Synthesis and Antiviral Activity of Carbocyclic Oxetanocin Analogues (C-OXT-A, C-OXT-G) and Related Compounds. II

Tokumi MARUYAMA,*,[a] Yasuaki HANAI,[a] Yoshiko SATO,[a] Robert SNOECK,[b] Graciela ANDREI,[b] Mitsuaki HOSOYA,[b] Jan BALZARINI[b] and Erik DE CLERCQ[b]

Department of Pharmaceutical Sciences, Tokushima Bunri University,[a] Yamashiro-cho, Tokushima 770, Japan and Rega Institute for Medical Research, Katholieke Universiteit Leuven,[b] Minderbroedersstraat 10, B-3000 Leuven, Belgium.
Received June 10, 1992

9-(*cis*-3-Hydroxymethyl-2-methylenecyclobutyl)guanine (3b) and 9-(3-methylene-*trans*-2-hydroxymethylcyclobutyl)guanine (4b) were prepared from N^2-isobutyryl-9-[*trans*-*trans*-2,3-bis(hydroxymethyl)cyclobutyl]guanine (2f) or 2,3-bis(hydroxymethyl)-1-cyclobutanol (7b). Carbocyclic oxetanocin analogues (A, 1d; G, 2d) and related compounds including 4b were assayed against a broad variety of viruses. It appeared that the activity of 2d against herpes simplex virus (HSV) and varicella-zoster virus (VZV) at least partially depends on phosphorylation by the virus-induced thymidine kinase (TK). Although 1d and 2d are inhibitory to the replication of human immunodeficiency virus (HIV), they are quite toxic to proliferating human T-lymphocytes.

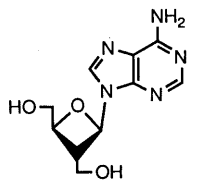
Keywords carbocyclic oxetanocin; methylenecyclobutane; antiviral activity; nucleoside

Several antiviral agents have been developed since the successful use of IDU (idoxuridine),[1] but the utility of these compounds for the treatment of herpes virus infections has been limited by their toxicity and lack of selectivity.[2] An exception to this rule is acyclovir (ACV), which is widely used in the topical and systemic treatment of herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections, because it is a selective inhibitor of these viruses.[2b,3] Problems encountered with ACV, however, are the emergence of ACV-resistant HSV and VZV mutants and its relative insolubility in water, which precludes its use as eye drops.[2b]

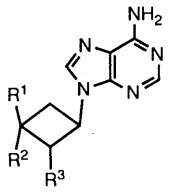
Recently, oxetanocin was discovered from *Bacillus megaterium* by Shimada *et al.*[4a] Oxetanocin is a nucleoside antibiotic in which the furanose ring has been replaced by an unusual oxetane ring. The activity of oxetanocin against human immunodeficiency virus (HIV)[4] has prompted several investigators to synthesize derivatives of oxetanocin.

We already have reported the synthesis of carbocyclic oxetanocins (C-OXT, **1d**, **2d**) as a racemate in an earlier report, in which the preparation of several C-OXT analogues (**1a**—**c**, **e**, **2e**) and their activity against HSV and HIV-1 *in vitro* were also included.[5] Here, we describe their effects against a broad variety of DNA and RNA viruses. Also reported here are the synthesis and antiviral activity of some racemic methylenecyclobutylguanines[6] related to decoyinine or DMDC.[7]

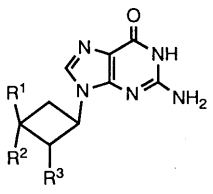
Synthesis of Methylenecyclobutylguanines Introduction of a bulky function such as a triphenylmethyl (trityl) group in N^6-benzoyl-C-OXT-A leads to non-specific protection of the primary alcohols, as shown earlier.[6] With N^2-isobutyryl-C-OXT-G **2f** as the starting material, two monotritylated products (**5a**, **6a**) were obtained in 15.6% and 28% yield, respectively; a ditritylated by-product and the starting material were also recovered. Alkaline treatment of the bis(benzoyloxymethyl)cyclobutanol **7a**[8c]



oxetanocin A

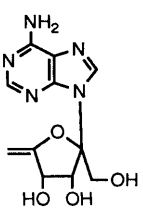


1a—e

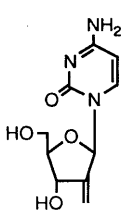


2d,e

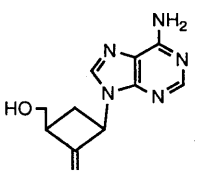
a series : $R^1 = R^2 = R^3 = H$
b series : $R^1 = CH_2OH, R^2 = R^3 = H$
c series : $R^1 = H, R^2 = CH_2OH, R^3 = H$
d series : $R^1 = R^3 = CH_2OH, R^2 = H$
e series : $R^1 = R^2 = CH_2OH, R^3 = H$



