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Graphical Abstract

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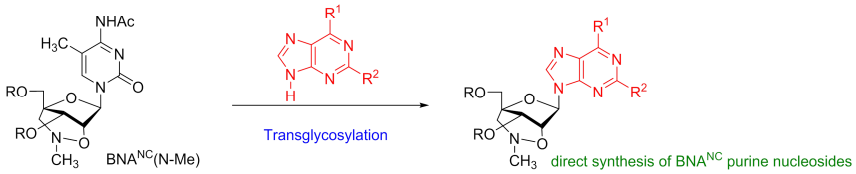

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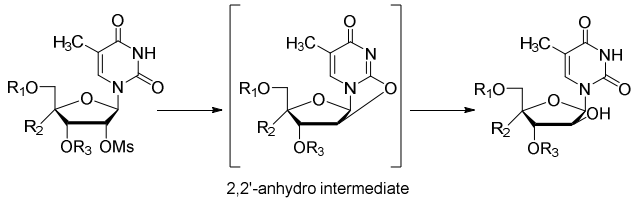
ABSTRACT

Purine nucleosides of 2′-*O*,4′-*C*-aminomethylene-bridged nucleic acid (BNA^{NC}) have been directly synthesized from pyrimidine BNA^{NC} using transglycosylation reaction with high stereoselectivity and excellent yield. The BNA^{NC} purine nucleosides (adenosine, guanosine, and inosine) prepared by the direct and practical procedure were derivatized to the corresponding phosphoramidites and were incorporated into DNA. A thermal denaturation study showed that BNA^{NC}-containing oligonucleotides hybridized to RNA selectively with excellent mismatch discrimination.

1. Introduction

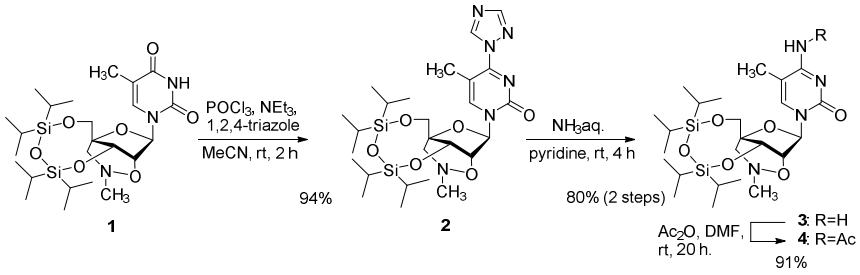
Modified nucleic acid derivatives have been widely investigated and have potential use in nucleic acid-based medicines, such as antisense oligonucleotides,[1] aptamers,[2] and siRNAs[3]. The modified nucleic acid confers thermal stability on duplexes and/or triplexes, resistance against nucleases, reduced immune stimulation, and other properties. Synthesis of modified nucleic acids often requires a multistep reaction to obtain monomer units for a solid-phase synthesizer. In particular, monomer units bearing purine nucleobases are always a source of concern for the synthesis of chemically modified nucleosides.

One of the most important classes of modified nucleotides is the LNA/2′,4′-BNA[4] family, which shows a restricted conformation of sugar puckering resulting from the addition of a bridge structure to the skeleton. Originally LNA/2′,4′-BNA was independently synthesized by the Imanishi et al. and Wengel et al.[4]. Since the publication of their articles, many research groups have reported novel derivatives of the family.[5–10] 2′-*O*,4′-*C*-aminomethylene-bridged nucleic acid (BNA^{NC}) has reported by Imanishi et al. as a novel BNA derivative containing N-C linkage in the six membered bridged strugure.[9] BNA^{NC} has excellent properties suitable for nucleic acid medicines, such as RNA-specific stable hybridization, nuclease resistance, and capability of chemical conjugation with the nitrogen atom in the bridge structure. However, no practical synthetic method has been reported for BNA^{NC} purine nucleosides.



Scheme 1. Inversion of 2′-hydroxyl group of pyrimidine nucleosides

The synthesis of BNA^{NC} pyrimidine nucleosides has been achieved using a 2,2′-anhydropyrimidine intermediates well as of other 2′-substituted nucleosides (Scheme 1), such as 2′-amino-2′-deoxynucleoside,[11] 2′-deoxy-2′-mercaptonucleoside,[12] 2′-methyl (2′-OMe),[13] 2′-methoxyethylnucleoside (2′-MOE),[14] α-L-LNA,[6] and 2′-amino/thio-LNAs.[5,15] However, the synthetic route is not applicable for purine nucleoside synthesis because the exocyclic oxygen atom of pyrimidine is essential for the key intermediate formation. For the epimerization of the 2′-hydroxyl group of purine nucleosides, two strategies have been reported as alternatives: the one is nucleophilic substitution with a carboxylate anion, followed by hydrolysis;[6b,16] and the other is oxidation followed by reduction from the less hindered α-face of the furanose ring.[17] However, both of the two strategies require multistep reactions, and the total yield is low. To avoid the epimerization of purine nucleosides for 2′-modified nucleoside synthesis, a transglycosylation reaction has been reported for 2′-trifluoroacetamido-2′-deoxyribonucleosides[18] and 2′-*O*-methylribonucleoside synthesis.[19] However, the reactions have not been applied to nucleosides possessing a bridged sugar



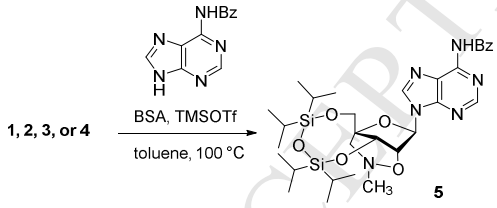
Scheme 2. Synthesis of substrates for the transglycosylation reactions

skeleton as a transglycosyl donor. Therefore, we set out to establish an efficient synthetic route for purine nucleosides having a bridged sugar skeleton. Herein, we wish to describe the direct and practical synthesis of BNA^{NC} purine nucleosides from the corresponding pyrimidine analogs by transglycosylation.

2. Results and Discussion

Nucleosides bearing uracilyl, thyminyl, cytosinyl, and their acylated derivatives have been used for transglycosylation reactions from pyrimidines to purines.[18–20] N^4-acetyl cytidine derivatives have been reported as for β-anomer selective reactions.[19,20] To achieve the direct synthesis of BNA^{NC} purine nucleosides by transglycosylation, thymin-1-yl (**1**), 4-(1,2,4-triazol-1-yl)pyrimidin-2-on-1yl (**2**), cytosin-1-yl (**3**), and N^4-acetylcytosin-1-yl (**4**) BNA^{NC} analogs were examined as glycosyl donors (Scheme 2). BNA^{NC} thymine (**1**) was synthesized by the previously reported procedure.[9b] BNA^{NC} cytidine (**3**) was synthesized by a conventional procedure *via* triazole intermediate (**2**) using 1,2,4-triazole and phosphorus oxychloride, followed by ammonia treatment. The nucleoside (**3**) was reacted with acetic anhydride to afford N^4-acetylated BNA^{NC} cytidine (**4**) in excellent yield.

Table 1. Transglycosylation reaction for the synthesis of BNA^{NC} adenosine

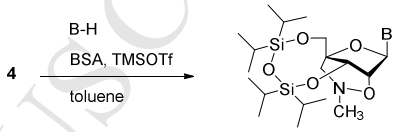


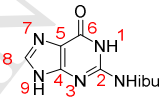
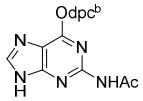
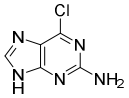
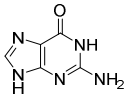
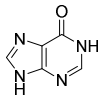
Entry	Substrate	Reaction Time	Yield %
1	**1**	40 min	29
2	**1**	4 h	0[a]
3	**4**	30 min	70
4	**3**	1 h	27
5	**2**	30 min	84

[a]N^6-Acetyl adenosine was collected.

