

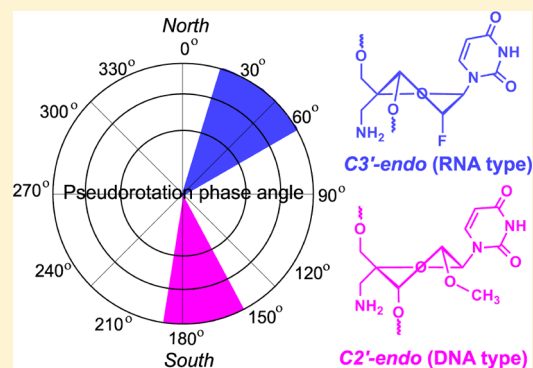
Influence of 2'-Fluoro versus 2'-O-Methyl Substituent on the Sugar Puckering of 4'-C-Aminomethyluridine

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S Supporting Information

ABSTRACT: Herein, we report the synthesis of 4'-C-aminomethyl-2'-deoxy-2'-fluorouridine, a therapeutically appealing RNA modification. Conformational analysis by DFT calculations and molecular dynamics simulations using trinucleotide model systems revealed that modified sugar adopts C3'-endo conformation. In this conformer, a weak intramolecular C–H...F H-bond between the hydrogen atom of the 4'-C-CH₂ group and the F atom at the 2' position is observed. Comparative studies with unmodified, 2'-fluoro-, 2'-O-methyl-, and 4'-C-aminomethyl-2'-O-methyluridine showed the chemical nature of 2'-substituent dictates the sugar puckering of 2',4'-modified nucleotides.



Various chemical modifications on the sugar moiety of nucleotides have been reported to improve the therapeutic potential of nucleic acids such as antisense oligonucleotides (AONs), aptamers, and small interfering RNAs (siRNAs).^{1–5} However, the chemical nature of modifications play a crucial role in retaining the functional efficacy of these agents.^{3–5} Among the plethora of sugar modifications reported, nucleic acids bearing a 2'-deoxy-2'-fluorine (2'-F) modification locks the sugar conformation in C3'-endo, increases the duplex stability, and enhances nuclease resistance properties of modified nucleic acids to a certain extent.^{6–8} Combining the 2'-F along with other sugar modifications such as 4'-C-aminomethyl or 4'-thio is an attractive strategy to further improve the therapeutic properties of nucleic acids, in particular, the nuclease resistance.^{9–11}

Chemical modifications in the furanose ring strongly influence the sugar puckering and the overall structure and stability of nucleic acids.¹² Like 2'-F,^{6,7} C2'-modifications such as 2'-O-methyl (2'-OMe),^{13,14} 2'-O-methoxyethyl (2'-MOE),^{14,15} 2'-azido,¹⁶ etc., tune the sugar toward the C3'-endo and stabilize both DNA/RNA and RNA/RNA duplexes. However, 2'-deoxy-2'-F-arabino nucleic acid (2'-F-ANA) modification adopts the O4'-endo conformation and renders moderate stability to duplexes.¹⁷ Nucleic acids containing 4'-carbon bridged to 2'-carbon as in C3'-endo-fused locked nucleic acid (LNA) and carbocyclic LNA (c-LNA) show the largest RNA binding affinity.^{18,19}

Toward the goal of harnessing the utilities of bifunctional sugar modifications, recently we have reported 4'-C-aminomethyl-2'-O-methyl (4'-AM-2'-OMe) uridine- and cytidine-modified siRNAs and 4'-AM-2'-OMe-thymidine triphosphate for potential applications in in vitro selection experiments.^{20,21} The modified siRNAs showed significant enhancement in

nuclease stability without compromising much in RNAi activity. However, the presence of this modification in an RNA duplex in general decreases the binding affinity ($\Delta T_m \sim 1$ °C/modification) by favoring the C2'-endo conformation, which was elucidated by molecular dynamics (MD) simulations.²⁰ These studies clearly illustrate that the conformational pre-organization of the sugar influences the H-bonding and stacking interactions between nucleobases.²² In this context, synthesis and conformational analysis of novel sugar-modified nucleosides can provide new insights for the design of therapeutically appealing nucleic acids.

Herein, we report the synthesis of 4'-C-aminomethyl-2'-deoxy-2'-fluoro (4'-AM-2'-F) uridine nucleoside (**D**) shown in Figure 1. The nature of sugar puckering and the Watson–Crick H-bonding of 4'-AM-2'-F-U in a trinucleotide model system were examined by DFT calculations, MD simulations, and utilizing umbrella sampling simulations. To distinguish the effect of 2'-modification from that of 4'-modification, comparative studies were also performed by using 2'-OH, 2'-OMe, 2'-F and 4'-AM-2'-OMe uridine (Figure 1) modified nucleotides.

