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Synthesis of novel C4-linked imidazole ribonucleoside phosphoramidites for probing general acid and base catalysis in ribozyme

Lisa Araki,[a] Shinya Harusawa,[a,*] Maho Yamaguchi,[a] Sumi Yonezawa,[a] Natsumi Taniguchi,[a] David M. J. Lilley,[b] Zheng-yun Zhao[b] and Takushi Kurihara[a]

[a]*Osaka University of Pharmaceutical Sciences, 4-20-1, Nasahara, Takatsuki, Osaka 569-1094, Japan*
[b]*Department of Biochemistry, MSI/WTB Complex, University of Dundee, Dundee DD1 5EH, UK*

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Abstract—We describe the synthesis of novel C4-linked imidazole ribonucleoside phosphoramidite (PA) **1a** by which the imidazole moiety is incorporated into VS ribozyme to study its role in general acid and base catalysis. Investigation of protecting groups for the imidazole-*N* first indicated that pivaloyloxymethyl (POM) was adequate as an *N*-protecting group for the imidazole nucleoside, which could be readily removed under mild basic conditions. Further, the synthetic method was extended to synthesis of 2′-deoxy- and 2′-*O*-allyl nucleoside PAs **1b** and **1c**.

1. Introduction

The VS ribozyme is the largest of a group of nucleolytic ribozymes that include hammerhead, hairpin, and HDV ribozymes, and catalyzes the site-specific cleavage of a phosphodiester linkage to generate products containing 2′, 3′-cyclic phosphate and 5′-OH termini.[1] We recently indicated that the A730 loop of VS ribozyme was an important component of the active site in general acid and base catalysis (Fig. 1).[2] In particular, the adenine (A756) in the A730 loop is a critical base in the cleavage,[3] because sequence variants at the position 756 are especially impaired in the cleavage and ligation activity. In these studies, we have used a trans-acting VS ribozyme, where a three-piece ribozyme-substrate system was used, as illustrated in Figure 1.

Been and co-workers previously reported a phenomenon called ‘imidazole rescue’: cleavage activity of the uracil-substituted mutant (αC76U) at the active site in HDV antigenomic ribozyme could be restored by addition of exogeneous imidazole.[4] This is probably due to rare circumstances in which the active site region of HDV is quite open to the solvent. By contrast, in the case of A756G

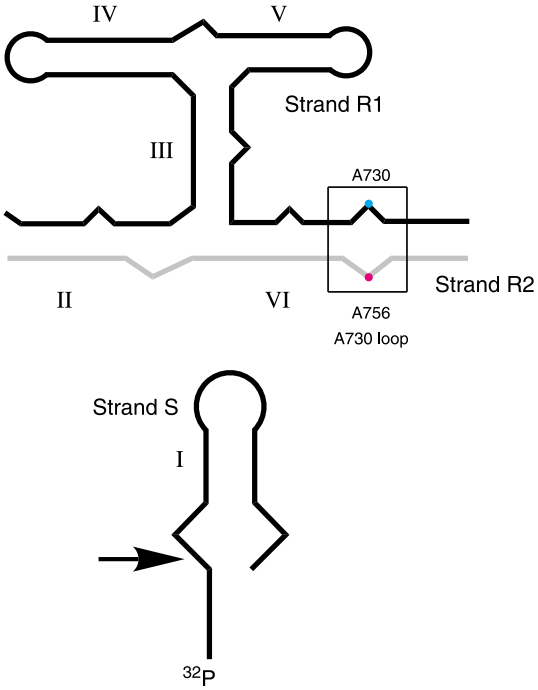

Figure 1. Schematic of a substrate and VS ribozyme, with helices numbered and the cleavage position arrowed. The trans-acting VS ribozyme was used in our studies where the substrate and ribozyme were separated. The A730 loop is boxed.

Keywords: Imidazole; Phosphoramidite; Ribozyme; POM; RNA.
* Corresponding author. Tel.: +81 72 690 1087; fax: +81 72 690 1086; e-mail: harusawa@gly.oups.ac.jp

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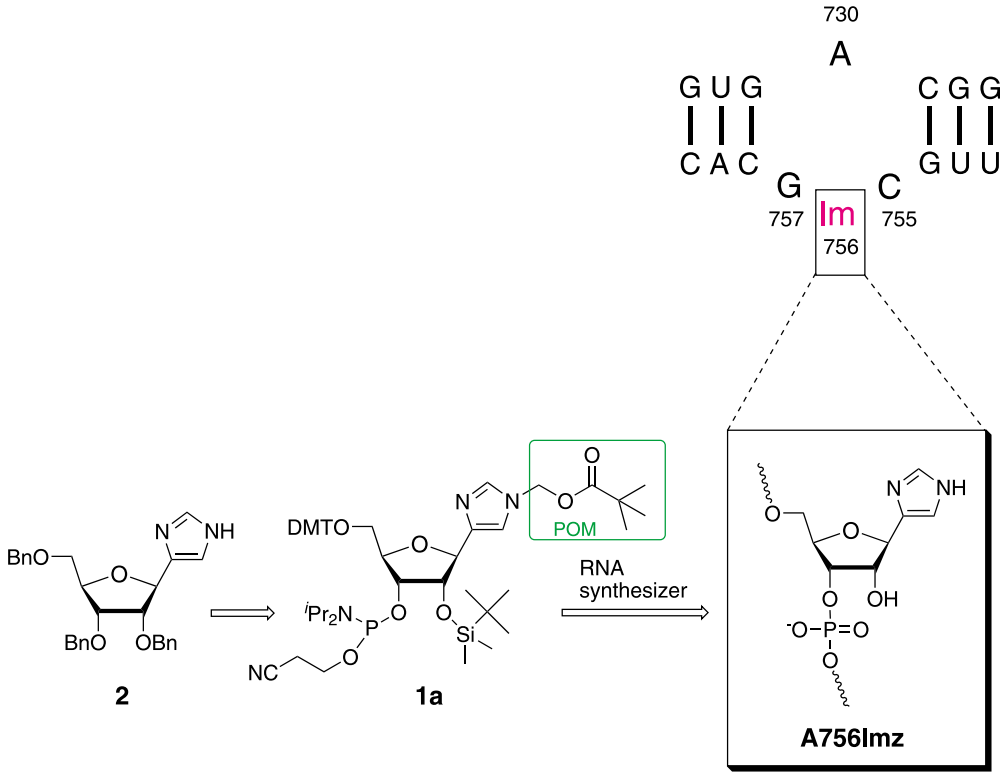


Figure 2. Incorporation of the imidazole into A756 of VS ribozyme using phosphoramidite **1a**.

or A756 abasic variants of VS ribozyme, addition of imidazole in the medium failed to restore the activity, possibly due to the fact that the active site is buried in a cliff between stem I and IV, therefore preventing free imidazole entering the active site.[3]

From these results, we have developed a new chemical strategy for determining the role of acid–base catalysis in a ribozyme function, in which C4-linked imidazole was placed into A756 of VS ribozyme covalently as a pseudonucleobase (Fig. 2).[5] In this study, a novel C4-linked imidazole ribonucleoside phosphoramide (PA) **1a** was subjected to a *t*BDMS approach of RNA automatic synthesis to provide an imidazole-substituted VS ribozyme (A756 Imz) in an average 99% coupling yield. The A756 Imz catalyzed the almost-complete cleavage of a substrate stem-loop at the correct position. Although the rate is slow ($K_{obs}=0.01\ min^{-1}$), it was comparable to that of the uracil-substituted HDV ribozyme in the presence of exogeneous imidazole.[4] The result powerfully supported a direct role of the nucleobase at position 756 in the chemistry of natural VS ribozyme.[5]

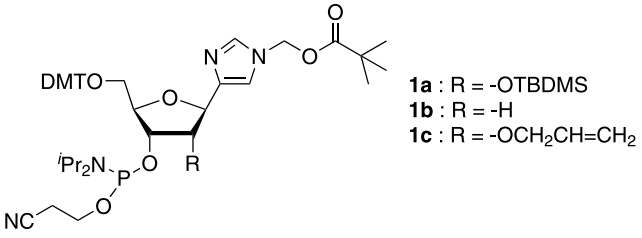


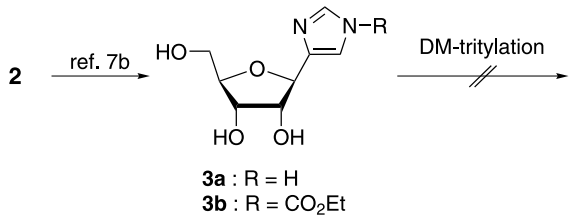
Figure 3. Novel C4-linked imidazole ribonucleoside PAs.