Facile conditions for the transglycosylation reaction to modified or unmodified nucleosides have been reported using *N,O*-bis-trimethylsilylacetamide (BSA), trimethylsilyl trifluoromethanesulfonate (TMSOTf), and corresponding nucleobases in 1,2-dichloroethane and/or acetonitrile.[18–20] We used the reported conditions except for solvent. We decided to use toluene as an alternative solvent for all transglycosylation reactions for the following two reasons: the one is that a higher reaction temperature is applicable owing to the relatively high boiling point, and the other is that the use of chlorinated solvents should be reduced for the purpose of green chemistry.

Table 2. Synthesis of BNA^{NC} purine derivatives by transglycosylation



Entry	Nucleobase (B-H)	Product	Yield %	Description
1		**6**	-	N^7:N^9=1:1[a] (not isolated)
2		**7**	23	N^2-deacylated (**7′**, 20%)
3		**8**	99	
4		**9**	50	
5		**10**	74	
6		**11**	77 (crude)	N^7-isomer[c] (~10%)

[a]Isomer ratio was determined by LC-MS analysis. [b]dpc: diphenylcarbamoyl [c]Isomer ratio was determined by the integrated area of anomer peak from ^{1}H NMR analysis.

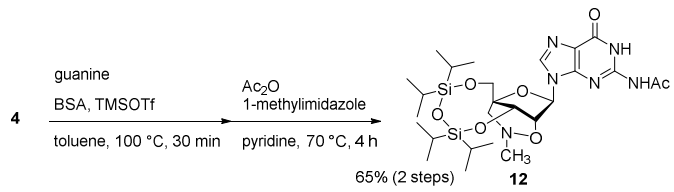
The transglycosylation reaction was optimized with N^6-benzoyladenine as a glycosyl acceptor (Table 1). The reaction was carried out with BSA and TMSOTf in toluene at 100 °C. At first, compound **1** was used as a glycosyl donor. The reaction after 40 min, BNA^{NC} N^6-benzoyladenosine (**5**) was obtained in 29% yield with unreacted starting material (entry 1). When the reaction time was extended to 4 h, the desired product **5** was not detected (entry 2). LC-MS analysis of the reaction mixture showed a new peak (rt = 1.68 min, *m/z* = 593.3). ^{1}H NMR

analysis indicated that an N^6-acetyladenosine analog and benzamide were present (see supporting information). These results suggested that the longer reaction time caused amido-exchange with acetamide derived from BSA. N^4-Acetylated cytidine nucleoside (**4**) as a glycosyl donor, providing **5** in 70% yield (entry 3). On the other hand, the reaction using N^4-unprotected cytidine nucleoside (**3**) proceeded more slowly than that using **4** (entry 4). Interestingly, transglycosylation reaction with **2** was completed within 30 min, affording **5** in 84% yield (entry 5). These results indicated that N^4-acetylcytosine and 4-(1,2,4-triazol-1-yl)primidin-2-one are appropriate nucleobases for the transglycosylation of BNA^{NC}. In all the cases, the reactions selectively afforded β-nucleosides. We speculate that the stereoselectivity results from not only steric hindrance by the 2′-*O*-4′-*C*-bridge on the α face of the furanose ring but also participation of the lone pair of the N–O bond, which regulates the direction of nucleophilic attack of the incoming nucleobases.

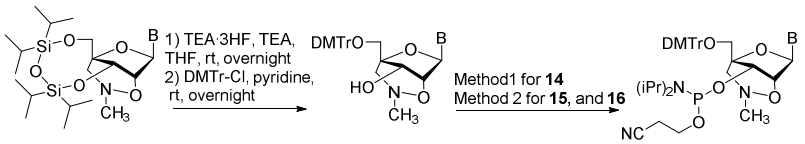
The optimized reaction conditions were applied to other purine nucleoside syntheses (Table 2). Compound **4** has been selected as a starting material to optimize other purine nucleoside synthesis since we had developed a procedure for large scale synthesis of **4** as an important intermediate for cytidine BNA^{NC}. Coupling N^2-isobutylprotected guanine with **4** resulted in inseparable mixtures of N^7/N^9 isomers of BNA^{NC} guanosine analogs (entry 1, see the supporting information). N^2-acetyl-O^6-diphenylcarbamoyl guanine, which has been reported for N^9-selective glycosylation in guanosine synthesis,[21] was used for the reaction (entry 2). BNA^{NC} N^2-acetyl-O^6-diphenylcarbamoylguanosine (**7**) was obtained in 23% yield with the N^2-deacylated by-product (**7′**, 20%). Reactions with 2-amino-6-chloropurine (entry 3), guanine (entry 4), 6-chloropurine (entry 5), and hypoxanthine (entry 6) proceeded without marked side reactions. In each case, the corresponding purine BNA^{NC} nucleosides were obtained in moderate or high yield. The isolated yield of BNA^{NC} guanosine (**9**) was relatively low due to the poor solubility to purify by silica-gel column chromatography. Additionally, the purification of BNA^{NC} inosine (**11**) was unsuccessful in this step due to the poor solubility of products. The purification BNA^{NC} inosine nucleoside was achieved after silyl deprotection to give 2′-*O*,4′-*C*-(*N*-methylaminomethylene)inosine (**13**). Inosine with BNA^{NC} skeleton would be useful as an universal base for the therapeutic use when the target RNA has single nucleotide polymorphism. To our knowledge, successful examples of transglycosylation or glycosylation using the unprotected guanine as a glycosyl acceptor have not been reported. We speculate that the difficulty of the glycosylation with unprotected guanine is caused from low conversion to persilylated guanine, due to its poor solubility in the early step of the reaction. We found that addition of a catalytic amount of TMSOTf along with BSA accelerated formation of the persilylated nucleobases before an addition of transglycosyldonor **4**. O^6-trimethylsilylguanine, supposed to be a major intermediate, has a 6 π-system in the purine ring. The intermediate acted similarly to the 2-amino-6-chloropurine (entry 3), leading to facilitation of the reaction as well as induction of N^9-selectivity. The reaction conditions were thus applied to BNA^{NC} inosine synthesis (entry 6). Finally, we applied the reaction conditions used in entry 4 to the synthesis of the BNA^{NC} guanosine monomer unit **12**. The practical synthesis of N^2-protected BNA^{NC} guanosine is described in Scheme 3. The transglycosylation reaction mixture was quenched and directly used for the subsequent acetylation to afford N^2-acetylated guanosine **12** in moderate yield (65%, 2 steps). Isolated yield was improved due to enhanced solubility of desire compound by N^2-acetylation.

Each of the silyl-protected BNA^{NC} purine nucleosides (**5**, **12**, **11**) was converted to the corresponding phosphoramidites by conventional deprotection of the silyl group, 4, 4′-dimethoxytrityl (DMTr) protection of the 5′-hydroxy group, and final 3′-phosphorylation (Scheme 4). Oligonucleotides were synthesized by the phosphite triester method using the standard phosphoramidite protocol. The coupling time for BNA^{NC} phosphoramidites was 20 min with 5-benzylthio-1*H*-tetrazole (BTT) as an activator.

