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Review

Potential of 4′-*C*-substituted nucleosides for the treatment of HIV-1

Hiroyuki Hayakawa[1]*, Satoru Kohgo[1], Kenji Kitano[1], Noriyuki Ashida[1], Eiichi Kodama[2], Hiroaki Mitsuya[3,4] and Hiroshi Ohrui[5]

[1]Biochemicals Division, Yamasa Corporation, Chiba, Japan
[2]Laboratory of Virus Immunology, Research Centre for AIDS, 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, Medicine Branch, Division of Clinical Sciences, National Cancer Institute, Bethesda, Md., USA
[5]Division of Life Science, Graduate School of Life Sciences, Tohoku University, Sendai, Japan

*Corresponding author: Tel: +81 479 22 0095; Fax: +81 479 22 9821; E-mail: hayakawa@yamasa.com

Extensive efforts have been made to identify nucleoside reverse transcriptase inhibitors (NRTIs). Eight NRTIs have now been approved for clinical use; however, variants of HIV-1 resistant to these antiviral agents have emerged in patients even when they are treated with combinations [highly active antiretroviral therapy (HAART)]. Thus, the development of novel compounds that are active against drug-resistant HIV-1 variants and that prevent or delay the emergence of resistant HIV-1 variants is urgently needed. Previously, 4′-*C*-substituted nucleosides (4′-SNs) were designed as new types of NRTIs. They were synthesized and examined as potential therapeutic agents against HIV infection. Among them, several 4′-substituted-2′-deoxynucleosides (4′-SdNs), especially those that bear an ethynyl group, were shown to be active against various laboratory and clinical HIV-1 strains including known drug-resistant variants. These results were recently reported by our collaborators. In this review, we summarize the design, synthesis and demonstrations of the anti-HIV activity of 4′-SNs, and then consider 4′-SNs as potential therapeutic agents for HIV-1.

Keywords: NRTIs, 4′-SNs, anti-HIV-1 agents, HAART, drug-resistant HIV-1 variants

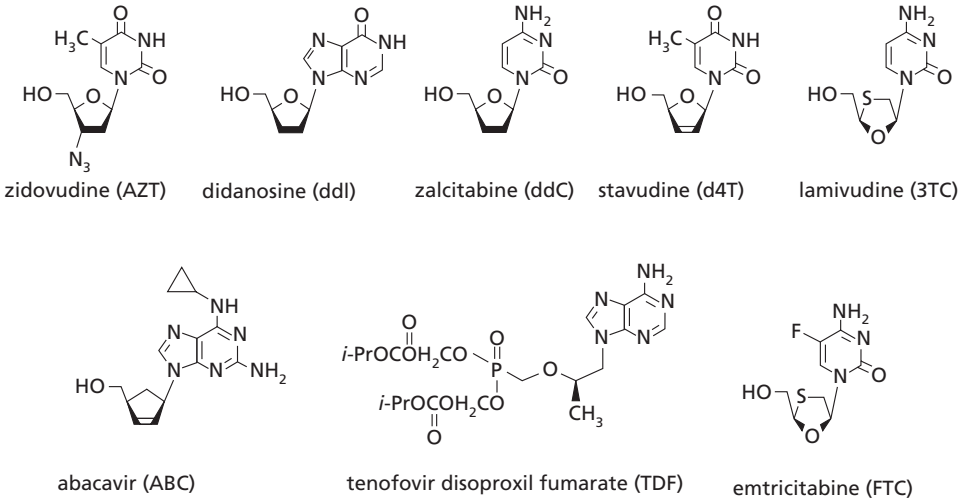
Introduction

The development of novel antiviral agents is essential in the battle against viruses such as HIV, because drug-resistant variants emerge. For the treatment of acquired immunodeficiency syndrome (AIDS), eight NRTIs have been approved for clinical use to date: 3′-azido-3′-deoxythymidine [zidovudine (AZT)], 2′,3′-dideoxyinosine [didanosine (ddI)], 2′,3′-dideoxycytdine [zalcitabine (ddC)], 2′,3′-dehydro-2′,3′-dideoxythymidine [stavudine (d4T)], L-1,3-oxathiolanylcytosine [lamivudine (3TC)], abacavir (ABC), tenofovir disoproxil fumarate (TDF), and L-1,3-oxathiolanyl-5-fluorocytosin [emtricitabine (FTC)] (Figure 1).

HAART using two, or more, NRTIs and protease inhibitors (PIs) has dramatically improved the quality of life and survival of patients infected with HIV-1. But the emergence of drug-resistant mutants has been a critical problem in using these chemotherapeutic agents; furthermore, some of these mutants show high levels of cross-resistance. Consequently, the development of structurally new nucleoside derivatives that are active against HIV-1 variants resistant to the existing 2′,3′-dideoxy nucleosides is urgently needed.

There are six classes of chemotherapeutic agent against HIV-1 so far: 1) NRTIs, mentioned above; 2) non-nucleoside reverse transcriptase inhibitors (NNRTIs); 3) protease inhibitors (PIs); 4) integrase inhibitors (INIs); 5) fusion inhibitors (FIs) and 6) chemokine receptor antagonists (CRAs). A number of NRTIs, NNRTIs and PIs are currently used clinically. Much progress has been made in the classes of FIs and CRAs, but INIs are still in the pre-clinical stage.

Figure 1. Structures of clinically used nucleoside analogues as NRTIs



During our exploration of novel NRTIs, we recently designed and synthesized a series of 4′-SdNs derivatives. Among these, 4′-*C*-ethynyl-2′-deoxynucleosides (4′-EdNs) showed promising features in both their biological activities and their structures (Kodama *et al*., 2001). They inhibited the replication of multidrug-resistant clinical HIV-1 strains carrying a wide variety of drug resistance-related amino acid substitutions isolated from HIV-1-infected individuals, for whom 10 or 11 different anti-HIV-1 agents had failed. These 4′-EdNs have a 2′-deoxyribose moiety, unlike all of the currently available NRTIs. Additionally, all of these 4′-EdNs blocked the replication of a wide spectrum of laboratory and clinical HIV-1 strains *in vitro* with low cellular toxicities. Therefore, we set out to search for promising new candidates.

Synthesis and anti-HIV activity of 4′-SNs

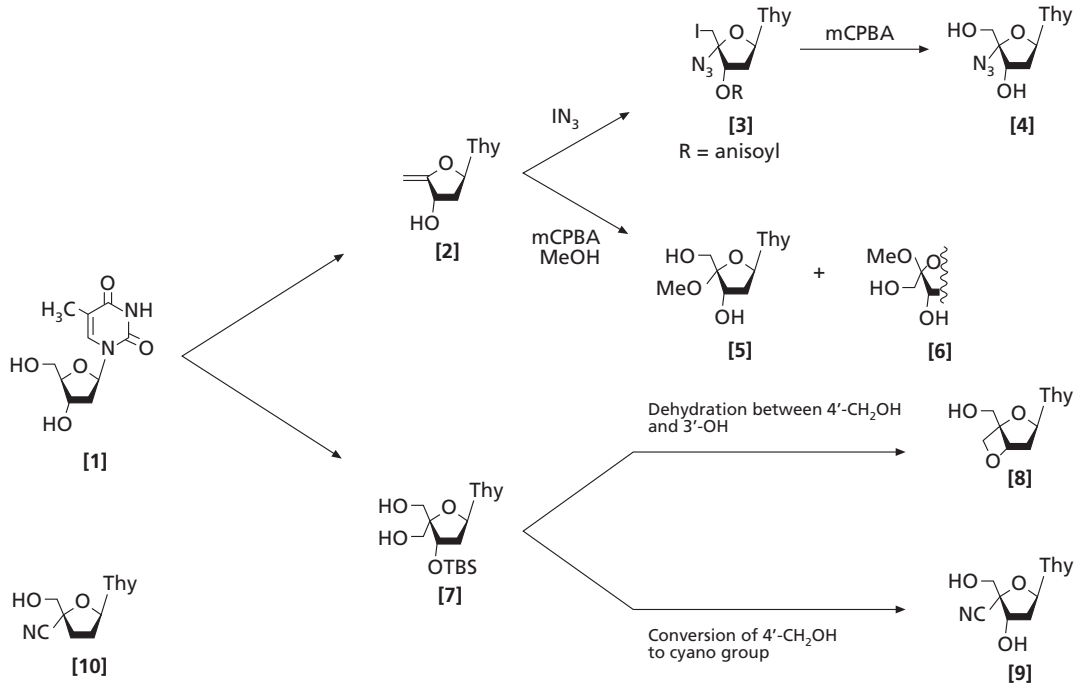
4′-Substituted nucleosides have been under development since JG Moffatt's group accomplished the synthesis of 4′-*C*-fluoro-5′-*O*-sulphamoyladenosine, the antibiotic nucleocidin and related nucleosides (Verheyden *et al*., 1975, Jenkins *et al*., 1976, Owen *et al*., 1976, Youssefyeh *et al*., 1977 & 1979, Jones *et al*., 1979).

