

Monitoring Chemical Reactions in Real Time with NMR Spectroscopy

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**Entering the forest he moves not the
grass; entering the water he makes
not a ripple.**

Zenrin Kushu



Introduction

- Many analytical techniques (IR, UV, Raman) are used to monitor chemical reactions.
 - For many reactions these techniques show important features of the reaction process and therefore provide a great deal of information to apply to a process.
 - Some features, such as structural isomers and low level impurities are not typically observed.
 - Utilization of NMR provides additional “molecular resolution” at the process development stage to provide many details about the process (e.g. mechanistic details, kinetics of impurity formation, etc...)
- On-line NMR is a very powerful tool for monitoring reaction progression as well as providing mechanistic insight into the synthesis.
- We are exploring ways to add detailed, real-time chemical information to better assess synthetic reactions and develop a better process understanding.
- **This data should be acquired in a non-invasive way to better understand the chemistry.**

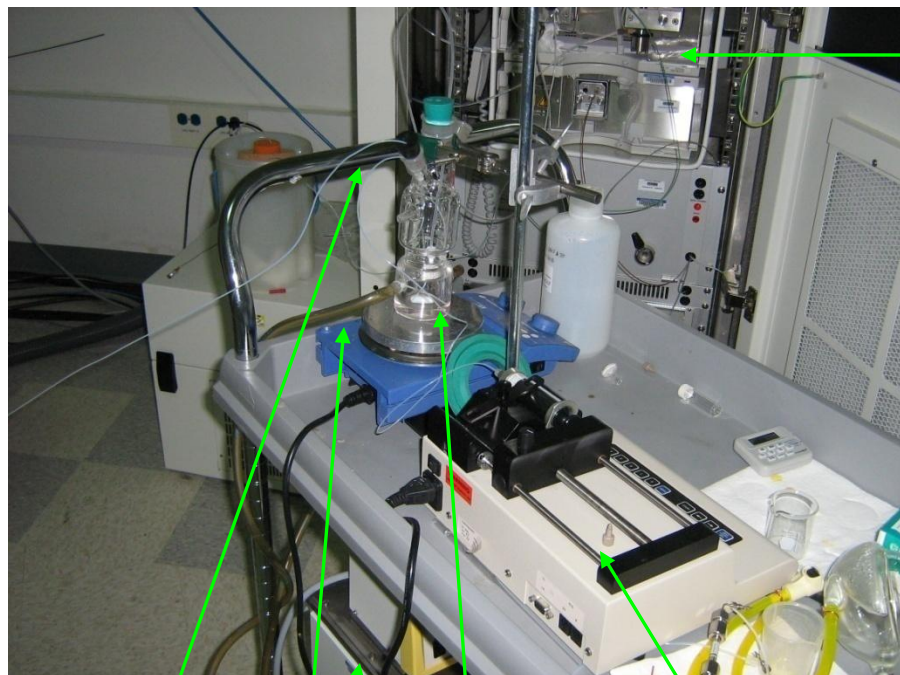


Introduction

Goals

- 👍 **Monitor chemical reactions via NMR Spectroscopy.**
- Develop a system that can provide a real-time reaction monitoring capability with value added information for better process understanding.

Pictures of the “first” System in our lab (~2006)



