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Synthesis and Structure of Novel Conformationally Constrained 1′,2′-Azetidine-Fused Bicyclic Pyrimidine Nucleosides: Their Incorporation into Oligo-DNAs and Thermal Stability of the Heteroduplexes

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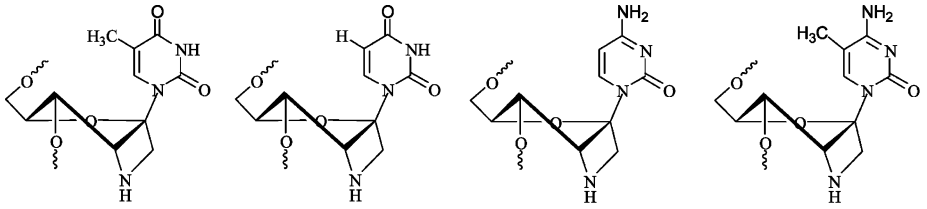

Azetidine-T **Azetidine-U** **Azetidine-C** **Azetidine-5-Me-C**

The synthesis of novel 1′,2′-aminomethylene bridged (6-aza-2-oxabicyclo[3.2.0]heptane) "azetidine" pyrimidine nucleosides and their transformations to the corresponding phosphoramidite building blocks (**20**, **39**, and **42**) for automated solid-phase oligonucleotide synthesis is reported. The novel bicyclo-nucleoside "azetidine" monomers were synthesized by two different strategies starting from the known sugar intermediate 6-*O*-benzyl-1,2:3,4-bis-*O*-isopropylidene-D-psicofuranose. Conformational analysis performed by molecular modeling (ab initio and MD simulations) and NMR showed that the azetidine-fused furanose sugar is locked in a North-East conformation with pseudorotational phase angle (*P*) in the range of 44.5−53.8° and sugar puckering amplitude (ϕ_m) of 29.3−32.6° for the azetidine-modified **T**, **U**, **C**, and 5-Me-**C** nucleosides. Thermal denaturation studies of azetidine-modified oligo-DNA/RNA heteroduplexes show that the azetidine-fused nucleosides display improved binding affinities when compared to that of previously synthesized *North-East* sugar constrained oxetane fused analogues.

Introduction

Conformationally constrained synthetic oligonucleotides having furanose fused bicyclic and tricyclic carbohydrate moieties[2,3] or modified pyranose derivative[4] in the monomer nucleotide units have been found to be useful for binding to the complementary mRNA, arresting its translation to protein products. The substitution of the natural nucleoside blocks with the North-type conformationally constrained units[5,6] results in conformational preorganization of the modified single strand antisense oligonucleotide (AON).[5,6] The induced control of the geometry in a single strand AON in turn dictates conformational preorganization[5−12] of the AON/RNA duplex to the rigid RNA/RNA-type duplex,[13−15] which can then modulate the strength of the target RNA affinity as well as the RNase H promoted cleavage efficiency of the target RNA strand in the hybrid duplex, thereby facilitating the engineering of potential gene-

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directed therapeutics and diagnostics.[2,3,7] Recently, we have synthesized novel 1′,2′-bridged oxetane nucleoside [1-(1′,3′-anhydro-β-D-psicofuranosyl) nucleoside] in which the sugar moiety is locked into a unique *North-East* type of conformation[1,8] in the sugar pseudorotational cycle.[31] The T_m of the oxetane-modified-AON/RNA hybrid with fully natural phosphodiester backbone in both strands drops by ∼5−6 °C/oxetane-**T** or ∼3 °C/oxetane-**C** modification, whereas the T_m of oxetane-**A** and -**G** modified-AON/RNA hybrids[8f] do not show any loss of stabilities compared to those of the native counterparts. It has been shown that any loss of the thermodynamic stability of the heteroduplexes could, however, be regained by the introduction of the appropriately tethered nontoxic DPPZ (dipyridophenazine) group[11,12] at the 3′-end, which also gives additional stability against 3′-exonucleases similar to that of the phosphorothioates AONs.[1,8c] The oxetane-modified appropriately tethered 3′-DPPZ mixmer AON/RNA hybrids were found to be excellent substrates for RNase H promoted cleavage.[8] The 4 nt gap after each oxetane modification in the AON made this gap resistant to RNase H promoted cleavage. However, the RNase H promoted cleavage of the phosphodiester bonds beyond the resistant 4 nt site in a mixmer with larger gaps (>4 nt) were very comparable to that of the native. The Michaelis−Menten kinetics of the RNase H cleavage showed that V_{max} and K_m increase with increase in the number (one to three) of **T**/**C**/**A** modifications (with the exception of **G**, which is slightly lower than that of the native) in the AONs, indicating higher catalytic activity and lower enzyme binding affinity of the oxetane-modified AON/RNA hybrids.[1,8e] In the β-D-LNA modified AONs, on the other hand, requires a gap size of 8−10 nt in order to get back the effective RNase H cleavage of the target.[9] It is likely that use of a lengthy unmodified phosphodiester oligonucleotide gaps in the gapmer AONs could be harmful in view of their endonuclease susceptibility.[10] The resistance to the endonuclease promoted cleavage of these oxetane-modified molecules was proportional to the number of the oxetane-modified nucleotides per AON molecule: single modification gave 2-fold protection to the cleavage, and double and triple modification gave 4-fold protection compared to that of the native phosphodiester oligonucleotide.[1,8c]

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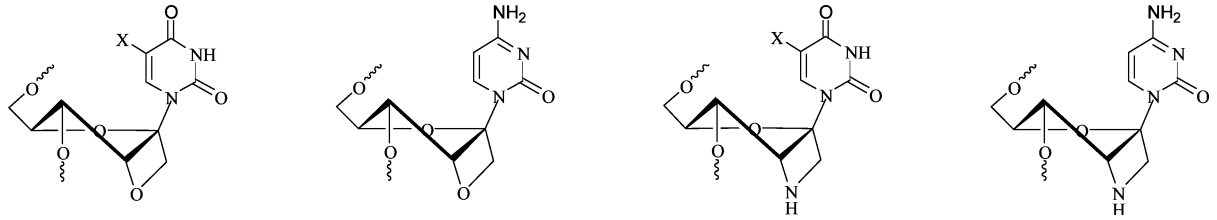
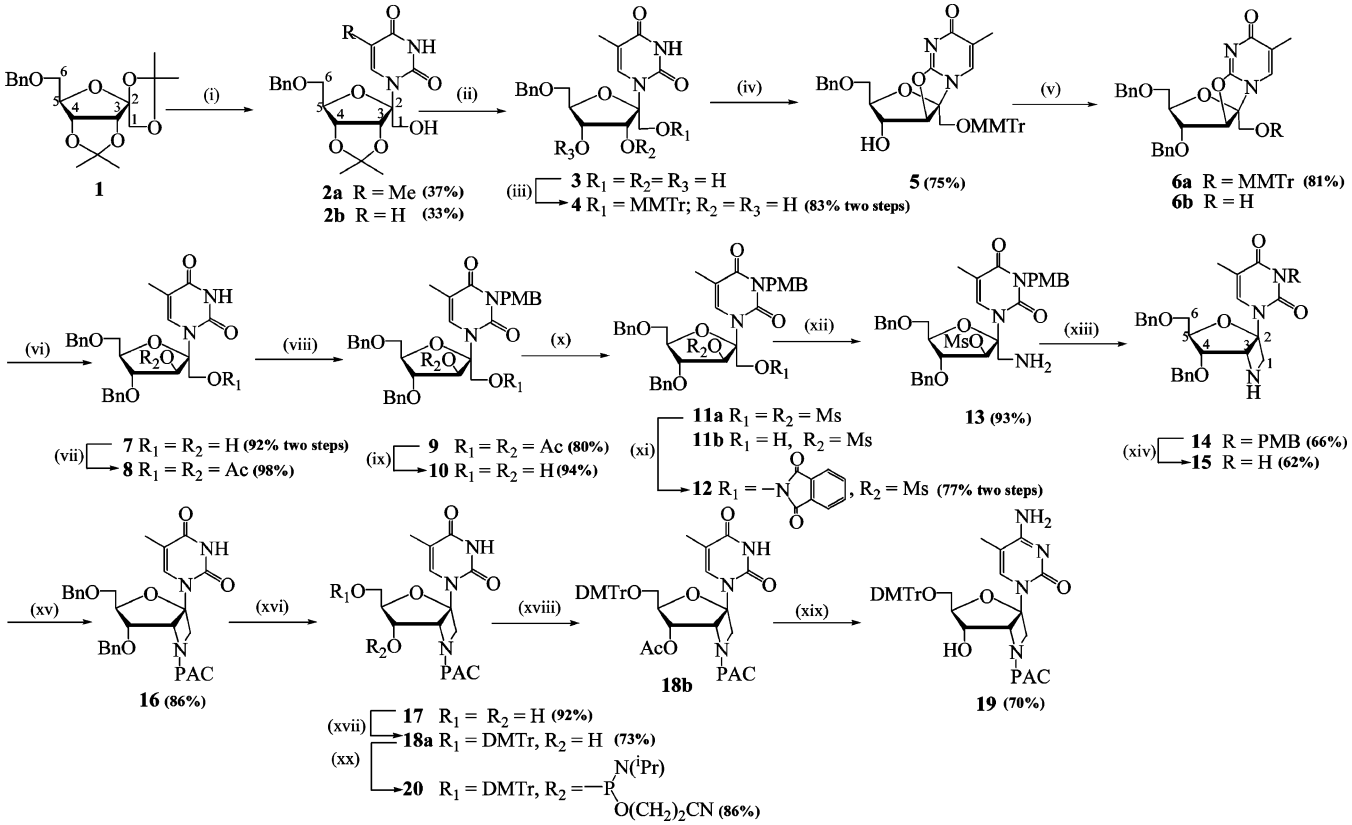


Oxe-T (X = CH_3) **Oxe-C** **Aze-T** (X = CH_3) **Aze-C**

Oxe-U (X = H) **Aze-U** (X = H)

FIGURE 1. Chemical structures of the oxetane- and azetidine-modified pyrimidine nucleosides building blocks incorporated into oligo-DNA.

SCHEME 1[a]

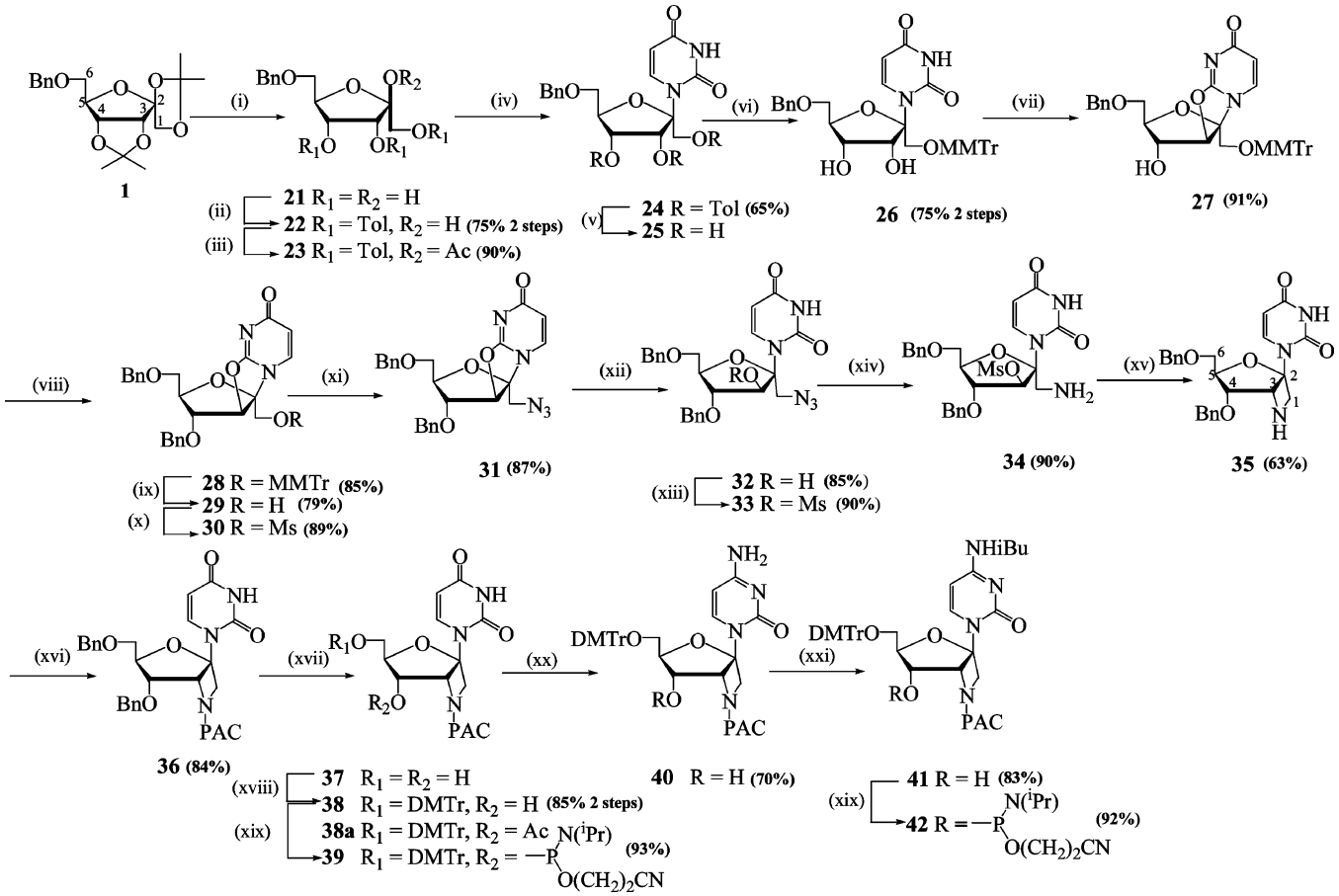


[a] Reagents and conditions: (i) (a) persilylated thymine, trimethylsilyl trifluoromethanesulfonate, CH_3CN, rt, 17 h, (b) persilylated uracil, trimethylsilyl trifluoromethanesulfonate, CH_3CN, rt, 17 h; (ii) 90% aq CF_3COOH, rt, 30 min; (iii) MMTrCl, pyridine, rt, 20 h; (iv) 1,1′-thiocarbonyldiimidazole, toluene, 120 °C, 3 h; (v) benzyl bromide, NaH, CH_3CN, 6 h; (vi) (a) 80% aq CH_3COOH, rt, 24 h; (b) 1 N aq NaOH in ethanol−water, rt, overnight; (vii) Ac_2O, pyridine, rt, 3 h; (viii) 4-methoxybenzyl bromide, NaH, DMF, 6 h; (ix) sodium methoxide, MeOH, rt, 30 min; (x) methanesulfonyl chloride, pyridine, 4 °C, overnight; (xi) potassium phthalimide, DMF, 110 °C, overnight; (xii) aq $MeNH_2$, MeOH, rt, 4 h; (xiii) Et_3N, pyridine, 90 °C, 48 h; (xiv) ceric ammonium nitrate, CH_3CN, H_2O, rt, 3 h; (xv) phenoxyacetyl chloride, pyridine, rt, 3 h; (xvi) ammonium formate, 20% $Pd(OH)_2/C$, methanol, reflux, 3 h; (xvii) DMTrCl, pyridine, rt, overnight; (xviii) Ac_2O, pyridine, rt, 28 h; (xix) (a) 2-chlorophenylphosphorodichloridate, 1,2,4-triazole, pyridine, rt, 6 h, (b) aq ammonia, rt, overnight; (xx) $NC(CH_2)_2OP(Cl)N(^iPr)_2$, $EtN(^iPr)_2$, THF, rt, 2 h.

It has also been recently shown that the rationally designed oxetane-**T** and -**C**-containing AONs effectively down-regulate the proto-oncogene c-myb mRNA in the K562 human leukemia cells[12] in a manner that is very comparable to the gene silencing efficiency of the phosphorothioate counterpart, thereby making these oxetane-locked analogues potentially important for therapeutic use. Thus, we became interested to examine the properties of 1′,2′-azetidine-fused AONs which are an isosteric analogue of the oxetane-fused counterpart.

