Pergamon

Tetrahedron, Vol. 52, No. 8, pp. 2863-2876, 1996
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0040-4020/96 $15.00 + 0.00

S0040-4020(96)00007-5

Nucleosides and Nucleotides. 147. Synthesis of DNA dodecamers containing Oxetanocin A and (2*R*, 3*R*)-2-*C*-(Adenin-9-yl)-1,4-anhydro-2,3-dideoxy-3-*C*-hydroxymethyl-D-arabitol[1]

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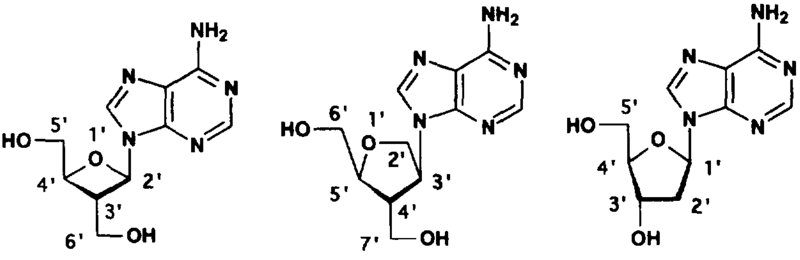
Abstract: Oxetanocin A (**1**) and (2*R*,3*R*)-2-*C*-(adenin-9-yl)-1,4-anhydro-2,3-dideoxy-3-*C*-hydroxy-methyl-D-arabitol (**2**), a ring-expanded oxetanocin analogue, were incorporated into DNA dodecamers, 5'-d(CGCG**1**ATTCGCG)-3' (**II**), 5'-d(CGCGA**1**TTCGCG)-3' (**III**), 5'-d(CGCG**2**ATTCGCG)-3' (**IV**), and 5'-d(CGCGA**2**TTCGCG)-3' (**V**). Thermally induced transitions of **II**, **III**, **IV**, and **V** together with a parent dodecamer, 5'-d(CGCGAATTCGCG)-3' (**I**) were monitored at 260 nm in a buffer containing 0.01 M sodium phosphate (pH 7.0), 0.02 M NaCl, and 6 μM of each dodecamer. The order of Tms was 40 °C (**I**) > 37 °C (**IV**) > 36 °C (**V**) > 34 °C (**II**) > 32 °C (**III**). The oligonucleotide containing **2** was more resistant to snake venom phosphodiesterase than unmodified DNA dodecamer (**I**).

INTRODUCTION

Various oligonucleotide analogues modified at base moieties, 5'- and 3'-ends, phosphodiester moieties, etc. have been synthesized and used for antisense studies.[2] Also, oligonucleotides containing modified sugars such as 2'-substituted ribose, 1'-substituted 2-deoxyribose, etc., have been synthesized and their biochemical and biophysical properties have been studied.[3-6] However, attempts to modify the five-membered ribose (or 2-deoxyribose) skeleton have been few.[7-10] Recently, it has been reported that oligonucleotide analogues containing hexopyranosyl nucleosides were resistant to nucleolytic digestion,[8] which is one of the requirements for antisense molecules. In contrast, duplexes formed by many of these oligonucleotide analogues with complimentary strands were less stable than corresponding unmodified duplexes, which is a drawback for antisense molecules. These studies indicated that proper modifications of the ribose skeleton could yield new oligonucleotide analogues that can form stable duplexes with target strands and will be useful for antisense studies.

In this study, we synthesized novel oligonucleotides containing two sugar-skeleton-modified 2'-deoxyadenosine analogues (Fig. 1). One of them is oxetanocin A (**1**),[11] which is a recently isolated antibiotic from *Bacillus megaterium*. Oxetanocin A consists of an adenine base and an oxetanose, a sugar having a four membered ring skeleton, which is in striking contrast with normal nucleosides having five membered ribose or 2-deoxyribose. Another nucleoside analogue is (2*R*,3*R*)-2-*C*-(adenin-9-yl)-1,4-anhydro-2,3-dideoxy-3-*C*-hydroxymethyl-D-arabitol (**2**),[12] a ring-expanded oxetanocin analogue, which contains a five-membered tetrahydrofuran ring in place of the 2-deoxyribose. One of the great differences between **2** and 2'-deoxyadenosine is the positions in which the five-membered rings are attached to adenine. In **2**, adenine is attached at the 3'-position of the tetrahydrofuran skeleton, but adenine is attached at the 1'-position of 2-deoxyribose in 2'-deoxyadenosine. Once incorporated into DNA strands, these structural differences between 2'-deoxyadenosine and the sugar-skeleton-modified nucleosides will affect the stability of duplexes. In this report, we describe the synthesis of DNA dodecamers **I**, **II**, **III**, **IV**, and **V** (Fig. 1). Thermal stability of these self-complementary duplexes and stability of these oligomers to nucleolytic digestion were also studied.



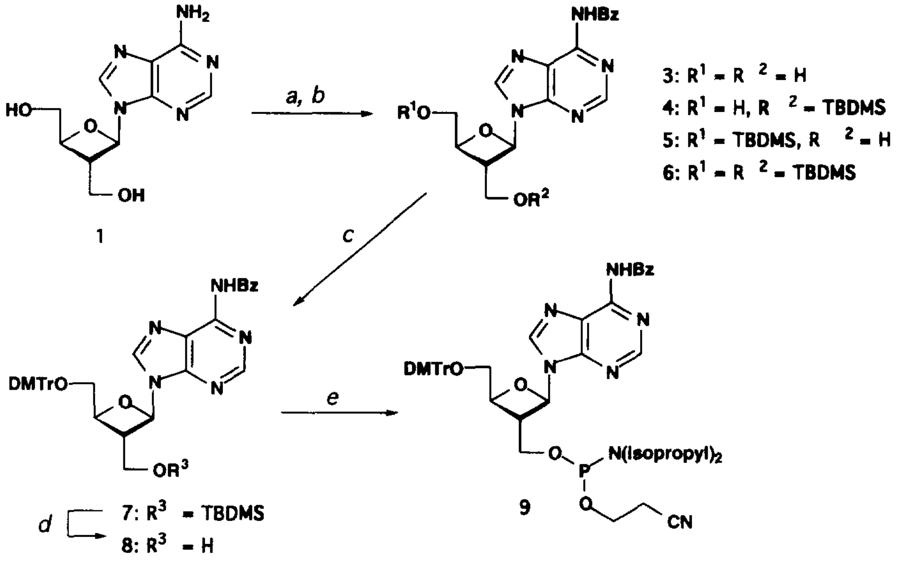
1 **2** **2'-deoxyadenosine**

I	5'-d(CGCGAATTCGCG)-3'
II	5'-d(CGCG1ATTCGCG)-3'
III	5'-d(CGCGA1TTCGCG)-3'
IV	5'-d(CGCG2ATTCGCG)-3'
V	5'-d(CGCGA2TTCGCG)-3'

Figure 1. Structures of sugar-skeleton-modified 2'-deoxyadenosine analogues and DNA dodecamers.