Synthesis of 4'-AM-2'-F nucleoside **8** was achieved from uridine nucleoside **1**²⁰ as shown in Scheme 1. Deacetylation of **1** was carried out using sodium methoxide in methanol to get crude compound **2**. Nucleoside **2** was directly mesylated using methanesulfonyl chloride (Ms-Cl) in pyridine to afford mesylated nucleoside **3** in 80% yield (after two steps from **1**).²³ Treatment of **3** with aqueous NaOH gave arabinonucleoside **4** in 91% yield.¹¹ 2'-Fluorination of compound **4** using fluorinating agents such as (diethylamino)sulfur trifluoride

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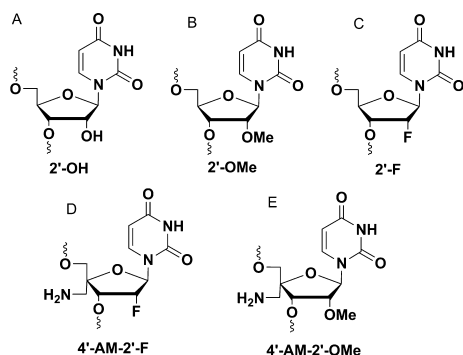


Figure 1. Structure of unmodified and modified uridine units: (A) 2'-hydroxyl (2'-OH), (B) 2'-O-methyl (2'-OMe), (C) 2'-fluoro (2'-F), (D) 4'-C-aminomethyl-2'-deoxy-2'-fluoro (4'-AM-2'-F), and (E) 4'-C-aminomethyl-2'-O-methyl (4'-AM-2'-OMe).

(DAST) and [bis(2-methoxyethyl)amino]sulfur trifluoride (MAST), under different reaction conditions, led to decomposition of starting nucleoside.^{24,25} To introduce an F atom at the 2'-position, arabino compound **4** was converted to its triflate **5** and then subsequently displaced by Et₃N·3HF in toluene at 45 °C to give the fluorinated nucleoside **6** in 45% yield.²⁶ Removal of benzyl groups, using 1 M BCl₃ in DCM by stirring at -78 to -30 °C, yielded the desired compound **7** in 83% yield.²⁷ Reduction of azide group of **7** with PPh₃ and H₂O in THF resulted in desired amine nucleoside **8** in 76% yield.²⁸

Having achieved the synthesis of 4'-AM-2'-F-U, the sugar conformation adopted by the nucleoside in solution was studied by utilizing NMR coupling constants and PSEUROT program.²⁹ A population distribution of sugar conformation showed the modified nucleoside strongly prefers C3'-endo (>90%) with a pseudorotational phase angle (*P*) of 3–21° and puckering amplitude (φ_m) of 15–41° (Figure S1, Supporting Information). Since the results emerge from the conformational analysis using just modified nucleosides can be different from the corresponding modified nucleotides,^{20,30} DFT calculations were performed in both gas phase (MP2/6-311++G*)³¹ and in solvent (water) phase (PBE/6-311++G*)³² using a model system where the modified nucleoside in the middle protected with sugar-phosphate moieties at the 3'- and 5'-ends (Figure S2, Supporting Information). When the conformational search was performed, sugars at both ends were fixed at C3'-endo

puckering. In the solvent phase, calculations resulted in total 720 conformations for each modification. The lowest energy conformers of 2'-OMe, 2'-F, and 4'-AM-2'-F adopt *P* values in the range of 9–28° that correspond to the C3'-endo conformation, which is usually found in A-type RNA (Figure 2).^{30,33} However, 4'-AM-2'-OMe-U showed lower energy

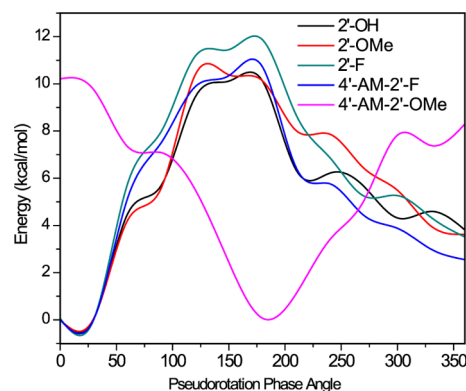
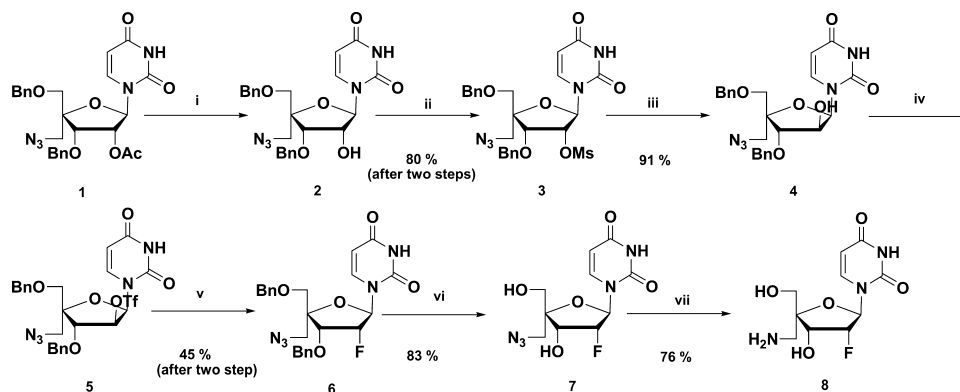


Figure 2. Pseudorotational phase angle (*P*) energy profile of modified and unmodified uridines. Energy values are from DFT calculations performed at PBE functional using 6-311++G* basis set in solvent (water).

