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Large Scale Synthesis of 2′-Amino-LNA Thymine and 5-Methylcytosine Nucleosides

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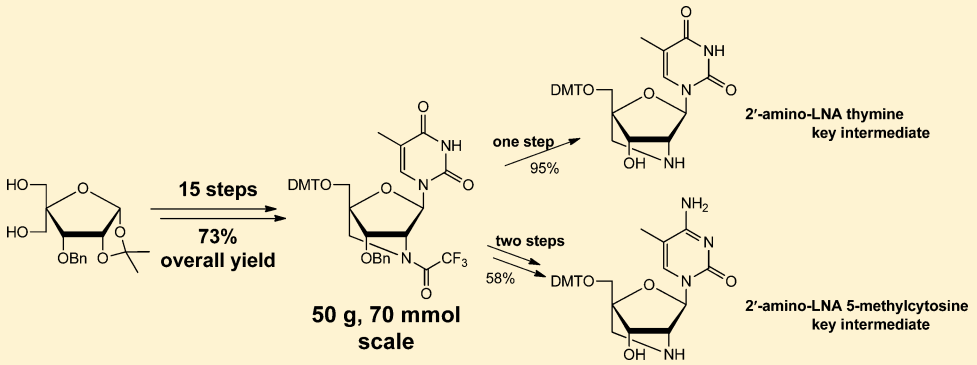
Supporting Information



ABSTRACT: Thymine intermediate **17** has been synthesized on a multigram scale (50 g, 70 mmol) from starting sugar **1** in 15 steps in an overall yield of 73%, with only 5 purification steps. The key thymine intermediate **18** was obtained from **17** in a single step in 96% yield, whereas the key 5-methylcytosine intermediate **20** was obtained from **17** in 2 steps in 58% yield. This highly efficient large scale route necessitates only 2 and 3 novel steps to obtain N2′-functionalized thymine and 5-methylcytosine amino-LNA phosphoramidites from these key intermediates, respectively.

INTRODUCTION

Incorporation of functionalized nucleosides into oligonucleotides (ONs) offers the opportunity of site specific conjugation of functional entities to nucleic acid structures. An enormous body of functionalized nucleotides has been synthesized and incorporated into ONs, including functionalization of the nucleobase with fluorescent moieties (recent examples include C5 of pyrimidines,[1−6] C8 of purines,[7,8] and C7 of 7-deazapurines[6,9,10]), substitution of the nucleobase with fluorescent moieties,[11−14] functionalization of the sugar moiety,[15−17] and functionalization of the phosphodiester moiety.[18−21] Non-nucleosidic structures have also been used to position functional groups in a duplex context, including forced intercalation probes (FIT, thiazole-orange modified PNAs),[22,23] intercalating nucleic acid (INA),[24,25] or twisted intercalating acid (TINA).[26,27]

2′-Amino-LNA compares favorably with other strategies for defined positioning of functional moieties in the duplex, by the unique combination of conformational restriction and a precisely positioned conjugation point. The bicyclic skeleton of 2′-amino-LNA leads to (a) a locked 3′-*endo* furanose conformation, (b) improved binding toward DNA and RNA complementary strands, and (c) pronounced mismatch discrimination. Functionalities that have successfully been introduced in the minor groove of duplexes via the N2′-atom of 2′-amino-LNA nucleotides include alkyl and acyl moieties,[28,29] metal chelators,[30−32] polar moieties like nucleobases[33] and amino acids,[29] and aromatic groups like pyrene and perylene derivatives.[28,34−41] Remarkably, these functionalities generally could be introduced without compromising the high thermal stability typical of LNA-containing duplexes. This is in contrast to related 2′-amino-uridine derivatives, where N2′-functionalization usually leads to significantly lower duplex thermal affinity.[31]

The first reports on 2′-amino-LNA nucleotides as conjugation site used synthetic strategies that were either not compatible with introduction of a wide variety of N2′-functionalities,[42] or demanded several steps for each introduced functionality.[28] Later syntheses have, however, used a slightly different strategy, where key intermediate **18** is first N2′-functionalized and then O3′-phosphitylated. This strategy thus necessitates only two novel steps to obtain a phosphoramidite derivative of each N2′-functionality (see Figure 1).

In our continued investigation of functionalized 2′-amino-LNA, we therefore wanted to establish a reliable and efficient large scale synthesis of key intermediate **18**. While the smaller scale synthesis of 3′-*O*-benzyl-2′-amino-LNA thymine derivative **15** has been described,[43] a large scale synthesis has never been reported. Furthermore, we wanted to amend the fact that the synthesis of amino alcohol **18** never has been reported. The majority of functionalized 2′-amino-LNA derivatives have been synthesized with thymine as the nucleobase, but we further wanted to pursue synthesis of a key 5-methylcytosine

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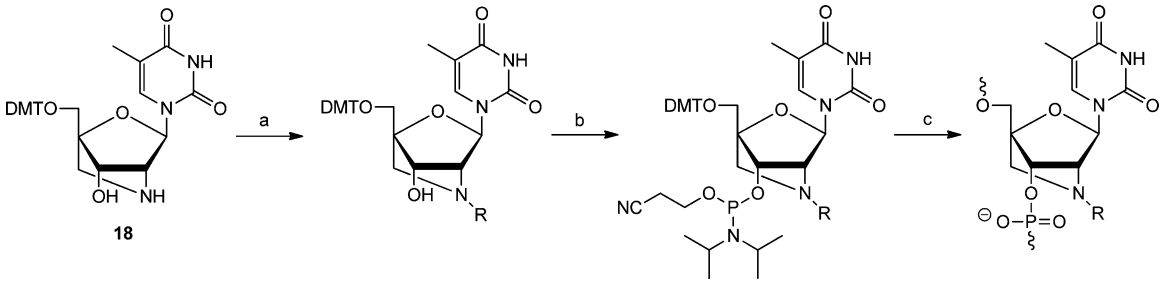
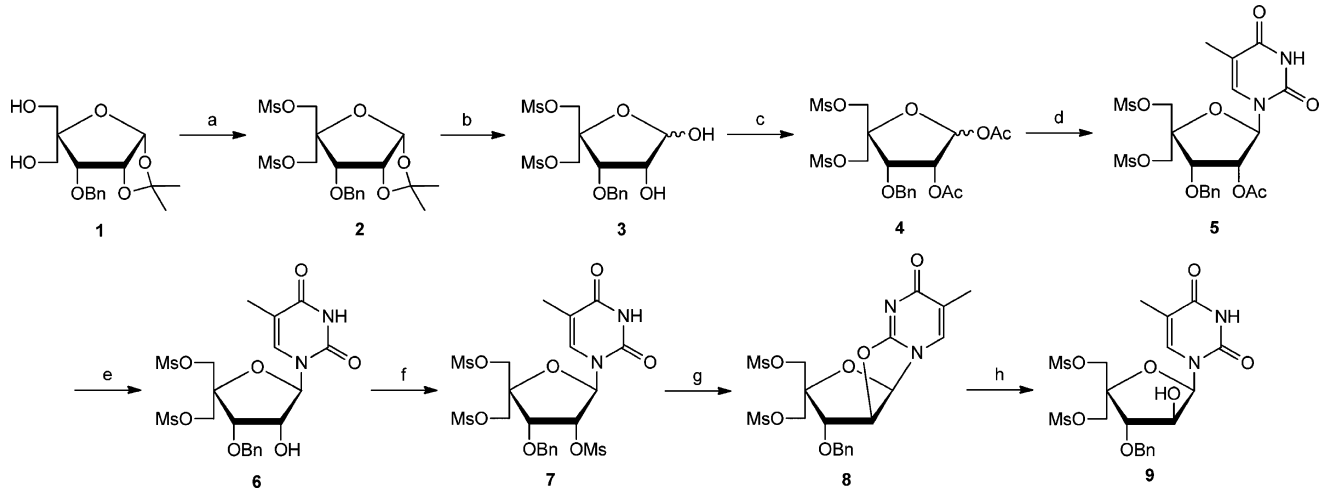


Figure 1. Synthesis of functionalized 2′-amino-LNA thymine. a) either aldehyde and $NaBH(OAc)_3$ or carboxylic acid and coupling reagent (e.g., HATU or EDC·HCl), (b) 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochlorodite and diisopropylethylamine or bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine and diisopropylammonium tetrazolide, c) DNA synthesis. See text for references to specific examples.

Scheme 1[a]



[a](a) MsCl, pyridine, 0 °C to rt, 20 h; (b) 80% aq F_3CCO_2H, 0 °C, 4 h; (c) Ac_2O, pyridine, 0 °C to rt, 40 h; (d) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMSOTf, MeCN, 60 °C, 96 h; (e) sat. NH_3 in MeOH, 0 °C, 4.5 h; (f) MsCl, pyridine/DCM (50:50, v/v), 0 °C, 5 h; (g) DBU, MeCN, 0 °C to rt, 18 h (86% from **1**); (h) H_2SO_4 (0.5 M), H_2O/acetone (50:50, v/v), reflux, 3 h (98%).

intermediate, thereby expanding the potential sequence context and applicability of functionalized 2′-amino-LNA oligonucleotides.

RESULTS AND DISCUSSION

Key Intermediate 17 and Thymine Derivative 18. Starting from known 4′-*C*-hydroxymethyl ribose derivative **1**,[44] mesylation, isopropylidene cleavage and acetylation was carried out on 275 mmol scale, to afford coupling sugar **4** without the need of purification. The two step procedure of isopropylidene cleavage using aqueous trifluoroacetic acid followed by acetylation using acetic anhydride in pyridine was chosen over the one step procedure of acetic anhydride in glacial acetic acid and catalytic amounts of sulfuric acid,[45] since the former conditions led to less colorization of the crude product. Subsequent introduction of the nucleobase was carried out on 100 mmol scale using the modified Vorbrüggen coupling conditions recently described for coupling of uracil to coupling sugar **4**.[46] The reduced temperature (60 °C compared to reflux (82 °C)) led to longer reaction time, but afforded nucleoside **5** in a purity sufficient to proceed without the need for purification. Nucleoside **5** was subsequently deacetylated in saturated methanolic ammonia affording alcohol **6** without any detectable ring formation between the O2′-atom and the C4′-hydroxymethyl group. Mesylation and treatment with anhydrous DBU afforded 2,2′-*O*-anhydro compound **8**, which was isolated after precipitation in 86% yield (calculated from starting sugar **1**, Scheme 1). This intermediate was subsequently converted to *β*-D-*threo*-configured nucleoside **9** in 98% yield by acidic hydrolysis. A more concentrated reaction mixture (0.5 M H_2SO_4 in H_2O/acetone (50:50, v/v) compared to 0.05 M H_2SO_4 in H_2O/acetone (50:50, v/v) previously reported) was more easy to handle on large scale and allowed a reduction in reaction time without compromising the yield.

To our surprise, the two step procedure of activation of the 2′-hydroxy group by 2 equiv of triflic anhydride, 4 equiv of DMAP in pyridine and dichloromethane (affording triflate **10**) followed by azidation using sodium azide in DMF did not afford the desired azide **11** in the high yields previously reported for a smaller scale transformation.[43] Instead, azide **11** was formed only in modest yields (~50%) along with formation of diazide **12** in >20% yield (Figure 2).

