Université de Montréal

Diastereoselective Synthesis of Ribo-like Nucleoside Analogues Bearing an All-Carbon C3′ Quaternary Center

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Résumé

Les analogues de nucléosides ont reçu une attention particulière en raison de leurs importantes applications anticancéreuses et antivirales. Dans cette thèse, de nouveaux analogues nucléosidiques de type 1′,2′-*cis* et 1′,2′-*trans* ribo portant un centre stéréogénique quaternaire fonctionnalisé en position C3′ ont été synthétisés par des réactions de *N*-glycosylation stéréosélectives, qui ont été contrôlées en installant différents types de groupes protecteurs sur le C2′ substituant hydroxyle. Le précurseur acyclique critique de 2,4-*syn* diol a été obtenu par réduction diastéréosélective d'une β-hydroxycétone en utilisant la délivrance d'hydrure intermoléculaire. Une approche pour une séparation facile des 2,4-*syn* et 2,4-*anti* diols par protection/déprotection acétonide a été établie, de sorte que le 2,4-*syn* diol pur puisse être rapidement accessible par oxydation allylique successive et protection acétonide.

Une stratégie alternative a également été développée pour la préparation d'analogues nucléosidiques en C1′-β de type ribo portant un centre quaternaire C3′ avec un groupe hydroxyle C5′ libre. Dans cette stratégie, les diacétates de type ribo ont servi de donneur de glycosyle qui ont été synthétisés à partir d'une époxydation diastéréosélective d'un précurseur de glycal. La réaction énantiosélective consécutive de Mukaiyama aldol et le transfert d'allyle intramoléculaire de radicaux libres catalysé par photoredox ont été établis et développés dans notre laboratoire pour installer le centre stéréogénique quaternaire. Des nucléosides 5′-triphosphates portant soit une purine soit une pyrimidine ont ensuite été synthétisés et sont testés contre le cancer et les infections virales.

De plus, l'analogue L-1′,2′-*cis*-4′-thionucléoside portant un centre quaternaire stéréogénique fonctionnalisé en position C3′ avec un substituant hydroxyle en C2′ a été synthétisé par une stratégie acyclique avec 1′,2′-*syn* thioaminal précurseur, qui a subi une cyclisation intramoléculaire de type S_N2 de type S1′→C4′. Le 1′,2′-*syn* thioaminal a été synthétisé par une addition de nucléobase diastéréosélective sur un dithioacétal.

Mots-clés: Analogues de nucléosides, synthèse diastéréosélective, centre stéréogène quaternaire, *N*-glycosylation, catalyse photorédox, 4′-thionucléoside, stratégie acyclique.

Abstract

Nucleoside analogues have received extensive attention due to their important anticancer and antiviral applications. In this thesis, novel 1′,2′-*cis* and *trans* ribo-like nucleoside analogues bearing an all-carbon C3′ quaternary stereogenic center were synthesized using stereoselective *N*-glycosylation reactions, which were controlled by installing different types of protecting groups on the C2′ hydroxyl substituent. The critical acyclic 2,4-*syn* diol precursor was obtained by diastereoselective reduction of a β-hydroxy ketone using intermolecular hydride delivery. An approach for easy separation of the 2,4-*syn* and 2,4-*anti* diols through acetonide protection/deprotection was established to rapidly access the pure 2,4-*syn* diol through successive allylic oxidation and acetonide protection.

An alternative strategy was also developed for the preparation of ribo-like C1′-β nucleoside analogues bearing an all-carbon C3′ quaternary center with a free C5′ hydroxyl group. In this strategy, ribo-like diacetates served as the glycosyl donors which were synthesized from a diastereoselective epoxidation of a glycal precursor. A consecutive enantioselective Mukaiyama aldol reaction followed by a photoredox catalyzed free radical intramolecular allyl transfer were established and developed in our lab to install the all-carbon quaternary stereogenic center. Nucleoside 5′-triphosphates bearing either a purine or a pyrimidine nucleobase were then synthesized and are currently being tested against cancer and viral infections.

In addition, L-1′,2′-*cis*-4′-thionucleoside analogues bearing an all-carbon C3′ stereogenic quaternary center along with a C2′ hydroxyl substituent were synthesized using an acyclic strategy from a 1′,2′-*syn* thioaminal precursor followed by a S1′→C4′ intramolecular S_N2-like cyclization. The 1′,2′-*syn* thioaminal was synthesized by a diastereoselective nucleobase addition onto a dithioacetal.

Keywords: Nucleoside analogues, diastereoselective synthesis, all-carbon quaternary stereogenic center, *N*-glycosylation, photoredox catalysis, 4′-thionucleoside, acyclic strategy.

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List of Abbreviations

[α]	Specific rotation
Ac	Acetyl
AIDS	Acquired immune deficiency syndrome
ALL	Acute lymphocytic leukemia
AML	Acute myeloid leukemia
aq.	Aqueous
Ar	Aryl
ATP	Adenosine triphosphate
ATRA	Atom transfer radical addition
Bu	Butyl
Bn	Benzyl
Bz	Benzoyl
c	Concentration in g/100 mL
calc.	calculated
CDA	Cytidine deaminase
^{13}C NMR	Carbon-13 nuclear magnetic resonance
conc.	concentrated
COSY	Proton-proton correlation spectroscopy
CSA	Camphorsulfonic acid
Cyt	Cytosine
d	doublet
°C	Degree Celsius
DCE	1,2-Dichloroethane
dCK	Deoxycytidine kinase
DCM	Dichloromethane
DCTD	Deoxycytidylate deaminase
DFT	Density functional theory
DIBAL-H	Diisobutylaluminum hydride

DIPA	Diisopropylamine
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-(*N*,*N*-Dimethylamino)pyridine
DMDO	Dimethyldioxirane
DMF	Dimethylformamide
DMP	Dess-Martin Periodinane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DPK	Diphosphate kinase
dr	diastereomeric ratio
dtbbpy	4,4′-di-tert-butyl-2,2′-bipyridine
ee	enantiomeric excess
eq.	equivalent
ESI	Electrospray ionization
Et	Ethyl
FDA	Food and Drug Administration
g	gram
h	hour
HBV	Hepatitis B virus
HCV	Hepatitis C virus
hENT	human equilibrative nucleoside transporter
HIV	Human immunodeficiency virus
HMBC	Heteronuclear multiple bond correlation
HMDS	Bis(trimethylsilyl)amine
^{1}H NMR	Proton nuclear magnetic resonance
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
HSV	Herpes simplex virus

Hz	Hertz
i-Pr	isopropyl
IR	Infrared spectroscopy
ISC	Intersystem crossing
J	Coupling constant
L	Liter
LA	Lewis acid
LC-MS	Liquid chromatography–mass spectrometry
LDA	Lithium diisopropylamide
LHMDS	Lithium hexamethyldisilazane
m	multiplet
M	Molar
m-CPBA	*meta*-Chloroperoxybenzoic acid
Me	Methyl
mg	microgram
MHz	Megahertz
min	minute
mL	milliliter
mmol	millimole
MPK	Monophosphate kinase
MRP	Multidrug resistance proteins
Ms	Methylsulfonyl
MS	Mass spectrometry
MW	Molecular weight
NA-C	Cytidine nucleoside analogue
NA-CDP	Cytidine nucleoside analogue diphosphate
NA-CMP	Cytidine nucleoside analogue monophosphate
NA-CTP	Cytidine nucleoside analogue triphosphate
NAs	Nucleoside analogues
NA-U	Uracil nucleoside analogue

NA-UMP	Uracil nucleoside analogue monophosphate
nm	nanometer
NOESY	Nuclear Overhauser effect spectroscopy
NRTIs	Nucleoside/nucleotide reverse transcriptase inhibitors
5′-NT	5′-Nucleotidase
NTP	Nucleotide triphosphate
Oxone	Potassium peroxymonosulfate
Ph	Phenyl
ppm	parts per million
PPTS	Pyridinium *para*-toluenesulfonate
ppy	2-phenylpyridine
Pr	Propyl
Pyr	Pyridine
q	quartet
R_f	Retention factor or frontal ratio
RNA	Ribonucleic acid
RNR	Ribonucleotide reductase
RT	Room temperature
s	singlet
satd.	saturated
SET	Single electron transfer
t	triplet
T	Temperature
TBAF	Tetra-*n*-butylammonium fluoride
TBDPS	*tert*-Butyldiphenylsilyl
TBHP	*tert*-Butyl hydroperoxide
TBS	*tert*-Butyldimethylsilyl
TES	Triethylsilyl
Tf	Trifluoromethanesulfonyl

TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Thy	Thymine
TLC	Thin layer chromatography
TMS	Trimethylsilyl
t_R	Retention time
Ts	*para*-Toluenesulfonyl
µL	microliter

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Nothing in life is to be feared, it is only to be understood.
Now is the time to understand more, so that we may fear less.

Marie Curie

Chapter 1 Nucleosides and Nucleotides

1.1 Introduction

Nucleotides are essential compounds in cellular metabolism serving as the monomeric components of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). They are phosphorylated nucleosides composed of a five-membered sugar ring and a nucleobase (Figure 1.1). The nucleobases include purines, adenine (A) and guanine (G), while the pyrimidine nucleobases include thymine (T), cytosine (C) and uracil (U). RNA contains a C2′ hydroxyl group and the four nucleobases: A, G, U and C. In DNA, the C2′ position contains only hydrogen atoms, and the nucleobases may be A, G, T and C. Pyrimidine nucleobases are attached to the sugar ring at the N^1 position, while purines are connected at the N^9 position. Nucleotides consist of the nucleoside component and a phosphate moiety (mono-, di- and triphosphate).

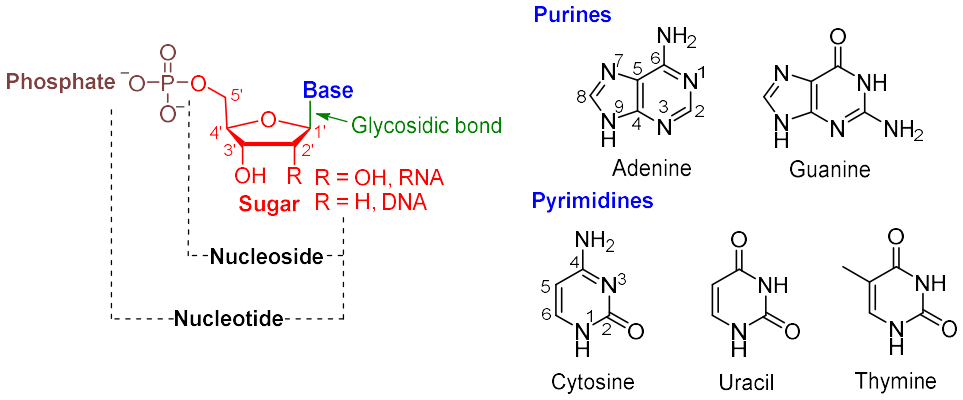


Figure 1.1. Basic structure of DNA and RNA monomers.

As shown in Figure 1.2, nucleotides are connected and extended through a 3′,5′-phosphodiester bond to form the primary structure of DNA or RNA.[1]

DNA exists in a double-stranded helix structure which stems from three interactions: hydrogen bonding, hydrophobic attractivity, and electrostatic forces between ions. Hydrogen bonds between the nucleobases, electrostatic repulsion of the phosphate ions and hydrophobic stacking of the nucleobases, all work to stabilize the DNA double helix structure in water. The hydroxyl group at the C3′ position attacks the C5′ triphosphate of an incoming nucleotide

forming a 5′-3′ phosphodiester bond. Adenine (A) can form two hydrogen bonds with thymine (T), while guanine (G) forms three hydrogen bonds with cytosine (C). In this way, two single-stranded deoxyribonucleotides are paired through base-packing forces and hydrogen bonds to form double-stranded DNA. Furthermore, the double-stranded DNA adopts a helical structure because the purine and pyrimidine nucleobases are not on the same plane with respect to the five-membered sugar.[2,3]

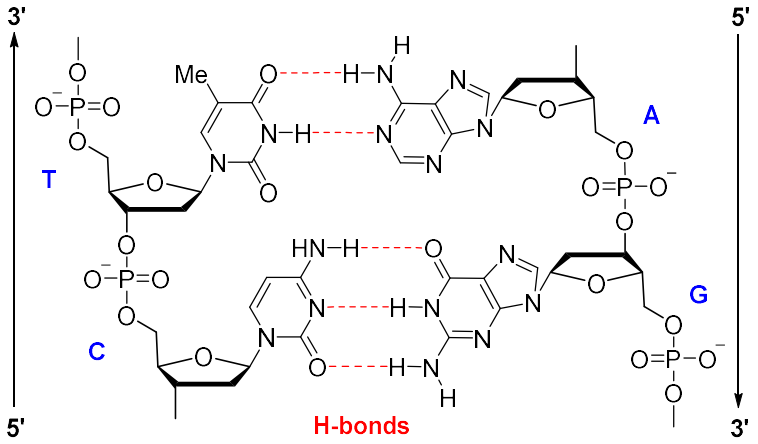


Figure 1.2. DNA double helix structure and nucleobase pairing.

The monomeric subunits of DNA possess D-configuration and β-relative stereochemistry. As illustrated in Figure 1.3, when the nucleobase at the anomeric C1′-carbon and the methylene hydroxyl at C4′ are present on the same side of the sugar plane, it is referred to as the β-isomer. On the contrary, the α-isomer exists when the nucleobase is on the opposite side of the group at C4′. The D- or L-configuration of the nucleoside relies on the configuration of the sugar as depicted in a Fischer projection. If the hydroxyl group at the penultimate carbon of the sugar in a Fischer projection is to the right of the molecule, it has the D-configuration, while if it is to the left of the molecule it has the L-configuration.[4–6]

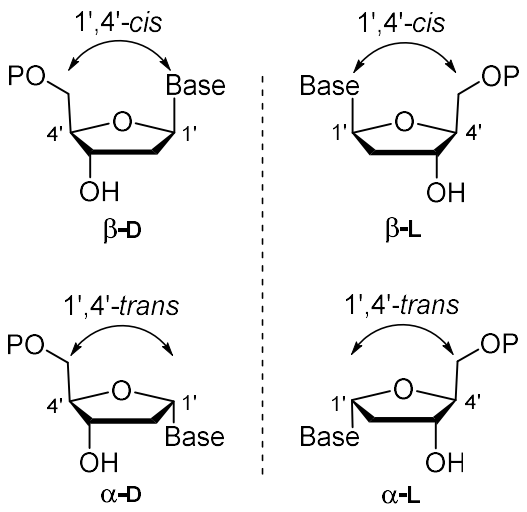


Figure 1.3. Configuration of nucleotides.

1.2 Nucleoside analogues

Nucleoside and nucleotide analogues are vital compounds used as antiviral and anticancer agents.[7] Such analogues can be modified at various positions including the nitrogenous base and the sugar moiety. Analogues are desired which are recognized by cells like natural nucleosides but sufficiently different to prevent cancer and viral cell proliferation.[8,9]

1.2.1 Nucleoside analogue sugar modifications

1.2.1.1 C2′-Hydroxyl and/or C3′-hydroxyl group modifications

C2′-Hydroxyl and/or C3′-hydroxyl modifications include the orientation of this moiety as can be seen in arabinose analogues such as Cytarabine (Ara-C, Figure 1.4) which contains a 2′-β hydroxyl rather than a 2′-α OH in ribose nucleosides.[10,11] This 1′,2′-*cis* stereochemistry has been shown to accelerate monophosphorylation by deoxycytidine kinase (dCK).[12] C2′-Cyano scaffolds such as CNDAC (2′-C-cyano-2′-deoxy-1-β-D-arabino-pentofuranosylcytosine) demonstrated that the 2′-β substitution of deoxycytidine enhances the stability of nucleosides susceptible to cytidine deaminase.[13,14] The mono- or di-fluorination of the C2′ position as in Clofarabine and Gemcitabine is an important mondification.[15,16] Fluorine has unique properties in terms of size and electronegativity. The presence of fluorine can effectively

restrict the nucleoside sugar pucker into a specific conformation, which may be important for the biological properties of nucleosides and their analogues.[17,18]

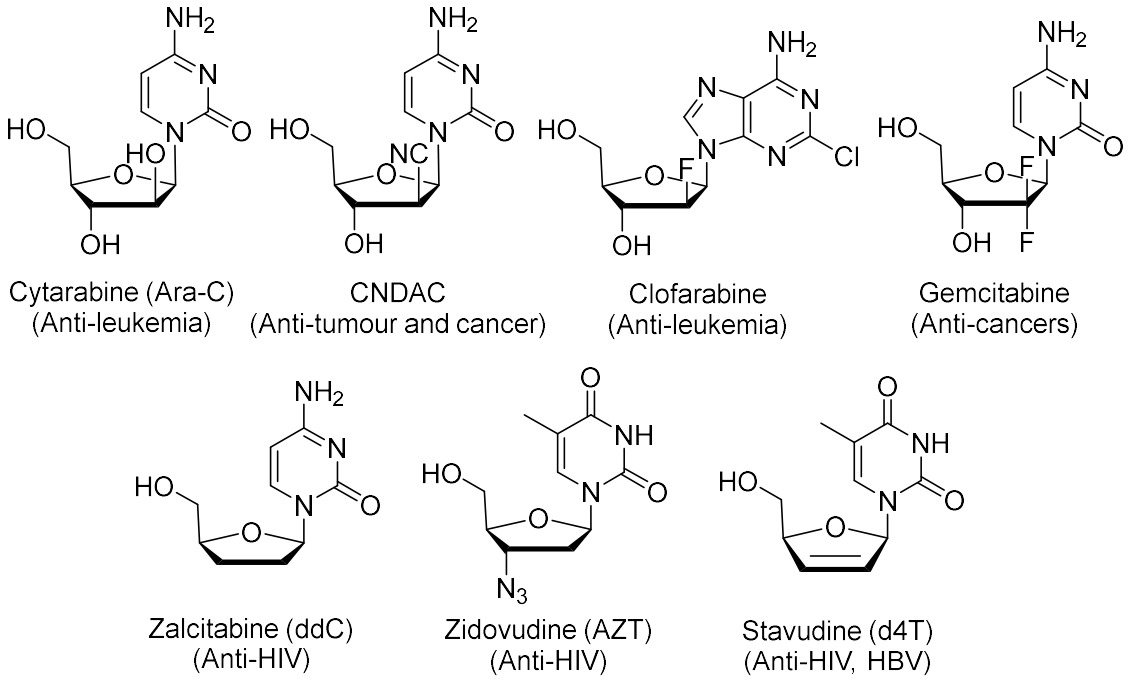


Figure 1.4. C2′-Hydroxyl and/or C3′-hydroxyl group modifications.

Fluorine at the C2′ position can increase the stability of the adjacent glycosidic bond which is susceptible to enzymatic cleavage resulting in inactivation of the nucleoside.[19] Thus, fluorination has become an effective modification for nucleoside analogues. Since the first-generation of anticancer and antiviral agents, some fluorine-modified nucleosides have demonstrated wide use in the treatment of various leukemias, and in the treatment and prevention of various carcinomas and tumours. For examples, Clofarabine has good clinical efficacy for leukemia, and Gemcitabine shows strong potential in the treatment of solid tumours, including various carcinomas, such as pancreatic, lung, breast, bladder and other cancers.[20–22]

Common C3′ position modifications such as deoxygenation, azide substitution and unsaturation (Figure 1.4) have been used in HIV treatment. The deoxygenation strategy at the C3′ position is an important nucleoside modification. As mentioned above, nucleic acids are connected through a 3'-5'-phosphodiester bond. After a nucleotide lacking a C3′ hydroxyl

group is incorporated into the DNA/RNA sequence, chain extension will be terminated. Zalcitabine (ddC), 2',3'-dideoxycytidine nucleoside, was the first deoxygenated analogue proven to be an effective nucleoside reverse transcriptase inhibitor against the human immunodeficiency virus (HIV).[23] Azide substitution at C3' of thymidine, Zidovudine (AZT) was the first FDA approved medication for the treatment of HIV.[24] Some other strategies have also been developed such as unsaturated sugars. Stavudine (d4T) contains a 2′,3′-unsaturated bond which increases the rigidity of the sugar ring. Stavudine demonstrated lower cytotoxicity and side effects, making it an effective antiviral agent against HIV and hepatitis B virus (HBV).[25]

As shown in Figure 1.5, nucleosides exist in an array of conformations including but not limited to north and south conformations (also known as C3′-*endo* and C2′-*endo*). These two (N) and (S) sugar puckering conformations are essential to the specific recognition of nucleosides and their analogues.[26] For examples, some enzymes such as DNA polymerases and reverse transcriptases prefer the C3′-*endo*-(N) conformation, while many kinases tend to react with a bias towards the C2′-*endo*-(S) conformation.[27,28]

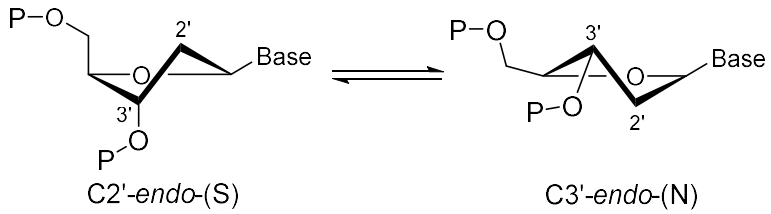


Figure 1.5. Nucleoside ring pucker.

1.2.1.2 Heterocyclic modifications of the nucleoside sugar moiety

Heterocyclic modifications of the nucleoside sugar moiety are also a common design strategy. Replacing the oxygen of the sugar ring with heteroatoms such as sulfur, nitrogen, or inserting a heteroatom at a different position of the sugar ring can modify the conformation of nucleoside and improve their metabolic characteristics (Figure 1.6).[29] Sulfur is considered as an isosteric oxygen atom because they have the same valence electrons and similar electronic configuration. However, many synthesized 4′-thionucleosides did not show anticancer nor antiviral activity, probably because they could not be recognized by cellular kinases. However,

replacement of the 4′-oxygen of cytarabine by sulfur gave 4′-thio-Ara-C, also known as T-Ara-C (Figure 1.6), which has entered in clinical trials as an anti-tumour agent. Compared with currently used Cytarabine, Clofarabine and Gemcitabine, Thiarabine has an enhanced potency for the treatment of solid tumors.[30–32]

Currently, the marketed heterocyclic antiviral drugs that exist include for example L-type nucleosides Lamivudine (anti-HBV and HIV)[33,34] and Emtricitabine (anti-HIV).[35] Other molecules such as Troxacitabine is currently used for renal cell carcinoma, in addition to potential use as an antiviral agent for HBV and HIV[36,37] (Figure 1.6).

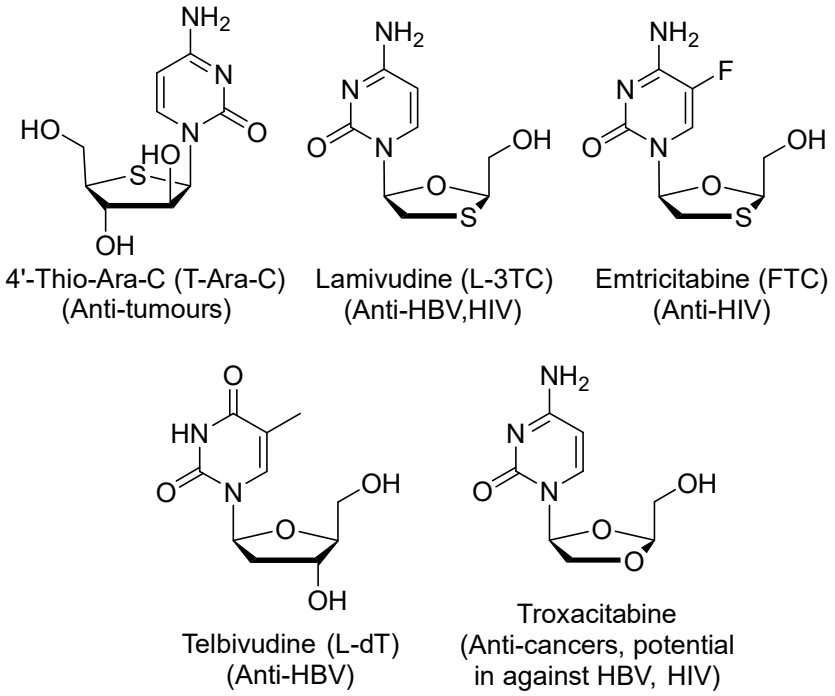


Figure 1.6. Heterocyclic modified sugar moiety and L-nucleosides.

1.2.1.3 L-Nucleosides and acyclic analogues

L-Type nucleosides have been demonstrated to have more potent antiviral activity with less cytotoxicity compared to their D-counterparts. For example, Lamivudine (L-3TC, Figure 1.6) is currently used to treat HBV, and as an antiretroviral medication, it is also effective against HIV.[33,34] Importantly, compared to the D-enantiomer, L-3TC exhibits reduced toxicity due to its strong resistance to cytidine deaminase (CDA), an enzyme that catalyzes the irreversible conversion of cytidine to uridine (shown in Figure 1.9). Telbivudine (L-dT) is the

L-enantiomer of its natural thymidine, which has been shown to be effective in treating chronic hepatitis B infection.[38] Troxacitabine that contains a heteroatom oxygen at C3′ has potential in the treatment of cancer, HBV and HIV.[36,37] All the L-nucleosides account for a considerable proportion of antiviral and antiretroviral drugs and play an irreplaceable role in the treatment of HBV and HIV.

Acyclic nucleosides with removal of the C2′ and/or C3′-carbon in the furanose sugar ring are also a typical modification seen for antiviral agents.[39,40] For example, Acyclovir and Ganciclovir (Figure 1.7), which are acyclic analogues of guanosine, show specific clinical efficacy in the treatment of herpes simplex virus (HSV) due to their selective monophosphorylation by the viral kinase enzyme.[41–44]

1.2.2 Nucleobase-modified nucleoside analogues

Various modifications of the nucleobase have been studied, including functionalization at the C5 position of pyrimidine scaffolds by fluorination, as seen with 5-Fluorouracil (Figure 1.7).[45,46] The nitrogen atoms of the nucleobases can also be removed or rearranged. 5-Fluorouracil (5-FU) is used in the treatment of breast and colon cancers. 6-Mercaptopurine (6-MP) is used as a medication for acute lymphocytic leukemia (ALL).[47] Nitrogen substitutions in purine and pyrimidine bases have been considered to introduce alternative hydrogen bond donors that could furnish new enzyme binding sites. For example, 5-Azacytidine in which a nitrogen atom is inserted at the 5-position of cytidine has shown anticancer efficacy.[48,49] Among the novel potential drugs, Sapacitabine is an orally available prodrug of the nucleoside CNDAC, in which a long acyl chain is introduced to reduce susceptibility to cytidine deaminase. Sapacitabine is currently under phase III clinical trials against leukemia, chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), non-small-cell lung cancer and other advanced solid tumours.[50,51]

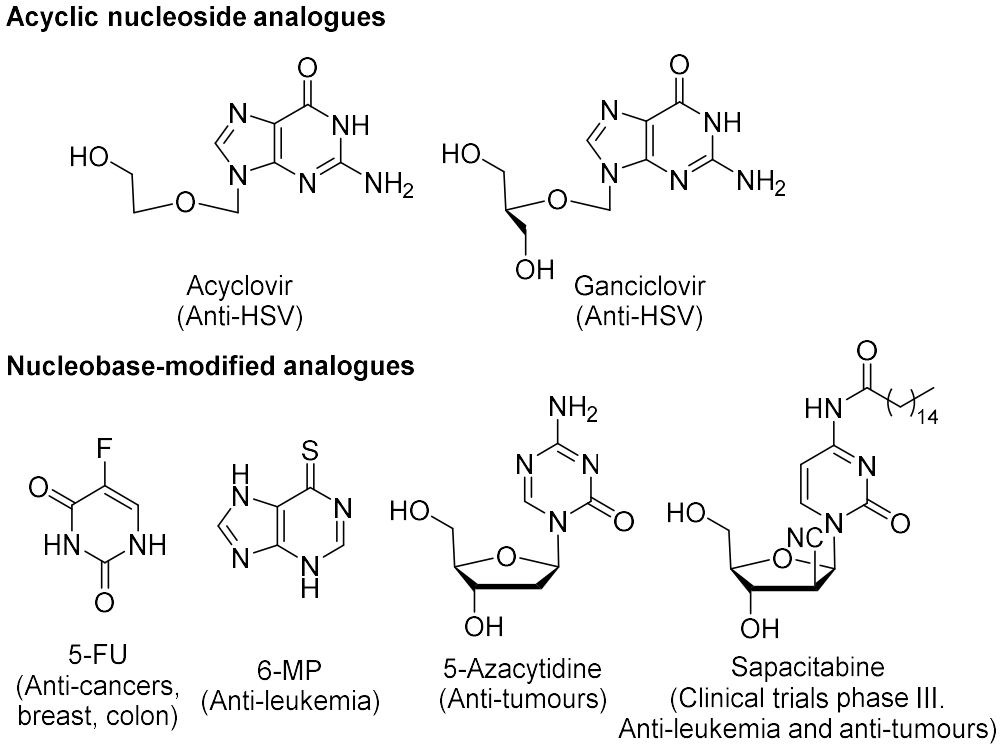


Figure 1.7. Acyclic nucleoside and nucleobase-modified analogues.

1.3 Nucleotide analogues

Nucleotides are nucleosides that contain one to three phosphate groups, some of which can be used as nucleoside reverse transcriptase inhibitors (NRTIs) that are commonly used as chemotherapeutic drugs for retroviruses. Retroviruses (such as HIV) use a reverse transcriptase to transcribe retrovirus RNA into DNA. Nucleoside triphosphates are the active species in the body. New nucleotide drugs are one of the hotspots in current research (Figure 1.8).[1] For example, Fludarabine, the monophosphate of Clofarabine, is also used against leukemia.[52] Gemcitabine can be used to treat a variety of tumors, but its efficacy is limited by resistance. NUC-1031 is a Gemcitabine phosphoramidate modification designed to overcome resistance mechanisms. NUC-1031 is well tolerated and exhibits significant clinical antitumor activity including tumours that were not responsive to Gemcitabine.[53] Sofosbuvir, which is a phosphoramidate uridine analogue, has been approved for hepatitis C viral infection.[54,55] Stampidine, a derivative of Stavudine (d4T), has been demonstrated to circumvent the rate-

limiting monophosphorylation step of Stavudine, and may have more potent antiretroviral activity for the treatment of HIV.[56–58]

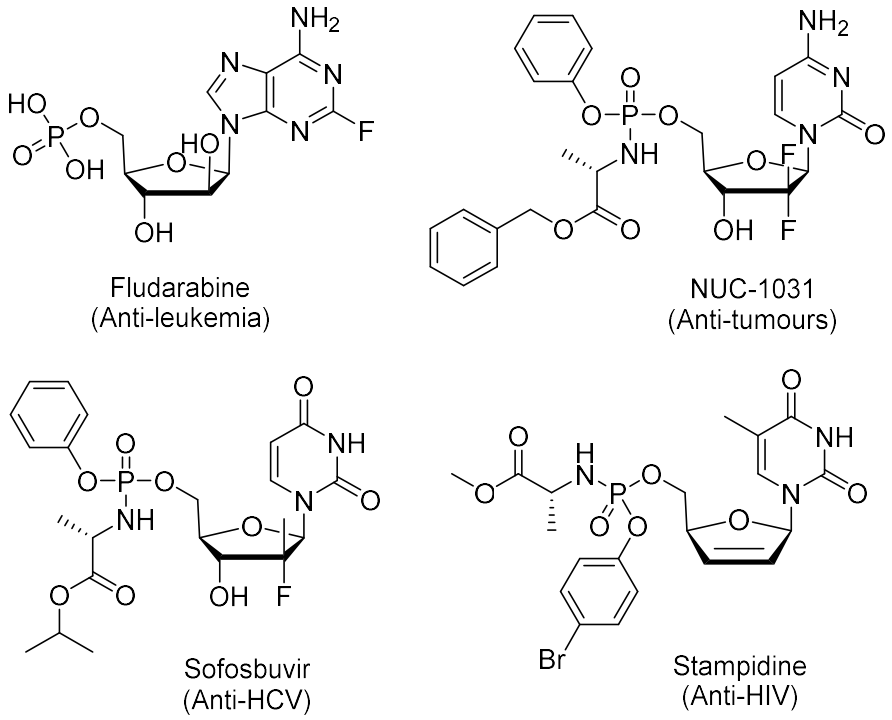


Figure 1.8. Phosphorylated nucleoside analogues.

1.4 Mechanism of action

Nucleoside analogues enter the cell with the assistance of specific membrane transporters, known as human equilibrative nucleoside transporter (hENTs) and human concentrative nucleoside transporters (hCNTs) (Figure 1.9).[59,60] After entering the cell, the nucleoside analogues undergo a first rate-limiting phosphorylation by deoxycytidine kinase (dCK) to generate the nucleoside monophosphate (NA-CMP).[61,62] Subsequently, the nucleoside monophosphates are then diphosphorylated (NA-CDP) and triphosphorylated (NA-CTP) by monophosphate kinase (MPK) and diphosphate kinase (DPK). These active metabolites will effectively inhibit essential enzymes, including ribonucleotide reductase (RNR) and DNA polymerase. Ribonucleotide reductase (RNR) catalyzes the critical intracellular transformation of nucleotide triphosphates (NTP) into deoxynucleotide triphosphates (dNTP), essential for DNA replication.[1,63]

Nucleotide diphosphate and triphosphate analogues that compete with physiological nucleotides can inhibit the activity of ribonucleotide reductase (RNR), which will lead to a decrease in the concentration of deoxynucleotide triphosphates. Therapeutic nucleoside analogue triphosphates can be directly integrated into newly synthesized DNA or RNA strands, which may lead to apoptosis in cancer or viral cells (Figure 1.9).[64]

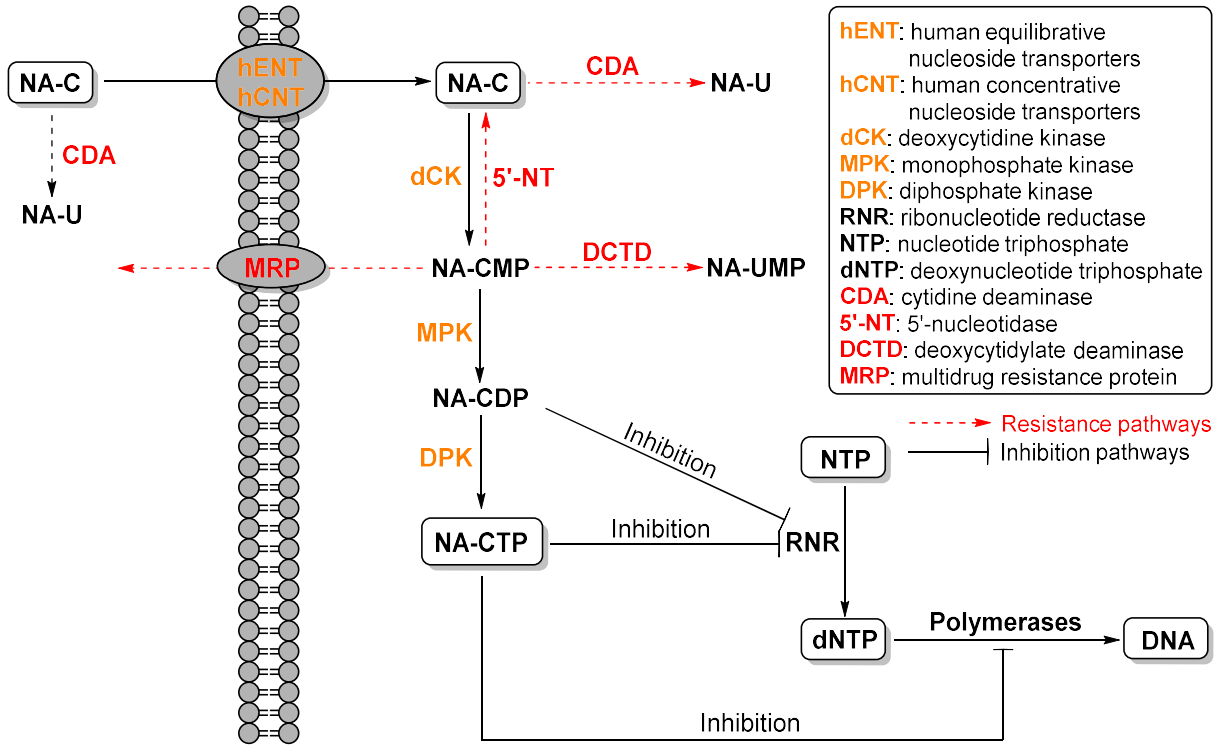


Figure 1.9. Schematic of nucleoside analogue mechanism of action and resistance pathways of recurrent resistance determinants.

Resistance to cytotoxic nucleoside analogues has largely suppressed the clinical efficacy of nucleoside analogue prodrugs (Figure 1.9).[65] Some catabolic enzymes can reduce the amount of active cytotoxic nucleoside analogues, including cytidine deaminase (CDA), 5′-nucleotidase (5′-NT) and deoxycytidylate deaminase (DCTD).[66,67] The existence of cytidine deaminase (CDA) in the cytosol may transform cytidine nucleoside analogues into the corresponding uracil analogue.[68] The cytidine nucleoside analogue monophosphate (NA-CMP) generated after monophosphorylation of NA-C with dCK could be involved in a reversely phosphorolytic cleavage in the presence of 5′-nucleotidase (5′-NT).[69] In addition, the generated NA-CMP could be deaminated into the uracil nucleoside analogue monophosphate

(NA-UMP) equivalent by deoxycytidylate deaminase (DCTD).[70] In addition, the multidrug resistance protein (MRP) can transport various organic molecules across the intracellular and extracellular membranes, including nucleoside analogue monophosphates (NA-CMP).[71,72] All of the above recurrent resistance determinants significantly reduce the amount of active nucleoside analogue diphosphates and triphosphates, thus affecting the activity of cytotoxic nucleoside analogue prodrugs in the clinical application.

1.5 Introduction of an all-carbon quaternary stereocenter

Conformational restriction of nucleoside analogues through the introduction of an all-carbon quaternary center has been an intense research topic in recent years in our lab. The methodology of installing an all-carbon quaternary center at the C2′ or C3′ position has been widely investigated.[73–77] In fact, the clinical efficacy of cytotoxic nucleoside analogue prodrugs relies not only on their molecular structures, but also significantly on their conformational bias. Research focused on methods to favor a specific bioactive conformation has attracted a lot of attention from chemists and biologists.[26,78] In our case, an all-carbon quaternary center installed at the C3′-position could be an effective way to favor one conformation while also improving the specificity of such scaffolds.

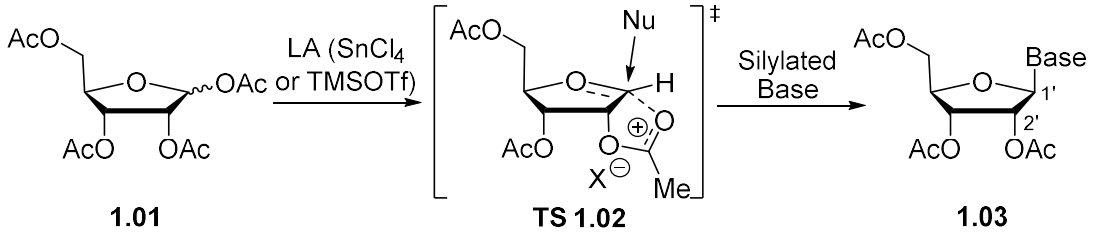
1.6 Synthesis of nucleoside analogues

In the development of stereoselective methods to synthesize nucleoside analogues, the commonly used method is the cyclic stereoselective *N*-glycosylation reaction, in which the stereochemistry of nucleoside scaffold is controlled by electrostatic and steric effects of the groups adjacent to the anomeric center. Alternatively, an acyclic strategy is also of great significance.

1.6.1 Cyclic stereoselective *N*-glycosylation

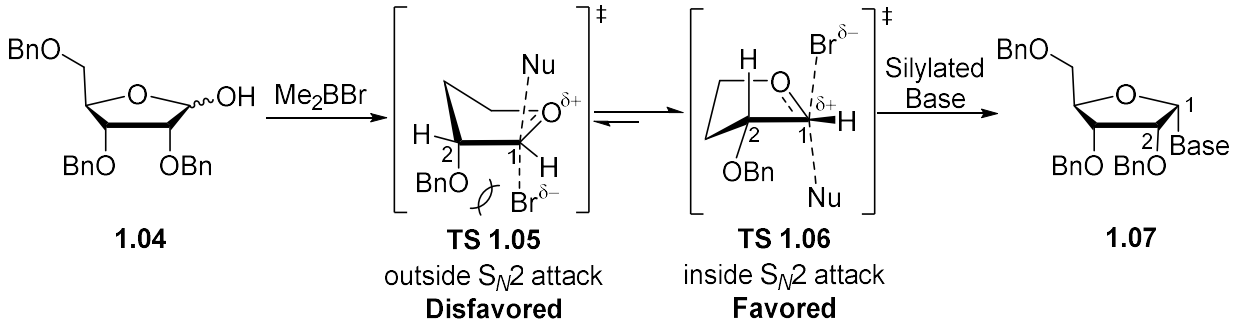
The stereoselective construction of 1′,2′-*trans* nucleoside analogues bearing a hydroxy group at the C2′ position is typically done through a classical Vorbrüggen *N*-glycosylation.[79–81] This strategy combines nucleophilic bases and electrophilic sugars activated by the presence of a Lewis acid such as $SnCl_4$ or TMSOTf. Acyl protecting group on the C2′ hydroxyl, such as

OBz, OAc (**1.01**) can be involved in anchimeric assistance resulting in the formation of a five-membered oxonium transition state **TS 1.02**. This leads to nucleophilic attack from the top face resulting in the formation of 1′,2′-*trans* analogues **1.03** (Scheme 1.1).



Scheme 1.1. Stereoselective *N*-glycosylation with anchimeric assistance.

The diastereoselective construction of 1′,2′-*cis* nucleoside analogues is more challenging and different approaches have been reported.[82,83] Previous work from the Guindon laboratory has demonstrated that benzyl (Bn) or silyl ether protection of the C2′ hydroxyl moiety can be used.[84] Activation of a lactol **1.04** with dimethylborane bromide (Me_2BBr), as the Lewis acid, results in the initial formation of a mixture of 1′,2′-*cis* and 1′,2′-*trans* bromosugars. A S_N2 attack of the nucleobase from the inside-envelop face, as in **TS 1.06**, is favored, providing 1′,2′-*cis* nucleoside analogues **1.07** (Scheme 1.2).[84]

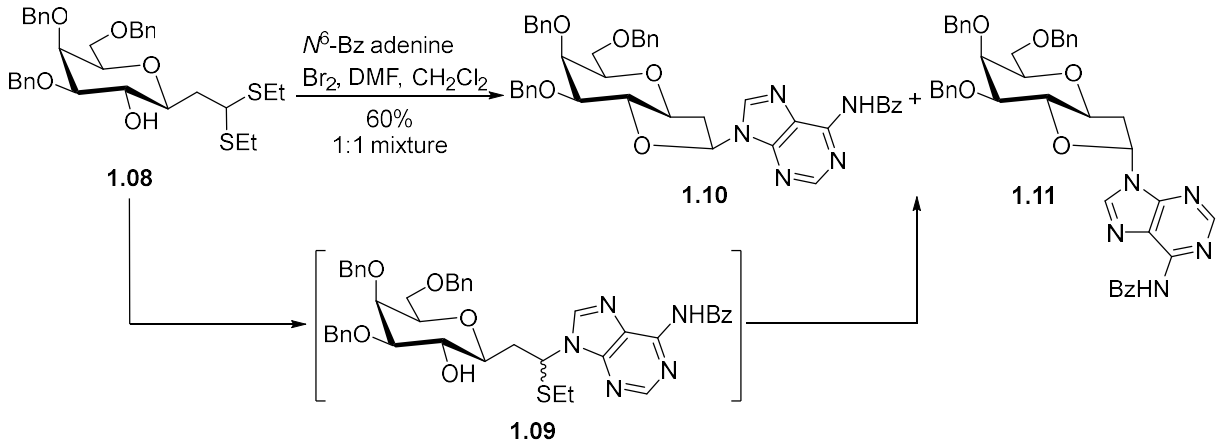


Scheme 1.2. Construction of 1′,2′-*cis* nucleoside analogues.