We assessed the effect of BNA^{NC} purine incorporation by measuring melting temperatures of the duplexes with target DNAs and RNAs using two different sequences for adenosine and guanosine derivatives (Table 3). Single BNA^{NC} incorporated oligonucleotides (**ON2** and **ON5**) formed stable duplexes with target RNAs (**ON2**. ΔT_m = +3.5 °C/mod.; **ON5**: ΔT_m = +7.3 °C/mod.). In contrast, the oligonucleotides didn't show marked duplex stabilizing effects against target DNAs. However, triple BNA^{NC} incorporation (**ON3** and **ON6**) contributed to increased duplex stability with both DNA and RNA targets. The stabilizing effects against target RNAs were higher than those against target



Scheme 3. practical synthesis of N^2-acetyl BNA^{NC} guanosine



5: B=6-*N*-benzoyladen-9-yl (A^{bz})
12: B=2-*N*-acetylguan-9-yl (G^{ac})
11: B=hypoxanth-9-yl (I)

14 (A^{bz}): 78% (2 steps)
15 (G^{ac}): 60% (2 steps)
16 (I): 70% (2 steps)

17 (A^{bz}): 91%
18 (G^{ac}): 88%
19 (I): 74%

Method 1: 2-cyanoethyl-*N*,*N*,*NN*-tetraisopropylphosphordiamidite, 2,3-dicyanoimidazole in MeCN
Method 2: 2-cyanoethyl-*N*,*N*,*NN*-tetraisopropylphosphordiamidite,1-methylimidazole, 1*H*-tetrazole in DMF

Scheme 4. BNA^{NC} purine phosphoramidites

DNAs. These results are consistent with those of the previous study using BNA^{NC} pyrimidines.[9]

Mismatch discrimination properties were measured for **ON2** and **ON5** (Table 4). We found that BNA^{NC} purines could discriminate the mismatches similarly to the parent oligonucleotides (**ON1** and **ON4**) in the duplexes with target DNAs and RNAs. In addition, **ON5** could make a wobble base pairing between BNA^{NC} G and DNA-T (X = T, +4.0 °C/mod.) probably owing to the local A-type structure induced by the sugar conformation of BNA^{NC}.

3. Conclusion

We have successfully established the efficient and practical synthetic route for BNA^{NC} adenosine, guanosine, and inosine directly from the corresponding pyrimidines by transglycosylation. In the reaction, N^4-acetylcytosine was used as an excellent leaving group, as in the case of the previous examples[19,20]. We also found that the 4-(1,2,4-triazol-1-yl)-pyrimidin-2-one nucleoside, which is commonly used as an intermediate of cytidine synthesis, can be used as a transglycosyl donor. The transglycosylation could be applied to introduce not only natural nucleobases but also unnatural nucleobases to the BNA^{NC} skeleton. Further, the transglycosylation can be a workable solution for synthesis of stereorestricted purine nucleosides.

Table 3. Melting temperature studies of BNA^{NC}-adenosine/guanosine incorporated oligonucleotides with complementary DNA and RNA

	Sequence	DNA			RNA		
		T_m (°C)[b]	ΔT_m (°C)	ΔT_m/mod. (°C)	T_m (°C)[b]	ΔT_m (°C)	ΔT_m/mod. (°C)
ON1	d(GCA TAT CAC T)	38.8	-	-	36.5	-	-
ON2	d(GCA T**A^B**T CAC T)	38.4	-0.4	-0.4	40.0	+3.5	+3.5
ON3	d(GC**A^B** T**A^B**T C**A^B**C T)	44.6	+6.1	+2.0	53.7	+17.2	+5.7
ON4	d(ATG CGC TGT T)	48.7	-	-	47.3	-	-
ON5	d(ATG C**G^B**C TGT T)	49.6	+0.9	+0.9	54.6	+7.3	+7.3
ON6	d(AT**G^B** C**G^B**C T**G^B**T T)	56.5	+7.8	+2.6	67.7	+20.4	+6.8

[a]**A^B** and **G^B** are adenin-9-yl and guanin-9-yl BNA^{NC} (*N*-Me) monomers respectively. [b]Thermal-denaturation temperature were measured as the maximum of the first derivative of the melting curve (A_{260} versus temperature; all curves appeared to be sigmoidal) observed for an equimolar mixture of oligonucleotides (1.0 μM) in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA. Complementary DNAs: d(AGT GAT ATG C) for ON1~3, d(AAC AGC GCA T) for ON4~6; Complementary RNAs: r(AGU GAU AUG C) for ON1~3, r(AAC AGC GCA U) for ON4~6.

Table 4. Mismatch discrimination properties of BNA^{NC} adenosine/guanosine-incorporated oligonucleotides

	Sequence	T_m (°C)[a] DNA[b]				RNA[c]			
		X = A	X = C	X = G	X = T	Y = A	Y = C	Y = G	Y = U
ON1	d(GCA TAT CAC T)	nt[d]	22.3	29.4	**38.8**	22.2	21.2	25.8	**36.5**
ON2	d(GCA T**A^B**T CAC T)	nt[d]	21.3	20.7	**38.4**	22.8	25.3	25.8	**40.0**
ON4	d(ATG CGC TGT T)	30.3	**48.7**	33.4	35.5	29.4	**46.0**	33.4	35.4
ON5	d(ATG C**G^B**C TGT T)	30.9	**49.6**	30.4	39.5	31.3	**54.6**	35.4	41.4

[a]Thermal-denaturation temperature were measured as the maximum of the first derivative of the melting curve (A_{260} versus temperature; all curves appeared to be sigmoidal) observed for an equimolar mixture of oligonucleotides (1.0 μM) in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA. [b]Target DNAs: d(AGT GA**X** ATG C) for ON1~3, d(AAC AG**X** GCA T) for ON4~6; [c]Target RNAs: r(AGU GA**Y** AUG C) for ON1~3, r(AAC AG**Y** GCA U) for ON4~6. [d]nt: No clear transition curve was observed.

4. Experimental section

Oligonucleotide (ON) synthesis: A MerMade192 DNA/RNA synthesizer was used for automated DNA synthesis. The BNA^{NC}(*N*-Me) purine-incorporated ONs were synthesized by the conventional solid-phase phosphite triester method using the corresponding phosphoramidites (activator: 0.3 M 5-benzylthio-1*H*-tetrazole in MeCN; coupling time 20 min; detritylation by 3% trichloroacetic acid in toluene (w/v); CapA, 20% 1-methylimidazole in MeCN (vol%); CapB, 20%Ac_2O, 30% 2,4,6-collidine in MeCN (vol%); oxidation by 0.1 M iodine in pyridine:H_2O (7:3, v/v)). The synthesized ONs were purified in the DMT-ON mode by reversed-phase HPLC (0.1 M triethylamine acetate–acetonitrile system), and their composition and purity were verified by ion-pair reversed-phase HPLC on an ACQUITY UPLC OST C18 Column (1.7 μm, 2.1 × 50 mm; A, 400 mM 1,1,1,3,3,3-hexafluoroisopropylalcohol(HFIP), 16 mM triethylamine(TEA) with 5% MeOH; B, 400 mM HFIP, 16 mM TEA in MeOH, gradient of B from 10% to 80% in 6 min, flow rate 0.2 mL/min). Identity of the synthesized ONs was verified by MALDI-TOF-MS recorded on an Applied Biosystems Voyager-DE™ spectrometer. Melting temperature studies: thermal-denaturation temperatures were measured as the

maximum of the first derivative of the melting curve (A260 versus temperature) observed for an equimolar mixture of oligonucleotides (1.0 μM) in 10 mM sodium phosphate buffer (pH 7.0) containing 100 mM NaCl and 0.1 mM EDTA. The melting curve measurement was performed on a Shimadzu UV-2450 (UV–vis spectrophotometer) with TMSPC-8 (TM analysis system).

