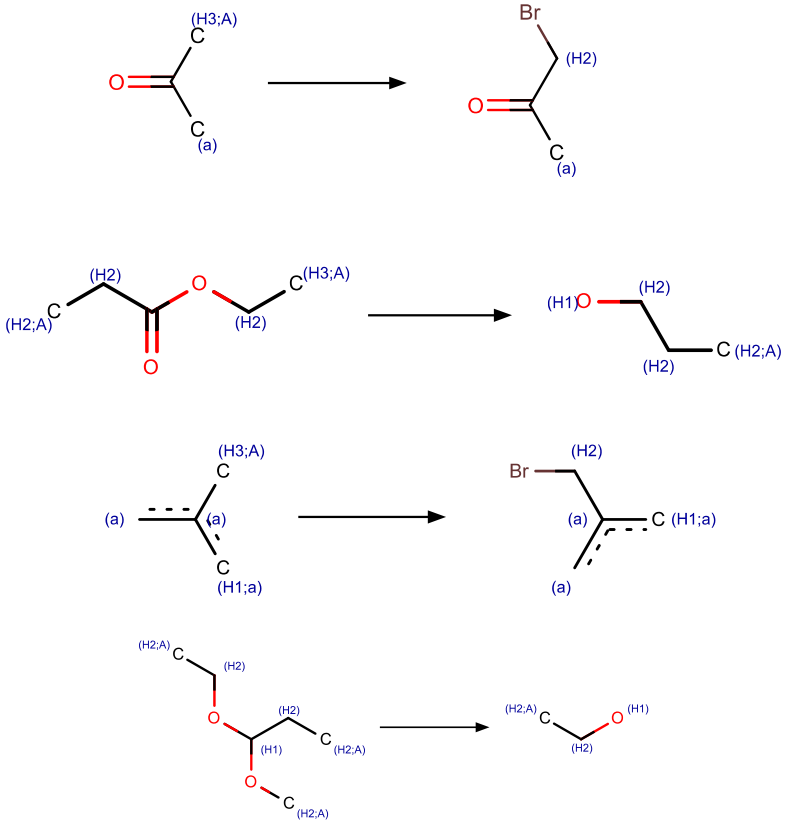
Iteratively, with the proper reaction centers, it leads to a general Amide N-alkylation transformation

New Transformations Identified by Atom Mapping Analysis

- Many of the most prevalent transformations were already covered by our Medicinal Chemists Toolbox (MCT) transformations

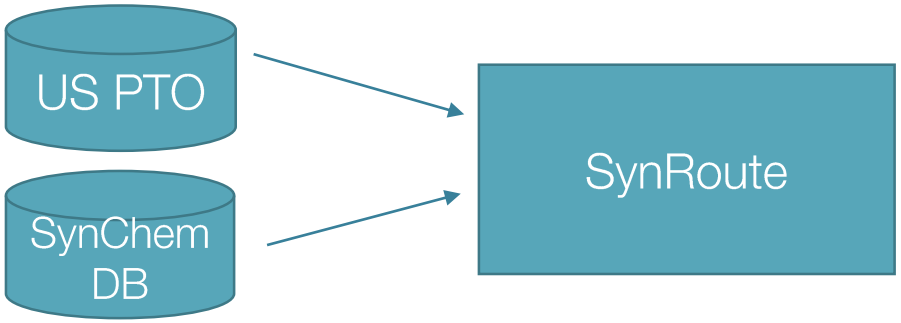
<u>Examples of New Extracted SMARTS Transformations with >40 examples:</u>



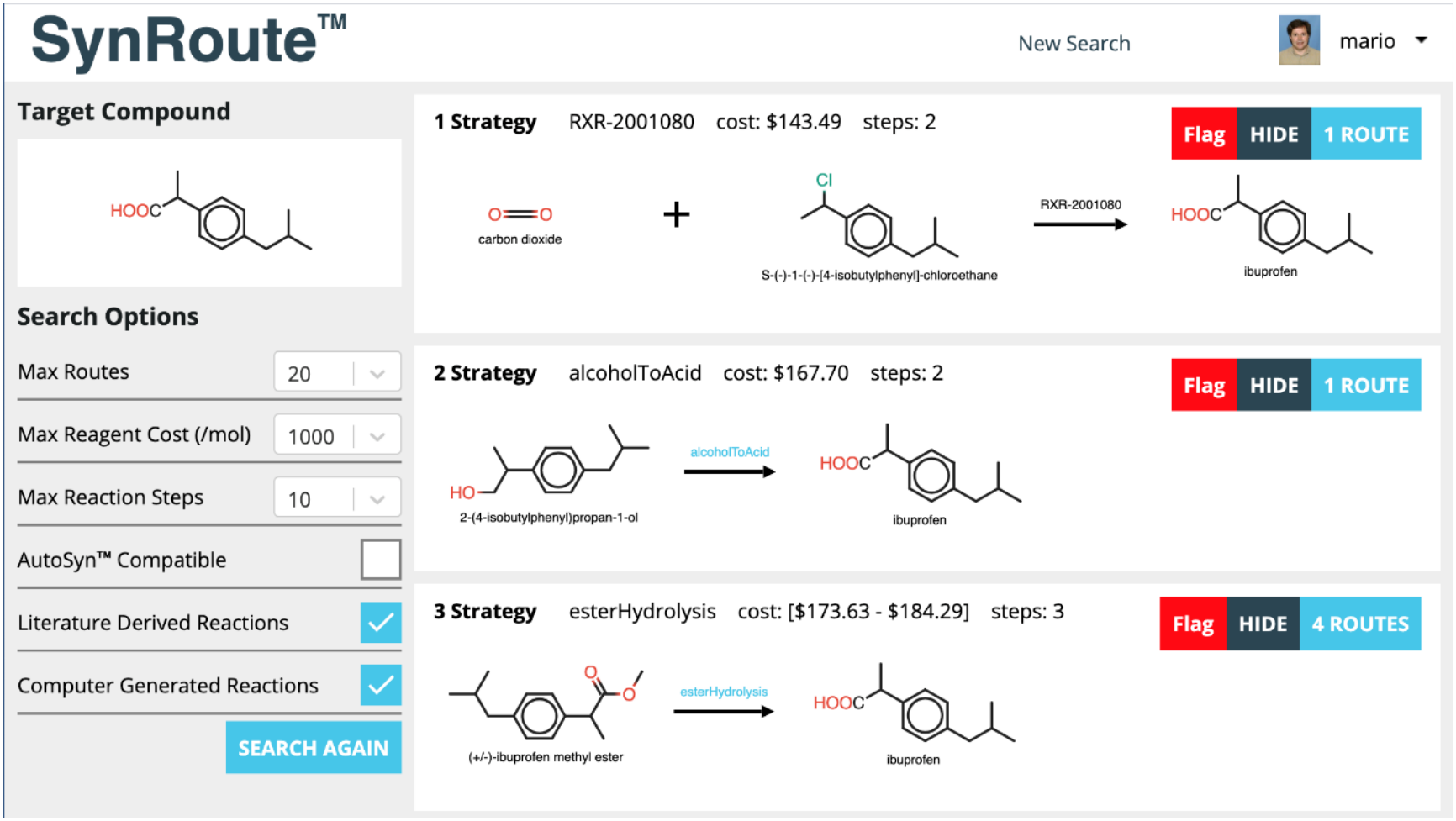


SynRoute without Reaxys data

- SynRoute is quite effective using the Reaxys database of reactions and compounds (17M reactions)
- However, no licensing is available to use Reaxys data directly in SynRoute for commercial use
- One answer is to use the US Patent data (1.5M reactions) combined with the SynChemDB data that will be generated at SRI
- The US Patent data was cleaned up to remove many inconsistencies, such as confusion between reagents, reactants and solvents
- The US Patent data is the initial stage to have SynRoute without Reaxys
  - Reaction data extraction from open literature materials & methods section also being tested through AMD program work
- With time, the SynChemDB data generated at SRI will provide experimentally verified reactions for SynRoute

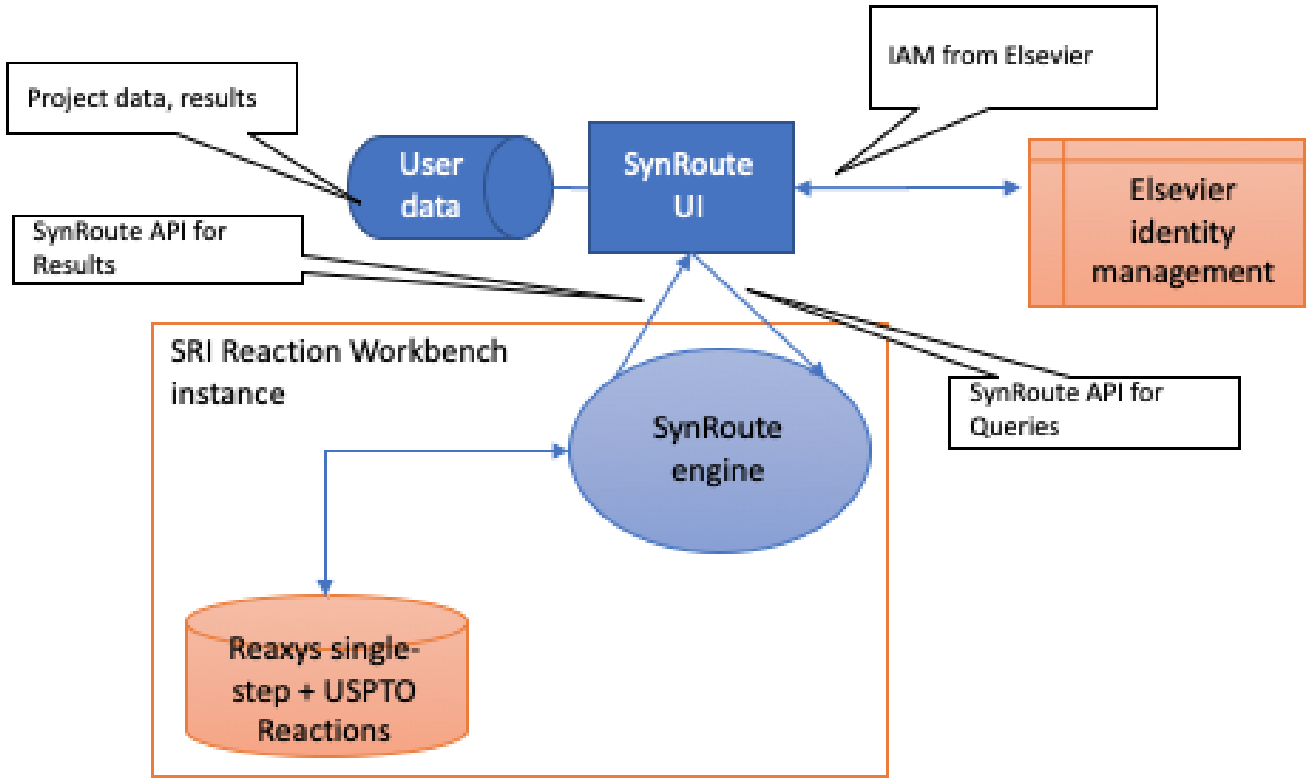


Complete New User Interface for SynRoute



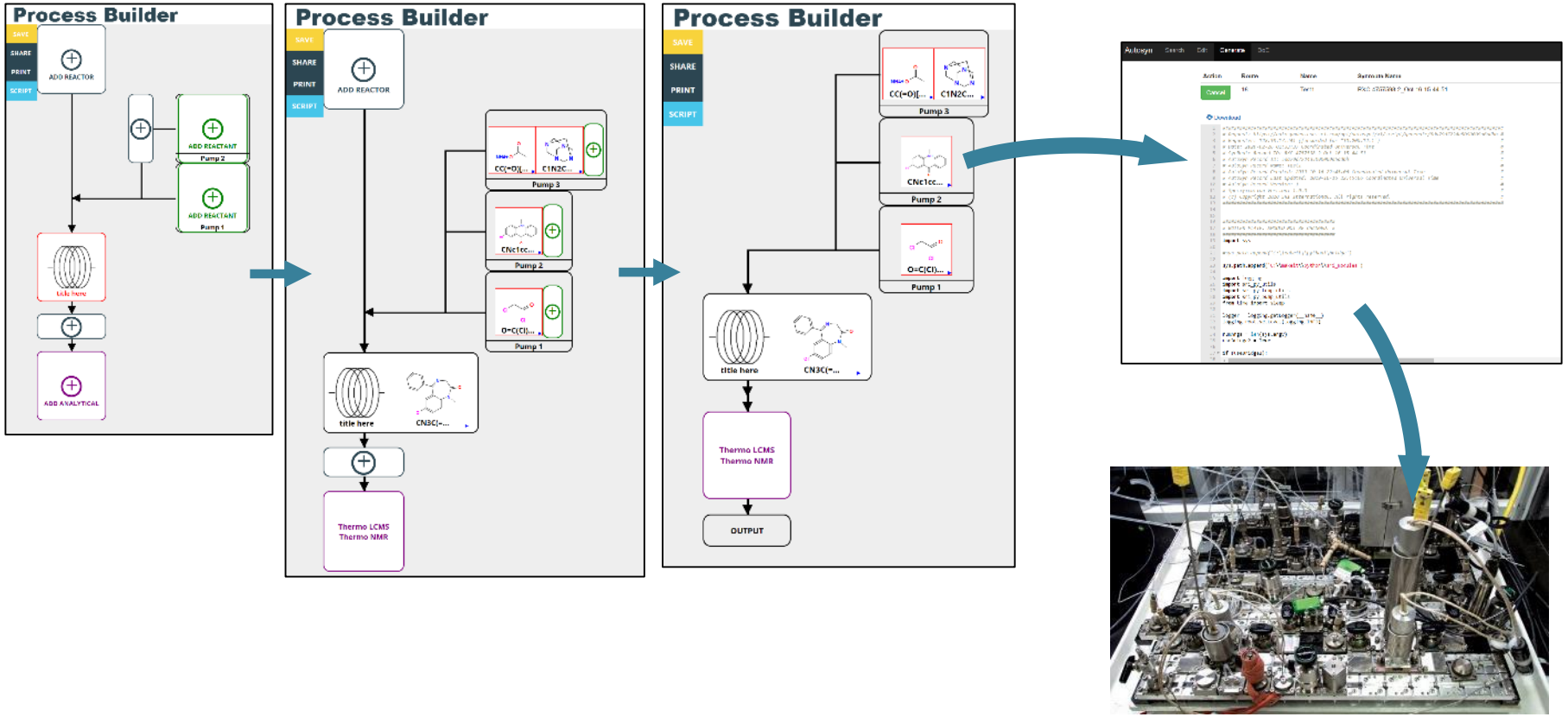
Porting SynRoute on Elsevier's Entellect Platform

- Difficulties in making Reaxys-based SynRoute available to third parties
- Planning with Elsevier to port SynRoute to Entellect platform
- That will provide high visibility of SynRoute to thousands of users



SynRoute to Process

- Developed web-based tool for manually annotating routes with a user-friendly GUI into SynRoute-compatible JSON schema
- Allows for direct integration into automated script generator



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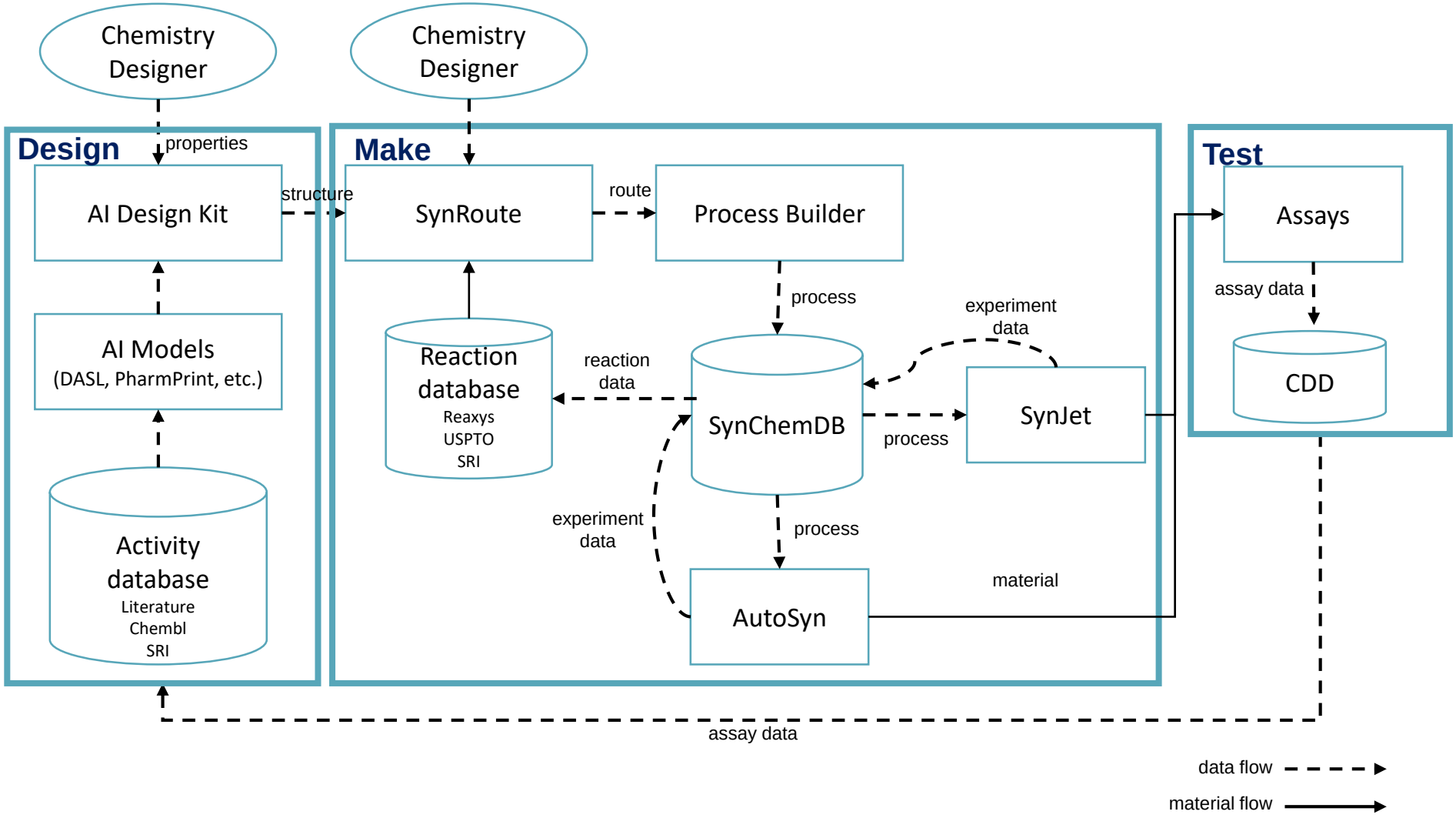
SynFini High Level Data Flow



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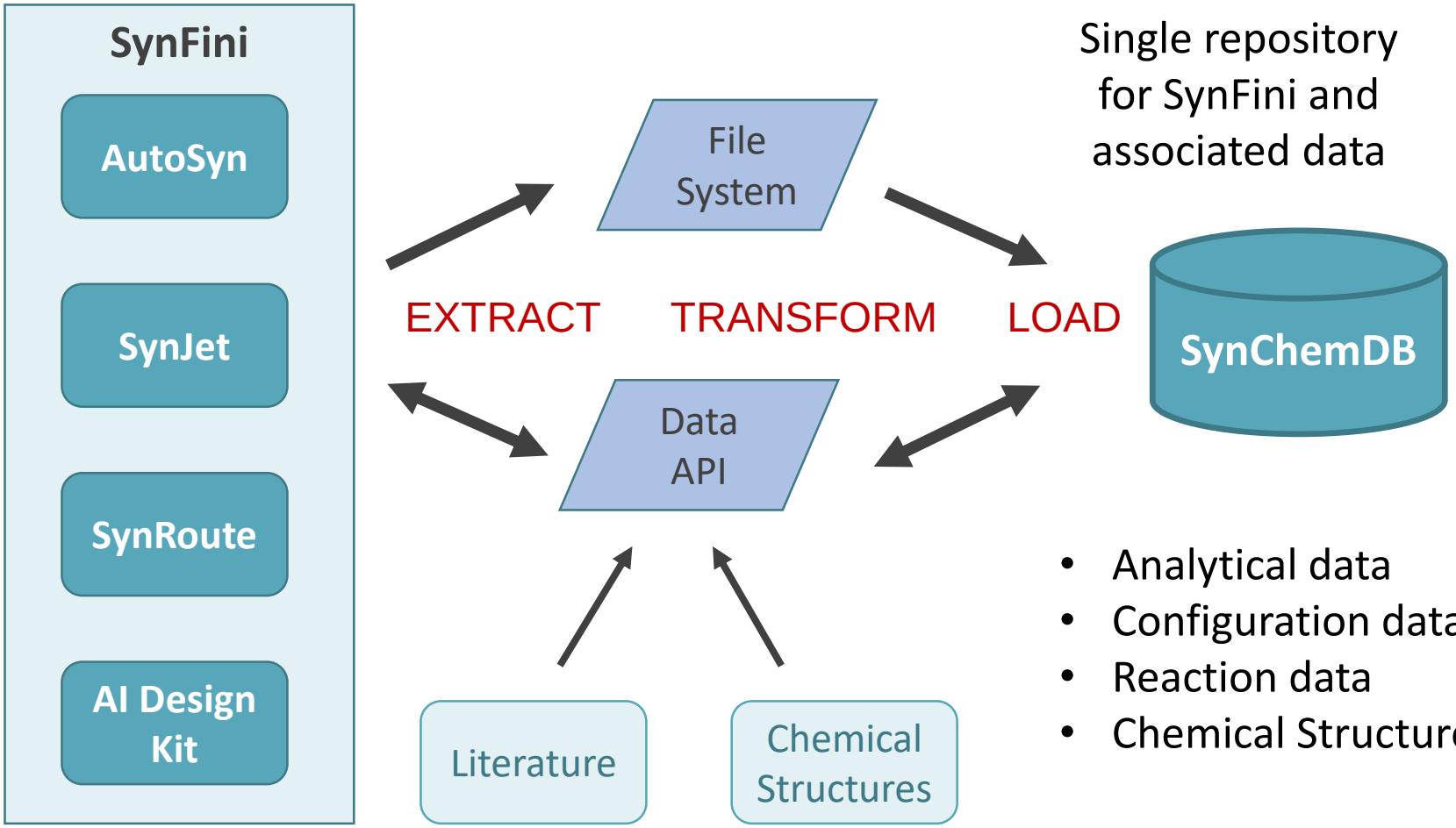
Interfacing to SynChemDB

- SynChemDB developed to receive route information, analytical information, experimental information, and data.
- Formatted with fixed file system for supporting future queries.
- Designed to accepts data from both SynJet and AutoSyn
  - *Currently* only takes AutoSyn data in real time.
- Key components
  - Linux VMs on SRI cloud infrastructure (IVI)
  - PostgreSQL version 11
  - Schemas for files, experiments, data, hardware components, compounds, reactions, routes, etc.
  - Data file loaders (Python)
  - APIs for database access (Python)
  - Code and Flask web server implemented in Docker container
  - Simple reporting UI
    - Charting with Python plotly, HTML templates

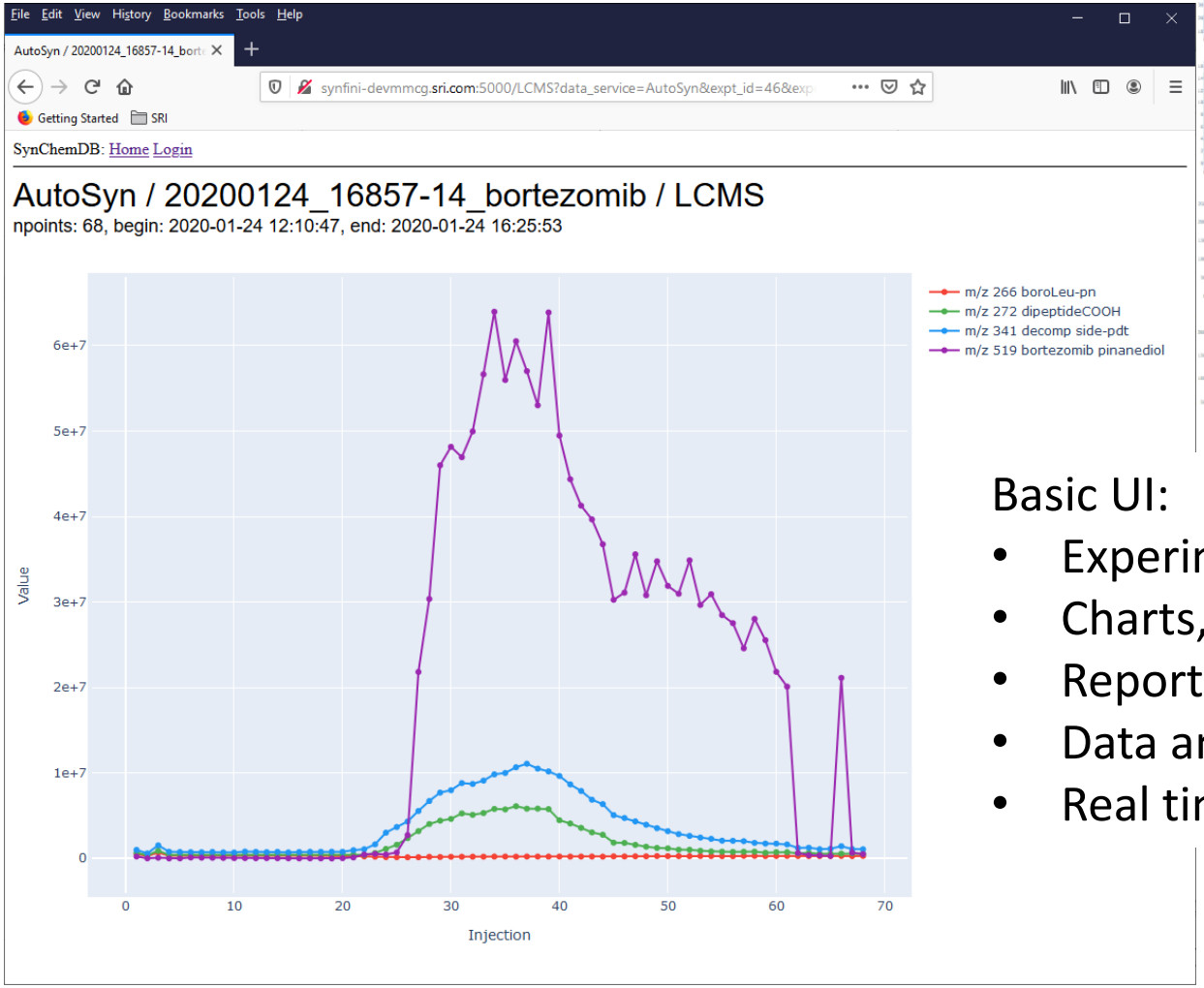
SynChemDB – Data Capture

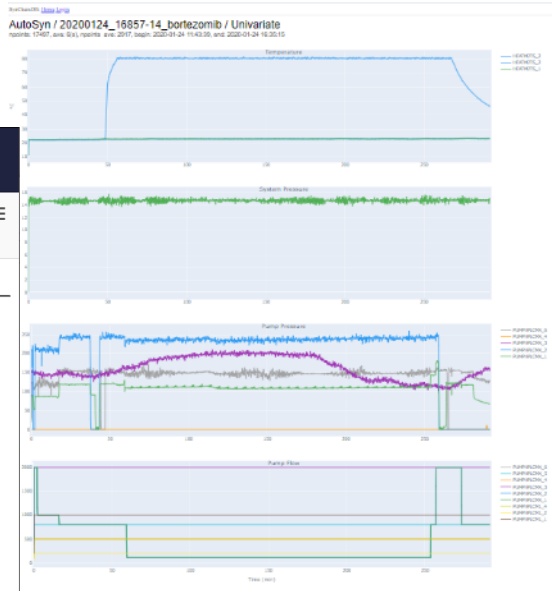


Single repository for SynFini and associated data

- Analytical data
- Configuration data
- Reaction data
- Chemical Structures

SynChemDB – Real-time Data Reporting





Basic UI:

- Experimental results
- Charts, tables, dashboard
- Reporting
- Data analysis
- Real time monitoring

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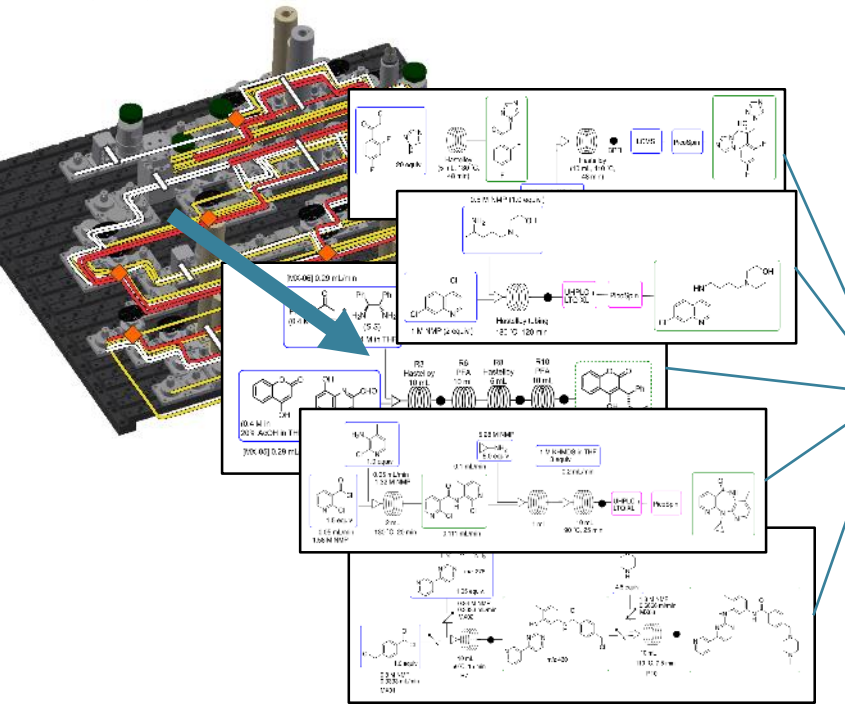
ELN to SynRoute

- Hardware-generated data is captured by SynChemDB, which will be provided to the reaction database for SynRoute for machine learning on flow chemistry experiments
  - JSON schema developed for passing reaction information

The AutoSyn platform runs via a script, generating data…



…which is captured in SynChemDB…

SynChemDB

…which can generate a structured JSON file containing reaction outcomes…

…which can be ingested into the SynRoute database.

