Review

Development of modified nucleosides that have supremely high anti-HIV activity and low toxicity and prevent the emergence of resistant HIV mutants

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Abstract: An idea to use 4′-C-substituted-2′-deoxynucleoside derivatives was proposed based on a working hypothesis to solve the problems of existing acquired immune deficiency syndrome chemotherapy (highly active antiretroviral therapy). Subsequent studies have successfully proved the validity of the idea and resulted in the development of 2′-deoxy-4′-C-ethynyl-2-fluoroadenosine and 2′-deoxy-4′-C-ethynyl-2-chloroadenosine, nucleoside reverse transcriptase inhibitors, which have supremely high activity against all human immunodeficiency viruses including multidrug-resistant HIV and low toxicity.

Keywords: AIDS, anti-HIV agent, nucleoside reverse transcriptase inhibitor, drug-resistant HIV, 4′-C-substituted-2′-deoxynucleoside

Preface

I would like to describe the development of 2′-deoxy-4′-C-ethynyl-2-fluoroadenosine and its 2-chloro congener that have high anti-HIV activity and low toxicity and prevent the emergence of resistant HIV mutants, because the development is one of my contributions to the collaboration studies, with Dr. Takeshi Kitahara, a Professor Emeritus of The University of Tokyo, on Invention and Application of Molecules with Novel Biological Functions for receiving The Japan Academy Prize in 2010.

Introduction

Since the discovery of 3′-C-azido-3′-deoxythymidine (AZT) as the anti-human immunodeficiency virus (HIV) agent by Dr. Hiroaki Mitsuya in 1985,1) many 2′,3′-dideoxy nucleoside (ddN) analogs have been developed as nucleoside reverse transcriptase inhibitors (NRTIs). However, resistant HIV mutants against all these NRTIs have emerged rapidly and easily.

A highly active antiretroviral therapy (HAART) using two or more nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors has dramatically improved the quality of life and prognosis of patients infected by HIV 2),3) However, the existing HAART has several critical problems that remain to be solved. These problems include: (i) the emergence of new drug-resistant HIV mutants, (ii) the need to take large dosages of drugs, and (iii) drug side effects. Therefore, the development of new, highly potent anti-HIV drugs that prevent the emergence of drug-resistant mutants and have few side effects is urgently needed.

These problems prompted me to speculate on the reason for the emergence of HIV mutant resistant to clinical NRTIs, which belong to the family of ddN. I proposed a working hypothesis for the chemical structure of a new highly anti-HIV active NRTIs that might prevent the emergence of drug-resistant mutants. The design of 4′-C-substituted-2′-deoxynucleoside (4′SdN) was based on the working hypothesis. The working hypothesis for the decrease of toxicity of nucleoside derivatives was also proposed based on my past findings of the relationship between the biological activity and structure of nucleoside derivatives. Study on the synthesis and biological evaluation of 4′SdNs have proved the validity of the working hypotheses and resulted in the development of 2′-deoxy-4′-C-ethynyl-2-fluoroadenosine (4′Ed2FA) and 2′-deoxy-4′-C-ethynyl-2-chloroadenosine (4′Ed2ClA) (Fig. 1). These NRTIs are...
supremely active against all HIVs and have prevented the emergence of drug-resistant mutants for many years since their development and expected to prevent the emergence of drug-resistant mutants, have a low toxicity, and stable to enzymatic catabolism. Hence, 4′Ed2FA and 4′Ed2ClA are important keys for their further development of potential therapeutics to solve the problems being encountered by the present HAART.

Working hypotheses of the study
1. Design of the nucleoside derivatives that might be effective for drug-resistant HIV-mutants and possibly prevent the emergence of new drug-resistant HIV mutants. The structures of the clinical NRTIs are shown in Fig. 2. All belong to the ddN family. The ddN structure has been considered essential for the nucleoside derivatives to be the chain terminator of the reverse transcriptase (RT)-catalyzed biosynthesis of proviral DNA. However, HIV mutants resistant to all ddN-NRTIs have emerged rapidly and easily.

The emergence of HIV-mutants resistant to ddNs indicates that HIV-mutants acquired the ability to distinguish ddNs from physiological 2′-deoxynucleosides (dNs) and do not accept ddNs into the active center of RT and/or cut off the incorporated ddNs from the proviral DNA terminus.

