SUMMER RESEARCH PROJECT
OHIO STATE UNIVERSITY
COLUMBUS, OHIO, USA

---

03 May, 2017 - 30 June 2017

PROJECT REPORT APPROVED:

Under the Supervision of:
Dr. Dennis Bong
Professor
Ohio State University
Columbus, OH, USA

Submitted by:
Ekroop Kaur Cheema
Summer Intern
Indian Institute of Technology
Kharagpur, WB, India

ACKNOWLEDGEMENT

This research project in itself is an acknowledgement to the inspiration and the technical assistance contributed to it by many individuals. The project and its report work would have never been completed without the guidance and assistance that I received from time to time through whole research process.

I express my sincere gratitude to my Supervisor **Dr. Dennis Bong** (Professor, Department of Chemistry and Biochemistry, Ohio State University) for giving me an opportunity and unconditional support to enhance my skills in the field of Organic Synthesis and BioChemistry. I am grateful to him for letting me explore the field of my project and learn the laboratory oriented work and also for providing every possible required resource during the project.

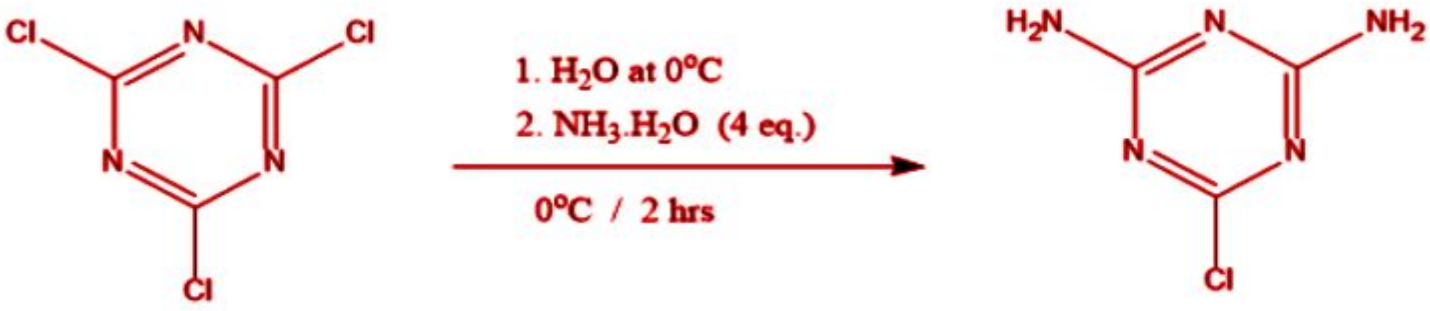
I would also like to thank **Yufeng Liang** (Phd, Department of Chemistry and Biochemistry, Ohio State University) and **Sarah Rundell** (Phd, Department of Chemistry and Biochemistry, Ohio State University) for their immense cooperation and support throughout the research project and for teaching me all the bits of the laboratory techniques and concepts. I shall always be grateful to them.

Ekroop Kaur Cheema
Summer Intern

Organic Synthesis

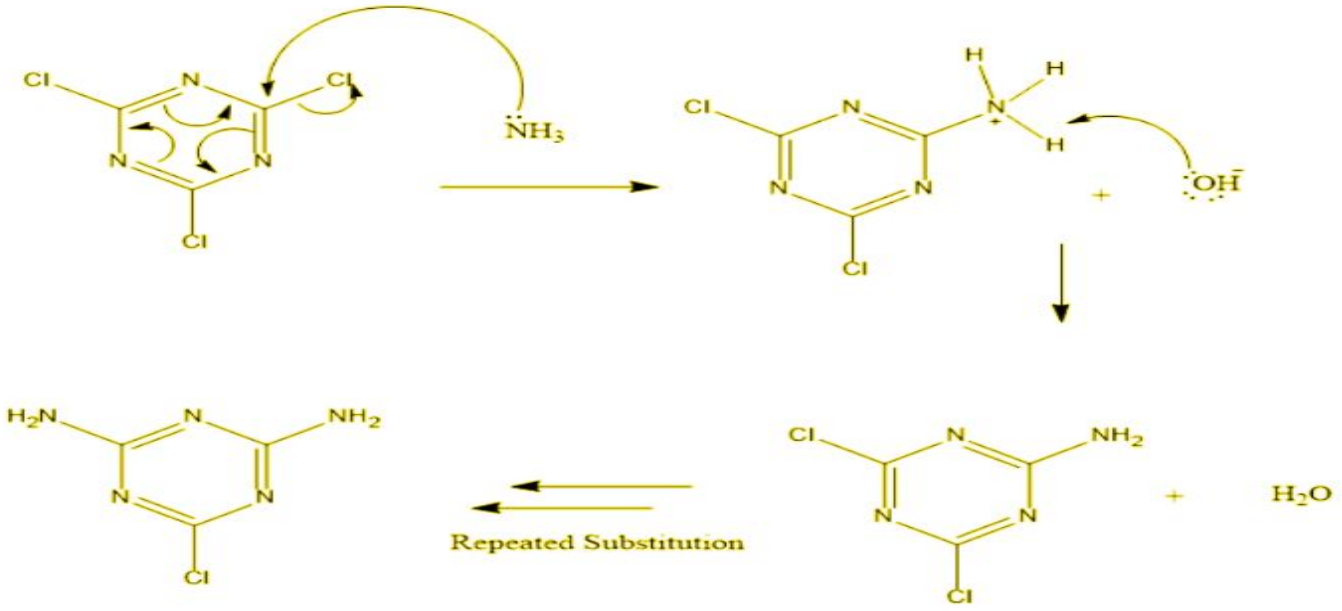
The summer internship included the organic synthesis followed by the NMR which included the following.

