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Nucleotide Analogues Bearing a C2′ or C3′-Stereogenic All-Carbon Quaternary Center as SARS-CoV-2 RdRp Inhibitors †

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† This paper is dedicated to Professor Stephen Hanessian—a mentor, colleague and friend.

Abstract: The design of novel nucleoside triphosphate (NTP) analogues bearing an all-carbon quaternary center at C2′ or C3′ is described. The construction of this all-carbon stereogenic center involves the use of an intramolecular photoredox-catalyzed reaction. The nucleoside analogues (NA) hydroxyl functional group at C2′ was generated by diastereoselective epoxidation. In addition, highly enantioselective and diastereoselective Mukaiyama aldol reactions, diastereoselective N-glycosylations and regioselective triphosphorylation reactions were employed to synthesize the novel NTPs. Two of these compounds are inhibitors of the RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2, the causal virus of COVID-19.

Keywords: SARS-CoV-2; COVID-19; RdRp; quaternary stereocenter; nucleoside analogues; epoxidation; glycosylation; triphosphorylation

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive-sense RNA virus and the causal agent of coronavirus (CoV) disease 2019 (COVID-19). CoVs employ a multi-subunit replication/transcription machinery. The virus enters the cell by endocytosis using the ACE 2 receptors and is then uncoated. ORF1a and ORF2 of the positive strand RNA are then translated to produce non-structural protein precursors (nsp), including proteins that further cleave the precursor to form mature functional helicase and RNA-dependent RNA polymerase [1] (RdRp, or alternatively nsp 12). The latter has been recognized as an optimal target for drug design, due to its crucial role in RNA synthesis, lack of host homologues and high structural conservation between coronaviruses.

The severity of SARS-CoV-2 disease has encouraged many laboratories to evaluate potential inhibitors of RdRp from related viruses. Sofosbuvir, a potent hepatitis c virus (HCV) polymerase inhibitor, was first studied. Inhibitors of single-stranded negative RNA viruses, such as Remdesivir (Figure 1A, Ebola) [2] and B-D-N4'-hydroxy-cytidine NHC [3] (Molnupiravir or EIDD-1931, influenza), along with other nucleotides, were also examined [4].
Remdesivir (RDV) was approved by the Food and Drug Administration (FDA) for the intravenous treatment of COVID-19 in hospitalized adult and pediatric patients [5]. RDV-TP (1), the active agent, is incorporated into RNA by the RdRp enzyme and then acts as a delayed chain termination of RNA synthesis at position i + 3 [6]. The Remdesivir-induced RdRp stalling is caused by a translocation barrier between the C1′-cyano group in the Remdesivir ribose moiety and the serine 861 side chain in the nsp 12 subunit of RdRp [7]. The truncation of serine 861 to alanine or glycine renders the RdRp less sensitive or insensitive to inhibition by Remdesivir [8]. An alternative mechanism was recently proposed involving the RdRp-dependent RNA proofreading. The mechanism of NHC (Molnupiravir), presently evaluated in clinical trials and is attributed to the triple level incorporation of NHC-TP, resulting in increased mutations and, ultimately, in a process known as “lethal mutagenesis”.

From a drug design standpoint, the antiviral activity of nucleoside analogues (NAs) depends on their efficient transport into cells where a first phosphorylation takes place (e.g., by cytidine kinase). These two critical steps are rate-limiting in the entire cascade that leads, through subsequent phosphorylation by other kinases, to the active nucleoside triphosphate (NTP) analogues that compete with natural nucleoside triphosphates. To circumvent these limitations, phosphoramidates were developed as monophosphate prodrugs that facilitate intracellular transport. The pro-drug is cleaved intracellularly, releasing the NA monophosphate that is then transformed into the corresponding bioactive triphosphorylated analogue. The main objective of the present study was to evaluate a novel series of triphosphorylated nucleotide analogues to test their activity
directly in vitro against RdRp [9]. If active, the corresponding phosphoramidate pro-drugs would then be installed on the parent NA for further evaluations. The nucleoside analogues reported in the literature thus far display additional substituents at C1′ (cyano for Remdesivir Figure 1A) and different C2′ or C3′ substituents (methyl or fluorine, Figure 1B). This was suggestive that further modification of the C2′ or C3′ positions would be tolerated.

Our laboratory has a long-standing interest in the development of new methodologies to improve the synthesis of nucleoside analogues. In parallel, we have been studying carbon-centered free radicals on acyclic molecules and their reactivity in atom transfer reactions, leading to the generation of all carbon stereogenic quaternary centers. Together, these findings [10–16] led to the conceptualization and synthesis of novel nucleoside analogues bearing a quaternary all-carbon stereogenic center at C3′ or C2′ [16–18].

The presence of these all-carbon quaternary centers also provides structural properties that may influence the recognition of these nucleosides or nucleotides by given targets (enzymes or a receptors). For instance, nucleosides and nucleotides are flexible molecules adopting conformations ranging between North (C3′ endo, Figure 1C) or South (C2′ endo, Figure 1C). Increasing the populations of nucleosides in their bio-active conformation may translate into a greater binding affinity to the target. We hypothesized that the presence of the quaternary center could induce a conformational bias when located at C2′ or C3′, the former would favor the North conformation (RNA-like) and the latter, the South (DNA-like). These conformational changes would be induced to minimize the steric effects (gauche effects) of the quaternary centers with their proximal substituents (Figure 1C). The X-ray analyses of some of our analogues having stereogenic quaternary centers at C2′ or C3′ supports these conformational biases. In solution, these molecules will, however, still possess some plasticity, allowing for conformational realignment during binding, contrary to locked nucleosides analogues [19]. The presence of the hydroxyl on this center could also potentially act as an extended pharmacophore, providing different proximal binding. On the other hand, binding to enzymes susceptible to steric hindrance at these positions could lead to inactivity. We thus embarked on the syntheses of a small library of nucleotides having stereogenic centers at C2′ or C3′ bearing adenine or cytosine nucleobases (Figure 1D) to investigate their inhibitory profile against RdRp.

2. Results and Discussion

The synthesis of the all-carbon quaternary stereogenic centers was based on our findings that a carbon-centered free radical flanked by an ester and a secondary carbon bearing an electronegative substituent (hydroxyl) could stereoselectively participate in kinetically controlled atom transfer reactions. Hydrogen and allylation transfer reactions have previously been studied both experimentally and theoretically by our group [20,21]. We recently prepared radical precursor 2 using an enantioselective Mukaiyama aldol reaction in good yield and with high diastereoselectivity in favor of the 3R isomer (Figure 2) [14]. The secondary alcohol was then transformed into dimethyallylsilyl ether 3. Acyclic intermediate 6, bearing the quaternary stereogenic center, was synthesized by a sequence involving an intramolecular atom transfer cyclization, an elimination reaction under photoredox catalysis and subsequent ester reduction with protection of alcohol generated.

Allylic oxidation of 6 using SeO₂ led to a modest 50% yield of the corresponding ketone 7 (Figure 2) [14]. The 2,4-syn diol 8 was then obtained from ketone 7 by reduction using catecholborane in the presence of cesium chloride. The key intermediate 8 was transformed to furanosides 9 and 10. The corresponding β-cytosine nucleoside analogue 11 was obtained from 9 by taking advantage of anchimeric participation by the acetate at C2′. On the other hand, α-cytosine NA 12 was accessed from 10 using Me2BBr [22].

Biological evaluation of the corresponding novel nucleotides necessitated an improvement in the overall synthesis of these C3′ quaternary substituted nucleosides. As reported herein (Figure 2), this was accomplished by introducing the hydroxyl at C2′ through a stereoselective epoxidation of glycal 13 [23,24] to the epoxide 14, followed by hydrolysis and stereoselective N-glycosylation to generate novel nucleoside analogues 16.
Synthesis of the requisite glycal intermediate began with secondary hydroxyl protection of methyl ester 5 with triethylsilyl ether (TES). Ester 17 was then reduced by DIBAL-H to the primary alcohol and further acetylated to give 18 in excellent yield (Scheme 1). Ozonolysis of the terminal alkene 18 provided aldehyde 19 in 85% yield.

Different pathways to prepare key intermediate 21 were explored. Cyclization of aldehyde 19 in the presence of PTSA in THF/H₂O led to lactols 20a,b with a 1.4:1 anomeric ratio (Scheme 2). Mesylation and elimination with Et₃N generated glycal 21 in 51% yield. Alternatively, methylfuranoside 22a,b was derived from aldehyde 19 in the presence of PTSA in anhydrous methanol. Methylfuranoside 22a,b were then treated with TMSOTf and 2,6-lutidine to give glycal 21 in an excellent yield.

Previous Work

This Work

Figure 2. Novel nucleoside analogues bearing a quaternary stereocenter at C3'.
Dondoni’s dimethyldioxirane (DMDO) [25], generated in situ with a catalytic amount of acetone and a stoichiometric amount of potassium peroxymonosulfate (oxone), was chosen as the oxidant. When glycal 21 was subjected to DMDO oxidation, the ribo-like epoxide 23a was obtained as the major epoxide in a 7:1 ratio relative to the arabino-like epoxide 23b (Scheme 3). The stereoselectivity is rationalized by spiro-like transition states TS A and TS B (Scheme 3), where DMDO approaches the olefin from the inside face of the glycals’ envelope-like conformations [26–28]. Attack from the bottom face in TS A avoids significant steric clash with the C5′ substituent in TS B (Scheme 3). Hydrolysis of the crude epoxides 23a and 23b in THF/H2O gave the ribo-like lactols 24a,b as the major compounds (63%, isolated by flash column chromatography). These lactols were then protected with acetyl groups to afford acetate ribofuranosides 25a,b in 85% yield.

With ribo-like diacetate furanosides 25a,b in hand, stereoselective N-glycosylations of 25a,b were performed in the presence of a silylated base (adenine or cytidine) and TMSOTf (Scheme 4). The 1′,2′-trans ribo-like nucleosides 26–28 were obtained in excellent diastereoselectivity (>20:1 dr) and yield (75–85%) in accordance with anchimeric assistance of the C2′ acetyl group. NMR spectroscopic analysis experiments confirmed the 1,2′-trans stereochemistries (2D NOESY) and, in the case of adenine coupling, N9 isomers (HMBC). Subsequent cleavage of the C5′-OTBDPS protecting groups with 3HF·NEt3 provided corresponding nucleoside analogues 29–31 (86–87%), which were the key precursors for the formation of the nucleoside C5′O-triphosphates. Further, cleavage of the C2′ and C3′ acetyl-
protecting groups with NaOMe provided 1′,2′-trans ribo-like nucleoside analogues 32-34 (80–82%).

Scheme 4. Stereoselective N-glycosylation with anichimeric assistance.

The synthesis of the C2′ quaternary series was then explored. The five-membered ring lactone 40, bearing the quaternary center at C2′, was prepared by a route previously reported by our group [13] (Scheme 5). The Mukaiyama aldol reaction of aldehyde 35 with tetrasubstituted silylated-enolether 36 provided 37a,b. The α-bromomethylesters 37a,b were subjected to lactonization, followed by installation of a TBS at the C5 primary alcohol. Installation of vinylidimethylsilane at the C3′ secondary alcohol provided lactones 39a,b, which were subjected to an atom transfer cyclization/elimination reaction using photoredox catalysis to give lactone 40. An alternative synthetic sequence was then optimized to reduce the number of steps to reach 43a,b [13]. A TBS-protecting group was introduced on the secondary hydroxyl of 40, followed by ozonolysis resulting in aldehyde 41 in excellent yield. After the simultaneous reduction of both the lactone and aldehyde using Red-Al, benzoylation provided furanosides 43a,b.