1.6.2 Acyclic stereoselective strategy

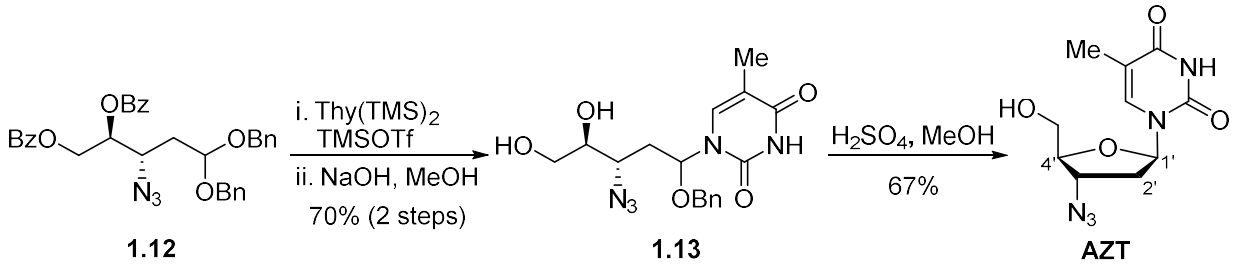
Since acyclic nucleoside analogues derived from appropriate dithioacetal derivatives were prepared for the first time by Wolfrom and co-workers in 1962,[85] acyclic strategies have been considered as alternatives to the cyclic glycosylation in the construction of nucleoside

analogues. In 1981, Hanessian and co-workers established an efficient method for the formation of bicyclic deoxyadenosine nucleoside analogues (Scheme 1.3).[86] The condensation of acyclic dithioacetal derivative **1.08** and N^6-benzoyladenine resulted in a thioaminal intermediate **1.09** in the presence of Br_2 in DMF, which resulted in the formation of a 1:1 mixture of **1.10** α and **1.11** β nucleosides.



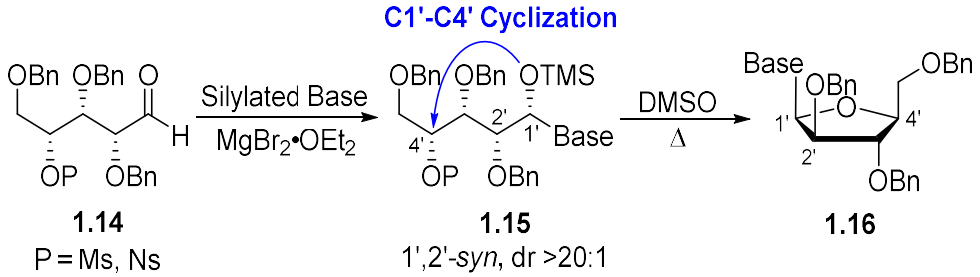
Scheme 1.3. Construction of bicyclic adenine nucleoside with dithioacetal.

Liotta and co-workers developed an acyclic methodology for controlling the stereoselective construction of nucleoside analogues in the synthesis of the antiviral agent AZT, in 1991 (Scheme 1.4).[87] The dibenzoate protected acetal **1.12**, under Vorbrüggen condensation conditions, was coupled to the nucleophilic persilylated thymine in the presence of TMSOTf. Subsequent cyclization of acyclic thymidine analogue **1.13** with concentrated H_2SO_4 under kinetic conditions (low temperature) generated exclusively the β-anomer of AZT.

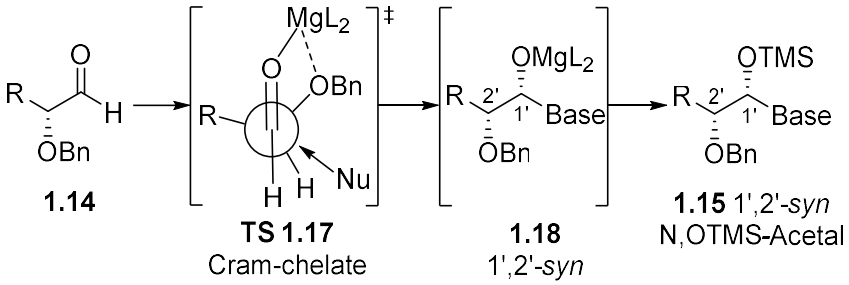


Scheme 1.4. Acyclic strategy for the synthesis of AZT.

In recent years, acyclic strategies were developed in our laboratory for the stereoselective construction of 1′,2′-*cis* and 1′,2′-*trans* nucleoside analogues.[88–91] Nucleoside analogues can be stereoselectively synthesized by O1′→C4′ cyclization of acyclic N,OTMS-acetals **1.15** where the C1′-OTMS participates in the nucleophilc substitution of the C4′ position that bears a good leaving group, such as a Ms or Ns. This cyclization proceeds with complete retention of the configuration of the acetal center (Scheme 1.5).[89] Acyclic N,OTMS-acetal **1.15** can be prepared from polybenzyloxyaldehyde **1.14** in the presence of Lewis acid $MgBr_2$•OEt_2, with excellent stereoselectivity. The high stereoselectivity of 1′,2′-*syn* N,OTMS-acetal can be well explained by a Cram-chelate bidentate transition state model **TS 1.17** (Scheme 1.6), in which the Lewis acid MgL_2 coordinated with the α-benzyloxyaldehyde oxygen is also slightly coordinated with the α-benzyloxy group, thus the silylated nucleobase attacks from the less sterically hindered face resulting in the 1′,2′-*syn* species **1.18**.



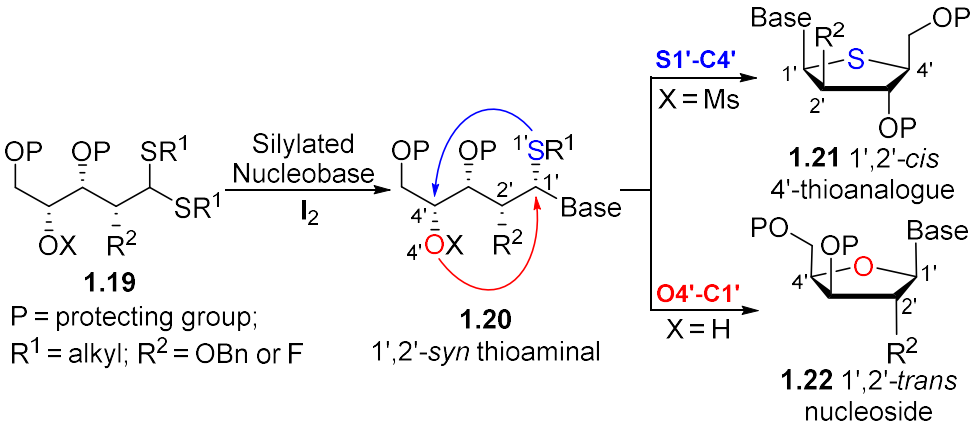
Scheme 1.5. Acyclic strategy with acyclic N,OTMS-acetal precursor.



Scheme 1.6. 1′,2′-*syn* N,OTMS-acetal controlled by a Cram chelate bidentate transition state.

Alternatively, acyclic stratagies involving thioaminal precursors to stereoselectively construct 4′-oxa- and 4′-thio-nucleoside analogues have been developed (Scheme 1.7).[90] The 1′,2′-*syn* thioaminal **1.20** can undergo two distinct intramolecular S_N2-like cyclizations. An O4′→C1′ cyclization (X = H) leads to an inversion of the C1′ stereocenter generating 1′,2′-

trans 4′-oxanucleoside analogue **1.22** with retention of the C4′ stereocenter. And alternative S1′→C4′ cyclization (X = Ms) results in 1′,2′-*cis* 4′-thioanalogue **1.21** with an inversion of the C4′ stereocenter while the stereochemistry of the thioaminal stereocenter is maintained. The thioaminal precursor **1.20** can be prepared from dithioacetal **1.19** in the presence of I_2 with excellent 1′,2′-*syn* stereoselectivity where R^2 is either a benzyloxy or fluorine substituent.

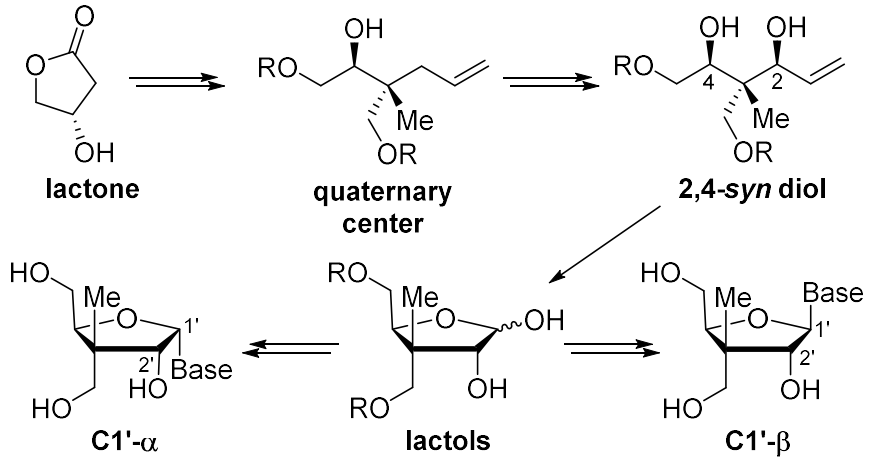


Scheme 1.7. Acyclic strategy with acyclic thioaminal.

1.7 Research objectives

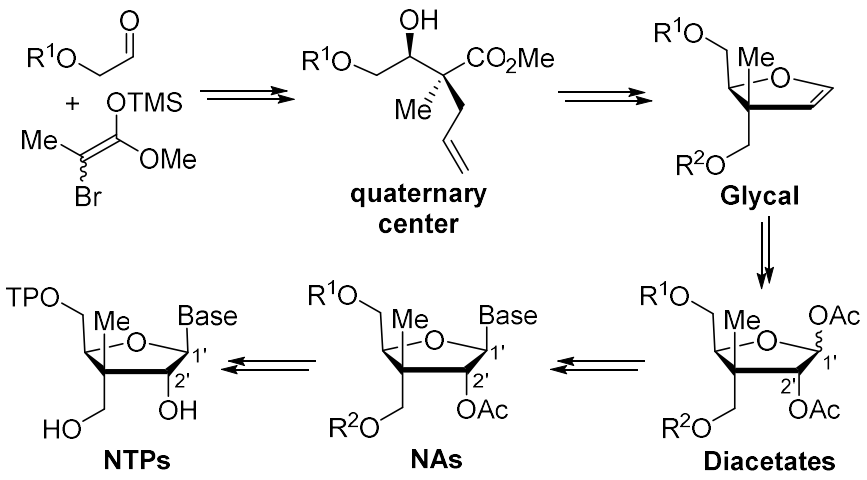
The diastereoselective synthesis of ribo-like nucleoside analogues bearing an all-carbon C3′ quaternary stereogenic center was the objective of this thesis.

As presented in Chapter 2, consecutive alkylations were employed for the construction of the all-carbon quaternary center (Scheme 1.8). An effective method for the diastereoselective synthesis of the requisite 2,4-*syn* diol was also established. Furthermore, stereoselective *N*-glycosylations were used to obtain both 1′,2′-*cis* and 1′,2′-*trans* ribo-like nucleoside analogues by installing different protecting groups on the C2′ hydroxyl.



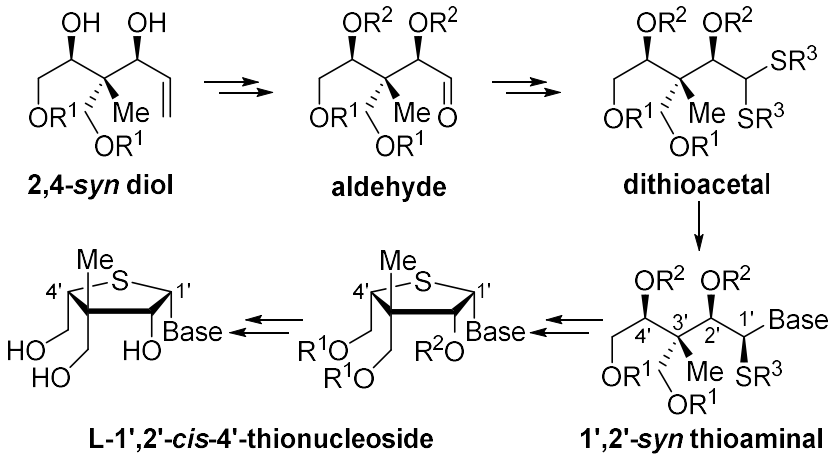
Scheme 1.8. Research Route in Chapter 2.

The synthesis of nucleoside 5'-triphosphates was the next objective, the results of which are presented in Chapter 3 (Scheme 1.9). A novel efficient method was developed to access the 1′,2′-*trans* ribo-like nucleoside scaffolds with a C5′ free hydroxyl as the key precursor for the synthesis of the desired nucleoside 5′-triphosphates. Therefore, orthogonal protecting groups were placed at the C5' and C3' hydroxyl groups to access such scaffolds. An efficient photocatalyzed protocol was used to construct the all-carbon quaternary center. Both purine and pyrimidine nucleoside scaffolds were synthesized and further used in the synthesis of purine and pyrimidine nucleoside 5′-triphosphates.



Scheme 1.9. Research Route in Chapter 3.

In Chapter 4, the diastereoselective synthesis of L-1′,2′-*cis*-4′-thionucleoside analogues bearing an all-carbon C3′ quaternary center and a C2′ hydroxyl group was realized (Scheme 1.10). The acyclic strategy that was previously developed in our laboratory was employed in this synthesis using a 1′,2′-*syn* thioaminal that can be obtained from a diastereoselective nucleobase addition onto a dithioacetal. The dithioacetal originated from a 2,4-*syn* diol.



Scheme 1.10. Research Route in Chapter 4.

Chapter 2 Diastereoselective Synthesis of Ribo-like Nucleoside Analogues Bearing an All-Carbon C3′ Quaternary Center

2.1 Introduction

In 2018, an efficient methodology for the diastereoselective synthesis of arabino-like nucleoside analogues with either a 1′,2′-*cis* or 1′,2′-*trans* stereochemistry bearing an all-carbon C3′ quaternary stereogenic center was established by Tommy Lussier, a former MSc student in the Guindon laboratory (Figure 2.1).[92] The 1′,2′-*cis* and 1′,2′-*trans* selectivities of these arabino-like scaffolds were controlled by stereoselective *N*-glycosylation reactions by installing different types of protecting groups on the C2′ hydroxyl substituent. Arabino-like lactols **2.01a,b** were derived from 2,4-*anti* diol **2.05a** (Scheme 2.1), the key precursor, which was obtained by a diastereoselective reduction of β-hydroxy ketone **2.06** using an Evans-Saksena protocol.[93] The work in this chapter is a continuation of the previous results obtained by Tommy Lussier, aiming at the diastereoselective synthesis of ribo-like nucleoside analogues with either a 1′,2′-*cis* or 1′,2′-*trans* stereochemistry (**2.02a** and **2.02b**) bearing an all-carbon C3′ quaternary center.[94]

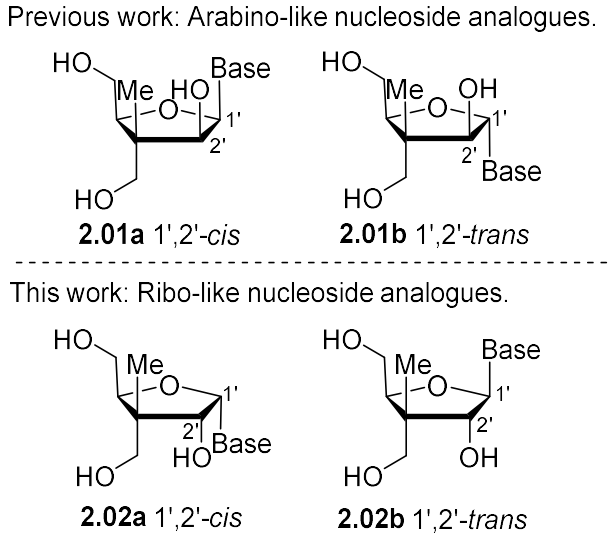
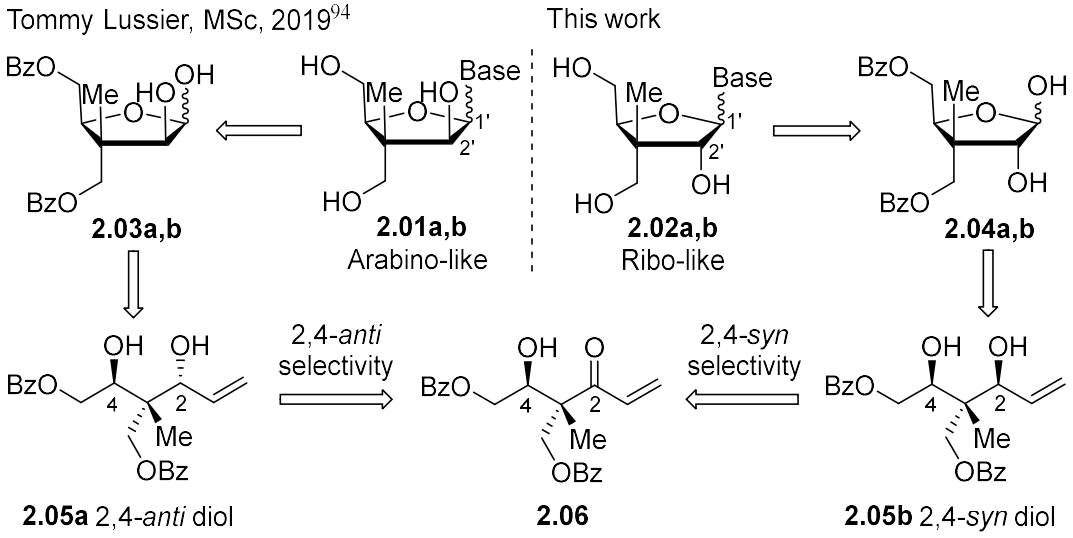


Figure 2.1. Nucleoside analogues in the arabino-like and ribo-like series.

The diastereoselective synthesis of arabino-like and ribo-like nucleoside analogues bearing an all-carbon C3′ quaternary center can be achieved from the same β-hydroxy ketone precursor **2.06.** For the diastereoselective synthesis of ribo-like nucleoside analogues, a 2,4-*syn* diastereoselective reduction of this β-hydroxy ketone is required.



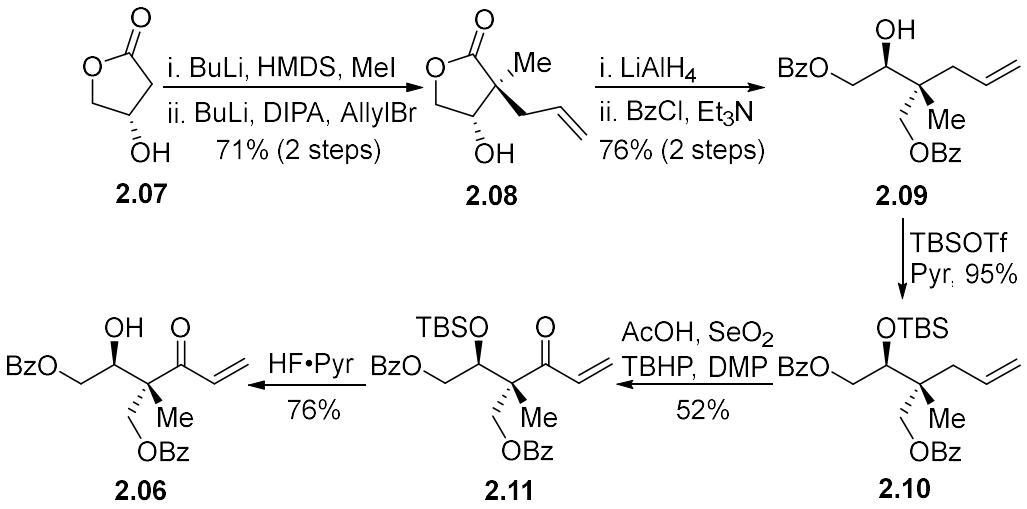
Scheme 2.1. Retrosysthetic analysis of ribo-like scaffolds.

2.2 Synthesis of ribo-like nucleoside analogues

2.2.1 Installation of the all-carbon quaternary center

Starting from the commercially available lactone **2.07** (Scheme 2.2), consecutive methylation and allylation steps were employed to give lactone **2.08**, in which the quaternary center was introduced with a 71% yield for the two steps. Following reduction of lactone **2.08** with $LiAlH_4$, and selective protection of the two primary alcohols with benzoate protecting groups, alkene **2.09** was formed with a 76% yield over the two steps. The secondary hydroxyl group of alkene **2.09** was then protected with a silyl ether TBS group (**2.10**), followed by an allylic oxidation using selenium dioxide (SeO_2) and *tert*-butyl hydroperoxide (TBHP).[94] The desired α,β-unsaturated carbonyl **2.11** was obtained with a small amount of α,β-unsaturated allylic alcohol by-product. This by-product was easily oxidized with Dess-Martin Periodinane (DMP), providing the α,β-unsaturated carbonyl compound **2.11** in a satisfactory yield.

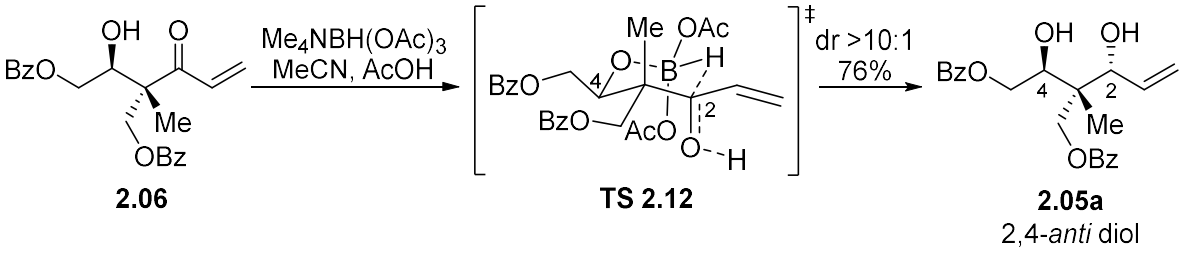
Removal of the secondary TBS group was done in the presence of HF•Pyr, providing β-hydroxy ketone **2.06** in a 76% yield.



Scheme 2.2. Synthesis of β-hydroxy ketone **2.06**.

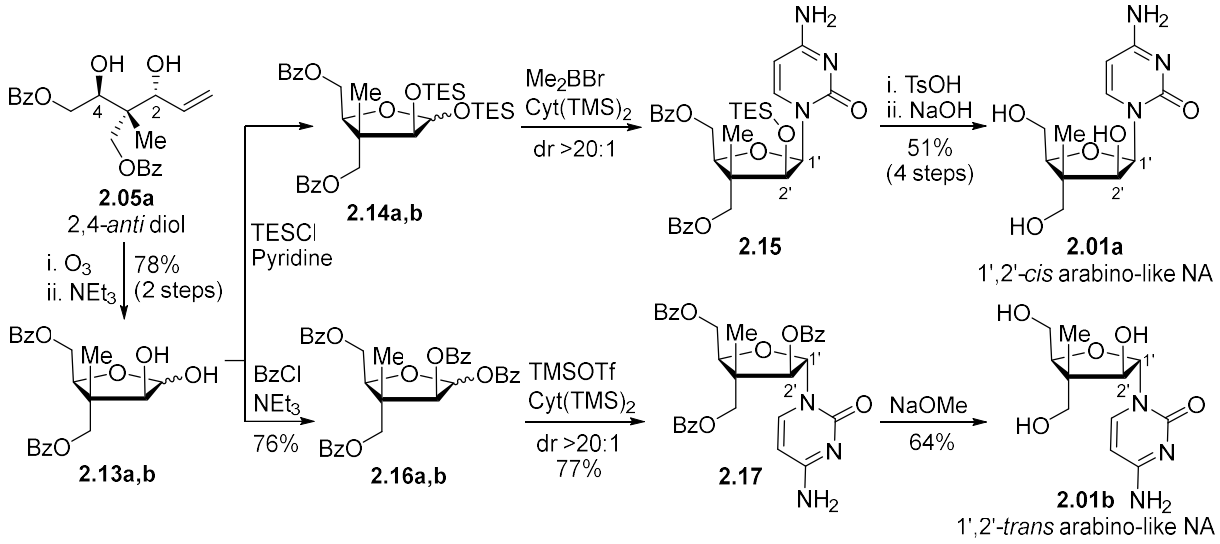
2.2.2 Diastereoselective reduction of β-hydroxy ketone

Using the Evans-Saksena protocol for diastereoselective reduction of β-hydroxy ketones, 2,4-*anti* diol **2.05a** was obtained from ketone **2.06** with excellent 2,4-*anti* diastereoselectivity (>10:1 dr) and yield (76%) (Scheme 2.3), which was achieved by Tommy Lussier. The reducing agent tetramethylammonium triacetoxyborohydride ($Me_4NBH(OAc)_3$) was employed in the reduction through a boron chelating transition state (**TS 2.12**), which undergoes intramolecular hydride delivery from the opposite direction as the chelated β-hydroxyl group to provide the 2,4-*anti* selectivity.[94]



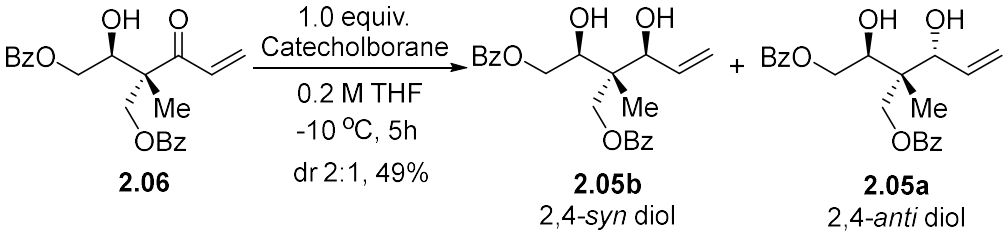
Scheme 2.3. Diastereoselective 2,4-*anti* reduction of β-hydroxy ketone **2.06**.

The 2,4-*anti* diol **2.05a** was then converted into the arabino-like lactols **2.13a,b** which were then protected either by bis-TES **2.14a,b** or dibenzoates **2.16a,b**.[94] Following stereoselective *N*-glycosylations and protecting group removal, the corresponding 1′,2′-*cis* and 1′,2′-*trans* arabino-like nucleoside analogues (**2.01a** and **2.01b**) were obtained (Scheme 2.4) (Tommy Lussier's thesis, 2019).



Scheme 2.4. Diastereoselective synthesis of 1′,2′-*cis* and 1′,2′-*trans* arabino-like NAs.[94]

Evans and co-workers reported the diastereoselective synthesis of 1,3-*syn* diols through reduction of β-hydroxy ketones using catecholborane.[95] When using these conditions in our case, a 2:1 selectivity in favor of the 2,4-*syn* diol **2.05b** was achieved (Scheme 2.5).

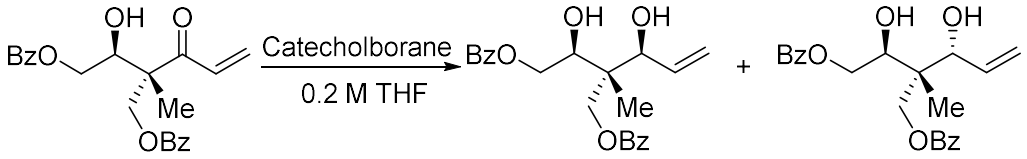


Scheme 2.5. Catecholborane induced the 2,4-*syn* selectivity.

Inspired by these preliminary results, optimization experiments were carried out to find the best reduction conditions. As illustrated in Table 2.1, different equivalents of catecholborane were investigated in THF at −10 °C. A similar result was achieved when

increasing the amount of catecholborane from 1.0 to 2.0 equivalents (entry 2). The diastereoselectivity in favor of the 2,4-*syn* diol **2.05b** was slightly enhanced (dr 3:1) when using 5.0 equivalents of catecholborane (entry 3) albeit with a lower yield. However, when ten equivalents of catecholborane was used, both the 2,4-*syn* diastereoselectivity (dr 1.5:1) and the reaction yield (23%) decreased significantly (entry 4). Interestingly, when one equivalent of cerium chloride ($CeCl_3 \bullet H_2O$) was added to the reaction mixture, both the diastereoselectivity (dr 7:1) and yield (72%) were significantly improved (entry 5). However, when the reaction was done at a lower temperature of −78 °C (entry 6), the yield was significantly reduced. Ultimately, employing 5.0 equivalents of catecholborane and 1.0 equivalent of $CeCl_3 \bullet H_2O$ in THF at −10°C was selected as the optimal conditions for diastereoselective reduction of β-hydroxy ketone **2.06** to 2,4-*syn* diol **2.05b**.

Table 2.1. Optimization of diastereoselective reduction conditions.

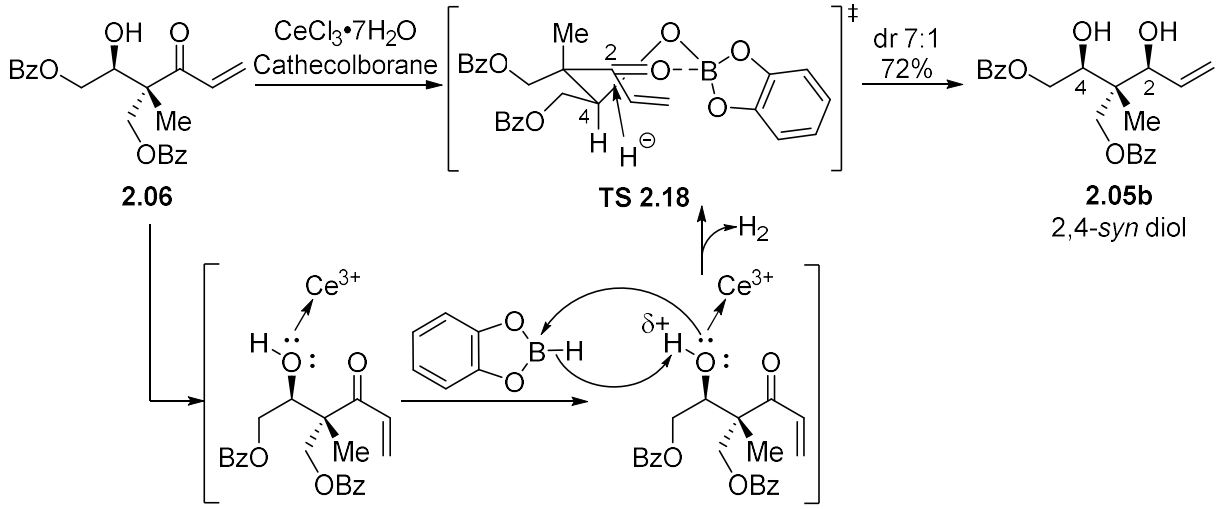


2.06 **2.05b** 2,4-*syn* diol **2.05a** 2,4-*anti* diol

Entry	Catecholborane (equiv.)	$CeCl_3 \bullet 7H_2O$ (equiv.)	Temp (°C)	dr (2,4-*syn* : 2,4-*anti*)[a]	Total yield (%)
1	1.0	–	−10	2: 1	49%
2	2.0	–	−10	2: 1	51%
3	5.0	–	−10	3: 1	36%
4	10.0	–	−10	1.5:1	23%
5	5.0	1.0	−10	7: 1	72%
6	5.0	1.0	−78	6: 1	39%

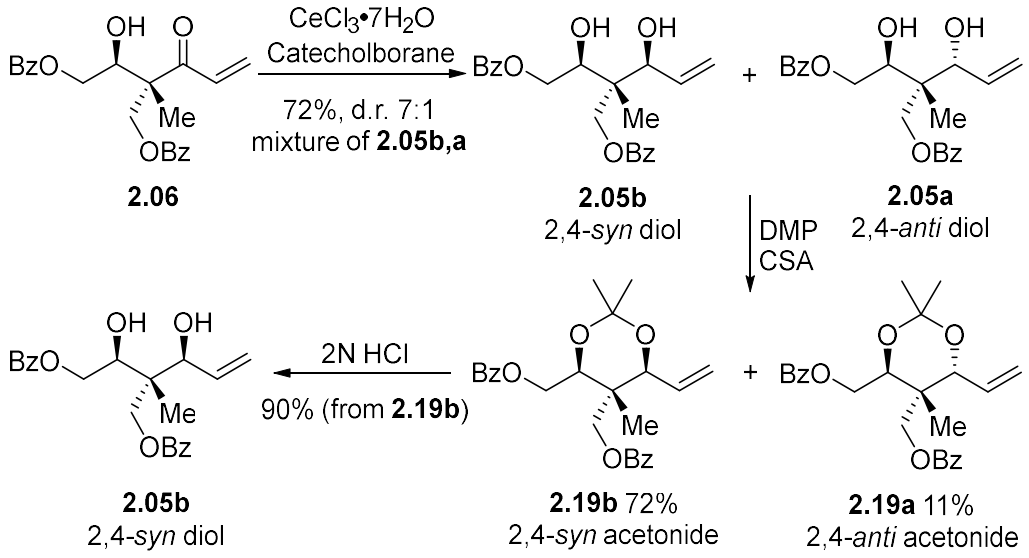
[a] dr ratio of 2,4-*syn*/*anti* diols was determined by ^{1}H NMR of the crude reaction mixture.

In the diastereoselective synthesis of ribo-like nucleoside analogues bearing an all-carbon C3′ quaternary center, an efficient protocol to achieve the 2,4-*syn* diol **2.05b** was established through the diastereoselective reduction of β-hydroxy ketone **2.06** using catecholborane and cerium trichloride ($CeCl_3•7H_2O$). It is suggested that the secondary alcohol firstly coordinates to the cerium cation making its proton more acidic. Subsequently, accompanied by the release of hydrogen, the coordination of the catecholborane with both the hydroxyl and carbonyl groups leads to the formation of a six-membered ring boron chelating transition state (Scheme 2.6, **TS 2.18**), where the larger substituents prefer equatorial positions. Intermolecular hydride delivered from the axial direction results in the 2,4-*syn* diastereoselectivity.



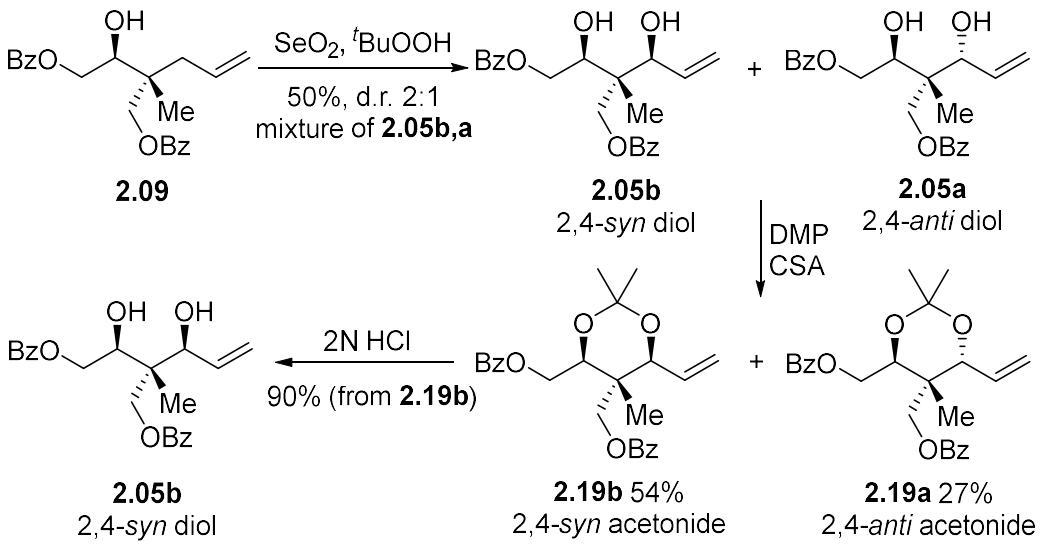
Scheme 2.6. Proposed mechanism of 2,4-*syn* diastereoselectivity.

An easy separation of 2,4-*syn* and 2,4-*anti* diols through an acetonide protection/deprotection protocol was established (Scheme 2.7). The mixture of 2,4-*syn* diol **2.05b** and 2,4-*anti* diol **2.05a** with 7:1 dr was treated with 2,2-dimethoxypropane (2,2-DMP) in the presence of camphorsulfonic acid (CSA), generating a 7:1 mixture of 2,4-*syn* and 2,4-*anti* acetonides **2.19b,a**. These were easily separated by silica gel flash chromatography, and subsequent cleavage of the isolated 2,4-*syn* acetonide **2.19b** provided the desired pure 2,4-*syn* diol **2.05b** with excellent yield (90%).



Scheme 2.7. Isolation of 2,4-*syn* diol through acetonide protection/deprotection.

An alternative method to construct the 2,4-*syn* diol **2.05b** was investigated using SeO_2 and TBHP (Scheme 2.8). Allylic oxidation of alkene **2.09** provided a mixture of 2,4-*syn* and 2,4-*anti* diols **2.05b,a** (50% yield) with a slight preference for the 2,4-*syn* diol **2.05b** (dr 2:1). Following the above acetonide protection and deprotection protocol, the mixture of 2,4-*syn* and 2,4-*anti* acetonides **2.19b,a** were separated by silica gel flash chromatography. Hydrolytic removal of the isopropylidene protecting group of 2,4-*syn* acetonide **2.19b** with 2N HCl gave the pure 2,4-*syn* diol **2.05b**. Transformation of alkene **2.09** directly to the 2,4-*syn* diol **2.05b** eliminates the low yielding allylic oxidation of alkene **2.10** (52%) and the subsequent removal of the secondary TBS group, which saves time and improves efficiency. In the subsequent preparation of 2,4-*syn* diol **2.05b**, this method was mainly exploited.

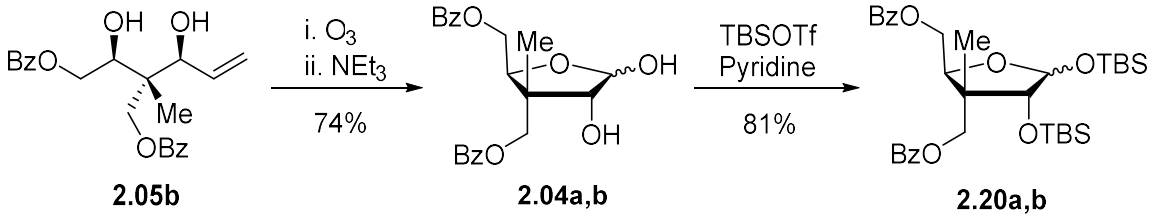


Scheme 2.8. Alternative method to rapidly construct the 2,4-*syn* diol.

2.2.3 Diastereoselective synthesis of ribo-like 1′,2′-*cis* nucleoside analogue

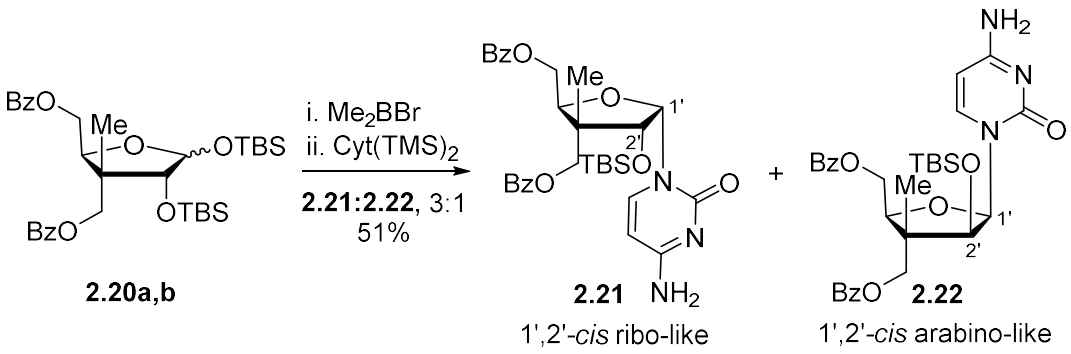
Diastereoselective synthesis of ribo-like nucleoside analogues bearing a C3′ stereogenic all-carbon quaternary center was achieved. The proper choice of C2′ hydroxyl protecting group was critical to control the 1′,2′-*cis*/*trans* diastereoselectivies.

Ozonolysis of 2,4-*syn* diol **2.05b** was conducted under an ozone atmosphere, followed by the treatment with triethylamine to generate the ribo-like lactols **2.04a,b**. Bis-protection of lactols **2.04a,b** with a TBS group provided a mixture of ribo-like bis-OTBS furanosides **2.20a,b** (Scheme 2.9).



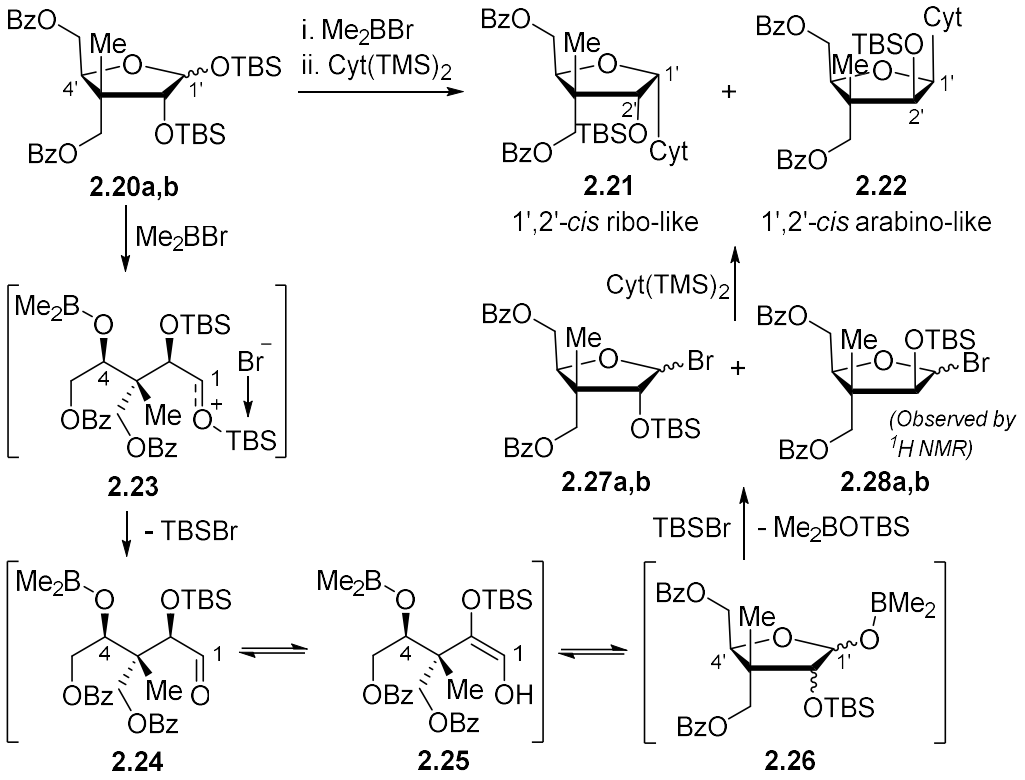
Scheme 2.9. Formation of bis-OTBS furanosides **2.20a,b**.

