Pergamon

Tetrahedron 54 (1998) 2031–2040

TETRAHEDRON

9-Hydroxymethylcyclopropylidenemethylenyladenine: The Design, Facile Synthesis, Isomer Separation and Anti-HIV-1 Activities.

Changmei Cheng,[a] Tetsuro Shimo,[a] Kenichi Somekawa[a]* and Masanori Baba[b]

[a]Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Kagoshima University, Korimoto, Kagoshmima 890, Japan; [b]Division of Human Retroviruses, Center for Chronic Viral Diseases, Faculty of Medicine, Kagoshima University, Sakuragaoka, Kagoshima 890, Japan

Received 4 November 1997; accepted 6 December 1997

Abstract: 9-Hydroxymethylcyclopropylidenemethylenyladenine was designed based on the analysis of the structure-activity relationship and synthesized by coupling reaction of a vicinal dibromocyclopropane derivative with adenine. The cis/trans isomers and an enantiomer of the cis-isomer of the cyclic α, β-unsaturated nucleosides derived by reduction were separated by reverse phase HPLC and chiral HPLC, respectively. The cis-isomer was effective against HIV-1 and the (-)cis-isomer was highly effective with EC50 13 μM.

Although many protease inhibitors,[1] antisense drugs[2] and other agents[3] have been developed, the reverse transcriptase inhibitors such as modified nucleosides AZT, DDI, D4T, etc., still play a more important role in the therapy of AIDS. For example, the most famous cocktail treatment[4] now being administered to patients is a combined method of protease inhibitors and reverse transcriptase inhibitors. However, HIV is also tolerant to such drugs. So, a more effective and simple drug is strongly demanded and the continuous study is necessary.

Modified nucleosides are a large family of reverse transcriptase inhibitors and have the longest history in the therapy of AIDS. Since the discovery of AZT, many five-membered,[5] four-membered[6] and acyclic nucleosides[7] have been proved to have anti-HIV activities, but all the three-membered carbocyclic nucleosides hitherto reported are ineffective.[8] We analyzed the structure-activity relationship and successfully designed a novel three-membered nucleoside lead compound, 9-hydroxymethylcyclopropylidenemethylenyladenine (CMA).

Design. It is very difficult to identify a single or a few structural parameters to correlate the activity with the structure, because of the diversity of compounds that show at least some activity, and the fact that apparently very similar compounds can have extremely different activities. In order to simplify the problem, we focused our study on the modified sugar units of the nucleosides, although the bases strongly affect the activities. In this way, we may extract some features from the modified sugar moieties regardless of the kind of bases that were bonded to them, because we believe that an active modified sugar unit is more necessary for the active nucleoside. In other words, if we can discover good modified sugar candidates, we can certainly obtain better nucleosides by modifying the bases.

Many structure-activity relationship studies have been carried out[9] mainly focusing on the conformations of the furanose ring, but no simple and explicit conclusion was obtained, perhaps due to the fact that the inhibitor's conformations in complex with enzyme and the conformations in crystal are different considering that the ener-

0040-4020/98/$19.00
PII: S0040-4020(97)10413-6

getical barriers between the different conformations are very low and the enzyme can change them by interacting with inhibitors in order to form the most stable complexes. Therefore, we believe the study of configurations is more important not only because the configurations in complex with enzyme and in crystal are the same but also because all kinds of nucleosides can be included in the study. Furthermore , the electronic properties of the substrates may also affect the activities.

The typical effective nucleosides are shown in Fig. 1. It is obvious that all kinds of nucleosides have a common chain of HO-C^e-C^d-X^c-C^b-N^a (X=C or O; the other valences of C^d, C^b can be filled with H, C in single bonds or double bonds; C^e always bonds with two hydrogen atoms) in the modified sugar moiety as shown at the end of Fig. 1. This chain can be viewed as a simple model of nucleoside. In other words, the steric and electronic properties of this chain are directly associated with activities. We then calculated the molecule structure and the steric and electronic properties of all kinds of nucleosides, using the Win MOPAC program by Fujitsu Co.. We discovered that, among the numerous data, three parameters are dominantly important: (i) the distance between the substituted nitrogen of bases N^a (N_1 for pyrimidines or N_9 for purines) and C^d, denoted by γ; (ii) the