*4.1. 1-[3′,5′-O-(Tetraisopropyldisiloxane)-2′-O,4′-C-(N-methylaminomethylene)-β-D-ribofuranosyl]-5-methyl-4-(1,2,3-triazol-1-yl)pyrimidin-2-one(**2**)*

Compound **1** (3.2 mmol, 1.8 g) was dissolved in acetonitrile (20 mL), triethylamine (38 mmol, 5.4 mL), 1,2,4-triazole (26 mmol, 1.8 g), and phosphorus oxychloride (6.4 mmol, 0.59 mL) were added, and the reaction mixture was stirred at room temperature for 2 h. Ice was added to the reaction mixture to quench the reaction, and the mixture was partitioned between ethyl acetate and water. The separated organic layer was washed with saturated brine and dried over sodium sulfate. The sodium sulfate was filtered off, and the organic layer was concentrated. The residue was purified by silica gel column chromatography (hexane–ethyl acetate, 30:70 to 0:100, v/v) to yield **2** (1.8 g, 94%) as a white foam; ^{1}H NMR (300 MHz, $CDCL_3$) δ 9.31 (s, 1H), 8.35 (s, 1H), 8.12 (s, 1H), 6.39 (s, 1H), 4.51 (d, *J*=3.0 Hz, 1H), 4.09 (d, *J*=13.2 Hz, 1H), 3.92 (d, *J*=3.0 Hz, 1H), 3.71 (d, *J*=13.2 Hz, 1H), 2.96 (d, *J*=11.3 Hz, 1H), 2.79 (s, 3H), 2.65 (d, *J*=10.9 Hz, 1H), 2.47 (s, 3H), 1.15 – 0.92 (m, 28H); ^{13}C NMR(75 MHz, $CDCL_3$) δ 158.36, 153.57, 153.43, 146.53, 145.21, 105.48, 87.52, 84.35, 79.92, 77.42, 76.99, 76.56, 63.92, 60.13, 57.53, 45.56, 17.47, 17.3, 17.23, 17.19, 17.13, 17.02, 16.95, 13.42, 12.9, 12.83, 12.41; HRMS (MALDI-TOF) calc. for $C_{26}H_{45}N_6O_6Si_2$+ $[M + H]^+$ m/z=593.2934, found 593.2932.

*4.2. 3′,5′-O-(Tetraisopropyldisiloxane)-5-methyl-2′-O,4′-C-(N-methylaminomethylene)cytidine (**3**)*

Compound **1** (75.3g, 139 mmol), 1,2,4-triazole (76.8 g, 1.11 mol) were suspended in acetonitrile (1.3 L) and triethylamine (168.8 g, 1.67 mol) was added. After stirring for 10 min at room temperature, the mixture was cooled to −10 °C and phosphorus oxychloride (42.6 g, 278 mmol) was added dropwise. The mixture was maintained at room temperature and then stirred for 1 h. Water (102 mL) was added to the reaction mixture to quench the reaction, and saturated NaCl (aq.) (1 L) and sodium hydrogen carbonate (aq.) (1 L) were added, followed by extraction with ethyl acetate (1.5 L). The organic layer was washed twice with the salt mixture (800 mL, 1:1 (v/v)) and then with brine (800 mL). The organic layer was concentrated to obtain a crude residue of **2** as pale yellow foam. The intermediate was dissolved in pyridine (820 mL) and 25% ammonia solution (410 mL) was added. The mixture was stirred for 4 h and evaporated. Saturated NaCl (800 mL), THF (550 mL), and ethyl acetate (1.1 L) were added to the residue. The resulting organic layer was dried over magnesium sulfate and evaporated. The residue was recrystallized by the addition of diisopropyl ether (100 mL) and cooled on ice. The crystals were washed with diisopropyl ether (twice of 50 mL) and dried *in vacuo* to afford **3** as white crystals (50.3 g). The filtrate was concentrated and recrystallized by diisopropyl ether treatment to afford **3** as white crystals (9.8 g). In total, 60.1 g of **3** was obtained (80%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.28 (s, 2H for tautomer A), 7.51 (s, 1H for tautomer B), 7.32 (br. s., 1H for tautomer B), 6.83 (br. s., 1H), 6.03 (s, 1H), 4.17 (d, *J*=3.4 Hz, 1H), 4.04 (d, *J*=13.2 Hz, 1H), 3.86 (d, *J*=3.4 Hz, 1H), 3.65 (d, *J*=13.2 Hz, 1H), 2.74 (s, 2H), 2.63 (s, 3H), 1.82 (s, 3H), 1.14 – 0.87 (m, 28H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.41, 154.64, 146.88, 136.36, 100.52, 85.39, 82.68, 79.93, 63.75, 59.8, 57.16, 45.35, 17.3, 17.25, 17.1, 17.05, 16.99, 16.89, 16.87, 16.74, 13.55, 12.69, 12.25, 12.16, 11.83; HRMS (MALDI-TOF) calc. for $C_{24}H_{45}N_4O_6Si_2$ $[M + H]^+$ m/z=541.2872, found 541.2888.

*4.3. N^4-Acetyl-3′,5′-O-(tetraisopropyldisiloxane)-5-methyl-2′-O,4′-C-(N-methylaminomethylene)cytidine (**4**)*

Compound **3** (60.1 g, 111.1 mmol) was dissolved in DMF (1 L) and acetic anhydride (13.6 g, 133.4 mmol) was added. The mixture was stirred at room temperature for 20 h and water (20 mL) was added. After stirring for 10 min, the mixture was diluted with ethyl acetate (2 L) and washed with a mixture of 1/4-saturated sodium hydrogen carbonate solution (2 L), 1/2-saturated sodium hydrogen carbonate (1 L), and water (1 L). The resulting organic layer was dried over magnesium sulfate and evaporated. The residue (85.1 g) was recrystallized with ethyl acetate–hexane (300 mL, 1:1, v/v) to afford **4** as a white powder (59.2 g, 91%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.92 (br. s., 1H), 7.82 (s, 1H), 6.09 (s, 1H), 4.32 (d, *J*=3.0 Hz, 1H), 4.07 (d, *J*=13.2 Hz, 1H), 3.87 (d, *J*=3.4 Hz, 1H), 3.67 (d, *J*=13.6 Hz, 1H), 2.77 (s, 2H), 2.66 (s, 3H), 2.26 (s, 3H), 1.95 (s, 3H), 1.12 – 0.87 (m, 28H); ^{13}C NMR(75 MHz, DMSO-d_6) δ 162.03, 153.29, 140.49, 105.18, 86, 83.26, 79.45, 63.53, 59.7, 57.05, 45.33, 25.06, 17.28, 17.25, 17.12, 17.04, 16.94, 16.92, 16.74, 14.12, 12.65, 12.19, 11.83; HRMS (MALDI-TOF) calc. for $C_{26}H_{47}N_4O_7Si_2$ $[M + H]^+$ m/z=583.2978, found 583.2977.