NMR Probe
Return

Reaction Vessel

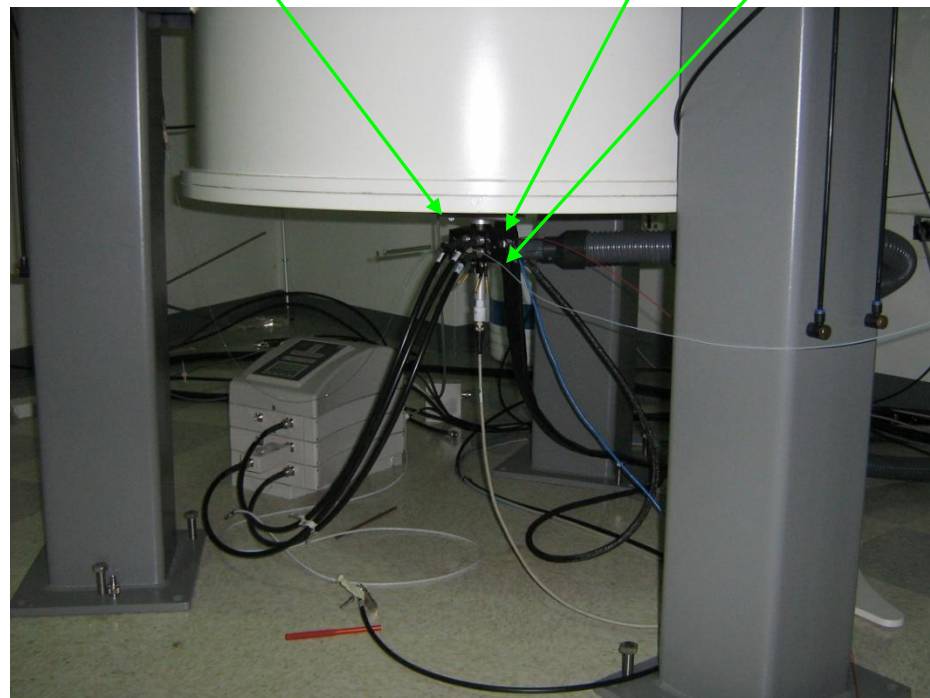
Temperature control

Syringe Pump

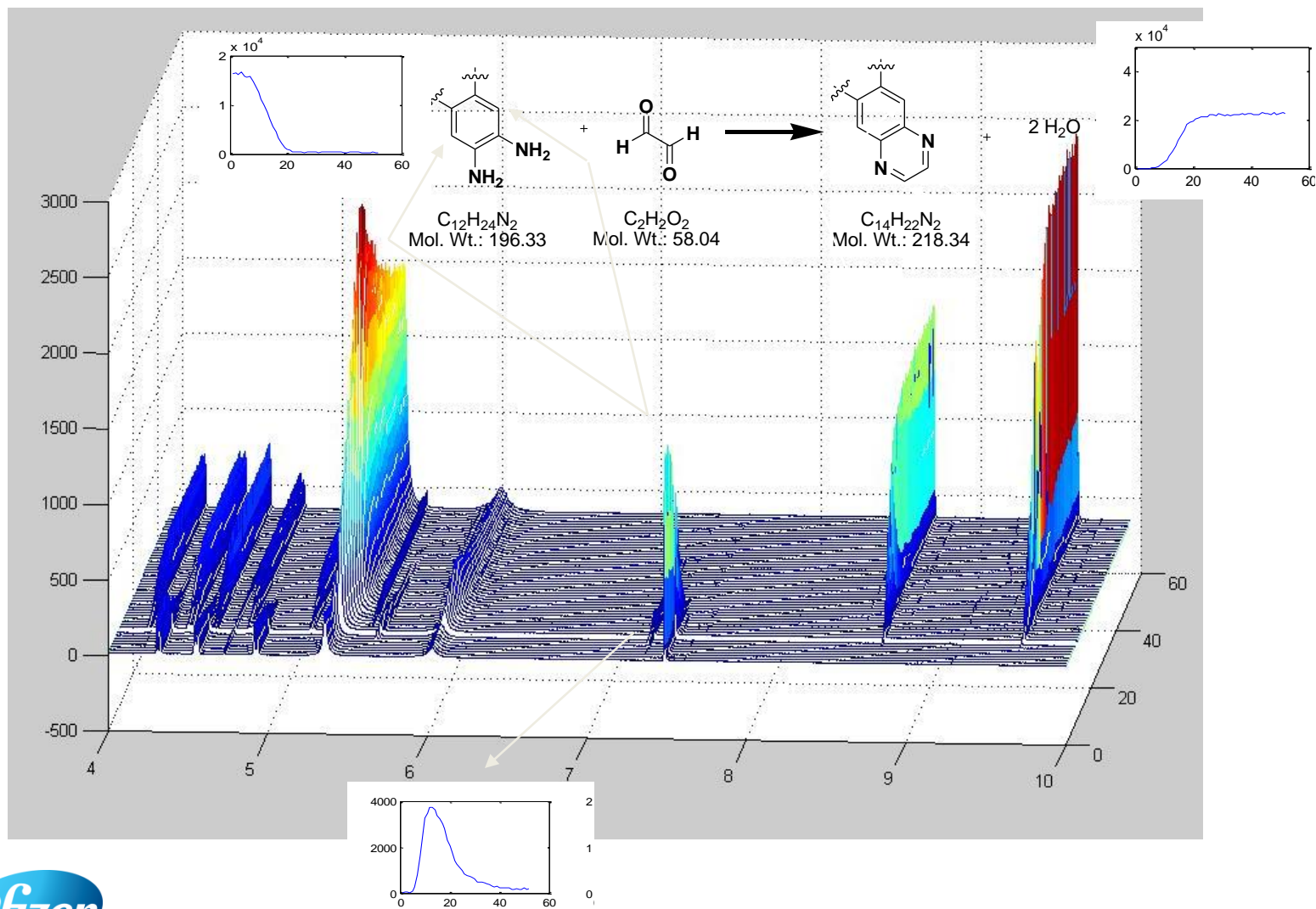
HPLC Pump

NMR Probe

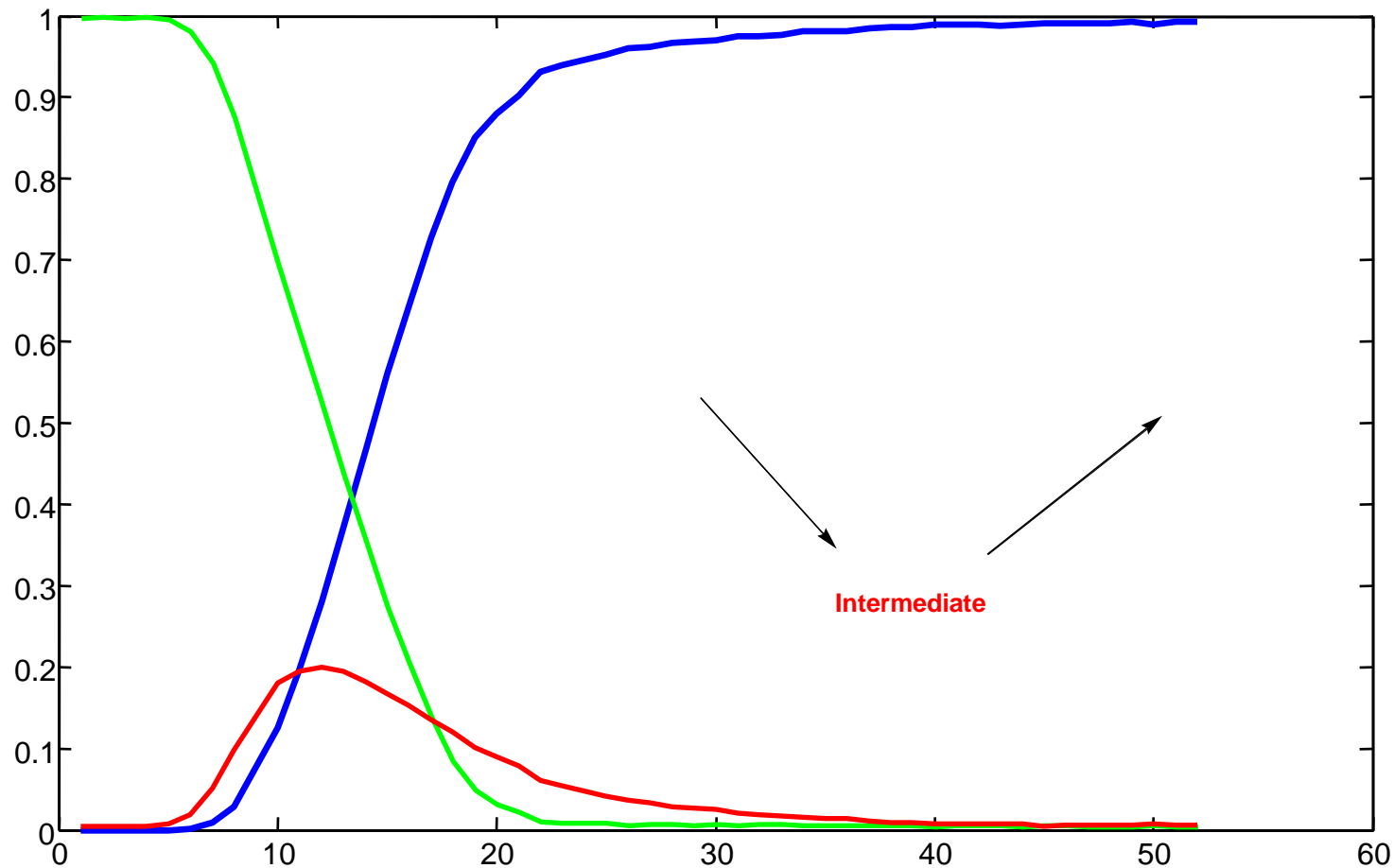
Inlet and outlet



A Real Life Example: Flow NMR Semi-Batch Reaction Profile for Alkylation of a Di-amine with Glyoxal



Reaction Profile -- Intermediates



Introduction

Goals

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Next Generation

- **Limitations with first system (HPLC/NMR flow probe):**
 - Fragile – 200 microliter small bore cell.
 - If (When) flow cell broke had to be sent back for repair
 - Flow rates had to be low to avoid pressure, easily plugged
 - No temperature control to/from cell
 - ^1H observe only
- **New Design in 2010:**
 - 👍 Flow control unit coupled with a flow cell that will fit in a standard NMR probe.
 - 👍 Easy to replace if flow cell is broken or plugged
 - 👍 Temperature control $\sim 0 - 80^\circ \text{C}$ (achieved -10°C to date), $\pm \sim 2^\circ \text{C}$
 - 👍 Goal is to reach $\sim -40^\circ \text{C}$
 - 👍 Standard ^1H - ^{19}F - ^{13}C TXO NMR probe (low gamma probe on order)



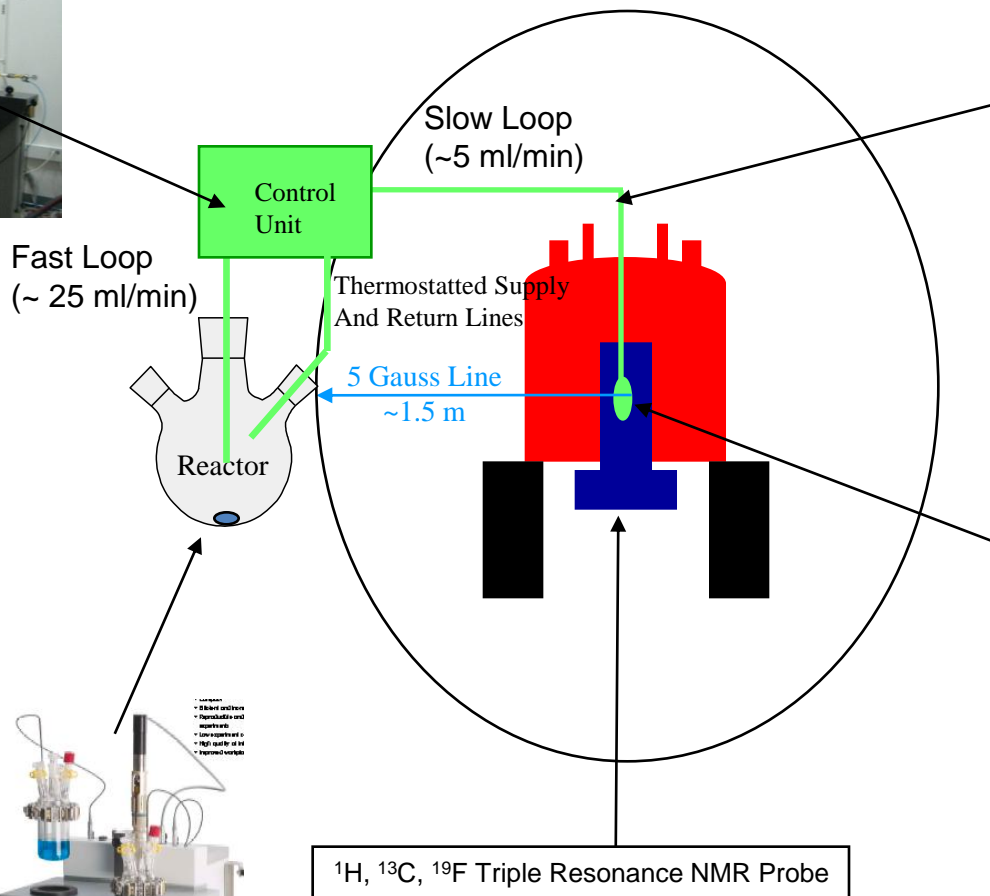
New and Improved!