The 1′,2′-azetidine-fused nucleoside (6-aza-2-oxabicyclo-[3.2.0]heptane) analogues (Figure 1) appealed to us primarily for two reasons. First, the endocyclic amino functionality of the azetidine moiety could be utilized as a well-defined conjugation site[13] in a conformationally restricted azetidine nucleoside, and thereby we can control the hydrophilic, hydrophobic, and steric requirements of a minor groove of the duplex. Second, the amine-derivatized AONs have displayed increased thermal affinities[14] toward complementary native AONs possibly because of the introduction of positively charged moieties at physiological pH and thus could influence partial neutralization of the negatively charged phosphates in the duplex.

SCHEME 2[a]



[a] Reagents and conditions: (i) 70% aq acetic acid, 80 °C, 5 h; (ii) 4-toluoyl chloride, pyridine, 0 °C, 4 h; (iii) Ac_2O, pyridine, rt, 48 h; (iv) persilylated uridine, TMSOTf, CH_3CN, 40 °C, 2 h; (v) NaOEt, ethanol, 10 min; (vi) MMTrCl, pyridine, rt, overnight; (vii) diphenyl carbonate, DMF, 110 °C, 2 h; (viii) benzyl bromide, NaH, CH_3CN, overnight; (ix) 80% aq CH_3COOH, rt, 48 h; (x) MsCl, pyridine, 0 °C, 1.5 h; (xi) NaN_3, DMF, 100 °C, 60 h; (xii) aq NaOH, rt, 2 h; (xiii) MsCl, pyridine, 0 °C, 24 h; (xiv) PMe_3, THF, water, rt, 1 h; (xv) Et_3N, pyridine, 90 °C, 48 h; (xvi) PACCl, pyridine, rt, 2.5 h; (xvii) BCl_3, CH_2Cl_2, −78 °C, 2 h, −20 °C 2 h; (xviii) DMTrCl, DMAP, pyridine, rt, 5 h; (xix) $NC(CH_2)_2OP(Cl)N(^iPr)_2$, $EtN(^iPr)_2$, THF, rt, 2 h; (xx) (a) Ac_2O, pyridine, rt, 24 h; (b) 2-chlorophenylphosphodichloridiate, 1,2,4-triazole, pyridine, rt, 6 h; (c) aq NH_3, 5 °C, overnight; (xxi) (a) TMSCl, pyridine, 30 min; (b) isobutyryl chloride, rt, 3 h.

We report here the synthesis of AONs incorporated with 1′, 2′-azetidine-fused nucleoside phosphoramidite building blocks **20**, **39**, and **42** with the desired 6-aza-2-oxabicyclo[3.2.0]heptane skeleton (Schemes 1 and 2) as well as their incorporation into AON/RNA heteroduplexes. A key step in the synthesis of these conformationally restricted azetidine-**C**, -**T**, -**U**, and -5-Me-**C** analogues appeared to be the nucleophilic displacement of a 3′-mesylate in **13** and **34** by intramolecular nucleophilic attack of the 1′-amino group (see compounds **1**, **2a**, and **14** for numbering in Scheme 1) leading to formation of the azetidine ring. The NMR and computational structural studies are also reported here, showing that by the introduction of the azetidine modification the sugar ring appears conformationally locked in the 3′-*exo*/4′-*endo North-East* conformation similar to that of the oxetane-modified analogues.[8f]

Results and Discussion

Synthesis of 1′, 2′-Azetidine-Fused Nucleoside Phosphoramidite Building Block 20. For the preparation of the D-*fructo* precursor **7** with β configuration, the 6′-*O*-benzyl-protected psicofuranoside **1** was condensed with persilylated thymine using trimethylsilyl triflate as the Lewis acid, which gave the desired β-anomer[15] **2a** in 37% yield (α:β, 1:1). The β-configuration at anomeric C2′ was confirmed by 1D NOE difference spectroscopy, which showed 2.3% NOE enhancement for H6−H3′. After removing the 3′,4′-*O*-acetonide protection of **2a**, using 90% aqueous trifluoroacetic acid at room temperature gave crude **3**, which was subsequently treated with 4-monomethoxytrityl (MMTr) chloride in dry pyridine to afford **4** in an overall 83% (yield in two steps from **2a**). Treatment of **4** with 1,1′-thiocarbonyldiimidazole in dry toluene for 3 h at 120 °C gave the 2,3′-anhydro derivative[16] **5** in 75% yield, and some traces of the starting material. Efforts to drive the reaction to completion by keeping for a longer time resulted in the formation of some undesired side products. The 4′-hydroxyl of **5** was protected with a second benzyl group using benzyl bromide and sodium hydride in dry acetonitrile to give **6a** (81%). The 1′-*O*-MMTr group from **6a** was deprotected without any identifiable side reactions by overnight treatment with 80% aqueous acetic acid at room temperature to give crude **6b** (>99% pure) which was directly taken to the next step. Nucleophilic opening[17] of the 2,3′-anhydro bridge in **6b** by 1 N aqueous sodium hydroxide in an ethanol−water mixture at room temperature gave the D-*fructo* derivative **7** in 92% yield after two steps from **6a**. Acetylation of **7** with excess of acetic anhydride (20 equiv) in

dry pyridine afforded 1′,3′-*O*-bisacetyl nucleoside **8** in 98% yield. The *N*-3 of thymine base in **8** was protected with 4-methoxybenzyl (PMB)[18,19] to give **9** (80%) in order to avoid 1′,2-anhydro formation during nucleophilic displacement of 1′-mesylate in subsequent steps. Deacetylation of **9** (94%) using sodium methoxide in methanol followed by mesylation at C1′ and C3′ hydroxyls in dry pyridine at 4 °C gave one major product, the desired 1′,3′-bismesyl product **11a** [^{1}H NMR ($CDCl_3$): δ 2.89 for 1′-OMs and 2.29 for 3′-OMs] along with the minor component, the 3′-mesyl product with a free 1′-OH group **11b** [^{1}H NMR ($CDCl_3$): δ 2.29 for 3′-OMs]. Since the bismesyl product was found to be unstable during column chromatography, we treated the crude reaction products **11a**/**11b** with an excess of potassium phthalimide in dry DMF at 110 °C overnight[20] to afford 1′-phthalimido-3′-*O*-Ms-derivative **12** in 77% yield (after two steps from **10**; the starting **11b** was recovered in 7% yield). The selective displacement of 1′-mesylate from **11a** to yield **12** keeping the 3′-mesylate group of **11a** intact was possible because the 3′-carbon is sterically more hindered in D-*fructo* sugar compared to that of the D-*arabino* analogue. Further experimental evidence of the 3′-center to be more sterically hindered than C1′ is provided by the selective displacement of 1′-mesylate in **30** by azide, resulting in **31** which has the 2,3′-anhydro bond left intact (Scheme 2).

The deprotection of 1′-phthalimido group from **12** by aqueous methylamine in methanol at room temperature[21] gave the corresponding 1′-amino derivative **13** (93%). Multiple attempts to perform azetidine cyclization at ambient temperature by intramolecular nucleophilic attack of the 1′-amino group with concomitant demesylation at C3′ were carried out under variety of conditions, such as 1 M NaOH in dioxane, NaOEt in ethanol, or 1 M NaHMDS in THF, all of which proved to be unsuccessful except for a mixture of Et_3N and pyridine at 90 °C for 48 h, which gave the azetidine-modified nucleoside **14** in 66% yield. Deprotection of N^3-PMB group was performed using ceric ammonium nitrate in aqueous acetonitrile (1:9, v/v)[18] at room temperature, giving **15** in 62% yield. Phenoxyacetyl (PAC) protection was employed to protect the azetidine-nitrogen to avoid *N*-branching during the solid-phase oligonucleotide synthesis using the phosphoramidite approach.[22] Other protecting groups such as acetyl and trifluoroacetyl were not useful in our case as acetyl deprotection was sluggish, whereas trifluoroacetyl group was found to be unstable. The PAC-protected nucleoside was obtained by treating **15** with PAC-Cl in pyridine, which afforded a mixture of diastereomers **16** (by ^{1}H and ^{13}C NMR) in 86% yield as a result of chiral tervalent azetidine-nitrogen atom,[23] which was also found for the nitrogen-derivatives of the LNA analogues.[24] Debenzylation of **16** using ammonium formate and 20% $Pd(OH)_2/C$[25] gave **17** in 92% yield. Treatment of **17** with 4,4′-dimethoxytrityl (DMTr) chloride in pyridine afforded **18a** in 73% yield. The 4′-hydroxyl of DMTr-protected nucleoside **18a** was acetylated and the product **18b** was converted into the 5-methylcytosine derivative via the *C*-4-triazolyl intermediate,[26] where thymine was initially activated by conversion into the 1,2,4-triazole derivative, which was subsequently displaced with ammonia along with deacetylation afforded **19** (70%). Phosphitylation of **18a** with 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite[8b] gave the desired amidite **20** in 86% yield [^{31}P NMR ($CDCl_3$): δ 154.7, 150.7, 149.9, 148.7] for the solid-phase synthesis.

Synthesis of 1′,2′-Azetidine-Fused Phosphoramidite Building Blocks 39 and 42. We have, however, envisaged a different strategy for the synthesis of 1′,2′-azetidine-fused uridine (**39**) and cytidine (**42**) phosphoramidite solid-phase oligodeoxynucleotide synthesis building blocks (Scheme 2) due to low yields of the desired β-anomer **24** in the coupling of the persilylated uracil with the 6-*O*-benzyl-1,2:3,4-di-*O*-isopropylidene-β-D-psicofurnose (**1**), as shown in Scheme 1, which gave a complex anomeric mixture (α:β, 1:0.8), from which only 23% of the β-anomer could be isolated. By utilizing 2-*O*-acetyl-6-*O*-benzyl-1,3,4-tri-*O*-(4-toluoyl)-D-psicofuranose **23** as a coupling precursor, the desired β-anomer **24** was obtained in 65% yield from an anomeric mixture in which the β-anomer was the predominant constituent (α:β, 1:9) (Scheme 2). The β-configuration at the anomeric C2′ center was confirmed by 1D NOE difference spectroscopy, which showed 1.9% enhancement for H6−H3′. Another advantage of this modified synthetic scheme, as shown in Scheme 2, was keeping the 2, 3′-anhydro bond intact until the 1′-mesylate in **30** was displaced with the azido group to give **31**, thereby avoiding a key nucleobase protection step at *N*-3 used earlier in Scheme 1. Thus the opening of the 2,3′-anhydro bond in **31** gave a direct entry to the desired β-D-*fructo* configured nucleoside **32** by bypassing acetylation/deacetylation and thyminyl *N*-3 protection/deprotection steps (in **8** → **10**/**15** in Scheme 1).

The psico-sugar **1** was treated with 70% aqueous acetic acid at 80 °C for 5 h followed by 4-toluoylation using 4-toluoyl chloride in dry pyridine to afford **22** as an anomeric mixture (∼1:1) in 75% yield. Acetylation of **22** in dry pyridine with a 20-fold excess of acetic anhydride in 48 h at room temperature gave **23** (90%) as an anomeric mixture. The coupling between **23** and the uracil nucleobase using Vorbruggen-type reaction[25,27] utilizing trimethylsilyltriflate as Lewis acid and *N*,*O*-bis-(trimethylsilyl)acetamide as silylating agent afforded **24** in 65% yield. Complete detoluoylation of nucleoside **24** was accomplished with 1 M NaOEt in ethanol to give intermediate **25**, which was subsequently monomethoxytritylated as for **4** and gave **26** in 75% yield in two steps from **24**. The use of 1,1′-thiocarbonyldiimidazole[16] in toluene at 120 °C to obtain the anhydro derivative **27**, as used in Scheme 1, gave some undesired side products. This problem was circumvented by treating **26** with diphenyl carbonate[28] and $NaHCO_3$ in DMF at 110 °C and afforded **27** in 91% yield. Benzylation of the 4′-hydroxyl of **27** went smoothly to give **28** (85%), from which 1′-*O*-MMTr was deprotected with 80% aqueous acetic acid to give **29** (79%) along with D-*fructo*-nucleoside as a byproduct (11%). Compound **29** upon treatment with Ms-Cl (3 equiv) in dry pyridine at 0 °C for 1.5 h gave the expected 1′-*O*-mesylated product **30** (89%) along with traces of starting material, while any prolongation of the reaction time in an attempt to drive the reaction to completion reduced the yield considerably. Displacement of 1′-mesylate of **30** with NaN_3 in DMF at 100 °C went smoothly with no traces of any side reactions to give **31** (87%). Nucleophilic opening of the 2,3′-anhydro bond in **31** with 1 M NaOH in THF gave the desired D-*fructo*-nucleoside **32** with 85% yield along with traces of nonnucleosidic sugar impurity. The nucleoside **32** was mesylated at C3′ with Ms-Cl in dry pyridine at 0 °C, which furnished a key precursor **33** in 90% yield. Reduction of the azide was carried out under Staudinger's conditions[29] (PMe_3, THF, and H_2O), which afforded **34** in 90% yield.

Cyclization of **34** by intramolecular nucleophilic attack of the 1′-amino group with concomitant demesylation at C3′, as performed in Scheme 1 (Et_3N and pyridine at 90 °C), gave

TABLE 1. Sequences Synthesized and Thermal Denaturation Studies toward Complementary RNA Targets and Comparison with Oxetane-Modified Sequences[a]

AON	Sequence	T_m (°C)	ΔT_m	MALDI-MS Found/calc $[M+H]^+$	ΔT_{m1}
1	3'-d(CTTCTTTTTTACTTC)-5'	44		4448.6/4448.7	
2	3'-d(CT**T**-azeCTTTTTTACTTC)	38.5	-5.5	4475.3/4475.7	+0.5
2a	3'-d(CT**T**-oxeCTTTTTTACTTC)	38	-6	nd	
3	3'-d(CTTCT**T**-azeTTTTACTTC)	40	-4	4475.6/4475.7	+1
3a	3'-d(CTTCT**T**-oxeTTTTACTTC)	39	-5	nd	
4	3'-d(CTTCTTT**T**-azeTTACTTC)	40	-4	4475.4/4475.7	0
4a	3'-d(CTTCTTT**T**-oxeTTACTTC)	40	-4	nd	
5	3'-d(CTTCTTTTT**T**-azeACTTC)	40	-4	4475.5/4475.7	+1.0
5a	3'-d(CTTCTTTTT**T**-oxeACTTC)	39	-5	nd	
6	3'-d(CT**U**-azeCTTTTTTACTTC)	39.5	-4.5	4461.7/4461.7	nd
7	3'-d(CTTCT**U**-azeTTTTACTTC)	40.5	-3.5	4461.6/4461.7	nd
8	3'-d(CTTCTTT**U**-azeTTACTTC)	41	-3	4461.9/4461.7	nd
9	3'-d(CTTCTTTTT**U**-azeACTTC)	40	-4	4461.8/4461.7	nd
10	3'-d(CTT**C**-azeTTTTTTACTTC)	43.0	-1.0	4475.9/4475.7	+2.0
10a	3'-d(CTT**C**-oxeTTTTTTACTTC)	41.0	-3	nd	
11	3'-d(CTTCTTTTTTA**C**-azeTTC)	42	-2	4475.6/4475.7	nd

[a] T_m values were measured as the maximum of the first derivative of the melting curve (A_{260} vs temperature) recorded in medium salt buffer (60 mM Tris-HCl at pH 7.5, 60 mM KCl, 0.8 mM $MgCl_2$, and 2 mM DTT) with temperature range 20−70 °C using 1 μM concentrations of the two complementary strands; $\Delta T_m = T_m$ relative to native duplex; $\Delta T_{m1} = T_m$ relative to oxetane-modified duplex; "nd" = not determined. Results of **2a**−**5a** and **10a** were taken from refs 8c and 9.

fused-azetidine-modified uracil nucleoside **35** (63%). Protection of the azetidine ring using PAC-Cl in dry pyridine, as used for **15**, gave **36** in 84% yield. This compound was found to be a mixture of diastereomers by ^{1}H and ^{13}C spectroscopy as it was previously found for **16**. Debenzylation of **36** with 20% Pd-$(OH)_2$/C, ammonium formate,[25] and 20% Pd$(OH)_2$/C, H_2, in ethanol did not work satisfactorily in our hands, but the reaction proceeded to completion very smoothly with 1 M BCl_3[5r] in CH_2Cl_2 at −78 °C. The crude product obtained was selectively dimethoxytritylated to **38** (85% in two steps from **36**) followed by phosphitylation in the same way as was done for **20** furnished the desired phosphoramidite building block **39** (93%) as a mixture of four diastereomers [^{31}P NMR ($CDCl_3$): δ 154.6, 150.6, 149.9, and 149.2]. The 6′-*O*-DMTr-protected uracil nucleoside **38** was acetylated using acetic anhydride in pyridine, and the product **38a** obtained was converted into the cytosine nucleoside **40** (70%) using the procedure used for **19** (Scheme 1).[26] This nucleoside was transiently protected by trimethylsilylation followed by treatment with isobutyryl chloride in pyridine and subsequent desilylation to afford **41** in 83% yield. Compound **41** thus obtained was transformed to its corresponding phosphoramidite **42** (92%) [^{31}P NMR ($CDCl_3$): δ 154.4, 150.6, 149.6, 149.0].