SynRoute reaction database

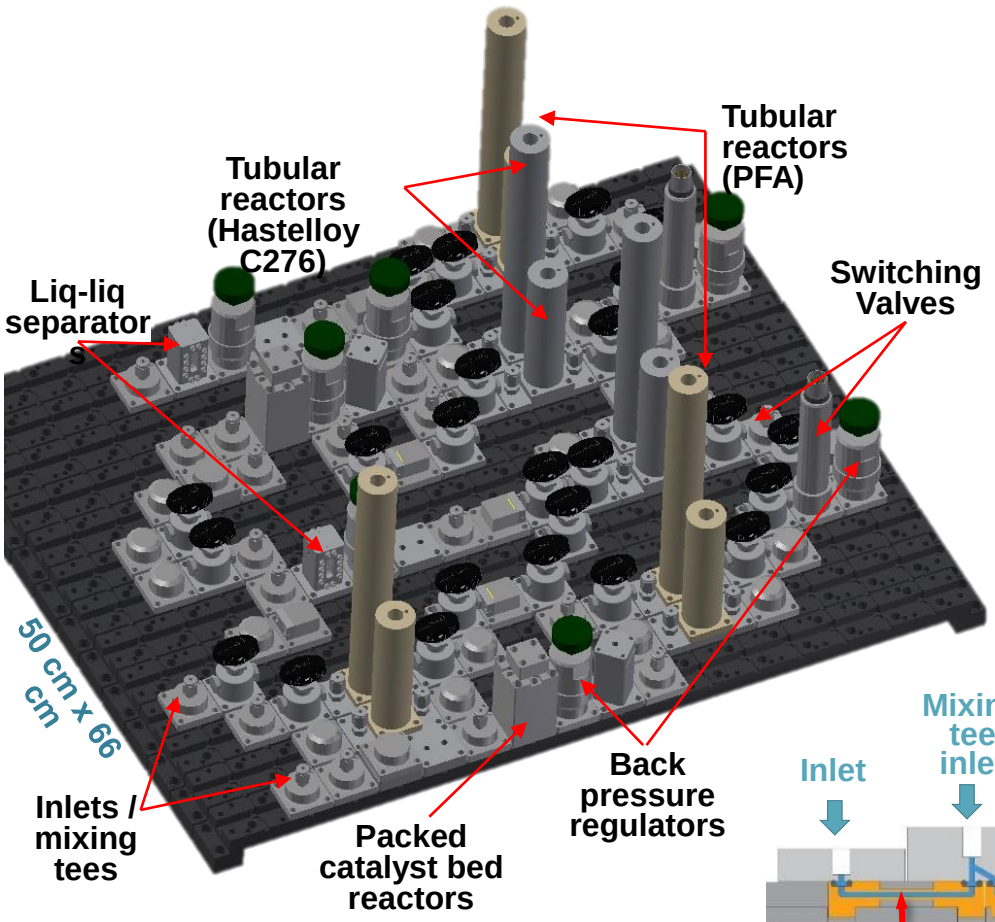
Task 2: AutoSyn Hardware Development

Multistep Flow Synthesis Platform Overview

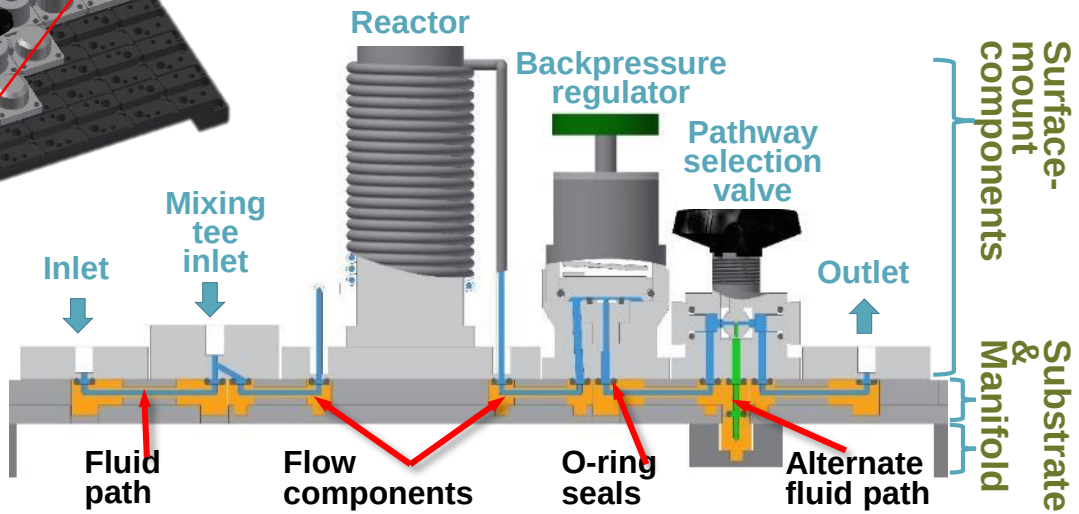
Summary of Phase 3 Updates

- Installed second system (“Beta”) at Menlo Park lab and demonstrated transfer of chemistry from previous system with high degree of reproducibility
- Integrated new SciEx LC-MS analytical with Beta system
- Built and revised novel syringe pump for best-in-class flow stability at elevated pressure
- Identified and acquired novel low-cost flow sensors for expanding potential feedback schemes
- Assembled cooling reactor solution for expanding capabilities of existing AutoSyn systems

Development of an Automated Synthesis Platform



- Adapted from Swagelok MPC, a surface-mount gas handling platform
- Reengineered to be compatible with flow chemistry



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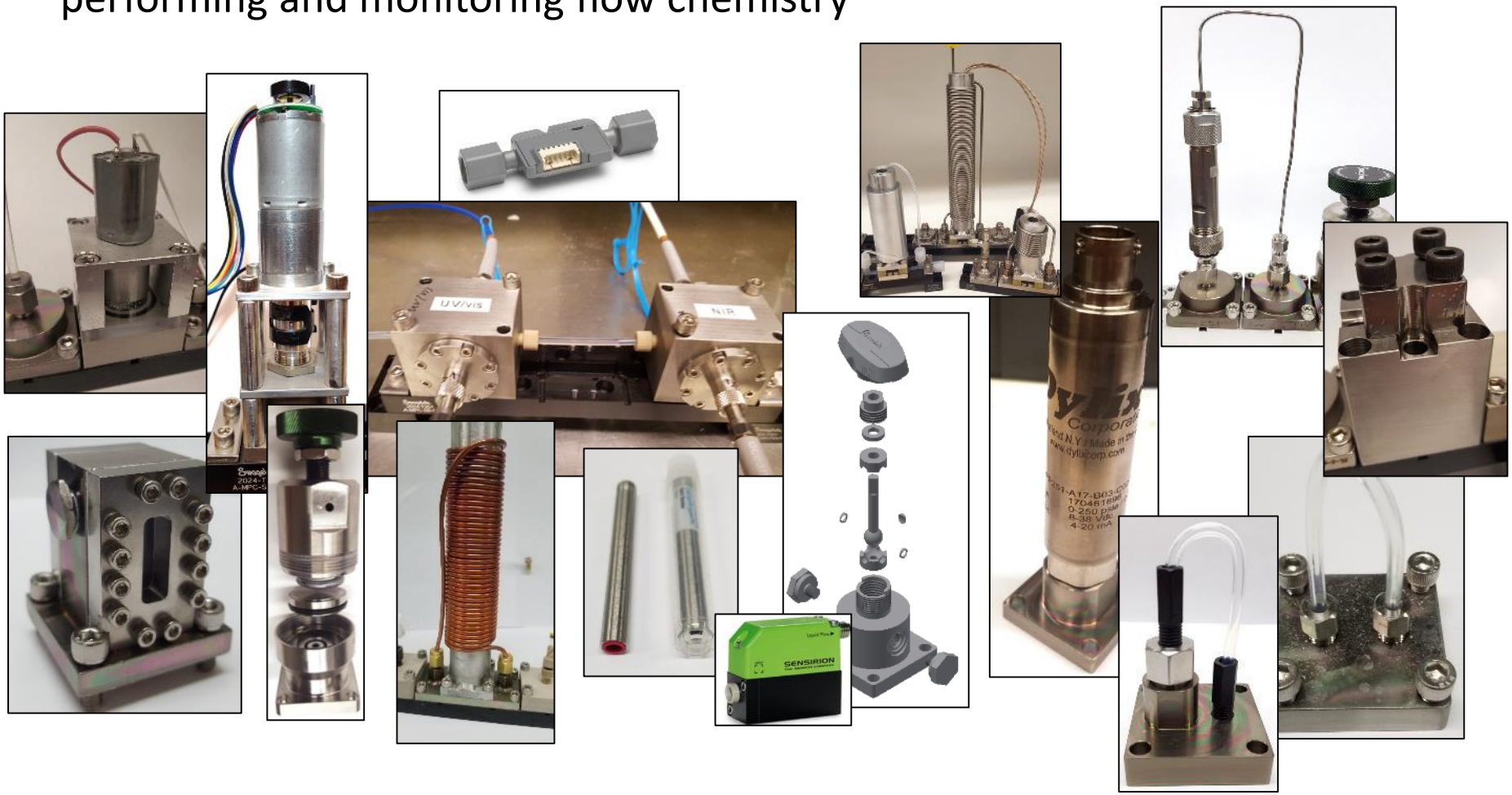
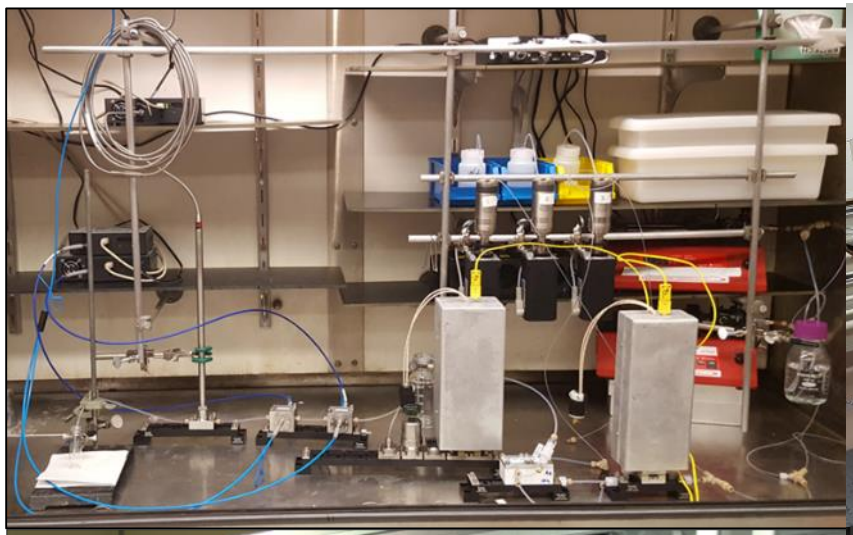
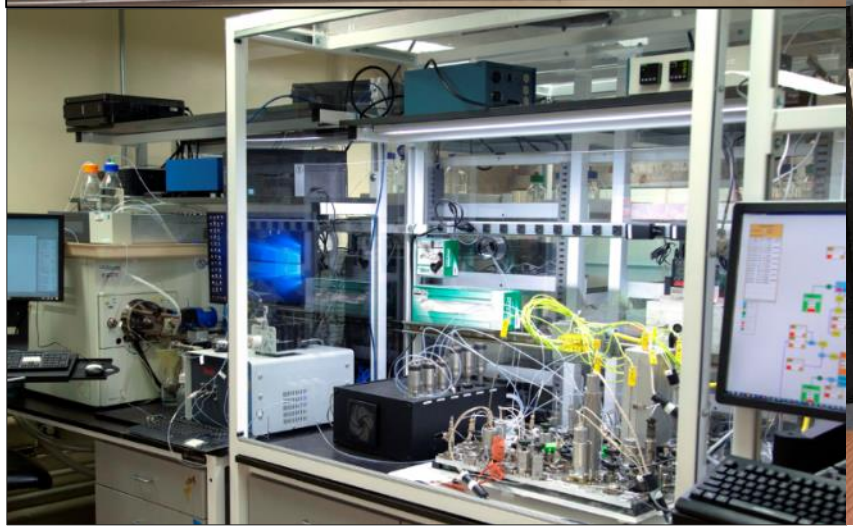
Custom Hardware Development

- Developed suite of custom MPC-compatible reaction hardware for performing and monitoring flow chemistry



Integration of Hardware

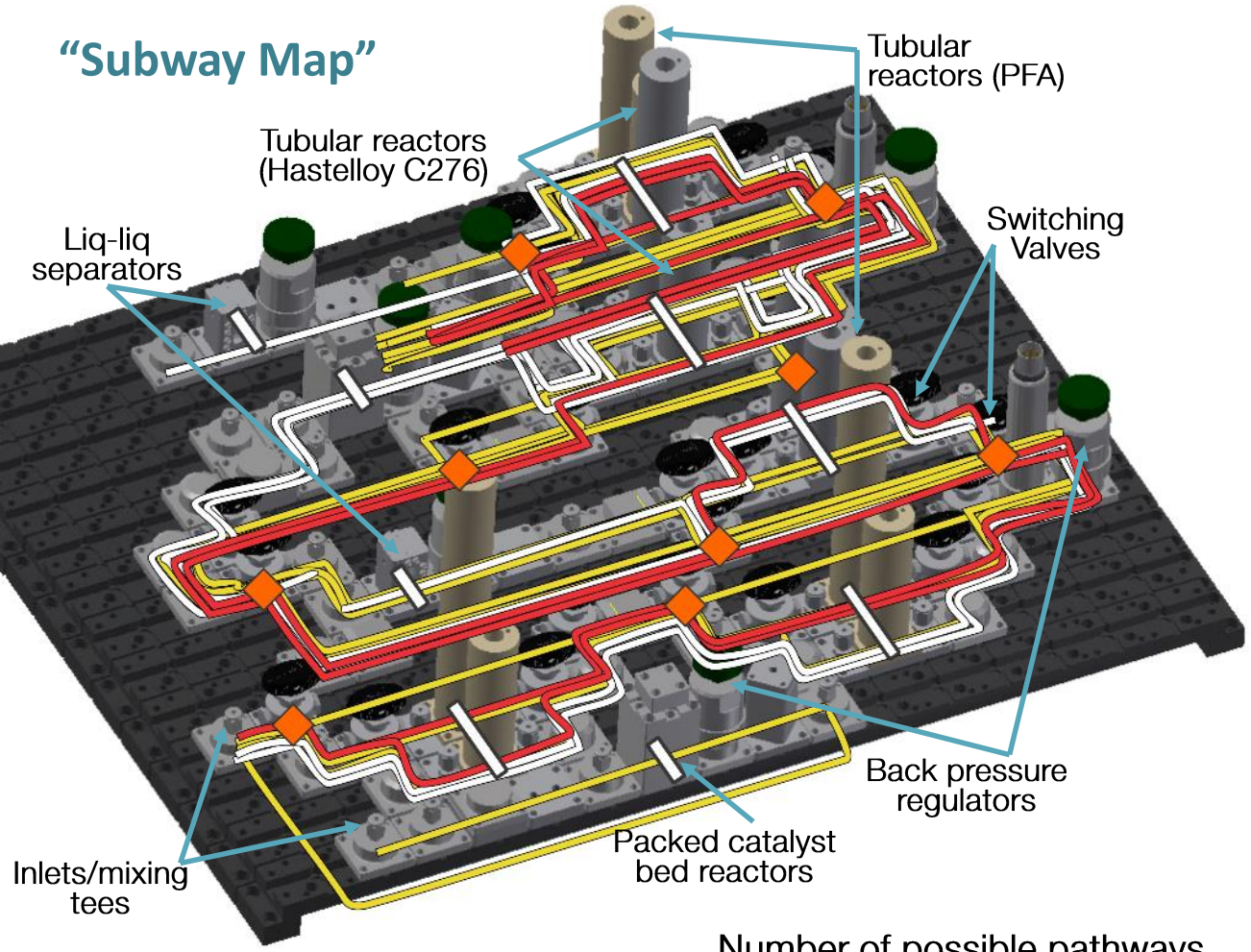




Mapping Synthetic Routes on the Baseline Configuration

Subway map with Phase 1-3 targets

"Subway Map"



Unit Operation Modules

Flow path selection point

Number of possible pathways

Not counting parallel reactors 511

Incl. different residence times 3,887

Mapped Routes

Phase 1
- Diphenhydramine
- Fluconazole
- Ibuprofen
- Diazepam
- Nevirapine
- Hydroxychloroquine
- Warfarin
- Tranexamic acid

Phase 2
- Imatinib
- Tramadol
- Pregabalin
- Naproxen
- Lamivudine
- Ribavirin
- Taribavirin
- Tiazofurin

Phase 3
- Bortezomib
- Itraconazole
- Quinapril

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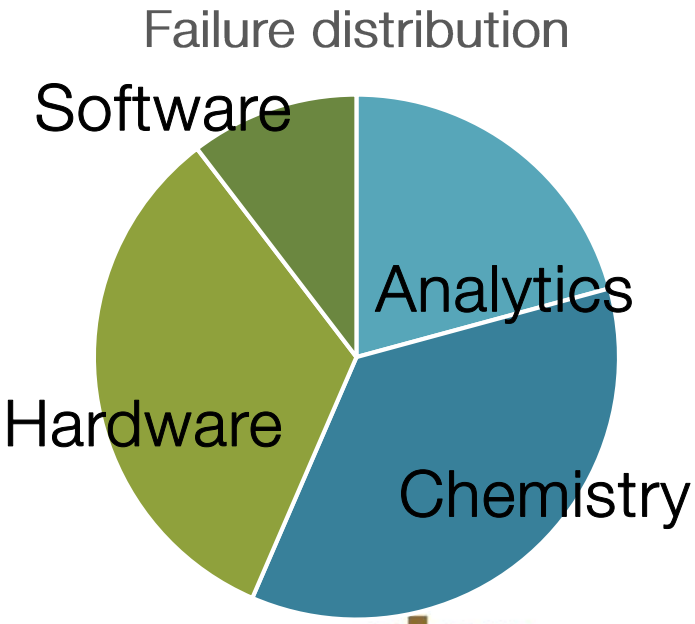
Usage and Failure Analysis

- Approach
  - Tabulated issues and failures from detailed logs
  - 262 runs, >2500 h of chemistry in 22 months
- Key issues
  - Pump robustness
  - Solid handling in flow
  - Software and data management
- Next steps
  - Incorporate additional sensors at key locatic
    to identify and predict failure
  - Evaluate pump modifications and alternativ
  - Develop methods to predict or measure precipitation and proclivity to clogging
  - Refine software and scripting



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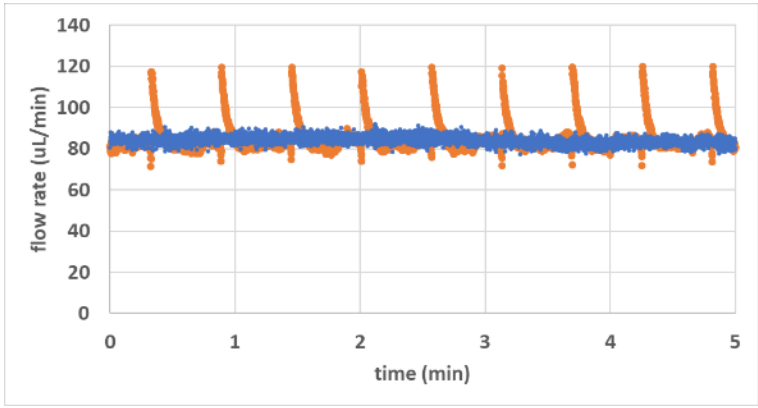
Evaluation of COTS Pumping Technologies

- Evaluation of state of the art in low flow, high pressure technologies
  - HPLC, syringe, rotary piston, etc.
- Modification of pumps required for flow chemistry
  - Chemical compatibility, clogging, higher pressures
- New type of pump (shown right) needed for flow
  - Chemical compatibility, pressure generation, delivery smoothness, mounting orientation, cost, footprint

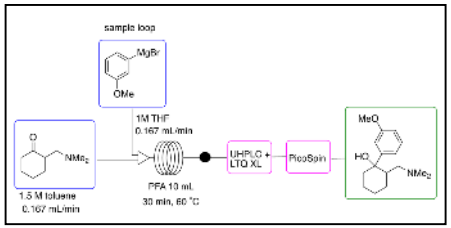
Some of the pumps evaluated and integrated into the AutoSyn platform. From left to right, OEM HPLC pumps, custom HPLC pumps, rotary piston pumps.

Development of Improved Pump

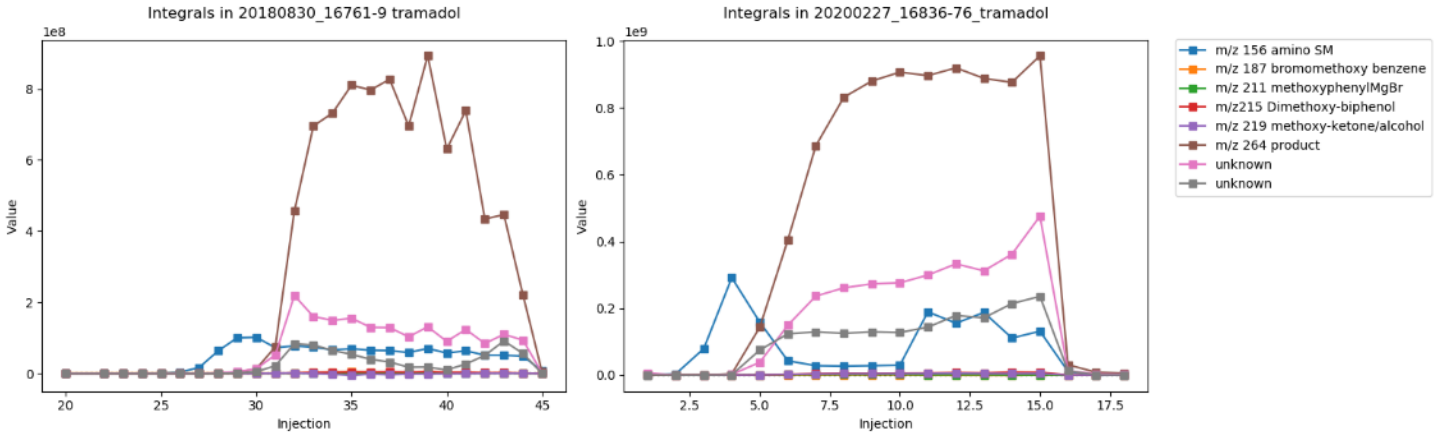
- Continued development of syringe pump to fill gap in pumping technologies
- Continued testing and refinement of design
  - Revised motor design based on user feedback for faster filling operation without reduction of pump performance
  - Tested Dursan-coated syringe with pump
- Tramadol test with Grignard reagent
  - Able to maintain slurry precipitation in flow throughout and get steady MS signal



Flow stability of state-of-the-art duplex HPLC pump with pulse dampener (orange) vs. SRI syringe pump (blue).



Tramadol synthesis with HPLC pumps (left) and syringe pumps (right). Syringe pump systems demonstrate very stable analytical signal.



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Improving Feedback Control

- Current state of the art for delivery and monitoring of reagents has no feedback loop
  - Existing pumps have no flow sensors for feedback on delivered flow
  - No solution for intermediate flows, i.e. between steps
  - Available flow sensors are too expensive at every step of the current CityScape and lack the needed range
- New OEM-style flow sensor assemblies recently released by Sensirion (SLF3F-1300F)
  - Enables capture of flow data at more locations
  - Downstream flow monitoring may enable more predictive control to identify and manage chemistry issues before they cause hardware failure
  - Additional chemical testing and integration required

New sensor released by Sensirion boasts lower integration costs and larger dynamic range

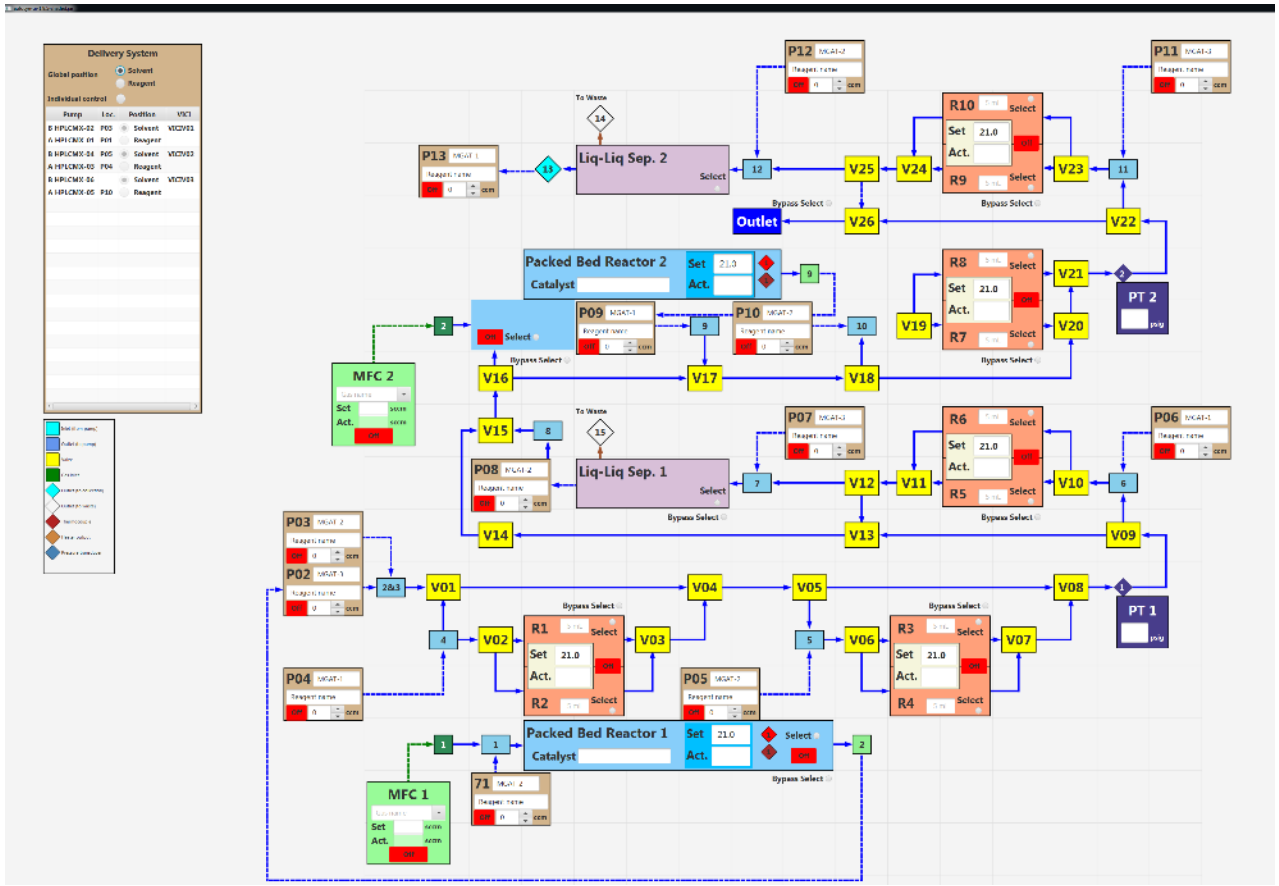
Comparison of new (left) and old (right) flow sensor specifications.

