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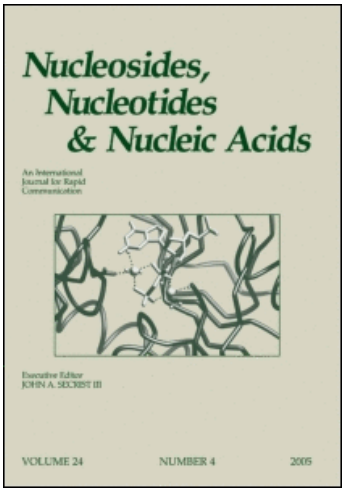

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SYNTHESIS OF SELENIUM-DERIVATIZED NUCLEOSIDES AND OLIGONUCLEOTIDES FOR X-RAY CRYSTALLOGRAPHY

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SYNTHESIS OF SELENIUM-DERIVATIZED NUCLEOSIDES AND OLIGONUCLEOTIDES FOR X-RAY CRYSTALLOGRAPHY

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ABSTRACT

We report here the synthesis of nucleoside and oligonucleotide analogs containing selenium, which serves as an anomalous scattering center to enable MAD phase determination in nucleotide X-ray crystallography. We have developed a phase transfer approach to introduce the selenium functionality in A, C, G, T, and U nucleosides at 5′-positions. In the incorporation of the selenium functionality, the leaving groups (bromide, mesyl, and tosyl) were readily displaced by sodium selenide, sodium diselenide, and sodium methyl selenide with yields higher than 90%. Selenium-derivatized oligonucleotides have been synthesized via phosphoramidite chemistry.

INTRODUCTION

Since nucleic acids play essential roles in fundamental biological processes, the determination of 3-D structures of functional RNAs, and RNA-protein and DNA-protein complexes by X-ray crystallography has recently received tremendous attention[1–4]. The determination of phase in nucleic acid

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X-ray crystallography is a long-standing problem. We endeavor to introduce heavy atoms as anomalous scatterers into nucleic acids to facilitate phase determination by the Multiwavelength Anomalous Dispersion (MAD) technique. As selenium and oxygen are from the same family in the periodic table, oxygen in nucleotides can be substituted with selenium. There are a variety of oxygen atoms positioned in different chemical environments in nucleotides, thus, selective replacement of oxygen with selenium may avoid structural perturbation, which can not always be avoided[5] in oligonucleotide structure determination by X-ray crystallography using halogenated nucleotides[6]. In addition, based on a rudimentary calculation on X-ray phasing power of a selenium scatterer, one selenium atom can enable phase determination for RNA or DNA up to 30 nucleotides[7]. Furthermore, selenium substitution has proven to be successful for MAD phasing and structural determination of proteins bearing selenomethionine[8–10]. Therefore, selenium replacement of oxygen in nucleotides is expected to provide an ideal scattering center, which may significantly advance 3-D structural determination of nucleic acids. In order to test this novel strategy for crystal structure determination, we have synthesized oligonucleotides containing selenium at the 5′-terminus (**1**, Fig. 1) by incorporating the building blocks (**2a** and **2b**), where the 5′-oxygen is substituted by selenium. As selenols are sensitive to air oxidation, **1** and **2b** are protected as methyl selenides. The hydrophobic nature of the methselenol functionality on **1** may enhance strand-strand stacking interaction in crystal lattice, which may assist crystallization.