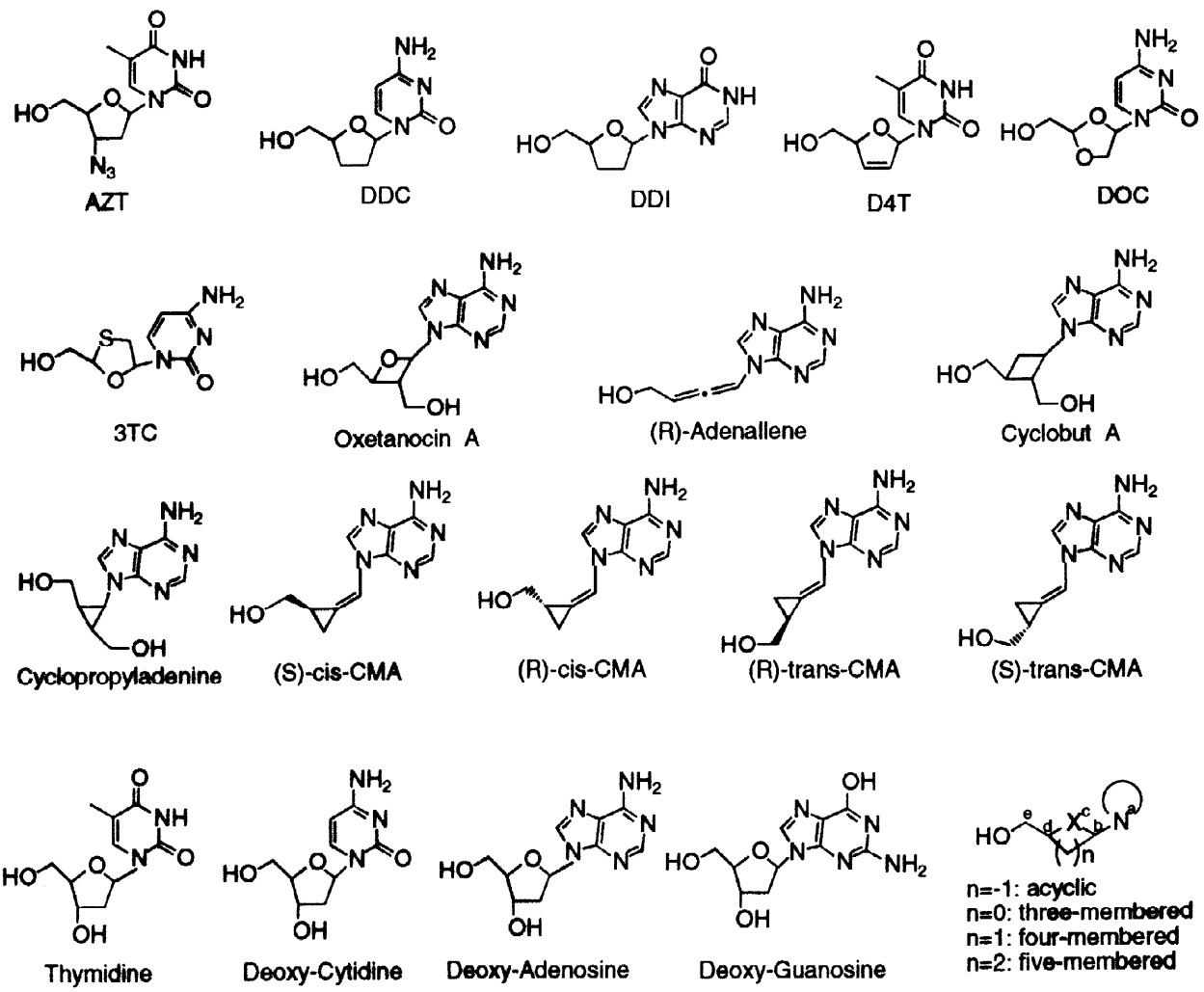


Fig. 1 Typical Nuleosides Possessing Anti-HIV-1 Activities and Related Compounds

torsion angle of N^a-C^b-C^d-C^e denoted by χ; and (iii) the atomic charge of X^c, denoted by σ. Table 1 lists the

representative effective nucleosides and other related species. Other calculated results for a large number of compounds and their QSAR study are also in progress. From Table 1, we can see that, for the effective species, the distance values of γ are in the range of 3.86Å to 4.35Å, the torsion angles χ are in the range of -79.8° to 11.4° and the atomic charges σ are in the range of -0.12 to -0.29. In order to compare the inhibitors with the original substrates of reverse transcriptase (RT), four 2'-deoxy-ribosides also were computed as shown in Table 1. If we define $\Delta P=P_{inhibitor}-P_{riboside}$ (P=γ, χ, σ), we can see that the low $|\Delta\gamma|$ inhibitors show high activities; the low Δχ inhibitors show low toxicities and the high ones show high toxicities; Δσ can not be simply correlated to activity and toxicity, but it had better be in minus value. From the viewpoint of the inhibition mechanism, γ may reflect the distance from a recognition site to the reaction site; χ may reflect the structure difference of reverse transcriptase of HIV from the cellular DNA polymerase of the host; the necessary minus value of σ may indicate that the atom X is also a recognition site in the modified sugar moiety besides the nucleobases.

As regards cyclopropyladenine, a typical three-membered nucleoside, the γ, χ and σ are 3.12, -2.86 and -0.17, respectively. The γ is much shorter than normal substrates and this is perhaps why this compound possesses no activity. In order to improve the activity of the three-membered nucleoside we designed the title compound in which we inserted a carbon atom between the base and the cyclopropane ring to lengthen the γ and led this atom bond to the three-membered ring in the double bond to decrease the σ and to fix the configuration. As expected, there exist four isomers (cis/trans and R/S); all of them were computed and listed in Table 1. We can see that, for all of the trans-isomers the γ are too long and χ are also out of the range, so they may have no activity. For

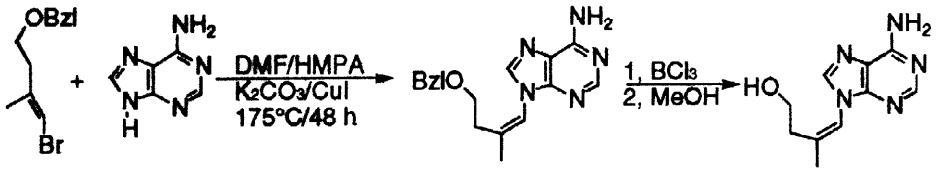
Table 1 Calculated Results of Effective Nucleosides and Related Compounds