*4.4. N^6-Benzoyl-3′,5′-O-(tetraisopropyldisiloxane)-2′-O,4′-C-(N-methylaminomethylene)adenosine (**5**)*

Compound **4** (680 mg, 1.17 mmol) and *N*^{6}-benzoyladenine (1.4 g, 5.9 mmol) were suspended in toluene (11 mL). To the suspension was added *N*,*O*-bis-trimethylsilylacetamide (BSA, 2.9 mL, 11.7 mmol), and the mixture was heated to 100 °C and stirred for 1 h until transparency. To the reaction mixture, trimethylsilyl trifluoromethanesulfonate (TMSOTf, 318 μL, 1.76 mmol) was added, and the mixture was stirred for an additional 30 min. The reaction mixture was cooled, diluted with ethyl acetate (200 mL), and washed with saturated aqueous sodium hydrogen carbonate solution, water, and saturated brine (100 mL each). The resulting organic layer was dried over sodium sulfate and concentrated. The mixture was purified by silica gel column chromatography (hexane–ethyl acetate) to afford compound **5** (533 mg, 0.813 mmol, 70%) as a white foam; ^{1}H NMR (600 MHz, $CDCL_3$) δ 9.17 (br. s., 1H), 8.81 (s, 1H), 8.35 (s, 1H), 8.03 (d, *J*=7.7 Hz, 2H), 7.65 – 7.47 (m, 3H), 6.79 (s, 1H), 4.77 (d, *J*=2.6 Hz, 1H), 4.50 (d, *J*=2.9 Hz, 1H), 4.04 (d, *J*=12.8 Hz, 1H), 3.73 (d, *J*=12.8 Hz, 1H), 3.01 (d, *J*=11.0 Hz, 1H), 2.82 (s, 3H), 2.68 (d, *J*=11.0 Hz, 1H), 1.15 – 0.93 (m, 28H); ^{13}C NMR (150 MHz, $CDCL_3$) δ 164.61, 152.74, 150.56, 149.48, 141.49, 133.69, 132.71, 128.8, 127.81, 123.35, 85.21, 83.32, 80.49, 64.96, 60.26, 57.76, 45.63, 17.39, 17.25, 17.23, 17.2, 17.18, 17.16, 17.04, 16.98, 13.35, 12.84, 12.74, 12.39; HRMS (MALDI-TOF) calc. for $C_{31}H_{46}N_6NaO_6Si_2$ $[M + H]^+$ m/z=677.2910, found 677.2907.

*4.5. N^2-Acetyl-3′,5′-O-(tetraisopropyldisiloxane)-2′-O,4′-C-(N-methylaminomethylene)-O^6-diphenylcarbamoylguanosine(**7**)*

Compound **4** (1.0 g, 1.7 mmol) and *N*^{2}-acetyl-*O*^{6}-diphenylcarbamoylguanine[21a] (3.6 g, 8.6 mmol) were suspended in toluene (10 mL). BSA (4.2 mL, 17.2 mmol) was added to the suspension, and the mixture was stirred at 100 °C for 30 min. TMSOTf (470 μL, 2.6 mmol) was added to the reaction mixture, and the mixture was stirred for an additional 30 min. The reaction mixture was cooled, diluted with ethyl acetate (50 mL), and washed with saturated aqueous sodium hydrogen carbonate solution, water, and saturated brine (50 mL each). The resulting organic layer was dried over sodium sulfate. The insoluble

material was removed by filtration and the filtrate was concentrated. The mixture was purified by silica gel column chromatography (hexane–ethyl acetate) to afford compound **7** (325 mg, 0.4 mmol, 23%) as a pale yellow foam; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.77 (s, 1H), 8.30 (s, 1H), 7.56 – 7.26 (m, 10H), 6.57 (s, 1H), 4.93 (d, *J*=3.0 Hz, 1H), 4.24 (d, *J*=3.0 Hz, 1H), 4.06 (d, *J*=13.2 Hz, 1H), 3.69 (d, *J*=13.2 Hz, 1H), 2.89 – 2.75 (m, 2H), 2.69 (s, 3H), 2.17 (s, 3H), 1.08 – 0.90 (m, 28H); ^{13}C NMR(75 MHz, DMSO-d_6) δ 168.52, 155.15, 153.64, 152.09, 149.97, 142.16, 141.59, 129.35, 127.27, 126.94, 120.16, 84.14, 82.38, 79.32, 65.03, 60.17, 57.25, 45.41, 24.37, 17.2, 17.07, 17.04, 16.99, 16.93, 16.8, 12.6, 12.24, 12.01, 11.93; HRMS (MALDI-TOF) calc. for $C_{39}H_{53}N_7NaO_8Si_2$ [M + H]$^+$ m/z=826.3386, found 826.3410. *N*^{2}-deacetylated compound 3,5′-*O*-(tetraisopropyldisiloxane)-2′-*O*,4′-*C*-(*N*-methylaminomethylene)-*O*^{6}-diphenylcarbamoylguanosine (**7′**) was obtained (275 mg, 0.36 mmol, 20%); ^{1}H NMR (300 MHz, $CDCL_3$) δ 8.05 (s, 1H), 7.53 – 7.17 (m, 10H), 6.57 (s, 1H), 5.41 (s, 2H), 4.54 (d, *J*=2.8 Hz, 1H), 4.30 (d, *J*=3.0 Hz, 1H), 4.03 (d, *J*=13.0 Hz, 1H), 3.70 (d, *J*=13.0 Hz, 1H), 2.98 (d, *J*=11.0 Hz, 1H), 2.81 (s, 3H), 2.65 (d, *J*=11.1 Hz, 1H), 1.14 – 0.93 (m, 28H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.59, 156.35, 154.62, 150.6, 141.86, 138.97, 128.93, 126.62, 118.38, 84.62, 82.87, 80.49, 64.75, 60.17, 57.78, 45.65, 17.35, 17.23, 17.16, 17, 16.94, 13.29, 12.78, 12.76, 12.31; HRMS (MALDI-TOF) calc. for $C_{37}H_{51}N_7NaO_7Si_2$ [M + Na]$^+$ m/z=784.3286, found 784.3283.

*4.6. 2-Amino-6-chloro-9-[3′,5′-O-(tetraisopropyldisiloxane)-2′-O,4′-C-(N-methylaminomethylene)-β-D-ribofuranosyl]purine (**8**)*

2-Amino-6-chloropurine (254 mg, 1.5 mmol) and **4** (290 mg, 0.5 mmol) were suspended in toluene (5 mL). To the resulting suspension, BSA (1.1 mL, 4.5 mmol) was added, and the suspension was stirred at 100 °C for 30 min. To the solution after stirring, TMSOTf (186 μL, 0.65 mmol) was added, and the resulting solution was stirred at 100 °C for 30 min. The solution after stirring was poured into saturated aqueous sodium hydrogen carbonate solution (30 mL). Ethyl acetate (40 mL) was added to the obtained solution, the resulting solution was filtered through Celite®, and the filtrate was recovered. The filtrate was separated to yield an organic layer. The resulting organic layer was washed with saturated aqueous sodium hydrogen carbonate solution (30 mL), water (30 mL), and 1 M hydrochloric acid (30 mL). Sodium sulfate was added to the organic layer after washing. The resulting solution was concentrated to dryness under reduced pressure to afford compound **8** (290 mg, 99%) as a pale yellow solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (s, 1H), 7.07 (s, 2H), 6.45 (s, 1H), 4.65 (d, *J*=3.4 Hz, 1H), 4.20 (d, *J*=3.4 Hz, 1H), 4.04 (d, *J*=13.2 Hz, 1H), 3.69 (d, *J*=13.2 Hz, 1H), 2.80 (s, 2H), 2.67 (s, 3H), 1.10 – 0.92 (m, 28H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.89, 152.94, 149.48, 138.82, 123.65, 83.66, 82.33, 79.56, 64.67, 59.98, 57.24, 45.4, 17.23, 17.12, 17.06, 17.04, 17, 16.94, 16.79, 12.61, 12.2, 11.88; HRMS (MALDI-TOF) calc. for $C_{24}H_{42}ClN_6O_5Si_2$ [M + Na]$^+$ m/z=585.2438, found 585.2443.

