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Synthesis of Novel C4-Linked C_2-Imidazole Ribonucleoside Phosphoramidite and Its Application to Probing the Catalytic Mechanism of a Ribozyme

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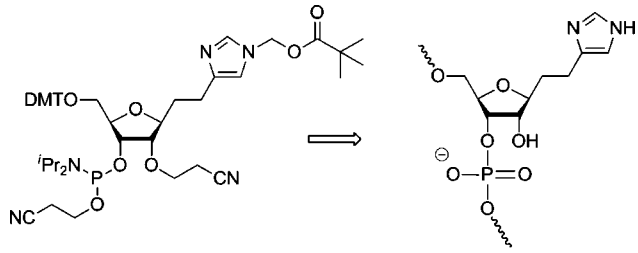

ICN-C_2-PA G638VSC_2Imz

The synthesis of a novel C4-linked C_2-imidazole ribonucleoside phosphoramidite (ICN-C_2-PA **1**) with a two-carbon linker between imidazole and ribose moieties is described. In the phosphoramidite, POM and 2-cyanoethyl groups were selected to protect the endocyclic amine function of imidazole and the 2′-hydroxyl function of D-ribose, respectively. The C_2-imidazole nucleoside, a flexible structural mimic of a purine nucleobase, was successfully incorporated using ICN-C_2-PA **1** into position 638 of the VS ribozyme through 2′-TBDMS chemistry to study the role of G638 in general acid−base catalysis. The modified VS ribozyme (G638C_2Imz) exhibited significantly greater catalytic activity than observed with the C_0-imidazole that has no carbon atoms linking the ribose and the C4-imidazole. Imidazole nucleoside analogues with variable spacer lengths could provide a valuable general methodology for exploring the catalytic mechanisms of ribozymes.

Introduction

Chemogenetic methods have proved to be powerful in the study of RNA function and especially in the mechanistic dissection of RNA catalysis.[1] A significant body of evidence now points to the direct role of nucleobases in ribozyme mechanism, and general acid−base catalysis appears to be a major contributor to rate enhancement in the nucleolytic ribozymes.[2] Guanine nucleobases are important in the hammerhead[3] and GlmS[4] ribozymes, and guanine and adenine bases act jointly in the hairpin[5] and VS[6] ribozymes. In the HDV ribozymes, a cytosine base appears to play a similar role.[1c,7] One way to investigate the suspected role of a given nucleobase is to seek restoration of activity when the nucleoside in question

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(1) (a) Ryder, S. P.; Strobel, S. A. *J. Mol. Biol.* **1999**, *291*, 295–311. (b) Lebruska, L. L.; Kuzmine, I.; Fedor, M. J. *Chem. Biol.* **2002**, *9*, 465–473. (c) Das, S. R.; Piccirilli, J. A. *Nat. Chem. Biol.* **2005**, *1*, 45–52.

(2) *Ribozymes and RNA Catalysis*; Lilley, D. M. J., Eckstein, F., Eds.; Royal Society of Chemistry: Cambridge, 2008.

(3) (a) Han, J.; Burke, J. M. *Biochemistry* **2005**, *44*, 7864–7870. (b) Martick, M.; Scott, W. G. *Cell* **2006**, *126*, 309–320.

(4) Klein, D. J.; Ferré-D'Amaré, A. R. *Science* **2006**, *313*, 1752–1756.

(5) Rupert, P. B.; Massey, A. P.; Sigurdsson, S. T.; Ferré-D'Amaré, A. R. *Science* **2002**, *298*, 1421–1424.

(6) Wilson, T. J.; McLeod, A. C.; Lilley, D. M. J. *EMBO J.* **2007**, *26*, 2489–2500.

(7) (a) Ferré-d'Amaré, A. R.; Zhou, K.; Doudna, J. A. *Nature* **1998**, *395*, 567–574. (b) Nakano, S.; Chadalavada, D. M.; Bevilacqua, P. C. *Science* **2000**, *287*, 1493–1497.

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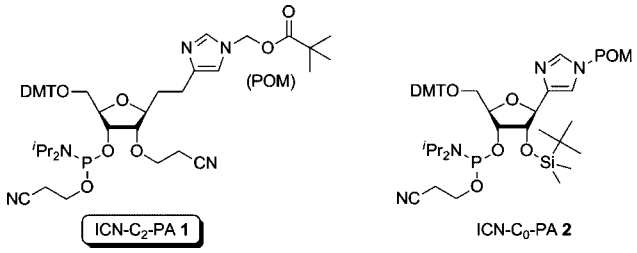


FIGURE 1. C4-linked C_2- and C_0-imidazole ribonucleoside PAs **1** and **2**.

is substituted, or the nucleobase ablated, by the addition of an exogenous base in the medium.[8] This approach provided convincing evidence for general acid−base catalysis in the HDV ribozyme, using exogenous imidazole bases.[9] However, success requires free access to the active center from the solvent, and that is not always possible. We therefore introduced an alternative approach, using a nucleoside analogue in which the nucleobase is replaced by imidazole. This may be incorporated as a phosphoramidite into RNA at a chosen site to investigate the role of the nucleobase normally residing at that position. If the substituted nucleobase participates in acid−base chemistry in the unmodified ribozyme, it is possible that imidazole assumes this role, leading to restoration of cleavage and ligation reactions. Moreover, the pH dependence of the reaction should change to reflect the pK_a of the imidazole nucleoside.

Introduction of a C-imidazole nucleoside provides a useful approach to study ribozyme function, as the pK_a values of the nucleobases are not optimal for general acid and base catalysis. Imidazole, having a pK_a close to 7, is an effective proton donor or acceptor in general acid and base catalysis. We previously reported the synthesis of a novel 2′-*O*-*tert*-butyldimethylsilyl (*t*BDMS) C4-linked imidazole ribonucleoside phosphoramidite (ICN-C_0-PA **2**)[10] (Figure 1). In that study, pivaloyloxymethyl (POM) was first introduced as an effective protecting group for the imidazole endocyclic amine that can be readily removed under mildly basic conditions [28% aq NH_3−EtOH (1:3, v/v)], compatible with 2′-*O*-*t*BDMS chemistry.[10] Using this method, we demonstrated the importance of a critical adenine base (A756) in VS ribozyme, showing that A756Imidazole ribozyme was active in both cleavage and ligation.[11] In another study, we showed that G8Imidazole hairpin ribozyme was active, exhibiting a rate that is pH dependent, consistent with proton transfer at the nucleobase at position 8.[12]

The VS ribozyme is the largest of all known nucleolytic ribozymes. It catalyzes the site-specific cleavage of a phosphodiester linkage to generate products containing 2′,3′-cyclic phosphate and 5′-OH termini.[13] Unfortunately, the VS ribozyme is the only member of the class of nucleolytic ribozymes for which there is no crystal structure at the present time, and therefore mechanistic investigation cannot be guided by structural data. In our ongoing studies of the catalytic mechanism

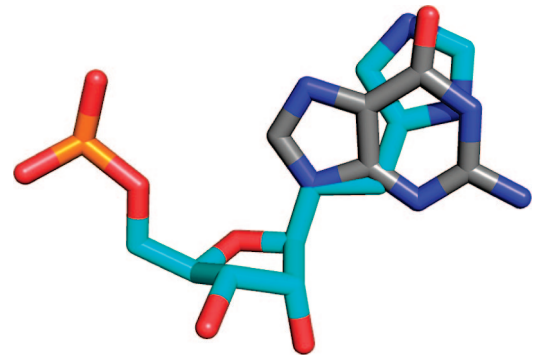


FIGURE 2. Superposition of molecular graphics structures of guanine and C_2-linked imidazole. The imidazole nucleoside was constructed so that the ethylene linker and imidazole nucleus were planar, and the ribose rings of both molecules were superimposed. This shows that the ring N of the imidazole readily achieves a similar position to that of guanine N1. By contrast, the imidazole of the C_0-linked species superimposes with the five-membered ring of the purine nucleus (not shown) so that the ring N is at least 2 Å from guanine N1. The structures are displayed using PyMol.

