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Recognition of T·A interruption by 2′,4′-BNAs bearing heteroaromatic nucleobases through parallel motif triplex formation

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Abstract—2′-*O*,4′-*C*-methylene bridged nucleic acid (2′,4′-BNA) monomers bearing novel unnatural nucleobases, 4-(3-benzamidophenyl)-2-pyridone and 2-(*N*-methylbenzamido)thiazole, were synthesized and successfully incorporated into oligonucleotides. UV melting experiments showed that the corresponding oligonucleotide derivatives formed stable triplexes with dsDNA targets even in the presence of a T·A interruption.

1. Introduction

Oligonucleotides with a homopyrimidine sequence are able to form triplexes with double-stranded DNA (dsDNA) in a sequence-specific manner via Hoogsteen-type hydrogen bonding.[1–3] Sequence-specific triplex formation would be useful in genetic therapy (e.g., the antigene approach) and/or diagnosis. In addition, it could be a fascinating and powerful tool in molecular biology. However, two major drawbacks in triplex formation limit the practical use of triplex-forming oligonucleotides (TFOs). One problem is the relatively low stability of the triplex under neutral pH conditions. To enhance the stability of the triplex, chemical modifications of TFOs have been carried out.[4] These studies have shown that the 2′,4′-BNA[5–10]/LNA,[11–14] which has a locked N-type sugar conformation, shows high binding affinity toward dsDNA target in a sequence-specific manner (Fig. 1).[10,15,16] The second problem is limitations of the target sequence. Pyrimidine nucleobases in TFOs interact with purine·pyrimidine base pairs in target dsDNA to form T*A·T and C$^+$*G·C base triads.[17] It is well known that the presence of pyrimidine·purine interruptions (C·G or T·A interruptions) in the dsDNA target drastically decrease the stability of the triplex.[1–3] To overcome this problem, considerable effort has been devoted to the synthesis of nucleobase analogues that recognize C·G interruptions.[4,18] We have developed novel 2′,4′-BNAs bearing unnatural nucleobases,[19–23] and found that the 2′,4′-BNA incorporating 2-pyridone (P^B: Fig. 1) effectively recognized C·G interruptions in the dsDNA target.[19,20] On the other hand, various base analogues have also been developed for recognizing T·A interruptions.[4,18,24–31] Among these nucleobase analogues, the phenylimidazole derivative, D_3,[24–26] and aminothiazole derivatives, S[27,29,30,32] and Bt,[28,29]

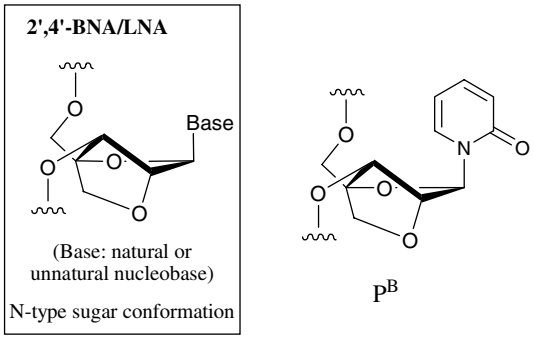

Figure 1. Structures of 2′,4′-BNA/LNA and P^B.

Keywords: Bridged nucleic acid (BNA); Locked nucleic acid (LNA); Triplex; Oligonucleotide.
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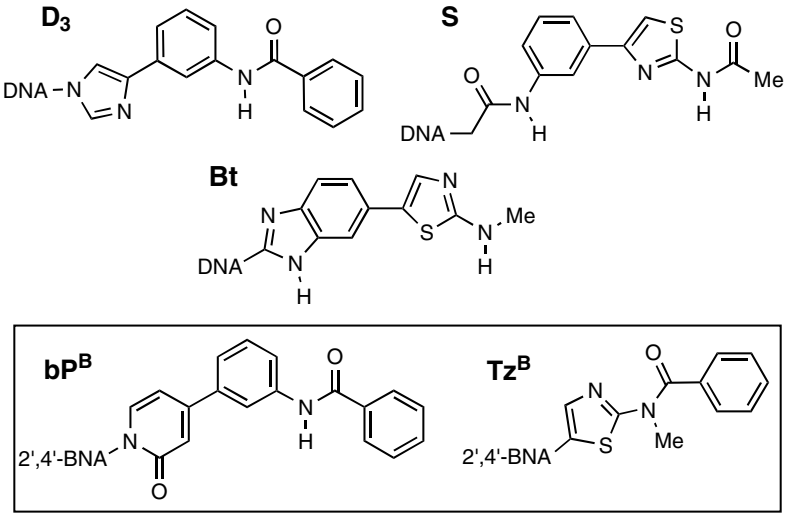
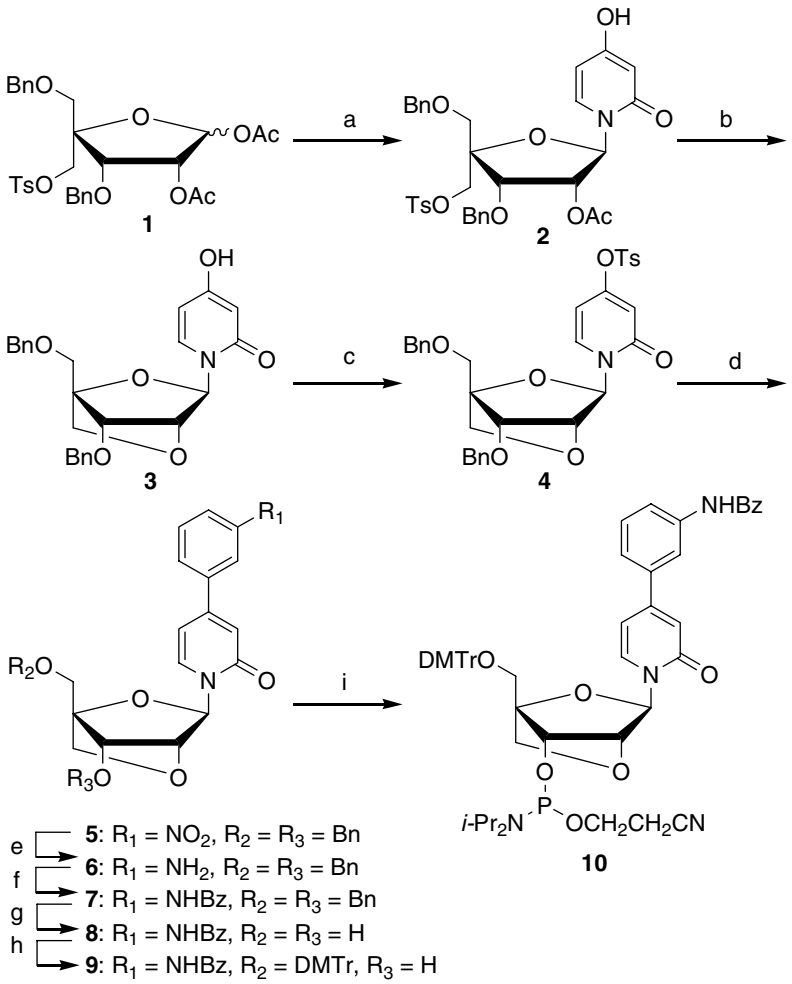
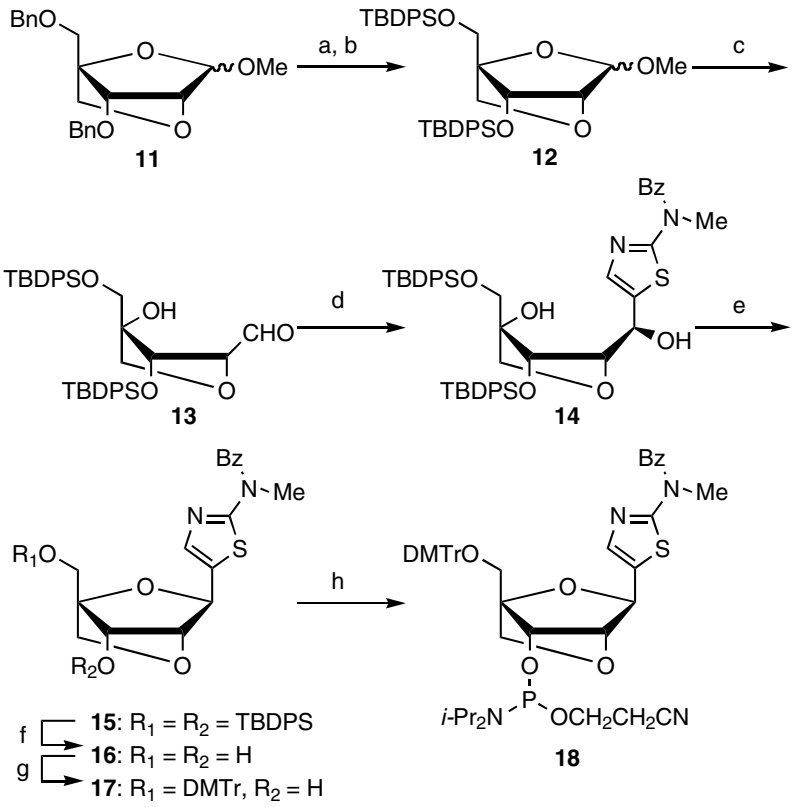


