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Synthesis of Pyridine, Pyrimidine and Pyridinone *C*-Nucleoside Phosphoramidites for Probing Cytosine Function in RNA

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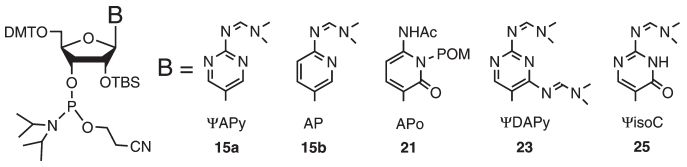

In the structures of the HDV ribozyme a cytosine nucleobase resides at the active site poised to participate directly in catalysis. Defining the functional role of the nucleobase requires nucleoside analogues that perturb the functional groups in a strategic manner. Herein, we have developed efficient methods for the synthesis of five *C*-nucleoside phosphoramidite derivatives that, when used in combination, provide strategies for probing the potential functional role of cytosine's keto group and imino nitrogen. Phosphoramidites **15a** and **15b** were synthesized in 11 steps starting from 2-amino-5-bromopyrimidine (**1a**) and 2-amino-5-bromopyridine (**1b**), respectively, with overall yields of 10.8% and 6.6%, respectively. Phosphoramidite **21** was prepared from intermediate **11b** in seven steps with an overall yield of 33.7%. Phosphoramidites **23** and **25** were prepared from 2,4-diamino-5-(β-D-ribofuranosyl)-1,3-pyrimidine (**22**) and pseudoisocytidine (**24**), respectively, with an overall yield of 15.9% (six steps) and 37.9% (four steps), respectively. These phosphoramidites were incorporated into oligonucleotides by solid-phase synthesis.

Introduction

C-Nucleosides are an important class of compounds distinguished by a carbon−carbon glycosidic bond.[1] Many *C*-nucleosides possess antiviral and antineoplastic activities and are of interest in medicinal chemistry.[2] In addition, the *C*-glycoside linkage expands the range of substituent patterns available to the aglycon heterocycle, engendering nucleosides with arrangements of hydrogen-bond donors and acceptors that are distinct from those of the natural nucleosides. For example, a carbon−carbon glycosidic linkage enables access to a cytidine analogue bearing a donor−donor−acceptor hydrogen-bonding pattern.[3] This hydrogen-bonding pattern facilitates the formation of a DNA triplex by circumventing the need for cytidine protonation. As described below and elsewhere, *C*-glycoside linkages also underlie novel strategies to investigate RNA structure and function.

In the HDV ribozyme, the nucleobase of a cytosine residue participates directly in catalysis as a general acid.[4] One mechanistic model that combines data from crystallography,[5] Raman spectroscopy,[6] and chemogenetic suppression[4c] proposes that the cytosine engages in multiple interactions during catalysis (Figure 1). The imino nitrogen bears a proton that transfers to the leaving group during the reaction.[4c] The N4 exocyclic amino group participates in a network of hydrogen bonds in the ribozyme,[7] and the exocyclic O2-keto group interacts with a magnesium-bound water molecule,[5] which may protonate (or donate a hydrogen bond to) a nonbridging oxygen at the scissile phosphate

(1) Miller, P. S. Antisense/Antigene Oligonucleotides. In *Bioorganic Chemistry-Nucleic Acids*; Hecht, S. M., Ed.; Oxford University Press: New York, Oxford, 1996; pp 347−374.

(2) (a) Franchetti, P.; Cappellacci, L.; Griffantini, M.; Barzi, A.; Nocentini, G.; Yang, H. Y.; O'Connor, A.; Jayaram, H. N.; Carrell, C.; Goldstein, B. M. *J. Med. Chem.* **1995**, *38*, 3829–3837. (b) Walker, J. A.; Liu, W.; Wise, D. S.; Drach, J. C.; Townsend, L. B. *J. Med. Chem.* **1998**, *41*, 1236–1241. (c) Guntaka, R. V.; Varma, B. R.; Weber, K. T. *Int. J. Biochem. Cell Biol.* **2003**, *35*, 22–31.

(3) (a) Hutter, D.; Benner, S. A. *J. Org. Chem.* **2003**, *68*, 9839–9842. (b) von Krosigk, U.; Benner, S. A. *J. Am. Chem. Soc.* **1995**, *117*, 5361–5362.

(4) (a) Nakano, S.-I.; Chadalavada, D. M.; Bevilacqua, P. C. *Science* **2000**, *287*, 1493–1497. (b) Shih, I. H.; Been, M. D. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1489–1494. (c) Das, S. R.; Piccirilli, J. A. *Nat. Chem. Biol.* **2005**, *1*, 45–52. (d) Cerrone-Szakal, A. L.; Siegfried, N. A.; Bevilacqua, P. C. *J. Am. Chem. Soc.* **2008**, *130*, 14504–14520.

(5) Chen, J. H.; Gong, B.; Bevilacqua, P. C.; Carey, P. R.; Golden, B. L. *Biochemistry* **2009**, *48*, 1498–1507.

(6) Ferré-D'Amaré, A. R.; Zhou, K.; Doudna, J. A. *Nature* **1998**, *395*, 567–574.

(7) Das, S. R.; Koo, S. C.; Piccirilli, J. A. Unpublished results.

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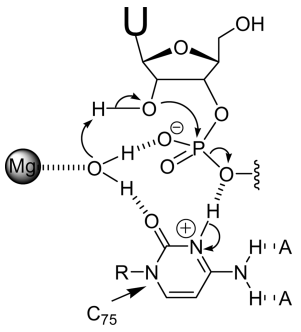


FIGURE 1. Proposed model for interactions of C75 in the HDV ribozyme. The N3 proton of C75 protonates the 5′-oxygen of the scissile phosphate. The O2 keto oxygen interacts with an outersphere water ligand of a divalent metal ion (Mg^{2+}, gray sphere) near the active site. That water ligand has been proposed to act as a general base that activates the 2′-hydroxyl nucleophile of the reaction. The N4 amino group of C75 interacts with other functional groups (A) in the ribozyme.

or activate the 2′-hydroxyl group for nucleophilic attack. Synthetic access to the cytidine analogues in Figure 2 would engender experimental strategies to probe nucleobase interactions. We describe here the synthesis of five phosphoramidite derivatives for *C*-nucleoside analogues of cytidine (Figure 2). When used in combination, these derivatives enable strategies for delineating the importance of the keto and imino groups of cytosine residues within RNA.

Strategies To Test Cytidine Nucleobase Interactions. As the active-site cytidine purportedly uses all of its substituents to engage in catalytic interactions, testing specific interactions in this model requires design and synthesis of multiple, carefully designed nucleoside analogues that, ideally, modify one functional group while introducing minimal changes elsewhere in the molecule. Synthetic access to the cytidine analogues in Figure 2 would engender experimental tests for nucleobase interactions in the manner described below.

(**a**)**. Testing the Role of the Keto Group.** Ribofuranoside derivatives of aminopyridine (AP), aminopyrimidine (ΨAPy), and diaminopyrimidine (ΨDAPy) provide a strategy to probe the functional importance of the O2 keto group at specific cytosine residues within RNA. They retain cytosine's $H_2N-C{=}N$ group and either remove the keto oxygen (AP and ΨAPy) or replace it with an amino group (ΨDAPy). To accommodate these modifications while remaining electrostatically neutral, the heterocycle must be linked to ribose through a carbon−carbon bond. The new glycosidic linkage in ΨAPy and ΨDAPy places N1 in the position equivalent to C5 of cytidine. Comparison of effects from the ΨAPy versus AP modification provides an independent control for the presence of this nitrogen.

(**b**)**. Testing the Role of Cytidine's Imino Nitrogen.** Some RNA species require protonation of cytidine's nucleobase (CH+) for function. These CH+ residues may serve a structural role, allowing formation of specific RNA conformations through tertiary interactions, or a catalytic role, transferring its N3 proton via general acid catalysis, as proposed for the HDV ribozyme. As shown previously, the nucleobase of pseudoisocytidine (ΨisoC) (in the N^3H tautomeric state;[9] Figure 3) mimics CH+ residues without the need for protonation.[10] This property provides a basis for biochemical detection of CH+ residues within RNA: at CH+ residues that make important contributions to RNA function, ΨisoC substitution would be expected to enhance function over the wild-type RNA at higher pH compared to lower pH due to a greater fraction of the wild type ribozyme lacking the N3 proton at higher pH relative to the variant ribozyme. Strobel and colleagues obtained proof-of-concept for this biochemical signature in the context of a variant of the *Tetrahymena* group I intron ribozyme.[11]

The APo derivative complements ΨisoC in this assay as it mimics CH+ and circumvents potential effects from the N1 imino nitrogen,[9] including formation of the N^1H tautomer and the capacity for protonation (Figure 3). The biochemical signature from ΨisoC substitution becomes less predictable when CH+ serves a role in general acid catalysis, transferring the imino proton to the leaving group during the reaction. The N^3H tautomer in the neutral form has little capacity for proton transfer due to its weak acidity; however, protonation of the nucleobase to form ΨisoCH+ could provide suitable general acid catalysis. Consequently, the biochemical signature for ΨisoC substitution at C residues that act as general acids will depend upon the pH-dependent contribution of ΨisoCH+ to the reaction. In contrast, APo substitution would be expected to impair catalysis severely at all pH values as the nucleobase has little capacity for proton transfer and has no additional imino nitrogens where protonation could enhance this capacity.

Here, we present the synthesis of the phosphoramidite derivatives for *C*-nucleoside analogues (Figure 2) to enable functional tests for contributions from the keto and imino groups at specific cytosine residues within RNA.

Results and Discussion

Synthetic routes for *C*-nucleosides are usually classified into two major approaches: (1) introduction of a functional group at the anomeric position of a sugar derivative followed by the construction of a heterocyclic base[12] and (2) direct attachment of a preformed aglycone unit to an appropriate carbohydrate moiety followed by dehydroxylation.[13] We chose the second

(8) (a) Chen, D. L.; McLaughlin, L. W. *J. Org. Chem.* **2000**, *65*, 7468–7474. (b) Miller, J. M.; Blackburn, A. C.; Shi, Y.; Melzak, A. J.; Ando, H. Y. *Electrophoresis* **2002**, *23*, 2833–2841. (c) Kan, L.-S.; Lin, W.-C.; Yadav, R. D.; Shih, J. H.; Chao, I. *Nucleosides Nucleotides* **1999**, *18*, 1091–1093. (d) Barlin, G. B.; Pfleiderer, W. *J. Chem. Soc. B* **1971**, 1425–1432. (e) Albert, A.; Goldacre, R.; Phillips, J. *J. Chem. Soc.* **1948**, 2240–2249.