conformers with *P* in the range of 169–180°, which corresponds to C2'-endo sugar pucker found in B-type DNA.^{30,33} The same trend is observed for all modifications in the gas-phase calculations as well (Figure S3A, Supporting Information). In addition, DFT calculations in the gas phase were carried out on three reference nucleotides: 2'-deoxy-2'-S-methyluridine (2'-SMe),³⁴ 4'-AM-2'-deoxyuridine, and 4'-AM-uridine. Results indicate that 2'-SMe-uridine and 4'-AM-2'-deoxyuridine adopt a C2'-endo sugar conformation (Figure S4, Supporting Information). A similar conformational preference has been reported for 2'-deoxy-2'-(trifluoroethyl)uridine (2'-SCF₃), a bulky 2' modification that destabilizes the RNA duplex due to the unfavorable steric interaction with the 3'-neighboring nucleotide.³⁵ Interestingly, 4'-AM-uridine mainly prefers the C3'-endo conformation; however, this modification is flexible enough to adopt the C2'-endo sugar conformation as well (Figure S4, Supporting Information)

Scheme 1. Synthesis of 4'-AM-2'-F-uridine Nucleoside **8**^a



^aReagents and conditions: (i) NaOMe, MeOH, rt, 2 h; (ii) MsCl, pyridine, 0 °C, 6 h; (iii) 1 M NaOH, EtOH–H₂O (2:1, v/v), rt, 2 h; (iv) Tf₂O, DCM–pyridine (9:1, v/v), 0 °C, 2 h; (v) Et₃N·3HF, Et₃N, toluene, 45 °C, 60 h; (vi) BCl₃, DCM, -78 °C, 3 h, then -30 °C, 3 h; (vii) PPh₃, H₂O, THF, 45 °C, 4 h.

In 4'-AM-2'-F, sugar contains two additional torsion angles such as C2'-C3'-C4'-C (Ω) and O3'-C3'-C4'-C (ω), which adopt values of $\sim 72^\circ$ and $\sim -51^\circ$, respectively. The two additional torsion angles Ω and ω in 4'-AM-2'-OMe adopt values entirely different than that of 4'-AM-2'-F, which are $\sim 170^\circ$ and $\sim 51^\circ$, respectively. The torsion angles in the sugar backbone γ (O5'-C5'-C4'-C3') and δ (C5'-C4'-C3'-O3') in 4'-AM-2'-F were able to adopt angles ($\sim 52^\circ$ and $\sim 86^\circ$) similar to those of native RNA (Table S1, Supporting Information).²² However, γ and δ of 4'-AM-2'-OMe adopt values $\sim 35^\circ$ and $\sim 110^\circ$, respectively, which is similar to those of native DNA (Table S1, Supporting Information).²²

To probe potential H-bonding interactions in the sugar moiety, the distance between the hydrogen atom of CH₂ in the 4'-C-aminomethyl group and the F atom at the C2' position has been measured from the conformations generated during the potential energy scan. For the lowest energy C3'-endo conformer, the distance between hydrogen and fluorine is found to be 2.3–2.5 Å with a C–H–F angle of 123–155°, which indicates the presence of a weak C–H...F H-bonding interaction (Figure 3).³⁶ The C–H...F H-bond was validated

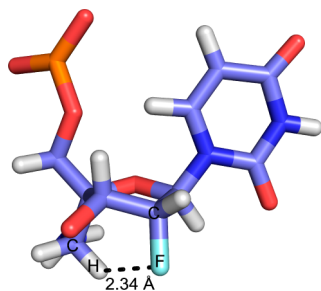


Figure 3. Low energy conformer of 4'-AM-2'-F-U from the potential energy scan utilizing PBE functional using 6-311++G* basis set in solvent. C3'-endo conformation in which C–H...F hydrogen bonding shown with dotted lines.

using noncovalent interactions (NCI) plot,³⁷ which showed the isosurface electron density between fluorine and hydrogen (Figure S5, Supporting Information). It should be noted that C–H...F hydrogen bonds in 2'-FANA at the pyrimidine–purine steps are shown to increase the duplex affinity of modified oligonucleotides.¹⁷ The combined strength of H-bond along with the gauche effect of the F2'-C2'-C1'-O4' torsion stabilize 4'-AM-2'-F-U in C3'-endo by reducing the conformational flexibility of endocyclic torsions (Tables S1 and S2, Supporting Information).³⁰

To compare the conformational dynamics of modifications, MD simulations (AMBER 12)³⁹ in explicit solvent were carried out with the same model system used in DFT calculations (Figure S2, Supporting Information). MD simulations started with 14 different sugar conformations of both modified and unmodified nucleotides.⁴⁰ Sugar torsions and conformations showed a similar trend, which was observed in the DFT calculations (Figure S3B, Supporting Information). RMSDs of all modifications showed maximum deviation up to 2.5 Å (Figure S6, Supporting Information).

