WHITE PAPER

# DIRECTINJECT-LC™

## REAL-TIME, QUANTITATIVE ANALYSIS OF CHEMICAL REACTIONS AND CRYSTALLIZATIONS

## FULL REACTION AND IMPURITY PROFILING

Pharmaceutical and fine chemical companies are under economic and social pressure to improve efficiency, reduce costs, and minimize the impact of their operations on the environment. The key to attaining these goals is defining and optimizing new synthetic processes via an in-depth understanding of reaction mechanisms and kinetics.

A plethora of instrument and software advances now support these efforts, most notably real-time, *in-situ* Process Analytical Technology (PAT). A gold-standard in analytical technology, high-performance liquid chromatography (HPLC), elucidates and quantitatively measures reaction species. However, specific requirements for sample removal, quenching, and dilution have prevented the deployment of chromatography as an online PAT.

**The solution to this quandary is found in Telescope Innovation's proprietary DirectInject-LC™ technology (Figure 1).** DirectInject-LC™ fully automates the sample extraction, quenching, and dilution process and transmits reaction samples to the HPLC in real-time (Figure 2). This approach eliminates any time-lapse related analysis issues of the sample such as post-reaction changes or loss of unstable intermediates. Key reaction species are identified and measured as the reaction proceeds, yielding unparalleled understanding of reaction kinetics, mechanism and the effect of variables on reaction outcome. True, time-course measurement of chemical syntheses and crystallizations via HPLC is now feasible.


**MONITOR REACTION PROGRESS IN REAL TIME**

Explore complete concentration profiles for nearly any chemical process, including impurity profiling

**UNRAVEL COMPLEX MECHANISTIC PATHWAYS**

Understand the behaviour of side-products and intermediates, catalytic activity, reaction kinetics, and pathways to optimization

**STUDY CHALLENGING CHEMICAL ENVIRONMENTS**

Handle solid-liquid slurries, biphasic systems, air/water-sensitive reactions, cryogenic chemistry, and reactions under high pressure

**GAIN DEEP UNDERSTANDING OF CRYSTALLIZATIONS**

Sample dynamically crystallizing systems, design and qualify chiral resolutions, selective crystallizations, and crystallization-induced asymmetric transformations

**Figure 1.** Highlights of DirectInject-LC™ technology.

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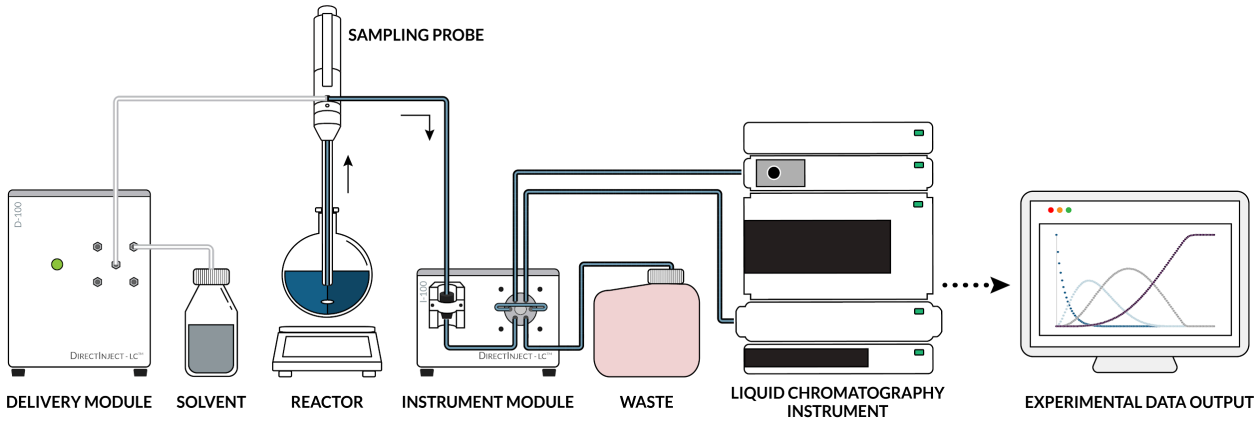


**Figure 2.** DirectInject-LC™ enables real-time, automated sampling and HPLC analysis of chemical reactions.

This white paper reviews the applications of DirectInject-LC™ in a variety of syntheses and crystallizations. In these examples, in-depth knowledge via online chromatographic analysis was key to successful outcomes.

## CONTENTS



## 1. REACTION PROFILING FOR CATALYSIS | KINUGASA REACTION

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See: Malig, T. C.; Yu, D. N.; Hein, J. E. "A revised mechanism for the Kinugasa reaction." *J. Am. Chem. Soc.* **2018**, 140(*29*), p. 9167-9173.

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| **SCIENTIFIC & INDUSTRIAL IMPORTANCE** | The catalytic Kinugasa reaction that produces high-value β-lactams is low-yielding, poorly understood, and generates many byproducts. |
| **ANALYTICAL CHALLENGES** | Many byproducts and potential mechanisms. |
| **DIRECTINJECT-LC™ SOLUTION** | Reaction profiling enabled reaction progress kinetic analysis (RPKA) and variable time normalization analysis (VTNA). These studies produced the first consistent mechanistic model accounting for the common byproducts of the Kinugasa reaction, and improved β-lactam yield up to 80%. |

β-Lactams are privileged scaffolds in organic synthesis that can be accessed with both enantio- and diastereoselectivity by the reaction of a copper acetylide with a nitrone (Figure 3).

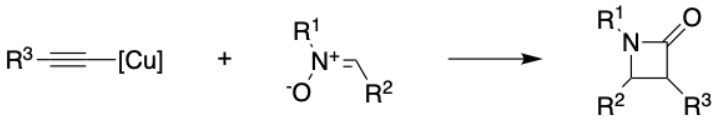


**Figure 3.** Kinugasa reaction for β-Lactam synthesis.

Nevertheless, the Kinugasa reaction is not widely used for β-Lactam synthesis because multiple byproducts are often formed and the reaction is poorly understood. Although a mechanism based on computational studies had been proposed, a detailed kinetic analysis had not been developed. Applying the DirectInject-LC™ technology to acquire real-time HPLC-MS measurements enabled the use of reaction progress kinetic analysis (RPKA) and variable time normalization analysis (VTNA) to elucidate the underlying reaction mechanism. The goal of this kinetic study was to increase β-lactam formation, while better understanding pathways leading to undesired byproducts.

*"The observed kinetic and chemoselectivity data allow us to propose a modified mechanism, involving a novel (3 + 2) cycloreversion followed by Lewis acid catalyzed (2 + 2) cycloaddition. Most importantly, our new catalytic pathway, which proceeds via a common ketene intermediate, provides the first consistent mechanistic model for the generation of all commonly observed byproducts of the Kinugasa reaction."*

### A. DETERMINE IMPORTANCE OF THE BASE ADDITIVE TO IMPROVE YIELD

Though sterically encumbered secondary amines such as diisopropylamine can provide the desired β-lactam, it was found that significant by-products formed in a model reaction. Based on additional mechanistic insight, the use of a stronger, non-nucleophilic amine base, such as DBU

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(1,8-diazabicyclo- (5.4.0)undec-7-ene) was proposed and this resulted in an increased yield of β-lactam (from 17% to 78%), with significant reduction in imine by-product formation.

### B. IDENTIFY THE CATALYST BEHAVIOR AND ORDER OF SPECIES VIA KINETIC ANALYSIS

The resting state and test for catalyst deactivation were investigated through a series of same and different excess experiments. The same excess experiments showed an overlap of the kinetic profiles for the decay of the nitrone over time via the application of a positive time shift (Figure 4a). This result suggested that neither product inhibition or catalyst deactivation was occurring. The driving force for the reaction was tested by different excess experiments. It was observed that varying the initial concentration of either the alkyne or nitrone does not affect the observed rate. A higher initial concentration of the nitrone speeds the reaction (i.e., positive order) whereas an increase in the alkyne decreases the rate (i.e., negative order). In a series of experiments in which the catalyst concentration was varied, the reaction showed a positive order, but the progress curves indicated the response was greater than first order. By analyzing the reaction progress data for both varied initial substrate and catalysts (Figure 4b, 4c), the order of each reaction component was elucidated. The kinetic data in Figure 4c indicates that the system is second order in copper concentration, as supported by the VTNA plot of this data in Figure 4d .

