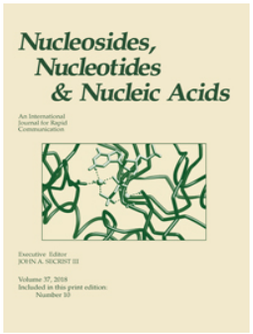

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Design And Synthesis Of An Azabicyclic Nucleoside Phosphoramidite For Oligonucleotide Antisense Constructs

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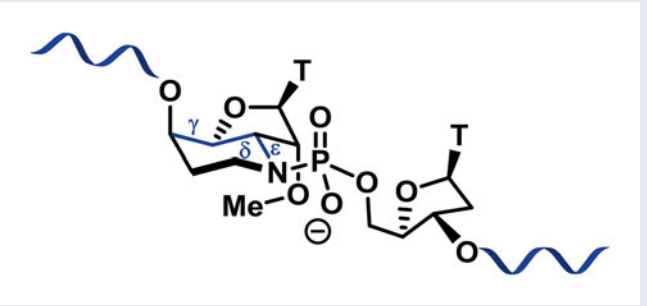
ABSTRACT

We report the synthesis and biophysical evaluation of an azabicycle dinucleotide with restricted γ, β, and ε torsion angles, featuring the introduction of a piperidine ring that locks the conformation of the nucleoside into an RNA-type nucleic acid. The conceptual basis of the design is predicated upon the notion that the conformation of the phosphate group linking two RNA nucleotides can be approximated with an azabicyclic phosphoramidite which may also benefit from a unique stereoelectronic effect.

GRAPHICAL ABSTRACT



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antisense; conformational restriction; azabicyclic nucleosides

Introduction

Oligonucleotides modified with conformationally restricted nucleic acid analogs, represents an important area of research in oligonucleotide therapeutics.[1–3] The introduction of one or more modified nucleosides into an oligonucleotide construct, reshapes the general conformation of the oligo, which in turn, impacts the affinity for complementary RNA or DNA strands. The conformationally constrained nucleosides, constitutes some of the most studied series of modifications. By restricting the pseudorotation

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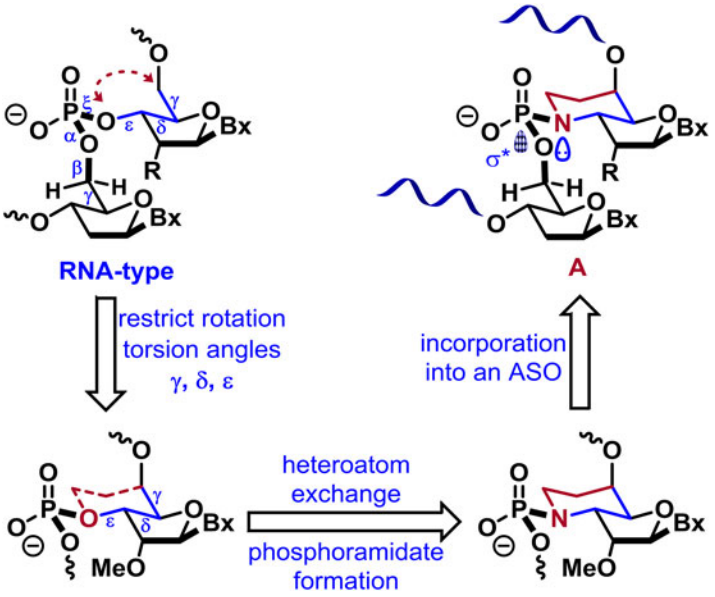


Figure 1. Conceptual design of an azabicyclic nucleoside phosphoramidite **A** as conformational surrogate of an RNA dinucleotide.

of the furanose ring, oligonucleotides containing constrained nucleosides have shown unprecedented affinity as demonstrated by LNA[4–6], TriNA[7], α-L-TriNA[8]. Over the years, several groups have focused their efforts in restricting a set of torsion angles in modified nucleosides. Escudier[9] demonstrated that restriction around α and β torsion angles improved affinity to complementary RNA and DNA without imposing a C3'-*endo* conformation in the furanose ring. In a series of reports, we used a double-constrain strategy to restrict torsion angles γ and δ. Once introduced into oligos, the resulting α-L-TriNA 1 and 2 nucleosides presented enhanced affinity versus RNA and DNA complementary strands.

In a recent report[10], we synthesized a oxabicyclic nucleic acid phosphonate as an internucleotide phosphate surrogate analog with restricted γ, β, and ε torsion angles. The thermal affinity measurements suggested that the applied constrain can mimic conformational preferences of RNA-like nucleic acids. Following a similar strategy, we report here the synthesis and biophysical evaluation of an azabicycle dinucleotide **A** (Figure 1) with restricted γ, β, and ε torsion angles, featuring the introduction of a piperidine ring that locks the conformation of the nucleoside into an RNA-type nucleic acid. The conceptual basis of the design is predicated upon the notion that the conformation of the phosphate group linking two RNA nucleotides can be approximated with an azabicyclic phosphoramidite which may also benefit from a unique stereoelectronic effect as depicted in Figure 1.

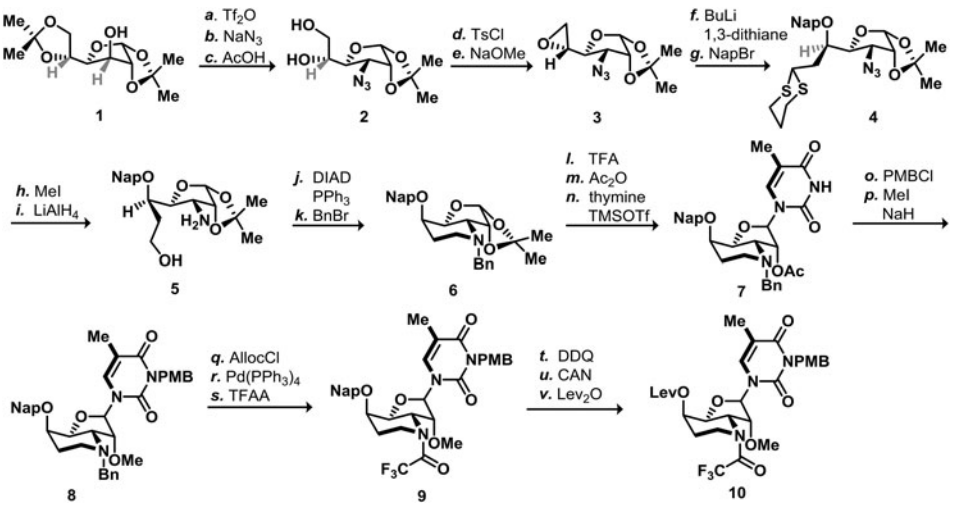
The synthesis of a 2′-deoxypiperidine related nucleoside has been previously reported by Wang[11,12] by using commercially available 3′-azido-

3'-deoxythymidine (AZT). In our target nucleoside **A**, the furanose ring is functionalized at the 2'-position with a 2'-OMe group which is intended to enforce the furanose ring into a C3'-*endo* conformation. In order to facilitate the installation of the different functionalities in **A**, we developed a carbohydrate-based route that secured stereocontrol in key transformations.

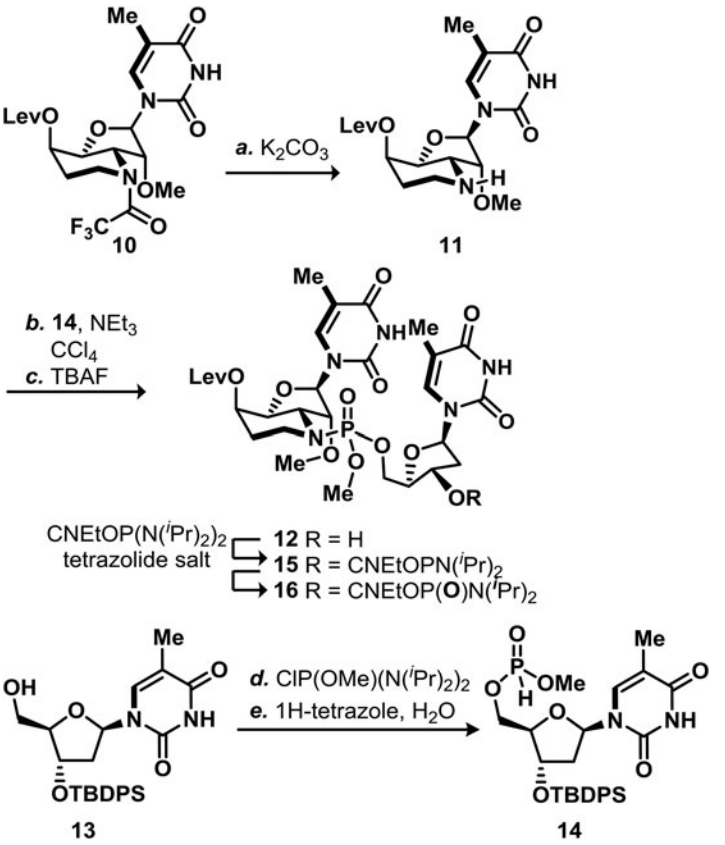
We started with known diol **2** which is readily available from diacetone-D-glucose (Scheme 1).[13,14] Selective tosylation of the primary alcohol in **2** and subsequent base-mediated cyclization provided epoxide **3** in 59% yield.