	SLF3S-1300F	SLI-1000
Price	$115	$1038
Footprint	0.61” x 1.89”	1.69” x 2.10”
Communications	I2C	USB
Response time	0.5 ms	40 ms
Failure mode detection	High flow, air-in-line	None
Dynamic range	±40 mL/min	±10 mL/min
Burst pressure	25 bar	30 bar
Chemical resistance	PPS, epoxy	PEEK, FEP

AutoSyn Control Software

- **Integrated UI developed for control and monitoring of CityScape, including the following features:**

- Single interface for hardware, sensors, and analytical
- Scripted experiments for reproducible chemistry
- Sensor fusion across platform for process feedback
- Robust structure
- Flexible data streaming and logging



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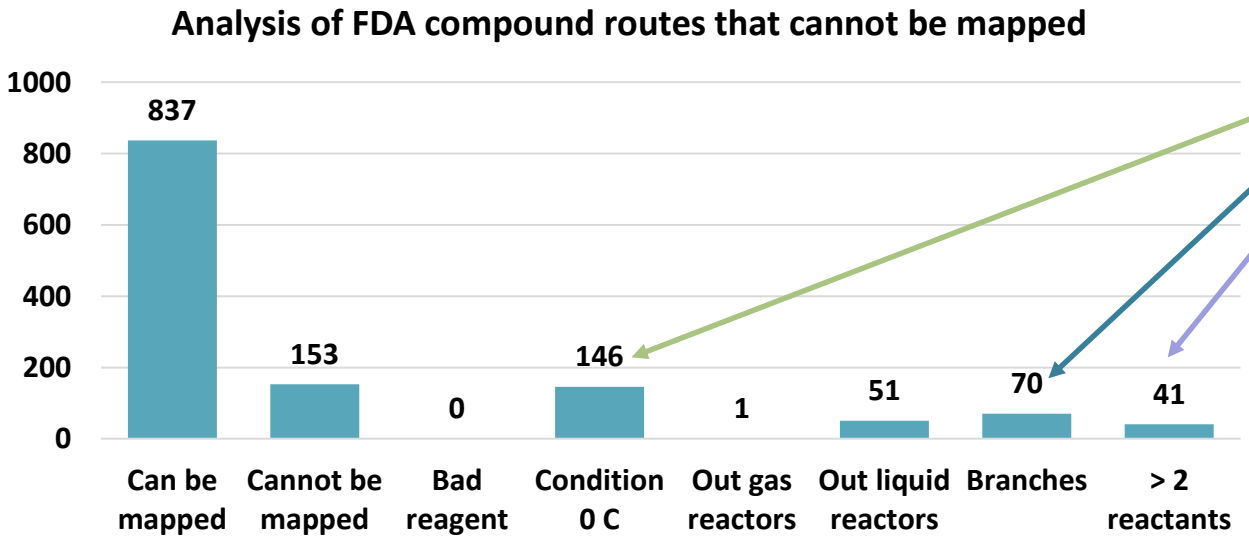
Expanding AutoSyn Capabilities

Previously reported analysis of AutoSyn's capability to synthesize FDA compounds

- 1,146 FDA compounds considered, after filtering out larger molecules (>1500 Da which are typically biologics), salt form variants of drugs and drugs that are simple gasses or salts
- Evaluated mapping of both cost-optimal routes and *any* mappable routes
- With no cost constraints 990 (86%) FDA compounds have routes on AutoSyn
  - Lack of low temperature reactor is the major cause of lack of mappability



Addition of:

- **Low temperature reactors**
- **Convergent synthesis**
- **Addition of >2 reactants per reactor**

All enable more routes

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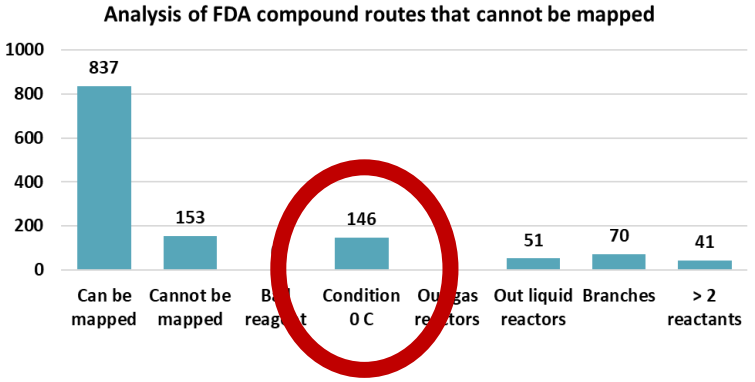
Enabling Low-Temperature Reactions

- Cooling previously identified as major opportunity for development
- Novel COTS solution identified from Solid State Cooling
  - Tested to enable 0 degC chemistry
- Testing miniature compressor-based chiller for direct cooling of tubular reactors
  - Improved SWAP compared to solid-state cooling, currently at 87% vs 20% efficiency

Left, ThermoWrap bottle chiller installed on plastic bottle. Right, mockup of ThermoCube Edge paired with ThermoWrap installed on a CityBlock.



Adding cooling capabilities to AutoSyn would enable >95% of the FDA-approved small molecule drugs to be synthesized

Proof of concept for miniature compressor-based cooler.

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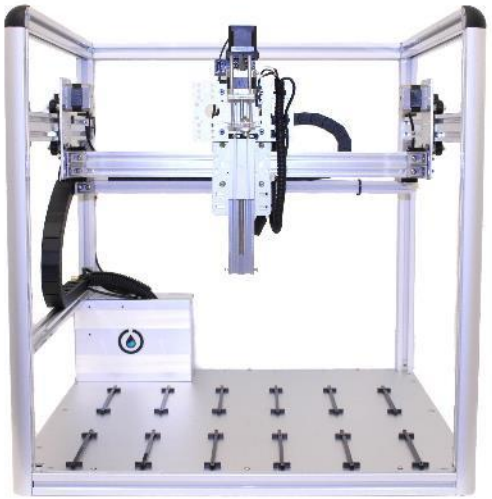
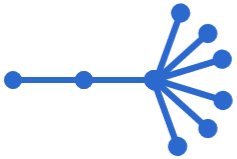
Multi-Reagent Delivery System

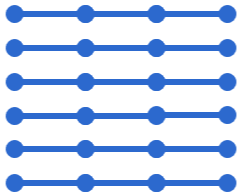
- Additional opportunities identified to expand range of operating modes
  - Reagent optimization:
    - Change reagents, solvents, and concentrations at multiple points
  - Library generation:
    - Add ability to switch delivery between multiple building blocks and diversity elements
    - Can do this at any step in AutoSyn to enable greater access to molecular diversity



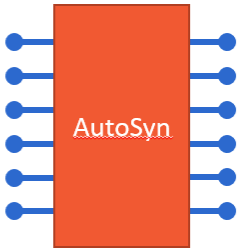
Modification of low-cost open-source liquid handler such as an Opentrons OT-1 will enable automated access to dozens of reagent inputs



Divergent synthesis
- 8 operations
- Diversity from common synthetic intermediate



Linear synthesis
- 18 operations
- Max chemical diversity



Automated synthesis
- 6 operations
- Diversity around common chemical processes, not common intermediates
- Access diverse targets by changing chemical feedstocks and flowpaths

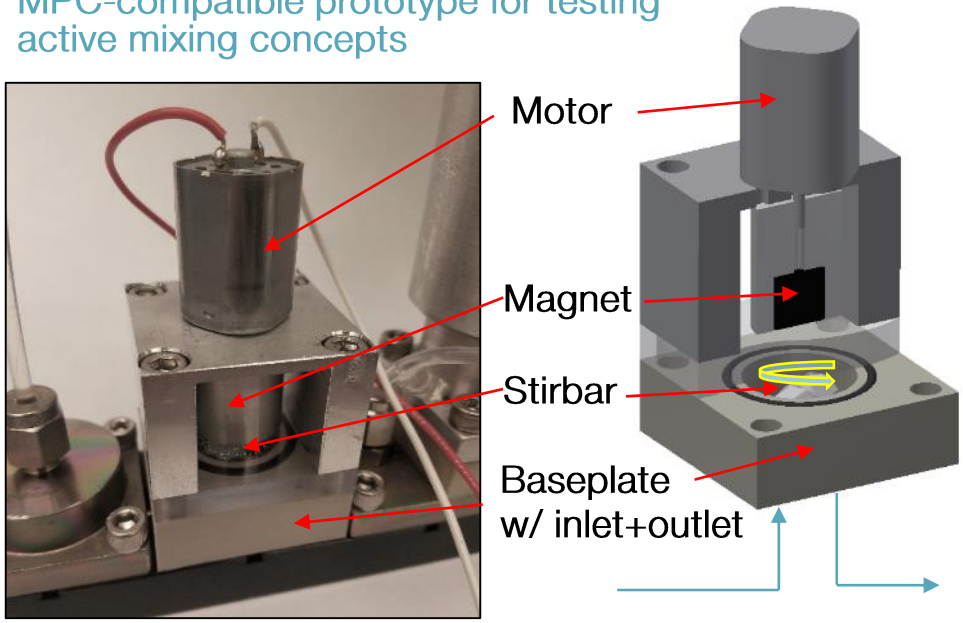
Concept drawing of modified liquid handler with standard vial racks

Inclusion of standard liquid handlers in AutoSyn will allow for diversification at any step, rather than late-stage diversification only.

Enhancing Reactions on AutoSyn

- Developed active mixing hardware for use on AutoSyn platform
- Can be used to further enhance multiphasic reactions and perform extended residence time reactions (>24 h) in a continuous fashion

MPC-compatible prototype for testing active mixing concepts



Cambridge Reactor Design's Chameleon reactor, a similar reactors-in-series approach.

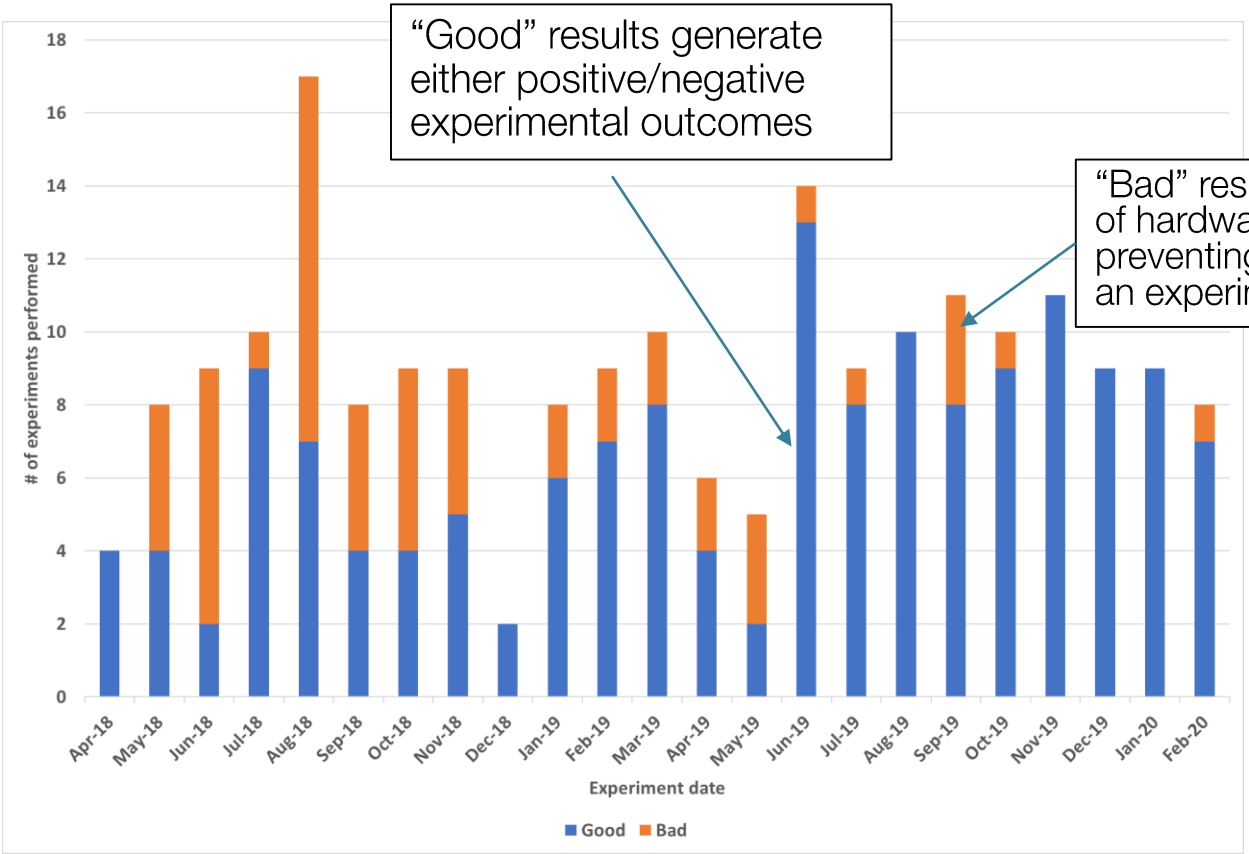
Test of extraction efficiency of propionic acid from an organic solvent by water. *Active mixer improved extraction by 5x.*

Configuration	% extraction
Tee to separator	53
Tee to 5 mL reactor to separator	74
Tee to 2 mL mixer to separator	96

Variable height baseline configurations

Hardware Performance

- Hardware robustness and chemistry familiarity of AutoSyn has improved dramatically over time
  - Major hardware issues have been identified and corrected
  - Identifying areas for improvement of user interaction



Transfer of Chemistry Between Systems

- Second system (Beta) built by SRI team in Princeton with no chemistry experience
  - Test knowledge transfer of hardware
- Tested with representative chemistry at Menlo Park
  - Diphenhydramine:
    - 91% purity on Beta vs 88% using Alpha
  - Imatinib:
    - 4.3 g/h on Beta vs 2.8 – 4.3 g/h using Alpha (range of optimized and non-optimized runs)
  - Additional data presented in analytical
- Integrated with alternative vendor of LC-MS to demonstrate robustness of integration and processing

Installation of Beta system in integrated lab.

Beta System: Analytical Hardware Installation

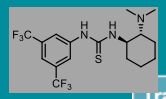
- Beta system and SciEx analytics deployed to SOA laboratory.
- SciEx methods developed and tested with imatinib (batch)
- Custom hardware being deployed for enabling online flow monitoring
- Python software updated to be agnostic to instrument vendor
  - Tested same processing code on Thermo and SciEx platforms.

Next Steps

- Verify operation to switch between flow and Autosampler
  - Supports versatility for AutoSyn, SynJet and other batch reactions
- Deploy updated Python code to all systems
- Implement daily operation of online monitoring with beta processes.

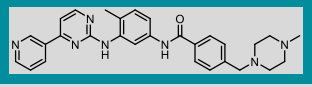
SynJet

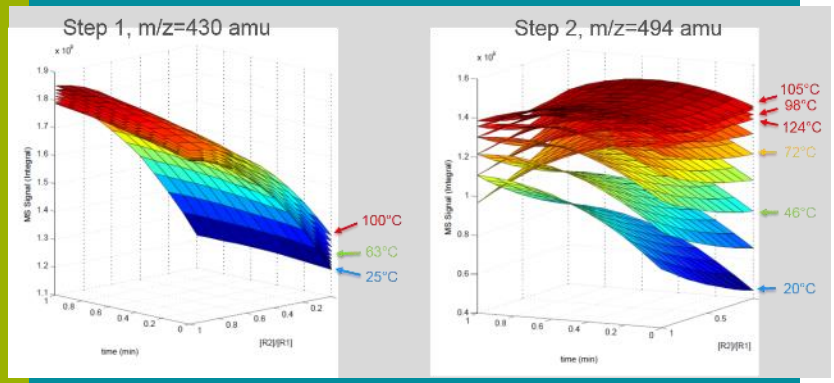
Reaction screening and optimization platform

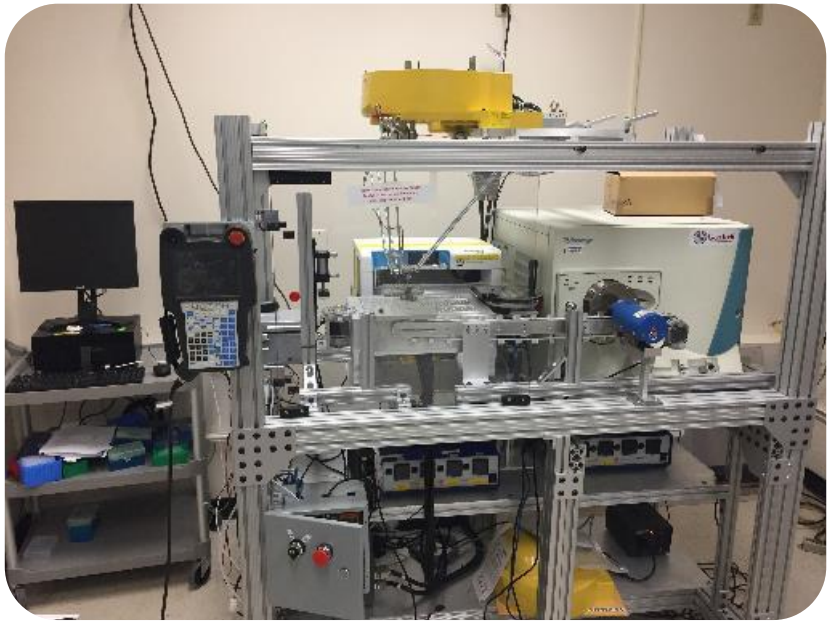


Translation of conditions

Step	SynJet optimum	Flow results
1	175 C, 10 min, 1.5 eq B	100%
2	98 C, 99 min, 0.9 eq B, 2.6 eq C	100%
3	175 C, 10 min, 6 eq B	>95%
4	70 C, 10 min, 1.2/1.3/1.4 eq B	42%







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SynJet: SNAr Profiling

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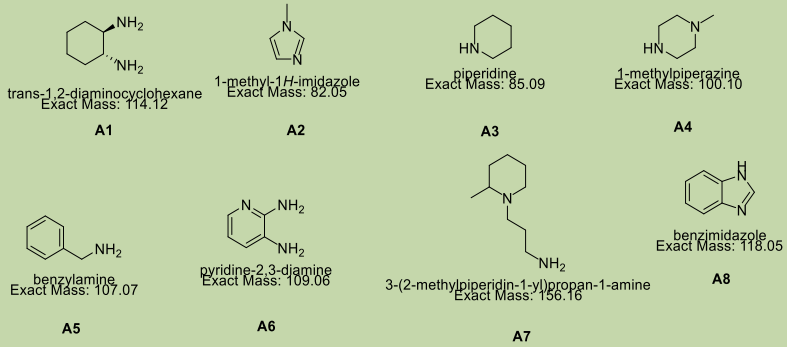

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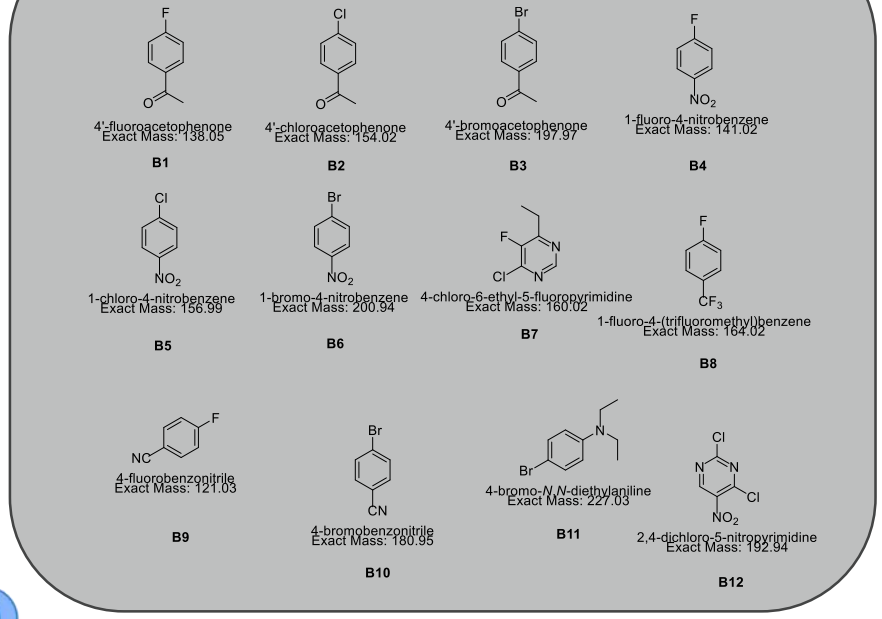

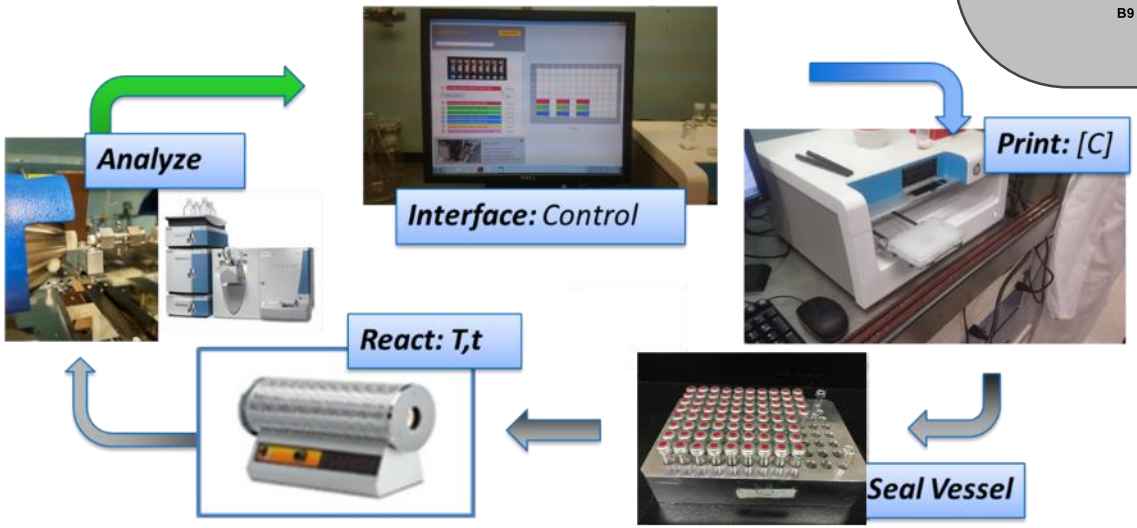

Test of SynJet platform to compare SNAr reaction profiling with Purdue's DESI approach

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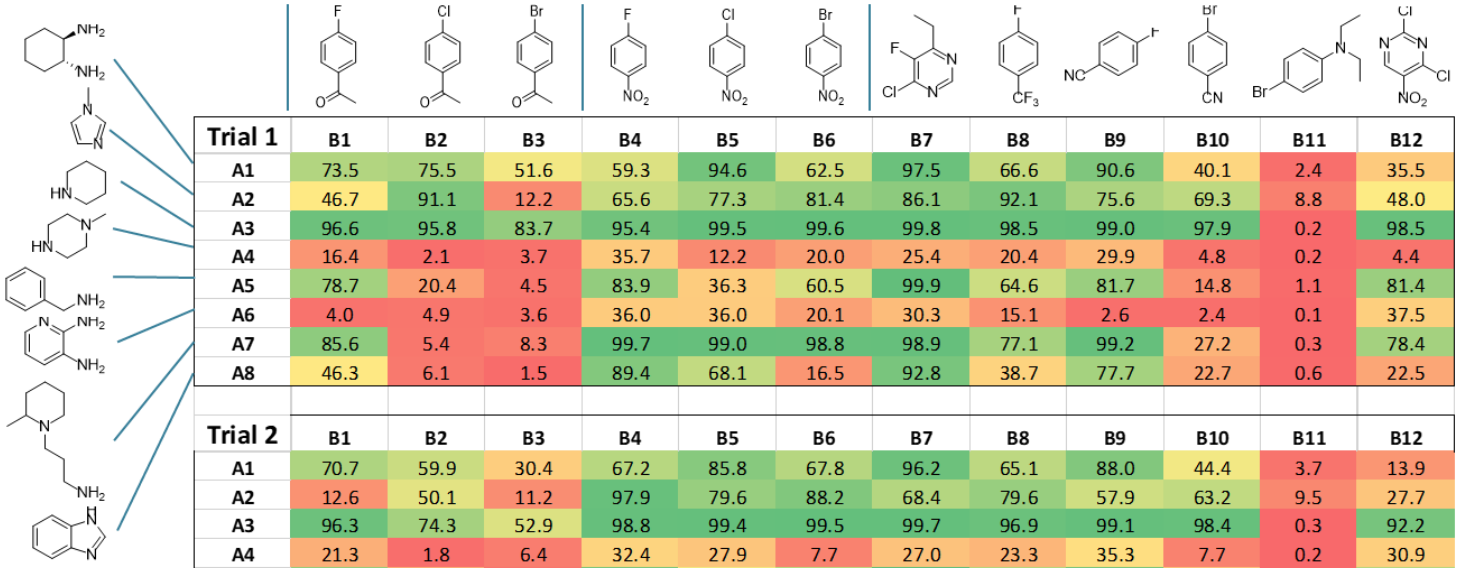
SRI SNAr results

- Three replicates over three days for product peak
- Reported as percentage of product $\frac{P_{int}}{\sum SM_{int} + P_{int}}$



Trial 1	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
A1	73.5	75.5	51.6	59.3	94.6	62.5	97.5	66.6	90.6	40.1	2.4	35.5
A2	46.7	91.1	12.2	65.6	77.3	81.4	86.1	92.1	75.6	69.3	8.8	48.0
A3	96.6	95.8	83.7	95.4	99.5	99.6	99.8	98.5	99.0	97.9	0.2	98.5
A4	16.4	2.1	3.7	35.7	12.2	20.0	25.4	20.4	29.9	4.8	0.2	4.4
A5	78.7	20.4	4.5	83.9	36.3	60.5	99.9	64.6	81.7	14.8	1.1	81.4
A6	4.0	4.9	3.6	36.0	36.0	20.1	30.3	15.1	2.6	2.4	0.1	37.5
A7	85.6	5.4	8.3	99.7	99.0	98.8	98.9	77.1	99.2	27.2	0.3	78.4
A8	46.3	6.1	1.5	89.4	68.1	16.5	92.8	38.7	77.7	22.7	0.6	22.5

Trial 2	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
A1	70.7	59.9	30.4	67.2	85.8	67.8	96.2	65.1	88.0	44.4	3.7	13.9
A2	12.6	50.1	11.2	97.9	79.6	88.2	68.4	79.6	57.9	63.2	9.5	27.7
A3	96.3	74.3	52.9	98.8	99.4	99.5	99.7	96.9	99.1	98.4	0.3	92.2
A4	21.3	1.8	6.4	32.4	27.9	7.7	27.0	23.3	35.3	7.7	0.2	30.9
A5	79.7	20.9	4.9	90.0	44.3	64.8	99.5	67.7	90.3	26.8	0.7	66.5
A6	3.3	3.6	1.4	9.6	3.3	4.8	28.1	12.6	2.8	2.6	0.1	19.2
A7	88.6	7.2	7.1	99.8	98.8	99.3	93.0	94.8	98.9	33.1	0.4	28.4
A8	39.6	2.2	2.9	91.8	41.7	29.0	86.1	38.7	77.0	23.7	15.4	12.4

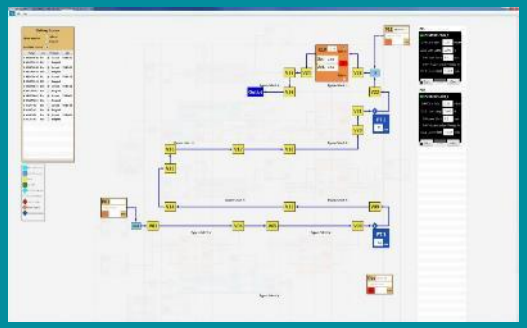
Trial 3	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
A1	63.5	73.4	39.4	48.1	97.2	62.1	96.4	64.7	89.0	42.2	2.7	21.0
A2	67.9	91.2	11.1	64.5	78.5	85.9	94.0	89.8	77.8	66.3	9.4	18.3
A3	97.1	96.0	71.3	94.5	99.2	99.7	99.8	98.7	99.0	79.8	0.1	93.4
A4	21.0	2.5	2.9	89.1	23.2	8.3	31.9	23.2	40.9	11.8	0.3	24.1
A5	83.1	15.2	4.7	85.1	39.5	56.0	100.0	63.3	80.8	19.4	0.5	36.6
A6	3.7	4.8	2.6	6.5	2.3	2.6	36.2	11.9	1.5	1.6	0.1	13.3
A7	85.3	9.4	5.7	99.8	98.4	99.3	99.7	92.2	97.2	74.1	0.3	32.2
A8	41.1	2.1	0.9	88.8	59.4	17.2	92.6	33.6	79.1	30.8	0.1	5.6

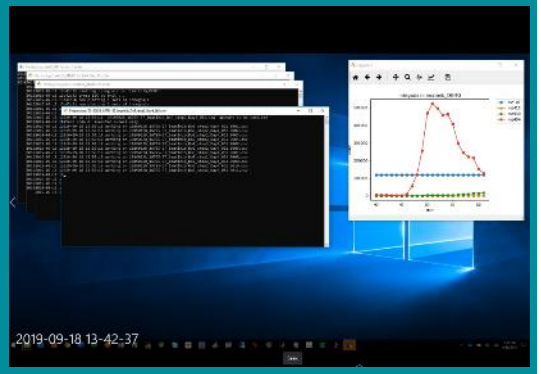
Ran replicate experiments on three separate days using SynJet system, which shows very good repeatability.

