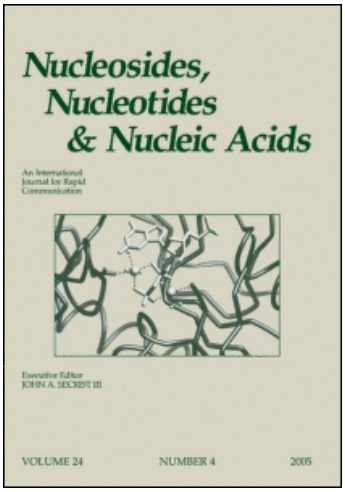

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Ohrui, Hiroshi(2009) 'A Proposed Structure of Modified Nucleosides Expected to Have High Antiviral Activity and Low Toxicity', Nucleosides, Nucleotides and Nucleic Acids, 28: 8, 772 — 775
To link to this Article: DOI: 10.1080/15257770903155675
URL: http://dx.doi.org/10.1080/15257770903155675

Nucleosides, Nucleotides and Nucleic Acids, 28:772–775, 2009

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770903155675

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A PROPOSED STRUCTURE OF MODIFIED NUCLEOSIDES EXPECTED TO HAVE HIGH ANTIVIRAL ACTIVITY AND LOW TOXICITY

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□ *This article describes modified nucleosides with deactivated 3′-OH and modified at two or more positions. These are expected to have high antiviral activity as well as have low toxicity.*

Keywords Antiviral; low toxicity; modified nucleoside; chain-terminator; DNA and RNA polymerase

Many emerging viral infectious diseases (e.g., AIDS, influenza, West Nile Virus, SARS, etc.) are causing major threats to global public health. Therefore, the development of antiviral drugs that are highly active and have low toxicity is urgent. In this article, the structure of a modified nucleoside expected to have high antiviral activity and low toxicity is proposed. The proposal is based mainly on the results of our study[1,2] on the development of high anti-HIV activity and low toxicity nucleosides, 2′-deoxy-4′-*C*-ethynyl-2-fluoroadenosine(**1**) and its 2-chloro congener(**2**), which are 2′-deoxyadenosines modified at two sites and have prevented the emergence of resistant HIV mutants for more than 4 years,[1–4] and Merck's study[5] on the development of the high anti-HCV activity and low toxicity plural site-modified adenosine, 7-deaza-2′-*C*-methyl-7-fluoro-adenosine(**6**) (Figure 1).

In our study on the development of high anti-HIV activity and low toxicity modified nucleosides, the following three working hypotheses were proposed.[1]

a. The way to prevent the emergence of resistant HIV mutants. A 4′-*C*-substituted-2′-deoxy nucleoside (**4′SdN**) was designed to be the chain-terminator of reverse transcriptase and prevent the emergence of

Received 25 November 2008; accepted 17 June 2009.
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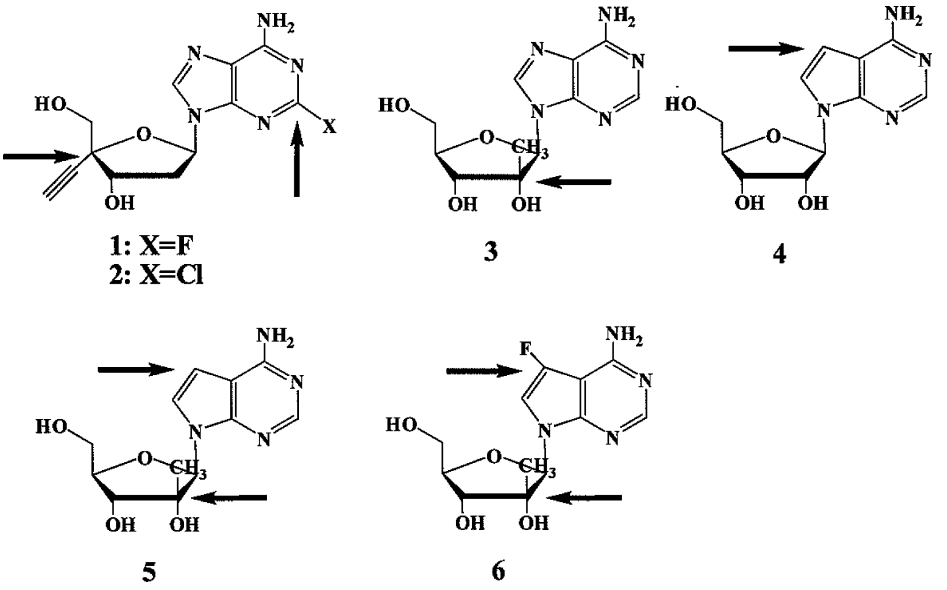