Scheme 5. Synthesis of key benzoylated furanosides 43a,b.

With benzoylated furanosides 43a,b in hand, stereoselective N-glycosidation of 43a,b with 2,6 dichloropurine in the presence of TMSOTf at −10 °C led to the regioselective formation of N9 nucleoside analogue 44 with high diastereoselectivity (β:α, 10:1) and good yield (73%, Scheme 6). The selective formation of the β-anomer is attributed to an anichimeric assistance from C2′ benzoate. The 1′,2′-trans stereochemistry was confirmed by 2D NOESY experiments, while the N9 regioselectivity was verified by the key indicative
three bond correlation between the H1’ of sugar and C4 of purine in $^{1}H/^{13}C$ 2D HMBC NMR experiments. Nucleoside 45 was formed from treatment with ammonia in methanol to give the 2-chloroadenosine and deprotection of the C2′ benzoate, followed by desilylation in the presence of 3HF-NEt$_3$. The 2-chloroadenosine analogue 45 was then hydrogenated to the adenosine derivative 46 in 78% yield. The cytidine analogue 47 was also prepared from the benzoylated furanoside, as reported by our group [13].

![Scheme 6](image)

**Scheme 6.** Synthesis of 1′,2′-trans ribo-like adenine nucleoside analogues.

2.1. **Synthesis of Nucleoside Triphosphates (NTPs)**

Having established synthetic routes to access nucleoside analogues bearing an all-carbon quaternary stereocenter at C2’ or C3’, we next investigated the synthesis of their C5′ triphosphate derivatives. The synthesis of NTPs represents a challenging task [29].

Initial triphosphorylation attempts using Taylor’s method, using trimetaphosphate and mesitylenesulfonyl chloride, were unsuccessful [30]. The one-pot synthesis approach of Huang and co-workers [31] was more appropriate for our substrates. The phosphorylation is accomplished under mild conditions using tributylammonium pyrophosphate in the presence of salicyl phosphorochloridite (SalPCI).

Phosphorylation of 1′,2′-trans ribo-like nucleoside analogues bearing either purine or pyrimidine nucleobases was carried out using SalPCI, (Bu$_3$HN)$_2$H$_2$P$_2$O$_7$ and Bu$_3$N in anhydrous DMF. Subsequent addition of protected nucleoside provided the five-cyclic triphosphate intermediates that were then subjected to iodine oxidation and hydrolysis. The cleavage of the C2’ and C3′acetyl-protecting groups with ammonium hydroxide (NH$_4$OH) then generated the corresponding 1′,2′-trans ribo-like nucleoside 5′-triphosphate (Scheme 7).

The final 1′,2′-trans ribo-like NTPs (2–4) were prepared in good yields (27–51%, Scheme 7) from the nucleoside analogues 29–31, respectively.

The phosphorylation of nucleoside analogues bearing a C2′ quaternary stereocenter was then conducted using the same strategy. Triphosphorylation of unprotected NAs 46–47 furnished the corresponding NTPs 5–7 in low, but acceptable yields, for this challenging transformation (5–13%, Scheme 8).
1',2'-trans ribo-like NAs 29–31

\[
\text{HO} \quad \text{Me} \quad \text{Base} \quad \text{AcO} \quad \text{OAc}
\]

\[
\text{1',2'-trans ribo-like NTPs 2–4}
\]

\[
\begin{align*}
\text{HO} & \quad \text{Me} \quad \text{Base} \\
\text{O} & \quad \text{O} \quad \text{O} \\
\text{AcO} & \quad \text{OAc}
\end{align*}
\]

\[
\begin{align*}
\text{i. (Bu}_3\text{HN})_2\text{H}_2\text{P}_2\text{O}_7 \\
\text{Bu}_3\text{N}, \text{DMF} \\
\text{ii. I}_2, \text{Pyr, H}_2\text{O} \\
\text{iii. NH}_4\text{OH}
\end{align*}
\]

\[
\begin{align*}
\text{NH}_2 & \quad \text{N} \\
\text{N} & \quad \text{X}
\end{align*}
\]

\[
\begin{align*}
\text{2 (LCB-2330), 28\%} \\
\text{3 X = H, (LCB-2332), 27\%} \\
\text{4 X = Cl, (LCB-2337), 51\%}
\end{align*}
\]

Scheme 7. Synthesis of 1',2'-trans ribo-like NTPs.

\[
\begin{align*}
\text{HO} & \quad \text{Me} \quad \text{Base} \\
\text{O} & \quad \text{O} \quad \text{O} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

\[
\begin{align*}
\text{5 (LCB-2289), 5\%} \\
\text{6 X = H, (LCB-2344), 5\%} \\
\text{7 X = Cl, (LCB-2279), 13\%}
\end{align*}
\]

Scheme 8. Synthesis of NTPs bearing C2' quaternary stereocenters.

2.2. SARS-CoV-2 RNA-Dependent RNA Polymerase (RdRp) Assay

Preliminary results for SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) inhibition suggested that the most active analogues were LCB-2344 (6) and LCB-2279 (7), which both bear purine nucleobases and the quaternary stereogenic center at C2' (Figure 3). These NTPs (6 and 7) appear to have just slightly lower activity than the commercial Remdesivir TP (1). We also prepared the REM-TP (1) using the approach described at Scheme 8, the activity against RdRp obtained with this product was similar to 6 (LCB-2344). NTP 5, bearing a cytosine nucleobase, was inactive. Nucleotide analogues (2–4), having the adenine, chloro-adenine and cytosine nucleobases at C1' and the quaternary center at C3', showed a lower activity profile.
was done on silica gel 60 (0.040–0.063 mm, Silicycle, QC, Canada) using an automated purification system. Thin-layer chromatography (TLC) was done on pre-coated (0.25 mm) Merck F-254 silica gel aluminum plates. Visualization was performed with short UV wavelength on a 500 MHz NMR spectrometer and the data is reported as follows: chemical shift in ppm referenced to residual solvent (CDCl₃ δ 7.26, CD3OD δ 3.31 and D2O δ 4.79 ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublet).

Figure 3. Inhibition of SARS-CoV-2 RdRp activity: (A) RNA 19-mer primer/43-mer template used in the SARS-CoV-2 RdRp reactions. (B) Full-length 43-mer RNA product syntheses by SARS-CoV-2 RdRp complex (nsp12/nsp7/nsp8) in the presence/absence of respective inhibitors. (–Pol) is the negative control without SARS-CoV-2 RdRp whereas (+Pol) is the no-treatment control. (T) is the 43-mer RNA template used in the assay. 5′ end 32P-labelled 20-mer DNA oligo served as the loading control (LC) for each reaction. Reduction in full-length 43-mer RNA synthesis by each inhibitor relative to the no-treatment control (+Pol) is presented as % inhibition normalized with the loading control (LC).

3. Materials and Methods
3.1. General Information

All the anhydrous solvents were purchased from Sigma Aldrich, Saint Louis, MO, USA. All the glassware was purchased from Pyrex USA. Reactions requiring anhydrous conditions were performed under an atmosphere of nitrogen in flame-dried glassware using standard syringe techniques. Molecular sieves (Sigma Aldrich, USA) were used to prepare the anhydrous solvents. The sieves (4 Å, 1–2 mm beads) were activated by heating at 180 °C for 48 h under vacuum prior to their addition into new bottles of solvent purged with argon. All commercially available reagents were used as received: TBSCI was purchased from TCI America, USA and TBSOTf was obtained from Oakwood Chemicals, USA. Remaining all reagents were purchased from Sigma Aldrich, USA. Flash chromatography was done on silica gel 60 (0.040–0.063 mm, Silicycle, QC, Canada) using an automated purification system. Thin-layer chromatography (TLC) was done on pre-coated (0.25 mm) Merck F-254 silica gel aluminum plates. Visualization was performed with short UV wave-
lengths and/or revealed with potassium permanganate solutions. See the $^{1}H$, $^{13}C$, and 2D NMR spectra data in the Supplementary Materials. The $^{1}H$ NMR spectra were recorded at room temperature on a 700 MHz, 500 MHz and 400 MHz NMR spectrometer and the data is reported as follows: chemical shift in ppm referenced to residual solvent (CDCl$_3$ $\delta$ 7.26, CD$_2$OD $\delta$ 3.31 and D$_2$O $\delta$ 4.79 ppm), multiplicity (s = singlet, d = doublet, dd = doublet of doublets, t = triplet, td = triplet of doublets, m = multiplet, app = apparent), coupling constants (Hz), and integration. The $^{13}C$ NMR spectra were recorded at room temperature using 176 MHz and 126 MHz with the data reported as follows: chemical shift in ppm referenced to residual solvent (CDCl$_3$ $\delta$ 77.16 and CD$_2$OD $\delta$ 49.00 ppm). The $^{31}P$ NMR spectra were recorded at room temperature using 162 MHz. Infrared spectra were recorded on a Fourier transform infrared spectrophotometer with water, dried over MgSO$_4$.

In a typical experiment, 1.0 M in hexanes) was added dropwise. The resulting mixture was stirred at 135.83 (2C), 135.78 (2C), 134.3, 133.5, 133.3, 129.80 (2C), 129.76 (2C), 127.80, 127.75, 118.0, 108.1, 98.9, 76.0, 61.1, 59.3, 50.9, 49.0, 47.7 ppm; HRMS (ESI) $m/z$ 77.3, 66.4, 51.5, 50.6, 42.1, 27.0 (3C), 19.3, 14.6, 7.0 (3C), 5.3 (3C) ppm; IR (neat) $\nu$ 1738 cm$^{-1}$. Mass spectra were recorded using electrospray ionization with 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 the formula: $[\alpha]_D = (100)\alpha_{obs} / (\ell(c))$, where $c$ = (g of substrate/100 mL of solvent) and $\ell$ = 1 dm. The sequence from 35 to 40 in the Scheme 5, and compounds 5 and 47 [13] were prepared as reported in our previous publications [13,14].

### 3.2. Synthesis

(−)-Methyl (S)-2-((S)-3,3-diethyl-9,9-dimethyl-8,8-diphenyl-4,7-dioxo-3,8-disiladecan-5-yl)-2-methylpent-4-enoate (17). To a solution of secondary alcohol 5 [14] (3.90 g, 22.9 mmol, 1.00 equiv.) in anhydrous DCM (45 mL, 0.20 M), imidazole (1.56 g, 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 h. The mixture was diluted with DCM (25 mL) and saturated NH$_4$Cl 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 17 (4.3 g, 87%) as a colorless oil. $R_f$ = 0.43 (Hexanes/EtOAc, 95:5); $[\alpha]_{25}^{D} = -27$ (c 0.7, CH$_2$Cl$_2$); Formula: C$_{31}$H$_{48}$O$_3$Si$_2$; MW: 540.82 g/mol; IR (neat) $\nu_{\text{max}}$ 3073, 3051, 2954, 2879, 1738 cm$^{-1}$; $^{1}H$ NMR (500 MHz, CDCl$_3$) $\delta$ 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, 2H), 3.52 (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 (q, $J$ = 7.9 Hz, 9H), 0.56 (q, $J$ = 8.0 Hz, 6H) ppm; $^{13}C$ NMR (126 MHz, CDCl$_3$) $\delta$ 175.5, 135.83 (2C), 135.78 (2C), 134.3, 133.5, 133.3, 129.80 (2C), 129.76 (2C), 127.80, 127.75, 118.0, 77.3, 66.4, 51.5, 50.6, 42.1, 27.0 (3C), 19.3, 14.6, 7.0 (3C), 5.3 (3C) ppm; HRMS (ESI) m/z [M + Na]$^+$ calcd for C$_{31}$H$_{48}$O$_3$Si$_2$Na: 563.2983; found 563.2985 (+0.4 ppm).

