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Anqi Wan April 14, 2020

Synthesis of a novel *C*-nucleoside prodrug for treatment of Hepatitis B

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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

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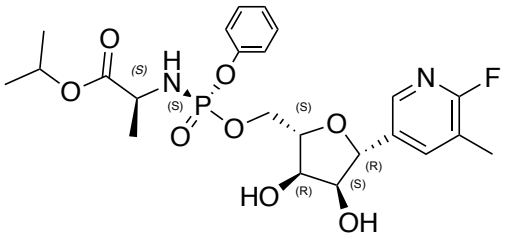
In an effort to treat newly emerging HBV viral strains effectively, there is a need to develop novel nucleoside analogs with improved biological properties. Nucleot(s)ide based anti-HBV agents suppress the HBV viral replication by inhibiting the key polymerase activity known as reverse transcriptase. *C*-nucleosides offer a more stable alternative to the conventional nucleoside analogs, due to their stable C-C linkage in place of common C-N glycosidic bond. We designed a novel *C*-nucleoside analog and its phosphoramidate prodrug for targeting HBV and the prodrug was synthesized successfully in 10 steps with overall yield of 2.84%.



Synthesis of a novel C-nucleoside analogue prodrug for treatment of Hepatitis B

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Introduction

Despite effective vaccines and other disease control measures in place, Hepatitis B remains a public health concern worldwide. According to the World Health Organization (WHO), as of 2015, 257 million individuals are chronically infected with hepatitis B,[1] and an estimated 0.8 million deaths were resulted from HBV-related diseases mainly from cirrhosis and hepatocellular carcinoma.[2] Currently, two types of inhibition strategies are used to treat HBV, injection of interferons and oral antivirals. In most countries, nucleoside or nucleotide analogues are the mainstream treatment including entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF).[2,3] Patients will be able to achieve very low viral load in short to medium term nucleoside treatment, and in the long term, it can reduce the chance of patients to develop hepatocellular carcinoma (HCC). However, for patients with consistent virological remission, HCC is still a risk.[4] Besides, if the patient discontinues their nucleoside analog therapy, viral relapse occurs consequently.[5] None of the current treatment options can eradicate the viral infection completely to achieve cure.[2] Therefore, There is an unmet medical need to develop novel therapeutics for the treatment of HBV infection.

In order to develop new therapeutics, it's important to understand the replication of HBV in the human body. The replication cycle of HBV is shown in figure 1 and it starts from the binding of hepatitis B virus to surface receptors on hepatocytes, and the virus is internalized in the cell through endocytosis. The HBV partially double-stranded relaxed circular DNA (rcDNA) enters the nucleus and converts to covalently closed circular DNA (cccDNA), which acts as the template for the transcription of mRNA.[13] The mRNA then reenters the cytoplasm, and the pregenomic RNA (pgRNA) shown in Figure 2 is used to translate to core proteins and virus polymerase while the subgenomic RNA is used to translate to three envelope proteins.[14] In addition, the pgRNA is

used as a template for reverse transcriptase to transcribe into DNA that can be either packaged for future progenies or reentry into nucleus to make more cccDNA. Since the viral polymerase plays an essential role in the replication process, the reverse transcriptase has been identified as the target for many nucleoside inhibitors.[15] By mimicking the structure of natural nucleotides, nucleoside inhibitors can bind competitively onto the HBV polymerase and no further nucleotides can be added to the transcription chain, leading to the termination of viral DNA transcription.[15] Therefore, nucleoside analogs lead to a reduced the load of cccDNA accumulating in infected hepatocytes and also formation of viral progenies.[16]

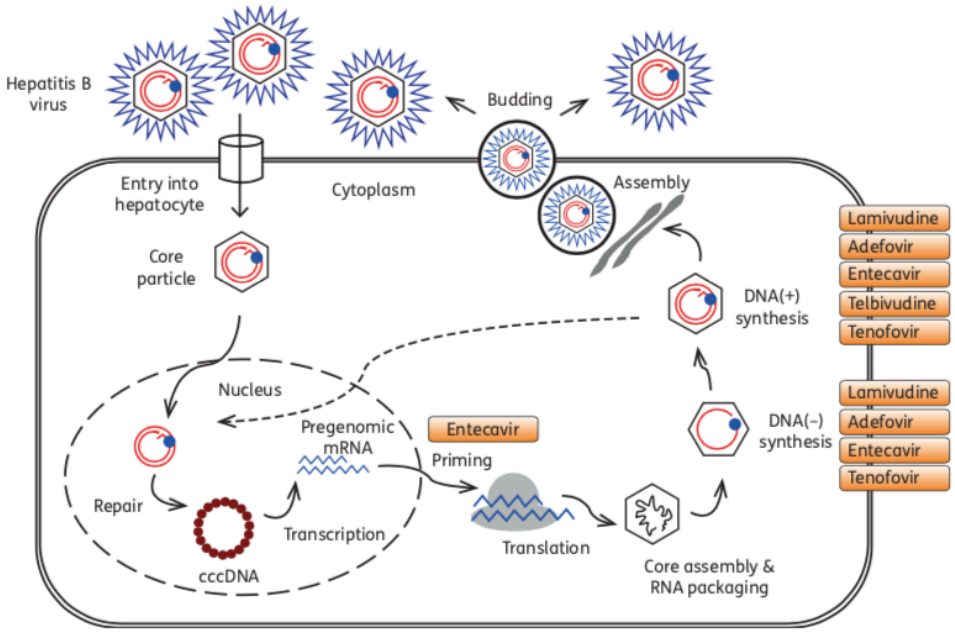


Figure 1. Life cycle of HBV. Reprinted from Fung, Lai, Seto, and Yuen.[14]

A common problem faced by many nucleoside inhibitors is that despite their promising profile in antiviral activity *in vitro*, they have shown poor oral bioactivity due to their high polarity and low membrane permeability.[10] Therefore, the concept of "the prodrug" is introduced to improve the lipophilicity and carry the drug to target tissue by covalently bind to a non-toxic

moiety through phosphoester bond, carboxylic ester bond, carbamate bond or amide bond.[11] In order for nucleosides to be active metabolites in physiological conditions, the molecule needs to be phosphorylated by a series of kinases to its triphosphate shown in Figure 5. However, the rate-limiting step in the intracellular conversion is the first step of phosphorylation, *i.e.*, the addition of monophosphate onto the nucleoside, mediated by nucleoside kinase to bypass this limitation, nucleoside monophosphate (nucleotide) prodrugs were designed by attaching the monophosphate group onto the nucleoside that can improve the effective concentration of the active drug.[11,12] Since phosphate group carries charges that make them cell impermeable, monophosphate groups were masked with lipophilic moieties for better membrane permeability.[12] Therefore, making "a nucleotide prodrug" is a hopeful strategy to improve the oral availability of the drug.

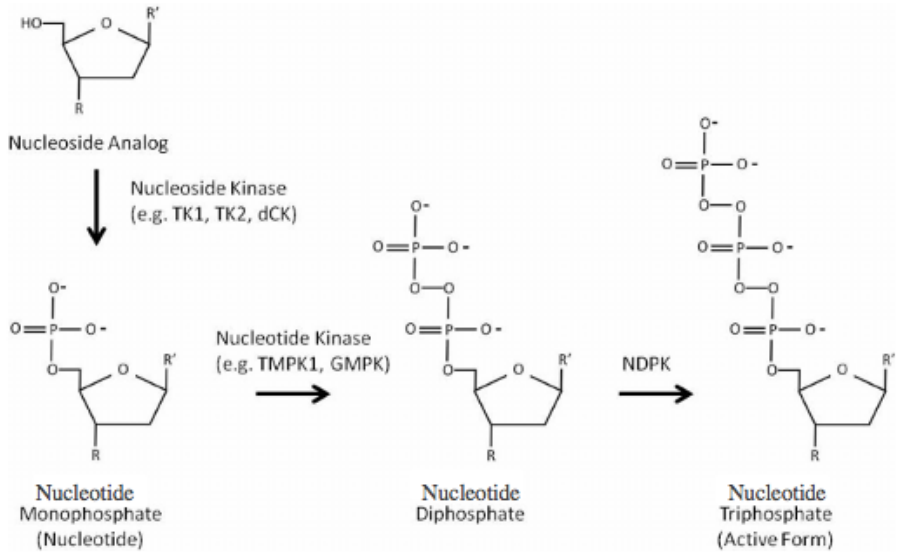


Figure 2. Phosphorylation of nucleoside analogs

Some of the most common nucleoside inhibitors used clinically are shown in Figure 2.[16] Lamivudine (LMV) has shown active viral inhibition activity due to lack of 3' OH functional group that lead to early termination of viral DNA polymerization. However, for many long-term Lamivudine users, they are more prone to develop a YMDD (tyrosine-methionine-aspartate-

