In search of a reagent for selective deprotection of N6-benzoyl deoxyadenosine for the solid-state synthesis of modified DNA with modified purines

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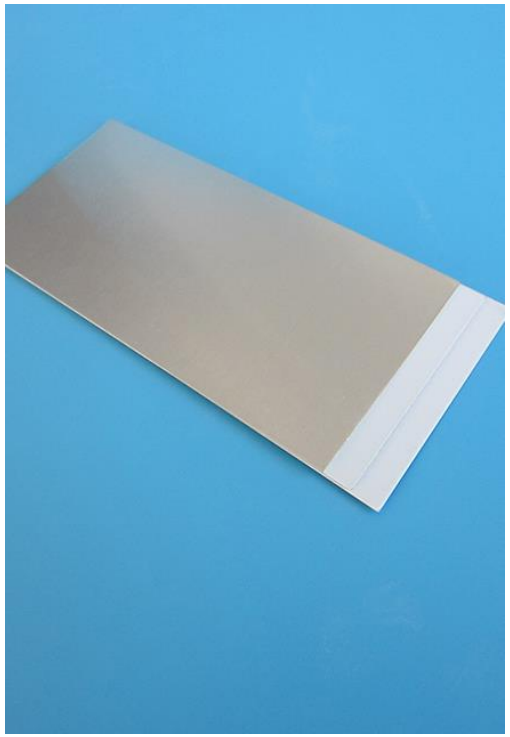
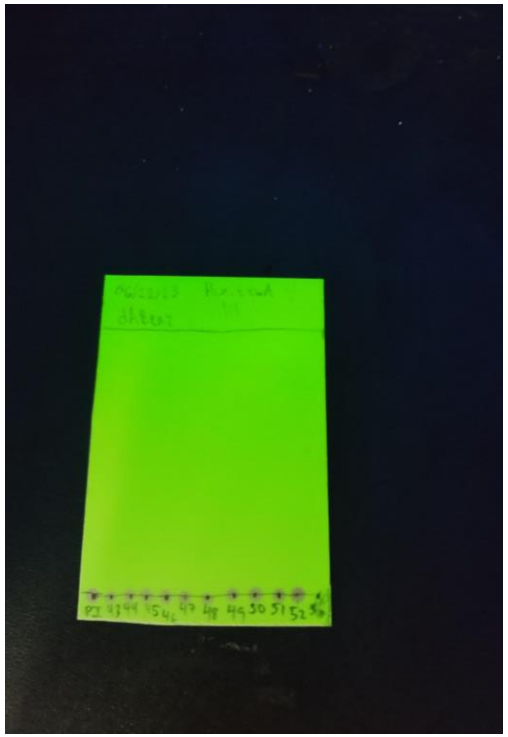
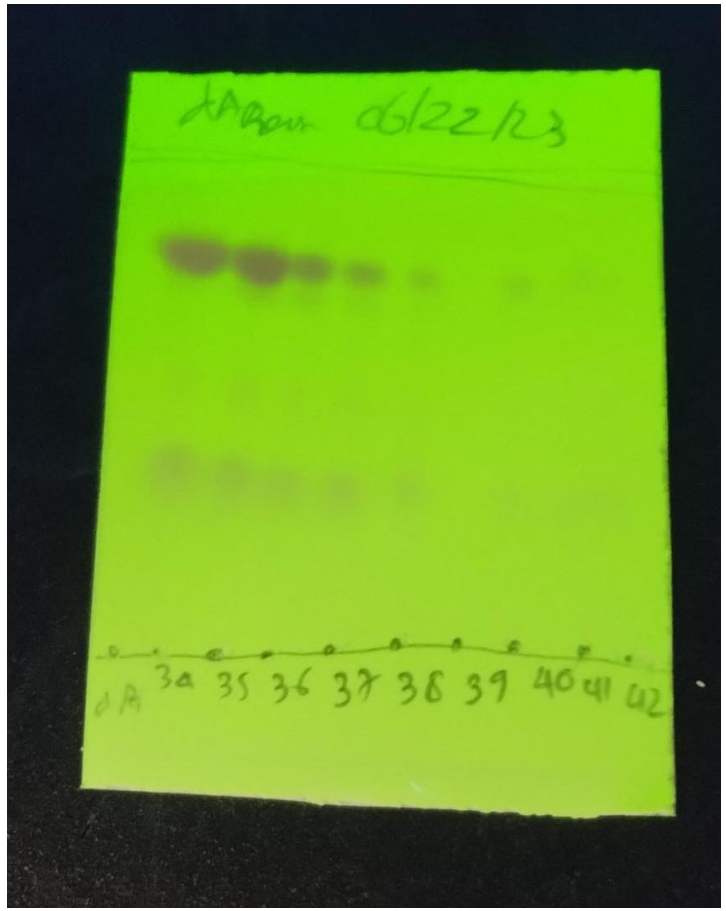