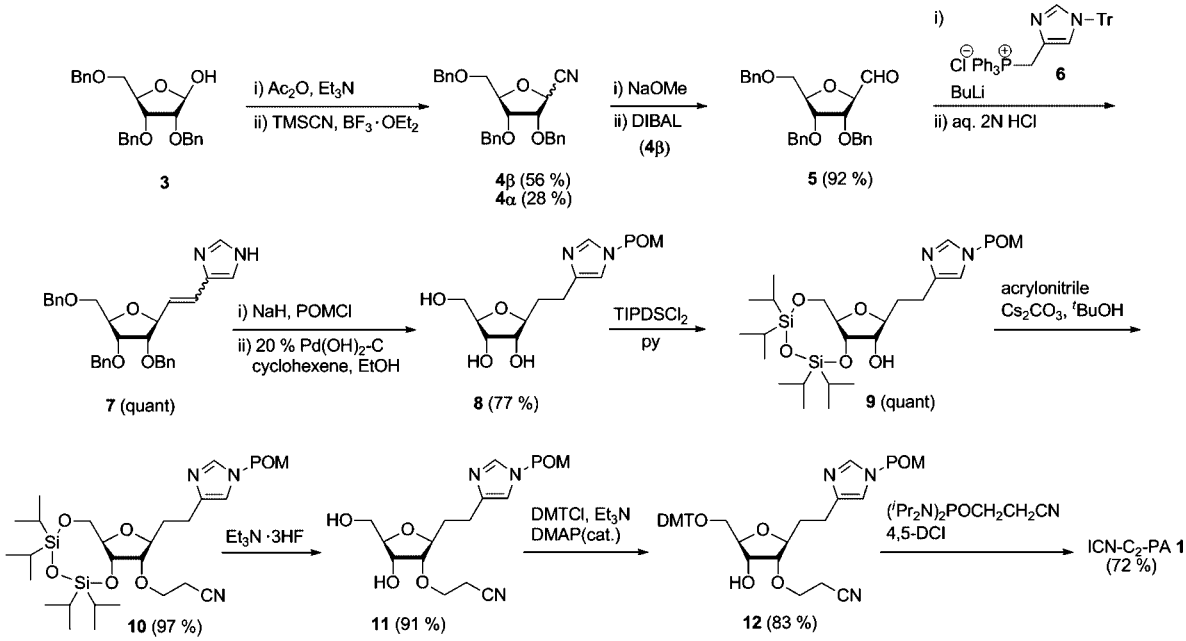
of VS ribozyme,[11,14] we recently identified a guanine (G638) in the substrate loop of the VS ribozyme as a second important nucleobase for general acid−base catalysis.[6] Functional group substitution at position 638 showed that the N1 position of the guanine base was critical, and the pH profile of the reaction reflected the pK_a of the base located at this position.[6] Thus the chemical mechanism appears to involve general acid−base catalysis via the combination of G638 and A756 nucleobases. These are juxtaposed with the scissile phosphate in our recent model of the VS ribozyme derived from small-angle X-ray scattering.[15]

In the course of these studies, we substituted our C_0-linked imidazole nucleoside at position 638 using ICN-C_0-PA **2** to probe the role of G638 in general acid−base catalysis by the VS ribozyme. However, the modified VS ribozyme (G638C_0Imz) exhibited virtually no restoration of cleavage activity, suggesting that neither of the two nitrogen atoms of the imidazole ring could be superimposed with the critical N1 position of the guanine base at position 638. This result was in contrast to the analogous substitution at position 756, suggesting that the environment at position 638 was more constrained, preventing the structural rearrangement that would be required to bring the imidazole base to the position normally occupied by the guanine N1. We therefore reasoned that the addition of methylene carbon atoms between the ribose and the imidazole might permit the base more readily to extend into the position that would allow it to participate in proton transfer with the RNA. Further, molecular graphics shows that the ring N atom of imidazole C_2-nucleoside and N1 of guanine can readily be superimposed (Figure 2), whereas these atoms are separated by ≥2 Å for the C_0-nucleoside in the absence of significant distortion.

Consequently, we designed and synthesized a two-carbon-elongated homologue (ICN-C_2-PA **1**) that may be a better structural mimic of purine nucleobase, as shown in Figure 1. In this article, we describe the chemical synthesis of the novel ICN-C_2-PA **1**. We have introduced a 2-cyanoethyl (CE) group

(8) Peracchi, A.; Beigelman, L.; Usman, N.; Herschlag, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11522–11527.
(9) Perrotta, A. T.; Shih, I.; Been, M. D. *Science* **1999**, *286*, 123–126.
(10) (a) Araki, L.; Harusawa, S.; Yamaguchi, M.; Yonezawa, S.; Taniguchi, N.; Lilley, D. M. J.; Zhao, Z.; Kurihara, T. *Tetrahedron Lett.* **2004**, *45*, 2657–2661. (b) Araki, L.; Harusawa, S.; Yamaguchi, M.; Yonezawa, S.; Taniguchi, N.; Lilley, D. M. J.; Zhao, Z.; Kurihara, T. *Tetrahedron* **2005**, *61*, 11976–11985.
(11) Zhao, Z.; McLeod, A.; Harusawa, S.; Araki, L.; Yamaguchi, M.; Kurihara, T.; Lilley, D. M. J. *J. Am. Chem. Soc.* **2005**, *127*, 5026–5027.
(12) Wilson, T. J.; Ouellet, J.; Zhao, Z.; Harusawa, S.; Araki, L.; Kurihara, T.; Lilley, D. M. J. *RNA* **2006**, *12*, 980–987.
(13) Saville, B. J.; Collins, R. A. *Cell* **1990**, *61*, 685–696.
(14) (a) Lafontaine, D. A.; Wilson, T. J.; Norman, D. G.; Lilley, D. M. J. *J. Mol. Biol.* **2001**, *312*, 663–674. (b) Lafontaine, D. A.; Wilson, T. J.; Zhao, Z.; Lilley, D. M. J. *J. Mol. Biol.* **2002**, *323*, 23–34.
(15) Lipfert, J.; Ouellet, J.; Norman, D. G.; Doniach, S.; Lilley, D. M. J. *Structure* **2008**, *16*, 1357–1367.

