Arrowhead's Approach to Oligonucleotide Scale-Up Manufacturing

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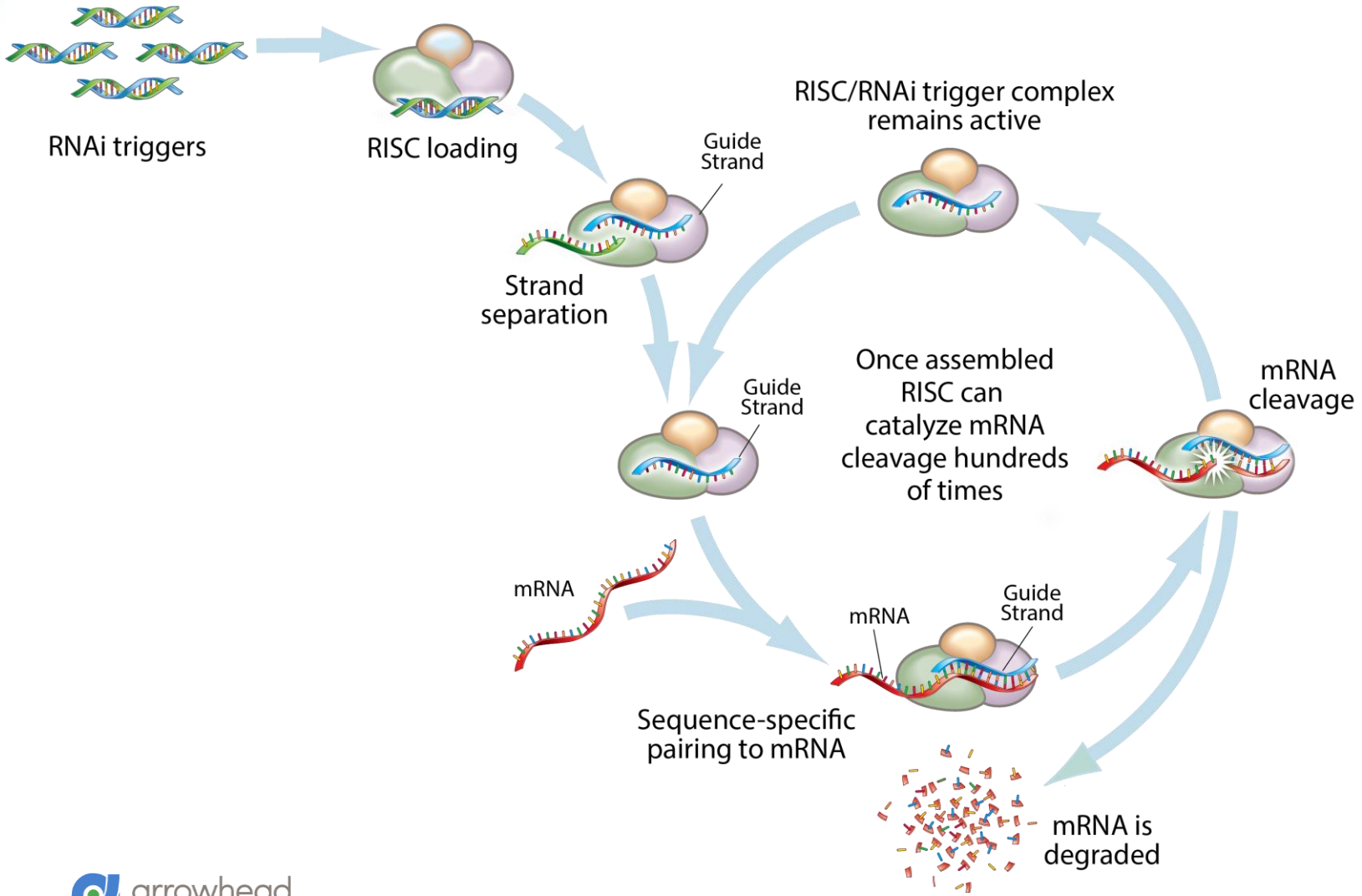
RNA Interference (RNAi) Mechanism



Advantages of RNAi

- Silences the expression of disease associated genes
- Potential to address previously "undruggable" targets
- High specificity
- Rapid path from idea to clinical candidate
- Positive record of clinical safety and tolerability

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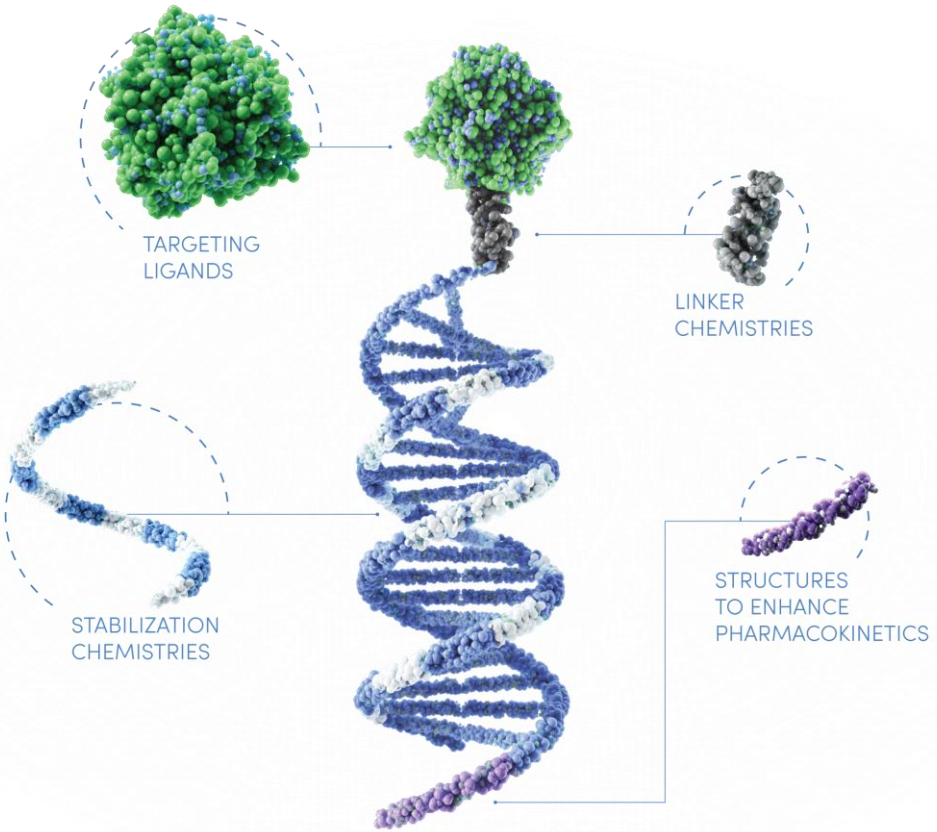
Targeted RNAi Molecules - TRiM™ Platform



TRiM™ – *Targeting the gene, to Silence the disease*

- ***Activity*** characterized by depth & duration of effect
  - Ability to unlock previously undruggable targets
- ***Specificity*** to maximize activity and innate stability with the potential for reduced off-target effects
- ***Versatility*** in formulation & ligand design offers multiple routes of administration, and access to multiple tissues
  - Facilitates rapid drug development and speed to patients
- ***Simplicity*** in design translates to relatively lower costs, and production at scale

Pipeline

THERAPEUTIC AREA		PRE-CLINICAL	PHASE 1	PHASE 2	PHASE 3	Product Rights
Cardiometabolic	**ARO-APOC3** FCS, sHTG					Arrowhead
	ARO-ANG3 Dyslipidemia					Arrowhead
	Olpasiran CVD					Amgen
Pulmonary	**ARO-ENAC2** Cystic fibrosis					Arrowhead
	ARO-RAGE Inflammatory					Arrowhead
	ARO-MUC5AC Muco-Obstructive					Arrowhead
Liver	**ARO-HSD** NASH					GSK
	ARO-AAT AATD					Arrowhead / Takeda
	JNJ-3989 HBV					Janssen
	ARO-XDH Gout					Horizon
	ARO-C3 PNH, others					Arrowhead
Oncology	**ARO-HIF2** RCC					Arrowhead
Muscular Dystrophy	**ARO-DUX4** FSHD					Arrowhead
With Janssen	**JNJ-75220795**					Janssen
	JNJ2					Janssen
	JNJ3					Janssen

Tissue Targets: Liver, Lung, Tumor, Muscle

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Why Internal Development and Manufacturing?

- Risk mitigation
  - Start developing a target prior to final nomination
  - Early understanding of purity profiles
  - Flexible manufacturing suite scheduling allows for quick reprioritization
- Innovation
  - Arrowhead takes on new chemistries and platforms routinely
  - We need to have internal development if we are to scale-up "firsts" in the RNA field
- Quality
  - Internal development allows us to meet/exceed regulatory guidances
- Speed
  - Accelerate GLP-tox studies
  - Ensure GMP (clinical) material is available at earliest possible time
  - Compress timelines of clinical candidates

Internal Chemistry Development Teams

- Process development
  - Small molecule development
  - Conjugation development
  - Initial scale-ups
  - New platform development
- RNA development
  - RNA synthesis understanding
  - Scale-down models
  - Early and late-stage yield/purity optimization
  - New platform development
- Manufacturing team
  - New platform development
  - Full engagement with Process and RNA development
  - Two GMP suites capable of rapid scale-ups ranging from 200 g up to multi-kilo deliveries of drug substance
  - Nomination to a first batch can be as rapid as two months!

