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A New DNA Building Block, 4′-Selenothymidine: Synthesis and Modification to 4′-Seleno-AZT as a Potential Anti-HIV Agent

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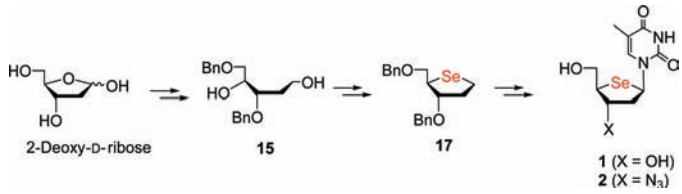
ABSTRACT



The first synthesis of 4′-selenothymidine (1), a novel DNA building block, and 4′-seleno-AZT (2) was accomplished from 2-deoxy-D-ribose via stereoselective formation of 2-deoxy-4-seleno-D-furanose 17 and a Pummerer-type base condensation as key steps. 4′-Selenothymidine (1) was discovered to adopt the same 2′-endo/3′-exo conformation as thymidine, which is unusual in that 4′-selenouridine has the opposite conformation to that of uridine.

Thymidine is a DNA building block and serves as a key element for replication of the cell. Therefore, modified thymidine or thymidylate derivatives have been extensively used as biochemical tools to study biochemical behaviors of enzymes related to cell replication or as antimetabolites to inhibit normal pathways of cells.[1] Several groups reported the second-generation DNA building block, 4′-thiothymidine, which is in a bioisosteric relationship with thymidine.[2] From this new template, many biologically active 2′-deoxy-4′-thionucleosides have been developed.[3,4] When compared with 4′-oxonucleosides, 4′-thionucleosides showed better glycosidic bond stability against metabolic enzymes or chemical hydrolysis.[3] The X-ray crystallographic analysis showed that 4′-thiothymidine adopts the same 2′-endo/3′-exo (South) conformation as thymidine.[2] This result indicates that the sulfur atom of 4′-thiothymidine behaves like the oxygen atom of thymidine, showing the electronic (gauche) effect.[5]

3′-Azido-3′-deoxythymidine (AZT)[6] is the representative of modified thymidine derivatives and has been a drug of

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(1) Breaker, R. R. *Nature* **2004**, *432*, 838–845.

(2) (a) Uenishi, J.; Takahashi, K.; Motoyama, M.; Akashi, H.; Sasaki, T. *Nucleosides Nucleotides* **1994**, *13*, 1347–1361. (b) Tiwari, K. N.; Montgomery, J. A.; Secrist, J. A., III *Nucleosides Nucleotides* **1993**, *12*, 841–846. (c) Rahim, S. G.; Trivedi, N.; Bogunovic-Batchelor, M. V.; Hardy, G. W.; Mills, G.; Selway, J. W. T.; Snowden, W.; Littler, E.; Coe, P. L.; Basnak, I.; Whale, R. F.; Walker, R. T. *J. Med. Chem.* **1996**, *39*, 789–795. (d) Secrist, J. A., III; Tiwari, K. N.; Riordan, J. M.; Montgomery, J. A. *J. Med. Chem.* **1991**, *34*, 2361–2366. (e) Dyson, M. R.; Coe, P. L.; Walker, R. T. *J. Med. Chem.* **1991**, *34*, 2782–2786. (f) Uenishi, J.; Takahashi, K.; Motoyama, M.; Akashi, H. *Chem. Lett.* **1993**, 255–256.

(3) Gunaga, P.; Moon, H. R.; Choi, W. J.; Shin, D. H.; Park, J. G.; Jeong, L. S. *Curr. Med. Chem.* **2004**, *11*, 2585–2637.

(4) Van Draanen, N. A.; Freeman, G. A.; Short, S. A.; Harvey, R.; Jansen, R.; Szczech, G.; Koszalka, G. W. *J. Med. Chem.* **1996**, *39*, 538–542.