Fig. 1. Structures of 2′-deoxy-4′-C-ethyl-2′-fluoroadenosine (4′Ed2FA) and its 2-chloro congener (4′Ed2ClA).

Fig. 2. Structures of clinical nucleosides reverse transcriptase inhibitors (NRTIs), physiologic 2′-deoxynucleoside (4′SdN). HIV = human immunodeficiency virus; dN = 2′-deoxynucleoside, 4′SdN = 4′-C-substituted-2′-deoxynucleoside.
Therefore, the anti-HIV nucleosides that might prevent the emergence of drug-resistant HIV mutants must satisfy the following two conditions.

1. To prevent the discrimination by HIV, the modified nucleosides should have a structure resembling those of physiologic nucleosides as closely as possible so that RT mistakes them for physiologic nucleosides. Since the striking difference of the ddN and dN is whether they have 3'-OH, the modified nucleosides must have 3'-OH.

2. In spite of having 3'-OH, the nucleoside must be the chain terminator of RT-catalyzed biosynthesis of proviral DNA.

Based on the following hypothesis, 4'SdN (Fig. 2) was designed as a nucleoside that could satisfy the above mentioned two conditions:

1. It would be difficult for HIV to discriminate 4'SdN from dN because 4'SdN has all the functional groups of dN.

2. The introduction of a substituent at 4'-position makes the 3'-OH into a very unreactive neopentyl-type secondary alcohol. Thus, the 3'-OH of 4'SdN will be used for HIV to mistake 4'SdN for dN, but is too unreactive to be used for the elongation of proviral DNA by RT. Therefore, 4'SdNs could be the chain terminators of proviral DNA biosynthesis.

3. Steric hindrance between 3'-OH and 4'-substituent changes the conformation of the furanose ring of 4'SdN preferably to the 3'-endo conformation (N-type). This results in 4'SdN being less susceptible to both acidic and enzymatic glycolysis than dN and ddN. (In glycolysis, the oxygen atom of the furanose ring participates to form a coplanar oxocarbonium ion, but the conformational change makes it difficult for the oxygen atom to form a coplanar oxocarbonium ion.)

4. Further, the electron-withdrawing 3'-OH makes 4'SdN more acid stable than does ddN even with purines. Thus, various purine derivatives can be made in this way.

5. The lipophilic substituent at the 4'-position imparts more lipophilicity to 4'SdNs, thus enabling them to penetrate the cell membrane efficiently. This possibly enhances their bioavailability.

2. Method of decreasing the toxicity of nucleoside drugs based on my past findings of the relationship between the biological activity and structure of nucleoside derivatives. If DNA polymerases also mistake 4'SdN for dN, 4'SdN should be highly toxic. However, ddNs have been used as anti-HIV drugs, meaning that DNA polymerases hardly accept ddNs as their substrates. Thus, the ability of DNA polymerases to distinguish substrate is superior to that of RT. (The substrate selectivity between DNA polymerases and RT must be different.)

The structures of the representative nucleoside antibiotic are shown in Fig. 3. Most of them are nucleoside derivatives modified at one site of the physiological nucleosides. Though they are highly active against microorganisms, they are highly toxic, too. Therefore, they can not be clinically used. In the 1960s and 1970s, many organic chemists synthesized nucleoside derivatives modified at two or more positions of physiologic nucleosides expecting to get nucleoside derivatives having new and/or higher...
biological activity. However, none of these modified nucleosides exhibited antimicrobial activity. These results indicated that intracellular enzymes do not recognize the nucleoside derivatives modified at two or more positions of physiologic nucleosides as substrates. Thus, there is a chance of decreasing the toxicity of 4'SdNs further by additional modification.

Results and discussion

Examination of the validity of the working hypotheses with 4'-C-methyl nucleosides. At first, to examine the validity of the working hypothesis, 4'-C-methyl-nucleosides (4'MNs), 4'-C-methyl-2'-deoxynucleosides (4'MdNs), 4'-C-methyl-2',3'-dideoxynucleosides (4'MddNs), and 4'-C-methyl-2',3'-didehydrodideoxynucleosides (4'Md4Ns) (Table 1) were synthesized and evaluated for their biological activity.5)6) 4'MdN showed remarkable biologoical activity (both anti-HIV activity and toxicity), but 4'MddN and 4'Md4N did not show notable biological activity (Table 1). 4'MNs did not show any anti-HIV activity at all.