Interestingly, when bis-OTBS furanosides **2.20a,b** were employed in the *N*-glycosylation, a 3:1 mixture of 1′,2′-*cis* ribo-like (**2.21**) and 1′,2′-*cis* arabino-like scaffolds (**2.22**) was generated (Scheme 2.10), thus suggesting an epimerization of the C2′-center.



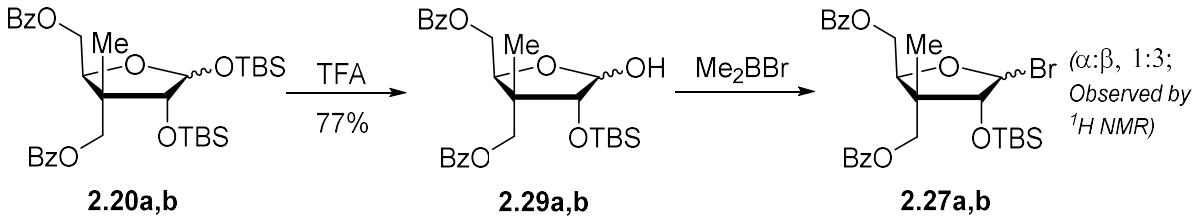
Scheme 2.10. Stereoselective *N*-glycosylation of bis-OTBS furanosides **2.20a,b**.

It was worth noting that, in the presence of dimethylborane bromide (Me_2BBr), the bis-OTBS furanosides **2.20a,b** could undergo an endocyclic C-O bond forming the acyclic bromoacetal **2.23**. The resulting aldehyde **2.24** generated upon removal of TMSBr would exist in equilibrium with its enol form **2.25**. Cyclization of this enol **2.25** would lead to C2′-epimerized furanosides **2.26**. Indeed, a mixture of bromofuranosides **2.27a,b** and **2.28a,b** was observed by ^{1}H NMR (Scheme 2.11).



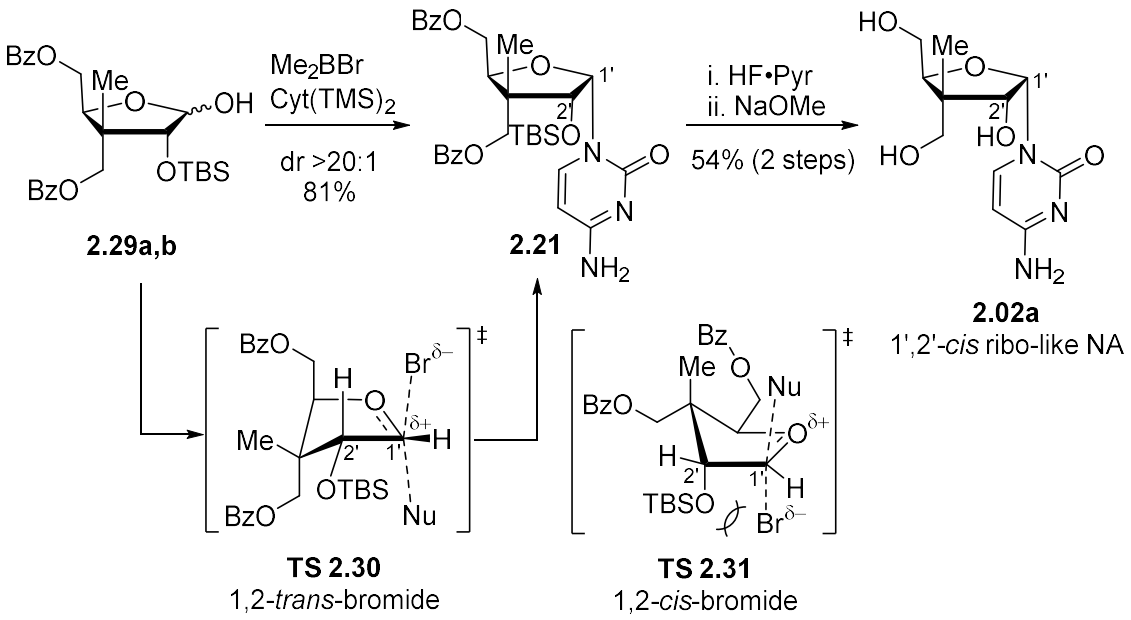
Scheme 2.11. Mechanistic illustration of epimerization process.

A mixture of C2′-TBS protected lactols **2.29a,b** was obtained through regioselective cleavage of the anomeric silyl protecting group of **2.20a,b** using trifluoroacetic acid (TFA) (Scheme 2.12). C2′-TBS protected lactols **2.29a,b** only generated a mixture of bromofuranosides **2.27a,b** in the presence of Me_2BBr as verified by ^1H NMR that lead to the exclusive formation of 1′,2′-*cis* ribo-like nucleoside analogue **2.21** (Scheme 2.13).



Scheme 2.12. ^1H NMR verification of bromofuranoside intermediate.

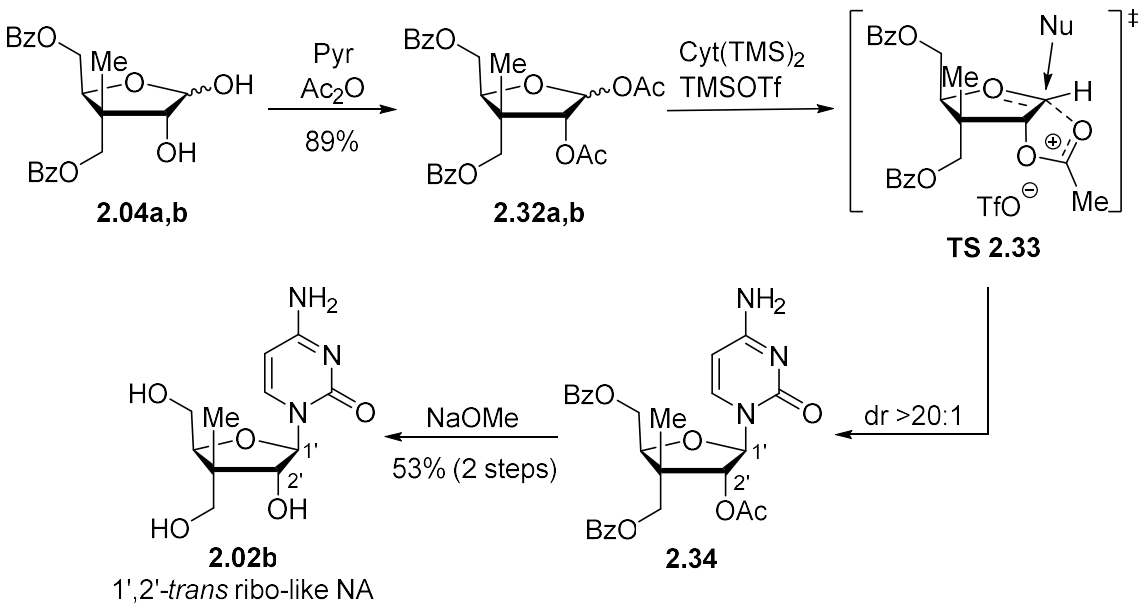
Upon treatment of C2′-TBS protected lactols **2.29a,b** with Me_2BBr, a mixture of 1′,2′-*cis* and 1′,2′-*trans* C1′-bromofuranosides **2.27a,b** was formed. Attack of the nucleobase would be favored through **TS 2.30** since it does not suffer from unfavorable electrostatic repulsion between the C1′-halide and C2′-alkoxy group as in **TS 2.31**. A high diastereoselectivity (>20:1 dr as determined by ^1H NMR of the crude reaction mixture) was thus obtained for 1′,2′-*cis* ribo-like scaffold **2.21** bearing an all-carbon quaternary center at the C3′ position. Subsequent deprotection of both the benzoate and silyl ether protecting groups provided 1′,2′-*cis* nucleoside analogue **2.02a** (C1′-α) in a 54% yield over two steps.



Scheme 2.13. Formation of 1′,2′-*cis* ribo-like nucleoside analogue **2.02a** (C1′-α).

2.2.4 Diastereoselective synthesis of ribo-like 1′,2′-*trans* nucleoside analogue

The bis-acetate protected furanosides **2.32a,b** were formed in excellent yield (89%) using acetic anhydride (Ac_2O) and pyridine from lactols **2.04a,b** (Scheme 2.14). Activation of **2.32a,b** with TMSOTf and glycosylation with silylated cytosine resulted in the 1′,2′-*trans* ribo-like nucleoside analogue with an excellent diastereoselectivity (>20:1 dr). This reaction proceeds through transition state **TS 2.33** which is stabilized by anchimeric assistance from the C2′-OAc protecting group. Cleavage of the benzoate and acetate protecting groups with NaOMe provided the final ribo-like 1′,2′-*trans* nucleoside analogue **2.02b** (C1′-β) in a 53% yield over two steps.



Scheme 2.14. Generation of 1′,2′-*trans* ribo-like nucleoside analogue **2.02b** (C1′-β).

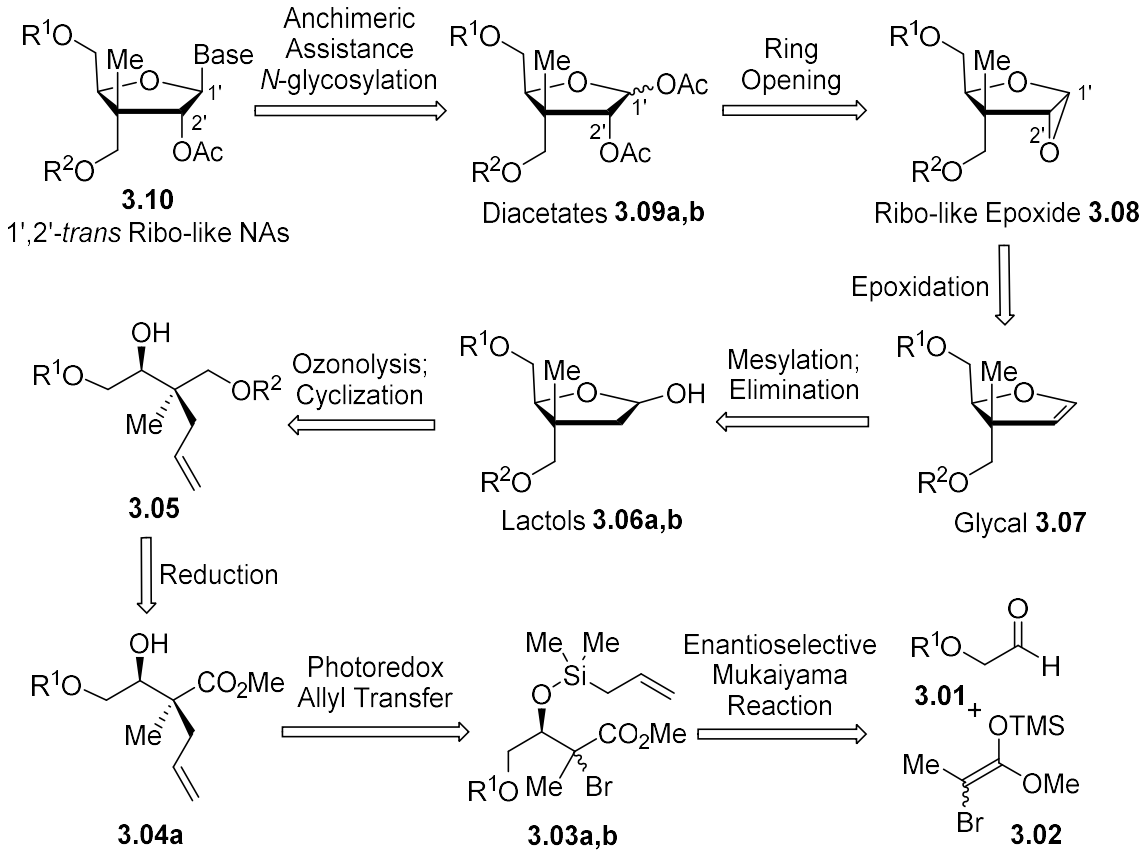
2.3 Conclusion

An efficient method was established to diatereoselectively synthesize 1′,2′-*cis* **2.02a** (C1′-α) and 1′,2′-*trans* **2.02b** (C1′-β) ribo-like nucleoside analogues bearing a C3′ all-carbon quaternary center and a C2′-hydroxyl group. This C3′ all-carbon quaternary center was constructed using consecutive alkylations. In order to access this series of novel nucleoside analogues, a 2,4-*syn* diastereoselective reduction of a β-hydroxy ketone was developed using catecholborane and $CeCl_3 \bullet 7H_2O$ through intermolecular hydride delivery. The diastereoselectivities for the *N*-glycosylation reactions were controlled by the proper selection of C2′ protecting groups.

Chapter 3 Development of an alternative synthetic pathway to obtain ribo-like nucleoside analogues bearing a C3′ all-carbon quaternary center

3.1 Introduction

The objective of this work was to selectively phosphorylate the primary C5′ hydroxyl group of our novel 1′,2′-*trans* ribo-like nucleosides. Thus, orthogonal protecting groups R^1 and R^2 (**3.10**, Scheme 3.1) were required on the C5′ and C3′ hydroxyls.



Scheme 3.1. Retrosynthesis of 1′,2′-*trans* ribo-like NAs using photoredox catalysis.

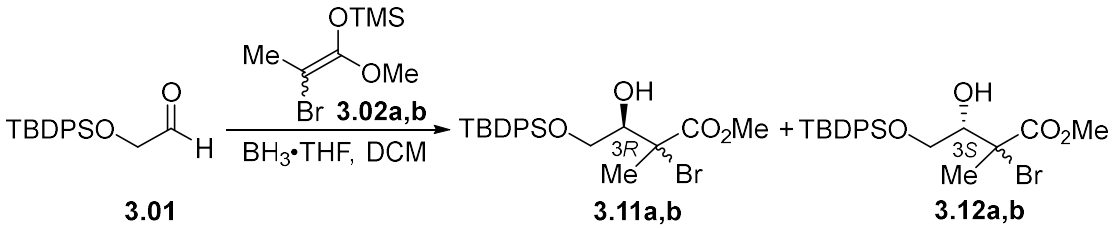
An alternative synthetic pathway was developed to diastereoselectively synthesize such 1′,2′-*trans* ribo-like nucleoside analogues previously described in Chapter 2. As described in Chapter 2, anchimeric assistance from the C2′-protecting group will be used to

diastereoselectively generate the 1′,2′-*trans* nucleoside analogues from the bis-acetate protected furanosides **3.09a,b**. The diacetates which were previously derived from a 2,4-*syn* diol could be alternatively synthesized through ring-opening of ribo-like epoxide **3.08**. Epoxide **3.08** can be provided through a stereoselective epoxidation of glycal **3.07**,[96] which could be synthesized by successive mesylation and elimination of lactol **3.06a,b**. Lactol **3.06a,b** can be obtained by ozonolysis of acyclic intermediate **3.05**, followed by cyclization. A photoredox catalyzed free radical intramolecular allyl transfer was employed to construct the all-carbon quaternary stereogenic center. The precursor of photoredox catalysis **3.03a,b** could be accessed through an enantioselective Mukaiyama aldol reaction of aldehyde **3.01** and bromoenolates **3.02a,b**.[92,97]

3.2 Enantioselective Mukaiyama aldol reaction

A new synthetic approach to construct such scaffolds began with an enantioselective Mukaiyama aldol reaction. This optimized protocol was used to generate methyl ester **3.11a,b**. A Mukaiyama aldol reaction of aldehyde **3.01** and bromoenolates **3.02a,b** was investigated by commencing with achiral reagent $TiCl_4$ providing a 1:1 mixture of 3*R* and 3*S* methyl esters **3.11a,b** and **3.12a,b** (entry 1). The use of (L)-tryptophane-Ts (Ts = *p*-Toluenesulphonyl) and BH_3•THF generated a chiral organoborane *in situ* and resulted in a 97:3 er as determined by chiral supercritical fluid chromatography and a 22% yield at −40 °C (entry 3) compared to only starting material at −78 °C (entry 2). When using (L)-valine-Ts instead of (L)-tryptophane as the chiral reagent, a better enantioselectivity was achieved as well as a significant increase in yield (entry 4). Upon changing Ts with Ns (Ns = *p*-Nitrophenylsulphonyl), methyl esters **3.11a,b** were obtained in excellent er (>99:1 er (3*R*/3*S*)) and yield (86%, entry 5). A 9:1 dr of 2,3-*anti* and 2,3-*syn* methyl esters were obtained. This work was done by Dr Amarender Manchoju (postdoctoral fellow in the Guindon lab).[92]

Table 3.1. Enantioselective Mukaiyama aldol reaction.[92]



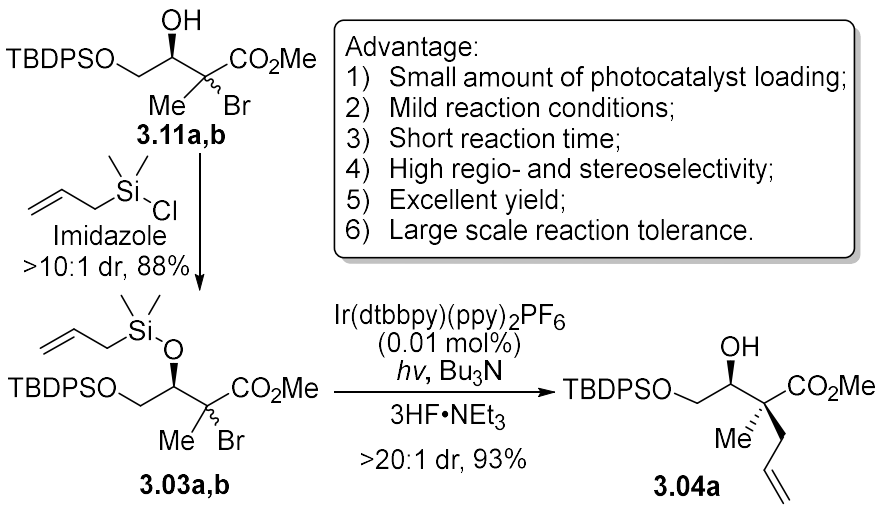
3.01 **3.11a,b** **3.12a,b**

Entry	Conditions	er (3*R*:3*S*)[a]	dr (2,3-*anti*:2,3-*syn*)[b]	Yield (%)[c]
1	$TiCl_4$, –78 °C	1:1	-	88
2	(L)-Tryptophane-Ts, –78 °C	-	-	S.M.
3	(L)-Tryptophane-Ts, –40 °C	97:3	-	22
4	(L)-Valine-Ts, –78 °C	99:1	-	65
5	(L)-Valine-Ns, –78 °C	>99:1	9:1	86

[a] Determined by chiral supercritical fluid chromatography. [b] Determined by ^{1}H NMR of crude mixtures. [c] Isolated yields following silica gel chromatography.

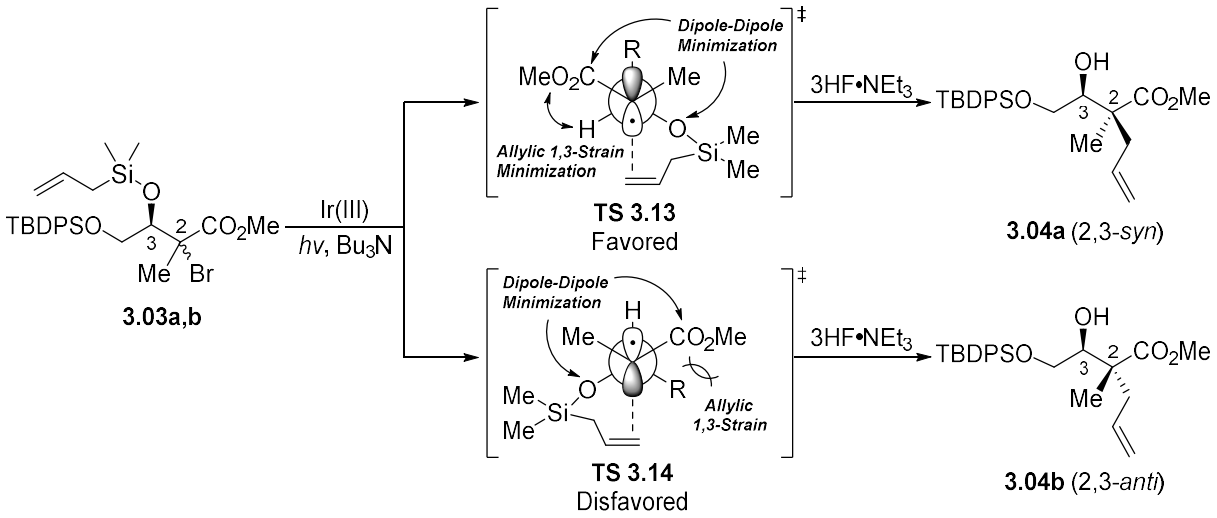
3.3 Photoredox catalyzed free radical-based allyl transfer

Taking advantage of a novel synthetic approach developed by Tommy Lussier (MSc 2019, Guindon lab), the all-carbon quaternary stereogenic center could be constructed using photoredox catalysis.[94] In view of the advantages of photoredox catalysis such as a small amount of photocatalyst loading, mild reaction conditions at room temperature, short reaction time, high stereo- and regio- selectivity, excellent yield and large scale reaction tolerance, photoredox catalysis has been widely used in C-C bond constructions and functionalizations over the past few decades.[98–101] Protection of methyl esters **3.11a,b** with allyldimethylchlorosilane provided the precursor (**3.03a,b**) necessary for the subsequent photocatalyzed free radical-based allyl transfer (Scheme 3.2).



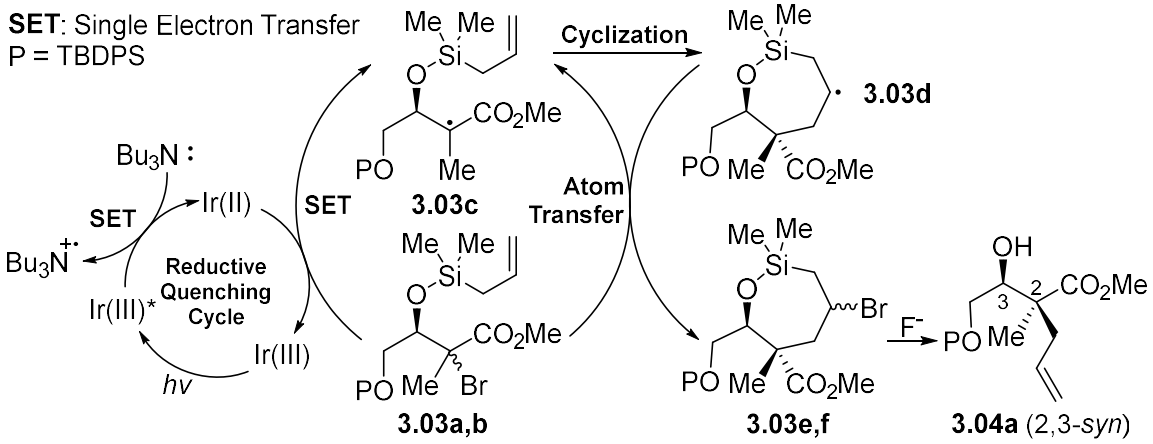
Scheme 3.2. Photoredox Ir(III)-catalyzed construction of quaternary stereogenic center.

The conditions for this photoredox catalyzed allyl group transfer were previously optimized in our group requiring only a catalytic amount of photocatalyst $Ir(dtbbpy)(ppy)_2PF_6$. The β-hydroxy ester **3.04a** bearing an all-carbon quaternary center was synthesized through a Ir(III)-catalyzed free radical reaction of dimethylallylsilyl β-hydroxy ester **3.03a,b** (Scheme 3.2).[92] The high diastereoselectivity (>20:1 dr) for this allyl group transfer can be explained through the two proposed transition states **TS 3.13** and **3.14** (Scheme 3.3). **TS 3.13** minimizes both intramolecular dipole-dipole interactions and allylic 1,3-strain providing the 2,3-*syn* stereochemistry.[92]



Scheme 3.3. Transition state illustration of 2,3-*syn* stereoselectivity.

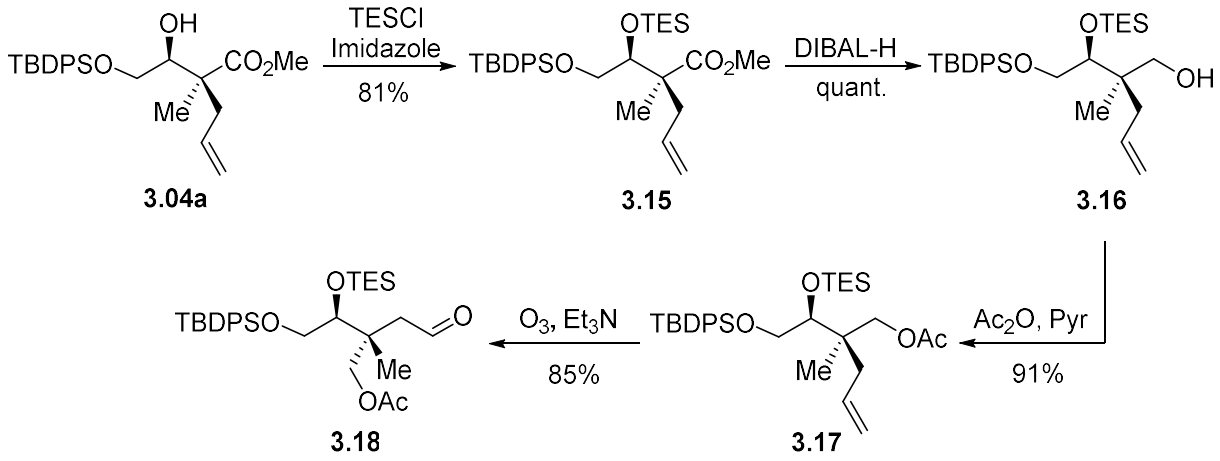
This photocatalytic process begins with excitation of Ir(III) using blue light providing active Ir(III)* (Scheme 3.4). This photoactive Ir(III)* can undergo a single electron transfer process (SET) with a tertiary amine (Bu_3N), which involves a reductive quenching cycle to reduce the Ir(III)* species to Ir(II). In the cycle of Ir(II) to Ir(III), the single electron transfers from **3.03a,b** to generate a tertiary free radical **3.03c**. The latter cyclizes to generate a seven-membered ring free radical **3.03d** bearing a quaternary stereogenic carbon. An atom transfer procedure provides the cyclic seven-membered ring bromide intermediates **3.03e,f** accompanied by regeneration of the tertiary free radical **3.03c**. Subsequent nucleophilic attack on silicon by a source of fluoride results in elimination, generating the 2,3-*syn* β-hydroxy ester **3.04a** bearing an all-carbon stereogenic quaternary center.[92]



Scheme 3.4. Mechanistic propose of photoredox Ir(III)-catalysis.

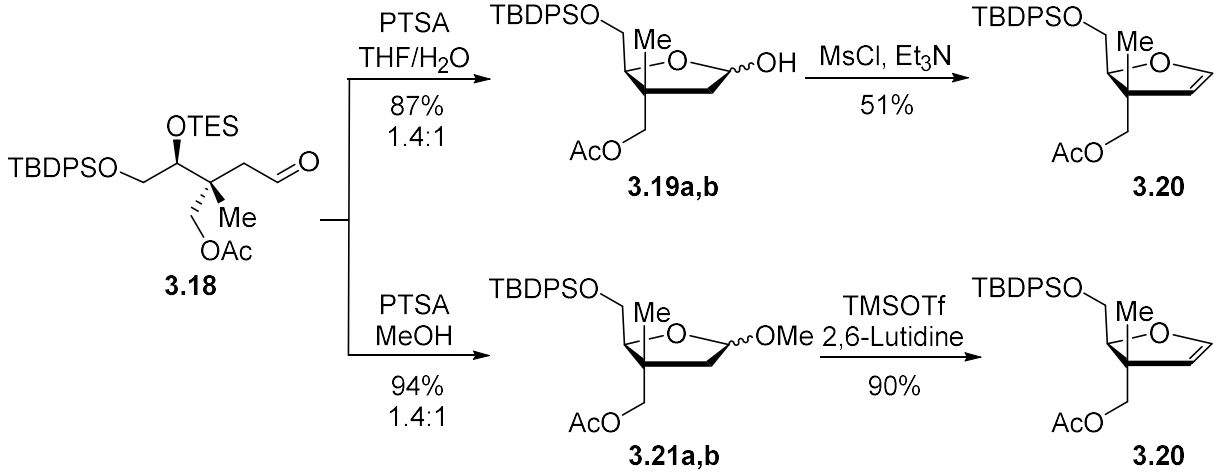
3.4 Preparation of *N*-glycosylation precursor

TES protection of β-hydroxy ester **3.04a** in the presence of imidazole provided the protected methyl ester **3.15**, which was then reduced to the primary alcohol **3.16** using DIBAL-H. Subsequently, the primary alcohol **3.16** was protected by an acetyl group (Ac) in the presence of acetic anhydride and pyridine in 91% yield. Alkene **3.17** was then oxidized using ozone providing aldehyde **3.18** in 85% yield (Scheme 3.5).



Scheme 3.5. Synthesis of aldehydes **3.18**.

Cyclization of aldehyde **3.18** in the presence of PTSA in THF/H_2O provided lactols **3.19a,b** with a 1.4:1 anomeric ratio (Scheme 3.6). Mesylation and elimination with Et_3N generated glycal **3.20** in 51% yield. In order to improve the yield of the key glycal intermediate **3.20**, another route was used to synthesize it through the methoxy lactols **3.21a,b**, which were derived from aldehyde **3.18** using PTSA in anhydrous methanol. The methoxy lactols **3.21a,b** were then treated with TMSOTf and 2,6-lutidine generating glycal **3.20**. Both steps had yields higher than 90%.

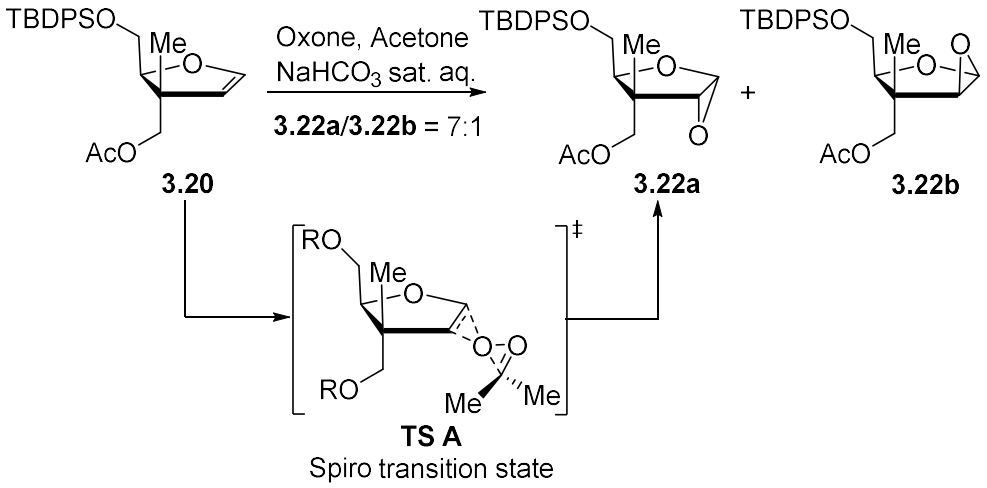


Scheme 3.6. Synthesis of glycals **3.20**.

With glycal **3.20** in hand, epoxidation using dimethyldioxirane (DMDO) was investigated. DMDO, also known as Murray's reagent, is widely used as an epoxidizing

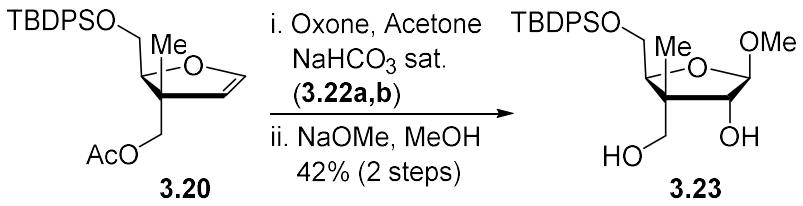
reagent due to its mild reaction conditions and generation of only acetone.[102] DMDO is usually generated *in situ* from a catalytic amount of acetone and a stoichiometric amount of potassium peroxymonosulfate (oxone).

When glycal **3.20** was subjected to DMDO epoxidation, the ribo-like epoxide **3.22a** was obtained as the major epoxide in a 7:1 ratio relative to the arabino-like epoxide **3.22b**, which was determined by ^{1}H NMR of crude epoxides **3.22a,b** (the chemical shifts at 5.16 and 5.07 correspond to the H1′ of the major and minor epoxides). The mechanism of the epoxidation with DMDO is believed to proceed through a spiro transition state **TS A** (Scheme 3.7),[102] with oxygen transfer occurring from the less hindered face of the alkene. For our scaffolds, the bulky TBDPS at the C5′ hydroxyl and the methyl group of the C3′ quaternary center, likely favor formation of the ribo-like epoxide **3.22a** as the major product.



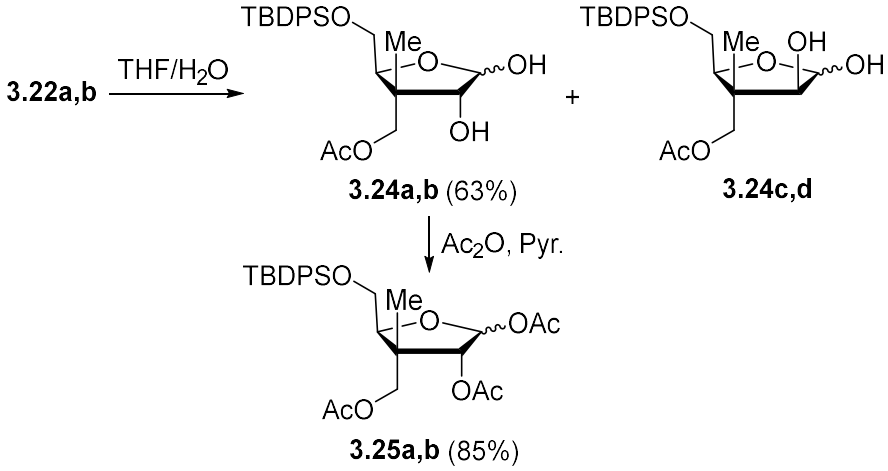
Scheme 3.7. Epoxidation of glycal **3.20** with DMDO.

The ring opening of crude epoxides **3.22a,b** using NaOMe in MeOH provided the major 1′-O-methoxy-β-ribofuranose **3.23**, which verified the epoxide stereochemistry (Scheme 3.8). The two-dimensional nuclear Overhauser effect experiment (2D NOESY) indicated the key through-space correlations between H1′ and H4′, as well as H2′ and quaternary methyl group, which confirmed the relative configuration of ribofuranose **3.23**.



Scheme 3.8. Verification of the epoxide stereochemistry.

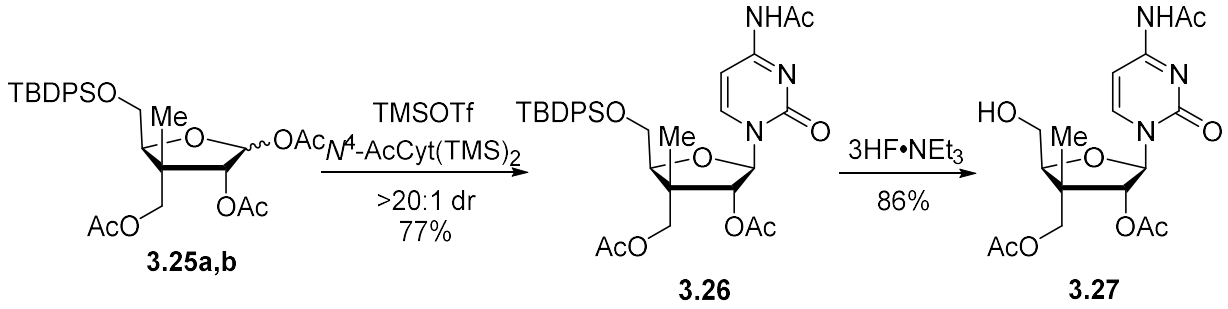
The crude epoxide **3.22a,b** was directly used to generate lactols **3.24a,b** (63%, isolated by flash chromatography), which were then protected with acetyl groups to generate acetate ribofuranosides **3.25a,b** in 85% yield (Scheme 3.9).



Scheme 3.9. Preparation of acetate ribofuranosides **3.25a,b**.

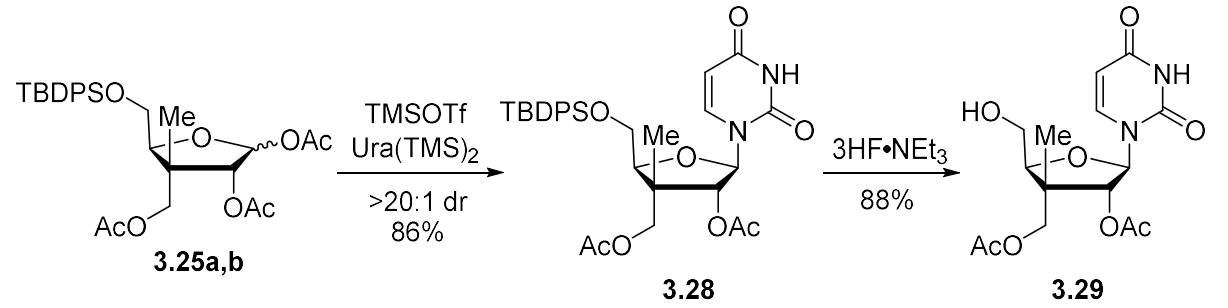
3.5 Stereoselective *N*-glycosylation

With ribo-like diacetate furanosides **3.25a,b** in hand, stereoselective *N*-glycosylation was performed in the presence of silylated N^4-acetylcytosine and TMSOTf (Scheme 3.10), 1′,2′-*trans* ribo-like nucleoside **3.26** was obtained in excellent diastereoselectivity (>20:1 dr) and yield (77%) in accordance with anchimeric assistance. The 1′,2′-*trans* stereochemistry was confirmed by relevant 2D NOESY experiment. Subsequent cleavage of the C5′-TBDPS protecting group with 3HF•NEt_3 provided 1′,2′-*trans* ribo-like nucleoside analogue **3.27** in 86% yield, which was the key precursor for formation of the nucleoside C5′ triphosphates.



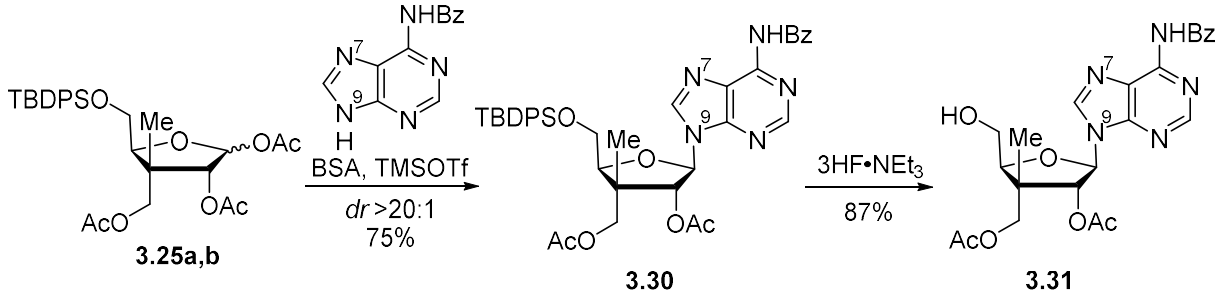
Scheme 3.10. Stereoselective *N*-glycosylation with anchimeric assistance.

Using the same *N*-glycosylation conditions, 1′,2′-*trans* ribo-like uracil nucleoside **3.28** was easily obtained in excellent diastereoselectivity (>20:1 dr) and yield (86%) using silylated uracil. The TBDPS was again cleaved with 3HF•NEt_3 to provide 1′,2′-*trans* ribo-like uracil nucleoside **3.29** with a free hydroxyl group at C5′ (Scheme 3.11).



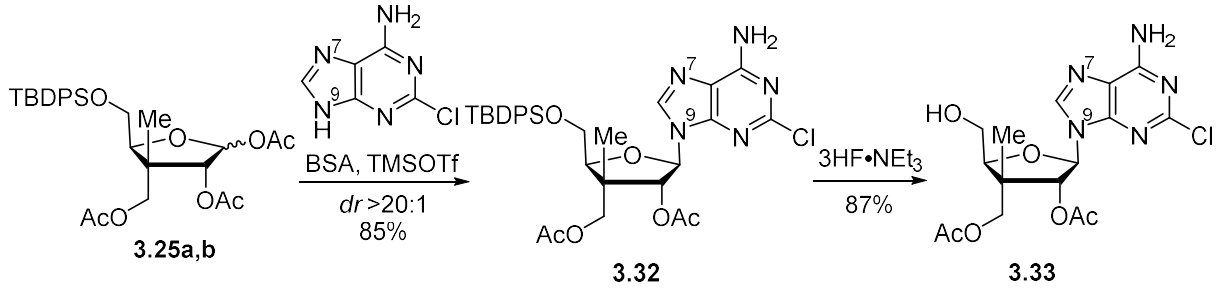
Scheme 3.11. Synthesis of 1′,2′-*trans* ribo-like uracil nucleoside **3.29**.

The diastereoselective synthesis of a 1′,2′-*trans* ribo-like nucleoside bearing an adenine nucleobase **3.30** was achieved by *in situ* silylation of N^6-benzoyladenine with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) following by glycosylation in the presence of TMSOTf (Scheme 3.12).[103–105] Regioselective N^9 glycosylated product **3.30** was obtained in excellent diastereoselectivity (>20:1 dr) and yield (75%). The 1′,2′-*trans* stereochemistry was confirmed by relevant 2D NOESY experiment, while the N^9 regioselectivity was verified by the key indicative three bond correlation between H1′ of sugar and C4 of purine in the $^1H/^{13}C$ 2D HMBC experiment. Subsequent cleavage of the TBDPS protecting group of **3.30** with 3HF•NEt_3 led to the formation of 1′,2′-*trans* ribo-like adenine nucleoside **3.31** with a free hydroxyl at C5′ in 87% yield.



Scheme 3.12. Synthesis of 1′,2′-*trans* ribo-like adenine nucleoside **3.31**.

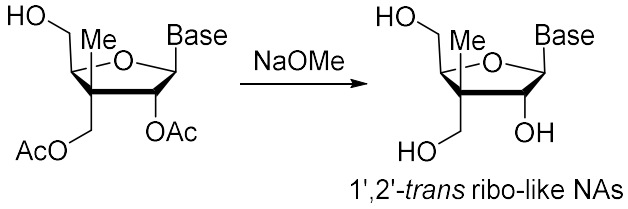
Using the same method above, 1′,2′-*trans* ribo-like 2-chloroadenine nucleoside **3.32** was obtained in excellent diastereoselectivity (>20:1 dr) and yield (85%). The cleavage of TBDPS protecting group gave 1′,2′-*trans* ribo-like 2-chloroadenine nucleoside **3.33** in 87% yield (Scheme 3.13).



Scheme 3.13. Synthesis of 1′,2′-*trans* ribo-like 2-chloroadenine nucleoside **3.33**.

With nucleoside **3.27** in hand, subsequent cleavage of the C2′ and C3′ acetyl protecting groups with NaOMe provided 1′,2′-*trans* ribo-like cytosine nucleoside **2.02b** in 81% yield (entry 1, Table 3.2). The same approach was employed for the formation of 1′,2′-*trans* ribo-like nucleoside bearing either a uracil (**3.34**, 80%, entry 2), an adenine (**3.35**, 82%, entry 3) or a 2-chloroadenine (**3.36**, 80%, entry 4) from the corresponding nucleosides **3.29**, **3.31** and **3.33**.

Table 3.2. Preparation of 1′,2′-*trans* ribo-like nucleosides.



