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6-Azathymidine-4′-thionucleosides: synthesis and antiviral evaluation

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Abstract

The synthesis of dideoxy-6-azathymidine 4′-thionucleoside 1-(2,3-dideoxy-4-thio-β-D-*erythro*-pentofuranosyl)-(6-azathymidine) (**2**), and the L-nucleoside, 1-(4-thio-β-L-*erythro*-pentofuranosyl)-(6-azathymidine) (**3**) and their evaluation against a wide panel of antiviral assays are described. The L-thionucleoside (**3**) was devoid of antiviral activity. The dideoxy-thionucleoside (**2**) was moderately active against vaccinia virus (VV) and the herpes simplex virus strains HSV-1 (strain KOS) and HSV-2 (strain G) (MIC $12\,\mu$M) and retained inhibitory activity *vs* a thymidine kinase-deficient strain HSV-1/TK$^-$, suggesting that (**2**) is not dependent on viral TK-catalysed phosphorylation for antiviral activity and/or may use an alternative metabolic activation pathway.

Keywords: *6-Azathymidine-4′-thionucleosides, L-nucleoside, dideoxynucleoside, antiviral, thymidine kinase deficient (TK-)*

Introduction

We have recently shown that 1-(2-deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-(6-azathymidine) (**1**) (Figure 1, R = CH$_3$) displayed pronounced activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV) and vaccinia virus [1]. Thionucleoside **1** displayed activity comparable with acyclovir (ACV) (EC$_{50}$ ∼ 1 μM) but was considerably less active than brivudin ((*E*)-5-(2-bromovinyl)-2′-deoxyuridine, BVDU) (EC$_{50}$: 0.003 μM). However, importantly **1** showed a good retention of antiviral activity against VZV/TK$^-$ strains, whereas BVDU lost activity by at least 4 orders of magnitude [1]. These observations suggest that the 4′-thionucleoside **1** may not entirely depend on viral TK-catalysed phosphorylation for antiviral activity and/or use an alternative metabolic activation pathway, and/or display a unique mechanism of antiviral action by the unmetabolised nucleoside analogue. 1-(2-Deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-(6-azauracil) (Figure 1, R = H), which has previously been described by us [2], was devoid of

antiviral activity. Likewise base modification at the 5-position generated the 5-ethyl and 5-propyl-6-azauridine 4′-thio-2′-deoxynucleosides (Figure 1, R = Et/Pr) that also lacked any antiviral activity [1].

This paper describes the synthesis and antiviral evaluation of two novel 4′-thiosugar modified derivatives of 6-azathymidine, namely the dideoxy, 1-(2,3-dideoxy-4-thio-β-D-*erythro*-pentofuranosyl)-(6-azathymidine) (**2**), and the L-nucleoside analogue, 1-(4-thio-β-L-*erythro*-pentofuranosyl)-(6-azathymidine) (**3**) (Figure 1).

Materials and methods

^{1}H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX500 spectrometer operating at 500 and 125 MHz, with Me$_4$Si as internal standard. Mass spectra were determined by the EPSRC mass spectrometry centre (Swansea, UK). Microanalyses were determined by Medac Ltd (Surrey, UK). Flash column chromatography was performed with silica gel 60 (230–400mesh) (Merck) and TLC was carried out

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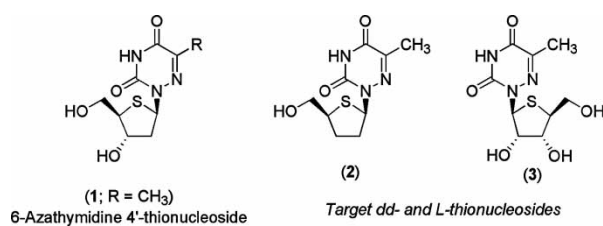
Figure 1. Structures of **1**, **2** & **3**.

on precoated silica plates (kiesel gel 60 F$_{254}$, BDH). Melting points were determined on an electrothermal instrument and are uncorrected. Compounds were visualised by illumination under UV light (254 nm) or by the use of vanillin stain followed by charring on a hotplate. All solvents were dried prior to use as described by the handbook Purification of Laboratory Chemicals [3] and stored over 4 Å molecular sieves, under nitrogen.