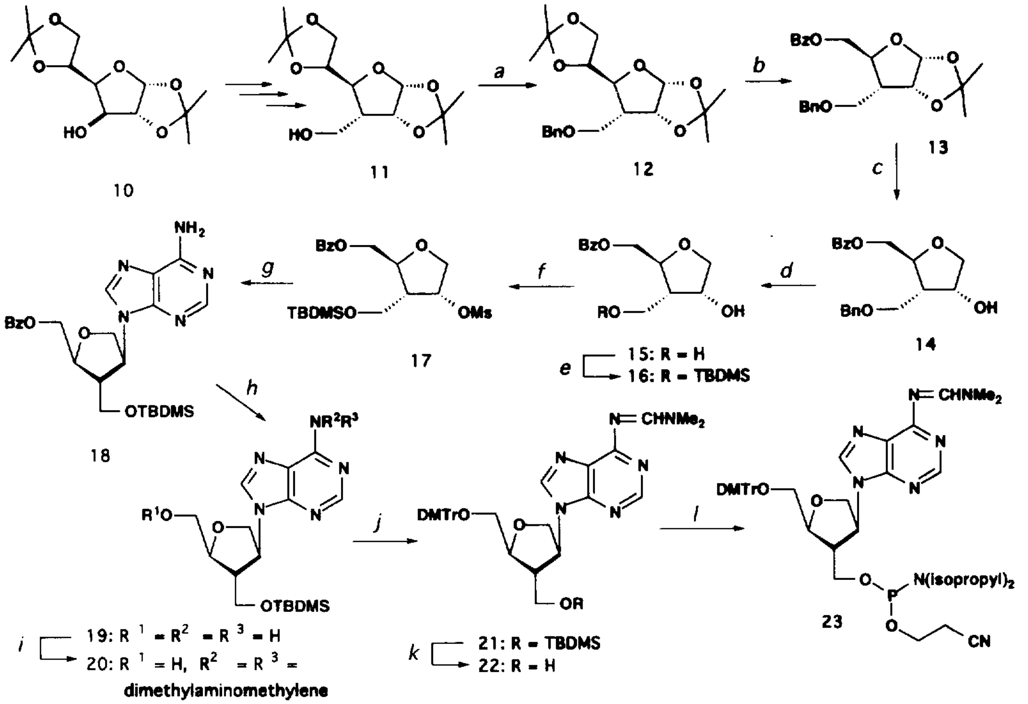
RESULTS AND DISCUSSION

Synthesis. To incorporate **1** and **2** into oligonucleotides, we examined the methods for selective protection of the two primary hydroxyl groups of **1** and **2**. In spite of a few attempts at selective protection of the two primary hydroxyl groups of **1**,[13] no practical method has been reported. Therefore, we examined the methods for separation of 5'- and 6'-protected derivatives of **1** (Scheme 1). Namely, treatment of N^6-benzoyloxetanocin A (**3**) with 1.1 equivalent of *tert*-butyldimethylsilyl chloride (TBDMSCl) in DMF gave, in approximately similar yields, 6'-*O*-TBDMS **4**, 5'-*O*-TBDMS **5**, and 5',6'-di-*O*-TBDMS **6** derivatives, which were separated by silica gel column chromatography. This method was useful for oxetanocin chemistry, since **4**, **5**, and **6** could be useful for synthesis of various oxetanocin analogues. In this study, only **4** was used for the next reactions. After the free hydroxyl group of **4** was protected by a DMTr group, the TBDMS group was removed, then the newly generated free hydroxyl group was phosphitylated[14] to yield nucleoside phosphoramidite derivative **9**, which was used for DNA oligomer synthesis.[15]



Scheme 1. *a*; 1) TBDMSCl, imidazole, DMF. 2) BzCl, pyridine. 3) 28% NH_4OH solution. 4) TBAF, THF. 73%. *b*; TBDMSCl, imidazole, DMF. 21% (**4**), 33% (**5**), 29% (**6**). *c*; DMTrCl, pyridine. 83%. *d*; TBAF, THF. 91%. *e*; 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, *N*,*N*-diisopropylethylamine. 78%.

A synthetic route for the ring-expanded oxetanocin analog **2** has been established in our laboratory.[12] Selective protection of the two primary hydroxyl groups of **2** was done in an early stage of the synthetic route (Scheme 2). Namely, a primary hydroxyl group of **11**, which was obtained from 1,2:5,6-di-*O*-isopropylidene-α-D-glucose (**10**) according to reported procedures,[16] was protected by a Bn group. After hydrolysis of the 5,6-isopropyridene group of **12** by 0.1 N H_2SO_4, a newly generated cis-diol was oxidized using $NaIO_4$ and a generated formyl group was converted to the primary hydroxyl group, which was protected by a Bz group to afford selectively protected intermediate **13** in 71% yield from **12**. Compound **13** was converted to tetrahydrofuran derivative **14** using TMSOTf and Et_3SiH,[12] and the Bn group was removed followed by protection of the resulting hydroxyl with a TBDMS group. Methanesulfonylation of **16** gave the corresponding mesylate **17** in 99% yield, which was then treated with adenine in the presence of K_2CO_3 and 18-crown-6 in DMF to afford **18** in 32% yield. Debenzoylation of **18**, followed by a selective protection of the N^6-amino group with a dimethylaminomethylene group, and the successive dimethoxytritylation gave **20** in 93% yield from **18**. After the TBDMS group of **20** was removed, the newly generated free hydroxyl group was phosphitylated[14] to yield phosphoramidite derivative **23**, which was used for DNA oligomer synthesis.[15]



Scheme 2. *a*; NaH, BnCl, DMF, 97%. *b*; 1) 0.1 N H_2SO_4, MeOH. 2) $NaIO_4$, MeOH-H_2O. 3) $NaBH_4$, MeOH. 4) BzCl, pyridine. 71%. *c*; 1) TMSOTf, CH_2Cl_2. 2) Et_3SiH. 74%. *d*; H_2, Pd-C, MeOH. *e*; TBDMSCl, pyridine. 88% from **14**. *f*; MsCl, Et_3N, DMAP, CH_2Cl_2. 99%. *g*; adenine, K_2CO_3, 18-crown-6, DMF. 32%. *h*; NH_3, MeOH. *i*; *N*,*N*-dimethylformamide dimethyl acetal. *j*; DMTrCl, pyridine. 93% from **18**. *k*; TBAF, THF. *l*; 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, *N*,*N*-diisopropylethylamine. 62% from **21**.

An unmodified DNA dodecamer, 5'-d(CGCGAATTCGCG)-3' (**I**), and dodecamers containing the sugar-skeleton-modified compounds, 5'-d(CGCG**1**ATTCGCG)-3' (**II**), 5'-d(CGCGA**1**TTCGCG)-3' (**III**), 5'-d(CGCG**2**ATTCGCG)-3' (**IV**), and 5'-d(CGCGA**2**TTCGCG)-3' (**V**) (Fig. 1) were synthesized on a DNA synthesizer. Coupling yields of **9** and **23** were 96% and 92% using a 0.1 M solution of the amidite derivatives in CH_3CN and 60 seconds for the coupling time, respectively (conditions for the coupling reaction were not optimized). Fully protected oligonucleotides were deprotected and purified by the same procedures as for the purification of unmodified oligonucleotides. Each oligonucleotide in this preparation showed a single peak by HPLC analysis with a C-18 silica gel column. Examples are shown in Fig. 2a,b.