(−)-(R)-2-((S)-3,3-Diethyl-9,9-dimethyl-8,8-diphenyl-4,7-dioxo-3,8-disiladecan-5-yl)-2-methylpent-4-enoate (S1). To a solution of methyl ester 17 (4.30 g, 7.95 mmol, 1.00 equiv.) in anhydrous DCM (40 mL, 0.20 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 h. Methanol (1.3 mL, 32 mmol, 4.0 equiv.) was added at $-40$ °C and the reaction was stirred for 10 min followed by addition of Et$_2$O (50 mL) and saturated Rochelle salt solution (50 mL). The solution was stirred vigorously until separation of both layers. The aqueous phase was extracted with Et$_2$O (2 × 50 mL) and the combined organic layers were washed with water, dried over MgSO$_4$, filtered and concentrated under reduced pressure. The $^{1}H$ NMR analysis indicated that alcohol S1 (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$_2$Cl$_2$); Formula: C$_{30}$H$_{48}$O$_3$Si$_2$; MW: 512.88 g/mol; IR (neat) $\nu_{\text{max}}$ 3461 (br), 3072, 3051, 2955, 2934, 2877 cm$^{-1}$; $^{1}H$ NMR (500 MHz, CDCl$_3$) $\delta$ 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); $^1C$ NMR (126 MHz, CDCl$_3$) $\delta$ 175.5, 135.83 (2C), 135.78 (2C), 134.3, 133.5, 133.3, 129.80 (2C), 129.76 (2C), 127.80, 127.75, 118.0, 77.3, 66.4, 51.5, 50.6, 42.1, 27.0 (3C), 19.3, 14.6, 7.0 (3C), 5.3 (3C) ppm; HRMS (ESI) m/z [M + Na]$^+$ calcd for C$_{30}$H$_{48}$O$_3$Si$_2$Na: 563.2983; found 563.2985 (+0.4 ppm).
1.50 equiv.). The resulting solution was stirred at 50
°C for 1.5 h. The solution was diluted with DCM (30 mL) and a saturated solution of NaHCO,
(3C) ppm; HRMS (ESI)

ν
MW: 442.63 g/mol; IR (neat)

ν
MW: 554.89 g/mol; IR (neat)

ν
MW: 555.2968; found 555.2953

+0.4 (c 0.6, CH₂Cl₂);

Formula: C₃₂H₅₀O₄Si₂;

MW: 556.89 g/mol; IR (neat)

ν
MW: 555.2953; found 555.2968; (−2.7 ppm).

(−)-(2R,3S)-4-((tert-Butyldiphenylsilyl)oxy)-2-methyl-2-(2-oxoethyl)-3-((triethylsilyl)oxy)
butyl acetate (19). To a solution of alkene 18 (2.21 g, 3.98 mmol, 1.0 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 min). The reaction was then purged with nitrogen to remove excess ozone. After addition of Et₃N (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 h. MgSO₄ 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
19 as a colorless oil (1.89 g, 85%). Rf = 0.42 (Hexanes/EtOAc, 90:10); [α]D

−5.5 (c 0.7,

CH₂Cl₂);

Formula: C₃₁H₄₈O₃Si₂;

MW: 556.89 g/mol; IR (neat) νmax
3072, 3050, 2955, 2935, 2877, 2859, 1745, 1720 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.89 (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, 3H), 1.03 (s, 3H), 0.86 (t, J = 8.0 Hz, 9H), 0.51 (q, J = 7.9 Hz,
6H) ppm; ¹³C NMR (126 MHz, CDCl₃) δ 201.6, 170.8, 135.9 (2C), 135.8 (2C), 133.12, 133.09,
130.01, 129.99, 127.9 (4C), 76.6, 68.9, 66.0, 48.5, 42.4, 27.0 (3C), 20.9, 19.6, 19.2, 7.0 (3C),
5.1 (3C) ppm; HRMS (ESI) m/z [M − H]⁺ calculated for C₃₁H₄₆O₃Si₂: 555.2968; found 555.2953
(−2.7 ppm).

((2S,3R)-2-(((tert-Butyldiphenylsilyl)oxy)methyl)-5-hydroxy-3-methyltetrahydrofurano[3,4-
l)methyl acetate (20a,b). Aldehyde 19 (1.88 g, 3.38 mmol, 1.0 equiv.) was dissolved in a THF
and H₂O mixture (4:1) (34 mL, 0.1 M) followed by the addition of PTS (963 mg, 5.06 mmol, 1.50 equiv.). The resulting solution was stirred at 50 °C for 1.5 h. The solution was diluted with DCM (30 mL) and a saturated solution of NaHCO₃ (20 mL) was added. The aqueous layer was extracted with DCM (3 × 25 mL) and the combined organic layers were dried
over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (Hexanes/EtOAc, 80:20) to provide lactols 20a,b (1.3 g,
87%, dr 1:4:1) as a colorless oil. Rf = 0.38 (Hexanes/EtOAc, 70:30); Formula: C₂₅H₃₄O₅Si;
MW: 442.63 g/mol; IR (neat) νmax
3428 (br), 3071, 3050, 2955, 2932, 2887, 2858, 1742 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) 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, minor), 1.90 (dd, J = 13.6, 2.7 Hz, 1H,
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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\(_3\)) \(\delta\) 171.10 (minor), 171.07 (major), 135.9 (minor, 2C), 135.8 (major, 2C), 135.7 (major and minor, 4C), 133.4 (major), 133.3 (major), 132.81 (minor), 132.77 (minor), 130.1 (minor), 130.0 (major), 129.9 (major, 2C), 127.98 (minor, 2C), 127.95 (minor, 2C), 127.86 (major, 2C), 127.84 (major, 2C), 98.7 (minor, 97.8 (major), 83.5 (minor), 82.4 (major, 2C), 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, 3C), 26.9 (major, 3C), 21.0 (major and minor), 19.3 (minor), 19.2 (major), 18.6 (major), 18.4 (minor) ppm; HRMS (ESI) m/z [M + Na\(^+\)] calculated for C\(_{25}\)H\(_{33}\)O\(_5\)SiNa\(^+\): 465.2068; found 465.2070 (+0.4 ppm).

\((-\cdots(2S,3R)-2-(((\text{tert-Butyldiphenylsilyl)}xy)methyl)-3-methyl-2,3-dihydrofuran-3-yl)methyl acetate (21).\) To a solution of lactols \(20a, b\) (1.27 g, 2.87 mmol, 1.0 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 min at room temperature, followed by 3 min 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\(_2\)O (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 21 (620 mg, 51%) as a colorless oil. R\(_f\) = 0.65 (Hexanes/EtOAc, 80:20); \([\alpha]\)\(_D\)\(^{25}\) +90.0 (c 0.8, CH\(_2\)Cl\(_2\)); Formula: C\(_{25}\)H\(_{33}\)O\(_5\)Si: MW: 424.61 g/mol; IR (neat) \(\nu_{\max}\) 3071, 3053, 2959, 2887, 2858, 1743 cm\(^{-1}\); \(^{1}H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 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\(_3\)) \(\delta\) 171.11, 145.6, 135.76 (2C), 135.75 (2C), 135.3, 135.4, 129.89, 129.87, 127.9 (4C), 107.4, 85.7, 70.9, 63.0, 48.1, 27.0 (3C), 21.0, 19.3, 18.0 ppm; HRMS (ESI) m/z [M + H\(^+\)] calculated for C\(_{25}\)H\(_{33}\)O\(_5\)SiH: 425.2143; found 425.2148 (+1.2 ppm).

\((-\cdots(2S,3R)-2-(((\text{tert-Butyldiphenylsilyl)}xy)methyl)-5-methoxy-3-methyltetrahydrofuran-3-yl)methyl acetate (22a, b).\) To a solution of aldehyde 19 (1.12 g, 2.00 mmol, 1.0 equiv.) in anhydrous MeOH (10 mL, 0.20 M), PTSA (0.19 g, 1.00 mmol, 0.50 equiv.) was added. The resulting mixture was stirred for 20 min until completion as indicated by TLC. The reaction was neutralized by addition of anhydrous Et\(_3\)N (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 \(22a, b\) (0.86 g, 94%, dr 1.4:1) as a colorless oil. R\(_f\) = 0.48 (Hexanes/EtOAc, 80:20); \([\alpha]\)\(_D\)\(^{25}\) +90.0 (c 0.8, CH\(_2\)Cl\(_2\)); Formula: C\(_{26}\)H\(_{34}\)O\(_5\)Si: MW: 456.65 g/mol; IR (neat) \(\nu_{\max}\) 3071, 3049, 2954, 2931, 2890, 2858, 1743 cm\(^{-1}\); \(^{1}H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 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 (3H, minor), 3.30 (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 = 13.5, 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\(_3\)) \(\delta\) 171.12 (major, 171.05 (major), 135.77 (major and minor, 4C), 135.75 (minor, 2C), 135.73 (major, 2C), 133.65 (minor), 133.56 (major), 133.52 (major), 133.47 (major), 129.9 (major), 129.84 (minor), 129.82 (major and minor), 127.8 (major and minor, 8C), 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, 6C), 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\(^+\)] calculated for C\(_{26}\)H\(_{34}\)O\(_5\)SiNa: 479.2224; found 479.2220 (−0.9 ppm).

\((-\cdots(2S,3R)-2-(((\text{tert-Butyldiphenylsilyl)}xy)methyl)-3-methyl-2,3-dihydrofuran-3-yl)methyl acetate (21).\) To a solution of methoxy lactols \(22a, b\) (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 [32]. The resulting solution was stirred for 30 min at room temperature. The mixture was then 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 MgSO₄, filtered and concentrated under reduced pressure. Purification by silica gel flash chromatography (Hexanes/EtOAc, 90:10) provided glycal 21 (96 mg, 90%) as a colorless oil, which was confirmed to be identical to the compound formed from 20a,b (see above).

((2S,3R,4R)-2-((tert-Butylidiphenylsilyloxy)methyl)-4,5-dihydroxy-3-methyltetrahydrofuran-3-yl)methyl acetate (24a,b). To a solution of glycal 21 (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 NaHCO₃ 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 min at 0 °C and 3 h at room temperature. After cooling and degassing the flask, the aqueous phase was extracted with DCM (3 × 5 mL), and the resulting organic phase was dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting crude epoxide 23a and 23b (dr 7:1, determined by 1H NMR of crude epoxide) was stirred in a THF and H₂O (1:1) mixture (5.5 mL, 0.05 M) for 1 h. The aqueous phase was extracted with EtOAc (3 × 5 mL), and the resulting organic phase was dried over MgSO₄, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel (DCM/EtOAc, 80:20) provided lactols 24a,b (80 mg, 63%, 4:1 mixture) as a white foam. 

