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Synthesis and Crystallographic Analysis of 5-Se-Thymidine DNAs

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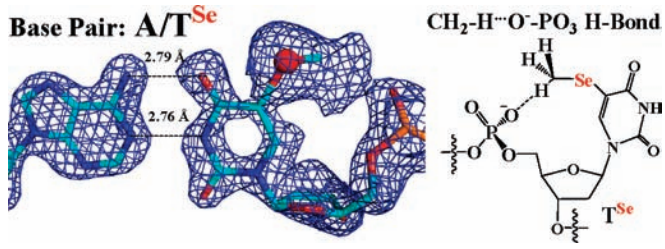
ABSTRACT



We investigated the possibility of the interaction of 5-CH_3 of thymidine and its 5′-phosphate backbone (C−H···O$^-$−PO_3 interaction) in DNA *via* the insertion of the atomic probe (a selenium atom) into the exo-5-position of thymidine (5-Se-T). 5-Se-T was synthesized for the first time, via $Mn(OAc)_3$ assisted electrophilic addition of $CH_3SeSeCH_3$ to 3′,5′-di-*O*-benzoyl-2′-deoxyuridine. The 5-Se-T phosphoramidite was subsequently synthesized and incorporated into DNA in over 99% coupling yield. Biophysical and structural investigations of the 5-Se-T DNAs revealed that the Se-modified and nonmodified DNAs are virtually identical. In addition, the crystallographic analysis of a 5-Se-T DNA strongly suggests a hydrogen-bond formation between the 5-CH_3 and 5′-phosphate groups (CH_3···PO_4^- interaction).

Hydrogen bonds are formed between hydrogen-bond acceptors and donors (X−H). In a classical hydrogen bond, X is an atom with strong electron-negativity (oxgen, nitrogen, etc.). However, recently hydrogen bonds where X is an atom with weak electron-negativity (e.g., carbon) are gaining more acceptance and importance, for instance carbon in C−H···O═C hydrogen bond.[1,2] The interactions between C−H and hydrogen-bond acceptors (electron donors), such as C−H···O═C hydrogen bond in proteins,[1a] C−H···O═C in uracil crystal,[1b] and C−H···Cl in a guest−host system,[3] and other nonconventional interactions (such as H···π interaction in RNA)[4a] have played critical roles in molecular recognition, catalysis, and DNA duplex stability within chemical and biological systems.[1−5] Since a negatively charged phosphate group is an excellent electron donor and the phosphorylation and dephosphorylation are common cellular regulation mechanisms,[5] we investigated whether a C−H (or CH_3) group is capable of forming a hydrogen bond with a phosphate group.

In DNA duplexes,[6] the 5-methyl group of thymidine is normally 4−5 Å away from the closest oxygen (pro-Sp oxygen) of the 5′-phosphate group (O$^-$−PO_3). In order to extend the CH_3 group closer to the phosphate and to give the CH_3 more rotational flexibility at the same time, we inserted an atomic linker between the methyl group and the C5 carbon of thymidine. If the CH_3 and phosphate (O$^-$−PO_3) groups are able to form a genuine hydrogen bond, they will

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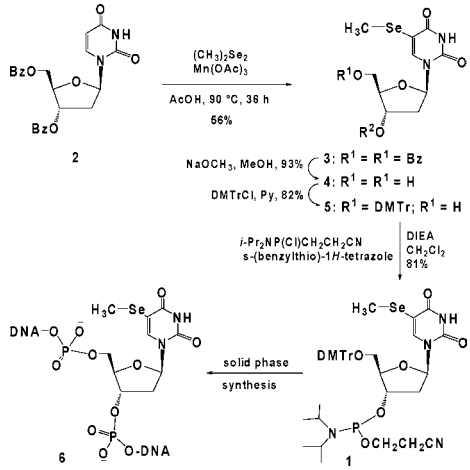
be observed in a closer proximity and with strong electron density between them in a crystal structure. If there is no such stabilizing interaction between them, the methyl group would prefer to turn away from the phosphate due to the steric-hindrance and point into the major groove. Since the size and geometry are essential requirements for this investigation, we thus inserted a selenium atom as an atomic probe[7] between the 5-methyl group and C5 carbon. Herein we report the first synthesis of 5-Se-thymidine phosphoramidite (**1**, Scheme 1), its chemical incorporation into DNAs,

Scheme 1. Synthesis of 5-Se-T Phosphoramidite **1** and Oligonucleotides (**6**) Containing 5-Se-T



and the biophysical and structural studies of the DNAs containing the Se-extended 5-CH_3. Excitingly, we have discovered a novel methyl/phosphate hydrogen bond ($CH\cdots O^--PO_3$) for the first time.

