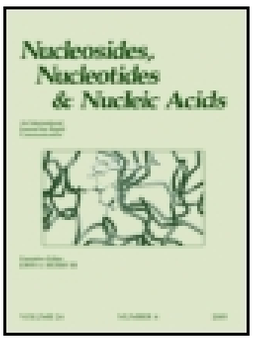

Nucleosides, Nucleotides and Nucleic Acids

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Synthesis and Antibacterial Activity of 5′-tetrachlorophthalimido and 5′-azido 5′-deoxyribonucleosides

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ABSTRACT

Reported is an efficient synthesis of adenyl and uridyl 5′-tetrachlorophthalimido-5′-deoxyribonucleosides, and guanylyl 5′-azido-5′-deoxyribonucleosides, which are useful in solid-phase synthesis of phosphoramidate and ribonucleic guanidine oligonucleotides. Replacement of 5′-hydroxyl with tetrachlorophthalimido group was performed via Mitsunobu reaction for adenosine and uridine. An alternative method was applied for guanosine which replaced the 5′-hydroxyl with an azido group. The resulting compounds were converted to 5′-amino-5′-deoxyribonucleosides for oligonucleotide synthesis. Synthetic intermediates were tested as antimicrobials against six bacterial strains. All analogs containing the 2′,3′-O-isopropylidine protecting group demonstrated antibacterial activity against Neisseria meningitidis, and among those analogs with 5′-tetrachlorophthalimido and 5′-azido demonstrated increased antibacterial effect.

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5′-Amino nucleosides; 2′,3′-*O*-isopropylidene nucleosides; modified nucleoside; mitsunobu reaction; ribonucleic guanidine; antibacterial activity of nucleoside analogs

Introduction

Many applications of modified nucleosides include incorporation into therapeutic oligonucleotides and usage as potent antibacterial and antiviral agents.[1–3] Azido and amino nucleosides, in particular, received considerable attention with new applications in chemistry, biology, and medicine.[4,5] Azidothymidine (AZT, Figure 1A), for example, is an antiretroviral nucleoside analog used for treatment of HIV.[6] Problems associated with AZT, such as immunodeficiency, lymphoma, muscle atrophy, and dementia, motivate synthetic chemists to develop alternative azido nucleosides that could provide the same benefits with less or no toxic effects. Azidoguanosine (AZG, Figure 1B) is another nucleoside drug which is a potent and selective inhibitor of HIV with a resistance profile superior to AZT.[7] A gemcitabine analog (Figure 1C) containing a 5′-amino modification has demonstrated inhibition

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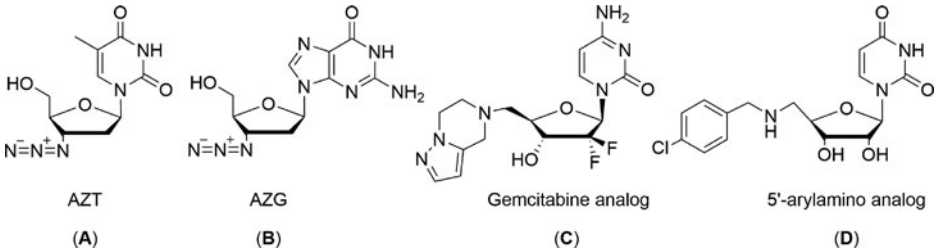


Figure 1. Modified nucleosides (**A**) 3′-azidothymidine (AZT); (**B**) 3′-azido-2′,3′-dideoxyguanosine (AZG); (**C**) Gemcitabine analog; (**D**) 5′-arylamino nucleoside.

of RRM1.[8] A 5′-arylamino-nucleoside (Figure 1D) exhibited significant inhibition of HIV-1 reverse transcriptase.[9] Moreover, therapeutic oligonucleotides which exhibit unique characteristics, such as sequence specific binding affinity, have been developed through chemical modification of the nucleoside sugar, nitrogenous base, and the phosphate backbone.[10–13] Among these, phosphoramidate and ribonucleic guanidine (RNG) oligonucleotides (Figure 2) have demonstrated capability as therapeutic nucleic acids that utilize 5′-amino-5′-deoxyribonucleoside monomers as building block components.[14–16]

Several experiments highlighted the potential of RNG to improve binding affinity, cellular uptake, and nuclease resistance. The positively charged guanidinium linkage within RNG is capable of overcoming electrostatic repulsion from native DNA/RNA.[17] The wide pH range through which the guanidinium group remains protonated allows binding to readily occur through both hydrogen bonding and charge pairing.[18] The positive charge facilitates interaction with softer anions such as phosphates and sulfates.[19] Prior work in vitro demonstrated pentameric uridyl RNG forms remarkably stable complexes with pentameric adenyl DNA, and no structures with DNA consisting of non-complementary bases were detected.[20]

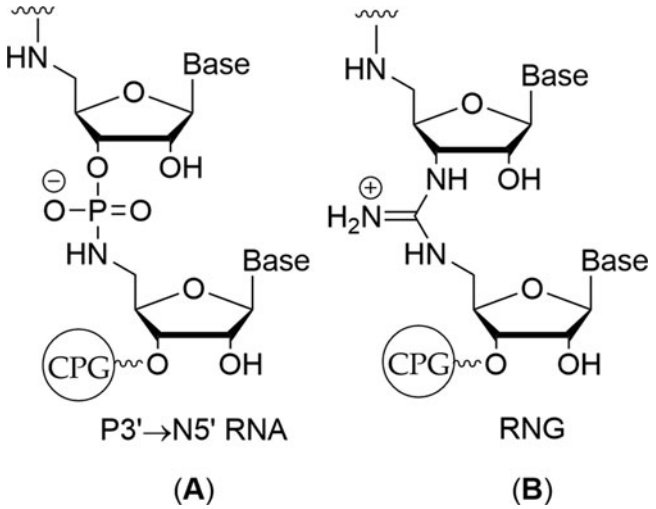


Figure 2. (**A**) P3′→N5′ phosphoramidate RNA; (**B**) Ribonucleic guanidine (RNG).