(9) Cytidine numbering used.

(10) In solution about 4% of psuedoisocytidine exists as the N1H tautomer, while 96% exits as the N3H tautomer, corresponding to a free energy difference of 2 kcal/mol (see ref 8c).

(11) Oyelere, A. K.; Kardon, J. R.; Strobel, S. A. *Biochemistry* **2002**, *41*, 3667–3675.

(12) (a) Chu, C. K.; Wempen, I.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1976**, *41*, 2793–2797. (b) Chu, C. K.; Reichman, U.; Watanabe, K. A.; Fox, J. J. *J. Org. Chem.* **1977**, *42*, 711–714. (c) Sato, T.; Hayakawa, Y.; Noyori, R. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2515–2525. (d) Townsend, L. B. *Chemistry of Nucleosides and Nucleotides*; Plenum Press: New York, 1994; pp 421−535. (e) Popsavin, M.; Torovic, L.; Svircev, M.; Kojic, V.; Bogdanovic, G.; Popsavin, V. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2773–2776. (f) Kamath, V. P.; Zhang, J.; Morris, P. E.; Babu, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2662–2665.

(13) (a) Watanabe, K. A. In *The Chemistry of Nucleosides and Nucleotides*; Towsend, L. B., Ed.; Plenum: New York, 1994; Vol. 3, pp 421−535. (b) Zhou, J.; Yang, M.; Schneller, S. W. *Tetrahedron Lett.* **2004**, *45*, 8233–8234. (c) Wu, Q.; Simons, C. *Synthesis* **2004**, 1533–1553. and references cited therein. (d) Benner, S. A.; Wellington, K. W. *Nucleosides, Nucleotides Nucl. Acids* **2006**, *25*, 1309–1333. (e) Urban, M.; Pohl, R.; Klepetarova, B.; Hocek, M. *J. Org. Chem.* **2006**, *71*, 7322–7328. (f) Sun, Z.; Ahmed, S.; McLaughlin, L. W. *J. Org. Chem.* **2006**, *71*, 2922–2925. (g) Joubert, N.; Pohl, R.; Klepetarova, B.; Hocek, M. *J. Org. Chem.* **2007**, *72*, 6797–6805. (h) Peyron, C.; Navarre, J. M.; Dubreuil, D.; Vierling, P.; Benhid, R. *Tetrahedron Lett.* **2008**, *49*, 6171–6174.

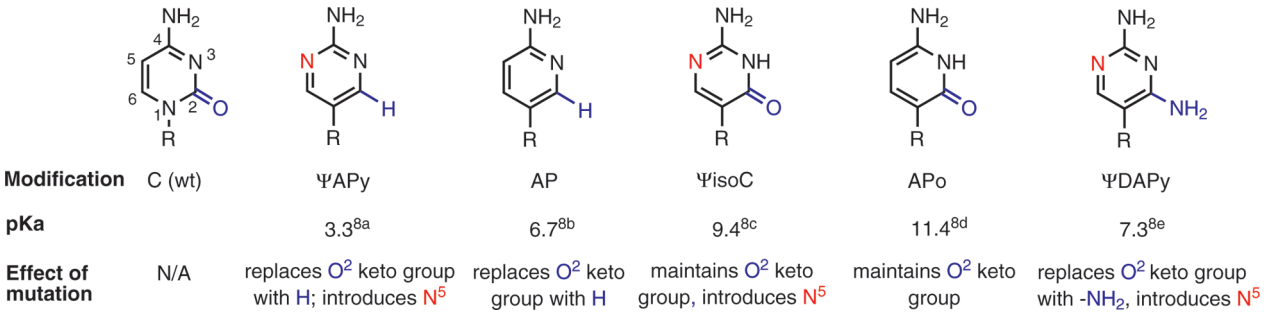


FIGURE 2. Nucleotide analogues for testing the role of cytidine's keto and imino groups. Atoms are numbered relative to cytidine.

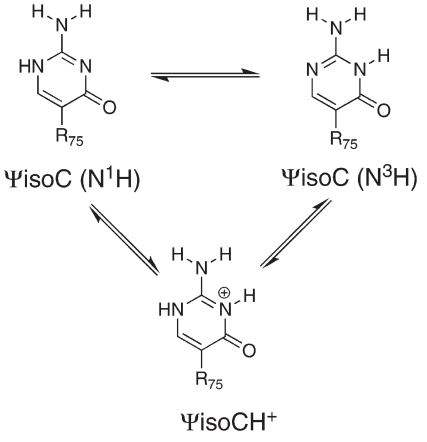


FIGURE 3. Tautomeric and protonated forms of pseudoisocytidine. Cytidine numbering used.

approach because of its flexibility and potential for higher yields. Previously, several 2′-deoxy-*C*-nucleoside phosphoramidites that bear a pyrimidine,[8a] pyridine,[13e,f,14] or a pyridinone[3a,15] ring as nucleobase have been synthesized. To the best of our knowledge, the synthesis of the corresponding *C*-ribonucleoside phosphoramidites has not been reported.

Synthesis of 2-Amino-5-(β-D-ribofuranosyl)pyridine, 2-Amino-5-(β-D-ribofuranosyl)-1,3-pyrimidine, and Their Phosphoramidites. The synthesis of the *C*-nucleosides **8a** and **8b** (Scheme 1) started from the commercially available 2-amino-5-bromopyrimidine (**1a**) or 2-amino-5-bromopyridine (**1b**) and the readily obtainable 5-[(*tert*-butyl)diphenylsilyl]-2,3-isopropylidene-D-ribono-l,4-lactone (**3**).[16] Protection of the amino group in **1a** or **1b** with benzyl bromide gave good yields of compounds **2a** and **2b** (**2a**: 92%; **2b**: 96%), which are suitable for coupling with the sugar component. The coupling essentially followed the syntheses of *C*-glycosides reported by Kraus et al.[17] Bromide−lithium exchange of **2** with *n*-BuLi at −78 °C and in situ reaction with 1,4-lactone **3** furnished hemiacetals **4a** and **4b**, which were subsequently dehydroxylated with Et_3SiH in the presence of a strong Lewis acid ($BF_3 \cdot Et_2O$) from −78 °C to room temperature. We found that these reaction conditions were effective in removing the 2′,3′-*O*-isopropylidine group, affording the corresponding *C*-nucleosides **5a** and **5b** in yields of 43% and 29%, respectively. We observed only β-configured products as confirmed by ^1H NMR−NOE spectroscopy, indicating that the glycosylation reaction proceeded stereoselectively. Desilylation of **5a** and **5b** with $Bu_4NF \cdot 3H_2O$ (TBAF) in THF gave the corresponding *N*,*N*-dibenzyl-protected *C*-nucleosides **6a** and **6b**. NOESY analysis further confirmed the β-anomeric configuration (Figure 4).

Relevant ^1H NMR spectroscopic data for compounds **6a** and **6b** are listed in Table 1 with through-space proton−proton interactions based on NOESY spectra as shown in Figure 4. The assignments in Table 1 are based primarily on two-dimensional $^1H-^1H$ COSY spectra. From the NOESY spectrum of the β-anomer **6a** and **6b**, we observe that H-4 and H-5′ interact strongly with H-2′, while H-2′ interacts strongly with H-3′. Additionally, H-1′ interacts strongly with H-6 and H-4′ but does not interact with H-2′ and H-3′. These NOESY spectroscopic data support the β-anomeric configurational assignments.

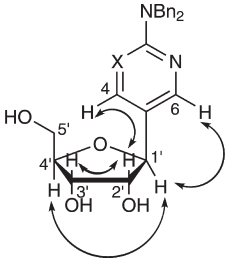


FIGURE 4. Through-space proton−proton interactions indicated by the NOESY spectra of **6a** (X = N) and **6b** (X = CH).

TABLE 1. ^1H NMR Spectra (CD_3OD) of *C*-Nucleoside 6a and 6b

compd	H-1′	H-2′	H-3′	H-4′	H-5′	H-4
6a	4.61 d	3.95 m	4.10 m	3.97 m	3.75 dd, 3.69 dd	8.44 s
6b	4.51 d	3.79 m	3.96 m	3.85 m	3.62 dd, 3.56 dd	6.41 s

To prepare the corresponding parent nucleosides, **6a** and **6b** were first acylated by treatment with acetic anhydride in acetonitrile to form **7a** and **7b**. Attempts to debenzylate **7a** and **7b** using 10% Pd−C/H_2, 20% Pd(OH)$_2$−C/H_2, or BCl_3 gave unsatisfactory yields. Ammonium cerium(IV) nitrate (CAN)[18] in wet acetonitrile at room temperature for 24 h removed only one benzyl group. However, when the temperature was increased to 50 °C, CAN in wet acetonitrile removed

(14) (a) Hsieh, H. P.; McLaughlin, L. W. *J. Org. Chem.* **1995**, *60*, 5356–5359. (b) Hildbrand, S.; Leumann, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1968–1970. (c) Hildbrand, S.; Blaser, A.; Parel, S. P.; Leumann, C. J. *J. Am. Chem. Soc.* **1997**, *119*, 5499–5511.

