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Synthesis and antiviral evaluation of thieno[3,4-*d*]pyrimidine *C*-nucleoside analogues of 2′,3′-dideoxy- and 2′,3′-dideoxy-2′,3′-didehydro-adenosine and -inosine

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ABSTRACT

Several thieno[3,4-*d*]pyrimidine derivatives, including four hitherto unknown 2′,3′-dideoxy- and 2′,3′-dideoxy-2′,3′-didehydro-*C*-nucleoside analogues of adenosine and inosine have been synthesized. When evaluated in cell culture experiments against human immunodeficiency virus, none of the tested compounds exhibited any significant antiviral effect, while two of them showed some cytotoxicity.

1. Introduction

To date, eight nucleoside analogues, namely didanosine (ddI), zidovudine (AZT), abacavir, lamivudine (3TC), zalcitabine (ddC), stavudine (d4T), tenofovir (PMPA) and emtricitabine (FTC) (Fig. 1) have been approved by the US Food and Drug Administration (FDA) for the treatment of human immunodeficiency virus (HIV) infection.[1] All these 2′,3′-dideoxynucleoside analogues share a common mechanism of action. They are metabolized by cellular kinases to their 5′-triphosphate forms, which then exert their biological effect as virus-specific polymerase (reverse transcriptase) competitive inhibitors or chain terminators because they lack a hydroxyl group at the C-3′ position.[2] Unfortunately, the use of 2′,3′-dideoxynucleosides as antiviral drugs is hampered by several limitations including delayed toxicities and/or emergence of drug-resistant HIV strains. Indeed 2′,3′-dideoxynucleoside triphosphates, as well as their mono- and diphosphate intermediates, often have affinity for other cellular enzymes that is thought to cause some of the undesired side effects observed in patients being treated with these drugs.[3]

The emergence and selection of mutations conferring resistance to anti-HIV-1 drugs is a major concern for these agents as well as for other drug classes in acquired immunodeficiency symptom (AIDS) treatment.[3] In addition to these toxicity and resistance issues, 2′,3′-dideoxynucleosides are reported to be particularly unstable under acidic conditions.[4] For example, in the stomach's acidic environment, didanosine and 2′,3′-dideoxy-adenosine are cleaved into the corresponding 2′,3′-dideoxyribose moiety and the free base. In order to discover new nucleoside derivatives with antiviral activity, modifications of the base and/or sugar moiety of natural nucleosides can be attempted. In this regard, special attention has been paid to *C*-nucleosides[5] (nucleosides with the C–N bond replaced by a carbon–carbon linkage) due to the increase of chemical and enzymatic stability of their glycosidic bond.[6]

As part of our ongoing research on new nucleoside analogues with potential antiviral activity, we have synthesized various *C*-nucleoside derivatives bearing a modified purine or pyrimidine base. Herein, we report the synthesis of several thieno[3,4-*d*]pyrimidine (7,9-dideaza-8-thiapurine) derivatives, including the four titled hitherto unknown 2′,3′-dideoxy- and 2′,3′-dideoxy-2′,3′-didehydro-*C*-nucleoside analogues of adenosine and inosine

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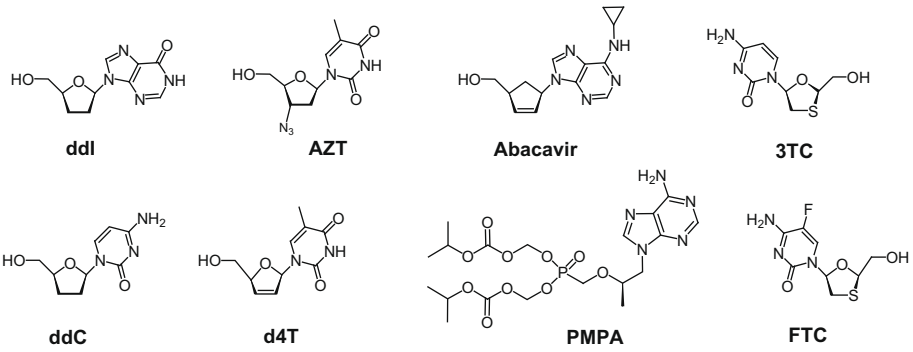


Figure 1. Structures of the nucleoside analogues currently approved by the US Food and Drug Administration (FDA) for the treatment of human immunodeficiency virus (HIV) infection.

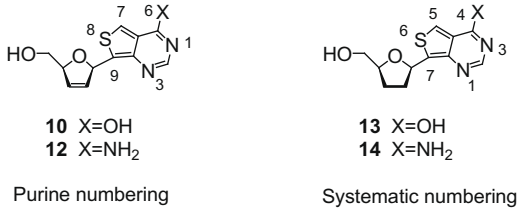


Figure 2. Novel 2′,3′-dideoxy- and 2′,3′-dideoxy-2′,3′-didehydro thieno[3,4-*d*]pyrimidine (7,9-dideaza-8-thiapurine) derivatives.

