The trifluoromethyl group contains three resonances degenerate in chemical shift, making the experiment more sensitive. The micromolar concentrations at which antisense DNA sequences are effective are detectable by ^{19}F NMR in reasonable acquisition times. A 10-μM sample of a 21 mer could be detected in 4 h with a 10:1 signal-to-noise ratio. For intracellular and in vivo studies this is crucial. The resonance is also a sharp singlet as a consequence of unhindered rotation about the trifluoromethyl group and the small four-bond coupling to H3 and H6.

The potency of 5-fluorouridine in antiviral and antitumor screening procedures has been explained in terms of a difference in the pK_A of the imino proton of 5-fU relative to uridine or thymidine, which results in base-pair mismatches during transcription.[20] In contrast the antiviral and antitumor activity of trifluorothymidine[21] is thought to result from inactivation of thymidilate synthetase via covalent complex formation to the α-methyl group, which is activated by fluorine substitution, and not at C6. There is no evidence that trifluorothymidine exerts toxicity by being incorporated into DNA and altering structure or duplex stability, and the studies described here argue against a structural role in the toxicity process. In fact, a number of points of evidence in the present study indicate that normal helix stability and geometry are conserved upon substitution of thymidine by trifluorothymidine. The base pairing study shows a similar change in chemical shift upon base pairing of trifluorothymidine to adenosine as in a normal A-T base pair, indicating that the base pairs are of similar stability. The detection of imino protons at appropriate chemical shifts for G-C, A-T, and A-T_f base pairs indicates that a stable duplex exists in solution although a broadening and lack of dispersion in this region indicates that the structural details of the helix are different from the control oligonucleotide. The similar thermal melting profiles and circular dichroism spectra for trifluorothymidine-containing duplexes as normal duplexes indicates that they are similar in stability and geometry. Finally, molecular modeling studies indicate that duplexes which potentially form intracellularly upon administration of antisense DNA are not disrupted by trifluoromethyl substitution of thymidine residues.

Oligonucleotides containing trifluorothymidine can be prepared by using the procedures applicable to the standard nucleosides and their physical and chemical properties are closely comparable. Trifluorothymidine does not disrupt the helix-forming properties of oligonucleotides containing it as demonstrated by imino proton studies and thermal melting and CD studies. The ^{19}F nucleus is easily detected, even at low concentrations, and the chemical shifts and relaxation times are indicative of molecular structure. The description of the chemical synthesis and physical properties of oligonucleotides containing trifluorothymidine contained here is the first of its kind and it should facilitate future studies of the mechanism of action of antisense oligonucleotides intracellularly. Work to incorporate trifluorothymidine into sequences of α-DNA, which are not degraded intracellularly by nuclease action, is in progress in an effort to detect antisense oligonucleotides by ^{19}F NMR intracellularly.

Acknowledgment. We wish to thank Ms. Jill Rudkowski for technical assistance and Mrs. Susan Gmeiner for help with the molecular modeling. W.H.G. wishes to thank the Alberta Heritage Foundation for Medical Research for a fellowship.

Registry No. 5'-dGAGGAT*CT*T*CAT*CT*T*CCCCGG, 133227-72-2; 5'-dCCGGGGAAGATGAAGATCCTC, 133227-73-3; 5'-*O*-(4,4'-dimethoxytrityl)-α,α,α-trifluorothymidine, 133128-06-0; trifluorothymidine, 70-00-8; 5'-*O*-(4,4'-dimethoxytrityl)-3'-*O*-(methyl phosphoramidite)-α,α,α-trifluorothymidine, 133100-90-0; *N*,*N*-diisopropylmethylphosphonamidic chloride, 110972-27-5; 3',5'-*O*-TPDS-α,α,α-trifluorothymidine, 133100-91-1; 3',5'-*O*-TPDS-2'-deoxyadenosine, 84828-84-2; 2-deoxyadenosine, 958-09-8.

Supplementary Material Available: 1D-^1H NMR spectrum of the hybrid duplex (1 page). Ordering information is given on any current masthead page.

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Pyridine Coenzyme Analogues. 3. Synthesis of Three NAD$^+$ Analogues Containing a 2'-Deoxy-2'-substituted Nicotinamide Arabinofuranosyl Moiety

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Received July 23, 1990 (Revised Manuscript Received February 11, 1991)

A general method for the preparation of 2'-deoxy-2'-substituted arabino-nicotinamide-adenine dinucleotide (NAD) analogues is described. Starting from 1,2:5,6-di-*O*-isopropylidine-α-D-allofuranose, the 2'-amino-, 2'-azido-, and 2'-fluoro-arabino-NAD analogues have been prepared. We report an improved phosphorylation procedure for nicotinamide nucleosides using pyrophosphoryl chloride in *m*-cresol. The selective reduction of azido substituents by aqueous dithiothreitol (DTT) in the presence of the readily reducible nicotinamide moiety is also reported. With both the 2'-azido and the 2'-fluoro substituents the cis configuration predominates for the incoming nicotinamide, thus allowing the stereoselective formation of the β anomer in high yield.

Introduction

The nicotinamide-adenine dinucleotide coenzyme, NAD$^+$, has two distinct physiological roles: first, as a redox coenzyme, it mediates aerobic and anaerobic energy metabolism, and second, it serves as a donor of the ADP ribose moiety in the ADP-ribosylation of proteins and the synthesis of poly ADP ribose.[1] The role of pyridine nu-

