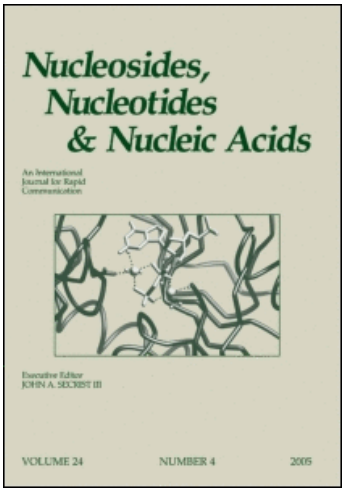


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Facile Synthesis and Anti-Tumor Cell Activity of Se-Containing Nucleosides

Lina Lin[ab]; Jia Sheng[a]; Razin K. Momin[a]; Quan Du[a]; Zhen Huang[ab]
[a] Department of Chemistry, Georgia State University, Atlanta, Georgia, USA [b] Department of Biology, Georgia State University, Atlanta, Georgia, USA

Online publication date: 04 January 2011

To cite this Article Lin, Lina , Sheng, Jia , Momin, Razin K. , Du, Quan and Huang, Zhen(2009) 'Facile Synthesis and Anti-Tumor Cell Activity of Se-Containing Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 28: 1, 56 — 66
To link to this Article: DOI: 10.1080/15257770802581765
URL: http://dx.doi.org/10.1080/15257770802581765

Nucleosides, Nucleotides and Nucleic Acids, 28:56–66, 2009

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770802581765

Taylor & Francis
Taylor & Francis Group

FACILE SYNTHESIS AND ANTI-TUMOR CELL ACTIVITY OF SE-CONTAINING NUCLEOSIDES

Lina Lin,[1, 2] Jia Sheng,[1] Razin K. Momin,[1] Quan Du,[1] and Zhen Huang[1, 2]

[1]*Department of Chemistry, Georgia State University, Atlanta, Georgia, USA*
[2]*Department of Biology, Georgia State University, Atlanta, Georgia, USA*

□ *Many organic compounds containing selenium have shown anticancer effects and some have been used in chemoprevention of cancers and other diseases. Though Se-containing amino acids are generally used for these purposes, the natural nucleosides may also be used as Se-carriers for these important applications. Therefore, we describe here the convenient synthesis of the new 3′-MeSe-thymidine nucleoside and the other uridine and thymidine derivatives modified with MeSe at the 2′ and 5′ positions, and report their anti-tumor activity against prostate cancer cell lines. Our work demonstrates for the first time anticancer activity of the methylseleno nucleosides.*

Keywords Selenium-modified nucleoside; methylseleno functionality; selenium chemoprevention; anticancer activity

INTRODUCTION

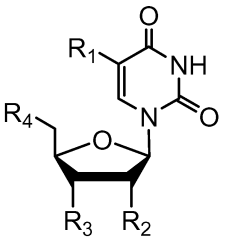
Due to the recent discovery that selenium is an essential trace element,[1–3] tremendous attention has been paid to the selenium research, including chemical, biochemical, and biological investigations.[3–6] Though selenium is the most noticeable for its antioxidant properties,[4] the biological activities of selenium are not just limited to its antioxidant abilities. The extensive research results have indicated an inverse relationship between the selenium level in body and the abnormalities of HIV and HPV infection, cardiomyopathy, rheumatoid arthritis, and asthma,[7–11] although most of the mechanisms are unclear. In addition, the clinic trial, conducted by Clark and coworkers using the

Received 7 July 2008; accepted 8 October 2008.
This work was supported by the GCC Distinguished Cancer Clinicians and Scientists Award, and the NSF (MCB-0517092 and CHE-0750235).
Address correspondence to Dr. Zhen Huang, Department of Chemistry, Georgia State University, Atlanta, GA, 30303, USA. E-mail: huang@gsu.edu

selenium-enriched yeast, revealed approximately 50% reduction of the occurrence and re-occurrence of many types of cancers.[3,12,13] Though selenium can be accessed from a variety of food sources,[14] such as broccoli, Brazil nut, and garlic,[15] Se-containing compounds have also been used as the supplements for people with infectious diseases, such as tuberculosis or AIDS,[16–18] as these diseases usually coincide with nutritional deficiencies.

