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SYNTHESIS OF IMIDAZOLE C_1- AND C_3-RIBONUCLEOSIDE PHOSPHORAMIDITES FOR PROBING CATALYTIC MECHANISM IN RIBOZYME

Shinya Harusawa,[1*] Kensuke Fujii,[1] Masayoshi Nishiura,[1] Lisa Araki,[1] Yoshihide Usami,[1] Zheng-yun Zhao,[2] and David M. J. Lilley[3]

[1]Osaka University of Pharmaceutical Sciences, 4-20-1, Nasahara, Takatsuki, Osaka 569-1094, Japan

[2]School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

[3]CR-UK Nucleic Acid Structure Research Group, MSI/WTB Complex, University of Dundee, Dundee DDI 5EH, UK

Abstract – Synthesis of C4-linked imidazole *N*-pivaloyloxymethyl (POM)-2'-*O*-cyanoethylated (CE) C_1- and C_3-ribonucleoside phosphoramidites **1a** and **1b** is described. These phosphoramidite products were incorporated into RNA sequence through solid phase phosphoramidite approach, providing RNA with imidazole linked through different length to sugar residue, to study the mechanism of a ribozyme.

INTRODUCTION

RNA catalysis is important in the processing and translation of RNA molecules, yet the mechanisms of catalysis are still unclear in most cases.[1] The VS ribozyme is the largest of a group of nucleolytic ribozymes that include hammerhead, hairpin, HDV, and GlmS, and catalyzes the site-specific cleavage of a phosphodiester linkage to generate products containing 2', 3'-cyclic phosphate and 5'-OH termini.[2] Imidazole with a p*K*a of 7.1 is both a good donor and acceptor of proton. It can be placed in the normal base position to study the general acid and base catalysis.[3] We have recently developed a new chemo-genetic strategy for examining the role of general acid-base catalysis in ribozyme function,

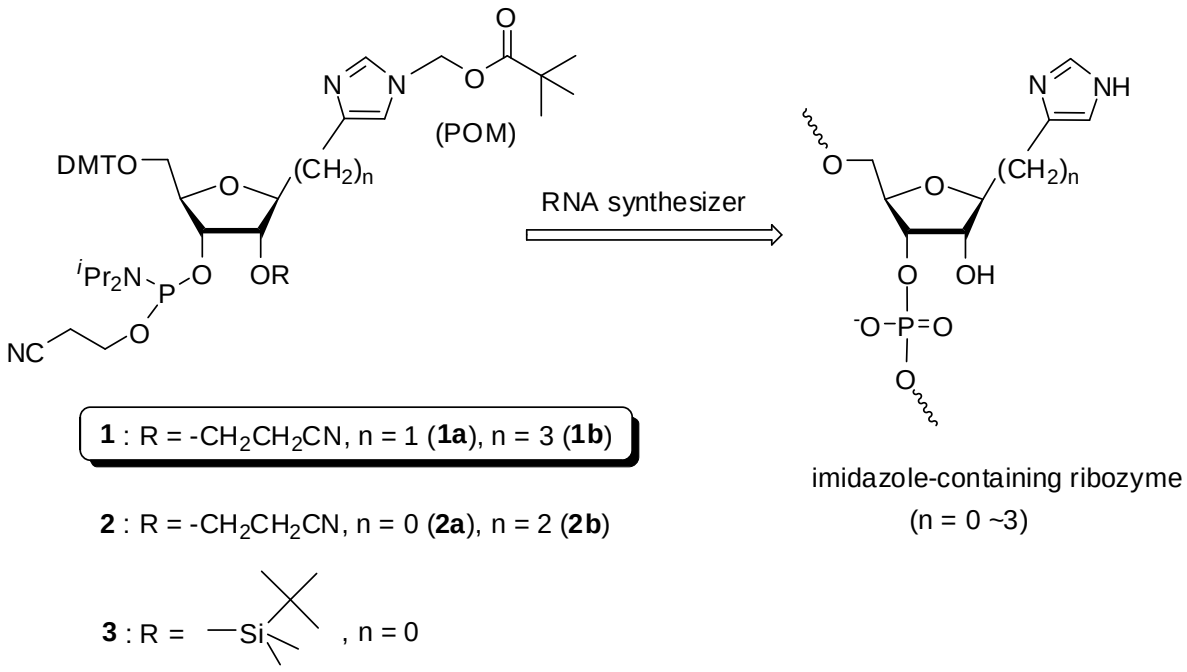


Figure 1. Imidazole C_n-ribonucleoside phosphoramidites for solid-phase RNA synthesis

whereby C4-linked imidazole was covalently placed as a pseudonucleobase at position 756 (replacing adenine) of the VS ribozyme[2e] and position 8 (replacing guanine) of the hairpin ribozyme[5] respectively. In the study, *N*-pivaloyloxymethyl (POM) 2'-*O*-*tert*-butyldimethylsilyl (*t*-BDMS)-imidazole C_0-phosphoramidite (PA) **3**[4a-c] was first employed to provide an imidazole-substituted ribozymes, which catalyzed the almost-complete cleavage of substrate stem-loops at the correct position.[2e,5] However, the synthetic route of PA **3** lacks the selective introduction of *t*-BDMS group at 2'-hydroxy group and in addition **3** itself has acid-labile character, eventually reducing the overall yield.[4a-b]

In our systematic studies on the catalytic mechanism of VS ribozyme,[2] we have recently introduced a new combination of protecting groups for the PAs **2a** (n = 0)[6b] and a two-carbon-elongated homologue **2b** (n = 2)[6a] of ribose-$(CH_2)_n$-imidazole species: POM for imidazole-*N* and cyanoethyl (CE) groups for the 2'-hydroxy group. They facilitated efficient synthesis of **2a** and **2b** and additionally provided considerable stability compared to the first labile 2'-*O*-*t*-BDMS-imidazole C_0-PA **3**. Using **2b**, a single imidazole base-moiety with two carbon linker was inserted at position 638 of VS ribozyme in *trans*-acting form to give a variant ($G638C_2Imz$). G638 is one of the nucleobases proposed to act in general acid-base catalysis in concert with A756 in the catalytic mechanism of the VS ribozyme.[2a]

