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RESEARCH ARTICLE

Synthesis and biological evaluation of 2′-substituted-4′-selenoribofuranosyl pyrimidines as antitumor agents

Varughese Alexander · Jayoung Song · Jinha Yu · Jung Hee Choi · Jin-Hee Kim · Sang Kook Lee · Won Jun Choi · Lak Shin Jeong

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Abstract The 2′-substituted-4′-selenoribofuranosyl pyrimidines **3a**–**3j** were synthesized from D-ribose and assayed for anticancer activity. The 2′-azido and 2′-fluoro groups with a *ribo* configuration were introduced by the regioselective opening of the O_2,2′-anhydronucleosides with sodium azide and $(HF)_x$-pyridine, respectively. Among the compounds tested, only 2′-fluoro derivative **3j** was found to exhibit significant anticancer activity, but was much less potent than the corresponding 2′-*arabino* analogue **2c**. This study will provide medicinal chemists with the insight into the identification of structural requirements for the anticancer activity for the developments of biologically active nucleosides.

Keywords Antitumor activity · 4′-Selenonucleosides · Regioselective opening · Azidation · Fluorination

Introduction

Modified nucleosides have been served as valuable resources of therapeutic agents such as antiviral and antitumor agents (Ichikawa 2001; Jordheim et al. 2013). Modifications have largely been made on the furanose ring, among which 2′-substitution resulted in potent antiviral and antitumor agents (Ichikawa 2001; Jordheim et al. 2013). For example, 1-β-D-arabinofuranosyl-cytosine (**1**, ara-C) is being clinically used for the treatment of leukemia (Ellison et al. 1968). Based on the bioisosteric rationale, we synthesized the 4′-seleno analogue, 4′-Se-ara-C of **1**, starting from D-ribose and evaluated it for cytotoxicity in human cancer cell lines, but 4′-Se-ara-C (**2**, X = NH_2, Y = H, R = OH) was less potent than **1** in human cancer cell lines tested (Jeong et al. 2009). However, the 2′-fluoro-4′-seleno analogue (**2**, X = NH_2, Y = H, R = F) exhibited more potent anticancer activity than **1** in a broad range of human cancer cell lines tested except leukemia (K562) cell lines (Jeong et al. 2009). Structure–activity relationships of 2′-modified-4′-selenoarabinofuranosyl pyrimidines demonstrated that the anticancer activity is in the following order: 2′-F > 2′-OH > 2′-N_3 (Kim et al. 2014). Thus, it is of great interest to synthesize the corresponding 2′-modified-4′-selenoribofuranosyl pyrimidines **3** and to compare the anticancer activities between *arabino* and *ribo* configurations at the 2′-position. Herein, we report the synthesis and anticancer activity of 2′-substituted-4′-selenoribofuranosyl pyrimidines **3**.

The 2′-azido and 2′-fluoro groups were stereoselectively introduced by the regioselective openings of the O_2,2′-anhydronucleoside with the azide and fluoride anions, respectively. Herein, we report the synthesis of 2′-substituted-4′-selenopyrimidine nucleosides **3** and their cytotoxic activity in various cancer cell lines (Fig. 1).

Results and discussion

For the synthesis of the 2′-azido-2′-deoxy-4′-selenoribofuranosyl pyrimidines **3a**–**3f** (Scheme 1), the ribo analogues

V. Alexander · J. Yu · J. H. Choi · J.-H. Kim
College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

J. Song · S. K. Lee · L. S. Jeong (✉)
Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Korea
e-mail: lakjeong@snu.ac.kr

W. J. Choi
College of Pharmacy, Dongguk University, Seoul 410-774, Kyungki-do, Korea

W. J. Choi (✉)
College of Pharmacy, Dongguk University, Seoul 410-820, Kyungki-do, Korea
e-mail: mp89@dongguk.edu

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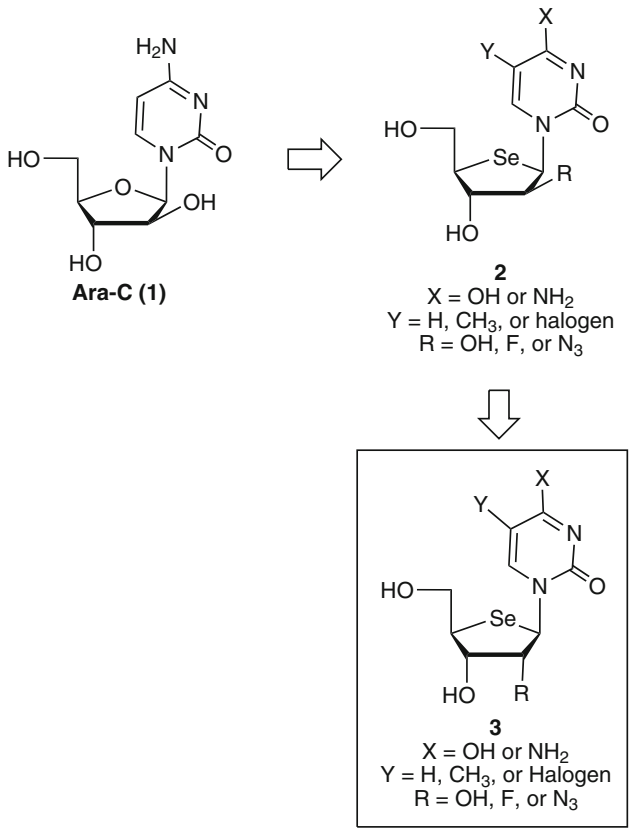


