JOC Featured Article

pubs.acs.org/joc

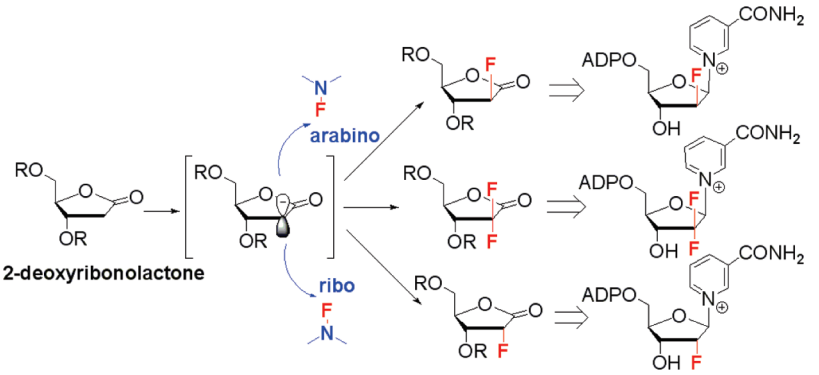
Diastereocontrolled Electrophilic Fluorinations of 2-Deoxyribonolactone: Syntheses of All Corresponding 2-Deoxy-2-fluorolactones and 2′-Deoxy-2′-fluoro-NAD$^+$s

Yana Cen and Anthony A. Sauve*

Department of Pharmacology, Weill Medical College of Cornell University, 1300 York Avenue, New York, New York 10065

aas2004@med.cornell.edu

Received March 25, 2009



Methods to construct 2′-deoxy-2′-fluoro nucleosides have undergone limited improvement in the last 20 years in spite of the substantially increased value of these compounds as pharmaceuticals and as tools for studying biological processes. We herein describe a consolidated approach to synthesize precursors to these commercially and scientifically valuable compounds via diastereocontrolled fluorination of the readily available precursor 2-deoxy-D-ribonolactone. With employment of appropriate sterically bulky silyl protecting groups at the 3 and 5 positions, controlled electrophilic fluorination of the Li−ribonolactone enolate by *N*-fluorodibenzenesulfonamide yielded the corresponding 2-deoxy-2-fluoroarabinolactone in high isolated yield (72%). The protected 2-deoxy-2,2-difluororibonolactone was obtained similarly in high yield from a second round of electrophilic fluorination (two steps, 51% from protected ribonolactone starting material). Accomplishment of the difficult ribofluorination of the lactone was achieved by the directive effects of a diastereoselectively installed α-trimethylsilyl group. Electrophilic fluorination of a protected 2-deoxy-2-trimethylsilylarabinolactone via enolate generation provided the protected 2-deoxy-2-fluororibolactone as the exclusive fluorinated product. The reaction also yielded the starting material, the desilylated protected 2-deoxyribonolactone, which was recycled to provide a 38% chemical yield of the fluorinated product (versus initial protected ribonolactone) after consecutive silylation and fluorination cycles. Using our fluorinated sugar precursors, we prepared the 2′-fluoroarabino-, 2′-fluororibo-, and 2′,2′-difluoronicotinamide adenine dinucleotides (NAD$^+$) of potential biological interest. These syntheses provide the most consolidated and efficient methods for production of sugar precursors of 2′-deoxy-2′-fluoronucleosides and have the advantage of utilizing an air-stable electrophilic fluorinating agent. The fluorinated NAD$^+$s are anticipated to be useful for studying a variety of cellular metabolic and signaling processes.

Introduction

The efficient introduction of fluorine atoms into bioactive organic molecules has attracted considerable attention in recent years owing to the unique properties of the fluorine substituent.[1−4] The selective replacement of hydrogen or oxygen with fluorine can change a compound's biological activity, metabolic stability, chemical stability, lipophilicity, acidity, and dipole properties with modest change in steric bulk.[2−4]

DOI: 10.1021/jo900637f Published on Web 07/07/2009

A broad class of particularly relevant compounds are the fluorinated carbohydrates[1,5,6] and nucleosides.[1,3,4] Selective fluorination in the sugar moiety of glycosides or nucleosides has proven useful to numerous investigations of enzyme mechanism where either sugars or nucleosides are substrates.[6−14] Some of these compounds are potent drugs. For example, gemcitabine (Gemzar, Eli Lilly, 2′-deoxy-2′,2′-difluorocytidine) is used clinically to treat numerous cancers including ovarian,[15] pancreatic,[16] and breast[17] cancers, with sales in excess of 1.6 billion dollars per year.[18] Of the monosubstituted 2′-fluoronucleosides, clofarabine (Clorar, Genzyme, 2-chloro-2′-deoxy-2′-fluoroarabinoadenosine), has been approved for pediatric patients with relapsed or refractory acute lymphocytic leukemia,[19] and annual sales now exceed 100 million dollars.[20]

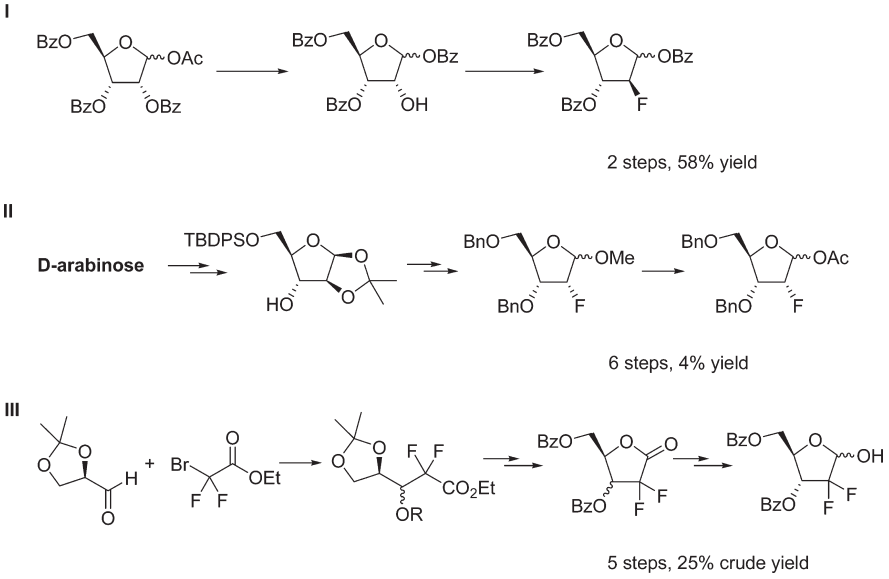
Our laboratory is interested in general and flexible methods to construct 2′-fluoronucleosides, nucleotides and dinucleotides, particularly derivatives of nicotinamide riboside. In previous studies, we demonstrated that 2′-deoxy-2′-fluoroarabinonicotinamide-mononucleotide is a potent mechanism-based inhibitor (apparent $K_i = 61$ nM) of the signaling enzyme cell developmental protein 38 (CD38).[10,12] The 2′-fluoro-NAD^+s and related compounds are likely to be valuable for the study of sirtuin enzyme mechanism[11,21] and for studying the chemical properties of poly-ADP-ribosylpolymerases.[22] Fluorinated NAD^+s could be useful for identifying ADP-ribosyltransfer sites on proteins as well. For example, we previously demonstrated that the 2′-deoxy-2′-fluoroarabinofuranosyl modification is suitably robust for MS/MS approaches used to characterize amino acid post-translational modifications.[10] ADP-ribosylation sites are poorly surveyed within the proteome, and yet these modifications are of heightened interest as they are implicated in important biological effects.[22−24] The synthesis of 2′-deoxy-2′-fluoroarabino-NAD^+ was previously described,[25] but syntheses of 2′-deoxy-2′-fluororibo-NAD^+ and 2′-deoxy-2′,2′-difluoro-NAD^+ or their nucleoside precursors have not appeared in the literature. The difluoro derivatives in particular could be quite interesting on the basis of their anticipated chemical stability and altered electronic properties.

Methods to synthesize the 2-deoxy-2-fluoro-D-furanose precursors to 2′-fluorinated nucleosides have not experienced substantial recent innovation,[1,3,26] despite the fact that interest and demand for these compounds have only increased in recent years. The synthetic methods currently available vary in efficiency, and all depend on routes relying on different initial precursors to the respective final products. For example, the best method to make a protected 2-deoxy-2-fluoroarabinofuranose requires only two steps (Scheme 1, part I) from a protected ribose (58% overall yield).[25,27−29] On the other hand, 2-deoxy-2-fluororibofuranose has no concise or efficient synthesis[30] and still requires six steps from arabinose (Scheme 1, part II; 4% overall yield).[31] Alternatively, it can be obtained via a 10-step nondiastereoselective method in 11% overall yield.[32−34] An efficient but nondiastereoselective route is established for the synthesis of protected 2-deoxy-2,2-difluororibonofuranose in five steps (Scheme 1, part III; crude yield 25%) via coupling of ethyl bromodifluoroacetate and isopropylidene glyceraldehyde. The route is not diastereoselective and depends upon crystallization of the preferred isomer.[35] This procedure was developed by Eli Lilly for commercial synthesis of gemcitabine. 2-Deoxy-2,2-difluororibofuranose can also be obtained stereoselectively from glucose or mannose,[36] but that method involves eight steps and very low overall yield (<15%). We wondered if it might be possible to improve and perhaps consolidate synthetic approaches to these desirable sugars by employing electrophilic fluorine as a means to modify protected 2-deoxyribonolactone, a starting material that can be obtained readily, abundantly, and cheaply.

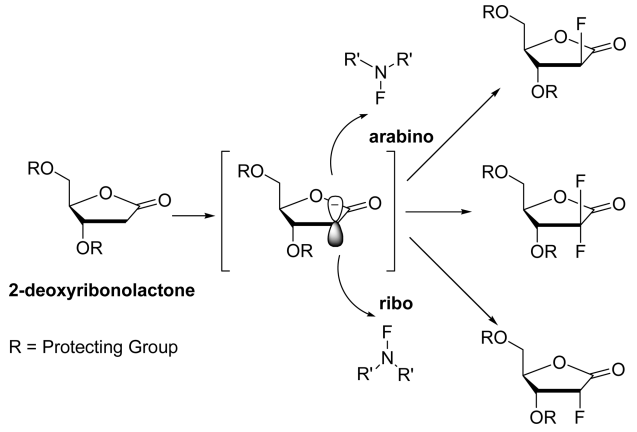
The strategy we considered is visually described in Scheme 2. We contemplated a general solution to the introduction of fluorine by controlling the reactivity of a 2-deoxyribonolactone enolate with an electrophilic fluorinating reagent. It is