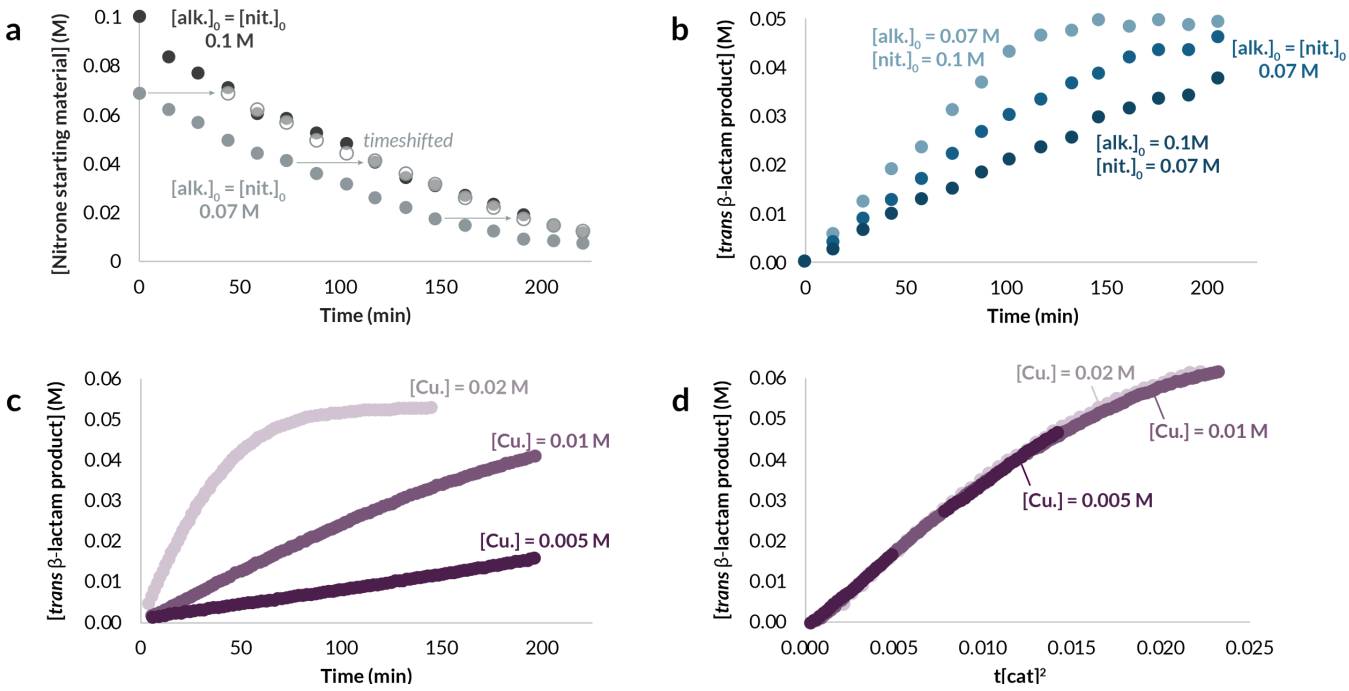


**Figure 4.** RPKA/VTNA experiment data generated by DirectInject-LC™.[1] (a) Same excess experiments. (b) Different excess experiments. (c) Kinetic data for reactions with various catalyst loadings. (d) VTNA plot for data from Figure 4c indicating that the system is second order in [Cu].

### C. PROPOSE A REVISED MECHANISM FOR THE KINUGASA REACTION

[1] See *J. Am. Chem. Soc.* **2018**, 140(*29*), p. 9167-9173 for original data and figures.

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From the data provided by the DirectInject-LC™ system in combination with the results of the RPKA and VTNA analysis, a complete mechanistic model is offered that explains both the productive and unproductive reaction pathways (Figure 5).

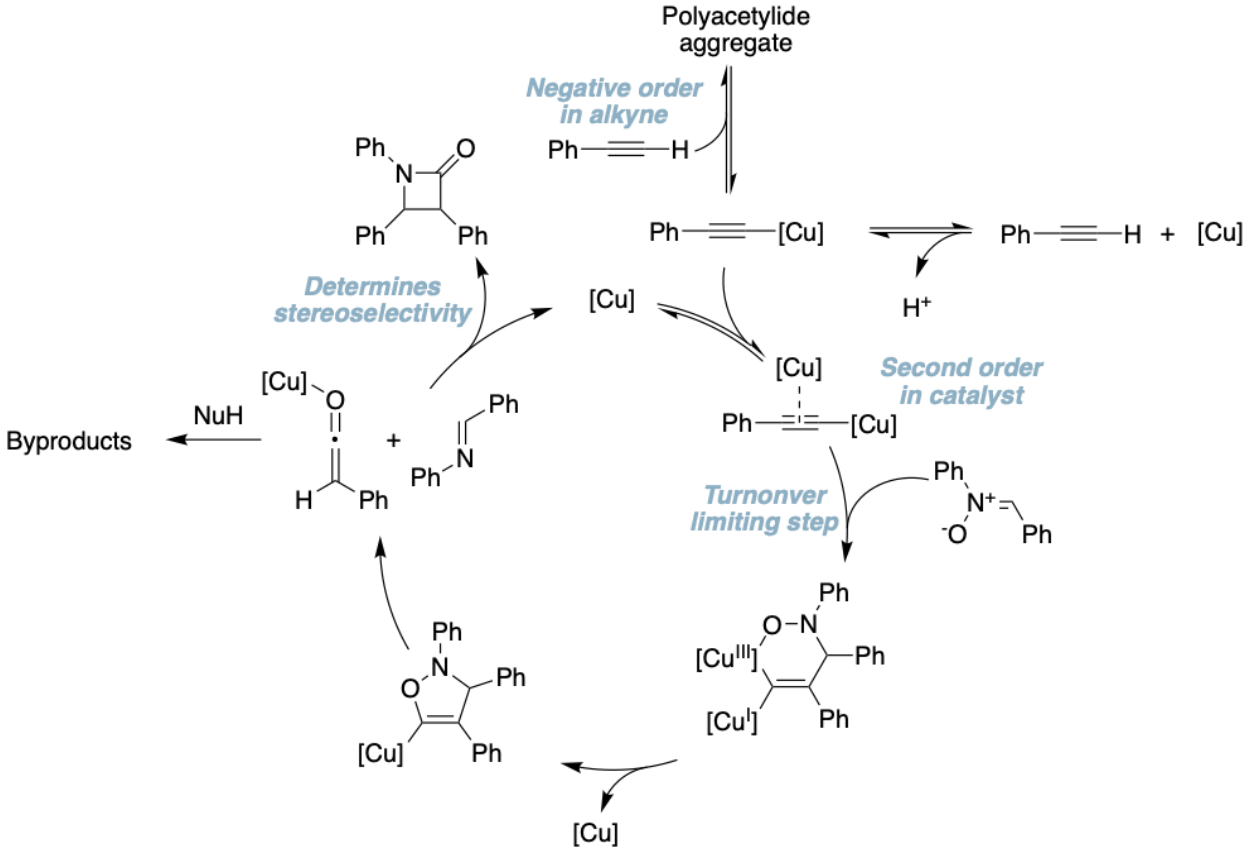


**Figure 5.** Proposed reaction mechanism based on kinetic data highlighting all key equilibria and binding events.[2]

This proposed mechanism also solves inconsistencies with previous models. First, presence of a ketene intermediate permits the rationalization for formation of both target β-lactams and common byproducts. Also, the mechanism suggests that the (2 + 2) cycloaddition between the ketene and imine sets the asymmetric center geometry in the β-lactam, not the initial (3 + 2) cycloaddition involving the nitrone and σ-Cu(I) acetylide, as described in earlier research.

[2] See *J. Am. Chem. Soc.* **2018**, 140(*29*), p. 9167-9173 for original data and figures.

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# 2. **AIR-SENSITIVE CHEMISTRY** | PD CATALYZED CROSS-COUPLING

See: Deem, M.C.; Derasp, J.S.; Malig, T.C.; Legard, K.; Berlinguette, C.P.; Hein, J.E. "Ring walking as a regioselectivity control element in Pd-catalyzed C-N cross-coupling." *Nat Commun.* **2022**, 13, 2869.