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choice in treating AIDS and AIDS-related complex (ARC). AZT is converted into AZT-triphosphate in the cell, which inhibits HIV-1 RT (reverse transcriptase) competitively and/or terminates viral DNA chain after being incorporated into proviral DNA.[7] However, the clinical use of AZT is hampered by severe toxicity such as bone marrow toxicity and anemia and appearance of AZT-resistant strains.[8] A similar analogue, 3′-azido-2′,3′-dideoxyuridine (AZDU),[9] was also discovered as a potent and selective anti-HIV agent. Although AZDU was less potent than AZT, it showed a better toxicity profile than AZT due to its different cellular metabolism.[10]

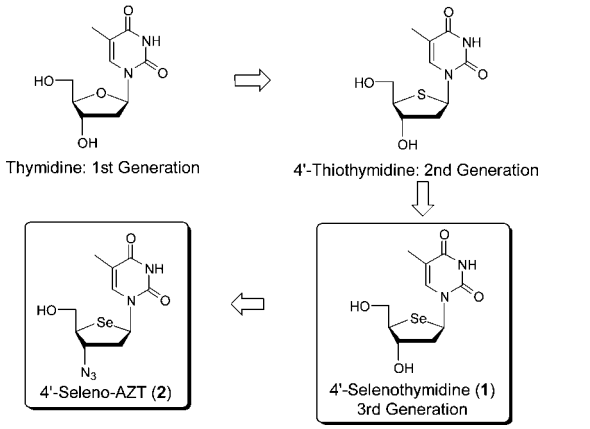
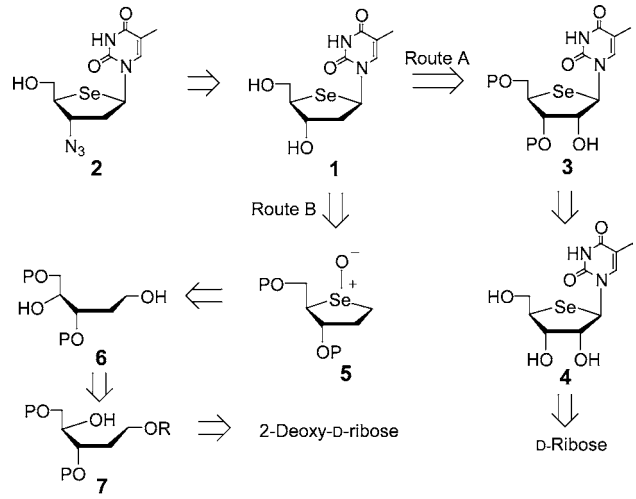


Figure 1. Rationale for the design of the target nucleosides.

Recently, we and other groups reported the synthesis of a novel RNA building block, 4′-selenouridine, and its oligonucleotides.[11] From this study, it was revealed that the bulky selenium atom played a key role in deciding the conformation of 4′-selenouridine. From this RNA building block, 2′-deoxy-2′-fluoro-4′-selenoarabinofuranosylcytosine (2′-F-4′-Se-ara-C) was discovered as a potent anticancer agent.[12] Thus, it was of great interest to synthesize the new DNA building block, 4′-selenothymidine, as a potential biochemical tool or as a template for the development of new drugs (Figure 1). It is also interesting to synthesize the 3′-azido-4′-selenothymidine (4′-Se-AZT) and to compare its anti-HIV activity with that of AZT. Herein, we describe the first synthesis of a new DNA building block, 4′-selenothymidine and its conformational study, as well as its modification to 4′-Se-AZT.

