Potential Synthesis Pathway for Redox-Active DNA Nucleoside

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Potential Synthesis Pathway for Redox-Active DNA Nucleoside

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Undergraduate Thesis
March 23rd, 2018

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Acknowledgements

I am incredibly thankful for the mentorship and experience I have received from Robert Kuchta, who gave me the opportunity to be an undergraduate research assistant and supported me during numerous independent projects. Likewise, I want to extend my thanks to the other members of the Kuchta lab that have been crucial in my development as a researcher and in the intricacies of this thesis: Sarah Dickerson, for her guidance through the research process and creation of a friendly working environment; Ayman Alawneh, for his mentorship in the art of organic synthesis from the bottom-up and for answering my numerous questions about the theory behind it all; and Michelle Ledru, for sharing the long synthesis days and learning the process with me. Additionally, thank you to my honors committee members Liu Xuedong and Levente Szentkirályi for volunteering their time to participate in this undergraduate thesis defense. A special thanks to Levente Szentkirályi, for helping me through the thesis writing process from the draft to the final and for instilling in me the responsibility we as a scientific community have to make science accessible to interdisciplinary readers and the general public. I extend my appreciation to Michael J. Rourke, for access to his honors thesis The Synthesis Of Stannylated C1 Nucleophilic Monosaccharides For Use In The Stille Coupling Reaction that helped me structure my thesis.
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Abstract

Science has failed those who die from the treatment of an illness rather than the illness itself. DNA nanomachines hold the promise of improving the site specificity of drug delivery, which can combat the toxicity of the drug to healthy cells and in turn, decrease unnecessary patient deaths due to treatment. We have found a novel synthesis pathway for a reversibly redox-active nucleoside (n-nucleoside) that has the promise to be incorporated in and create redox-active DNA. Synthesis of 5′-deazalloxazine-ribonolactone was achieved via sodium hydride catalyzed glycosylation of 1-α-chloro-3,5-ditoluoyl-2-deoxy-D-ribose (5′,3′-protected-chloro-ribose). We were not able to reproduce results obtained by Wang & Rizzo that showed N-3-β selectively as the thermodynamically favored product using the Vorbrüggen glycosylation of alloxazine. Aspects that need to be further optimized in continuation of the sodium hydride pathway and future synthesis pathways for the n-nucleoside were found to be the stability of the glycosidic bond, 2′ position for use in phosphoramidite chemistry and leaving group reactivity at the C-1 glycosylation site. The N-3-β regioisomer will allow for the hydrogen bonding face to change and base pair either with adenine or the p-nucleotide when placed in an oxidizing or reducing environment. Through the synthesis of the redox-active n-nucleotide, the creation of autonomous DNA nanomachines for drug trafficking is one step closer to preventing unnecessary patient deaths.
Introduction

When facing debilitating illnesses, not only do patients have to treat the illness itself but also the symptoms of the toxic treatments involved. Cancer patients are especially at risk; the use of cytotoxic cancer drugs (those in chemotherapy) kills both cancer and healthy cells. The possible side-effects of chemotherapy range from less than serious anemia and hair loss, to more serious infertility and cardiovascular damage. A study conducted by O’Brien et al. found that at 30 days post-chemotherapy, 7.5% of reported deaths were related to the chemotherapy treatment, including sepsis (infection) and organ failure.\(^1\) The most susceptible patients to chemotherapy toxicity are geriatric (>50); Muss et al. found that 2% of geriatric patients in comparison to 0.02% of all other age groups died due to chemotherapy treatment.\(^2\) To reduce these treatment symptoms and unnecessary mortality from treatment, toxicity can potentially be addressed via drug localization and dosage adjustment. Nanomachines can potentially improve the site specificity of drug delivery. With a more targeted approach, lower dosages and decreased side effects should be a benefit.