Presumably, the reaction conditions employed during the first of the two steps mentioned above leads to activation of the 4-OH enol tautomer of the nucleobase as well as the 2′-hydroxy group, allowing incorporation of two azido moieties. This is in line with the observation that thymine nucleosides can be activated by phosphorus oxychloride, as known from the widespread thymine to 5-methylcytosine, or uracil to cytosine, conversion. Furthermore, it is known that activation of 2-pyrimidones by 4-chlorophenylphosphorodichloridate in the presence of pyridine lead to formation of fluorescent

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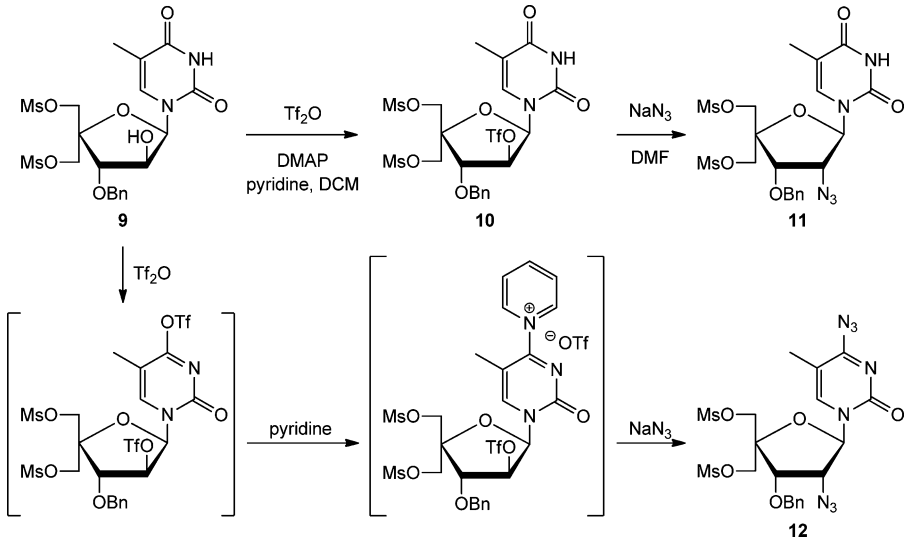
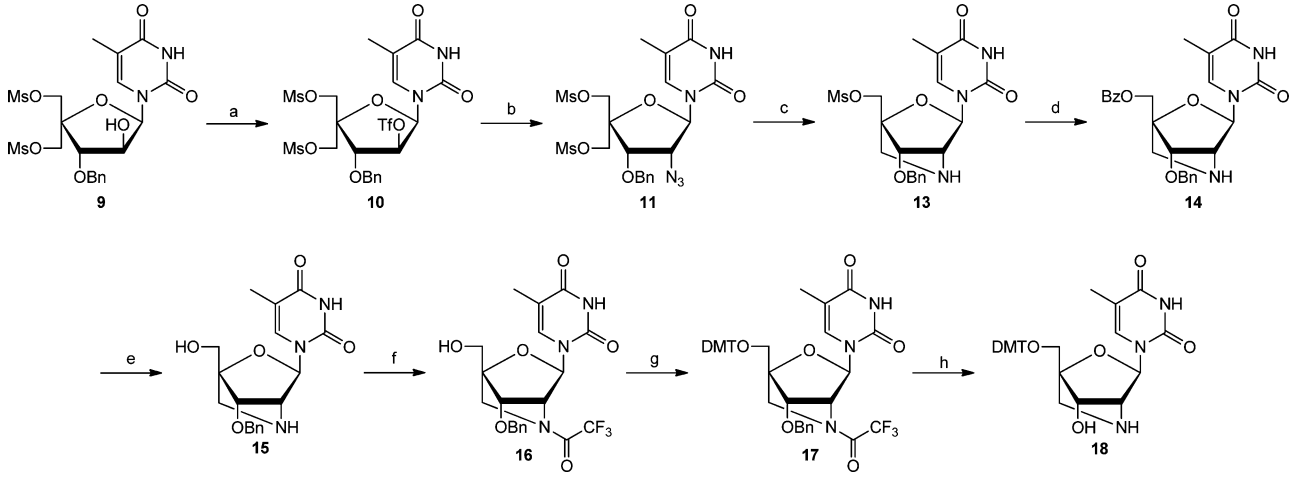


Figure 2. Formation of azides **11** and **12** from alcohol **9** under suboptimal conditions.

Scheme 2[a]



[a](a) Tf_2O, DMAP, pyridine, DCM, −70 to 0 °C, 4 h (97%); (b) NaN_3, DMF, rt, 18 h; (c) PMe_3, NaOH, THF, H_2O, 0 °C to rt, 7 h; (d) KOBz, 18-crown-6, dioxane, 80 °C, 33 h (94% from **10**); (e) satd NH_3/MeOH, 0 °C to rt, 36 h; (f) $(CF_3CO)_2O$, pyridine/DCM (50:50, v/v), −70 to 0 °C, 30 min; (g) DMTCl, pyridine/DCM (50:50, v/v), rt, 18 h (95% from **14**); (h) NH_4HCO_2, 20% $Pd(OH)_2/C$, MeOH, reflux, 3 h (96%).

pyrimidinyl-pyridinium salts which can then serve as intermediates for the synthesis of various 4-substituted 2-pyrimidinones including 4-azido-2-pyrimidones.[47] In agreement with these results, a very polar fluorescent compound could be identified by TLC (as evaluated by irradiation at 365 nm). The fluorescence of the unwanted intermediate allowed us to qualitatively evaluate the amount formed under various activation conditions (1.3−2.0 equiv of Tf_2O, 0.05−4.0 equiv of DMAP and 0−10 equiv of pyridine). In our hands, we found that 1.4 equiv of triflic anhydride, 2 equiv of DMAP and 10 equiv of pyridine and adding the anhydride at −70 °C resulted in almost no formation of the fluorescent compound. Surprisingly, aqueous workup of the reaction mixture after the first step did not efficiently remove the polar fluorescent compound. However, column chromatography using ethyl acetate as eluent resulted in easy removal of any polar intermediates and isolation of the surprisingly stable triflate **10** in 97% yield on 85 mmol scale, thereby omitting the need for cumbersome separation of azides **11** and **12** (Scheme 2).

Azidation, Staudinger reduction under basic conditions to ensure immediate ring closure, and exchange of the 5′-mesyloxy group to benzoate afforded nucleoside **14** in 94% yield on a 80 mmol scale from triflate **10** without the need of purification of intermediates. The use of a more concentrated reaction solution than previously reported in the azidation step and the use of dioxane as solvent rather than DMF in the last step simplified the large scale workup procedures significantly. Ammonolysis of the benzoate ester afforded amino alcohol **15**, which could be reacted with trifluoroacetic anhydride in pyridine and dichloromethane to give N2′-trifluoroacetyl derivative **16** followed by dimethoxytritylation to afford fully protected nucleoside **17** in 95% yield from nucleoside **14** on 75 mmol scale. Finally, debenzylation by transfer hydrogenation using ammonium formate as hydrogen source and $Pd(OH)_2/C$ as catalyst on a 26 mmol scale, also resulting in removal of the N2′-trifluoroacetyl group, furnished the desired key intermediate **18** in 96% yield. This corresponds to an overall yield of 70% for the 16 step conversion **1**→**18** involving only six purification procedures.

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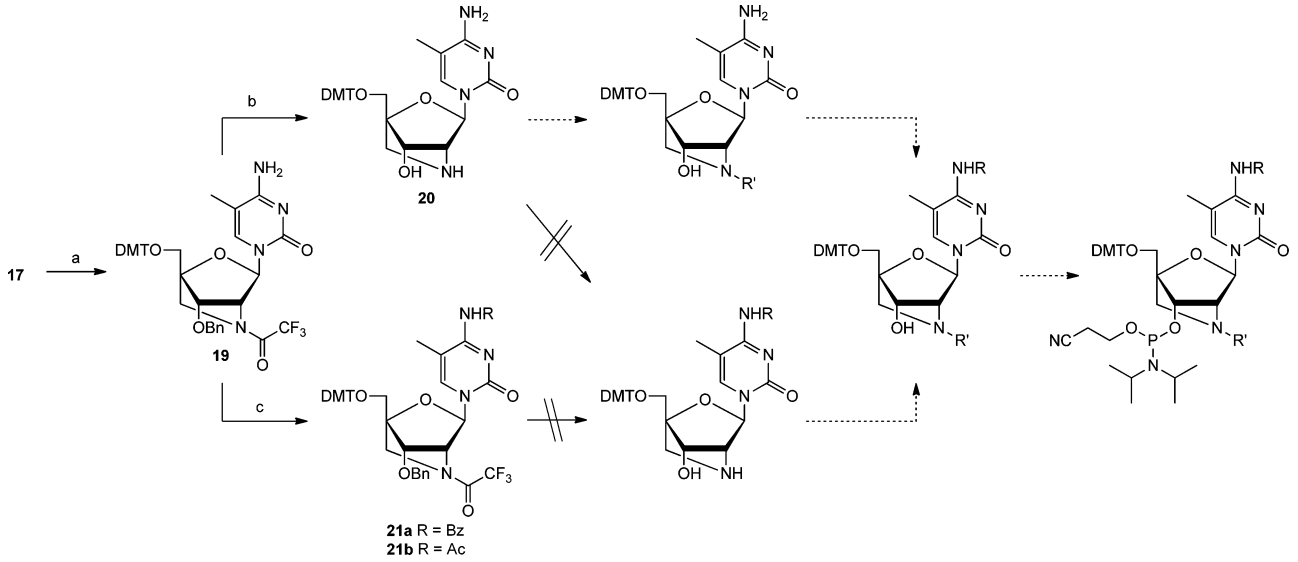
Scheme 3. [a]



[a](a) (i) $POCl_3$, 1,2,4-triazole, NEt_3, MeCN, 0 °C to rt, 75 min, (ii) NH_3, H_2O, THF, rt, 70 min (quantitative); (b) NH_4HCO_2, 20% $Pd(OH)_2/C$, MeOH, reflux, 3 h (58%); (c) **21a**: BzCl, pyridine, rt 24 h (74%) or **21b**: Ac_2O, DMAP, pyridine/DCM (50:50, v/v), rt, 5 days (82%.

5-Methylcytosine Derivatives. While thymine derivative **18** is highly useful as divergence point in the synthesis of functionalized amino-LNA thymine derivative, synthesis of 5-methylcytosine derivatives are slightly more demanding, owing to N4-protection which is necessary for oligonucleotide synthesis. A single N2′-functionalized 5-methylcytosine derivative has previously been prepared and incorporated into ONs by Hrdlicka and co-workers.[48] Initially following the same route, key intermediate **17** was conveniently and in quantitative yield converted to 5-methylcytosine derivative **19** on a 7 mmol scale via the well-established two-step triazolo/ammonolysis methodology.[49] Thymine derivative **17** was thus treated with $POCl_3$, 1,2,4-triazole and Et_3N to afford the 4-triazolothymine derivative, which was converted to the corresponding 5-methylcytosine derivative **19** by treatment with aqueous ammonia in THF (Scheme 3).

Debenzylation and trifluoroacetamide cleavage by transfer hydrogenation using ammonium formate as hydrogen source and $Pd(OH)_2/C$ as catalyst, then afforded diamino alcohol **20** in 58% yield. We did not pursue isolation and identification of byproducts; however, the relatively low yield of **20** probably reflects several aspects. While small scale conversion of thymine derivative **17** to 5-methylcytosine derivative **19** proceeded smoothly, upscaling to 25 mmol scale and the use of crude **19** in the subsequent hydrogenation reaction (concomitant debenzylation and trifluoroacetamide cleavage) might affect yields. Furthermore, purification of the very polar diamino alcohol **20** was severely complicated by residual ammonium formate, which might also negatively affect the isolated yield. Interestingly, crude diamino alcohol **20** was insoluble in DCM, whereas purified diamino alcohol **20** was readily soluble. We hypothesize that crude compound **20** precipitated as a formate salt, whereas the laborious workup broke the salt formation, leaving diamino alcohol **20** after purification. In summary, in view of the four-step procedure with no intermediate purifications and a final purification of the highly polar product, compound **20** is obtained from key intermediate **17** in an acceptable yield. This key intermediate is set up for a three step sequence of N2′-functionalization, N4-protection and O3′-phosphitylation to afford phosphoramidite building blocks ready for solid phase oligonucleotides synthesis.

As a consequence, three steps are needed for each N2′-functionalization of the 5-methylcytosine derivative. Alternative routes that could reduce the number of diverged steps were therefore explored. 5-Methylcytosine derivative **19** is well-suited for instalment of an appropriate 4-amino protection group (e.g., benzoyl or acetyl). Both protection groups were easily introduced affording acylated nucleosides **21a** and **21b**. Subsequent debenzylation, however, proved problematic; hydrogenation under a H_2 atmosphere using Pd/C or $Pd(OH)_2/C$ as catalyst gave a complex mixture of products, whereas *in situ* hydrogen generation using cyclohexene as hydrogen source and either Pd/C or $Pd(OH)_2/C$ as catalyst led to O5′-detritylation while leaving the O3′-benzyl group intact. Transfer hydrogenation using ammonium formate as hydrogen source and Pd/C as catalyst resulted in debenzylation but with concomitant removal of the 2′-*N*- and 4-*N*-acyl groups, affording diamino alcohol **20**, where to a more efficient synthesis was established above. Furthermore, selective N4-benzoylation of diamino alcohol **20** was attempted by a transient protection strategy using two equivalents of TMSCl followed by addition of BzCl. This procedure however resulted in N2′-benzoylation in agreement with the expected higher nucleophilicity of the secondary aliphatic 2′-*C*-amino group over the exocyclic 4-*C*-amino group. These results are in agreement with the previous report, where selective N4-protection also failed.[48] Alternative routes, including instalment of an orthogonal N2′-protection group prior to N4-acylation, followed by N2′-deprotection to allow a two-step divergent strategy, as used by Hrdlicka and co-workers, could be envisioned. However, since the N2′-/N4-selectivity of such protection group would not be expected to be superior to direct instalment of the desired functionality, we conclude, also in light of the extra synthetic and purification steps needed, that the three-step diverged strategy (N2′-functionalization, N4-protection and O3′-phosphitylation) most likely is the most viable route toward N2′-functionalized 5-methylcytosine amino-LNA phosphoramidites. This is corroborated by the

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results obtained by Hrdlicka and co-workers, where the trifluoroacetyl group serves as orthogonal N2′-protection group, affording the N4-benzoylated derivative in 30% yield from **20**, followed by N2′-functionalization and phosphitylation. To confirm the practicability of the three-step route, N2′-palmitoylation of 5-methylcytosine intermediate **20**, followed by N4-benzoylation and O3′-phosphitylation was used to afford phosphoramidite **23**. Reaction scheme can be found in the Supporting Information. In agreement with the expected N2′-selectivity of 5-methylcytosine derivative **20**, acylation gave predominantly the N2′-palmitoylated intermediate, which upon benzoylation afforded N2′-palmitoyl,4-*N*-benzoyl intermediate **22** in 45% yield from diamino alcohol **20**. The relatively low yield of intermediate **22** is likely a consequence of some dipalmitoylation at N2′ and 4-N in the first step. In agreement herewith, formation of a less polar compound was observed, but not further analyzed. While selectivity of the first step might be improved, phosphoramidite **23** was obtained in 38% yield from intermediate **20**, thereby demonstrating the feasibility of the three-step route.