The distributions of sugar puckering were calculated using their endocyclic torsional angle from the MD simulation trajectories and are plotted against P for every 5 ps. From these analyses it became evident that, as expected, 2'-OH, 2'-OMe, and 2'-F preferred the C3'-endo sugar conformation (Figure S7, Supporting Information). The 4'-AM-2'-F-U favored the

C3'-endo sugar conformation along with 23% of C4'-exo pucker. The C–H...F H-bond observed in the 4'-AM-2'-F modification from the DFT calculations was retained during the course of MD simulations with H-bond occupancy of 96%. As revealed from DFT studies, 4'-AM-2'-OMe favored the C2'-endo pucker along with $\sim 5\%$ of the population distributed in C3'-exo (Figure S7, Supporting Information).

The distribution of backbone torsions (α to ζ) for the 2'-OMe, 2'-F, and 4'-AM-2'-F did not show any substantial differences from those of a C3'-endo pucker (Table S3 and Figures S8–S10, Supporting Information). However, 4'-AM-2'-OMe-U deviates significantly in the γ , δ , ϵ , and ζ angles from that of the C3'-endo sugar pucker (Figures S9 and S10, Supporting Information). This deviation is due to the flexibility of the 4'-C-aminomethyl group evident from their two additional flexible torsional angles Ω ($\sim 166^\circ \pm 33^\circ$) and ω ($\sim 57^\circ \pm 40^\circ$), whereas in 4'-AM-2'-F Ω ($\sim 75^\circ \pm 11^\circ$) and ω ($\sim -56^\circ \pm 13^\circ$) torsions were less fluctuating due to the presence of the C–H...F H-bond.

Sugar conformations determine the base-pairing geometry.⁴¹ Here, to probe the H-bonding interactions, RNA duplex model systems (5'-GUC-3'/3'-CAG-5') in which the middle uridine was replaced with modified nucleotides were utilized. To predict appropriate W–C H-bond distance and free energy of formation (PMF) between the bases, umbrella sampling simulations were exploited.⁴² Free energy variation as a function of H-bond distance is represented in Figure 4. Each of the curves showed the global minimum (m_1) where the distance between the two bases adopt its most stable conformation.

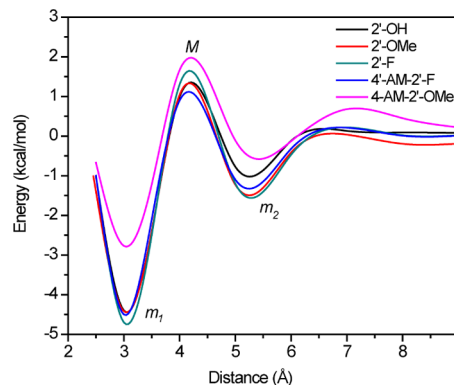


Figure 4. Free energy variations as a function of H-bond distance between nitrogenous bases. The free energy formation of the H-bond is calculated using 35 ns umbrella sampling simulations for each modification. Here, m_1 , m_2 are the global minimum and local minimum, respectively, and M corresponds to the energy maximum.

Structures at the m_1 for all of the modifications are shown in Figure 5 and Figure S11 (Supporting Information). At the local minimum m_2 , W–C H-bonding atoms in bases were bridged by a water molecule, and distances between two bases are ~ 5.7 Å. The 2'-F-U-A pair was found to be most stable with a standard free energy of $\Delta G^\circ -4.83$ kcal/mol at a H-bond distance of 2.89 Å. This is in agreement with the reports that C2'-F nucleotides have higher H-bonding strength and stacking interactions compared to unmodified nucleotides.⁴³ Nucleotide pairs with next lowest energy are 2'-OH-U-A, 2'-OMe-U-A, and 4'-AM-2'-F-U-A at a H-bond distance of ~ 2.9 Å (Figure S11, Supporting Information). The C1'–C1' distances of 2'-

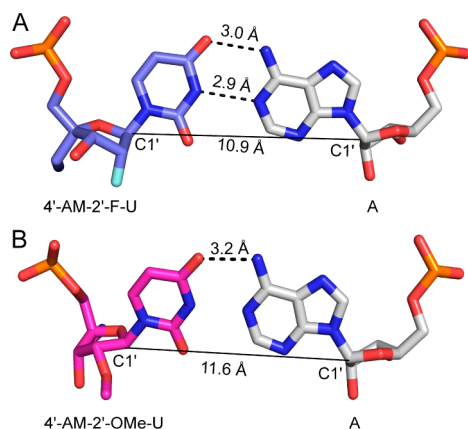


Figure 5. MD snapshot at m_1 of modified base pairs from the 35 ns of umbrella sampling simulations. The sugar conformations of (A) 4'-AM-2'-F and (B) 4'-AM-2'-OMe are represented with base pairing adenosine. Black dotted line represents the H-bond and their distances between the nitrogenous bases are shown in Å. The solid line represents the distance between C1'–C1' of the U–A base pair.

OMe and 4'-AM-2'-F-U were found to be ~ 10.9 Å. The 4'-AM-2'-OMe-U modification has larger C1'–C1' distance of 11.6 Å, with a nonplanar base-pair geometry. Thus, this modification from only one H-bond (C–O \cdots H–N) with base pair stabilization of -2.6 kcal/mol at 3.2 Å distance.²⁰

It is known that hydration of bases is important for the stabilization of H-bonding between the bases.⁴⁴ At m_1 , for the unmodified system, the W–C base pair was hydrated by five water molecules, of which three are present in the major groove and two in the minor groove position (Figure S12, Supporting Information). Similarly, 2'-F, 2'-OMe, and 4'-AM-2'-F-U were hydrated by five water molecules. But 4'-AM-2'-OMe-U lost hydration completely in the minor groove. This loss in hydration attributes to the unstabilized base pairs and absence of a water mediated hydrogen bond at m_2 .