Fig. 1 The rationale for the design of the target nucleoside **3**

4a–**4f** (Kim et al. 2014) were protected with TIPDS group to give the 3′,5′-di-*O*-TIPDS protected nucleosides **5a**–**5f**, (Kim et al. 2014) respectively. Treatment of **5a**–**5f** with DAST or MsCl/DBU afforded the O_2,2′-anhydronucleosides **6a**–**6f**, (Kim et al. 2014) respectively. After the removal of the TIPDS groups of **6a**–**6f** with 3HF-Et_3N, the resulting diols **7a**–**7f** (Kim et al. 2014) were treated with sodium azide in DMF at 120 °C to yield the final 2′-azido-2′-deoxy-4′-selenoribofuranosyl pyrimidines **3a**-**3f**. The configuration of the 2′-azido group of **3a**–**3f** was unambiguously confirmed by ^{1}H NOE experiments, as shown in Fig. 2. Strong NOE between 2′-H and H-6 was observed, but no NOE between 2′-H and 4′-H was observed, confirming that 2′-azido group possesses the *ribo* configuration.

Synthesis of the 2′-deoxy-2′-fluoro-4′-selenoribofuranosyl uracil (**3g**) is illustrated in Scheme 2. 4′-Selenouridine (**4a**) was treated with trityl chloride to give the 5′-*O*-trityl derivative **8** (Jeong et al. 2009), which was treated with 1,1′-thiocarbonyl diimidazole (TCI) in toluene to yield the O_2,2′-anhydronucleoside **9** (Jeong et al. 2009) as a single regioisomer. Treatment of **9** with 3HF-pyridine in 1,4-dioxane afforded the 2′-fluoro analogue **3g** with a *ribo* configuration.

Synthesis of the cytosine derivatives **3h**-**3j** is depicted in Scheme 3. The uracil derivatives, **4a**, **3a**, and **3g** were peracetylated to yield **10**, **11**, and **12**, which were converted to the corresponding triazole derivatives **13**, **14**, and **15**, respectively by treating with $POCl_3$, 1,2,4-triazole, and Et_3N. Treatment of **13**, **14**, and **15** with 28 % NH_4OH afforded the cytosine derivatives, which were further treated with methanolic ammonia to yield the final cytosine derivatives **3h**–**3j**, respectively.

All the synthesized 2′-substituted-4′-selenonucleosides **3a**–**3j** were tested for cytotoxic effects in several human cancer cell lines such as colon cancer (HCT116), lung cancer (A549), stomach cancer (SNU638), breast cancer (T47D), prostate cancer (PC-3), and leukemia (K562) cells, using sulforhodamine B (SRB) protein staining method (Lee et al. 2002; Table 1).

All the uracil, thymine, and 5-halouracil derivatives **3a**–**3g** did not exhibit significant anticancer activity up to 100 μM in human tumor cell lines tested, irrespective of any substitution at 2′-position. In the case of the cytosine derivatives **3h**–**3j**, only 2′-fluor derivative **3j** exhibited significant anticancer activity, but was less potent than the corresponding *arabino* derivative **2c** (Kim et al. 2014). In general, the *arabino* derivatives were much more potent than the corresponding *ribo* derivatives.