Phosphoramidites **20**, **39**, and **42** were incorporated at different positions of mixed 15-mer AON sequences as depicted in Table 1. Their structural integrity was confirmed by mass measurements by MALDI-TOF spectroscopy. All compounds were spectroscopically pure and their properties are documented in the Experimental Section. Structure determinations of these compounds were based upon ^{1}H NMR, COSY, HETCORR, and NOE experiments as well as by MALDI-TOF mass spectrometry.

Thermal Stabilities (T_m) of Azetidine-Modified AON/RNA Hybrids. The thermostability of the azetidine-modified AONs and their oxetane counterparts measured at pH 7.5 are shown in Table 1. Single azetidine-**T** incorporation enhances the T_m of the AON/RNA duplex by ∼1 °C compared to the isosequential oxetane-**T** modified hybrid duplex. However aze-**C** incorporation enhanced the T_m by ∼2 °C with respect to oxetane-**C**-modified AON/RNA duplexes. Aze-**U** incorporation did not improve the target binding affinity in comparison with aze-**T** modified hybrid duplexes. Although the moderate improvement in binding affinity of azetidine-modified AONs at pH 7.5 was encouraging, we argued that lowering of the pH to 6 might enhance the target affinity even further owing to the efficient protonation of the azetidine-nitrogen (pK_a = 6.07 for 3′,5′-bisethyl phosphate of azetidine-locked **T**) leading to partial neutralization of the negative charge of the phosphate backbone. To check this possibility we have measured the T_m of azetidine-modified AON/RNA hybrids at pH 6 (Tables 2). Surprisingly, there was no significant T_m increment observed at this pH compared to the one at pH 7.5. This suggests that the protonation of azetidine-nitrogen does not lead to effective phosphate charge

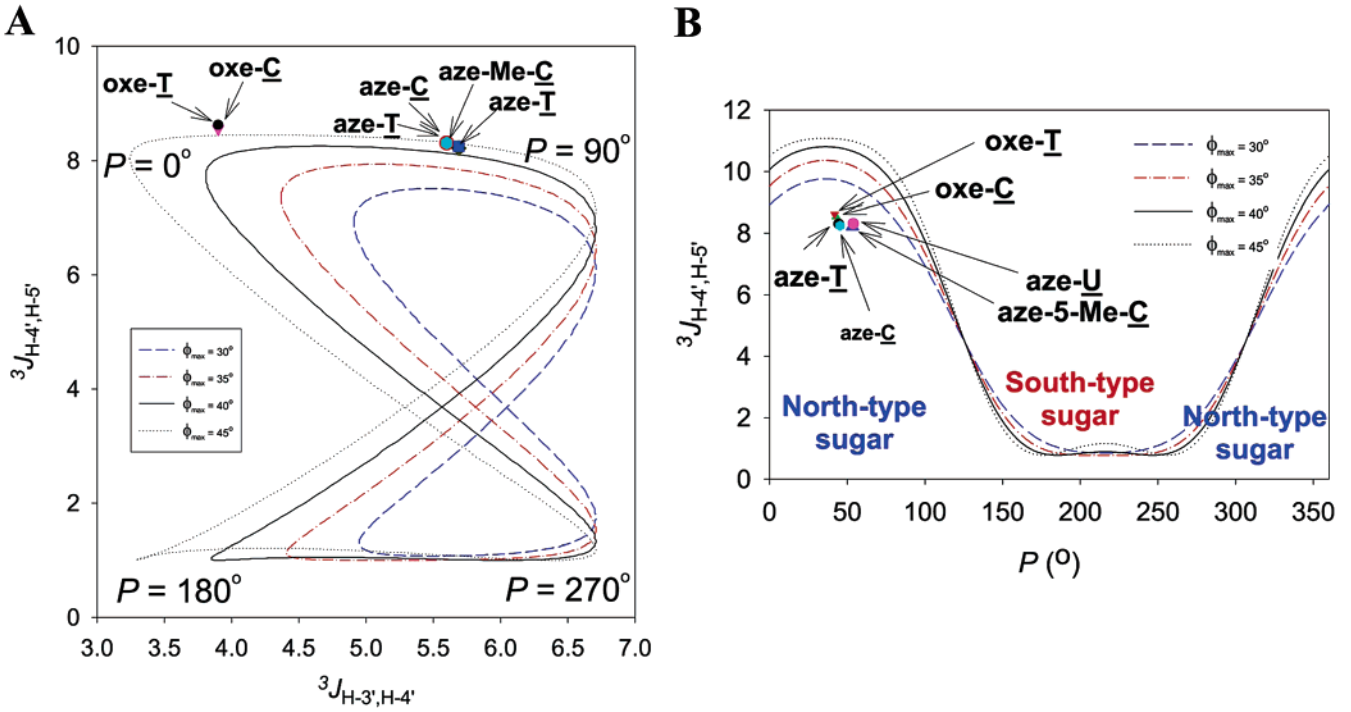


FIGURE 2. Experimental $^3J_{H-3',H-4'}$ and $^3J_{H-4',H-5'}$ vicinal proton coupling constants (Hz) (panel A) and experimental $^3J_{H-4',H-5'}$. vs calculated pseudorotational phase angle P (panel B) for the oxetane-modified **T** and **C** and azetidine-modified **T**, **C**, 5-Me-**C**, and **U** nucleosides compared to the theoretical dependences (colored lines) of the $^3J_{H,H}$ calculated for $0 \leq P \leq 360°$ employing a generalized Karplus-type equation ($P_1 = 13.24°$, $P_2 = -0.91°$, $P_3 = 0$, $P_4 = 0.53°$, $P_5 = -2.41°$, $P_6 = 15.5°$) developed by Altona et al.[31] at fixed puckering amplitudes of 30° (blue dashed line), 35° (red dot−dashed line), 40° (black line), and 45° (black dotted line). The experimental coupling constants for the oxetane- and azetidine-modified nucleosides were obtained through 600 MHz ^{1}H NMR spectra (DMSO-d_6 + CD_3OD) at 298 K.

TABLE 2. Thermal Denaturation Studies at 110 and 40 mM [Na^+]

	pH 6, 110 mM [Na^+][a]		pH 6, 40 mM [Na^+][b]	
AON	T_m	ΔT_m[c]	T_m	ΔT_m[c]
1	43.5		37	
2	39	−4.5	32	−5.0
3	39.5	−4.0	32	−5.0
4	39.5	−4.0	31.5	−5.5
5	39	−4.5	30	−7.0
6	39.5	−4.0	29.5	−7.5
7	39.5	−4.0	31.5	−5.5
8	39	−4.5	33	−4.0
9	39	−4.0	31.5	−5.5
10	42	−1.5	36	−1.0
11	42	−1.5	36	−1.0

[a] 0.1 mM EDTA, 100 mM sodium chloride, 10 mM sodium phosphate, pH 6.0. [b] 0.1 mM EDTA, 30 mM sodium chloride, 10 mM sodium phosphate, pH 6.0. [c] ΔT_m is melting temperature of the azetidine-modified duplex relative to its native counterpart.

neutralization, presumably because both entities are far apart from each other.

To check the role of electrostatics in RNA binding affinity of azetidine-modified AONs, we have determined the T_m values at high (100 mM) and low (40 mM) salt concentration at pH 6 (Table 2). In these conditions the thermostability of the AON/RNA hybrids increases at higher salt concentration but decreases at the lower salt concentration, which means that the intramolecular electrostatics-modulated stabilization is negligible compared to the external salt effect.[30]

Conformational Analysis of the Azetidine-Fused Sugar Moiety Based on Experimental $^3J_{H,H}$ Coupling Data. Fusion of the azetidine ring into sugar moiety removes the H1′ proton from the native pentose and, correspondingly, the important endocyclic vicinal proton−proton coupling constant, thus denying straightforward approach to determine sugar conformation from three related endocyclic $^3J_{H,H}$ of the sugar moiety. However, the conformation adopted by the pentose in the bicyclic azetidine-modified nucleosides (azetidine-modified **T**, **U**, **C**, and 5-Me-**C**, Figure 1), can still be estimated with high accuracy from the only remaining experimental $^3J_{H-3',H-4'}$ and $^3J_{H-4',H-5'}$ (Figure 2) coupling constants. These estimations can further be verified by direct theoretical modeling using a generalized Karplus-type equation developed by Altona et al.,[31,32] which sets empirical rules relating $^3J_{H,H'}$ coupling constants of pentose with values of the respective torsion angles. A combination of the $^3J_{H-2',H-3'}$ and $^3J_{H-3',H-4'}$ provides a good quantitative measure of the pseudorotational phase angle (see Figure 2) except for the region of relative ambiguity when simultaneously the $^3J_{H-2',H-3'}$ constant is in the range of 3 to 5 Hz while the $^3J_{H-3',H-4'}$ is in the range of 5−6 Hz.

The $^3J_{H-3',H-4'}$ and $^3J_{H-4',H-5'}$ experimental NMR coupling constants of the azetidine-modified **T**, **U** **C**, and 5-Me-**C** nucleosides (Figure 2, panel A) are in the ranges 5.6−5.7 and 8.2−8.4 Hz (Table 3), respectively, compared to $^3J_{H-3',H-4'}$ = 3.9 Hz and $^3J_{H-4',H-5'}$ = 8.5 Hz observed for the oxetane-modified **T** and **C**.[10] Comparison of the estimated $^3J_{H-3',H-4'}$ vs $^3J_{H-4',H-5'}$ dependence calculated using the standard Karplus equation[31] with the experimentally obtained proton coupling constants suggests that the sugar conformations of the azetidine-**C**, -**T**, -**U**, and -5-Me-**C** nucleosides is of the *North*-type (Figure 2, panel A) with its pseudorotational phase angle (P) being in a range of 55−75°, which is apparently much higher compared

(32) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205 and references therein.

TABLE 3. Sugar Moiety Conformational Parameters (ν_0–ν_4 Torsions, Pseudorotational Phase Angle *P*, Puckering Amplitude ϕ_m) and Related Experimental and Calculated $^3J_{H-3',H-4'}$ and $^3J_{H-4',H-5'}$ Vicinal Proton NMR Coupling Constants of the Azetidine-C, -T, -U, and -5-Me-C Nucleosides[a]

sugar conformational parameters	azetidine-T	azetidine-U	azetidine-C	azetidine-5-Me-C
ν_0: C5′–O5′–C2′–C3′	−13.22° (−6.4 ± 12.2°)	−19.33° (−5.3 ± 12.5°)	−13.91° (−5.8 ± 12.3°)	−19.25° (−6.81 ± 11.4°)
ν_1: O5′–C2′–C3′–C4′	−6.16° (-8.9 ± 6.3°)	−1.39° (−9.9 ± 6.5°)	−5.83° (−9.0 ± 6.6°)	−1.73° (−10.2 ± 6.5°)
ν_2: C2′–C3′–C4′–C5′	20.94° (19.3 ± 11.9°)	19.21° (19.8 ± 11.1°)	21.03° (18.9 ± 11.7°)	19.64° (20.9 ± 9.8°)
ν_3: C3′–C4′–C5′–O5′	−28.96° (−24.6 ± 18.1°)	−30.90° (−24.2 ± 17.1°)	−29.45° (−23.7 ± 17.7°)	−31.32° (−25.8 ± 14.9°)
ν_4: C4′–C5′–O5′–C2′	27.18° (19.8 ± 19.4°)	33.06° (18.9 ± 18.0°)	27.97° (18.8 ± 18.1°)	32.32° (20.5 ± 15.8°)
phase angle *P*	44.45° (37.2 ± 27.0°)	53.84° (33.6 ± 31.7°)	45.36° (35.9 ± 29.6°)	53.33° (32.4 ± 29.7°)
puckering amplitude ϕ_m	29.33° (25.1 ± 18.2°)	32.56° (25.5 ± 16.5°)	29.93° (24.5 ± 17.6°)	32.88° (27.0 ± 13.9°)

vicinal proton coupling constants	azetidine-T	azetidine-U	azetidine-C	azetidine-5-Me-C
H3′–C3′–C4′–H4′ calc	35.22	32.86	35.48	33.39
$^3J_{H-3',H-4'}$ calc, Hz	5.68	5.95	5.65	5.89
$^3J_{H-3',H-4'}$ exptl, Hz	5.60	5.69	5.69	5.60
error, Hz	0.08	0.26	−0.04	0.29
H4′–C4′–C5′–H5′ calc	−152.98	−155.54	−153.38	−155.82
$^3J_{H-4',H-5'}$ calc, Hz	8.54	8.83	8.58	8.86
$^3J_{H-4',H-5'}$ exptl, Hz	8.31	8.25	8.22	8.31
error, Hz	0.23	0.58	0.36	0.55

sugar conformational parameters	oxetane-C	oxetane-T
ν_0: C5′–O5′–C2′–C3′	−15.20° (−9.7 ± 8.5°)	−14.30° (−10.5 ± 9.1°)
ν_1: O5′–C2′–C3′–C4′	−8.57° (−10.7 ± 5.9°)	−8.89° (−10.2 ± 6.2°)
ν_2: C2′–C3′–C4′–C5′	26.34° (25.0 ± 6.7°)	26.03° (24.9 ± 7.5°)
ν_3: C3′–C4′–C5′–O5′	−35.76° (−32.0 ± 9.5°)	−34.88° (−32.4 ± 10.7°)
ν_4: C4′–C5′–O5′–C2′	32.66° (26.7 ± 10.5°)	31.52° (27.5 ± 11.6°)
phase angle *P*	42.79° (35.3 ± 18.2°)	41.87° (36.6 ± 20.7°)
puckering amplitude, ϕ_m	36.55° (32.3 ± 8.7°)	35.60° (32.9 ± 9.5°)

[a] Corresponding sugar conformation parameters of the oxetane-modified **C** and **T**[10] are also show for comparison. Theoretical values were calculated using optimized parameters for the standard Karplus equation[30] without electronegativity corrections: ($^3J_{H',H'} = A\ cos^2\ \phi + B\ cos\ \phi + C$, $A = 7.76$, $B = -1.10$, $C = 1.4$) and corresponding H3′–C3′–C4′–H4′ and H4′–C4′–C5′–H5′ torsions from (a) ab initio 6-31G** Hartree–Fock optimized by GAUSSIAN 98[35] molecular geometries and (b) from unrestrained 2 ns MD simulation by Amber 7[36] averaged in 1.5–2 ns time interval of the respective simulations (shown in parentheses).

to 39.8° < *P* < 42.8° observed for the oxetane-modified nucleosides.[8f] However, only preliminary conclusions can be drawn at this point as the theoretical dependences by standard Karplus equation[31] shown in Figure 2A provide only rough estimation. Further analysis of the sugar conformation in azetidine pyrimidines is provided in the next section discussing conformational parameters obtained from the ab initio and molecular dynamics (MD) simulations.

