Pergamon

Tetrahedron 56 (2000) 2615–2627

TETRAHEDRON

Synthesis and Hybridization Property of Oligonucleotides Containing Carbocyclic Oxetanocins

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Received 24 January 2000; accepted 21 February 2000

Abstract—Oligonucleotides containing enantiomeric carbocyclic oxetanocins possessing adenine, thymine, guanine and cytosine were synthesized from an optically active cyclobutane derivative. Their hybridization properties with the complementary oligonucleotides were studied using melting point method, CD spectroscopy, and mixing curve method (Job plots). The artificial nucleotides possessing adenine, guanine and cytosine bases show tendency to form complexes more strongly with ribonucleotide than deoxyribonucelotide. These complexes are triplexes consisting of purine and pyrimidine in 1:2 ratio. Such triplex formation is observed both under high (1 M NaCl) and low salt conditions (0.1 M NaCl).

Hybridization properties of artificial oligonucleotides with complementary natural oligonucleotides are attracting much attention in the field of nucleic acid chemistry. Various oligonucleotides modified at the base, phosphate, and sugar moiety have been prepared.[1] Oxetanocin A, isolated from the culture filtrate of *Bacillus megaterium*, is an isomer of deoxyadenosine with an oxetane sugar moiety (Fig. 1),[2] and shows potent antiviral, antitumor, and antibacterial activities. Carbocyclic oxetanocin A is later developed to improve chemical and physiological stability of the oxetanocin A.[3] The unique structure and potent biological activity of these nucleosides led some researchers to study property of oligonucleotides containing four-membered sugar moiety. Baschang synthesized nucleotides possessing achiral 3,3-bis(hydroxymethyl)cyclobutane sugar with adenine and thymine bases, and found high affinity of the adenine containing oligomer to poly-rU.[4] Matsuda studied oligonucleotides containing an oxetanocin A.[5] Described here are the synthesis and hybridization properties of oligonucleotides containing carbocyclic oxetanocins (Fig. 2).[6,7] Enantiomeric carbocyclic oxetanocins possessing four bases were synthesized, and their hybridization property was studied systematically.

Phosphoramidite derivatives were prepared in order to apply the solid phase oligonucleotide synthesis. The strategy involves the introduction of bases to cyclobutyl tosylate **1**[8] and the differentiation of two primary hydroxy groups. Optically active tosylate **1** was converted to adenosine **2** according to the method of Bisacchi (Scheme 1).[9] Since reaction of **1** with thymine resulted in a lower yield (44%), a stepwise method was employed: Reaction of **1** with

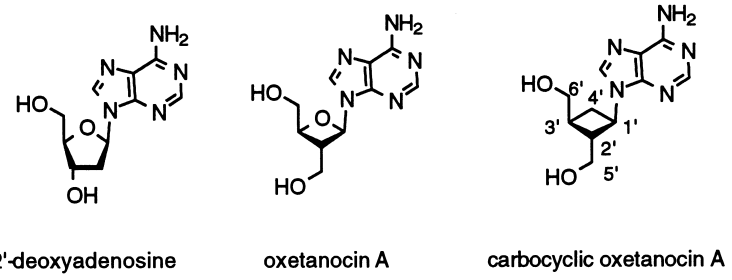

Figure 1.

Keywords: oligonucleotides; carbocyclic oxetanocins; hybridization.
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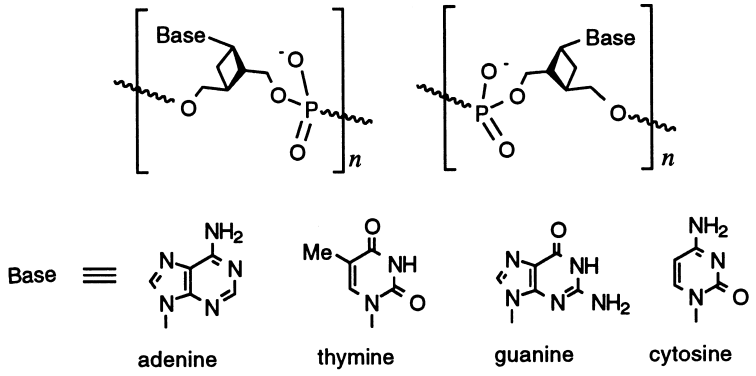
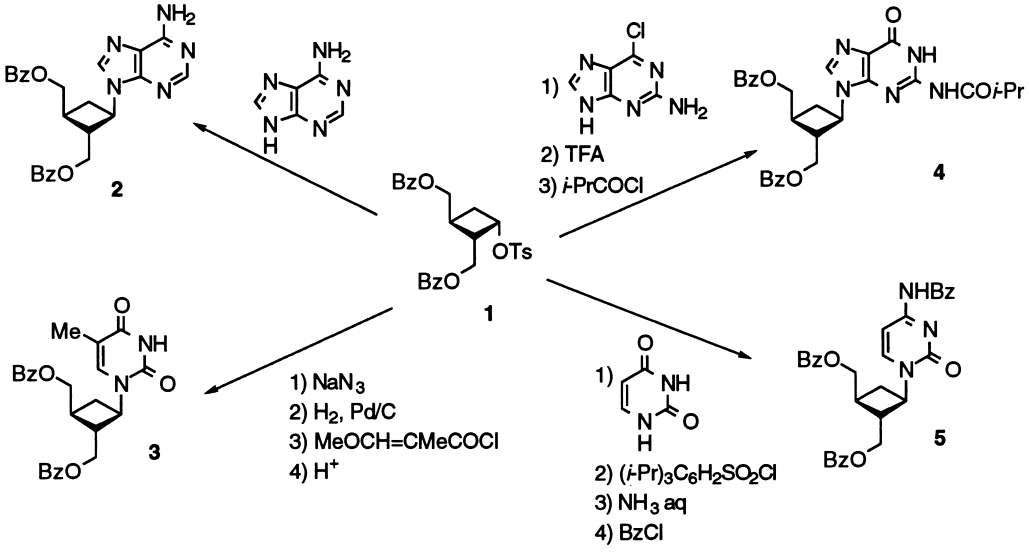
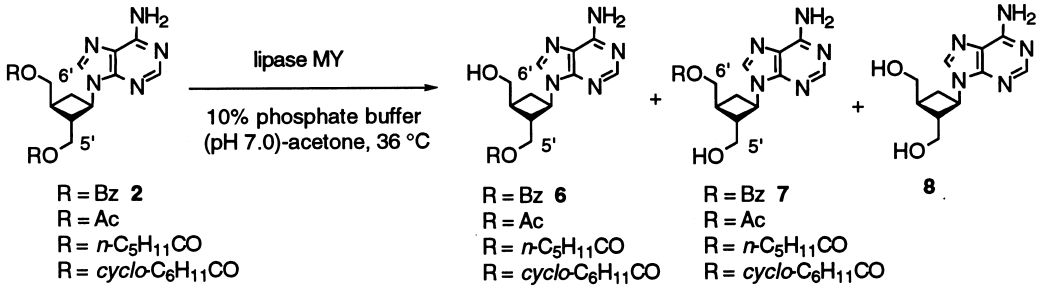


Figure 2.

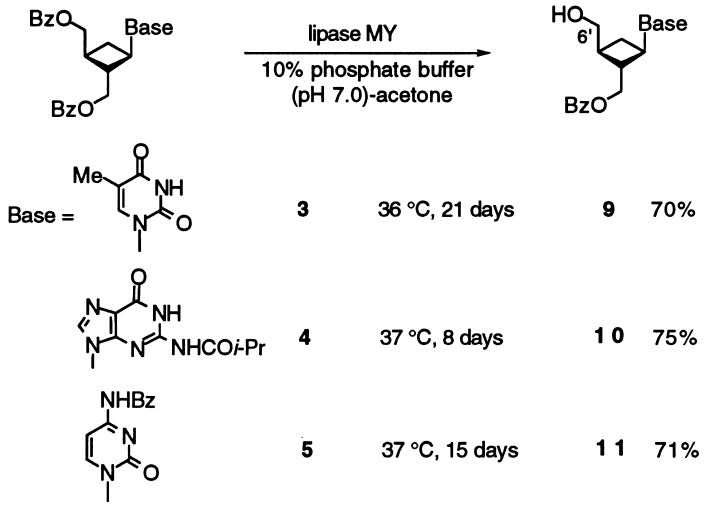


Scheme 1.

Table 1. Enzymatic hydrolysis of adenosine diester (nd: not detected)



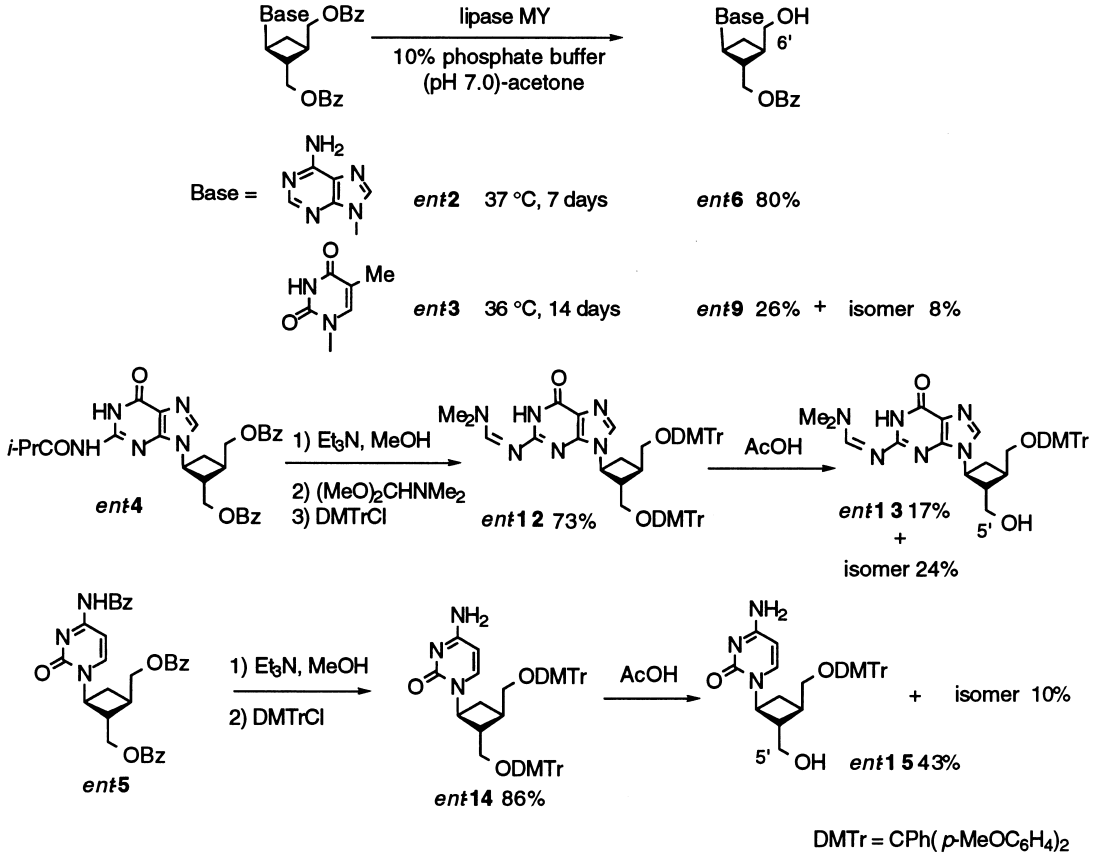
Entry	R	Time (day)	Yield (%)			
			5′-ester	6′-ester	**8**	Recovered diester
1	CH_3CO	7	51	Trace	nd	38
2		21	70	Trace	4	24
3	C_6H_5CO	6	87	nd	2	nd
4	n-$C_5H_{11}CO$	6	2	4	4	90
5	*cyclo*-$C_6H_{11}CO$	6	2	3	1	95



Scheme 2.

