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Discovery of a New Template for Anticancer Agents: 2′-deoxy-2′-fluoro-4′-selenoarabinofuranosyl-cytosine (2′-F-4′-Seleno-ara-C)

Lak Shin Jeong,* Dilip K. Tosh, Won Jun Choi, Sang Kook Lee, You-Jin Kang, Sun Choi, Jin Hee Lee, Hankil Lee, Hyuk Woo Lee, and Hea Ok Kim

Department of Bioinspired Science and Division of Life and Pharmaceutical Sciences, Laboratory of Medicinal Chemistry, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea

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Abstract: The first synthesis of 2′-deoxy-2′-fluoro-4′-selenoarabinofuranosyl pyrimidines as potent anticancer agents was accomplished using the DAST fluorination as a key step. It was first revealed that selenium atom participated in the DAST fluorination of 4′-selenonucleosides and that conformational bias induced by bulky selenium acted as a decisive factor in the DAST fluorination. Among compounds tested, 2′-F-4′-seleno-ara-C (**4a**) exhibited highly potent anticancer activity in all cancer cell lines tested and was more potent than ara-C (**1**).

The carbohydrate moieties of nucleosides have been used as good modification sites for the development of therapeutically useful antiviral and antitumor agents. Among those, nucleosides with an *arabino* configuration have been shown to be a good template for antiviral and antitumor agents. For example, 1-β-D-arabinofuranosyl cytosine (ara-C,[a] **1**)[1] is being used clinically as an anticancer agent and its 2′-fluoro-analogue **2**[2] was also reported to have antiherpetic activity (Figure 1).

On the basis of a bioisosteric rationale, the 4′-thio analogue **3**[3,4] was also reported to be a potent anticancer agent. While synthesizing this compound, the fluorination reaction proceeded via an episulfonium intermediate due to the participation of the sulfur in diethylaminosulfur trifluoride (DAST) fluorination.[4]

Jeong et al.[5–7] first reported that sulfur behaved differently during the DAST fluorination of a 2′-hydroxyl group with an *arabino* or *ribo* configuration, as shown in Scheme 1. In the case of 2′-hydroxyl with *arabino* configuration,[5,7] DAST fluorination of compound **5** proceeded via an episulfonium intermediate, giving the 2′-fluoro compound **5a** with an *arabino* configuration as the sole product without intramolecular participation of the C2 oxygen of the uracil. However, in case of 2′-hydroxyl with the *ribo* configuration,[6] DAST fluorination of compound **6** produced 2,2′-anhydro nucleoside **6b** directly from an attack of the C2 oxygen as an exclusive product instead of forming an episulfonium intermediate.

Recently, we have reported the synthesis of 4′-selenoribofuranosyl pyrimidines and their unusual conformations.[8] X-ray single crystallographic analysis indicated that 4′-selenouridine adopted unusual 2′-*endo*/3′-*exo* (south) conformation unlike uridine, which showed a 2′-*exo*/3′-*endo* (north) conformation. Very recently, we have also reported the synthesis of novel 2′,3′-didehydro-2′,3′-dideoxy-4′-selenopyrimidine nucleosides as potential antiviral agents.[9] Therefore, it would be of great interest to synthesize the 4′-selenoarabinofuranosyl analogues **4** (X=OH or F), combining the properties of 4′-selenonucleosides and arabinofuranosyl nucleosides and to compare their anticancer activity with that of 4′-oxonucleoside **1**. It would be also very interesting to compare the behavior of selenium in DAST fluorination with that of sulfur because selenium has a larger empty d orbital than sulfur and is more nucleophilic. Herein, we report the first asymmetric synthesis of 4′-selenoarabinofuranosyl pyrimidines (**4**) as highly potent anticancer agents as well as the unusual behavior of selenium in DAST fluorination.

For the introduction of the fluorine at the 2′-position, substrate **11** for DAST fluorination was first synthesized from 4′-selenouridine (**7**),[8] as shown in Scheme 2. Treatment of 4′-selenouridine (**7**) with trityl chloride afforded the 5′-*O*-trityl derivative **8**. Refluxing compound **8** with 1,1′-thiocarbonyl diimidazole in toluene gave the 2,2′-anhydro nucleoside **9** in good yield. The remaining hydroxyl group in **9** was protected with a tetrahydropyran (THP) group by treating with dihydropyran (DHP) in the presence of *p*-TsOH to produce compound **10**. Compound **10** was treated with 1 *N* NaOH to give the 2′-hydroxyl derivative **11** with an *arabino* configuration.