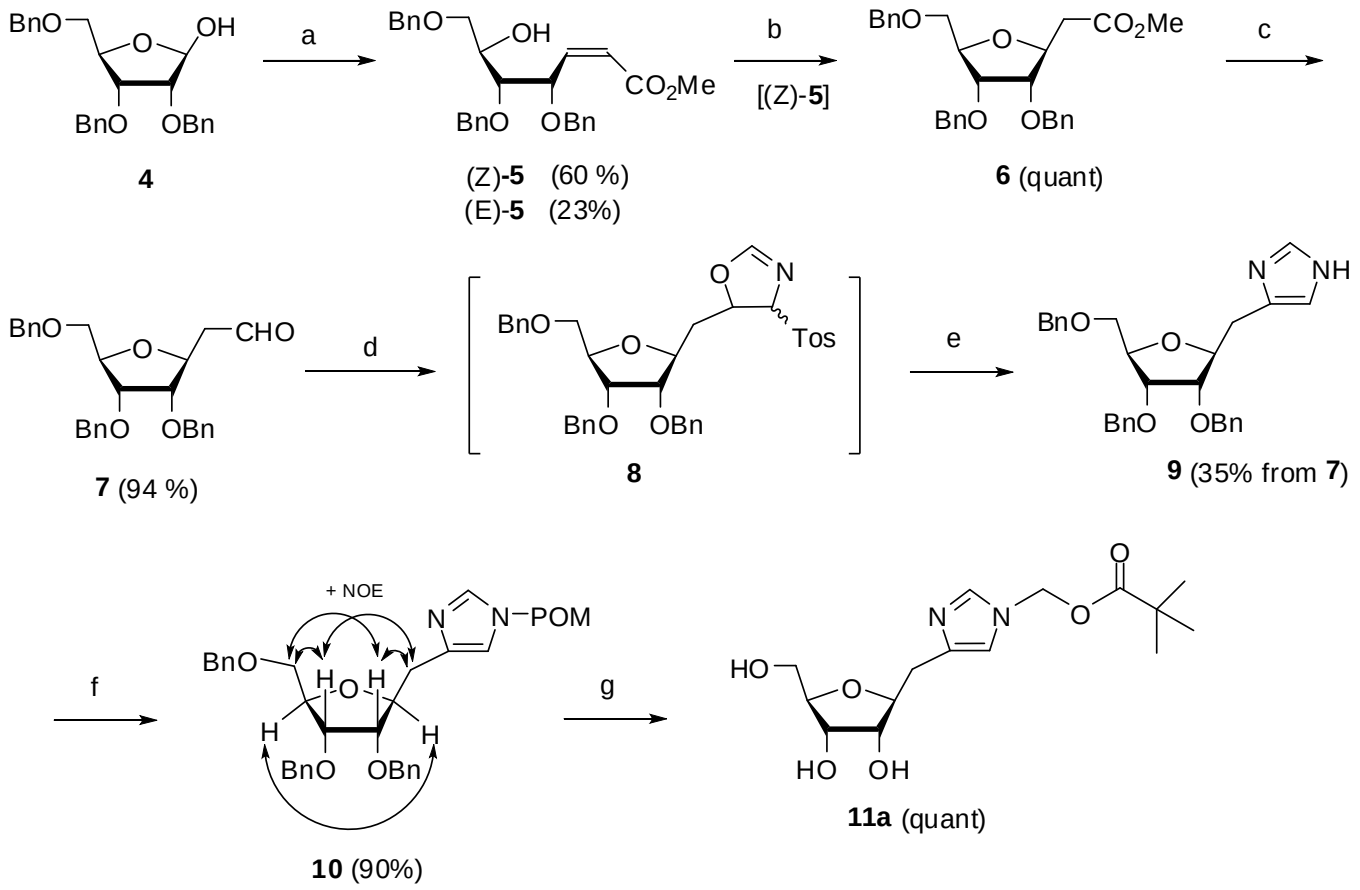
It might be anticipated that the manner of linking the imidazole group to the ribose would affect its ability to function in general acid base catalysis. If the linker is too short then its ring *N* cannot be superimposed

with that of *N*1 of adenine or guanine. On the other hand, if it is too long then it may spend too little time in the position required for its potential catalytic function. It is interesting to note that the cleavage rate of G638C_2Imz was found to be 15-fold greater than that achieved with the ribozyme (G638C_0Imz) containing a C4-linked imidazole at the same position.[6] The result suggests that imidzole nucleoside analogues with flexible spacer lengths provide a valuable general methodology for further exploring the catalytic mechanisms of ribozymes as well as other RNA functions as better structural mimics of purine nucleobase. Further, the whole series of ribose-$(CH_2)_n$-imidazole PAs (n = 0 ~ 3) could make an interesting series of compounds for systematically tracing out the geometry of the active site of a ribozyme. As the VS ribozyme is the only member of the class of nucleolytic ribozymes for which there is no crystal structure at the present time, mechanistic investigation cannot be guided by structural data.[2d] We therefore required C_1- and C_3-PAs **1a** and **1b** in order to complete and extend our series, allowing us to compare imidazole C_1- and C_3-ribozymes with those of the C_0- and C_2 forms. We herein describe the chemical synthesis of novel C4-linked C_1- and C_3-imidazole ribonucleoside PAs **1a** and **1b**.

RESULTS AND DISCUSSION

Starting from commercially available 2,3,5-tri-*O*-benzyl-D-ribose **4**,[7] a two-step synthesis of methyl 3,6-anhydro-4,5,7-tri-*O*-benzyl-2-deoxy-*β*-D-allo-heptonate **6** was at first carried out according to the synthetic procedure of Ohrui and co-workers.[8] Wittig olefination of **4** with methyl (triphenylphosphoranylidene)acetate afforded (*Z*)-alkene product **5** (60 %), together with (*E*)-isomer (23%). Although the reaction required 6 hours (h) using the original procedure,[8] use of benzoic acid (0.1 eq) as an additive shortened the reaction time to 50 min in our hands. Treatment of (*Z*)-**5** with sodium methoxide provided ester **6** (quant) and then DIBAL reduction of **6** afforded a new aldehyde **7** (94%) which is a mutual intermediate for synthesis of C_1- and C_3-PAs **1a** and **1b** (Scheme 1 and 2).

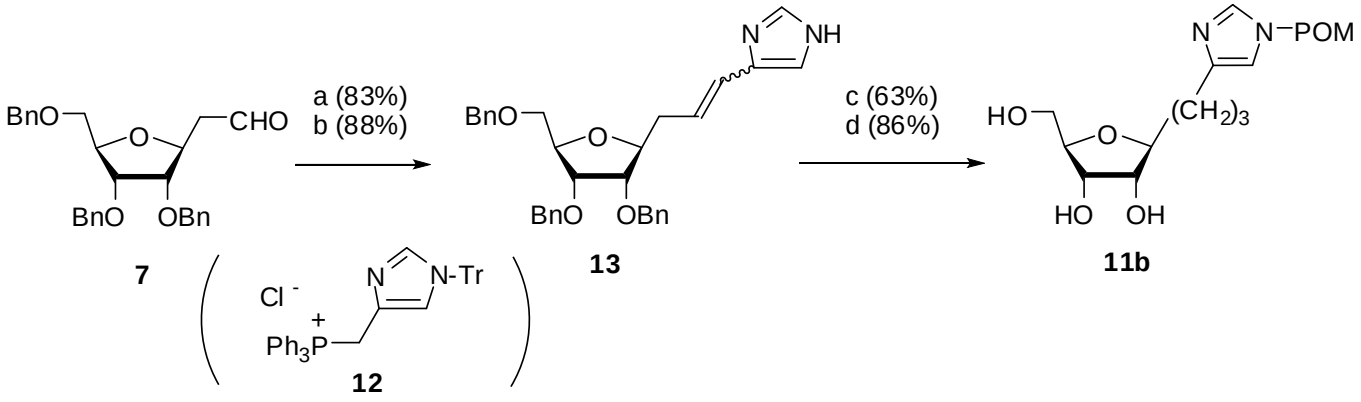
We next tried to prepare the imidazole ring from aldehyde **7** by Büchi procedure.[9] Reaction of **7** with (*p*-tolylsulfonyl)methylisocyanide (TosMIC) in the presence of catalytic amount of NaCN in a [3+2] cycloaddition led to the formation of the 4-tosyloxazoline **8**, which was subsequently heated with a solution of saturated ammonia in ethanol at 125 ^oC in a stainless steel tube for 2 h to give a one-carbon-elongated homo-*C*-nucleoside **9** in 35% yield from **7**. Introduction of a POM group at the imidazole-*N* position of **9** produced 2',3',5'-tri-*O*-benzyl-*N*-POM-nucleoside **10** (90%).[4] The relative *β*-stereochemistry of **10** was confirmed from a NOESY experiment, as summarized in Scheme 1. Debenzylation of **10** with $Pd(OH)_2$-C/cyclohexene afforded ^{im}*N*-POM-imidazole C_1-D-ribonucleoside **11a** (quant).