Opening of epoxide **3** with lithiated 1,3-dithiane[15,16] produced a secondary alcohol which was protected with 2-naphthylmethyl bromide to give ether **4**. Unmasking of the dithiane in **4** with MeI/K_2CO_3, led to an aldehyde that was reduced together with the azide to give aminoalcohol **5**. In order to avoid several steps involving activation/protection/deprotection for the formation of piperidine **6**, we explore the possibility to use Mitsunobu conditions, being cognizant that there are only few precedents in literature for the formation of phosphine-mediated azacycles.[17,18] Gratifyingly, piperidine formation under standard Mitsunobu conditions with subsequent protection with DIPEA/BnBr[19] gave azabicyclic sugar **6** in good yield. The thymine nucleobase was installed following a three steps sequence starting from TFA[20] mediated cleavage of the 1,2-isopropylidene group, acetylation of the anomeric mixture of alcohols (not shown) and Vorbrüggen glycosylation[21,22] to give nucleoside **7**. Protection of the nucleobase under phase-transfer conditions resulted in the installation of a PMB with concomitant cleavage of the acetate group and subsequent methylation at the 2'-position providing nucleoside **8**.

Removal of the N-benzyl group in **8** was problematic under several hydrogenolysis conditions. Fortunately, the exchange of the benzyl for an



Scheme 1. Synthesis of azabicyclic nucleoside **10**.



Scheme 2. Synthesis of azabicyclic phosphoramidite **15**.

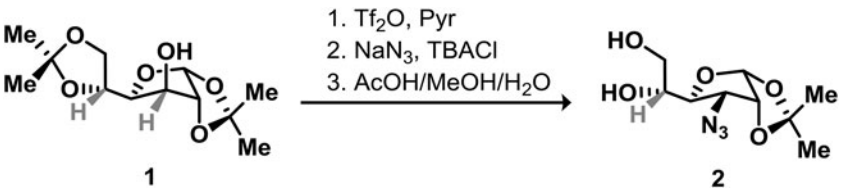
alloc group was successfully achieved by refluxing nucleoside **8** with allyl chloroformate in toluene. Pd-mediated[23] cleavage of the alloc carbamate released the free amine which was later protected as a trifluoroacetamide[24] providing nucleoside **9**. At this juncture, we cleaved the 5′-O naphthyl ether with DDQ[25] and the N-PMB using CAN.[26] Esterification of the 5′-secondary alcohol afforded the levulinic ester[27] at the 5′-position (Scheme 2). Selective cleavage of the N-trifluoroacetyl group in **10** provided the free amine **11**, which was engaged in an Atherton-Todd[12] reaction with H-phosphonate **14** prepared in two steps from the known[28] nucleoside **13**. The resulting nucleotide was deprotected using TBAF giving phosphoramidate **12.** Conversion of the dimer **12** to the corresponding phosphoramidite **15** was accomplished using 2-cyanoethyl tetraisopropylphosphordiamidite. Unfortunately, the dimer phosphoramidite **15** underwent spontaneous air oxidation after column chromatography to yield the phosphoramidate dimer **16**, which was not suitable for incorporation into oligonucleotides.

In conclusion, we describe the design and synthesis of an azabicycle dinucleotide with restricted γ, β, and ε torsion angles, featuring the introduction

of a piperidine ring that locks the conformation of the nucleoside into an RNA-type nucleic acid. Further incorporation into oligonucleotides will reveal its utility for oligonucleotide medicinal chemistry.

Experimental section

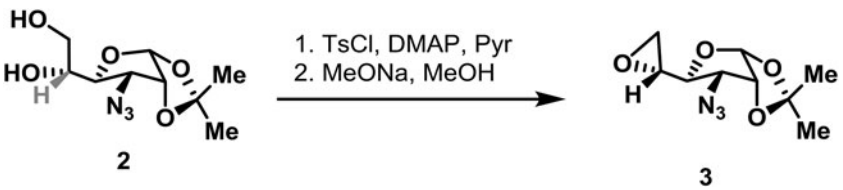
General Information: All non-aqueous reactions were run in oven dried (120°C) glassware under a positive pressure of argon, with exclusion of moisture from reagents and glassware, using standard techniques for manipulating air-sensitive compounds, unless otherwise stated. Anhydrous tetrahydrofuran, diethyl ether, toluene, and CH_2Cl_2 were obtained by passing these solvents through activated columns of alumina, while all other solvents were used as received from chemical suppliers. Reagents were purchased and used without further purification. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous material, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Machery-Nagel silica plates (SIL 60, G-25, UV254) that were visualized using a UVP UVG-11 compact UV lamp (254 nm) and developed with an aqueous solution of cerium ammonium molybdate, or an ethanolic solution of p-anisaldehyde. Flash chromatography was performed using SiliaFlash P60 40-63 μm (230-400 mesh) silica gel. NMR spectra were recorded on Bruker AV-300, ARX-400, or AV-400 instruments, calibrated using residual undeuterated solvent as an internal reference ($CHCl_3$, $\delta = 7.26$ ppm), and reported in parts per million relative to trimethylsilane (TMS $\delta = 0.00$ ppm) as follows: chemical shift (multiplicity, coupling constant (Hz), integration). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets, dt = doublet of triplets, ddt = doublet of doublet of triplets. High resolution mass spectra (HRMS) were recorded at the Center Régional de Spectrométrie de Masse de l'Université de Montréal on an Agilent LC-MSD TOF mass spectrometer by electrospray ionization time of flight reflectron experiments. IR spectra were recorded on a Perkin Elmer Spectrum One spectrometer and are reported in reciprocal centimeters (cm^{-1}).



1 2

Diol 2

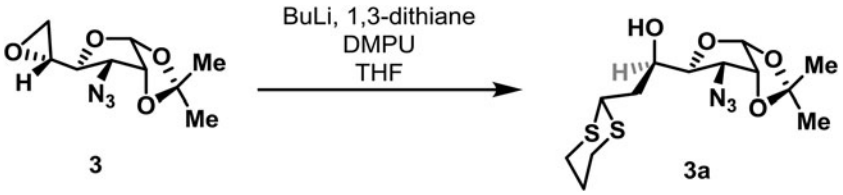
Pyridine (38 mL, 0.473 mol) was added to an stirred solution of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **1** (50.0 g, 0.192 mol) in CH_2Cl_2 (800 mL) at 0°C. Trifluoromethanesulfonic anhydride (33 mL, 0.196 mol) was added to the previous solution during a period of 30 min keeping the temperature under 5°C. The coldbath was removed and the reaction mixture was stirred for 3 h. The organic phase was washed with 1 M HCl (2 × 100 mL) then dried over Na_2SO_4. The solvent was removed under reduced pressure and coevaporated with toluene (2 × 50 mL) to furnish the corresponding triflate. Crude product was dissolved in anhydrous DMF (250 mL), then mixed subsequently with tetrabutyl ammonium chloride (270 mg, 0.97 mmol) and sodium azide (25 g, 0.38 mol). The reaction mixture was heated at 60°C for 4 h and then cooled down. The organic phase was partitioned with the addition of water (750 mL) and extracted with diethyl ether (4 × 100 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. A mixture of methanol (210 mL), water (260 mL), acetic acid (160 mL) was added to the crude azide and warmed to 60°C for 6 h, then cooled down. The mixture was neutralized via addition of solid sodium bicarbonate followed by extraction with ethyl acetate (3 × 200 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (7:3 ethyl acetate/hexanes) to give diol **2** as an oil (18.6 g, 40% over 3 steps); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.75 (d, J = 3.7 Hz, 1 H), 4.72 – 4.67 (m, 1 H), 4.00 (dd, J = 9.3, 4.1 Hz, 1 H), 3.92 (dd, J = 7.2, 3.7 Hz, 1 H), 3.65 (dd, J = 10.3, 5.4 Hz, 2 H), 3.56 (dd, J = 9.3, 4.8 Hz, 1 H), 1.51 (s, 3 H), 1.30 (s, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 113.22, 104.04, 80.57, 77.84, 71.57, 62.96, 60.26, 26.41, 26.38; **HRMS** (ESI) calc'd for $C_9H_{15}N_3NaO_5$ $[M+Na]^+$ m/z = 268.09039, found = 268.08939; **FT-IR** (azide) = 2109.0 cm^{-1}.