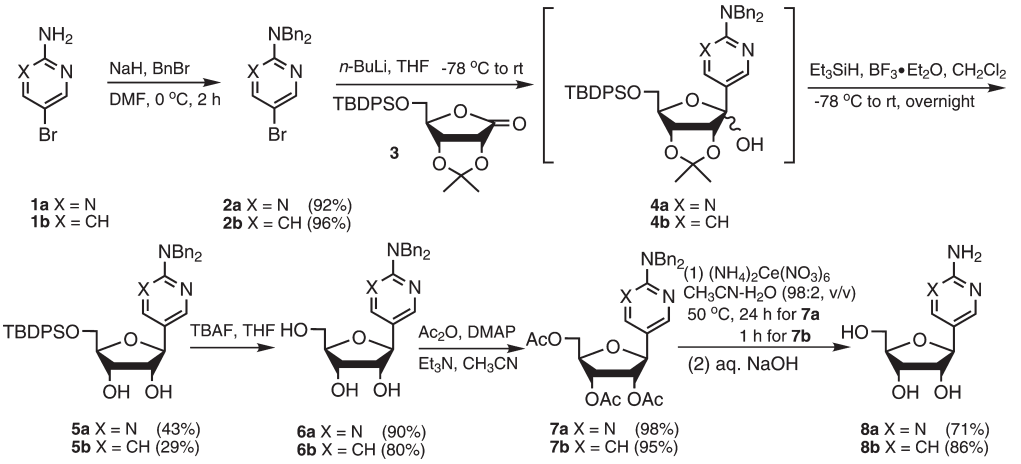
(15) Yang, Z.; Hutter, D.; Sheng, P.; Sismour, A. M.; Benner, S. A. *Nucleic Acids Res.* **2006**, *34*, 6095–6101.

(16) Piccirilli, J. A.; Krauch, T.; MacPherson, L. J.; Benner, S. A. *Helv. Chim. Acta* **1991**, *74*, 397–406.

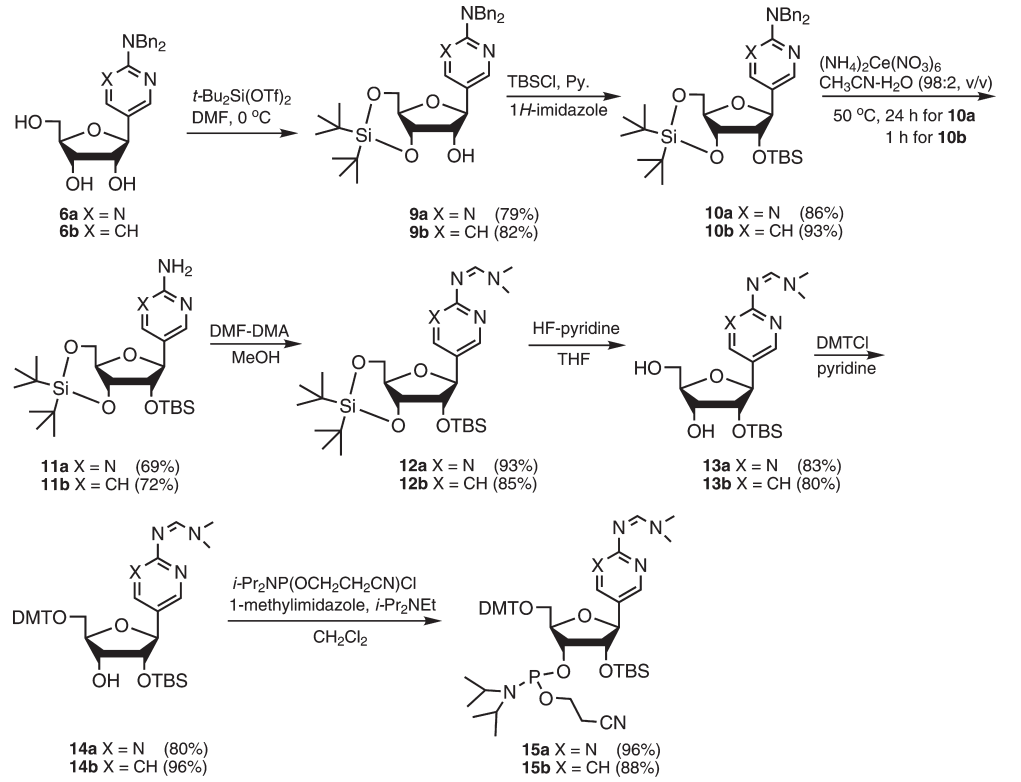
(17) Kraus, G. A.; Molina, M. T. *J. Org. Chem.* **1988**, *53*, 752–753.

(18) Reese, C. B.; Wu, Q. *Org. Biomol. Chem.* **2003**, *1*, 3160–3172.

SCHEME 1. Synthesis of 2-Amino-5-(β-D-ribofuranosyl)pyridine and 2-Amino-5-(β-D-ribofuranosyl)-1,3-pyrimidine



SCHEME 2. Synthesis of 2-Amino-5-(β-D-ribofuranosyl)pyridine and 2-Amino-5-(β-D-ribofuranosyl)-1,3-pyrimidine Phosphoramidites



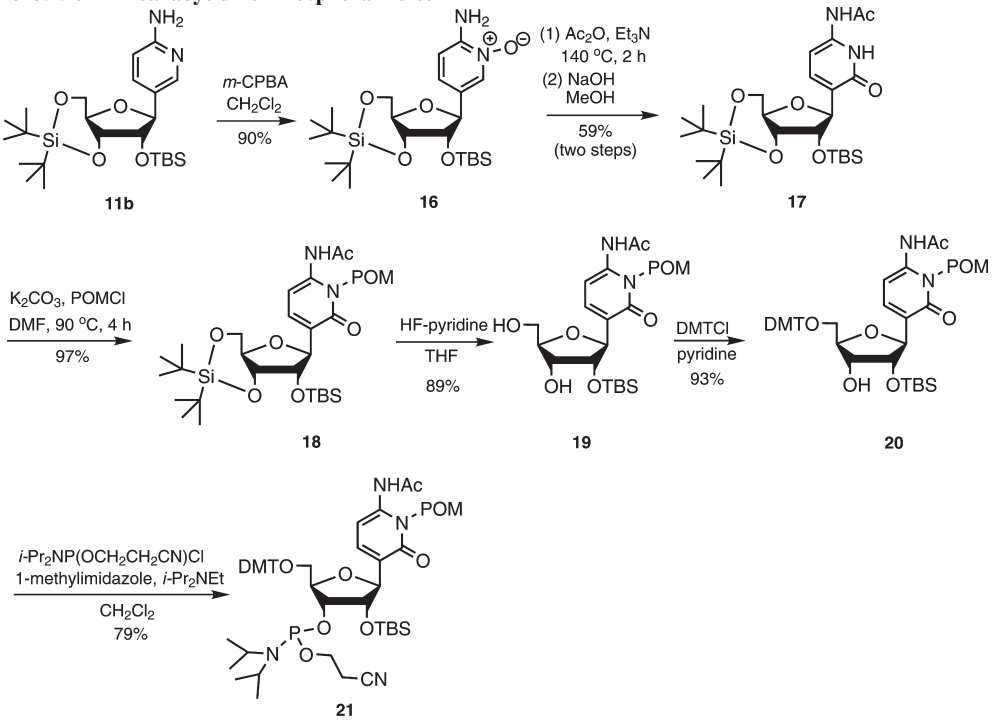
both benzyl groups from **7a** within 24 h and from **7b** within 1 h to give *C*-nucleosides 2-amino-5-(β-D-ribofuranosyl)-1,3-pyrimidine (**8a**, 71% yield) and 2-amino-5-(β-D-ribofuranosyl)-pyridine (**8b**, 86% yield), respectively.

Next, we prepared suitably protected phosphoramidites of **8a** and **8b** starting from synthetic intermediates **6a** and **6b** (Scheme 2). To allow regioselective protection of the 2′-hydroxyl group as the 2′-*O*-TBS ether, **6a** and **6b** were reacted with di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (*t*-$Bu_2Si(OTf)_2$) in DMF to give the corresponding 3′,5′-*O*-(di-*tert*-butylsilanediyl) derivatives **9a** (79% yield) and **9b** (82% yield). Subsequent reaction of **9a** and **9b** with *tert*-butyldimethylsilyl chloride (TBSCl) gave silyl ethers **10a** (86% yield) and **10b** (93% yield), respectively. Debenzylation of **10a** and **10b** with CAN in wet acetonitrile at 50 °C gave **11a** (69% yield) and **11b** (72% yield), respectively. Protection of the exocyclic amino group of **11a** and **11b** with *N*,*N*-dimethylformamide dimethyl acetal gave compounds **12a** (93% yield) and **12b** (85% yield). Pyridinium poly(hydrogen fluoride) removed the di-*tert*-butylsilylene group efficiently to afford **13a** and **13b** in yields of 83% and 80%, respectively. No migration of the 2′-*O*-TBS group to the 3′-oxygen was observed under the reaction conditions.[19] Using standard procedures, **13a** and **13b** were converted efficiently via **14a** and **14b** to the corresponding phosphoramidites **15a** and **15b**.

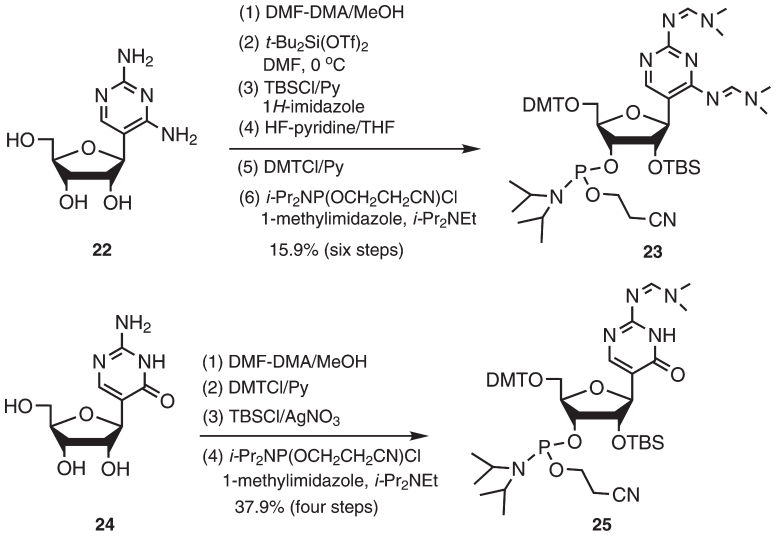
Synthesis of 1-Deazacytidine Phosphoramidite. In 2002, Sollogoub et al. reported the synthesis of 1-deazacytidine

(19) Trost, B. M.; Caldwell, C. G. *Tetrahedron Lett.* **1981**, *22*, 4999–5002.