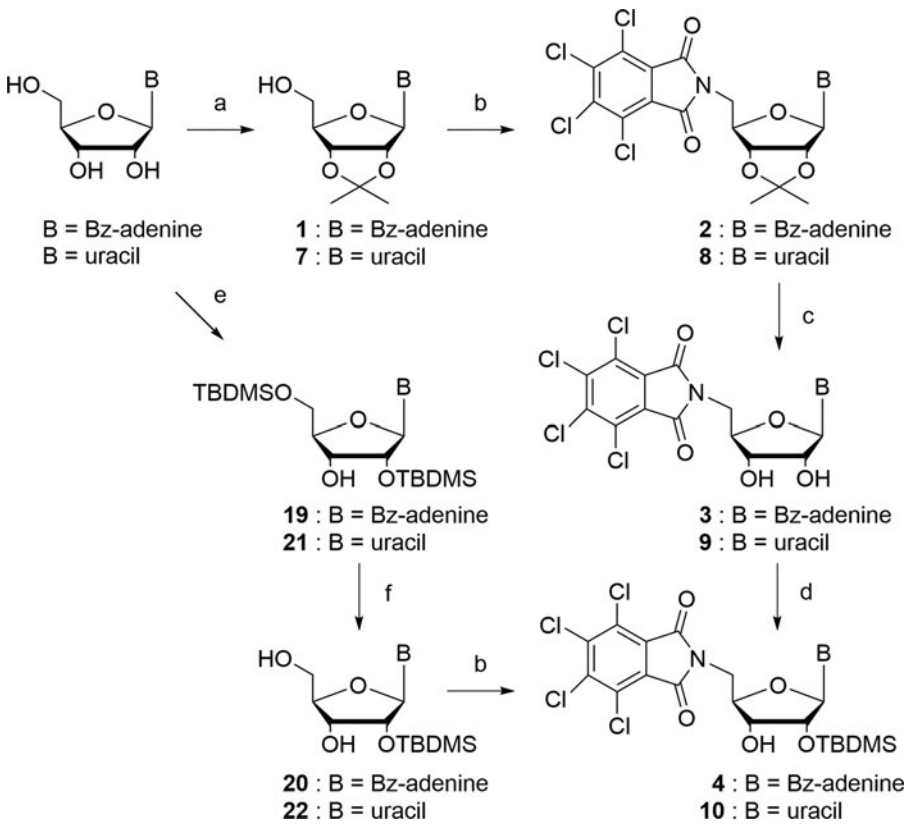
Proposing novel and more efficient synthetic methods to develop amino and azido nucleosides is crucial in nucleic acids chemistry. The precedent synthetic method for 5′-azido-5′-deoxyribonucleosides involves nucleophilic displacement of 5′-*O*-sulfonate esters with azide, which can then be reduced to the 5′-amino derivative.[21,22] In this work, we describe convenient and efficient methods to synthesize adenyl and uridyl 5′-tetrachlorophthalimido-5′-deoxyribonucleosides, and guanylyl 5′-azido-5′-deoxyribonucleosides. Subsequent preparation of the corresponding 5′-amino-5′-deoxyribonucleoside analogs is also reported. The target compounds may serve as precursors to synthesize the building blocks for automated solid-phase synthesis of P3′→N5′ phosphoramidate RNA or the 3′-terminal monomers in RNG oligonucleotides. In addition, adenyl, uridyl, and guanylyl synthetic intermediates were tested for antibacterial activity against three gram positive and three gram negative strains of bacteria. Six of the tested compounds demonstrated significant inhibition of the growth of bacteria *Neisseria meningitidis*.

Results and discussion

Synthesis

Preparation of monomers for oligonucleotide synthesis first required protection of the nitrogenous base for adenosine and guanosine, which was accomplished by using well-established protocols to produce N^6-benzoyladenosine and N^2-isobutyrylguanosine.[23] Protection of the nucleoside 2′,3′-hydroxyls was then achieved using 2,2-dimethoxypropane for adenosine, uridine, and guanosine analogs, utilizing the isopropylidene protecting group to produce compounds **1**, **7**, and **13**, respectively. 5′-hydroxyl group of the nucleosides was then converted to 5′-tetrachlorophthalimido in high yield for adenosine and uridine analogs **2** (91%) and **8** (78%) via Mitsunobu reaction (Scheme 1) using 3,4,5,6-tetrachlorophthalimide.[24] This reaction did not produce the desired guanylyl analog, perhaps due to the necessary protection of the O^6-position of guanine.[25] The guanylyl nucleoside 5′-hydroxyl was instead converted to 5′-azido according to a previously published method to produce **14** (Scheme 2) in 80% yield.[26]

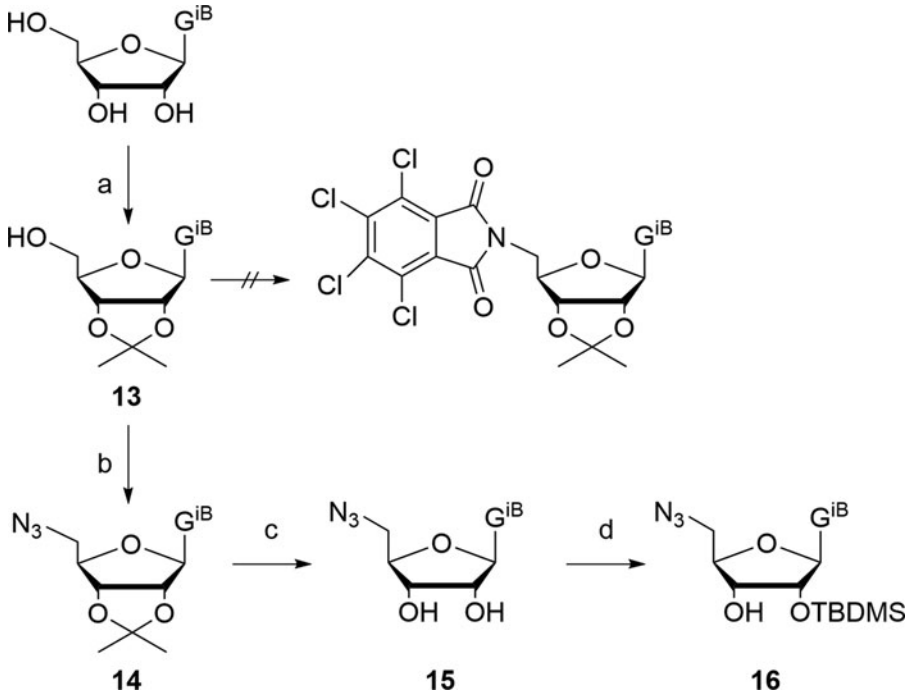
The 2′,3′-*O*-isopropylidene protecting group was then removed by stirring in 75% TFA to reveal the free 2′,3′-hydroxyls on compounds 3, 9, and 15 at yields of 94%, 91%, and 82%, respectively. Next, TBDMS was selectively added to the 2′-hydroxyl, leaving the 3′-hydroxyl exposed for its linker on analogs 4, 10, and 16. This reaction was performed with TBDMSCl and imidazole in anhydrous pyridine for the uridyl analog, while TBDMSCl, $AgNO_3$, and pyridine in anhydrous DMF provided higher reaction yields for adenyl and guanylyl analogs. In this silylation step, the undesired 3′-*O*-TBDMS nucleoside derivatives were separated from the desired 2′-*O*-TBDMS nucleoside analogs by silica gel flash column chromatography. The nitrogen-containing groups at the nucleoside 5′-position on adenyl and uridyl analogs were then converted to 5′-amino using ethylenediamine via modified Gabriel synthesis.[27] A catalytic reduction of the azido guanylyl analog



Scheme 1. Synthesis of 5′-tetrachlorophthalimido-5′-deoxyribonucleosides: (a) 2,2-dimethoxypro pane, *p*-toluenesulfonic acid, acetone, 24 h reflux; (b) Mitsunobu reaction, 3,4,5,6-tetrachlorophthalimide, PPh_3, THF, 15 h reflux; (c) 75% aq TFA; (d) TBDMSCl, $AgNO_3$, pyridine, DMF or TBDMSCl, imidazole, pyridine; (e) TBDMSCl, $AgNO_3$, pyridine, THF (f) 90% aq TFA, 0°C.

was performed using 10% Pd/C. The formed 5′-amino group was then protected with MMTr, which is useful during oligonucleotide synthesis cycles, resulting in compounds 5, 11, and 17 (Scheme 3). The last step involved the addition of the 3′-*O*-succinyl linker, required to facilitate the attachment to controlled-pore glass solid support. This was performed using succinic anhydride, leading to the formation of 2′-*O*-(*tert*-butyldimethylsilyl)-5′-(4-monomethoxytritylamino)-3′-*O*-succinyl-5′-deoxyribonucleosides 6, 12, and 18 in respective yields of 79%, 84%, and 76%.

