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Synthesis and Crystal Structure of 2′-Se-Modified Guanosine Containing DNA

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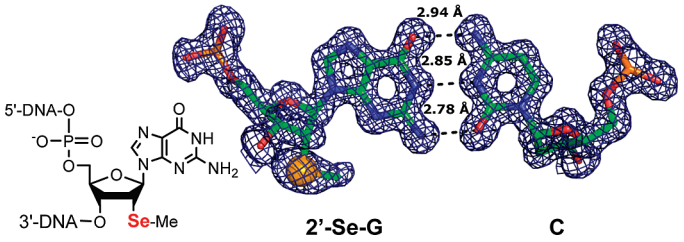

Selenium modification of nucleic acids is of great importance in X-ray crystal structure determination and functional study of nucleic acids. Herein, we describe a convenient synthesis of a new building block, the 2′-SeMe-modified guanosine (G_{Se}) phosphoramidite, and report the first incorporation of the 2′-Se-G moiety into DNA. The X-ray crystal structure of the 2′-Se-modified octamer DNA (5′-GTG_{Se}TACAC-3′) was determined at a resolution of 1.20 Å. We also found that the 2′-Se modification points to the minor groove and that the modified and native structures are virtually identical. Furthermore, we observed that the 2′-Se-G modification can significantly facilitate the crystal growth with respect to the corresponding native DNA.

Introduction

X-ray crystallography plays a leading role in providing structure information about nucleic acids with atomic resolution.[1] In this field, however, there are two long-standing challenges: crystallization and the phase problem. Besides molecular replacement, other strategies have been developed to calculate the experimental phase of nucleic acid structures, including heavy-atom soaking and cocrystallization in combination with MIR or SIR (multiple- and single-isomorphous replacement), respectively.[2−4] Halogen derivatization and indirect protein derivatization have also been used in conjunction with MAD or SAD (multi- and single-wavelength anomalous dispersion), respectively.[2−4] Halogen derivatization strategy (especially with bromine)[2,5,6] was thought to be a convenient method for the determination of novel nucleic acid structures. However, the lack of multiple choices of derivatization positions, light sensitivity,[7] and potential structure perturbations[8] have limited its application in this field. Determination of three-dimensional structures of nucleic acids through indirect protein derivatization is apparently labor intensive. Therefore, it is necessary to develop an alternative method for nucleic acid structure determination.

Inspired by the Se-methionine derivatization of proteins, which plays the important role in novel protein crystal structure determination,[9,10] the Se modification of nucleic acids[11,12] has recently been recognized as an important tool in phasing X-ray diffraction data. We pioneered this research