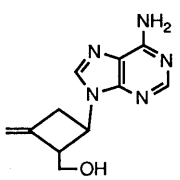
decoyinine



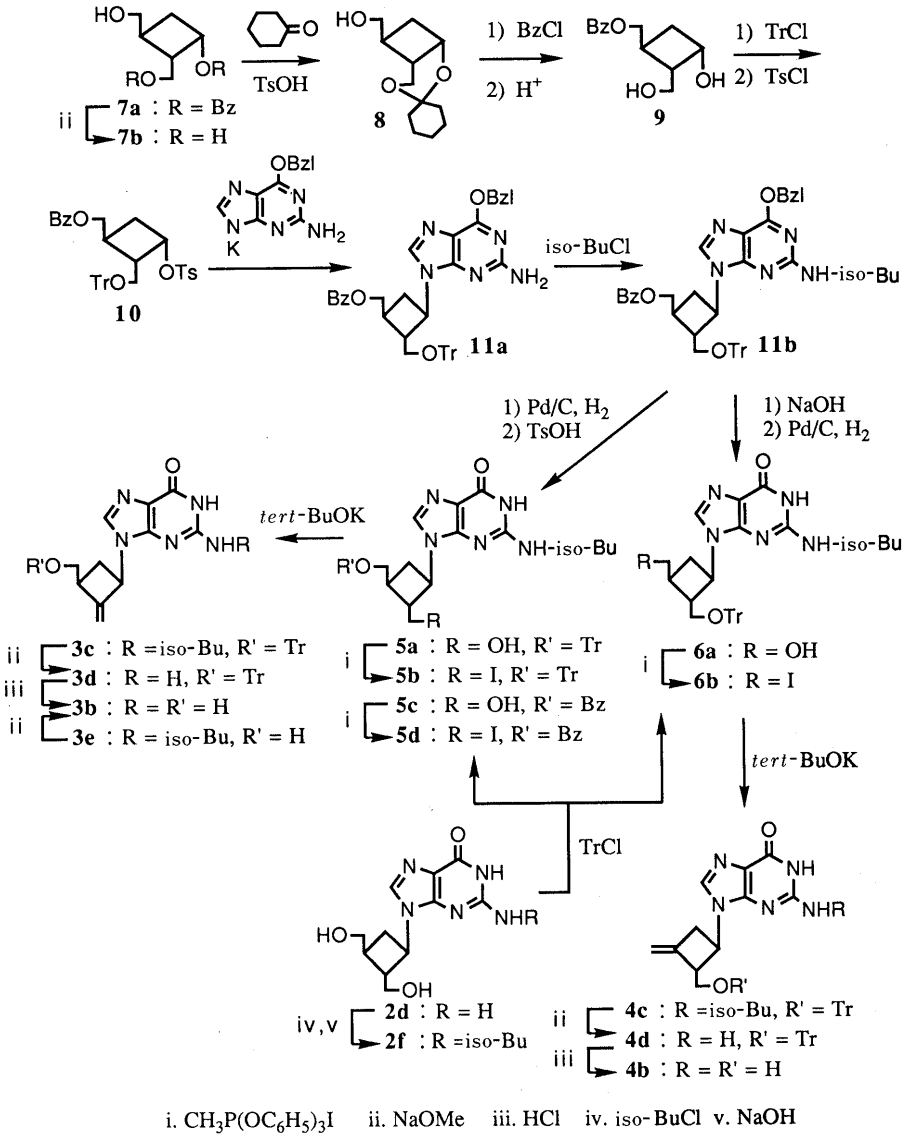
DMDC



3a



4a



i. $CH_3P(OC_6H_5)_3I$ ii. NaOMe iii. HCl iv. iso-BuCl v. NaOH

Chart 1

gave the triol **7b**, which was reacted with cyclohexanone in the presence of acid to give the ketal **8**. The ^{1}H-NMR spectrum of **8** showed that the 2-methylene protons which participate in the new ring behave as if they were conformationally restricted (ABX-type signals at 3.58 and 3.97 ppm). Successive treatment with benzoyl chloride and *p*-toluenesulfonic acid gave the 3-*O*-benzoylated triol **9**. After further trityl protection of the 2-methanol function of **9**, tosylation was achieved on the oxygen at l-carbon to afford the tosylate **10**. Nucleophilic substitution of **10** with 2-amino-6-benzyloxypurinyl anion and acylation with isobutyryl chloride gave the C-OXT-G derivative **11b**. To obtain the 2′-*O*-protected **6a**, the key intermediate **11b** was treated with alkali to remove the *O*-benzoyl group from the cyclobutyl ring and reduced with hydrogen using 5% Pd/C as a catalyst. Catalytic hydrogenation of **11b** and acid treatment with *p*-toluenesulfonic acid afforded the 3′-*O*-protected **5c**. Although this synthetic route to obtain *O*-protected C-OXT-G includes more steps than the non-specific protection procedure described previously, each step proceeds with good yield and without difficulty of separation. Dehydration of C-OXT-G was achieved using a method established in our laboratory.[6] Thus compound **5a**, **5c** or **6a** was reacted with methyltriphenoxyphosphonium iodide to afford the iodomethyl derivative, and elimination of the product with potassium *tert*-butoxide gave, after removal of the protecting group, methylenecyclobutylguanines **3b**, **4b**.

Antiviral Activity The antiviral effects[8] of C-OXT-A (**1d**) and C-OXT-G (**2d**) and related compounds including the methylenecyclobutylpurines were assayed according to previously established procedures[9] and the results are presented in Tables I—VI. Both **1d** and **2d** displayed potent activity against HSV-1 and HSV-2 (Table I). To elucidate the role of the HSV-encoded thymidine kinase (TK) in the antiherpetic activities of **1d** and **2d**, the compounds were also evaluated against TK-deficient (TK^-) strains of HSV. C-OXT-A was equally active against TK^- and TK^+ strains but C-OXT-G **2d** proved more than 100-fold less active against TK^- HSV-1 than TK^+ HSV-1 or HSV-2. This means that the activity of C-OXT-G at least partially depends on phosphorylation by the virus-induced TK, whereas C-OXT-A must for its phosphorylation depend entirely on cellular deoxynucleoside kinases.

TABLE I. Activity of **1**—**4** against HSV (Types 1 and 2) and Vaccinia Virus

Compound	EC_{50} (μg/ml)[a]					MCC_{50} (μg/ml)[b]
	HSV-1 (Strain KOS)	HSV-2 (Strain G)	TK⁻ HSV-1 (Strain B2006)	TK⁻ HSV-1 (Strain VMW1837)	Vaccinia virus	E_6SM
1a	70	70	70	>100	70	≥400
1b	150	100	125	150	150	≥400
1c	>10	>10	>40	>40	>10	40
1d (C-OXT-A)	1	1	2	2	40	>400
1e	150	>200	>400	>200	>200	≥400
2d (C-OXT-G)	0.007	0.007	2	0.4	70	>400
2e	40	>400	>400	>400	>400	>400
3a	>400	200	>400	>400	150	>400
4a	300	150	200	>200	300	>400
4b	>200	300	>200	>200	>200	>400
IDU	0.2	0.7	20	10	0.2	>400
BVDU	0.02	40	>200	>200	0.2	>400
Ribavirin	>400	300	100	20	70	>400

a) 50% effective concentration, or concentration required to reduce virus-induced cytopathogenicity by 50%. Virus-induced cytopathogenicity was recorded at day 3 post infection. *b*) Minimum cytotoxic concentration, or concentration required to cause a microscopically detectable alteration of normal cell morphology.

TABLE II. Activity of **1**—**4** against VZV in Human Embryonic Lung (HEL) Cells

Compound	EC_{50} (μg/ml)[a]				CC_{50} (μg/ml)[b]
	TK⁺ VZV		TK⁻ VZV		
	Strain OKA	Strain YS	Strain 07/1	Strain YS/R	
1a	24	8	32	16	80
1b	50	10	39	31	150
1c	30	22	19	21	40
1d (C-OXT-A)	0.2	0.1	—	0.1	8
1e	70	70	70	70	>200
2d (C-OXT-G)	0.03	0.03	0.4	0.1	59
2e	>400	>400	>400	>400	>200
3a	47	32	7	17	>50
4a	34	29	8	13	>50
4b	>100	>100	>100	>100	>50
BVDU	0.001	0.0007	>10	>10	>200
ACV	0.20	0.10	23	17	200

a) 50% effective concentration, or concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU). *b*) Cytotoxic concentration, or concentration required to reduce host cell growth by 50%.