An alternate protective strategy was developed to synthesize compounds 4 and 10, which involved silyl protection of the 2′,5′-hydroxyls to produce compounds 19 and 21 (Scheme 1). The 5′-*O*-TBDMS group was then removed via selective deprotection by stirring in 90% TFA at 0°C to produce 20 or 22 (93%, 91%), which allowed high yield conversion to 4 or 10 (85%, 79%) via Mitsunobu reaction. This was another convenient method providing 2′-*O*-(*tert*-butyldimethylsilyl)-5′-tetrachlorophthalimido-5′-deoxyribonucleosides in high yield, and further demonstrated the versatility of the herein reported Mitsunobu reaction in which no necessity for the protection of the 3′-hydroxyl was required. However, it is worth

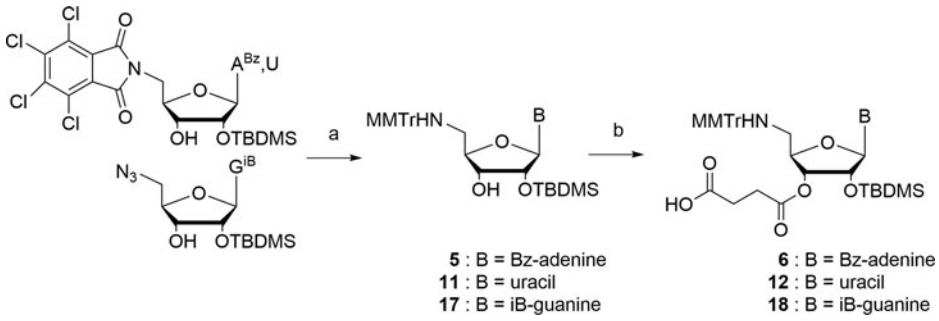


Scheme 2. Synthesis of 5′-azido-5′-deoxyguanosine: (a) 2,2-dimethoxypropane, *p*-toluenesulfonic acid, acetone, 24 h reflux; (b) DMF, PPh_3, CBr_4, NaN_3, 90°C; (c) 75% aq TFA; (d) TBDMSCl, $AgNO_3$, pyridine, DMF.

mentioning that the Mitsunobu reaction failed when applied directly on N^6-benzoyladenosine with both 2′ and 3′-hydroxyls remain unprotected, even when the THF solvent was replaced by DMF to overcome the low solubility of the unprotected analog.

Antibacterial activity

With an increasing number of antibiotic resistant pathogens, and a shortening supply of new antibiotics, it is critical to design new, effective antibacterial



Scheme 3. Oligonucleotide monomer preparation: (a) (i) ethylenediamine, THF or Pd/C, H_2, CH_2Cl_2; (ii) MMTrCl, Et_3N, CH_2Cl_2; (b) succinic anhydride, Et_3N, CH_2Cl_2.

agents, and to find new applications of existing compounds that act as antibacterial agents. Modified nucleosides resemble naturally occurring RNA analogs, and therefore may interfere with numerous biological processes, so it is not surprising that many nucleoside-based analogs, including 5′-amino derivatives, have demonstrated antimicrobial activity.[28] Considering this, intermediates containing 5′-tetrachlorophthalimido, 5′-azido, and respective precursors made suitable candidates for antibacterial screening. Synthetic intermediates were tested for antibacterial activity against six strains of bacteria, including three gram positive and three gram negative strains. Bacteria tested includes *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Enterococcus faecalis, Proteus vulgaris*, and *Neisseria meningitidis*. Disc diffusion was implemented to determine percent inhibition and to obtain quantitative susceptibility data. Known antibacterial agents ampicillin, kanamycin, and erythromycin were used as positive controls, and DMSO was used as a negative control. No antibacterial activity could be observed with five strains of bacteria. However, a total of six compounds, two of each nucleobase, demonstrated inhibition against *Neisseria meningitidis* serogroup B. *Neisseria meningitidis* is a gram negative diplococcus that colonizes the nasopharyngeal mucosa and inhabits approximately 10% of the population on a continual basis, but the bacteria can cause devastating diseases with high fatality rates and is prone to avoid detection by the immune system.[29]

Percent inhibition results revealed an observable trend. One of six bacteria, *Neisseria meningitidis*, was inhibited by synthetic intermediates 1, 2, 7, 8, 13, and 14, as seen in Table 1. All six compounds that demonstrated inhibition against *N. meningitidis* possess the 2′,3′-*O*-isopropylidene protecting group. Adenyl, uridyl, and guanylyl 2′,3′-*O*-isopropylidene-nucleosides 1, 7, and 13, each possessing

Table 1. Antibacterial activity.

	Percent inhibition of selected bacterial strains					
Compound	*S. aureus* (G^+)	*B. cereus* (G^+)	*E. faecalis* (G^+)	*P. vulgaris* (G^-)	*E. coli* (G^-)	*N. meningitidis* (G^-)
DMSO (Negative Control)	0	0	0	0	0	0
N^6-Benzoyladenosine	0	0	0	0	0	0
N^2-Isobutyrylguanosine	0	0	0	0	0	0
1	0	0	0	0	0	10
2	0	0	0	0	0	12
3	0	0	0	0	0	0
4	0	0	0	0	0	0
7	0	0	0	0	0	8
8	0	0	0	0	0	9
9	0	0	0	0	0	0
10	0	0	0	0	0	0
13	0	0	0	0	0	9
14	0	0	0	0	0	10
15	0	0	0	0	0	0
19	0	0	0	0	0	0
20	0	0	0	0	0	0
21	0	0	0	0	0	0
22	0	0	0	0	0	0
Ampicillin (10 μg)	13	7	12	13	0	20
Kanamycin (30 μg)	20	25	16	27	20	19
Erythromycin (15 μg)	23	26	19	7	9	27

Table 2. Quantitative susceptibility determination.

Mass loaded on disc (μg)	Volume pipetted onto disc (μL)	Observed inhibition by varied concentrations of compounds				
		Compound **2**	Compound **8**	Compound **14**	Ampicillin	Kanamycin
256	5	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition
128	5	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition
64	5	Inhibition	No inhibition	Inhibition	Inhibition	Inhibition
32	5	No inhibition	No inhibition	No inhibition	Inhibition	Inhibition

a 5′-hydroxyl, demonstrated inhibition against *N. meningitidis*. The presence of a nitrogen-containing group at the 5′-position in addition to the 2′,3′-*O*-isopropylidene protecting group increased antibacterial activity against *N. meningitidis* for all three tested nucleobases in compounds 2, 8, and 14. Analogs with a 5′-amino or 5′-azido derivative modification, but lacking the 2′,3′-*O*- isopropylidene protecting group (3, 9, and 15), failed to inhibit *N. meningitidis* at tested quantities. This indicates the 2′,3′-*O*- isopropylidene protecting group was critical for bacterial inhibition against *Neisseria meningitidis*. Nucleosides possessing 5′-tetrachlorophthalimido and 2′-*O*-TBDMS (4 and 10) did not exhibit antibacterial activity, reinforcing the importance of the presence of the 2′,3′-*O*-isopropylidene protecting group to achieve antibacterial activity against *N. meningitidis*. Enzymes that utilize nucleoside substrates, such as adenosine deaminase, tolerate some steric hindrance of modified substrates as seen in prior work with 5′-substituted-2′,3′-*O*-isopropylidene adenosine.[30] One potential target of inhibition is the rhamnosylation enzyme EarP, which is crucial for cell viability and has been identified as a promising target for antibacterial drug design specific to *Neisseria meningitidis*.[31]

Comparable antibiotic susceptibility results have been previously reported using both disc diffusion and the agar dilution method commonly used to determine minimum inhibitory concentration.[32] Compounds 2, 8, and 14 were tested against *Neisseria meningitidis* using disc diffusion to determine the minimum amount of compound necessary to prevent growth of the bacteria. The working amounts of known antibiotics ampicillin and kanamycin for disc diffusion tests are 10 μg and 30 μg, respectively.[33] As shown in Table 2, compounds 2 and 14 inhibited the growth of *N. meningitidis* using as little as 64 μg, and compound 8 inhibited *N. meningitidis* using 128 μg.

These results could be useful in future drug design of nucleoside antimicrobial agents, by utilizing the 2′,3′-*O*-isopropylidene group in combination with nitrogen-containing or other possible effective substituents located at the 5′-position. The isopropylidene group should also be investigated for use with other organic molecules being examined as antibiotics, as it may provide additional efficacy against *Neisseria meningitidis* and other bacteria.