These results indicate the importance of the 3'-OH for biological activity. Further, we demonstrated that 5'-O-triphosphate of both 4'-C-methyl-2'-deoxy-cytidine (4'MdCTP) and 4'-C-methyl-D-arabinofuranosyl cytidine (4'MaraCTP) are the chain terminator of calf thymus DNA polymerase α and recombinant rat DNA polymerase β.7) These results indicate that 4'SdN was an NRTI, although further study of 4'MdCTP with RT was not performed.

They were synthesized by glycosidation of 1,2-di-O-acetyl-3,5-di-O-benzyl-4-C-methyl-D-ribo-furanose (4), prepared from D-glucose through 3-O-benzyl-4-C-hydroxymethyl-1,2-O-isopropylidene-α-D-ribo-furanose (1),8) and nucleobases and then deoxygenation of the hydroxyl groups of 4'MNs (Scheme 1).

It should be noted that the 4'-C-methyl ribo-furanosyl derivatives took longer reaction time to complete the glycosidation reaction with silylated nucleoside bases than that of normal ribo-furanoside derivatives, indicating the lower reactivity of the 4'-C-methyl ribo-furanoses than normal ribo-furanoses. In addition, the 3J1',2' values of 4'-C-methyl ribo-furanosyl nucleoside were larger (4–7 Hz) than those of the normal β-ribose-nucleoside (0–1 Hz), indicating the change of the conformation of the furanose ring of the 4'-C-substituted nucleosides.

Structure–activity relationship (SAR) of 4'SdNs. Next, to study the SAR of 4'SdNs and develop 4'SdNs having more potent anti-HIV activity and less toxicity than 4'MdNs, 4'SdNs having various kinds of 4'-C-substituents and nucleobases were synthesized and evaluated for their biological activity.5)10) While we were working on our project, the anti-HIV activity of several 4'SdNs

Table 1. Anti-HIV activity of 4'-C-methyl-2'-deoxynucleosides

| Structure | Base       | EC50 (µM) | CC50 (µM) | SI (CC50/EC50) |
|-----------|------------|-----------|-----------|----------------|
| 4'MedN    | Ad         | 2.6       | 2.6       | 1.0            |
|           | Th         | 7.2       | 104       |                |
|           | Cy         | 0.072     | 0.13      | 1.8            |
|           | Purine     | 1.9       | >200      | >100           |
| 4'Med4N   | Ad         | >500      | >500      | ~1             |
|           | Th         | 21        | 330       | 16             |
|           | Cy         | 350       | 350       |                |
| 4'MeddN   | Ad         | 30        | 400       | 13             |
|           | Th         | >500      | >500      | ~1             |
|           | Cy         | 27        | 27        | 1              |

EC50 = 50% effective concentration; CC50 = cytotoxic concentration; SI = selectivity index (CC50/EC50); 4'MdN = 4'-C-methyl-2'-deoxynucleoside; 4'Md4N = 4'-C-methyl-2',3'-didehydrodideoxynucleoside; 4'MddN = 4'-C-methyl-2',3'-dideoxynucleoside; AZT = 3'-azido-3'-deoxythymidine; ddA = 2',3'-dideoxycadenosine; d4T = 2',3'-didehydro-3'-deoxythymidine.
Scheme 1. General synthetic scheme of 4′-C-substituted-2′-deoxynucleoside (4′SdNs) from D-glucose. 4′SdN = 4′-C-substituted-2′-deoxynucleoside; 4′MN = 4′-C-methylnucleoside; 4′Md4N = 4′-C-methyl-2′,3′-didehydrodeoxynucleoside; 4′MddN = 4′-C-methyl-2′,3′-dideoxynucleoside.