aspartate) mutation at the HBV reverse transcriptase active site where methionine residue is replaced by either isoleucine or valine at amino acid 204.[17] Many other compensatory mutations are observed by long-term Lamivudine use including rtL80V/I, 58rtI169T, 59rtV173L, rtL180M, rtT184S, rtS202I, and rtQ215S.[18] Telbivudine (LdT) is a L-isomer of thymidine, but it promotes similar mutations developed in LMV including rtM204I in at YMDD loop and co-mutation at rtL180M and rtL80I/V.[19] Adefovir dipivoxil is a prodrug that can be phosphorylated by nucleotide kinases to convert to the active metabolite adefovir diphosphate which can cause premature termination of DNA polymerization.[20] Despite ADV's effectiveness against LMV-resistance, long-term ADV users develop rtA181V/T at domain B of the HBV polymerase and rtN236T and rtI233V at domain D.[21,22] Entecavir is a 2'-deoxy-guanosine analog, and it can be efficiently phosphorylated by kinase into a triphosphate and it actively competes with natural 2′-deoxy-guanosine triphosphate to binds to the reverse transcriptase to terminate transcription of viral DNA.[23] ETV is very efficient in anti-HBV activity, and it becomes one of the first-line treatment for HBV with less than 1% resistant rate even after five-year treatment.[24] Tenofovir disoproxil fumarate (TDF) is structurally very similar to adefovir dipivoxil with an additional methyl group, and it shares a similar potency, effectiveness against LMV-resistance and the same mechanism of action with ADV.[25,26] In addition, after seven-year treatment of TDF, no resistance was detected with low occurrence of body density loss and renal failure.[27] Clevudine (CLV) is a pyrimidine analog with very similar structure to Telbivudine with an additional fluorine on the 2′ carbon of the sugar backbone. It exhibits similar resistance as to LMV and LdT with rtM204I be the most common mutation.[28] It was approved in South Korea and Philippines for HBV treatment, later revoked due to complications associated with myopathy. Tenofovir alafenamide (TAF) is a nucleoside analog that used a different prodrug moiety for Tenofovir. It shares comparable efficacy

against HBV and high barrier to resistance to TDF, but it has improved the bone and renal safety profile.[30] TAF has better intrahepatic levels than TDF, so a low dose of TAF is needed to achieve viral suppression and off-target effects are reduced.[31] Currently, TAF and TDF are the first-line therapy for treating Hepatitis B virus due to their high resistance profile and potency.[2] However, due to the emergence of multi-resistance viral strains and the development of HCC under nucleoside therapy, it warrants new drug therapy that could completely eradicate the viral load from the system.

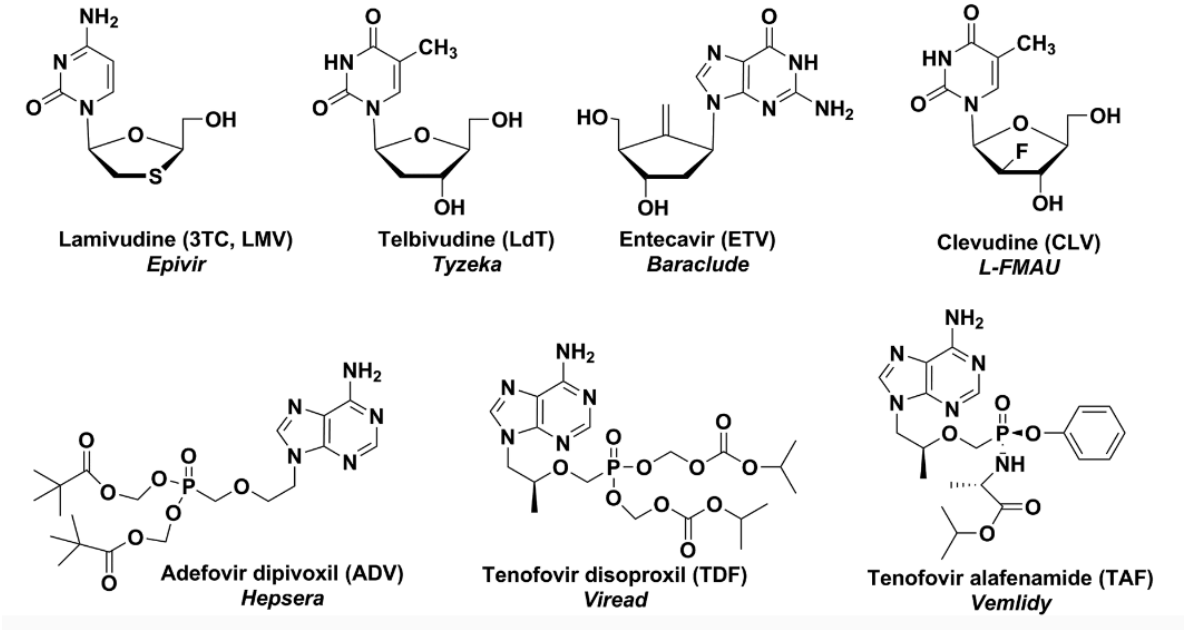


Figure 3. Structures of current nucleoside analogs to HBV infection, reprinted from Singh, Mulamoottil, and Chu[16]

C-nucleoside was an idea brought up about half a century ago, and in theory, it can target enzymes used in nucleic acid metabolism.[6] It uses carbon instead of nitrogen to link the sugar base of the nucleoside to their heterocyclic moiety shown in Figure 3. *C*-nucleoside can change the acid-basic properties and tautomeric population in the active site of the enzyme. Therefore, it has been experimentally proven that some *C*-nucleoside analogs are great inhibitors of purine

nucleoside phosphorylase (PNP) and nucleosidase.[6,7] In addition, it has an advantage over the conventional *N*-nucleoside that it remains stable against the phosphorolysis that cleaves the *N*-glycosidic linkage that degrades the compound.[7] By changing different heterocyclic bases attached to the sugar, the efficacy towards antiviral activity and safety profile can be improved.[7] The first *C*-nucleoside HCV polymerase inhibitor GS-6602 (Figure 4) went into clinical trial showing promising inhibitory activity and selectivity against HCV, but it's limited by the high pharmacokinetics and pharmacodynamic variability.[9] However, this provides a promising approach for the development of other novel nucleoside inhibitors to treat other viral infections.

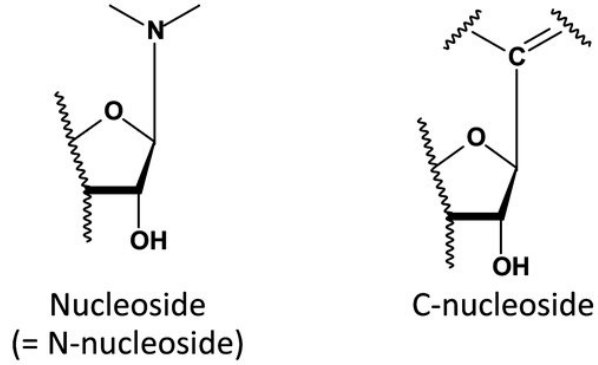


Figure 4. *N*-nucleoside and *C*-nucleoside

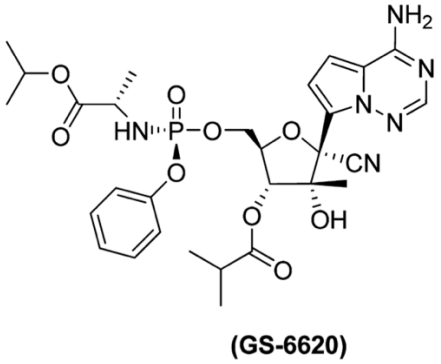


Figure 5. Structure of GS-6620

The stable Carbon-Carbon bond of C-nucleoside gives us new hope to synthesis more stable nucleoside inhibitors that can bind to HBV polymerase and terminate transcription with higher potency. Our proposed target *C*-nucleoside and its prodrug are shown in Figure 6. A cytosine equivalent base is proposed to link to the sugar moiety to design a novel nucleoside analog (**13**), and a phosphoramidite prodrug moiety is attached to the 5' of nucleoside to convert to the desired prodrug (**15**).

