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Synthesis and anti-HIV activity of 2′-deoxy-2′-fluoro-4′-*C*-ethynyl nucleoside analogues

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Abstract

Based on the favorable antiviral profiles of 4′-substituted nucleosides, novel 1-(2′-deoxy-2′-fluoro-4′-*C*-ethynyl-*β*-D-arabinofuranosyl)-uracil (**1a**), -thymine (**1b**), and – cytosine (**2**) analogues were synthesized. Compounds **1b** and **2** exhibited potent anti-HIV-1 activity with IC_{50} values of 86 and 1.34 nM, respectively, without significant cytotoxicity. Compound **2** was 35-fold more potent than AZT against wild-type virus, and also retained nanomolar antiviral activity against resistant strains, NL4-3(K101E) and RTMDR. Thus, **2** merits further development as a novel NRTI drug.

Keywords

2′-Deoxy-2′-fluoro-4′-*C*-ethynyl nucleosides; anti-HIV activity

Human immunodeficiency virus type-1 (HIV-1) infection affects approximately 40 million individuals worldwide. The HIV-1 reverse transcriptase (RT) enzyme is responsible for converting the genomic single-strand RNA of HIV into double-strand DNA; therefore, it is a major target for anti-HIV drug discovery.1 HIV-1 RT inhibitors fall into two classes: nucleoside RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs). Since the discovery of zidovudine (AZT),2 many nucleoside analogues have been designed and synthesized. Currently, seven NRTIs have been approved by the US FDA for the treatment of HIV infections, including zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC), and emtricitabine [(−)FTC].3 These drugs block the synthesis of double-strand viral DNA from the newly made single-strand DNA, and thus terminate or abort the polymerization process catalyzed by HIV RT.4

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Supplementary Data:
Supplementary data (synthesis, ^{1}H NMR data, bioassay methods, and HPLC/mass spectral purity analyses of final compounds) associated with this article can be found, in the online version, at doi:.

Emerging drug-resistant viral strains as well as long-term toxicity are the main problems in current antiviral chemotherapy.5 Although there are many RT inhibitor-resistant viral strains generated clinically, NRTIs remain the most potent and efficient antiviral drugs and are still used as first-line clinical therapies. Therefore, structurally modified novel nucleosides are needed to overcome the treatment drawbacks.

From a structure-activity relationship (SAR) standpoint, the emergence of highly drug-resistant HIV-1 variants suggests that RT is capable of discriminating physiologic 2′-deoxynucleosides (dNs) from 2′,3′-dideoxynucleosides (ddNs), at least by recognizing the difference in the 3′-position.6 To overcome the resistance issues, novel nucleoside analogues that retain the 3′-*α*-OH were designed in our study to exert antiviral activity against HIV-1. RT might not be able to discriminate such analogues or, if it does, it may do so less effectively. In addition, a fluorine moiety was also incorporated into the 2′ position of the ribose. Thus, in our continued research on NRTIs,7‒9 we report herein the synthesis of 2′-deoxy-2′-fluoro-4′-*C*-ethynyl nucleoside analogues (Figure 1) and their potent anti-HIV-1 activity.

1-(2′-Deoxy-2′-fluoro-4′-*C*-ethynyl-*β*-D-arabinofuranosyl)-uracil (**1a**) and -thymine (**1b**) were synthesized from uracil (**5a**) and thymine (**5b**) in 15 steps. Compound **18a**, the precursor to **1a**, was converted to the corresponding 4′-*C*-ethynyl-cytosine analogue **2** in two steps (Scheme 1).