The frequency distribution of χ angle (O4'–C1'–N1–C6) shows that 4'-AM-2'-F-U adopts a value of 169.5°, which is similar to that of unmodified (163.4°), 2'-OMe (160.7°), and 2'-F (165.7°) modifications (Figure S13, Supporting Information). Deviating from native C3'-endo conformation, 4'-AM-2'-OMe-U adopts C2'-endo like χ of 118.9°.²⁰ The deviation of χ disrupts the base pair geometry, and due to this 4'-AM-2'-OMe-U-A pair lost one H-bond, which was observed during umbrella sampling simulations (Figure 5).

In summary, we have carried out the synthesis of 4'-AM-2'-F-U with reasonable yields. From the NMR analysis it was evident that modified nucleoside adopts C3'-endo conformation in solution. DFT calculations and MD simulations using nucleotide model systems revealed that in 4'-AM-2'-F-U, a stronger gauche effect and C–H \cdots F H-bond stabilizes C3'-endo sugar conformation, which results in normal W–C base pair formation. In contrast, 4'-AM-2'-OMe modification prefers C2'-endo sugar conformation and loses one W–C H-bond between the bases. These results also highlight the importance of 2'-substituent in dictating conformational preference of 4'-C-aminomethyl nucleotides. Because of its C3'-endo conformational preference, 4'-AM-2'-F modification may find application in therapeutically appealing nucleic acids.

EXPERIMENTAL SECTION

General Methods. All chemicals and dry solvents (THF, MeOH) were obtained from commercial sources and used without any further purification. Acetonitrile, DCM, Et₃N, and pyridine were dried using calcium hydride. Toluene was dried using calcium chloride. Thin-layer chromatography (TLC) was performed on silica gel plates precoated with fluorescent indicator with visualization by UV light or by dipping into a solution of 5% concd H₂SO₄ in ethanol (v/v) and heating. Silica gel (100–200 mesh) was used for column chromatography. ¹H NMR (400 or 300 MHz), ¹³C NMR (100 MHz), and ¹⁹F NMR (376.5 MHz) were recorded on a 400 or 300 MHz instrument. The chemical shifts in parts per million (δ) are reported downfield from TMS (0 ppm) and referenced to the TMS signal or residual proton signal of the deuterated solvent as follows: TMS (0 ppm) or CD₃OD (3.31 ppm) for ¹H NMR spectra, and CDCl₃ (77.2 ppm) or CD₃OD (49.1 ppm) for ¹³C NMR spectra. Multiplicities of ¹H NMR spin couplings are reported as s for singlet, bs for broad singlet, d for doublet, dt for doublet of triplets, dd for doublet of doublets, ddd for doublet of doublet of doublets, ABq for AB quartet, AXq for AX quartet, or m for multiplet and overlapping spin systems. Values for apparent coupling constants (J) are reported in Hz. High-resolution mass spectra (HRMS) were obtained in positive-ion electrospray ionization (ESI) mode using a Q-TOF analyzer.

4'-C-Azidomethyl-3',5'-di-O-benzyl-2'-O-methanesulfonyluridine (3). Nucleoside 1 (0.2 g, 0.38 mmol) was dissolved in dry methanol (2 mL). To this 1 M sodium methoxide in methanol (0.6 mL) was added and the mixture stirred at room temperature for 2 h. Solvent was partially evaporated under reduced pressure and extracted with DCM (2 \times 30 mL). The combined organic layer was dried over anhydrous Na₂SO₄, evaporated, co-evaporated with dry pyridine (2 \times 20 mL), and dissolved in 2 mL of the same solvent. The reaction mixture was cooled in an ice bath, and methanesulfonyl chloride (0.06 mL, 0.77 mmol) was added dropwise and stirred at 4 °C for 6 h. The reaction was quenched with saturated aqueous NaHCO₃ (20 mL) and extracted with DCM (2 \times 30 mL). The organic phase was dried over anhydrous Na₂SO₄, evaporated under reduced pressure, and coevaporated with toluene (2 \times 20 mL) and DCM (2 \times 20 mL). The crude compound was purified by column chromatography (40% ethyl acetate in hexane) to give 3 as a white solid (183 mg, 80%): R_f = 0.72 (20% ethyl acetate in petroleum ether); mp 75–76 °C; ¹H NMR (400 MHz, CDCl₃) δ : 9.77 (s, 1H), 7.77 (d, J = 7.9 Hz, 1H), 7.39–7.32 (m, 7H), 7.22–7.20 (m, 2H), 6.09 (d, J = 3.4, 1H), 5.27–5.22 (m, 2H), 4.86, 4.48 (AXq, J = 11.6 Hz, 2H), 4.43, 4.36 (ABq, J = 10.7, 2H), 4.36 (d, J = 6.04 Hz, 1H), 3.87, 3.47 (AXq, J = 10.6 Hz, 2H), 3.76, 3.35 (AXq, J = 13.6 Hz, 2H), 3.18 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 162.9, 150.5, 139.8, 136.8, 128.8, 128.7, 128.6, 128.4, 128.2, 102.7, 88.1, 87.8, 79.7, 76.3, 74.2, 74.0, 70.8, 52.9, 39.0. HRMS (ESI) calcd for C₂₅H₂₈N₅O₈S [M + H]⁺ 558.1659, found [M + H]⁺ 558.1678 (Δm +0.002, error, +3.6 ppm).

