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A bridged nucleic acid, 2′,4′-BNA^{COC}: synthesis of fully modified oligonucleotides bearing thymine, 5-methylcytosine, adenine and guanine 2′,4′-BNA^{COC} monomers and RNA-selective nucleic-acid recognition

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ABSTRACT

Recently, we synthesized pyrimidine derivatives of the 2′-*O*,4′-*C*-methylenoxymethylene-bridged nucleic-acid (2′,4′-BNA^{COC}) monomer, the sugar conformation of which is restricted in N-type conformation by a seven-membered bridged structure. Oligonucleotides (BNA^{COC}) containing this monomer show high affinity with complementary single-stranded RNA and significant resistance to nuclease degradation. Here, BNA^{COC} consisting of 2′,4′-BNA^{COC} monomers bearing all four bases, namely thymine, 5-methylcytosine, adenine and guanine was efficiently synthesized and properties of duplexes containing the 2′,4′-BNA^{COC} monomers were investigated by UV melting experiments and circular dichroism (CD) spectroscopy. The UV melting curve analyses showed that the BNA^{COC}/BNA^{COC} duplex possessed excellent thermal stability and that the BNA^{COC} increased thermal stability with a complementary RNA strand. On the other hand, BNA^{COC}/DNA heteroduplexes showed almost the same thermal stability as RNA/DNA heteroduplexes. Furthermore, mismatched sequence studies showed that BNA^{COC} generally improved the sequence selectivity with Watson–Crick base-pairing compared to the corresponding natural DNA and RNA. A CD spectroscopic analysis indicated that the BNA^{COC} formed duplexes with complementary DNA and RNA in a manner similar to natural RNA.

INTRODUCTION

Antisense oligonucleotides are now attracting interest for their potential to be developed as a new class of drugs for treatment of inveterate diseases such as cancer and viral diseases. For practical application of antisense methodology, it is essential to develop modified oligonucleotides, which strongly interact with single-stranded RNA (ssRNA) in a sequence-specific manner (1–3). The sugar moiety of natural nucleosides and single-stranded oligonucleotides exists in an individual equilibrium mixture between S-type and N-type conformations. However, it is well known that the B-form DNA duplex possesses the S-type sugar conformation, and that a range of N-type sugar conformation (pseudorotation phase angle, $0 \leq P \leq 36^{\circ}$) are adapted to the A-form RNA duplex structure (Figure 1) (4–6). Therefore, modified oligonucleotides, which have sugar moiety restricted to the S-type or N-type conformations in advance, are expected to have high binding affinity with complementary ssDNA or ssRNA respectively. We have so far developed various kinds of bridged nucleic acids (BNAs) (7–19), the sugar conformation of which is restricted or locked by introduction of an additional bridged structure to the furanose skeleton. It has been observed that 2′,4′-BNA (9,10)/LNA [The 2′,4′-BNA was independently synthesized by the group of Wengel *et al*. immediately after our first report (9), and it is called a locked nucleic acid (LNA). See refs (3,20–22)] (Figure 2), which is a BNAs with its sugar moiety fixed to an N-type conformation by five-membered ring, prominently hybridizes to ssRNA targets. An X-ray crystallographic analysis of 2′,4′-BNA showed that the maximum out-of-plane (ν_{max}) value was 57°, and

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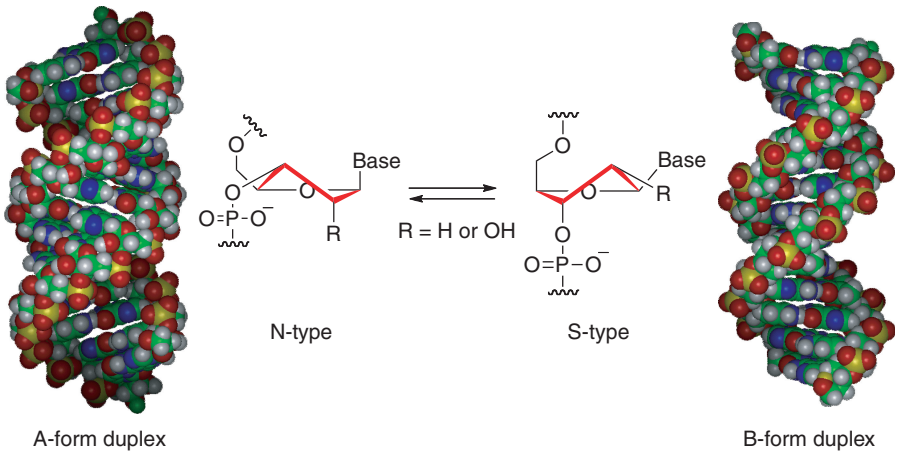


Figure 1. Sugar conformations and helix structures of double-stranded nucleic acids.

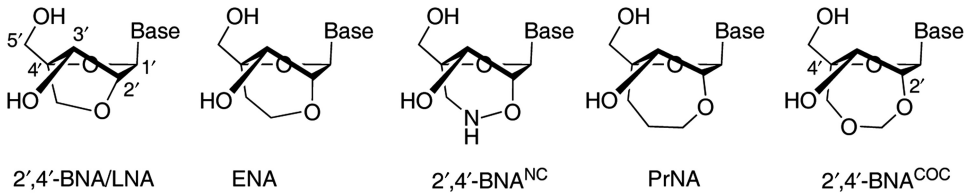


Figure 2. Structures of 2′,4′-BNA/LNA, ENA, 2′,4′-BNA^{NC}, PrNA and 2′,4′-BNA^{COC} monomers.

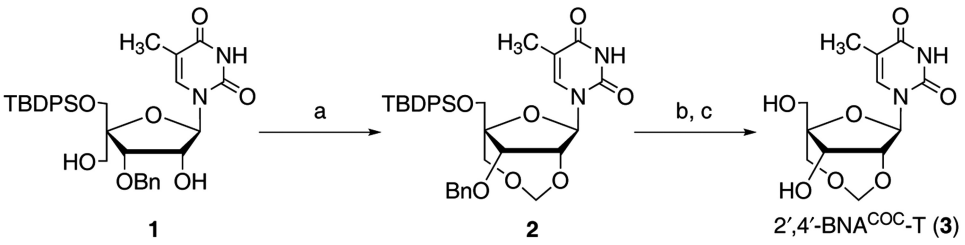
that this value is larger than an adapted value ($\nu_{max} = 38.6° \pm 3°$) to natural A-form RNA duplex (4–6), so the N-type nature of 2′,4′-BNA was emphasized due to the restriction of the sugar moiety by the small five-membered ring. Other nucleic-acid analogues with a different type of bridged structure between the 2′- and 4′-positions, which have six- and seven-membered ring, have been reported (16–19,23–29). In one of these studies, we described the synthesis and properties of 2′-*O*,4′-*C*-aminomethylene BNA (2′,4′-BNA^{NC}) (Figure 2), and showed that oligonucleotides containing 2′,4′-BNA^{NC} have high hybridizing affinity with RNA compliments (16–19). X-ray crystallographic analysis revealed that the *P* and ν_{max} values of 2′,4′-BNA^{NC}[NMe] were 23° and 49°, respectively (19). These values indicated that the conformation of 2′,4′-BNA^{NC}[NMe] is restricted to the N-type conformation as seen in natural A-form RNA duplex. This suggested that the sugar conformation restricted by the bridged structure between the 2′- and 4′-positions approximated to the adapted sugar conformation of the natural A-form RNA duplex because of increased ring size. Recently, we designed 2′,4′-BNA^{COC}, bearing a seven-membered bridged structure, and successfully synthesized the 2′,4′-BNA^{COC} monomers having pyrimidine nucleobases (Figure 2) (30). The oligonucleotides containing these monomers show high affinity with complementary ssRNA. An X-ray crystallographic analysis of 2′,4′-BNA^{COC} bearing thymine showed that the *P* and ν_{max} values were 17° and 38°, respectively. This revealed that the sugar conformation of 2′,4′-BNA^{COC}, which is restricted by a large seven-membered ring, is identical with the N-type sugar puckering fit to canonical A-form RNA duplex, and that 2′,4′-BNA^{COC} has the closest sugar conformation among nucleic-acid analogues with a different type of bridged structure between the 2′- and 4′-positions.

It is of great interest to synthesize 2′,4′-BNA^{COC} bearing a purine nucleobase for potential use in a wide variety of applications and to investigate the conformations of oligonucleotides containing 2′,4′-BNA^{COC}. Moreover, conformational analysis of duplex, which is formed from fully modified oligonucleotides consisted of 2′,4′-BNA^{COC}, is very fascinating. In general, a purine glycosidic linkage is more acid-labile than a pyrimidine glycosidic linkage (31). Therefore, it is readily expected that the acidic conditions used for the synthesis of 2′,4′-BNA^{COC} with a pyrimidine nucleobase are not suitable for the purine congener (Scheme 1). Here, we report the synthesis of 2′,4′-BNA^{COC} monomers having a purine nucleobase *via* formation of a COC linkage under mild conditions, and we describe the properties of the corresponding oligonucleotide derivatives.

MATERIALS AND METHODS

General aspects and instrumentation

All reactions were performed under an atmosphere of nitrogen. Unless otherwise mentioned, all chemicals from commercial sources were used without further purification. Acetonitrile (MeCN), dichloromethane (CH_2Cl_2), dichloroethane (DCE), triethylamine and pyridine were distilled from CaH_2. Tetrahydrofuran (THF) was distilled from CaH_2 followed by $LiAlH_4$ just



Scheme 1. Reagents and conditions: (**a**) *p*-TsOH·H_2O, $(CH_2O)_n$, DCE, reflux, 3 h, 81%; (**b**) TBAF, THF, rt, 3 h, 91%; (**c**) 20% $Pd(OH)_2$-C, cyclohexene, EtOH, reflux, 3 h, 89%.

before use. All melting points were measured with a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H NMR (270 or 300 MHz), ^{13}C NMR (67.8 or 75.5 MHz) and ^{31}P NMR (202.4 MHz) spectra were recorded on JEOL EX-270, JEOL-AL-300 and JEOL GX-500 spectrometers, respectively. Chemical shifts are reported in parts per million downfield from tetramethylsilane or deuterated solvent as internal standard for ^{1}H and ^{13}C spectra, and 85% H_3PO_4 as external standard for ^{31}P spectra. IR spectra were recorded on a JASCO FT/IR-200 spectrometer. Optical rotations were recorded on a JASCO DIP-370 instrument. Mass spectra were measured on JEOL JMS-600 or JMS-700 mass spectrometers. Column chromatography was carried out using Fuji Silysia PSQ100B or FL-100D. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectra were recorded on Bruker Daltonics® Autoflex II TOF/TOF instruments.