The dynamics of proton exchange with water, which has direct effect on the observed vicinal proton constants, can be used as a measure of proton exposure to the solvent. As the $^3J_{H-3',H-4'}$ and $^3J_{H-4',H-5'}$ of the azetidine-modified **C**, **T**, **U**, and 5-Me-**C** have been found to be temperature-independent, the ^{1}H NMR data suggest that the sugar moiety of the azetidine-modified nucleosides is *locked* in the *North-East* conformation,[33a–c,34,35] as found for the oxetane-modified nucleosides.[8f]

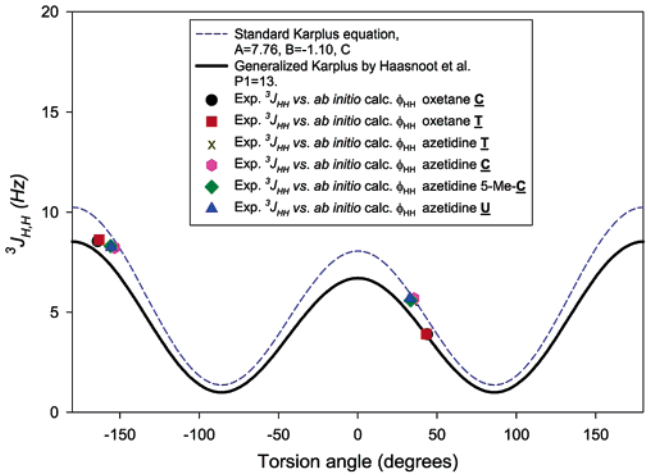


FIGURE 3. Empirical standard and generalized Karplus dependencies[37] compared to the experimental $^3J_{H,H}$ constants (Hz) for the H-3′,H-4′ and H-4′,H-5′ proton–proton interactions in the azetidine-modified pyrimidine nucleosides plotted against the values of the H3′–C3′–C4′–H4′ and H4′–C4′–C5′–H5′ torsions from the 6-31G** ab initio calculated molecular structures of the azetidine-modified **C**, **T**, **U**, and 5-Me-**C** nucleosides.

Theoretical Conformational Analysis (ab Initio and MD Simulations) of Azetidine-Fused Nucleosides. Conformational

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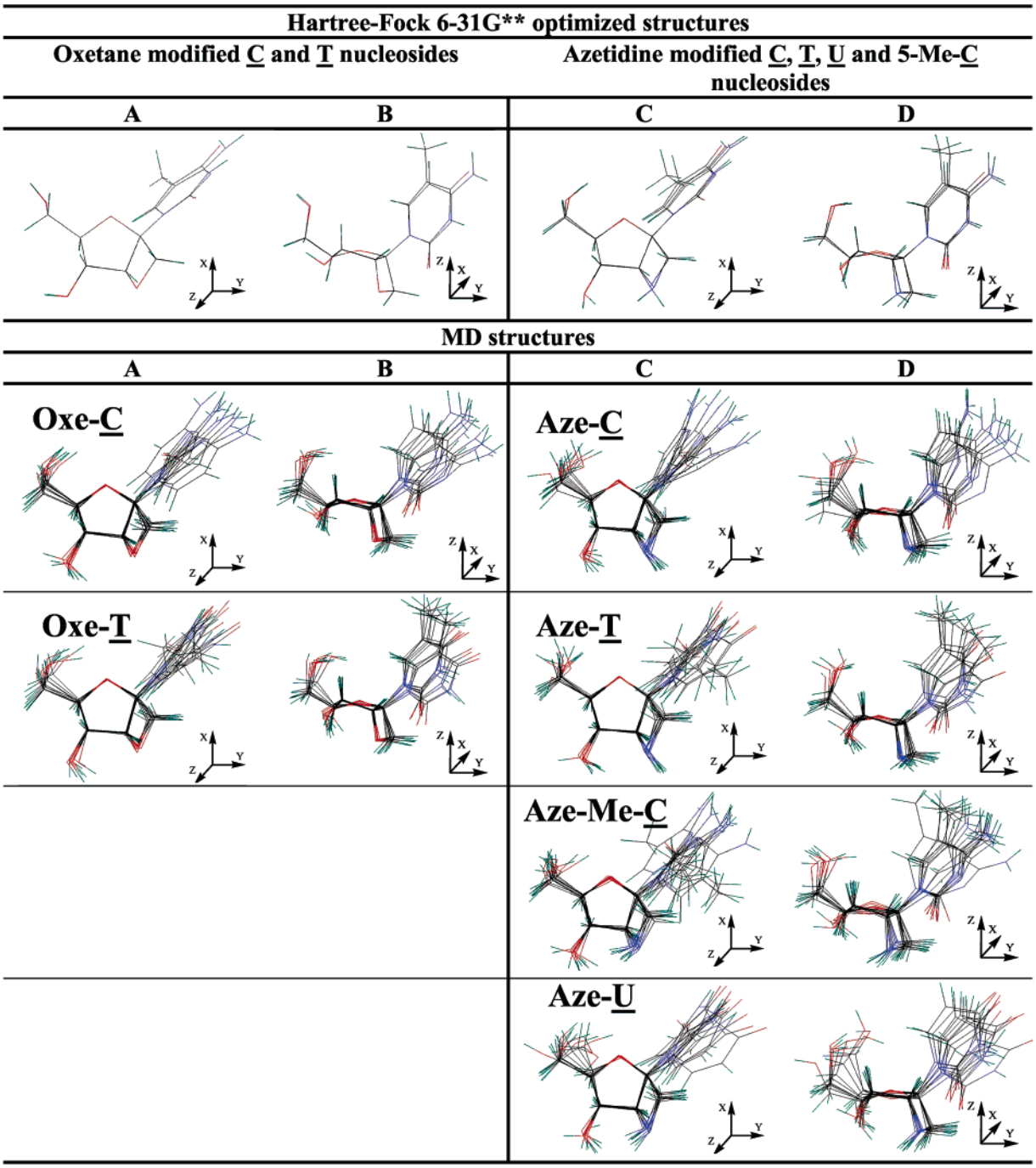

FIGURE 4. Superimposed molecular 3D ab initio and MD (10 snapshots taken from 1.5 to 2 ns time interval of respective MD trajectories) structures of the oxetane-**C** and -**T** (left panels **A** and **B**) and azetidine-**C**, -**T**, -5-Me-**C**, and -**U** (right panels **C** and **D**) shown in two projections (see included axis schemes): (1) from the top (columns **A** and **C**) and (2) vertically rotated by 90° around the *y*-axis (columns **B** and **D**).

analysis of the azetidine-modified **C**, **T**, **U**, and 5-Me-**C** nucleosides has been also performed on the basis of (a) ab initio gas-phase geometry optimizations using 6-31G** Hartree-Fock in the Gaussian 98[36] program package and (b) results of 2 ns AMBER 7[37] molecular dynamics (MD) simulations in the explicit aqueous medium to probe a conformational hyperspace available for these systems (see details in Experimental section). $^3J_{H-3',H-4'}$ and $^3J_{H-4',H-5'}$ coupling constants have been back-calculated from the ab initio obtained torsions employing standard and generalized Karplus equations[31,32] and compared with the experimental ones (Figure 3, Table 3).

(i) Sugar Pucker Conformation. Contrary to the rough estimation of the sugar pucker from the $^3J_{H-3',H-4'}$ vs $^3J_{H-4',H-5'}$ dependence (Figure 2, panel A), which showed *North*-type but much closer to the *East* than to the *North* sugar conformation, both ab initio and MD obtained geometries have demonstrated that the sugar in the azetidine-modified pyrimidine

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nucleosides assumes *North-East* conformation (44° < *P* < 54°, 29° < ϕ_m < 33° from ab initio results) similar to that of the oxetane-modified **T** and **C** nucleosides[8f] with *P* being within 2° of the corresponding value for the oxetane-**C** and **T** (Table 3) and puckering amplitude (ϕ_m) being ~7° lower compared to the oxetane-**T** and -**C** values. As expected, lower electronegativity of nitrogen atom compared to the oxygen led to about 0.02 Å increase in C2′−C3′ and C3′−C4′ bonds and weakened restraining power of the azetidine moiety compared to the oxetane. This resulted in broader conformational subspace available for the azetidine-modified sugar moiety, which has been reflected in higher by ~10° amplitude of the *P* variation during MD trajectories (compare average $P = 34.8 \pm 29.5°$, $\phi_m = 34.8 \pm 29.5°$ for the azetidine-modified pyrimidine series with corresponding $P = 36.0 \pm 19.7°$, $\phi_m = 32.6 \pm 9.1°$ for the oxetanes). Although for the azetidine-**U** and -5-Me-**C** the ab initio obtained sugar puckers both the *P* and ϕ_m were found to be ~10° higher than that of the azetidine-**T** and -**C**, the apparent gas phase conformational preference disappeared in the MD dynamic model (Table 3) resulting in very close corresponding average values of *P* and ϕ_m and similar variations along the trajectories for all azetidine-modified pyrimidines in question.

(ii) RMSd Differences. RMS differences of the sugar atoms along MD trajectories during simulations of azetidine-modified nucleosides have been found to be 0.09 ± 0.07 Å (0.32 ± 0.14 Å for all heavy atoms), 0.09 ± 0.05 Å (0.38 ± 0.15 Å), 0.09 ± 0.03 Å (0.34 ± 0.10 Å), and 0.11 ± 0.06 Å (0.40 ± 0.12 Å) for the azetidine-**C**, -**T**, -**U** and -5-Me-**C** respectively. The RMS difference in positioning of the sugar atoms in the ab initio optimized geometries of these nucleosides has only been 0.01 Å. Figure 4 compares superimposed ab initio and MD (10 snapshots) structures of the oxetane-**C** and -**T** (left panels **A** and **B**) and azetidine-**C**, -**T**, -5-Me-**C**, and -**U** (right panels **C** and **D**) nucleosides. The major movements have been observed for the aglycon part of the azetidine-modified nucleosides similar to conclusions drawn for the oxetane-modified nucleosides.[10] However, significantly more dynamics can be seen for the backbone atoms and the azetidine moiety (up to 0.5 Å) compared to the oxetane-modified nucleosides. However, overall sugar conformation is very similar in azetidine and oxetane-modified nucleosides (sugar atoms RMSd = 0.06 Å).

(iii) Coupling Constants. The empirical Karplus equation[31,32] provided us means to back-calculate the $^3J_{H,H}$ vicinal coupling constants from the ab initio geometries. Theoretical values calculated using optimized parameters for the standard Karplus equation[31] without electronegativity corrections ($^3J_{H',H'} = A\cos^2\phi + B\cos\phi + C$; $A = 7.76$, $B = -1.10$, $C = 1.4$) applied to ab initio calculated H3′−C3′−C4′−H4′ and H4′−C4′−C5′−H5′ torsions appeared to provide somewhat better agreement with the experimental $^3J_{H-3',H-4'}$ and $^3J_{H-4',H-5'}$ values than obtained employing the modified Karplus equation[32] (P1 = 7.76, P2 = 1.1, P3 = 1.4, P4 = P5 = P6 = 0). Maximum absolute error of the calculated $^3J_{H-3',H-4'}$ and $^3J_{H-4',H-5'}$ constants has been 0.58 Hz compared to experimental value (Table 3, Figure 3). The experimental values of $^3J_{H-4',H-5'}$ for the azetidine-modified nucleosides fit well with the corresponding values of the oxetane-**C** and -**T** (Figure 2, panel B) while the difference in the experimental $^3J_{H-3',H-4'}$ directly derived from the H3′−C3′−C4′−H4′ torsions explains the ~7° decrease in the puckering amplitude of the azetidine-modified pyrimidines.

Conclusions

Fused azetidine derivatives of **T**, **U**, and **C** phosphoramidites building blocks were synthesized from **6**-*O*-benzyl-1,2:3,4-bis-*O*-isopropylidene-D-psicofuranose and incorporated into 15 mer AONs. The thermal denaturation studies revealed that the azetidine-modified AONs showed improved target affinity when compared to the isosequential oxetane-modified AON/RNA hybrids. The molecular structures of these monomer units have been studied by means of high-field NMR and theoretical ab initio and MD simulations. The combined experimental NMR data and theoreticalsimulations show that azetidine modification leads sugar moiety into locked *North-East* conformation with the puckering amplitudes and phase angles similar to those observed in the class of oxetane-modified nucleosides.[8f] However, the relatively less electronegative nature of the nitrogen atom compared to the oxygen atom decreases the strength of restrains introduced by the azetidine moiety on the sugar conformation, and although it leads to very similar average sugar conformation, the dynamics of the sugar pucker in the azetidine-modified nucleoside increases *P* fluctuation amplitude by almost 30%. Broader conformational subspace available for the azetidine modification can potentially be a decisive factor for the in vivo and in vitro applications, making these oligos better substrate for a selected target. Further work is in progress.

Experimental Section

General experimental methods are given in Supporting Information. All NMR data are given in δ scale.

1-[6-*O*-Benzyl-3,4-*O*-isopropylidene-β-D-psicofuranosyl]thymine (2a). Thymine (7.1 g, 56.4 mmol) was suspended in hexamethyldisilazane (140 mL); trimethylchlorosilane (13.9 mL) was added and stirred at 120 °C under a nitrogen atmosphere for 16 h. The volatile material was evaporated and the residue was dried for 20 min. Sugar **1** (14.1 g, 40.3 mmol) dissolved in dry acetonitrile was added to the persilylated nucleobase. The mixture was cooled in an ice bath and trimethylsilyl trifluoromethanesulfonate (9 mL, 52.3 mmol) was added dropwise under nitrogen atmosphere. After being stirred for 1 h the reaction was warmed to room temperature and stirred for 17 h. Saturated aqueous NH_4Cl was added with stirring for additional 30 min, followed by filtration. To this residue was added saturated aqueous $NaHCO_3$ and the mixture was extracted with CH_2Cl_2 (4 times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The resultant oil was chromatographed using 0 to 3% MeOH in CH_2Cl_2 as eluent to furnish **2a** (6.23 g, 14.9 mmol, 37%). $R_f = 0.45$ (CH_2Cl_2/CH_3-OH 92:8 v/v); MALDI-TOF *m*/*z* $[M - H]^-$ found 417.1, calcd 417.1; ^{1}H NMR (270 MHz, $CDCl_3$) 7.56 (d, $J = 1.11$ Hz, 1H, H-6), 7.34−7.24 (m, 3H, benzyl), 7.15−7.11 (m, 2H, benzyl), 5.30 (d, $J_{H-3',\ H-4'} = 5.94$ Hz, 1H, H-3′), 4.75 (dd, $J_{H-4',\ H-5'} = 0.87$ Hz, 1H, H-4′), 4.63 (m, 1H, H-5′), 4.43 (d, $J_{gem} = 11.63$ Hz, 1H, CH_2Ph), 4.32 (d, 1H, CH_2Ph), 4.25 (d, $J_{gem} = 12.25$ Hz, 1H, H-1′), 3.80 (d, 1H, H-1″), 3.66−3.51 (m, 2H, H-6′, H-6″), 2.86 (br s, 1H, OH), 1.78 (s, 3H, CH_3, thymine), 1.56 (s, 3H, CH_3, isopropyl), 1.35 (s, 3H, CH_3, isopropyl); ^{13}C NMR (67.9 MHz, $CDCl_3$) 164.4 (C-4), 150.1 (C-2), 138.0 (C-6), 136.7, 128.4, 128.0, 127.0, 112.9 (C-5), 107.7, 101.1 (C-2′), 86.1 (C-3′), 84.6 (C-5′), 82.0 (C-4′), 73.5 (CH_2Ph), 70.4 (C-6′), 64.5 (C-1′), 25.7 (CH_3, isopropyl), 24.3 (CH_3, isopropyl), 12.4 (CH_3, thymine).