Epoxide 3

4-(dimethylamino)pyridine (930 mg, 7.58 mmol), and p-toluensulfonyl chloride (17.5 g, 90.3 mmol) were added sequentially to an stirred solution of diol **2** (18.6 g, 75.8 mmol) in pyridine (200 mL). After 6 h the reaction

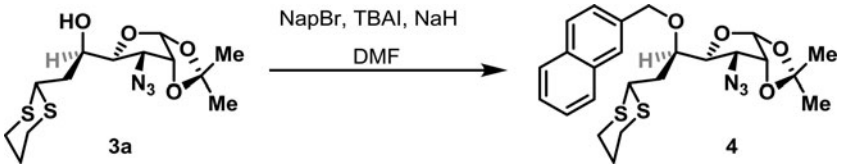
was quenched with 1 M HCl (300 mL) and the resulting solution was extracted with ethyl acetate (4 × 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude tosylate was dissolved in methanol (300 mL) and cooled down to 0°C. A 0.5 M solution of sodium methoxide (150 mmol, 300 mL) was added to the previous solution and let to warm up to room temperature for 1 h. The reaction was carefully quenched with water and the methanol was removed under reduced pressure. The remaining aqueous phase was extracted with ethyl acetate (4 × 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (15:85 ethyl acetate/hexanes) to give epoxide **3** as an oil (8.9 g, 52% over 2 steps); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.79 (d, J = 3.7 Hz, 1 H), 4.71 (t, J = 4.2 Hz, 1 H), 4.14 (dd, J = 9.5, 3.6 Hz, 1 H), 3.29 (dd, J = 9.5, 4.7 Hz, 1 H), 3.21 (dd, J = 6.7, 3.6 Hz, 1 H), 2.84 (t, J = 4.5 Hz, 1 H), 2.79 – 2.75 (m, 1 H), 1.54 (s, 3 H), 1.33 (s, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 113.41, 104.33, 80.44, 77.61, 60.64, 50.47, 44.74, 26.59, 26.56**;** **HRMS** (ESI) calc'd for $C_9H_{13}N_3NaO_4$ $[M + Na]^+$ m/z = 250.07983, found = 250.08086, calc'd for $C_9H_{17}N_4O_4$ $[M + NH_4]^+$ m/z = 245.12443, found = 245.12549; **FT-IR** (azide) = 2105.5 cm^{-1}.



Alcohol 3a

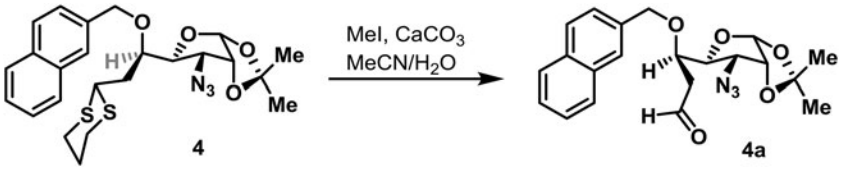
To a cooled (−20°C) solution of 1,3-dithiane (6.25 g, 50.9 mmol) in THF (200 mL), was added dropwise a 1.6 M solution of *n*-butyllithium (31.8 mL, 50.9 mmol), then stirred at the same temperature for 0.5 h. A previously prepared solution of epoxide **3** (8.9 g, 39.2 mmol) in THF (200 mL) was added dropwise keeping the temperature at −20°C. The reaction mixture was allowed to warm to 0°C and then DMPU (24.0 mL, 198 mmol) was added slowly. After stirring for 3 h at room temperature, the reaction mixture was quenched with NH_4Cl saturated and the aqueous layer was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (2:8 ethyl acetate/hexanes) to give alcohol **3a** as an oil (6.8 g, 50%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 5.79 (d, J = 3.6 Hz, 1 H), 4.78 – 4.71 (m, 1 H), 4.27 (dd, J = 9.4,

4.9 Hz, 2 H), 4.01 (dd, $J = 9.3$, 3.4 Hz, 1 H), 3.57 (dd, $J = 9.3$, 4.8 Hz, 1 H), 2.96 – 2.82 (m, 4 H), 2.43 (s, 1 H), 2.18 – 1.83 (m, 4 H), 1.58 (s, 3 H), 1.36 (s, $J = 11.6$ Hz, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 113.39, 104.11, 80.98, 80.12, 67.46, 59.50, 43.75, 37.65, 30.17, 29.79, 26.63, 26.61, 25.99; **HRMS** (ESI) calc'd for $C_{13}H_{22}N_3O_4S_2$ $[M + H]^+$ $m/z = 348.10462$, found = 348.10428; **FT-IR** (azide) = 2102.0 cm^{-1}.



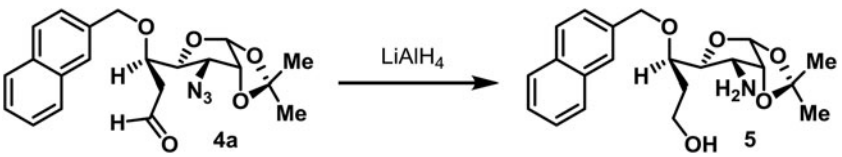
Azide 4

Sodium hydride (1.17 g, 29.4 mmol) was added to a stirred solution of alcohol **3a** (6.8 g, 19.6 mmol) in DMF (100 mL) at− 20°C. After stirring at the same temperature for 20 min, tetrabutylamonium iodide (730 mg, 1.96 mmol) and 2-(bromomethyl)-naphthalene (6.62 g, 29.4 mmol) were added sequentially. The reaction mixture was warmed to 0°C and stirred for 1 h. The reaction was carefully quenched with water (400 mL) and the aqueous layer was extracted with diethyl ether (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (1:9 ethyl acetate/hexanes) to give azide **4** as an oil (7.7 g, 81%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.88 – 7.78 (m, 4 H), 7.53 – 7.44 (m, 3 H), 5.76 (d, $J = 3.7$ Hz, 1 H), 4.93 (d, $J = 11.8$ Hz, 1 H), 4.83 (d, $J = 11.8$ Hz, 1 H), 4.72 – 4.67 (m, 1 H), 4.28 – 4.14 (m, 2 H), 4.07 (dd, $J = 10.2$, 4.2 Hz, 1 H), 3.62 (dd, $J = 9.3$, 4.8 Hz, 1 H), 2.86 – 2.62 (m, 3 H), 2.55 – 2.44 (m, 1 H), 2.23 – 2.11 (m, 1 H), 2.07 – 1.88 (m, 2 H), 1.88 – 1.72 (m, 1 H), 1.59 (s, 3 H), 1.37 (s, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 135.96, 133.24, 132.92, 128.07, 127.91, 127.63, 126.71, 126.13, 126.07, 125.93, 113.06, 103.82, 80.66, 80.42, 74.52, 74.44, 59.63, 43.66, 37.04, 30.18, 29.53, 26.51, 26.47, 25.82; **HRMS** (ESI) calc'd for $C_{24}H_{30}N_3O_4S_2$ $[M + H]^+$ $m/z = 488.16722$, found = 488.165960; **FT-IR** (azide) = 2105.0 cm^{-1}



Aldehyde 4a

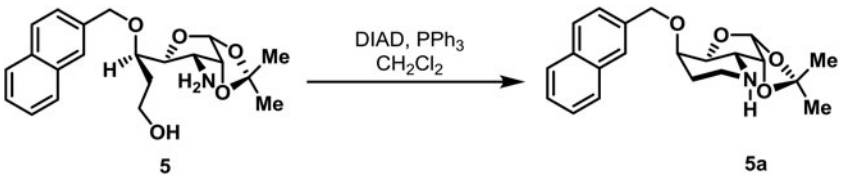
Iodomethane (10.0 mL, 157.9 mmol) and calcium carbonate (7.9 g, 78.9 mmol) were added sequentially to a stirred solution of azide **4** (7.7 g, 15.8 mmol) in 10:1 acetonitrile/water (350 mL). The resulting mixture was warmed to 45°C for 24 h. The reaction mixture was partitioned with the addition of water (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (2:8 ethyl acetate/hexanes) to give aldehyde **4a** as an oil (3.8 g, 61%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.76 (s, 1 H), 7.88 – 7.79 (m, 3 H), 7.76 (s, 1 H), 7.54 – 7.39 (m, 3 H), 5.78 (d, J = 3.6 Hz, 1 H), 4.91 – 4.80 (m, 2 H), 4.78 – 4.70 (m, 1 H), 4.39 (dt, J = 7.8, 3.8 Hz, 1 H), 4.19 (dd, J = 9.4, 3.2 Hz, 1 H), 3.58 (dd, J = 9.4, 4.7 Hz, 1 H), 2.89 (ddd, J = 17.4, 8.3, 1.9 Hz, 1 H), 2.75 – 2.64 (m, 1 H), 1.58 (s, 3 H), 1.37 (s, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 199.76, 135.40, 133.33, 133.17, 128.40, 128.06, 127.83, 126.89, 126.33, 126.17, 126.00, 113.46, 104.00, 80.79, 79.81, 74.09, 73.03, 60.51, 45.53, 26.65, 26.61; **HRMS** (ESI) calc'd for $C_{21}H_{23}N_3O_5Na$ $[M + Na]^+$ m/z = 420.15299, found = 420.15313; **FT-IR** (azide) = 2107.4 cm^{-1}



Aminoalcohol 5

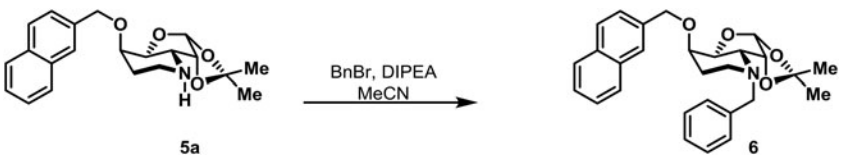
Lithium aluminum hydride (1.45 g, 38.3 mmol) was added portionwise to a stirred solution of aldehyde **4a** (3.8 g, 9.6 mmol) in diethyl ether (160 mL) at 0°C. After stirring for 20 min at the same temperature, the reaction mixture was carefully quenched with water and subsequently treated with 1 M NaOH (100 mL). The formed solid was filtrated through a celite pad, the organig layer was separated and the remaining aqueous layer was extracted with CH_2Cl_2 (4 × 50 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (0% to 6% MeOH in CH_2Cl_2) to give aminoalcohol **5** as an oil (3.5 g, 98%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.87 – 7.75 (m, 8 H), 7.52 – 7.42 (m, 6 H), 5.77 (d, J = 3.7 Hz, 2 H), 4.81 (s, 4 H), 4.50 (dd, J = 4.9, 3.8 Hz, 2 H), 3.95 – 3.87 (m, 4 H), 3.84 – 3.67 (m, 4 H), 3.37 – 3.29 (m, 2 H), 2.23 – 1.86 (m, 11 H), 1.53 (s, 6 H), 1.35 (s,