4'-C-Azidomethyl-3',5'-di-O-benzylarabinouridine (4). Compound 3 (1.45 g, 2.59 mmol) was dissolved in ethanol–H₂O (2:1, v/v, 36 mL). To this was added 1 M NaOH (5.5 mL), and the reaction mixture was stirred at room temperature for 2 h. The reaction was neutralized with 6 M HCl (4–6 drops) and extracted with EtOAc (3 \times 80 mL). The organic phase was dried over anhydrous Na₂SO₄ and evaporated under reduced pressure. The compound was purified by column chromatography (40% ethyl acetate in hexane) to afford nucleoside 4 as white solid (1.13 g, 91%): R_f = 0.42 (70% ethyl acetate in petroleum ether); mp 90–91 °C; ¹H NMR (400 MHz, CDCl₃) δ 10.6 (bs, 1H), 7.61 (d, J = 8 Hz, 1H), 7.37–7.25 (m, 10H), 6.23 (d, J = 3.8 Hz, 1H), 5.29 (d, J = 8.2 Hz, 1H), 5.01 (bs, 1H), 4.77, 4.55 (AXq, J = 11.3 Hz, 2H), 4.76 (d, J = 4.2 Hz, 1H), 4.52, 4.48 (ABq, J = 11.3 Hz, 2H), 4.16 (d, J = 2.2 Hz, 1H), 3.64, 3.45 (AXq, J = 12.6 Hz, 2H), 3.69, 3.66 (ABq, J = 9.6 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 165.6, 150.7, 142.9, 137.5, 137.4, 128.7, 128.6, 128.2, 128.1, 128.1, 100.7, 86.6, 86.1, 83.8, 74.3, 73.8, 72.5, 70.0, 52.2; HRMS (ESI) calcd for C₂₄H₂₆N₅O₆ [M + H]⁺ 480.1883, found [M + H]⁺ 480.1882 (Δm –0.0001, error, –0.2 ppm).

4'-C-Azidomethyl-3',5'-di-O-benzyl-2'-deoxy-2'-fluorouridine (6). Compound 4 (200 mg, 0.42 mmol) was dissolved in DCM/

pyridine (3:1, v/v, 4 mL) and stirred in an ice bath. Ti_2O_3 (0.08 mL, 0.50 mmol) was added dropwise under nitrogen atmosphere and the mixture stirred for 2 h. The reaction was quenched with cold saturated aqueous NaHCO_3 (50 mL) and extracted with DCM (3×80 mL). The organic phase was dried over anhydrous Na_2SO_4 , evaporated under reduced pressure followed by co-evaporation with toluene (2×30 mL), and dried. The crude compound **5** was dissolved in dry toluene (10 mL). $\text{Et}_3\text{N} \cdot 3\text{HF}$ (0.07 mL, 1.67 mmol) and Et_3N (0.12 mL, 0.83 mmol) were added and the mixture heated at 45°C . After 12 h, an additional batch of $\text{Et}_3\text{N} \cdot 3\text{HF}$ (0.14 mL, 0.83 mmol) and Et_3N (0.12 mL, 0.83 mmol) were added, and the reaction mixture was heated for 48 h. Toluene was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate (70 mL) and washed with satd NaHCO_3 (40 mL). The aqueous layer was extracted with ethyl acetate (3×70 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude compound was purified by column chromatography (25% ethyl acetate in hexane) to give compound **6** as yellow sticky solid (90 mg, 45%): $R_f = 0.52$ (40% ethyl acetate in petroleum ether); ^1H NMR (300 MHz, CDCl_3) δ 9.25 (bs, 1H), 7.69 (d, $J = 8.1$ Hz, 1H), 7.36–7.22 (m, 11 H), 6.2 (dd, $J = 15.5$ Hz, 2.6 Hz, 1H), 5.3 (d, $J = 8.1$ Hz, 1H), 5.10 (ddd, $J = 53$ Hz, 5.2 Hz, 2.87 Hz, 1H), 4.80, 4.52 (AXq, $J = 11.6$ Hz, 2H), 4.50, 4.46 (ABq, $J = 11.6$ Hz, 2H), 4.33 (dd, $J = 16.7$ Hz, 4.6 Hz, 1H), 3.87, 3.56 (AXq, $J = 10.4$ Hz, 2H), 3.71, 3.42 (AXq, $J = 13.5$ Hz, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 163.3, 150.2, 140.3, 136.9, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 102.5, 92.5 (d, $J = 194.5$, 1C), 88.3 (d, $J = 33.2$ Hz, 1C), 87.5, 76.5 (d, $J = 14.2$ Hz, 1C), 74.0, 73.6, 70.8, 52.9; ^{19}F NMR (376.5 MHz, CDCl_3) δ -199.1 (dt, $J = 52.8$ Hz, 17.3 Hz, 1F); HRMS (ESI) calcd for $\text{C}_{24}\text{H}_{25}\text{N}_5\text{O}_5\text{F}$ $[\text{M} + \text{H}]^+$ 482.1840, found $[\text{M} + \text{H}]^+$ 482.1832 ($\Delta m = -0.0008$, error, -1.7 ppm).