Rf = 0.33 (DCM/EtOAc, 85:15); Formula: C₂₃H₂₉O₆Si; MW: 458.63 g/mol; IR (Neat) νmax 3421 (br), 2931, 2857, 1741, 1720 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) 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, minor), 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; OH signals are missing possibly due to exchange in CDCl₃ ppm; ¹³C NMR (126 MHz, CDCl₃) δ 172.3 (major), 172.0 (minor), 135.74 (minor, 2C), 135.69 (minor, 2C), 135.67 (major, 2C), 135.65 (major, 2C), 133.1 (major), 133.0 (major, 2C), 127.94 (minor, 2C), 127.90 (major, 2C), 127.89 (major, 2C), 103.9 (minor), 96.8 (major), 83.6 (minor) 83.0 (minor), 80.1 (major), 77.3 (major), 66.8 (major), 66.6 (major), 63.9 (minor), 63.0 (major), 48.4 (major), 47.4 (minor, 2C), 27.0 (minor, 3C), 26.9 (major, 3C), 21.0 (major and minor), 19.3 (minor), 19.2 (major), 15.7 (major), 15.4 (major) ppm; HRMS (ESI) m/z [M + NH₄]⁺ calcld for C₂₅H₂₉O₆NSi: 476.2463; found 476.2462 (±0.2 ppm).

(3R,4R,5S)-4-(Acetoxy)methyl-5-(((tert-Butyldiphenylsilyloxy)methyl)-4-methyltetrahydrofuran-2,3-diyl diacetate (25a,b). Lactols 24a,b (65 mg, 0.14 mmol) were stirred in a solution of Ac₂O/Pyr (2:1, 1.0 mL, 0.14 M) at room temperature for 18 h and then concentrated. Purification by flash chromatography on silica gel (Hexanes/EtOAc, 60:40), provided acetates 25a,b (65 mg, 85%, 4:1 mixture) as a colorless oil. Rf = 0.53 (Hexanes/EtOAc, 70:30); Formula: C₂₅H₃₀O₈Si; MW: 542.70 g/mol; IR (Neat) νmax 2933, 2858, 1746 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) 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, 3H, minor), 1.08 (s, 9H, major) ppm; ¹³C NMR (126 MHz, CDCl₃) δ 170.9 (major), 170.7 (minor), 169.8 (major), 169.7 (major), 169.64 (minor), 169.61 (major), 135.8 (major, 2C), 135.72 (major, 2C), 135.71 (minor, 2C), 135.69 (minor, 2C), 133.03 (minor), 132.97 (major), 132.8 (minor), 132.7 (major), 130.01 (minor), 129.98 (major, 2C), 129.95 (minor), 127.94 (major, 2C), 127.92 (major and minor, 4C), 127.89 (minor, 2C), 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, 3C), 26.88...
(major, 3C), 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 + NH₄⁺] calcld for C₉₂H₄₁O₈NSi: 560.2674; found 560.2673 (−0.2 ppm).

(+)-(2R,3R,4R,5S)-2-(4-Acetamido-2-oxopyrimidin-1(2H)-yl)-4-(acetoxyethyl)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-3-methyltetrahydrofuran-3-yl acetate (26). To a solution of acetates 25a,b (70 mg, 0.13 mmol, 1.0 equiv.) in anhydrous MeCN (0.6 mL, 0.2 M), silylated N₄-acycetylcysteine (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 min and TMSOTf (0.10 mL, 0.52 mmol, 4.0 equiv.) was added. The reaction was stirred at 60 °C for 3.5 h and then cooled to room temperature and quenched with saturated aqueous NaHCO₃ (1 mL). The aqueous layer was extracted with DCM (3 × 5 mL) and the combined organic layers were washed with brine, dried over MgSO₄, 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 26 (63 mg, 77%, dr 20:1) as a white foam. Rf = 0.34 (DCM/MeOH, 95:5); [α]₂⁵⁰_D +35 (c 0.2, MeOH); Formula: C₃₃H₄₃N₃OSi; MW: 635.79 g/mol; IR (neat) ν_max 2957, 2932, 2893, 2858, 1746, 1671 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) 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; NH signals are missing possibly due to exchange in CD₃OD; ¹³C NMR (126 MHz, CD₃OD) δ 173.0, 172.2, 171.3, 164.3, 158.1, 145.5, 134.7, 128.6, 121.9, 108.9, 95.4, 90.1, 85.4, 81.4, 76.5, 64.9, 47.2, 27.6 (3C), 24.5, 20.7, 20.6, 20.1, 16.8 ppm; HRMS (ESI) m/z [M + H⁺] calcld for C₃₃H₄₃N₃OSi: 636.2736; found 636.2726 (−1.5 ppm).

(−)-(2S,3R,4R,5R)-4-Acetoxy-5-(6-benzamido-9H-purin-9-yl)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-3-methyltetrahydrofuran-3-yl acetate (27). To a suspension of N⁶-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 [33]. The resulting mixture was vigorously stirred for 2 h until a clear solution was obtained. The solution was concentrated under high vacuum and treated with a solution of TMSOTf (27 µL, 0.15 mmol, 2.0 equiv.), the resulting solution was heated at reflux for 4 h. The solution was diluted in DCM (5 mL) and washed with a saturated NaHCO₃ solution (2 mL). The aqueous phase was extracted with DCM (3 × 5 mL) and the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The ¹H NMR spectrum of the crude reaction mixture indicated a >20:1 dr and only the N⁶-ribo-isomer. Purification by flash chromatography on silica gel (EtOAc/MeOH, 97:3) provided N⁶-benzoyladenine nucleoside analogue 27 (40 mg, 75%) as a white foam. Rf = 0.37 (DCM/MeOH, 95:5); [α]₂⁵⁰_D −15 (c 0.8, MeOH); Formula: C₃₀H₄₃N₃O₇Si; MW: 721.89 g/mol; IR (neat) ν_max 3268 (br), 3070, 3053, 2956, 2931, 2892, 2857, 1745 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) 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; NH signals are missing possibly due to exchange in CD₃OD; ¹³C NMR (126 MHz, CD₃OD) δ 172.3, 171.5, 168.0, 153.4, 153.3, 151.1, 143.9, 136.73 (2C), 136.65 (2C), 135.0, 134.3, 133.9, 133.8, 131.2, 131.1, 129.8 (2C), 129.4 (2C), 128.9 (2C), 128.5, 88.5, 85.4, 81.4, 67.5, 65.2, 47.4, 27.5 (3C), 20.8, 20.4, 20.1, 17.1 ppm; HRMS (ESI) m/z [M + H⁺] calcld for C₃₀H₄₃N₃O₇Si: 722.3005; found 722.3011 (+0.9 ppm).

(+)-(25R,3R,4R,5R)-4-Acetoxy-5-(6-amino-2-chloro-9H-purin-9-yl)-2-(((tert-butyldiphenylsilyl)oxy)methyl)-3-methyltetrahydrofuran-3-yl acetate (28). 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 h until a clear solution was obtained. The solution was concentrated...
under high vacuum and treated with a solution of 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 h. The solution was diluted in DCM (5 mL) and washed with a saturated NaHCO₃ solution (2 mL). The aqueous phase was extracted with DCM (3 × 5 mL) and the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The ¹H NMR spectrum of the crude reaction mixture indicated a >20:1 dr and only the N⁹-regiosomer. Purification by flash chromatography on silica gel (DCM/MeOH, 95:5) provided 2-chloroadenine nucleoside analogue 28 (61 mg, 85%) as a white foam. Rₙ = 0.32 (DCM/MeOH, 95:5); [α]D²⁰ +6.7 (c 1.0, MeOH); Formula: C₃₂H₃₂ClN₂O₅Si; MW: 652.22 g/mol; IR (neat) νₘᵋₓ 3319 (br), 3170 (br), 2957, 2932, 2893, 2859, 1744, 1646 cm⁻¹; ¹H NMR (500 MHz, CD₂OD) 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; NH signals are missing possibly due to exchange in CD₂OD; ¹³C NMR (126 MHz, CD₂OD) δ 172.4, 171.6, 158.0, 155.4, 151.8, 140.8, 136.8 (2C), 136.7 (2C), 134.2, 133.7, 131.12, 131.11, 129.0 (2C), 128.9 (2C), 119.3, 88.2, 85.4, 81.5, 67.7, 65.2, 47.4, 27.6 (3C), 20.8, 20.5, 20.1, 17.0 ppm; HRMS (ESI) m/z [M + H⁺] calcd for C₃₂H₃₂ClN₂O₅Si: 652.2353; found 652.2344 (1.3 ppm).

(+)-(2R,3R,4R,5S)-2-(4-Acetamido-2-oxoprimidin-1(2H)-yl)-4-(acetoxyethyl)-5-(hydroxymethyl)-4-methyltetrahydrofuran-3-yl acetate (29). Representative Procedure A: To a solution of 1',2'-trans ribo-nucleoside 26 (50 mg, 77 µmol, 1.0 equiv.) in anhydrous THF (0.8 mL, 0.1 M), 3HF-NET₃ (0.13 mL, 0.79 mmol, 10 equiv.) was added at room temperature and the resulting mixture was stirred for 16 h. 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-nucleoside 29 (27 mg, 86%) as a white foam. Rₙ = 0.30 (DCM/MeOH, 95:5); [α]D²⁰ +26 (c 0.2, MeOH); Formula: C₁₇H₂₃N₃O₆; MW: 397.38 g/mol; IR (neat) νₘᵋₓ 3307 (br), 2927, 1742, 1650 cm⁻¹; ¹H NMR (500 MHz, CD₂OD) 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 (dt, 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; OH and NH signals are missing possibly due to exchange in CD₂OD; ¹³C NMR (126 MHz, CD₂OD) δ 173.0, 172.4, 171.5, 164.4, 158.3, 146.3, 148.6, 93.0, 84.9, 82.5, 67.7, 62.5, 47.3, 24.5, 20.8, 20.6, 16.4 ppm; HRMS (ESI) m/z [M + H⁺] calcd for C₁₇H₂₃N₃O₆: 398.1558; found 398.1552 (1.6 ppm).

(+)-(2S,3R,4R,5R)-4-Acetoxy-5-(6-benzamido-9H-purin-9-yl)-2-(hydroxymethyl)-3-methyltetrahydrofuran-3-yl methyl (30). Following the Representative Procedure A, N⁹-benzoyladine nucleoside analogue 27 (40 mg, 55 µmol, 1.0 equiv.) in anhydrous THF (0.55 mL, 0.10 M), 3HF-NET₃ (0.14 mL, 0.83 mmol, 15 equiv.) was added and the mixture was stirred for 16 h. Triethylamine (0.77 mL, 5.55 mmol, 100 equiv.) was then added. Purification by flash chromatography on silica gel (DCM/MeOH, 95:5), provided the 1',2'-trans ribo-like N⁹-benzoyladine nucleoside analogue 30 (23 mg, 86%) as a white foam. Rₙ = 0.54 (DCM/MeOH, 90:10); [α]D²⁰ +51 (c 0.7, MeOH); Formula: C₂₃H₂₅N₃O₇; MW: 483.48 g/mol; IR (neat) νₘᵋₓ 3300 (br), 3069, 2972, 2855, 1742 cm⁻¹; ¹H NMR (500 MHz, CD₂OD) δ 8.82 (s, 1H), 8.70 (s, 1H), 8.09–8.08 (m, 2H), 7.67–7.64 (m, 4H), 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; OH and NH signals are missing possibly due to exchange in CD₂OD; ¹³C NMR (126 MHz, CD₂OD) δ 172.4, 171.5, 168.1, 153.4, 153.1, 151.2, 144.5, 135.0, 133.9, 129.8 (2C), 129.4 (2C), 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 C₂₃H₂₅N₃O₇: 484.1827; found 484.1831 (+0.8 ppm).