Next, we confirmed the existence of **1** and **2** in the dodecamers **II** and **IV**. Each dodecamer was completely hydrolyzed to the corresponding nucleosides by a mixture of snake venom phosphodiesterase and alkaline phosphatase, then the nucleoside composition of each mixture was analyzed by HPLC (Fig. 2c,d).

The peaks corresponding to **1** and **2**, confirmed by coelution with authentic samples, were observed. The result indicated that **1** and **2** in DNA strands were stable to NH_4OH treatment.

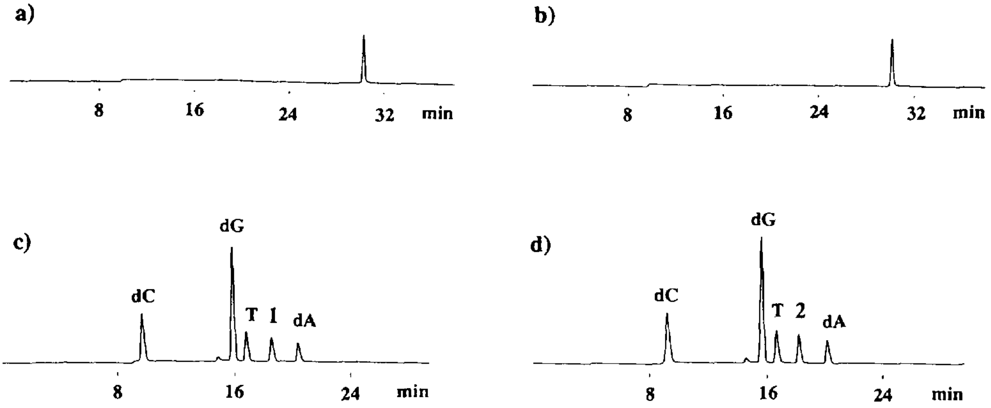


Figure 2. HPLC profiles (reversed phase silica gel column) of **II** (a) and **IV** (b); a nucleoside mixture obtained by enzymatic hydrolysis of **II** (c); a nucleoside mixture obtained by enzymatic hydrolysis of **IV** (d). A linear gradient of CH_3CN from 0 to 40% (40 min) in 0.1 M triethylammonium acetate (pH 6.8) was used for (a) and (b), and a linear gradient of CH_3CN from 0 to 50% (50 min) in the same buffer was used for (c) and (d). The peaks were detected at 260 nm.

Duplex Formation of the Dodecamers. DNA dodecamers **I**, **II**, **III**, **IV**, and **V** have self-complementary sequences and form duplexes in solutions containing some amount of salts.[17] Normalized thermal denaturation profiles of the dodecamers (6 μM) in a buffer containing 10 mM sodium phosphate (pH 7.0) and 20 mM NaCl are shown in Fig. 3. An order of Tms determined from these profiles was 40 °C (**I**) > 37 °C (**IV**) > 36 °C (**V**) > 34 °C (**II**) > 32 °C (**III**). The sugar-skeleton-modified analogues **1** and **2**, which

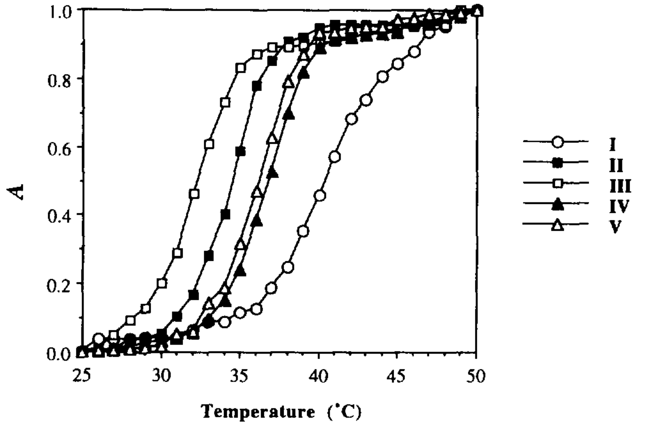


Figure 3. Normalized thermal denaturation profiles of the dodecamers.

have four- and five-membered rings as the sugar moiety, were not stabilized but had slightly destabilized duplex formation. This is a striking contrast with the previously reported result that substitution with hexopyranosyl nucleosides in the middle of an oligonucleotide largely destabilized duplex formation.[8] In two analogues used in this study, **2** carrying the five-membered ring stabilized duplex formation more than **1** carrying the four-membered ring. Since CD spectra of **I**, **II**, **III**, **IV**, and **V** did not indicate large conformational alterations (data not shown), the analogues **1** and **2** could be accommodated in the DNA duplex.

Partial Digestion of the Dodecamers with Snake Venom Phosphodiesterase. The potency of any given antisense oligonucleotides is highly dependent on its resistance to nucleolytic digestion. Several studies have demonstrated that 3'-exonuclease activities were the major cause of degradation of unmodified oligonucleotides in serum.[18] We examined the stability of the dodecamers containing **1** and **2** to snake venom phosphodiesterase, a 3'-exonuclease. The dodecamers **III** and **V** containing **1** and **2** were labeled at the 5'-end with ^{32}P, and incubated with the nuclease, and the reactions were analyzed by polyacrylamide gel electrophoresis under denaturing conditions (Fig. 4).[19] Although the control **I** and the oligonucleotide **III** containing **1** were hydrolyzed almost completely by the nuclease after 2 h of incubation (Fig. 4, lanes 4 and 10), the dodecamer **V** containing **2** was hydrolyzed only at the 3'-side from the nucleoside analogue (Fig. 4, lane 16). The phosphodiester linkage at the 3'-side of **2** was more resistant to the nuclease than the phosphodiester linkages at the 3'-sides of **1** and dA.

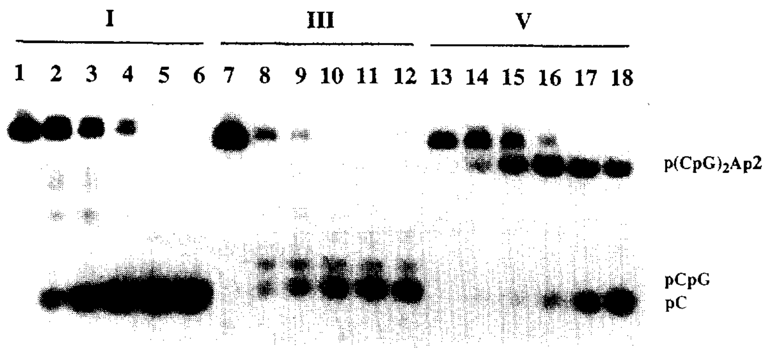


Figure 4. Polyacrylamide gel electrophoresis of oligonucleotides hydrolyzed by snake venom phosphodiesterase: **I** (lanes 1-6), **III** (lanes 7-12) and **V** (lanes 13-18) were incubated with snake venom phosphodiesterase at 37 °C for 0 h (lanes 1, 7, and 13), 0.5 h (lanes 2, 8, and 14), 1 h (lanes 3, 9, and 15), 2 h (lanes 4, 10, and 16), 4 h (lanes 5, 11, and 17), and 6 h (lanes 6, 12, and 18). See Experimental Section for the conditions.