Compared with 2′- and 3′-substituted nucleoside derivatives, methods for the synthesis of 4′-SNs were very difficult. However, since the 1990s, several research groups have attempted the synthesis of 4′-SNs and the results, including biological activities, published.

Initially, the Syntex group, led by JG Moffat, pioneered the exploration for an improved method (Figure 2). Maag *et al*. reported the synthesis and anti-HIV activity of 4′-*C*-azidothymidine (4′-AZT 4) and 4′-*C*-methoxynucleosides (**5**) (Maag *et al*., 1992). The key steps in the synthesis of the 4′-azido analogues were the stereo- and regioselective addition of iodine azido to a 4′,5′-unsaturated nucleoside precursor **[2]** followed by an oxidatively assisted displacement of the 5′-iodo group. 4′-AZT **[4]** led to potent activity against HIV-1 *in vitro*, especially its activity against HIV mutants which were resistant to AZT. IC_{50} was 0.01 μM against HIV-1 (LAV-IIIb) replication in A301 cells. Structure–activity relationships among HIV inhibitory 4′-*C*-substituted nucleosides were published by the Syntex research group (Prisbe *et al*., 1993).

O-Yang, of Syntex, also reported two interesting findings: that 1) the fused oxetane derivative of thymidine **[8]** inhibited HIV replication in A301 cells with remarkably low bone marrow toxicity (O-Yang *et al*., 1992); and

Figure 2. Synthesis of various 4′-SNs by Syntex research groups



2) 4′-*C*-cyanothymidine (4′-CNT **[9]**) inhibited HIV in A301 cells with an IC_{50} of 0.002 μM (O-Yang *et al.*, 1992). 4′-*C*-cyano-3′-deoxythymidine **[10]** was also synthesized from 3′-deoxythymidine, but it was not active against HIV (O-Yang *et al.*, 1992). Additionally, both oxetane fused **[8]** and 4′-*C*-cyano **[9]** derivatives were prepared via similar intermediates **[7]** bearing a hydroxymethyl group at the *C*-4′ position of the sugar moiety.

Subsequently, Chen and colleagues reported the mechanism of action of 4′-AZT **[4]** against HIV-1 to be through its DNA chain-terminating activity (Chen *et al.*, 1993).

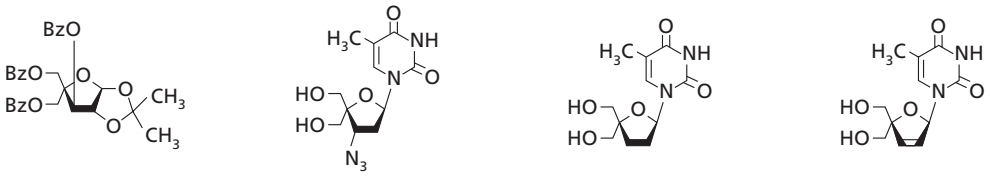
Results similar to those of the Syntex research group were published by A Holy's group (Hrebabecky *et al.*, 1993). They reported the synthesis of 4′α-*C*-hydroxymethyl thymidine derivatives of AZT **[12]**, ddT **[13]** and d4T **[14]** related Ns starting from 1,2-*O*-isopropylidene-3,5-di-*O*-benzoyl-4-*C*-benzoyloxymethyl-β-L-arabinofuranose **[11]** (Figure 3).

JG Moffatt's group introduced a hydroxymethyl group at the 4′α-position of nucleosides using the Cannizzaro reaction 25 years ago, using an appropriately protected ribose 5-aldehyde **[15]** (Figure 4) (Youssefyeh *et al.*, 1979).

Since oxetane-fused derivatives of thymidine **[8]** and 4′-CNT **[9]** showed potent anti-HIV activity as mentioned in the Syntex report, A Matsuda's group reported that they adopted Moffatt's method to synthesize their target nucleosides via 4′-*C*-formyl derivatives **[19]**: 4′-*C*-ethynyl, -vinyl, -ethyl, -chlorovinyl, -cyano, and -methyl derivatives of pyrimidine nucleosides **[20–28]** (Figure 5) (Nomura *et al.*, 1999), (Sugimoto *et al.*, 1999). Moreover, they have recently reported the synthesis of 4′-*C*-branched thymidine **[31–34]** by the use of an intramolecular radical cyclization reaction (Figure 5) (Sugimoto *et al.*, 1999). They showed that 4′-*C*-substituted thymidine **[31–34]** including 4′-ET **[28]** exhibited potent activity against not only HIV-1 but also herpes simplex type 1. Besides the foregoing, the biological activities of these derivatives have also been reported in a 2′-deoxycytidine, cytidine and uridine series (Nomura *et al.*, 1999).

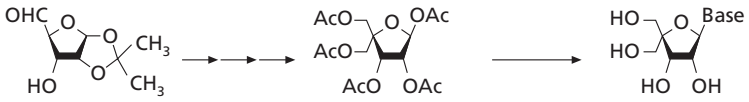
A new synthetic method leading to a series of 4′-*C*-branched 2′,3′-didehydro-2′,3′-dideoxyuridine (4′-Sd4U

Figure 3. Structures of 4-hydroxymethyl sugar **[11]** and nucleosides **[12–14]**



[11] **[12]** **[13]** **[14]**

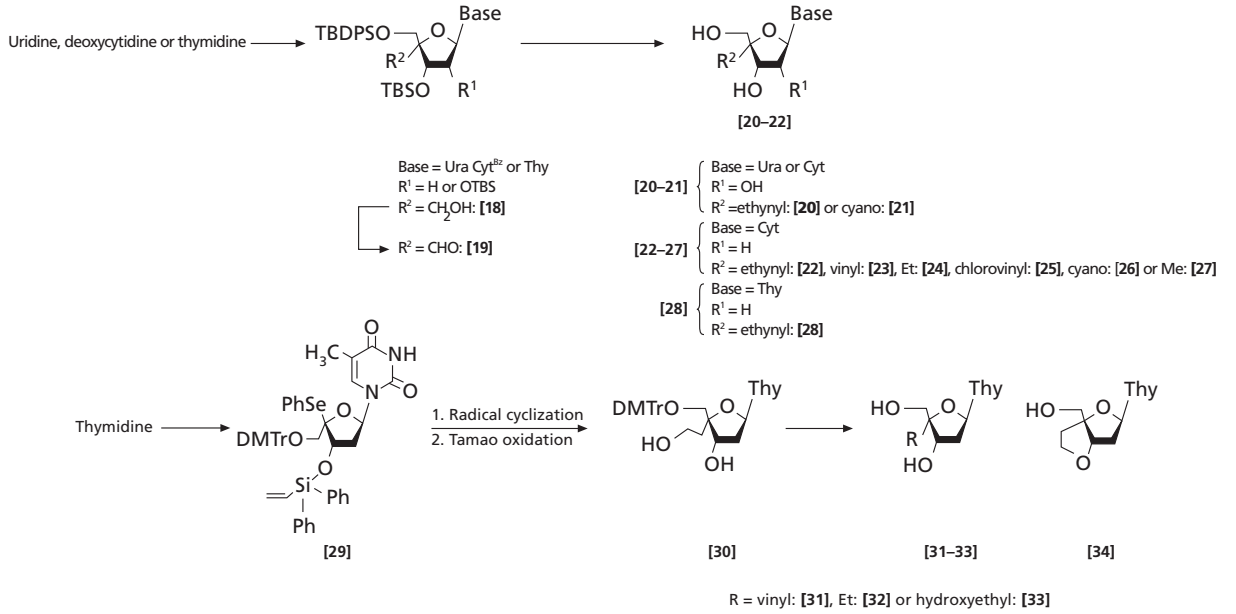
Figure 4. Synthesis of 4′-*C*-hydroxymethyl nucleosides by Cannizzaro reaction aldehyde **[15]** with formaldehyde