We started novel synthesis of the 5-Se-thymidine phosphoramidite (**1**) from 3′,5′-di-*O*-benzyol-2′-deoxyuridine (**2**).[8a] Though the arylselenylation at the 5-position of pyrimidines was reported over a decade ago,[8b] the incorporation of alkylselenyl substitutions (such as CH_3-Se) at the 5-position has not been reported in the literature, probably due to the instability of the alkylselenyl intermediate. After several attempts of screening Lewis acids as electrophile activators, $Mn(OAc)_3$ was found effective to promote the methyselenation at the 5-position of **2** with dimethyldiselenide $CH_3SeSeCH_3$. Treatment of **2** with $CH_3SeSeCH_3$ (in the presence of $Mn(OAc)_3$ in AcOH at 90 °C) gave 5-Se-thymidine derivative **3** in good yield. NMR analysis of **3** showed the disappearance of the H-5 peak and the appearance of the H-6 singlet peak, and displayed the characteristic ^1H and ^{13}C chemical shifts of the 5-$SeCH_3$ moiety at 2.06 and 7.24 ppm, respectively. Deprotection of **3** with $NaOCH_3$ in MeOH gave **4** in a quantitative yield. UV spectrum of **5** shows λ_{max} absorption at 309 nm, red-shifted by 44 nm when compared with thymidine (Supporting Information). This large red-shift is caused by the electron-donating effect of 5-$SeCH_3$ on the nucleobase π-system. Following the tritylation of **4**, **5** was converted to **1** by the standard phosphoramidite synthesis.[7c]

5-Se-thymidine (5-Se-T) phosphoramidite **1** was found compatible with the conditions of the solid-phase synthesis, including the coupling reaction, acetylation capping, I_2 oxidation, and trichloroacetic acid and concentrated ammonia treatments. The stability of the 5-Se-T moiety allows us to successfully synthesize the Se-oligonucleotides using the normal phosphoramidites. A typical HPLC profile of the synthesized crude Se-DNAs is shown in Figure 1, which is

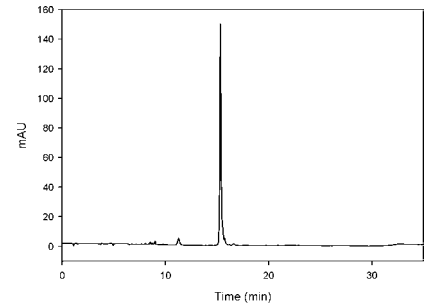


Figure 1. HPLC analysis of crude 5′-DMTr-TT-$^{5\text{-Se}}$T-T-3′ (15.3 min).

virtually identical to that of the corresponding native DNA (Supporting Information). We determined that the coupling yield of the 5-Se-T phosphoramidite was over 99%. The synthesized Se-DNAs were purified and analyzed by HPLC and MS (Table 1 and Supporting Information).

Table 1. MALDI-TOF MS Data of the 5-Se-T DNAs

entry	Se-oligonucleotides	measured (calcd.) *m/z*
a	5′-TT-$^{5\text{-Se}}$T-T-3′	$[M]^+$: 1234.5 (1234.1)
b	5′-G-$^{5\text{-Se}}$T-GTACAC-3′	$[M - H^+]^-$: 2487.4 (2487.4)
c	5′-GCG-$^{5\text{-Se}}$T-ATACGC-3′	$[M - H^+]^-$: 3106.7 (3105.5)
d	5′-ATGG-$^{5\text{-Se}}$T-GCTC-3′	$[M - H^+]^-$: 2808 (2808.4)
e	5′-CTCCCA-$^{5\text{-Se}}$T-CC-3′	$[M + H^+]^+$: 2674.5 (2674.4)
f	5′-CTTCT-$^{5\text{-Se}}$T-GTCCG-3′	$[M + H^+]^+$: 3352.4 (3352.6)
g	$GdU_{2'\text{-Se}}$G-$^{5\text{-Se}}$T-ACAC	$[M + K^+ - 2H^+]^-$: 2605.1 (2605.2)