Scheme 1. Synthesis of imidazole C_1 triol **11a**.
Reagents and conditions: (a) $Ph_3P{=}CHCO_2Me$ (1 eq), benzoic acid (0.1 eq), reflux 50 min, MeCN; (b) NaOMe, rt, 10 min; (c) DIBAL, -78 °C; (d) TosMIC, NaCN (cat.), EtOH, 0.5h; (e) NH_3 in EtOH, 125 °C, 2 h; (f) NaH, POMCl, rt, 3 h, THF; (g) 20% $Pd(OH)_2$-C, cyclohexene, EtOH, reflux, 4 h.

Alternatively, Wittig olefination (83%) of aldehyde **7** using *N*-trityl-4-imidazolylmethylphosphonium chloride **12**[6a] followed by acid treatment to remove the trityl group gave three-carbon-elongated imidazole **13** (88%) (Scheme 2). POM protection (63%) of **13** and subsequent debenzylation and reduction of double bond with $Pd(OH)_2$-C/cyclohexene (86%) afforded ^{im}*N*-POM-C_3-imidazole **11b**.

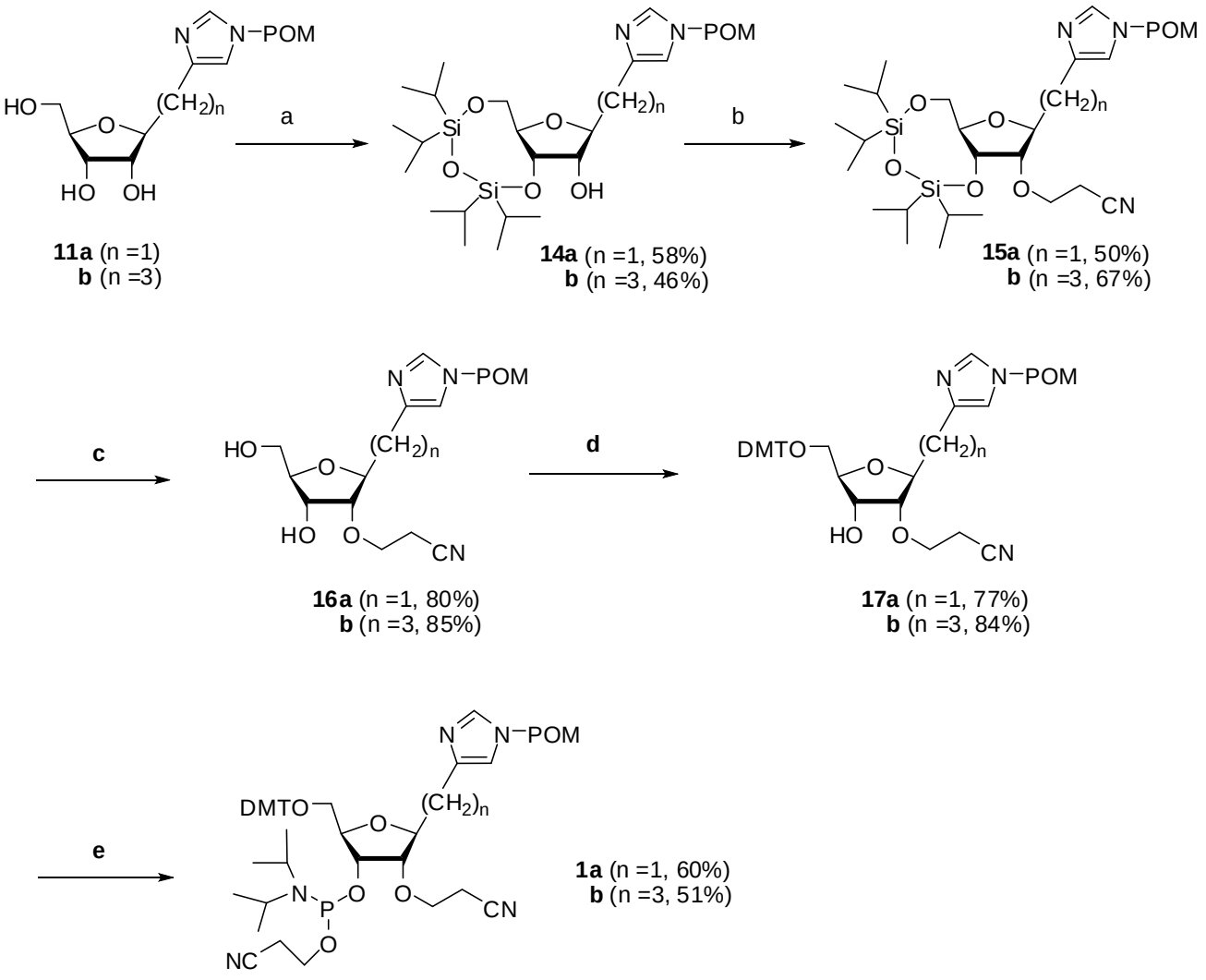
With C_1-and C_3-triol intermediates **11a** and **11b** in hand, we addressed the synthesis of imidazole-C_1- and C_3-PAs **1a** and **1b**, as shown in Scheme 3. 3',5'-*O*-TIPDS-protection (TIPDS = 1,1,3,3-tetra-isopropyldisiloxanediyl) of **11a** (n = 1) gave **14a** (58%), which allowed selective introduction of the 2'-hydroxy protecting group in a moderate yield. Cyanoethylation of **14a** with acrylonitrile did not proceed at room temperature (rt),[10] while the reaction gave fully protected intermediate **15a** at 40 °C in 50% yield, causing partial removal of POM group. The TIPDS group of **15a** was selectively removed by treatment with $Et_3N{\cdot}3HF$ to give 3',5'-*O*-unprotected ribonucleoside derivative **16a** (80%). After



Scheme 2. Synthesis of imidazole C_3 triol **11b**.
Reagents and conditions: (a) **12**, n-BuLi, -78 °C then rt; (b) 2*N* HCl, reflux, EtOH; (c) NaH, POMCl, rt, THF; (d) 20% $Pd(OH)_2$-C, cyclohexene, EtOH, reflux.