10, **12**, **13** and **14** (Fig. 2).[7] The antiviral evaluation of these compounds against HIV replication in cell culture experiments is presented.

2. Results and discussion

2.1. Chemistry

The synthesis of 2′,3′-dideoxynucleosides and 2′,3′-dideoxy-2′,3′-didehydronucleosides starting from their corresponding ribofuranonucleosides has been extensively reviewed.[8] For instance, 2′,3′-unsaturated nucleosides have been mainly obtained through reductive elimination of 2′(3′)-acetoxy-3′(2′)-halogeno derivatives,[9] fragmentation of cyclic orthoformates in the presence of acid catalyst,[10] or through removal of the 3′-hydroxy group of 2′-deoxyribonucleoside by Barton deoxygenation.[11]

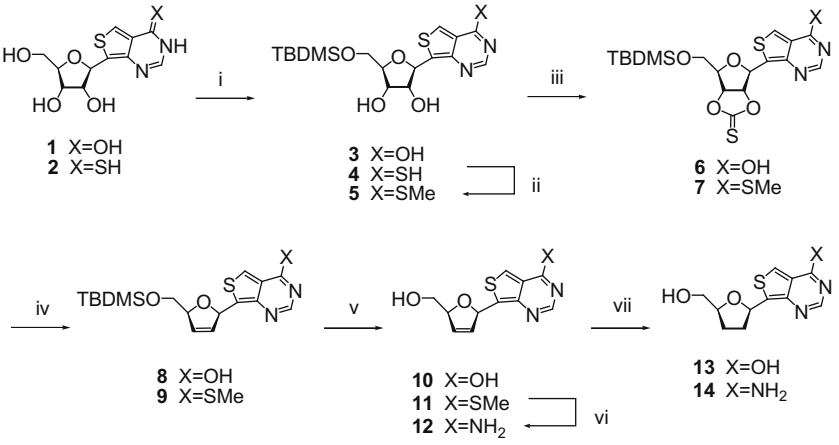
We elected to prepare the hitherto unknown 2′,3′-dideoxy- and 2′,3′-dideoxy-2′,3′-didehydro-*C*-nucleosides **10**, **12**, **13** and **14** from the 2′,3′-cyclic thienocarbonates of their related *C*-ribonucleosides following a Corey–Winter reaction.[12]

The starting *C*-ribonucleosides **1**[13] and **2**[13c] were synthesized according to previously reported procedures.[13a–c]

For the conversion of **1** and **2** into the hitherto unknown 2′,3′-dideoxy- and 2′,3′-dideoxy-2′,3′-didehydro-*C*-nucleosides **10**, **12**, **13** and **14** (Scheme 1), selective protection of their 5′-hydroxyl function was carried out with *tert*-butyldimethylsilyl chloride and imidazole in anhydrous dimethylformamide or pyridine, affording **3** and **4**, respectively. The 4-thione compound **4** was converted into its corresponding 4-methylthio derivative **5** by treatment with methyl iodide in an aqueous sodium hydroxide solution. Compounds **3** and **5** were then converted into the 1,3-dioxolane-2-thione (cyclic thienocarbonate) derivatives **6** and **7** using 1,1′-thiocarbonyldiimidazole in dimethylformamide. Heating the cyclic thienocarbonate derivatives **6** and **7** with triethylphosphite at 140 °C led to olefination, and compounds **8** and **9** were isolated in good yields. Deprotection of **8** and **9** with tetrabutylammonium fluoride gave the 2′,3′-dideoxy-2′,3′-didehydro-*C*-nucleosides **10** and **11**. 4-Amino-7-(2,3-didehydro-2,3-dideoxy-β-D-ribofuranosyl)thieno[3,4-*d*]pyrimidine **12** was obtained by reaction of the methylthio derivative **11** with methanolic ammonia in the microwave at 120 °C for 20 min. Finally, hydrogenation using hydrogen and catalytic amount of Pd/C in ethanol led to 2′,3′-dideoxynucleosides **13** and **14**.

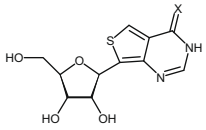
2.2. Antiviral evaluation

The title 2′,3′-dideoxy- and 2′,3′-dideoxy-2′,3′-didehydro-*C*-nucleosides **10**, **12**, **13** and **14,** as well as their precursors **1**, **2**,

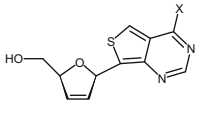


Scheme 1. Reagents and conditions: (i) TBDMSCl, imidazole in DMF or pyridine, rt, 69–72%; (ii) NaOH 0.1 N, MeI, RT 96%; (iii) 1,1′-thiocarbonyl diimidazole, DMF, 80 °C, 79%; (iv) triethyl phosphite, 140 °C, 94%; (v) TBAF 1 N, THF, 0 °C, 57–85%; (vi) ammonia 7 N in methanol, 120 °C, microwave, 56%; (vii) EtOH, H_2, Pd/C 10%, rt, 23–56%.