Our UV-melting data (Table 2) show that the melting temperature differences of the native and Se-modified DNA duplexes are small (less than ±1.0 °C per modification), whereas the melting temperature of the S-modified (5-Me-S-T) DNA duplex is the lowest stable (approximately 3 °C lower than that of the native[8c]). Lower stability of the 5-S-T DNA duplex is probably due to the shorter S−C bond length compared with the Se−C one, which may better facilitate the 5-methyl/phosphate backbone interaction. In most cases that we examined (Table 2), the native duplexes are slightly more stable than the corresponding Se duplexes. Our results

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Table 2. UV Melting Temperatures of the 5-Se-T DNAs

entry	DNA/DNA pairs	melting temp. (°C, native)
a	5′-G-$^{5\text{-Se}}$T-GTACAC-3′ (self-complementary)	27.9 (27.5)
b	5′-GCG-$^{5\text{-Se}}$T-ATACGC-3′ (self-complementary)	26.5 (28.0)
c	5′-ATGG-$^{5\text{-Se}}$T-GCTC-3′ 3′-TACC---A-CGAG-5′	39.3 (40.3)
d	5′-CTCCCA-$^{5\text{-Se}}$T-CC-3′ 3′-GAGGGT---A-GG-5′	36.3 (36.5)
e	5′-CTTCT-$^{5\text{-Se}}$T-GTCCG-3′ 3′-GAAGA---A-CAGGC-5′	43.9 (44.9)

suggest that the insertion of a Se atom at the exo-5-position of thymidine does not cause significant structure perturbation. It is counterintuitive that the larger selenium atom causes less destabilization than smaller sulfur atom, although both disrupt the 5-CH_3/π interaction with the preceding 5′-nucleobase. It appears that the larger Se-linker allows the methyl/phosphate stabilizing interaction.

Similar to other Se-derivatized nucleic acids (SeNA),[7,9] these novel 5-Se-T DNAs are also stable. The crystal growth of the self-complementary Se-DNA (5′-G-$dU_{2'\text{-Se}}$-G-$^{5\text{-Se}}$T-ACAC-3′)$_2$ was successfully facilitated by the utilization of 2′-Me-Se-dU.[6c] Superimposition of the determined Se-DNA crystal structure (1.80 Å) over the corresponding native in the same tetragonal space group[6b] reveals that these two structures are virtually identical (Figure 2A), which is

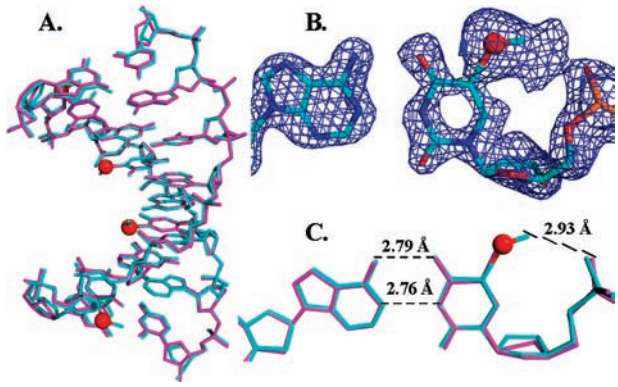

Figure 2. Global and local structures of the 5-Se-T-DNA [(5′-$GdU_{2'\text{-Se}}$-G-$^{5\text{-Se}}$T-ACAC-3′)$_2$]. (A) Duplex structure of the modified DNA (3BM0, in cyan) is superimposed over the native (1DNS, in pink). (B) The experimental electron density map of the $^{5\text{-Se}}$T4:A5 base pair and CH_3···O^-−PO_3 hydrogen-bond formation. (C) The superimposition of the Se-modified (in cyan) and native (in pink) local $^{5\text{-Se}}$T4:A5 structures.

consistent with our UV-melting study. Moreover, we observed that the 5-Se-T and A form a base pair as well as the native T-A pair (Figure 2, B and C). The lengths of these two hydrogen bonds of the $^{5\text{-Se}}$T-A are 2.79 and 2.76 Å.