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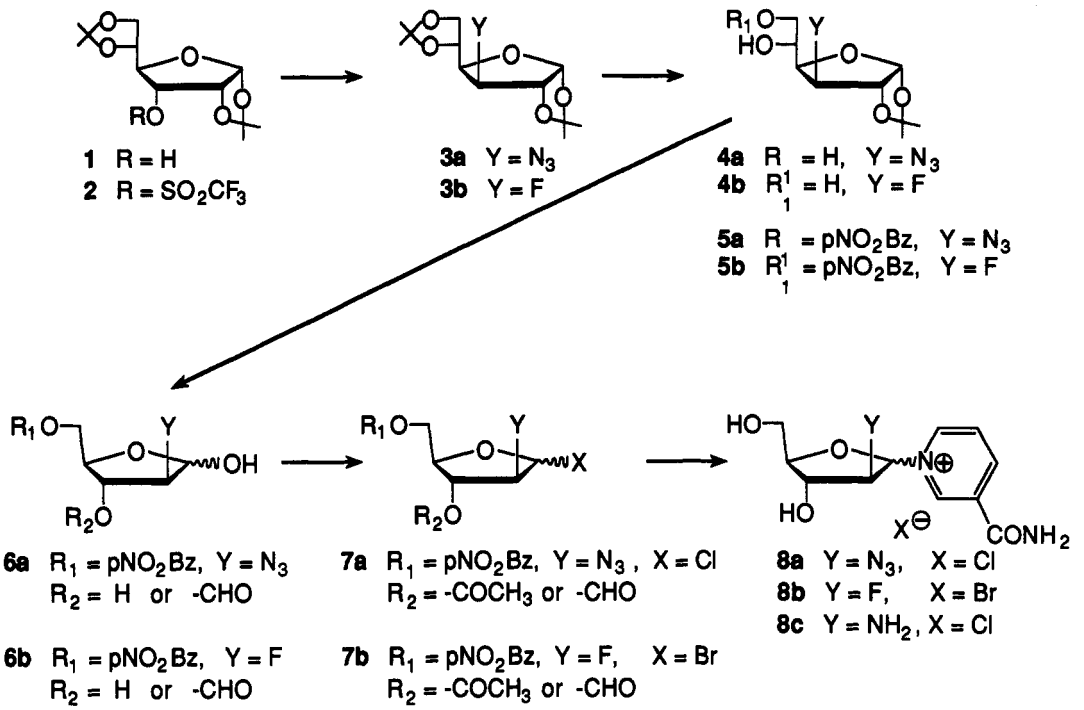
Scheme I



cleotides as redox coenzymes has been extensively studied using NAD$^+$ analogues as probes of the structural and steric constraints necessary for their function with dehydrogenases.[2] In the past, focus has been on the preparation of pyridine coenzymes with modified pyridine rings,[2] whereas few analogues have featured alterations in the nicotinamide ribose moiety.[3] This has been a significant omission since there is now ample evidence that dehydrogenases have a general ability to accommodate major configurational alterations in the sugar moiety without loss of redox function.[4] On the other hand, preliminary evidence for enzymes that conduct the cleavage of the nicotinamide–glycosyl bond indicates that small changes at the 2′-position of the nicotinamide sugar moiety abolish their ability to serve as substrates while enhancing their binding by upwards of 2 orders of magnitude.[5] Furthermore, the ribose diol plays a role in the base-catalyzed hydrolysis of NAD$^+$ and may also participate in the enzymatic cleavage of the nicotinamide–glycosyl bond of NAD$^+$.[6] Enzymes catalyzing the latter reaction include the ADP-ribosyltransferases (e.g., cholera toxin and diphtheria toxin), poly ADP ribose synthases, and NAD-glycohydrolases.[1]

In order to investigate the involvement of the nicotinamide sugar moiety in the redox reactions of the coenzyme and in both the chemical and enzyme-mediated cleavage of the glycosyl bond of NAD$^+$, we have synthesized three 2′-substituted arabino-nicotinamide-adenine dinucleotide analogues. The syntheses take advantage of a common starting material, 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose, which allows the stereospecific introduction of the fluoro, azido, and amino substituents. The severe constraints imposed by the chemical properties of the quaternary pyridinium nucleosides (including instability, insolubility, susceptibility to nucleophilic attack, and redox activity) require that the synthetic steps carried out after the introduction of the pyridinium ring use polar solvents while excluding strong bases, nucleophiles, and reducing agents. These methods should have wide applicability for nucleotide synthesis of similarly labile compounds. Detailed chemical and biochemical evaluation of the properties of these analogues is underway and will be reported in subsequent publications.

Results and Discussion

The syntheses outlined in this paper allow the preparation of three NAD$^+$ analogues with a 2′-arabino substituent in the nicotinamide ribosome moiety, starting from the common precursor, 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**1**). The first step in the synthesis (Scheme I) is the introduction of a substituent into the 3-position of this hexose. The azido moiety is introduced by activation of the 3-hydroxyl with trifluoromethanesulfonic anhydride[7] followed by displacement with lithium azide to give **3a**. Similarly, a fluoro moiety is introduced using anhydrous tetrabutylammonium fluoride as the source of incoming nucleophile.[8] However, difficulties with generating and handling the anhydrous fluoride salt[9] lead to our use of (diethylamino)sulfur trifluoride (DAST)[10] to generate **3b**.

Once the substituent has been introduced into the 3-position, the 5,6-isopropylidene group is selectively removed with dilute acid to give **4a** and **4b**[11] and the 6-

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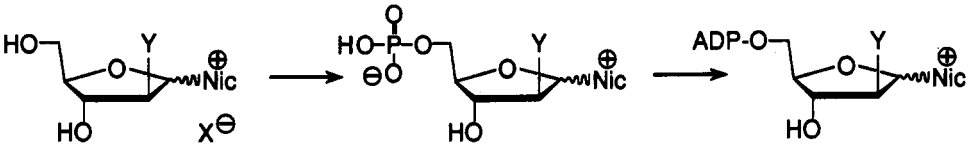
Scheme II



8a Y = N_3, X = Cl
8b Y = F, X = Br

9a Y = N_3
9b Y = F

10a Y = N_3
10b Y = F
10c Y = NH_2

position is then protected with the *p*-nitrobenzoyl moiety to give **5a** and **5b**. The latter protecting group is preferred over the more commonly used benzoyl derivative since pyridine nucleotides are unstable in the more vigorously basic conditions needed to remove the benzoyl group.

The 1,2-isopropylidene group can be removed either by the procedure of Bobek[12] or by using trifluoroacetic acid as the proton source. Oxidative cleavage of the C1–C2 bond with sodium periodate is followed by spontaneous ring closure to generate the 2-substituted sugars **6a** and **6b**.[12,13] The 3-*O*-formyl group resulting from the periodate oxidation is only partially hydrolyzed off under the reaction conditions that lead to **6a**, while it is fully hydrolyzed in the case of **6b**. The resulting mixture of **6a** and its 3-*O*-formyl derivative were not separated but directly acetylated with pyridine/acetic anhydride to give **7a** and its 3-*O*-formyl derivative. The 1′-halo sugar **7b** was prepared using 30% hydrogen bromide in acetic acid.[13] This method has proven inappropriate for the synthesis of **7a** because alkyl azides are readily reduced by HBr; thus, the titanium tetrachloride procedure of Bobek[12] was used instead to prepare the 1-chloro sugar **7a**.

Coupling of the 1-halo sugars with nicotinamide was conducted in anhydrous acetonitrile.[14] The 3′-acetyl and the 5′-*p*-nitrobenzoyl groups were removed by treating the nucleosides with methanolic ammonia at 0 °C for 2 h[14,15] (longer incubation leads to partial destruction of the nucleosides). The overall yields of the nucleosides (based on 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose) were as follows: **8a**, 34%; **8b**, 24%.

