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**Modified Nucleosides**

# Synthesis and Duplex-Stabilizing Properties of Fluorinated N-Methanocarbathymidine Analogues Locked in the C3′-endo Conformation**

*Michael E. Jung,* Timothy A. Dwight, Frederic Vigant, Michael E. Østergaard, Eric E. Swayze, and Punit P. Seth*

*Abstract: The efficient synthesis, antiviral activity, and duplex-stabilizing properties of both isomers of the 2′-fluoro analogue of Northern methanocarbathymidine (N-MCT), 2 and 3, are reported. We show that 2′-F incorporation on the N-MCT scaffold has a strong stabilizing effect on duplex thermal stability.*
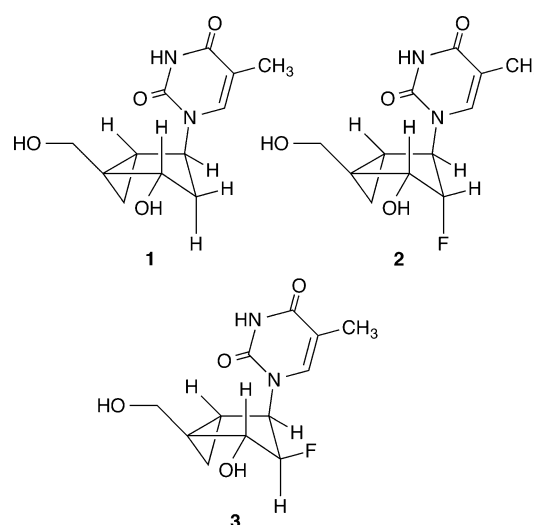
Oligonucleotides (ONs) modified with fluorinated nucleic acid monomers show excellent properties for the modulation of gene expression through multiple antisense mechanisms.[1] Furthermore, fluorinated nucleoside analogues been applied as antiviral[2] and anticancer agents.[3] We recently characterized the antisense properties of both isomers of 2′-fluorohexitol nucleic acid (FHNA)[4,5] and 2′-fluorocyclohexenyl nucleic acid (F-CeNA).[6] We found that fluorine incorporation can indeed have a beneficial effect on the properties of modified oligonucleotides as compared to those of the parent nonfluorinated counterparts. As a continuation of that program and our interest in the synthesis of carbocyclic nucleoside analogues,[7] we investigated the synthesis and duplex-stabilizing properties of both isomers of 2′-F-modified N-methanocarbathymidine (N-MCT) oligonucleotides.

Almost two decades ago, Altmann et al.[8] and Marquez and co-workers[9] independently showed that the bicyclo-[3.1.0]hexyl scaffold can serve as an effective structural mimic of the C3′-endo sugar pucker. The appended cyclopropyl ring essentially "locks" the conformation of the cyclopentane ring without the need for an electron-withdrawing group at the 2′-position (Scheme 1). As a result, Northern-methanocarba-cyclic (NMC)-modified ONs displayed enhanced RNA-bind-
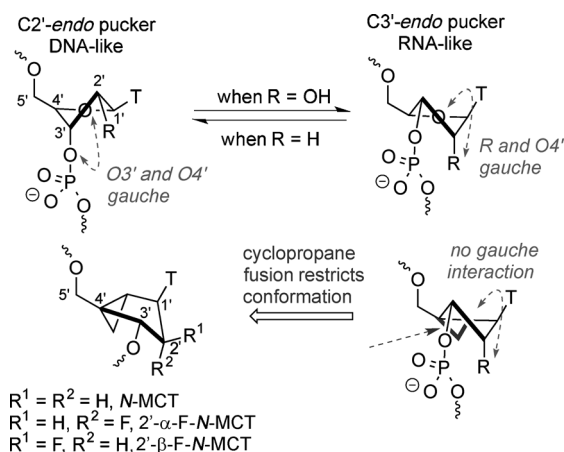