dimethoxytritylation (77 %) of **16a** in the usual manner, 3'-free derivative **17a** was subjected to phosphitylation condition. Treatment of **17a** 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 1h to give the final product imidazole-C_1-PA **1a**. Purification of the crude product was readily carried out on basic silica gel column chromatography to yield phosphoramidite **1a** (60%). The overall yield of **1a** is 1.9% in 12 steps from the starting tribenzyl D-ribose **4**. ^{31}P-NMR of **1a** reaveled two P-diastereomers at δ 149.3 and 149.9 ppm ($CDCl_3$). Although MS measurement of PAs has been problematic owing to their labile properties, we recently reported MS measurement of nucleoside and non-nucleoside PAs, with the new matrix system [triethanolamine (TEOA)-NaCl] on LSIMS or FABMS using a double-focusing mass spectrometer.[11] The present method was consistent with the composition formula {$C_{48}H_{62}N_5O_9Na$ [$(M+Na)^+$]; calculated: 906.4182, observed: 906.4191}. In a similar manner, conversion of ^{im}*N*-POM-imidazole C_3-ribonucleoside **11b** into imidazole-C_3-PA **1b** was performed according to that of C_1-homologue **11a** into **1a**, as shown in Scheme 3. The structure of **1b** was characterized by ^{31}P-NMR [δ 149.8 and 150.7 ppm ($CDCl_3$)] and MS measurement {$C_{50}H_{66}N_5O_9Na$ [$(M+Na)^+$]; calculated: 934.4495, observed: 934.4490}.[11] The overall yield of **1b** is 2.5% in 12 steps from the starting material **4**.

Studies of modified VS ribozymes and other imidazole oligonucleotides using **1a** and **1b** are under way, and will be published in due course.



Scheme 3. Synthesis of 2'-*O*-cyanoethylimidazole C_1 and C_3-nucleoside phosphoramidites **1a** and **1b**. Reagents and conditions: (a) $TIPDSCl_2$, py; (b) CH_2=CHCN, $CsCO_3$, ^{t}BuOH; (c) $Et_3N \cdot 3HF$, Et_3N, THF; (d) DMTCl, DMAP, Et_3N, py; (e) $(^iPr_2N)_2POCH_2CH_2CN$, 4,5-DCI, $ClCH_2CH_2Cl$, 40 ^oC.

EXPERIMENTAL

General

IR spectra were recorded using a Shimadzu IR-435 spectrometer. ^{1}H- and ^{13}C-spectra were measured with tetramethylsilane as an internal standard on a Varian Mercury-300 or Varian UNITY INOVA-500 spectrometers. ^{31}P-NMR spectra were recorded at 121 MHz (Varian UNITY INOVA-500) and the chemical shifts were measured relative to 85% H_3PO_4 as an external standard. Fast atom bombardment mass spectrometric studies (FAB-MS) were performed using a JMS-700 (JEOL Ltd.) instrument in the positive ion mode with a TEOA-NaCl or 3-nitrobenzylalcohol (NBA) matrix. 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 solvents were removed in a rotary evaporator under reduced pressure. Anhydrous solvents were purchased from Wako Chemical Co. and Nacalai Chemical Co. Fuji Silysia FL-60D silica gel, Fuji Silysia BW-127ZH silica gel, and Merck $60F_{254}$ 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 Silysia Chemical Ltd.) was used.

2-(2,3,5-Tri-*O*-benzyl-*β*-D-ribofuranosyl)acetaldehyde (7)

To a solution of **6** (88 mg, 0.19 mmol) in dry toluene (2 mL) at -70 ^oC was added dropwise a 1M solution of DIBAL in toluene (0.56 mL, 0.56 mmol) over 5 min. After stirring for 0.5 h at the same temperature, the reaction mixture was quenched with MeOH (0.6 mL) and further stirred for 0.5 h at rt. Saturated aq. $NaHCO_3$ (0.6 mL) was added and the reaction mixture was stirred for another 0.5 h. After anhydrous magnesium sulfate was added to the resulting suspension, the reaction mixture was filtered through Celite pad, and washed with AcOEt. The filtrate was evaporated to give a crude oil, which was purified by column chromatography (25% AcOEt in hexane) to give **7** (80 mg, 94%) as a colorless oil. IR (film) cm^{-1} 1720 (CO); ^{1}H-NMR ($CDCl_3$) δ 2.54 (ddd, 1H, *J* = 16.3, 7.8, 2.2 Hz), 2.64 (ddd, 1H, *J* = 16.3, 5.2, 1.9 Hz), 3.47 (d, 2H, *J* = 4.4 Hz), 3.65 (dd, 1H, *J* = 7.7, 5.5 Hz), 3.92 (dd, 1H, *J* = 5.5, 3.3 Hz), 4.17-4.25 (m, 1H), 4.40-4.65 (m, 7H), 7.20-7.40 (m, 15H), 9.74 (t, 1H, *J* = 2.3 Hz); ^{13}C-NMR ($CDCl_3$) δ 47.1, 70.1, 71.7, 72.1, 73.4, 75.5, 76.6, 80.6, 82.1, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3,128.4, 137.4, 137.6, 137.9, 200.6; MS of **7** could not be measured owing to its thermal instability.

4-(2,3,5-Tri-*O*-benzyl-*β*-D-ribofuranosyl)methyl-1*H*-imidazole (9)

To a suspension of TosMIC (372 mg, 1.87 mmol) and **7** (837 mg, 1.87 mmol) in EtOH (4 mL) was added NaCN (14 mg, 0.28 mmol) at rt, and the mixture was stirred for 30 min. The resulting solution was transferred to a saturated NH_3 in EtOH (30 mL) in a stainless steel tube at 0 ^oC, and the sealed system was then heated at 125 ^oC for 2 h. After the mixture was cooled, the solvent was evaporated to give a residue which was purified by column chromatography (15% MeOH in AcOEt) to give the imidazole compound **9** (295 mg, 35%) as a pale brown oil. R*f* (15% MeOH in AcOEt): 0.67; ^{1}H NMR($CDCl_3$) δ 2.67 (dd, 1H, *J* = 16.0, 4.8 Hz), 2.92 (dd, 1H, *J* = 16.0, 4.8 Hz), 3.50-3.58 (m, 2H), 3.62 (t, 1H, *J* = 6.8 Hz), 3.80 (dd, 1H, *J* = 10.6, 2.9 Hz), 4.02-4.17 (m, 1H), 4.26-4.64 (m, 7H), 6.57 (s, 1H), 6.66 (s, 1H), 7.15-7.47 (m, 15H). ^{13}C-NMR ($CDCl_3$) δ 28.4, 68.7, 70.2, 72.3, 73.2, 73.7, 76.4, 77.2, 77.8, 78.1, 79.8, 80.1, 82.0, 124.9, 127.0, 127.6, 127.8, 127.9 (127.89), 127.9 (127.94), 128.1 (128.06), 128.1 (128.12), 128.3, 128.4 (128.38), 128.4 (128.41), 128.6, 134.9, 137.1, 137.5, 137.6. HRMS (FABMS) calcd for $C_{30}H_{33}N_2O_4$

$(M+H)^+$ 485.2440, found 485.2448.