Conclusion

This work provides a convenient and high yield synthesis for adenyl, uridyl, and guanylyl 5′-amino-5′-deoxynucleosides which may be used as precursors

to synthesize the building blocks required for the assembly of phosphoramidate or ribonucleic guanidine oligonucleotides. Also, antimicrobial activity against *Neisseria meningitidis* was exhibited by six intermediates containing the 2′,3′-*O*-isopropylidene group. The antibacterial activity of 2′,3′-*O*-isopropyldiene-nucleosides was improved when a nitrogen-containing group replaced the nucleoside 5′-hydroxyl, as demonstrated by the reported 5′-tetrachlorophthalmido-5′-deoxyribonucleosides and 5′-azido-5′-deoxyribonucleosides.

Experimental

General procedures

^{1}H-NMR spectra were recorded on a 500 MHz Varian instrument, using $CDCl_3$ or DMSO-d_6 as solvents. Chemical shifts were reported in δ ppm and coupling constants (J) are given in Hz. Thin-layer chromatography (TLC) was carried out on Silica Gel 60 F_{254} pre-coated plates (EMD Millipore, Merck KGaA, Darmstadt, Germany) and visualization of the products was performed under UV light. Silica gel used for flash column chromatography was Scientific Silica Gel (particle size 60–200, Acros Organics, Geel, Belgium). ESI mass spectra were recorded on QSTAR Pulsar Quadrupole/time-of-flight mass spectrometer with Turbo Ion Spray Ionization Source (Applied Biosystems, Foster City, CA, USA). Reactions were performed using anhydrous solvents under N_2 atmosphere.

Synthesis

General procedure for 2′,3′-O-isopropylidenenucleosides. To a suspension of N^6-benzoyladenosine, uridine, or N^2-isobutyrylguanosine (15.70 mmol) in anhydrous acetone (100 mL), *p*-toluenesulfonic acid monohydrate (0.30 g, 1.57 mmol) and 2,2-dimethyoxypropane (17.3 mL, 141.19 mmol) were added. After 24 h reflux, the mixture was filtered to remove any unreacted starting material and the filtrate was concentrated under vacuum. The residue was dissolved in EtOAc, washed with 5% $NaHCO_3$, brine, and was dried over sodium sulfate anhydrous. The crude mixture was washed with anhydrous diethyl ether and filtered to afford pure compound **1**, **7**, or **13**, respectively.

N^6-Benzoyl-2′,3′-O-isopropylideneadenosine (**1**). 5.75 g, yield 89%. ^{1}HNMR (500 MHz, DMSO-d_6): 11.19 s, 1H (NH); 8.76 s, 1H (H-8); 8.66 s, 1H (H-2); 8.03 d, 2H, $J = 7.4$ (H-Bz); 7.63 t, 1H, $J = 7.4$ (H-Bz); 7.53 t, 2H, $J = 7.8$ (H-Bz); 6.26 d, 1H, $J = 2.9$ (H-1′); 5.43 dd, 1H, $J = 2.7, 6.1$ (H-2′); 5.12 t, 1H, $J = 5.2$ (OH-5′); 4.99 dd, 1H, $J = 2.5, 6.4$ (H-3′); 4.26 m, 1H (H-4′); 3.58 – 3.52 m, 2H (H-5′); 1.55 s, 3H (CH_3); 1.33 s, 3H (CH_3). ^{13}C NMR (500 MHz, DMSO-d_6): 166.1, 152.3, 152.2, 150.9, 143.6, 133.8, 132.9, 128.9, 128.9, 126.2, 113.5, 90.3, 87.3, 84.0, 81.9, 62.0, 27.5, 25.6.

2′,3′-O-Isopropylideneuridine (**7**). 3.53 g, yield 79%. ^{1}H NMR (500 MHz, DMSO-d_6): 11.35 s, 1H (NH); 7.77 d, 1H, $J = 8.3$ (H-6); 5.81 d, 1H, $J = 2.9$ (H-1′); 5.61 d,

1H, $J = 8.3$ (H-5); 5.06 t, 1H, $J = 5.4$ (OH-5′); 4.88 dd, 1H, $J = 2.7, 6.1$ (H-2′); 4.72 dd, 1H, $J = 3.4, 6.4$ (H-3′); 4.07 q, 1H, $J = 5.4$ (H-4′); 3.57–3.52 m, 2H (H-5′); 1.47 s, 3H (CH_3); 1.27 s, 3H (CH_3). ^{13}C NMR (500 MHz, DMSO-d_6): 163.6, 150.8, 142.4, 113.4, 102.2, 91.6, 87.0, 84.1, 80.9, 61.7, 27.5, 25.6.

N^2-Isobutyryl-2′,3′-O-isopropylideneguanosine (**13**). 5.37 g, yield 87%. ^{1}H NMR (500 MHz, $CDCl_3$): 7.86 s, 1H (H-8); 5.84 d, 1H, $J = 3.8$ (H-1′); 5.13 dd, 1H, $J =$ 4.0, 6.2 (H-2′); 5.01 dd, 1H, $J = 2.3, 6.2$ (H-3′); 4.40 q, 1H, $J = 2.5$ (H-4′); 3.93 – 3.76 m, 2H (H-5′); 2.71 m, 1H (CH); 1.58 s, 3H (CH_3); 1.34 s, 3H (CH_3); 1.24, 6H, $J = 6.9$ ($CH(CH_3)_2$). ^{13}C NMR (500 MHz, DMSO-d_6): 180.6, 155.2, 148.6, 138.5, 120.8, 113.6, 89.2, 87.4, 84.2, 81.5, 62.0, 35.2, 27.5, 25.7, 24.7, 19.3, 19.3.

General procedure for 5′-tetrachlorophthalimido-2′,3′-O-isopropylidene-5′-deoxyribonucleosides. A suspension was made of **1** or **7** (3.10 mmol), PPh_3 (1.02 g, 3.88 mmol), and 3,4,5,6-tetrachlorophthalimide (1.06 g, 3.72 mmol) in anhydrous THF (50 mL). Diisopropyl azodicarboxylate (0.73 mL, 3.72 mmol) was added dropwise over 15 min, and the mixture was refluxed for 15 h. The solvent was concentrated under vacuum, and the crude mixture was purified by silica gel column chromatography to produce **2** or **8**, respectively.

N^6-Benzoyl-5′-tetrachlorophthalimido-2′,3′-O-isopropylidene-5′-deoxyadenosine (**2**). 1.91 g, yield 91%, purified using 1:1 ratio of hexanes to EtOAc. HRMS (ESI) *m/z* Calcd for $C_{28}H_{20}N_6O_6Cl_4$ $(M + Na)^+$ 699.0096. Found 699.0084. ^{1}HNMR (500 MHz, $CDCl_3$): 9.04 s, 1H (NH); 8.73 s, 1H (H-8); 8.07 s, 1H (H-2); 8.03 d, 2H, $J = 7.3$ (H-Bz); 7.69 – 7.44 m, 3H (H-Bz); 6.12 d, 1H, $J = 1.4$ (H-1′); 5.58 dd, 1H, $J = 1.7, 6.1$ (H-2′); 5.26 dd, 1H, $J = 3.4, 6.4$ (H-3′); 4.58 m, 1H (H-4′); 4.06 – 3.95 m, 2H (H-5′); 1.59 s, 3H (CH_3); 1.39 s, 3H (CH_3). ^{13}C NMR (500 MHz, DMSO-d_6): 166.0, 163.7, 152.0, 151.9, 151.0, 144.2, 138.8, 133.8, 133.6, 132.9, 132.8, 132.5, 132.5, 129.0, 128.9, 128.6, 128.4, 126.1, 114.0, 89.4, 84.4, 84.0, 82.1, 55.4, 27.5, 25.8.