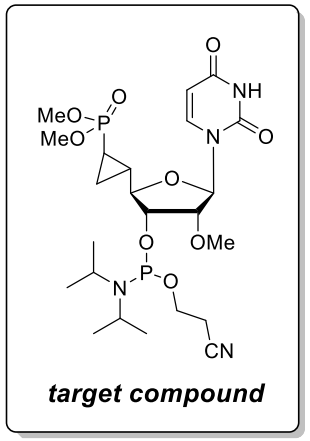
Goals

Presentation Goals: highlight Arrowhead's approach to managing risks in manufacturing through case studies

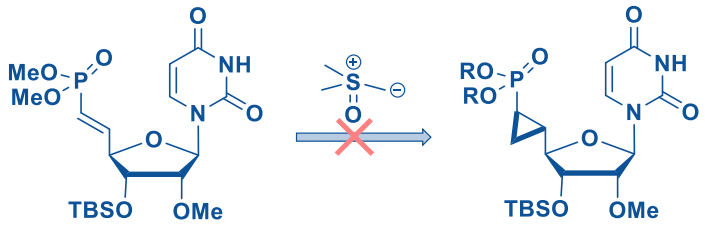
- Case study 1: challenging cyclopropanation reaction (fixing an intrinsically difficult reaction)
- Case study 2: designing a manufacturing friendly linker (designing around potential issues)
- Case study 3: simple fix to an unstable amidite (designing processes to avoid impurities)

Cyclopropyl Uridine (CpU) – A Surprisingly Tough Synthetic Target

- Initial route to make target compound resulted in extremely low yields due to poor cyclopropanation
- Vendor A could not make 10 g of CpU amidite
- Vendor B struggled to even produce 100 mg of clean material (requested 20 g on proposal)

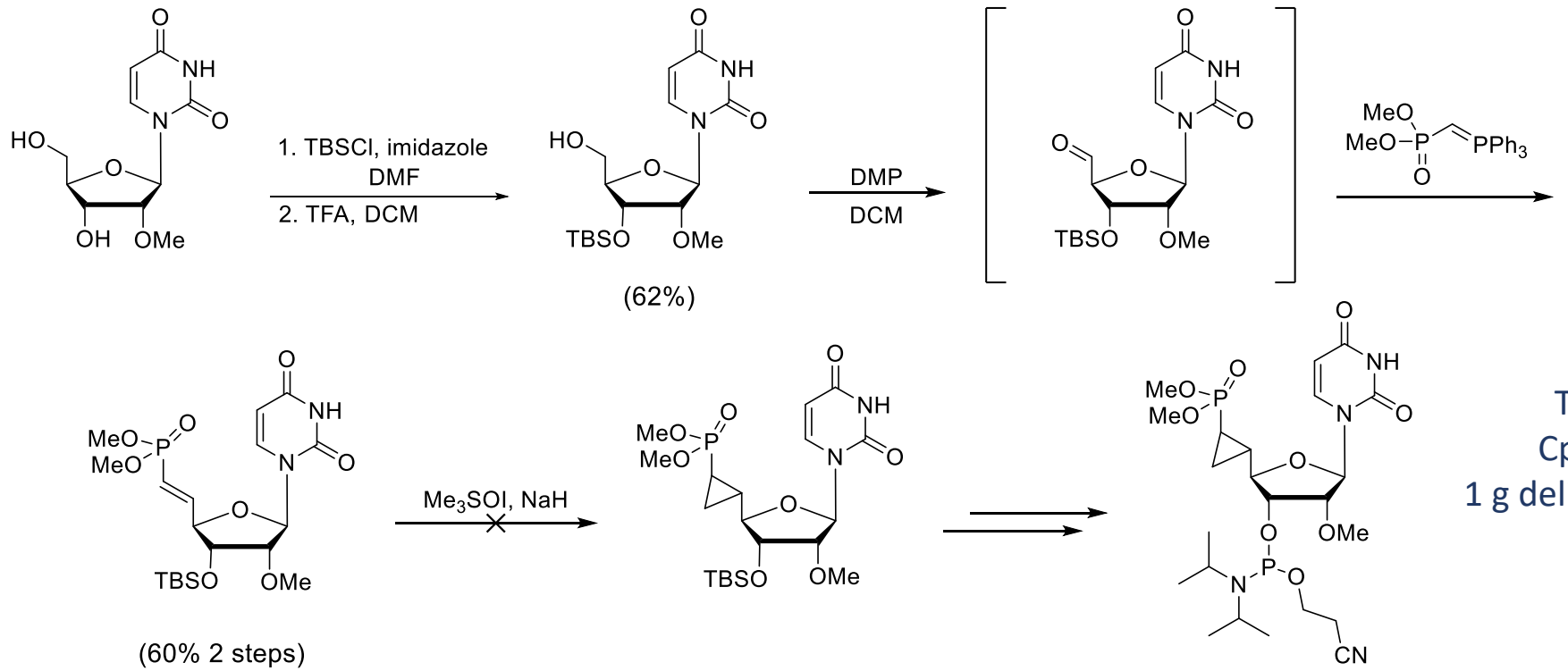


target compound



Two separate outsourcing campaigns failed to produce any cyclopropanated product

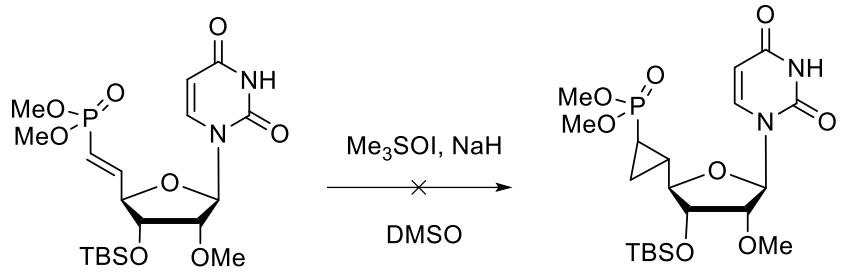
Original Synthetic Scheme



Target compound:
Cp phosphoramidite
1 g delivery to discovery team

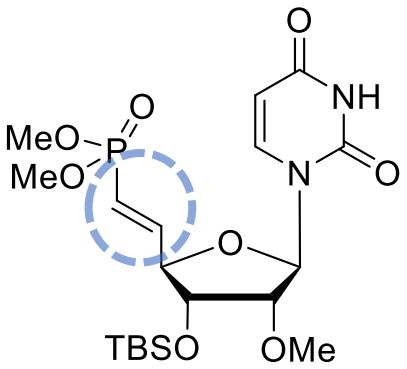
- A proposed 10-step synthesis from 2'-OMe-uridine
- Most of the chemical transformations at 5'-position
- Cyclopropanation was unsuccessful

In-House Attempts to Cyclopropanate

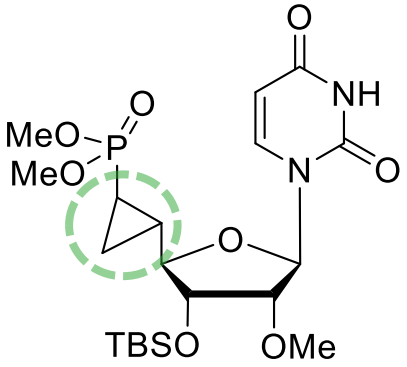


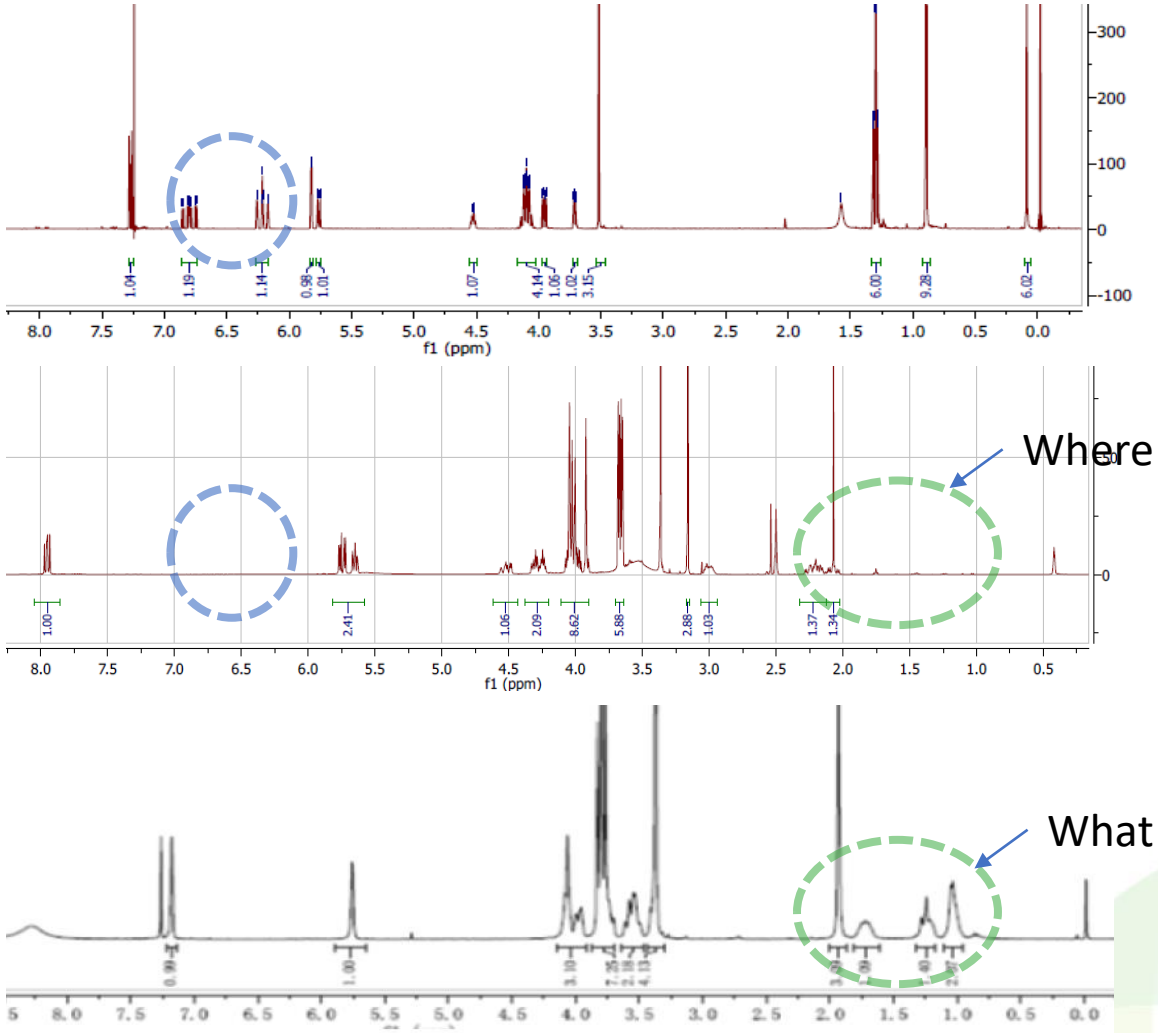
- Early in-house attempts at cyclopropanation appeared promising
  - Observed formation of new early eluting peak and disappearance of vinyl phosphonate on HPLC
  - LC-MS seemed to show correct mass
  - Vinyl peaks gone in proton NMR
- Critical look at the data
  - Never saw [M]+ ion, only [M]+78
  - Unusually polar, no retention on C18 column.
  - Proton NMR did not show CP protons in correct region (~1 ppm)

NMR Looks Incorrect



?