4'-C-Azidomethyl-2'-deoxy-2'-fluorouridine (7). Nucleoside **6** (50 mg, 0.103 mmol) was dissolved in DCM (2 mL). The solution was cooled to -78°C , BCl_3 (0.7 mL, 1 M solution in DCM) was added, and the mixture was stirred at -78°C for 3 h. The temperature was increased to -30°C and the mixture stirred again for 3 h. The reaction mixture was quenched using a 1:1 mixture of DCM–MeOH (v/v, 1 mL). Solvent was evaporated and purified using column chromatography (5% methanol in DCM) to afford compound **7** as a white sticky solid (25 mg, 83%): $R_f = 0.3$ (5% methanol in DCM); ^1H NMR (400 MHz, CD_3OD) δ 8.01 (d, $J = 8.0$ Hz, 1H), 6.2 (dd, $J = 14.8$ Hz, 4.5 Hz, 1H), 5.72 (d, $J = 8$ Hz, 1H), 5.22 (dt, $J = 53.5$ Hz, 4.6 Hz, 1H), 4.51 (dd, $J = 12.2$ Hz, 5 Hz, 1H), 3.78, 3.68 (ABq, $J = 11.7$ Hz, 2 H), 3.60, 3.44 (ABq, $J = 13.2$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.2, 152.3, 142.8, 103.2, 95 (d, $J = 190.5$ Hz, 1C), 89.7, 88.7 (d, $J = 33.1$ Hz, 1C), 71.3 (d, $J = 15.4$ Hz, 1C), 64.2, 53.3; ^{19}F NMR (376.5 MHz, CD_3OD) δ -205.2 (dt, $J = 52.1$ Hz, 13.9 Hz, 1F); HRMS (ESI) calcd for $\text{C}_{10}\text{H}_{13}\text{N}_5\text{O}_5\text{F}$ $[\text{M} + \text{H}]^+$ 302.0901, found $[\text{M} + \text{H}]^+$ 302.0887 ($\Delta m = 0.0013$, error, -4.4 ppm).

4'-C-Aminomethyl-2'-deoxy-2'-fluorouridine (8). Nucleoside **7** (20 mg, 0.07 mmol) was dissolved in THF (1.5 mL). To this, water (0.05 mL) and PPh_3 (34 mg, 0.132 mmol) were added. The reaction mixture was stirred at 45°C for 3 h. Solvent was evaporated and the mixture purified by preparative TLC (80% ethyl acetate in petroleum ether) to give fluoro nucleoside **8** as a pale yellow solid (14 mg, 76%): $R_f = 0.15$ (40% MeOH in DCM); mp 160 – 161°C ; ^1H NMR (400 MHz, CD_3OD) δ 7.98 (d, $J = 8.0$ Hz, 1H), 6.2 (dd, $J = 14.8$ Hz, 4.8 Hz, 1H), 5.72 (d, $J = 8$ Hz, 1H), 5.22 (dt, $J = 53.4$ Hz, 5.0 Hz, 1H), 4.51 (dd, $J = 11.1$ Hz, 5.3 Hz, 1H), 3.71, 3.68 (ABq, $J = 11.9$ Hz, 2 H), 2.99, 2.84 (ABq, $J = 13.7$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.8, 152.8, 143.0, 103.2, 95 (d, $J = 191.2$ Hz, 1C), 89.2, 89 (d, $J = 33.6$ Hz, 1C), 72.5 (d, $J = 15.4$ Hz, 1C), 65.3, 42.3. ^{19}F NMR (376.5 MHz, CD_3OD) δ -205.6 (dt, $J = 54.8$ Hz, 12.9 Hz, 1F); HRMS (ESI) calcd for $\text{C}_{10}\text{H}_{15}\text{N}_5\text{O}_5\text{F}$ $[\text{M} + \text{H}]^+$ 276.0996, found $[\text{M} + \text{H}]^+$ 276.0986 ($\Delta m = -0.001$, error, -3.6 ppm).

Sugar Conformational Analysis Using PSEUROT Program. The coupling constant (J) derived from ^1H NMR of the free nucleoside in methanol- d_4 was used for the sugar conformational analysis with PSEUROT program.²⁹ Using the electronegativity editor in the program, the nature of the substituent such as fluorine at C2' and aminomethyl at C4' of the five-membered ring was assigned. The

scanning was performed with ν_{max} and started from 10° to 60° and P from 0° to 360° ; a total of 3600 grid points were scanned to find the minimum energy surface of the nucleoside in P . The temperature was set to 22°C , which was used for recording NMR; all other parameters were retained as default in the program.²⁹