As in the case of 4′-thionucleosides,[4–6] it was expected that treatment of compound **11** with DAST would yield the desired 2′-fluoro compound **12** as the sole product with the retention of stereochemistry, which would proceed via the episelenium intermediate (Scheme 3).

However, the 2,2′-anhydro nucleoside **10**, which was formed from the abstraction of the amide hydrogen by the fluoride anion was unexpectedly produced as the major product. This finding has never been observed in the 4′-thionucleosides.[4–6] To confirm the participation of the C2 oxygen of the uracil, the N-3 position of compound **11** was protected with an electron withdrawing benzoyl group. As expected, DAST fluorination of N^3-benzoyl compound **13** afforded the 2′-fluoro compound **14** as the major product with concomitant formation of **10**.

The protecting groups of compound **12** were removed using 80% acetic acid to give the 2′-fluoro-4′-seleno-ara-U (**15**), which was converted to the final 2′-fluoro-4′-seleno-ara-C (**4a**) using the conventional method (Scheme 4).[10]

The stereochemistry of the fluorine in compound **15** was confirmed by the typical long-range couplings shown in 2′-“UP”-fluoronucleosides and 2D NOESY experiments. The long-range coupling constant between 2′-fluorine and H-6 in compound **15** was observed as 1.2 Hz and the same coupling constant between 2′-fluorine and 5′-H was also measured, confirming that the fluorine possessed the *arabino* configuration. 2D NOESY experiments between H-6 and 2′-H also confirmed the stereochemistry of the fluorine atom (see

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*To whom correspondence should be addressed. Phone: 82 2 3277 3466. Fax: 82 2 3277 2851. E-mail: lakjeong@ewha.ac.kr.

[a] Abbreviations: ara-C, 1-β-D-arabinofuranosyl cytosine; DAST, diethylaminosulfur trifluoride; THP, tetrahydropyran; DHP, dihydropyran; NOESY, nuclear Overhauser effect spectroscopy; SRB, sulforhodamine B; TCI, 1,1′-thiocarbonyl diimidazole.

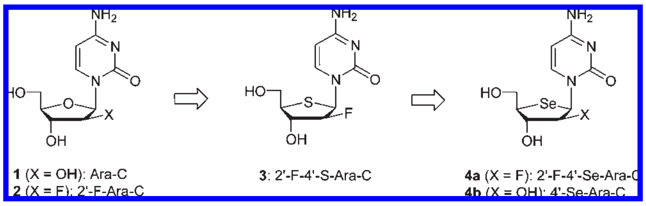
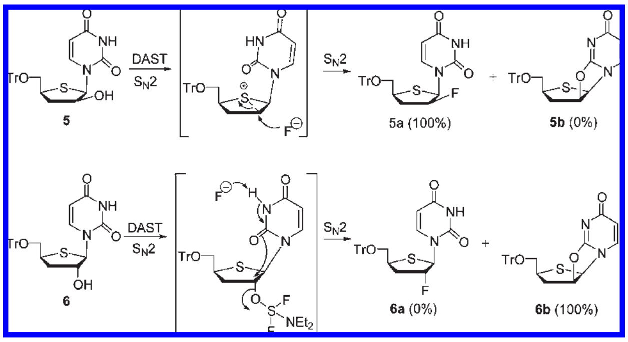
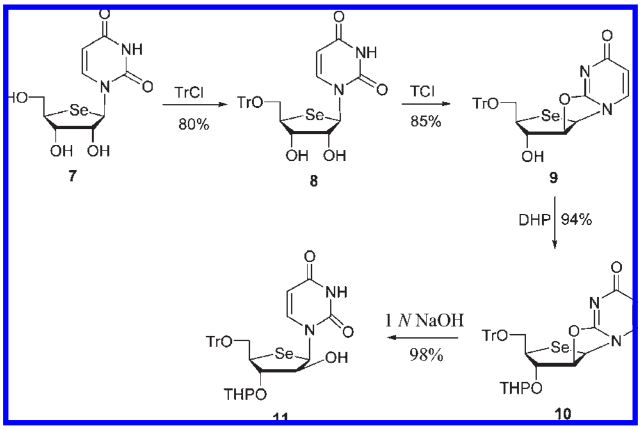


Figure 1. Rationale for the design of the target nucleosides.