SCHEME 1. Synthesis of ICN-C_2-PA 1



recently developed by Sekine and co-workers[16] as a suitable protecting group for the 2′-hydroxyl function in RNA, together with the POM group that we developed earlier for the endocyclic amine of imidazole. With this compound in hand, we demonstrate the efficient syntheses of four RNA oligonucleotides using PA **1** and present preliminary results showing cleavage activity of G638C_2Imz-substituted VS ribozyme.

Results and Discussion

Synthesis of ICN-C_2-PA 1. Although the useful intermediate β-D-ribofuranosyl carbaldehyde **5** is a known compound,[17] we employed an alternative feasible approach (51.5% overall yield) starting from commercially available 2,3,5-tri-*O*-benzyl-D-ribose (**3**)[18] involving four steps. Acetylation of **3** and subsequent treatment with TMSCN/BF_3OEt_2 gave β-D-ribofuranosyl cyanide **4β** (56%)[19b] and its α-epimer **4α** (28%), which were easily separated by column chromatography using a modification of Yokoyama's procedure[19] (Scheme 1). Two-step conversion of the desired cyanide **4β** into aldehyde **5** was carried out via the methyl ester according to Koert's procedure.[20] In this case, direct DIBAL reduction of the cyanide **4β** into the aldehyde caused partial epimerization at the C1 position of **5**. Wittig olefination of **5** using *N*-1-trityl-4-imidazolylmethylphosphonium chloride **6**[21] followed by acid treatment to remove the trityl group provided two-carbon-elongated vinylimidazole **7** (*E*/*Z* = 2/1; quant). Introduction of a POM group at the ^{im}N position of **7** and subsequent debenzylation and reduction of double bond with $Pd(OH)_2$-C/cyclohexene produced *N*-POM-imidazole C_2-ribonucleoside **8** (77%), which was a partially protected derivative of ICN-C_2-PA **1**. Further, 3′,5′-*O*-TIPDS-protection (TIPDS = 1,1,3,3-tetraisopropyldisiloxanediyl) gave **9** (quant) that allowed selective introduction of the 2′-hydroxyl protecting group.[22]

We expended significant effort to find an appropriate 2′-*O*-protecting group (Scheme 2). Whereas the 4,4′-dimethoxytrityl (DMT) group is generally used to protect the 5′-OH of nucleoside 3′-*O*-phosphoramidites,[23] the selection of a 2′-*O*-protecting group was crucial in the chemical synthesis. Various 2′-*O*-protecting groups have been reported in the last ∼30 years.[24] Whereas the *t*BDMS group is the most widely used protecting group for the 2′-hydroxyl function, our initial approach was the selective protection of the 5′-OH of **8** to give the 5′-*O*-DMT compound **13**, followed by silylation with *t*BDMS triflate (Scheme 2, eq 1). However, this resulted in an inseparable 1:1 mixture **14ab** of 2′-*O*-*t*BDMS and 3′-*O*-*t*BDMS isomers arising from facile interconversion of the two isomers, as in the previous synthesis of ICN-C_0-PA **2**.[10] On the other hand, bis(2-acetoxyethoxy)methyl (ACE)[25] and triisopropylsilyloxymethyl (TOM)[26] groups have emerged recently as 2′-hydroxyl-protecting groups in practical RNA chemical synthesis.

(16) (a) Saneyoshi, H.; Ando, K.; Seio, K.; Sekine, M. *Tetrahedron* **2007**, *63*, 11195–11203. (b) Saneyoshi, H.; Seio, K.; Sekine, M. *J. Org. Chem.* **2005**, *70*, 10453–10460.

(17) (a) Dondoni, A.; Formaglio, P.; Marra, A.; Massi, A. *Tetrahedron* **2001**, *57*, 7719–7727. (b) Dondoni, A.; Scherrmann, M.-C. *J. Org. Chem.* **1994**, *59*, 6404–6412. (c) Dondoni and co-workers reported a synthetic method[17a,b] to generate aldehyde **5** from 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone by three-step route in 44% overall yield.

(18) 2,3,5-Tri-*O*-benzyl-D-ribofuranose **3** was purchased from Carbosynth Ltd.

(19) (a) Togo, H.; Ishigami, S.; Fujii, M.; Ikuma, T.; Yokoyama, M. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2931–2942. (b) Yokoyama and co-workers reported a cyanation giving an anomeric mixture (88%) of **4α** and **4β** in 75:25 ratio at 0 °C.[19a] When we carried out the same reaction at −48 °C, **4β** was obtained in 56% yield.

(20) Koert, U.; Stein, M.; Wagner, H. *Liebigs Ann* **1995**, 1415–1426.

(21) (a) Harusawa, S.; Kawamura, M.; Koyabu, S.; Hosokawa, T.; Araki, L.; Sakamoto, Y.; Hashimoto, T.; Yamamoto, Y.; Yamatodani, A.; Kurihara, T. *Synthesis* **2003**, 2844–2850. (b) Recrystallized phosphonium salt **6** (mp 264−265 °C, MeOH/EtOAc = 1:1) should be used to sustain the reproducibility of this reaction.

(22) Markiewicz, W. T. *J. Chem. Res. (S)* **1979**, 24–25.

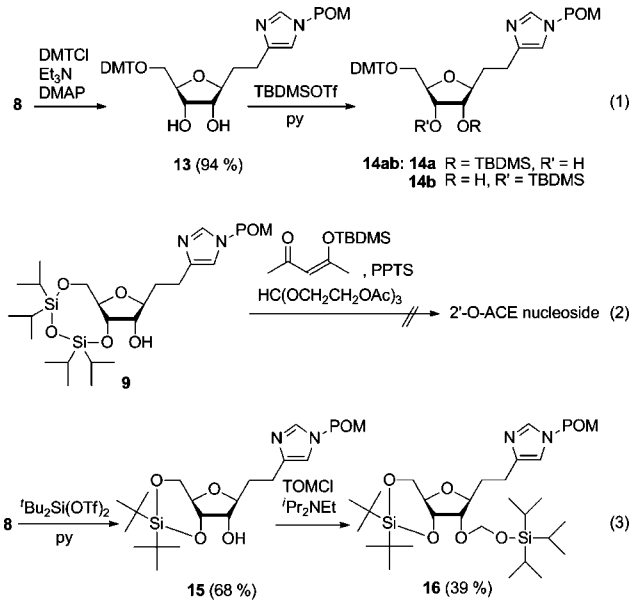
(23) Seliger, H. In *Current Protocols in Nucleic Acid Chemistry*; Beaucage, S. L., Bergstrom, D. E., Glick, G. D., Jones, R. A., Eds.; John Wiley & Sons: New York, 2000; pp 2.3.1−2.3.34.