1. Cyanuric Chloride to 2-Chloro-4,6-diamino-[1,3,5]-triazine



Compound	*CYANURIC CHLORIDE*	*NH_4OH*
Molecular Weight (g/mole)	184.41	35.04
Weight Taken (g)	5	3.78
Milli Moles	27	108
Equivalents	1	4
Density (g/ml)	-	0.88
Volume (ml)	-	4.33

MECHANISM



PROCEDURE:

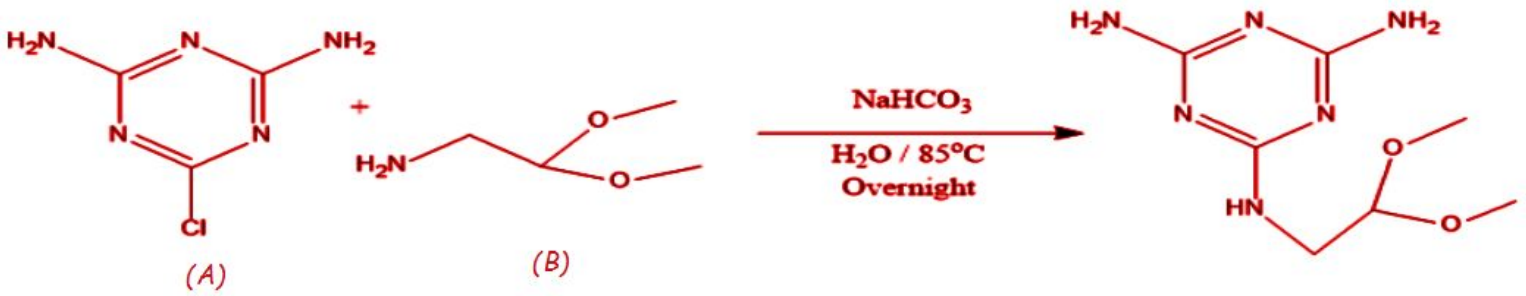
Cyanuric Chloride (5gm) was added to 40 ml of acetone in a round bottom flask and stirred for 5 minutes to let it dissolve completely. Then it was poured into a rb filled with 40 ml of water at 0^0C. Then NH_4OH was added to the flask dropwise and the reaction was continued for 2 hours and then the rb was kept in a sand bath for overnight at a temperature of 55^0C. The orange colored product was filtered using vacuum filtration and washed with water to remove the color completely. NMR spectra was taken of the compound.

Amount of product : 3.3345g

Yield of the reaction : (3.3345/(0.027*145.55)) = 0.848

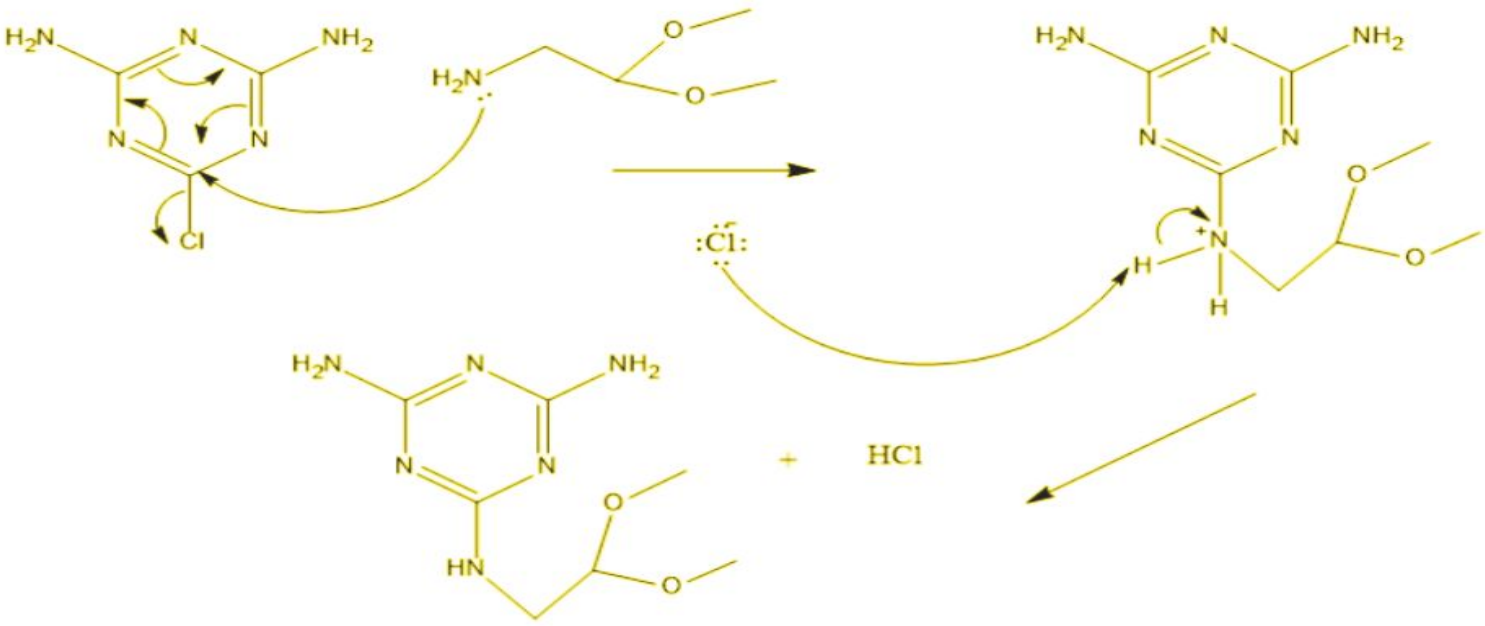
84.8 % yield

2.