Selenium level in the body is basically dependent on its absorption,[19,20] though the amount of selenium in diet also plays a role. The form and oxidative state of selenium in diet can also significantly affect its absorption and activity.[21,22] Various selenium compounds, in both inorganic and organic forms, have been reported to have biological activities.[1,23] For instance, selenite and selenomethionine are the most commonly used Se-containing compounds in the experimental and clinic studies.[3] Development of Se-containing organic compounds that can be used as micronutrient supplements is an important approach to improve the disease prevention and treatment. Considering the toxicity and absorption, it is better to synthesize organic seleno-compounds in the forms of the naturally occurring products and the derivatives. Furthermore, Ip and coworkers have demonstrated that methylselenol is an active metabolite of the Se-containing compounds, such as methylselenocysteine and seleno-methionine.[1,24–26] Methylselenol, offering the high anticancer activity, can be generated via in vivo reduction of the methylseleno-containing compounds.[25,27] Therefore, the organic compounds containing methylseleno (MeSe) functionality are potential agents for anticancer application and chemoprevention.[25]

It has been well-demonstrated that selenomethionine, containing MeSe functionality, is better absorbed and retained in body than inorganic selenite.[22] Though selenomethionine is commonly used as a food additive and Se-carrier, its cytotoxicity is relatively high.[28] In addition to the Se-amino acids, it was shown that several selenonucleobases and 8-seleno-cGMPs had anticancer activities,[29,30] and that many natural tRNAs contain selenium-modified nucleosides.[31,32] Recently, our research group has pioneered and developed selenium-derivatized nucleic acids (SeNA) for function and x-ray crystal structure studies.[33–37] Therefore, by taking advantage of our experiences in selenium chemistry, we have designed and synthesized novel nucleosides derivatized with MeSe functionality at various positions (Figure 1) in order to evaluate the MeSe effect and to develop better Se-carriers for anticancer application and chemoprevention. We describe here the simple synthesis of the new 3′-MeSe-thymidine nucleoside and the other uridine and thymidine derivatives modified with MeSe at the 2′ and 5′ positions, and report their activity against prostate tumor cell lines.



1: R_1= H, R_2= MeSe-, R_3= OH, R_4= OH
2: R_1= CH_3, R_2= H, R_3= MeSe-, R_4= OH
3: R_1= CH_3, R_2= H, R_3= OH, R_4= MeSe-

FIGURE 1 Se-modified nucleosides.

RESULTS AND DISCUSSION

Chemistry

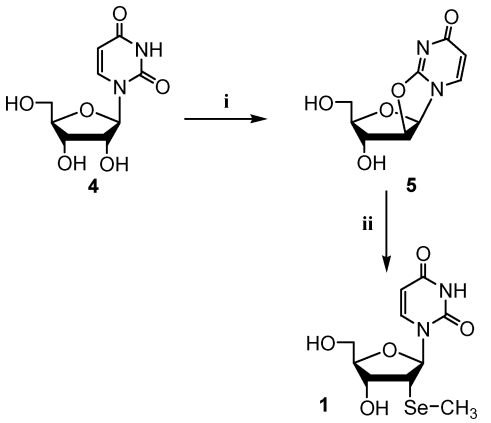
In this work, we have developed convenient synthetic routes for synthesis of the new 3′-MeSe-thymidine nucleoside and the other uridine and thymidine derivatives modified with MeSe at the 2′ and 5′ positions, and their overall synthetic yields are high (MeSe-nucleosides, **1–3**; Table 1).

The synthetic route for 2′-methylseleno-2′-deoxyuridine (**1**) is shown in Scheme 1. After converting uridine (**4**) to 2,2′-anhydrouridine (**5**) using diphenyl carbonate in hot DMF,[38,39] we normally protected the 5′-OH of **5** to increase the anhydrouridine solubility for the MeSe incorporation, followed by removal of the 5′-protection. In order to avoid the 5′ protection and deprotection steps, we explored several conditions, including hot alcohol solvent, to increase the solubility of 2,2′-anhydrouridine while controlling the reaction regioselectivity at the 2′-α-position instead of the 2-position. Without the formation of the 2-MeSe-uridine isomer, methylselenol formed by reduction of dimethyl diselenide with $NaBH_4$ was incorporated, in high yield, into the anhydrouridine (**5**) at the 2′ α-position under the elevated temperature. Thus, the target compound (**1**) was conveniently synthesized in two steps (78% overall yield).

Because of the successful MeSe incorporation to the 2′ position of 2,2′-anhydrouridine without the 5′-protection, we planned to use the similar strategy to introduce MeSe functionality to the 3′-position of the

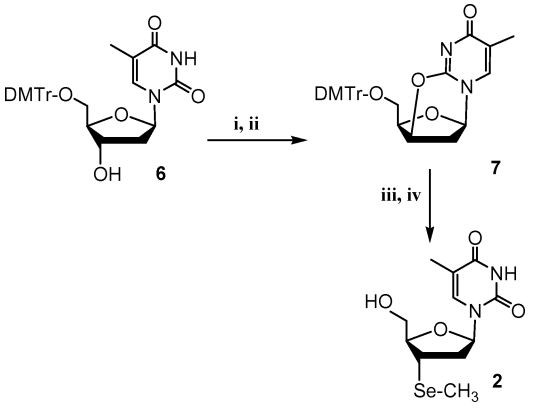
TABLE 1 Synthesized MeSe-nucleosides (**1–3**)

Compound	R_1	R_2	R_3	R_4	Over-all Yield%
1	-H	-$SeCH_3$	-OH	-OH	78
2	-CH_3	-H	-$SeCH_3$	-OH	81
3	-CH_3	-H	-OH	-$SeCH_3$	80