6 H); ^{13}**C NMR** (101 MHz, $CDCl_3$) δ 135.75, 133.38, 133.14, 128.46, 128.05, 127.84, 126.95, 126.33, 126.14, 126.07, 112.23, 104.08, 83.22, 81.32, 78.24, 73.11, 59.51, 55.71, 34.02, 26.95, 26.68; **HRMS** (ESI) calc'd for $C_{21}H_{28}N_1O_5$ $[M+H]^+$ $m/z = 374.1962$, found = 374.1967;



Piperidine 5a

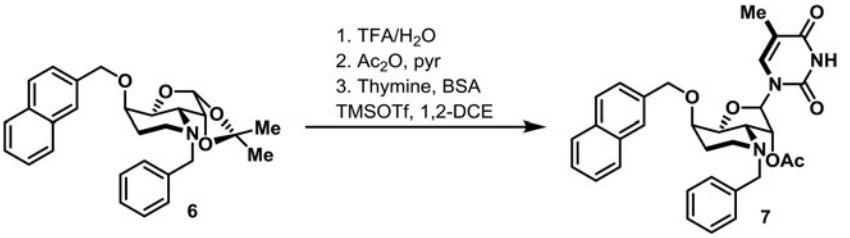
To a solution of triphenylphosphine (4.92 g, 18.7 mmol) and aminoalcohol **5** (3.5 g, 9.4 mmol) in CH_2Cl_2 (500 mL), diisopropyl azodicarboxylate (3.7 mL, 18.7 mmol) was added slowly at 0°C. The cold bath was removed and the reaction mixture was stirred for 1 h. The reaction mixture was pre-adsorbed in silica and purified by flash chromatography (20% acetone in CH_2Cl_2) to give piperidine **5a** as an oil (2.06 g, 62%); ^{1}**H NMR** (400 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 4 H), 7.54 – 7.39 (m, 3 H), 5.84 (d, $J = 3.4$ Hz, 1 H), 4.91 (d, $J = 12.5$ Hz, 1 H), 4.77 (d, $J = 12.5$ Hz, 1 H), 4.60 (t, $J = 3.6$ Hz, 1 H), 4.27 (s, 1 H), 3.70 (dd, $J = 10.1$, 2.0 Hz, 1 H), 3.17 (dd, $J = 10.1$, 4.2 Hz, 1 H), 3.04 (td, $J = 13.1$, 2.6 Hz, 1 H), 2.91 (dd, $J = 13.3$, 4.3 Hz, 1 H), 1.89 (d, $J = 14.2$ Hz, 1 H), 1.54 (s, $J = 9.3$ Hz, 3 H), 1.50 – 1.41 (m, 1 H), 1.33 (s, 3 H); **13 C NMR** (75 MHz, $CDCl_3$) δ 136.32, 133.27, 132.91, 128.08, 127.88, 127.69, 126.08, 126.05, 125.79, 125.61, 112.60, 104.62, 78.71, 78.07, 72.22, 57.34, 41.57, 30.50, 26.28, 26.06; **HRMS** (ESI) calc'd for $C_{21}H_{26}NO_4$ $[M+H]^+$ $m/z = 356.185635$, found = 356.18594.



Amine 6

Benzyl bromide (0.76 mL, 6.4 mmol) and K_2CO_3 (0.88 g, 6.4 mmol) were added sequentially to a stirred solution of piperidine **5a** (2.06 g, 5.80 mmol) in ethanol (23 mL) at room temperature. After stirring for 4 h, the solvent

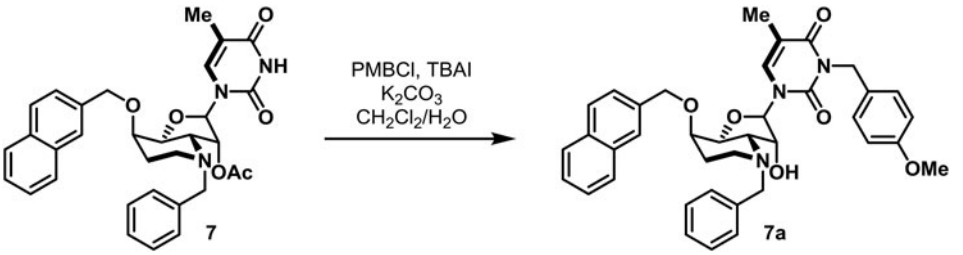
was removed under reduced pressure, and the residue dissolved in water (100 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (2:8 Ethyl acetate/hexane) to give amine **6** as an oil (1.45 g, 56%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.90 – 7.77 (m, 4 H), 7.54 – 7.44 (m, 3 H), 7.42 – 7.27 (m, 5 H), 5.90 (d, J = 3.5 Hz, 1 H), 4.94 (d, J = 12.6 Hz, 1 H), 4.85 – 4.73 (m, 2 H), 4.20 – 4.13 (m, 2 H), 4.08 (dd, J = 9.9, 2.4 Hz, 1 H), 3.39 (d, J = 12.7 Hz, 1 H), 2.76 – 2.63 (m, 2 H), 2.41 – 2.28 (m, 1 H), 1.87 (dd, J = 14.5, 2.5 Hz, 1 H), 1.71 – 1.57 (m, 4 H), 1.40 (s, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 137.47, 136.52, 133.28, 132.89, 129.78, 128.19, 128.02, 127.89, 127.68, 127.20, 126.01, 125.72, 125.63, 112.49, 104.73, 78.88, 77.54, 71.97, 71.38, 63.86, 59.59, 47.42, 29.01, 26.53, 26.03; **HRMS** (ESI) calc'd for $C_{28}H_{31}NO_4$ $[M+H]^+$ m/z = 446.23258, found = 446,23335.



Nucleoside 7

Amine **6** (1.45 g, 3.32 mmol) was dissolved in a 3:2 mixture of trifluoroacetic acid/water (20 mL) and heated to 60°C for 4 h, then the solvent was removed under reduced pressure and co-evaporated with toluene. The crude residue was dissolved in pyridine (20 mL) followed by slow addition of acetic anhydride (3 mL). The resulting solution was stirred for 1 h, then the solvent was removed under reduced pressure and co-evaporated with toluene and the crude diacetate was dissolved in anhydrous 1,2-dichloroethane (5 mL). N,*O*-bis(trimethylsilyl)acetamide (8.2 mL, 33.3 mmol) was added to an stirred solution of thymine (1.26 g, 9.99 mmol) in 1,2-dichloroethane (17 mL) and the resulting suspension was heated at 80°C. After 1 h, the reaction mixture was cooled down to 0°C and the previously prepared solution of the diacetate was transferred via cannula followed by addition of trimethylsilyl trifluoromethanesulfnate (1.81 mL, 9.99 mmol). The resulting solution was heated to 60°C for 2 h, then quenched via addition of saturated solution of $NaHCO_3$. The aqueous layer was extracted with with CH_2Cl_2 (3 × 50 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was

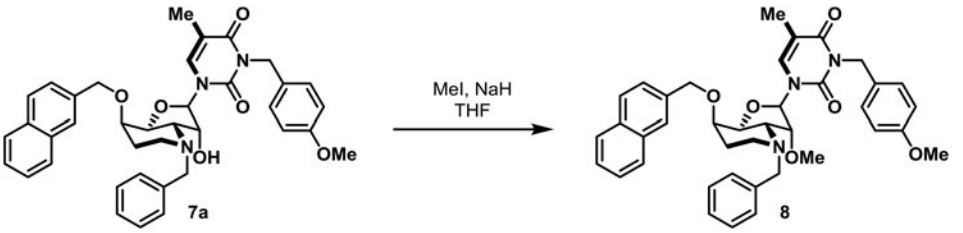
purified by flash chromatography (1:1 ethyl acetate/hexane) to give nucleoside **7** as an oil (0.99 g, 54% over 3 steps); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.46 (s, 1 H), 7.90 – 7.76 (m, 4 H), 7.62 (d, $J = 1.1$ Hz, 1 H), 7.53 – 7.43 (m, 3 H), 7.35 – 7.20 (m, 7 H), 5.95 (s, 1 H), 5.59 (d, $J = 4.3$ Hz, 1 H), 4.86 (d, $J = 11.6$ Hz, 1 H), 4.73 (d, $J = 11.6$ Hz, 1 H), 4.31 (s, 1 H), 4.15 (dd, $J = 10.5$, 2.5 Hz, 1 H), 4.06 (d, $J = 13.0$ Hz, 1 H), 3.15 (d, $J = 12.9$ Hz, 1 H), 2.88 (dd, $J = 10.5$, 4.3 Hz, 1 H), 2.76 – 2.68 (m, 1 H), 2.36 – 2.26 (m, 1 H), 2.20 (s, 3 H), 2.07 – 1.97 (m, 1 H), 1.67 – 1.51 (m, 1 H), 1.23 (s, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.28, 164.03, 150.03, 137.81, 135.43, 135.27, 133.29, 133.11, 128.89, 128.53, 128.34, 127.80, 127.75, 127.26, 126.56, 126.42, 126.20, 125.63, 110.63, 89.57, 81.14, 74.26, 72.21, 71.49, 60.76, 60.15, 47.69, 27.32, 20.93, 11.80; **HRMS** (ESI) calc'd for $C_{32}H_{34}N_3O_6$ $[M+H]^+$ $m/z = 556.244212$, found = 556.24490.