Compound	(A)	(B)	$NaHCO_3$
Molecular Weight (g/mole)	145.55	105.14	84.00
Weight Taken (g)	3.33	3.122	2.125
Milli Moles	23	29.7	25.3

MECHANISM

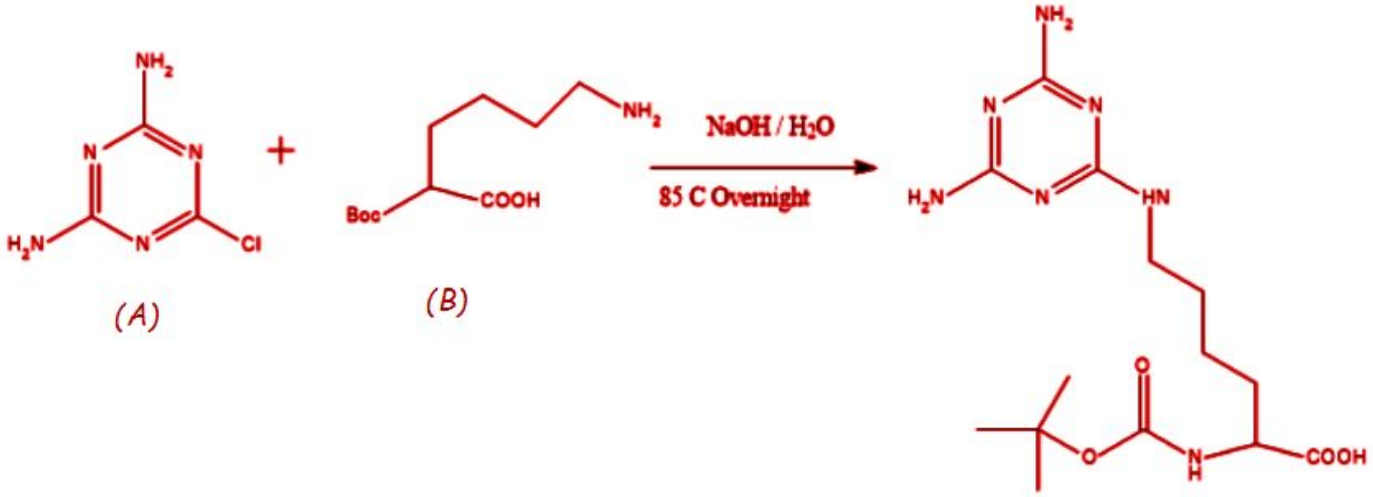


PROCEDURE:

Amino acetaldehyde dimethyl acetal was dissolved in H_2O at which point (A) and $NaHCO_3$ were added to the solution. The reaction was heated to 85^0C and stirred overnight. After cooling to RT the reaction was filtered and solid was washed with water twice. After drying under vacuum, the product is collected as a white solid.

Amount of product : 4.67g
Yield of the reaction : (4.67/(0.023*214.12)) = 0.950
95 % yield

3.



Compound	(A)	(B)	NaOH
Molecular Weight (g/mole)	145.55	246.31	40
Weight Taken (g)	3.33	4.73	1.54

Milli Moles	23	19.2	38.3

PROCEDURE:

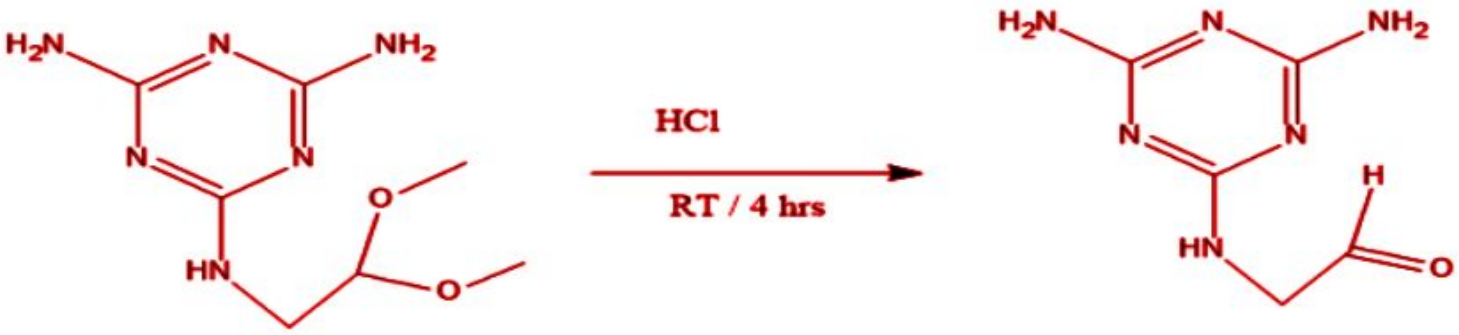
Boc-Lys-OH was dissolved in water and was added to a suspension of compound (A). 38.3 mmols of NaOH was added to it. 60 ml of water was slowly added to the mixture and the reaction was stirred overnight at 85^{0}C. The reaction was then cooled down to room temperature and the white solid was filtered off. Aqueous solution was acidified to a pH 5 with HCl at 0^{0}C. Resulting precipitates were filtered and washed with water (2*15 ml) and dried in vacuum to give 93.4% yield.