CONCLUSION

A multigram scale synthesis of thymine intermediate **17** has been realized from starting sugar **1** in fifteen steps in an overall yield of 73%, with only five purification steps necessary. One step conversion to key thymine intermediate **18** was achieved in 95% yield, whereas the two step conversion to 5-methylcytosine key intermediate **20** proceeded in 58% yield. With the procedures disclosed herein, highly efficient larger scale routes have been established allowing convenient and high-yielding syntheses of N2′-functionalized thymine and 5-methylcytosine amino-LNA phosphoramidites in only two and three divergent steps, respectively.

EXPERIMENTAL SECTION

General Experimental Section. All reagents and solvents were of analytical grade and used without further purification as obtained from commercial suppliers, except for DCM and $POCl_3$ which were distilled before use. Petroleum ether of distillation range 60−80 °C was used. Acetonitrile for use in anhydrous reactions was dried through storage over activated 3 Å molecular sieves. DCM, 1,2-DCE, 1,4-dioxane, DMF, DBU, triethylamine, and pyridine for use in anhydrous reactions were dried through storage over activated 4 Å molecular sieves. Reactions were conducted under an atmosphere of argon or nitrogen whenever anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates (analytical SiO_2-60, F-254). The TLC plates were visualized under UV light and by dipping in either a 5% conc. solution of sulfuric acid in abs. ethanol (v/v) or a solution of molybdato-phosphoric acid (12.5 g/L) and cerium(IV)sulfate (5 g/L) in 3% conc. sulfuric acid in water (v/v), followed by heating with a heat gun. Silica gel column chromatography was performed using an automated purification system or manually with silica gel 60 (particle size 0.040−0.063 mm, Merck) using moderate pressure (pressure ball). After column chromatography, appropriate fractions were pooled, evaporated under reduced pressure and dried at high vacuum for at least 12 h to give products in >95% purity unless otherwise stated. Evaporation of solvents was carried out under reduced pressure at a temperature below 40 °C. ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and ^{31}P NMR were recorded at 400 MHz, 101 MHz, 376 MHz, and 162 MHz respectively. Chemical shifts are reported in ppm relative to deuterated solvent as internal standard (δ_H: DMSO-d_6 2.50 ppm; δ_C: DMSO-d_6 39.52 ppm) or external standard (δ_F and δ_P). Coupling constants are reported in Hz. Exchangeable protons were detected by disappearance of peaks upon addition of D_2O. Assignments of NMR spectra are based on correlation spectroscopy (COSY, HSQC, and HMBC spectra) and follow standard nucleoside nomenclature. Systematic compound names are given according to von Baeyer nomenclature. High resolution mass spectra (HRMS) were performed on a TOF (time-of-flight) analyzer and were recorded in positive ion mode using electrospray ionization (ESI).

*1,2-Di-O-acetyl-3-O-benzyl-4-C-methanesulfonoxymethyl-5-O-methanesulfonyl-D-erythro-pentofuranose (**4**).*[50] Two reaction batches (274 mmol each) were used for the steps **1**→**4**, a representative protocol is given here: Diol **1** (85.0 g, 274 mmol) was coevaporated with pyridine (100 and 50 mL) and then dissolved in anhydrous pyridine (430 mL) and cooled to 0 °C. MsCl (53 mL, 685 mmol) was added dropwise over 20 min. The reaction mixture was stirred for 20 h, during which the reaction was allowed to heat to rt and the clear reaction mixture turned first green and then brown upon stirring. Ice (400 mL) was added and the reaction mixture was extracted with EtOAc (400 mL and 2 × 200 mL). The org. phase was dried over $MgSO_4$, and evaporated to dryness to afford dimesylate **2** as a brownish syrup which was used directly in the next step. TLC (50% EtOAc in PE, v/v): R_f (**1**) = 0.3, R_f (**2**) = 0.5. Crude isopropylidene derivative **2** (128 g) was suspended in ice-cold aq trifluoroacetic acid (80%, v/v, 550 mL) and then stirred at 0 °C for 4 h, whereupon the mixture was evaporated to dryness. The crude residue was coevaporated with 1,2-DCE (2 × 100 mL) affording crude hemiacetal **3** which was used directly in the next step. TLC (50% EtOAc in PE, v/v): R_f (**2**) = 0.5, R_f (**3**) = 0.3. Crude diol **3** (117 g) was dissolved in anhydrous pyridine (500 mL) and then cooled to 0 °C. After dropwise addition of Ac_2O (100 mL, 1.10 mol) over 30 min, stirring was continued for 40 h during which the reaction was allowed to heat to rt. Ice (150 mL) and EtOAc (400 mL) were added. The organic phase was evaporated to dryness, and then coevaporated with toluene (2 × 100 mL). The resulting residue was taken up in EtOAc (400 mL) and the resulting mixture washed with satd aq $NaHCO_3$ (2 × 500 mL). The combined aq phase was back-extracted with EtOAc (2 × 200 mL). The combined org. phase was dried over $MgSO_4$ and then evaporated to dryness. After workup, analytical TLC showed some remaining diol **3**. The residue was dissolved in anhydrous pyridine (500 mL) and then cooled to 0 °C. Ac_2O (25 mL, 274 mmol) was dropwise added over 10 min and the reaction mixture stirred for 18 h. The reaction mixture was cooled to 0 °C and then partitioned between ice (150 mL) and EtOAc (200 mL). The separated org phase was concentrated, then coevaporated with toluene (3 × 200 mL). The resulting residue was partitioned between EtOAc (400 mL) and satd aq $NaHCO_3$ (300 mL). The aq phases of the two reactions (i.e., from reacting a total of 334 g of crude diol **3**) were combined and extracted with EtOAc (2 × 200 mL). The org. phases were combined, evaporated to dryness and then coevaporated with MeCN (2 × 150 mL) to afford diacetate **4** (274.82 g, two anomers, 98% from **1**) as a brown syrup which was used directly in the next step. TLC (3% MeOH in DCM, v/v): R_f (**3**) = 0.1, R_f (**4**) = 0.6 and 0.7. Analytical data were identical to those previously reported.[50]

*1-(2-O-Acetyl-3-O-benzyl-4-C-methanesulfonoxymethyl-5-O-methanesulfonyl-β-D-erythro-pentofuranosyl)thymine (**5**).*[50] Five reaction batches (94−118 mmol) were used for the steps **4**→**8**, a representative protocol is given here: Coupling sugar **4** (57.28 g, 112 mmol) and thymine (28.30 g, 224 mmol) were coevaporated with MeCN (2 × 100 mL) and then suspended in anhydrous MeCN (450 mL). *N*,*O*-bis(Trimethylsilyl)acetamide (97 mL, 393 mmol) was added at rt, and the stirred reaction mixture was heated under reflux for 20 min during which time the reaction mixture became homogeneous. The reaction mixture was cooled to 0 °C and TMSOTf (51 mL, 280 mmol) was added dropwise over 20 min whereupon the resulting mixture was stirred at 60 °C for 92 h. After cooling to 0 °C, the reaction mixture was poured into satd aq $NaHCO_3$ (250 mL) and then concentrated to approx 250 mL which led to precipitation. To the resulting suspension was added DCM (400 mL) and brine (200 mL). The mixture was filtered and the precipitate washed with DCM (2 × 75 mL). The combined org. phase was washed with satd aq $NaHCO_3$ (250 mL) and the combined aq phase back-extracted with DCM (2 × 75 mL). The combined org. phase was evaporated to dryness and the residue subsequently coevaporated with MeCN (100

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mL) to afford crude nucleoside **5** (64.18 g) as a brown foam, which was used directly in the next step. TLC (5% MeOH in DCM, v/v): R_f (**4**) = 0.7, R_f (**5**) = 0.4. Analytical data were identical to those previously reported.[49]

*1-(3-O-Benzyl-5-O-methanesulfonyl-4-C-methanesulfonyloxymethyl-β-D-erythro-pentofuranosyl)thymine (**6**).*[43] Crude acetate **5** (64.18 g) was dissolved in satd methanolic ammonia (560 mL) at 0 °C and then stirred 5 h. The reaction mixture was evaporated to dryness whereupon EtOAc (400 mL) and brine (200 mL) were added. The aqueous phase was back-extracted with EtOAc (2 × 100 mL) and the combined organic phase evaporated to dryness affording crude alcohol **6** (63.57 g) as a brown foam, which was used directly in the next step. TLC (10% MeOH in DCM, v/v): R_f (**5**) = 0.6, R_f (**6**) = 0.5. Analytical data were identical to those previously reported.[43]

*1-(3-O-Benzyl-2,5-di-O-methanesulfonyl-4-C-methanesulfonyloxymethyl-β-D-erythro-pentofuranosyl)thymine (**7**).*[51] Crude alcohol **6** (63.57 g) was dissolved in a mixture of anhydrous DCM (300 mL) and anhydrous pyridine (300 mL). After cooling to 0 °C, MsCl (11.0 mL, 143 mmol) was added dropwise over 5 min with stirring whereupon the reaction mixture was heated to rt. After stirring at rt for 5 h the reaction mixture was poured into satd aq $NaHCO_3$ (200 mL) and concentrated to approximately 200 mL. DCM (400 mL), brine (200 mL) and water (100 mL) were added, the org phase separated and the aq phase back-extracted with DCM (2 × 100 mL). The combined organic phase was evaporated to dryness and the residue coevaporated with toluene (2 × 100 mL) to give a mixture of desired mesylate **7** and 2,2′-anhydro derivative **8** (76.88 g) as a brown foam, which was used directly in the next step. TLC (10% MeOH in DCM, v/v): R_f (**6**) = 0.6, R_f (**7**) = 0.5, R_f (**8**) = 0.4. Analytical data were identical to those previously reported.[51]

*2,2′-Anhydro-1-(3-O-benzyl-5-O-methanesulfonyl-4-C-methanesulfonyloxymethyl-β-D-threo-pentofuranosyl)thymine (**8**).*[43] The crude mixture of mesylate **7** and 2,2′-anhydro derivative **8** (76.88 g) was coevaporated with acetonitrile/toluene (2 × 150 mL, 67:33 v/v) and then dissolved in anhydrous MeCN (550 mL). The resulting mixture was cooled to 0 °C under mechanical stirring, DBU (20.6 mL, 138 mmol) was added and the reaction mixture was stirred for 24 h during which time the reaction was allowed to heat to rt. Extensive precipitation was observed and further facilitated by subsequently concentrating the reaction mixture to approximately 250 mL and cooling to −20 °C The precipitate was filtered off and washed with ice-cold MeCN (2 × 25 mL). The precipitate was dried under vacuum affording the desired anhydro derivative **8** (33.37 g, 57%) as a white solid. The mother liquors from the five reaction batches were combined and then concentrated leading to extensive precipitation. The precipitate was filtered off to afford the desired anhydro derivative **8** (92.64 g) as a white solid. The combined mother liquor was further concentrated affording a residue which was purified by silica gel column chromatography (0−10% ^{i}PrOH in $CHCl_3$, v/v) affording the desired anhydro derivative **8** (5.65 g) as a white foam. In total, 242.89 g (470 mmol, 86% from **1**) of the desired anhydro derivative **8** was isolated. TLC (10% MeOH in DCM, v/v): R_f (**7**) = 0.5, R_f (**8**) = 0.4. Analytical data were identical to those previously reported.[43]