Synthesis

5'-O-tert-Butyldimethylsilyl-2'-deoxy-4'-thio-β-D-erythro-pentofuranosyl-1'-(6-azathymine) (4). To a solution of 1-(2-deoxy-4-thio-β-D-*erythro*-pento-furanosyl)-(6-azathymidine) (**1**) [1] (0.30 g, 1.16 mmol) and imidazole (0.20 g, 2.89 mmol) in dry *N,N*-dimethylformamide (DMF) (10 mL) was added a solution of *tert*-butyldimethylsilyl chloride (TBDMSCl) (0.22 g, 1.45 mmol) in dry DMF (2 mL) and the reaction stirred at room temperature under nitrogen for 3.5 h. The reaction mixture was quenched with MeOH (6 mL), diluted with EtOAc (12 mL) and washed with H$_2$O (6 × 12 mL). Then the organic layer was dried (MgSO$_4$), filtered and concentrated under reduced pressure to give the crude product as a pale yellow solid. Purification by column chromatography (EtOAc-petroleum ether 1:1 v/v) yielded 0.23 g (53%) of compound **4** as a white solid. R_f 0.38 (EtOAc-petroleum ether 3:2 v/v); m.p. 36–38 C; ^{1}H-NMR (CDCl$_3$): δ 8.85 (bs, 1, NH), 6.27 (dd, 1, J = 7.6, 3.3 Hz, H-1'), 4.75 (m, 1, OH), 3.84 (dd, 1, J = 10.0, 5.4 Hz, H-5'), 3.73 (t, 1, J = 9.8 Hz, H-5'), 3.35 (quintet, 1, J = 5.2 Hz, H-3'), 2.65 (m, 1, H-4'), 2.57 (ddd, 1, J = 13.6, 5.1, 3.4 Hz, H-2'), 2.37 (dd, 1, J = 13.6, 7.9 Hz, H-2'), 2.26 (s, 3, CH$_3$), 0.88 (s, 9, 3 × CH$_3$-Si), 0.07 (s, 3, CH$_3$-Si), 0.06 (s, 3, CH$_3$-Si). ^{13}C-NMR (CDCl$_3$): δ 156.08 (C=O, C-2), 148.25 (C, C-5), 144.85 (C=O, C-4), 78.43 (CH, C-3'), 67.10 (CH$_2$, C-5'), 62.28 (CH, C-1'), 55.60 (CH, C-4'), 41.31 (CH$_2$, C-2'), 26.28 (CH$_3$, 3 × CH$_3$-Si), 18.67 (C, SiCCH$_3$), 17.08 (CH$_3$), −4.92 (CH$_3$, 2 × CH$_3$-Si). IR$_{Vmax/cm}^{-1}$ (NaCl, film): 1690.1 (C=O). HRMS (ES +) calcd for C$_{15}$H$_{28}$N$_3$O$_4$SSi (M + H)$^+$ 374.1564, found, 374.1565.

3'-O-Thiocarbonylimidazole-5'-O-tert-butyldimethyl-silyl-2'-deoxy-4'-thio-β-D-erythro-pentofuranosyl-1'-(6-azathymine) (5). Thiocarbonyldiimidazole (0.43 g, 2.40 mmol) was added to a solution of **4** (0.45 g, 1.20 mmol) in dry CH$_2$Cl$_2$ (35 mL) and the reaction stirred at 40°C for 8 h and at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure to give the crude product as a yellow syrup. Purification by column chromatography (EtOAc-petroleum ether 1:1 v/v) yielded 0.80 g (69%) of compound **5** as a white solid. R_f 0.24 (EtOAc-petroleum ether 1:1 v/v); m.p. 160°C (sharp); ^{1}H-NMR (CDCl$_3$): δ 9.17 (bs, 1, NH), 8.36 (s, 1, H-5''), 7.61 (s, 1, H-2''), 7.04 (s, 1, H-3''), 6.47 (t, 1, J = 6.6 Hz, H-1'), 6.40 (q, 1, J = 4.0 Hz, H-3'), 3.79 (d, 2, J = 8.9 Hz, H-5'), 3.72 (q, 1, J = 8.0 Hz, H-4'), 3.00 (dd, 1, J = 15, 5.3 Hz, H-2'), 2.61 (ddd, 1, J = 14.3, 7.3, 4.7 Hz, H-2'), 2.29 (s, 3, CH$_3$), 0.85 (s, 9, 3 × CH$_3$-Si), 0.03 (s, 6, 2 × CH$_3$-Si). ^{13}C-NMR (CDCl$_3$): δ 183.24 (C=S, OCSN), 156.40 (C=O, C-2), 148.78 (C, C-5), 148.78 (C=O, C-4), 137.43 (CH, C-3''), 131.25 (CH, C-2''), 118.44 (CH, C-5''), 87.05 (CH, C-3'), 65.57 (CH$_2$, C-5'), 62.87 (CH, C-1'), 54.62 (CH, C-4'), 37.56 (CH$_2$, C-2'), 26.22 (CH$_3$, 3 × CH$_3$-Si), 18.67 (C, SiCCH$_3$), 17.15 (CH$_3$), −4.90 (CH$_3$, CH$_3$-Si), −4.98 (CH$_3$, CH$_3$-Si). IR$_{Vmax/cm}^{-1}$ (NaCl, film): 3423.0 (N-H stretch), 1701.7 (C=O), 1388.0-1232.0 (C-O stretch). Anal. Calcd. for C$_{19}$H$_{29}$N$_5$O$_4$S$_2$Si (483.6747): C, 47.18%, H, 6.04%, N, 14.48%. Found: C, 47.05%, H, 6.12%, N, 14.35%.