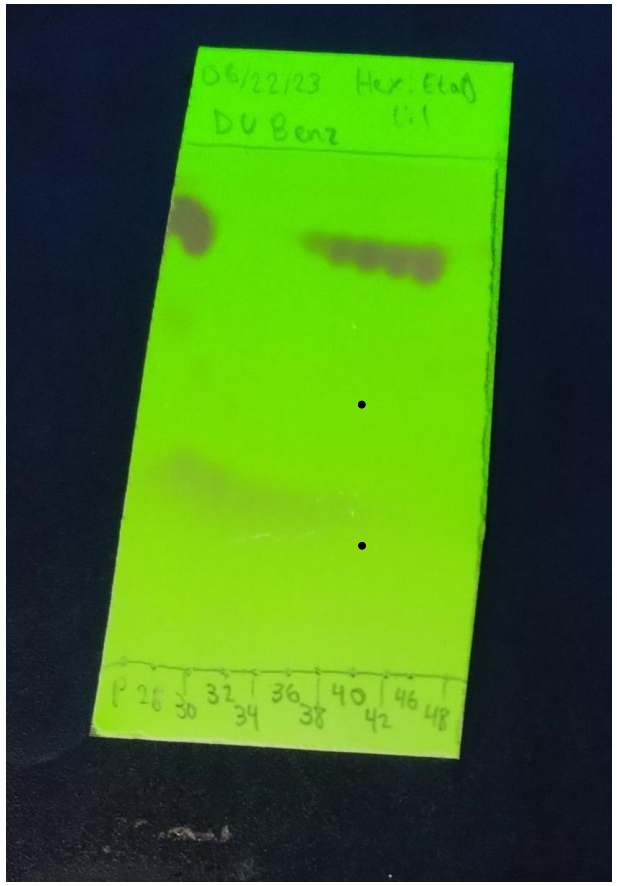


TLC

- Thin Layer Chromatography
- How does it work?
  - highly polar silica powder on aluminum plate
  - Stationary phase
    -spot product or reaction mixture
    -spot sample mixture
    -shows up under UV light
  - Mobile phase
    -paper is dipped in solvent
    -different solvents and different ratios are used for different reactions
  - Less polar will travel farther up

Mixture with more product in tubes 34, 35
Some in 36, 37, 38





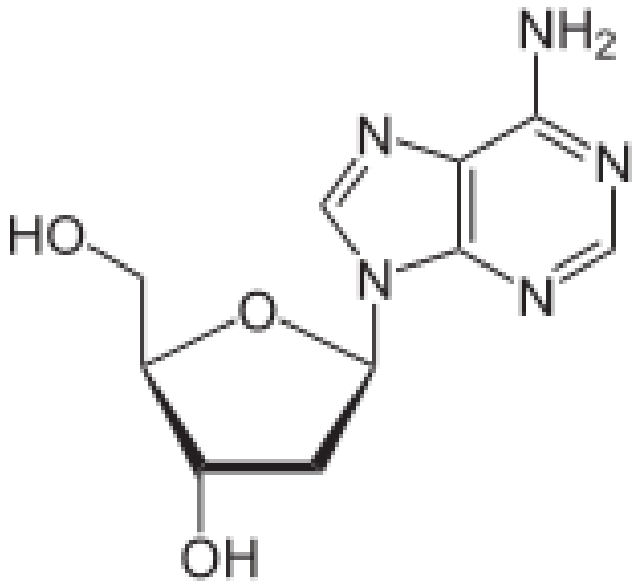
Research Update

Modification of Organic Nucleosides

Introduction

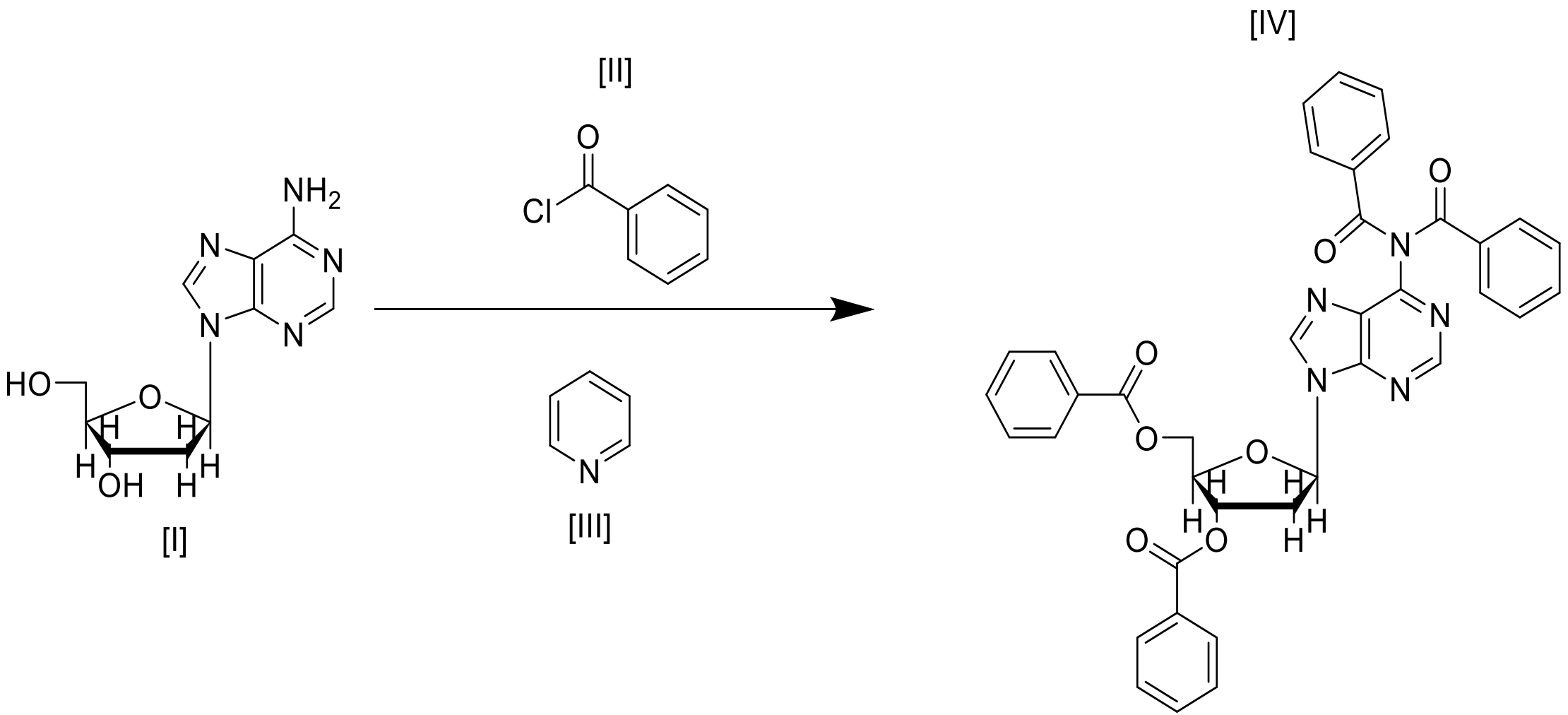
- All nucleosides contain a 5-carbon sugar and a nitrogenous base.
- In this project we are synthesizing nucleosides that contain R groups.
- We intend to use them to modify aptamers.
- It is necessary for the N-6 to be protected, otherwise it will interfere with the phosphodiester bonding.
- In this project we protected and then partially deprotected adenosine molecules.