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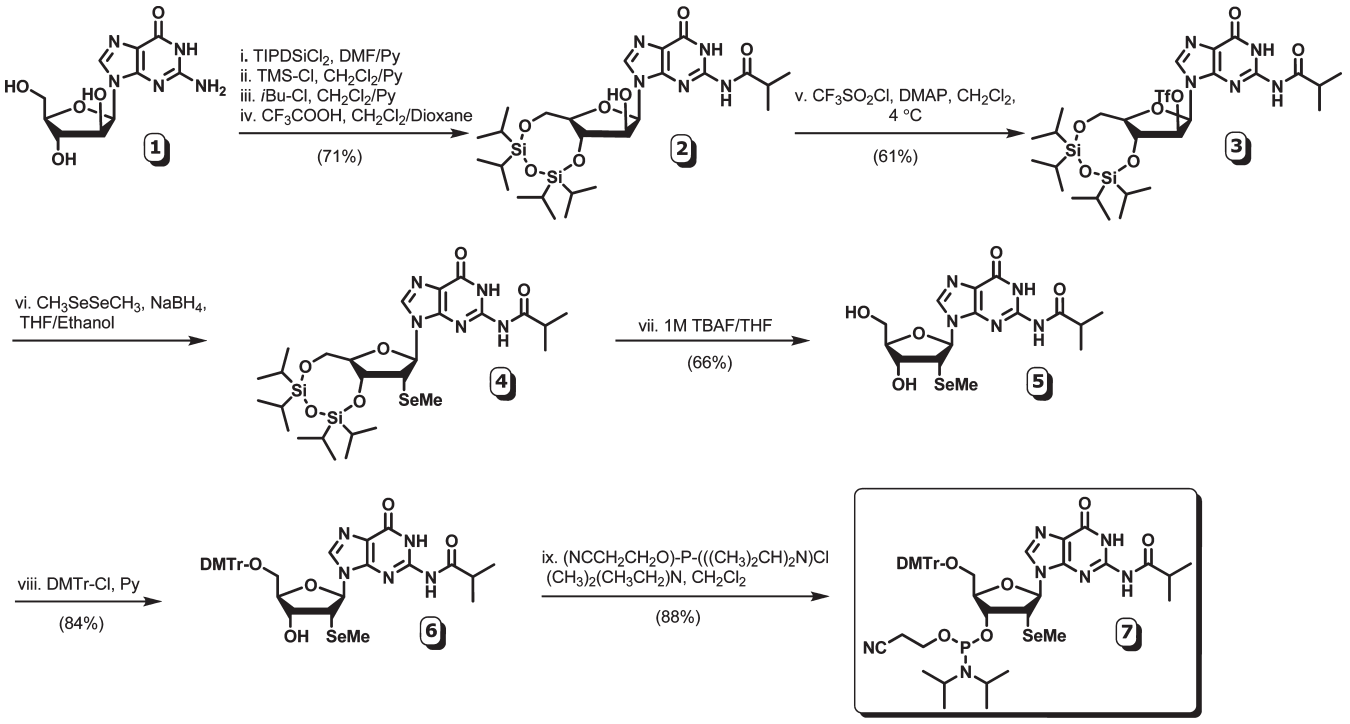
SCHEME 1. Synthesis of the 2′-Selenomethyl-2′-deoxyguanosine Phosphoramidite Building Block



area of selenium-derivatized nucleic acid (SeNA) for nucleic acid crystallography. We and others have developed the chemical and enzymatic syntheses of SeNAs.[13−21] Among several different Se modifications, whether on the sugar or on the nucleobase,[13−21] the 2′-SeMe derivatization (including the 2′-SeMe-G in RNA)[19] is quite stable and preferred for its genuine ability to dramatically facilitate crystal growth.[8,22−24] To further explore the 2′-Se-G derivatization of nucleic acids, we report here a convenient synthesis of a new 2′-Se-guanosine phosphoramidite for DNA and RNA derivatization, describe the first incorporation of the 2′-Se-guanosine moiety into DNA, and report the crystallization and 3D crystal structure of 2′-SeMe-G-derivatized DNA.

Results and Discussion

In our approach, we first protected both the 3′- and 5′-hydroxyl groups of commercially available 9-[β-D-arabinofuranosyl]guanine using the tetraisopropyldisiloxanyl (TIPDS) group. Then, we used a modified *Beigelman* procedure to protect the guanosine amino group (N^2) by the isobutyryl group in excellent yield. The procedure utilizes TMS transient protection of the 2′-hydroxyl group and O^6, which is known to be prone to acylation and sulfonylation reactions.[25] Silylation of the O^6 not only hinders O^6 acylation, but also drives N^2 acylation, as it only requires 1.1 equiv of isobutyryl chloride reagent.[26] Upon the successful acylation, we proceeded with a selective deprotection of the 2′-*O*-TMS group using 2 equiv of neat trifluoroacetic acid to furnish intermediate **2**. The next step did not require protection of the O^6 because we, unlike others,[19] found it to be nonreactive toward trifluoromethanesulfonyl chloride (CF_3SO_2Cl). In addition to a 61% reaction yield, we were able to recover ca. 27% of the unreacted starting material. Isolated derivative **3** was treated with in situ generated sodium methylselenide, followed by deprotection of the TIPDS group with tetrabutylammonium fluoride (TBAF) to yield diol **5**. Compound **5** was actually synthesized without purification of **4** (66% yield in two steps). This key intermediate was allowed to react with dimethoxytrityl chloride to produce compound **6**, which was subsequently converted into phosphoramidite **7** by reaction with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite in the presence of dimethylethyl amine (Scheme 1). Our novel approach supplies phosphoramidite **7** in a 21% overall yield in nine steps with only five steps of chromatographic purification. We should mention that the synthesis is scalable, allowing preparation of phosphoramidite

