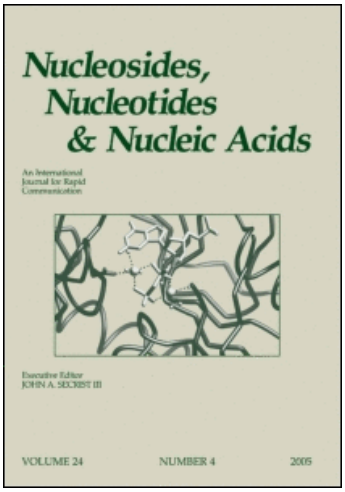


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Design, Efficient Synthesis, and Anti-HIV Activity of 4'-*C*-Cyano- and 4'-*C*-Ethynyl-2'-deoxy Purine Nucleosides

Satoru Kohgo[a]; Kohei Yamada[a]; Kenji Kitano[a]; Yuko Iwai[a]; Shinji Sakata[a]; Noriyuki Ashida[a]; Hiroyuki Hayakawa[a]; Daisuke Nameki[b]; Eiichi Kodama[b]; Masao Matsuoka[b]; Hiroaki Mitsuya[cd]; Hiroshi Ohrui[e]
[a] Chemistry and Pharmacology Laboratory, Biochemicals Division, Yamasa Corporation, Choshi, Chiba-ken, Japan [b] Laboratory of Virus Immunology, Institute for Virus Research, Kyoto University, Sakyo-Ku, Kyoto, Japan [c] Department of Internal Medicine II, Kumamoto University School of Medicine, Honjo, Kumamoto, Japan [d] Experimental Retrovirology Section, Department of Developmental Therapeutics, Medicine Branch, National Cancer Institute, Bethesda, Maryland, USA [e] Division of Life Science, Graduate School of Life Sciences, Tohoku University, Aoba-Ku, Sendai, Japan

Online publication date: 05 June 2004

To cite this Article Kohgo, Satoru , Yamada, Kohei , Kitano, Kenji , Iwai, Yuko , Sakata, Shinji , Ashida, Noriyuki , Hayakawa, Hiroyuki , Nameki, Daisuke , Kodama, Eiichi , Matsuoka, Masao , Mitsuya, Hiroaki and Ohrui, Hiroshi(2004) 'Design, Efficient Synthesis, and Anti-HIV Activity of 4'-*C*-Cyano- and 4'-*C*-Ethynyl-2'-deoxy Purine Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 23: 4, 671 — 690
To link to this Article: DOI: 10.1081/NCN-120037508
URL: http://dx.doi.org/10.1081/NCN-120037508

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, No. 4, pp. 671–690, 2004

Design, Efficient Synthesis, and Anti-HIV Activity of 4′-*C*-Cyano- and 4′-*C*-Ethynyl-2′-deoxy Purine Nucleosides

Satoru Kohgo,[1] Kohei Yamada,[1] Kenji Kitano,[1] Yuko Iwai,[1] Shinji Sakata,[1] Noriyuki Ashida,[1] Hiroyuki Hayakawa,[1,*] Daisuke Nameki,[2] Eiichi Kodama,[2] Masao Matsuoka,[2] Hiroaki Mitsuya,[3,4] and Hiroshi Ohrui[5]

[1]Biochemicals Division, Yamasa Corporation, Chiba, Japan
[2]Laboratory of Virus Immunology, Institute for Virus Research, Kyoto University, Kyoto, Japan
[3]Department of Internal Medicine II, Kumamoto University School of Medicine, Kumamoto, Japan
[4]Experimental Retrovirology Section, Department of Developmental Therapeutics, Medicine Branch, National Cancer Institute, Bethesda, Maryland, USA
[5]Division of Life Science, Graduate School of Life Sciences, Tohoku University, Sendai, Japan

ABSTRACT

Some 4′-*C*-ethynyl-2′-deoxy purine nucleosides showed the most potent anti-HIV activity among the series of 4′-*C*-substituted 2′-deoxynucleosides whose 4′-*C*-substituents were methyl, ethyl, ethynyl and so on. Our hypothesis is that the smaller the substituent at the *C*-4′ position they have, the more acceptable biological activity they show. Thus, 4′-*C*-cyano-2′-deoxy purine nucleosides, whose substituent is smaller than the ethynyl group, will have more potent antiviral activity. To prove our

*Correspondence: Hiroyuki Hayakawa, Ph.D., Chemistry and Pharmacology Laboratory, Biochemicals Division, Yamasa Corporation, 10-1, Araoicho 2-Chome Choshi, Chiba-ken 288-0056, Japan; Fax: +81-479-22-9821; E-mail: hayakawa@yamasa.com.

DOI: 10.1081/NCN-120037508 1525-7770 (Print); 1532-2335 (Online)

hypothesis, we planned to develop an efficient synthesis of 4′-*C*-cyano-2′-deoxy purine nucleosides (4′-CNdNs) and 4′-*C*-ethynyl-2′-deoxy purine nucleosides (4′-EdNs). Consequently, we succeeded in developing an efficient synthesis of six 2′-deoxy purine nucleosides bearing either a cyano or an ethynyl group at the *C*-4′ position of the sugar moiety from 2′-deoxyadenosine and 2,6-diaminopurine 2′-deoxyriboside. Unfortunately, 4′-*C*-cyano derivatives showed lower activity against HIV-1, and two 4′-*C*-ethynyl derivatives suggested high toxicity in vivo.

Key Words: NRTIs; Efficient synthesis; 4′-CNdNs; 4′-EdNs; Anti-HIV-1 agents.

INTRODUCTION

There are mainly two ways for the preparation of our 4′-*C*-substituted-2′-deoxynucleosides (4′-SdNs). One is a condensation method and the other is modification of nucleosides. During our exploration of novel nucleoside reverse transcriptase inhibitors (NRTIs), we have prepared many 4′-SdNs by the use of glycosidation reaction of 4-*C*-substituted sugar derivatives with nucleobases. This method was effective to make various kinds of 4′-SdNs. However, this synthetic route incurs some problems in making 4′-*C*-cyano-2′-deoxy purine nucleosides, because 4-*C*-substituted sugars have low reactivity in glycosidation reaction, especially when its substituent is an electron withdrawing group like a cyano group (data not shown). These problems encouraged us to develop a preparation method of 4′-*C*-cyano-2′-deoxy purine nucleosides from natural 2′-deoxynucleosides, which were so far difficult to be synthesized by condensation of sugars with nucleobases. The synthetic routes of 4′-SdNs from the corresponding nucleosides as starting materials were already reported. Matsuda et al. synthesized various 4′-*C*-substituted pyrimidine nucleosides by intramolecular radical cyclization reaction of 3′-*O*-diphenylvinylsilyl nucleosides with their 4′-radical and then conversion of 4′-*C*-hydroxyethyl nucleosides.[1] O-Yang et al. also reported an interesting article, which described the preparation of 4′-cyanothymidine (4′-CNT) from thymidine via 5′-aldehyde.[2] Haraguchi et al. recently reported ring opening reaction of 4′,5′-epoxy-nucleosides to make 4′-*C*-branched nucleosides.[3] These methods were useful for the preparation of 4′-SdNs, but there were few cases of the preparation of 4′-*C*-cyano purine nucleosides.

In this report, we describe the design and efficient synthesis of 4′-*C*-substituted 2′-deoxy purine nucleosides bearing a cyano and an ethynyl group at the *C*-4′ position of the sugar moiety from purine 2′-deoxynucleosides, and their anti-HIV activity. Furthermore, a preliminary toxicity test using 4′-*C*-ethynyl derivatives in vivo will be reported.

RESULTS AND DISCUSSIONS

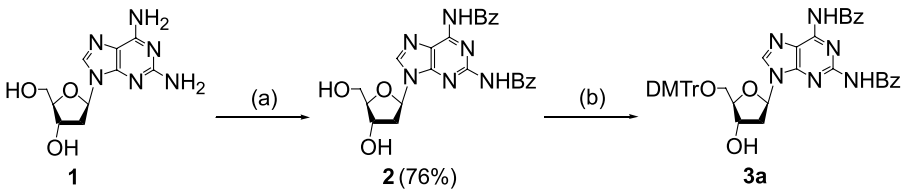
From previous studies on structure-activity relationships of 4′-*C*-substituted nucleosides,[1,2,4–10] it is expected that a smaller substituent at the *C*-4′ position will give more acceptable biological activity against HIV-1. Actually, 4′-*C*-cyano-2′-deoxycytidine showed highest activity compared to 4′-*C*-ethynyl and 4′-*C*-methyl derivatives. From these results, we speculated that there was relationship between the

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

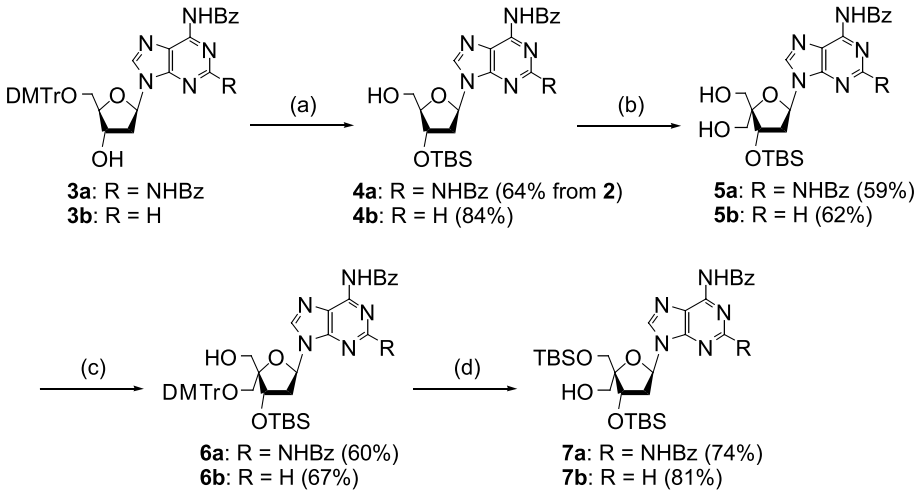
biological activity and a parameter, namely, $-\Delta G^0$ values[11] between equatorial and axial substituents on a cyclohexane rings (CN < F < C ≡ CH < CH ═ CH_2 < Me ≦ Et <*tert*-Bu). On the other hand, we have already found that 4′-*C*-ethynyl-2′-deoxy purine nucleosides exhibited potent anti-HIV activity and moderate cytotoxicity compared to the corresponding pyrimidine derivatives.[6,7,10] Therefore, we were interested in the synthesis and biological activity of 2′-deoxy purine nucleosides bearing a cyano group at *C*-4′ position. However, the glycosidation reaction will have drawbacks as described in the above introduction on synthetic problems. These problems prompted us to develop a preparation method of 4′-*C*-substituted purine nucleosides from the corresponding nucleosides, and this approach would enable us to synthesize 4′-*C*-cyano purine nucleoside derivatives, which were so far difficult to be synthesized by condensation of sugars with nucleobases.