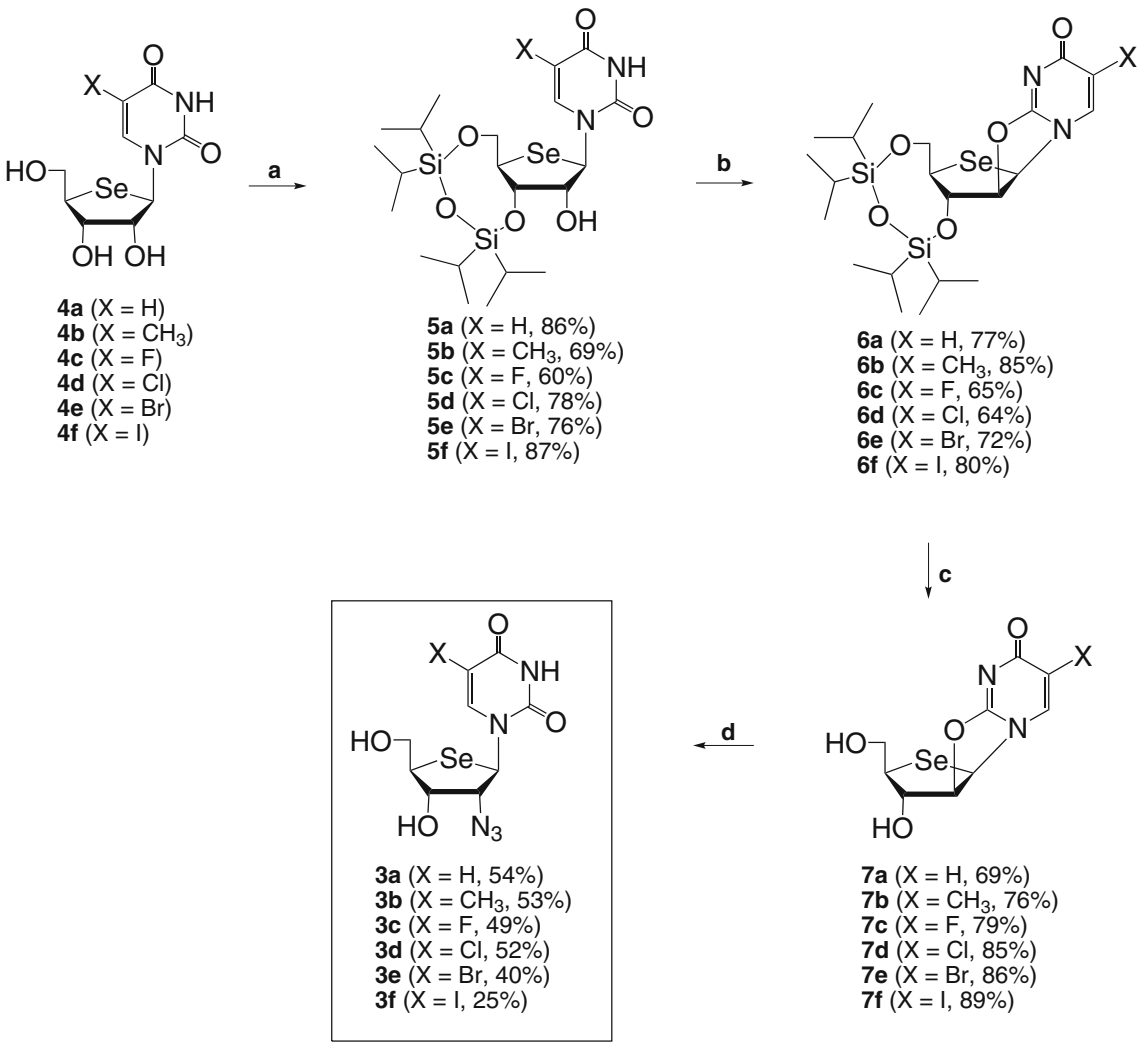
Conclusions

Based on the structure–activity relationship of the 2′-substituted-4′-selenoarabinofuranosyl pyrimidines **2** (Kim et al. 2014) as anticancer agents, novel 2′-substituted-4′-selenoribofuranosyl pyrimidines **3a**–**3j** were synthesized from D-ribose and evaluated for anticancer activity. The highlight of our synthetic endeavor is the regioselective formation of O_2,2′-anhydronucleosides using DAST or MsCl/DBU and the regioselective opening of the resulting O_2,2′-anhydronucleosides with sodium azide and $(HF)_x$-pyridine for the introduction of the 2′-azido and 2′-fluoro group with a *ribo* configuration, respectively. From this study, 2′-fluoro derivative **3j** only exhibited significant anticancer activity among the compounds synthesized and the 2′-*ribo* analogues were much less potent than the corresponding 2′-*arabino* analogues. Although we could not discover promising anticancer agents from this study, the identification of structural requirements for the anticancer activity will be extensively utilized for the developments of biologically active nucleosides.