(24) Reese, C. B. In *Current Protocols in Nucleic Acid Chemistry*; Beaucage, S. L., Bergstrom, D. E., Glick, G. D., Jones, R. A., Eds.; John Wiley & Sons: New York, 2000; pp 2.2.1−2.2.24.

(25) (a) Scaringe, S. A.; Wincott, F. E.; Caruthers, M. H. *J. Am. Chem. Soc.* **1998**, *120*, 11820–11821. (b) Scaringe, S. A. *Methods Enzymol.* **2000**, *317*, 3–19.

(26) (a) Pitsch, S.; Weiss, P. A.; Jenny, L.; Stutz, A.; Wu, X. *Helv. Chim. Acta* **2001**, *84*, 3773–3795. (b) Pitsch, S.; Weiss, P. A. In *Current Protocols in Nucleic Acid Chemistry*; Beaucage, S. L., Bergstrom, D. E., Glick, G. D., Jones, R. A., Eds.;John Wiley & Sons: New York, 2001; pp 3.8.1−3.8.15.

SCHEME 2. Studies of 2′-*O*-Protecting Group for ICN-C_2-PA 1



Unfortunately, the reaction of 3′,5′-*O*-protected compound **9** with tris(2-acetoxyethoxy)orthoformate under standard conditions did not proceed (Scheme 2, eq 2). Furthermore, the reaction of 3′,5′-*O*-di-*tert*-butylsilanediyl (DTBS) protected precursor **15**[27] with TOMCl in the presence of *N*,*N*-diisopropylethylamine provided 2′-*O*-TOM nucleoside **16** in only low yield (eq 3). From these results, it appeared that the introduction of bulky protecting groups to the ICN-PAs is responsible for the reaction failures, unlike in the cases of conventional *N*-nucleosides. Consequently, there is a need for small temporary substituents with little steric hindrance for 2′-*O*-protection.

During the course of our investigation, Sekine and co-workers reported a new method for the synthesis of oligoribonucleotides using the CE group, that is, the smallest of the available protecting groups for the 2′-hydroxyl group.[16] Migration of the protecting group between 2′- and 3′-positions is inhibited, and it can be removed using 1 M TBAF in THF. Therefore, our attention was directed to the synthesis of novel 2′-*O*-CE-protected PA **1** (Scheme 1). Cyanoethylation of **9** with acrylonitrile in *t*-BuOH in the presence of Cs_2CO_3 at room temperature (rt) successfully afforded fully protected intermediate **10** in excellent yield (97%). The TIPDS group of **10** was selectively removed by treatment with $Et_3N \cdot 3HF$ to give 3′,5′-*O*-unprotected ribonucleoside derivative **11** (91%).[16] Although the ^{im}*N*-POM group was readily removed under the mildly basic conditions as described previously, it remained intact during cyanoethylation and removal of the TIPDS group, showing the added advantage of using this group. After dimethoxytritylation of **11**, 3′-hydroxyl derivative **12** was subjected to phosphitylation. Treatment of **12** with 2-*O*-cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphodiamidite in the presence of 4,5-dicyanoimidazole (DCI) in dichloroethane proceeded smoothly at 40 °C for 20 min to give the final product ICN-C_2-PA **1**. Purification of the crude product was easily carried out on basic column chromatography to yield phosphoramidite **1** (72%) as a white foam. An overall yield of 23% was achieved in 13 steps from readily available starting material 2′,3′,5′-*O*-tribenzyl D-ribose **3**, making it a feasible approach for scaling up synthesis of **1**.

(27) Furusawa, K.; Ueno, K.; Katsura, T. *Chem. Lett.* **1990**, 97–100.

^{31}P NMR data of **1** indicated a pair of P diastereoisomers at δ 149.2 and 150.0 ppm ($CDCl_3$). We recently reported a reliable MS measurement of nucleoside or non-nucleoside phosphoramidites using a matrix system [triethanolamine (TEOA)−NaCl] on LSIMS or FABMS equipped with a double-focusing mass spectrometer.[28] The present method successfully revealed the molecular-related ion of **1** at *m*/*z* 920.4336, leading to the chemical formula $C_{49}H_{64}N_5O_9P$ + Na. To check the stability of PA **1** in CH_3CN used in automated RNA synthesis, a CD_3CN solution of **1** was allowed to stand at rt in an NMR tube. After 1 week, ^{1}H, ^{13}C, and ^{31}P NMR measurements of the solution did not show any significant decomposition, indicating that the solution of **1** in CH_3CN is stable. Furthermore, phosphoramidite **1** could be stored as a white foam in a vial at rt for nearly 1 month without significant spectral changes.

Synthesis of Oligoribonucleotides Containing C_2-Imidazole Substitution. The efficiency of ICN-C_2-PA **1** in automated RNA synthesis was examined. Four RNA species of lengths between 25 and 36 nt were synthesized, each containing a single C_2-linked imidazole nucleotide at a given position. The RNA was synthesized using *t*-BDMS-protected phosphoramidites,[29] using a DNA/RNA synthesizer, with a coupling time of 12 min for the imidazole nucleoside phosphoramidite. Measurement of trityl cation conductivity indicated a step yield of >95% for the coupling of the C_2-linked imidazole phosphoramidite. Gel electrophoretic separation under denaturing conditions of the crude synthesis following deprotection showed substantial full-length products with no evidence of strong bands that would correspond to failure at the position of the modified nucleotide (Figure 3).

The RNA sequences synthesized were (1) hairpin ribozyme a strand G8C_2Z (CCGACAGAZAAGUCAACCAGAGAAA-CACACUUGCGg), (2) hairpin ribozyme b strand A38C_2Z (CCGCAAGUGGUZUAUUACCUGGUACGCG-UUCACGg), (3) VS substrate G638C_2Z (GCGCGAAGGGCGUC-GUCGCCCCZAt), (4) VS lower strand A756C_2Z (AACG-CAGUAUUGCZGCACAGCACAAGCCCGCUUGc), where Z designates the C_2-imidazole nucleoside, and letters shown in lower case are 3′-deoxyribonucleosides arising from the support.

Ribozyme Activity of G638C_2Imz. One of the RNA oligonucleotides synthesized was based on the substrate of the VS ribozyme, with a single imidazole substitution at position 638. G638 is one of the nucleobases proposed to act in general acid−base catalysis in the catalytic mechanism of the ribozyme.[6] After purification, we tested the ribozyme activity of the modified substrate in a cleavage reaction with unmodified VS ribozyme in *trans*. The C_2-linked imidazole-containing substrate was incubated with 1 μM *trans* VS ribozyme under single turnover conditions, in the presence of 50 mM MES-HCl (pH 6.5), 200 mM $MgCl_2$, 25 mM KCl at 37 °C, and the products were analyzed by gel electrophoresis (Figure 4A). Extended incubation under these conditions resulted in 70% ribozyme cleavage at the normal site. Reaction progress is plotted in Figure

(28) (a) Fujitake, M.; Harusawa, S.; Araki, L.; Yamaguchi, M.; Lilley, D. M. J.; Zhao, Z.; Kurihara, T. *Tetrahedron* **2005**, *61*, 4689–4699. (b) Fujitake, M.; Harusawa, S.; Zhao, Z.; Kurihara, T. *Bull. Osaka Univ. Pharm. Sci.* **2007**, *1*, 107–112. (c) Harusawa, S.; Fujitake, M.; Kurihara, T.; Zhao, Z.; Lilley, D. M. J. In *Current Protocols in Nucleic Acid Chemistry*; Beaucage, S. L., Bergstrom, D. E., Herdewijn, P., Matsuda, A., Eds.; John Wiley & Sons: New York, 2006; pp 10.11.1−10.11.15.