Synthesis of 4′-*C*-cyano-2′-deoxy purine nucleosides starting from 2′-deoxyadenosine and 2,6-diaminopurine 2′-deoxyriboside (dDAP) is shown in Schemes 1–3. To begin with, 9-(2-deoxy-5-*O*-dimethoxytrityl-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine **3a** was prepared from dDAP **1** by way of *N*-benzoylation and 5′-*O*-dimethoxytritylation (Scheme 1). Similarly, N^6-benzoyl-2′-deoxy-5′-*O*-dimethoxytrityladenosine **3b**, which was commercially available from Yamasa Corporation, was also used as the starting material for the synthesis of the target compounds.

The key intermediates **7a**,**b** were obtained according to Scheme 2. In the process for the synthesis of 3′-protected nucleosides **4b**, we initially expected that protection of the 3′-hydroxyl group as *tert*-butyldimethylsilyl ether (TBS) and the following deprotection of the 5′-*O*-dimethoxytrityl group (DMTr) would give the compound **4b** in good yield. However, when deprotection of the dimethoxytrityl group with 80% AcOH was tried at room temperature, the isolated yield of compound **4b** was very low because of acid sensitivity of *N*-acylated 2′-deoxyadenosine. On the contrary, when 2% *p*-toluenesulphonic acid (TsOH) in $CHCl_3$–MeOH was utilized for removal of DMTr group, this treatment gave 3′-TBS-derivative **4b** in 84% yield. To give 4′-*C*-hydroxymethyl derivative **5b**, compound **4b** was oxidized to the corresponding 5′-carboaldehyde by Moffatt oxidation, and then the aldehyde was treated under aldol reaction conditions with formaldehyde. The 4′-α-hydroxyl group of **5b** was selectively protected to give DMTr ether **6b** in 67% yield. Compound **6b** was converted to the key intermediate **7b** in 81% yield by *tert*-butyldimethylsilylation of the 5′-hydroxyl group and the following deprotection of the 4′-hydroxymethyl group. These reactions were also effective to prepare protected dDAP derivative **7a**. The overall yields were 12.7% in 6 steps.

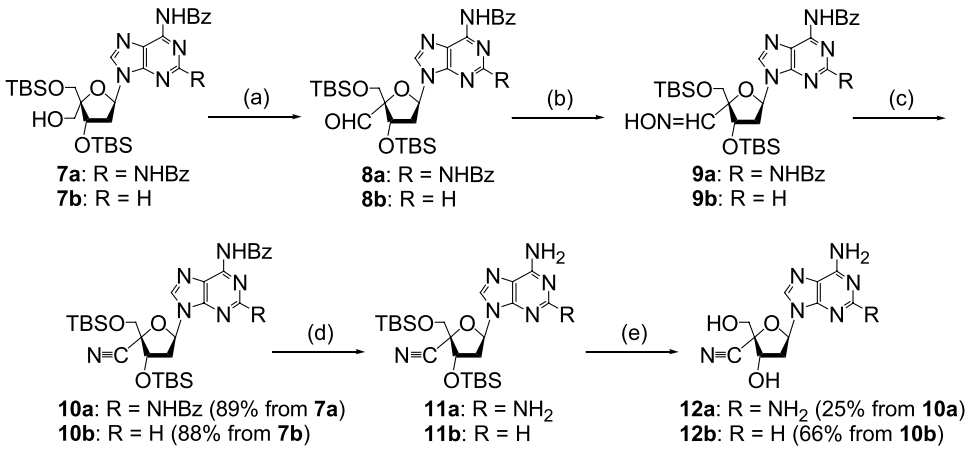


Scheme 1. *Reagents and conditions:* dDAP 1 was obtained by enzymatic base exchange reaction from 2′-deoxyuridine. (a) 1. TMSCl, pyridine, 0°C, 30 min; 2. BzCl, pyridine, 0°C, 2 h; 3. NH_4OH, H_2O, 0°C, 30 min; (b) DMTrCl, pyridine, rt., 3 h.

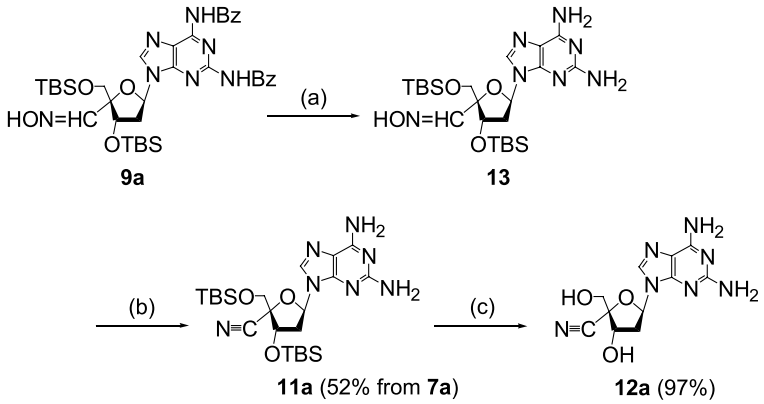


Scheme 2. *Reagents and conditions:* (a) 1. TBSCl, imidazole, DMF, rt., overnight; 2. TsOH · H_2O, MeOH, $CHCl_3$, 0°C, 15–30 min; (b) 1. EDC · HCl, pyridine, TFA, toluene, DMSO, rt., 2 h; 2. aq CH_2O, 1N NaOH, dioxane, rt., 1–3 h; 3. $NaBH_4$, EtOH, 0°C, 30 min; (c) DMTrCl, Et_3N, DMF, rt., 1 h, for **5a**; DMTrCl Et_3N, CH_2Cl_2, 0°C, 30 min, for **5b**; (d) 1. TBSCl, imidazole, DMF, rt., overnight; 2. TsOH · H_2O, MeOH, $CHCl_3$, 0°C, 20–30 min.

Conversion of key intermediates **7a**,**b** to the corresponding 4′-*C*-cyano derivatives **12a**,**b** is shown in Scheme 3. 4′-*C*-Formyl derivatives **8a**,**b**, which were obtained by Moffatt oxidation of 4′-*C*-hydroxymethyl derivatives **7a**,**b**, were derived to 4′-*C*-aldoxime derivatives **9a**,**b** by treatment with hydroxylamine hydrochloride in pyridine, and were further dehydrated by methanesulfonyl chloride and triethylamine in CH_2Cl_2



Scheme 3. *Reagents and conditions:* (a) EDC · HCl, pyridine, TFA, toluene, DMSO, rt., 1 h; (b) NH_2OH · HCl, pyridine, rt., 30 min; (c) MsCl, Et_3N, CH_2Cl_2, 0°C, 30 min; (d) aq $MeNH_2$, MeOH, rt., overnight, for **10a**; NH_4OH, MeOH, rt., overnight, for **10b**; (e) TBAF, THF, rt., 15 min.

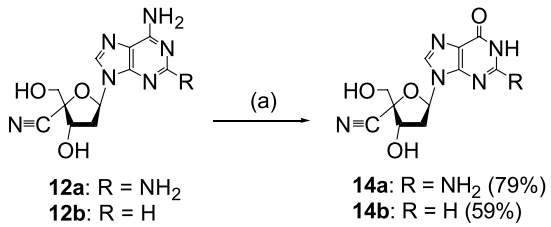


Scheme 4. *Reagents and conditions:* (a) aq $MeNH_2$, MeOH, rt., 46 h; (b) 1,1′-carbonyldiimidazole, MeCN, rt., 4 h; (c) TBAF, THF, rt., 10 min.

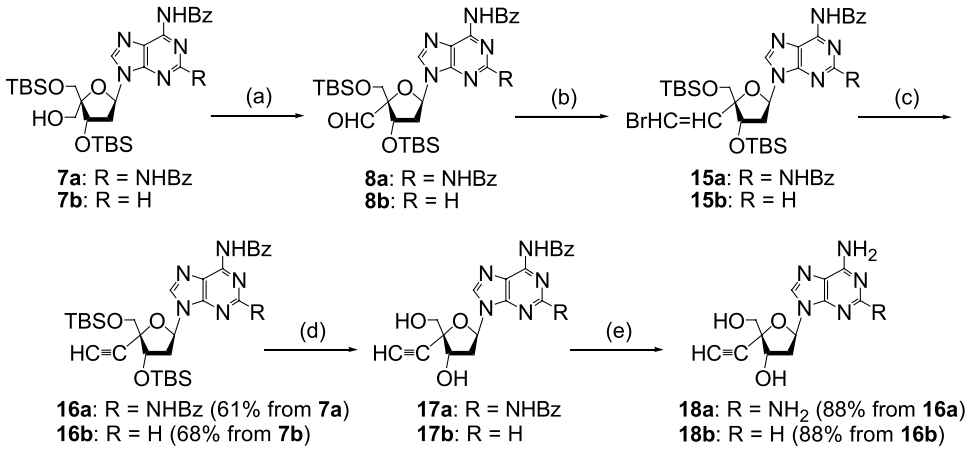
to give 4′-*C*-cyano derivatives **10a**,**b** in good yields through 3 steps. These 4′-*C*-cyano derivatives **10a** and **10b** were deblocked under aq $MeNH_2$ or NH_4OH in MeOH, respectively, to give crude debenzoylated products **11a** and **11b**. Treatment of **11a** and **11b** with tetra-*n*-butylammonium fluoride (TBAF) in tetrahydrofuran (THF) gave 4′-*C*-cyano-2′-deoxy-2,6-diaminopurine-ribonucleoside **12a** and 2′-deoxyadenosine nucleoside **12b** in 25% and 66% yield, respectively.

