Synthesis and Antiviral Activities of Carbocyclic Oxetanocin Analogues[1)]

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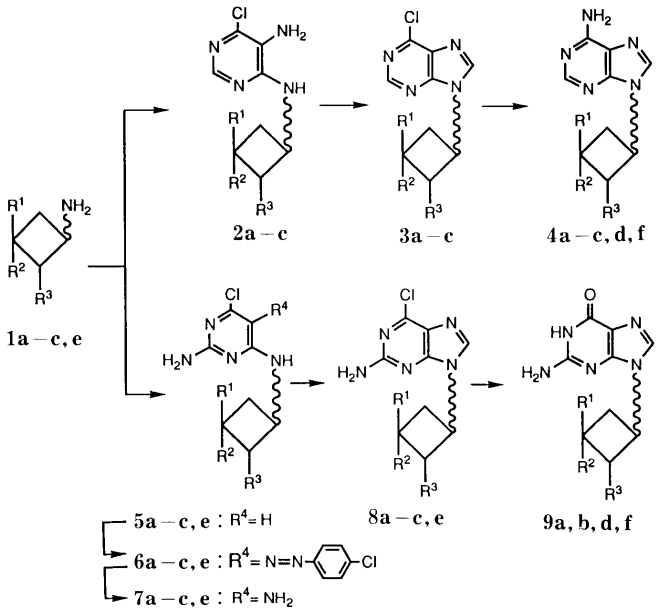
9-Cyclobutyladenine (4a), *cis*- and *trans*-9-[3-(hydroxymethyl)cyclobutyl]adenine (4b) and 9-[3,3-bis(hydroxymethyl)cyclobutyl]adenine(4d) were prepared from the corresponding cyclobutylamine derivatives (1a, 1b and 1d). Guanine congeners (9a, *cis*- and *trans*-9b and 9d) and carbocyclic oxetanocin G (1′,2′-*trans*-9f) were also prepared. Carbocyclic oxetanocin A(1′,2′-*trans*-4f), the preparation of which we have already published, and G were found to be active against herpes simplex virus (type 1 and 2) *in vitro*, while *cis*-4b and *cis*-9b showed an *in vitro* antiretroviral activity against human immunodeficiency virus (type 1).

Keywords oxetanocin; carbocyclic nucleoside; antiviral activity; carbocyclic oxetanocin; cyclobutane; herpes simplex virus; human immunodeficiency virus; adenine; guanine

The carbocyclic nucleosides refer to nucleoside analogues, in which the oxygen of the furanose ring is replaced by a methylene or methine group. These nucleosides benefit from greater chemical or metabolic stability than their furanose counterparts and many of them are endowed with remarkable antiviral or anticancer activities.[2)] Aristeromycin,[3)] neplanocins (A—D, F)[4,5)] and adecypenol[6)] are members of the carbocyclic nucleoside antibiotics. Recently there have been reports on the isolation, biological evaluation[7)] and chemical synthesis[8)] of (−)-oxetanocin, which contains adenine and a unique oxetane ring and exhibits antiviral and antitumor activities. As part of our studies directed towards exploration of new antiviral agents, we prepared carbocyclic oxetanocin analogues.

Synthesis Reaction of cyclobutylamine (**1a**) with 5-amino-4,6-dichloropyrimidine, followed by successive treatments with triethyl orthoformate and with liquid ammonia[9)] afforded 9-cyclobutyladenine (**4a**) (16%). Reduction of ethyl 3-azidocyclobutanecarboxylate[10)] with lithium aluminum hydride gave 3-(hydroxymethyl)cyclobutylamine (**1b**) (79%). Coupling of **1b** with 5-amino-4,6-dichloropyrimidine, followed by cyclization with triethyl orthoformate provided an equimolar mixture of the *cis*- and *trans*-isomers of 6-chloro-9-[3-(hydroxymethyl)cyclobutyl]purine (**3b**) (50%) (Chart 1). The presence of the two isomers was disclosed by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. A slight difference between the *Rf* values of the isomers was detected by thin layer chromatography (TLC), but their separation by silica gel column chromatography was found to be difficult. Tritylation of the mixture brought about an increase in the difference of the *Rf* values and thus, silica gel column chromatography resulted in successful separation of the isomers (*cis*- and *trans*-**10**) in 31 and 32% yields, respectively. Detritylation of each isomer with hydrochloric acid, followed by amination with liquid ammonia provided the respective 9-[3-(hydroxymethyl)cyclobutyl]adenine (*cis*- and *trans*-**4b**). It was difficult to assign the stereochemistry of the isomers by the nuclear Overhauser effect (NOE) difference experiment. One isomer (mp 151—155 °C) was subjected to bromination at C-8 with *N*-bromosuccinimide, and then to cyclization with sodium hydride in dimethyl formamide (DMF) to give the 8,3′-*O*-cyclic compound[11)] (**12**), while similar treatment of the other isomer (mp 208—209 °C) resulted in no cyclization (Chart 2). The structure of **12** was confirmed by ultraviolet (UV), ^{1}H-NMR and high resolution mass spectroscopies. This fact indicates that the former isomer has the *cis* configuration which enables it to undergo an intramolecular nucleophilic substitution, and the latter isomer has the *trans* configuration.

Reduction of diisoamyl 3-benzyloxycyclobutane-1,1-dicarboxylate[12)] (**14**) with lithium aluminum hydride gave the diol (**15**) (45%), which was subsequently acylated with cyclohexanecarbonyl chloride to yield the diester (**16**). Hydrogenation of **16** with palladium black, followed by treatment with chromium trioxide–pyridine afforded the ketone (**18**) (72%). Condensation of **18** with hydroxylamine



a series : R^1=R^2=R^3= H
b series : R^1= CH_2OH, R^2= R^3= H
c series : R^1=R^2= $CH_2OOCC_6H_{11}$, R^3= H
d series : R^1=R^2= CH_2OH, R^3= H
e series : R^1=R^3= $CH_2OOCC_6H_{11}$, R^2= H
f series : R^1=R^3= CH_2OH, R^2= H