SCHEME 3. Synthesis of 1-Deazacytidine Phosphoramidite



SCHEME 4. Synthesis of 2,4-Diamino-5-(β-D-ribofuranosyl)-1,3-pyrimidine and Pseudoisocytidine Phosphoramidites



through the coupling of a PMB (*p*-methoxybenzyl)-protected 2-amino-5-bromopyridine with perbenzylated ribonolactone followed by transformation of the pyridine ring to the desired substituted pyridinone.[20] However, synthesis of the corresponding phosphoramidite has not been reported. Starting from **11b**, we developed an efficient synthetic route to synthesize 1-deazacytidine phosphoramidite as shown in Scheme 3. Compound **11b** was oxidized to the corresponding *N*-oxide **16** with *m*-CPBA (*m*-chloroperoxybenzoic acid) in 90% yield.[21] Subsequent refluxing in acetic anhydride for 2 h accomplished the Katada rearrangement.[22] Treatment with methanolic sodium hydroxide gave **17** in 59% yield (two steps). To prepare the 1-deazacytidine phosphoramidite **21**, the pivaloyloxymethyl (POM) was used as a *N*-protecting group for the pyridinone ribonucleoside.[23] Without protection, the synthesis of the phosphoramidite was unsuccessful. Treatment of **17** with a mixture of chloromethyl

(20) Sollogoub, M.; Fox, K. R.; Powersa, V. E. C.; Brown, T. *Tetrahedron Lett*. **2002**, *43*, 3121–3123.

(21) Sato, N.; Miwa, N.; Suzuki, H.; Sakakibara, T. *J. Heterocycl. Chem*. **1994**, *31*, 1229–1233.

(22) Katada, M. *J. Pharm. Soc. Jpn*. **1947**, *67*, 51–52.

(23) (a) Li, S.; Nair, M. G.; Edwards, D. M.; Kisliuk, R. L.; Gaumont, Y.; Dev, I. K.; Duch, D. S.; Humphreys, J.; Smith, G. K.; Ferone, R. *J. Med. Chem*. **1991**, *34*, 2746–54. (b) Araki, L.; Harusawa, S.; Yamaguchi, M.; Yonezawa, S.; Taniguchi, N.; Lilley, D.M. J.; Zhao, Z.; Kurihara, T. *Tetrahedron Lett*. **2004**, *45*, 2657–2661. (c) Araki, L.; Harusawa, S.; Yamaguchi, M.; Yonezawa, S.; Taniguchi, N.; Lilley, D.M. J.; Zhao, Z.; Kurihara, T. *Tetrahedron* **2005**, *61*, 11976–11985.

TABLE 2. Mass of 3′-ACCGAGAGGGAAUC*GGU-5′ and 3′-AUC*GG-5′

	3′-ACCGAGAGGGAAUC*GGU-5′				3′-AUC*GG-5′		
C*	C (wt)	AP	APO	ΨisoC	C (wt)	ΨAPy	ΨDAPy
calcd mass	5525.8	5508.8	5524.8	5526.8	1568.3	1552.3	1567.3
MALDI mass	5525.0[a]	5509.5[a]	5525.6[a]	5526.5[a]	1566.5[b]	1550.7[b]	1566.0[b]

[a]Positive ($M + H^+$). [b]Negative ($M - H^+$).

pivaloate and K_2CO_3 in DMF produced the 1-*N*-POM derivative **18** in 97% yield. The di-*tert*-butylsilylene group of **18** was then removed by treatment with pyridinium poly(hydrogen fluoride) to afford **19**, which was subsequently converted via **20** to the corresponding POM-protected 1-deazacytidine phosphoramidite **21** using standard procedures.

Synthesis of 2,4-Diamino-5-(β-D-ribofuranosyl)-1,3-pyrimidine and Pseudoisocytidine Phosphoramidites. We prepared 2,4-diamino-5-(β-D-ribofuranosyl)-1,3-pyrimidine[12b,24] (**22**) in eight steps from D-ribose and converted it to phosphoramidite **23** (Scheme 4) in six steps with 15.9% overall yield. We also prepared phosphoramidite **25** from commercially available pseudoisocytidine (**24**) in four steps with 37.9% overall yield.

We subsequently incorporated these five new *C*-nucleoside phosphoramidites into the RNA oligonucleotides as described before.[25] The C-nucleoside phosphoramidites (~100 mg) were dissolved in dry acetonitrile (1 mL) and coupled twice during the appropriate synthesis cycle; sequences were 3′-ACCGAGAGGGAAUC*GGU-5′ [C*: **15b** (AP), **21** (APo), **25** (ψDAPy)] and 3′-AUC*GG-5′ [C*: **15a** (ΨAPy), **23** (ΨisoC)]. Based on trityl yield data, coupling efficiencies throughout oligonucleotide synthesis were excellent (data not shown). Following standard oligonucleotide deprotection conditions [3:1 NH_4OH/EtOH, 55 °C, 2−4 h (for **21** NH_3/MeOH in sealed tube, 55 °C, 24 h); TEA·3HF/NMP, 65 °C, 1.5 h] and purification, MALDI mass spectra were obtained for 3′-ACCGAGAGGGAAUC*GGU-5′ [C*: **15b** (AP), **21** (APo), **25** (ΨDAPy)] and 3′-AUC*GG-5′ [C*: **15a** (ΨAPy), **23** (ΨisoC)]. Each gave the peaks expected from the calculated molecular exact mass (Table 2). The structures of modified oligonucleotides were also confirmed by enzymatic digestion according to established protocol.[26]

In summary, we have described efficient methods for the synthesis of phosphoramidites of five cytidine analogues. The phosphoramidites of 2-amino-5-(β-D-ribofuranosyl)pyrimidine and 2-amino-5-(β-D-ribofuranosyl)pyridine were prepared with 10.8% and 6.6% overall yield, respectively, from lactone **3** and the corresponding 2-amino-5-bromopyrimidine (**1a**) or 2-amino-5-bromopyridine (**1b**). 1-Deazacytidine phosphoramidite **21** was prepared from **11b** via Katada rearrangement of *N*-oxide **16** with an overall yield of 33.7%. The other two *C*-nucleoside phosphoramidites **23** and **25** were prepared from their corresponding nucleoside derivatives, 2,4-diamino-5-(β-D-ribofuranosyl)-1,3-pyrimidine (**22**) and pseudoisocytidine, with overall yields of 37.9% and 15.9%, respectively. Using solid-phase methods, we achieved efficient incorporation of these five new *C*-nucleoside phosphoramidites into RNA oligonucleotides. This work expands the spectrum of nucleoside analogues with which to investigate the structural and energetic contribution of functionally important cytidine residues in RNA.

Experimental Section

5-Bromo-2-dibenzylaminopyrimidine (2a). To a solution of 2-amino-5-bromopyrimidine (**1a**) (2.12 g, 12.2 mmol) in dry DMF (40 mL) under argon was added NaH (877 mg, 36.6 mmol), and the mixture was stirred at 0 °C. After the hydrogen gas generation had ceased, benzyl bromide (5.21 g, 30.5 mmol) was added, and the mixture was stirred at 0 °C for 2 h. The reaction was quenched with MeOH (10 mL); the solvent was removed, and the residue was partitioned between CH_2Cl_2 (60 mL) and H_2O (60 mL). The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After the solvent was removed, the residue was purified by silica gel chromatography, eluting with 0−3% ethyl acetate in hexane, to give the product **2a** as a white foam: 3.98 g (92% yield); ^{1}H NMR (CD_3Cl) δ 8.33 (s, 2H), 7.22−7.32 (m, 12 H), 4.82 (s, 4H); ^{13}C NMR (CD_3Cl): δ 160.7, 158.2, 137.9, 128.7, 127.6, 127.3, 106.2, 49.5; HRMS (FAB^+) m/z calcd for $C_{18}H_{17}BrN_3$ [$M + H^+$] 354.0606, found 354.0614.

5-Bromo-2-dibenzylaminopyridine (2b). Compound **2b** was prepared from 2-amino-5-bromopyridine (**1b**) (8.65 g, 50 mmol), NaH (3.6 g, 150 mmol), and benzyl bromide (21.4 g, 125 mmol) as described for **2a**. Silica gel chromatography (0−3% ethyl acetate in hexane) of the residue yielded 16.96 g (96% yield) of **2b** as a white foam: ^{1}H NMR (CD_3Cl) δ 8.20 (dd, 1H, J = 0.4, 2.4 Hz), 7.41 (dd, 1 H, J = 2.8, 9.2 Hz), 7.19−7.33 (m, 10 H), 6.34 (dd, 1 H, J = 0.4, 9.2 Hz), 4.75 (s, 4H); ^{13}C NMR (CD_3Cl) δ 157.3, 148.7, 139.8, 138.0, 128.8, 127.3, 127.1, 107.6, 106.8, 51.3; HRMS (FAB^+) m/z calcd for $C_{19}H_{18}BrN_2$ [$M + H^+$] 353.0653, found 353.0659.

