

# Synthesis of 3-fluoro-6-S-(2-S-pyridyl) nucleosides as potential lead cytostatic agents

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## ABSTRACT

The 3-deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio-β-D-glucopyranosyl nucleoside analogs **7** were prepared via two facile synthetic routes. Their precursors, 3-fluoro-6-thio-glucopyranosyl nucleosides **5a–e**, were obtained by the sequence of deacetylation of 3-deoxy-3-fluoro-β-D-glucopyranosyl nucleosides **2a–e**, selective tosylation of the primary OH of **3** and finally treatment with potassium thioacetate. The desired thiopyridine protected analogs **7a–c,f,g** were obtained by the sequence of deacetylation of **5a–c** followed by thiopyridinylation and/or condensation of the corresponding heterocyclic bases with the newly synthesized peracetylated 6-S-(2-S-pyridyl) sugar precursor **13**, which was obtained via a novel synthetic route from glycosyl donor **12**. None of the compounds **6** and **7** showed antiviral activity, but the 5-fluorouracil derivative **7c** and particularly the uracil derivative **7b** were endowed with an interesting and selective cytostatic action against a variety of murine and human tumor cell cultures.

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## 1. Introduction

Nucleoside analogs markedly contribute to the chemotherapy of cancer and viral diseases [1–4]. However, novel applications are still being explored and new nucleoside structures have reached the clinic as effective agents for the treatment of AIDS [5], herpes virus infections [6] and viral hepatitis [7].

Fluorine-containing nucleosides and their analogs are attractive compounds and have drawn special attention [8–11], with the fluorine incorporated either on the base moiety or on the sugar part frequently leading to a drastic change in biological activity, stability and bioavailability [8,12,13]. Our recent data have revealed, that new classes of uncommon fluorinated pyranonucleosides have a promising potential in combating the rotaviral infections, in the treatment of colon cancer and are efficient antitumor growth inhibitors [10,11,14,15]. Experimental data also suggested, that human Poly(A)-specific ribonuclease is among the molecular targets of these compounds and could act therapeutically by lowering the mRNA turnover rate [16].

In the field of nucleosides, sulfur chemistry appears particularly fruitful in the development of therapeutic agents [17,18]. Considerable interest has been drawn in the synthesis of thionucleoside analogs [18–21] and nucleosidyl disulfides [22–26], attractive

candidates for use as both antiviral and antineoplastic agents [21,24,27], with the latter being assessed as prodrugs able to interfere with HIV reverse transcription [21,22,26]. Particularly, nucleosides in which the primary hydroxyl group is replaced by a thiol- or an alkylthio-group, are promising antiviral agents since they disrupt essential viral macromolecular methylation processes [28,29].

In view of the above data and our recent findings on the biology of modified nucleosides, as templates for antiviral and anticancer activity [10,11,14–16,30–32], we turned our attention to the possible improvement of the biological properties of such molecules. We thus synthesised a series of glucopyranosyl nucleoside analogs, where we combined both a pharmacologically compatible fluorine and a sulfur/thiopyridine moiety in the sugar part. In this paper, we report on two efficient methods for their preparation and supply data on their biological activity.

## 2. Experimental

### 2.1. General methods

Melting points were recorded in a Mel-Temp apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on Merck precoated 60F<sub>254</sub> plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with sulfuric acid. Flash column chromatography was performed using silica gel (240–400 mesh, Merck). <sup>1</sup>H, <sup>19</sup>F and <sup>13</sup>C NMR spectra were obtained at room temperature with a Bruker 400

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spectrometer at 400, 376 and 100 MHz, respectively, using  $\text{CDCl}_3$  and methanol- $d_4$  ( $\text{CD}_3\text{OD}$ ) with internal tetramethylsilane (TMS) for  $^1\text{H}$  and  $^{13}\text{C}$  and internal trifluorotoluene (TFT) for  $^{19}\text{F}$ .

The chemical shifts are expressed in parts per million ( $\delta$ ) and following abbreviations were used: s = singlet, d = doublet, dd = doublet doublet, dtr = doublet triplet and m = multiplet. Mass spectra were obtained with a Micromass Platform LC (ESI-MS). Infrared spectra were obtained with a Perkin–Elmer Model 1600 FT-IR spectrophotometer. Optical rotations were measured using an Autopol I polarimeter. All reactions were carried out in dry solvents. Acetonitrile was distilled from calcium hydride and stored over 3E molecular sieves. Dimethylformamide (DMF) was also stored over 3E molecular sieves, and pyridine was stored over potassium hydroxide pellets.

## 2.2. Synthesis of 1-[3-deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl] nucleosides (**7a–c**) from **5a–c** and/or **6a–c**

### 2.2.1. 1-(3-Deoxy-3-fluoro- $\beta$ -D-glucopyranosyl)cytosine (**3f**)

A mixture of methanolic ammonia (140 mL) and  $N^4$ -benzoyl-cytosine derivative **2d** [11] (1.70 g, 3.36 mmol) stirred for 4 h at room temperature. The reaction mixture was concentrated and crude **3f** was obtained (0.83 g, 90%,  $R_f$  = 0.15 in AcOEt) as colorless oil. Product **3f** was used without further purification.  $[\alpha]_D^{22} + 5.00$  (c 0.1, MeOH);  $\lambda_{\text{max}}$  268 nm ( $\epsilon$  7162); ESI-MS ( $m/z$ ): 276.26 ( $M+H^+$ ).

### 2.2.2. 9-(3-Deoxy-3-fluoro- $\beta$ -D-glucopyranosyl)adenine (**3g**)

Adenine derivative **3g** was synthesized from  $N^6$ -benzoyl adenine derivative **2e** [11] by the similar procedure as described for **3f**. Compound **3g** was obtained (0.85 g, 88%,  $R_f$  = 0.12 in AcOEt) as colorless oil and it was used without further purification.  $[\alpha]_D^{22} + 4.00$  (c 0.1, MeOH);  $\lambda_{\text{max}}$  259 nm ( $\epsilon$  4930); ESI-MS ( $m/z$ ): 300.27 ( $M+H^+$ ).

### 2.2.3. 1-(2,4-Di-O-acetyl-3-deoxy-3-fluoro-6-O-p-toluenesulfonyl- $\beta$ -D-glucopyranosyl)thymine (**4a**)

A solution of compound **3a** [15] (1.10 g, 3.70 mmol) in 4.50 mL of dry pyridine was cooled to 0 °C, and *p*-toluenesulfonyl chloride (1.10 g, 5.58 mmol) in 4.50 mL of dry pyridine was added dropwise with stirring. The reaction mixture was stored at room temperature overnight, and then was concentrated. The residual gum was dissolved in pyridine (49 mL), acetic anhydride was added (25 mL) and the resulted mixture stirred for 3 h at room temperature. MeOH (0.40 mL) was added to quench the reaction and the mixture was concentrated under high vacuum to remove the solvents. The mixture was extracted with two 200 mL portions of ethyl acetate and neutralized with aqueous  $\text{NaHCO}_3$ . The organic layer was washed with  $\text{NaHSO}_4$  (20 mL), dried over anhydrous sodium sulfate and evaporated to dryness. Purification by flash chromatography (hexane/AcOEt, 4:6), gave **4a** (1.20 g, 62%,  $R_f$  = 0.35 in hexane/AcOEt, 4:6) as white solid. m.p. 145–147 °C;  $[\alpha]_D^{22} + 10.52$  (c 0.1,  $\text{CHCl}_3$ );  $\lambda_{\text{max}}$  260 nm ( $\epsilon$  8836);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  8.60 (br s, NH), 7.37–7.20 (m, 4H, tosyl group), 7.19 (s, 1H, H-6), 5.75 (d, 1H,  $J_{1',2'} = 9.5$  Hz, H-1'), 5.29–5.20 (m, 2H, H-2' and H-4'), 4.70 (dtr, 1H,  $J_{F,3'} = 51.7$  Hz,  $J_{2',3'} = 9.1$ ,  $J_{3',4'} = 9.0$  Hz, H-3'), 4.21–4.07 (m, 2H, H-6a',6b'), 3.84 (m, 1H, H-5'), 2.45 (s, 3H,  $\text{ArCH}_3$ ), 2.10 and 2.05 (2s, 6H, 2OAc), 1.96 (s, 3H, 5- $\text{CH}_3$ ); Anal. Calcd for  $\text{C}_{22}\text{H}_{25}\text{FN}_2\text{O}_{10}\text{S}$ : C, 50.00; H, 4.77; N, 5.30. Found: C, 50.26; H, 4.92; N, 5.47. ESI-MS ( $m/z$ ): 529.51 ( $M+H^+$ ).

### 2.2.4. 1-(2,4-Di-O-acetyl-3-deoxy-3-fluoro-6-O-p-toluenesulfonyl- $\beta$ -D-glucopyranosyl)uracil (**4b**)

Uracil derivative **4b** was synthesized from **3b** [15] by the similar procedure as described for **4a**. Purified by flash chromatography (hexane/AcOEt, 3:7) compound **4b** was obtained (1.10 g, 58%,

$R_f$  = 0.4 in hexane/AcOEt, 3:7) as white solid. m.p. 175–177 °C;  $[\alpha]_D^{22} + 5.26$  (c 0.1,  $\text{CHCl}_3$ );  $\lambda_{\text{max}}$  260 nm ( $\epsilon$  6760);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  8.20 (br s, NH), 7.76 (d, 1H,  $J_{5,6} = 8.2$  Hz, H-6), 7.38–7.22 (m, 4H, tosyl group), 5.79 (d, 1H, H-5), 5.73 (d, 1H,  $J_{1',2'} = 9.6$  Hz, H-1'), 5.26–5.15 (m, 2H, H-2' and H-4'), 4.70 (dtr, 1H,  $J_{F,3'} = 51.7$  Hz,  $J_{2',3'} = J_{3',4'} = 9.0$  Hz, H-3'), 4.22–4.06 (m, 2H, H-6a',6b'), 3.85 (m, 1H, H-5'), 2.45 (s, 3H,  $\text{ArCH}_3$ ), 2.10 and 2.06 (2s, 6H, 2OAc); Anal. Calcd for  $\text{C}_{21}\text{H}_{23}\text{FN}_2\text{O}_{10}\text{S}$ : C, 49.03; H, 4.51; N, 5.45. Found: C, 49.19; H, 4.65; N, 5.62. ESI-MS ( $m/z$ ): 515.49 ( $M+H^+$ ).