(1) Ma, J. A.; Cahard, D. *Chem. Rev.* **2008**, *108*, PR1–PR43.
(2) Hagmann, W. K. *J. Med. Chem.* **2008**, *51*, 4359–4369.
(3) Kirk, K. L. *Org. Process Res. Dev.* **2008**, *12*, 305–321.
(4) O'Hagan, D. *Chem. Soc. Rev.* **2008**, *37* (2), 308–319.
(5) Nicolaou, K. C.; Mitchell, H. J. *Angew. Chem., Int. Ed.* **2001**, *40*, 1576–1624.
(6) Williams, S. J.; Withers, S. G. *Carbohydr. Res.* **2000**, *327*, 27–46.
(7) Vocadlo, D. J.; Withers, S. G. *Carbohydr. Res.* **2005**, *340*, 379–388.
(8) Vocadlo, D. J.; Davies, G. J.; Laine, R.; Withers, S. G. *Nature* **2001**, *412*, 835–838.
(9) Zechel, D. L.; Withers, S. G. *Curr. Opin. Chem. Biol.* **2001**, *5*, 643–649.
(10) Sauve, A. A.; Deng, H. T.; Angeletti, R. H.; Schramm, V. L. *J. Am. Chem. Soc.* **2000**, *122*, 7855–7859.
(11) Jackson, M. D.; Schmidt, M. T.; Oppenheimer, N. J.; Denu, J. M. *J. Biol. Chem.* **2003**, *278*, 50985–50998.
(12) Liu, Q.; Kriksunov, I. A.; Jiang, H.; Graeff, R.; Lin, H.; Lee, H. C.; Hao, Q. *Chem. Biol.* **2008**, *15*, 1068–1078.
(13) Porter, D. J.; Merrill, B. M.; Short, S. A. *J. Biol. Chem.* **1995**, *270*, 15551–15556.
(14) Clark, J. L.; Hollecker, L.; Mason, J. C.; Stuyver, L. J.; Tharnish, P. M.; Lostia, S.; McBrayer, T. R.; Schinazi, R. F.; Watanabe, K. A.; Otto, M. J.; Furman, P. A.; Stec, W. J.; Patterson, S. E.; Pankiewicz, K. W. *J. Med. Chem.* **2005**, *48*, 5504–5508.
(15) Ferrandina, G.; Ludovisi, M.; Lorusso, D.; Pignata, S.; Breda, E.; Savarese, A.; Del Medico, P.; Scaltriti, L.; Katsaros, D.; Priolo, D.; Scambia, G. *J. Clin. Oncol.* **2008**, *26*, 890–896.
(16) Vulfovich, M.; Rocha-Lima, C. *Expert Rev. Anticancer Ther.* **2008**, *8*, 993–1002.
(17) Silvestris, N.; Cinieri, S.; La Torre, I.; Pezzella, G.; Numico, G.; Orlando, L.; Lorusso, V. *Breast* **2008**, *17*, 220–226.
(18) Eli Lilly. *Eli Lilly 2007 Annual Report*, 2007.
(19) Bonate, P. L.; Arthaud, L.; Cantrell, W. R., Jr.; Stephenson, K.; Secrist, J. A. III; Weitman, S. *Nat. Rev. Drug Discov.* **2006**, *5*, 855–863.
(20) Genzyme. *Genzyme 2007 Annual Report*, 2007.
(21) Sauve, A. A.; Wolberger, C.; Schramm, V. L.; Boeke, J. D. *Annu. Rev. Biochem.* **2006**, *75*, 435–465.
(22) Hakme, A.; Wong, H. K.; Dantzer, F.; Schreiber, V. *EMBO Rep.* **2008**, *9*, 1094–1100.
(23) Smith, B. C.; Hallows, W. C.; Denu, J. M. *Chem. Biol.* **2008**, *15*, 1002–1013.
(24) Haigis, M. C.; Mostoslavsky, R.; Haigis, K. M.; Fahie, K.; Christodoulou, D. C.; Murphy, A. J.; Valenzuela, D. M.; Yancopoulos, G. D.; Karow, M.; Blander, G.; Wolberger, C.; Prolla, T. A.; Weindruch, R.; Alt, F. W.; Guarente, L. *Cell* **2006**, *126*, 941–954.
(25) Sleath, P. R.; Handlon, A. L.; Oppenheimer, N. J. *J. Org. Chem.* **1991**, *56*, 3608–3613.
(26) Dax, K.; Albert, M.; Ortner, J.; Paul, B. J. *Carbohydr. Res.* **2000**, *327*, 47–86.
(27) Reichman, U.; Watanabe, K. A.; Fox, J. J. *Carbohydr. Res.* **1975**, *42*, 233–240.
(28) Tewson, T. J.; Welch, M. J. *J. Org. Chem.* **1978**, *43*, 1090–1092.
(29) Watts, J. K.; Damha, M. J. *Can. J. Chem.* **2008**, *86*, 641–656.
(30) Pankiewicz, K. W. *Carbohydr. Res.* **2000**, *327*, 87–105.
(31) Larsen, C. H.; Ridgway, B. H.; Shaw, J. T.; Smith, D. M.; Woerpel, K. A. *J. Am. Chem. Soc.* **2005**, *127*, 10879–10884.
(32) Mikhailopulo, I. A.; Poopeiko, N. E.; Sivets, G. G.; Khripach, N. B. *Nucleosides Nucleotides* **1995**, *14*, 383–384.
(33) Wright, J. A.; Taylor, N. F.; Fox, J. J. *J. Org. Chem.* **1969**, *34*, 2632–2636.
(34) Anderson, C. D.; Goodman, L.; Baker, B. R. *J. Am. Chem. Soc.* **1958**, *80*, 5247–5252.
(35) Chou, T. S.; Heath, P. C.; Patterson, L. E.; Poteet, L. M.; Lakin, R. E.; Hunt, A. H. *Synthesis* **1992**, *6*, 565–570.
(36) Fernandez, R.; Matheu, M. I.; Echarri, R.; Castillon, S. *Tetrahedron* **1998**, *54*, 3523–3532.

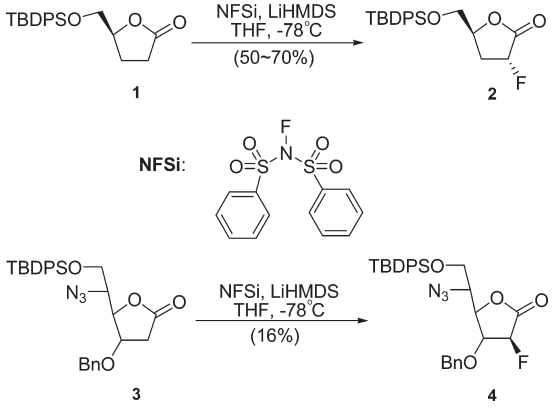
SCHEME 1. Existing Methods for the Syntheses of 2-Deoxy-2-fluoroarabino/ribofuranoses



SCHEME 2. General Strategy for Diastereoselective Electrophilic Fluorination of Protected 2-Deoxyribonolactone

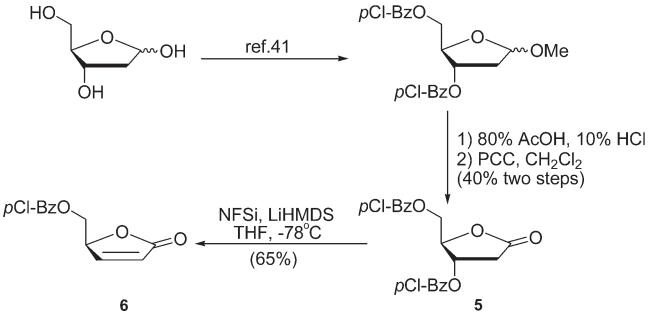


SCHEME 3. Electrophilic Fluorination of 2,3-Dideoxylactone 1 and 2-Deoxylactone 3

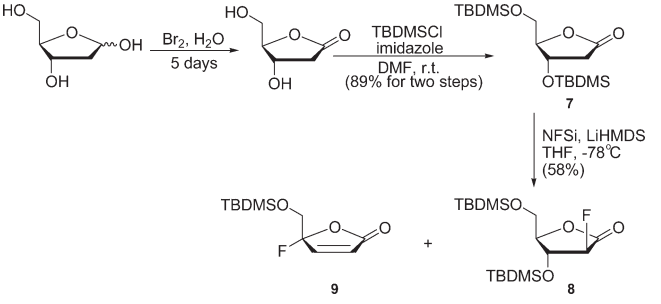


well-known that a lactone enolate can be functionalized in this manner, although stereocontrol is an issue and the studied systems predominantly are not subject to elimination.[1] To be

SCHEME 4. Attempted Fluorination of Lactone 5

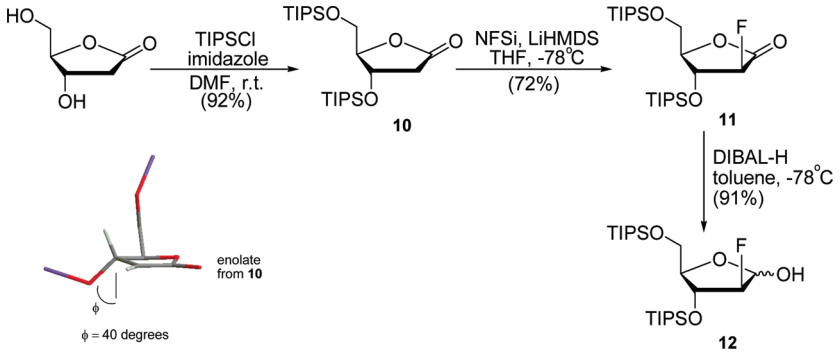


SCHEME 5. Synthesis of TBDMS-Protected 2-Deoxy-2-fluoroarabinolactone



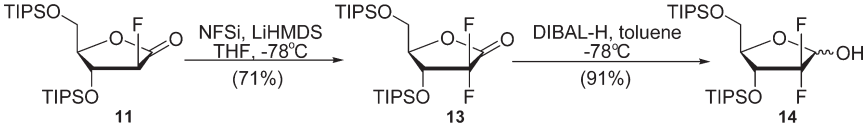
successful, we needed (1) to solve the competing β-elimination of the enolate species and (2) to rigorously control stereochemistry for the electrophilic substitution. We herein describe a successful solution to these problems with significant attenuation of the β-elimination as the undesired side reaction by utilization of bulky silyl protecting groups. Furthermore, we establish a novel approach to the *ribo*-isomer using an α-silyl group. These methods allow us to synthesize the corresponding 2-deoxy-2-fluoroarabino-, 2-deoxy-2-fluororibo-, and 2-deoxy-2,2-difluorolactones and furanoses in a diastereoselective manner with markedly improved synthetic efficiency. We also report the successful

SCHEME 6. Synthesis of Silyl-Protected 2-Deoxy-2-fluoroarabinofuranose[a]

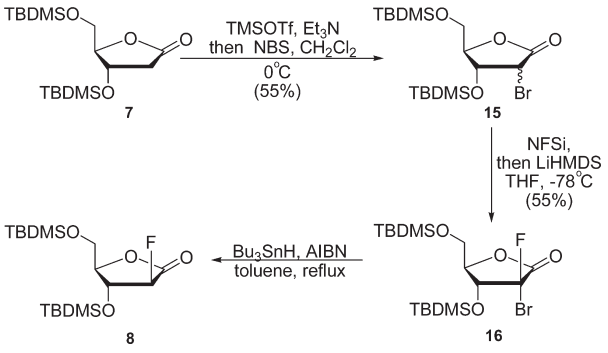


[a]The inset shows the Li-enolate of compound **10** as minimized by MM2 calculations. The bond angle is shown after minimization. Triisopropyl groups, lithium atom, and other hydrogens have been omitted for clarity.

SCHEME 7. Synthesis of Silyl-Protected 2-Deoxy-2,2-difluororibofuranose



SCHEME 8. Debromination Approach to 2-Deoxy-2-fluoroarabinolactone



synthesis of the corresponding 2′-fluoro-NAD$^+$s. Importantly, the successful development of a consolidated and flexible synthetic methodology to obtain all three types of 2-deoxy-2-fluoro(ribo or arabino)furanoses is anticipated to improve access to a large family of medicinally important 2′-fluoro-substituted nucleosides.