5'-O-tert-Butyldimethylsilyl-2',3'-dideoxy-4'-thio-β-D-erythro-pentofuranosyl-1'-(6-azathymine) (6). Tributyltin hydride (0.13 g, 0.45 mmol) and 1,1'-azobis(cyclohexene-carbonitrile) (0.07 g, 0.30 mmol) was added to a solution of **5** (0.15 g, 0.31 mmol) in dry toluene (14 mL) and stirred at 100°C for 30 min. The reaction mixture was concentrated under reduced pressure to give the crude product as a clear syrup. Purification by column chromatography (EtOAc-petroleum ether 3:7 v/v) yielded 0.07 g (64%) of compound **6** as a clear syrup. R_f 0.77 (EtOAc-petroleum ether 1:1 v/v); ^{1}H-NMR (CDCl$_3$): δ 8.56 (bs, 1, NH), 5.57 (t, 1, J = 4.0 Hz, H-1'), 3.93 (q, 2, J = 7.3 Hz, H-3'), 3.75 (dd, 1, J = 10.0, 7.9 Hz, H-5'), 3.67 (dd, 1, J = 10.0, 6.4 Hz, H-5'), 3.52 (quintet, 1, J = 7.0 Hz, H-4'), 2.43 (m, 1, H-2'), 2.38 (m, 1, H-2'), 1.51 (s, 3, CH$_3$), 0.84 (s, 9, 3 × CH$_3$-Si), 0.02 (s, 3, CH$_3$-Si), 0.00 (s, 3, CH$_3$-Si). ^{13}C-NMR (CDCl$_3$): δ 156.34 (C, C-5), 148.78 (C=O, C-2), 143.94 (C=O, C-4), 66.86 (CH$_2$, C-5'), 65.44 (CH, C-1'), 52.20 (CH, C-4''), 34.55 (CH$_2$, C-2'), 31.94 (CH$_2$, C-3'), 25.95 (CH$_3$, CH$_3$-Si), 25.88 (CH$_3$, CH$_3$-Si), 25.85 (CH$_3$, CH$_3$-Si), 18.40 (C, SiCCH$_3$), 16.20 (CH$_3$), −5.20 (CH$_3$, CH$_3$-Si), −5.29 (CH$_3$, CH$_3$-Si). IR$_{Vmax/cm}^{-1}$ (NaCl, film): 3450.7 (N-H stretch), 1651.6 (C=O), 1462.3-1100.1

(C–O stretch). HRMS (ES+) calcd for $C_{15}H_{28}N_3O_3SSi$ (M + H)$^+$358.1615, found, 358.1614.