FIGURE 1 The structures of compounds **1–9**.

resistant HIV mutants; the importance of the 3′-OH to prevent the emergence of resistant HIV mutants was emphasized.

b. The way to decrease the toxicity of nucleosides. If DNA polymerases mistake **4′SdN** for a physiologic 2′-deoxynucleoside (**dN**), **4′SdN** should be highly toxic. However, there will be a chance of decreasing the toxicity of **4′SdN** by additional modification.

c. The way to provide nucleosides with resistance to both enzymatic and acidic cleavage of the glycosyl bond. The conformational change of furanose ring of nucleoside by the introduction of a substituent at 4′-position will make it difficult for the lone pair of the ring oxygen to form an oxocarbonium ion. Therefore, the introduction of a substituent at 4′-position will provide the nucleoside with resistance to both enzymatic and acidic cleavage of the glycosyl bond.

The studies based on these hypotheses have proved the validity of all the three hypotheses and resulted in the development of the high anti-HIV activity and low toxicity two-site modified nucleosides **1** and **2.**[1–4] It was also found by these studies that the two-site modified 2′-deoxy-4′-*C*-ethynyl-5-fluorocytidine is much less toxic than the one-site modified 2′-deoxy-4′-*C*-ethynyl cytidine.[1]

On the other hand, Olsen and his coworkers[5] synthesized the hybrid, 7-deaza-2′-*C*-methyl adenosine(**5**), derived from the one-site modified highly active and toxic nucleosides, 2′-*C*-methyl adenosine(**3**) and 7-deazaadenosine(**4**), and found that the two-site

modified nucleoside **5** has high anti-HCV activity and low toxicity. Finally, they developed a more anti-HCV active and less toxic plural site-modified nucleoside, 7-deaza-2′-*C*-methyl-7-fluoroadenosine(**6**)[5] (Figure1).

These results indicated that the introduction of a substituent at the 2′ or 4′ position of a nucleoside deactivates its 3′-hydroxy group and makes the 2′-*C* and 4′-*C*-substituted nucleosides into chain terminators of DNA and RNA polymerases. Further, the results indicated that human DNA and RNA polymerases are more sophisticated than viral polymerases in the point that they can differentiate the nucleosides modified at two or more sites of physiologic nucleosides from physiologic ones and do not accept the modified nucleosides as their substrates, but viral polymerases cannot do it. Thus, they indicated that by taking advantage of the difference of the substrate selectivity between human DNA and RNA polymerases and viral polymerases, it will be possible to develop antiviral modified nucleosides that are highly active and lowly toxic.

Besides, Hattori et al.[6] reported that 3′-*C*-ethynyl-D-*ribo*nucleosides are chain terminators of RNA polymerases and therefore antitumor active. These results indicate that the introduction of a substituent at the 2′, 3′, or 4′ position of a nucleoside deactivates its 3′-OH and makes the nucleosides into a chain terminator of DNA or RNA polymerases.

Based on the above findings, the structures of modified nucleosides expected to have high antiviral activity and low toxicity could be deduced as follows.

1. To be a chain terminator of DNA and RNA polymerase, the modified nucleosides should have a deactivated 3′-OH. The introduction of a substituent at the 2′ or 4′ position that makes the 3′-OH into a sterically hindered unreactive neopentyl type secondary alcohol and at the 3′ position that makes the 3′-OH into an unreactive tertiary alcohol will achieve the purpose.
2. To have high antiviral activity and prevent the emergence of resistant viral mutants, the modified nucleosides should have a structure resembling those of physiologic nucleosides as closely as possible so that viral polymerases mistake them for physiologic nucleosides. Therefore, the sterically less-demanding substituent will be more preferred as was shown in the case of **4′SdN**.[1] The hydroxyl groups of *D*-ribofuranose and 2′-deoxy-*D*-ribofuranose moieties will be needed. It should be noted that a fluorine atom, isostere of a hydroxyl group, could be a replacement for the hydroxyl group, as was shown in the case of anti-HCV active 2′-α-fluoro-2′-β-methylcytidine(**7**).[7]
3. Additional modification of a toxic modified nucleoside will decrease the toxicity of the nucleoside. Here, it should be noted that one-site modified highly anti-HCV active 4′-*C*-azidocytidine(**8**) did not show significant

cytotoxic or cytostatic properties[8] and is being investigated in the clinic for HCV, though highly anti-HIV active one-position modified nucleosides including 4′-*C*-azido-2′-deoxycytidine(**9**),[9] showed significant toxicity.[1,9]

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