1-[6-*O*-Benzyl-1-*O*-(4-methoxytrityl)-β-D-psicofuranosyl]thymine (4). Compound **2a** (6.23 g, 14.9 mmol) was stirred with 75 mL of 90% aqueous CF_3COOH at room temperature for 30 min. The reaction mixture was evaporated to give **3**. The crude reaction mixture was coevaporated with pyridine (4 times) and dissolved in 125 mL of the same solvent, and 4-methoxytrityl chloride (5.29

g, 17.15 mmol) was added and stirred at room temperature for 20 h under nitrogen atmosphere. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue was coevaporated with toluene (4 times) and CH_2Cl_2 (3 times) followed by purification by silica gel column chromatography using 0 to 3% MeOH in CH_2Cl_2 as eluent to afford **4** (8.1 g, 12.46 mmol, 83% after 2 steps). R_f = 0.35 (CH_2Cl_2/CH_3-OH 95:5 v/v); MALDI-TOF *m*/*z* $[M + Na]^+$ found 673.1, calcd 673.2; ^{1}H NMR (270 MHz, $CDCl_3$) 8.13 (br s, 1H, NH), 7.74 (d, J = 1.11, Hz, 1H, H-6), 7.37−7.10 (m, 17H, MMTr, benzyl), 6.77 (d, 2H, MMTr), 4.60 (dd, $J_{H-4',\ H-5'}$ = 2.85 Hz, $J_{H-3',\ H-4'}$ = 5.20 Hz, 1H, H-4′), 4.51−4.42 (m, 2H, CH_2Ph, H-5′), 4.33 (d, J_{gem} = 11.50 Hz, 1H, CH_2Ph), 4.30 (d, 1H, H-3′), 3.76 (s, 3H, OCH_3), 3.66−3.52 (m, 3H, H-1′, H-6′, H-6″), 3.45 (d, J = 10.39, Hz, 1H, H-1″), 2.86 (s, 1H, OH), 1.92 (s, 3H, CH_3, thymine); ^{13}C NMR (67.9 MHz, $CDCl_3$) 163.4 (C-4), 158.5, 151.0 (C-2), 143.9, 143.7, 138.7 (C-6), 137.1, 134.9, 130.1, 128.3, 128.2, 128.2, 127.7, 127.2, 126.9, 113.0 (C-5), 108.4, 99.4 (C-2′), 86.7, 85.7 (C-5′), 77.9 (C-4′), 74.0 (C-3′), 73.6 (CH_2Ph), 70.4 (C-6′), 63.5 (C-1′), 55.1 (OCH_3), 12.3 (CH_3, thymine).

2,3′-Anhydro-1-[6-*O*-benzyl-1-*O*-(4-methoxytrityl)-β-D-fructofuranosyl]thymine (5). 1,1′-Thiocarbonyldiimidazole (2.73 g, 15.33 mmol) was added to the stirred solution of compound **4** (8.1 g, 12.46 mmol) in dry toluene (120 mL). The reaction mixture was heated at 120 °C for 3 h under nitrogen atmosphere. Solvent was evaporated, the residue was dissolved in CH_2Cl_2, and saturated aqueous $NaHCO_3$ was added and extracted with CH_2Cl_2 (4 times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography using 0 to 3% MeOH in CH_2Cl_2 as eluent to afford **5** (5.9 g, 9.33 mmol, 75%). R_f = 0.47 (CH_2Cl_2/CH_3OH 92:8 v/v); MALDI-TOF *m*/*z* $[M - H]^-$ found 631.1, calcd 631.2; ^{1}H NMR (270 MHz, $CDCl_3$) 7.37−7.07 (m, 17H, MMTr, benzyl), 6.80 (d, 2H, MMTr), 6.69 (d, J = 1.24, Hz, 1H, H-6), 5.38 (d, $J_{H-3',\ H-4'}$= 1.36 Hz, 1H, H-3′), 4.62 (br s, 1H, H-4′), 4.36−4.30 (m, 3H, CH_2Ph, H-5′), 3.74 (s, 3H, OCH_3), 3.68 (d, J_{gem} = 10.39 Hz, 1H, H-1′), 3.60 (d, 1H, H-1″), 3.38 (dd, J_{gem} = 10.52 Hz, $J_{H-5',\ H-6'}$ = 3.46 Hz, 1H, H-6′), 3.26 (dd, $J_{H-5',\ H-6''}$ = 5.07 Hz, 1H, H-6″), 1.88 (s, 3H, CH_3, thymine); ^{13}C NMR (67.9 MHz, $CDCl_3$) 172.9 (C-4), 159.8 (C-2), 158.8, 143.0, 142.7, 137.0, 133.9, 130.2, 129.2 (C-6), 128.3, 128.1, 128.0, 127.7, 127.7, 127.3, 118.2 (C-5), 113.3, 99.8 (C-2′), 90.1 (C-3′), 87.6 (C-5′), 87.2, 76.9 (C-4′), 73.4 (CH_2Ph), 69.0 (C-6′), 62.0 (C-1′), 55.1 (OCH_3, MMTr), 14.0. (CH_3, thymine).

2,3′-Anhydro-1-[4,6-*O*-benzyl-1-*O*-(4-methoxytrityl)-β-D-fructofuranosyl]thymine (6a). Compound **5** (5.9 g, 9.33 mmol) was dissolved in 95 mL of dry acetonitrile and cooled to 0 °C and sodium hydride (0.336 g, 14 mmol) was added. The reaction mixture was stirred at 0 °C for 20 min under nitrogen atmosphere. To this solution was added benzyl bromide (1.44 mL, 12.135 mmol), and the mixture was allowed to warm slowly to room temperature and stirred for 6 h. The reaction was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (eluent 0 to 3% MeOH in CH_2Cl_2) to give **6a** (5.47 g, 7.57 mmol, 81%). R_f = 0.39 (CH_2Cl_2/CH_3OH 95:5 v/v); MALDI-TOF *m*/*z* $[M - H]^-$ found 721.1, calcd 721.2; ^{1}H NMR (270 MHz, $CDCl_3$) 7.37−7.11 (m, 22H, MMTr, benzyl), 6.81 (d, 2H, MMTr), 6.65 (d, J = 1.24 Hz, 1H, H-6) 5.13 (s, 1H, H-3′), 4.63 (d, J_{gem} = 11.50 Hz, 1H, CH_2-Ph), 4.54 (d, 1H, CH_2Ph), 4.41−4.26 (m, 4H, CH_2Ph, H-4′, H-5′), 3.77 (s, 3H, CH_3, MMTr), 3.58 (ABq, J_{gem} = 10.64 Hz, 2H, H-1′, H-1″), 3.33−3.20 (ddd, J_{gem} = 10.52 Hz, $J_{H-5',\ H-6'}$ = 4.58 Hz, 2H, H-6′, H-6″), 1.91 (s, 3H, CH_3, thymine); ^{13}C NMR (67.9 MHz, $CDCl_3$) 172.3 (C-4), 159.6 (C-2), 158.8, 142.9, 142.7, 136.7, 136.1, 133.9, 130.2, 128.6 (C-6), 128.2, 128.0, 127.9, 127.8, 127.6, 127.3, 118.5 (C-5), 113.3, 100.1 (C-2′), 87.3 (C-3′), 87.1, 85.9 (C-5′), 83.8 (C-4′), 73.5 (CH_2Ph), 72.2 (CH_2Ph), 68.7 (C-6′), 61.7 (C-1′), 55.1 (OCH_3), 14.1 (CH_3, thymine).

1-[4,6-*O*-Benzyl-β-D-fructofuranosyl]thymine (7). Compound **6a** (5.47 g, 7.57 mmol) was dissolved in 220 mL of 80% aqueous acetic acid and stirred at ambient temperature for 24 h. The acid was removed under reduced pressure, coevaporated with toluene (4 times) and CH_2Cl_2 (3 times) to give **6b**. The product obtained without further purification was dissolved in 70 mL of EtOH−H_2O solution (v/v 1:1), and 13 mL of 1 N NaOH was added, followed by dropwise addition of dioxane till the solution becomes homogeneous. The mixture was stirred overnight, then poured into saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2 (4 times). The organic phase was dried over $MgSO_4$, filtered, evaporated, and coevaporated with toluene (4 times) and CH_2Cl_2 (3 times). The residue was subjected to column chromatography using 0 to 5% MeOH in CH_2Cl_2 as eluent to give **7** (3.26 g, 6.96 mmol, 92% after two steps). R_f = 0.37 (CH_2Cl_2/CH_3OH 92:8 v/v); MALDI-TOF *m*/*z* $[M - H]^-$ found 467.1, calcd 467.1; ^{1}H NMR (270 MHz, $CDCl_3$) 7.75 (s, 1H, H-6), 7.39−7.20 (m, 10H, benzyl), 4.72−4.62 (m, 2H, 1 × CH_2Ph, H-3′), 4.57−4.46 (m, 3H, 2 × CH_2Ph, 1 × CH_2Ph), 4.41 (m, 1H, H-5′), 4.29−4.13 (m, 2H, H-1′, OH), 4.04−3.93 (m, 2H, H-4′, H-1″), 3.70−3.54 (m, 2H, H-6′, H-6″), 1.80 (s, 3H, CH_3, thymine); ^{13}C NMR (67.9 MHz, $CDCl_3$) 164.8 (C-4), 151.0 (C-2), 138.2 (C-6), 136.9, 136.8, 128.5, 128.1, 127.9, 127.7, 127.6, 108.9 (C-5), 101.2 (C-2′), 85.1 (C-4′), 83.6 (C-5′), 76.0 (C-3′), 73.5 (CH_2Ph), 71.7 (CH_2Ph), 69.3 (C-6′), 63.8 (C-1′), 12.4 (CH_3, thymine).

1-[1,3-*O*-Acetyl-4,6-*O*-benzyl-β-D-fructofuranosyl]thymine (8). See Supporting Information (SI) page S3 for details.

1-[1,3-*O*-Acetyl-4,6-*O*-benzyl-β-D-fructofuranosyl]3-*N*-(4-methoxybenzyl)thymine (9). Compound **8** (3.3 g, 5.98 mmol) was dissolved in 70 mL of dry DMF and cooled to 0 °C and sodium hydride (0.216 g, 9 mmol) was added. The reaction mixture was stirred at 0 °C for 1 h under nitrogen atmosphere. To this solution was added 4-methoxybenzyl chloride (PMBCl) (1.08 mL, 7.96 mmol), and the mixture was allowed to warm slowly to room temperature and stirred for 6 h. The reaction was quenched by saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography using 0 to 20% ethyl acetate in cyclohexane as eluent to furnish **9** (3.2 g, 4.76 mmol, 80%). R_f = 0.71 (CH_2Cl_2/CH_3OH 98:2 v/v); MALDI-TOF *m*/*z* $[M - H]^-$ found 671.1, calcd 671.2; ^{1}H NMR (270 MHz, $CDCl_3$) 7.57 (s, 1H, H-6), 7.45−7.16 (m, 12H, benzyl, PMB), 6.80 (d, 2H, PMB), 5.67 (s, H-3′), 5.19 (d, J_{gem} = 13.36 Hz, 1H, PMB), 4.88−4.80 (m, 2H, 1 × CH_2Ph, 1 × PMB), 4.79−4.68 (ABq, J_{gem} = 12.00 Hz, 2H, H-1′, H-1″), 4.62 (d, 1H, CH_2Ph), 4.53−4.37 (m, 3H, 2 × CH_2Ph, H-5′), 3.82 (d, $J_{H-4',\ H-5'}$ = 2.60 Hz, 1H, H-4′), 3.75 (s, 3H, OCH_3), 3.52 (dd, J_{gem} = 10.39 Hz, $J_{H-5',\ H-6'}$ = 5.81 Hz, 1H, H-6′), 3.42 (dd, $J_{H-5',\ H-6''}$ = 5.57 Hz 1H, H-6″), 1.99 (s, 3H, CH_3, *O*-acetyl), 1.93 (s, 3H, CH_3, thymine), 1.32 (s, 3H, CH_3, *O*-acetyl); ^{13}C NMR (67.9 MHz, $CDCl_3$) 169.9 (C═O), 168.7 (C═O), 163.6 (C-4), 159.0, 149.9 (C-2), 137.4, 137.0, 134.8 (C-6), 130.8, 128.3, 127.8, 127.7, 127.4, 113.5, 108.4 (C-5), 96.7 (C-2′), 83.4 (C-5′), 82.6 (C-4′), 76.9 (C-3′), 73.1 (CH_2Ph), 71.6 (CH_2Ph), 68.9 (C-6′), 62.2 (C-1′), 55.1 (OCH_3), 43.5 (CH_2, PMB), 20.5 (CH_3, O-acetyl), 19.8 (CH_3, O-acetyl), 13.3 (CH_3, thymine).

1-[4,6-*O*-Benzyl-β-D-fructofuranosyl]-3-*N*-(4-methoxybenzyl)-thymine (10). See SI page S3 for details.

1-[4,6-*O*-Benzyl-3-*O*-methanesulfonyl-1-phthalimido-β-D-fructofuranosyl]-3-*N*-(4-methoxybenzyl)thymine (12). Compound **10** (1.0 g, 1.7 mmol) was coevaporated with dry pyridine (3 times) and dissolved in 50 mL of the same solvent, the solution was cooled to 0 °C, and methanesulfonyl chloride (0.8 mL, 10.22 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h and then at 4 °C overnight. The crude reaction mixture was poured into ice-cooled saturated aqueous $NaHCO_3$ and extracted with CH_2-Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, and evaporated to give **11a** along with traces of **11b**. The residue was coevaporated with toluene (3 times) and kept for drying on an oil pump for 20 min. Crude compound **11a** was dissolved in 50

mL of dry DMF, and potassium phthalimide (1.89 g, 10.2 mmol) was added followed by stirring at 110 °C overnight under nitrogen atmosphere. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue was coevaporated with toluene (5 times) and CH_2Cl_2 (3 times). The mixture was purified by silica gel column chromatography using 0 to 30% ethyl acetate in cyclohexane as eluent to afford **12** (1.04 g, 1.3 mmol, 77% after two steps and recovered **11b**, 7%). $R_f = 0.69$ (CH_2Cl_2/CH_3OH 98:2v/v); MALDI-TOF *m*/*z* $[M - H]^-$ found 794.0, calcd 794.2; ^1H NMR (270 MHz, $CDCl_3$) 7.91−7.70 (m, 4H, phthalimido), 7.41−7.24 (m, 13H, benzyl, 2 × PMB, H-6), 6.81 (d, 2H, PMB), 5.56−5.47 (m, 2H, 1 × PMB, H-3′), 4.84−4.71 (m, 4H, 2 × CH_2Ph, 1 × PMB, H-5′), 4.65 (d, $J_{gem} = 14.23$ Hz, 2H, H-1′), 4.53−4.45 (m, 3H, 2 × CH_2Ph, H-1″), 4.35 (d, $J_{H-4',\ H-5'} = 2.35$ Hz, 1H, H-4′), 3.77 (s, 3H, OCH_3), 3.51 (ddd, $J_{gem} = 10.27$ Hz, $J_{H-5',\ H-6'} = 6.19$ Hz, $J_{H-5',\ H-6''} = 5.94$ Hz, 2H, H-6′, H-6″), 2.27 (s, 3H, CH_3, *O*-mesyl), 1.77 (s, 3H, CH_3, thymine); ^{13}C NMR (67.9 MHz, $CDCl_3$) 167.8 (C═O), 163.7 (C-4), 158.9, 150.6 (C-2), 137.5, 136.9, 134.1, 133.7 (C-6), 131.5, 130.1, 129.5, 128.3, 127.8, 127.6, 123.4, 113.6, 109.4 (C-5), 96.7 (C-2′), 83.8 (C-4′), 83.4 (C-3′), 83.1 (C-5′), 73.1 (CH_2Ph), 72.1 (CH_2Ph), 69.3 (C-6′), 55.2 (OCH_3), 44.1 (CH_2, PMB), 38.5 (C-1′), 37.4 (CH_3, *O*-mesyl), 13.2 (CH_3, thymine).

1-[1-Amino-4,6-*O*-benzyl-3-*O*-methanesulfonyl-β-D-fructofuranosyl]-3-*N*-(4-methoxybenzyl)thymine (13). Compound **12** (0.8 g, 1.01 mmol) was dissolved in 10 mL of methanol, 50 mL of 40% aqueous methylamine was added, followed by dropwise addition of methanol till the solution becomes homogeneous. The reaction mixture was stirred at room temperature for 4 h, then was poured into saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2 (4 times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue was coevaporated with toluene (3 times) and CH_2Cl_2 (3 times). The mixture was purified by silica gel column chromatography using 0 to 5% MeOH in CH_2Cl_2 as eluent gave **13** (0.62 g, 0.93 mmol, 93%). $R_f = 0.55$ (CH_2Cl_2/CH_3OH 92:8 v/v); MALDI-TOF *m*/*z* $[M - H]^-$ found 664.1, calcd 664.2; ^1H NMR (270 MHz, $CDCl_3$) 7.65 (d, $J = 1.24$ Hz, 1H, H-6), 7.39−7.25 (m, 12H, benzyl, PMB), 6.80 (d, 2H, PMB), 5.32 (d, $J_{gem} = 14.47$ Hz 1H, 1 × PMB), 5.24 (s, 1H, H-3′), 4.80−4.70 (m, 2H, 1 × CH_2Ph, 1 × PMB), 4.57 (d, $J_{gem} = 11.75$ Hz, 1H, 1 × CH_2Ph), 4.52 (s, 2H, CH_2Ph), 4.36−4.29 (m, 1H, H-5′), 4.26 (d, $J_{H-4',\ H-5'} = 2.10$ Hz, 1H, H-4′), 3.76 (s, 3H, OCH_3), 3.64−3.47 (m, 3H, H-1′, H-6′, H-6″), 3.27 (d, $J_{gem} = 14.23$ Hz, 1H, H-1″), 2.29 (s, 3H, CH_3, *O*-mesyl), 1.96 (s, 3H, CH_3, thymine); ^{13}C NMR (67.9 MHz, $CDCl_3$) 163.6 (C-4), 159.0, 150.0 (C-2), 137.4, 136.8, 135.5 (C-6), 130.2, 129.3, 128.4, 128.0, 127.8, 127.6, 113.7, 108.6 (C-5), 99.0 (C-2′), 83.9 (C-4′), 83.5 (C-5′), 83.1 (C-3′), 73.4 (CH_2Ph), 72.3 (CH_2Ph), 69.6 (C-6′), 55.2 (OCH_3), 43.9 (CH_2, PMB), 37.5 (CH_3, *O*-mesyl), 13.5 (CH_3, thymine).