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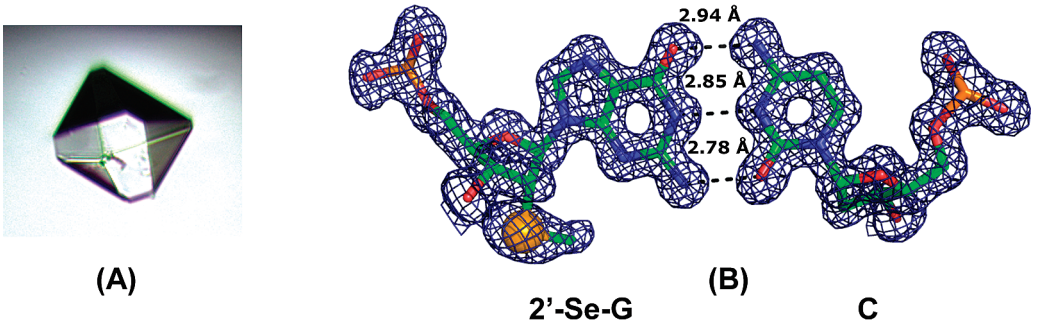

FIGURE 1. Crystal picture and structure of the Se-DNA (5′-GTG_{Se}TACAC-3′)$_2$: (A) typical crystal image and (B) Se-G:C base pair shown with the electron density map contoured at the 1σ level.

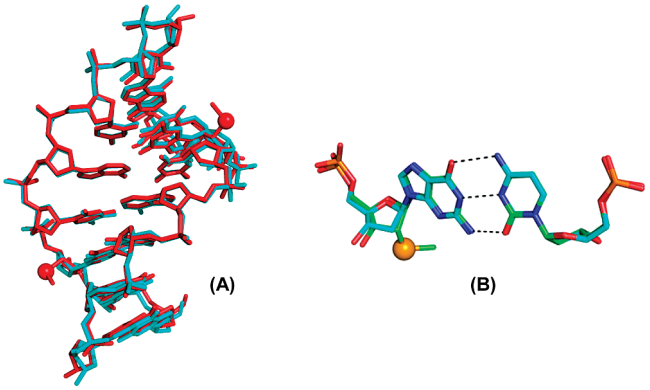

FIGURE 2. Crystal structure of the 2′-Se-modified DNA (5′-GTG_{Se}TACAC-3′): (A) Superimposition of the 2′-Se-modified (red) and nonmodified (cyan) double-stranded DNAs with rms 0.39; the two red balls represent the selenium atoms. (B) Superimposition of the Se-G:C base pair (green) over the native G:C base pair (cyan).

7 on a multigram scale, and is suitable not only for modification of DNA but also RNA.

Similar to the 2′-SeMe-T and 2′-SeMe-U research,[8,22−24] we performed a screening to study the crystallization behavior of the modified oligonucleotide d(5′-GTG_{Se}TACAC-3′) as a model. We found that the 2′-Se-G-DNA could generate high-quality crystals in all the buffer conditions of the Hampton kit (24 buffers) within 3 days (up to approximate size 0.1 × 0.1 × 0.1 mm^3). This size is sufficient for X-ray diffraction data collection. In contrast, we found that the native counterpart did not form crystals in any buffer of the kit over several months.[8] Interestingly, the crystal structure of the 2′-Se-G-modified DNA has the highest resolution of any other SeNAs. In addition, the 2′-Se-T- and 2′-Se-U-modified DNAs with the same sequence did not crystallize in all of the Hampton buffers,[8,24] and their crystal structure resolution was not as high as that of the 2′-Se-G-modified DNA (1.20 Å). Therefore, we conclude that the 2′-SeMe-G modification can greatly facilitate crystal growth of the DNA (5′-GTG_{Se}TACAC-3′) and that the 2′-Se-G modification may particularly be useful for crystallization facilitation and preparation of crystals with high diffraction quality.