Results and Discussion

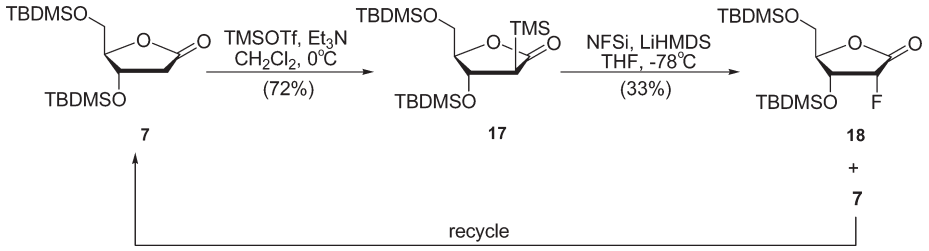
Diastereoselective electrophilic fluorination of protected γ-lactone was encouraged by existing precedents wherein β-elimination is not a problem. For example, Liotta and co-workers reported fluorination of 2,3-dideoxylactone **1** with *N*-fluorodibenezenesulfonimide (NFSi) to furnish the 2-fluorolactone **2** in 50−70% yield (Scheme 3).[37] However, very limited success has been achieved when the β-position is substituted with oxygen. In one of the very few examples, Dehoux et al. applied these conditions to compound **3** and obtained the fluorinated compound **4** in only 16% yield (Scheme 3).[38] Although the authors did not specifically address the reason for the poor outcome, we suspected competing elimination was likely the cause, since the alkoxide anion is significantly less basic than the enolate (Δ p$K_a \approx 10$) providing a strong thermodynamic driving force for elimination. We began our study by using the *p*-chlorobenzoyl-protected deoxyribonolactone **5**, which was reacted under Liotta's procedure (NFSi, LiHMDS in THF at −78 °C) and furnished only the α,β-unsaturated γ-lactone **6** in 65% yield (Scheme 4). Since the driving force for elimination in our case was so much greater than in the Dehoux case, we were not surprised by this result.

We speculated that the elimination process could be diminished by moving to silyl protecting groups, which form leaving groups less basic than alkoxides (alcohol pK_a = 16 versus silanol pK_a = 11[39,40]) but can be potentially bulkier and more readily installed. We hypothesized that a sufficiently bulky group might sterically demand a 3-endo-ring pucker of the deoxyribonolactone ring, which would minimize steric interactions of the bulky group. Such a ring conformation would organize the 3-oxygen into a pseudoequatorial position, thus diminishing the tendency of the silanoate to undergo elimination upon enolate formation.

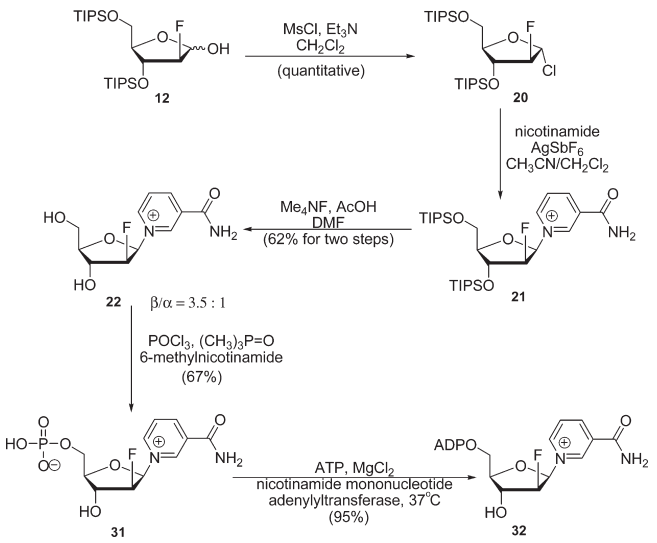
Protection of deoxyribonolactone with *tert*-butyldimethylsilyl (TBDMS) groups provided lactone **7**. When **7** and NFSi were dissolved in THF and cooled to −78 °C, slow addition of LiHMDS resulted in the formation of **8** and **9** (Scheme 5). 2-Deoxy-2-fluoroarabinolactone **8** was obtained in 58% yield after silica gel chromatography, supplying a more than 3-fold improvement in yield for electrophilic fluorination versus the Dehoux result, encouraging our strategy. Favorably, the reaction yielded only the arabino isomer, which can be attributed to the sterically bulky TBDMS group preventing a *syn* approach of the bulky fluorinating agent to the enolate. Although promising, the bulky TBDMS did not completely inhibit β-elimination as

(37) McAtee, J. J.; Schinazi, R. F.; Liotta, D. C. *J. Org. Chem.* **1998**, *63*, 2161–2167.
(38) Dehoux, C.; Gorrichon, L.; Baltas, M. *Eur. J. Org. Chem.* **2001**, *6*, 1105–1113.
(39) Tripp, C. P.; Hair, M. L. *Langmuir* **1995**, *11*, 149–155.
(40) Damrauer, R.; Simon, R.; Krempp, M. *J. Am. Chem. Soc.* **1991**, *113*, 4431–4435.

SCHEME 9. Synthesis of 2-Deoxy-2-fluororibolactone



SCHEME 10. Synthesis of 2′-Deoxy-2′-fluoroarabino-NAD$^+$



evident by the formation of **9**. To increase silyl bulk, we turned to the triisopropylsilyl (TIPS) group and synthesized the TIPS-protected lactone **10**. Lactone **10** was reacted as before and β-elimination was diminished, enabling diastereoselective fluorination to give only the arabino isomer **11** in 72% isolated yield (Scheme 6). Stereochemistry was confirmed by NOE measurements (see the Experimental Section). Subsequently, **11** was reduced with DIBAL-H to obtain the TIPS-protected 2-deoxy-2-fluoroarabinofuranose **12** in 91% yield.

These results demonstrate that β-elimination in electrophilic fluorination of 2-deoxyribonolactone can be successfully mitigated by silyl steric bulk at the susceptible β-position. An MM2 minimization of the lithium enolate provided evidence that the steric bulk of the TIPS group organizes the 3-siloxy group into a geometry that is 40 degrees from the ideal angle for elimination (inset Scheme 6). The effect of the TIPS on preventing the elimination is impressive, as approximately 14 orders of magnitude of anion stability (ΔpK_a) favor elimination of the silanoate from the enolate. Finally, in two steps from a protected 2-deoxyribonolactone, diastereoselective production of a 2-deoxy-2-fluoroarabinofuranoside was achieved in 66% yield, surpassing the yield of the previous methodology (58% yield in two steps)[25,28,29] and avoiding the use of the caustic substance diethylaminosulfur trifluoride (DAST).

We considered the monofluoro-substituted lactone **11** to be a possible precursor to the corresponding 2-deoxy-2,2-difluororibonolactone. We felt it was only required that we repeat the fluorination procedure to achieve this valuable compound. Favorably, the second fluorination under the same reaction conditions provided the desired 2-deoxy-2,2-difluororibonolactone **13** in 71% yield (Scheme 7). Reduction with DIBAL-H provided lactol **14** in three steps with 47% yield from **10**. This straightforward method surpassed the nondiastereoselective method of Chou (five steps, 25% yield, Scheme 1) for selectivity, brevity, and yield.

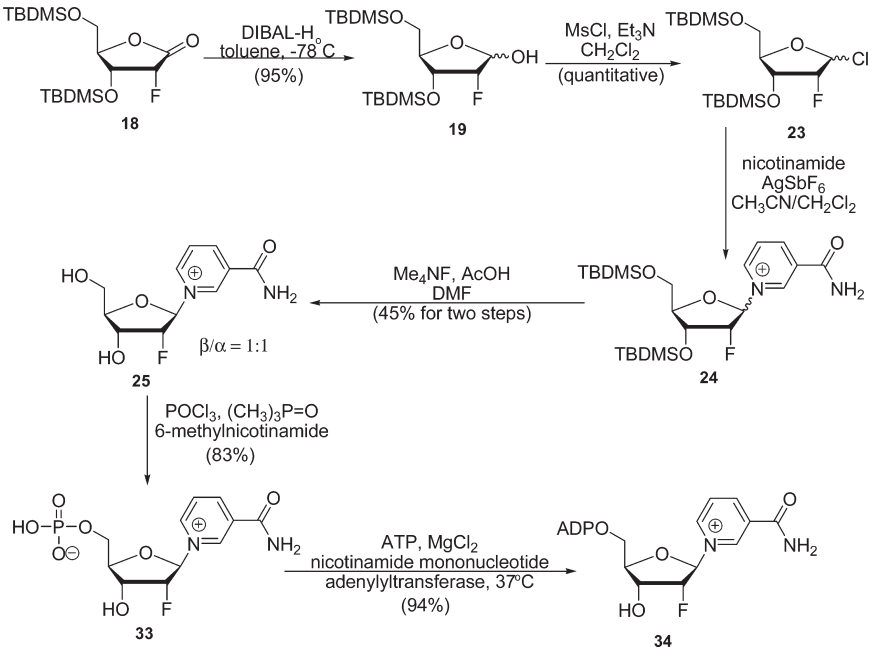
The absence from the literature of a convenient synthesis of 2-deoxy-2-fluororibofuranose[30] prompted us to investigate the possibility of converting the 2-deoxyribonolactone to the 2-deoxy-2-fluororibo isomer. We supposed that a bulky protecting group at the 3 position would enforce steric arrangements in enolate reactions with electrophiles. To force stereochemistry of the fluoro-electrophile into the ribo configuration we thought of using a removable directing group introduced at the α position. One attempt along these lines is shown in Scheme 8 and is based on a previous strategy of Kirk.[41] 2-Deoxy-2-bromolactone **15** was prepared by direct bromination of **7** (Scheme 8). Fluorination of **15** occurred stereoselectively, providing **16**, of which the stereochemistry was assigned based upon coupling constants. Somewhat surprisingly, debromination of **16** with tributyltin hydride gave only the undesired arabinofluoro compound **8** as the major product with no ribo isomer formed (Scheme 8).

This failure led us to consider an α-silyl lactone as a means for diastereocontrolled formation of the 2-deoxy-2-fluororibolactone. Purified α-silyl ketones have been used as substrates in electrophilic fluorinations,[42,43] mostly for regiocontrolled fluorination away from the silyl group, although fluorination at the silylated carbon suggested the potential workability of our strategy.[43] We imagined that fluorination of an α-silyl lactone would generate the desired ribofluoro configuration as a consequence of the tendency of the bulky α-silyl and 3 position protecting group to move into a *trans* disposition in the enolate, thus permitting electrophilic fluorination *syn* to the 3-substitutent at the α-carbon. Subsequent proteo-desilylation with retention of stereochemistry was envisioned to provide the desired compound.

α-Silyl lactone **17** was generated diastereoselectively (the arabino configuration was determined by NOE correlation between H_2 and H_4) from **7** in 71% yield (Scheme 9). Interestingly, treatment of **17** with NFSi afforded the desired ribofluoro product **18** in 33% yield with 2-deoxyribonolactone **7** in 61% yield. The formation of 2-fluoroarabinolac-

(41) Ge, P.; Kirk, K. L. *J. Org. Chem.* **1997**, *62*, 3340–3343.
(42) Enders, D.; Potthoff, M.; Raabe, G.; Runsink, J. *Angew. Chem., Int. Ed.* **1997**, *36*, 2362–2364.
(43) Enders, D.; Faure, S.; Potthoff, M.; Runsink, J. *Synthesis* **2001**, *15*, 2307–2319.

SCHEME 11. Synthesis of 2′-Deoxy-2′-fluororibo-NAD$^+$



tone **8** was not observed. The recovered compound **7** was resubmitted to silylation−fluorination conditions to give **18** in 38% combined yield after one recycle (Scheme 9). Subsequent reduction of **18** to lactol **19** was effected in 95% yield. The three-step silylation, fluorination, and reduction sequence afforded the 2-deoxy-2-fluororibofuranose in 22% yield. This method is by far the most efficient chemical synthesis of 2-deoxy-2-fluororibofuranose ever reported.