[4-(2,3,5-Tri-*O*-benzyl-*β*-D-ribofuranosyl)methylimidazolyl]methyl 2,2-dimethylpropionate (10)

Under stirring, 60% NaH (37 mg, 0.91 mmol) in mineral oil was added to THF (3 ml) to give a suspension. A solution of **9** (295 mg, 0.61 mmol) in THF (5 mL) was added to the suspension, and the resulting mixture was stirred at rt for 0.5 h. Then, a solution of chloromethyl pivaloate (138 mg, 0.91 mmol) in THF (4 mL) was added. After 3 h, H_2O (1 mL) was added and the mixture was evaporated to give a residue, which was subsequently dissolved in AcOEt. The organic layer was washed with water, brine, dried, and evaporated. The residue was purified by column chromatography on silica gel using AcOEt in hexane (50% to 100%) to give compound **10** (328 mg, 90%) as an oil; ^{1}H-NMR ($CDCl_3$) δ 1.14 (s, 9H), 2.78 (dd, 1H, *J* = 14.3, 6.5 Hz), 2.86 (dd, 1H, *J* = 14.3, 6.5 Hz), 3.49 (dd, 1H, *J* = 10.4, 3.9 Hz), 3.55 (dd, 1H, *J* = 10.4, 3.9 Hz), 3.76-3.83 (m, 2H), 4.20 (dd, 1H, *J* = 9.1, 3.9 Hz), 4.32 (dd, 1H, *J* = 11.7, 5.2 Hz), 4.38-4.62 (m, 6H), 5.65 (q, 2H, *J* = 9.5 Hz), 6.83 (s, 1H), 7.16-7.40 (m, 15H), 7.53 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ : 26.8, 32.8, 38.7, 67.6, 70.2, 71.7, 73.3, 77.3, 79.7, 80.7, 81.0, 117.0, 127.6, 127.9, 128.0, 128.3, 137.3, 137.9, 138.0, 138.2, 139.5; HRMS(FABMS) calcd for $C_{36}H_{43}N_2O_6$ $(M+H)^+$ 599.3121, found 599.3123.

[4-(*β*-D-Ribofuranosyl)methylimidazolyl]methyl 2,2-dimethylpropionate (11a)

A mixture of **10** (328 mg, 0.55 mmol), 20% $Pd(OH)_2$-C (197 mg), and cyclohexene (1.7 mL, 16.43 mmol) in EtOH (12 mL) was refluxed for 4 h. After filtration through Celite, the filtrate was evaporated to give a residue, which was purified by column chromatography (20% MeOH-EtOAc) to give **11a** (180 mg, quant) as a colorless oil; ^{1}H-NMR (CD_3OD) δ 1.17 (s, 9H), 2.72 (dd, 1H, *J* = 15.5, 7.1 Hz), 2.86 (dd, 1H, *J* = 15.5, 5.2 Hz), 3.51 (dd, 1H, *J* = 17.8, 6.6 Hz), 3.65 (dd, 1H, *J* = 17.8, 6.6 Hz), 3.71-4.03 (m, 4H), 5.89 (s, 2H), 7.09 (s, 1H), 7.76 (s, 1H); ^{13}C-NMR (CD_3OD) δ 27.2, 32.8, 39.7, 63.4, 69.3, 72.6, 75.7, 83.7, 85.5, 118.8, 139.1, 179.0; HRMS (FABMS) calcd for $C_{15}H_{25}N_2O_6$: 329.1712 $(M+H)^+$, found 329.1711.

4-[(*EZ*)-3-(2,3,5-Tri-*O*-benzyl-*β*-D-ribofuranos-1-yl)prop-1-enyl]-1*H*-imidazole [(*EZ*)-13]

A 1.6 M BuLi solution in hexane (2.9 ml, 4.6 mmol) was added dropwise over a period of 15 min to a white suspension of phosphonium salt **12** (2.90 g, 4.6 mmol) in dry THF (20 mL) at -70 °C. The resulting yellow suspension was stirred for 30 min at the same temperature, and a solution of aldehyde **7** (1.02 g, 2.30 mmol) in THF (10 mL) was added slowly to keep the temperature of the suspension at approximately -70 °C. The reaction mixture was elevated to rt and continued to stir for 3 h. The reaction was quenched by the addition of H_2O and was evaporated. $CHCl_3$ was added to the residue, and the

organic layer was washed with H_2O and brine, dried, and then evaporated to give a residue. It was again diluted with AcOEt, mixed with a small amount of silica gel, and evaporated to obtain coated silica gel for use in column chromatography. Chromatography with 60% AcOEt-hexane as eluent gave a pale yellow foam (1.42 g, 83%) of 4-[(*EZ*)-2-(2,3,5-tri-*O*-benzyl-*β*-D-ribofuranos-1-yl)prop-1-enyl]-1-trityl-1*H*-imidazole; ^{1}H-NMR ($CDCl_3$) δ 2.30-2.48 (m, 2H), 3.46 (d, 0.33H, *J* = 4.3 Hz), 3.51 (d, 1.67H, *J* = 4.3 Hz), 3.63-3.70 (m, 1H), 3.85 (t, 1H, *J* = 5.1 Hz), 4.07-4.22 (m, 2H), 4.42-4.58 (m, 6H), 6.19-6.35 (m, 2H), 6.68 (s, 0.83H), 6.78 (s, 0.17H), 7.08-7.40 (m, 31H); ^{13}C-NMR ($CDCl_3$) δ 14.1, 21.0, 32.9, 36.9, 60.3, 70.3, 70.4, 71.7 (71.66), 71.7 (71.71), 73.3, 75.1, 77.3, 77.6, 79.5, 79.7, 80.8, 80.9, 81.0; HRMS (FABMS) calcd for $C_{51}H_{49}N_2O_4$ $(M+H)^+$: 753.3692, found 753.3687. Aqueous 2*N* HCl (12 mL) was added to the solution of the propenylimidazole (1.49 g, 1.89 mmol) in EtOH (15 mL) and the solution was refluxed for 2.5 h. After cooling to rt, the precipitated material was removed by filtration. The filtrate was evaporated to give a residue that was subsequently diluted with water and neutralized by the addition of saturated aq. $NaHCO_3$ solution. The aqueous was extracted with AcOEt, and the combined organic layers were washed with H_2O, dried, and evaporated to yield a residue. Chromatography purification on silica with 10% MeOH in AcOEt as eluent gave a mixture (0.85 g, 88%) of **13** as a yellow foam. ^{1}H-NMR ($CDCl_3$) δ 2.26-2.64 (m, 2H), 3.40-3.74 (m, 3H), 3.82-3.97 (m, 1H), 3.98-4.26 (m, 2H), 4.40-4.67 (m, 6H), 5.53 (dt, 0.3H, *J* = 10.8, 8.4 Hz), 6.01 (dt, 0.7H, *J* = 18.1, 8.4 Hz), 6.23 (d, 0.7H, *J* = 18.1 Hz), 6.40 (d, 0.3H, *J* = 10.8 Hz), 6.81 (s, 0.7H), 6.92 (s, 0.3H), 7.19-7.48 (m, 16H); ^{13}C-NMR ($CDCl_3$) δ 32.5, 36.8, 69.8, 70.4, 71.8, 72.0 (71.95), 72.0 (72.03), 72.3, 73.4 (73.38), 73.4 (73.44), 77.1, 120.9, 121.7, 123.8, 124.7, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1 (128.09), 128.1 (128.12), 128.3, 128.4, 128.5, 134.9, 135.3, 137.5, 137.7 (137.68), 137.7 (137.73), 138.1; HRMS (FABMS) calcd for $C_{32}H_{34}N_2O_4$ $(M+H)^+$: 511.2597, found 511.2592.