Sugar Conformational Analysis Using DFT Calculations. The model used in this study contains modified nucleosides in the middle and is capped with sugar phosphate at the 3' and 5' end (Figure S1, Supporting Information). To avoid the complexity from stacking interactions between the bases, bases were removed from both ends of the sugar and replaced with methyl groups. The sugar conformation at both ends was frozen to the C3'-endo conformer, and the potential energy for the sugar conformations of the modified nucleotides were calculated using DFT level (MP2/6-311++G*) in Gaussian 09.⁴⁵ For each modification, different sugar–phosphate dihedrals were chosen from the Nucleic Acid Database (NDB),⁴⁰ and calculations were performed in the gas phase and in implicit solvent. A potential energy scan was performed around the pseudorotation phase angle (P) with increments of 0.5° yielding 720 conformations for each modification. For each of these conformations, initially the structures were first optimized in the HF/6-31G* level, and then energies were obtained at MP2/6-311++G* level.³¹ To investigate the effect of solvent (water) on the sugar conformation, the structures were initially optimized at HF/6-31G* level, and energies were obtained at the PBE density functional, 6-311++G* basis set.³⁰ For solvation in DFT calculations the PCM model was used.⁴⁶

C–H...F H-Bond Analysis Using NCI Plot. A low energy conformer obtained from DFT calculations in the solvent phase of 4'-AM-2'-F-uridine was used for plotting reduced density gradient(s) against electron density (ρ).³⁷ To identify only the hydrogen bonds and electrostatic interactions, excluding the van der Waals interactions, the ρ value was set from 0.02–0.06 au. The output from the NCI program was plotted using gnuplot, and the H-bond surface was rendered using VMD 1.9.1.

Sugar Conformational Analysis Using MD Simulations. The same model system used for the DFT calculations was employed for the MD simulations using AMBER 12.³⁹ Modified nucleotides 2'-F and 4'-AM-2'-F uridine were optimized using Gaussian 09 at the HF/6-31G* level, and the force field was obtained using the protocol published by Aduri and co-workers⁴⁷ (Figures S14 and S15, Supporting Information). Initial coordinates for the model system were chosen from the 14 different sugar conformers with different P values, which are obtained from NDB.³⁰ For each sugar conformation, 5 ns of production run were carried out yielding 70 ns ($14 \times 5 = 70$) simulations for each modification. Using van der Waals and 1–4 electrostatic interactions, the energy of the system at each sugar conformation was calculated from every 5 ns of the total 70 ns MD simulations. In all simulations, the AMBER force field with revised torsional parameters χ and α/γ was used,⁴⁸ and charges of the systems were neutralized using Na^+ ions. A TIP3P water box was used to solvate the system around 6 Å from any atom of the solute. Structures were minimized in three steps: (i) by fixing the model system, solvent and ions were minimized using steepest descent of 2500 steps; (ii) while the modified nucleotide was detained with a restraint force of 500 kcal/mol Å², steepest descent minimization of 5000 steps was followed by 5000 steps of conjugate gradient minimization; and (iii) without any restraints, steepest descent minimization of 1000 steps was followed by 1000 steps of conjugate gradient minimization. After the minimization, pressure equilibration were carried out in two steps, in which the modified nucleotide was fixed with a restraint force 25 kcal/mol Å² and a long-range cutoff of 8 Å. SHAKE was used for bonds involving hydrogen atoms. The temperature was raised from 0 to 300 K. In the second step, all of the above conditions were maintained except the temperature was kept constant and pressure dynamics was used. Minimization and equilibration were carried out using the SANDER module, and production runs were carried out with the PMEMD in AMBER 12.³⁹

H-Bonding Analysis Using Umbrella Sampling Simulations. Trinucleotide RNA duplex (5'-GUC-3'/3'-CAG-5') was generated using the *nucgen* module in AMBER12.³⁹ Uridine was replaced with

modified nucleotides such as 2'-OMe, 2'-F, 4'-AM-2'-F, and 4'-AM-2'-OMe. The AMBER force field with revised torsional parameters for χ and α/γ was used.⁴⁸ The system was initially minimized at vacuum to reach low energy geometries and further used for the restraint angle determination. All of five trinucleotide systems with different modifications, including an unmodified system, were solvated in an octahedral box using TIP3P water and neutralized with and Na⁺ ions.⁴⁹ The SHAKE algorithm was used to constrain the bonds involving hydrogen atoms. Solvated systems were initially minimized in two steps; at first the positional restraints were applied to the nucleotides to minimize the solvent and ions. Finally, unrestrained minimizations were applied to the whole solvated system. Various sampling times were performed for each system to predict the error in the final energy calculation (Tables S4–S8, Supporting Information), and the free energy errors were within the acceptable limit⁴¹ (Table S9, Supporting Information). All of the parameters for performing the final equilibrations and MD simulations were obtained from the published protocol.⁴¹ The reaction co-ordinates were recorded every five steps, and the WHAM algorithm potential mean force was obtained as a function of H-bond distance with a tolerance of 0.00001.⁵⁰ Trajectories were visualized using UCSF Chimera, and the figures were rendered using PyMOL v0.99.

■ ASSOCIATED CONTENT

Supporting Information

Additional data from DFT calculations, MD simulations, and NMR spectra of all the new compounds. 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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■ DEDICATION

Dedicated to Professor Krishna N. Ganesh on the occasion of his 60th birthday.

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