Scheme 1. Retrosynthetic Analysis of the Desired Nucleosides **1** and **2**



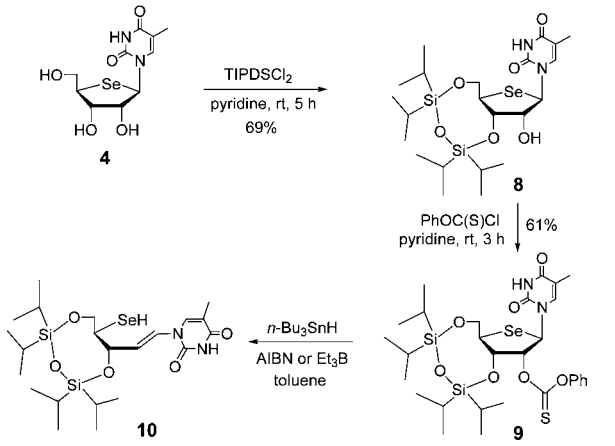
Scheme 1 illustrates the retrosynthetic analysis of the desired nucleosides **1** and **2**. 4′-Se-AZT (**2**) can be synthesized from 4′-selenothymidine (**1**) by opening the 2,3′-anhydrothymidine derivative with sodium azide. It was thought that 4′-selenothymidine (**1**) might be synthesized via two routes (A and B). The first route was to utilize the Barton−McCombie deoxygenation[13] of the 2′-hydroxyl derivative **3** which could be easily derived from 4′-selenoribosyl derivative **4**[14] (route A). The second route was to use the Pummerer-type condensation of selenoxide **5** with thymine (route B). The selenoxide **5** would be synthesized from the diol **6** by mesylation followed by ring closure with Na_2Se. The diol **6** might be derived from **7** using a Mitsunobu reaction as a key step. Thus, it was realized that compound **7** could be easily synthesized from 2-deoxy-D-ribose.

Route A using radical deoxygenation[13,15] as a key step was first tried (Scheme 2). 4′-Selenoribosyl derivative **4**[14] was treated with $TIPDSCl_2$ to give 3′,5′-di-*O*-protected derivative **8**. Treatment of **8** with phenyl chlorothionoformate gave the thiocarbonate **9**. Radical deoxygenation[13,15] of **9** with *n*-Bu_3SnH in the presence of AIBN or Et_3B afforded the ring-cleaved product **10**.

Even under milder conditions, the desired 2′-deoxy derivative could not be obtained. These observations are in agreement with those observed during the synthesis of 2′-

(5) Altona, C.; Sundaranlingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205.
(6) Mitsuya, H.; Weinhold, K. J.; Furman, P. A.; St. Clair, M. H.; Lehrmann, S. N.; Gallo, R. C.; Bolognesi, D.; Barry, D. W.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096–7100.
(7) Arts, E. J.; Wainberg, M. A. *Antimicrob. Agents Chemother.* **1996**, *40*, 527–540.
(8) Styrt, B. A.; Piazza-Hepp, T. D.; Chikami, G. K. *Antiviral Res.* **1996**, *31*, 121–135.
(9) Chu, C. K.; Schinazi, R. F.; Ahn, M. K.; Ullas, G. V.; Gu, Z. P. *J. Med. Chem.* **1989**, *32*, 612–617.
(10) Zhu, Z.; Schinazi, R. F.; Chu, C. K.; Williams, G. J.; Colby, C. B.; Sommadossi, J. P. *Mol. Pharmacol.* **1990**, *38*, 929–938.
(11) (a) Jeong, L. S.; Tosh, D. K.; Kim, H. O.; Wang, T.; Hou, X.; Yun, H. S.; Kwon, Y.; Lee, S. K.; Choi, J.; Zhao, L. X. *Org. Lett.* **2008**, *10*, 209–212. (b) Jayakanthan, K.; Johnston, B. D.; Pinto, B. M. *Carbohydr. Res.* **2008**, *343*, 1790–1800. (c) Watts, J. K.; Johnston, B. D.; Jayakanthan, K.; Wahba, A. S.; Pinto, B. M.; Damha, M. J. *J. Am. Chem. Soc.* **2008**, *130*, 8578–8579.
(12) Jeong, L. S.; Tosh, D. K.; Choi, W. J.; Lee, S. K.; Kang, Y.-J.; Choi, S.; Lee, J. H.; Lee, H.; Lee, H. W.; Kim, H. O. *J. Med. Chem.* **2009**, *52*, 5303–5306.
(13) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, *16*, 1574–1585.
(14) Tosh, D. K.; Choi, W. J.; Kim, H. O.; Lee, Y.; Pal, S.; Hou, X.; Choi, J.; Choi, S.; Jeong, L. S. *J. Org. Chem.* **2008**, *73*, 4259–4262.
(15) Nozaki, K.; Oshima, K.; Utimoto, K. *Tetrahedron Lett.* **1988**, *29*, 6125–6126. (b) Crich, D.; Quintero, L. *Chem. Rev.* **1989**, *89*, 1413–1432.