### 2.2.5. 1-(2,4-Di-O-acetyl-3-deoxy-3-fluoro-6-O-p-toluenesulfonyl- $\beta$ -D-glucopyranosyl)5-fluorouracil (**4c**)

5-Fluorouracil derivative **4c** was synthesized from **3c** [15] by the similar procedure as described for **4a**. Purified by flash chromatography (hexane/AcOEt, 4:6) derivative **4c** was obtained (1.18 g, 60%,  $R_f$  = 0.4 in hexane/AcOEt, 4:6) as white solid. m.p. 198–200 °C;  $[\alpha]_D^{22} + 5.32$  (c 0.1,  $\text{CHCl}_3$ );  $\lambda_{\text{max}}$  260 nm ( $\epsilon$  7486);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  8.28 (br s, NH), 7.50 (d, 1H,  $J_{6,F5} = 5.85$  Hz, H-6), 7.37–7.20 (m, 4H, tosyl group), 5.74 (d, 1H,  $J_{1',2'} = 9.4$  Hz, H-1'), 5.27–5.15 (m, 2H, H-2' and H-4'), 4.75 (dtr, 1H,  $J_{F,3'} = 51.5$  Hz,  $J_{2',3'} = 9.2$ ,  $J_{3',4'} = 9.0$  Hz, H-3'), 4.21–4.06 (m, 2H, H-6a',6b'), 3.90 (m, 1H, H-5'), 2.45 (s, 3H,  $\text{ArCH}_3$ ), 2.11 and 2.07 (2s, 6H, 2OAc); Anal. Calcd for  $\text{C}_{21}\text{H}_{22}\text{F}_2\text{N}_2\text{O}_{10}\text{S}$ : C, 47.37; H, 4.16; N, 5.26. Found: C, 47.54; H, 4.29; N, 5.42. ESI-MS ( $m/z$ ): 533.48 ( $M+H^+$ ).

### 2.2.6. 1-(2,4-Di-O-acetyl-3-deoxy-3-fluoro-6-O-p-toluenesulfonyl- $\beta$ -D-glucopyranosyl)- $N^4$ -benzoyl cytosine (**4d**)

$N^4$ -benzoyl cytosine derivative **4d** was synthesized from **3d** [11] by the similar procedure as described for **4a**. Purified by flash chromatography (hexane/AcOEt, 4:6) derivative **4d** was obtained (1.41 g, 62%,  $R_f$  = 0.4 in hexane/AcOEt, 4:6) as white foam.  $[\alpha]_D^{22} + 12.00$  (c 0.5,  $\text{CHCl}_3$ );  $\lambda_{\text{max}}$  263 nm ( $\epsilon$  21,906);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  8.66 (br s, NH), 7.88–7.31 (m, 11H, Bz, H-6, H-5 and tosyl group), 6.01 (d, 1H,  $J_{1',2'} = 9.4$  Hz, H-1'), 5.30–5.14 (m, 2H, H-2' and H-4'), 4.74 (dtr, 1H,  $J_{F,3'} = 51.6$  Hz,  $J_{2',3'} = 9.1$ ,  $J_{3',4'} = 9.0$  Hz, H-3'), 4.24–4.08 (m, 2H, H-6a',6b'), 3.86 (m, 1H, H-5'), 2.45 (s, 3H,  $\text{ArCH}_3$ ), 2.12 and 2.04 (2s, 6H, 2OAc); Anal. Calcd for  $\text{C}_{28}\text{H}_{28}\text{FN}_3\text{O}_{10}\text{S}$ : C, 54.45; H, 4.57; N, 6.80. Found: C, 54.84; H, 4.29; N, 6.62. ESI-MS ( $m/z$ ): 618.62 ( $M+H^+$ ).

### 2.2.7. 9-(2,4-Di-O-acetyl-3-deoxy-3-fluoro-6-O-p-toluenesulfonyl- $\beta$ -D-glucopyranosyl)- $N^6$ -benzoyl adenine (**4e**)

$N^6$ -benzoyl adenine derivative **4e** was synthesized from **3e** [11] by the similar procedure as described for **4a**. Purified by flash chromatography (hexane/AcOEt, 3:7) compound **4e** was obtained (1.52 g, 64%,  $R_f$  = 0.3 in hexane/AcOEt, 3:7) as white foam.  $[\alpha]_D^{22} - 2.00$  (c 0.2,  $\text{CHCl}_3$ );  $\lambda_{\text{max}}$  278 nm ( $\epsilon$  17,393);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  9.05 (br s, NH), 8.84 and 8.15 (2s, 2H, H-2,8), 8.14–7.51 (m, 9H, Bz and tosyl group), 5.84 (d, 1H,  $J_{1',2'} = 9.5$  Hz, H-1'), 5.64 (m, 1H, H-2'), 5.35 (m, 1H, H-4'), 4.81 (dtr, 1H,  $J_{F,3'} = 51.5$  Hz,  $J_{2',3'} = J_{3',4'} = 9.1$  Hz, H-3'), 4.23–4.11 (m, 2H, H-6a',6b'), 4.01 (m, 1H, H-5'), 2.42 (s, 3H,  $\text{ArCH}_3$ ), 2.16 and 1.84 (2s, 6H, 2OAc); Anal. Calcd for  $\text{C}_{29}\text{H}_{28}\text{FN}_5\text{O}_9\text{S}$ : C, 54.29; H, 4.40; N, 10.92. Found: C, 54.34; H, 4.59; N, 10.78. ESI-MS ( $m/z$ ): 642.64 ( $M+H^+$ ).

### 2.2.8. 1-(2,4-Di-O-acetyl-6-S-acetyl-3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)thymine (**5a**)

The tosylate **4a** (1.20 g, 2.30 mmol) was heated with potassium thioacetate (0.36 g, 3.10 mmol) in DMF (8.70 mL) at 100 °C for 2 h. The reaction mixture was neutralized with aqueous  $\text{NaHCO}_3$ . After that, the mixture was concentrated under high vacuum pump to eliminate the DMF. The residue was partitioned between water and EtOAc, the organic extract was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was purified by flash chromatography (hexane/AcOEt, 4:6) to give compound **5a** (0.75 g, 78%,  $R_f$  = 0.4 in hexane/AcOEt, 4:6) as white solid.

m.p. 204–206 °C;  $[\alpha]_D^{22} + 5.21$  (c 0.1, CHCl<sub>3</sub>);  $\lambda_{\max}$  260 nm ( $\epsilon$  9219); IR (Nujol): 1697 (SAC) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.10 (br s, NH), 7.12 (s, 1H, H-6), 5.75 (d, 1H,  $J_{1',2'} = 9.5$  Hz, H-1'), 5.29–5.18 (m, 2H, H-2' and H-4'), 4.71 (dtr, 1H,  $J_{F,3'} = 51.8$  Hz,  $J_{2',3'} = 9.1$  Hz,  $J_{3',4'} = 9.0$  Hz, H-3'), 3.78 (m, 1H, H-5'), 3.30–3.11 (m, 2H, H-6a', 6b'), 2.37 (s, 3H, SAC), 2.21 and 2.08 (2s, 6H, 2OAc), 1.98 (s, 3H, 5-CH<sub>3</sub>); Anal. Calcd for C<sub>17</sub>H<sub>21</sub>FN<sub>2</sub>O<sub>8</sub>S: C, 47.22; H, 4.89; N, 6.48. Found: C, 47.39; H, 4.78; N, 6.54. ESI-MS ( $m/z$ ): 433.44 (M+H<sup>+</sup>).

#### 2.2.9. 1-(2,4-Di-O-acetyl-6-S-acetyl-3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)uracil (**5b**)

Uracil derivative **5b** was synthesized from **4b** by the similar procedure as described for **5a**. Purified by flash chromatography (hexane/AcOEt, 3:7) and obtained (0.64 g, 76%,  $R_f = 0.45$  in hexane/AcOEt, 3:7) as white solid. m.p. 158–160 °C;  $[\alpha]_D^{22} + 5.18$  (c 0.1, CHCl<sub>3</sub>);  $\lambda_{\max}$  260 nm ( $\epsilon$  6283); IR (Nujol): 1697 (SAC) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.30 (br s, NH), 7.30 (d, 1H,  $J_{5,6} = 8.3$  Hz, H-6), 5.82 (d, 1H, H-5), 5.75 (d, 1H,  $J_{1',2'} = 9.5$  Hz, H-1'), 5.26–5.15 (m, 2H, H-2' and H-4'), 4.69 (dtr, 1H,  $J_{F,3'} = 51.8$  Hz,  $J_{2',3'} = J_{3',4'} = 9.1$  Hz, H-3'), 3.80 (m, 1H, H-5'), 3.27–3.11 (m, 2H, H-6a', 6b'), 2.35 (s, 3H, SAC), 2.19 and 2.07 (2s, 6H, 2OAc); Anal. Calcd for C<sub>16</sub>H<sub>19</sub>FN<sub>2</sub>O<sub>8</sub>S: C, 45.93; H, 4.58; N, 6.70. Found: C, 45.89; H, 4.64; N, 6.82. ESI-MS ( $m/z$ ): 419.41 (M+H<sup>+</sup>).