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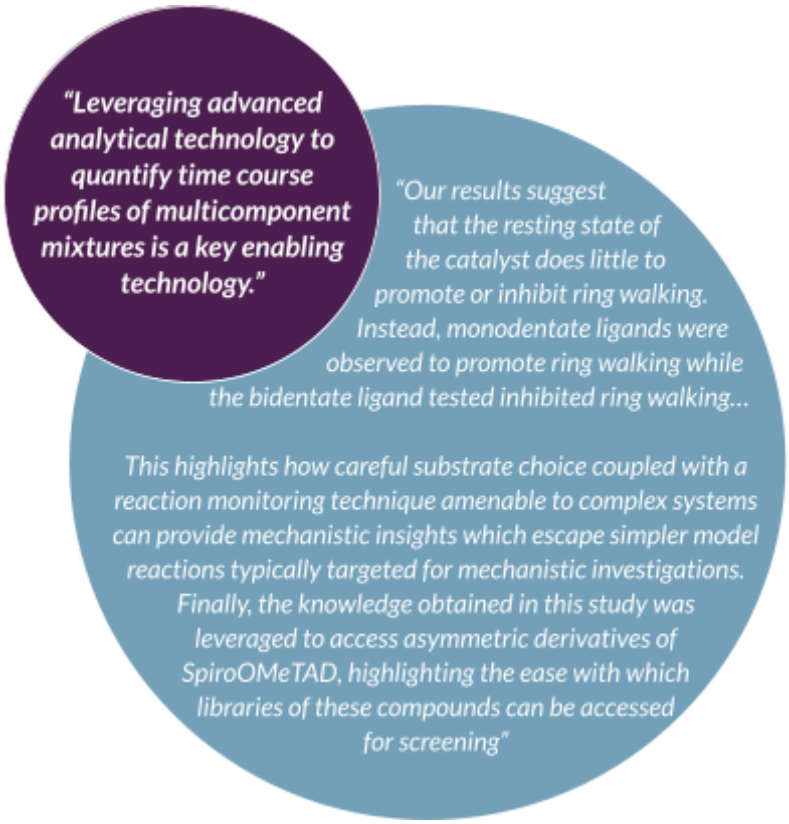
| **SCIENTIFIC & INDUSTRIAL IMPORTANCE** | Spiro-OMeTAD is a best-in-class organic conductor for photoelectronics. High manufacturing costs prevent technology deployment . |
| **ANALYTICAL CHALLENGES** | The reaction is air-sensitive and involves a product and 4 intermediates that are structurally similar. Several mechanistic pathways are possible. |
| **DIRECTINJECT-LC™ SOLUTION** | Insights from online reaction profiling accelerated reaction times by 30x and showed that catalyst ligands direct formation of asymmetric intermediates. This work provides synthetic control of valuable, asymmetric SpiroOMeTAD derivatives. |

SpiroOMeTAD is used in perovskite solar cells, molecular modifications are being sought to enhance its properties as a hole transport material. Though controlling substitution patterns in SpiroOMeTAD derivatives could improve performance, it is challenging to use the Buchwald-Hartwig Amination (BHA) to access asymmetric species from polyhalogenated precursors. Ring walking is one method for gaining regioselectivity in small molecules, however, ring walking under BHA conditions has not been extensively studied. In this work, DirectInject-LC™ is leveraged to perform an in-depth mechanistic investigation of ligand effects on the synthesis of SpiroOMeTAD via BHA. This study reveals the drastic effect that catalyst ligand choice has on the evolution of intermediates throughout the reaction, leading to the synthesis of a range of asymmetric SpiroOMeTAD derivatives (Figure 6).

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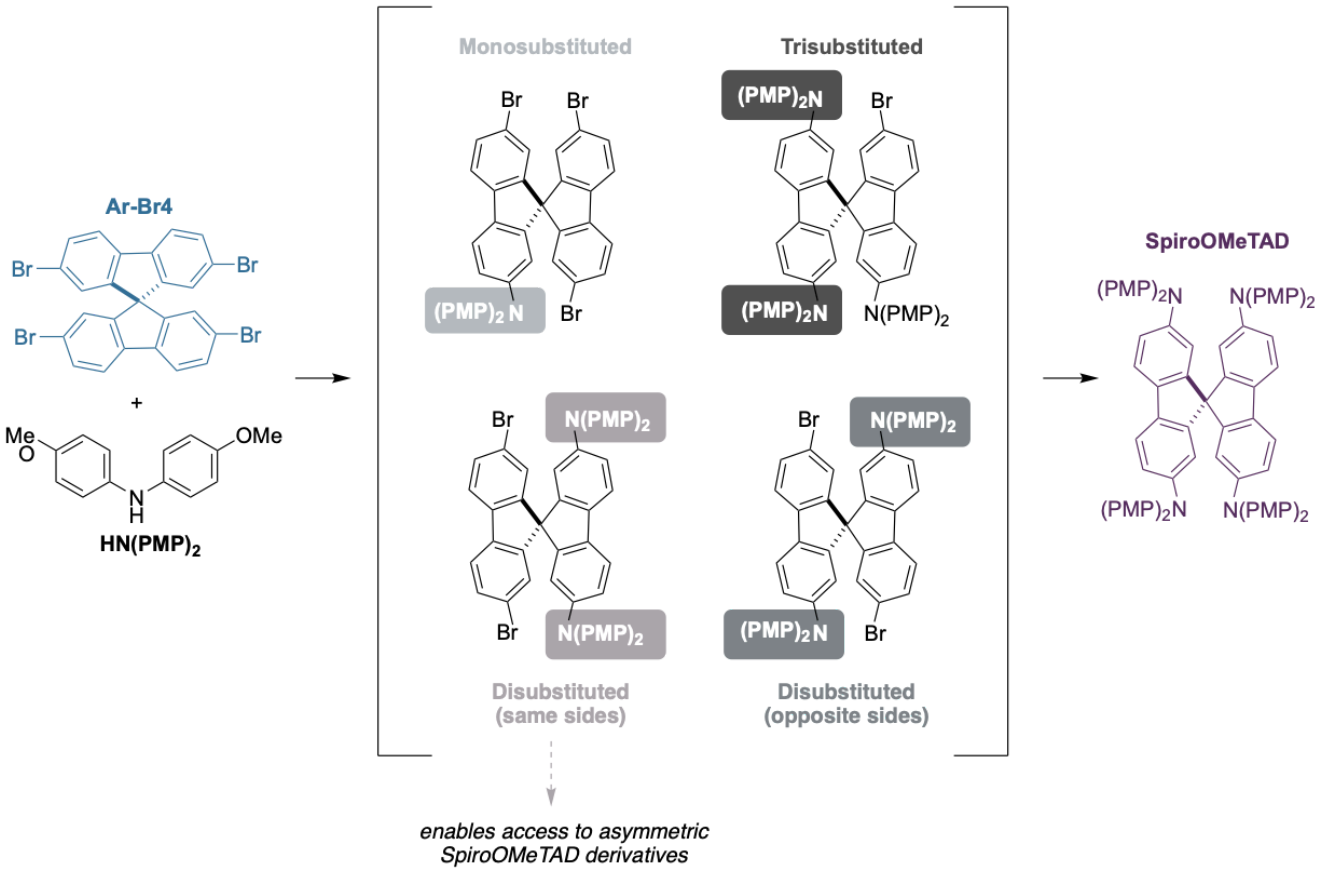


**Figure 6.** Buchwald-Hartwig Amination produces SpiroOMeTAD in addition to symmetric and asymmetric products.

Along with the product, the air-sensitive, complex model reaction could produce up to 4 intermediate compounds (Figure 6). Various scenarios are possible for a SpiroOMeTAD synthesis via BHA, which can involve ring walking and/or diffusion control (Figure 7). Each of these scenarios would lead to different outcomes relative to intermediates produced. The model system was investigated using the DirectInject-LC™ platform by examining four ligands often used for palladium-catalyzed synthesis of triarylamines: $Pd(OAc)_2/P(tBu)_3$, XantPhos Pd G4, RuPhos Pd G4, and PEPPSI-IPr. The goals of this study were to: (A) understand the effect of catalyst ligand on the ring-walking behavior of intermediates, (B) generate kinetic models based on reaction profile data, and (C) leverage these insights to produce a variety of asymmetric SpiroOMeTAD derivatives.