Chart 1

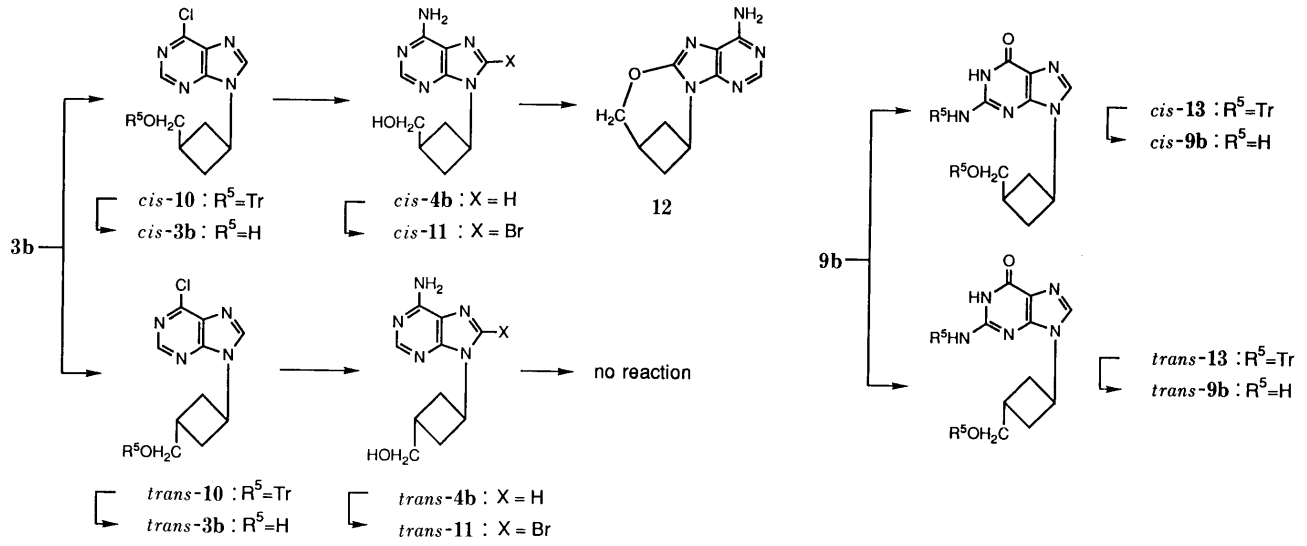


Chart 2

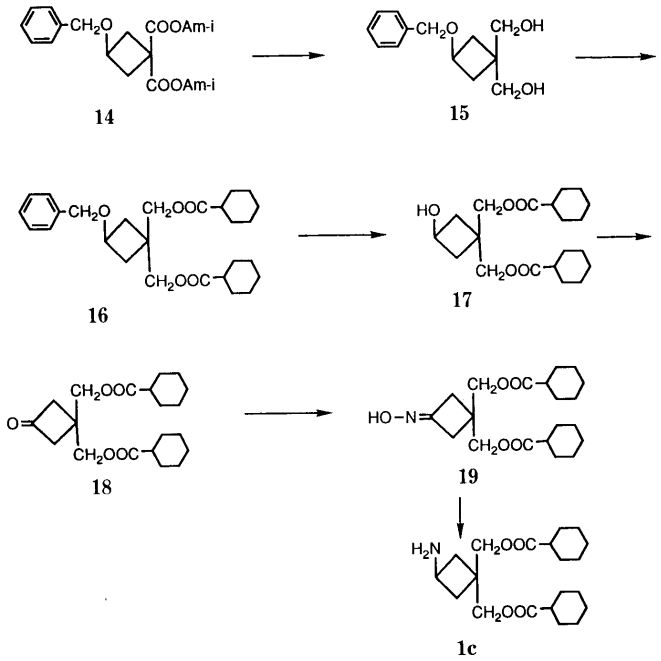


Chart 3

followed by hydrogenation with platinum oxide provided the amine (**1c**) (70%) (Chart 3). A series of analogous reactions of **1c** to that of **1a** furnished 9-[3,3-bis(hydroxymethyl)cyclobutyl]adenine (**4d**) in four steps.

In a previous paper[13] we reported the synthesis of carbocyclic oxetanocin A (1′,2′-*trans*-**4f**) and its diastereoisomer (1′,2′-*cis*-**4f**) as the respective racemate in four steps from (1α,2β,3α)-1-amino-2,3-bis(cyclohexylcarbonyloxymethyl)cyclobutane (1,2-*trans*-**1e**). Reaction of 1,2-*trans*-**1e** with 2-amino-4,6-dichloropyrimidine furnished 1′,2′-*trans*-**5e** (71%), which was coupled with *p*-chlorophenyldiazonium chloride to afford the intermediary azo compound (1′,2′-*trans*-**6e**) (59%). Reduction of 1′,2′-*trans*-**6e** with zinc powder yielded the triamine (1′,2′-*trans*-**7e**) (56%). Cyclization of 1′,2′-*trans*-**7e** with triethyl orthoformate followed by successive treatments with sodium hydroxide and subsequently with hydrochloric acid[14] provided carbocyclic oxetanocin G (1′,2′-*trans*-**9f**) as a racemate (34%).

A series of analogous reactions of **1a**, **1b** or **1c** to that of **1e** afforded 9-cyclobutylguanine (**9a**), 9-[3-(hydroxymethyl)cyclobutyl]guanine (*cis*-**9b** and *trans*-**9b**) or 9-[3,3-bis(hydroxymethyl)cyclobutyl]guanine (**9d**), respectively (Chart 1). A separation of the isomers of **9b** was achieved by similar treatment to that of **3b** (Chart 2) and the respective configuration was assigned by a comparison of their ^{1}H-NMR spectra with those of **4b** isomers.

Antiviral Activities Carbocyclic oxetanocin A (1′,2′-*trans*-**4f**) showed more potent *in vitro* activity ($ID_{50} = 4.2\ \mu M$) than adenine arabinoside against herpes simplex virus type 1 (HSV-1) (PH strain) in VERO cells, but was less active ($ID_{50} = 2.5 \times 10\ \mu M$) in type 2 (UW strain) than in type 1. Carbocyclic oxetanocin G (1′,2′-*trans*-**9f**) exhibited nearly equal activity ($ID_{50} = 2.5 \times 10^{-1}\ \mu M$) to acyclovir against HSV-1 (Fig. 1). Compounds **4a**, *cis*- and *trans*-**4b**, **4d**, 1′,2′-*cis*-**4f** and **9d** had no detectable activity against HSV-1.