#### 2.2.10. 1-(2,4-Di-O-acetyl-6-S-acetyl-3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)5-fluorouracil (**5c**)

5-Fluorouracil derivative **5c** was synthesized from **4c** by the similar procedure as described for **5a**. Purified by flash chromatography (hexane/AcOEt, 4:6) and obtained (0.65 g, 75%,  $R_f = 0.47$  in hexane/AcOEt, 4:6) as white solid. m.p. 200–202 °C;  $[\alpha]_D^{22} + 5.26$  (c 0.1, CHCl<sub>3</sub>);  $\lambda_{\max}$  260 nm ( $\epsilon$  8232); IR (Nujol): 1697 (SAC) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  8.28 (br s, NH), 7.36 (d, 1H,  $J_{6,F5} = 5.3$  Hz, H-6), 5.71 (d, 1H,  $J_{1',2'} = 9.3$  Hz, H-1'), 5.23–5.11 (m, 2H, H-2' and H-4'), 4.70 (dtr, 1H,  $J_{F,3'} = 51.5$  Hz,  $J_{2',3'} = 9.2$ ,  $J_{3',4'} = 9.0$  Hz, H-3'), 3.80 (m, 1H, H-5'), 3.29–3.10 (m, 2H, H-6a', 6b'), 2.36 (s, 3H, SAC), 2.19 and 2.08 (2s, 6H, 2OAc); Anal. Calcd for C<sub>16</sub>H<sub>18</sub>F<sub>2</sub>N<sub>2</sub>O<sub>8</sub>S: C, 44.04; H, 4.16; N, 6.42. Found: C, 44.28; H, 4.29; N, 6.34. ESI-MS ( $m/z$ ): 437.41 (M+H<sup>+</sup>).

#### 2.2.11. 1-(2,4-Di-O-acetyl-6-S-acetyl-3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)-N<sup>4</sup>-benzoyl cytosine (**5d**)

N<sup>4</sup>-benzoyl cytosine derivative **5d** was synthesized from **4d** by the similar procedure as described for **5a**. Purified by flash chromatography (hexane/AcOEt, 2:8) to give compound **5d** (0.82 g, 68%,  $R_f = 0.32$  in hexane/AcOEt, 2:8) as solid. m.p. 277–279 °C;  $[\alpha]_D^{22} + 16.00$  (c 0.1, CHCl<sub>3</sub>);  $\lambda_{\max}$  263 nm ( $\epsilon$  20,626); IR (Nujol): 1697 (SAC) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.88 (d, 1H,  $J_{5,6} = 7.2$  Hz, H-6), 7.84–7.48 (m, 6H, Bz and H-5), 6.03 (d, 1H,  $J_{1',2'} = 9.4$  Hz, H-1'), 5.27–5.17 (m, 2H, H-2' and H-4'), 4.75 (dtr, 1H,  $J_{F,3'} = 51.8$  Hz,  $J_{2',3'} = 9.1$  Hz,  $J_{3',4'} = 9.0$  Hz, H-3'), 3.83–3.77 (m, 1H, H-5'), 3.29–3.11 (m, 2H, H-6a', 6b'), 2.35 (s, 3H, SAC), 2.20 and 2.05 (2s, 6H, 2OAc); Anal. Calcd for C<sub>23</sub>H<sub>24</sub>FN<sub>3</sub>O<sub>8</sub>S: C, 52.97; H, 4.64; N, 8.06. Found: C, 52.70; H, 4.80; N, 8.22. ESI-MS ( $m/z$ ): 522.54 (M+H<sup>+</sup>).

#### 2.2.12. 9-(2,4-Di-O-acetyl-6-S-acetyl-3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)-N<sup>6</sup>-benzoyl adenine (**5e**)

N<sup>6</sup>-benzoyl adenine derivative **5e** was synthesized from **4e** by the similar procedure as described for **5a**. Finally it was purified by flash chromatography (hexane/AcOEt, 2:8) to give compound **5e** (0.61 g, 60%,  $R_f = 0.24$  in hexane/AcOEt, 2:8) as solid. m.p. 118–120 °C;  $[\alpha]_D^{22} + 4.00$  (c 0.1, CHCl<sub>3</sub>);  $\lambda_{\max}$  279 nm ( $\epsilon$  12,567); IR (Nujol): 1697 (SAC) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  9.07 (br s, NH), 8.77 and 8.19 (2s, 2H, H-2,8), 7.98–7.42 (m, 5H, Bz), 5.88 (d, 1H,  $J_{1',2'} = 9.5$  Hz, H-1'), 5.70–5.61 (m, 1H, H-2'), 5.40–5.31 (m, 1H, H-4'), 4.81 (dtr, 1H,  $J_{F,3'} = 51.7$  Hz,  $J_{2',3'} = 9.3$  Hz,  $J_{3',4'} = 8.9$  Hz), 3.95–3.89 (m, 1H, H-5'), 3.32–3.15 (m, 2H, H-6a', 6b'), 2.35 (s, 3H, SAC),

2.23 and 1.84 (2s, 6H, 2OAc); Anal. Calcd for C<sub>24</sub>H<sub>24</sub>FN<sub>5</sub>O<sub>7</sub>S: C, 52.84; H, 4.43; N, 12.84. Found: C, 52.68; H, 4.38; N, 13.09. ESI-MS ( $m/z$ ): 546.56 (M+H<sup>+</sup>).

#### 2.2.13. 1-[3-Deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl]thymine (**7a**) from **5a**

To a solution of thioacetate **5a** (1.10 g, 2.50 mmol) in methanolic ammonia (104 mL), 2,2-dipyridyl-disulfide (DTDP) (2.60 g, 12 mmol) dissolved in MeOH/H<sub>2</sub>O (1:1, 22 mL) was added dropwise and the mixture was stirred at room temperature. After stirring for 20 h, the reaction mixture was concentrated. The crude was finally purified by flash chromatography (AcOEt) to give compound **7a** (0.71 g, 68%,  $R_f = 0.30$  in AcOEt) as white foam.  $[\alpha]_D^{22} + 16.00$  (c 0.5, MeOH);  $\lambda_{\max}$  266 nm ( $\epsilon$  9093); <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.09 (br s, NH), 8.41 (d, H-6 of pyridine), 7.83–7.58 (m, H-4 and H-5 of pyridine), 7.29–7.12 (m, H-3 of pyridine and H-6), 5.40 (d, 1H,  $J_{1',2'} = 9.4$  Hz, H-1'), 4.29 (dtr, 1H,  $J_{F,3'} = 52.3$  Hz,  $J_{2',3'} = 8.5$  Hz,  $J_{3',4'} = 8.4$  Hz, H-3'), 3.92–3.79 (m, 1H, H-2'), 3.56–3.45 (m, 2H, H-6a' and H-4'), 3.44–3.35 (m, 1H, 6b'), 2.98–2.89 (m, 1H, H-5'); <sup>19</sup>F NMR:  $\delta$  -65.00; <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  163.73, 160.52, 150.11, 148.90, 136.95, 132.92, 121.11, 119.18, 110.23, 92.18, 85.49, 72.36, 70.12, 67.95, 33.63, 12.41; Anal. Calcd for C<sub>16</sub>H<sub>18</sub>FN<sub>3</sub>O<sub>5</sub>S<sub>2</sub>: C, 46.26; H, 4.37; N, 10.11. Found: C, 46.41; H, 4.51; N, 10.32. ESI-MS ( $m/z$ ): 416.47 (M+H<sup>+</sup>).

#### 2.2.14. 1-[3-Deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl]uracil (**7b**) from **5b**

Uracil derivative **7b** was synthesized from thioacetate **5b** by the similar procedure as described for **7a**. Purified by flash chromatography (AcOEt) and obtained (0.70 g, 70%,  $R_f = 0.27$  in AcOEt) as white foam.  $[\alpha]_D^{22} + 6.00$  (c 0.1, MeOH);  $\lambda_{\max}$  262 nm ( $\epsilon$  6478); <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.53 (br s, NH), 8.32 (dd, H-6 of pyridine), 7.76–7.73 (m, H-4 and H-5 of pyridine), 7.66 (m, H-3 of pyridine), 7.57 (d, 1H,  $J_{5,6} = 8.1$  Hz, H-6), 5.68 (d, 1H, H-5), 5.46 (d, 1H,  $J_{1',2'} = 9.4$  Hz, H-1'), 4.29 (dtr, 1H,  $J_{F,3'} = 52.3$  Hz,  $J_{2',3'} = 8.7$ ,  $J_{3',4'} = 8.6$  Hz, H-3'), 3.83–3.77 (m, 1H, H-2'), 3.64–3.60 (m, 1H, H-6a'), 3.57–3.52 (m, 1H, H-4'), 3.42–3.39 (m, 1H, 6b'), 3.01–2.96 (m, 1H, H-5'); <sup>19</sup>F NMR:  $\delta$  -63.20; <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  163.34, 160.43, 150.19, 147.96, 142.56, 132.74, 120.95, 119.26, 101.76, 91.98, 84.77, 71.83, 70.31, 67.62, 33.64; Anal. Calcd for C<sub>15</sub>H<sub>16</sub>FN<sub>3</sub>O<sub>5</sub>S<sub>2</sub>: C, 44.88; H, 4.02; N, 10.47. Found: C, 44.71; H, 4.22; N, 10.52. ESI-MS ( $m/z$ ): 402.46 (M+H<sup>+</sup>).

#### 2.2.15. 1-[3-Deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl]5-fluorouracil (**7c**) from **5c**

5-Fluorouracil derivative **7c** was synthesized from corresponding thioacetate **5c** by the similar procedure as described for **7a**. Purified by flash chromatography (AcOEt) and obtained (0.72 g, 69%,  $R_f = 0.25$  in AcOEt) as white foam.  $[\alpha]_D^{22} + 2.00$  (c 0.5, CHCl<sub>3</sub>);  $\lambda_{\max}$  263 nm ( $\epsilon$  5885); <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.34–8.32 (m, H-6 of pyridine), 8.28 (br s, NH), 7.83–7.78 (m, H-4 and H-5 of pyridine), 7.17–7.13 (m, H-3 of pyridine), 5.44 (d, 1H,  $J_{1',2'} = 9.0$  Hz, H-1'), 4.28 (dtr, 1H,  $J_{F,3'} = 52.3$  Hz,  $J_{2',3'} = 8.7$ ,  $J_{3',4'} = 8.6$  Hz, H-3'), 3.82–3.76 (m, 1H, H-2'), 3.64–3.60 (m, 1H, H-6a'), 3.56–3.51 (m, 1H, H-4'), 3.43–3.39 (m, 1H, H-6b'), 3.01–2.96 (m, 1H, H-5'); <sup>19</sup>F NMR:  $\delta$  -64.30, -63.20; <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  162.34, 157.89, 150.41, 148.76, 138.11, 132.91, 127.45, , 120.32, 119.07, 94.41, 85.29, 72.37, 70.11, 68.65, 33.47; Anal. Calcd for C<sub>15</sub>H<sub>15</sub>F<sub>2</sub>N<sub>3</sub>O<sub>5</sub>S<sub>2</sub>: C, 42.95; H, 3.60; N, 10.02. Found: C, 42.81; H, 3.48; N, 10.28. ESI-MS ( $m/z$ ): 420.44.