5′-Tetrachlorophthalimido-2′,3′-O-isopropylidene-5′-deoxyuridine (**8**). 1.33 g, yield 78%, purified using 1:1 ratio of hexanes to EtOAc. HRMS (ESI) *m/z* Calcd for $C_{20}H_{15}Cl_4N_3O_7$ $(M + Na)^+$ 573.9509, found 573.9485. ^{1}H NMR (500 MHz, DMSO-d_6): 11.34 d, 1H, $J = 2.0$ (NH); 7.71 d, 1H, $J = 8.3$ (H-6); 5.67 d, 1H, $J = 1.0$ (H-1′); 5.59 dd, 1H, $J = 2.2, 8.1$ (H-5); 5.12 dd, 1H, $J = 1.5, 6.3$ (H-2′); 4.86 dd, 1H, $J = 4.4, 6.4$ (H-3′); 4.20 dd, 1H, $J = 2.0, 4.5$ (H-4′); 3.97–3.85 m, 2H (H-5′); 1.45 s, 3H (CH_3); 1.27 s, 3H (CH_3). ^{13}C NMR (500 MHz, $CDCl_3$): 164.0, 163.3, 150.7, 144.0, 140.0, 129.5, 127.4, 114.1, 103.0, 97.3, 86.1, 84.6, 82.5, 60.4, 40.3, 26.9, 25.2, 14.2.

General procedure for 5′-tetrachlorophthalimido-5′-deoxyribonucleosides. To compound **2** or **8** (1.59 mmol) in methylene chloride (5 mL), aqueous trifluoroacetic acid (75%, 20 mL) was added, and the mixture was stirred at room temperature for 2 hours. The solvent was evaporated under reduced pressure, and the residue was filtered and washed with anhydrous diethyl ether to produce compound **3** or **9**, respectively.

N^6-Benzoyl-5′-tetrachlorophthalimido-5′-deoxyadenosine (**3**). 0.95 g, yield 94%. HRMS (ESI) *m/z* Calcd for $C_{25}H_{16}N_6O_6Cl_4$ $(M + Na)^+$ 658.9783, found 658.9767.

HNMR (500 MHz, DMSO-d_6): 8.72 s, 1H (H-8); 8.61 s, 1H (H-2); 8.04 d, 2H, J = 7.3 (H-Bz); 7.63 t, 1H, J = 7.3 (H-Bz); 7.54 t, 2H, J = 7.6 (H-Bz); 6.01 d, 1H, J = 5.3 (H-1′); 4.83 t, 1H, J = 5.2 (H-2′); 4.30 t, 1H, J = 4.4 (H-3′); 4.19 m, 1H (H-4′); 4.01 – 3.90 m, 2H (H-5′). ^{13}C NMR (500 MHz, DMSO-d_6): 163.8, 152.5, 151.9, 150.8, 144.1, 138.8, 133.8, 132.9, 132.5, 132.0, 132.0, 129.3, 129.2, 129.0, 128.9, 128.6, 128.6, 126.2, 88.4, 81.8, 73.1, 71.9.

5′-Tetrachlorophthalimido-5′-deoxyuridine (**9**). 0.74 g, yield 91%. HRMS (ESI) *m/z* Calcd for $C_{17}H_{11}N_3O_7Cl_4$ $(M + Na)^+$ 531.9249, found 531.9240. ^{1}H NMR (500 MHz, DMSO-d_6): 11.33 d, 1H, J = 1.9 (NH); 7.73 d, 1H, J = 8.3 (H-6); 5.67 d, 1H, J = 5.4 (H-1′); 5.63 dd, 1H, J = 2.2, 8.1 (H-5); 4.18 t, 1H, J = 5.2 (H-2′); 4.01–3.95 m, 2H (H-3′ & H-4′); 3.89 – 3.82 m, 2H (H-5′). ^{13}C NMR (500 MHz, DMSO-d_6): 163.7, 163.5, 151.0, 142.0, 138.7, 128.6, 128.5, 102.3, 89.6, 80.9, 72.7, 71.5.

N^6-Benzoyl-2′-O-(tert-butyldimethylsilyl)-5′-tetrachlorophthalimido-5′-deoxyadenosine (**4**). To a solution of **3** (0.40 g, 0.63 mmol) in anhydrous DMF (20 mL), anhydrous pyridine (0.25 mL, 3.15 mmol) and $AgNO_3$ (0.19 g, 1.13 mmol) were added. The mixture was stirred for 10 min, and *tert*-butyldimethylsilyl chloride (0.17 g, 1.13 mmol) was added and the mixture was stirred at room temperature for 48 h. The mixture was filtered through celite, washed with ethanol, and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc, washed with H_2O, brine, and dried over sodium sulfate anhydrous. The solvent was removed under vacuum and the crude mixture was purified using silica gel column chromatography (1.5:1 ratio hexanes to EtOAc) to obtain compound **4** (0.29 g, 61%). HRMS (ESI) *m/z* Calcd for $C_{31}H_{30}N_6O_6SiCl_4$ $(M + Na)^+$ 773.0648, found 773.0638. ^{1}HNMR (500 MHz, $CDCl_3$): 9.13 s, 1H (NH); 8.63 s, 1H (H-8); 8.21 s, 1H (H-2); 8.02 d, 2H, J = 7.4 (H-Bz); 7.60 t, 1H, J = 7.4 (H-Bz); 7.52 t, 2H, J = 7.6 (H-Bz); 5.90 d, 1H, J = 3.4 (H-1′); 5.04 t, 1H, J = 4.2 (H-2′); 4.42 – 4.35 m, 2H (H-3′, H-4′); 4.21 – 4.03 m, 2H (H-5′); 0.86 s, 9H ($SiC(CH_3)_3$); 0.04 s, 3H ($SiCH_3$); −0.06 s, 3H ($SiCH_3$). ^{13}C NMR (500 MHz, DMSO-d_6): 170.8, 165.9, 163.8, 152.4, 151.9, 150.9, 144.0, 138.8, 133.8, 132.9, 128.9, 128.9, 128.6, 128.6, 126.2, 88.7, 82.0, 74.9, 71.8, 60.2, 26.0, 21.2, 18.2, 14.5, −4.36, −4.85.

2′-O-(tert-Butyldimethylsilyl)-5′-tetrachlorophthalimido-5′-deoxyuridine (**10**). To a mixture of **9** (0.33 g, 0.64 mmol) and imidazole (0.061 g, 0.90 mmol) in anhydrous pyridine (10 mL), TBDMSCl (0.135 g, 0.90 mmol) was added, and the mixture was stirred under N_2 overnight. The solvent was evaporated under vacuum, and the residue dissolved in EtOAc and washed with H_2O, brine, then was dried over sodium sulfate anhydrous. The crude was purified using silica gel column chromatography (1.75:1 ratio hexanes to EtOAc) to afford compound **10** (0.30 g, 76%). HRMS (ESI) *m/z* Calcd for $C_{23}H_{25}N_3O_7SiCl_4$ $(M + Na)^+$ 648.01, found 648.01. ^{1}H NMR (500 MHz, DMSO-d_6): 11.36 s, 1H (NH); 7.78 d, 1H, J = 7.8 (H-6); 5.68 d, 1H, J = 4.9 (H-1′); 5.66 d, 1H, J = 7.4 (H-5); 5.20 d, 1H, J = 5.9 (OH-3′); 4.33 t, 1H, J = 5.2 (H-2′); 4.02–4.01 m, 1H (H-3′); 3.92–3.89 m, 3H (H-4′ & H-5′); 0.81 s, 9H

($SiC(CH_3)_3$); 0.04 s, 3H ($SiCH_3$); 0.00 s, 3H ($SiCH_3$). ^{13}C NMR (500 MHz, DMSO-d_6): 163.8, 163.4, 150.9, 141.6, 138.7, 128.6, 102.3, 89.6, 81.2, 74.7, 71.4, 26.1, 18.3, −4.3, −4.7.