Table 2. Anti-HIV activity of 4′-C-substituted-2′-deoxynucleosides

| Compound                                      | EC50 (mM)a | CC50 (mM) | S.I. |
|-----------------------------------------------|------------|-----------|------|
| 4′-C-cyano-2′-deoxycytidine                   | 0.002      | 1         | 500  |
| 4′-C-azido-2′-deoxycytidine                   | 0.004      | 0.17      | 142  |
| 4′-C-ethynyl-2′-deoxycytidine                 | 0.0048     | 2.2       | 458  |
| 4′-C-cyano-2′-deoxy-5- fluorocytidine         | 0.030      | 100       | <333 |
| 4′-C-ethynyl-2′-deoxy-5- fluorocytidine       | 0.043      | 2.0       | 46.5 |
| 4′-C-ethyl-2′-deoxycytidine                   | 0.015      | 1.0       | 66.7 |
| 4′-C-ethyl-2′-deoxycytidine                   | 0.0068     | 0.12      | 18   |
| 2′,3′-dideoxy-3′-thio-L-cytidine (3TC)        | 0.10       | 100       | >1000|
| 3′-azido-3′-deoxythymidine (AZT)              | 0.0032     | 29.4      | 9190 |

a) Anti-HIV activity was determined by MTT assay. MT-4 cells and HIV-1 LAI were employed. ND: not determined.
was reported by the Syntex group\(^{17-21}\) and others.\(^{22,23}\) Therefore, the anti-HIV activities of 4'SdNs that we studied together with those reported by other groups are listed in Table 2.

The SARs of 4'-C-substituted nucleosides against HIV are summarized as follows:

1. The estimated relative order of anti-HIV activity is as follows: CN ≥ C≡CH > N≡N > CH≡CH > Me = Et > C≡C–CH₃. Interestingly, the order is the reverse of the \(\Delta G^*\) values between equatorial and axial substituents on a cyclohexane ring: CN < F < C≡CH < CH≡CH₂ < Me ≤ Et < ¹Bu. Thus, these results indicate that the sterically less demanding substituent at the 4'-position gives more potent anti-HIV activity.

2. Purine analogs are generally less toxic than pyrimidine. Although 2'-deoxy-4'-C-ethyl-5-fluorocytidine, which is a nucleoside derivative modified at two positions of physiologic 2'-deoxycytidine, gave a very acceptable Selectivity Index (SI = CC₅₀/EC₅₀) with MT-4 cells, it was toxic with other cells (Kohgo, Yamasa Corporation, private communication).

3. *Arabino* analogs are less active and less toxic compared with their corresponding 2'-deoxy counterparts.

4. 4'SsdNs do not show anti-HIV activity.

5. The L-isomers of 4'SdN have no anti-HIV activity,\(^{24}\) although it is known that the L-enantiomer of 2',3'-dideoxy-3'-thia-L-cytidine (3TC) is as active as the D-enantiomer and less toxic than the D-isomer.\(^{24}\) This may be due to the fact that the L-isomers are too much modified to be recognized by RT as its substrates.

**Synthesis of purine derivatives of 4'-C-cyano-2'-deoxynucleoside and 4'-C-ethyl-2'-deoxynucleoside from 2'-deoxynucleosides.** TFA = trifluoroacetic acid; DMF = dimethylformamide; rt = room temperature.

**Scheme 2. Synthetic scheme of purine derivatives of 4'-C-cyano-2'-deoxynucleoside and 4'-C-ethyl-2'-deoxynucleoside from 2'-deoxynucleosides.** TFA = trifluoroacetic acid; DMF = dimethylformamide; rt = room temperature.
The biological activities of the purine derivatives of 4′-C-acyano-2′-deoxy (4′CNdN) and 4′-C-ethynyl-2′-deoxynucleosides are summarized as follows (Table 3):

1. Some of the purine derivatives of 4′CNdN have high anti-HIV activity, but none of them gives an acceptable SI.
2. All the purine derivatives of 4′EdN have both high anti-HIV activity and an acceptable SI.

### Table 3. Anti-HIV activity of purine derivatives of 4′-C-acyano-2′-deoxy (4′CNdN) and 4′-C-ethynyl-2′-deoxynucleosides

| Structure | Base | EC50 (µM)a | CC50 (µM) | S.I. |
|----------|------|------------|-----------|------|
| ![Structure](image1.png) | A    | 0.051      | 12        | 235  |
|          | I    | 0.051      | 23        | 451  |
|          | 2AAb | 0.00079    | 0.034     | 43   |
|          | G    | 0.000188   | 0.034     | 181  |
| ![Structure](image2.png) | A    | 0.098      | 16        | 1630 |
|          | I    | 0.15       | 216       | 1440 |
|          | 2AA  | 0.0003     | 0.82      | 2733 |
|          | G    | 0.0014     | 1.5       | 975  |
| AZT      |      | 0.0032     | 29.4      | 9190 |

a) Anti-HIV activity was determined by MTT assay. MT-4 cells and HIV-1_LAI were employed.
b) 2-aminoadenine.