TABLE III. Activity of **1d**, **2**, **3** and **4** against CMV in Human Embryonic Lung (HEL) Cells

Compound	EC_{50} (μg/ml)[a]				CC_{50} (μg/ml)[b]
	Strain AD-169		Davis strain		
	20 PFU	100 PFU	20 PFU	100 PFU	
1a	—	—	—	—	80
1b	—	—	—	—	150
1c	—	—	—	—	40
1d (C-OXT-A)	0.2	1.5	0.4	2	8
1e	—	—	—	—	>200
2d (C-OXT-G)	1	2	1	1.5	59
2e	>400	>400	>400	>400	>200
3a	—	>100	—	>100	>50
4a	—	40	—	70	>50
4b	—	70	—	70	>50
ACV	11	40	17	—	>200
BVDU	>100	>100	>100	—	>200

a) 50% effective concentration, or concentration required to reduce virus plaque formation by 50%. Virus input was 20 or 100 plaque forming units (PFU), as indicated. *b*) 50% cytotoxic concentration, or concentration required to reduce host cell growth by 50%.

This assumption was further supported by the fact that C-OXT-A was equally active against TK⁻ and TK⁺ VZV, whereas C-OXT-G was definitely less active against TK⁻ VZV (Table II). C-OXT-A and C-OXT-G were found to inhibit VZV replication at a concentration that was 40- to 2000-fold lower than the concentration required to inhibit host cell growth (Table II).

C-OXT-G also inhibited cytomegalovirus (CMV) replication at a concentration that was 30- to 60-fold lower than the cytostatic concentration (Table III). The methylenecyclobutylpurines **3a**, **4a** and **4b** showed some activity against VZV, but this activity was seen only at a concentration that was markedly higher than the concentration at which **2d** was found active (Table II). Compounds **3a**, **4a** and **4b** exhibited little activity against CMV and were also virtually inactive against HSV-1 and HSV-2 (Table I).[10]

The **1** series of compounds demonstrated an appreciable activity against adenovirus (Table IV): *i.e.*, **1b** inhibited the replication of adenovirus types 2 and 3 at a concentration

TABLE IV. Activity of **1** and **2** against Adenoviruses in HeLa Cells

Compound	EC_{50} (μg/ml)[a]		MCC (μg/ml)[b]
	Adenovirus		HeLa
	Type 2	Type 3	
1a	20	20	100
1b	8	8	200
1c	20	8	100
1d (C-OXT-A)	≥200	20	>200
1e	20	20	>200
2d (C-OXT-G)	—	>200	>200
2e	≥200	>200	>200
Ribavirin	20	20	>200
ACV	≥200	>200	>200
Ara-C	100	—	>200

a) 50% effective concentration, or concentration required to reduce virus-induced cytopathogenicity by 50%. Virus-induced cytopathogenicity was recorded at day 5 post infection. *b*) Minimum cytotoxic concentration, or concentration required to cause a microscopically detectable alteration of normal cell morphology. Ara-C: cytosine arabinoside.

TABLE V. Activity of **1** to **4** against HIV (Types 1 and 2) in Human T-Lymphocyte (MT-4) Cells

Compound	ED_{50} (μM)[a]		CC_{50} (μM)[b]
	HIV-1 Strain III_B	HIV-2 Strain ROD	MT-1
1a	>100	>100	277±104
1b	16±12	34±23	353±171
1c	131±47	228±51	354±169
1d (C-OXT-A)	1.8±1.1	4.4±4.8	12±9
1e	>500	>500	>500
2d (C-OXT-G)	30±28	17±9.5	29
2e	>500	>500	>500
3a	22	63	>100
4a	>100	>100	>100
4b	>100	>100	>100
AZT	0.0019±0.0001	0.0020±0.0001	4.24±1.61

a) 50% effective concentration, or concentration required to protect MT-4 cells against virus-induced cytopathogenicity by 50%. Virus-induced cytopathogenicity was recorded at day 5 post infection. *b*) Minimum cytotoxic concentration, or concentration required to reduce MT-4 cell viability by 50%. AZT: 3′-azidothymidine.

TABLE VI. Activity of **1** and **2** against Arenaviruses in Vero Cells

Compound	EC_{50} (μg/ml)[a]		MCC (μg/ml)[b]
	Junin virus	Tacarbe virus	Vero
1a	24	34	400
1b	10	12	400
1c	4	5	40
1d (C-OXT-A)	>400	>400	>400
1e	>400	>400	>400
2d (C-OXT-G)	>100	>100	>100
2e	>100	>100	>100
Ribavirin	7	8	>400

a) 50% effective concentration, or concentration required to reduce virus-induced cytopathogenicity by 50%. Virus-induced cytopathogenicity was recorded at day 3 post infection. *b*) Minimum cytotoxic concentration, or concentration required to cause a microscopically detectable alteration of normal cell morphology.

that was 25-fold lower than the cytotoxic concentration. In contrast, **2d** and **2e** proved inactive against adenovirus. Compound **1b** could be considered as a lead compound for development of anti-adenovirus agents and its further modification should be attempted so as to increase its anti-adenovirus activity.

The activity of **1** and **2** against HIV-1 and HIV-2 was evaluated in human T-lymphocyte (MT-4) cells (Table V). Compound **2d** (C-OXT-G) was effective only at a concentration that coincided with its 50% cytotoxicity concentration. The adenine congener **1d** (C-OXT-A) was found to inhibit HIV replication at a concentration that was 3-to 7-fold below the cytotoxicity threshold. Thus, although C-OXT-A and C-OXT-G are inhibitory to the replication of HIV,[11] they are quite toxic to proliferating human T-lymphocytes, and this should be taken into account in assessing the potential utility of these compounds for the AIDS therapy.[11a]

The activity of the test compounds **1**, **2**, **3** and **4** was also examined against RNA viruses other than HIV, such as Sindbis, Coxsackie B4, Semliki forest virus, polio-1, parainfluenza-3, respiratory syncytial, vesicular stomatitis, reo-1, and arenaviruses (Junin, Tacaribe). Except for **1a**—**c**, which showed an appreciative activity against the arenaviruses (Table VI), no activity was noted with any of the compounds against any of the other RNA viruses tested.