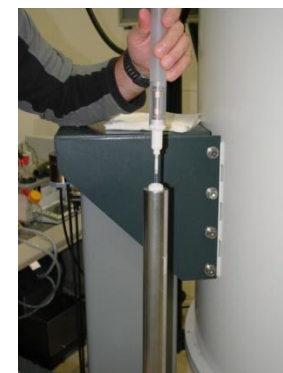
Coming Soon!

ReactIR

Bruker IonTrap MS

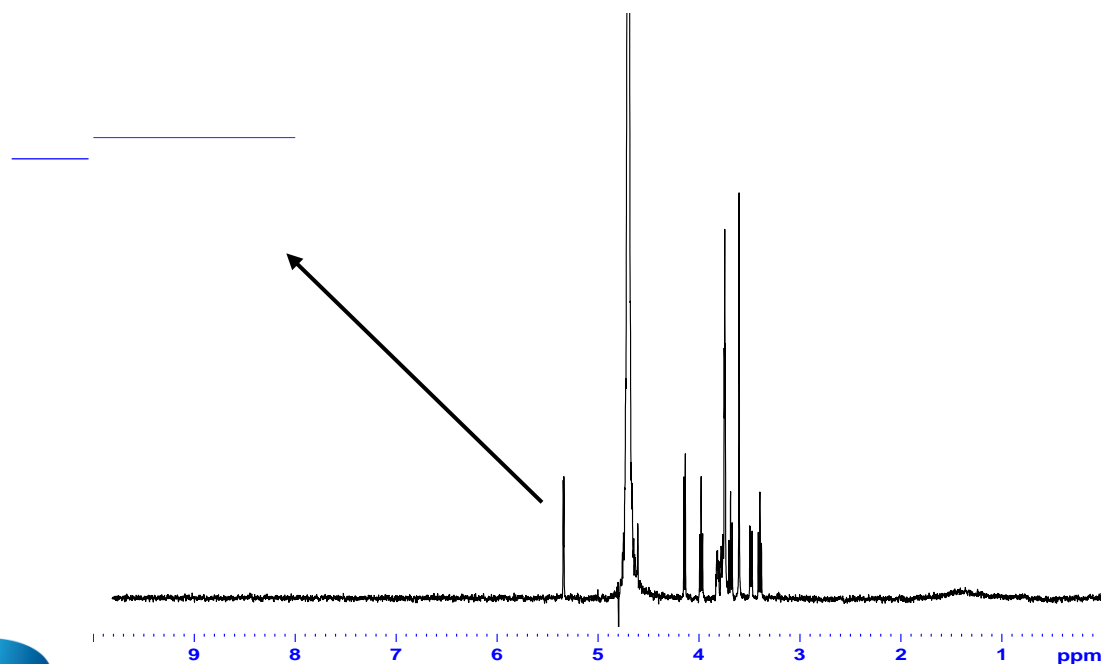


• Shorter analysis
 • Reproducible and
 accurate
 • Low equipment
 • High quality
 • Improved workflow



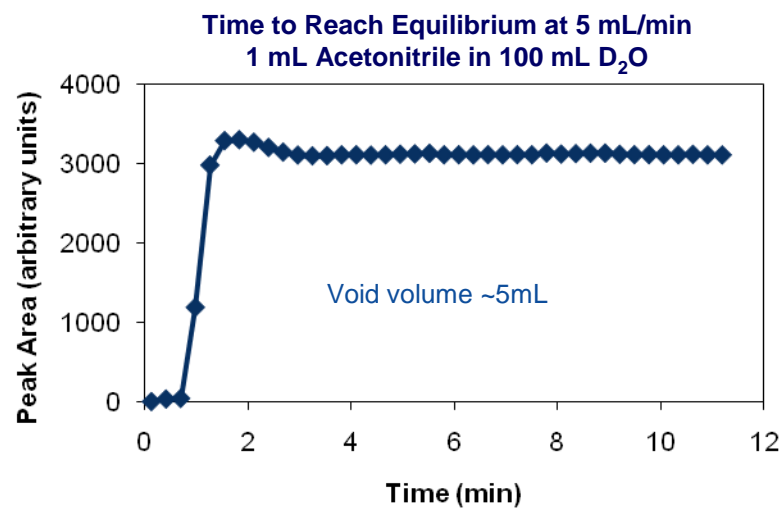
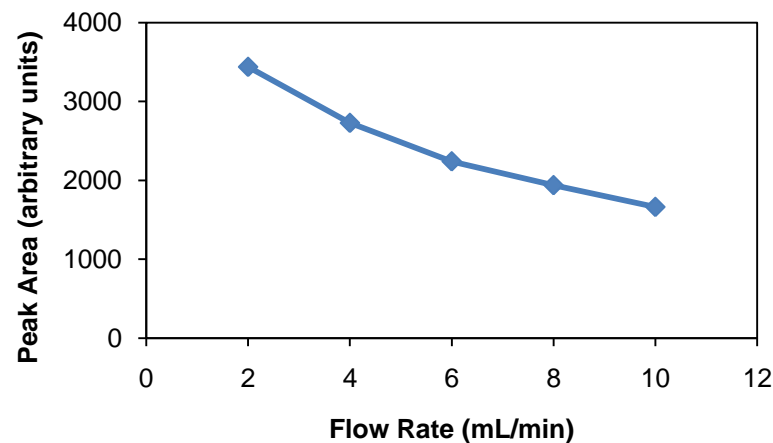
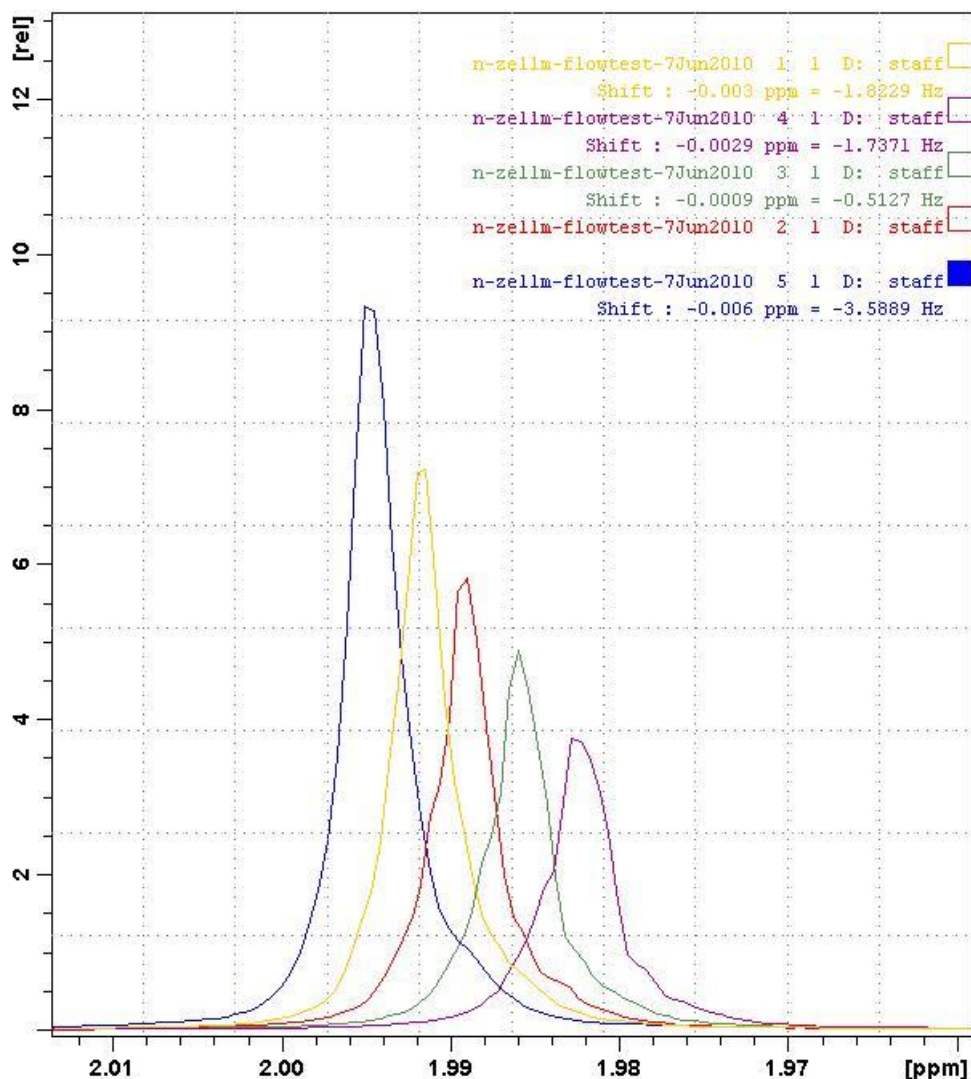
New Flow Cell Design

First data acquired on new flow cell at Bruker. 2 mM sucrose sample. Spectrum demonstrates good NMR lineshape even with tubing extended into flowcell.