Next, an alternative route was investigated because the isolated yield of **12a** was very low (Scheme 4). The cyano group might be unstable under $MeNH_2$ in MeOH for debenzoylation. 4′-*C*-Aldoxime **9a**, which was similarly derived from **7a**, was deblocked by treatment with aq $MeNH_2$ in MeOH to give the debenzoylated aldoxime derivative **13**. Crude **13** was then dehydrated with 1,1′-carbonyldiimidazole in MeCN to give **11a** in 52% yield from **7a**. In the case of compound **13** whose amino group is not protected, when dehydration of oxime was performed with MsCl, sulfonylation of the amino groups also occurred. Finally, desilylation of **11a** gave the desired nucleosides **12a** in 97% yield. The isolated yield of **12a** from **7a** was improved to 50% (as compared to 22% yield in Scheme 3).

We have already reported the synthesis of 4′-*C*-ethynyl-2′-deoxyinosine and 4′-*C*-ethynyl-2′-deoxyguanosine from the corresponding 2′-deoxyadenosine and 2,6-diaminopurine 2′-deoxyriboside derivatives by enzymatic deamination with adenosine



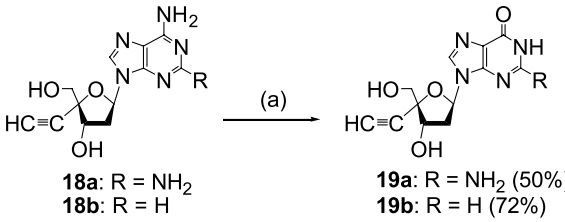
Scheme 5. *Reagents and conditions:* (a) adenosine deaminase, Tris-HCl buffer, 40°C, 1–2 h.



Scheme 6. *Reagents and conditions:* (a) EDC · HCl, pyridine, TFA, toluene, DMSO, rt., 90 min–3 h; (b) PPh_3 = CHBr, THF, −40°C, 90 min–2 h; (c) *tert*-BuOK, THF, −40°C, 1–2 h; (d) TBAF, THF, rt., 30 min; (e) aq $MeNH_2$, MeOH, rt., overnight, for **17a**; NH_4OH, MeOH, rt., overnight, for **17b**.

deaminase.[6] This procedure was utilized to synthesize 4′-*C*-cyano-2′-deoxyguanosine **14a** and 4′-*C*-cyano-2′-deoxyinosine **14b** (Scheme 5). 4′-*C*-cyano nucleosides **12a** and **12b** were readily hydrolyzed to give **14a** and **14b**, respectively, by treatment with adenosine deaminase in Tris-HCl buffer (pH 7.5).

The 4′-*C*-hydroxymethyl derivatives **7a**,**b** are versatile intermediates for the synthesis of various 4′-*C*-substituted nucleosides. Therefore, we also utilized these intermediates **7a**,**b** to synthesize 4′-*C*-ethynyl-2′-deoxy purine nucleosides **18a**,**b**. Scheme 6 shows the synthesis of 4′-*C*-ethynyl derivatives **18a**,**b** from the key intermediates **7a**,**b**. Matsuda et al. synthesized 4′-*C*-ethynyl-2′-deoxycytidine by treatment of the corresponding 4′-*C*-chlorovinyl derivative with *n*-BuLi in THF in good yield.[5] However, conversion of N^6-benzoyl-4′-*C*-chlorovinyl-2′-deoxyadenosine to the corresponding 4′-*C*-ethynyl derivative **16b** under this condition resulted in lower isolated yield than that of 4′-*C*-ethynyl-2′-deoxycytidine derivative because of decomposition of desired product. After investigating reaction conditions, we obtained 4′-*C*-ethynyl derivatives **16a**,**b** by conversion of the 4′-*C*-formyl group of **8a**,**b** to a more reactive 4′-*C*-bromovinyl group with bromomethylenetriphenylphosphorane[12] in



Scheme 7. *Reagents and conditions:* (a) adenosine deaminase, Tris-HCl buffer, 40°C, 1–2 h.

Table 1. Anti-HIV activity of 4′-*C*-ethynyl and 4′-*C*-cyano 2′-deoxy purine nucleosides.

	Anti-HIV activity[a]		
Compound no.	EC_{50} (μM)	CC_{50} (μM)	Selectivity index
4′-*C*-ethynyl dAdo **18b**	0.0098	16	1633
4′-*C*-ethynyl dDAP **18a**	0.00034	0.9	2647
4′-*C*-ethynyl dIno **19b**	0.13	137	1054
4′-*C*-ethynyl dGuo **19a**	0.0015	1.4	933
4′-*C*-cyano dAdo **12b**	0.051	12	235
4′-*C*-cyano dDAP **12a**	0.00079	>0.034	>43
4′-*C*-cyano dIno **14b**	0.051	23	451
4′-*C*-cyano dGuo **14a**	0.000188	>0.034	>181
3′-azidothymidine (AZT)	0.0032	29.4	9188

[a]Anti-HIV activity was determined by MTT assay.[13,14] MT-4 cells and HIV-1_{LAI}[15] were employed.

THF, and then the following dehydrobromination of bromoolefin **15a**,**b** with *tert*-BuOK. After removal of the TBS groups of **16a**,**b** by treatment with TBAF in THF, benzamide groups at the base moiety of **17a**,**b** were cleaved by aq $MeNH_2$ or NH_4OH in MeOH, respectively, to give 4′-*C*-ethynyl-2′-deoxy-2,6-diaminopurine 2′-deoxyriboside **18a** and 2′-deoxyadenosine derivative **18b**.

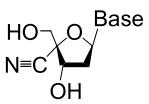
The preparation of 4′-*C*-ethynyl-2′-deoxyguanosine **19a** and 4′-*C*-ethynyl-2′-deoxyinosine **19b** (Scheme 7) was already reported.[6]

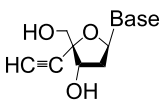
Consequently, we were able to synthesize four 4′-*C*-cyano- and two 4′-*C*-ethynyl-2′-deoxy- purine ribonucleosides by a more useful procedure using 2′-deoxyadenosine and 2, 6-diaminopurine 2′-deoxyriboside as the starting materials.

Antiviral Evaluation

Table 1 shows a summary of anti-HIV activity of 4′-*C*-cyano-2′-deoxy purine nucleosides together with that of 4′-*C*-ethynyl-2′-deoxy purine nucleosides. The structures are summarized in Scheme 8.

4′-*C*-Ethynyl dAdo **18b**, 4′-*C*-ethynyl dDAP **18a**, and 4′-*C*-ethynyl dGuo **19a** were highly potent against HIV-1, with subnanomolar to nanomolar EC_{50}s. 4′-*C*-Ethynyl dIno **19b** was only moderately active against the virus. It is noteworthy that both 4′-*C*-





12a: Base = 2,6-diaminopurin-9-yl
12b: Base = adenin-9-yl
14a: Base = guanin-9-yl
14b: Base = hypoxanthin-9-yl

18a: Base = 2,6-diaminopurin-9-yl
18b: Base = adenin-9-yl
19a: Base = guanin-9-yl
19b: Base = hypoxanthin-9-yl

Scheme 8. The structures of 4′-*C*-cyano- and 4′-*C*-ethynyl-2′-deoxy purine nucleosides.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 2. Anti-HIV activity of 4′-*C*-ethynyl and 4′-*C*-cyano 2′-deoxy purine nucleosides against drug-resistant infectious clones.

	Anti-HIV activity[a], EC_{50} (μM)		
Compound no.	Wild type (HXB2)	MDR[b]	M184V[c]
4′-*C*-ethynyl dAdo **18b**	0.008	0.0062	0.047
4′-*C*-ethynyl dDAP **18a**	0.0014	0.001	0.0059
4′-*C*-ethynyl dIno **19b**	0.81	0.51	16.6
4′-*C*-ethynyl dGuo **19a**	0.007	0.0048	0.008
4′-*C*-cyano dAdo **12b**	0.043	0.083	2.28
4′-*C*-cyano dIno **14b**	0.242	0.296	6.06
3′-azidothymidine(AZT)	0.022	15.3	0.01
3′-thiacytidine (3TC)	0.71	1.1	>100

[a]Anti-HIV activity was determined by MAGI assay.[16-18] HeLa-CD4-LTR-β-gal cells were employed.
[b]An infectious molecular clone (HIV-1_{MDR}) which contains five amino acid substitutions (Ala62Val, Val75Leu, Phe77Leu, Phe116Tyr and Gln115Met) and shows a high level of resistance against a variety of 2′,3′-dideoxy nucleoside analogues (AZT, ddI, ddC, d4T).[19]
[c]An infectious molecular clone (HIV-1_{M184V}) which contains an amino acid substitution (Met184-Val) and shows a high level of resistance against 3TC.

ethynyl dDAP **18a** and 4′-*C*-ethynyl dAdo **18b** had a favorable toxicity profile, with selectivity indices of 2647 and 1633, respectively. On the contrary, 4′-*C*-cyano dDAP **12a** and 4′-*C*-cyano dGuo **14a** showed remarkable cytotoxicity. Additionally, anti-HIV activity of 4′-*C*-cyano dAdo **12b** and 4′-*C*-cyano dIno **14b** decreased in comparison to that of 4′-*C*-ethynyl derivatives. Interestingly, 4′-*C*-cyano dIno **14b** was as potent as 4′-*C*-cyano dAdo **12b** against HIV-1 in spite of showing the low activity of 4′-*C*-ethynyl dIno **19b**.