{4-[3-(*β*-D-Ribofuranosyl)propyl]imidazolyl}methyl 2,2-dimethylpropionate (11b)

Imidazole **13** (220 mg, 0.43 mmol) was first converted into (*EZ*)-^{im}*N*-POM-propenylimidazoles (168 mg, 63%, *E/Z* = 20:1) as a colorless oil, according to the synthetic procedure of **10**. Although the separation (*E*) and (*Z*)-POM products was not required for the following reduction, they could be resolved by silica gel chromatography (50% AcOEt in hexane); {4-[(*E*)-3-(2,3,5-Tri-*O*-benzyl-*β*-D-ribofuranosyl)prop-1-enyl]imidazolyl}methyl 2,2-dimethylpropionate; colorless oil; R_f (60% AcOEt in hexane); 0.28; ^{1}H-NMR ($CDCl_3$) δ 1.15 (s, 9H), 2.70-2.90 (m, 2H), 3.48-3.60 (m, 2H), 3.73 (t, 1H, *J* = 5.7 Hz), 3.92 (t, 1H, *J* = 5.0 Hz), 4.17-4.27 (m, 2H), 4.46-4.60 (m, 6H), 5.63-5.74 (m, 3H), 6.35 (d, 1H, *J* = 11.7 Hz), 7.04 (s, 1H), 7.22-7.37 (m, 15H), 7.59 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 26.8, 33.0, 38.7, 67.6, 70.5, 71.7, 71.8, 73.3, 77.5, 80.1, 80.8, 81.2, 118.0, 122.9, 126.1, 127.5 (127.49), 127.5 (127.52), 127.6, 127.7, 127.8,

127.9, 128.0, 128.1 (128.09), 128.1 (128.14), 128.2 (128.18), 128.2 (128.24), 128.3 (128.26), 128.3 (128.28), 137.6, 137.9, 138.0, 138.2, 140.4, 177.6; HRMS(FABMS) calcd for $C_{38}H_{46}N_2O_6$ $(M+H)^+$ 625.3277, found 625.3279. {4-[(*Z*)-3-(2,3,5-Tri-*O*-benzyl-*β*-D-ribofuranosyl)prop-1-enyl]imidazolyl}-methyl 2,2-dimethylpropionate; colorless oil; R_f (60% AcOEt in hexane); 0.14. ^{1}H-NMR ($CDCl_3$) δ 1.17 (s, 9H), 2.34-2.53 (m, 2H), 3.52 (d, 2H, *J* = 4.2 Hz), 3.67 (t, 1H, *J* = 6.0 Hz), 3.87 (t, 1H, *J* = 4.8 Hz), 4.06-4.25 (m, 2H), 4.44-4.65 (m, 6H), 5.78 (s, 2H), 6.27 (d, 1H, *J* = 15.6 Hz), 6.28-6.40 (m, 1H), 6.89 (s, 1H), 7.19-7.40 (m, 15H), 7.60 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 26.6, 29.5, 30.1, 36.7, 38.5, 67.4, 70.2, 71.5, 71.6, 73.2, 77.2, 79.5, 80.6, 80.9, 115.7, 123.8, 124.9, 127.3, 127.4, 127.5, 127.6, 127.8, 127.9 (127.88), 127.9 (127.94), 128.1, 128.2, 137.7, 138.0, 138.1, 141.0, 177.5; HRMS(FABMS: NBA) calcd for $C_{38}H_{46}N_2O_6$ $(M+H)^+$ 625.3277, found 625.3276. Next, a mixture of (*EZ*)-^{im}*N*-POM-propenylimidazole (173 mg, 0.28 mmol), 20% $Pd(OH)_2$-C (104 mg), and cyclohexene (1.0 mL, 8.40 mmol) in EtOH (9 mL) was refluxed for 2.5 h. After filtration through Celite, the filtrate was evaporated to give a residue, which was purified by silica column chromatography (20% MeOH in AcOEt) to give **11b** (86 mg, 86%) as a colorless oil. ^{1}H-NMR (CD_3OD) δ 1.16 (s, 9H), 1.44-1.89 (m, 4H), 2.57 (t, 2H, *J* = 9.6 Hz), 3.50-3.83 (m, 5H), 3.87 (t, 1H, *J* = 6.0 Hz), 5.90 (s, 2H), 6.98 (s, 1H), 7.73 (s, 1H); ^{13}C-NMR (CD_3OD) δ 26.7, 27.4, 28.9, 34.3, 39.9, 48.6, 48.9, 49.5, 49.8, 63.8, 69.4, 73.0, 76.5, 84.1, 85.7, 117.4, 139.4, 143.9, 179.1; HRMS(FABMS) calcd for $C_{17}H_{29}N_2O_6$ $(M+H)^+$ 357.2025, found 357.2029.

{4-[(3,5-*O*-TIPDS-*β*-D-ribofuranosyl)methyl]imidazolyl}methyl 2,2-dimethypropionate (14a)

1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (0.14 mL, 0.43 mmol) was added dropwise to a solution of **11a** (141 mg, 0.43 mmol) in pyridine (10 mL) at 0 °C. The resulting mixture was stirred for 1 h at the same temperature and evaporated to a crude product (white wax), which was subsequently purified by column chromatography (AcOEt then 10% MeOH in AcOEt) to yield **14a** (141 mg, 58%) as an oil. ^{1}H-NMR (CD_3OD) δ 0.80-1.17 (m, 28H), 1.98 (s, 9H), 2.75-3.20 (m, 2H), 3.50-4.20 (m, 6H), 6.09 (s, 2H), 7.57 (brs, 1H), 9.04 (s, 1H); ^{13}C-NMR (CD_3OD) δ 13.8, 13.9, 14.0, 14.1, 14.4, 14.7, 17.4, 17.5, 17.6 (17.57), 17.6 (17.64), 17.8, 27.1, 30.1, 39.8, 63.1, 64.3, 70.7, 73.0, 75.1, 79.5, 81.1, 83.5, 83.8, 86.9, 120.6, 137.9, 178.7; HRMS(FABMS) calcd for $C_{27}H_{51}N_2O_7Si_2$ $(M+H)^+$ 571.3234, found 571.3240.

{4-[(2-*O*-CE-3,5-*O*-TIPDS-*β*-D-ribofuranosyl)methyl]imidazolyl}methyl 2,2-dimethylpropionate (15a)

Acrylonitrile (0.12 mL, 1.8 mmol) and cesium carbonate (29 mg, 0.09 mmol) were added to the solution of **14a** (50 mg, 0.09 mmol) in *t*-BuOH (0.4 mL). After being vigorously stirred at 40 °C for 15 h, the mixture was diluted with AcOEt (25 mL). Filtration through Celite and evaporation gave a residue, which

was purified by column chromatography (50% AcOEt in hexane) to obtain **15a** (27 mg, 50%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ 0.89-1.13 (m, 28H), 1.19 (s, 9H), 2.40-3.00 (m, 4H), 3.50-4.25 (m, 8H), 5.79 (s, 2H), 6.93 (s, 1H), 7.60 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 12.6, 12.7, 13.0, 13.4, 17.0, 17.1, 17.3 (17.27), 17.3 (17.32), 18.8, 19.1, 26.8, 27.1, 33.1, 38.7, 60.2, 65.4, 65.9, 67.7, 71.6, 80.1, 82.2, 83.0, 117.0, 117.9, 137.7, 138.8; HRMS(FABMS) calcd for $C_{30}H_{54}N_3O_7Si_2(M+H)^+$ 624.3500, found 624.3501.