Several nanomaterials have been functionalized into machines, such as liposomes, polymeric nanoparticles, dendrimers, polymersomes and nanoemulsions.\(^3\) Indeed, many proteins serve as high efficiency biological nanomachines (helicases, e.g.). DNA has been a relatively new target for nanomachine technology, over the already well understood and natural protein nanomachines mentioned above. Also, due to DNA’s robust nature and malleability in its structural dependence on sequence, it can become a highly specified tool. One of the properties that makes DNA an excellent candidate is how it forms predictable structures based off of Watson-Crick (W-C) hydrogen bonding as well as a triplex formation with Hoogsteen hydrogen
bonds (H). Therefore, through sequence manipulation, defined structures can be designed depending on the desired function.

Synthesizing 5-deazalloxazine-ribonolactone (n-nucleoside) via sodium hydride catalyzed glycosylation with 1-α-chloro-3,5-ditoluoyl-2-deoxy-D-ribose (5’,3’-protected-chloro-ribose) was the most successful synthesis pathway thus far in comparison with other synthesis approaches (which will be discussed later in the thesis). Key aspects that need to be further considered in continuation of the sodium hydride pathway and future pathways for the n-nucleoside include stability of starting material and glyosidic bond, the stereo-chemical selectivity for the β-regioisomer and leaving group reactivity at the C-1 glycosylation site. The sequence manipulation of DNA using the n-nucleoside, in this case, is eliciting a change in base-pairing partners to potentially alter DNA double-helix conformation upon either oxidative or reductive stimulus.

**Novel contribution to DNA nanotechnology**

DNA nanotechnologies have been developed to allow the DNA to change conformation, but are not efficiently reversible, nor does the machine move autonomously. For instance, DNA molecular tweezers utilizing several fuel strands can open and close a fork conformation, but leave much to be desired with the complexity of movement and efficacy (Fig.1). Adding each strand sequentially

Fig.1: DNA molecular tweezers using fuel strands to change confirmation, ultimately resulting in accumulation of waste products for subsequent rounds.
results in waste products created by the release of subsequent fuel strands, which have been a major concern, as they can be competitive inhibitors to the desired reaction and they can reduce the efficiency of the machine.° Modeling a system more closely to how the body naturally utilizes some protein motors such as kinesin may be more promising. Kinesin is used to traffic cargo around the cell by “walking” on actin filaments. The chemical energy created through hydrolysis of ATP is utilized by kinesin and turned into mechanical energy.° This transfer of chemical energy into mechanical energy in DNA has been utilized in the creation of a chemical oscillator DNA nanomachine system. One functionalized chemical oscillator was used to switch a pH-sensitive DNA construct between two different conformations, random coil and i-motif.° Although this more closely resembles the body’s own mechanism, it is very limited in the complexity of the movement and number of cycles. The chemical oscillator works using a non-equilibrium variation of the Landolt chemical reaction to vary the pH in a continuously-fed chemical reactor that cycles between two products, but the reactants eventually diminish, thus limiting the number of cycles.° Therefore, redox-active DNA can bridge these gaps found in current DNA nanomachines. Redox-active DNA can utilize the natural redox potential difference between the cell’s cytosol and extracellular milieu to change conformation by changing redox nucleoside binding partners. Theoretically, this activation would not, therefore, have a cycle limit, nor produce possible competitive waste products.

Redox-active nucleotide synthesis and base-pairing abilities

Non-DNA specific redox cofactors that are found in several metabolic pathways are flavin and F420 (5-deazaalloxazine). The redox-active functional group involved in both of these cofactors is the isoalloxazine ring system, which allows for hyper-conjugation of hydrogens in
the benzylic positions.\textsuperscript{9} Flavin is involved in both one and two electron transfers in the ground state; whereas, F420 only transfers two electrons when in the ground state.\textsuperscript{10} The biologically relevant forms of these cofactors have glycosidic bonds extending from the N-10 position.\textsuperscript{11} Alloxazine, a redox-active heterocycle moiety in the Flavin family\textsuperscript{12}, in previous studies has been glycosylated at the N-1 and N-3 positions with furan sugars.\textsuperscript{13} Both these nucleosides have W-C hydrogen bonding faces mimicking that of a pyrimidine, although only that of the glycosylated N-3 position has a W-C hydrogen bonding face that can be altered when oxidized or reduced to mimic a natural pyrimidine/purine. Oxidized N-3 glycosylated alloxazine mimics thymine, thus it will pair with adenine by contributing one proton-donating and one proton-accepting group. Reduced N-3 glycosylated alloxazine will hydrogen bond with the non-natural P-nucleotide, with two proton-donating groups and one proton-accepting group (Fig. 2).\textsuperscript{14} F420 has yet to be glycosylated by researchers aside from its biologically relevant N-10 site, but given the structural similarities to alloxazine it is assumed to occur in the same fashion.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{On the left, is the potential functional n-nucleoside W-C base pairing with the P-nucleoside, under reducing conditions. On the right, is the other potential W-C base pairing partner, adenine, of the n-nucleoside under oxidizing conditions. The 10 position is the natural glycosylated position of Flavin’s and position 3 is the altered glycosylation site to allow for duel bonding face.}
\end{figure}
Redox-active DNA synthesis