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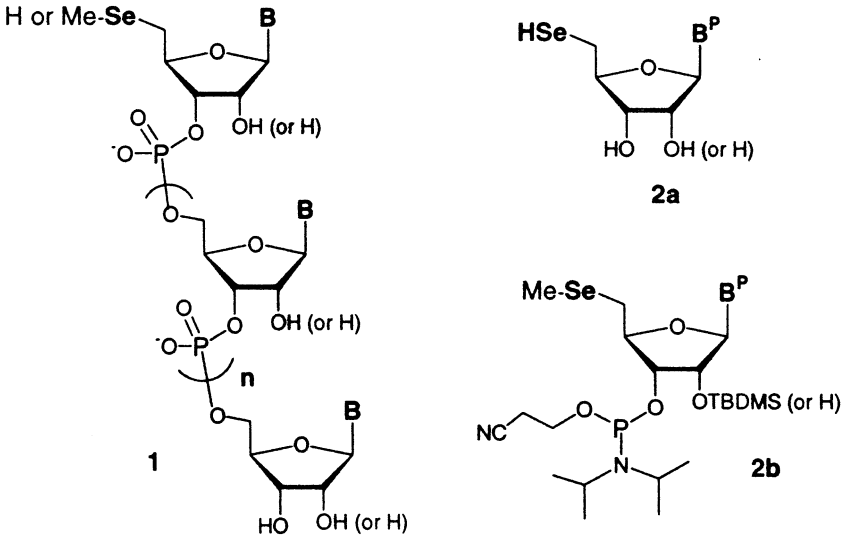
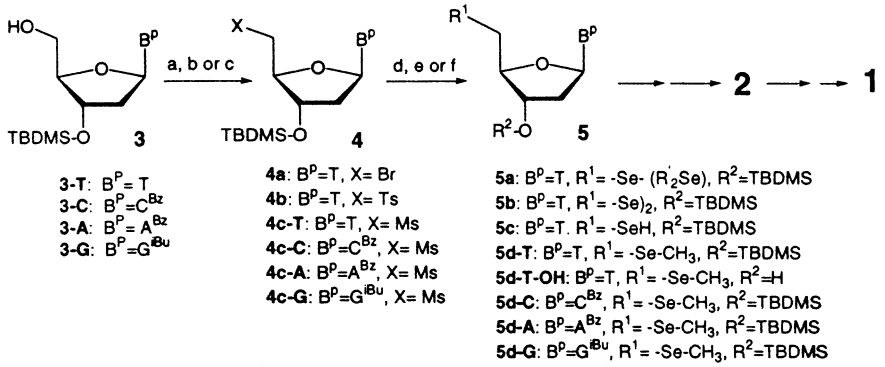


Figure 1. OTBDMS: *tert*-butyldimethylsilyloxyl.

RESULTS AND DISCUSSION

Incorporation of the selenium functionality is commonly done by nucleophilic substitution chemistry in ethanol or DMF solvent using sodium selenide made by $NaBH_4$ reduction of selenium metal[11–13]. However, this conventional approach proved unsatisfactory in the case of acyl protected nucleosides, given the fact the strong base in the nucleophilic substitution induces removal of the acyl protecting groups on the nucleobases, along with salt formation. To overcome this problem, we have developed the two-phase system (H_2O-toluene) to incorporate selenium using a phase transfer catalyst. To our knowledge, this is the first example of selenide alkylation using a phase transfer method. The half-time of the nucleophilic reaction was less than 10 min. when Ms- (mesyl) and Br- groups were used as the leaving groups and sodium selenide was used as the nucleophile. As this nucleophilic substitution was fast in the organic phase, the selenide anions transferred into the organic phase did not cause removal of the acyl groups from the nucleosides. For C, A, and G nucleosides with acyl protection, the two-phase reactions were conducted at pH 8[14], which avoid the base deprotection. These reactions assisted by the phase transfer catalyst are fast, easy to workup, and give high yields (usually higher than 90% yields after purification by silica gel chromatography).

The 5′-hydroxy groups of partially protected nucleosides **3** (T, U, C, A, and G) were activated for nucleophilic substitution with the leaving groups, Br-, Ts- (tosyl), and Ms- (Scheme 1). Compound **4a** was synthesized by the Mitsunobu reaction[15], and **4b** and **4c** (T, C, A, and G) were synthesized by standard procedures[16]. In order to introduce the selenium functionality, we initially attempted to displace the leaving groups (Br, Ms, or Ts) with sodium selenide (Na_2Se), which was generated by reduction of selenium metal with



Scheme 1. (a) CBr_4/Ph_3P/DIAD/THF. (b) Ts-Cl/Py. (c) Ms-Cl/TEA/THF. (d) Na_2Se, (e) Na_2Se_2, or (f) $NaSeCH_3$ plus H_2O/Toluene and Tetrahexylammonium Hydrogen Sulfate. TBDMS: *tert*-butyldimethylsilyl.

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$NaBH_4$[17]. Because of side reactions and poor solubility of the inorganic sodium selenide salt in organic solvents, it was difficult to develop a satisfactory procedure for this substitution reaction in organic solvents or in aqueous solvents, or even in mixed solvents. Finally, a two-phase system (H_2O-toluene) was developed for this substitution using a phase-transfer catalyst (a quaternary ammonium ion).

A phase-transfer catalyst (tetrahexyl-ammonium hydrogen sulfate) was used to shuttle the selenide anions from the aqueous phase to the organic phase where the reaction took place. As the selenide ions are not solvated and are highly reactive in the organic phase, when Na_2Se was used as a nucleophile, the nucleophilic reaction did not stop at the selenol, which was further alkylated by another alkylating molecule, forming dialkylated product (**5a**). Attempts to prevent formation of the dialkylation product by increasing the amount of the catalyst were not successful. When a Ts- group (in case of **4b**) was applied as the leaving group instead of Br- and Ms- groups, the substitution reaction was slowed down. Nevertheless, the formation of dialkylation product was still observed.