The result confirmed our expectation that the α-trimethylsilyl (TMS) substituent can control the stereochemistry of the fluorine substituent. The coincident desilylation observed in the process, although desirable and stereoselective, raises the question of the origin of the selectivity. Is it determined at the enolate reaction to the fluorine, or in the desilylation process, or both? At this point, we do not have a clear answer. Additionally, we have limited insight into the cause of the desilylation (without fluorination) that limits yield. We speculate that the reaction conditions stimulate a significant percentage of the *C*-silyl starting material to isomerize to the *O*-silyl ketene acetal, before it can react with NFSi, and when quenched the *O*-silyl derivative decomposes to **7**. Low-temperature interconversions of *C*-silylated esters and *O*-silylated ketene acetals have been reported.[44] Further investigations into this reaction are ongoing in our laboratory.

At this point, with all possible 2-deoxy-2-fluorofuranoses in hands, we were prepared to couple nicotinamide to sugars to obtain the corresponding 2′-deoxy-2′-fluoronicotinamide nucleosides and 2′-deoxy-2′,2′-difluoronucleosides, preferably with stereocontrol to yield the desired β-isomers. Our main concern was that the silyl groups restrict the levels of acidity we could employ in the coupling reaction.

The only reported synthesis of 1-(2′-deoxy-2′-fluoroarabinofuranosyl)nicotinamide was from 1,2:5,6-di-*O*-isopropylidene-D-allofuranose (eight steps in 24% yield).[25] We here describe the synthesis of the 1-(2′-deoxy-2′-fluoroarabinofuranosyl)nicotinamide in five steps, 41% isolated yield from protected 2-deoxyribonolactone. Compound **12** was activated with methanesulfonylchloride and triethylamine, which formed only the α-chloro sugar **20** (Scheme 10). Compound **20** was coupled with nicotinamide in acetonitrile and dichloromethane mixed solvent with stoichiometric amount of $AgSbF_6$. Crude product **21** was a mixture of both isomers with β being the major isomer. After deprotection with fluoride, β- and α-isomers were separated by preparative HPLC ($\beta/\alpha = 3.5$). The ^{1}H NMR spectrum obtained for **22** (β) agreed with literature data.[25] The yield of **22** (β) from **12** was 62% and required only one purification step.

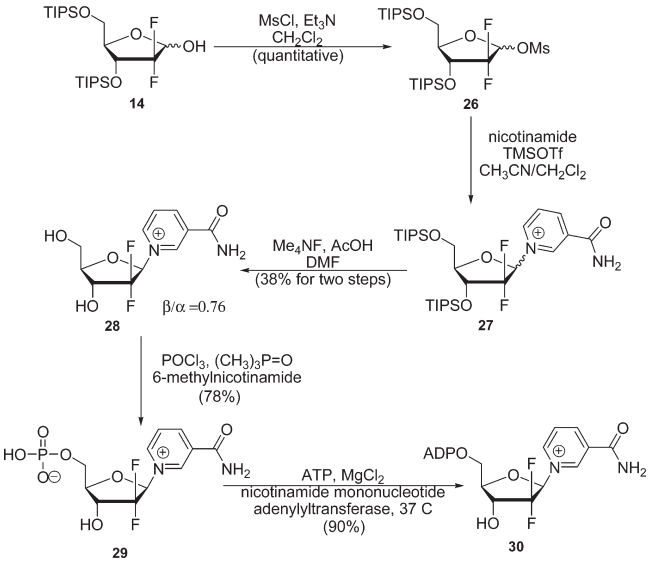
A description of the synthesis of 1-(2′-deoxy-2′-fluororibofuranosyl)nicotinamide has not appeared in the chemical literature, although the compound's stability to hydrolysis has been described.[45] We herein describe the synthesis of this compound in six steps and 15% isolated yield from 2-deoxyribonolactone. The furanose **19** was converted to the chloro sugar **23** by treatment with methanesulfonylchloride and triethylamine, and the reaction occurred quantitatively but produced both α- and β-isomers (Scheme 11). Coupling of the chloro sugar to nicotinamide with $AgSbF_6$ provided a mixture of nicotinamide adducts **24**, followed by deprotection and HPLC purification to provide the desired nicotinamide-substituted 2′-deoxy-2′-fluororibonucleoside **25** in 45% yield from the lactol **19**. The corresponding α isomer was isolated in 46% yield. Stereochemistries were assigned by NOEs (see the Experimental Section).

The synthesis of the 1-(2′-deoxy-2′,2′-difluororibofuranosyl)nicotinamide has never been reported. The completion of the synthesis of the β-isomer in six steps with 18% isolated yield and 23% for the α-isomer are reported here. We prepared the 1-mesylate **26** similarly to a reported

(44) Welch, J. T.; Plummer, J. S.; Chou, T. S. *J. Org. Chem.* **1991**, *56*, 353–359.

(45) Handlon, A. L.; Xu, C.; Mullersteffner, H. M.; Schuber, F.; Oppenheimer, N. J. *J. Am. Chem. Soc.* **1994**, *116*, 12087–12088.

SCHEME 12. Synthesis of 2′-Deoxy-2′,2′-difluoro-NAD$^+$



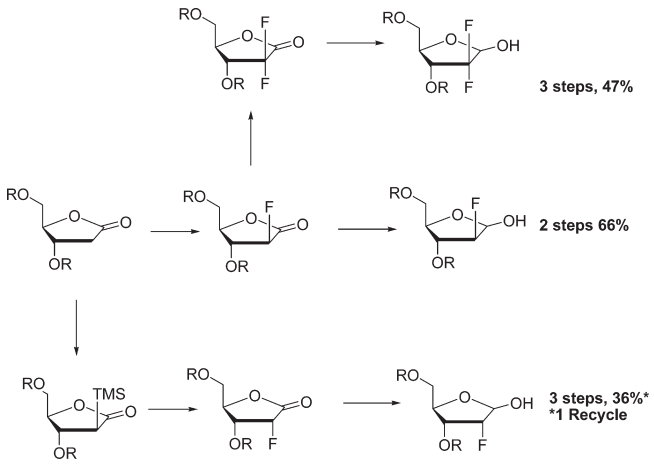
method.[35] It appears that the additional fluorine at the 2-position deactivates the mesylate from nucleophilic chlorination which occurs for the arabino- and ribofluoro analogues (Schemes 10 and 11). Reaction with nicotinamide yielded nucleoside **27** in both α and *β*-configurations. Subsequent deprotection and HPLC purification provided *β*-**28** in 38% yield and the α-anomer in 50% yield. Poor stereochemical control from the mesylate is known, with few preferable alternatives.[35] NOEs between the $H_{1'}$ and $H_{4'}$ in the *β*-nucleoside and NOEs between the $H_{1'}$ and $H_{3'}$ and nicotinamide H_2 and $H_{4'}$ for the α isomer confirmed the stereochemistries.

The complete syntheses of the nicotinamide-substituted mononucleotides and dinucleotides were straightforward, and only the syntheses of the difluoronucleotide and difluorodinucleotide are described. The monofluoro derivatives were prepared by similar methods, and the preparation of these compounds is described in the Experimental Section and in Schemes 10 and 11. 1-(2-Deoxy-2,2-difluororibosyl) nicotinamide **28** (*β*-isomer) was phosphorylated with $POCl_3$ in trimethylphosphate in the presence of 6-methylnicotinamide, a hindered weak base which controls acidity, to yield 2′-deoxy-2′,2′-difluoronicotinamide mononucleotide (2′-deoxy-2′,2′-difluoro-NMN) **29** in 78% isolated yield (Scheme 12). Although previously unstudied, we found that the 2-fluoro-NMN compounds could be adenylated enzymatically with yeast nicotinamide mononucleotide adenylyltransferase[46] (NMNAT-1, see the Supporting Information). In this case, reaction with ATP furnished 2′-deoxy-2′,2′-difluoro-NAD$^+$ **30** in 90% yield versus ATP, which was limiting, with recovery of unreacted **29**. These steps complete the first reported syntheses of 2′,2′-difluoro-NMN and 2′,2′-difluoro-NAD$^+$. The dinucleotide was completed in 8 steps with 12.4% overall yield from 2-deoxyribonolactone **10**. Similarly, with these methods 2′-fluoroarabino-NMN and NAD$^+$ **31**, **32** (seven steps, 26% yield from **10**) were

(46) Natalini, P.; Ruggieri, S.; Raffaelli, N.; Magni, G. *Biochemistry* **1986**, *25*, 3725–3729.

(47) Fox, J. J.; Hoffer, M.; Wempen, I.; Yung, N. C. *J. Am. Chem. Soc.* **1961**, *83*, 4066–4070.

SCHEME 13. Syntheses of Protected 2-Deoxy-2-fluorofuranose from 2-Deoxyribonolactone



synthesized as well as 2′-fluororibo-NMN and NAD$^+$ **33**, **34** (eight steps 13% yield from **7**).

Conclusions

We have developed general methods of achieving diastereoselective electrophilic fluorination of 2-deoxyribonolactone to produce each of the corresponding α-fluoro-substituted isomers (Scheme 13). Fluorinated furanoses, appropriate for nucleoside synthesis, are made in increased yield and efficiency versus previously reported methods with the advantage of avoiding use of DAST. In addition, consolidation and synthetic brevity are achieved with complete control of diastereoselectivity of fluorination. In the case of 2-deoxy-2,2-difluororibofuranose, the yield almost doubles the previously reported yield (47% versus 25%) while the number of required synthetic steps decreases from five to three (Scheme 13). Moreover, the synthesis here is diastereochemically pure. With respect to the ribofluoro derivatives, we found that we could control stereochemistry into the difficult ribo configuration by utilization of an α-silyl group. The corresponding ribofuranose was made in three steps (with one recycle), slashing in half the number of steps from the previous shortest synthesis while increasing yield by 32% (from 4% to 36%, Scheme 13).

Central to the success of our strategy was the ability of bulky silyl protecting groups at the 3 and 5 positions of the lactone to attenuate the tendency of the lactone enolate to undergo elimination prior to reacting with the electrophilic fluorinating agent. The silyl groups could have forced late-stage manipulations of the protecting groups for synthesis of nucleosides, but we found that the silyl-protected lactols were easily activated to chloro sugars (in arabino and ribo), and in the arabino case, only the α-isomer was generated, allowing preferential synthesis of the *β*-nicotinamide substituted nucleoside. The *gem*-difluoro sugar was activated to mesylate.[35] We anticipate that the methods reported here will improve and simplify synthetic access to a variety of 2′-fluorosubstituted nucleosides, particularly the 2′-deoxy-2′-fluororibofuranoside derivatives. In addition, we are exploring other types of diastereocontrolled electrophilic modification of 2-deoxyribonolactones and are investigating introduction of chlorine, nitrogen, and other heteroatoms.

We expect to report the chemical and biochemical properties of the 2′-fluorinated NAD^+ derivatives in future publications.

Experimental Section

2-deoxy-3,5-di-*O*-(*p*-chlorobenzoyl)-D-ribonolactone (5). A solution of methyl 2-deoxy-3,5-di-*O*-(*p*-chlorobenzoyl)-D-ribofuranoside[47] (500 mg, 1.18 mmol) in 20 mL of 80% acetic acid aqueous solution and 2 mL of 10% aqueous HCl was heated to reflux for 1 h and then cooled to room temperature. Water was added, and the organic phase was washed with water, saturated $NaHCO_3$ solution, and brine and dried over anhydrous Na_2SO_4. Solvent was removed under vacuo, and crude product was dissolved in 10 mL of CH_2Cl_2; to this solution was added PCC (294 mg, 1.36 mmol). The reaction was stirred at room temperature and monitored by TLC; once it was completed, PCC was filtered off, and filtrate was concentrated and purified by column chromatography (hexanes/ethyl acetate 4:1) to afford **5** (190 mg, 0.46 mmol, 40% yield for two steps) as a white solid: mp = 89−90 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 0.2.81 (dd, J = 2.1, 18.8 Hz, 1H), 3.12 (dd, J = 7.5, 18.9 Hz, 1H), 4.60 (dd, J = 3.7, 12.3 Hz, 1H), 4.68 (dd, J = 3.8, 12.3 Hz, 1H), 4.92 (m, 1H), 5.58 (dt, J = 1.8, 7.5 Hz, 1H), 7.42 (m, 4H), 7.93 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 34.9, 63.9, 71.9, 82.2, 127.0, 127.4, 129.09, 129.11, 131.0, 131.2, 140.3, 140.6, 164.98, 165.02, 173.5; HRMS (ESI) calcd for $C_{19}H_{14}C_{l2}O_6$ 408.0167, found 408.017.