**Scheme 1.** Conformational preference of bicyclo[3.1.0]hexane nucleosides versus normal nucleosides.

ing affinity and stability towards nuclease-mediated degradation.[9] Also, **1** (Scheme 2) was shown to have very potent antiviral activity against a number of viruses.[10] We postulated that the introduction of a fluorine substituent at the 2′-position of the NMC scaffold could impart interesting biological properties for antiviral and antisense applications. In particular, we predicted that it would further improve the antisense properties of modified oligonucleotides by

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**Scheme 2.** Structure of nucleoside analogues.

increased polarization of the nucleobase[11] and/ or by improved interactions with proteins which facilitate antisense effects.[1d,e] Herein, we present the synthesis of both isomers, **2** and **3**, of the 2′F-N-MCT nucleoside, their evaluation as antiviral agents, and the duplex-stabilizing properties of the modified oligonucleotides.

The original syntheses of the N-methanocarbacyclic nucleosides both started from a natural sugar, and the existing functionality was altered to prepare **1** and its close analogues. We decided to begin with achiral material and carry out an enantioselective synthesis (Scheme 3). The well-known enone alcohol **4**[12] was protected as the TBS ether **5** (TBSCl/ImH/dichloromethane) in 96% yield. The key enantioselective reduction of **5** was accomplished by using the Corey–Bakshi–Shibata (CBS) catalyst,[13] which gave the desired allylic alcohol **6** in 88% yield. The ee value was calculated to be 93% by integration of the relevant peaks in the $^1$H NMR spectrum of the corresponding Mosher esters.[14] Indeed, this mixture of diastereomeric Mosher esters could be readily separated by column chromatography to give an essentially enantiomerically pure sample of **6**. Hydroxy-directed cyclopropanation[15] of **6** afforded the desired cis-cyclopropyl alcohol **7** in excellent yield and diastereoselectivity (essentially none of the opposite diastereomer was observed), and oxidation with Dess–Martin periodinane (DMP) furnished the ketone **8** in 92% yield.[16] The fluorine substituent was then introduced by formation of the enolate and trapping with N-fluorodibenzenesulfonimide (NFSI) to give a mixture of the two diastereomeric fluorides **9a,b** in 72% yield. Since the two-step introduction of the alkene (phenylselenylation followed by oxidative elimination) gave the desired α-fluoroenone **10** in 86% yield, the formation of **9** as a mixture of diastereomers was inconsequential.

With the enone **10** in hand, we next examined various nucleophilic 1,4-addition reactions. In a model study, we showed that certain nucleobases, such as N-benzoyladenine (N-Bz-Ade), could be added to the unsubstituted enone **15** (prepared by a Saegusa–Ito oxidation[17] of the ketone **8**), but the yield of **16** was not extremely high (Scheme 4). Although silyl azide and even ammonia could be added to **15**, again disappointing, poor yields prompted us to examine other nucleophiles. We finally settled on dibenzylamine. When added to the enone **10**, it gave a mixture of two diastereomers at the carbon center bearing the fluorine atom, with the α-fluoro compound **11a** (59%) predominating over the β-fluoro compound **11b** (12%).[18] Attack of the nucleophile on the β face of the enone was expected, since the cyclopropyl hydrogen atoms hinder attack on the α face. Presumably, the configuration α to the ketone in **11a,b** is determined by
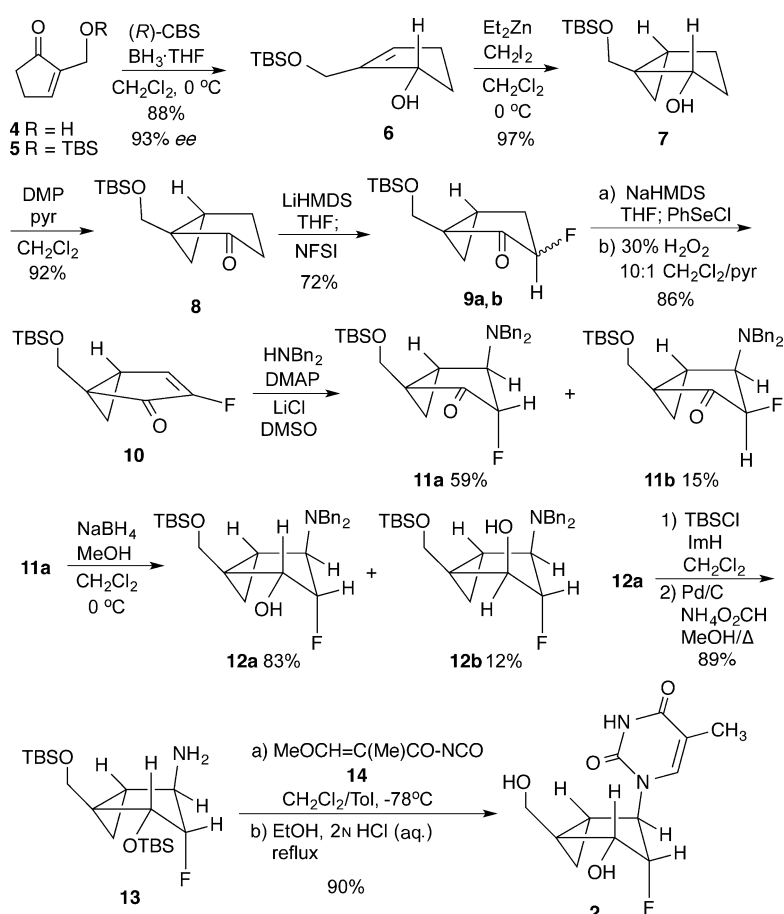