Anomeric Ratios. In the halo sugar **7b**, the α anomer predominated over the β anomer by a 9:1 ratio, whereas in the final product **8b**, the ratio was 1:9 with the β anomer predominant. Thus, halogenation favors formation of the trans configuration of **7b** with subsequent inversion of configuration by the incoming nicotinamide; i.e., the 2′-fluoro group causes no overt influence on the stereochemistry of the reaction. In the case of the azido analogue, however, we observed unusual selectivity of attack by nicotinamide on **7a**. The α and β anomers of the 1-chloro sugar **7a** can be separated by flash chromatography. Regardless of the initial configuration of **7a**, the formation of the nicotinamide nucleoside **8a** gave the identical 6:1 ratio of isomers. Nuclear Overhauser enhancement (NOE) experiments demonstrated that the predominant isomer had the nicotinamide moiety cis to the bulky 2′-azido group. The observed stereochemistry results from a combination of two factors: the selective reaction of nicotinamide with the α-chloro anomer and faster anomerization of the β-chloro sugar than its displacement by nicotinamide.[16] The origin of the differences in reaction rates remains unclear. Formation of a charge-transfer complex or an electrostatic interaction between the azido group and the incoming nicotinamide moiety may be responsible for the cis addition.

Phosphorylation and Coupling. The nicotinamide nucleosides were phosphorylated using *m*-cresol and pyrophosphoryl chloride following the procedure of Imai et al.[17] (Scheme II). This procedure was preferred over the conventional Yoshikawa method utilizing trimethyl phosphate and phosphorus oxychloride[18] because of the greater solubility of nicotinamide nucleosides in *m*-cresol than in trimethyl phosphate and because water is readily removed from *m*-cresol by azeotropic distillation with benzene. The pyrophosphate linkage of the dinucleotide was formed using a modified Michelson coupling procedure[4a,19] in which the nicotinamide nucleotide was activated with diphenyl chlorophosphate in dioxane and then reacted with AMP in pyridine.

Azide Reduction. The 2′-amino analogues were prepared from the corresponding azido analogues. The standard procedures in the literature for the reduction of azido groups to amines, e.g., catalytic hydrogenation, various forms of hydride reduction, or one-electron reductants,[20] are all inappropriate because they would cause a concomitant reduction of the nicotinamide moiety. Indeed, the reductive lability of the nicotinamide moiety combined with the general insolubility of pyridine nucleoside/-tides in solvents other than water places severe limitations on the chemistry that can be used and precludes most standard methods of azide reduction.[21] Given the resistance of NAD^+ to direct chemical reduction by thiols,[22] we explored their use as selective reductants. Knowles et al. have reported the reduction of aryl azides with dithiothreitol (DTT),[23] and we have recently demonstrated the facile reduction of 3′-azido-3′-deoxythymidine (AZT) using aqueous DTT.[24] When **8a** is incubated with DTT, it is transformed to **8c** without any detectable reduction of the nicotinamide moiety. Therefore, we can use the azido group as a cryptic amine that is unmasked at the last step, thus avoiding working with an amino group (or an amine blocking group) during the earlier phases of the synthesis. The DTT-mediated reduction of azides in aqueous solution at neutral pH should

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be of significant utility in the manipulation of functional groups on biomolecules.

Enzymatic Activity. Preliminary studies with horse liver alcohol dehydrogenase using ethanol as a reductant indicate that β-2'-fluoro-ara-NAD$^+$ has kinetic parameters comparable to those of β-NAD$^+$. The β-2'-amino-ara-NAD$^+$ was also active with horse liver alcohol dehydrogenase but with decreased V_{max} (interference with coenzyme function from reactions between the product, acetaldehyde, and the 2'-amino group of the analogue cannot be ruled out at this time). There was no detectable enzymatic reduction of β-2'-azido-ara-NAD$^+$. Detailed evaluation of the biochemical and chemical properties of these analogues is currently underway and will be reported at a later time.

Experimental Section

General Procedures. Proton NMR chemical shifts (25 °C) are reported relative to internal 3-(trimethylsilyl)propionate (TSP) for aqueous samples or to tetramethylsilane (TMS) for samples in organic solvents. Samples of nucleosides, nucleotides, and dinucleotides (1 mM) were lyophilized twice from D_2O before spectra were acquired. Anomeric configurations were determined from NOE experiments.[25] Liquid secondary-ion mass spectra were obtained using 1% trifluoroacetic acid in thioglycerol as the matrix. Solutions were dried over $MgSO_4$, and volatile solvents were evaporated by rotary evaporation. All reagents used for synthesis were obtained from Aldrich Chemical Co., Inc. unless otherwise specified. Thin-layer chromatography (TLC) plates were visualized by UV light, sulfuric acid charring, or with an acetone/ammonia spray.[26]

1,2:5,6-Di-*O*-isopropylidene-3-(trifluoromethanesulfonyl)-α-D-allofuranose (2). 1,2:5,6-Di-*O*-isopropylidene-α-D-allofuranose (4.80 g, 18.4 mmol, Pfanstiehl Labs Inc.) was reacted with trifluoromethanesulfonic anhydride following the method of Hall and Miller.[7] The product was purified by flash chromatography with dichloromethane as eluant and isolated as a colorless glass (6.6 g, 16.9 mmol, 92%): ^{1}H NMR ($CDCl_3$) δ 5.83 (d, 1 H, H1), 5.04–4.63 (m, 2 H, H2, H3), 4.30–3.75 (m, 4 H, H4, -5, -6, -6').

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (3a). 2 (6.60 g, 16.9 mmol) was dissolved in dimethylformamide (25 mL), and lithium azide (2.48 g, 50.7 mmol, Eastman Kodak) was added before the mixture was stirred at 25 °C for 24 h. The solvent was removed, and the residue was partitioned between water (20 mL) and dichloromethane (20 mL). The aqueous layer was extracted with dichloromethane (2 × 20 mL) and evaporated to give a yellow oil (4.48 g, 15.7 mmol, 93%): ^{1}H NMR ($CDCl_3$) δ 5.85 (d, 1 H, H1), 4.62 (d, 1 H, H2), 4.19–4.00 (m, 5 H, H3, -4, -5, -6, -6'), 1.51, 1.43, 1.37, 1.32 (4s, 12 H, four isopropylidene methyls).