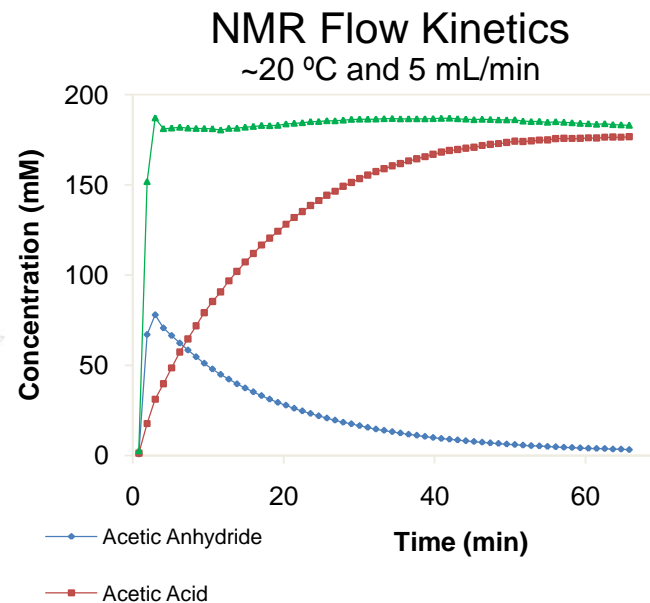
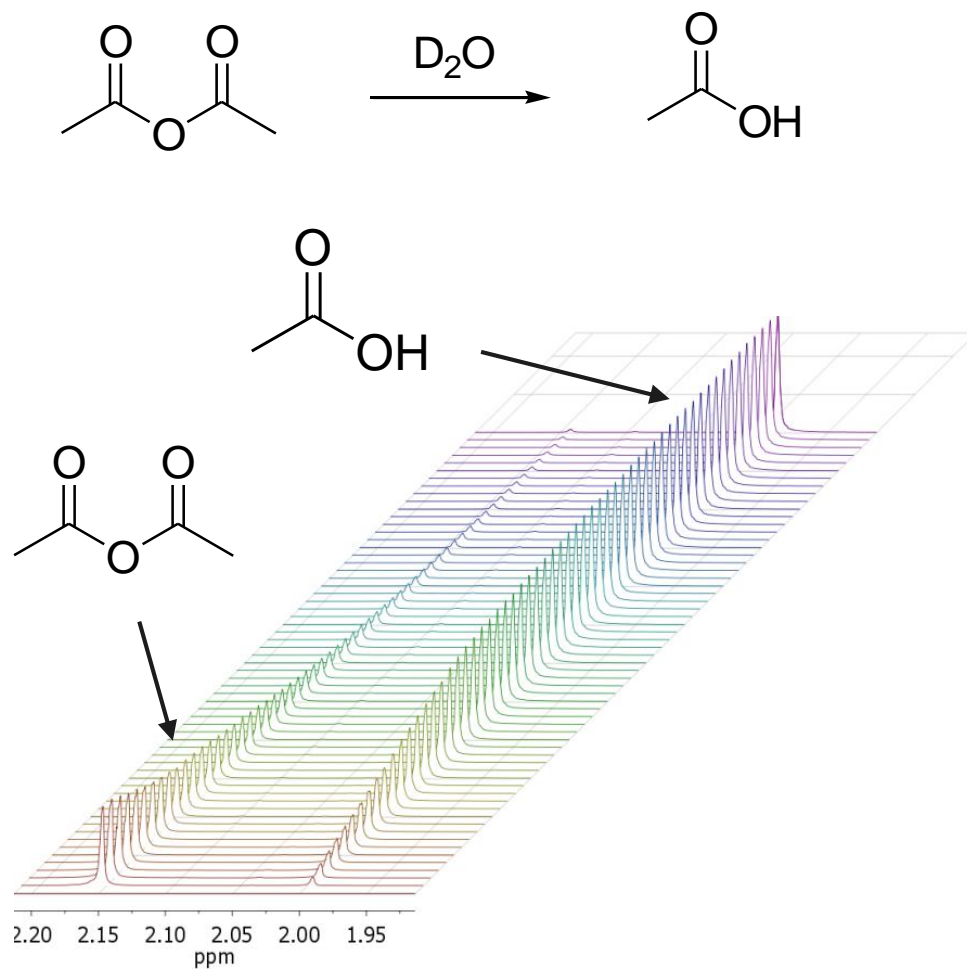


Optimal Flow rate minimizes lag from reaction vessel

Acetic Acid 1H Signal depends on the flow rate through the NMR cell



Acetic Anhydride



	Temp (°C)	$10^3 \cdot k_D$ (sec ⁻¹)
NMR tube	25	0.94
Literature	?	0.89
NMR flow	~20	0.82

Quantitative NMR Spectroscopy

- **Electronic REFerence To access In vivo CONcentrations (ERETIC) Method**
 - Prior to the ERETIC method
 - Internal standards were necessary for absolute quantitation
 - Potential problems included signal overlap, chemical interaction, and errors in addition of the internal standard
 - ERETIC
 - Electronically generated signal injected into the spectrometer to replace the internal standard
 - Requires additional hardware and the reference signal can't be moved once the data is collected
 - Digital ERETIC (QANTUS, Quantification by Artificial Signal, Upton, et.al.)
 - Synthetic signal added after data collection as part of the data processing
 - Reference signal can be moved anywhere in the spectrum without recollecting the data and does not require additional hardware