Figure 2. Structures of D_3, S, Bt, bP^B, and Tz^B.

showed effective interaction with a T·A interruption in a parallel motif triplex. The non-fused heteroaromatic structure of these nucleobase analogues is likely to contribute to base stacking, hydrogen bonding and/or inter-



Scheme 1. Reagents and conditions: (a) 4-hydroxy-2-pyridone, *N*,*O*-bis(trimethylsilyl)acetamide, TMSOTf, 1,2-dichloroethane, reflux, 2.5 h, 96%; (b) K_2CO_3, MeOH, rt, 9 h, 98%; (c) TsCl, Et_3N, CH_2Cl_2, −50 °C, 3 h, 98%; (d) *m*-nitrophenylboronic acid, $Pd(PPh_3)_4$, K_3PO_4, KBr, dioxane, 90 °C, 24 h, 64%; (e) H_2, PtO_2, EtOH–AcOEt, rt, 18 h, quant.; (f) BzCl, pyridine, rt, 2 h, 99%; (g) 20% $Pd(OH)_2$–C, cyclohexene, EtOH, reflux, 8 h, 86%; (h) DMTrCl, pyridine, rt, 2.5 h, 95%; (i) $(i\text{-}Pr_2N)_2POCH_2CH_2CN$, diisopropylammonium tetrazolide, MeCN–THF, rt, 21 h, 94%.



Scheme 2. Reagents and conditions: (a) 20% $Pd(OH)_2$–C, H_2, rt, 1.5 h; (b) TBDPSCl, imidazole, DMF, rt, 12 h, 79% over 2 steps; (c) 10% HCl aq, THF, rt, 4 h, 96%; (d) 2-(*N*-methylbenzamido)thiazole, LDA, THF, −78 °C, 1 h, 53%; (e) 1,1′-azobis(*N*,*N*-dimethylformamide), *n*-Bu_3P, THF, rt, 2 h, 64%; (f) TBAF, THF, rt, 2 h, 72%; (g) DMTrCl, pyridine, rt, 12 h, 96%; (h) *i*-$Pr_2NP(Cl)OCH_2CH_2CN$, *i*-Pr_2NEt, CH_2Cl_2, rt, 3 h, 89%.

calation in the process of recognizing of the T·A base pair.

We have designed heteroaromatic compounds, 4-(3-benzamidophenyl)-2-pyridone and 2-(*N*-methylbenzamido)thiazole, as novel artificial nucleobases. Here, we describe the synthesis of 2′,4′-BNAs bearing the unnatural nucleobases (bP^B and Tz^B: Fig. 2) and demonstrate successful recognition of the T·A interruption by bP^B and Tz^B through parallel motif triplex formation.

2. Results and discussion

Synthesis of the phosphoramidite derivative of bP^B was accomplished as shown in Scheme 1. As per Vorbrüggen's method,[33] diacetate **1**[34] was coupled with 4-hydroxy-2-pyridone in the presence of *N*,*O*-bis(trimethylsilyl)acetamide and TMSOTf to give the desired β-anomer **2**. Treatment of **2** with K_2CO_3 resulted in deacetylation and subsequent ring-closure reaction to afford **3** in good yield.[35] The obtained 2′,4′-BNA derivative **3** bearing 4-hydroxy-2-pyridone nucleobase was tosylated to give **4**, which was subjected to a palladium-catalyzed cross-coupling reaction with *m*-nitrophenylboronic acid in the presence of K_3PO_4 and KBr to provide **5**.[36] Catalytic hydrogenation of **5** over PtO_2 furnished the corresponding amino compound **6**, which was then treated with BzCl in pyridine to give **7**. Hydrogenolysis of **7** followed by dimethoxytritylation provided **9**. Compound **9** was phosphitylated to give the phosphoramidite **10**.

The other 2′,4′-BNA analogue, Tz^B, was synthesized as shown in Scheme 2. The Bn groups of compound **11**[37] were converted to the TBDPS groups,[38] and treated with 10% HCl aq in THF to give aldehyde **13**. 2-(*N*-Methylbenzamido)thiazole was treated with LDA in THF and the resulting litho-derivative was allowed to react with aldehyde **13** to give the coupling product **14** stereoselectively. Mitsunobu reaction of **14** gave bicyclic nucleoside **15**, and TBDPS groups were removed by treatment with TBAF to give **16**. Dimethoxytritylation of **16** followed by phosphitylation afforded the phosphoramidite **18**.

The obtained phosphoramidites **10** and **18** were incorporated into 15-mer oligonucleotides by a standard phosphoramidite protocol on an automated DNA synthesizer. The obtained oligonucleotides were purified by reversed-phase HPLC, and the composition of the oligonucleotides was confirmed by MALDI-TOF-MS analysis (Table 1).