### Table 4. Anti-HIV activity of selected 4′-C-substituted-2′-deoxynucleoside against wild type HIV and drug-resistant HIVs

| Compound | EC50 (µM)a | HXBb | KH65R | L74V | 41/215 | M184V | M184I | 41/69 | 125/SG | MDRc | Y181C | CC50 (µM) |
|----------|------------|------|-------|------|--------|-------|-------|-------|--------|-------|--------|-----------|
| 4′EdC    | 0.0012     | 0.0008 | 0.0013 | 0.006 | 0.0004 | 0.0025 | 0.0015 | 0.0007 | 0.0012 | 0.0021 | >200   | 59        |
| 4′EaraC  | 0.0071     | 0.015  | 0.026  | 0.026 | 0.071  | 0.48   | 0.17   | 0.079  | 0.016  | >200   | >200   | 59        |
| 4′MdC    | 0.0058     | 0.0071 | 0.0062 | ND   | 0.0004 | 0.74   | ND     | 0.0003 | ND     | >200   | >200   | 59        |
| 4′EdA    | 0.008      | 0.0033 | 0.004  | 0.012 | 0.047  | 0.022  | 0.065  | 0.0068 | 0.011  | >200   | >200   | 59        |
| 4′Ed2AA  | 0.0014     | 0.00035 | 0.0007 | 0.0017 | 0.0059 | 0.0027 | 0.0041 | 0.0001 | 0.0008 | >200   | >200   | 59        |
| 4′EdG    | 0.007      | 0.001  | 0.0012 | 0.0019 | 0.008  | 0.0041 | 0.0068 | 0.0048 | 0.01   | 52     |        | 59        |
| 4′EdI    | 0.81       | 0.25   | 0.61   | 1.3   | 1.6    | 1.5    | 2.2    | 0.51   | ND     | >200   | >200   | 59        |
| AZT      | 0.022      | 0.02   | 0.02   | 0.3   | 0.01   | 0.017  | 1.6    | 15.3   | 0.014  | >100   | >100   | >100      |
| 3TC      | 0.71       | ND     | ND     | ND    | >100   | >100   | 9.9    | 1.1    | ND     | >100   | >100   | >100      |
| ddC      | 0.2        | 3.0    | 1.5    | 2.2   | ND     | 1.3    | 5.5    | ND     | >100   | >100   | >100   | >100      |
| ddI      | 3.9        | 12.7   | 19.5   | 3.6   | 10.1   | ND     | 12.2   | 25     | ND     | >100   | >100   | >100      |

a) Anti-HIV activity was determined with MIG assay. ND: not determined. b) wild type HIV. c) multidrug-resistant HIV.

The biological activities of the purine derivatives of 4′CNdN and 4′EdN are summarized as follows (Table 3):

1. Some of the purine derivatives of 4′CNdN have high anti-HIV activity, but none of them gives an acceptable SI.
2. All the purine derivatives of 4′EdN have both high anti-HIV activity and an acceptable SI.

### Anti-HIV activity of 4′SdNs against drug-resistant HIV mutants. 13,14,25  
Many 4′SdNs showed very high anti-HIV activity against wild-type HIV. However, the most important point of our study is whether they are active against drug-resistant HIV-mutants. The anti-HIV activity of selected 4′SdNs against HIV mutants resistant to various NRTIs is listed in Table 4.