Scheme 1. Participation of the Sulfur in DAST Fluorination

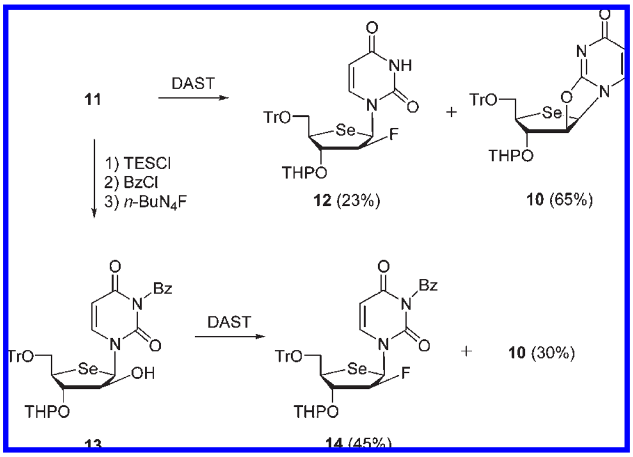


Scheme 2. Synthesis of the Substrate **11** for DAST Fluorination.

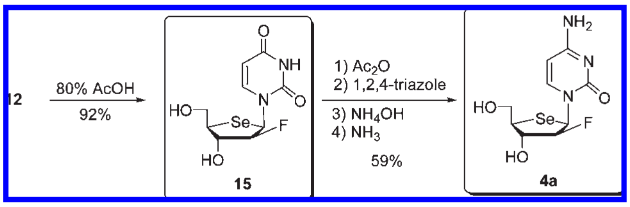


Supporting Information). Next, the conformation of compound **15** was compared with that of the corresponding 4′-thio analogue,[11] which has the 2′-*exo*/3′-*endo* (north) conformation. The coupling constants ($J_{1'H,2'F}$) of both compounds were compared because the coupling constants in the 2′-*endo*/3′-*exo* (south) conformer of an *arbino* sugar was reported to be larger than that in the 2′-*exo*/3′-*endo* (North) conformer.[11] The coupling constant ($^3J_{1'H,2'F} = 11.2$ Hz) in compound **15** was larger than that ($^3J_{1'H,2'F} = 7.9$ Hz) in the corresponding 4′-thio analogue, which was reported to adopt the northern conformation,[11] indicating that compound **15** has the unusual 2′-*endo*/3′-*exo* (south) conformation. The stereochemitry of the fluorine and the conformation of compound **15** were finally confirmed by the X-ray crystal structure (data not shown). This conformational bias was induced by the steric effects of the selenium atom in the 4-selenofuranose, which overwhelmed the electronic effects (gauche effects) of sulfur or oxygen in the corresponding 4-thiofuranose or furanose.

Scheme 3. DAST fluorination through the Participation of the Selenium



Scheme 4. Synthesis of 2′-Fluoro-4′-selenoarabinofuranosyl-nucleosides (**4a** and **15**)



To determine why 4′-thionucleosides and 4′-selenonucleosides gave different results on DAST fluorination, the reaction mechanism was examined using ab initio calculation as shown in Figure 2. Using the Gaussian 03 program,[12] the geometry optimizations and vibration frequency calculations for 4′-thionucleoside and 4′-selenonucleoside intermediates were performed at B3LYP/6-311+G(d,p) level.