Entry	Substrate	Base	Product	Yield[a]
1	**3.27**	Cytosine	**2.02b**	81%
2	**3.29**	Uracil	**3.34**	80%
3	**3.31**	Adenine	**3.35**	82%
4	**3.33**	2-Chloroadenine	**3.36**	80%

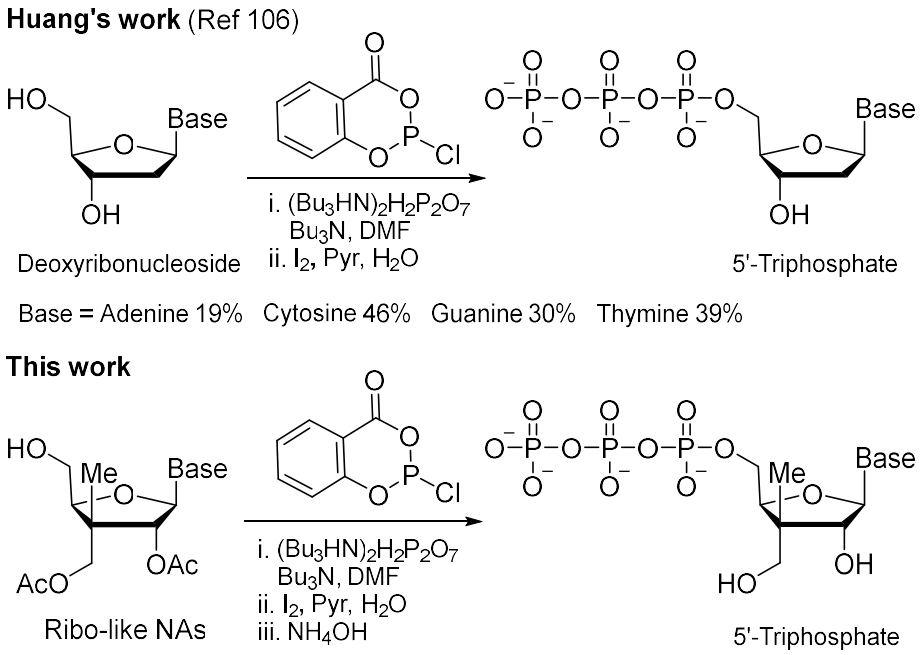
[a] Isolated yields following C18 reverse phase flash chromatography.

3.6 Comparison of the two synthetic approaches

The novel synthetic method combining an enantioselective Mukaiyama aldol reaction and an efficient photocatalytic free radical allyl transfer leads to an excellent diastereoselectivity in the construction of the all-carbon quaternary center. This approach involving glycal and epoxide intermediates is much more efficient for achieving the desired 1′,2′-*trans* ribo-like nucleosides with either purine or pyrimidine nucleobases. Compared with the low yield (50% – 70%) for diastereoselective synthesis of the 2,4-*syn* diol in the previous method (Chapter 2), each step of this novel method has a high yield (80% – >90%). Additionally, the necessary extra separation of the 2,4-*syn* and 2,4-*anti* diols through protection and deprotection procedures was essential to obtain pure 2,4-*syn* diol in Chapter 2. More importantly, this method allows for the formation of the nucleoside scaffolds bearing a C5′-free hydroxyl group for subsequent phosphorylation.

3.7 Nucleoside Triphosphates (NTPs)

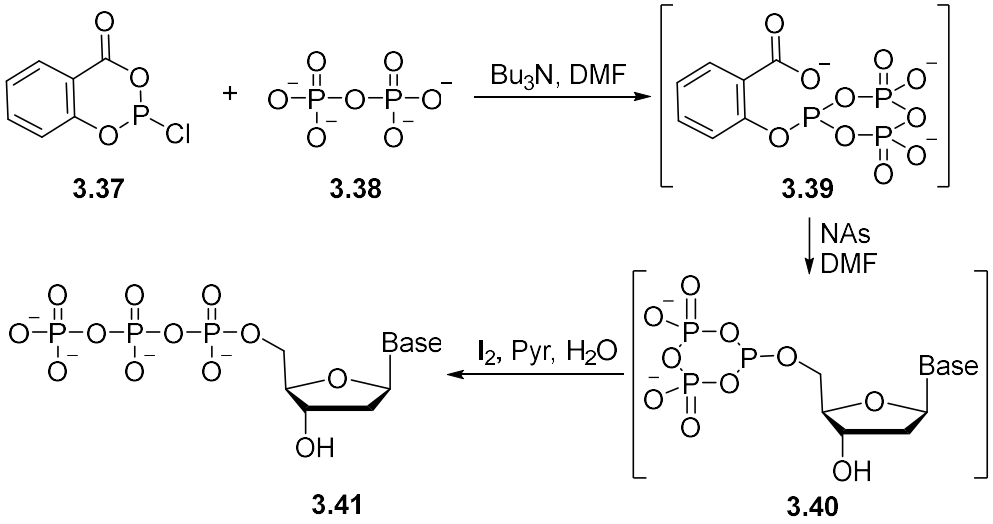
Synthesis of 1′,2′-*trans* ribo-like nucleotide bearing a 5′-triphosphate was investigated. These triphosphate species can be used in enzymatic assays to assess their antiviral or anticancer properties. The synthesis of such species is very challenging, and the optimization of the phosphorylation conditions was done by Dr Amarender Manchoju (postdoctoral fellow in the Guindon laboratory). Huang and co-workers have reported a one-pot synthesis of nucleoside 5′-triphosphates (Scheme 3.14) in 2011.[106] The phosphorylation can be easily accomplished with tributylammonium pyrophosphate in the presence of salicyl phosphorochloridite (SalPCl). Due to the mild reaction conditions, it is considered a good approach and has been applied in our triphosphate project.



Scheme 3.14. One-pot synthesis of nucleoside 5′-triphosphates.

The proposed mechanism of phosphorylation is depicted in Scheme 3.15. In the presence of salicyl phosphorochloridite (SalPCl, **3.37**), pyrophosphate (**3.38**) and tributylamine (NBu_3), the phosphitylating reagent **3.39** is formed through two consecutive nucleophilic substitution reactions. Upon addition of the nucleoside analogue in DMF, the phosphorylation of the free C5′ hydroxyl leads to the formation of the key intermediate 5′-

cyclic triphosphite **3.40**, followed by iodine oxidation of P(III) to P(V) and hydrolysis to generate the nucleoside 5′-triphosphate **3.41**.[106]

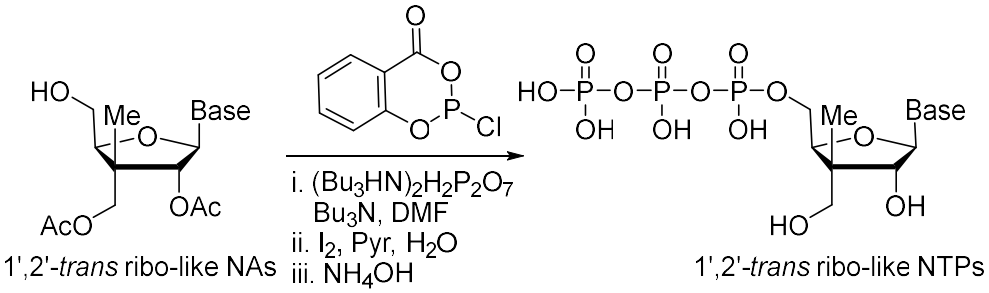


Scheme 3.15. Mechanism of phosphorylation.[106]

3.7.1 Phosphorylation of nucleoside analogues

Phosphorylation of 1′,2′-*trans* ribo-like nucleoside analogues bearing either purine or pyrimidine was investigated. Using SalPCl, $(Bu_2HN)_2H_2P_2O_7$ and Bu_3N in anhydrous DMF, followed by addition of nucleoside **3.27**, a solution of I_2 was used in the oxidation of the 5′-cyclic triphosphite. Subsequent cleavage of the C2′ and C3′ acetyl protecting groups with ammonium hydroxide (NH_4OH) generated 1′,2′-*trans* ribo-like cytosine nucleoside 5′-triphosphate **LCB-2330** in 28% yield (entry 1, Table 3.3, an overall yield after multiple steps, isolated by reverse phase C18 flash chromatography using 0-20% gradient of MeCN in 20 mM triethylammonium acetate buffer). The same phosphorylation approach was employed for the formation of 1′,2′-trans ribo-like nucleoside 5′-triphosphate bearing a uracil (**LCB-2331**, 24%, entry 2) or an adenine (**LCB-2332**, 27%, entry 3) from the corresponding 1′,2′-trans ribo-like nucleosides **3.29** and **3.31**. The purities of the final nucleoside triphosphates **LCB-2330**, **LCB-2331** and **LCB-2332** were examined by high-performance liquid chromatography (HPLC).

Table 3.3. Preparation of 1′,2′-*trans* ribo-like nucleoside 5′-triphosphates.



Entry	Substrate	Base	Product	Yield[a]
1	**3.27**	Cytosine	**LCB-2330**	28%
2	**3.29**	Uracil	**LCB-2331**	24%
3	**3.31**	Adenine	**LCB-2332**	27%

[a] Overall yield after multiple steps.

3.8 Conclusion

The synthesis of 1′,2′-*trans* ribo-like nucleoside analogue C5′-triphosphates bearing an all-carbon C3′ quaternary stereogenic center was achieved using diastereoselective *N*-glycosylations of ribo-like diacetate furanosides with anchimeric assistance. The novel synthetic pathway to obtain such ribo-like scaffolds involved diastereoselective epoxidation of a glycal. For installation of the all-carbon C3′ quaternary stereogenic center, an alternative photocatalyzed allyl transfer avenue was utilized to give excellent stereoselectivity and yield. An enantioselective Mukaiyama aldol reaction was used to form the photoredox precursor with exceptional enantioselectivity.

Chapter 4 Diastereoselective Synthesis of L-1′,2′-*cis*-4′-Thionucleoside Analogues Using an Acyclic Approach

4.1 Introduction

Since the first synthesis of a 4′-thionucleoside in the early 1970s by Bobek et al[107,108], this class of nucleoside analogues has attracted extensive attention in their synthesis and biological evaluation as potential therapeutic agents for treating viral infections and malignant tumors.[109,110] Yoshimura and co-workers, in the 1990s, reported potent anti-HSV and CMV activity for 4′-thioarabinoguanine (4′-Thio-Ara-G, Figure 4.1) compared to its inactive 4′-oxa congener.[111] 4′-Thio-Ara-C, compared to its 4′-oxa congener, Cytarabine, exhibits enhanced antitumor activity. This has been attributed to a longer half-life and decreased degradation from cytidine deaminase as compared to Ara-C.[29,30]

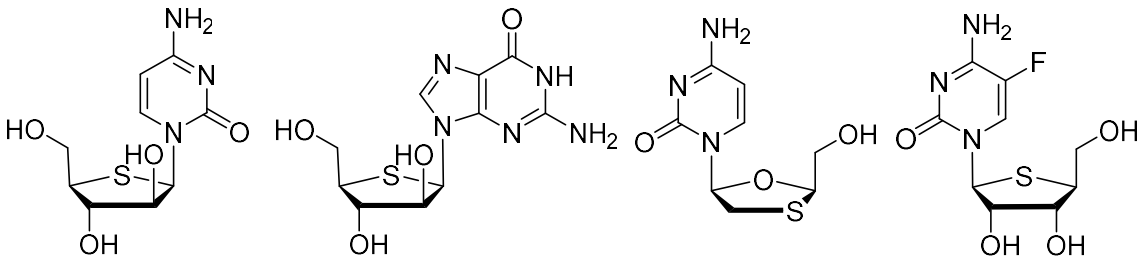
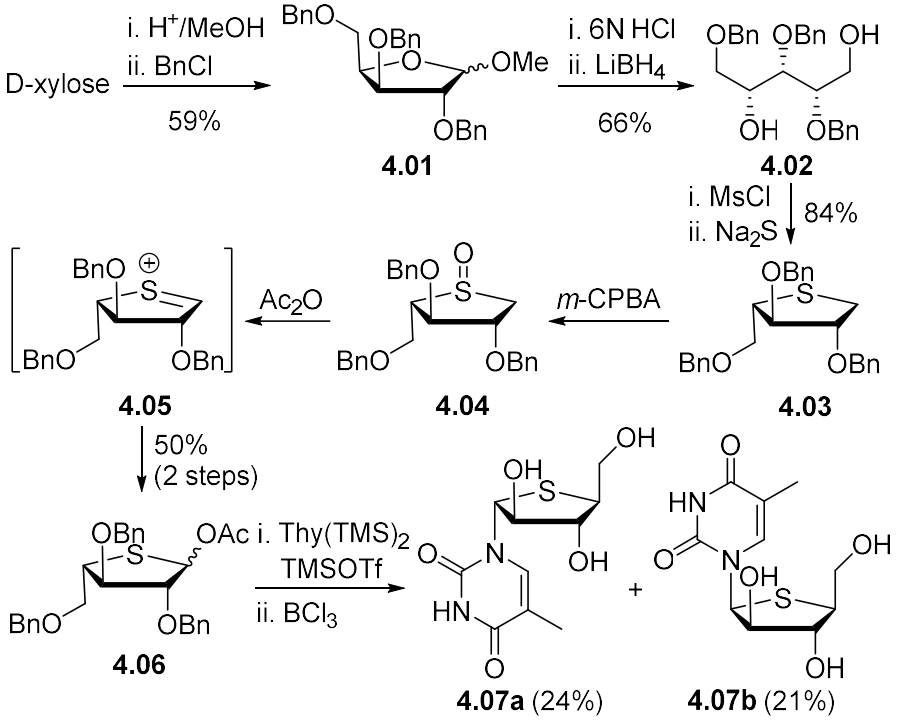


Figure 4.1. 4′-Thionucleoside analogues.

The development of nucleoside analogues in the L-series has also been an area of interest since they demonstrate less host cytotoxicity in comparison to their corresponding D-enantiomers. This is due to the fact that they are not recognized by normal cellular enzymes.[112,113] Lamivudine (L-3TC, Figure 4.1) is one of the antiretroviral medications approved by the FDA in the treatment of HIV and HBV.[31,32] As for 4′-thionucleosides, L-4′-thio-5-FU has been shown to have growth inhibitory activity against acute promyelocytic leukemia (APL) and breast cancer.[113]

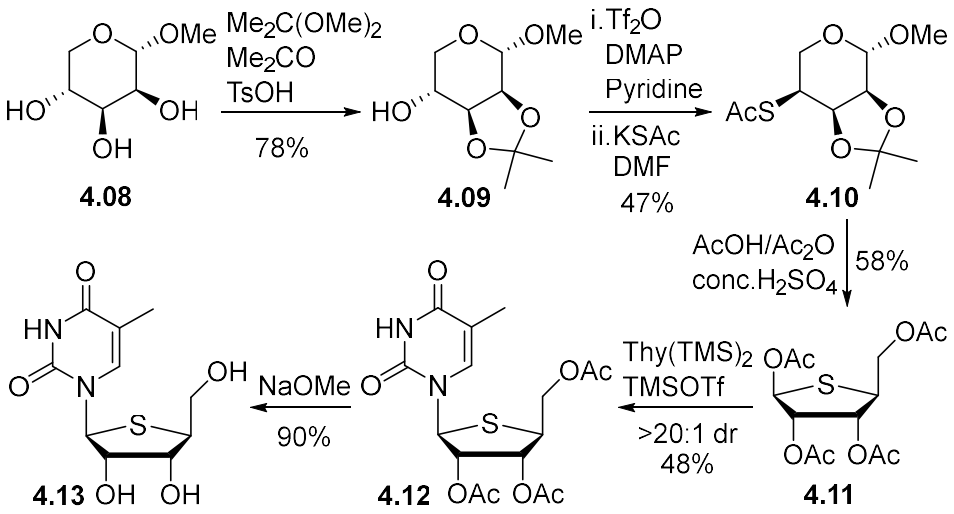
The synthesis of such 4′-thionucleoside scaffolds typically makes use of a Pummerer-type thioglycosylation[114,115] as demonstrated in 1998 by Matsuda and co-workers[116] in their synthesis of L-4′-thioarabinosylthymine (Scheme 4.1). In this approach, methyl 2,3,5-tri-O-benzyl-D-xylofuranoside **4.01** was prepared from D-xylose in two steps. Acidic treatment of **4.01** followed by reduction provided diol **4.02**. Dimesylate installation followed by treatment with sodium sulfide gave L-4′-thioarabitol **4.03**. Sulfoxide **4.04** was obtained with *m*-chloroperbenzoic acid (*m*-CPBA), and subsequent Pummerer rearrangement with Ac_2O, via a thiacarbenium ion **4.05**, provided an anomeric mixture of L-4′-thioarabinose **4.06**. *N*-glycosylation using silylated thymine and TMSOTf followed by benzyl group deprotection gave a mixture of α- and β-L-4′-thioarabinosyl thymine **4.07a** and **4.07b** with a poor diastereoselectivity.



Scheme 4.1. Pummerer-type thioglycosylation.

Alternatively, Pejanovic and co-workers[113] reported an efficient method in less steps to synthesize L-4′-thionucleoside starting with methyl α-D-lyxopyranoside **4.08**, which was easily prepared from D-xylose. Compound **4.08** reacted with dimethoxypropane to provide acetonide **4.09**. Thioacetyl derivative **4.10** was synthesized from **4.09** via a triflate

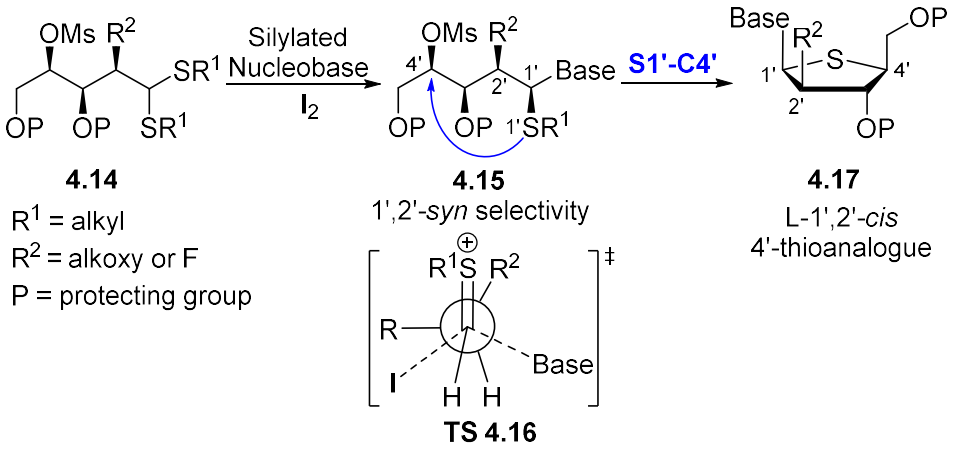
intermediate followed by a S_N2 displacement with potassium thioacetate (KSAc). Treatment of thioacetyl **4.10** with concentrated H_2SO_4 in a mixture of AcOH and Ac_2O provided L-4′-thioribofuranose **4.11**. *N*-Glycosylation of **4.11** with silylated thymine in the presence of TMSOTf provided L-4′-thioribonucleoside **4.12** with an excellent diastereoselectivity due to anchimeric assistance. Subsequent deacetylation with NaOMe formed L-4′-thioribonucleoside **4.13** (Scheme 4.2).



Scheme 4.2. Diastereoselective synthesis of L-4′-thioribonucleosides.

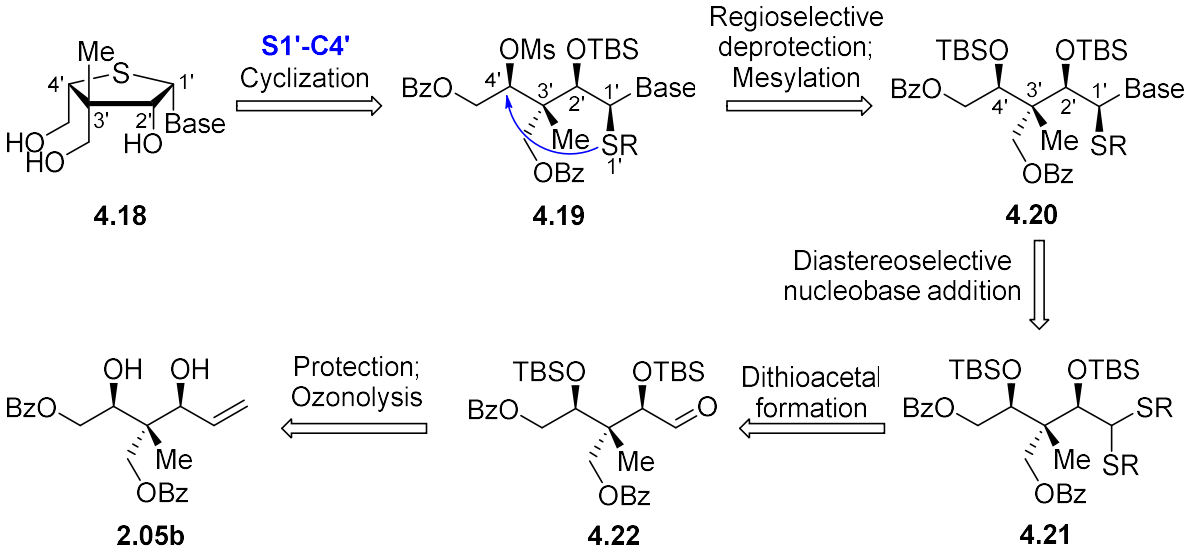
Our laboratory has previously designed an acyclic approach to construct 4′-thionucleoside scaffolds with a high diastereoselectivity starting from a thioaminal precursor bearing either a C2′ alkoxy or fluoro group (Scheme 4.3).[76,88,117] An intramolecular S_N2-like S1′→C4′ cyclization with displacement of a C4′ leaving group provides the L-1′,2′-*cis*-4′-thioanalogues **4.17** with an inversion of the C4′ stereocenter while maintaining the stereochemistry of the thioaminal stereocenter. The thioaminal precursor **4.15** can be synthesized from dithioacetal **4.14** with an excellent 1′,2′-*syn* selectivity. This 1′,2′-*syn* selectivity is proposed to occur through an initially formed halothioether that adapts a conformational preference in which the C2′ substituent and the thioether moiety are in close proximity to maximize R-C2′ and H-C2′ sigma donation to the electron poor thiacarbenium intermediate in **TS 4.16**. DFT calculations supported the stabilization of this transition state by the presence of the I^- counteranion which prefers to be located on the opposite side of the

incoming nucleobase.[90] This strategy has also been used for the synthesis of novel thionucleoside analogues bearing a C3′ all-carbon quaternary center and a C2′-F substituent.[76]



Scheme 4.3. Acyclic approach to 1′,2′-*cis*-4′-thioanalogues.

This acyclic strategy has been used herein to construct L-4′-thioanalogues bearing an all-carbon quarternary center at C3′ with an alkoxy substituent at C2′. As shown in Scheme 4.4, L-1′,2′-*cis*-4′-thionucleoside analogue **4.18** can be constructed from the 1′,2′-*syn* thioaminal precursor **4.19**. Thioaminal **4.19** can be prepared through regioselective C4′ silyl ether deprotection of precursor **4.20** with subsequent installation of a mesylate.

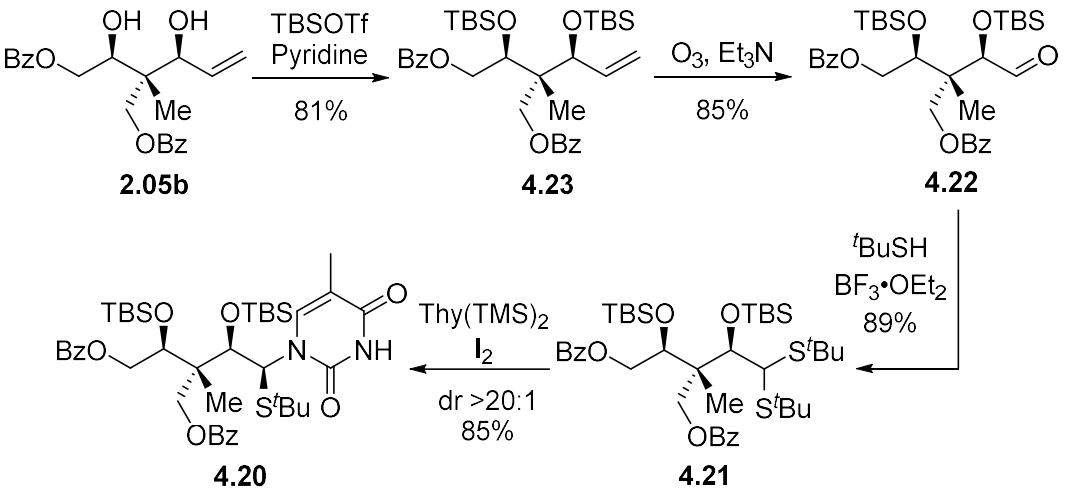


Scheme 4.4. Retrosynthetic analysis of L-1′,2′-*cis*-4′-thionucleoside analogue **4.18**.

Thioaminal **4.20** can be obtained by diastereoselective nucleobase addition onto dithioacetal **4.21** that originates from aldehyde **4.22**. Protection of 2,4-*syn* diol **2.05b** (reported in Chapter 2) with silyl ether followed by ozonolysis allows for the formation of aldehyde **4.22**.

4.2 Preparation of thioaminal precursor

Starting from 2,4-*syn* diol **2.05b**, bis-silyl ether hydroxyl group protection was carried out in the presence of TBSOTf and pyridine to generate bis-TBS protected alkene **4.23** (81%), followed by ozonolysis and subsequent hydrolysis with triethylamine to provide aldehyde **4.22** (85%) (Scheme 4.5). Dithioacetal **4.21** was then prepared by condensation of aldehyde **4.22** with ^{t}BuSH in the presence of BF_3•OEt_2 (89%). Subsequently, nucleobase addition of dithioacetal **4.21** in the presence of silylated thymine and iodine provided 1′,2′-*syn* thioaminal **4.20** with excellent diastereoselectivity (>20:1 dr) and yield (85%).

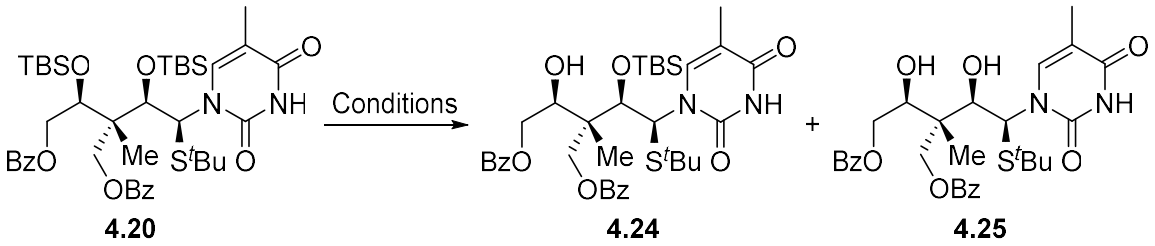


Scheme 4.5. Preparation of 1′,2′-*syn* thioaminal precursor **4.20**.

The regioselective cleavage of the C4′-TBS protecting group of 1′,2′-*syn* thioaminal **4.20** was very challenging. As shown in Table 4.1, various fluoride reagents were first considered. Regioselective C4′-TBS deprotected thioaminal **4.24** was obtained in low yield (8–20%) when using 50 equivalents of HF•Pyr or 3HF•NEt_3 in THF at room temperature for 96 hours (entries 1 and 2), with the majority of the reaction mixture being starting thioaminal **4.20** (29–57%). When TBAF was used as the deprotecting reagent, diol **4.25** was obtained in a high yield (81%) with no traces of the desired thioaminal **4.24** (entry 3). Subsequently, the use

of PTSA instead of fluoride reagents in the regioselective deprotection was investigated. Compound **4.24** was again formed in poor yield (15–26%) when using 10 to 50 equivalents of PTSA in a DCM/MeOH (0.02 M) at 50 °C (entries 4–6), as well as recovery of starting material (44%–85%). Unfortunately, increasing the reaction concentration to 0.1 M (entry 7) did not increase the yield of the desired thioaminal **4.24**.

Table 4.1. Regioselective Cleavage of C4′-TBS.



Entry	Reagent	Sol, Temp, Time	**4.24**[a]	**4.25**[a]	Recovered **4.20**[a]
1	50 eq. HF•Pyr	THF (0.1 M), rt, 96 h	8%	–	29%
2	50 eq. 3HF•NEt_3	THF (0.1 M), rt, 96 h	20%	–	57%
3	1.0 eq. TBAF	THF (0.1 M), rt, 24 h	–	81%	–
4	10 eq. PTSA	DCM/MeOH (0.02 M) 50 °C, 40 h	15%	–	85%
5	20 eq. PTSA	DCM/MeOH (0.02 M) 50 °C, 40 h	26%	–	72%
6	50 eq. PTSA	DCM/MeOH (0.02 M) 50 °C, 40 h	25%	–	44%
7	20 eq. PTSA	DCM/MeOH (0.1 M) 50 °C, 40 h	22%	–	34%

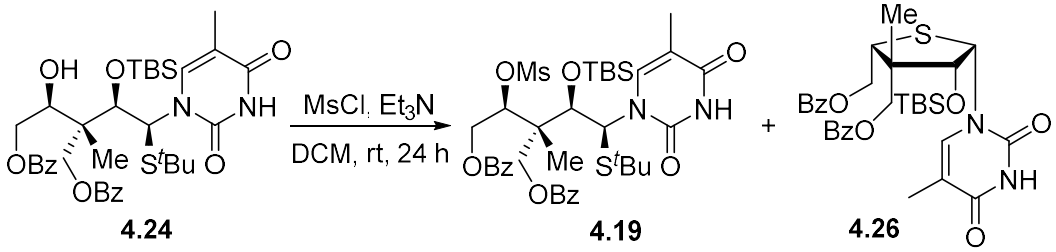
[a] Isolated yields following silica gel chromatography.

Based on these preliminary results, 20 equivalents of PTSA in a 0.02 M DCM/MeOH solution at 50 °C for 40 hours (entry 5) was chosen for the regioselective deprotection. It should be noted that these deprotection conditions were successful using a slightly different thioaminal substrate.[91]

4.3 Synthesis of L-1′,2′-*cis*-4′-Thionucleoside

With thioaminal **4.24** in hand, a mesylate was installed at C4′ providing the thioaminal intermediate **4.19** (17%) along with the desired cyclized thionucleoside **4.26** (32%) when using 3.0 equivalents of MsCl and 4.0 equivalents of Et_3N at room temperature (entry 1, Table 4.2). The formation of cyclized product **4.26** under such mild conditions might be explained by the steric effect of the C3′ quaternary center. It should be noted that such S1′-C4′ cyclizations usually require reflux conditions.[76] Interestingly, when using 5.0 equivalents of MsCl and 5.0 equivalents of Et_3N, L-1′,2′-*cis*-4′-thionucleoside **4.26** was obtained in a 50% yield (entry 2).

Table 4.2. Mesylate installation and intramolecular S_N2-like cyclization.

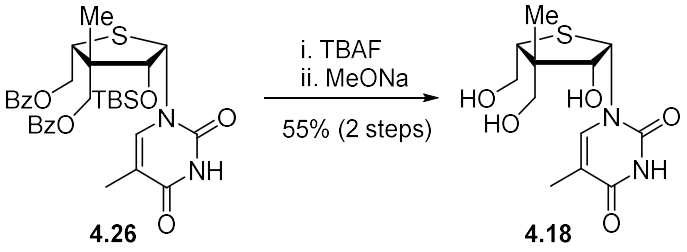


4.24 **4.19** **4.26**

Entry	MsCl	Et_3N	Ratio[a] of **4.26** and **4.19**	**4.19**[b]	**4.26**[b]
1	3.0 equiv.	4.0 equiv.	2:1	17%	32%
2	5.0 equiv.	5.0 equiv.	15:1	–	50%

[a] Determined by ^1H NMR of the crude reaction mixture. [b] Isolated yields following silica gel chromatography.

Deprotection of the C2′-TBS along with the C3′ and C5′-Bz groups was achieved through the successive addition of TBAF and MeONa providing L-1′,2′-*cis*-4′-thionucleoside analogue **4.18** in a 55% yield over two steps (Scheme 4.6).

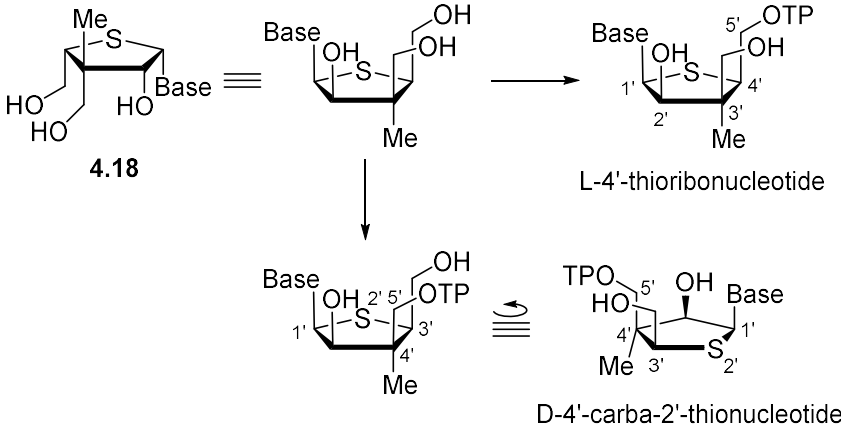


Scheme 4.6. Synthesis of L-1′,2′-*cis*-4′-thionucleoside **4.18**.

These preliminary results have demonstrated that the diastereoselective synthesis of a L-1′,2′-*cis*-4′-thionucleoside scaffold bearing an all-carbon C3′ quaternary center and a C2′-hydroxyl group can be achieved using our acyclic approach. This approach makes use of a S1′→C4′ intramolecular S_N2-like cyclization of a 1′,2′-*syn* thioaminal precursor obtained from diastereoselective nucleobase addition onto a dithioacetal.

4.4 Perspectives

With L-1′,2′-*cis*-4′-thionucleoside analogue **4.18** in hand, subsequent regioselective phosphorylation may result in the formation of either L-1′,2′-*cis*-4′-thionucleotide or D-4′-carba-2′-thionucleotide (Scheme 4.7). L-1′,2′-*cis*-4′-thionucleotide may have the potential activity as an antiviral agent.[112,113] The latter D-4′-carba-2′-thionucleotide may exhibit inhibitory activity against some retroviruses such as HIV. Some carbocyclic nucleoside analogues have been shown an increased metabolic stability against nucleoside phosphorylase and 5′-nucleotidase, as well as an increased oral bioavailability.[118,119]



Scheme 4.7. Prospective regioselective phosphorylation.

General Conclusions

In this thesis, the diastereoselective synthesis of 1′,2′-*cis* and 1′,2′-*trans* ribo-like nucleoside analogues bearing an all-carbon C3′ quaternary stereogenic center was accomplished using stereoselective *N*-glycosylations which were controlled by different C2′ hydroxyl protecting groups. For the diastereoselective construction of ribo-like diacetate furanosides, two synthetic pathways were developed. The novel method which employed diastereoselective epoxidation of a glycal proved to be more efficient than diastereoselective synthesis of a 2,4-*syn* diol.

Two different strategies were investigated for the formation of the all-carbon quaternary stereogenic center which involved either successive alkylations of a lactone or a consecutive enantioselective Mukaiyama aldol reaction followed by a photocatalytic free radical allyl transfer, both of which demonstrated excellent diastereoselectivity and yield.

In addition, the efficient phosphorylation of 1′,2′-*trans* ribo-like nucleoside analogues with a free C5′ hydroxyl group resulted in the formation of 1′,2′-*trans* ribo-like nucleoside triphosphates bearing either a purine or pyrimidine nucleobase. The biological analysis of these nucleoside 5′-triphosphates is underway.

Furthermore, diastereoselective synthesis of L-1′,2′-*cis*-4′-thionucleoside analogue bearing a C3′ all-carbon quaternary center and a C2′ hydroxyl substituent was achieved by an intromolecular S1′→C4′ S_N2-like cyclization of a 1′,2′-*syn* thioaminal.

The characterization of the 43 new compounds synthesized throughout this thesis as well as the determination of the stereochemical structure is presented in the subsequent experimental section. The work from Chapter 2 contributed to an article published in the Journal of Organic Chemistry in 2019.[92]

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Experimental Section

General Comments. All reactions requiring anhydrous conditions were carried out under an atmosphere of nitrogen in flame-dried glassware using standard syringe techniques. All anhydrous solvents were dried with 4 Å molecular sieves (1–2 mm beads) prior to use. The 4 Å molecular sieves were activated by heating in a sand bath at 180 °C for 48 hours under vacuum prior to adding to new bottles of solvent purged with nitrogen. Commercially available reagents were used as received.

Flash chromatography[1] was performed on silica gel 60 (0.040 – 0.063 mm) using forced flow of the indicated solvent system or an automated flash purification system. Analytical thin-layer chromatography (TLC) was carried out on pre-coated (0.25 mm) silica gel aluminum plates. Visualization was performed with U.V. short wavelength and/or revealed with ammonium molybdate or potassium permanganate solutions.

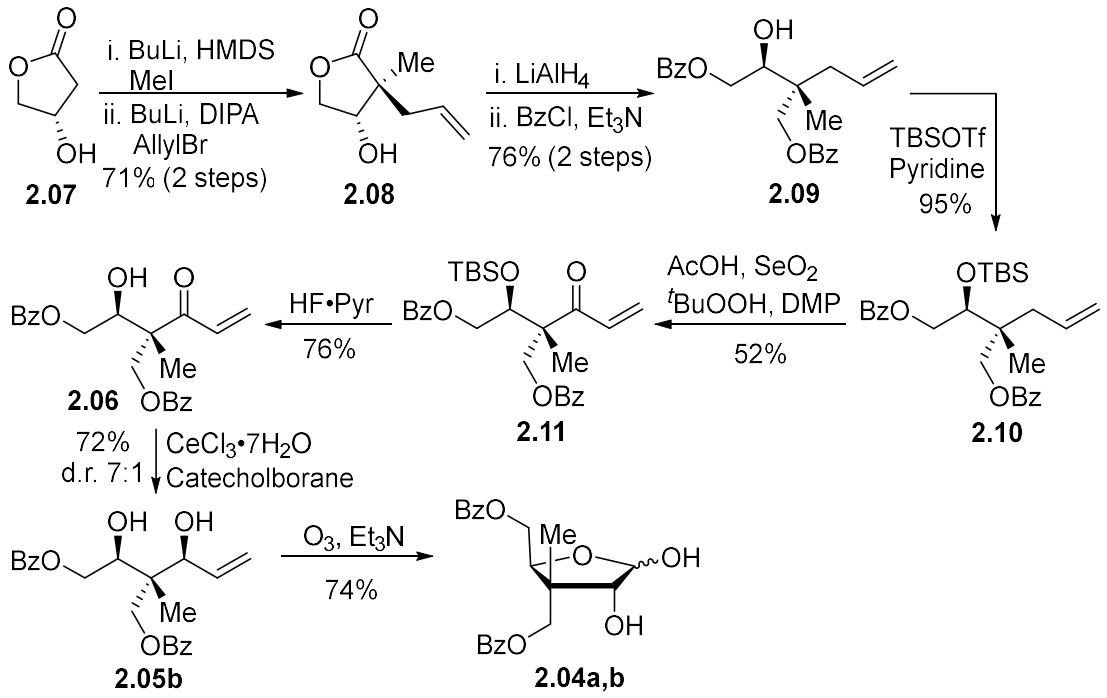
^{1}H NMR spectra were recorded at room temperature on a 500 MHz NMR spectrometer. The data are reported with chemical shifts in ppm that are referenced to residual solvent ($CDCl_3$ δ 7.26 ppm, CD_3OD δ 3.31 ppm, D_2O δ 4.79 ppm) with multiplicity (s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, t = triplet, td = triplet of doublets, m = multiplet, br = broad, app = apparent), coupling constants (Hz), and integration. ^{13}C NMR spectra were recorded at room temperature using 126 MHz. The data are reported with chemical shifts in ppm referenced to residual solvent ($CDCl_3$ δ 77.16 ppm, CD_3OD δ 49.00 ppm).

Infrared spectra were recorded on a Fourier transform infrared spectrophotometer from a thin film of purified product or with single reflection diamond attenuated total reflection module and signals are reported in cm^{-1}. Mass spectra were recorded through electrospray ionization positive ion mode. A Hybrid Quadrupole Orbitrap mass analyzer was used for high-resolution mass spectrometry (HRMS) measurements. Optical rotations were measured at room temperature from the sodium D line (589 nm) using CH_2Cl_2 as solvent unless otherwise noted and calculated using the formula: $[\alpha]_D = (100)\alpha_{obs}//(\ell \cdot (c))$, where c = (g of substrate/100 mL of solvent) and ℓ = 1 dm.

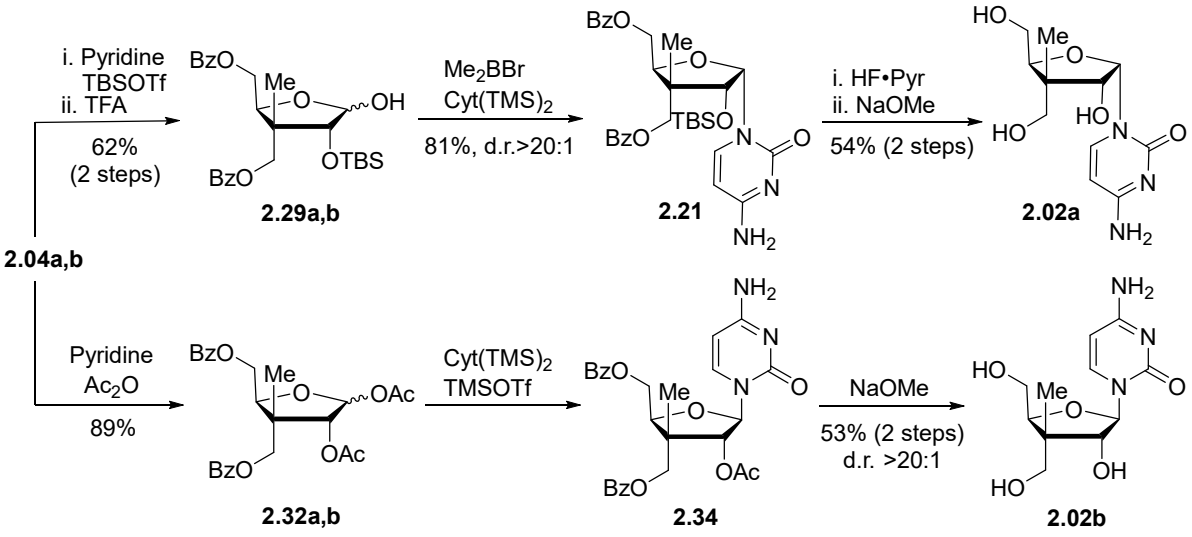
The purities of the final nucleoside triphosphates **LCB-2330**, **LCB-2331** and **LCB-2332** were examined by high-performance liquid chromatography (HPLC). HPLC conditions to assess purity were as follows: Thermo Fisher Scientific 50 × 2.1 mm HyPURITY C18 column; 0-100% gradient of methanol in water/methanol (95:5, 0.1% formic acid); flow rate, 0.5 mL/min; acquisition time, 5 min; wavelength, UV 273 nm.

Experimental Procedures: Chapter 2

1′,2′-*cis* and *trans* Ribo-like nucleoside analogues **2.02a** (C1′-α) and **2.02b** (C1′-β) were synthesized according to Schemes S1 and S2.