Amount of product : 6.67g

Yield of the reaction : (6.67/(0.023*355.20)) = 0.934

93.4 % yield

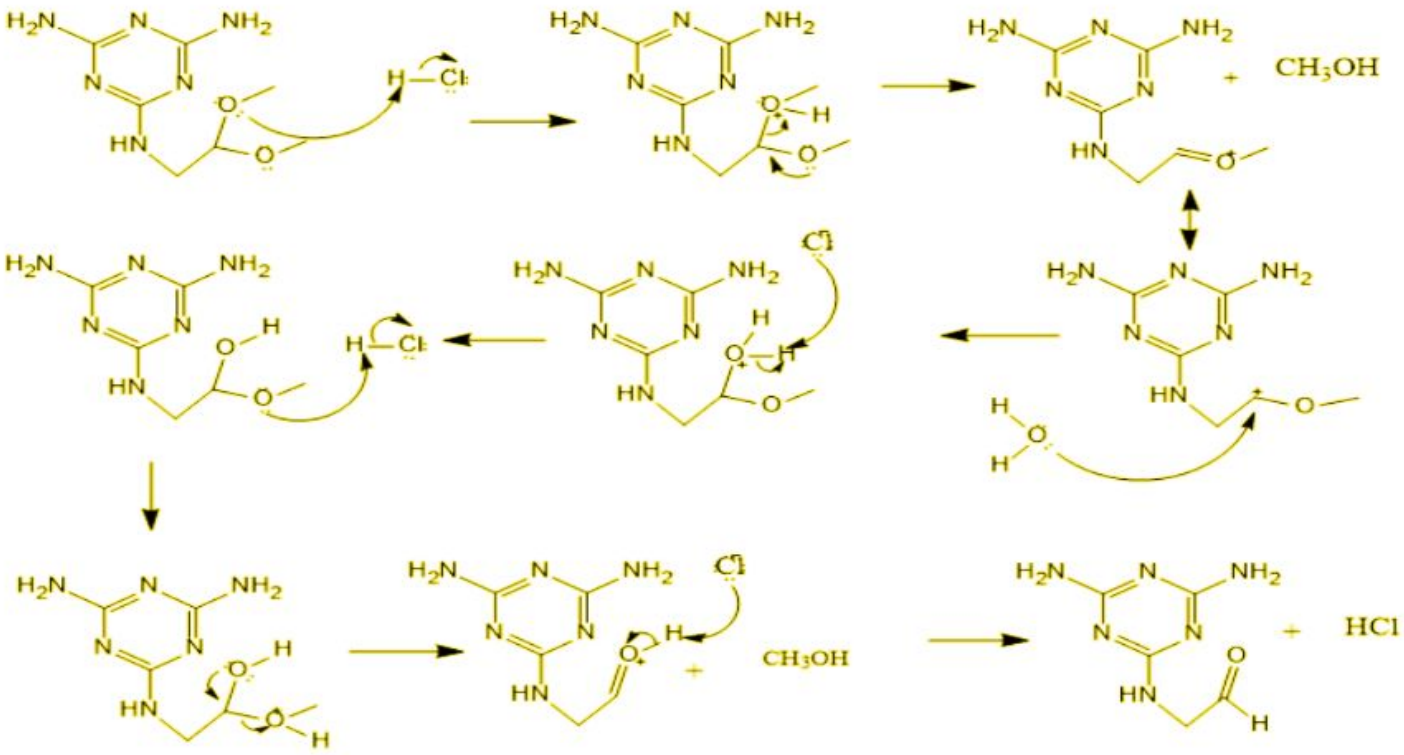
4. *HYDROLYSIS OF ACETAL TO ALDEHYDE*



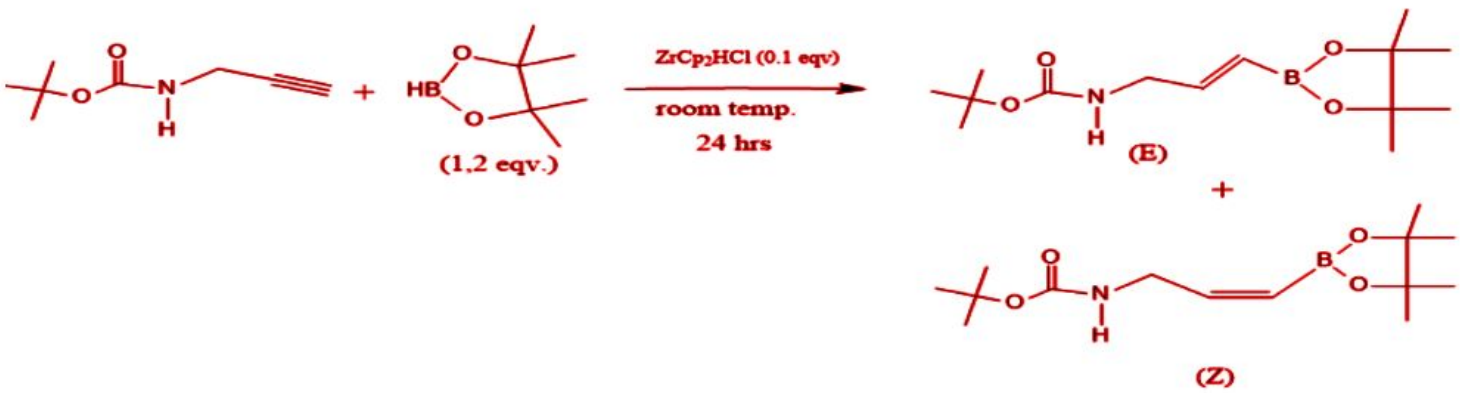
PROCEDURE:

The acetal is added to a round bottom flask containing around 50 ml of HCl and the reaction was continued for 4hrs at room temperature (25^{0}C). The byproducts of the reaction were HCl and methanol which were removed by evaporating using high pressure oil pump.