Conclusion. In this study, we would like to find some answers to the question, "can an oligonucleotide containing a sugar-skeleton-modified nucleoside simultaneously satisfy conflicting requirements such as stable duplex formation and resistance to nucleases, since modifications at sugar moiety may increase resistance to nucleases but may destabilize duplex formation."[8] We chose two sugar-skeleton-modified nucleosides, oxetanocin A (**1**) carrying a four-membered ring with two primary hydroxyl groups and a ring-expanded oxetanocin analogue **2** carrying a five-membered ring with two primary hydroxyl groups. The structural differences between **1**, **2**, and 2'-deoxyadenosine are not large, thus introduction of **1** and **2** into the oligonucleotides did not significantly destabilize duplex formation. However the dodecamer containing **2** was more resistant to snake venom phosphodiesterase than the unmodified dodecamer and the dodecamer containing **1**. These results indicated that small structural modifications at sugar moieties in oligonucleotides can increase resistance to nucleases without reducing duplex stability, thus these two important requirements for antisense applications, duplex stability and resistance to nucleases, can be satisfied simultaneously by using appropriately designed sugar-skeleton-modified nucleosides.

EXPERIMENTAL SECTION

General Methods. Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus (Yanagimoto, Japan) and are uncorrected. TLC was done on Merck Kieselgel F254 precoated plates (Merck, Germany). The silica gel used for column chromatography was YMC gel 60A (70-230 mesh) (YMC Co., Ltd., Japan). The neutralized silica gel was ICN silica 60A (ICN Biochemicals). Mass spectra (EI-MS) and Fast Atom Bombardment Mass Spectrometry (FAB-MS) were done on a Jeol JMX-DX303 (JEOL) or a Jeol JMS-HX110 (JEOL), respectively, at an ionizing voltage of 70 eV. The ^{1}H-NMR spectra were recorded on a Jeol JNM-GX 270 (270 MHz) spectrometer (JEOL) with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet), or br (broad). All exchangeable protons were detected by disappearance on the addition of D_2O. UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer.

(2'*R*,3'*R*,4'*S*)-9-[3',4'-Bis(hydroxymethyl)oxetan-2'-yl]-*N*^{6}-benzoyladenine (*N*^{6}-benzoyloxetanocin A) (3). TBDMSCl (5.7 g, 38 mmol) was added to a DMF (50 mL) solution containing **1** (3.8 g, 15 mmol) and imidazole (3.1 g, 45 mmol), and the solution was stirred at room temperature under an Ar atmosphere. After 6.5 h, again, imidazole (2.6 g, 38 mmol) and TBDMSCl (4.6 g, 30 mmol) were added to the reaction mixture and the whole was stirred for 2 h at room temperature. MeOH (5 mL) was added to the mixture, and the solution was kept for 0.5 h at room temperature, then the solvents were evaporated. The residue was partitioned between $CHCl_3$ and H_2O, and the organic layer was washed successively with sat. $NaHCO_3$ solution, then brine, dried (Na_2SO_4), and concentrated. After coevaporation three times with anhydrous pyridine, the residue was dissolved in anhydrous pyridine (50 mL). BzCl (3.5 mL, 30 mmol) was added to the solution at 0 °C, then the reaction mixture was stirred for 5 h at room temperature. MeOH (10 mL) was added to the solution, and the whole was stirred for 0.5 h at room temperature. The solution was concentrated, and the residue was partitioned between EtOAc and H_2O, then the organic layer was washed successively with sat. $NaHCO_3$ solution, then brine, dried (Na_2SO_4), and concentrated. After coevaporation twice with toluene, the residue was dissolved in a mixture of MeOH (50 mL) and conc. NH_4OH solution (8 mL), and the whole was stirred for 0.5 h at room temperature. The solution was concentrated, and the residue was coevaporated successively with EtOH (x2) then toluene (x2). The residue was dissolved in THF (50 mL) and TBAF (1 M in THF, 33 mL, 33 mmol) was added to the solution, then the reaction mixture was stirred for 4 h at room temperature. The solution was concentrated, and the residue was purified on a silica gel column (6 x 15 cm) with 12% MeOH in $CHCl_3$ to give **3** (3.9 g, 73% as a white solid): EI-MS *m/z* 355 (M^+). ^{1}H-NMR (270 MHz, DMSO-d_6) 11.22 (1 H, br s, H-N6), 8.99 and 8.77 each (1 H, s, H-2 or H-8), 8.07-8.03 (2 H, m, Ph-2,6), 7.68-7.53 (3 H, m, Ph-3,4,5), 6.56 (1 H, d, H-2', $J_{2',3'}$ = 5.5 Hz), 5.39 (1 H, t, 5'-OH, J = 4.9 Hz), 5.06 (1 H, t, 6'-OH, J = 4.9 Hz), 4.60-4.56 (1 H, m, H-4'), 3.79-3.70 (5 H, H-3', H-5'a,b, H-6'a,b).

N^6-Benzoyl-6'-*O*-(TBDMS)oxetanocin A (4), N^6-benzoyl-5'-*O*-(TBDMS)oxetanocin A (5), and N^6-benzoyl-5',6'-di-*O*-(TBDMS)oxetanocin A (6). TBDMSCl (0.14 g, 3.1 mmol) was added to a DMF (20 mL) solution containing **3** (1.0 g, 2.8 mmol) and imidazole (0.48 g, 7.0 mmol), and the reaction mixture was stirred for 4 h at room temperature under an Ar atmosphere. MeOH (5 mL) was added to the reaction mixture, and the whole was kept for 0.5 h at room temperature, then the solution was concentrated. The residue was partitioned between $CHCl_3$ and H_2O, and the organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified on a silica gel column (3.7 x 12.5 cm) with 2-4% EtOH in $CHCl_3$ to give **4** (0.27g, 21%), **5** (0.43g, 33%), and **6** (0.48 g, 29%). Physical data for **4**: EI-MS *m/z* 469 (M^+), 454 (M^+-CH_3), 412 (M^+-t-Bu). ^{1}H-NMR (270 MHz, $CDCl_3$) 9.05 (1 H, br s, H-N6), 8.84 and 8.21 (each 1 H, s, H-2 or H-8), 8.05-8.02 (2H, m, Ph-2,6), 7.65-7.51 (3 H, m, Ph-3,4,5), 6.48 (1 H, d, H-2', $J_{2',3'}$ = 6.0 Hz), 5.39 (1 H, br s, 5'-OH), 4.84-4.82 (1 H, m, H-4'), 4.23-4.10 (2 H, m, H-6'a,b), 3.83 (2 H, d, H-5'a,b, $J_{5',4'}$ = 3.8 Hz), 3.82-3.75 (1 H, m, H-3'), 0.93 (9 H, s, t-Bu), 0.11 and 0.10 each (3 H, s, $SiMe_2$). Physical data for **5**: EI-MS *m/z* 454 (M^+), 412 (M^+-t-Bu). ^{1}H-NMR (270 MHz, $CDCl_3$) 9.11 (1 H, br s, H-N6), 8.78 and 8.64 (each 1 H, s, H-2 or H-8), 8.05-8.02 (2 H, m, Ph-2,6), 7.65-7.50 (3 H, m, Ph-3,4,5), 6.59 (1 H, d, H-2', $J_{2',3'}$ = 5.5 Hz), 4.73 (1 H, ddd, H-4', $J_{4',5'a}$ = 3.3, $J_{4',5'b}$ = 2.8, $J_{4',3'}$ = 6.0 Hz), 4.08-3.92 (3 H, m, H-5'a, H-6'a,b), 3.80 (1 H, dd, H-5'b, J_{gem} = 12.1, $J_{5'b,4'}$ = 2.8 Hz), 3.69-3.60 (1 H, m, H-3'), 3.22 (1 H, br s, 6'-OH), 0.90 (9 H, s, t-Bu), 0.13 and 0.11 (each 3 H, s, $SiMe_2$). Physical data for **6**: EI-MS *m/z* 538 (M^+). ^{1}H-NMR (270 MHz, DMSO-d_6) 11.22 (1 H, br s, H-N6), 8.99 and 8.77 (each 1 H, s, H-2 or H-8), 8.07-8.03 (2 H, m, Ph-2,6), 7.68-7.53 (3 H, m, Ph-3,4,5), 6.56 (1 H, d, H-2', $J_{2',3'}$ = 5.5 Hz), 5.39 (1 H, t, 5'-OH, J = 4.9 Hz), 5.06 (1 H, t, 6'-OH, J = 4.9 Hz), 4.60-4.56 (1 H, m, H-4'), 3.79-3.70 (5 H, H-3', H-5'a,b, H-6'a,b).