The disadvantage of undesired dialkylation reaction was turned into an advantage by using sodium diselenide (Na_2Se_2) instead of sodium selenide. Sodium diselenide was prepared by fully reducing selenium metal to sodium selenide with 0.3 eq. of $NaBH_4$, then adding another equivalent of selenium metal to the sodium selenide solution. The phase transfer catalyst shuttled sodium diselenide into the organic phase, where sodium diselenide was dialkylated. The dialkyl diselenide compound (**5b**) was stable, and reduction of the diselenide gave the corresponding selenol in quantitative yield. As the selenol was not stable in air, it was oxidized to the diselenide again. Therefore, the freshly prepared selenol was used for conducting the next transformation. A selenol can also be permanently protected with a stable protecting group, such as a methyl group; this protection was achieved by treatment with methyl iodide. As a permanent protection of the hydroseleno group was desired in synthesis of **1** and **2b**, sodium methyl selenide (prepared by reduction of dimethyl diselenide with $NaBH_4$) was used as the nucleophile to react with **4c** (T, C, A, and G) using the phase transfer catalyst. Although the aqueous phase was basic, this two-phase system has completely prevented the hydrolysis of the protecting acyl groups on A, C and G during the reaction, which were otherwise hydrolyzed in the basic medium.

We have prepared selenium-labeled phosphoramidites **2b** and oligonucleotides **1** using standard phosphoramidite procedures[18]. The MS data of the Se-dinucleotide (5′-$^{Se-}$TT) in Fig. 2A shows the selenium satellite isotopic peaks of the molecular anion generated by the electrospray experiment, which is consistent with the structure. The MS data of the Se-hexanucleotide (5′-$^{Se-}$TGCGCA-3′) shows the molecular anions carrying from one to three negative charges (Fig. 2B); dimerization of the self-complementary sequence carrying three charges is also revealed by the

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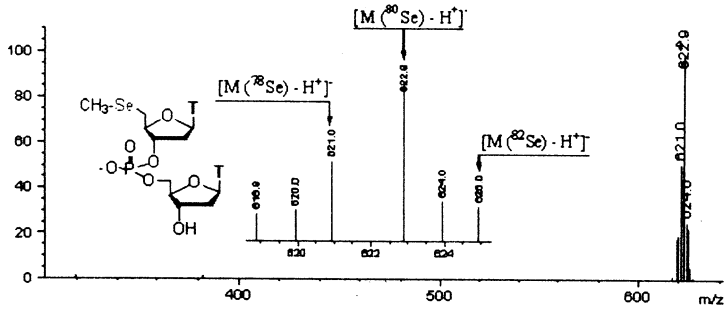
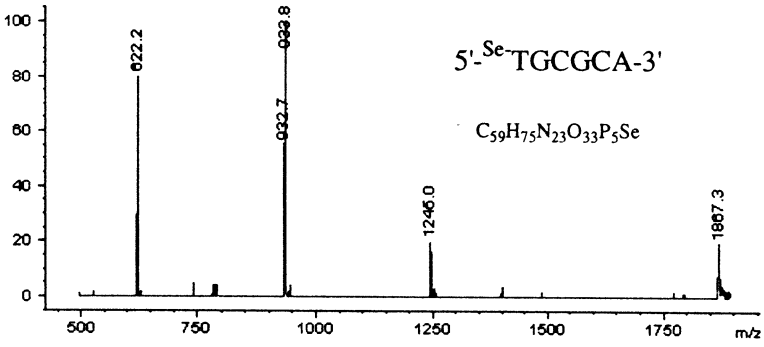


A. MS spectrum of 5'-^{Se}-TT by electrospray (negative ion experiment). The molecular weight ($C_{21}H_{29}N_4O_{11}PSe$) is 624 with adjustment of ^{80}Se isotope. Selenium isotopes 76 (9%), 77 (7%), 78 (23%), 80 (49%), 82 (9.2%). MS peaks: 619 [M (^{76}Se) – H^+]$^-$, 620 [M (^{77}Se) – H^+]$^-$, 621 [M (^{78}Se) – H^+]$^-$, 623 [M (^{80}Se) – H^+]$^-$, 625 [M (^{82}Se) – H^+]$^-$.



B. MS spectrum of 5'-^{Se}-TGCGCA-3' by electrospray (negative ion experiment). The molecular weight ($C_{59}H_{75}N_{23}O_{33}P_5Se$) is 1868 for ^{80}Se. Measured (expected) m/z: [M-H^+]$^-$ = 1867.3 (1867), [M-$2H^+$]$^{2-}$ =933.8 (933), [M-$3H^+$]$^{3-}$ =622.2 (621.7), [2M-$3H^+$]$^{3-}$ =1245.0 (1244.3).