We herein describe the chemical synthesis of the imidazole ribonucleoside PA **1a**,[6] which is a crucial building block in the construction of the imidazole-containing oligonucleotides, starting from 4(5)-2,3,5-tri-*O*-benzyl-β-ribofuranosylimidazole **2**.[7] The key feature of the synthesis is the use of the pivaloyloxymethyl (POM) group as an efficient *N*-protecting group of imidazole. In connection with this study, 2′-deoxy-3′-*O*-PA **1b** and 2′-*O*-allylribonucleoside-3′-*O*-PA **1c** were synthesized, since removal of the 2′-hydroxy group from the ribose of A756 led to a small (10-fold slower) reduction in the rate of cleavage.[3] (Fig. 3)

2. Results and discussion

2.1. An adequate protecting group for imidazole-*N* in the RNA synthesis

We reported an efficient and stereoselective synthesis of tri-*O*-benzyl-β-ribofuranosylimidazole **2**, 4(5)-β-D-ribofuranosylimidazole **3a**, its N^{im}-ethoxycarbonyl compound **3b**, which were useful intermediates to supply related nucleosides.[7,8] First, 5′-*O*-dimethoxytritylation of non-protected or N^{im}-ethoxycarbonylated imidazole C-nucleosides (ICNs) **3a** or **3b** was attempted according to the ordinary PA synthesis.[9] Although the ethoxycarbonyl group of imidazole-*N* could be removed by aqueous ammonia,[7b] dimethoxytritylation of **3a** or **3b** failed to give the desired compounds (Scheme 1). Instability of 5′-*O*-dimethoxytrityl (DMT) products seems responsible for the failure, but the result emphasized the need for a suitable protecting group of imidazole-*N*.



Scheme 1. Attempted direct tritylation of **3a** and **3b**.

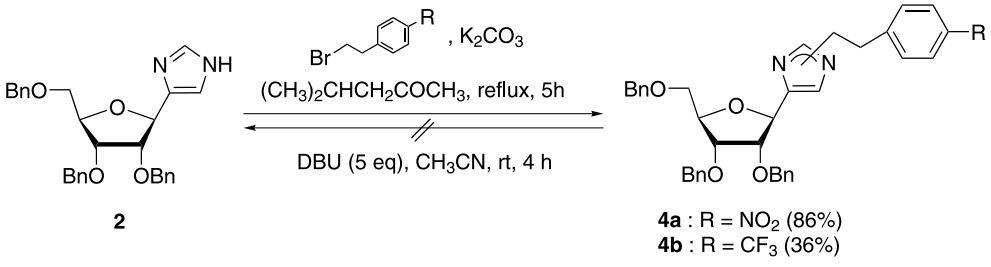
Bergstrom et al.[10] previously reported the synthesis of 2′-deoxy-β-ribofuranosylimidazole with *p*-nitrophenylethyl (PNPE)[11] as a protecting group at imidazole-*N*. They described that PNPE could be removed by treatment with DBU, but they did not give any experimental details. We thus examined whether the PNPE group at the imidazole-*N* could be easily removed by DBU in the ribo-situation. As a nitro group could be reduced under debenzylation-condition such as catalytic hydrogenation, we assumed a protecting group, *p*-trifluoromethylphenylethyl group, which could be stable under such reductive conditions. Reaction of tribenzyl compound **2** with PNPE bromide afforded PNPE-protected ICN **4a** (86%) as an 8:1 isomeric mixture at the N^{im} position (Scheme 2). Treatment of **4a** with DBU (5 equiv) in acetonitrile at room temperature (rt) did not remove the PNPE group, but prolonged refluxing (20 h) of the reaction mixture gave **2** in only 22% yield. These results indicated that the PNPE group was not appropriate for protection of imidazole-*N* in the RNA synthesis. *p*-Trifluoromethylphenylethyl-protected ICN **4b** (36%) was similarly obtained from **2**, but reaction of **4b** with DBU was ineffective.

We envisaged the indispensable factors as a protecting group for imidazole-*N* in RNA synthesis by the *t*BDMS approach: (1) The factor, which might contribute to the stability of the synthetic intermediates in the whole process via the building block **1a** from **2**. (2) The factor, which should be compatible with the deprotection-condition [28% aqueous ammonia–ethanol (3/1, v/v)] at the end of the solid-phase RNA synthesis by the *t*BDMS–phosphoramidite approach. (3) The factor, which could be tolerated under the debenzylation-condition of 2′,3′,5′-tri-*O*-benzyl-*N*-protected ICNs **5**.[7b] (4) Further, the factor which might increase the lipophilicity of imidazole, which facilitated the extraction and isolation of each synthetic intermediate. We investigated the protecting group from the viewpoint of the factors (2) and (3), and focused our attention on *p*-anisoyl (An),[12] 2,2,2-trichloroethoxycarbonyl (Troc),[13] and the POM groups[14] as candidates. They were introduced into the imidazole-*N* of **2** via the corresponding chlorides to give **5a** (74%), **5b** (89%), and **5c** (94%) as single isomers (Table 1). The location of the protecting groups was tentatively assigned to be τ-nitrogen because *N*-acylation of the imidazole ring occurs regioselectively on the less-hindered τ-nitrogen.[15] The substituents on the imidazole-*N* could be readily removed to give back **2** by aqueous ammonia–MeOH (1/3, v/v) at rt after 3 h in 73–92% yields.

Next, the protected imidazoles **5a–c** were evaluated under catalytic debenzylation as shown in Table 2. Hydrogenolysis of **5a** (An) or **5b** (Troc) over Pd–C cleaved their N^{im}-substituted groups as well as benzyl groups to give a non-protected imidazole C-nucleoside **3a** (R^1, R^2, R^3=H, quant) (Table 2, entries 1 and 2). However, POM-protected imidazole **5c** interestingly maintained the POM group under the reduction conditions to give partial debenzylated products **6** and **7** (Table 2, entry 3). Further, treatment of **5c** with $Pd(OH)_2$–C/cyclohexene in refluxing ethanol produced *N*-POM–ICN **8** in quantitative yield. Accordingly, the POM group satisfied the factors (2) and (3) required for the protecting group of imidazole-*N*.

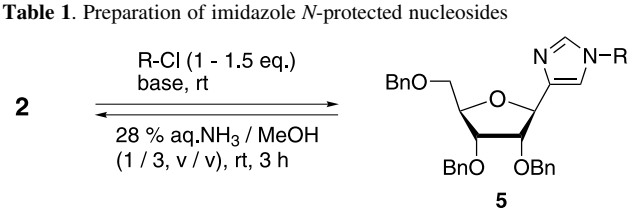
Zaramella and co-workers recently reported a convenient method for positioning of the imidazole-protecting group, in which ^1H–^{15}N heteronuclear multiple bond correlation (HMBC) in the NMR method was used for several histidine derivatives.[16] We then applied the method to **8** to determine the location of the POM group at the τ-nitrogen: a $^1H(C1')/^{15}N(\pi)$ cross-peak could clearly be seen in ^1H–^{15}N HMBC signals [δ (ppm) ^{15}N (τ) 176.1, ^{15}N (π) 247.1, and ^1H (C1′) 4.66] (Scheme 3), since the signal of the substituted imidazole-*N* always appeared at lower chemical shift (δ) than that of the unsubstituted one.

The utility of the POM group may be pointed out in RNA



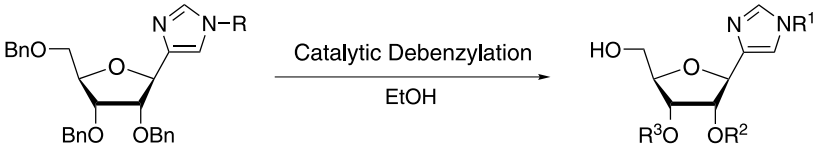
Scheme 2. Attempted imidazole *N*-protection and deprotection.