(*S*)-4-Hydroxymethyl-2-buten-4-olide (6). To a flame-dried round-bottom flask were added **5** (155 mg, 0.38 mmol) and NFSi (120 mg, 0.38 mmol) in 5 mL of anhydrous THF. The solution was cooled to −78 °C, and LiHMDS in THF solution (0.456 mL, 0.456 mmol) was added dropwise. The reaction mixture was allowed to stir at −78 °C for an additional 1 h and then quenched with saturated NH_4Cl solution. The organic layer was washed with saturated $NaHCO_3$ solution, water, and brine and dried over anhydrous Na_2SO_4. Column chromatography (hexanes/ethyl acetate 4:1−2:1) gave **6** (62 mg, 0.24 mmol, 65%) as a white solid: mp = 115−116 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 4.55 (dd, J = 4.8, 12.0 Hz, 1H), 4.61 (dd, J = 3.7, 12.1 Hz, 1H), 5.34 (m, 1H), 6.22 (dd, J = 2.1, 5.8 Hz, 1H), 7.40 (d, J = 8.6 Hz, 2H), 7.48 (dd, J = 1.5, 5.7 Hz, 1H), 7.91 (d, J = 8.6 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 63.1, 76.9, 80.8, 123.6, 127.5, 128.91, 128.96, 131.1, 131.6, 140.2, 152.1, 165.2, 172.1; HRMS (ESI) calcd for $C_{12}H_9ClO_4$ 252.0189, found 252.0188.

2-Deoxy-3,5-di-*O*-(*tert*-butyldimethylsilyl)-D-ribonolactone (7). To a solution of 2-deoxy-D-ribose (1.0 g, 7.45 mmol) in 6 mL of water was added Br_2 (2 mL). The flask was sealed, and the contents were stirred at room temperature for 5 days. The resulting mixture was neutralized by adding silver carbonate until the pH was 7. The mixture was filtered, and the filtrate was concentrated under reduced pressure to yield 2-deoxyribonolactone as a yellow oil. Without further purification, the crude product was dissolved in 20 mL of anhydrous DMF, and imidazole (2.53 g, 37.3 mmol) and *tert*-butyldimethylsilyl chloride (4.5 g, 29.8 mmol) were added. The resulting solution was stirred at room temperature for 24 h and quenched by addition of water. The water layer was extracted with ethyl acetate (3 × 10 mL), and the organic layers were combined, washed with brine, and dried over anhydrous Na_2SO_4. The crude product was concentrated in vacuo. Flash chromatography (hexanes/ethyl acetate 20:1) afforded **7** (3.2 g, 8.9 mmol, 89% yield after two steps) as a white solid: mp = 72−73 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 0.038 (s, 3H), 0.051 (s, 3H), 0.062 (s, 6H), 0.085 (s, 18H), 2.36 (dd, J = 2.6, 17.7 Hz, 1H), 2.79 (dd, J = 6.7, 17.7 Hz, 1H), 3.73 (dd, J = 2.5, 11.5 Hz, 1H), 3.78 (dd, J = 3.4, 11.5 Hz, 1H), 4.30 (dd, J = 2.5, 5.2 Hz, 1H), 4.48 (dt, J = 2.3, 6.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) −5.7, −5.5, −4.9, −4.8, 17.9, 18.2, 25.7, 25.8, 39.0, 62.5, 69.6, 88.1, 175.8; HRMS (ESI) calcd for $C_{17}H_{36}O_4Si_2$ 360.2152, found 360.2155.

2-Deoxy-2-fluoro-3,5-di-*O*-(*tert*-butyldimethylsilyl)-D-ribonolactone (8). To a flame-dried 100 mL round-bottom flask were added **7** (1.8 g, 5 mmol) and NFSi (2.36 g, 7.5 mmol) in 20 mL of anhydrous THF. The solution was cooled to −78 °C, and 6.5 mL (6.5 mmol) of a 1 M solution of LiHMDS in THF was added dropwise over a period of 10 min. This was allowed to stir at −78 °C for an additional 1 h and was quenched by saturated NH_4Cl. The mixture was allowed to warm to room temperature, the water layer was extracted by ethyl acetate (3 × 10 mL), and the organic layers were combined, washed with saturated $NaHCO_3$, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography (hexanes/ethyl acetate 20:1) to afford both **8** (1.1 g, 29 mmol, 58%) and **9** (0.5 g, 1.9 mmol, 38%) also as a white solid. **8**: mp = 49−50 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 0.060 (s, 3H), 0.065 (s, 3H), 0.107 (s, 3H), 0.128 (s, 3H), 0.87 (s, 9H), 0.89 (s, 9H), 3.76 (dd, J = 2.5, 12.4 Hz, 1H), 3.95 (dt, J = 2.1, 12.4 Hz, 1H), 4.10 (dt, J = 2.0, 7.7 Hz, 1H), 4.70 (dt, J = 7.8, 18.9 Hz, 1H), 5.09 (dd, J = 8.0, 51.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) −5.5, −5.4, −5.2, −4.8, 17.9, 18.2, 25.5, 25.7, 59.4, 71.3, 71.5, 80.6, 80.7, 91.3, 93.3, 168.5, 168.7; HRMS (ESI) calcd for $C_{17}H_{35}FO_4Si_2$ 378.2058, found 378.206.

4-[(*tert*-Butyldimethylsiloxy)methyl]-4-fluoro-2-buten-4-olide (9): mp = 85−87 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 0.045 (s, 3H), 0.057 (s, 3H), 0.85 (s, 9H), 3.86 (dd, J = 11.3, 15.9 Hz, 1H), 4.04 (dd, J = 7.8, 11.3 Hz, 1H), 6.25 (d, J = 5.7 Hz, 1H), 7.33 (d, J = 5.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) −5.62, −5.58, 18.1, 25.6, 63.5, 63.8, 114.4, 116.3, 125.01, 125.05, 149.7, 149.8, 168.39, 168.41; HRMS (ESI) calcd for $C_{11}H_{19}FO_3Si$ 246.1087, found 246.1085.

2-Deoxy-3,5-di-*O*-(triisopropylsilyl)-D-ribonolactone (10). 2-Deoxy-D-ribonolactone (1.1 g, 8.3 mmol) was dissolved in 10 mL of anhydrous DMF, and to this solution were added imidazole (3.4 g, 50 mmol) and triisopropylsilyl chloride (6.4 g, 33 mmol). The resulting solution was stirred at room temperature for 24 h and quenched by addition of water. The water layer was extracted with ethyl acetate (3 × 10 mL), and the organic layers were combined, washed with brine, and dried over anhydrous Na_2SO_4. The crude product was concentrated in vacuo. Column chromatography (hexanes/ethyl acetate 25:1−20:1) provided **10** (3.4 g, 7.7 mmol, 92%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.05 (stack, 42H), 2.41 (dd, J = 1.9, 17.6 Hz, 1H), 2.86 (dd, J = 6.6, 17.6 Hz, 1H), 3.83 (dd, J = 2.6, 9.4 Hz, 1H), 3.91 (dd, J = 3.0, 11.4 Hz, 1H), 4.39 (s, 1H), 4.65 (d, J = 6.5, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 11.9, 12.1, 18.0, 39.7, 63.4, 70.1, 88.9, 176.1; HRMS (ESI) calcd for $C_{23}H_{48}O_4Si_2$ 444.3091, found 444.3094. MM2 calculations on the lithium enolate of **10** were performed using CHEM3D version 10.0 with a minimized energy to minimum rms gradient of 0.100.

2-Deoxy-2-fluoro-3,5-di-*O*-(triisopropylsilyl)-D-ribonolactone (11). Compound **11** was obtained according to the fluorination procedure to synthesize **8**, using **10** (2 g, 4.5 mmol) as the starting material. Column chromatography (hexanes/ethyl acetate 30:1) provided **11** (1.5 g, 3.25 mmol, 72%) as a white solid: mp = 38−39 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.07 (stack, 42H), 3.91 (dd, J = 2.4, 12.1 Hz, 1H), 4.08 (dt, J = 2.1, 12.1 Hz, 1H), 4.16 (dt, J = 2.1, 7.0 Hz, 1H), 4.92 (dt, J = 7.2, 18.8 Hz, 1H), 5.10 (dd, J = 7.4, 51.3 Hz, 1H); C NMR (100 MHz, $CDCl_3$) δ (ppm) 11.9, 12.1, 17.7, 17.80, 17.84, 17.86, 60.3, 71.6, 71.8, 81.8, 81.9, 91.7, 93.7, 168.6, 168.8; NOE identified between H_2 and H_4 in NOESY; HRMS (ESI) calcd for $C_{23}H_{47}FO_4Si_2$ 462.2997, found 462.2993.

2-Deoxy-2-fluoro-3,5-di-*O*-(triisopropylsilyl)-D-arabinofuranose (12). Compound **11** (200 mg, 0.43 mmol) was dissolved in 1.5 mL of anhydrous toluene and cooled to −78 °C. To this solution was

added 3.02 mL of DIBAL-H in THF solution (3.02 mmol). The reaction mixture was held at −78 °C at all times. Two hours later, the mixture was quenched with methanol at −20 °C, and additional cold methanol was added. The mixture was then allowed to warm slowly to room temperature and was washed with 0.1 M HCl. The aqueous layer was extracted with ether, and the combined organic layer was washed with saturated $NaHCO_3$, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Column chromatography (hexanes/ethyl acetate 15:1) afforded **12** (181 mg, 0.39 mmol, 91%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.07 (stack, 54H), 3.46 (d, J = 10.9 Hz, 1H), 3.60 (m, 1.27H), 3.78 (m, 1.57H), 3.95 (q, J = 3.7 Hz, 0.28H), 4.32 (m, 1H), 4.49 (dd, J = 0.9, 12.6 Hz, 1H), 4.63 (t, J = 4.0 Hz, 0.14H), 4.67 (t, J = 4.0 Hz, 0.14 H), 4.81 (t, J = 4.1 Hz, 0.28H), 4.83 (dd, J = 0.8, 50.2 Hz, 1H), 5.31 (m, 0.3H), 5.35 (t, J = 10.1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 12.0, 12.2, 18.0, 63.2, 75.4, 75.5, 75.6, 87.6, 87.7, 95.9, 97.5, 97.7, 98.8, 98.9, 100.8, 101.0; HRMS (ESI) calcd for $C_{23}H_{49}FO_4Si_2$ 464.3153, found 464.3162.

2-Deoxy-2,2-difluoro-3,5-di-*O*-(triisopropylsilyl)-D-ribonolactone (13). Compound **13** was obtained according to the fluorination procedure to synthesize **8**, using **11** (92 mg, 0.2 mmol) as the starting material. Column chromatography (hexanes/ethyl acetate 40:1) provided **13** (68 mg, 0.14 mmol, 71%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.08 (stack, 42H), 3.96 (dd, J = 2.4, 12.0 Hz, 1H), 4.08 (dt, J = 2.6, 12.1 Hz, 1H), 4.31 (m, 1H), 4.76 (dt, J = 6.0, 11.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 11.4, 11.45, 11.8, 12.1, 17.2, 17.3, 17.36, 17.43, 59.6, 68.3, 68.5, 68.6, 68.7, 82.5, 82.6, 109.9, 112.4, 115.0, 163.3, 163.6, 164.0; HRMS (ESI) calcd for $C_{23}H_{46}F_2O_4Si_2$ 480.2903, found 480.2901.