3-Azido-3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (4a). 3a (4.48 g, 15.7 mmol) was converted to 4a (3.70 g, 15 mmol, 95%) following the procedure of Bobek:[12] ^{1}H NMR ($CDCl_3$) δ 5.88 (d, 1 H, H1), 4.64 (d, 1 H, H2), 4.20–3.70 (m, 5 H, H3, -4, -5, -6, -6'), 2.75 (s, 1 H, 5-OH), 2.30 (s, 1 H, 6-OH), 1.50, 1.32 (2s, 6 H, two isopropylidene methyls).

3-Azido-3-deoxy-6-*O*-(*p*-nitrobenzoyl)-1,2-*O*-isopropylidene-α-D-glucofuranose (5a). 4a (3.70 g, 15 mmol) was dissolved in dichloromethane (25 mL) and pyridine (7 mL). The solution was cooled to −30 °C before *p*-nitrobenzoyl chloride (2.92 g, 15.7 mmol) was added slowly. The mixture was maintained at −20 °C for 12 h before evaporation of the solvents and the residue partitioned between toluene (20 mL) and water (20 mL). The aqueous phase was extracted with toluene (2 × 10 mL), and the combined organic layers were washed with water (3 × 10 mL) and evaporated. The residue was purified by flash chromatography (chloroform/ether (3:1), R_f product 0.7) to give 5a (5.30 g, 13.4 mmol, 89%): ^{1}H NMR ($CDCl_3$) δ 8.27 (A_2B_2, 4 H, aromatics), 5.91 (d, 1 H, H1, $J_{1,2}$ = 3.4 Hz), 4.76 (d, 1 H, H6, $J_{6,6'}$ = 11.9 Hz), 4.68 (d, 1 H, H2, $J_{2,1}$ = 3.4 Hz), 4.50 (dd, 1 H, H6', $J_{6',6}$ = 11.9 Hz), 4.24–4.21 (m, 3 H, H3, -4, -5), 2.59 (s, 1 H, 5-OH), 1.50, 1.34 (2s, 6 H, two isopropylidene methyls).

2-Azido-2-deoxy-5-*O*-(*p*-nitrobenzoyl)-D-arabinofuranose (6a). 5a (5.30 g, 13.4 mmol) was dissolved in 200 mL of 50% triflouroacetic acid solution (water:dioxane:trifluoroacetic acid = 1:1:2). The reaction was monitored by TLC ($CHCl_3$/ether (2:1)); after 20 h at 25 °C, all starting material had been hydrolyzed. The solvents were evaporated to give a cream-colored solid that was not further purified before oxidation.

The solid was dissolved in acetonitrile (50 mL) and water (50 mL), and then sodium bicarbonate (1.00 g) was added to buffer the solution. Sodium periodate (3.15 g, 14.7 mmol) was added and the mixture stirred overnight at 25 °C. The mixture was then filtered and evaporated before being partitioned between water (20 mL) and ethyl acetate (20 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL), and the combined organic layers were dried and evaporated. TLC of the product ($CHCl_3$/ethyl acetate (2:1)) showed two spots (R_f 0.40 and 0.50) that were identified by ^{1}H NMR as 6a and its 3-*O*-formyl derivative, respectively (combined yield ca. 10.7 mmol, 80%). The mixture was used without further purification, although the compounds were separable by flash chromatography using the previous solvent system. ^{1}H NMR ($CDCl_3$) data for the anomeric protons of the two compounds: δ 5.52 (d, 1 H, H1 β anomer of 3-*O*-formyl derivative of 6a), 5.47 (s, 1 H, H1 α anomer of 3-*O*-formyl derivative of 6a), 5.46 (d, 1 H, H1 β anomer of 6a), 5.43 (s, 1 H, H1 α anomer of 6a).

2-Azido-2-deoxy-3-*O*-acetyl-5-*O*-(*p*-nitrobenzoyl)-D-arabinofuranosyl Chloride (7a). 6a and its 3-*O*-formyl derivative were dissolved in pyridine (20 mL) and acetic anhydride (20 mL), and the reaction mixture was maintained at 25 °C for 1 h before the solvents were removed. The residue was coevaporated with toluene (2 × 20 mL) to remove traces of acetic acid; the 1,3-diacetylated sugar (4.40 g 10.7 mmol) was then dissolved in dichloromethane (60 mL) at 0 °C. Titanium tetrachloride (1.75 mL, 16 mmol) was added dropwise under nitrogen. The reaction mixture was maintained at 4 °C for 4 h before being added slowly to saturated sodium bicarbonate solution (100 mL). The heterogeneous mixture was filtered through a bed of silica; the organic layer was separated and dried and the solvent removed by rotary evaporation (3.20 g, 8.3 mmol, 78%). Four compounds were obtained: the α and β anomers of 7a and the α and β anomers of the 3-*O*-formyl derivative of 7a. TLC of the products (toluene/ethyl acetate (8:1)) allowed separation of the α and β anomers of the two compounds with R_f's of 0–.54 and 0.37, resp. The ratio of 3-*O*-formyl to 3-*O*-acetyl compounds was 1:3 on the basis of integration of the ^{1}H NMR spectrum. The ratio of α to β anomers of the two compounds was 1:6, respectively: ^{1}H NMR ($CDCl_3$ β anomer of 7a) δ 8.33–8.24 (m, 4 H, aromatics), 6.26 (d, 1 H, H1), 5.58 (t, 1 H, H2), 4.87–4.34 (m, 4 H, H3 -4, -5, -5') 2.17 (s, 3 H, acetyl); (α anomer of 7a) 8.33–8.25 (m, 4 H, aromatics), 6.12 (s, 1 H, H1), 5.06 (d, 1 H, H2), 4.80–4.47 (m, 4 H, H3, -4, -5, -5'), 2.18 (s, 3 H, acetyl); (β anomer of 3-*O*-formyl derivative of 7a) 8.33–8.24 (m, 4 H, aromatics), 8.16 (s, 1 H, formyl), 6.28 (d, 1 H, H1), 5.70 (t, 1 H, H2), 4.87–4.34 (m, 4 H, H3, -4, -5, -5'); (α anomer of 3-*O*-formyl derivative of 7a) 8.33–8.25 (m, 4 H, aromatics), 8.15 (s, 1 H, formyl), 6.15 (d, 1 H, H1), 5.18 (t, 1 H, H_2), 4.80–4.47 (m, 4 H, H3, -4, -5, -5').

2'-Azido-2'-deoxy-Substituted Nicotinamide Arabinofuranoside Chloride 8a. Nicotinamide (2.00 g, 16.4 mmol) was dissolved in acetonitrile (80 mL) by refluxing under nitrogen. The solution was allowed to cool to room temperature before being added to the anomeric mixture of 7a (3.20 g, 8.3 mmol). The mixture was maintained at room temperature overnight, and the solvent was evaporated, keeping the temperature below 35 °C.