Compounds	EC_{50}(μM)	CC_{50}(μM)	Cells	γ(angstrom)	χ(degree)	σ	Ref.
AZT	0.005	53	MT-4	3.86	-8.79	-0.25	10
DDC	0.20	35	ATH8	3.88	-1.16	-0.26	11
DDI	2.10	>100	ATH8	4.13	11.41	-0.27	12
D4T	0.01	1.2	MT-4	4.12	-4.89	-0.28	13
DOC	0.0047	>200	PBM	3.94	-12.00	-0.29	14
3TC	0.0018	>50	PBM	3.87	4.57	-0.24	15
Oxetanocin A	2.78	63.7	MT-4	4.11	-0.56	-0.27	16
Cyclobut A	1.8	12.0	ATH8	4.23	-1.36	-0.12	17
(R)-Adenallene	5.8	>200	MT-4	4.35	-79.78	-0.24	18
Cyclopropyladenine	No Activity			3.12	-2.86	-0.17	19
(S)-cis-CMA*				4.17	-67.11	-0.18	
(R)-cis-CMA*				4.17	67.11	-0.18	
(S)-trans-CMA				4.81	116.32	-0.18	
(R)-trans-CMA				4.81	-116.32	-0.18	
2'-Deoxy-Cytidine				3.90	-16.35	-0.29	
Thymidine				3.89	-15.75	-0.29	
2'-Deoxy-Adenosine				3.95	-10.67	-0.27	
2'-Deoxy-Guanosine				3.97	-10.19	-0.27	

* (S)-cis-CMA and (R)-cis-CMA were inferred to be (-)-cis-CMA and (+)-cis-CMA respectively in Fig. 4.

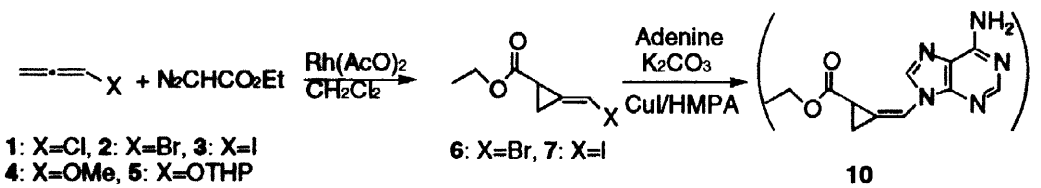
the cis-isomer, the χ of (R)-isomer is out of the range and it may have low activity; the (S)-cis isomer has good values of γ, χ, and σ is also in minus value, so this will be a promising compound.

Synthesis. Recently, we successfully coupled a vinly bromide derivative with adenine in the presence of CuI to afford acyclic nucleoside as shown in Scheme 1.[20] Therefore, in our first trial to synthesize the title



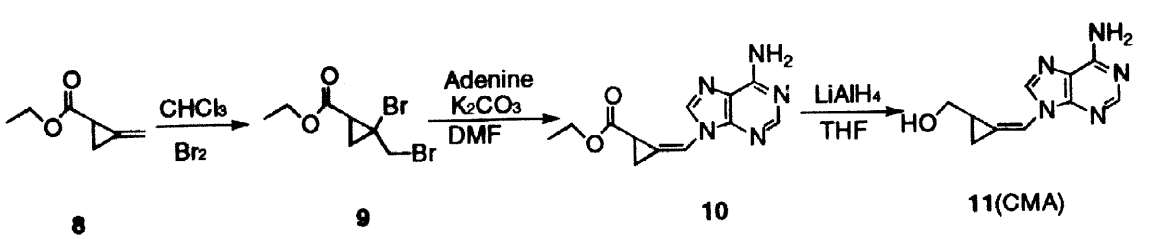
Scheme 1

compound, we adopted the route 1 as shown in Scheme 2. All the haloallenes and alkyloxyallenes **1**, **2**, **3**, **4**, **5** were prepared according to procedures described in the literature,[21-25] and then they were reacted with ethyl diazoacetate in the presence of $Rh(AcO)_2$ under different temperatures, reaction times and molar ratios, but only bromoallene and iodoallene gave the corresponding halomethylenecyclopropane derivatives **6**, **7** in 62.6% and 4% yields with trans/cis ratio of 5:1 and 1:1, respectively, according to the ^1H NMR and NOE measurements. Ethyl 2-bromomethylenecyclopropane-1-carboxylate **6** was subjected to the coupling reaction with adenine in the presence of CuI[26] under conditions of different temperatures, solvents, molar ratios and reaction times, but failed to give the target molecule.



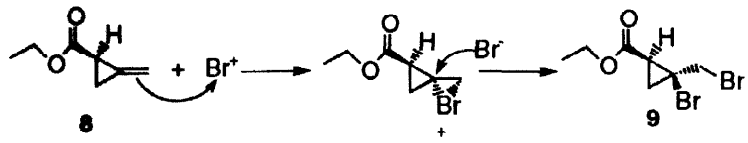
Scheme 2 The Synthetic Route 1

In our other trial of synthesizing spiro-nucleosides[27] we successfully obtained an analogue of the title compound by coupling vicinal dibromocyclopropane derivatives with adenine. The success prompted us to propose route 2 as shown in Scheme 3. Ethyl 2-methylenecyclopropane-1-carboxylate **8** was prepared by procedure described in the literature,[28] and then **8** was converted to ethyl cis-2-bromo-2-bromomethylcyclopropane-1-carboxylate **9** by bromination in almost quantitative yield. The cis-isomer structure was confirmed by ^1H NMR spectrum and NOE measurement. The fact that the more steric-hindranced cis-isomer was specifically



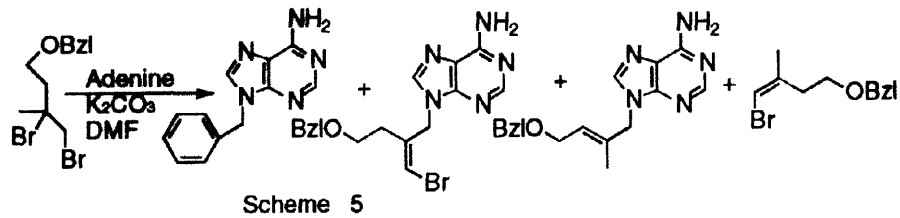
Scheme 3 The Synthetic Route 2

formed revealed a dynamically controlled mechanism as inferred in Scheme 4. Compound **9** was smoothly converted to 9-ethoxycarbonylcyclopropylidenemethylenyladenine **10** by coupling with adenine in 54% yield in one-pot reaction and a small amount of **6** was also isolated (10%). The trans/cis ratio of **10** is 1:2 according to ^{1}H NMR spectrum and NOE measurement.