It is noteworthy that the three cytidine derivatives maintained their activity against the drug-resistant HIV mutants, although the activity of 4′-C-ethynyl D-arabino-furanosyl cytosine (4′EaraC) and 4′MdC decreased significantly against M184V, M184I, and...
Table 5. Toxicity of purine derivatives of 4′-C-ethyl-2′-deoxynucleosides to mice

| Intravenous administration | Oral administration |
|----------------------------|---------------------|
| Dose (mg/Kg) | Mortality (%) | Dose (mg/Kg) | Mortality (%) |
| 4′EdA | 100 | 0 | 100 | 0 |
|       | 10 | 0 | 10 | 0 |
| and | 3 | 0 | 3 | 0 |
| 4′EdI | 1 | 0 | 1 | 0 |
| 4′Ed2AA | 100 | 100 (1 day) | 100 | 100 (1 day) |
|       | 10 | 100 (2 days) | 10 | 100 (2 days) |
|       | 3 | 0 | 3 | 100 (2 days) |
|       | 1 | 0 | 1 | 0 |
| 4′EdG | 100 | 100 (1 day) | 100 | 100 (1 day) |
|       | 10 | 100 (2 days) | 10 | 100 (4 days) |
|       | 3 | 100 (4 days) | 3 | 100 (4 days) |
|       | 1 | 0 | 1 | 0 |

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

41/69/125/SQ. The three purine derivatives, 2′-deoxy-4′-C-ethynyladenosine (4′EdA), 2′-deoxy-4′-C-ethyl-2-aminoadenosine (4′Ed2AA), and 2′-deoxy-4′-C-ethylguanosine (4′EdG) except for 2′-deoxy-4′-C-ethynylinosine (4′EdI) were highly potent against all drug-resistant HIV-mutants (4′EdI) was much less active than the former three derivatives, especially against M184V. Additionally, the three were also active against a non-nucleoside reverse transcriptase inhibitor-resistant Y181C. Further, the three purine derivatives were highly potent against the HIVs isolated from seven heavily drug-experienced patients with acquired immune deficiency syndrome (AIDS) as efficiently as against wild-type HIV.

These results let us suppose that the three purine 4′EdNs could even prevent the emergence of drug-resistant HIVs. It should be noted that 4′EdG showed toxicity to Hela cells at 52 μM.

Mouse toxicity of purine 4′EdNs. Because the three purine derivatives of 4′EdNs showed high activity against all HIVs and acceptable SIs, the mouse toxicity of these 4′EdNs was next examined (Table 5).

All eight mice survived after a single dosage of 3–100 mg kg⁻¹ of 4′EdA and 4′EdI by both intravenous and oral administrations, but all mice died after a single dosage of 3 mg kg⁻¹ of 4′Ed2AA and 4′EdG irrespective of the administration method (Table 5). Thus, it seemed that 4′EA and 4′EI were not toxic, but 4′E2AA and 4′EdG were highly toxic.

However, in mice, it was found that 4′EdA and 4′Ed2AA were easily converted to 4′EdI and 4′EdG, respectively, by adenosine deaminase. These results showed that the actual toxicity of 4′EdA and 4′Ed2AA to animals is hard to estimate.

Anti-HIV activity of 4′EdA derivatives stable to adenosine deaminase. The fact that both 4′EdA and 4′Ed2AA are deaminated by adenosine deaminase prompted us to prepare 4′EdA derivatives stable to the enzyme. It has been known that the adenine derivatives having a halogen atom at the 2-position of the base are stable to adenosine deaminase. Therefore, at first, 4′Ed2FA (Fig. 1) was synthesized and evaluated for anti-HIV activity and stability to both adenosine deaminase and acidic conditions.

Because 4′Ed2FA is a nucleoside derivative modified at two positions (4′-position and 2-position) of physiologic 2′-deoxyadenosine, the toxicity of 4′Ed2FA is expected to be lower than that of 4′EdA. As shown in Table 6, 4′Ed2FA is highly potent against all HIVs including multidrug-resistant and M184V HIV mutants and has an acceptable SI (110,000). Expectedly, 4′Ed2FA was completely stable to adenosine deaminase under the conditions where 4′EdA was completely deaminated in 60 min and, further, fairly stable under acidic conditions. Thus, in 120 min only a small part (3%) of 4′Ed2FA was decomposed under the acidic conditions of gastric juices (pH 1.06) at 24 °C, while 2′,3′-dideoxyadenosine (ddA) was completely decomposed in 5 min.
Appearance of two papers claiming that 3′-OH of 4′EdNs is the cause of the toxicity of 4′EdNs. While we were working on our project, two papers on the role of 3′-OH of 4′SdNs appeared in which the authors claimed that 3′-OH was the cause of the toxicity of 4′SdNs. Tanaka and coworkers reported\(^\text{29}\) that 4′-C-ethyl-2′,3′-dideoxy-3′-deoxythymydine (4′Ed4T) was more potent against wild-type HIV and multi-drug resistant HIV mutant but a little less potent against M184V-mutant and much less than d4T. Further, they claimed that 3′-OH of 4′SdNs was the cause of the toxicity.