(+)-(2S,3R,4R,5R)-4-Acetoxy-5-(6-amino-2-chloro-9H-purin-9-yl)-2-(hydroxymethyl)-3-methyltetrahydrofuran-3-yl methyl acetate (31). Following the Representative Procedure A, Nucleoside analogue 28 (60 mg, 92 µmol, 1.0 equiv.) in anhydrous THF (0.9 mL, 0.1 M), 3HF-NET₃ (0.23 mL, 1.44 mmol, 15 equiv.) was added and the mixture was stirred for 16 h. Tri-
ethylamine (1.3 mL, 9.2 mmol, 100 equiv.) was then added. Purification by flash chromatography on silica gel (DCM/MeOH, 95:5), provided the 1',2'-trans ribo-like 2-chloroadenine nucleoside analogue 31 (33 mg, 87%) as a white foam. $R_f = 0.50$ (DCM/MeOH, 90:10; $[\alpha]_{D}^{25} = -75$ (c 0.2, MeOH); Formula: C$_{16}$H$_{20}$ClN$_{2}$O$_{6}$; MW: 413.82 g/mol; IR (neat) $\nu_{\text{max}}$ 3326 (br), 2942, 1743, 1618 cm$^{-1}$; $^1$H NMR (500 MHz, CD$_2$OD) $\delta$ 8.41 (s, 1H), 6.12 (d, $J = 6.8$ Hz, 1H), 5.80 ($J = 6.8$ 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; OH and NH signals are missing possibly due to exchange in CD$_2$OD; $^{13}$C NMR (126 MHz, CD$_2$OD) $\delta$ 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$_{23}$ClN$_{2}$O$_{6}$: 414.1175; found 414.1169 ($-1.5$ ppm).

(+)-4-Amino-1-(2R,3R,4S,5S)-3-hydroxy-4,5-bis(hydroxymethyl)-4-methyltetrahydro furan-2-yl)pyrimidin-2(1H)-one (32). Representative Procedure B: To a solution of nucleoside 29 (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 h, quenched with amberlite acidic resin (~50 mg) and stirred for 10 min. The mixture was filtered with MeOH (~5 mL) and concentrated under reduced pressure. Purification by C18 reverse phase flash chromatography (MeOH/H$_2$O) provided 1',2'-trans ribo-like cytosine nucleoside analogue 32 [14] (7 mg, 81%) as a white foam. $[\alpha]_{D}^{25} +28$ (c 0.4, CH$_3$OH); Formula: C$_{11}$H$_{12}$N$_{2}$O$_{5}$: MW: 271.27 g/mol; IR (neat) $\nu_{\text{max}}$ 3345, 3217, 2967, 2949, 1649, 1607 cm$^{-1}$; $^1$H NMR (500 MHz, CD$_2$OD) $\delta$ 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, $J = 11.5$ 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; OH and NH signals are missing possibly due to exchange in CD$_2$OD; $^{13}$C NMR (126 MHz, CD$_2$OD) $\delta$ 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$_{12}$O$_{5}$N$_{2}$Na$^+$: 294.1060; found 294.1063 (+0.8 ppm).

(--)(2S,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-4-hydroxy-3-methyltetrahydrofuran-2,3-diyl dimethanol (33). Following Representative Procedure B, NaOMe (25 µL, 25 µmol, 1.0 equiv., 1.0 M in MeOH) was added to a solution of nucleoside 30 (12 mg, 25 µmol, 1.0 equiv.) in MeOH (0.25 mL, 0.10 M). Purification by C18 reverse phase flash chromatography (MeOH/H$_2$O) provided 1',2'-trans ribo-like adenine nucleoside analogue 33 (6 mg, 82%) as a white foam. $R_f = 0.30$ (DCM/MeOH, 80:20); $[\alpha]_{D}^{25} = -42$ (c 0.2, MeOH); Formula: C$_{12}$H$_{11}$N$_{2}$O$_{4}$: MW: 295.30 g/mol; IR (neat) $\nu_{\text{max}}$ 3332 (br), 3193 (br), 2928, 2882, 1649 cm$^{-1}$; $^1$H NMR (500 MHz, CD$_2$OD) $\delta$ 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; OH and NH signals are missing possibly due to exchange in CD$_2$OD; $^{13}$C NMR (126 MHz, CD$_2$OD) $\delta$ 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$_{15}$N$_{3}$O$_{4}$: 296.1353; found 296.1356 (+1.0 ppm).

(--)(2S,3S,4R,5R)-5-(6-amino-2-chloro-9H-purin-9-yl)-4-hydroxy-3-methyltetrahydrofuran-2,3-diyl dimethanol (34). Following Representative Procedure B, NaOMe (24 µL, 24 µmol, 1.0 equiv., 1.0 M in MeOH) was added to a solution of nucleoside 31 (10 mg, 24 µmol, 1.0 equiv.) in MeOH (0.24 mL, 0.10 M). Purification by C18 reverse phase flash chromatography (MeOH/H$_2$O) provided 1',2'-trans ribo-like 2-chloroadenine nucleoside analogue 34 (6.4 mg, 80%) as a white foam. $R_f = 0.50$ (DCM/MeOH, 80:20); $[\alpha]_{D}^{25} = -25$ (c 0.2, MeOH); Formula: C$_{12}$H$_{16}$ClN$_{2}$O$_{4}$: MW: 329.74 g/mol; IR (neat) $\nu_{\text{max}}$ 3322 (br), 3186 (br), 2940, 2884, 1653 cm$^{-1}$; $^1$H NMR (500 MHz, CD$_2$OD) $\delta$ 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; OH and NH signals are missing possibly due to exchange in CD$_2$OD; $^{13}$C NMR (126 MHz, CD$_2$OD) $\delta$ 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$_{2}$O$_{4}$: 330.0964; found 330.0958 (−1.7 ppm).
(-)-(3R,4S,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-((((tert-butylidemethylsilyl)oxy)methyl)-3-methyl-3-vinylidihydrofuran-2(3H)-one (S2). To a solution of secondary alcohol 40 (4.00 g, 14.0 mmol, 1.00 equiv.) in anhydrous DCM (42 mL, 0.33 M), 2,6 Lutidine (4.04 mL, 34.9 mmol, 2.50 equiv.) and TBSOTf (4.81 mL, 2.09 mmol, 1.50 equiv.) were added at 0 °C. The resulting mixture was gradually warmed to room temperature and stirring was continued for overnight. A saturated aqueous solution of NaHCO₃ (20 mL) was added and the mixture was extracted with CH₂Cl₂ (3 x 40 mL). The combined organic layers were washed with brine, dried over MgSO₄, filtered and condensed under reduced pressure. The residue was purified by flash chromatography on silica gel (Hexanes/EtOAc, 90:10) to provide bis-silylated ethers S2 (4.2 g, 75%) as a colorless oil. Rf = 0.28 (Hexanes/EtOAc, 9:1); [α]D +60 (c 1.2, CH₂Cl₂); IR (neat) νmax 2954, 2930, 2858, 1786 cm⁻¹; Formula: C₂₀H₄₀O₅Si₂; MW: 400.70 g/mol; ¹H NMR (500 MHz, CDCl₃) δ 5.94 (dd, J = 17.7, 10.7 Hz, 1H), 5.24 (dd, J = 10.7, 0.9 Hz, 1H), 5.18 (d, J = 17.6 Hz, 1H), 4.27 (d, J = 8.1 Hz, 1H), 4.28–3.99 (m, 1H), 3.98 (dd, J = 12.3, 18 Hz, 1H), 3.74 (dd, J = 12.3, 2.5 Hz, 1H), 1.33 (s, 3H), 0.90 (s, 9H), 0.88 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H), 0.07 (s, 3H), 0.06 (s, 3H) ppm; NMR (126 MHz, CDCl₃) δ: 176.8, 134.1, 116.8, 82.1, 75.0, 59.6, 51.6, 25.9 (3C), 25.8 (3C), 21.3, 18.4, 18.1, −4.2, −4.7, −5.2, −5.4 ppm; HRMS (ESI) m/z [M + H]⁺ calc for C₂₀H₄₀O₅Si₂: 401.2543; found 401.2539 (+0.2 ppm) and [M+NH₄]⁺ calcd for C₂₀H₄₄NO₅Si₂: 418.2809; found 418.2800 (−0.7 ppm).

(+)-(3S,4R,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-methyl-3-vinylidihydrofuran-2-carbaldehyde (41). The crude was purified by flash chromatography on silica gel (Hexanes/EtOAc, 100:0 to 70:30) to provide the aldehyde 41 (663 mg, 87%) as colourless oil. Rf = 0.67 (EtOAc/Hexanes, 1:4); [α]D +37 (c 5.4, in CH₂Cl₂); IR (neat) νmax 2954, 2930, 2886, 2858, 1789, 1729, 1472, 1463, 1254 cm⁻¹; Formula: C₁₉H₂₇O₃Si₂; MW: 402.67 g/mol; ¹H NMR (500 MHz, CDCl₃) δ 9.59 (s, 1H), 4.54 (d, J = 6.8 Hz, 1H), 4.35 (apppt, J = 6.8, 2.2 Hz, 1H), 4.00 (dd, J = 12.3, 2.1 Hz, 1H), 3.77 (dd, J = 12.3, 2.3 Hz, 1H), 1.49 (s, 3H), 0.88 (s, 9H), 0.86 (s, 9H), 0.11 (s, 3H), 0.09 (s, 3H), 0.08 (s, 3H), 0.06 (s, 3H) ppm; NMR (126 MHz, CDCl₃) δ 195.9, 172.7, 83.7, 76.8, 60.5, 59.9, 25.9 (3C), 25.6 (3C), 18.4, 17.9, 15.4, −4.5, −4.8, −5.2, −5.4 ppm; HRMS (ESI) m/z [M + H]⁺ calc for C₁₉H₂₉O₃Si₂: 403.2331, found: 403.2329 (−0.50 ppm).