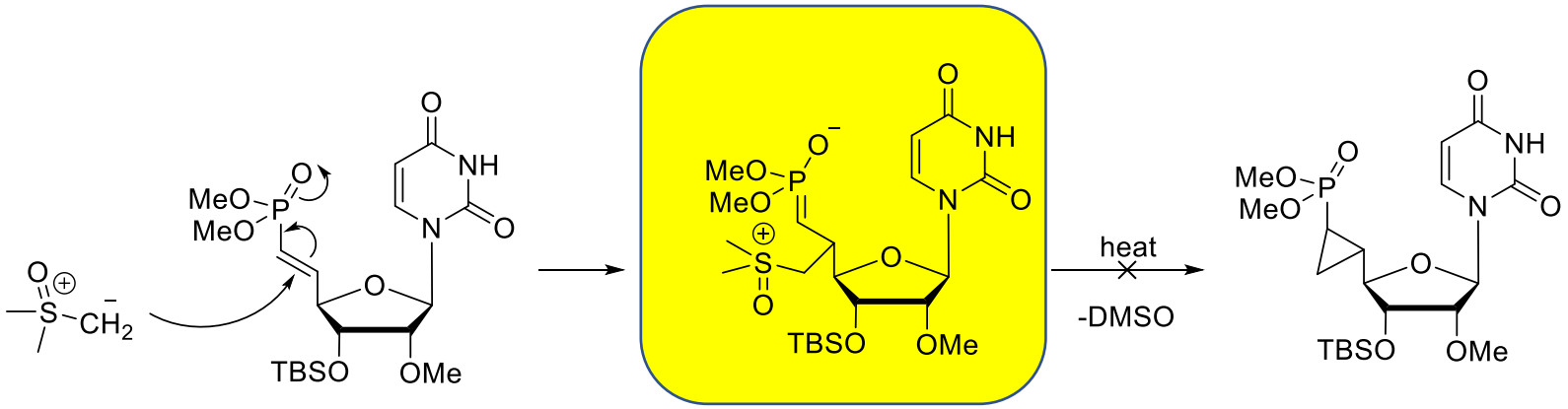




What Did We Make?

- Mechanistically, DMSO is not only the solvent, but also a leaving group, leading to our +78 mystery mass

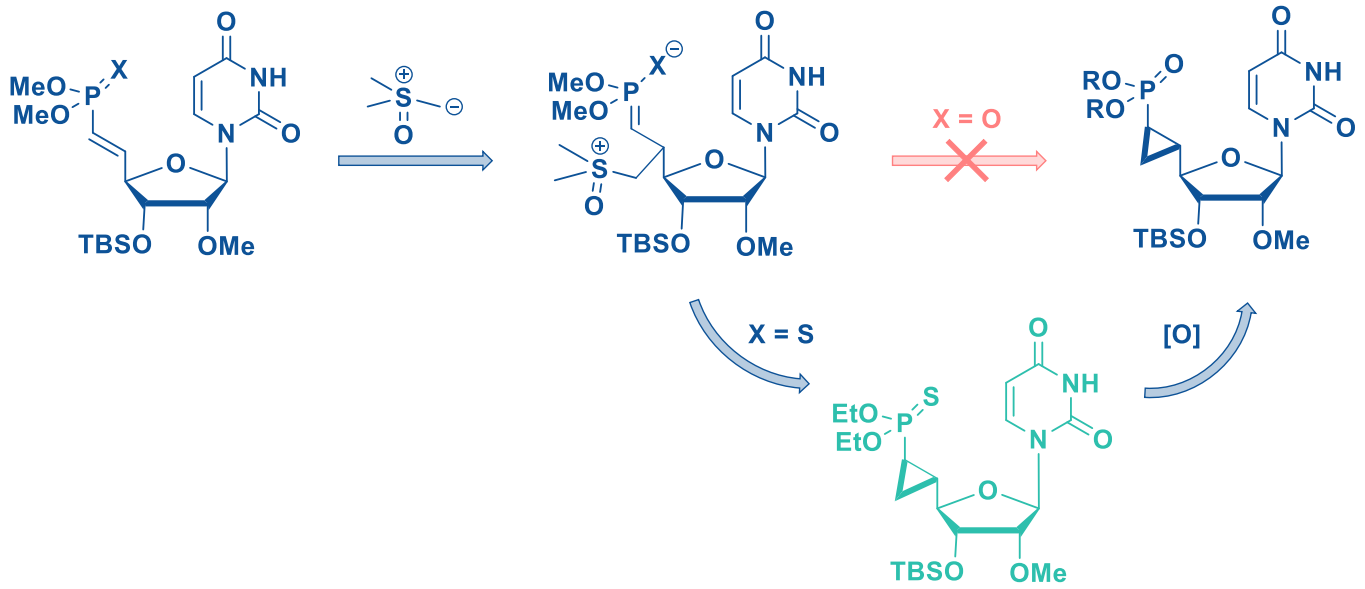
- The problem behind the stalled reaction:
  - Vinyl phosphonate underwent 1,4-addition rapidly with nucleophilic sulfur ylide
  - Stabilized intermediate **did not convert** to product despite heating



How Can We Fix the Cyclopropanation?

An understanding of the mechanism allowed for possible solutions

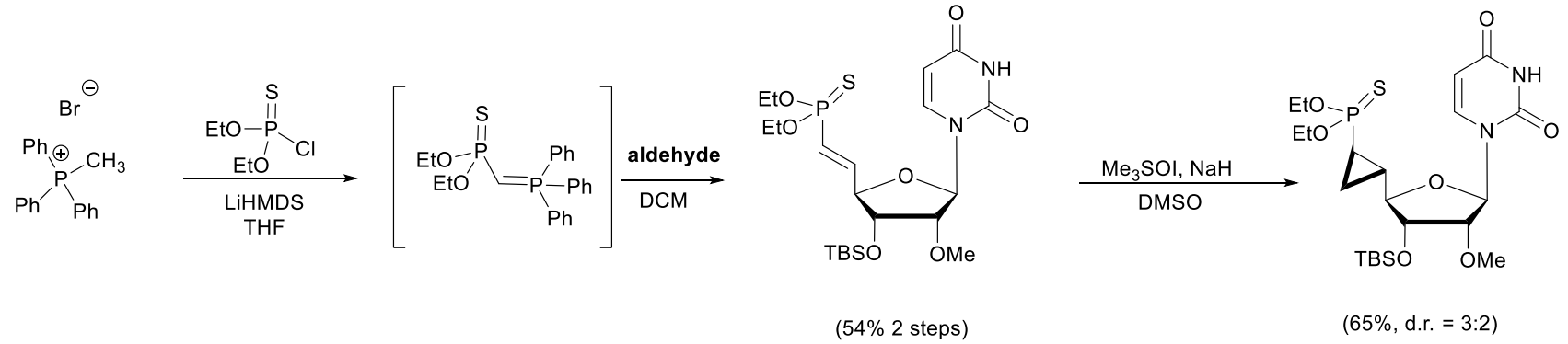
- Oxygen can stabilize the negative charge, thus decreasing nucleophilic character of adjacent carbon nucleophile
- Can we tune the electronics of the intermediate to facilitate cyclization?
- Substitute oxygen with a less electronegative sulfur atom



Can a less electronegative sulfur help?

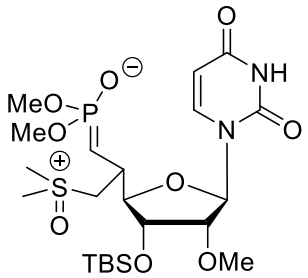
Synthesizing Sulfur Analogue

- New sulfur ylide is synthesized in a one-pot process
- No precedence in the literature

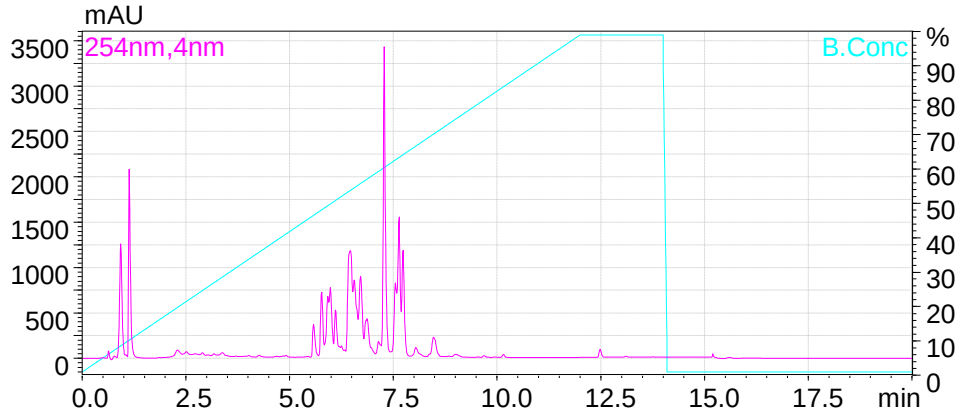


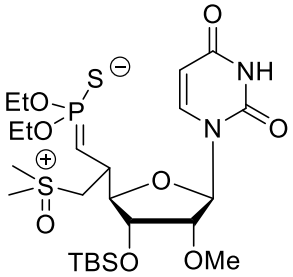
- Vinyl thiophosphonate (VTP) formed in comparable yield to VP — 54% over two steps
- Cyclopropanation of VTP was successful!