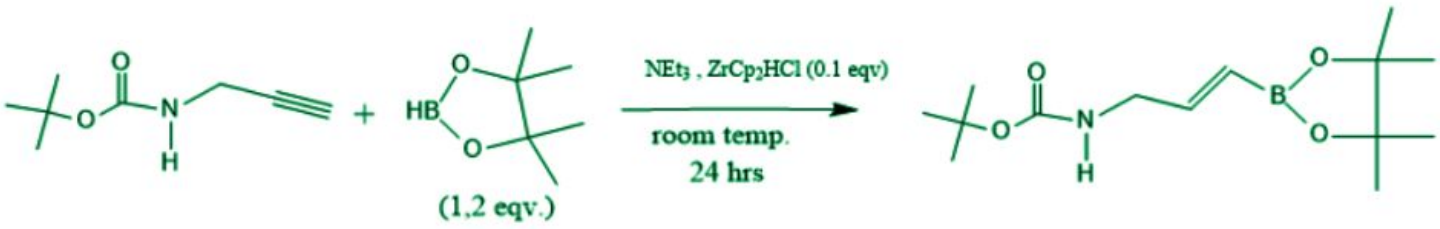
MECHANISM



5. Zr based hydroboration : Stereoselective synthesis

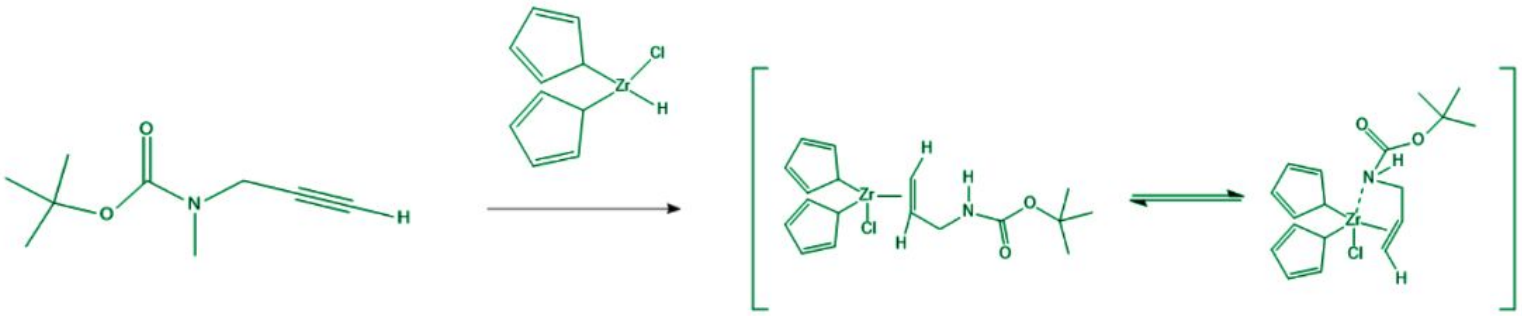


This reaction gives both E and Z conformers, though E enantiomer is the major product but Z enantiomers are also present. The aim was to get pure E enantiomer for this small amount of base such as trimethylamine is added and this results in the desired product.

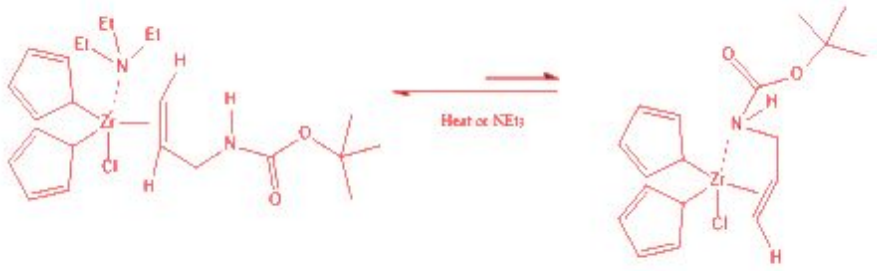


Mechanism:

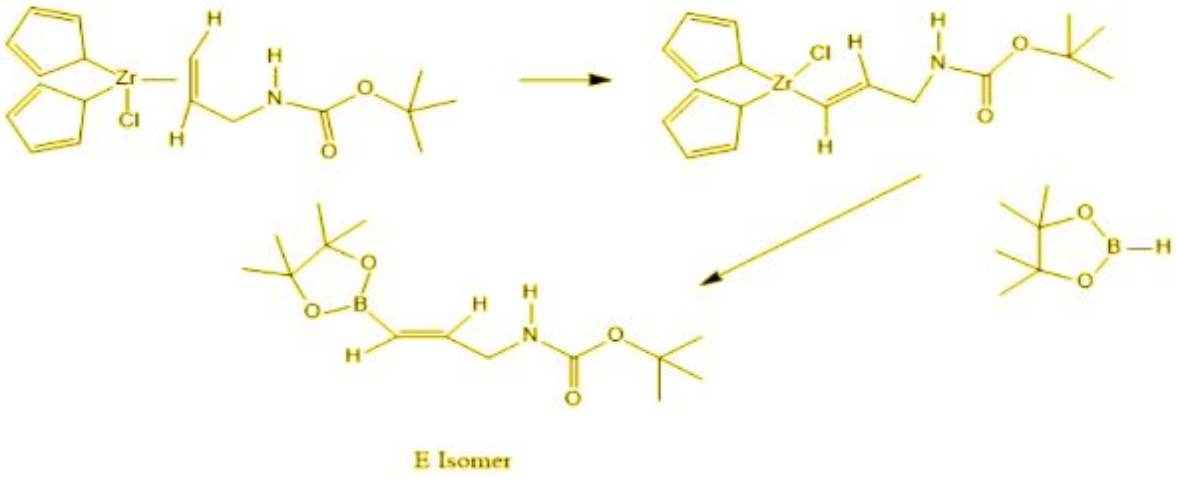
The first step in the mechanism is the coordination of Zr metal with the tripple bond. This coordination can occur in 2 ways as shown below



In the first case when only the triple bond coordinates with Zr, the E enantiomer is formed and the second case where electronegative atom such as N forms a coordinate bond with Zr, the Z enantiomer is formed. So in order to get the only E enantiomer a strong base such as triethylamine is added so that the N of the base coordinates with Zr and the electronegative atom of the substrate does not hence resulting in the desired product.



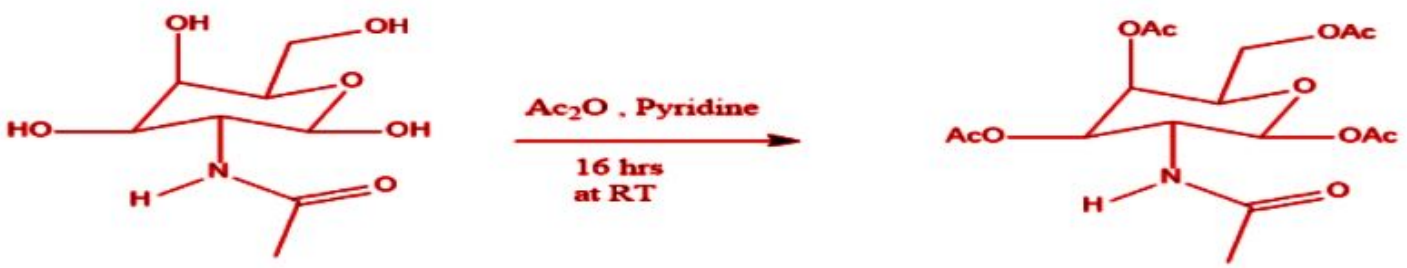
The next step is the simple reduction by Boronic ester as depicted in the reaction below.