5-[5′-*O*-(*tert*-Butyldiphenylsilyl)-β-D-ribofuranosyl]-2-dibenzylamino-1,3-pyrimidine (5a). To a solution of 5-bromo-2-dibenzylaminopyrimidine **2a** (1.417 g, 4.0 mmol) in dry THF (30 mL) was added *n*-butyllithium (3.0 mL, 1.6 M solution in hexane, 4.8 mmol) at −78 °C under argon, and the mixture was stirred for 1 h. A solution of lactone **3** (1.42 g, 3.3 mmol) in THF (10 mL) was added at −78 °C. The mixture was stirred for 2 h and then allowed to warm to room temperature over 3 h. The reaction was quenched with H_2O (40 mL), and the mixture was extracted with Et_2O (3 × 80 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The resulting yellow residue **4a** was dried and dissolved in dry CH_2Cl_2 (20 mL), Et_3SiH (1.60 mL, 10.0 mmol) was added at −78 °C, and the mixture was stirred for 10 min. $BF_3 \cdot Et_2O$ (1.27 mL, 10.0 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature overnight and quenched with satd $NaHCO_3$ (10 mL). The mixture was partitioned between Et_2O (60 mL) and H_2O (60 mL). The organic layer was washed with brine and dried over anhydrous Na_2SO_4. The sodium sulfate was filtered off, and the solvent was removed by evaporation under vacuum. The residue was purified by silica gel chromatography, eluting with 1% methanol in dichloromethane, to give the product **5a** as a white foam: 922 mg (43% yield); ^{1}H NMR (CD_3Cl) δ 8.37 (s, 2H), 7.65−7.68 (m, 4H), 7.34−7.42 (m, 6H), 7.20−7.29 (m, 10H), 4.85 (dd, 4H, J = 15.8, 20.5 Hz), 4.58 (d, 1H, J = 7.1 Hz), 4.21 (t, 1H, J = 4.3 Hz), 4.04 (m, 2H), 3.80 (d, 2H, J = 4.0 Hz), 1.06 (s, 9H); ^{13}C NMR

(24) Ohrui, H.; Jones, G. H.; Moffatt, J. G.; Maddox, M. L.; Christensen, A. T.; Byram, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 4602–4613.
(25) Sun, S.; Yoshida, A.; Piccirilli, J. A. *RNA* **1997**, *3*, 1352–1363.
(26) Andrus, A.; Kuimelis, R. G. Base composition analysis of nucleosides using HPLC. In *Current Protocols in Nucleic Acid Chemistry*; Beaucage, S. L., Ed.; Wiley: New York, 2001; unit 10.6.

(CD_3Cl) δ 162.4, 156.9, 138.2, 135.7, 133.1, 133.0, 130.0, 129.9, 128.6, 127.9, 127.6, 127.1, 120.7, 84.8, 80.6, 76.8, 72.3, 64.3, 49.3, 27.0, 19.4; HRMS (FAB^+) m/z calcd for $C_{39}H_{44}N_3O_4Si$ [M + H^+] 646.3101, found 646.3117.

5-[5′-*O*-(*tert*-Butyl)diphenylsilyl-β-D-ribofuranosyl]-2-dibenzylaminopyridine (5b). Compound **5b** was prepared from **2b** (2.83 g, 8 mmol), lactone **3** (3.41 g, 8 mmol), and *n*-butyllithium (6.0 mL, 1.6 M solution in hexane, 9.6 mmol) as described for **5a**. Silica gel chromatography (10% ethyl acetate in hexane) of the residue yielded 1.50 g (29% yield) of **5b** as a white foam: ^1H NMR (CD_3Cl) δ 8.18 (s, 1H), 7.65−7.63 (m, 4H), 7.19−7.46 (m, 16H), 6.40 (d, 1H, J = 7.2 Hz), 4.77 (s, 4H), 4.63 (d, 1H, J = 7.2 Hz), 4.26 (m, 1H), 3.99−4.02 (m, 2H), 3.85 (m, 1H), 1.05 (s, 9H); ^{13}C NMR (CD_3Cl) δ 158.7, 146.8, 138.3, 136.3, 136.2, 135.8, 135.7, 133.2, 133.1, 130.0, 129.9, 128.7, 128.1, 127.9, 127.8, 127.2, 127.16, 127.12, 123.1, 123.0, 84.5, 82.4, 82.4, 77.1, 72.4, 64.4, 51.1, 27.0, 19.4; HRMS (FAB^+) m/z calcd for $C_{40}H_{45}N_2O_4Si$ [M + H^+] 645.3149, found 645.3151.

5-β-D-Ribofuranosyl-2-dibenzylamino-1,3-pyrimidine (6a). To the solution of **5a** (1.81 g, 2.80 mmol) in THF (40 mL) was added $Bu_4NF\cdot 3H_2O$ (1.10 g, 4.20 mmol). The mixture was stirred at room temperature for 6 h. TLC showed that the reaction was complete. Solvent was removed, and the resulting residue was purified by silica gel chromatography, eluting with 5% methanol in dichloromethane, to give product as a white foam. **6a** (1.03 g, 90% yield): ^1H NMR (CD_3OD) δ 8.44 (s, 2H), 7.16−7.27 (m, 10H), 4.82 (s, 4H), 4.61 (d, 1H, J = 7.6 Hz), 4.10 (m, 1H), 3.97 (m, 1H), 3.95 (m, 1H), 3.75 (dd, 1H, J = 3.8, 12.0 Hz), 3.69 (dd, 1H, J = 4.5, 12.0 Hz); ^{13}C NMR (CD_3COD) δ 163.4,158.1, 139.4, 129.4, 128.5, 128.0, 122.8, 86.8, 81.3, 78.3, 73.0, 63.5, 50.4; HRMS (FAB^+) m/z calcd for $C_{23}H_{26}N_3O_4$ [M + H^+] 408.1923, found 408.1937.

5-β-D-Ribofuranosyl-2-dibenzylaminopyridine (6b). Compound **6b** was prepared from **5b** (0.583 g, 0.90 mmol) and TBAF (1.50 mL, 1 M in THF, 1.5 mmol) as described for **6a**. Silica gel chromatography (5% methanol in dichloromethane) of the residue yielded 295 mg (80% yield) of **6b** as a white foam: ^1H NMR (CD_3COD) δ 8.04 (d, 1H, J = 2.0 Hz), 7.40 (dd, 1H, J = 2.0, 9.0 Hz), 7.03−7.14 (m, 10H), 6.41 (d, 1H, J = 9.0 Hz), 4.61 (s, 4H), 4.51 (d, 1H, J = 7.0 Hz), 3.96 (m, 1H), 3.85 (m, 1H), 3.79 (m, 1H), 3.62 (dd, 1H, J = 4.0, 12.0 Hz), 3.56 (dd, 1H, J = 4.5, 12.0 Hz); ^{13}C NMR (CD_3OD) δ 159.8, 147.5, 139.6, 137.7, 129.7, 128.2, 128.1, 125.1, 107.7, 86.5, 83.3, 78.4, 73.1, 63.7, 52.4; HRMS (FAB^+) m/z calcd for $C_{24}H_{27}N_2O_4$ [M + H^+] 407.1971, found 407.1979.

5-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-2-dibenzylamino-1,3-pyrimidine (7a). To the solution of **6a** (89 mg, 0.218 mmol) in CH_3CN (10 mL) were added Et_3N (214 μL, 1.53 mmol), DMAP (27 mg, 0.218 mmol), and Ac_2O (74 μL, 0.785 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h and then warmed to room temperature for 1 h. TLC showed that the reaction was complete. The reaction mixture was quenched with MeOH (1 mL), and the solvent was removed. The residue was purified by silica gel chromatography, eluting with 1% methanol in dichloromethane, to give the product as a white foam. **6a** (114 m g, 98% yield): ^1H NMR (CD_3Cl) δ 8.38 (s, 2H), 7.22−7.32 (m, 10H), 5.32 (m, 1H), 5.12 (m, 1H), 4.87 (s, 4H), 4.86 (m, 1 H), 4.38 (m, 1H), 4.27−4.31 (m, 2H), 2.13 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (CD_3Cl) δ 170.6, 169.9, 169.8, 162.7, 156.9, 138.0, 128.6, 127.6, 127.2, 118.9, 80.2, 78.8, 75.6, 71.8, 63.7, 49.2, 20.9, 20.7, 20.6; HRMS (FAB^+) m/z calcd for $C_{29}H_{32}N_3O_7$ [M + H^+] 534.2240, found 534.2231.

5-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-2-dibenzylaminopyridine (7b). Compound **7b** was prepared from **6b** (167 mg, 0.41 mmol), DMAP (50 mg, 0.41 mmol), and Ac_2O (150 mg, 1.47 mmol) as described for **7a**. Silica gel chromatography (1% methanol in dichloromethane) of the residue yielded 207 mg (95% yield) of **7b** as a white foam: ^1H NMR (CD_3COD) δ 8.19 (d, 1H, J = 2.4 Hz), 7.41 (dd, 1H, J = 2.4, 8.8 Hz), 7.19−7.40 (m, 10H), 6.48 (d, 1H, J = 8.8 Hz), 5.30 (m, 1H), 5.10 (m, 1H), 4.88 (d, 1H, J = 7.2 Hz), 4.78 (s, 4H), 4.37 (m, 1H), 4.25−4.29 (m, 2H), 2.12 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (CD_3OD) δ 170.7, 169.9, 169.8, 159.1, 147.0, 138.2, 135.7, 128.7, 128.6, 127.1, 121.1, 106.1, 80.4, 80.0, 75.8, 71.9, 63.9, 51.1, 20.9, 20.8, 20.7; HRMS (FAB^+) m/z calcd for $C_{30}H_{33}N_2O_7$ [M + H^+] 533.2288, found 533.2291.

2-Amino-5-(β-D-ribofuranosyl)-1,3-pyrimidine (8a). Ammonium cerium(IV) nitrate (586 mg, 1.07 mmol) was added to a solution of **7a** (114 mg, 0.214 mmol) in acetonitrile−water (98:2 v/v, 10 mL), and the mixture was stirred overnight at 50 °C under Ar. The reaction was quenched with 1 N NaOH until the pH reached a value of about 8 and stirred at room temperature for 1 h. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 20% methanol in dichloromethane, to give the product **8a** as a white foam: 35 mg (71% yield); ^1H NMR (CD_3COD) δ 8.33 (s, 2H), 4.56 (d, 1H, J = 7.6 Hz), 4.08 (m, 1H), 3.95 (m, 1H), 3.89 (m, 1 H), 3.75 (dd, 1 H, J = 3.6, 12.0 Hz), 3.68 (dd, 1 H, J = 4.4, 12.0 Hz); ^{13}C NMR (CD_3COD) δ 164.4, 158.2, 123.8, 87.0, 81.0, 78.5, 73.0, 63.5; HRMS (FAB^+) m/z calcd for $C_9H_{14}N_3O_4$ [M + H^+] 228.0984, found 228.0988.