Experimental

Melting points (mp) were determined using a Yanagimoto micromelting point apparatus (hot stage type) and are uncorrected. UV spectra were recorded with a Shimadzu UV-190 digital spectrometer. Low resolution mass spectra were obtained on a Shimadzu-LKB 9000B mass spectrometer in the direct-inlet mode. High resolution mass spectra were obtained on a JMS AX-500 spectrometer in the direct-inlet mode. ^{1}H-NMR spectra were recorded on either JEOL FX-90Q (90 MHz) or JEOL GX-400 (400 MHz) in $CDCl_3$ (or dimethyl sulfoxide (DMSO)-d_6) with tetramethylsilane as an internal standard. Merck Art 5554 plates precoated with Silica gel 60 containing fluorescent indicator F_{254} were used for thin-layer chromatography and Silica gel 60 (Merck 7734, 60—200 mesh) was employed for column chromatography.

1,2-*O*-Cyclohexylidene-*trans-cis*-2,3-bis(hydroxymethyl)-1-cyclobutanol (8) *trans-cis*-2,3-Bis(hydroxymethyl)-1-cyclobutanol (**7b**) was obtained by alkaline hydrolysis of **7a**.[8c] Thus, **7b** (1.42 g, 10.7 mmol) was dissolved in cyclohexanone (18 ml) and *p*-toluenesulfonic acid (580 mg) was added. After stirring for 20 min at 0 °C, the solution was neutralized with triethylamine (0.5 ml) and the solvent was evaporated. The residue was dissolved in $CHCl_3$ (120 ml) and the organic layer was washed with water (60 ml), dried over $MgSO_4$ and concentrated to a small volume. The solution was chromatographed on a column of Silica gel 60 (i.d. 3.0×35 cm) with a 10% AcOEt in $CHCl_3$ (1.01) to give a syrup (1.95 g, 86%). ^{1}H-NMR ($CDCl_3$) δ: 4.36 (1H, m, H1), 3.97 (1H, dd, J=11.6, 5.3 Hz, 2-CH_2O-), 3.63 (3H, m, 3-CH_2OH), 3.58 (1H, d, J=11.6 Hz, 2-CH_2O-), 2.98 (1H, m, H2), 2.05 (1H, m, H3), 1.96 (1H, dd, J=8.0, 1.0 Hz, H4a), 1.79 (1H, d, J=8.0 Hz, H4b), 1.6—2.1 (*ca.* 10H, -$(CH_2)_5$-).

***trans*-3-Benzoyloxymethyl-*cis*-2-hydroxymethyl-1-cyclobutanol (9)** Benzoyl chloride (1.2 ml, 10.3 mmol) was added to a solution of **8** (1.39 g, 6.55 mmol) in pyridine (50 ml) and the mixture was stirred for 1 h at room temperature. The usual workup of the resulting solution gave the benzoylated product of **8** as a syrup, which was dissolved in MeOH (25 ml). Amberlite IR 120B (H^+ form, 6 ml) was added to the solution and stirred for 1 h at room temperature. The resin was separated off by the filtration and the filtrate was evaporated to give a residue, which was chromatographed on a column of Silica gel 60 (i.d. 3.0×30 cm) with a gradient of 0—15% EtOH in $CHCl_3$ (1.01). The syrup thus obtained was crystallized from hexane to give white crystals (1.04 g, 67%). mp 70—73 °C. *Anal.* Calcd for $C_{13}H_{16}O_4$. C, 66.08; H, 6.83. Found: C, 66.15; H, 6.90. ^{1}H-NMR ($CDCl_3$) δ: 7.2—8.05 (5H, m, -COC_6H_5), 4.65 (1H, quintet, J=5.0 Hz, H1), 4.33 (2H, d, J=5.1 Hz, 3-CH_2OBz), 3.94 (2H, m, 2-CH_2OH), 2.1—2.75 (6H, H2, H3, H4a, H4b, 1-OH, 2-CH_2OH).

***trans*-3-Benzoyloxymethyl-*cis*-2-trityloxymethyl-1-tosyloxycyclobutane (10)** Trityl chloride (1.98 g, 7.10 mmol) and **9** (1.38 g, 5.84 mmol) were dissolved in pyridine (20 ml) and the mixture was stirred overnight at room temperature. The resulting solution was subjected to the standard workup to give a foam which was dissolved in pyridine (20 ml). *p*-Toluenesulfonyl chloride (1.36 g, 7.1 mmol) was added to the solution and stirred overnight at room temperature. After addition of water (2 ml), the solution was evaporated and the residue was dissolved in a mixture of benzene (20 ml) and ether (10 ml). The organic layer was washed twice with water (10 ml), dried over $MgSO_4$ and evaporated to give a syrup. Pyridine was removed completely by the azeotropical evaporation with toluene (10 ml) and the solution was chromatographed on a column of silica gel 60 (i.d. 2.5×25 cm) with benzene (1.01) to afford a foam (2.56 g, 69%). ^{1}H-NMR ($CDCl_3$) δ: 8.00, 7.69 (each 2H, d, -$SO_2C_6H_5CH_3$), 7.2—8.1 (*ca.* 20H, m, -COC_6H_5, -$C(C_6H_5)_3$), 5.07 (1H, quintet, J=6.8 Hz, H1), 4.30 (2H, m, 3-CH_2O-), 3.31 (2H, m, 2-CH_2O-), 2.67 (2H, m, H2, H3), 2.42 (3H, s, -$SO_2C_6H_5CH_3$), 2.38 (1H, m, H4a), 2.20 (1H, m, H4b).

2-Amino-6-benzyloxy-9-(*trans-trans*-3-benzoyloxymethyl-2-trityloxymethylcyclobutyl)purine (11a) To a suspension of 2-amino-6-benzyloxypurine (1.40 g, 5.80 mmol) in dimethylformamide (DMF, 100 ml) was added potassium carbonate (400 mg, 2.89 mmol) and the solution was stirred for 30 min at 120 °C, then **10** (2.20 g, 3.48 mmol) was added. After stirring for 5 h, the solution was neutralized by acetic acid and evaporated to dryness. Benzene (200 ml) was added to the residue and the insoluble portion was filtered off. The remainder was washed with water, dried over $MgSO_4$ and concentrated to a small volume. The solution was chromatographed on a column of Silica gel 60 (i.d. 3.0×19 cm) with a

gradient of 0—50% AcOEt in benzene (1.2 l) to give a syrup (1.08 g, 44%). UV λ_{max}^{MeOH} nm: 282.