2-Deoxy-2,2-difluoro-3,5-di-*O*-(triisopropylsilyl)-D-ribofuranose (14). Compound **14** was obtained according to the reduction procedure to synthesize **12**, using **13** (160 mg, 0.33 mmol) as the starting material. Column chromatography (hexanes/ethyl acetate 10:1) provided **14** (146 mg, 0.3 mmol, 91%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.04 (stack, 78H), 3.48 (d, J = 11.3 Hz, 1H), 3.67 (m, 1.8H), 3.81 (m, 1.8H), 3.88 (dt, J = 2.1, 11.2 Hz, 1H), 4.02 (m, 0.8H), 4.24 (m, 1H), 4.39 (dt, J = 2.0, 10.7 Hz, 1H), 4.67 (m, 0.8H), 5.02 (dd, J = 5.1, 9.8 Hz, 0.8H), 5.11 (dd, J = 6.1, 11.3 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 11.87, 11.90, 12.1, 12.3, 17.72, 17.73, 17.78, 17.83, 17.86, 17.89, 62.2, 62.34, 62.36, 69.6, 69.7, 69.8, 70.0, 71.9, 72.0, 72.1, 72.3, 83.95, 84.02, 85.3, 95.3, 95.5, 95.6, 95.8, 96.0, 96.2, 96.3, 96.5, 119.5, 120.1, 121.5, 121.6, 122.1, 123.6, 124.2; HRMS (ESI) calcd for $C_{23}H_{48}F_2O_4Si_2$ 482.3059, found 482.3054.

2-Deoxy-2-bromo-3,5-di-*O*-(*tert*-butyldimethylsilyl)-D-ribono, arabino-lactones (15). To a solution of **7** (180 mg, 0.5 mmol) and triethylamine (303 mg, 3 mmol) in 6 mL of CH_2Cl_2 at 0 °C was added TMSOTf (333 mg, 1.5 mmol), and the solution was stirred at this temperature for 30 min. A solution of NBS (134 mg, 0.75 mmol) in 1.5 mL of CH_2Cl_2 was added, and stirring was continued for 1 h at 0 °C. The reaction mixture was poured into saturated $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. Flash chromatography (hexanes/ethyl acetate 35:1) afforded a mixture of two isomers of **15** (120 mg, 0.27 mmol, 55%, 1:1.4, arabino/ribono) as pure pale yellow liquid. A small amount of this mixture was separated to obtain the pure compounds. The stereochemistry was determined by an observed NOE between the 2- and 4-protons of the arabino isomer. **Arabino-15:** ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 0.041 (s, 3H), 0.053 (s, 3H), 0.10 (s, 3H), 0.13 (s, 3H), 0.85 (s, 9H), 0.90 (s, 9H), 3.76 (dd, J = 2.0, 12.2 Hz, 1H), 3.93 (dd, J = 2.2, 12.2 Hz, 1H), 4.31 (m, 1H), 4.39 (t, J = 5.0 Hz, 1H), 4.47 (d, J = 5.7 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) −5.6, −5.5, −5.0, −4.8, 18.1, 18.2, 25.6, 25.8, 46.1, 60.3, 68.8, 85.0, 170.9; HRMS (ESI) calcd for $C_{17}H_{35}BrO_4Si_2$ 438.1257, found 438.1261. **Ribono-15:** ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 0.058 (s, 3H), 0.062 (s, 3H), 0.116 (s, 3H), 0.172 (s, 3H), 0.87 (s, 9H), 0.88 (s, 9H), 3.79 (dd, J = 3.0, 12.0 Hz, 1H), 3.92 (dd, J = 3.3, 12.0 Hz, 1H), 4.21 (m, 1H), 4.37 (d, J = 6.9 Hz, 1H), 4.67 (dd, J = 6.1, 6.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) −5.5, −5.4, −5.0, −4.1, 17.8, 18.2, 25.6, 25.7, 46.1, 60.2, 75.6, 85.3, 170.1; HRMS (ESI) calcd for $C_{17}H_{35}BrO_4Si_2$ 438.1257, found 438.1258.

(2*R*)-2-Deoxy-2-bromo-2-fluoro-3,5-di-*O*-(*tert*-butyldimethylsilyl)-D-ribonolactone (16). Compound **16** was obtained according to the fluorination procedure to synthesize **8**, using **15** (400 mg, 0.91 mmol) as the starting material. Column chromatography (hexanes/ethyl acetate 30:1) provided **16** (230 mg, 0.5 mmol, 55%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 0.058 (s, 3H), 0.065 (s, 3H), 0.13 (s, 3H), 0.17 (s, 3H), 0.86 (s, 9H), 0.93 (s, 9H), 3.77 (dd, J = 1.9, 12.7 Hz, 1H), 4.00 (m, 2H), 4.53 (dd, J = 8.0, 15.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) −5.5, −5.4, −5.2, −4.6, 18.0, 18.2, 25.5, 25.7, 58.3, 72.0, 72.2, 80.6, 80.7, 98.2, 100.5, 165.6, 165.8; HRMS (ESI) calcd for $C_{17}H_{34}BrFO_4Si_2$ 456.1163, found 456.1178.

Debromination of 16 Yielding 8. Compound **16** (60 mg, 0.13 mmol), tributyltin hydride (83 mg, 0.28 mmol), and AIBN (3 mg, 0.018 mmol) were dissolved in 1 mL of toluene and stirred at 90 °C for 24 h. Solvent was evaporated, and the residue was dissolved in acetonitrile and washed with hexanes to remove organotin compounds. The solvent was again concentrated in vacuo, and an ^{1}H NMR spectrum identified it as compound **8** described previously.

2-Deoxy-2-trimethylsilyl-3,5-di-*O*-(*tert*-butyldimethylsilyl)-D-arabinolactone (17). To a solution of **7** (1 g, 2.78 mmol) and triethylamine (1.68 g, 16.68 mmol) in 28 mL of CH_2Cl_2 at 0 °C was added TMSOTf (1.85 g, 8.34 mmol) dropwise. The solution was stirred at this temperature for another 2 h and then quenched with saturated NH_4Cl. The mixture was allowed to warm to room temperature, the water layer was extracted by CH_2Cl_2 (3 × 10 mL), and the organic layers were combined, washed with saturated $NaHCO_3$, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography (hexanes/ethyl acetate 25:1) to afford unreacted **7** (240 mg, 0.67 mmol) and **17** (850 mg, 1.97 mmol, 71%) as a white solid: mp = 70−71 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 0.067 (s, 6H), 0.074 (s, 6H), 0.19 (s, 9H), 0.85 (s, 9H), 0.89 (s, 9H), 2.17 (d, J = 2.5 Hz), 3.50 (dd, J = 7.7, 10.7 Hz, 1H), 3.76 (dd, J = 7.1, 12.2 Hz, 1H), 4.27 (m, 1H), 4.45 (t, J = 2.2 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) −5.4, −5.3, −4.7, −4.2, −1.7, −0.8, 17.7, 18.4, 25.6, 25.8, 25.90, 25.94, 42.2, 62.2, 71.8, 87.4, 177.7; HRMS (ESI) calcd for $C_{20}H_{44}O_4Si_3$ 432.2547, found 432.2553.

2-Deoxy-2-fluoro-3,5-di-*O*-(*tert*-butyldimethylsilyl)-D-ribonolactone (18). Compound **18** was obtained according to the fluorination procedure to synthesize **8**, using **17** (780 mg, 1.81 mmol) as the starting material. Column chromatography (hexanes/ethyl acetate 30:1−10:1) provided both **18** (226 mg, 0.60 mmol, 33%) and **7** (400 mg,1.11 mmol, 61%) as white solid: mp = 75−77 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 0.046 (s, 3H), 0.061 (s, 3H), 0.087 (s, 6H), 0.86 (s, 9H), 0.87 (s, 9H), 3.77 (dd, J = 1.7, 11.9 Hz, 1H), 3.84 (dd, J = 2.6, 12.0 Hz, 2H), 4.37 (d, J = 2.1 Hz, 1H), 4.43 (d, J = 5.2 Hz, 1H), 5.21 (dd, J = 5.3, 50 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) −5.7, −5.6, −5.3, −4.9,-4.8, 18.2, 18.3, 25.6, 25.8, 62.3, 70.3, 70.4, 71.8, 84.2, 85.8, 86.4, 88.1, 171.1; HRMS (ESI) calcd for $C_{17}H_{35}FO_4Si_2$ 378.2058, found 378.2059.

2-Deoxy-2-fluoro-3,5-di-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranose (19). Compound **19** was obtained according to the reduction procedure to synthesize **12**, using **18** (100 mg, 0.22 mmol) as the starting material. Column chromatography (hexanes/ethyl acetate 15:1) provided **19** (95 mg, 0.205 mmol, 95%) as a

colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 1.02 (stack, 96 H), 3.28 (d, J = 7.3 Hz, 2.2H), 3.68 (dd, J = 3.7, 11 Hz, 1H), 3.76 (dd, J = 2.7, 11 Hz, 1H), 3.80 (dd, J = 1.8, 11 Hz, 2.2H), 3.92 (dd, J = 2.2, 11 Hz, 2.2H), 4.09 (dt, J = 1.9, 6.7 Hz, 2.2H), 4.14 (dd, J = 1.2, 12.3 Hz, 1H), 4.23 (s, 1H), 4.49 (dd, J = 1.7, 2.8 Hz, 1H), 4.59 (dd, J = 3.7, 53.4 Hz, 2.2H), 4.71 (ddd, J = 3.8, 10.4, 23.5 Hz, 2.2H), 4.85 (dt, J = 4.4, 51.7 Hz, 1H), 5.24 (dd, J = 4.2, 8 Hz, 1H), 5.27 (t, J = 6.1 Hz, 2.2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 11.83, 11.85, 11.9, 12.2, 17.71, 17.72, 17.81, 17.85, 17.87, 17.88, 61.8, 63.5, 69.8, 69.9, 71.9, 72.0, 84.0, 85.67, 85.70, 87.6, 89.2, 93.5, 95.0, 95.7, 95.9, 99.1, 99.3; HRMS (ESI) calcd for $C_{17}H_{37}FO_4Si_2$ 380.2214, found 380.2212.

1-Chloro-2-deoxy-2-fluoro-3,5-di-*O*-(triisopropylsilyl)-D-arabinofuranose (20). Compound **12** (40 mg, 0.086 mmol) was dissolved in 0.5 mL of CH_2Cl_2 and triethylamine (12.2 mg, 0.12 mmol). To this solution was added at 0 °C methanesulfonyl chloride (11.5 mg, 0.1 mmol). After 3 h of stirring under argon at room temperature, the mixture was evaporated in vacuo, and the residue was taken up in ethyl acetate. The solution was washed with saturated $NaHCO_3$, followed by 1 M HCl, water, and brine. Solvent was concentrated under reduced pressure to afford **20** (40 mg, 0.086 mmol, almost quantitatively) as a yellow liquid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.08 (stack, 42H), 3.89 (dd, J = 3.7, 11.7 Hz, 1H), 3.96 (dd, J = 2.9, 11.7 Hz, 1H), 4.30 (dd, J = 3.4, 8.3 Hz, 1H), 4.58 (dd, J = 5.1, 14.8 Hz, 1H), 5.12 (d, J = 51.7 Hz, 1H), 6.15 (d, J = 12.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 11.9, 12.0, 17.80, 17.84, 17.9, 61.4, 75.1, 75.4, 88.61, 88.64, 95.3, 95.6, 103.8, 105.3; HRMS (ESI) calcd for $C_{23}H_{48}ClFO_3Si_2$ 482.2815, found 482.2822.