*1-(3-O-Benzyl-5-O-methanesulfonyl-4-C-methanesulfonyloxymethyl-β-D-threo-pentofuranosyl)thymine (**9**).*[43] 2,2′-Anhydro derivative **8** (44.01 g, 85.2 mmol) was suspended in a stirred mixture of acetone (250 mL) and aq H_2SO_4 (1.0 M, 220 mL, 220 mmol). The resulting mixture was heated under reflux for 3 h resulting in a transparent yellow solution. Subsequent cooling to 0 °C induced extensive precipitation. The reaction mixture was then neutralized by careful addition of sat, aq, $NaHCO_3$ (250 mL) under stirring. The suspension was filtered and the mother liquor concentrated to approximately 500 mL, leading to further precipitation. The combined precipitate was washed with water (3 × 400 mL). The combined aq phase was extracted with DCM (100 mL) and the precipitate and org. phase evaporated to dryness and coevaporated with 1,2-DCE (4 × 200 mL). The resulting solid was adsorbed on silica gel and purified by DCVC (dry column vacuum chromatography) (0−6% MeOH in DCM, v/v), affording the desired alcohol **9** (44.82 g, 98%) as a white foam. TLC (10% ^{i}PrOH in $CHCl_3$, v/v): R_f (**8**) = 0.3, R_f (**9**) = 0.5. Analytical data were identical to those previously reported.[43]

*1-(3-O-Benzyl-5-O-methanesulfonyl-4-C-methanesulfonyloxymethyl-2-O-trifluoromethanesulfonyl-β-D-threo-pentofuranosyl)thymine (**10**).*[43] Alcohol **9** (44.82 g, 83.8 mmol) was coevaporated with 1,2-DCE (100 mL) and then dissolved in anhydrous DCM (1250 mL). DMAP (20.47 g, 168 mmol) and anhydrous pyridine (70 mL, 865 mmol) were added and the resulting solution cooled to −70 °C with stirring. Triflic anhydride (19.9 mL, 117 mmol) was added dropwise over 20 min, and the reaction mixture was stirred at −70 °C for additional 10 min and then heated to 0 °C. After stirring for 4 h at 0 °C, aq HCl (2 M, 500 mL, 1.0 mol) was added, and the org phase extracted with DCM (100 mL). The combined org phase was washed with aq HCl (2 M, 500 mL, 1.0 mol) and the aq phase back-extracted with DCM (100 mL). The combined organic phase was washed with satd aq $NaHCO_3$ (200 mL) and the aq phase back-extracted with DCM (100 mL). The combined organic phase was dried over $MgSO_4$ and then adsorbed directly onto silica gel and purified by DCVC (EtOAc) to give the desired triflate **10** (53.96 g, 97%) as a white foam. TLC (10% ^{i}PrOH in $CHCl_3$, v/v): R_f (**9**) = 0.5, R_f (**10**) = 0.6. Analytical data were identical to those previously reported.[43]

*1-(2-Azido-3-O-benzyl-2-deoxy-2,5-di-O-methanesulfonyl-4-C-methanesulfonyloxymethyl-β-D-erythro-pentofuranosyl)thymine (**11**).*[43] Triflate **10** (53.96 g, 80.9 mmol) was dissolved in anhydrous DMF (400 mL) and NaN_3 (7.91 g, 122 mmol) was added at 0 °C. The reaction mixture was stirred at rt for 16 h and then slowly added to 10% brine (v/v, 3000 mL) which induced extensive precipitation. The precipitate was filtered off and washed with water (2 × 400 mL). The combined aqueous phase was extracted with EtOAc (2 × 500 mL). The precipitate was taken up in the organic phase which was washed with 10% brine (v/v, 2 × 300 mL), dried over $MgSO_4$ and evaporated to dryness to afford crude azide **11** (46.22 g, DMF as impurity) as a white foam. TLC (70% EtOAc in PE, v/v): R_f (**10**) = 0.3, R_f (**11**) = 0.25. Analytical data were identical to those previously reported.[43]

*1-(5-O-Methanesulfonyl-4-C-methanesulfonyloxymethyl-3-O-benzyl-2-deoxy-2-azido-β-D-erythro-pentofuranosyl)-4-azido-5-methylpyrimidin-2(1H)-one (**12**).* Alcohol **9** (5.19 g, 9.71 mmol) was coevaporated with pyridine (2 × 25 mL), then suspended in anh DCM (50 mL) and anh pyridine (16 mL, 198 mmol) and DMAP (4.73 g, 38.7 mmol) was added under stirring at 0 °C, resulting in a clear solution, followed by addition of triflic anhydride (3.3 mL, 19.4 mmol). After stirring for 2 h at 0 °C the reaction mixture was poured into sat aq $NaHCO_3$ (50 mL) and the organic phase washed consecutively with aq HCl (1.0 M, 2 × 50 mL) and sat aq $NaHCO_3$ (50 mL), then dried over $MgSO_4$, and evaporated to dryness to afford a residue which was dried over high vacuum for 48 h, then dissolved in anh DMF (50 mL) under argon. 15-crown-5 (1.93 mL, 9.72 mmol) and NaN_3 (694 mg, 10.7 mmol) was added, then the reaction mixture heated to 80 °C. After stirring for 19 h, the reaction mixture was poured into water (100 mL) and EtOAc (100 mL) was added. The organic phase was washed with sat. aq. $NaHCO_3$ (2 × 100 mL), then dried over $MgSO_4$, and evaporated to dryness. The resulting residue was purified by column chromatography (0−7% MeOH in DCM) to afford azide **11** (2.59 g, 48%) and diazide **12** (1.19 g, 21%) as white foams. Data for diazide **12**: TLC (70% EtOAc in PE): R_f (**10**) = 0.3, R_f (**11**) = 0.25, R_f (**12**) = 0.3; TLC (10% MeOH in DCM): R_f (**10**) = 0.3, R_f (**11**) = 0.3, R_f (**12**) = 0.5. ^{1}H NMR (DMSO-d_6) δ 7.64 (d, J = 1.2, 1H, H6), 7.31−7.47 (m, 5H, $H2_{Bn}$, $H3_{Bn}$, $H4_{Bn}$, $H5_{Bn}$, $H6_{Bn}$), 6.20 (d, J = 4.9, 1H, H1′), 4.83 (dd, J = 4.9, 6.5, 1H, H2′), 4.77 (s, 2H, CH_2Ph), 4.69 (d, J = 6.5, 1H, H3′), 4.58 (d, J = 11.4, 1H, $H5'_A$/$H5''_A$), 4.57 (d, J = 10.9, 1H, $H5'_A$/$H5''_A$), 4.51 (d, J = 10.9, 1H, $H5'_B$/$H5''_B$), 4.39 (d, J = 11.4, 1H, $H5'_B$/$H5''_B$), 3.33 (s, 3H, SCH_3), 3.23 (s, 3H, SCH_3), 2.31 (d, J = 1.2, 3H, 5-CH_3). ^{13}C NMR (DMSO-d_6) δ 151.8 (C4), 142.6 (C2), 137.0 ($C1_{Bn}$), 131.4 (C6), 128.4 ($C2_{Bn}$, $C6_{Bn}$), 128.1 ($C4_{Bn}$), 128.0 ($C3_{Bn}$, $C5_{Bn}$), 103.4 (C5), 88.5 (C1′), 84.2 (C4′), 78.2 (C3′), 73.9 (CH_2Ph), 68.0 (C5′/C5″), 67.7 (C5′/C5″), 63.5 (C2′), 37.0 (SCH_3), 36.9 (SCH_3), 12.5 (5-CH_3). ESI-HRMS m/z 585.1187 ($[M + H]^+$, $C_{20}H_{25}N_8O_9S_2^+$ Calcd 585.1180).

*(1R,3R,4R,7S)-7-Benzyloxy-1-methanesulfonyloxymethyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (**13**).*[43] Crude azide **11**

(46.22 g) was dissolved in THF (400 mL), and then aq NaOH (2M, 100 mL, 200 mmol) was added. The resulting mixture was cooled to 0 °C with stirring under an atmosphere of argon. PMe_3 in THF (1M, 100 mL, 100 mmol) was added slowly leading to distinct effervescence. The resulting mixture was allowed to heat to rt and stirred for 14 h. The reaction mixture was concentrated to approximately 100 mL and then 33% brine (v/v, 300 mL) and 20% MeOH in DCM (v/v, 800 mL) were added. The resulting mixture was neutralized by addition of aq HCl (2 M, 75 mL) and satd aq $NaHCO_3$ (10 mL). The phases were separated and the aq phase extracted with 20% MeOH in DCM (v/v, 2 × 200 mL). The combined org phase was evaporated to dryness affording crude derivative **13** (35.26 g, $POMe_3$ as impurity) as a slightly yellow solid which was used in the next step without purification. TLC (10% MeOH in EtOAc, v/v): R_f (**11**) = 0.7, R_f (intermediate) = 0.4, R_f (**13**) = 0.3. Analytical data were identical to those previously reported.[43]

*(1R,3R,4R,7S)-1-Benzoyloxymethyl-7-benzyloxy-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (**14**).*[43] A three-neck RB equipped with reflux condenser and mechanical stirring was charged with 18-crown-6 (25.47 g, 97 mmol). Crude mesylate **13** (35.26 g) was suspended in dioxane (800 mL) and added to the crown ether with stirring. KOBz (25.82 g, 161 mmol) was added and the reaction mixture heated at 80 °C for 18 h. The reaction mixture was cooled to rt, evaporated to dryness and to the resulting residue was added EtOAc (500 mL) and water (500 mL). The aq phase was extracted with EtOAc (2 × 100 mL) and the combined organic phase dried over $MgSO_4$ and evaporated to dryness. The resulting residue was purified by DCVC (0−6% MeOH in DCM, v/v) to afford the desired benzoate ester **14** (35.35 g, 94% from **10**) as a white foam. TLC (10% ^{i}PrOH in $CHCl_3$, v/v): R_f (**13**) = 0.2, R_f (**14**) = 0.3. Analytical data were identical to those previously reported.[43]

*(1R,3R,4R,7S)-7-Benzyloxy-1-hydroxymethyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (**15**).*[43] Benzoate ester **14** (35.35 g, 76.3 mmol) was dissolved in freshly prepared methanolic ammonia (760 mL) at 0 °C and stirred at 6 °C for 4 days. Silica gel (∼100 mL) was added and the reaction mixture was then evaporated to dryness. The residue was purified by DCVC (0−10% MeOH in DCM, v/v) to afford the desired alcohol **15** (25.64g, 94%) as a white foam. TLC (10% MeOH in EtOAc, v/v): R_f (**14**) = 0.3, R_f (**15**) = 0.2. Analytical data were identical to those previously reported.[43]