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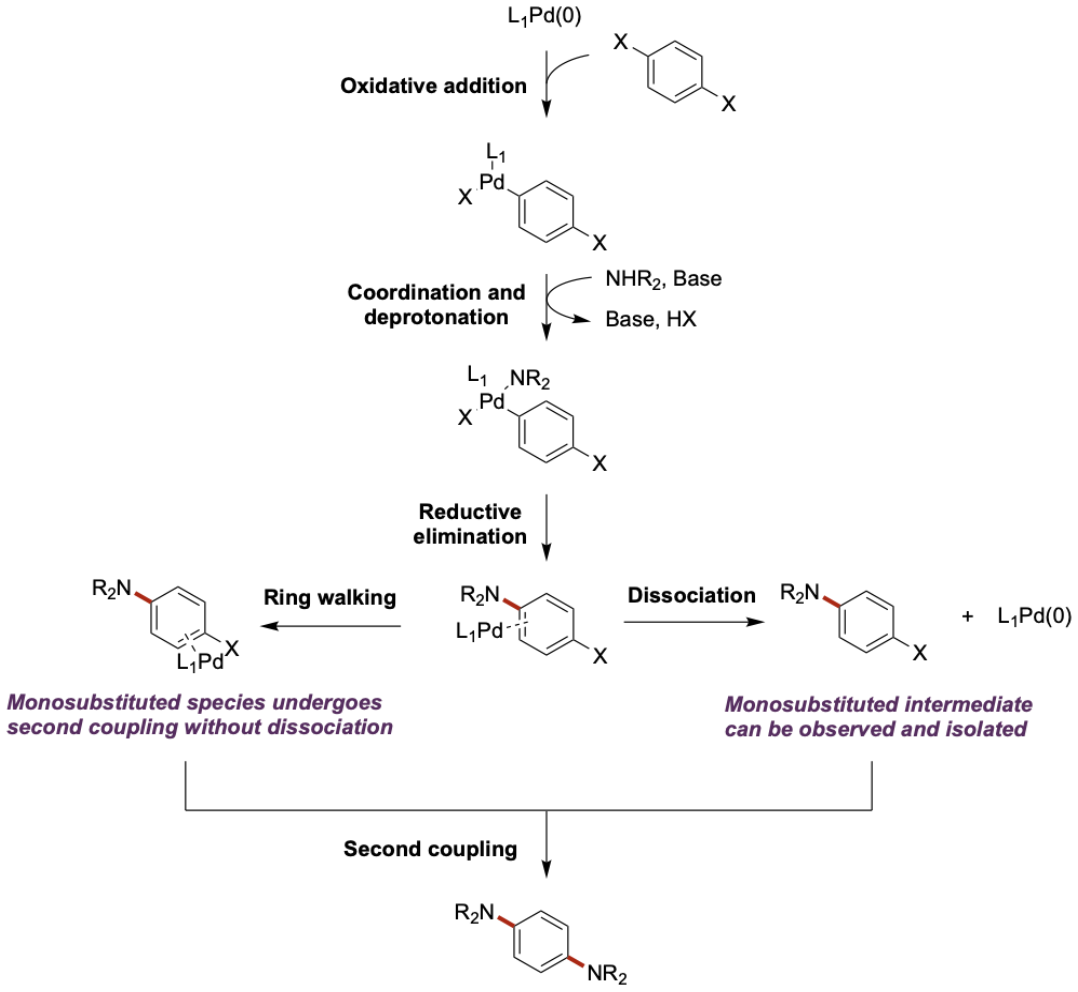


**Figure 7.** Buchwald-Hartwig diamination with ring walking (left path) in which the catalyst remains bound to the pi system in between the first and second coupling, and without ring walking (right path) in which the monosubsituted intermediate dissociates from the catalyst prior to undergoing a second round of coupling.

## A. EFFECT OF CATALYST LIGAND ON THE PRODUCTION OF INTERMEDIATES

BHA were performed for various catalyst/ligand pairs. The $Pd(OAc)_2$/ P(*t*-Bu)$_3$ system was studied first because it is often the catalyst and ligand of choice for SpiroOMeTAD synthesis. The time course measurements acquired using the DirectInject-LC™ technology revealed the presence of just one intermediate (Figure 8a), which was fully characterized as the disubstituted intermediate with amine groups on the same side of the Spiro core. The presence of this single intermediate provided strong evidence for the ring walking mechanism. Kinetic analysis of the reaction was performed to obtain information about the resting state of the catalyst and turnover-limiting step.

To represent a bidentate phosphine system, XantPhos was selected as the ligand. In contrast to the results of the P(*t*-Bu)$_3$ ligand, four intermediates were revealed by the time course measurements (Figure 8b), and this observation strongly supports the inhibition of the ring walking in this system. As a result of the difference observed for the effect of monodentate and

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bidentate phosphines on ring walking, dialkylbiarylphosphines ligands were next chosen for investigation, since they are frequently used in BHA reactions. The time course measurements revealed just one intermediate and in spite of the weak chelating nature of this ligand, a ring walking mechanism is likely for RuPhos ligand. Lastly, N-heterocyclic carbenes (NHC) were investigated, and PEPPSI-IPr was chosen as the ligand since the compound is commercially available and frequently used in BHA reactions. The time course data revealed a similar intermediate profile as was observed for the P(*t*-Bu)3 and RuPhos ligands, again supporting ring walking behavior.

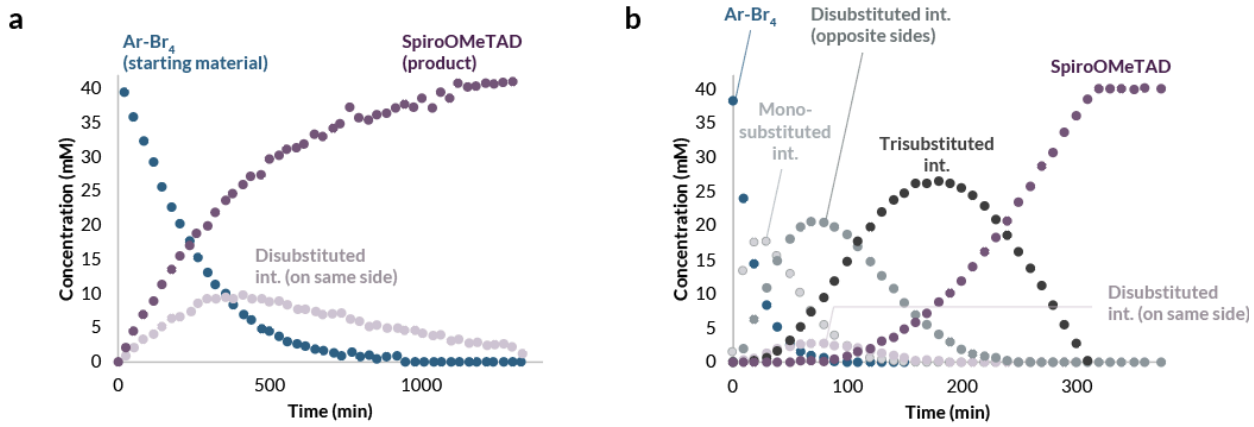


**Figure 8**. DirectInject-LC™ reaction profile data for SpiroOMeTAD synthesis through BHA with (a) $Pd(OAc)_2$/P(*t*-Bu)$_3$ and (b)XantPhos Pd G4.[3]

## B. KINETIC ANALYSIS AND MODELING

A thorough analysis of the kinetic data gives further insight into the ring walking behavior. When comparing RuPhos and XantPhos data, both show turnover-limiting reductive elimination, yet only the RuPhos demonstrated ring walking. Thus, the tendency to ring-walk does not appear to be related to the resting state of the catalyst, but rather to the denticity of the ligand. To gain further insight about the distribution of intermediates, COPASI modeling was employed on the time course profile for each ligand. For a simple model for P(*t*-Bu)$_3$ data, in which starting material and catalyst are converted directly to product, the modeled and the fitted data were not in agreement. Through further investigation it was determined that enhancing the model to allow for diffusion-controlled coupling behavior improved the agreement and this implied that both ring-walking and diffusion-controlled coupling may be present for this ligand.

## C. ACCESSING ASYMMETRIC DERIVATIVES OF SpiroOMeTAD

The information and understanding acquired in this study enabled the synthesis of a series of asymmetric SpiroOMeTAD derivatives. Due to its lack of diffusion-controlled coupling behavior, PEPPSI-IPr ligand was used to access the disubstituted intermediate with amine groups on the same side of the Sprio core. This compound was the derivatized via a Suzuki-Miyaura cross coupling to synthesize a series of four asymmetric SpiroOMeTAD derivatives.

[3] See *Nat Commun.* **2022**, 13, 2869 for original data and figures.

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# 3. SOLID-LIQUID SLURRIES | OPTIMIZED SYNTHESIS OF TETRABENAZINE

See: Sato, Y.; Liu, J.; Kukor, A.J.; Culhane, J.C.; Tucker, J.L.; Kucera, D.J.; Cochran, B.M.; Hein, J.E. "Real-Time Monitoring of Solid–Liquid Slurries: Optimized Synthesis of Tetrabenazine." *J. Org. Chem.* **2021**, 86(*20*), p. 14069–14078.