[15] **[16]** **[17]**

[36–42] was developed (Haraguchi *et al.*, 1992). This method was based on $SnCl_4$-promoted allylic rearrangement to give 4′-Sd4U **[36–42]** (Figure 6). However, they did not mention the biological activities of these derivatives. H Tanaka's group also very recently published a paper describing the synthesis and activity of 2′,3′-didehydro-3′-deoxy-4′-ethynyl-thymidine (4′-Ed4T) **[47]** (Haraguchi *et al.*, 2003). The key step of this reaction consisted of the ring-opening of 4′,5′-epoxy precursors **[43]** with aluminium reagents resulting in the formation of 4′-*C*-substituted nucleoside analogues **[45–47]** (Figure 6). In this reaction, the 3′-configuration of 4′,5′-epoxide **[43]** was very important to form 4′-SNs having the expected 4′-configuration. Very interestingly, 4′-Ed4T **[47]** was active against HIV-1 with an EC_{50} value of 0.20 μM, which was 14-fold more potent than that of d4T (EC_{50}=2.8 μM); 4′-Ed4T's cytotoxicity was low in comparison. In order to determine SAR, H Tanaka's group went on to prepare 4′-*C*-cyano-2′,3′-didehydro-3′-deoxythymidine (4′-CNd4T) **[42]** (Haraguchi *et al.*, 2003) by allylic substitution of the

Figure 5. Synthesis of 4′-*C*-substituted nucleosides by conversion of 4′-*C*-formyl derivatives and by radical cyclization reaction



3′,4′-unsaturated nucleoside **[35]**, having a leaving group at the 2′-position, with cyanotrimethylsilane in the presence of stannic chloride ($SnCl_4$) (Figure 6). Unfortunately, 4′-CNd4T **[42]**' s activity was only one-fifth that of d4T. One of their derivatives, 4′-Ed4T **[47]**, is expected to become a promising new NRTI candidate

4′-Trifluoromethylthymidine derivatives **[52, 53, 55]** and related purine nucleosides **[54, 56**, **57]** were synthesized by Johnson (Figure 7) (Johnson *et al.* 1998). A strategy based on the use of (trifluoromethyl)trimethylsilane for introduction of a trifluoromethyl group at the *C*-4 of ribose was developed. Unfortunately, these nucleosides were not active against HIV.

Compared to 4′-*C*-substituted nucleosides, there are few reports on the synthesis of 4′α-carbon substituted carbocyclic nucleosides, the most common method being transformation from a natural product. The functionalization of the cyclopentene moiety is restricted in these cases. Interestingly, Kato reported that enantio- and diastereoselective synthesis of 4′-α-alkylcarbovir derivatives was achieved based on Sakai's asymmetric alkylation of β-keto esters (Kato *et al.*, 1998). This method and the related papers cited in his report will enable us to make many carobocyclic derivatives.

For the readers' reference, we cite related reports known to us for the synthesis of various 4′-*C*-substituted nucleosides: (Secrist III *et al.*, 1978; Johnson *et al.*, 1994; Thrane *et al.*, 1995; Marx *et al.*, 1996; Wang *et al.*, 1996; Kozak *et al.*, 1998; Singh *et al.*, 1998; Imanishi *et al.*, 1998; Wang *et al.*, 1999; Crich *et al.*, 1999; Jung *et al.*, 2001; Summerer *et al.*, 2001).

Chemistry and biological activity of 4′-SNs

Two principle methods were employed for the preparation of 4′-SNs: 1) condensation and 2) modification starting from natural nucleosides. The first approach used for the preparation of 4′-SNs was the condensation method; this is an efficient route to various derivatives. Modification starting from natural nucleosides readily scaled up, creating several candidates.

Therefore, initially we started our chemistry by the condensation method to explore the seeds, and then we utilized

Figure 6. Synthesis of 4′-*C*-substituted nucleosides by $SnCl_4$-promoted allylic rearrangement reaction and by ring-opening reaction of 4′,5′-epoxy nucleosides **[43]**

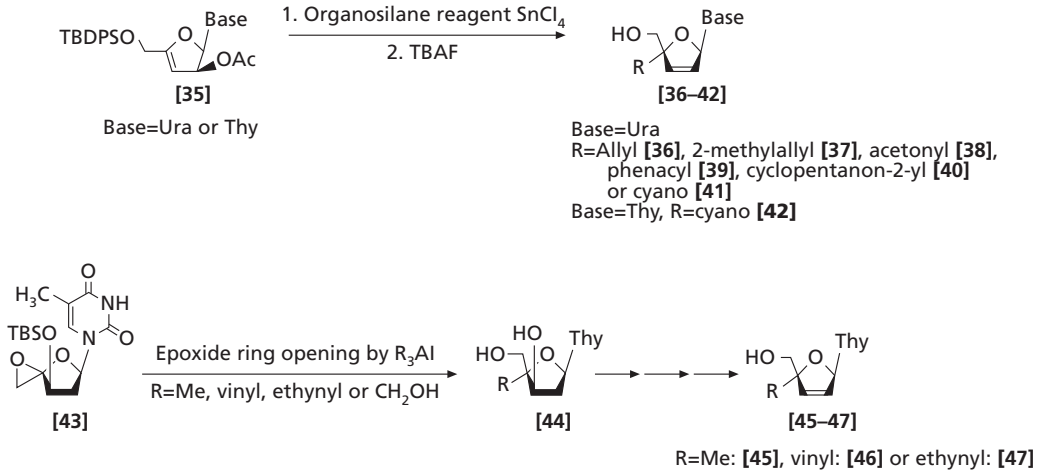


Figure 7. Synthesis of 4′-*C*-trifluoromethyl nucleosides **[52–57]**

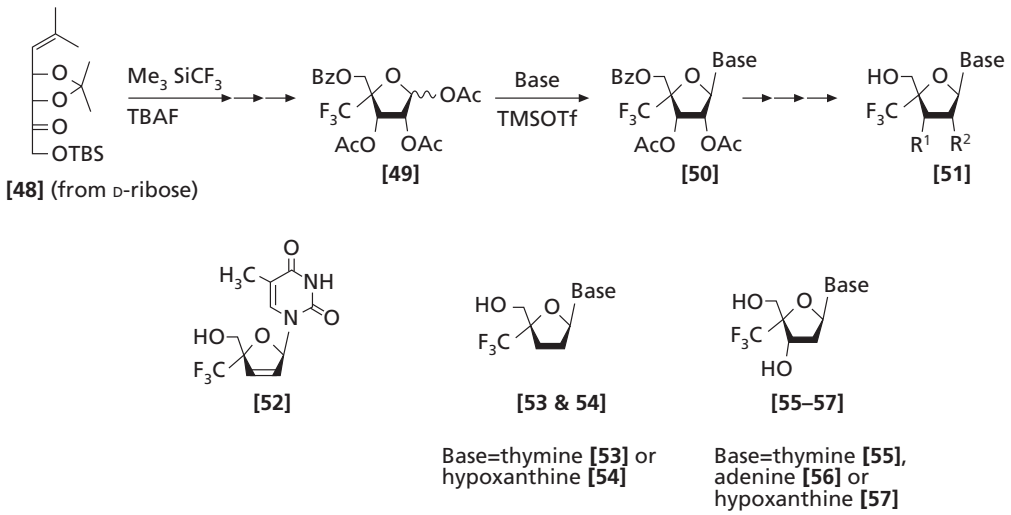
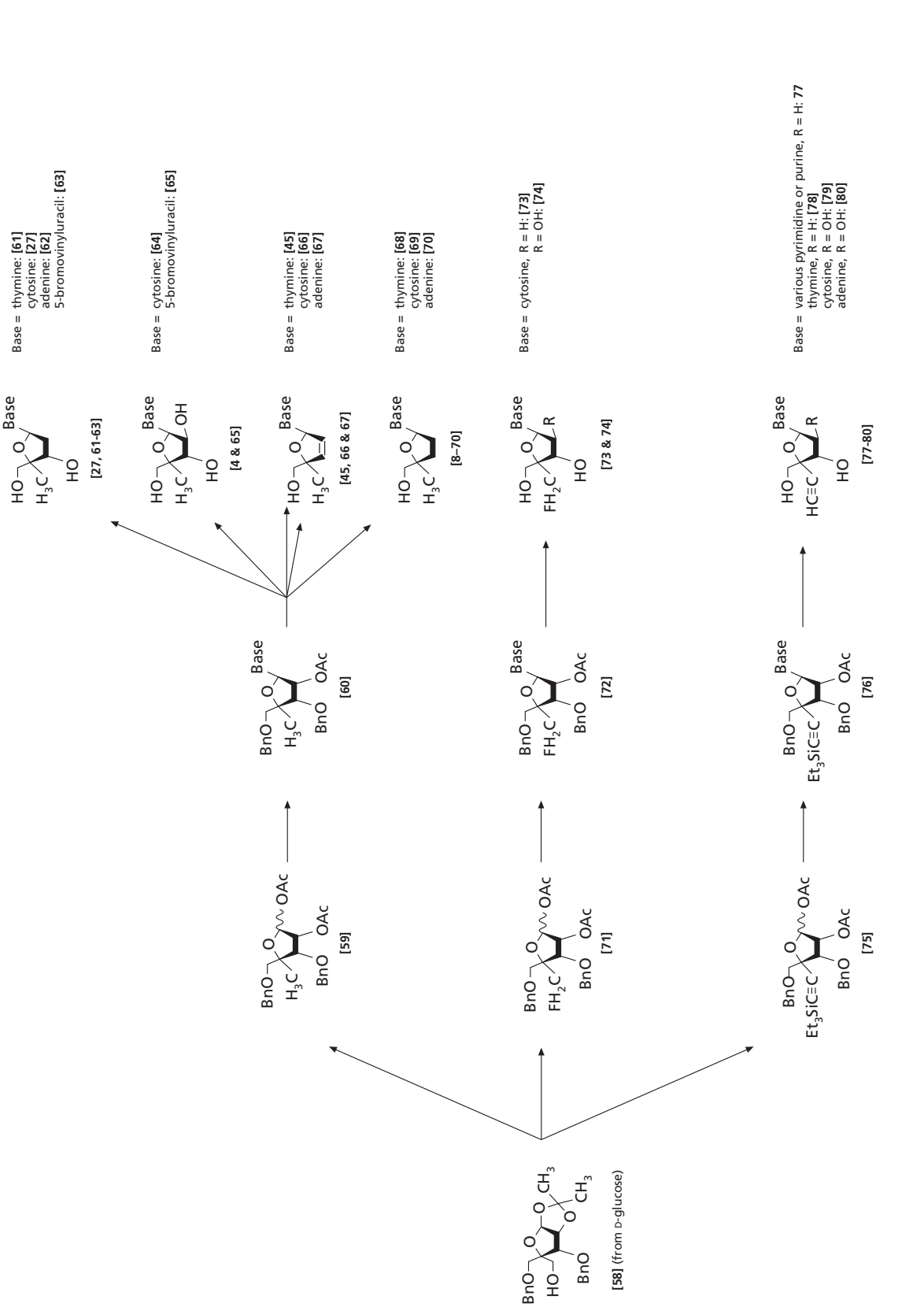