1-(2′-Deoxy-2′-fluoro-3′,5′-di-*O*-(triisopropylsilyl)arabinofuranosyl)nicotinamide (21). Compound **20** (25 mg, 0.052 mmol) and nicotinamide (15 mg, 0.12 mmol) were dissolved in 1 mL of CH_2Cl_2. To this solution at 0 °C was added an ice-cold solution of nicotinamide (15 mg, 0.12 mmol) and $AgSbF_6$ (36 mg, 0.104 mmol) in 1.5 mL of acetonitrile. The reaction mixture was kept at room temperature overnight. Solvent was evaporated under reduce pressure, and the residue was redissolved in methanol and passed through a short pad of Celite. Concentrated crude product (which contained a mixture of α and β isomers) was examined by NMR and used for the next step without further purification.

1-(2′-Deoxy-2′-fluoroarabinofuranosyl)nicotinamide (22). To a solution of **21** (25 mg, 0.044 mmol) in 1 mL of DMF were added acetic acid (10.6 mg, 0.176 mmol) and tetramethylammonium fluoride (16.4 mg, 0.176 mmol). The reaction was stirred at room temperature overnight and then was concentrated and purified by HPLC on a Waters RP-18 XBridge PrepShield 19 × 50 mm column (solvent was 20 mM ammonium acetate, compounds were eluted at a flow rate of 2 mL/min) to afford **22** (β-isomer, t_R = 8 min, 7 mg, 0.027 mmol, 62%) and α-isomer (t_R = 6.7 min, 2 mg, 0.008 mmol, 18%). ***β*-Isomer:** ^{1}H NMR (D_2O, 400 MHz) δ (ppm) 3.88 (dd, J = 4.8, 13.0 Hz, 1H), 4.0 (dd, J = 1.9, 12.9 Hz, 1H), 4.28 (dd, J = 4.7, 8.3 Hz, 1H), 4.51 (dt, J = 5.5, 17.6 Hz, 1H), 5.5 (dt, J = 4.6, 51.3 Hz, 1H), 6.68 (dd, J = 4.7, 9.8 Hz, 1H), 8.23, (t, J = 6.6 Hz, 1H), 8.95 (d, J = 8.1 Hz, 1H), 9.18 (d, J = 6.2 Hz, 1H), 9.57 (s, 1H); ^{13}C NMR (125 MHz, D_2O) δ (ppm) 59.4, 71.1, 71.3, 85.07, 85.11, 93.9, 94.0, 94.1, 95.6, 128.1, 133.8, 141.5, 143.8, 146.1, 165.7; HRMS (ESI) calcd for $C_{11}H_{13}FN_2O_4$ 256.0859, found 256.0865.

(1*R*,2*R*)-1-Chloro-2-deoxy-2-fluoro-3,5-di-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranose (23). Compound **23** was obtained according to the chlorination procedure to synthesize **20** using **19** (10 mg, 0.021 mmol) as the starting material to afford **23** (10 mg, 0.021 mmol, almost quantitatively) as yellow liquid: ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) 1.02 (stack, 51 H), 3.86 (stack, 3.4H), 4.02 (dd, J = 1.8, 11.8 Hz, 0.7H), 4.10 (m, 0.7H), 4.31 (d, J = 2.1 Hz, 1H), 4.55 (quintet, J = 3 Hz, 1H), 4.82 (dt, J = 4.7, 49 Hz, 1H), 4.95 (dd, J = 3.3, 45 Hz, 0.7H), 6.06 (d, J = 11.1 Hz, 0.7H), 6.21 (d, J = 4.1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 11.8, 11.9, 12.1, 12.2, 17.77, 17.81, 17.84, 17.86, 17.95, 17.96, 31.5, 52.5, 61.6, 62.3, 68.6, 68.8, 85.8, 87.9, 88.7, 89.6, 92.8, 93.0, 93.4, 93.7, 95.6, 97.2; HRMS (ESI) calcd for $C_{17}H_{36}ClFO_3Si_2$ 398.1876, found 398.1880.

1-(2′-Deoxy-2′-fluoro-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-D-ribofuranosyl)nicotinamide (24). Compound **23** (11 mg, 0.026 mmol) and nicotinamide (8 mg, 0.065 mmol) were dissolved in 1 mL of CH_2Cl_2. To this solution at 0 °C was added an ice-cold solution of nicotinamide (8 mg, 0.065 mmol) and $AgSbF_6$ (8.9 mg, 0.026 mmol) in 1.5 mL of acetonitrile. The reaction mixture was kept at room temperature overnight. Solvent was evaporated under reduced pressure, and the residue was redissolved in methanol and passed through a short pad of Celite. Concentrated crude product was examined by NMR and used for the next step without further purification.

1-(2-Deoxy-2-fluoro-D-ribofuranosyl)nicotinamide (25). Compound **25** was obtained according to the deprotection procedure to synthesize **22**, using **24** (12.6 mg, 0.026 mmol) as the starting material. Crude product was purified by HPLC on a Waters RP-18 XBridge PrepShield 19 × 50 mm column (solvent was 20 mM ammonium acetate, compounds were eluted at a flow rate of 2 mL/min) to afford **25** (β-isomer, t_R = 14.5 min, 3 mg, 0.012 mmol, 45%) and α-isomer (t_R = 10.6 min, 3.1 mg, 0.012 mmol, 46%). ***β*-Isomer:** ^{1}H NMR (D_2O, 600 MHz) δ (ppm) 3.75 (dd, J = 2.4, 13.2 Hz, 1H), 3.97 (dd, J = 2.4, 13.8 Hz, 1H), 4.31 (m, 1H), 4.35 (m, 1H), 5.20 (dd, J = 4.2, 49.2 Hz, 1H), 6.47 (d, J = 14.4 Hz, 1H), 8.10 (t, J = 7.8 Hz, 1H), 8.80 (dd, J = 1.2, 7.8 Hz, 1H), 9.16 (d, J = 6.6 Hz, 1H), 9.54 (s, 1H); ^{13}C NMR (125 MHz, D_2O) δ (ppm) 55.15, 55.18, 55.22, 58.8, 67.3, 67.4, 85.8, 94.2, 95.7, 97.2, 97.5, 128.5, 134.1, 140.8, 142.9, 145.9, 165.2; NOESY NOE correlation between sugar $H_{3'}$ and nicotinamide H_2; HRMS (ESI) calcd for $C_{11}H_{13}FN_2O_4$ 256.0859, found 256.0863. **α-Isomer:** ^{1}H NMR (D_2O, 500 MHz) δ (ppm) 3.82 (dd, J = 4.5, 10.5 Hz, 1H), 4.0 (dd, J = 2.0, 10.5 Hz, 1H), 4.61 (m, 1H), 4.81 (m, 1H), 5.65 (dt, J = 4.5, 43.5 Hz, 1H), 6.79 (dd, J = 3.5, 8.5 Hz, 1H), 8.29, (dd, J = 5.5, 7.0 Hz, 1H), 9.01 (d, J = 6.5 Hz, 1H), 9.17 (d, J = 5.5 Hz, 1H), 9.40 (s, 1H); NOESY NOE correlations between sugar $H_{4'}$ and nicotinamide H_2, sugar $H_{4'}$ and nicotinamide H_4, and sugar $H_{1'}$ and $H_{3'}$.

1-Methylsulfonyl-2-deoxy-2,2-difluoro-3,5-di-*O*-(triisopropylsilyl)-D-ribofuranose (26). Compound **14** (146 mg, 0.3 mmol) was dissolved in 1.1 mL of CH_2Cl_2 and triethylamine (42 mg, 0.42 mmol). To this solution was added at 0 °C methanesulfonyl chloride (41 mg, 0.35 mmol). After 3 h of stirring under argon at room temperature, the mixture was evaporated in vacuo, and the residue was taken up in ethyl acetate. The solution was washed with saturated $NaHCO_3$, followed by 1 M HCl, water, and brine. Solvent was concentrated under reduced pressure to afford **26** (165 mg, 0.3 mmol, almost quantitatively) as a yellow liquid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.07 (stack, 74H), 3.07 (s, 1.9H), 3.08 (s, 3H), 3.83 (dd, J = 3.8, 11.4 Hz, 0.64H), 3.89 (m, 2H), 4.00 (m, 1.6H), 4.26 (dd, J = 4.0, 8.1 Hz, 1H), 4.47 (dd, J = 4.7, 16.5 Hz, 1H), 4.59 (m, 0.64H), 5.83 (d, J = 7.0 Hz, 0.64H), 5.92 (d, J = 6.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 11.84, 11.85, 12.1, 12.2, 17.67, 17.73, 17.76, 17.79, 17.81, 17.87, 17.91, 40.0, 40.2, 46.3, 61.5, 61.8, 69.0, 69.2, 69.4, 70.98, 71.12, 71.2, 71.4, 84.7, 84.8, 88.0, 99.4, 99.6, 99.9, 100.1, 100.3, 100.5; HRMS (ESI) calcd for $C_{24}H_{50}F_2O_6SSi_2$ 560.2835, found 560.284.

1-(2′-Deoxy-2′,2′-difluoro-3′,5′-di-*O*-(triisopropylsilyl)ribofuranosyl)nicotinamide (27). Compound **26** (420 mg, 0.75 mmol) and nicotinamide (732 mg, 6 mmol) were dissolved in 20 mL of CH_3CN/CH_2Cl_2 (1:1). To this solution was added TMSOTf (167 mg, 0.75 mmol) under argon, and the reaction mixture was allowed to reflux overnight. Solvent was evaporated in vacuo, and concentrated crude product was examined by NMR and used for the next step without further purification.

1-(2′-Deoxy-2′,2′-difluororibofuranosyl)nicotinamide (28). Compound **28** was obtained according to the deprotection procedure to

synthesize **22**, using **27** (440 mg, 0.75 mmol) as the starting material. Crude product was purified by HPLC on a Waters RP-18 XBridge PrepShield 19 × 50 mm column (solvent was 20 mM ammonium acetate, compounds were eluted at a flow rate of 2 mL/min) to afford **28** (β-isomer, t_R = 17.8 min, 78 mg, 0.28 mmol, 38%) and the α-isomer (t_R = 14.8 min, 103 mg, 0.37 mmol, 50%). **β-Isomer:** ^{1}H NMR (D_2O, 600 MHz) δ (ppm) 3.83 (d, J = 11.4 Hz, 1H), 4.01 (d, J = 12.6 Hz, 1H), 4.24 (d, J = 7.8 Hz, 1H), 4.46 (dd, J = 10.8, 20.4 Hz, 1H), 6.56 (d, J = 8.4 Hz, 1H), 8.20 (t, J = 6.6 Hz, 1H), 8.92 (d, J = 7.8 Hz, 1H), 9.20 (d, J = 6.6 Hz, 1H), 9.61 (s, 1H); ^{13}C NMR (125 MHz, D_2O) δ (ppm) 58.4, 67.3, 67.5, 67.6, 83.2, 83.3, 93.3, 93.4, 93.5, 93.6, 119.4, 121.7, 124.0, 128.6, 134.3, 141.4, 143.6, 146.9, 165.4; HRMS (ESI) calcd for $C_{11}H_{12}F_2N_2O_4$ 274.0765, found 274.0771. **α-Isomer:** ^{1}H NMR (D_2O, 500 MHz) δ (ppm) 3.78 (dd, J = 4.5, 11.0 Hz, 1H), 3.91 (dd, J = 1.5, 11 Hz, 1H), 4.59 (m, 1H), 6.75 (t, J = 5.0 Hz, 1H), 8.24, (dd, J = 5.0, 6.5 Hz, 1H), 8.98 (d, J = 6.5 Hz, 1H), 9.11 (d, J = 4.5 Hz, 1H), 9.34 (s, 1H).