6-Benzyloxy-2-isobutyrylamino-9-(*trans-trans*-3-benzoyloxymethyl-2-trityloxymethylcyclobutyl)purine (11b) Isobutyryl chloride (0.9 ml, 8.6 mmol) was added to a solution of **11a** (1.07 g, 1.52 mmol) in pyridine (15 ml) and the mixture was stirred for 1 h at 0 °C. The usual workup of the resulting solution gave a gum (1.15 g, 98%). UV λ_{max}^{MeOH} nm: 282. MS *m/z*: 771 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 7.1—8.0 (*ca.* 26H, H8, 2'-$CH_2OC(C_6\underline{H}_5)_3$, 3'-$CH_2OCOC_6\underline{H}_5$), 6-$OCH_2C_6\underline{H}_5$), 5.61 (2H, s, 6-$OC\underline{H}_2C_6H_5$), 4.77 (1H, m, H1'), 4.46 (2H, m, 3'-$C\underline{H}_2$OBz), 3.32 (2H, m, 2'-$C\underline{H}_2$OTr, 3.2—3.3 (5H, m, H2', H3', H4'a, H4'b, -$COC\underline{H}(CH_3)_2$), 1.10 (6H, m, -$COCH(C\underline{H}_3)_2$).

***N*^{2}-Isobutyryl-9-[*trans-trans*-2,3-bis(hydroxymethyl)cyclobutyl]-guanine (2f)** Isobutyryl chloride (0.9 ml, 8.6 mmol) was added to a solution of (±)-**2d** (398 mg, 1.50 mmol) in pyridine (15 ml) and the mixture was stirred for 45 min at 0 °C. The usual workup of the resulting solution gave the fully acylated product of **2d** as a syrup, to which 2 M NaOH (8 ml) and EtOH (8 ml) was added. After stirring for 1 h at 0 °C. The solution was neutralized with 2 N HCl and concentrated to a small volume to afford crystals (388 mg, 77%). mp 238—241 °C. *Anal.* Calcd for $C_{15}H_{21}N_5O_4$. C, 53.72; H, 6.31; N, 20.89. Found: C, 53.24; H, 6.31; N, 20.89. UV λ_{max}^{MeOH} nm: 261,280 (sh).

Tritylation of 2f Trityl chloride (167 mg, 0.6 mmol) and **2f** (184 mg, 0.55 mmol) were dissolved in pyridine (20 ml) and the mixture was stirred overnight at room temperature. After addition of water (3 ml), the solution was evaporated to give a pale brownish gum which was dissolved in $CHCl_3$ (50 ml). The organic solution was washed twice with water (30 ml), dried over $MgSO_4$, and concentrated to a small volume. Pyridine was removed completely by the azeotropical evaporation with toluene (20 ml). The solution was chromatographed on a column of Silica gel 60 (i.d. 2.2 × 30 cm) with a gradient of 0—10% EtOH in $CHCl_3$ (1.0 l). The first fraction was collected and evaporated to dryness to give *N*^{2}-isobutyryl-9-[*trans-trans*-2,3-bis(trityloxymethyl)cyclobutyl]guanine (79 mg, 18%) as a foam. UV λ_{max}^{MeOH} nm: 261.

From th second fraction, *N*^{2}-isobutyryl-9-(*trans*-2-hydroxymethyl-*trans*-3-trityloxymethylcyclobutyl)guanine **5a** (90 mg, 28%) was obtained as white crystals. mp 217—219 °C. *Anal.* Calcd for $C_{34}H_{35}N_5O_4$. C, 70.69; H, 6.11; N, 12.12. Found: C, 71.11; H, 6.23; N, 12.17. UV λ_{max}^{MeOH} nm: 260, 280 (sh). ^{1}H-NMR ($CDCl_3$) δ: 12.01 (1H, s, *N*^{1}-H), 8.67 (1H, s, N$\underline{H}$COCH$(CH_3)_2$), 7.67 (1H, s, H8), 7.2—7.5 (*ca.* 15H, m, 3'-$CH_2OC(C_6\underline{H}_5)_3$), 4.45 (1H, q, J=8.6 Hz, H1'), 3.76 (3H, m, 2'-$C\underline{H}_2O\underline{H}$), 3.30 (1H, dd, J=5.0, 9.3 Hz, one of -$C\underline{H}_2$OTr), 3.14 (1H, dd, J=6.5, 9.3 Hz, one of -$C\underline{H}_2$OTr), 2.73 (1H, m, H2'), 2.54 (2H, m, H4'a, -$COC\underline{H}(CH_3)_2$), 2.32 (1H, q, J=9.8 Hz, H4'b), 2.22 (1H, m, H3'), 1.22 (6H, d, J=7.2 Hz, -$COCH(C\underline{H}_3)_2$).

The third fraction was evaporated to afford *N*^{2}-isobutyryl-9-(*trans*-3-hydroxymethyl-*trans*-2-trityloxymethylcyclobutyl)guanine **6a** (49.4 mg, 15.6%) as a foam. UV λ_{max}^{MeOH} nm: 261,280 (sh). ^{1}H-NMR ($CDCl_3$) δ: 11.96 (1H, s, *N*^{1}-H), 8.61 (1H, s, N$\underline{H}$COCH$(CH_3)_2$), 7.65 (1H, s, H8), 7.2—7.4 (*ca.* 15H, m, 2'-$CH_2OC(C_6\underline{H}_5)_3$), 4.49 (1H, q, J=8.6 Hz, H1'), 3.68 (2H, m, 3'-$C\underline{H}_2$OH), 3.33 (1H, dd, J=9.3, 5.7 Hz, one of $C\underline{H}_2$OTr), 3.24 (1H, dd, J=9.3, 6.8 Hz, one of $C\underline{H}_2$OTr), 2.86 (1H, q, J=7.0 Hz, H2'), 2.57 (1H, m, J=6.4 Hz, -$COC\underline{H}(CH_3)_2$), 2.36—2.50 (2H, m, H4'a, H4'b), 2.22 (1H, m, H3'), 1.20 (6H, t, -$COCH(C\underline{H}_3)_2$).