TLC of the reaction mixture (*n*-BuOH:H_2O:AcOH = 5:3:2) showed two major spots (R_f's 0.68 and 0.57); the former corresponded to nicotinamide, the latter gave a bright fluorescent spot upon treatment with ammonia/acetone.[26] The 3',5'-protecting groups were removed by treatment with saturated methanolic ammonia (50 mL) at 0 °C for 4 h, and the mixture was concentrated under reduced pressure. The product was coevaporated with methanol (2 × 10 mL) and then dissolved in a minimum volume of methanol and added to cold (0 °C), stirred ether (200 mL). The resulting brown precipitate was allowed to settle before

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the ether was decanted off and the reprecipitation repeated to remove traces of nicotinamide still present in the product. The product from the second precipitation was dissolved in methanol and the solvent removed to give **8a** (1.96 g, 6.2 mmol, 75%). TLC (*n*-BuOH:H_2O:AcOH = 5:3:2) showed one spot (R_f 0.45) that gave a positive acetone/ammonia test.[26] The anomeric ratio of product was ca. 1:6 α/β.

The mixture of the α and β anomers of **8a** was used in subsequent steps without further purification. The resolution of the anomers can be achieved by use of a reversed-phase HPLC column (Rainin Microsorb C_{18} analytical). The anomers were eluted with a linear gradient from 0–10% acetonitrile in ammonium phosphate buffer (20 mM, pH 2.8) over 10 min. The retention times were 6.3 min for α-**8a** and 7.6 min for β-**8a** (flow rate 1 mL/min.): ^{1}H NMR (β-**8a**, D_2O, pD 2.53) δ 9.69 (s, 1 H, N2), 9.20 (d, 1 H, N6, J = 6.3 Hz), 8.99 (d, 1 H, N4, J = 8.1 Hz), 8.27 (t, 1 H, N5, J = 6.6 Hz), 6.65 (d, 1 H, 1′, J = 6.6 Hz), 4.89 (t, 1 H, 2′, J = 8 Hz), 4.41 (t, 1 H, 3′, J = 8.3 Hz), 4.26 (dt, 1 H, 4′, $J_{3-4'}$ = 8.4 Hz, $J_{4'-5'}$ = 2.5 Hz), 4.12 (dd, 1 H, 5′, $J_{4'-5'}$ = 2.5 Hz, $J_{5'-5''}$ = 13.5 Hz), 3.97 (dd, 1 H, 5″, $J_{4'-5'}$ = 2.5 Hz, $J_{5'-5''}$ = 13.5 Hz); (α-**8a**; D_2O, pD 2.59) 9.46 (s, 1 H, N2), 9.23 (d, 1 H, N6, J = 6.3 Hz), 9.01 (d, 1 H, N4, J = 8.2 Hz), 8.30 (t, 1 H, N5, J = 6.7 Hz), 6.34 (d, 1 H, 1′, J = 4.6 Hz), 4.71 (t, 1 H, 2′), 4.55 (t, 1 H, 3′), 3.98 (dd, 1 H, 5′, $J_{4'-5'}$ = 3.1 Hz, $J_{5'-5''}$ = 12.7 Hz), 3.87 (dd, 1 H, 5″, $J_{4'-5''}$ = 5.7 Hz, $J_{5'-5''}$ = 12.7 Hz).

2′-Amino-2′-deoxy-Substituted Nicotinamide Arabinofuranoside Chloride 8c. **8a** (152 mg, 0.48 mmol) was dissolved in water (30 mL), and dithiothreitol (215 mg, 2.40 mmol) was added. The solution was adjusted to pH 7.1 with 0.1 M sodium hydroxide, and the mixture was stirred for 4 h at 25 °C, until evolution of nitrogen had ceased. The mixture was then diluted to 200 mL with water, degassed, and applied to a Bio-Rex 70 (200–400 mesh, 10 mL of resin) column generated in the pyridinium form. The amino nucleosides were eluted with a 0–1 M pyridinium acetate gradient at pH 6.0 with a flow rate of 0.9 mL/min. The eluant was collected in 8-mL fractions. Each fraction was assayed for the pyridinium moiety by monitoring the absorbance at 326 nm following addition of cyanide.[27] The β anomer eluted in fractions 58–70 and the α anomer in fractions 88–98. Hydrochloric acid (2 equiv with respect to the nucleoside) was added to the combined fractions for each anomer, and the water and pyridinium acetate were removed by rotary evaporation. The residue was coevaporated with methanol to remove traces of water and volatile salts. Yield of α anomer of **8c**, 17 mg (0.06 mmol, 12.5%), β anomer of **8c**, 84 mg (0.29 mmol, 60.4%): ^{1}H NMR (α-**8c**; D_2O, pD 4.8) δ 9.49 (s, 1 H, N2), 9.27 (d, 1 H, N6), 9.01 (d, 1 H, N4), 8.31 (dd, 1 H, N5), 6.72 (d, 1 H, 1′), 4.92 (q, 1 H, 4′), 4.57 (t, 1 H, 3′) 4.25 (t, 1 H, 2′), 4.04 (dd, 1 H, 5′), 3.89 (dd, 1 H, 5″); (β-**8c**; D_2O, pD 3.7) 9.80 (s, 1 H, N2, J = 8 Hz), 9.31 (d, 1 H, N6, J = 6 Hz), 9.03 (d, 1 H, N4, J = 8 Hz), 8.34 (t, 1 H, N5, J = 7 Hz), 6.71 (d, 1 H, 1′, J = 6 Hz), 4.46 (t, 1 H, 3′, J = 7 Hz), 4.37 (t, 1 H, 2′, J = 6 Hz), 4.31 (d, 1 H, 4′, J = 8 Hz), 4.16 (d, 1 H, 5′, J = 13.5 Hz), 3.99 (d, 1 H, 5″, J = 13.5 Hz).

2′-Azido-2′-deoxy-Substituted Nicotinamide Arabinomononucleotide 9a. **8a** (514 mg, 1.63 mmol) was lyophilized from water before being dissolved in *m*-cresol (20 mL). Traces of water were removed by adding dry benzene (10 mL) and the azeotrope removed by rotary evaporation (repeated three times). The solution was cooled to 5 °C and pyrophosphoryl chloride (1.64 g, 6.52 mmol, 4 equivalents, Alfa Products, Morton Thiokol Inc.) was added. The reaction was monitored by TLC of hydrolyzed aliquots; after 18 h at 5 °C, all nucleoside **8b** was judged to have been phosphorylated. The reaction mixture was poured into ice–water (30 mL) and extracted with ether (4 × 10 mL) to remove the *m*-cresol. The water was evaporated, and the residual oil was dissolved in a minimum volume of methanol and reprecipitated with ice-cold acetone (150 mL). The product was centrifuged down and dried after washing with ether to give **9a** (504 mg, 1.40 mmol, 86.1%): ^{1}H NMR (β-**9a**; D_2O) δ 9.37 (s, 1 H, N2), 9.34 (d, 1 H, N6), 9.00 (d, 1 H, N4), 8.30 (t, 1 H, N5), 6.65 (d, 1 H, 1′), 4.90 (t, 1 H, 2′), 4.49 (t, 1 H, 3′), 4.39 (m, 2 H, 4′,5′), 4.24 (m, 1 H, 5″).