Experimental section

General methods

^{1}H-NMR Spectra ($CDCl_3$, CD_3OD or DMSO-d_6) were recorded on Varian Unity Invoa 400 MHz. The ^{1}H-NMR



Scheme 1 Reagents and conditions: (*a*) $TIPSCl_2$, pyridine, rt, 3 h; (*b*) DAST, MC, −78 °C, 1 h or MsCl, then DBU; (*c*) 3HF–Et_3N, THF, 0 °C, 1 h; (*d*) NaN_3, DMF, 120 °C, overnight

data are reported as peak multiplicities: s for singlet, d for doublet, dd for doublet of doublets, t for triplet, q for quartet, br s for broad singlet and m for multiplet. Coupling constants are reported in hertz. ^{13}C-NMR spectra ($CDCl_3$, CD_3OD or DMSO-d_6) were recorded on Varian Unity Inova 100 MHz. ^{19}F-NMR spectra ($CDCl_3$, CD_3OD) were recorded on Varian Unity Inova 376 MHz. The chemical shifts were reported as parts per million (δ) relative to the solvent peak. Optical rotations were determined on Jasco III in appropriate solvent. UV spectra were recorded on U-3000 made by Hitachi in methanol or water. Infrared spectra were recorded on FT-IR (FTS-135) made by Bio-Rad. Melting points were measured on B-540 made by Buchi. Elemental analyses (C, H, and N) were used to determine purity of all synthesized compounds, and the results were within ±0.4 % of the calculated values, confirming ≥95 % purity. Reactions were checked with TLC (Merck precoated $60F_{254}$ plates). Spots were detected by viewing under a UV light, colorizing with charring after dipping in anisaldehyde solution with acetic acid, sulfuric acid and methanol. Column chromatography was performed on silica gel 60 (230–400 mesh, Merck). Reagents were purchased from Aldrich Chemical Company. Solvents were obtained from local suppliers. All the anhydrous solvents were distilled over CaH_2, P_2O_5 or sodium/benzophenone prior to the reaction.

General procedure for the synthesis of **5a**–**5f** (Kim et al. 2014)

To a solution of **4a**–**4f** in pyridine was added $TIPDSCl_2$ (1.5 equiv.) at 0 °C and the mixture was allowed to stir at room temperature for 3 h. The reaction mixture was concentrated, and the residue co-evaporated three times with

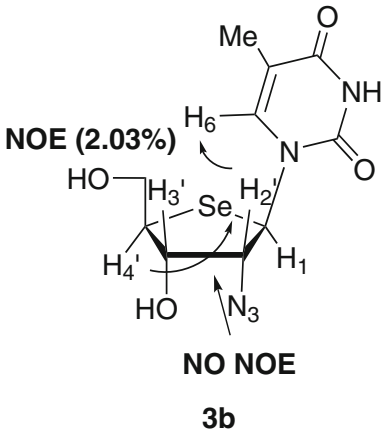
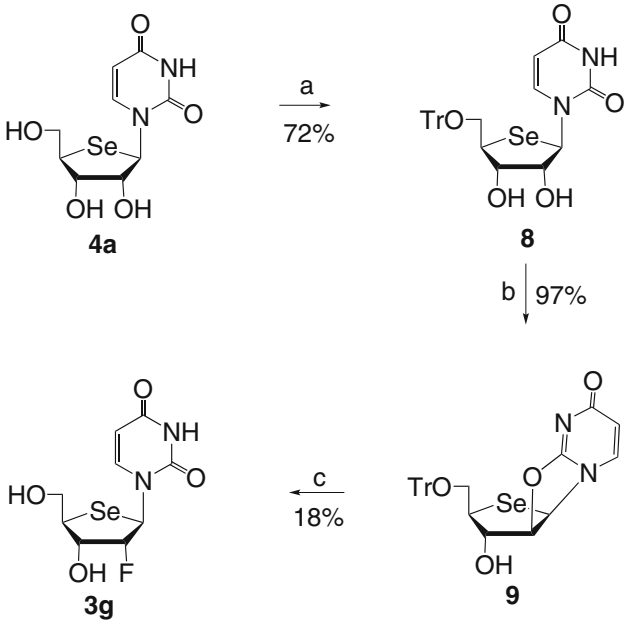


Fig. 2 ^{1}H NOE of 2′-azido-4′-selenoribofuranosyl thymine (**3b**)