Other structurally similar tricyclic nucleosides that were incorporated into DNA were found to be very stable. The tricyclic nucleosides tC and tC° formed normal W-C base-pairs with guanine. Because of the inclusion of two additional aromatic rings in the major groove of the DNA helix, Engman et al. found the only structural difference was that thermal stability increased.\(^\text{15}\) It is likely that the increased pi-bond stacking due to the extra two rings resulted in this characteristic. These stacked aromatic rings of the nucleotide bases are also how DNA conducts charge over its length.\(^\text{16}\) Guanine, in particular, is an important but dangerous electron carrier due to its ability to be oxidized.\(^\text{17}\) However, guanine oxidation is a major form of DNA damage. In response to oxidative stress, high energy oxidizing species oxidize guanine to 8-oxoguanine (8-oxoG) creating either irreversible strand breaks or base lesions.\(^\text{18}\) N-10 alloxazine was found to cause guanine oxidation when placed either in the DNA duplex or at the end. This phenomenon occurred more readily when O\(_2\) and light were introduced into the system (Fig.3).\(^\text{19}\)\(^\text{20}\) Since, 5- deazalloxazine will not undergo single electron transfers in the ground state, the use of 5-deazalloxazine may help limit the possibility of guanine oxidation and DNA damage in redox-active DNA.

Fig. 3: Oxidative damage that occurs when guanine is converted to 8-oxoG and causes DNA strand breaks.
Methods
*No NMR data is provided because no extensive purification steps were performed successfully.

Materials
All chemicals were purchased as reagent grade and used without further purification. Molecular sieves were added to solvents that easily retained water after exposure to air. All reactions were carried out under inert gas, Ar, and in oven-dried glassware. All chemicals were ordered from Sigma-Aldrich unless otherwise specified.

Deazaalloxazine
2-aminobenzaldehyde (0.9 g, stored at -20°C and in white powder form) and Barbituric Acid (1.1 g, stored at rt and in yellow powder form) was combined in a 100mL round bottom flask with 50 mL ddH2O. The reaction was heated to 70°C and refluxed for 4 hours. Vacuum filtration was performed immediately after, while the reaction was still hot. Digestion with diethylether to improve yield. The yield was 1.4 g of white powdered product, the percent yield was 89%. Presence of product was confirmed by mass spectrometry and was consistent with the literature.

Bis-silylated D-Ribonolactone
D-Ribonolactone was combined with pyridine in excess, then bis-silylation agent from ChemGenes (1:1.1 eq.) was added dropwise until a white precipitate was produced. The reaction was allowed to stir overnight and was light sensitive. The reaction was rotovapped. The workup included, re-dissolving the product in acetonitrile and washing the organic layer with sodium carbonate and collecting the organic layer via gravity filtration and drying it over magnesium sulfate. TLC visualized using 5% anis aldehyde in 3 M ethanol stain. Crude yield. The presence of the product was confirmed by mass spectrometry and is consistent with the literature.

Bis-silylated Deazalloxazine
Benzene in excess was cooled along with deazaalloxazine in an ice bath at 0°C. Then, the trimethylamine was added all at once (1: 3 eq.) and the chlorotrimethylsilane was added dropwise (1: 2.25 eq.). The reaction stirred for 48 hours. The workup included vacuum filtration to remove the salt, then washed filtrate with more benzene to extract product. The product was then rotovapped until dry. Crude yield. The presence of the product was confirmed by mass spectrometry and is consistent with the literature.