Figure 2. MS Spectra of Se-Oligonucleotides.

peak (1245.0 m/z). Crystallization of these oligonucleotides (the dimer and the hexamer) for X-ray crystallography is under way. The successful synthesis of the Se-containing oligonucleotides indicates that the selenium functionality is compatible with the phosphoramidite solid-phase synthesis, and large-scale Se-containing oligonucleotides can be prepared for structure determination.

EXPERIMENTAL SECTION

1-[(2R, 4S, 5R)-4-*tert*-butyldimethylsilyloxy-5-bromomethyl-tetrahydrofuran-2-yl]-thymidine (4a). 3-T (261.1 mg, 0.733 mmol) and Ph_3P (577.5 mg, 2.2 mmol, 3 eq.) were placed in a 25-mL round-bottom flask and dried on high vacuum for 1 hr THF (7.33 mL, final conc. 0.1 M), TEA (614 μL, 6 eq.), and CBr_4 (729.72 mg, 2.2 mmol, 3 eq.) were then added

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sequentially. The reaction mixture was stirred at RT under dry argon. The reaction was completed after 15 min as indicated by silica gel TLC (5% $MeOH/CH_2Cl_2R_f = 0.46$). MeOH (0.5 mL) was then added to consume any excess reagent, and the reaction mixture was stirred for another 15 min. All the solvents were removed by rotary evaporation under reduced pressure at 30 °C. The crude product was then dissolved in EtOAc, the salt was removed by filtration, and the solvent was evaporated. The residue was directly applied to a silica gel column (25 g of silica gel), and the column was eluded with EtOAc/Hexane (3:7). This solution precipitated the majority of triphenylphosphoxide, which facilitated this purification. The fractions containing the pure product were combined and evaporated under reduced pressure, and the resultant product was dried on high vacuum overnight to give a brownish foamy product (298 mg, 97% yield).

^{1}H-NMR ($CDCl_3$) δ:0.11 [(s, 6H, $(CH_3)_2Si$], 0.90 (s, 9H, t-Bu), 1.94 (s, 3H, 5-CH_3), 2.11–2.39 (m, 2H, 3′-H), 3.58–3.72 (m, 2H, 5′′-H), 4.0–4.13 (m, 1H, 5′-H), 4.35–4.48 (m, 1H, 3′-H), 6.29 (t, J = 6.75 Hz, 1H, 2′-H), 7.45 (s, 1H, 6-H), 9.65–9.78 (b, 1H, NH, exchangeable by D_2O).

^{13}C-NMR ($CDCl_3$) δ: 12.60 (5-CH_3), 17.81 (CH_3-Si), 25.81 [($\underline{CH_3}$)$_3$C], 33.03 ($C_{5''}$), 40.52 ($C_{3'}$), 73.13 ($C_{5'}$), 84.60 ($C_{4'}$), 84.76 ($C_{2'}$), 111.20 (C_5), 135.58 (C_6), 150.35 (C_2), 163.95 (C_4).

IR (KBr): 3162, 3040, 2951, 2929, 2857, 1692, 1470, 1426, 1276, 1198, 1054, 993, 904, 838, 782, 671, 561 cm^{-1}.

UV (in acetonitrile): 263.8 nm.

N^6-Benzoyl-1-[(2R, 4S, 5R)-4-***tert*-butyldimethylsilyloxy-5-methanesulfonyl-methyl-tetrahydrofuran-2-yl]-adenine (4c-A). 3-A** (54.0 mg, 0.115 mmol) was placed in 10-mL flask and dried on high vacuum for 2 h. THF (2.3 mL) and TEA (47 μL, 0.345 mmol, 3 eq) were then added, and the flask was placed on an ice-water bath and kept under dry argon. Methanesulfonyl chloride (13 μL, 0.17 mmol, 1.5 eq) was added and the reaction was completed in 15 min. (silica gel TLC in 5% $MeOH/CH_2Cl_2$, **4c-A** $R_f = 0.35$, **3-A** $R_f = 0.30$). MeOH (1 mL) was added to consume the excess reagent and the reaction was stirred for another 15 min. The solvents were removed by rotary evaporation at 40 °C; the residue was dissolved in EtOAc (15 mL), and the solution was filtered. The filtrate was then evaporated, and the residue was purified on silica gel G60 column (gradient, from 0 to 5% $MeOH/CH_2Cl_2$). The collected fractions were evaporated under reduced pressure and dried on high vacuum overnight. A colorless foamy product (**4c-A**) was obtained (60 mg, 98% yield).