NaN_3 followed by hydrogenation, amidation, and acid catalyzed cyclization gave **3** (65% from **1**).[10] Guanylation was conducted by the alkylation with 2-amino-6-chloropurine[11] followed by acid hydrolysis and amine protection giving **4** (32% from **1**). Uracil derivative synthesized from **1** was converted to cytosine **5** by amination (36% from **1**).[12] Antipodes *ent*-**2**, *ent*-**3**, *ent*-**4**, and *ent*-**5** were synthesized from the enantiomeric tosylate *ent*-**1** employing the same synthetic procedures.

Discrimination of the two primary hydroxy groups was next examined.[13] While alkaline hydrolysis of the adenosine **2** showed no selectivity, enzymatic method turned out to be effective (Table 1). Treatment of **2** with lipase MY (Meito) in acetone–phosphate buffer (pH 7.0) gave 5′-*O*-monobenzoate **6** selectively (entry 3). Very small amounts of the regioisomer **7** and diol **8** were obtained. The structures of the isomers were determined unambiguously by 2D-NMR studies. Notably, diacetate as well as dihexanoate



Scheme 3.

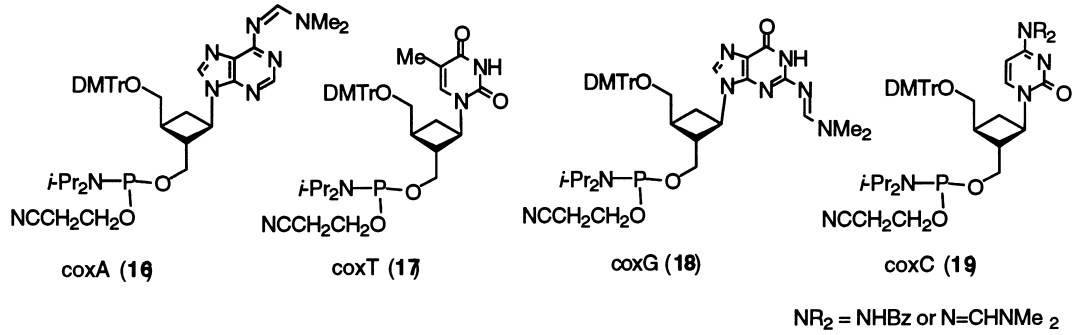


Figure 3.

Table 2. Artificial oligonucleotides

Oligonucleotide		MALDI-TOF MS *m/z*		
		Calcd		Found
5′-coxA$_{15}$dA-3′	**20**	4917.3	$C_{175}H_{223}N_{80}O_{63}P_{15}$	4918.2
5′-(*ent*-coxA)$_{14}$dA-3′	**21**	4606.2	$C_{164}H_{209}N_{75}O_{59}P_{14}$	4606.7
5′-coxT$_{15}$dT-3′	**22**	4773.1	$C_{172}H_{239}N_{32}O_{95}P_{15}$	4772.9
5′-(*ent*-coxT)$_{15}$dT-3′	**23**	4773.1	$C_{172}H_{239}N_{32}O_{95}P_{15}$	4773.7
5′-dA$_4$(coxGdA)$_4$-3′	**24**	3750.8	$C_{124}H_{152}N_{60}O_{58}P_{11}$	3749.9
5′-dA$_4$[(*ent*-coxG)dA]$_4$-3′	**25**	3750.8	$C_{124}H_{152}N_{60}O_{58}P_{11}$	3751.4
5′-(dTcoxC)$_4$dT$_4$-3′	**26**	3518.7	$C_{120}H_{161}N_{28}O_{74}P_{11}$	3518.1
5′-[dT(*ent*-coxC)]$_4$dT$_4$-3′	**27**	3518.7	$C_{120}H_{161}N_{28}O_{74}P_{11}$	3518.6

and dicyclohexanecarboxylate reacted more slowly (entries 1, 2, 4 and 5). The enzyme hydrolyzed benzoate faster than acetate, although the former is chemically less reactive. As for the organic solvent, acetone was superior to 1,4-dioxane, pyridine, and ethanol. Acetonitrile gave comparable results with acetone.

Thymidine **3**, guanosine **4** and cytidine **5** were also selectively monodebenzoylated at 6′-*O*-position giving **9**, **10**, and **11**, respectively (Scheme 2). Dibenzoate again reacted in the case of **3** faster than diacetate. The lipase MY turned out to be insensitive to the structure of bases in this series.

The enantiomeric adenosine *ent*-**2** was also hydrolyzed to *ent*-**6** by lipase MY in high selectivity at 6′-*O*-position (Scheme 3). In contrast, thymidine *ent*-**3** was not a good substrate, and slow reaction gave a mixture of the regioisomers containing *ent*-**9**. Reactions of guanosine *ent*-**4** and cytidine *ent*-**5** were too slow to be practical. The lipase MY appears to recognize the base part in this enantiomeric series. Then, *ent*-**4** and *ent*-**5** were converted to bistrityl derivative *ent*-**12** and *ent*-**14**, and monodetritylation was conducted. Using acetic acid *ent*-**14** was converted to 5′-*O*-deprotected *ent*-**15** predominantly. In contrast, *ent*-**12** showed no selectivity in the deprotection giving *ent*-**13**.

Alcohols **6**, *ent*-**6**, **9**, *ent*-**9**, **10**, and **11** were transformed to coxA (**16**), *ent*-coxA (*ent*-**16**), coxT (**17**), *ent*-coxT (*ent*-**17**), coxG (**18**), and coxC (**19**) (NR_2=BzNH), respectively, by

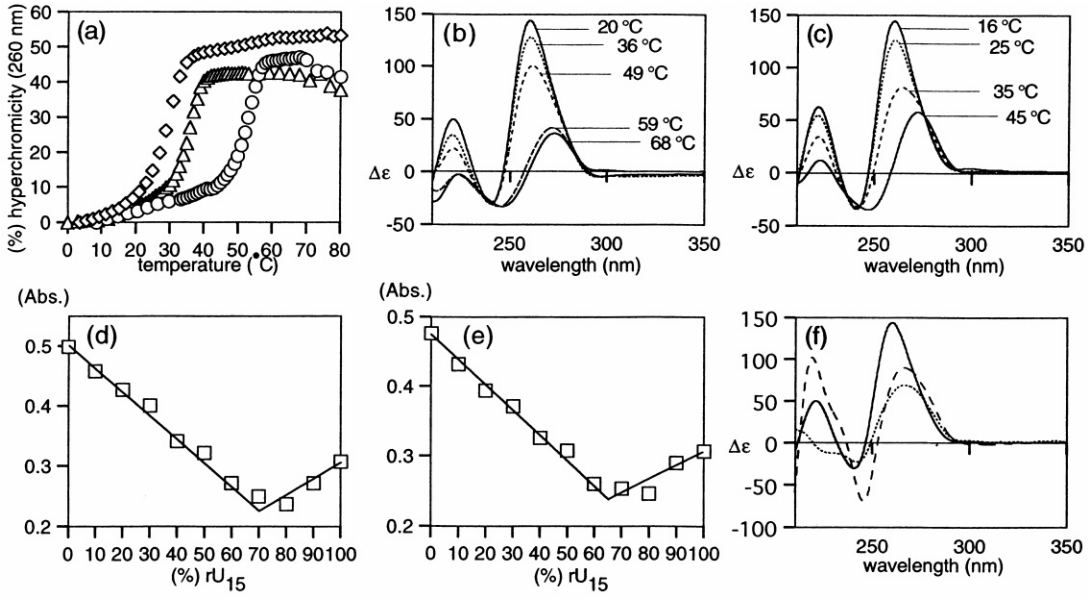


Figure 4. Hybridization property of **20** and **21**. All the experiments were conducted in 10 mM phosphate buffer at pH 7.0. The concentration of total strands is 2.1 μM. (a) Melting profiles of **20**/rU$_{15}$ (1:1) in 1 M NaCl (○), **20**/rU$_{15}$ (1:1) in 0.1 M NaCl (△), and **20**/dT$_{15}$ (1:1) in 1 M NaCl (◇). The profiles were recorded at 260 nm with a temperature ramp of 0.5°C/min. (b) Variable temperature CD spectra of **20**/rU$_{15}$ (1:2) in 1 M NaCl. (c) Variable temperature CD spectra of **20**/rU$_{15}$ (1:2) in 0.1 M NaCl. (d) Job plots of **20**/rU$_{15}$ at 5°C in 1 M NaCl. (e) Job plots of **20**/rU$_{15}$ at 5°C in 0.1 M NaCl. (f) CD spectra of dA$_{15}$/rU$_{15}$ (1:2) at 20°C in 1 M NaCl (—), **20**/rU$_{15}$ (1:2) at 16°C in 1 M NaCl (···), and **21**/rU$_{15}$ (1:2) at 5°C in 1 M NaCl (– – –).

Table 3. Complexation of **20**, **21**, **22** and **23** with complementary oligonucleotides. Shown are T_m at 1 M NaCl. Shown in ( ) are T_m at 0.1 M NaCl. Shown in [ ] are complex composition of adenine and thymine (uracil) derivative obtained by Job plots. nd: No determinable T_m

	coxA$_{15}$dA (**20**)		(*ent*-coxA)$_{14}$dA (**21**)	dA$_{15}$		rA$_{15}$	
coxT$_{15}$dT (**22**)	34 [1:2]			nd		nd	
(*ent*-coxT)$_{15}$dT (**23**)			34 [1:2]	nd		nd	
dT$_{15}$	30 [1:2]		11	51[a] [1:2]	(42) [1:1]	43 [1:2]	(32) [1:1]
rU$_{15}$	54 [1:2]	(36) [1:2]	36 [1:2]	34 [1:2]	(12)	40[a] [1:2]	(25) [1:2]

[a] T_m of dA$_{15}$/rU$_{15}$ and rA$_{15}$/rU$_{15}$ in our previous report should be corrected.[7]

tritylation, debenzoylation and amiditization including appropriate protection steps (Fig. 3). The protected *ent*-coxG (*ent*-**18**) and *ent*-coxC (*ent*-**19**) (NR_2=N=CH–NMe_2) were synthesized from *ent*-**13** and *ent*-**15** by amiditization and protection. These eight optically active amidites were converted to oligonucleotides **20** to **27** shown in Table 2 by the solid phase synthesis. Their structures were confirmed by MALDI-TOF MS.

Hybridization of the artificial oligonucleotides **20** to **23** with the complementary natural and artificial oligonucleotides were studied by melting point method, CD spectroscopy, and mixing curve method (Job plots). The melting behaviors were first examined under high salt conditions (1 M NaCl). The compound **20** containing coxA forms complexes with both natural dT$_{15}$ and rU$_{15}$ (Fig. 4a). Variable temperature CD spectra support such behaviors (Fig. 4b and 4c). The melting point of the complex **20**/rU$_{15}$ (T_m=54°C) is much higher than **20**/dT$_{15}$ (T_m=30°C) indicating that **20** prefers RNA derivative than DNA derivative.[14] It should also be