We finally summarized anti-HIV activity of 4′-CNdNs and 4′-EdNs against drug-resistant infectious clones as shown in Table 2. The 4′-EdNs analogs (**18a**, **18b**, and **19a**) were active against the infectious clones tested, including HIV-1_{M184V}. However, the activity of 2′-deoxyinosine derivatives (**14b** and **19b**) was low against, especially, clone HIV-1_{M184V}. Taken together, although both 4′-*C*-cyano and 4′-*C*-ethynyl derivatives were active against HIV-1, the 4′-*C*-ethynyl substitution appears to be more acceptable for activity against 3TC-resistant HIV-1_{M184V}. On the other hand, we have not explained any relationship between cytotoxicity and biological activity on 4′-SdNs including both 4′-CNdNs and 4′-EdNs, but these biological data were already reported.[6,7,10]

Preliminary Toxicity Test In Vivo

A preliminary toxicity test for three 4′-*C*-ethynyl derivatives (**18a**, **18b**, and **19a**) was conducted, and the results are summarized in Table 3. Two of the three candidates (**18a** and **19a**) were toxic, but 4′-*C*-ethynyl dAdo **18b** was not in mice.

We developed an efficient preparation method for purine 2′-deoxyribonucleoside derivatives bearing a cyano group at the *C*-4′ position from the corresponding

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Table 3. Preliminary toxicity test of three 4′-*C*-ethynyl-2′-deoxy purine nucleosides.[a]

	Intravenous administration		Oral administration	
Compound no.	Dose (mg/kg)	Mortality (%)	Dose (mg/kg)	Mortality (%)
4′-*C*-ethynyl dAdo **18b**	100	0	100	0
	10	0	10	0
	3	0	3	0
	1	0	1	0
4′-*C*-ethynyl dDAP **18a**	100	100 (1 d)[b]	100	100 (1 d)[b]
	10	100 (2 d)[b]	10	100 (2 d)[b]
	3	0	3	100 (2 d)[b]
	1	0	1	0
4′-*C*-ethynyl dGuo **19a**	100	100 (1 d)[b]	100	100 (1 d)[b]
	10	100 (2 d)[b]	10	100 (4 d)[b]
	3	100 (4 d)[b]	3	100 (4 d)[b]
	1	0	1	0

[a]Six-week-old ICR male mice were employed.
[b]Numbers in parentheses represent survival days of mice after administration.

2′-deoxynucleosides as the starting materials. This method was also applicable to effective preparation of 4′-*C*-ethynyl-2′-deoxy purine nucleosides. The total yields of 4′-*C*-substituted derivatives were improved (3.4 $\sim$ 17%) compared to that of the method by condensation of sugars with nucleobases (< 1%[6]) by the use of these routes. Subsequently, it became easy for us to make derivatives bearing an electron withdrawing substitutent like a cyano group.

We initially expected that the target derivatives having a smaller 4′-*C*-substituent would lead to potent anti-HIV activity. However, we found that 4′-*C*-ethynyl derivatives were superior to 4′-*C*-cyano derivatives in terms of anti-HIV activity. Additionally, it turned out that 4′-*C*-ethynyl dDAP **18a** and dGuo **19a** were toxic in the preliminary toxicity test, but 4′-*C*-ethynyl dAdo **18b** was not.

Further investigations to discover novel NRTIs are in progress in our laboratory.

EXPERIMENTALS

Chemistry

General Method for Chemistry. All melting points were determined on a Yanagimoto MP-500D micro melting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded on a JEOL JNM-GSX-400 spectrometer or a Bruker Avance 500 spectrometer in $CDCl_3$ or DMSO-d_6 as the solvent with tetramethylsilane (TMS) as reference. UV spectra were recorded with a Shimazu UV-160A spectrophotometer. Low-resolution mass spectra (MS) were taken on a JEOL JMS-AX500 spectrometer. Merck precoated plates (Kieselgel 60F254) were used for thin-layer chromatography (TLC) and spots were examined with ultraviolet light and sulfuric acid/anisaldehyde solution. Merck Kieselgel 60 was used for column chromatography. Reversed-phase

column chromatography was performed on Fuji Silysia DM1020T. Purity of all compounds obtained was verified by TLC or high-performance liquid chromatography (HPLC), and identity was verified by MS, ^{1}H-NMR, and elementary analysis.

9-(2-Deoxy-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (2). 9-(2-Deoxy-*ribo*-pentofuranosyl)-2,6-diaminopurine **1** (26.6 g, 100 mmol) was coevaporated with pyridine and then it was suspended in pyridine (400 mL). Chlorotrimethylsilane (88.0 mL, 700 mmol) was added dropwise to the suspension at 0°C. After stirring for 30 min at 0°C, the mixture was added dropwise benzoyl chloride (82.0 mL, 700 mmol) and was stirred for 2 h. The reaction mixture was added ice-water. After stirring for 15 min, the mixture was added conc. NH_4OH and was stirred for 30 min. The mixture was evaporated under reduced pressure. The residue was added AcOEt (600 mL) and saturated $NaHCO_3$ (600 mL) and was stirred for 1 h at 0°C. The precipitate formed was collected by filtration to give **2** (36.2 g, 76.3 mmol, 76.3%).

^{1}H-NMR (DMSO-d_6) δ 11.21, 11.00 (each 1H, s, NH), 8.62 (1H, s, H-8), 8.08–7.51 (10H, m, aromatic), 6.42 (1H, t, H-1′, J = 6.5), 5.34 (1H, d, 3′-OH, J = 4.0), 4.94 (1H, t, 5′-OH, J = 6.0), 4.44 (1H, m, H-3′), 3.88 (1H, q, H-4′, J = 5.0), 3.62, 3.54 (each 1H, m, H-5′), 2.79, 2.34 (each 1H, m, H-2′). FABMS m/z: 475 (MH^+). Anal. Found: C, 57.82; H, 4.80; N, 16.76. Calcd. for $C_{24}H_{22}N_6O_5 \cdot 1.4H_2O$: C, 57.69; H, 5.00; N, 16.82.

9-(3-*O*-*tert*-Butyldimethylsilyl-2-deoxy-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (4a). Compound **2** (36.2 g, 76.4 mmol) which was dried by coevaporation with pyridine was dissolved in pyridine (300 mL). The solution was added dimethoxytrityl chloride (37.6 g, 111 mmol) and stirred for 3 h at room temperature. After addition of EtOH, the reaction mixture was evaporated. The residue was suspended to AcOEt and water and the precipitate formed was removed by filtration. The organic layer was washed successively water and brine. The organic layer was dried over Na_2SO_4 and was evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: AcOEt/*n*-hexane, 3:1–4:1–5:1) to give crude **3a** (69.9 g), which was used for the synthesis of **4a** without further purification.

To a solution of crude **3a** (69.9 g) in DMF (370 mL) was added imidazole (8.80 g, 129 mmol) and *tert*-butylchlorodimethylsilane (16.5 g, 109 mmol) and the solution was stirred at room temperature overnight. After addition of water, the reaction mixture was evaporated under reduced pressure. The residue was dissolved to AcOEt and washed successively with water and brine. The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure.

The residue was dissolved in $CHCl_3$ (510 mL) and was added dropwise toluenesulfonic acid hydrate (14.6 g) in MeOH (220 mL) at 0°C. After addition, the solution was stirred for 15 min at same temperature. The reaction mixture was neutralized by addition of saturated $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The residue was dissolved to AcOEt and stirred for 30 min. The precipitate formed was collected by filtration to give **4a** (28.8 g, 48.9 mmol, 64.0%).

^{1}H-NMR ($CDCl_3$) δ 9.30, 9.13 (each 1H, s, NH), 7.95 (1H, s, H-8), 8.06–7.45 (10H, m, aromatic), 6.29 (1H, dd, H-1′, J = 6.5, 8.0), 4.96 (1H, dd, 5′-OH, J = 4.5, 9.0), 4.85 (1H, m, H-3′), 4.04 (1H, m, H-4′), 3.99–3.83 (2H, m, H-5′), 3.10, 2.29 (each 1H, m, H-2′), 0.91 (9H, s, *tert*-Bu), 0.12, 0.10 (each 3H, s, Me). FABMS m/z: 589

(MH^+). Anal. Found: C, 60.96; H, 6.14; N, 13.99. Calcd. for $C_{30}H_{36}N_6O_5Si$: C, 61.20; H, 6.16; N, 14.27.

9-(3-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*C*-hydroxymethyl-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (5a). The solution of **4a** (50.0 g, 85.0 mmol) in toluene (190 mL) and DMSO (290 mL) was suspended 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (24.6 g, 128 mmol). Pyridine (6.90 mL) and trifluoroacetic acid (3.30 mL) were added and stirred for 2 h at room temperature. The reaction mixture was added AcOEt (750 mL) and ice-water (750 mL). The mixture was stirred and precipitate formed was collected by filtration. The filtrate was washed successively with water and brine and was dried over Na_2SO_4 and evaporated under reduced pressure. This residue was combined with the precipitate to give crude aldehyde.

The crude aldehyde was dissolved in dioxane (240 mL) and added 37% formaldehyde (45.0 mL) and 2 N NaOH (45.0 mL). After stirring for 3 h at room temperature, the reaction mixture was neutralized by addition of AcOH. The mixture was diluted with AcOEt and washed successively with water, saturated $NaHCO_3$ and brine. The solution was dried over Na_2SO_4 and evaporated under reduced pressure.

The residue was dissolved in EtOH (360 mL) and added $NaBH_4$ (3.20 g, 85.0 mmol) at 0°C. After stirring for 30 min, the reaction mixture was neutralized by addition of AcOH. The mixture was diluted with mixture of $CHCl_3$ and MeOH and washed successively with water and brine. The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. After coevaporation of the residue with AcOEt, it was dissolved to AcOEt (500 mL) and stirred at room temperature. Precipitate formed was collected by filtration to give **5a** (30.83 g, 49.83 mmol, 58.6%).