N^6-Benzoyl-5'-*O*-dimethoxytrityl-6'-*O*-(TBDMS)oxetanocin A (7). After coevaporation three times with anhydrous pyridine, **4** (260 mg, 0.56 mmol), DMTrCl (380 mg, 1.0 mmol), and DMAP (14 mg, 0.12 mmol) were dissolved in anhydrous pyridine, and the solution was stirred for 31 h at room temperature under an Ar atmosphere. The solution was partitioned between $CHCl_3$ and H_2O, and the organic layer was separated and washed successively with sat. $NaHCO_3$ solution then brine, dried (Na_2SO_4), and concentrated. After coevaporation with toluene, the residue was purified on a neutralized silica gel column (2.3

x 8.5 cm) with a mixture of hexane and EtOAc (1:1) to give **7** (360 mg, 83% as a yellow gum): EI-MS *m/z* 469 (M^+-DMTr). ^{1}H-NMR (270 MHz, $CDCl_3$) 9.02 (1 H, br s, H-N6), 8.74 and 8.49 (each 1 H, s, H-2 or H-8), 8.04-8.02 (2 H, m, Bz, Ph-2,6), 7.64-6.81 (16 H, m, Bz and DMTr), 6.62 (1 H, d, H-2', $J_{2',3'}$ = 5.5 Hz), 4.86-4.82 (1 H, m, H-4'), 3.93-3.71 (9 H, m, H-3', H-5'a or b, H-6'a or b, -OMe x 2), 3.56 (2 H, d, H-5'a or b, $J_{5',4'}$ = 3.8 Hz), 3.82-3.75 (1 H, m, H-3'), 0.89 (9 H, s, t-Bu), 0.11 and 0.09 (each 3 H, s, $SiMe_2$).

N^6-Benzoyl-5'-*O*-dimethoxytrityloxetanocin A (8). TBAF (1 M in THF, 0.69 mL, 0.69 mmol) was added to a THF solution (8 mL) containing **7** (350 mg, 0.46 mmol), and the reaction mixture was stirred for 4 h at room temperature. The solution was concentrated, and the residue was purified on a neutralized silica gel column (2.3 x 7.5 cm) with 2-4% EtOH in $CHCl_3$ to give **8** (270 mg, 91% as a yellow foam): EI-MS *m/z* 355 (M^+-DMTr). ^{1}H-NMR (270 MHz, $CDCl_3$) 9.07 (1 H, br s, H-N6), 8.75 and 8.44 (each 1 H, s, H-2 or H-8), 8.05-8.02 (2 H, m, Bz, Ph-2,6), 7.65-7.50 (3 H, m, Bz, Ph-3, 4, 5), 7.40-6.78 (13 H, m, DMTr), 6.56 (1 H, d, H-2', $J_{2',3'}$ = 6.0 Hz), 4.79-4.76 (1 H, m, H-4'), 4.02 (1 H, dd, H-6'a, J_{gem} = 11.5, $J_{6'a,3'}$ = 4.4 Hz), 3.77 (6 H, s, -OMe x 2), 3.71-3.66 (1 H, m, H-3'), 3.53 (1 H, dd, H-5'a, J_{gem} = 11.5, $J_{5'a,4'}$ = 3.3 Hz), 3.43 (1 H, dd, H-5'b, J_{gem} = 11.5, $J_{5'b,4'}$ = 4.4 Hz), 3.28 (1 H, br, 6'-OH).

β-Cyanoethyl (N^6-benzoyl-5'-*O*-dimethoxytrityloxetanocin)-*N*,*N*-diisopropylphosphoramidite (9). After coevaporation twice with anhydrous pyridine, **8** (270 mg, 0.40 mmol) was dissolved in CH_2Cl_2 (5 mL) and diisopropylethylamine (140 μL, 0.81 mmol), and 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (135 μL, 0.60 mmol) were added to the solution. The reaction mixture was stirred for 0.5 h at room temperature. The solution was partitioned between CH_2Cl_2 and sat. $NaHCO_3$ solution, then the organic layer was dried (Na_2SO_4), and concentrated. The residue was purified on a neutralized silica gel column (2.8 x 5.5 cm) with 50 to 63% EtOAc in hexane to give **9** (270 mg,78% as a pale yellow foam): FAB-MS *m/z* 858 (M^++1). ^{31}P-NMR (202 MHz, $CDCl_3$, 85% H_3PO_4 as an internal standard) 149.60, 149.37. HR-FAB Calcd for $C_{47}H_{53}O_7N_7P$ (M^++1); 858.3744. Found: 858.3779.

3-[(Benzyloxy)methyl]-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (12). A DMF (70 mL) solution containing **11** (13 g, 48 mmol) was added to a suspension of NaH (60%, 3.4 g, 86 mmol) in DMF (150 mL), and the whole was stirred for 40 min at room temperature under an Ar atmosphere. BnBr (12.6 mL, 106 mmol) was added to the mixture in an ice bath, and the whole was stirred for 13 h at room temperature under an Ar atmosphere. 1 N NH_4Cl (100 mL) was added to the reaction mixture, and the whole was stirred for 10 min. Water (300 mL) was added to the mixture, and the whole was extracted with EtOAc (200 mL) three times. The organic layers were combined and washed successively three times with H_2O (200 mL), then with brine (300 mL), dried (Na_2SO_4), and concentrated. The residue was purified on a silica gel column (7.6 x 17 cm) with 10-20% EtOAc in hexane to give **12** (16.8 g, 97% as a yellow gum): EI-MS *m/z* 364 (M^+), 349 (M^+-CH_3). ^{1}H-NMR (270 MHz, $CDCl_3$) 7.36-7.26 (5 H, m, Ph), 5.76 (1 H, d, H-1, $J_{1,2}$ = 3.8 Hz), 4.78 (1 H, dd, H-2, $J_{2,3}$ = 4.4, $J_{2,1}$ = 3.8 Hz), 4.58 and 4.54 (each 1 H, d, $PhCH_2O$, J = 12.1 Hz), 4.09-3.74 (6 H, m, H-4, H-5, H-6a,b, and H-7a,b), 2.28-2.19 (1 H, m, H-3).

