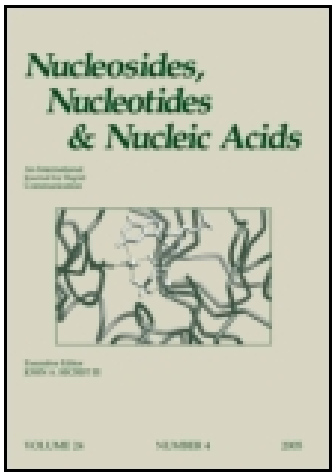

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

2′-Fluoro-4′-thio-2′,3′-unsaturated Nucleosides: Anti-HIV Activity, Resistance Profile, and Molecular Modeling Studies

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Published online: 31 Aug 2006.

To cite this article: Youhoon Chong , Hyunah Choo , Raymond F. Schinazi & Chung K. Chu (2003) 2′-Fluoro-4′-thio-2′,3′-unsaturated Nucleosides: Anti-HIV Activity, Resistance Profile, and Molecular Modeling Studies, Nucleosides, Nucleotides and Nucleic Acids, 22:5-8, 611-615, DOI: 10.1081/NCN-120021965

To link to this article: http://dx.doi.org/10.1081/NCN-120021965

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 611–615, 2003

2′-Fluoro-4′-thio-2′,3′-unsaturated Nucleosides: Anti-HIV Activity, Resistance Profile, and Molecular Modeling Studies

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ABSTRACT

Both D- and L-2′-fluoro-4′-thio-2′,3′-unsaturated nucleosides were synthesized and their anti-HIV activity against the drug sensitive virus and lamivudine-resistant mutant (M184V) were evaluated. In vitro antiviral evaluation indicated that the L-isomers are more potent than the D-isomers, but unfortunately all were cross-resistant with 3TC. Molecular modeling studies revealed that the unnatural sugar moiety of the L-nucleosides as well as 4′-sulfur atom of the D-isomer has a steric conflict with the bulky side chain of valine 184, resulting in cross-resistance.

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DOI: 10.1081/NCN-120021965
1525-7770 (Print); 1532-2335 (Online)

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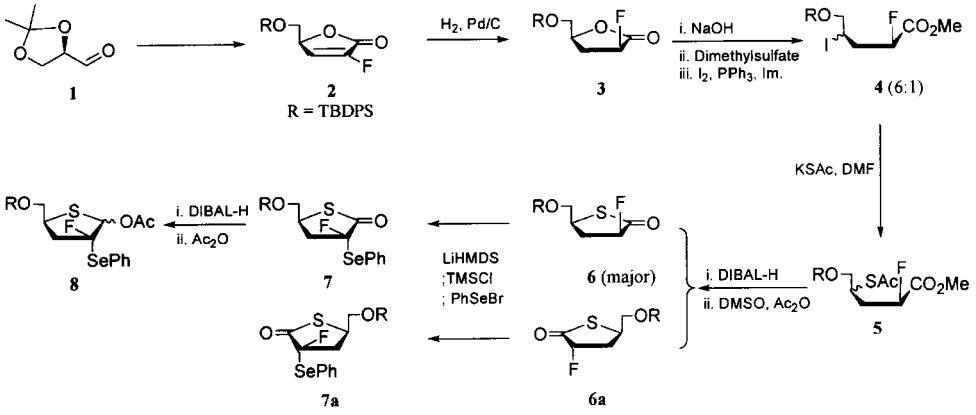
The past decade has witnessed the discovery of a class of 2′,3′-unsaturated nucleosides such as d4T,[1,2] L-d4C,[3,4] L-d4FC[3,4] and abacavir[5,6] as interesting therapeutic agents for anti-HIV therapy because of their potent antiviral activity. However, these unsaturated nucleosides are inheritably unstable in acidic environment, which reduce the bioavailability and antiviral efficacy. In order to alleviate these drawbacks, 2′-F moiety was introduced to increase acid stability while maintaining the antiviral potency of several purine and pyrimidine nucleosides.[7] Therefore, agents containing the 2′,3′-unsaturated sugar moiety with 2′-fluoro substitution has become one of the rational targets in search for safe, effective and chemically stable antiviral agents. Thus, it was of interest to further modify the structure to 2′F-4′S-2′,3′-unsaturated nucleosides with isosteric replacement of the 4′-oxygen by a sulfur atom.