Triplex-forming ability of the TFOs containing bP^B and Tz^B was evaluated by UV melting experiments under physiological pH conditions (Tables 2 and 3). As shown in Table 2, the triplexes containing bP^B*T·A and Tz^B*T·A triads showed T_m values of 37 °C and 30 °C, respectively, which are higher than those of the triplexes containing G*T·A and G^B*T·A triads. Thus, bP^B and Tz^B effectively stabilized the triplex containing a T·A interruption. However, bP^B showed only moderate ability to discriminate between the T·A base pair and other

Table 1. Sequences and MALDI-TOF-mass data of TFOs

TFO	MALDI-TOF-mass [M−H]⁻	
	Calcd	Found
5′-TTTTTCTbP^B TCTCTCT-3′	4688.3	4688.6
5′-TTTTTCTTz^B TCTCTCT-3′	4616.5	4617.2
5′-TTTTTCTbP^B CTCTCT-3′	4687.3	4686.4
5′-TTTTTCbP^B TCTCTCT-3′	4687.3	4686.8
5′-TTTTTCTTz^B CTCTCT-3′	4612.8	4612.8
5′-TTTTTCTz^B TCTCTCT-3′	4612.8	4612.2

C means 2′-deoxy-5-methylcytidine.

base pairs (ΔT_m values for the C·G, A·T, and G·C base pairs were −4 °C, −6 °C, and −9 °C, respectively). In contrast, the T_m value of the triplex containing Tz^B*T·A is much higher than those of the triplexes containing Tz^B*C·G, Tz^B*A·T, and Tz^B*G·C triads (ΔT_m = −11 °C, −14 °C, and −7 °C, respectively). Electrostatic surface potential analysis of the Tz^B*T·A triad suggested that the selective affinity of Tz^B to the T·A base pair was likely to involve favorable van der Waals interactions between Tz^B and the T·A base pair, although there is no appropriate hydrogen bonding between Tz^B and T·A base pair (Fig. 3, below). However, the shape of the polyheteroaromatic ring of bP^B is a poor fit to the T·A base pair. A part of the polyheteroaromatic ring would be overlapped with the T·A base pair (Fig. 3, above), which suggests that the heteroaromatic part of bP^B acts as an intercalator to stabilize the triplex involving the bP^B*T·A triad. Here, it is also noteworthy that the stability of the triplex containing the G^B*T·A triad was similar to that of the triplex including the G*T·A triad. Thus, the locked N-type sugar conformation and G nucleobase were found to be an inadequate combination for T·A recognition. On the other hand, the triplex containing the bP^B*C·G triad had a T_m value of 33 °C, which is the same as that of the triplex involving the P^B*C·G triad. This means that the additional benzoylaminophenyl group in bP^B had no effect on the interaction with a C·G base pair. It was previously reported that D_3 intercalates between the T·A base pair and the 3′ neighboring triad when D_3 recognizes the T·A interruption in a pyrimidine motif triplex.[26] In addition, it was also demonstrated that the 3′ neighboring triad affected triplex stability.[25] Such effects of a neighbor base have been discussed with respect to G*T·A[25] and S*T·A[29] triads. To investigate the stabilization effect of bP^B in more detail, we examined the neighbor effect with the triplexes containing bP^B*T·A and Tz^B*T·A triads. As shown in Table 3, replacement of the T*A·T triad with C*G·C at the 5′ or 3′ neighboring site (X_1*Y_1Z_1 or X_2*Y_2Z_2) decreases the triplex's stability, even if the triplex contains no T·A interruption (X = T and YZ = A·T: ΔT_m = −14 to −12 °C). A similar decrease in T_m value was observed for the triplexes containing Tz^B*T·A (ΔT_m = −14 to −13 °C) and G*T·A triads (ΔT_m = −13 to −12 °C). In these cases, no obvious 3′- or 5′-C*G·C side effect was observed. However, the adjacent 3′-C*G·C resulted in greater destabilization of the triplex in the case of bP^B (ΔT_ms for 3′- and 5′-C*G·C = −15 and −10 °C, respec-

Table 2. T_m values (°C) of triplex[a,b]

TFO	dsDNA targets (YZ)			
(X)	T·A	C·G	A·T	G·C
bP^B	37	33 (−4)	31 (−6)	28 (−9)
Tz^B	30	19 (−11)	16 (−14)	23 (−7)
G	27	20 (−7)	16 (−9)	23 (−4)
G^B	26	19, 28[c]	16, 22[c]	24 (−2)
P^B	14[d]	33[d]	23[d]	19[d]

5′-TTTTTCTXTCTCTCT-3′ TFO.
5′-GCTAAAAAGAYAGAGAGATCG-3′ dsDNA.
3′-CGATTTTTCTZTCTCTCTAGC-5′.

[a] UV melting profiles were measured in 7 mm sodium phosphate buffer (pH 7.0) containing 140 mm KCl and 10 mm $MgCl_2$ at a scan rate of 0.5 °C/min with detection at 260 nm. T_m values were obtained as the maxima of the first derivative of the melting curves. Concentration of each oligonucleotide was 1.5 μm. C and G^B mean 2′-deoxy-5-methylcytidine and 2′,4′-BNA/LNA-G, respectively.
[b] ΔT_m values (T_m value of the triplex involving the X*Y·Z triad—that which contains X*T·A) are shown in parentheses.
[c] Two transitions were observed for triplex dissociation.
[d] Our previous work. See Refs. 19 and 20.

Table 3. Neighbor effect of the triplexes containing bP^B *T·A and Tz^B*T·A triad[a,b]

X_1*Y_1·Z_1	X_2*Y_2·Z_2	T_m (°C)			
		X = bP^B (Y = T, Z = A)	X = Tz^B (Y = T, Z = A)	X = G (Y = T, Z = A)	X = T (Y = A, Z = T)
T*A·T	T*A·T	37	30	27	44
T*A·T	C*G·C	22 (−15)	16 (−14)	14 (−13)	30 (−14)
C*G·C	T*A·T	27 (−10)	17 (−13)	15 (−12)	32 (−12)

5′-TTTTTCX_1XX_2CTCTCT-3′ TFO.
5′-GCTAAAAAGY_1YY_2GAGAGATCG-3′ dsDNA.
3′-CGATTTTTCZ_1ZZ_2CTCTCTAGC-5′.

[a] See footnote in Table 2.
[b] ΔT_m values [T_m value of the triplex (X_1XX_2 = TXC or CXT)—that of the triplex (X_1XX_2 = TXT)] are shown in parentheses.