4′-*C*-Acetoxymethyl-2′-*O*-acetyl-6-*N*-benzoyl-3′-*O*-benzyl-5′-*O*-*tert*-butyldiphenylsilyladenosine (5)

Trimethylsilyl chloride (TMSCl) (0.5 ml) was added to a suspension of N^6-benzoyladenine (2.17 g, 9.07 mmol) in anhydrous hexamethyldisilazane (HMDS) (35 ml) at room temperature and the mixture was refluxed for 24 h. After the resulting clear solution was evaporated under reduced pressure, to the residue a solution of compound **4** (30) (4.80 g, 7.56 mmol) in anhydrous DCE (25 ml) and trimethylsilyl trifluorimethanesulfonate (TMSOTf) (0.14 ml, 0.756 mmol) were added and the mixture was refluxed for 11 h. After addition of saturated aqueous $NaHCO_3$ solution at 0°C, the mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (3:4→1:2, v/v)] to give compound 5 (4.68 g, 76%). White powder; m.p. 71–73°C. $[\alpha]_D^{26}$ − 6.80 (*c* 1.03, $CHCl_3$). IR ν_{max} (KBr): 3065, 2939, 2862, 1744, 1703, 1607, 1458, 1368, 1235, 1106 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.06 (9H, s), 1.97 (3H, s), 2.05 (3H, s), 3.81(1H, d, *J* = 11 Hz), 3.96 (1H, d, *J* = 11 Hz), 4.30 (1H, d, *J* = 12 Hz), 4.66 (1H, d, *J* = 12 Hz), 4.62 (2H, s), 4.91 (1H, d, *J* = 6 Hz), 6.08 (1H, t, *J* = 6 Hz), 6.25 (1H, d, *J* = 5 Hz), 7.31–7.66 (18H, m), 8.01 (2H, d, *J* = 7 Hz), 8.09 (1H, s), 8.59 (1H, s), 9.04 (1H, brs). ^{13}C NMR ($CDCl_3$): δ 19.3, 20.7, 20.9, 26.9, 26.9, 26.9, 62.7, 64.0, 74.1, 74.7, 78.1, 86.6, 87.0, 123.4, 127.7, 127.7, 127.7, 127.7, 127.7, 127.7, 127.8, 127.8, 128.0, 128.4, 128.4, 128.7, 128.7, 129.8, 129.8, 132.3, 132.4, 132.6, 133.4, 135.4, 135.4, 135.4, 135.4, 136.9, 142.1, 149.4, 151.2, 152.4, 164.4, 169.7, 170.4. Mass (FAB): *m/z* 814 (MH^+). Anal. Calcd for $C_{45}H_{47}N_5O_8Si·1/2H_2O$: C, 65.67; H, 5.88; N, 8.51. Found: C, 65.44; H, 5.71; N, 8.42.

6-*N*-Benzoyl-3′-*O*-benzyl-5′-*O*-*tert*-butyldiphenylsilyl-4′-*C*-(hydroxymethyl)adenosine (6)

K_2CO_3 (98 mg, 0.712 mmol) was added to a solution of compound **5** (193 mg, 0.237 mmol) in MeOH (3.4 ml) at 0°C, and the mixture was stirred at the same temperature for 1.5 h. After neutralization with a 10% aqueous HCl solution (0.26 ml), the mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (1:3, v/v)] to give compound **6** (170 mg, 98%). White powder; m.p. 88–90°C. $[\alpha]_D^{26}$ + 4.43 (*c* 1.43, $CHCl_3$). IR ν_{max} (KBr): 3287, 3062, 2937, 2862, 1702, 1612, 1461, 1328, 1253, 1109 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.00 (9H, s), 2.57 (1H, dd, *J* = 5, 7 Hz), 3.71 (1H, d, *J* = 11 Hz), 3.76 (1H, d, *J* = 11 Hz), 3.86 (1H, dd, *J* = 7, 12 Hz), 4.02 (1H, dd, *J* = 5, 12 Hz), 4.52 (1H, d, *J* = 8 Hz), 4.66 (1H, d, *J* = 6 Hz), 4.68 (1H, d, *J* = 11 Hz), 4.85 (1H, d, *J* = 11 Hz), 4.90 (1H, m), 6.10 (1H, d, *J* = 4 Hz), 7.22–7.44 (11H, m), 7.50–7.65 (7H, m), 8.02 (2H, d, *J* = 7 Hz), 8.08 (1H, s), 8.64 (1H, s), 8.99 (1H, brs). ^{13}C NMR ($CDCl_3$): δ 19.3, 26.9, 26.9, 26.9, 63.0, 65.8, 73.8, 74.2, 78.7, 89.3, 91.0, 123.1, 127.6, 127.6, 127.7, 127.7, 127.7, 128.0, 128.0, 128.2, 128.2, 128.5, 128.5, 128.7, 128.7, 129.8, 129.8, 132.2, 132.5, 132.7, 133.4, 135.3, 135.3, 135.4, 135.4, 137.0, 142.0, 149.4, 150.7, 152.3, 164.4. Mass (FAB): *m/z* 730 (MH^+). Anal. Calcd for $C_{41}H_{43}N_5O_6Si·3/2H_2O$: C, 65.06; H, 6.13; N, 9.25. Found: C, 65.30; H, 5.86; N, 9.19.

4′-*C*-Acetoxymethyl-2′-*O*-acetyl-6-*N*-benzoyl-3′-*O*-benzyl-6-*N*-benzyloxymethyl-5′-*O*-*tert*-butyldiphenylsilyladenosine (8)

DBU (0.39 ml, 2.58 mmol) was added to a solution of compound **5** (1.05 g, 1.29 mmol) in anhydrous DMF (6.4 ml) at 0°C and the mixture was stirred for 10 min. Benzyloxymethyl chloride (BOMCl) (0.27 ml, 1.93 mmol) was added to the reaction mixture at 0°C and the mixture was stirred at the same temperature for 1 h. After addition of water at 0°C, the mixture was extracted with Et_2O. The organic extracts were washed with 0.5 M aqueous $KHSO_4$ solution, water and brine, dried over $MgSO_4$,

and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (2:1, v/v)] to give compound **8** (769 mg, 64%). White foam. $[\alpha]_D^{26} - 16.4$ (*c* 1.23, $CHCl_3$). IR ν_{max} (KBr): 3064, 2939, 2863, 2251, 1962, 1894, 1745, 1680, 1581, 1456, 1366, 1227, 1105 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.04 (9H, s), 1.96 (3H, s), 2.03 (3H, s), 3.79 (1H, d, $J = 11$ Hz), 3.92 (1H, d, $J = 11$ Hz), 4.28 (1H, d, $J = 12$ Hz), 4.62 (1H, d, $J = 12$ Hz), 4.59 (2H, s), 4.76 (2H, s), 4.87 (1H, d, $J = 6$ Hz), 5.87 (2H, s), 5.98 (1H, t, $J = 6$ Hz), 6.18 (1H, d, $J = 5$ Hz), 7.14–7.46 (19H, m), 7.50 (2H, d, $J = 7$ Hz), 7.60-7.64 (4H, m), 7.99 (1H, s), 8.38 (1H, s). ^{13}C NMR ($CDCl_3$): δ 19.3, 20.7, 20.9, 26.9, 26.9, 26.9, 62.8, 63.9, 71.3, 74.0, 74.7, 76.7, 78.0, 86.5, 87.0, 127.1, 127.5, 127.5, 127.7, 127.7, 127.7, 127.8, 127.8, 127.8, 127.9, 127.9, 128.1, 128.1, 128.1, 128.1, 128.4, 128.4, 128.7, 128.7, 129.9, 129.9, 131.0, 132.3, 132.4, 135.3, 135.4, 135.4, 135.5, 135.5, 136.9, 137.6, 142.8, 151.9, 152.1, 153.2, 169.7, 170.4, 172.2. Mass (FAB): *m/z* 934 (MH^+). Anal. Calcd for $C_{53}H_{55}N_5O_9Si \cdot 1/3H_2O$: C, 67.71; H, 5.97; N, 7.45. Found: C, 67.64; H, 5.88; N, 7.40.

6-*N*-Benzoyl-3′-*O*-benzyl-6-*N*-benzyloxymethyl-5′-*O*-*tert*-butyldiphenylsilyl-4′-*C*-(hydroxymethyl)adenosine (9)

K_2CO_3 (1.61 g, 11.6 mmol) was added to a solution of compound **8** (3.62 g, 3.88 mmol) in MeOH (80 ml) at 0°C and the mixture was stirred for 50 min. After neutralization with a 10% aqueous HCl solution (4.3 ml), the mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (3:2 to 1:1, v/v)] to give compound **9** (3.21 g, 97%). White powder; m.p. 54–57°C. $[\alpha]_D^{26} - 1.94$ (*c* 1.23, $CHCl_3$). IR ν_{max} (KBr): 3326, 3064, 2940, 2864, 2247, 1962, 1894, 1820, 1677, 1581, 1458, 1281, 1210, 1070 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.99 (9H, s), 2.51 (1H, dd, $J = 5$, 7 Hz), 3.70 (2H, s), 3.80 (1H, dd, $J = 7$, 12 Hz), 3.97 (1H, dd, $J = 5$, 12 Hz), 4.47 (1H, d, $J = 9$ Hz), 4.58 (1H, d, $J = 6$ Hz), 4.63 (1H, d, $J = 11$ Hz), 4.80 (1H, d, $J = 11$ Hz), 4.77 (1H, m), 4.78 (2H, s), 5.88 (2H, s), 6.04 (1H, d, $J = 4$ Hz), 7.10-7.44 (19H, m), 7.48–7.56 (6H, m), 8.00 (1H, s), 8.43 (1H, s). ^{13}C NMR ($CDCl_3$): δ 19.3, 26.9, 26.9, 26.9, 63.2, 65.7, 71.3, 73.9, 74.2, 76.7, 78.7, 89.1, 90.9, 127.1, 127.5, 127.5, 127.7, 127.7, 127.7, 127.7, 127.9, 127.9, 128.0, 128.0, 128.1, 128.1, 128.3, 128.3, 128.6, 128.6, 128.7, 128.7, 129.9, 129.9, 131.1, 132.2, 132.4, 135.3, 135.3, 135.3, 135.4, 135.4, 136.9, 137.6, 142.6, 151.6, 151.8, 153.2, 172.2. Mass (FAB): *m/z* 850 (MH^+). Anal. Calcd for $C_{49}H_{51}N_5O_7Si \cdot 3/4H_2O$: C, 68.15; H, 6.13; N, 8.11. Found: C, 68.30; H, 6.06; N, 8.07.