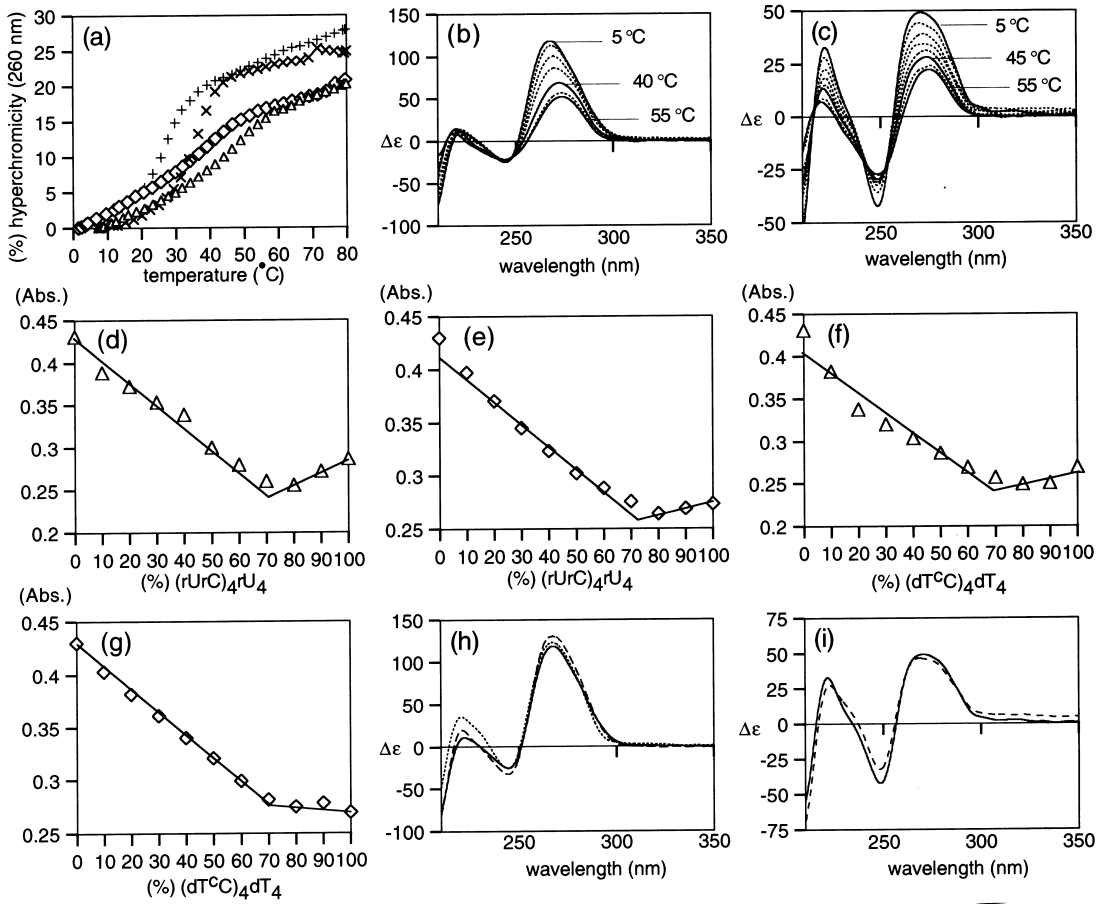


Figure 5. Hybridization properties of **24** and **26**. All the experiments were conducted in 10 mM phosphate buffer at pH 7.0. The concentration of total strands is 2.6 μM. (a) Melting profiles of **24**/(rUrC)$_4$rU$_4$ (1:1) in 1 M NaCl (×), **24**/(rUrC)$_4$rU$_4$ (1:1) in 0.1 M NaCl (+), **26**/rA$_4$(rGrA)$_4$ (1:1) in 1 M NaCl (△), and **26**/rA$_4$(rGrA)$_4$ (1:1) in 0.1 M NaCl (◇). The profiles were recorded at 260 nm with a temperature ramp of 0.5°C/min. (b) Variable temperature CD spectra of **24**/(rUrC)$_4$rU$_4$ (1:2) in 1 M NaCl. (c) Variable temperature CD spectra of **26**/rA$_4$(rGrA)$_4$ (1:2) in 1 M NaCl. (d) Job plots of **24**/(rUrC)$_4$rU$_4$ at 5°C in 1 M NaCl. (e) Job plots of **24**/(rUrC)$_4$rU$_4$ at 5°C in 0.1 M NaCl. (f) Job plots of **26**/rA$_4$(rGrA)$_4$ at 5°C in 1 M NaCl. (g) Job plots of **26**/rA$_4$(rGrA)$_4$ at 5°C in 0.1 M NaCl. (h) CD spectra of **24**/(rUrC)$_4$rU$_4$ (1:2) in 1 M NaCl (···), in 0.1 M NaCl (– – –), and dA$_4$(dGdA)$_4$/(rUrC)$_4$rU$_4$ (1:2) in 1 M NaCl (—) all at 5°C. (i) CD spectra of **26**/rA$_4$(rGrA)$_4$ (2:1) in 1 M NaCl (—) and 0.1 M NaCl (– – –) at 5°C.

Table 4. Complexation of **24**, **25**, **26** and **27** with complementary oligonucleotides. Shown are T_m in 1 M NaCl. Shown in ( ) are T_m in 0.1 M NaCl. Shown in [ ] are complex compositions of guanine and cytosine derivative obtained by Job plots. nd: No determinable T_m

	dA_4(coxGdA)$_4$ (**24**)		dA_4[(*ent*-coxG)dA]$_4$ (**25**)	dA_4(dGdA)$_4$		rA_4(rGrA)$_4$	
(dTcoxC)$_4$dT$_4$ (**26**)	24		nd	30	(24)	45	(33)
	[1:2]			[1:2]		[1:2]	[1:2]
[dT(*ent*-coxC)]$_4$dT$_4$ (**27**)	nd		nd	nd		nd	
(dTdC)$_4$dT$_4$	36	(26)	7	45	(32)	55	(45)
	[1:2]	[1:2]	[1:2]	[1:2]	[1:1]	[1:1]	[1:1]
(rUrC)$_4$rU$_4$	39	(27)	31	33	(19)	60	(49)
	[1:2]	[1:2]	[1:2]	[1:2]		[1:1]	[1:1]

noted that T_m of **20**/rU_{15} is even higher than those of dA_{15}/rU_{15} and rA_{15}/rU_{15}, T_m=34 and 40°C, respectively (Table 3). Thus, coxA possesses higher affinity to rU than the natural dA and rA. The complexation of **20**/rU_{15} is strong enough that the binding is observed even under low salt conditions (0.1 M NaCl) with T_m=36°C. Mixing curve study (Job plots) of the complex **20**/rU_{15} at the high-salt conditions and even at the low salt conditions indicates the formation of 1:2 complex probably with the triple helix structure (Fig. 4d and e). The CD spectra shows that the structure of the triplex **20**/rU_{15} under the two salt conditions are identical (Fig. 4b and c). This contrasts with the observation that the natural dA_{15}/rU_{15} forms triplex at high salt conditions and duplex at low salt conditions.[15]

Enantiomeric oligonucleotide **21** containing *ent*-**16** also shows tendency to form complex with RNA more strongly than DNA, although the melting points are lower than **20**, T_m=36 and 11°C, respectively (Table 3). The combination of **21**/rU_{15} again gives 1:2 complex in 0.1 M NaCl. The similarlity of the CD spectra of **20**/rU_{15} (1:2) and **21**/rU_{15} (1:2) suggests the similar structure of the triplex in spite of the enantiomeric nature of **20** and **21** (Fig. 4f). It appears that the absolute configuration of the four membered sugar moiety has rather small effect on the hybridization property. While adenosine derivatives **20** and **21** strongly bind to RNA, thymidine derivatives **22** and **23** containing **17** and *ent*-**17** do not bind to the complementary natural dA_{15} and rA_{15} even under the high salt conditions.

Hybridization behaviors of the artificial oligonucleotides **24** to **27** containing coxG and coxC are examined using the same methodologies (Fig. 5 and Table 4). In order to avoid self-aggregation of homooligomers,[16] oligonucleotides with alternating sequences are employed. The oligonucleotide **24** containing coxG shows a slightly higher affinity to RNA derivative (rUrC)$_4$rU$_4$ than DNA derivative (dTdC)$_4$dT$_4$, T_m=39 and 36°C in 1 M NaCl, respectively (Fig. 5a and b). The melting point of the RNA complex **24**/(rUrC)$_4$rU$_4$ is even higher than T_m=33°C of the natural combination dA_4(dGdA)$_4$/(rUrC)$_4$rU$_4$. Thus, coxG binds to rC more strongly than the natural dG. Both under the high and low salt conditions **24** and (rUrC)$_4$rU$_4$ form 1:2 complex (Fig. 5d and e). The structure of the triplex may be similar to that of the natural triplex formed from (rUrC)$_4$rU$_4$ and dA_4(dGdA)$_4$ as indicated by CD spectra (Fig. 5h). It has now become clear that that coxA and coxG, which possess cyclobutane sugar moiety and purine bases, bind very strongly to natural rU and rC in 1:2 ratio. Oligonucleotide **25** containing *ent*-coxG also shows higher affinity to RNA than DNA, T_m=31 and 7°C in 1 M NaCl, respectively. As was in the case of coxA and *ent*-coxA the hybridization properties of coxG and *ent*-coxG are somehow similar.

Oligonucleotide **26** containing coxC also prefers RNA derivative rA_4(rGrA)$_4$ to DNA derivative dA_4(dGdA)$_4$, T_m=45 and 30°C in 1 M NaCl, respectively (Fig. 5a and c). In addition, **26**/rA_4(rGrA)$_4$ exhibits T_m more clearly than **26**/dA_4(dGdA)$_4$ (data not shown). Triplex is formed both at the high and low salt concentration (Fig. 5f and g) with the same structure as indicated by CD spectra (Fig. 5i). The natural combination rA_4(rGrA)$_4$/(dTdC)$_4$dT$_4$ gives T_m=55°C forming 1:1 complex under the high salt conditions. Thus, affinity of coxC to rG is weaker than dC, although coxC still appears to possess substantial hybridization ability. It should also be emphasized that, as was coxA and coxG, triplex formation is a characteristic aspect of coxC. Oligonucleotide **27** which contains *ent*-coxC does not form complex with the complementary oligonucleotides.

To summarize, carbocyclic oxetanocins A, G and C show a tendency to form stronger complexes with ribonucleotide rather than with deoxyribonucleotide. All these nucleotides prefer triplex formation consisting of purine and pyrimidine nucleotides in 1:2 ratio. It is highly likely that various sequences of oligonucleotides with the cyclobutane sugar can strongly bind to the complementary natural RNA.

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus without correction. Optical rotations were measured on a JASCO DIP-340 digital polarimeter. IR spectra were measured on JASCO FT/IR-400 spectrophotometer. UV spectra were measured on BECKMAN DU 640 and Hitachi U-3000 UV–VIS spectrophotometer. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were recorded on a Varian Mercury NMR (400 MHz) or a Brucker AM-600 (600 MHz) with Me_4Si as an internal standard or PPh_3 (δ −6.0) as an external standard. Mass spectra were recorded on a JEOL JMS-DX-303 or JMS-AX-500 spectrometer. FAB mass spectra were measured using *m*-nitrobenzyl alcohol matrix. MALDI TOF mass spectra were obtained by PerSeptive Biosystems Voyager DE using 3-hydroxy-2-picolinic acid as a matrix.