2-Deoxy-2-fluoro-1,3,5-*O*-tribenzoyl-D-arabinofuranoside (**3**) was first converted to 1′-α-bromide **4** with HBr-HOAc in 45% yield. Bromide **4** was then glycosylated with silylated pyrimidines **5** and **6** in $CHCl_3$ to give the desired dibenzoylated β-nucleoside analogues **7a** and **7b** in over 80% yield. Deprotection of the benzoyl groups with saturated methanolic ammonia afforded 2′-deoxy-2′-fluoro-*β*-D-arabinofuranosyl-uracil (**8a**) and -thymine (**8b**) in 90% and 97% yields, respectively. Compounds **8a** and **8b** were reacted sequentially with 4,4′-dimethoxytrityl chloride (DMTrCl) and *tert*-butyldimethylsilyl chloride (TBDMSCl) to protect the 5′- and 3′-hydroxy groups, respectively. The 5′-DMTr protecting group of the resulting compounds **10a** and **10b** was then selectively removed with trifluoroacetic acid (TFA) in CH_2Cl_2 to provide the 3′-silyated analogues **11a** and **11b**. The 5′-hydroxymethyl group of **11a** and **11b** was oxidized to an aldehyde by Pfitzner-Moffatt oxidation. The resulting compounds **12a** and **12b** were then treated with formaldehyde under basic conditions in 1,4-dioxane, followed by sodium borohydride, to yield the corresponding 4′-α-*C*-hydroxymethyl analogues 13a and 13b. To differentiate the two hydroxymethyl groups of 13a and 13b, the 4′-*α*-hydroxymethyl group was selectively protected with DMTr, and the remaining *β*-hydroxymethyl group was then protected with TBDMS.10 Compounds **14a** and **14b** were obtained in high yields of 71% and 95%. Selective removal of the DMTr group with TFA afforded **16a** and **16b**, which now had one *α*-C-hydroxymethyl group open for further modification. Oxidation of the 4′-*α*-hydroxymethyl group of **16a** and **16b** to the formyl derivatives, followed by Wittig olefination with chloromethyl triphenyl phosphonium chloride, afforded chlorovinyl derivatives **17a** and **17b**. The chlorovinyl group of these compound**s** was directly converted into an ethynyl group by treatment with *n*-butyllithium in THF to provide 4-ethynyl analogues **18a** and **18b**. Finally, removal of the protecting groups with ammonium fluoride in refluxing MeOH provided the target compounds **1a** (R = H) and **1b** (R = CH_3). The uridine analogue **18a** was converted to the cytidine derivative **19** by a traditional approach. Deprotection of **19** with the same method as for **1** yielded compound **2**.

The chlorovinyl compound **17** from the classical Wittig olefination was predominantly in a *Z*-configuration. In the ^{1}H NMR spectrum of **17a**, δ 5.95 (1H, d, *J* = 8.05 Hz) was assigned to the *Z*-configured vinyl-H, and δ 6.01 (1H, d, *J* = 13.54 Hz) to the *E*-configured vinyl-H, based on the coupling constants. The integration values of the two peaks were 0.73 (δ 5.95) and 0.27 (δ 6.01), indicating that the ratio of *Z*- to *E*-isomers was approximately 2.7:1. Both isomers could be converted to **18**.

Compounds **1a**, **1b**, and **2** were evaluated in an anti-HIV (wild type) replication assay and the *in vitro* anti-HIV activity results are listed in Table 1. Cytotoxicity was evaluated by MTT assay. All three compounds did not exhibit significant cytotoxicity at concentrations up to 10 M.

Compound **1b** exhibited potent anti-HIV-1 replication activity with an IC_{50} value of 86 nM, and thus, was ten-fold more potent than 1-(2′-deoxy-4′-*C*-ethynyl-*β*-D-arabinofuranosyl)-thymine without a fluorine atom at the 2′-*β*-position, which had an IC_{50} value of 830 nM[11] (equivalent to that of AZT). This result confirmed that insertion of an electron-withdrawing atom, such as fluorine, into the nucleoside deoxyribose moiety can lead to dramatically improved anti-HIV activity. Such a modification can greatly affect the electronic properties and conformational shape of the nucleoside,[12–15] which often results in better biological activity.