**Scheme 3.** Synthesis of analogue **2**. Bn = benzyl, DMAP = 4-dimethylaminopyridine, DMSO = dimethyl sulfoxide, HMDS = hexamethyldisilazide, ImH = imidazole, pyr = pyridine, TBS = tert-butyldimethylsilyl, Tol = toluene.
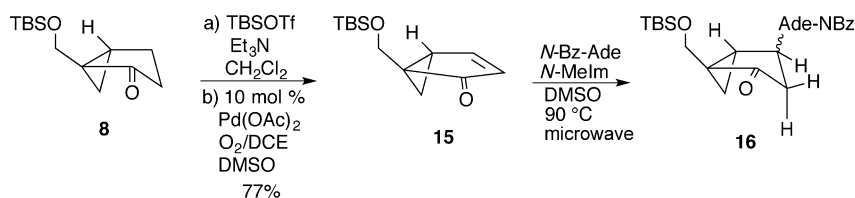


**Scheme 4.** Synthesis of **15** and **16**. DCE = 1,2-dichloroethane, Tf = trifluoromethanesulfonyl. N-MeIm = N-methylimidazole.

protonation of the α-fluoroenolate, which occurs preferentially from the more hindered top face, possibly as a result of internal protonation from the protonated amine group on the β face of the molecule. The structures of these compounds were assigned on the basis of $^1$H NMR spectroscopy, and especially the vicinal coupling constants. It turns out that in **11a**, the angles between the proton Hb and both Ha and Hc are nearly 85°, and thus the coupling constants $J_{a,b}$ and $J_{b,c}$ are nearly zero according to the Karplus equation[19] (Scheme 5).

Reduction of the α-fluoroketone **11a** with sodium borohydride afforded mainly the desired α-alcohol **12a** (83%) with a minor amount of the epimeric alcohol **12b**. This result was expected, since in conformationally rigid α-fluorocyclohexanone systems in which the fluorine substituent is held
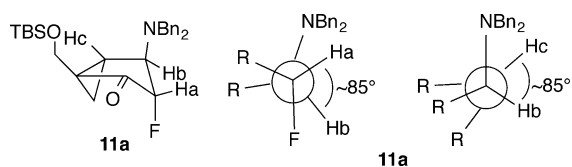
**Scheme 5.** Stereoview of **11 a**.