E Isomer

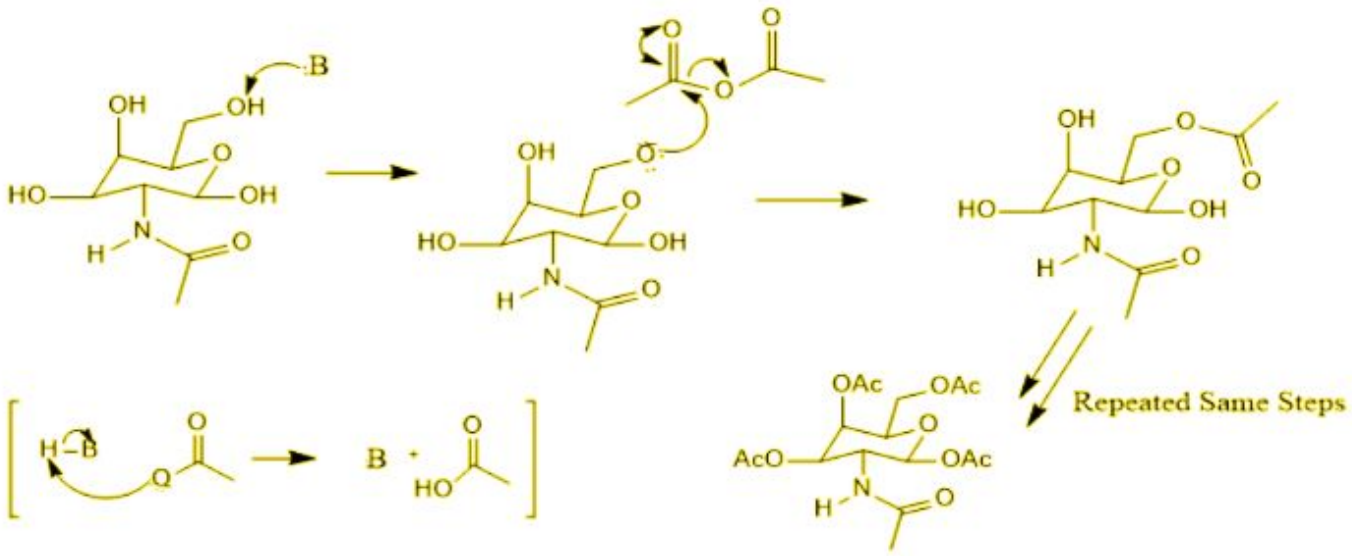
Compound	Substrate	Pinacolborane	NEt_3	$ZrCp_2HCl$
Molecular Wt.	167.25g	127.09g	101.2	
Wt. taken	2.68	1.23g		0.165g
Milli moles	17	9.6	0.64	0.64
equivalents	1	1.5	0.1	0.1
Density		0.882 g/ml	0.726	
Volume		1.4ml	100ul	

6. (a) Acetyl Protection of N-acetylglucosamine



Compound	Substrate	Ac_2O	Pyridine
Molecular Wt.	221.21g	102.09	79.1g
Wt. taken	0.884g	24g	0.491g
Milli moles	4	-	6.2
equivalents	1	-	1.5
Density	-	1.08g/ml	0.982g/ml
Volume	-	22ml	0.5ml

Acetyl protection of N-acetylglucosamine was done using acetyl acetone as a reagent as well as the solvent with small amount of pyridine that acts as a base. Mechanism is as following:

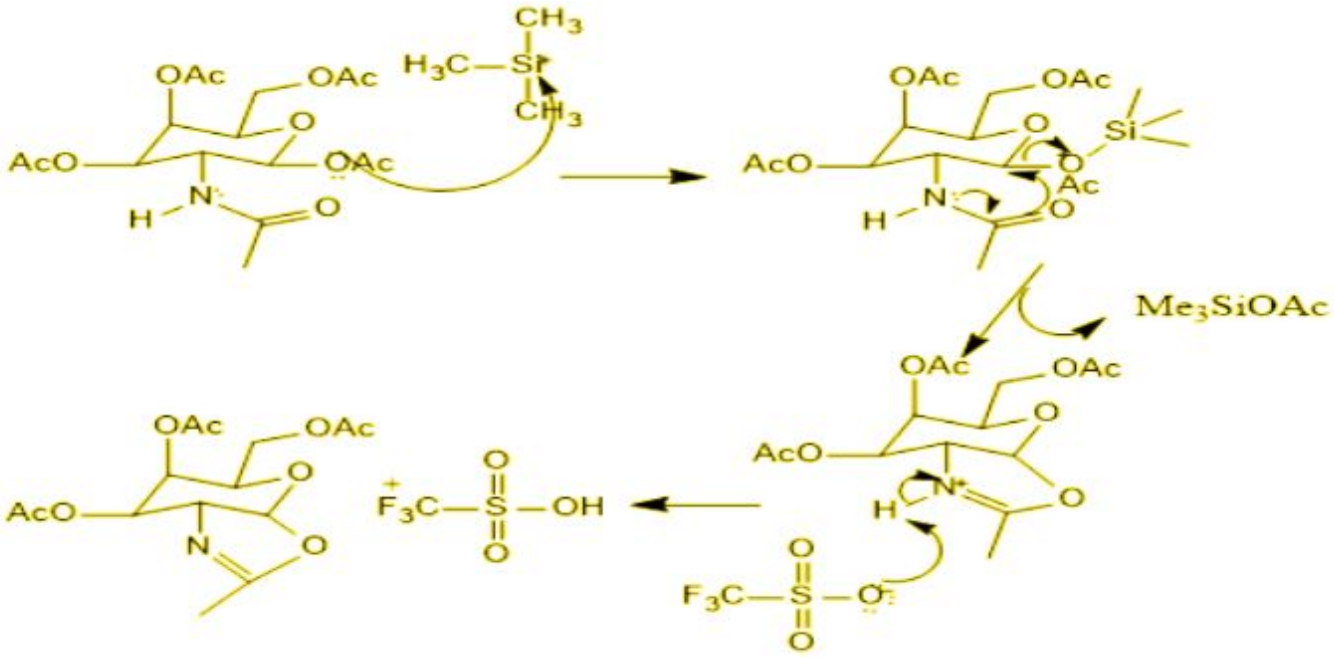


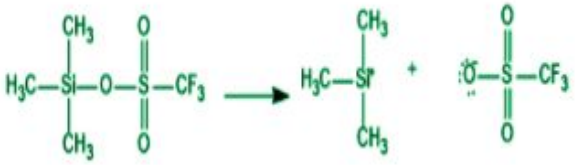
6. (b)