Deazaalloxazine-N-3/N-5-5’-ditoluoyl-2-deoxy-d-ribose
Deazaalloxazine was first dried on a vacuum for 4 hours and then put under Ar. A 100 mL RBF was dried at least overnight at 200°C then put under argon. Deazaalloxazine (0.5 g.) was dissolved in 20 mL DMSO and NaH (1:1 eq.) was added, the reaction was allowed to stir at rt. for 2 hours. The solution was clear yellow. The 1-α-Chloro-3’,5’-Ditoluoyl-2-Deoxy-D-Ribose from ChemGenes (0.9 g, highly reactive, stored at -20°C and in white powder form) was added in and ran for 1 hour at rt., creating a cloudier darker yellow solution. The workup included pouring 100 mL of chilled 0.01 M Phosphate Buffer pH ~ 7.2 over the reaction and vacuum filtrating the precipitate. The precipitate was then dried overnight on the vacuum. Crude yield. The presence of product was found using TLC; it showed a newly formed fluorescent band between the ribose and the deazaalloxazine standards. Product presence was then confirmed using MS and is consistent with the modeled value.
Results

Exploration of Williamson Ether Synthesis for 2’-methoxy-D-ribonolactone

To create a stable ribose electrophile for the creation of the glycosidic bond, a functional group at C-2 was imperative. Previous studies by Handlon et al. have found a 10-fold increased stabilization of the oxocarbenium intermediates involved in glycosidic bond formation via electron donating or withdrawing groups at the C-2 position, rather than the naturally occurring deoxy/hydrogen.\textsuperscript{21} For use in standard DNA phosphoramidite chemistry the nucleoside cannot contain base labile protecting groups, therefore a hydroxyl would not work at C-2. In previous unpublished research we found that a methoxy group meets these criteria. Therefore, several alkylating agents were tested on 2’-D-ribonolactone with a hydroxyl at C-2. The C-3 secondary and C-4 primary hydroxyl groups on 2’-D-ribonolactone were first protected via base-catalyzed bis-silylation. Williamson ether synthesis involves an SN2 reaction with a primary alkylhalide, iodomethane (MeI) and alkoxide, C-2 de-protonated hydroxyl of 2’-D-ribonolactone.\textsuperscript{22} Even with stoichiometric amounts of MeI and optimal heating conditions this method did not yield any product. In an attempt to push the reaction, dry silver oxide (Ag\textsubscript{2}O) was used as a catalyst along with the MeI. The silver would provide a larger crystalline surface area for the reaction to occur on. Unfortunately, the reaction did not produce any desired product. This led us to use a stronger alkylating agent, dimethyl sulfate (DMS). Again, the reaction did not produce any product. Although, all reactions were performed under anhydrous conditions and the starting materials were also stored under anhydrous conditions, it is possible that any introduced atmospheric water destroyed the alkylating agent.\textsuperscript{24}
Synthesis of Bis-silylated 2’D-Ribonolactone

Tert-Butyldimethylsilyl (TBDMS) was used for the protection of the 5’ and 3’ hydroxyls of 2’D-ribonolactone.\textsuperscript{25} Stoichiometric amounts of the silylating agent was used to add protecting groups to the 3’ and 5’ hydroxyls. The Oglive group found that the 5’ was the favored silylated hydroxyl due to the reduced steric hindrance it offers for the bulky butyl groups, and the second silyl group adds to the more accessible 3-OH.\textsuperscript{26} This step was necessary to allow for exclusive methylation of 2’ hydroxyl later on. The TBDMS sialylation produced a product that was purified on a silica column. Mass spectrometry after purification showed the bis-silylated
2’D-ribonolactone had started to decompose on the column. This could have been due to the light sensitivity of the product, even though the column was wrapped with foil. The bis-silylated 2’D-ribonolactone was then used in the Vorbrüggen reaction. The mass spectrometry (MS) after the Vorbrüggen reaction showed a loss of one protecting group and subsequent re-cyclization of one of the bis-silyl groups with a neighboring hydroxyl. This rearrangement could have created a less specific reaction, where the 3’ hydroxyl could compete for glycosylation sites with 1’ position if it is able to pick up another hydrogen and undergo dehydration. Not only was it important to exclusively glycosylate the 1’ ribose position, but reduction of 3’ and 5’ hydroxyl was imperative so that hydrogen ions could not be lost to the reaction solution, which could easily destroy the catalytic TinCl (IV) as HCl after combination with the released 1’-Cl.