Nucleoside 7a

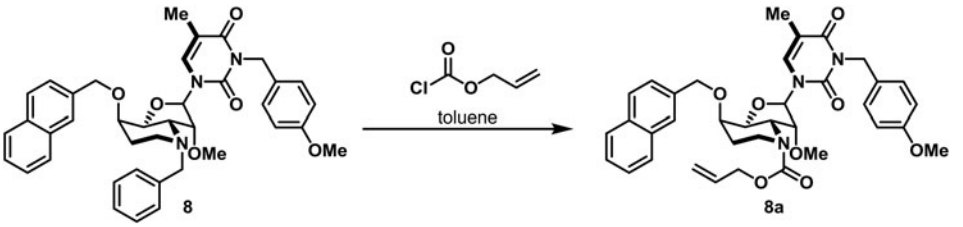
Tetrabutylammonium iodide (49 mg, 0.13 mmol) and *p*-methoxybenzyl chloride (0.12 mL, 0.86 mmol) were added to a stirred solution of nucleoside **7** (367 mg, 0.66 mmol) in CH_2Cl_2 (3.3 mL). To the previous solution was added K_2CO_3 (460 mg, 3.3 mmol) in water (3.3 mL), and the resulting biphasic mixture was stirred vigorously for 3 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (4:6 Ethyl acetate/hexane) to give nucleoside **7a** as an oil (270 mg, 60%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.89 – 7.77 (m, 4 H), 7.67 (d, $J = 1.0$ Hz, 1 H), 7.55 – 7.42 (m, 5 H), 7.37 – 7.27 (m, 5 H), 6.85 – 6.77 (m, 2 H), 5.91 (s, 1 H), 5.03 (q, $J = 13.5$ Hz, 2 H), 4.87 (d, $J = 11.7$ Hz, 1 H), 4.73 (d, $J = 11.7$ Hz, 1 H), 4.34 – 4.26 (m, 2 H), 4.20 (dd, $J = 10.4$, 2.4 Hz, 1 H), 4.00 (d, $J = 12.9$ Hz, 1 H), 3.75 (s, 3 H), 3.31 – 3.14 (m, 2 H), 2.83 – 2.72 (m, 2 H), 2.42 – 2.29 (m, 1 H), 2.08 – 1.97 (m, 1 H), 1.66 – 1.51 (m, 1 H), 1.26 (d, $J = 0.8$ Hz, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 163.46, 159.14, 150.88, 137.46, 135.55, 133.68, 133.35, 133.16, 131.04, 129.31, 129.11,

128.59, 127.87, 127.81, 127.62, 126.50, 126.46, 126.23, 125.59, 113.74, 109.58, 92.07, 80.73, 74.18, 72.47, 71.39, 61.59, 59.98, 55.30, 47.78, 43.76, 27.34, 12.62; **HRMS** (ESI) calc'd for $C_{38}H_{40}N_3O_6$ $[M+H]^+$ $m/z = 634.29116$, found = 634.29024.



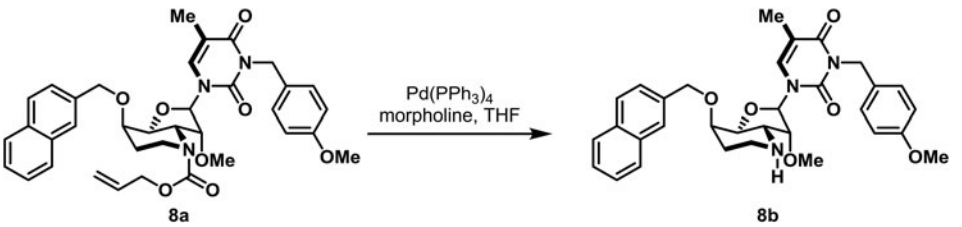
Nucleoside 8

To a stirred solution of **7a** (270 mg, 0.426 mmol) in THF (2.1 mL) at 0°C was added 60% sodium hydride (26 mg, 0.85 mmol). After stirring at 0°C for 30 min, methyl iodide (54 µL, 0.85 mmol) was added and the reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was quenched with water (10 mL) and the resulting mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (4:6 Ethyl acetate/hexane) to give nucleoside **8** as a white foam (260 mg, 94%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.88 – 7.73 (m, 5 H), 7.54 – 7.27 (m, 11 H), 6.84 (d, $J = 8.4$ Hz, 2 H), 6.03 (s, 1 H), 5.11 (d, $J = 13.6$ Hz, 1 H), 5.01 (d, $J = 13.6$ Hz, 1 H), 4.86 (d, $J = 11.6$ Hz, 1 H), 4.70 (d, $J = 11.6$ Hz, 1 H), 4.33 – 4.19 (m, 2 H), 3.95 (d, $J = 12.6$ Hz, 1 H), 3.84 (d, $J = 4.1$ Hz, 1 H), 3.77 (s, 3 H), 3.64 (s, 3 H), 3.27 (d, $J = 12.6$ Hz, 1 H), 2.80 – 2.60 (m, 2 H), 2.32 (dd, $J = 23.5$, 12.4 Hz, 1 H), 2.04 (d, $J = 16.2$ Hz, 1 H), 1.69 – 1.56 (m, 1 H), 1.18 (s, $J = 11.7$ Hz, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 163.41, 159.10, 150.70, 137.43, 135.49, 133.77, 133.31, 133.13, 130.77, 129.54, 129.32, 128.55, 128.33, 127.82, 127.76, 127.40, 126.58, 126.41, 126.18, 125.64, 113.73, 109.50, 88.71, 83.22, 80.97, 72.59, 71.18, 60.91, 60.06, 58.13, 55.26, 47.55, 43.62, 27.12, 12.45; **HRMS** (ESI) calc'd for $C_{39}H_{42}N_3O_6$ $[M+H]^+$ $m/z = 648.30681$, found = 648.30851.



Nucleoside 8a

Allyl chloroformate (2 mL, 28.3 mmol) was added to a stirred solution of nucleoside **8** (260 mg, 0.40 mmol) in toluene (10 mL) and the resulting solution was stirred for 2 days at 100°C. After the reaction was complete, the solvent was removed under reduced pressure and the crude residue was passed through a silica pad eluted with ethyl acetate to give nucleoside **8a** (184 mg, 72%).; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.86 – 7.76 (m, 4 H), 7.63 (d, $J = 1.1$ Hz, 1 H), 7.52 – 7.38 (m, 5 H), 6.85 – 6.78 (m, 2 H), 5.99 – 5.88 (m, 2 H), 5.37 – 5.19 (m, 2 H), 5.07 (d, $J = 13.6$ Hz, 1 H), 4.97 (d, $J = 13.6$ Hz, 1 H), 4.87 (d, $J = 11.5$ Hz, 1 H), 4.74 (d, $J = 11.5$ Hz, 1 H), 4.69 – 4.61 (m, 1 H), 4.56 (ddt, $J = 13.2$, 5.7, 1.3 Hz, 1 H), 4.32 (dd, $J = 17.7$, 3.1 Hz, 2 H), 4.16 (dd, $J = 11.0$, 2.6 Hz, 1 H), 3.93 (ddd, $J = 13.0$, 4.9, 3.0 Hz, 1 H), 3.76 (s, $J = 3.6$ Hz, 3 H), 3.63 (s, 3 H), 3.36 (dd, $J = 11.0$, 4.1 Hz, 1 H), 3.26 (td, $J = 12.7$, 3.5 Hz, 1 H), 2.13 (dq, $J = 14.6$, 3.2 Hz, 1 H), 1.82 – 1.70 (m, 1 H), 1.16 (d, $J = 1.0$ Hz, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 163.29, 159.05, 156.51, 150.60, 135.03, 133.28, 133.27, 133.14, 132.56, 130.64, 129.20, 128.62, 127.79, 127.75, 126.62, 126.48, 126.27, 125.52, 118.28, 113.71, 109.52, 88.77, 84.06, 80.11, 77.10, 71.56, 71.54, 66.55, 57.92, 55.23, 53.30, 43.60, 40.89, 26.83, 12.44; **HRMS** (ESI) calc'd for $C_{36}H_{40}N_3O_8$ $[M+H]^+$ $m/z = 642.28099$, found = 642.28125.