6-*N*-Benzoyl-3′-*O*-benzyl-6-*N*-benzyloxymethyl-5′-*O*-*tert*-butyldiphenylsilyl-2′-*O*,4′-*C*- (methylenoxymethylene) adenosine (10)

N-bromosuccinimide (NBS) (312 mg, 1.75 mmol) was added to a solution of compound **9** (373 mg, 0.438 mmol) in anhydrous dimethylsulfoxide (DMSO) (5.5 ml) at room temperature and the mixture was stirred at 60°C for 1.5 h. After addition of saturated aqueous $NaHCO_3$ solution at 0°C, the mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (3:1, v/v)] to give compound **10** (281 mg, 74%). White foam. $[\alpha]_D^{27} - 3.97$ (*c* 1.01, $CHCl_3$). IR ν_{max} (KBr): 3064, 2955, 2865, 1679, 1580, 1456, 1362, 1281, 1209, 1176, 1117, 1074 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.97 (9H, s), 3.68 (1H, d, $J = 12$ Hz), 3.81 (1H, d, $J = 12$ Hz), 3.72 (1H, d, $J = 12$ Hz), 3.88 (1H, d, $J = 12$ Hz), 4.62 (1H, d, $J = 11$ Hz), 4.81 (1H, d, $J = 11$ Hz), 4.79 (2H, s), 4.83 (1H, d, $J = 6$ Hz), 4.99 (1H, d, $J = 6$ Hz), 5.25 (1H, d, $J = 6$ Hz), 5.40 (1H, d, $J = 6$ Hz), 5.91 (2H, s), 6.42 (1H, s), 7.10-7.42 (19H, m), 7.50 (2H, d, $J = 7$ Hz), 7.54–7.59 (4H, m), 8.14 (1H, s), 8.50 (1H, s). ^{13}C NMR ($CDCl_3$): δ 19.2, 26.8, 26.8, 26.8, 62.7, 71.3, 71.7, 72.8, 75.8, 76.0, 76.7, 89.0, 91.8, 96.0, 127.3, 127.5, 127.5, 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 127.9, 127.9, 127.9, 127.9, 128.1, 128.1, 128.4, 128.4, 128.7, 128.7, 129.8, 129.8, 130.9, 132.2, 132.3, 135.3, 135.3, 135.4, 135.4, 135.4, 136.8, 137.7, 142.6, 151.5, 151.9, 153.1, 172.2. Mass (FAB): *m/z* 862 (MH^+). Anal. Calcd for $C_{50}H_{51}N_5O_7Si \cdot 1/10H_2O$: C, 69.52; H, 5.97; N, 8.11. Found: C, 69.29; H, 5.86; N, 7.96.

3′-*O*-Benzyl-6-*N*-benzyloxymethyl-5′-*O*-*tert*-butyldiphenylsilyl-2′-*O*,4′-*C*-(methylenoxymethylene)adenosine (11)

Twenty-eight percent aqueous NH_3 (1.2 ml) was added to a solution of compound **10** (209 mg, 0.242 mmol) in THF (2.4 ml) at room temperature. The flask was sealed and the mixture was stirred at 50°C for 48 h. The mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (2:1, v/v)] to give compound **11** (186 mg, quant.). White foam. $[\alpha]_D^{27} + 2.62$ (*c* 1.33, $CHCl_3$). IR ν_{max} (KBr): 3266, 3037, 2938, 2246, 1959, 1897, 1615, 1475, 1418, 1357, 1292, 1176, 1116 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.99 (9H, s), 3.69 (1H, d, $J = 11$ Hz), 3.73 (1H, d, $J = 12$ Hz), 3.83 (1H, d, $J = 11$ Hz), 3.90 (1H, d, $J = 12$ Hz), 4.62 (1H, d, $J = 11$ Hz), 4.66 (2H, s), 4.82 (1H, d, $J = 11$ Hz), 4.92 (1H, d, $J = 6$ Hz), 5.04 (1H, d, $J = 6$ Hz), 5.27 (1H, d, $J = 6$ Hz), 5.28 (2H, brd, $J = 7$ Hz), 5.41 (1H, d, $J = 6$ Hz), 6.43 (1H, s), 6.52 (1H, brs), 7.21–7.43 (16H, m), 7.57–7.60 (4H, m), 8.03 (1H, s), 8.38 (1H, s). ^{13}C NMR ($CDCl_3$): δ 19.2, 26.7, 26.7, 26.7, 62.9, 70.0, 71.6, 72.7, 75.9, 76.0, 88.7, 91.8, 95.9, 120.5, 127.5, 127.5, 127.6, 127.6, 127.6, 127.6, 127.7, 127.7, 127.8, 127.8, 128.0, 128.0, 128.2, 128.2, 128.4, 128.4, 129.7, 129.7, 132.2, 132.4, 135.3, 135.3, 135.4, 135.4, 136.9, 137.8, 139.5, 152.7, 154.3. Mass (FAB): *m/z* 758 (MH^+). Anal. Calcd for $C_{43}H_{47}N_5O_6Si$: C, 68.14; H, 6.25; N, 9.24. Found: C, 68.11; H, 6.29; N, 9.10.

3′-*O*-Benzyl-6-*N*-benzyloxymethyl-2′-*O*,4′-*C*-(methylenoxymethylene)adenosine (12)

Tetrabutylammonium fluoride (TBAF) (1.0 M solution in THF, 0.19 ml, 0.19 mmol) was added to a solution of

compound **11** (128 mg, 0.169 mmol) in THF (1.7 ml) at room temperature and the mixture was stirred at room temperature for 3 h. After addition of hexane/AcOEt (1:1, v/v, 2.0 ml) at room temperature, the mixture was evaporated under reduced pressure to one-tenth of its volume. The residue was purified by silica gel column chromatography [hexane/AcOEt (2:5, v/v)] to give compound **12** (80 mg, 91%). White foam. $[\alpha]_D^{27} - 8.40$ (*c* 1.17, $CHCl_3$). IR ν_{max} (KBr): 3286, 2919, 2245, 1959, 1616, 1484, 1358, 1293, 1213, 1176, 1116, 1056 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.21 (1H, brs), 3.69 (1H, d, $J = 12$ Hz), 3.82 (1H, d, $J = 12$ Hz), 3.79 (2H, s), 4.52 (1H, d, $J = 6$ Hz), 4.65 (2H, s), 4.70 (1H, d, $J = 11$ Hz), 4.78 (1H, d, $J = 11$ Hz), 5.25 (1H, d, $J = 6$ Hz), 5.26 (2H, brs), 5.26 (1H, d, $J = 6$ Hz), 5.43 (1H, d, $J = 6$ Hz), 6.35 (1H, s), 7.14 (1H, brs), 7.22–7.38 (10H, m), 7.86 (1H, s), 8.35 (1H, s). ^{13}C NMR ($CDCl_3$): δ 61.1, 70.1, 71.7, 73.0, 74.9, 77.5, 89.7, 93.1, 96.0, 120.5, 127.6, 127.7, 127.7, 127.8, 127.8, 127.8, 128.0, 128.3, 128.3, 128.3, 128.3, 128.3, 136.9, 137.7, 139.5, 152.5, 154.5. Mass (FAB): *m/z* 520 (MH^+). Anal. Calcd for $C_{27}H_{29}N_5O_6 \cdot 1/6H_2O$: C, 62.06; H, 5.66; N, 13.40. Found: C, 61.79; H, 5.63; N, 13.29.

2′-*O*,4′-*C*-(Methylenoxymethylene)adenosine (13)

Twenty percent $Pd(OH)_2$-C (750 mg) and ammonium formate (3.00 g, 47.43 mmol) were added to a solution of compound **12** (493 mg, 0.949 mmol) in EtOH/AcOH (100:3, v/v, 24 ml) at room temperature and the mixture was refluxed for 3.5 h. The hot solution was filtrated through a Celite pad and Celite was washed with boiling MeOH (200 ml). After addition of SiO_2 (3.0 g) to the filtrate, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography [$CHCl_3$/MeOH (15:1→12:1, v/v)] to give compound **13** (162 mg, 55%). Colorless crystals; m.p. 255–257°C (MeOH). $[\alpha]_D^{23} - 26.8$ (*c* 0.86, DMSO). IR ν_{max} (KBr): 3132, 1683, 1611, 1477, 1418, 1330, 1175, 1119, 1080 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.53 (1H, dd, $J = 6$, 12 Hz), 3.61 (1H, dd, $J = 6$, 12 Hz), 3.65 (1H, d, $J = 12$ Hz), 3.80 (1H, d, $J = 12$ Hz), 4.41 (1H, d, $J = 6$ Hz), 4.85 (1H, t, $J = 6$ Hz), 5.09 (1H, d, $J = 6$ Hz), 5.30 (1H, d, $J = 6$ Hz), 5.14 (1H, t, $J = 6$ Hz), 6.09 (1H, brd, $J = 4$ Hz), 6.23 (1H, s), 7.27 (2H, s), 8.13 (1H, s), 8.32 (1H, s). ^{13}C NMR (DMSO-d_6): δ 60.9, 68.9, 70.7, 78.7, 88.8, 90.0, 94.9, 119.2, 139.0, 148.5, 152.5, 156.0. Mass (FAB): *m/z* 310 (MH^+). Anal. Calcd for $C_{12}H_{15}N_5O_5$: C, 46.60; H, 4.89; N, 22.64. Found: C, 46.39; H, 4.99; N, 22.38.

6-*N*-Benzoyl-2′-*O*,4′-*C*-(methylenoxymethylene) adenosine (14)

Benzoyl chloride (0.016 µl, 0.136 mmol) was added to a solution of compound **13** (8.4 mg, 0.027 mmol) in anhydrous pyridine (0.3 ml) at room temperature and the mixture was stirred at room temperature for 12 h. After addition of saturated aqueous $NaHCO_3$ solution at 0°C, the mixture was extracted with AcOEt. The organic extracts were washed with saturated aqueous $NaHCO_3$ solution twice, followed by washes with water and brine, then dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in MeOH/1,4-dioxane (2:1, v/v, 0.81 ml) and NaOMe (28% solution in MeOH, 0.052 ml) was added. After stirring for 5 min at room temperature, the mixture was neutralized with DOWEX 50W-X2 and filtrated. After addition of SiO_2 (1.0 g) to the filtrate, the mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography [$CHCl_3$/MeOH (40:1→20:1, v/v)] to give compound **14** (7.7 mg, 68% over two steps). White powder; m.p. 133–136°C. $[\alpha]_D^{24} - 17.7$ (*c* 0.88, DMSO). IR ν_{max} (KBr): 3289, 2924, 1692, 1610, 1517, 1457, 1400, 1255, 1176, 1110 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.55 (1H, dd, $J = 6$, 12 Hz), 3.63 (1H, dd, $J = 6$, 12 Hz), 3.66 (1H, d, $J = 12$ Hz), 3.81 (1H, d, $J = 12$ Hz), 4.55 (1H, d, $J = 6$ Hz), 4.87 (1H, t, $J = 6$ Hz), 5.12 (1H, d, $J = 6$ Hz), 5.32 (1H, d, $J = 6$ Hz), 5.20 (1H, t, $J = 6$ Hz), 6.19 (1H, brd, $J = 5$ Hz), 6.36 (1H, s), 7.52–7.67 (3H, m), 8.04 (2H, d, $J = 7$ Hz), 8.66 (1H, s), 8.74 (1H, s), 11.24 (1H, brs). ^{13}C NMR (DMSO-d_6): δ 60.6, 68.8, 70.6, 78.5, 89.1, 90.2, 95.0, 126.0, 128.5, 128.5, 128.5, 128.5, 132.4, 133.6, 142.5, 150.7, 151.3, 151.5, 165.7. Mass (FAB): *m/z* 414 (MH^+). High-resolution MS (FAB): Calcd for $C_{19}H_{20}N_5O_6$ (MH^+): 414.1414. Found: 414.1397.