(1′*S*,2′*S*,3′*R*)-9-[2′,3′-Bis(benzoyloxymethyl)cyclobutyl]-adenine (*ent*-2). Prepared according to Ref. 9 starting from *ent*-**1**. $[\alpha]_D^{23}$=+31.7 (*c* 0.99, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-9-[2′,3′-Bis(acetoxymethyl)cyclobutyl]-adenine. A mixture of **8**[9] (249 mg, 1.0 mmol) and acetic anhydride (0.6 mL) in pyridine (5 mL) was stirred at room temperature for 1 h. After addition of excess methanol, the volatile materials were removed in vacuo, and the residue was chromatographed over silica gel ($CHCl_3$–MeOH, 5:1) giving diacetate (312 mg, 94%). Mp 140–141°C ($CHCl_3$–hexane). IR ($CHCl_3$) 3440, 1740, 1635, 1220 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 2.01 (3H, s), 2.13 (3H, s), 2.32–2.44 (1H, m), 2.55 (1H, dt, J=11.0, 9.5 Hz), 2.65 (1H, dt, J=11.2, 8.2 Hz), 3.19 (1H, apparent tt, J=8.8, 5.5 Hz), 4.17–4.31 (4H, m), 4.70 (1H, apparent q, J=9.0 Hz), 5.72 (2H, brs), 7.85 (1H, s), 8.34 (1H, s). HRMS *m/z* Calcd for $C_{15}H_{19}N_5O_4$: 333.1437. Found: 333.1431. $[\alpha]_D^{26}$=−33.6 (*c* 1.00, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-9-[2′,3′-Bis(hexanoyloxymethyl)cyclobutyl]-adenine. IR ($CHCl_3$) 3412, 1732, 1631, 1247, 1221 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 0.82–0.95 (6H, m), 1.16–1.44 (6H, m), 1.46–1.56 (2H, m), 1.58–1.84 (4H, m), 2.18–2.25 (2H, m), 2.30–2.42 (3H, m), 2.48–2.68 (2H, m), 3.12–3.24 (1H, m), 4.16–4.31 (4H, m), 4.69 (1H, apparent q, J=8.6 Hz), 5.59 (2H, brs), 7.84 (1H, s), 8.33 (1H, s). HRMS *m/z* Calcd for $C_{23}H_{35}N_5O_4$: 445.2689. Found: 445.2703. $[\alpha]_D^{20}$=−27.6 (*c* 1.00, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-9-[2′,3′-Bis(cyclohexanecarbonyloxymethyl)-cyclobutyl]adenine. Mp 128–129°C ($CHCl_3$–hexane). IR ($CHCl_3$) 3413, 1727, 1631, 1248, 1221 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.10–1.37 (6H, m), 1.40–1.53 (2H, m), 1.55–1.82 (10H, m), 1.89–1.98 (2H, m), 2.14–2.24 (1H, m), 2.30–2.42 (2H, m), 2.46–2.67 (2H, m), 3.14–3.24 (1H, m), 4.15–4.30 (4H, m), 4.70 (1H, apparent q, J=8.1 Hz), 5.57 (2H, brs), 7.84 (1H, s), 8.34 (1H, s). HRMS *m/z* Calcd for $C_{25}H_{35}N_5O_4$: 469.2689. Found: 469.2722. $[\alpha]_D^{21}$=−25.5 (*c* 1.00, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-1-[2′,3′-Bis(benzoyloxymethyl)cyclobutyl]-thymine (3) by one-pot synthesis. Under an argon atmosphere, a mixture of **1** (267 mg, 0.54 mmol), thymine (111 mg, 0.88 mmol), K_2CO_3, (89 mg, 0.64 mmol), and 18-crown-6 (212 mg, 0.80 mmol) in dry DMF (5 mL) was heated at 100°C for 20 h. After removal of the solvent in vacuo the residue was diluted with AcOEt, and the solution was washed with saturated aqueous NH_4Cl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, and concentrated in vacuo. Silica gel chromatogrophy (AcOEt–hexane, 1:1) followed by recrystallization from AcOEt gave **3** (99 mg, 44%). Mp 112–113°C. IR ($CHCl_3$) 3400, 1712, 1685, 1270 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.84 (3H, d, J=1.1 Hz), 2.19 (1H, apparent q, J=10.6 Hz), 2.42–2.53 (1H, m), 2.58 (1H, dt, J=10.8, 7.9 Hz), 3.01 (1H, tt, J=9.0, 5.7 Hz), 4.41–4.52 (4H, m), 4.75 (1H, apparent q, J=9.1 Hz), 7.38–7.50 (4H, m), 7.52–7.62 (2H, m), 7.94–8.10 (4H, m), 8.25 (1H, brs). HRMS *m/z* Calcd for $C_{25}H_{24}N_2O_6$: 448.1634. Found: 448.1645. $[\alpha]_D^{26}$=−23.0 (*c* 1.00, $CHCl_3$). **Stepwise synthesis:** Under an argon atmosphere, a mixture of **1** (2.88 g, 5.82 mmol) and lithium azide (314 mg, 6.41 mmol) in dry DMF (50 mL) was heated at 100°C for 15 h. The solvent was removed in vacuo, and the residue was chromatographed over silica gel (AcOEt–hexane, 1:5) to give (1*R*,2*S*,3*S*)-1-azido-2,3-bis-(benzoyloxymethyl)cyclobutane (2.02 g, 95%). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.97 (1H, dt, J=10.8, 9.0 Hz), 2.30–2.52 (2H, m), 2.71 (1H, tt, J=11.4, 5.5 Hz), 3.74 (1H, apparent q, J=8.2 Hz), 4.35 (1H, dd, J=11.4, 5.9 Hz), 4.41 (1H, dd, J=11.4, 5.1 Hz), 4.43 (2H, apparent d, J=6.1 Hz), 7.40–7.47 (m, 4H), 7.53–7.59 (m, 2H), 8.02–8.07 (m, 4H). Under a hydrogen atmosphere, a suspension of the azide (1.96 g, 5.38 mmol) and 5% Pd/C in ethanol (20 mL) was stirred at room temperature for 10 h. Insoluble materials were filtered by passing through celite, and the filtrate was concentrated in vacuo. Silica gel chromatography (AcOEt–MeOH, 10:1) gave (1*R*,2*R*,3*S*)-1-amino-2,3-bis(benzoyloxymethyl)cyclobutane (1.75 g, 96%). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.54 (1H, apparent q, J=10.5 Hz), 2.15–2.32 (2H, m), 2.46 (1H, dt, J=10.6, 7.5 Hz), 3.25 (1H, apparent q, J=8.3 Hz), 4.33 (1H, dd, J=11.2, 5.7 Hz), 4.39 (1H, dd, J=11.2, 5.3 Hz), 4.43 (2H, d, J=5.0 Hz), 7.34–7.46 (m, 4H), 7.52–7.58 (m, 2H), 8.00–8.06 (m, 4H). HRMS *m/z* Calcd for $C_{20}H_{21}NO_4$: 339.1471. Found: 339.1456. Under an argon atmosphere, to a toluene (80 mL) suspension of AgOCN (2.99 g, 21.9 mmol) was added 3-methoxy-2-methyl-2-butenoyl chloride (2.91 g, 19.3 mmol), and the mixture was stirred at 80°C for 1 h. After cooled to room temperature, the supernatant was added to the aminocyclobutane (1.55 g, 4.57 mmol) in dry toluene (50 mL) under an argon atmosphere. The mixture was stirred at room temperature for 4 h. Then the solvent was removed in vacuo, and the residue was chromatographed over silica gel (AcOEt–hexane, 1:4 to 1:1) giving *N*-[(1*R*,2*R*,3*S*)-2,3-bis(benzoyloxymethyl)cyclobutyl]-*N*′-(3-methoxy-2-methyl-2-butenoyl)urea (2.17 g, 99%). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.77 (3H, d, J=1.1 Hz), 1.85 (1H, apparent q, J=10.8 Hz), 2.35–2.46 (1H, m), 2.55 (1H, dt, J=10.8, 7.9 Hz), 2.61 (1H, tt, J=8.6, 5.7 Hz), 3.84 (3H, s), 4.22 (1H, apparent dq, J=8.3 Hz), 4.35 (1H, dd, J=11.4, 6.1 Hz), 4.40 (1H, dd, J=11.5, 5.5 Hz), 4.43 (1H, dd, J=11.9, 5.7 Hz), 4.49 (1H, dd, J=11.5, 5.5 Hz), 7.31 (2H, d, J=1.1 Hz), 7.36–7.45 (m, 4H), 7.50–7.58 (m, 2H), 8.00–8.06 (m, 4H), 8.30–8.42 (br, 1H), 9.04 (1H, brd). A mixture of the urea (2.01 g, 4.19 mmol) in 1,4-dioxane (100 mL) and 1 M H_2SO_4 (20 mL) was stirred at 100°C for 100 min. The solvents were removed in vacuo, and the residue was chromatographed over silica gel (AcOEt–hexane, 1:1 to AcOEt–MeOH, 10:1) giving **3** (1.35 g, 72%).

(1′*S*,2′*S*,3′*R*)-1-[2′,3′-Bis(benzoyloxymethyl)cyclobutyl]-thymine (*ent*-3). $[\alpha]_D^{28}$=+23.9 (*c* 1.05, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-9-[2′,3′-Bis(benzoyloxymethyl)cyclobutyl]-N^2-isobutyrylguanine (4). Under an argon atmosphere to a solution of **1** (3.00 g, 8.8 mmol) in DMF (50 mL) was added ammonium salt of 2-amino-6-chloropurine[11] (4.1 g, 9.6 mmol), and the mixture was stirred for 10 h at 100°C. After cooling to room temperature, the solvent was removed in vacuo, and the residue was diluted with AcOEt. The solution was washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was dissolved in trifluoroacetic acid–water (50 mL, 3:1), and the mixture was stirred at room temperature for 12 h. The solvent was removed in vacuo, and the residue was diluted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was dissolved in pyridine (12 mL), to which was added isobutyryl chloride (300 μL, 11.0 mmol), and the mixture was stirred at 0°C for

30 min. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ (50 mL) at 0°C, and the organic materials were extracted with CH_2Cl_2. The organic layer was washed with aqueous 10 % citric acid, water and brine, dried over $MgSO_4$, and concentrated. Purification by silica gel flash column chromatography (AcOEt) gave **4** (1.53 g, 32%). Mp 102.5–103.0°C (AcOEt–hexane). ^{1}H NMR ($CDCl_3$) δ 1.31 (6H, d, J=6.8 Hz), 2.54–2.80 (4H, m), 3.12–3.21 (1H, m), 4.50 (1H, dd, J=5.6, 11.6 Hz), 4.62–4.71 (3H, m), 4.75 (1H, dd, J=6.4, 11.2 Hz), 7.39–7.49 (4H, m), 7.54–7.62 (2H, m), 7.67 (2H, m), 7.87–7.92 (2H, m), 8.02–8.06 (2H, m), 8.8 (1H, brs), 11.8 (1H, brs). ^{13}C NMR ($CDCl_3$) δ 19.0, 19.1, 25.8, 30.8, 36.4, 45.9, 48.5, 64.8, 66.3, 110.1, 121.6, 128.36, 128.43, 129.2, 129.36, 129.4, 133.2, 137.1, 147.1, 148.1, 155.4, 166.3, 166.5, 178.5. IR (KBr) 3551, 3471, 3414, 1717, 1676, 1616, 1271 cm^{-1}. HRMS (EI) Calcd for $C_{29}H_{29}N_5O_6$: 543.2118. Found: 543.2112. $[\alpha]_D^{24}=-0.1$ (c 1.0, $CHCl_3$).

(1′*S*,2′*S*,3′*R*)-9-[2′,3′-Bis(benzoyloxymethyl)cyclobutyl]-*N*^{2}-isobutyrylguanine (*ent*-4). $[\alpha]_D^{24}=+0.1$ (c 1.0, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-1-[2′,3′-Bis(benzoyloxymethyl)cyclobutyl]-*N*^{4}-benzoylcytosine (5). Under an argon atmosphere to a solution of **1** (2.00 g, 4.04 mmol) in DMF (50 mL) was added K_2CO_3 (0.62 g, 4.4 mmol), uracil (0.91 g, 8.08 mmol), and 18-crown-6 (1.28 g, 8.08 mmol). The mixture was stirred for 10 h at 100°C. After cooling to room temperature, the solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate. The organic solution was washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was dissolved in CH_3CN (65 mL), to which Et_3N (0.75 mL) and DMAP (650 mg) were added. Then, under an argon atmosphere 2,4,6-triisopropylbenzenesulfonyl chloride (1.6 g, 8.1 mmol) was added, and the mixture was stirred at room temperature for 4.5 h. The reaction mixture was cooled to 0°C, and 28% aqueous NH_3 (27 mL) was added. After stirring for 6.5 h, the solvent was removed under reduced pressure. Under an argon atmosphere to a solution of this crude material in pyridine (20 mL) was added benzoyl chloride (317 μL, 4.44 mmol), and the mixture was stirred for 1 h at room temperature. Water was added, and the reaction mixture was diluted with AcOEt. The organic solution was washed with saturated aqueous $NaHCO_3$, water, and brine. After dried over Na_2SO_4, the solution was concentrated. Purification by silica gel chromatography (AcOEt–hexane, 2:1 to 1:1) gave **5**, which was recrystallized from $CHCl_3$–hexane (0.78 g, 36%). Mp 154.0–155.0°C. ^{1}H NMR ($CDCl_3$) δ 2.19 (1H, q, J= 10.1 Hz), 2.51–2.61 (1H, m), 2.73 (1H, dt, J=7.9, 10.7 Hz), 3.03–3.12 (1H, m), 4.42–4.50 (2H, m), 4.55 (1H, d, J=6.0 Hz), 4.84 (1H, q, J=8.9 Hz), 7.38–7.64 (10H, m), 7.38 (1H, d, J=7.2 Hz), 8.01 (2H, d, J=8.0 Hz), 7.96–8.01 (2H, m), 8.01–806 (2H, m). ^{13}C NMR ($CDCl_3$) δ 29.2, 31.2, 44.3, 52.8, 65.2, 66.3, 127.4, 128.3, 128.4, 128.9, 129.41, 129.44, 129.6, 133.08, 133.12, 145.2, 161.6, 166.1, 166.2. IR (KBr) 3551, 3477, 3141, 3062, 2951, 1718, 1661, 1271 cm^{-1}. HRMS (EI) Calcd for $C_{31}H_{27}N_3O_6$: 537.1900. Found: 537.1881. $[\alpha]_D^{22}=-39$ (c 1.0, $CHCl_3$).