Reaction Progress – Heated

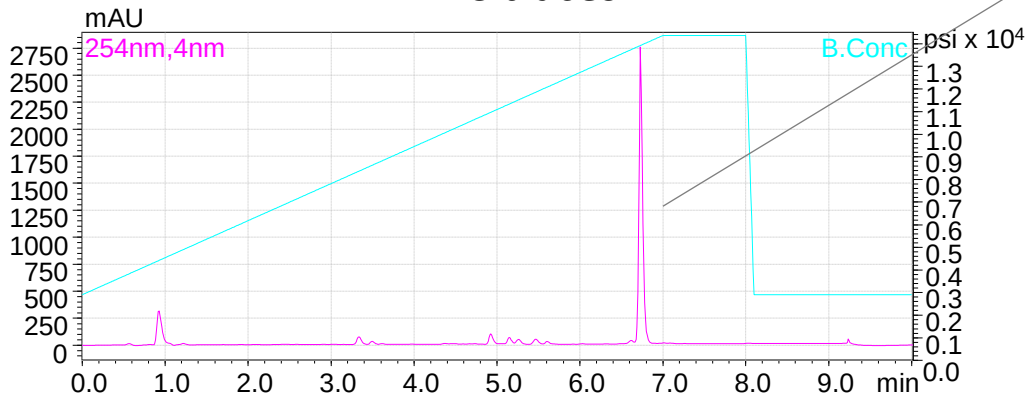


heat

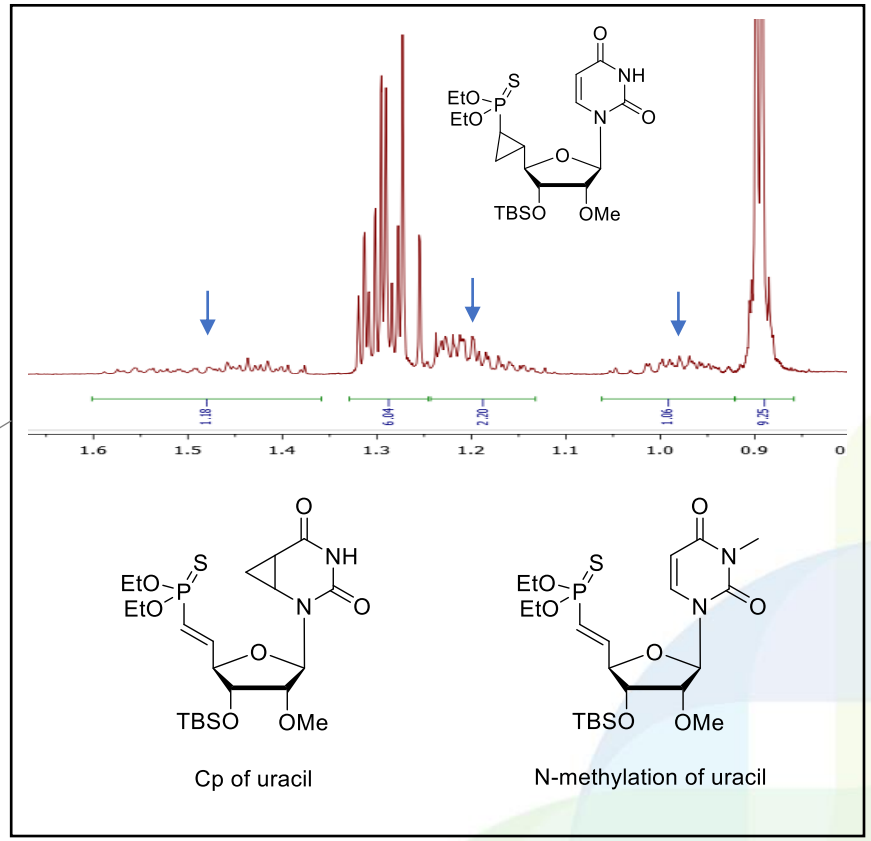




heat



HPLC traces



- LCMS confirms mass, also [M]+78 no longer seen.
- Observed four cyclopropyl protons in correct region
- Did not detect two critical impurities

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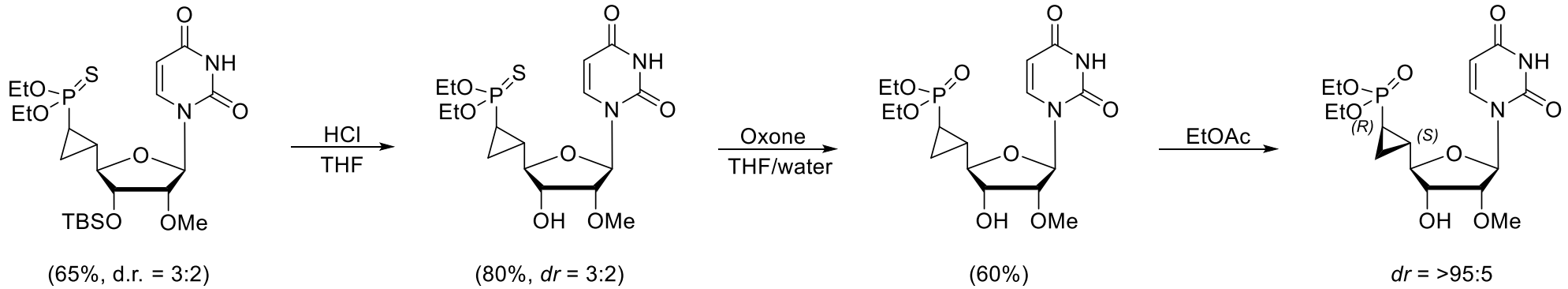
Further Innovation Required for the Final Steps

- Still need to obtain a phosphonate, not thiophosphonate
- Can a "reverse" Lawesson's transformation be performed?

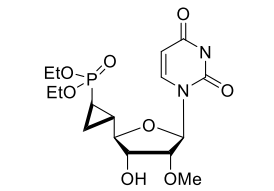


(65%, d.r. = 3:2) (80%, *dr* = 3:2) (60%) *dr* = >95:5

- Oxone is mild, inexpensive and led to a cleaner reaction compared to *m*-CPBA
- This was reported as the first use Oxone in converting P=S to P=O
- Secondly, we found a way to separate the Cp diastereomers during EtOAc workup

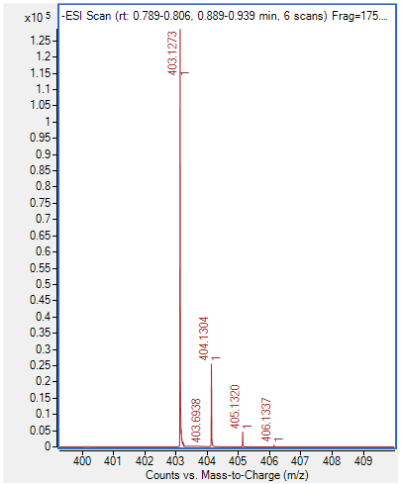
Characterization of the Major Product

- Molecular weight (HR-MS)

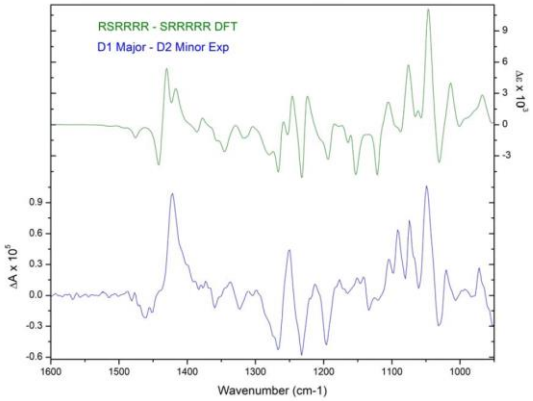


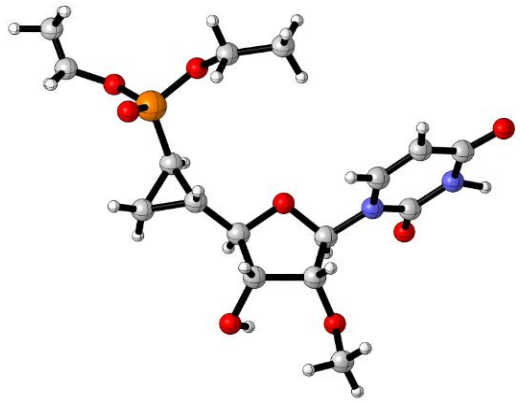
Chemical Formula: $C_{16}H_{24}N_2O_8P$
Exact Mass: 403.1270

0.74 ppm difference

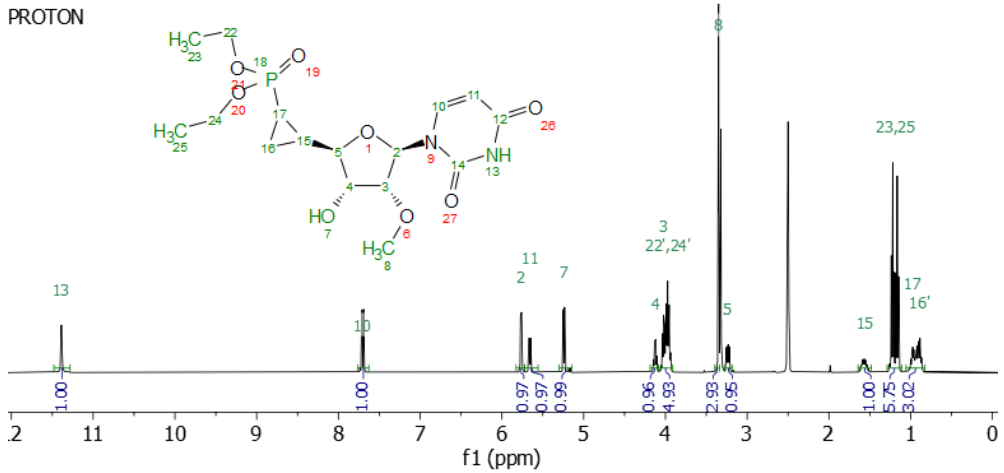


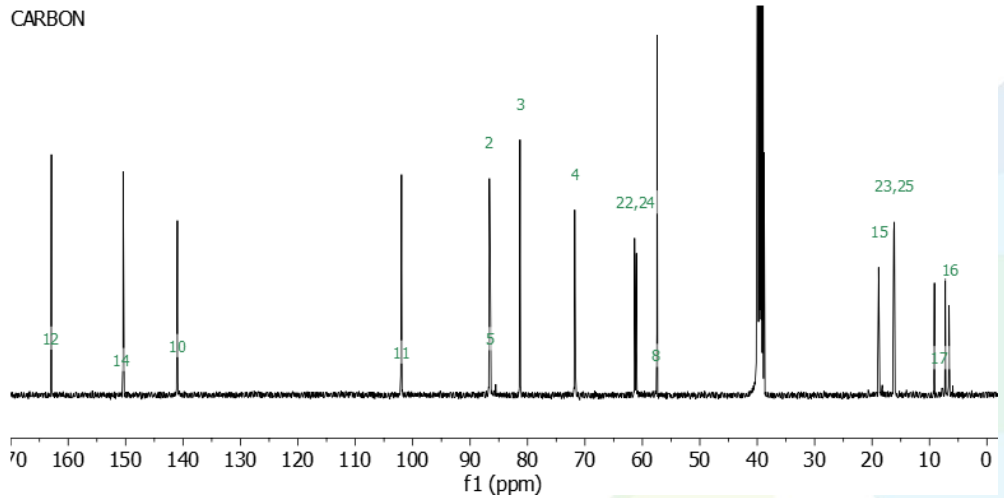
- Absolute configuration (VCD and SCXRD)





- Molecular connectivity (full 2D NMR)





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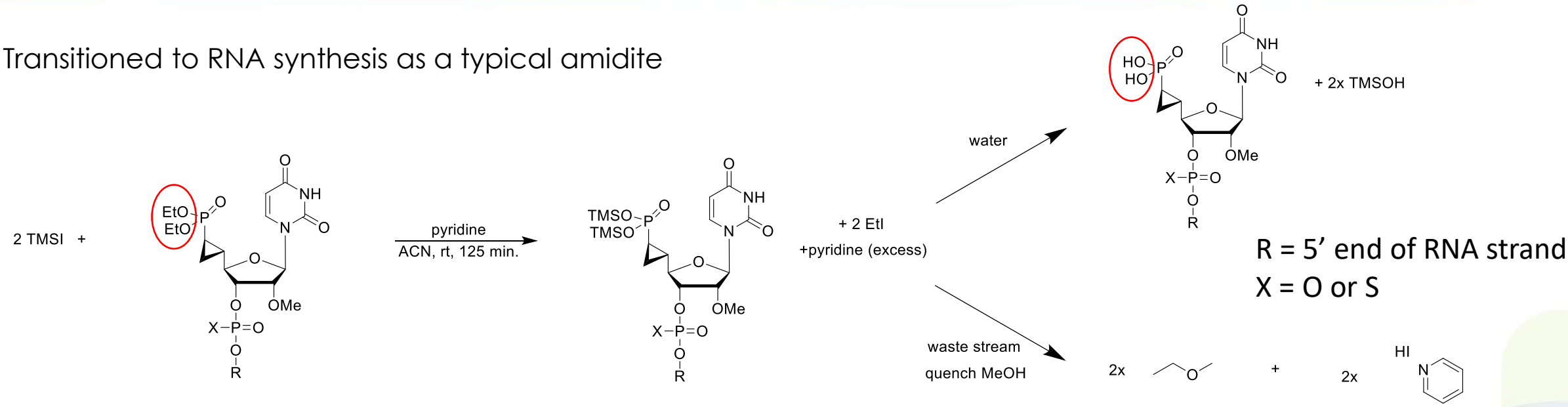
Deprotecting the Phosphonate: TMSI-Pyridine Safety

- Transitioned to RNA synthesis as a typical amidite



- Ethyl groups still need to be removed (on-column)
- Leading up to Manufacturing on 32-64 mmol scale we evaluated safety issues for the flow-through TMSI deprotection
  - Balanced equation shows ethyl iodide (alkylator) – basic waste stream quenched with methanol
  - Required ~10L of a TMSI/pyridine/ACN mixture
  - What will happen when we combine this hot Lewis acid and base?