*(1R,3R,4R,7S)-7-Benzyloxy-1-hydroxymethyl-3-(thymin-1-yl)-5-trifluoroacetyl-2-oxa-5-azabicyclo[2.2.1]heptane (**16**).* Amino alcohol **15** (25.68 g, 71.5 mmol) was coevaporated with pyridine (50 mL) and then dissolved in a mixture of anhydrous pyridine (180 mL) and anhydrous DCM (180 mL). After cooling to −70 °C with stirring, trifluoroacetic anhydride (22.5 mL, 159 mmol) was added dropwise over 30 min and the resulting mixture was stirred at −70 °C for 10 min, whereafter the reaction mixture was heated to 0 °C and further stirred for 30 min. The reaction mixture was evaporated to dryness and the residue coevaporated with toluene (2 × 50 mL). To the resulting residue was added DCM (300 mL) and satd aq $NaHCO_3$ (175 mL), and the resulting biphasic mixture was then stirred at rt for 10 min which resulted in distinct effervescence. The two phases were separated and the org phase washed with satd aq $NaHCO_3$ (175 mL). The resulting biphasic mixture was stirred for 10 min and then separated. The combined aq phase was extracted with DCM (2 × 50 mL), and the combined organic phase then evaporated to dryness. The residue was coevaporated with pyridine to afford a rotameric mixture of crude alcohol **16** (40:60 ratio according to ^{1}H NMR) as a brown foam which was used without further purification in the next step. TLC (10% MeOH in DCM, v/v): R_f (**15**) = 0.2, R_f (**16**) = 0.5. Found: C, 52.4; H, 4.4; N, 9.1. Calcd for $C_{20}H_{20}N_3O_6F_3$: C, 52.7; H, 4.4; N, 9.1. ^{1}H NMR (DMSO-d_6) δ 11.39 (s, $1H_{II}$, ex, N3−H), 11.37 (s, $1H_I$, N3−H), 7.68 (d, J = 0.9, $1H_I$, H6), 7.66 (d, J = 0.9, $1H_{II}$, H6), 7.26−7.35 (m, 3H, $H3_{Bn}$, $H4_{Bn}$, $H5_{Bn}$), 7.21−7.25 (m, 2H, $H2_{Bn}$, $H6_{Bn}$), 5.66 (s, 1H, H1′), 5.45−5.52 (m, 1H, ex, 5′−OH), 5.09 (s, $1H_{II}$, H2′), 4.92 (s, $1H_I$, H2′), 4.63 (d, J = 11.8, $1H_I$, CH_2Ph_A), 4.62 (d, J = 11.7, 1H, $1H_{II}$, CH_2Ph_A), 4.56 (d, J = 11.8, $1H_I$, CH_2Ph_B), 4.53 (d, J = 11.7, 1H, $1H_{II}$, CH_2Ph_B), 4.18 (s, $1H_I$, H3′), 4.17 (s, $1H_{II}$, H3′), 3.77−3.91 (m, 2H, $H5'_{A+B}$), 3.75 (d, J = 10.0, $1H_{II}$, $H5''_A$), 3.67 (d, J = 10.0, $1H_{II}$, $H5''_B$), 3.59 (d, J = 11.6, $1H_I$, $H5''_A$), 3.42 (d, J = 11.6, $1H_I$, $H5''_B$), 1.79 (d, J = 0.9, 3H, 5-CH_3). ^{13}C NMR (DMSO-d_6) δ 164.0 ($C4_I$), 163.9 ($C4_{II}$), 155.0 (q, J = 36.4, $COCF_{3,II}$), 154.9 (q, J = 36.8, $COCF_{3,I}$), 149.86 ($C2_{II}$), 149.85 ($C2_I$), 137.44 ($C1_{Bn,II}$), 137.42 ($C1_{Bn,I}$), 134.7 ($C6_{II}$), 134.6 ($C6_I$), 128.30 ($C3_{Bn,I}$, $C5_{Bn,I}$), 128.27 ($C3_{Bn,II}$, $C5_{Bn,II}$), 127.7 ($C4_{Bn}$), 127.3 ($C2_{Bn,II}$, $C6_{Bn,II}$), 127.2 ($C2_{Bn,I}$, $C6_{Bn,I}$), 115.9 (q, J = 288.5, $CF_{3,II}$), 115.8 (q, J = 287.8, $CF_{3,I}$), 108.4 ($C5_{II}$), 108.3 ($C5_I$), 88.5 ($C4'_{II}$), 87.1 ($C4'_I$), 85.6 ($C1'_I$), 85.1 ($C1'_{II}$), 75.7 ($C3'_I$), 74.2 ($C3'_{II}$), 71.44 (CH_2Ph_{II}), 71.43 (CH_2Ph_I), 61.9 ($C2'_I$), 60.3 ($C2'_{II}$), 56.4 (C5′), 52.6 ($C5''_I$), 52.4 ($C5''_{II}$), 12.50 (5-$CH_{3,I}$), 12.47 (5-$CH_{3,II}$). ^{19}F NMR (DMSO-d_6) δ −70.1 ($CF_{3,I}$), −71.6 ($CF_{3,II}$). ESI-HRMS m/z 456.1386 ($[M + H]^+$, $C_{20}H_{21}F_3N_3O_6^+$ Calcd 456.1377).

*(1R,3R,4R,7S)-7-Benzyloxy-1-(4,4′-dimethoxytrityl)oxymethyl-3-(thymin-1-yl)-5-trifluoroacetyl-2-oxa-5-azabicyclo[2.2.1]heptane (**17**).* Crude alcohol **16** was dissolved in a mixture of anhydrous pyridine (180 mL) and anhydrous DCM (180 mL). DMTCl (29.03 g, 85.7 mmol) was added and the resulting mixture was stirred for 18 h at rt. MeOH (5 mL) was added and the reaction mixture evaporated to dryness. The resulting residue was coevaporated with toluene (2 × 50 mL) whereupon DCM (300 mL) was added. After washing with satd aq $NaHCO_3$ (150 mL) and brine (150 mL), the combined aqueous phase was extracted with DCM (2 × 50 mL). The combined organic phase was evaporated to dryness and the resulting residue purified by DCVC (0−4% MeOH in DCM, v/v) to afford a rotameric mixture of the desired nucleoside **17** (51.64 g, 95% from **15**, 2:3 ratio between rotamers according to ^{1}H NMR) as a yellow foam. TLC (5% ^{i}PrOH in $CHCl_3$, v/v): R_f (**16**) = 0.1, R_f (**17**) = 0.5. Found: C, 64.6; H, 5.1; N, 5.6. Calcd for $C_{41}H_{38}F_3N_3O_8$: C, 65.0; H, 5.1; N, 5.5. ^{1}H NMR (DMSO-d_6) δ 11.46 (s, $1H_{II}$, ex, N3−H), 11.44 (s, $1H_I$, ex, N3−H), 7.56 (d, J = 1.2, $1H_{II}$, C6), 7.55 (d, J = 1.2, $1H_I$, C6), 7.34−7.38 (m, 2H, $H2''_{DMT}$, $H6''_{DMT}$), 7.28−7.33 (m, 5H, $H3''_{DMT}$, $H5''_{DMT}$, $H3_{Bn}$, $H4_{Bn}$, $H5_{Bn}$), 7.22−7.27 (m, 5H, $H2_{DMT}$, $H6_{DMT}$, $H2'_{DMT}$, $H6'_{DMT}$, $H4''_{DMT}$), 7.18−7.22 (m, 2H, $H2_{Bn}$, $H6_{Bn}$), 6.84−6.91 (m 4H, $H3_{DMT}$, $H5_{DMT}$, $H3'_{DMT}$, $H5'_{DMT}$), 5.700 (s, $1H_I$, H1′), 5.679 (s, $1H_{II}$, H1′), 5.22 (s, $1H_{II}$, H2′), 5.06 (s, $1H_I$, H2′), 4.63 (d, J = 11.9, $1H_I$, CH_2Ph_A), 4.62 (d, J = 11.9, 1H, $1H_{II}$, CH_2Ph_A), 4.57 (d, J = 11.9, 1H, CH_2Ph_B), 4.45 (s, 1H, H3′), 3.733 (s, $3H_I$, OCH_3), 3.729 (s, $3H_{II}$, OCH_3), 3.723 (s, $3H_I$, OCH_3), 3.720 (s, $3H_{II}$, OCH_3), 3.61−3.70 (m, 1H, $H5''_A$), 3.41−3.52 (m, 3H, $H5'_{A+B}$, $H5''_B$), 1.47−1.50 (m, 3H, 5-CH_3). ^{13}C NMR (DMSO-d_6) δ 163.94 ($C4_I$), 163.86 ($C4_{II}$), 158.24 ($C4_{DMT,I}$, $C4'_{DMT,I}$), 158.20 ($C4_{DMT,II}$, $C4'_{DMT,II}$), 155.0 (q, J = 36.6, $COCF_{3,II}$), 154.8 (q, J = 37.1, $COCF_{3,I}$), 149.81 ($C2_{II}$), 149.80 ($C2_I$), 144.4 ($C1''_{DMT}$), 137.1 ($C1_{Bn,II}$), 137.0 ($C1_{Bn,I}$), 134.88 ($C1_{DMT,I}$/$C1'_{DMT,I}$), 134.86 ($C1_{DMT,II}$/$C1'_{DMT,II}$), 134.77 ($C1_{DMT,II}$/$C1'_{DMT,II}$), 134.76 ($C1_{DMT,I}$/$C1'_{DMT,I}$), 133.92 ($C6_{II}$), 133.86 ($C6_I$), 129.7 ($C2_{DMT}$, $C6_{DMT}$, $C2'_{DMT}$, $C6'_{DMT}$), 128.33 ($C3_{Bn,I}$, $C5_{Bn,I}$), 128.29 ($C3_{Bn,II}$, $C5_{Bn,II}$), 128.0 ($C3''_{DMT}$, $C5''_{DMT}$), 127.9 ($C4_{Bn,I}$), 127.8 ($C4_{Bn,II}$), 127.53 ($C2_{Bn,II}$, $C6_{Bn,II}$, $C2''_{DMT,II}$, $C6''_{DMT,II}$), 127.51 ($C2_{Bn,I}$, $C6_{Bn,I}$, $C2''_{DMT,I}$, $C6''_{DMT,I}$), 126.9 ($C4''_{DMT}$), 115.80 (q, J = 288.7, $CF_{3,II}$), 115.76 (q, J = 287.3, $CF_{3,I}$), 113.38 ($C3_{DMT,I}$, $C5_{DMT,I}$/$C3'_{DMT,I}$, $C5'_{DMT,I}$), 113.36 ($C3_{DMT,II}$, $C5_{DMT,II}$/$C3'_{DMT,II}$, $C5'_{DMT,II}$), 113.34 ($C3_{DMT,I}$, $C5_{DMT,I}$/$C3'_{DMT,I}$, $C5'_{DMT,I}$), 113.33 ($C3_{DMT,II}$, $C5_{DMT,II}$/$C3'_{DMT,II}$, $C5'_{DMT,II}$), 108.7 ($C5_{II}$), 108.6 ($C5_I$), 87.1 ($C4'_{II}$), 86.00 ($CAr_{3,II}$), 85.97 ($CAr_{3,I}$), 85.9 ($C1'_I$), 85.6 ($C4'_I$), 85.4 ($C1'_{II}$), 75.8 ($C3'_I$), 74.3 ($C3'_{II}$), 71.3 (CH_2Ph_{II}), 71.2 (CH_2Ph_I), 61.7 ($C2'_I$), 60.1 ($C2'_{II}$), 58.5 ($C5'_I$), 58.4 ($C5'_{II}$), 55.0 (OCH_3), 52.9 ($C5''_I$), 52.7 ($C5''_{II}$), 12.47 (5-$CH_{3,I}$), 12.45 (5-$CH_{3,II}$). ^{19}F NMR (DMSO-d_6) δ −70.1 ($CF_{3,I}$), −71.6 ($CF_{3,II}$). ESI-HRMS m/z 780.2507 ($[M + Na]^+$, $C_{41}H_{38}F_3N_3O_8Na^+$ Calcd 780.2503).

*(1R,3R,4R,7S)-7-Hydroxy-1-(4,4′-dimethoxytrityl)oxymethyl-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (**18**).* Nucleoside **17** (20.04 g, 26.4 mmol) was dissolved in MeOH (500 mL) and the resulting mixture was evacuated and an atmosphere of nitrogen was applied. Evacuation and nitrogen atmosphere application was repeated three times. 20% $Pd(OH)_2$/C (1.39 g) and ammonium formate (11.63 g, 184 mmol) were added and the reaction heated under reflux with stirring for 3 h. The reaction mixture was cooled to rt and filtered through a short Celite pad. The Celite pad was washed thoroughly with MeOH (3 × 50 mL) and DCM (3 × 50 mL) and the resulting