*4.7. 3′,5′-O-(Tetraisopropyldisiloxane)-2′-O,4′-C-(N-methylaminomethylene)guanosine(**9**)*

Guanine (775 mg, 5.13 mmol) was suspended in BSA (10 mL, 40.9 mmol) and toluene (10 mL), and the resulting suspension was refluxed for 1 h. To the solution after refluxing, TMSOTf (60 μL, 0.33 mmol) was added, and the resulting suspension was refluxed until it became transparent. The solution after refluxing was cooled to 100 °C, and **4** (1.0 g, 1.71 mmol) and TMSOTf (300 μL, 1.65 mmol) were added to it. The mixture was stirred for 1.5 h and quenched with sodium hydrogen carbonate. Silica gel was added to the mixture, followed by evaporation. The resulting residue was purified by silica gel column chromatography (ethyl acetate–methanol 0%–30%) to afford compound **9** (484 mg, 50%) as a white powder; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.67 (br. s., 1H), 7.63 (s, 1H), 6.62 (br. s., 2H), 6.35 (s, 1H), 4.49 (d, *J*=3.0 Hz, 1H), 4.20 (d, *J*=3.0 Hz, 1H), 4.02 (d, *J*=13.2 Hz, 1H), 3.67 (d, *J*=13.2 Hz, 1H), 2.78 (s, 2H), 2.66 (s, 3H), 1.10 – 0.91 (m, 28H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.68, 153.98, 150.23, 133.17, 116.85, 83.24, 82.09, 79.96, 64.66, 59.99, 57.29, 45.4, 17.23, 17.14, 17.08, 17.07, 16.99, 16.96, 16.88, 16.77, 12.65, 12.23, 12.2, 11.9; HRMS (MALDI-TOF) calc. for $C_{24}H_{42}N_6NaO_6Si_2$ [M + Na]$^+$ m/z=589.2597, found 589.2586.

*4.8. 6-Chloro-9-[3′,5′-O-(tetraisopropyldisiloxane)-2′-O,4′-C-(N-methylaminomethylene)-β-D-ribofuranosyl]purine (**10**)*

A mixture of **4** (1.50 mmol, 874 mg), 6-chloropurine (1.95 mmol, 301 mg), and BSA (5.85 mmol, 1.43 mL) in toluene (15 mL) was heated at 100 °C. After the mixture became clear, TMSOTf (1.95 mmol, 0.35 mL) was added, and the reaction mixture was stirred at 100 °C for a further 30 min. The reaction mixture was cooled, diluted with ethyl acetate (50 mL), and washed with saturated aqueous sodium hydrogen carbonate solution, water, and saturated brine (50 mL each). The combined aqueous phases were back-extracted with ethyl acetate. The resulting organic layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography (0 to 30% AcOEt in hexane) to afford **10** (631 mg, 74%) as a white foam; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.75 (s, 1H), 8.63 (s, 1H), 6.70 (s, 1H), 4.90 (d, J=3.4 Hz, 1H), 4.51 (d, J=3.4 Hz, 1H), 4.02 (d, J=13.2 Hz, 1H), 3.69 (d, J=13.2 Hz, 1H), 2.90 – 2.77 (m, 2H), 2.71 (s, 3H), 1.10 – 0.90 (m, 28H); ^{13}C NMR(75 MHz, DMSO-d_6) δ 151.46, 150.7, 149.22, 145.16, 131.55, 84.54, 82.59, 79.35, 64.92, 60.07, 57.24, 45.35, 17.17, 17.08, 17.01, 16.95, 16.86, 16.81, 12.67, 12.21, 12.01, 11.85; HRMS (MALDI-TOF) calc. for $C_{24}H_{40}ClN_5NaO_5Si_2$ [M + Na]$^+$ m/z=592.2154, found 592.2152.

*4.9. 3′,5′-O-(Tetraisopropyldisiloxane)-2′-O,4′-C-(N-methylaminomethylene)inosine (**11**)*

A mixture of hypoxanthine (3.50 g, 25.74 mmol), BSA (12.59 mL, 51.47 mmol), and TMSOTf (0.934 mL, 5.15 mmol) in toluene (50.0 mL) was stirred at 80 °C for 40 min under an argon atmosphere. **4** (10.00 g, 17.16 mmol) and TMSOTf (3.11 mL, 17.16 mmol) were added to the solution, and the mixture was stirred at 80 °C for 3 h. After cooling (~25 °C), the mixture was diluted with ethyl acetate (50 mL) and quenched with saturated sodium hydrogen carbonate solution (50 mL, temperature was ~35 °C). After stirring for 10 min, the precipitate was collected by filtration, washed with water and ethyl acetate, and dried *in vacuo* to afford **11** (7.30 g, 13.23 mmol, 77%) as a colorless solid. This solid contains ~10% of *N*^{7}-isomer of compound **11**; HRMS (MALDI-TOF) calc. for $C_{24}H_{41}N_5NaO_6Si_2$ [M + Na]$^+$ m/z=574.2488, found 574.2483.

*4.10. N^2-Acetyl-2′-O,4′-C-(N-methylaminomethylene)guanosine(**12**)*

Guanine (775 mg, 5.13 mmol) was suspended in BSA (10 mL, 40.9 mmol) and toluene (10 mL), and the resulting suspension was heated to 140 °C and refluxed for 1 h. TMSOTf (60 μL, 0.33 mmol) was added to the aforementioned suspension, and the resulting suspension was refluxed until it became transparent.

The solution after refluxing was cooled to 100 °C (the temperature of oil bath), **4** (1.0 g, 1.71 mmol) and TMSOTf (300 μL, 1.65 mmol) were added, and the resulting solution was reacted for 30 min. The resulting reaction mixture was poured into a mixed solution (130 mL) of pyridine-methanol-water-triethylamine (100:10:20:1, v/v/v/v). The resulting suspension was stirred at room temperature for 30 min. The suspension after stirring was concentrated and dried by co-evaporation with dry pyridine (three times) to yield a residue. Dry pyridine (30 mL), acetic anhydride (20 mL), and 1-methylimidazole (50 μL) were added to the obtained residue, and the reaction mixture was stirred with heating at 70 °C for 4 h. The reaction mixture was cooled to room temperature, water (100 mL) was added, and the mixture was stirred for 30 min. To the solution after stirring, 25% aqueous ammonia (30 mL) was added, and the mixture was stirred for 15 min. The resulting solution was concentrated under reduced pressure to yield a residue. Ethyl acetate (200 mL) was added to the residue, and the resulting solution was washed with saturated brine–water (1:1, v/v). The resulting solution was separated to yield an organic layer. The resulting organic layer was concentrated, and the residue was purified by silica gel column chromatography (hexane–ethyl acetate=85:15, v/v) to afford **12** (680 mg, 65% (2 steps)) as a white foam; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.10 (s, 1H), 11.88 (s, 1H), 7.90 (s, 1H), 6.44 (s, 1H), 4.59 (d, *J*=3.3 Hz, 1H), 4.20 (d, *J*=2.9 Hz, 1H), 4.05 (d, *J*=12.8 Hz, 1H), 3.69 (d, *J*=12.8 Hz, 1H), 2.80 (s, 2H), 2.67 (s, 3H), 2.18 (s, 3H), 1.09 – 0.91 (m, 28H); ^{13}C NMR(150 MHz, DMSO-d_6) δ 173.67, 154.8, 148.18, 147.74, 135.53, 120.59, 83.72, 82.45, 79.95, 64.55, 59.99, 57.27, 45.47, 23.75, 17.29, 17.2, 17.15, 17.12, 17.07, 17.03, 16.97, 16.84, 12.66, 12.26, 12.24, 11.92; HRMS (MALDI-TOF) calc. for $C_{26}H_{44}N_6NaO_7Si_2$ $[M + Na]^+$ m/z=631.2702, found 631.2702.