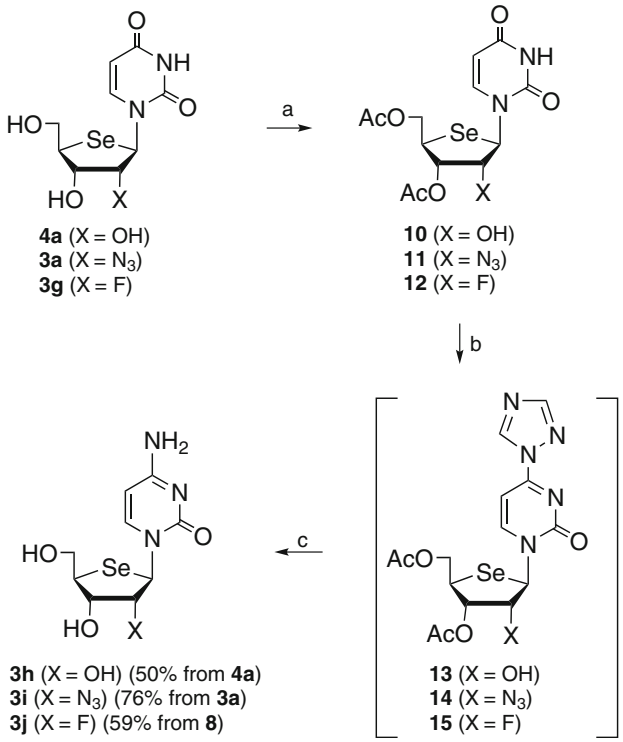


Scheme 2 Reagents and conditions: (*a*) TrCI, pyridine, rt, 15 h; (*b*) TCI, toluene, 100 °C, 15 h; (*c*) $(HF)_x$-pyridine, 1,4-dioxane, 125 °C, 2 d

toluene. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 2:1) to give **5a**–**5f**.

General procedure for the synthesis of O_2,2′-anhydro nucleosides **6a**–**6f** (Kim et al. 2014)

To a stirred solution of **5a**–**5f** (1 equiv.) in dichloromethane was added DAST (2 equiv.) at −78 °C, and the reaction mixture was stirred at the same temperature for 1.5 h. Then saturated $NaHCO_3$ solution was added at 0 °C and the reaction mixture was partitioned between dichloromethane and water. The organic layer was washed with saturated



Scheme 3 Reagents and conditions: **a** Ac_20, rt, 15 h; **b** 1,2,4-triazole, Et_3N, $POCl_3$, CH_3CN, rt, 15 h; **c** NH_4OH, 1,4-dioxane, rt, 15 h, then, NH_3, MeOH, rt, 15 h

$NaHCO_3$ solution, dried ($MgSO_4$), filtered, and evaporated. The residue was purified by flash silica gel column chromatography (dichloromethane:ethyl acetate:methanol = 20:20:1) to give the anhydro derivatives **6a**–**6f**.

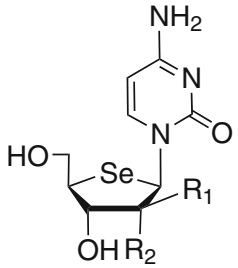
General procedure for the synthesis of **7a**–**7f** (Kim et al. 2014)

To a solution of **6a**–**6f** (1 equiv.) in dry THF were added $Et_3N{\cdot}3HF$ (3 equiv.) and triethylamine (3 equiv.) at 0 °C. After being stirred at the same temperature for 30 min, the reaction mixture was allowed to warm to room temperature and stirred for additional 30 min. The reaction mixture was evaporated and the residue was purified by silica gel column chromatography (dichloromethane:ethyl acetate:methanol = 10:10:1) to give **7a**–**7f**.

General procedure for the synthesis of **3a**–**3f**

To a stirred solution of compound **7a**–**7f** (Kim et al. 2014) (1 equiv.) in dry *N*,*N*-dimethylformamide was added sodium azide (2 equiv.) and the mixture was stirred at 120 °C for 15 h. The reaction mixture was evaporated and the residue was purified by silica gel column chromatography (dichloromethane/methanol = 8:1) to give **3a**–**3f**.

Table 1 Anticancer activity of the synthesized 2′-substituted-4′-selenonucleosides, **3h**–3**j** in several human cancer cell lines



Compound no.	IC_{50} (μM)[a]					
	HCT116[b]	A549[c]	SNU638[d]	T47D[e]	PC–3[f]	K562 [g]
3h (R_1 = H, R_2 = OH)	>100	>100	>100	>100	>100	>100
3i (R_1 = H, R_2 = N_3)	>100	>100	>100	>100	>100	>100
3j (R_1 = H, R_2 = F)	27.8	92.9	19.1	20.1	15.5	10.3
2a (R_1 = OH, R_2 = H)[h]	7.13	8.83	4.72	8.91	4.54	86.6
2b (R_1 = N_3, R_2 = H)[i]	78.3	80.1	85.2	78.4	75.1	62.3
2c (R_1 = F, R_2 = H)[h]	1.1	0.47	0.14	0.79	0.58	0.63
Ara-C (1)[h]	5.3	1.90	0.15	2.72	55.9	0.05

[a] Measured using SRB method

[b] Human colon cancer cell lines

[c] Human lung cancer cell lines

[d] Human stomach cancer cell lines

[e] Human breast cancer cell lines

[f] Human prostate cancer cell lines

[g] Human leukemia cell lines

[h] Jeong et al. (2009)