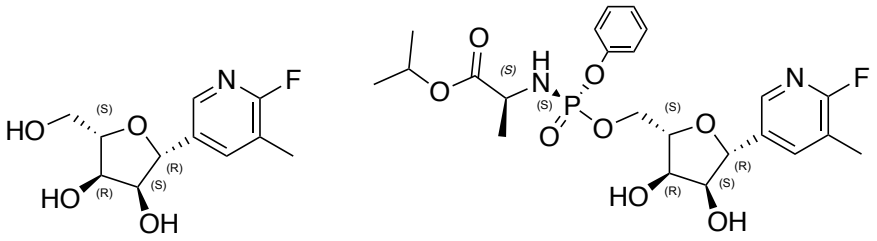
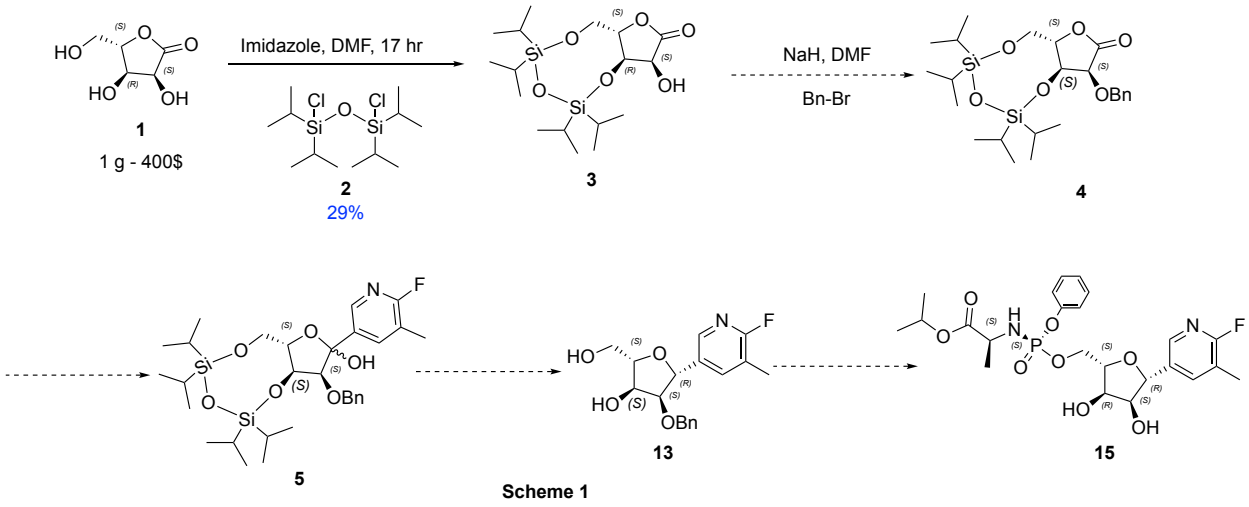


Figure 6. Novel C-nucleoside and its prodrug

Results and Discussion

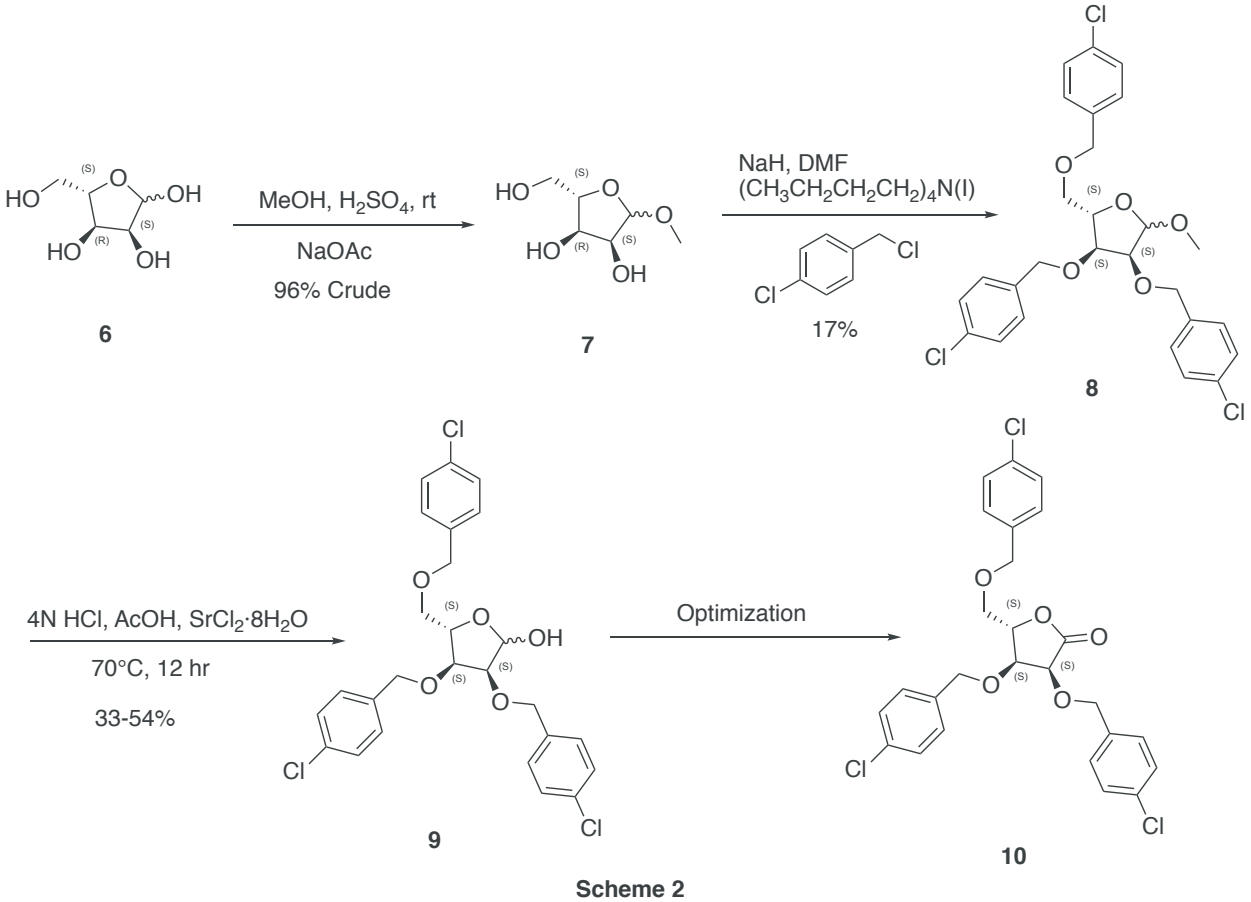
Initially attempted approach to make *C*-nucleoside (**13**) is described in scheme 1, which includes 4 steps to obtain proposed nucleoside **13**. The commercially available L-lactone **1** (1g - 400$) was used as a starting material and it's 4' and 5' hydroxyl groups were protected as tetraisopyldisiloxane to obtain **3**. However, the step was problematic due to the formation of lots of side-products resulting in the required product with a yield of 29%. Then, the plan was to protect 2' hydroxyl as benzoate. However, the reaction failed with no product formed, and scheme 1 was abandoned mainly due to difficulty with the synthesis as well as not being able to scale-up due to expensive starting material lactone **1**.