The data set of the best crystals, grown in buffer No. 12 (10% MPD, 40 mM Sodium Cacodylate pH 6.0, 12 mM Spermine tetra-HCl, 80 mM KCl, and 20 mM $BaCl_2$), was chosen to determine the Se-DNA structure (Figure 1), which was resolved at 1.20 Å. As shown in Figure 2, this A-form Se-DNA structure (3IFI, in red) is superimposed over the native A-form DNA structure (1DNS, in cyan), which has the same tetragonal space group $P4_32_12$. The overall rmsd of the Se-DNA over the native DNA is low (0.39) and mainly contributed by the first and last bases, and the main structure is considered the same as the native structure. It is clear that the 2′-SeMe derivatization points into the minor groove and does not cause significant structure perturbation with respect to the native structure. The detailed data statistics for the structural analysis of this structure are summarized in Table S1 (see the Supporting Information). Probably due to higher molecular dynamics at the minor groove region, the ordered water molecules are not observed in both the native and Se-DNA structures. Consistent with the previous 2′-Se-dU- and T-DNA structures,[8,24] we did not find any interactions between the selenium atom (or the Se-methyl group) and other atoms. Furthermore, we have analyzed the duplex packing pattern, but no significant intermolecular and/or molecular packing interactions were found. Thus, the facilitated crystallization is likely due to the intrinsic sugar pucker after the Se modification.

Conclusion

In summary, we have developed a new and scalable synthesis of 2′-selenomethyl-2′-deoxyguanosine phosphoramidite **7**, described the first incorporation of the 2′-Se-G moiety into DNA, and reported the X-ray crystal structure of the 2′-Se-G modified DNA at a resolution of 1.20 Å, which is the highest structural resolution among the Se-derivatized nucleic acids. It has also been found that this modification does not cause any significant structure perturbation and that the Se-modified and native structures are virtually identical. Furthermore, we have observed that the 2′-Se-G modification speeds up crystal growth. Consistent with our previous work on the 2′-Se-U and 2′-Se-T modifications, the 2′-Se-G-assisted crystallization success suggests that the crystal growth is mainly reinforced by the 2′-SeMe functionality itself instead of the nucleobases. Our crystallization study, therefore, has potential to aid in the structural and functional studies of nucleic acids as well as nucleic acid−protein complexes.

Experimental Section

***N*^{2}-Isobutyryl-9-[3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]guanine (2).** 9-[3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]guanine (2.7 g, 9.5 mmol), prepared according to the literature,[20] was coevaporated with dry pyridine (50 mL). Dry dichloromethane

(200 mL) and pyridine (50 mL) were added under argon, and the mixture was cooled in an ice bath with stirring. Trimethylsilyl chloride (7.2 mL, 57 mmol, 6 equiv) was added, and the flask was removed from the ice bath and stirred for 2 h. The reaction mixture was cooled again in an ice bath, and isobutyryl chloride (1.1 mL, 10.5 mmol, 1.1 equiv) was added over 5 min. The mixture was stirred at room temperature for 1.5 h. The solution was then poured into 300 mL of 5% aq $NaHCO_3$, and the organic layer was isolated. The mixture was evaporated to a thick oil followed by coevaporation with toluene (2 × 50 mL). The fully protected intermediate was crystallized by addition of diethyl ether (25 mL) and hexanes (25 mL). The crystalline compound (5.6 g) was dissolved in dry CH_2Cl_2 (100 mL) by means of ultrasonication. Into this solution, trifluoroacetic acid (1.2 mL, 2 equiv) in dry dioxane (25 mL) was injected slowly. The reaction mixture was stirred for 6 h. Neat triethylamine (2.3 mL) was added and the mixture was filtered and concentrated. The crude product was purified by flash column chromatography on SiO_2 (CH_2Cl_2/MeOH, 99.5/0.5−98/2 v/v). Yield: 4.03 g (71% over four steps) of **2** as a colorless foam. ^{1}H NMR (DMSO-d_6): δ 1.02−1.16 (m, 34H), 2.77 (m, 1H), 3.82 (m, 1H), 3.94 (dd, J = 2.8, 12.8 Hz, 1H), 4.04 (dd, J = 3.6, 12.8 Hz, 1H), 4.33 (t, J = 8.0 Hz, 1H), 4.50 (m, 1H), 5.85 (d, J = 6.4 Hz, 1H), 6.05 (d, J = 6.4 Hz, 1H), 7.91 (s, 1H), 11.75 (s, 1H), 12.08 (s, 1H) ppm.