^{1}H-NMR (DMSO-d_6) δ 11.20, 11.00 (each 1H, s, NH), 8.63 (1H, s, H-8), 8.08–7.51 (10H, m, aromatic), 6.44 (1H, t, H-1′, J = 6.5), 4.90 (1H, t, OH, J = 5.5), 4.78 (1H, t, H-3′, J = 5.5), 4.46 (1H, t, OH, J = 5.5), 3.65–3.53 (4H, m, H-5′ and H-6′), 2.96, 2.43 (each 1H, m, H-2′), 0.89 (9H, s, *tert*-Bu), 0.10, 0.097 (each 3H, s, Me). FABMS m/z: 619 (MH^+). Anal. Found: C, 58.99; H, 6.14; N, 13.08. Calcd. for $C_{31}H_{38}N_6O_6Si \cdot 0.8H_2O$: C, 58.81; H, 6.30; N, 13.27.

9-(3-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*C*-dimethoxytrityloxymethyl-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (6a). To a solution of **5a** (24.8 g, 40.0 mmol) in DMF (200 mL) was added triethylamine (11.2 mL, 80.0 mmol) and dimethoxytrityl chloride (20.3 g, 60.0 mmol) and stirred for 1 h at room temperature. The reaction mixture was diluted with AcOEt and was washed with water. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica-gel column chromatography (Eluent: *n*-hexane/AcOEt, 2:1–1:1) to give **6a** (22.2 g, 24.1 mmol, 60.3%).

^{1}H-NMR ($CDCl_3$) δ 9.33, 9.23 (each 1H, s, NH), 8.07 (1H, s, H-8), 8.13–6.91 (23H, m, aromatic), 6.27 (1H, t, H-1′, J = 6.5), 5.14 (1H, dd, H-3′, J = 3.5, 6.0), 4.59 (1H, dd, 5′-OH, dd, J = 5.5, 8.5), 4.30 (1H, dd, H-5′a, J = 5.5, 12.5), 3.81 (1H, d, H-5′b, J = 9.0, 12.5), 3.59 (1H, d, H-6′a, J = 10.5), 3.28 (1H, m, H-2′a), 3.17 (1H, d, H-6′b, J = 11.0), 2.48 (1H, m, H-2′b), 0.84 (9H, s, *tert*-Bu), 0.09, 0.07 (each 3H, s, Me). FABMS m/z: 921 (MH^+). Anal. Found: C, 67.87; H, 6.35; N, 8.80. Calcd. for $C_{52}H_{56}N_6O_8Si$: C, 67.80; H, 6.13; N, 9.12.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

9-(3,5-Di-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*C*-hydroxymethyl-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (7a). To a solution of **6a** (29.9 g, 32.5 mmol) in DMF (100 mL) was added imidazole (3.30 g, 48.8 mmol) and *tert*-butylchlorodimethylsilane (5.90 g, 39.0 mmol) and stirred at room temperature overnight. The reaction mixture was diluted with AcOEt and was washed with water. The organic layer was dried over $MgSO_4$ and evaporated.

The residue was dissolved to $CHCl_3$ (620 mL) and was added dropwise TsOH hydrate (6.20 g) in MeOH (190 mL) at 0°C. After addition, the solution was stirred for 20 min at same temperature. The reaction mixture was neutralized by addition of saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica-gel column chromatography (Eluent: *n*-hexane/AcOEt, 3:1) to give **7a** (17.5 g, 23.9 mmol, 73.5%).

^{1}H-NMR ($CDCl_3$) δ 9.14, 9.05 (each 1H, s, NH), 8.13 (1H, s, H-8), 8.02–7.53 (10H, m, aromatic), 6.45 (1H, t, H-1′, *J* = 6.5), 4.99 (1H, dd, H-3′, *J* = 4.5, 6.5), 3.91–3.74 (4H, m, H-5′ and H-6′), 3.12, 2.54 (each 1H, m, H-2′), 2.70 (1H, dd, 6′-OH, J = 5.0, 8.5), 0.93, 0.86 (each 9H, s, *tert*-Bu), 0.17, 0.15, 0.02, 0.01 (each 3H, s, Me). FABMS m/z: 733 (MH^+). Anal. Found: C, 60.51; H, 7.19; N, 11.37. Calcd. for $C_{37}H_{52}N_6O_6Si_2$: C, 60.63; H, 7.15; N, 11.46.

9-(3,5-Di-*O*-*tert*-Butyldimethylsilyl-4-*C*-cyano-2-deoxy-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (10a). The solution of **7a** (2.00 g, 2.73 mmol) in toluene (6.00 mL) and DMSO (12.0 mL) was suspended 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (1.57 g, 8.19 mmol). The mixture was added pyridine (0.22 mL) and trifluoroacetic acid (0.11 mL) and was stirred for 1 h at room temperature. The reaction mixture was diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated to give crude aldehyde **8a**.

To a solution of crude aldehyde **8a** in pyridine (20.0 mL) was added hydroxylamine hydrochloride (0.28 g, 4.03 mmol) and stirred for 30 min at room temperature. The reaction mixture was evaporated and the residue was partitioned between AcOEt and water. The organic layer was dried over $MgSO_4$ and evaporated to give crude oxime **9a**.

To a solution of crude oxime **9a** in CH_2Cl_2 (20.0 mL) was added triethylamine (0.76 mL, 5.45 mmol) and methanesulfonyl chloride (0.32 mL, 4.13 mmol) at 0°C and stirred for 30 min at same temperature. The reaction mixture was diluted with $CHCl_3$ and washed with saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica-gel column chromatography (Eluent: *n*-Hexane/AcOEt, 1:1) to give **10a** (1.77 g, 2.43 mmol, 89.0%).

^{1}H-NMR ($CDCl_3$) δ 9.54, 9.29 (each 1H, s, NH), 7.97 (1H, s, H-8), 8.03–7.49 (10H, m, aromatic), 6.43 (1H, t, H-1′, *J* = 6.5), 5.21 (1H, t, H-3′, *J* = 5.5), 4.22, 3.99 (each 1H, d, H-5′, *J* = 11.0), 3.42, 2.53 (each 1H, m, H-2′), 0.97, 0.86 (each 9H, s, *tert*-Bu), 0.24, 0.17, 0.054, 0.051 (each 3H, s, Me). FABMS m/z: 728 (MH^+). Anal. Found: C, 57.87; H, 6.97; N, 12.78. Calcd. for $C_{37}H_{49}N_7O_5Si_2 \cdot 2H_2O$: C, 58.17; H, 6.99; N, 12.83.

9-(4-*C*-Cyano-2-deoxy-*ribo*-pentofuranosyl)-2,6-diaminopurine (12a). The mixture of **10a** (1.00 g, 1.37 mmol) in MeOH (10.0 mL) and 40% $MeNH_2$ (10.0 mL)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

was stirred at room temperature overnight. Precipitate formed was collected by filtration to give crude debenzoylated product **11a** (0.25 g).

To a solution of crude **11a** (0.25 g) in THF (9.00 mL) was added tetra-*n*-butylammonium fluoride (1M solution in THF, 1.00 mL, 1.00 mmol) and stirred for 15 min at room temperature. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (Eluent: AcOEt/MeOH,10:1). The residue was triturated with 2-propanol to give **12a** (0.10 g, 0.34 mmol, 24.8% from **10a**).

^{1}H-NMR (DMSO-d_6) δ 7.93 (1H, s, H-8), 6.77 (2H, s, NH_2), 6.34 (1H, t, H-1′, J = 7.0), 6.28 (1H, d, 3′-OH, J = 4.5), 5.87 (1H, t, 5′-OH, J = 6.5), 5.85 (2H, s, NH_2), 4.64 (1H, q, H-3′, J = 4.5), 3.78 (1H, dd, H-5′a, J = 6.0, 12.0), 3.65 (1H, dd, H-5′b, J = 6.5, 12.0), 2.87, 2.37 (each 1H, m, H-2′). FABMS m/z: 292 (MH^+). Anal. Found: C, 43.18; H, 4.62; N, 31.80. Calcd. for $C_{11}H_{13}N_7O_3 \cdot 0.8H_2O$: C, 43.22; H, 4.81; N, 32.07.

Improved Route for the Synthesis of 9-(4-*C*-Cyano-2-deoxy-*ribo*-pentofuranosyl)-2,6-diaminopurine (12a) from 7a. The solution of **7a** (1.00 g, 1.40 mmol) in toluene (3.00 mL) and DMSO (6.00 mL) was suspended 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.79 g, 4.10 mmol). Pyridine (0.11 mL) and trifluoroacetic acid (60.0 μL) were added and stirred for 90 min at room temperature. The reaction mixture was diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated to give crude aldehyde **8a**.

To a solution of crude aldehyde **8a** in pyridine (10.0 mL) was added hydroxylamine hydrochloride (0.19 g, 2.70 mmol) and stirred for 1 h at room temperature. The reaction mixture was evaporated and the residue was partitioned between AcOEt and water. The organic layer was dried over $MgSO_4$ and evaporated to give crude oxime **9a**.

The mixture of crude oxime **9a** in MeOH (10.0 mL) and 40% $MeNH_3$ (10.0 mL) was stirred for 46 h at room temperature. Precipitate formed was collected by filtaration to give crude debenzoylated 4′-*C*-oxime derivative **13** (0.61 g).

To a suspension of crude **13** (0.61 g) in MeCN (5 mL) was added 1,1′-carbonyldiimidazole (0.40 g, 2.50 mmol) and stirred for 4 h at room temperature. The reaction mixture was diluted with AcOEt and washed successively with water and brine. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: $CHCl_3$/MeOH, 100:1–50:1) to give **11a** (0.38 g, 0.73 mmol, 52.1% from **9a**).