General procedure for 2′-O-(tert-Butyldimethylsilyl)-5′-(4-monomethoxytritylamino)-5′-deoxyribonucleosides. To a solution of compound **4** or **10** (1.90 mmol) in anhydrous THF (25 mL), ethylenediamine (0.15 mL, 2.28 mmol) was added and the mixture was stirred for 2 hours at room temperature. The solvent was concentrated under vacuum. The crude 5′-amino nucleoside product was then dissolved in anhydrous methylene chloride (45 mL), Et_3N (1.15 mL, 8.25 mmol) and MMTrCl (0.77 g, 2.48 mmol) were added, and the mixture was stirred under N_2 at room temperature for 2 h. The mixture was then cooled to 0°C, and quenched with MeOH (10 mL). The solvent was removed under vacuum, and the residue was dissolved in methylene chloride, washed with H_2O and brine, dried over sodium sulfate anhydrous. The crude was purified by silica gel column chromatography pre-washed with 2% Et_3N to afford **5** or **11**, respectively.

N^6-Benzoyl-2′-O-(tert-butyldimethylsilyl)-5′-(4-monomethoxytritylamino)-5′-deoxyadenosine (**5**). 1.04 g, yield 72%, purified using 1:1 ratio hexanes to EtOAc. HRMS (ESI) *m/z* Calc for $C_{43}H_{48}N_6O_5Si$ $(M + Na)^+$ 779.3353, found 779.3334. ^{1}HNMR (500 MHz, $CDCl_3$): 9.03 s, 1H (NH); 8.01 d, 2H, $J = 7.4$ (H-Bz); 7.96 s, 1H (H-8); 7.73 s, 1H (H-2); 7.62 t, 1H, $J = 7.6$ (H-Bz); 7.55 – 7.17 m, 14H (H-Ar and H-Bz); 6.79 d, 2H, $J = 8.3$ (H-Ar); 5.77 d, 1H, $J = 7.3$ (H-1′); 5.67 t, 1H, $J = 5.9$ (H-2′); 4.72 d, 1H, $J = 4.4$ (H-3′); 4.37 s, 1H (H-4′); 3.85 – 3.81 m, 1H (H-5′); 3.54 – 3.46 m, 1H (H-5′); 3.77 s, 3H (MMTr-OCH_3); 0.85 s, 9H ($SiC(CH_3)_3$); −0.03 s, 3H ($SiCH_3$); −0.27 s, 3H ($SiCH_3$).

2′-O-(tert-Butyldimethylsilyl)-5′-(4-monomethoxytritylamino)-5′-deoxyuridine (**11**). 0.89 g, yield 74%, purified using 1:1 ratio hexanes to EtOAc. HRMS (ESI) *m/z* Calcd for $C_{35}H_{43}N_3O_6Si$ $(M + Na)^+$ 652.2819, found 652.2797. ^{1}HNMR (500 MHz, $CDCl_3$): 7.47 – 7.09 m, 13H (ArH, BzH, and H-6); 6.81 d, 2H, $J = 8.8$ (ArH); 5.70 d, 1H, $J = 3.9$ (H-1′); 5.66 d, 1H, $J = 8.3$ (H-5); 4.14 – 4.05 m, 4H (H-2′, H-3′, H-4′, & H-5′); 3.78 m, 4H (MMTr-OCH_3 & 5′-H); 0.88 s, 9H ($SiC(CH_3)_3$); 0.06 s, 3H ($SiCH_3$); 0.01 s, 3H ($SiCH_3$).

2′-O-(tert-Butyldimethylsilyl)-N^2-isobutyryl-5′-(4-monomethoxytritylamino)-5′-deoxyguanosine (**17**). Compound **16** (0.40 g, 0.81 mmol) was dissolved in anhydrous methylene chloride (8 mL), and Pd/C (0.04 g, 0.41 mmol) was added, and the mixture was stirred under H_2 overnight. The resulting precipitate was dissolved in methanol, filtered, and concentrated under vacuum. The crude 5′-amino nucleoside product was then dissolved in anhydrous methylene chloride (5 mL), Et_3N (1.06 mL, 7.64 mmol) and MMTrCl (0.28 g, 0.92 mmol) were added, and the mixture was stirred under N_2 at room temperature for 2 h. The mixture was then cooled to 0°C, and quenched with MeOH (5 mL). The solvent was removed under vacuum, and the residue was dissolved in methylene chloride, washed with H_2O and brine, dried over sodium sulfate anhydrous. The crude was purified by silica gel column chromatography pre-washed with 2% Et_3N using 1:1 ratio hexanes to

EtOAc to afford **17** (0.32 g, 53%). ^{1}H NMR (500 MHz, $CDCl_3$): 11.97 s, 1H (NH); 7.66 s, 1H (H-8); 7.51 – 7.21 m, 12H (Ar-H); 6.83 d, 2H, $J = 8.8$ (Ar-H); 5.65 d, 1H, $J = 6.4$ (H-1′); 4.68 q, 1H, $J = 6.9$ (H-2′); 4.37 q, 1H, $J = 2.9$ (H-3′); 4.27 – 4.05 m, 3H (H-4′ & H-5′); 3.78 s, 3H (OCH_3); 2.73 m, 1H (CH); 1.56 s, 6H ($CH(CH_3)_2$); 0.89 s, 9H ($SiC(CH_3)_3$); 0.05 s, 3H ($SiCH_3$); −0.03 s, 3H ($SiCH_3$).

General procedure for 2′-O-(tert-Butyldimethylsilyl)-5′-(4-monomethoxytrity lamino)-3′-O-succinyl-5′-deoxyribonucleosides. To a solution of **5, 11,** or **17** (0.25 mmol) in anhydrous methylene chloride (5 mL), Et_3N (0.34 mL, 2.45 mmol) and succinic anhydride (0.08 g, 0.75 mmol) were added and the mixture was stirred for 24 h at room temperature. The solvent was evaporated under reduced pressure, and the residue was dissolved in EtOAc and washed with 5% citric acid, brine, then was dried over sodium sulfate anhydrous. The crude was purified by silica gel column chromatography pre-washed with 2% Et_3N to afford **6**, **12**, or **18**, respectively.

N^6-Benzoyl-2′-O-(tert-butyldimethylsilyl)-5′-(4-monomethoxytritylamino)-3′-O-succinyl-5′-deoxyadenosine (**6**). 0.17 g, yield 79%, purified using 4:1 ratio EtOAc to MeOH. HRMS (ESI) *m/z* Calcd for $C_{47}H_{52}N_6O_8Si$ $(M + Na)^+$ 879.3514, found 879.3487. ^{1}HNMR (500 MHz, DMSO-d_6): 11.17 s, 1H (NH); 8.70 s, 1H (NH); 8.01 d, 2H, $J = 7.3$ (H-Bz); 7.64 – 7.18 m, 17H (H-8, H-2, H-Ar, and H-Bz); 6.85 d, 2H, $J = 8.8$ (H-Ar); 5.98 d, 1H, $J = 7.8$ (H-1′); 5.80 d, 1H, $J = 5.4$ (H-3′); 5.58 dd, 1H, $J = 2.4, 7.8$ (H-2′); 4.29 s, 1H (H-4′); 4.07 – 4.04 m, 2H (H-5′); 3.71 s, 3H (MMTr-OCH_3); 2.60 – 2.54 m, 4H ($CO(CH_2)_2$); 0.65 s, 9H ($SiC(CH_3)_3$); −0.09 s, 3H ($SiCH_3$); −0.31 s, 3H ($SiCH_3$).