Compound	Substrate	TMSOTf
Molecular Wt.	389.13g	222.26g
Wt. taken	1.52g	1.73g
Milli moles	3.9	7.8
equivalents	1	2
Density	-	1.02g/ml
Volume	-	1.42ml

Second step was the formation of oxime from the acetyl protected N-acetylglucosamine. The Substrate dissolved in 25 ml of DCM followed by addition of TMS otf and telling the reaction mixture at 40 degrees celsius for 24 hours. The reaction was quenched with triethylamine, diluted in DCM and extracted with saturated sodium bicarbonate solution. The organic layer was washed with water and Brine and dried over sodium sulphate before evaporation of solvent under reduced pressure. the Residue was purified by Silica Gel column chromatography using DCM:EtOAc:MeOH (7.5:2.0:0.5) as a mobile phase to obtain the compound 2.

The Mechanism of the reaction is as follows:





In this reaction TMSotf acts as Lewis acid

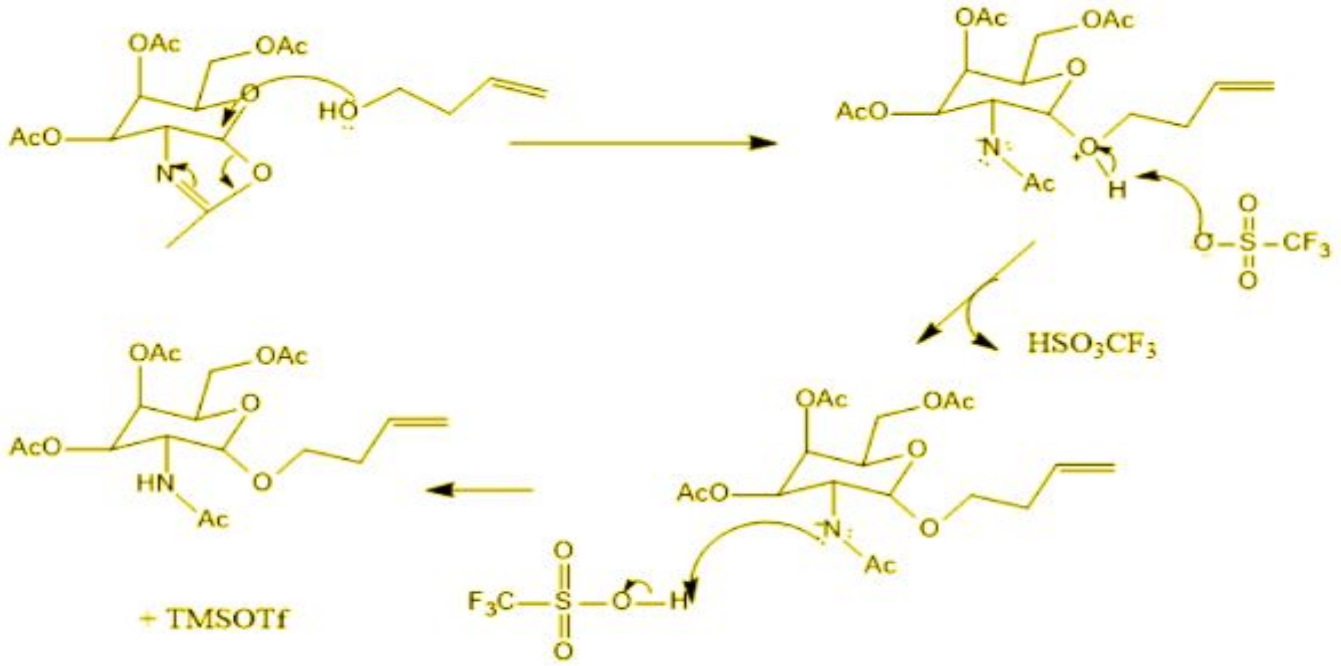
6. (c) Alkenol addition step

Compound	Substrate	TMSOTf	Alkenol
Molecular Wt.	329g	222.26g	72g
Wt. taken	0.845g	0.286g	0.221g
Milli moles	2.57	1.29	3.08
equivalents	1	0.5	1.2
Density	-	1.02g/ml	0.898g/ml
Volume	-	233.8ul	246ul

The substrate in anhydrous 1 2 dichloroethane was stirred with $4A^0$ molecular sieves 45 minutes at room temperature. But-4-en-1-ol was added and stirring was continued for 30 minutes. TMS-triflate was added dropwise under constant stirring over 10 minutes and stirring was continued for 2 hours at room temperature. The reaction mixture was quenched with cold saturated ammonium carbonate solution and organic layer was separated, the product was extracted into dichloromethane and the combined organic layers for washed with water, dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The crude product obtained was triturated with hexane. The solid was

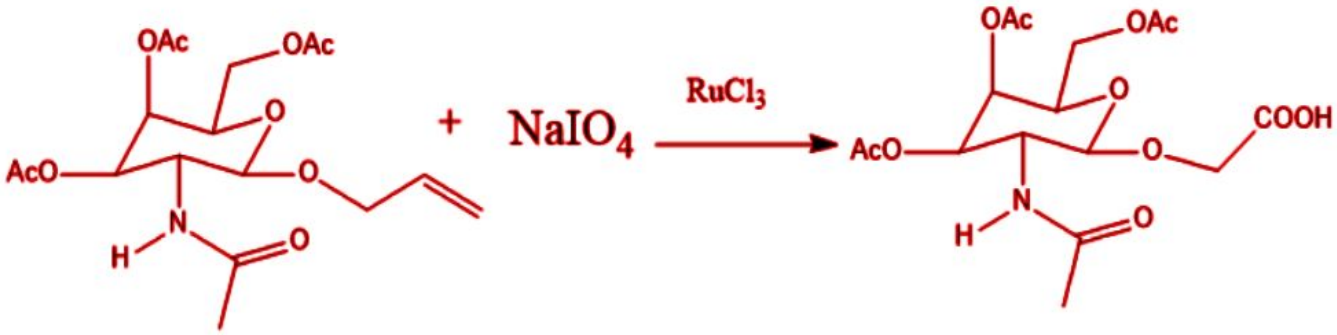
filtered and dried under reduced pressure to obtain the desired product as a pale brown solid.