*4.11. 2′-O,4′-C-(N-methylaminomethylene)inosine (**13**)*

To a suspension of **11** in DMF(dry) (9.31 mL), triethylamine (0.113 mL, 0.81 mmol) and triethylamine trihydrofluoride (0.197 mL, 1.21 mmol) were added. The mixture was stirred at room temperature for 16 h and concentrated under reduced pressure. The residue was crystallized from 2-propanol and the solvent was removed under reduced pressure to yield a crude solid. The solid was recrystallized with 9% aq 2-PrOH (4.5 mL) at 60 °C to yield 2′-*O*,4′-*C*-(*N*-methylaminomethylene)inosine (**13**, 300 mg, 0.974 mmol, 80%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.73 – 11.95 (m, 1H), 8.23 (s, 1H), 8.07 (s, 1H), 6.50 (s, 1H), 5.59 – 5.28 (m, 1H), 5.24 – 4.90 (m, 1H), 4.35 (d, *J*=3.0 Hz, 1H), 4.06 (d, *J*=3.0 Hz, 1H), 3.60 (d, *J*=3.4 Hz, 2H), 2.82 (s, 2H), 2.69 (s, 3H); ^{13}C NMR(75 MHz, DMSO-d_6) δ 156.57, 147.29, 145.92, 137.6, 124.44, 83.88, 83.03, 80.81, 64.24, 60.03, 56.97, 45.25; HRMS (MALDI-TOF) calc. for $C_{12}H_{15}N_5NaO_5$ $[M + Na]^+$ m/z=332.0971, found 332.0965.

*4.12. N^6-Benzoyl-5′-O-(4,4′-dimethoxytriphenyl)methyl-2′-O,4′-C-(N-methylaminomethylene)adenosine (**14**)*

Compound **5** (4.3 g, 6.57 mmol) was dissolved in THF (40 mL), triethylamine (1.61 mL, 9.85 mmol) and triethylamine trihydrofluoride (1.83 mL, 13.1 mmol) were added, and the mixture was stirred for 2 h at 50 °C. Diisopropylether (40 mL) was added to the reaction mixture, followed by stirring for 0.5 h at 0 °C to form a precipitate. The precipitate was collected by filtration and dried by co-evaporation with dry pyridine. The residue was dissolved in pyridine (60 mL). To the mixture, 4,4′-dimethoxytrityl chloride (DMTr-Cl, 2.56 g, 7.57 mmol) was added, and the mixture was stirred at room temperature overnight.The mixture was concentrated to 1/3 of its volume. The residue was diluted with ethyl acetate–THF (80 mL, 1:1 (v/v)) and washed with sodium hydrogen carbonate solution (80 mL, twice) and brine (80 mL). The resulting organic layer was dried over anhydrous magnesium sulfate and concentrated. The residue was purified by silica gel column chromatography (hexane–ethyl acetate, 40–80%, (v/v)) to afford compound **14** (4.3 g, 92% (2 steps)) as a white foam; ^{1}H NMR (300 MHz, $CDCL_3$) δ 9.19 (s, 1H), 8.80 (s, 1H), 8.33 (s, 1H), 8.01 (d, *J*=7.5 Hz, 2H), 7.62 – 7.16 (m, 12H), 6.89 – 6.77 (m, 5H), 4.68 (d, *J*=3.0 Hz, 1H), 4.41 (d, *J*=4.1 Hz, 1H), 3.78 (s, 6H), 3.39 (s, 2H), 3.07 – 2.83 (m, 3H), 2.78 (s, 3H); ^{13}C NMR(75 MHz, $CDCL_3$) δ 164.59, 158.6, 158.58, 152.75, 150.76, 149.52, 144.31, 141.17, 135.54, 135.32, 133.62, 132.72, 130.03, 129.97, 128.8, 128.05, 127.94, 127.84, 126.99, 123.52, 113.25, 86.5, 84.84, 82.83, 80.93, 66.73, 62.63, 58.5, 55.19, 45.63; HRMS (MALDI-TOF) calc. for $C_{40}H_{39}N_6O_7$ $[M + H]^+$ m/z=715.2875, found 715.2880.

*4.13. N^2-Acetyl-5′-O-(4,4′-dimethoxytriphenyl)methyl-2′-O,4′-C-(N-methylaminomethylene)guanosine(**15**)*

Compound **12** (1.5 g, 2.5 mmol) was dissolved in THF (25 mL), triethylamine (700 μL, 5 mmol) and TEA·3HF (triethylamine trihydrofluoride, 410 μL, 2.5 mmol) were added, and the mixture was stirred at 50 °C for 2 h. After stirring, THF was evaporated under reduced pressure, and diisopropyl ether (50 mL) was added to form a precipitate. The precipitate was collected by filtration. The resulting compound **15** was dried by co-evaporation with dry pyridine (three times), and dissolved in pyridine (10 mL). DMTr-Cl (1.3 g, 3.75 mmol) was then added, and the mixture was stirred overnight at room temperature. Water (20 mL) was added to the reaction mixture to quench the reaction. The target substance was extracted with ethyl acetate (200 mL). The ethyl acetate layer was successively washed with water (100 mL), saturated sodium hydrogen carbonate (100 mL), and saturated brine (100 mL), and concentrated. The residue was purified by diol-silica gel column chromatography (hexane–ethyl acetate=1:9, v/v) to afford **15** (1 g, yield 60%, 2 steps) as white foam; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.20 – 11.67 (m, 2H), 8.03 (s, 1H), 7.49 – 7.13 (m, 9H), 6.97 – 6.75 (m, 4H), 6.48 (s, 1H), 5.51 (d, *J*=5.3 Hz, 1H), 4.43 (d, *J*=3.0 Hz, 1H), 4.15 (br. s., 1H), 3.74 (s, 6H), 3.32 – 3.27 (m, 1H), 3.16 (d, *J*=10.5 Hz, 1H), 2.98 (d, *J*=11.3 Hz, 1H), 2.78 (d, *J*=11.3 Hz, 1H), 2.67 (s, 3H), 2.19 (s, 3H); ^{13}C NMR(75 MHz, DMSO-d_6) δ 173.6, 158.1, 154.8, 148.06, 147.85, 144.7, 136.47, 135.42, 135.15, 129.77, 129.71, 127.82, 127.68, 126.74, 120.48, 113.17, 85.45, 83.6, 82.06, 80.37, 65.37, 62.57, 57.13, 55, 45.2, 23.71; HRMS (MALDI-TOF) calc. for $C_{35}H_{36}N_6NaO_8$ $[M + Na]^+$ m/z=691.2487, found 691.2488.

*4.14. 5′-O-(4,4′-Dimethoxytriphenyl)methyl-2′-O,4′-C-(N-methylaminomethylene)inosine(**16**)*

Compound **13** was co-evaporated twice with dry pyridine and dissolved in pyridine (dry) (4.9 mL). To the solution, DMTr-Cl (362 mg, 1.07 mmol) was added, and the mixture was stirred at room temperature for 20 h. The solvent was removed under reduced pressure, and the residue was mixed with ethyl acetate and sodium hydrogen carbonate solution. The organic layer was purified by column chromatography (silica gel, eluted with 0%–10% methanol in ethyl acetate) to afford **16** (514 mg, 0.840 mmol, 87% (70%, 2 steps from **11**)) as a colorless foam; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.40 (br. s., 1H), 8.13 (s, 1H), 8.09 (s, 1H), 7.45 – 7.18 (m, 9H), 6.92 – 6.81 (m, 4H), 6.58 (s, 1H), 5.49 (d, *J*=3.4 Hz, 1H), 4.45 (d, *J*=3.0 Hz, 1H), 4.23 (br. s., 1H), 3.74 (s, 6H), 3.29 – 3.09 (m, *J*=10.9 Hz, 2H), 2.99 (d, *J*=11.3 Hz, 1H), 2.78 (d, *J*=11.3 Hz, 1H), 2.69 (s, 3H); ^{13}C NMR(75 MHz, DMSO-d_6) δ 158.09, 156.57, 147.4, 145.95, 144.72, 137.53, 135.45, 135.2, 129.75, 129.69, 127.8, 127.67, 126.71, 124.52, 113.16, 85.39, 83.96, 82.12, 80.48, 65.44, 62.58,

57.08, 55, 45.14; HRMS (MALDI-TOF) calc. for $C_{33}H_{33}N_5NaO_7$ $[M + Na]^+$ m/z=634.2272, found 634.2271.