***N*^{2}-Isobutyryl-9-(*trans*-3-benzoyloxymethyl-*trans*-2-hydroxymethylcyclobutyl)guanine (5c)** A solution of **11b** (908 mg, 1.178 mmol) in EtOH (30 ml) was stirred vigorously for 6 h at room temperature in the presence of 5% Pd/C (450 mg) under H_2 atmosphere. The catalyst was filtered off and the solvent was evaporated to give a residue, which was dissolved in $CHCl_3$ (30 ml). The insoluble portion was removed by the filtration and the remainder was evaporated leaving a foam, which was dissolved in a mixture of $CHCl_3$ (7 ml) and MeOH (7 ml). *p*-Toluenesulfonic acid (200 mg) was added to the solution and the mixture was stirred for 5 h at room temperature. The solution was neutralized with triethylamine and evaporated to dryness. The residue was dissolved in $CHCl_3$, washed with water (100 ml) twice, dried over $MgSO_4$ and chromatographed on a column of Silica gel 60 (i.d. 2.2 × 15 cm) with a gradient of 0—15% EtOH in $CHCl_3$ (0.7 l). Evaporation of the eluent and crystallization of the resulting syrup afforded white crystals (363 mg, 70%). mp 186.5—189 °C. *Anal.* Calcd for $C_{22}H_{25}N_5O_5 \cdot 0.2H_2O$: C, 59.63; H, 5.78; N, 15.81. Found: C, 59.32; H, 5.88; N, 15.36. UV λ_{max}^{MeOH} nm: 261. ^{1}H-NMR (DMSO-d_6) δ: 8.23 (1H, s, H8), 8.00, 7.50—7.70 (2H and 3H, m, $C_6\underline{H}_5$CO-), 4.78 (1H, t, 2'-$CH_2O\underline{H}$), 4.68 (1H, m, H1'), 4.43 (2H, d, 3'-$C\underline{H}_2$OBz), 3.55 (2H, m, 2'-$C\underline{H}_2$OH), 2.20—3.30 (5H, m, H2', H3', H4'a, H4'b, -$COC\underline{H}(CH_3)_2$), 1.17 (6H, d, -$COCH(C\underline{H}_3)_2$).

***N*^{2}-Isobutyryl-9-(*trans*-2-iodomethyl-*trans*-3-trityloxymethylcyclobutyl)guanine (5b)** Methyltriphenoxyphosphonium iodide (1.2 g, 2.65 mmol) was added to the solution of **5a** (659 mg, 1.14 mmol) in DMF (60 ml) and the mixture was stirred for 1 h at room temperature. After dilution with benzene (200 ml), the organic layer was washed successively with water (150 ml), 5% $Na_2S_2O_3$ (200 ml) and water (150 ml). The solution was dried over $MgSO_4$, concentrated to a small volume, then chromatographed on a column of Silica gel 60 (i.d. 2.2 × 20 cm) with a gradient of 0—5% EtOH in $CHCl_3$ (500 ml) to afford a gum (545 mg, 70%). UV λ_{max}^{MeOH} nm: 261.

***N*^{2}-Isobutyryl-9-(*trans*-3-benzoyloxymethyl-*trans*-2-iodomethylcyclobutyl)guanine (5d)** Methyltriphenoxyphosphonium iodide (850 mg, 1.88 mmol) was added to a solution of **5c** (343 mg, 0.78 mmol) in DMF (25 ml) and the solution was stirred for 1 h at room temperature. The resulting solution was subjected to the standard workup to give a foam (387 mg, 90%). mp 108 °C (dec.). *Anal.* Calcd for $C_{22}H_{24}IN_5O_4$. C, 48.10; H, 4.40; N, 12.75. Found: C, 47.76; H, 4.22; N, 12.41. UV λ_{max}^{MeOH} nm: 261.

***N*^{2}-Isobutyryl-9-[*trans*-3-iodomethyl-*trans*-2-trityloxymethylcyclobutyl]guanine (6b)** Methyltriphenoxyphosphonium iodide (2.4 g, 5.3 mmol) was added to a solution of **6a** (575 mg, 1.00 mmol) in DMF (25 ml) and the solution was stirred for 1 h at room temperature. The resulting solution was subjected to the standard workup to give a foam (599 mg, 87%). UV λ_{max}^{MeOH} nm: 260.

***N*^{2}-Isobutyryl-9-(2-methylene-*cis*-3-trityloxymethylcyclobutyl)guanine (3c)** Potassium *tert*-butoxide (840 mg, 7.49 mmol) was added to a solution of **5b** (538 mg, 0.783 mmol) in pyridine (15 ml)–*tert*-butanol (28 ml) and the mixture was stirred for 1 h at room temperature. The solution was neutralized with acetic acid, and evaporated to dryness. The residual syrup was dissolved in $CHCl_3$ (150 ml), and after washing with water (100 ml) twice, evaporated azeotropically with toluene (30 ml) twice. The solution was chromatographed on a column of silica gel 60 (i.d. 2.0 × 19 cm) with a gradient of 0—5% EtOH in $CHCl_3$ (600 ml). Evaporation of the eluent to dryness and crystallization from MeOH afforded white crystals (362 mg, 83%). UV λ_{max}^{MeOH} nm: 260. MS *m/z*: 559 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 8.18 (1H, s, *N*^{1}-H), 7.72 (1H, s, H8), 7.2—7.5 (*ca.* 15H, m, 3'-$CH_2OC(C_6\underline{H}_5)_3$), 5.30 (1H, t, H1'), 5.10, 4.90 (each 1H, s, $=CH_2$), 3.32 (2H, m, 3'-$C\underline{H}_2$OTr), 3.15 (1H, m, H3'), 2.63 (1H, m, H4'a), 2.3—2.5 (2H, m, H4'b, -$COC\underline{H}(CH_3)_2$), 1.15—1.35 (6H, m, -$COCH(C\underline{H}_3)_2$).

***N*^{2}-Isobutyryl-9-(*cis*-3-hydroxymethyl-2-methylenecyclobutyl)guanine (3e)** Potassium *tert*-butoxide (725 mg, 6.46 mmol) was added to a solution of **5b** (371 mg, 0.68mmol) in pyridine (12 ml)–*tert*-butanol (24 ml) and the mixture was stirred for 1 h at room temperature. The solution was treated in the manner described above to give a foam (112 mg, 52%). mp 120 °C (dec.). *Anal.* Calcd for $C_{15}H_{19}N_5O_3 \cdot 0.5H_2O$. C, 55.20; H, 6.18; N, 21.46. Found: C, 55.30; H, 6.06; N, 21.11. UV λ_{max}^{MeOH} nm: 261. MS *m/z*: 559 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 8.08 (1H, s, H8), 5.30 (1H, m, H1'), 5.08, 4.89 (each 1H, m, $=CH_2$), 4.87 (1H, m, 3'-$CH_2O\underline{H}$), 3.63 (2H, m, 3'-$C\underline{H}_2$OH), 2.2—3.10 (4H, m, H3', H4'a, H4'b, -$COC\underline{H}(CH_3)$), 1.05 (6H, m, -$COCH(C\underline{H}_3)_2$).