Figure 8. Synthesis of 4′-C-methyl, fluoromethyl and ethynyl nucleosides by condensation of sugars with bases



both methods depending on the structure of the target derivatives. We summarize our synthesis of 4′-*C*-methyl, fluoromethyl and ethynyl nucleosides using the condensation method (Figure 8). We started our chemistry with the synthesis of 4′-*C*-methyl nucleosides (Ohrui *et al.*, 1991). These 4′-SNs **[27**, **45**, **61–70]** were prepared by the condensation method, which utilized a key intermediate, 4-*C*-methyl-D-ribofuranose derivative **[59]** (Waga *et al.*, 1993). During the preparation of a series of 2′-deoxynucleoside **[27**, **61**, **62]**, 2′,3′-unsaturated nucleoside **[45**, **66**, **67]**, 2′,3′-dideoxynucleoside **[68–70]** and ara-*C* analogues **[64]** by the above method, we found that 4′-*C*-methyl-2′-deoxycytidine (4′-MdC) **[27]** showed inhibitory activity against HIV in MT-4 cells (Waga *et al.*, 1996).

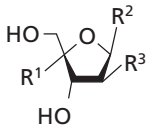
Although 4′-MdC **[27]** was 100-fold more potent than the thymidine derivative 4′-MT **[61]**, it was the most cytotoxic compound among the 4′-*C*-methyl nucleosides tested. Interestingly, the 4′-MdC **[27]** was also tested for its ability to inhibit the growth of P388 mouse leukaemia cells, and it proved to be markedly effective (IC_{50}=1.7 μM). The mechanism of action of **[27]** was also studied (Yamaguchi *et al.*, 1997).

Since the Yamasa Corporation developed BVaraU as an anti-HSV-1 drug, we prepared 4′-*C*-methyl-BVaraU **[65]** and 4′-*C*-methyl-BVDU **[63]** as antiviral agents. Compound **[63]** exhibited particularly potent anti-HSV-1 and anti-varicella-zoster virus (VZV) activity (Kitano *et al.*, 1999). Additionally, 4′-*C*-fluoromethyl nucleosides such as 2-deoxy-D-erythro- and *arabino*-pentophuranosyl cytosine **[73,74]** were previously synthesized by us using an analogous method (Kitano *et al.*, 1997). Interestingly, 4′-*C*-fluoromethyl-2′-deoxycytidine (4′-FMdC) **[73]** exhibited not only potent anti-HIV activity but also antineoplastic activity.

As mentioned above, 4′-ethynyl-pyrimidine-nucleosides showed anti-HIV activity. However, there were only a few reports on the preparation of 4′-ENs. Moreover, those papers did not mention any synthetic method of 4′-ENs, especially in the case of purine nucleosides. Thus, we started the chemistry for modifying the 4′-*C*-position of purine nucleosides with an ethynyl group. 4′-ENs could be prepared from the corresponding nucleosides, but we usually used 4-*C*-hydroxymethyl-3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*ribo*-furanose **[75]** as a versatile starting material for the synthesis of D-arabino and 2′-deoxy-D-ribo analogues of 4′-ENs **[77–80]**. The outline of our study for the synthesis of 4′-ENs is shown in Figure 8 (Kohgo *et al.*, 1999), and the anti-HIV activity is summarized in Figure 9 and Table 1 (Maag *et al.*, 1992; O-Yang *et al.*, 1999; Nomura *et al.*, 1999; Sugimoto *et al.*, 1999; Ohrui *et al.*, 2000; Ohri *et al.*, 2001).

Since 4′-*C*-substituted nucleosides showed anti-HIV activity (Table 1), we decided to explore the novel NRTIs that are active against drug-resistant HIV-1 variants and that prevent or delay the emergence of resistant HIV-1.

Figure 9. Structures of 4′-*C*-substituted nucleosides*



*See Table 1 for anti-HIV activity of R^1, R^2 and R^3.

Summary of SARs of 4′-SNs against HIV-1

As described in other research groups' investigations, the syntheses and anti-HIV activity of 4′-*C*-methyl-thymidine (4′-MT) **[61]**, 4′-*C*-ethynyl-2′-deoxycytidine (4′-EdC) **[22]**, 4′-*C*-ethyl-2′-deoxycytidine (4′-EtdC) **[24]**, 4′-*C*-ethynyl-thymidine (4′-ET) **[28]** and some other 4′-SdNs were reported while we were working on our project. Therefore, SAR of various 4′-*C*-substituted nucleosides against HIV-1 are summarized together with our data:

1) The estimated relative order of anti-HIV-1 potency is as follows: $CN \geq N_3 \geq C{\equiv}CH > CH{=}CH_2 > Me = Et > C{\equiv}C-Me$. This is based on published data. Interestingly, the order is the reverse of the $-\Delta G^0$ values between equatorial and axial substituents on a cyclohexane ring: $CN < F < C{\equiv}CH < CH{=}CH_2 < Me \leq Et < t\text{-}Bu$. Thus, these results indicate that the structure of 4′-SNs with a less sterically demanding substituent at the 4′-position is closer to the structure of dNs and has greater anti-HIV activity.

2) Purine analogues are generally less toxic than pyrimidine analogues; the former generally have greater selectivity indices (SI).

3) Ribo-derivatives are inactive, and arabino-derivatives are inactive or weakly active compared with 2′-deoxyribo counterparts.

4) 2′,3′-Dideoxyribo derivatives, including d4 type derivatives, with some exceptions do not show anti-HIV activity.

Design for creating novel NRTIs

One of our collaborators (Ohrui, 2001) speculated that the expected chemical and biological mechanisms of novel NRTIs were as follows:

1) The expected properties come from the presence of a 3′α-OH group.