*4.15. N^6-Benzoyl-3′-O-[2-cyanoethoxy(diisopropylamino)phosphino]-5′-O-(4,4′-dimethoxytriphenyl)methyl-2′-O,4′-C-(N-methylaminomethylene)adenosine (**17**)*

Compound **14** (410 mg, 0.57 mmol) was dried by co-evaporation (twice) with anhydrous acetonitrile and dissolved in anhydrous acetonitrile (2.5 mL). *N*,*N*,*N*′,*N*′-Tetraisopropyl-2-cyanoethylphosphorodiamidite (200 μL, 0.63 mmol) and 4,5-dicyanoimidazole (DCI, 71 mg, 0.6 mmol) were successively added. The mixture was stirred at room temperature for 2 h, and the reaction mixture was diluted with ethyl acetate (20 mL). Saturated aqueous sodium hydrogen carbonate solution (10 mL) was added to quench the reaction. The organic layer was washed with saturated aqueous sodium hydrogen carbonate solution (10 mL), dried over anhydrous sodium sulfate, and concentrated. The residue was purified by silica gel column chromatography (diol-silica gel, hexane-acetone) to afford **17** (480 mg, 0.52 mmol, 91%) as a white foam; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.24 (br. s., 1H), 8.78 – 8.70 (m, 1H), 8.60 – 8.49 (m, 1H), 8.09 – 7.98 (m, 2H), 7.69 – 7.50 (m, 3H), 7.42 – 7.11 (m, 10H), 6.90 – 6.76 (m, 5H), 4.97 – 4.82 (m, 2H), 3.76 – 3.67 (m, 6H), 3.62 – 3.47 (m, 3H), 3.31 – 3.20 (m, 3H), 3.14 – 2.94 (m, 1H), 2.77 – 2.66 (m, 4H), 2.56 (dt, *J*=2.3, 5.9 Hz, 1H), 1.15 – 0.93 (m, 12H); ^{31}P NMR(121 MHz, DMSO-d_6) δ 148.32, 147.63; HRMS (MALDI-TOF) calc. for $C_{49}H_{56}N_8O_8P$ $[M + H]^+$ m/z=915.3953, found 915.3948.

*4.16. N^2-Acetyl-3′-O-[2-cyanoethoxy(diisopropylamino)phosphino]-5′-O-(4,4′-dimethoxytriphenyl)methyl-2′-O,4′-C-(N-methylaminomethylene)guanosine (**18**)*

Compound **15** (870 mg, 1.3 mmol) was dried by co-evaporation (three times) with dry acetonitrile, and dissolved in DMF (9 mL). *N*,*N*,*N*′,*N*′-tetraisopropyl-2-cyanoethylphosphorodiamidite (784 mg, 2.6 mmol) and 1-methylimidazole (51.8 μL, 0.65 mmol) were added, and then 1*H*-tetrazole (91 mg, 1.3 mmol) was added. The mixture was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (40 mL), and washed twice with saturated brine (40 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. The residue was purified by diol-silica gel column chromatography (0.5% triethylamine containing hexane-ethyl acetate) to afford **18** (1.0 g, 1.15 mmol, 88%) as a white foam; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.93 (br. s., 2H), 8.13 – 7.93 (m, 1H), 7.49 – 7.19 (m, 9H), 6.96 – 6.78 (m, 4H), 6.59 – 6.50 (m, 1H), 4.69 – 4.59 (m, 1H), 4.44 – 4.23 (m, 1H), 3.79 – 3.41 (m, 11H), 3.30 – 3.28 (m, 1H), 3.13 – 2.92 (m, 1H), 2.75 – 2.53 (m, 6H), 2.19 (s, 3H), 1.13 – 0.90 (m, 12H); ^{31}P NMR(121 MHz, DMSO-d_6) δ 148.62, 147.49; HRMS (MALDI-TOF) calc. for $C_{44}H_{53}N_8NaO_9P$ $[M + Na]^+$ m/z=891.3571, found 891.3574.

*4.17. 3′-O-[2-Cyanoethoxy(diisopropylamino)phosphino]-5′-O-(4,4′-dimethoxytriphenyl)methyl-2′-O,4′-C-(N-methylaminomethylene)inosine (**19**)*

Compound **16** (1.22 g, 1.99 mmol) was co-evaporated with toluene (dry) twice and dissolved with DMF(dry) (8 mL). To the solution, 1-methylimidazole (0.159 mL, 1.99 mmol), 1*H*-tetrazole (0.070 g, 1.00 mmol), and *N*,*N*,*N*′,*N*′-tetraisopropyl-2-cyanoethylphosphorodiamidite (0.127 mL, 0.40 mmol) were added. After stirring for 4 h, additional 1-methylimidazole (0.016 mL, 0.20 mmol), 1*H*-tetrazole (6.99 mg, 0.10 mmol), and *N*,*N*,*N*′,*N*′-tetraisopropyl-2-cyanoethylphosphorodiamidite (0.127 mL, 0.40 mmol) were added to the solution. The mixture was stirred at room temperature under a dry atmosphere ($CaCl_2$ tube) for overnight. The mixture was poured into saturated sodium hydrogen carbonate aq. and extracted with ethyl acetate. The organic layer was separated, washed with brine, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluted with 0%–50% ethyl acetate (+0.5% triethylamine) in hexane (+0.5% triethylamine)) and (diol silica gel. eluted with 0%–30% acetone (0.5% triethylamine) in hexane (+0.5% triethylamine)) to afford **19** (1.2 g, 1.5 mmol, 74%) as a colorless oil. **19** was precipitated from ethyl acetate (0.6 mL)–hexane (25 mL) to afford a white solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.61 – 12.15 (m, 1H), 8.26 – 7.90 (m, 2H), 7.44 – 7.34 (m, 2H), 7.34 – 7.15 (m, 7H), 6.86 (dt, *J*=1.7, 8.8 Hz, 4H), 6.68 – 6.60 (m, *J*=5.7 Hz, 1H), 4.74 – 4.66 (m, *J*=7.0 Hz, 1H), 4.63 – 4.49 (m, 1H), 3.73 (d, *J*=3.2 Hz, 7H), 3.59 – 3.45 (m, 3H), 3.30 – 3.20 (m, 2H), 3.11 – 2.92 (m, 1H), 2.74 – 2.62 (m, 5H), 2.58 – 2.53 (m, 1H), 1.13 – 0.90 (m, 12H); ^{31}P NMR(121 MHz, DMSO-d_6) δ 148.36, 147.47; HRMS (MALDI-TOF) calc. for $C_{42}H_{50}N_7NaO_8P$ $[M + Na]^+$ m/z=834.3351, found 834.3347.

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Supplementary Material

Supporting information; ^{1}H and ^{13}C NMR spectra of new compounds, LC-MS analysis data for the reaction mixture entry 2 of Table 1 and MALDI-TOF MS data for ODNs.