^{1}H-NMR ($CDCl_3$) δ 7.62 (1H, s, H-8), 6.32 (1H, t, H-1′, J = 6.25), 5.35 (2H, s, NH_2), 4.93 (1H, t, H-3′, J = 5.75), 4.66 (2H, s, NH_2), 4.00, 3.87 (each 1H, d, H-5′, J = 11.0), 3.08, 2.50 (each 1H, m, H-2′), 0.96, 0.89 (each 9H, s, *tert*-Bu), 0.20, 0.17, 0.086, 0.046 (each 3H, s, Me). FABMS m/z: 520 (MH^+). Anal. Found: C, 52.50; H, 7.84; N, 18.39. Calcd. for $C_{23}H_{41}N_7O_3Si_2 \cdot 0.4H_2O$: C, 52.42; H, 7.99; N, 18.60.

To a solution of **11a** (0.272 g, 0.523 mmol) in THF (4.80 mL) was added tetra-*n*-butylammonium fluoride (1M solution in THF, 1.10 mL, 1.10 mmol) and stirred for 10 min at room temperature. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (Eluent: AcOEt/MeOH,10:1–5:1) to give **12a** (0.147 mg, 0.506 mmol, 96.7%). Total isolated yield of **12a** from **7a** by this procedure was 50.4%.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

4′-*C*-Cyano-2-deoxyguanosine (14a). To a solution of **12a** (70.0 mg, 0.24 mmol) in 50 mM Tris-HCl buffer (pH, 7.50, 13.3 mL) was added adenosine deaminase (0.13 mL, 58.5 units) and stirred for 1 h at 40°C. The precipitate formed was collected by filtaration and recrystallized from water to give **14a** (56.0 mg, 0.19 mmol, 79.2%).

^{1}H-NMR (DMSO-d_6) δ 10.68 (1H, s, NH), 7.93 (1H, s, H-8), 6.53 (2H, s, NH_2), 6.29 (1H, t, H-1′, J = 6.8), 6.27 (1H, d, 3′-OH, J = 4.9), 5.73 (1H, t, 5′-OH, J = 5.8), 4.60 (1H, dd, H-3′, J = 4.9, 10.8), 3.75 (1H, dd, H-5′a, J = 5.8, 11.7), 3.64 (1H, dd, H-5′b, J = 5.9, 11.7), 2.80, 2.40 (each 1H, m, H-2′). FABMS m/z: 293 (MH^+). Anal. Found: C, 41.54; H, 4.37; N, 26.58. Calcd. for $C_{11}H_{12}N_6O_4 \cdot 0.6H_2O$: C, 41.41; H, 4.17; N, 26.34.

***N*^{6}-Benzoyl-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxyadenosine (4b).** To a solution of **3b** (2.00 g, 3.04 mmol) in DMF (6.00 mL) was added imidazole (0.83 g, 12.2 mmol) and *tert*-butylchlorodimethylsilane (0.92 g, 6.10 mmol) and the solution was stirred at room temperature overnight. After addition of MeOH, the reaction mixture was diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure.

The residue was dissolved in $CHCl_3$ (70.0 mL) and added dropwise toluenesulfonic acid hydrate (0.60 g) in MeOH (30.0 mL) at 0°C. After addition, the solution was stirred for 30 min at same temperature. The reaction mixture was neutralized by addition of saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: AcOEt/*n*-hexane, 3:1) to give **4b** (1.20 g, 2.56 mmol, 84.2%).

^{1}H-NMR ($CDCl_3$) δ 9.02 (1H, s, NH), 8.79, 8.10 (each 1H, s, H-2 and H-8), 8.03–7.52 (5H, m, aromatic), 6.37 (1H, dd, H-1′, J = 5.5, 9.5), 5.78 (1H, dd, 5′-OH, J = 1.5, 11.5), 4.74 (1H, d, H-3′, J = 6.5), 4.17 (1H, s, H-4′), 4.00–3.74 (2H, m, H-5′), 3.07, 2.27 (each 1H, m, H-2′), 0.94 (9H, s, t-Bu), 0.13 (6H, s, Me). FABMS m/z: 470 (MH^+). Anal. Found: C, 58.45; H, 6.56; N, 14.76. Calcd. for $C_{23}H_{31}N_5O_4Si$: C, 58.83; H, 6.65; N, 14.91.

***N*^{6}-Benzoyl-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxy-4′-*C*-hydroxymethyladenosine (5b).** The solution of **4b** (2.55 g, 5.43 mmol) in toluene (10.0 mL) and DMSO (15.0 mL) was suspended 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (3.12 g, 16.3 mmol). The mixture was added pyridine (0.41 mL) and trifluoroacetic acid (0.21 mL) and stirred for 2 h at room temperature. The reaction mixture was diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure.

The residue was dissolved in dioxane (15.0 mL) and added 37% aqueous formaldehyde solution (2.86 mL) and 2 N sodium hydroxide (2.86 mL). After stirring for 1 h at room temperature, the reaction mixture was neutralized by addition of AcOH, diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated under reduce pressure.

The residue was dissolved in EtOH (25.0 mL) and added $NaBH_4$ (0.21 g, 5.55 mmol) at 0°C. After stirring for 30 min, the reaction mixture was neutralized by addition of AcOH. The mixture was diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: AcOEt/*n*-hexane, 3:1–4:1–5:1) to give **5b** (1.68 g, 3.36 mmol, 61.9%).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

^{1}H-NMR ($CDCl_3$) δ 9.03 (1H, s, NH), 8.79, 8.08 (each 1H, s, H-2 and H-8), 8.03–7.52 (5H, m, aromatic), 6.45 (1H, dd, H-1′, *J* = 6.0, 9.0), 5.61 (1H, dd, OH, *J* = 2.0, 11.0), 4.94 (1H, dd, H-3′, *J* = 1.5, 6.0), 3.87–3.66 (4H, m, H-5′ and H-6′), 3.24 (1H, m, H-2′a), 2.68 (1H, dd, OH, *J* = 4.0, 9.0), 2.37 (1H, m, H-2′b), 0.96 (9H, s, *tert*-Bu), 0.19, 0.18 (each 3H, s, Me). FABMS m/z: 500 (MH^+). Anal. Found: C, 55.41; H, 6.50; N, 13.32. Calcd. for $C_{24}H_{33}N_5O_5Si \cdot 1.1H_2O$: C, 60.63; H, 7.15; N, 11.46.

N^6-Benzoyl-3′-*O*-*tert*-butyldimethylsilyl-2′-deoxy-4′-*C*-dimethoxytrityloxymethyladenosine (6b). To a solution of **5b** (0.84 g, 1.68 mmol) in CH_2Cl_2 (17.0 mL) was added triethylamine (0.47 mL, 3.37 mmol) and dimethoxytrityl chloride (0.85 g, 2.51 mmol) and stirred for 30 min at 0°C. After addition of MeOH, the reaction mixture was diluted with $CHCl_3$ and washed with saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: *n*-hexane/AcOEt, 2:1–1:1) to give **6b** (0.91 g, 1.13 mmol, 67.3%).

^{1}H-NMR ($CDCl_3$) δ 9.17 (1H, s, NH), 8.96, 8.24 (each 1H, s, H-2 and H-8), 8.23–6.94 (18H, m, aromatic), 6.41 (1H, dd, H-1′, *J* = 6.0, 8.5), 5.44 (1H, dd, 5′-OH, *J* = 2.5, 11.0), 4.84 (1H, dd, H-3′, *J* = 1.5, 5.5), 4.43 (1H, dd, H-5′a, *J* = 3.0, 12.5), 3.92 (6H, s, OMe), 3.78 (1H, t, H-5′b, *J* = 12.0), 3.67 (1H, d, H-6′a, *J* = 11.0), 3.30 (1H, m, H-2′a), 3.20 (1H, d, H-6′b, *J* = 11.0), 2.39 (1H, m, H-2′b), 0.90 (9H, s, *tert*-Bu), 0.13, 0.11, (each 3H, s, Me). FABMS m/z: 802 (MH^+). Anal. Found: C, 67.02; H, 6.52; N, 8.55. Calcd. for $C_{45}H_{51}N_5O_7Si$: C, 67.39; H, 6.41; N, 8.73.

N^6-Benzoyl-3′,5′-di-*O*-*tert*-butyldimethylsilyl-2′-deoxy-4′-*C*-hydroxymethyladenosine (7b). To a solution of **6b** (1.61 g, 2.01 mmol) in DMF (8.00 mL) was added imidazole (0.41 g, 6.02 mmol) and *tert*-butylchlorodimethylsilane (0.45 g, 2.99 mmol) and stirred at room temperature overnight. After addition of MeOH, the reaction mixture was diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated.

The residue was dissolved to $CHCl_3$ (70.0 mL) and added dropwise 1% TsOH hydrate in MeOH (30.0 mL) at 0°C. After addition, the solution was stirred for 30 min at same temperature. The reaction mixture was neutralized by addition of saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: *n*-hexane/AcOEt, 3:1) to give **7b** (1.00 g, 1.63mmol, 81.1%).

^{1}H-NMR ($CDCl_3$) δ 9.04 (1H, s, NH), 8.81, 8.28 (each 1H, s, H-2 and H-8), 8.04–7.52 (5H, m, aromatic), 6.52 (1H, t, H-1′, *J* = 6.5), 5.78, 4.88 (1H, dd, H-3′, *J* = 4.5, 6.5), 3.89–3.73 (4H, m, H-5′ and H-6′), 3.04, 2.58 (each 1H, m, H-2′), 2.48 (1H, dd, 6′-OH, *J* = 5.5, 8.5) 0.95, 0.89 (each 9H, s, *tert*-Bu), 0.16, 0.06 (each 6H, s, Me). FABMS m/z: 614 (MH^+). Anal. Found: C, 58.33; H, 7.80; N, 11.14. Calcd. for $C_{30}H_{47}N5O5Si2$: C, 58.69; H, 7.72; N, 11.41.