Impressively, compound **2** showed extremely potent antiviral activity with an IC_{50} value of 1.34 nM, and was 35-fold more potent than AZT, suggesting that dissimilar nucleobase moieities may contribute differently towards the antiviral potency of these nucleoside analogs. We concluded that the base component of NRTIs has a moderate influence on activity, and the anti-HIV-1 activity of our compounds followed the rank order of cytidine > thymidine > uridine. The results with **1b** and **2** also confirmed that compounds carrying a 3′-α-OH could still show significant anti-HIV activity. In the further evaluation of **2**, we discovered that it retained its nanomolar activity against drug-resistant HIV strains including NL4-3 (K101E) and RTMDR (Table 2). K101E tends to decrease viral susceptibility to all nucleoside RT inhibitors, while RTMDR is a multiple RT inhibitor-resistant strain, which is insensitive to AZT, ddI, nevirapine, and other NNRTIs. In our screening, **2** exhibited extremely potent anti-HIV activity against NL4-3 (wild-type), NL4-3 (K101E), and RTMDR, with IC_{50} values of 0.46, 1.52, and 1.45 nM respectively. These findings indicate that **2** has a great potential to be developed as a novel NRTI that could overcome drug-resistance issues.

In summary, new 2′-deoxy-2′-fluoro-4′-*C*-ethynyl nucleoside analogues were designed, synthesized, and evaluated for *in vitro* antiviral activity in this study. Compound **2** was extremely potent against HIV-1 wild-type strain without obvious cytotoxicity. It retained nanomolar activity against NRTI-resistant and multi-resistant HIV strains, and merits further development as an anti-AIDS clinical trial candidate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HIV-1	human immunodeficiency virus type-1

RT	reverse transcriptase
NRTI	nucleoside reverse transcriptase inhibitor
NNRTI	non-nucleoside reverse transcriptase inhibitor
dN	2′-deoxynucleoside
ddN	2′,3′-dideoxynucleoside
AZT	zidovudine
SAR	structure-activity relationship
DMTrCl	4,4′-dimethoxytrityl chloride
TBDMSCl	*tert*-butyldimethylsilyl chloride
TLC	thin-layer chromatography

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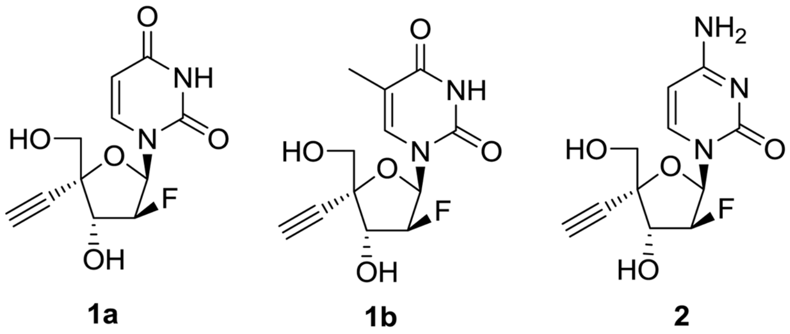
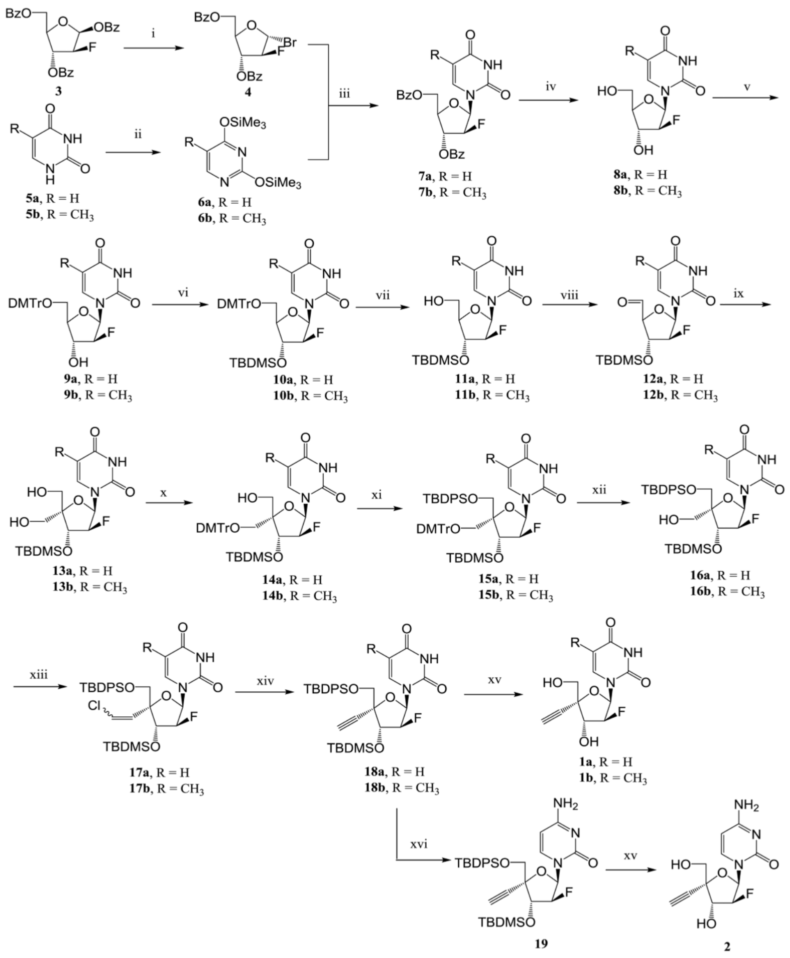