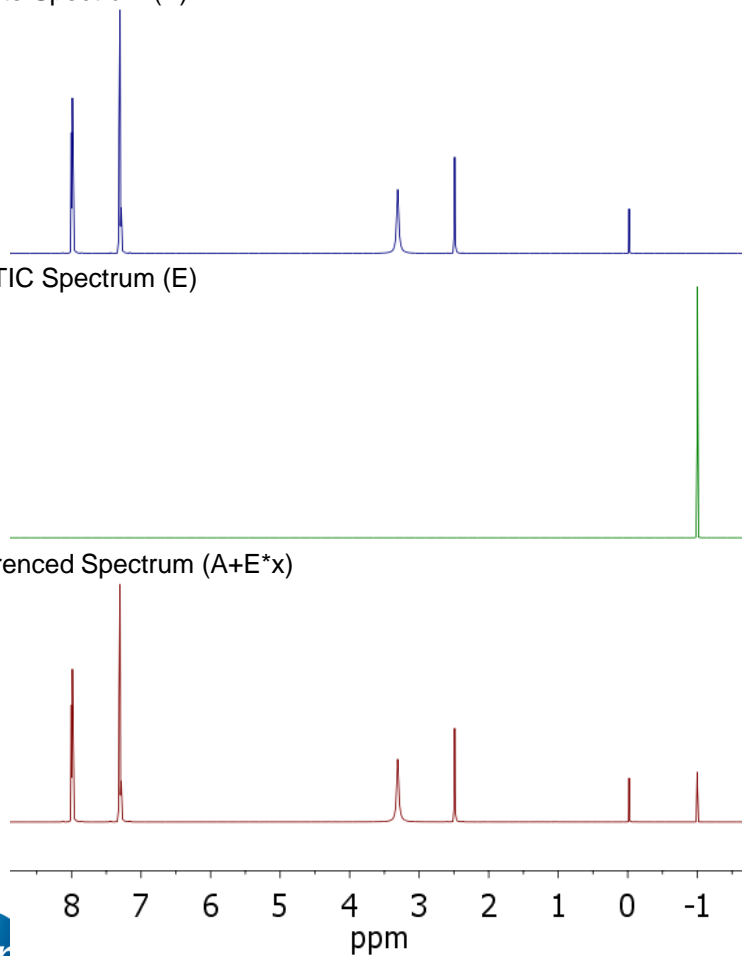


Digital ERETIC Method

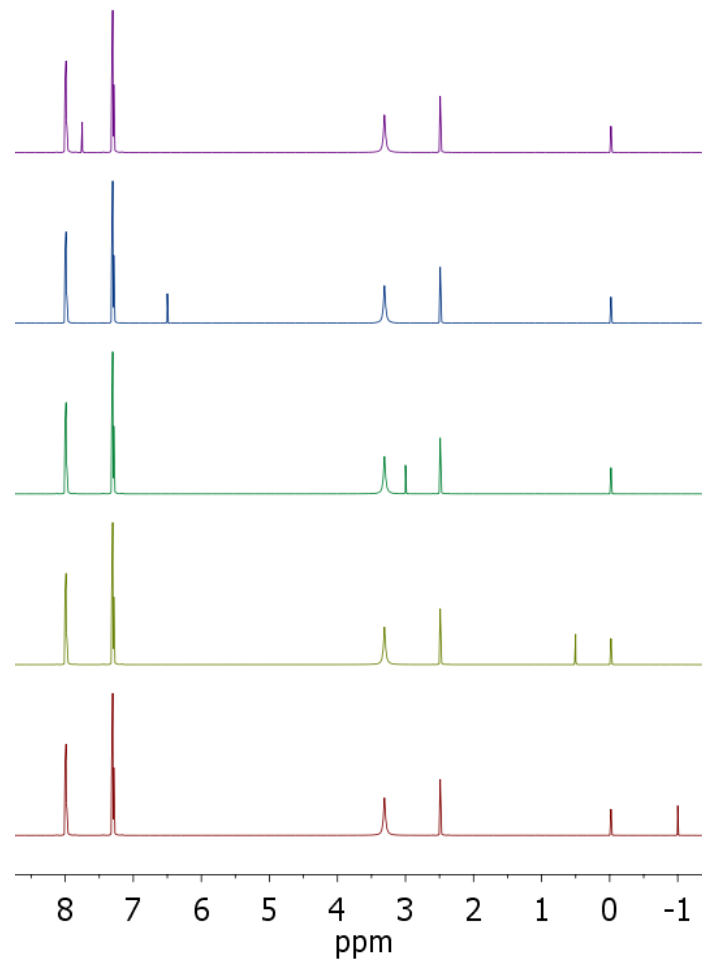
Analyte Spectrum (A)

ERETIC Spectrum (E)

Referenced Spectrum (A+E*x)



The ERETIC signal can be placed anywhere in the spectrum without changing the calibration.



QNMR Potency Measurements

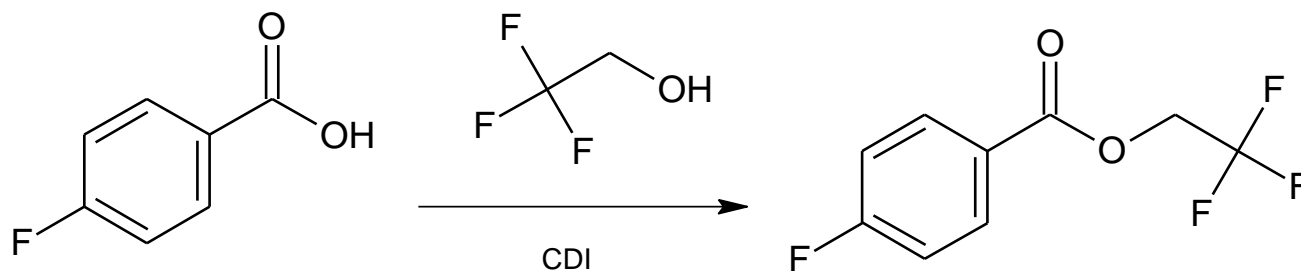
Compound	Lot #	Theoretical Potency	Traditional QNMR	Digital ERETIC QNMR	
				Analyte	Maleic acid
Benzoic acid	NIST 39j	0.999996	---	1.016	---
Maleic acid	1296124	>0.99	0.990	---	0.990
p-Fluorobenzoic acid	NIST 2143	0.9995	0.987	1.024	0.994
PF-04971729-G3 L-Pyroglutamic acid	GR02878	0.755 0.235	0.733 0.242	0.736 0.241	0.996
3-TMS-1-propylsulfonic acid sodium salt	23021EB	1.000	0.917	0.868	0.941
1,4-Bis(TMS)benzene	04202KC	0.995	0.453*	0.460*	1.003
α-D-Glucose, anhydrous	12926AC	0.999	0.768	0.981	1.262

Digital ERETIC signal calibrated against the Maleic acid peaks in the Benzoic acid sample, assuming the purity determined by the Traditional QNMR method

* = Sonicated several times but not all of the sample dissolved



Esterification reaction



Reaction Conditions:

First reaction:

20 mM para-fluorobenzoic acid circulating in reactor. CDI and trifluoroethanol both added in excess. Acquired proton data only (top spectra).

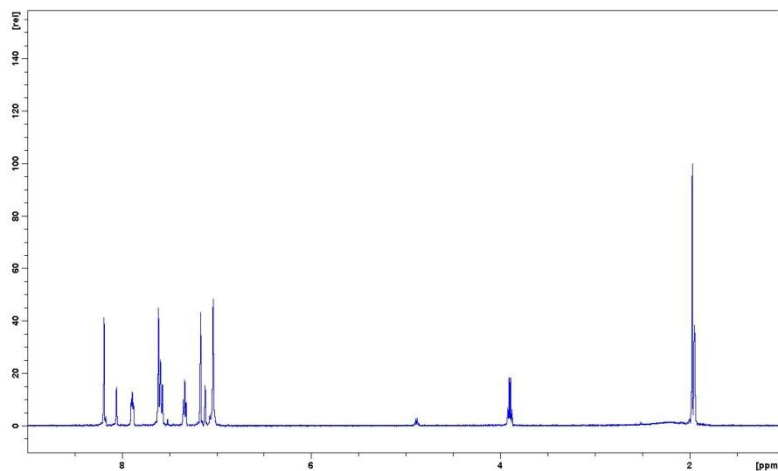
Second Reaction:

20 mM para-fluorobenzoic acid circulating in reactor. Added CDI and allowed to stir over lunch. Added trifluoroethanol and no reaction occurred. Added additional (non-stoichiometric) amount of CDI and reaction proceeded. Reaction did not go to completion. ^{19}F (and ^1H – not shown) data acquired during this reaction (bottom spectra)

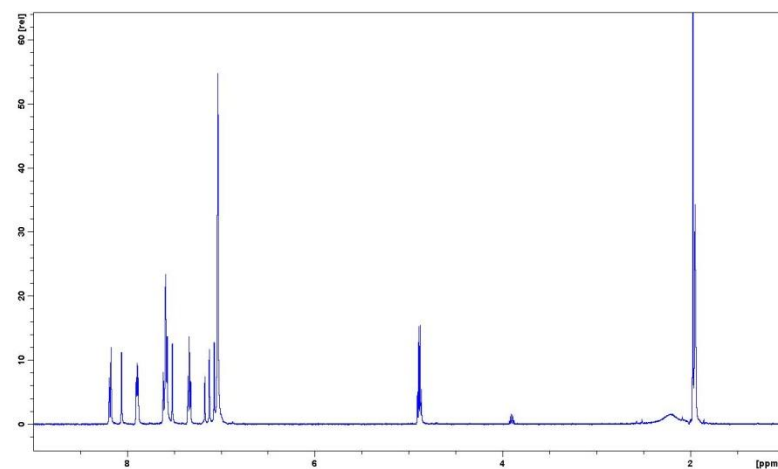
Reaction to be repeated again in the next couple of weeks after pump variability is resolved.