Table 1. Anti-HIV activity of various 4′-*C*-substituted pyrimidine and purine nucleosides

Compound			Anti-HIV activity		
R^1	**R^2**	**R^3**	**EC_{50} (μM)**	**CC_{50} (μM)**	**SI**
CN	thymine	H	0.002	1	500
N_3	thymine	H	0.01	8	800
ethynyl	thymine	H	0.61	>380	>623
ethynyl	thymine	OH	>350	>350	–
ethynyl	5-ethyluracil	H	>360	>360	–
ethynyl	uracil	H	>100	>100	–
ethynyl	5-fluorouracil	H	>10	3.4	–
ethynyl	5-chlorouracil	H	6.0	81.7	13.6
ethynyl	5-bromouracil	H	2.3	>100	>43.5
ethynyl	5-iodouracil	H	0.34	>260	>765
CH_2N_3	thymine	H	2.1	333	159
Me	thymine	H	7.2	104	14.4
Et	thymine	H	16.1	>100	6.21
OMe	thymine	H	8.49	200	23.6
vinyl	thymine	H	6.1	>100	>16.4
hydroxyethyl	thymine	H	>4.7	4.7	–
propynyl	thymine	H	>100	>100	–
CN	cytosine	H	0.0012	0.17	142
N_3	cytosine	H	0.01	8	800
ethynyl	cytosine	H	0.0048	0.92	192
ethynyl	cytosine	OH	0.0048	1.74	363
ethynyl	5-methylcytosine	H	0.011	0.70	63
ethynyl	5-fluorocytosine	H	0.030	>100	>3333
ethynyl	5-chlorocytosine	H	>100	>100	–
ethynyl	5-bromocytosine	H	>100	>100	–
ethynyl	5-iodocytosine	H	>100	>100	–
Me	cytosine	H	0.015	1.0	66.7
CH_2F	cytosine	H	0.0068	0.12	18
Et	cytosine	H	0.013	0.77	59
vinyl	cytosine	H	0.0086	0.18	21
chlorovinyl	cytosine	H	2.1	4.6	2.2
N_3	adenine	H	0.13	50	385
ethynyl	adenine	H	0.098	16	1630
ethynyl	adenine	OH	0.78	248	318
ethynyl	2,6-diaminopurine	H	0.00034	0.9	2600
ethynyl	hypoxanthine	H	0.13	137	1053
ethynyl	guanine	H	0.0015	1.4	933
ethynyl	purine	H	135	>400	>3
methyl	adenine	H	2.6	2.6	–
	zidovudine (AZT)		0.0032	29.4	9190
	lamivudine (3TC)		0.10	>100	933

Figure 10. Speculations on how to overcome NRTIs' problems

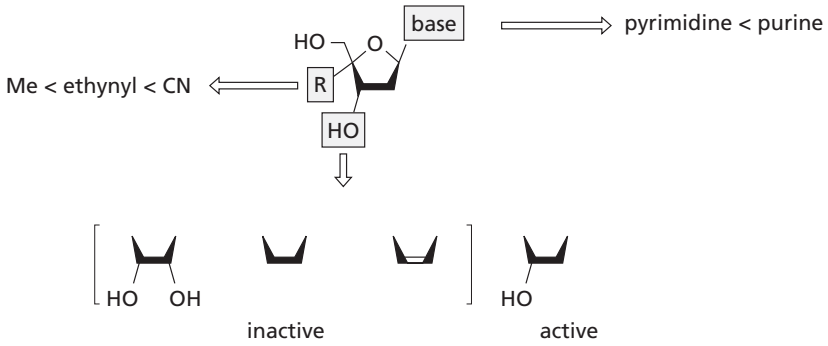
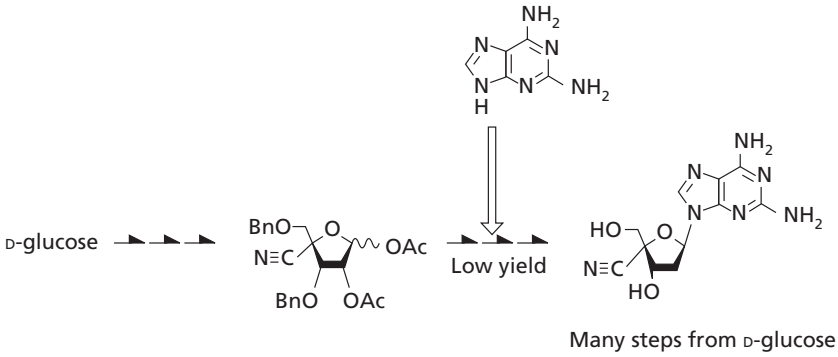


Figure 11. Synthetic problems of 4′-*C*-substituted nucleosides by condensation method



2) The presence of 3′α-OH in 4′-SdNs makes it acceptable by RTs and, therefore, 4′-SdNs are incorporated into the proviral DNA chains. The electron-withdrawing 3′α-OH group makes 4′-SdNs acid-stable even with purines. Thus, various purine derivatives can be made.

3) The expected properties come from the presence of 4′-substituents.

4) The 4′-substituents cause severe steric hindrance to the neighbouring *cis* 3′α-OH group due to restricted rotation around the C3′-C4′ single bond. Thus, the reactivity of 3′α-OH sharply decreases. Therefore, it was expected that enzymatic chain elongation of DNA would not proceed by using the very unreactive 3′α-OH. Consequently, 4′-SdNs could be chain terminators for proviral DNA biosynthesis. The steric repulsion between 3′α-OH and 4′-substituents changes the conformation of the furanose ring of 4′-SdNs, preferably to 3′-endo conformation (N-Type); this results in 4′-SdNs being less susceptible to enzymatic degradation. Therefore, 4′-SdNs would be more stable than 2′-deoxynucleosides (dN) and 2′,3′-dideoxynucleosides (ddN) against catabolism. The lipophilic substituent at the 4′-position imparts more lipophilicity to 4′-SdNs thus enabling them to penetrate the cell membrane efficiently. Possibly, this may enhance oral bioavailability and penetration through the

blood–brain barrier, although formal testing is needed to ascertain these issues. The emergence of highly resistant HIV-1 variants may not readily arise since the active anti-HIV 4′-SdNs with a smaller substituent at the 4′-position have structures more similar to dN.

If this speculation is true, we can design and discover novel NRTIs which are able to prevent, or delay, emergence of drug-resistant virus.

Design and synthesis of 4′-CNdNs from natural 2′-deoxynucleosides

From previous studies on SAR of 4′-*C*-substituted nucleosides, it was expected that a smaller substituent at the *C*-4′ position would give more acceptable biological activity. This is based on the parameter $-\Delta G^0$ values between equatorial and axial substituents on the cyclohexane rings. Thus, purine 2′-deoxynucleoside derivatives bearing a cyano group, which might be smaller than an ethynyl one (at the *C*-4′-position), will have more potent antiviral activity. During our synthesis of 4′-*C*-substituted nucleosides, we have been utilizing a glycosidation reaction of 4-*C*-substituted sugar derivatives with nucleobases. However, this synthetic route incurs some problems (Figure 11).

Synthetic problems in the condensation method are summarized as follows:

1) Preparation of 4′-*C*-substituted sugars and their derivation to the desired nucleosides require multi-step reactions and their total yields are low.

2) 4′-*C*-Substituted sugars have low reactivity in glycosidation reaction, especially when their substituent is an electron-withdrawing group like a cyano group.

These problems prompted us to develop a preparation method of 4′-*C*-substituted purine nucleosides from the corresponding nucleosides. This approach enabled us to synthesize 4′-*C*-cyano purine nucleoside derivatives, which were difficult to synthesize by condensation of sugars with nucleobases.

Synthesis of 4′-C-cyano-2′-purine-nucleosides. The synthesis of 4′-*C*-cyano-2′-purine-nucleosides starting from 2′-deoxyadenosine and dDAP is shown (Figure 12). The key intermediates **[81a,b]** for the synthesis of 4′-*C*-ethynyl- and 4′-*C*-cyano-purine-2′-deoxynucleosides were prepared according to Matsuda's procedure, with some modifications (Nomura *et al.*, 1999). The hydroxymethyl group of the key intermediate **[81a]** was converted to a cyano group. A 4′-*C*-formyl derivative, which was obtained by Moffatt oxidation of the hydroxymethyl group in **[81a]**, was converted to a 4′-*C*-aldoxime derivative and then further dehydrated to give 4′-*C*-cyano derivative **[82a]**. The protecting groups of compound **[82a]** were removed to give 2′-deoxyadenosine derivative (4′-CNdA) **[83a]**. Additionally, 4′-*C*-cyano-2,6-diaminopurine 2′-deoxyriboside (4′-CNdDAP) **[83b]** was also synthesized by a similar procedure to that described for compound **[83a]**. The cyano derivatives **[83a,b]** were readily converted to 2′-deoxyinosine (4′-CNdI) **[84a]** and 2′-deoxyguanosine derivatives (4′-CNdG) **[84b]** by enzymatic deamination.