5-*O*-Benzyl-3-[(benzyloxy)methyl]-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose (13). 0.8 N H_2SO_4 (20 mL) was added to a MeOH (120 mL) solution containing **12** (16.8 g, 46 mmol), and the mixture was stirred for 48 h at room temperature. The solution was neutralized by the addition of $NaHCO_3$, then precipitated salts were filtered off. The filtrate was concentrated, and the residue was dissolved in MeOH (80 mL). An MeOH solution (80 mL) containing $NaIO_4$ (14.8 g, 69 mmol) was added to the solution, then the mixture was stirred for 2.5 h at room temperature. Ethylene glycol (3 mL) was added to the reaction mixture, and the whole was stirred for 0.5 h, then white precipitates were filtered off, and the filtrate was concentrated. The residue was dissolved in MeOH (80 mL) and a solution (50 mL) containing $NaBH_4$ (2.6 g, 69 mmol) was added to the solution in an ice bath. The whole was stirred for 2 h at room temperature, then the solution was neutralized with AcOH. The neutralized solution was concentrated, and the residue was partitioned between CH_2Cl_2 and H_2O, then the organic layer was washed twice with brine, dried (Na_2SO_4), and concentrated. The residue was purified on a silica gel column (7.6 x 15 cm) with 33-50% EtOAc in hexane as eluents. The fractions were concentrated, and the residue was coevaporated three times with toluene, then the residue was dissolved in pyridine (100 mL). BzCl (6.2 mL, 54 mmol) was added to the solution in an ice bath, and the mixture was stirred at room temperature under an Ar atmosphere. After 2 h, sat. $NaHCO_3$ solution was added to the mixture in an ice bath, and the whole was stirred for 0.5 h at room temperature, then the mixture was concentrated. The residue was partitioned between Et_2O and H_2O, then the organic layer was washed successively with sat. $NaHCO_3$ solution then with brine, dried (Na_2SO_4), and concentrated. The residue was purified on a silica gel column (7.6 x 16 cm) with 16-25% EtOAc in hexane to give **13** (13 g, 71% as a pale yellow gum): EI-MS *m/z* 398 (M^+), 383 (M^+-CH_3). ^{1}H-NMR (270 MHz, $CDCl_3$) 8.07-8.03 (2 H, m, Bz, Ph-2,6), 7.59-7.43 (3 H, m, Bz, Ph-3,4,5), 7.41-7.27 (5 H, m, *Ph*-CH_2-O), 5.87 (1 H, d, H-1, $J_{1,2}$ = 3.8 Hz), 4.75 (1 H, dd, H-2, $J_{2,3}$ = 4.4, $J_{2,1}$ = 3.8 Hz), 4.71 (1 H, dd, H-5a, $J_{5a,b}$ = 11.5, $J_{5a,4}$ = 2.2 Hz), 4.54 (2 H, s, PhCH_2O), 4.34 (1 H, dd, H-5b, $J_{5b,a}$ = 11.5, $J_{5b,4}$ = 5.5 Hz), 4.29-4.22 (1 H, m, H-4), 3.85 (1 H, dd, H-7a, $J_{7a,b}$= 9.3, $J_{7a,3}$ = 6.6 Hz), 3.62 (1 H, dd, H-7b, $J_{7b,a}$ = 9.3, $J_{7b,3}$ = 7.1 Hz), 2.40-2.30 (1 H, m, H-3).

(3*R*,4*S*,5*R*)-5-[(Benzoyloxy)methyl]-4-[(benzyloxy)methyl]tetrahydrofuran-3-ol (14). A CH_2Cl_2 solution (40 mL) containing TMSOTf (870 μL, 5 mmol) was added dropwise to a CH_2Cl_2 solution (40 mL) containing **13** (980 mg, 2.5 mmol), and the whole was stirred for 0.5 h at room temperature under an Ar atmosphere. Et_3SiH (2 mL, 12 mmol) was added to the solution in an ice bath, and the mixture was stirred for 18 h at room temperature under an Ar atmosphere. The solution was partitioned between CH_2Cl_2 and sat. $NaHCO_3$ solution, and the organic layer was washed successively with sat. $NaHCO_3$ solution then brine, dried (Na_2SO_4), and concentrated. The residue was purified on a silica gel column (3.7 x 9 cm) with 50-66% EtOAc in hexane to give **14** (620 mg, 73% as a colorless gum): EI-MS *m/z* 343 (M^++1), 237 (M^+-Bz). ^{1}H-NMR (270 MHz, $CDCl_3$) 8.05-8.01 (2 H, m, Bz, Ph-2,6), 7.65-7.40 (3 H, m, Bz, Ph-3,4,5), 7.39-7.26 (5 H, m, *Ph*-CH_2O), 4.59 and 4.52 (each 1 H, d, PhCH_2O, J = 12.1 Hz), 4.56-4.51 (1 H, m, H-3), 4.48 (1 H, dd, H-6a, $J_{6a,b}$ = 11.5, $J_{6a,5}$ = 3.3 Hz), 4.34 (1 H, dd, H-6b, $J_{6b,a}$ = 11.5, $J_{6b,5}$ = 5.5 Hz), 4.30-4.25 (1 H, m, H-5), 4.06 (1 H, dd, H-2a, $J_{2a,3}$ = 4.4, $J_{2a,b}$ = 9.9 Hz), 3.84 (1 H, dd, H-2b, $J_{2b,a}$ = 9.9, $J_{2b,3}$ = 1.7 Hz), 3.80-3.73 (2 H, m, H-7a,b), 2.61 (1 H, d, 3-OH, J = 4.4 Hz), 2.41-2.31 (1 H, m, H-4).