Table 1. Preparation of imidazole *N*-protected nucleosides



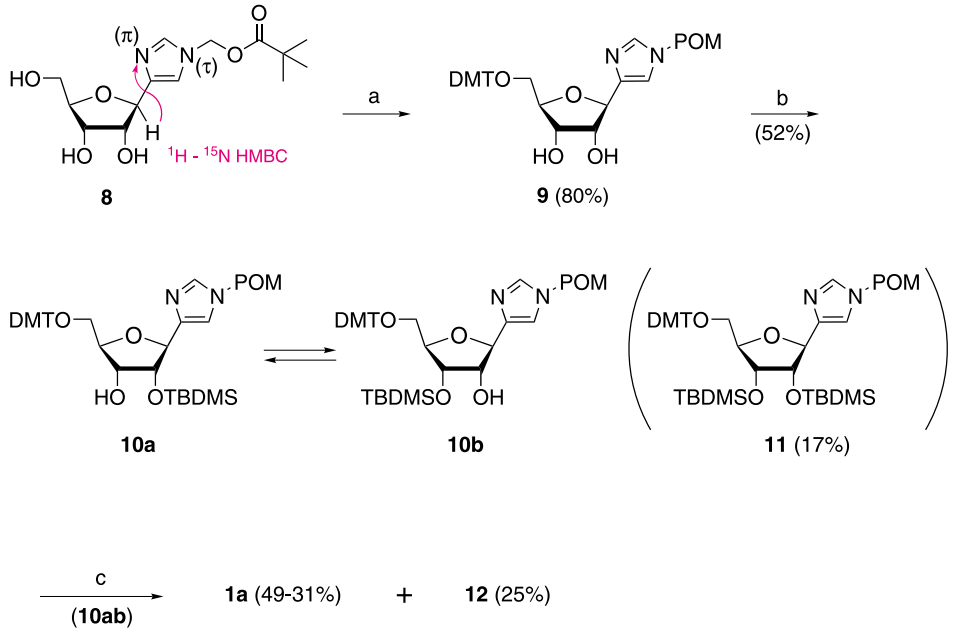
Entry	Reaction condition (equiv, h)	**5** (%)	**5** to **2** (%)
1	*p*-$CH_3OC_6H_4COCl$ (1.1), $^iPr_2NEt$, pyridine, 1.5	**5a**, 74	73
2	Cl_3CCH_2OCOCl (1.1), DMAP, benzene, 4	**5b**, 89	89
3	$(CH_3)_3CCO_2CH_2Cl$ (1.5), NaH, THF, 3	**5c**, 94	92

Table 2. Catalytic debenzylation of *N*-protected imidazoles



5a-c **3a, 6 - 8**

Entry	Reaction conditions	Product	Yield (%)
1	**5a** (An), H_2/10% Pd–C (3 kg/cm^2), 16 h	**3a**, R^1, R^2, R^3=H	Quant
2	**5b** (Troc), H_2/5% Pd–C (1 kg/cm^2), 6 h	**3a**, R^1, R^2, R^3=H	Quant
3	**5c** (POM), H_2/10% Pd–C (3 kg/cm^2), 16 h	**6**, R^1=POM, R^2, R^3=Bn	22
		7, R^1=POM, R^2=H, R^3=Bn or R^1=POM, R^2=Bn, R^3=H	33
4	**5c** (POM), 20% $Pd(OH)_2$–C, cyclohexene, reflux, 3 h	**8**, R^1=POM, R^2, R^3=H	Quant



8 **9** (80%) **10a** **10b** **11** (17%)

Scheme 3. Preparation of imidazole PA **1a**. Reagents and conditions: (a) DMTCl, Et_3N, cat. DMAP, pyridine; (b) TBDMSOTf, pryidine, −40 °C, MS 4 Å; (c) $(^iPr_2N)_2POCH_2CH_2CN$, 4,5-DCl, $ClCH_2CH_2Cl$, 40 °C, 15 h.

automatic synthesis: (1) In the capping step, the unreacted 5′-hydroxy group is acetylated with acetic anhydride to prevent the growing oligonucleotide chain with a nucleoside deletion,[9] whereas activated N^{im}-carbonyl groups (as in **5a**,**b**) may be susceptible to potential exchange-reactions at this stage, leading to complexities. (2) Base-protecting groups are conventionally removed in the final step of RNA synthesis by an ammonia and ethanol mixture [28% aqueous NH_3–EtOH (3/1, v/v)] at 60 °C for 16 h. As the POM group can be removed under faster and milder conditions, it is particularly attractive to sensitive RNA such as Cy5 labelled RNA where deprotection is recommended at rt to minimize the destruction of cyanine dye. Obviously, the very mild conditions require other bases to be protected with more labile groups such as PAC or TAC for RNA monomers A, G, and C. Hence, the *N*-POM group is the most suitable and practical protecting group for the imidazole RNA.

2.2. Synthesis of imidazole ribonucleoside PA 1a

5′-*O*-DMT-2′-*O*-*tert*-butyldimethylsilyl(TBDMS)-3′-*O*-cyanoethyldiisopropylphosphoramidite **1a** was synthesized in three steps from the *N*-POM-imidazole **8** (Scheme 3). After DM-tritylation of **8**, 5′-*O*-DMT-ribonucleoside **9** (80%) was isolated immediately through a basic silica gel bed, because the DMT group of **9** was removed by neutral or standard silica gel chromatography. In this note, the basic silica gel was used for the purification and isolation of the labile compounds containing the DMT group. Although the silylation reaction of the 2′-hydroxy group can be controlled to some extent by using $AgNO_3$ as an additive,[17] silylation of **9** with TBDMSCl did not proceed in the presence of $AgNO_3$ and pyridine in THF. On the other hand, treatment of **9** with TBDMSOTf in pyridine led to an inseparable 1:1 mixture **10ab** (52%) of 2′-*O*-TBDMS and 3′-*O*-TBDMS isomers, together with a 2′,3′-bis-*O*-substituted derivative

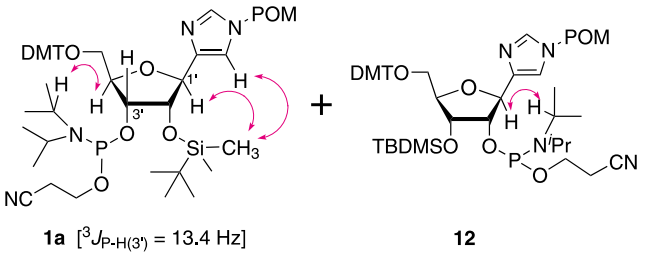
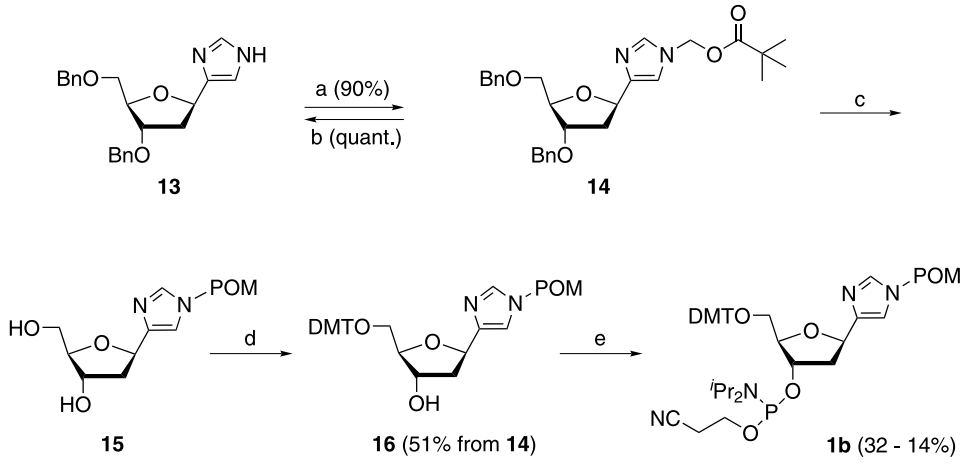


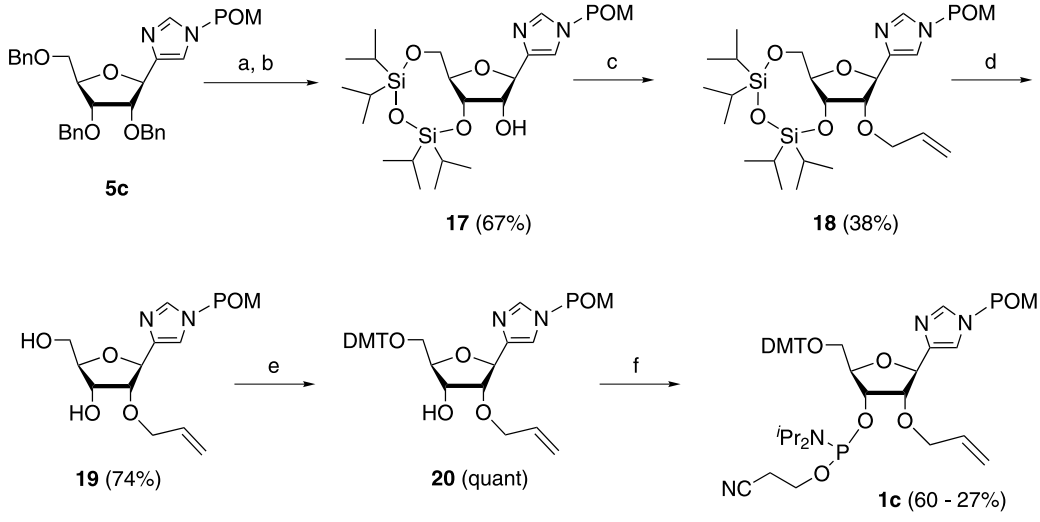
Figure 4. The NOESY experiments of phosphoramidites **1a** and **12**. Arrows indicate interactions between sets of two protons.