N^6-Benzoyl-3′,5′-di-*O*-*tert*-butyldimethylsilyl-4′-*C*-cyano-2′-deoxyadenosine (10b). The solution of **7b** (1.00 g, 1.63 mmol) in toluene (3.00 mL) and DMSO (6.00 mL) was suspended 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.94 g, 4.90 mmol). Pyridine (0.13 mL) and trifluoroacetic acid (62.8 μL) were added and stirred for 1 h at room temperature. The reaction mixture was diluted with

AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated to give crude aldehyde **8b**.

To a solution of crude aldehyde **8b** in pyridine (10.0 mL) was added hydroxylamine hydrochloride (0.17 g, 2.45 mmol) and stirred for 30 min at room temperature. The reaction mixture was evaporated and the residue was partitioned between AcOEt and water. The organic layer was dried over $MgSO_4$ and evaporated to give crude oxime **9b**.

To a solution of crude oxime **9b** in CH_2Cl_2 (10.0 mL) was added triethylamine (0.45 mL, 3.23 mmol) and methanesulfonyl chloride (0.19 mL, 2.45 mmol) at 0°C and stirred for 30 min at same temperature. The reaction mixture was diluted with $CHCl_3$ and washed with saturated $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica-gel column chromatography (Eluent: $CHCl_3$/MeOH, 100:1) to give **10b** (0.87 g, 1.41 mmol, 87.7%).

^{1}H-NMR ($CDCl_3$) δ 9.10 (1H, s, NH), 8.79, 8.14 (each 1H, s, H-2 and H-8), 8.04–7.52 (5H, m, aromatic), 6.52 (1H, dd, H-1′, J = 6.0, 6.5), 5.02 (1H, t, H-3′, J = 6.0), 4.05, 3.89 (each 1H, d, H-5′, J = 11), 3.22, 2.62 (each 1H, m, H-2′), 0.98, 0.87 (each 9H, s, *tert*-Bu), 0.21, 0.19, 0.08, 0.03 (each 3H, s, Me). FABMS m/z: 609 (MH^+). Anal. Found: C, 58.64; H, 7.38; N, 13.60. Calcd. for $C_{30}H_{44}N_6O_4Si_2 \cdot 0.2H_2O$: C, 58.83; H, 7.31; N, 13.72.

4′-*C*-Cyano-2′-deoxyadenosine (12b). The mixture of **10b** (0.70 g, 1.15 mmol) in MeOH (10.5 mL) and conc. NH_4OH (3.50 mL) was stirred at room temperature overnight. Precipitate formed was collected by filtration to give crude debenzoylated product **11b** (0.50 g).

To a solution of crude **11b** (0.50 g) in THF (7.80 mL) was added tetra-*n*-butylammonium fluoride (1 M solution of THF, 2.18 mL, 2.18 mmol) and stirred for 15 min at room temperature. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (Eluent: $CHCl_3$/MeOH, 20:1–10:1). The residue was recrystallized from water to give **12b** (0.21 g, 0.76 mmol, 66.1% from **10b**).

^{1}H-NMR (DMSO-d_6) δ 8.34 (1H, s, H-8), 8.16 (1H, s, H-2), 7.36 (2H, s, NH_2), 6.52 (1H, t, H-1′, J = 6.5), 6.32 (1H, d, 3′-OH, J = 5.0), 5.83 (1H, t, 5′-OH, J = 6.0), 4.74 (1H, dd, H-3′, J = 5.5, 11.5), 3.82 (1H, dd, H-5′a, J = 5.0, 11.5), 3.66 (1H, dd, H-5′b, J = 6.0, 12), 3.01, 2.47 (each 1H, m, H-2′). FABMS m/z: 277 (MH^+). Anal. Found: C, 45.90; H, 4.42; N, 29.12. Calcd. for $C_{11}H_{12}N_6O_3 \cdot 0.6H_2O$: C, 46.02; H, 4.63; N, 29.28.

4′-*C*-Cyano-2′-deoxyinosine (14b). To a solution of **12b** (0.15 g, 0.54 mmol) in Tris-HCl buffer (pH, 7.50, 30.0 mL) was added adenosine deaminase (0.30 mL, 135 units) and stirred for 2 h at 40°C. The reaction mixture was concentrated and purified reverse phase column chromatography (0–2.5% EtOH). The residue was recrystalized from water to give **14b** (90.0 mg, 0.32 mmol, 59.3%).

^{1}H-NMR (DMSO-d_6) δ 12.46 (1H, s, NH), 8.32 (1H, s, H-8), 8.10 (1H, s, H-2), 6.49 (1H, t, H-1′, J = 6.5), 6.36 (1H, d, 3′-OH, J = 5.0), 5.76 (1H, t, 5′-OH, J = 6.0), 4.69 (1H, dd, H-3′, J = 5.5, 11.5), 3.78 (1H, dd, H-5′a, J = 6.0, 12.0), 3.66 (1H, dd, H-5′b, J = 6.0, 12.0), 2.92, 2.49 (each 1H, m, H-2′). FABMS m/z: 278 (MH^+). Anal. Found: C, 44.91; H, 4.06; N, 24.06. Calcd. for $C_{11}H_{11}N_5O_4 \cdot 1H_2O$: C, 44.75; H, 4.44; N, 23.72.

9-(3,5-Di-*O*-*tert*-Butyldimethylsilyl-2-*deoxy*-4-C-ethynyl-*ribo*-pentofuranosyl)-2,6-dibenzamidopurine (16a). The solution of **7a** (2.00 g, 2.73 mmol) in toluene

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

(6.00 mL) and DMSO (12.0 mL) was suspended 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (1.57 g, 8.19 mmol). Pyridine (0.22 mL) and trifluoroacetic acid (0.11 mL) were added and stirred for 90 min at room temperature. The reaction mixture was diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated to give crude aldehyde **8a**.

To a suspension of bromomethyltriphenylphosphonium bromide (2.40 g, 5.50 mmol) in THF (32.0 mL) was added potassium *tert*-butoxide (0.93 g, 8.29 mmol) at −40°C and stirred for 90 min at same temperature to prepare bromomethylene triphenylphosphorane. The solution was added crude aldehyde **8a** in THF (32.0 mL) and stirred for 2 h at −40°C. The reaction mixture was added saturated NH_4Cl and stirred. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: *n*-hexane/AcOEt, 3:1–2:1–1:1) to give crude bromoethene **15a** (1.96 g).

To a solution of crude **15a** (1.96 g) in THF (50.0 mL) was added potassium *tert*-butoxide (0.91 g, 8.11 mmol) at −40°C and the solution was stirred for 1 h at same temperature. After addition of saturated NH_4Cl, the mixture was stirred at room temperature. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: *n*-hexane/AcOEt, 2:1) to give **16a** (1.21 g, 1.66 mmol, 60.8% from **7a**).

^{1}H-NMR ($CDCl_3$) δ 9.38, 9.37 (each 1H, s, NH), 8.19 (1H, s, H-8), 8.04–7.49 (10H, m, aromatic), 6.54 (1H, dd, H-1′, J = 4.5, 7.0), 4.84 (1H, t, H-3′, J = 6.5), 4.03, 3.83 (each 1H, d, H-5′, J = 11.0), 2.86, 2.66 (each 1H, m, H-2′), 2.55 (1H, s, ethynyl), 0.94, 0.90 (each 9H, s, *tert*-Bu), 0.14, 0.13, 0.08, 0.06 (each 3H, s, Me). FABMS m/z: 727 (MH^+). Anal. Found: C, 61.91; H, 6.94; N, 11.21. Calcd. for $C_{38}H_{50}N_6O_5Si_2 \cdot 0.5H_2O$: C, 62.01; H, 6.98; N, 11.42.

9-(2-Deoxy-4-*C*-ethynyl-*ribo*-pentofuranosyl)-2,6-diaminopurine (18a). To a solution of **16a** (1.24 g, 1.71 mmol) in THF (30.0 mL) was added tetra-*n*-butylammonium fluoride (1 M solution of THF, 4.30 mL, 4.30 mmol) and stirred for 30 min at room temperature. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (Eluent: $CHCl_3$/MeOH, 20:1) to give **17a** (0.70 g, 1.40 mmol).

The mixture of **17a** (0.65 g, 1.30 mmol) in MeOH (15.0 mL) and 40% $MeNH_2$ aqueous solution (30.0 mL) was stirred at room temperature overnight. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (Eluent: $CHCl_3$/MeOH, 10:1). The residue was recrystallized from water to give **18a** (0.33 g, 1.14 mmol, 87.7%), whose structure was confirmed by comparing spectroscopic data of **18a** with these of previously reported 9-(2-deoxy-4-*C*-ethynyl-*ribo*-pentofuranosyl)-2,6-diaminopurine.[6]

***N*^{6}-Benzoyl-3′,5′-di-*O*-*tert*-butyldimethylsilyl-4′-*C*-ethynyl-2′-deoxyadenosine (16b).** The solution of **7b** (1.80 g, 2.93 mmol) in toluene (6.00 mL) and DMSO (12.0 mL) was suspended 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.69 g, 8.82 mmol). Pyridine (0.24 mL) and trifluoroacetic acid (0.12 mL) were added and stirred for 3 h at room temperature. The reaction mixture was diluted with AcOEt and washed with water. The organic layer was dried over $MgSO_4$ and evaporated to give crude aldehyde **8b**.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

To a suspension of bromomethyltriphenylphosphonium bromide (2.56 g, 5.87 mmol) in THF (35.0 mL) was added potassium *tert*-butoxide (1.00 g, 8.91 mmol) at −40°C and stirred for 2 h at same temperature to prepare bromomethylene triphenylphosphorane. The solution was added crude aldehyde **8b** in THF (35.0 mL) and stirred for 2 h at −40°C. The reaction mixture was added saturated NH_4Cl and stirred. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: *n*-hexane/AcOEt, 3:1–2:1–1:1) to give crude bromoethene **15b**.