combined mixture was evaporated to dryness. The resulting residue was suspended in MeOH (100 mL) and filtered through a glass filter. The white precipitate was washed with cold MeOH (2 × 100 mL) and dried under high vacuum to afford the desired amino alcohol **18** (10.37 g) as a white solid. The methanolic phase was evaporated to dryness affording a white solid, which was purified by DCVC (0−12% MeOH in DCM, v/v) to afford the desired amino alcohol **18** (4.21 g) as a white solid (total yield of amino alcohol **18**: 14.58 g, 96%). TLC (10% MeOH in DCM, v/v): R_f (**17**) = 0.5, R_f (**18**) = 0.3. ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H, ex, N3−H), 7.62 (d, J = 0.9, 1H, H-6), 7.42−7.45 (m, 2H, H2″$_{DMT}$, H6″$_{DMT}$), 7.28−7.35 (m, 6H, H2$_{DMT}$, H6$_{DMT}$, H2′$_{DMT}$, H6′$_{DMT}$, H3″$_{DMT}$, H5″$_{DMT}$), 7.22−7.27 (m, 1H, H4″$_{DMT}$), 6.89−6.92 (m, 4H, H3$_{DMT}$, H5$_{DMT}$, H3′$_{DMT}$, H5′$_{DMT}$), 5.40 (d, J = 4.5, 1H, ex, 3′−OH), 5.35 (s, 1H, H1′), 4.06 (d, J = 4.5, 1H, H3′), 3.74 (s, 6H, OCH$_3$), 3.35 (d, J = 10.8, 1H, H5′$_A$), 3.31 (s, 1H, H2′), 3.28 (d, J = 10.8, H5′$_B$), 2.84 (d, J = 9.9, 1H, H5″$_A$), 2.63 (d, J = 9.9, 1H, H5″$_B$), 1.50 (d, J = 0.9, 3H, 5-CH$_3$). ^{13}C NMR (DMSO-d_6) δ 163.8 (C4), 158.2 (C4$_{DMT}$, C4′$_{DMT}$), 149.9 (C2), 144.8 (C1″$_{DMT}$), 135.5 (C1$_{DMT}$/C1′$_{DMT}$), 135.2 (C1$_{DMT}$/C1′$_{DMT}$), 134.7 (C6), 129.8 (C2$_{DMT}$, C6$_{DMT}$/C2′$_{DMT}$, C6′$_{DMT}$), 129.7 (C2$_{DMT}$, C6$_{DMT}$/C2′$_{DMT}$, C6′$_{DMT}$), 127.9 (C3″$_{DMT}$, C5″$_{DMT}$), 127.7 (C2″$_{DMT}$, C6″$_{DMT}$), 126.8 (C4″$_{DMT}$), 113.26 (C3$_{DMT}$, C5$_{DMT}$/C3′$_{DMT}$, C5′$_{DMT}$), 113.25 (C3$_{DMT}$, C5$_{DMT}$/C3′$_{DMT}$, C5′$_{DMT}$), 108.1 (C5), 88.1 (C1′), 87.9 (C4′), 85.6 (CAr$_3$), 69.5 (C3′), 61.9 (C2′), 59.7 (C5′), 55.0 (OCH$_3$), 49.9 (C5″), 12.3 (5-CH$_3$). ESI-HRMS m/z 572.2375 ([M + H]$^+$, $C_{32}H_{34}N_3O_7^+$ Calcd 572.2391).

*(1R,3R,4R,7S)-7-Benzyloxy-1-(4,4′-dimethoxytrityl)oxymethyl-3-(5-methylcytosin-1-yl)-5-trifluoroacetyl-2-oxa-5-azabicyclo[2.2.1]-heptane (**19**).* Thymine nucleoside **17** (5.01 g, 6.61 mmol) was coevaporated with MeCN (20 mL) and then dissolved in anhydrous MeCN (120 mL). Anhydrous Et$_3$N (16.0 mL, 114 mmol) and 1,2,4-triazole (5.46 g, 79.1 mmol) were added and the resulting mixture was cooled to 0 °C with stirring. Freshly distilled phosphorus oxychloride (1.85 mL, 19.8 mmol) was added dropwise and after stirring for 10 min at 0 °C the reaction mixture was heated to r.t and stirred for additional 60 min when analytical TLC indicated full conversion to a fluorescent intermediate tentatively assigned as 1-((1*R*,3*R*,4*R*,7*S*)-7-benzyloxy-1-(4,4′-dimethoxytrityl)oxymethyl-5-trifluoroacetyl-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl)-5-methyl-4-(1*H*-1,2,4-triazol-1-yl)-pyrimidin-2(1*H*)-one [TLC (50% EtOAc in PE, v/v): R_f (**17**) = 0.3, R_f (intermediate) = 0.1]. The reaction mixture was partitioned between half satd aq NaHCO$_3$ (200 mL) and EtOAc (200 mL). The aq phase was extracted with EtOAc (50 mL) and the combined org phase washed with brine (50 mL) and dried over MgSO$_4$ followed by evaporation to dryness. The resulting residue was dissolved in THF (60 mL) and satd aq NH$_3$ (2.8 mL) was added. After stirring for 60 min, the reaction mixture was evaporated to dryness and the resulting residue purified by DCVC (0−7% MeOH in DCM, v/v) to afford a rotameric mixture of 5-methylcytosine nucleoside **19** (5.01 g, quantitative yield, 2:3 ratio of rotamers according to ^{1}H NMR) as a white foam. TLC (5% MeOH in DCM, v/v): R_f (intermediate) = 0.4, R_f (**19**) = 0.2. ^{1}H NMR (DMSO-d_6) δ 7.56 (s, 1H, H6), 7.36−7.40 (m, 2H, H2″$_{DMT}$, H6″$_{DMT}$), 7.22−7.34 (m, 10H, H3$_{Bn}$, H4$_{Bn}$, H5$_{Bn}$, H2$_{DMT}$, H6$_{DMT}$, H2′$_{DMT}$, H6′$_{DMT}$, H3″$_{DMT}$, H4″$_{DMT}$, H5″$_{DMT}$), 7.15−7.19 (m, 2H, H2$_{Bn}$, H6$_{Bn}$), 6.85−6.92 (m, 4H, H3$_{DMT}$, H5$_{DMT}$, H3′$_{DMT}$, H5′$_{DMT}$), 5.664 (s, 1H$_{II}$, H1′), 5.655 (s, 1H$_I$, H1′), 5.20 (s, 1H$_{II}$, H2′), 5.04 (s, 1H$_I$, H2′), 4.62 (d, J = 11.8, 1H$_I$, CH_2Ph$_A$), 4.60 (d, J = 11.7, 1H$_{II}$, CH_2Ph$_A$), 4.53 (d, J = 11.8, 1H, CH_2Ph$_B$), 4.42 (s, 1H$_I$, H3′), 4.41 (s, 1H$_{II}$, H3′), 3.732 (s, 3H$_I$, OCH$_3$), 3.728 (s, 3H$_{II}$, OCH$_3$), 3.725 (s, 3H$_I$, OCH$_3$), 3.72 (s, 3H$_{II}$, OCH$_3$), 3.61−3.70 (m, 1H, H5″$_A$), 3.41−3.53 (m, 3H, H5′$_{A+B}$, H5″$_B$), 1.56 (s, 3H$_{II}$, 5-CH$_3$), 1.55 (s, 3H$_I$, 5-CH$_3$). ^{13}C NMR (DMSO-d_6) δ 165.7 (C4$_I$), 165.6 (C4$_{II}$), 158.23 (C4$_{DMT,I}$, C4′$_{DMT,I}$), 158.21 (C4$_{DMT,II}$, C4′$_{DMT,II}$), 155.1 (q, J = 37.5, COCF$_{3,I}$), 154.9 (q, J = 36.4, COCF$_{3,II}$), 154.6 (C2$_I$), 154.5 (C2$_{II}$), 144.36 (C1″$_{DMT,II}$), 144.35 (C1″$_{DMT,I}$), 137.02 (C1$_{Bn,II}$), 136.95 (C1$_{Bn,I}$), 136.3 (C6), 135.0 (C1$_{DMT,I}$/C1′$_{DMT,I}$), 134.9 (C1$_{DMT,II}$/C1′$_{DMT,II}$), 134.9 (C1$_{DMT,II}$/C1′$_{DMT,II}$), 134.8 (C1$_{DMT,I}$/C1′$_{DMT,I}$), 129.7 (C2$_{DMT}$, C6$_{DMT}$, C2′$_{DMT}$, C6′$_{DMT}$), 128.32 (C3$_{Bn,I}$, C5$_{Bn,I}$), 128.29 (C3$_{Bn,II}$, C5$_{Bn,II}$), 128.0 (C3″$_{DMT}$, C5″$_{DMT}$), 127.9 (C4$_{Bn,I}$), 127.8 (C4$_{Bn,II}$), 127.6 (C2″$_{DMT}$, C6″$_{DMT}$), 127.5 (C2$_{Bn}$, C6$_{Bn}$), 126.9 (C4″$_{DMT}$), 115.85 (q, J = 288.7, CF$_{3,II}$), 115.78 (q, J = 287.9, CF$_{3,I}$), 113.39 (C3$_{DMT,I}$, C5$_{DMT,I}$/C3′$_{DMT,I}$, C5′$_{DMT,I}$), 113.37 (C3$_{DMT,I}$, C5$_{DMT,I}$/C3′$_{DMT,I}$, C5′$_{DMT,I}$), 113.35 (C3$_{DMT,II}$, C5$_{DMT,II}$, C3′$_{DMT,II}$, C5′$_{DMT,II}$), 101.10 (C5$_{II}$), 101.06 (C5$_I$), 86.8 (C4′$_{II}$), 86.4 (C1′$_I$), 86.01 (C1′$_{II}$), 85.99 (CAr$_{3,II}$), 85.96 (CAr$_{3,I}$), 85.4 (C4′$_I$), 75.6 (C3′$_I$), 74.1 (C3′$_{II}$), 71.35 (CH$_2$Ph$_{II}$), 71.28 (CH$_2$Ph$_I$), 61.6 (C2′$_I$), 60.0 (C2′$_{II}$), 58.54 (C5′$_I$), 58.45 (C5′$_{II}$), 55.0 (OCH$_3$), 52.9 (C5″$_I$), 52.7 (C5″$_{II}$), 13.59 (5-CH$_{3,I}$), 13.57 (5-CH$_{3,II}$). ^{19}F NMR (DMSO-d_6) δ −69.9 (CF$_{3,I}$), −71.6 (CF$_{3,II}$) ESI-HRMS m/z 757.2872 ([M + H]$^+$, $C_{41}H_{40}F_3N_4O_7^+$ Calcd 757.2844).

*(1R,3R,4R,7S)-7-Hydroxy-1-(4,4′-dimethoxytrityl)oxymethyl-3-(5-methylcytosin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (**20**).* Thymine nucleoside **17** (19.05 g, 25.1 mmol) was converted to crude 5-methylcytosine nucleoside **19** using the conditions described above, but proceeding with the following step without purification. Crude nucleoside **19** was dissolved in MeOH (500 mL) followed by evacuation and introduction of an N$_2$ atmosphere. Evacuation and nitrogen atmosphere application was repeated three times. 20% Pd(OH)$_2$/C (1.46 g) and ammonium formate (11.34 g, 180 mmol) were added with stirring and the reaction mixture was heated under reflux for 2.5 h. Then the reaction mixture was cooled to rt and filtered through a short Celite pad. The Celite pad was washed thoroughly with MeOH (3 × 50 mL) and DCM (3 × 50 mL) and the resulting mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (10−18% MeOH in DCM, v/v) affording a light yellow solid (9.88 g). This solid was dissolved in 2% MeOH in CHCl$_3$ (v/v, 500 mL) and the org. phase was washed with aq K$_2$CO$_3$ (0.1 M, 1.0 L). The aq phase was back-extracted with CHCl$_3$ (6 × 100 mL) and 10% MeOH in CHCl$_3$ (v/v, 2 × 100 mL). The combined org phase was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (10−20% MeOH in DCM, v/v) affording the desired nucleoside **20** (8.37 g, 58%), as a white foam. TLC (20% MeOH in DCM, v/v): R_f (**19**) = 0.8, R_f (**20**) = 0.1. ^{1}H NMR (DMSO-d_6) δ 7.59 (s, 1H, H6), 7.42−47 (m, 2H, H2″$_{DMT}$, H6″$_{DMT}$), 7.37−7.22 (m, 7H, H2$_{DMT}$, H6$_{DMT}$, H2′$_{DMT}$, H6′$_{DMT}$, H3″$_{DMT}$, H4″$_{DMT}$, H5″$_{DMT}$), 6.95−6.88 (m, 4H, H3$_{DMT}$, H5$_{DMT}$, H3′$_{DMT}$, H5′$_{DMT}$), 6.75 (br s, 1H, NH), 5.38 (d, J = 4.1, 1H, 3′−OH), 5.34 (s, 1H, H1′), 4.03 (d, J = 4.1, 1H, H3′), 3.74 (s, 6H, OCH$_3$), 3.35−3.26 (m, 3H, H2′, H5′$_{A+B}$), 2.83 (d, J = 9.9, 1H, H5″$_A$), 2.64 (d, J = 9.9, 1H, H5″$_B$), 1.56 (s, 3H, 5-CH$_3$). ^{13}C NMR (DMSO-d_6) δ 165.4 (C4), 158.1 (C4$_{DMT}$, C4′$_{DMT}$), 154.8 (C2), 144.8 (C1″$_{DMT}$), 137.0 (C6), 135.5 (C1$_{DMT}$/C1′$_{DMT}$), 135.3 (C1$_{DMT}$/C1′$_{DMT}$), 129.8 (C2$_{DMT}$, C6$_{DMT}$/C2′$_{DMT}$, C6′$_{DMT}$), 129.7 (C2$_{DMT}$, C6$_{DMT}$/C2′$_{DMT}$, C6′$_{DMT}$), 127.9 (C3″$_{DMT}$, C5″$_{DMT}$), 127.8 (C2″$_{DMT}$, C6″$_{DMT}$), 126.8 (C4″$_{DMT}$), 113.3 (C3$_{DMT}$, C5$_{DMT}$, C3′$_{DMT}$, C5′$_{DMT}$), 100.2 (C5), 88.3 (C1′), 87.5 (C4′), 85.6 (CAr$_3$), 69.3 (C3′), 61.8 (C2′), 59.7 (C5′), 55.0 (OCH$_3$), 50.0 (C5″), 13.4 (5-CH$_3$). ESI-HRMS m/z 571.2562 ([M + H]$^+$, $C_{32}H_{35}N_4O_6^+$ Calcd 571.2551).