(3*R*,4*S*,5*R*)-5-[(Benzoyloxy)methyl]-4-{[(TBDMS)oxy]methyl}-tetrahydrofuran-3-ol (16). A suspension containing 10% Pd-C (62 mg) in MeOH (10 mL) was stirred vigorously for 0.5 h under

H_2 atmosphere, then a MeOH solution (10 mL) containing **14** (620 mg, 1.8 mmol) was added to the suspension. The whole was stirred vigorously for 20 h at room temperature under H_2 atmosphere. The suspension was filtered through a Celite pad, and the filtrate was concentrated to give crude **15**, which was used for the next reaction without further purification. After coevaporation three times with pyridine, **15** was dissolved in anhydrous pyridine (5 mL) containing DMAP (22 mg, 0.18 mmol) and TBDMSCl (330 mg, 2.2 mmol). The mixture was stirred for 23 h at room temperature under an Ar atmosphere, then the solution was concentrated. The residue was partitioned between EtOAc and H_2O, then the organic layer was washed successively with sat. $NaHCO_3$ solution then brine, dried (Na_2SO_4), and concentrated. The residue was purified on a silica gel column (3.7 x 7 cm) with a 33% EtOAc in hexane to give **16** (580 mg, 88% as a colorless gum): EI-MS *m/z* 367 (M^++1), 351 (M^+-CH3), 309 (M^+-t-Bu). ^{1}H-NMR (270 MHz, $CDCl_3$) 8.06-8.03 (2 H, m, Bz, Ph-2,6), 7.60-7.41 (3 H, m, Bz, Ph-3,4,5), 4.54-4.51 (1 H, m, H-3), 4.48 (1 H, dd, H-6a, $J_{6a,b}$ = 11.5, $J_{6a,5}$ = 3.3 Hz), 4.37 (1 H, dd, H-6b, $J_{6b,a}$ = 11.5, $J_{6b,5}$ = 5.5 Hz), 4.36-4.29 (1 H, m, H-5), 4.05 (1 H, dd, H-2a, $J_{2a,3}$ = 4.4, $J_{2a,b}$ = 9.9 Hz), 4.02 (1 H, dd, H-7a, $J_{7a,b}$ = 10.4, $J_{7a,4}$ = 4.9 Hz), 3.93 (1 H, dd, H-7b, $J_{7b,a}$ = 10.4, $J_{7b,4}$ = 6.1 Hz), 3.83 (1 H, dd, H-2b, $J_{2b,a}$ = 9.9, $J_{2b,3}$ = 2.2 Hz), 3.15 (1 H, d, 3-OH, *J* = 4.4 Hz), 2.28-2.18 (1 H, m, H-4), 0.90 (9 H, s, t-Bu), 0.09 (6 H, s, SiMe).

(2*S*,3*S*,4*R*)-2-[(Benzoyloxy)methyl]-3-{[(TBDMS)oxy]methyl}-4-[(methylsulfonyl)-oxy]tetrahydrofuran (17). Et_3N (488 µL, 3.5 mmol), DMAP (17 mg, 0.14 mmol), and MsCl (220 µL, 2.8 mmol) were added to a CH_2Cl_2 solution (20 mL) containing **16** (507 mg, 1.4 mmol) in an ice bath, and the mixture was stirred for 2 h at room temperature. The solution was partitioned between H_2O and CH_2Cl_2, and the organic layer was washed successively with sat. $NaHCO_3$ solution then brine, dried (Na_2SO_4), and concentrated. The residue was purified on a silica gel column (2.8 x 14 cm) with 33-50% EtOAc in hexane to give **17** (623 mg, quant. as a colorless gum): EI-MS *m/z* 429 (M^+-CH_3), 387 (M^+-t-Bu). ^{1}H-NMR (270 MHz, $CDCl_3$) 8.06-8.02 (2 H, m, Bz, Ph-2,6), 7.58-7.42 (3 H, m, Bz, Ph-3,4,5), 5.33-5.30 (1 H, m, H-3), 4.56 (1 H, dd, H-6a, $J_{6a,b}$ = 11.5, $J_{6a,5}$ = 3.3 Hz), 4.37 (1 H, dd, H-6b, $J_{6b,a}$ = 11.5, $J_{6b,5}$ = 5.5 Hz), 4.23-4.17 (2 H, m, H-5, H-7a), 4.12 (1 H, dd, H-7b, $J_{7b,a}$ = 11.5, $J_{7b,4}$ = 3.3 Hz), 3.93 (1 H, dd, H-2a, $J_{2a,3}$ = 7.7, $J_{2a,b}$ = 10.4 Hz), 3.78 (1 H, dd, H-2b, $J_{2b,a}$ = 10.4, $J_{2b,3}$ = 6.6 Hz), 3.07 (3 H, s, MsO-), 2.54-2.43 (1 H, m, H-4), 0.88 (9 H, s, t-Bu), 0.07 and 0.06 (each 3 H, s, SiMe).

(3'*S*,4'*S*,5'*R*)-9-{5'-[(Benzyloxy)methyl]-4'-{[(TBDMS)oxy]methyl}tetrahydro-furan-3'-yl}adenine (18). Adenine (380 mg, 2.8 mmol), K_2CO_3 (390 mg, 2.8 mmol), and 18-crown-6 ether (370 mg, 1.4 mmol) were added to a DMF solution (40 mL) containing **17** (600 mg, 1.4 mmol), and the mixture was stirred for 20 h at 120 °C under an Ar atmosphere. The solution was concentrated, and the residue was partitioned between $CHCl_3$ and H_2O, then the organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified on a silica gel column (3.3 x 11 cm) with 4 % EtOH in $CHCl_3$ to give **18** (208 mg, 32% as a white solid): EI-MS *m/z* 468 (M^+-CH_3), 426 (M^+-t-Bu). UV λ_{max} (MeOH) 260 nm. ^{1}H-NMR (270 MHz, $CDCl_3$) 8.31 and 8.01 (each 1 H, s, H-2 or H-8), 8.05-8.01 (2 H, m, Bz, Ph-2,6), 7.61-7.42 (3 H, m, Bz, Ph-3,4,5), 5.62 (2 H, br s, 6-NH_2), 5.18-5.13 (1 H, m, H-3'), 4.67 (1 H, dd, H-6'a, $J_{6'a,b}$ = 12.1, $J_{6'a,5'}$ = 3.3 Hz), 4.55 (1 H, dd, H-6'b, $J_{6'b,a}$ = 12.1, $J_{6'b,5'}$ = 5.5 Hz), 4.35 (1 H, dd, H-7'a, $J_{7'a,b}$ = 9.9, $J_{7'a,4'}$ = 3.9 Hz), 4.29 (1 H, ddd, H-5', $J_{5',6'a}$ = 3.3, $J_{5',6'b}$ = 5.5, $J_{5',4'}$ = 1.7 Hz), 4.18 (1

H, dd, H-7'b, $J_{7'b,a}$ = 9.9, $J_{7'b,4'}$ = 6.0 Hz), 3.91 (1 H, dd, H-2'a, $J_{2'a,3'}$ = 5.0, $J_{2'a,b}$ = 10.4 Hz), 3.83 (1 H, dd, H-2'b, $J_{2'b,a}$ = 10.4, $J_{2'b,3'}$ = 4.4 Hz), 2.75-2.69 (1 H, m, H-4'), 0.89 (9 H, s, t-Bu), 0.08 and 0.07 (each 3 H, s, SiMe).