**Scheme 2**: Synthetic pathway for bis-silylated-2’-D-ribonolactone under basic-catalytic conditions.

---

**Friedlander Reaction for Synthesis of Deazaalloxazine**

The Friedlander Reaction was utilized under aqueous conditions to synthesize deazaalloxazine from 2-amino benzylaldehyde and barbituric acid. The first step in this reaction takes place relatively rapidly; the most acidic proton attached to the carbon between the two carbonyl groups is extracted by the water molecules. The resulting carbanion is a strong nucleophile and attacks the aldehyde group of benzylaldehyde to create an open ring isoalloxazine system. The second step can be relatively slow if the nucleophilic amine group
starts taking up protons left in solution, therefore TEA was included to extract these free protons in solution. This pushed the ring-closure step to occur via nucleophilic attack of the amine group on the adjacent carbonyl group. To increase yield and purity I used a digestion with MeOH. The MeOH did not increase yield significantly, therefore we tried a diethylether digestion. Given that the diethylether would not dissolve high molecular weight products such as the deazaalloxazine nor salts, it was more ideal for purification.

**Scheme 3:** Synthetic pathway to create deazalloxazine from 2-amino-benzylaldehyde and barbituric acid using the Friedlander reaction that was successful in producing product.

---

**Exploration of Vorbrüggen Glycosylation of Deazaalloxazine and Alloxazine**

Initial reaction schemes for the synthesis of n-nucleoside were based on the regioisomer selectivity found by Wang & Rizzo in their synthesis of β–N-1 and β–N-3 alloxazine nucleosides. They theorized that the glycosylation of alloxazine would mirror that of guanine, which would lead them to conclude the N-1 product was thermodynamically favored over the kinetically favored N-3 regioisomer after the formation of the N1, N3-glycosylated intermediate. Therefore, we followed the procedure for a Vorbrüggen glycosylation of silylated alloxazine using tin-tetrachloride to extract an acidic proton at C-1 in the ribose ring to create a strong nucleophile that extracted a Cl from the SnCl₄ (IV) creating a halogen sugar that became an ideal leaving group during the glycosylation. At longer reaction times they found the N-1 product was
exclusively favored; at shorter reaction times (before 15 minutes) the N-3 position was exclusively favored. This result was not reproducible in our hands for either deazaalloxazine or alloxazine with 1,3,5-Tri-O-benzoyl-2-o-methyl-α-D-ribose (1,3,5'-acetyl-2'-methyl-ribose). Not only were the N-3 and N-1 products produced in equal amounts when the reaction was stopped at the earlier time point, but the isomers were unable to be easily purified independently of each other by column chromatography. Silylating the carbonyls at the 2 and 4 positions of alloxazine was key to ensure the reactivity of TinCl (IV) with the silyl- 2’-D-ribonolactone over interacting with the nitrogen groups of alloxazine. The silyl groups were essential to eliminate the undesired flow of free electrons between N-1 and N-3 through aromatic π-bonds for deazalloxazine as well. The deazalloxazine without alkylation of the N-10 position did not allow for as much delocalization as with the alloxazine, thus increasing the nucleophilic nature of the desired N-3 position for glycosylation.

**Scheme 4**: Proposed Vorbrüggen Glycosylation of bis-silylated Deazaalloxazine and Alloxazine with 2’methoxy-ribose, no product produced.
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Table 3: Conditions for Vorbrüggen Glycosylation of bis-silylated Deazaalloxazine and Alloxazine with 2′methoxy-ribose that were unsuccessful.