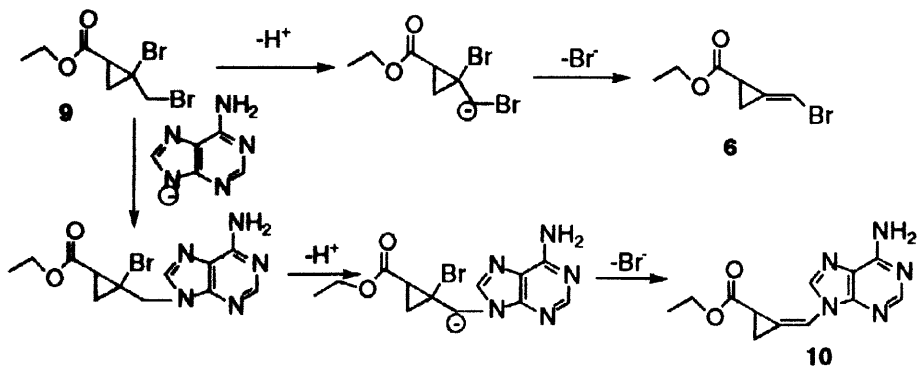
Scheme 4 The Addition Mechanism of **8** with Br_2

Similar coupling attempt between an acyclic vicinal dibromide and adenine however gave two β, γ-unsaturated acyclic nucleosides and a vinyl bromide derivative as shown in Scheme 5.[20]



Scheme 5

These results suggest that in the reaction of acyclic vicinal dibromo-intermediate with adenine/K_2CO_3, the elimination reaction preferentially took place in terms of the easy formation of a tertiary carbonium ion by dissociating a bromide anion from the quaternary carbon, but in the reaction of cyclic vicinal dibromo-intermediate with adenine/K_2CO_3, the substitution preferentially took place because of the stability of cyclopropyl carbinyl cation due to the dramatic π-acceptor/π-attractor substituent effect.[29] On the other hand, once the cyclopropyl carbinyl bromide was substituted by adenine, the stronger inductive effect of N (than Br) and conjugative effect of the aromatic adenine made the 1'-C easily form carbanion, and then led to the formation of an α, β-double-bond by dissociating a bromide anion from the cyclopropane ring as shown in Scheme 6. Contrastively, cyclic vinyl bromide **6** can not be converted to **10** through the catalytic coupling reaction due to its unstability under high temperature and in the presence of catalyst whereas the acyclic vinyl bromide can tolerate such condition (in Scheme 1 and Scheme 2).



Scheme 6 The Reaction Mechanism of **9** with Adenine

The ester-type nucleoside **10** was converted to the target molecule 9-hydroxymethylcyclopropylidenemethylenyladenine **11** in 93% yield by reducing with $LiAlH_4$ at 0℃. The trans/cis ratio of **11** is also 1:2 according to ^1H NMR spectrum and NOE measurement.

Structure and NMR Spectra. The cis/trans isomers of **6**, **9**, **10** and **11** were easily identified by NOE measurements as shown in Fig. 2. Here we only listed one of the isomer pair of each compound because the counterpart can be easily differentiated. All substituted methylenecyclopropane derivatives show a long-distance coupling of the olefin proton H_d with three other protons H_a, H_b, H_c in the three-membered ring in low coupling constants and H_a, H_b and H_c also couple each other. The proton H_d of saturated compound **9** does not show long-distance coupling but couple each other to form a typical AB pattern. In terms of the effect of the chiral center, the protons in methylene of the hydroxylmethyl group of compound trans-**11** show an ABX pattern whereas the chemical shifts of the corresponding two protons in cis-**11** were completely separated due to the effect of the purine ring and they form two dd patterns. The protons in methylene of the ethoxyl group in compound **10** and one of the isomers of compound **7** even display a complex ABX_3 pattern. It is notable that the proton H_a in trans-**11** shows a complex dddt pattern and the corresponding H_a in cis-**11** even shows a unique ddddd pattern.