N^2-Isobutyryl-9-{2′-*O*-[(trifluoromethyl)sulfonyl]-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl}-guanine (3). A suspension of compound **2** (4.03 g, 6.8 mmol) in CH_2Cl_2 (50 mL) was cooled to 4 °C in an ice water bath. DMAP (2.50 g, 17 mmol, 2.5 equiv) was added, followed by dropwise addition of trifluoromethanesulfonyl chloride (0.65 mL, 10.2 mmol, 1.5 equiv) over 2 min. The reaction mixture was then stirred at 4 °C for 10 min. It was diluted with dichloromethane, washed with saturated sodium bicarbonate solution, dried over $MgSO_4$, and evaporated. The crude product was purified by flash column chromatography on SiO_2 (CH_2Cl_2/MeOH, 99.5/0.5−98/2 v/v). The yield was 3.0 g (61%) of **3** as a colorless solid. Additionally, 1.10 g (27%) of the starting material was isolated. ^{1}H NMR (DMSO-d_6): δ 0.94−1.15 (m, 34H), 2.80 (m, 1H), 3.95 (d, J = 12.4 Hz, 1H), 4.03 (m, 1H), 4.21 (dd, J = 4.8, 12.4 Hz, 1H), 5.00 (t, J = 8.0 Hz, 1H), 6.10 (m, 1H), 6.38 (d, J = 6.4 Hz, 1H), 8.26 (s, 1H), 11.69 (s, 1H), 12.16 (s, 1H) ppm.

N^2-Isobutyryl-2′-methylseleno-2′-deoxyguanosine (5). Sodium borohydride (0.32 g, 2 equiv) was placed in a sealed 100 mL round-bottomed flask, dried on a high vacuum for 5 min to deplete oxygen, kept under argon, and suspended in dry THF (25 mL). Dimethyl diselenide (0.77 mL, 2 equiv) was slowly injected into this suspension, followed by the addition of anhydrous ethanol (2.5 mL). The solution was stirred at room temperature for 1 h. To this slightly yellow solution, compound **3** (3.0 g, 4.1 mmol) in dry THF (25 mL) was injected. The reaction mixture was stirred at room temperature for 20 min. Then, cold water (50 mL) was added and the solution was reduced to half of its volume by evaporation. Dichloromethane (100 mL) was added and the organic layer was separated. The water layer was extracted twice with dichloromethane. The combined organic layers were dried over $MgSO_4$ and the solvent was evaporated to dryness. A small portion of an oily substance was purified by flash column chromatography on SiO_2 (CH_2Cl_2/MeOH, 99/0−99/1 v/v) to obtain compound **4** as a colorless foam. The oily substance was dissolved in THF (20 mL) and treated with 1 M TBAF in THF (6 mL). The solution was stirred at room temperature for 5 min. The solvent was evaporated and the product was isolated by flash column chromatography on SiO_2 (CH_2Cl_2/MeOH, 99/1−95/5 v/v). The yield was 1.20 g (66% over two steps) of **5** as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 1.12, 1.14 (2 × s, 6H), 1.64 (s, 3H), 2.78 (m, 1H), 3.56 (m, 2H), 3.95 (m, 1H), 4.03 (m, 1H), 4.32 (m, 1H), 5.04 (t, J = 5.6 Hz, 1H), 5.84 (d, J = 4.8 Hz, 1H), 6.14 (d, J = 9.2 Hz, 1H), 8.31 (s, 1H), 11.70 (s, 1H), 12.11 (s, 1H), ppm. ^{13}C NMR (DMSO-d_6): δ 3.0, 19.3, 35.2, 46.8, 62.1, 73.3, 87.8, 88.8, 120.5, 138.2, 148.8, 149.5, 155.3, 180.6 ppm. ESI-TOF high-acc (m/z): calcd for $C_{15}H_{21}N_5O_5Se$ $[M + H]^+$ 432.0781, found 432.0777.