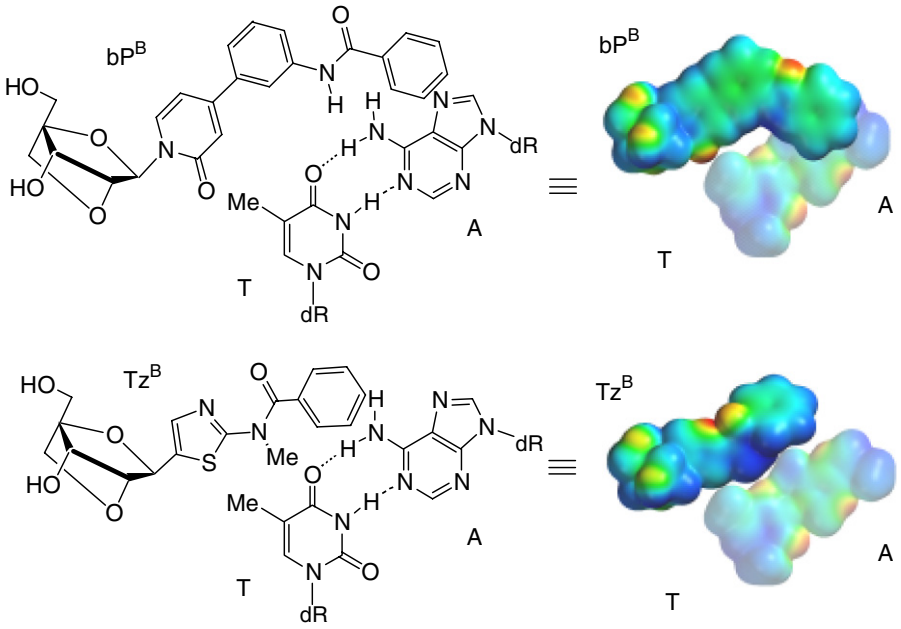


Figure 3. Proposed structures and electrostatic surface potential of bP^B*T·A and Tz^B*T·A. Calculated with Spartan'04 (Wavefunction, Inc.).

tively). This result indicates a similar nearest neighbor effect with regard to stabilization of bP^B*T·A and D_3*T·A[25] triads, and it is therefore probable that bP^B, like D_3, intercalates between the T·A base pair and the 3′ flanking base triad.

3. Conclusion

In conclusion, 2′,4′-BNA analogues bearing novel unnatural nucleobases, 4-(3-benzamidophenyl)-2-pyridone and 2-(*N*-methylbenzamido)thiazole, were synthesized and incorporated into oligonucleotides. The TFOs containing the 2′,4′-BNA analogues, bP^B and Tz^B, formed a stable triplex with the dsDNA targets incorporating a T·A interruption under physiological pH conditions. The stability of the triplexes containing a bP^B*T·A triad was much higher than that of the corresponding triplexes containing a G*T·A triad. Tz^B effectively discriminated the T·A interruption from other base pairs. Electrostatic surface potential analysis and observation of the 3′-C*G·C side effect suggest that bP^B, like D_3, works as an intercalator when it recognizes a T·A interruption, whereas specific binding of Tz^B with a T·A base pair involves shape recognition, i.e., van der Waals interaction.

4. Experimental

4.1. General

All melting points were measured on a Yanagimoto micro melting points apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP-370 instrument. IR spectra were recorded on a JASCO FT/IR-200 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL EX-270 (^{1}H, 270 MHz; ^{13}C, 67.8 MHz) and ^{31}P NMR spectra were recorded on a Varian VXR-200 (^{31}P, 81.0 MHz). Mass spectra of nucleoside analogues were recorded on a JEOL JMS-D300 or JMS-600 mass spectrometer. Fuji Silysia BW-300 (200–400 mesh) was used for flash chromatography. MALDI-TOF-Mass spectra were recorded on an Applied Biosystems Voyager®-DE.

4.1.1. 1-(2-*O*-Acetyl-3,5-di-*O*-benzyl-4-*p*-toluenesulfonyloxymethyl-β-D-ribofuranosyl)-4-hydroxy-2-pyridone (2). Under a N_2 atmosphere, 4-hydroxy-2-pyridone (300 mg, 2.70 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (1.47 ml, 5.95 mmol) were added to a solution of compound **1** (1.08 g, 1.80 mmol) in anhydrous 1,2-dichloroethane (20 ml) at room temperature and the mixture was refluxed for 1 h. TMSOTf (0.26 ml, 1.44 mmol) was added to the reaction mixture at room temperature and the mixture was refluxed for 2.5 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution. The mixture was extracted with AcOEt. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [*n*-hexane/AcOEt (1:4, v/v)] to give compound **2** (1.13 g, 96%). White powder. Mp 72–74 °C. $[\alpha]_D^{22}$ +27.9 (*c* 0.81, $CHCl_3$). IR ν_{max} (KBr): 1749, 1650, 1552, 1494, 1364, 1227, 1180, 1106 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.01 (3H, s), 2.40 (3H, s), 3.46, 3.82 (2H, AB, J = 10 Hz), 4.14, 4.26 (2H, AB, J = 11 Hz), 4.31, 4.41 (2H, AB, J = 12 Hz), 4.31, 4.51 (2H, AB, J = 12 Hz), 4.38 (1H, d, J = 6 Hz), 5.24 (1H, dd, J = 3, 6 Hz), 5.63 (1H, dd, J = 2, 8 Hz), 5.88 (1H, d, J = 2 Hz), 5.91 (1H, d, J = 3 Hz), 7.18–7.34 (12H, m), 7.62 (1H, d, J = 8 Hz), 7.76 (2H, d, J = 8 Hz), 11.20 (1H, br s). ^{13}C NMR ($CDCl_3$) δ 20.7, 21.7, 69.3, 69.4, 73.5, 74.1, 75.1, 76.1, 85.4, 88.1, 98.7, 103.0, 127.8, 127.9, 128.0, 128.3, 128.4, 129.7, 132.0, 133.9, 136.9, 137.0, 145.1, 164.0, 168.9, 169.1. Mass (FAB):

m/*z* 650 (MH^+). Anal. Calcd for $C_{34}H_{35}NO_{10}S$: C, 62.85; H, 5.43; N, 2.16; S, 4.94. Found: C, 62.73; H, 5.49; N, 2.10; S, 4.85.

4.1.2. 1-(3,5-Di-*O*-benzyl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-4-hydroxy-2-pyridone (3). To a solution of compound **2** (700 mg, 1.08 mmol) in MeOH (15 ml) was added K_2CO_3 (434 mg, 0.52 mmol) at room temperature and the mixture was stirred for 9 h. After neutralization by addition of 10% HCl aqueous solution, the mixture was extracted with AcOEt. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [*n*-hexane/AcOEt (1:4, v/v)] to give compound **3** (462 mg, 98%). Colorless crystals. Mp 117–118 °C (AcOEt). $[\alpha]_D^{24}$ +171.5 (*c* 1.07, $CHCl_3$). IR ν_{max} (KBr): 1648, 1546, 1489, 1244, 1094, 1052 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.81, 3.83 (2H, AB, *J* = 11 Hz), 3.88, 4.04 (2H, AB, *J* = 8 Hz), 3.97 (1H, s), 4.43, 4.58 (2H, AB, *J* = 12 Hz), 4.60 (1H, s), 4.60 (2H, s), 5.86 (1H, s), 5.94 (1H, s), 5.96 (1H, d, *J* = 7 Hz), 7.20–7.36 (10H, m), 7.70 (1H, d, *J* = 7 Hz), 11.86 (1H, br s). ^{13}C NMR ($CDCl_3$) δ 64.7, 72.1, 72.2, 73.7, 75.7, 76.7, 87.2, 87.9, 98.9, 103.2, 127.4, 127.6, 127.8, 127.9, 128.3, 128.4, 132.9, 136.9, 137.5, 164.0, 169.2. Mass (FAB): *m*/*z* 436 (MH^+). Anal. Calcd for $C_{25}H_{25}NO_6$: C, 68.95; H, 5.79; N, 3.22. Found: C, 68.85; H, 5.85; N, 3.23.