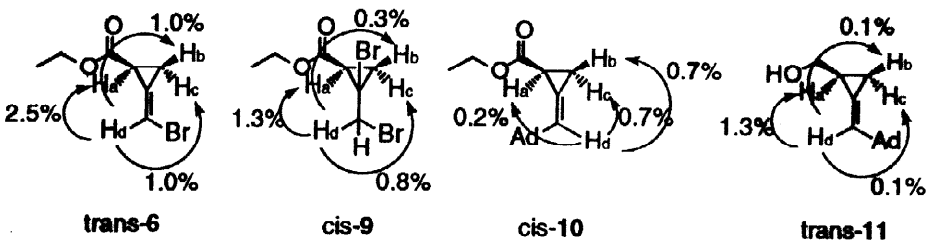


Fig. 2 The identification of trans or cis isomer by NOE measurements

Separation and Resolution. The cis/trans isomers of **11** were separated by silica gel column chroma-tography with an eluent of CH_2Cl_2/MeOH (8:1, v/v) and purified by reverse phase HPLC (μ Bondasphare column) with an eluent of methanol/water (42:58, v/v). The effective cis-isomer wasresolved by using CHIRALPAC AD column silica gel coated with amylose tris (3, 5-dimethylphenylcarbamate) as a chiral stationary phase, Daicel Chemical Industries, LTD.] with an eluent of hexane/iso-propanol (7:1, v/v). The flow rate is 0.7 ml/min.; operation temperature is 25℃ and the retention times are 33.1 min.for (+)-cis-enantiomer and 42.3 min. for (-)-cis-enantiomer, respectively , as shown in Fig. 3. The optical rotations are $[\alpha]^{28}_D$ =+26.3 (c 1.32, MeOH) and $[\alpha]^{28}_D$=-26.3 (c 1.32, MeOH) respectively.

Fig. 3 The Resolution of cis-CMA by HPLC

Biological Activities. The biological tests of the cis-isomer and trans-isomer against Human Immunodeficiency Virus Type 1 (HIV-1) were carried out in MT-4 cells culture. All the inhibition activities are shown in Fig. 4. As was predicted, only the cis-isomer revealed significant biological activity (EC_{50}>100 μM, CC_{50}=79.8μM for trans-isomer and EC_{50}=26 μM, CC_{50}=79 μM for cis-isomer). Then the effective cis-isomer was resolved and tested. The (-)-cis enantiomer revealed high activity (EC_{50}=13 μM) and low toxicity (CC_{50}>100 μM). It is notable that, at the concentration of 100 μM, 100% mock MT-4 cells are viable whereas near 90% HIV-1 infected MT-4 cells are protected; the (+)-cis enantiomer show lower activity (EC_{50}=50 μM) and higher toxicity (CC_{50}>100 μM, but at the concentration of 100 μM, only 72% mock MT-4 cells are viable).

Considering the similar configurations of CMA with adenallene and the structure-acivity relationship as shown in Table 1, it may be inferred that the similar minus χ isomers show higher activities than the plus χ isomers; in other words, active (-)-cis-CMA is a (S)-cis enantiomer.

We previously reported synthesis of 9-(3', 4'-dihydroxymethylcyclopropylidenemethylenyl)adenine,[27] but it did not show anti-HIV-1 activity. The extra hydroxymethyl group may disturb the binding with receptor RT, and/or decrease the penetratability to the target cell in terms of the enhanced water solubility.

We also subjected these compounds to tests against other viruses and tumor cells and will report these results elsewhere.

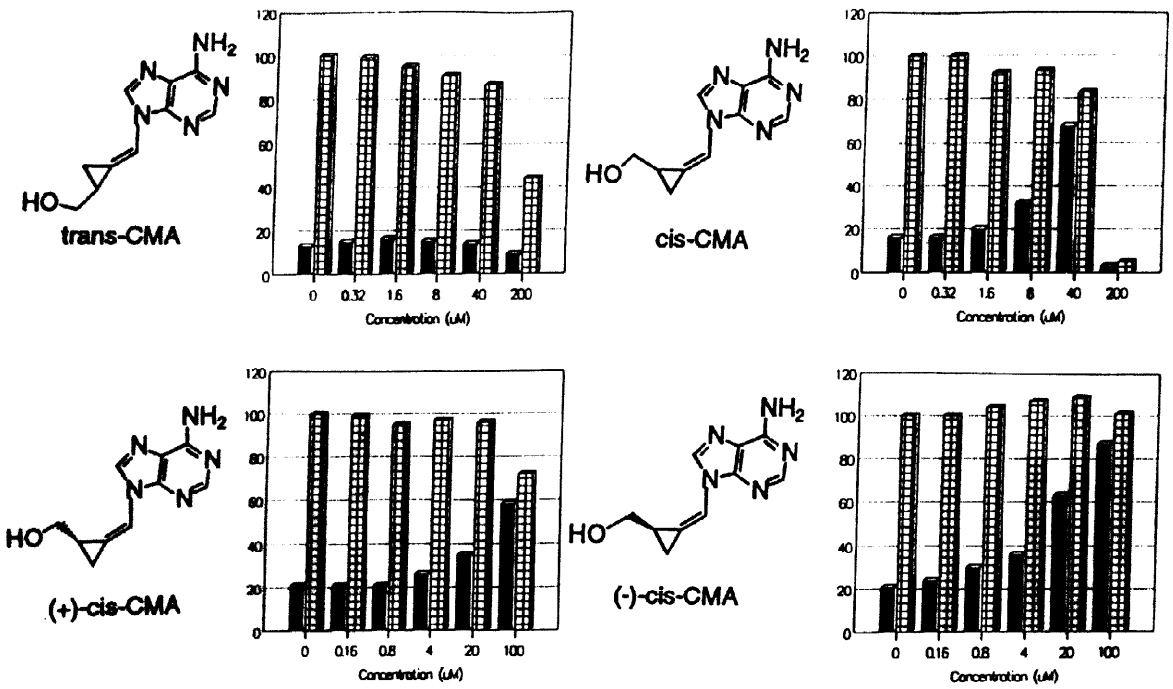


Fig. 4 The Inhibition Effectivity and Toxicity of trans-CMA, cis-CMA, (+)-cis-CMA and (-)-cis-CMA. The MT-4 cells were exposed to HIV-1 and incubated in the presence of the above inhibitors. Infected cells are indicated as solid bars and controlled cells without the virus as open bars. The vertical is number of viable cells (%) and the horizontal is the concentration of inhibitors.