(29) (a) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859–1862. (b) Usman, N.; Pon, R. T.; Ogilvie, K. K. *Tetrahedron Lett.* **1985**, *26*, 4567–4570. (c) Damha, M. J.; Ogilvie, K. K. In *Methods in Molecular Biology. Protocols for Oligonucleotides and Analogs*; AgrawalS., Ed.; Humana Press: Totowa, NJ, 1993; Vol. 20, Chapter 5.

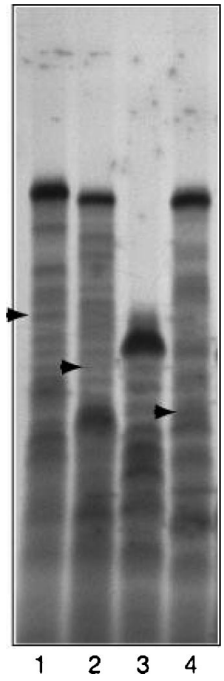


FIGURE 3. Analysis of the crude products of RNA synthesis by gel electrophoresis. After deprotection, ~120 μg aliquots of the synthetic RNA were applied to a 16% polyacrylamide gel containing 7 M urea in 90 mM Tris-borate (pH 8.3), 10 mM EDTA. The RNA was visualized by UV shadowing. The arrowheads mark the position corresponding to the incorporation of C_2-linked imidazole. The RNA species were (1) hairpin ribozyme strand a, 36 nt; (2) hairpin ribozyme strand b, 35 nt; (3) VS ribozyme substrate, 25 nt (C_2-linked imidazole is located three nucleotides from the 3 terminus, which is not resolved by the gel and therefore not observable in the image); and (4) VS ribozyme 6-2 strand, 35 nt.

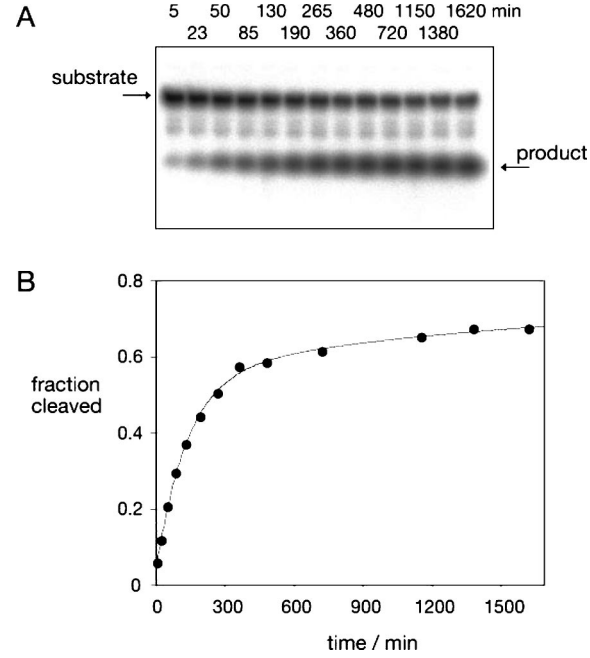


FIGURE 4. Cleavage activity of a VS ribozyme substrate with C_2-linked imidazole replacing guanine at position 638. (A) Electrophoretic separation of substrate and product during the course of the incubation with VS ribozyme over a time course of 27 h. Times of incubation (min) are indicated over each track. Radioactive 5′-^{32}P-labeled substrate RNA was incubated with 1 μM VS ribozyme in 50 mM MES-HCl (pH 6.5), 200 mM $MgCl_2$, and 25 mM KCl at 37 °C; aliquots were removed at the times indicated, and the reaction was terminated with EDTA. These were separated by electrophoresis in a 20% polyacrylamide gel. The substrate and product were visualized and quantified by phosphorimage analysis. (B) Plot of the extent of cleavage as a function of time, using the data from (A). The data (points) have been fitted (line) to a rate equation with two exponential functions, from which rates of 0.0076 and 0.0007 min^{-1} were calculated.

4B. Fitting the data required two exponential functions, corresponding to rates of 0.0076 and 0.0007 min^{-1} (Table 1). The faster rate is 15-fold greater than that achieved with ribozyme containing the C_0-linked imidazole at the same position. Incubations were also performed in 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 25 mM KCl, and 2 mM spermidine. Rates of cleavage were 10-fold slower, but the ratio of rates between the two imidazole substituents was closely similar at 14. Thus the additional linker length between the ribose and imidazole has resulted in significantly greater catalytic activity of the ribozyme.

TABLE 1. Comparison of Cleavage Rates between G638C_2Imz and G638C_0Imz

	rates/min^{-1} natural	G638C_2Imz	G638C_0Imz	C_2Imz/C_0Imz
10 mM Mg^{2+} pH 8				
- Rz		6×10^{-5}	5×10^{-5}	
+ 1 μM Rz	0.61	0.001	7×10^{-5}	14
200 mM Mg^{2+} pH 6.5				
- Rz		1×10^{-5}	2×10^{-5}	
+ 1 μM Rz	6.2	0.008	5×10^{-4}	15

Conclusions

ICN-C_2-PA **1** having a two-carbon linker between imidazole and ribose moieties was designed as a novel purine nucleobase mimic and was successfully synthesized from 2,3,5-tri-*O*-benzylribofuranose **3**. We have demonstrated the feasibility of preparing stable 2′-*O*-cyanoethylated PA **1**, and we see no impediment to increasing the scale of the synthesis to meet the needs of RNA structural studies. Furthermore, we have clarified that the CE group is an efficient protecting group for the 2′-hydroxyl group and reinforced that the POM group could be used for the protection of imidazole endocyclic amino function of ICN-PAs. The phosphoramidites with these modifications are compatible with 2′-TBDMS chemistry for RNA synthesis. The synthetic method using the CE and POM protecting groups may be generally applicable to the PAs of ribose-$(CH_2)_n$-imidazole species. When ICN-C_2-PA **1** was subjected to automated RNA synthesis using TBDMS methodology, it provided C_2-imidazole modified ribozymes in >95% single stepwise coupling yield with other RNA bases coupling over 98% on average. This promises to open a more practical pathway to this chemical strategy for investigating RNA functionality. Of particular interest is that the modified VS ribozyme (G638C_2Imz) incorporated from ICN-C_2-PA **1** showed significantly greater catalytic activity than G638C_0Imz. Further work on the application of imidazole oligonucleotides is underway, and the characteristics of the modified VS ribozyme will be published in due course.