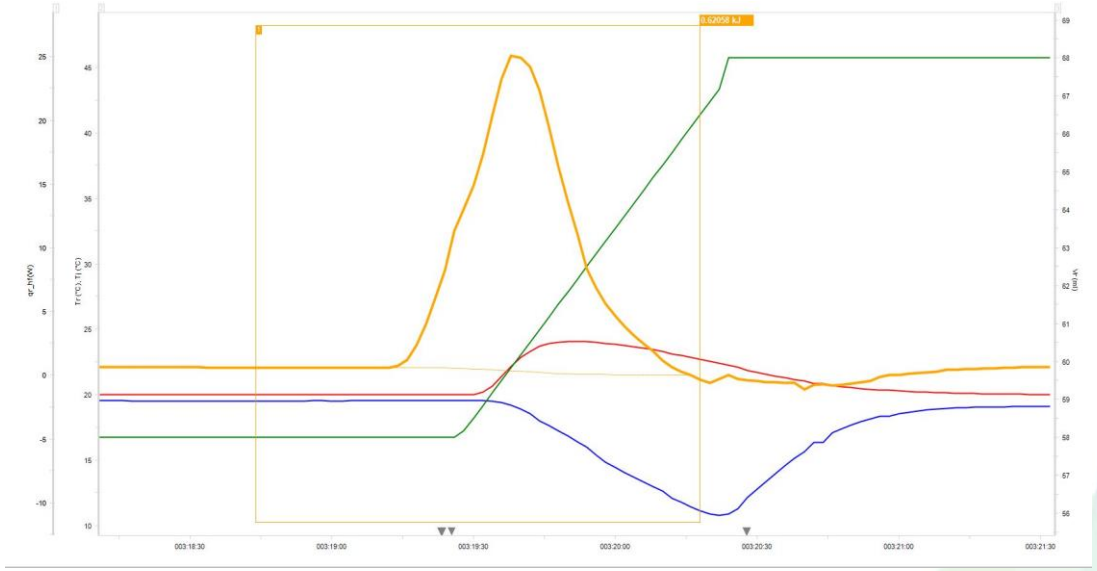
TMSI-Pyridine Safety

Objective

- Using an EasyMax with HFCal, find the heat of reaction for the addition of pyridine to a mixture of ACN and TMSI
- The final solution composition consists of a 56:10:2 mixture of ACN:pyridine:TMSI

Findings

- The calculated heat of reaction is 44 kJ/mol for the pyridine addition
- No off-gassing was noted during the pyridine addition
- The calculated change in temperature under adiabatic conditions is 5.59 K; which is very mild and doesn't even require cooling



Key

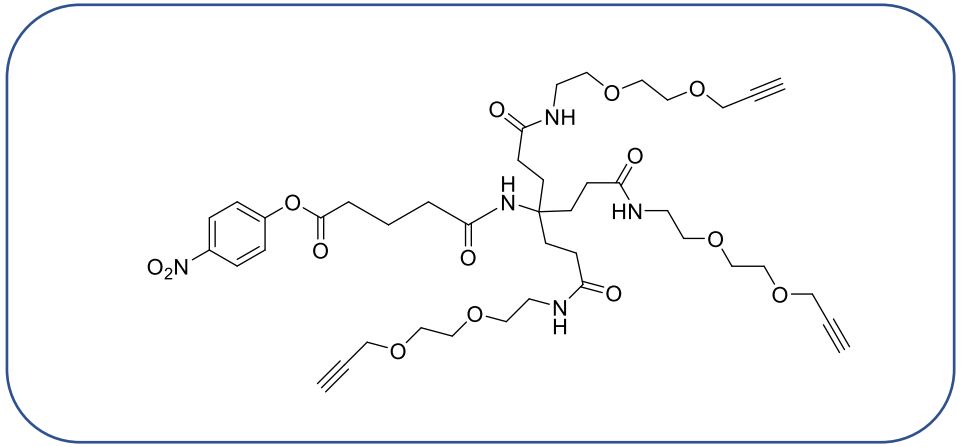
Heat Flow
Reactor Volume
Reaction Temperature
Jacket Temperature

Performed on 64 mmol scale with internal Arrowhead manufacturing team

CpU Summary

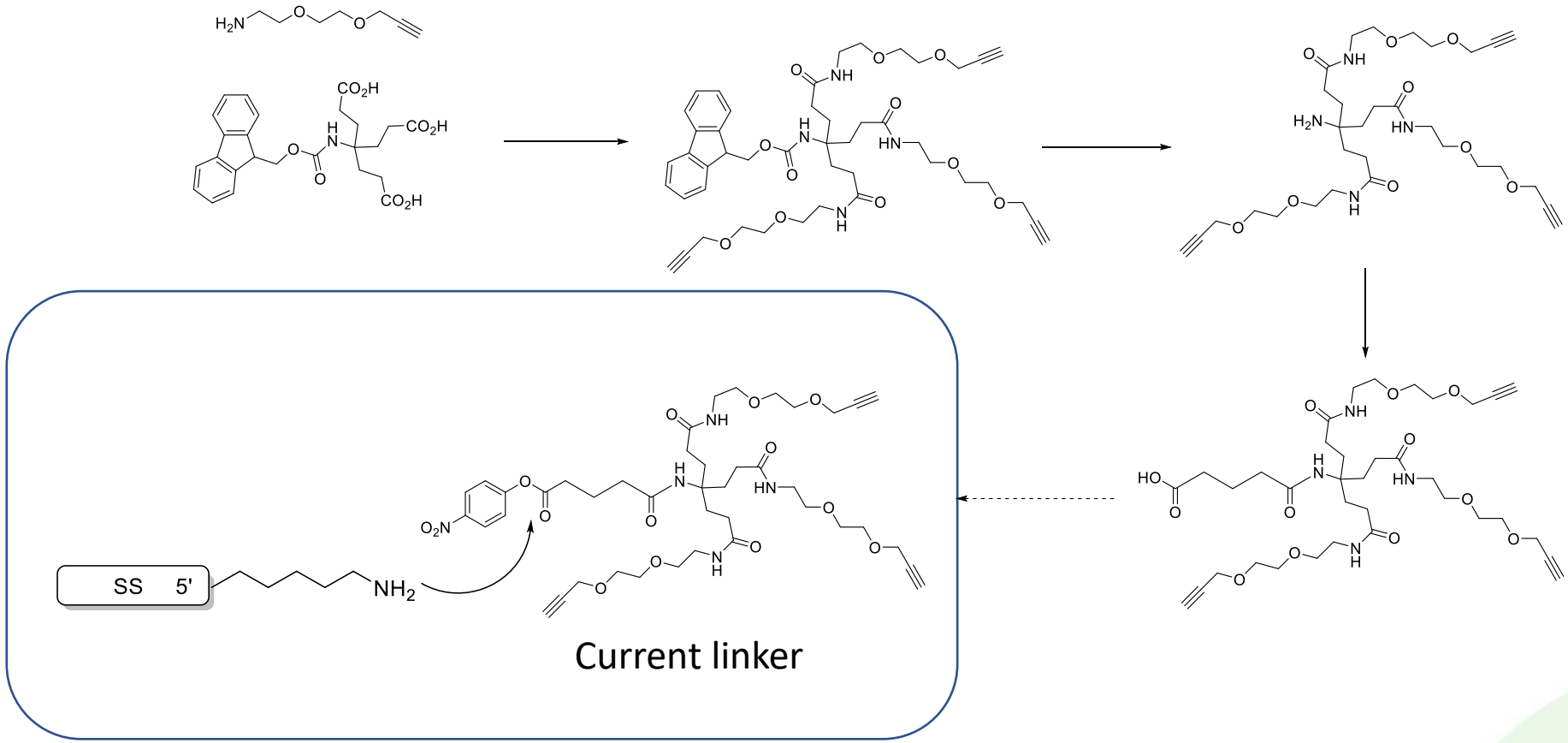
- Original route was not feasible and led to potential program delays
- Mechanistic understanding led to a novel idea allowing a successful cyclopropanation
- 1.4 g amidite initially delivered to mid-scale RNA team; new route is currently used to make 4 kg batches of amidite in support of large oligonucleotide campaigns
- The deprotection of the diethyl phosphonate led to interesting heat-flow question prior to scale-up, but ultimately a safe, scalable process
- Overall message: Develop challenging chemistry internally for the best chance of success!

How to Design a Manufacturing Friendly Linker



Should a target be redesigned to help streamline manufacturing?

Is this a Friendly Linker?

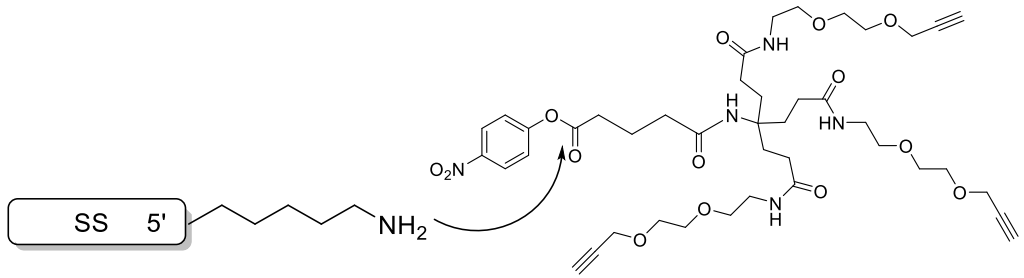


Is this OK to carry through manufacturing?