Compound *cis*-**4b** exerted antiretroviral activity against human immunodeficiency virus type 1(HIV-1) *in vitro*. This compound gave partial protection at 10 and 20 μM and virtually complete protection at 50 μM of the MT2 cells against the infectivity and cytopathic effect of HIV-1 without affecting the growth of the cells. However, the compound slightly suppressed the growth of the target cells at 200 μM, and at 500 μM the compound was very toxic to the cells (Fig. 2). We also tested the antiviral activity of *cis*-**9b**. This compound, at > 10 μM, protected ATH8 cells against the infectivity and cytopathic effect of HIV-1 and enabled them to survive and grow comparably to the virus-unexposed ATH8 cell populations. The compound *cis*-**9b**, however, suppressed the growth of the cells at > 100 μM (Fig. 3). Compounds **4a**, *trans*-**4b**, **4d**, 1′,2′-*cis*-**4f** and **9d** failed to show activity inhibiting the viral infectivity and replication in MT2 cells. Antiretroviral activity of carbocyclic oxetanocin A and G against HIV was reported by S. Hayashi *et al.*[15a,16]

Discussion

We have already presented part of this paper in

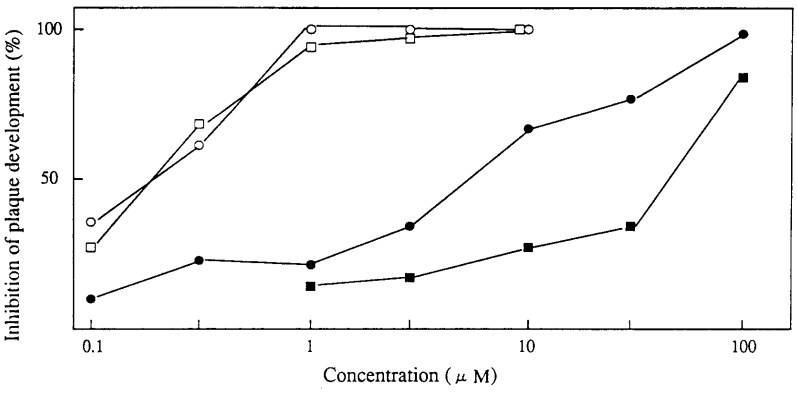


Fig. 1. Antiviral Activity of Carbocyclic Oxetanocin A and G against HSV-1 in VERO Cells

●—●, carbocyclic oxetanocin A; ○—○, carbocyclic oxetanocin G; ■—■, adenine arabinoside; □—□, acyclovir.

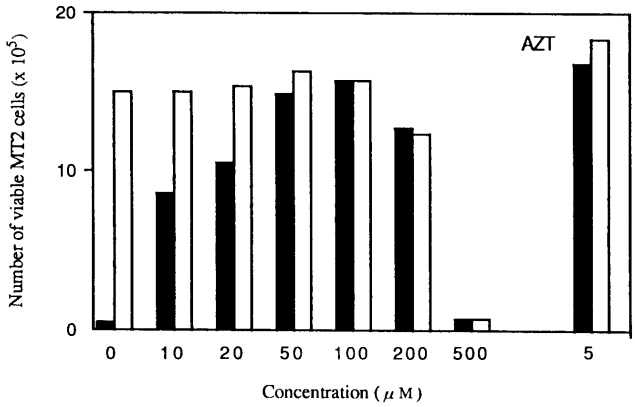


Fig. 2. Inhibition of Cytopathic Effect of HIV by *cis*-**4b** against MT Cells *in Vitro*

These cells were exposed to HIV in culture tubes (solid columns) in the presence or absence of the compound. Control cells (open columns) were similarly treated, but were not exposed to the virus. The antiviral activity of 3′-azido-2′,3′-dideoxythymidine (AZT) is shown as a reference.

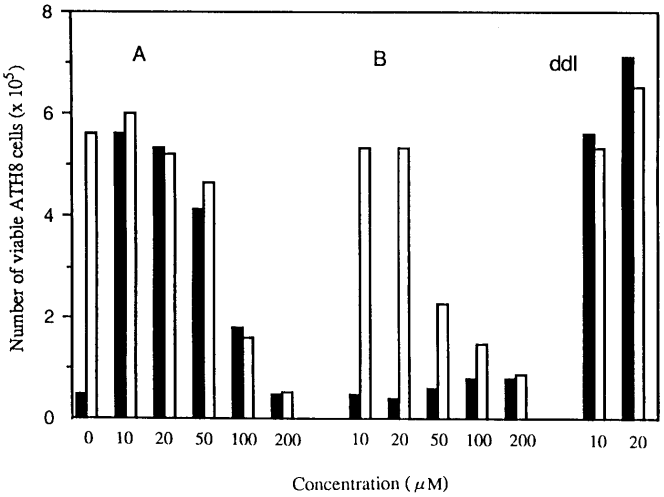


Fig. 3. Inhibition of Cytopathic Effect of HIV by *cis*-**9b** (A) and *cis*-**4b** (B) against ATH8 Cells *in Vitro*

The cells were exposed to HIV (solid columns) in the presence or absence of various concentrations of A and B. Control cells (open columns) were not exposed to the virus. The antiviral activity of 2′,3′-dideoxyinosine (DDI) is shown as a reference.

preliminary form.[1] Recently, independent works in the same field were reported from other laboratories.[15,17-19] At this time, five studies have been published on the synthesis of carbocyclic oxetanocin analogues, including ours.[1b,13,15b,18,19] The use of cyclobutylamine derivatives (**1a—c, e**) as key intermediates is characteristic of our synthetic method. The anti-HSV activity of analogues was also reported by three groups[1b,c,15b,c,17]: our group used PH strain for HSV-1 and UW strain for HSV-2, which differed from those strains used by other groups. The anti-HIV-1 activity of analogues was published by two groups[15a,c,16]: these groups detected the antiviral activity of carbocyclic oxetanocin A and G, and one of the groups[15c] published the activity of *cis*-**4b**. Our group reported the antiviral activity of *cis*-**4b** and *cis*-**9b** in this paper.

As to the structure–activity relationship, data on the *in vitro* anti-HSV (type 1) activity suggest that the presence of each hydroxymethyl group at C-2′ and C-3′, and the 1′,2′-*trans* configuration of a purine base and the hydroxymethyl group might be necessary for the activity. Guanine analogue exhibits stronger activity than its adenine counterpart. Results on the *in vitro* anti-HIV (type 1) activity also suggest that the presence of a single hydroxymethyl group at C-3′ and the 1′,3′-*cis* juxtaposition of a purine base and hydroxymethyl group would be mandatory, while the presence of hydroxymethyl group at C-2′ would maintain or increase the activity.

The mechanism of the antiviral activity of carbocyclic oxetanocin analogues against HIV-1 are as yet incompletely understood. It is possible that following triphosphorylation, these compounds may preferentially bind to reverse transcriptase and compete with normal nucleotides and/or serve as deoxyribonucleic acid (DNA)-chain terminators. To address these issues, more research is required.