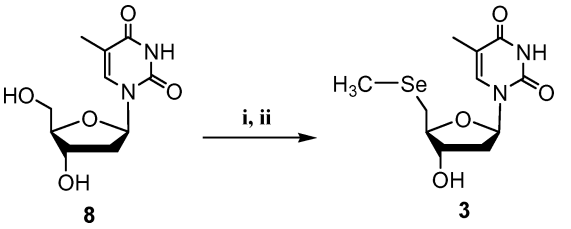


SCHEME 1 Synthesis of 2′-MeSe-uridine (**1**). Reagents: (i) diphenyl carbonate and $NaHCO_3$ in DMF, heated at 120°C for 2 hours; (ii) dimethyl diselenide and $NaBH_4$ in EtOH:MeOH (8:1), heated at 50°C for 3–4 hours.

2,2′-anhydrothymidine (**7**). We started the synthesis of 3′-methylseleno-3′-deoxythymidine (**2**) from the partially protected thymidine, 5′-DMTr-thymidine (**6**; Scheme 2). After activating the 3′-hydroxyl group of **6** through a quantitative mesylation reaction, the mesylate intermediate (without purification) was converted to the 2,3′-anhydrothymidine derivative (**7**) under reflux in ethanol solvent. This intramolecular cyclization was almost quantitative when a low concentration of the mesylate intermediate (10 mM) was used in the presence of dilute NaOH (10.4 mM). Via the intramolecular S_N2 substitution, the α-3′-mesyl group was displaced by the 2-oxide formed under the basic condition to give 2,3′-anhydrothymidine (**7**). Under higher concentrations of the mesylate or NaOH, many by-products were formed



SCHEME 2 Synthesis of 3′-MeSe-thymidine (**2**). Reagents: (i) MsCl/TEA/THF, at room temperature for 30 minutes; (ii) dilute NaOH (aq) in EtOH, reflux for 2.5 hours; (iii) dimethyl diselenide and $NaBH_4$ in dioxane, heated at 93°C for 2 hours; (iv) trichloroacetic acid treatment.



SCHEME 3 Synthesis of 5′-MeSe-thymidine (**3**). Reagents: (i) p-toluenesulfonyl chloride, DMAP, anhydrous pyridine, 22 hours; (ii) dimethyl diselenide, $NaBH_4$.

probably due to the 3′,2′- or 3′,4′-mesylate elimination. After purification of the generated 2,3′-anhydrothymidine (**7**), thymidine was functionized with MeSe functionality using a procedure analogous to the synthesis of 2′-MeSe-U (**1**). Due to the higher stability of 2,3′-anhydrothymidine, which has much less ring strain comparing with 2,2′-anhydrouridine, higher reaction temperature was required to allow successful incorporation of the methylseleno functionality. Without the formation of the 2-MeSe-thymidine isomer, MeSe functionality was almost quantitatively introduced to the 3′-α-position of **7**. Without further purification of this Se-containing intermediate, its 5′-dimethoxytrityl group was quantitatively removed by 80% acetic acid, offering the target compound (**2**) with 81% overall yield (four steps).

5′-Methylseleno-5′-deoxythymidine (**3**) was synthesized, in the pyridine-ethanol mixed solution, through a two-step reaction shown in Scheme 3. Since it is possible to differentiate the primary and secondary hydroxyl groups, we used p-toluenesulfonyl chloride to selectively activate the 5′ position in pyridine. The 5′ p-toluenesulfonyl group, a good leaving group, was displaced by methylselenol via a S_N2 substitution, thereby generating target compound **3** (80% yield in two steps).

Anti-Prostate Tumor Cell Evaluation

Compounds **1–3**, with relatively good solubility in aqueous solution, were screened for their anticancer activity against prostate cancer cell lines (DU145, CWR22, PC3, and LNCaP). We found that these MeSe-nucleosides have anticancer effects and can generally inhibit cancer cell growth. Their IC_{50} values for the cell growth inhibition and the average cell death caused by these nucleosides at 10 μM concentration are shown in Table 2. Their IC_{50} values against these prostate cancer cells varied from 15 to 200 nM. Our experimental results indicated that 5′-Se-thymidine (**3**) is generally more active than the other 2′ and 3′ Se-nucleosides against these prostate cancer cell lines. It is probably easier to metabolize the Se-nucleoside containing the primary MeSe than the secondary MeSe, thereby generating more methylselenol from **3**. It was observed that the inhibition of the tumor cell growth was proportional to the concentrations of these MSe-nucleosides.