6-*N*-Benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*,4′-*C*-(methylenoxymethylene)adenosine (15)

Synthesis of compound 15 from compound 14 (path A). To a suspension of AgOTf (622 mg, 2.42 mmol) in CH_2Cl_2 (2.0 ml) was added dropwise DMTrCl (820 mg, 2.42 mmol) in CH_2Cl_2 (8.0 ml) at room temperature, and the mixture was stirred for 1 h at room temperature. The supernatant fluid (2.5 ml, 2.5 eq.) was added to a solution of compound **14** (101 mg, 0.244 mmol) in CH_2Cl_2/pyridine (1:1, v/v, 4.8 ml) at room temperature and the mixture was stirred for 1 h. After addition of saturated aqueous $NaHCO_3$ solution at 0°C, the mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (1:2, v/v)] to give compound **15** (183 mg, quant.). White powder; m.p. 125–127°C. $[\alpha]_D^{24} - 27.4$ (*c* 0.71, $CHCl_3$). IR ν_{max} (KBr): 3325, 3062, 2922, 2245, 2050, 1901, 1703, 1609, 1509, 1456, 1251, 1178, 1073 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.37 (1H, d, $J = 10$ Hz), 3.42 (1H, d, $J = 10$ Hz), 3.39 (1H, d, $J = 9$ Hz), 3.77 (6H, s), 4.01 (1H, d, $J = 13$ Hz), 4.06 (1H, d, $J = 13$ Hz), 4.92 (1H, d, $J = 6$ Hz), 4.99 (1H, dd, $J = 6$, 9 Hz), 5.23 (1H, d, $J = 7$ Hz), 5.33 (1H, d, $J = 7$ Hz), 6.39 (1H, s), 6.78 (2H, d, $J = 9$ Hz), 6.79 (2H, d, $J = 9$ Hz), 7.19–7.27 (7H, m), 7.34–7.38 (2H, m), 7.48–7.64 (3H, m), 8.01 (2H, d, $J = 7$ Hz), 8.10 (1H, s), 8.74 (1H, s), 9.07 (1H, s). ^{13}C NMR ($CDCl_3$): δ 55.2, 55.2, 64.5, 72.8, 73.4, 78.9, 86.6, 88.6, 88.9, 93.5, 113.1, 113.1, 113.1, 113.1, 123.3, 126.9, 127.7, 127.7, 127.8, 127.8, 127.8, 127.8, 128.7, 128.7, 129.8, 129.8, 129.8, 129.8, 132.7, 133.4, 135.0, 135.1, 141.3, 143.9, 149.4, 150.5, 152.4, 158.4, 158.4, 164.5. Mass (FAB): *m/z* 716 (MH^+). High-resolution MS (FAB): Calcd for $C_{40}H_{38}N_5O_8$ (MH^+): 716.2720. Found: 716.2716.

Synthesis of compound 15 from compound 13 (path B). To a suspension of AgOTf (1.06 g, 4.13 mmol) in CH_2Cl_2 (3.0 ml) was added dropwise DMTrCl (1.40 g, 4.13 mmol) in CH_2Cl_2 (7.0 ml) at room temperature and the mixture was stirred for 1 h. The supernatant fluid (3.0 ml, 3.0 eq.) was added to a solution of compound **13** (128 mg, 0.413 mmol) in CH_2Cl_2/pyridine (1:2, v/v, 8.4 ml) at room temperature and the mixture was stirred for 1.5 h. Benzoyl chloride (0.24 ml, 2.07 mmol) was added to the mixture at room temperature and the mixture was stirred for 24 h. After addition of saturated aqueous $NaHCO_3$ solution at 0°C, the mixture was extracted with AcOEt. The organic extracts were washed with saturated aqueous $NaHCO_3$ solution twice, followed by washes with water and brine, then dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in THF (5.6 ml) and 1 M aqueous LiOH (2.8 ml, 2.8 mmol) was added. After stirring for 5 h at room temperature, the mixture was extracted with AcOEt. The organic extracts were washed with saturated aqueous $NaHCO_3$ solution twice, followed by washes with water and brine, then dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (1:2, v/v)] to give compound **15** (272 mg, 92% over two steps).

6-*N*-Benzoyl-3′-*O*-[2--cyanoethoxy(diisopropylamino)phosphino]-5′-*O*-(4,4′--dimethoxytrityl)-2′-*O*,4′-*C*-(methylenoxymethylene) adenosine (17)

To a solution of compound **15** (283 mg, 0.396 mmol) in MeCN (3.0 ml) were added 4,5-dicyanoimidazole (0.25 M in MeCN, 1.9 ml, 0.48 mmol) and $(i\text{-}Pr_2N)_2POCH_2CH_2CN$ (0.19 ml, 0.59 mmol) at room temperature and the mixture was stirred at room temperature for 16 h. After removal of the solvent under reduced pressure, the residue was diluted with AcOEt, washed with saturated aqueous $NaHCO_3$ solution, water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (2:3, v/v)] followed by the precipitation from hexane/AcOEt to give compound **17** (304 mg, 84%). White powder; m.p. 99–102°C. ^{31}P NMR ($CDCl_3$): δ 150.7, 151.2. MS (FAB): *m/z* 916 (MH^+). High-resolution MS (FAB): Calcd for $C_{49}H_{55}N_7O_9P$ (MH^+): 916.3799. Found: 916.3808.

4′-*C*-Acetoxymethyl-2′-*O*-acetyl-3′-*O*-benzyl-5′-*O*-*tert*-butyldiphenylsilyl-6-*O*-diphenylcarbamoyl-2-*N*-isobutyryl-guanosine (18)

To a suspension of O^6-(diphenylcarbamoyl)-N^2-isobutyrylguanine (121 mg, 0.291 mmol) in DCE (1.9 ml) was added *N*,*O*-bis(trimethylsily)acetamide (0.17 ml, 0.678 mmol) at room temperature, and the mixture was stirred at 80°C for 30 min. After the resulting clear solution was evaporated under reduced pressure, to the residue a solution of compound **4** (123 mg, 0.194 mmol) in anhydrous toluene (1.9 ml) and TMSOTf (0.04 ml, 0.233 mmol) were added and the mixture was stirred at 80°C for 2 h. After addition of saturated aqueous $NaHCO_3$ solution at 0°C, the mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (2:1 to 3:2, v/v)] to give compound **18** (161 mg, 84%). White powder; m.p. 86–88°C. $[\alpha]_D^{25} + 15.1$ (*c* 0.97 $CHCl_3$). IR ν_{max} (KBr): 3327, 3063, 2961, 2866, 2463, 2251, 1959, 1744, 1588, 1500, 1453, 1408, 1227, 1106 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.01 (9H, s), 1.15 (3H, d, $J = 7$ Hz), 1.19 (3H, d, $J = 7$ Hz), 1.94 (3H, s), 2.06 (3H, s), 2.77 (1H, m), 3.85 (2H, s), 4.31 (1H, d, $J = 12$ Hz), 4.66 (1H, d, $J = 12$ Hz), 4.65 (1H, d, $J = 11$ Hz), 4.74 (1H, d, $J = 11$ Hz), 5.23 (1H, d, $J = 6$ Hz), 5.88 (1H, dd, $J = 4$, 6 Hz), 6.09 (1H, d, $J = 4$ Hz), 7.18–7.45 (21H, m), 7.54–7.61 (4H, m), 7.82 (1H, brs), 7.98 (1H, s). ^{13}C NMR ($CDCl_3$): δ 19.3, 19.3, 19.3, 20.8, 20.9, 26.9, 26.9, 26.9, 36.0, 62.5, 64.9, 74.3, 74.6, 78.3, 87.0, 87.2, 121.3, 127.4, 127.4, 127.6, 127.6, 127.6, 127.6, 127.7, 127.7, 127.7, 127.7, 127.7, 128.2, 128.2, 128.2, 128.2, 129.0, 129.0, 129.0, 129.0, 129.7, 129.7, 132.5, 132.6, 135.4, 135.4, 135.4, 135.4, 137.7, 137.7, 141.6, 142.9, 150.1, 151.7, 154.0, 156.0, 169.7, 170.5, 174.3. Mass (FAB): *m/z* 991 (MH^+). Anal. Calcd for $C_{55}H_{58}N_6O_{10}Si \cdot 1/10H_2O$: C, 66.53; H, 5.91; N, 8.46. Found: C, 66.26; H, 5.96; N, 8.43.

3′-*O*-Benzyl-5′-*O*-*tert*-butyldiphenylsilyl-6-*O*-diphenylcarbamoyl-4′-*C*-hydroxymethyl-2-*N*-isobutyrylguanosine (19)

A mixture of **18** (100 mg, 0.10 mmol) and K_2CO_3 (42 mg, 0.30 mmol) in MeOH (1.4 ml) was stirred at 0°C for 40 min. After neutralization with a 10% aqueous HCl solution (0.11 ml), the mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (3:2 to 1:1, v/v)] to give compound **19** (88 mg, 96%). White powder; m.p. 94–96°C. $[\alpha]_D^{24} + 58.0$ (*c* 0.91, $CHCl_3$). IR ν_{max} (KBr): 3311, 3063, 2958, 2865, 2248, 1958, 1890, 1744, 1588, 1499, 1452, 1407, 1330, 1180 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.91 (9H, s), 1.24 (3H, d, $J = 7$ Hz), 1.25 (3H, d, $J = 7$ Hz), 2.57 (1H, m), 3.55 (1H, d, $J = 11$ Hz), 3.67 (1H, d, $J = 11$ Hz), 3.80 (1H, d, $J = 12$ Hz), 3.96 (1H, d, $J = 12$ Hz), 4.66 (1H, d, $J = 5$ Hz), 4.74 (1H, d, $J = 12$ Hz), 5.16 (1H, d, $J = 12$ Hz), 4.80 (1H, t, $J = 5$ Hz), 6.02 (1H, d, $J = 5$ Hz), 7.18–7.49 (25H, m), 8.05 (1H, brs), 8.11 (1H, s). ^{13}C NMR ($CDCl_3$): δ 19.2, 19.3, 19.4, 26.8, 26.8, 26.8, 36.9, 63.7, 66.1, 74.3, 76.2, 80.2, 89.6, 91.5, 121.2, 127.5, 127.5, 127.6, 127.6, 127.6, 127.6, 127.9, 127.9, 128.0, 128.0, 128.0, 128.5, 128.5, 128.5, 128.5, 129.1, 129.1, 129.1, 129.1, 129.7, 129.7, 132.3, 132.4, 135.3, 135.3, 135.3, 135.3, 137.8, 137.8, 141.5, 142.9, 150.1, 151.1, 153.3, 155.9, 174.4. Mass (FAB): *m/z* 907 (MH^+). Anal. Calcd for $C_{51}H_{54}N_6O_8Si \cdot H_2O$: C, 66.21; H, 6.10; N, 9.08. Found: C, 66.15; H, 5.93; N, 9.04.