2-Amino-5-(β-D-ribofuranosyl)pyridine (8b). Compound **8b** was prepared from **7b** (205 mg, 0.39 mmol) and ammonium cerium(IV) nitrate (1.05 g, 1.95 mmol) as described for **8a**. Silica gel chromatography (20% methanol in dichloromethane) of the residue yielded 75 mg (86% yield) of **8b** as a white foam:^1H NMR (CD_3COD) δ 7.89 (d, 1H, J = 2.0 Hz), 7.79 (dd, 1H, J = 2.0, 8.8 Hz), 6.80 (d, 1H, J = 9.2 Hz), 4.60 (d, 1H, J = 7.6 Hz), 4.08 (m, 1H), 3.99 (m, 1H), 3.88 (m, 1H), 3.77 (dd, 1H, J = 3.6, 11.6 Hz), 3.70 (dd, 1H, J = 4.4, 12.0 Hz); ^{13}C NMR (CD_3OD) δ 158.3, 140.8, 140.3, 126.4, 112.4, 86.9, 82.3, 78.3, 72.9, 63.5; HRMS (FAB^+) m/z calcd for $C_{10}H_{15}N_2O_4$ [M + H^+] 227.1032, found 227.1031.

5-[3′,5′-*O*-(Di-*tert*-butylsilylene)-β-D-ribofuranosyl]-2-dibenzylamino-1,3-pyrimidine (9a). Compound **6a** (0.998 g, 2.45 mmol) was dried three times by azeotropic evaporation of pyridine and then was dissolved in dry DMF (20 mL). *t*-$Bu_2Si(OTf)_2$ (1.10 g, 2.5 mmol) was added to the solution at 0 °C under Ar. The solution was stirred for 30 min and warmed to room temperature for 15 min. Then Et_3N (1.02 mL, 7.35 mmol) was added and the mixture stirred for another 10 min. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 2% methanol in dichloromethane, to give the product **9a** as a white foam: 1.06 g (79% yield); ^1H NMR ($CDCl_3$) δ 8.31 (s, 2H), 7.21−7.32 (m, 10H), 4.88 (m, 5H), 4.48 (m, 1H), 4.17 (d, 1H, J = 4.2 Hz), 3.99−4.02 (m, 3H), 2.63 (s, 1H, disappeared with D_2O), 1.05 (s, 9H), 1.07 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 162.2, 156.6, 138.0, 128.5, 127.6, 127.4, 120.7, 85.0, 77.8, 75.7, 74.4, 68.0, 49.2, 27.4, 27.3, 22.7, 20.4; HRMS (FAB^+) m/z calcd for $C_{31}H_{42}N_3O_4Si$ [M + H^+] 548.2945, found 548.2958.

5- [3′,5′-*O*-(Di-*tert*-butylsilylene)-β-D-ribofuranosyl]-2-dibenzylaminopyridine (9b). Compound **9b** was prepared from **6b** (2.645 g, 6.50 mmol) and *t*-$Bu_2Si(OTf)_2$ (2.92 g, 6.63 mmol) as described for **9a**. Silica gel chromatography (10% ethyl acetate in hexane) of the residue yielded 2.92 g (82% yield) of **9b** as a white foam: ^1H NMR ($CDCl_3$) δ 8.17 (d, 1H, J = 2.4 Hz), 7.19−7.30 (m, 10H), 6.43 (d, 1H, J = 8.8 Hz), 4.90 (m, 1H), 4.77 (d, 4H, J = 1.6 Hz), 4.46 (m, 1H), 4.12 (d, 1H, J = 4.4 Hz), 3.98−4.01 (m, 3H), 1.06 (s, 9H), 1.04 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 158.5, 146.5, 138.3, 136.0, 128.7, 127.1, 123.2, 105.8, 86.9, 78.0, 76.0, 74.4, 68.2, 51.1, 27.5, 27.4, 22.8, 20.5; HRMS (FAB^+) m/z calcd for $C_{32}H_{43}N_2O_4Si$ [M + H^+] 547.2992, found 547.2998.

5-[2′-*O*-(*tert*-Butyldimethylsilyl)-3′,5′-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-2-dibenzylamino-1,3-pyrimidine (10a). To a solution of **9a** (1.06 g, 1.94 mmol) in dry pyridine (40 mL) were

added 1*H*-imidazole (3.95 g, 58.1 mmol) and *t*-BuMe$_2$SiCl (7.29 g, 48.4 mmol). The mixture was stirred overnight and then evaporated to a syrup. The residue was partitioned between CH_2Cl_2 and H_2O; the organic layer was washed with water followed by brine and dried over anhydrous Na_2SO_4. The sodium sulfate was filtered off, and the solvent was removed by evaporation under vacuum. The residue was purified by silica gel chromatography, eluting with 5% ethyl acetate in hexane, to give the product **10a** as a white foam: 1.103 g (86% yield); ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 2H), 7.21−7.31 (m, 10H), 4.87 (d, 4H, J = 2.4 Hz), 4.78 (s, 1H), 4.47 (m, 1H), 4.15 (d, 1H, J = 4.8 Hz), 4.10 (m, 1H), 3.88−3.97 (m, 2H), 1.05 (s, 9H), 1.04 (s, 9H), 0.93 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 162.4, 156.5, 138.1, 128.7, 127.6, 127.2, 121.5, 87.0, 77.9, 77.7, 74.1, 68.5, 49.3, 27.7, 27.2, 26.1, 22.9, 20.5, 18.5, −4.0, −4.7; HRMS (FAB$^+$) m/z calcd for $C_{37}H_{56}N_3O_4Si_2$ [M + H$^+$] 662.3809, found 662.3825.

5-[2′-*O*-(*tert*-Butyldimethylsilyl)-3′,5′-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-2-dibenzylaminopyridine (10b). Compound **10b** was prepared from **9b** (2.02 g, 3.70 mmol), 1*H*-imidazole (7.50 g, 110.32 mmol), and *t*-BuMe$_2$SiCl (13.90 g, 92.29 mmol) as described for **10a**. Silica gel chromatography (5% ethyl acetate in hexane) of the residue yielded 2.26 g (93% yield) of **10b** as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.14 (d, 1H, J = 2.4 Hz), 7.20−7.32 (m, 11H), 6.45 (d, 1H, J = 8.8 Hz), 4.81 (s, 1H), 4.79 (s, 4H,), 4.46 (m, 1H), 4.13 (d, 1H, J = 4.8 Hz), 4.08 (m, 1H), 3.95 (m, 1H), 3.90 (m, 1H), 1.04 (s, 9H), 1.03 (s, 9H), 0.92 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.4, 146.2, 138.2, 135.7, 128.6, 127.0, 123.7, 105.8, 88.7, 77.7, 73.9, 68.5, 51.0, 27.5, 27.1, 26.0, 22.8, 20.4, 18.4, −4.1, −4.8; HRMS (FAB$^+$) m/z calcd for $C_{38}H_{57}N_2O_4Si_2$ [M + H$^+$] 661.3857, found 661.3867.

5-[2′-*O*-(*tert*-Butyldimethylsilyl)-3′,5′-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-2-amino-1,3-pyrimidine (11a). Ammonium cerium(IV) nitrate (4.52 g, 8.25 mmol) was added to a solution of **10a** (1.09 g, 1.65 mmol) in acetonitrile−water (98:2 v/v, 50 mL) and stirred overnight at 50 °C under Ar. The reaction was quenched with 1 N NaOH until the pH reached about 8. The mixture was partitioned between Et_2O and H_2O; the organic layer was washed with brine and dried over anhydrous Na_2SO_4. The sodium sulfate was filtered off, and the solvent was removed by evaporation under vacuum. The residue was purified by silica gel chromatography, eluting with 15% ethyl acetate in hexane and then 2% methanol in dichloromethane, to give the product **11a** as a white foam: 546 mg (69% yield); ^{1}H NMR ($CDCl_3$) δ 8.21 (s, 2H), 5.33 (s, 2H, disappeared with D_2O), 4.76 (s, 1H), 4.78 (m, 1H), 4.06−4.13 (m, 2H), 3.95 (m, 1H), 3.84 (m, 1H), 1.05 (s, 9H), 1.04 (s, 9H), 0.91 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 162.9, 156.6, 123.3, 86.7, 77.7, 77.6, 74.2, 68.4, 27.6, 27.2, 26.1, 22.9, 20.5, 18.5, −4.0, −4.8; HRMS (FAB$^+$) m/z calcd for $C_{23}H_{44}N_3O_4Si_2$ [M + H$^+$] 482.2870, found 482.2878.

5-[2′-*O*-(*tert*-Butyldimethylsilyl)-3′,5′-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-2-aminopyridine (11b). Compound **11b** was prepared from **10b** (2.04 g, 3.08 mmol) and ammonium cerium-(IV) nitrate (8.45 g, 15.40 mmol) as described for **11a**. Silica gel chromatography (2% methanol in dichloromethane) of the residue yielded 1.06 g (72% yield) of **11b** as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.01 (d, 1H, J = 2.4 Hz), 7.32 (dd, 1H, J = 2.4, 8.8 Hz), 6.49 (d, 1H, J = 8.8 Hz), 4.80 (s, 1H), 4.50 (b, 2H), 4.47 (m, 1H), 4.06−4.12 (m, 2H), 3.97 (m, 1H), 3.86 (m, 1H), 1.05 (s, 9H), 1.04 (s, 9H), 0.92 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.2, 146.3, 135.9, 125.8, 108.6, 88.6, 77.9, 77.6, 74.1, 68.5, 27.6, 27.2, 26.1, 22.9, 20.5, 18.5, −4.0, −4.8; HRMS (FAB$^+$) m/z calcd for $C_{24}H_{45}N_2O_4Si_2$ [M + H$^+$] 481.2918, found 481.2922.