11 (17%). Unfortunately, since the TBDMS group readily migrated between the 2′- and the 3′-hydroxy function, the desired 2′-protected ribonucleoside **10a** was not separated as a stable compound.[18] Thus, the mixture **10ab** was subjected to phosphitylation. Treatment of **10ab** with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphodiamidite in the presence of diisopropylammonium tetrazolide (DIPT) in dichloromethane at rt (90 h) generated 3′- and 2′-phosphoramidites **1a** and **12** in low yields. Alternatively, use of 4,5-dicyanoimidazole (DCI) as an activator and heating at 40 °C for 15 h in 1,2-dichloroethane improved the reaction to provide **1a** and **12** in the range of 49–31 and 25% yields, respectively. Since the final phosphoramidite **1a** was decomposed on a standard TLC plate (Merck 60 F_{254}), the purification of the crude product was not straightforward. However, further efforts clarified that the phosphoramidite **1a** could be purified by a basic preparative TLC plate.

The stereochemical assignment of phosphoramidites **1a** and **12** was indicated by the observation of an NOESY between the C1′ and Si-*Me* protons of **1a**, as illustrated in Figure 4, while NOE enhancement between C1′ and NC*H*Me_2 protons in **12** was observed. It was further supported by observation of a ^{31}P–^{1}H (C3′) coupling constant [$^3J_{P-H(C3')}$ = 13.4 Hz] with the aid of the ^{31}P-decoupled ^{1}H NMR spectrum of **1a** [^{31}P NMR: δ 148.9 ppm (CD_3CN) as a singlet-like peak for two P-diastereoisomers]. Although MS measurement of PAs has been problematic owing to their labile properties, we have very recently found that the accurate molecular weight (MW) of PAs may be generally



Scheme 4. Preparation of imidazole PA **1b**. Reagents and conditions: (a) NaH, POMCl, rt; (b) 28% NH_3–MeOH (1/4, v/v), rt, 3 h; (c) 20% $Pd(OH)_2$–C, cyclohexene, EtOH, reflux, 3 h; (d) DMTCl·Et_3N, DMAP (cat.), py, rt; (e) (^{i}Pr$_2$N)$_2$POCH$_2$CH$_2$CN, DIPT, 40 °C, 46 h.



Scheme 5. Preparation of imidazole 2′-allyl ribonucleoside PA **1c**. Reagents and condition: (a) Table 2, entry 4; (b) $TIPDSCl_2$, 0 °C then py, rt, 3 h; (c) allyl ethyl carbonate, $Pd_2(dba)_3$, Ph_3P, reflux, 15 h; (d) HF, TMEDA, 0 °C then rt, 2.5 h; (e) DMTCl, Et_3N, DMAP (cat.), py, rt, 19 h; (f) (^{i}Pr$_2$N)$_2$POCH$_2$CH$_2$CN, 4, 5-DCl, rt, 24 h.

determined by using a suitable matrix system, triethanolamine (TEOA)–NaCl, on liquid secondary ion (LSI) MS equipped with a double-focusing mass spectrometer. The present method successfully revealed the molecular-related ion $[M+Na]^+$ of **1a** at *m/z* 953.4625, leading to the composition formula ($C_{50}H_{71}N_4O_9PSi$).[19] Purified imidazole phosphoramidite **1a** was stored for several months at −20 °C to check its long-term stability, but ^{31}P and ^{1}H NMR measurements of the stored compound did not show any substantial decomposition.

2.3. Synthesis of 2′-deoxy- and 2′-*O*-allyl nucleoside PAs 1b and 1c

The synthetic method using POM protection of imidazole-*N* was extended to the preparation of 2′-deoxy- and 2′-*O*-allyl imidazole C-nucleoside PAs **1b** and **1c** (Schemes 4 and 5). It is noted that 2′-*O*-allyl nucleosides are potent antisense molecules because of their high nuclease resistance.[20] The imidazole-*N* of 3′,5′-di-*O*-benzyl-2′-deoxy-D-ribonucleosides **13**[7b] was analogously protected by the POM group to give 3′,5′-di-*O*-benzyl-*N*-protected ICN **14** (90%). Subsequent debenzylation followed by DM-tritylation gave 5′-*O*-DMT-2′-deoxyribonucleoside **16** (51%). Phosphitylation of **16** produced the fully protected 2′-deoxy nucleoside PA **1b** (32–14%). The moderate yield of **1b** was due to isolation problems caused by column chromatography. The feasibility of **1b** as a protecting group of the N^{im}-POM group on 2′-deoxy-D-ribonucleosides was examined by restoration of *N*-protected imidazole **14** into unsubstituted imidazole **13** in aqueous ammonia–MeOH.

For the synthesis of 2′-*O*-allyl imidazole C-nucleoside PAs **1c**, 3′,5′-*O*-TIPDS-protection (TIPDS =1,1,3,3-tetraisopropyldisiloxanediyl) was carried out to give **17** (67%), starting from tribenzylated POM–ICN **5c** (Scheme 5), as the regioselective formation for 2′-*O*-allyl ribonucleosides had been reported.[21a] Palladium-catalyzed allylation[21a,b] of the 2′-hydroxy group of **17** produced a 2′-*O*-allyl compound **18** (38%). Desilylation of **18** gave a 3′, 5′-dihydroxy compound **19** (74%). Further, DM-tritylation (quant) followed by phosphitylation provided the desired 2′-*O*-allyl nucleoside PA **1c** (60–27%).

3. Conclusion

A novel C4-linked ICN PA **1a** was designed and synthesized starting from tribenzylribofuranosylimidazole **2**. The imidazole PA enabled incorporation of the imidazole moiety into VS ribozyme in order to elucidate its roles in the general acid and base catalysis of ribozyme. In the synthetic study, POM was first introduced as a protecting group of imidazole-*N* and it could be readily removed under mild basic conditions. Further, the imidazole nucleoside **1a** places the base at the natural C1-atom, while other modified nucleosides with imidazole attached to the nucleobase have been reported.[22] As the imidazole base may be incorporated at any site in an RNA sequence using **1a**, the present approach would be applied to other situations in which nucleobase participation is suspected. 2′-Deoxy and 2′-*O*-allyl analogues **1b** and **1c** could be applicable to insert the imidazole into modified DNA and RNA oligonucleotides. Further work on application of the imidazole oligonucleotides is under way and will be published in due course.