TABLE 2 Inhibition results of tumor cell growth by compounds **1–3**

Tumor cell lines	IC_{50} of cell growth inhibition and average cell death (ACD) at the Se-nucleoside concentration (10 μM)	1	2	3	Control ACD (%) (no Se-nucleosides)
DU145	IC_{50} (nM)	25	22	15	3.2
	ACD (%)	3.9	3.4	2.6	
CWR22	IC_{50} (nM)	63	58	25	4.6
	ACD (%)	3.6	2.1	3.7	
PC3	IC_{50} (nM)	76	21	15	5.0
	ACD (%)	6.9	7.4	7.4	
LNCaP	IC_{50} (nM)	200	150	50	1.8
	ACD (%)	2.1	1.1	2.2	

The percentage of the dead cells almost did not change over the nucleoside concentrations from 10 nM to 10 μM, and the mean percentages of the dead cells were not significantly different from those of the control groups where the Se-nucleosides were not used.

In addition, there appeared no significant toxicity to these cells at the higher concentration of these Se-nucleosides (10 μM). The cell culture experiments at the US National Cancer Institute, using the same Se-nucleosides, have also shown the growth inhibition of cancer cell lines without significant toxicity. Using bacteria cell culture, recently we have also tested the Se-nucleosides at higher concentration (200 μM), and no significant toxicity to E. coli were observed. These results encourage us to further study the natural nucleosides and derivatives as potential selenium carriers for anticancer and chemoprevention applications in the future.

EXPERIMENTAL SECTION

Most solvents and reagents were purchased and used without purification unless mentioned otherwise. Pyridine was dried over KOH (s) and distilled under argon. When necessary, solid reagents were dried under high vacuum. Reactions with compounds sensitive to air or moisture were performed under argon. Solvent mixtures are indicated as volume/volume ratios. Thin layer chromatography (TLC) was run on Merck 60 F_{254} plates (0.25 mm thick; R_f values in the text are for the title products), and visualized under UV-light or by a Ce-Mo staining solution (phosphomolybdate, 25 g; $Ce(SO_4)2.4H_2O$, 10 g; H_2SO_4, 60 mL, conc.; H_2O, 940 mL) with heating. Preparative TLC was performed using Merck 60 F_{254} pre-coated plates (2 mm thick). Flash chromatography was performed using Fluka silica gel 60 (mesh size 0.040–0.063 mm) using a silica gel:crude compound weight ratio of ca. 30:1. ^1H and ^{13}C-NMR spectra were recorded using

Bruker-300 or 400 (300 or 400 MHz). All chemical shifts (δ) are in ppm relative to tetramethylsilane and all coupling constants (J) are in Hz.

2′-methylseleno-2′-deoxyuridine (1). Uridine (4.17 g, 17.1 mmol) and diphenyl carbonate (4.0 g, 18.7 mmol) were placed in a round-bottom flask, and N,N-dimethylformamide (5.0 mL) was added. The slurry was heated in an oil bath at 100°C. Dry sodium bicarbonate (33 mg) was then added, and a watch glass was used to cover the flask. The reaction mixture was heated at 120–130°C for 1 hour while being monitored on TLC (methanol/methylene chloride, 2:8). After completion, the reaction was cooled to room temperature. The precipitated product was filtered and washed by cold methanol three times (each time 1.5 mL). The white powdered product (**5**) was dried on high vacuum overnight. (3.31 g, 86%). Compound [**5**, 2,2′-O-anhydro-1-(β-D-arabinofuranosyl)-uracil]: ^{1}H-NMR (d_6-DMSO) δ (ppm): 3.11–3.23 (m, 2H, H-5′), 3.97–4.11 (m, 1H, H-4′), 4.25–4.41 (m, 1H, H-3′), 4.91 (m, HO), 5.18 (d, J = 5.8 Hz, 1H, H-2′), 5.81 (m, HO), 5.96 (d, J = 7.5 Hz, 1H, H-5), 6.22 (d, J = 5.8 Hz, 1H, H-1′), 7.78 (d, J = 7.5 Hz, 1H, H-6). ^{1}H-NMR spectrum is consistent with the literature.[38,39] A suspension of sodium borohydride (148 mg) in ethanol (1 mL) was added dropwisely to dimethyldiselenide (0.33 g, 1.75 mmol) dissolved in ethanol (3 mL) under argon. After the yellow solution turned colorless and there was no more bubble formation in the reaction, the suspension of 2,2′-anhydrouridine (**5**, 200 mg, 0.88 mmol) in ethanol (4 mL) was added slowly, followed by addition of methanol (2 mL). The reaction was heated at 50°C for 3–4 hours before it was completed (monitored by TLC, 10% MeOH in CH_2Cl_2). After evaporating all solvents, the crude product was purified by silica-gel column chromatography (7% MeOH in CH_2Cl_2). Pure product (258.5 mg, 91%) was obtained. Compound **1**: ^{1}H-NMR (MeOD, 400 MHz): δ 1.99 (s, 3H, 2′-Se-CH_3), 3.18–3.55 (m, 1H, 2′-H), 3.74–3.83 (m, 2H, 5′-H), 4.03–4.06 (m, 1H, 3′-H), 4.35–4.37 (m, 1H, 4′-H), 5.76 (d, 1H, J = 8.1 Hz, 5-H), 6.28 (d, 1H, J = 8.4 Hz, 1′-H), 7.99 (d, 1H, J = 8.1 Hz, 6-H); ^{13}C-NMR (100 MHz, $CDCl_3$+DMSO-d6) δ: 8.21 ($SeCH_3$), 53.10 (C-2′), 67.28 (C-5′), 77.46 (C-3′), 91.48 (C-4′), 94.38 (C-1′), 107.39 (C-5), 145.51 (C-6), 155.66 (C-2), 168.51 (C-4); ESI-MS: molecular formular $C_{10}H_{14}N_2O_5Se$, observed ($M+Na^+$): 344.9950 (calc. 344.9966).