(3R,4S,5R)-4-((tert-Butyldimethylsilyl)oxy)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-(hydroxymethyl)-3-methyltetrahydrofuran-2-ol (42a,b). To a solution of aldehyde 41 (2.30 g, 5.71 mmol, 1.00 equiv.) in anhydrous THF (57 mL, 0.10 M), Red-Al (3.57 mL, 11.4 mmol, 2.00 equiv.) was added at −40 °C and stirring was continued for 40 min. at −40 °C. The reaction was then cooled to −78 °C and was quenched by the addition of few drops (−0.5 mL) of saturated Rochelle salt solution. The stirring was continued for 10 min at −78 °C and it was then gradually warmed to room temperature. THF (−20 mL) and saturated Rochelle salt solution (−15 mL) were added. The resulting biphasic mixture was stirred vigorously for an hour. The layers were separated, the aqueous layer was extracted with Et₂O (3 x 40 mL), and the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (Hexanes/EtOAc, 4:1) to give the lactol 42a,b (1.6 g, 69%, dr 1:2) as a colorless oil. Rf = 0.24 (Hexanes/EtOAc, 4:1); IR (neat) νmax 3329, 3200, 2930, 1645, 1600 cm⁻¹; Formula: C₁₀H₁₂O₂Si₂; MW: 406.70 g/mol; ¹H NMR (500 MHz, CDCl₃) δ: 5.36 (dd, J = 8.9, 2.3 Hz, 1H, minor (OH, D₂O exchange)), 5.04 (d, J = 8.6 Hz, 1H, minor), 4.92 (d, J = 8.8 Hz, 1H, major), 4.25 (d, J = 5.5 Hz, 1H, major), 4.08 (d, J = 6.3 Hz, 1H, minor), 4.02–3.95 (m, 2H, major and minor), 3.89 (d, J = 11.8 Hz, 1H, minor), 3.83 (dd,
J = 11.6, 2.8 Hz, 1H, minor), 3.80–3.74 (m, 2H, major and minor), 3.74–3.67 (m, 2H, minor and major), 3.65 (dd, J = 11.0, 2.3 Hz, 1H, major), 3.59–3.52 (m, 2H, major and OH (D$_2$O exchange)), 1.11 (s, 3H, major), 1.01 (s, 3H, minor), 0.93 (s, 9H, major), 0.90 (s, 9H, minor), 0.89 (s, 18H, major and minor), 0.12 (s, 6H, major), 0.11 (s, 12H, minor), 0.07 (s, 3H, major), 0.06 (s, 3H, major) ppm; OH signals are missing possibly due to exchange in CDCl$_3$ ppm. 13C NMR (126 MHz, CDCl$_3$) δ 105.7 (minor), 102.2 (major), 85.4 (major), 84.6 (minor), 78.9 (minor), 78.7 (major), 66.7 (major), 65.8 (minor), 62.6 (minor), 61.6 (major), 51.4 (major), 49.0 (minor), 26.00 (3C, minor), 25.96 (3C, major), 25.84 (3C, minor), 25.80 (3C, major), 21.2 (minor), 18.43 (minor), 18.40 (major), 18.0 (major), 17.9 (major), 16.0 (major), −4.3 (major), −4.39 (minor), −4.43 (major), −4.6 (major), −5.1 (minor), −5.39 (major), −5.40 (2C, minor) ppm; HRMS (ESI) m/z [M+NH$_4$]$^+$ calcld for C$_{19}$H$_{46}$NO$_3$Si$_2$: 424.2915; found 424.2904 (−1.6 ppm).

(3R,4S,5R)-2-(Benzoyloxy)-4-((tert-butyldimethylsilyl)oxy)-3-methyltetrahydrofuran-3-yl)methyl benzoate (43a,b). To a solution of lactol 42a,b (3.30 g, 8.11 mmol, 1.00 equiv.) in CH$_2$Cl$_2$ (45 mL, 0.18 M), pyridine (3.94 mL, 48.7 mmol, 6.00 equiv.) and DMAP (99 mg, 0.81 mmol, 0.10 equiv.) were added. After cooling the resulting mixture to 0 °C, BzCl (4.71 mL, 40.6 mmol, 5.00 equiv.) was added dropwise. The reaction mixture was gradually warmed to room temperature and stirred for 16 h. The mixture was cooled to 0 °C and ethylenediamine (1.36 mL, 20.3 mmol, 2.50 equiv.) was added and stirring was continued for 1 h at 0 °C. The reaction mixture was then diluted with hexanes (40 mL) and passed through a celite using Et$_2$O. The filtrates were concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (Hexanes/EtOAc, 4:1) to afford the 43a,b (3.8 g, 76%, dr 1.2:1) as a mixture of anomers as a white foam. R$_f$ = 0.34 (Hexanes/EtOAc, 4:1); IR (neat) ν$_{max}$ 2954, 2929, 2857, 1722, 1261 cm$^{-1}$; Formula: C$_{33}$H$_{50}$O$_3$Si$_2$: MW: 614.92 g/mol; $^1$H NMR (500 MHz, CDCl$_3$): δ 8.11–8.03 (m, 4H, major), 7.62–7.50 (m, 5H, minor), 7.49–7.36 (m, 9H, major and minor), 6.54 (s, 1H, major), 6.37 (s, 1H, minor), 6.41 (d, J = 11.1 Hz, 1H, minor), 4.56 (d, J = 11.1 Hz, 1H, minor), 4.58–4.54 (m, 2H, major and minor), 4.40 (d, J = 7.4 Hz, 1H, major), 4.27–4.23 (m, 1H, minor), 4.19 (d, J = 3.1 Hz, 1H, major), 4.09–4.03 (m, 1H, major), 3.91 (dd, J = 11.6, 4.4 Hz, 1H, major), 3.88–3.84 (m, 2H, major and minor), 3.72 (dd, J = 11.6, 4.4 Hz, 1H, major), 1.32 (s, 6H, major and minor), 0.97 (s, 9H, minor), 0.94 (s, 9H, major), 0.93 (s, 9H, minor), 0.79 (s, 9H, major), 0.19 (s, 3H, major), 0.14 (s, 6H, major and minor), 0.13 (s, 3H, minor), 0.11 (s, 6H, major and minor), −0.01 (s, 3H, minor), −0.08 (s, 3H, minor) ppm. 13C NMR (126 MHz, CDCl$_3$) δ 166.6 (minor), 166.5 (major), 165.6 (major), 165.5 (minor), 133.34 (major), 133.31 (minor), 133.2 (major), 133.0 (minor), 130.3 (minor), 130.13 (major), 130.06 (minor), 130.0 (3C, 2minor and 1major), 129.9 (2C, major), 129.8 (2C, major), 129.61 (2C, minor), 128.6 (2C, major), 128.5 (4C, major and minor), 128.4 (2C, minor), 102.4 (minor), 100.5 (major), 89.9 (minor), 85.5 (major), 77.8 (minor), 67.6 (major), 66.3 (major), 65.4 (minor), 63.1 (major), 62.6 (minor), 50.1 (minor), 49.8 (major), 26.1 (3C, minor), 26.0 (3C, major), 25.9 (3C, major), 25.8 (3C, minor), 20.8 (minor), 18.6 (major), 18.5 (major), 18.08 (major), 18.07 (minor), 16.9 (major), −4.1 (major), −4.2 (major), −4.3 (major), −4.8 (major), −5.2 (minor), −5.28 (2C major and minor) ppm HRMS (ESI) m/z [M-OBz]$^+$ calcld for C$_{26}$H$_{45}$O$_2$Si$_2$: 493.2806; found 493.2805; and [M+NH$_4$]$^+$ calcld for C$_{33}$H$_{50}$O$_3$Si$_2$: 632.3439; found 632.3439 (−0.2 ppm).

(−)(2R,3R,4S,5R)-4-((tert-butyldimethylsilyl)oxy)-5-((tert-butyldimethylsilyl)oxy)methyl)-2-(2,6-dichloro-9H-purin-9-yl)-3-methyltetrahydrofuran-3-yl)methyl benzoate (44). To a solution of the benzoylated furanosides 43a,b (434 mg, 0.706 mmol, 1.00 equiv.) in anhydrous MeCN (2.8 mL, 0.25 M), 2,6-dichloropurine (147 mg, 0.776 mmol, 1.10 equiv.) was added at room temperature. The resulting mixture was cooled to −10 °C and DBU (0.316 mL, 2.12 mmol, 3.00 equiv.) was added followed by dropwise addition of TMSOTf (0.520 mL, 2.82 mmol, 4.00 equiv.). The stirring was continued at −10 °C for 3 h. The mixture was warmed to room temperature, and a saturated solution of NaHCO$_3$ (~8 mL) was added. The aqueous layer was extracted with CH$_2$Cl$_2$ (3 × 20 mL) and the combined organic layers were washed with brine, dried over MgSO$_4$, filtered and concentrated under reduced pressure. The crude was...
purified by flash chromatography on silica gel (CH$_2$Cl$_2$/MeOH, 100:0 to 70:30) to provide the pure product 44 (375 mg, 78%, β:α = 10:1) as brown gum. R$_f$ = 0.87 (MeOH/CH$_2$Cl$_2$, 1:9); [α]$^D_{25}$: −10 (c 4.9, MeOH); IR (neat) ν$_{max}$ 2955, 2930, 2988, 2858, 1724, 1590, 1553, 1470, 1464, 1452, 1356, 1255, 1214 cm$^{-1}$; Formula: C$_{31}$H$_{46}$Cl$_2$N$_4$O$_5$Si$_2$; MW: 680.80 g mol$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 8.90 (s, 1H), 8.20 (s, 2H), 7.61 (t, J = 7.4 Hz, 1H), 7.52 (t, J = 7.7 Hz, 2H), 6.61 (s, 1H), 4.60 (d, J = 11.7 Hz, 1H), 4.50 (d, J = 7.2 Hz, 1H), 4.48 (d, J = 11.6 Hz, 1H), 4.15 (d, J = 12.1 Hz, 1H), 4.07 (appd, J = 8.4 Hz, 1H), 3.90 (d, J = 12.1 Hz, 1H), 1.01 (s, 9H), 0.93 (s, 9H), 0.87 (s, 3H), 0.21 (s, 3H), 0.14 (s, 3H), 0.12 (s, 3H) ppm; NMR $^{13}$C: (126 MHz, CDCl$_3$) δ 166.7, 153.1, 152.8, 151.9, 145.0, 133.5, 131.0, 129.9 (2C), 129.7, 128.8 (2C), 88.2, 84.3, 74.6, 66.5, 61.0, 50.1, 26.4 (3C), 25.9 (3C), 18.8, 18.1, 17.5, −4.1, −4.3, −5.0, −5.1 ppm; HRMS (ESI) m/z: [M + Na]$^+$ calc'd for C$_{31}$H$_{47}$Cl$_2$N$_4$O$_5$Si$_2$: 681.2457, found: 681.2450 (−1.0 ppm).

(+)(2R,3R,4S,5R)-2-(6-Amino-2-chloro-9H-purin-9-yl)-4-((tert-butyl(dimethyl)silyl)oxy)-5-(((tert-butyl(dimethyl)silyl)oxy)methyl)-3-methyltetrahydrofuran-2,4-diyl)dimethanol (45). To a solution of nucleoside 44 (199 mg, 0.292 mmol, 1.00 equiv.) in anhydrous MeOH (1.2 mL, 0.20 M) in a high-pressure flask, NH$_3$(g) was bubbled until saturation at room temperature. The reaction mixture was then stirred at 80 °C for 24 h. The mixture was diluted with MeOH and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (CH$_2$Cl$_2$/MeOH, 100:0 to 94:6) to provide the nucleoside analogue S3 (141 mg, 67%) as white foam. R$_f$ = 0.66 (EtOAc/Hexamnes, 1:4); [α]$^D_{25}$: +12 (c 0.8, in MeOH); IR (neat) ν$_{max}$ 3324, 3189, 2955, 2930, 2896, 2858, 1642, 1549, 1463, 1346, 1316, 1253, 1215 cm$^{-1}$; Formula: C$_{32}$H$_{44}$Cl$_2$N$_4$O$_4$Si$_2$; MW: 558.26 g mol$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 8.40 (s, 1H), 6.30 (s, 1H), 4.37 (d, J = 7.6 Hz, 1H), 4.06 (dd, J = 11.8, 2.0 Hz, 1H), 4.01 (dt, J = 7.6, 2.3 Hz, 1H), 3.89 (s, 2H), 3.86 (dd, J = 11.8, 2.5 Hz, 1H), 0.97 (s, 3H), 0.95 (s, 9H), 0.71 (s, 3H), 0.17 (s, 3H), 0.15 (s, 3H), 0.14 (s, 3H), 0.13 (s, 3H) ppm; OH and NH signals are missing possibly due to exchange in CDCl$_3$. NMR $^{13}$C: (126 MHz, CDCl$_3$) δ 155.6, 150.3, 145.8, 139.3, 114.2, 89.3, 85.0, 77.5, 66.2, 61.1, 50.6, 26.3 (3C), 25.9 (3C), 18.7, 18.0, 17.3, −4.1, −4.4, −5.1, −5.1 ppm; HRMS (ESI): m/z: [M + H]$^+$ calc'd for C$_{29}$H$_{36}$Cl$_2$N$_4$O$_4$Si$_2$: 558.2693, found: 558.2686 (−0.15 ppm).