5-[2′-*O*-(*tert*-Butyldimethylsilyl)-3′,5′-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-2-*N*-(dimethylformamidino)-1,3-pyrimidine (12a). To a solution of compound **11a** (540 mg, 1.12 mmol) in methanol (20 mL) was added *N,N*-dimethylformamide dimethyl acetal (1.5 mL, 11.2 mmol), and the reaction mixture was stirred at room temperature overnight. The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 2% methanol in dichloromethane, to give the product **12a** as a white foam: 558 mg (93% yield); ^{1}H NMR ($CDCl_3$) δ 8.65 (s, 1H), 8.41 (s, 2H), 4.84 (s, 1H), 4.50 (m, 1H), 4.08−4.14 (m, 2H), 3.98 (m, 1H), 3.87 (m, 1H), 3.17 (s, 3H), 3.15 (s, 3H), 1.05 (s, 9H), 1.04 (s, 9H), 0.93 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.6, 158.2, 156.2, 126.3, 86.6, 77.8, 77.5, 74.2, 68.3, 41.1, 35.1, 27.5, 27.1, 26.0, 22.8, 20.4, 18.4, −4.1, −4.9; HRMS (FAB$^+$) m/z calcd for $C_{26}H_{49}N_4O_4Si_2$ [M + H$^+$] 537.3292, found 537.3301.

5-[2′-*O*-(*tert*-Butyldimethylsilyl)-3′,5′-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-2-*N*-(dimethylformamidino)pyridine (12b). Compound **12b** was prepared from **11b** (390 mg, 0.81 mmol) and *N,N*-dimethylformamide dimethyl acetal (1.13 mL, 8.10 mmol) as described for **12a**. Silica gel chromatography (2% methanol in dichloromethane) of the residue yield 370 mg (85% yield) of **12b** as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.42 (s, 1H), 8.18 (d, 1H, J = 2.0 Hz), 7.43 (dd, 1H, J = 2.4, 8.4 Hz), 6.93 (d, 1H, J = 8.4 Hz), 4.87 (s, 1H), 4.49 (m, 1H), 4.08−4.12 (m, 2H), 4.00 (m, 1H), 3.90 (m, 1H), 3.08 (s, 6H), 1.05 (s, 9H), 1.04 (s, 9H), 0.92 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 161.9, 158.5, 146.1, 135.6, 129.5, 117.9, 88.6, 78.0, 77.5, 74.1, 68.5, 40.8, 34.8, 27.6, 27.2, 26.1, 22.8, 20.5, 18.4, −4.0, −4.8; HRMS (FAB$^+$) m/z calcd for $C_{27}H_{50}N_3O_4Si_2$ [M + H$^+$] 536.3340, found 536.3348.

5-[2′-*O*-(*tert*-Butyldimethylsilyl)-β-D-ribofuranosyl]-2-*N*-(dimethylformamidino)-1,3-pyrimidine (13a). HF·pyridine (445 μL, 3.42 mmol) was carefully diluted with pyridine (0.50 mL) and then added dropwise to a solution of **12a** (344 mg, 0.64 mmol) in THF (10 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 10 min. TLC showed that the reaction was complete. The reaction mixture was diluted with pyridine (2 mL). The mixture was partitioned between CH_2Cl_2 and H_2O; the organic layer was washed with 5% aq $NaHCO_3$ followed by brine and dried over anhydrous Na_2SO_4. The sodium sulfate was filtered off, and the solvent was removed by evaporation under vacuum. The residue was purified by silica gel chromatography, eluting with 5% methanol in dichloromethane, to give the product **13a** as a white foam: 212 mg (83% yield); ^{1}H NMR ($CDCl_3$) δ 8.65 (s, 1H), 8.53 (s, 2H), 4.62 (d, 1H, J = 7.1 Hz), 4.15 (m, 1H), 4.12 (m, 1H), 4.06 (m, 1H), 3.93 (dd, 1H, J = 3.2, 11.8 Hz), 3.81 (dd, 1H, J = 3.7, 11.8 Hz), 3.17 (s, 3H), 3.15 (s, 3H), 0.87 (s, 9H), −0.04 (s, 3H), −0.15 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.8, 158.3, 157.1, 125.8, 85.5, 80.5, 78.8, 72.3, 62.9, 41.2, 35.1, 27.5, 18.0, −4.7, −4.9; HRMS (FAB$^+$) m/z calcd for $C_{18}H_{33}N_4O_4Si$ [M + H$^+$] 397.2271, found 397.2268.

5-[2′-*O*-(*tert*-Butyldimethylsilyl)-β-D-ribofuranosyl]-2-*N*-(dimethylformamidino)pyridine (13b). Compound **13b** was prepared from **12b** (100 mg, 0.19 mmol) and HF·pyridine (130 μL, 0.95 mmol) as described for **13a**. Silica gel chromatography (5% methanol in dichloromethane) of the residue yielded 59 mg (80% yield) of **13b** as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.41 (s, 1H), 8.21 (d, 1H, J = 1.6 Hz), 7.57 (dd, 1H, J = 2.4, 8.4 Hz), 6.97 (d, 1H, J = 8.4 Hz), 4.62 (d, 1H, J = 6.8 Hz), 4.05−4.10 (m, 2H), 3.99 (m, 1H), 3.89 (dd, 1H, J = 3.2, 12.0 Hz), 3.78 (dd, 1H, J = 4.0, 12.0 Hz), 3.09 (s, 6H), 0.86 (s, 9H), −0.08 (s, 3H), −0.18 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 162.3, 155.7, 147.1, 136.3, 128.5, 117.9, 85.3, 82.4, 78.8, 72.1, 63.0, 40.9, 34.8, 25.7, 18.0, −4.7, −5.1; HRMS (FAB$^+$) m/z calcd for $C_{19}H_{34}N_3O_4Si$ [M + H$^+$] 396.2319, found 396.2326.

5-[2′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(4,4-dimethoxytrityl)-β-D-ribofuranosyl]-2-*N*-(dimethylformamidino)-1,3-pyrimidine (14a). To a solution of **13a** (209 mg, 0.527 mmol) in dry pyridine (10 mL) was added dimethoxytrityl chloride (535 mg, 1.581 mmol). The mixture was stirred at room temperature overnight. The reaction

was quenched with methanol (1.0 mL) and partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with 5% aq $NaHCO_3$ followed by brine and dried over anhydrous Na_2SO_4. The sodium sulfate was filtered off, and the solvent was removed by evaporation under vacuum. The residue was purified by silica gel chromatography, eluting with 1% MeOH/CH_2Cl_2 + 0.5% Et_3N, to give the product **14a** as a white foam: 294 mg (80% yield); ^{1}H NMR ($CDCl_3$) δ 8.68 (s, 1H), 8.58 (s, 2H), 7.20−7.47 (m, 9 H), 6.82 (d, 4H, J = 8.4 Hz), 4.66 (d, 1H, J = 8.0 Hz), 4.19−4.23 (m, 2H), 4.11 (m, 1H), 3.78 (s, 6H), 3.48 (dd, 1H, J = 2.8, 10.4 Hz), 3.25 (dd, 1H, J = 3.2, 10.4 Hz), 3.18 (s, 3H), 3.14 (s, 3H), 2.77 (d, 1H, J = 2.0 Hz, disappeared with D_2O), 0.87 (s, 9H), −0.04 (s, 3H), −0.13 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 167.0, 158.6, 158.4, 157.1, 144.9, 136.0, 135.9, 130.2, 130.1, 128.2, 128.0, 126.9, 125.8, 113.33, 113.31, 86.4, 84.7, 79.6, 79.0, 73.1, 64.1, 55.4, 41.2, 35.2, 25.8, 18.0, −4.6, −4.9; HRMS (FAB$^+$) m/z calcd for $C_{39}H_{51}N_4O_6Si$ [M + H$^+$] 699.3578, found 699.3583.

5-[2′-*O*-(*tert*-Butyl)dimethylsilyl-5′-*O*-(4,4-dimethoxytrityl)-β-D-ribofuranosyl]-2-*N*-(dimethylformamidino)pyridine (14b). Compound **14b** was prepared from **13b** (132 mg, 0.33 mmol) and dimethoxytrityl chloride (339 mg, 1.00 mmol) as described for **14a**. Silica gel chromatography (1% methanol in dichloromethane + 0.5% Et_3N) of the residue yielded 224 mg (96% yield) of **14b** as a white foam: ^{1}H NMR ($CDCl_3$) δ 8.43 (s, 1H), 8.25 (d, 1H, J = 2.4 Hz), 7.71 (dd, 1H, J = 2.4, 8.0 Hz), 7.18−7.49 (m, 9H), 6.93 (d, 1H, J = 8.0 Hz), 6.82 (m, 4H), 4.68 (d, 1H, J = 7.6 Hz), 4.16−4.20 (m, 2H), 4.10 (m, 1H), 3.783 (s, 3H), 3.780 (s, 3H), 3.46 (dd, 1H, J = 3.2, 10.4 Hz), 3.26 (dd, 1H, J = 3.6, 10.0 Hz), 3.09 (s, 6H), 2.80 (d, 1H, J = 2.4 Hz), 0.85 (s, 9H), −0.09 (s, 3H), −0.18 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 162.5, 158.6, 155.6, 147.3, 145.0, 136.3, 136.2, 136.0, 130.3, 130.2, 128.8, 128.3, 127.9, 126.9, 118.0, 113.3, 86.4, 84.5, 81.5, 79.2, 73.1, 64.3, 55.3, 40.9, 34.8, 25.8, 18.1, −4.7, −5.0; HRMS (FAB$^+$) m/z calcd for $C_{40}H_{52}N_3O_6Si$ [M + H$^+$] 698.3625, found 698.3630.

5-[2′-*O*-(*tert*-Butyl)dimethylsilyl-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphino)-5′-*O*-(4,4-dimethoxytrityl)-β-D-ribofuranosyl]-2-*N*-(dimethylformamidino)-1,3-pyrimidine (15a). To a solution of **14a** (95 mg, 0.136 mmol) in dry dichloromethane (5 mL) under argon were added *N*,*N*-diisopropylethylamine (117 μL, 0.68 mmol), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (95 mg, 0.41 mmol), and 1-methylimidazole (11 μL, 0.136 mmol). The reaction mixture was stirred at room temperature for 1 h. TLC indicated that the reaction was complete. The reaction mixture was quenched with MeOH (1 mL) and stirred for 5 min. After the solvent was removed, the residue was purified by silica gel chromatography, eluting with 0−5% acetone in dichloromethane containing 0.5% triethylamine, to give the corresponding phosphoramidite **15a** as a white foam: 118 mg (96% yield); ^{31}P NMR (CD_3CN) δ 153.2, 150.6; HRMS (FAB$^+$) m/z calcd for $C_{48}H_{68}N_6O_7PSi$ [M + H$^+$] 899.4656, found 899.4637.