2′-Azido-2′-deoxy-Substituted Nicotinamide Arabinoside Adenine Dinucleotide 10a. **9a** (359 mg, 1.00 mmol) was dissolved in a minimum volume of water and added dropwise to a cold (0 °C), rapidly stirred mixture of pyridine (15 mL) and acetic anhydride (15 mL). The solution was stirred for 90 min before the solvents were removed by rotary evaporation and the residue coevaporated with water to remove traces of acetic acid. The 3′-acetylated mononucleotide was then treated with water/pyridine (1:1, 10 mL) for 2 h to hydrolyze any mixed anhydride that may have formed.[28] Removal of the water/pyridine yielded 3′-acetylated mononucleotide that was lyophilized for 1 h before being dissolved in dimethylformamide (8 mL). Diphenyl chlorophosphate (311 μL, 403 mg, 1.50 mmol) and freshly distilled tri-*n*-butylamine (357 mL, 278 mg, 1.50 mmol) were added to the solution at ambient temperature under nitrogen. The mixture was allowed to stand for 1 h before the solvents were removed and the residue washed with dry ether (50 mL) to remove the excess amine.

The bis(tetrabutylammonium) salt of AMP was prepared following a standard procedure,[19] and 2 equiv (1.70 g, 2.00 mmol) of this compound were dissolved in dimethylformamide (7 mL). This solution was then added to the activated mononucleotide from the diphenyl chlorophosphate reaction before pyridine (7 mL) was added. The mixture was stirred overnight at ambient temperature before the solvents were evaporated and the residue dissolved in saturated methanolic ammonia at 0 °C for 2 h. The methanol/ammonia was removed under reduced pressure and the residue coevaporated twice with methanol before being dissolved in a minimum volume of water. The pH of the solution was adjusted to pH 2 with 10% aqueous nitric acid and then added dropwise to a stirred solution of acetone (500 mL) at 0 °C. The precipitated product was centrifuged and washed twice with ether before being dried under a stream of nitrogen.

The crude product was then dissolved in water (200 mL) and the solution adjusted to pH 6.0 with 1 M potassium hydroxide solution before being loaded onto a Dowex AG 1X-8 (100–200 mesh) column (2.6 × 41 cm) generated in the formate form. The column was flushed with water to recover unreacted mononucleotide before a 0–0.5 M formic acid gradient (2-L total volume) was applied. **10a** was eluted with ca. 0.2 M formic acid. The fractions containing **10a** were pooled, evaporated, acidified to pH 2.0 with 10% nitric acid, and precipitated from ice-cold acetone (100 mL) to give **10a** (185 mg, 0.27 mmol, 27%).

Separation of the α and β anomers of **10a** (25 mg) was achieved on a Whatman SAX M9/25 column using ammonium phosphate buffer (pH 3.5, 10 mM). The fractions containing each anomer were pooled, evaporated, and acidified to pH 2.0 with 10% nitric acid before being precipitated from ice-cold acetone. The products were assayed by cyanide addition;[27] a good correlation was obtained between the results of this assay and the weights of the dinucleotides obtained by precipitation. Yield of α anomer of **10a**, 5 mg, β anomer of **10a**, 18 mg. Mass spectra of both anomers are identical with MH^+ ion at m/z 689. Each anomer was shown to be >99% pure by HPLC and ^{1}H NMR: ^{1}H NMR (β-**10a**, D_2O, pD 8.0) δ 9.20 (s, 1 H, N2), 9.16 (d, 1 H, N6), 8.82 (d, 1 H, N4), 8.39 (s, 1 H, A8), 8.20 (t, 1 H, N5), 8.18 (s, 1 H, A2), 6.52 (d, 1 H, N1′), 6.00 (d, 1 H, A1′), 4.69 (t, 1 H, N2′), 4.20–4.50 (m, 6 H), 3.88 (m, 1 H), 3.49 (m, 2 H); (α **10a**; D_2O, pD 8.0) 9.34 (s, 1 H, N2), 9.10 (d, 1 H, N6), 8.88 (d, 1 H, N4), 8.46 (s, 1 H, A8), 8.18 (m, 2 H, N5 and A2), 6.18 (d, 1 H, N1′), 5.99 (d, 1 H, A1′), 4.41 (t, 1 H, N2′), 4.26–3.91 (m, 6 H), 3.87 (m, 1 H), 3.55 (m, 1 H), 3.45 (m, 1 H).

2′-Amino-2′-deoxy-Substituted Nicotinamide Arabinoside Adenine Dinucleotide 10c. **10a** (3.38 mg, 4.92 μmol, based on cyanide addition assay[27] at 327 nm, $\epsilon = 7.25 \times 10^3$ M^{-1} cm^{-1}) was dissolved in water (1.0 mL) at 25 °C, and dithiothreitol (20 mg, 0.13 mmol) was added. The pH of the reaction mixture was maintained between 7–8 by adding 0.1 M sodium hydroxide. The mixture was allowed to sit overnight at room temperature. NMR analysis showed complete conversion to the amine. DTT was removed by precipitating the product with acetone yielding **10c** (3.11 mg, 4.71 μmol, based on cyanide addition assay[27] at 327 nm, $\epsilon = 6.27 \times 10^3$ M^{-1} cm^{-1}).

Larger quantities of **10c** were purified on a Dowex AG 1X-8 column generated in the formate form. The product was eluted

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at a formic acid concentration of ca. 0.2 M and was isolated in the same manner as **10a** except that acetonitrile rather than acetone was used for the precipitation procedures.

Separation of the two anomers of **10c** was achieved using the conditions outlined for **10a**, except that the concentration of ammonium phosphate buffer used was 7 mM. The mass spectrum of the β anomer of **10c** showed the MH^+ ion at m/z 663: ^1H NMR (β-**10c**; D_2O, pD 2.0) 9.54 (s, 1 H, N2), 9.48 (d, 1 H, N6, J = 5.9 Hz), 9.04 (d, 1 H, N4, J = 8.0 Hz), 8.61 (s, 1 H, A8), 8.44 (s, 1 H, A2), 8.42 (t, 1 H, N5, J = 7.8 Hz), 6.80 (d, 1 H, N1′, J = 5.0 Hz), 6.18 (d, 1 H, A1′, J = 5.2 Hz), 4.75 (t, 1 H, A2′), 4.6–4.2 (m, 9 H).