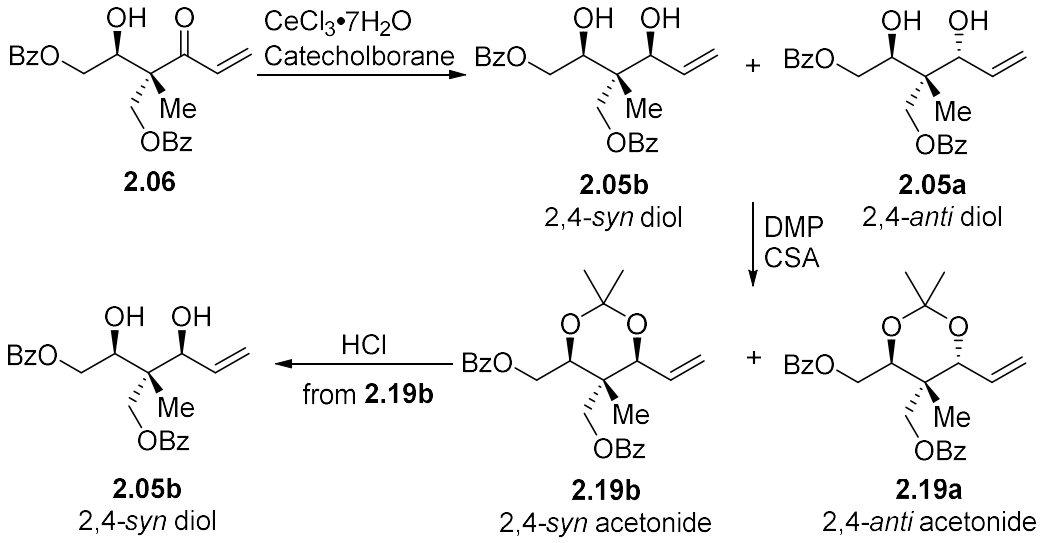


Scheme S1. Preparation of lactols **2.04a,b**.



Scheme S2. Preparation of nucleoside analogues **2.02a** and **2.02b**.

(−)-(2*S*,3*S*)-3-Hydroxy-2-((*S*)-1-hydroxyallyl)-2-methylbutane-1,4-diyl dibenzoate (2.05b)

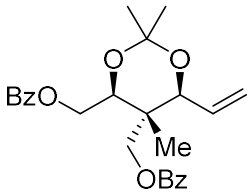


To a stirred solution of *β*-hydroxy ketone **2.06** (Tommy Lussier's MSc thesis, 2019[2]) (68 mg, 0.18 mmol, 1.0 equiv.) in anhydrous THF (0.9 mL, 0.2 M), $CeCl_3$•$7H_2O$ (66 mg, 0.18 mmol, 1.0 equiv.) was added. The resulting heterogeneous mixture was stirred vigorously for 10 minutes at room temperature, cooled to −10 °C and treated with catecholborane (0.9 mL, 0.9 mmol, 5 equiv., 1 M in THF). The resulting mixture was stirred for 4 hours at −10 °C. Then the reaction was quenched by addition of anhydrous methanol (0.7 mL) and saturated aqueous sodium potassium tartrate (0.7 mL). The resulting mixture was stirred vigorously for 2 hours at room temperature and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (5 mL), washed with water (5 mL) and the aqueous phases were extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product (7:1 *d.r.*) was purified by flash chromatography on silica gel (Hexanes/EtOAc, 75:25) to provide a mixture of 2,4-*syn* and 2,4-*anti* diols **2.05b,a** (49 mg, 72%) as a colorless oil.

To a solution of 2,4-*syn* and 2,4-*anti* diols **2.05b,a** (7:1 *d.r.*, 95 mg, 0.25 mmol, 1.0 equiv.) in acetone (2.5 mL, 0.10 M), dimethoxypropane (150 µL, 1.25 mmol, 5.00 equiv.) and (1*R*)-(-)-10-CSA (0.1 mL, 0.005 mmol, 0.02 equiv. 0.05 M in acetone) were successively added at room temperature. The resulting mixture was stirred for 1 hour and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel

(DCM/EtOAc, 99:1) to provide the pure 2,4-*syn* acetonide **2.19b** (75 mg, 72%) as a white foam and 2,4-*anti* acetonide **2.19a** (12 mg, 11%) as a colorless oil.

(+)-((4*S*,5*S*,6*S*)-2,2,5-Trimethyl-6-vinyl-1,3-dioxane-4,5-diyl) *bis*(methylene) dibenzoate (2.19b)



R_f = 0.38 (CH_2Cl_2/EtOAc, 98:2);

$[\alpha]^{25}_D$ +8.6 (*c* 0.9, CH_2Cl_2);

Formula: $C_{25}H_{28}O_6$; **MW:** 424.49 g/mol;

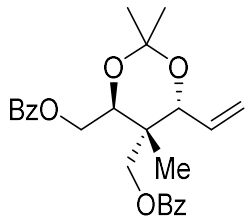
IR (neat) ν_{max} 2990, 2939, 1720 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 – 7.97 (m, 2H), 7.94 – 7.91 (m, 2H), 7.59 – 7.54 (m, 1H), 7.52 – 7.47 (m, 1H), 7.47 – 7.42 (m, 2H), 7.36 – 7.31 (m, 2H), 5.85 (ddd, *J* = 17.4, 10.7, 6.9 Hz, 1H), 5.33 – 5.27 (m, 2H), 4.62 (br d, *J* = 6.9 Hz, 1H), 4.60 – 4.55 (m, 2H), 4.35 (td, *J* = 4.5, 9.0 Hz, 1H), 4.28 (d, *J* = 12.1 Hz, 1H), 4.22 (d, *J* = 12.1 Hz, 1H), 1.58 (s, 3H), 1.50 (s, 3H), 1.06 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5, 166.0, 133.3, 133.2, 133.1, 130.0, 129.9, 129.7, 129.6, 128.7, 128.4, 119.6, 99.2, 75.2, 71.7, 66.2, 64.5, 39.0, 29.9, 19.6, 10.0 ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{25}H_{28}O_6Na^+$: 447.1778; found 447.1776 (–0.5 ppm).

(–)-((4*S*,5*S*,6*R*)-2,2,5-Trimethyl-6-vinyl-1,3-dioxane-4,5-diyl)bis(methylene) dibenzoate (2.19a)



R_f = 0.62 (CH_2Cl_2/EtOAc, 98:2);

[α]$^{25}_{D}$ −5.8 (*c* 0.5, CH_2Cl_2);

Formula: $C_{25}H_{28}O_6$; **MW:** 424.49 g/mol;

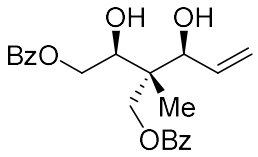
IR (neat) ν_{max} 2986, 1720 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 – 8.06 (m, 2H), 8.04 – 8.00 (m, 2H), 7.58 – 7.53 (m, 2H), 7.45 – 7.40 (m, 4H), 5.91 (ddd, *J* = 17.5, 10.5, 7.0 Hz, 1H), 5.39 – 5.34 (m, 1H), 5.29 – 5.25 (m, 1H), 4.58 (dd, *J* = 11.5, 3.0 Hz, 1H), 4.38 (dd, *J* = 11.5, 9.0 Hz, 1H), 4.29 – 4.26 (m, 1H), 4.27 (d, *J* = 11.5 Hz, 1H), 4.22 (d, *J* = 11.5 Hz, 1H), 4.10 (d, *J* = 7.0 Hz, 1H), 1.49 (s, 3H), 1.43 (s, 3H), 1.07 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.6, 166.5, 133.2, 133.1, 132.84, 132.81, 130.14, 130.07, 129.81, 129.75, 128.6, 128.5, 118.4, 101.8, 70.7, 67.5, 64.5, 43.0, 24.2, 23.9, 14.9 ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{25}H_{28}O_6Na^+$: 447.1778; found 447.1775 (–0.8 ppm).

(−)-(2*S*,3*S*)-3-Hydroxy-2-((*S*)-1-hydroxyallyl)-2-methylbutane-1,4-diyl dibenzoate (2.05b)



To a solution of 2,4-*syn* acetonide **2.19b** (296 mg, 697 μmol, 1.00 equiv.) in THF (7 mL, 0.1 M), 2M HCl (1.7 mL, 3.5 mmol, 5.0 equiv.) was added at room temperature.[3] The resulting reaction mixture was heated to 50 °C and stirred for 16 hours. The reaction was cooled to room temperature and quenched with saturated aqueous $NaHCO_3$ (10 mL). The mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography on silica gel (Hexanes/EtOAc, 70:30) to give 2,4-*syn* diol **2.05b** (240 mg, 90%) as a colorless oil.

R_f = 0.57 (Hexanes/EtOAc, 60:40);

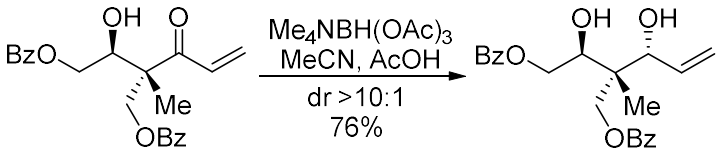
[α]$^{25}_{D}$ −0.7 (*c* 1.3, CH_2Cl_2);

Formula: $C_{22}H_{24}O_6$; **MW:** 384.43 g/mol;

IR (neat) ν_{max} 3482, 3067, 2977, 1718 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 – 7.99 (m, 4H), 7.60 – 7.52 (m, 2H), 7.47 – 7.38 (m, 4H), 6.02 (ddd, *J* = 17.5, 10.5, 7.0 Hz, 1H), 5.36 (dt, *J* = 17.0, 1.5 Hz, 1H), 5.28 (dt, *J* = 10.5, 1.5 Hz, 1H), 4.70 (dd, *J* = 11.5, 2.5 Hz, 1H), 4.55 – 4.52 (m, 1H), 4.52 (dd, *J* = 11.7, 8.0 Hz, 1H), 4.37 – 4.31 (m, 3H), 3.24 (d, *J* = 3.5 Hz, 1H), 2.79 (d, *J* = 3.5 Hz, 1H), 1.14 (s, 3H) ppm;
^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2, 166.5, 136.4, 133.38, 133.35, 129.90, 129.89, 129.8, 129.7, 128.7, 128.6, 118.3, 76.5, 74.5, 67.4, 67.2, 44.6, 13.2 ppm;
HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{22}H_{24}O_6Na^+$: 407.1465; found 407.1453 (–2.9 ppm).

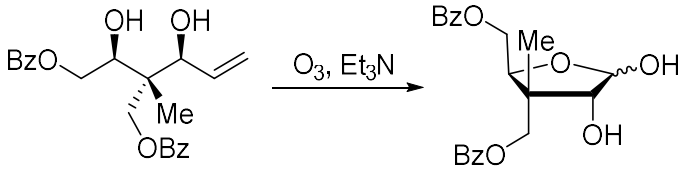
(+)-(2*S*,3*S*)-3-Hydroxy-2-((*R*)-1-hydroxyallyl)-2-methylbutane-1,4-diyl dibenzoate (2.05a)



2.06 **2.05a**

2,4-*anti* diol **2.05a** was previously characterized by Tommy Lussier, MSc 2019[2].

((2*S*,3*S*,4*R*)-4,5-Dihydroxy-3-methyltetrahydrofuran-2,3-diyl)bis(methylene) dibenzoate (2.04a,b)



2.05b **2.04a,b**

The 2,4-*syn* diol **2.05b** (195 mg, 507 μmol, 1.00 equiv.) was dissolved in anhydrous DCM (15 mL, 0.030 M) and the mixture was cooled to −78 °C. Ozone was bubbled through the reaction mixture until a slight blue color appeared at which point the ozone inlet was changed for a N_2 inlet and bubbling was continued for 15 minutes. Triethylamine (212 μL, 1.52 mmol, 3.00 equiv.) was added and the mixture was stirred for 30 minutes at −78 °C followed by warming to room temperature and stirring for 1 hour. The reaction mixture was filtered over $MgSO_4$ and condensed under reduced pressure. The crude was purified by flash chromatography on silica gel (Hexanes/EtOAc, 60:40) to afford lactols **2.04a,b** (144 mg, 74%, *α*/*β* = 1:2) as a white foam.

R_f = 0.40 (Hexanes/ EtOAc, 40:60);

Formula: $C_{21}H_{22}O_7$; **MW:** 386.40 g/mol;

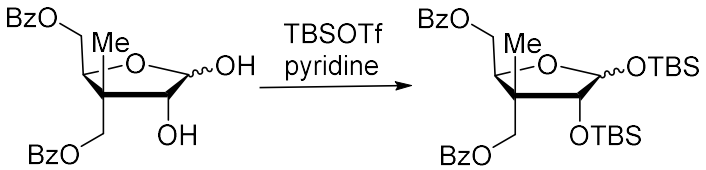
IR (neat) ν_{max} 3434, 2970, 2949, 2897, 1715 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 – 8.03 (m, 8H, major and minor), 7.63 – 7.55 (m, 4H, major and minor), 7.50 – 7.42 (m, 8H, major and minor), 5.57 (dd, *J* = 9.0, 4.0 Hz, 1H, major), 5.39 (s, 1H, minor), 4.86 (d, *J* = 11.5 Hz, 1H, major), 4.76 (d, *J* = 11.5 Hz, 1H, minor), 4.57 – 4.49 (m, 2H, major), 4.47 – 4.43 (m, 1H, major), 4.42 – 4.36 (m, 3H, major and minor), 4.24 (d, *J* = 11.0 Hz, 1H, major), 4.22 (d, *J* = 11.5 Hz, 1H, minor), 4.18 (d, *J* = 9.0 Hz, 1H, major), 4.03 (d, *J* = 4.0 Hz, 1H, major), 3.97 (br s, 1H, minor), 3.85 (t, *J* = 4.0 Hz, 1H, major), 3.31 (br d, *J* = 3.0 Hz, 1H, minor), 3.01 (s, 1H, minor), 1.35 (s, 3H, minor), 1.17 (s, 3H, major) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 167.5 (major), 167.3 (minor), 166.6 (minor), 166.5 (major), 133.7 (major), 133.6 (minor), 133.4 (2C, major and minor), 129.89 (2C, major), 129.87 (2C, major), 129.86 (2C, major), 129.8 (minor), 129.5 (minor), 128.74 (major) 128.72 (minor), 128.61 (minor), 128.58 (major), 104.1 (minor), 97.1 (major), 83.0 (minor), 81.0 (minor), 78.3 (major), 77.3 (major), 66.8 (minor), 66.5 (major), 65.3 (minor), 64.4 (major), 48.8 (major), 47.6 (minor), 16.0 (minor), 15.8 (major) ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{21}H_{22}O_7Na^+$: 409.1258; found 409.1272 (+3.6 ppm).

((2*S*,3*R*,4*R*)-4,5-Bis((*tert*-butyldimethylsilyl)oxy)-3-methyltetrahydrofuran-2,3-diyl)bis(methylene) dibenzoate (2.20a,b)



2.04a,b **2.20a,b**

To a solution of lactols **2.04a,b** (77 mg, 0.20 mmol, 1.0 equiv.) in anhydrous DCM (2 mL, 0.1 M), pyridine (322 µL, 4.00 mmol, 20.0 equiv.) was added. After cooling the mixture to 0 °C, TBSOTf (137 µL, 600 µmol, 3.00 equiv.) was added. The resulting mixture was stirred for 16 hours at room temperature. A saturated aqueous solution of NH_4Cl (4 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and condensed under reduced pressure.

Purification by flash chromatography on silica gel (Hexanes/EtOAc, 90:10) provided bis-silylated ethers **2.20a,b** (99 mg, 81%, α/β = 1:2) as a colorless oil.

$\mathbf{R_f}$ = 0.60 (Hexanes/EtOAc, 90:10);

Formula: $C_{33}H_{50}O_7Si_2$; **MW:** 614.93 g/mol;

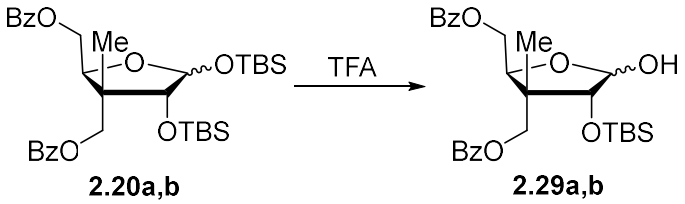
IR (neat) ν_{max} 2955, 2930, 2887, 2857, 1722 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 – 8.08 (m, 2H, minor), 8.06 – 8.01 (m, 6H, major and minor), 7.58 – 7.52 (m, 4H, major), 7.47 – 7.38 (m, 8H, major), 5.26 (d, *J* = 4.0 Hz, 1H, major), 5.23 (d, *J* = 1.5 Hz, 1H, minor), 4.76 (d, *J* = 11.5 Hz, 1H, major), 4.58 – 4.50 (m, 4H, minor and major), 4.46 – 4.32 (m, 5H, minor and major), 4.03 (d, *J* = 1.5 Hz, 1H, minor), 3.94 (d, *J* = 4.0 Hz, 1H, major), 1.31 (s, 3H, minor), 1.29 (s, 3H, major), 0.94 (s, 9H, major), 0.93 (s, 9H, major), 0.892 (s, 9H, minor), 0.886 (s, 9H, minor), 0.16 (s, 3H, major), 0.13 (s, 3H, major), 0.11 (s, 9H, minor and major), 0.10 (s, 3H, minor), 0.09 (s, 3H, major), 0.05 (s, 3H, minor) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.62 (major), 166.58 (minor), 166.54 (minor), 166.50 (major), 133.18 (minor), 133.17 (major), 133.1 (minor), 133.0 (major), 130.5 (minor), 130.3 (minor), 130.13 (major), 130.09 (minor), 129.9 (minor), 129.8 (major), 129.68 (minor), 129.65 (major), 128.6 (2C, major and minor), 128.52 (minor), 128.49 (major), 104.7 (minor), 96.3 (major), 85.7 (minor), 81.5 (minor), 81.4 (major), 79.5 (major), 70.6 (major), 67.8 (minor), 65.6 (minor), 65.4 (major), 47.2 (minor), 44.3 (major), 26.0 (major), 25.92 (major), 25.88 (minor), 25.8 (minor), 18.6 (major), 18.23 (major), 18.15 (minor), 18.1 (major), 18.0 (minor), 16.4 (minor), −4.0 (minor), −4.1 (major), −4.19 (major), −4.21 (minor), −4.8 (minor), −4.9 (major), −5.0 (major), −5.1 (minor) ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{33}H_{50}O_7Si_2Na^+$: 637.2987; found 637.2978 (–1.5 ppm).

((2*S*,3*R*,4*R*)-4-((*tert*-Butyldimethylsilyl)oxy)-5-hydroxy-3-methyltetrahydrofuran-2,3-diyl)bis(methylene) dibenzoate (2.29a,b)



2.20a,b **2.29a,b**

To a solution of bis-silylated ethers **2.20a,b** (40 mg, 65 μmol, 1.0 equiv.) in chloroform (1.3 mL, 0.050 M), TFA (0.65 mL, 0.10 M) was added at room temperature. The resulting mixture was stirred for 20 minutes. Then the mixture was added dropwise to a solution of ammonium hydroxide (1.5 mL) and methanol (5 mL) at −20 °C and stirred for 5 minutes followed by warming to room temperature and stirring for another 5 minutes. The mixture was poured into a solution of water (10 mL) and chloroform (10 mL), and then extracted with DCM (3 × 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Purification by flash chromatography on silica gel (Hexanes/EtOAc, 50:50) provided the C2-TBS protected lactols **2.29a,b** (25 mg, 77%, α/β = 1:2) as a colorless oil.

R_f = 0.39 (Hexanes/EtOAc, 80:20);

Formula: $C_{27}H_{36}O_7Si$; **MW:** 500.66 g/mol;

IR (neat) ν_{max} 3475, 3435, 2954, 2930, 2888, 2857, 1721 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 – 8.02 (m, 8H, major and minor), 7.60 – 7.54 (m, 4H, major and minor), 7.47 – 7.40 (m, 8H, major and minor), 5.48 (dd, *J* = 10.0, 4.5 Hz, 1H, minor), 5.29 – 5.27 (m, 1H, major), 4.63 – 4.37 (m, 10H, major and minor), 4.09 (d, *J* = 2.0 Hz, 1H, major), 4.03 (d, *J* = 4.0 Hz, 1H, minor), 3.72 (d, *J* = 9.5 Hz, 1H, minor), 2.90 (d, *J* = 4.0 Hz, 1H, major), 1.33 (s, 3H, major), 1.22 (s, 3H, minor), 0.95 (s, 9H, minor), 0.90 (s, 9H, major), 0.14 (s, 6H, minor), 0.12 (s, 3H, major), 0.08 (s, 3H, major) ppm;

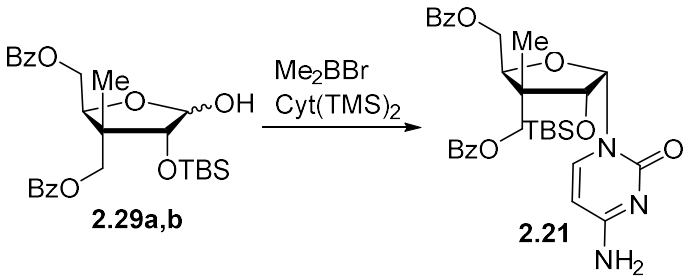
^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7 (major), 166.6 (major), 166.52 (minor), 166.49 (minor), 133.4 (minor), 133.30 (major), 133.27 (minor), 133.2 (major), 130.2 (minor), 130.0 (major), 129.9 (4C, major and minor), 129.67 (major), 129.65 (minor), 128.7 (minor), 128.62 (major), 128.57 (major), 128.5 (minor), 104.4 (major), 96.9 (minor), 84.7 (minor), 81.7 (major), 79.9

(minor), 78.6 (major), 67.7 (major), 67.1 (minor), 65.6 (major), 64.8 (minor), 47.33 (major), 47.28 (minor), 25.91 (minor), 25.85 (major), 18.3 (minor), 18.2 (major), 16.9 (minor), 16.5 (major), −4.4 (major), −4.6 (minor), −4.7 (minor), −5.0 (major) ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{27}H_{36}O_7SiNa^+$: 523.2123; found 523.2129 (+1.3 ppm).

Preparation of silylated cytosine[4]: To a suspension of cytosine (1.0 g, 9.0 mmol, 1.0 equiv.) in HMDS (4.72 mL, 22.5 mmol, 2.50 equiv.) under nitrogen atmosphere, $(NH_4)_2SO_4$ (12 mg, 0.090 mmol, 0.010 equiv.) was added. The reaction mixture was heated at reflux until a clear solution was obtained (~1 hour). Upon cooling to room temperature, the solution was placed under high vacuum overnight to remove excess HMDS. A 0.4 M solution of the silylated cytosine ($Cyt(TMS)_2$) was prepared in anhydrous DCE (22 mL).

(−)-((2*S*,3*R*,4*R*,5*S*)-5-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-4-((tert-butyldimethylsilyl) oxy)-3-methyltetrahydrofuran-2,3-diyl)bis(methylene) dibenzoate (2.21)



To a solution of lactols **2.29a,b** (45 mg, 90 μmol, 1.0 equiv.) in anhydrous DCM (0.60 mL, 0.15 M), Me_2BBr (0.12 mL, 0.18 mmol, 2.0 equiv., 1.5 M in DCM) was added at 0 °C. The resulting mixture was slowly warmed to room temperature and stirred for 80 minutes for full activation of the starting material. The freshly prepared silylated cytosine (0.45 mL, 0.18 mmol, 2.0 equiv. 0.40 M in DCE) was added to the reaction mixture which was heated to 35 °C and stirred for 3 hours. The mixture was cooled to room temperature and quenched with a few drops of methanol and triethylamine. The resulting mixture was concentrated under reduced pressure and the crude (>20:1 d.r.) was purified by flash chromatography on silica gel (DCM/MeOH, 95:5) to provide 1′,2′-*cis* ribo-like α-anomer **2.21** (43 mg, 81%) as a white foam.

R_f = 0.74 (DCM/MeOH, 90:10);

$[\alpha]^{25}_D$ –39 (*c* 0.5, CH_2Cl_2);

Formula: $C_{31}H_{39}N_3O_7Si$; **MW:** 593.75 g/mol;

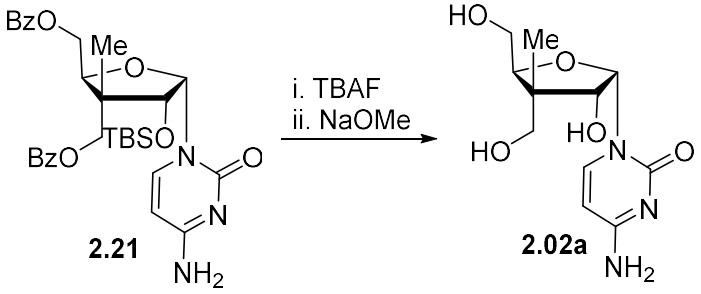
IR (neat) ν_{max} 3306, 3123, 2952, 2928, 2857, 1718 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 – 8.01 (m, 4H), 7.60 – 7.55 (m, 2H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.48 – 7.43 (m, 4H), 6.48 (d, *J* = 3.0 Hz, 1H), 5.72 (d, *J* = 7.5 Hz, 1H), 4.69 (dd, *J* = 11.5, 3.5 Hz, 1H), 4.57 (dd, *J* = 8.0, 3.5 Hz, 1H), 4.51 – 4.45 (m, 3H), 4.36 (d, *J* = 2.5 Hz, 1H), 1.33 (s, 3H), 0.86 (s, 9H), 0.02 (s, 3H), –0.23 (s, 3H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.42, 166.41, 165.4, 155.8, 144.0, 133.5, 133.4, 129.9 (2C), 129.8, 129.7, 128.7, 128.6, 93.0, 87.9, 81.2, 77.9, 66.2, 64.7, 49.7, 26.4, 18.1, 16.1, –4.98, –5.01 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{31}H_{40}N_3O_7Si^+$: 594.2630; found 594.2632 (+0.4 ppm).

(–)-4-Amino-1-((2*S*,3*R*,4*S*,5*S*)-3-hydroxy-4,5-bis(hydroxymethyl)-4-methyltetrahydro furan-2-yl)pyrimidin-2(1*H*)-one (2.02a)



To a solution of protected α-anomer **2.21** (43 mg, 72 μmol, 1.0 equiv.) in anhydrous THF (0.45 mL, 0.16 M), TBAF (0.29 mL, 0.29 mmol, 4.0 equiv., 1.0 M in THF) was added at 0 °C. The resulting mixture was stirred for 18 hours at room temperature. Saturated aqueous NH_4Cl (~3 mL) was added and the mixture was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure.

To a solution of crude bis-benzoate protected nucleoside in anhydrous MeOH (0.36 mL, 0.20 M), NaOMe (17 μL, 72 μmol, 1.0 equiv., 4.4 M in MeOH) was added at room

temperature. The resulting mixture was stirred for 3 hours. Amberlite acidic resin (~100 mg) was added and the mixture was stirred for 10 minutes, filtered, and concentrated under reduced pressure to provide the crude nucleoside. The residue was purified by C18 reverse phase flash chromatography (MeOH/H_2O) to give 1′,2′-*cis* ribo like nucleoside analogue **2.02a** (11 mg, 54% over two steps) as a white foam.

[α]$^{25}_D$ –53 (*c* 0.08, CH_3OH);

Formula: $C_{11}H_{17}N_3O_5$; **MW:** 271.27 g/mol;

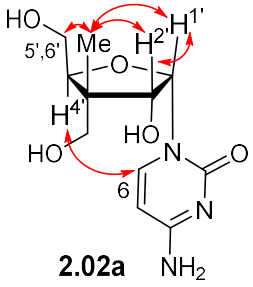
IR (neat) ν_{max} 3341, 3211, 2927, 1647, 1601 cm^{-1};

^{1}H NMR (500 MHz, CD_3OD) δ 7.65 (d, *J* = 7.5 Hz, 1H), 6.18 (d, *J* = 3.0 Hz, 1H), 5.87 (d, *J* = 7.5 Hz, 1H), 4.28 (t, *J* = 6.0 Hz, 1H), 4.09 (d, *J* = 3.0 Hz, 1H), 3.74 (d, *J* = 11.1 Hz, 1H), 3.69 (d, *J* = 6.0 Hz, 2H), 3.67 (d, *J* = 11.1 Hz, 1H), 1.08 (s, 3H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, CD_3OD) δ 167.8, 158.4, 144.4, 94.4, 89.4, 85.6, 78.8, 65.2, 62.5, 50.8, 15.6 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{11}H_{18}O_5N_3^+$: 272.1241; found 272.1242 (+0.3 ppm).

The relative configuration of 1′,2′-*cis* ribo-like nucleoside analogue C1′-α (**2.02a**) was determined by relevant nuclear Overhauser effect (nOe) enhancements (2D NOESY). The peaks in the ^{1}H NMR spectra were assigned using ^{1}H/^{1}H 2D COSY experiment, chemical shifts and coupling constants.

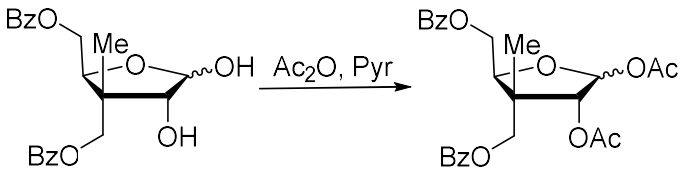


2.02a

From 2D-NOESY

$H^{1'}$ (6.18 ppm) ⟷ $H^{2'}$ (4.09 ppm)
$H^{1'}$ (6.18 ppm) ⟷ H^{Me} (1.08 ppm)
$H^{2'}$ (4.09 ppm) ⟷ H^{Me} (1.08 ppm)
$H^{4'}$ (4.28 ppm) ⟷ H^{6} (7.65 ppm)
$H^{5'}$ (3.69 ppm) ⟷ H^{Me} (1.08 ppm)
$H^{6'}$ (3.69 ppm) ⟷ H^{Me} (1.08 ppm)

((2*S*,3*R*,4*R*)-4,5-Diacetoxy-3-methyltetrahydrofuran-2,3-diyl)bis(methylene) dibenzoate (2.32a,b)



2.04a,b **2.32a,b**

To a solution of lactols **2.04a,b** (74.0 mg, 192 μmol, 1.00 equiv.) in anhydrous pyridine (1 mL, 0.2 M), AcOH (91.0 μL, 958 μmol, 5.00 equiv.) was added at 0 °C. The mixture was warmed to room temperature and stirred for 16 hours. The mixture was then concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (Hexanes/EtOAc, 80:20) to provide bis-acetated lactols **2.32a,b** (80 mg, 89%, α/β = 1:1.2) as a colorless gum.

R_f = 0.49 (Hexanes/EtOAc, 70:30);

Formula: $C_{25}H_{26}O_9$; **MW:** 470.47 g/mol;

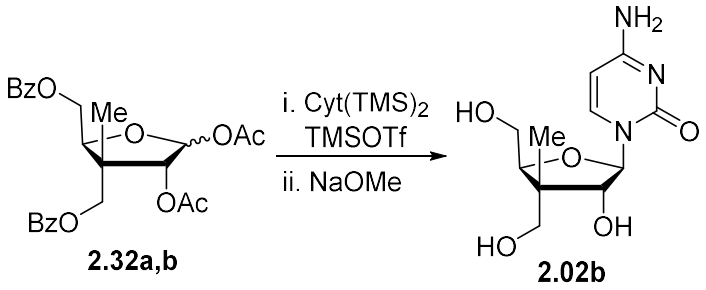
IR (neat) ν_{max} 2978, 2926, 1749, 1720 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 – 8.00 (m, 8H, major and minor), 7.60 – 7.55 (m, 4H, major and minor), 7.48 – 7.42 (m, 8H, major and minor), 6.50 (d, *J* = 5.0 Hz, 1H, minor), 6.13 (d, *J* = 1.0 Hz, 1H, major), 5.37 (d, *J* = 1.0 Hz, 1H, major), 5.29 (d, *J* = 4.5 Hz, 1H, minor), 4.67 – 4.52 (m, 5H, major and minor), 4.51 – 4.38 (m, 5H, major and minor), 2.08 (s, 6H, minor and minor), 2.07 (s, 3H, major), 2.06 (s, 3H, major), 1.43 (s, 3H, major), 1.36 (s, 3H, minor) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 169.8 (minor), 169.6 (major), 169.5 (minor), 169.4 (major), 166.34 (major), 166.28 (major), 166.27 (major), 166.2 (major), 133.49 (major), 133.46 (minor), 133.45 (minor), 133.4 (major), 129.89 (major), 129.85 (major), 129.8 (minor), 129.72 (2C, major and minor), 129.68 (major), 129.66 (minor), 129.6 (minor), 128.72 (major), 128.71 (minor), 128.65 (minor), 128.6 (major), 100.2 (major), 94.2 (major), 83.1 (major), 82.5 (major), 80.8 (major), 78.3 (major), 66.9 (minor), 66.5 (major), 64.3 (major), 64.0 (minor), 46.4 (major), 45.3 (minor), 21.2 (major), 21.1 (minor), 20.8 (major), 20.5 (minor), 17.6 (minor), 16.3 (major) ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{25}H_{26}O_9Na^+$: 493.1469; found 493.1470 (+0.3 ppm).

(+)-4-Amino-1-((2*R*,3*R*,4*S*,5*S*)-3-hydroxy-4,5-bis(hydroxymethyl)-4-methyltetrahydro furan-2-yl)pyrimidin-2(1*H*)-one (2.02b)



2.32a,b **2.02b**

To a solution of anomeric acetates **2.32a,b** (50.0 mg, 106 μmol, 1.00 equiv.) in anhydrous MeCN (0.5 mL, 0.2 M), freshly prepared silylated cytosine (0.43 mL, 0.17 mmol, 1.6 equiv. 0.40 M in DCE) and TMSOTf (77.0 μL, 424 μmol, 4.00 equiv.) were added. The resulting reaction mixture was stirred at 60 °C for 5 hours. The reaction was cooled to room temperature, quenched with saturated aqueous $NaHCO_3$ (1 mL) and extracted with EtOAc (3 × 8 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure.

To a solution of the crude in anhydrous MeOH (0.5 mL, 0.2 M), NaOMe (24 μL, 98 μmol, 1.0 equiv., 4.4 M in MeOH) was added at room temperature. The reaction was stirred for 3 hours and was quenched with amberlite acidic resin (~100 mg) and stirred for 10 minutes followed by filtration with MeOH (~6 mL) and concentration under reduced pressure. The crude nucleoside analogue (>20:1 *d.r.*) was purified by C18 reverse phase flash chromatography ($MeOH/H_2O$) to give 1′,2′-*trans* ribo-like β-anomer **2.02b** (14 mg, 53% over two steps) as a white foam.

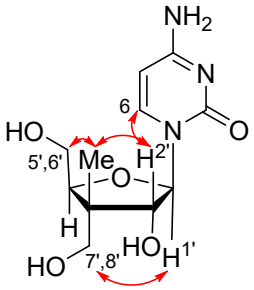
$[\alpha]^{25}_D$ +28 (*c* 0.4, CH_3OH);
Formula: $C_{11}H_{17}N_3O_5$; **MW:** 271.27 g/mol;
IR (neat) ν_{max} 3345, 3217, 2967, 2949, 1649, 1607 cm^{-1};
^{1}H NMR (500 MHz, CD_3OD) δ 8.03 (d, *J* = 7.5 Hz, 1H), 5.91 (d, *J* = 7.5 Hz, 1H), 5.83 (d, *J* = 5.5 Hz, 1H), 4.18 (dd, *J* = 4.5, 3.5 Hz, 1H), 4.11 (d, *J* = 5.5 Hz, 1H), 3.78 (dd, *J* = 11.5, 3.5

Hz, 1H), 3.70 (d, *J* = 11.1 Hz, 1H), 3.67 (dd, *J* = 11.8, 4.7 Hz, 1H), 3.63 (d, *J* = 11.1 Hz, 1H), 1.09 (s, 3H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, CD_3OD) δ 167.7, 159.1, 143.1, 95.9, 92.8, 85.5, 83.1, 66.3, 63.1, 48.4, 16.6 ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{11}H_{17}O_5N_3Na^+$: 294.1060; found 294.1063 (+0.8 ppm).

The relative configuration of 1′,2′-*trans* ribo-like nucleoside analogue C1′-β (**2.02b**) was determined by relevant nuclear Overhauser effect (nOe) enhancements (2D NOESY). The peaks in the ^{1}H NMR spectra were assigned using ^{1}H/^{1}H 2D COSY experiment, chemical shifts and coupling constants.



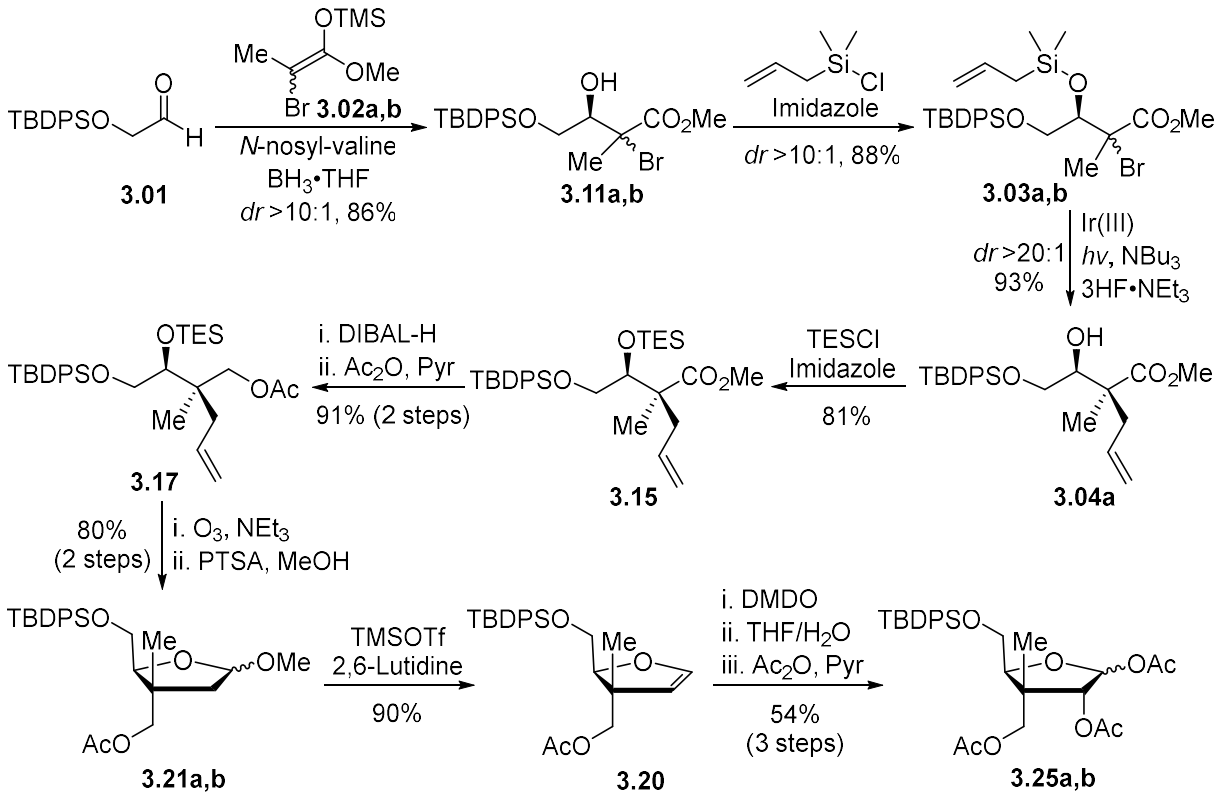
2.02b

From 2D-NOESY

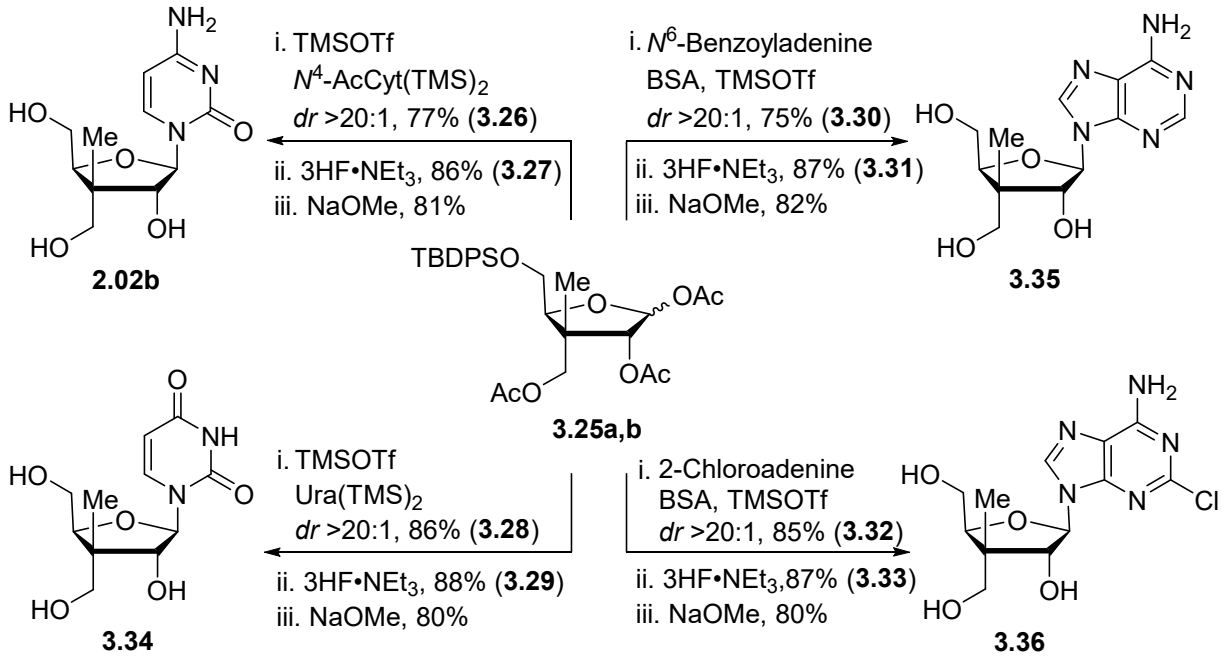
$H^{1'}$ (5.83 ppm) ⟷ $H^{7'}$ (3.70 ppm)
$H^{1'}$ (5.83 ppm) ⟷ $H^{8'}$ (3.63 ppm)
$H^{2'}$ (4.11 ppm) ⟷ H^{6} (8.03 ppm)
$H^{2'}$ (4.11 ppm) ⟷ H^{Me} (1.09 ppm)
$H^{5'}$ (3.78 ppm) ⟷ H^{Me} (1.09 ppm)
$H^{6'}$ (3.67 ppm) ⟷ H^{Me} (1.09 ppm)

Experimental Procedures: Chapter 3

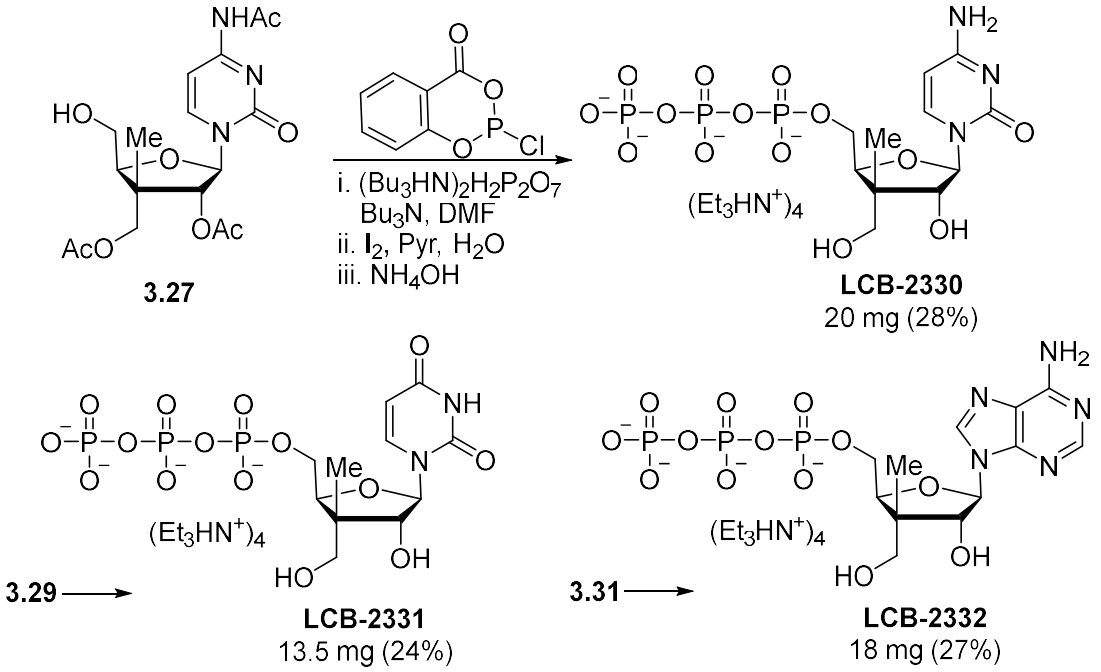
1′,2′-*trans* Ribo-like nucleoside analogues and their corresponding triphosphates were synthesized according to Schemes S3, S4 and S5.