^{1}H-NMR ($CDCl_3$). δ: 0.13 [s, 6H, $(CH_3)_2Si$], 0.92 (s, 9H, t-Bu), 2.45-2.55 (m, 2H, 3′-H), 2.98 (s, 3H, CH_3SO_3), 4.20-4.26 (m, 1H, 5′-H), 4.40–4.52 (m, 2H, 5′′-H), 4.70-4.78 (m, 1H, 4′-H), 6.45–6.53 (t, J = 6.6 Hz, 1H, 2′-H), 7.49-7.65 (m, 3H, Ar), 8.01–8.08 (m, 2H, Ar), 8.20 (s, 1H, 8-H), 8.80 (s, 1H, 2-H), 8.95–9.20 (b, 1H, NH, exchangeable with D_2O).

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Mass spectrum. The molecular weight of 4c-A is 547. Electrospray experiment showed molecular peaks at 548 $[M + H]^+$, 570 $[M + Na]^+$.

N^2-*iso*Butyryl-1-[(2R, 4S, 5R)-4-tert-butyldimethylsilyloxy-5-methanesulfonyl-methyl-tetrahydrofuran-2-yl]-guanine (4c-G). The same procedure for **4c-A** was used to prepare **4c-G**. The mesylation reaction was complete in 1.5 hours. After the column chromatography over silica gel, **4c-G** was obtained in 94% yield.

^{1}H-NMR ($CDCl_3$) δ: 0.15 [s, 6H, $(CH_3)_2Si$], 0.93 (s, 9H, t-Bu), 1.25 [s, 6H, $(CH_3)_2C$], 2.35–2.64 (m, 2H, 3′-H), 2.76 [sept, J = 6.9 Hz, 1H, $\underline{CH}(CH_3)_2$], 3.07 (s, 3H, CH_3-SO_3), 4.12–4.25 (m, 1H, 5′-H), 4.31–4.52 (m, 1H, 4′-H), 4.58–4.72 (m, 2H, 5″-H), 6.21 (dd, J = 6.0, 6.8 Hz, 1H, 2′-H), 7.73 (s, 1H, 8-H), 8.87-8.95 (br, 1H, NH, exchangeable by D_2O).

^{13}C-NMR ($CDCl_3$) δ: 17.84 (CH_3-Si), 18.82 [$(CH(\underline{C}H_3)_2)$], 25.60 [$(\underline{C}H_3)_3C$], 27.59 (CH_3SO_3), 36.49 [$(\underline{C}H(CH_3)_2)$], 41.46 ($C_{3'}$), 66.35 ($C_{5''}$), 71.36 ($C_{5'}$), 86.81 ($C_{4'}$), 89.27 ($C_{2'}$), 122.48 (C_5), 138.47 (C_8), 146.89 (C_4) 147.36 (C_2), 155.86 (C_6).

UV (in acetonitrile): 266.8 nm.

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1-[(2R, 4S, 5S)-4-***tert*-butyldimethylsilyloxy-5-diselenomethyl-tetrahydrofuran-2-yl]-thymidine (5b).** Sodium borohydride (45.1 mg, 1.2 mmol) dissolved in water (1.2 mL) was added to a 25-mL flask containing a suspension of selenium metal powder (94.3 mg, 1.19 mmol) in water (1.2 mL). The reaction was placed in an ice-bath for the first few minutes to slow down the reaction; the reaction mixture was later stirred at room temperature under argon. After the vigorous reaction had subsided (approximately 10 min), additional selenium metal powder (94.3 mg, 1.19 mmol) was added. The mixture was stirred for another 10 min and then warmed on a steam bath for 5 min to completely dissolve all the selenium and to decompose the excess $NaBH_4$. The color of the solution was brownish red and its pH was about 10–11. This aqueous solution was injected into a 25-mL flask containing **4a** (100 mg, 0.239 mmol), tetrahexylammonium hydrogen sulfate (10.7 mg, 0.0239 mmol, 0.1 eq.), and toluene (4.8 mL). The reaction was closely monitored by silica gel TLC (5% $MeOH/CH_2Cl_2$, product $R_f = 0.33$). After 45 min the reaction was complete. A current of air was then passed through the reaction mixture to oxidize any excess of sodium diselenide to selenium metal, which precipitated.