Both D- and L-isomers were synthesized starting from D- and L-glyceraldehyde derivatives, respectively (Sch. 1 and 2). The 2-fluorobutenolactone, which can be readily prepared from glyceraldehyde derivative, was converted to 2-fluoro-4-thiolactone in four steps, from which the key intermediate, 2-fluoro-4-thio-1-acetate was prepared for condensation with various pyrimidine and purine bases.

The antiviral activity of the synthesized compounds were evaluated against HIV-1 in human peripheral blood mononuclear (PBM) cells, among which the cytidine, 5-fluorocytidine, adenosine and 2-fluoroadenosine analogues showed moderate to potent anti-HIV activities. The isosteric substitution of the 4′-oxygen with a 4′-sulfur, however, resulted in cross-resistance with the M184V mutant. The cytidine and 5-fluorocytidine analogues showed significantly decreased antiviral activity against the clinically important lamivudine-resistant variants (HIV-1_{M184V}), whereas the corresponding D-2′-Fd4 nucleosides showed only limited cross-resistance.

According to our molecular modeling studies using the crystal structure of HIV-1 RT, we found that the M184V mutation in HIV-1 RT causes a serious problem in positioning the unnatural L-configured nucleoside triphosphates at the active site because the side chain of Val184 tend to occupy the space where the sugar moiety



Scheme 1. Synthesis of the key intermediate 2-fluoro-4-thio-2-phenylselenyl-1-acetate 8.

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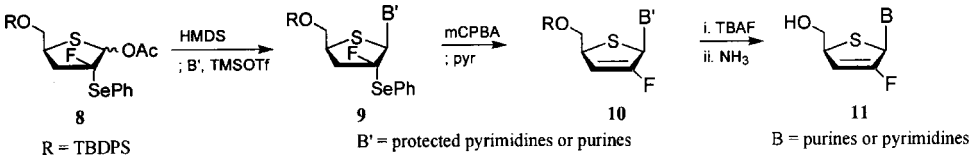

Scheme 2. Synthesis of various pyrimidine and purine nucleosides.

of L-2′F-2′,3′-unsaturated nucleosides projected (Fig. 1). The resulting steric hindrance destabilized the L-2′F-2′,3′-unsaturated nucleoside triphosphate/RT complex. On the other hand, the structure of D-2′F-d4CTP/RT complex does not show any significant steric crash between Val184 and the sugar moiety of nucleoside triphosphate because the natural D-configured sugar moiety was located at the opposite side (Fig. 1).

The potent anti-HIV-1_{M184V} activity of the cytidine analog, D-2′F-d4C, supports this result. In this context, it is interesting that M184V RT is cross-resistant to the natural D-2′F-4′S-d4C. Molecular modeling studies demonstrated that the larger van der Waals radius as well as the close proximity to Met184 of the 4′-sulfur atom of D-2′-F-4′-Sd4C may be the reasons for the decreased antiviral potency of synthesized 4′-thio nucleosides against the lamivudine-resistant variants (HIV-1_{M184V}). The energy-minimized structures[8] of D-2′-Fd4C and D-2′-F-4′-Sd4C bound to HIV-1 RT shows that nucleoside inhibitors are located in a well-defined binding pocket formed by Arg72, Met184 and 3′-OH pocket residues. However, because of its out-of-plane location and large van der Waals radius, the 4′-sulfur atom in the D-2′-F-4′-Sd4N sugar moiety is in close contact to Met184 (Fig. 2). As a result, the mutation of Met184 to Val184, which has a bulky side chain, results in a significant steric hindrance between the 4′-sulfur atom in the D-2′-F-4′-Sd4N sugar moiety and the side chain of Val184, which might be one of the reasons for the high cross-resistance of HIV-1_{M184V} to D-2′-F-4′-Sd4C and D-2′-F-4′-Sd4FC.