Scheme S3. Preparation of acetates **3.25a,b**.

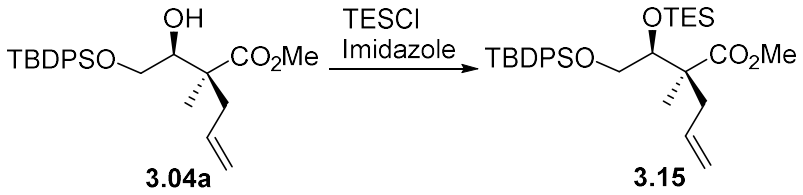


Scheme S4. Preparation of 1′,2′-*trans* ribo-like nucleoside analogues.



Scheme S5. Preparation of nucleoside C5′ triphosphates.

(–)-Methyl (*S*)-2-((*S*)-3,3-diethyl-9,9-dimethyl-8,8-diphenyl-4,7-dioxa-3,8-disiladecan-5-yl)-2-methylpent-4-enoate (3.15)



3.04a **3.15**

To a solution of secondary alcohol **3.04a**[5] (3.90 g, 9.14 mmol, 1.00 equiv.) in anhydrous DCM (45 mL, 0.20 M), imidazole (1.56g, 22.9 mmol, 2.50 equiv.) was added immediately followed by triethylchlorosilane (2.30 mL, 13.7 mmol, 1.50 equiv.). The resulting mixture was stirred at room temperature for 16 hours. The mixture was diluted with DCM (25 mL) and saturated NH_4Cl solution (25 mL). The aqueous phase was extracted with DCM (3 × 25 mL) and the combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (Hexanes/EtOAc, 97:3) provided the TES-protected methyl ester 3.15 (4.3 g, 87%) as a colorless oil.

R_f = 0.43 (Hexanes/EtOAc, 95:5);

$[\alpha]^{25}_D$ –27 (*c* 0.7, CH_2Cl_2);

Formula: $C_{31}H_{48}O_4Si_2$; **MW:** 540.82 g/mol;

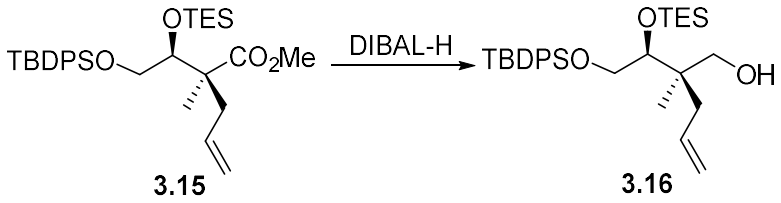
IR (neat) ν_{max} 3073, 3051, 2954, 2879, 1738 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 – 7.63 (m, 4H), 7.44 – 7.36 (m, 6H), 5.71 – 5.63 (m, 1H), 5.02 (s, 1H), 5.01 – 4.98 (m, 1H), 4.15 (t, *J* = 5.9 Hz, 1H), 3.52 (d, *J* = 5.9 Hz, 2H), 3.46 (s, 3H), 2.39 (dd, *J* = 13.6, 7.3 Hz, 1H), 2.25 (dd, *J* = 13.3, 7.7 Hz, 1H), 1.06 (s, 3H), 1.04 (s, 9H), 0.89 (t, *J* = 7.9 Hz, 9H), 0.56 (q, *J* = 8.0 Hz, 6H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 175.5, 135.83, 135.78, 134.3, 133.5, 133.3, 129.80, 129.76, 127.80, 127.75, 118.0, 77.3, 66.4, 51.5, 50.6, 42.1, 27.0, 19.3, 14.6, 7.0, 5.3 ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{31}H_{48}O_4Si_2Na^+$: 563.2983; found 563.2985 (+0.4 ppm).

(–)-(*R*)-2-((*S*)-3,3-Diethyl-9,9-dimethyl-8,8-diphenyl-4,7-dioxa-3,8-disiladecan-5-yl)-2-methylpent-4-en-1-ol (3.16)



3.15 **3.16**

To a solution of methyl ester **3.15** (4.30 g, 7.95 mmol, 1.00 equiv.) in anhydrous DCM (40 mL, 0.2 M) at –40 °C, DIBAL-H (20 mL, 20 mmol, 2.5 equiv., 1.0 M in hexanes) was added dropwise. The resulting mixture was stirred at –40 °C for 2 hours. Methanol (1.3 mL, 32 mmol, 4.0 equiv.) was added at –40 °C and the reaction was stirred for 10 minutes followed by addition of Et_2O (50 mL) and saturated Rochelle salt solution (50 mL). The solution was stirred vigorously until separation of both phases. The aqueous phase was extracted twice with Et_2O (2 × 50 mL), and the combined organic phases were washed with water, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. ^{1}H NMR analysis indicated that alcohol **3.16** (4.08 g, quant., colorless oil) was clean enough to be directly used in the next step without further purification.

R_f = 0.45 (Hexanes/EtOAc, 90:10);

$[\alpha]^{25}_D$ –7.4 (*c* 1.1, CH_2Cl_2);

Formula: $C_{30}H_{48}O_3Si_2$; **MW:** 512.88 g/mol;

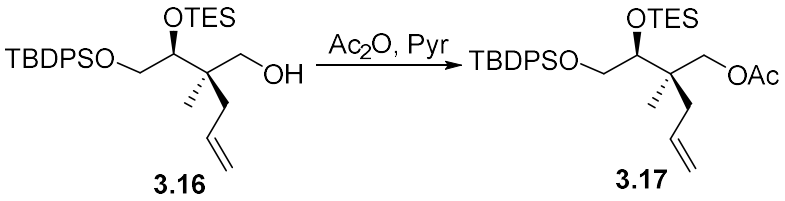
IR (neat) ν_{max} 3461 (br), 3072, 3051, 2955, 2934, 2877 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 – 7.66 (m, 4H), 7.46 – 7.39 (m, 6H), 5.83 – 5.74 (m, 1H), 5.03 – 4.97 (m, 2H), 3.78 (dd, *J* = 10.9, 5.8 Hz, 1H), 3.70 (dd, *J* = 5.5, 4.4 Hz, 1H), 3.56 (dd, *J* = 10.9, 4.3 Hz, 1H), 3.52 (dd, *J* = 11.5, 6.3 Hz, 1H), 3.44 (dd, *J* = 11.4, 6.2 Hz, 1H), 3.18 (t, *J* = 6.3 Hz, 1H), 2.14 (dd, *J* = 13.6, 7.4 Hz, 1H), 1.97 (dd, *J* = 13.6, 7.5 Hz, 1H), 1.07 (s, 9H), 0.87 (t, *J* = 8.0 Hz, 9H), 0.85 (s, 3H), 0.52 (q, *J* = 7.9 Hz, 6H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 135.90, 135.85, 134.7, 132.94, 132.87, 130.04, 130.02, 127.92, 127.91, 117.7, 79.6, 68.1, 66.0, 42.1, 39.1, 27.0, 19.2, 18.4, 7.0, 5.1 ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{30}H_{48}O_3Si_2Na^+$: 535.3034; found 535.3042 (+1.4 ppm).

(+)-(*R*)-2-((*S*)-3,3-Diethyl-9,9-dimethyl-8,8-diphenyl-4,7-dioxa-3,8-disiladecan-5-yl)-2-methylpent-4-en-1-yl acetate (3.17)



3.16 **3.17**

To a solution of primary alcohol **3.16** (2.25 g, 4.39 mmol, 1.00 equiv.), a 1:2 mixture of pyridine and Ac_2O (18 ml, 0.25 M) was added at 0 °C and the resulting mixture was stirred at room temperature for 1 h. The solution was concentrated under reduced pressure and the crude product was purified by silica gel flash chromatography (Hexanes/EtOAc, 95:5), providing the acetate product **3.17** as a colorless oil (2.21 g, 91% over two steps).

R_f = 0.65 (Hexanes/EtOAc, 90:10);

$[\alpha]^{25}_D$ +0.4 (*c* 0.6, CH_2Cl_2);

Formula: $C_{32}H_{50}O_4Si_2$; **MW:** 554.92 g/mol;

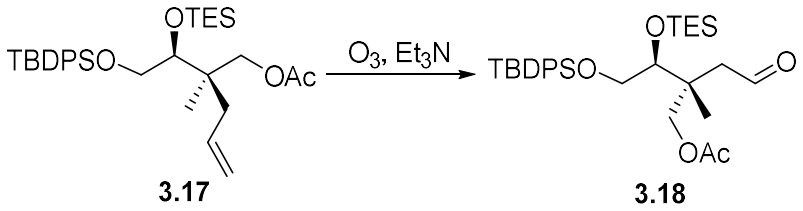
IR (neat) ν_{max} 3073, 3051, 2956, 2934, 2877, 2859, 1744 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 – 7.66 (m, 4H), 7.45 – 7.37 (m, 6H), 5.76 – 5.69 (m, 1H), 5.00 – 4.92 (m, 2H), 3.95 – 3.90 (m, 2H), 3.79 – 3.74 (m, 2H), 3.54 (dd, *J* = 10.0, 4.7Hz, 1H), 2.15 (dd, *J* = 13.9, 7.4 Hz, 1H), 2.01 (dd, *J* = 13.8, 7.6 Hz, 1H), 1.97 (s, 3H), 1.07 (s, 9H), 0.91 (t, *J* = 7.9 Hz, 9H), 0.83 (s, 3H), 0.62 – 0.54 (m, 6H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.0, 135.9, 135.8, 134.5, 133.5, 133.4, 129.9, 129.8, 127.8 (2C), 117.7, 77.4, 68.3, 66.5, 41.1, 38.5, 27.0, 21.0, 19.3, 18.9, 7.1, 5.3 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{32}H_{51}O_4Si_2^+$: 555.3320; found 555.3328 (+1.4 ppm).

(–)-(2*R*,3*S*)-4-((*tert*-Butyldiphenylsilyl)oxy)-2-methyl-2-(2-oxoethyl)-3-((triethylsilyl)oxy) butyl acetate (3.18)



3.17 **3.18**

To a solution of alkene **3.17** (2.21 g, 3.98 mmol, 1.00 equiv.) in anhydrous DCM (60 ml, 0.070 M) at –78 °C, ozone was bubbled under vacuum until the solution turned pale blue (about 25 minutes). The reaction was then purged with nitrogen to remove excess ozone. After addition of Et_3N (1.67 ml, 11.9 mmol, 3.00 equiv.), the solution was kept at –78 °C for 30 min and then warmed to room temperature for 1 hour. $MgSO_4$ was added and the resulting mixture was filtered and concentrated *in vacuo*. The crude product was purified by silica gel flash chromatography (Hexanes/EtOAc, 90:10) to provide aldehyde **3.18** as a colorless oil (1.89 g, 85%).

R_f = 0.42 (Hexanes/EtOAc, 90:10);

$[\alpha]^{25}_D$ –5.5 (*c* 0.7, CH_2Cl_2);

Formula: $C_{31}H_{48}O_5Si_2$; **MW:** 556.89 g/mol;

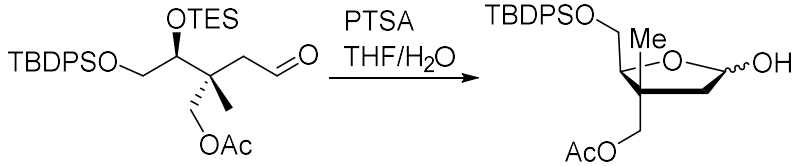
IR (neat) ν_{max} 3072, 3050, 2955, 2935, 2877, 2859, 1745, 1720 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.78 (t, *J* = 2.9 Hz, 1H), 7.66 – 7.65 (m, 4H), 7.44 – 7.38 (m, 6H), 4.15 (d, *J* = 11.0 Hz, 1H), 4.10 (d, *J* = 11.0 Hz, 1H), 3.79 – 3.74 (m, 2H), 3.56 (dd, *J* = 10.1, 3.7 Hz, 1H), 2.45 (dd, *J* = 15.3, 3.2 Hz, 1H), 2.32 (dd, *J* = 15.3, 2.6 Hz, 1H), 2.03 (s, 3H), 1.07 (s, 9H), 1.03 (s, 3H), 0.86 (t, *J* = 8.0 Hz, 9H), 0.51 (q, *J* = 7.9 Hz, 6H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 201.6, 170.8, 135.9, 135.8, 133.12, 133.09, 130.01, 129.99, 127.9 (2C), 76.6, 68.9, 66.0, 48.5, 42.4, 27.0, 20.9, 19.6, 19.2, 7.0, 5.1 ppm;

HRMS (ESI–) *m/z* $[M - H]^-$ calcd for $C_{31}H_{47}O_5Si_2^-$: 555.2968; found 555.2953 (–2.7 ppm).

((2*S*,3*R*)-2-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-5-hydroxy-3-methyltetrahydrofuran-3-yl)methyl acetate (3.19a,b)



3.18 **3.19a,b**

Aldehyde **3.18** (1.88 g, 3.38 mmol, 1.00 equiv.) was dissolved in a THF and H_2O mixture (4:1) (34 ml, 0.1 M) followed by the addition of PTSA (963 mg, 5.06 mmol, 1.50 equiv.). The resulting solution was stirred at 50 °C for 1.5 hours. The solution was diluted with DCM (30 mL) and a saturated solution of $NaHCO_3$ (20 mL) was added. The aqueous layer was extracted with DCM (3 × 25 mL) and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by silica gel flash chromatography (Hexanes/EtOAc, 80:20) provided lactols **3.19a,b** (1.3 g, 87%) as a colorless oil (1.4:1).

R_f = 0.38 (Hexanes/EtOAc, 70:30);

Formula: $C_{25}H_{34}O_5Si$; **MW:** 442.63 g/mol;

IR (neat) ν_{max} 3428 (br), 3071, 3050, 2955, 2932, 2887, 2858, 1742 cm^{-1};

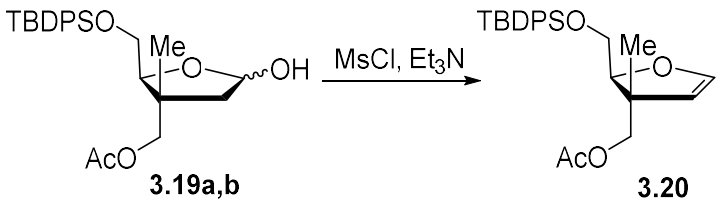
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 – 7.67 (m, 8H, major and minor), 7.46 – 7.37 (m, 12H, major and minor), 5.58 – 5.55 (m, 1H, major), 5.46 – 5.43 (m, 1H, minor), 4.11 – 4.09 (m, 3H, major), 3.92 – 3.85 (m, 3H, minor), 3.73 – 3.66 (m, 4H, major and minor), 3.23 (d, *J* = 7.5 Hz, 1H, minor), 2.74 (d, *J* = 4.3 Hz, 1H, major), 2.21 (dd, *J* = 13.7, 6.1 Hz, 1H, minor), 2.06 (dd, *J* = 13.0, 5.8 Hz, 1H, major), 2.033 (s, 3H, minor), 2.027 (s, 3H, major), 1.90 (dd, *J* = 13.6, 2.7 Hz, 1H, major), 1.81 (dd, *J* = 14.0, 3.1 Hz, 1H, minor), 1.18 (s, 3H, minor), 1.09 (s, 9H, minor), 1.06 (s, 3H, major), 1.05 (s, 9H, major) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.10 (minor), 171.07 (major), 135.9 (minor), 135.8 (major), 135.7 (major and minor), 133.4 (major), 133.3 (major), 132.81 (minor), 132.77 (minor), 130.1 (minor), 130.0 (minor), 129.9 (major), 127.98 (minor), 127.95 (minor), 127.86 (major), 127.84 (major), 98.7 (minor), 97.8 (major), 83.5 (minor), 82.4 (major), 70.8 (minor), 70.4 (major), 64.8 (minor), 63.9 (major), 45.3 (major), 45.2 (minor), 44.4 (minor), 44.2 (major), 27.1

(minor), 26.9 (major), 21.0 (major and minor), 19.3 (minor), 19.2 (major), 18.6 (major), 18.4 (minor) ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{25}H_{34}O_5SiNa^+$: 465.2068; found 465.2070 (+0.4 ppm).

(+)-((2*S*,3*R*)-2-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-3-methyl-2,3-dihydrofuran-3-yl)methyl acetate (3.20)



3.19a,b **3.20**

To a solution of lactols **3.19a,b** (1.27 g, 2.87 mmol, 1.00 equiv.) in anhydrous DCE (72 mL, 0.04 M), MsCl (0.78 mL, 10 mmol, 3.5 equiv.) was added. The resulting solution was stirred 3 minutes at room temperature followed by 3 minutes at 75 °C. Triethylamine (3.0 ml, 22 mmol, 7.5 equiv.) was then added and the resulting solution was stirred 3 minutes at 75 °C. After cooling to room temperature, a saturated solution of $NaHCO_3$ (20 mL) was added and the aqueous layer was extracted with Et_2O (3 × 25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by silica gel flash chromatography (Hexanes/EtOAc, 90:10) provided glycal **3.20** (620 mg, 51%) as a colorless oil.

R_f = 0.65 (Hexanes/EtOAc, 80:20);

$[\alpha]^{25}_D$ +59 (*c* 0.8, CH_2Cl_2);

Formula: $C_{25}H_{32}O_4Si$; **MW:** 424.61 g/mol;

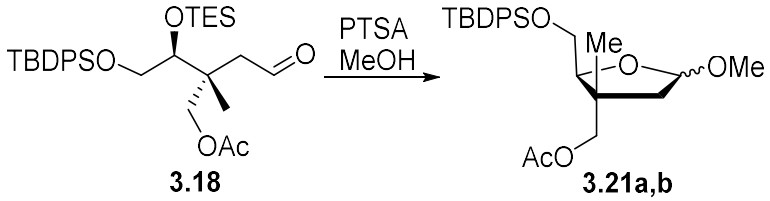
IR (neat) ν_{max} 3071, 3050, 2958, 2932, 2887, 2858, 1743 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 – 7.68 (m, 4H), 7.45 – 7.37 (m, 6H), 6.28 (d, *J* = 2.7 Hz, 1H), 4.74 (d, *J* = 2.7 Hz, 1H), 4.33 (t, *J* = 6.1 Hz, 1H), 4.06 (d, *J* = 10.8 Hz, 1H), 3.89 (d, *J* = 10.8 Hz, 1H), 3.87 – 3.80 (m, 2H), 2.02 (s, 3H), 1.08 (s, 3H), 1.07 (s, 9H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.1, 145.6, 135.76, 135.75, 133.5, 133.4, 129.89, 129.87, 127.9 (2C), 107.4, 85.7, 70.9, 63.0, 48.1, 27.0, 21.0, 19.3, 18.0 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{25}H_{33}O_4Si^+$: 425.2143; found 425.2148 (+1.2 ppm).

((2*S*,3*R*)-2-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-5-methoxy-3-methyltetrahydrofuran-3-yl)methyl acetate (3.21a,b)



3.18 **3.21a,b**

To a solution of aldehyde **3.18** (1.12 g, 2.00 mmol, 1.00 equiv.) in anhydrous MeOH (10 mL, 0.20 M), PTSA (0.19 g, 1.0 mmol, 0.50 equiv.) was added at room temperature. The resulting mixture was stirred for 20 minutes until completion as indicated by TLC. The reaction was neutralized by addition of anhydrous Et_3N (0.56 mL, 4.0 mmol, 2.0 equiv.) and the resulting mixture was concentrated under reduced pressure. Purification by silica gel flash chromatography (Hexanes/EtOAc, 90:10) provided methoxy lactols **3.21a,b** (0.86 g, 94%) as a colorless oil (1.4:1).

R_f = 0.48 (Hexanes/EtOAc, 80:20);

Formula: $C_{26}H_{36}O_5Si$; **MW:** 456.65 g/mol;

IR (neat) ν_{max} 3071, 3049, 2954, 2931, 2890, 2858, 1743 cm^{-1};

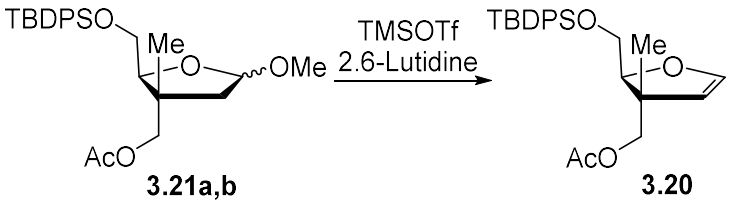
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 – 7.68 (m, 8H, major and minor), 7.45 – 7.36 (m, 12H, major and minor), 5.03 (dd, *J* = 5.7, 2.9 Hz, 1H, major), 4.94 (dd, *J* = 6.0, 2.3 Hz, 1H, minor), 4.10 (d, *J* = 10.8 Hz, 1H, major), 4.00 – 3.93 (m, 5H, major and minor), 3.80 – 3.72 (m, 4H, major and minor), 3.36 (s, 3H, major), 3.30 (s, 3H, minor), 2.12 (dd, *J* = 13.5, 5.9 Hz, 1H, minor), 2.05 (s, 3H, minor), 1.99 (s, 3H, major), 1.97 (dd, *J* = 13.6, 5.8 Hz, 1H, major), 1.88 (dd, *J* = 13.6, 2.9 Hz, 1H, major), 1.74 (dd, *J* = 13.5, 2.2 Hz, 1H, minor), 1.18 (s, 3H, minor), 1.06 (s, 18H, major and minor), 1.01 (s, 3H, major) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 171.12 (minor), 171.05 (major), 135.77 (major and minor), 135.75 (minor), 135.73 (major), 133.65 (minor), 133.56 (major), 133.52 (minor), 133.47 (major), 129.9 (major), 129.84 (minor), 129.82 (major and minor), 127.8 (major and minor), 105.0 (minor), 104.2 (major), 83.6 (minor), 82.0 (major), 70.8 (minor), 70.3 (major), 64.4 (minor), 63.8 (major), 55.4 (minor), 55.2 (major), 44.5 (major), 43.9 (minor), 43.7

(minor), 43.5 (major), 26.9 (major and minor), 20.99 (minor), 20.96 (major), 19.33 (minor), 19.30 (major), 18.6 (major), 18.4 (minor) ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{26}H_{36}O_5SiNa^+$: 479.2224; found 479.2220 (–0.9 ppm).

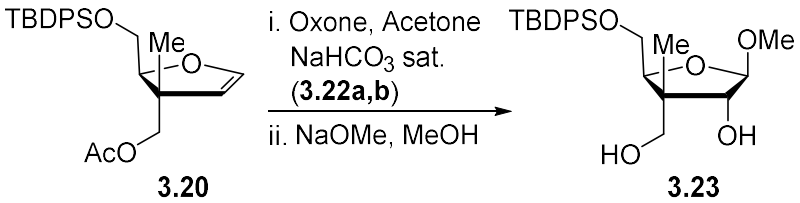
(+)-((2*S*,3*R*)-2-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-3-methyl-2,3-dihydrofuran-3-yl)methyl acetate (3.20)



3.21a,b **3.20**

To a solution of methoxy lactols **3.21a,b** (0.12 g, 0.25 mmol, 1.0 equiv.) in anhydrous DCM (1.3 mL, 0.2 M), 2,6-lutidine (0.12 mL, 1.0 mmol, 4.0 equiv.) and TMSOTf (0.09 mL, 0.5 mmol, 2 equiv.) were added at 0 °C.[6] The resulting solution was stirred 30 minutes at room temperature. Then the mixture was diluted in DCM (5 mL) followed by washing with water (5 mL), and the aqueous layer was extracted with DCM (3 × 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by silica gel flash chromatography (Hexanes/EtOAc, 90:10) provided glycal **3.20** (96 mg, 90%) as a colorless oil, the full characterization of which was reported above.

(–)-(2*R*,3*R*,4*S*,5*S*)-5-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-4-(hydroxymethyl)-2-methoxy-4-methyltetrahydrofuran-3-ol (3.23)



3.20 **3.23**

To a solution of glycal **3.20** (48 mg, 0.11 mmol, 1.0 equiv.) in anhydrous DCM (0.5 mL, 0.2 M) at 0 °C, acetone (50 µL, 0.60 mmol, 6.0 equiv.) and a saturated $NaHCO_3$ solution (1.0 mL) were added. To the resulting biphasic mixture, a 0.37 mM solution of oxone in water

(0.6 mL) was added.[7] After sealing the flask, the resulting mixture was stirred for 30 minutes at 0 °C and 3 hours at room temperature. After degassing the flask, the aqueous phase was extracted with DCM (3 × 5 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude epoxide **3.22a,b** (*dr* 7:1, determined by ^{1}H NMR of crude epoxide) was dissolved in MeOH (1.1 mL, 0.10 M). A 1.0 M solution of NaOMe in MeOH (0.11 mL, 0.11 μmol, 1.0 equiv.) was added at room temperature. The resulting mixture was stirred for 3 hours, quenched with amberlite acidic resin (~20 mg), filtered and evaporated under reduced pressure. Purification by flash chromatography on silica gel (DCM/EtOAc, 80:20) provided 1′-O-methoxy-β-ribofuranose **3.23** (20 mg, 42%) as a colorless oil.

R_f = 0.15 (Hexanes/EtOAc, 70:30);

$[\alpha]^{25}_D$ –3.0 (*c* 1.0, CH_2Cl_2);

Formula: $C_{24}H_{34}O_5Si$; **MW:** 430.62 g/mol;

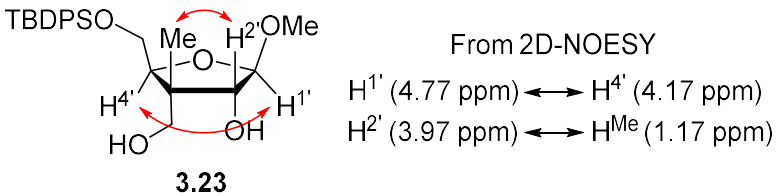
IR (neat) ν_{max} 3410 (br), 3071, 3049, 2955, 2931, 2889, 2858 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 – 7.66 (m, 4H), 7.44 – 7.39 (m, 6H), 4.77 (s, 1H), 4.17 (dd, *J* = 7.7, 5.6 Hz, 1H), 3.97 (d, *J* = 3.2 Hz, 1H), 3.83 – 3.74 (m, 3H), 3.71 (dd, *J* = 10.4, 7.8 Hz, 1H), 3.33 (s, 3H), 2.62 (d, *J* = 4.8 Hz, 1H), 2.31 (t, *J* = 5.5 Hz, 1H), 1.17 (s, 3H), 1.05 (s, 9H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 135.75, 135.72, 133.3, 133.2, 130.0, 129.9, 127.92, 127.90, 110.5, 85.0, 83.0, 67.6, 63.6, 55.8, 47.5, 26.9, 19.3, 16.1 ppm;

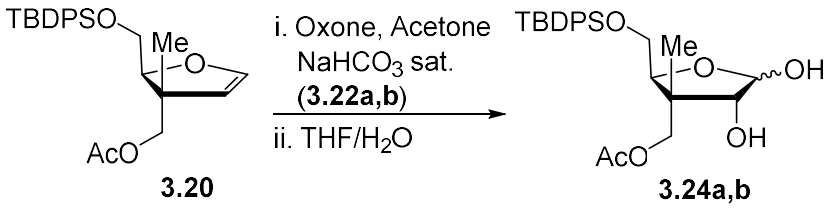
HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{24}H_{34}O_5SiNa^+$: 453.2068; found 453.2067 (–0.2 ppm).

The relative configuration of 1′-O-methoxy-β-ribofuranose **3.23** was determined by relevant nuclear Overhauser effect (nOe) enhancements (2D NOESY). The peaks in the ^{1}H NMR spectra were assigned using ^{1}H/^{1}H 2D COSY experiment, chemical shifts and coupling constants.



3.23

((2*S*,3*S*,4*R*)-2-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-4,5-dihydroxy-3-methyltetra hydrofuran-3-yl)methyl acetate (3.24a,b)



To a solution of glycal **3.20** (118 mg, 0.278 mmol, 1.00 equiv.) in anhydrous DCM (1.3 mL, 0.22 M) at 0 °C, acetone (0.13 mL, 1.6 mmol, 6.0 equiv.) and a saturated $NaHCO_3$ solution (2.5 mL) were added. To the resulting biphasic mixture, a 0.37 mM solution of oxone in water (1.5 mL) was added. After sealing the flask, the resulting mixture was stirred for 30 minutes at 0 °C and 3 hours at room temperature. After degassing the flask, the aqueous phase was extracted with DCM (3 × 5 mL), and the resulting organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude epoxide **3.22a,b** (*dr* 7:1, determined by ^{1}H NMR of crude epoxide) was stirred in a THF and H_2O (1:1) mixture (5.5 mL, 0.050 M) for 1 hour. The aqueous phase was extracted with EtOAc (3 × 5 mL), and the resulting organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (DCM/EtOAc, 80:20) provided lactols **3.24a,b** (80 mg, 63%, 4:1 mixture) as a white foam.

R_f = 0.33 (DCM/EtOAc, 85:15);

Formula: $C_{25}H_{34}O_6Si$; **MW:** 458.63 g/mol;

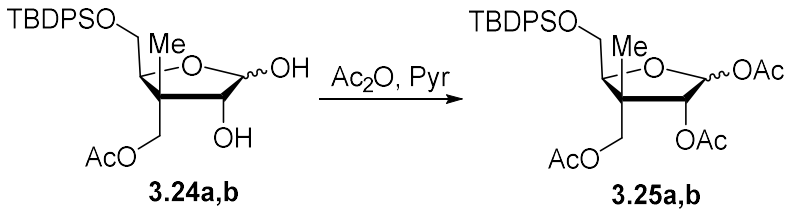
IR (Neat) ν_{max} 3421 (br), 2931, 2857, 1741, 1720 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 – 7.63 (m, 8H, major and minor), 7.45 – 7.37 (m, 12H, major and minor), 5.42 (dd, *J* = 9.6, 3.9 Hz, 1H, major), 5.23 (dd, *J* = 5.4, 1.1 Hz, 1H, minor), 4.53 (d, *J* = 11.4 Hz, 1H, major), 4.39 (d, *J* = 11.4 Hz, 1H, minor), 4.18 (d, *J* = 11.4 Hz, 1H, major), 4.06 (d, *J* = 11.4 Hz, 1H, minor), 4.11 (dd, *J* = 7.5, 4.9 Hz, 1H, minor), 4.02 (dd, *J* = 6.0, 4.9 Hz, 1H, minor), 3.89 (d, *J* = 4.2 Hz, 1H, major), 3.87 (d, *J* = 2.9 Hz, 1H, minor), 3.80 – 3.73 (m, 3H, major and minor), 3.64 (dd, *J* = 10.6, 7.5 Hz, 1H, major), 2.10 (s, 3H, major), 2.09 (s, 3H, minor), 1.18 (s, 3H, minor), 1.08 (s, 9H, minor), 1.05 (s, 9H, major), 1.03 (s, 3H, major) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, $CDCl_3$) δ 172.3 (major), 172.0 (minor), 135.74 (minor), 135.69 (minor), 135.67 (major), 135.65 (major), 133.1 (major), 133.0 (major), 132.9 (minor), 132.8 (minor), 130.04 (minor), 130.02 (minor), 129.95 (major), 128.0 (minor), 127.94 (minor), 127.90 (major), 127.89 (major), 103.9 (minor), 96.8 (major), 83.6 (minor) 83.0 (minor), 80.1 (major), 77.3 (major), 66.8 (minor), 66.6 (major), 63.9 (minor), 63.0 (major), 48.4 (major), 47.4 (minor), 27.0 (minor), 26.9 (major), 21.0 (major and minor), 19.3 (minor), 19.2 (major), 15.7 (minor), 15.4 (major) ppm;

HRMS (ESI+) *m/z* $[M + NH_4]^+$ calcd for $C_{25}H_{38}O_6NSi^+$: 476.2463; found 476.2462 (–0.2 ppm).

(3*R*,4*R*,5*S*)-4-(Acetoxymethyl)-5-(((*tert*-butyldiphenylsilyl)oxy)methyl)-4-methyltetra hydrofuran-2,3-diyl diacetate (3.25a,b)



3.24a,b **3.25a,b**

Lactols **3.24a,b** (65 mg, 0.14 mmol) were stirred in a solution of Ac_2O:Pyr (2:1, 1.0 ml, 0.14 M) at room temperature for 18 hours and then concentrated. Purification by flash chromatography on silica gel (Hexanes/EtOAc, 60:40), provided acetates **3.25a,b** (65 mg, 85%, 4:1 mixture) as a colorless oil.

R_f = 0.53 (Hexanes/EtOAc, 70:30);

Formula: $C_{29}H_{38}O_8Si$; **MW:** 542.70 g/mol;

IR (Neat) ν_{max} 2933, 2858, 1746 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 – 7.65 (m, 8H, major and minor), 7.46 – 7.37 (m, 12H, major and minor), 6.41 (d, *J* = 4.8 Hz, 1H, major), 6.00 (d, *J* = 1.6 Hz, 1H, minor), 5.35 (d, *J* = 4.8 Hz, 1H, major), 5.31 (d, *J* = 1.6 Hz, 1H, minor), 4.29 (d, *J* = 11.2 Hz, 1H, major), 4.25 – 4.23 (m, 2H, major and minor), 4.24 (d, *J* = 11.2 Hz, 1H, major), 4.16 – 4.10 (m, 2H, minor), 3.77 – 3.74 (m, 3H, major and minor), 3.68 (dd, *J* = 11.3, 3.5 Hz, 1H, major), 2.11 (s, 3H, minor), 2.10 (s, 3H, major), 2.07 (s, 3H, major), 2.03 (s, 3H, minor), 2.03 (s, 3H, major), 1.98

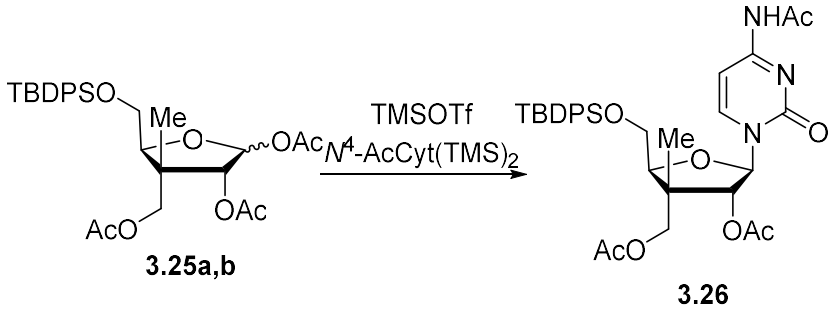
(s, 3H, minor), 1.28 (s, 3H, minor), 1.25 (s, 3H, major), 1.08 (s, 9H, minor), 1.08 (s, 9H, major) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 170.9 (major), 170.7 (minor), 169.8 (major), 169.7 (minor), 169.64 (minor), 169.61 (major), 135.8 (major), 135.72 (major), 135.71 (minor), 135.69 (minor), 133.03 (minor), 132.97 (major), 132.8 (minor), 132.7 (major), 130.01 (minor), 129.98 (major), 129.95 (minor), 127.94 (major), 127.93 (minor), 127.92 (major), 127.89 (minor), 100.0 (minor), 94.2 (major), 84.9 (minor), 82.9 (major), 82.0 (minor), 77.9 (major), 66.8 (major), 66.4 (minor), 63.4 (major), 63.2 (minor), 46.1 (minor), 45.2 (major), 26.90 (minor), 26.88 (major), 21.21 (minor), 21.19 (major), 20.93 (major), 20.89 (minor), 20.85 (minor), 20.6 (major), 19.3 (minor), 19.2 (major), 16.8 (major), 15.8 (minor) ppm;

HRMS (ESI+) *m/z* $[M + NH_4]^+$ calcd for $C_{29}H_{42}O_8NSi^+$: 560.2674; found 560.2673 (–0.2 ppm).

Preparation of silylated *N*^{4}-acetylcytosine: To a suspension of the *N*^{4}-acetylcytosine (1.00 g, 6.53 mmol, 1.00 equiv.) in HMDS (3.4 mL, 16 mmol, 2.5 equiv.), $(NH_4)_2SO_4$ (8.6 mg, 0.065 mmol, 0.010 equiv.) was added. The reaction mixture was heated at reflux until a clear solution was obtained (~1 hour). Upon cooling to room temperature, the solution was placed under high vacuum overnight to remove excess HMDS. A 0.4 M solution of the silylated *N*^{4}-acetylcytosine was prepared in anhydrous DCE (16.3 mL).

(+)-(2*R*,3*R*,4*R*,5*S*)-2-(4-Acetamido-2-oxopyrimidin-1(2*H*)-yl)-4-(acetoxymethyl)-5-(((*tert*-butyldiphenylsilyl)oxy)methyl)-4-methyltetrahydrofuran-3-yl acetate (3.26)



3.25a,b **3.26**

To a solution of acetates **3.25a,b** (70 mg, 0.13 mmol, 1.0 equiv.) in anhydrous MeCN (0.6 mL, 0.2 M), silylated *N*^{4}-acetylcytosine (0.52 mL, 0.21 mmol, 1.6 equiv. 0.40 M in DCE) was added at room temperature. The resulting mixture was stirred for 10 minutes and

TMSOTf (0.10 mL, 0.52 mmol, 4.0 equiv.) was added. The reaction was stirred at 60 °C for 3.5 hours and then cooled to room temperature and quenched with saturated aqueous $NaHCO_3$ (1 mL). The aqueous layer was extracted with DCM (3 × 5 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (DCM/MeOH, 95:5) provided the 1′,2′-*trans* ribo-like nucleoside analogue **3.26** (63 mg, 77%) as a white foam.

R_f = 0.34 (DCM/MeOH, 95:5);

$[\alpha]^{25}_D$ +35 (*c* 0.2, MeOH);

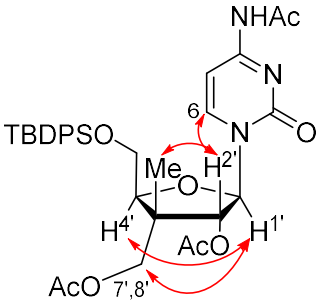
Formula: $C_{33}H_{41}N_3O_8Si$; **MW:** 635.79 g/mol;

IR (neat) ν_{max} 2957, 2932, 2893, 2858, 1746, 1671 cm^{-1};

^{1}H NMR (500 MHz, CD_3OD) δ 8.17 (d, *J* = 7.6 Hz, 1H), 7.72 – 7.68 (m, 4H), 7.50 – 7.41 (m, 6H), 7.11 (d, *J* = 7.5 Hz, 1H), 6.06 (d, *J* = 5.3 Hz, 1H), 5.37 (d, *J* = 5.3 Hz, 1H), 4.27 (t, *J* = 3.9 Hz, 1H), 4.18 (d, *J* = 11.2 Hz, 1H), 4.13 (d, *J* = 11.2 Hz, 1H), 4.09 (dd, *J* = 11.9, 4.0 Hz, 1H), 3.94 (dd, *J* = 11.8, 3.9 Hz, 1H), 2.16 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.18 (s, 3H), 1.11 (s, 9H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, CD_3OD) δ 173.0, 172.2, 171.3, 164.3, 158.1, 145.5, 136.9, 136.7, 134.1, 133.5, 131.35, 131.32, 129.12, 129.09, 98.2, 90.1, 85.4, 82.5, 67.5, 64.9, 47.2, 27.6, 24.5, 20.7, 20.6, 20.1, 16.8 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{33}H_{42}N_3O_8Si^+$: 636.2736; found 636.2726 (–1.5 ppm).

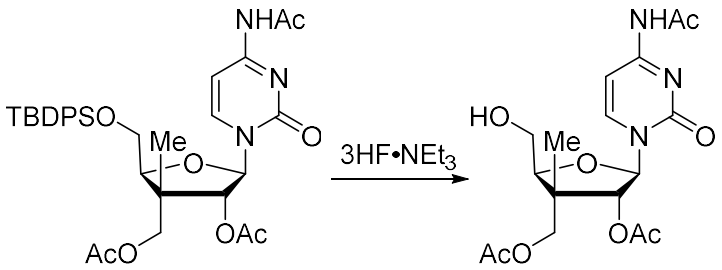


3.26

From 2D-NOESY

$H^{1'}$ (6.06 ppm) ⟷ $H^{4'}$ (4.27 ppm)
$H^{1'}$ (6.06 ppm) ⟷ $H^{7'}$ (4.18 ppm)
$H^{1'}$ (6.06 ppm) ⟷ $H^{8'}$ (4.13 ppm)
$H^{2'}$ (5.37 ppm) ⟷ H^{6} (8.17 ppm)
$H^{2'}$ (5.37 ppm) ⟷ H^{Me} (1.18 ppm)

(+)-(2*R*,3*R*,4*R*,5*S*)-2-(4-Acetamido-2-oxopyrimidin-1(2*H*)-yl)-4-(acetoxymethyl)-5-(hydroxymethyl)-4-methyltetrahydrofuran-3-yl acetate (3.27)



To a solution of 1′,2′-*trans* ribo-like nucleoside **3.26** (50 mg, 77 μmol, 1.0 equiv.) in anhydrous THF (0.8 mL, 0.1 M), 3HF•NEt_3 (0.13 mL, 0.79 mmol, 10 equiv.) was added at room temperature and the resulting mixture was stirred for 16 hours. Triethylamine (1.10 mL, 7.90 mmol, 100 equiv.) was then added and the mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (DCM/MeOH, 95:5) provided the 1′,2′-*trans* ribo-like nucleoside **3.27** (27 mg, 86%) as a white foam.