Conclusions. According to the analysis of the structure-activity relationship, we designed a novel three-membered nucleoside; by our newly developed unique coupling reaction, we successfully synthesized it in only 3 steps in 50.2% overall yield. As had been predicted, the cis-isomer revealed anti-HIV-1 activity, especially the (-)-cis enantiomer shows high activity and low toxicity. As a good novel lead compound, we believe that it should lead to discovery of more effective anti-HIV agents in the future by changing and/or modifying the nucleobases.

Experimental section. General methods. NMR spectra were measured with JEOL JNM-GSX 400; HPLC was run on Shimazu CL 6A with UV detector of Shimazu SPD-6A. High resolution mass spectra were

determined by Hitachi M-2000 AM and optical rotations were determined with JASCO J-20A spectrometer. Compounds **1**, **2**, **3**, **4**, **5** and **8** were prepared according to the literature and other reagents are commercially available. The compounds subjected to an anti-HIV-1 test were purified by reverse phase HPLC and their purities were checked by the ^{1}H NMR mearsurements.

Ethyl 2-bromomethylenecyclopropane-1-carboxylate **6**. To a stirred mixture of bromoallene (11.4 g, 96.0 mmol.) and $Rh(AcO)_2$ (60.0 mg, 0.136 mmol.), N_2CHCO_2Et (21.9 g, 192 mmol. in 60 ml methylene chloride) was added dropwise under N_2 atmosphere at refluxing temperature for 6 hours. The solution was refluxed and stirred overnight and then was evaporated. After silica gel column chromatography with an eluent of 1:10 ethyl acetate/hexane, the colorless liquid **6** was obtained in 62.6% yield. The trans/cis isomers were separated by further column chromatography with an eluent of 1:20 ethyl acetate/hexane. trans-**6**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm), δ6.56 (dt, 1H, J=1.6, 3.0 Hz), 4.17 (q, 2H, J=7.2 Hz), 2.49 (ddd, 1H, J=8.0, 5.0, 1.6 Hz), 1.70 (ddd, 1H, J=9.2, 8.0, 3.0 Hz), 1.26 (t, 3H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, ppm), δ171.56, 128.36, 98.77, 62.12, 24.68, 15.38, 14.26; HRMS calcd for $C_7H_9BrO_2$ 203.9786, found 203.9791. cis-**6**: ^{1}H NMR (400MHz, $CDCl_3$, ppm), δ6.48 (dt, 1H, J=1.6, 3.0 Hz), 4.23 (q, 2H, J=7.2 Hz), 2.40 (ddd, 1H, J=8.0, 5.0, 1.6 Hz), 2.01 (ddd, 1H, J=9.2, 8.0, 3.0 Hz), 1.26 (t, 3H, J=7.2 Hz); ^{13}C NMR (100MHz, $CDCl_3$, ppm), δ 172.76, 128.00, 97.77, 62.82, 24.38, 15.88, 13.26; HRMS calcd for $C_7H_9BrO_2$ 203.9786, found 203.9791.

Ethyl 2-iodomethylenecyclopropane-1-carboxylate **7**. To a stirred mixture of iodoallene (2.0 g, 12.8 mmol. in 25 ml methylene chloride) and $Rh(AcO)_2$ (9.0 mg, 0.02 mmol.), N_2CHCO_2Et (1.37 g, 12.8 mmol. in 25 ml methylene chloride) was added dropwise under N_2 atmosphere at -10℃ for 9 hours. The solution was stirred overnight at -10℃ and then was evaporated. After twice silica gel column chromatography with an eluent of 1:5 ethyl acetate/petroleum ether and an eluent of 4:6 petroleum ether/benzene and once preparative thin layer chromatography with an eluent of benzene, the colorless liquid (gradually became red in solution), trans/cis (1:1) isomer mixture of **7** was obtained in 4% yield. trans/cis (1:1)-**7**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm), because there were two isomers, we calculated the protons based on two molecules. δ6.51 (q, 1H, J=2.4 Hz), 6.47 (q, 1H, J=1.8 Hz), 4.22 (ABX_3, 2H, J=7.2, 7.2, 14.4 Hz), 4.15 (q, 2H, J=7.2 Hz), 2.55 (ddd, 1H, J=8.8, 4.9, 1.8 Hz); 2.29 (ddd, 1H, J=8.8, 5.0, 2.4 Hz), 2.10 (ddd, 1H, J=8.8, 4.9, 1.8 Hz), 1.92 (dt, 1H, J=8.8, 1.8 Hz), 1.80 (ddd, 1H, J=8.8, 5.0, 2.4 Hz), 1.61 (dt, 1H, J=8.8, 2.4 Hz), 1.30 (t, 3H, J=7.2 Hz), 1.27 (t, 3H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, ppm), δ 170.56, 169.91, 136.00, 134.70, 67.48, 67.30, 61.44, 61.42, 26.73, 23.51, 19.59, 15.37, 14.66, 14.46; anal. calcd for $C_7H_9IO_2$ C 33.36, H 3.60, found C 33.67, H 3.82; MS 125 $(M-I)^+$.