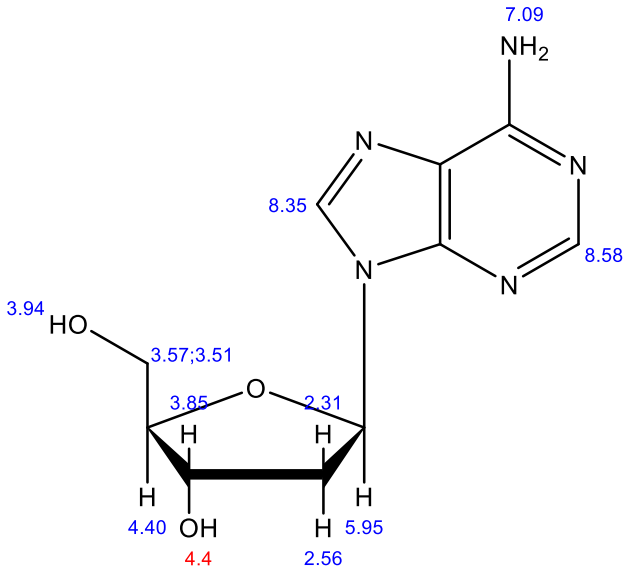
deoxyAdenosine



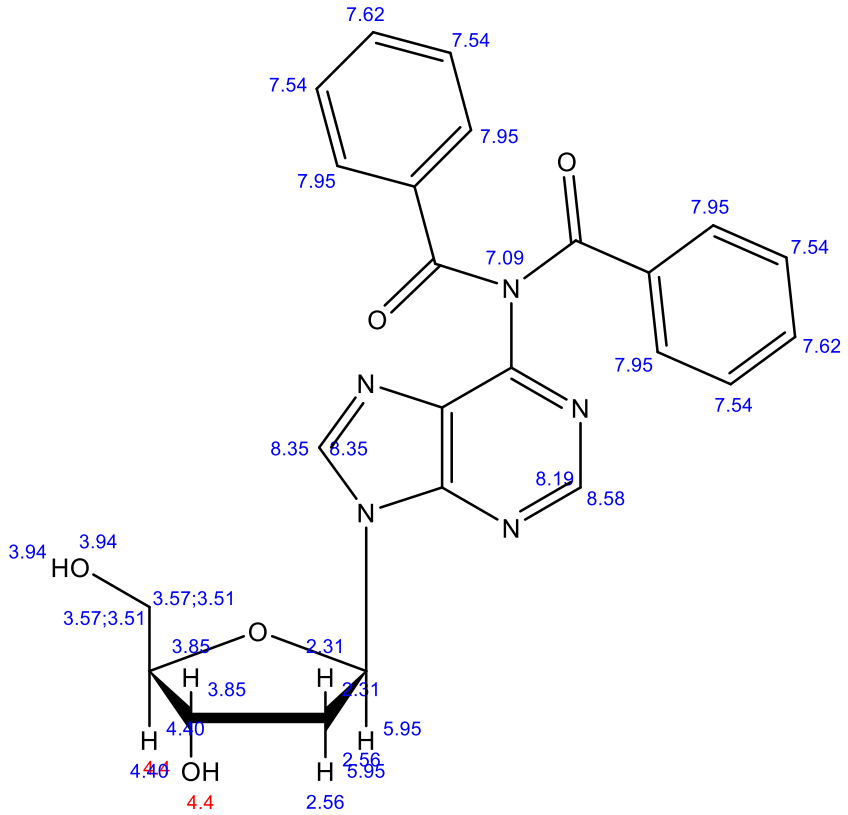
- Contains deoxyribose sugar
- Contains H at the 2’ instead of OH in Adenosine
- Abbreviated as dA