The crude product mixture was centrifuged to remove the precipitated selenium metal, followed by extraction twice with toluene (15 mL), and the respective organic layers were combined. This organic phase was then washed with $NaHCO_3$ (15 mL, sat.) and NaCl (15 mL, sat.). The resultant yellowish organic phase was dried over anhydrous $mgSO_4$ for 30 min, the solution was filtered, and the solvents were removed by rotary evaporation under reduced pressure at 40 °C. The crude residue was then dissolved in CH_2Cl_2, and the

solution was loaded onto a silica gel column (6.0 g silica gel). The column was eluted with CH_2Cl_2 (50 mL) followed by a stepwise gradient of MeOH (0–3%). The fractions containing the product were combined and the solvents were removed by rotary evaporation at 30 °C. After drying on high vacuum overnight, 96 mg of the yellowish diselenide product (**5b**) was obtained (96% yield).

^{1}H-NMR ($CDCl_3$) δ: 0.11 [s, 6H, $(CH_3)_2Si$], 0.93 (s, 9H, *t*-Bu), 1.94 (s, 3H, 5-CH_3), 2.25-2.4 (m, 2H, 3′-H), 3.33-3.36 (m, 2H, 5″-H), 4.03–4.12 (m, 1H, 5′-H), 4.28–4.35 (m, 1H, 4′-H) 6.19 (t, J = 6.6 Hz, 1H, 2′-H) 7.25 (s, 1H, 6-H). 8.80–8.92 (b, 1H, NH, exchangeable by D_2O).

^{13}C-NMR ($CDCl_3$) δ: 12.66 (CH_3-Si), 17.92 [($\underline{CH_3}$)$_3$C], 25.71 (5-CH_3), 40.41 ($C_{3'}$), 52.79 ($C_{5''}$), 74.14 ($C_{5'}$), 85.38 ($C_{4'}$), 86.04 ($C_{2'}$), 111.18 (C_5), 135.72 (C_6), 150.16 (C_2), 164.10 (C_4).

IR (KBr): 3428, 3179, 3057, 2962, 2934, 2862, 2363, 1703, 1476, 1370, 1281, 1204, 1104, 838, 782, 666 cm^{-1}.

UV (in acetonitrile): 264.4 nm.

N^6-Benzoyl-1-[(2R, 4S, 5R)-4-tert-butyldimethylsilyloxy-5-methselenomethyl-tetrahydrofuran-2-yl]-adenine (5d-A). $NaBH_4$ (20.0 mg, 0.525 mmol) was placed in 10mL-round flask under nitrogen. Water (1.5 mL) and dimethyl diselenide (17.0 μL, 0.175 mmol) were sequentially and slowly injected into the flask. Vigorous stirring helped to dissolve dimethyl diselenide completely, forming a colorless homogeneous solution after 5–10 min; the pH of the solution was higher than 11. Since high pH caused the hydrolysis of the protecting benzoyl group on adenine base, and pH 7.0 or lower made the following selenide substitution very slow, the pH was adjusted to 8.0 by adding dilute HCl dropwise. **4c-A** (19.2 mg, 0.0350 mmol) and tetrahexylammonium hydrogen sulfate (0.5 mg) dissolved in toluene (0.7 mL) were then added to the sodium methylselenide solution (pH 8.0) described above, and the two-phase mixture (toluene and water) was stirred under nitrogen. The reaction was complete after 5 h, forming **5d-A** (silica gel TLC, 5% MeOH/CH_2Cl_2, $R_f = 0.52$). Longer reaction time caused slow hydrolysis of the benzoyl group (the hydrolyzed product $R_f = 0.33$ on TLC, 5% MeOH/CH_2Cl_2). The organic phase was removed and the aqueous phase was extracted twice with EtOAc; the combined organic phase was washed twice with saturated NaCl solution. The solvents were removed by rotary evaporation under reduced pressure at 40 °C. The crude product was dissolved in CH_2Cl_2 and loaded on a silica gel TLC plate (5% MeOH/CH_2Cl_2). Colorless product was recovered from this purification (18.2 mg, 95% yield).

^{1}H-NMR ($CDCl_3$). δ: 0.12 [s, 6H, $(CH_3)_2Si$], 0.92 (s, 9H, t-Bu), 2.02 (s, 3H, CH_3Se), 2.43–2.53 (m, 1H, 3′-H), 2.78–3.00 (m, 3H, 3′-H and 5″-H), 4.15–4.23 (m, 1H, 5′-H), 4.55–4.63 (m, 1H, 4′-H), 6.42–6.50 (t, J = 6.6 Hz, 1H, 2′-H), 7.48–7.57 (m, 2H, Ar), 7.57–7.65 (m, 1H, Ar), 8.00–8.07 (m, 2H, Ar), 8.27 (s, 1H, 8-H), 8.80 (s, 1H, 2-H), 8.94–9.00 (b, 1H, NH, exchangeable by D_2O).