#### 2.2.16. Bis-[1-(3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)thymine]-6,6-disulfide (**6a**)

A mixture of methanolic ammonia (72.30 mL) and thionucleoside **5a** (0.75 g, 1.73 mmol) stirred for 4 h at room temperature. The reaction mixture was concentrated and disulfide **6a** was

obtained (0.95 g, 90%,  $R_f = 0.20$  in AcOEt) as white foam.  $[\alpha]_D^{22} + 2.00$  (c 0.5, MeOH);  $\lambda_{\max}$  262 nm ( $\epsilon$  19,126);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  7.88 (br s, NH), 7.49 (s, 1H, H-6), 5.55 (d, 1H,  $J_{1',2'} = 9.4$  Hz, H-1'), 4.37 (dtr, 1H,  $J_{F,3'} = 52.3$  Hz,  $J_{2',3'} = 8.5$ ,  $J_{3',4'} = 8.3$  Hz, H-3'), 4.14–4.04 (m, 1H, H-2'), 3.72–3.55 (m, 1H, H-4'), 3.35–3.23 (m, 2H, H-6a',6b'), 2.92–2.80 (m, 1H, H-5'), 1.89 (s, 3H, 5- $\text{CH}_3$ ); Anal. Calcd for  $\text{C}_{22}\text{H}_{28}\text{F}_2\text{N}_4\text{O}_{10}\text{S}_2$ : C, 43.27; H, 4.62; N, 9.18. Found: C, 43.18; H, 4.84; N, 9.07. ESI-MS ( $m/z$ ): 611.64.

#### 2.2.17. Bis-[1-(3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)uracil]-6,6-disulfide (**6b**)

Uracil derivative **6b** was synthesized from thionucleoside **5b** following similar procedure as described for **6a**. Disulfide **6b** was obtained (0.89 g, 88%,  $R_f = 0.16$  in AcOEt) as white foam.  $[\alpha]_D^{22} + 3.00$  (c 0.5, MeOH);  $\lambda_{\max}$  258 nm ( $\epsilon$  14,339);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  7.66 (d, 1H,  $J_{5,6} = 8.2$  Hz, H-6), 7.24 (br s, NH), 5.64 (d, 1H, H-5), 5.56 (d, 1H,  $J_{1',2'} = 9.5$  Hz, H-1'), 4.38 (dtr, 1H,  $J_{F,3'} = 52.0$  Hz,  $J_{2',3'} = 8.6$  Hz,  $J_{3',4'} = 8.1$  Hz, H-3'), 3.95–3.81 (m, 1H, H-2'), 3.74–3.53 (m, 1H, H-4'), 3.34–3.22 (m, 2H, H-6a',6b'), 2.92–2.81 (m, 1H, H-5'); Anal. Calcd for  $\text{C}_{20}\text{H}_{24}\text{F}_2\text{N}_4\text{O}_{10}\text{S}_2$ : C, 41.23; H, 4.15; N, 9.62. Found: C, 41.38; H, 4.34; N, 9.78. ESI-MS ( $m/z$ ): 583.57.

#### 2.2.18. Bis-[1-(3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)5-fluorouracil]-6,6-disulfide (**6c**)

5-Fluorouracil derivative **6c** was synthesized from thionucleoside **5c** by the similar procedure as described for **6a**. Compound **6c** was obtained (0.95 g, 89%,  $R_f = 0.13$  in AcOEt) as white foam.  $[\alpha]_D^{22} + 2.00$  (c 0.5, MeOH);  $\lambda_{\max}$  263 nm ( $\epsilon$  15,056);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  7.89 (d, 1H,  $J_{6,F5} = 6.4$  Hz, H-6), 7.35 (br s, NH), 5.56 (d, 1H,  $J_{1',2'} = 9.3$  Hz, H-1'), 4.38 (dtr, 1H,  $J_{F,3'} = 52.5$  Hz,  $J_{2',3'} = 8.7$ ,  $J_{3',4'} = 8.6$  Hz, H-3'), 3.90–3.84 (m, 1H, H-2'), 3.70–3.66 (m, 1H, H-6a'), 3.61–3.56 (m, 1H, H-4'), 3.29–3.26 (m, 1H, H-6b'), 2.88–2.83 (m, 1H, H-5'); Anal. Calcd for  $\text{C}_{20}\text{H}_{22}\text{F}_4\text{N}_4\text{O}_{10}\text{S}_2$ : C, 38.84; H, 3.59; N, 9.06. Found: C, 38.72; H, 3.47; N, 9.12. ESI-MS ( $m/z$ ): 619.56.

#### 2.2.19. 1-[3-Deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl]thymine (**7a**) from **6a**

To a solution of **6a** (1.83 g, 3.00 mmol) in MeOH (26 mL), DTDP (3.16 g, 14.46 mmol) dissolved in MeOH/ $\text{H}_2\text{O}$  (1:1, 26 mL) was added dropwise. The reaction was carried out at room temperature and within 12 h. After disappearance of the starting material (TLC), the solvents were removed under diminished pressure and the yellow residue was purified by flash column chromatography (AcOEt) to give compound **7a** (0.88 g, 71%,  $R_f = 0.30$  in AcOEt) as white foam.

#### 2.2.20. 1-[3-Deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl]uracil (**7b**) from **6b**

Uracil derivative **7b** was synthesized from **6b** by the similar procedure as described for **7a**. Purified by flash chromatography (AcOEt) and obtained (0.83 g, 69%,  $R_f = 0.27$  in AcOEt) as white foam.

#### 2.2.21. 1-[3-Deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl]5-fluorouracil (**7c**) from **6c**

5-Fluorouracil derivative **7c** was synthesized from **6c** by the similar procedure as described for **7a**. Purified by flash chromatography (AcOEt) and obtained (0.86 g, 68%,  $R_f = 0.25$  in AcOEt) as white foam.

### 2.3. Synthesis of 1-[3-deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl] nucleosides (**7a-c,f,g**) from glycosyl donor **13**

#### 2.3.1. 3-Deoxy-3-fluoro-1,2-O-isopropylidene-6-O-p-toluenesulfonyl- $\alpha$ -D-glucufuranose (**9**)

To a solution of diol **8** [33] (4.00 g, 18 mmol) in dry pyridine (46.20 mL) was added *p*-toluenesulfonyl chloride (4.60 g, 24 mmol) and kept for 2 h at room temperature. After neutralization ( $\text{NaHCO}_3$ ) and extraction with ethyl acetate ( $4 \times 500$  mL), the combined extracts were dried over anhydrous sodium sulfate and evaporated to dryness. Purification of the residue by flash chromatography (hexane/AcOEt, 1:1) yielded the title compound (5.08 g, 74%,  $R_f = 0.45$  in hexane/AcOEt, 1:1) as colorless oil.  $[\alpha]_D^{22} - 8.00$  (c 0.5,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.38 and 7.82 (dd, 4H, tosyl group), 5.92 (d, 1H,  $J_{1',2'} = 3.2$  Hz, H-1'), 5.09 (dd, 1H,  $J_{3',F} = 49.3$  Hz, H-3'), 4.70 (dd, 1H,  $J_{2',F} = 10.3$  Hz, H-2'), 4.34 (d, 1H, H-4'), 4.13–4.06 (m, 2H, H-5a',5b'), 2.47 (s, 3H,  $\text{ArCH}_3$ ), 1.48 (s, 3H,  $\text{CH}_3$ ), 1.33 (s, 3H,  $\text{CH}_3$ ); Anal. Calcd for  $\text{C}_{16}\text{H}_{21}\text{FO}_5\text{S}$ : C, 51.06; H, 5.62. Found: C, 51.14; H, 5.46. ESI-MS ( $m/z$ ): 377.42 ( $\text{M}+\text{H}^+$ ).

#### 2.3.2. 3-Deoxy-3-fluoro-1,2-O-isopropylidene-5-O-tetrahydropyranyl-6-O-p-toluenesulfonyl- $\alpha$ -D-glucufuranose (**10**)

To a 0 °C solution of **9** (5.00 g, 13.30 mmol) and dried *p*-toluenesulfonic acid (0.25 g, 1.33 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (26.60 mL) was added 3,4-dihydro-2H-pyran (1.80 mL, 19.90 mmol). After being kept for 2 h in room temperature, the reaction mixture was neutralized with aqueous  $\text{NaHCO}_3$  and extracted with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 1$  L). The organic layers were combined, dried over  $\text{Na}_2\text{SO}_4$  and concentrated in vacuo. The residue was chromatographed through a column of silica gel (hexane/AcOEt, 3:7). Fractions containing the product were combined and concentrated to afford **10** (4.78 g, 78%,  $R_f = 0.48$  in hexane/AcOEt, 3:7) as off-white oil.  $[\alpha]_D^{22} - 6.00$  (c 0.5,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.84–7.80 (four separate s, 2H,  $\text{HC}_8 + \text{HC}_2$ ), 7.35–7.28 (m, 5H, Ph), 5.98 (d, 1/2H,  $J_{1',2'} = 2.8$  Hz, H-1'), 5.89 (d, 1/2H,  $J_{1',2'} = 2.9$  Hz, H-1'), 2.47 (s, 3H,  $\text{ArCH}_3$ ), 1.50 (s, 3H,  $\text{CH}_3$ ), 1.33 (s, 3H,  $\text{CH}_3$ ); Anal. Calcd for  $\text{C}_{21}\text{H}_{29}\text{FO}_6\text{S}$ : C, 54.77; H, 6.35. Found: C, 54.64; H, 6.42. ESI-MS ( $m/z$ ): 461.53 ( $\text{M}+\text{H}^+$ ).