dA Benzoylation Reaction

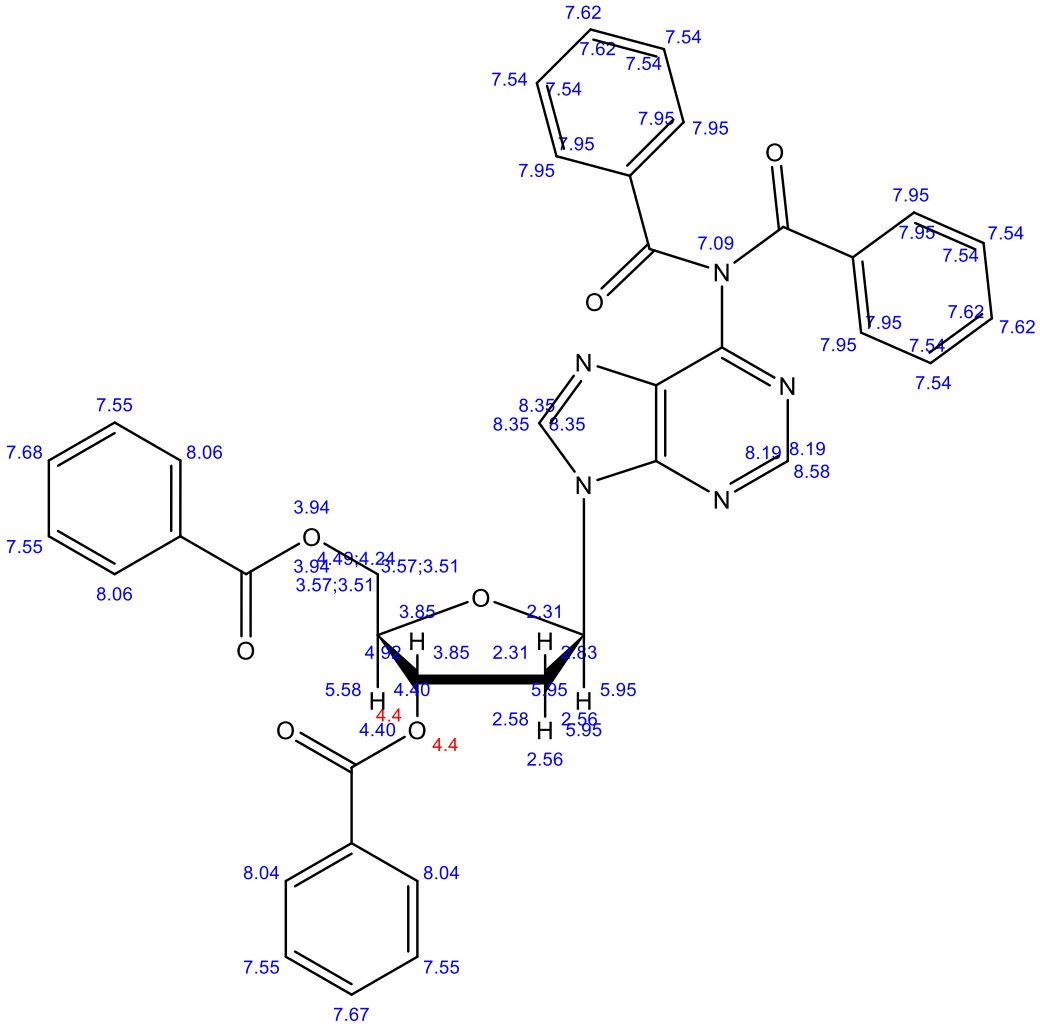




Fully deprotected dA with predicted NMR shifts

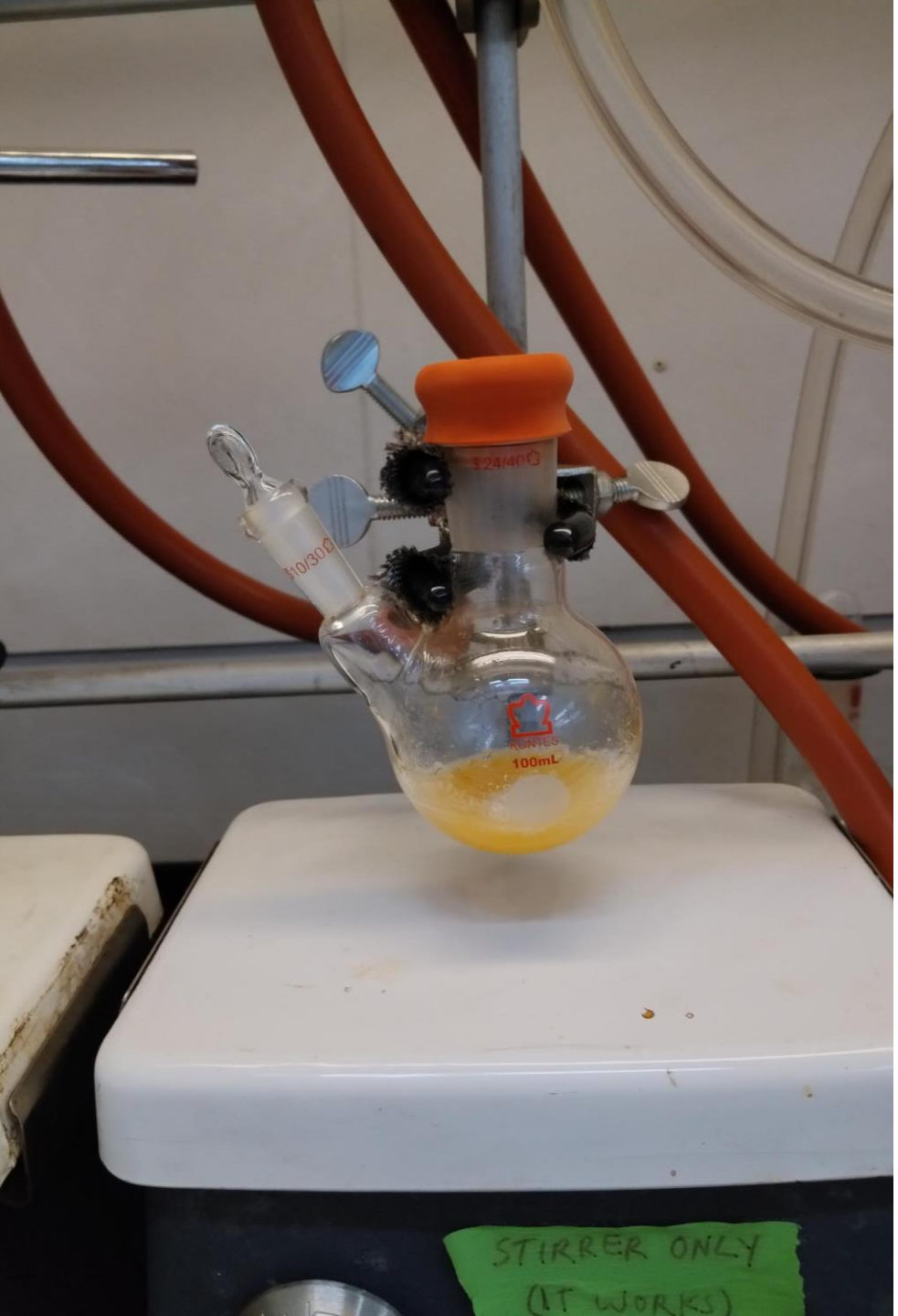


Partially deprotected dA with predicted NMR shifts



Fully benzoylated dA with predicted NMR shifts

Materials & Methods



Reaction Setup:

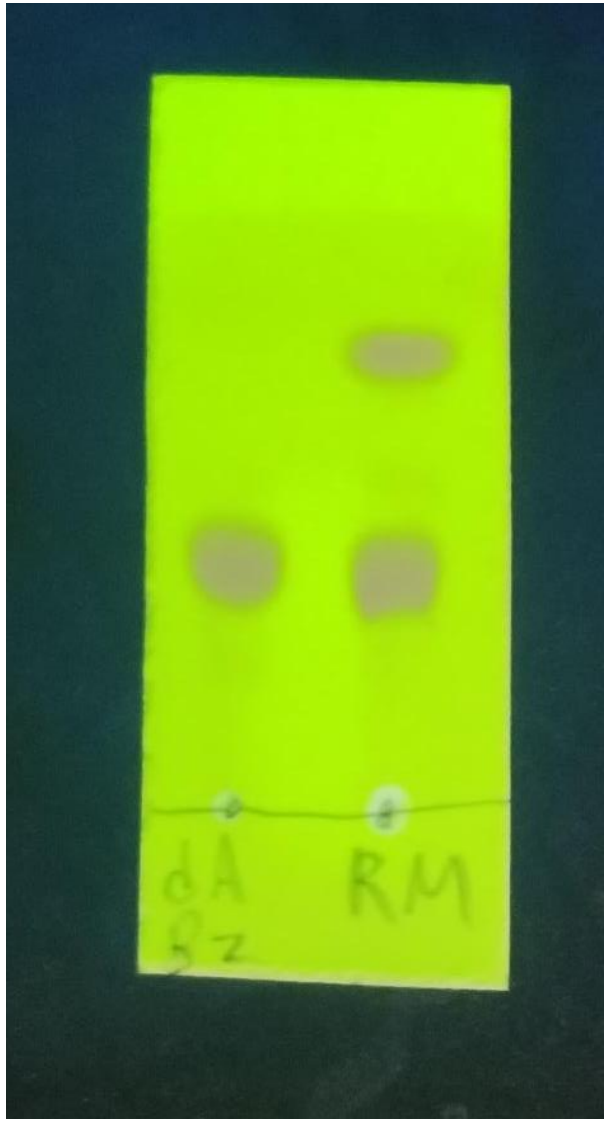
0.5 g dA

1.8 mL benzoyl chloride

4.9 mL pyridine

Placed under fume hood with magnetic stirrer

3 hours

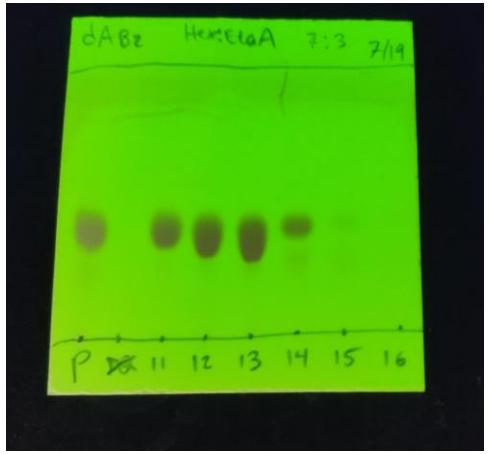


- Separate organic & water layers using separatory funnel
- Add sodium sulfide to remove water
- TLC organic layer