Esterification reaction

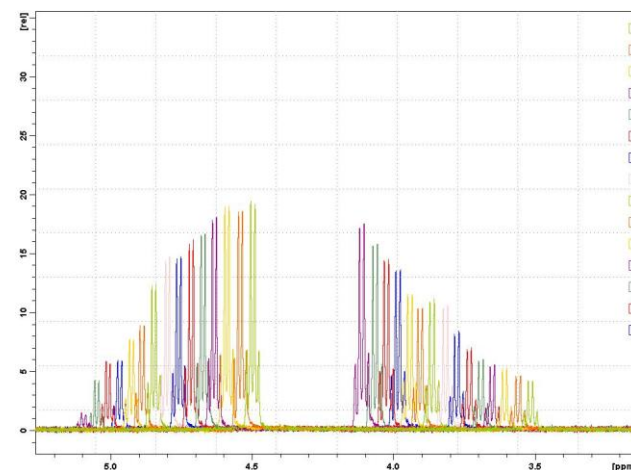
Primarily Unreacted – ^1H



Primarily Reacted – ^1H

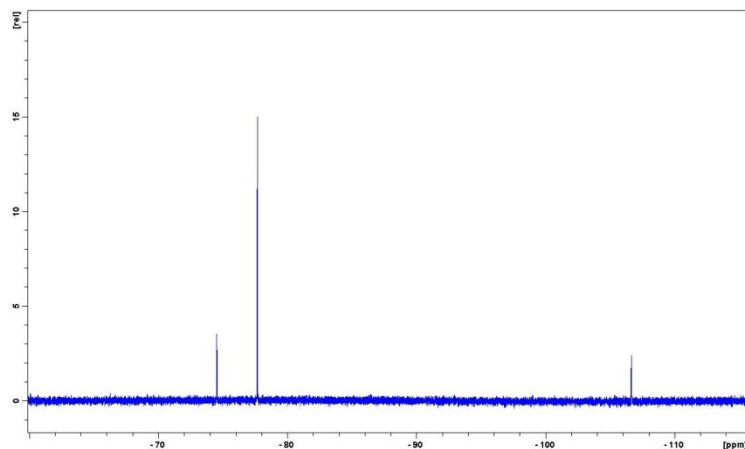


Methylene Protons

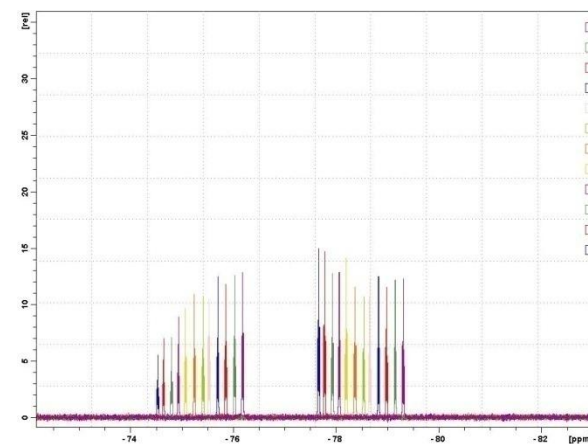
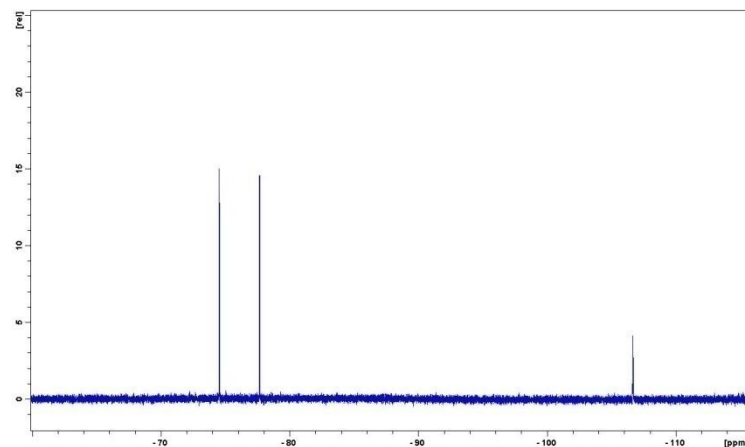


Esterification reaction

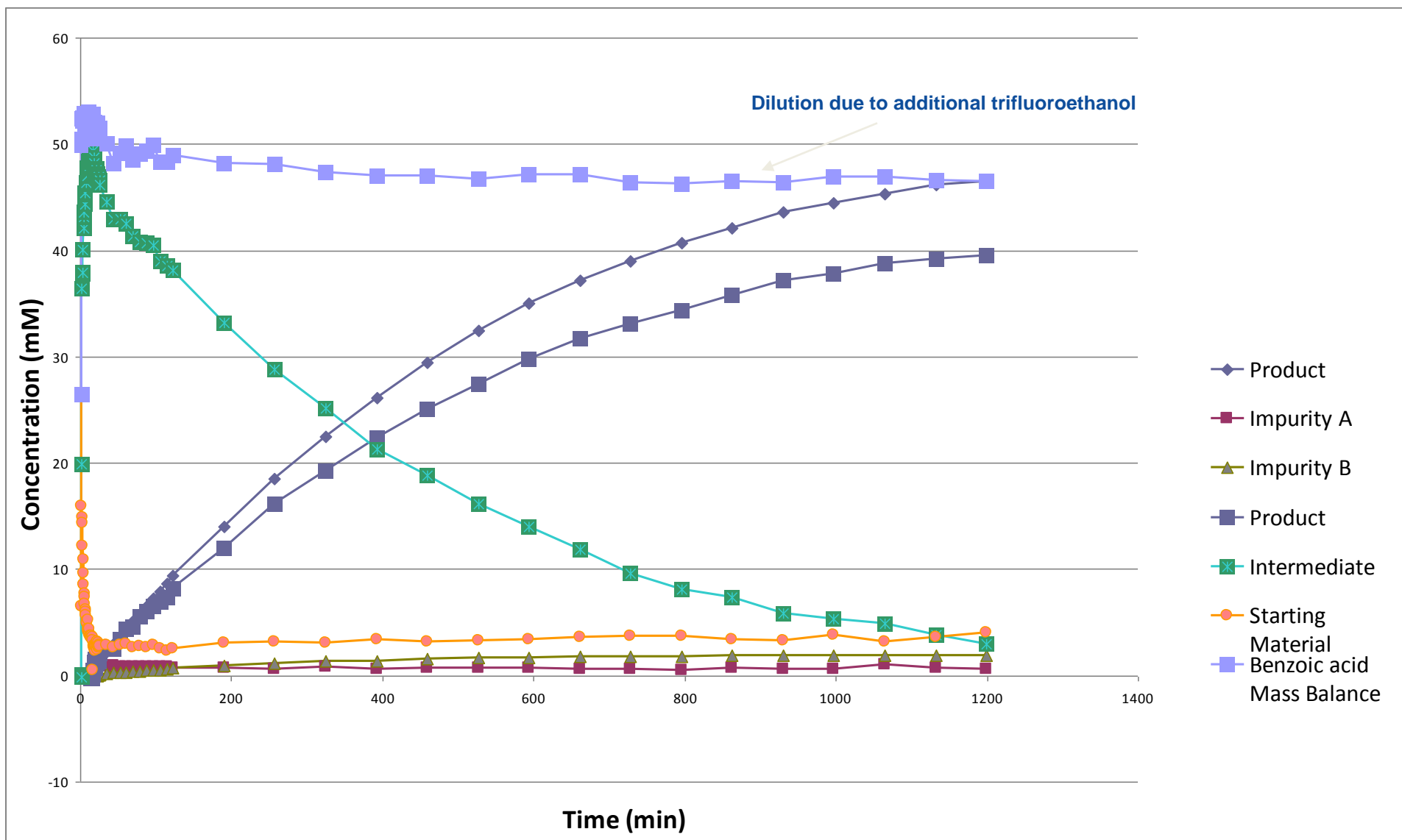
Primarily Unreacted – ^{19}F



Partially Reacted – ^{19}F



Esterification reaction



Applicability to Pfizer projects

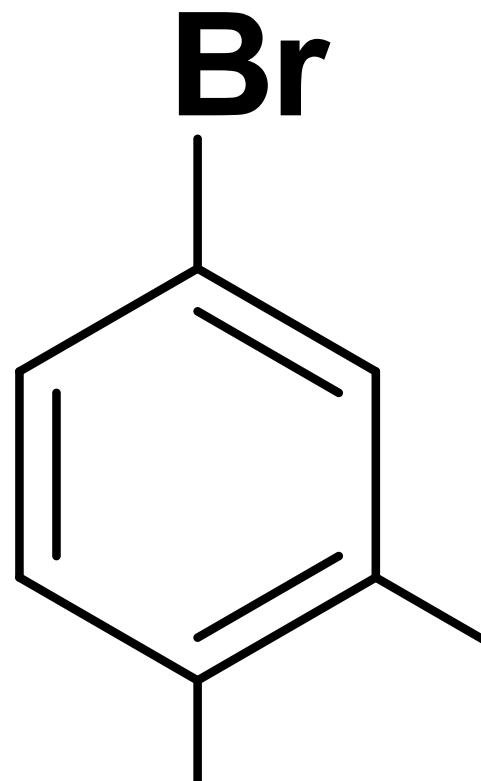
- This technology is directly applicable to a wide range of internal synthetic projects.
- Previously showed the use of our early flow-NMR system to identify the presence of a transient intermediate in the synthesis of an active ingredient in a currently marketed product.
- The data shown in the following slides is from a current internal project, and is just one of many projects that could benefit from advances in the area of flow NMR technology.
 - Step 1: Formation of an aryl-metal species
 - Step 2: The addition of this aryl-metal to a cyclobutanone.
- These reactions are sensitive to moisture and require a careful control of the conditions. Our current understanding of the chemistry is limited by the use of off-line analytical tools.