(1′*S*,2′*S*,3′*R*)-1-[2′,2′-Bis(benzoyloxymethyl)cyclobutyl]-*N*^{4}-benzoylcytosine (*ent*-5). $[\alpha]_D^{24}=+37.6$ (c 0.95, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-9-[2′-Benzoyloxymethyl-3′-hydroxymethylcyclobutyl]adenine (6) and (1′*R*,2′*R*,3′*S*)-9-{3′-benzoyloxymethyl-2′-hydroxymethylcyclobutyl}adenine (7) by chemical hydrolysis. To a stirred dioxane (1 mL) solution of **2** (241 mg, 0.53 mmol) was added 1 M NaOH (0.5 mL) at 0°C. Then the mixture was warmed to room temperature, and stirred for 12 h. The solvent was removed in vacuo, and the residue was chromatographed over silica gel (AcOEt–hexane, 1:1 to AcOEt–MeOH, 3:1) giving **6** (19 mg, 10%), **7** (33 mg, 18%), and **8** (25 mg, 19%). **6**: IR ($CHCl_3$) 3400, 1718, 1630, 1590, 1270 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.41 (1H, apparent qt, J=8.8, 4.4 Hz), 2.63 (1H, dt, J=11.6, 8.8 Hz), 2.72 (1H, dt, J=11.6, 9.3 Hz), 3.45 (1H, apparent tt, J=8.6, 5.7 Hz), 3.81 (1H, dd, J=11.2, 4.4 Hz), 3.86 (1H, dd, J=11.2, 4.0 Hz), 4.06 (1H, brs), 4.48 (1H, dd, J=11.7, 5.5 Hz), 4.52 (1H, dd, J=11.5, 6.1 Hz), 4.82 (1H, apparent q, J=8.7 Hz), 6.32 (2H, brs), 7.34–7.40 (2H, m), 7.48–7.54 (1H, m), 7.84–7.88 (2H, m), 7.92 (1H, s), 8.30 (1H, s). HRMS m/z Calcd for $C_{18}H_{19}N_5O_3$: 353.1488. Found: 353.1488. $[\alpha]_D^{24}=-49.2$ (c 1.00, $CHCl_3$). **7**: Mp 170–171°C ($CHCl_3$–hexane). IR ($CHCl_3$) 3412, 1717, 1632, 1471, 1274 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.38–2.55 (2H, m), 2.68–2.85 (2H, m), 3.82 (2H, d, J=6.1 Hz), 4.37 (1H, dd, J=11.4, 5.5 Hz), 4.45 (1H, dd, J=11.4, 5.0 Hz), 4.55 (1H, apparent q, J=8.4 Hz), 5.00–5.80 (1H, brs), 6.09 (2H, brs), 7.38–7.44 (2H, m), 7.51–7.57 (1H, m), 7.81 (1H, s), 7.96–8.02 (2H, m), 8.26 (1H, s). HRMS m/z Calcd for $C_{18}H_{19}N_5O_3$: 353.1488. Found: 353.1451. $[\alpha]_D^{24}=+34.4$ (c 1.00, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-9-[2′-Benzoyloxymethyl-3′-(hydroxymethyl)-cyclobutyl]adenine (6) by enzymatic hydrolysis. A mixture of **2** (1.61 g, 3.53 mmol) and lipase MY (12 g, 3.6×10^5 units) in acetone (250 mL) and phosphate buffer (1/15 M, pH 7.0, 25 mL) was vigorously shaken at 36°C for 7 days. The insoluble materials were filtered, and the solvents were removed in vacuo. The residue was flash chromatographed over silica gel (AcOEt to AcOEt–MeOH, 4:1) giving **6** (1.08 g, 87%).

(1′*S*,2′*S*,3′*R*)-9-[2′-Benzoyloxymethyl-3′-(hydroxymethyl)-cyclobutyl]adenine (*ent*-6). $[\alpha]_D^{24}=+44.5$ (c 1.03, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-9-[2′-Acetoxymethyl-3′-(hydroxymethyl)-cyclobutyl]adenine. Mp 158–159°C ($CHCl_3$–hexane). IR ($CHCl_3$) 3240, 1735, 1630, 1220 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.99 (3H, s), 2.25–2.35 (1H, m), 2.58 (1H, dt, J=11.7, 8.6 Hz), 2.71 (1H, dt, J=11.9, 9.2 Hz), 3.34 (1H, apparent tt, J=8.4, 5.5 Hz), 3.76 (1H, dd, J=11.0, 4.0 Hz), 3.80 (1H, dd, J=11.2, 4.0 Hz), 4.16 (1H, dd, J=11.6, 5.1 Hz), 4.28 (1H, dd, J=11.5, 6.2 Hz), 4.68 (1H, apparent q, J=9.0 Hz), 5.70 (2H, brs), 7.81 (1H, s), 8.32 (1H, s). HRMS m/z Calcd for $C_{13}H_{17}N_5O_3$: 291.1331. Found: 291.1296. $[\alpha]_D^{26}=-29.4$ (c 1.00, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-1-[2′-Benzoyloxymethyl-3′-(hydroxymethyl)-cyclobutyl]thymine (9). IR ($CHCl_3$) 3400, 1705, 1690, 1600, 1470, 1450, 1275 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.90 (3H, s), 2.12–2.26 (2H, m), 2.38–2.48 (1H, m), 2.95–3.04 (1H, m), 3.71 (1H, dd, J=11.0, 5.5 Hz), 3.78 (1H, dd, J=10.6, 3.3 Hz), 4.43 (1H, dd, J=11.6, 5.5 Hz), 4.47 (1H, dd, J=12.6, 5.1 Hz), 4.66–4.75 (1H, m), 7.26 (1H, s), 7.38–7.48 (2H, m), 7.52–7.60

(1H, m), 7.88–8.02 (2H, m), 8.84–9.10 (1H, br). HRMS *m/z* Calcd for $C_{18}H_{20}N_2O_5$: 344.1372. Found: 344.1386. $[\alpha]_D^{27}=-38.0$ (*c* 0.50, $CHCl_3$).

(1′*S*,2′*S*,3′*R*)-1-[2′-Benzoyloxymethyl-3′-(hydroxymethyl)-cyclobutyl]thymine (*ent*-9). $[\alpha]_D^{26}=+37.4$ (*c* 1.03, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-9-[2′-Benzoyloxymethyl-3′-(hydroxymethyl)-cyclobutyl]-*N*^{2}-isobutyrylguanine (10). Mp 102–103°C ($CHCl_3$–hexane). ^{1}H NMR ($CDCl_3$) δ 1.23 (2H, d, *J*=6.8 Hz), 2.38 (1H, brs), 2.50–2.61 (2H, m), 2.65–2.71 (3H, m), 3.09–3.16 (1H, m), 3.76–3.85 (2H, m), 4.45–4.59 (3H, m), 7.35 (2H, t, *J*=7.6 Hz), 7.52 (2H, t, *J*=6.8 Hz), 7.81 (1H, s), 7.83 (1H, s), 9.45 (1H, brs), 11.95 (1H, brs). ^{13}C NMR ($CDCl_3$) δ 19.0, 19.1, 27.9, 34.1, 36.0, 44.9, 48.6, 63.3, 65.4, 120.7, 128.2, 129.0, 133.0, 137.8, 147.4, 148.6, 155.7, 166.2, 179.5. IR (KBr) 2974, 2936, 2874, 1677, 1607, 1557, 1477, 1400, 1314, 1272 cm^{-1}. HRMS (EI) Calcd for $C_{22}H_{25}N_5O_5$: 439.1856. Found: 439.1855. $[\alpha]_D^{25}=+2.15$ (*c* 1.02, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-1-[2′-Benzoyloxymethyl-3′-(hydroxymethyl)-cyclobutyl]-*N*^{4}-benzoylcytosine (11). Mp 90–91°C ($CHCl_3$–hexane). ^{1}H NMR (CD_3OD) δ 1.99–2.18 (m, 4H), 2.50 (1H, dt, *J*=7.7, 10.0 Hz), 2.57–2.65 (1H, m), 3.57–3.68 (2H, m), 3.70 (2H, d, *J*=6.0 Hz), 4.60 (1H, q, *J*=8.4 Hz), 7.50–7.56 (2H, m), 7.58–7.67 (2H, m), 7.94–7.99 (2H, m), 8.25 (1H, d, *J*=7.2 Hz). ^{13}C NMR (CD_3OD) δ 29.6, 34.5, 54.6, 64.0, 64.8, 98.3, 128.9, 129.1, 129.6, 130.5, 133.8, 134.4, 147.8, 158.5, 163.9, 168.7. IR (KBr) 3417, 1714, 1650, 1480, 1271 cm^{-1}. HRMS (EI) Calcd for $C_{17}H_{19}N_3O_4$: 329.1376. Found: 329.1360. $[\alpha]_D^{24}=+57.4$ (*c* 1.0, $CHCl_3$).

(1′*S*,2′*S*,3′*R*)-9-{2′,3′-Bis[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}-*N*^{2}-(*N*,*N*-dimethylaminomethylene)guanine (*ent*-12). To a solution of *ent*-**4** (191 mg, 0.35 mmol) in MeOH (5 mL) was added NEt_3 (5 mL) and water (1 mL), and the mixture was stirred at room temperature for 12 h. Then the solvent was removed in vacuo. The residue was dissolved in MeOH (1 mL), and *N*,*N*-dimethylformamide dimethyl acetal (280 μL, 1.40 mmol) was added. The mixture was stirred at room temperature for 12 h. The solvent was removed in vacuo, and the residue was dissolved in pyridine (3 mL). Under an argon atmosphere bis(*p*-methoxyphenyl)phenylmethyl chloride (300 mg, 0.88 mmol) was added, and the mixture was stirred at room temperature for 12 h. The reaction was quenched by addition of MeOH, and the solvent was removed in vacuo. The residue was diluted with CH_2Cl_2, and the organic solution was washed with saturated aqueous $NaHCO_3$ and brine. After dried over Na_2SO_4 the solution was concentrated. Purification by silica gel flash column chromatography (AcOEt–hexane, 20: 1) gave *ent*-**12** (191 mg, 73%). Mp 125.0–125.5°C ($CHCl_3$–hexane). ^{1}H NMR ($CDCl_3$) δ 2.29–2.32 (2H, m), 2.53–2.56 (1H, m), 2.59 (3H, s), 2.83–2.85 (1H, m), 2.98 (3H, s), 3.11–3.15 (3H, m), 3.32 (1H, dd, *J*=4.4, 9.6 Hz), 3.73 (6H, s), 3.78 (6H, s), 4.90 (1H, q, *J*=8.0 Hz), 6.67–6.73(4H, m), 6.76–6.79 (4H, m), 7.15–7.25 (14H, m), 7.35–7.42 (4H, d, *J*=8.4 Hz), 7.72 (1H, s), 8.29 (1H, s), 8.44 (1H, brs). ^{13}C NMR ($CDCl_3$) δ 14.2, 22.7, 30.1, 31.6, 35.1, 40.8, 47.6, 55.15, 55.17, 62.8, 65.2, 85.7, 112.86, 112.9, 126.4, 127.5, 127.6, 127.8, 127.9, 129.67, 129.7, 135.8, 135.96, 136.01, 136.03, 144.7, 144.8, 156.2, 157.7, 158.0, 158.1, 166.2. IR (KBr) 2930, 1684, 1630, 1537, 1509, 1250 cm^{-1}. FAB-MS *m/z* 925 (M^++1). Anal. Calcd for $C_{56}H_{56}N_6O_7\cdot1.5H_2O$: C, 70.64; H, 6.25; N, 8.83%. Found: C, 70.93; H, 6.55; N, 8.76%. $[\alpha]_D^{23}=-31.3$ (*c* 1.01, $CHCl_3$).