Activated Ester Linker Potential Issues

Where do we see potential issues?

- Potential PNP-ester stability issues
- Need for solution-phase conjugation
- Solution-phase chemistry may necessitate a second purification
- Method needed to detect *p*-nitro phenol
- Long term this would prove a challenging RSM to justify



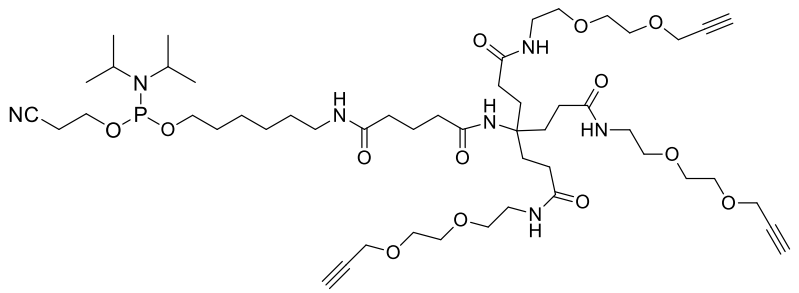
Current linker

Manufacturing Strategy: Can we take advantage of the 5’ end and change this into an amidite?

Amidite Potential Issues

Amidites don't come without risks

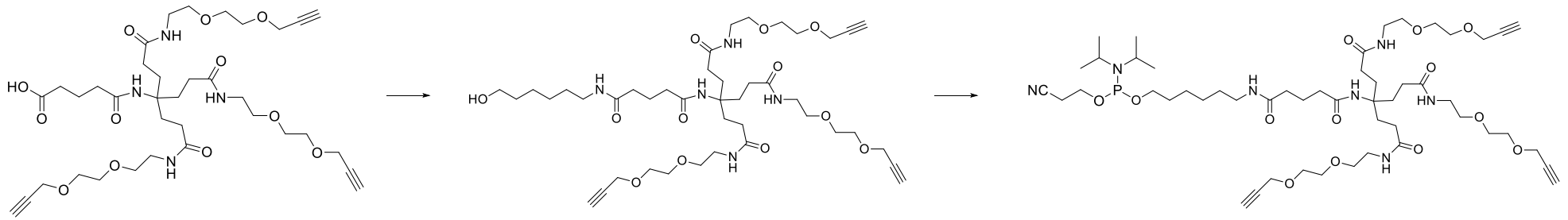
- Primary alcohol derived amidites may have stability/synthesis issues (hydrolysis)
- Unknown coupling efficiency
- One extra step to manufacture



Amidite Goal

Early goal: produce a few hundred grams internally to test out in manufacturing campaign

Synthetic Route Was Straightforward



1st Scale-up campaign 169 g in 3 batches!

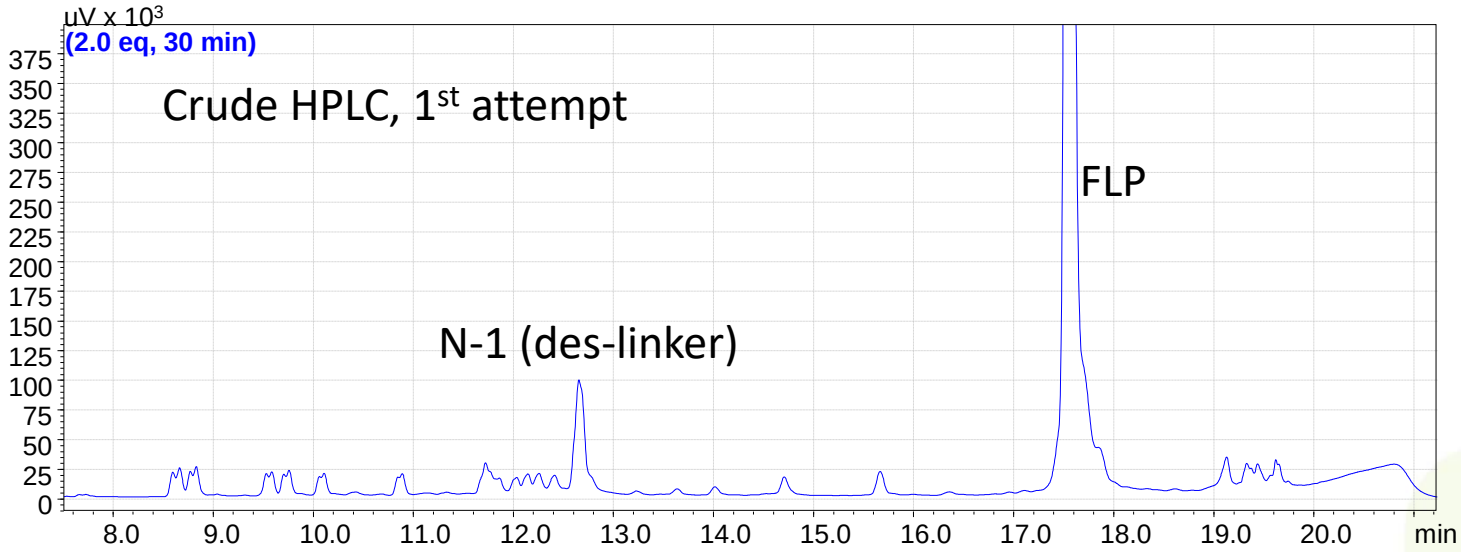
Risks seem mitigated

- Primary alcohol-derived amidite didn't impact synthesis
- Stable in ACN solution for duration of oligo synthesis
- Concept to scale-up in one month time (from intermediate)
- Extra synthesis step will be worth avoiding solution-phase activated ester if the amidite coupling is efficient

Linker Coupling/Summary

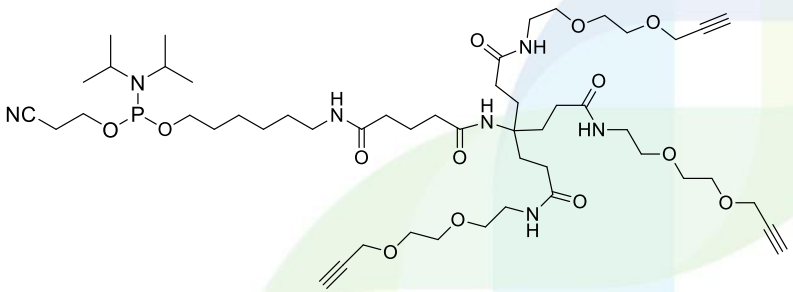
Initial 0.2 mmol scale:

- 0.2 mmol scale
- 2 equivalents of amidite
- 92% conversion (FLP vs N-1)

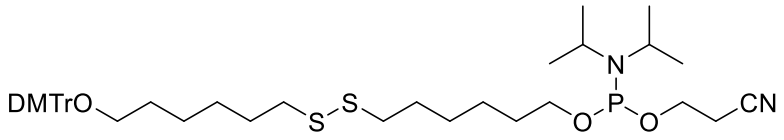


Summary:

- Idea through proof of concept took less than 2 months
- Amidite has shown to be an attractive alternative to solution-phase activated ester chemistry
- Fast forward to present day: Amidite is being scaled to multi-kilo batches with crude oligo synthesis purities of ~80% and ~97-99% conversion



Impurities During Disulfide Scale-Ups

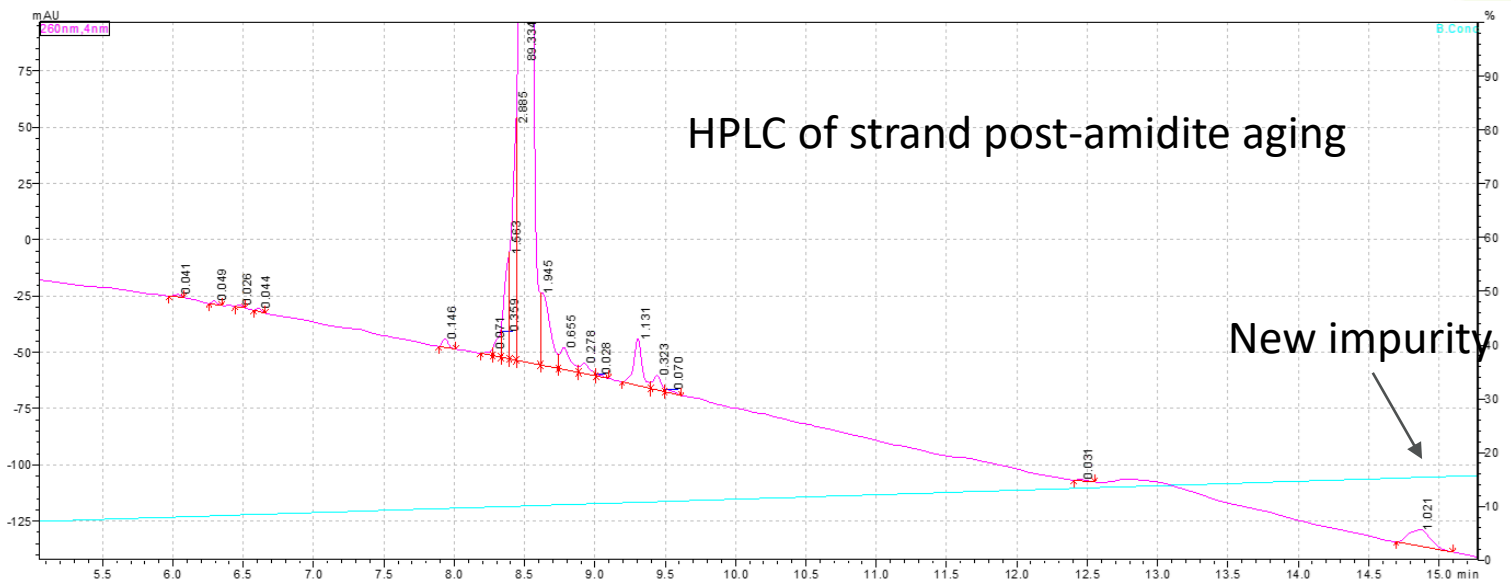


C6-SS-C6 phosphoramidite

- Discovery team relayed the amidite was unstable and should be prepped right before coupling
- This was acceptable for initial scale-ups (32mmol scale)
- Long-term goals:
  - Understand how long we have stability in solution
  - Understand how the amidite is decomposing
  - Try to find a more scale-friendly fix than just-in-time mixing/use