#### 2.3.3. 3-Deoxy-3-fluoro-1,2-O-isopropylidene-5-O-tetrahydropyranyl-6-S-acetyl-6-thio- $\alpha$ -D-glucufuranose (**11**)

The tosylate **10** (4.50 g, 9.80 mmol) was heated with potassium thioacetate (1.50 g, 13.50 mmol) in DMF (39.20 mL) at 100 °C for 1 h. The reaction mixture was neutralized with aqueous  $\text{NaHCO}_3$ . After that, the mixture was concentrated under high vacuum pump to eliminate the DMF. The residue was partitioned between water and EtOAc, the organic extract was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was purified by flash chromatography (hexane/AcOEt, 4:6) to give compound **11** (2.71 g, 76%,  $R_f = 0.42$  in hexane/AcOEt, 4:6) as yellow syrup.  $[\alpha]_D^{22} - 8.00$  (c 0.1,  $\text{CHCl}_3$ ); IR (Nujol): 1697 (SAC)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.84–7.80 (4 separate s, 2H,  $\text{HC}_8 + \text{HC}_2$ ), 7.35–7.28 (m, 5H, Ph), 5.96 (d, 1H,  $J_{1',2'} = 3.7$  Hz, H-1'), 2.37 (s, 3H, SAC), 1.52 (s, 3H,  $\text{CH}_3$ ), 1.34 (s, 3H,  $\text{CH}_3$ ); Anal. Calcd for  $\text{C}_{16}\text{H}_{25}\text{FO}_6\text{S}$ : C, 52.73; H, 6.91. Found: C, 52.84; H, 6.72. ESI-MS ( $m/z$ ): 365.47 ( $\text{M}+\text{H}^+$ ).

#### 2.3.4. 1,2,4-Tri-O-acetyl-3-deoxy-3-fluoro-6-S-acetyl-6-thio- $\alpha$ -D-glucopyranose (**12**)

A solution of thioacetate **11** (2.50 g, 6.90 mmol) in 90% TFA (172.50 mL) was stirred for 20 min at room temperature. The reaction mixture was concentrated under diminished pressure and crude was obtained as a colorless oil. Acetylation of the residue with  $\text{Ac}_2\text{O}$ -pyridine (1:2, 36.50 mL) and chromatography of the



product (hexane/AcOEt, 4:6) gave compound **12** (1.77 g, 70%,  $R_f = 0.45$  in hexane/AcOEt, 4:6) as colorless syrup.  $[\alpha]_D^{22} + 12.00$  (c 0.1, CHCl<sub>3</sub>); IR (Nujol): 1697 (SAc) cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  6.30 (br s, 1H, H-1'), 5.62 (d, 1H, H-4'), 5.33–5.04 (m, 1H, H-2'), 4.80 (dtr, 1/2H,  $J_{3',F} = 53.3$  Hz,  $J_{3',4'} = 9.2$  Hz, H-3'), 4.58 (dtr, 1/2H,  $J_{3',F} = 51.9$  Hz,  $J_{3',4'} = 9.0$  Hz, H-3'), 4.03–3.98 (m, 1/2H, H-5'), 3.70–3.64 (m, 1/2H, H-5'), 3.28–3.21 and 3.15–3.08 (2 m, 2H, H-6a',6b'), 2.36 (s, 3H, SAc), 2.17, 2.14, 2.12, 2.10 (4s, 12H, 4OAc); Anal. Calcd for C<sub>14</sub>H<sub>19</sub>FO<sub>8</sub>S: C, 45.90; H, 5.23. Found: C, 46.04; H, 5.16. ESI-MS ( $m/z$ ): 367.38 (M+H<sup>+</sup>).

### 2.3.5. 1,2,4-Tri-O-acetyl-3-deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio-D-glucopyranose (**13**)

To a solution of thiosugar **12** (2.00 g, 5.46 mmol) in methanolic ammonia (228 mL), DTDP (9.55 g, 43.70 mmol) dissolved in MeOH:H<sub>2</sub>O (1:1, 47 mL) was added dropwise and the mixture was stirred at room temperature. After stirring for 20 h, the reaction mixture was concentrated. Acetylation of the residue with Ac<sub>2</sub>O-pyridine (1:2, 109 mL) and chromatography of the product (hexane/AcOEt, 4:6) gave compound **13** (1.67 g, 70%,  $R_f = 0.45$  in hexane/AcOEt, 4:6) as colorless syrup.  $[\alpha]_D^{22} + 4.00$  (c 0.1, CHCl<sub>3</sub>);  $\lambda_{max}$  283 nm ( $\epsilon$  5510); <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  5.62 (d, 1/2H, H-1'), 5.26–5.03 (m, 2H, H-2' and H-4'), 4.79 (dtr, 1/2H,  $J_{3',F} = 51.9$  Hz,  $J_{3',4'} = 9.2$  Hz, H-3'), 4.53 (dtr, 1/2H,  $J_{3',F} = 51.7$  Hz,  $J_{3',4'} = 9.0$  Hz, H-3'), 4.19–4.14 (m, 1/2H, H-5'), 3.75–3.70 (m, 1/2H, H-5'), 3.42–3.35 (m, 2H, H-6a',6b'), 2.14, 2.12, 2.10, 2.08, 2.07, 2.05 (6s, 18H, 6OAc); Anal. Calcd for C<sub>17</sub>H<sub>20</sub>FNO<sub>7</sub>S<sub>2</sub>: C, 47.10; H, 4.65; N, 3.23. Found: C, 47.24; H, 4.46; N, 3.35. ESI-MS ( $m/z$ ): 434.49.

### 2.3.6. 1-[3-Deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl]thymine (**7a**)

A mixture of thymine (2.33 g, 18.55 mmol), HMDS (4.80 mL, 23 mmol) and saccharine (0.16 g, 0.85 mmol) in anhydrous CH<sub>3</sub>CN (69 mL) was refluxed for 30 min. Triacetylated thiopyridyl sugar **13** (5.74 g, 13.25 mmol) and tin chloride (1.94 mL, 17.22 mmol) were then added and the reaction mixture was refluxed for 5 h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (500 mL). The organic layer was washed with water (3  $\times$  10 mL) and dried over anhydrous sodium sulfate, evaporated to dryness. The crude underwent deacetylation with methanolic ammonia (550 mL) within 4 h, concentrated and finally purified by flash chromatography (AcOEt) to give compound **7a** (3.53 g, 64%,  $R_f = 0.30$  in AcOEt) as white foam.

### 2.3.7. 1-[3-Deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl]uracil (**7b**)

A mixture of uracil (2.08 g, 18.55 mmol), HMDS (4.80 mL, 23 mmol) and saccharine (0.16 g, 0.85 mmol) in anhydrous CH<sub>3</sub>CN (69 mL) was refluxed for 30 min. Triacetylated thiopyridyl sugar **13** (5.74 g, 13.25 mmol) and trimethylsilyl trifluoromethane-sulfonate (3.10 mL, 17.22 mmol) were then added and the reaction mixture was refluxed for 5 h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (500 mL). The organic layer was washed with water (3  $\times$  10 mL) and dried over anhydrous sodium sulfate, evaporated to dryness. The crude underwent deacetylation with methanolic ammonia (550 mL) within 4 h, concentrated and finally purified by flash chromatography (AcOEt) to give compound **7b** (3.29 g, 62%,  $R_f = 0.27$  in AcOEt) as white foam.

### 2.3.8. 1-[3-Deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl]5-fluorouracil (**7c**)

A mixture of 5-fluorouracil (2.41 g, 18.55 mmol), HMDS (4.80 mL, 23 mmol) and saccharine (0.16 g, 0.85 mmol) in anhydrous CH<sub>3</sub>CN (69 mL) was refluxed for 30 min. Triacetylated thiopyridyl sugar **13** (5.74 g, 13.25 mmol) and trimethylsilyl trifluoromethane-sulfonate (3.10 mL, 17.22 mmol) were then

added and the reaction mixture was refluxed for 5 h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (500 mL). The organic layer was washed with water (3  $\times$  10 mL) and dried over anhydrous sodium sulfate, evaporated to dryness. The crude underwent deacetylation with methanolic ammonia (550 mL) within 4 h, concentrated and finally purified by flash chromatography (AcOEt) to give compound **7c** (3.89 g, 70%,  $R_f = 0.25$  in AcOEt) as white foam.

