Nucleoside Analogues: Efficient, Cost Effective, And Flexible Optimization Synthesis

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Nucleoside Analogue Overview

Pre Existing Nucleoside Analogues

What is a nucleoside analogue?

• Important classes of antiviral agents used for treatment and therapy of HIV, hepatitis B, hepatitis C, cytomegalovirus, herpes simplex virus and varicella-zoster infection.
• Synthesized to resemble naturally occurring nucleosides and aim to terminate DNA chains by attacking viral polymerase/binding to enzyme sites.
• Tolerated by the body because they are not a part of human polymerases in DNA replication.
• Contain a sugar and nucleobase
• Used in development of drugs for **cancer and rheumatologic diseases** (inflammation in bones, muscles and joints)

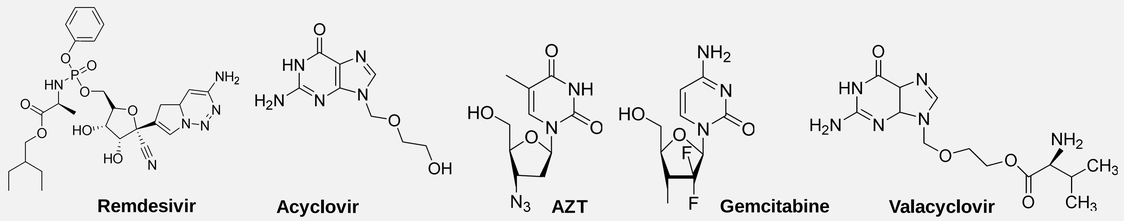


Figure 1. Nucleoside Analogue Antivirals

Common nucleoside analogues:

- **Remdesivir:** Inhibits viral RNA polymerases, affective against SARS-CoV-2 (virus causing covid 19), originally designed to fight against hepatitis C, Ebola sickness and Marburg virus infections.
- **Acyclovir:** Treats herpes simplex virus, genital herpes and HSV encephalitis. Incorporates itself into viral DNA preventing synthesis.
- **AZT:** First NA affective against HIV, originally developed in the 1960s to thwart cancer, interferes with enzyme reverse transcriptase causing HIV cells to produce fewer viruses
- **Gemcitabine:**Treats ovarian, bladder, lung, pancreatic, and breast cancer. Activated within tumor cells.
- **Valacyclovir:** Treats cold sores in children(HSV) and chicken pox (varicella-zoster), treats adults of herpes zoster (shingles), prodrug version of acyclovir, turns into it after being taken into the body. Interferes with multiplication of viruses. Does not cure the previously mentioned diseases but alleviates pain and helps with healing of sores.

Existing Methods

Negative Aspects:

- Time Consuming (multi step processes can take weeks/months)
- Costly (starting materials, solvents, reagents, product)
- Lack of modification to numerous sites along the nucleoside at once (less diversification)
- Variation leads to broad spectrum antivirals that treat various patients efficiently and effectively (dire due to recent pandemic and increase in viral outbreaks)

Early Synthesis:

Completed by Nishimura in 1964 who protected nucleobases and then created an a-anomer (variation at the c1 of the sugar with nucleobase at the bottom of nucleoside)

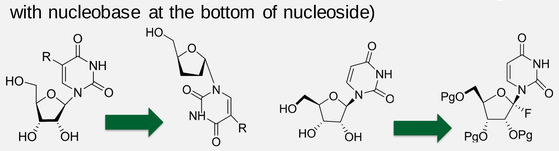


Figure 2. Early nucleoside synthesis and fluorinated nucleoside synthesis at 1'

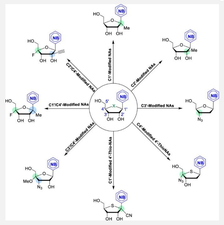
Recent Synthesis (Fluorine Nucleosides):

Shuto and coworkers reported the first synthesis of a 1' fluoronucleoside in 2006. Wasn't attempted before due to assumed instability, high electronegativity of fluorine making the 1' carbon reactive, and possible elimination of the nucleobase.

Our Research

Objectives:

- **Discovery:** Finding nucleoside analogues that will form through shorter processes, less expensive SMs and be more convenient to modify
- **Optimization:** Refining synthesis methods
- **Cyclization:** Creating the correct stereochemistry for a nucleoside (want a specific diastereomer)
- **Modification:** Occurring at different ' positions



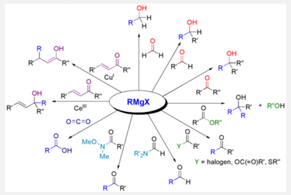


Figure 3,4. Nucleoside modifications and potential carbonyl groups for Grignard reaction

Grignard Reaction (Organometallic):

- Used to develop nucleoside analogues.
- Grignard reagent contains a halogen (X)
- Organic material (R)
- Reagent has to be in an "ether" solution
- Organic material is more negative in nature so it easily binds to a carbonyl
- Carbonyl becomes negative and hydrogen from a water or other ion source binds to the carbonyl turning it into an alcohol

Methods

- Separation of compounds due to density, solubility and polarity for organic extraction:
- Practiced with iodine + potassium iodide, water and DCM (dichloromethane). After mixing we would extract the bottom layer (organic material) and add it to a separate test tube. DCM would be added once again and the organic layer would be extracted. The original process was repeated 3 times.