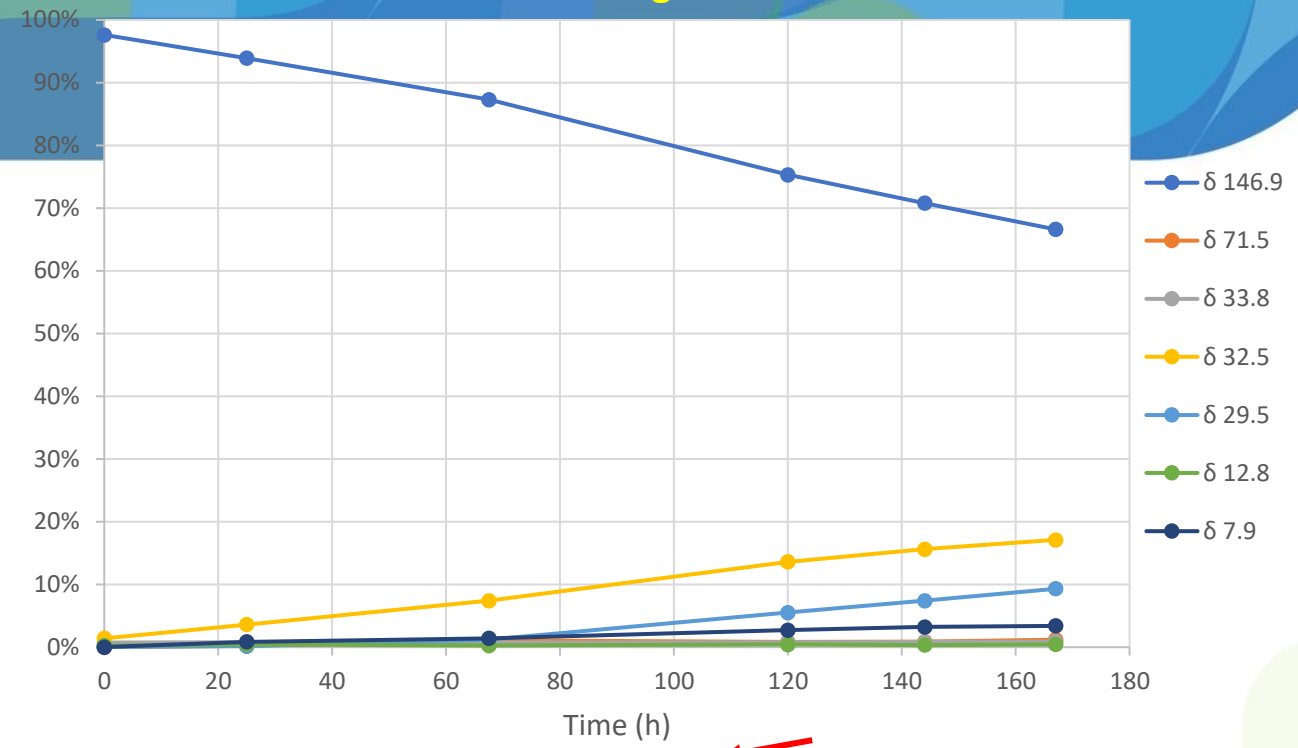
Can We Measure the Instability?

- Just-in-time mixing of the amidite leads to normal syntheses
- When purposely leaving the amidite in ACN for 24 hours, a new peak shows up in the synthesis and yields are lower
- High risk for a new impurity above qualification limits!
- Very late eluting on RP chromatography
  - Hints of extra "grease" or incorporations of C6 fragments
  - Ranges between 1 and 3 area percent
- Time to look at the actual amidite



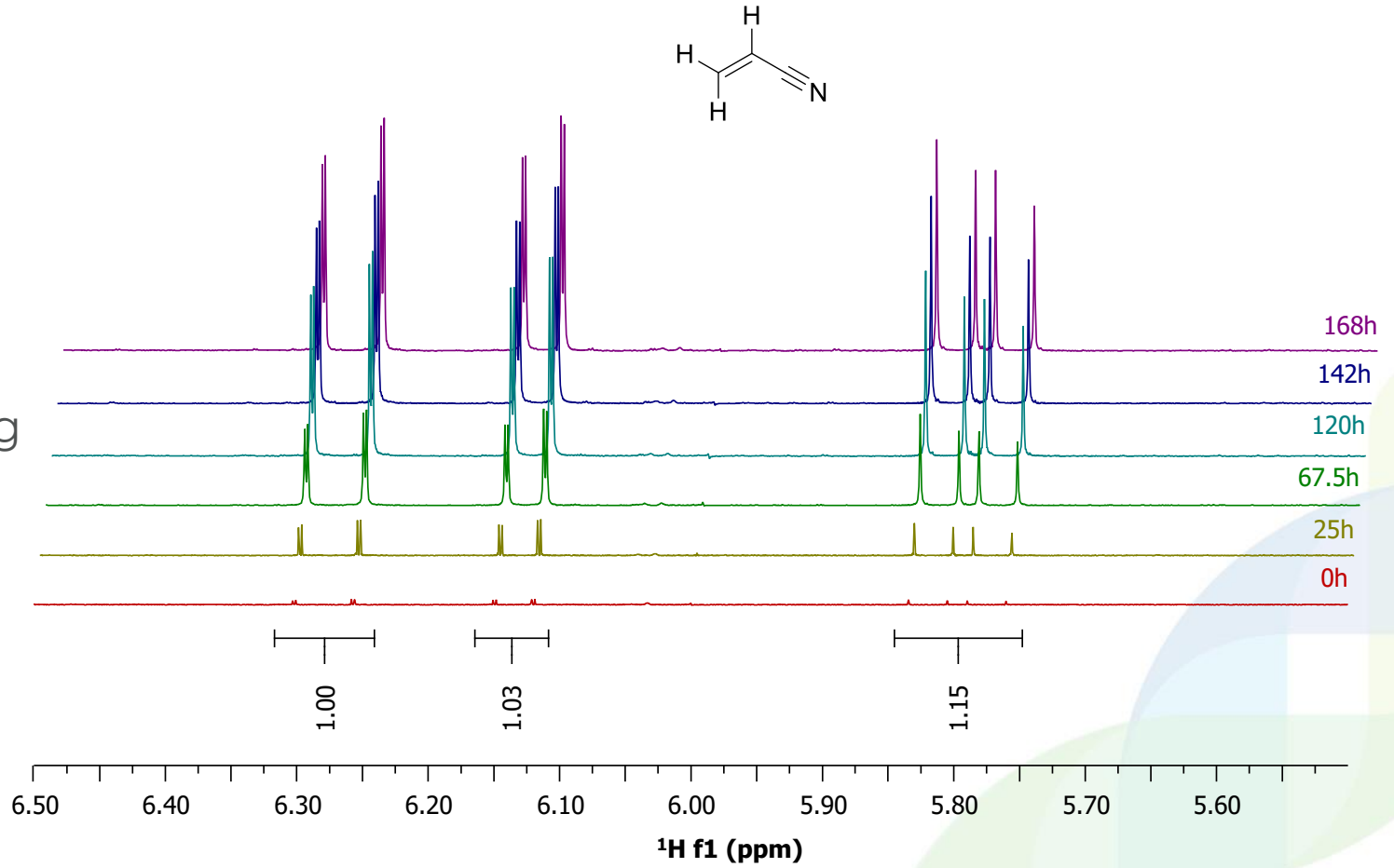
C6-SS-C6 Amidite in CD_3CN: ^{31}P NMR

- Even after 1 day, significant decomp.
- δ 32.5, 29.5 grow in quickly
- δ 71.5, 7.9 grow in slowly
- δ 33.8, 12.8 don't change much



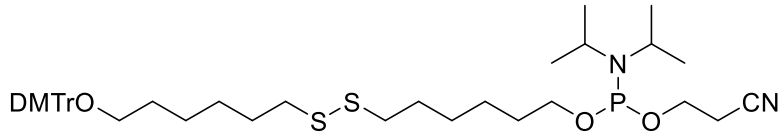
C6-SS-C6 Amidite in CD_3CN: ^1H NMR

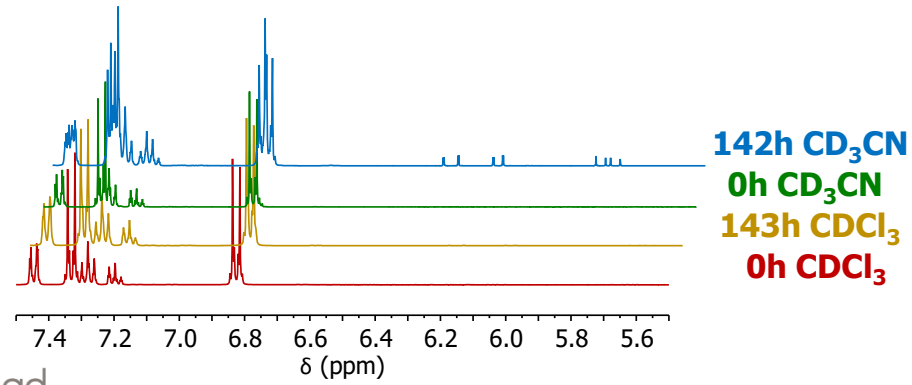
- In CD_3CN: Acrylonitrile grows in significantly
- Leads to a multitude of mechanistic pathways for impurity growth
- We wondered if ACN could be acting as a basic/nucleophilic solvent in the presence of the disulfide
- Next: try non-nucleophilic solvents