4.1.3. 1-(3,5-Di-*O*-benzyl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-4-*p*-toluenesulfonyloxy-2-pyridone (4). Under a N_2 atmosphere, TsCl (175 mg, 0.92 mmol) and Et_3N (148 μl, 1.06 mmol) were added to a solution of compound **3** (308 mg, 0.71 mmol) in anhydrous CH_2Cl_2 (8 ml) at −50 °C and the mixture was stirred at −50 °C for 3 h. After addition of water, the mixture was extracted with AcOEt. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [*n*-hexane/AcOEt (3:2, v/v)] to give compound **4** (413 mg, 98%). Colorless oil. $[\alpha]_D^{22}$ +156.4 (*c* 0.64, $CHCl_3$). IR ν_{max} (KBr): 1663, 1598, 1543, 1379, 1087 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.46 (3H, s), 1.32 (3H, s), 3.80, 3.83 (2H, AB, *J* = 11 Hz), 3.85, 4.03 (2H, AB, *J* = 8 Hz), 3.91 (1H, s), 4.45, 4.58 (2H, AB, *J* = 12 Hz), 4.56 (1H, s), 4.61, 4.62 (2H, AB, *J* = 12 Hz), 5.78 (1H, s), 6.03–6.07 (2H, m), 7.19–7.40 (12H, m), 7.77–7.81 (3H, m). ^{13}C NMR ($CDCl_3$) δ 21.8, 64.4, 72.0, 72.1, 73.6, 75.4, 76.2, 87.3, 88.0, 101.8, 109.7, 127.3, 127.6, 127.9, 127.9, 128.2, 128.3, 128.4, 130.0, 131.9, 133.8, 136.7, 137.4, 146.0, 158.9, 161.9. Mass (EI): *m*/*z* 589 (M^+, 6.3), 91 (100). Anal. Calcd for $C_{32}H_{31}NO_8S$: C, 65.18; H, 5.30; N, 2.38; S, 5.44. Found: C, 64.84; H, 5.41; N, 2.31; S, 5.33.

4.1.4. 1-(3,5-Di-*O*-benzyl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-4-(3-nitrophenyl)-2-pyridone (5). Under a N_2 atmosphere, *m*-nitrophenylboronic acid (146 mg, 0.87 mmol), K_3PO_4 (232 mg, 1.09 mmol), KBr (104 mg, 0.87 mmol), and $Pd(PPh_3)_4$ (84 mg, 73 μmol) were added to a solution of compound **4** (508 mg, 0.99 mmol) in anhydrous dioxane (10 ml) at room temperature and the mixture was stirred at 90 °C for 24 h. The mixture was filtered through a pad of Celite® and the filtrate was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [*n*-hexane/AcOEt (1:1, v/v)] to give compound **5** (253 mg, 64%). Pale brown powder. Mp 49–50 °C. $[\alpha]_D^{22}$ +145.1 (*c* 0.74, $CHCl_3$). IR ν_{max} (KBr): 1661, 1592, 1531, 1350, 1098 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.86, 3.89 (2H, AB, *J* = 11 Hz), 3.91, 4.08 (2H, AB, *J* = 8 Hz), 4.01 (1H, s), 4.48, 4.62 (2H, AB, *J* = 12 Hz), 4.66, 4.68 (2H, AB, *J* = 12 Hz), 4.71 (1H, s), 5.93 (1H, s), 6.34 (1H, dd, *J* = 2, 7 Hz), 6.75 (1H, d, *J* = 2 Hz), 7.21–7.39 (10H, m), 7.66 (1H, dd, *J* = 8, 8 Hz), 7.86–7.95 (2H, m), 8.28–8.32 (1H, m), 8.40–8.42 (1H, m). ^{13}C NMR ($CDCl_3$) δ 64.5, 72.0, 72.1, 73.6, 75.6, 76.2, 87.3, 88.0, 104.5, 117.2, 121.5, 123.9, 127.3, 127.4, 127.7, 128.2, 128.3, 130.0, 132.3, 132.8, 136.8, 137.4, 138.9, 148.4, 149.1, 161.4. Mass (EI): *m*/*z* 540 (M^+, 7.5), 91 (100). Anal. Calcd for $C_{31}H_{28}N_2O_7$: C, 68.88; H, 5.22; N, 5.18. Found: C, 68.52; H, 5.27; N, 5.17.

4.1.5. 4-(3-Aminophenyl)-1-(3,5-di-*O*-benzyl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-2-pyridone (6). Under a H_2 atmosphere PtO_2 (8.0 mg, 35 μmol) was added to a solution of compound **5** (100 mg, 0.18 mmol) in anhydrous EtOH/AcOEt (1:1, 2 ml) at room temperature and the mixture was stirred at room temperature for 18 h. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [*n*-hexane/AcOEt (1:2, v/v)] to give compound **6** (94 mg, quant.). Pale yellow powder. Mp 52–54 °C. $[\alpha]_D^{22}$ +149.2 (*c* 1.48, $CHCl_3$). IR ν_{max} (KBr): 3446, 3353, 2944, 2877, 1656, 1585, 1523, 1455, 1268, 1205, 1097, 1052 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.08 (2H, br s), 3.85, 3.87 (2H, AB, *J* = 11 Hz), 3.91, 4.06 (2H, AB, *J* = 8 Hz), 4.00 (1H, s), 4.46, 4.60 (2H, AB, *J* = 12 Hz), 4.65, 4.66 (2H, AB, *J* = 12 Hz), 4.70 (1H, s), 5.93 (1H, s), 6.35 (1H, dd, *J* = 2, 7 Hz), 6.69 (1H, d, *J* = 1 Hz), 6.80 (1H, dd, *J* = 1, 8 Hz), 6.92 (1H, s), 6.96 (1H, d, *J* = 7 Hz), 7.21–7.36 (11H, m), 7.81 (1H, d, *J* = 7 Hz). ^{13}C NMR ($CDCl_3$) δ 64.7, 72.0, 73.6, 75.7, 76.4, 87.2, 88.0, 105.6, 113.1, 116.1, 116.4, 116.9, 127.4, 127.6, 127.8, 127.9, 128.3, 128.5, 129.9, 131.8, 136.9, 137.6, 138.4, 146.9, 152.1, 162.1. Mass (EI): *m*/*z* 510 (M^+, 5.2), 91 (100). Anal. Calcd for $C_{31}H_{30}N_2O_5 \cdot 1/4H_2O$: C, 72.29; H, 5.97; N, 5.44. Found: C, 72.34; H, 6.00; N, 5.42.