axial, hydride attack occurs preferentially antiperiplanar to the C–F bond to give predominately the *cis* fluoroalcohol (91:9 with LiAlH$_4$).[20] In this case, electronic preference outweighs the considerable steric hindrance of the dibenzyl-amino substituent on the β face of the molecule, and the α-alcohol predominates. Again, the configuration was assigned on the basis of the same type of coupling-constant analysis. However, in this case, after chromatographic purification, the major alcohol **12 a** crystallized, and we were able to obtain an X-ray crystal structure that confirmed the stereostructure (Figure 1). Protection of the hydroxy group of **12 a** as the silyl ether and subsequent hydrogenolytic removal of the benzyl groups gave the amine **13**. This secondary amine was treated with the known acyl isocyanate **14**[21] at low temperature, and the product was heated at reflux in ethanolic acid to afford the desired thymidine nucleoside analogue **2** in 90% yield.

To prepare the diastereomeric analogue **3** (Scheme 6), we reduced the ketone **11b** with borohydride and obtained only the β-alcohol **17** in 94% yield. In this case, the steric hindrance of the β-dibenzylamino substituent completely dominates the much weaker electronic preference.[20] Mitsu-nobu inversion with 4-nitrobenzoic acid gave the inverted ester, which was hydrolyzed with carbonate in hot methanol to afford the desired α-alcohol **18** in 75% yield for the two steps. This secondary alcohol was converted into the *S* Mosher ester to both protect it for the final steps of the synthesis and to guarantee the optical purity of the material. Reductive removal of the benzyl groups by transfer hydrogenation gave the amino ester **19** in 84% yield. The final steps were analogous to those used for the synthesis of **2**, namely, condensation of the amine with the isocyanate **14**, followed by cyclization and desilylation with ethanol acid to give the ester derivative of the nucleoside. Final cleavage of the ester with
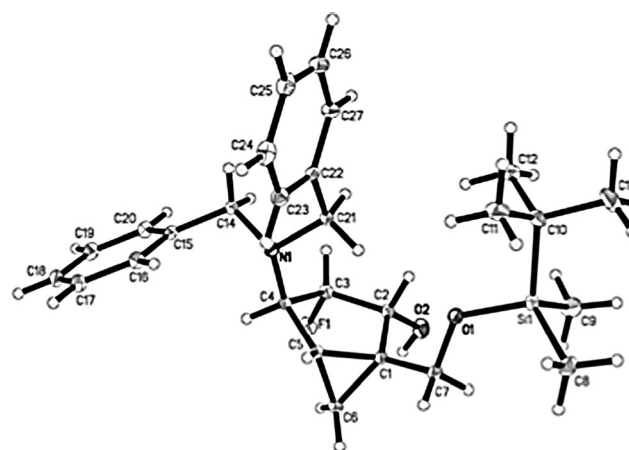


**Figure 1.** X-ray crystal structure of **12 a**.

potassium carbonate in warm methanol gave the desired 3′-β-fluoro nucleoside analogue **3** in 70% yield for the last two operations.

The new analogues **2** and **3** were subjected to antiviral testing; both N-MCT **1** and ganciclovir were used as positive controls. Both analogues are active antivirals but significantly less active than the control compounds: **2** is 100–1000-fold less active than N-MCT **1**, and **3** is 10–100-fold less active than N-MCT **1**.

The duplex-stabilizing properties of 2′-F-NMC **2** and *ara*-2′-F-NMC **3** were measured by the single incorporation of the modified nucleotide in a previously described oligonucleotide sequence[22] and compared to those found for F-RNA (Table 1). The modified nucleotides were inserted at four different locations and were flanked on either side by different nucleobases, thus providing a position and sequence context for the evaluation. A single incorporation of the F-NMC nucleotide showed good duplex-stabilization properties versus RNA (+2.2°C per modification). The duplex stabilization was superior to that exhibited by F-RNA (+0.8°C/mod vs. RNA), but both modifications were slightly destabilizing versus DNA (−1.2 and −1.9°C/mod, respectively). In contrast, *ara*-2′-F-NMC had a moderate destabilizing effect on duplex thermal stability versus RNA (−2.8°C/mod) and a strong destabilization effect versus DNA complements (−6.9°C/mod).