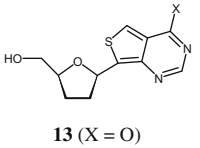
Table 1
Activity of the synthesized compounds against HIV-1



1 (X = O)
2 (X = S)



10 (X = OH)
11 (X = SCH_3)
12 (X = NH_2)



13 (X = O)
14 (X = NH_2)

Compound	EC_{50}[a]	CC_{50}[b]
1	>100	>100
2	>2.9	2.9
10	>100	>100
11	>70	70
12	>7.3	7.3
13	>100	>100
14	>75	>75
AZT (Reference)	0.009	61

[a] Compound concentration (μM) required to achieve 50% protection of MT-4 cell lines from virus-induced cytopathogenicity, as determined by the MTS method.
[b] Compound concentration (μM) required to reduce MT-4 cell proliferation by 50%, as determined by the MTS method, under conditions that allow untreated controls to undergo at least three consecutive rounds of multiplication.

and **11** were evaluated in a cell-based assay against HIV-1. As shown in Table 1, none of them showed any antiretroviral activity. On the other hand, the known 7-β-D-ribofuranosyl)thieno[3,4-*d*]pyrimidine-4(3*H*)-thione (**2**)[13c] and the hitherto unknown 4-amino-7-(2,3-didehydro-2,3-dideoxy-β-D-ribofuranosyl)thieno[3,4-*d*]pyrimidine (**12**) were moderately toxic to MT-4 cells, with CC_{50} values of 2.9 and 7.3 μM, respectively. The cytotoxicity of the thiono derivative **2** in MT-4 cells (exponentially growing $CD4^+$ human T-cells derived from human haematological tumors and containing an integrated human T-lymphotropic virus genome) is in accordance with its previously reported in vitro growth inhibitory activities against a number of tumor cell lines, including L1210-C1 (IC_{50} = 17.6 μM), Sarcoma 180 (IC_{50} = 29.1 μM) and HL60 cell lines (IC_{50} = 8.17 μM).[13c]

The synthesized *C*-nucleoside analogues **1**, **2**, **10**–**14** were also evaluated in cell-based assays against viruses representative of two genera of the $ssRNA^+$ *Flaviviridae*, that is, Flavivirus (Yellow Fever, Dengue and West Nile viruses) and *Hepacivirus* (HCV), following methods described in Ref. 14. However, none of them showed either significant activity or toxicity at the highest concentration tested, generally 75 μM (data not shown).

3. Summary and conclusion

Two novel 2′,3′-dideoxy- (**13**, **14**) and two novel 2′,3′-dideoxy-2′,3′-didehydro- (**10**, **12**) *C*-nucleoside analogues in the thieno[3,4-*d*]pyrimidine (7,9-dideaza-8-thiapurine) series were synthesized from the corresponding *C*-ribonucleosides **1** and **2** and evaluated for their potential inhibition of HIV-1 replication in cell culture experiments. No antiretroviral activity was observed. The *C*-nucleoside derivatives **2** and **12** showed moderate toxicity against the MT-4 host cells. Several factors could be responsible for the inactivity of these *C*-nucleoside analogues against HIV including their inability to enter cells or to serve as substrates for intracellular enzymes catalyzing phosphorylation, as well as a lack of inhibition of the viral reverse transcriptase by their triphosphate forms. Further research would be needed to differentiate between these hypotheses, but these studies have not been pursued further in the absence of promising results on the class of thieno[3,4-*d*]pyrimidine *C*-nucleoside analogues.

4. Experimental

4.1. General methods for chemistry

All reaction involving moisture sensitive reagents were conducted in oven dried glassware under nitrogen atmosphere. All chemicals and solvents were of reagent grade unless otherwise specified. ^1H and ^{13}C NMR spectra were recorded at ambient temperature on Buker AC 200 and 300 or Avance II 400 MHz spectrometers. ^1H and ^{13}C NMR chemical shift (δ) are quoted in parts per million (ppm) referenced to the residual solvent peak [DMSO-d_6] set at 2.49 ppm or [$CDCl_3$] set at 7.26 ppm or [D_2O] set at 4.72 ppm. The accepted abbreviations are as followed: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. LC/MS spectra were recorded on a WATERS unit [Alliance 2695, Photodiode Array detector 2996, ZQ 2000 (ESCI source), Mass Lynx 4.1] using a reverse phase analytical column Synergi 4 μm Fusion 80A 50x2.0 mm (Phenomenex 100B-4424-BO). The compound to be analyzed was eluted using a linear gradient of 2.5–100% acetonitrile in 0.05% formic acid programmed over an 8 min period with a flow rate of 0.4 mL/min. Mass spectra were recorded on a Q-TOF micromass mass spectrometer (ESIMS) or on a Jeol JMS DX 300 mass spectrometer (HRFABMS). Thin layer chromatography (TLC) was performed on precoated aluminum sheets of Silica Gel 60 F254 (Merck, Art. 5554), visualization of products being accomplished by UV absorbance at 254 nm and by charring with a solution of $(NH_4)_2SO_4$ (150 g) in $EtOH/H_2SO_4/H_2O$ (300:30:450 mL) with heating. Column chromatography was carried out on Silica Gel 60 40–63 μm (Merck, Art. 11567). Reverse phase column chromatography was done on Biotage C18 packing column (Biotage, Art. KP-C18-HS, 35–70 μm, 90 Å). Evaporation of the solvent was carried out in a rotary evaporator under reduce pressure. Reactions in microwave were done on a Biotage Initiator Eight Exp.