3′-*O*-Benzyl-5′-*O*-*tert*-butyldiphenylsilyl-6-*O*-diphenylcarbamoyl-2-*N*-isobutyryl-2′-*O*,4′-*C*-(methylenoxymethylene)-guanosine (20)

To a solution of **19** (123 mg, 0.136 mmol) in anhydrous DMSO (1.7 ml) was added NBS (145 mg, 0.816 mmol) at room temperature and the mixture was stirred at 60°C for 40 min. After addition of saturated aqueous $NaHCO_3$ solution at 0°C, the mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (3:1, v/v)] to give compound **20** (56 mg, 45%). White powder; m.p. 97–99°C. $[\alpha]_D^{23} + 39.1$ (*c* 0.94, $CHCl_3$). IR ν_{max} (KBr): 3313, 3062, 2962, 2865, 2246, 1958, 1890, 1745, 1588, 1500, 1451, 1405, 1328, 1176, 1116, 1068 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.97 (9H, s), 1.11 (3H, d, $J = 7$ Hz), 1.17 (3H, d, $J = 7$ Hz), 2.69 (1H, m), 3.80 (1H, d, $J = 12$ Hz), 4.12 (1H, d, $J = 12$ Hz), 3.93 (2H, s), 4.79 (1H, d, $J = 6$ Hz), 4.82 (2H, s), 5.23 (1H, d, $J = 6$ Hz), 5.47 (1H, d, $J = 6$ Hz), 5.41 (1H, d, $J = 6$ Hz), 6.32 (1H, s), 7.05–7.10 (2H, m), 7.20–7.59 (23H, m), 7.84 (1H, brs), 8.07 (1H, s). ^{13}C NMR ($CDCl_3$): δ 19.2, 19.2, 19.3, 26.8, 26.8, 26.8, 36.2, 63.9, 71.6, 72.8, 76.4, 77.1, 89.1, 92.3, 96.0, 121.8, 127.2, 127.2, 127.5, 127.5, 127.5, 127.5, 127.7, 127.7, 127.7, 127.7, 127.7, 128.3, 128.3, 128.3, 128.3, 129.1, 129.1, 129.1, 129.1, 129.5, 129.6, 132.4, 132.8, 135.3, 135.3, 135.5, 135.5, 137.8, 137.8, 141.6, 143.3, 150.2, 151.3, 153.5, 155.9, 174.0. Mass (FAB): *m/z* 919 (MH^+). Anal. Calcd for $C_{52}H_{54}N_6O_8Si \cdot 3/4H_2O$: C, 66.97; H, 6.00; N, 9.01. Found: C, 66.92; H, 5.91; N, 8.94.

3′-*O*-Benzyl-2-*N*-isobutyryl-2′-*O*,4′-*C*-(methylenoxymethylene)guanosine (21)

To a solution of **20** (801 mg, 0.871 mmol) in anhydrous DMSO (7.2 ml) was added $NaNO_2$ (1.20 g, 17.42 mmol) at room temperature and the mixture was stirred at 70°C for 19 h. After addition of saturated aqueous $NaHCO_3$ solution at 0°C, the mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in THF (8.7 ml) and TBAF (1.0 M in THF, 1.05 ml, 1.05 mmol) was added. After stirring for 15 h at room temperature, the solvent was removed under reduce pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (1:2, v/v) and AcOEt/EtOH (50:1, v/v)] to give compound **21** (264 mg, 62% over two steps). Pale brown powder; m.p. 144–146°C. $[\alpha]_D^{26} - 30.0$ (*c* 1.04, $CHCl_3$). IR ν_{max} (KBr): 3157, 2975, 2923, 2248, 1682, 1607, 1559, 1473, 1406, 1315, 1252, 1169, 1117, 1074 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.25 (6H, d, $J = 5$ Hz), 2.72 (1H, m), 3.75 (1H, d, $J = 12$ Hz), 3.97 (1H, d, $J = 12$ Hz), 3.83 (1H, d, $J = 12$ Hz), 3.92 (1H, d, $J = 12$ Hz), 4.44 (1H, d, $J = 5$ Hz), 4.62 (1H, d, $J = 11$ Hz), 4.72 (1H, d, $J = 11$ Hz), 4.94 (1H, d, $J = 5$ Hz), 5.16 (1H, d, $J = 6$ Hz), 5.36 (1H, d, $J = 6$ Hz), 6.18 (1H, s), 7.24–7.33 (5H, m), 8.04 (1H, s), 9.92 (1H, brs), 12.09 (1H, brs). ^{13}C NMR ($CDCl_3$): δ 19.1, 19.1, 36.1, 61.1, 71.7, 72.4, 75.9, 89.4, 91.6, 96.0, 120.6, 127.2, 127.2, 127.7, 128.3, 128.3, 128.3, 137.2, 138.4, 146.9, 147.7, 154.9, 179.3. Mass (FAB): *m/z* 486 (MH^+). High-resolution MS (FAB): Calcd for $C_{23}H_{28}N_5O_7$ (MH^+): 486.1989. Found: 486.1980.

2-*N*-Isobutyryl-2′-*O*,4′-*C*-(methylenoxymethylene) guanosine (22)

Twenty percent of $Pd(OH)_2$-C (170 mg) was added to a solution of compound **21** (171 mg, 0.351 mmol) in MeOH (7.0 ml) at room temperature and the mixture was stirred under an H_2 atmosphere for 40 h. After filtration of the mixture, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography [$CHCl_3$/MeOH (15:1 to 10:1, v/v)] to give compound **22** (118 mg, 85%). White powder; m.p. 182–184°C. $[\alpha]_D^{24} - 12.2$ (*c* 1.09, MeOH). IR ν_{max} (KBr): 3239, 2978, 2932, 1681, 1606, 1566, 1473, 1405, 1316, 1253, 1181, 1111 cm^{-1}. ^1H NMR (CD_3OD): δ 1.22 (6H, d, $J = 7$ Hz), 2.71 (1H, sept, $J = 7$ Hz), 3.67 (1H, d, $J = 12$ Hz), 3.74 (1H, d, $J = 12$ Hz), 3.75 (2H, s), 4.39 (1H, d, $J = 6$ Hz), 4.73 (1H, d, $J = 6$ Hz), 5.07 (1H, d, $J = 7$ Hz), 5.35 (1H, d, $J = 7$ Hz), 6.23 (1H, s), 8.44 (1H, brs). ^{13}C NMR (DMSO-d_6): δ 19.1, 19.1, 34.9, 60.2, 68.3, 70.6, 79.0, 89.0, 89.3, 94.9, 120.2, 136.7, 147.8, 148.2, 154.8, 180.2. Mass (FAB): *m/z* 396 (MH^+). High-resolution MS (FAB): Calcd for $C_{16}H_{22}N_5O_7$ (MH^+): 396.1519. Found: 396.1530.

5′-*O*-(4,4′-Dimethoxytrityl)-2-*N*-isobutyryl-2′-*O*,4′-*C*-(methylenoxymethylene)guanosine (23)

To a solution of **22** (265 mg, 0.670 mmol) in anhydrous pyridine (5.2 ml) was added DMTrCl (454 mg, 1.340 mmol) at room temperature and the mixture was stirred for 9 h. After addition of saturated aqueous $NaHCO_3$ solution at 0°C, the mixture was extracted with AcOEt. The organic extracts were washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [$CHCl_3$/MeOH (50:1 to 20:1, v/v)] to give compound **23** (442 mg, 95%). White powder; m.p. 173–175°C. $[\alpha]_D^{24} + 24.4$ (*c* 0.97, $CHCl_3$). IR ν_{max} (KBr): 3146, 2973, 2250, 2051, 1899, 1680, 1605, 1560, 1507, 1407, 1305, 1250, 1179, 1115, 1072, 1033 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.23 (3H, d, $J = 7$ Hz), 1.27 (3H, d, $J = 7$ Hz), 2.75 (1H, sept, $J = 7$ Hz), 3.37 (1H, d, $J = 10$ Hz), 3.56 (1H, d, $J = 10$ Hz), 3.70 (6H, s), 3.90 (1H, d, $J = 13$ Hz), 4.19 (1H, d, $J = 13$ Hz), 4.57 (1H, d, $J = 5$ Hz), 5.10 (1H, brd, $J = 5$ Hz), 5.14 (1H, brs), 5.17 (1H, d, $J = 7$ Hz), 5.37 (1H, d, $J = 7$ Hz), 6.19 (1H, s), 6.70 (4H, d, $J = 9$ Hz), 7.11–7.18 (3H, m), 7.24 (4H, d, $J = 9$ Hz), 7.32–7.36 (2H, m), 7.67 (1H, s), 9.58 (1H, brs), 12.34 (1H, brs). ^{13}C NMR ($CDCl_3$): δ 19.0, 19.1, 36.3, 55.1, 55.1, 64.6, 71.6, 72.5, 79.0, 86.2, 89.1, 91.3, 95.2, 112.8, 112.8, 112.8, 112.8, 121.1, 126.7, 127.5, 127.5, 128.1, 128.1, 129.9, 129.9, 129.9, 129.9, 135.4, 135.5, 138.8, 144.1, 147.7, 147.7, 155.5, 158.2, 158.2, 180.1. Mass (FAB): *m/z* 698 (MH^+). High-resolution MS (FAB): Calcd for $C_{37}H_{40}N_5O_9$ (MH^+): 698.2826. Found: 698.2813.

3′-*O*-[2-Cyanoethoxy(diisopropylamino)phosphino]-5′-*O*-(4,4′-dimethoxytrityl)-2-*N*-isobutyryl-2′-*O*,4′-*C*-(methylenoxymethylene)guanosine (24)

To a solution of **23** (440 mg, 0.630 mmol) in MeCN/THF (1:1, v/v, 5.0 ml) were added 4,5-dicyanoimidazole (0.25 M in MeCN, 3.0 ml, 0.75 mmol) and $(i\text{-}Pr_2N)_2POCH_2CH_2CN$ (0.30 ml, 0.945 mmol) at room temperature and the mixture was stirred for 19 h. Then, 4,5--dicyanoimidazole (0.25 M in MeCN, 2.6 ml, 0.65 mmol) and $(i\text{-}Pr_2N)_2POCH_2CH_2CN$ (0.30 ml, 0.945 mmol) were added, and the mixture was further stirred at room temperature for 6 h. After removal of the solvent under reduced pressure, the residue was diluted with AcOEt, washed with saturated aqueous $NaHCO_3$ solution, water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography [hexane/AcOEt (2:3, v/v)] followed by precipitation from hexane/AcOEt to give compound **24** (360 mg, 64%). White powder; m.p. 124–127°C. ^{31}P NMR ($CDCl_3$): δ 150.5, 150.6. MS (FAB): m/z 898 (MH^+). High-resolution MS (FAB): Calcd for $C_{46}H_{57}N_7O_{10}P$ (MH^+): 898.3905. Found: 898.3887.

Synthesis of BNA^{COC} 27 and 30

Syntheses of 2′,4′-BNA^{COC}-modified oligonucleotides (BNA^{COC}) **27** and **30** were performed at a 0.2 μmol scale on an automated DNA synthesizer (Applied Biosystems Expedite™ 8909) using the standard phosphoramidite protocol except that the coupling time was increased from the standard 1.5–20 min. As an activator, 5-ethylthio-1*H*-tetrazole was used for every coupling step. Oligonucleotide synthesis was performed on DMTr-OFF mode. Cleavage from the universal CPG support was accomplished by using a 2 M ammonia methanol solution at room temperature for 1.5 h and removal of the protecting groups was accomplished by using a 28% ammonia solution at 55°C for 15 h. The obtained crude oligonucleotides were purified with Sephadex™ G-25 (Amersham Biosciences, NAP™ 10 Column) followed by reversed-phase HPLC (Waters XTerra® MS C_{18} 2.5 μm, 10 × 50 mm) [buffer A, 0.1 M TEAA; buffer B, 0.1 M TEAA/MeCN = 1:1, B 12% to 24%/30 min, linear gradient; flow rate, 3.0 ml/min]. The composition of the oligonucleotides was confirmed by MALDI-TOF-MS analysis. MALDI-TOF-MS data ($[M–H]^-$) for oligonucleotides **27** and **30**: For **27**, found 4354.19 (calcd 4354.71); for **30**, found 4391.12 (calcd 4391.80).