Scheme 2. Synthetic Approach to 4′-Selenothymidine via 2′-Deoxygenation



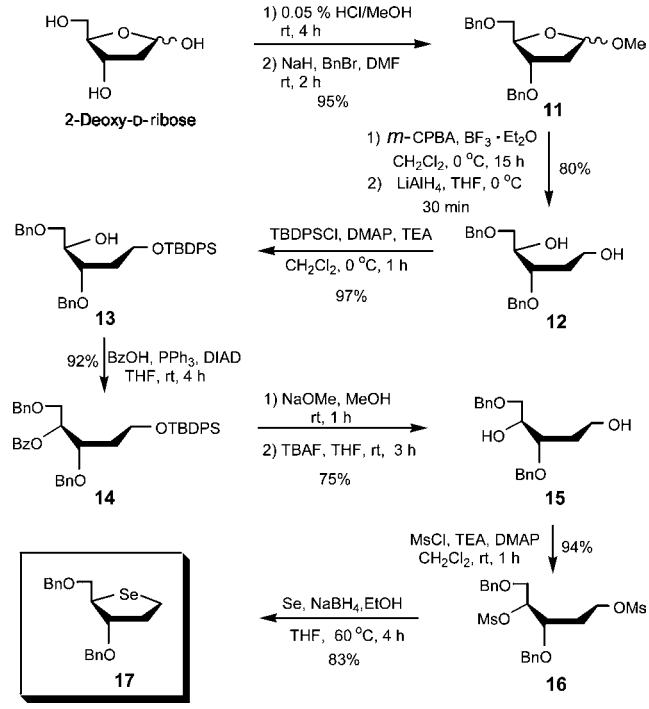
deoxy-4′-thioribonucleosides.[16] In the case of 4′-thionucleosides, the desired 2′-deoxy derivative after deoxygenation reaction was obtained as a major product, along with the ring-cleaved derivative. However, we observed the exclusive formation of **10** under our set of conditions. These observations led to the postulate that homolytic cleavage of the C−Se bond occurs rapidly along with the C−O bond, leading to the ring-cleaved radical intermediate **10** as the exclusive product. This route was abandoned as it appeared that the desired 2′-deoxy analogues could not be synthesized under these conditions. Thus, we opted for an alternative strategy (route B) which would synthesize the key 4-selenosugar **17** prior to condensing it with thymine, as illustrated in Scheme 3.

Treatment of 2-deoxy-D-ribose with 0.05% HCl in MeOH followed by benzylation of the remaining hydroxyl groups afforded the anomeric methoxide **11**.[17] For the conversion of **11** into the diol **12**, various conventional conditions (acid-catalyzed hydrolysis and reduction) were attempted, but the desired diol **12** was obtained in poor yield. As the low yield resulted from the acid-catalyzed hydrolysis, we decided to use nonhydrolysis conditions. Oxidation of the anomeric methoxide **11** with *m*-CPBA in the presence of BF_3•Et_2O yielded the lactone which was reduced with LAH to produce the diol **12**[17] in excellent yield. Inversion of stereochemistry of the secondary hydroxyl group of **13** was achieved by the Mitsunobu reaction, giving the benzoate **14** after the primary hydroxyl group of **12** was protected with TBDPS group. Removal of the benzoyl and TBDPS protecting groups of **14** gave the diol **15**, which was converted to the dimesylate **16**. Treatment of **16** with selenium and $NaBH_4$ in EtOH at reflux afforded the key 2-deoxy-4-seleno-D-ribose derivative **17** in very good yield.[11] To the best of our knowledge, this is the first example of a sugar of the 2-deoxy-4-selenoribofuranose series.