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>Electrophile</th>
<th>Catalyst</th>
<th>Solvents</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis-Silylated Deazaalloxazine</td>
<td>OAc-ribose</td>
<td>SnCl₄</td>
<td>TEA</td>
<td>Cool to -70°C, then slowly to rt. Anhydrous Under inert gas</td>
<td>No Product</td>
</tr>
<tr>
<td>Bis-Silylated Alloxazine</td>
<td>OAc-ribose</td>
<td>SnCl₄</td>
<td>TEA</td>
<td>Cool to -70°C, then slowly to rt. Anhydrous Under inert gas</td>
<td>No Product</td>
</tr>
</tbody>
</table>

Synthesis of 5-deazaalloxazine-ribose nucleoside (n-nucleoside)

Highly reactive sodium hydride was used for the glycosylation of 5-deazalloxazine. Characterizing the solubility of the deazalloxazine, DMSO was the ideal solvent. The sodium-hydride was introduced into the solution of DMSO and deazalloxazine and allowed to stir at room temperature for several hours to allow for deprotonation of the aromatic nitrogens and producing H₂ gas. Excess protons from the reduced aromatic nitrogens were not necessary to remove via silylation before glycosylation using NaH. Additionally, Yari et al. found the treatment of ribose with a silylation agent anomerized the α-ribose to create a mixture of α/β ribose and thus created a mixture of α/β nucleosides if purification is not done between these steps.²⁹ The 1-α-chloro-3,5-ditoluoyl-2-deoxy-D-ribose (chloro-ribose) was added once the nitrogen anion was created in excess. The SN2 reaction proceeded rapidly, with the Cl leaving group in the α configuration favoring attack to give the desired β configuration, thus producing the n-nucleoside and NaCl. A phosphate buffer was used for the work-up because the excess H⁻ anion from the unreacted sodium hydride would combine with water to make a highly basic
solution that could de-glycosylate the product. A phosphate buffer was used instead of bicarbonate because of its triprotic nature allowing for a large buffer range if needed, since the amount of unreacted sodium hydride was difficult to assess. The buffer was made using equal ratios of the monoprotic and diprotic species to allow a pH ~7.2. The solution was then dried on a vacuum pump overnight. Product was identified using MS and the values were consistent with the literature although yields were very low. Glycosylation of alloxazine via this method was also successful. To improve yield, silver hexafluoroantimonate (V) (silver antimony) was added as a catalyst. The silver will react with the Cl to create an insoluble salt, precipitating out of solution to drive the reaction. The antimony will attack the C1 position from below given the chloride is attached in a α-configuration. As the SN2 reaction proceeds, given that most of the antimony mass (six flourines) is now blocking the α position, the nucleophile will favor attack of the β-face and produce the wanted N-3-β deazalloxazine glycosylated product. Unfortunately, the silver antimony did not yield significant changes in yield. The use of 1-α-chloro-3,5-ditolyoyl-2-deoxy-D-ribose (chloro-sugar) was previously found to be very reactive and relatively stable for glycosylation reactions, especially stable after glycosylation.\textsuperscript{30} Chloro-sugar with a methoxy group at C-2 was unable to be commercially found, henceforth the Williamson ether synthesis described earlier. The chloro-sugar in our case was found to degrade even under inert gas, storage at -20°C in containers with anhydrous calcium sulfate, and with minimal exposure to air. This was most likely due to the reactivity of Cl in the presence of atmospheric water that could be present even under anhydrous conditions.
**Exploration of 1-α-mesylate-D-ribonolactone for ideal leaving group**

Due to the stability issues I encountered with the chloro-sugar, the sodium hydride glycosylation reaction was attempted using a less reactive but more stable mesylate group at the α-C-1 position with difluoride at C-2. The mesylate group allows for resonance delocalization of the developing negative charge on the leaving oxygen and in the α position will favor attack to generate the desired β position. The 2-deoxy-2,2-difluoro-D-ribofuranose-3,5-dibenzolate-1-methanesulfonate (α-1’mesylated-2’difluoro-ribose) was not able to produce any product when combined with deazaalloxazine and sodium hydride, even when its reactivity was pushed under heating conditions.
**Scheme 6:** Proposed synthetic pathway for the creation of n-nucleoside using 1′-α-mesylated-2′difluoro-ribose and catalytic amounts of sodium hydride in DMSO that did not produce any product.