Scheme 1

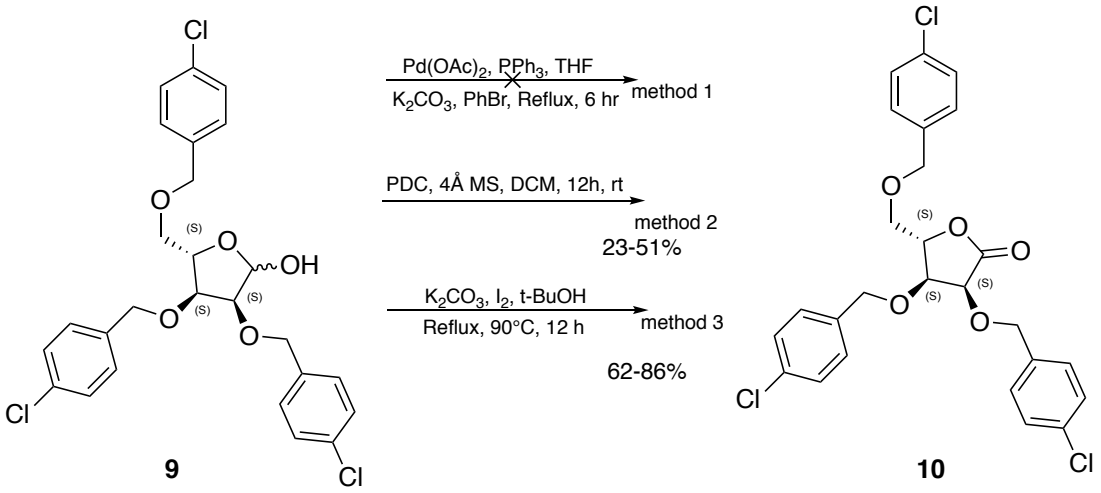
Scheme 2 was proposed as an alternative approach to making C-nucleoside **13** in which we used a different protection group strategy using commercially available lactol **6**. L-(+)-Ribose (**6**) was a relatively cheaper starting material compared to L-ribonic acid-1,4-lactone (**1**) which makes Scheme 2 more cost-efficient. The lactol **6** underwent acid-mediated addition of methoxide to make a methoxy derivative **7**, and the reaction was carried out as a crude mixture. Then, **7** was protected with 4-chlorobenzyl chloride on the 3', 4' and 5' hydroxyls to form benzyl ethers. The

reaction in a 20 g scale reaction resulted in 17% yield, because the global protection didn't go to completion and side-products formed. In addition, the work-up environment wasn't ideal. During the extraction of products in dichloromethane from the water layer, large amount of precipitation formed, and the filtration of the precipitation was difficult to perform due to the large volume. The reaction was repeated again in 50g scale, and the crude mixture was used to carry on the next reaction due to the difficulty to do column chromatography in large scale. Even though the yield for the reaction was low, but around 15 g of product were produced from the reaction to continue the synthesis. However, in the future, this step is in need of optimization. The compound **8** was subjected to replace the methoxy to a hydroxide in order to gain access to the 1' alcohol **9**. The oxidation of **9** was challenging given by three methods used in scheme 3.

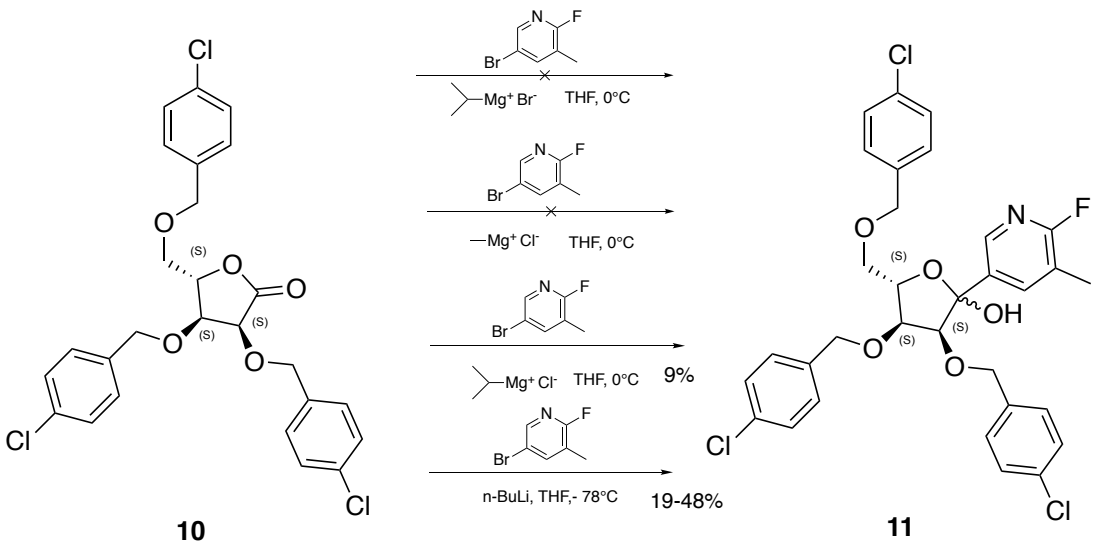


Scheme 2

The oxidation of 1' alcohol **9** to a carbonyl to obtain **10** was difficult, three methods were used to find a higher yielding method. **Method 1** used Palladium(II) acetate with triphenylphosphine and reflux with potassium carbonate and bromobenzene, but the reaction did not lead to the desired product. **Method 2** used pyridinium dichromate and molecular sieves in DCM which gave **10**, yields varying between 23-51%. Eventually, **Method 3** was used with potassium carbonate and iodine in *tert*-butanol with a promising yield ranging from 62-86% to obtain **10**.

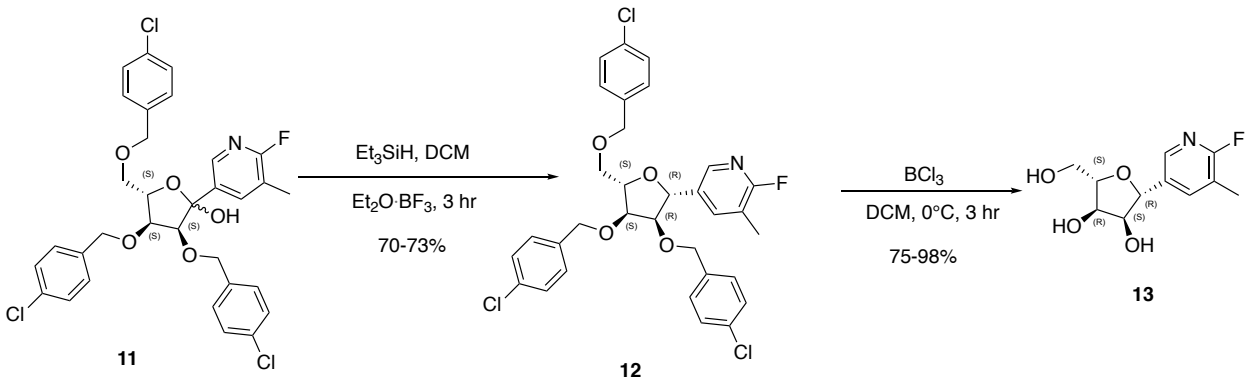


Scheme 3



Scheme 4

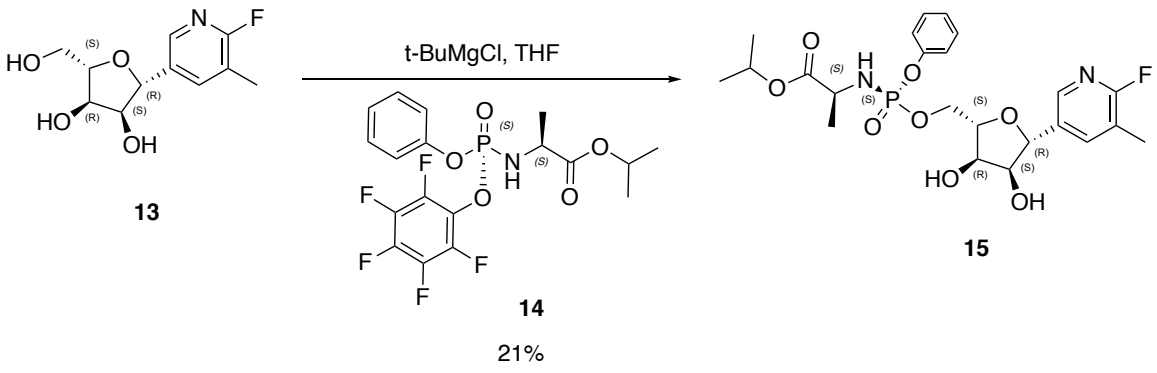
The coupling of commercially available pyridine base on the lactone sugar core **10** to obtain **11** was also a challenging step (Scheme 4). Initially, Grignard reagents were used for the coupling, however, among the three reagents tested, only isopropyl magnesium chloride successfully made the carbon-carbon bond with only 9% yield in addition to the long reaction time needed for the experiment. The two other Grignard reagents, methyl magnesium chloride and isopropyl magnesium bromide didn't work leaving starting material in the reaction mixture. Eventually, n-butyllithium worked best for the coupling reaction and obtained **11** with yield from 19% to 48%. The products **11** are mixture of anomers, but both isomers can be carried on to the subsequent reaction.