3-Fluoro-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (3b). DAST was reacted with 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (10.41 g, 40.0 mmol, Pfanstiehl Labs Inc.) following the method of Tewson and Welch.[10] The material obtained by distillation was partitioned between water (10 mL) and dichloromethane (10 mL) and the organic layer was separated, dried ($MgSO_4$), and evaporated down to give **3b** (5.70 g, 21.4 mmol, 53%): ^1H NMR ($CDCl_3$) δ 5.95 (d, 1 H, H1, $J_{1,2}$ = 4.5 Hz), 4.70 (dd, 1 H, H2, $J_{2,1}$ = 3.7 Hz, $J_{2,F}$ = 10.6 Hz), 5.02 (dd, 1 H, H3, $J_{3,2}$ = 2.2 Hz, $J_{3,F}$ = 49.8 Hz), 4.29 (m, 1 H, H5), 4.16–4.02 (m, 3 H, H4, -6, -6′), 1.51, 1.48, 1.44, 1.42, (4s, 12 H, isopropylidene methyls).

3-Fluoro-3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose (4b). **3b** (5.70 g, 21.4 mmol) was dissolved in p-dioxane/ethanol/5 M sulfuric acid (60:30:10 mL), kept at room temperature, and monitored by TLC (benzene/ether (9:1) according to Foster et al.[8] until the starting material disappeared completely (ca. 4 h). The mixture was neutralized with saturated sodium bicarbonate solution and concentrated by evaporation before being extracted with dichloromethane (6 × 20 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent evaporated to give **4b** (4.75 g, 21 mmol, 98%): ^1H NMR (CD_2Cl_2) δ 5.95 (d, 1 H, H1, $J_{1,2}$ = 3.8 Hz), 5.07 (dd, 1 H, H3, $J_{3,4}$ = 2.2 Hz, $J_{3,F}$ = 49.9 Hz), 4.70 (dd, 1 H, H2, $J_{2,1}$ = 3.8 Hz, $J_{2,F}$ = 10.9 Hz), 4.14 (dq, 1 H, H4, $J_{4,F}$ = 29.7 Hz, $J_{4,3}$ = 2.2 Hz, $J_{4,5}$ = 8.7 Hz), 3.92 (m, 1 H, H5), 3.83 (dd, 1 H, H6, $J_{6,6'}$ = 11.4 Hz, $J_{6,5}$ = 3.2 Hz), 3.72 (dd, 1 H, H6, $J_{6',6}$ = 11.4 Hz, $J_{6',5}$ = 5.2 Hz), 1.47, 1.32 (2s, 6 H, two isopropylidene methyls).

3-Fluoro-3-deoxy-6-*O*-(*p*-nitrobenzoyl)-1,2-*O*-isopropylidene-α-D-glucofuranose (5b). **4b** (4.75 g, 21.0 mmol) was dissolved in pyridine (10 mL) and dichloromethane (25 mL) and the mixture was cooled to −30 °C. p-Nitrobenzoyl chloride (4.29 g, 23.1 mmol) was added slowly with vigorous stirring, and then the mixture was stored overnight at −20 °C. The solvent was evaporated and the residue partitioned between water (100 mL) and dichloromethane (100 mL). The organic layer was washed with water (2 × 50 mL), dried, and then removed by rotary evaporation to give crude **5b**. The product was purified by flash chromatography, eluting first with dichloromethane and then with methanol to give pure **5b** (6.31 g, 16.8 mmol, 80%): ^1H NMR ($CDCl_3$) δ 8.25 (A_2B_2, 4 H, aromatics), 5.99 (d, 1 H, H1), 5.14 (dd, 1 H, H3), 4.78–4.20 (m, 5 H, H2, -4, -5, -6, -6′), 1.49 and 1.34 (2s, 6 H, isopropylidene methyls).

2-Fluoro-2-deoxy-5-*O*-(*p*-nitrobenzoyl)-α-D-arabinofuranose (6b). **5b** (6.31 g, 16.8 mmol) was dissolved in dioxane (100 mL) and water (100 mL) at 80 °C before Dowex-50 (H^+) resin (25 mL) was added. The mixture was stirred at 80 °C for ca. 20 h; TLC indicated hydrolysis of the isopropylidene function was complete (R_f products 0.55, benzene/ethanol 7:1). The solvents were evaporated and the residue coevaporated with methanol (2 × 50 mL).

The product was dissolved in acetonitrile (75 mL) and water (125 mL) containing sodium bicarbonate (2.00 g). Sodium periodate (1.1 equiv, 18.5 mmol, 3.95 g) was added slowly with stirring at room temperature. The mixture was allowed to stand overnight before the acetonitrile was removed, and the residue was extracted with chloroform (5 × 30 mL). The extracts were dried and solvent evaporated to give **6b** (4.08 g, 13.6 mmol, 81%): ^1H NMR ($CDCl_3$) δ 8.26 (A_2B_2, 4, aromatics), 5.62 (d, 1 H, H1), 4.94 (d, 1 H, H2), 4.65–4.50 (m, 3 H, H4, -5, -5′), 4.33 (dd, 1 H, H3).

2-Fluoro-2-deoxy-3-*O*-acetyl-5-*O*-(*p*-nitrobenzoyl)-D-arabinofuranosyl Bromide (7b). **6b** (4.08 g, 13.6 mmol) was dissolved in pyridine (50 mL) and acetic anhydride (25 mL), and the mixture was left at room temperature for 2 h. The solvents were then removed, and the residue was coevaporated with toluene (2 × 25 mL) to remove traces of pyridine. The 1,3-diacetylated sugar was dissolved in dichloromethane (50 mL), and 30% hydrogen bromide in acetic acid (5 mL) was added. The mixture was maintained at room temperature overnight before the solvents were removed; the residue was coevaporated with toluene (2 × 50 mL) to give **7b** (5.48 g, 13.5 mmol, 99%). Product, >95% α anomer: ^1H NMR ($CDCl_3$) δ 8.27 (A_2B_2, 4 H, aromatics), 6.55 (d, 1 H, H1, $J_{1,F}$ = 12.3 Hz), 5.47 (d, 1 H, H2, $J_{2,F}$ = 50.1 Hz), 5.24 (dd, 1 H, H3, $J_{3,F}$ = 23.0 Hz, $J_{3,4}$ = 4.2 Hz), 4.84 (dd, 1 H, H5, $J_{5,5'}$ = 12.2 Hz, $J_{5,4}$ = 2.1 Hz), 4.69 (dd, 1 H, H5′, $J_{5',5}$ = 12.2 Hz, $J_{5',4}$ = 4.0 Hz), 4.65 (m, 1 H, H4) and 2.22 (s, 3 H, acetyl).