4. Experimental

4.1. General

The melting points were determined on a hot-stage apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IR-435 spectrometer. ^{1}H and ^{13}C NMR spectra were taken with tetramethylsilane as an internal standard on a Varian Gemini-200, Varian Mercury-300, and Varian UNITY INOVA-500 spectrometers. Reactions with air- and moisture-sensitive compounds were carried out under an argon atmosphere. Unless otherwise noted, all extracts were dried over Na_2SO_4, and the solvent was removed in a rotary evaporator under reduced pressure. Unless otherwise stated, Fuji Silysia FL-60D silica gel, Fuji Silysia BW-127ZH silica gel, and Merck 60 F_{254} were used for flash column chromatography, column chromatography and thin-layer chromatography (TLC), respectively. As for the basic (N–H) silica gel, Chromatorex NH-DM 1020 (Fuji Silisia Chemical Ltd) was used. The accurate molecular weight measurements of nucleoside PAs **1a**, **1b**, and **1c** were determined by MS spectrometry using a novel matrix system, triethanolamine (TEOA)–NaCl, on LSIMS equipped with a double-focusing MS spectrometer.[19]

4.1.1. 4(5)-(2,3,5-Tri-*O*-benzyl-β-D-ribofuranosyl)-1-[2-(4-nitrophenyl)ethyl]imidazole (4a). To a solution of **2** (81 mg, 0.17 mmol) in 4-methyl-2-pentanone (0.5 ml) was added a solution of *p*-nitrophenethyl bromide (56 mg, 0.24 mmol) in 4-methyl-2-pentanone (0.5 ml) followed by potassium carbonate (50 mg, 0.36 mmol). The resulting mixture was refluxed for 5 h and then evaporated. The residue was dissolved with EtOAc and the resulting solution was washed with water, brine. The organic layers were dried, and evaporated. The crude oil was purified by flash column chromatography [80% EtOAc in hexane] to give **4a** (90 mg, 86%) as a colorless oil; IR (film, cm^{-1}) 1600, 1510, 1450, 1345 (NO_2); ^{1}H NMR ($CDCl_3$) δ 2.95 (t, 6/4H, J= 7.2 Hz), 3.09 (t, 2/4H, J=7.2 Hz), 3.50 (dd, 1H, J=9.5, 3.8 Hz), 3.77 (dd, 1H, J=9.5, 3.8 Hz), 3.98 (t, 2H, J= 3.6 Hz), 4.02–4.72 (m, 8H), 4.96 (d, 1/4H, J=6.5 Hz), 5.10 (d, 3/4H, J=3.8 Hz), 6.86 (s, 1H), 7.08 (d, 2H, J=8.6 Hz), 7.15 (s, 1H), 7.25–7.35 (m, 15H), 8.08 (d, 2H, J=8.6 Hz); HRMS(EIMS) calcd for $C_{37}H_{37}N_3O_6$ [$(M)^+$]: 619.2680, found 619.2681.

4.1.2. 4(5)-(2,3,5-Tri-*O*-benzyl-β-D-ribofuranosyl)-1-[2-(4-trifluoromethylphenyl)ethyl]imidazole (4b). The same procedure (**4a**) was used for the preparation of **4b** (44 mg, 36%, a colorless oil) from **2** (90 mg, 0.19 mmol); ^{1}H NMR ($CDCl_3$) δ 2.92 (t, 6/4H, J=6.8 Hz), 3.05 (t, 2/4H, J=7.2 Hz), 3.62 (dd, 1H, J=10.3, 3.5 Hz), 3.76 (dd, 1H, J=10.3, 3.5 Hz), 3.97 (t, 2H, J=6.8 Hz), 4.02–4.68 (m, 9H), 4.98 (d, 1/4H, J=6.8 Hz), 5.10 (d, 3/4H, J=3.5 Hz), 6.85 (s, 1H), 7.07 (d, 2H, J=7.2 Hz), 7.12–7.39 (m, 16H), 7.48 (d, 2H, J=7.2 Hz).

Conversion of **4a** *into* **2**. A mixture of **4a** (45 mg, 0.07 mmol) and DBU (56 mg, 0.37 mmol) in acetonitrile

(2 ml) was refluxed for 20 h. After cooling, acetic acid (28 mg) was then added. The reaction mixture was evaporated to give a residue, which was subsequently dissolved in CH_2Cl_2. The organic layer was washed with water, dried over anhydrous $MgSO_4$, and evaporated to give a residue. Chromatography using EtOAc in hexane (60–100%) gave a mixture (colorless oil, 37 mg), showing ca. 1:3 ratio of **2** (22%) and **4a** (65%) from ^{1}H NMR.

4.1.3. 4-(2,3,5-Tri-*O*-benzyl-β-D-ribofuranosyl)-1-(4-methoxybenzoyl)imidazole (5a). To a mixture of **2** (101 mg, 0.22 mmol) and diisopropylethylamine (56 mg, 0.43 mmol) in pyridine (1 ml) was added *p*-anisoyl chloride (74 mg, 0.43 mmol) in pyridine (1 ml). The resulting mixtue was stirred for 1.5 h and then evaporated to give a residue, which was subsequently diluted with EtOAc. The organic layer was washed with 1.5 N HCl followed by brine. The organic layers were dried, and evaporated. The resulting crude oil was purified by flash column chromatography on silica gel using 50% EtOAc in hexane to give compound **5a** (96 mg, 74%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 3.61 (dd, 1H, J=10.3, 4.8 Hz), 3.70 (dd, 1H, J=10.3, 4.0 Hz), 3.88 (s, 3H), 4.06 (t, 1H, J=5.6 Hz), 4.25 (t, 1H, J=4.8 Hz), 4.33 (dt, 1H, J=5.6, 4.0 Hz), 4.47–4.69 (m, 6H), 5.11 (d, 3/4H, J=4.0 Hz), 6.98 (d, 2H, J=9.1 Hz), 7.21–7.35 (m, 15H), 7.48 (s, 1H), 7.76 (d, 2H, J=9.1 Hz), 8.09 (d, 2H, J=1.4 Hz); HRMS(EIMS) calcd for $C_{37}H_{36}N_2O_6$ [$(M)^+$]: 604.2571, found 604.2571.

4.1.4. 2,2,2-Trichloroethyl 4-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)imidazole-1-carboxylate (5b). A mixture of **2** (57 mg, 0.12 mmol), trichloroethoxycarbonyl chloride (29 mg, 0.13 mmol), pyridine (12 mg, 0.16 mmol), and a catalytic amount of 4-DMAP (1 mg) in benzene (3.5 ml) was stirred for 3 h. After H_2O (0.5 ml) was added to the mixture, the solvent was evaporated to give a residue, which was subsequently dissolved with EtOAc. The organic layer was washed with water, brine, dried, and evaporated to give a crude oil. It was purified by flash column chromatography using 20% EtOAc in hexane to give **5b** (69 mg, 89%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 3.60 (dd, 1H, J=10.9, 4.3 Hz), 3.70 (dd, 1H, J=10.9, 3.9 Hz), 4.05 (t, 1H, J=5.8 Hz), 4.21 (t, 1H, J=4.8 Hz), 4.32 (dt, 1H, J=5.8, 4.3 Hz), 4.45–4.65 (m, 6H), 4.91 (d, 1H, J=11.6 Hz), 5.00 (d, 1H, J=11.6 Hz), 5.07 (d, 1H, J=4.8 Hz), 7.18–7.36 (m, 15H), 7.44 (s, 1H), 8.12 (s, 1H).

4.1.5. [4-(2,3,5-Tri-*O*-benzyl-β-D-ribofuranosyl)imidazolyl]methyl 2,2-dimethylpropiolate (5c). Under stirring, 60% NaH (72 mg, 1.80 mmol) in mineral oil was added to THF (7 ml) to give a suspension. A solution of **2** (562 mg, 1.20 mmol) in THF (3 ml) was added to the suspension, and the resulting mixture was stirred at rt for 3 h. Then, a solution of chloromethyl pivaloate (271 mg, 1.80 mmol) in THF (8 ml) was added. After 1 h, H_2O (0.5 ml) was added and the whole was evaporated to give a residue, which was subsequently dissolved with EtOAc. The organic layer was washed with water, brine, dried, and evaporated. The crude product was purified by flash column chromatography on silica gel using 50% EtOAc in hexane to give compound **5c** (654 mg, 94%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.15 (s, 9H), 3.63 (dd, 1H, J=7.3, 3.1 Hz), 3.71 (dd, 1H, J=7.3, 3.1 Hz), 4.03 (t, 1H, J=3.1 Hz), 4.20 (t, 1H, J=2.9 Hz), 4.26 (m, 1H), 4.44–4.67 (m, 6H), 5.06 (d, 1H, J=2.9 Hz), 5.64 (dd, 1H, J=19.5, 7.3 Hz), 7.03 (s, 1H), 7.21–7.38 (m, 15H), 7.61 (s, 1H).

Conversion of **5c** *into* **2**. A mixture of **5c** (20 mg, 0.04 mmol) in methanol (1.5 ml) and 28% aqueous NH_3 (0.5 ml) was stirred 3 h at rt. After evaporation, the resulting residue was purified by flash column chromatography using AcOEt to give **2** (15 mg, 92%) as a colorless oil. By the same procedure, **5a** and **5b** were converted into **2** in 74 and 89% yields, respectively.