4.1.6. 4-(3-Benzamidophenyl)-1-(3,5-di-*O*-benzyl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-2-pyridone (7). Under a N_2 atmosphere, BzCl (0.17 ml, 1.46 mmol) was added to a solution of compound **6** (508 mg, 0.99 mmol) in anhydrous pyridine (5 ml) at room temperature and the mixture was stirred at room temperature for 2 h. After addition of water, the mixture was extracted with AcOEt. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [*n*-hexane/AcOEt (2:3, v/v)] to give compound **7** (606 mg, 99%). White powder. Mp 80–83 °C. $[\alpha]_D^{22}$ +113.4 (*c* 1.68, $CHCl_3$). IR ν_{max} (KBr): 3296, 3062, 3030, 2946, 2876, 1656, 1588, 1544,

1257, 1098, 1052 cm^{-1}. ^1H NMR ($CDCl_3$) δ 3.83, 3.86 (2H, AB, J = 11 Hz), 3.88, 4.05 (2H, AB, J = 8 Hz), 4.00 (1H, s), 4.44, 4.57 (2H, AB, J = 11 Hz), 4.64, 4.67 (2H, AB, J = 12 Hz), 4.66 (1H, s), 5.90 (1H, s), 6.44 (1H, d, J = 7 Hz), 6.70 (1H, s), 7.20–7.53 (15H, m), 7.75–8.00 (5H, m), 8.60 (1H, s). ^{13}C NMR ($CDCl_3$) δ 64.5, 72.1, 72.2, 73.6, 75.7, 76.4, 87.2, 88.0, 105.8, 116.6, 118.7, 121.4, 122.6, 127.2, 127.5, 127.6, 127.9, 127.9, 128.4, 128.5, 128.7, 129.6, 131.9, 132.1, 134.7, 136.9, 137.6, 138.1, 139.0, 151.8, 162.0, 166.1. Mass (FAB): m/z 615 (MH^+). Anal. Calcd for $C_{38}H_{34}N_2O_6 \cdot 1/4H_2O$: C, 73.71; H, 5.62; N, 4.52. Found: C, 73.64; H, 5.76; N, 4.46.

4.1.7. 4-(3-Benzamidophenyl)-1-(2-*O*,4-*C*-methylene-β-D-ribofuranosyl)-2-pyridone (8). A solution of compound **7** (520 mg, 0.85 mmol), 20% $Pd(OH)_2$–C (300 mg), and cyclohexene (4.30 ml, 42.5 mmol) in EtOH (25 ml) was refluxed for 8 h. After filtration of the mixture, silica gel (2 g) was added to the filtrate and the mixture was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [$CHCl_3$/MeOH (12:1, v/v)] to give compound **8** (315 mg, 86%). White powder. Mp 277–278 °C. $[\alpha]_D^{28}$ +94.7 (c 1.10, DMSO). IR ν_{max} (KBr): 3303, 2970, 1675, 1570 cm^{-1}. ^1H NMR (DMSO-d_6) δ 3.72, 3.88 (2H, AB, J = 8 Hz), 3.82 (2H, d, J = 6 Hz), 3.95 (1H, d, J = 4 Hz), 4.22 (1H, s), 5.22 (1H, dd, J = 6, 6 Hz), 5.64 (1H, d, J = 4 Hz), 5.68 (1H, s), 6.64 (1H, d, J = 2 Hz), 6.67 (1H, dd, J = 2, 7 Hz), 7.47–7.64 (5H, m), 7.89–8.00 (4H, m), 8.16 (1H, s), 10.37 (1H, s). ^{13}C NMR (DMSO-d_6) δ 56.1, 68.3, 71.2, 78.8, 87.1, 89.0, 104.4, 115.2, 118.5, 121.5, 122.1, 127.7, 128.5, 129.6, 131.8, 133.0, 134.8, 137.0, 139.9, 150.9, 161.1, 165.8. Mass (FAB): m/z 435 (MH^+). Anal. Calcd for $C_{24}H_{22}N_2O_6$: C, 66.35; H, 5.10; N, 6.45. Found: C, 65.98; H, 5.21; N, 6.31.

4.1.8. 4-(3-Benzamidophenyl)-1-[5-*O*-(4,4′-dimethoxy-trityl)-2-*O*,4-*C*-methylene-β-D-ribofuranosyl]-2-pyridone (9). Under a N_2 atmosphere, DMTrCl (62 mg, 0.18 mmol) was added to a solution of compound **8** (43 mg, 99 μmol) in anhydrous pyridine (3 ml) at room temperature and the mixture was stirred at room temperature for 2.5 h. After addition of saturated aqueous $NaHCO_3$ solution, the mixture was extracted with AcOEt. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [$CHCl_3$/AcOEt (4:5, v/v)] to give compound **9** (69 mg, 95%). White powder. Mp 153–155 °C. $[\alpha]_D^{21}$ +32.2 (c 0.80, $CHCl_3$). IR ν_{max} (KBr): 3301, 1655, 1577, 1512, 1301, 1253, 1179, 1046 cm^{-1}. ^1H NMR (acetone-d_6) δ 3.55, 3.63 (2H, AB, J = 11 Hz), 3.78 (6H, s), 3.82, 3.96 (2H, AB, J = 8 Hz), 4.43 (1H, s), 4.47 (1H, d, J = 5 Hz), 4.90 (1H, d, J = 5 Hz), 5.82 (1H, s), 6.55 (1H, dd, J = 2, 7 Hz), 6.65 (1H, d, J = 2 Hz), 6.91–6.94 (4H, m), 7.27–7.58 (14H, m), 7.96–8.05 (3H, m), 8.18 (1H, d, J = 7 Hz), 8.28 (1H, s), 9.74 (1H, s). ^{13}C NMR (acetone-d_6) δ 55.4, 59.5, 70.3, 72.4, 79.9, 87.1, 88.6, 88.6, 105.1, 113.8, 116.6, 119.1, 121.7, 122.6, 127.6, 128.2, 128.5, 128.8, 129.1, 130.1, 130.8, 132.3, 133.5, 135.8, 136.3, 136.5, 138.4, 140.8, 145.7, 152.0, 159.5, 162.1, 166.3. Mass (FAB): m/z 737 (MH^+). Mass (FAB): m/z 759 (MNa^+). Anal. Calcd for $C_{45}H_{40}N_2O_8H_2O$: C, 71.60; H, 5.61; N, 3.71. Found: C, 71.41; H, 5.62; N, 3.61.

4.1.9. 4-(3-Benzamidophenyl)-1-[3-*O*-[2-cyanoethoxy-(diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxy-trityl)-2-*O*,4-*C*-methylene-β-D-ribofuranosyl]-2-pyridone (10). Under a N_2 atmosphere, (i-$Pr_2N)_2POCH_2CH_2CN$ (38 μl, 0.12 mmol) was added to a solution of compound **9** (30 mg, 41 μmol), diisopropylammonium tetrazolide (12 mg, 70 μmol) in anhydrous MeCN/THF (1:1, 2 ml) at room temperature and the mixture was stirred at room temperature for 21 h. The solvent was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography [n-hexane/AcOEt (1:1, v/v)] to give compound **10** (36 mg, 94%). Colorless oil. ^{31}P NMR (acetone-d_6) δ 148.8, 149.6.