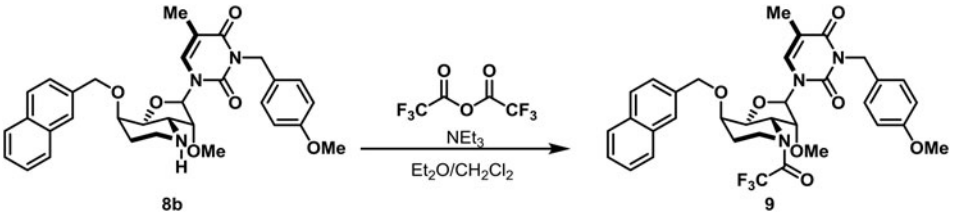


8a 8b

Nucleoside 8 b

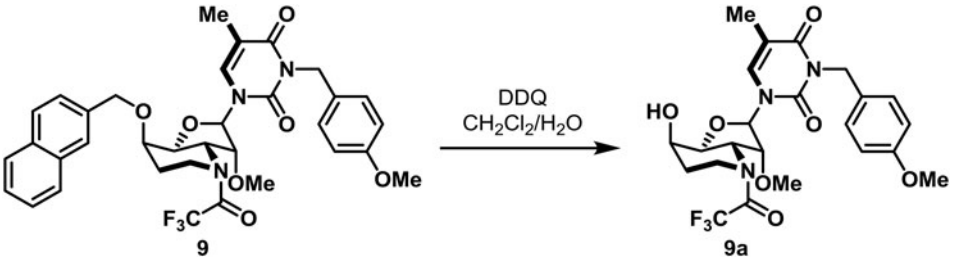
Morpholine (27 µL, 0.31 mmol) and $Pd(PPh_3)_4$ (9 mg, 7.8 µmol) were added sequentially to an stirred solution of nucleoside **8a** (98 mg, 0.15 mmol) in THF (1 mL). After 30 min, the reaction mixture was diluted with CH_2Cl_2 and silica gel was added to the reaction mixture. The volatiles were removed under reduced pressure and the dry residue was purified by flash chromatography (0% to 5% MeOH in CH_2Cl_2) to give nucleoside **8 b** as a white foam (87 mg, 99%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.84 – 7.76 (m, 4 H), 7.65 (d, $J = 1.0$ Hz, 1 H), 7.52 – 7.36 (m, 5 H), 6.80 (d, $J = 8.7$ Hz, 2 H), 5.93 (s, 1 H), 5.07 (d, $J = 13.6$ Hz, 1 H), 4.97 (d, $J = 13.6$ Hz, 1 H), 4.88 (d, $J = 11.6$ Hz, 1 H), 4.74 (d, $J = 11.6$ Hz, 1 H), 4.41 (s, 1 H), 3.87 (dd,

$J = 10.8$, 2.5 Hz, 1 H), 3.76 (s, 3 H), 3.74 – 3.70 (m, 2 H), 3.65 (d, $J = 4.6$ Hz, 1 H), 3.61 (s, 3 H), 3.19 (dd, $J = 10.8$, 4.6 Hz, 1 H), 2.07 (d, $J = 13.8$ Hz, 1 H), 1.53 – 1.41 (m, 1 H), 1.15 (d, $J = 0.8$ Hz, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 163.40, 159.10, 150.70, 135.54, 134.55, 133.66, 133.36, 133.17, 130.66, 129.30, 128.59, 127.85, 127.80, 126.55, 126.47, 126.22, 125.63, 118.53, 113.77, 109.64, 88.43, 84.19, 81.41, 73.84, 71.59, 67.03, 62.23, 58.24, 55.31, 54.80, 53.63, 43.66, 41.47, 29.06, 12.47.; **HRMS** (ESI) calc'd for $C_{32}H_{36}N_3O_6$ $[M + H]^+$ $m/z = 558.25986$, found = 558.26053



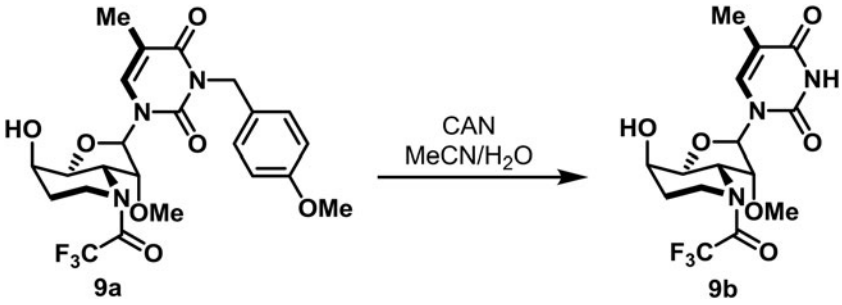
Nucleoside 9

Triethylamine (63 µL, 0.45 mmol) and trifluoroacetic anhydride (63 µL, 0.45 mmol) were added to a stirred solution of nucleoside **8 b** (201 mg, 0.38 mmol) in a 1:1 mixture of Et_2O/CH_2Cl_2 (2 mL) at 0°C. After stirring for 30 min, an aqueous saturated solution of $NaHCO_3$ was added, and the resulting mixture was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic extractions were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (2:8 EtOAc/hexane) to give nucleoside **9** (208 mg, 85%) as a white foam.; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.88 – 7.77 (m, 4 H), 7.58 (d, $J = 0.8$ Hz, 1 H), 7.55 – 7.39 (m, 5 H), 6.82 (d, $J = 8.7$ Hz, 2 H), 5.97 (s, 1 H), 5.09 (d, $J = 13.6$ Hz, 1 H), 4.98 (d, $J = 13.6$ Hz, 1 H), 4.88 (d, $J = 11.5$ Hz, 1 H), 4.79 (d, $J = 11.6$ Hz, 1 H), 4.45 (d, $J = 4.0$ Hz, 1 H), 4.41 (d, $J = 2.8$ Hz, 1 H), 4.22 (dd, $J = 11.2$, 2.6 Hz, 1 H), 3.89 – 3.79 (m, 1 H), 3.77 (s, 3 H), 3.59 (s, $J = 7.8$ Hz, 3 H), 3.54 – 3.44 (m, 2 H), 2.20 – 2.09 (m, 1 H), 1.96 – 1.82 (m, 1 H), 1.22 (s, 3 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 163.27, 159.16, 157.37 (d, $J = 36.2$ Hz), 150.63, 134.72, 133.32, 133.26, 132.97, 130.73, 129.18, 128.81, 127.87, 127.84, 126.77, 126.65, 126.46, 125.50, 117.68, 113.81, 109.87, 89.17, 82.70, 79.13, 72.10, 70.94, 57.93, 55.29, 53.82, 43.73, 41.12, 29.79, 27.59, 12.62; **^{19}F NMR** (376 MHz, $CDCl_3$) δ −69.59; **HRMS** (ESI) calc'd for $C_{34}H_{35}F_3N_3O_7$ $[M + H]^+$ $m/z = 654.24216$, found = 654.24315.



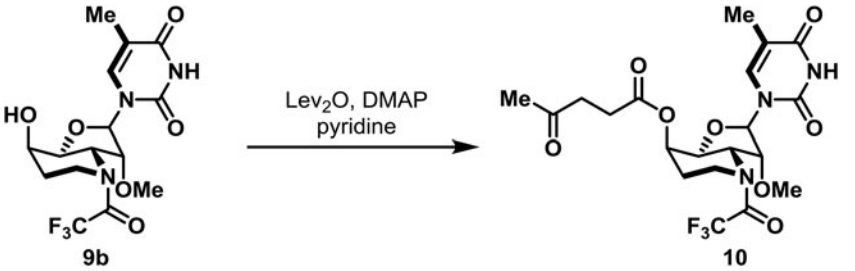
Nucleoside 9a

To an stirred solution of nucleoside **9** (208 mg, 0.318 mmol) in a 9:1 mixture of CH_2Cl_2/water (0.2 mL), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (217 mg, 0.954 mmol) was added. The resulting mixture was stirred at room temperature for 3 h followed by addition of aqueous 10% $NaHSO_3$. The resulting biphasic mixture was stirred for 10 minutes and then diluted by addition of CH_2Cl_2 (10 mL). The layers were separated, then the aqueous layer was extracted with CH_2Cl_2 (3×10 mL). The combined organic extractions were dried over Na2SO4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (4:6 Ethyl acetate/hexane) to give nucleoside **9a** as a white solid (153 mg, 94%); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.68 (s, 1 H), 7.41 (d, $J = 8.7$ Hz, 2 H), 6.86 – 6.78 (m, 2 H), 5.93 (s, 1 H), 5.10 (d, $J = 13.7$ Hz, 1 H), 5.01 (d, $J = 13.7$ Hz, 1 H), 4.57 (s, 1 H), 4.45 (d, $J = 4.1$ Hz, 1 H), 3.81 – 3.71 (m, 4 H), 3.55 (s, 3 H), 3.53 – 3.42 (m, 2 H), 2.96 (t, $J = 16.0$ Hz, 1 H), 2.03 – 1.89 (m, 2 H), 1.87 (s, 3 H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 163.64, 159.21, 157.44 (d, $J = 36.0$ Hz), 150.61, 133.73, 133.70, 130.63, 129.02, 116.25 (d, $J = 288.2$ Hz), 113.87, 109.71, 89.48, 82.73, 79.27, 63.40, 57.95, 55.34, 53.05, 43.95, 40.88, 40.84, 31.09, 13.55; **^{19}F NMR** (376 MHz, $CDCl_3$) δ −69.66; **HRMS** (ESI) calc'd for $C_{23}H_{27}F_3N_3O_7$ $[M + H]^+$ $m/z = 514.17956$, found = 514.17967.