Experimental

Biological Procedures Monolayer cultures of VERO cells were prepared in plastic petri dishes 6.0 cm in diameter at 37 °C in a 5% CO_2 incubator in Eagle's minimum essential medium (MEM) with 10% calf serum and 0.0292% L-glutamine. After the medium was discarded and washed with phosphate buffer saline once, 0.2 ml of the PH strain of HSV, type 1, at 5×10^2 plaque forming units/ml was innoculated over the VERO cells. One hour later the cell sheets were overlaid with a solution of the test compounds at various concentrations, prepared in MEM with 0.5% methyl cellulose and 1% calf serum. The cultures were incubated at 37 °C in a 5% CO_2 incubator for 2 d and then the cells were stained with crystal violet and the plaques were counted. Petri dishes without an added compound served as controls, and the percentage inhibition of plaque development by test compounds was calculated. Adenine arabinoside (and acyclovir) served as

TABLE I. Physical Properties of Cyclobutane Analogues

Compd. No.	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	UV (λ max) (nm)	^{1}H-NMR
1b	79	—	$C_5H_{11}NO \cdot 0.2H_2O$	57.33 (57.20	10.97 10.84	13.37 13.23)	—	(DMSO-d_6) δ: 3.30, 3.37 (2H, d, C$\underline{H}_2$OH), 3.10, 3.35 (0.5H each, m, H1), 2.85 (3H, br s, NH_2, $CH_2O\underline{H}$), 1.86, 2.15 (0.5H each, m, H3), 2.15, 1.93, 1.66, 1.30 (1H each, m, H2, H4)
1c	74	—	—		—		—	
2a	34	179—182	$C_8H_{11}ClN_4 \cdot 0.2H_2O$	47.51 (47.49	5.68 5.48	27.70 27.91)	298, 269	($CDCl_3$) δ: 8.07 (1H, s, H2), 5.02 (1H, br s, N^4-H), 4.55 (1H, sestet, H1′), 3.37 (2H, br s, 5-NH_2), 2.47 (2H, m, H2′a, H4′a), 1.76—1.94 (4H, m, H2′b, H4′b, H3′)
2b	73	156—158	$C_9H_{13}ClN_4O$	47.27 (47.50	5.73 5.88	24.50 24.40)	300, 271	($CDCl_3$) δ: 7.70 (1H, s, H2), 6.98, 6.92 (0.5H each, d, N^4-H), 5.06 (2H, s, NH_2), 4.60, 4.53 (0.5H each, dd, $CH_2O\underline{H}$), 4.46, 4.33 (0.5H, each, ddd, H1′), 3.47 (2H, dd, C$\underline{H}_2$OH), 1.16—2.51 (5H, m, H2′, H3′, H4′)
2c	62	—	—		—		298, 267	
3a	65	122—124	$C_9H_9ClN_4$	51.81 (51.56	4.35 4.33	26.85 26.90)	265	($CDCl_3$) δ: 8.75 (1H, s, H8), 8.23 (1H, s, H2), 5.10 (1H, quintet, H1′), 2.62—2.75 (4H, m, H2′a, H4′a, H3′), 1.95—2.10 (2H, m, H2′b, H4′b)
3b	69	99—102	$C_{10}H_{11}ClN_4O$	50.32 (50.18	4.65 4.68	23.47 23.61)	266	(DMSO-d_6) δ: 8.75 (1H, s, H8), 8.26, 8.24 (0.5H, each, s, H2), 5.24, 5.02 (0.5H each, quintet, H1′), 3.87, 3.79 (1H each, dd, C$\underline{H}_2$OH)
cis-**3b**	73	106—108	—		—		265	(DMSO-d_6) δ: 8.83 (1H, s, H8), 8.77 (1H, s, H2), 5.00 (1H, quintet, H1), 4.64 (1H, dd, $CH_2O\underline{H}$), 3.51 (2H, m, C$\underline{H}_2$OH)
trans-**3b**	85	129—131	—		—		266	(DMSO-d_6) δ: 8.90 (1H, s, H8), 8.78 (1H, s, H2), 5.21 (1H, quintet, H1′), 4.82 (1H, dd, $CH_2O\underline{H}$), 3.58 (2H, m, C$\underline{H}_2$OH), 2.78 (2H, m, H2′a, H4′a), 2.53 (1H, m, H3′), 2.40 (2H, m, H2′b, H4′b)
3c	91	—	—		—		266	
4a	74	175—178	$C_9H_{11}N_5 \cdot 0.75H_2O$	53.05 (53.33	6.24 6.21	34.77 34.54)	261	(DMSO-d_6) δ: 8.31 (1H, s, H8), 8.13 (1H, s, H2), 7.28 (2H, br s, 6-NH_2), 4.97 (1H, quintet, H1′), 2.67 (2H, m, H2′a, H4′a), 2.43 (2H, m, H3′), 1.86 (2H, m, H2′b, H4′b)
cis-**4b**	76	145—146	$C_{10}H_{13}N_5O \cdot 1.33H_2O$	49.39 (49.67	6.49 6.24	28.80 28.51)	261	(DMSO-d_6) δ: 8.32 (1H, s, H8), 8.27 (1H, s, H2), 7.19 (2H, s, 6-NH_2), 4.85 (1H, quintet, H1′), 4.63 (1H, m, $CH_2O\underline{H}$), 3.50 (2H, m, C$\underline{H}_2$OH), 2.47—2.50 (2H, m, H2′a, H4′a), 2.30—2.39 (3H, m, H2′b, H4′b, H3′)
trans-**4b**	78	195—197	$C_{10}H_{13}N_5O \cdot 0.1H_2O$	54.34 (54.12	6.02 6.00	31.68 31.58)	262	(DMSO-d_6) δ: 8.33 (1H, s, H8), 8.13 (1H, s, H2), 7.20 (2H, s, 6-NH_2), 5.07 (1H, quintet, H1), 4.77 (1H, m, $CH_2O\underline{H}$), 3.57 (2H, dd, C$\underline{H}_2$OH), 2.75—2.68 (2H, m, H2′a, H4′a), 2.47—2.50 (1H, m, H3′), 2.29—2.34 (2H, m, H2′b, H4′b)
4c	60	153—155	$C_{25}H_{35}N_5O_4$	63.94 (63.83	7.51 7.56	14.91 14.92)	261	($CDCl_3$) δ: 8.34 (1H, s, H8), 7.93 (1H, s, H2), 6.25 (2H, br s, NH_2), 4.8—5.3 (1H, m, H1′), 4.25 (4H, d, 3′-C$\underline{H}_2$O– × 2), 1.0—2.9 (m, –OCOC$_6\underline{H}_{11}$ × 2, H2′, H4′)
4d	65	225—227	$C_{11}H_{15}N_5O_2 \cdot 1.2H_2O$	48.77 (48.91	6.47 6.50	25.85 25.76)	262	(DMSO-d_6) δ: 8.29 (1H, s, H8), 8.13 (1H, s, H2), 7.20 (2H, s, NH_2), 4.94—4.98 (1H, m, H1′), 4.75—4.84 (2H, m, 3′-$CH_2O\underline{H}$ × 2), 3.41—3.52 (4H, m, 3′-C$\underline{H}_2$OH × 2), 2.30—2.51 (4H, m, H2′, H4′)
5a	69	145—147	$C_8H_{11}ClN_4$	48.37 (48.17	5.58 5.51	28.20 28.41)	286	
5b	73	—	—		—		286	
5c	78	161—163	$C_{24}H_{35}ClN_4O_4$	60.18 (60.69	7.36 7.48	11.70 11.57)	285	($CDCl_3$) δ: 7.3 (1H, s, H5), 5.2—5.5 (1H, m, H1′), 5.0—5.25 (2H, br s, NH_2), 4.1 (4H, d, 2′ and 3′-C$\underline{H}_2$O–), 1.1—2.6 (m, H2′, H4′, –OCOC$_6\underline{H}_{11}$ × 2)
5e	71	—	—		—		289	($CDCl_3$) δ: 5.84 (1H, s, H5), 5.27 (2H, m, 2-NH_2), 3.9—4.6 (5H, m, H1′, 2′- and 3′-C$\underline{H}_2$O–), 1.0—2.6 (*ca.* 26H, H2′, H3′, H4′, OCOC$_6\underline{H}_{11}$)
6a	74	269—271	$C_{14}H_{14}Cl_2N_6$	49.87 (49.64	4.18 4.15	24.92 24.72)	389	
6b	48	262	$C_{15}H_{16}Cl_2N_6O \cdot 0.5H_2O$	47.89 (47.51	4.55 4.21	22.34 22.07)		