(16) Naonori, I.; Daisuke, K.; Noriaki, M.; Akira, M. *J. Org. Chem.* **2005**, *70*, 8597–8600.

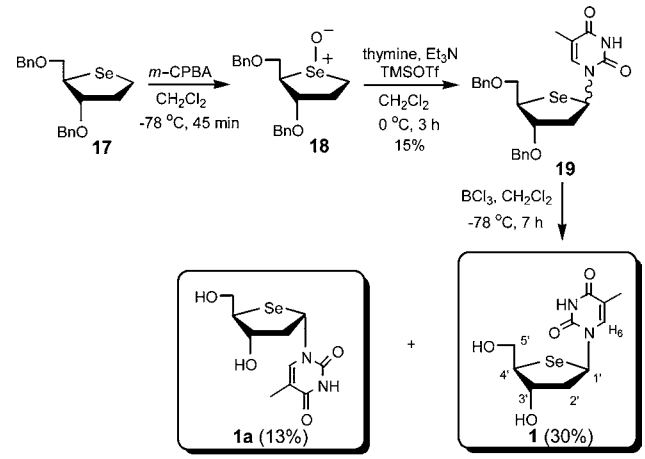
(17) Takahashi, T.; Hirose, Y.; Iwamoto, H.; Doi, T. *J. Org. Chem.* **1998**, *63*, 5742–5743.

Scheme 3. Synthesis of the Key 4-Selenosugar **17**



The 2-deoxy-4-seleno-D-ribose derivative **17** was oxidized to the 4-selenoxide **18** which was condensed with thymine in the presence of TMSOTf and Et_3N in CH_2Cl_2 to give the thymidine analogue **19** (15%) as an inseparable anomeric mixture (Scheme 4). Treatment of **19** with BCl_3 afforded the 4′-selenothymidine (**1**) and its α-anomer (**1a**). Anomeric configurations of **1** and **1a** were assigned on the basis of ^{1}H NOE experiments. Irradiation on 1′-H of compound **1** gave no NOE effect on 3′-H, while an NOE effect (1.48%) was observed in the same experiment on compound **1a**. The NOE effect (1.4%) between 3′-H and H-6 in compound **1** was also

Scheme 4. Synthesis of 4′-Selenothymidine (**1**) and Its α-Anomer **1a**



measured, but no NOE was observed in case of **1a**, indicating that compound **1** is the β-anomer. Anomeric configuration of compound **1** was finally confirmed by the X-ray crystal structure,[18] as shown in Figure 2.

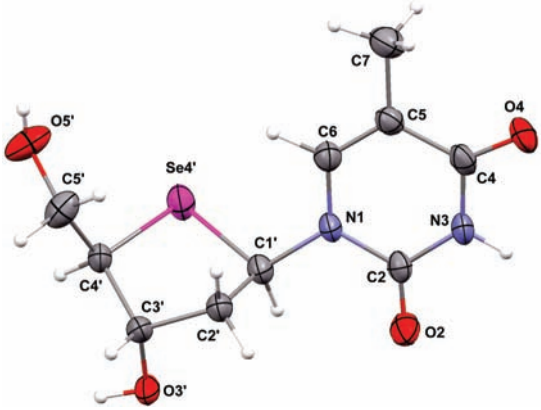


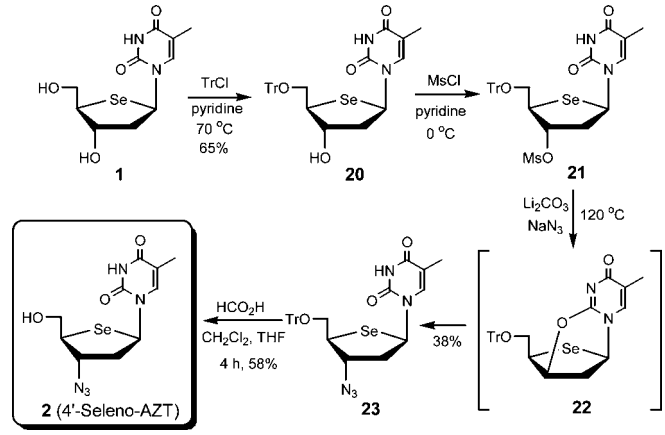
Figure 2. X-ray crystal structure of **1**.