3′-methylseleno-3′-deoxythymidine (2). This compound was synthesized from 5′-DMT thymidine in four steps. The first step (**I**) was mesylation of the 3′ position of 5′-DMT thymidine. Without purification of the mesylate intermediate, the second step (**II**) allowed the intramolecular cyclization, thereby forming compound **7**. This cyclic compound was purified by flash chromatography. The third step (**III**) involved the introduction of the methylseleno group at the 3′ position. After the detritylation with trichloroacetic acid (**IV**), the target compound **2** was purified by chromatography. Step (**I**): After placing **6** (1.44 g, 2.69 mmol) in a round-bottom flask and drying it under high vacuum for 30 minutes, dry THF (26.4 mL) and

triethylamine (1.1 mL, 7.95 mmol, 3 equiv.) were injected into the flask under argon. Methanesulfonyl chloride (247 μL, 3.18 mmol, 1.2 equiv.) was slowly injected into the flask in an ice bath and under dry argon. After stirring the reaction for 10 minutes at 0°C, the reaction flask was placed and stirred at room temperature for 30 minutes. After monitoring the reaction by TLC to confirm its completion, methanol (1 mL) was added to quench the reaction. After evaporation of all solvents under reduced pressure, the crude product was redissolved in ethyl acetate and the salts were filtered out. The solvent was evaporated again under reduced pressure to afford the crude product.

Step (**II**): The crude product from step (**I**) was redissolved in ethanol (250 mL), and NaOH (13.75 mL, 0.2 M aqueous solution, 2.75 mmol, 1.04 eq.) was added subsequently. The reaction was completed after 2.5-hour reflux (monitored by TLC, 5% MeOH in CH_2Cl_2). The 90% solvent was evaporated under reduced pressure, and water (100 mL) was subsequently added. The crude product was then extracted with EtOAc (3 × 25 mL). The organic phases were combined and dried over anhydrous $MgSO_4$ for 20 minutes. After the filtration and solvent evaporation under reduced pressure, the crude product was purified by flash chromatography (0 to 5% methanol in CH_2Cl_2, gradient). The pure product was dried on high vacuum to afford compound **7** as a fine white powder (1.25 g, 2.42 mmol, 90% yield over two steps).

Step (**III** and **IV**): **7** (50 mg, 0.096 mmol) and $NaBH_4$ (18 mg) were dried separately on high vacuum. Dry dioxane (2.0 mL) was injected into each round bottom flask. A brown liquid of dimethyl diselenide (13 μL, 13.4 mg, 0.071 mmol, 1.5 equiv.) was carefully injected into the flask containing $NaBH_4$. Afterward, a few drops of ethanol were injected into the same flask dropwise until bubble appeared. The reaction was kept running at room temperature for 15 minutes until the solution became colorless, followed by injecting the dioxane solution of compound **7** into this reaction. The mixture was refluxed under argon for 2 hours before its completion (monitoring by TLC). The solvent was evaporated under reduced pressure and the residue was dissolved in CH_2Cl_2 (25 mL), followed by washing with brine (3 × 10 mL). The combined organic layers were dried with anhydrous $MgSO_4$, filtered and evaporated to dryness. Without further purifications, the crude product was redissolved in CH_2Cl_2 (5 mL) and treated with trichloroacetic acid (0.19 g, 1.14 mmol). The mixture was stirred (0.5–1 hour) and followed by adding several drops of CH_3CH_2SH to quench the DMTr cation. The reaction was monitored by TLC, and triethylamine was added to neutralize the acid. After evaporation of CH_2Cl_2, the crude product was purified by silica gel column chromatography using 10% methanol in methylene chloride as eluent. The pure product (27.8 mg) was obtained with 89% yield over two steps. Compound **2**: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.90 (s, 5 -CH_3), 2.07 (s, 3H, $SeCH_3$), 2.46–2.56 (m, 2H, 2′-H), 3.44–3.55 (m, 1H, 3′-H), 3.86–4.10 (m, 2H, 5′-H), 3.96–4.00 (m, 1H, 4′-H),

6.10 (m, 1H, 1′-H), 7.59 (s, 1H, 6-H); ^{13}C-NMR ($CDCl_3$, 100 MHz), δ 3.52 (3′-$SeCH_3$), 12.69 (5-CH_3), 32.48 (C-3′), 40.14 (C-2′), 61.18 (C-5′), 85.66 (C-1′) 86.71 (C-4′), 110.82 (C-5), 136.65 (C-6), 150.48 (C-2), 164.04 (C-4); ESI-MS: molecular formular $C_{11}H_{16}N_2O_4Se$, observed ($M + Na^+$): 343.0167 (calc. 343.0173).