2′-O-(tert-Butyldimethylsilyl)-5′-(4-monomethoxytritylamino)-3′-O-succinyl-5′-deoxyuridine (**12**). 0.15 g, yield 84%, purified using 1:1.5 ratio hexanes to EtOAc. HRMS (ESI) *m/z* Calcd for $C_{39}H_{47}N_3O_9Si$ $(M + Na)^+$ 752.3192, found 752.3118. ^{1}H NMR (500 MHz, DMSO-d_6): 11.36 s, 1H (NH); 7.63 d, 1H, $J = 7.9$ (H-6); 7.38 – 7.14 m, 12H (ArH); 6.82 d, 2H, $J = 8.8$ (ArH); 5.72 d, 1H, $J = 3.9$ (H-1′); 5.59 d, 1H, $J = 8.3$ (H-5); 5.24 t, 1H, $J = 4.9$ (H-3′); 4.31 t, 1H, $J = 5.9$ (H-2′); 4.06 – 3.91 m, 3H (H-4′ & H-5′); 3.70 s, 3H (MMTr-OCH_3); 2.62 – 2.60 m, 4H ($CO(CH_2)_2$); 0.74 s, 9H, ($SiC(CH_3)_3$); −0.06 s, 3H ($SiCH_3$); −0.11 s, 3H ($SiCH_3$).

2′-O-(tert-Butyldimethylsilyl)-N^2-isobutyryl-5′-(4-monomethoxytritylamino)-3′-O-succinyl-5′-deoxyguanosine (**18**). 0.16 g, yield 76%, purified using 4:1 ratio EtOAc to MeOH. ^{1}H NMR (500 MHz, $CDCl_3$): 12.10 s, 1H (NH); 7.63 s, 1H (H-8); 7.47 – 7.16 m, 12H (Ar-H); 6.81 d, 2H, $J = 8.8$ (Ar-H); 5.87 d, 1H, $J = 5.4$ (H-1′); 5.62 s, 1H, (H-3′); 4.27 s, 1H (H-2′); 4.15 s, 1H (H-4′); 3.80 – 3.61 m, 2H (H-5′); 3.77 s, 3H (OCH_3); 2.80 – 2.61 m, 5H (CH & $CO(CH_2)_2$); 1.08 d, 3H, $J = 6.8$ ($CH(CH_3)_2$); 0.98 s, 3H, $J = 6.8$ ($CH(CH_3)_2$); 0.82 s, 9H ($SiC(CH_3)_3$); −0.05 s, 3H ($SiCH_3$); −0.12 s, 3H ($SiCH_3$).

5′-Azido-N^2-isobutyryl-2′,3′-O-isopropylidene-5′-deoxyguanosine (**14**). To a stirred solution of compound **13** (1.40 g, 3.56 mmol) in anhydrous DMF (8 mL), PPh_3 (1.52 g, 5.81 mmol) and CBr_4 (2.31 g, 6.97 mmol) were added. The mixture was stirred under N_2 at room temperature for 5 min, then excess NaN_3 (1.13 g, 17.42 mmol) was added, and the reaction mixture was stirred at 90°C for 24 h.

The reaction was quenched by adding H_2O (5 mL). After stirring for 5 min, the mixture was diluted with EtOAc, washed with 5% sodium bicarbonate, brine, and the organic layer was dried over sodium sulfate anhydrous. The solvent was evaporated under vacuum and the crude product was purified by silica gel column chromatography (1:1 ratio hexanes to EtOAc) to produce **14** (1.19 g, 80%). ^{1}H NMR (500 MHz, $CDCl_3$): 12.23 s, 1H (NH); 7.81 d, 1H, $J = 1.4$ (H-8); 5.95 s, 1H (H-1′); 5.19 m, 1H (H-2′); 4.95 m, 1H (H-3′); 4.29 m, 1H (H-4′); 3.59 – 3.51 m, 2H (H-5′); 2.71 m, 1H (CH); 1.57 s, 3H (CH_3); 1.33, 3H (CH_3); 1.25 m, 6H ($CH(CH_3)_2$). ^{13}C NMR (500 MHz, DMSO-d_6): 180.6, 155.2, 148.6, 138.9, 129.3, 121.1, 114.0, 89.0, 85.4, 83.8, 81.8, 52.2, 35.3, 27.4, 25.8, 19.3, 19.3.

5′-Azido-N^2-isobutyryl-5′-deoxyguanosine (**15**). To compound **14** (0.67 g, 1.59 mmol) in methylene chloride (4 mL), aqueous trifluoroacetic acid (75%, 20 mL) was added, and the mixture was stirred at room temperature for 2 hours. The solvent was evaporated under reduced pressure, and the residue was filtered and washed with anhydrous diethyl ether to produce compound **15** (0.49 g, 82%). HRMS (ESI) *m/z* Calcd for $C_{14}H_{18}N_8O_5$ $(M + Na)^+$ 401.1298, found 401.1294. ^{1}H NMR (500 MHz, DMSO-d_6): 12.07 s, 1H (NH); 8.23 s, 1H (H-8); 5.82 d, 1H, $J = 5.9$ (H-1′); 5.60 d, 1H, $J = 5.9$ (OH-2′); 5.33 d, 1H, $J = 4.9$ (OH-3′); 4.58 q, 1H, $J = 5.5$ (H-2′); 4.08 q, 1H, $J = 4.6$ (H-3′); 4.00 q, 1H, $J = 3.7$ (H-4′); 3.66 – 3.53 m, 2H (H-5′); 2.75 m, 1H (CH); 1.11 dd, 6H, $J = 1.3, 6.6$ ($CH(CH_3)_2$). ^{13}C NMR (500 MHz, DMSO-d_6): 158.9, 120.8, 87.6, 83.4, 73.2, 71.4, 65.3, 56.4, 52.3, 35.3, 19.5, 19.4, 15.6.

5′-Azido-2′-O-(tert-butyldimethylsilyl)-N^2-isobutyryl-5′-deoxyguanosine (**16**). To a solution of **15** (0.20 g, 0.53 mmol) in anhydrous DMF (5 mL), anhydrous pyridine (0.16 mL, 2.12 mmol) and $AgNO_3$ (0.12 g, 0.69 mmol) were added. The mixture was stirred for 10 min, and *tert*-butyldimethylsilyl chloride (0.10 g, 0.69 mmol) was added and the mixture was stirred at room temperature for 48 h. The mixture was filtered through celite, washed with ethanol, and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc, washed with H_2O, brine, and dried over sodium sulfate anhydrous. The solvent was concentrated under vacuum and the crude mixture was purified using silica gel column chromatography (1:1 ratio hexanes to EtOAc) to obtain compound **16** (0.13 g, 48%).^{1}H NMR (500 MHz, $CDCl_3$): 11.99 s, 1H (NH); 7.88 s, 1H (H-8); 5.84 s, 1H (H-1′); 5.29 s, 1H (OH-3′); 4.49 s, 1H (H-2′); 4.41 s, 1H (H-3′); 4.11 s, 1H (H-4′); 3.72 – 3.49 m, 2H (H-5′); 2.64 m, 1H (CH); 1.28 m, 6H ($CH(CH_3)_2$); 0.94 s, 9H ($SiC(CH_3)_3$); 0.17 s, 3H ($SiCH_3$); −0.01 s, 3H ($SiCH_3$).