Synthesis of 4′-EdNs from 2′-deoxynucleosides. These synthetic methods using nucleosides as the starting material were also an effective route to the various 4′-*C*-ethynyl derivatives 4′-EdA **[86a]**, 4′-EdI **[87a]**, 4′-EdDAP **[86b]** and 4′-EdG **[87b]** (Figure 12). It will be easy for us to scale up the process for the preparation of 4′-*C*-ethynyl derivatives.

In summary, we developed a method for preparing purine 2′-deoxynucleoside derivatives bearing an ethynyl or a cyano group at the 4′ position from the corresponding 2′-deoxynucleosides as the starting materials. The total yields of 4′-*C*-substituted purine nucleosides were improved (compared to that of the condensation method of sugars with bases) by using this synthetic route. Furthermore, it became easy to make derivatives bearing an electron-withdrawing substituent like a cyano group.

Anti-HIV activity of 4′-CNdNs. The activity of 4′-CNdNs, together with that of 4′-EdNs, is shown in Table 2. Unfortunately, the anti-HIV activity of 4′-CNdNs did not meet our expectations. In the case of 4′-EdA **[86a]**, it was easily hydrolysed to give 4′-EdI **[87a]**, which was less active than the parent 4′-EdA **[86a]**. In contrast, 4′-CNdI **[84a]** was as potent as 4′-CNdA **[83a]** against HIV-1 despite the low activity of 4′-EdI **[87a]**.

Attempts to synthesize less toxic and/or stable analogues

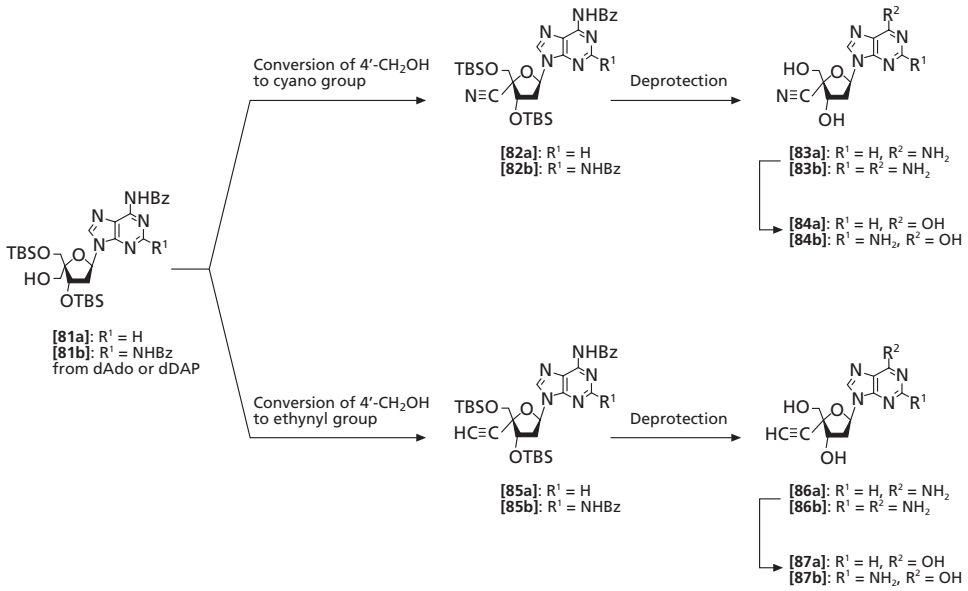
Design and synthesis of L-4′-EdNs (L-4′-EdDAP [92], dG [93], and dC [98]). During our exploration of novel NRTIs, we selected 4′-ethynyl-2′-deoxy-purine-nucleoside (2,6-diaminopurine derivative **[86b]** and guanine derivative **[87b]**) for *in-vivo* assay because of their high biological activity *in vitro*. However, they showed high toxicity in mice (Ashida, Yamasa Corporation, personal communication). Additionally, 4′-*C*-cyano-2′-deoxy-purine-nucleoside (2,6-diaminopurine derivative **[83b]** and guanine derivative **[84b]**) were very toxic in the *in-vitro* assay (Ashida, Yamasa Corporation, personal communication). On the other hand, Chu *et al.* reported that the enantiomer of 3TC (D-enantiomer) was very toxic, but 3TC itself (L-enantiomer) was less toxic (Beach *et al.*, 1992). Therefore, we designed and synthesized L-4′-ethynyl derivatives such as **[92]** and **[93]** in an effort to reduce toxicity (Figure 13) (Kitano, Yamasa Corporation, private communication). Synthesis of the L-ribose unit bearing an ethynyl group at the 4-*C*-position and the glycosidation are outlined (Figure 13).

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

Compound	Anti-HIV activity*		
	EC_{50} (μM)	**CC_{50} (μM)**	**Selectivity index**
4′-ethynyl dA **[86a]**	0.0098	16	1633
4′-ethynyl dDAP **[86b]**	0.00034	0.9	2647
4′-ethynyl dI **[87a]**	0.13	137	1054
4′-ethynyl dG **[87b]**	0.0015	1.4	933
4′-cyano dA **[83a]**	0.051	12	235
4′-cyano dDAP **[83b]**	0.00079	>0.034	>43
4′-cyano dI **[84a]**	0.051	23	451
4′-cyano dG [**84b]**	0.000188	>0.034	>181
zidovudine (AZT)	0.0032	29.4	9188

*Anti-HIV activity was determined by MTT assay. MT-4 cells and HIV-1_{LAI} were employed.

Figure 12. Synthesis of 4′-*C*-cyano and 4′-*C*-ethynyl purine 2′-deoxynucleosides by modification of natural nucleosides



*4-C-Hydroxymethyl-3,5-di-O-benzyl-1,2-O-isopropylidene-α-L-ribo-pentofuranose **[89]**.* The key intermediate, was obtained from D-arabinose in nine steps. Silylation and isopropylidenation of D-arabinose gave 5-*O*-TBDPS-1,2-*O*-isopropylidene-α-D-*arabino*-pentofuranose **[88]** in two steps. The inversion of the 3-hydroxyl group in compound **[88]** gave D-lyxo-pentofuranose derivative, which was derived to the key intermediate **[89]** by introduction of a hydroxymethyl group into the *C*-4 position by Moffatt's procedure and the following selective benzylation of the 5-hydroxyl group. L-Enantiomers of 4′-*C*-ethynyl dDAP **[92]** and dG **[93]** were obtained from the key intermediate **[89]** by a known procedure (Kohgo *et al*., 1999; Ohrui *et al*., 2000).

On the other hand, 4′-*C*-ethynyl-2′-deoxycytidine (4′-EdC) **[22]** had very potent anti-HIV activity, but this compound also showed cytotoxicity. Therefore, L-4′-*C*-ethynyl-2′-deoxycytidine (L-4′-EdC) **[98]** was also prepared from D-glucose by us (Figure 14) (Kohgo *et al*., 2001). The synthetic scheme is summarized (Figure 14).

The L-enantiomers of 4′-*C*-ethynyl-2′-deoxynucleosides **[92, 93, 98]** were evaluated for anti-HIV activity toward MT-2 or MT-4 cells by an MTT assay. However, all these nucleosides were inactive against HIV-1 at concentrations up to 100 μM. It is worth noting none of the L-isomers of 4′-EdNs showed significant antiviral activity against HIV-1 *in vitro*.

Design and synthesis of 4′-C-substituted-6-chloropurine nucleosides (4′-S-6-CldNs) [101a,b] and 4′-C-substituted-6-mercapto-purine nucleosides (4′-S-6-SHdNs) [103a,b]. Additionally, we also chose 4′-EdA **[86a]** as another candidate because of its activity in the *in vitro* assay, but it was metabolized immediately *in vivo* to give the less active 4′-EdI **[87a]**. Thus, we attempted to resolve the above metabolic problem by a different approach. As mentioned above, while the anti-HIV activity of 4′-CNdNs did not meet our expectations, 4′-CNdI **[84a]** did prove active against HIV-1. Therefore, we focused on the modification at the 6-position of purine nucleosides in the case of 4′-ethynyl- and cyano-derivatives.