Scheme 5

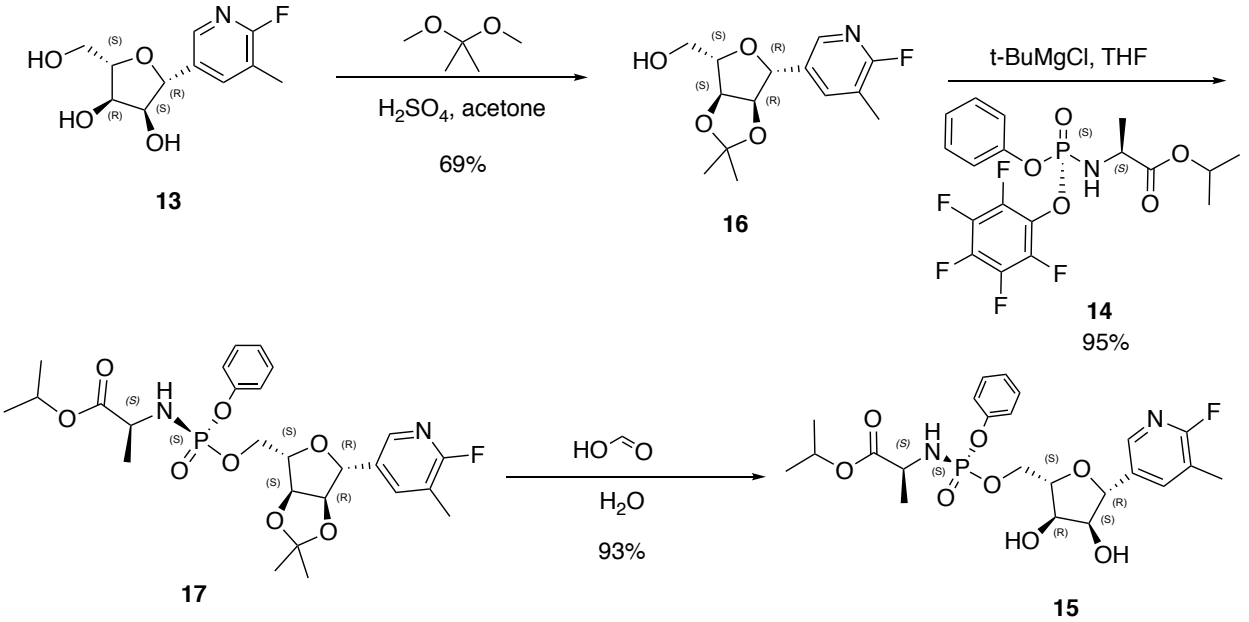
In Scheme 5, after the formation of the carbon-carbon bond from the coupling of nucleobase onto the sugar core, the tertiary alcohol **11** on the 1' of sugar was removed with triethylsilane and boron trifluoride etherate. The dehydroxylation reaction produced product **12** in a single diastereomer with a yield of around 70%. The following deprotection of 4-chlorobenzyl groups was accomplished with boron trichloride in DCM at 0 °C to obtain the final *C*-nucleoside **13** in 75-98% yield.

However, in order to target the hepatocytes, the *C*-nucleoside **13** was converted to its monophosphate prodrug **14** (Scheme 6). For this reaction, the 5' hydroxyl group of the sugar core was coupled with phosphoramidate moiety **14** (provided by Dr. Manohar Saindane) using t-butyl magnesium chloride. Even though the final product **15** was successfully synthesized in Scheme 6, the yield was close to 21% with 70% purity (purity analyzed by ^{1}H-NMR). The separation of mixture by silica gel column chromatography wasn't very successful, resulting in a mixture of product and reagent in 7:3 ratio. In order to achieve product **15** with high purity and in higher yield, scheme 7 was employed.



Scheme 6

In scheme 7, 2' and 3' hydroxyl groups were protected with 2,2-dimethoxypropane to form acetonide **16** in 69% yield which was promising in improving the final yield of the product. The coupling of **16** with phosphoramidate moiety **14** using t-butyl magnesium chloride as a base produced **17** in 95% yield. In the final step, formic acid was used to deprotect the acetonide of compound **17** to give a pure final prodrug **15** after column chromatography with 93% yield. This concludes the successful synthesis of *C*-nucleoside **13** and its phosphoramidate prodrug **15.** Both compounds (**13** and **15**) will be sent out to evaluate its anti-HBV activity.



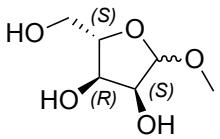
Scheme 7

Experimental

General:

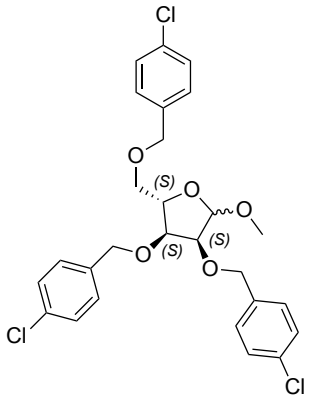
All ^{1}H-NMR, ^{19}F-NMR, ^{31}P-NMR spectrums were obtained on a 400 MHz-Varian INOVA 400 spectrometer at 20 °C, and all the ^{13}C-NMR spectrums were obtained on a 600 MHz- Bruker Avance 600WB solid spectrometer at room temperature. All NMR spectra were reported in parts per million (ppm) in δ scale. All NMR samples were prepared with either deuterated chloroform ($CDCl_3$) with solvent peak at 7.26 ppm for ^{1}H spectrum and 77.16 ppm for ^{13}C spectrum or deuterated methanol (CD_3OD) with solvent peak at 3.31 ppm for ^{1}H spectrum and 49.15 ppm for ^{13}C spectrum. ^{1}H-NMR data were reported as following: chemical shift [multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets), coupling constant (J) in Hz, integration]. All the high-resolution mass spectrometry (HRMS) were obtained from Emory University Mass Spectrometry Center with either a Thermo LTQ-FTMS or Thermo Exactive Plus Mass Spectrometer. All automated flash column chromatography was performed on a Teledyne ISCO CombiFlash Rf + instrument with silica gel cartridge (RediSep). Analytical thin-layer chromatography (TLC) was carried out on glass silica-gel plate and visualized under UV light (254 nm). The starting material, D(-)-Ribose (**6**) was obtained commercially from Sigma Aldrich, and the 5-bromo-2-fluoro-3-methyl-pyridine was obtained commercially from Combi-Blocks, Inc. isopropyl (2S)-2-[[(2,3,4,5,6-pentafluorophenoxy)-phenoxy-phosphoryl]amino]propanoate (**14**) was made by Dr. Manohar Saindane from Emory Institute for Drug Development.