Applicability to Pfizer projects

Temperature

- Reactor = -20 °C
- Line temperature = -10 °C
- NMR probe temperature = -10 °C
- The reaction mixture was circulated through the NMR probe at 5 mL/min.
- ^1H , ^{19}F , and ^{13}C spectra were collected at various points during the reaction
- Further steps were not investigated due to solvent-induced pump leak!



Applicability to Pfizer projects

F

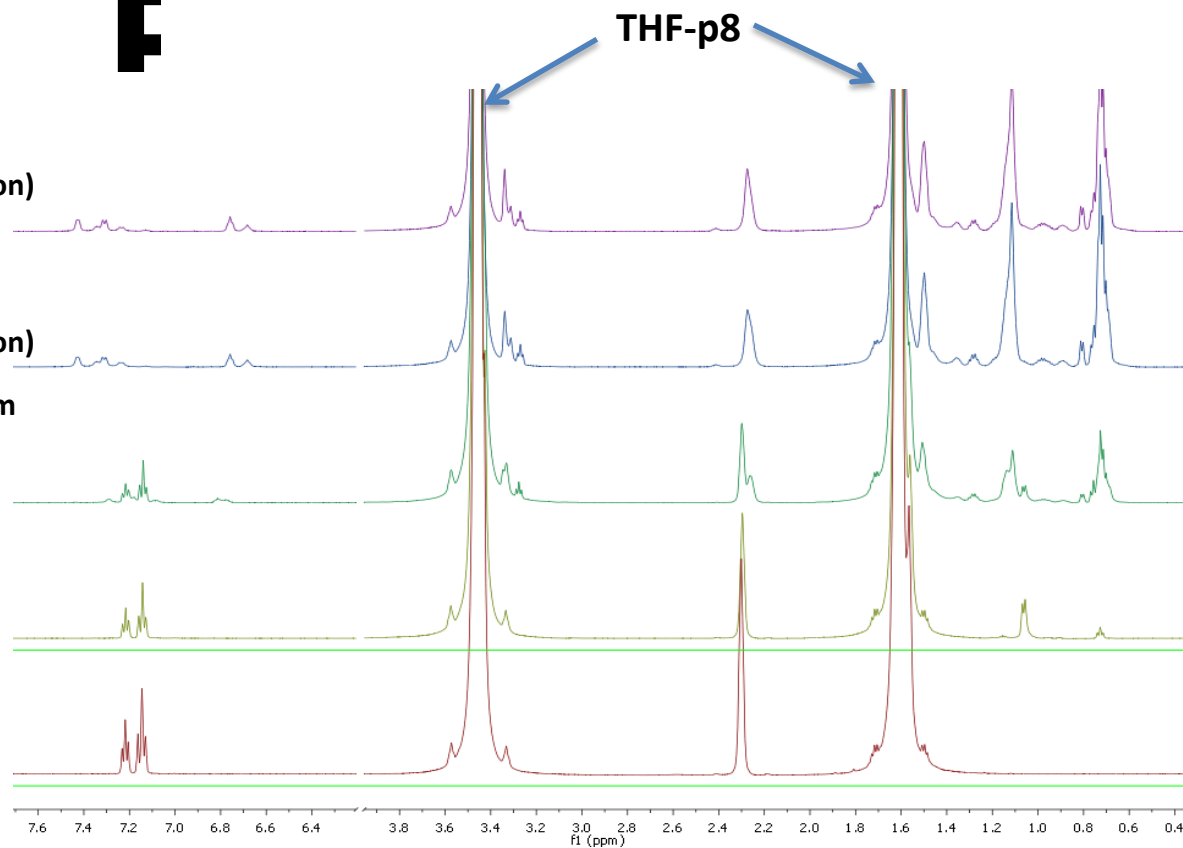
Aryl Bromide +
Isopropylmagnesium Chloride +
Butyl Lithium (~20 min after addition)

Aryl Bromide +
Isopropylmagnesium Chloride +
Butyl Lithium (~10 min after addition)

Aryl Bromide + Isopropylmagnesium
Chloride +
Butyl Lithium (during addition)

Aryl bromide +
isopropylmagnesium chloride

Aryl bromide



Applicability to Pfizer projects

F

^{19}F Spectra (^1H Decoupled)

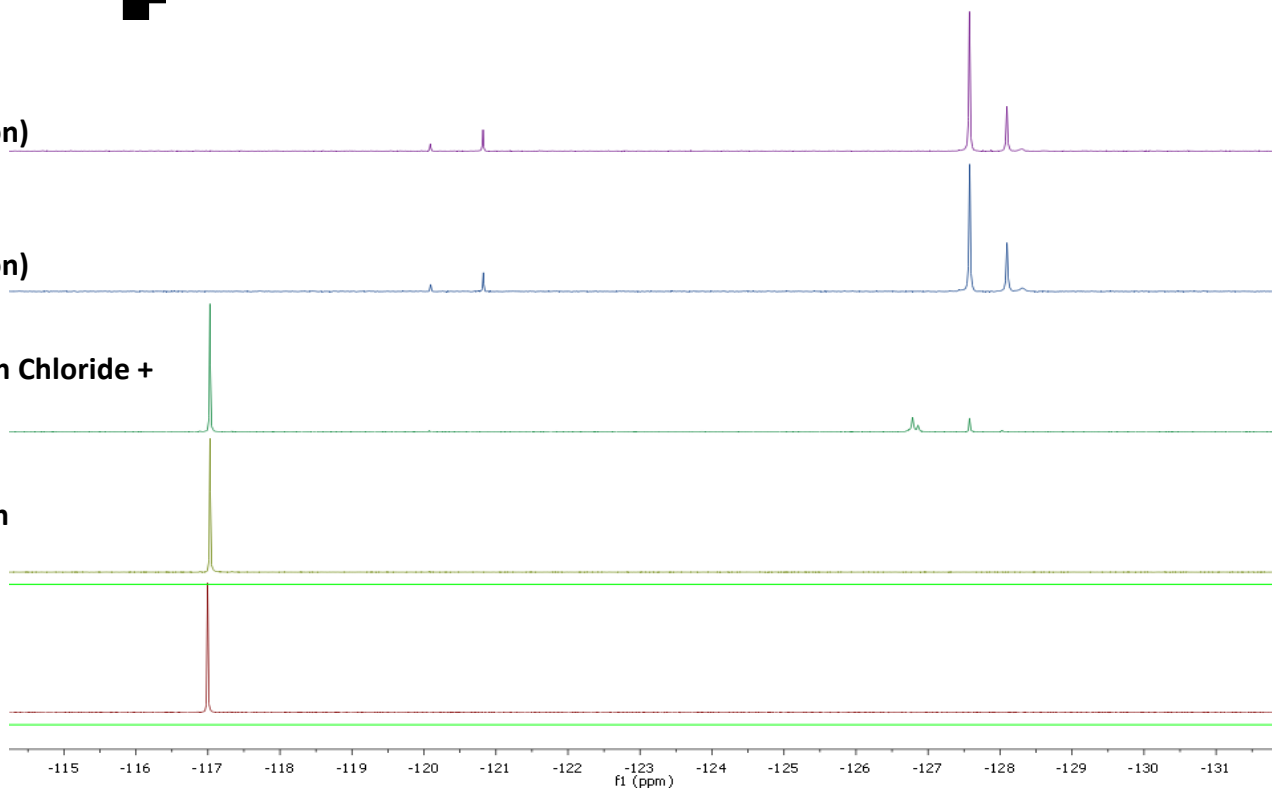
Aryl Bromide +
Isopropylmagnesium Chloride +
Butyl Lithium (~20 min after addition)