2′,3′-Dideoxy-4′-thio-β-D-erythro-pentofuranosyl-1′-(6-azathymine) (**2**). To a solution of **6** (0.13 g, 0.36 mmol) in MeOH (10 mL), was added Dowex 50 W (H$^+$) (0.50 g) [Dowex 50 W (H$^+$) was washed with 10% aq. HCl (10 mL), MeOH (10 mL), CH_2Cl_2 (10 mL) and another 10% aq. HCl (10 mL) prior to use] and the reaction mixture was stirred at room temperature for 24 h. The resulting light yellow solution was filtered to remove Dowex 50W (H$^+$) and washed with MeOH. The filtrate was concentrated *in vacuo* to give the crude product as a light yellow syrup. Purification by column chromatography (MeOH-CH_2Cl_2 1:19 v/v) yielded 0.06 g (67%) of compound **2** as a clear syrup. R_f 0.28 (EtOAc-petroleum ether 1:1 v/v); ^{1}H-NMR (DMSO-d$_6$): δ 12.01 (bs, 1, NH), 6.15 (dd, 1, J = 7.0, 3.5 Hz, H-1′), 4.90 (bs, 1, OH), 3.81 (m, 1, H-3′), 3.60 (m, 1, H-3′), 3.48 (dd, 1, J = 13.6, 6.9 Hz, H-5′), 3.44 (m, 1, H-5′), 3.38 (m, 1, H-4′), 2.37 (m, 1, H-2′), 2.24 (m, 1, H-3′), 2.08 (s, 3, CH$_3$). ^{13}C-NMR (DMSO-d$_6$): δ 148.47 (C, C-5), 143.23 (2 × C=O, C-2 & C-4), 65.35 (CH$_2$, C-5′), 64.38 (CH, C-1′), 52.31 (CH, C-4′), 33.91 (CH$_2$, C-2′), 31.82 (CH$_2$, C-3′), 16.41 (CH$_3$). LRMS (CI+) *m/z*: 117.0 (M-heterocyclic base)$^+$, 128.0 (M-2,3-dideoxy-thiosugar)$^+$, 244.1 (M + H)$^+$. HRMS (ES+) calcd for $C_9H_{14}N_3O_3S$ (M + H)$^+$244.0750, found, 244.0751.

2′,3′,5′-Tri-O-acetyl-4′-thio-β-L-6-azathymidine (**8**). To a suspension of 6-azathymine (0.17 g, 1.35 mmol) in dry CH$_3$CN (5 mL) was added BSA (0.67 mL, 2.70 mmol) and the reaction mixture was stirred at room temperature under nitrogen for 30 min. A solution of 1,2,3,5-tetra-O-acetyl-4-thio-β-L-ribofuranose **7** [4] (0.52 g, 1.55 mmol) in dry CH$_3$CN (5 mL) was then added. The reaction mixture was cooled in an ice bath before TMSOTf (0.57 mL, 2.56 mmol) was added dropwise. The reaction mixture was stirred at 50°C under nitrogen for 8 h, then at room temperature for 12 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous sodium bicarbonate (2 × 30 mL). The organic layer was dried (MgSO$_4$) and concentrated under reduced pressure to give a yellow syrup. Purification by column chromatography (EtOAc-petroleum ether 2:3 v/v) yielded 0.35 g (65%) of compound **8** as a thick syrup. R_f 0.62 (EtOAc-petroleum ether 2:1 v/v); ^{1}H-NMR (CDCl$_3$): δ 8.86 (bs, 1, NH), 6.25 (d, 1, J = 4.4 Hz, H-1′), 5.83 (t, 1, J = 4.2 Hz, H-2′), 5.73 (dd, 1, J = 5.6, 4.1 Hz, H-3′), 4.40 (dd, 1, J = 11.6, 6.8 Hz, H-5′), 4.31 (dd, 1, J = 11.6, 6.6 Hz, H-5′),

3.77 (q, 1, J = 6.3 Hz, H-4′), 2.14 (s, 3, CH$_3$Ac), 2.12 (s, 3, CH$_3$Ac), 2.11 (s, 3, CH$_3$Ac), 2.07 (s, 3, CH$_3$). ^{13}C-NMR (CDCl$_3$): δ 170.46 (C=O, Ac), 169.81 (C=O, Ac), 169.64 (C=O, Ac), 155.46 (C=O, C-2), 147.94 (C=O, C-5), 145.54 (C, C-4), 75.56 (CH, C-2′), 74.07 (CH, C-3′), 64.70 (CH$_2$, C-5′), 64.01 (CH, C-1′), 47.10 (CH, C-4′), 20.64 (CH$_3$, CH$_3$Ac), 20.42 (CH$_3$, CH$_3$Ac), 16.67 (CH$_3$, CH$_3$Ac), 14.20 (CH$_3$). IR$_{Vmax/cm}^{-1}$ (NaCl, film): 1642.7 (C=O), 1226.8 (C–O stretch). HRMS (ES+) calcd for $C_{15}H_{20}O_8N_3S$ (M + H)$^+$ 419.1231, found, 419.1229.