### 2.3.9. 1-[3-Deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl]cytosine (**7f**)

A mixture of N<sup>4</sup>-benzoyl cytosine (3.99 g, 18.55 mmol), HMDS (4.80 mL, 23 mmol) and saccharine (0.16 g, 0.85 mmol) in anhydrous CH<sub>3</sub>CN (69 mL) was refluxed for 30 min. Triacetylated thiopyridyl sugar **13** (5.74 g, 13.25 mmol) and trimethylsilyl trifluoromethane-sulfonate (3.10 mL, 17.22 mmol) were then added and the reaction mixture was refluxed for 5 h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (500 mL). The organic layer was washed with water (3  $\times$  10 mL) and dried over anhydrous sodium sulfate, evaporated to dryness. The crude underwent deacetylation with methanolic ammonia (550 mL) within 4 h, concentrated and finally purified by flash chromatography (AcOEt) to give compound **7f** (3.60 g, 68%,  $R_f = 0.32$  in AcOEt) as white foam.  $[\alpha]_D^{22} + 2.00$  (c 0.5, MeOH);  $\lambda_{max}$  269 nm ( $\epsilon$  7400); <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.23 (m, H-6 of pyridine), 7.78–7.69 (m, H-4 and H-5 of pyridine), 7.51 (d, 1H, H-5), 7.46 (d, 1H,  $J_{5,6} = 7.3$  Hz, H-6), 7.08–7.03 (m, H-3 of pyridine), 5.52 (d, 1H,  $J_{1',2'} = 9.4$  Hz, H-1'), 4.22 (dtr, 1H,  $J_{F,3'} = 52.5$  Hz,  $J_{2',3'} = 9.0$  Hz,  $J_{3',4'} = 8.9$  Hz, H-3'), 3.73–3.66 (m, 1H, H-2'), 3.53–3.42 (m, 2H, H-4' and H-6a'), 3.34–3.30 (m, 1H, H-6b'), 2.92–2.87 (m, 1H, H-5'); <sup>19</sup>F NMR:  $\delta$  –64.33; <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  165.54, 160.32, 154.28, 148.46, 137.56, 132.91, 122.03, 120.12, 99.36, 92.38, 85.62, 72.39, 70.64, 70.22, 33.63; Anal. Calcd for C<sub>15</sub>H<sub>17</sub>FN<sub>4</sub>O<sub>4</sub>S<sub>2</sub>: C, 44.99; H, 4.28; N, 13.99. Found: C, 44.82; H, 4.42; N, 13.52. ESI-MS ( $m/z$ ): 401.48.

### 2.3.10. 9-[3-Deoxy-3-fluoro-6-S-(2-S-pyridyl)-6-thio- $\beta$ -D-glucopyranosyl]adenine (**7g**)

A mixture of N<sup>6</sup>-benzoyl adenine (2.93 g, 12.25 mmol), HMDS (3.10 mL, 15.19 mmol) and saccharine (0.10 g, 0.56 mmol) in anhydrous CH<sub>3</sub>CN (46 mL) was refluxed for 30 min. Triacetylated thiopyridyl sugar **13** (3.80 g, 8.75 mmol) and tin chloride (1.38 mL, 12.25 mmol) were then added and the reaction mixture was refluxed for 5 h, cooled, neutralized with aqueous sodium bicarbonate, and extracted with CH<sub>2</sub>Cl<sub>2</sub> (500 mL). The organic layer was washed with water (3  $\times$  10 mL) and dried over anhydrous sodium sulfate, evaporated to dryness. The crude underwent deacetylation with methanolic ammonia (512 mL) within 4 h, concentrated and finally purified by flash chromatography (AcOEt) to give compound **7g** (3.32 g, 64%,  $R_f = 0.32$  in AcOEt) as white foam.  $[\alpha]_D^{22} + 4.00$  (c 0.5, MeOH);  $\lambda_{max}$  263 nm ( $\epsilon$  9860); <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  8.58 and 8.35 (2s, 2H, H-2,8), 8.33–8.25 (m, H-6 of pyridine), 7.63–7.18 (m, H-4, H-5, H-3 of pyridine), 6.96 (br s, NH<sub>2</sub>), 5.88 (d, 1H,  $J_{1',2'} = 9.1$  Hz, H-1'), 4.01 (dtr, 1H,  $J_{F,3'} = 52.3$  Hz,  $J_{2',3'} = 8.7$  Hz,  $J_{3',4'} = 8.6$  Hz, H-3'), 3.79–3.67 (m, 1H, H-2'), 3.59–3.53 (m, 1H, H-6a'), 3.51–3.47 (m, 1H, H-4'), 3.42–3.37 (m, 1H, H-6b'), 2.96–2.81 (m, 1H, H-5'); <sup>19</sup>F NMR:  $\delta$  –63.20; <sup>13</sup>C NMR (CD<sub>3</sub>OD):  $\delta$  160.54, 155.75, 152.31, 149.86, 147.75, 145.76, 136.71, 120.37, 120.23, 116.98, 91.76, 89.64, 72.19, 69.70, 66.64, 33.87; Anal. Calcd for C<sub>16</sub>H<sub>17</sub>FN<sub>6</sub>O<sub>3</sub>S<sub>2</sub>: C, 45.27; H, 4.04; N, 19.80. Found: C, 45.31; H, 4.12; N, 19.62. ESI-MS ( $m/z$ ): 425.51.

## 2.4. Biological assays

The antiviral assays were based on the inhibition of virus-induced cytopathicity in confluent cell cultures, and the cytostatic

assays on inhibition of tumor cell proliferation in exponentially growing tumor cell cultures according to previously described methodology [15,32].

#### 2.4.1. Antiviral activity assays

The antiviral assays, other than the anti-HIV assays, were based on inhibition of virus-induced cytopathicity in HEL [herpes simplex virus type 1 (HSV-1) (KOS), HSV-2 (G), vaccinia virus, vesicular stomatitis virus, cytomegalovirus (HCMV) and varicella-zoster virus (VZV)], Vero (parainfluenza-3, reovirus-1, Sindbis virus and Coxsackie B4), HeLa (vesicular stomatitis virus, Coxsackie virus B4, and respiratory syncytial virus) or MDCK [influenza A (H1N1; H3N2) and influenza B] cell cultures. Confluent cell cultures (or nearly confluent for MDCK cells) in microtiter 96-well plates were inoculated with 100 CCID<sub>50</sub> of virus (1 CCID<sub>50</sub> being the virus dose 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 (200, 40, 8, ...  $\mu$ M) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were not treated with the test compounds. The minimal cytotoxic concentration (MCC) of the compounds was defined as the compound concentration that caused a microscopically visible alteration of cell morphology. The methodology of the anti-HIV assays was as follows: human CEM ( $\sim 3 \times 10^5$  cells/cm<sup>3</sup>) cells were infected with 100 CCID<sub>50</sub> of HIV(III<sub>B</sub>) or HIV-2(ROD)/mL and seeded in 200  $\mu$ L wells of a microtiter plate containing appropriate dilutions of the test compounds. After 4 days of incubation at 37 °C, HIV-induced CEM giant cell formation was examined microscopically.

#### 2.4.2. Cytostatic/toxic activity assays

Murine leukemia L1210, murine mammary carcinoma FM3A, and human lymphocyte CEM and human cervix carcinoma HeLa cells were seeded in 96-well microtiter plates at 50,000 (L1210, FM3A), 75,000 (CEM) or 20,000 (HeLa) cells per 200  $\mu$ L-well in the presence of different concentrations of the test compounds. After 2 (L1210, FM3A), 3 (CEM) or 4 (HeLa) days, the viable cell number was counted using a Coulter counter apparatus. The 50% cytostatic concentration (CC<sub>50</sub>) was defined as the compound concentration required to inhibit tumor cell proliferation by 50%.

#### 2.4.3. Thymidine and uridine phosphorylase assays

The conversion of dThd to thymine, Urd to uracil and compounds **13b** and **13c** to the free base by human recombinant thymidine phosphorylase and uridine phosphorylase type 1 (kindly provided by Dr. T.P. Roosild, Las Vegas, Nevada, USA) was measured by high-pressure liquid chromatography (HPLC) analysis. To determine the conversion activity, a high amount of recombinant enzyme was incubated with 100  $\mu$ M of dThd or **13b** and **13c** in TP-buffer (10 mM Tris.HCl, pH 7.6, 1 mM EDTA, 2 mM KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub> and 150 mM NaCl) or UP-buffer (same as TP-buffer, but 300 mM NaCl instead of 150 mM). At 20, 40 and 60 min, 100  $\mu$ L aliquots of the reaction mixtures were withdrawn and heated at 95 °C for 3 min to inactivate the enzyme. dThd and Urd were separated from thymine and uracil and **13b** and **13c** were separated from 5-FU and uracil on a reverse-phase RP-8 column (Merck, Darmstadt, Germany) and quantified by high-pressure liquid chromatography (HPLC) analysis (Alliance 2690, Waters, Milford, MA). The separation was performed by a linear gradient from 98% buffer B (50 mM NaH<sub>2</sub>PO<sub>4</sub> and 5 mM heptane sulfonic acid, pH 3.2), to 20% buffer B + 80% acetonitrile (8 min 98% buffer B + 2% acetonitrile; 5 min linear gradient of 98% buffer B + 2% acetonitrile to 20% buffer B + 80% acetonitrile; 10 min 20% buffer B + 80% acetonitrile, followed by equilibration at 98% buffer B + 2% acetonitrile). Retention times for thymine and dThd were, respectively, 5.1 and 10.8 min, for uracil and Urd, respectively, 2.1 and 2.4 min and for