![Scheme 6](image)

**Table 4:** Conditions for Sodium Hydride catalyzed glycosylation of Deazaalloxazine with α-1 mesylated-2′difluoro-ribose -ribose that were unsuccessful.

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>Electrophile</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deazaalloxazine</td>
<td>α-1′mesylated-2′difluoro-ribose</td>
<td>NaH</td>
<td>DMSO</td>
<td>1. Let NaH and Deaza stir at rt. for 3 hrs 2. Add Mesylated sugar and reflux 15-60 min.</td>
<td>No Product</td>
</tr>
</tbody>
</table>

**Exploration of Zemplén Conditions**

Purification of the n-nucleotide was more difficult with the protecting groups still attached to D-ribose. Large plate TLC did not allow separation of the deazaalloxazine starting material for the n-nucleoside due to their similar Rf values. Column chromatography was employed but given the sticky nature of the protected product it was difficult to elute all of the
product off the column. Therefore, we attempted to deprotect the n-nucleotide prior to purification. The deprotection was carried out under catalytic Zemplén conditions\textsuperscript{31} including sodium methoxide and methanol. The reaction was stirred at room temperature for no longer than 30 minutes to minimize additional side products. To extract the Na\textsuperscript{+} ions in the solution a H\textsuperscript{+} - exchange resin was added until a pH of \textasciitilde 7 was reached. It was found that the solution retained the starting material and no de-protected n-nucleoside product was present. The n-nucleoside crude product was oily and retained water very easily, even if left on the vacuum pump overnight and dried with petroleum ether. This proved disastrous for the deprotection reaction. The water when in contact with sodium methoxide initiated a de-purination reaction.

**Scheme 7**: Theoretical synthesis pathway for deprotection of 1’ & 2’ ethyl phenol ether of n-nucleoside using sodium methoxide and acidic workup.

![Scheme 7](image)

**Table 5**: Conditions for deprotection of 1’ & 2’ ethyl phenol ether of n-nucleoside using sodium methoxide and H\textsuperscript{+} exchange resin that showed no desired results.

<table>
<thead>
<tr>
<th>Nucleophile</th>
<th>Electrophile</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaMeO</td>
<td>n-nucleoside (crude)</td>
<td>MeOH</td>
<td>Stir at rt. for 30 min., add H\textsuperscript{+} exchange resin until pH \textasciitilde 7</td>
<td>No Product</td>
</tr>
</tbody>
</table>
Discussion

Establishing a stable and selective glycosidic bond between the n-nucleoside and the ribose sugar was difficult due to stability issues, generation of the appropriate regioisomer and deprotection to obtain the desired product. Several methods were considered including functional groups at C-2 to aid in stabilization of the oxocarbenium (anything but hydrogen) and that were not base labile for use of the nucleoside in standard DNA phosphoramidite chemistry, hence the methoxy and di-fluoro. Unfortunately, Williamson Ether synthesis of the methoxy group was unsuccessful and the stable di-fluoro compound contained the relatively unreactive mesylate group. The Vorbrüggen glycosylation was unable to produce the same published results of the kinetically favored β-N-3 product at early time points as Wang & Rizzo with alloxazine and when applied to deaza-alloxazine. The reaction produced a mixture of both regioisomers (α/B) at both the desired N-3 position and undesired N-1 position, due to their similar reactivity, in low yield and unable to be separated from each other by column chromatography due to close Rf values. The excess hydrogens that had to be eliminated before using the TinCl (IV) complicated the reaction, requiring protecting hydroxyls and aromatic amines with bis-silylation and adding trimethylamine in order to extract any free hydrogens in solution. The only successful synthesis pathway used stoichiometric amounts of sodium hydride to glycosylate unprotected deaza-alloxazine with the chloro-ribose. The crude yield was very low and high amounts of starting material were present despite efforts to improve yield. The chloro-ribose was a difficult starting material to work with given its high reactivity with atmospheric water and was found to degrade even under ideal storage conditions. The chloro-sugar glycosylated product was also found to be unstable, possibly due to the destabilizing effects of the protons at C-2. Therefore, we opted to de-protect before purifying. Under Zemplén conditions, the deprotection did not
yield any positive results. This could have been due to excess atmospheric water present in the product during the deprotection step that created de-purination. Additionally, Sleath et al. found that benzoyl protecting groups when in the basic conditions, as that of the deprotection reaction, destabilized the glycosidic bond. They suggest the use of the p-nitrobenzoyl moiety to avoid this problem. The purification step was tried once before deprotection. However, given the light sensitivity and general instability of the n-nucleotide it was found to degrade on the silica column. The scope of this study only allowed for analysis of product presence via mass spectrometry, so purification and yield optimization were not taken into consideration. All of the chemical reagents, catalysts and functional groups found to be essential for stabilization, for stereo-selectivity and for leaving group reactivity will be an important consideration in the synthesis pathways constructed for optimization of the sodium hydride glycosylation synthesis pathway and in future synthesis pathways for the n-nucleoside.