(−)(2R,3S,4R,5R)-5-(6-Amino-2-chloro-9H-purin-9-yl)-3-hydroxy-4-methyltetrahydrofuran-2,4-diyl)dimethanol (46). To a solution of nucleoside 45 (30 mg, 75%) as white foam. R$_f$ = 0.17 (CH$_2$Cl$_2$/CH$_3$OH, 90:10); [α]$^D_{25}$: −10 (c 1.0, CH$_3$OH); Formula: C$_{12}$H$_{16}$ClN$_5$O$_2$; MW: 329.74 g/mol; IR (neat) ν$_{max}$ 3339, 3199, 2936, 1653, 1594 cm$^{-1}$; 1H NMR (500 MHz, CD$_2$OD) δ 8.48 (s, 1H), 6.27 (s, 1H), 4.36 (d, J = 8.8 Hz, 1H), 4.07 (dd, J = 8.8, 3.6, 2.2 Hz, 1H), 3.97 (dd, J = 12.4, 2.2 Hz, 1H), 3.89–3.84 (m, 2H), 3.77 (d, J = 11.3 Hz, 1H), 0.70 (s, 3H) ppm; OH and NH signals are missing possibly due to exchange in CD$_2$OD; $^{13}$C NMR (125 MHz, CD$_2$OD) δ 158.1, 155.3, 151.5, 140.9, 119.0, 90.6, 85.6, 76.3, 65.4, 61.7, 51.7, 17.3 ppm; HRMS (ESI): m/z: [M + Na]$^+$ calc'd for C$_{12}$H$_{16}$Cl$_2$N$_3$O$_2$: 352.0783; found: 352.0783 (−0.096 ppm).

(−)(2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3-hydroxy-4-methyltetrahydrofuran-2,4-diyl)dimethanol (46). To a solution of nucleoside 45 (70 mg, 0.21 mmol, 1.0 equiv) in methanol (10 mL, 0.021 M), palladium (10 wt.%) on activated carbon (90 mg, 85 μmol, 0.40 equiv.) was added. The reaction mixture was degassed and flushed using a hydrogen-filled balloon. The resulting reaction was stirred for overnight at 40 °C. The reaction mixture was filtered through Celite®, washed with methanol, and filtrates were concentrated under reduced pressure to provide 46 (49 mg, 78%). The crude was pure by NMR and used for the next step without purification. R$_f$ = 0.27 (CH$_2$Cl$_2$/CH$_3$OH, 4:1); [α]$^D_{25}$: −60 (c 0.4, CH$_3$OH); IR (neat) ν$_{max}$ 3427, 2934, 2929, 2886, 2857, 1472, 1252 cm$^{-1}$; Formula: C$_{12}$H$_{15}$Cl$_2$N$_4$O$_4$; MW: 295.29 g/mol; $^1$H NMR (500 MHz, CD$_2$OD) δ 8.51 (s, 1H), 8.18 (s, 1H), 6.35 (s, 1H), 4.37 (d, J = 8.7 Hz, 1H), 4.08 (dd, J = 8.7, 3.1, 2.3 Hz, 1H), 3.99 (dd, J = 12.4, 2.3 Hz, 1H), 3.89–3.82
(m, 2H), 3.77 (d, J = 11.2 Hz, 1H), 0.67 (s, 3H) OH and NH signals are missing possibly due to exchange in CD$_2$OD ppm; $^{13}$C NMR (126 MHz, CD$_2$OD) δ 157.4, 153.7, 150.3, 141.6, 120.1, 90.7, 85.6, 76.1, 65.5, 61.5, 51.7, 17.3 ppm; HRMS (ESI) m/z [M + H]$^+$ calcd for C$_{12}$H$_{18}$N$_3$O$_4$: 296.1359; found 296.1348 (−1.8 ppm).

General Procedure C: Prior to the reaction, nucleoside analogue (29–31 and 45–47), salicyl phosphorochloridite (SalPCI) and tributylammonium pyrophosphate [(Bu$_3$H)N$_2$H$_2$P$_2$O$_7$] were respectively dried under reduced pressure in 10 mL, 5 mL, 5 mL flasks for 1 h. To a solution of (Bu$_3$H)N$_2$H$_2$P$_2$O$_7$ (1.2–2.5 equiv.) in anhydrous DMF (0.1 M), NBu$_3$ (0.25 M) was added under nitrogen atmosphere and the mixture was stirred until (5 min) it became homogenous. The reaction mixture then was injected into a 5 mL flask containing SalPCI (1.2–2.5 equiv.) and the resulting mixture was stirred at room temperature for 30 min. The mixture was then transferred to a flask containing nucleoside 29–31 and 45–47 (1.0 equiv.) and the resulting mixture was stirred for 1.5 h. A solution of iodine (3% in Pyr: H$_2$O 9:1 w/v) was injected dropwise into the solution until a permanent brown color was persisted (~0.5 mL) and the resulting mixture was stirred for 15 min. Water (1.5 mL) was added and the solution was stirred for 1.5 h to provide the desired C$_5$'-triphosphate which was detected by TLC (i-PrOH: NH$_4$OH: H$_2$O, 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 h. Centrifugation was conducted at 10 °C with 3200 rpm for 20 min and the resulting liquid phase was then transferred to a 50 mL Erlenmeyer flask. The resulting solid (residue) inside the centrifuge tube was air dried for 15 min. The residue was purified by reverse phase C18 flash chromatography (MeCN in 20 mM triethylammonium acetate (TEAAc) buffer, pH = 7) to provide corresponding nucleoside triphosphate triethylammonium salt, which was then lyophilized to provide pure solid nucleoside triphosphate as a white powder.

General Procedure D: If the nucleoside analogues hydroxyl or amine functional groups are protected with acetyl (Ac) or benzoyl (Bz) follow the General Procedure C until centrifugation. Residue (solid) inside the centrifuge tube was dissolved in NH$_4$OH (0.20 M) and was stirred for overnight. The mixture was concentrated under reduced pressure. Purification by reverse phase C18 flash chromatography (flow rate of 10 mL/min., gradient run of acetonitrile from 0 to 40% in 20 mM TEAAc, pH = 7) provided nucleoside triphosphate triethylammonium acetate buffer, pH = 7) to provide corresponding nucleoside triphosphate triethylammonium salt, which was then lyophilized to provide pure solid nucleoside triphosphate as a white powder.

((2S,3S,4R,5R)-5-(4-Amino-2-oxopyrimidin-1(2H)-yl)-4-hydroxy-3-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate 2 (LCB-2330). Following general procedure D, (Bu$_3$H)N$_2$H$_2$P$_2$O$_7$ (0.10 g, 0.18 mmol, 2.5 equiv.), NBu$_3$ (0.28 mL, 0.25 M), SalPCI (40 mg, 0.20 mmol, 2.5 equiv.) and nucleoside analogue 29 (36.0 mg, 0.079 mmol, 1.2–2.5 equiv.) in anhydrous DMF (0.8 mL, 0.1 M) were employed in the phosphorylation. The final mixture was concentrated under reduced pressure. Purification by reverse phase C18 flash chromatography (flow rate of 10 mL/min., gradient run of acetonitrile from 0 to 10% in 20 mM TEAAc, pH = 7) provided nucleoside triphosphate triethylammonium salt 2 (LCB-2330) (20 mg, 28%) as a white powder. Formula: C$_{11}$H$_{20}$N$_3$O$_{14}$P$_3$; MW: 511.21 g/mol; $^1$H NMR (500 MHz, CD$_3$OD) 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, 1H), 1.29 (t, J = 7.3 Hz, 2H), 1.17 (s, 3H) ppm; $^13$C NMR (126 MHz, CD$_3$OD,$^2$H$_2$O 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$_2$O) δ −10.69 (br s, 1P), −11.94 (s, 1P), −23.14 (br s, 1P) ppm; HRMS (ESI) m/z [M − H]$^-$ calcd for C$_{11}$H$_{19}$N$_3$O$_{14}$P$_3$: 510.0085; found 510.0087 (+0.4 ppm).

((2S,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-4-hydroxy-3-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)methyl tetrahydrogen triphosphate 3 (LCB-2332). Following general procedure D, (Bu$_3$H)N$_2$H$_2$P$_2$O$_7$ (0.10 g, 0.18 mmol, 2.5 equiv.), NBu$_3$ (0.29 mL, 0.25 M), SalPCI (37 mg,
0.18 mmol, 2.5 equiv.) and nucleoside analogue 30 (35.0 mg, 0.072 mmol, 1.00 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 (flow rate of 10 mL/min., gradient run of acetonitrile from 0 to 10% in 20 mM TEAAc, pH =7) provided nucleoside triphosphate 3 (LCB-2332) triethylammonium salt (18 mg, 27%) as a white powder. Formula: C_{12}H_{20}N_{4}O_{13}P_{3}; MW: 535.24 g/mol; 1H NMR (500 MHz, D_{2}O, 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; OH and NH signals are missing possibly due to exchange in D_{2}O; 13C NMR (126 MHz, D_{2}O, 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, 8.2* ppm; 31P NMR (162 MHz, D_{2}O) 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_{4}O_{13}P_{3}: 534.0198; found 534.0203 (+1.0 ppm).

((2S,3S,4R,5R)-5-[(6-Amino-2-chloro-9H-purin-9-yl)-4-hydroxy-3-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)methyl tetraphosphate 4 (LCB-2337). Following general procedure D, (Bu_{3}HN)H_{2}PO_{4} (73.0 mg, 0.133 mmol, 2.20 equiv.) and nucleoside analogue 31 (25.0 mg, 0.06 mmol, 1.00 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 (flow rate of 10 mL/min., gradient run of acetonitrile from 0 to 10% in 20 mM TEAAc, pH =7) provided nucleoside triphosphate 4 (LCB-2337) triethylammonium salt (30 mg, 51%) as a white powder. Formula: C_{12}H_{19}ClN_{5}O_{13}P_{3}; MW: 569.68 g/mol; 1H NMR (500 MHz, D_{2}O, signals for triethylammonium denoted by * ) δ 8.60 (s, 1H), 6.12 (d, J = 7.1 Hz, 1H), 4.75–4.70 (m, 1H), 4.46 (q, J = 3.3 Hz, 1H), 4.26–4.12 (m, 2H), 3.84 (d, J = 11.5 Hz, 1H), 3.72 (d, J = 11.5 Hz, 1H), 3.20 (q, J = 7.3 Hz, 22H*), 1.28 (t, J = 7.3 Hz, 31H*), 1.24 (s, 3H) ppm; OH and NH signals are missing possibly due to exchange in D_{2}O; 13C NMR (126 MHz, D_{2}O, signals for triethylammonium denoted by * ) δ 156.3, 153.8, 150.7 (Brs), 140.2, 117.54 (BrS), 86.9, 82.9 (d, J = 10.1 Hz), 80.2, 66.23 (d, J = 6.2 Hz), 64.5, 47.1, 46.6*, 15.2, 8.2* ppm (J values result from 13C−31P coupling and were assigned when possible); 31P NMR (162 MHz, D_{2}O) δ −6.34 (d, J = 18.7 Hz, 1P), −11.71 (d, J = 19.4 Hz, 1P), −22.58 (t, J = 19.0 Hz, 1P) ppm; HRMS (ESI) m/z [M − H]− calcd for C_{12}H_{18}ClN_{5}O_{13}P_{3}: 567.9808; found 567.9808 (−0.09 ppm).