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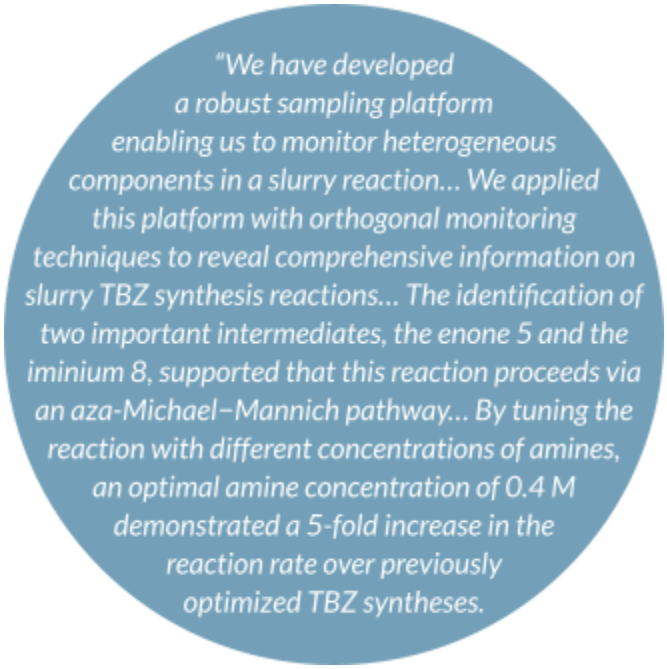
| **SCIENTIFIC & INDUSTRIAL IMPORTANCE** | Solid-liquid slurries are crucial in process chemistry scale-up, providing high reaction rates due to saturation and economized solvent volumes. |
| **ANALYTICAL CHALLENGES** | Clogs occur, dissolution must occur in real-time to avoid post-sampling decomposition, and dissolution causes changes in sample volumes, impacting kinetic analysis. |
| **DIRECTINJECT-LC™ SOLUTION** | Real-time reaction monitoring was achieved using an in-line dynamic mixer between the sampling probe and LC injection valve. Volume changes were calibrated for slurry dissolution. Combined with orthogonal PAT, the mechanism of tetrabenazine synthesis was determined. Mechanistic insights resulted in 5-fold increase in reaction rates |

Chemical and pharmaceutical processes frequently feature solid-liquid heterogeneous systems; the capability for in-depth characterization and measurement of slurry process batch reactions is critical. Analyzing these systems is a challenge due to the need to accurately sample and dissolve the heterogeneous mixture, as well as physical issues such as clogging. Regardless of these issues, real-time HPLC analysis measurements could bypass typical off-line analysis problems such as the occurrence of post-sampling reactions or the loss of transient intermediates. Herein, the DirectInject-LC™ technology platform is adapted to enable reaction monitoring and true time course measurement of these dual-phase systems, employing methodology that addresses the efficient sampling and dissolution of heterogeneous components.

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The synthesis of tetrabenazine (TBZ) was selected to test the automated platform. (±)-Tetrabenazine is synthesized by formal annulation between 3,4-dihydroisoquinoline and β-amino ketone (Figure 9). TBZ is quite insoluble in currently optimized reaction solvents, however, which results in a heterogeneous reaction mixture that is difficult to address with typical reaction monitoring methods. Though the industrial process for TBZ is established, a detailed mechanistic investigation had not been reported. Mechanisms have been proposed such as Mannich–aza-Michael and an aza-Michael–Mannich pathways, or elimination from the β-amino ketone to generate an enone and dimethylamine, preceding a 4 + 2 cycloaddition.

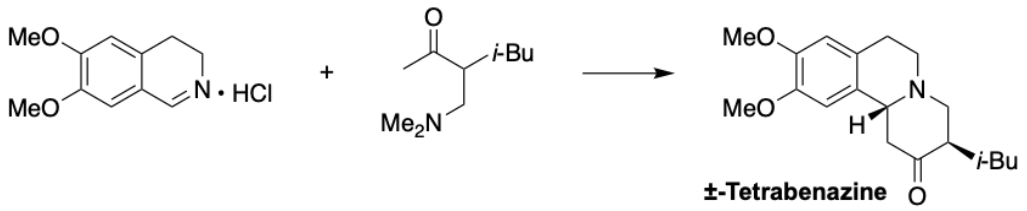


**Figure 9.** (a) Standard synthesis of racemic TBZ.

To access mechanistic understanding, DirectInject-LC™ was applied to address several areas of interest: slurry reaction composition analysis; the role of pH in the reaction, since Michael reactions can be either acid or base catalyzed; and real-time crystallization information such as morphology, size, nucleation etc. To acquire the data for this study, an automated reaction platform was set up involving DirectInject-LC™ (which includes an online sampling probe), a Mettler-Toledo EasyViewer, and a pH probe (Figure 12) . Following DirectInject-LC™ protocol, the sampling probe takes a slurry sample from the reaction mixture for presentation to the HPLC. An in-line mixer was installed between the reaction sampler and the LC injection valve to aid in homogenizing the slurry and minimizing clogging to ensure accurate measurement of reaction species. The EasyViewer provided real-time video imaging of the particles in the reaction.

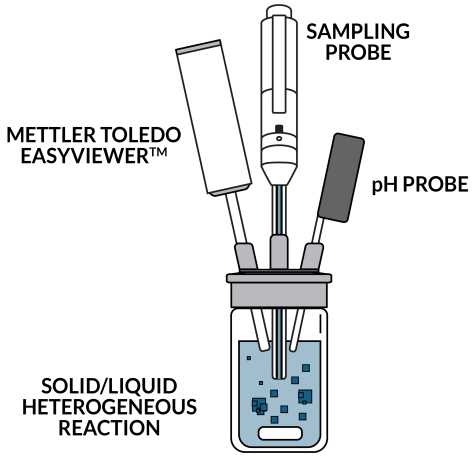


**Figure 12**. Reaction and analysis setup for the solid-liquid heterogeneous synthesis of tetrabenazine.

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## MONITORING THE REACTION VIA DirectInject-LC™

An isoquinoline salt was reacted with a β-amino ketone using NaI in iPrOH/ $H_2O$ and monitored by DirectInject-LC™ (Figure 13). Three new species were identified including the major product TBZ, a minor product diastereomer, and an enone intermediate. The presence of the latter species is proof the reaction proceeds by elimination of dimethylamine, as opposed to direct C−C bond formation via a Mannich-like coupling involving a substrate enolate.

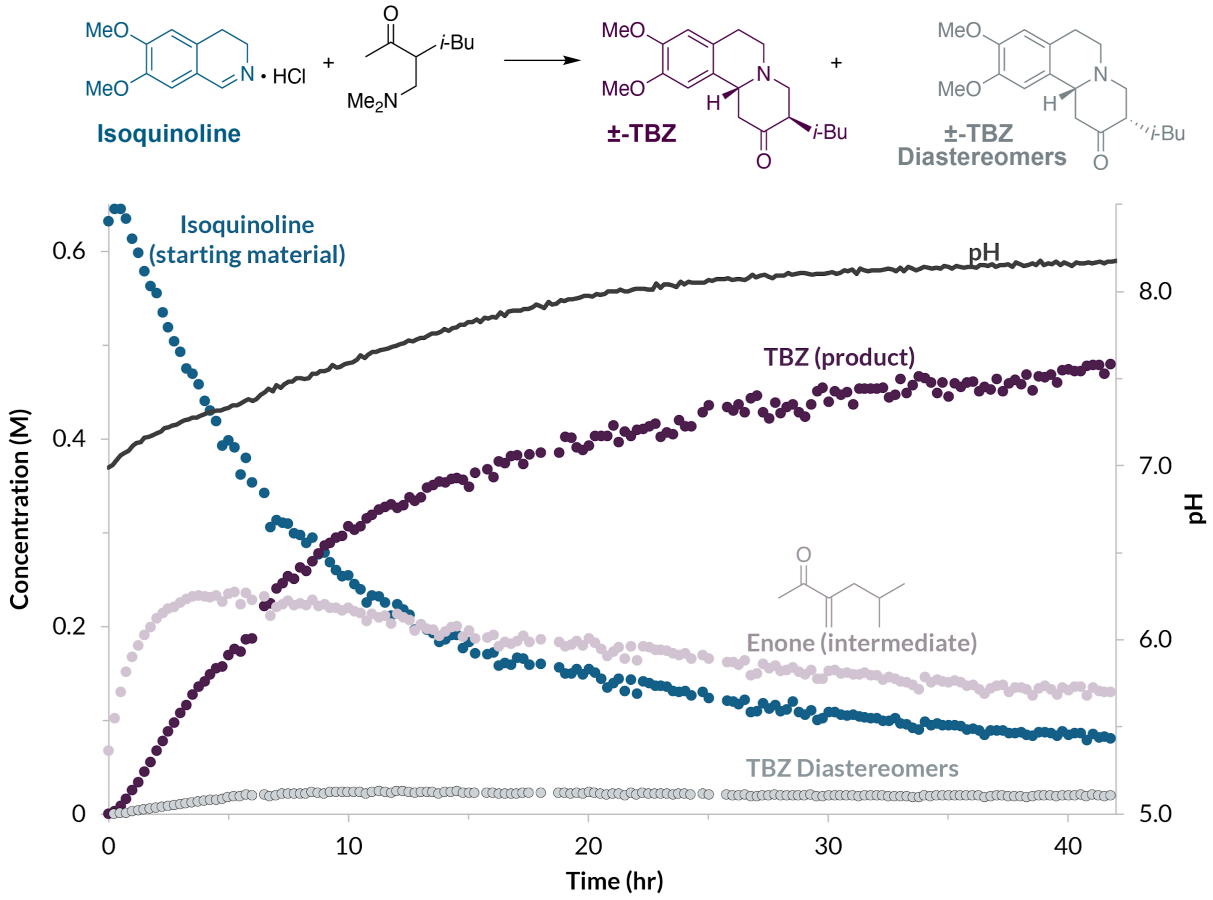


**Figure 13.** DirectInject-LC™ time-course reaction profile and pH data for solid-liquid heterogeneous synthesis of tetrabenazine.[4]

## MECHANISTIC INSIGHT

DirectInject-LC time-course data and kinetic analysis revealed that the enone intermediate has a positive order, but there were deviations from first-order behavior, and that the kinetic order was influenced by pH of the system. In further experiments a new intermediate was observed that was identified as an iminium. This insight suggested that TBZ likely synthesis proceeds by a sequential aza-Michael addition, followed by Mannich cyclization. Further examination indicated that the rate-determining step of the reaction is likely the aza-Michael addition

[4] See *J. Org. Chem.* **2021**, 86(*20*), p. 14069–14078 for original data and figures.