TABLE I. (continued)

Compd. No.	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	UV (λmax) (nm)	^{1}H-NMR
6c	67	185—187	$C_{30}H_{38}Cl_2N_6O_4$	58.35 (58.45	6.20 6.17	13.61 13.65)		($CDCl_3$) δ: 7.3—8.0 (4H, m, –N=N–$C_6\underline{H}_4$–Cl), 5.7 (2H, br s, NH_2), 4.4—4.7 (1H, m, H1′), 4.15 (4H, d, 2′ and 3′-$C\underline{H}_2O$–), 1.0—2.6 (m, H2′, H4′, –$OCOC_6\underline{H}_{11}$ ×2).
6e	59	161—163	$C_{30}H_{38}Cl_2N_6O_4$	58.35 (58.28	6.20 6.17	13.61 13.70)	390, 283	($CDCl_3$) δ: 7.71, 7.44 (2H each, d, –N=N–$C_6\underline{H}_4$–Cl), 6.15 (2H, br s, 2-NH_2), 4.73, 3.96 (1H, each, q, 2′-CH_2O–), 4.15 (2H, m, 3′-CH_2O–), 0.9—2.7 (*ca.* 26H, H2′, H3′, H4′, –$OCOC_6\underline{H}_{11}$)
7a	68	159—161	$C_8H_{12}ClN_5$	44.97 (44.81	5.66 5.70	32.78 32.72)	307, 247	
7b	58	172	$C_9H_{14}ClN_5O$	44.36 (44.22	5.79 5.81	28.74 28.68)		
7c	77	159—161	$C_{24}H_{36}ClN_5O_4$	58.35 (58.55	7.35 7.38	14.18 14.11)		
7e	56	106—109	$C_{24}H_{36}ClN_5O_4$	58.35 (58.53	7.35 7.40	14.18 14.32)	308	($CDCl_3$) δ: 5.67 (1H, br s, –N^6H), 4.88 (2H, br s, 2-NH_2), 4.50, 4.05 (1H each, q, 3′-$C\underline{H}_2O$–), 4.20 (1H, m, H1′), 4.08—4.16 (2H, m, 2′-CH_2O–), 2.2 (2H, br s, 5-NH_2), 1.15—2.5 (*ca.* 26H, H2′, H3′, H4′, –$OCOC_6\underline{H}_{11}$)
8a	74	—	—		—		310	
8b	60	—	—		—		309	
8c	73	157—158	—		—		309, 249, 231	
8e	55	127—134	—		—		310, 248	($CDCl_3$) δ: 9.51, 8.14 (1H each, d, J=10.16, 10.71, 2-NH_2), 8.04 (1H, s, H8), 4.60 (5H, m, 2′- and 3′-CH_2O–, H1′), 1.1—3.1 (*ca.* 26H, H2′, H3′, H4′, –$OCOC_6\underline{H}_{11}$).
9a	56	>300	$C_9H_{11}N_5O$ ·0.6H_2O	50.04 (49.91	5.69 5.40	32.42 32.23)	253	
cis-**9b**	70	288—290	$C_{10}H_{13}N_5O_2$ ·H_2O	47.42 (47.32	5.97 5.75	27.65 27.36)	253.5	(DMSO-d_6) δ: 10.54 (1H, s, N^1-H), 7.86 (1H, s, H8), 6.39 (2H, s, 2-NH_2), 4.62 (2H, m, H1, $CH_2O\underline{H}$), 3.46 (2H, m, $C\underline{H}_2OH$), 2.4—2.5 (2H, m, H2′a, H4′a), 2.15—2.3 (3H, m, H2′b, H4′b, H3′)
trans-**9b**	70	>300	$C_{10}H_{13}N_5O_2$ ·1.2H_2O	46.76 (46.96	6.04 5.87	27.26 27.07)	253	(DMSO-d_6), δ: 10.53 (1H, s, N^1-H), 7.95 (1H, s, H8), 6.41 (2H, s, 2-NH_2), 4.85 (1H, m, H1′), 4.76 (1H, t, $CH_2O\underline{H}$), 3.53 (2H, t, $C\underline{H}_2OH$), 2.45—2.6 (2H, m, H2′a, H4′a), 2.35—2.45 (1H, m, H3′), 2.2—2.3 (2H, m, H2′b, H4′b)
9d	81	>300	$C_{11}H_{15}N_5O_3$ ·1.3H_2O	45.76 (45.52	6.14 5.91	24.26 24.04)	270 (sh), 253	(DMSO-d_6) δ: 7.90 (1H, s, H8), 6.41 (2H, s, NH_2), 4.82—4.73 (3H, m, H1′, 3′-$CH_2O\underline{H}$ ×2), 3.34—3.47 (4H, m, 3′-$C\underline{H}_2OH$ ×2), 2.23—2.34 (4H, m, H2′, H4′)
9f	61	269—270	$C_{11}H_{15}N_5O_3$	49.67 (49.80	6.03 5.70	26.51 26.40)	253	(DMSO-d_6) δ: 7.84 (1H, s, H8), 6.40 (2H, s, –NH_2), 4.68, 3.50 (1H, each, dd, 2′-$C\underline{H}_2OH$), 4.62 (1H, dd, 2′-$CH_2O\underline{H}$), 3.50 (3H, m, 3′-$C\underline{H}_2O\underline{H}$), 2.69 (1H, m, H2′), 2.35 (1H, m, H3′), 2.04 (2H, m, H4)
cis-**10**	32	151—155	$C_{29}H_{25}ClN_4O$	72.42 (72.24	5.24 5.36	11.65 11.57)	265	($CDCl_3$) δ: 8.71 (1H, s, H8), 8.20 (1H, s, H2), 7.24—7.50 (15H, m, $C(C_6\underline{H}_5)_3$), 4.99 (1H, quintet, H1′), 3.24 (2H, m, –$C\underline{H}_2O$–), 2.71 (2H, m, H2′a, H4′a), 2.52 (3H, m, H2′b, H4′b, H3′)
trans-**10**	31	208—209	$C_{29}H_{25}ClN_4O$	72.42 (72.64	5.24 5.29	11.65 11.78)	266	($CDCl_3$) δ: 8.75 (1H, s, H8), 8.21 (1H, s, H2), 7.22—7.52 (15H, m, $C(C_6\underline{H}_5)_3$), 5.18 (1H, quintet, H1′), 3.32 (2H, m, –$C\underline{H}_2O$–), 2.82 (3H, m, H2′a, H4′a, H3′), 2.58 (2H, m, H2′b, H4′b)
cis-**11**	20	151—153	$C_{10}H_{12}BrN_5O$ ·0.7H_2O	38.42 (38.65	4.48 4.35	22.29 22.54)	265.5	($CDCl_3$) δ: 8.33 (1H, s, H2), 5.7 (2H, br s, 6-NH_2), 5.04 (1H, m, H1), 4.2 (1H, m, $CH_2O\underline{H}$), 3.82 (2H, s-like, $C\underline{H}_2OH$), 3.1—3.4 (2H, m, H2′a, H4′a), 2.3—2.8 (3H, m, H2′b, H4′b, H3′)
trans-**11**	34	163—165	—		—		265.5	
12	22	220	—		—		263	($CDCl_3$) δ: 8.25 (1H, s, H2), 5.94 (1H, br s, NH_2), 5.14 (1H, m, H1′), 4.68 (2H, s, –$C\underline{H}_2O$–), 2.94—3.05 (3H, m, H2′a, H3′, H4′a), 2.13 (2H, d-like, H2′b, H4′b)
cis-**13**	30	258–260	$C_{48}H_{41}N_5O_2$ ·H_2O	78.13 (78.50	5.87 6.16	9.49 9.00)	—	
trans-**13**	19	286—288	$C_{48}H_{41}N_5O_2$	80.09 (79.96	5.74 5.85	9.73 9.58)	—	
15	45	66—69	$C_{13}H_{18}O_3$	70.24 (70.33	8.16 8.31	— −0.06)	—	($CDCl_3$) δ: 7.35 (5H, s-like, $C_6\underline{H}_5CH_2O$–), 4.43 (2H, s-like, $C_6H_5C\underline{H}_2O$–), 3.9—4.3 (1H, m, H1), 3.6—3.8 (2H each, s, 3-$C\underline{H}_2OH$ ×2), 1.6—2.4 (4H, m, H2, H4)