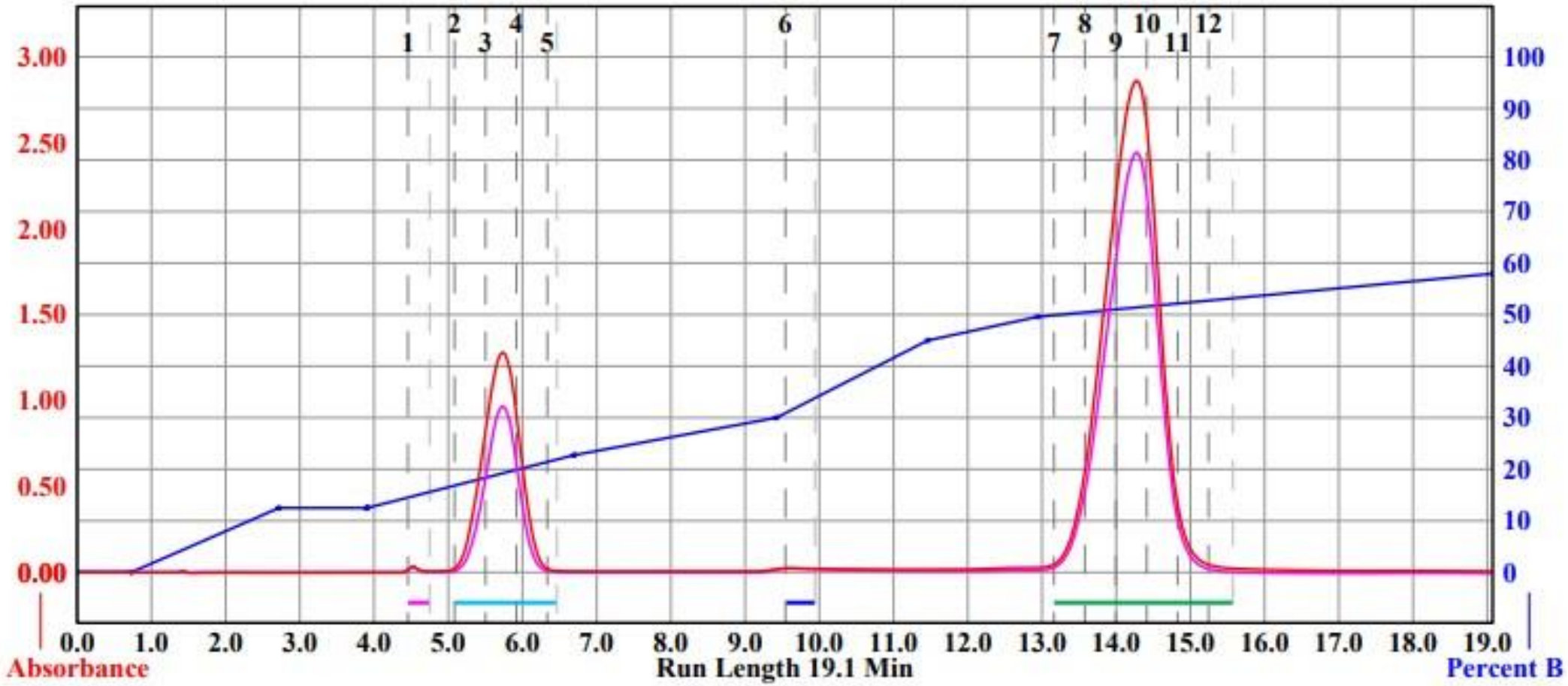
- Dry in Rotovap
- Purify using flash chromatography
- TLC tubes that comprise peak