UV melting experiments (T_m measurements)

UV melting experiments were carried out on a SHIMADZU UV-1650PC spectrometer equipped with T_m analysis accessory quartz cuvettes of 1 cm optical path length. The UV melting profiles were recorded in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl at scan rate of 0.5°C/min with detection at 260 nm. The final concentration of each oligonucleotide was 4 μM. The melting temperatures were obtained as the maxima of the first derivative of the melting curves.

CD spectroscopy

CD spectra were measured with a JASCO J-720W spectrophotometer. Spectra were recorded at 12°C in a quartz cuvette of 0.1 cm optical path length. The samples were prepared in the same manner as described for the UV melting experiments. The molar ellipticity was calculated from the equation $[\theta] = \theta/cl$, where θ is the relative intensity, c is the sample concentration, and l is the cell path length in centimeters.

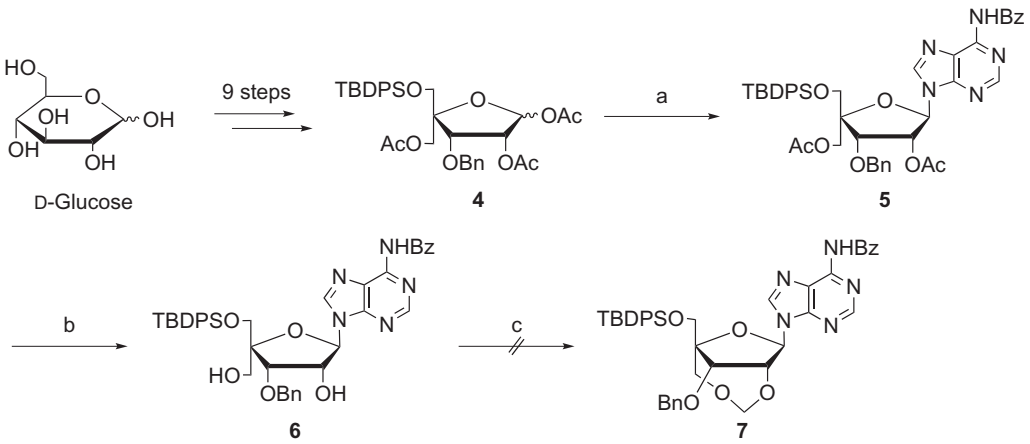
X-ray crystallographic data

Crystallographic data (excluding structure factors) of **13** has been deposited at the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 634527. Copy of the data can be obtained, free of charge via www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi (or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or email: deposit@ccdc.cam.ac.uk).

RESULTS

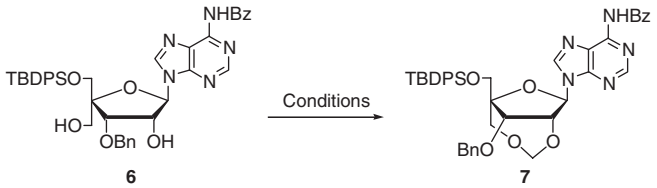
Synthesis of 2′,4′-BNA^{COC}-A

As shown in Scheme 2, the synthesis of the protected 2′,4′-BNA^{COC}-A^{Bz} (**7**), bearing a seven-membered bridged structure, from **4** (30) was performed. According to Vorbrüggen's procedure (32,33), triacetate **4** was coupled with N^6-benzoyladenine to give **5**. Two acetyl groups of **5** were removed to give diol **6** sequentially. At first, diol **6** was treated with paraformaldehyde under acidic conditions; the same reaction conditions for the synthesis of the pyrimidine congener (Scheme 1) (30). However, the methyleneacetal formation did not proceed, while cleavage of the glycosidic linkage was predominantly observed. Then, several reaction conditions were attempted for formation of the methylenoxymethylene (COC) linkage (Table 1). Basic reaction conditions were also employed; however, the desired product **7** was not obtained at all (Table 1, Run 2 and 3). At last, we found that the reaction of diol **6** with NBS in DMSO (34) slightly proceeded to give a trace amount of the target compound **7** bearing a seven-membered bridged structure (Table 1, Run 4). The methyleneacetal formation using NBS in DMSO was dramatically improved by protection with the benzyloxymethyl (BOM) group of the N^6-amide moiety, which might cause many side reactions, to give the desired compound **10** bearing a seven-membered bridged structure in 74% yield as shown in Scheme 3. Because all our attempts for $O^{3'}$-debenzylation of 2′,4′-BNA^{COC}-adenine (2′,4′-BNA^{COC}-A) in the presence of the N^6-benzoyl group failed, we alternatively attempted to remove the N^6-benzoyl group with 28% ammonia aqueous solution to afford **11**. The *tert*-butyldiphenylsilyl (TBDPS) group was removed by treatment with TBAF to give **12**. Hydrogenation of **12** with palladium hydroxide on carbon and ammonium formate resulted in removal of both benzyl and BOM groups to finally afford the desired 2′,4′-BNA^{COC}-A monomer **13**. As shown in Scheme 4, after perbenzoylation of **13**, the obtained compound was



Scheme 2. Reagents and conditions: (**a**) silylated N^6-benzoyladenine, TMSOTf, DCE, reflux, 11 h, 76%; (**b**) K_2CO_3, MeOH, 0°C, 1.5 h, 98%; (**c**) *p*-TsOH·H_2O, $(CH_2O)_n$, DCE, reflux.

Table 1. Attempt at cyclization of compound **6**



Run	Reagents	Solvent	Temperature	Result
1	$(CH_2O)_n$, *p*-TsOH	DCE	Reflux	Complex mixture
2	CH_2I_2, *t*-BuOK	DMF	60°C	Complex mixture
3	CH_2I_2, NaHMDS	THF	60°C	Complex mixture
4	NBS	DMSO	60°C	Trace of **7**

treated with sodium methoxide to give diol **14**. Reaction of a primary hydroxyl group in **14** with 4,4′-dimethoxytrityl trifluolomethanesulfonate (35) gave **15** in 68% yield from **13** (path A). We attempted another synthetic route to improve the yield of **15**. We found that reaction of 2′,4′-BNA^{COC}-A monomer **13** with 4,4′-dimethoxytrityl trifluolomethanesulfonate followed by treatment with benzoyl chloride by a one-pot operation gave **16**. Selective removal of both the $O^{3'}$-benzoyl group and one of the N^6-benzoyl groups gave **15** in 92% yield from **13** (path B). At last, phosphitylation of **15** led to the corresponding amidite **17**.

Synthesis of 2′,4′-BNA^{COC}-G

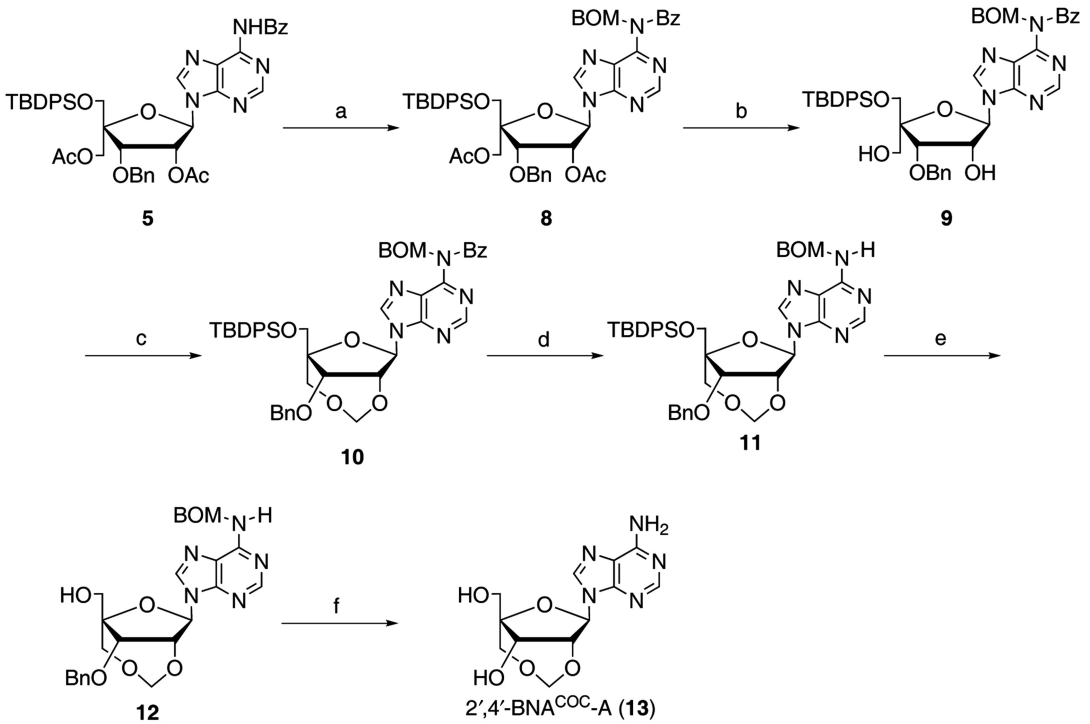
As shown in Scheme 5, an amidite **24** was synthesized by using a common intermediate **4** as the starting material. To circumvent the formation of an N^7/N^9-diastereo-mixture during nucleosidation of **4**, we chose O^6-(diphenylcarbamoyl)-N^2-isobutyrylguanine as the base moiety (36) to give **18** in 84% yield. After removal of the acetyl groups of **18**, the bridge structure formation was accomplished by the same reaction conditions as for the synthesis of the adenine congener to afford **20** in a moderate yield. The O^6-diphenylcarbamoyl group was removed by treatment with sodium nitrite (37) and subsequent desilylation by TBAF leading to **21**. Hydrogenolysis of **21** with palladium hydroxide on carbon afforded the desired 2′,4′-BNA^{COC}-G$^{i\text{-Bu}}$ monomer **22**. Finally, dimethoxytritylation of **22** with 4,4′-dimethoxytrityl chloride followed by phosphitylation gave the phosphoroamidite **24**.