When compound **11**, which adopts the 2′-*endo*/3′-*exo* (south) conformation, was treated with DAST, selenium first attacked the C2 position via a S_N2 reaction, forming an episelenium ion intermediate **11a**, whose optimized geometry is shown in Figure 2a. In this model, nucleophiles (fluoride or the oxygen atom of the uracil) can only attack axially from the convex side of **11a**. The intramolecular S_N2 reaction product **10** was obtained as a major product because the oxygen atom of the uracil is quite close (with the distance of 2.724 Å) to the C2 position of the episelenium ion intermediate and is oriented to the axial position, while axial attack of the fluoride anion to the C2 position of the episelenium ion is hindered by the axial 5′-substituent, giving the intermolecular S_N2 reaction product **12** as a minor product. On the other hand, the corresponding 4′-thio analogue **11′** adopts the 2′-*exo*/3′-*endo* (north) conformation.[11] The same DAST reaction of the model compound **11′** would form an episulfonium ion (**11a′**)[4-7] similarly to **11a**, whose optimized geometry is shown in Figure 2b. In this model, nucleophiles (fluoride or the oxygen atom of the uracil) could attack from the convex side but only in the equatorial direction, making an intramolecular reaction from the convex side by the oxygen atom of the uracil impossible. Hence, no 2,2′-anhydronucleoside could be formed. Instead, an intermolecular S_N2 reaction by the fluoride anion occurs exclusively to form 2′-arabino-fluoronucleoside **12′** as the sole product. From this study, it was revealed that the steric effect by bulky selenium overhelmed the electronic effect (gauche effect) by sulfur, forcing the conformation of 4′-seleno-arabinonucleosides to adopt the

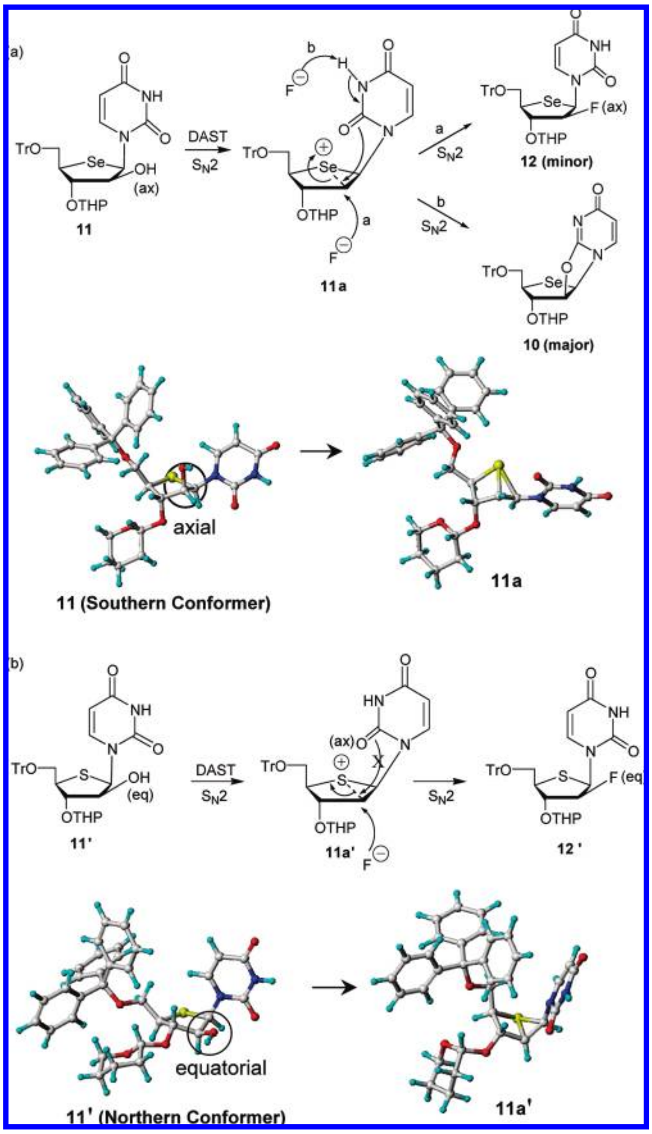


Figure 2. Geometry optimized structures of (a) 4′-selenonucleoside intermediate and (b) 4′-thionucleoside intermediate using the ab initio calculations and their proposed reaction mechanisms.

2′-*endo*/3′-*exo* (south) conformation. This is opposite to the corresponding 4′-thio analogue which adopted the opposite North conformation. This conformational bias induced by selenium acted as a decisive factor in the DAST fluorination of 4′-selenonucleosides. To the best of our knowledge, this is the first example that selenium participates during the DAST fluorination and DAST fluorination of the 4-thio- or 4-selenosugar is absolutely controlled by the sugar conformation.