In contrast, Marquez and coworkers reported\(^\text{33}\) that 3′-OH of 4′SdNs played an important role in the phosphorylation of 5′-OH by cellular kinases, but was the cause of the toxicity of 4′SdNs. This determination was based on their results that 4′-C-ethyl-2′,3′-dideoxyxycytidine (4′EddC) was inactive against HIV in cellular systems, but its 5′-O-triphosphate (4′EddCTP) was more active than 3′-azido-3′-deoxythymidine-5′-O-phosphate (AZTTP) against the RT of wild-type HIV. They also reported that 4′EddCTP was much less active against RT of the M184V mutant than against the RT of wild-type HIV. In addition, the L-isomer of 4′EddCTP was not active against the RT of the M184V mutant.

Anti-HIV activity of 2′,3′-dideoxy (dd-) and 2′,3′-didehydrodideoxy (d4) analogs of 4′Ed2FA. The two papers previously cited caused us to synthesize the dd- and d4-analogs of 4′Ed2FA and evaluate their anti-HIV activity.\(^\text{33}\) The anti-HIV activities of 2′,3′-dideoxy-4′-C-ethyl-2′-fluoroadenosine (4′Edd2FA) and 2′,3′-didehydrodideoxy-4′-C-ethyl-2′-fluoroadenosine (4′Edd4FA) together with that of 2′-deoxy-4′-C-ethyl-2′-chloroadenosine (4′Ed2ClA) are listed in Table 7.

Although both 4′Ed2FA and 4′Edd2FA showed some activity against wild-type HIV, they significantly lost any activity against drug-resistant HIVs. 4′Ed2ClA is highly active against all HIVs, however, its activity is lower than that of 4′Ed2FA. These results indicated that the 3′-OH played important roles not only for the phosphorylation of 5′-OH, but also for the activity against drug-resistant HIVs. Further, these results together with those reported by Tanaka and coworkers\(^\text{29}\) indicated that the 4′-C-ethyl group played an important role for the anti-HIV activity. Recently, it was reported that the active center of the RT of HIVs has a narrow lipophilic cavity to accept preferably the ethynyl group, thus 4′-C-ethyl nucleoside derivatives have potent anti-HIV activity.\(^\text{34}\)

| HIV-1 | Compound | EC\(_{50}\) (nM) | CC\(_{50}\) (nM) | SI |
|-------|----------|----------------|----------------|----|
| Wild-type | 4′Ed2FA | 0.068 | 7,500 | 110,000 |
|        | AZT     | 3.2    | 29,400 | 9,190 |
| M184V  | 4′Ed2FA | 3.1    | 900   | 2,600 |
|        | AZT     | 16.0   |       |      |
| MDR    | 4′Ed2FA | 0.15   |       |      |
|        | AZT     | 15,300.00 |       |      |

| 4′Ed2FA |
|---------|
| m.p. (decomp.) |
| 224.4–224.6°C |
| [\(\beta\)]\(_{D}\) +4.64 |
| (c = 0.5, DMSO) |

While we were working on our project, two papers previously cited caused us to synthesize the dd- and d4-analogs of 4′Ed2FA and evaluate their anti-HIV activity.\(^\text{33}\) The anti-HIV activities of 2′,3′-dideoxy-4′-C-ethyl-2′-fluoroadenosine (4′Edd2FA) and 2′,3′-didehydrodideoxy-4′-C-ethyl-2′-fluoroadenosine (4′Edd4FA) together with that of 2′-deoxy-4′-C-ethyl-2′-chloroadenosine (4′Ed2ClA) are listed in Table 7.