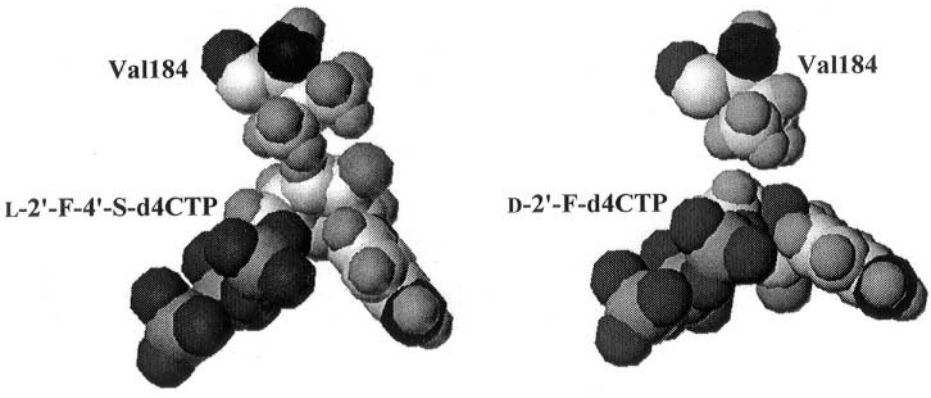

Figure 1. L-2′-F-4'-S-d4CTP/RT complex after mutation from M184 to V184 showing a steric hindrance between the sugar moiety of L-2′-F-4′-S-d4CTP and the side chain of V184 (left). D-2′-F-d4CTP/RT complex after mutation showing no steric hindrance (right).

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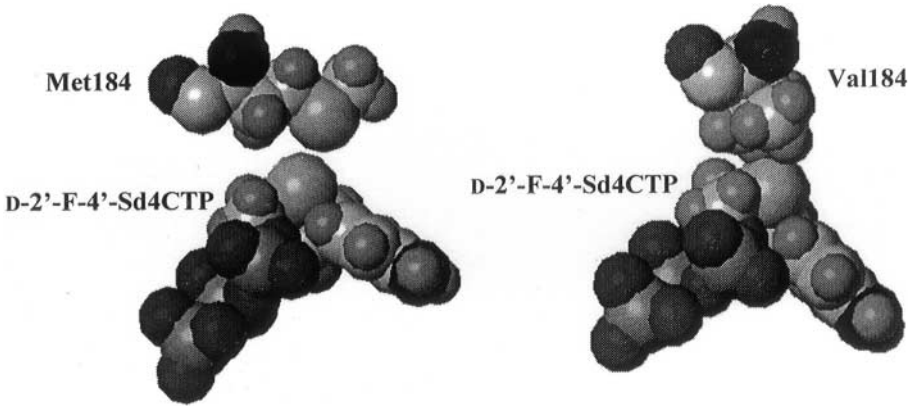

Figure 2. Energy minimized structure of D-2′-F-4′-Sd4C, complexed with the wild-type HIV-1 RT (left) and M184V mutant RT (right).

Table 1. Activity of selected nucleosides against lamivudine-resistant virus (HIV-1_{M184V}) in human PBM cells.

Compound	xxBRU (EC_{90}, μM)	M184V (EC_{90}, μM)	FI[a]
D-2′-F-4′-Sd4C[b]	5.0	≈125	25
L-2′-F-4′-Sd4C[b]	1.4	>100	>100
D-2′-F-d4C[b]	8.0	1.8	0.2
L-2′-F-d4C[b]	2.1	>100	>100
AZT	0.01	0.003	0.3
3TC	0.08	535	6,688

In summary, the synthesized 2′-fluoro-4′-thio-2′,3′-unsaturated nucleosides showed potent anti-HIV activity. In vitro antiviral evaluation indicated that the L-isomers are more potent than the D-isomers, but unfortunately all were cross-resistant with 3TC. Molecular modeling studies showed that the steric hindrance of the bulky side chain of valine 184 with unnatural sugar moiety of the L-nucleoside as well as 4′-sulfur atom of the D-isomer can be one of the reasons for the crossresistance.

ACKNOWLEDGMENTS

This research was supported by the U.S. Public Health Service Grant (AI32351 & AI25899) from the National Institutes of Health and the Department of Veterans Affairs.

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