**13b**, **13c** and 5-FU, respectively, 20.1, 20.1 and 2.4 min. UV-based detection was performed at 267 nm.

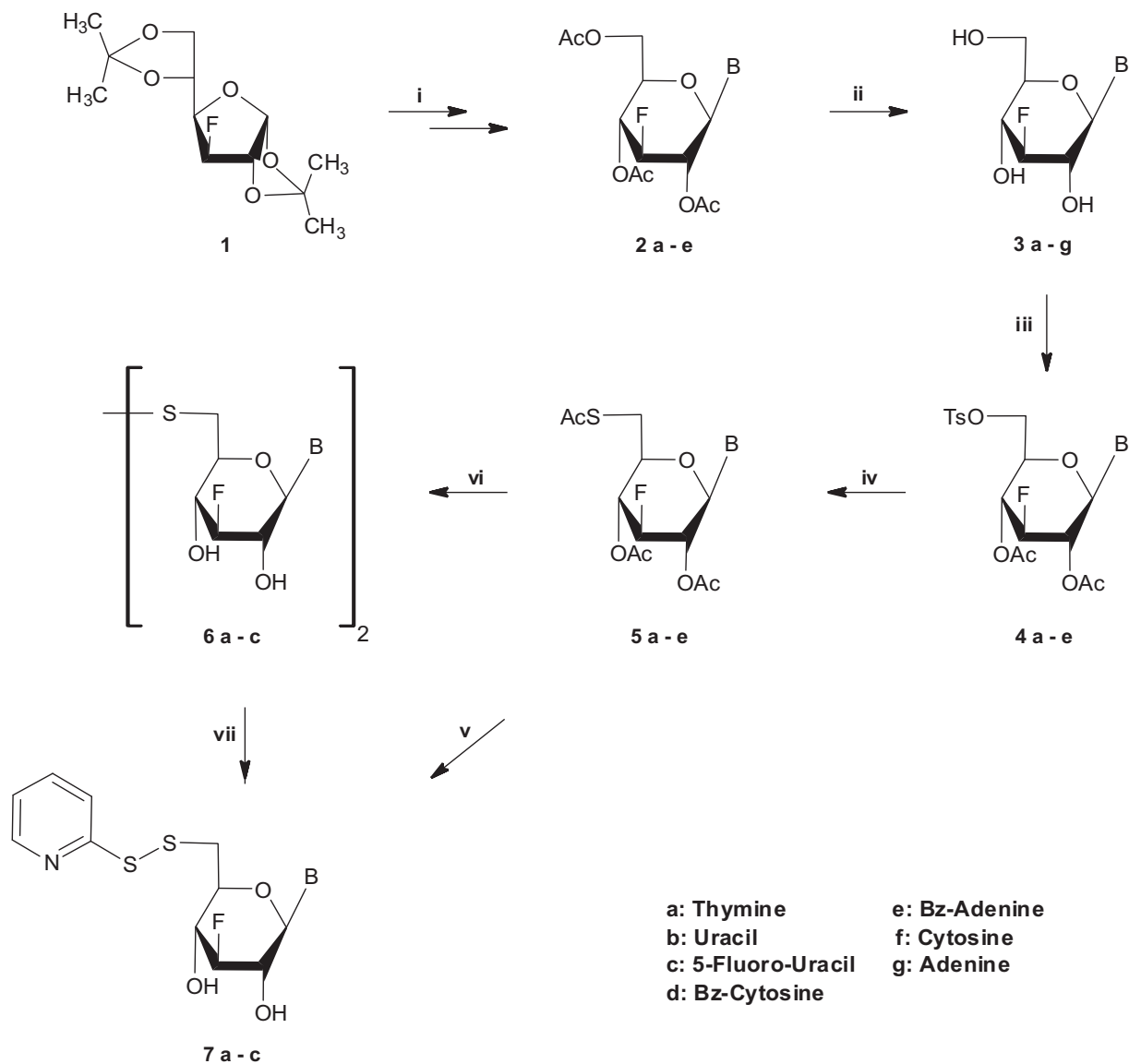
### 3. Results and discussion

#### 3.1. Synthesis

Two main strategies were used for the synthesis of 3-fluoro-6-S-(2-S-pyridyl) nucleosides **7a-c.f.g**.

Our first approach (Scheme 1) was focused on the preparation of 3-fluoro-6-thio-glucopyranosyl nucleosides of thymine **5a**, uracil **5b**, 5-fluorouracil **5c**, N<sup>4</sup>-benzoyl cytosine **5d** and N<sup>6</sup>-benzoyl adenine **5e** as useful precursors toward the synthesis of 6-S-nucleosidyl S-pyridine disulfides. 1,2:5,6-di-O-isopropylidene-3-deoxy-3-fluoro- $\alpha$ -D-glucofuranose (**1**) [34] was readily transformed into the corresponding 1,2,4,6-tetra-O-acetyl-3-deoxy-3-fluoro-glucopyranose [35], which upon condensation with purine and pyrimidine bases afforded 3-deoxy-3-fluoro- $\beta$ -D-glucopyranosyl nucleosides **2a-e** [14]. Deprotection of nucleosides **2a-e** in methanolic ammonia gave the fully unprotected derivatives **3a-c.f.g**, while the base protected benzoylated derivatives **3d,e** were obtained when **2d,e** were treated with NaOH-ethanol-pyridine [10,11,14]. Treatment of **3a-e** with *p*-toluenesulphonyl chloride in dry pyridine [35] and direct acetylation of the free hydroxyls at C-2' and C-4' with acetic anhydride/pyridine afforded the desired acetylated 6-O-tosylated derivatives **4a-e** in very good yields. Subsequently, the tosylated derivatives **4a-e**, after treatment with potassium thioacetate in hot *N,N*-dimethylformamide (DMF) [36–38], were converted into the corresponding thioacetates, 1-(2,4-di-O-acetyl-6-S-acetyl-3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)thymine (**5a**), 1-(2,4-di-O-acetyl-6-S-acetyl-3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)uracil (**5b**), 1-(2,4-di-O-acetyl-6-S-acetyl-3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)5-fluorouracil (**5c**), 1-(2,4-di-O-acetyl-6-S-acetyl-3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)-N<sup>4</sup>-benzoyl cytosine (**5d**) and 9-(2,4-di-O-acetyl-6-S-acetyl-3-deoxy-3-fluoro-6-thio- $\beta$ -D-glucopyranosyl)-N<sup>6</sup>-benzoyl adenine (**5e**), respectively. Deprotection of the  $\beta$ -protected thionucleosides **5a-e** by methanolic ammonia and *in situ* thiopyridinylation in H<sub>2</sub>O, MeOH and 2,2-dipyridyl disulfide (DTDP) at room temperature [22], led to the desired derivatives **7a-c**. These reactions were completed within 20 h and the isolated yields of the 6-S-nucleosidyl S-pyridine disulfides were generally higher than 65%. Attempts to fully unprotect thionucleosides **5a-c** resulted to the formation of their corresponding symmetrical disulfides **6a-c**, the thiopyridinylation of which also led to the desired 6-S-nucleosidyl S-dipyridine disulfides **7a-c**. It is noteworthy that only decomposition products were obtained after deprotection/thiopyridinylation of N<sup>4</sup>-benzoyl cytosine and N<sup>6</sup>-benzoyl adenine derivatives, **5d** and **5e**, respectively.

In order to circumvent this difficulty we chose to investigate a route in which the thionucleosides could be generated from a suitable thiopyridine sugar precursor. Our alternative synthetic pathway, in which compound **12** was envisioned as an appropriate glycosyl donor for the preparation of the suitable sugar, is described in Scheme 2. Initially, selective removal of the 5,6-O-isopropylidene group of starting material **1** [34] led to the formation of diol **8** [33]. Selective sulfonylation of **8** with *p*-toluenesulphonyl chloride in pyridine led to compound **9**, which was treated with dihydropyran (DHP) and *p*-toluenesulphonic acid monohydrate in dry CH<sub>2</sub>Cl<sub>2</sub> in order to give compound **10** in very satisfactory overall yield. Displacement of the tosyl group with the thioacetyl moiety proceeded without difficulties to afford compound **11**. Precursor **12** was readily available from glucofuranose **11** through hydrolysis with 90% aqueous trifluoroacetic acid at room temperature and direct acetylation. Deprotection of the glycosyl donor **12** using methanolic ammonia, direct thiopyridinylation



**Scheme 1.** (i) (a) MeOH/H<sub>2</sub>O/amberlite IR 120(H<sup>+</sup>); (b) Ac<sub>2</sub>O/pyridine; (c) silylated base, CH<sub>3</sub>CN, trimethylsilyl trifluoromethane-sulfonate or tin chloride; (ii) pyridine/MeOH/amberlite IR 120(H<sup>+</sup>)/NaOH or methanolic ammonia; (iii) (a) pyridine, *p*-toluenesulfonyl chloride; (b) Ac<sub>2</sub>O/Pyridine; (iv) KSAc/DMF/100 °C; (v) methanolic ammonia/H<sub>2</sub>O/MeOH/2,2-dipyridine-disulfide; (vi) methanolic ammonia; (vii) H<sub>2</sub>O/MeOH/2,2-dipyridine-disulfide.

tion [22] (8 mol excess of DTDP/MeOH/H<sub>2</sub>O) followed by acetylation led to the desired thiopyridine sugar **13**. In order to prepare not only the target 6-S-nucleosidyl S-dipyridine disulfides **7f,g**, but the previously synthesized 6-S-(2-S-pyridyl) nucleosides **7a-c** as well, glycosylation reactions with the corresponding purine and pyrimidine bases and the 6-thiopyridine sugar **13** followed by direct deacetylation were employed.