We previously confirmed that 6-chloro-purine-nucleosides were easily hydrolysed by adenosine deaminase (ADA) to give inosine derivatives. However, it was reported that 6-mercaptopurine-nucleosides were tolerant to this reaction (Murakami *et al*., 1991). Furthermore, they also reported the modification method at the 6-position of purine dideoxynucleosides by chloro and mercapto groups to increase lipophilicity for these compounds (Murakami *et al*., 1991). Therefore, we designed the modification at the 6-position of purine nucleosides utilizing their method. The preparation method for 4′-*C*-substituted 6-chloro-purine and 6-mercapto-purine derivatives is summarized in Figure 15.

We selected 4′-EdA derivative **[99a]** and 4′-CNdA derivative **[99b]** as key intermediates. The 6-amino group in 4′-EdA **[99a]** or 4′-CNdA derivatives **[99b]** were converted to a chloro group with Et_4NCl-tBuONO to give protected 6-chloro derivative **[100a,b]**. These derivatives **[100a,b]** were deprotected with ammoniumfluoride hydrogen fluoride (NH_4F-HF) in methanol or tetrabuthylammonium fluoride (TBAF) in THF to give the desired nucleosides **[101a,b]**.

Protected 6-chloro-purine derivatives **[100a,b]** were also used to prepare 6-mercapto-purine derivatives **[103a,b]**. Protected 4′-E- and 4′-CN-6-mercaptopurine analogues **[102a,b]** were obtained by the reaction of **[100a,b]** with sodium hydrosulphide in distilled water/ethanol or thiourea in refluxing ethanol, and the following deprotection of **[102a,b]** gave 4′-E- and 4′-CN-6-mercaptopurine derivatives **[103a,b].**

Unfortunately, while 6-Cl purine derivative **[101a,b]** still showed weak activity, 6-mercapto derivatives **[103a,b]** exerted no anti-HIV-1 activity *in vitro*. The lack of antiretroviral activity of these nucleosides **[101a,b; 103a,b]** may be due to them not being converted to the desired analogues of 4′-*C*-substituted-dG or dI. According to Murakami *et al*., (1991) 2-amino-6-mercapto-ddNs are not substrates for ADA nor is it converted to ddG at all in the presence of an excess of isolated ADA.

Design and synthesis of α-L-4′-CNdNs (Kohgo, Yamasa Corporation, private communication). Inversion of the configuration at the 4′-*C* of the sugar leads to the α-L-series of nucleosides; therefore, we made alpha-L-4′-*C*-cyano-2′-deoxyadenosine. Unfortunately, α-L-4′-CNdNs did not show anti-HIV activity. Synthesis in our laboratory of α-L-4′-EdNs is in progress.

As can be seen from the results mentioned above, 4′-*C*-ethynyl- and 4′-cyano-nucleosides are promising candidates among various 4′-*C*-substituted nucleosides. Therefore, finally we describe the crucial factors (drug resistance, stability, mode of actions, toxicity and bulkiness) which will influence the development of the 4′-*C*-ethynyl-nucleosides and other 4′-SdNs.

Drug resistance

Activity of 4′-SdNs against drug-resistant infectious HIV-1 clones

The activity of selected 4′-SdNs against HIV-1 variants resistant to various NRTIs using MAGI assay is listed (Table 3). It is noteworthy that the three cytosine analogues, 4′-EdC **[22]**, 4′-EaraC **[79]** and 4′-MdC **[27]** suppressed the replication of HIV-1_{K65R}, HIV-1_{L74V}, HIV-$1_{M41L/T215Y}$ and multi-dideoxynucleoside-resistant HIV-$1_{A62V/V75I/F77L/F116Y/Q151M}$ (MDR) at EC_{50} values ranging

Figure 13. Synthesis of L-4′-*C*-ethynyl purine 2′-deoxynucleosides from D-arabinose

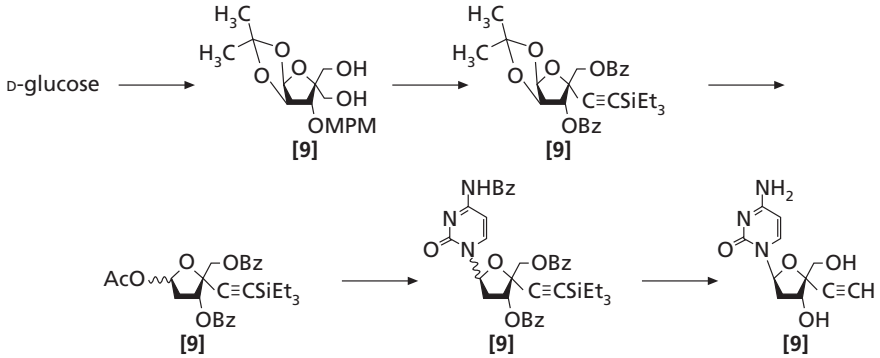


Figure 14. Synthesis of L-enantiomer of 4′-*C*-ethynyl-2′-deoxycytidine from D-glucose

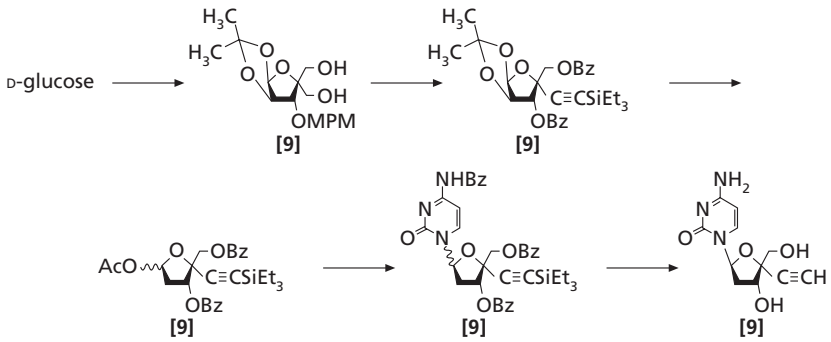


Table 3. Antiviral activity of 4′-*C*-substituted nucleosides against drug-resistant infectious clones

Compound	EC_{50} (μM)*									CC_{50} (μM)
	HXB2	K65R	L74V	M41L/T215Y	M184V	M184I	M41L/T69S-S-G/T215Y	MDR	Y181C	
Pyrimidine analogues										
4′-ET										
[28]	0.36	0.53	0.68	0.43	0.18	0.14	0.44	0.12	0.13	>200
4′-EdC										
[22]	0.0012	0.0008	0.0013	0.006	0.0024	0.0026	0.015	0.0012	0.0021	>200
4′-EaraC										
[79]	0.0071	0.015	0.026	0.026	0.71	0.48	0.17	0.0079	0.016	>200
4′-MdC										
[27]	0.0058	0.0071	0.0062	ND	0.2	0.74	ND	0.0033	ND	>200
4′-FMdC										
[73]	0.0046	0.065	0.0019	0.0035	2.0	ND	0.066	0.0039	ND	>200
Purine analogues										
4′-EdA										
[86a]	0.008	0.033	0.004	0.012	0.047	0.022	0.065	0.0062	0.011	>200
4′-EdDAP										
[86b]	0.0014	0.00035	0.0007	0.0017	0.0059	0.0027	0.0041	0.001	0.0008	>200
4′-EdI										
[87a]	0.81	0.25	0.61	1.3	16.6	1.5	2.2	0.51	ND	>200
4′-EdG										
[87b]	0.007	0.001	0.0012	0.019	0.008	0.0041	0.0068	0.0048	0.01	52
4′-CNdA										
[83a]	0.043	ND	ND	ND	2.28	ND	ND	0.083	ND	ND
4′-CNdI										
[84a]	0.242	ND	ND	ND	6.06	ND	ND	0.296	ND	ND
AZT	0.022	0.02	0.02	0.3	0.01	0.017	1.6	15.3	0.014	>100
ddC	0.2	3.0	1.5	ND	2.2	ND	1.3	5.5	ND	>100
3TC	0.71	ND	ND	ND	>100	>100	9.9	1.1	ND	>100
ddI	3.9	12.7	19.5	3.6	10.1	ND	12.2	25	ND	>100

*Anti-HIV activity was determined with the MAGI assay. ND, not determined.