[i] Kim et al. (Kim et al. 2014)

1-((2R,3R,4S,5R)-3-Azido-tetrahydro-4-hydroxy-5-(hydroxymethyl) selenophen-2-yl) pyrimidine-2,4(1H,3H)-dione (**3a**)

White solid; yield: 54 %; mp 202–206 °C (decomposed); $[\alpha]_D^{20}$ −100.80 (c 0.25, CH_3OH); UV (CH_2Cl_2) λ_{max} 266 nm; IR (KBr) 2 107 cm^{-1} (N_3); ^1H NMR (400 MHz, CD_3OD) δ 8.14 (d, J = 8.0 Hz, 1H), 6.48 (d, J = 8.4 Hz, 1H), 5.80 (d, J = 8.0 Hz, 1H), 4.47 (t, J = 3.2 Hz, 1H), 4.14 (dd, J = 3.6, 8.4 Hz, 1H), 3.89–3.80 (m, 2H), 3.63–3.59 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 152.5, 143.8, 103.7, 76.9, 71.1, 64.8, 55.4, 51.8; MS (ESI) m/z 371.9613 ($M+K^+$); Calc. for $C_9H_{11}N_5O_4Se$: C, 32.54; H, 3.34; N, 21.08. Found: C, 32.14; H, 3.45; N, 19.98.

1-((2R,3R,4S,5R)-3-Azido-tetrahydro-4-hydroxy-5-(hydroxymethyl) selenophen-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**3b**)

White solid; yield: 53 %: mp 88–92 °C; $[\alpha]_D^{20}$ −78.06 (c 0.36, CH_3OH); UV (CH_2Cl_2) λ_{max} 271 nm; IR (KBr) 2112 cm^{-1} (N_3); ^1H NMR (400 MHz, CD_3OD) δ 7.95 (s, 1H), 6.51 (d, J = 8.8 Hz, 1H), 4.47 (t, J = 2.8 Hz, 1H), 4.16 (dd, J = 3.6, 8.8 Hz, 1H), 3.89–3.81 (m, 2H), 3.62–3.58 (m, 1H), 1.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 152.7, 139.1, 112.7, 76.9, 70.8, 64.8, 55.2, 51.8, 12.5; MS (ESI) m/z 348.0211 ($M+H^+$); Calc. for $C_{10}H_{13}N_5O_4Se$:C, 34.69; H, 3.78; N, 20.23. Found: C, 34.87; H, 3.87; N, 20.43.

1-((2R,3R,4S,5R)-3-Azido-tetrahydro-4-hydroxy-5-(hydroxymethyl) selenophen-2-yl)-5-fluoropyrimidine-2,4(1H,3H)-dione (**3c**)

White solid; Yield: 49 %; mp 213-216 °C (decomposed); $[\alpha]_D^{20}$ −48.5 (c 1.07, CH_3OH); UV (CH_3OH) λ_{max} 272 nm; IR (KBr) 2 115 cm^{-1} (N_3); ^1H NMR (400 MHz, CD_3OD) δ 8.35 (d, J = 6.8 Hz, 1H), 6.46 (dd, J = 2.0, 8.4 Hz, 1H), 4.46 (t, J = 3.2 Hz, 1H), 4.13 (dd, J = 3.2, 8.4 Hz, 1H), 3.85 (d, J = 5.6 Hz, 2H), 3.59–3.62 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 159.2 (d, J_{C-F} = 26.2 Hz), 151.2, 141.7 (d, J_{C-F} = 233.3 Hz), 127.6 (d, J_{C-F} = 34.9 Hz),

77.0, 71.2, 64.5, 56.1, 51.9; ^{19}F NMR (CD_3OD) δ 162.7; MS (ESI) m/z 351.9955 ($M+H^+$); Calc. for $C_9H_{10}FN_5O_4Se$: C, 30.87; H, 2.88; N, 20.00. Found: C, 30.98; H, 2.48; N, 20.04.

1-((2R,3R,4S,5R)-3-Azido-tetrahydro-4-hydroxy-5-(hydroxymethyl)selenophen-2-yl)-5-chloropyrimidine-2,4(1H,3H)-dione (**3d**)

White solid; Yield: 52 %; mp 144–146 °C; $[\alpha]_D^{20}$ −32.85 (c 0.7, CH_3OH); UV λ_{max} (CH_3OH) 271 nm; IR (KBr) 2 115 cm^{-1} (N_3); ^{1}H NMR (400 MHz, CD_3OD) δ 8.47 (s, 1H), 6.44 (d, J = 8.0 Hz, 1H), 4.46 (t, J = 3.2 Hz, 1H), 4.17 (dd, J = 3.6, 8 Hz, 1H), 3.86 (d, J = 5.2 Hz, 2H), 3.64–3.60 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.3, 151.7, 140.8, 110.0, 77.1, 71.4, 64.3, 56.0, 52.0; MS (ESI): m/z 389.9471 ($M+Na^+$); Calc. for $C_9H_{10}ClN_5O_4Se$: C, 29.48; H, 2.75; N, 19.10. Found: C, 29.76; H, 2.45; N, 19.09.