Aryl Bromide +
Isopropylmagnesium Chloride +
Butyl Lithium (~10 min after addition)

Aryl Bromide + Isopropylmagnesium Chloride +
Butyl Lithium (during addition)

Aryl bromide + isopropylmagnesium
chloride

Aryl bromide



Applicability to Pfizer projects

F

¹³C Spectra

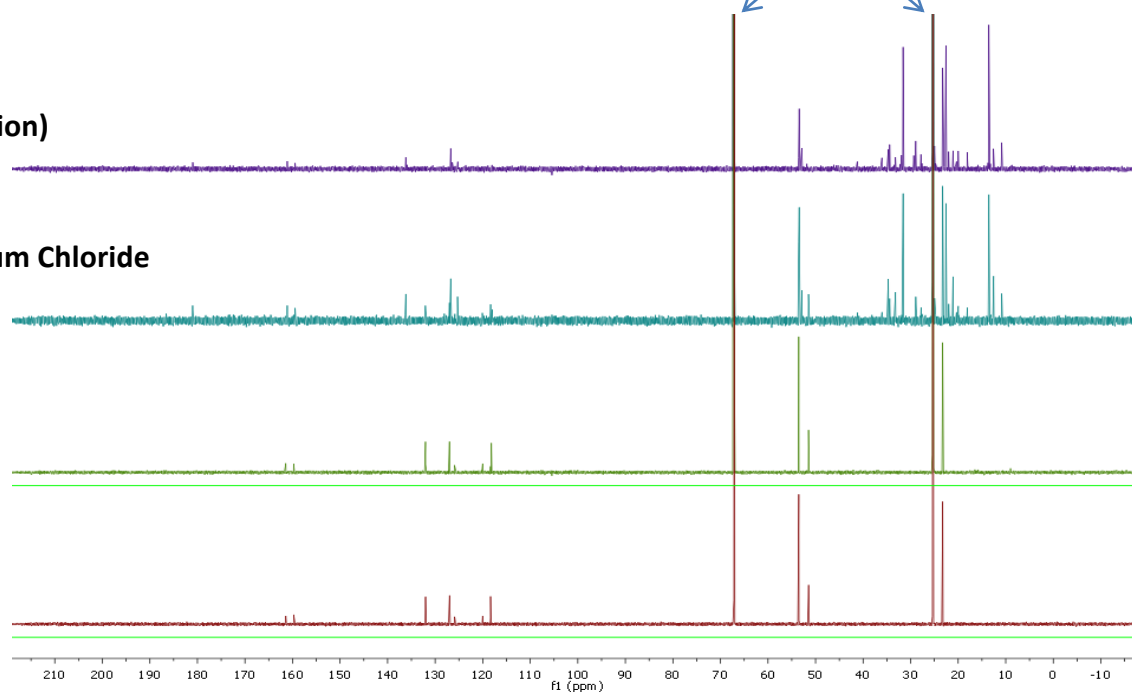
THF-p8

Aryl Bromide +
Isopropylmagnesium Chloride +
Butyl Lithium (~20 min after addition)

Aryl Bromide + Isopropylmagnesium Chloride
+
Butyl Lithium (during addition)

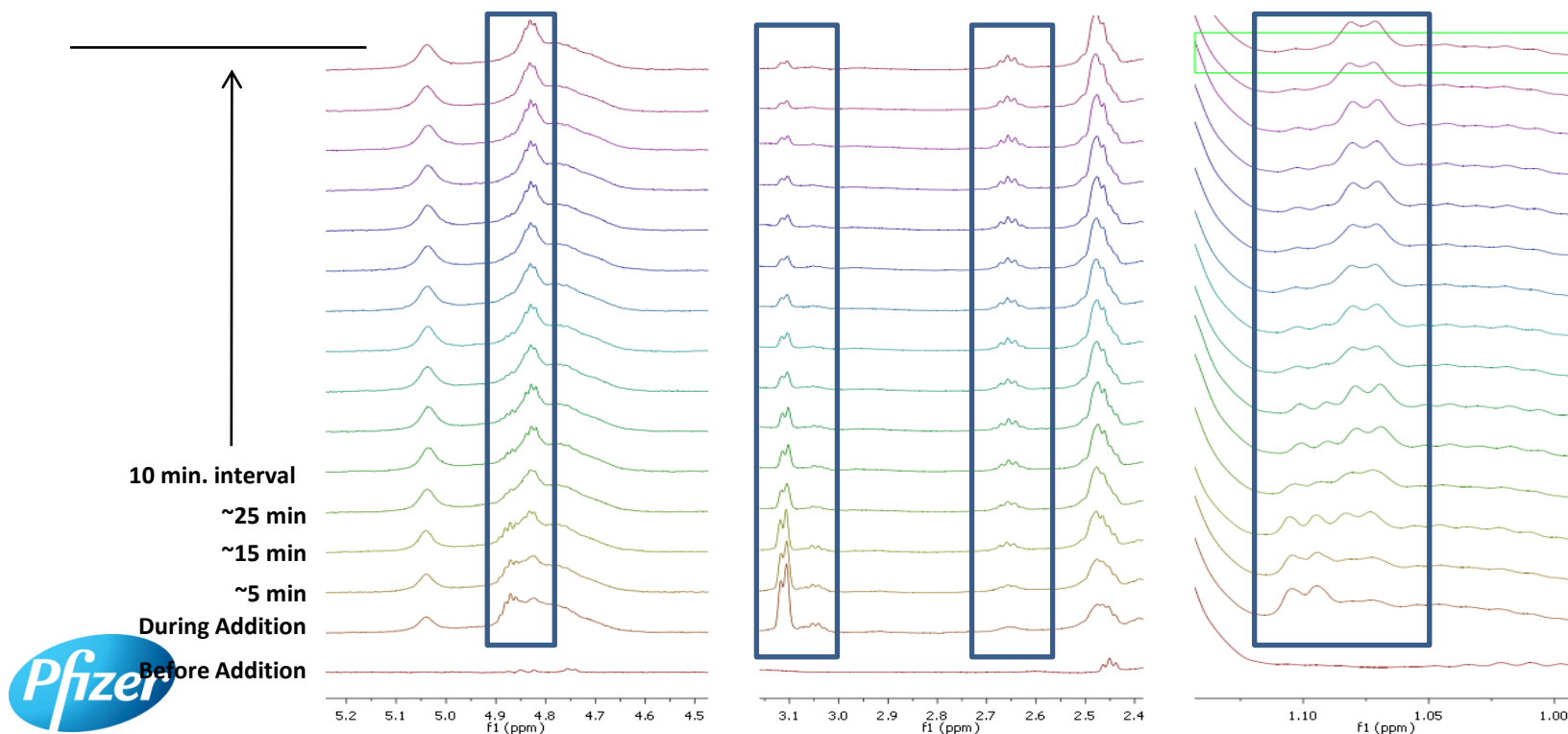
Aryl bromide +
isopropylmagnesium chloride

Aryl bromide



Applicability to Pfizer projects

^1H Spectra



Next Steps

- **Reduce the lag time to 30 seconds or less.**
 - Use of a pump with appropriate seals is critical. (so far using slow loop at 5 ml/min through 1/16" tubing)
 - Improve fast loop operation while keeping approx 5 ml/min thru the slow loop.
- **Further characterize the system at sub-ambient conditions and ensure adequate thermostating of the lines.**
- **Extend to catalytic hydrogenations, follow reaction kinetics without disrupting the gas-liquid equilibrium.**
- **Continue to extend to new chemistries, unit operations (distillations, pKa determinations, extractions etc)**
- **Enable as facilitated walk-up platform**



Acknowledgements

- **Bruker-Biospin**
- **Pfizer Groton and Sandwich SE groups**
- **Jason Mustakis**
- **Carrie Wager**
- **Steve Brown**
- **Geraldine Taber**
- **Andreas Kaerner – Eli Lilly & Company**
- **John Edwards – Process NMR**