(1*R*,3*R*,4*S*,5*R*)-3-Benzyloxymethyl-4-benzyloxy-1-(3-*N*-(4-methoxybenzyl)thymine-1-yl)-6-aza-2-oxabicyclo[3.2.0]heptane (14). Compound **13** (2.45 g, 3.68 mmol) was dissolved in 55 mL of pyridine, 2.6 mL of triethylamine was added and heated at 90 °C for 48 h under nitrogen atmosphere. The solvent was removed under reduced pressure. The residue was coevaporated with toluene (3 times) and CH_2Cl_2 (3 times) and subjected to column chromatography using 0 to 5% MeOH in CH_2Cl_2 as eluent to give **14** (1.38 g, 2.42 mmol, 66%). $R_f = 0.43$ (CH_2Cl_2/CH_3OH 92:8 v/v); MALDI-TOF *m*/*z* $[M - H]^-$ found 568.1, calcd 568.2; ^1H NMR (270 MHz, $CDCl_3$) 7.44−7.25 (m, 12H, benzyl, 2 × PMB), 6.84−6.75 (m, 3H, 2 × PMB, H-6), 5.06−4.93 (ABq, $J_{gem} = 13.73$ Hz, 2H, PMB), 4.69 (d, $J_{H-3',\ H-4'} = 5.32$ Hz, 1H, H-3′), 4.59−4.38 (m, 5H, 2 × CH_2Ph, 2 × CH_2Ph, H-5′), 4.30 (dd, $J_{H-4',\ H-5'} = 7.67$ Hz, 1H, H-4′), 4.08 (d, $J_{gem} = 10.39$ Hz, 1H, H-1′), 3.86 (d, 1H, H-1″), 3.81−3.73 (m, 4H, OCH_3, H-6′), 3.67 (dd, $J_{gem} = 10.70$ Hz, $J_{H-5',\ H-6''} = 6.43$ Hz, 1H, H-6″), 1.88 (s, 3H, CH_3, thymine); ^{13}C NMR (67.9 MHz, $CDCl_3$) 163.3 (C-4), 159.0, 149.8 (C-2), 137.8, 137.3, 133.7 (C-6), 130.5, 128.4, 128.2, 127.9, 127.6, 113.6, 110.4 (C-5), 94.1 (C-2′), 82.2 (C-5′), 78.9 (C-4′), 73.4 (CH_2Ph), 72.8 (CH_2Ph), 70.1 (C-6′), 65.2 (C-3′), 55.2 (C-1′), 55.1 (OCH_3), 43.5 (CH_2, PMB), 13.0 (CH_3, thymine).

(1*R*,3*R*,4*S*,5*R*)-3-Benzyloxymethyl-4-benzyloxy-1-(thymine-1-yl)-6-aza-2-oxabicyclo[3.2.0]heptane (15). Compound **14** (0.38 g, 0.668 mmol) was dissolved in 7.6 mL CH_3CN-H_2O mixture (v/v 9:1), ceric ammonium nitrate (1.36 g, 2.48 mmol) was added, and the reaction mixture was stirred at room temperature for 3 h. Saturated aqueous $NaHCO_3$ was added, and the mixture was stirred for an additional 20 min, then filtered and extracted with CH_2Cl_2 (3 times). The extract was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography using 0 to 5% MeOH in CH_2Cl_2 as eluent to give **15** (0.187 g, 0.416 mmol, 62%). $R_f = 0.35$ (CH_2Cl_2/CH_3OH 92:8 v/v); MALDI-TOF *m*/*z* $[M - H]^-$ found 448.1, calcd 448.1; ^1H NMR (270 MHz, $CDCl_3$) 8.55 (br s, 1H, NH), 7.41−7.24 (m, 10H, benzyl), 6.81 (d, $J = 0.99$ Hz, 1H, H-6), 4.72 (d, $J_{H-3',\ H-4'} = 5.32$ Hz, 1H, H-3′), 4.61−4.50 (ABq, 2H, CH_2Ph), 4.49 (s, 2H, CH_2Ph), 4.47−4.39 (m, 1H, H-5′), 4.22 (dd, $J_{H-4',\ H-5'} = 7.79$ Hz, 1H, H-4′), 4.11 (d, $J_{gem} = 10.39$ Hz, 1H, H-1′), 3.86 (d, 1H, H-1″), 3.71 (ddd, $J_{gem} = 10.64$ Hz, $J_{H-5',\ H-6'} = 2.47$ Hz, $J_{H-5',\ H-6''} = 6.80$ Hz, 2H, H-6′, H-6″), 1.88 (s, 3H, CH_3, thymine); ^{13}C NMR (67.9 MHz, $CDCl_3$) 163.5 (C-4), 149.0 (C-2), 137.7, 137.2, 135.8 (C-6), 128.4, 128.3, 127.9, 127.7, 111.0 (C-5), 93.5 (C-2′), 82.1 (C-5′), 78.7 (C-4′), 73.4 (CH_2Ph), 72.8 (CH_2Ph), 70.2 (C-6′), 65.1 (C-3′), 55.0 (C-1′), 12.2 (CH_3, thymine).

(1*R*,3*R*,4*S*,5*R*)-3-Benzyloxymethyl-4-benzyloxy-6-*N*-phenoxyacetyl-1-(thymine-1-yl)-6-aza-2-oxabicyclo[3.2.0]heptane (16). Compound **15** (0.32 g, 0.71 mmol) was coevaporated with dry pyridine (3 times) and dissolved in 12 mL of the same solvent. Phenoxyacetyl chloride (0.13 mL, 0.93 mmol) was added dropwise and stirred at room temperature for 3 h. The reaction mixture was poured into ice-cooled saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue was coevaporated with toluene (4 times) and CH_2Cl_2 (3 times) followed by purification by silica gel column chromatography using 0 to 3% MeOH in CH_2Cl_2 as eluent to afford **16** (0.36 g, 0.62 mmol, 86%). $R_f = 0.53$ (CH_2Cl_2/CH_3OH 92:8 v/v); MALDI-TOF *m*/*z* $[M - H]^-$ found 582.1, calcd 582.2; ^{13}C NMR (67.9 MHz, $CDCl_3$) 169.6, 163.3, 157.1, 149.0, 137.6, 129.6, 128.3, 127.6, 121.9, 121.5, 114.2, 103.2, 111.7, 90.6, 89.7, 81.6, 78.0, 73.9, 73.3, 73.0, 70.7, 68.9, 68.1, 67.8, 67.3, 61.1, 57.5, 12.2.

(1*R*,3*R*,4*S*,5*R*)-3,4-Hydroxy-6-*N*-phenoxyacetyl-1-(thymine-1-yl)-6-aza-2-oxabicyclo[3.2.0]heptane (17). Compound **16** (0.03 g, 0.051 mmol) was dissolved in 1.5 mL of methanol, and $Pd(OH)_2$ on charcoal (20% moist, 0.01 g) and ammonium formate (0.042 g, 0.66 mmol) were added to the solution of nucleoside. The resulting suspension was heated under reflux for 3 h. The reaction mixture was filtered through silica gel bed and washed with hot methanol. The filtrate was concentrated to dryness under reduced pressure and purified by silica gel column chromatography using 0 to 10% MeOH in CH_2Cl_2 as eluent to afford **17** (0.019 g, 0.047 mmol, 92%). $R_f = 0.28$ (CH_2Cl_2/CH_3OH 90:10 v/v); MALDI-TOF *m*/*z* $[M - H]^-$, found 402.0, calcd 402.1; ^{13}C NMR (67.9 MHz, CD_3OD): 172.6, 166.7, 159.9, 159.4, 151.6, 138.5, 131.0, 130.8, 123.1, 122.7, 116.0, 112.3, 91.4, 85.5, 85.2, 73.8, 73.0, 71.7, 71.2, 68.0, 66.5, 62.7, 62.4, 59.7, 12.5.

(1*R*,3*R*,4*S*,5*R*)-3-(4,4′-Dimethoxytrityloxymethyl)-4-hydroxy-6-*N*-phenoxyacetyl-1-(thymine-1-yl)-6-aza-2-oxabicyclo[3.2.0]-heptane (18a). See SI page S4 for details.

(1*R*,3*R*,4*S*,5*R*)-3-(4,4′-Dimethoxytrityloxymethyl)-4-hydroxy-1-(5-methylcytosin-1-yl)-6-*N*-phenoxyacetyl-6-aza-2-oxabicyclo-[3.2.0]heptane (19). Compound **18a** (0.35 g, 0.496 mmol) was dissolved in 10 mL of dry pyridine, and acetic anhydride (0.47 mL, 4.96 mmol) was added and stirred at room temperature for 28 h. Then reaction was quenched with methanol (0.95 mL) and stirred overnight at room temperature. Saturated aqueous $NaHCO_3$ was added to the reaction mixture and extracted with CH_2Cl_2 (3 times).

The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure to give **18b**. The residue was coevaporated with dry pyridine (3 times) and dissolved in 5 mL of the same solvent. To this solution was added 1,2,4-triazole (0.342 g, 4.96 mmol), and the mixture was cooled to 0 °C. 2-Chlorophenylphosphodichloridate (0.41 mL, 2.48 mmol) was added dropwise to the reaction mixture under nitrogen atmosphere. After being stirred at 0 °C for 30 min the reaction mixture was warmed to room temperature and stirred for 6 h. Aqueous ammonia (32%, 10 mL) was added, and the reaction mixture was stirred at room temperature overnight. The resulting solution was treated with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (4 times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography using 0 to 5% MeOH in CH_2Cl_2 as eluent to give **19** (0.245 g, 0.348 mmol, 70%). R_f = 0.37 (CH_2Cl_2/CH_3OH 92:8 v/v); MALDI-TOF *m/z* $[M - H]^-$ found 703.2, calcd 703.2; ^{13}C NMR (67.9 MHz, $CDCl_3$) 170.5, 169.7, 165.5, 158.3, 157.7, 157.1, 155.2, 144.6, 137.9, 137.7, 135.7, 129.9, 129.6, 129.2, 127.9, 127.6, 126.6, 121.8, 121.0, 114.5, 114.2, 112.9, 103.3, 103.1, 89.8, 89.6, 86.0, 85.9, 83.1, 81.5, 72.8, 72.4, 71.6, 70.9, 67.1, 66.0, 63.2, 62.6, 62.1, 55.0, 12.9.

(1*R*,3*R*,4*S*,5*R*)-4-(2-Cyanoethoxy(diisopropylamino)-phosphinoxy)-3-(4,4′-dimethoxytrityloxy-methyl)-6-*N*-phenoxyacetyl-1-(thymine-1-yl)-6-aza-2-oxabicyclo[3.2.0]heptane (20). Compound **18a** (0.054 g, 0.0765 mmol) was dissolved in 0.8 mL of dry THF, diisopropylethylamine (0.066 mL, 0.382 mmol) was added at 0 °C under nitrogen atmosphere, and the reaction mixture was stirred for 15 min. To this solution was added 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite (0.034 mL, 0.153 mmol)dropwise, the mixture was stirred at this temperature for 20 min, and thereafter the reaction was warmed to room temperature and stirred for 2 h. The reaction was quenched with methanol (0.3 mL) and continued stirring for another 30 min. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The crude residue was purified by silica gel column chromatography using 0 to 1.5% MeOH in CH_2Cl_2 as eluent containing 0.5% Et_3N to afford **20** (0.059 g, 0.065 mmol, 86%). R_f = 0.55 (CH_2Cl_2/CH_3OH 92:8 v/v); MALDI-TOF *m/z* $[M - H]^-$ found 904.2, calcd 904.3; ^{31}P NMR (67.9 MHz, $CDCl_3$) 154.7, 150.7, 149.9, 148.6.

6-*O*-Benzyl-1,3,4-tri-*O*-(4-toluoyl)-D-psicofuranose (22). The sugar **1** (7 g, 20 mmol) was refluxed with 70% aqueous CH_3COOH (120 mL) at 80 °C for 5 h. The reaction mixture was cooled, acid was evaporated, and residue was coevaporated with water (5 times) to give compound **21**. The crude product was coevaporated with dry pyridine (3 times), dissolved in dichloromethane/pyridine (7:1, v/v, 100 mL), at 0 °C, under nitrogen atmosphere, 4-toluoyl chloride (8.2 mL, 62 mmol) was added dropwise, and the reaction was stirred for 4 h at this temperature. The reaction mixture was poured into saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, evaporated, and coevaporated with toluene (3 times) and CH_2Cl_2 (3 times). The residue was purified by column chromatography (eluent ethyl acetate 0−30% in cyclohexane), which afforded **22** (9.4 g, 15 mmol, 75% after two steps) as a mixture of anomers. R_f = 0.57 (cyclohexane/EtOAc 65:35 v/v); MALDI-TOF *m/z* $[M + Na]^+$ found 647.0, calcd 647.2; ^{13}C NMR (125.7 MHz, $CDCl_3$) 171.2, 166.4, 166.1, 165.5, 165.4, 164.9, 164.8, 148.7, 144.3, 144.2, 144.1, 144.0, 143.7, 143.6, 137.6, 136.9, 136.8, 130.1, 129.8,129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.5, 128.4, 128.0, 127.9, 127.6, 127.4, 126.7, 126.7, 126.4, 126.2, 126.1, 124.0, 104.3, 102.0, 81.8, 81.6, 76.5, 73.8, 73.5, 72.6, 72.4, 71.9, 69.8, 69.4, 65.5, 64.9, 21.7, 21.6, 21.5.

2-*O*-Acetyl-6-*O*-benzyl-1,3,4-tri-*O*-(4-toluoyl)-D-psicofuranose (23). The sugar **22** (9.4 g, 15 mmol) after coevaporation with pyridine (3 times), was dissolved in 75 mL of this same solvent and treated with acetic anhydride (28 mL, 300 mmol). The reaction mixture was stirred at room temperature for 48 h under nitrogen atmosphere. The reaction mixture was poured into saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, filtered, evaporated, and coevaporated with toluene (3 times). Column chromatography (gradient 0−30% ethyl acetate in cyclohexane) afforded **23** as an anomeric mixture (9 g, 13.5 mmol, 90%). R_f = 0.61 (cyclohexane/EtOAc 65:35 v/v); MALDI-TOF *m/z* $[M + Na]^+$ found 689.0, calcd 689.2; ^{13}C NMR (125.7 MHz, $CDCl_3$) 171.3, 168.8, 168.7, 165.6, 165.5, 165.4, 164.7, 164.6, 148.8, 144.4, 144.2, 144.1, 143.9, 143.7, 143.6, 137.7, 137.6, 136.8, 130.2, 129.8, 129.7, 129.6, 129.2, 129.1, 129.0, 128.9, 128.4, 128.3, 127.8, 127.7, 127.6, 127.4, 126.7, 126.6, 126.2, 126.1, 108.5, 106.5, 83.8, 82.3, 74.9, 73.6, 73.5, 72.9, 71.4, 70.8, 68.9, 68.8, 63.3, 61.3, 21.8, 21.7, 21.6, 21.5.