(1′*S*,2′*S*,3′R*S*)-9-{2′-Hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxy]cyclobutyl}-*N*^{2}-(*N*,*N*-dimethylaminomethylene)guanine (*ent*-13). To a solution of *ent*-**12** in MeOH (3 mL) and $CHCl_3$ (3 mL) was added CH_3CO_2H (1 mL), and the mixture was stirred for at room temperature for 3 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ (3 mL), and the organic layer separated was washed with saturated aqueous $NaHCO_3$ and brine. After dried over Na_2SO_4 the solution was concentrated. Purification by silica gel flash column chromatography ($CHCl_3$–hexane–MeOH, 10:10:1) gave *ent*-**13** (10 mg, 17%), its regioisomer (14 mg, 24%), and recovery of *ent*-**12** (51 mg, 58%). *ent*-**13**: Mp 127.0–127.2°C ($CHCl_3$–Hexane). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.00 (1H, brs), 2.16–2.20 (1H, m), 2.28 (1H, q, *J*=9.9 Hz), 2.52–2.59 (1H, m), 2.62–2.66 (1H, m), 3.07 (3H, s), 3.13 (3H, s), 3.10–3.13 (1H, m), 3.25 (1H, dd, *J*=4.7, 9.3 Hz), 3.78 (6H, s), 4.43 (1H, q, *J*=8.3 Hz), 4.70 (1H, brs), 6.82 (4H, d, *J*=9.0 Hz), 7.20 (2H, t, *J*=8.6 Hz), 7.27–7.31 (5H, m), 7.40 (2H, d, *J*=8.6 Hz), 7.59 (2H, s), 8.46 (1H, s), 9.30 (1H, brs). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 28.3, 32.1, 35.2, 41.4, 49.4, 50.1, 55.2, 60.0, 65.6, 86.1, 113.0, 136.1, 144.7, 149.7, 156.6, 157.6, 158.3. IR (KBr) 3404, 2931, 1681, 1631, 1541, 1509, 1425, 1349, 1250 cm^{-1}. FAB-MS *m/z* 623 (M^++1). Anal. Calcd for $C_{35}H_{38}N_6O_5\cdot0.6H_2O$: C, 66.36; H, 6.21; N, 13.22%. Found: C, 66.36; H, 6.24; N, 13.27%. $[\alpha]_D^{23}=-68.8$ (*c* 1.02, $CHCl_3$).

(1′*S*,2′*S*,3′*R*)-1-{2′,3′-Bis[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}cytosine (*ent*-14). To a solution of *ent*-**5** (193 mg, 0.44 mmol) in MeOH (5 mL) was added NEt_3 (5 mL) and water (1 mL), and the mixture was stirred at room temperature for 12 h. The solvent was removed in vacuo, and the residue was dissolved in dry pyridine (3 mL). Then under an argon atmosphere added was bis(*p*-methoxyphenyl)phenylmethyl chloride (375 mg, 0.88 mmol), and the mixture was stirred at room temperature for 12 h. The reaction was quenched by addition of MeOH (2 mL), and the solvent was removed in vacuo. The residue was diluted with CH_2Cl_2, and the solution was washed with saturated aqueous $NaHCO_3$ and brine. After dried over Na_2SO_4 the solution was concentrated. Purification by silica gel flash column chromatography (AcOEt–hexane, 20:1) gave *ent*-**14** (317 mg, 86%). Mp 132.0–132.5°C ($CHCl_3$–hexane). ^{1}H NMR ($CDCl_3$) δ 1.70–1.77 (1H, m), 2.01–2.04 (1H, m), 2.51–2.62 (2H, m), 3.07 (1H, dd, *J*=6.0, 9,6 Hz), 3.15 (1H, dd, *J*=4.8, 9.2 Hz), 3.20 (1H, dd, *J*=8.0, 9.6 Hz), 3.35 (1H, dd, *J*=5.2, 9.6 Hz), 3.73 (6H, s), 3,76 (6H, s), 4.53 (1H, q, *J*=8.8 Hz), 5.61 (1H, d, *J*=7.2 Hz), 6.74–6.78 (8H, m), 7.16–7.24 (15H, m), 7.31–7.36 (4H, m), 7.66 (1H, d, *J*=7.2 Hz). ^{13}C NMR ($CDCl_3$) δ 14.3, 30.7, 31.8, 32.2, 44.8, 52.9, 55.28, 55.3, 65.4, 65.7, 85.9, 86.3, 113.0, 113.1, 126.6, 127.7, 128.0, 128.1, 129.9, 135.9, 136.8, 142.8, 144.8, 145.0, 156.3, 158.3, 165.1. IR (KBr) 2931, 1648, 1607, 1508, 1298, 1250, 1175, 1033 cm^{-1}. FAB-MS

m/z 831 (M^++2). Anal. Calcd for $C_{52}H_{51}N_3O_7 \cdot 1.3H_2O$: C, 73.19; H, 6.33; N, 4.92%. Found: C, 73.26; H, 6.15; N, 4.71%. $[\alpha]_D^{24}$=+6.21 (*c* 1.03, $CHCl_3$).

(1′*S*,2′*S*,3′*R*)-1-{2′-Hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxy]cyclobutyl}cytosine (*ent*-15). To a solution of *ent*-**14** (230 mg, 0.28 mmol) in MeOH (15 mL) and $CHCl_3$ (15 mL) was added CH_3CO_2H (3.5 mL), and the mixture was stirred for 3 h at room temperature. The reaction was quenched by addition of saturated aqueous $NaHCO_3$, and the separated organic layer was washed with saturated aqueous $NaHCO_3$ and brine. After dried over Na_2SO_4 the solution was concentrated. Purification by silica gel flash column chromatography ($CHCl_3$–MeOH, 100:1 to 25:1) gave *ent*-**15** (63 mg, 43%) and its regioisomer (14 mg, 10%) as well as recovery of *ent*-**14** (90 mg, 39%). *ent*-**15**: Mp 118.5–119.5°C ($CHCl_3$–hexane). ^{1}H NMR ($CDCl_3$) δ 1.95–2.11 (2H, m), 2.23–2.34 (2H, m), 3.05 (1H, dd, *J*=5.6, 9.6 Hz), 3.18 (1H, dd, *J*=4.4, 9.6 Hz), 3.65–3.73 (2H, m), 3.79 (6H, s), 4.42 (1H, q, *J*=9.2 Hz), 5.77 (1H, d, *J*=7.6 Hz), 6.83 (4H, d, *J*=11.6 Hz), 7.20 (1H, t, *J*=7.2 Hz), 7.26–7.30 (7H, m), 7.39–7.42 (3H, m). ^{13}C NMR ($CDCl_3$) δ 26.6, 30.9, 51.0, 53.4, 55.2, 64.1, 65.4, 86.0, 94.8, 113.0, 126.6, 127.7, 128.0, 130.0, 135.8, 135.9, 141.3, 144.7, 157.5, 158.2, 165.4. IR (KBr) 2931, 1644, 1607, 1508, 1250 cm^{-1}. FAB-MS *m/z* 528 (M^++1). Anal. Calcd for $C_{31}H_{33}N_3O_5 \cdot 1.6H_2O$: C, 67.13; H, 6.54; N, 7.58%. Found: C, 67.03; H, 6.74; N, 7.38%. $[\alpha]_D^{20}$=−76.7 (*c* 0.98, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-9-{2′-Hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}-N^6-(*N*,*N*-dimethylaminomethylene)adenine 5′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (16). Under an argon atmosphere, to a dry pyridine (10 mL) solution of **6** (1.04 g, 2.95 mmol) was added bis(*p*-methoxyphenyl)-phenylmethyl chloride (1.20 g, 3.50 mmol) in pyridine (10 mL), and the mixture was stirred at room temperature for 2 days. The solvent was removed in vacuo, and the residue was diluted with $CHCl_3$. The organic solution was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. To the residue were added 1,4-dioxane (5 mL), methanol (5 mL), and 1 M NaOH (5 mL), and the mixture was stirred at room temperature for 12 h. After removal of the solvents in vacuo the residue was diluted with $CHCl_3$, and the organic solution was washed with saturated aqueous $NaHCO_3$ and brine. The solvents were removed in vacuo, and flash silica gel chromatography (AcOEt–hexane, 1:1 to AcOEt–MeOH, 10:1) gave (1′*R*,2′*R*,3′*S*)-9-{2′-hydroxymethyl-3′-[bis-(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}-adenine (1.07 g, 66%). IR ($CHCl_3$) 3600–3100, 1720, 1630, 1603, 1580, 1510, 1250, 1210 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.14–2.26 (1H, m), 2.34 (1H, apparent q, *J*=9.6), 2.58–2.74 (2H, m), 3.14 (1H, dd, *J*=9.5, 6.4 Hz), 3.24 (1H, dd, *J*=9.5, 5.0 Hz), 3.78 (6H, s), 3.82 (2H, d, *J*=6.2 Hz), 4.49 (1H, apparent q, *J*=8.6 Hz), 5.00–5.60 (1H, brs), 5.92 (2H, brs), 6.80–6.85 (4H, m), 7.18–7.32 (7H, m), 7.37–7.42 (2H, m), 7.82 (1H, s), 8.32 (1H, s). HRMS *m/z* Calcd for $C_{32}H_{33}N_5O_4$: 551.2533. Found: 551.2556. $[\alpha]_D^{24}$=+29.8 (*c* 1.00, $CHCl_3$). Under an argon atmosphere, to a methanol (8 mL) solution of the trityl ether (1.07 g, 1.94 mmol) was added *N*,*N*-dimethylformamide dimethyl acetal (941 mg, 7.9 mmol), and the mixture was stirred at room temperature for 12 h. The solvent was removed in vacuo, and flash chromatography over silica gel (AcOEt–hexane, 1:1 to AcOEt–MeOH, 5:1) gave (1′*R*,2′*R*,3′*S*)-9-{2′-hydroxymethyl-3′-[bis(*p*-methoxyphenyl)-phenylmethyloxymethyl]cyclobutyl}-N^6-(*N*,*N*-dimethyl-aminomethylene)adenine (1.10 g, 93%). IR ($CHCl_3$) 3300, 1635, 1610, 1580, 1565, 1510, 1445, 1415, 1398, 1350 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.14–2.26 (1H, m), 2.37 (1H, apparent q, *J*=9.6 Hz), 2.50–2.74 (2H, m), 3.15 (1H, dd, *J*=9.5, 6.4 Hz), 3.21 (3H, s), 3.24 (1H, dd, *J*=9.5, 5.0 Hz), 3.27 (3H, s), 3.78 (6H, s), 3.83 (2H, brd, *J*=6.6 Hz), 4.49 (1H, apparent q, *J*=8.6 Hz), 6.79–6.85 (4H, m), 7.17–7.32 (7H, m), 7.37–7.43 (2H, m), 7.89 (1H, s), 8.51 (1H, s), 8.96 (1H, s). HRMS Calcd for $C_{35}H_{38}N_6O_4$: 606.2955. Found: 606.2963. $[\alpha]_D^{25}$=+22.4 (*c* 1.00, $CHCl_3$). Under an argon atmosphere, the alcohol (1.10 g, 1.81 mmol) was thoroughly dried by azeotropically evaporating with dry pyridine twice and dry toluene twice, and was dissolved in dry CH_2Cl_2 (10 mL). The mixture was cooled to 0°C, and *i*-Pr_2NEt (1.58 mL, 1.82 mmol) was added. After stirred for 5 min, 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.61 mL, 2.72 mmol) was added. The mixture was stirred 30 min at 0°C and 30 min at room temperature. After cooled to 0°C the mixture was diluted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. Silica gel chromatography (AcOEy–hexane, 1:1 to AcOEt–MeOH, 5:1) gave **16** (1.06 g, 73%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.07 (6H, dd, *J*=6.8, 2.4 Hz), 1.13 (6H, dd, *J*=6.8, 2.0 Hz), 2.28–2.44 (2H, m), 2.48 (2H, dt (apparent q), *J*=6.4 Hz), 2.56–2.66 (1H, m), 3.02–3.18 (1H, m), 3.20 (3H, s), 3.21–3.32 (5H, m), 3.42–3.57 (2H, m), 3.60–3.93 (10H, m), 4.76–4.86 (1H, m), 6.80–6.86 (4H, m), 7.18–7.36 (7H, m), 7.42–7.47 (2H, m), 8.01 (1H, d, *J*=2.9 Hz), 8.51 (1H, s), 8.94 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.2 (d, *J*=6.9 Hz), 24.5 (d, *J*=7.6 Hz), 29.9 (d, *J*=4.6 Hz), 31.4 (d, *J*=7.6 Hz), 35.0, 41.1, 42.89 (d, *J*=12.2 Hz) and 42.92 (d, *J*=12.2 Hz), 47.2 (d, *J*=7.6 Hz), 48.2 and 48.6, 55.1, 58.1 (d, *J*=19.1 Hz), 63.7 (d *J*=15.3 Hz), 85.7, 112.8, 117.4, 126.0, 126.4, 127.5, 127.9, 129.7, 135.9, 140.17 and 140.19, 144.7, 151.6, 151.95 and 151.97, 157.7, 158.1, 159.1. ^{31}P NMR ($CDCl_3$, 162 MHz) δ 145.8, 146.2. FAB-MS *m/z* 807 (M^++1).