5′-methylseleno-5′-deoxythymidine (3). Step (**I**): Thymidine (60.6 mg, 0.25 mmol), p-toluenesulfonyl chloride (97.3 mg) and DMAP (2 mg) were dried under high vacuum in a 10-mL flask for 1 hour, and then dry pyridine (1.25 mL) was added into the flask under argon. The reaction mixture was stirred under argon at room temperature overnight. Step (**II**): To a round-bottom flask containing $NaBH_4$ (111 mg) under argon, ethanol (1.0 mL) was added, followed by addition of dimethyl diselenide (47.0 μL, 93 mg, 0.50 mmol). This reaction was stirred for 30 minutes in an ice-water bath and under dry argon, and the solution turned from yellow to colorless. The tosylate suspension from Step **I** was injected to the methyl selenol solution, followed by stirring at room temperature overnight. The solvents were evaporated under reduced pressure. The crude product was purified by silica gel plate using 7% methanol in methylene chloride as the eluent. The white foamy product (53.1 mg) was obtained with 80% yield (recovered thymidine, 10.4 mg). Compound **3**: ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.96 (s, 3H, 5-CH_3), 2.09 (s, 3H, 2′-$SeCH_3$), 2.26–2.33 (m, 2H, 2′-H), 2.79–2.96 (m, 2H, 5′-H), 4.05–4.10 (m, 1H, 3′-H), 4.38–4.45 (m, 1H, 4′-H), 6.26 (t, 1H, J = 6.7 Hz, 1′-H), 7.29 (s, 1H, 6-H), 8.13–8.18 (br, 1H, NH, exchangeable in D_2O); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 5.63 (2′-$SeCH_3$), 12.46 (5-CH_3), 27.69 (C-2′), 40.04 (C-5′), 73.39 (C-3′), 84.52 (C-4′), 85.21 (C-1′), 111.19 (C-5), 135.64 (C-6), 150.57 (C-2), 164.15 (C-4); ESI-MS: molecular formular $C_{11}H_{16}N_2O_4Se$, observed $(M\text{-}H)^-$: 319.0194 (calc. 319.0197).

Anti-prostate tumor cell assays. Approximately 100,000 cells of each tumor cell line were plated onto six-well plates in duplicate and allowed 24 hours to adhere. Old media was aspirated and the fresh media (2 mL, containing various concentrations (1.0, 10, 100, 1000, 10,000 nM) of compound **1**, **2**, or **3**) was added. A control group with no additives was also prepared. These plates were incubated at 37°C in an environment containing 5% CO_2. After 96-hour incubation, the cells were trypsinized and counted. For each test sample with a Se-nucleoside, the number of live cells was normalized to that of the control group and plotted against the concentration of the Se-nucleoside. IC_{50} values of cell growth inhibition were determined by least squares curve fitting.

CONCLUSIONS

We have developed facile synthesis of the nucleosides containing the methylseleno functionality at the 2′, 3′, and 5′ positions. The new 3′-MeSe-thymidine nucleoside and the other uridine and thymidine derivatives

modified with MeSe at the 2′ and 5′ positions can be synthesized conveniently. Their synthetic routes are short, and the overall yields are high. Excitingly, we observed for the first time the anticancer activity of the MeSe-nucleosides. Our experimental results encourage us to synthesize more Se-modified nucleosides and to further study the anticancer activity and cytotoxicity of the Se-nucleosides at the cellular and animal levels in the future.