Catalytic debenzylation of **5a** (Table 2, entry 1). A solution of **5a** (52 mg, 0.09 mmol) in EtOH (8 ml) was hydrogenated over 10% Pd on carbon[23] (35 mg) at 3.0 kg/cm^2 for 16 h. After filtration through Celite, the filtrate was evaporated to give a residue, which was purified by column chromatography to give **3a**[7b] (17 mg, quant).

Catalytic debenzylation of **5b** (Table 2, entry 2). By the same procedure as above (Table 2, entry 1), **5b** (30 mg, 0.05 mmol) in EtOH (5 ml) was hydrogenated over 5% Pd on carbon[23] (20 mg) at 1.0 kg/cm^2 for 6 h to give **3a**[7b] (10 mg, quant).

Catalytic debenzylation of **5c** (Table 2, entry 3). By the same procedure as above (Table 2, entry 1), **5c** (36 mg, 0.06 mmol) in EtOH (6 ml) was hydrogenated over 5% Pd on carbon[23] (25 mg) at 3.0 kg/cm^2 for 16 h to give **6** (7 mg, 22%) and **7** (8 mg, 33%).[7b]

4.1.6. [4-(β-D-Ribofuranosyl)imidazolyl]methyl 2,2-dimethylpropiolate (8) (Table 2, entry 4). A mixture of **5c** (206 mg, 0.35 mmol), 20% $Pd(OH)_2$–C[23] (124 mg), and cyclohexene (1.1 ml, 10.56 mmol) in EtOH (10 ml) was refluxed for 3 h. After filtration through Celite, the filtrate was evaporated to give a residue, which was purified by column chromatography [MeOH–EtOAc (1/20)] to give **8** (111 mg, quant) as a colorless oil; ^{1}H NMR (CD_3OD) δ 1.18 (s, 9H), 3.52–4.19 (m, 5H), 4.66 (d, 1H, J=5.4 Hz), 6.02 (s, 2H), 7.45 (s, 1H), 8.36 (s, 1H). LSIMS *m/z*: 315 [$(M+H)^+$]. The τ positioning of POM group on the imidazole ring was determined by means of a ^{1}H–^{15}N HMBC experiment [conditions: 25 °C, 499.7 MHz for ^{1}H and 50.7 MHz for ^{15}N; δ (ppm) relative to external DMF (103.2 ppm)]; ^{1}H–^{15}N HMBC (CD_3OD) δ 176.1 [^{15}N (τ)], 247.1 [^{15}N (π)], and the cross peak coordinate 247.1/4.66 [^{1}H(C1′)].[16]

4.1.7. [4-(5-*O*-DMT-D-β-ribofuranosyl)imidazolyl]methyl 2,2-dimethylpropiolate (9). Compound **8** (111 mg, 0.35 mmol) was coevaporated with pyridine (2 ml) three times and redissolved in pyridine (2 ml) again. DMTCl (188 mg, 0.51 mmol), Et_3N (0.07 ml, 0.51 mmol), and DMAP (1 mg, 0.01 mmol) were added to the pyridine solution of **8**. After the mixture was stirred overnight, methanol (1 ml) was added to the reaction mixture. The solvent was removed to give a residue, which was purified through NH-silica gel bed with chloroform to give compound **9** (174 mg, 80%) as white amorphous product; ^{1}H NMR (CD_3OD) δ 1.09 (s, 9H), 3.15–3.38 (overlapped with CD_3OD), 3.76 (s, 6H), 3.99–4.13 (m, 3H), 4.79 (d, 1H, J=5.3 Hz), 5.73–5.92 (m, 2H),

6.82 (d, 4H, J=8.3 Hz), 7.12–7.49 (m, 10H), 7.81 (s, 1H); LSIMS m/z 617 [$(M+1)^+$].

4.1.8. [4-(5-*O*-DMT-2(3)-*O*-TBDMS-β-D-ribofuranosyl) imidazolyl]methyl 2,2-dimethylpropiolate (10ab). Compound **9** (314 mg, 0.51 mmol) was coevaporated with pyridine (2 ml) three times and dissolved in pyridine (5 ml) again. TBDMSOTf (0.13 ml, 0.56 mmol) was added to a solution of **9** at −40 °C. The reaction mixture was stirred for 5 min and then evaporated. The crude product was chromatographied by NH-silica gel chromatography using gradient solvent system [20–30% EtOAc in hexane] to give compound **11** (33 mg, 8%) and **10ab** (250 mg, 67%) in that order. *Compound* **10ab**. A pale yellow amorphous product; ^{1}H NMR ($CDCl_3$) δ 0.00 (s, 3H), 0.06 (s, 3H), 0.88 (s, 9H), 1.16 (s, 9H), 2.70 (m, 1H), 3.16 (dd, 1/3H, J=11.0, 4.3 Hz), 3.25 (dd, 2/3H, J=11.0, 4.3 Hz), 3.37 (dd, 1/3H, J=11.0, 3.1 Hz), 3.38 (dd, 2/3H, J=11.0, 3.1 Hz), 3.78 (s, 6H), 4.07 (m, 2/3H), 4.24 (m, 2/3H), 4.46 (t, 1H, J=4.3 Hz), 4.76 (d, 2/3H, J=5.1 Hz), 4.82 (d, 1/3H, J=5.1 Hz), 5.73 (q, 2H, J=10.2 Hz), 6.80 (d, 4H, J=10.2 Hz), 7.08 (s, 2/3H), 7.12 (s, 1/3H), 7.16–7.52 (m, 9H), 7.62 (s, 1H); HRMS(EIMS) calcd for $C_{41}H_{54}N_2O_8Si$ [$(M)^+$] 730.3647, found 730.3649. *Compound* **11**. A pale yellow amorphous; ^{1}H NMR ($CDCl_3$) δ −0.10 (s, 3H), −0.08 (s, 3H) −0.04 (s, 6H), 0.79 (s, 9H), 0.82 (s, 9H), 1.10 (s, 9H), 3.18 (dd, 1H, J=10.3, 4.5 Hz), 3.37 (dd, 1H, J=10.3, 4.5 Hz), 3.76 (s, 6H), 4.04 (t, 1H, J=4.3 Hz), 4.09 (m, 1H), 4.22 (t, 1H, J=4.3 Hz), 4.84 (d, 1H, J=4.3 Hz), 5.61 (d, 1H, J=10.8 Hz), 5.72 (d, 1H, J=10.8 Hz), 6.80 (d, 4H, J=9.0 Hz), 7.08 (s, 1H), 7.14–7.51 (m, 9H), 7.59 (s, 1H).