Task 3: Automated Analysis

Automating processes and feedback from AutoSyn

	HEATER 1						PUMP B (1-methylpiperazine)	PUMP A (amide int)	
	R10						MXD5	MXD6	
Condition	T (deg C)	t (min)	Temp	Time	B	A	B (mL/min)	A (mL/min)	Total Flow (mL/min)
1	20	15	LOW	LOW	HIGH	FIXED	0.57	0.10	0.67
2	20	15	LOW	LOW	LOW	FIXED	0.44	0.23	0.67
3	20	30	LOW	MED	MED	FIXED	0.27	0.06	0.33
4	20	60	LOW	HIGH	HIGH	FIXED	0.14	0.03	0.17
5	20	60	LOW	HIGH	LOW	FIXED	0.11	0.06	0.17
6	100	15	MED	LOW	MED	FIXED	0.53	0.14	0.67
7	100	30	MED	MED	HIGH	FIXED	0.29	0.04	0.33
8	100	30	MED	MED	MED	FIXED	0.27	0.06	0.33
9	100	30	MED	MED	LOW	FIXED	0.22	0.11	0.33
10	100	30	MED	MED	MED	FIXED	0.27	0.06	0.33
11	100	60	MED	HIGH	MED	FIXED	0.13	0.04	0.17
12	150	15	HIGH	LOW	LOW	FIXED	0.44	0.23	0.67
13	150	15	HIGH	LOW	HIGH	FIXED	0.57	0.10	0.67
14	150	30	HIGH	MED	MED	FIXED	0.27	0.06	0.33
15	150	60	HIGH	HIGH	LOW	FIXED	0.11	0.06	0.17
16	150	60	HIGH	HIGH	HIGH	FIXED	0.14	0.03	0.17





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PAT Overview

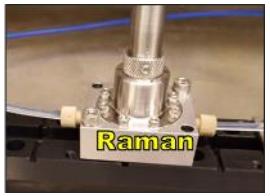
> *"(In PAT)…there is no one in situ analytical tool that will work for all applications."*
> Chanda et. al. 2015. Org. Process Rev.Dev. **19**, 63-83

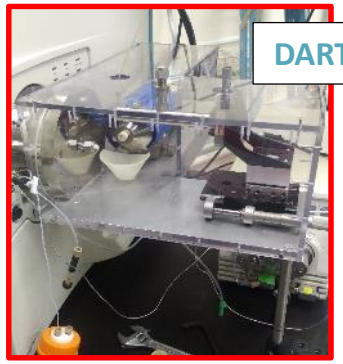
- Process Control Sensors
  - COTS temperature, pressure, flow, etc.
- DART-MS (Online)
- UHPLC-MS (Online-chiral separation)
- NMR (Inline)

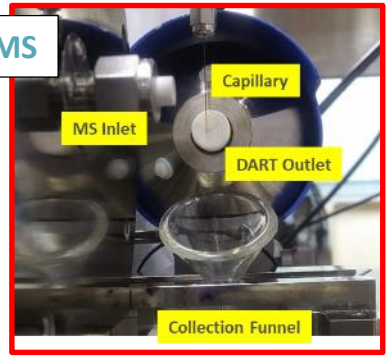
- *Optional Optical*
  - *Raman* (Ocean Optics)
  - *UV/Vis* (Ocean Optics)
  - *NIR* (Ocean Optics)
  - *ATR-FTIR System* (currently offline)

(in-line)

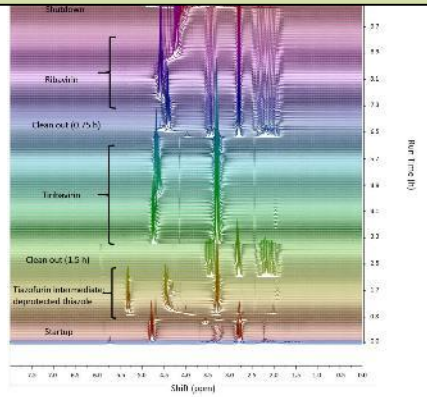
Custom Optical Interfaces



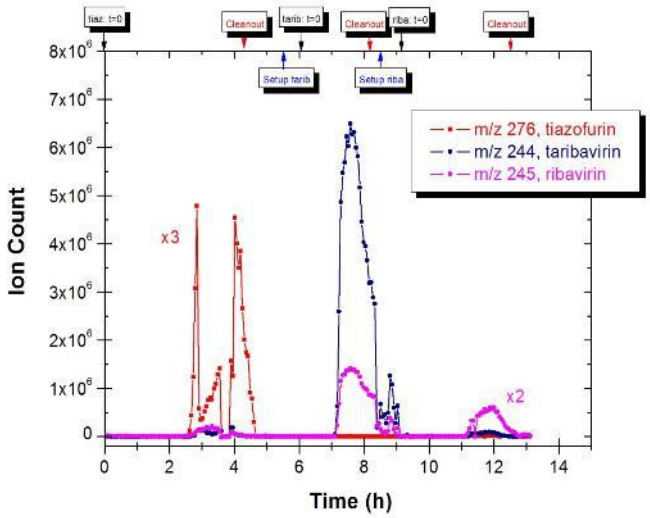




Thermo Flow NMR
Current



UHPLC-MS
Current



Final findings: Most of our operations have relied only on the LC-MS due to sensitivity and selectivity deficiencies of other instruments.

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Analytical Achievements: Phase 1

- **Innovated solution to minimize solvent effects for LSP on DART**
  - Installation of DART 90° from normal
  - Liquid droplets versus steady-state flow
  - Injection from CityScape coupled with diverter valve and column
- **Online generation of data for feedback**
  - Semi-quantitative yield of target on city block during process optimization
  - Demonstration of change-over from one synthesis to another
- **Tested automated analysis procedure**
  - Generate .RAW data
  - Extract and convert to ASCII
  - Fit against pre-generated libraries

Analytical Achievements: Phase 2

- **Installed LC-MS and NMR for online monitoring**
  - *Switched to exclusive use of LC-MS over DART-MS*
- **Online generation of data for feedback**
  - COTS software for NMR.
  - Custom software for LC-MS that only requires target ion inputs, without additional user configuration. Supports immediate visualization
  - Demonstrated ability to generate univariate data log
- **Routine operation of analytical instrumentation for repeat of all Phase 1 chemistry, and generation of Phase 2 data.**
- **Developed requirements for process monitoring**
  - Demonstrated ability for chemical optimization.
- **Installation of chiral column for EE separation**

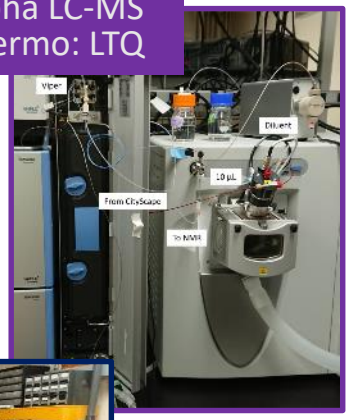
Analytical Achievements: Phase 3

- Shifted focus to LC-MS operation
- Installed analytics into beta system. System is now online and able to collect data (shown later).
  - The beta system is more advanced than the alpha system also in the ability to easily switch between flow and autosampler without moving tubing.
- Feedback and Control
  - Demonstrated feedback at DARPA/APL visit in December for LC and Univariate data
  - Implemented control protocols for software to identify and remedy deviation.
    - Software also provides notification to user
- Working on schema for addressing auto-optimization

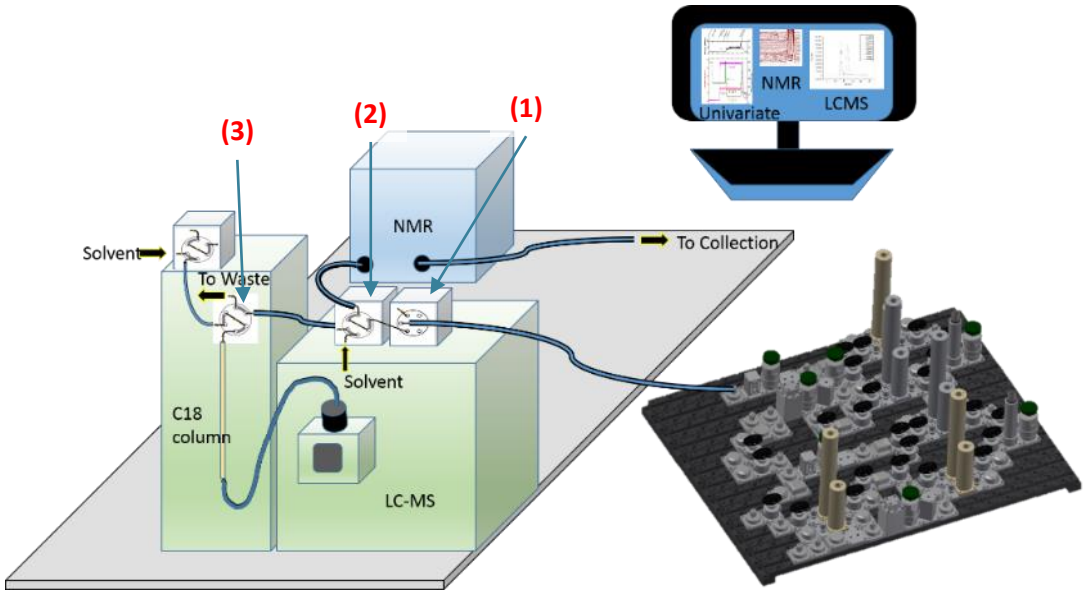
AutoSyn Hardware Interfacing with Analytical Instruments

- Output of Cityscape enters diverter valve (1) either to analytics (sampling) or waste (during cleanout with high flow)
- Second valve (2) removes 10 mL reaction sample from flow.
  - Remainder of sample passes to NMR, and then to collection
  - Solvent ACN/H_2O solvent pushes to sample loop on LC (3)
  - Sample from here is injected onto column and analyzed
- Similar configurations are setup for both Alpha and Beta system.

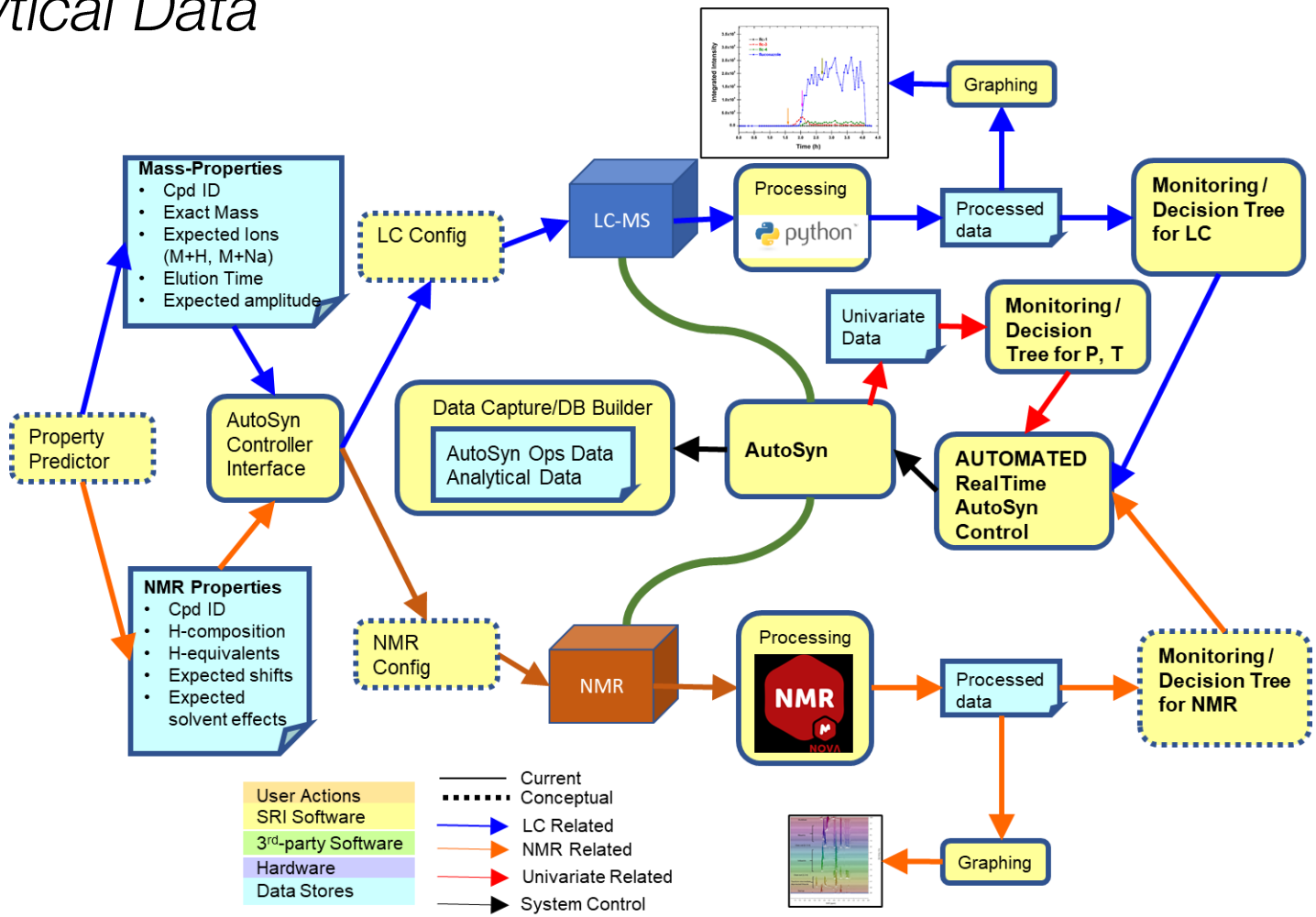
Alpha LC-MS
Thermo: LTQ



Beta LC-MS
SciEx: 3500

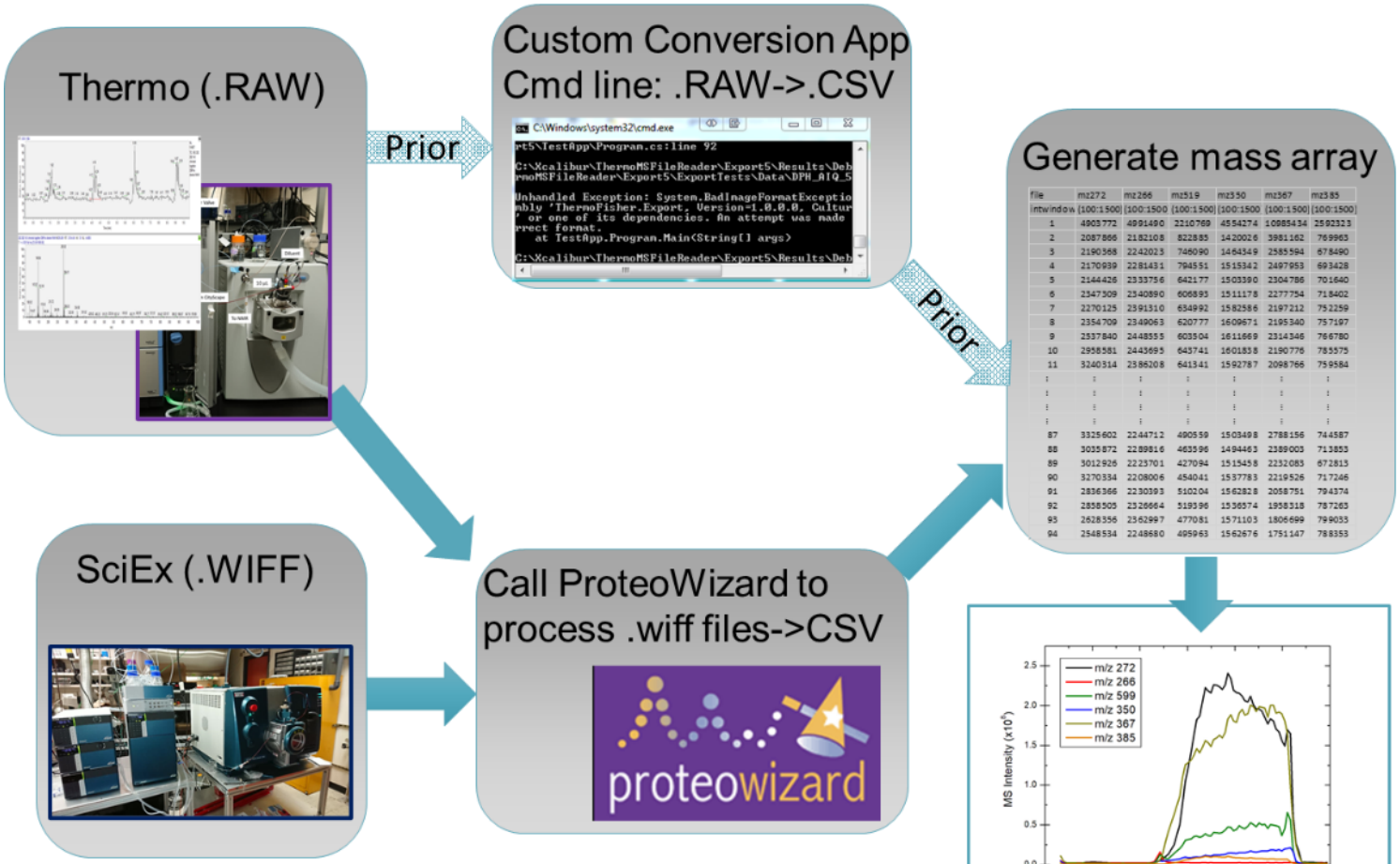


SynFini Information Flow Schematic
Analytical Data



We achieved all goals to implement automated monitoring and control. Remote configuration of analytical instrumentation and monitoring NMR data was unable to be automated.

Automated Processing with Python: Generalization

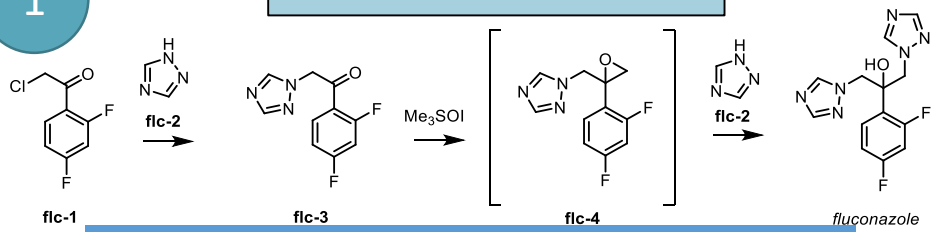


- We developed automated processing procedures using open source software (Python, Proteowizard) that supply real-time information to the user.
- This provided the foundation for process monitoring and control.

AutoSyn Analytical

Details on Automation Software: Focus on Fluconazole

1 User defines masses

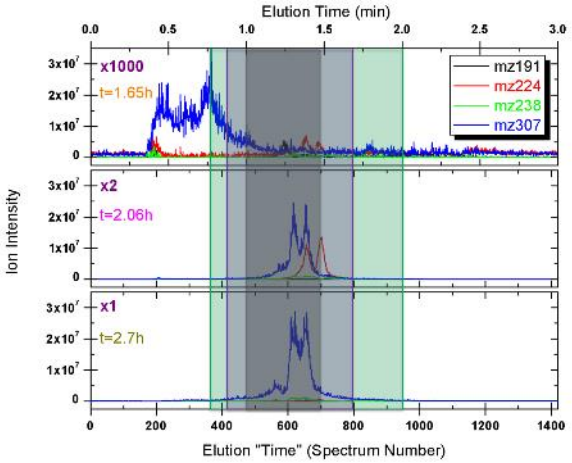


	Compound (main text number)	Exact Ma
flc-1	2-chloro-1-(2,4-difluorophenyl)ethan-1-one	190.00
flc-2	1,2,4-triazole	69.03
flc-3	triazole intermediate	223.06
flc-4	epoxide intermediate	237.07
	fluconazole	306.10

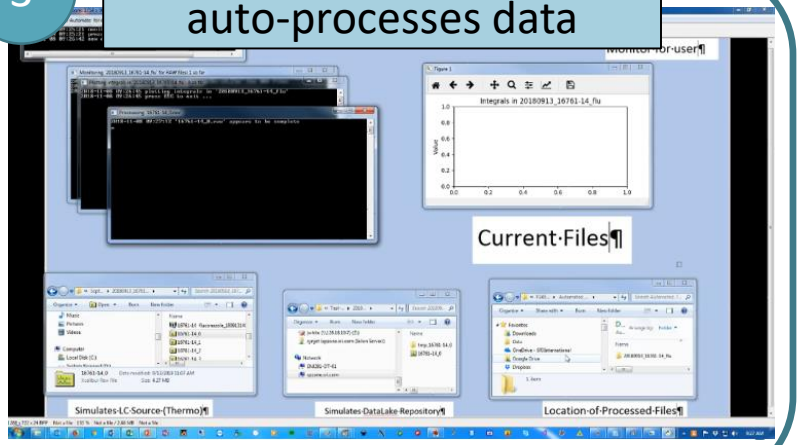
2 User defines LC integration Software uses boundaries

mass	intstart	intend	delta
190	490	700	210
191	490	700	210
224	420	800	380
238	375	950	575
378	620	890	270
379	620	890	270
307	420	800	380

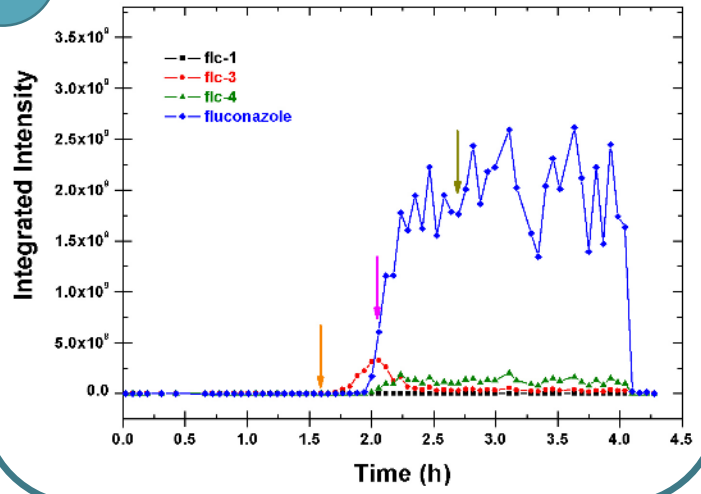
**default is entire LC profile*



3 Software detects new files auto-processes data



4 Data plotted in real-time, available for post-processing



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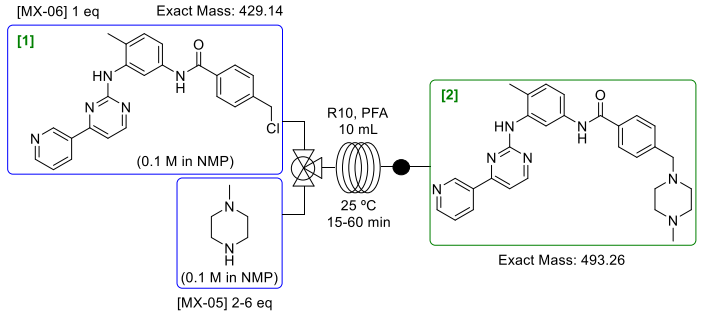
Automated DoE on AutoSyn

1. Decide Temperature, Residence Time, stoichiometry



2. Calculate Flow

Low, Low, High

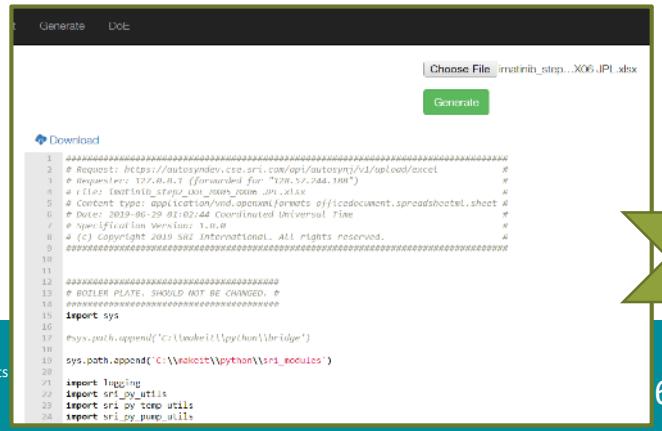
	MX02	*MX01*	*R7*
type	***FA****	***FB****	***TF****
flow rate (mL/min)	0.444	0.222	0.667
ratio new reagent {RnR}		0.5	
volume (mL) {V}			10
residence time (min) {RT}			15
Temp C			25
output			

* FA=TF / (1 + RnR), ** FB = TF – FA, *** TF = V / RT

3. Fill out DoE table

	HEATER 1						PUMP B (1-methylpiperazine)	PUMP A (amide int)	
	R10						MX05	MX06	
Condition	T (deg C)	t (min)	Temp	Time	B	A	B (mL/min)	A (mL/min)	Total Flow (mL/min)
1	20	15	LOW	LOW	HIGH	FIXED	0.57	0.10	0.67
2	20	15	LOW	LOW	LOW	FIXED	0.44	0.23	0.67
3	20	30	LOW	MED	MED	FIXED	0.27	0.06	0.33
4	20	60	LOW	HIGH	HIGH	FIXED	0.14	0.03	0.17
5	20	60	LOW	HIGH	LOW	FIXED	0.11	0.06	0.17
6	100	15	MED	LOW	MED	FIXED	0.53	0.14	0.67
7	100	30	MED	MED	HIGH	FIXED	0.29	0.04	0.33
8	100	30	MED	MED	MED	FIXED	0.27	0.06	0.33
9	100	30	MED	MED	LOW	FIXED	0.22	0.11	0.33
10	100	30	MED	MED	MED	FIXED	0.27	0.06	0.33
11	100	60	MED	HIGH	MED	FIXED	0.13	0.04	0.17
12	150	15	HIGH	LOW	LOW	FIXED	0.44	0.23	0.67
13	150	15	HIGH	LOW	HIGH	FIXED	0.57	0.10	0.67
14	150	30	HIGH	MED	MED	FIXED	0.27	0.06	0.33
15	150	60	HIGH	HIGH	LOW	FIXED	0.11	0.06	0.17
16	150	60	HIGH	HIGH	HIGH	FIXED	0.14	0.03	0.17