4.1.1. 7-(5-*O*-*tert*-Butyldimethylsilyl-β-D-ribofuranosyl) thieno[3,4-*d*]pyrimidine-4(3*H*)-one (3)

tert-Butyldimethylsilyl chloride (403 mg, 2.7 mmol) was added under stirring to a solution of **1**[13] (1.9 g, 6.7 mmol) and imidazole (455 mg, 6.7 mmol) in anhydrous dimethylformamide (15 mL) at 0 °C. The mixture was warmed to room temperature and subsequently stirred overnight. After evaporation of the solvent, the residue was purified by flash chromatography, eluting with 10% methanol in dichloromethane, to afford compound **3** (1.93 g, 72% yield). ^1H NMR (300 MHz, DMSO-d_6): δ 11.63 (s, 1H, NH, D_2O exchangeable), 8.36 (s, 1H, H-7), 7.72 (s, 1H, H-2), 5.30 (d, 1H, H-1′, $J_{1'\text{-}2'}$ = 6.2 Hz), 5.17 (d, 1H, OH-2′, $J_{OH\text{-}2'\text{-}2'}$ = 5.4 Hz, D_2O exchangeable), 4.99 (d, 1H, OH-3′, $J_{OH\text{-}3'\text{-}3'}$ = 4.5 Hz, D_2O exchangeable), 3.95–4.03 (m, 2H, H-2′ and H-3′), 3.80 (q, 1H, H-4′, $J_{4'\text{-}5'}$ = 3.2 Hz), 3.72–3.74 (m, 2H, H-5′ and H-5″), 0.82 (s, 9H, $SiC(CH_3)_3$), 0.01 (s, 6H, $Si(CH_3)_2$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.7 (C-6), 145.4 (C-4), 143.4 (C-2), 135.7 (C-9), 126.4 (C-7), 126.0 (C-5), 84.4 (C-4′), 77.7 (C-2′), 76.8 (C-1′), 71.2 (C-3′), 63.5 (C-5′), 25.8 ($SiC(CH_3)_3$), 18.0 ($SiC(CH_3)_3$), −5.3 ($SiCH_3$), -5.5 ($SiCH_3$); MS (ESI) *m/z* 398 $(M+H)^+$, 396 $(M-H)^-$.

4.1.2. 7-(2,3-*O*-Thiocarbonylene-5-*O*-*tert*-butyldimethyl silyl-β-D-ribofuranosyl)thieno[3,4-*d*] pyrimidine-4(3*H*)-one (6)

A solution of **3** (1.8 g, 4.5 mmol) and 1,1′-thiocarbonyldiimidazole (1.2 g, 6.8 mmol) in anhydrous dimethylformamide (20 mL) was stirred at 80 °C for 15 h. After evaporation of the solvent, the residue was purified by flash chromatography using 5% methanol in dichloromethane as eluant to give **6** (1.8 g, 92% yield). ^1H NMR (300 MHz, DMSO-d_6): δ 11.75 (s, 1H, NH, D_2O exchangeable), 8.43 (s, 1H, H-7), 7.80 (s, 1H, H-2), 5.80 (dd, 1H, H-2′, $J_{1'\text{-}2'}$ = 3.9 Hz, $J_{2'\text{-}3'}$ = 7.5 Hz), 5.67 (d, 1H, H-1′, $J_{1'\text{-}2'}$ = 4.2 Hz), 5.55

(dd, 1H, H-3′, $J_{4'-3'}$ = 3.0 Hz, $J_{2'-3'}$ = 7.5 Hz), 4.39 (m, 1H, H-4′), 3.74 (m, 2H, H-5′, H-5″), 0.82 (s, 9H, SiC(CH_3)$_3$), 0.01 (s, 3H, SiCH_3), 0.00 (s, 3H, SiCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 190.7 (C=S), 157.5 (C-6), 149.0 (C-4), 144.3 (C-2), 131.0 (C-9), 127.4 (C-7), 126.5 (C-5), 89.5 (C-2′), 86.7 (C-3′), 84.0 (C-4′), 79.7 (C-1′), 62.2 (C-5′), 25.6 (SiC(CH_3)$_3$), 17.8 (SiC(CH_3)$_3$), −5.6 (SiCH_3), -5.6 (SiCH_3); MS (ESI) *m/z* 441 (M+H)$^+$, 439 (M−H)$^-$.