{4-[(2-*O*-CE-*β*-D-ribofuranosyl)methyl]imidazolyl}methyl 2,2-dimethylpropionate (16a)

Compound **15a** (53 mg, 0.085 mmol) was dissolved in anhydrous THF (0.5 mL). To the solution were added $Et_3N \cdot 3HF$ (50 μL, 0.30 mmol) and triethylamine (21 μL, 0.15 mmol). After stirring at rt for 1.5 h, the reaction mixture was evaporated. The residue was subjected to chromatography on a silica gel column using MeOH-$CHCl_3$ (3:97 to 5:95 v/v) as eluent to give compound **16a** (26 mg, 80%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ 1.19 (s, 9H), 2.63 (t, 2H, *J* = 7.3 Hz), 2.83 (dd, 1H, *J* = 16.9, 6.0 Hz), 2.95 (dd, 1H, *J* = 16.9, 6.0 Hz), 3.56-4.07 (m, 6H), 4.15-4.27 (m, 2H), 5.79 (s, 2H), 6.96 (s, 1H), 7.62 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 19.1, 26.8, 30.5, 38.7, 62.0, 64.9, 67.7, 70.8, 79.7, 81.9, 84.6, 117.5, 138.0, 138.5; HRMS(FABMS) calcd for $C_{18}H_{28}N_3O_6$ $(M+H)^+$ 382.1978, found 382.1981.

[4-(2-*O*-CE-5-*O*-DMT-*β*-D-ribofuranosyl)methylimidazolyl]methyl 2,2-dimethylpropionate (17a)

Compound **16a** (26 mg, 0.068 mmol) was co-evaporated three times with pyridine (1 mL) and redissolved in dry pyridine (0.3 mL). 95% DMTCl (36 mg, 0.10 mmol), Et_3N (14 μL, 0.10 mmol), and DMAP (0.2 mg, 0.0017 mmol) were added to the solution, and the mixture stirred at rt for 20 h. MeOH (2 mL) was added and the mixture stirred for further 5 min. The solvents were removed to leave a residue that was purified by the column chromatography (NH-silica gel) with $CHCl_3$-benzene (0:100, 1:1, 2:1, to 100:0 v/v) as eluent to give the foam of compound **17a** (36 mg, 77%). ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 9H), 2.64 (td, 2H, *J* = 5.8, 2.0 Hz), 2.86 (dd, 1H, *J* = 14.6, 6.6 Hz), 2.96 (dd, 1H, *J* = 14.6, 5.9 Hz), 3.14 (dd, 1H, *J* = 10.1, 4.4 Hz), 3.30 (dd, 1H, *J* = 10.1, 4.0 Hz), 3.68-3.76 (m, 2H), 3.78 (s, 6H), 3.89 (t, 1H, *J* = 4.9 Hz), 3.92-3.97 (m, 1H), 4.01 (t, 1H, *J* = 5.3 Hz), 4.21 (q, 1H, *J* = 4.4 Hz), 5.65 (s, 2H), 6.82 (d, 4H, *J* = 8.8 Hz), 6.92 (s, 1H), 7.19-7.36 (m, 7H), 7.43 (d, 2H, *J* = 7.3 Hz), 7.56 (s, 1H) ; ^{13}C NMR ($CDCl_3$) δ 18.9, 26.8, 32.4, 38.7, 55.2, 64.0, 64.8, 67.9, 71.4, 77.4, 80.5, 82.0, 82.7, 86.0, 113.0, 117.3, 117.5, 138.9, 144.9, 158.4, 177.6; HRMS(FABMS) calcd for $C_{39}H_{46}N_3O_8$ $(M+H)^+$ 684.3284, found 684.3284.

[4-{5-*O*-DMT-2-*O*-CE-3-*O*-(2-cyanoethoxy-*N*,*N*-diisopropylaminophosphino)-*β*-D-ribofuranos-1-yl}-methylimidazol-1-yl]methyl 2,2-dimethylpropionate (1a)

Compound **17a** (36 mg, 0.053 mmol) was dissolved in dry dichloroethane (0.5 mL) and 4,5-DCI (8.0 mg,

0.064 mmol) and 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite (36 μL, 0.11 mmol) were added. The resulting mixture was stirred at 40 °C for 1 h and then evaporated. The residual oil was subjected to chromatography on NH-silica gel with the solvent system; AcOEt in hexane (0:100, 30:70, 40:60 to 50:50 v/v) to give **1a** (28 mg, 60%) as a white foam. ^{1}H NMR ($CDCl_3$) δ 0.92 (d, 4H, *J* = 6.6 Hz), 1.04-1.12 (m, 19H), 2.21 (t, 1H, *J* = 6.6 Hz), 2.49-2.59 (m, 3H), 2.88 (m, 2H), 2.96 (dd, 1H, *J* = 10.2, 4.2 Hz), 3.17 (dd, 0.5H, *J* = 10.2, 4.2 Hz), 3.26 (dd, 0.5H, *J* = 9.9, 3.3Hz), 3.39-3.66 (m, 4H), 3.73 (s, 6H), 3.75-3.83 (m, 1H), 4.01-4.20 (m, 3H), 5.58 (s, 2H), 6.71-6.80 (m, 4H), 6.86 (s, 1H), 7.13-7.27 (m, 7H), 7.33-7.38 (m, 2H), 7.49 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 18.77, 18.83, 19.94, 20.03, 20.35, 20.42, 24.47, 24.56, 24.68, 26.78, 29.65, 32.42, 38.65, 42.87, 43.03, 43.22, 43.28, 55.12, 57.81, 58.22, 58.45, 63.51, 64.75, 65.03, 67.66, 71.95, 72.17, 72.38, 80.00, 80.03, 81.87, 82.22, 82.50, 82.79, 85.91, 85.99, 112.99, 117.26, 117.55, 117.89, 126.67, 127.71, 127.84, 128.21, 128.27, 130.11, 135.89, 136.04, 136.15, 137.52, 139.14, 144.81, 144.87, 158.38, 158.41, 177.64; ^{31}P NMR ($CDCl_3$) δ 149.3 and 149.9. HRMS (FABMS: TEOA+NaCl) calcd for $C_{48}H_{62}N_5O_9P$+Na [$(M+Na)^+$] 906.4182, found 906.4191.