((2R,3R,4R,5S)-5-[(4-Amino-2-oxopyrimidin-1(2H)-yl)-3-hydroxy-4-(hydroxymethyl)-3-methyltetrahydrofuran-2-yl)methyl tetraphosphate 5 (LCB-2289). Following general procedure C, (Bu_{3}HN)H_{2}PO_{4} (162 mg, 295 µmol, 2.00 equiv.) and nucleoside analogue 47 [13] (40.0 mg, 147 µmol, 1.00 equiv.) in anhydrous DMF (1 mL, 0.1 M) were employed in the phosphorylation. The final mixture was concentrated under reduced pressure. Purification by reverse phase C18 flash chromatography (flow rate of 8 mL/min., gradient run of acetonitrile from 0 to 6% in 20 mM TEAAc, pH =7) provided nucleoside triphosphate 5 (LCB-2289) triethylammonium salt (7 mg, 5%) as a white powder. Formula: C_{11}H_{16}N_{3}O_{14}P_{3}; MW: 511.21 g/mol; 1H NMR (700 MHz, D_{2}O, signals for triethylammonium denoted by * ) δ 8.14 (d, J = 7.8 Hz, 1H), 6.25 (d, J = 7.5 Hz, 1H), 6.24 (d, J = 7.8 Hz, 1H), 4.37–4.33 (m, 1H), 4.29–4.20 (m, 1H), 3.84 (d, J = 11.5 Hz, 1H), 3.72 (d, J = 11.5 Hz, 1H), 3.20 (q, J = 7.3 Hz, 24H*), 1.28 (t, J = 7.3 Hz, 36H*), 0.88 (s, 3H) ppm, OH and NH signals are missing possibly due to exchange in D_{2}O; 13C NMR (176 MHz, D_{2}O, signals for triethylammonium denoted by * ) δ 163.1, 153.8, 143.4, 95.8, 89.3, 81.8 (d, J_{CP} = 8.9 Hz), 73.7, 64.0, 63.4 (d, J_{CP} = 5.0 Hz), 49.6, 46.6*, 16.0, 8.2* ppm; 31P NMR (162 MHz, D_{2}O) δ −10.88 (d, J = 10.6 Hz, 1P), −11.34 (d, J = 19.6 Hz, 1P), −23.24 (brs, 1P) ppm; HRMS (ESI) m/z [M + H]± calcd for C_{11}H_{16}N_{3}O_{14}P_{3}: 510.0085; found 510.0081 (−0.07 ppm) and [M + Na − H]− calcd for C_{11}H_{18}NaO_{14}P_{3}: 531.9905; found 531.9900 (−0.88 ppm).

((2R,3S,4R,5R)-5-[(6-Amino-9H-purin-9-yl)-3-hydroxy-4-(hydroxymethyl)-4-methyltetrahydrofuran-2-yl)methyl tetraphosphate 6 (LCB-2344). Following general procedure
C, (Bu₃HN)₂H₂P₂O₇ (51.0 mg, 0.092 mmol, 1.30 equiv.), NBu₃ (0.28 mL, 0.25 M), SalPcI (17.0 mg, 0.085 mmol, 1.20 equiv.) and nucleoside analogue 46 (21.0 mg, 0.071 mmol, 1.00 equiv.) in anhydrous DMF (0.7 mL, 0.1 M) were employed in the phosphorylation. Purification by reverse phase C18 flash chromatography (flow rate of 8 mL/min, gradient run of acetonitrile from 0 to 8% in 20 mM TEAAc, pH = 6) provided nucleoside triphosphate 6 (LCB-2344) triethylammonium salt (3.4 mg, 5%) as a white powder. Formula: C₁₂H₂₃N₅O₁₃P₃; MW: 535.23 g/mol; ¹H NMR (500 MHz, D₂O, signals for triethylammonium denoted by *) δ 8.60 (Br s, 1H), 8.28 (s, 1H), 6.43 (s, 1H), 4.50 (Br d, J = 8.6 Hz, 1H), 4.43–4.30 (m, 3H), 3.93 (d, J = 11.9 Hz, 1H), 3.83 (d, J = 11.6 Hz, 1H), 3.21 (q, J = 7.3 Hz, 21H*), 1.29 (t, J = 7.3 Hz, 30H*), 0.72 (s, 3H) ppm; OH and NH₂ signals are missing possibly due to exchange in D₂O; ¹³C NMR (176 MHz, D₂O, signals for triethylammonium denoted by *) δ 152.8, 148.7, 148.4, 118.2, 88.4, 82.11 (d, J = 21.2 Hz, 1P), −11.35 (d, J = 19.9 Hz, 1P), −22.54 (t, J = 20.6 Hz, 1P) ppm; HRMS (ESI) m/z [M + Na]⁺ calcd for C₁₂H₁₉N₅O₁₃P₃: 534.0198; found 534.0197 (−0.08 ppm).

((2R,3S,4R,5R)-5-(6-Amino-2-chloro-9H-purin-9-yl)-3-hydroxy-4-(hydroxymethyl)-4-methyltetrahydrofuran-2-yl)methyl triphosphate: 7 (LCB-2279). Following general procedure C, (Bu₃HN)₂H₂P₂O₇ (100 mg, 0.182 mmol, 2.00 equiv.), NBu₃ (0.36 mL, 0.25 M), SalPcI (37.0 mg, 0.182 mmol, 2.00 equiv.) and nucleoside analogue 45 (30.0 mg, 0.091 mmol, 1.00 equiv.) in anhydrous DMF (1 mL, 0.1 M) were employed in the phosphorylation. Purification by reverse phase C18 flash chromatography (flow rate of 12 mL/min, gradient run of acetonitrile from 0 to 20% in 20 mM TEAAc, pH = 7) provided nucleoside triphosphate 7 (LCB-2279) triethylammonium salt (10 mg, 11%) as a white powder. Formula: C₁₂H₁₅ClN₅O₁₃P₃; MW: 569.68 g/mol; ¹H NMR (500 MHz, D₂O, signals for triethylammonium denoted by *) δ 8.52 (Br s, 1H), 6.29 (s, 1H), 4.48 (d, J = 8.4 Hz, 1H), 4.41–4.26 (m, 3H), 3.91 (d, J = 11.7 Hz, 1H), 3.82 (d, J = 11.6 Hz, 1H), 3.19 (q, J = 6.2 Hz, 20H*), 1.27 (t, J = 7.3 Hz, 30H*), 0.72 (s, 3H) ppm; OH and NH₂ signals are missing possibly due to exchange in D₂O; ¹³C NMR (176 MHz, D₂O, signals for triethylammonium denoted by *) δ 156.3, 153.7, 149.7, 140.9, 117.3, 88.1, 82.1 (d, JCP = 8.8 Hz), 73.8, 63.9 (d, JCp = 5.3 Hz), 63.5, 49.9, 46.6*, 15.7, 8.2* ppm (J values result from ¹³C⋯¹¹P coupling and were assigned when possible); ³¹P NMR (162 MHz, D₂O) δ −6.43 (d, J = 21.2 Hz, 1P), −11.35 (d, J = 19.9 Hz, 1P), −22.54 (t, J = 20.6 Hz, 1P) ppm; HRMS (ESI) m/z [M − H]− calcd for C₁₂H₁₅ClN₅O₁₃P₃: 567.9808; found 567.9819 (+1.86 ppm) and [M + Na − H]− calcd for C₁₂H₁₇ClN₅NaO₁₃P₃: 589.9627; found 589.9637 (+1.62 ppm). A minor and inseparable impurity was isolated with the compound. ³¹P NMR signals at −11.04 (d) and −6.49 (d) ppm suggest that this side product could be the corresponding diphosphate.

3.3. Biology

SARS-CoV-2 RdRp complex (nsp12/nsp7/nsp8) was prepared as published [9]. The RNA synthesis activity of the RdRp complex was evaluated in a reaction mixture comprising a 19-mer RNA primer, 43-mer RNA template, 25 mM TRIS-HCl (pH8), cold NTPs (50 µM ATP, CTP and UTP; 25 µM GTP), 0.1 µM [α-³²P]-GTP and different concentrations of each inhibitor of interest. Nuclease-free water was added in place of the RdRp or the inhibitors for the negative control (−Pol) or no-treatment control (+Pol), respectively. After 10 min incubation at 30 °C, 5mM MnCl₂ was added into each reaction mix to initiate the RdRp reaction. After another 30 min incubation at 30 °C, the RdRp reactions were terminated with formamide containing 40 mM EDTA, and were heated at 95 °C for 10 min. The resulting reaction products were resolved on 20% polyacrylamide-urea denaturing gels (Sequagel, National Diagnostics, Atlanta, GA, USA) and visualized using the Amersham Typhoon IP (Cytiva, Marlborough, MA, USA). Analyses were subsequently conducted with ImageQuant TL 8.2 (Cytiva) [6].
4. Conclusions

The syntheses of novel NTP analogues bearing a quaternary all-carbon stereogenic center at C3′ and C2′ have been achieved. The stereogenic quaternary center at C2′ or C3′ were generated by photocatalyzed cyclization/elimination free-radical-based reactions through five-exo-trig cyclization in the C2′ quaternary series and seven-endo trig cyclization in the C3′ quaternary series. The installation of the hydroxyl at C2′ was significantly improved through a stereoselective epoxidation, providing access to NA-containing quaternary carbon at C3′. A modified approach was also presented for the synthesis of NAs of C2′ quaternary center series. The synthesis and purification of the corresponding nucleoside triphosphates are reported for the first time. Optimization for NTPs bearing C2′ quaternary stereogenic center is under development.

Finding novel molecules that could act as antiviral agents against SARS-CoV-2, or other emerging viruses, is an important venue for the present and future treatment of these infections. We have reported herein two inhibitors, 6 (LCB-2344) and 7 (LCB-2279), against SARS-CoV-2 RdRp, which are lead molecules for further optimization. The stereogenic quaternary center at C2′ will be further modified to improve the potency of the novel series of molecules. Currently the study is underway to explore the monophosphorylated pro-drugs of these molecules and their antiviral efficacy.

Supplementary Materials: The following supporting information can be downloaded at Supplementary Materials: HSQC, HMBC, nOe correlations of compounds 26–28, 29, 30–31 and 33–34. 1H and 13C NMR spectra of compounds 2–7, 17–34 and S2–46. 31P NMR spectra of NTPs 2–7.

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Conflicts of Interest: The compounds described herein are the subject matter of patents and patent application [16–18]. The rights to them are owned by LCB-Pharma Inc. in accordance with an institute by the Institut de Recherches Cliniques de Montréal (IRCM) and the University of Ottawa. Yvan Guindon and Michel Prevost are shareowners of LCB-Pharma Inc. The other authors declare no conflict of interest.

Sample Availability: Samples of the compounds are not available from the authors.

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