*(1R,3R,4R,7S)-7-Benzyloxy-1-(4,4′-dimethoxytrityl)oxymethyl-3-(4-N-benzoyl-5-methylcytosin-1-yl)-5-trifluoroacetyl-2-oxa-5-azabicyclo[2.2.1]heptane (**21a**).* Nucleoside **19** (4.99 g, 6.59 mmol) was coevaporated with pyridine (2 × 10 mL) and then dissolved in anhydrous pyridine (60 mL) at rt. BzCl (1.00 mL, 8.56 mmol) was added with stirring and the reaction mixture was stirred for 20 h. Then BzCl (1.00 mL, 8.61 mmol) was added and stirring was continued for additional 4 h whereupon the reaction mixture was evaporated to dryness. DCM (200 mL) was added and washed was performed with satd aq NaHCO$_3$ (100 mL) and brine (100 mL). The org phase was separated, dried over MgSO$_4$ and evaporated to dryness. The resulting residue was purified by DCVC (0−50% [30% EtOAc in PE, v/v] in [50% DCM in PE, v/v], v/v), to afford a rotameric mixture of the desired protected nucleoside **21a** (4.22 g, 74%, 2:3 ratio of rotamers according to ^{1}H NMR) as a white foam. TLC (5% MeOH in CHCl$_3$, v/v): R_f (**19**) = 0.2, R_f (**21a**) = 0.8. ^{1}H NMR (DMSO-d_6) δ 13.14 (br s, 1H, ex, 4-NH), 8.18−8.25 (m, 2H, H2$_{Bz}$, H6$_{Bz}$), 7.82 (s, 1H, H6), 7.57−7.63 (m, 1H, H4$_{Bz}$), 7.48−7.54 (m, 2H, H3$_{Bz}$, H5$_{Bz}$), 7.36−7.41 (m, 2H, H2″$_{DMT}$, H6″$_{DMT}$), 7.24−7.35 (m, 10H, H3$_{Bn}$, H4$_{Bn}$, H5$_{Bn}$, H2$_{DMT}$, H6$_{DMT}$, H2′$_{DMT}$, H6′$_{DMT}$, H3″$_{DMT}$, H4″$_{DMT}$, H5″$_{DMT}$), 7.18−7.23 (m, 2H, H2$_{Bn}$, H6$_{Bn}$), 6.85−6.93 (m, 4H, H3$_{DMT}$, H5$_{DMT}$, H3′$_{DMT}$, H5′$_{DMT}$), 5.80 (s, 1H$_I$, H1′), 5.79 (m, 1H$_{II}$, H1′), 5.30 (s,

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$1H_{II}$, H2′), 5.15 (s, $1H_I$, H2′), 4.63 (d, $1H_I$, J = 12.0, CH_2Ph_A), 4.62 (d, $1H_{II}$, J = 12.0, CH_2Ph_A), 4.58 (d, 1H, J = 12.0, CH_2Ph_B), 4.50 (s, 1H, H3′), 3.744 (s, $3H_I$, OCH_3), 3.741 (s, $3H_{II}$, OCH_3), 3.733 (s, $3H_I$, OCH_3), 3.729 (s, $3H_{II}$, OCH_3), 3.64−3.73 (m, 1H, H5″$_A$), 3.46−3.55 (m, 3H, H5′$_{A+B}$, H5″$_B$), 1.733 (s, $3H_I$, 5-CH_3), 1.725 (s, $3H_{II}$, 5-CH_3). ^{13}C NMR (DMSO-d_6) δ 178.2 (COPh), 159.2 (C4), 158.3 ($C4_{DMT}$/$C4'_{DMT}$), 158.2 ($C4_{DMT}$/$C4'_{DMT}$), 155.0 (q, J = 36.5, $COCF_{3,I}$), 154.8 (q, J = 37.0, $COCF_{3,II}$), 146.9 (C2), 144.4 ($C1''_{DMT}$), 137.1 ($C1_{Bn,II}$), 137.0 ($C1_{Bn,I}$), 136.7 (C6, $C1_{Bz}$), 134.84 ($C1_{DMT,I}$/$C1'_{DMT,I}$), 134.83 ($C1_{DMT,II}$/$C1'_{DMT,II}$), 134.75 ($C1_{DMT,II}$/$C1'_{DMT,II}$), 134.73 ($C1_{DMT,I}$/$C1'_{DMT,I}$), 132.6 ($C4_{Bz}$), 129.72 ($C2_{DMT,II}$, $C6_{DMT,II}$, $C2'_{DMT,II}$, $C6'_{DMT,II}$), 129.69 ($C2_{DMT,I}$, $C6_{DMT,I}$, $C2'_{DMT,I}$, $C6'_{DMT,I}$), 129.4 ($C2_{Bz}$, $C6_{Bz}$), 128.33 ($C3_{Bz}$, $C5_{Bz}$, $C3_{Bn,I}$, $C5_{Bn,I}$), 128.29 ($C3_{Bn,II}$, $C5_{Bn,II}$), 128.1 ($C3''_{DMT}$, $C5''_{DMT}$), 127.9 ($C4_{Bn,I}$), 127.8 ($C4_{Bn,II}$), 127.5 ($C2''_{DMT}$, $C6''_{DMT}$), 127.4 ($C2_{Bn}$, $C6_{Bn}$), 126.9 ($C4''_{DMT}$), 115.8 (q, J = 288.5, $CF_{3,II}$), 115.7 (q, J = 287.7, $CF_{3,I}$), 113.40 ($C3_{DMT,I}$, $C5_{DMT,I}$/$C3'_{DMT,I}$, $C5'_{DMT,I}$), 113.38 ($C3_{DMT,II}$, $C5_{DMT,II}$/$C3'_{DMT,II}$, $C5'_{DMT,II}$), 113.36 ($C3_{DMT,I}$, $C5_{DMT,I}$/$C3'_{DMT,I}$, $C5'_{DMT,I}$), 113.35 ($C3_{DMT,II}$, $C5_{DMT,II}$/$C3'_{DMT,II}$, $C5'_{DMT,II}$), 109.4 (C5), 87.4 ($C4'_{II}$), 86.3 ($C1'_{II}$), 86.06 ($CAr_{3,II}$), 86.03 ($CAr_{3,I}$), 85.98 ($C4'_I$), 75.8 ($C3'_I$), 74.3 ($C3'_{II}$), 71.4 (CH_2Ph_{II}), 71.3 (CH_2Ph_I), 61.3 ($C2'_I$), 59.8 ($C2'_{II}$), 58.5 ($C5'_I$), 58.4 ($C5'_{II}$), 55.0 (OCH_3), 52.9 ($C5''_I$), 52.8 ($C5''_{II}$), 13.4 (5-CH_3).[52] ^{19}F NMR (DMSO-d_6) δ −70.1 ($CF_{3,II}$), −71.5 ($CF_{3,I}$). ESI-HRMS m/z 861.3104 ([M + H]$^+$, $C_{48}H_{44}F_3N_4O_8^+$ Calcd 861.3106).

*(1R,3R,4R,7S)-7-Benzyloxy-1-(4,4′-dimethoxytrityl)oxymethyl-3-(4-N-acetyl-5-methylcytosin-1-yl)-5-trifluoroacetyl-2-oxa-5-azabicyclo[2.2.1]heptane (**21b**).* Nucleoside **19** (1.00 g, 1.32 mmol) was coevaporated with pyridine (3 mL) and then dissolved in a mixture of anhydrous pyridine (7 mL) and anhydrous DCM (7 mL). Ac_2O (0.14 mL, 1.48 mmol) was added. The reaction mixture stirred at rt for 24 h, whereafter Ac_2O (0.07 mL, 0.74 mmol) and DMAP (79 mg, 0.65 mmol) were added, and the reaction mixture stirred for additional 72 h. Ac_2O (0.07 mL, 0.74 mmol) was added, and the reaction mixture stirred for additional 24 h, whereafter the reaction mixture was evaporated to dryness and the residue coevaporated with toluene (3 × 10 mL). To the resulting residue was added DCM (50 mL) whereupon washing was performed with satd aq $NaHCO_3$ (50 mL) and brine (50 mL). The combined aq phase was extracted with DCM (25 mL), and the combined organic phase dried over $MgSO_4$ and evaporated to dryness. The resulting residue was purified by DCVC (0−6% MeOH in DCM, v/v) to afford a rotameric mixture of the desired nucleoside **21b** (868 mg, 82%, 2:3 ratio of rotamers according to ^{1}H NMR) as a white foam. TLC (5% MeOH in EtOAc, v/v): R_f (**19**) = 0.1, R_f (**21b**) = 0.6. ^{1}H NMR (DMSO-d_6) δ 9.90 (s, 1H, ex, 4-NH), 7.85−7.88 (m, 1H, H6), 7.36−7.40 (m, 2H, H2″$_{DMT}$, H6″$_{DMT}$), 7.23−7.43 (m, 10H, $H3_{Bn}$, $H4_{Bn}$, $H5_{Bn}$, $H2_{DMT}$, $H6_{DMT}$, H2′$_{DMT}$, H6′$_{DMT}$, H3″$_{DMT}$, H4″$_{DMT}$, H5″$_{DMT}$), 7.14−7.19 (m, 2H, $H2_{Bn}$, $H6_{Bn}$), 6.85−6.91 (m, 4H, $H3_{DMT}$, $H5_{DMT}$, H3′$_{DMT}$, H5′$_{DMT}$), 5.75 (s, 1H, H1′), 5.30 (s, $1H_{II}$, H2′), 5.14 (s, $1H_I$, H2′), 4.61 (d, J = 11.8, $1H_I$, CH_2Ph_A), 4.60 (d, J = 11.8, $1H_{II}$, CH_2Ph_A), 4.54 (d, J = 11.8, 1H, CH_2Ph_B), 4.45 (s, 1H, H3′), 3.74 (s, $3H_I$, OCH_3), 3.733 (s, $3H_{II}$, OCH_3), 3.729 (s, $3H_I$, OCH_3), 3.726 (s, $3H_{II}$, OCH_3), 3.63−3.72 (m, 1H, H5″$_A$), 3.45−3.55 (m, 3H, H5′$_{A+B}$, H5″$_B$), 2.30 (s, 3H, $COCH_3$), 1.69 (s, 3H, 5-CH_3). ^{13}C NMR (DMSO-d_6) δ 170.6 ($COCH_{3,I}$), 170.5 ($COCH_{3,II}$), 162.7 (C4), 158.24 ($C4_{DMT,I}$, $C4'_{DMT,I}$), 158.20 ($C4_{DMT,II}$, $C4'_{DMT,II}$), 155.0 (q, J = 36.4, $COCF_{3,I}$), 154.9 (q, J = 37.3, $COCF_{3,II}$), 153.6 ($C2_I$), 153.5 ($C2_{II}$), 144.3 ($C1''_{DMT}$), 140.7 (C6), 137.04 ($C1_{Bn,II}$), 136.94 ($C1_{Bn,I}$), 134.92 ($C1_{DMT,I}$/$C1'_{DMT,I}$), 134.83 ($C1_{DMT,II}$/$C1'_{DMT,II}$), 134.82 ($C1_{DMT,II}$/$C1'_{DMT,II}$), 134.77 ($C1_{DMT,I}$/$C1'_{DMT,I}$), 129.7 ($C2_{DMT}$, $C6_{DMT}$, $C2'_{DMT}$, $C6'_{DMT}$), 128.31 ($C3_{Bn,I}$, $C5_{Bn,I}$), 128.28 ($C3_{Bn,II}$, $C5_{Bn,II}$), 128.0 ($C3''_{DMT}$, $C5''_{DMT}$), 127.9 ($C4_{Bn,I}$), 127.8 ($C4_{Bn,II}$), 127.6 ($C2''_{DMT}$, $C6''_{DMT}$), 127.51 ($C2_{Bn,II}$, $C6_{Bn,II}$), 127.50 ($C2_{Bn,I}$, $C6_{Bn,I}$), 126.9 ($C4''_{DMT}$), 115.82 (q, J = 288.3, $CF_{3,II}$), 115.76 (q, J = 287.8, $CF_{3,I}$), 113.40 ($C3_{DMT,I}$, $C5_{DMT,I}$/$C3'_{DMT,I}$, $C5'_{DMT,I}$), 113.38 ($C3_{DMT,II}$, $C5_{DMT,II}$/$C3'_{DMT,II}$, $C5'_{DMT,II}$), 113.36 ($C3_{DMT,I}$, $C5_{DMT,I}$/$C3'_{DMT,I}$, $C5'_{DMT,I}$), 113.35 ($C3_{DMT,II}$, $C5_{DMT,II}$/$C3'_{DMT,II}$, $C5'_{DMT,II}$), 105.5 ($C5_{II}$), 105.4 ($C5_I$), 87.3 ($C4'_{II}$), 86.8 ($C1'_I$), 86.4 ($C1'_{II}$), 86.03 ($CAr_{3,II}$), 85.99 ($CAr_{3,I}$), 85.9 ($C4'_I$), 75.6 ($C3'_I$), 74.0 ($C3'_{II}$), 71.4 (CH_2Ph_{II}), 71.3 (CH_2Ph_I), 61.2 ($C2'_I$), 59.6 ($C2'_{II}$), 58.5 ($C5'_I$), 58.4 ($C5'_{II}$), 55.0 (OCH_3), 52.9 ($C5''_I$), 52.7 ($C5''_{II}$), 24.90 ($COCH_{3,I}$), 24.87 ($COCH_{3,II}$), 14.2 (5-CH_3). ^{19}F NMR (DMSO-d_6) δ −70.0 ($CF_{3,I}$), −71.6 ($CF_{3,II}$). ESI-HRMS m/z 799.2947 ([M + H]$^+$, $C_{43}H_{42}F_3N_4O_8^+$ Calcd 799.2949).