4. Import to Internal Website



Scripts are written using custom human-readable Python interpreter language

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Script generation

```
#################################################
# SPREADSHEET REACTIONS. SHOULD NOT BE CHANGED. #
#################################################
###########################################
# Spreadsheet 'DoE 1 Reaction 2 Reagents' #
###########################################
##########################################################################################################################################################################################
# |               |    HEATER 1    |           |        |        |        |        |PUMP B (1-methylpiperazine)| PUMP A (amide int) |                      |
# |               |      R10       |           |        |        |        |        |          MX05             |       MX06         |                      |
# |   Condition   |   T (deg C)    |  t (min)  |        |        |        |   A    |       B (ml/min)          |     A (ml/min)     |  Total Flow (ml/min) |
# |      1.0      |      20.0      |   15.0    |        |        |        | FIXED  |         0.57              |       0.1          |        0.67          |
# |      2.0      |      20.0      |   15.0    |        |        |        | FIXED  |         0.44              |       0.23         |        0.67          |
# |      3.0      |      20.0      |   30.0    |        |        |        | FIXED  |         0.27              |       0.06         |        0.33          |
# |      4.0      |      20.0      |   60.0    |        |        |        | FIXED  |         0.14              |       0.03         |        0.17          |
# |      5.0      |      20.0      |   60.0    |  LOW   |  HIGH  |  LOW   | FIXED  |         0.11              |       0.06         |        0.17          |
# |      6.0      |     100.0      |   75.0    |  MED   |  LOW   |  MED   | FIXED  |         0.53              |       0.14         |        0.67          |
# |      7.0      |     100.0      |   30.0    |  MED   |  MED   |  HIGH  | FIXED  |         0.29              |       0.04         |        0.33          |
# |      8.0      |     100.0      |   30.0    |  MED   |  MED   |  MED   | FIXED  |         0.27              |       0.06         |        0.33          |
# |      9.0      |     100.0      |   30.0    |  MED   |  MED   |  LOW   | FIXED  |         0.22              |       0.11         |        0.33          |
# |     10.0      |     100.0      |   30.0    |  MED   |  MED   |  MED   | FIXED  |         0.27              |       0.06         |        0.33          |
# |     11.0      |     100.0      |   60.0    |  MED   |  HIGH  |  MED   | FIXED  |         0.13              |       0.04         |        0.17          |
# |     12.0      |     150.0      |   75.0    |  HIGH  |  LOW   |  LOW   | FIXED  |         0.44              |       0.23         |        0.67          |
# |     13.0      |     150.0      |   15.0    |  HIGH  |  LOW   |  HIGH  | FIXED  |         0.57              |       0.1          |        0.67          |
# |     14.0      |     150.0      |   30.0    |  HIGH  |  MED   |  MED   | FIXED  |         0.27              |       0.06         |        0.33          |
# |     15.0      |     150.0      |   60.0    |  HIGH  |  HIGH  |  LOW   | FIXED  |         0.11              |       0.06         |        0.17          |
# |     16.0      |     150.0      |   60.0    |  HIGH  |  HIGH  |  HIGH  | FIXED  |         0.14              |       0.03         |        0.17          |
##########################################################################################################################################################################################
logger.info('##########################################################################################################################################################################
reactionchoice = sri_py_utils.sri_get_user_input_number('\n\nEnter 1 to run the above reactions from spreadsheet DoE 1 Reaction 2 Reagents.\nOtherwise enter 0 ... ')
if (reactionchoice == 1):
  logger.info("\n*********REACTIONS STARTED***********\n")

  logger.info("\nSwitching diverter valve to PAT")
  sri_py_pump_utils.sri_pump_set_vici(VALVE_DIVERTER,VALVE_DIVERTER_TO_PAT)
  HEATERS_TEMPS = [20.0] if QUICK_TEST else [20.0]
  sri_py_temp_utils.sri_set_temperatures(HEATERS, HEATERS_TEMPS, True)
  if QUICK_TEST:
    sri_py_utils.sri_block_for_user_input_or_sleep('\n\nWaiting 1.0 minute for a reactor temperature of 20.0 degrees Celsius to be reached\n\n',1.0)
  else:
    sri_py_utils.sri_block_for_user_input_or_sleep('\n\nWaiting 5.0 minutes for a reactor temperature of 20.0 degrees Celsius to be reached\n\n',5.0)
  logger.info("\nSwitching valves to REAGENT")
  for i in range(0, len(VALVES)):
    using(VALVES[i]).set_position(REAGENT).run()
  logger.info("\nSetting pump B flow rate to 1000.0 ul/min")
  logger.info("\nSetting pump A flow rate to 1000.0 ul/min")
  sri_py_pump_utils.sri_pump_dispense(PUMPS_B,[1000.0]*len(PUMPS_B))
  sri_py_pump_utils.sri_pump_dispense(PUMPS_A,[1000.0]*len(PUMPS_A))
  sri_py_utils.sri_block_for_user_input_or_sleep('\n\nWaiting for 3.0 minutes to introduce reagents to reactor\n\n',3.0)

  ########################################################################################################################################################################
  # |               |    HEATER 1    |           |        |        |        |        |PUMP B (1-methylpiperazine)| PUMP A (amide int) |                      | #
  # |               |      R10       |           |        |        |        |        |                           |       MX06         |                      | #
  # |   Condition   |   T (deg C)    |  t (min)  |        |        |        |        |                           |     A (ml/min)     |  Total Flow (ml/min) | #
  # |      1.0      |      20.0      |   15.0    |        |        |        |        |                           |       0.1          |        0.67          | #
  ########################################################################################################################################################################
  logger.info('\n########################################################################################################################################################################
  logger.info("\nSetting pump B flow rate to 570.0 ul/min")
  logger.info("\nSetting pump A flow rate to 100.0 ul/min")
  sri_py_pump_utils.sri_pump_dispense(PUMPS_B,[570.0]*len(PUMPS_B))
  sri_py_pump_utils.sri_pump_dispense(PUMPS_A,[100.0]*len(PUMPS_A))
  if QUICK_TEST:
    sri_py_utils.sri_block_for_user_input_or_sleep('\n\nWaiting 1.0 minute to equalibriate\n\n',1.0)
  else:
    sri_py_utils.sri_block_for_user_input_or_sleep('\n\nWaiting 5.0 minutes to equalibriate\n\n',5.0)
  if QUICK_TEST:
    sri_py_utils.sri_block_for_user_input_or_sleep('\n\nWaiting until a reaction time of 1.0 minute and a collection time of 1.0 minute is reached\n\n',2.0)
  else:
    sri_py_utils.sri_block_for_user_input_or_sleep('\n\nWaiting until a reaction time of 15.0 minutes and a collection time of 22.39 minutes is reached\n\n',37.39)
```

1. Parse of DoE table.

2. Modular startup/shutdown.

3. Automated progression through reaction conditions.

4. Calculation of expected equilibration and transfer times to PAT.

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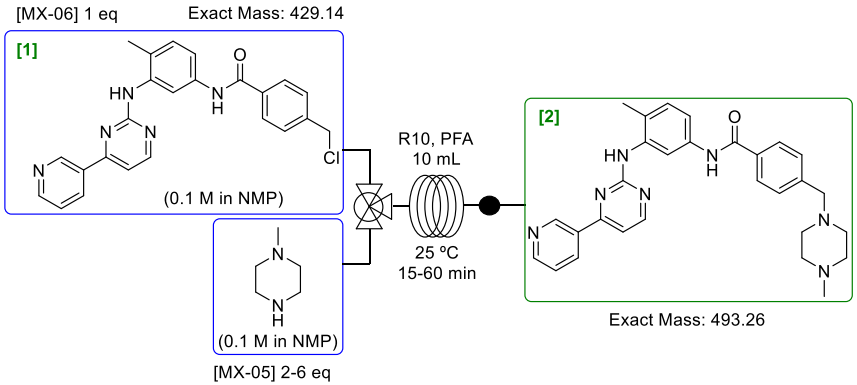
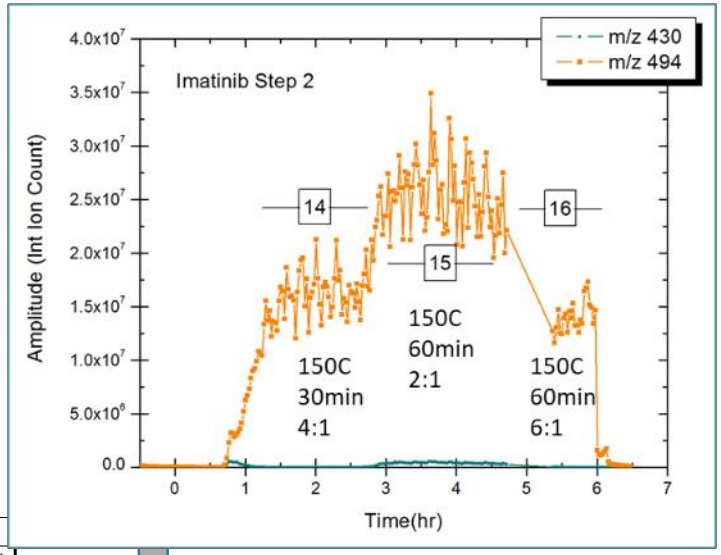
DoE Optimization AutoSyn and SynJet





				AutoSyn			SynJet		
Condition	Temperature	Time	Volume	mz430	mz494	PerYield	mz430	mz494	PerYield
1	20	15	36.585	3.88E+07	1.60E+07	29%	1.3E+09	5.86E+08	31%
2	20	15	12.195	2.44E+07	1.32E+07	35%	1.165E+09	2.63E+08	18%
3	20	30	24.39	1.34E+07	1.39E+07	51%	1.027E+09	7.12E+08	41%
4	20	60	36.585	8.77E+06	1.45E+07	62%	570316617	9.95E+08	64%
5	20	60	12.195	1.10E+07	1.99E+07	64%	1.193E+09	6.27E+08	34%
6	100	15	24.39	1.55E+06	1.95E+07	93%	102682892	1.04E+09	91%
7	100	30	36.585	3.79E+04	1.30E+07	100%	319680	9.52E+05	75%*
8	100	30	24.39	7.34E+04	1.72E+07	100%	661763670	1.13E+09	63%**
9	100	30	12.195	4.23E+06	2.60E+07	86%	645921145	9.58E+08	60%
10	100	30	24.39	1.30E+05	1.78E+07	99%	6513278	9.98E+08	99%
11	100	60	24.39	9.27E+03	1.62E+07	100%	1065921	1.02E+09	100%
12	150	15	12.195	8.63E+05	2.64E+07	97%	336359790	9.05E+08	73%
13	150	15	36.585	1.04E+04	1.57E+07	100%	6112443	9.20E+08	99%
14	150	30	24.39	2.27E+03	1.63E+07	100%	21280462	8.57E+08	98%
15	150	60	12.195	4.12E+05	2.59E+07	98%	239098680	8.28E+08	78%
16	150	60	36.585	2.76E+03	1.42E+07	100%	2329428	8.18E+08	100%

* Anomalously low LC sample
** Condition 8 is replicate of Condition 10

- Optimization on AutoSyn and SynJet agree, indicating translation between µL and flow.
- The next slide compares statistics between the two approaches.

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Reaction Optimization Comparison: Imatinib

Parameter	AutoSyn	SynJet
Temperature	Highest temperature	Highest temperature
Reaction Time	Time independent (Short generally better)	Short time
Stoichiometry	2:1	4:1
Time to complete	28 hours	2.5 hours
Reagent Consumed	19 g	0.43 g
Solvent Usage	1300 mL	< 0.5 mL

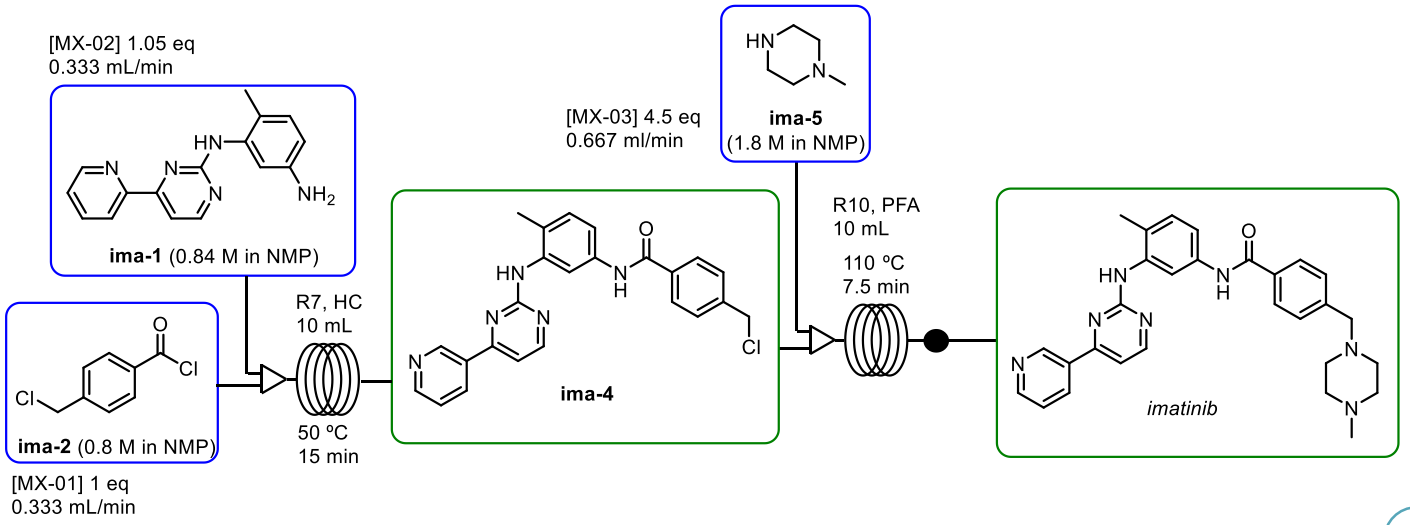
- Data is qualitatively similar for most conditions
- SynJet would benefit from additional replicates of conditions to verify statistics.
  - *Provides starting point for chemists.*

Optimization has been demonstrated on AutoSyn.

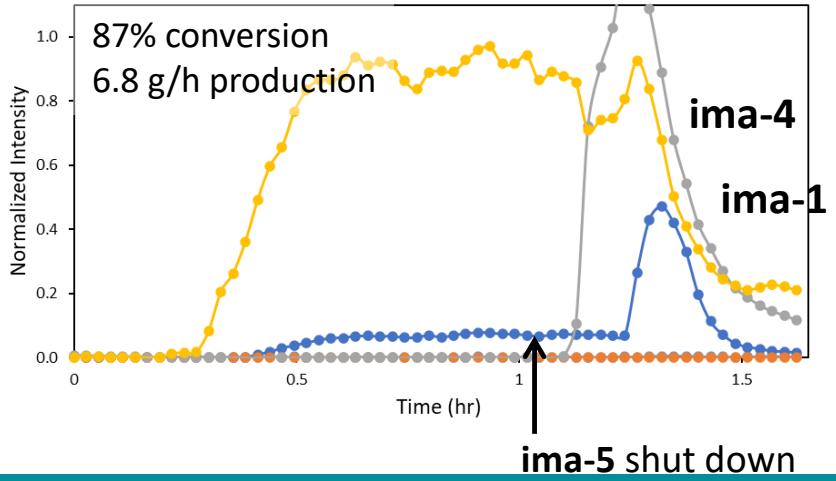
SynFini workflow to include a full DoE on SynJet, and refined (narrower) DoE on AutoSyn around SynJet-suggested conditions

Translation of reaction chemistry

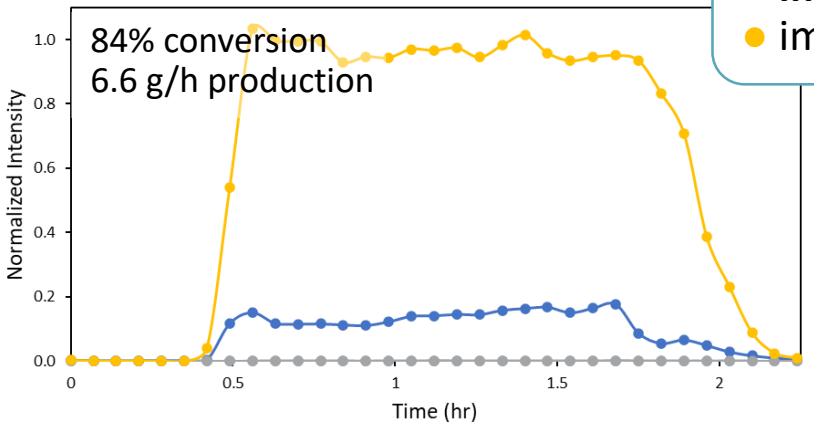
Translation of imatinib from AutoSyn alpha to AutoSyn beta

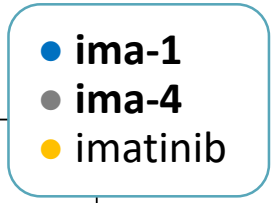


Online LC-MS trace from alpha



Online LC-MS trace from beta





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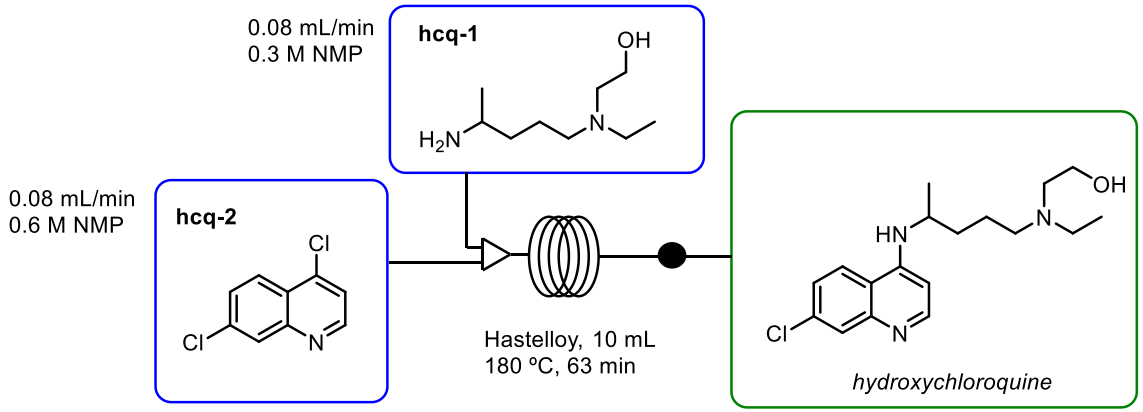
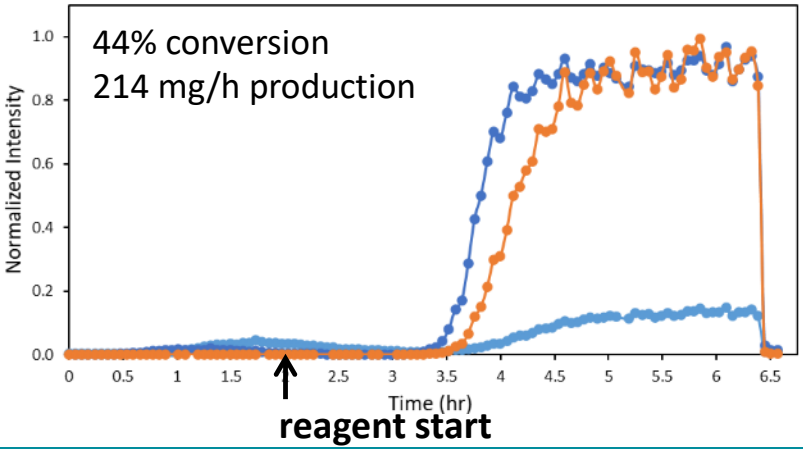
Translation of reaction chemistry

Translation of hydroxychloroquine from AutoSyn alpha to AutoSyn beta

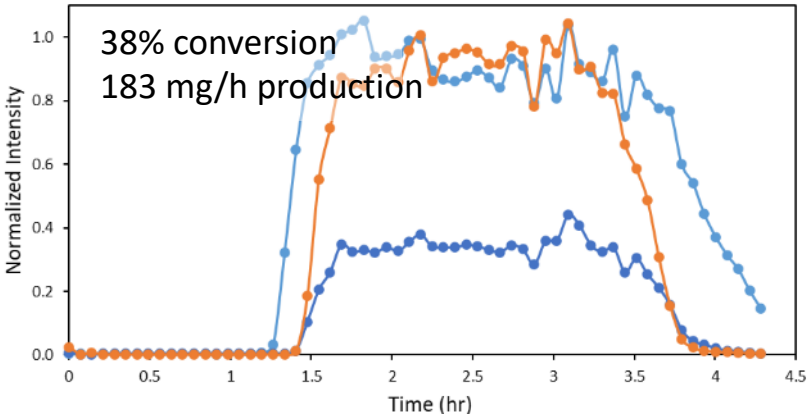


- **hcq-1**
- **hcq-2**
- hydroxychloroquine

Online LC-MS trace from alpha



Online LC-MS trace from beta



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AutoSyn Data Capture and Reporting: In standard use

SynFini ELN: Word Doc on Sharepoint, used for Alpha, Beta, SynJet

Sheets 1, 2: Instrument configuration, ion identification, Cityscape map

20181112_16760-49_taribavirin_to_ribavirin_change-over - Last Saved 11/15/2018 9:20 AM — Table Tools — Jason White

File Home Insert Draw Design Layout References Mailings Review View Help ACROBAT PKZIP Design Layout Tell me what you want to do — Share History Comments

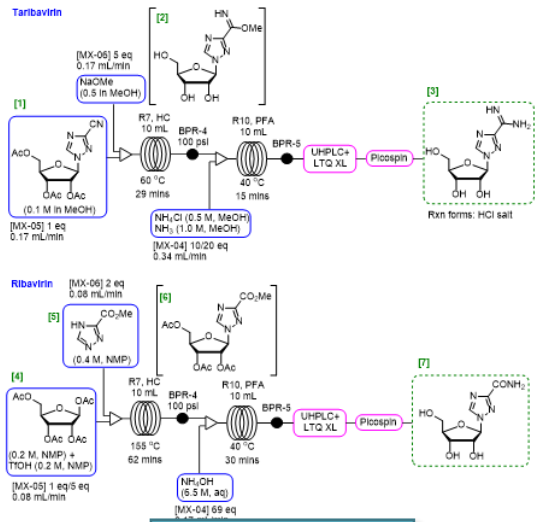
Experiment Information

Target compound: Taribavirin to ribavirin **Date:** 11/12/2018

Chemist: Vi-Anh **Notebook page:** 16760-49

Experiment description: Taribavirin to ribavirin change over experiment

Scheme:



Planned Results

Level 2 Diagram

Figure 1: How to fill out the table

1. Cpd ID is a integer and correlates to expected reagents, intermediates, products in Scheme.

2. Frag ID is number of fragments associated with specific compound. For example, loss of chloride would expect two total fragments

3. Parent exact mass can be calculated from ChemDraw or chemcalc.org. It is related to C=12 amu, it is not the molecular weight.

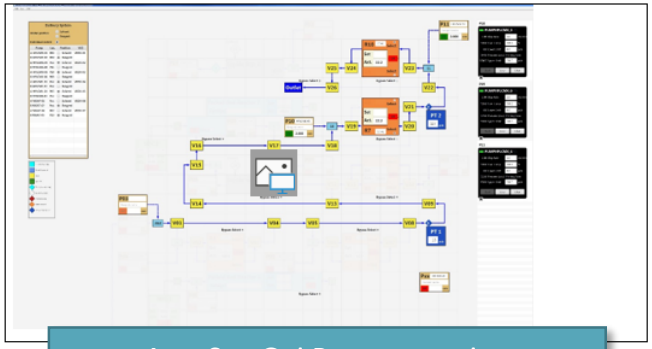
4. Mass modification is the expected ionization production. For example, M+H is defined as +1, M-Cl is defined and -35.

User/SynRoute Defined ions

5. The ion to … should auto … modification. It

Cmpd ID	Cmpd Name	FragID	Parent Exact Mass	Mass Modification	Ion Target	Pump ID	Solvent
1	cyano-triazole intermediate	1	352	-93	259	MX-05	MeOH
		2	352	1	353		
2	imidate intermediate	1	258	1	259	MX-06	MeOH
3	taribavirin	1	243	1	244	MX-04	MeOH
		2	243	23	266		
4	ribofuranose tetraacetate	1	318	-59	259	MX-05	NMP
5	ester triazole	1	127	1	128	MX-06	NMP
6	ester triazole intermediate	1	385	1	386		
		2	385	23	408		
7	NH_4OH	1	244	1	245	MX-04	water
		2	244	23	267		

Setup (screenshot of GUI):



AutoSyn Gui Representation

Page 1 of 8 1114 words — Display Settings — 85%

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Automated Operation

Project Goal: AutoSyn system provides real-time feedback and control during operation

Feedback

- Data from processed real-time LC-MS (3.5min) and Univariate streams of pressure, flow, and temperature sensors (~5 seconds)

- Algorithm evaluates in-spec/out-of-Spec for LC-MS data
  - Spec may be pre-defined, but most likely calculated as system operates.
  - Visual indicator for user to show Feedback status
    - Baseline: blue Ramp/Transition: Yellow in-spec: green out-of-spec: red
- Separate algorithm for univariate with similar color scheme.
- For in-spec, system operates according to script/user
- For out-of-spec, visual warning appears on computer.
  - During automated operation, this enables Control

Automated Operation

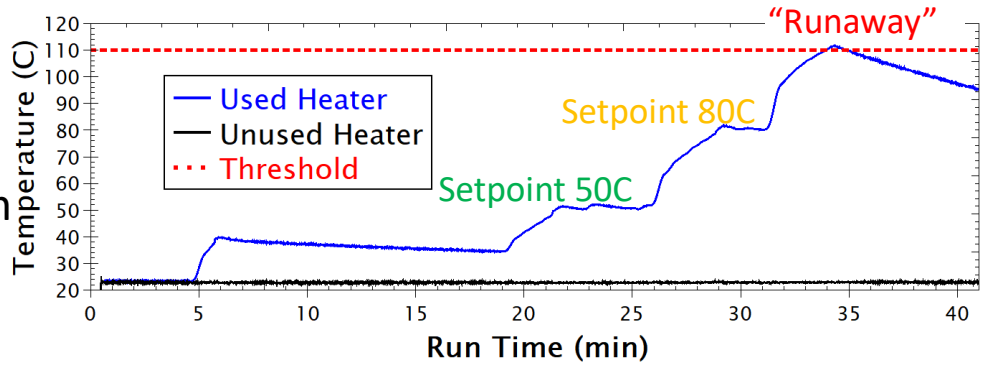
Control

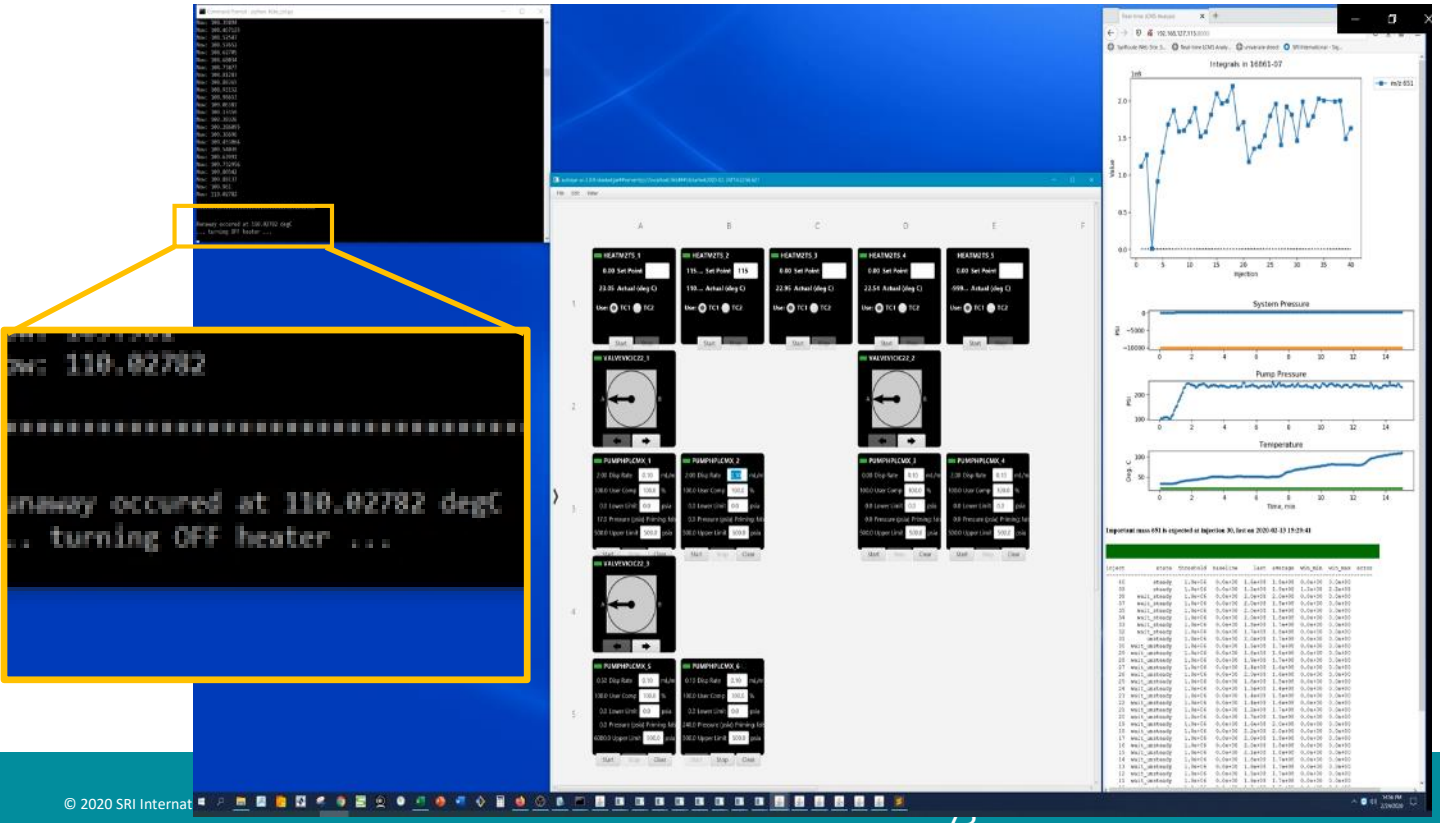
Python code monitors pre-defined conditions, watches to exceed setpoints. Code breaks into operation and takes control when needed

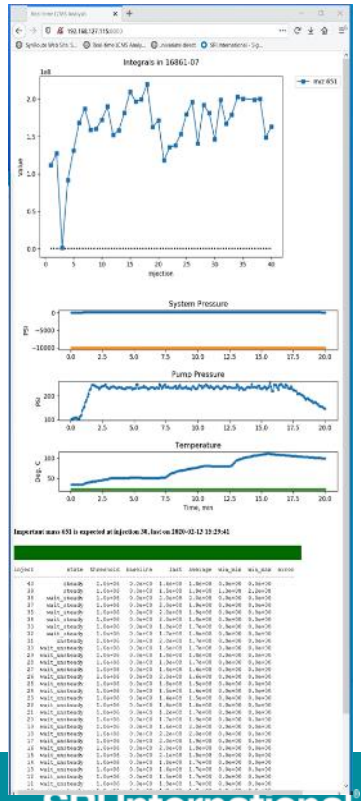
- For in-spec operation, automated script (if used) controls system settings
- For out-of-spec operation,
  - Classify issue using Univariate or LC analytics.
    - E.g. overpressure, low flow, etc.
  - Unidentified issue: system shutdown
    - Turn off reactors, (Optional) Switch to solvent, wait to cool, shutoff flow.
  - Identified issue (approved automated control from engineering team)
    - Enable established remedy, e.g. higher flow, lower T, etc.
    - Monitor for operation back to specification
    - Resume process

Control Demo: Runaway Reactor Situation

- Components: 1 Pump,1 Reactor, Univariate monitoring
- System monitors for ‘overtemperature’, then shuts off Heater. 2 min later, system shuts off pump.