4.1.10. Methyl 3,5-di-*O*-*tert*-butyldiphenylsilyl-2-*O*,4-*C*-methylene-D-ribofuranoside (12). Under a H_2 atmosphere at room temperature, a solution of compound **11** (847 mg, 2.38 mmol) and 20% $Pd(OH)_2$–C (400 mg) in AcOEt (15 ml) was stirred for 1.5 h. The mixture was filtered and concentrated under reduced pressure. Under a N_2 atmosphere, the residue was dissolved in anhydrous DMF (5 ml), and TBDPSCl (1.3 ml, 5.00 mmol) and imidazole (1.20 g, 18.2 mmol) were added at room temperature. After stirring for 12 h, saturated aqueous $NaHCO_3$ was added to the mixture, and the organic phase was extracted with ethyl acetate, washed with water and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [n-hexane/$CHCl_3$ (4:1, v/v)] to give compound **12** (1.23 g, 79% 2 steps from **11**). Colorless oil. $[\alpha]_D^{26}$ −45.2 (c 2.67, $CHCl_3$). IR ν_{max} (KBr): 3063, 2939, 1468, 1368, 1106 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.03 (9H, s), 1.04 (9H, s), 3.23 (3H, s), 3.60 (1H, s), 3.77, 4.10 (2H, AB, J = 7 Hz), 3.94 (2H, s), 4.32 (1H, s), 4.67 (1H, s), 7.23–7.42 (12H, m), 7.57–7.72 (8H, m). ^{13}C NMR ($CDCl_3$) δ 19.2, 19.4, 26.8, 26.9, 54.9, 60.8, 72.1, 73.4, 79.1, 86.8, 104.5, 127.6, 127.6, 127.6, 129.5, 129.6, 129.7, 129.8, 132.5, 133.1, 133.3, 133.3, 135.5, 135.5, 135.6. Mass (FAB): m/z 675 (MNa^+). HRMS Calcd for $C_{39}H_{48}O_5Si_2Na$: 675.2938. Found: 675.2938.

4.1.11. 3,5-Di-*O*-*tert*-butyldiphenylsilyl-2-*O*,4-*C*-methylene-D-ribofuranose (13). At room temperature, 10% HCl aqueous solution (0.2 ml) was added to a solution of compound **12** (1.00 g, 1.53 mmol) in THF (15 ml) and the mixture was stirred for 4 h. After the addition of saturated aqueous $NaHCO_3$ solution, the organic phase was extracted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [n-hexane/$CHCl_3$ (2:1, v/v)] to give compound **13** (940 mg, 96%). Colorless oil. $[\alpha]_D^{26}$ −3.4 (c 1.83, $CHCl_3$). IR ν_{max} (KBr): 3434, 3063, 2939, 2862, 1728, 1467, 1108 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.97 (9H, s), 1.07 (9H, s), 2.64 (1H, br s), 3.79, 3.90 (2H, AB, J = 10 Hz), 3.99, 4.07 (2H, AB, J = 9 Hz), 4.06

(1H, s), 4.29 (1H, s), 7.25–7.47 (12H, m), 7.54–7.61 (8H, m), 9.35 (1H, s). ^{13}C NMR ($CDCl_3$) δ 19.2, 19.4, 26.9, 27.0, 63.3, 76.0, 80.9, 82.3, 89.6, 127.7, 127.8, 127.8, 129.9, 130.0, 130.0, 131.9, 132.3, 132.4, 132.9, 135.4, 135.6, 135.6. Mass (FAB): *m*/*z* 661 (MNa^+). HRMS Calcd for $C_{38}H_{46}O_5Si_2Na$: 661.2781. Found: 661.2780.

4.1.12. 2-(*N*-Methylbenzamido)-5-(3,5-di-*O*-tert-butyldiphenylsilyl-2-*O*,4-*C*-methylene-D-ribitol-1-yl)thiazole (14). Under a N_2 atmosphere at −78 °C, a solution of 2-(*N*-methylbenzamido)thiazole (1.71 g, 7.85 mmol) in anhydrous THF (10 ml) was added to lithium diisopropylammonium (67.0 mM in THF, 130 ml, 8.64 mmol). After stirring for 30 min, the mixture was added to a solution of compound **13** (1.0 g, 1.57 mmol) in anhydrous THF (10 ml) at −78 °C and stirred for 1 h. After the addition of a saturated aqueous NH_4Cl solution, the organic phase was extracted with AcOEt, washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [*n*-hexane/$CHCl_3$ (2:1, v/v)] to give compound **14** (710 mg, 53%). White powder. Mp 100–102 °C. $[\alpha]_D^{26}$ +72.2 (*c* 1.04, $CHCl_3$). IR ν_{max} (KBr): 3330, 3064, 2938, 2861, 1495, 1372, 1107 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.83 (9H, s), 1.02 (9H, s), 3.28 (1H, broad s), 3.59 (3H, s), 3.63 (1H, s), 3.78 (1H, s), 3.80, 3.89 (2H, AB, *J* = 12 Hz), 3.88 (2H, s), 4.16 (1H, s), 6.50 (1H, s), 7.16–7.21 (3H, m), 7.29–7.46 (16H, m), 7.54–7.57 (4H, m), 9.35 (1H, dd, *J* = 2, 6 Hz). ^{13}C NMR ($CDCl_3$) δ 19.1, 19.3, 26.8, 26.9, 35.5, 64.7, 67.4, 76.1, 79.5, 82.5, 90.6, 123.4, 126.8, 127.7, 127.7, 127.8, 127.9, 129.0, 129.8, 129.9, 130.0, 130.1, 131.1, 132.0, 132.3, 132.8, 135.3, 135.3, 135.5, 135.6, 136.7, 167.2, 173.2. Mass (FAB): *m*/*z* 857 (MH^+). HRMS Calcd for $C_{49}H_{57}N_2O_6SSi_2$: 857.3476. Found: 857.3491.

4.1.13. 2-(*N*-Methylbenzamido)-5-(3,5-di-*O*-*tert*-butyldiphenylsilyl-2-*O*,4-*C*-methylene-β-D-ribofuranosyl)thiazole (15). Under a N_2 atmosphere at room temperature, 1,1′-azobis(*N*,*N*-dimethylformamide) (370 mg, 2.13 mmol) and tri-*n*-butylphosphine (0.53 ml, 2.13 mmol) were added to a solution of compound **14** (1.22 g, 1.42 mmol) in anhydrous THF (15 ml) and the mixture was stirred for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography [*n*-hexane/AcOEt (6:1, v/v)] to give compound **15** (763 mg, 64%). White powder. Mp 79–82 °C. $[\alpha]_D^{26}$ +23.9 (*c* 0.56, $CHCl_3$). IR ν_{max} (KBr): 3063, 2940, 2862, 1600, 1565, 1506, 1364, 1108 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 1.04 (9H, s), 1.06 (9H, s), 3.50 (3H, s), 3.76 (1H, s), 4.01 (2H, s), 4.01, 4.19 (2H, AB, *J* = 8 Hz), 4.14 (1H, s), 4.91 (1H, s), 6.42 (1H, d, *J* = 1 Hz), 7.17–7.75 (23H, m), 8.34 (1H, dd, *J* = 2, 8 Hz). ^{13}C NMR ($CDCl_3$) δ 19.1, 19.4, 26.8, 27.0, 35.4, 60.2, 72.8, 73.3, 78.4, 81.2, 87.3, 122.8, 124.0, 127.6, 127.7, 127.7, 127.9, 129.1, 129.7, 129.8, 130.0, 131.4, 132.3, 132.7, 133.0, 135.4, 135.5, 135.5, 135.6, 136.7, 166.9, 173.4. Mass (FAB): *m*/*z* 839 (MH^+). HRMS Calcd for $C_{49}H_{55}N_2O_5SSi_2$: 839.3370. Found: 839.3387.