Ethyl 2-bromo-2-bromomethylcyclopropane-1-carboxylate **9**. To a stirred solution of ethyl 2-methylenecyclopropane-1-carboxylate **8** (2.8 g, 20 mmol. in 50 ml chloroform), bromine (3.2 g, 20 mmol. in 50 ml chloroform) was added dropwise at 0℃ within 4 hours. After being stirred further for 3 hours at 0℃, the colorless solution was evaporated and the light yellow liquid **9** was obtained in almost quantitative yield with almost specific cis-isomer. The compound was used in the following reaction without further purification. cis-**9**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm), δ4.24 (q, 2H, J=7.2 Hz), 3.77 (AB, 2H, J=7.2 Hz), 2.12 (dd, 1H, J=9.2, 7.2 Hz), 1.77 (t, 1H, J=7.2 Hz), 1.52 (dd, 1H, J=9.2, 7.2 Hz), 1.31 (t, 3H, J=7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, ppm), δ168.18, 61.51, 41.93, 37.13, 28.98, 22.28, 14.08; HRMS calcd for $C_7H_{10}BrBr^*O_2$ 285.9027, found 285.9013.

9-Ethoxycarbonylcyclopropylidenemethylenyladenine **10**. A mixture of vicinal dibromo-compound **9** (1.2 g, 4.0 mmol.), adenine (2.2 g, 16.0 mmol.), K_2CO_3 (2.2 g, 16.0 mmol.) and DMF (70 ml) was stirred at 85℃ (oil bath temperature) under nitrogen atmosphere for 16 hours. The mixture was filtrated and the resolution was evaporated by a water rotary vacuum pump, the white solid compound **10** was obtained after silica gel chromatography with an eluent of 8:1 ethyl acetate/ethanol in 54% yield. cis-**10**: ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD, ppm), δ8.31 (s, 1H), 8.17 (s, 1H), 7.60 (q, 1H, J=2.4 Hz), 4.22 (ABX_3, 2H, J=7.2, 7.2, 14.4 Hz), 2.72 (ddd, 1H, J=8.4, 5.2, 2.4 Hz), 2.14 (ddd, 1H, J=8.8, 5.2, 2.4 Hz), 1.99 (ddd, 1H, J=8.8, 8.4, 2.4 Hz), 1.33 (t, 3H, J= 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, ppm), δ 170.26, 155.49, 153.16, 148.00, 137.04, 119.00, 112.61, 111.01, 61.53, 19.47, 13.89, 10.10; HRMS calcd for $C_{12}H_{13}N_5O_2$ 259.1071, found 259.1109; anal. calcd for $C_{12}H_{13}N_5O_2$ C 55.60, H 5.02, N 27.03, found C 55.49, H 5.08, N 26.69. trans-**10**: ^{1}H NMR 400 MHz, $CDCl_3$/ CD_3OD, ppm), δ8.32 (s, 1H), 8.31 (s, 1H), 7.65(q, 1H, J=2.4Hz), 4.22 (ABX_3, 2H, J=7.2, 7.2, 14.4 Hz), 2.65 (ddd, 1H, J=8.8,4.8 , 2.4 Hz), 2.19 (ddd, 1H, J=8.8, 4.8, 2.4 Hz), 2.07 (dt, 1H, J=8.8, 2.4 Hz), 1.29 (t, 3H, J= 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD, ppm), δ170.01, 155.49, 153.16, 148.00, 137.04, 118.00, 112.96, 111.80, 61.37, 17.38, 13.87,12.23; HRMS calcd for $C_{12}H_{13}N_5O_2$ 259.1071, found 259.1109; anal. calcd for $C_{12}H_{13}N_5O_2$ C 55.60, H 5.02, N 27.03, found C 55.49, H 5.08, N 26.69.

9-Hydroxymethylcyclopropylidenemethylenyladenine **11**. To a stirred mixture of $LiAlH_4$ (0.15 g, 4.0 mmol.) and anhydrous THF (50 ml) a solution of ester-type nucleoside **10** (0.539 g, 2.0 mmol.) in 50 ml anhydrous THF was added dropwise within 5 hours at 0℃. After being stirred overnight at 0℃, the excess $LiAlH_4$ was neutralizated by water, then the mixture was filtrated, evaporated and the white solid compound **11** was obtained in 93% yield after the silica gel chromatography with 8:1 methylene chloride/methanol. The cis/trans isomers and the enantiomers of cis-isomer were separated by HPLC as described above. trans-**11**: ^{1}H NMR (400 MHz, CD_3OD, ppm), δ8.50 (s, 1H), 8.23 (s, 1H), 7.56 (q, 1H, J=2.0 Hz), 3.60 (ABX, 2H, J=11.2, 6.4, 6.4 Hz), 2.12 (dddt, 1H, J=6.4, 8.8, 6.8, 2.0 Hz), 1.79 (dt, 1H, J=2.0, 6.8 Hz), 1.46 (ddd, 1H, J=8.8, 6.8, 2.0 Hz); ^{13}C NMR (100 MHz, CD_3OD, ppm), δ157.49, 154.23, 149.62, 139.28, 119.86, 118.29, 111.82, 65.03, 18.78, 10.11; HRMS calcd for $C_{10}H_{11}N_5O$ 217.0965, found 217.0929. cis-**11**: ^{1}H NMR(400MHz, CD_3OD, ppm), δ8.80 (s, 1H), 8.22 (s, 1H), 7.44 (q, 1H, J=2.0 Hz), 3.90 (dd, 1H, J=11.2, 5.6 Hz), 3.45 (dd, 1H, J=11.2, 7.6 Hz), 2.22 (ddddd, 1H, J=8.8, 7.6, 5.6, 5.2, 2.0 Hz), 1.60 (dt, 1H, J=8.8, 2.0 Hz), 1.30 (ddd, 1H, J=8.8, 5.2, 2.0 Hz); ^{13}C NMR (100 MHz, CD_3OD, ppm), δ 157.50, 154.22, 149.24, 140.28, 119.90, 117.62, 111.79, 65.09, 20.84, 7.36; HRMS calcd for $C_{10}H_{11}N_5O$ 217.0965, found 217.0939.