R_f = 0.30 (DCM/MeOH, 95:5);

$[\alpha]^{25}_D$ +26 (*c* 0.2, MeOH);

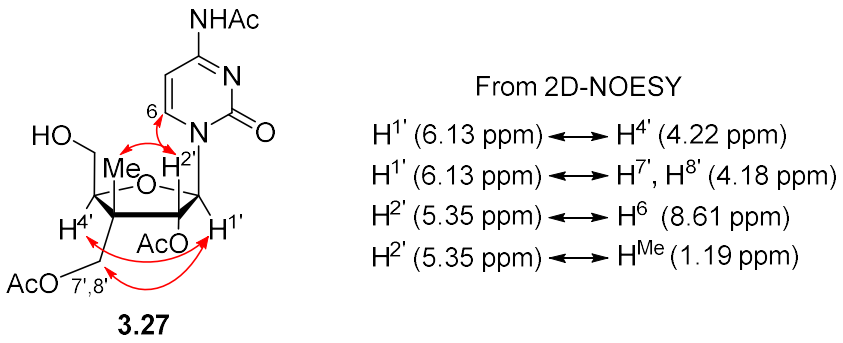
Formula: $C_{17}H_{23}N_3O_8$; **MW:** 397.38 g/mol;

IR (neat) ν_{max} 3307 (br), 2927, 1742, 1650 cm^{-1};

^{1}H NMR (500 MHz, CD_3OD) δ 8.61 (d, *J* = 7.6 Hz, 1H), 7.45 (d, *J* = 7.5 Hz, 1H), 6.13 (d, *J* = 5.7 Hz, 1H), 5.35 (d, *J* = 5.7 Hz, 1H), 4.22 (t, *J* = 3.6 Hz, 1H), 4.18 (s, 2H), 3.89 (dd, *J* = 12.1, 3.4 Hz, 1H), 3.79 (dd, *J* = 12.0, 3.8 Hz, 1H), 2.18 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 1.19 (s, 3H) ppm; Labile protons could not be observed;

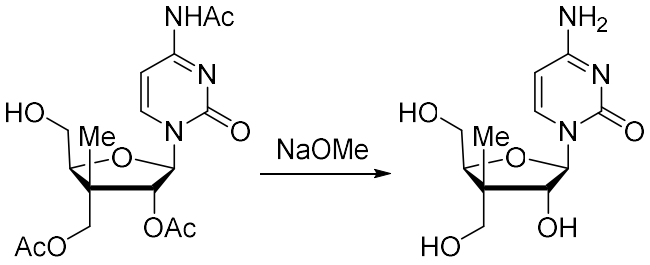
^{13}C NMR (126 MHz, CD_3OD) δ 173.0, 172.4, 171.5, 164.4, 158.3, 146.3, 98.2, 90.0, 85.9, 82.6, 67.7, 62.5, 47.3, 24.5, 20.8, 20.6, 16.4 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{17}H_{24}N_3O_8^+$: 398.1558; found 398.1552 (–1.6 ppm).



3.27

(+)-4-Amino-1-((2*R*,3*R*,4*S*,5*S*)-3-hydroxy-4,5-bis(hydroxymethyl)-4-methyltetrahydro furan-2-yl)pyrimidin-2(1*H*)-one (2.02b)

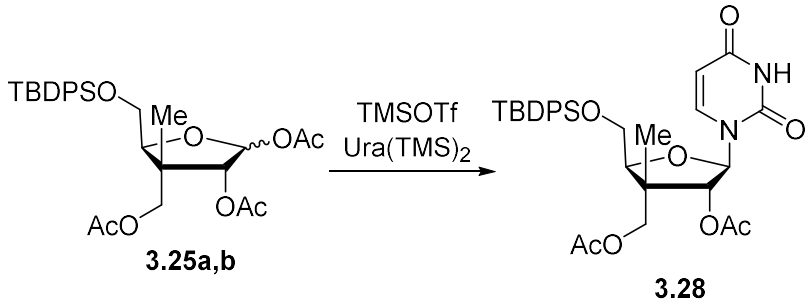


3.27 **2.02b**

Representative procedure A: To a solution of nucleoside **3.27** (13 mg, 32 µmol, 1.0 equiv.) in anhydrous MeOH (0.3 mL, 0.1 M), NaOMe (32 µL, 32 µmol, 1.0 equiv., 1.0 M in MeOH) was added at room temperature. The reaction was stirred for 3 hours, quenched with amberlite acidic resin (~50 mg) and stirred for 10 minutes. The mixtrue was filtered with MeOH (~5 mL) and concentrated under reduced pressure. Purification by C18 reverse phase flash chromatography (MeOH/H_2O) provided 1′,2′-*trans* ribo-like cytosine nucleoside analogue **2.02b** (7 mg, 81%) as a white foam. The full characterization of **2.02b** was reported in the experimental section Chapter 2.

Preparation of silylated uracil: To a suspension of uracil (1.00 g, 8.92 mmol, 1.00 equiv.) in HMDS (4.7 mL, 22 mmol, 2.50 equiv.), $(NH_4)_2SO_4$ (12 mg, 0.089 mmol, 0.010 equiv.) was added. The reaction mixture was heated at reflux for 5 hours until a clear solution was obtained. Upon cooling to room temperature, the solution was placed under high vacuum overnight to remove excess HMDS. A 0.4 M solution of the silylated uracil was prepared in anhydrous DCE (22.3 mL).

(+)-((2*S*,3*R*,4*R*,5*R*)-4-Acetoxy-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3-methyltetrahydrofuran-3-yl)methyl acetate (3.28)



3.25a,b **3.28**

To a solution of acetates **3.25a,b** (88 mg, 0.16 mmol, 1.0 equiv.) in anhydrous MeCN (0.8 mL, 0.2 M), uracil(TMS)$_2$ (1.0 mL, 0.40 mmol, 2.5 equiv. 0.40 M in DCE) was added at room temperature. The resulting mixture was stirred for 10 minutes and TMSOTf (60 μL, 0.32 mmol, 2.0 equiv.) was added. The reaction was stirred at 60 °C for 4 hours, cooled to room temperature and quenched with saturated aqueous $NaHCO_3$ (2 mL). The aqueous layer was extracted with DCM (3 × 5 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The ^{1}H NMR of the crude reaction mixture indicated a >20:1 dr. Purification by flash chromatography on silica gel (DCM/MeOH, 90:10) provided nucleoside uracil analogue **3.28** (83 mg, 86%) as a white foam.

R$_f$ = 0.61 (DCM/MeOH, 90:10);

[α]$^{25}_D$ +14 (*c* 0.6, MeOH);

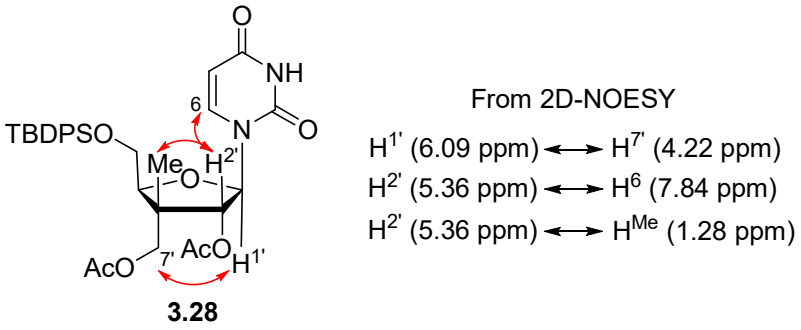
Formula: $C_{31}H_{38}N_2O_8Si$; **MW:** 594.74 g/mol;

IR (neat) ν_{max} 3195 (br), 3070, 3054, 2959, 2932, 2890, 2859, 1744, 1688 cm^{-1};

^{1}H NMR (500 MHz, CD_3OD) δ 7.84 (d, *J* = 8.1 Hz, 1H), 7.72 – 7.67 (m, 4H), 7.50 – 7.41 (m, 6H), 6.09 (d, *J* = 6.6 Hz, 1H), 5.36 (d, *J* = 6.6 Hz, 1H), 5.16 (d, *J* = 8.1 Hz, 1H), 4.22 (d, *J* = 11.3 Hz, 1H), 4.20 (t, *J* = 3.5 Hz, 1H), 4.10 (d, *J* = 11.3 Hz, 1H), 4.07 (dd, *J* = 11.8, 3.8 Hz, 1H), 3.90 (dd, *J* = 11.8, 3.3 Hz, 1H), 2.08 (s, 3H), 2.06 (s, 3H), 1.28 (s, 3H), 1.12 (s, 9H) ppm; Labile protons could not be observed;

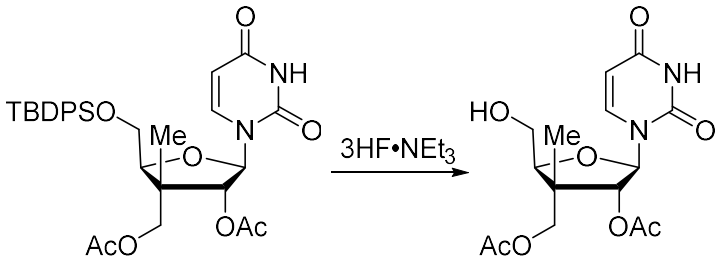
^{13}C NMR (126 MHz, CD_3OD) δ 172.2, 171.6, 165.8, 152.4, 141.9, 136.9, 136.7, 134.3, 133.4, 131.4, 131.3, 129.12, 129.10, 102.8, 87.5, 84.6, 81.1, 67.8, 65.2, 46.6, 27.7, 20.8, 20.4, 20.2, 17.1 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{31}H_{39}N_2O_8Si^+$: 595.2470; found 595.2478 (+1.2 ppm).



3.28

(+)-((2*S*,3*R*,4*R*,5*R*)-4-Acetoxy-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-(hydroxymethyl)-3-methyltetrahydrofuran-3-yl)methyl acetate (3.29)



3.28 **3.29**

To a solution of nucleoside uracil analogue **3.28** (78 mg, 0.13 mmol, 1.0 equiv.) in anhydrous THF (2.6 mL, 0.050 M), 3HF•NEt_3 (0.44 mL, 2.6 mmol, 20 equiv.) was added at room temperature and the resulting mixture was stirred for 40 hours. Triethylamine (1.8 mL, 13 mmol, 100 equiv.) was then added and the mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (DCM/MeOH, 95:5), provided the 1′,2′-*trans* ribo-like nucleoside uracil analogue **3.29** (41 mg, 88%) as a white foam.

R_f = 0.58 (DCM/MeOH, 90:10);

$[\alpha]^{25}_D$ +11 (*c* 1.0, MeOH);

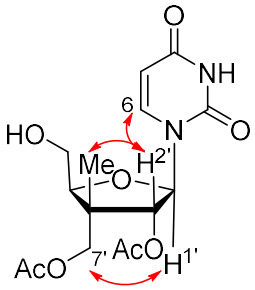
Formula: $C_{15}H_{20}N_2O_8$; **MW:** 356.33 g/mol;

IR (neat) ν_{max} 3441 (br), 3207 (br), 3098, 3064, 2939, 2889, 1742, 1688 cm^{-1};

^{1}H NMR (500 MHz, CD_3OD) δ 8.28 (d, *J* = 8.1 Hz, 1H), 6.11 (d, *J* = 6.7 Hz, 1H), 5.72 (d, *J* = 8.1 Hz, 1H), 5.32 (d, *J* = 6.7 Hz, 1H), 4.22 (d, *J* = 11.2 Hz, 1H), 4.15 – 4.13 (m, 2H), 3.86 (dd, *J* = 12.0, 3.2 Hz, 1H), 3.75 (dd, *J* = 12.1, 3.3 Hz, 1H), 2.10 (s, 3H), 2.06 (s, 3H), 1.24 (s, 3H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, CD_3OD) δ 172.3, 171.7, 166.1, 152.7, 142.5, 102.9, 87.8, 85.2, 81.4, 68.0, 62.7, 46.8, 20.8, 20.4, 16.6 ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{15}H_{20}N_2O_8Na^+$: 379.1112; found 379.1108 (–0.9 ppm).



3.29

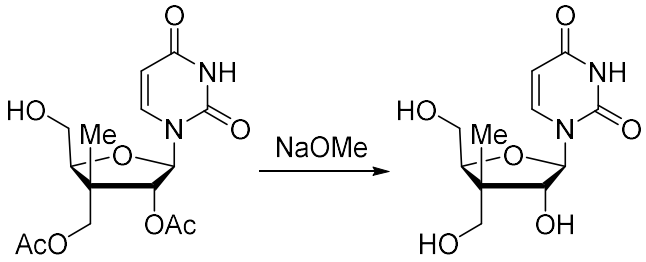
From 2D-NOESY

$H^{1'}$ (6.11 ppm) ⟷ $H^{7'}$ (4.22 ppm)

$H^{2'}$ (5.32 ppm) ⟷ H^{6} (8.28 ppm)

$H^{2'}$ (5.32 ppm) ⟷ H^{Me} (1.24 ppm)

(+)-1-((2*R*,3*R*,4*S*,5*S*)-3-Hydroxy-4,5-bis(hydroxymethyl)-4-methyltetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione (3.34)



3.29 **3.34**

Following procedure A, NaOMe (42 µL, 42 µmol, 1.0 equiv., 1.0 M in MeOH) was added to a solution of nucleoside **3.29** (15 mg, 42 µmol, 1.0 equiv.) in MeOH (0.4 mL, 0.1 M). Purification by C18 reverse phase flash chromatography ($MeOH/H_2O$) provided 1′,2′-*trans* ribo-like uracil nucleoside analogue **3.34** (9 mg, 80%) as a white foam.

R_f = 0.20 (DCM/MeOH, 90:10);

$[\alpha]^{25}_D$ +13 (*c* 0.5, MeOH);

Formula: $C_{11}H_{16}N_2O_6$; **MW:** 272.26 g/mol;

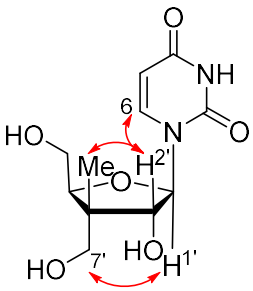
IR (neat) ν_{max} 3355 (br), 2935, 2885, 1682 cm^{-1};

^{1}H NMR (500 MHz, CD_3OD) δ 8.17 (d, *J* = 8.1 Hz, 1H), 5.97 (d, *J* = 6.7 Hz, 1H), 5.71 (d, *J* = 8.1 Hz, 1H), 4.15 – 4.12 (m, 2H), 3.79 (dd, *J* = 11.9, 3.3 Hz, 1H), 3.70 (d, *J* = 11.0 Hz, 1H),

3.68 (dd, J = 11.9, 3.8 Hz, 1H), 3.58 (d, J = 11.1 Hz, 1H), 1.15 (s, 3H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, CD_3OD) δ 166.2, 152.9, 143.0, 102.7, 90.2, 85.0, 81.9, 66.4, 63.2, 48.0, 16.6 ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{11}H_{16}N_2O_6Na^+$: 295.0901; found 295.0900 (–0.03 ppm).



3.34

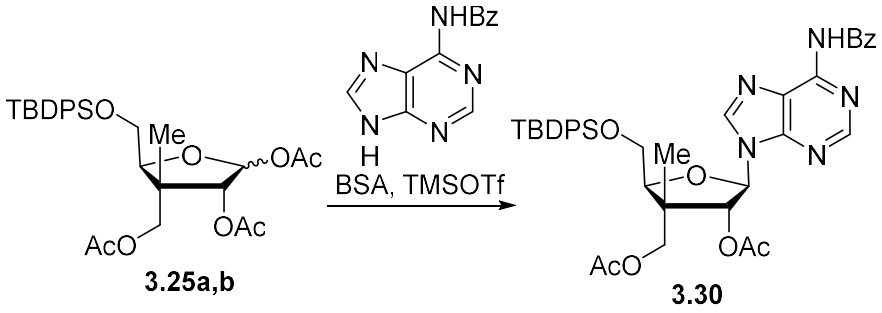
From 2D-NOESY

$H^{1'}$ (5.97 ppm) ⟷ $H^{7'}$ (3.70 ppm)

$H^{2'}$ (4.14 ppm) ⟷ H^{6} (8.17 ppm)

$H^{2'}$ (4.14 ppm) ⟷ H^{Me} (1.15 ppm)

(–)-((2*S*,3*R*,4*R*,5*R*)-4-Acetoxy-5-(6-benzamido-9*H*-purin-9-yl)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-methyltetrahydrofuran-3-yl)methyl acetate (3.30)



3.25a,b **3.30**

To a suspension of N^6-benzoyladenine (44 mg, 0.18 mmol, 2.5 equiv.) in anhydrous DCE (0.9 mL, 0.08 M), bis(trimethylsilyl)acetamide (0.10 mL, 0.40 mmol, 5.5 equiv.) was added at room temperature.[8] The resulting mixture was vigorously stirred for 2 hours until a clear solution was obtained. The solution was concentrated under high vacuum and treated with a solution of **3.25a,b** (40 mg, 74 µmol, 1.0 equiv.) in anhydrous DCE (0.9 mL, 0.08 M). After adding TMSOTf (27 µL, 0.15 mmol, 2.0 equiv.), the resulting solution was heated at reflux for 4 hours. The solution was diluted in DCM (5 mL) and washed with a saturated $NaHCO_3$ solution (2 mL). The aqueous phase was extracted with DCM (3 × 5 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and

concentrated under reduced pressure. The ^{1}H NMR spectrum of the crude reaction mixture indicated a >20:1 dr and only the N9-regioisomer. Purification by flash chromatography on silica gel (EtOAc/MeOH, 97:3) provided N^6-benzoyladenine nucleoside analogue **3.30** (40 mg, 75%) as a white foam.

R_f = 0.37 (DCM/MeOH, 95:5);

[α]$^{25}_{D}$ −15 (*c* 0.8, MeOH);

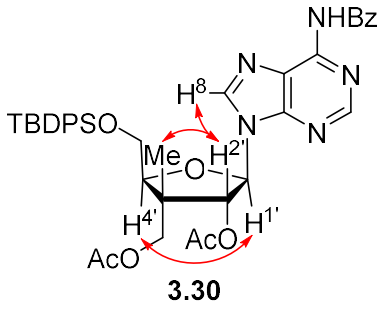
Formula: $C_{39}H_{43}N_5O_7Si$; **MW:** 721.89 g/mol;

IR (neat) ν_{max} 3268 (br), 3070, 3053, 2956, 2931, 2892, 2857, 1745 cm^{-1};

^{1}H NMR (500 MHz, CD_3OD) δ 8.66 (s, 1H), 8.44 (s, 1H), 8.08 (d, *J* = 7.9 Hz, 2H), 7.69 – 7.63 (m, 5H), 7.56 (t, *J* = 7.7 Hz, 2H), 7.47 – 7.38 (m, 4H), 7.31 (t, *J* = 7.5 Hz, 2H), 6.28 (d, *J* = 6.2 Hz, 1H), 6.02 (d, *J* = 6.2 Hz, 1H), 4.34 (t, *J* = 4.4 Hz, 1H), 4.30 (d, *J* = 11.3 Hz, 1H), 4.22 (d, *J* = 11.3 Hz, 1H), 4.08 (dd, *J* = 11.6, 4.3 Hz, 1H), 3.99 (dd, *J* = 11.6, 4.7 Hz, 1H), 2.11 (s, 3H), 2.07 (s, 3H), 1.34 (s, 3H), 1.07 (s, 9H) ppm; Labile proton could not be observed;

^{13}C NMR (126 MHz, CD_3OD) δ 172.3, 171.5, 168.0, 153.4, 153.3, 151.1, 143.9, 136.7, 136.6, 135.0, 134.3, 133.9, 133.8, 131.2, 131.1, 129.8, 129.4, 129.0, 128.9, 125.0, 88.5, 85.4, 81.4, 67.7, 65.2, 47.4, 27.5, 20.8, 20.4, 20.1, 17.1 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{39}H_{44}N_5O_7Si^+$: 722.3005; found 722.3011 (+0.9 ppm).



3.30

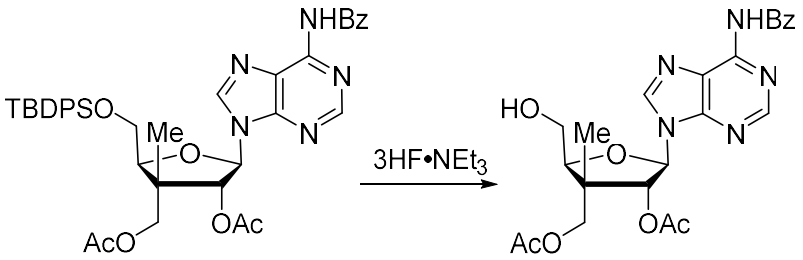
From 2D-NOESY

$H^{1'}$ (6.28 ppm) ⟷ $H^{4'}$ (4.34 ppm)

$H^{2'}$ (6.02 ppm) ⟷ H^{8} (8.44 ppm)

$H^{2'}$ (6.02 ppm) ⟷ H^{Me} (1.34 ppm)

(–)-((2*S*,3*R*,4*R*,5*R*)-4-Acetoxy-5-(6-benzamido-9*H*-purin-9-yl)-2-(hydroxymethyl)-3-methyltetrahydrofuran-3-yl)methyl (3.31)



To a solution of N^6-benzoyladenine nucleoside analogue **3.30** (40 mg, 55 μmol, 1.0 equiv.) in anhydrous THF (0.55 mL, 0.10 M), 3HF•NEt_3 (0.14 mL, 0.83 mmol, 15 equiv.) was added at room temperature and the resulting mixture was stirred for 16 hours. Triethylamine (0.77 mL, 5.5 mmol, 100 equiv.) was then added and the mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (DCM/MeOH, 95:5), provided the 1′,2′-*trans* ribo-like N^6-benzoyladenine nucleoside analogue **3.31** (23 mg, 86%) as a white foam.

R_f = 0.54 (DCM/MeOH, 90:10);

$[\alpha]^{25}_D$ –51 (*c* 0.7, MeOH);

Formula: $C_{23}H_{25}N_5O_7$; **MW:** 483.48 g/mol;

IR (neat) ν_{max} 3300 (br), 3069, 2927, 2855, 1742 cm^{-1};

^{1}H NMR (500 MHz, CD_3OD) δ 8.82 (s, 1H), 8.70 (s, 1H), 8.09 – 8.08 (m, 2H), 7.67 – 7.64 (m, 1H), 7.58 – 7.55 (m, 2H), 6.35 (d, *J* = 6.7 Hz, 1H), 5.92 (d, *J* = 6.7 Hz, 1H), 4.31 – 4.25 (m, 3H), 3.93 (dd, *J* = 12.2, 3.1 Hz, 1H), 3.84 (dd, *J* = 12.2, 3.3 Hz, 1H), 2.16 (s, 3H), 2.03 (s, 3H), 1.34 (s, 3H) ppm; Labile protons could not be observed;

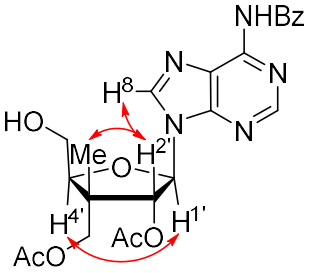
^{13}C NMR (126 MHz, CD_3OD) δ 172.4, 171.5, 168.1, 153.4, 153.1, 151.2, 144.5, 135.0, 133.9, 129.8, 129.4, 125.2, 88.4, 86.2, 81.6, 67.9, 63.0, 47.3, 20.8, 20.4, 16.6 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{23}H_{26}N_5O_7^+$: 484.1827; found 484.1831 (+0.8 ppm).

The relative configuration of ribo-like adenine nucleoside analogue **3.31** was determined by relevant nuclear Overhauser effect (nOe) enhancements (2D NOESY) confirming the 1′,2′-*trans* stereoselectivity. ^{1}H/^{13}C 2D HMBC was used to confirm the N^9-

regioselective glycosylation. The peaks in the ^{1}H NMR and ^{13}C NMR spectra were assigned using $^{1}H/^{1}H$ 2D COSY, $^{1}H/^{13}C$ 2D HSQC, chemical shifts and coupling constants.

Interactions observed by NOESY experiment

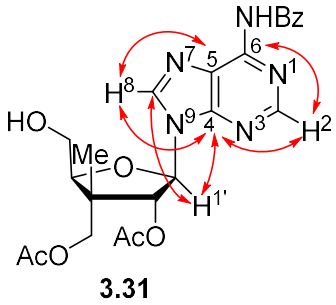


3.31

From 2D-NOESY

$H^{1'}$ (6.35 ppm) ⟷ $H^{4'}$ (4.28 ppm)
$H^{2'}$ (5.92 ppm) ⟷ H^{8} (8.82 ppm)
$H^{2'}$ (5.92 ppm) ⟷ H^{Me} (1.34 ppm)

HSQC and HMBC experiments confirmed the N9-regiochemistry



3.31

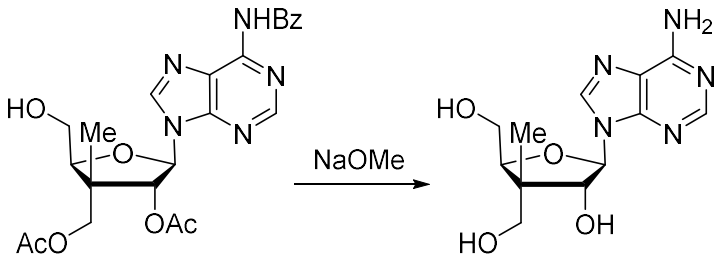
From HSQC

H^{8} (8.82 ppm) ⟷ C^{8} (144.5 ppm)
H^{2} (8.70 ppm) ⟷ C^{2} (153.1 ppm)
$H^{1'}$ (6.35 ppm) ⟷ $C^{1'}$ (88.4 ppm)

From HMBC

H^{8} (8.82 ppm) ⟷ C^{5} (125.2 ppm)
H^{8} (8.82 ppm) ⟷ C^{4} (153.4 ppm)
H^{2} (8.70 ppm) ⟷ C^{6} (151.2 ppm)
H^{2} (8.70 ppm) ⟷ C^{4} (153.4 ppm)
$H^{1'}$ (6.35 ppm) ⟷ C^{4} (153.4 ppm)
$H^{1'}$ (6.35 ppm) ⟷ C^{8} (144.5 ppm)

(–)-((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxy-3-methyltetrahydrofuran-2,3-diyl)dimethanol (3.35)



3.31 **3.35**

Following procedure A, NaOMe (25 μL, 25 μmol, 1.0 equiv., 1.0 M in MeOH) was added to a solution of nucleoside **3.31** (12 mg, 25 μmol, 1.0 equiv.) in MeOH (0.25 mL, 0.10 M). Purification by C18 reverse phase flash chromatography (MeOH/H_2O) provided 1′,2′-*trans* ribo-like adenine nucleoside analogue **3.35** (6 mg, 82%) as a white foam.

R_f = 0.30 (DCM/MeOH, 80:20);

$[\alpha]^{25}_D$ –42 (*c* 0.2, MeOH);

Formula: $C_{12}H_{17}N_5O_4$; **MW:** 295.30 g/mol;

IR (neat) ν_{max} 3332 (br), 3193 (br), 2928, 2882, 1649 cm^{-1};

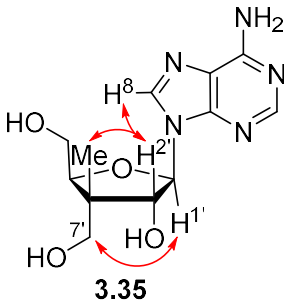
^{1}H NMR (500 MHz, CD_3OD) δ 8.30 (s, 1H), 8.18 (s, 1H), 6.03 (d, *J* = 7.4 Hz, 1H), 4.66 (d, *J* = 7.5 Hz, 1H), 4.23 (t, *J* = 2.6 Hz, 1H), 3.92 (dd, *J* = 12.6, 2.7 Hz, 1H), 3.80 (d, *J* = 11.0 Hz, 1H), 3.73 (dd, *J* = 12.6, 2.6 Hz, 1H), 3.61 (d, *J* = 11.0 Hz, 1H), 1.26 (s, 3H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, CD_3OD) δ 157.6, 153.3, 149.9, 142.4, 121.1, 91.4, 85.9, 80.9, 66.5, 63.9, 48.1, 16.4 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{12}H_{18}N_5O_4^+$: 296.1353; found 296.1356 (+1.0 ppm).

The relative configuration of ribo-like adenine nucleoside analogue **3.35** was determined by relevant nuclear Overhauser effect (nOe) enhancements (2D NOESY) confirming the 1′,2′-*trans* stereoselectivity. ^{1}H/^{13}C 2D HMBC was used to confirm the N^9-regioselective glycosylation. The peaks in the ^{1}H NMR and ^{13}C NMR spectra were assigned using ^{1}H/^{1}H 2D COSY, ^{1}H/^{13}C 2D HSQC, chemical shifts and coupling constants.

Interactions observed by NOESY experiment

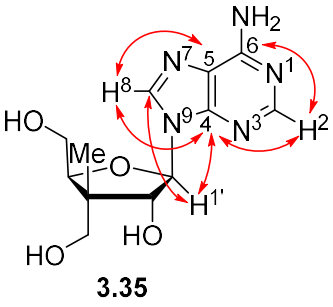


3.35

From 2D-NOESY

$H^{1'}$ (6.03 ppm) ⟷ $H^{7'}$ (3.80 ppm)
$H^{2'}$ (4.66 ppm) ⟷ H^{8} (8.30 ppm)
$H^{2'}$ (4.66 ppm) ⟷ H^{Me} (1.26 ppm)

HSQC and HMBC experiments confirmed the N9-regiochemistry



3.35

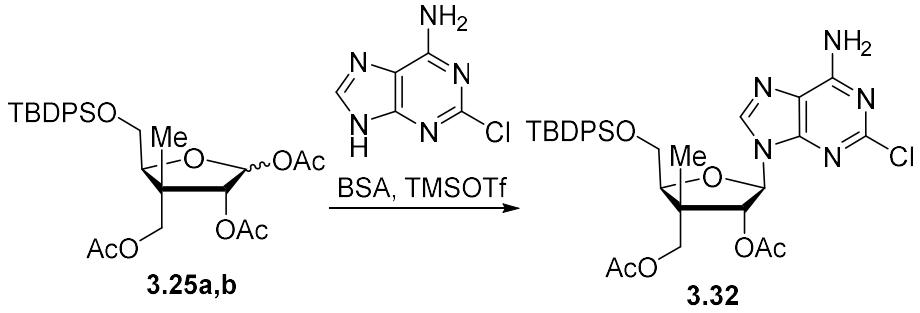
From HSQC

H^{8} (8.30 ppm) ⟷ C^{8} (142.4 ppm)
H^{2} (8.18 ppm) ⟷ C^{2} (153.3 ppm)
$H^{1'}$ (6.03 ppm) ⟷ $C^{1'}$ (91.4 ppm)

From HMBC

H^{8} (8.30 ppm) ⟷ C^{5} (121.1 ppm)
H^{8} (8.30 ppm) ⟷ C^{4} (149.9 ppm)
H^{2} (8.18 ppm) ⟷ C^{6} (157.6 ppm)
H^{2} (8.18 ppm) ⟷ C^{4} (149.9 ppm)
$H^{1'}$ (6.03 ppm) ⟷ C^{4} (149.9 ppm)
$H^{1'}$ (6.03 ppm) ⟷ C^{8} (142.4 ppm)

(+)-((2*S*,3*R*,4*R*,5*R*)-4-Acetoxy-5-(6-amino-2-chloro-9*H*-purin-9-yl)-2-(((*tert*-butyldiphenylsilyl)oxy)methyl)-3-methyltetrahydrofuran-3-yl)methyl acetate (3.32)



To a suspension of 2-chloroadenine (47 mg, 0.28 mmol, 2.5 equiv.) in anhydrous DCE (1.4 mL, 0.08 M), bis(trimethylsilyl)acetamide (0.15 mL, 0.61 mmol, 5.5 equiv.) was added at room temperature. The resulting mixture was vigorously stirred for 2 hours until a clear solution was obtained. The solution was concentrated under high vacuum and treated with a

solution of **3.25a,b** (60 mg, 0.11 mmol, 1.0 equiv.) in anhydrous DCE (1.4 mL, 0.08 M). After adding TMSOTf (40 µL, 0.22 mmol, 2.0 equiv.), the resulting solution was heated at reflux for 4 hours. The solution was diluted in DCM (5 mL) and washed with a saturated $NaHCO_3$ solution (2 mL). The aqueous phase was extracted with DCM (3 × 5 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The ^1H NMR spectrum of the crude reaction mixture indicated a >20:1 dr and only the N9-regioisomer. Purification by flash chromatography on silica gel (DCM/MeOH, 95:5) provided 2-chloroadenine nucleoside analogue **3.32** (61 mg, 85%) as a white foam.

R_f = 0.32 (DCM/MeOH, 95:5);

$[\alpha]^{25}_D$ +6.7 (*c* 1.0, MeOH);

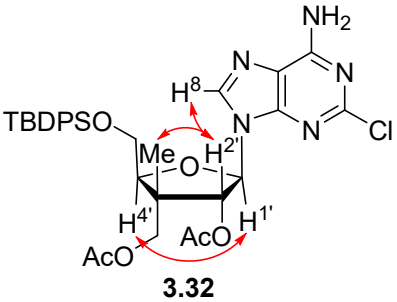
Formula: $C_{32}H_{38}ClN_5O_6Si$; **MW:** 652.22 g/mol;

IR (neat) ν_{max} 3319 (br), 3170 (br), 2957, 2932, 2893, 2859, 1744, 1646 cm^{-1};

^1H NMR (500 MHz, CD_3OD) δ 8.10 (s, 1H), 7.68 – 7.63 (m, 4H), 7.46 – 7.31 (m, 6H), 6.08 (d, *J* = 6.3 Hz, 1H), 5.89 (d, *J* = 6.4 Hz, 1H), 4.30 – 4.27 (m, 2H), 4.19 (d, *J* = 11.3 Hz, 1H), 4.06 (dd, *J* = 11.6, 4.1 Hz, 1H), 3.94 (dd, *J* = 11.7, 4.3 Hz, 1H), 2.10 (s, 3H), 2.08 (s, 3H), 1.31 (s, 3H), 1.06 (s, 9H) ppm; Labile proton could not be observed;

^{13}C NMR (126 MHz, CD_3OD) δ 172.4, 171.6, 158.0, 155.4, 151.8, 140.8, 136.8, 136.7, 134.2, 133.7, 131.12, 131.11, 129.0, 128.9, 119.3, 88.2, 85.4, 81.5, 67.7, 65.2, 47.4, 27.6, 20.8, 20.5, 20.1, 17.0 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{32}H_{39}ClN_5O_6Si^+$: 652.2353; found 652.2344 (–1.3 ppm).



3.32

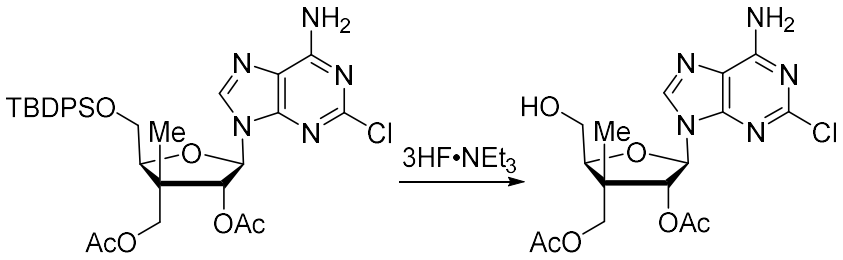
From 2D-NOESY

$H^{1'}$ (6.08 ppm) ⟷ $H^{4'}$ (4.29 ppm)

$H^{2'}$ (5.89 ppm) ⟷ H^{8} (8.10 ppm)

$H^{2'}$ (5.89 ppm) ⟷ H^{Me} (1.31 ppm)

(–)-((2*S*,3*R*,4*R*,5*R*)-4-Acetoxy-5-(6-amino-2-chloro-9*H*-purin-9-yl)-2-(hydroxymethyl)-3-methyltetrahydrofuran-3-yl)methyl acetate (3.33)



To a solution of silylether **3.32** (60 mg, 92 μmol, 1.0 equiv.) in anhydrous THF (0.9 mL, 0.1 M), 3HF•NEt_3 (0.23 mL, 1.4 mmol, 15 equiv.) was added at room temperature and the resulting mixture was stirred for 16 hours. Triethylamine (1.3 mL, 9.2 mmol, 100 equiv.) was then added and the mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (DCM/MeOH, 95:5), provided the 1′,2′-*trans* ribo-like 2-chloroadenine nucleoside analogue **3.33** (33 mg, 87%) as a white foam.

R_f = 0.50 (DCM/MeOH, 90:10);

$[\alpha]^{25}_D$ –75 (*c* 0.2, MeOH);

Formula: $C_{16}H_{20}ClN_5O_6$; **MW:** 413.82 g/mol;

IR (neat) ν_{max} 3326 (br), 2942, 1743, 1618 cm^{-1};

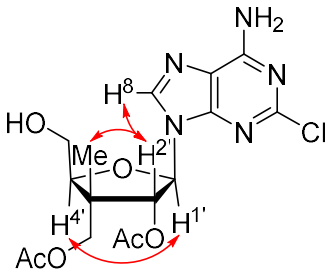
^{1}H NMR (500 MHz, CD_3OD) δ 8.41 (s, 1H), 6.12 (d, *J* = 6.8 Hz, 1H), 5.80 (d, *J* = 6.8 Hz, 1H), 4.27 (d, *J* = 11.2 Hz, 1H), 4.24 – 4.21 (m, 2H), 3.91 (dd, *J* = 12.4, 3.0 Hz, 1H), 3.80 (dd, *J* = 12.4, 3.2 Hz, 1H), 2.15 (s, 3H), 2.05 (s, 3H), 1.32 (s, 3H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, CD_3OD) δ 172.5, 171.5, 158.1, 155.2, 151.7, 141.6, 119.4, 88.4, 86.0, 81.4, 67.9, 63.0, 47.2, 20.8, 20.4, 16.6 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{16}H_{21}ClN_5O_6^+$: 414.1175; found 414.1169 (–1.5 ppm).

The relative configuration of ribo-like 2-chloroadenine nucleoside analogue **3.33** was determined by relevant nuclear Overhauser effect (nOe) enhancements (2D NOESY) confirming the 1′,2′-*trans* stereoselectivity. ^{1}H/^{13}C 2D HMBC was used to confirm the N^9-regioselective glycosylation. The peaks in the ^{1}H NMR and ^{13}C NMR spectra were assigned using ^{1}H/^{1}H 2D COSY, ^{1}H/^{13}C 2D HSQC, chemical shifts and coupling constants.

Interactions observed by NOESY experiment



3.33

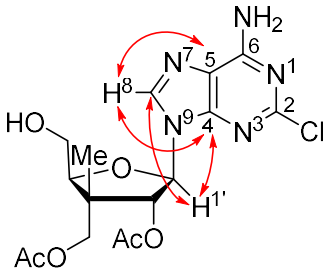
From 2D-NOESY

$H^{1'}$ (6.12 ppm) ⟷ $H^{4'}$ (4.24 ppm)

$H^{2'}$ (5.80 ppm) ⟷ H^{8} (8.41 ppm)

$H^{2'}$ (5.80 ppm) ⟷ H^{Me} (1.32 ppm)

HSQC and HMBC experiments confirmed the N9-regiochemistry



3.33

From HSQC

H^{8} (8.41 ppm) ⟷ C^{8} (141.6 ppm)

$H^{1'}$ (6.12 ppm) ⟷ $C^{1'}$ (151.7 ppm)

From HMBC

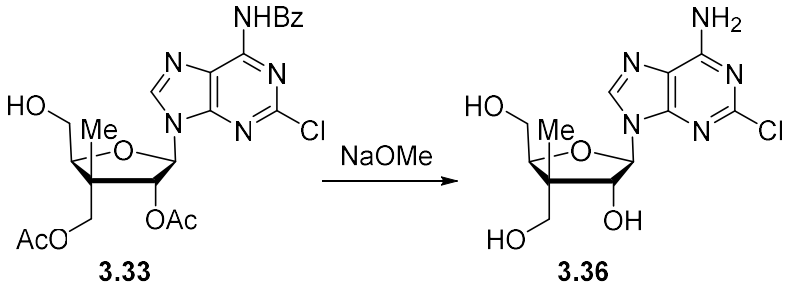
H^{8} (8.41 ppm) ⟷ C^{5} (119.4 ppm)

H^{8} (8.41 ppm) ⟷ C^{4} (151.7 ppm)

$H^{1'}$ (6.12 ppm) ⟷ C^{8} (141.6 ppm)

$H^{1'}$ (6.12 ppm) ⟷ C^{4} (151.7 ppm)

(–)-((2*S*,3*S*,4*R*,5*R*)-5-(6-amino-2-chloro-9*H*-purin-9-yl)-4-hydroxy-3-methyltetrahydrofuran-2,3-diyl)dimethanol (3.36)



3.33 **3.36**

Following procedure A, NaOMe (24 µL, 24 µmol, 1.0 equiv., 1.0 M in MeOH) was added to a solution of nucleoside **3.33** (10 mg, 24 µmol, 1.0 equiv.) in MeOH (0.24 mL, 0.10 M). Purification by C18 reverse phase flash chromatography (MeOH/H_2O) provided 1′,2′-*trans* ribo-like 2-chloroadenine nucleoside analogue **3.36** (6.4 mg, 80%) as a white foam.

R_f = 0.50 (DCM/MeOH, 80:20);

$[\alpha]^{25}_D$ –25 (*c* 0.2, MeOH);

Formula: $C_{12}H_{16}ClN_5O_4$; **MW:** 329.74 g/mol;

IR (neat) ν_{max} 3322 (br), 3186 (br), 2940, 2884, 1653 cm^{-1};

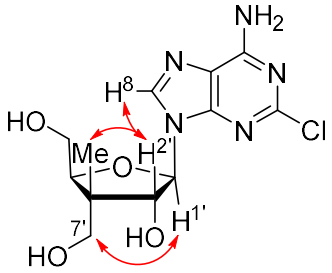
^{1}H NMR (500 MHz, CD_3OD) δ 8.30 (s, 1H), 5.98 (d, *J* = 7.3 Hz, 1H), 4.61 (d, *J* = 7.3 Hz, 1H), 4.22 (t, *J* = 2.9 Hz, 1H), 3.90 (dd, *J* = 12.5, 2.9 Hz, 1H), 3.79 (d, *J* = 11.0 Hz, 1H), 3.74 (dd, *J* = 12.5, 3.0 Hz, 1H), 3.60 (d, *J* = 11.0 Hz, 1H), 1.24 (s, 3H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, CD_3OD) δ 158.2, 155.0, 151.4, 142.4, 119.9, 91.1, 85.8, 81.1, 66.4, 63.8, 48.1, 16.4 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{12}H_{17}ClN_5O_4^+$: 330.0964; found 330.0958 (−1.7 ppm).

The relative configuration of ribo-like 2-chloroadenine nucleoside analogue **3.36** was determined by relevant nuclear Overhauser effect (nOe) enhancements (2D NOESY) confirming the 1′,2′-*trans* stereoselectivity. ^{1}H/^{13}C 2D HMBC was used to confirm the N^9-regioselective glycosylation. The peaks in the ^{1}H NMR and ^{13}C NMR spectra were assigned using ^{1}H/^{1}H 2D COSY, ^{1}H/^{13}C 2D HSQC, chemical shifts and coupling constants.