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## EPIMERIZATION AND CRYSTALLIZATION MONITORING

A Mettler-Toledo EasyViewer was used to measure the physical properties of the reaction mixture such as dissolution, primary crystallization, and crystal growth. EasyViewer data indicated that primary crystallization occurred when the TBZ concentration reached approximately 0.25 M (as seen by DirectInject-LC™) and that there was a relationship between the crystallization and the ratio of TBZ to its diastereomers.

Further it was found that this ratio could be affected by kinetic control of the Mannich cyclization step, epimerization between TBZ and TBZ diastereomers, and crystallization of TBZ. The kinetically obtained TBZ/diastereomer ratio decreased the epimerization process. This process required time to reach equilibrium and was significantly suppressed by crystallization events, indicating that crystallization at an early reaction time can increase the ratio of TBZ to its diastereomers (Figure 15).

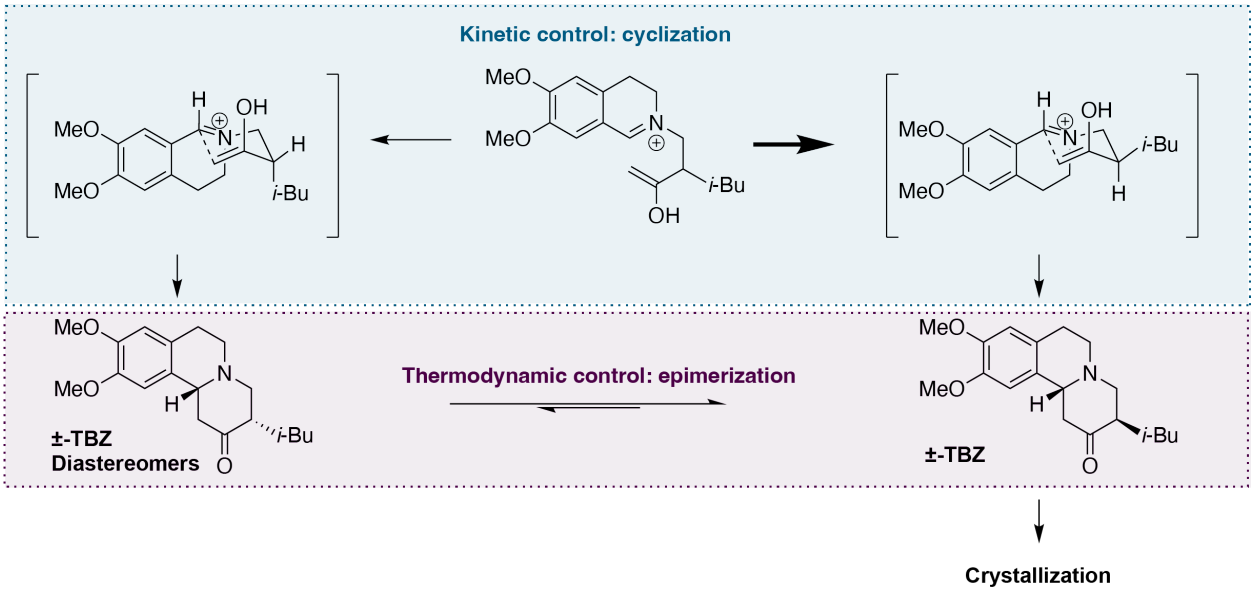


**Figure 15.** Proposed kinetic control of the Mannich cyclization and thermodynamic epimerization mechanisms.

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# 4. **CONTINUOUS CRYSTALLIZATION** | ENANTIOSELECTIVE SYNTHESIS

See: Kukor, A.J.; Depner, N.; Cai, I.; Tucker, J.L.; Culhane, J.C.; Hein, J.E. "Enantioselective synthesis of (−)-tetrabenazine via continuous crystallization-induced diastereomer transformation." *Chem. Sci.*, **2022**, 13, p. 10765-10772.

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| **SCIENTIFIC & INDUSTRIAL IMPORTANCE** | Crystallization-induced diastereomer transformations (CIDT) provide access to valuable, enantiopure compounds (e.g. Tetrabenazine, TBZ). Continuous CIDT can increase yields & facilitate product isolation if it can be carefully analyzed and controlled. |
| **ANALYTICAL CHALLENGES** | Solid-liquid heterogeneous system is challenging to analyze. There are 5 dynamic chemical equilibria at play. |
| **DIRECTINJECT-LC™ SOLUTION** | Solution-phase sampling with filter frit with DirectInject-LC™ was coupled to chiral chromatography. System enabled control over continuous crystallization for large yields of enantiopure (−)-TBZ·(−)-CSA. |

*"...we have demonstrated the control over continuous crystallization afforded by physically separating the processes of racemization, dissolution and crystallization. We highlighted the utility of monitoring such processes with PATs to inform rational experimental design and real-time control of processes, using two novel PATs to track solid and solution phase behaviour of a continuous CIDT. With this data, we were able to selectively crystallize large quantities of enantiopure (−)-TBZ·(−)-CSA..."*

Crystallization-induced diastereomer transformations (CIDTs) are used to access enantiopure crystals from interconverting stereoisomers in solution, and methods involving one pot or two pot procedures are often utilized. Both methodologies have specific issues that can cause suboptimal performance. For example, because racemization and crystallization require inherently different conditions, the one-pot method may negatively affect crystallization yield. The two-pot method is less useful for multicomponent systems where multiple solid phases are possible. This work describes a new approach with separate dissolution and crystallization vessels that are connected via inline racemization. This work utilizes DirectInject-LC™ to develop and analyze a CIDT methodology for the production of enantiopure tetrabenazine salt, (−)-TBZ·(−)-CSA.

## FINE CONTROL OVER CIDT VIA IN-LINE RACEMIZATION

Under continuous operation, one dissolver and one crystallization container allows precise control over crystallization (Figure 16). In this system, the solubilities and therefore solute concentrations

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in each vessel are controlled by temperature. Racemic TBZ is added to the dissolver flask with enantiopure camphorsulfonic acid (CSA) to form diastereomeric salts. The salts racemize inline between the dissolver flask and the crystallizer, where the enantiopure salt is harvested. The separate crystallizer and dissolver flasks enable additional crystallization control *via* seeding and ensure that undesired solids (ie., racemic starting material) are not in the crystallizer.