To a solution of crude **15b** in THF (70.0 mL) was added potassium *tert*-butoxide (1.00 g, 8.91 mmol) at −40°C and the solution was stirred for 2 h at same temperature. After addition of saturated NH_4Cl, the mixture was stirred at room temperature. The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by silica-gel column chromatography (Eluent: *n*-hexane/AcOEt, 2:1–1:1) to give **16b** (1.21 g, 1.99 mmol, 67.9% from **7b**).

^{1}H-NMR ($CDCl_3$) δ 8.99 (1H, s, NHBz), 8.81, 8.30 (each 1H, s, H-2 and H-8), 8.04–7.51 (5H, m, aromatic), 6.54 (1H, dd, H-1′, J = 4.9, 7.3), 4.83 (1H, t, H-3′, J = 6.8), 3.97 (1H, d, H-5′a, J = 11.2), 3.81 (1H, d, H-5′b, J = 11.2), 2.79, 2.68 (each 1H, m, H-2′), 2.57 (1H, s, ethynyl), 0.94, 0.89 (each 9H, s, *tert*-Bu), 0.14, 0.13, 0.08, 0.04 (each, 3H, s, Me). FABMS m/z: 608 (MH^+). Anal. Found: C, 60.25; H, 7.50; N, 11.10. Calcd. for $C_{31}H_{45}N_5O_4Si_2 \cdot 0.5H_2O$: C, 60.36; H, 7.52; N, 11.35.

2′-Deoxy-4′-*C*-ethynyladenosine (18b). To a solution of **16b** (0.118 g, 0.235 mmol) in THF (3.2 mL) was added tetra-*n*-butylammonium fluoride (1 M solution of THF, 0.7 mL, 0.7 mmol) and stirred for 30 min at room temperature. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (Eluent: $CHCl_3$/MeOH, 20:1) to give crude **17b** (0.122 g).

The mixture of **17b** (0.122 g) in MeOH (2.1 mL) and conc. NH_4OH (0.7 mL) was stirred at room temperature overnight. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (Eluent: $CHCl_3$/MeOH, 20:1–10:1). The residue was recrystallized from water to give **18b** (0.057 g, 0.207 mmol, 88.1% from **16b**), whose structure was confirmed by comparing spectroscopic data of **18b** with these of previously reported 2′-deoxy-4′-*C*-ethynyladenosine.[6]

Antiviral Evaluation

Antiviral Agents. 3′-Azido-3′-deoxythymidine (AZT or zidovudine), 2′,3′-dideoxyinosine (ddI or didanosine), and 2′,3′-dideoxycytidine (ddC or zalcitabine) were purchased from Sigma (St. Louis, MO). (-)-2′,3′-Dideoxy-3′-thiacytidine (3TC or lamivudine) was a kind gift from Dr. R. F. Schinazi (Atlanta, GA). A series of 4′-position substituted nucleosides were designed and synthesized as described by us.

Determination of Drug Susceptibility of HIV-1. The inhibitory effects of test compounds on HIV-1 replication were monitored by the inhibition of virally induced cytopathicity in MT-4 cells. Briefly, MT-4 cells were suspended at 10^5 cells/mL and exposed to $HIV\text{-}1_{LAI}$ at 100 50% tissue culture infectious doses ($TCID_{50}$s). Immediately after viral exposure, the cell suspension (10^4 cells in 100 μL) was brought into each well of a 96-well flat microtiter culture plate (Costar, Cambridge,

Mass.) containing various concentrations of test compounds. After incubation for 5 days, the number of viable cells was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method as previously described.[13,14]

The sensitivity of infectious clones to various RTIs was determined in the multinuclear activation of the galactosidase indicator (MAGI) assay,[17] with some modifications using the viral preparations titrated as previously described.[18] Briefly, target cells (HeLa CD4-LTR/β-gal; 104/well) were plated in 96-well flat microtiter culture plates. On the following day, the medium was aspirated and the cells were inoculated with HIV-1 clones (70 MAGI units/well, which gave 70 blue cells after 48 h of incubation) and cultured in the presence of various concentrations of drug in fresh medium. Forty-eight hours after viral exposure, all blue cells in each well were counted. The cytotoxicity of the compound was determined by the MTT method as previously described.[13] All experiments were performed in triplicate.

Preliminary Toxicity Test for Mice. Six-week-old, random-bred, Swiss albino ICR male mice, (Jcl:ICR) were purchased from Clea Japan. The drugs were dissolved in saline and administered once to the mice either orally (*p.o.*) or intravenously (*i.v.*). The mice were observed twice daily for 7 days for their death.

ACKNOWLEDGMENT

The authors are grateful to Dr. K. Kodama of Yamasa Corporation for his encouragement throughout this work.

REFERENCES

1. Sugimoto, I.; Shuto, S.; Mori, S.; Shigeta, S.; Matsuda, A. Nucleosides and nucleotides. 183. Synthesis of 4′-α-*C*-branched thymidine as a new type of antiviral agent. Bioorg. Med. Chem. Lett. **1999**, *9*, 385–388.
2. O-Yang, C.; Wu, H.Y.; Fraser-Smith, W.B.; Walker, K.A.M. Synthesis of 4′-cyanothymidine and analogues as potent inhibitors of HIV. Tetrahedron Lett. **1992**, *33*, 37–40.
3. Haraguchi, K.; Takeda, S.; Tanaka, H. Ring opening of 4′,5′-epoxynucleosides: a novel stereoselective entry to 4′-*C*-branched nucleosides. Org. Lett. **2003**, *5*, 1399–1402.
4. Waga, T.; Ohrui, H.; Meguro, H. Synthesis and biological evaluation of 4′-*C*-methyl nucleosides. Nucleosides Nucleotides **1996**, *15*, 287–304.
5. Nomura, N.; Shuto, S.; Tanaka, M.; Sasaki, T.; Mori, S.; Shigeta, S.; Matsuda, A. Nucleosides and nucleotides. 185. Synthesis and biological activities of 4′-α-*C*-branched-chain sugar pyrimidine nucleosides. J. Med. Chem. **1999**, *42*, 2901–2908.
6. Ohrui, H.; Kohgo, S.; Kitano, K.; Sakata, S.; Kodama, E.; Yoshimura, K.; Matsuoka, M.; Shigeta, S.; Mitsuya, H. Synthesis of 4′-*C*-ethynyl-β-D-*arabino*- and 4′-*C*-ethynyl-2′-deoxy-β-D-*ribo*-pentofuranosylpyrimidines and -purines and evaluation of their anti-HIV activity. J. Med. Chem. **2000**, *43*, 4516–4525.
7. Kodama, E.; Kohgo, S.; Kitano, K.; Machida, H.; Gatanaga, H.; Shigeta, S.;

Matsuoka, M.; Ohrui, H.; Mitsuya, H. 4′-Ethynyl nucleosides analogs: potent inhibitors of multidrug-resistant human immunodeficiency virus variants in vitro. Antimicrob. Agents Chemother. **2001**, *45*, 1539–1546.
8. Maag, H.; Rydzewski, R.M.; McRoberts, M.J.; Crawford-Ruth, D.; Verheyden, J.P.H.; Prisbe, E.J. Synthesis and anti-HIV activity of 4′-azido- and 4′-methoxynucleosides. J. Med. Chem. **1992**, *35*, 1440–1451.
9. Kozak, J.; Johnson, C.R. Synthesis of 4′-trifluoromethyl nucleoside analogs. Nucleosides Nucleotides **1998**, *17*, 2221–2239.
10. Ohrui, H.; Mitsuya, H. 4′-*C*-Substituted-2′-deoxynucleosides: a family of anti-retroviral agents which are potent against drug-resistant HIV variants. Curr. Drug Targets. Infect. Disord. **2001**, *1*, 1–10.
11. March, J. *Advanced Organic Chemistry*, 4th Ed.; John Wiley & Sons: New York, 1992; 143–146.
12. Matsumoto, M.; Kuroda, K. A convenient synthesis of 1-bromoolefins and acetylenes by a chain extension of aldehydes. Tetrahedron Lett. **1980**, *21*, 4021–4024.
13. Kodama, E.; Shigeta, S.; Suzuki, T.; De Clercq, E. Application of a gastric cancer cell line (MKN-28) for anti-adenovirus screening using the MTT method. Antivir. Res. **1996**, *31*, 159–164.
14. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J. Virol. Methods **1988**, *20*, 309–321.
15. Wain-Hobson, S.; Vartanian, J.-P.; Henry, M.; Chenciner, N.; Cheynier, R.; Delassus, S.; Martins, L.P.; Sala, M.; Nugeyre, M.-T.; Guetard, D.; Klatzmann, D.; Gluckman, J.-C.; Rozenbaum, W.; Barre-Sinoussi, F.; Montagnier, L. LAV revisited: origins of the early HIV-1 isolates from Institut Pasteur. Science **1991**, *252*, 961–965.
16. Uchida, H.; Maeda, Y.; Mitsuya, H. HIV-1 protease does not play a critical role in the early steps of HIV-1 infection. Antivir. Res. **1997**, *36*, 107–113.
17. Kimpton, J.; Emerman, M. Detection of replication-competent and pseudotyped human immunodeficiency virus with a sensitive cell line on the basis of activation of an integrated β-galactosidase gene. J. Virol. Methods **1992**, *66*, 2232–2239.
18. Maeda, Y.; Venzon, D.; Mitsuya, H. Evolution and fitness of HIV-1 with the pol gene mutations conferring multi-dideoxynucleoside resistance. J. Infect. Dis. **1998**, *177*, 1207–1213.
19. Shirasaka, T.; Kavlick, M.F.; Ueno, T.; Gao, W.Y.; Kojima, E.; Alcaide, M.L.; Chokekijchai, S.; Roy, B.M.; Arnold, E.; Yar-Choan, R.; Mitsuya, H. Emergence of human immunodeficiency virus type 1 variants with resistance to multiple dideoxynucleosides in patients receiving therapy with dideoxynucleosides. Proc. Natl. Acad. Sci. U. S. A. **1995**, *92*, 2398–2402.

Received January 5, 2004
Accepted February 23, 2004

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!