REFERENCES

1. Ip, C.; Hayes, C.; Budnick, R.M.; Ganther, H.E. Chemical form of selenium, critical metabolites, and cancer prevention. *Cancer Res.* **1991**, 51, 595–600.
2. Barceloux, D.G. Selenium. *J. Toxicol. Clin. Toxicol.* **1999**, 37, 145–172.
3. El-Bayoumy, K.; Sinha, R. Mechanisms of mammary cancer chemoprevention by organoselenium compounds. *Mutat. Res.* **2004**, 551, 181–197.
4. Gladyshev, V.N.; Hatfield, D.L. Selenocysteine-containing proteins in mammals. *J. Biomed. Sci.* **1999**, 6, 151–160.
5. Burk, R.F.; Hill, K.E.; Motley, A.K. Selenoprotein metabolism and function: evidence for more than one function for selenoprotein. *P. J. Nutr.* **2003**, 133, 1517S–1520S.
6. Sinha, R.; Pinto, J.T.; Facompre, N.; Kilheffer, J.; Baatz, J.E.; El–Bayoumy, K. Effects of naturally occurring and synthetic organoselenium compounds on protein profiling in androgen responsive and androgen independent human prostate cancer cells. *Nutr. Cancer* **2008**, 60, 267–275.
7. Cirelli, A.; Ciardi, M.; de Simone, C.; Sorice, F.; Giordano, R.; Ciaralli, L.; Costantini, S. Serum selenium concentration and disease progress in patients with HIV infection. *Clin. Biochem.* **1991**, 24, 211–214.
8. Thompson, F.E.; Patterson, B.H.; Weinstein, S.J.; McAdams, M.; Spate, V.L.; Hamman, R.F.; Levine, R.S.; Mallin, K.; Stolley, P.D.; Brinton, L.A.; Morris, J.S.; Ziegler, R.G. Serum selenium and the risk of cervical cancer among women in the United States. *Cancer Causes Control* **2002**, 13, 517–526.
9. Cheng, T.O. Selenium deficiency and cardiomyopathy. *J. R. Soc. Med.* **2002**, 95, 219–220.
10. Tarp, U. Selenium and the selenium-dependent glutathione peroxidase in rheumatoid arthritis. *Dan. Med. Bull.* **1994**, 41, 264–274.
11. Allam, M.F.; Lucane, R.A. Selenium supplementation for asthma. *Cochrane Database Syst. Rev.* 2004, CD003538.
12. Clark, L.C.; Combs, G.F.; Jr.; Turnbull, B.W.; Slate, E.H.; Chalker, D.K.; Chow, J.; Davis, L.S.; Glover, R.A.; Graham, G.F.; Gross, E.G.; Krongrad, A.; Lesher, J.L.; Jr.; Park, H.K.; Sanders, B.B.; Jr.; Smith, C.L.; Taylor, J.R. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA* **1996**, 276, 1957–1963.
13. Duffield–Lillico, A.J.; Reid, M.E.; Turnbull, B.W.; Combs, G.F.; Jr.; Slate, E.H.; Fischbach, L.A.; Marshall, J.R.; Clark, L.C. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial. *Cancer Epidemiol. Biomarkers Prev.* **2002**, 11, 630–639.
14. Dumont, E.; Vanhaecke, F.; Cornelis, R. Selenium speciation from food source to metabolites: a critical review. *Anal. Bioanal. Chem.* **2006**, 385, 1304–1323.
15. El-Bayoumy, K.; Sinha, R.; Pinto, J.T.; Rivlin, R.S. Cancer chemoprevention by garlic and garlic-containing sulfur and selenium compounds. *J. Nutr.* **2006**, 136, 864S–869S.
16. Hurwitz, B.E.; Klaus, J.R.; Llabre, M.M.; Gonzalez, A.; Lawrence, P.J.; Maher, K.J.; Greeson, J.M.; Baum, M.K.; Shor–Posner, G.; Skyler, J.S.; Schneiderman, N. Suppression of human immunodeficiency virus type 1 viral load with selenium supplementation: a randomized controlled trial. *Arch. Intern. Med.* **2007**, 167, 148–154.
17. Kupka, R.; Mugusi, F.; Aboud, S.; Msamanga, G.I.; Finkelstein, J.L.; Spiegelman, D.; Fawzi, W.W. Randomized, double-blind, placebo-controlled trial of selenium supplements among HIV-infected pregnant women in Tanzania: effects on maternal and child outcomes. *Am. J. Clin. Nutr.* **2008**, 87, 1802–1808.