Nucleoside 9 b

To an stirred solution of nucleoside **9a** (153 mg, 0.297 mmol) in a 10:1 mixture of MeCN:H_2O (3.3 mL), Cerium ammonium nitrate (326 mg, 0.65 mmol) was added. After 24 h, water (5 mL) was added and the aqueous layer was extracted with Et_2O (3 x 10 mL). The combined organic extractions were dried over Na_2SO_4 and the volatiles were removed under reduced pressure. The residue was purified by flash chromatography (1:1 EtOAc:hexanes) to give nucleoside **9 b** (60 mg, 51%) as a white solid; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 9.43 (s, 1 H), 7.64 (d, $J = 1.3$ Hz, 1 H), 5.93 (s, 1 H), 4.61 (q, $J = 3.6$ Hz, 1 H), 4.46 (d, $J = 4.2$ Hz, 1 H), 4.13 (dd, $J = 10.8$, 2.3 Hz, 1 H), 3.80 – 3.73 (m, 1 H), 3.54 – 3.48 (m, 5 H), 3.18 (s, 1 H), 2.01 – 1.91 (m, 2 H), 1.85 (d, $J = 1.2$ Hz, 3 H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 164.27, 157.52 (q, $J = 36.1$ Hz), 150.12, 135.68, 116.25 (q, $J = 288.3$ Hz), 110.66, 89.02, 82.73, 79.32, 63.26, 57.97, 53.15, 40.93, 30.97, 12.73; **^{19}F NMR** (471 MHz, $CDCl_3$) δ -69.71; **HRMS** (ESI) calc'd for $C_{15}H_{19}F_3N_3O_6$ $[M + H]^+$ $m/z = 394.12205$, found = 394.12257.

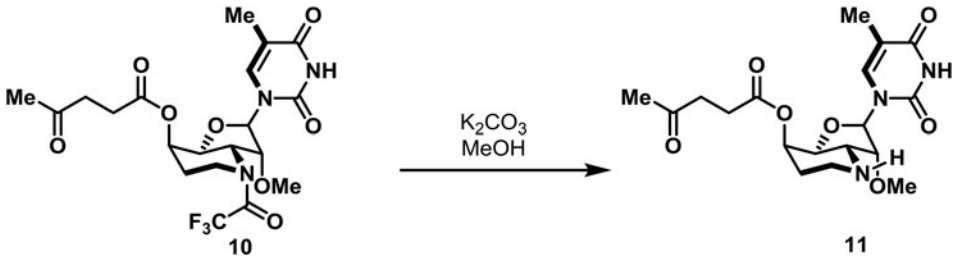


Nucleoside 10

Preparation of the levulinic anhydride solution: Levulinic acid (0.2 mL, 1.963 mmol) was added to a solution of N,N'-dicyclohexylcarbodiimide (197 mg, 0.954 mmol) in ether (2 mL) at r.t. After 3 h, the reaction mixture was filtered through a sintered glass keeping all the system under argon. The remaining solid was washed with ether (3 mL) giving a solution of Lev_2O (≈ 0.19 M).

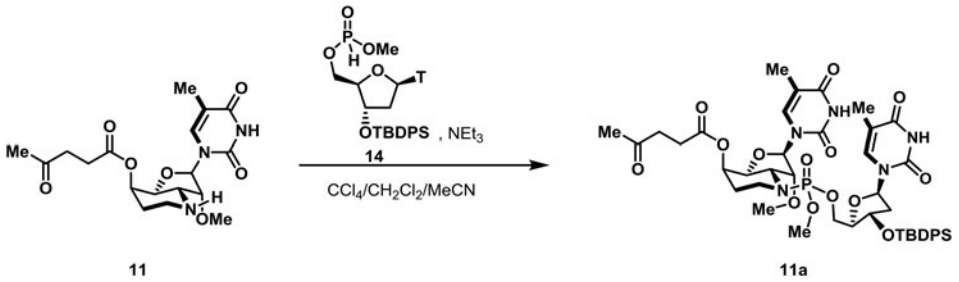
To a solution of nucleoside **9 b** (46.6 mg, 0.118 mmol) in pyridine (0.3 mL) was added sequentially DMAP (1 mg, 0.012 mmol) and ≈0.19 M levulinic anhydride solution (1.6 mL, 0.30 mmol). After stirring for 2 h, the volatiles were removed under reduced pressure and the solid residue was dissolved in CH_2Cl_2 (30 mL). The solution was washed with sat $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography (0% MeOH to 4% CH_2Cl_2 using Fluorosil as stationary phase) to give nucleoside **10** (56.2 mg, 97%) as a white foam.; **^{1}H NMR** (500 MHz,

$CDCl_3$) δ 8.72 (s, 1 H), 7.49 – 7.45 (m, 1 H), 5.90 (s, 1 H), 5.55 (q, J = 3.5 Hz, 1 H), 4.52 (d, J = 4.2 Hz, 1 H), 4.27 (dd, J = 11.2, 2.9 Hz, 1 H), 3.87 – 3.80 (m, 1 H), 3.54 (d, J = 0.8 Hz, 3 H), 3.51 – 3.42 (m, 2 H), 2.95 – 2.77 (m, 2 H), 2.70 – 2.51 (m, 2 H), 2.20 (s, 3 H), 2.18 – 2.02 (m, 2 H), 1.93 – 1.89 (m, 3 H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 206.46, 171.90, 163.54, 149.80, 134.62, 110.57, 89.30, 82.47, 66.42, 58.10, 54.33, 41.08, 38.13, 29.80, 28.26, 12.85; **^{19}F NMR** (471 MHz, $CDCl_3$) δ -69.68.; **HRMS** (ESI) calc'd for $C_{20}H_{24}F_3N_3O_8$ $[M+H]^+$ $m/z = 492.15883$, found = 492.16003.



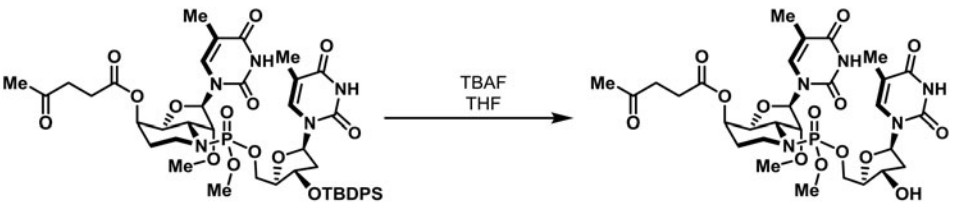
Nucleoside 11

To a stirred solution of nucleoside **10** (56.2 mg, 0.114 mmol) in a 5:1 mixture of MeOH:H_2O (0.6 mL), K_2CO_3 (16.1 mg, 0.114 mmol) was added. The resulting mixture was stirred at room temperature for 30 min, then silica gel was added to the flask and the volatiles were removed under reduced pressure. The solid residue was charged on the top of a pre-equilibrated chromatography column and eluted with a gradient of 0% to 8% MeOH in CH_2Cl_2 to give nucleoside **11** (31.6 mg, 70%) as a yellowish solid; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.47 (d, $J = 1.4$ Hz, 1 H), 5.86 (s, 1 H), 5.57 (q, $J = 2.9$ Hz, 1 H), 3.86 (dd, $J = 10.8$, 2.7 Hz, 1 H), 3.75 (d, $J = 4.8$ Hz, 1 H), 3.57 (s, 3 H), 3.11 – 3.00 (m, 2 H), 2.93 (td, $J = 13.4$, 3.1 Hz, 1 H), 2.88 – 2.75 (m, 2 H), 2.68 (ddd, $J = 16.9$, 7.7, 5.1 Hz, 1 H), 2.59 (ddd, $J = 16.9$, 6.9, 5.4 Hz, 1 H), 2.19 (s, 3 H), 2.02 (ddq, $J = 13.7$, 3.6, 1.7 Hz, 2 H), 1.91 (d, $J = 1.2$ Hz, 3 H), 1.66 – 1.56 (m, 1 H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 206.39, 171.95, 164.07, 150.14, 135.12, 110.48, 88.47, 83.69, 79.22, 69.25, 58.34, 55.56, 41.29, 38.02, 29.88, 29.78, 28.35, 12.75; **HRMS** (ESI) calc'd for $C_{18}H_{25}N_3O_7$ $[M+H]^+$ $m/z = 396.17653$, found = 396.17645.