4′-Thio-β-L-6-azathymidine (**3**). Aqueous methylamine (7 mL) was added to compound **8** (0.30 g, 0.75 mmol) and the resulting clear solution was stirred at 50°C for 30 min. On completion, the clear solution changed colour to a light brown solution and was concentrated *in vacuo* to give a thick light brown syrup. Purification by column chromatography (MeOH-CH_2Cl_2 1:9 v/v) yielded 0.19 g (90%) of compound **3** as a light yellow syrup. R_f 0.46 (MeOH-CH_2Cl_2 1:9 v/v); ^{1}H-NMR (DMSO-d$_6$): δ 12.13 (bs, 1, NH), 5.87 (d, 1, J = 5.5 Hz, H-1′), 5.32 (d, 1, J = 5.0 Hz, OH, H-2′), 5.13 (d, 1, J = 4.8 Hz, OH, H-3′), 4.97 (m, 1, OH, H-5′), 4.36 (q, 1, J = 3.9 Hz, H-2′), 4.18 (q, 1, J = 3.8 Hz, H-3′), 3.69 (ddd, 1, J = 13.2, 6.6, 3.9 Hz, H-5′), 3.45 (ddd, 1, J = 17.9, 10.6, 6.5 Hz, H-5′), 3.24 (ddd, 1, J = 14.4, 7.0, 4.4 Hz, H-4′), 2.12 (s, 3, CH$_3$). ^{13}C-NMR (DMSO-d$_6$): δ 156.53 (C=O, C-2), 148.89 (C, C-5), 143.69 (C=O, C-4), 75.63 (CH, C-2′), 73.54 (CH, C-3′), 64.65 (CH, C-1′), 64.40 (CH$_2$, C-5′), 53.16 (CH, C-4′), 16.45 (CH$_3$). LRMS (ES+) *m/z*: 298.05 (M + Na)$^+$; LRMS (ES-) *m/z*: 274.1 (M−H)$^+$, 275.1 (M)$^+$. HRMS (ES+) calcd for $C_9H_{17}N_4O_5S$ (M + NH$_4$)$^+$293.0914, found, 293.0912.

Thymidine kinase assay [1]

The radiolabeled substrate [methyl-^{3}H]dThd (70 Ci/mmol) was obtained from Amersham Pharmacia Biotech. The thymidine kinase activity using purified cytosolic TK-1, recombinant mitochondrial TK-2, recombinant herpes simplex virus type 1 TK, and recombinant varicella-zoster virus TK was assayed in a 50 μL reaction mixture containing 50 mM Tris HCl, pH 8.0, 2.5 mM MgCl$_2$, 10 mM DTT, 0.5 mM CHAPS, 3 mg/mL bovine serum albumin, 2.5 mM ATP, 1 μM [methyl-^{3}H]dThd, and varying concentrations of compound **2** or **3** and enzyme. The samples were incubated at 37°C for 30 min. Aliquots of 45 μL of the reaction mixtures were spotted on Whatman DE-81 filter paper disks. The filters were washed three times for 5 min in 1 mM HCOONH$_4$ and once for 5 min in ethanol. The radioactivity was determined by scintillation counting.

Cell cultures

The antiviral assays were based on an inhibition of virus-induced cytopathicity in either E_6SM, HeLa, Vero, or HEL cell cultures, following previously established procedures [5–8]. Briefly, confluent cell cultures in 96-well microtiter plates were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100,... μg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

The following viruses were included in the study: herpes simplex virus type 1 (HSV-1, strain KOS), HSV-2 (strain G), a thymidine kinase (TK)-deficient HSV-1 strain (HSV-1/TK-ACVr), vaccinia virus and vesicular stomatitis virus (VSV) in E_6SM cell cultures, cytomegalovirus (strain AD169 and Davis), varicella-zoster virus (strains YS and OKA) and TK-deficient VZV (strains 07/1 and YS/R) in HEL cell cultures, human immunodeficiency virus (HIV) type 1 and 2, vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytial virus (RSV) in HeLa cell cultures, and parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, and Punta Toro virus in Vero cell cultures.