N^2-Isobutyryl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-methylseleno-2′-deoxyguanosine (4). ^{1}H NMR ($CDCl_3$): δ 1.11 (m, 28H), 1.28, 1.30 (2 × s, 6H), 1.92 (s, 3H), 2.69 (m, 1H), 3.94 (dd, J = 4.4, 6.8 Hz, 1H), 4.05 (m, 2H), 4.16 (m, 1H), 4.74 (t, J = 6.8 Hz, 1H), 6.21 (d, J = 4.4 Hz, 1H), 7.93 (s, 1H), 8.57 (s, 1H), 12.11 (s, 1H), ppm. ^{13}C NMR ($CDCl_3$): δ 3.5, 12.6, 13.1, 13.2, 13.5, 16.9, 16.99, 17.0, 17.2, 17.3, 17.3, 17.4, 17.5, 19.0, 19.04, 34.7, 47.7, 61.8, 71.6, 84.5, 89.2, 121.6, 136.6, 147.7, 147.8, 155.6, 178.9 ppm. ESI-TOF high-acc (m/z): calcd for $C_{27}H_{47}N_5O_6SeSi_2$ $[M + H]^+$ 674.2303, found 674.2304; $[M + Na]^+$ 696.2122, found 696.2126.

N^2-Isobutyryl-5′-*O*-(4,4′-dimethoxytrityl)-2′-methylseleno-2′-deoxyguanosine (6). Compound **5** (1.20 g, 2.79 mmol) was coevaporated with dry pyridine and then dissolved in pyridine (10 mL). The solution was treated with dimethoxytrityl chloride (1.23 g, 3.63 mmol, 1.3 equiv) in two portions over a period of 45 min. The reaction mixture was stirred for 1 h. The solvents were removed under vacuum and the residue was dissolved in dichloromethane, washed with 5% citric acid, water, and 5% $NaHCO_3$, and then dried over $MgSO_4$ and evaporated. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/MeOH/Et_3N, 99/0/1−98/1/1 v/v/v). Yield: 1.71 g of **6** as a colorless foam (84%). ^{1}H NMR (DMSO-d_6): δ 1.13, 1.14 (2 × s, 6H), 1.68 (s, 3H), 2.76 (m, 1H), 3.10 (m, 1H), 3.38 (m, 1H), 3.74 (s, 6H), 4.07 (m, 1H), 4.20 (m, 1H), 4.25 (m, 1H), 5.89 (d, J = 4.0 Hz, 1H), 6.22 (d, J = 7.6 Hz, 1H), 6.84, 7.20−7.38 (m, 13H), 8.20 (s, 1H), 11.60 (s, 1H), 12.10 (s, 1H), ppm. ^{13}C NMR ($CDCl_3$): δ 4.1, 18.5, 18.6, 36.0, 48.1, 55.2, 64.1, 72.7, 85.5, 86.3, 89.3, 113.1, 121.5, 127.0, 127.9, 128.1, 130.0, 135.7, 135.9, 138.3, 145.0, 147.9, 148.9, 155.7, 158.8, 179.6 ppm. ESI-TOF high-acc (m/z): calcd for $C_{36}H_{39}N_5O_7Se$ $[M + H]^+$ 734.2087, found 734.2085; $[M + Na]^+$ 756.1907, found 756.1908.