Nucleoside 11a

To a stirred solution of nucleoside **11** (21.6 mg, 0.0546 mmol) and H-phosphonate **14** (40 mg, 0.071 mmol) in a 1:1:1 mixture of CCl_4:MeCN:CH_2Cl_2 (2.1 mL), triethylamine (10 µL, 0.071 mmol) was added. After 3 h, the volatiles were removed under reduced pressure and the dry residue was purified by flash chromatography (100% EtOAc) to give nucleoside **11a** (42.6 mg, 82%) as a ≈ 1:1 mixture of P-isomers (^{31}P NMR); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.59 (s, 1 H), 9.10 (d, J = 14.1 Hz, 1 H), 7.65 – 7.58 (m, 4 H), 7.49 – 7.30 (m, 8 H), 6.46 – 6.34 (m, 1 H), 5.82 (d, J = 4.3 Hz, 1 H), 5.39 (d, J = 52.2 Hz, 1 H), 4.40 – 4.26 (m, 1 H), 4.22 – 3.97 (m, 4 H), 3.95 – 3.79 (m, 1 H), 3.73 – 3.55 (m, 5 H), 3.55 – 3.42 (m, 2 H), 3.08 – 2.74 (m, 5 H), 2.69 – 2.48 (m, 2 H), 2.33 (ddd, J = 13.4, 5.6, 2.1 Hz, 1 H), 2.18 (d, J = 8.3 Hz, 3 H), 2.04 (s, 1 H), 1.92 – 1.79 (m, 7 H), 1.07 (d, J = 3.4 Hz, 9 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 206.44, 206.43, 171.99, 171.87, 164.08, 163.97, 163.83, 150.40, 150.08, 135.85, 135.85, 135.81, 135.68, 135.33, 134.67, 134.57, 133.14, 133.11, 133.02, 132.90, 130.36, 130.33, 130.25, 128.16, 128.13, 128.08, 128.06, 111.25, 110.98, 110.13, 110.09, 88.55, 88.50, 85.94, 85.85, 85.70, 85.63, 85.41, 85.39, 82.72, 78.52, 78.40, 74.05, 73.24, 67.64, 67.58, 60.52, 57.54, 57.45, 54.23, 54.22, 54.22, 53.48, 53.43, 53.43, 53.22, 53.17, 41.03, 41.01, 40.93, 40.90, 40.71, 38.07, 29.81, 29.77, 28.30, 26.96, 26.91, 21.17, 19.13, 19.10, 14.32, 12.84, 12.52, 12.40; **^{31}P NMR** (162 MHz, $CDCl_3$) δ 8.38, 7.73; **HRMS** (ESI) calc'd for $C_{45}H_{58}N_5O_{14}PSi$ $[M+H]^+$ m/z = 952.35599, found = 952.35676.

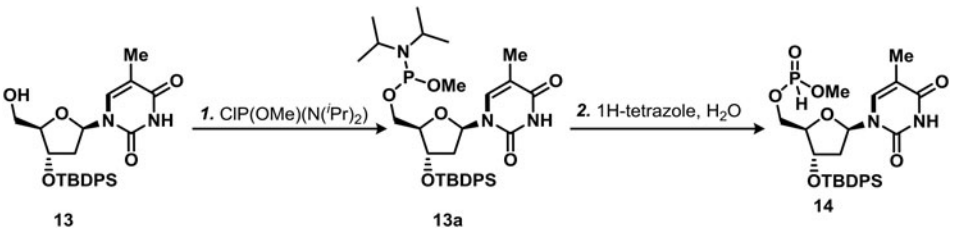


11a 12

Nucleoside 12

To a stirred solution of nucleoside **11a** (36 mg, 0.038 mmol) in THF (2.0 mL), tetrabutylammonium fluoride (1 M, 46 µL, 0.045 mmol) in THF was added. After 20 min, solid silica gel was added to the reaction mixture and the volatiles were removed under reduced pressure. The solid residue was charged in on the top of a pre-equilibrated chromatography column and eluted with a gradient of 0% to 8% MeOH in CH_2Cl_2, to give nucleoside **12** (25 mg, 92%) as a ≈ 1:1 mixture of P-isomers (^{31}P NMR). **^{1}H NMR** (300 MHz, $CDCl_3$) δ 10.09 – 9.48 (m, 2 H), 7.56 – 7.29 (m, 2 H), 6.23 (dt,

$J = 18.1$, 6.5 Hz, 1 H), 5.82 (d, $J = 4.3$ Hz, 1 H), 5.48 (s, 1 H), 4.67 – 4.56 (m, 1 H), 4.53 – 4.05 (m, 6 H), 3.75 (d, $J = 11.3$ Hz, 3 H), 3.63 (d, $J = 6.0$ Hz, 3 H), 3.39 – 3.24 (m, 3 H), 3.19 – 2.99 (m, 2 H), 2.95 – 2.79 (m, 2 H), 2.72 – 2.52 (m, 2 H), 2.44 – 2.32 (m, 1 H), 2.19 (d, $J = 1.2$ Hz, 3 H), 1.93 – 1.85 (m, 6 H), 1.44 (h, $J = 7.3$ Hz, 2 H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 206.76, 206.63, 172.00, 171.92, 164.60, 164.19, 164.16, 164.09, 150.62, 150.60, 150.51, 150.28, 135.82, 135.53, 135.06, 134.73, 111.19, 111.15, 110.22, 109.94, 88.71, 88.47, 85.26, 85.15, 85.09, 85.02, 84.94, 82.61, 82.47, 78.75, 78.63, 78.50, 71.25, 70.63, 67.73, 66.54, 66.48, 65.89, 65.84, 60.52, 59.13, 57.58, 57.49, 54.41, 54.20, 54.18, 53.76, 53.71, 53.62, 53.57, 41.45, 41.11, 40.28, 39.98, 38.10, 29.83, 28.36, 24.19, 19.88, 13.78, 12.87, 12.84, 12.64, 12.52; **^{31}P NMR** (121 MHz, $CDCl_3$) δ 9.23, 8.51; **HRMS** (ESI) calc'd for $C_{29}H_{40}N_5O_{14}P$ $[M+H]^+$ $m/z = 714.23821$, found = 714.23811.

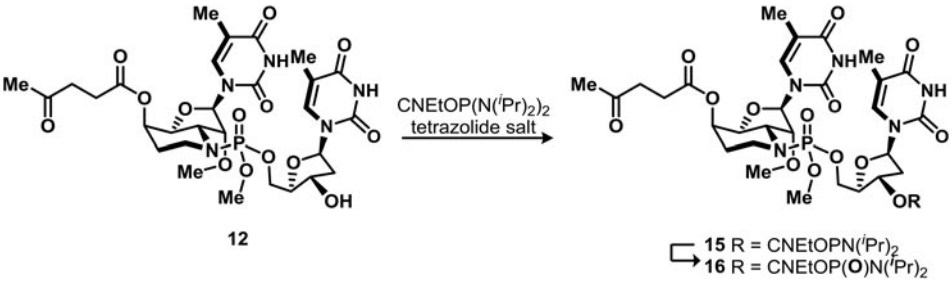


13 13a 14

H-phosphonate 14

To a solution of nucleoside **13** (0.26 mg, 0.53 mmol) and diisopropylethylamine (0.38 mL, 2.14 mmol) at 0°C was added N,N-diisopropylmethylchlorophosphoramidite (0.21 mL, 1.1 mmol). The resulting solution was stirred at rt for 30 min, cooled to 0°C, then diluted with EtOAc, washed with cold 10% sodium bicarbonate, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (1:2 EtOAc;hexanes + 1% NEt_3) to give phosphoramidite **13a** (0.29 mg, 97%). To a solution of phosphoramidite **13a** (0.29 mg, 0.45 mmol) in MeCN (4 mL), a 0.45 M solution of 1 H-tetrazole (3 mL, 1.36 mmol) and water (0.8 mL) were added. After 30 min, the reaction mixture was diluted with EtOAc (50 mL) and washed sequentially with water, 5% $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc) to givephosphoramidate **14** (0.23 mg, 91%) as a 1:1 mixture of compounds (^{31}P NMR); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.53 (s, 1 H), 7.68 – 7.58 (m, 4 H), 7.55 – 7.50 and 5.78 – 5.73 (H-P, m, 1 H), 7.48 – 7.34 (m, 6 H), 7.31 – 7.24 (m, 2 H), 6.49 – 6.41 (m, 1 H), 4.41 – 4.30 (m, 1 H), 4.08 – 4.01 (m, 1 H), 3.97 – 3.87 (m, 1 H), 3.72 – 3.56 (m, 4 H), 2.39 – 2.30 (m,

1 H), 1.95 – 1.83 (m, 4 H), 1.07 (s, 9 H).; **^{13}C NMR** (101 MHz, $CDCl_3$) δ 163.97, 150.56, 135.76, 135.72, 135.27, 135.22, 133.02, 132.97, 132.75, 132.73, 130.33, 130.30, 130.26, 128.08, 128.06, 128.05, 111.47, 85.24, 85.17, 84.88, 84.81, 72.89, 72.72, 64.75, 64.70, 64.63, 64.57, 52.30, 52.24, 52.18, 52.13, 40.56, 40.49, 26.89, 19.03, 12.41; **^{31}P NMR** (162 MHz, $CDCl_3$) δ 10.10, 9.05; **HRMS** (ESI) calc'd for $C_{27}H_{36}N_2O_7PSi$ $[M+H]^+$ $m/z = 559.20239$, found = 559.20271.



Nucleoside dimer 16

2-Cyanoethyl tetraisopropylphosphordiamidite (0.084 mmol, 0.025 g, 0.027 mL) was added to a suspension of the nucleoside dimer **12** (0.035 mmol, 0.025 g) and diisopropylammonium tetrazolide (0.011 mmol, 0.0018 g) in dry acetonitrile (0.19 mL). The reaction was stirred at room temperature for 4 hours at which time analysis of the reaction by LCMS showed conversion to **15** (calc'd for $C_{38}H_{56}N_7O_{15}P_2$, $[M\text{-}H]^-$ $m/z = 912.3$, found 912.3). The reaction was diluted with ethyl acetate and loaded on silica gel prewashed with 50% THF/3% triethylamine/ethyl acetate. The product was eluted from the column using 70% THF/3% triethylamine/ethyl acetate and concentrated under reduce pressure to yield **16** (0.012 g, 36%) as a white foam (calc'd for $C_{38}H_{56}N_7O_{16}P_2$, $[M\text{-}H]^-$ $m/z = 928.3$, found 928.3). **^{31}P NMR** (121 MHz, $CDCl_3$) δ 8.92, 8.15, 7.95, 7.83.

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