Figure 1.

Structures of 1-(2′-deoxy-2′-fluoro-4′-*C*-ethynyl-*β*-D-arabinofuranosyl)-uracil (**1a**), -thymine (**1b**), and -cytosine (**2**).



Scheme 1.

Reagents and conditions: (i) HBr.HOAc, CH_2Cl_2; (ii) HMDS, $(NH_4)_2SO_4$; (iii) $CHCl_3$, reflux; (iv) saturated NH_3/CH_3OH, r.t.; (v) DMTrCl, Pyr, 0 °C; (vi) imidazole, TBDMSCl, CH_2Cl_2; (vii) TFA, CH_2Cl_2; (viii) Pyr, TFA, EDC.HCl, DMSO; (ix) 1) 37% HCHO, 2N NaOH, 1,4-dioxane; 2) HOAc, $NaBH_4$, EtOH; (x) DMTrCl, CH_2Cl_2, Pyr; (xi) imidazole, TBDPSCl, CH_2Cl_2; (xii) TFA, CH_2Cl_2; (xiii) 1) Pyr, TFA, EDC.HCl, DMSO; 2) chloromethyl triphenyl phosphonium chloride, *n*-BuLi, −78 °C, THF; (xiv) *n*-BuLi, −78 °C, THF; (xv) NH_4F, MeOH, reflux; (xvi) 1) 1,2,4-triazole, $POCl_3$, Pyr, CH_2Cl_2; 2) NH_4OH, THF.

Table 1

Anti-HIV-1 replication activity in MT-2 lymphocytes

compound	**IC_{50} (μM)[a]**	**CC_{50} (μM)**
1a	6.53	> 10
1b	0.086	> 10
2	0.00134	> 10
AZT	0.047	> 200

[a] IC_{50} (μM) is the concentration that inhibits HIV by 50%.

Table 2

Anti-HIV activity of **2** against wild-type virus and resistant strains

viral strain	**compound 2 (IC_{50}, μM)**[a]
NL4-3 (wild-type)	0.00046
NL4-3 (K101E)	0.00152
RTMDR[b]	0.00145

[a] IC_{50} (μM) is the concentration that inhibits HIV by 50%.

[b] RTMDR is a multiple RT inhibitor-resistant strain, has RT mutations - M41L, L74V, V106A and T215Y, and is resistant to AZT, ddI, Nevirapine, and other NNRTIs.