4.1.3. 7-(2,3-Didehydro-2,3-dideoxy-5-*O*-*tert*-butyl dimethylsilyl-β-D-ribofuranosyl)thieno[3,4-*d*]pyrimidine-4(3*H*)-one (8)

A mixture of **6** (1.7 g, 3.9 mmol) in triethyl phosphite (20 mL) was stirred at 140 °C for one night. After completion of the reaction, the excess of triethyl phosphite was removed in vacuum. The residue was purified by silica gel column chromatography (5% methanol in dichloromethane) to give **8** (1.5 g, quantitative yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.75 (s, 1H, NH, D_2O exchangeable), 8.41 (s, 1H, H-7), 7.81 (s, 1H, H-2), 6.44 (d, 1H, H-1′, $J_{1'-2'}$ = 3.3 Hz), 6.10 (m, 2H, H-2′, H-3′), 4.84 (s, 1H, H-4), 3.69–3.70 (d, 2H, H-5′, H-5″, $J_{4'-5'}$ = 5.4 Hz), 0.86 (s, 9H, SiC(CH_3)$_3$), 0.017 (s, 6H, Si(CH_3)$_2$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.7 (C-6), 144.4 (C-4), 143.8 (C-2), 137.1 (C-9), 130.2 (C-3′), 128.9 (C-2′), 126.4 (C-5 and C-7), 86.9 (C-4′), 79.5 (C-1′), 66.4 (C-5′), 25.8 CH_3 (SiC(CH_3)$_3$), 18.0 (SiC(CH_3)$_3$), −5.3 (SiCH_3), −5.4 (SiCH_3); MS (ESI) *m/z* 365 (M+H)$^+$, 363 (M−H)$^-$.

4.1.4. 7-(2,3-Didehydro-2,3-dideoxy-β-D-ribofuranosyl) thieno [3,4-*d*]pyrimidine-4(3*H*)-one (10)

To a solution of **8** (0.680 g, 1.9 mmol) in anhydrous tetrahydrofuran (20 mL) at 0 °C, was added tetrabutylammonium fluoride (1.0 M in tetrahydrofuran, 3.7 mL, 3.8 mmol) with stirring. The mixture was warmed to room temperature and stirred for 1 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (5% methanol in dichloromethane) to afford compound **10** as a yellow solid (266 mg, 57% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.68 (s, 1H, NH, D_2O exchangeable), 8.41 (s, 1H, H-7), 7.80 (s, 1H, H-2), 6.43 (t, 1H, H-1′, $J_{1'-2'}$ = 2.1 Hz), 6.14 (s, 1H, H-2′, $J_{2'-3'}$ = 5.8 Hz), 6.07 (s, 1H, H-3′, $J_{2'-3'}$ = 5.9 Hz), 4.87 (s, 1H, OH-5′, D_2O exchangeable), 4.81 (m, 1H, H-4′), 3.50 (dd, 1H, H-5′, H-5″, $J_{4'-5'}$ = 5.1 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.7 (C-6), 144.4 (C-4), 143.7 (C-2), 137.4 (C-9), 129.7 (C-2′), 129.5 (C-3′), 126.4 (C-5), 126.4 (C-7), 87.4 (C-4′), 79.4 (C-1′), 64.8 (C-5′); MS (ESI) *m/z* 251 (M+H)$^+$, 249 (M−H)$^-$. HRFABMS: calcd for $C_{11}H_{11}N_2O_3S$ 251.0490 [M+H]$^+$; found (*m/z*) 251.0498.

4.1.5. 7-(2,3-Dideoxy-β-D-ribofuranosyl)thieno[3,4-*d*]-pyrimidine-4(3*H*)-one (13)

Palladium on carbon (10%) was added to a solution of **10** (0.1 g, 0.4 mmol) in methanol (6 mL). The reaction was exposed to hydrogen (atmospheric pressure) and stirred for 24 h. The mixture was filtered through Celite, and the Celite was washed twice with methanol. Filtrate was concentrated in vacuum to dryness. The residue was purified by flash chromatography eluting with 10% methanol in dichloromethane to give **13** as a white foam (56 mg, 56% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.65 (br s, 1H, NH, D_2O exchangeable), 8.23 (s, 1H, H-7), 7.75 (s, 1H, H-2), 5.50 (t, 1H, H-1′, $J_{1'-2'}$ = 6.9 Hz), 4.78 (br s, 1H, OH-5′, D_2O exchangeable), 3.99 (q, 1H, H-4′, $J_{4'-5'}$ = 6.0 Hz), 3.48 (m, 1H, H-5′and H-5″), 2.33 (m, 1H, H-2′), 1.87 (s, 1H, H-3′, $J_{2'-3'}$ = 5.2 Hz), 1.71–1.62 (m, 2H, H-2″ and H-3″); ^{13}C NMR (75 MHz, DMSO-d_6): δ 157.7 (C-6), 143.7 (C-4), 143.2 (C-2), 138.3 (C-9), 126.5 (C-5), 124.9 (C-7), 80.2 (C-1′), 74.0 (C-4′), 63.9 (C-5′), 33.9 (C-2′), 27.9 (C-3′); MS (ESI) *m/z* 253 (M+H)$^+$, 251(M−H)$^-$. HRFABMS: calcd for $C_{11}H_{13}N_2O_3S$ 253.0647 [M+H]$^+$; found (*m/z*) 253.0642.