*(1R,3R,4R,7S)-1-(4,4′-Dimethoxytrityloxymethyl)-5-(hexadecanoyl)-7-hydroxy-3-(4-N-benzoyl-5-methyl-cytosine-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (**22**).* 5-Methylcytosine intermediate **20** (2.00 g, 3.50 mmol) was coevaporated with anhydrous pyridine (2 × 10 mL) and then dissolved in anhydrous DCM (35 mL) and anhydrous pyridine (1.4 mL, 17.3 mmol) under stirring at 0 °C. Palmitoyl chloride (1.05 mL, 3.46 mmol) was added dropwise and the resulting mixture stirred at 0 °C for 3 h. DCM (35 mL) was added and the resulting mixture was washed consecutively with satd aq $NaHCO_3$ (2 × 40 mL) and water (2 × 40 mL). The aqueous phases were back-extracted with DCM (150 mL in total). The combined organic phase was dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The resulting residue was purified by silica gel column chromatography (0−7% MeOH in DCM, v/v) to afford a white foam (1.56 g, R_f = 0.5 (10% MeOH in DCM, v/v)), tentatively assigned as (1*R*,3*R*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-5-(hexadecanoyl)-7-hydroxy-3-(5-methyl-cytosine-1-yl)-2-oxa-5-azabicyclo-[2.2.1]heptane (N2′-palmitoyl,5′-*O*-dimethoxytrityl-5-methylcytosine-amino-LNA). A portion of this intermediate (78 mg, 5% of the total amount) was dissolved in anhydrous DMF (2.0 mL). Anhydrous pyridine (24 μL, 0.30 mmol) and benzoic anhydride (25 mg, 0.11 mmol) were added and the reaction mixture stirred at rt for 20 h. Additional anhydrous pyridine (24 μL, 0.30 mmol) and benzoic anhydride (29 mg, 0.13 mmol) were added, and the reaction mixture stirred for another 78 h. The reaction mixture was diluted with EtOAc (30 mL) and then successively washed with satd aq $NaHCO_3$ (2 × 20 mL) and water (2 × 20 mL). The aqueous phases were back-extracted with EtOAc (30 mL in total). The combined organic phase was dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The resulting residue was purified by silica gel column chromatography (0−2% MeOH and 0.1% pyridine in DCM, v/v/v) to afford a rotameric mixture of desired nucleoside **22** (71 mg, 45% from **20**, 7:3 ratio according to ^{1}H NMR) as a white foam. TLC (5% MeOH in DCM, v/v): R_f = 0.4; ^{1}H NMR ($CDCl_3$) δ 13.43 (br s, 1H, 4-NH), 8.32−8.25 (m, $2H_I$, $H2_{Bz}$, $H6_{Bz}$), 8.17−8.23 (m, $2H_{II}$, $H2_{Bz}$, $H6_{Bz}$), 7.84 (s, $1H_I$, H6), 7.77 (s, $1H_{II}$, H6), 7.55−7.22 (m, 12H, $H2_{DMT}$, $H6_{DMT}$, H2′$_{DMT}$, H6′$_{DMT}$, H2″$_{DMT}$, H3″$_{DMT}$, H4″$_{DMT}$, H5″$_{DMT}$, H6″$_{DMT}$, $H3_{Bz}$, $H4_{Bz}$, $H5_{Bz}$), 6.90−6.83 (m, 4H, $H3_{DMT}$, $H5_{DMT}$, H3′$_{DMT}$, H5′$_{DMT}$), 5.49 (s, 1H, H1′), 5.12 (s, $1H_{II}$, H2′), 4.52 (s, $1H_I$, H2′), 4.37 (s, $1H_{II}$, H3′), 4.29 (s, $1H_I$, H3′), 3.81−3.78 (m, 6H, 2 × OCH_3), 3.73 (d, J = 11.0, $1H_{II}$, H5′$_A$), 3.67 (d, J = 11.2, $1H_I$, H5′$_A$), 3.58−3.37 (m, 3H, H5′$_B$, H5″$_{A+B}$), 2.55−2.34 (m, $2H_I$, $COCH_2$), 2.12−2.19 (m, $2H_{II}$, $COCH_2$), 1.82 (s, $3H_I$, 5-CH_3), 1.76 (s, $3H_{II}$, 5-CH_3), 1.72−1.49 (m, 2H, $COCH_2CH_2$), 1.38−1.12 (m, 24H, $(CH_2)_{12}CH_3$), 0.87 (t, J = 6.8, 3H, $(CH_2)_{14}CH_3$). ^{13}C NMR ($CDCl_3$) δ 179.7 (COPh), 173.3 ($CO(CH_2)_{14,I}$), 173.2 ($CO(CH_2)_{14,II}$), 159.9 (C4), 158.93 ($C4_{DMT}$/$C4'_{DMT}$), 158.87 ($C4_{DMT}$/$C4'_{DMT}$), 148.0 (C2), 144.5 ($C1''_{DMT}$), 137.0 ($C1_{Bz}$), 136.0 (C6), 135.6 ($C1_{DMT,II}$/$C1'_{DMT,II}$), 135.43 ($C1_{DMT}$/$C1'_{DMT}$), 135.40 ($C1_{DMT,I}$/$C1'_{DMT,I}$), 132.7 ($C4_{Bz,I}$), 132.5 ($C4_{Bz,II}$), 130.3 ($C2_{DMT,I/II}$, $C6_{DMT,I/II}$/$C2'_{DMT,I/II}$, $C6'_{DMT,I/II}$), 130.2 ($C2_{DMT,I/II}$, $C6_{DMT,I/II}$/$C2'_{DMT,I/II}$, $C6'_{DMT,I/II}$), 130.1 ($C2_{Bz}$, $C6_{Bz}$), 128.29 ($C2''_{DMT,I/II}$, $C3''_{DMT,I/II}$, $C5''_{DMT,I/II}$, $C6''_{DMT,I/II}$, $C3_{Bz,I/II}$, $C5_{Bz,I/II}$), 128.24 ($C2''_{DMT,I/II}$, $C3''_{DMT,I/II}$, $C5''_{DMT,I/II}$, $C6''_{DMT,I/II}$, $C3_{Bz,I/II}$, $C5_{Bz,I/II}$), 128.19 ($C2''_{DMT,I/II}$, $C3''_{DMT,I/II}$, $C5''_{DMT,I/II}$, $C6''_{DMT,I/II}$, $C3_{Bz,I/II}$, $C5_{Bz,I/II}$), 127.4 ($C4''_{DMT,I}$), 127.3 ($C4''_{DMT,II}$), 113.58 ($C3_{DMT,I}$, $C5_{DMT,I}$/$C3'_{DMT,I}$, $C5'_{DMT,I}$), 113.56 ($C3_{DMT}$, $C5_{DMT}$/$C3'_{DMT}$, $C5'_{DMT}$), 113.52 ($C3_{DMT,II}$, $C5_{DMT,II}$/$C3'_{DMT,II}$, $C5'_{DMT,II}$), 111.8 (C5), 89.1 ($C4'_{II}$), 88.3 ($C4'_I$), 87.7 ($C1'_I$), 87.5 ($C1'_{II}$), 87.1 ($CAr_{3,I}$), 87.0 ($CAr_{3,II}$), 70.3 ($C3'_I$), 69.0 ($C3'_{II}$), 63.5 ($C2'_I$), 61.4 ($C2'_{II}$), 59.6 ($C5'_{II}$), 59.1 ($C5'_I$), 55.4 (OCH_3), 52.4 ($C5''_{II}$), 51.1 ($C5''_I$), 34.4 ($COCH_{2,I}$), 34.1 ($COCH_{2,II}$), 32.1 ($CH_2CH_2CH_3$), 29.9, 29.8, 29.7, 29.63, 29.57, 29.5, 25.3 ($COCH_2CH_{2,I}$), 24.8 ($COCH_2CH_{2,II}$), 22.8 (CH_2CH_3), 14.3 ($(CH_2)_{14}CH_3$), 13.8 (5-CH_3);[53] HRMS-ESI m/z: 913.5085 ([M + H]$^+$, $C_{55}H_{68}N_4O_8$−H$^+$ calcd 913.5110). When the benzoylation reaction was run on 1.83 mmol scale, the overall yield from **20** to

22 dropped to 32%, but no further optimization of the reaction conditions was performed.

*(1R,3R,4R,7S)-7-(2-Cyanoethoxy(diisopropylamino)-phosphinoxy)-1-(4,4′-dimethoxytrityloxymethyl)-5-(hexadecanoyl)-7-hydroxy-3-(4-N-benzoyl-5-methyl-cytosine-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (**23**).* Nucleoside **22** (530 mg, 0.58 mmol) was coevaporated with anhydrous 1,2-DCE (2 × 5 mL) and subsequently mixed with *N*,*N*-diisopropylammonium tetrazolide (201 mg, 1.17 mmol). The solid mixture was dissolved in anhydrous DCM (8 mL) under stirring and 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphane (370 μL, 1.16 mmol) was added dropwise. The reaction mixture was stirred at rt for 21 h whereupon EtOH (1 mL) was added and the resulting mixture stirred for 40 min. The mixture was diluted with DCM (25 mL), then washed with satd aq $NaHCO_3$ (2 × 25 mL). The combined aqueous phase was back-extracted with DCM (3 × 15 mL), and the combined organic phase dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure. The resulting residue was purified by silica gel column chromatography (0−100% EtOAc in petroleum ether, v/v) to afford a rotameric mixture of diastereomeric phosphoramidite **23** as a white foam (544 mg, 84%). R_f = 0.5 (50% EtOAc in petroleum ether, v/v); ^{31}P NMR (162 MHz, $CDCl_3$) δ_P 149.90, 149.87, 149.4, 148.4; HRMS-ESI *m*/*z*: 1113.6143 ($[M + H]^+$, $C_{64}H_{85}N_6O_9P-H^+$ calcd 1113.6188).

■ ASSOCIATED CONTENT

Supporting Information

Scheme S1. Synthesis of compounds **22** and **23**. NMR data (^{1}H, ^{13}C, ^{19}F, and/or ^{31}P) of compounds **12** and **16**−**23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(52) Peaks for C2, C4, C5, 5-CH_3, C6, COPh and $C1_{Bz}$ were broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei and rotameric change of the benzamide bond. The peak for $C1'_I$, which is of low intensity, probably as a combination of quadrupolar relaxation and long distance coupling with ^{19}F, overlaps with CAr_3 and $C4'_I$ peaks at 86.0 ppm.
(53) Peaks for C2, C4, C5, 5-CH_3, C6, COPh and $C1_{Bz}$ were broad and of low intensity in ^{13}C NMR, probably due to fast quadrupolar relaxation via the nearby ^{14}N-nuclei and rotameric change of the benzamide bond.