4.1.9. {4-[5-*O*-DMT-2-*O*-TBDMS-3-*O*-(2-CE-*N*,*N*-diisopropylphosphoramidite)-β-D-ribofuranosyl]imidazolyl}-methyl 2,2-dimethylpropiolate (1a) and {4-[5-*O*-DMT-3-*O*-TBDMS-2-*O*-(2-CE-*N*,*N*-diisopropylphosphoramidite)-β-D-ribofuranosyl]imidazolyl}methyl 2,2-dimethylpropiolate (12). Compound **10ab** (93 mg, 0.13 mmol) was coevaporated with dichloroethane (2 ml) three times and dissolved in dichloroethane (1.5 ml) again. To the solution was added 4,5-DCI (19 mg, 0.16 mmol) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphodiamidite (0.09 ml, 0.26 mmol). The resulting mixture was stirred at 40 °C for 15 h and then evaporated. The residual oil was chromatographed (25% EtOAc in hexane) by NH-silica gel chromatography to give partially purified **12** (25%) and **1a** (49%) in that order. The desired PA **1a** was further carefully purified on a preparative NH-TLC with 50% EtOAc in hexane to give **1a** (37 mg, 31%), while PA **12** remained without further purification owing to its instability. *Compound* **1a**. A white foam; R_f =0.69 (50% EtOAc/hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ −0.09 (s, 3H), −0.03 (s, 3H), 0.82 (s, 9H), 1.00 (d, 6H, J=7.0 Hz), 1.14 (s, 9H), 1.16 (d, 6H, J=7.0 Hz), 2.55–2.68 (m, 2H), 3.16 (dd, 1H, J=10.0, 4.0 Hz), 3.40 (dd, 1H, J=10.0, 5.0 Hz), 3.52–3.60 (m, 2H), 3.78 (s, 6H), 3.82–3.88 (m, 1H), 3.90–3.96 (m, 1H), 4.21 (t, 3/2H, J=3.5 Hz), 4.49 (t, 1/2H, J=3.0 Hz), 4.49 (dd, 1H, J=7.0, 4.0 Hz), 4.80 (d, 1H, J=7.0 Hz), 5.69 (d, 1H, J=10.5 Hz), 5.75 (d, 1H, J=10.5 Hz), 6.79 (dd, 4H, J=9.0 Hz), 7.11 (d, 1H, J=2.5 Hz), 7.16–7.20 (m, 1H), 7.26–7.28 (m, 3H), 7.39 (dd, 3H, J=6.0 Hz), 7.49–7.52 (m, 2H), 7.62 (d, 1H, J=1.5 Hz); ^{13}C NMR ($CDCl_3$) δ −4.8, −4.7, 18.2, 20.4, 24.5, 24.6, 24.6, 24.7, 25.9, 26.9, 27.0, 29.7, 38.7, 42.8, 42.9, 55.2, 58.6, 58.7, 64.3, 67.7, 73.9, 76.5, 78.5, 82.7, 86.2, 113.0, 113.1, 117.7, 118.4, 126.6, 127.7, 128.4, 130.3, 130.3, 136.1, 136.1, 138.0, 141.7, 145.0, 158.4, 177.6; ^{31}P NMR (202 MHz, CD_3CN) δ 148.9; HRMS(LSIMS)[19] calcd for $C_{50}H_{71}N_4O_9SiP$+Na [$(M+Na)^+$] 953.4621, found 953.4625. *Compound* **12**. A white powder; R_f =0.75 (50% EtOAc/hexane); ^{1}H NMR (500 MHz, CD_3CN) δ −0.10 (s, 3H), 0.02 (s, 3H), 0.77 (s, 9H), 1.07 (s, 9H), 1.08 (d, 3H, J=5.0 Hz), 1.16 (d, 9H, J=8.3 Hz), 2.52–2.53 (m, 2H), 3.00 (dd, 1H, J=11.0, 7.4 Hz), 3.30 (dd, 1H, J=7.4, 3.7 Hz), 3.55–3.65 (m, 2H), 3.75 (s, 6H), 3.70–3.85 (m, 2H), 3.95–4.05 (m, 1H), 4.30–4.35 (m, 2H), 4.92 (s, 1H), 5.75 (s, 2H), 6.84 (d, 4H, J=8.0 Hz), 7.14 (s, 1H), 7.2–7.5 (br m, 9H), 7.61 (s, 1H); ^{31}P NMR (202 MHz, CD_3CN) δ 149.1, 150.7; HRMS (LSIMS)[19] calcd for $C_{50}H_{71}N_4O_9SiP$+Na [$(M+Na)^+$] 953.4621, found 953.4618.

4.1.10. [4-(3,5-Di-*O*-benzyl-2-deoxy-β-D-ribofuranosyl) imidazolyl]methyl 2,2-dimethylpropiolate (14). By the same procedure as used for the preparation of **5c**, 2-deoxy compound **13**[7b] (1480 mg, 4.06 mmol) was converted to **14** (1750 mg, 90%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.14 (s, 9H), 2.20–2.38 (m, 2H), 3.53 (dd, 1H, J=7.0, 5.8 Hz), 3.64 (dd, 1H, J=7.0, 4.7 Hz), 4.08–4.26 (m, 2H), 4.55 (d, 4H, J=6.25 Hz), 5.13 (dd, 1H, J=5.8, 4.7 Hz), 7.00 (s, 1H), 5.75 (s, 2H), 7.20–7.40 (m, 11H), 7.60 (s, 1H); HRMS(EIMS) calcd for $C_{28}H_{34}N_2O_5$ [$(M)^+$] 478.2466, found 478.2464.

Conversion of **14** *into* **13**. To a solution of **14** (114 mg, 0.24 mmol) in methanol (10 ml) 28% aqueous NH_3 (2.5 ml) was added and then the whole was stirred for 3 h at rt. After evaporation, the resulting residue was purified by flash column chromatography (70–100% AcOEt) to give **13** (87 mg, quant) as a colorless oil.

4.1.11. [4-(5-*O*-DMT-2-deoxy-D-β-ribofuranosyl)imidazolyl]methyl 2,2-dimethylpropiolate (16). By the same procedures as used for the preparations of **8** and **9**, 2-deoxy compound **14** (229 mg, 0.48 mmol) was converted to a crude diol **15**, which was subsequently tritylated to give **16** (146 mg, 51%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 9H), 2.10–2.44 (m, 2H), 3.12–3.26 (m, 1H), 3.32–3.45 (m, 1H), 3.80 (s, 6H), 3.92–4.08 (m, 1H), 4.38–4.50 (m, 1H), 5.10–5.22 (m, 1H), 6.78 (s, 2H), 6.80 (d, 4H, J=8.0 Hz) 7.00 (s, 1H), 7.16–7.40 (m, 9H), 7.60 (s, 1H).

4.1.12. {4-[5-*O*-DMT-2-deoxy-3-*O*-(2-CE-*N*,*N*-diisopropylphosphoramidite)-β-D-ribo-furanosyl]imidazolyl}methyl 2,2-dimethylpropiolate (1b). By the same procedure for **1a**, compound **16** (129 mg, 0.22 mmol) was coevaporated with dichloroethane (2 ml) three times and dissolved in dichloroethane (1.0 ml) again. To the solution of **16** was added DIPT (18 mg, 0.11 mmol) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphodiamidite (0.08 ml, 0.24 mmol). The resulting mixture was stirred at 40 °C for 46 h and then evaporated. The residual oil was chromatographed by NH-silica gel chromatography to give partially purified **1b** (56 mg, 32%). PA **1b** was further purified on a preparative NH-TLC with 65% EtOAc in hexane to give **1b** (25 mg, 14%) as a colorless oil; **1b**: ^{1}H

NMR (500 MHz, $CDCl_3$) δ 1.13 (s, 12H), 1.26 (s, 9H), 2.27–2.30 (m, 2H), 2.45 (dt, 2H, $J=6.5$, 2.8 Hz), 3.23 (d, 2H, $J=5.0$ Hz), 3.57–3.63 (m, 2H), 3.66–3.74 (m, 2H), 3.79 (s, 6H), 4.15–4.19 (m, 1H), 4.50–4.56 (m, 1H), 5.15 (dd, 1H, $J=9.2$, 5.8 Hz), 5.75 (d, 2H, $J=2.6$ Hz), 6.81 (d, 4H, $J=8.4$ Hz), 7.04–7.06 (m, 1H), 7.17–7.22 (m, 1H), 7.23–7.29 (m, 3H), 7.30–7.36 (m, 4H), 7.43–7.47 (m, 2H), 7.61 (d, 1H, $J=1.0$ Hz); ^{13}C NMR ($CDCl_3$) δ 20.2, 24.5, 24.7, 26.8, 29.7, 38.7, 43.3, 55.2, 59.4, 64.2, 67.7, 75.1, 76.5, 86.1, 97.5, 113.0, 116.6, 126.7, 127.7, 128.3, 130.2, 136.2, 138.0, 143.4, 158.4, 177.6; ^{31}P NMR (202 MHz, CD_3CN) δ: 148.7; HRMS (LSIMS)[19] calcd for $C_{44}H_{57}N_4O_8P+Na$ [$(M+Na)^+$] 823.3809, found 823.3801.

4.1.13. {4-[3,5-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]imidazolyl}methyl 2,2-dimethylpropiolate (17). A mixture of **5c** (755 mg, 1.29 mmol), 20% $Pd(OH)_2$–C[23] (453 mg), and cyclohexene (3.9 ml, 38.80 mmol) in EtOH (30 ml) was refluxed for 3 h. After filtration through Celite, the filtrate was evaporated to give triol **8** (437 mg), which was subsequently dissolved with dry pyridine (18 ml). 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (0.4 ml) was added dropwise to the pyridine solution of **8** at 0 °C. The resulting mixture was stirred for 1 h at the same temperature and then at rt for 3 h. After evaporation, the crude product was purified by flash column chromatography (30–50% EtOAc in hexanes) to obtain **17** (481 mg, 67%) as a colorless oil; ^1H NMR ($CDCl_3$) δ 0.94–1.12 (m, 28H), 1.18 (s, 9H), 3.00 (br s, 1H), 3.94–4.10 (m, 3H), 4.27 (dd, 1H, $J=12.0$, 7.7 Hz), 4.46 (t, 1H, $J=12.0$ Hz), 4.77 (d, 1H, $J=7.7$ Hz), 5.78 (s, 2H), 7.10 (s, 1H), 7.66 (s, 1H); HRMS(EIMS) calcd for $C_{26}H_{48}N_2O_7Si_2$ [$(M)^+$] 556.2997, found 556.2988.