Experimental Section

Chemical Synthesis of Oligoribonucleotides. Oligoribonucleotides were synthesized using *t*-BDMS-protected phosphoramidites[29] implemented on an DNA/RNA synthesizer. LV200 columns

(Applied Biosystems) introduced a deoxyribonucleotide at the 3′ end of each oligonucleotide. Imidazole phosphoramidites were dissolved in anhydrous acetonitrile as a 0.1 M solution less than 30 min before coupling. An equal volume of 0.25 M 4,5-dicyanoimidazole was employed as activator, and coupling times of 12 min were used. A 0.02 M iodine/water solution was used for phosphorus oxidation at each step of synthesis. RNA was deprotected using ammonia/ethanol (3:1, v/v) solution at 55 °C for 2 h, followed by treatment with 1 M TBAF in THF under standard conditions. RNA oligonucleotides were separated by electrophoresis in a 15% polyacrylamide gel containing 7 M urea with 90 mM Tris-borate (pH 8.3), 10 mM EDTA buffer, for 2.5 h at 20 W. RNA was visualized by UV shadowing.

Preparation of VS Ribozyme by Transcription. Templates for transcription of ribozymes were made by recursive PCR from synthetic DNA oligonucleotides. RNA was synthesized using T7 RNA polymerase and purified by electrophoresis in 5% polyacrylamide gels containing 7 M urea. RNA was recovered by electroelution into 8 M ammonium acetate. The sequence of the *trans*-acting ribozyme[30] was GCGGUAGUAAGCAGGGAACUCACCUCCAAUUUCAGUACUGAAAUUGUCGUAGCAGUUGACUACUGUUAUGUGAUUGGUAGAGGCUAAGUGACGGUAUUGGCGUAAGUCAGUAUUGCAGCACAGCACAAGCCCGCUUGCGAGAAU.

Analysis of VS Ribozyme Kinetics. Cleavage kinetics were studied under single-turnover conditions.[6] Ribozyme and 5′-^{32}P-labeled substrate were incubated separately at 37 °C for 20 min in reaction buffer, and the reaction was initiated by adding an equal volume of ribozyme to the substrate tube. The final reaction contained 1 μM ribozyme and 10 nM substrate. Two buffer conditions were used, either 50 mM Tris (pH 8), 10 mM $MgCl_2$, 25 mM KCl, 2 mM spermidine, or 50 mM MES (pH 6.5), 200 mM $MgCl_2$, 25 mM KCl. Mineral oil was layered on top to prevent evaporation. Two microliter aliquots were removed at intervals, and the reaction was terminated by addition to 8 μL of a mixture containing 95% (v/v) formamide, 50 mM EDTA, and electrophoresis dyes. Substrate and product were separated by electrophoresis in 20% polyacrylamide gels containing 7 M urea and quantified by phosphorimaging. Progress curves were fitted by nonlinear regression analysis to exponential functions using Kalaidagraph.

4-[(*E*,*Z*)-2-(2,3,5-Tri-*O*-benzyl-β-D-ribofuranos-1-yl)vinyl]-1*H*-imidazole [(*E*,*Z*)-7]. A 1.6 M BuLi solution in hexane (4.4 mL, 7.0 mmol) was added dropwise over a period of 15 min to a white suspension of phosphonium salt **6**[21b] (4.34 g, 7.0 mmol) in dry THF (60 mL) at −70 °C. The resulting yellow suspension was stirred for 30 min at the same temperature, and a solution of aldehyde **5** (1.51 g, 3.5 mmol) in THF (20 mL) was added slowly to keep the temperature of the suspension at approximately −70 °C. The reaction mixture was elevated to rt, where it was transformed into a yellow solution, and continued to stir for 1 h at rt. The reaction was quenched by the addition of water (1 mL) and evaporated. The residue was subsequently dissolved in $CHCl_3$ (50 mL), and the organic layer was washed with water, dried over anhydrous $MgSO_4$, and evaporated. The residue was subjected to column chromatography with EtOAc/hexane (40:60) as eluent using the coated silica gel technique to give a crude pale yellow oil {ca. 2.60 g; 4-[(*E*,*Z*)-2-(2,3,5-tri-*O*-benzyl-β-D-ribofuranos-1-yl)vinyl]-1-tritylimidazole containing a small amount of $Ph_3P=O$}. Aqueous 2 N HCl (20 mL) was added to the solution of partially purified oil in EtOH (24 mL), and the solution was refluxed for 2 h. After cooling to rt, the precipitated material ($Ph_3P=O$) was removed by filtration. The filtrate was evaporated to give a residue that was subsequently diluted with water (20 mL) and neutralized by the addition of saturated aq $NaHCO_3$ solution. The mixture was extracted with EtOAc (2 × 50 mL), and the combined organic layers were washed with H_2O, dried, and evaporated to yield a residue. Chromatography purification on silica with EtOAc as eluent gave a 2:1 mixture (1.74 g, quant) of (*E*)-**7** and (*Z*)-**7**. Although the separation of (*E*)-**7** and (*Z*)-**7** was not required for the following experiment, they could be partially isolated by chromatography. **(*E*)-7:** oil; R_f = 0.52 (12% MeOH/EtOAc); ^{1}H NMR ($CDCl_3$) δ 3.55 (dd, 1H, J = 10.3, 3.4 Hz), 3.59 (dd, 1H, J = 10.3, 3.4 Hz), 3.76 (t, 1H, J = 5.1 Hz), 3.93 (t, 1H, J = 3.4 Hz), 4.24 (q, 1H, J = 3.4 Hz), 4.44−4.60 (m, 7H), 6.08 (dd, 1H, J = 15.5, 6.8 Hz, C<u>H</u>=CH−Im), 6.54 (d, 1H, J = 15.5 Hz, CH=C<u>H</u>−Im), 6.80 (s, 1H), 7.20−7.35 (m, 15H), 7.40 (s, 1H); HRMS calcd for $C_{31}H_{32}N_2O_4$ (M^+) 496.2360, found 496.2364. **(*Z*)-7:** oil; R_f = 0.58 (12% MeOH/EtOAc); ^{1}H NMR ($CDCl_3$) δ 3.52 (dd, 1H, J = 10.0, 2.5 Hz), 3.58 (dd, 1H, J = 10.0, 2.5 Hz), 3.86 (dd, 1H, J = 7.5, 5.0 Hz), 4.02 (dd, 1H, J = 5.0, 2.5 Hz), 4.26 (q, 1H, J = 2.5 Hz), 4.46−4.69 (m, 6H), 4.83 (t, 1H, J = 7.5 Hz), 5.49 (dd, 1H, J = 11.6, 7.5 Hz, C<u>H</u>=CH−Im), 6.46 (d, 1H, J = 11.6 Hz, CH=C<u>H</u>−Im), 7.00 (s, 1H), 7.20 (s, 1H), 7.25−7.40 (m, 15H); HRMS calcd for $C_{31}H_{32}N_2O_4$ (M^+) 496.2360, found 496.2363. ^{13}C NMR of a 2:1 mixture of (*E*)-**7** and (*Z*)-**7** was measured: ^{13}C NMR ($CDCl_3$) of (*E*,*Z*)-**7** δ 70.4, 72.0, 72.2, 72.8, 73.4, 73.6, 77.2, 77.6, 78.1, 80.9, 81.4, 81.9, 82.2, 82.3, 118.6, 120.1, 121.7, 125.2, 126.0, 127.2, 127.3 (127.28), 127.3 (127.31), 127.4 (127.37), 127.4 (127.40), 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 134.4, 134.9, 135.4, 136.4, 137.1, 137.2, 137.3, 137.4, 137.7.