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The molecular weight of **5d-A** ($C_{24}H_{33}N_5O_2SiSe$) is 547 with adjustment for ^{80}Se isotope [average atomic weight of Se is 79, including 76 (9%), 77 (7%), 78 (23%), 80 (49%), 82 (9.2%)]. The molecular peaks are: 546 [M(^{78}Se)+H]$^+$, 548 [M(^{80}Se)+H]$^+$, 550 [M(^{82}Se)+H]$^+$, 568 [M(^{78}Se)+Na]$^+$, 570 [M(^{80}Se)+Na]$^+$, 572 [M(^{82}Se)+Na]$^+$.

1-[(2R,4S,5R)-4-tert-butyldimethylsilyloxy-5-methselenomethyl-tetrahydro-furan-2-yl]-thymidine (5d-T)

Method 1. $NaBH_4$ (63 mg, 1.65 mmol) was dissolved in 1.5 ml water and sealed in a 10-mL flask under nitrogen. Dimethyl diselenide (54 μL, 0.55 mmol) was slowly injected into the flask. Addition of 0.2 mL of ethanol with vigorous stirring helped to dissolve dimethyl diselenide completely; a colorless homogeneous solution was formed in 5 min. The solution of **4c-T** (47.7 mg, 0.110 mmol) and tetrahexylammonium hydrogen sulfate (1 mg) in toluene (1.1 mL) was then added to the sodium methyl selenide solution described above. The two-phase mixture (toluene and water) was stirred under nitrogen. The reaction was complete in 3 h as indicated silica gel TLC (5% $MeOH/CH_2Cl_2$, $R_f = 0.40$). The organic phase was removed, the aqueous phase was extracted twice with EtOAc (10 mL each time), and the combined organic phase was washed twice with saturated NaCl solution (10 mL each time). The solvents were removed by rotary evaporation under reduced pressure at 40 °C. The crude product was purified on TLC (5% $MeOH/CH_2Cl_2$). The pure product (**5d-T**) was dried on high vacuum overnight to afford 44.2 mg (93% yield).

Method 2. The title compound was also made by reduction of the diselenide compound **5b** with $NaBH_4$ in EtOH. After the yellow solution of the diselenide turned into colorless, indicating production of the selenol (**5c**) (approximately 5 min), CH_3I was added to protect **5c**. This approach gave quantitative yield.

^{1}H-NMR ($CDCl_3$) δ: 0.05 [s, 6H, $(CH_3)_2Si$], 0.88 (s, 9H, t-Bu), 1.94 (s, 3H, 5-CH_3), 2.08 (s, 3H, CH_3-Se), 2.05–2.18 and 2.28–2.40 (2m, 2H, 3′-H), 2.78–2.93 (m, 2H, 5′′-H), 4.02–4.09 (m, 1H, 5′-H), 4.28–4.38 (m, 1H, 4′-H), 6.26 (t, J = 6.5 Hz, 1H, 2′-H), 7.42 (s, 1H, 6-H), 8.95–9.06 (b, 1H, NH, exchangeable in D_2O).

^{13}C-NMR ($CDCl_3$) δ: 5.66 (CH_3-Se), 12.55 (5-CH_3), 17.68 (CH_3-Si), 25.68 [$(CH_3)_3C$], 27.63 ($C_{5''}$), 40.63 ($C_{3'}$), 73.94 ($C_{5'}$), 84.57 ($C_{4'}$), 85.60 ($C_{2'}$), 111.11 (C_5), 135.59 (C_6), 150.18 (C_2), 163.69 (C_4).

The MS spectrum of **5d-T** is shown in Figure 3 (electrospray, positive ion experiment). The molecular weight ($C_{17}H_{30}N_2O_4SiSe$) is 434 with adjustment for ^{80}Se isotope. Because of binding of H or Na ions, two sets of molecular peaks are observed, in which all selenium isotopic peaks [Se 76 (9%), 77 (7%), 78 (23%), 80 (49%), 82 (9.2%)] are also observed: 431 [M (^{76}Se)+H]$^+$, 432 [M (^{77}Se)+H]$^+$, 433 [M (^{78}Se)+H]$^+$, 435 [M (^{80}Se)+H]$^+$, 437