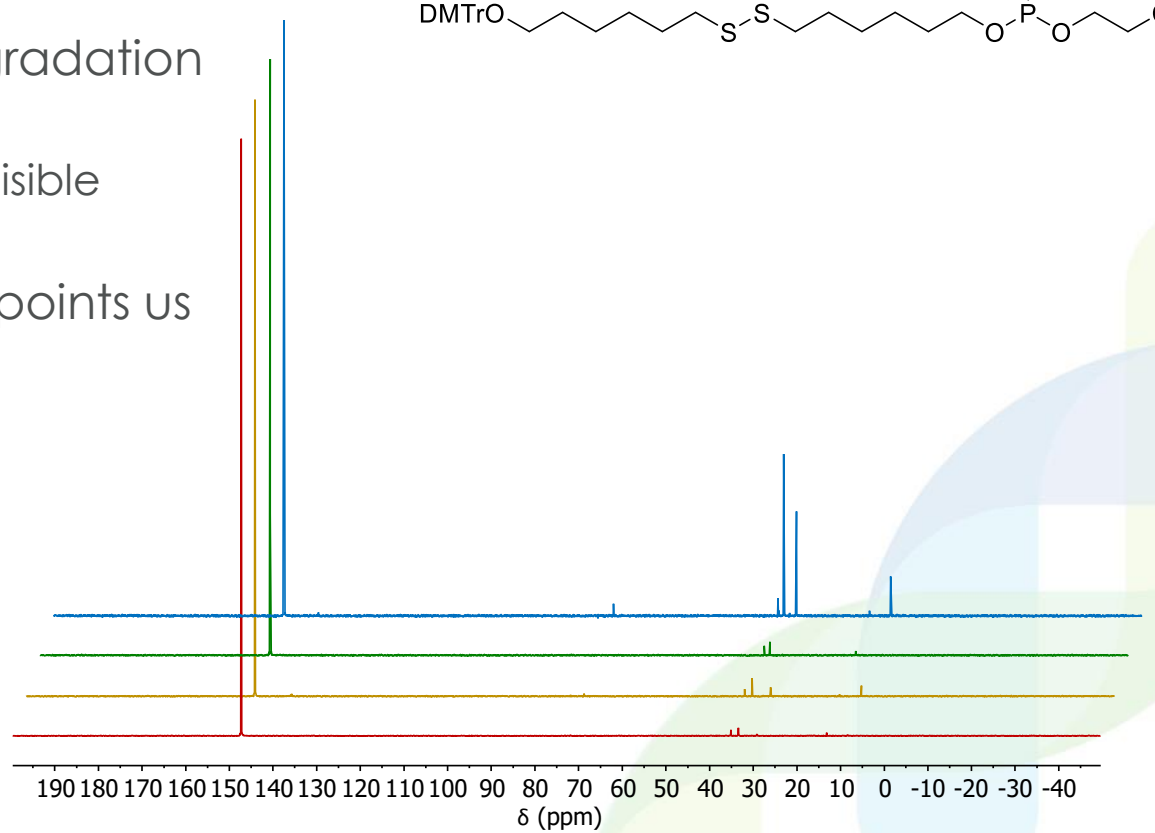


NMR Comparison: CD_3CN vs $CDCl_3$

- Over 7 days, $CDCl_3$ spectra largely unchanged
  - ^{31}P: Slight increase in δ ~32, ~30, and ~13 peaks
  - ^{1}H: One set of DMT (shown) and OMe peaks; can only see acrylonitrile if you zoom in
- Over 7 days, CD_3CN spectra show significant degradation
  - ^{31}P: Large δ ~32, ~30 peaks
  - ^{1}H: Two clear sets of DMT and OMe peaks; clearly visible acrylonitrile
- Developing analytics around the decomposition points us towards a long-term solution







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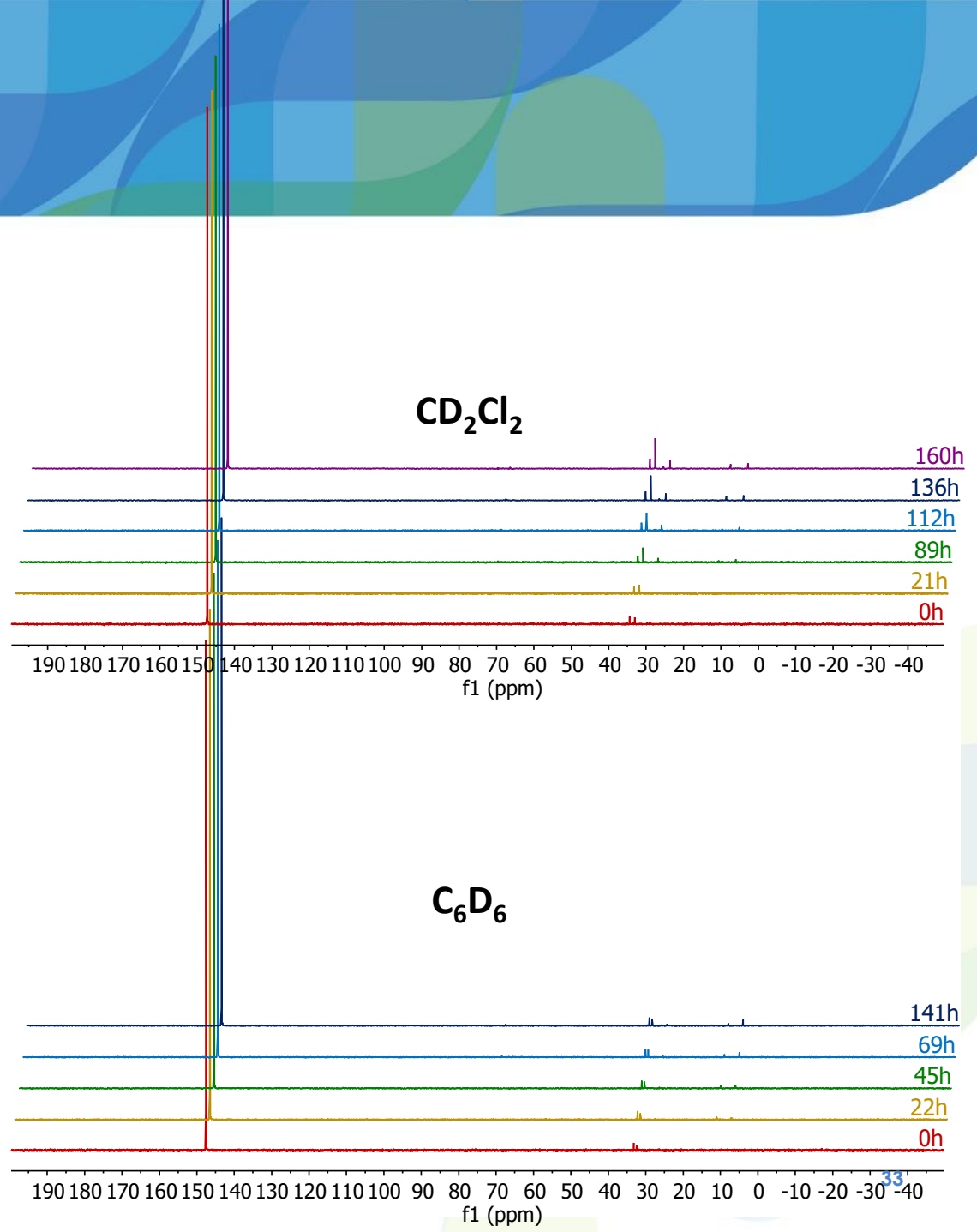
Other Solvents: C6-SS-C6 in CD_2Cl_2 and C_6D_6

- Slightly less stable in CD_2Cl_2
  - Small δ 33 ppm peak grows in
  - DCM isn't a very process friendly solvent

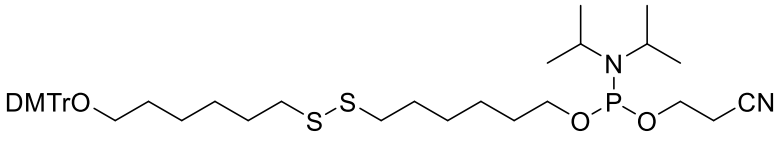
- Toluene: Used C_6D_6 as proxy for NMR expt.
  - Best one yet: δ 30-35 ppm peaks do not change
  - Still >95% pure in solution after 6 days!
  - Already part of our residual solvents method!

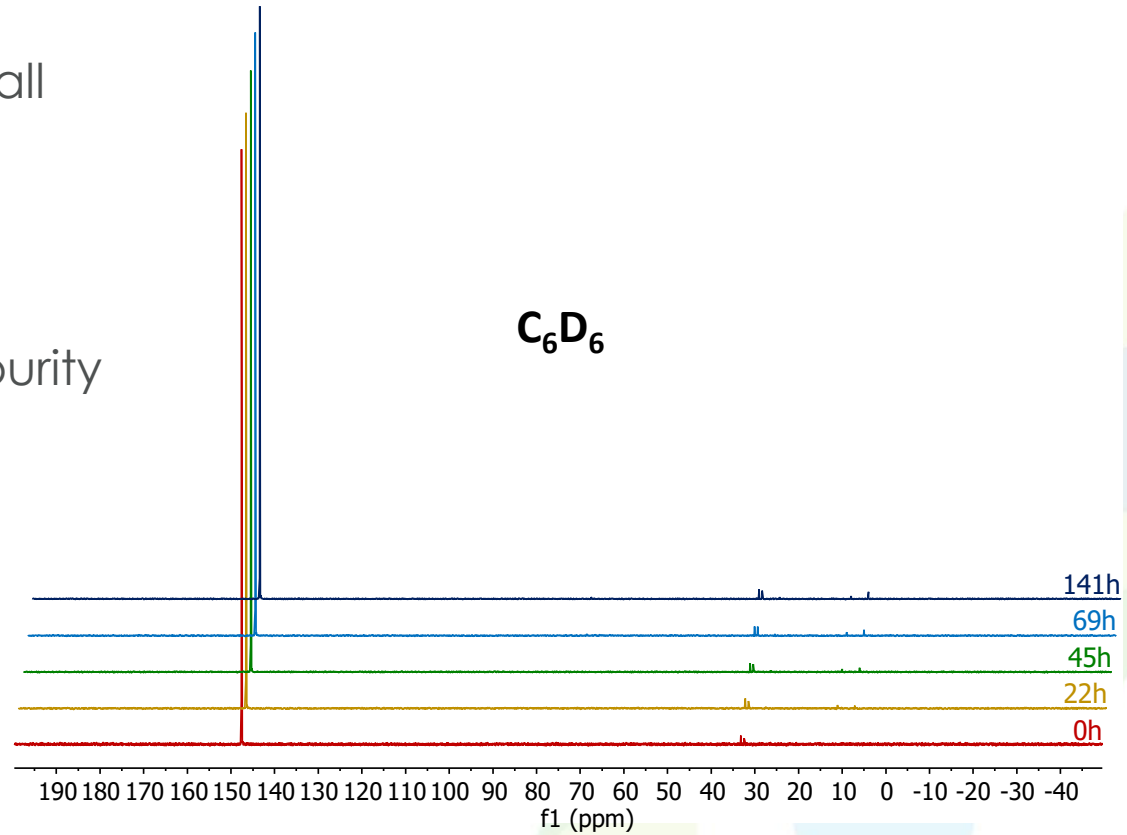


Finalizing the Fix

- With good solubility and stability, a toluene amidite solution was chosen to scale-up 48 mmol syntheses
- Disulfide amidite is now prepped at the same time as all standard amidites without timing constraints
- Impurity risks have been mitigated

- Future work: finalize mechanistic understanding of impurity formation





Tying the Case Studies Together

Arrowhead manufacturing strategy

- Process chemistry understanding
- Internally develop chemistry across teams
- Understand mechanisms
- Design scalable molecules
- Early manufacturing engagement
- Speed of development enabled by scientific understanding
- Collaboration, innovation and speed are part of our corporate culture

Arrowhead Team Thanks!

- CpU
  - Erich Altenhofer, Pankaj Kumar, Leo Joyce, Matthew Fowler-Watters, Tao Pei, Zhen Li, Nathan Logic, Josh Pletzke
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  - Erich Altenhofer, Brendan Mowery
- Disulfide
  - Brendan Mowery, Angie Fenrick, Josh Pletzke, Fred Fleitz, Zach Trilling
- Analytical Development, QA, QC, project management
- Arrowhead Senior Management
- Audience