In addition to 2′-fluoro-4′-seleno-ara-U (**15**) and 2′-fluoro-4′-seleno-ara-C (**4a**), 4′-seleno-ara-U (**16**) and 4′-seleno-ara-C (**4b**) were also synthesized from **11** as shown in Scheme 5. Treatment of **11** with 80% AcOH at 60 °C afforded the 4′-seleno-ara-U (**16**), which was smoothly converted to the 4′-seleno-ara-C (**4b**) using the same procedure used in Scheme 4.

Anticancer activity of all synthesized 1-β-D-arabinofuranosyl-4′-selenonucleosides (**4a**, **4b**, **15**, and **16**) were measured 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.[13] As shown in Table 1, 2′-fluoro-4′-seleno-ara-C (**4a**) exhibited the most potent anticancer activity in all human cancer cell lines among compounds tested. In general, the cytosine derivatives **4a** and **4b** exhibited very potent anticancer activity, while the uracil derivatives **15** and **16** did not show any anticancer activity up to 100 μM. It was also found that the 2′-fluoro analogue **4b** was more potent than the 2′-hydroxyl derivative **4a**. 2′-Fluoro-4′-seleno-ara-C (**4a**) was more potent than the control, ara-C (**1**), in all tumor cells lines except leukemia cell lines. 4′-Seleno-ara-C (**4b**) is slightly less potent than ara-C (**1**). The anticancer activity of the 2′-fluoro analogue **4b** was compared with that of gemcitabine approved clinically for the treatment of lung cancer. As shown in Table 1, the 2′-fluoro analogue **4b** was found to be less potent than gemcitabine. The comformation of **4b** was also compared with that of gemcitabine. Unlike compound **4b**, which adopts the 3′-*exo* (south) conformation, gemcitabine adopts the 3′-*endo* (north) conformation,[14] indicating that they might have different mechanisms of action for anticancer activity, but mechanism of action of **4b** should be further studied.

Scheme 5. Synthesis of 4′-Selenoarabinofuranosyl-nucleosides (**4b** and **16**)

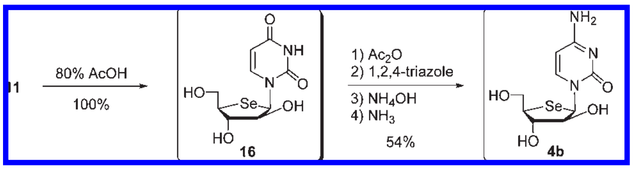


Table 1. Anticancer Activity of the Final 4′-Selenoarabinofuranosyl-nucleosides, **4a**, **4b**, **15**, and **16**, in Several Human Cancer Cell Lines

	IC_{50} (μM)[a]					
compd	HCT116[b]	A549[c]	SNU638[d]	T47D[e]	PC-3[f]	K562[g]
4a	1.1	0.47	0.14	0.79	0.58	0.63
4b	7.13	8.83	4.72	ND	ND	86.6
15	> 100	> 100	> 100	> 100	> 100	> 100
16	> 100	> 100	> 100	> 100	> 100	> 100
1	5.3	1.9	0.15	2.7	55.9	0.05
gemcitabine[h]	0.005	0.09	ND	ND	0.04	ND

[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] Approved clinically for the treatment of lung cancer; ND, not determined.

In summary, this paper reports the first synthesis of 2′-deoxy-2′-fluoro-4′-selenoarabinofuranosyl cytosine as a potent anticancer agent, starting from 4′-selenouridine using DAST fluorination as a key step. It was revealed that selenium participates in the DAST fluorination like sulfur, but unlike sulfur or oxygen, bulky selenium induced conformational bias because steric effects by selenium overwhelmed electronic effects (gauche effects) by sulfur or oxygen. 2′-Fluoro-4′-seleno-ara-C (**4a**) exhibited very potent anticancer activity in all human cancer cell lines tested. We are sure that this novel template as well as unusual chemistry discovered in this study will contribute greatly to the development of novel biologically active nucleosides.

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Supporting Information Available: Complete experimental procedures and characterization data for all compounds described herein. ^{1}H and ^{13}C NMR copies of **4a**, and **15**, and 2D NOESY spectrum of compound **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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