Table 4. Antiviral activity of 4′-*C*-ethynyl nucleosides against clinical isolates

Strain	Amino acid substitution(s)		EC_{50} (μM)*			
	RT region	Protease region	4′-EdC **[22]**	4′-EdA **[86a]**	4′-EdDAP **[86b]**	AZT
ERS_{104pre}	None	None	0.0012	0.013	0.00083	0.0056
IVR_{205}	None	K20M, M36I, D60E	0.0023	0.0034	0.0001	0.0015
IVR_{207}	G190Q	V77I	0.0046	0.0022	<0.0001	0.0036
Pt1	T69G, K70R, L74V, A98G, K103N, V179D, M184V, T215F, K219F	L10I, L33I, M36I, M46I, L36P, A71V, G73S, V82A, L90M	0.00064 (0.5-fold)	0.00054 (0.4-fold)	0.0011 (1.3-fold)	0.029 (52-fold)
Pt6	M41L, D67N, M184V, L210W, T215Y	L10I, K20R, L24I, M36I, M46L, I54V, L63P, V82A, L89M	0.013 (11-fold)	0.040 (3-fold)	0.0001 (0.1-fold)	0.28 (50-fold)
Pt7	M41L, D67N, T69D, M184V, T215F	L10I, K45R, I54V, L63P, A71V, V82T, L90M	0.0016 (1.3-fold)	0.009 (0.7-fold)	0.0005 (0.6-fold)	1.9 (340-fold)
Pt9	M41L, M184V, T215Y	M46I, L63P, A71V, V77I, I84V, N88D, L90M	0.023 (19-fold)	0.029 (2.2-fold)	0.0031 (3.7-fold)	0.97 (170-fold)

*EC_{50}s were determined with PHA-PBMC. Numbers in parentheses represent fold changes of EC_{50}s against each HIV-1 isolate compared to those against the wild-type clinical HIV-1 strain, $ERS_{104pre+}$.

from 0.001–0.015 μM. Among these nucleosides, only 4′-EdC **[22]** remained active against 3TC-resistant HIV-1_{M184I} and HIV-1_{M184V} but was less active against MDR-HIV-$1_{M41L/T69S\text{-}S\text{-}G/T215Y}$ than other variants, while 4′-EdC **[22]** remained potent against MDR-HIV-$1_{M41L/T69S\text{-}S\text{-}G/T215Y}$. The three purine analogues (4′-EdA) **[86a]**, 4′-EdDAP **[86b]** and 4′-EdG **[87b]** that were potent against wild-type HIV-1 were also highly active against all the infectious clones. Additionally, they were also active against a non-nucleoside RTI-resistant (NNRTI) infectious HIV-1_{Y181C}.

Activity of 4′-SdNs against HIV-1 isolated from heavily drug-experienced patients with AIDS

The *in vitro* activity of the three most potent 4′-E-nucleosides (Table 4), 4′-EdC **[22]**, 4′-EdA **[86a]** and 4′-EdDAP **[86b]** was tested against multi-drug resistant clinical HIV-1 variants isolated from heavily drug-experienced patients with AIDS. All three 4′-E-nucleosides suppressed replication of these highly drug-resistant clinical strains isolated from patients 1 and 7 as effectively as those of the wild-type clinical strain HIV-$1_{ERS104pre}$.

Properties and pharmacokinetics of 4′-EdNs and 4′-CNdNs

Stability of 4′-SdNs against enzymatic catabolism. It took 4 h to completely deaminate 4′-EdA **[86a].** Under the same conditions, the enzyme, adenosine deaminase, deaminated ddA in 2 h. Pyrimidine phosphorylase hydrolysed 4′-*C*-methyl-2′-deoxy-5-ethyluridine by only 6% under conditions where the enzyme hydrolysed arabinofuranosyl-5-ethyluridine by 53% and 2′-deoxy-5-ethyl-uridine completely. All attempts for enzymatic exchange of the base of 4′-ET **[28]** with adenine were unsuccessful because the glycosyl linkage of 4′-ET **[28]** was too stable to be cleaved by the enzymes used. 4′-SdNs are fairly stable under physiological conditions, although the catabolism of 4′-SdNs following triphosphorylation as yet remains to be determined.

Stability of 4′-SdNs (mainly 4′-EdNs) under acidic conditions. When three 4′-EdNs (4′-EdC 22, 4′-EdA) **[86a]**, and 4′-EdDAP **[86b]** and two ddNs (ddI and AZT) were exposed to 1M HCl for up to 20 min and then to 1M NaOH, their anti-HIV-1 activity was then tested. All 4′-EdNs were active although the acid-labile ddI completely lost its antiviral activity. The acid-stable property of these 4′-SdNs may contribute favourably to their oral bioavailability, if they are ultimately administered orally.

Mode of actions of 4′-SdNs. 4′-SdNs act as NRTIs and terminators of viral DNA biosynthesis in spite of the presence of the 3′α-OH group. Although a variety of side effects of NRTIs, some of which are often lethal, are well known and attributed to the inhibitory effect of ddN-TPs on mitchondrial polymerase γ activity, such effect has yet to be determined for 4′-SdNs. Such data regarding polymerase γ inhibition of 4′-SdNs-TPs are essential before 4′-SdNs may be considered as potential therapeutics for HIV-1 infection.

4′-E-nucleosides examined in our study retain the 3′-OH moiety like natural substrates, which may enable 4′-E-nucleosides to interact with the mutated 3′-OH binding site of various types of drug-resistant HIV.

Toxicity. It should be noted, however, since 4′-SdNs have the 3′-OH moiety, that the incorporation of 4′-SdNs to cellular and mitochondrial DNA may be more likely to happen, thus causing higher levels of unacceptable toxicity compared with ddNs. 4′-EdDAP **[86b]**, 4′-EdG **[87b]**, 4′-CNdDAP **[83b]** and 4′-CNdG **[84b]** were very toxic. Fortunately, 4′-EdA **[86a]**, 4′-EdI **[87a]**, 4′-CNdA **[83a]** and 4′-CNdI **[84a]** were less toxic.

Bulkiness. The results indicate that the structures of 4′-SdNs with a less sterically demanding substituent at the 4′-position are closer to the structures of dNs and have greater anti-HIV activity. However, the 4′-CN derivative did not meet our expectations. The expected properties come from the presence of 4′-substituents.

Conclusions

As can be seen from the research histories of various 4′-SdNs, novel NRTIs such as either 4′-EdNs (by us) and 4′-Ed4T **[47]** (by M Tanaka's group) appear to have sufficient anti-HIV-1 potency and favourable pharmacological properties. Therefore, they may be promising candidate therapeutics for HIV-1 infection and may be active against presently existing drug-resistant HIV-1 strains.

It should be noted that several issues, such as inhibition of mitochondrial DNA synthesis by 4′-SdNs-TPs (due to polymerase γ inhibition by 4′-SdNs-TPs) and pharmacokinetics, remain to be addressed before these analogues are considered for further development. However, we believe that NRTIs, especially 4′-EdNs, 4′-CNdNs and 4′-Ed4T **[47]**, may be considered as potential therapeutics for HIV-1 infection. It is also noteworthy that 4′-Ed4T **[47]** is less toxic to CEM cell growth and less inhibitory to mitochondrial DNA synthesis than d4T. Further searches for NRTIs are in progress in our laboratory.

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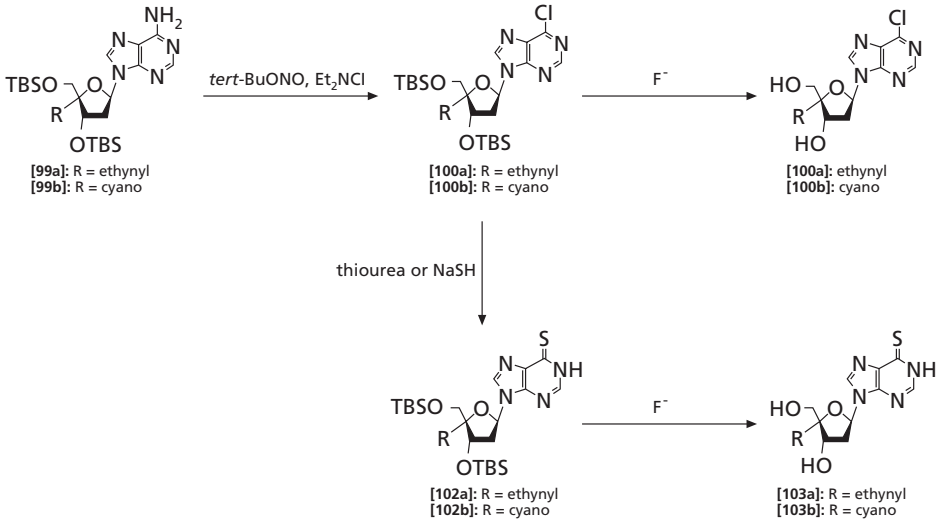
Figure 15. 4′-*C*-substituted 6-chloro and 6-mercapto purine nucleosides



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