4.1.6. 7-(5-*O*-*tert*-Butyldimethylsilyl-β-D-ribofuranosyl) thieno[3,4-*d*]pyrimidine-4(3*H*)-thione (4)

To a solution of **2**[13c] (1 g, 3.33 mmol) in anhydrous pyridine (45 mL) at room temperature was added *tert*-butyldimethylsilyl chloride (553 mg, 3.67 mmol) with stirring. The mixture was stirred for overnight at room temperature. After evaporation of the solvent, the residue was purified by flash chromatography using 5% methanol in dichloromethane as eluant to give **4** (0.954 g, 69% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.17 (s, 1H, NH, D_2O exchangeable), 8.48 (s, 1H, H-7), 7.78 (s, 1H, H-2), 5.32 (d, 1H, H-1′, $J_{1'-2'}$ = 8.0 Hz), 5.12 (d, 1H, OH-2′, $J_{OH\text{-}2'-2'}$ = 4.0 Hz, D_2O exchangeable), 4.99 (d, 1H, OH-3′, $J_{OH\text{-}3'-3'}$ = 8.0 Hz, D_2O exchangeable), 3.86–3.95 (m, 2H, H-2′ and H-3′), 3.80 (q, 1H, H-4′, $J_{4'-5'}$ = 4.0 Hz), 3.62–3.71 (m, 2H, H-5′ and H-5″), 0.82 (s, 9H, SiC(CH_3)$_3$), 0.01 (s, 6H, Si(CH_3)$_2$); MS (ESI) *m/z* 415 (M+H)$^+$, 413 (M−H)$^-$.

4.1.7. 4-(Methylthio)-7-(5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)thieno[3,4-*d*]pyrimidine (5)

A solution of **4** (950 mg, 2.30 mmol) in 0.1 N aqueous sodium hydroxide solution (24 mL) was treated with methyl iodide (7 mL) and the resulting mixture was stirred vigorously at room temperature for 1 h. Water was added and the reaction mixture was extracted twice with dichloromethane. The aqueous layer was washed with dichloromethane and the combined organic layers were dried over anhydrous sodium sulfate. After filtration and removal of the solvent in vacuum, the residue was flash chromatographed over silica gel using 5% methanol in dichloromethane as eluant to give **5** (0.949 g, 96% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.53 (s, 1H, H-2), 8.40 (s, 1H, H-7), 5.48 (d, 1H, H-1′, $J_{1'-2'}$ = 6.0 Hz), 5.24 (d, 1H, OH-2′, $J_{OH\text{-}2'-2'}$ = 8.0 Hz, D_2O exchangeable), 5.02 (d, 1H, OH-3′, $J_{OH\text{-}3'-3'}$ = 4.0 Hz, D_2O exchangeable), 4.03 (d, 1H, H-2′, $J_{OH\text{-}2'-2'}$ = 8.0 Hz, D_2O exchangeable), 3.91 (d, 1H, H-3′, $J_{3'-4'}$ = 3.1 Hz), 3.84 (d, 1H, H-4′, $J_{3'-4'}$ = 3.2 Hz), 3.63-3.72 (m, 2H, H-5′, H-5″), 2.60 (s, 3H, CH_3, SMe), 0.82 (s, 9H, SiC(CH_3)$_3$), 0.00 (s, 6H, Si(CH_3)$_2$); ^{13}C NMR (75 MHz, DMSO-d_6): δ 167.6 (C-6), 150.7 (C-2), 144.4 (C-4), 136.7 (C-9), 126.9 (C-5), 120.3 (C-7), 84.5 (C-4′), 77.9 (C-2′), 76.9 (C-1′), 71.2 (C-3′), 63.5 (C-5′), 25.8-25.6 (SiC(CH_3)$_3$), 18.0 (SiC(CH_3)$_3$), 11.4 CH_3 (SMe), −5.3 (SiCH_3), −5.5 (SiCH_3); MS (ESI) *m/z* 429 (M+H)$^+$.