Structure of 2′,4′-BNA^{COC}-A and 2′,4′-BNA^{COC}-G

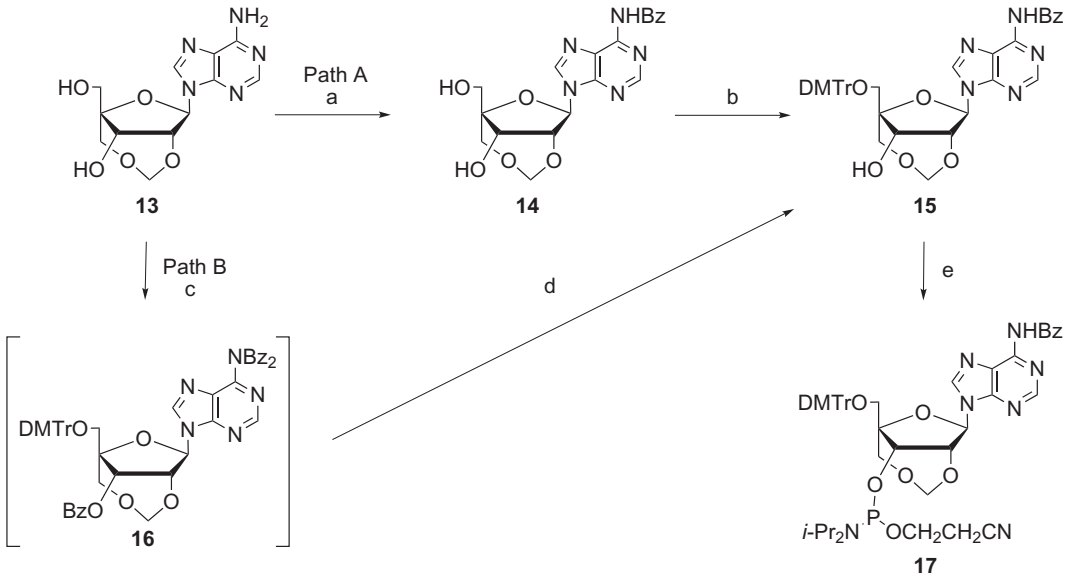
The structural features of 2′,4′-BNA^{COC}-A monomer **13** were well confirmed by ^{1}H NMR measurement and X-ray crystallography (Figure 3, Table 4, and also see experimental sections). No scalar coupling between H1′ and H2′ in the ^{1}H NMR spectra of **13** and **22** strongly suggested the North-type (N-type) sugar puckers, as observed in a series of 2′,4′-BNA (LNA). The crystal structure of 2′,4′-BNA^{COC}-A (**13**) revealed the pseudorotation phase angle *P* (32°) supporting its N-type sugar pucker and COC linkage configuration surrounded the O3′ atom. Moreover, the ν_{max} and δ values of **13** were 38° and 78°, respectively. These values are in good agreement with the parameters of the $C_{3'}$-*endo* structure which is the typical N-type seen in the A-form RNA duplex (4–6).

BNA^{COC} oligonucleotide synthesis

2′,4′-BNA^{COC} phosphoramidites were incorporated into oligonucleotides by conventional phosphoramidite chemistry on an automated DNA synthesizer. The coupling efficiency of 2′,4′-BNA^{COC} phosphoramidites was decreased by using 4,5-dicyanoimidazole as an activator due to steric hindrance of the seven-membered ring structure with a methylenoxymethylene linkage. After several trials, we found that the coupling efficiency was remarkably improved (over 95% yield) when the coupling time was prolonged to 20 min from 90 s for 2′,4′-BNA^{COC} phosphoramidites and 5-ethylthio-1*H*-tetrazole was used as the activator [In the case of PrNA which also has a seven-membered ring structure, the coupling efficiency



Scheme 3. Reagents and conditions: (**a**) BOMCl, DBU, DMF, 0°C, 1 h, 64%; (**b**) K_2CO_3, MeOH, 0°C, 50 min, 97%; (**c**) NBS, DMSO, 60°C, 1.5 h, 74%; (**d**) 28% NH_3aq, THF, 50°C, 48 h, quant.; (**e**) TBAF, THF, rt, 3 h, 91%; (**f**) 20% $Pd(OH)_2$-C, $HCOONH_4$, EtOH-AcOH, reflux, 3.5 h, 55%.

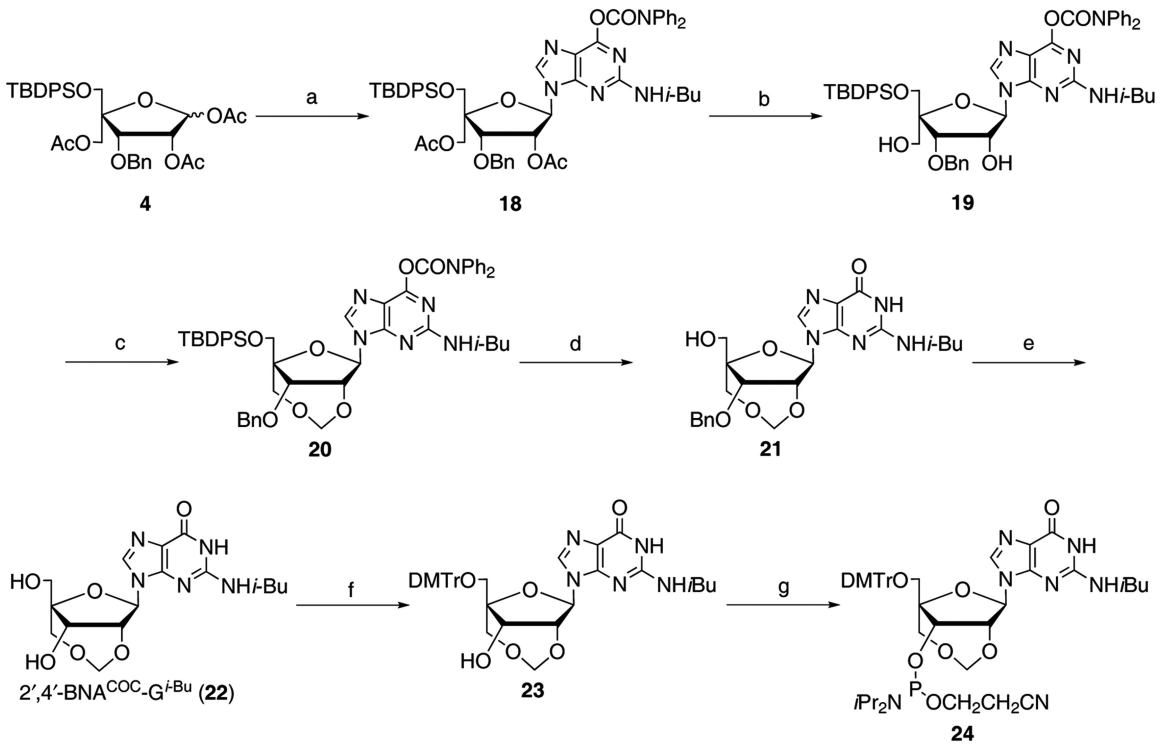


Scheme 4. Reagents and conditions: (**a**) (**1**) BzCl, pyridine, rt, 12 h, (**2**) NaOMe, MeOH-1,4-dioxane, rt, 15 min, 68% over two steps; (**b**) DMTrOTf, pyridine-CH_2Cl_2, rt, 1 h, quant.; (**c**) DMTrOTf then BzCl, pyridine-CH_2Cl_2, rt, 21.5 h; (**d**) LiOH·H_2O, THF-H_2O, rt, 5 h, 92% over two steps; (**e**) $(i\text{-}Pr_2N)_2POCH_2CH_2CN$, DCI, MeCN, rt, 16 h, 84%.

was not good (~50–60%) even with the use of 5-ethylthio-1*H*-tetrazole. See ref. (25)].

Duplex formation abilities of fully modified BNA^{COC} oligonucleotides with DNA and RNA

The duplex forming abilities of fully 2′,4′-BNA^{COC}-modified oligonucleotides (BNA^{COC}) containing all four bases (thymine, 5-methylcytosine, adenine and guanine) were examined by means of UV melting experiments. Base pairing experiments of BNA^{COC} **27** and **30** were carried out with complementary DNA, RNA and BNA^{COC}. The melting temperatures (T_m) for the duplexes are depicted in Table 2. In its own series, the BNA^{COC} duplex showed an extremely high T_m value compared with the corresponding DNA duplex ($\triangle T_m > +43$°C) and the RNA



Scheme 5. Reagents and conditions: (**a**) silylated O^6-(diphenylcarbamoyl)-N^2-isobutyrylguanine, TMSOTf, toluene, 80°C, 2 h, 84%; (**b**) K_2CO_3, MeOH, 0°C, 40 min, 96%; (**c**) NBS, DMSO, 60°C, 40 min, 45%; (**d**) (**1**) $NaNO_2$, DMSO, 70°C, 19 h, (**2**) TBAF, THF, rt, 15 h, 62% over two steps; (**e**) 20% $Pd(OH)_2$-C, H_2, MeOH, rt, 40 h, 85%; (**f**) DMTrCl, pyridine, rt, 9 h, 95%; (**g**) $(i\text{Pr}_2\text{N})_2\text{POCH}_2\text{CH}_2\text{CN}$, DCI, MeCN-THF, rt, 25 h, 64%.

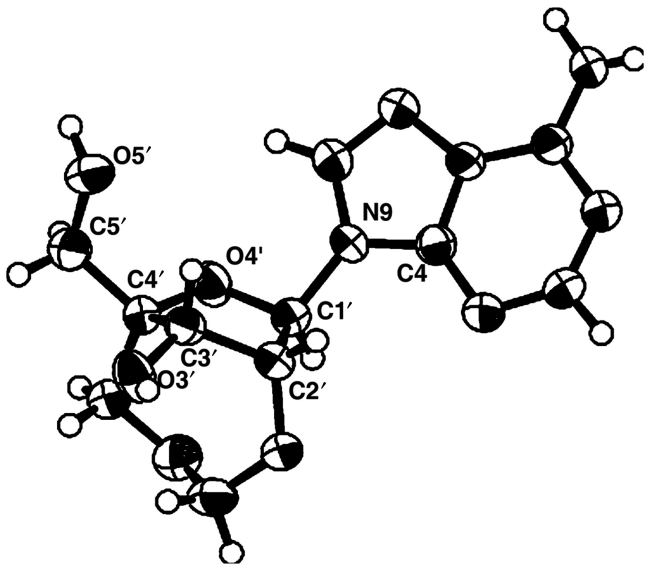


Figure 3. X-ray structure of 2′,4′-BNA^{COC}-A (**13**).

duplex ($\triangle T_m > +44$°C). With the RNA complement, BNA^{COC} constituted more stable pairing systems for **30**/**26** and **27**/**29** than the RNA/RNA duplex ($\triangle T_m$ of +9°C and +27°C, respectively) and the DNA/RNA heteroduplex ($\triangle T_m$ of +30°C each). On the other hand, BNA^{COC}/DNA heteroduplexes **30**/**25** and **27**/**28** showed lower T_m values compared with the DNA/DNA duplex ($\triangle T_m$ of −7°C and −13°C, respectively). BNA^{COC} **27** formed stable duplex with complementary DNA **28** compared with the corresponding RNA/DNA duplex (**26**/**28**), probably due to an effect of 5-methyl group of 2′,4′-BNA^{COC}-T in **27** [Probably, thymine in place of uracil nucleobase should cause higher thermal stability of BNA^{COC} **27** with the DNA complement than that of natural RNA/DNA duplex. See ref. (7) describing the effect of displacement of thymine with uracil in 2′,4′-BNA duplex.] Remarkably, BNA^{COC} clearly distinguished complementary RNA from complementary DNA ($\triangle T_m$ of +39°C for **27** and +15°C for **30**, respectively), whereas natural RNAs, **26** and **29**, showed relatively poor discrimination between complementary RNA and complementary DNA ($\triangle T_m$ of +21°C for **26** and +3°C for **29**, respectively).