***N*^{2}-Isobutyryl-9-(3-methylene-*cis*-2-trityloxymethylcyclobutyl)guanine (4c)** Potassium *tert*-butoxide (784 mg) was added to a solution of **6b** (590 mg, 0.86 mmol) in pyridine (18 ml)–*tert*-butanol (30 ml) and the mixture was stirred for 2 h at room temperature. The solution was treated in the manner as described above to give a foam (487 mg, quantitative). UV λ_{max}^{MeOH} nm: 261. MS *m/z*: 559 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 7.85 (1H, s, N^1-H), 7.80 (1H, s, H8), 7.18—7.38 (*ca.* 15H, m, 2'-$CH_2OC(C_6\underline{H}_5)_3$), 4.97, 4.88 (each 1H, m, $=CH_2$), 4.74 (1H, m, H1'), 3.17—3.64 (5H, m, 2'-$C\underline{H}_2$OTr, H2', H4'a, H4'b), 2.42 (1H, m, -$COC\underline{H}(CH_3)_2$), 1.08—1.20 (6H, m, -$COCH(C\underline{H}_3)_2$).

Removal of Protecting Group. 9-(2-Methylene-*cis*-3-trityloxymethylcyclobutyl)guanine (3d) Compound **3c** (195 mg, 0.35 mmol) was treated with 0.02 M NaOMe in MeOH (6 ml) for 2 h at 50°C, then cooled. The solution was neutralized with 1 M HCl (0.15 ml) and, after evaporation of the solvent, the residue was dissolved in $CHCl_3$ (20 ml). The organic layer was washed with water (10 ml), dried over $MgSO_4$, and evaporated to dryness. The resulting syrup was crystallized from a small amount of MeOH to give white crystals (164 mg, 88%). mp 244—246 °C. *Anal.* Calcd for $C_{30}H_{27}N_5O_2 \cdot 0.5H_2O$. C, 72.27; H, 5.66; N, 14.05. Found: C, 72.24; H, 5.35; N, 13.68. UV λ_{max}^{MeOH} nm: 256.

9-(3-Methylene-*trans*-2-trityloxymethylcyclobutyl)guanine (4d) Compound **4c** (480 mg, 0.86 mmol) was treated with 0.02 M NaOMe in MeOH (15 ml) for 3 h at 50 °C and the solution was subjected to the standard processing. Crystallization of the product from MeOH gave white crystals (384 mg, 91%). mp 244—248 °C. *Anal.* Calcd for $C_{30}H_{27}N_5O_2 \cdot 0.2H_2O$: C, 73.06; H, 5.60; N, 14.20. Found: C, 73.00; H, 5.30; N, 13.89. MS *m/z*:

489 (M^+). UV λ_{max}^{MeOH} nm: 260.5.

9-(*cis*-3-Hydroxymethyl-2-methylenecyclobutyl)guanine (3b) Method 1: 1 M HCl (1 ml) was introduced to a solution of **3d** (185 mg, 0.38 mmol) in MeOH (15 ml), then stirred for 1.5 h at 60 °C. After cooling, Amberlite IR 400 (OAc^-, 10 ml) was added to the mixture to capture HCl. The resin was removed by filtration and the filtrate was evaporated leaving a residue which was partitioned between $CHCl_3$ (5 ml) and water (10 ml). The aqueous layer was washed with $CHCl_3$ and concentrated to a small volume to afford white crystals (60 mg, 64%). mp 282—285 °C. UV $\lambda_{max}^{0.1\ N\ HCl}$ nm: 259.5, UV λ_{max}^{MeOH} nm: 253. ^{1}H-NMR (DMSO-d_6) δ: 10.58 (1H, s, N^1-H), 7.78 (1H, s, H8), 6.42 (2H, br s, 2-NH_2), 5.23 (1H, t, J=9.1, 2.3 Hz, H1′), 5.06, 4.76 (each 1H, m, =CH_2), 4.73 (1H, t, J=5.7 Hz, 3′-CH_2O<u>H</u>), 3.65 (2H, m, 3′-C<u>H</u>$_2$OH), 2.97 (1H, m, H3′), 2.55 (1H, ddt, J=10.8, 4.0, 8.8 Hz, H4′a), 2.23 (1H, ddt, J=10.2, 4.0, 8.8 Hz, H4′b).

Method 2: Compound **3e** (50 mg, 0.16 mmol) was treated with 0.02 M NaOMe in MeOH (5 ml) for 2.5 h at 50 °C, then cooled. The solution was neutralized with 1 M HCl (0.10 ml) and concentrated to a small volume to give white crystals (30 mg, 76%), which was identical in all respects to the sample obtained as described above.

9-(3-Methylene-*trans*-2-hydroxymethylcyclobutyl)guanine (4b) 1 M HCl (0.2 ml) was added to a solution of **4d** (373 mg, 0.76 mmol) in MeOH (30 ml) and the solution was stirred for 1.5 h at 60 °C. The mixture was treated in the manner as described above to give white crystals (132.5 mg, 70%). mp 176—178 °C. *Anal.* Calcd for $C_{11}H_{13}N_5O$. C, 57.13; H, 5.67; N, 30.28. Found: C, 56.94; H, 5.64; N, 29.99. UV λ_{max}^{MeOH} nm: 254. ^{1}H-NMR (DMSO-d_6) δ: 10.59 (1H, br s, N^1-H), 7.78 (1H, s, H8), 6.44 (2H, br s, 2-NH_2), 5.00, 4.95 (each 1H, m, =CH_2), 4.71 (1H, t, J=4.6 Hz, 2′-CH_2O<u>H</u>), 4.58 (1H, q, J=8.0 Hz, H1′), 3.55—3.70 (3H, m, H2′, 2′-C<u>H</u>$_2$OH), 3.20 (1H, dd, J=8.0, 12.6 Hz, H4′a), 3.00 (1H, dd, J=8.5, 12.6 Hz, H4′b).