4.1.8. 4-(Methylthio)-7-(2,3-*O*-thiocarbonylene-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)thieno[3,4-*d*]-pyrimidine (7)

Compound **7** (815 mg, 79% yield) has been synthesized from **5** according to the same procedure as for compound **6**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.69 (s, 1H, H-2), 8.61 (s, 1H, H-7), 5.93–5.99 (m, 2H, H-1′, H-2′), 5.65 (dd, 1H, H-3′, $J_{2'-3'}$ = 7.0 Hz, $J_{3'-4'}$ = 3.0 Hz), 4.51 (m, 1H, H-4′), 3.75–3.84 (m, 2H, H-5′, H-5″), 2.71 (s, 3H, S–CH_3), 0.84 (s, 9H, SiC(CH_3)$_3$), −0.01 (s, 3H, SiCH_3), −0.04 (s, 3H, SiCH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 190.7 (C=S), 168.0 (C-6), 151.2 (C-2), 143.8 (C-4), 132.0 (C-9), 126.9 (C-5), 121.8 (C-7), 89.5 (C-2′), 87.1 (C-3′), 84.3 (C-4′), 80.0 (C-1′), 62.3 (C-5′), 25.7–25.6 (SiC(CH_3)$_3$), 17.8 (SiC(CH_3)$_3$), 11.5 (S–CH_3), −5.7 (SiCH_3), −5.3 (SiCH_3); MS (ESI) *m/z* 471(M+H)$^+$.

4.1.9. 4-(Methylthio)-7-(2,3-didehydro-2,3-dideoxy-5-*O*-*tert*-butyldimethylsilyl-β-D-ribofuranosyl)thieno[3,4-*d*]pyrimidine (9)

Compound **9** (641 mg, 94% yield) has been obtained from **7** following the same procedure as for compound **8**. ^{1}H NMR (DMSO-d_6): δ 8.61 (s, 1H, H-2), 8.47 (s, 1H, H-7), 6.61 (dd, 1H H-1′, $J_{1'-4'}$ = 2.1 Hz, $J_{2'-1'}$ = 3.9 Hz), 6.21 (dd, 1H, H-3′, $J_{2'-3'}$ = 1.8 Hz, $J_{3'-4'}$ = 3.9 Hz), 6.14 (dd, 1H, H-2′, $J_{2'-3'}$ = 1.5 Hz, $J_{2'-1'}$ = 3.9 Hz), 4.89 (m, 1H, H-4′), 3.74-3.78 (m, 2H, H-5′, H-5″), 2.67 (s, 3H, S–CH_3), 0.83 (s, 9H, SiC(CH_3)$_3$), −0.05 (s, 6H, Si(CH_3)$_2$); ^{13}C NMR (DMSO-d_6): δ 167.6 (C-6), 150.9 (C-2), 143.5 (C-4), 138.1 (C-9), 130.1

(C-3′), 129.0 (C-2′), 126.8 (C-5), 120.7 (C-7), 87.0 (C-4′), 79.5 (C-1′), 66.3 (C-5′), 25.8–25.6 (SiC(CH_3)$_3$), 18.0 (SiC(CH_3)$_3$), 11.4 (S–CH_3), −5.4 (SiCH_3), −5.3 (SiCH_3); MS (ESI) *m/z* 395 $(M+H)^+$, 417 $(M+Na)^+$.

4.1.10. 4-(Methylthio)-7-(2,3-didehydro-2,3-dideoxy-β-D-ribofuranosyl)thieno[3,4-*d*]pyrimidine (11)

Compound **11** (360 mg, 85% yield) has been prepared from **9** according to the same procedure as for compound **10**. ^{1}H NMR (DMSO-d_6): δ 8.61 (s, 1H, H-2), 8.45 (s, 1H, H-7), 6.61 (dd, 1H H-1′, $J_{1'-4'}$ = 1.6 Hz, $J_{2'-1'}$ = 3.6 Hz), 6.14–6.16 (m, 2H, H-2′, H-3′), 4.89–4.84 (m, 2H, H-4′, OH-5′), 3.52–3.53 (m, 2H, H-5′, H-5″), 2.66 (s, 3H, S–CH_3); ^{13}C NMR (DMSO-d_6): δ 168.1 (C-6), 151.4 (C-2), 143.9 (C-4), 138.9 (C-9), 130.2–130.1 (C-2′ and C-3′), 127.3 (C-5), 121.2 (C-7), 88.0 (C-4′), 79.9 (C-1′), 65.1 (C-5′), 11.9 (S–CH_3); MS (ESI) *m/z* 281 $(M+H)^+$, 303$(M+Na)^+$.