18. Villamor, E.; Mugusi, F.; Urassa, W.; Bosch, R.J.; Saathoff, E.; Matsumoto, K.; Meydani, S.N.; Fawzi, W.W. A trial of the effect of micronutrient supplementation on treatment outcome, t cell counts, morbidity, and mortality in adults with pulmonary tuberculosis. *J. Infect. Dis.* **2008**, 197, 1499–1505.
19. Sohni, O.S.; Li, H.; Surace, A.; El–Bayoumy, K.; Upadhyaya, P.; Fiala, E.S. Contrasting patterns of selenium excretion by female CD rats treated with chemically related chemopreventive organic selenocyanate compounds. *Anticancer Res.* **1995**, 15, 1849–1856.
20. Sohn, O.S.; Fiala, E.S.; Upadhyaya, P.; Chae, Y.H.; El–Bayoumy, K. Comparative effects of phenylenebis(methylene)selenocyanate isomers on xenobiotic metabolizing enzymes in organs of female CD rats. *Carcinogenesis* **1999**, 20, 615–621.
21. Deagen, J.T.; Butler, J.A.; Beilstein, M.A.; Whanger, P.D. Effects of dietary selenite, selenocystine and selenomethionine on selenocysteine lyase and glutathione peroxidase activities and on selenium levels in rat tissues. *J. Nutr.* **1987**, 117, 91–98.
22. Swanson, C.A.; Patterson, B.H.; Levander, O.A.; Veillon, C.; Taylor, P.R.; Helzlsouer, K.; McAdam, P.A.; Zech, L.A. Human [74Se]selenomethionine metabolism: a kinetic model. *Am. J. Clin. Nutr.* **1991**, 54, 917–926.
23. Brown, K.M.; Pickard, K.; Nicol, F.; Beckett, G.J.; Duthie, G.G.; Arthur, J.R. Effects of organic and inorganic selenium supplementation on selenoenzyme activity in blood lymphoctyes, granulocytes, platelets and erythrocytes. *Clin. Sci. (Lond.)* **2000**, 98, 593–599.
24. Ip, C.; Thompson, H.J.; Zhu, Z.; Ganther, H.E. In vitro and in vivo studies of methylseleninic acid: evidence that a monomethylated selenium metabolite is critical for cancer chemoprevention. *Cancer Res.* **2000**, 60, 2882–2886.
25. Ip, C.; Dong, Y.; Ganther, H.E. New concepts in selenium chemoprevention. *Cancer Metastasis Rev.* **2002**, 21, 281–289.
26. Lee, J.S.; Ma, Y.B.; Choi, K.S.; Park, S.Y.; Baek, S.H.; Park, Y.M.; Zu, K.; Zhang, H.; Ip, C.; Kim, Y.H.; Park, E.M. Neural network-based analysis of thiol proteomics data in identifying potential selenium targets. *Prep. Biochem. Biotechnol.* **2006**, 36, 37–64.
27. Letavayova, L.; Vlckova, V.; Brozmanova, J. Selenium: from cancer prevention to DNA damage. *Toxicology* **2006**, 227, 1–14.
28. Kajander, E.O.; Harvima, R.J.; Eloranta, T.O.; Martikainen, H.; Kantola, M.; Karenlampi, S.O.; Akerman, K. Metabolism, cellular actions, and cytotoxicity of selenomethionine in cultured cells. *Biol. Trace Elem. Res.* **1991**, 28, 57–68.
29. Chu, S.H.; Shiue, C.Y.; Chu, M.Y. Synthesis and biological activity of some 8-substituted selenoguanosine cyclic 3′,5′-phosphates and related compounds. *J. Med. Chem.* **1975**, 18, 559–564.
30. Mautner, H.G.; Chu, S.H.; Jaffe, J.J.; Sartorelli, A.C. The synthesis and antineoplastic properties of selenoguanine, selenocytosine and related compounds. *J. Med. Chem.* **1963**, 6, 36–39.
31. Ching, W.M.; Alzner–DeWeerd, B.; Stadtman, T.C. A selenium-containing nucleoside at the first position of the anticodon in seleno-tRNAGlu from Clostridium sticklandii. *Proc. Natl. Acad. Sci. USA* **1985**, 82, 347–350.
32. Caton–Williams, J.; Huang, Z. Biochemistry of selenium-derivatized naturally occurring and unnatural nucleic acids. *Chem. Biodivers.* **2008**, 5, 396–407.
33. Carrasco, N.; Ginsburg, D.; Du, Q.; Huang, Z. Synthesis of selenium-derivatized nucleosides and oligonucleotides for x-ray crystallography. *Nucleosides Nucleotides Nucleic Acids* **2001**, 20, 1723–1734.
34. Du, Q.; Carrasco, N.; Teplova, M.; Wilds, C.J.; Egli, M.; Huang, Z. Internal derivatization of oligonucleotides with selenium for x-ray crystallography using MAD. *J. Am. Chem. Soc.* **2002**, 124, 24–25.
35. Salon, J.; Sheng, J.; Jiang, J.; Chen, G.; Caton–Williams, J.; Huang, Z. Oxygen replacement with selenium at the thymidine 4-position for the Se base pairing and crystal structure studies. *J. Am. Chem. Soc.* **2007**, 129, 4862–4863.
36. Jiang, J.; Sheng, J.; Carrasco, N.; Huang, Z. Selenium derivatization of nucleic acids for crystallography. *Nucleic Acids Res.* **2007**, 35, 477–485.
37. Caton–Williams, J.; Huang, Z. Synthesis and DNA-polymerase incorporation of colored 4-selenothymidine triphosphate for polymerase recognition and DNA visualization. *Angew. Chem. Int. Ed. Engl.* **2008**, 47, 1723–1725.
38. McGee, D.P.; Vaughn–Settle, A.; Vargeese, C.; Zhai, Y. 2′-Amino-2′-deoxyuridine via an intramolecular cyclization of a trichloroacetimidate. *J. Org. Chem.* **1996**, 61, 781–785.
39. Carrasco, N.; Buzin, Y.; Tyson, E.; Halpert, E.; Huang, Z. Selenium derivatization and crystallization of DNA and RNA oligonucleotides for x-ray crystallography using multiple anomalous dispersion. *Nucleic Acids Res.* **2004**, 32, 1638–1646.