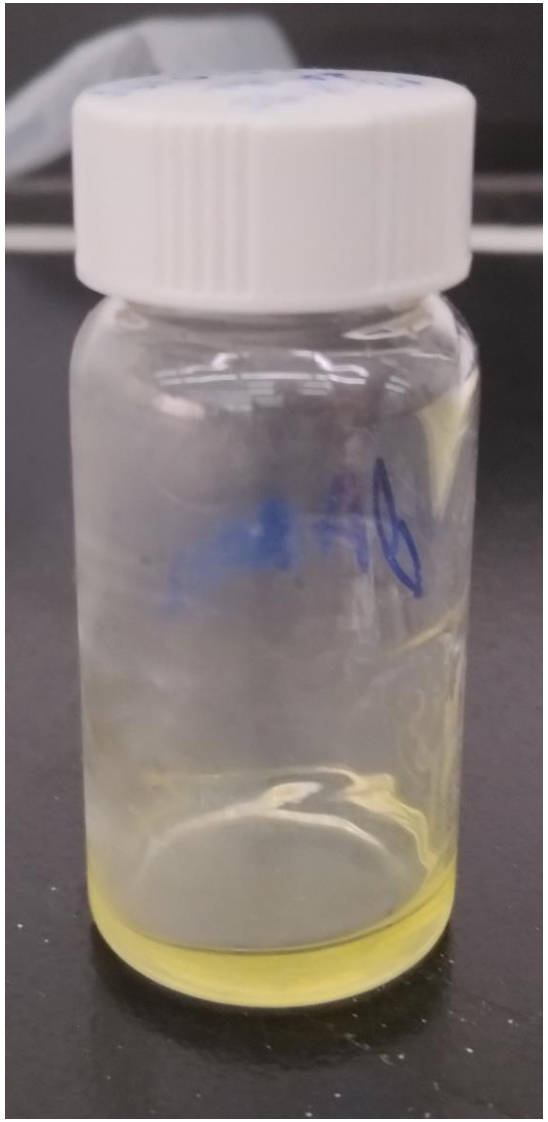
Combiflash

TLC



Combiflash Graph





- Tubes containing the compound were combined
- Dried in Rotovap
- Product was weighed
- 90% yield was obtained

Deprotection:

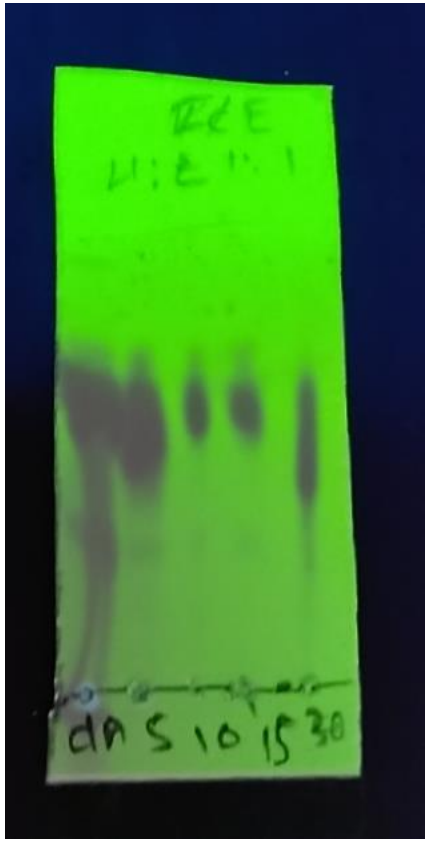
- vial 1: 28.4 mg dA & 0.5 mL K_2CO_3 at 0 °C
  TLC at different time intervals to check for cleavage

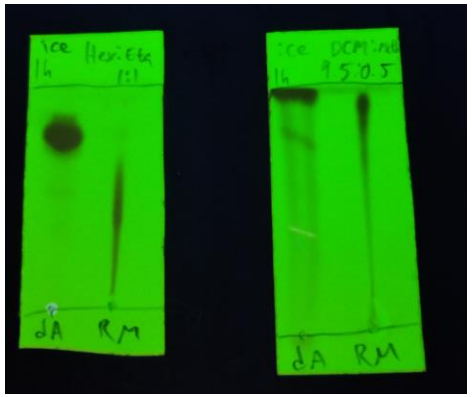
- vial 2: 26.8 mg dA & 0.5 mL K_2CO_3 at room temperature
  TLC at different time intervals to check for cleavage

- vial 3: 23.7 mg dA & 0.5 mL lithium diisopropylamide overnight on ice
  TLC to check for cleavage

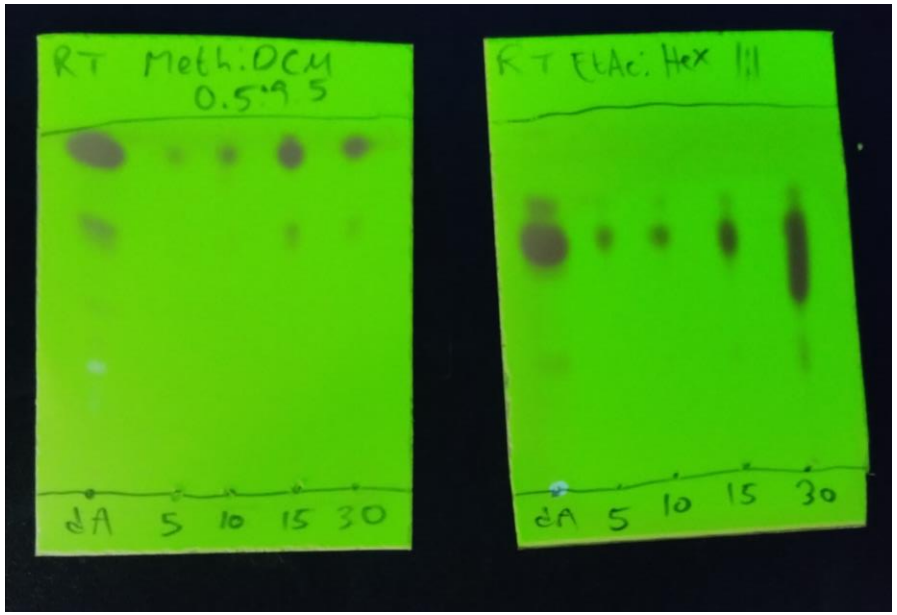
Data Analysis

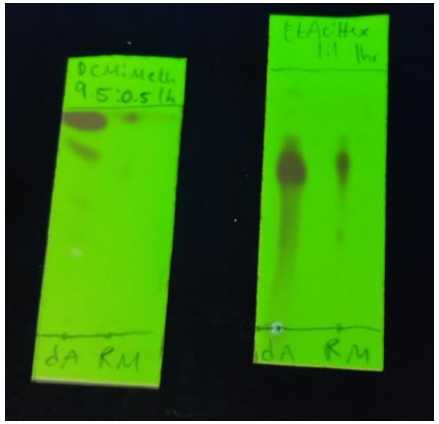
Vial 1:


dA 5 10 15 30

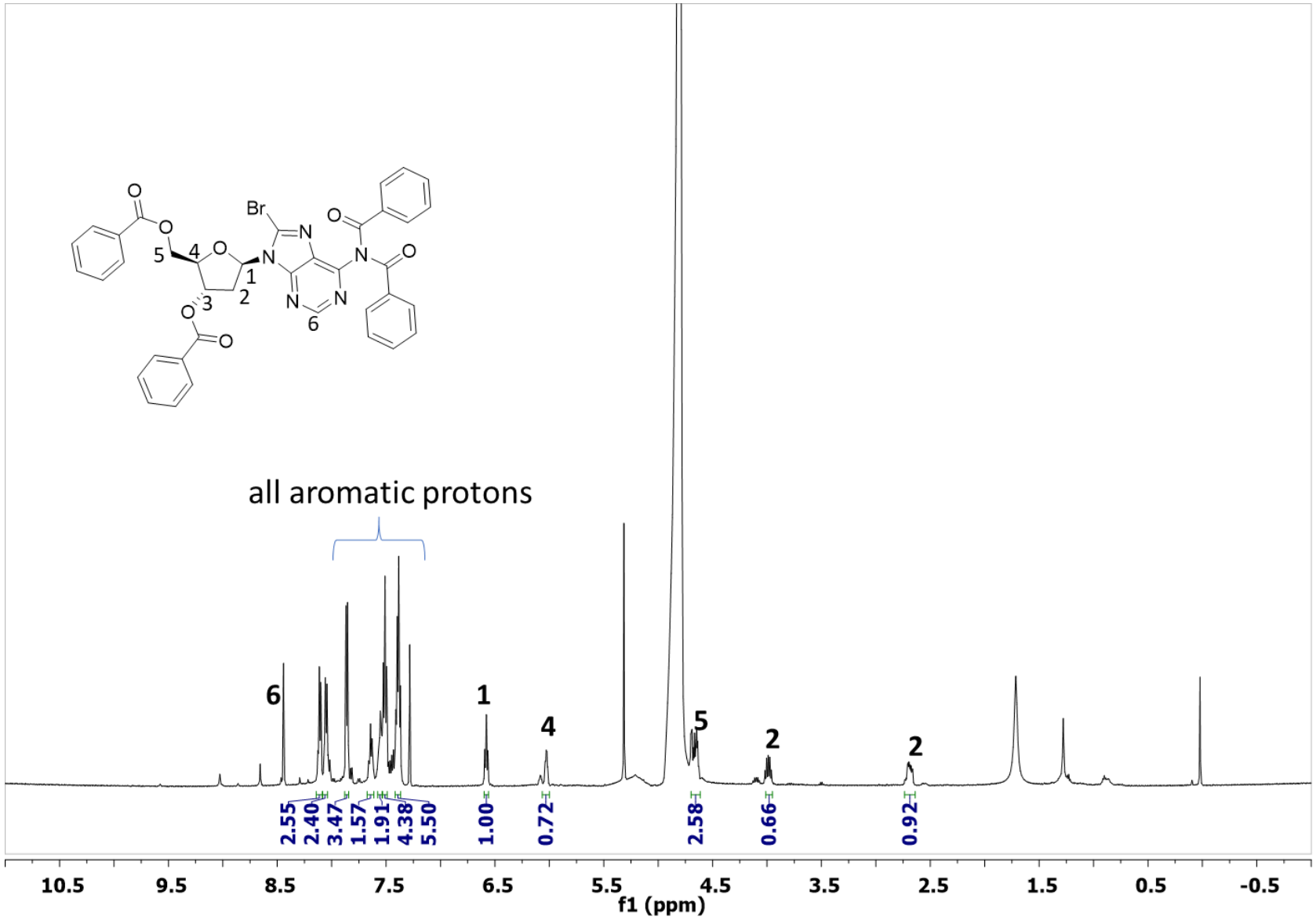

dA RM
dA RM

Vial 2:

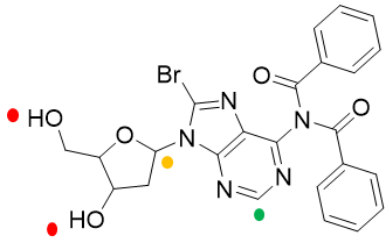

RT Meth:DCM 0.5:95
dA 5 10 15 30
RT EtAc:Hex 1:1
dA 5 10 15 30


dA RM
dA RM

Potassium Carbonate deprotection experiment NMR:



Lithium diisopropylamide deprotection experiment NMR:



500 MHz, DMSO-d6, 298 K

Conclusion:

- Potassium Carbonate did not deprotect the molecule at all, as shown in the NMR
- The product obtained from the lithium diisopropyl amide deprotection reaction is a mixture
- Must be performed again and purified to ascertain whether red dot's peak is indeed free OH

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.