Interactions observed by NOESY experiment



3.36

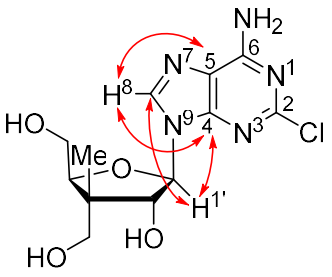
From 2D-NOESY

$H^{1'}$ (5.98 ppm) ⟷ $H^{7'}$ (3.79 ppm)

$H^{2'}$ (4.61 ppm) ⟷ H^8 (8.30 ppm)

$H^{2'}$ (4.61 ppm) ⟷ H^{Me} (1.24 ppm)

HSQC and HMBC experiments confirmed the N9-regiochemistry



3.36

From HSQC

H^8 (8.30 ppm) ⟷ C^8 (142.4 ppm)

$H^{1'}$ (5.98 ppm) ⟷ $C^{1'}$ (91.1 ppm)

From HMBC

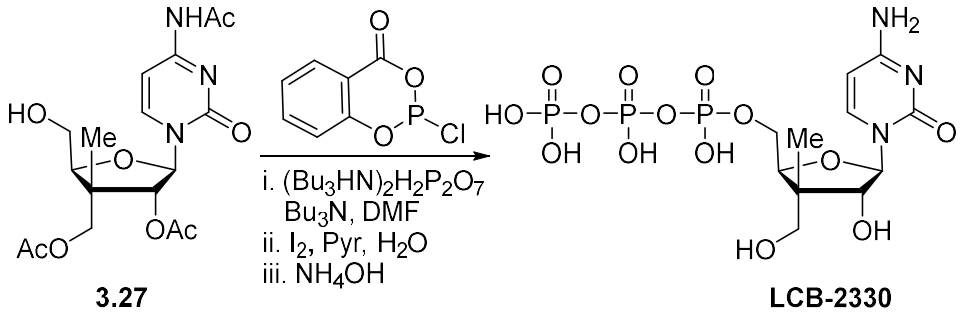
H^8 (8.30 ppm) ⟷ C^5 (119.9 ppm)

H^8 (8.30 ppm) ⟷ C^4 (151.4 ppm)

$H^{1'}$ (5.98 ppm) ⟷ C^8 (142.4 ppm)

$H^{1'}$ (5.98 ppm) ⟷ C^4 (151.4 ppm)

((2*S*,3*S*,4*R*,5*R*)-5-(4-Amino-2-oxopyrimidin-1(2*H*)-yl)-4-hydroxy-3-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate (LCB-2330)



Representative procedure B[9]: Prior to the reaction, nucleoside analogue **3.27**, salicyl phosphorochloridite (SalPCl) and tributylammonium pyrophosphate [$(Bu_3HN)_2H_2P_2O_7$] were respectively dried under reduced pressure in 10 mL, 5 mL, 5 mL flasks for 1 hour. To a solution of $(Bu_3HN)_2H_2P_2O_7$ (0.11 g, 0.20 mmol, 2.5 equiv.) in anhydrous DMF (0.8 mL, 0.1 M), NBu_3 (0.48 mL, 0.17 M) was added under nitrogen atmosphere. The reaction mixture was injected into a 5 mL flask containing SalPCl (40 mg, 0.20 mmol, 2.5 equiv.) and the resulting mixture was stirred at room temperature for 30 minutes. The mixture was then transferred to a flask containing nucleoside **3.27** (36 mg, 0.079 mmol, 1.0 equiv.) and the resulting mixture was stirred for 1.5 hours. A solution of iodine (3% in Pyr: H_2O 9:1 wV) was injected dropwise into the solution until a permanent brown color was persisted (~0.5 mL) and the resulting mixture was stirred for 15 minutes. Water (1.5 mL) was added and the solution was stirred for 1.5 hours to provide the desired C5'-triphosphate which was detected by TLC: R_f = 0.72 (*i*-PrOH: NH_4OH: H_2O, 5:3:2). The reaction mixture was transferred into a centrifuge tube using 15 mL of EtOH. A solution of 3M NaCl was added dropwise until the reaction mixture became cloudy (~0.5 mL) and was cooled to –78 °C for 1 hour. Centrifugation was conducted at 10 °C with 3200 rpm for 20 minutes and the resulting liquid phase was then transferred to a 10 mL Erlenmeyer flask. The resulting solid inside the centrifuge tube was air dried for 30 minutes, treated with NH_4OH (4.0 mL, 0.02 M) and stirred overnight. The mixture was concentrated under reduced pressure and the residue was purified by reverse phase C18 flash chromatography (0-20% MeCN in 20 mM triethylammonium acetate buffer) to provide cytosine nucleoside triphosphate **LCB-2330** triethylammonium salt (20 mg, 28%, 2.7 equiv triethylammonium) as a white powder.

Formula: $C_{11}H_{20}N_3O_{14}P_3$; **MW:** 511.21 g/mol;

^{1}H NMR (500 MHz, D_2O, signals for triethylammonium denoted by *) δ 8.38 (d, *J* = 8.0 Hz, 1H), 6.42 (d, *J* = 8.0 Hz, 1H), 6.11 (d, *J* = 6.5 Hz, 1H), 4.42 – 4.41 (m, 1H), 4.32 (d, *J* = 6.8 Hz, 1H), 4.30 – 4.27 (m, 1H), 4.16 – 4.13 (m, 1H), 3.78 (d, *J* = 11.6 Hz, 1H), 3.64 (d, *J* = 11.5 Hz, 1H), 3.22 (q, *J* = 7.3 Hz, 16H*), 1.29 (t, *J* = 7.3 Hz, 24H*), 1.17 (s, 3H) ppm; Labile protons could not be observed;

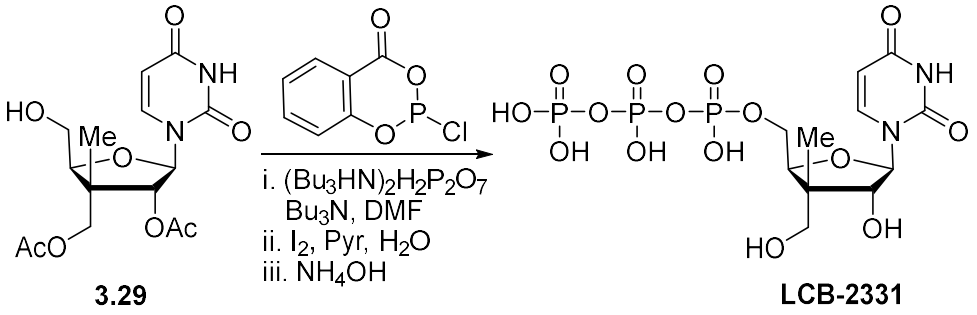
^{13}C NMR (126 MHz, D_2O, signals for triethylammonium denoted by *) δ 164.4, 155.9, 142.3, 88.9, 82.5 (d, *J* = 9.3 Hz), 80.4, 66.2, 64.5, 46.9, 46.6*, 42.2, 15.2, 8.2* ppm;

^{31}P NMR (162 MHz, D_2O) δ –10.69 (br s), –11.94 (s), –23.14 (br s) ppm;

HRMS (ESI–) *m/z* $[M - H]^-$ calcd for $C_{11}H_{19}N_3O_{14}P_3^-$: 510.0085; found 510.0087 (+0.4 ppm).

HPLC: t_R = 0.547 min.

((2*S*,3*S*,4*R*,5*R*)-5-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-hydroxy-3-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate (LCB-2331)



3.29 **LCB-2331**

Following general procedure B, $(Bu_3HN)_2H_2P_2O_7$ (85 mg, 0.15 mmol, 2.5 equiv.), NBu_3 (0.3 mL, 0.2 M), SalPCl (30 mg, 0.15 mmol, 2.5 equiv.) and uracil nucleoside analogue **3.29** (22 mg, 0.062 mmol, 1.0 equiv.) in anhydrous DMF (0.5 mL, 0.1 M) were employed in the phosphorylation. The final mixture was concentrated under reduced pressure. Purification by reverse phase C18 flash chromatography (0-20% MeCN in 20 mM triethylammonium acetate buffer) provided uracil nucleoside triphosphate **LCB-2331** triethylammonium salt (14 mg, 24%, 4.0 equiv triethylammonium) as a white powder.

Formula: $C_{11}H_{19}N_2O_{15}P_3$; **MW:** 512.19 g/mol;

^{1}H NMR (500 MHz, D_2O, signals for triethylammonium denoted by *) δ 8.12 (d, *J* = 8.2 Hz, 1H), 6.09 (d, *J* = 6.9 Hz, 1H), 6.02 (d, *J* = 8.0 Hz, 1H), 4.39 – 4.37 (m, 1H), 4.30 (d, *J* = 6.9 Hz, 1H), 4.27 – 4.23 (m, 1H), 4.17 – 4.13 (m, 1H), 3.77 (d, *J* = 11.5 Hz, 1H), 3.65 (d, *J* = 11.5 Hz, 1H), 3.22 (q, *J* = 7.3 Hz, 24H*), 1.29 (t, *J* = 7.3 Hz, 36H*), 1.17 (s, 3H) ppm; Labile protons could not be observed;

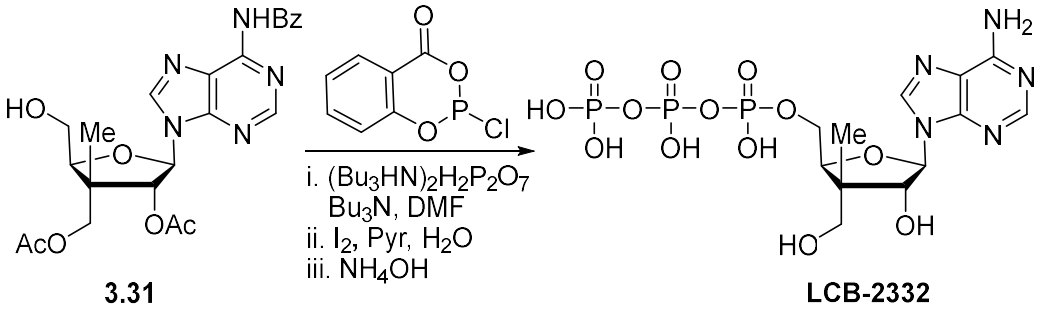
^{13}C NMR (126 MHz, D_2O, signals for triethylammonium denoted by *) δ 166.2, 152.2, 141.8, 102.7, 87.8, 82.3 (d, *J* = 10.4 Hz), 79.6, 66.2, 64.5, 46.6*, 42.2, 15.2, 8.2* ppm;

^{31}P NMR (162 MHz, D_2O) δ –9.80 (br s), –11.82 (d, *J* = 16.2 Hz), –23.15 (br s) ppm;

HRMS (ESI–) *m/z* $[M - H]^-$ calcd for $C_{11}H_{18}N_2O_{15}P_3^-$: 510.9920; found 510.9926 (+0.2 ppm).

HPLC: t_R = 0.473 min.

((2*S*,3*S*,4*R*,5*R*)-5-(6-Amino-9*H*-purin-9-yl)-4-hydroxy-3-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate (LCB-2332)



Following general procedure B, $(Bu_3HN)_2H_2P_2O_7$ (0.10 g, 0.18 mmol, 2.5 equiv.), NBu_3 (0.36 mL, 0.2 M), SalPCl (37 mg, 0.18 mmol, 2.5 equiv.) and adenine nucleoside analogue **3.31** (35 mg, 0.072 mmol, 1.0 equiv.) in anhydrous DMF (0.6 mL, 0.1 M) were employed in the phosphorylation. The final mixture was concentrated under reduced pressure. Purification by reverse phase C18 flash chromatography (0-20% MeCN in 20 mM triethylammonium acetate buffer) provided adenine nucleoside triphosphate **LCB-2332** triethylammonium salt (18 mg, 27%, 3.0 equiv triethylammonium) as a white powder.

Formula: $C_{12}H_{20}N_5O_{13}P_3$; **MW:** 535.24 g/mol;

^{1}H NMR (500 MHz, D_2O, signals for triethylammonium denoted by *) δ 8.66 (br s, 1H), 8.25 (s, 1H), 6.20 (d, *J* = 7.0 Hz, 1H), 4.76 – 4.73 (m, 1H), 4.46 (s, 1H), 4.25 – 4.14 (m, 1H), 3.85

(d, J = 11.5 Hz, 1H), 3.73 (d, J = 11.5 Hz, 1H), 3.19 (q, J = 7.3 Hz, 18H*), 1.27 (t, J = 7.3 Hz, 27H*), 1.24 (s, 3H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, D_2O, signals for triethylammonium denoted by *) δ 154.7, 151.6, 149.4, 140.5, 118.8, 86.9, 82.8 (d, J = 10.2 Hz), 80.1, 66.3 (d, J = 6.4 Hz), 64.5, 47.0, 46.5*, 15.1, 8.1* ppm;

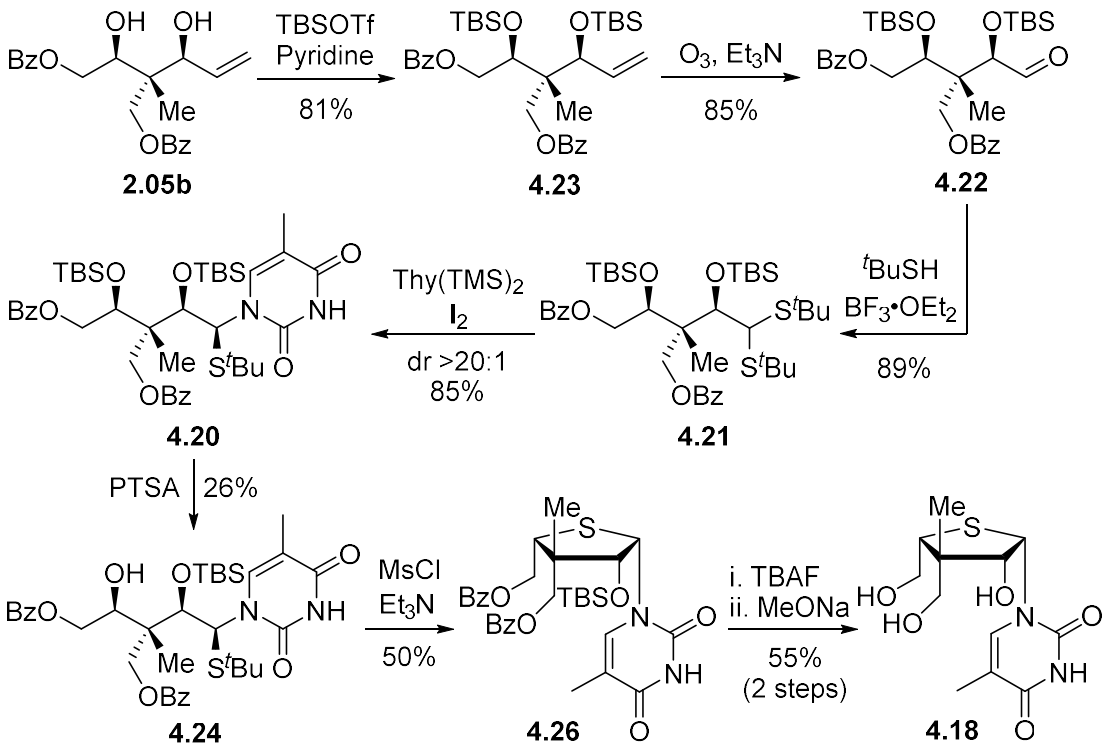
^{31}P NMR (162 MHz, D_2O) δ –11.68 (br s, 2P), –23.60 (br s, 1P) ppm;

HRMS (ESI–) *m/z* $[M - H]^-$ calcd for $C_{12}H_{19}N_5O_{13}P_3^-$: 534.0198; found 534.0203 (+1.0 ppm).

HPLC: t_R = 0.438 min.

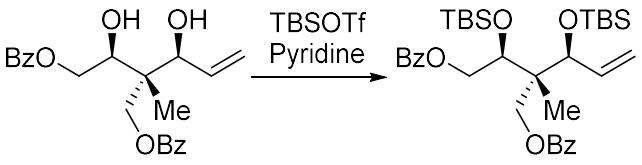
Experimental Procedures: Chapter 4

L-1′,2′-*cis*-4′-Thionucleoside analogue **4.18** was synthesized according to Scheme S6.



Scheme S6. Synthesis of L-1′,2′-*cis*-4′-thioanalogue **4.18**.

(+)-(2*S*,3*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-((*S*)-1-((*tert*-butyldimethylsilyl)oxy)allyl)-2-methylbutane-1,4-diyl dibenzoate (4.23)



2.05b **4.23**

To a solution of 2,4-*syn* diol **2.05b** (Chapter 2) (0.27 g, 0.70 mmol, 1.0 equiv.) in anhydrous DCM (7.0 mL, 0.1 M), pyridine (0.28 mL, 3.5 mmol, 5.0 equiv.) and TBSOTf (0.48 mL, 2.1 mmol, 3.0 equiv.) were added at 0 °C. The resulting mixture was stirred for 16 hours at room temperature. The reaction was quenched by addition of a saturated NH_4Cl solution (3 mL). The aqueous phase was extracted with DCM (3 × 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (Hexanes/EtOAc, 95:5) provided alkene **4.23** (0.35 g, 81%) as a colorless oil.

R_f = 0.42 (Hexanes/EtOAc, 95:5);

$[\alpha]^{25}_D$ +10 (*c* 0.8, CH_2Cl_2);

Formula: $C_{34}H_{52}O_6Si_2$; **MW:** 612.95 g/mol;

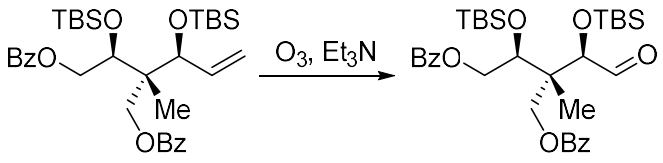
IR (neat) ν_{max} 2955, 2930, 2887, 2857, 1722 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 – 8.03 (m, 4H), 7.58 – 7.54 (m, 2H), 7.47 – 7.43 (m, 4H), 5.96 – 5.89 (m, 1H), 5.21 – 5.18 (m, 2H), 4.85 (dd, *J* = 12.0, 1.9 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.41 (dd, *J* = 12.0, 6.8 Hz, 1H), 4.37 (d, *J* = 11.2 Hz, 1H), 4.27 (dd, *J* = 6.7, 1.9 Hz, 1H), 4.26 (d, *J* = 11.2 Hz, 1H), 1.13 (s, 3H), 0.89 (s, 9H), 0.88 (s, 9H), 0.08 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H), 0.02 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.9, 166.5, 137.8, 133.04, 133.03, 130.43, 130.39, 129.8, 129.6, 128.6, 128.5, 117.7, 75.9, 74.1, 68.7, 66.2, 46.8, 26.2, 26.1, 18.5, 18.3, 16.7, −3.4, −3.7, −4.7, −4.8 ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{34}H_{52}O_6Si_2Na^+$: 635.3195; found 635.3202 (+1.2 ppm).

(+)-(2*R*,3*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-2-oxoethyl)-2-methylbutane-1,4-diyl dibenzoate (4.22)



4.23 **4.22**

To a solution of alkene **4.23** (0.30 g, 0.49 mmol, 1.0 equiv.) in anhydrous DCM (25 mL, 0.02 M) at −78 °C, ozone was bubbled through the reaction mixture until a slight blue color appeared at which point the ozone inlet was changed for a N_2 inlet and bubbling was continued for 15 minutes. Triethylamine (0.20 mL, 1.5 mmol, 3.0 equiv.) was added to the mixture which was stirred for 30 minutes at −78 °C. The cooling bath was removed, and the reaction mixture was warmed to room temperature with stirring for an additional 1 hour. The reaction mixture was filtered through a pad of $MgSO_4$ and evaporated under reduced pressure. Purification by flash chromatography on silica gel (Hexanes/EtOAc, 95:5) provided aldehyde **4.22** (0.26 g, 85%) as a colorless oil.

R_f = 0.32 (Hexanes/EtOAc, 95:5);

$[\alpha]^{25}_D$ +9.7 (*c* 1.0, CH_2Cl_2);

Formula: $C_{33}H_{50}O_7Si_2$; **MW:** 614.93 g/mol;

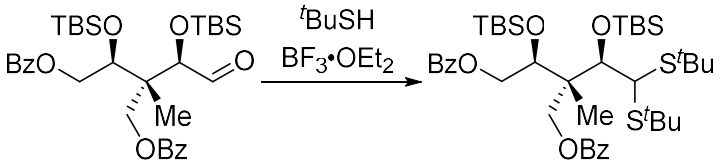
IR (neat) ν_{max} 2956, 2929, 2895, 2857, 1721 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.67 (d, *J* = 2.8 Hz, 1H), 7.94 – 7.89 (m, 4H), 7.56 – 7.49 (m, 2H), 7.41 (t, *J* = 7.8 Hz, 2H), 7.35 (t, *J* = 7.8 Hz, 2H), 4.58 – 4.54 (m, 1H), 4.50 (d, *J* = 11.5 Hz, 1H), 4.43 – 4.38 (m, 1H), 4.41 (s, 1H), 4.33 (d, *J* = 11.5 Hz, 1H), 4.23 (d, *J* = 2.7 Hz, 1H), 1.21 (s, 3H), 0.93 (s, 9H), 0.90 (s, 9H), 0.17 (s, 3H), 0.16 (s, 3H), 0.06 (s, 3H), 0.04 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 202.9, 166.5, 166.1, 133.22, 133.18, 129.82, 129.80, 129.72, 129.66, 128.6, 128.5, 78.8, 70.9, 66.7, 65.3, 48.3, 26.1, 25.9, 18.5, 18.3, 15.5, −4.0, −4.2, −4.6, −4.9 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{33}H_{51}O_7Si_2^+$: 615.3168; found 615.3172 (+0.6 ppm).

(–)-(2*R*,3*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-((*R*)-1-((*tert*-butyldimethylsilyl)oxy)-2,2-bis(*tert*-butylthio)ethyl)-2-methylbutane-1,4-diyl dibenzoate (4.21)



To a solution of aldehyde **4.22** (84 mg, 0.14 mmol, 1.0 equiv.) in anhydrous DCM (0.7 mL, 0.2 M), ^{t}BuSH (64 μL, 0.55 mmol, 4.0 equiv.) and BF_3•OEt_2 (42 μL, 0.34 mmol, 2.5 equiv.) were added at −78 °C. After stirring for 5 hours, the resulting mixture was treated with Et_3N (95 μL, 0.68 mmol, 5.0 equiv.). A saturated solution of $NaHCO_3$ (2 mL) was added slowly and the biphasic mixture was warmed to room temperature. After stirring vigorously for 30 minutes, the layers were separated, and the aqueous layer was extracted with DCM (3 × 5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (Hexanes/EtOAc, 95:5) provided dithioacetal **4.21** (94 mg, 89%) as a white foam.

R_f = 0.39 (Hexanes/EtOAc, 95:5);

$[\alpha]^{25}_D$ –0.3 (*c* 1.1, CH_2Cl_2);

Formula: $C_{41}H_{68}O_6S_2Si_2$; **MW:** 777.28 g/mol;

IR (neat) ν_{max} 2957, 2928, 2896, 2857, 1720 cm^{-1};

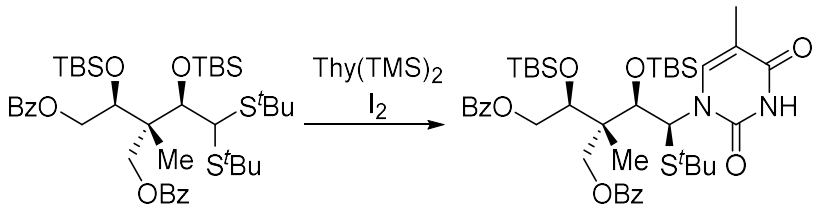
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 – 8.11 (m, 2H), 8.05 – 8.03 (m, 2H), 7.58 – 7.53 (m, 2H), 7.48 – 7.41 (m, 4H), 5.03 (dd, *J* = 11.7, 2.3 Hz, 1H), 4.79 – 4.78 (m, 1H), 4.72 (d, *J* = 10.9 Hz, 1H), 4.67 (d, *J* = 10.9 Hz, 1H), 4.63 (dd, *J* = 11.8, 4.9 Hz, 1H), 4.60 (s, 1H), 4.48 (s, 1H), 1.45 (s, 18H), 1.37 (s, 3H), 0.90 (s, 9H), 0.84 (s, 9H), 0.28 (s, 3H), 0.12 (s, 3H), 0.042 (s, 3H), 0.039 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 166.6, 133.1, 132.8, 130.7, 130.5, 129.8, 129.6, 128.48, 128.46, 83.0, 73.6, 68.7, 66.4, 49.1, 48.6, 45.9, 43.8, 32.1, 31.9, 26.6, 26.1, 22.4 (br), 19.2, 18.4, −2.5, −3.6, −4.0, −4.2 ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{41}H_{68}O_6S_2Si_2Na^+$: 799.3888; found 799.3872 (−2.0 ppm).

Preparation of silylated thymine: To a suspension of thymine (1.0 g, 7.9 mmol, 1.0 equiv.) in HMDS (5.0 mL, 24 mmol, 3.0 equiv.), $(NH_4)_2SO_4$ (23 mg, 0.17 mmol, 0.020 equiv.) was added. The reaction mixture was heated at reflux until a clear solution was obtained (~3.5 hours). Upon cooling to room temperature, the solution was placed under high vacuum overnight to remove excess HMDS. A 0.40 M solution of the silylated thymine ($Thy(TMS)_2$) was prepared in anhydrous THF (20 mL).

(–)-(2*R*,3*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-((1*R*,2*S*)-1-((*tert*-butyldimethylsilyl)oxy)-2-(*tert*-butylthio)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)ethyl)-2-methyl butane-1,4-diyl dibenzoate (4.20)



4.21 **4.20**

To a solution of dithioacetal **4.21** (45 mg, 60 μmol, 1.0 equiv.) in anhydrous THF (0.6 mL, 0.1 M), $Thy(TMS)_2$ (0.45 mL, 0.18 mmol, 3.0 equiv., 0.40 M in THF) and iodine (30 mg, 0.12 mmol, 2.0 equiv.) were added.[10] The resulting mixture was stirred for 16 hours at room temperature. The reaction was quenched by addition of a saturated solution of $Na_2S_2O_3$ (2 mL) followed by addition of a few drops of 2N NaOH solution. The biphasic mixture was stirred vigorously until a clear solution was obtained at which point DCM (5 mL) was added and the layers were separated. The aqueous phase was extracted with DCM (3 × 5 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (Hexanes/EtOAc, 95:5) provided 1′,2′-*syn* thioaminal **4.20** (40 mg, 85%) as a white foam.

R_f = 0.40 (Hexanes/EtOAc, 70:30);

$[\alpha]^{25}_D$ –24 (*c* 1.0, CH_2Cl_2);

Formula: $C_{42}H_{64}N_2O_8SSi_2$; **MW:** 813.21 g/mol;

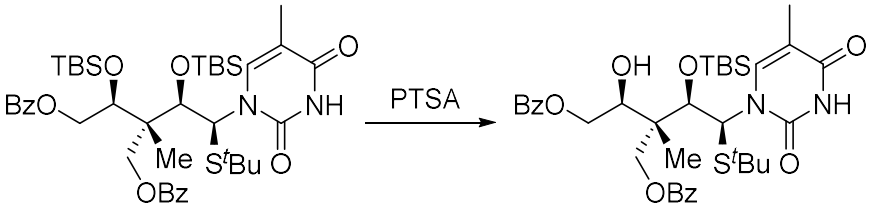
IR (neat) ν_{max} 3173 (br), 3064, 3038, 2956, 2930, 2896, 2858, 1719, 1699, 1677 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70 (br s, 1H), 8.08 (d, *J* = 7.3 Hz, 2H), 8.03 – 8.01 (m, 3H), 7.58 – 7.54 (m, 2H), 7.49 – 7.43 (m, 4H), 6.15 (s, 1H), 4.96 (dd, *J* = 11.9, 2.2 Hz, 1H), 4.81 (d, *J* = 11.1 Hz, 1H), 4.64 – 4.62 (m, 1H), 4.54 – 4.47 (m, 2H), 4.42 (s, 1H), 1.98 (s, 3H), 1.40 (s, 3H), 1.29 (s, 9H), 0.96 (s, 9H), 0.86 (s, 9H), 0.12 (s, 3H), 0.06 (s, 3H), 0.02 (s, 3H), –0.08 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.7, 166.4, 164.0, 150.5, 139.3, 133.2, 133.0, 130.4, 130.2, 129.7, 129.6, 128.7, 128.6, 109.7, 81.1 (br), 73.7, 68.4, 65.6, 62.4, 48.1, 44.7, 31.4, 26.6, 26.0, 21.5 (br), 19.2, 18.4, 12.4, −2.5, −3.6, −4.2, −5.1 ppm;

HRMS (ESI+) *m/z* $[M + NH_4]^+$ calcd for $C_{42}H_{68}O_8N_3SSi_2^+$: 830.4260; found 830.4251 (−1.2 ppm).

(–)-(2*R*,3*S*)-2-((1*R*,2*S*)-1-((*tert*-Butyldimethylsilyl)oxy)-2-(*tert*-butylthio)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)ethyl)-3-hydroxy-2-methylbutane-1,4-diyl dibenzoate (4.24)



4.20 **4.24**

To a solution of thioaminal **4.20** (32 mg, 40 μmol, 1.0 equiv.) in anhydrous DCM/MeOH (1:1, 2 mL, 0.02 M), PTSA (0.15 g, 0.80 mmol, 20 equiv.) was added at room temperature. The resulting solution was stirred at 50 °C for 40 hours. The mixture was then cooled to room temperature followed by addition of Et_3N (0.12 mL, 0.87 mmol, 22 equiv.). After evaporation of the volatiles, purification by flash chromatography on silica gel (Hexanes/EtOAc, 65:35) provided thioaminal **4.24** (10 mg, 26%) as a colorless oil.

R_f = 0.48 (Hexanes/EtOAc, 60:40);

$[\alpha]^{25}_D$ –22 (*c* 0.5, CH_2Cl_2);

Formula: $C_{36}H_{50}N_2O_8SSi$; **MW:** 698.95 g/mol;

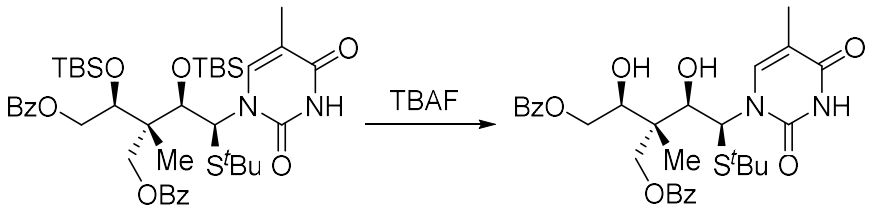
IR (neat) ν_{max} 3434 (br), 3180 (br), 3064, 3034, 2958, 2929, 2900, 2858, 1720, 1678 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.06 – 8.01 (m, 5H), 7.57 – 7.52 (m, 2H), 7.45 – 7.39 (m, 4H), 6.25 (s, 1H), 4.80 (d, *J* = 9.6 Hz, 1H), 4.66 (q, *J* = 12.4 Hz, 2H), 4.52 – 4.48 (m, 2H), 4.27 (s, 1H), 4.15 (d, *J* = 4.0 Hz, 1H), 1.97 (s, 3H), 1.29 (s, 9H), 1.25 (s, 3H), 1.01 (s, 9H), 0.12 (s, 3H), –0.03 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.9, 166.6, 163.4, 151.5, 139.2, 133.2, 133.1, 130.24, 130.18, 129.9, 129.7, 128.7, 128.4, 110.2, 82.0, 72.8, 66.9, 66.3, 63.0, 47.1, 44.8, 31.3, 26.5, 19.2, 17.2, 12.5, –1.8, –3.5 ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{36}H_{50}O_8N_2SSiNa^+$: 721.2949; found 721.2940 (−1.2 ppm).

(−)-(2*R*,3*S*)-2-((1*R*,2*S*)-2-(*tert*-Butylthio)-1-hydroxy-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)ethyl)-3-hydroxy-2-methylbutane-1,4-diyl dibenzoate (4.25)



4.20 **4.25**

To a solution of thioaminal **4.20** (65 mg, 80 μmol, 1.0 equiv.) in anhydrous THF (0.8 mL, 0.1 M), TBAF (80 μL, 80 μmol, 1.0 equiv.) was added at 0 °C. The resulting solution was stirred at room temperature for 24 hours. The mixture was quenched with saturated NH_4Cl solution (2 mL). The aqueous layer was extracted with EtOAc (3 × 5 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (Hexanes/EtOAc, 60:40) provided diol **4.25** (38 mg, 81%) as a white foam.

R_f = 0.30 (Hexanes/EtOAc, 50:50);

$[\alpha]^{25}_D$ –29 (*c* 1.0, CH_2Cl_2);

Formula: $C_{30}H_{36}N_2O_8S$; **MW:** 584.68 g/mol;

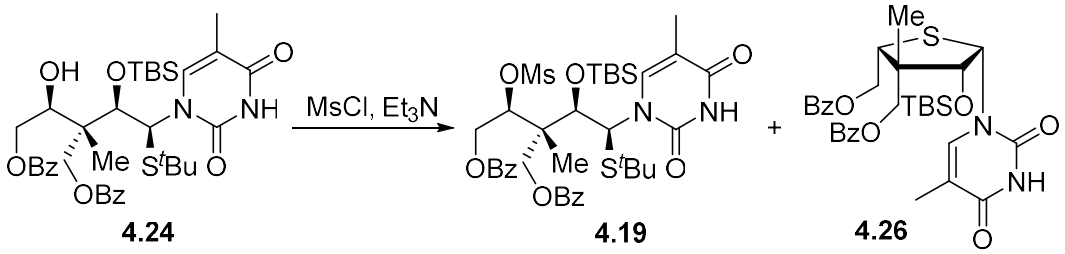
IR (neat) ν_{max} 3431 (br), 3219 (br), 3064, 3034, 2962, 2928, 2901, 2866, 1696, 1677 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.01 (d, *J* = 7.2 Hz, 4H), 7.82 (d, *J* = 1.0 Hz, 1H), 7.58 – 7.53 (m, 2H), 7.46 – 7.39 (m, 4H), 6.25 (d, *J* = 2.0 Hz, 1H), 4.70 – 4.65 (m, 2H), 4.50 – 4.46 (m, 2H), 4.34 (dd, *J* = 7.8, 2.3 Hz, 1H), 4.17 (s, 1H), 3.63 (s, 2H), 1.86 (d, *J* = 0.7 Hz, 3H), 1.33 (s, 3H), 1.31 (s, 9H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2, 166.5, 163.9, 150.7, 139.1, 133.5, 133.4, 129.9, 129.8, 129.74, 129.70, 128.7, 128.5, 110.6, 79.2, 74.0, 66.75, 66.74, 60.1, 45.3, 45.1, 31.2, 14.6, 12.6 ppm;

HRMS (ESI+) *m/z* $[M + Na]^+$ calcd for $C_{30}H_{36}N_2O_8SNa^+$: 607.2085; found 607.2073 (−1.8 ppm).

(−)-(2*S*,3*S*)-2-((1*R*,2*S*)-1-((*tert*-Butyldimethylsilyl)oxy)-2-(*tert*-butylthio)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)ethyl)-2-methyl-3-((methylsulfonyl)oxy)butane-1,4-diyl dibenzoate (4.19)



4.24 **4.19** **4.26**

To a solution of thioaminal **4.24** (38 mg, 54 μmol, 1.0 equiv.) in anhydrous DCM (0.2 mL, 0.3 M), Et_3N (30 μL, 0.22 mmol, 4.0 equiv.) and MsCl (13 μL, 0.16 mmol, 3.0 equiv.) were added at 0 °C. The resulting mixture was stirred for 24 hours at room temperature. Saturated aqueous NH_4Cl (~0.5 mL) was added to the mixture which was extracted with DCM (3 × 2 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography (Hexanes/EtOAc, 75:25) provided thioaminal **4.19** (7 mg, 17%) as a white foam and 4′-thionucleoside **4.26** (11 mg, 32%) as a white foam.

Thioaminal **4.19**

R_f = 0.41 (Hexanes/EtOAc, 60:40);

$[\alpha]^{25}_D$ –10 (*c* 0.1, CH_2Cl_2);

Formula: $C_{37}H_{52}N_2O_{10}S_2Si$; **MW:** 777.03 g/mol;

IR (neat) ν_{max} 3185 (br), 2957, 2926, 2855, 1723, 1688 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, *J* = 1.2 Hz, 1H), 8.08 – 8.06 (m, 2H), 8.04 (s, 1H), 8.00 – 7.98 (m, 2H), 7.61 – 7.57 (m, 2H), 7.48 – 7.44 (m, 4H), 6.41 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.10 (s, 1H), 5.19 (dd, *J* = 10.9, 2.5 Hz, 1H), 4.83 (d, *J* = 11.1 Hz, 1H), 4.48 – 4.44 (m, 2H), 4.27 (d, *J* = 11.2 Hz, 1H), 2.92 (s, 3H), 2.00 (d, *J* = 1.1 Hz, 3H), 1.42 (s, 9H), 1.25 (s, 3H), 1.02 (s, 9H), 0.09 (s, 3H), –0.12 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.3, 165.9, 163.4, 150.5, 139.1, 133.7, 133.6, 130.0, 129.64, 129.60, 129.4, 128.9, 128.8, 109.5, 80.6, 73.4, 68.8, 65.2, 62.8, 47.0, 45.6, 37.9, 31.4, 29.9, 26.6, 19.1, 12.5, –2.6, –5.4 ppm;

HRMS (ESI+) *m/z* $[M + NH_4]^+$ calcd for $C_{37}H_{56}N_3O_{10}S_2Si^+$: 794.3171; found 794.3157 (−1.7 ppm).

4′-Thionucleoside **4.26**

R_f = 0.21 (Hexanes/EtOAc, 70:30);

$[\alpha]^{25}_D$ –37 (*c* 0.5, DCM);

Formula: $C_{32}H_{40}N_2O_7SSi$; **MW:** 624.82 g/mol;

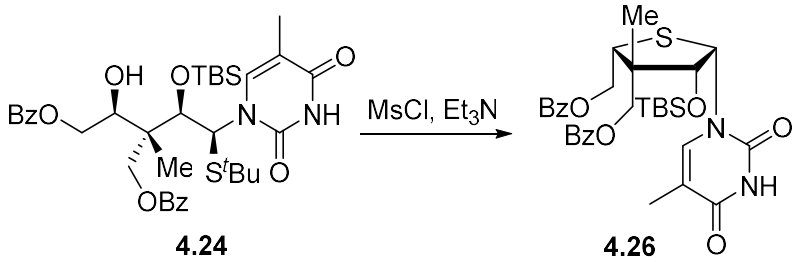
IR (neat) ν_{max} 3191 (br), 3070, 2954, 2929, 2898, 2857, 1720, 1688 cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 – 7.95 (m, 5H), 7.60 – 7.52 (m, 2H), 7.47 – 7.38 (m, 4H), 6.52 (d, *J* = 4.6 Hz, 1H), 5.29 (t, *J* = 10.3 Hz, 1H), 4.58 (dd, *J* = 10.9, 5.8 Hz, 1H), 4.52 (d, *J* = 11.1 Hz, 1H), 4.45 (d, *J* = 11.1 Hz, 1H), 4.29 (d, *J* = 4.6 Hz, 1H), 3.67 (dd, *J* = 9.7, 5.8 Hz, 1H), 1.99 (s, 3H), 1.48 (s, 3H), 0.98 (s, 9H), 0.10 (s, 3H), –0.20 (s, 3H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, $CDCl_3$) δ 166.33, 166.30, 163.1, 151.1, 141.3, 133.5, 133.4, 129.8, 129.7, 129.6, 128.7, 128.6, 109.9, 80.1, 66.2, 65.6, 64.1, 55.3, 53.0, 29.9, 26.1, 22.7, 18.2, 12.7, –4.7, –5.1 ppm;

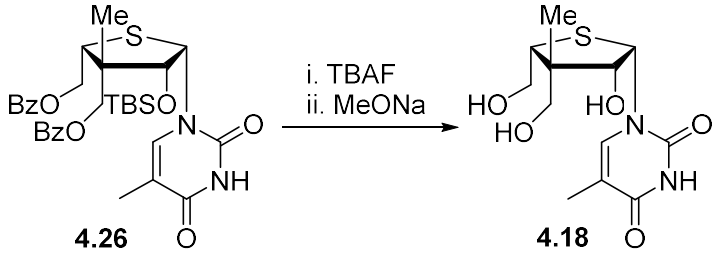
HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{32}H_{41}N_2O_7SSi^+$: 625.2398; found 625.2393 (–0.9 ppm).

(–)-((2*R*,3*S*,4*R*,5*S*)-4-((*tert*-Butyldimethylsilyl)oxy)-3-methyl-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrothiophene-2,3-diyl)bis(methylene) dibenzoate (4.26)



To a solution of thioaminal **4.24** (45 mg, 64 μmol, 1.0 equiv.) in anhydrous DCM (0.2 mL, 0.3 M), Et_3N (45 μL, 0.32 mmol, 5.0 equiv.) and MsCl (25 μL, 0.32 mmol, 5.0 equiv.) were added at 0 °C. The resulting mixture was stirred for 24 hours at room temperature. Saturated aqueous NH_4Cl (~0.5 mL) was added and the mixture was extracted with DCM (3 × 2 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography (Hexanes/EtOAc, 75:25) provided L-1′,2′-*cis*-4′-thionucleoside analogue **4.26** (20 mg, 50%) as a white foam, the full characterization of which was reported above.

(–)-1-((2*S*,3*R*,4*S*,5*R*)-3-Hydroxy-4,5-bis(hydroxymethyl)-4-methyltetrahydrothiophen-2-yl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (4.18)



To a solution of compound **4.26** (20 mg, 32 μmol, 1.0 equiv.) in anhydrous THF (0.32 mL, 0.10 M), TBAF (32 μL, 32 μmol, 1.0 equiv., 1.0 M in THF) was added at 0 °C. The resulting mixture was stirred for 24 hours at room temperature. Saturated aqueous NH_4Cl solution (~2 mL) was added and the mixture was extracted with EtOAc (3 × 5 mL). The

combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure.

To a solution of crude bis-benzoate protected nucleoside in anhydrous MeOH (0.32 mL, 0.10 M), NaOMe (32 µL, 32 µmol, 1.0 equiv., 1.0 M in MeOH) was added at room temperature. The resulting mixture was stirred for 3 hours. Amberlite acidic resin (~50 mg) was added and the mixture was stirred for 10 minutes, filtered, and concentrated under reduced pressure. The residue was purified by C18 reverse phase flash chromatography ($MeOH/H_2O$) to give L-1′,2′-*cis*-4′-thionucleoside analogue **4.18** (5.3 mg, 55% over two steps) as a white foam.

R_f = 0.29 (DCM/MeOH, 90:10);

$[\alpha]^{25}_D$ –68 (*c* 0.1, MeOH);

Formula: $C_{12}H_{18}N_2O_5S$; **MW:** 302.35 g/mol;

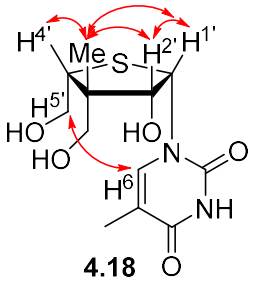
IR (neat) ν_{max} 3362 (br), 2976, 2927, 1691, 1674 cm^{-1};

^{1}H NMR (500 MHz, CD_3OD) δ 8.13 (s, 1H), 6.35 (d, *J* = 4.5 Hz, 1H), 3.96 – 3.93 (m, 2H), 3.89 (dd, *J* = 11.4, 4.5 Hz, 1H), 3.76 (d, *J* = 11.1 Hz, 1H), 3.73 (d, *J* = 10.9 Hz, 1H), 3.27 (t, *J* = 5.3 Hz, 1H), 1.90 (s, 3H), 1.32 (s, 3H) ppm; Labile protons could not be observed;

^{13}C NMR (126 MHz, CD_3OD) δ 166.5, 153.1, 142.3, 109.2, 79.7, 65.4, 64.3, 63.7, 60.4, 54.8, 22.9, 12.5 ppm;

HRMS (ESI+) *m/z* $[M + H]^+$ calcd for $C_{12}H_{19}N_2O_5S^+$: 303.1009; found 303.1007 (–0.7 ppm).

The relative configuration of L-1′,2′-*cis*-4′-thionucleoside analogue **4.18** was determined by relevant nuclear Overhauser effect (nOe) enhancements (2D NOESY). The peaks in the ^{1}H NMR spectra were assigned using ^{1}H/^{1}H 2D COSY experiment, chemical shifts and coupling constants.



4.18

From 2D-NOESY

H^6 (8.13 ppm) ⟷ $H^{5'}$ (3.89 ppm)

$H^{1'}$ (6.35 ppm) ⟷ $H^{2'}$ (3.94 ppm)

$H^{1'}$ (6.35 ppm) ⟷ H^{Me} (1.32 ppm)

$H^{2'}$ (3.94 ppm) ⟷ H^{Me} (1.32 ppm)

$H^{4'}$ (3.27 ppm) ⟷ H^{Me} (1.32 ppm)

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