

## Supplementary Material

### New NTP Analogs: The Synthesis of 4'-ThioUTP and 4'-ThioCTP and Their Utility for SELEX.

Yuka Kato, Noriaki Minakawa, Yasuo Komatsu, Hiroyuki Kamiya, Naoki Ogawa, Hideyoshi Harashima, and Akira Matsuda

**1-[5-*O*-(4-Methoxytrityl)-2,3-di-*O*-acetyl-4-thio- $\beta$ -D-ribofuranosyl]uracil (3).**  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ 8.07 (br s, 1 H), 7.77 (d, 1 H,  $J = 7.9$  Hz),  $\square\square$ 76.85 (m, 14 H), 6.37 (d, 1 H,  $J = 7.3$  Hz), 5.68 (dd, 1 H,  $J = 4.0$  and 7.3 Hz), 5.53 (m, 1 H), 5.36 (dd, 1 H,  $J = 1.8$  and 7.9 Hz), 3.81 (s, 3 H), 3.60 (m, 1 H), 3.57 (m, 1 H), 3.37 (dd, 1 H,  $J = 4.1$  and 10.0 Hz), 2.11 and 2.06 (each s, each 3 H);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ 169.7, 169.6, 162.3, 158.8, 150.6, 143.4, 143.2, 140.3, 134.1, 130.6, 128.5, 128.0, 127.3, 113.3, 103.4, 88.0, 75.8, 74.1, 63.8, 61.4, 55.3, 49.3, 20.9, 20.6; FAB-LRMS  $m/z$  617 ( $\text{MH}^+$ ); FAB-HRMS calad for  $\text{C}_{33}\text{H}_{33}\text{N}_2\text{O}_8\text{S}$  ( $\text{MH}^+$ ) 617.1981 found. 617.1983.

**1-(2,3-Di-*O*-acetyl-4-thio- $\beta$ -D-ribofuranosyl)uracil (5).**  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ 8.23 (br s, 1 H), 8.08 (d, 1 H,  $J = 7.9$  Hz), 6.36 (d, 1 H,  $J = 7.3$  Hz), 5.85 (br d, 1 H,  $J = 7.9$  Hz), 5.69 (dd, 1 H,  $J = 4.0$  and 7.3 Hz), 5.49 (m, 1 H), 4.10 (m, 1 H), 3.84 (m, 1 H), 3.55 (m, 1 H), 2.42 (br s, 1 H), 2.17 and 2.06 (each s, each 3 H);  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ 170.1, 169.8, 162.7, 150.6, 140.9, 103.6, 76.1, 74.6, 63.0, 62.1, 51.1, 21.0, 20.6; FAB-LRMS  $m/z$  345 ( $\text{MH}^+$ ); FAB-HRMS calad for  $\text{C}_{13}\text{H}_{17}\text{N}_2\text{O}_7\text{S}$  ( $\text{MH}^+$ ) 345.0769 found. 345.0771.

**4'-Thiouridine 5'-triphosphate (7).**  $^1\text{H-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ 8.29 (d, 1 H,  $J = 7.9$  Hz), 6.00 (m, 2 H), 4.45 (m, 2 H), 4.27 (m, 2 H), 4.14 (m, 1 H), 3.60 (m, 1 H);  $^{31}\text{P-NMR}$  ( $\text{D}_2\text{O}$ )  $\delta$ -5.59 (d,  $J = 20.0$  Hz), -10.48 (d,  $J = 20.0$  Hz), -21.12 (t,  $J = 20.0$  Hz); FAB-LRMS (negative)  $m/z$  565 ( $\text{M}^-$ ); FAB-HRMS (negative) calad for  $\text{C}_9\text{H}_{12}\text{N}_2\text{Na}_3\text{O}_{14}\text{P}_3\text{S}$  ( $\text{M}^-$ ) 565.8915 found. 565.8892.

***N*<sup>4</sup>-Benzoyl-1-[5-*O*-(4-methoxytrityl)-2,3-di-*O*-acetyl-4-thio- $\beta$ -D-ribofuranosyl]cytosine**

**(4).** <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ 8.86 (br s, 1 H), 8.32 (d, 1 H, *J* = 7.6 Hz),  $\tilde{\square}$ 89 (m, 2 H), 7.61–7.27 (m, 15 H), 6.89 (m, 2 H), 6.53 (d, 1 H, *J* = 6.2 Hz), 5.69 (m 1 H), 5.17 (m, 1 H), 3.82 (s, 3 H), 3.62 (m, 1 H), 3.55 (m, 1 H), 3.45 (m, 1 H), 2.08 and 2.06 (each s, each 3 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ 169.6, 169.4, 161.8, 158.6, 145.5, 143.2, 143.1, 134.3, 133.0, 130.4, 128.8, 128.5, 127.9, 127.4, 127.2, 97.7, 87.7, 76.5, 63.1, 62.7, 55.2, 48.9, 20.7, 20.5; FAB-LRMS *m/z* 720 (MH<sup>+</sup>); FAB-HRMS calad for C<sub>40</sub>H<sub>38</sub>N<sub>3</sub>O<sub>8</sub>S (MH<sup>+</sup>) 720.2380 found. 720.2375.

***N*<sup>4</sup>-Benzoyl-1-(2,3-di-*O*-acetyl-4-thio- $\beta$ -D-ribofuranosyl)cytosine (6).**

<sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ 9.11 (br s, 1 H), 8.75 (d, 1 H, *J* = 7.6 Hz),  $\tilde{\square}$ 85 (m, 2 H), 7.60–7.43 (m, 4 H), 6.89 (m, 2 H), 6.45 (d, 1 H, *J* = 6.7 Hz), 5.82 (m 1 H), 5.58 (m, 1 H), 4.45 (br s, 1 H), 4.05 (m, 1 H), 3.88 (m, 1 H), 3.60 (m, 1 H), 2.11 and 2.01 (each s, each 3 H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>)  $\delta$ 170.1, 169.7, 162.1, 146.5, 133.0, 132.8, 128.7, 127.5, 98.1, 76.8, 74.7, 63.4, 62.4, 51.3, 20.8, 20.6; FAB-LRMS *m/z* 448 (MH<sup>+</sup>); FAB-HRMS calad for C<sub>20</sub>H<sub>22</sub>N<sub>3</sub>O<sub>7</sub>S (MH<sup>+</sup>) 448.1155 found. 448.1198.

**4<sup>2</sup>-Thiocytidine 5<sup>2</sup>-triphosphate (8).**

<sup>1</sup>H-NMR (D<sub>2</sub>O)  $\delta$ 8.33 (d, 1 H, *J* = 7.9 Hz), 6.16 (m, 1 H), 6.01 (d, 1 H, *J* = 4.6 Hz), 4.40 (m, 2 H), 4.30 (m, 1 H), 4.18 (m, 1 H), 3.62 (m, 1 H); <sup>31</sup>P-NMR (D<sub>2</sub>O)  $\delta$ -6.28 (d, *J* = 20.0 Hz), -10.48 (d, *J* = 20.0 Hz), -21.23 (t, *J* = 20.0 Hz); FAB-LRMS (negative) *m/z* 564 [(M-H)<sup>-</sup>]; FAB-HRMS (negative) calad for C<sub>9</sub>H<sub>12</sub>N<sub>3</sub>Na<sub>3</sub>O<sub>13</sub>P<sub>3</sub>S [(M-H)<sup>-</sup>] 563.8997 found. 563.8992.