4.1.11. 4-Amino-7-(2,3-didehydro-2,3-dideoxy-β-D-ribofuranosyl)thieno[3,4-*d*]pyrimidine (12)

Compound **11** (130 mg, 0.464 mmol) dissolved in a 7 N solution of ammonia in methanol (5 mL) was stirred for 20 min at 120 °C under microwave. After evaporation of the solvent, the residue was purified by silica gel column chromatography using 10% methanol in dichloromethane. The collected fractions were evaporated to dryness and lyophilized to give **12** as a yellow powder (66 mg, 56% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.39 (s, 1H, H-2), 8.05 (s, 1H, H-7), 7.93 (br s, 1H, NH_2, D_2O exchangeable), 7.77 (br s, 1H, NH_2, D_2O exchangeable), 6.52 (dd, 1H, H-1′, $J_{1'-4'}$ = 1.8 Hz, $J_{2'-1'}$ = 3.9 Hz), 6.08–6.14 (m, 2H, H-2′, H-3′), 4.97 (br s, 1H, OH-5′, D_2O exchangeable), 4.81 (m, 1H, H-4′), 3.53 (m, 2H, H-5′, H-5″); ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.2 (C-6), 154.4 (C-2), 147.0 (C-4), 134.9 (C-9), 130.1 (C-2′), 129.2 (C-3′), 121.1 (C-5), 120.3 (C-7), 87.2 (C-4′), 79.6 (C-1′), 64.9 (C-5′); MS (ESI) *m/z* 250 $(M+H)^+$, 248 $(M-H)^-$.

4.1.12. 4-Amino-7-(2,3-dideoxy-β-D-ribofuranosyl)thieno[3,4-*d*]pyrimidine (14)

Compound **14** (13 mg, 23% yield) was obtained (as a yellow lyophilized powder) from **12** following the same procedure as for compound **13**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.30 (s, 1H, H-7), 8.13 (s, 1H, H-2), 7.89 (br s, 1H, NH_2, D_2O exchangeable), 7.73 (br s, 1H, NH_2, D_2O exchangeable), 5.55 (d, 1H, H-1′, $J_{1'-2'}$ = 4.0 Hz), 4.92 (s, 1H, OH-5′, D_2O exchangeable), 4.00 (m, 1H, H-4′), 3.50–3.47 (m, 2H, H-5′and H-5″), 2.33–2.30 (m, 1H, H-2′), 2.06–1.81 (m, 3H, H-2″, H-3′ and H-3″); ^{13}C NMR (75 MHz, DMSO-d_6): δ 154.4 (C-2), 135.5 (C-9), 121.3 (C-5), 119.2 (C-7), 84.5 (C-4′), 74.8 (C-1′), 64.5 (C-5′), 34.5 (C-2′), 28.5 (C-3′); MS (ESI) m/z 252 $(M+H)^+$, 250 $(M-H)^-$. HRFABMS: calcd for $C_{11}H_{14}N_3O_2S$ 252.0807 $[M+H]^+$; found (*m/z*) 252.0790.

4.2. Materials and methods for virology

4.2.1. Compounds

Compounds were dissolved in DMSO at 15 mM and then diluted in culture medium RPMI 1640 supplemented with 10% fetal calf serum (FCS), 2 g/L $NaHCO_3$, 100 units/mL penicillin, 100 μg/mL streptomycin and 1.2% DMSO.

4.2.2. Cells

The used cell line supporting the replication of HIV-1 was MT-4. This cell line was purchased from NIH (Bethesda, MD).

4.2.3. HIV strain

HIV-1 strain BH10 was purchased from NIH (Bethesda, MD).

4.2.4. Anti-HIV assay

The activity of compounds against HIV was measured by the inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected with HIV (subtype B, BH10 strain) at a multiplicity of infection (m.o.i.) of about 0.05, which typically gives a 90% cytopathic effect. Briefly, MT-4 cells were seeded into 96-well cell culture plates at a concentration of 10^4 cells per well in 50 μL of RPMI 1640 medium supplemented with 10% FCS, 100 units/mL penicillin and 100 μg/mL streptomycin. Then, serial twofold dilutions of test compounds in 50 μL (final concentrations 0.29–75 μM) were added and the cells were infected with a 20 μL-aliquot of an HIV suspension at a dilution that gives 90% cytopathic effect. The final DMSO concentration in the assay was 0.5% in 120 μL. Cell cultures were then incubated at 37 °C in a humidified 5% CO_2 atmosphere for 4 days. Cell Titer 96 AQ_{ueous} One Solution Cell Proliferation Assay (Promega) was used to measure cell viability. The assay is based on a biochemical reaction in which yellow 3-(4,5-dimethylthiazol-2-yl-)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) is reduced to purple formazan by a mitochondrial reductase enzyme. The rate of the conversion is directly related to the number of viable cells. Briefly, the One Solution Reagent was added directly to culture wells (20 μL/well), incubated for 4 h, and the absorbance was recorded at 492 nm using the Sunrise Tecan Spectrophotometer. The EC_{50} values were determined from the percent inhibition versus concentration data using a sigmoidal non-linear regression analysis based on four parameters with Tecan Magellan software.

4.2.5. Cytotoxicity assay

Cytotoxicity assays were run in parallel with antiviral assays. MT-4 cells were treated exactly as described for the anti-HIV assay but not infected with virus. Growth medium was used as the inoculum for the mock infection. The CC_{50} values were determined from the percent cytotoxicity versus concentration data using a sigmoidal non-linear regression analysis based on four parameters with Tecan Magellan software.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.02.011.

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