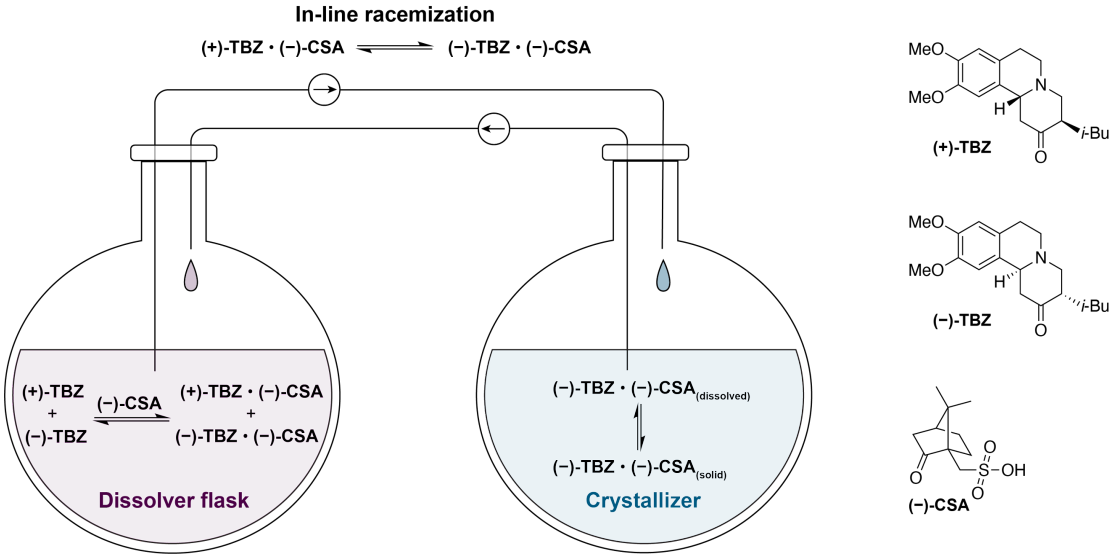


**Figure 16.** Continuous CIDT setups for synthesis of a tetrabenazine-camphorsulfonic acid salt.

Successful application of continuous crystallization requires fine-tuned control and optimization. This is especially true when multiple solid phases are possibly present that have similar solubilities as the target enantiopure crystals. Thus, the ability to monitor continuous crystallizations via real-time analysis is extremely valuable to enable actionable decisions by measuring slight differences in solution and solids composition. To accommodate real-time monitoring and mitigate the challenges of sampling crystallizations, a filter frit device was developed that attaches to a Mettler Toledo EasySampler probe. This modification to the probe eliminates clogging or probe fouling, enabling sampling of the solution phase. This facilitates solution-phase equilibria and solid phase composition to be determined by DirectInject-LC™ analysis. When chiral chromatography is used for analyzing a CIDT, the modification allows simultaneous calculation of solid phase yield and enantiomeric excess (e.e.). In addition to the use of the modified EasySampler-based system described above, other PAT tools can be used for online measurements for both solid and solution phases. On-line turbidity devices and *in-situ* microscopic imaging via EasyViewer can be added to the system, providing further analytical handles to study and control the crystallization.

## IDENTIFYING RUN CONDITIONS

By adding an additional 1.0 equiv. of (−)-CSA to the (−)-TBZ and (−)-CSA solution and then passing the solution through the flow system at 100 °C, racemization increased significantly without TBZ decomposition. Thus, these conditions were selected for optimal racemization in the continuous CIDT process. For a useful CIDT process, the target solid phase must be the only polymorph formed and consist of a single enantiomer. The TBZ system proved to be complex with regard to enantiomers formed and a developing a detailed understanding of solubility curves was required.

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It was determined that high TBZ concentrations tend to crystallize two TBZ enantiomers, and that CIDT conditions must be carefully controlled to ensure enantiopure solid formation.

Once it was determined that crystallization of the (−)-TBZ·(−)-CSA diastereomeric salt could be controlled in the presence of (+)-TBZ, inline racemization was added to convert undesired (+)-TBZ into the desired enantiomer. (−)-TBZ·(−)-CSA seed crystals were added to the crystallizer and DirectInject-LC™ measurements indicated successful control of the crystallization by seeding. As the CIDT continued and (+)-TBZ-enriched solution flowed through the racemizer, the (+)-TBZ concentration decreased over time, indicating that the undesired (+)-TBZ was being racemized into (−)-TBZ.

## DEMONSTRATING CONTINUOUS CIDT TO ACCESS 18g OF ENANTIOPURE PRODUCTS

Finally, a continuous CIDT was used to produce enantiopure (−)-TBZ·(−)-CSA as racemic starting material was fed into the dissolver flask. Higher temperatures were selected for both the dissolver and the crystallizer in order to increase the rate of racemization, however the concentration of (+)-TBZ elevated temperatures was too high for enantiopure (−)-TBZ·(−)-CSA to form. It was determined that by reducing the amount of solid doses of the starting material to the dissolver, the undesired nucleation of solids nucleation was minimized. This controlled dosing was made possible by real-time, on-line HPLC concentration data, which was indicative of rate of solution transfer (Figure 17). The three well CIDT system was run continually over a 2 week period and produced 18.39 g of enantiopure (−)-TBZ·(−)-CSA with >98% e.e. If necessary, optimizing conditions for increasing the rate of racemization could be developed to accelerate process speed.

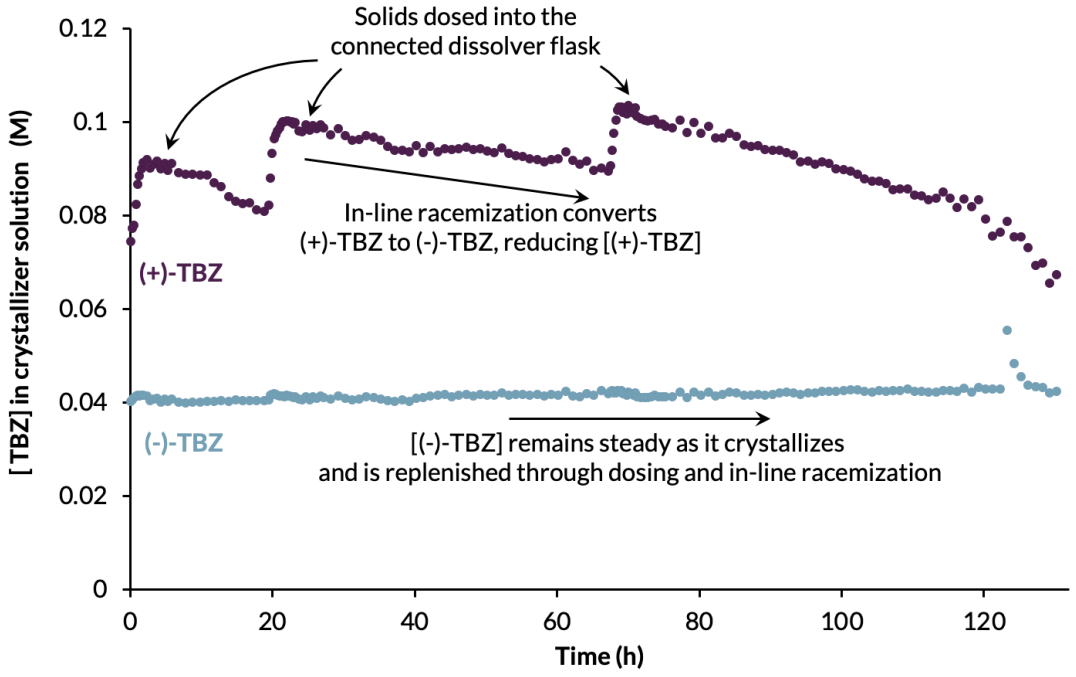


**Figure 17.** Continuous CIDT monitored by DirectInject-LC™. [5]

[5] See *Chem. Sci.*, **2022**, 13, p. 10765-10772 for original data and figures.

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# FURTHER READING

## DirectInject-LC™ TECHNOLOGY PLATFORM DEVELOPMENT

Malig, T. C.; Koenig, J. D. B.; Situ, H.; Chehal, N. K.; Hultin, P. G.; Hein, J. E. "Real-Time HPLC-MS reaction progress monitoring using an automated analytical platform." *React. Chem. Eng.* **2017**, 2(3), p. 309-314.

> "An automated reaction monitoring system capable of unattended sample aliquoting and dilution, as well as immediate quantification and identification of reaction components *via* HPLC-MS has been developed. The device allows for facile reaction progress analysis, enabling mechanistic studies and serving as a primary process analytical technology (PAT) for reaction monitoring…progress curves have been acquired for…a Cu(I)-catalysed azide-alkyne cycloaddition…a Suzuki cross-coupling reaction…and a complex multicomponent cascade reaction that generates multiple regioisomers…".

Malig, T. C.; Yunker, L. P. E.; Steiner, S.; Hein, J. E. "Online HPLC analysis of Buchwald-Hartwig aminations from within an inert environment." *ACS Cat.* **2020**, 10, p. 13236-13244.

> "…a reaction monitoring platform capable of automated sampling and online HPLC analysis to generate temporal profiles for reactions performed from within a glovebox. The device allows for facile reaction progress analysis to aid in mechanistic studies of air-sensitive chemical transformations…We employed the sampling platform to acquire temporal profiles for a series of Buchwald-Hartwig aminations."

Sato, Y.; Liu, J.; Kukor, A.; Culhane, J.; Tucker, J.; Kucera, D.; Cochran, B.; Hein, J.E. "Real-Time Monitoring of Solid-Liquid Slurries: Optimized Synthesis of Tetrabenazine." *J. Org. Chem.* **2021**, 86(20), p. 14069–14078.

> "…an online HPLC monitoring platform enabling access to real-time compositional information on slurries. We demonstrate the system by investigating the heterogeneous synthesis reaction of tetrabenazine."

Kukor, A. J.; Guy, M. A.; Hawkins, J. M.; Hein, J. E. "A robust new tool for online solution-phase sampling of crystallizations." *React. Chem. Eng.* **2021**, 6(11), p. 2042-2049.

> "…a dynamically flushed *in situ* filtration device that attaches to the tip of Mettler-Toledo's EasySampler probe and makes use of its mechanical motion to avoid surface fouling. Filter functionality was tested under both increasing saturation…as well as decreasing saturation...The utility of the filter tip was highlighted by monitoring the classical resolution of TBZ, an important drug precursor."