N^2-Isobutyryl-5′-*O*-(4,4′-dimethoxytrityl)-2′-methylseleno-2′-deoxyguanosine 3′-(2-Cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (7). Compound **6** (1.50 g, 2.05 mmol) was dissolved in a mixture of dimethylethyl amine (2.0 mL, 13.2 mmol, 6 equiv) and dry dichloromethane (10 mL) under argon. After 10 min, 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.73 g, 3.1 mmol, 1.5 equiv) was slowly added and the solution was stirred at room temperature for 2 h. The crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/MeOH/Et_3N, 98/0/2−97.5/0.5/2 v/v/v). Yield: 1.68 g of **7** as a colorless foam (88%). ^{1}H NMR ($CDCl_3$, two sets of peaks as a mixture of two diastereoisomers): δ 0.56, 0.66, 0.78, 0.83 (d, 12H); 0.98−1.32 (m, 24H); 1.47 (m, 2H); 1.69, 1.74 (s, 6H); 2.16, 2.33 (2*m*, 2H); 2.66, 2.78 (t, m, 2H); 3.12 (m, 2H); 3.50−3.64 (m, 8H); 3.78, 3.79 (2s, 12H); 3.91−4.02, 4.11−4.24 (2 m, 2H); 4.27, 4.35 (m, 2H); 4.50 (m, 2H); 4.73, 4.78 (2 m, 2H); 6.00, 6.06 (2d, J = 9.6 Hz, 2H); 6.82, 7.26, 7.46, 7.58 (4m, 26H); 7.82, 7.83 (2s, 2H); 11.96 (s, 2H) ppm. ^{13}C NMR ($CDCl_3$): δ 3.5, 4.1, 18.3, 18.4, 18.4, 18.5, 20.4, 20.5, 24.4, 24.5, 24.6, 24.7, 35.9, 36.0, 43.2, 43.3, 47.0, 46.1, 55.3, 55.3, 57.5, 57.7, 63.3, 63.5, 74.7, 75.8, 84.9, 85.3, 86.1, 86.3, 91.1, 92.1, 113.3, 113.3, 122.7, 122.9, 127.2, 128.0, 128.10, 128.1, 129.97, 130.00, 130.04, 135.6, 135.8, 135.9, 136.2, 117.2, 117.6, 139.0, 144.9, 145.1, 147.0, 147.1, 148.3, 148.3, 155.4, 158.8, 178.3 ppm. ^{31}P NMR ($CDCl_3$): δ 149.3, 150.5 ppm. ESI-TOF high-acc (m/z): calcd for $C_{45}H_{56}N_7O_8PSe$ $[M + H]^+$ 934.3166, found 934.3170; $[M + Na]^+$ 956.2985, found 956.2983.

Synthesis of 2′-SeMe-G Containing DNA. The sequence of our target oligonucleotide (5′-GTG_{Se}TAC AC-3′) was selected from the PDB (protein data bank)[27] and chemically synthesized

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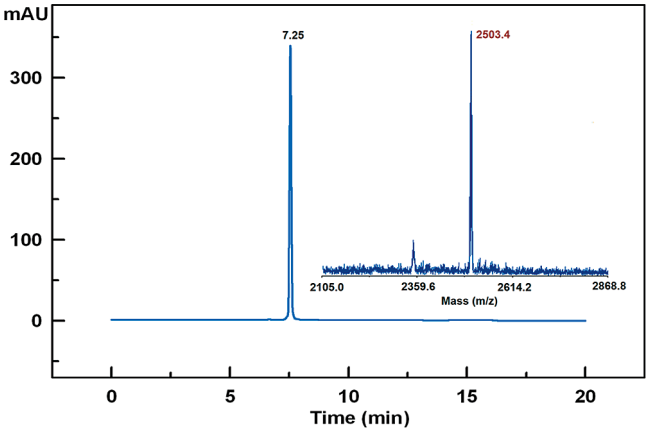

FIGURE 3. Representative HPLC analysis of the 2′-Se-G-modified DNA (5′-GTG_{Se}TACAC-3′). HPLC conditions: Welchrom XB-C18 column (4.6 × 250 mm) with a gradient of 5−60% buffer B in 10 min; flow rate 1 mL/min, 25 °C: (A) 10 mM TEAAc (pH 7.6); (B) 60% acetonitrile in 10 mM TEAAc (pH 7.6). Inset: MALDI-TOF (*m*/*z*): the Se-DNA (molecular formula: $C_{79}H_{101}N_{30}P_7O_{46}Se$), found $[M + H]^+$ 2503.4 (calcd. 2503.4).