In conclusion, we have reported the efficient enantioselective synthesis of both isomers of 2′-F-NMC-modified T nucleosides, **2** and **3**, their evaluation in antiviral assays, and the duplex-stabilizing properties of the modified oligonucleotides. We showed that whereas the 2′-F-NMC modification has a strong stabilizing effect upon duplex stability, the 2′-*ara*-fluoro isomer is destabilizing. This result is in contrast to the duplex-stabilizing properties of 2′-fluoroarabino nucleic acids (F-ANA), in which the 2′-F atom stabilizes oligonucleotide duplexes by engaging in H⋯F interactions with H8 of the 3′-adjacent purine
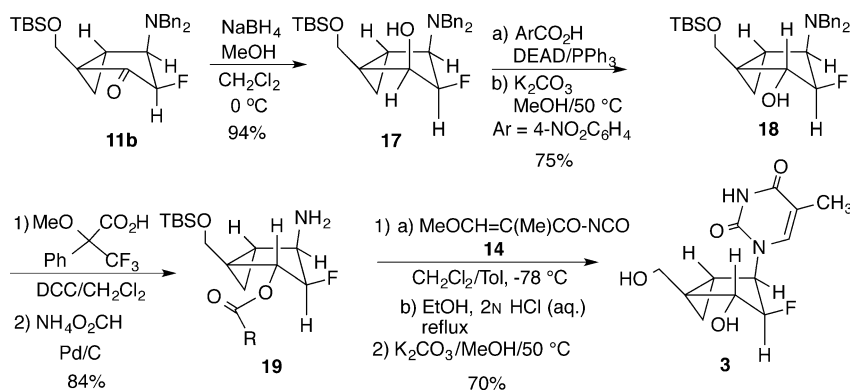


**Scheme 6.** Synthesis of analogue **3**. DCC = *N,N′*-dicyclohexylcarbodiimide, DEAD = diethyl azodicarboxylate.

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***Table 1:*** Relative melting temperature ($T_m$) of modified oligonucleotide duplexes.[a]

| Sequence (5′ to 3′) | $\Delta T_m$/mod [°C] vs. RNA | | | $\Delta T_m$/mod [°C] vs. DNA | | |
|---|---|---|---|---|---|---|
| | F-RNA U | **2** | **3** | F-RNA U | **2** | **3** |
| GGA*T*GTTCTCGA | +1.1 | +2.2 | −2.6 | −0.5 | −2.7 | −7.3 |
| GGATG*T*TCTCGA | −0.3 | +2.7 | −2.2 | −1.3 | −0.9 | −4.6 |
| GGATGT*T*CTCGA | +1.3 | +2.0 | −4.3 | −1.9 | −3.0 | −8.1 |
| GGATGTTC*T*CGA | +1.2 | +2.6 | −2.1 | −1.0 | −1.1 | −4.9 |
| average $\Delta T_m$/mod values | +0.8 | +2.2 | −2.8 | −1.2 | −1.9 | −6.2 |

[a] $T_m$ values were measured in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl and 0.1 mM ethylenediaminetetraacetic acid by using the RNA complement 5′-r(UCGAGAACAUCC)-3′. Capital letters represent DNA monomers, whereas red letters indicate the position of modification.

nucleobases.[23] Structural studies have shown that the furanose ring in F-ANA adopts the O4′-*endo* conformation, which presumably facilitates the above interactions.[24] In contrast, the sugar rings of 2′-F-NMC and *ara*-2′-F-NMC are locked in the C3′-*endo* conformation. Thus, our data show that a *trans*-diaxial orientation between the fluorine atom and the nucleobase is critical for nucleic acid modifications in which the sugar ring is locked in the C3′-*endo* conformation.[11] Further investigation of the biological properties of 2′-F-NMC-modified ONs are ongoing.

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a mixture of diastereomers at the fluorine atom, but in only moderate yield (26%).

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