Results and discussion

Chemistry

Synthesis of the 2′,3′-dideoxy-4′-thionucleoside (**2**) was achieved using the Barton-McCombie deoxygenation procedure [9] (Scheme 1), a method previously employed successfully with 4′-thionucleosides [10]. 1-(2-Deoxy-4-thio-β-D-*erythro*-pentofuranosyl)-(6-azathymidine) (**1**) [1] was selectively silylated at the 5′-position on reaction with TBDMSCl and imidazole in dry DMF [11]. The 5′-protected nucleoside (**4**) was then transformed into a 3′-thiocarbonyl derivative (**5**) by reaction with thiocarbonyldiimidazole in dry CH_2Cl_2 at 40°C for 8 h and then at room temperature overnight. Radical deoxygenation was achieved on treatment of **5** with tributyltin hydride and the radical initiator, 1,1′-azobis(cyclohexane carbonitrile) at 100°C for 30 min to give the 2′,3′-dideoxy nucleoside (**6**) in 64% yield. Removal of the 5′-protecting group using TBAF was successful as shown by t.l.c. However, removal of the excess TBAF proved problematic, with traces still observed on NMR after chromatographic purification. Treatment with Dowex 50W (H^+) however gave the required product (**2**) cleanly in 67% yield.

Synthesis of the L-4′-thionucleoside (**3**) involved coupling of the L-thiosugar (**7**), prepared as previously reported from D-lyxose [4], with 6-azathymine using the Vorbrüggen coupling procedure (Scheme 2) [12] 6-Azathymine was silylated using bis(trimethylsilyl)acetamide (BSA), the silylated pyrimidine was then reacted with the L-thiosugar in the presence of the Lewis acid catalyst trimethylsilyl trifluoromethane sulfonate (TMSOTf) at 50°C for 12 h to give the β-L-thionucleoside (**8**). Deacylation of the protected β-L-thionucleoside (**8**) with aqueous methylamine at 50°C for 30 min gave the required product (**3**) in 90% yield.
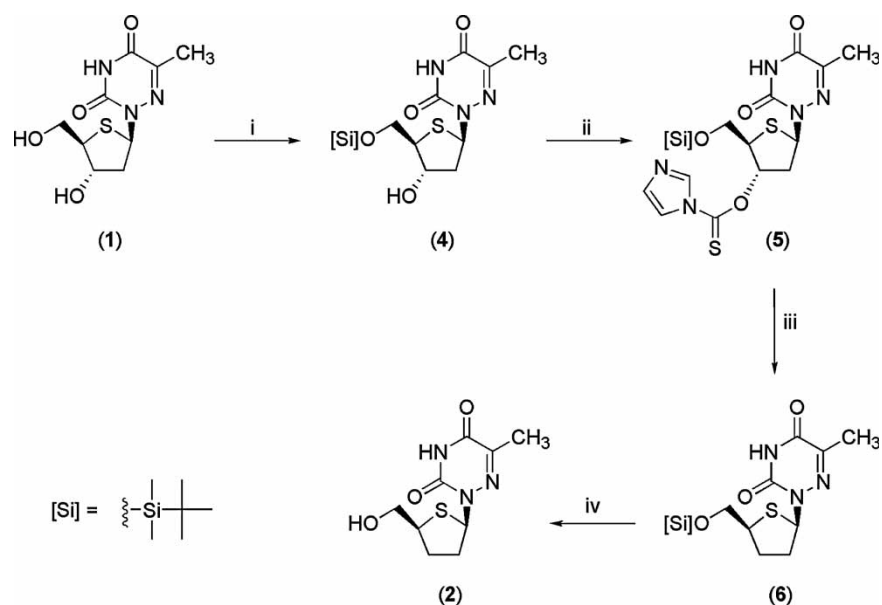
Scheme 1. *Reagents and Conditions*: (i) TBDMSCl, imidazole, DMF, 3.5 h (ii) thiocarbonyldiimidazole, CH_2Cl_2, 40°C, 8 h then r.t. 12 h (iii) Bu_3SnH, 1,1′-azobis(cyclohexene-carbonitrile), toluene, 100°C, 30 min (iv) Dowex 50W (H^+), MeOH, 24 h.
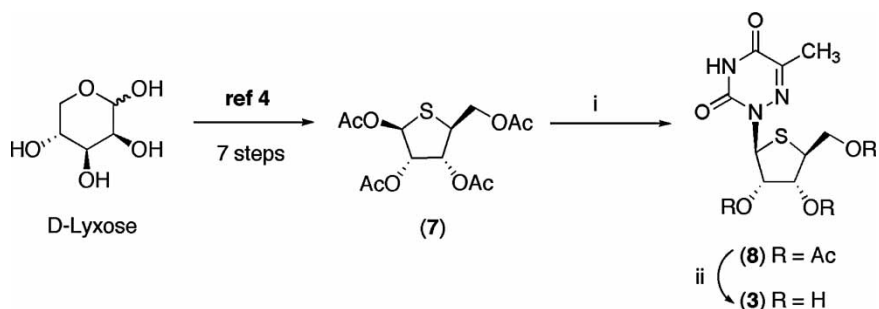
Scheme 2. *Reagents and Conditions*: (i) 6-Azathymine, BSA, CH$_3$CN, 30 min then 7, TMSOTf, CH$_3$CN, 50°C, 12 h (ii) CH$_3$NH$_2$ (35% aqueous), 50°C, 30 min.