4.1.14. 2-(*N*-Methylbenzamido)-5-(2-*O*,4-*C*-methylene-β-D- ribofuranosyl)thiazole (16). Under a N_2 atmosphere at room temperature, 1 M tetra-*n*-butyl ammonium fluoride in THF (2.4 ml, 2.40 mmol) was added to a solution of compound **15** (680 mg, 3.22 mmol) in anhydrous THF (30 ml) and the mixture was stirred for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography [AcOEt/EtOH (30:1, v/v)] to give compound **16** (840 mg, 72%). Colorless crystals. Mp 180–182 °C. $[\alpha]_D^{26}$ −23.7 (*c* 0.55, MeOH). IR ν_{max} (KBr): 3041, 2949, 1496, 1378, 1027 cm^{-1}. ^{1}H NMR (MeOH-d_4) δ 3.82 (3H, s), 3.89 (2H, s), 3.93, 4.03 (2H, AB, *J* = 8 Hz), 4.16 (1H, s), 4.26 (1H, s), 5.06 (1H, s), 7.32 (1H, d, *J* = 1 Hz), 7.40–7.50 (3H, m), 8.22–8.26 (2H, m). ^{13}C NMR (MeOH-d_4) δ 36.6, 59.9, 73.1, 73.8, 80.1, 84.2, 89.1, 102.3, 126.1, 126.4, 129.4, 130.4, 133.1, 138.2, 169.0, 175.6. Mass (EI): *m*/*z* 362 (M^+, 87.2), 247 (26.4), 105 (100). HRMS Calcd for $C_{17}H_{18}N_2O_5S$: 362.0936. Found: 362.0933.

4.1.15. 2-(*N*-Methylbenzamido)-5-[5-*O*-(4,4′-dimethoxytrityl)-2-*O*,4-*C*-methylene-β-D-ribofuranosyl]thiazole (17). Under a N_2 atmosphere, DMTrCl (282 mg, 2.48 mmol) was added to a solution of compound **16** (201 mg, 1.65 mmol) in anhydrous pyridine (5 ml) at room temperature and the mixture was stirred at room temperature for 12 h. After addition of saturated aqueous $NaHCO_3$ solution, the mixture was extracted with AcOEt. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [*n*-hexane/AcOEt (1:1, v/v)] to give compound **17** (352 mg, 96%). White powder. Mp 104–107 °C. $[\alpha]_D^{22}$ +9.3 (*c* 1.70, $CHCl_3$). IR ν_{max} (KBr): 3332, 3063, 2943, 1492, 1372, 1105 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 2.88 (1H, d, *J* = 5 Hz), 3.48 (2H, s), 3.72 (3H, s), 3.77 (6H, s), 4.00 (2H, s), 4.17 (1H, s), 4.45 (1H, d, *J* = 5 Hz), 5.08 (1H, s), 6.84 (4H, d, *J* = 7 Hz), 6.88 (1H, d, *J* = 1 Hz), 7.18–7.50 (11H, m), 8.31 (2H, dd, *J* = 1, 8 Hz). ^{13}C NMR ($CDCl_3$) δ_C: 35.7, 55.1, 59.6, 72.3, 72.6, 78.4, 82.0, 85.9, 86.2, 113.0, 123.7, 126.7, 127.7, 127.8, 127.9, 129.0, 129.8, 131.4, 135.4, 136.3, 144.3, 158.3, 167.0, 173.6. Mass (FAB): *m*/*z* 665 (MH^+). HRMS Calcd for $C_{38}H_{37}N_2O_7S$: 665.2243. Found: 665.2321.

4.1.16. 2-(*N*-Methylbenzamido)-5-[3-*O*-[2-cyanoethoxy (diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,4-*C*-methylene-β-D-ribofuranosyl] thiazole (18). Under a N_2 atmosphere, *i*-Pr_2NEt (0.17 ml, 0.96 mmol) and *i*-$Pr_2NP(Cl)OCH_2CH_2CN$ (40 μl, 0.19 mmol) were added to a solution of compound **9** (64 mg, 96 μmol) in anhydrous CH_2Cl_2 (4 ml) at 0 °C and the mixture was stirred at room temperature for 3 h. After addition of saturated aqueous $NaHCO_3$ solution, the mixture was extracted with AcOEt. The organic phase was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [*n*-hexane/AcOEt (3:1, v/v)] to give compound **18** (84 mg, 89%). Colorless oil. ^{31}P NMR ($CDCl_3$) δ 148.2, 149.0.

4.1.17. Oligonucleotide synthesis. Synthesis of oligonucleotides containing bP^B and Tz^B was performed on a 0.2 μmol scale on an automated DNA synthesizer (Applied Biosys-

tems, Expedite® 8909) using the standard phosphoramidite protocol. The oligonucleotide synthesis was performed on DMTr-ON mode. Cleavage from the CPG support and removal of protecting groups were accomplished using 28% aqueous ammonia (55 °C for 12 h). The crude oligonucleotides bearing a DMTr group were detritylated and purified with NENSORB™ PREP according to the manufacturer's protocol. The obtained oligonucleotides were again purified by reversed-phase HPLC (ChemcoPak® CHEMCOSORB 300-5C18, 4.6 mm × 250 mm or Waters Xterra® MS C_{18} 2.5 μm, 10 mm × 50 mm). The composition of the oligonucleotides was confirmed by MALDI-TOF-Mass analysis: 5′-TTTTTCTbP^B TCTCTCT-3′: $[M-H]^-$ calcd 4688.3; found: 4688.6; 5′-TTTTTCTTz^B TCTCTCT-3′: $[M-H]^-$ calcd 4616.5; found: 4617.2; 5′-TTTTTCTbP^B CTCTCT-3′: $[M-H]^-$ calcd 4687.3; found: 4686.4; 5′-TTTTTCTTz^B CTCTCT-3′: $[M-H]^-$ calcd 4612.8; found: 4612.8; 5′-TTTTTCbP^B TCTCTCT-3′: $[M-H]^-$ calcd 4687.3; found: 4686.8; 5′-TTTTTCTz^B TCTCTCT-3′: $[M-H]^-$ calcd 4612.8; found: 4612.2.

4.2. T_m measurements

The UV melting experiments were carried out on a Beckman DU-650 spectrophotometer equipped with a T_m analysis accessory using quartz cuvettes with 1 cm optical path length. The UV melting profiles were recorded in 7 mm sodium phosphate buffer (pH 7.0) containing 140 mm KCl and 10 mm $MgCl_2$ at a scan rate of 0.5 °C/min with detection at 260 nm. The final concentration of each oligonucleotide was 1.5 μm. The melting temperatures were obtained as the maxima of the first derivative of the melting curves.

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