{4-[3-(3,5-*O*-TIPDS-β-D-ribofuranosyl)propyl]imidazolyl}methyl 2,2-dimethylpropionate (14b)

By the same procedure as used for the preparation of **14a**, **11b** (118 mg, 0.33 mmol) was converted to **14b** (60 mg, 46%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ 0.92-1.13 (m, 28H), 1.98 (s, 9H), 1.50-1.87 (brs, 4H), 2.59 (t, 2H, *J* = 8.4 Hz), 3.70-3.90 (m, 4H), 4.01 (dd, 1H, *J* = 11.6, 2.9 Hz), 4.18 (t, 1H, *J* = 6.7 Hz), 5.77 (s, 2H), 6.79 (s, 1H), 7.57 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 12.6, 12.7, 13.2, 13.4, 16.9, 17.0 (17.00), 17.0 (17.03), 17.2, 17.3, 17.4, 25.1, 26.8, 28.1, 33.2, 38.7, 63.0, 67.7, 72.5, 74.5, 77.2, 82.2, 84.0, 115.4, 137.5, 143.5, 177.7; HRMS (FABMS:NBA) calcd for $C_{29}H_{55}N_2O_7Si_2$ $(M+H)^+$ 599.3547, found 599.3549.

{4-[3-(2-*O*-CE-3,5-*O*-TIPDS-β-D-ribofuranosyl)propyl]imidazolyl}methyl 2,2-dimethylpropionate (15b)

By the same procedure as used for the preparation of **15a**, **14b** (121 mg, 0.2 mmol) was converted to **15b** (87 mg, 67%) as an oil. IR (film) cm^{-1}: 2200 (CN), 1735 (COO); ^{1}H-NMR ($CDCl_3$) δ 0.90-1.13 (brs, 28H), 1.18 (s, 9H), 1.50-1.83 (m, 4H), 2.50-2.69 (m, 4H), 3.55-4.21 (m, 8H), 5.78 (s, 2H), 6.79 (s, 1H), 7.60 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 12.5, 12.7, 13.0, 13.4, 16.9, 17.0, 17.2 (17.19), 17.2 (17.23), 17.3, 18.8, 19.3, 24.9, 26.8, 27.3, 27.9, 28.5, 34.0, 38.7, 60.3, 62.3, 65.5, 65.8, 67.6, 72.0, 80.0, 83.2, 90.0, 115.5, 117.7, 137.5, 143.0, 177.6; HRMS (FABMS) calcd for $C_{32}H_{58}N_3O_7Si_2$ $(M+H)^+$ 652.3813, found 652.3817.

{4-[3-(2-*O*-CE-β-D-ribofuranosyl)propyl]imidazolyl}methyl 2,2-dimethylpropionate (16b)

By the same procedure as used for the preparation of **16a**, **15b** (87 mg, 0.13 mmol) was converted to **16b** (47 mg, 85%) as an oil.^{1}H-NMR ($CDCl_3$) δ 1.18 (s, 9H), 1.58-1.90 (m, 4H), 2.47-2.73 (m, 4H), 3.59-3.70 (m, 2H), 3.71-3.88 (m, 4H), 3.94 (q, 1H, *J* = 6.0 Hz), 4.16 (t, 1H, *J* = 6.0 Hz), 5.78 (s, 2H), 6.80 (s, 1H), 7.60 (s, 1H). ^{13}C-NMR ($CDCl_3$) δ 19.0, 25.3, 26.8, 27.6, 32.6, 38.7, 62.0, 65.0, 67.7, 70.7, 77.2, 81.2, 83.3, 83.5, 84.1, 115.6, 117.6, 137.6, 143.0, 177.7; HRMS (FABMS) calcd for $C_{20}H_{32}N_3O_6$ $(M+H)^+$ 410.2293, found 410.2293.

{4-[2-(5-DMT-2-*O*-CE-β-D-ribofuranosyl)propyl]imidazolyl}methyl 2,2-dimethylpropionate (17b)

By the same procedure as used for the preparation of **17a**, **16b** (47 mg, 0.11 mmol) was converted to **17b** (68 mg, 84%) as a foam. ^{1}H-NMR ($CDCl_3$) δ 1.14 (s, 9H), 1.60-1.93 (m, 4H), 2.55-2.68 (m, 4H), 3.14 (dd, 1H, *J* = 10.3, 4.3 Hz), 3.28 (dd, 1H, *J* = 11.1, 3.4 Hz), 3.62 (t, 1H, *J* = 6.0 Hz), 3.68-3.84 (m, 2H), 3.78 (s, 6H), 3.86-3.99 (m, 2H), 4.06 (t, 1H, *J* = 6.0 Hz), 5.69 (s, 2H), 6.78 (s, 1H), 6.81 (d, 4H, *J* = 8.6 Hz), 7.15-7.38 (m, 7H), 7.40-7.48 (m, 2H), 7.55 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 19.0, 25.3, 26.7, 28.1, 33.6, 38.6, 55.1, 64.2, 65.0, 67.6, 71.6, 77.2, 80.4, 82.9, 83.6, 86.0, 113.0, 115.5, 117.4, 126.6, 127.7, 128.1, 128.3, 130.0, 135.9, 136.0, 137.5, 143.2, 144.9, 149.0, 158.3, 177.6; HRMS (FABMS) calcd for $C_{41}H_{50}N_3O_8$ $(M+H)^+$ 712.3597, found 712.3599.

(4-{3-[5-*O*-DMT-2-*O*-CE-3-*O*-(2-cyanoethoxy-*N*,*N*-diisopropylaminophosphino)-β-D-ribofuranos-1-yl]propyl}imidazol-1-yl)methyl 2,2-dimethylpropionate (1b)

By the same procedure as used for the preparation of **1a**, **17b** (20 mg, 0.028 mmol) was converted to **1b** (13 mg, 51%) as a colorless oil. R_f(AcOEt) 0.57; ^{1}H-NMR ($CDCl_3$) δ 1.01 (d, 4H, *J* = 6.9 Hz), 1.09-1.18 (m, 20H), 1.94-2.08 (m, 4H), 2.27 (t, 1H, *J* = 6.5 Hz), 2.55-2.70 (m, 5H), 3.07 (dt, 1H, *J* = 10.3, 3.4 Hz), 3.24 (dd, 0.7H, *J* = 10.4, 3.9 Hz), 3.32 (dd, 0.3H, *J* = 10.4, 2.6 Hz), 3.46-4.04 (m, 6H), 3.78 (s, 6H), 4.06-4.17 (m, 1H), 4.17-4.28 (m, 1H), 5.69 (s, 2H), 6.75-6.84 (m, 5H), 7.17-7.39 (m, 7H), 7.39-7.49 (m, 2H), 7.57 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ 18.9, 19.9, 20.3, 20.4, 24.4, 24.5, 24.6, 24.7, 25.4, 26.8, 28.2, 33.6, 38.7, 42.9, 43.0, 43.1, 43.3, 55.2, 57.8, 58.1, 58.3, 63.9, 65.0, 67.7, 72.3, 72.6, 72.8, 77.2, 80.1, 80.2, 82.6, 83.0, 83.5, 85.9, 86.0, 113.0, 115.4, 117.5, 117.8, 117.9, 126.7, 127.7, 128.2, 128.3, 130.1, 135.9, 136.1, 137.5, 143.5, 144.8, 158.4, 177.7; ^{31}P-NMR ($CDCl_3$) δ 149.8, 150.7; HRMS (FABMS: TEOA+NaCl) calcd for $C_{50}H_{66}N_5O_9P$+Na $[(M+Na)^+]$ 934.4495, found 934.4490.

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