1-[1,3,4-*O*-(4-Toluoyl)-6-*O*-benzyl-β-D-psicofuranosyl]uracil (24). The sugar **23** (6.6 g, 10 mmol) and uracil (1.3 g, 12 mmol) were dissolved in dry acetonitrile (100 mL). *N*,*O*-Bis(trimethylsilyl)-acetamide (4.95 mL, 20 mmol) was added and the reaction was heated at 90 °C for 1.5 h (the reaction becomes clear solution) under nitrogen atmosphere. The reaction mixture was cooled to room temperature; trimethylsilyl trifluoromethanesulfonate (1 mL, 5.5 mmol) was added and again heated at 40 °C for 2 h under nitrogen atmosphere. After cooling the reaction mixture was poured into saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, and evaporated. The crude residue after column chromatography (0 to 2% MeOH in CH_2Cl_2) afforded **24** (α:β, 1:9; 4.65 g, 6.5 mmol, 65%). R_f = 0.57 (CH_2Cl_2/CH_3OH 96:4 v/v); MALDI-TOF *m/z* $[M + Na]^+$ found 741.6, calcd 741.2; ^1H NMR (270 MHz, $CDCl_3$) 8.67 (br s, 1H, NH), 7.91 (d, $J_{H-5,\ H-6}$ = 8.16 Hz, 1H, H-6), 7.87−7.76 (m, 6H, 4-toluoyl), 7.37−7.25 (m, 5H, benzyl), 7.19−7.12 (m, 6H, 4-toluoyl), 6.42 (d, $J_{H-3',\ H-4'}$ = 5.44 Hz, 1H, H-3′), 5.83 (dd, $J_{H-4',\ H-5'}$ = 2.85 Hz, 1H, H-4′), 5.14 (d, J_{gem} = 11.75 Hz, 1H, H-1′), 4.99 (d, 1H, H″), 4.71 (m, 1H, H-5′), 4.55 (d, J_{gem} = 11.26 Hz, 1H, CH_2Ph), 4.40 (d,1H, CH_2Ph), 3.80−3.70 (m, 2H, H-6′, H-6″), 2.37 (s, 6H, CH_3, 4-toluoyl), 2.36 (s, 3H, CH_3, 4-toluoyl); ^{13}C NMR (67.9 MHz, $CDCl_3$) 165.55 (C═O), 165.5 (C═O), 164.4 (C═O), 163.6 (C-4), 149.8 (C-2), 144.4, 144.2, 141.2 (C-6), 136.5, 129.8, 129.3, 128.7, 127.6, 100.4 (C-5), 97.5 (C-2′), 84.4 (C-5′), 76.8 (C-3′), 74.0 (CH_2Ph), 73.6 (C-4′), 69.0 (C-6′), 64.4 (C-1′), 21.7 (CH_3, 4-Toluoyl).

1-[6-*O*-Benzyl-1-*O*-(4-monomethoxytrityl)-β-D-psicofuranosyl]-uracil (26). See SI page S4 for details.

2′,3-Anhydro-1-[6-*O*-benzyl-1-*O*-(4-monomethoxytrityl)-β-D-fructofuranosyl]uracil (27). Compound **26** (3.1 g 4.8 mmol) was dissolved in dry DMF (20 mL), and diphenyl carbonate (1.13 g, 5.28 mmol) and $NaHCO_3$ (806 mg, 9.6 mmol) were added. The mixture was heated at 110 °C for 2 h under nitrogen atmosphere. The solvent was evaporated under reduced pressure, the residue was dissolved in CH_2Cl_2, and brine was added and extracted with CH_2Cl_2 (3 times). The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was purified using column chromatography (eluent 0 to 3% MeOH in CH_2Cl_2), which afforded **27** (2.35 g, 3.8 mmol, 91%). R_f = 0.3 (CH_2Cl_2/CH_3OH 96:4 v/v); MALDI-TOF *m/z* $[M + H]^+$ found 619.5, calcd 619.2; ^1H NMR (270 MHz, $CDCl_3$) 7.36−7.07 (m, 17H, MMTr, Benzyl), 6.90 (d, $J_{H-6,\ H-5}$ = 7.55 Hz, 1H, H-6), 6.79 (d, 2H, MMTr), 6.07 (br s, 1H, OH-4′), 5.92 (d, $J_{H-5,\ H-6}$ = 7.55 Hz, 1H, H-5), 5.41 (s, 1H, H-3′), 4.65 (br s, 1H, H-4′), 4.36 (m, 1H, H-5′), 4.27 (ABq, J_{gem} = 12.49 Hz, 2H, CH_2Ph), 3.76 (s, 3H, OCH_3, MMTr), 3.65 (ABq, J_{gem} = 10.64 Hz, 2H, H-1′, H-1″), 3.36−3.19 (ddd, J_{gem} = 10.89 Hz, $J_{H-5',\ H-6'}$ = 3.22 Hz, 2H, H-6′, H-6″); ^{13}C NMR (67.9 MHz, $CDCl_3$) 172.7 (C-4), 160.4 (C-2), 158.7, 143.0, 142.7, 137.0, 133.9 (C-6), 133.7, 130.1, 128.2, 128.1, 128.03, 127.9, 127.7, 127.2, 113.3, 109.3(C-5), 99,9 (C-2′), 90.5 (C-3′), 88.4 (C-5′), 87.1, 76.4(C-4′), 73.4 (CH_2Ph), 69.9 (C-6′), 62.2 (C-1′), 55.1 (OCH_3).

2′,3-Anhydro-1-[4,6-*O*-benzyl-1-*O*-(4-monomethoxytrityl)-β-D-fructofuranosyl]uracil (28). Compound **27** (2.3 g, 3.8 mmol) was dissolved in 40 mL of dry CH_3CN and cooled to 0 °C, NaH (140 mg, 3.45 mmol) was added, and the mixture was stirred for

15 min under nitrogen atmosphere. To this turbid solution was added benzyl bromide (0.54 mL, 4.56 mmol), and the mixture was allowed to warm slowly to room temperature. It was then stirred overnight under nitrogen atmosphere. The solvent was removed under reduced pressure; saturated aqueous $NaHCO_3$ was added and extracted with CH_2Cl_2 (3 times). The organic phase was dried using $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (eluent 0 to 3% MeOH in CH_2Cl_2) to give **28** (2.28 g, 3.23 mmol, 85%). $R_f = 0.38$ (CH_2Cl_2/CH_3OH 96:4 v/v); MALDI-TOF *m/z* $[M + H]^+$ found 709.6, calcd 709.2; ^{1}H NMR (270 MHz, $CDCl_3$) 7.33−7.12 (m, 22H, MMTr, benzyl), 6.90 (d, $J_{H-6,\ H-5} = 8.29$ Hz, 1H, H-6), 6.79 (d, 2H, MMTr), 5.99 (d, $J_{H-5,\ H-6} = 8.16$ Hz, 1H, H-5), 5.08 (s, 1H, H-3′), 4.52 (ABq, $J_{gem} = 11.75$ Hz, 2H, CH_2Ph), 4.47−4.22 (m, 4H, CH_2-Ph, H-4′, H-5′), 3.76 (s, 3H, CH_3, MMTr), 3.58 (ABq, $J_{gem} = 10.64$ Hz, 2H, H-1′, H-1″), 3.32−3.20 (ddd, $J_{gem} = 10.51$ Hz, $J_{H-5',\ H-6'} = 4.08$ Hz, 2H, H-6′, H-6″); ^{13}C NMR (67.9 MHz, $CDCl_3$) 171.7 (C-4), 159.9 (C-2), 158.7, 142.8, 142.7, 136.6, 136.0, 133.8, 132.5 (C-6), 130.0, 128.5, 128.2, 128.1, 128.0, 127.9, 127.6, 127.2 113.3, 109.8(C-5), 99.9 (C-2′), 87.5(C-3′), 87.1, 86.1 (C-5′), 83.7 (C-4′), 73.5 (CH_2Ph), 72.1(CH_2Ph), 68.5 (C-6′), 61.9 (C-1′), 55.1 (OCH_3).

2′,3-Anhydro-1-[4,6-*O*-benzyl-β-D-fructofuranosyl]uridine (29). Compound **28** (2.2 g, 3.2 mmol) was dissolved in 22 mL of 80% aqueous acetic acid and stirred at room temperature for 48 h. The acid was removed under vacuum and coevaporated with toluene 4 times and CH_2Cl_2 (3 times). The residue was subjected to column chromatography (eluent 0 to 3% MeOH in CH_2Cl_2) to give **29** (1.1 g, 2.5 mmol, 79%). $R_f = 0.27$ (CH_2Cl_2/CH_3OH 95:5 v/v); MALDI-TOF *m/z* $[M + 2H]^+$ found 438.7, calcd 438.1; ^{1}H NMR (270 MHz, $CDCl_3$) 7.36−7.26 (m, 9H, benzyl, H-6), 7.12−7.09 (m, 2H, benzyl), 5.89 (d, $J_{H-5,\ H-6} = 7.42$ Hz, 1H, H-5), 5.45 (s, 1H, H-3′), 4.70 (d, $J_{gem} = 11.75$ Hz, CH_2Ph), 4.52 (d, $J_{gem} = 11.75$ Hz, CH_2-Ph), 4.43−4.40 (m, 1H, H-5′), 4.33−4.17 (m, $J_{gem} = 12.25$ Hz, CH_2Ph, 4H, CH_2Ph, H-1′, H-4′), 3.86 (d, $J_{H-1',\ H-1''} = 13.24$ Hz, 1H, H-1″), 3.30−3.17 (ddd, $J_{gem} = 10.52$ Hz, $J_{H-5',\ H-6'} = 3.59$ Hz, 2H, H-6′, H-6″); ^{13}C NMR (67.9 MHz, $CDCl_3$) 173.3 (C-4), 160.6 (C-2), 136.5, 136.1, 134.4 (C-6), 128.4, 128.2, 128.1, 127.9, 127.8, 108.8 (C-5), 102.2 (C-2′), 87.6 (C-3′), 86.5 (C-5′), 86.6 (C-4′), 73.5 (CH_2Ph), 71.9 (CH_2Ph), 68.6 (C-6′), 61.5 (C-1′).

2′,3-Anhydro-1-[4,6-*O*-benzyl-1-*O*-methanesulfonyl-β-D-fructofuranosyl]uracil (30). Compound **29** (5 g, 11.4 mmol) was coevaporated with dry pyridine (3 times) and dissolved in 55 mL of the same solvent. The reaction mixture was cooled in an ice bath, and methanesulfonyl chloride (2.66 mL, 34.2 mmol) was added dropwise and stirred under nitrogen atmosphere for 1.5 h. The reaction was quenched by saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, concentrated in vacuo, and coevaporated with toluene (3 times). The residue was purified using column chromatography (eluent 0 to 3% MeOH in CH_2Cl_2) to give **30** (5.21 g, 10.1 mmol, 89%). $R_f = 0.35$ (CH_2Cl_2/CH_3OH 95:5 v/v); MALDI-TOF *m/z* $[M + H]^+$ found 515.5, calcd 515.1; ^{1}H NMR (270 MHz, $CDCl_3$) 7.36−7.26 (m, 9H, benzyl, H-6), 7.18−7.14 (m, 2H, benzyl), 6.08 (d, $J_{H-5,\ H-6} = 7.55$ Hz, 1H, H-5), 5.21 (s, 1H, H-3′), 4.69−4.46 (m, 5H, 2 × CH_2Ph, H-1′, H-1″, H-5′), 4.41 (d, $J_{gem} = 12.37$ Hz, 1H, CH_2Ph,), 4.30 (ABq, $J_{H-5',H-4'} = 1.48$ Hz), 4.22 (d, 1H, CH_2Ph), 3.36−3.20 (ddd, $J_{gem} = 10.64$ Hz, $J_{H-5',\ H-6'} = 3.59$ Hz, 2H, H-6′, H-6″), 3.03 (s, 3H, CH_3, *O*-mesyl); ^{13}C NMR (67.9 MHz, $CDCl_3$) 171.4 (C-4), 159.9 (C-2), 136.4, 135.8, 132.6 (C-6), 128.5, 128.3, 128.1, 127.9, 110.3 (C-5), 98.3 (C-2′), 87.5 (C-5′), 87.1 (C-3′), 83.8 (C-4′), 73.5 (CH2Ph), 72.3 (CH2Ph), 68.2 (C-6′), 65.0 (C-1′), 38.0 (*O*-mesyl).

2′,3-Anhydro-1-[1-azido-4,6-*O*-benzyl-1-deoxy-β-D-fructofuranosyl]uracil (31). Compound **30** (5.2 g, 10 mmol) was dissolved in 60 mL of dry DMF, NaN_3 (3.25 g, 50 mmol) was added, and the reaction was heated at 100 °C for 60 h under nitrogen atmosphere. The solvent was removed in vacuo, and the residue was diluted with CH_2Cl_2 and washed with brine. The aqueous phase was extracted with CH_2Cl_2 (3 times). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified using column chromatography (eluent 0 to 2.5% MeOH in CH_2Cl_2) to give **31** (4.01 g, 8.7 mmol, 87%). $R_f = 0.33$ (CH_2Cl_2/CH_3OH 95:5 v/v); MALDI-TOF *m/z* $[M + Na]^+$ found 484.7, calcd 484.1; IR (KBr) ν 2100 cm^{-1} (strong); ^{1}H NMR (270 MHz, $CDCl_3$) 7.41 (d, $J_{H6-H5} = 7.55$ Hz, 1H, H-6), 7.33−7.26 (m, 8H, benzyl), 7.18−7.14 (m, 2H, benzyl), 6.05 (d, $J_{H-5,\ H-6} = 7.55$ Hz, 1H, H-5), 5.12 (s, 1H, H-3′), 4.65−4.50 (ABq, $J_{gem} = 11.75$ Hz, 2H, 2 × CH2Ph), 4.48 (m, 1H, H-5′), 4.38 (d, $J_{gem} = 12.37$ Hz, CH_2Ph), 4.28 (d, $J_{H-4',\ H-5'} = 1.61$ Hz, 1H, H-4′), 4.21 (d, 1H, CH_2Ph), 3.87 (ABq, $J_{gem} = 13.36$ Hz), 3.34−3.18 (ddd, $J_{gem} = 10.64$ Hz, $J_{H-5',\ H-6'} = 3.46$ Hz, 2H, H-6′, H-6″); ^{13}C NMR (67.9 MHz, $CDCl_3$) 171.4 (C-4), 159.6 (C-2), 136.4, 135.9, 132.9 (C-6), 128.4, 128.1, 128.0, 127.9, 127.7, 127.6, 110.4 (C-5), 99.4 (C-2′), 87.4 (C-3′), 86.8 (C-5′), 83.9 (C-4′), 73.3 (CH_2Ph), 72.0 (CH_2Ph), 68.2 (C-6′), 52.6 (C-1′).

1-[1-Azido-4,6-*O*-benzyl-1-deoxy-β-D-fructofuranosyl]uracil (32). To a solution of compound **31** (4 g, 8.7 mmol) in 50% aqueous ethanol was added distilled THF to dissolve the compound completely, followed by 1 M NaOH (15.5 mL). The reaction mixture was stirred for 2 h and then concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and washed with brine. The aqueous phase obtained was extracted 3 times with CH_2Cl_2. The combined organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. It was then subjected to column chromatography (eluent 0 to 3% MeOH in CH_2Cl_2), which afforded **32** (3.54 g, 7.39 mmol, 85%). $R_f = 0.41$ (CH_2Cl_2/CH_3OH 95:5 v/v); MALDI-TOF *m/z* $[M + Na]^+$ found 502.6, calcd 502.1; ^{1}H NMR (270 MHz, $CDCl_3$) 7.80 (d, $J_{H-6,\ H-5} = 8.29$ Hz, 1H, H-6), 7.40−7.22 (m, 10H, benzyl), 5.66 (d, $J_{H-5,\ H-6} = 8.29$ Hz, 1H, H-5), 4.69−4.43 (m, 6H, 2 × CH_2Ph, 2 × CH_2Ph, H-3′, H-5′), 4.04 (br s, 1H, H-4′), 3.99 (d, $J_{gem} = 13.11$ Hz, 1H, H-1′), 3.84 (d, 1H, H-1″), 3.72−3.54 (ddd, $J_{gem} = 10.39$ Hz, $J_{H-5',\ H-6'} = 3.46$ Hz, 2H, H-6′, H-6″); ^{13}C NMR (67.9 MHz, $CDCl_3$) 164.4 (C-4), 150.7 (C-2), 141.8 (C-6), 137.0, 136.7, 128.7, 128.0, 127.9, 127.7, 127.6, 101.1 (C-5), 100.9 (C-2′), 85.3 (C-4′), 84.4 (C-5′), 76.7 (C-3′), 73.7 (benzyl), 72.1 (benzyl), 69.4 (C-6′), 53.6 (C-1′).