(3'*S*,4'*S*,5'*R*)-9-{4'-{[(TBDMS)oxy]methyl}-5'-[(4,4'-dimethoxytrityl)oxy]methyl]-tetrahydrofuran-3'-yl}-*N*^{6}-(*N*,*N*-dimethylaminomethylene)adenine (21). Methanolic ammonia (saturated at 0 °C, 10 mL) was added to **18** (206 mg, 0.43 mmol), and the suspension was kept for 2 days at room temperature. The mixture was concentrated, and the residue was purified on a silica gel column (2.3 x 11 cm) with 8-12% EtOH in $CHCl_3$ to give **19** as white solids, which were used for the next reaction without further purification. $(MeO)_2CHNMe_2$ (270 μL, 2.0 mmol) was added to a MeOH solution (8 mL) containing **19**, and the mixture was stirred for 11 h at room temperature. The mixture was concentrated to give **20**, which was coevaporated three times with pyridine and dissolved in anhydrous pyridine (8 mL). DMTrCl (206 mg, 0.61 mmol) and DMAP (5 mg, 0.041 mmol) were added to the solution, and the mixture was stirred for 2 h at room temperature under an Ar atmosphere. The mixture was partitioned between H_2O and $CHCl_3$, and the organic layer was washed successively with sat. $NaHCO_3$ solution then with brine, dried (Na_2SO_4), and concentrated. The residue was purified on a silica gel column (2.3 x 8 cm) with 2% EtOH in $CHCl_3$ to give **21** (283 mg, 93% as a pale yellow gum): FAB-MS *m/z* 737 (M^++1), 679 (M^+-t-Bu). ^{1}H-NMR (270 MHz, $CDCl_3$) 8.98 (1 H, s, -N=C*H*-NMe_2), 8.53 and 8.07 (each 1 H, s, H-2 or H-8), 7.46-6.80 (13 H, m, DMTr), 5.20-5.15 (1 H, m, H-3'), 4.25 (1 H, dd, H-7'a, $J_{7'a,b}$ = 9.9, $J_{7'a,4'}$ = 3.9 Hz), 4.15 (1 H, dd, H-7'b, $J_{7'b,a}$ = 9.9, $J_{7'b,4'}$ = 6.0 Hz), 4.11-4.06 (1 H, m, H-5'), 3.80 (1 H, dd, H-2'a, $J_{2'a,3'}$ = 5.5, $J_{2'a,b}$ = 10.4 Hz), 3.78 (6 H, s, OMe x 2), 3.65 (1 H, dd, H-2'b, $J_{2'b,a}$ = 10.4, $J_{2'b,3'}$ = 4.4 Hz), 3.40 (1 H, dd, H-6'a, $J_{6'a,b}$ = 9.9, $J_{6'a,5'}$ = 4.4 Hz), 3.35 (1 H, dd, H-6'b, $J_{6'b,a}$ = 9.9, $J_{6'b,5'}$ = 5.0 Hz), 3.26 and 3.20 (each 3 H, s, -NMe_2), 2.51-2.47 (1 H, m, H-4'), 0.83 (9 H, s, t-Bu), 0.01 and 0.00 (each 3 H, s, SiMe).

(3'*S*,4'*S*,5'*R*)-9-{4'-{[(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite]oxy}-methyl}-5'-[(4,4'-di-methoxytrityl)oxy]methyl]tetrahydrofuran-3'-yl}-*N*^{6}-(*N*,*N*-dimethylaminomethylene)adenine (23). TBAF (1 M in THF, 0.56 mL, 0.56 mmol) was added to a THF solution (8 mL) containing **21** (262 mg, 0.36 mmol), and the mixture was stirred for 1 h at room temperature. The solution was concentrated, and the residue was purified on a silica gel column (2.3 x 8.5 cm) with 2-8% EtOH in $CHCl_3$ to give **22** as colorless gum, which was used for the next reaction without further purification. After coevaporation three times with anhydrous pyridine, and diisopropylethylamine (110 μL, 0.66 mmol) and 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (106 μL, 0.48 mmol) were added to a CH_2Cl_2 solution of **22**. The mixture was stirred for 0.5 h at room temperature, then partitioned with CH_2Cl_2 and sat. $NaHCO_3$ solution. The organic layer was separated, washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified on a neutralized silica gel column (2.8 x 6.5 cm) with 50-66% acetone in EtOAc to give **23** (182 mg, 62% as a pale yellow gum): FAB-MS *m/z* 823 (M^++1). ^{31}P-NMR (202 MHz, $CDCl_3$, 85% H_3PO_4 as an internal standard) 149.04, 148.68. HR-FAB Calcd for $C_{44}H_{56}O_6N_8P$ (M^++1); 823.4060. Found: 823.4072.

Synthesis of Oligonucleotides. Oligonucleotides were synthesized on a DNA synthesizer (Applied Biosystem Model 381A) by the phosphoramidite method.[15] Then fully protected oligonucleotides were deblocked and purified by the same procedure as for the purification of normal oligonucleotides. Each oligonucleotide linked to the resin was treated with conc. NH_4OH at 55 °C for 5 h, and the released oligonucleotide was purified on a C-18 silica gel column (1 x 10 cm, Waters) with a linear gradient of CH_3CN from 0 to 30% in 0.1 M TEAA buffer (pH 7.0). The fractions containing the desired product were concentrated, and the residue was treated with aqueous 80% AcOH at room temperature for 20 min, then the solution was concentrated, and the residue was coevaporated several times with water. The residue was dissolved in water, and the solution was washed with Et_2O, then the H_2O layer was concentrated to give a deprotected oligonucleotide. The purity of the dodecamers were higher than 99% according to HPLC analysis with a C-18 silica gel column (Inertsil ODS-2, GL Science Inc.).

Complete Hydrolysis of Oligonucleotides. A solution containing alkaline phosphatase (0.4 units) (Takara Shuzo Co.) in 0.1 M Tris-HCl (pH 7.7) and 2 mM $MgCl_2$ (total 130 μL) was heated in boiling water for 10 min to inactivate adenosine deaminase activity contaminating in the enzyme solution. The above solution and snake venom phosphodiesterase (10 μg) (Boehringer Mannheim) were added to each dodecamer (0.2 OD units), and the whole was incubated at 37 °C for 24 h. After it was heated in boiling water for 5 min, cold EtOH (313 μL) was added to the mixture, and the whole was kept at -20 °C for 1 h. The cold solution was centrifuged for 20 min (12,000 rpm), then the supernatant was separated and concentrated. The residue was analyzed by HPLC with the C-18 column.

Thermal Denaturation. Each solution contained the dodecamer (6 μM) in a buffer containing 0.01 M sodium phosphate, 0.02 M NaCl (pH 7.0). Thermally induced transition of each mixture was monitored at 260 nm on a Perkin Elmer Lambda2S.

Partial Hydrolysis of Oligonucleotide with Snake Venom Phosphodiesterase. Each oligonucleotide labeled with ^{32}P at the 5' end (10 pmol)[19] was incubated with snake venom phosphodiesterase (0.8 μg) in the presence of Torula RNA (0.3 OD units at 260 nm) in a buffer containing 37.5 mM Tris-HCl (pH 8.0) and 7.5 mM $MgCl_2$ (total 20 μL) at 37 °C. Samples of the reaction mixture were separated and added to a solution of EDTA (5 mM, 20 μL) at appropriate times, and then the mixtures were heated at 100 °C for 3 min. The solutions were analyzed by electrophoresis on 20% polyacrylamide gel containing 8 M urea.[19]

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(*Received in Japan* 27 *November* 1995; *accepted* 25 *December* 1995)