on a 1.0 μmol scale using a DNA Synthesizer. The 2′-SeMe-modified guanosine phosphoramidite **7** was incorporated into oligonucleotides by using the standard protocol for solid-phase synthesis with the additional step of treatmeant with DTT;[14] coupling: phosphoramidites in dry acetonitrile (0.1 M) were activated by 0.3 M benzylthiotetrazole in dry acetonitrile; capping: (A) Ac_2O/2,6-lutidine/THF, (B) 16% 1-methylimidazole/THF; oxidation: 0.02 M I_2/THF/Py/H_2O; detrytilation: 3% CCl_3COOH in CH_2Cl_2; manual DTT treatment between each coupling step (2 min): 0.1 M DTT (1 mL each time) in EtOH/H_2O (2/3). Solid-phase synthesis was performed on control pore glass (CPG-500) immobilized with the appropriate nucleoside. The oligonucleotide was made in the DMTr-on mode. After the synthesis, the Se-DNA oligonucleotide was cleaved from the solid support and fully deprotected by concd NH_4OH at 55 °C.

HPLC Analysis and Purification. DNA oligonucleotides were analyzed and purified by reverse-phase high-performance liquid chromatography (RP-HPLC) in both DMTr-on and -off forms (Figure 3). Purification was carried out with an XB-C18 column (Welchrom, 21.2 × 250 mm) at a flow rate of 6 mL/min. Buffer A consisted of 30 mM triethylammonium acetate (TEAA, pH 7.6), while buffer B contained 50% acetonitrile in 30 mM TEAA (pH 7.6). Analysis was performed on an XB-C18 column (Welchrom, 4.6 × 250 mm) at a flow rate of 1.0 mL/min, using buffers A and B. DMTr-on oligonucleotide was purified by eluting with up to 100% B in 20 min in a linear gradient starting at 5% B, while analysis for both the DMTr-on and -off oligonucleotides was carried out with up to 70% of B in a linear gradient in 10 min, starting at 5% B as well. The collected fractions were combined, lyophilized, and desalted by a Sep-Pak C18 cartridge.

Crystallization. The purified DNA oligonucleotide (5′-GTG_{Se}-TACAC-3′) at a concentration of 1 mM was heated to 80 °C for 2 min, and then cooled slowly to room temperature. Both native buffer and Nucleic Acid Mini Screen Kit (Hampton Research) were applied to screen the crystallization conditions at different temperatures (5, 10, and 20 °C), using the hanging drop method by vapor diffusion.

Data Collection. The cryoprotectants 30% glycerol, PEG 400, or perfluoropolyether were used during the crystal mounting, and data collection was taken under a stream of liquid nitrogen at 99 K. The 2′-SeMe-dG-DNA crystal data were collected at beamline X12B and X12C in the NSLS of the Brookhaven National Laboratory. A number of crystals were scanned to find the one with strong anomalous scattering at the K-edge absorption of selenium. The distance of the detector to the crystals was set to 150 mm. The wavelength 0.9795 Å was chosen for selenium SAD phasing. The crystals were exposed for 10 to 15 s per image with one degree oscillation, and a total of 180 images were taken for each data set. All the data were processed with HKL2000 and DENZO/ SCALEPACK.[28]

Structure Determination and Refinement. The structure of Se-DNA was solved by molecular replacement with both CNS[29] and Phaser.[30] The refinement protocol includes simulated annealing, positional refinement, restrained B-factor refinement, and bulk solvent correction. The stereochemical topology and geometrical restraint parameters of DNA/RNA[31] have been applied. The topologies and parameters for the modified guanosine with 2′-SeMe (XUG) were constructed and applied. After several cycles of refinement, a number of highly ordered waters were added. Finally, the occupancies of selenium were adjusted. Cross-validation[32] with a 5−10% test set was monitored during the refinement. The σA-weighted maps[33] of the $(2m|F_o| - D|F_c|)$ and the difference $(m|F_o| - D|F_c|)$ density maps were computed and used throughout the model building.

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Note Added after ASAP Publication. This paper was published on the Web on January 4, 2010, with an error in reference 24. The corrected version was reposted on January 29, 2010.

Supporting Information Available: ^1H NMR spectra of compounds **2**−**7**, ^{13}C NMR spectra of compounds **4**−**7**, ^{31}P NMR spectrum of compound **7**, MALDI-TOF spectrum of the 2′-Se-DNA (dGTACG_{Se} CGTAC), and ESI-TOF high-acc mass spectra of compounds **4**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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