1-[1-Azido-4,6-*O*-benzyl-3-*O*-methanesulfonyl-1-deoxy-β-D-fructofuranosyl]uracil (33). See SI page S5 for details.

1-[1-Amino-4,6-*O*-benzyl-3-*O*-methanesulfonyl-1-deoxy-β-D-fructofuranosyl]uracil (34). To a solution of **33** (3.7 g, 6.65 mmol) in 100 mL of THF and 33 mL of water was added 1 M PMe_3 in THF (13.3 mL, 13.3 mmol), and the mixture was stirred at room temperature for 1 h. To this solution was added brine, and the mixture was extracted with CH_2Cl_2 (5 times). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting oil was purified using column chromatography (eluent 0 to 5% MeOH in CH_2Cl_2) to give **34** (3.17 g, 5.98 mmol, 90%). $R_f = 0.63$ (CH_2Cl_2/CH_3OH 85:15 v/v); MALDI-TOF *m/z* $[M + Na]^+$ found 554.4, calcd 554.1; ^{1}H NMR (270 MHz, $CDCl_3$) 7.78 (d, $J_{H-6,\ H-5} = 8.29$ Hz, 1H, H-6), 7.34−7.25 (m, 10H, benzyl), 5.63 (d, $J_{H-5,\ H-6} = 8.29$ Hz, 1H, H-5), 5.29 (s, 1H, H-3′), 4.77 (d, $J_{gem} = 11.88$ Hz, 1H, CH_2Ph), 4.61 (d, 1H, CH_2-Ph), 4.51(s, 2H, 2 × CH_2Ph), 4.37−4.32 (m, 1H, H-5′), 4.28 (d, $J_{H-4',\ H-5'} = 2.23$ Hz), 3.63−3.49 (m, 3H, H-6′, H-6″, H-1′), 3.30 (d, $J_{H-1',\ H-1''} = 14.23$ Hz, 1H, H-1″), 2.95 (s, 3H, CH_3, *O*-mesyl); ^{13}C NMR (67.9 MHz, $CDCl_3$) 163.9 (C-4), 149.7 (C-2), 141.6 (C-6), 137.2, 136.6, 128.4, 128.0, 127.8, 127.7, 100.8 (C-5), 98.9 (C-2′), 83.6 (C-5′), 83.5 (C-4′), 83.0 (C-3′), 73.3 (benzyl), 72.2 (benzyl), 69.3 (C-6′), 44.0 (C-1′), 38.3 (*O*-mesyl).

(1*R*,3*R*,4*S*,5*R*)-3-(Benzyloxymethyl)-4-(benzyloxy)-1-(uracil-1-yl)-6-aza-2-oxabicyclo[3.2.0]heptane (35). Compound **34** (3.17 g, 5.98 mmol) was dissolved in 90 mL pyridine, 4 mL triethylamine was added, and the mixture was heated at 90 °C for 48 h under nitrogen atmosphere. The solvent was removed under reduced pressure and coevaporated with toluene (3 times). The residue obtained was purified by column chromatography (eluent 0 to 5% MeOH in CH_2Cl_2) to give **35** (1.64 g, 3.76 mmol, 63%). $R_f = 0.71$ (CH_2Cl_2/CH_3OH 85:15 v/v); MALDI-TOF *m/z* $[M + 2H]^+$ found

437.7, calcd 437.1; ^{1}H NMR (270 MHz, $CDCl_3$) 7.32−7.26 (m, 10H, benzyl), 6.98 (d, $J_{H-6,\ H-5}$ = 8.04 Hz, 1H, H-6), 5.65 (d, $J_{H-5,\ H-6}$ = 8.04 Hz, 1H, H-5), 4.71 (d, $J_{H-3',\ H-4'}$ = 5.44 Hz, 1H, H-3′), 4.58−4.41 (m, 5H, 2 × CH_2Ph, 2 × CH_2Ph, H-5′), 4.22 (dd, $J_{H-3',\ H-4'}$ = 5.44 Hz, $J_{H-4',\ H-5'}$ = 7.67 Hz, 1H, H-4′), 4.12 (d, J_{gem} = 10.52 Hz, 1H, H-1′), 3.85 (d, 1H, H-1″), 3.76−3.62 (ddd, J_{gem} = 10.64 Hz, $J_{H-5',\ H-6'}$ = 2.60 Hz, 2H, H-6′, H-6″); ^{13}C NMR (67.9 MHz, $CDCl_3$) 163.5 (C-4), 149.2 (C-2), 139.9 (C-6), 128.3, 128.2, 127.9, 127.6, 102.4 (C-5), 93.5 (C-2′), 82.2 (C-5′), 78.5 (C-4′), 73.3 (benzyl), 72.7 (benzyl), 70.1 (C-6′), 65.0 (C-3′), 54.6 (C-1′).

(1*R*,3*R*,4*S*,5*R*)-3-(Benzyloxymethyl)-4-(benzyloxy)-6-*N*-(phenoxyacetyl)-1-(uracil-1-yl)-6-aza-2-oxabicyclo[3.2.0]heptane (36). Compound **35** (1.64 g, 3.76 mmol) was coevaporated with dry pyridine (3 times) and dissolved in 37 mL of the same solvent, and phenoxyacetyl chloride (0.67 mL, 4.88 mmol) was added dropwise and stirred at room temperature for 2.5 h. The reaction was quenched by saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 times). The residue was purified by column chromatography (eluent 0 to 3% MeOH in CH_2Cl_2) to give **36** (1.8 g, 3.15 mmol, 84%). R_f = 0.39 (CH_2Cl_2/CH_3OH 95:5 v/v); MALDI-TOF *m*/*z* $[M + H]^+$ found 570.5, calcd 570.2; ^{13}C NMR (67.9 MHz, $CDCl_3$) 169.9, 169.0, 163.1, 157.4, 157.3, 149.2, 139.7, 137.7, 137.6, 136.9, 129.8, 128.4, 127.8, 127.6, 122.0, 121.7, 114.4, 103.3, 103.2, 91.0, 90.1, 82.0, 81.8, 78.2, 73.8, 73.5, 73.2, 71.0, 69.0, 68.4, 67.9, 67.4, 61.3, 57.7, 29.7.

(1*R*,3*R*,4*S*,5*R*)-3-(4,4′-Dimethoxytrityloxymethyl)-4-hydroxy-6-*N*-(phenoxyacetyl)-1-(uracil-1-yl)-6-aza-2-oxabicyclo[3.2.0]-heptane (38). Compound **36** (1.8 g, 3.15 mmol) was dissolved in anhydrous CH_2Cl_2 (31 mL) at −78 °C and BCl_3 (26.5 mL, 1 M solution in CH_2Cl_2) was added dropwise under nitrogen atmosphere. The reaction mixture was kept at this temperature for 2 h and then slowly warmed to −20 °C and stirred at this temperature for 2 h. MeOH (30 mL) was added and the reaction was slowly warmed to room temperature and stirred for 10 min. The solvent was removed under reduced pressure and coevaporated with MeOH (3 times) to give **37**. The residue without further purification was coevaporated with dry pyridine (3 times) and dissolved in 30 mL of the same solvent. To this reaction mixture were added DMTrCl (1.38 g, 4 mmol) and DMAP (385 mg, 3.15 mmol), and the mixture was stirred at room temperature for 5 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, concentrated in vacuo, and purified by column chromatography to give **38** (1.85 g, 2.67 mmol, 85%). R_f = 0.37 (CH_2Cl_2/CH_3OH 95:5 v/v); MALDI-TOF *m*/*z* $[M + Na]^+$ found 714.6, calcd 714.2; ^{13}C NMR (125.7 MHz, $CDCl_3$) 170.5, 169.1, 162.5, 162.6, 158.4, 158.5, 157.3, 157.1, 149.0, 144.6, 144.4, 139.5, 135.7, 135.6, 130.1, 130.0, 129.9, 129.8, 129.0, 128.2, 128.1, 128.0, 127.8, 127.7, 126.9, 126.8, 122.3, 122.1, 114.4, 114.1, 113.1, 113.0, 103.3, 103.2, 90.1, 89.5, 86.5, 86.2, 84.1, 82.2, 73.1, 72.5, 71.7, 71.6, 67.7, 67.4, 62.9, 62.5, 62.2, 58.0, 55.1, 53.4.

(1*R*,3*R*,4*S*,5*R*)-4-(2-Cyanoethoxy(diisopropylamino)-phosphinoxy)-3-(4,4′-dimethoxytrityloxymethyl)-6-*N*-(phenoxyacetyl)-1-(uracil-1-yl)-6-aza-2-oxabicyclo[3.2.0]heptane (39). Compound **38** (0.92 g, 1.33 mmol) was dissolved in 13 mL of dry THF, and diisopropylethylamine (1.35 mL, 7.75 mmol) was added at 0 °C followed by 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite. After 30 min the reaction was warmed to room temperature and stirred for 2 h. MeOH (0.5 mL) was added and stirring was continued for 5 min. Thereafter saturated aqueous $NaHCO_3$ was added and extracted with freshly distilled CH_2Cl_2 (3 times). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography using 40% to 100% CH_2Cl_2 in cyclohexane containing 1% Et_3N as the eluent, which afforded **39** (1.1 g, 1.23 mmol, 93%) as a mixture of four isomers. R_f = 0.3 (CH_2Cl_2/CH_3OH 96:4 v/v); MALDI-TOF *m*/*z* $[M + H]^+$ found 892.9, calcd 892.3;^{31}P NMR (109.4 MHz, $CDCl_3$) 154.6, 150.6, 149.9, 149.2.

(1*R*,3*R*,4*S*,5*R*)-1-(Cytosin-1-yl)-3-(4,4′-dimethoxytrityloxymethyl)-4-hydroxy-6-*N*-(phenoxyacetyl)-6-aza-2-oxabicyclo-[3.2.0]heptane (40). Compound **38** (0.92 g, 1.33 mmol) was dissolved in dry pyridine(13 mL), and acetic anhydride (1.25 mL, 13.3 mmol) was added and stirred at ambient temperature for 24 h. Thereafter the reaction was quenched with MeOH (1 mL) and stirred overnight at room temperature. To this reaction mixture was added saturated aqueous $NaHCO_3$, and the mixture was extracted with CH_2Cl_2 (3 times). The organic phase was dried ($MgSO_4$), filtered, and evaporated under reduced pressure to get **38a**. To this residue was added 1,2,4-triazole (0.91 g, 13.3 mmol) and coevaporated with dry pyridine (3 times), and 13 mL of the same solvent was added and cooled to 0 °C. To the resulting clear solution was added 2-chlorophenylphosphodichloridate (1.1 mL, 6.65 mmol) under nitrogen atmosphere. After 30 min the reaction was allowed to reach room temperature and stirred at this temperature for 5 h. Thereafter aqueous NH_3 (15 mL 32%aqueous NH_3) was added, and the reaction was cooled to 5 °C and kept overnight at this temperature. The resulting solution was extracted with CH_2Cl_2 (5 times) and the organic phase was dried ($MgSO_4$), filtered, and evaporated under reduced pressure. Purification by column chromatography (eluent 0 to 5% MeOH in CH_2Cl_2) afforded **40** (640 g, 0.93 mmol, 70%). R_f = 0.73 (CH_2Cl_2/CH_3OH 85:15 v/v); MALDI-TOF *m*/*z* $[M + Na]^+$ found 713.6, calcd 713.2; ^{13}C NMR (125.7 MHz, $CDCl_3$) 170.6, 169.6, 165.8, 158.4, 157.2, 154.8, 144.7, 144.6, 141.0, 140.8, 135.8, 130.1, 130.0, 129.8, 129.7, 129.6, 128.2, 128.1, 127.8, 127.7, 126.8, 122.1, 121.6, 114.5, 114.2, 113.1, 95.3, 90.3, 89.9, 86.1, 83.6, 81.7, 73.2, 72.7, 72.0, 71.6, 67.5, 66.7, 63.5, 62.7, 62.4, 55.2.

(1*R*,3*R*,4*S*,5*R*)-3-(4,4′-Dimethoxytrityloxymethyl)-4-hydroxy-1-(4-*N*-isobutyrylcytosin-1-yl)-6-*N*-(phenoxyacetyl)-6-aza-2-oxabicyclo[3.2.0]heptane (41). Compound **40** (640 mg, 0.93 mmol) was dried by coevaporating with dry pyridine (3 times) and suspended in 15 mL of the same solvent. To this solution was added trimethylchlorosilane (0.58 mL, 4.65 mmol) under nitrogen atmosphere, and the mixture was stirred for 30 min. Thereafter isobutyryl chloride (0.2 mL, 1.86 mmol) was added and stirred for 3 h. The reaction was quenched by adding MeOH (0.5 mL) and stirred overnight. Saturated aqueous $NaHCO_3$ was added to the reaction and extracted with CH_2Cl_2 (3 times); the organic layer was collected, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue obtained was purified by column chromatography (eluent 0 to 2% MeOH in CH_2Cl_2), which afforded **41** (585 mg, 0.77 mmol, 83%). R_f = 0.45 (CH_2Cl_2/CH_3OH 95:5 v/v); MALDI-TOF *m*/*z* $[M + H]^+$ found 761.7, calcd 761.3; ^{13}C NMR (150.9 MHz, $CDCl_3$) 176.9, 170.7, 169.6, 162.9, 162.9, 158.5, 158.5, 157.2, 154.2, 144.6, 144.3, 135.7, 135.6, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 128.2, 128.1, 128.0, 127.8, 126.8, 122.1, 121.7, 114.5, 114.3, 114.2, 113.1, 97.1, 96.9, 90.9, 90.3, 86.2, 84.2, 82.5, 73.2, 72.5, 71.9, 71.6, 67.5, 66.9, 63.1, 62.5, 62.1, 58.2, 55.2, 36.7, 29.6, 19.0, 18.9.

(1*R*,3*R*,4*S*,5*R*)-4-(2-Cyanoethoxy(diisopropylamino)-phosphinoxy)-3-(4,4′-dimethoxytrityloxymethyl)-1-(4-*N*-isobutyrylcytosin-1-yl)-6-*N*-(phenoxyacetyl)-6-aza-2-oxabicyclo[3.2.0]heptane (42). See SI page S6 for details.

Theoretical Calculations. The structural parameters of the azetidine- and oxetane-modified nucleosides and conformational hyperspace available for the compounds have been determined by the ab initio geometry optimizations followed by 2 ns molecular dynamics (MD) simulations. The geometry optimizations of the modified nucleosides have been carried out by the GAUSSIAN 98 program package[36] at the Hartree−Fock level using 6-31G** basis set. The atomic charges and optimized geometries of azetidine-modified **C**, **T**, **U**, and 5-Me-**C** nucleosides were then used in AMBER[37] force field parameters employed in the MD simulations. The protocol of this MD simulations is based on Cheathan-

Kollman's[38] procedure employing modified version of Amber 1994 force field as it is implemented in AMBER 7 program package.[37] The TIP3P water model was used to introduce explicit solvent molecules in the MD calculations. Periodic boxes containing 1048, 1125, 1084, and 1122 water molecules were created around azetidine-modified **C**, **T**, **U**, and 5-Me-**C**, respectively, extending 10.0 Å from these molecules in three dimensions.

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Supporting Information Available: General experimental methods and experimental conditions for the preparation of compounds **8**, **10**, **18a**, **26**, **33**, and **42**; ^{13}C NMR spectra of compounds **2a**, **4**, **5**, **6a**, **7**−**10**, **12**−**17**, **18a**, **19**, **22**−**24**, **26**−**36**, **38**, **40**,and **41**; ^{31}P NMR spectra of compounds **20**, **39**, and **42**; Cartesian coordinates of azetidine-**C**, -**T**, -**U**, and -5-Me-**C** nucleosides. This material is available free of charge via the Internet at http://pubs.acs.org.

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