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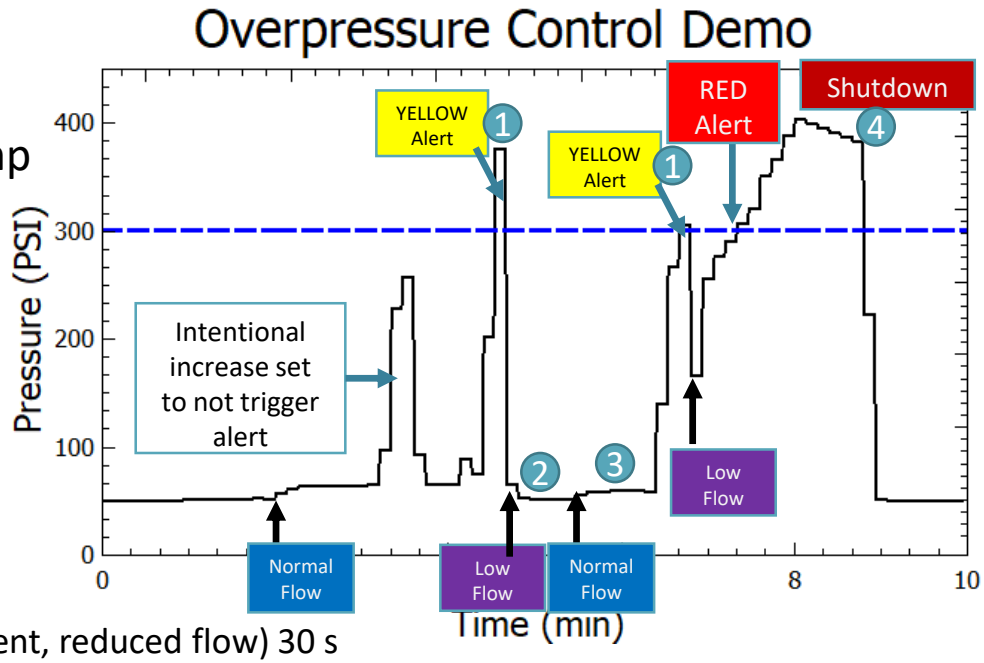
Control Demo: System Pumps Over Pressure

Setup

- Components: 2 Pump (LS01 and LS02)1 Reactor
- Univariate monitoring
- HW pressure fault threshold set at 300 PSI
- System monitors backpressure at each pump

Simulation

- Manual Increase pump backpressure
- Control uses following logic



- **Process and Logic**
  - Pressure exceeds Setpoint: Yellow Alert 1
  - CHK_RED Process: System Switches to flush (solvent, reduced flow) 30 s
    - System checks pressure at low flow. 2
    - If P<300 PSI, Orange Alert→check at high flow (CHK_ORN Process)
      - If P<300 PSI at high flow, system resumes process (reagent, normal flow) RESUME 3
      - If P>300 PSI at high flow, Repeat CHK_RED, then CHK_ORN
        - If system fails CHK_ORN twice, Orange Alert issued and system shuts down
    - If P>300 PSI , Red Alert → Shutdown 4

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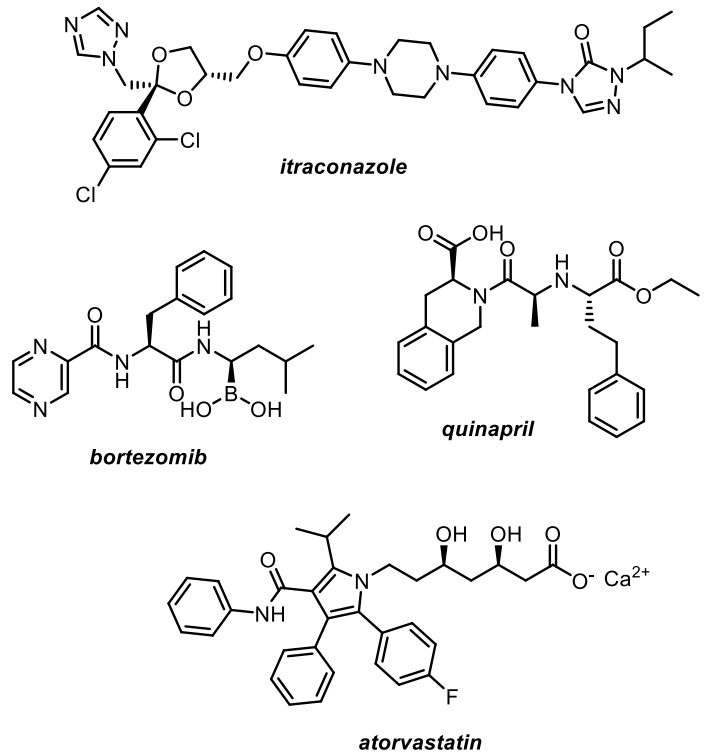
Auto Optimization Schema

- Receive SynJet Conditions for
  - Temperature
  - Reaction Time
  - Stoichiometry
- Statistical analysis of conditions to ID driving parameters
  - Assessment of Regression analysis from DoE
  - Dynamic Mode Decompression to find cross-correlations
- Run flow process and use flow rate and flow path to calculate when to activate monitoring
- Monitor system operations using analytics (LC-MS, univariate, other)
  - Continue operation as long as system has no failure detection (e.g. Pump Pressure)
- Implement temperature or flow controls to modify parameters with coarse and fine steps of driving parameters.
- Observe and Monitor changes.
- Iterate until steady state with highest yield is measured.

Task 4: Chemistry and Process Development



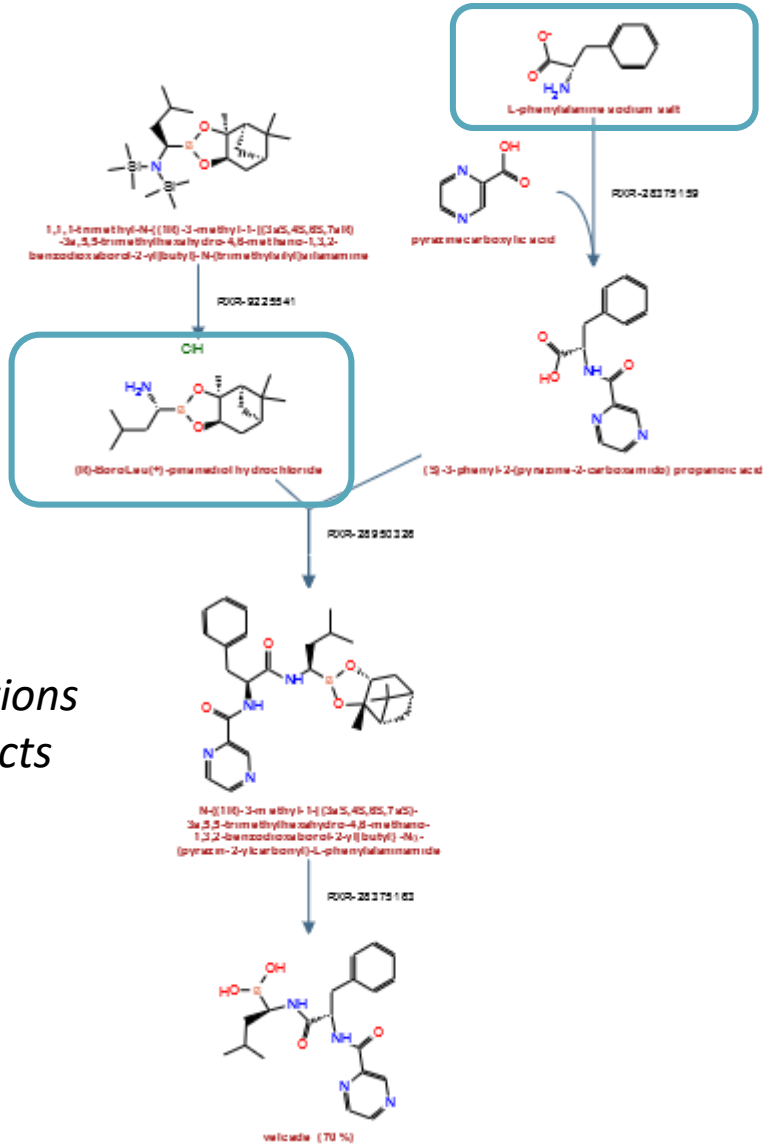
Synthesis of Phase 3 Demonstration Compounds

Bortezomib

SynRoute analysis

Commercially available, or 4 step asymmetric synthesis (requires low temp)

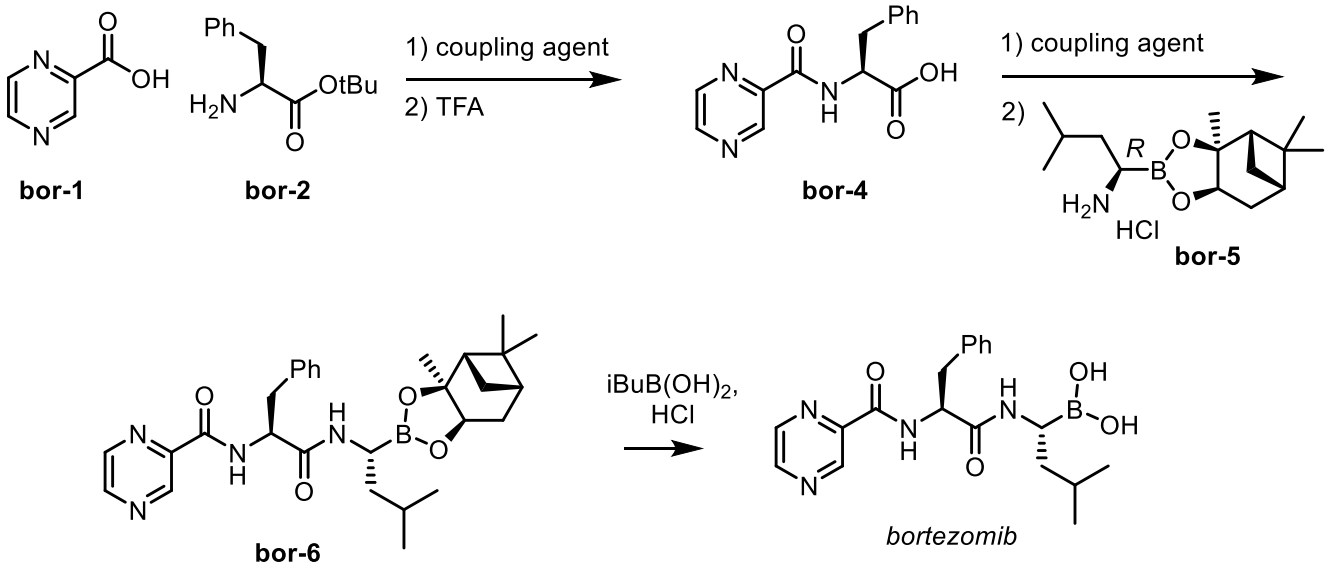
Desire amide coupling conditions avoiding urea/HOBt byproducts



Use tBu protected version for solubility; Requires addition of deprotection step

Bortezomib

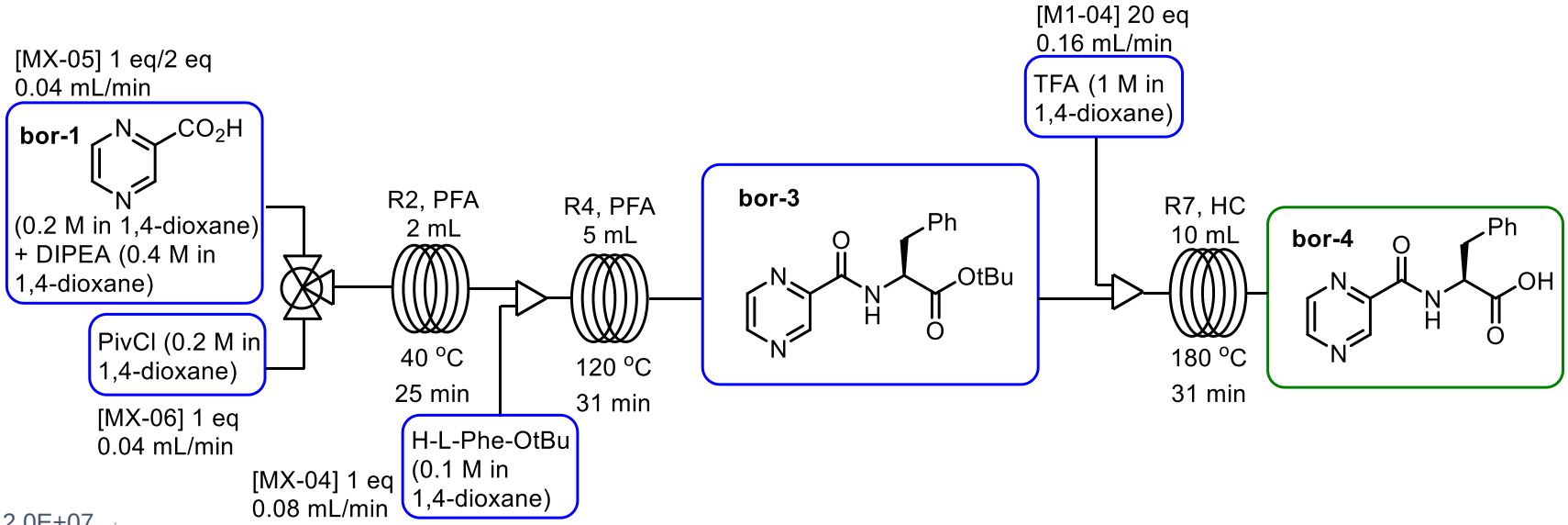
Phase 3 Target Scheme

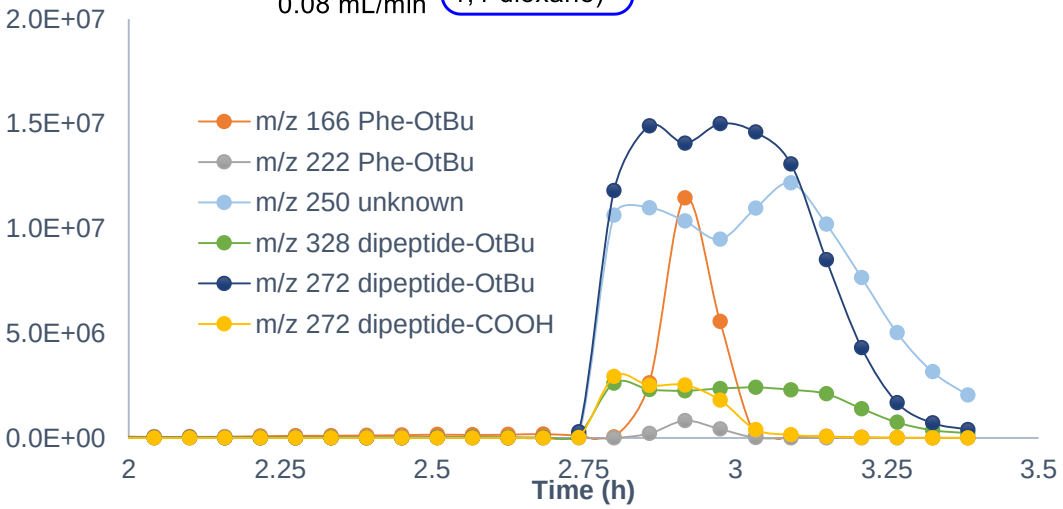


Revised scheme based on chemist evaluation of SynRoute output

Bortezomib

3-Step Activation/Amide Coupling/Deprotection

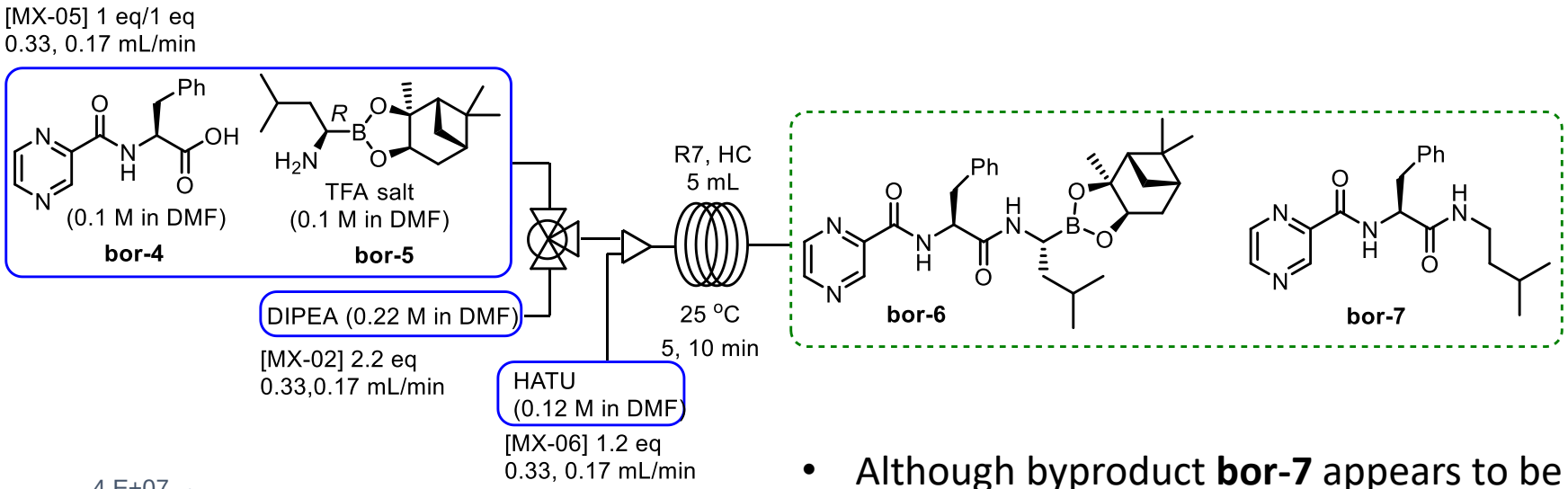


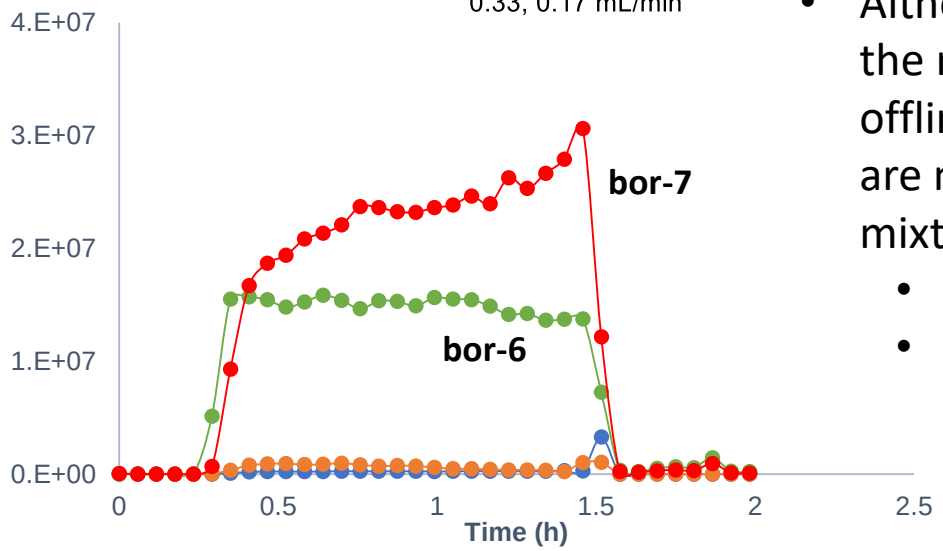


- Activation and acylation quickly developed to give full conversion to amide as 2-step process
- Amide partially deprotected (yellow trace)
- Addition of water improves deprotection

Bortezomib

2nd Coupling Step

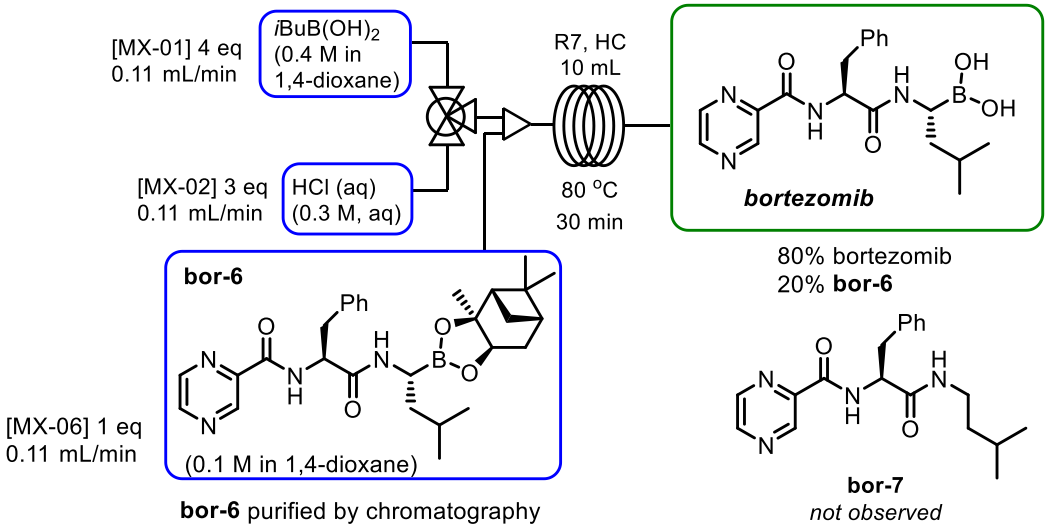


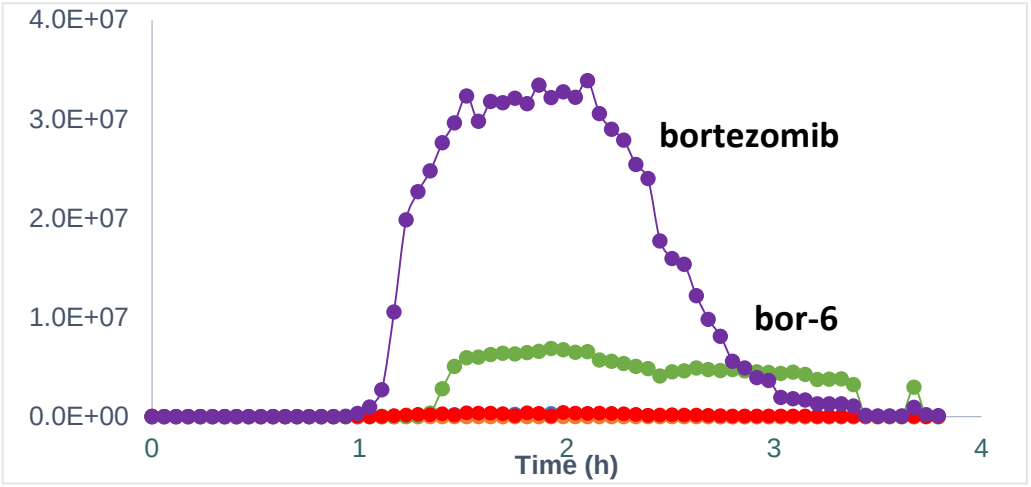


- Although byproduct **bor-7** appears to be the major component of the mixture, offline NMR indicates the LC-MS results are not representative of the true mixture
  - Offline LC-MS: 40:60 (**bor-6**:**bor-7**)
  - Offline NMR: 72:28 (**bor-6**:**bor-7**)

Bortezomib

Deprotection Step

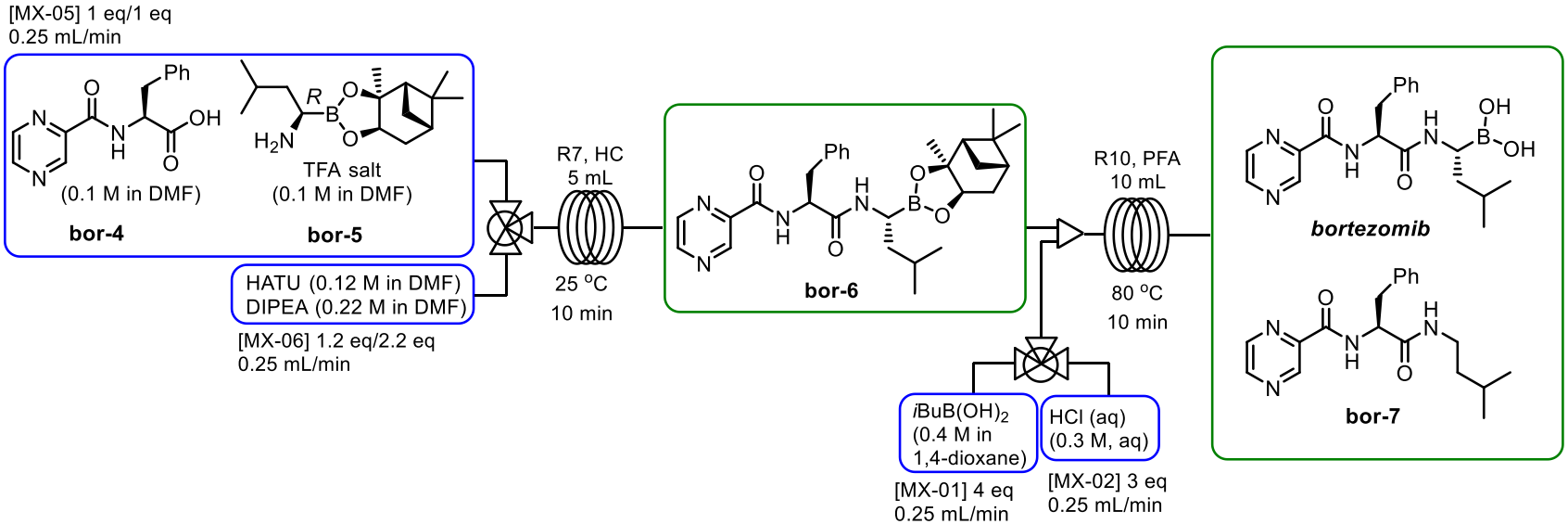




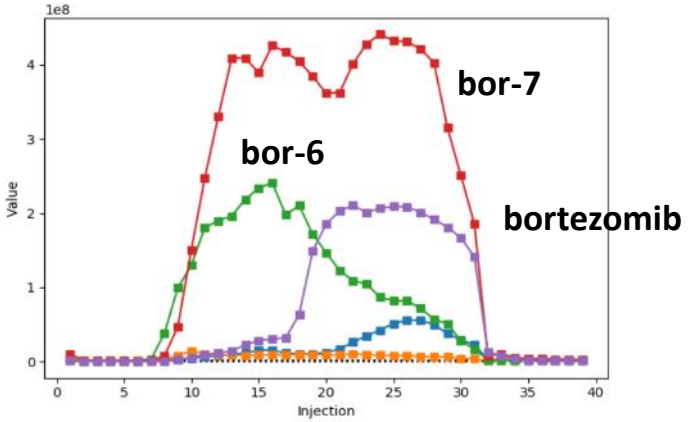
- Starting with purified **bor-6**, the deprotection to give bortezomib proceeds cleanly
- Byproduct **bor-7** is not observed

Bortezomib

2nd Coupling Step + Deprotection



- Experiment run in two stages
  - Initially, the reagents for the coupling are run, without the deprotection reagents.
  - As expected, amid **bor-6** is observed, along with byproduct **bor-7**.
  - The reagents for the deprotection are introduced, and signal for amide **bor-6** decreases as bortezomib is observed.