Mismatch discrimination of 2′,4′-BNA^{COC}-modified oligonucleotides

To analyze the base selectivity of BNA^{COC}, we investigated the duplex stabilities between BNA^{COC} **27** and mismatched DNA or RNA complements. The melting temperatures (T_m) of duplexes are shown in Table 3. With the mismatched DNA complement, the T_m values of natural DNA **25** decreased by −11°C to −15°C compared to that of the fully-matched duplex, while both natural RNA **26** and BNA^{COC} **27** showed T_m values lower than room temperature. In the case of duplexes with a mismatched RNA complement, BNA^{COC} **27** recognized one base mismatch ($\triangle T_m = -6$°C to −21°C) by a similar manner to that observed in natural DNA **25**

Table 2. T_m values (°C) of BNA^{COC} with complementary DNA, RNA and 2′,4′- BNA^{COC} oligonucleotides

	Target strands		
	28 5′-d(AGCAAAAAACGC)-3′	**29** 5′-r(AGCAAAAAACGC)-3′	**30** 5′-ag^{m}caaaaaa^{m}cg^{m}c-3′
25 5′-d(GCGTTTTTTGCT)-3′	52	48	45
26 5′-r(GCGUUUUU1UGCU)-3′	30	51	60
27 5′-g ^{m}c g t t t t t t g ^{m}c t-3′	39	78	>95

The UV melting profiles were measured in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl at a scan rate of 0.5°C/min at 260 nm. The concentration of the oligonucleotide used was 4 μM for each strand. BNA^{COC} oligonucleotides are shown in small letters.

Table 3. T_m values (°C) of oligonucleotides **25**–**27** with their complementary DNA or RNA with or without a one base mismatch

	Target sequence 5′-d(AGCAAA**N**AACGC)-3′				Target sequence 5′-r(AGCAAA**N**AACGC)-3′			
	N = A	T	G	C	N = A	U	G	C
25 5′-d(GCGTTTTTTGCT)-3′	52	38 (−14)	41 (−11)	37 (−15)	48	33 (−15)	43 (−5)	32 (−16)
26 5′-r(GCGUUUUUUGCU)-3′	30	<25	<25	<25	51	42 (−9)	48 (−3)	39 (−12)
27 5′-g ^{m}c g t t t t t t g ^{m}c t-3′	39	<25	<25	<25	78	62 (−16)	72 (−6)	57 (−21)

Conditions: 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl at a scan rate of 0.5°C/min at 260 nm. The concentration of the oligonucleotide used was 4 μM for each strand. The values in parentheses are differences in T_m values between mismatched and matched duplexes.

($\triangle T_m = -5$°C to −16°C). The differences in T_m values between matched and mismatched duplexes were larger than those observed for duplexes containing RNA **26** ($\triangle T_m = -3$°C to −12°C). As expected, the T−G wobble base pair was more stable than the other mismatched base pairs in all cases investigated.

Analysis of the helical structure of 2′,4′-BNA^{COC}-modified oligonucleotide

Circular dichroism spectroscopic analyses of BNA^{COC}-hybridizing duplexes were performed to investigate the structural preferences. CD spectra were recorded at 10°C for the BNA^{COC} duplex **27**/**30**, the BNA^{COC}/DNA heteroduplexes **27**/**28** and **30**/**25**, the BNA^{COC}/RNA heteroduplexes **27**/**29** and **30**/**26**, as well as for the control DNA duplex **25**/**28**, the RNA duplex **26**/**29** and the DNA/RNA duplexes **25**/**29** and **28**/**26** (Figure 4). The CD spectra of BNA^{COC} duplex **27**/**30** and the BNA^{COC}/RNA heteroduplexes **27**/**29** and **30**/**26** were similar to that of the natural RNA duplex. These spectra showed a large positive Cotton band around 265 nm and a large negative Cotton band at 210 nm, indicating a typical A-form helix formation (Figure 4A). In the case of the BNA^{COC}/DNA and the RNA/DNA heteroduplexes, all spectra were very similar to each other and the shoulder band around 280 nm suggested an intermediate between A- and B-form geometry. This revealed that BNA^{COC} formed duplexes with complementary DNA in the same manner the natural RNA did. Additionally, the CD profile of the natural DNA duplex belonged to no other types of duplexes in this study.

DISCUSSION

X-ray crystallography established the relationship between the sugar conformation and the ring size of the bridged structure between the 2′- and 4′-positions. The values of selected torsion angles are summarized in Table 4. In making a comparison between 2′,4′-BNA^{COC}-A monomer **13** and 2′,4′-BNA^{COC}-T monomer **3**, even though the pseudorotation phase angle P are slightly different from each other, they are in the range preferred by the $C_{3'}$-*endo* conformation ($0° \leq P \leq 36°$) which is the typical N-type geometry fit in a natural A-form RNA duplex (4–6). From another point of view, non-observed H1′–H2′ coupling in both ^{1}H NMR spectra suggested very similar N-type sugar puckering in the solution phase. These data indicate that the sugar conformation of 2′,4′-BNA^{COC} is fixed to an N-type, but still slightly flexible. Molecular dynamics simulations supported its conformational features showing larger *P*-amplitude of 2′,4′-BNA^{COC} than that of 2′,4′-BNA (LNA) and 2′,4′-BNA^{NC} (Figure S1). Concerning the ν_{max} values, which represent the maximum degree of the sugar puckering mode (N/S-type), the sugar conformation of 2′,4′-BNA^{COC} (ν_{max} 38°) is very similar to that of a natural A-form RNA duplex (ν_{max} 38°) compared with that of 2′,4′-BNA (LNA), ENA and 2′,4′-BNA^{NC}[NMe] [ν_{max} 57° (7), 48° (23) and 49° (19), respectively]. These results showed that the sugar conformation of 2′,4′-BNA^{COC} is closest to that of A-form RNA among nucleic-acid analogues with a different type of bridged structure between the 2′- and 4′-positions.

NMR and molecular dynamics studies of the 2′,4′-BNA(LNA)-modified oligonucleotide (BNA) demonstrated that the conformation of the complementary DNA in BNA/DNA heteroduplexes is changed adaptively into a suitable one to form A-form duplexes and results in larger populations of N-type sugar puckers (38,39). On the other hand, the 2′,4′-BNA^{COC}-modified oligonucleotide (BNA^{COC}) duplex and the BNA^{COC}/RNA heteroduplexes

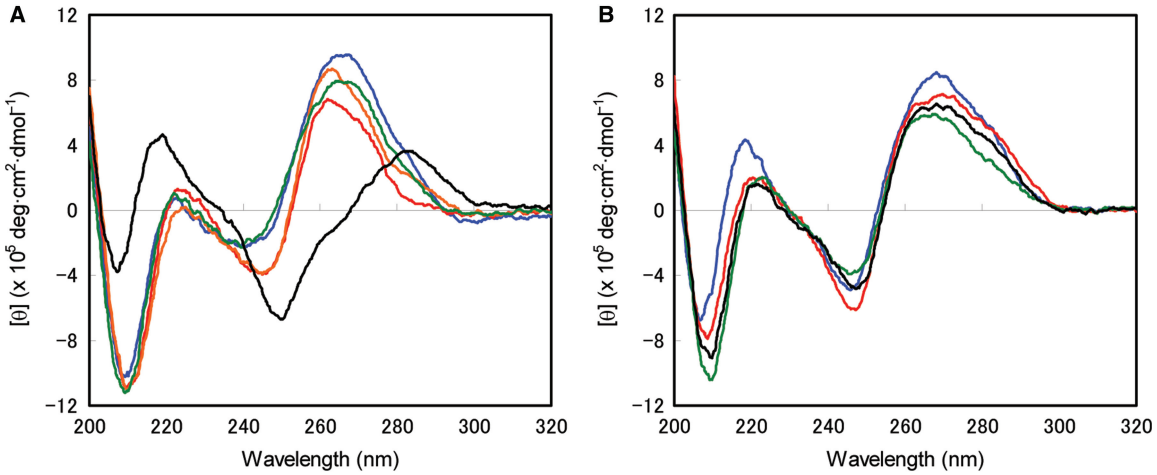


Figure 4. (**A**) CD spectra of BNA^{COC} duplex **27**/**30** (red line), BNA^{COC}/RNA heteroduplexes **27**/**29** (orange line) and **26**/**30** (green line), DNA duplex **25**/**28** (black line), RNA duplex **26**/**29** (blue line); (**B**) CD spectra of duplexes the BNA^{COC}/DNA heteroduplexes **27**/**28** (red line) and **25**/**30** (green line) and DNA/RNA duplexes **25**/**29** (black line) and **26**/**28** (blue line). The spectra were recorded in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl at 12°C. The concentration of each strand was 4 μM.

Table 4. Selected parameters by X-ray analysis

		δ	ν_{max}	*P*
2′,4′-BNA[7]		66°	57°	17°
ENA[24]		76°	48°	15°
2′,4′-BNA^{NC}[NMe][19]		75°	49°	23°
2′,4′-BNA^{coc}-T (**3**)[30]		78°	38°	17°
2′,4′-BNA^{coc}-A (**13**)	this work	78°	38°	32°
$C_{3'}$-*endo*[4-6]	(A-form RNA)	83°	38.6° ± 3°	0°–36°

showed the typical A-form features on the CD spectra, while the BNA^{COC}/DNA heteroduplexes showed the same aspect with the natural RNA/DNA duplex (Figure 4). These differences between BNA and BNA^{COC} probably correlate with the above-mentioned conformational features of each monomer, that is to say, the oligonucleotide bearing a fixed N-type sugar conformation and larger ν_{max} value than that of the typical A-form duplex could force the complementary DNA to take an N-type sugar conformation in the duplex.

Concerning the thermal stability, BNA^{COC} forms a very stable duplex with complementary RNA, while it moderately stabilizes the duplex with complementary DNA. On the other hand, BNA formed extremely stable duplexes with both complementary DNA and RNA (9,20–22), and PrNA, which possesses an alternative –$CH_2CH_2CH_2$– group to –CH_2OCH_2– of 2′,4′-BNA^{COC} within the bridge structure, leads to a decrease in duplex stabilities with both complementary DNA and RNA (24). These differences in hybridization properties would probably result from a balance between the entropic advantages derived from the conformational restriction of the sugar moiety (10) and the disadvantages caused by the additional bridge structure. BNA possesses a rigid N-type sugar restricted with a small bridge to make a great entropic advantage over any other disadvantages. In the case of BNA^{COC}, the entropic advantage by the sugar restriction would be smaller than that of BNA because of the large perturbation of 2′,4′-BNA^{COC} come from the larger bridge structure compared with 2′,4′-BNA (LNA) (40; Supplementary Figure S1). However, the hydrophilic bridge structure of BNA^{COC} could somewhat positively influence the network of hydration around the duplex. Consequently, BNA^{COC} makes the duplex more thermally stable than PrNA bearing a hydrophobic bridge structure.

BNA^{COC} has interesting features, such as high binding affinity towards the RNA complement and discrimination between complementary RNA and DNA. Moreover, it has been revealed that the modification of 2′,4′-BNA^{COC} leads to excellent nuclease resistance (30). These results indicate a high potential for 2′,4′-BNA^{COC} in genome technology. Further biological studies of 2′,4′-BNA^{COC} are now in progress.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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