TABLE I. (continued)

Compd. No.	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	UV (λ max) (nm)	^{1}H-NMR
16	Quantitative	—	—	—			—	($CDCl_3$) δ: 7.3 (5H, s-like, $C_6\underline{H}_5CH_2O$–), 4.40 (2H, s, $C_6H_5C\underline{H}_2O$–), 3.9—4.25 (5H, m, H1, 3-$C\underline{H}_2O$– × 2), 1.0—2.5 (m, –$OCOC_6\underline{H}_{11}$ × 2, H2, H4)
17	89	—	—	—			—	($CDCl_3$) δ: 4.1—4.5 (1H, m, H1), 3.9—4.1 (4H, m, 3-$C\underline{H}_2O$– × 2), 1.0—2.5 (H2′, H4′, –$OCOC_6\underline{H}_{11}$ × 2)
18	81	54—55	$C_{20}H_{30}O_5$	68.54 (68.71	8.63 8.63)		—	($CDCl_3$) δ: 4.25 (4H, s, 3-$C\underline{H}_2O$– × 2), 3.0 (4H, s, H2, H4), 1.0—2.5 (m, –$OCOC_6\underline{H}_{11}$ × 2)
19	94	72.5—73.5	$C_{20}H_{31}NO_5$	65.73 (65.96	8.55 8.66	3.83 3.76)	—	($CDCl_3$) δ: 7.4 (1H, br s, =N–O$\underline{H}$), 4.2 (4H, s, 3-$C\underline{H}_2O$– × 2), 2.8 (4H, m, H2, H4), 1.0—2.5 (m, –$OCOC_6\underline{H}_{11}$ × 2)

a positive control for comparison.

HIV cytopathic effect inhibition assay was performed as previously described.[20,21] Briefly, MT2 cells or ATH8 cells (2×10^5) were exposed to an 8 or 21 $TCID_{50}$ dose of HIV-1, respectively, and cultured in the presence or absence of various concentrations of the compound. Control cells were not exposed to the virus. On day 8 in culture, viable cells were counted.

Chemical Procedures All melting points were determined on a Yanagimoto micromelting point apparatus (hot stage type) and are uncorrected. The UV spectra were recorded with a Shimadzu UV-190 digital spectrometer. The ^{1}H-NMR spectra were recorded with a JEOL GX-400 (400 MHz) spectrometer in $CDCl_3$ (or dimethyl sulfoxide (DMSO)-d_6) with a tetramethylsilane as an internal standard. Mass spectra (MS) were measured with a Shimadzu-LKB 9000B. TLC was carried out on Merck Art 5554 plates (2 × 10 cm) precoated with Silica gel 60 including fluorescent indicator F_{254}. All data are listed in Table I.

3-(Hydroxymethyl)cyclobutylamine (1b) A solution of ethyl 3-azidocyclobutanecarboxylate[9] (4.00 g, 23.7 mmol) in dry ether (20 ml) was added by drops to an ice-cooled suspension of lithium aluminum hydride (1.18 g, 31 mmol) in dry ether (90 ml), and the mixture was heated under reflux for 2 h, then cooled. The solution was treated with AcOEt (12 ml) and water (1 ml) to give a gel. The solid was filtrated and washed successively with ether (100 ml) and $CHCl_3$ (150 ml). The filtrate and washings were combined, dried over $MgSO_4$, evaporated to a small volume and distilled under a reduced pressure to obtain a syrup (1.88 g, 79%).