(1′*S*,2′*S*,3′*R*)-9-{2′-Hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}adenine. $[\alpha]_D^{22}$=−28.6 (*c* 1.07, $CHCl_3$).

[(1′*S*,2′*S*,3′*R*)-9-{2′-Hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}-N^6-(*N*,*N*-dimethylaminomethylene)adenine. $[\alpha]_D^{26}$=−24.2 (*c* 1.00, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-1-{2′-Hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}thymine 5′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (17). Under an argon atmosphere to a pyridine (4 mL) solution of **9** (666 mg, 1.94 mmol) was added bis(*p*-methoxyphenyl)-phenylmethyl chloride (788 mg, 2.32 mmol) in pyridine (4 mL), and the mixture was stirred at room temperature

for 2 days. The solvent was removed in vacuo, and the residue was diluted with $CHCl_3$. The solution was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. To the residue were added 1,4-dioxane (3 mL), MeOH (3 mL), and 1 M NaOH (3 mL), and the mixture was stirred at room temperature for 12 h. The solvents were removed in vacuo, and the residue was diluted with $CHCl_3$. The organic solution was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. Silica gel flash chromatography (AcOEt–hexane, 1:5 to AcOEt–MeOH, 10:1) gave (1′*R*,2′*R*,3′*S*)-1-{2′-hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}-thymine (790 mg, 75%). IR ($CHCl_3$) 3400, 1685, 1607, 1509 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.91 (3H, s), 1.94–2.12 (2H, m), 2.26–2.35 (1H, m), 2.41–2.51 (1H, m), 3.10 (1H, dd, *J*=9.9, 6.2 Hz), 3.26 (1H, dd, *J*=9.7, 4.0 Hz), 3.24–3.33 (1H, m), 3.60–3.74 (2H, m), 3.79 (6H, s), 4.55 (1H, apparent q, *J*=8.4 Hz), 6.80–6.87 (4H, m), 7.18–7.35 (7H, m), 7.39–7.45 (2H, m), 9.51 (1H, brs). HRMS Calcd for $C_{32}H_{34}N_2O_6$: 542.2417. Found: 542.2413. $[\alpha]_D^{22}=+19.6$ (*c* 1.00, $CHCl_3$). Under an argon atmosphere, the alcohol (750 mg, 1.38 mmol) was thoroughly dried by azeotropically evaporating with dry pyridine twice and dry toluene twice, and was dissolved in dry CH_2Cl_2 (13 mL). The mixture was cooled to 0°C, and *i*-Pr_2NEt (1.21 mL, 6.92 mmol) was added. After stirred for 5 min, 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.47 mL, 2.08 mmol) was added. The mixture was stirred 30 min at 0°C and 30 min at room temperature. The mixture was diluted with CH_2Cl_2, and the organic solution was washed with saturated aqueous $NaHCO_3$ and brine. After dried over $MgSO_4$ the solution was concentrated in vacuo. Silica gel chromatography (AcOEt–hexane, 3:1 to AcOEt–MeOH, 5:1) gave **17** (514 mg, 50%). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.12 (6H, dd, *J*=6.6, 4.0 Hz), 1.16 (6H, d, *J*=6.8 Hz), 1.90 (3H, d, *J*=3.7 Hz), 1.92–2.04 (1H, m), 2.11–2.24 (1H, m), 2.34–2.44 (1H, m), 2.53–2.63 (2H, m), 2.68–2.80 (1H, m), 3.12–3.26 (2H, m), 3.48–3.92 (12H, m), 4.66–4.80 (1H, m), 6.80–6.86 (4H, m), 7.20–7.34 (8H, m), 7.40–7.44 (2H, m), 8.22–8.54 (1H, brs). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 12.6, 20.36 (d, *J*=6.9 Hz) and 20.42 (d, *J*=6.8 Hz), 21.4, 24.61 (d, *J*=6.9 Hz) and 24.66 (d, *J*=6.8 Hz), 28.90 and 28.93, 30.93 and 31.27, 43.1 (d, *J*=12.0 Hz), 45.6 (d, *J*=7 Hz), 49.49 and 50.17, 55.2, 58.23 (d, *J*=19.0 Hz) and 58.33 (d, *J*=18.0 Hz), 63.6 and 64.3, 64.5, 86.0, 110.17 and 110.25, 113.0, 117.59 and 117.70, 126.6, 127.6, 127.9, 129.8, 135.9, 136.92 and 137.01, 144.81, 150.76 and 150.90, 158.2, 163.9. ^{31}P NMR ($CDCl_3$, 162 MHz) δ 145.8, 146.5. FAB-MS *m*/*z* 743 (M^++1).

(1′*S*,2′*S*,3′*R*)-1-{2′-Hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}thymine. $[\alpha]_D^{24}=-19.6$ (*c* 1.05, $CHCl_3$).

(1′*R*,2′*R*,3′*S*)-9-{2′-Hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl-N^2-(*N*,*N*-dimethylaminomethylene}guanine 5′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (18). Under an argon atmosphere to a solution of **10** (178 mg, 0.40 mmol) in pyridine (3 mL) was added bis(*p*-methoxyphenyl)phenylmethyl chloride (169 mg 0.81 mmol) in pyridine (2 mL), and the mixture was stirred at room temperature for 12 h. The reaction was quenched by addition of MeOH, and the solvent was removed in vacuo. The residue was diluted with CH_2Cl_2, and the organic solution was washed with saturated aqueous $NaHCO_3$ and brine. After dried over Na_2SO_4 the solution was concentrated. The residue was dissolved in MeOH (3 mL), to which was added 1 M aqueous NaOH (3 mL). The mixture was stirred at room temperature for 12 h, and diluted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine. After dried over Na_2SO_4, the solution was concentrated. The residue was dissolved in MeOH (3 mL), and *N*,*N*-dimethylformamide dimethyl acetal (192 μL, 1.62 mmol) was added. After stirred at room temperature for 12 h, the reaction mixture was concentrated. Purification by silica gel flash column chromatography ($CHCl_3$–hexane–MeOH, 10:10:1) gave (1′*R*,2′*R*,3′*S*)-9-{2′-hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}-N^2-(*N*,*N*-dimethylaminomethylene)guanine (181 mg, 72%). Mp 127.0–127.2°C ($CHCl_3$–hexane). ^1H NMR ($CDCl_3$) δ 2.00 (1H, brs), 2.16–2.20 (1H, m), 2.28 (1H, q, *J*=9.9 Hz), 2.52–2.59 (1H, m), 2.62–2.66 (1H, m), 3.07 (3H, s), 3.13 (3H, s), 3.10–3.13 (1H, m), 3.25 (1H, dd, *J*=4.7, 9.3 Hz), 3.78 (6H, s), 4.43 (1H, q, *J*=8.3 Hz), 4.70 (1H, brs), 6.82 (4H, d, *J*=9.0), 7.20 (2H, t, *J*=8.6 Hz), 7.27–7.31 (5H, m), 7.40 (2H, d, *J*=8.6 Hz), 7.59 (2H, s), 8.46 (1H, s), 9.30 (1H, brs). ^{13}C NMR ($CDCl_3$) δ 28.3, 32.1, 35.2, 41.4, 49.4, 50.1, 55.2, 60.0, 65.6, 86.1, 113.0, 136.1, 144.7, 149.7, 156.6, 157.6, 158.3. IR (KBr) 3404, 2931, 1681, 1631, 1541, 1509, 1425, 1349, 1250 cm^{-1}. FAB-MS *m*/*z* 623 (M^++1). Anal. Calcd for $C_{35}H_{38}N_6O_5\cdot 0.6H_2O$: C, 66.36; H, 6.21; N, 13.22%. Found: C, 66.36; H, 6.24; N, 13.27%. $[\alpha]_D^{22}=+68.7$ (*c* 1.01, $CHCl_3$). Under an argon atmosphere to a solution of the alcohol (167.2 mg, 0.268 mmol) in CH_2Cl_2 (1.6 mL) was added *i*-Pr_2NEt (240 μL, 1.34 mmol), and the mixture was stirred for 5 min at 0°C. Then 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (91 μL, 1.34 mmol) was added, and stirring was continued for 30 min at 0°C and 30 min at room temperature. The reaction was quenched by adding saturated aqueous $NaHCO_3$, and the mixture was diluted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. Purification by silica gel flash column chromatography (AcOEt–hexane–MeOH, 12:8:1) gave **18** (176 mg, 79%). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.08 (6H, dd, *J*=1.6, 6.8 Hz), 1.45 (6H, dd, *J*=2.8, 6.8 Hz), 2.26–2.42 (2H, m), 2.48–2.55 (3H, m), 2.97 (3H, d, *J*=4.4 Hz), 3.01–3.06 (1H, m), 3.07 (3H, s), 3.20–3.24 (2H, m), 3.46–3.58 (2H, m), 3.64–3.77 (5H, m), 3.79 (6H, s), 4.60–4.70 (1H, m), 6.81–6.84 (4H, m), 7.20–7.23 (1H, m), 7.28–7.33 (5H, m), 7.42–7.44 (2H, m), 7.70 (1H, d, *J*=1.2 Hz), 8.53 (1H, d, *J*=4.8 Hz), 9.20 (1H, brs). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.47 and 20.54, 24.69 (d, *J*=3.1 Hz) and 24.73 (d, *J*=3.8 Hz), 30.0 and 30.1, 31.46 and 31.56, 35.3, 41.2, 43.1 (d, *J*=12.2 Hz) and 43.2 (d, *J*=12.1 Hz), 47.50 (d, *J*=6.8 Hz) and 47.57 (d, *J*=7.6 Hz), 48.1 and 48.5, 55.3, 58.3 (d, *J*=18.9 Hz), 63.7 (d, *J*=15.1 Hz), 65.34 and 65.37, 85.8, 113.0, 117.6, 120.7, 126.7, 127.7, 128.0, 129.9, 136.1, 136.89 and 136.92, 144.9, 150.3, 156.04 and 156.08, 157.67 and 157.71, 158.0, 158.3. ^{31}P NMR ($CDCl_3$, 162 MHz) δ 145.8, 146.0. IR (KBr) 2965, 1685, 1630, 1541, 1348, 1250, 1178 cm^{-1}. FAB-MS *m*/*z* 823 (M^++1).