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| 4′Ed2FA |
|---------|
| m.p. (decomp.) |
| 224.4–224.6°C |
| [\(\beta\)]\(_{D}\) +4.64 |
| (c = 0.5, DMSO) |

Table 6. Anti-HIV activity of 2′-deoxy-4′-C-ethyl-2′-fluoroadenosine (4′Ed2FA)
metabolites, (4′Ed2FA-monophosphate, 4′Ed2FA-diphosphate, and 4′Ed2FATP) increased proportionately with an increase in the concentration of intracellular 4′Ed2FA, while compared to AZT-diphosphate and AZTTP, only AZT-monophosphate markedly increased with an increase in intracellular AZT concentration. The intracellular half-life (T1/2) of 4′Ed2FATP was ~18 h in complete expansion media (CEM) cells, MT4 cells, and multinuclear activation of galactosidase indicator (MAGI)-CCR5 cells (T1/2 of AZTTP was 3 h). About 50% of the cells were protected against the infection of HIV for 24 h after removal of extracellular 4′Ed2FA in both MT4 cells and MAGI cells cultured in the presence of 0.1 μM of 4′Ed2FA.[30,31,36] These results indicate that 4′Ed2FA, 2′-deoxy-4′-C-ethyl-2-fluoroadenosine-5-O-diphosphate (4′Ed2FADP), and 4′Ed2FATP are very stable against intracellular enzymatic catabolism.

### Summary

A study of the synthesis and biological evaluation of 4′SdNs was conducted according to the proposed working hypotheses based on the fundamentals of both organic chemistry and biochemistry. All scientific evidences proved the validity of the working hypotheses and resulted in the development of 4′Ed2FA, which is highly potent against all HIVs, is stable to intracellular enzymatic catabolism and acidic degradation, has a very long

Table 7. Anti-HIV activity of 4′-C-substituted-2′-deoxy-2-haloadenosines

| Compound       | Anti-HIV activity (MAGI assay, µg) | HIV-1n Cl | HIV-1MDR | HIV-1M184V | SI     |
|----------------|-----------------------------------|----------|----------|------------|--------|
| 4′Ed2FA        | 0.00020                           | 0.00014  | 0.01     | 110,000    |        |
| 4′Ed2CIA       | 0.0019                            | 0.0084   | 0.13     | 330,000    |        |
| 4′Ed42FA       | 0.80                              | 0.15     | 97       | >100       |        |
| 4′Ed4dFA       | 0.94                              | 8.7      | >100     | >50,000    |        |
| AZT            | 0.17                              | 74.3     | 17       | >50,000    |        |
| 3TC            | 1.0                               | 2.8      | >100     | >50,000    |        |
| 4′Ed4T         | 1.5                               | 1.1      | 17       | >50,000    |        |
| d4T            | 7.6                               | 64       | 5.6      |           |        |

MAGI = multinuclear activation of galactosidase indicator; HIV = human immunodeficiency virus; AZT = 3′-azido-3′-deoxythymidine; 3TC = 2′,3′-dideoxy-3′-thia-L-cytidine, d4T = 2′,3′-didehydrodideoxythymidine.

Table 8. Toxicity of 2′-deoxy-4′-C-ethynyl-2-fluoroadenosine (4′Ed2FA) after a single dosage to ICR mice

| Dose (mg Kg⁻¹) | Survivors/total | p.o | i.v. |
|----------------|-----------------|-----|------|
| Placebo        | 8/8             |     |      |
| 1              | 8/8             | 8/8 |     |
| 3              | 8/8             | 8/8 |     |
| 10             | 8/8             | 8/8 |     |
| 30             | 8/8             | 8/8 |     |
| 100            | 8/8             | 8/8 |     |

Fig. 4. Body weight change of mice after a single dosage of 2′-deoxy-4′-C-ethyl-2-fluoroadenosin, administrated orally or intravenously to ICR mice.
intracellular T₁/₂, does not greatly inhibit DNA polymerase γ, and does not have acute mouse toxicity.

These results strongly suggest that 4′Ed2FA deserves further study for the development of a highly potent therapeutic agent for HIV infection (AIDS), and which may solve the problems of the existing HAART.

It should be noted that 4′Ed2CIA is also highly potent against all HIVs, though it is a little less potent than 4′Ed2FA, and has better SI than 4′Ed2FA. Therefore, 4′Ed2CIA deserves also for further study.

The above study together with the recent studies by others on the development of anti-HCV active and/or anti-tumor active modified nucleosides which are chain-terminators of RNA polymerase, prompted the author to propose the structure of modified nucleosides expected to have high antiviral activity and low toxicity.

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Profile

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