3,3-Bis(cyclohexylcarbonyloxymethyl)cyclobutylamine (1c) A mixture of **19** (5.36 g, 14.7 mmol) and platinum (IV) oxide (540 mg) in EtOH (150 ml) was stirred vigorously at room temperature overnight under a hydrogen atmosphere showing the presence of a ninhydrin-positive spot by TLC ($CHCl_3$–EtOH = 10 : 1, *Rf* 0.30). After removal of the catalyst, the solution was concentrated to a small volume and chromatographed over a column of silica gel G (i.d. 2.5 × 30 cm) with a gradient (1 l) of 0—15% EtOH in $CHCl_3$ to give a syrup (3.84 g, 74%).

5,6-Diamino-4-chloro-N^6-[3-(hydroxymethyl)cyclobutyl]pyrimidine (2b). General Procedure for 2a and 2c 5-Amino-4,6-dichloropyrimidine (1.64 g, 10 mmol) was added to a solution of **1b** (0.33 g, 3.3 mmol) in a mixture of triethylamine (1.5 ml) and *n*-butanol (10.5 ml). After refluxing overnight, a dark brownish solution was evaporated to dryness *in vacuo* and the residue was partitioned between $CHCl_3$ (40 ml) and water (20 ml). The organic layer was washed with water (10 ml), dried over $MgSO_4$ and concentrated to 10 ml. The solution was chromatographed over a column of silica gel G (i.d. 2.6 × 25 cm) with a gradient (1.5 l) of 0—5% EtOH in $CHCl_3$ to give white crystals (0.56 g, 73%).

6-Chloro-9-[3-(hydroxymethyl)cyclobutyl]purine (3b). General Procedure for 3a and 3c A solution of **2b** (1.10 g, 4.8 mmol) in triethyl orthoformate (16 ml) and concentrated HCl (0.4 ml) was stirred at room temperature for 6 h. After neutralization with triethylamine, the solution was evaporated to dryness and the residue was dissolved in AcOEt (180 ml). The organic layer was washed with water (20 ml), dried over $MgSO_4$ and evaporated to give a mixture of **3b** and an acid-labile by-product. Thus, the mixture was dissolved in EtOH (10 ml) and concentrated HCl (2 drops) was added to decompose the by-product, giving almost one spot of **3b** by TLC. Chromatographic separation (Silica gel G) in a usual manner afforded white crystals (788 mg, 69%). In the case of **3a** and **3c**, no acid-labile by-product was observed.

***cis*- and *trans*-6-Chloro-9-(3-trityloxymethylcyclobutyl)purine (*cis*- and *trans*-10). General Procedure for *cis*-13 and *trans*-13** A solution of **3b** (0.50 g, 2 mmol) in pyridine (50 ml) was concentrated to 25 ml, to which was added trityl chloride (3.40 g). The mixture was kept at 50 °C for 3 h. After evaporation of the solvent, the reaction was stopped by the addition of water (8 ml) and the solution was evaporated to give a brownish gum, which was partitioned between $CHCl_3$ (30 ml) and water (20 ml). The organic layer was washed with water (10 ml), dried over $MgSO_4$ and evaporated to dryness. The residue was evaporated azeotropically with toluene (20 ml) twice and chromatographed repeatedly over a column of Silica gel G as follows: (1) (i.d. 4.4 × 20 cm) with $CHCl_3$ as an eluent (2) (i.d. 2.0 × 75 cm) with 0—20% AcOEt in benzene (2 l) as an eluent. The first fraction was evaporated and crystallized from MeOH to give white crystals (*trans*-**10**) (325 mg, 32%). The second fraction was evaporated and crystallized from benzene to give white crystals (*cis*-**10**) (315 mg, 31%).

6-Chloro-9-[*cis*-3-(hydroxymethyl)cyclobutyl]purine (*cis*-3b). General Procedure for *trans*-3b, *cis*-9b and *trans*-9b Concentrated HCl (1 ml) was added by drops to a solution of *cis*-**10** (1.08 g, 2.2 mmol) in $CHCl_3$ (20 ml) and EtOH (20 ml), and the solution was kept at room temperature for 1 h, then neutralized with triethylamine. After evaporation of the solvent, the residue was treated in a usual manner to obtain the crude *cis*-**3b**, which was chromatographed on a column of Silica gel G (i.d. 3.5 × 30 cm) with a gradient of 0—17% EtOH in $CHCl_3$ (11) to give white crystals (456 mg, 85%).

9-[*cis*-3-(Hydroxymethyl)cyclobutyl]adenine (*cis*-4b). General Procedure for 4a, *trans*-4b and 4c A mixture of *cis*-6-chloro-9-[3-(hydroxymethyl)cyclobutyl]purine (*cis*-**3b**) (170 mg, 0.71 mmol) and liquid NH_3 (*ca.* 3 ml) was warmed in a steel bomb at 40 °C overnight. After cooling, NH_3 was vaporized carefully and a solution of the residue in water (2 ml) was desalted with a column of charcoal (2.0 g) to obtain white crystals (118 mg, 76%).

2,6-Diamino-4-chloro-N^6-[*trans-trans*-2,3-bis(cyclohexylcarbonyloxymethyl)cyclobutyl]pyrimidine (1′,2′-*trans*-5e). General Procedure for 5a—c A solution of 1′,2′-*trans*-**1e** (431 mg, 1.23 mmol) and 2-amino-4,6-dichloropyrimidine (202 mg, 1.23 mmol) in a mixture of *n*-butanol (5 ml), MeOH (0.5 ml) and triethylamine (1.5 ml) was refluxed for 11 h, then cooled. The solution was treated in a manner similar to that described in section **2b** to afford a syrup (383 mg, 71%).

2,6-Diamino-4-chloro-5-[(4-chlorophenyl)azo]-N^6-[*trans-trans*-2,3-bis(cyclohexylcarbonyloxymethyl)cyclobutyl]pyrimidine (1′,2′-*trans*-6e). General Procedure for 6a—c A solution of sodium nitrite (471 mg) in water (4.1 ml) was added by drops to an ice-cooled solution of *p*-chloroaniline (828 mg) in concentrated HCl (4 ml) and water (10.8 ml) to give *p*-chlorobenzenediazonium salt. A solution of 1′,2′-*trans*-**5e** (1.22 g, 2.55 mmol) in a mixture of $NaOAc \cdot 3H_2O$ (6.15 g), AcOH (4.8 ml) and water (5.9 ml) was added during 30 min to the ice-cooled solution of the diazonium salt and stirred at room temperature for 1 d. After evaporation of the solution to dryness, the residue was dissolved in $CHCl_3$ (100 ml) and the organic layer was washed with saturated $NaHCO_3$ (50 ml) twice and water (50 ml), dried over $MgSO_4$ and concentrated to 10 ml. The solution was chromatographed over a column of Silica gel G (i.d. 2.5 × 35 cm) with a gradient (1 l) of 0—25% AcOEt in benzene to give yellowish crystals (930 mg, 59%).