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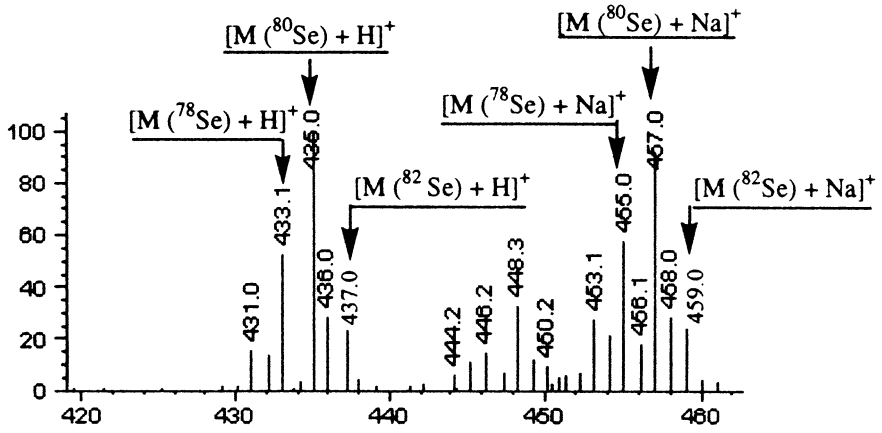

Figure 3. MS of 1-[(2R, 4S, 5R)-4-*tert*-butyldimethylsilyloxy-5-methselenomethyl-tetrahydro-furan-2-yl]-thymidine (**5d-T**).

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[M (^{82}Se)+H]$^+$, 453 [M (^{76}Se)+Na]$^+$, 454 [M (^{77}Se)+Na]$^+$, 455 [M (^{78}Se)+Na]$^+$, 457 [M (^{80}Se)+Na]$^+$, and 459 [M (^{82}Se)+Na]$^+$.

IR (KBr): 3157, 3034, 2960, 2940, 2862, 2363, 1698, 1470, 1426, 1370, 1276, 1198, 1120, 1089, 1049, 827, 777, 682, 621 cm^{-1}.

UV (in acetonitrile): 265.2 nm.

1-[(2R, 4S, 5R)-4-hydroxy-5-methselenomethyl-tetrahydrofuran-2-yl]-thymidine (5d-T-OH). Following the standard procedure, compound **5d-T** (50 mg, 0.115 mmol) was dissolved in THF (345 μL), and *tert*-butyl

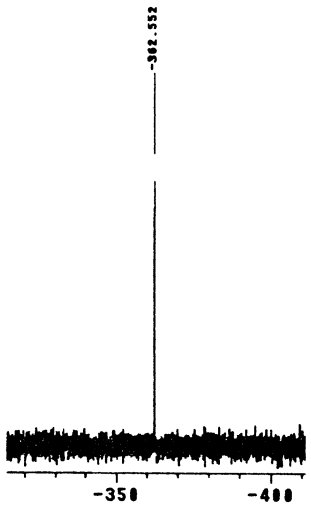

Figure 4. ^{77}Se-NMR of 1-[(2R, 4S, 5R)-4-hydroxy-5-methseleno-methyl-tetrahydrofuran-2-yl]-thymidine in $CDCl_3$; reference (dibenzyl diselenide, 133.25 ppm).

ammonium fluoride 1 M in THF (230 μL, 2 eq.) was added. The deprotection reaction was complete in 2 hours (monitored by TLC, 7.5% $MeOH/CH_2Cl_2$). The product was purified on TLC to give quantitative yield, and the structure of this product was confirmed by spectroscopy analysis, including ^{77}Se-NMR (Fig. 4).

^{1}H-NMR ($CD_3OD/CDCl_3 = 1{:}1$) δ: 1.93 (s, 3H, 5-CH_3), 2.06 (s, 3H, CH_3-Se), 2.15–2.28 and 2.32–2.46 (2m, 2H, 3′-H), 2.82–2.96 (m, 2H, 5″-H), 4.05–4.15 (m, 1H, 5′-H), 4.28–4.38 (m, 1H, 4′-H), 6.26 (t, J = 6.7 Hz, 1H, 2′-H), 7.49 (s, 1H, 6-H), 8.95–9.06 (b, 1H, NH, exchangeable in D_2O).

^{13}C-NMR ($CDCl_3$) δ: 5.31 (CH_3-Se), 12.11 (5-CH_3), 27.49 ($C_{5''}$), 39.74 ($C_{3'}$), 72.88 ($C_{5'}$), 84.21 ($C_{4'}$), 84.14 ($C_{2'}$), 110.89 (C_5), 135.70 (C_6), 150.44 (C_2), 163.69 (C_4).

As ^{77}Se NMR active ($M_I = 1/2$), a Se-NMR was done (Fig. 4). ^{77}Se-NMR ($CDCl_3$) δ: 362.55 ppm (reference: dibenzyl diselenide, 133.25 ppm)

IR (KBr): 3473, 3167, 3095, 2960, 2923, 2812, 2679, 1703, 1476, 1410, 1259, 1071, 1015, 950, 888, 816, 632, 570 cm^{-1}.

UV (in acetonitrile): 265.0 nm.

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