2′-Deoxy-2′,2′-difluororibonicotinamide Mononucleotide (29). To a flame-dried round-bottom flask were added **28** (5 mg, 0.018 mmol), 6-methylnicotinamide (12.4 mg, 0.091 mmol), and 0.5 mL of trimethyl phosphate. At 0 °C, 13.9 mg (0.091 mmol) of phosphorus oxychloride was added to the reaction mixture. This solution was stirred at 0 °C for another 2 h. Ice was added to quench the reaction, and the pH was adjusted to 7 by adding NaOH solution and phosphate buffer. The crude product was concentrated and purified by HPLC on a Waters RP-18 XBridge PrepShield 19 × 50 mm column (solvent was 20 mM ammonium acetate, compound was eluted at a flow rate of 2 mL/min) to afford **29** (t_R = 6.2 min, 5 mg, 0.014 mmol, 78%): ^{1}H NMR (D_2O, 500 MHz) δ (ppm) 4.02 (dd, J = 3.2, 13.4 Hz, 1H), 4.19 (dt, J = 2.4, 13.4 Hz, 1H), 4.43 (d, J = 8.4 Hz, 1H), 4.65 (stack, 2H), 6.75 (dd, J = 2.3, 8.9 Hz, 1H), 8.38 (t, J = 6.5 Hz, 1H), 9.10 (d, J = 8.2 Hz, 1H), 9.36 (d, J = 5.8 Hz, 1H), 9.78 (s, 1H); ^{13}C NMR (125 MHz, D_2O) δ (ppm) 59.7, 69.4, 69.5, 69.6, 69.8, 86.80, 86.84, 94.0, 94.2, 94.4, 94.5, 115.9, 118.4, 120.9, 123.4, 128.5, 134.1, 140.9, 143.2, 146.8, 165.4; HRMS (ESI) calcd for $C_{11}H_{14}F_2N_2O_7P$ 355.0501, found 355.0503.

2′-Deoxy-2′,2′-difluoro-NAD$^+$ (30). A single reaction (50 μL) containing 6 mM **29**, 2 mM ATP, 10 mM $MgCl_2$, 1 μL of pyrophosphatase (1 unit), 5 μL of NMNAT-1 (13.5 μM), and 50 mM phosphate buffer (pH ~7.4) was incubated at 37 °C for 1 h. The reaction was terminated by addition of 3 μL of 10% TFA and purified by HPLC on a Waters RP-18 XBridge PrepShield 19 × 50 mm column (solvent was 20 mM ammonium acetate, compound was eluted at a flow rate of 2 mL/min, t_R = 12.7 min, 90% versus ATP with recovery of unreacted **30**): ^{1}H NMR (500 MHz, D_2O) δ (ppm) 4.28 (stack, 2H), 4.36 (m, 1H), 4.42 (s, 1H), 4.55 (stack, 3H), 4.73 (stack, 2H), 6.16 (d, J = 5.6 Hz, 1H), 6.69 (d, J = 9.3 Hz, 1H), 8.38 (dd, J = 6.5, 7.9 Hz, 1H), 8.41 (s, 1H), 8.60 (s, 1H), 9.04 (d, J = 8.2 Hz, 1H), 9.38 (d, J = 6.3 Hz, 1H), 9.54 (s, 1H); ^{13}C NMR (125 MHz, D_2O) δ (ppm) 59.7, 65.18, 65.22, 69.3, 69.49, 69.55, 69.7, 70.3, 74.3, 83.9, 84.0, 86.76, 86.81, 94.0, 94.2, 94.3, 94.5, 118.4, 119.7, 121.8, 123.8, 128.4, 133.9, 140.2, 140.8, 143.1, 146.7, 148.9, 151.6, 154.7, 165.2; HRMS (ESI) calcd for $C_{21}H_{26}F_2N_7O_{13}P_2$ 684.1026, found 684.1019.

2′-Deoxy-2′-fluoroarabinonicotinamide Mononucleotide (31). Compound **31** was obtained according to the phosphorylation procedure to synthesize **29** using **22** (7 mg, 0.027 mmol) as the starting material. Crude product was concentrated and purified by HPLC on a Waters RP-18 XBridge PrepShield 19 × 50 mm column (solvent was 20 mM ammonium acetate, compound was eluted at a flow rate of 2 mL/min) to afford **31** (t_R = 5.4 min, 6 mg, 0.018 mmol, 67%): ^{1}H NMR (D_2O, 400 MHz) δ (ppm) 4.04 (m, 1H), 4.19 (m, 1H), 4.34 (m, 1H), 4.56 (dt, J = 5.0, 17.8 Hz, 1H), 5.51 (dt, J = 4.6, 51.4 Hz, 1H), 6.67 (dd, J = 4.8, 8.8 Hz, 1H), 8.24 (t, J = 7.1 Hz, 1H), 8.92 (d, J = 7.9 Hz, 1H), 9.31 (d, J = 6.1 Hz, 1H), 9.39 (s, 1H); ^{13}C NMR (125 MHz, D_2O) δ (ppm) 55.17, 55.20, 55.23, 60.60, 60.62, 72.6, 72.8, 90.17, 90.19, 98.5, 98.8, 99.2, 100.7, 128.3, 133.9, 140.3, 142.6, 145.8, 165.6; MS (M^+) calcd 337.06, found 337.45.

2′-Deoxy-2′-fluoroarabino-NAD$^+$ (32). A single reaction (50 μL) containing 6 mM **31**, 2 mM ATP, 10 mM $MgCl_2$, 1 μL of pyrophosphatase (1 unit), 5 μL of NMNAT-1 (13.5 μM), and 50 mM phosphate buffer (pH ~7.4) was incubated at 37 °C for 1 h. The reaction was terminated by addition of 3 μL of 10% TFA and purified by HPLC on a Waters RP-18 XBridge PrepShield 19 × 50 mm column (solvent was 20 mM ammonium acetate, compound was eluted at a flow rate of 2 mL/min, t_R = 14.7 min, 95% versus ATP with recovery of unreacted **32**): ^{1}H NMR (D_2O, 600 MHz) δ (ppm) 4.41 (m, 1H), 4.46 (stack, 2H), 4.57 (stack, 3H), 4.67 (dd, J = 3.6, 5.4 Hz, 1H), 4.80 (dt, J = 4.8, 17.4 Hz, 1H), 4.90 (t, J = 6 Hz, 1H), 5.71 (dt, J = 4.8, 51 Hz, 1H), 6.20 (d, J = 6 Hz, 1H), 6.81, (dd, J = 4.8, 9.6 Hz, 1H), 8.37, (s, 1H), 8.40 (dd, J = 6.6, 7.8 Hz, 1H), 9.05, (d, J = 7.8 Hz, 1H), 9.38 (d, J = 6.6 Hz, 1H), 9.51 (s, 1H); ^{13}C NMR (125 MHz, D_2O) δ (ppm) 63.4, 65.4, 70.3, 70.8, 71.0, 74.1, 83.5, 83.8, 83.9, 86.8, 93.68, 93.73, 93.9, 95.3, 118.4, 128.3, 133.2, 140.0, 141.2, 143.4, 146.03, 148.9, 152.2, 165.1; MS (M^+) calcd 666.11, found 666.60.

2′-Deoxy-2′-fluororibonicotinamide Mononucleotide (33). Compound **33** was obtained according to the phosphorylation procedure to synthesize **29** using **25** (3.5 mg, 0.014 mmol) as the starting material. Crude product was concentrated and purified by HPLC on a Waters RP-18 XBridge PrepShield 19 × 50 mm column (solvent was 20 mM ammonium acetate, compound was eluted at a flow rate of 2 mL/min) to afford **33** (t_R = 5.9 min, 3.8 mg, 0.011 mmol, 83%): ^{1}H NMR (D_2O, 600 MHz) δ (ppm) 3.95 (d, J = 12.6 Hz, 1H), 4.16 (d, J = 13.2 Hz, 1H), 4.52 (stack, 2H), 5.40 (d, J = 50.4 Hz, 1H), 6.67 (d, J = 13.8 Hz, 1H), 8.31 (s, 1H), 9.00 (s, 1H), 9.35 (s, 1H), 9.74 (s, 1H); ^{13}C NMR (150 MHz, D_2O) δ (ppm) 55.16, 55.20, 60.1, 60.6, 69.3, 69.4, 86.97, 86.98, 90.0, 91.5, 94.6, 94.8, 127.8, 133.4, 141.0, 143.4, 145.9, 165.7; HRMS (ESI) calcd for $C_{11}H_{15}FN_2O_7P$ 337.0595, found 337.0599.

2′-Deoxy-2′-fluororibo-NAD$^+$ (34). A single reaction mixture (50 μL) containing 3.8 mM **33**, 10 mM ATP, 10 mM $MgCl_2$, 1 μL of pyrophosphatase (1 unit), 10 μL of NMNAT-1 (27 μM), and 50 mM phosphate buffer (pH ~7.4) was incubated at 37 °C for 1 h. The reaction was terminated by addition of 3 μL of 10% TFA and purified by HPLC on a Waters RP-18 XBridge PrepShield 19 × 50 mm column (solvent was 20 mM ammonium acetate, compound was eluted at a flow rate of 2 mL/min, t_R = 18.6 min, 94% versus **33**): ^{1}H NMR (D_2O, 600 MHz) δ (ppm) 3.44 (dd, J = 7.2, 12 Hz, 1H), 3.54 (dd, J = 4.2, 11.4 Hz, 1H), 4.10 (m, 1H), 4.15 (dd, J = 4.8, 11.4 Hz, 2H), 4.27 (t, J = 2.4 Hz, 1H), 4.37 (dd, J = 1.8, 14.4 Hz, 1H), 4.40 (t, J = 3.6 Hz, 1H), 4.49 (stack, 2H), 5.30, (dt, J = 3.0, 51 Hz, 1H), 5.95, (d, J = 6.0 Hz, 1H), 6.41 (dd, J = 2.4, 13.8 Hz, 1H), 8.11, (s, 1H), 8.13 (dd, J = 6.6, 7.8 Hz, 1H), 8.74 (d, J = 8.4 Hz, 1H), 9.13 (d, J = 6.6 Hz, 1H), 9.32 (s, 1H); ^{13}C NMR (125 MHz, D_2O) δ (ppm) 62.5, 63.45, 63.49, 65.36, 65.40, 67.7, 67.8, 70.4, 72.1, 73.9, 83.8, 83.9, 85.1, 85.2, 86.6, 94.1, 95.7, 97.2, 97.5, 102.4, 118.4, 128.7, 133.8, 139.2, 139.8, 140.3, 142.5, 145.99, 146.02, 149.0, 152.8, 155.4, 156.5, 165.0; HRMS (ESI) calcd for $C_{21}H_{27}FN_7O_{13}P_2$ 666.1121, found 666.1132.

Acknowledgment. We thank Ellison Medical Foundation for a New Scholar in Aging Award 2007 to A.A.S. We also thank Cornell University for funding. This work was supported by NIH funds for purchase of a new NMR spectrometer to Weill Cornell Medical College. We thank Cliff Soll of Hunter College of New York City for HRMS measurements. We thank Rockefeller Proteomics Resource for use of MS facilities.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for all new compounds described herein. This material is available free of charge via the Internet at http://pubs.acs.org.