{4-[2-(β-D-Ribofuranos-1-yl)ethyl]imidazolyl}methyl 2,2-dimethylpropionate (8). NaH (60%, 161 mg, 4.0 mmol) in mineral oil was added to THF (20 mL) while stirring to give a suspension. A solution of (*E*,*Z*)-**7** (1.33 g, 2.7 mmol) in THF (30 mL) was added to the suspension, and the resulting mixture was stirred at rt for 40 min. A solution of chloromethyl pivaloate (607 mg, 4.0 mmol) in THF (30 mL) was then added. After stirring for 2 h, H_2O (0.5 mL) was added followed by evaporation to a residue that was subsequently dissolved in EtOAc. The organic layer was washed with water and brine, dried, and evaporated. The crude product was purified by column chromatography on silica gel using 50% EtOAc in hexane to give (*E*,*Z*)-{4-[(*E*,*Z*)-2-(2,3,5-tri-*O*-benzyl-β-D-ribofuranos-1-yl)vinyl]imidazolyl}methyl 2,2-dimethylpropionate [**17**, 1.49 g, 92%, *E*/*Z* = 2/3] as a colorless oil. The 2:3 ratio of the *E* and *Z* isomers of **17** was assigned based on the following ^{1}H NMR data: ^{1}H NMR ($CDCl_3$) δ 1.04 [s, 5.4H, $C(CH_{3a})_3$], 1.17 [s, 3.6H, $C(CH_{3b})_3$], 3.52−3.64 (m, 2H), 3.81 (t, 0.4H, J = 5.6 Hz), 3.86 (t, 0.6H, J = 5.6 Hz), 3.94 (t, 0.4H, J = 5.6 Hz), 4.01 (t, 0.6H, J = 5.6 Hz), 4.22−4.30 (m, 1H), 4.47−4.76 (m, 6.4H), 5.31 (dd, 0.6H, J = 9.6, 5.6 Hz), 5.57 (dd, 0.6H, J = 12.4, 9.6 Hz, C<u>H</u>$_a$=CH−Im), 5.72 (q, 1.2H, J = 11.5 Hz), 5.76 (s, 0.8H), 6.34 (dd, 0.4H, J = 15.3, 7.6 Hz, C<u>H</u>$_b$=CH−Im), 6.45 (d, 0.6H, J = 12.4 Hz, CH=C<u>H</u>$_a$−Im), 6.58 (d, 0.4H, J = 15.3 Hz, CH=C<u>H</u>$_b$−Im), 6.83 (s, 0.4H), 7.24−7.35 (m, 15.6H), 7.61 (s, 0.4H), 7.64 (s, 0.6H); ^{13}C NMR ($CDCl_3$) δ 27.2, 39.0, 67.7, 67.8, 70.3, 70.4, 71.9, 72.0, 72.1, 72.2, 73.4, 73.5, 77.4, 77.6, 78.1, 80.8, 81.1, 81.2, 81.8, 82.1, 108.9, 117.0, 118.7, 122.6, 123.9, 127.1, 127.1, 127.2, 127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 127.9, 127.9, 128.8, 137.4, 137.5, 137.8, 137.9, 138.4, 139.7, 176.9 (COO, overlapped); HRMS calcd for $C_{37}H_{42}N_2O_6$ (M^+) 610.3040, found 610.3032. Next, a mixture of (*E*,*Z*)-methyl 2,2-dimethylpropionate (165 mg, 0.27 mmol), 20% $Pd(OH)_2$−C (99 mg), and cyclohexene (0.82 mL, 8.1 mmol) in EtOH (7 mL) was refluxed for 1 h. After filtration through Celite, the filtrate was evaporated to give a residue, which was purified by silica column chromatography [MeOH/EtOAc (1/9)] to give **8** (77 mg, 84%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.15 (s, 9H), 1.72−1.86 (m, 1H), 1.86−2.00 (m, 1H), 2.55−2.77 (m, 2H), 3.56 (dd, 1H, J = 11.4, 5.2 Hz), 3.64−3.80 (m, 4H), 3.92 (t, 1H, J = 5.2 Hz), 5.89 (s, 2H), 6.99 (s, 1H), 7.72 (s, 1H); ^{13}C NMR (CD_3OD) δ 25.3, 27.3, 34.5, 39.8, 63.5, 69.1, 72.7, 76.1, 83.1, 85.3, 116.7, 138.7, 142.8, 178.0 (COO); HRMS (EIMS) calcd for $C_{16}H_{27}N_2O_6$ $(M + H)^+$ 343.1867, found 343.1863.

{4-[2-(3,5-*O*-TIPDS-β-D-ribofuranos-1-yl)ethyl]imidazolyl}methyl 2,2-dimethylpropionate (9). 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (0.064 mL, 0.2 mmol) was added dropwise to a solution of

(30) Beattie, T. L.; Olive, J. E.; Collins, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4686–4690.

8 (35 mg, 0.1 mmol) in pyridine (1.5 mL) at 0 °C. The resulting mixture was stirred for 1 h at the same temperature and then at rt for 2 h. After evaporation, the crude product was purified by column chromatography [EtOAc/hexane (4/1)] to yield **9** (60 mg, quant) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.95−1.10 (br s, 28H), 1.17 (s, 9H), 1.78−2.07 (m, 2H), 2.60−2.77 (m, 2H), 3.77−3.91 (m, 4H), 4.01 (dd, 1H, J = 11.3, 3.3 Hz), 4.21 (t, 1H, J = 6.6 Hz), 5.77 (s, 2H), 6.81 (s, 1H), 7.60 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.2, 13.6, 13.8, 17.40, 17.46, 17.49, 17.6, 17.7, 17.9, 24.6, 27.1, 33.4, 39.0, 63.1, 67.8, 72.6, 74.6, 77.2, 82.0, 83.5, 115.2, 137.1, 142.4, 176.9; HRMS (EIMS) calcd for $C_{28}H_{52}N_2O_7Si_2$ (M^+) 584.3310, found 584.3317.