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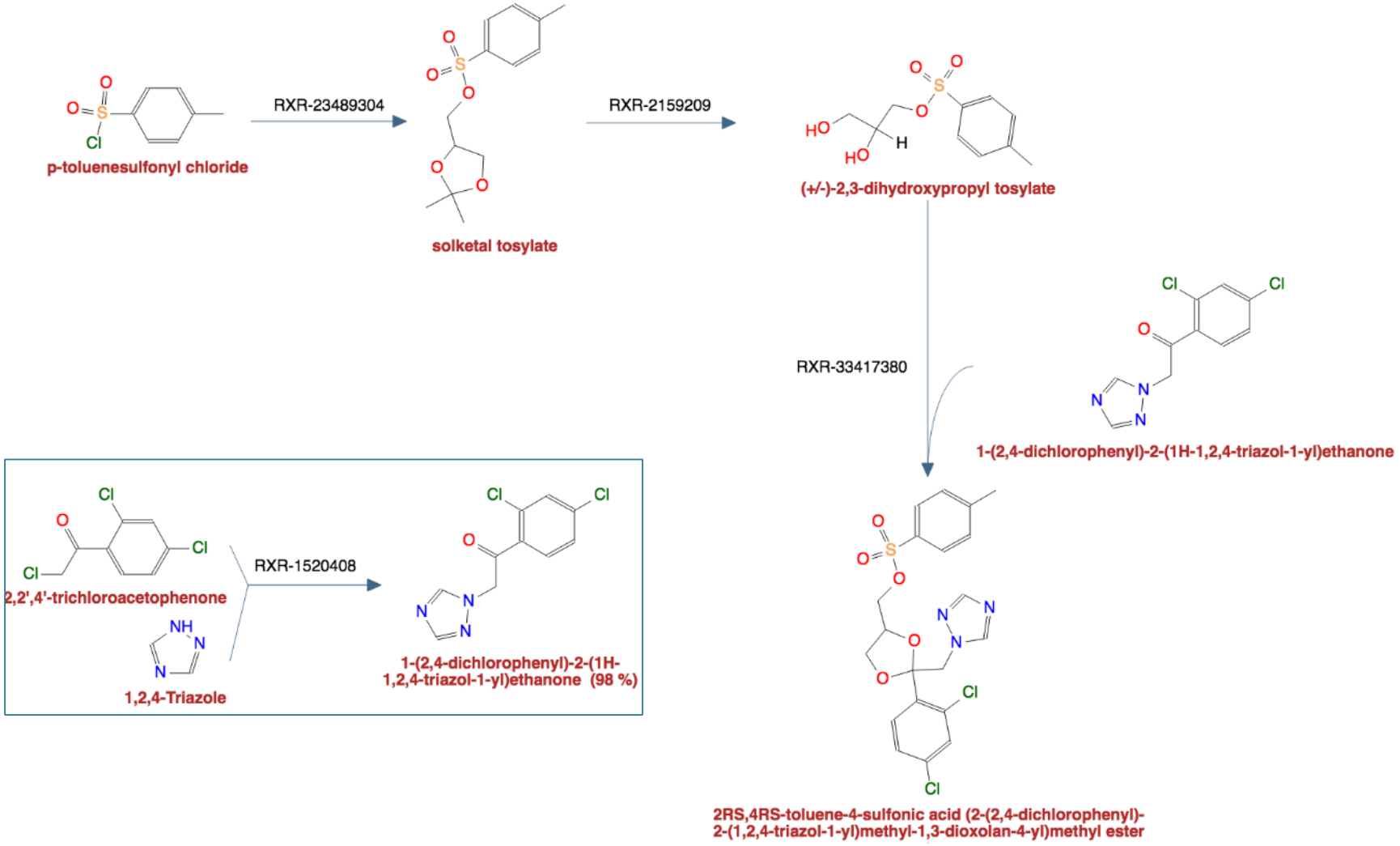
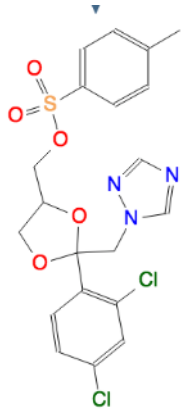
Itraconazole

Output from SynRoute

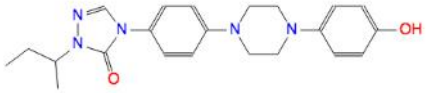


Itraconazole

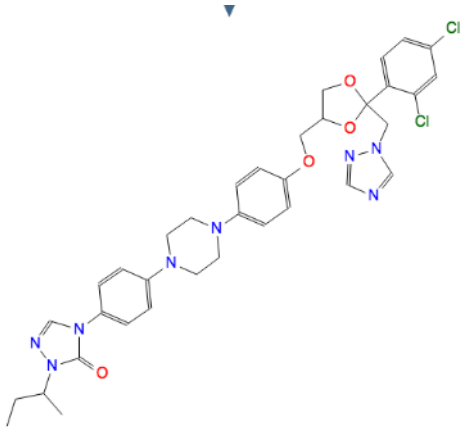
Output from SynRoute (continued)



2RS,4RS-toluene-4-sulfonic acid (2-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-yl)methyl-1,3-dioxolan-4-yl)methyl ester



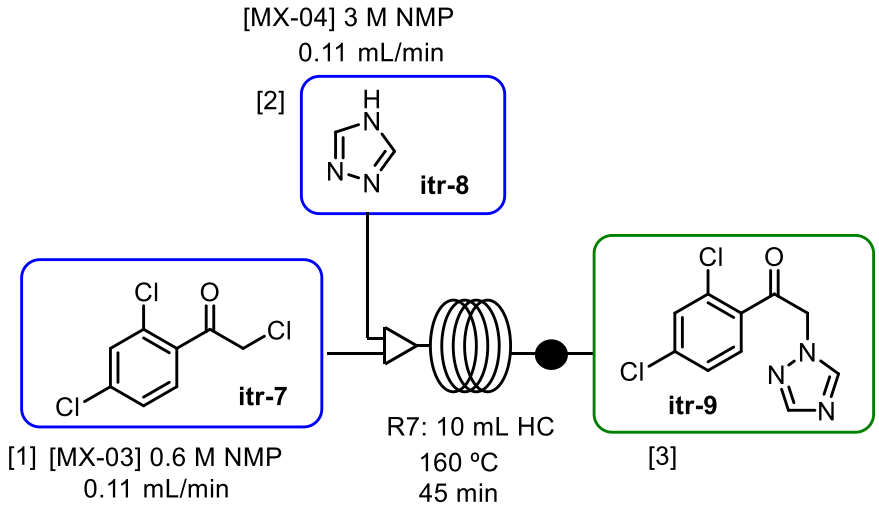
(-)-(R)-2,4dihydro-4-[4-[4-(4-hydroxyphenyl)-1-piperazinyl]phenyl]-2-(1-methylpropyl)-3H-1,2,4-triazol 3-one



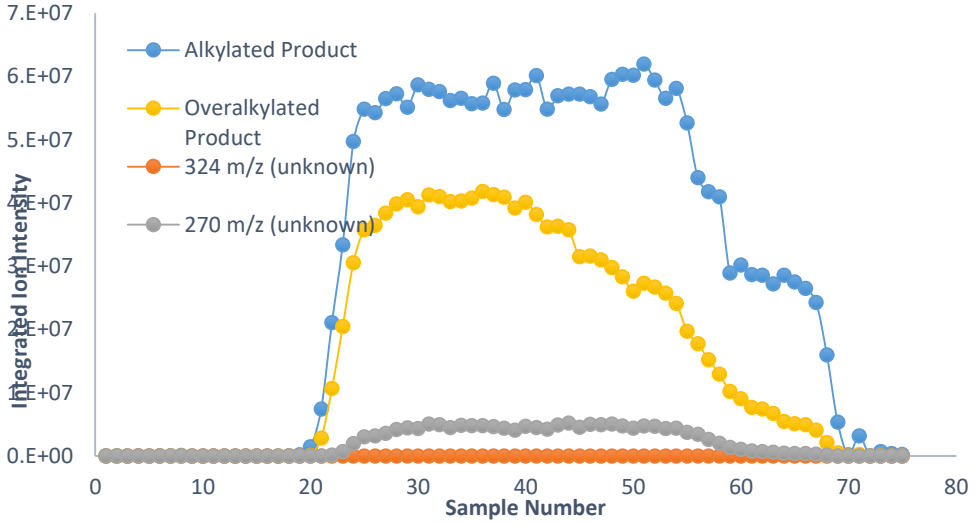
itraconazole

Itraconazole synthesis

Triazole alkylation



- Offline NMR: 85% product, 12% over-alkylated, <5% starting material
- Production rate: 550 mg / h (59%)
- Offline aqueous workup



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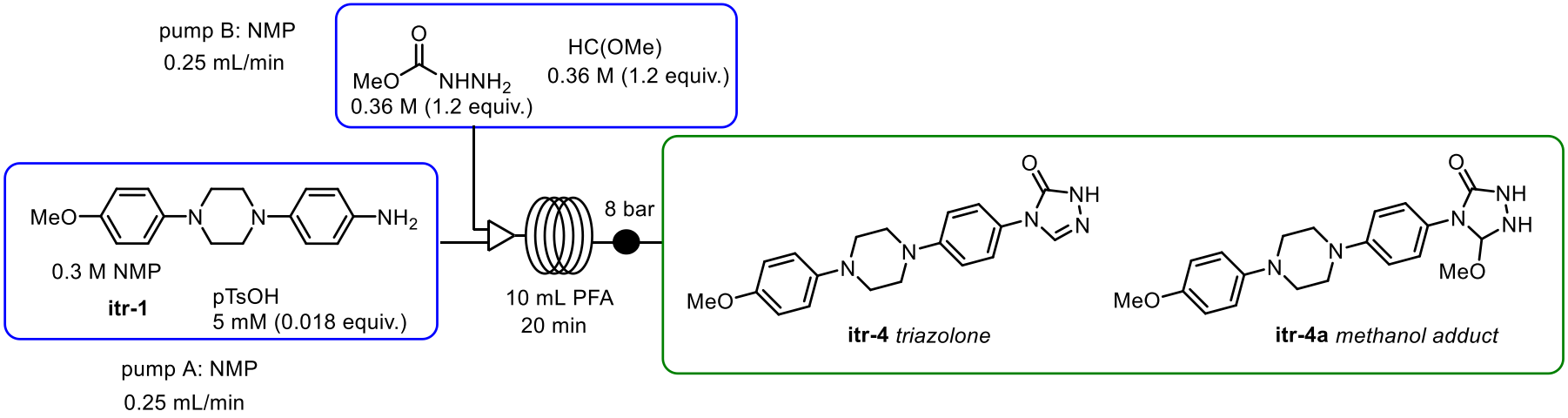
Itraconazole synthesis

Single step triazolone formation

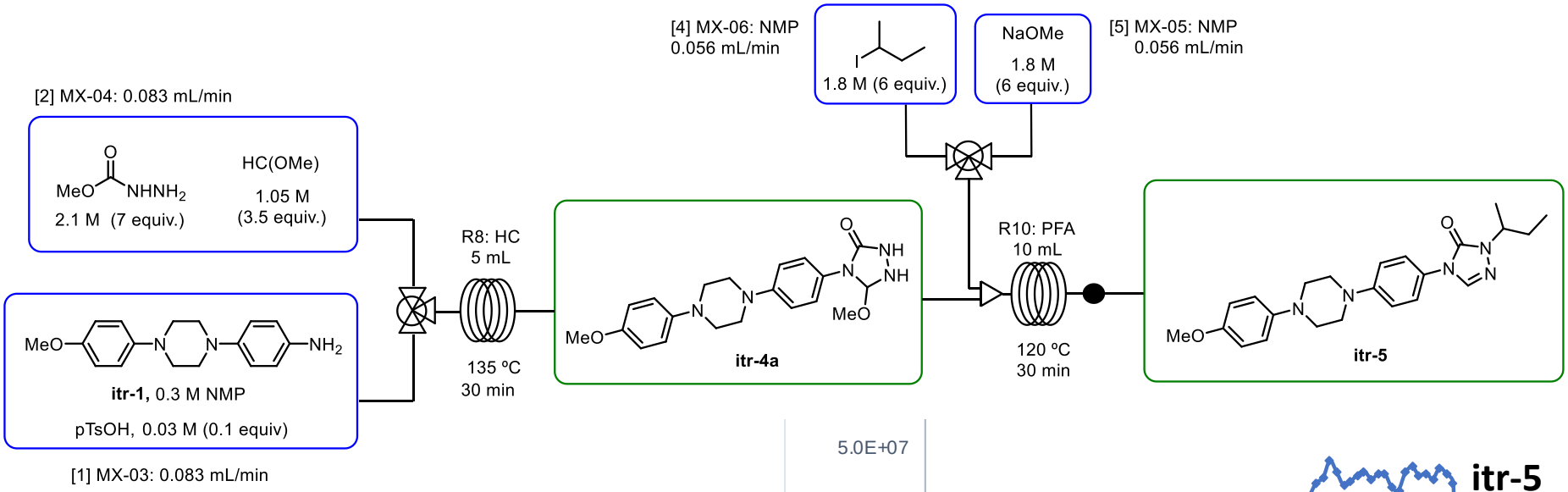


- Previous syntheses of the triazolone are stepwise (by acylation followed by cyclization)
- Here we build the heterocycle in a single operation
- The methanol adduct **itr-4a** is produced as the major product
- This is inconsequential, as the subsequent step eliminates methanol

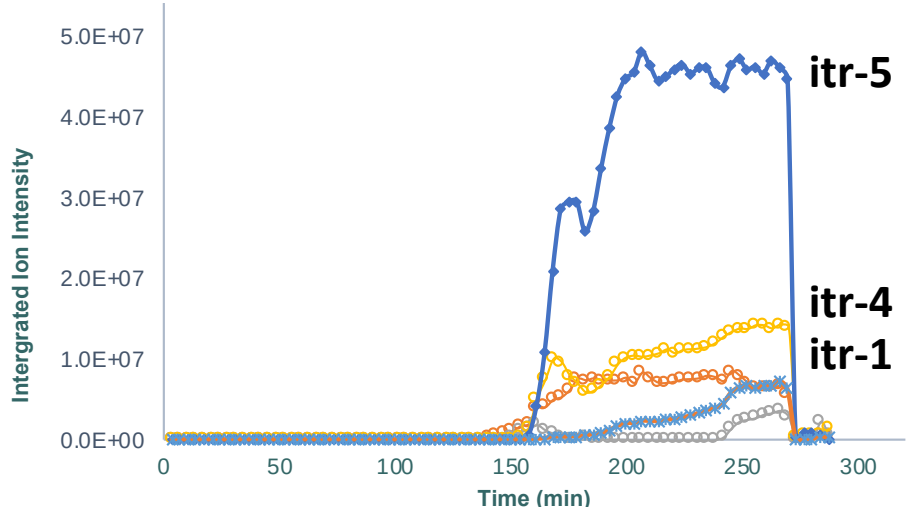
Temp (˚C)	Results (LCMS)
50	100% **itr-1**
90	70% **itr-1**, 19% **itr-4a**, 11% unknown
120	33% **itr-1**, 53% **itr-4a**, 14% unknown
150	23% **itr-1**, 32% **itr-4a**, 24% **itr-4**, 20% unknown

Itraconazole synthesis

3-Step Synthesis of Alkylated Triazololone



- Addition of the alkylation reagents gives access to **itr-5**
- Mixture composition:
  - 52% alkylated product **itr-5**
  - 19% eliminated triazololone **itr-4**
  - 14% **itr-1**
- Demethylation must be performed offline, as necessary reagent (BBr_3) is incompatible with AutoSyn



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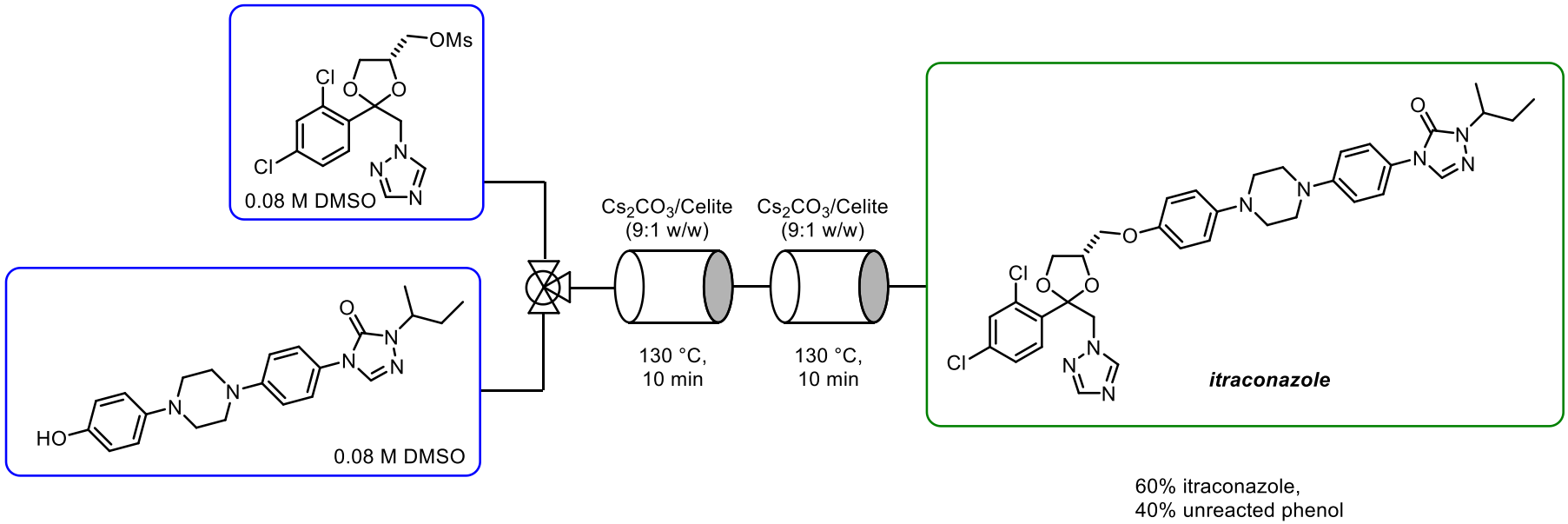
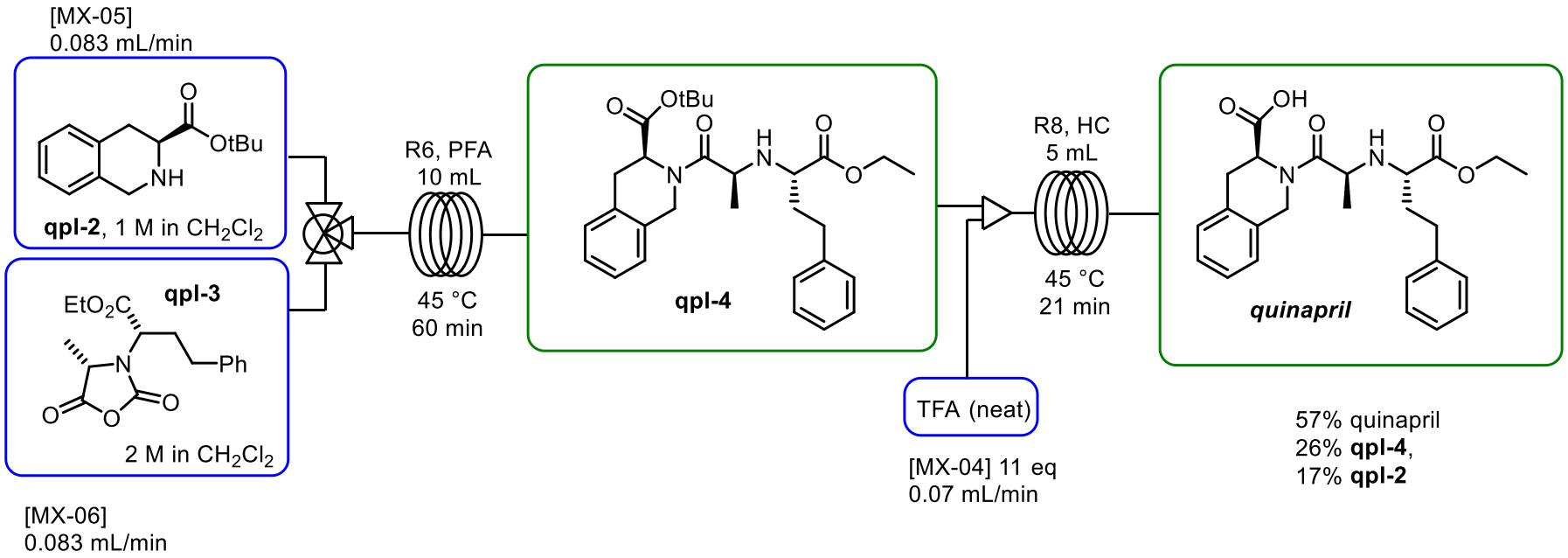
Itraconazole synthesis

Packed bed base mediated alkylation

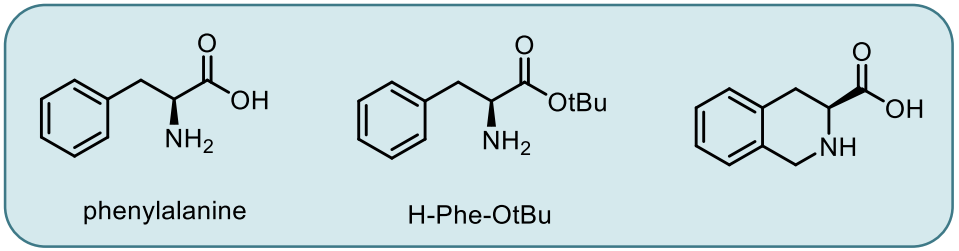


- Soluble bases (e.g., Et3N, iPr2NEt, alkoxides) performed poorly
- All reported procedures use a carbonate base
- With packed bed of Cs2CO3, the reaction proceeds cleanly, with only unreacted phenol remaining
- Additional capacity in the packed bed reactors would enable complete conversion

Quinapril synthesis



- SynRoute suggested strategies start with compounds shown at right
- Each of these is insoluble in suitable solvents for necessary chemistry
- Using **qpl-2** proved successful
- Acylation with **qpl-3** gives amide **qpl-4**, and subsequent deprotection of the tBu ester produces quinapril



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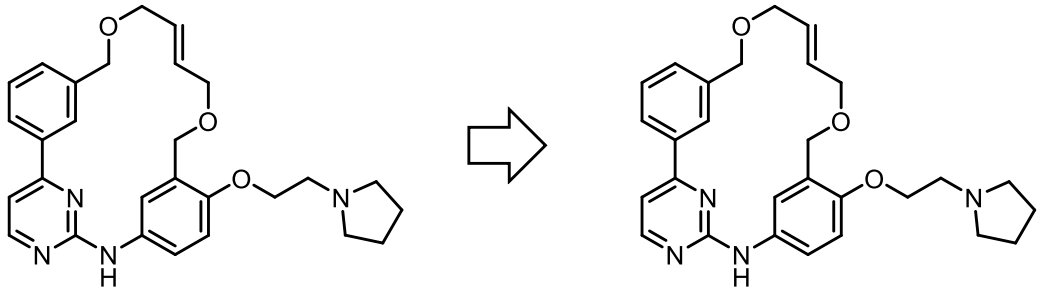
Pacritinib

Phase 3 Challenge

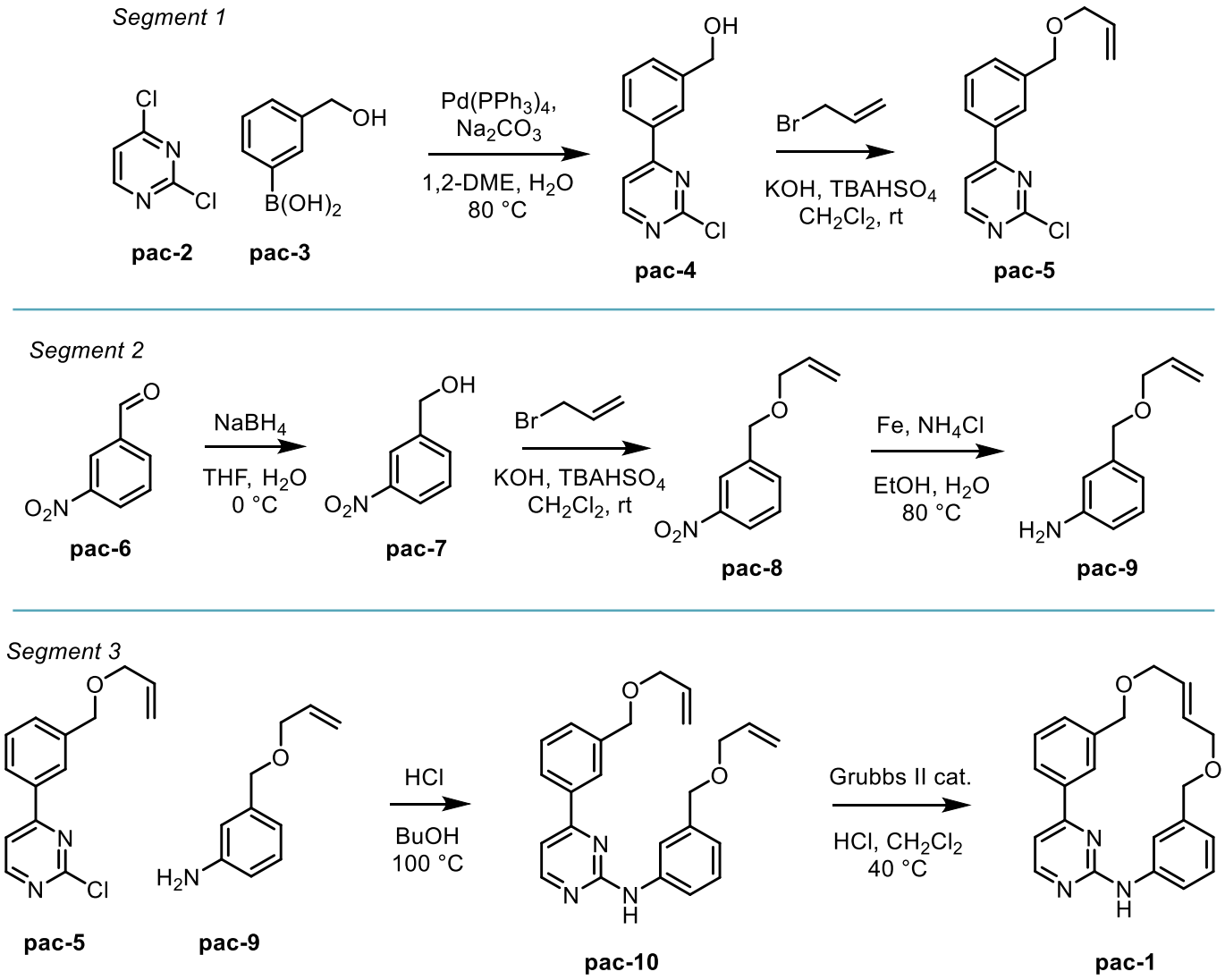


- We sought a complex compound with current pharmaceutical relevance
- Medium sized rings (macrocycles) are found in many drugs and drug candidates
- These compounds offer a new synthetic challenge and occupy a new chemical space relative to most small molecules and compounds addressed by SynFini to date
- Pacritinib: JAK2 (kinase) inhibitor under development for myelofibrosis
- Synthesis requires modern methods such as cross-coupling and olefin metathesis
- As a demonstration, synthesis of the macrocyclic core **pac-1** was chosen as the target