Synthesis:



(2S,3R,4S)-2-(hydroxymethyl)-5-methoxytetrahydrofuran-3,4-diol (7)

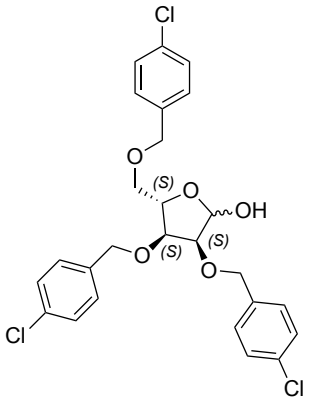
L-(+)-Ribose (**6**) (6.00g, 39.965 mmol, 1 eq.) was dissolved in 100 mL of methanol, and sulfuric acid (0.25 mL, 4.56 mmol, 0.11 eq.) was added to the solution. The solution was stirred overnight, and sodium acetates (0.72 g, 8.78 mmol, 0.22 eq.) was added and stirred for an hour. The solvent was evaporated with rotary evaporator, and vacuum dried to obtain crude product **7**.



(2S,3S,4S)-3,4-bis((4-chlorobenzyl)oxy)-2-(((4-chlorobenzyl)oxy)methyl)-5-methoxytetrahydrofuran (8)

Sodium hydride (5.54 g, 172.92 mmol, 3.5 eq.) in mineral oil was washed by 50 mL of hexane, and hexane was extracted out of the flask into isopropanol. 30 mL of dimethylformamide was added into the flask under argon along with (2S,3R,4S)-2-(hydroxymethyl)-5-methoxytetrahydrofuran-3,4-diol (**7**) (6.5 g, 36.55 mmol, 1 eq.). Then, tetrabutylammonium iodide (0.15 g, 0.37 mmol, 0.01eq.) and 4-Chlorobenzyl chloride (22.3 g, 127.9 mmol, 3.5 eq.) were added into the mixture. The solution was stirred overnight, and 5 mL of ammonium chloride was added at 0 °C to quench the reaction. After evaporation of dimethylformamide using rotary evaporator, the reaction mixture was extracted with dichloromethane and washed with water and dried over Na_2SO_4. The crude material was loaded on to silica gel column (80 g RediSep Rf silica column) and purified to get product **8** (15-25% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 – 7.22 (m, 10H), 7.22 – 7.17 (m, 2H), 4.91 (s, 1H), 4.67 – 4.39 (m, 6H), 4.34 – 4.26 (m, 1H),

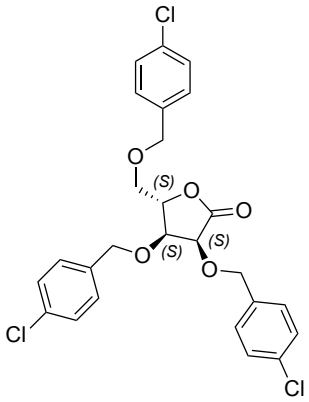
4.00 (d, J = 11.8 Hz, 1H), 3.82 (d, J = 4.6 Hz, 1H), 3.59 (dd, J = 10.6, 3.8 Hz, 1H), 3.50 (dd, J = 10.6, 5.6 Hz, 1H), 3.32 (s, 3H); HRMS m/z calculated for $C_{27}H_{27}Cl_3O_5$ $[M + Na]^+$: 560.09, found 559.08.



(3S,4S,5S)-3,4-bis((4-chlorobenzyl)oxy)-5-(((4-chlorobenzyl)oxy)methyl)tetrahydrofuran-2-ol (9)

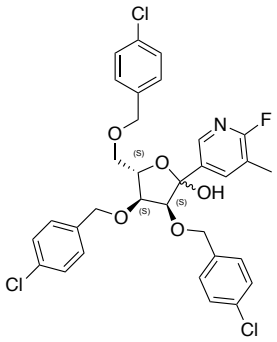
Acetic acid (50 mL, 868.61 mmol, 56.3 eq.) was added to (2S,3S,4S)-3,4-bis((4-chlorobenzyl)oxy)-2-(((4-chlorobenzyl)oxy)methyl)-5-methoxytetrahydrofuran (**8**) (8.3 g, 15.43 mmol, 1 eq.) under argon. Then, Strontium chloride hexahydrate (0.52 g, 1.94 mmol, 0.13 eq.) and 4N HCl (16.5 mL, 65.9 mmol, 4.3 eq.) was added to the mixture. The solution was heated to 70 °C overnight, and the reaction was allowed to cool to room temperature and the acetic acid was removed using rotary evaporator. The concentrated reaction mixture was loaded onto silica gel column through dry loading (120 g RediSep Rf silica column) to get the purified product **9** (35-42% EtOAc in hexane). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34 – 7.27 (m, 16H, two isomers), 7.22 – 7.18 (m, 4H, one isomer), 7.18 – 7.14 (m, 4H, other isomer), 5.31 (m,, 2H, two isomers), 4.70 – 4.33 (m, 12H, two isomers), 4.29 (dt, *J* = 6.2, 2.9 Hz, 1H, one isomer), 4.18 (dd, *J* = 6.8, 4.7 Hz, 1H, other isomer), 3.84 – 3.83 (d, *J* = 4.4 Hz, 1H, one isomer), 3.65 (dd, *J* = 10.4, 2.8 Hz, 1H,

other isomer), 3.54 – 3.41 (m, 4H, two isomers); HRMS m/z calculated for $C_{26}H_{25}Cl_3O_5$ $[M + Na]^+$: 546.08, found 545.1.



(3S,4S,5S)-3,4-bis((4-chlorobenzyl)oxy)-5-(((4-chlorobenzyl)oxy)methyl)dihydrofuran-2(3H)-one (10)

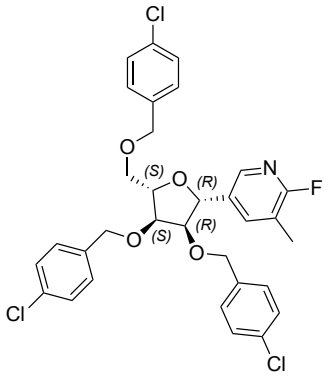
(3S,4S,5S)-3,4-bis((4-chlorobenzyl)oxy)-5-(((4-chlorobenzyl)oxy)methyl) tetrahydrofuran-2-ol (**9)** (2.16 g, 4.12 mmol, 1 eq.) was dissolved in 11 mL of tert-butanol under argon. Iodine (2.1 g, 8.35 mmol, 2 eq.) and potassium carbonate (1.14 g, 8.35 mmol, 2 eq.) was added into the solution, and the solution was heated to 90 °C under reflux and stirred overnight. The reaction was quenched by addition of sodium sulfite at 0 °C. The mixture was extracted with ethyl acetate and washed with brine solution, dried over Na_2SO_4, filtered and concentrated. The mixture was purified by silica gel chromatography (40 g RediSep Rf silica column) to obtain (25-35% EtOAc in hexane) product **10** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 – 7.21 (m, 10H), 7.20 – 7.15 (m, 2H), 7.09 – 7.03 (m, 2H), 4.90 (d, J = 12.1 Hz, 1H), 4.65 (t, J = 12.3 Hz, 3H), 4.53 – 4.47 (m, 2H), 4.39 (q, J = 11.9 Hz, 2H), 4.32 (d, J = 5.6 Hz, 1H), 3.65 (dd, J = 11.0, 2.8 Hz, 1H), 3.56 (dd, J = 11.0, 2.6 Hz, 1H); HRMS m/z calculated for $C_{26}H_{23}Cl_3O_5$ $[M + H]^+$: 519.06, found 519.05.