1-((2R,3R,4S,5R)-3-Azido-tetrahydro-4-hydroxy-5-(hydroxymethyl) selenophen-2-yl)-5-bromopyrimidine-2,4(1H,3H)-dione (**3e**)

White solid; yield, 40 %; mp 116–121 °C; $[\alpha]_D^{20}$ −21.86 (c 0.7, CH_3OH); UV λ_{max} (CH_3OH) 269 nm; IR (KBr) 2 113 cm^{-1} (N_3); ^{1}H NMR (400 MHz, CD_3OD) δ 8.57 (s, 1H), 6.42 (d, J = 8.0 Hz, 1H), 4.46 (t, J = 3.2 Hz, 1H), 4.18 (dd, J = 3.2, 8.0 Hz, 1H), 3.86 (d, J = 5.2 Hz, 2H), 3.64–3.61 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.4, 151.9, 143.4, 97.8, 77.1, 71.5, 64.2, 56.0, 52.0; MS (ESI): m/z 409.9006 $[M-H]^-$; Calc. for $C_9H_{10}BrN_5O_4Se$: C, 26.30; H, 2.45; N, 17.04. Found: C, 26.54; H, 2.55; N, 17.01.

1-((2R,3R,4S,5R)-3-Azido-tetrahydro-4-hydroxy-5-(hydroxymethyl) selenophen-2-yl)-5-iodopyrimidine-2,4(1H,3H)-dione (**3f**)

White solid; yield, 25 %; mp 198–202 °C (decomposed); $[\alpha]_D^{20}$ −53.20 (c 0.10, CH_3OH); UV λ_{max} (CH_3OH) 289 nm; IR (KBr) 2 115 cm^{-1} (N_3); ^{1}H NMR (400 MHz, CD_3OD) δ 8.61 (s, 1H), 6.39 (d, J = 8.0 Hz, 1H), 4.44 (t, J = 3.2 Hz, 1H), 4.16 (dd, J = 3.4, 7.8 Hz, 1H), 3.85 (d, J = 5.2 Hz, 2H), 3.63–3.59 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 162.6, 152.3, 148.5, 77.1, 71.6, 69.4, 64.2, 55.9, 52.0; MS (ESI): m/z 481.8838 ($M+Na^+$); Calc. for $C_9H_{10}IN_5O_4Se$: C, 23.60; H, 2.20; N, 15.29. Found: C, 23.61; H, 2.43; N, 15.09.

1-((2R,3R,4S,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl) tetrahydroselenophen-2-yl)pyrimidine-2,4(1H,3H)-dione (**3g**)

To a solution of **9** (Jeong et al. 2009) (314 mg, 0.591 mmol) in 1,4-dioxane (6 mL) was added $(HF)_x$-pyridine (0.15 mL, 5.91 mmol) and the mixture was heated to 125 °C for 2 d. The mixture was evaporated and the residue was purified by silica gel column chromatography (dichloromethane:methanol = 10:1) to give **3g** (33.4 mg, 18 %) as a white solid: UV (CH_2Cl_2) λ_{max} 265 nm; ^{1}H NMR (400 MHz, CD_3OD) δ 8.17 (d, 1H, J = 8.0 Hz), 6.46 (dd, 1H, J = 14.0, 6.0 Hz), 5.79 (d, 1H, J = 8.0 Hz), 5.21 (ddd, 1H, J = 50.0, 6.0 and 3.2 Hz), 4.39 (ddd, 1H, J = 13.2, 4.4 and 3.2 Hz), 3.93 (dd, 1H, J = 12.0, 5.6 Hz), 3.85 (dd, 1H, J = 12.0, 5.6 Hz), 3.62 (m, 1H); FAB-MS m/z 310 ($M+H^+$).

General procedure for the synthesis of cytosine derivatives **3h**, **3i**, and **3j**

A solution of **4a**, **3a**, and **8** (1 equiv.) in anhydrous pyridine was treated with acetic anhydride (10 equiv.), and the mixture was stirred at room temperature for 15 h. The mixture was evaporated and the residue was diluted with dichloromethane. This solution was washed consecutively with dilute HCl, saturated $NaHCO_3$ solution and brine. The organic layer was dried ($MgSO_4$), filtered and evaporated. The residue was purified by silica gel column chromatography (hexane:ethyl acetate = 1:1) to give pereacetylated compound **9**, **10**, and **11**, respectively as white foams.