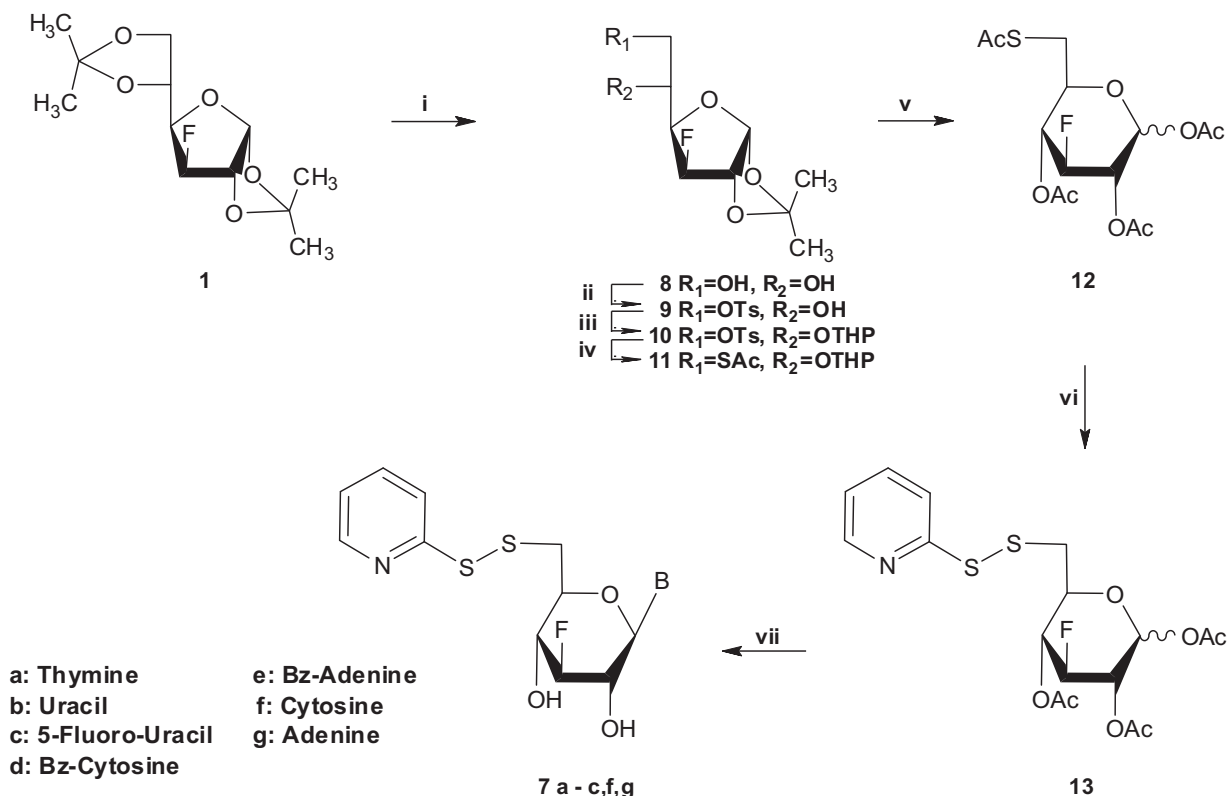
All new compounds were well-characterized by NMR and UV spectroscopy, mass spectrometry and elemental analysis. The <sup>1</sup>H NMR data obtained for the newly synthesized **5a-e** revealed that these compounds had the β configuration (*J*<sub>1,2'</sub> >> 8.0 Hz). It must also be mentioned that the desired compounds **5a-e**, showed strong infra-red absorptions at 1750 cm<sup>-1</sup> (OAc) and 1697 cm<sup>-1</sup> (SAC), while in their <sup>1</sup>H NMR spectra prominent 3-proton methyl signals appeared, which were ascribed to the acetylthio moiety and 6-proton methyl peaks, which correspond to the acetoxyl group. Moreover, data in the <sup>1</sup>H NMR spectra, obtained for the newly synthesized 6-S-(2-S-pyridyl) nucleosides **7a-c,f,g**, revealed the absence of 6-proton methyl peaks, which correspond to the

acetoxyl group and the presence of 2-S-pyridyl moiety, whose proton peaks appeared between 8.4 and 7.1 ppm.

The stability of the target compounds was assigned after 3 months storage, either in organic solution or in solid phase, by performing NMR spectroscopy. Comparison of the primarily NMR spectra of the target molecules with those of the same storable compounds could clearly reveal identical proton NMR spectra.

### 3.2. Antiviral and cytostatic activity

Compounds **6a-c** and **7b,c,f,g** were evaluated for their antiviral activity against a wide variety of DNA and RNA viruses, including herpes simplex virus type 1 (HSV-1) (strain KOS), HSV-2 (strain G), vaccinia virus, vesicular stomatitis virus (VSV), varicella-zoster virus (VZV) strains OKA and 07/1, and human cytomegalovirus (HCMV) strains AD-169 and Davis in HEL cell cultures, VSV, Coxsackie B4 and respiratory syncytial virus (RSV) in HeLa cell cultures, parainfluenza-3 virus, reovirus, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures, influenza A



**Scheme 2.** (i) 70% AcOH; (ii) pyridine, *p*-toluenesulfonyl chloride; (iii)  $CH_2Cl_2$ , *p*-TsOH, DHP; (iv) KSAc/DMF/100 °C; (v) (a) 90% TFA (b)  $Ac_2O$ /pyridine; (vi) (a) methanolic ammonia/ $H_2O$ /MeOH/2,2-dipyridine-disulfide (b)  $Ac_2O$ /pyridine; (vii) (a) silylated base,  $CH_3CN$ , trimethylsilyl trifluoromethane-sulfonate or tin chloride, (b) methanolic ammonia.

(H1N1, H3N2) and B virus in MDCK cell cultures and feline corona virus (FIPV) and feline herpesvirus in CRFK cell cultures. None of the compounds tested showed an appreciable inhibitory activity at subtoxic concentrations (i.e. 4–20  $\mu M$  for **7b**, 20–100  $\mu M$  for **7c** and >100  $\mu M$  for the other compounds). The new molecules were also examined for their cytostatic activity against murine leukemia L1210, murine mammary carcinoma FM3A, human lymphocyte Molt4/C8 and CEM and human cervix carcinoma HeLa cells. Compounds **3a–g** (Table 1) and the acetyl-substituted **5a–e** analogs (data not shown) did not show appreciable antiproliferative activity. Likewise, the 6,6-disulfide glucopyranosyl pyrimidine dinucleoside derivatives **6a–c** did not show appreciable cytostatic action against the evaluated tumor cell lines ( $IC_{50}$ : 71 to >500  $\mu M$ ) (Table 1). However, their corresponding 2-S-pyridyl 6-thioglucofuranosyl mononucleosides **7** were endowed with a more pronounced cytostatic activity in cell culture. In particular, the 5-fluorouracil (**7c**) and especially the uracil (**7b**) derivative showed considerable cytostatic potency against the murine L1210, and human Molt4/C8, CEM and HeLa cells. Also, **7c** and **7b** were cytostatic against proliferating human embryonic lung HEL fibroblasts ( $IC_{50}$ : 36  $\mu M$  and 9.2  $\mu M$ , respectively), Crandell feline kidney cells (CRFK) ( $IC_{50}$ : 6.2 and 39  $\mu M$ ) and canine Madin-Darby kidney cells (MDCK) ( $IC_{50}$ : 50  $\mu M$  and 11  $\mu M$ , respectively). Conversely, the adenine (**7g**) and cytosine (**7f**) derivatives showed poor, if any cytostatic activity (192 – >500  $\mu M$ ) (Table 1). It is somewhat intriguing that among the 2-S-pyridyl 6-thioglucofuranosyl mononucleotides, only the derivatives containing an uracil/5-fluorouracil base were markedly more cytostatic than those, which bear a thymine, cytosine or adenine base. The fluorouracil derivatives proved equally cytostatic against murine and human cells, as well as against leukemia, mammary carcinoma, cervix carcinoma and lymphoma tumor cells. The molecular basis of the antiproliferative activity of

**Table 1**

Inhibitory effects of the test compounds on the proliferation of murine leukemia cells (L1210), murine mammary carcinoma cells (FM3A), human T-lymphocyte cells (Molt4/C8, CEM) and human cervix carcinoma (HeLa) cells.

Compounds	$IC_{50}^a$ ( $\mu M$ )				
	L1210	FM3A	Molt4/C8	CEM	HeLa
<b>3a</b>	>200	>200	>200	>200	
<b>3b</b>	>200	>200	>200	>200	
<b>3c</b>	>200	>200	>200	>200	
<b>3d</b>	>200	>200	>200	>200	
<b>3e</b>	$\geq 200$	>200	$81 \pm 7$	$120 \pm 15$	
<b>3f</b>	$159 \pm 58$	>200	$163 \pm 21$	$155 \pm 64$	
<b>3g</b>	$166 \pm 47$	>200	$183 \pm 24$	$124 \pm 47$	
<b>6a</b>	>500	>500		>500	>500
<b>6b</b>	$302 \pm 36$	$123 \pm 39$		>500	$320 \pm 40$
<b>6c</b>	$231 \pm 34$	$71 \pm 18$		>500	$280 \pm 0$
<b>7a</b>	$120 \pm 56$	>200	$84 \pm 4$	$106 \pm 21$	
<b>7b</b>	$9.5 \pm 0.0$	$17 \pm 4$		$9.2 \pm 1.1$	$8.8 \pm 0.7$
<b>7c</b>	$54 \pm 19$	$35 \pm 2$		$43 \pm 3$	$35 \pm 3$
<b>7f</b>	>500	>500		>500	$369 \pm 7$
<b>7g</b>	$238 \pm 41$	$\geq 500$		$228 \pm 36$	$192 \pm 9$

<sup>a</sup> 50% Inhibitory concentration or compound concentration required to inhibit tumor cell proliferation in cell culture by 50%.

the fluorouracil derivatives is currently unknown. When compounds **7b** and **7c** were exposed to purified recombinant thymidine phosphorylase (TPase) or uridine phosphorylase type I (UPase), they were not hydrolyzed to the free pyrimidine base under conditions where thymidine (exposed to TPase) and uridine (exposed to UPase) were readily converted to thymine and uracil, respectively (data not shown). These findings indicate that these molecules exert their potency *via* their intact nucleoside moiety (or anabolite derived thereof), rather than a catabolic metabolite



(i.e. free 2-S-pyridyl 6-thioglucofuranose). Also, it is clear from the inactivity of the **3a-g** series of compounds that the 2-S-pyridyl group plays an important role, in concert with the uracil base, to express pronounced cytostatic activity. In fact, Decout has recently reported on 2',3'-dideoxyribonucleoside 3'-disulfides [22] and found that those derivatives, which contain a rather bulky moiety on the disulfide part of the molecule (i.e. nitrophenyl, butyl, hexyl, acetyl) were also most cytostatic than others. This is in agreement with the presence of a rather bulky entity on the disulfide in compounds **7b** and **7c** as well. The exact molecular mechanism of cytostatic activity is currently subjected to further studies. *Mutatis mutandis*, compounds **7b** and **7c** could be viewed as potential lead compounds for further modification in order to redesign and synthesize more potent cytostatic agents.

#### 4. Conclusion

We have prepared a series of 2-S-pyridyl-6-thionucleosides, bearing natural and modified purines and pyrimidines, by developing highly efficient synthetic routes. None of the new compounds showed antiviral potency at subtoxic concentrations, but compound **7c**, and in particular **7b**, were endowed with significant cytostatic potency and can be viewed as novel lead compounds for further modifications. It would therefore be interesting to further explore the structure–activity relationships by modifying the base part as well as the sugar portion by incorporating different chemical entities.

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