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# ADDITIONAL APPLICATIONS OF DirectInject-LC™

Wilkerson-Hill, S.M.; Yu, D.; Painter, P.P.; Fisher, E.L.; Tantillo, D.J.; Sarpong, R.; Hein. J.E. "Mechanism of a No-Metal-Added Heterocycloisomerization of Alkynylcyclopropylhydrazones: Synthesis of Cycloheptane-Fused Aminopyrroles Facilitated by Copper Salts at Trace Loadings." *JACS*, 2017, 139(*30*), p. 10569-10577

> "A mechanistic study of a new heterocycloisomerization reaction that forms annulated aminopyrroles is presented. Density functional theory calculations and kinetic studies suggest the reaction is catalyzed by trace copper salts and that a Z- to E-hydrazone isomerization occurs through an enehydrazine intermediate before the rate-determining cyclization of the hydrazone onto the alkyne group…A new automated sampling technique was developed to obtain robust mechanistic data."

Rougeot C.; Situ, H.; Cao, B.H.; Vlachos, V.; Hein, J.E. "Automated reaction progress monitoring of heterogeneous reactions: crystallization-induced stereoselectivity in amine-catalyzed aldol reactions." *React. Chem. Eng.*, **2017**, 2, p. 226-231

> "A prototype automated system has been developed, which is capable of acquiring accurate kinetic reaction profiles from heterogeneous reactions. The device is capable of monitoring the composition and concentration of either the dissolved components (solution phase) or slurry (solid and solution phases) in parallel. This prototype was used to study the diastereo- and enantioselectivity of the aldol reaction between 4-*tert*-butyl cyclohexanone and *p*-nitrobenzaldehyde, catalyzed by either pyrrolidine or L-proline."

Chung, R.; Vo, A.; Fokin, V.V.; Hein, J.E. "Catalyst Activation, Chemoselectivity, and Reaction Rate Controlled by the Counterion in the Cu(I)-Catalyzed Cycloaddition between Azide and Terminal or 1-Iodoalkynes." *ACS Catal.* **2018**, 8(*9*), p. 7889–7897

> "A comprehensive mechanistic analysis of the copper-catalyzed azide–alkyne cycloaddition to form 5-protio-1,2,3-triazoles (from terminal alkynes) or 5-iodo-1,2,3-triazoles (from 1-iodoalkynes) is presented. Through various mechanistic probes, we elucidate several salient features… An expanded reaction manifold is offered to provide the most comprehensive image to date of the different copper-catalyzed processes active during triazole synthesis"

Christensen, M.; Adedeji, F.; Grosser, S.; Zawatzky, K.; Ji, Y.; Liu, J.; Jurica, J.A.; Naber, J.R.; Hein, J.E. "Development of an automated kinetic profiling system with online HPLC for reaction Optimization." *React. Chem. Eng.*, **2019**, 4, p. 1555-1558

> "In an attempt to optimize a palladium-catalyzed Suzuki cross-coupling reaction, automated kinetic profiling was utilized with offline liquid chromatography to monitor reaction progress… Upon uncovering analytical sample instability issues, an online HPLC capability was developed… this capability resulted in the observation that precatalyst activation was a key factor influencing the reaction rate."

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Daponte, J.A.; Guo, Y.; Ruck, R.T.; Hein, J.E. "Using an Automated Monitoring Platform for Investigations of Biphasic Reactions." *ACS Catal.* **2019**, 9(*12*), p. 11484–11491

"…development and application of an automated monitoring platform capable of delineating time course reaction progress of biphasic reactions. The system was applied toward a case study of an enantioselective biphasic spirocyclization catalyzed by a doubly quaternized cinchona alkaloid. A combination of heterogeneous and phase-selective sampling, coupled with high-performance liquid chromatography–mass spectrometry (HPLC–MS), allowed for the detection, quantification, and identification of reactive species in either the heterogeneous liquid–liquid mixture or isolated organic phase throughout the reaction."

Chung, R.; Hein, J.E. "Automated solubility and crystallization analysis of non-UV active compounds: integration of evaporative light scattering detection (ELSD) and robotic sampling." *React. Chem. Eng.* 2019, 4(*9*), p. 1674-1681

"…the integration of evaporative light scattering detection (ELSD) with automated robotic sampling to obtain reliable and data-rich solubility and crystallization profiles of minimally- or non-UV active compounds. The new technology allowed for the thermodynamic solubilities of various compounds to be profiled over broad temperature ranges without experimenter intervention. Two case studies are presented that illustrate the ability of the automated system to furnish solution phase composition of systems actively undergoing crystallization…"

Malig, T.C.; Tan, Y.; Wisniewski, S.R.; Higman, C.S.; Carrasquillo-Flores, R.; Ortiz, A.; Purdum, G.E.; Kolotuchin, S.; Hein, J.E. "Development of a telescoped synthesis of 4-(1H)-cyanoimidazole core accelerated by orthogonal reaction monitoring." *React. Chem. Eng.* **2020**, 5(*8*), p. 1421-1428.

"…a convenient two-step, one-pot method for the facile synthesis of cyanoimidazoles. By integrating a suite of reaction-monitoring techniques, we were able to interrogate each transformation independently. We observed that formation of a key hemiaminal intermediate is complicated *via* many equilibrium processes, creating oligomers and eventually resulting in unproductive dimerization…By leveraging kinetic information gathered from each step independently, we report reaction conditions to achieve high yields of the cyanoimidazole from carbonyl-containing substrate directly in one pot."

Forget, S.M.; Xia, F.; Hein, J.E.; Brumer, H. "Determination of biocatalytic parameters of a copper radical oxidase using real-time reaction progress monitoring." *Org. Biomol. Chem.*, **2020**,18, 2076-2084.

"An Auxiliary Activity Family 5 (AA5) copper-radical alcohol oxidase (AlcOx) with promiscuous activity towards simple alkyl and aromatic alcohols was evaluated using real-time reaction progress monitoring. Reaction kinetics using variable time normalization analysis (VTNA) were determined from reaction progress curves…a detailed view of the entire reaction time course under various conditions was obtained and used to identify parameters that will inform further process optimization development."

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Christensen, M.; Yunker, L.P.E.; Shiri, P.; Zepel, T.; Prieto, P.L.; Grunert, S.; Bork, F.; Hein, J.E. "Automation isn't automatic." *React. Chem. Eng.*, **2021**, 6, p. 1497-1507.

"Recent advances in robotics and computer science have led to the emergence of automated systems that execute common laboratory procedures including parallel synthesis, reaction discovery, reaction optimization, time course studies, and crystallization development… This perspective provides an overview of the current state of automation of synthetic chemistry at the benchtop scale with a particular emphasis on core considerations and the ensuing challenges of deploying a system."

Kukor, A.J.; Depner, N.; Cai, I; Tucker, J.; Culhane, J.C.; Hein, J.E. "Enantioselective synthesis of (−)-tetrabenazine via continuous crystallization-induced diastereomer transformation." *Chem. Sci.*, **2022**, 13, p. 10765-10772.

"A multi-well continuous CIDT approach with inline racemization of the solution phase is presented. Using two in-house built PATs and a flow reactor, we were able to successfully crystallize an enantiopure salt of TBZ… Despite discovering an undesired racemic solid phase, inline racemization combined with careful control of crystallization conditions allowed for multigram quantities of enantiopure material to be harvested using our setup. Critically, this control was made possible by the use of PATs to observe and quantify the composition of both the solid and solution phases.

Deem, M.C.; Hein, J.E. "A Method for Converting HPLC Peak Area from Online Reaction Monitoring to Concentration Using Nonlinear Regression." *J. Org. Chem.*, **2023**, 88(*2*), 1292–1297.

"Online HPLC reaction progress monitoring provides detailed data-rich profiles; however, extracting kinetic information requires ultraviolet–visible response factors to determine concentrations from peak areas. If the reaction's overall mass balance is known and some analytical trend for all relevant species can be recorded, it is possible to estimate the absolute response factors of all species using a system of linear equations."

Deem, M.C.; Cai, I.; Derasp, J.S.; Prieto, P.L.; Sato, Y.; Liu, J.; Kukor, A.J.; Hein, J.E. "Best Practices for the Collection of Robust Time Course Reaction Profiles for Kinetic Studies." *ACS Catal.* **2023**, 13(*2*), p. 1418–1430

"…Modern kinetic analyses such as RPKA and VTNA provide many advantages over traditional initial rate methods and are especially powerful when coupled with reaction monitoring technologies. While these are robust analytical methods, the lack of careful observation and optimization can lead to misinterpretation of the data. In this Perspective, we highlight some commonly overlooked considerations in kinetic studies based on our experiences and present a general guide to proper optimization of reactions and analytics prior to acquiring kinetic data."

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