(3S,4S,5S)-3,4-bis((4-chlorobenzyl)oxy)-5-(((4-chlorobenzyl)oxy)methyl)-2-(6-fluoro-5-methylpyridin-3-yl)tetrahydrofuran-2-ol (11)

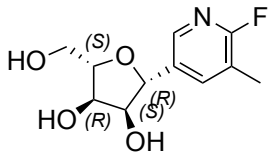
5-bromo-2-fluoro-3-methyl-pyridine (1.09 g, 5.75 mmol, 3 eq.) was dissolved in 10 mL of anhydrous tetrahydrofuran under argon, and the solution was cooled to -78 °C. *n*-Butyllithium (2.53 mL, 6.32 mmol, 3.3 eq.) was added dropwise and stirred for 30 min. To this mixture, (3S,4S,5S)-3,4-bis((4-chlorobenzyl)oxy)-5-(((4-chlorobenzyl)oxy)methyl)dihydrofuran-2(3H)-one (**10**) (1.0 g, 1.91 mmol, 1 eq.) in 10 mL of anhydrous tetrahydrofuran was added dropwise. The mixture was stirred for 3 hours, and the reaction was quenched by the addition of saturated aqueous ammonium. chloride. The solution was extracted with ethyl acetate and washed with brine solution, dried over Na_2SO_4, filtered, and concentrated. The mixture was purified by silica gel chromatography (24 g Gold RediSep Rf silica column) to obtain (30-35% EtOAc in hexane) product **11**. ^{1}H NMR (400 MHz, $CDCl_3$): 8.25 (s, 1H), 7.80 – 7.73 (m, 0.3H, isomer), 7.73 – 7.67 (m, 0.7H, isomer), 7.31 (m, 6H), 7.25 – 7.12 (m, 8H), 7.01 (d, *J* = 8.3 Hz, 1.4H, isomer), 6.88 (d, *J* = 8.3 Hz, 0.6H, isomer), 5.05 (td, *J* = 5.1, 2.1 Hz, 0.3H, isomer), 4.97 (d, *J* = 3.5 Hz, 0.7 H, isomer), 4.82 (s, 0.6 H, isomer), 4.62 (s, 1.4 H, isomer), 4.57 – 4.31 (m, 4H), 4.15 – 3.96 (m, 1H), 3.91 (d, *J* = 4.9 Hz, 0.7H, isomer), 3.82 (d, *J* = 4.2 Hz, 0.3H, isomer), 3.76 – 3.48 (m, 4H), 2.23 (s, 0.9H, isomer), 2.18 (d, *J* = 1.0 Hz, 2.1H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -73.40 (d, *J* =

9.7 Hz), -73.53 (d, *J* = 9.4 Hz); HRMS m/z calculated for $C_{32}H_{29}Cl_3FNO_5$ $[M + H]^+$: 632. 11, found 632.12.



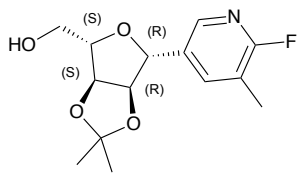
5-((3*R*,4*S*,5*S*)-3,4-bis((4-chlorobenzyl)oxy)-5-(((4-chlorobenzyl)oxy)methyl)tetrahydrofuran-2-yl)-2-fluoro-3-methylpyridine (12)

(3S,4S,5S)-3,4-bis((4-chlorobenzyl)oxy)-5-(((4-chlorobenzyl)oxy)methyl)-2-(6-fluoro-5-methylpyridin-3-yl)tetrahydrofuran-2-ol (**11**) (0.48 g, 0.76 mmol, 1 eq.) was dissolved in 11 ml anhydrous dichloromethane under argon. Triethylsilane (0.48 mL, 3.03 mmol, 4 eq.) was added to the solution. After the solution was cooled to 0°C with water and ice, boron trifluoride-diethyl ether complex (0.28 mL, 2.28 mmol, 3 eq.) was added to solution. The reaction was left at room temperature to stir for an hour, and 1 mL of triethylamine was used to stop the reaction. The solvent was evaporated with rotary evaporator and loaded on normal phase chromatography (12 g RediSep Rf silica column) to purify (25-30% EtOAc in hexane) product **7**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (s, 1H), 7.49 (dd, J = 9.4 Hz, 1H), 7.34-7.29 (m, 4H), 7.27-7.24 (m, 2H), 7.21-7.18 (m, 4H), 7.04 (d, J = 8.5 Hz, 2H), 4.92 (d, J = 7.7 Hz, 1H), 4.58 (s, 2H), 4.53 – 4.44 (m, 3H), 4.36 – 4.29 (m, 2H), 3.99 (dd, J = 5.1, 2.9 Hz, 1H), 3.73 (dd, J = 7.6, 5.1 Hz, 1H), 3.68 – 3.57 (m, 2H), 2.15 (s, 3H); ^{19}F NMR (400 MHz, $CDCl_3$): δ -73.55 (d, J = 9.5 Hz); HRMS m/z calculated for $C_{32}H_{29}Cl_3FNO_4$ $[M + H]^+$: 616.11, found 616.12.



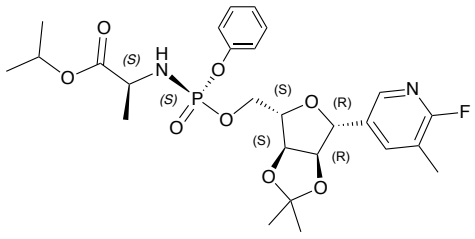
(2R,3S,4R,5S)-2-(6-fluoro-5-methylpyridin-3-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (13)

5-((2R,3R,4S,5S)-3,4-bis((4-chlorobenzyl)oxy)-5-(((4-chlorobenzyl)oxy)methyl) tetrahydrofuran-2-yl)-2-fluoro-3-methylpyridine (**12**) (0.10 g, 0.162 mmol, 1 eq.) was dissolved in 1 mL DCM and cooled to 0 °C. Boron trichloride (1.62 mL, 1.62 mmol, 10 eq.) was added dropwise, and the reaction was quenched after 45 mins with 1 mL of methanol. 1 mL of 1:1 dichloromethane and methanol were added to dilute the solution, and 7N ammonium methanol was added to neutralize the reaction. All salt was filtered off , and the solvent was evaporated using rotary evaporator. The crude mixture was purified by silica gel chromatography (4 g RediSep Rf silica column) to obtain (15-20% MeOH in DCM) product **13**. ^{1}H NMR (400 MHz, CD_3OD): δ 8.06 (s, 1H), 7.90 (d, J = 9.7 Hz, 1H), 4.70 (d, J = 7.7 Hz, 1H), 4.08 (dd, J = 5.5, 3.5 Hz, 1H), 3.99 (q, J = 3.9 Hz, 1H), 3.86 – 3.81 (m, 1H), 3.81 – 3.68 (m, 2H), 2.30 (s, 3H); ^{13}C NMR (600 MHz, CD_3OD): δ 163.27 (d, J = 238.2 Hz), 143.57 (d, J = 13.8 Hz), 141.81 (d, J = 6.1 Hz), 136.21 (d, J = 4.5 Hz), 120.92 (d, J = 31.9 Hz), 87.30, 82.30, 79.15, 73.10, 63.61,14.42; ^{19}F NMR (400 MHz, CD_3OD): δ -77.77 (d, J= 9.3 Hz); HRMS m/z calculated for $C_{11}H_{14}FNO_4$ $[M + H]^+$: 244.09, found 244.1.



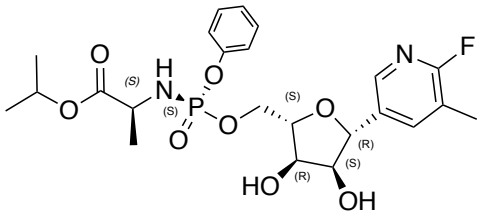
((3aS,4S,6R,6aR)-6-(6-fluoro-5-methylpyridin-3-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol (16)

(2R,3S,4R,5S)-2-(6-fluoro-5-methylpyridin-3-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (**13**) (80 mg, 0.329 mmol, 1 eq.) was dissolved in 2.5 ml acetone. 2,2-dimethoxypropane (0.19 mL, 1.58 mmol, 4.8 eq.) was added to the solution followed by sulfuric acid (0.023 mL, 0.428 mmol, 1.3 eq.). After the mixture was stirred at room temperature for 30 minutes, the reaction was warmed to 45°C for 30 minutes. To stop the reaction, the reaction was cooled to 0°C, and 0.5 mL of ammonium hydroxide was added. The solvent was evaporated using rotary evaporator and the crude mixture was purified by silica gel chromatography (4 g RediSep Rf silica column, solid loading) to obtain (4-7% MeOH in DCM) product **16**. ^{1}H NMR (400 MHz, CD_3OD): δ 8.03 (s, 1H), 7.84 (d, J = 9.5 Hz, 1H), 4.82 (d, J = 5.7 Hz, 1H), 4.75 (dd, J = 6.8, 3.9 Hz, 1H), 4.54 – 4.48 (m, 1H), 4.11 (q, J = 4.0 Hz, 1H), 3.76 (qd, J = 12.0, 4.3 Hz, 1H), 2.30 (s, 3H), 1.59 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 162.09 (d, J = 238.9 Hz), 142.37 (d, J = 14.7 Hz), 139.53 (d, J = 6.1 Hz), 132.94 (d, J = 4.5 Hz), 119.74 (d, J = 32.6 Hz), 115.48, 86.53, 84.71, 83.36, 81.61, 62.57 (d, J = 20.0 Hz), 27.55, 25.53, 14.53; ^{19}F NMR (400 MHz, CD_3OD): δ -77.13 (d, J= 9.6 Hz). HRMS m/z calculated for $C_{14}H_{18}FNO_4$ $[M + H]^+$: 284.12, found 284.1.