(1′*S*,2′*S*,3′*R*)-9-{2′-Hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl-N^2-(*N*,*N*-dimethylaminomethylene}guanine 5′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (*ent*-18). Under an argon atmosphere to a solution of *ent*-**13** (88 mg, 0.14 mmol) in dry CH_2Cl_2 (0.8 mL) was added *i*-Pr_2NEt (126 μL, 0.70 mmol), and the mixture was stirred for 5 min at 0°C. Then 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (48 μL, 0.21 mmol) was added, and stirring was continued for 30 min at 0°C and 30 min at room temperature. Then the mixture was diluted with CH_2Cl_2, and the solution was washed with saturated aqueous $NaHCO_3$ and brine. After dried over Na_2SO_4, the solution was concentrated. Purification by silica gel flash column chromatography (AcOEt–hexane–MeOH, 12: 8: 1) gave *ent*-**18** (74 mg, 64%).

(1′*R*,2′*R*,3′*S*)-1-{2′-Hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}-N^4-benzoylcytosine 5′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (19, NR_2=NHBz). Under an argon atmosphere to a solution of **11** (455 mg, 1.06 mmol) in pyridine (6 mL) was added bis(*p*-methoxyphenyl)phenylmethyl chloride (716 mg, 2.12 mmol), and the mixture was stirred at room temperature for 12 h. The reaction was quenched by addition of MeOH, and the solvent was removed in vacuo. The residue was diluted with CH_2Cl_2, and the organic solution was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was in dissolved in MeOH (8 mL), and NaOMe (561 mg, 21.2 mmol) was added. The mixture was stirred at room temperature for 10 min, and diluted with CH_2Cl_2. The organic solution was washed with brine, dried over Na_2SO_4, and concentrated. Purification by silica gel flash column chromatography (AcOEt, then AcOEt–MeOH, 50:1 to 10:1) gave (1′*R*,2′*R*,3′*S*)-1-{2′-hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxy-methyl]cyclobutyl}-N^4-benzoylcytosine (482 mg, 72%). Mp 110.0–111.0°C ($CHCl_3$–hexane). ^{1}H NMR ($CDCl_3$) δ 2.13 (2H, m), 2.38–2.43 (2H, m), 3.10 (1H, dd, *J*=4.8, 9.6 Hz), 3.22–3.26 (1H, dd, *J*=4.8, 9.6 Hz), 3.68–3.74 (2H, m), 3.80 (6H, s), 4.57 (1H, q, *J*=9.2 Hz), 6.82–6.86 (4H, m), 7.20–7.32 (7H, m), 7.39–7,42 (2H, m), 7.51–7.55 (2H, m), 7.61–7.65 (2H, m), 7.83 (1H, d, *J*=7.2 Hz), 7.89 (1H, d, *J*=7.6 Hz). ^{13}C NMR ($CDCl_3$) δ 21.5, 27.1, 31.1, 50.1, 50.2, 53.9, 55.2, 63.8, 65.0, 86.0, 96.9, 113.0, 126.6, 127.5, 127.6, 128.0, 128.7, 128.8, 129.8, 132.8, 135.8, 144.6, 145.3, 156.3, 158.2, 161.7, 166.5. IR (KBr) 3399, 2932, 1487, 1250 cm^{-1}. FAB-MS *m*/*z* 632 (M^++1). Anal. Calcd for $C_{38}H_{37}N_3O_6 \cdot 2H_2O$: C, 68.35; H, 6.19; N, 6.29%. Found: C, 68.55; H, 6.18; N, 5.80%. $[\alpha]_D^{26}$=+54.9 (*c* 1.10, $CHCl_3$). Under an argon atmosphere a mixture of the alcohol (166 mg, 0.263 mmol) and *i*-Pr_2NEt (232 μL, 1.31 mmol) in CH_2Cl_2 (1.5 mL) was stirred for 5 min at 0°C. Then 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (87 μL, 0.394 mmol) was added, and the mixture was stirred for 30 min at 0°C and 30 min at room temperature. The reaction mixture was cooled to 0°C, and saturated aqueous $NaHCO_3$ was added. After diluted with CH_2Cl_2, the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. Purification by silica gel flash column chromatography (AcOEt–hexane–MeOH, 12:8:1) gave **19** (NR_2=NHBz) (174 mg, 79%). ^{1}H NMR ($CDCl_3$) δ 1.12 (6H, d, *J*=6.4 Hz), 1.16 (6H, dd, *J*=1.6, 6.8 Hz). 1.85–1.93 (1H, m), 2.17–2.26 (1H, m), 2.56 (2H, q, *J*=6.4 Hz), 2.60–2.67 (1H, m), 2.73–2.80 (1H, m), 3.12–3.17 (1H, m), 3.21–3.25 (1H, m), 3.49–3.60 (2H, m), 3.62–3.82 (4H, m), 3.80 (6H, s), 4.76 (1H, quint, *J*=8.8 Hz), 6.82–6.86 (4H, m), 7.20–7.31 (8H, m), 7.40–7.42 (2H, m), 7.49–7.53 (2H, m), 7.58–7.63(1H, m), 7.90(2H, d, *J*=7.6 Hz), 7.98 (1H, t, *J*=7.6 Hz). ^{13}C NMR ($CDCl_3$) δ 20.37 (d, *J*=6.8 Hz) and 20.41 (d *J*=6.8 Hz), 24.64 (d, *J*=7.5 Hz) and 24.71 (d, *J*=6.9 Hz), 29.83 and 29.87, 31.37 and 31.53, 43.05 (d, *J*=12.1 Hz) and 43.10 (d, *J*=12.1 Hz), 45.66 (d, *J*=10.6 Hz) and 45.74 (d, *J*=10.6 Hz), 52.83, 53.32, 55.19, 58.23 (d, *J*=18.9 Hz) and 58.28 (d, *J*=19.7 Hz), 64.62 (d, *J*=16.7 Hz) and 64.78 (d, *J*=15.9 Hz), 65.0, 85.93, 96.18, 112.9, 117.50 and 117.55, 126.63, 127.36, 127.63, 127.92, 128.77, 129.8, 132.9, 135.83 and 135.86, 144.7, 145.8, 158.2, 161.2. ^{31}P NMR ($CDCl_3$) δ 147.1, 148.5. IR (KBr) 2965, 1666, 1623, 1554, 1508, 1486, 1303, 1251, 1178 cm^{-1}. FAB-MS *m*/*z* 832 (M^++1).

(1′*S*,2′*S*,3′*R*)-1-{2′-Hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}-N^4-(*N*,*N*-dimethylaminomethylene)cytosine 5′-*O*-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) (*ent*-19, NR_2=N=CHNMe$_2$). Under an argon atmosphere to a solution of *ent*-**15** (210 mg, 0.40 mmol) in MeOH (5 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (320 μL, 1.60 mmol) at room temperature, and the mixture was stirred for 12 h. The reaction mixture was concentrated in vacuo, and purification by silica gel flash column chromatography ($CHCl_3$–hexane–MeOH, 10:10:1) gave (1′*S*,2′*S*,3′*R*)-1-{2′-hydroxymethyl-3′-[bis(*p*-methoxyphenyl)phenylmethyloxymethyl]cyclobutyl}-N^4-(*N*,*N*-dimethylaminomethylene)cytosine (195 mg, 84%). Mp 79.5–80.3°C ($CHCl_3$–hexane). ^{1}H NMR ($CDCl_3$) δ 2.09–2.14 (1H, m), 2.30–2.36 (2H, m), 3.08 (1H, dd, *J*=5.2, 7.6 Hz), 3.13 (3H, s), 3.15 (3H, s), 3.19 (1H, dd, *J*= 4.4, 9.2 Hz), 3.71 (2H, d, *J*=6.0 Hz), 3.78 (6H, s), 4.50 (1H, q, *J*=8.4 Hz), 6.13 (1H, d, *J*=7.2 Hz), 6.80–6.84 (4H, m), 7.18–7.22 (1H, m), 7.26–7.31 (7H, m), 7.39–7.41 (2H, m), 7.55 (1H, d, *J*=7.6 Hz), 8.82 (1H, s). ^{13}C NMR ($CDCl_3$) δ 26.0, 30.8, 35.0, 41.2, 51.1, 53.8, 55.0, 64.0, 64.9, 85.7, 102.8, 112.8, 126.4, 127.4, 127.7, 127.8, 135.6, 135.7, 141.7, 144.6, 157.6, 158.0, 170.9. IR (KBr) 2931, 1655, 1593, 1509, 1440, 1414, 1342 cm^{-1}. FAB-MS *m*/*z* 583 (M^++1). $[\alpha]_D^{29}$=−123.6 (*c* 0.89, $CHCl_3$). Under an argon atmosphere, to a solution of the alcohol (98 mg, 0.17 mmol) in dry CH_2Cl_2 (1 mL) was added *i*-Pr_2NEt (148 μL, 0.85 mmol), and the mixture was stirred for 5 min at 0°C. Then 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (38 μL, 0.25 mmol) was added, and the mixture was stirred for 30 min at 0°C and 30 min at room temperature. The mixture was diluted with CH_2Cl_2, and saturated aqueous $NaHCO_3$ was added. The organic layer separated was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. Purification by silica gel flash column chromatography (AcOEt–hexane–MeOH, 12: 8: 1) gave *ent*-**19** (NR_2=N=CHNMe$_2$) (78 mg, 60%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.11 (6H, t, *J*=6.8 Hz), 1.15 (6H, d, *J*=6.4 Hz), 1.84 (1H, dq, *J*=5.2, 9.6 Hz), 2.12–2.21 (1H, m), 2.46–2.64 (2H, m), 2.66–2.76 (1H, m), 3.07–3.14 (7H, m), 3.18 (1H, dd, *J*=4.4, 13.2 Hz), 3.48–3.59 (2H, m), 3.60–3.66 (1H, m), 3.69–6.80 (9H, m),

4.80 (1H, dq, J=9.2, 13.6 Hz), 6.06 (1H, dd, J=2.0, 7.2 Hz), 6.23 (4H, dd, J=1.2, 8.8 Hz), 7.19–7.23 (1H, m), 7.28–7.31 (6H, m), 7.41 (2H, d, J=8.4 Hz), 7.68 (1H, dd, J=2.8, 7.2 Hz), 8.81 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.41 (d, J=6.8 Hz) and 20.44 (d, J=6.0 Hz), 24.69 (d, J=6.8 Hz) and 24.72 (d, J=6.8 Hz), 29.96 and 30.05, 31.6 and 31.7, 35.2, 41.4, 43.07 (d, J=12.2 Hz) and 43.11 (d, J=12.2 Hz), 45.8 (d, J=7.6 Hz), 51.7, 52.3, 55.2, 58.4 (d, J=18.2 Hz), 64.6 (d, J=16.0 Hz) and 65.0 (d, J=15.1 Hz), 65.18 and 65.22, 85.9, 102.06 and 102.09, 113.0, 117.60 and 117.66, 126.6, 127.6, 128.0, 129.9, 136.00 and 136.03, 142.54 and 142.57, 144.9, 156.68 and 156.73, 158.2, 171.0. ^{31}P NMR ($CDCl_3$, 162 MHz) δ 145.5, 145.9. IR (KBr) 2963, 1656, 1593, 1509, 1442, 1416, 1342, 1250 cm^{-1}. FAB-MS m/z 783 (M^++1).

Oligonucleotides. Oligonucleotides were synthesized by Nippon Gene Co., Ltd. The solid-phase synthesis was carried out on a PerSeptive Biosystems Expedite Model 8909 DNA synthesizer. Purification was done by PAGE (15% polyacrylamide gel).

Acknowledgements

This work was supported by the Terumo Science Foundation and Novartis Foundation (Japan) for the Promotion of Science.

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