{4-[2-(2-*O*-CE-3,5-*O*-TIPDS-β-D-ribofuranos-1-yl)ethyl]imidazolyl}methyl 2,2-dimethylpropionate (10). Acrylonitrile (0.27 mL, 4.0 mmol) and cesium carbonate (67.3 mg, 0.203 mmol) were added to a solution of **9** (118 mg, 0.2 mmol) in *t*-butanol (1.0 mL). After vigorously stirring at rt for 6 h, the mixture was filtered through Celite. The filtrate was evaporated to a residue that was purified by column chromatography with EtOAc/hexane (50:50 to 100:0, v/v) as eluent to give **10** (124.6 mg, 97%) as an oil: IR (film, cm^{-1}) 1735 (CO−O), 2250 (CN); ^{1}H NMR ($CDCl_3$) δ 0.86−1.08 (m, 28H), 1.12 (s, 9H), 1.71−1.96 (m, 2H), 2.48−2.64 (m, 4H), 3.52−4.17 (m, 8H), 5.70 (s, 2H), 6.75 (s, 1H), 7.51 (s,1H); ^{13}C NMR ($CDCl_3$) δ 13.0, 13.2, 13.5, 13.9, 17.3, 17.5, 17.6, 17.67, 17.73, 17.8, 19.6, 24.5, 27.1, 34.1, 38.9, 60.5, 65.6, 67.7, 72.1, 80.0, 82.6, 83.2, 115.3, 117.4, 137.1, 142.0, 176.8; HRMS (FABMS: NBA) calcd for $C_{31}H_{56}N_3O_7Si_2$ ($M + H$)$^+$ 638.3656, found 638.3658.

{4-[2-(2-*O*-CE-β-D-ribofuranos-1-yl)ethyl]imidazolyl}methyl 2,2-dimethypropionate (11). $Et_3N\cdot 3HF$ (0.11 mL, 0.7 mmol) and Et_3N (0.05 mL, 0.35 mmol) were added to a solution of compound **10** (125 mg, 0.2 mmol) in THF (2.0 mL). The mixture was stirred at rt for 1.5 h and was evaporated. The residue was subjected to chromatography with $CHCl_3$/MeOH (100:0 to 95:5, v/v) to give **11** (70 mg, 91%) as an oil: IR (film, cm^{-1}) 1735 (CO−O), 2250 (CN); ^{1}H NMR ($CDCl_3$) δ 1.18 (s, 9H), 1.93 (m, 2H), 2.57−2.80 (m, 2H), 2.66 (t, 2H), 3.60−3.98 (m, 7H), 4.20 (t, 1H, J = 5.2 Hz), 5.78 (s, 2H), 6.83 (s, 1H), 7.61 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.0, 24.9, 27.7, 34.0, 39.5, 62.5, 65.6, 68.3, 71.2, 80.9, 83.8, 84.6, 115.9, 117.9, 137.6, 142.5, 177.5; HRMS (FABMS: NBA) calcd for $C_{19}H_{30}N_3O_6$ ($M + H$)$^+$ 396.2135, found 396.2134.

{4-[2-(5-*O*-DMT-2-*O*-CE-β-D-ribofuranos-1-yl)ethyl]imidazolyl}-methyl 2,2-dimethylpropionate (12). Compound **11** (30 mg, 0.08 mmol) was coevaporated three times with pyridine (1 mL) and redissolved in dry pyridine (0.5 mL). DMTCl (41 mg, 0.12 mmol), Et_3N (0.02 mL, 0.115 mmol), and DMAP (0.2 mg, 0.002 mmol) were added to the solution. After stirring at rt for 2.5 h, methanol (0.5 mL) was added. The solvents were removed to give a residue that was purified by column chromatography (NH/silica gel) using a gradient solvent system [$CHCl_3$/benzene (30:70, 50:50, 70:30 to 100:0)] as eluent to give compound **12** (44.3 mg, 83%) as white foam: ^{1}H NMR ($CDCl_3$) δ 1.15 (s, 9H), 1.84−2.12 (m, 2H), 2.44−2.85 (m, 2H), 2.63 (t, 2H, J = 6.0 Hz), 3.16 (dd, 1H, J = 10.1, 4.7 Hz), 3.29 (dd, 1H, J = 10.1, 3.4 Hz), 3.62−3.84 (m, 2H), 3.67 (t, 1H, J = 5.4 Hz), 3.78 (s, 6H), 3.94 (m, 2H), 4.09 (t, 1H, J = 5.4 Hz), 5.76 (s, 2H), 6.81 (d, 4H, J = 8.3 Hz), 7.14−7.48 (m, 10H), 7.62 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 19.5, 24.7, 27.2, 30.0, 33.8, 39.0, 55.3, 64.2, 65.1, 67.7, 71.7, 79.8, 82.8, 83.5, 85.9, 112.7, 115.4, 117.2, 126.3, 127.4, 127.8, 129.7, 135.5, 135.6, 137.1, 141.9, 144.4, 157.8, 176.9; IR (film, cm^{-1}) 1735 (CO−O), 2250 (CN); HRMS (FABMS: NBA) calcd for $C_{40}H_{48}N_3O_8$ ($M + H$)$^+$ 698.3441, found 698.3445.

[4-(2-{5-*O*-DMT-2-*O*-(2-cyanoethyl)-3-*O*-[(2-cyanoethyl)-(*N*,*N*-diisopropylamino)phosphoramidyl]-β-D-ribofuranos-1-yl}ethyl)imidazolyl]methyl 2,2-dimethylpropionate (1). 2-Cyanoethyl *N,N,N′,N′*-tetraisopropylphosphodiamidite (0.04 mL, 0.121 mmol) and 4,5-DCI (9.0 mg, 0.073 mmol) were added to a solution of compound **12** (42.2 mg, 0.061 mmol) in dry dichloroethane (0.7 mL) under an argon atmosphere. The resulting mixture was stirred at 40 °C for 20 min and then evaporated. The residual oil was subjected to NH/silica gel chromatography using a solvent system (30% and then 50% EtOAc in hexane) to give **1** (39.1 mg, 72%) as a white foam: R_f = 0.73, 0.63 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.11−1.18 (overlapping peaks, 23H), 1.94 (m, 1H), 2.08 (m, 1H), 2.28 (t, 0.8H, J = 6.2 Hz), 2.50−2.90 (m, 5.2H), 3.07 (dt, 1H, J = 10.3, 3.4 Hz), 3.26 (dd, 0.6H, J = 10.3, 3.4 Hz), 3.34 (dd, 0.4H, J = 10.3, 3.4 Hz), 3.46−3.91 (m, 5H), 3.78 (s, 6H), 3.98 (m, 1H), 4.13 (m, 1H), 4.26 (m, 1H), 5.76 (s, 2H), 6.82 (m, 4H), 7.15−7.49 (m, 10H), 7.59 (s, 1H); ^{31}P NMR ($CDCl_3$) δ 149.2, 150.0; IR (film, cm^{-1}) 1735 (CO−O), 2250 (CN); HRMS (FABMS: TEOA+NaCl)[28] calcd for $C_{49}H_{65}N_5O_9P$ ($M + H$)$^+$ 898.4520, found 898.4515; calcd for $C_{49}H_{64}N_5O_9PNa$ ($M + Na$)$^+$ 920.4339, found 920.4336.

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Supporting Information Available: General procedure and the procedures for the synthesis of **4β**, **5**, **13**, **14ab**, **15**, and **16**. Copies of NMR spectra (^{1}H, ^{13}C, and/or ^{31}P) of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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