2′-Fluoro-2′-deoxy-Substituted Nicotinamide Arabinofuranoside Bromide 8b. **7b** (5.48 g, 13.5 mmol) was treated following the procedure described for **7a** to yield **8b** (3.28 g, 9.7 mmol, 72%). TLC (n-BuOH:H_2O:AcOH = 5:3:2) showed one spot (R_f 0.51) that gave a positive acetone/ammonia test:[26] ^1H NMR (β-**8b**; CD_3OD) δ 9.80 (s, 1 H, N2), 9.47 (d, 1 H, N6, J = 6 Hz), 9.07 (d, 1 H, N4, J = 8 Hz), 8.32 (dd, 1 H, N5, $J_{4'-5'}$ = 8 Hz, $J_{5'-6'}$ = 6 Hz), 6.72 (dd, 1 H, 1′, $J_{1'-2'}$ = 5 Hz, $J_{1'-F}$ = 8.1 Hz), 5.50 (dt, 1 H, 2′, $J_{2'-3'}$ = 5 Hz, $J_{2'-F}$ = 51.9 Hz), 4.52 (dt, 1 H, 3′, $J_{3'-4'}$ = 5 Hz, $J_{3'-F}$ = 18.5 Hz), 4.23 (m, 1 H, 4′, $J_{3'-4'}$ = 5 Hz, $J_{4'-5'}$ = 2.6 Hz, $J_{4-5''}$ = 3.7 Hz), 4.03 (dd, 1 H, 5′, $J_{4'-5'}$ = 2.6 Hz, $J_{5'-5''}$ = 12.5 Hz), 3.90 (dd, 1 H, 5″, $J_{4'-5''}$ = 3.7 Hz, $J_{5'-5''}$ = 12.5 Hz).

2′-Fluoro-2′-deoxy-Substituted Nicotinamide Arabinomononucleotide 9b. **8b** (1.80 g, 7.2 mmol) was phosphorylated following the procedure described for **8a** to give **9b** (1.95 g, 5.8 mmol, 80%): ^1H NMR (β-**9b**; D_2O) δ 9.48 (s, 1 H, N2), 9.36 (d, 1 H, N6), 9.02 (d, 1 H, N4), 8.33 (t, 1 H, N5), 6.77 (dd, 1 H, 1′), 5.59 (dt, 1 H, 2′), 4.70 (dt, 1 H, 3′), 4.45 (m, 1 H, 4′), 4.44 (m, 1 H, 5′) 4.31 (m, 1 H, 5″).

2′-Fluoro-2′-deoxy-Substituted Nicotinamide Arabinoside Adenine Dinucleotide 10b. **9b** (0.30 g, 0.89 mmol) was coupled with AMP following the procedure for the synthesis of the azido analogue. Purification of the crude product was achieved using a Dowex AG 1X-8 formate column and a gradient of 0–0.5 M formic acid. **10b** (133 mg, 0.20 mmol, 22%) eluted with ca. 0.2 M formic acid.

Separation of the α and β anomers of **10b** was carried out on a Whatman SAX M9/25 column using ammonium phosphate buffer (pH 3.5, 7 mM). The workup procedure is as reported for **10a** giving the β anomer of **10b** (115 mg) and the α anomer of **10b** (11 mg). Mass spectra of both anomers are identical with the MH^+ ion at m/z 666. Each anomer was shown to be >99% pure by ^1H NMR and HPLC. The molar absorption coefficient for the cyanide adduct is 5.70 × 10^3 M^{-1} cm^{-1} at 323 nm (λ_{max}): ^1H NMR (β-**10b**; D_2O, pD 8.0) δ 9.32 (s, 1 H, N2), 9.19 (d, 1 H, N6, J = 6.0 Hz), 8.87 (d, 1 H, N4, J = 8.0 Hz), 8.41 (s, 1 H, A8), 8.22 (t, 1 H, N5, J = 8.0 Hz), 8.16 (s, 1 H, A2), 6.62 (dd, 1 H, N1′, J_{HH} = 4.78 Hz, J_{HF} = 9.28 H_2O, 6.01 (d, 1 H, A1′, J = 5.88 Hz), 5.53 (dt, 1 H, N2′, J_{HH} = 4.75, J_{HF} = 51.3 Hz), 4.72 (t, 1 H, A2′, J = 5.5 Hz), 4.62 (dt, 1 H, N3′, J_{HH} = 5.0 Hz, J_{HF} = 18.0 Hz), 4.50 (t, 1 H, A3′, J = 4.82 Hz), 4.44–4.21 (m, 6 H); (α-**10b**; D_2O, pD 8.0) 9.35 (s, 1 H, N2), 9.14 (d, 1 H, N6), 8.89 (d, 1 H, N4), 8.48 (s, 1 H, A8), 8.18 (m, 2 H, A2 and N5), 6.67 (d, 1 H, N1′), 6.04 (d, 1 H, A1′), 5.42–5.31 (m, 1 H, N2′), 4.65 (t, 1 H), 4.54 (t, 1 H), 4.41 (t, 1 H), 4.27–4.17 (m, 4 H), 3.56–3.42 (m, 2 H).

Acknowledgment. This research was supported in part by National Institutes of Health Grant GM-22982. The UCSF Magnetic Resonance Laboratory was partially funded by grants from the National Science Foundation (DMB 8406826) and the National Institutes of Health (RR-01668). The UCSF Mass Spectrometry Facility (A. L. Burlingame, Director) was supported by the National Institutes of Health Division of Research Resources Grant P41 RR-01614. We thank Neil Buckley for comments on the manuscript.

Supplementary Material Available: ^1H NMR spectra of compounds α-**8a**, β-**8a**, β-**8b**, α-**8c**, β-**8c**, **9a**, α-**9b**, β-**9b**, α-**10a**, β-**10a**, α-**10b**, β-**10b**, β-**10c**; liquid secondary-ion mass spectra of compounds α-**10a**, β-**10a**, α-**10b**, β-**10b**, β-**10c**; and summary of NOE results for β-**8b**, α-**8b**, β-**8c**, α-**8c** (20 pages). Ordering information is given on any current masthead page.