5-[2′-*O*-(*tert*-Butyldimethylsilyl)-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphino)-5′-*O*-(4,4-dimethoxytrityl)-β-D-ribofuranosyl]-2-*N*-(dimethylformamidino)pyridine (15b). Compound **15b** was prepared from **14b** (117 mg, 0.17 mmol), *N*,*N*-diisopropylethylamine (155 μL, 0.90 mmol), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (128 mg, 0.55 mmol), and 1-methylimidazole (16 μL, 0.20 mmol) as described for **15a**. Silica gel chromatography (0−5% acetone in dichloromethane + 0.5% Et_3N) of the residue yielded 132 mg (88% yield) of **15b** as a white foam: ^{31}P NMR (CD_3CN) δ 150.6, 148.2; HRMS (FAB$^+$) m/z calc for $C_{49}H_{69}N_5O_7PSi$ [M + H$^+$] 898.4704, found 898.4707.

5-[2′-*O*-(*tert*-Butyldimethylsilyl)-3′,5′-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-2-aminopyridine 1-Oxide (16). To a solution of 5-[2′-*O*-(*tert*-Butyldimethylsilyl)-3′,5′-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-2-aminopyridine **11b** (173 mg, 0.36 mmol) in dry CH_2Cl_2 (10 mL) was added *m*-CBPA (94 mg, 0.54 mmol), and the reaction mixture was stirred at room temperature for overnight under Ar. The solvent was removed by evaporation under vacuum, and the residue was purified by silica gel chromatography, eluting with 2−5% MeOH in dichloromethane, to give the product **16** as a white foam: 160 mg (90% yield); ^{1}H NMR ($CDCl_3$) δ 8.09 (d, 1H, J = 2.0 Hz), 7.03 (dd, 1H, J = 2.0, 8.8 Hz), 6.77 (d, 1H, J = 8.4 Hz), 5.82 (b, 2H), 4.75 (s, 1H), 4.47 (m, 1H), 4.05−4.11 (m, 2H), 3.97 (m, 1H), 3.80 (m, 1H), 1.03 (s, 9H), 1.02 (s, 9H), 0.93 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 149.5, 135.7, 126.9, 126.5, 109.7, 87.2, 77.8, 77.3, 74.2, 68.3, 27.6, 27.2, 26.1, 22.8, 20.5, 18.4, −3.9, −4.7; HRMS (FAB$^+$) m/z calcd for $C_{24}H_{45}N_2O_5Si_2$ [M + H$^+$] 497.2867, found 497.2872.

6-Acetamido-3-[2′-*O*-(*tert*-butyldimethylsilyl)-3′,5′-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-1*H*-pyridin-2-one (17). To a solution of 5-[2′-*O*-(*tert*-butyldimethylsilyl)-3′,5′-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-2-aminopyridine 1-oxide (**16**) (160 mg, 0.32 mmol) in Ac_2O (5 mL) was added Et_3N (161 μL, 1.12 mmol), and the reaction mixture was stirred at 140 °C for 2 h. The solvent was removed by evaporation under vacuum, and the residue was dissolved in MeOH (5 mL). NaOH (38 mg, 0.96 mmol) was added, and the reaction mixture was stirred at room temperature for 2 h under Ar. The solvent was removed by evaporation under vacuum, and the residue was purified by silica gel chromatography, eluting with 3% MeOH in dichloromethane, to give the product **17** as a white foam: 102 mg (59% yield); ^{1}H NMR ($CDCl_3$) δ 10.58 (b, 1H), 7.40 (d, 1H, J = 8.0 Hz), 6.19 (d, 1H, J = 7.6 Hz), 4.88 (s, 1H), 4.46 (m, 1H), 4.25 (m, 1H), 4.08 (m, 1H), 3.90−3.95 (m, 2H), 2.17 (s, 3H), 1.02 (s, 9H), 1.01 (s, 9H), 0.89 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.7, 160.0, 143.3, 140.6, 122.9, 93.7, 86.2, 77.3, 75.6, 74.0, 68.5, 27.7, 27.2, 26.1, 24.5, 22.8, 20.5, 18.4, −3.9, −4.8; HRMS (FAB$^+$) m/z calcd for $C_{26}H_{47}N_2O_6Si_2$ [M + H$^+$] 539.2973, found 539.2977.

6-Acetamido-3-[2′-*O*-(*tert*-butyldimethylsilyl)-3′,5′-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-1-[(pivaloyloxy)methyl]pyridin-2-one (18). To a solution of **17** (214 mg, 0.40 mmol) in DMF (10 mL) were added K_2CO_3 (83 mg, 0.60 mmol) and chloromethyl pivaloate (73 mg, 0.48 mmol), and the reaction mixture was stirred at 90 °C for 4 h. The solvent was removed by evaporation under vacuum, and the residue was purified by silica gel chromatography, eluting with 1% MeOH in dichloromethane, to give the product **18** as a white foam: 223 mg (86% yield); ^{1}H NMR ($CDCl_3$) δ 7.83 (d, 1H, J = 7.6 Hz), 7.78 (s, 1H), 7.63 (d, 1H, J = 8.0 Hz), 6.13 (d, 1H, J = 5.6 Hz), 6.01 (d, 1H, J = 5.6 Hz), 5.04 (s, 1H), 4.51 (m, 1H), 4.12−4.18 (m, 2H), 4.02 (m, 1H), 3.89 (m, 1H), 2.21 (s, 3H), 1.16 (s, 9H), 1.04 (s, 18H), 0.94 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 177.4, 168.6, 156.1, 148.2, 139.4, 118.5, 107.4, 84.5, 81.9, 77.0, 76.6, 73.9, 68.5, 38.9, 27.7, 27.2, 27.0, 26.0, 24.8, 22.8, 20.4, 18.4, −3.9, −5.0; HRMS (FAB$^+$): m/z calcd for $C_{32}H_{57}N_2O_8NaSi_2$ [M + Na$^+$] 675.3473, found 675.3475.

6-Acetamido-3-[2′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-1-[(pivaloyloxy)methyl]pyridin-2-one (19). Compound **19** was prepared from **18** (274 mg, 0.42 mmol) and HF·pyridine (279 μL, 2.10 mmol) as described for **13a**. Silica gel chromatography (3% methanol in dichloromethane) of the residue yielded 192 mg (89% yield) of **19** as a white foam: ^{1}H NMR ($CDCl_3$ + D_2O) δ.17 (s, 1H), 7.84 (d, 1H, J = 8.8 Hz), 7.68 (d, 1H, J = 8.8 Hz), 6.10 (d, 1H, J = 5.6 Hz), 5.98 (d, 1H, J = 5.6 Hz), 4.78 (d, 1H, J = 7.0 Hz), 4.32 (m, 1H), 4.12 (m, 1H), 4.02 (m, 1H), 3.91 (dd, 1H, J = 2.8, 12.0 Hz), 3.78 (dd, 1H, J = 3.6, 12.0 Hz), 2.21 (s, 3H), 1.17 (s, 9H), 0.86 (s, 9H), −0.04 (s, 3H), −0.14 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 177.7, 168.8, 157.3, 148.7, 142.1, 116.4, 107.2, 84.9, 82.0, 81.9, 76.0, 71.8, 62.8, 38.9, 27.0, 25.7, 24.8, 18.0, −4.7, −5.1; HRMS (FAB$^+$) m/z calcd for $C_{24}H_{41}N_2O_8Si$ [M + H$^+$] 513.2632, found 513.2641.

6-Acetamido-3-[5′-*O*-(4,4-dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-1-[(pivaloyloxy)methyl]pyridin-2-one (20). Compound **20** was prepared from **19** (192 mg, 0.37 mmol) and dimethoxytrityl chloride (382 mg, 1.12 mmol) as described for **14a**. Silica gel chromatography (1% methanol in dichloromethane + 0.5% Et_3N) of the residue yielded 283 mg (93% yield) of **20** as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.99 (d, 1H, J = 8.0 Hz), 7.22−7.81 (m, 11H), 6.86 (d, 4H, J = 8.8 Hz), 6.14 (d, 1H, J = 5.2 Hz), 5.95 (d, 1H, J = 5.2 Hz), 5.10 (d, 1H, J = 5.2 Hz), 4.23 (m, 1H), 4.14 (m, 2H), 3.82 (s, 6H), 3.51 (dd, 1H, J = 2.4, 10.4 Hz), 3.39 (dd, 1H, J = 4.0, 10.4 Hz), 2.62 (d, 1H, J = 6.0 Hz), 2.22 (s, 3H), 1.20 (s, 9H), 0.93 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 177.4, 168.5, 158.6, 156.9, 148.3, 145.0, 140.4, 136.1, 136.0, 130.3, 130.2, 128.3, 127.9, 126.9, 118.0, 113.2, 107.5, 86.4, 83.0, 82.2, 79.0, 78.0, 72.0, 63.8, 55.3, 38.9, 27.0, 25.8, 24.8, 18.1, −4.6, −5.1; HRMS (FAB^+) m/z calcd for $C_{45}H_{59}N_2O_{10}NaSi$ [M + Na^+] 837.3758, found 837.3767.

6-Acetamido-3-[5′-*O*-(4,4-dimethoxytrityl)-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphino)-2′-*O*-(*tert*-butyl)dimethylsilyl-β-D-ribofuranosyl]-1-[(pivaloyloxy)methyl]pyridin-2-one (21). Compound **21** was prepared from **20** (96 mg, 0.12 mmol), *N*,*N*-diisopropylethylamine (109 μL, 0.63 mmol), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (90 mg, 0.39 mmol), and 1-methylimidazole (11 μL, 0.14 mmol) as described for **15a**. Silica gel chromatography (0−5% acetone in dichloromethane + 0.5% Et_3N) of the residue yielded 94 mg (79% yield) of **21** as a white foam: ^{31}P NMR (CD_3CN) δ 148.7, 149.6; HRMS (FAB^+) m/z calcd for $C_{54}H_{76}N_4O_{11}NaPsi$ 1037.4837, found 1037.4846.

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Supporting Information Available: Proton, carbon, and phosphorus NMR spectra as appropriate for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.