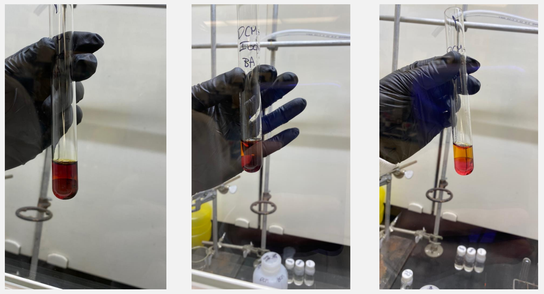


Figure 5,6,7. Iodine density separation with DCM and water

Purification Steps:

After extracting organic material created for producing diversified nucleosides, purification methods must be to gain the desired molecule

1. Extracting organic material
2. Mixing the organic material with specific chemicals in a separatory funnel to create 2 layers (one organic, one aqueous)
3. Extracting organic and aqueous layer and putting aqueous layer back into the separatory funnel and washing with solvents as deemed necessary
4. Roto Vap organic material to discard any remaining impurities
5. Set up column and collect fractions of organic material
6. Conduct a TLC to determine fractions where product is present
7. Roto Vap concise fractions
8. Conduct an NMR to solidify organic material

Other Lab Methods For Analyzing Nucleosides

- TLC (Thin Layer Chromatography)
- NMR(Nuclear Magnetic Resonance)

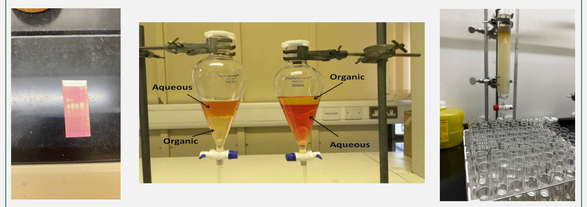


Figure 8,9,10. TLC, Separation of organic and aqueous solutions in separatory funnel, column used to determine what fractions contain desired organic product

Aldol Condensation:

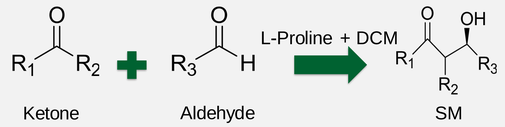


Figure 11. Aldol Condensation to develop starting material

Results

Discovery Optimization:

- Synthesized starting material:

Figure 12,13,14. Synthetization of starting material

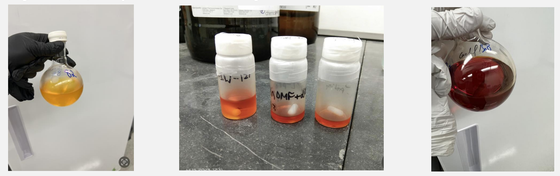


Table 1. Discovery Optimization Data

Grignard	Solvent	Temperature and Time	Concentration (mol/L)	Mass of SM	Yield (%)
Dodecyl Magnesium Bromide	THF	-78°C	0.15, 0.2	25mg	50.09, 62.18
	DCM	-78°C	0.1, 0.15	25mg	58.33, 52.35
		-78°C	0.2	25mg	50.95
		-10°C	0.15	30mg	37.85
		0°C	0.15	35.7mg	30.20
		-24°C	0.15	35.5mg	37.96
		Room Temp 2 Hours	0.15	35.5mg	63.59
		(-78°C)	0.15	25mg	34.17
		(-24°C) Overnight	0.15	25mg	16.60
Allyl Magnesium Bromide	THF	-78°C	0.15, 0.2	25mg	40.99, 37.33
	DCM	-78°C	0.1, 0.15	25mg	55.19, 47.45
		-78°C	0.2	25mg	57.17
		-10°C	0.15	32.5mg	38.15
		0°C	0.15	34.7mg	68.29
		-24°C	0.15	35mg	26.51
		Room Temp 2 Hours	0.15	25mg	17.62
		(-78°C)	0.15	16mg	61.71
		(-24°C) Overnight	0.15	16mg	60.49

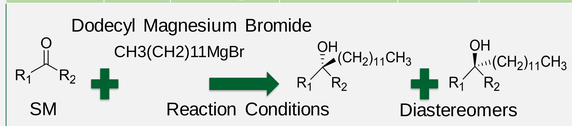


Figure 15. Grignard reaction with dodecyl magnesium bromide and SM

Future Directions:

- Cyclization to collect desired diastereomer
- Possibly sending off nucleoside to virology lab

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Resources

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