X-ray crystallographic analysis indicated that 4′-selenothymidine (**1**) adopts the same 2′-endo/3′-exo (South) conformation as thymidine (Figure 2). This result is surprising in that 4′-selenouridine adopted opposite conformation to that of uridine.

As mentioned earlier, as AZT is a potent anti-HIV agent, 4′-selenothymidine (**1**) was converted to the 4′-seleno-AZT (**2**), as illustrated in Scheme 5. Tritylation of **1** gave 5′-trityl derivative **20** which was converted to the mesylate **21**.

Treatment of **21** with NaN_3 in the presence of Li_2CO_3 afforded 3′-azido derivative **23** via the intermediate **22**.[19] Removal of the trityl group of **23** with formic acid yielded the 4′-seleno-AZT (**2**). The configuration of azido group of **2** was confirmed by ^{1}H NOE experiments (400 MHz NMR)

Scheme 5. Synthesis of 4′-Seleno-AZT (**2**)



in CD_3OD. An NOE effect (1.44%) between 3′-H and H-6 was observed, but no NOE effect between 1′-H and 3′-H was measured, indicating that the azido substituent possesses the desired α-configuration. Anti-HIV activity of **1** and **2** was measured in MT-4 cells infected with the HIV-1 IIIB strain, but these compounds showed neither anti-HIV activity nor cytotoxicity up to 100 μM.

In summary, we have accomplished the first synthesis of 4′-selenothymidine (**1**), which is a novel DNA building block, and 4′-seleno-AZT (**2**) as a potential antiviral agent from 2-deoxy-D-ribose. It was also discovered that 4′-selenothymidine (**1**) showed the same 2′-endo/3′-exo (South) conformation as thymidine. The 4′-selenothymidine (**1**) reported here is expected to serve as a good template for the development of new drugs as well as new biochemical tools. Experiments to identify if 4′-selenothymidine is phosphorylated by cellular kinases or incorporated into DNA chain are underway.

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Note Added after ASAP Publication. Two references were added to reference 11 in the version reposted April 29, 2010.

Supporting Information Available: Complete experimental procedure and ^{1}H and ^{13}C NMR copies of all compounds described herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) $C_{10}H_{14}N_2O_4Se$: M_r = 305.19, monoclinic, space group $P21$ (no. 4), a = 9.2745(7) Å, b = 5.2059(3) Å, c = 12.1233(7) Å, V = 584.97(6) Å^3, T = 290(2) K, Z = 2, ρ_{calc} = 1.733 g cm^{-3}, $F(000)$ = 308, crystal dimension 0.40 × 0.30 × 0.20 mm^3, μ(MoKα) = 3.22 mm^{-1}, MoKα radiation (λ = 0.71073 Å). Of 5697 reflections collected in the 2θ range from 3.4 to 27.5° using an ω scan on a Rigaku Rapid R-axis diffractometer, 2410 were unique reflections (R_{int} = 0.028). The structure was solved and refined against F_2 using SHELXS97 and SHELXL97, 184 variables, wR_2 = 0.055, R_1 = 0.025 (the 2196 reflections having $F_o^2 > 2\sigma(F_o^2)$), GOF = 1.14, and max/min residual electron density 0.35/−0.53 e Å^{-3}. Flack x parameter = 0.025(9). Further details of the crystal structure investigation(s) may be obtained from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge, CB2 1EZ (U.K.); Tel: (+44)1223-336-408, Fax: (+44)1223-336-033, e-mail: deposit@ccdc.cam.ac.uk) on quoting the depository no. CCDC-761848.

(19) Chen, B.-C.; Quinlan, S. L.; Reid, J. G. *Tetrahedron Lett.* **1995**, *36*, 7961–7964.