2,5,6-Triamino-4-chloro-N^6-[*trans-trans*-2,3-bis(cyclohexylcarbonyl-

oxymethyl)cyclobutyl]pyrimidine (1′,2′-*trans*-7e). General Procedure for 7a—c 1′,2′-*trans*-**6e** (500 mg, 0.81 mmol) and zinc powder (590 mg) were suspended in a mixture of AcOH (2.7 ml), EtOH (67 ml) and water (5 ml), and heated under reflux for 1.5 h, then cooled. The insoluble material was removed by filtration and the filtrate was evaporated to dryness. The residue was dissolved in benzene (10 ml) and chromatographed over a column of Silica gel G (i.d. 2.6 × 25 cm) with a gradient (1.2 l) of 0—5% AcOEt in benzene to obtain white crystals (222 mg, 56%).

6-Chloro-9-[*trans*-*trans*-2,3-bis(cyclohexylcarbonyloxymethyl)cyclobutyl]purine (1′,2′-*trans*-8e). General Procedure for 8a—c A solution of 1′,2′-*trans*-**7e** (390 mg, 0.79 mmol) in triethyl orthoformate (3.6 ml) and concentrated HCl (0.5 ml) was stirred at room temperature for 3 h and the mixture was treated in a manner similar to that described in section **3b** to afford white crystals (220 mg, 55%).

9-[*trans*-*trans*-2,3-Bis(hydroxymethyl)cyclobutyl]guanine (1′,2′-*trans*-9f). General Procedure for 9a, 9b, 9d 0.5 N NaOH (4 ml) was added slowly to a solution of 1′,2′-*trans*-**8e** (220 mg, 0.44 mmol) in dioxane (15 ml) and the solution was stirred at room temperature for 3 h, then acidified by adding 1 N HCl (3 ml). The solution was concentrated to 7 ml and washed with $CHCl_3$ (3 ml) three times, then refluxed for 10 h. After cooling, the mixture was treated with a column of charcoal (5 g) to give white crystals (71 mg, 61%).

8-Bromo-9-[*cis*-3-(hydroxymethyl)cyclobutyl]adenine (*cis*-11). General Procedure for *trans*-11 A mixture of *cis*-**4b** (30 mg, 0.14 mmol) and *N*-bromosuccinimide (45 mg, 0.25 mmol) in DMF (1 ml) was kept at room temperature for 1 h, then evaporated to dryness. The residue was dissolved in a small amount of EtOH and chromatographed over a column of Silica gel G (i.d. 2.5 × 25 cm) with a gradient (800 ml) of 0—15% EtOH in $CHCl_3$ to give pale yellowish crystals (8.2 mg, 20%).

8,3′-Anhydro-9-[*cis*-3′-(hydroxymethyl)cyclobutyl]-8-oxyadenine (12) A solution of *cis*-**11** (20 mg, 0.067 mmol) in DMF (1.0 ml) was treated with NaH (13.4 mg, 5 eq) at 75—80 °C for 3 h when disappearance of the starting material (*Rf* 0.49) and appearance of the product (*Rf* 0.43) was observed by TLC. After removal of the insoluble material by decantation, the solution was neutralized with AcOH and evaporated to dryness. The residue was dissolved in a small amount of EtOH and chromatographed over a column of Silica gel G (i.d. 2.0 × 20 cm) to give a white powder (3.2 mg, 22%). HR-MS: Calcd for $C_{10}H_{11}N_5O$ (mol. weight 217.0964). Found: 217.0976 (M^+). A similar reaction was examined in the case of *trans*-**11**, but the spots observed by TLC were strating material and its decomposed by-product at the original point.

1-Benzyloxy-3,3-bis(hydroxymethyl)cyclobutane (15) Diisoamyl 3-benzyloxycyclobutane-1,1-dicarboxylate (**14**)[11] (168.9 g, 0.43 mol) was added by drops to a suspension of lithium aluminum hydride (27.0 g, 0.71 mol) in a mixture of dry ether (620 ml) and dry tetrahydrofuran (THF) (600 ml) and stirred at room temperature for 1 h. The mixture was treated in a manner similar to that described in section **1b** to give an oil. The liquid with a high-boiling point was removed by distillation under reduced pressure to give a residue, which was crystallized from benzene to afford white crystals (43.55 g, 45%).

1-Benzyloxy-3,3-bis(cyclohexylcarbonyloxymethyl)cyclobutane (16) Cyclohexanecarbonyl chloride (52.34 g, 0.36 mmol) was added by drops to an ice-cooled solution of **15** (33.0 g, 0.15 mol) in dry pyridine (300 ml), and kept at room temperature for 1 h. The mixture was treated in a usual manner to give a syrup (65.7 g, quantitative).

3,3-Bis(cyclohexylcarbonyloxymethyl)cyclobutanol (17) Palladium-black (200 mg) was suspended to a solution of **16** (15.84 g, 36 mmol) in dry EtOH (200 ml) and stirred vigorously under H_2 atmosphere at 60 °C overnight, then the catalyst was removed by filtration. Evaporation of the solvent gave a syrup (11.19 g, 89%).

3,3-Bis(cyclohexylcarbonyloxymethyl)cyclobutanone (18) Chromium (VI) oxide (70.0 g) was carefully added to ice-cooled pyridine (1 l) by stirring, and a solution of **17** (52.5 g, 0.15 mol) in pyridine was added by drops to the solution. After stirring at room temperature overnight, ice-water (1 l) was added to the reaction mixture and the brownish solution was extracted with benzene–ether (1 : 1, 1 l) twice. The organic layer was washed with water (1 l), dried over $MgSO_4$ and evaporated to obtain a syrup, which was crystallized from hexane (200 ml) to give white crystals (42.46 g, 81%).

3,3-Bis(cyclohexylcarbonyloxymethyl)cyclobutanone Oxime (19) A solution of **18** (46.0 g, 0.13 mol) and hydroxylamine hydrochloride (25 g) in pyridine (460 ml) was kept at 35 °C for 1.5 h and the solvent was removed *in vacuo*. The residue was dissolved in benzene–ether (1 : 1, 500 ml) and the organic layer was washed with water (200 ml) twice, dried over $MgSO_4$ and evaporated. The residue thus obtained was evaporated azeotropically with toluene (50 ml) three times and crystallized from hexane (200 ml) to give white crystals (44.94 g, 94%).

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References and Notes

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