Acknowledgments We thank Anita Van Lierde, Frieda De Meyer, Anita Camps and Ann Absillis for excellent technical assistance. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas, No. 03242213, from the Ministry of Education, Science and Culture, Japan, grants from the AIDS Basic Research Programme of the European Community and by grants from the Belgian Fonds voor Geneeskundig Weten-schappelijk Onderzoek (projects no. 3.0026.91 and 3.0097.87) and the Belgian Geconcerteerde Onderzoeksacties (project no. 90/94-2).

References and Notes

1) *a*) W. H. Prusoff, *Biochim. Biophys. Acta*, **32**, 295 (1959); *b*) E. C. Herrmann, Jr., *Proc. Soc. Exp. Biol. Med.*, **107**, 142 (1961); *c*) H. E. Kaufman and E. D. Maloney, *Arch. Ophthalmol.*, **68**, 396 (1962); *d*) H. E. Kaufman, *Proc. Soc. Exp. Biol. Med.*, **109**, 251 (1962).

2) *a*) W. H. Prusoff and T.-S. Lin, "Antiviral Drug Development," ed. by E. De Clercq and R. T. Walker, Plenum Press, New York, 1988; *b*) R. K. Robins, *Chemistry and Engineering News*, January 27, 28 (1986).

3) J. D. Karkas, W. T. Ashton, L. F. Canning, R. Liou, J. Germershausen, R. Bostedor, B. Arison, A. K. Field and R. L. Tolman, *J. Med. Chem.*, **29**, 842 (1986) and references cited therein.

4) *a*) N. Shimada, S. Hasegawa, T. Harada, T. Tomisawa, A. Fujii and T. Takita, *J. Antibiotics*, **39**, 1623 (1986); *b*) H. Hoshino, N. Shimada, T. Takita and T. Takeuchi, *ibid.*, **40**, 1077 (1987).

5) *a*) M. Honjo, T. Maruyama, Y. Sato and T. Horii, *Chem. Pharm. Bull.*, **37**, 1413 (1989); *b*) T. Maruyama, T. Sato, T. Horii, H. Shiota, K. Nitta, T. Sirasaka, H. Mitsuya and M. Honjo, *ibid.*, **38**, 2719 (1990).

6) Synthesis of methylenecyclobutyladenines **3a**, **4a** has been reported in the following reports: T. Maruyama, Y. Hanai and Y. Sato, *Nucleoxides & Nucleotides*, in press and the 18th Symposium on Nucleic Acids Chemistry, Sendai, Oct. 1991, *Nucleic Acids Research Symposium Series*, No. 25, p. 87 (1991).

7) *a*) K. Takenuki, A. Matsuda, T. Ueda, T. Sasaki, A. Fujii and K. Yamagami, *J. Med. Chem.*, **31**, 1063 (1991); *b*) A. Matsuda, K. Takenuki, M. Tanaka, T. Sasaki and T. Ueda, *ibid.*, **34**, 812 (1991); *c*) T. Lin, M. Luo, M. Liu, R. H. Clarke-Katzenburg, Y. Cheng, W. H. Prusoff, W. R. Mancini, G. I. Birnbaum, E. J. Gabe and J. Giziewicz, *ibid.*, **34**, 2607 (1991).

8) Synthesis and antiviral activity of C-OXT-A and C-OXT-G have been reported by *a*) Y. Ichikawa, A. Narita, A. Shiozawa, Y. Hayashi and K. Narasaka, *J. Chem. Soc., Chem. Commun.*, **1989**, 1919; *b*) Y. Nishiyama, N. Yamamoto, Y. Yamada, T. Daikoku, Y. Ichikawa and K. Takahashi, *J. Antibiot.*, **XLII**, 1854 (1989); *c*) G. S. Basacchi, A. Braitman, C. W. Cianci, J. M. Clark, A. K. Field, M. E. Hagen, D. R. Hockstein, M. F. Malley, T. Mitt, W. A. Slusarchyk, J. E. Sundeen, B. J. Terry, A. V. Tuomari, E. R. Weaver, M. G. Young and R. Zahler, *J. Med. Chem.*, **34**, 1415 (1991); *d*) C. N. Hsiao and S. M. Hannick, *Tetrahedron Lett.*, **31**, 6609 (1990); *e*) B. J. Terry, K. E. Mazina, A. V. Tuomari, M. E. Hagen, M. L. Haffey, G. A. Jacobs, R. Zahler and A. K. Field, *Antiviral Chem. Chemother.*, **1**, 263 (1990); *f*) A. K. Field, A. V. Tuomari, B. McGeever-Rubin, B. J. Terry, K. E. Mazina, M. L. Haffey, M. E. Hagen, J. M. Clark, A. Braitman, W. A. Slusarchyk, M. G. Young and R. Zahler, *Antiviral Res.*, **13**, 41 (1990).

9) *a*) E. De Clercq, J. Descamps, G. Verhelst, R. T. Walker, A. S. Jones, P. F. Torrence and D. Shugar, *J. Infect. Dis.*, **141**, 563 (1980); *b*) E. De Clercq, A. Holy, I. Rosenberg, T. Sakuma, J. Balzarini and P. C. Maudgal, *Nature* (London), **323**, 464 (1986); *c*) D. Schols, E. De Clercq, J. Balzarini, M. Baba, M. Witvrouw, M. Hosoya, G. Andrei, R. Snoeck, J. Neyts, R. Pauwels, M. Nagy, J. Gyorgyi-Edelenyi, R. Machovich, I. Horvath, M. Low and S. Gorog, *Antiviral Chem. Chemother.*, **1**, 233 (1990).

10) Compound **3a** inhibited the replication of hepatitis B virus (HBV) with 50% effective concentration at 10—100 μg/ml, that is equal to 9-(β-D-arabinofuranosyl)adenine (Ara-A); private communication from T. Nagahara, Research Laboratories, Nippon Kayaku Co., Ltd., Tokyo 115, Japan.

11) *a*) D. W. Norbeck, E. Kern, S. Hayashi, W. Rosenbrook, H. Sham, T. Herrin, J. P. Plattner, J. Erickson, J. Clement, R. Swanson, N. Shipkowitz, D. Hardy, K. Marsh, G. Arnett, W. Shannon, S. Broder and H. Mitsuya, *J. Med. Chem.*, **33**, 1281 (1990); *b*) S. Hayashi, D. W. Norbeck, W. Rosenbrook, R. L. Fine, M. Matsukura, J. J. Plattner, S. Broder and H. Mitsuya, *Antimicrob. Agents Chemother.*, **34**, 287 (1990).