4.1.14. {4-[2-*O*-Allyl-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]imidazolyl}methyl 2,2-dimethylpropiolate (18). Compound **17** (481 mg, 0.87 mmol) was rendered anhydrous by coevaporation with dry pyridine three times. The residue was repeated coevaporated with dry toluene and finally dissolved in dry THF (9 ml). Triphenylphosphine (45 mg, 0.17 mmol) and tris(dibenzylideneacetone)-dipalladium (0) (16 mg, 0.017 mmol) were added. Finally, allyl ethyl carbonate (0.2 ml, 1.74 mmol) was added dropwise with stirring, and the reaction mixture was refluxed for 15 h and then evaporated. The residue was purified by flash column chromatography (10–30% EtOAc in hexanes) to obtain **18** (197 mg, 38%) as a colorless oil; ^1H NMR ($CDCl_3$) δ 0.92–1.12 (m, 28H), 1.16 (s, 9H), 3.97 (dd, 1H, $J=12.7$, 2.4 Hz), 4.00–4.06 (m, 3H), 4.11 (dd, 1H, $J=12.7$, 2.4 Hz), 4.16–4.43 (m, 2H), 4.97 (s, 1H), 5.15 (dq, 1H, $J=10.1$, 1.9 Hz), 5.32 (dq, 1H, $J=17.5$, 1.9 Hz), 5.76 (dd, 1H, $J=15.9$, 10.6 Hz), 5.87–6.01 (ddt, 1H, $J=17.5$, 10.1, 5.0 Hz), 7.10 (s, 1H), 7.62 (s, 1H); HRMS(EIMS) calcd for $C_{29}H_{52}N_2O_7Si_2$ [$(M)^+$] 596.3310, found 596.3308.

4.1.15. [4-(2-*O*-Allyl-β-D-ribofuranosyl)imidazolyl]methyl 2,2-dimethylpropiolate (19). A solution of TMEDA (0.3 ml, 1.98 mmol) in CH_3CN (0.3 ml) and HF (46% aqueous solution, 57 μl) was added to a small round-bottom flask at 0 °C. The HF/TMEDA mixture was stirred at 0 °C for 10 min, and a solution of **18** (197 mg, 0.33 mmol) in CH_3CN (2.3 ml) was added dropwise over 5 min. The resulting mixture was stirred at 0 °C for 30 min and then at rt for 2.5 h. The solvent was evaporated to give a residue, which was subsequently purified by flash column chromatography (5–15% MeOH in EtOAc) to obtain **19** (86 mg, 74%) as a colorless oil; ^1H NMR ($CDCl_3$) δ 1.17 (s, 9H), 3.62 (dd, 1H, $J=12.6$, 4.2 Hz), 3.77 (dd, 1H, $J=12.6$, 3.1 Hz), 3.90–3.97 (m, 2H), 3.98 (ddt, 1H, $J=13.1$, 5.3, 1.6 Hz), 4.08 (ddt, 1H, $J=13.1$, 5.3, 1.6 Hz), 4.19 (dd, 1H, $J=5.2$, 4.5 Hz), 4.77 (d, 1H, $J=6.6$ Hz), 5.07 (ddt, 1H, $J=10.3$, 1.6, 1.2 Hz), 5.16 (dq, 1H, $J=17.4$, 1.6 Hz), 5.82 (ddt, 1H, $J=17.4$, 10.3, 5.3 Hz), 5.94 (s, 2H), 7.32 (s, 1H), 7.84 (s, 1H); HRMS(EIMS) calcd for $C_{17}H_{27}N_2O_6$ [$(M+H)^+$] 355.1867, found 355.1869.

4.1.16. [4-(2-*O*-Allyl-5-*O*-DMT-D-β-ribofuranosyl)imidazolyl]methyl 2,2-dimethylpropiolate (20). By the same procedure as used for the preparation of **9**, a mixture of allyl compound **19** (43 mg, 0.12 mmol), DMTCl (66 mg, 0.18 mmol), Et_3N (0.03 ml, 0.18 mmol), and DMAP (0.4 mg, 0.003 mmol) was stirred at rt for 19 h to give **20** (81 mg, quant) as an amorphous product; ^1H NMR ($CDCl_3$) δ 1.12 (s, 9H), 3.28 (dd, 1H, $J=10.0$, 4.8 Hz), 3.38 (dd, 1H, $J=10.0$, 3.9 Hz), 3.78 (s, 6H), 4.00–4.28 (m, 5H), 4.97 (d, 1H, $J=3.8$ Hz), 5.17 (dd, 1H, $J=10.3$, 1.4 Hz), 5.26 (dd, 1H, $J=17.1$, 3.0, 1.4 Hz), 5.69 (q, 2H, $J=10.8$ Hz), 5.90 (ddt, 1H, $J=17.1$, 15.7, 5.4 Hz), 6.81 (d, 4H, $J=9.0$ Hz), 7.07 (br s, 1H), 7.14–7.50 (m, 9H), 7.62 (br d, 2H, $J=1.3$ Hz); ^{13}C NMR ($CDCl_3$) δ 27.1, 55.3, 64.2, 67.7, 71.2, 71.4, 78.2, 82.1, 82.9, 86.0, 112.7, 117.2, 126.2, 127.3, 127.9, 129.8, 133.5, 135.6, 137.7, 141.6, 144.5, 157.8, 176.8 (CO); HRMS(EIMS) calcd for $C_{38}H_{44}N_2O_8$ [$(M)^+$] 656.3095, found 656.3091.

4.1.17. {4-[2-O-Allyl-5-*O*-DMT-3-*O*-(2-CE-*N*,*N*-diisopropylphosphoramidite)-β-D-ribofuranosyl]imidazolyl}methyl 2,2-dimethylpropiolate (1c). By the same procedure for **1a**, compound **20** (43 mg, 0.07 mmol) was dissolved in dichloromethane (0.3 ml). To the solution was added a solution of DCI (6 mg, 0.05 mmol) in CH_3CN (0.05 ml) followed by CETPA (21 μl, 0.07 mmol). After the mixture was stirred at rt for 12 h, DCI (6 mg, 0.05 mmol) in CH_3CN (0.05 ml) and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphodiamidite (21 μl, 0.07 mmol) were added. The resulting mixture was further stirred for 12 h at rt and then evaporated. The residual oil was chromatographed using benzene by NH-silica gel chromatography to give partially purified **1c** (33 mg, 60%). The semi-purified **1c** was further purified by NH-silica gel (35% EtOAc in hexane) to give **1c** (15 mg, 27%) as a colorless oil; ^1H NMR (500 MHz, $CDCl_3$) δ 1.00 (s, 2H), 1.08–1.40 (m, 19H), 2.32 (t, 1H, $J=6.5$ Hz), 2.61 (q, 1H, $J=5.9$ Hz), 3.20 (td, 2H, $J=8.8$, 3.8 Hz), 3.33–3.65 (m, 5H), 3.78 (s, 6H), 4.00–4.45 (m, 5H), 4.98 (t, 1H, $J=5.3$ Hz), 5.11 (br d, 1H, $J=9.5$ Hz), 5.23 (d, 1H, $J=17.3$ Hz), 5.60–5.77 (m, 2H), 5.80–5.95 (m, 1H), 6.80 (dd, 4H, $J=8.3$, 6.4 Hz), 7.10 (br d, 1H, $J=3.7$ Hz), 7.16–7.30 (m, 3H), 7.31–7.39 (m, 4H), 7.44–7.50 (m, 2H), 7.61 (s, 1H); ^{31}P NMR (202 MHz, $CDCl_3$) δ 149.7, 150.4; HRMS(LSIMS)[19] calcd for $C_{47}H_{62}N_4O_9P$ [$(M+H)^+$] 857.4251, found 857.4252; calcd for $C_{47}H_{61}N_4O_9PNa$ [$(M+Na)^+$] 879.4070, found 879.4069; calcd for $C_{47}H_{61}N_4O_9PK$ [$(M+K)^+$] 895.3810, found 895.3811.

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