References

1. Wilson, E. K. *C &EN.* **1996**, July 29, 42
2. Agrawal, S. and Tang, J. Y. *Antisense Research and Development* **1992**, *2*, 261.
3. Ohkawa, J., Koguma, T., Kohda, T. and Taira, K. *J. Biochem* **1995**, *118*, 251.
4. Makowitz, M. *XI International Conference on AIDS (Abstract)*: **1996**, Th B 933.
5. Agrofoglio, L., Suhas, E., Farese, A., Condom, R., Challand, R., Earl, R. A. and Guedj, R. *Tetrahedron* **1994**, *50(36)*, 1061
6. Somekawa, K., Hara, R., Kinnami, K., Muraoka, F., Suishu, T. and Shimo, T. *Chemistry. Lett.* **1995**, 407.

7. Tsai, C.-C., Follis, K. E., Sabo, A., Beck, T. W., Grant, R. F., Bischofberger, N., Benveniste, R. E. and Black, R. *Science* **1995**, *270*, 1197.
8. Norbeck, D. W., Sham, H. L., Herrin, T., Rosenbrook, W. and Plattner, J. J. *J. Chem. Soc., Chem. Commun.* **1992**, 128. Katagiri, N., Sato, H. and Kaneko, C. *Chem. Pharm. Bull.* **1990**, *38(11)*, 3184. Csuk, R. and von Scholz, Y. *Tetrahedron* **1994**, *50(35)*, 10431.
9. Roey, R. V. and Chu, C. K. 'Nucleosides and Nucleotides as Antitumor and Antiviral Agents', ed. by Chu, C. K. and Baker, D. C., Plenum Press, New York, **1993**, pp285.
10. Watanabe, K. A., Harada, K., Zeidler, J., Matulic-Adamic, J., Takahashi, K., Ren, W.-Y., Cheng, L.-C., Fox, J. J., Chou, T.-C., Zhu, Q.-Y., Polsky, B., Gold, J. W. M. and Armstrong, D. *J. Med. Chem.* **1990**, *33*, 2145.
11. Herdewijn, P., Balzarini, J., de Clercq, E., Pauwels, R., Baba, M., Broder, S. and Vanderhaege, H. *J. Med. Chem.* **1987**, *30*, 1270.
12. Mitsuya, H. and Broder, S. *Proc. Nat. Acad. Sci. USA*, **1986**, *83*, 1991.
13. Baba, M., Pauwels, Herdewijn, P., de Clercq, E., Desmyter, J. and Vandeputte, M. *Biochem. Biophys. Res, Commun.* **1987**, *142*, 128.
14. Liotta, D. C. and Schinazi, R. F. **PCT WO** 92/14729 (C07D 411/04, C07F 5/04, A61K 31/505).
15. Schinazi, R. F., Chu, C. K., Peck, A., McMillan, R., Mathis, R., Cannon, D., Jeong, L. S., Beach, J. W., Choi, W. B., Yeola, S. and Liotta, D. C. *Antimicrob. Agents Chemother.* **1992**, *36*, 672.
16. Hoshino, H., Shimizu, N., Shimada, N., Takita, T. and Takeuchi, T. *J. Antibiot.* **1987**, *40*, 1077.
17. Hayashi, S., Norbeck, D. W., Rosenbrook, W., Fine, R. L., Matsukura, M., Plattner, J. J., Broder, S. and Mitsuya, H. *Antimicrob. Agents Chemother.* **1990**, *34*, 287.
18. Megati, S., Goren, Z., Silverton, J. V., Orlina, J., Nishimura, H., Shirasaki, T., Mitsuya, H. and Zemlicka, J. *J. Med. Chem.* **1992**, *35*, 4098.
19. Katagiri, N. *Yakugaku Zasshi* **1993**, *113(4)*, 285.
20. Cheng, C., Shimo, T., Somekawa, K. and Baba, M. *Heterocycles.* submitted.
21. Brandsma, L. and Verkruijsse, H. D. 'Synthesis of Acetylenes, Allenes and Cumulenes' ed. by Brandsma, L. and Verkruijsse, H. D., Elsevier Scientific Publishing Company, Amsterdam-Oxford-New York, **1981**, pp176.
22. Jacobs, T. L. and Brill, W. F. *J. Am. Chem. Soc.* **1953**, *75*, 1314.
23. Cheng, C., Somekawa, K. and Shimo, T. Improved synthetic approach of bromoallene, Unpublished.
24. Brandsma, L. and Verkruijsse, H. D. 'Synthesis of Acetylenes, Allenes and Cumulenes' ed. by Brandsma, L. and Verkruijsse, H. D., Elsevier Scientific Publishing Company, Amsterdam-Oxford-New York, **1981**, pp92.
25. Cheng, C., Somekawa, K. and Shimo, T. The synthesis of tetrahydropyranyloxyallene, Unpublished.
26. Ogawa, T., Kiji, T., Hayami, K. and Suzuki, H. *Chemistry Lett.* **1991**, 1443.
27. Cheng, C., Shimo, T., Somekawa, K. and Kawaminami, M. *Tetrahedron Lett.* in press.
28. Lai, M.-T. and Liu, H.-W. *J. Am. Chem. Soc.* **1990**, *112*, 4034.
29. Saunders, M., Laidig, K. E., Wiberg, K. B. and Schleyer, P. V. R. *J. Am. Chem. Soc.* **1988**, *110*, 7652.