Pacritinib

Synthetic scheme

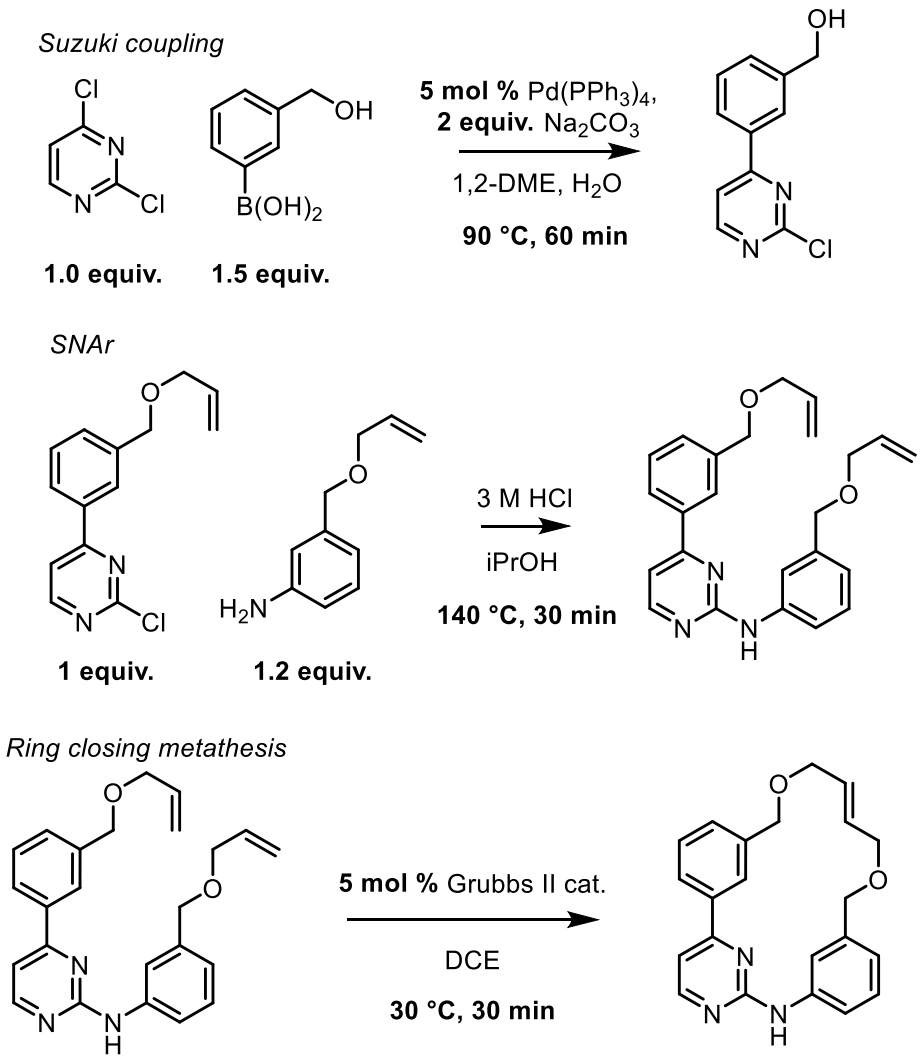
- The route to **pac-1** is convergent, requiring the pyrimidyl chloride **pac-5** and aniline **pac-9**
- Conditions listed are from previous reports toward pacritinib
- Key transformations of interest include: Suzuki coupling, Williamson ether synthesis of an unactivated hydroxyl, SNAr, and olefin metathesis



Pacritinib

SynJet Studies

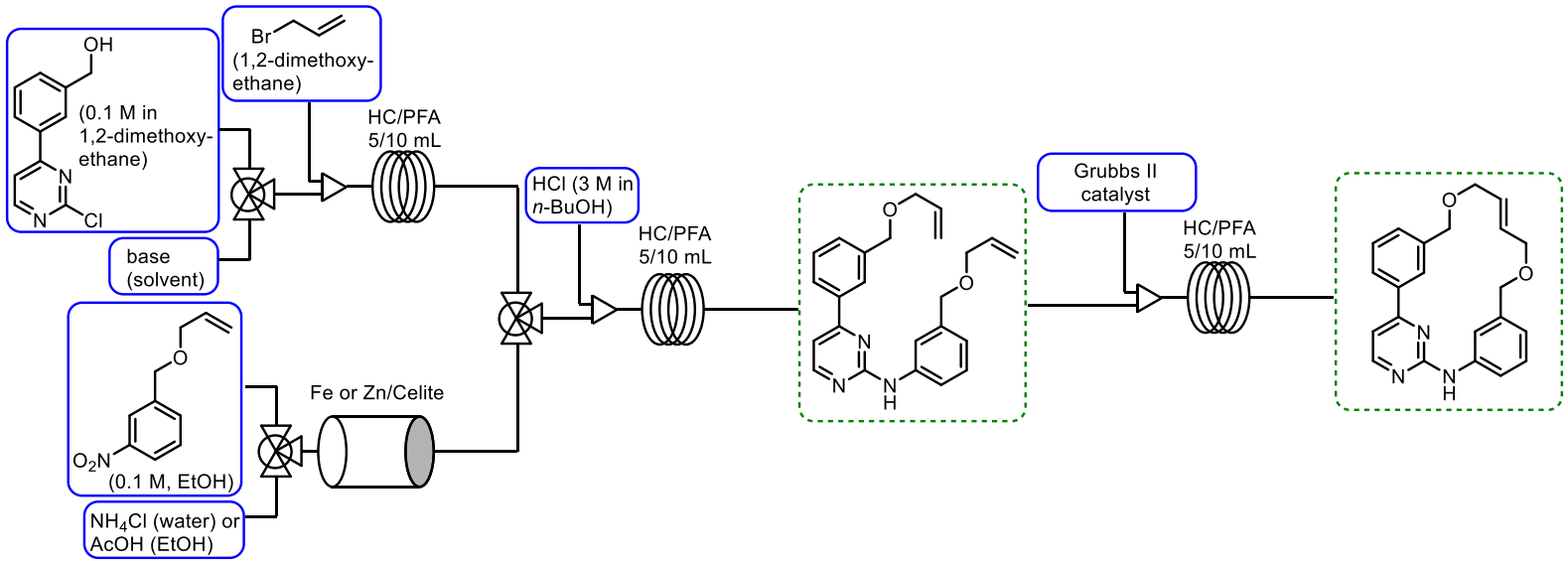
- Key reactions were optimized using SynJet
- Conditions determined are listed in **bold**
- These results represent the first examples of Suzuki coupling and olefin metathesis on SynJet



Pacritinib

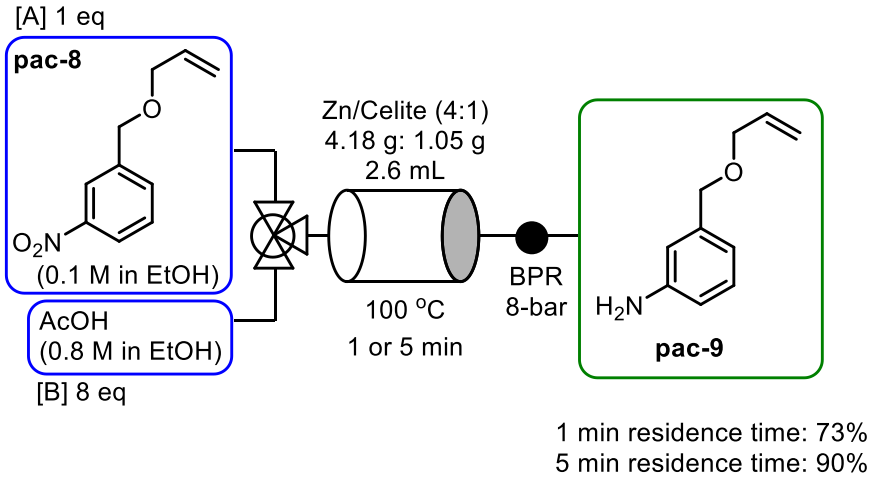
Toward a convergent Synthesis

- Designed flow path



Pacritinib

Toward a convergent synthesis

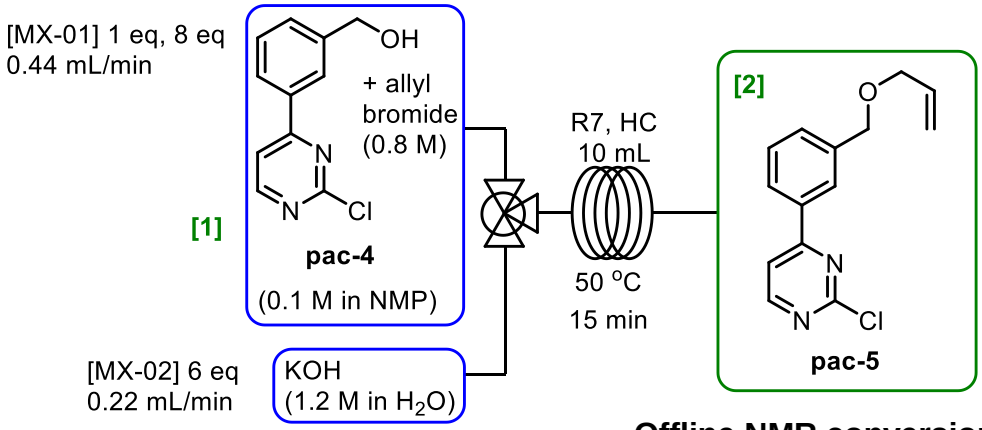


- Reduction of the nitro group of **pac-8** was demonstrated on the Vapourtec using a packed bed of zinc

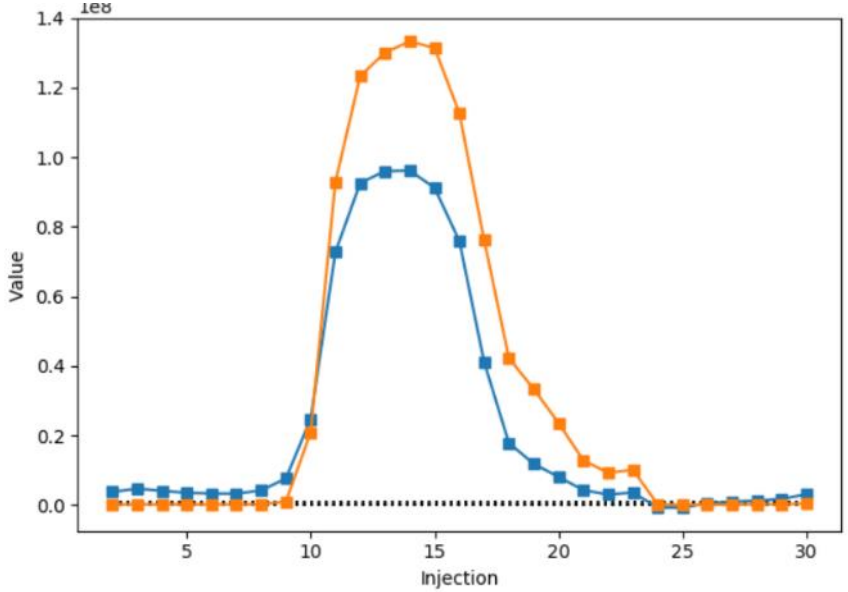
Pacritinib

Toward a convergent synthesis

- Typical conditions for this reaction would utilize sodium hydride, which would be very difficult to handle with AutoSyn
- A method using KOH was developed and is working well

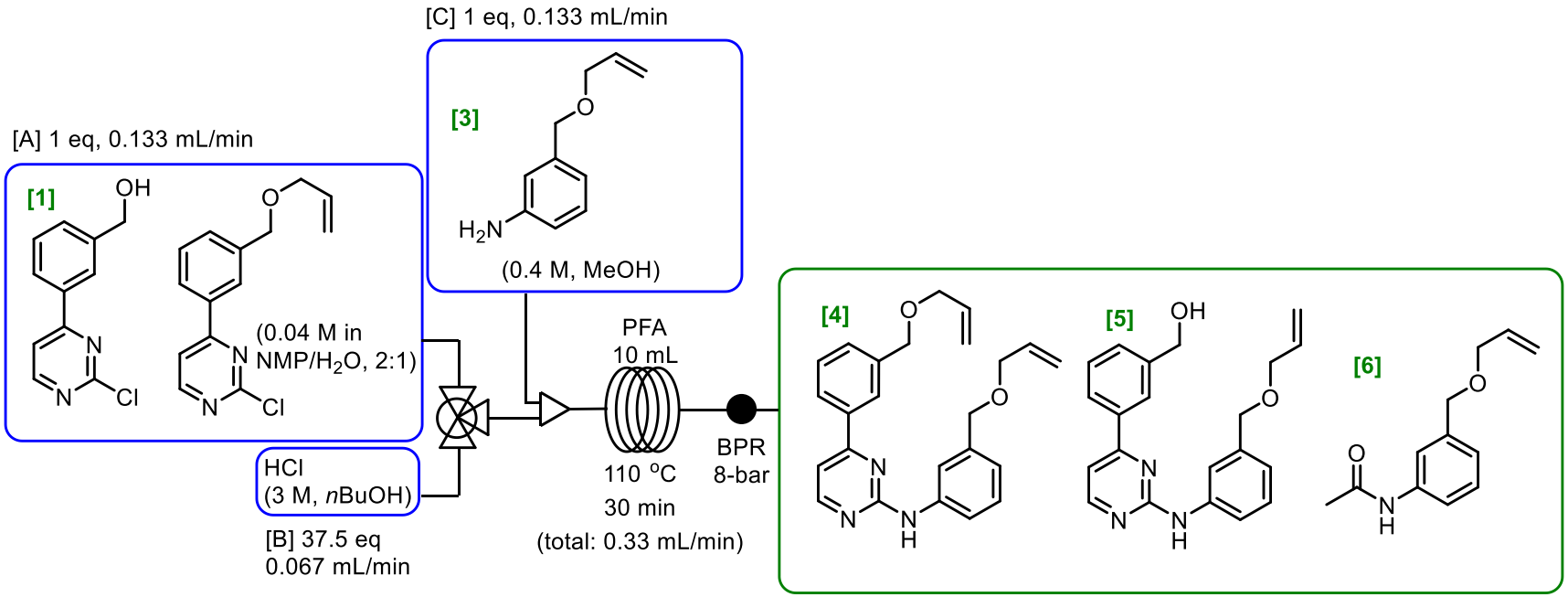


Offline NMR conversion: 76%



Pacritinib

Toward a convergent synthesis

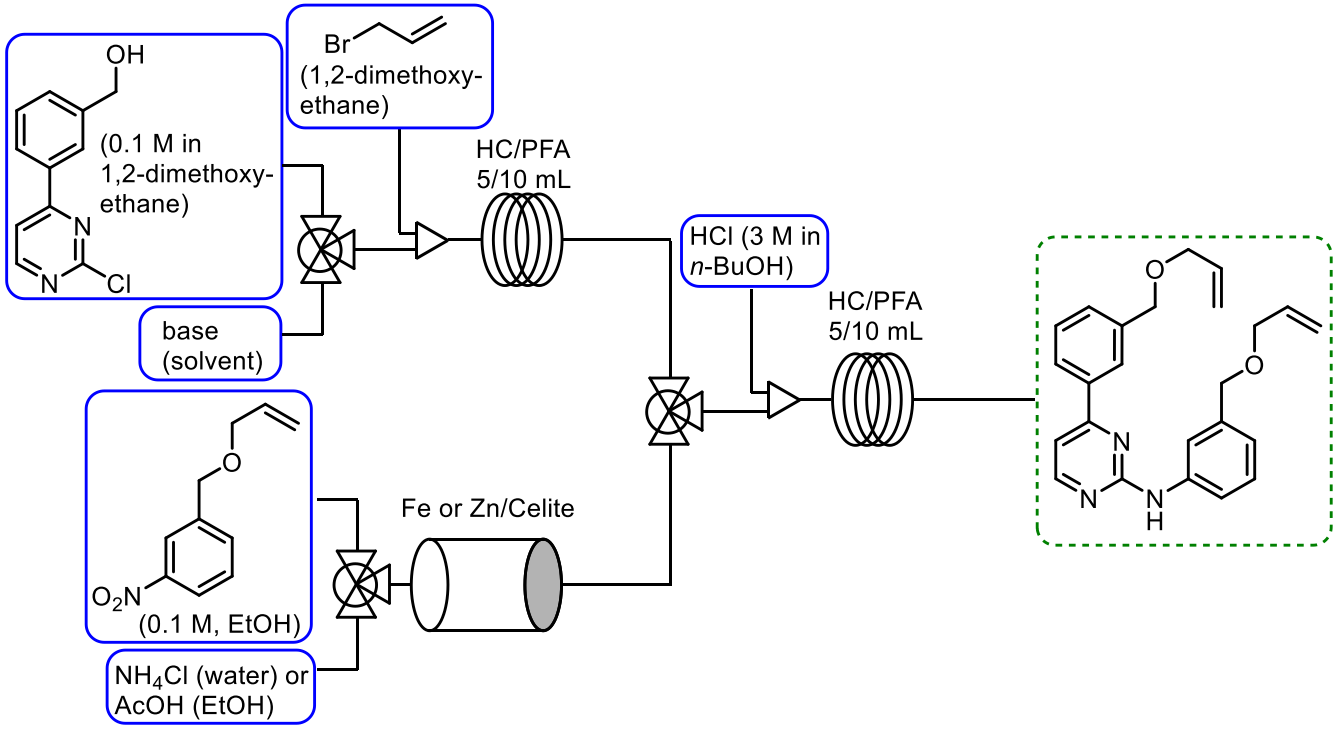


- To simulate a telescoped, convergent process, reaction mixtures from each of the preceding reactions were pumped together at 110 C
- The mixture of **pac-5** contained significant amounts of **pac-4**
- The mixture of aniline **pac-9** contained residual acetic acid from the preceding reduction step
- Results: 50% conversion (for each of the pyrimidyl chlorides), with traces of acetylated **pac-9**

Pacritinib

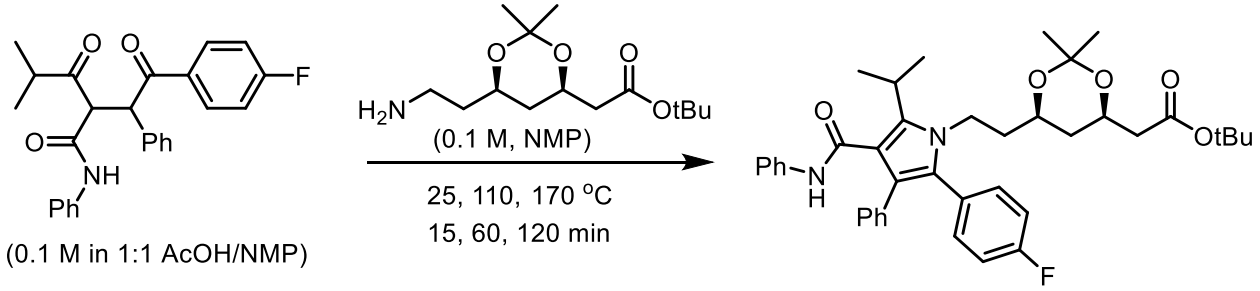
Toward a convergent Synthesis

- When performed as a convergent process, precipitation has occurred, preventing the full process
- Changing from HCl to TFA keeps the reaction in solution, however SNAr conversion is low (<20%)
- Development is continuing

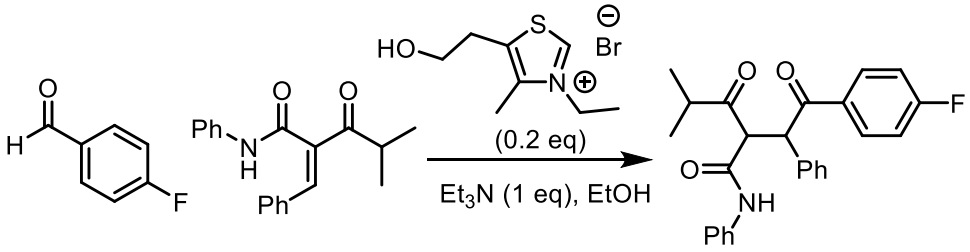


Atorvastatin

SynJet Screening Paal-Knorr Pyrrole Synthesis & Stetter Reaction

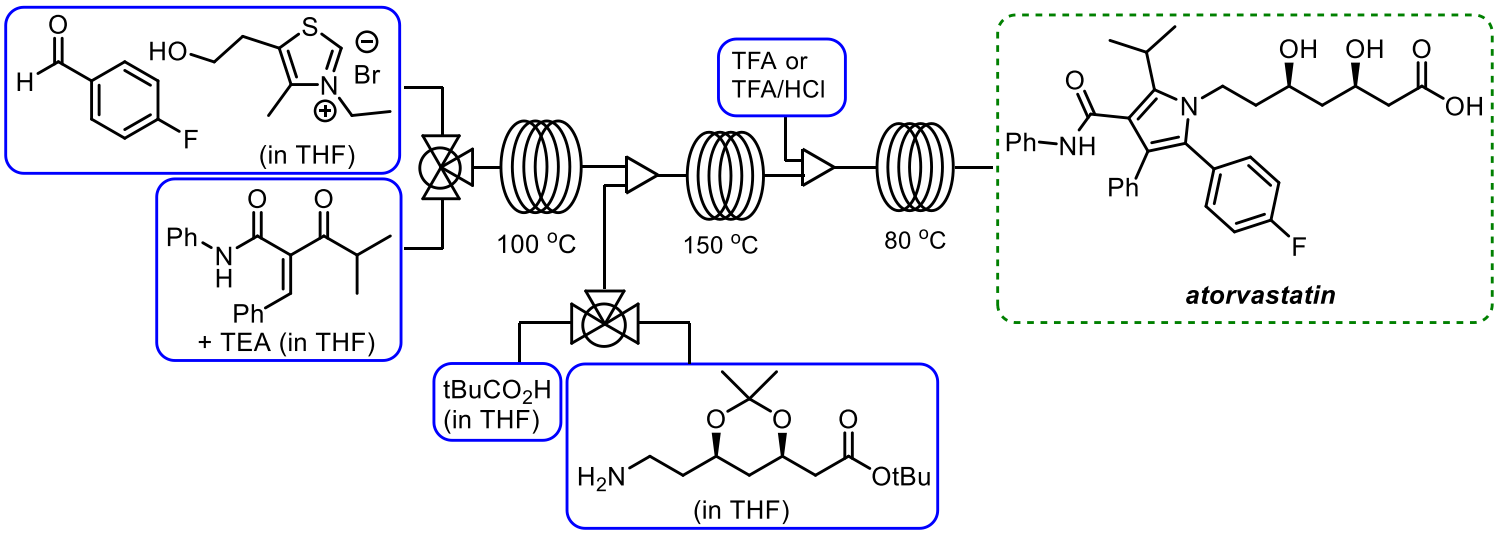


Standard SynJet optimization run gave:
1:2 dione:amine, 110 C, 2 h, 22%
1:2 dione:amine, 170 C, 1 h, 20%



Standard SynJet optimization run gave:
1.2:1 aldehyde:enone, 80 C, 19 h, 20%

Atorvastatin
Flow Strategy



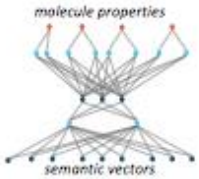
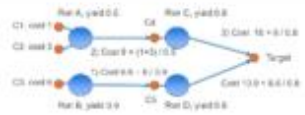
- Consecutive slow reactions preclude direct translation to AutoSyn (SynJet results on next slide)
- Hardware solution under evalaution: series of CSTRs as described in hardware section (Enhancing Reactions on AutoSyn – Slide 48) - ongoing

Summary

Target	Completed	Remaining	New AutoSyn capabilities
Itraconazole	• Triazolininone building block • Triazole alkylation • Offline ketal formation		• Multicomponent heterocycle formation • Solid phase base
Bortezomib	• Amide coupling/deprotection – 2 rounds		• Amide coupling
Quinapril	• Amide coupling • Pictet-Spengler • Deprotection		
Atorvastatin	• SynJet reaction screening	• Overcoming slow Stetter & Paal-Knorr reaction	
Pacritinib	• SynJet reaction screening • Individual steps in flow	• Convergent synthesis	• Alcohol allylation

SynFini – Transition Planning

Automated Chemical Discovery

AI structure design	Route design	Reaction screening & optimization	Multi-step synthesis development	Rapid *in vitro* bioassays and ADMET
				
DASL™	**SynRoute™**	**SynJet™**	**AutoSyn™**	**Data Generation**
• AI platforms for molecular design	• Design multistep routes ~10 sec	• Print ~rxn/secs • >3,000 rxn/hr	• Multistep synthesis • New chemical in hrs	• Medium throughout • Full compound assessment

Radical paradigm shift in synthetic chemistry, enabling more inventors, more innovation

Integrating core SynFini components into AI driven molecular design at the front end and property testing at the back end to create a rapid iterative chemical discovery platform

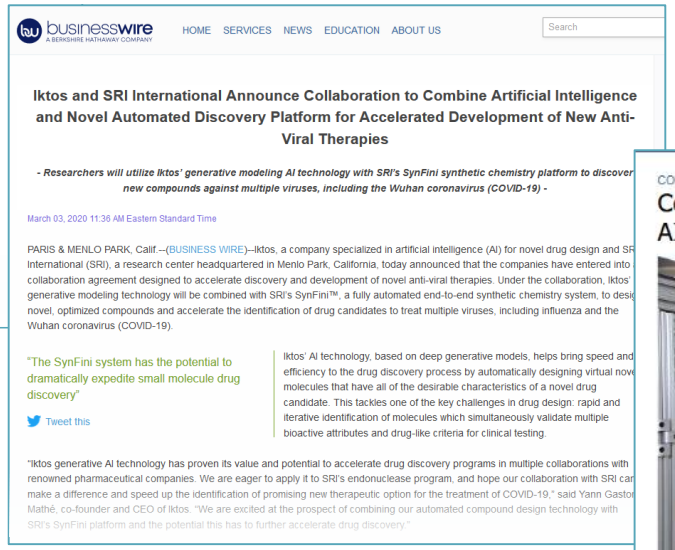
SRI International®

SynFini-In the News.....

Appeared January 29, 2020
[C&EN, Volume 98, Issue 5]


c&en CHEMICAL & ENGINEERING NEWS
TOPICS MAGAZINE COLLECTIONS VIDEOS JOBS
INSTRUMENTATION
AI and robotics come together for synthesis
SRI's SynFini system integrates machine learning with automated flow chemistry
by Craig Bettenhausen
JANUARY 29, 2020 | APPEARED IN VOLUME 98, ISSUE 5
Credit: SRI International
The AutoSyn module of SRI International's system uses flow chemistry informed by AI to prepare molecules at the gram scale.
AI-assisted synthesis planning is on the march. Flow chemistry is maturing and scaling up. Now the race is on to bring the two together.

Appeared March 3, 2020
[Businesswire.com Press Release]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Iktos and SRI International Announce Collaboration to Combine Artificial Intelligence and Novel Automated Discovery Platform for Accelerated Development of New Anti-Viral Therapies
- Researchers will utilize Iktos' generative modeling AI technology with SRI's SynFini synthetic chemistry platform to discover new compounds against multiple viruses, including the Wuhan coronavirus (COVID-19) -
March 03, 2020 11:36 AM Eastern Standard Time
PARIS & MENLO PARK, Calif.--(BUSINESS WIRE)--Iktos, a company specialized in artificial intelligence (AI) for novel drug design and SRI International (SRI), a research center headquartered in Menlo Park, California, today announced that the companies have entered into a collaboration agreement designed to accelerate discovery and development of novel anti-viral therapies. Under the collaboration, Iktos' generative modeling technology will be combined with SRI's SynFini™, a fully automated end-to-end synthetic chemistry system, to design novel, optimized compounds and accelerate the identification of drug candidates to treat multiple viruses, including influenza and the Wuhan coronavirus (COVID-19).
"The SynFini system has the potential to dramatically expedite small molecule drug discovery"
Tweet this
Iktos' AI technology, based on deep generative models, helps bring speed and efficiency to the drug discovery process by automatically designing virtual novel molecules that have all of the desirable characteristics of a novel drug candidate. This tackles one of the key challenges in drug design: rapid and iterative identification of molecules which simultaneously validate multiple bioactive attributes and drug-like criteria for clinical testing.
"Iktos generative AI technology has proven its value and potential to accelerate drug discovery programs in multiple collaborations with renowned pharmaceutical companies. We are eager to apply it to SRI's endonuclease program, and hope our collaboration with SRI can make a difference and speed up the identification of promising new therapeutic option for the treatment of COVID-19," said Yann Gaston-Mathé, co-founder and CEO of Iktos. "We are excited at the prospect of combining our automated compound design technology with SRI's SynFini platform and the potential this has to further accelerate drug discovery."

Appeared March 4, 2020
[abc 7 Bay Area Local News]


CORONAVIRUS
Coronavirus research: Menlo Park lab using robots, AI to find COVID-19 medication
DEVELOPING NEWS
PETER MADRID, PH.D.
SRI APPLIED BIOSCIENCES DIRECTOR
EMBED MORE VIDEOS
A Menlo Park lab is using robots and artificial intelligence to find medicine to combat the symptoms of the novel coronavirus.
By David Louie
Wednesday, March 4, 2020
MENLO PARK, Calif. (KGO) -- As the number of cases of COVID-19 increases, so does the pressure to develop an anti-viral to treat its symptoms. A lab on the Peninsula appears to have developed the right process at the right time to speed up that process.
"We're hoping to be able to take the full discovery of a candidate drug from roughly two years down to six months," said Peter Madrid, senior director of applied biosciences at SRI Biosciences in Menlo Park

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