Isopropyl((S)-(((3aS,4S,6R,6aR)-6-(6-fluoro-5-methylpyridin-3-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (17)

((3aS,4S,6R,6aR)-6-(6-fluoro-5-methylpyridin-3-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol (**16**) (60 mg, 0.211 mmol, 1 eq.) was dissolved in 3 mL tetrahydrofuran, and the solution was cooled to 0°C. Then, t-butylmagnesium chloride (0.52 mL, 0.53 mmol, 2.5 eq.) was added to the solution, and the mixture was brought back to room temperature and stirred for an hour. Isopropyl(2S)-2-[[(2,3,4,5,6-pentafluorophenoxy)-phenoxy-phosphoryl]amino]propanoate (**11**) (0.1440 g, 0.318 mmol, 1.5 eq.) dissolved in 1 mL of tetrahydrofuran was added to the reaction mixture dropwise, and the mixture was stirred overnight. The reaction was quenched by cooling to 0°C with the addition of 1 mL of methanol. After the solvent was evaporated using rotary evaporator, the crude mixture was purified by silica gel chromatography (4 g RediSep Rf silica column) to obtain (50-60% EtOAc in Hexane) product **17**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.57 (d, J = 9.4 Hz, 1H), 7.26 (m, 2H), 7.21-7.18 (m, 1H), 7.16-7.11 (m, 1H), 5.00 (p, J = 6.3 Hz, 1H), 4.77 (d, J = 5.7 Hz, 1H), 4.63 (dd, J = 6.7, 3.4 Hz, 1H), 4.35 – 4.24 (m, 3H), 4.17 – 4.09 (m, 1H), 4.03 – 3.92 (m, 1H), 3.64(t, J= 11.4 Hz, 1H), 2.29 (s, 3H), 1.38 (d, J = 7.0 Hz, 3H), 1.30 (s, 3H), 1.21 (t, J= 7.5 Hz, 6H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 173.00 (d, J = 7.8 Hz), 162.22 (d, J = 238.3 Hz), 150.90 (d, J = 6.8 Hz), 142.53 (d, J = 14.9 Hz), 139.40 (d, J = 6.2 Hz), 132.88 (d, J = 4.6 Hz), 129.80, 125.17, 120.36 (d, J = 4.8 Hz), 119.65 (d, J = 32.9 Hz), 115.32, 86.35, 83.67, 82.59 (d, J = 7.9 Hz), 81.59, 69.49, 66.17 (d, J = 5.1 Hz), 50.43, 27.60, 25.53, 21.81, 21.76, 21.21 (d, J = 4.5 Hz), 14.59; ^{19}F NMR (400 MHz, $CDCl_3$): δ -73.54 (d, J= 9.6 Hz); ^{31}P NMR (400 MHz, $CDCl_3$): δ 2.55 (s). HRMS m/z calculated for $C_{26}H_{34}FN_2O_8P$ $[M + H]^+$: 553.20, found 553.2.



Isopropyl((((2S,3R,4S,5R)-5-(6-fluoro-5-methylpyridin-3-yl)-3,4-dihydroxy tetrahydrofuran -2-yl)methoxy)(phenoxy)phosphoryl)-D-alaninate (15)

Scheme 6:

(2R,3S,4R,5S)-2-(6-fluoro-5-methylpyridin-3-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (**13**) (20 mg, 0.0822 mmol, 1 eq.) was dissolved in 1 mL of tetrahydrofuran, and the solution was cooled to -5°C (ice and sodium chloride bath). t-Butyl magnesium chloride (0.1 mL, 0.0987 mmol, 1.2 eq.) was added to the solution, and the solution was brought to room temperature and stirred for an hour and thirty minutes. Isopropyl (2S)-2-[[(2,3,4,5,6-pentafluorophenoxy)-phenoxy-phosphoryl]amino]propanoate (**14**) (0.0373 g, 0.0822 mol, 1 eq.) in 1 mL of tetrahydrofuran was added dropwise to the mixture, and the solution was stirred overnight. The reaction was quenched by the addition of 1 mL of methanol at 0°C. The solvent was evaporated with rotary evaporator and the crude mixture was purified by silica gel chromatography (4 g RediSep Rf silica column) to obtain product **15** (5-6% MeOH in DCM). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.59 (d, J = 11.0 Hz, 1H), 7.31-7.12 (m, 5H), 5.30 (s, 1H), 5.02 (p, J = 6.3 Hz, 1H), 4.71 (d, J = 6.2 Hz, 1H), 4.39 – 4.25 (m, 2H), 4.13 (s, 2H), 3.99 – 3.92 (m, 1H), 3.80 – 3.70 (m, 1H), 2.27 (s, 3H), 1.37 (d, J = 7.0 Hz, 3H), 1.26 – 1.22 (m, 6H); ^{19}F NMR (400 MHz, $CDCl_3$): δ 19F NMR (376 MHz, Chloroform-d) δ -73.89 (d, J = 9.2 Hz); ^{31}P NMR (400 MHz, $CDCl_3$): δ 3.28 (s).

Scheme 7:

Isopropyl((S)-(((3aS,4S,6R,6aR)-6-(6-fluoro-5-methylpyridin-3-yl)-2,2-dimethyl tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methoxy)(phenoxy)phosphoryl)-L-alaninate (**17**) (100 mg, 0.1810 mmol, 1 eq.) was added formic acid (0.34 mL, 9.05 mmol, 50 eq.) in 0.085 mL of water. The reaction was stirred overnight and stopped by evaporating solvent with rotary evaporator. The crude mixture was purified by silica gel chromatography (4 g RediSep Rf silica column) to obtain product **15** (2-3% MeOH in DCM). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (s, 1H), 7.60 (d, J = 10.9 Hz, 1H), 7.29 – 7.23 (m, 2H), 7.19 – 7.09 (m, 3H), 5.00 (p, J = 6.3 Hz, 1H), 4.70 (d, J = 6.4 Hz, 1H), 4.57 (s, 1H), 4.29 (dd, J = 7.8, 3.1 Hz, 2H), 4.16 – 4.07 (m, 3H), 4.00 – 3.90 (m, 1H), 3.85 (s, 1H), 3.72 (t, J = 5.6 Hz, 1H), 2.24 (s, 3H), 1.35 (d, J = 7.0 Hz, 4H), 1.21 (d, J = 6.2 Hz, 7H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 173.16 (d, J = 7.2 Hz), 162.08 (d, J = 238.3 Hz), 150.60 (d, J = 6.8 Hz), 142.58 (d, J = 14.7 Hz), 139.66 (d, J = 6.1 Hz), 133.39 (d, J = 4.4 Hz), 129.89, 125.32, 120.26 (d, J = 4.9 Hz), 119.51 (d, J = 32.6 Hz), 82.93 (d, J = 6.6 Hz), 81.97, 77.09, 71.15, 69.66, 66.54 (d, J = 5.2 Hz), 50.43, 21.77 (d, J = 9.6 Hz), 20.98 (d, J = 5.0 Hz), 14.57; ^{19}F NMR (400 MHz, $CDCl_3$): δ -74.21 (d, J = 9.3 Hz); ^{31}P NMR (400 MHz, $CDCl_3$): δ 3.00. HRMS m/z calculated for $C_{23}H_{30}FN_2O_8P$ $[M + H]^+$: 513.17, found 513.2.

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