To a solution of **9**, **10**, and **11** in acetonitrile were added 1,2,4-triazole (1 equiv.), phosphorus oxychloride (1.1 equiv.), and triethylamine (1.1 equiv.) and the mixture was stirred at room temperature for 15 h. The mixture was diluted with dichloromethane and the organic layer was washed with saturated $NaHCO_3$ solution, brine, dried ($MgSO_4$), filtered and evaporated to give **12**, **13**, and **14**, respectively. To a solution of crude **12**, **13**, and **14** in 1,4-dioxane was added ammonium hydroxide (28 %), and the mixture was stirred at room temperature for 15 h. After removal of all volatiles, the residue was dissolved in methanolic ammonia (3 mL) and stirred again for 15 h. The reaction mixture was evaporated, and the residue was purified by silica gel column chromatography (dichloromethane:methanol = 6:1) to give **3 h, 3i,** and **3j**, respectively.

4-Amino-1-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl) tetrahydroselenophen-2-yl)pyrimidin-2(1H)-one (**3h**) (Jeong et al. 2008)

White solid; yield, 50 %; mp 150-153 °C; UV (MeOH) λ_{max} 277 nm; MS (FAB) m/z 307 (M^+); $[\alpha]_D^{20}$ −260.0; (*c* 0.17, CH_3OH); ^{1}H NMR (CD_3OD) δ 3.56–3.63 (m, 1H), 3.80 (dd, 1H, J = 5.6, 11.6 Hz), 3.90 (dd, 1H, J = 6.4, 11.6 Hz), 4.21 (t, 1H, J = 3.6 Hz); 4.32 (dd, 1H, J = 3.0, 6.8 Hz); 5.95 (d, 1H, J = 7.6 Hz); 6.27 (d, 1H, J = 7.2 Hz); 8.13 (d, 1H, J = 7.6 Hz); ^{13}C NMR

(CD_3OD) δ 59.1, 66.2, 76.5, 80.5, 96.7, 144.7, 159.0, 167.4; Anal. Calcd for $C_9H_{13}N_3O_4Se$: C, 35.31; H, 4.28; N, 13.72. Found: C, 35.13; H, 3.98; N, 13.38.

4-Amino-1-((2R,3R,4S,5R)-3-azido-tetrahydro-4-hydroxy-5-(hydroxymethyl) selenophen-2-yl)pyrimidin-2(1H)-one (**3i**)

White solid; yield, 76 %; mp 115–120 °C; $[\alpha]_D^{20}$ −83.42 (c 0.36, CH_3OH); UV (CH_3OH) λ_{max} 276 nm; IR (KBr) 2 114 cm^{-1} (N_3); ^1H NMR (400 MHz, CD_3OD) δ 8.16 (d, J = 7.6 Hz, 1H), 6.53 (d, J = 8.0 Hz, 1H), 5.98 (d, J = 7.6 Hz, 1H), 4.43 (t, J = 3.6 Hz, 1H), 4.14 (dd, J = 3.2, 8.2 Hz, 1H), 3.90–3.80 (m, 2H), 3.63–3.59 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 167.4, 158.5, 144.3, 97.1, 76.8, 71.6, 64.8, 54.8, 51.2; MS (ESI) m/z 333.0207 ($M+H^+$); Calc. for $C_9H_{12}N_6O_3Se$: C, 32.64; H, 3.65; N, 25.38. Found: C, 32.54; H, 3.65; N, 25.46.

4-amino-1-((2R,3R,4S,5R)-3-fluoro-4-hydroxy-5-(hydroxymethyl) tetrahydroselenophen-2-yl)pyrimidin-2(1H)-one (**3j**)

White solid; Yield, 59 %; UV (CH_3OH) λ_{max} 276 nm; ^1H NMR (400 MHz, CD_3OD) δ 8.19 (d, 1H, $J = 7.2$ Hz), 6.48 (dd, 1H, $J = 14.4$, 5.6 Hz), 5.97 (d, 1H, $J = 7.2$ Hz), 5.17 (ddd, 1H, $J = 50.0$, 5.6 and 3.2 Hz), 4.35 (ddd, 1H, $J = 15.6$, 5.2 and 3.2 Hz), 3.95 (dd, 1H, $J = 11.6$, 5.2 Hz), 3.85 (dd, 1H, $J = 11.6$, 5.6 Hz), 3.63 (dq, 1H, $J = 5.6$, 2.0); FAB-MS m/z 307 ($M-H^+$); Calcd for $C_9H_{12}FN_3O_3Se$: C, 35.08; H, 3.92; N, 13.64. Found: C, 34.88; H, 3.99; N, 13.99.

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