General procedure for 2′,5′-bis-O-(tert-butyldimethylsilyl)-ribonucleosides. N^6-benzoyladenosine or uridine (9.79 mmol), $AgNO_3$ (4.59 g, 29.37 mmol), and anhydrous pyridine (5.57 mL, 68.53 mmol) were suspended in anhydrous THF (100 mL) and stirred for 10 minutes. TBDMSCl (4.42 g, 29.37 mmol) was added, and the suspension was stirred for 48 hours. The reaction was quenched by addition of ethanol (8 mL), and the mixture was filtered through celite and washed with ethanol. The filtrate was concentrated under vacuum and the residue was dissolved in EtOAc, washed with H_2O, brine, and dried over sodium sulfate anhydrous. The crude mixture was purified by silica gel column chromatography to afford **19** or **21**.

N^6-Benzoyl-2′,5′-bis-O-(tert-butyldimethylsilyl)adenosine (**19**). 3.82 g, yield 65%, purified using 1.75:1 ratio of hexanes to EtOAc. ^{1}HNMR (500 MHz, $CDCl_3$): 9.30 s, 1H (NH); 8.80 s, 1H (H-8); 8.43 s, 1H (H-2); 8.03 d, 2H, $J = 7.8$ (H-Bz); 7.58 t, 1H, $J = 7.4$ (H-Bz); 7.50 t, 2H, $J = 7.9$ (H-Bz); 6.18 d, 1H, $J = 4.9$ (H-1′); 4.64 t, 1H, $J = 4.9$ (H-2′); 4.29 q, 1H, $J = 4.4$ (H-3′); 4.22 d, 1H, $J = 3.4$ (H-4′); 4.03 – 3.85 m, 2H (H-5′); 0.94 s, 9H ($SiC(CH_3)_3$); 0.84 s, 9H ($SiC(CH_3)_3$); 0.15 s, 3H ($SiCH_3$); 0.13 s, 3H ($SiCH_3$); −0.05 s, 3H ($SiCH_3$); −0.12 s, 3H ($SiCH_3$). ^{13}C NMR (500 MHz, $CDCl_3$): 164.7, 152.8, 151.6, 149.5, 141.2, 133.8, 132.7, 128.8, 127.9, 122.9, 88.2, 85.3, 71.1, 63.0, 30.9, 26.0, 25.5, 18.5, 17.9, −5.0, −5.3, −5.4, −5.5.

2′,5′-Bis-O-(tert-butyldimethylsilyl)uridine (**21**). 6.66 g, yield 69%, purified using 2:1 ratio of hexanes to EtOAc. ^{1}HNMR (500 MHz, DMSO-d_6): 7.79 d, 1H, $J = 8.3$ (H-6); 5.79 d, 1H, $J = 4.9$ (H-1′); 5.57 dd, 1H, $J = 7.8, 7.8$ (H-5); 5.06 d, 1H, $J = 5.3$ (OH-3′); 4.07 t, 1H, $J = 4.9$ (H-2′); 3.93 m, 1H (H-3′); 3.90 m, 1H (H-4′); 3.85 – 3.72 m, 1H (H-5′); 0.88 s, 9H ($SiC(CH_3)_3$); 0.82 s, 9H ($SiC(CH_3)_3$); 0.07 s, 6H ($Si(CH_3)_2$); 0.01 s, 3H ($SiCH_3$); −0.01 s, 3H ($SiCH_3$). ^{13}C NMR (500 MHz, DMSO-d_6): 163.3, 150.9, 140.2, 102.1, 88.1, 85.0, 76.3, 70.0, 63.0, 31.1, 26.2, 26.0, 18.5, 18.3, −4.4, −4.8, −5.2.

General procedure for 2′-O-(tert-butyldimethylsilyl)-ribonucleosides

Compound **19** or **21** (6.37 mmol) was stirred in aqueous trifluoroacetic acid (90%, 25 mL) at 0°C for 2 hours. The solvent was removed under reduced pressure, and the crude was purified by silica gel column chromatography (EtOAc) to produce **20** or **22**.

N^6-Benzoyl-2′-O-(tert-butyldimethylsilyl)adenosine (**20**). 2.87 g, yield 93%, purified using EtOAc. HRMS (ESI) *m/z* Calcd for $C_{23}H_{31}N_5O_5Si$ $(M + Na)^+$ 508.1992, found 508.1971. HNMR (500 MHz, DMSO-d_6): 11.17 s, 1H (NH); 8.74 s, 2H (H-8, H-2); 8.03 d, 2H, $J = 7.3$ (H-Bz); 7.63 t, 1H, $J = 7.6$ (H-Bz); 7.53 t, 2H, $J = 7.9$ (H-Bz); 6.06 d, 1H, $J = 5.4$ (H-1′); 5.17 t, 1H, $J = 5.4$ (OH-5′); 5.12 d, 1H, $J = 5.4$ (OH-3′); 4.72 t, 1H, $J = 5.2$ (H-2′); 4.18 q, 1H, $J = 4.7$ (H-3′); 4.01 m, 1H (H-4′); 3.76 – 3.58 m, 2H (H-5′); 0.74 s, 9H ($SiC(CH_3)_3$); −0.06 s, 3H ($SiCH_3$); −0.16 s, 3H ($SiCH_3$). ^{13}C NMR (500 MHz, DMSO-d_6): 166.0, 152.5, 152.1, 150.9, 143.4, 133.8, 132.9, 128.9, 128.9, 126.3, 88.4, 86.3, 76.1, 70.6, 61.5, 60.2, 31.1, 26.0, 18.3, 14.5, −4.4, −4.8.

2′-O-(tert-Butyldimethylsilyl)uridine (**22**). 3.06 g, yield 91%. HNMR (500 MHz, DMSO-d_6): 7.93 d, 1H, $J = 8.3$ (H-6); 5.77 d, 1H, $J = 4.9$ (H-1′); 5.63 dd, 1H, $J = 8.3$ (H-5), 7.8; 4.12 t, 1H, $J = 4.9$ (H-2′); 3.93 t, 1H, $J = 4.9$ (H-3′); 3.86 m, 1H (H-4′); 3.65–3.54 m, 1H (H-5′); 0.82 s, 9H ($SiC(CH_3)_3$); 0.02 s, 3H ($SiCH_3$); 0.00 s, 3H ($SiCH_3$). ^{13}C NMR (500 MHz, DMSO-d_6): 163.5, 151.1, 140.8, 102.2, 88.1, 85.4, 76.1, 70.0, 60.9, 31.1, 26.1, 26.0, 18.3, −4.4, −4.7.

Antibacterial testing

Percent inhibition

Percent inhibition was determined using disc diffusion. DMSO (5 μL) was pipetted onto a 5 mm sterile filter paper disc, and the wet disc had 1.0 mg of solid compound applied. Test discs, along with control discs, were loaded on sterile Mueller

Hinton agar plates or 5% sheep's blood agar plates that had been inoculated with the bacteria of interest. Plates were then incubated for 24 hours at 37°C, and *Neisseria meningitidis* required a 5% CO_2 supplement for reliable growth. The diameter of the zone of inhibition provided by a compound was measured to demonstrate inhibitory effectiveness. Percent inhibition is found by dividing the measured diameter of a compound's zone of inhibition by the total diameter of the test plate (100 mm), and multiplying the result by 100. DMSO was used as a negative control and did not show any inhibitory activity. The reported experimental results were compared to reference antibiotic compounds.

Quantitative susceptibility determination

Disc diffusion was used to resolve minimum susceptibility limits. A known amount of compound was dissolved in DMSO and diluted so that amounts of 256 μg, 128 μg, 64 μg, and 32 μg of compound were delivered in 5 μL of solution onto a 6 mm sterile test disc. Test discs and control discs containing the compounds of interest, ampicillin, and kanamycin of varied dilutions were applied to 5% sheep's blood agar plates inoculated with *Neisseria meningitidis*. Plates were then incubated at 37°C with a 5% CO_2 supplement for 24 hours. The lowest concentration at which bacterial growth was inhibited by a given test disc was then observed.

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Conflicts of interest

The authors declare no conflict of interest.

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