Table I.   Cytotoxicity and antiviral activity of novel 4'thionucleosides **2** and **3**.

Compound	Minimum Inhibitory Concentration[a] (µM)				
	HSV-1 (KOS)	HSV-2 (G)	HSV-1/TK$^-$	Vaccinia virus	MCC[b] (µM)
1 [1]	1.3	3.2	18	2.2	>200
2	12 ± 0	12 ± 0	12 ± 0	12 ± 0	100
3	>100	>100	>100	>100	>100
Acyclovir	0.4	0.4	150	>250	>250
Ganciclovir	0.032	0.032	50	>100	>100
Brivudin	0.048	30	250	10	>250

[a] Required to reduce virus-induced cytopathogenicity by 50%; [b] Required to cause a microscopically detectable alteration of normal cell morphology

Antiviral activity

The L-thionucleoside (**3**) was devoid of antiviral activity, however the dideoxy-thionucleoside (**2**) was moderately active against vaccinia virus (MIC 12 µM) and herpes simplex virus strains, HSV-1 (strain KOS) and HSV-2 (strain G) (MIC 12 µM) (Table I). Interestingly, it fully retained inhibitory activity against the thymidine kinase-deficient HSV-1 TK$^-$ strain, suggesting that **2** is not dependent on viral TK-catalysed phosphorylation for antiviral activity and/or may use an alternative metabolic activation pathway. This result is consistent with the (partial) retention of inhibitory activity *vs* HSV-1 TK$^-$ observed for 1-(2-deoxy-4-thio-β-D-*erythro*-pento-furanosyl)-(6-azathymidine) (**1**) [1].

In fact, compound **2** had poor, if any affinity for cytosolic TK-1 and mitochondrial TK-2, but it showed an IC$_{50}$ of 34 µM and 427 µM for HSV-1 TK- and VZV TK-catalysed dThd (1 µM) phosphorylation (Table II). Therefore, HSV-1

TK-directed phosphorylation of **2** cannot be excluded but it is unclear at this moment to what extent the metabolism of the compound, if it occurs at all, contributes to the eventual antiviral activity.

The compounds were also evaluated against a broad range of other viruses, including varicella zoster virus, cytomegalovirus, human immunodeficiency virus types 1 and 2, reovirus-1, Coxsackie virus B4, Sindbis virus, parainfluenza-3 virus, Punta Toro virus, vesicular stomatitis virus, and respiratory syncytial virus, but were found to be inactive at subtoxic concentrations.

Acknowledgements

Table II.   Thymidine kinase affinity of dideoxynucleoside **2**.

Compound	IC$_{50}$[a] (µM)			
	TK-1	TK-2	HSV-1 TK	VZV TK
2	> 500	> 500	34 ± 2	427 ± 27

[a] 50% Inhibitory concentration required to inhibit dThd kinase-catalyzed conversion of [^{3}H]dThd (1 µM) to its 5'-monophosphate derivative.

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