The Mechanism is as follows:



Without further purification the product was used for next reaction.

6. (d)



Compound	Substrate	$NaIO_4$	$RuCl_3$
Molecular Wt.	407g	213.84g	207.43g
Wt. taken	1.046g	2.2g	0.016g
Milli moles	2.57	10.28	0.077

equivalents	1	5	Catalytic amount

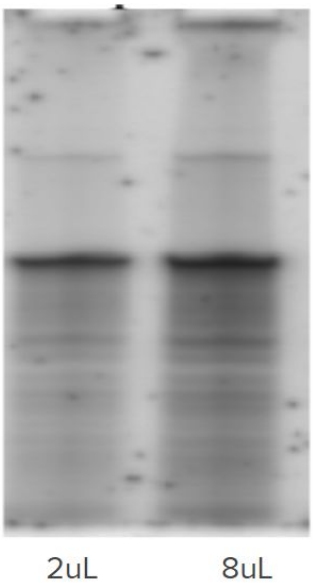
To a solution of the substrate in DCM and MeCN 1 mole equivalent of sodium iodate was added as a solution in water. The mixture was cooled to 10 degree centigrade in a cold water bath and stirred for 15 minutes. Ruthenium chloride was added to the cold mixture. During addition, the temperature was maintained at or below 35 degrees celsius by external cooling over the water bath. The reaction mixture was stored at room temperature for 1 hour and additional 1 mole equivalent of sodium periodate was added and stirring was continued for 1 hour at room temperature. Completion of the reaction was confirmed by a TLC. The reaction mixture was diluted with water (2 litres) and the pH was adjusted to 7. 5 by adding solid sodium carbonate. The DCM layer was removed and the aqueous layer was washed 3 times with DCM around 2 litre and the organic extracts was discarded pH of aqueous layer was adjusted to 3 by addition of citric acid and carboxylic acid was extracted into DCM the organic layer was stirred with saturated brine followed by dropwise addition of 3% dry sodium sulphate solution until the dark green organic phase turned to a pale yellow colour. The layers was separated and the organic layer was right over anhydrous sodium sulphate and evaporated under reduced pressure to get the desired product as an of white solid.

BIOCHEMISTRY

I also got an opportunity to explore the field of Biochemistry in The Bong Laboratory. I learnt basic techniques which included PCR, DNA purification and transcriptions and translations.

PURIFICATION OF DNA TEMPLATE [TL-TLR-U6]

DNA Sample before purification



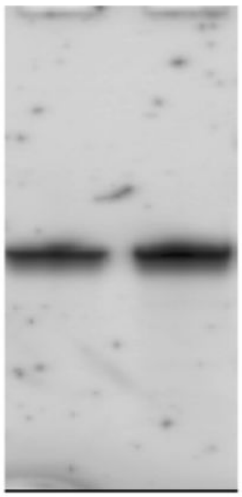
lane 1 is 2 uL of ~250 nM TL-TLR-U6 template
Lane 2 is 8 uL of ~250 nM TL-TLR-U6 template

- ❏ Concentration of the sample : 900 uM
- ❏ Diluted Sample was poured into acrylamide gel and run on 250V for 2hrs.
- ❏ Staining was done with SYBR-Gold stain and then scan using typhon.

Purification Protocol
Initial concentration of TL-TLR-U6 template was ~900uM. The template was diluted (used 6.7uL of template) into 120uL so that each well held 50uM.
To it 120uL of TBE/Urea sample buffer was added.

Tube was heated at 95^0C for 10 minutes followed by cooling on ice.
15% denaturing gel was made with 5 well comb and gel was pre-run for 20min at 250V. After this sample gel was loaded and ran at 250V for 2 hrs. The bands were visualised with UV then cut out. The band which was cut was added into Dialysis tube with 1mL of 1X TBE then added another 1mLand the tube was closed. Electroelution was carried out for 4hrs at 120V
The buffer was removed into Eppendorf tubes where there was 300uL per tube To it 100uL of 10M NH 4 OH was added and then 2.5X 100% EtOH (1mL) to each tube. Tubes were inverted, placed in 0^0C freezer overnight.
After that they were centrifuged at 4C for 30min and supernatant was removed carefully. 200uL of 75% EtOH was added, mixed, centrifuged for 10min at 4C, and again the supernatant was removed. Sample was then dried on speed vacuum for 20 mins. The concentration of the sample was measured on Nanodrop using UV-Vis mode (ended with 60uL of ~12.4uM TR-TLR-U6 purified template). Purity check was done on a 10 well, 15% denaturing gel at 250V with ~250nM template. 5uL of ~250nm template was made, added 5uL of TBE/Urea sample buffer, followed same heating and cooling procedure as before.

Post Purification:



ane 1 is 2 uL of ~250 nM TL-TLR-U6 template
Lane 2 is 8 uL of ~250 nM TL-TLR-U6 template

- ❏ Concentration of the sample : 900 uM
- ❏ Diluted Sample was poured into acrylamide gel and run on 250V for 2hrs.
- ❏ Staining was done with SYBR-Gold stain and then scan using typhon.

The Purified DNA sample was further transcribed using RNA polymerase and enzymes along with suitable Magnesium concentration.