Further Investigation of Synthesis Pathways

*Improve selectivity of glycosylated N-3 position over N-1 position*

To solve the issue of producing the unwanted N-5 glycosylated deazalloxazine using the Vorbrüggen Glycosylation we utilized the approach used by Kanhed et al. and Vlaar Tjostil et al., where an electron donating/protecting group was placed at N-10 in their synthesis of isoalloxazine derivatives. We have since tried adding an electron reconfiguring group, 1-ethyl-4-methoxybenzene attached, to the N-10 amine group of alloxazine. This resulted in a highly favored oxidation of the N-1 position, therefore making the only viable electrophile N-3 for the nucleophilic attack of the carbocation formed at the C-1 position of the ribose. This gave the desired product of N-3-β alloxazine nucleoside. The crude yield was relatively high because
there was no need to protect the surrounding carbonyl groups, which minimized a protection and deprotection step as well as avoidance of steric hindrance surrounding the N-3 position.

Theoretically, the N-10 electron re-configuring group should be easily removed after addition of the ribose through reduction of the N-10 amine group and loss of the 1-ethyl-4-methoxybenzene. The next step will be synthesizing deazalloxazine with the same N-10 electron re-configuring group.

*Separating the mixture of regioisomers (α/β)*

One of the key issues with Vorbrüggen Glycosylation synthesis pathways was the inability to produce the β-regioisomer exclusively over the α-regioisomer at the shorter time points, especially difficult with the presence of the silyl-groups that have shown anomerization of α-carbohydrates. A recent patent by Yari et al. found a means for the selectivity of the β-regioisomer over the α-regioisomer, that they found to be in a ratio range of 1:1-5:1 in their preparation of gemcitabine hydrochloride a potent chemotherapy agent. By removing the silyl protecting groups on the nucleoside base and precipitate out the mixed products using dilute HCl. The product was then dissolved in a polar and protic solvent and allowed to reflux with an alkyl amine to obtain just the β-regioisomer.²⁹

*Increasing Reactivity of 1-α-mesylate-2-diflouro-D-ribonolactone*

The power of this sugar is the stability that it offers after glycosylation. Due to the increased stability the reactivity of the leaving group is lower than the chloro-ribose and even under heating conditions the reaction did not proceed. Therefore, consideration of treatments of the mesylate group to make it a better leaving group need to be considered. Silver carbonate has
been shown to interact with the mesylate group and withdraw electrons from the less electronegative carbon the mesylate group is attached to and allow the carbon to assume a partial positive charge and be more susceptible to nucleophilic attack. This phenomenon was found by Hashimoto et al. in the context of interaction between the mesylate group and silver carbonate, which allowed for what is thought to be partial cleavage of the kinetically favored mesylate epoxide ring to allow for an unusual ring expansion reaction.\textsuperscript{34} Therefore, the addition of silver carbonate to the mesylate-ribose during the sodium hydride glycosylation reaction with deazaalloxazine may improve reactivity and drive the reaction to favor the desired n-nucleotide product.
Citations


(22) Li, J. J. Williamson Ether Synthesis. In Name Reactions; Springer International Publishing: Cham, 2014; pp 628–628.