We also closely examined the geometry of the 5-CH_3 moiety extended by the selenium insertion (Figures 2 and 3). Excitingly, the 5-methyl group clearly points to the 5′-

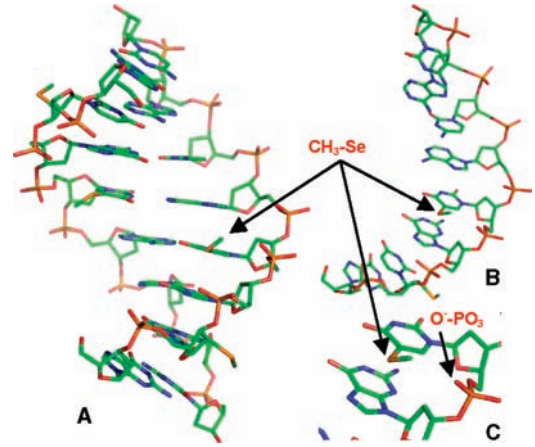

Figure 3. Global and local structures of the 5-Se-T-DNA [(5′-$GdU_{2'\text{-Se}}$-G-$^{5\text{-Se}}$T-ACAC-3′)$_2$]. (A) The duplex structure of the modified DNA (3BM0, in cyan). (B) Single-strand structure. (C) Methyl group and pro-Sp-oxygen in the local structure.

phosphate backbone (Figure 3), and strong experimental electron density exists between them (Figure 2B), although the major groove is wide open and available to accommodate the 5-Me-Se moiety. As a typical hydrogen-bond length (X−H···Y) is between 2.8 and 3.2 Å, the distance (2.93 Å) between the carbon of the 5-methyl group and the pro-Sp oxygen of the 5′-phosphate (Figure 2C) indicates formation of a methyl/phosphate hydrogen bond (CH_3···O^-−PO_3). In addition, our observation on the CH_3···O^-−PO_3 interaction is consistent with the interactions between C−H and other electron donors, such as C−H···O═C hydrogen bond in proteins [bond length (3.14 Å) and angle (149°)][1a] and C−H···O═C interaction in uracil crystal [hydrogen-bond length (3.21 Å) and angle (161°)].[1b] The angle of the C−H···O in the 5-Me-Se-T DNA structure is 144°, which further supports the C−H···O hydrogen-bond formation. The CH_3 rotation about the single bond between the Se and C allows the three hydrogen atoms to have equal opportunity to interact with the pro-Sp oxygen (Figure 2B), which strengthens the C−H···O^-−PO_3 interaction.

Furthermore, in our structural comparison with the native structure, we found that the Se insertion disrupts the weak 5-CH_3/π interaction[4e] in the native structure,[6b] which results in slight reduction of the duplex stability. On the other hand, the intramolecular hydrogen bond slightly stabilizes the intermolecular interaction (the duplex stability) due to the possible preorganization (slight entropy decrease of the Se-modified single strand). Therefore, our UV-melting observa-

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tion on the slight destabilization or stabilization of the Se-DNA duplexes (±1.0 °C per modification) is consistent with these two conflicting interactions, with the sequence and modification dependences, in the presence of the Se-modification and the C−H···O^-−PO_3 hydrogen bond. Furthermore, lower stability of the S-DNA duplex[8c] is consistent with this C−H···O^-−PO_3 interaction in the Se-DNA duplex. As showned in Figure 3, negatively charged oxide orientates the 5-methyl group toward DNA backbone, instead of the major groove, for better interaction. Thus, our results indicate the first observation of the C−H···O^-−PO_3 hydrogen bond.

In conclusion, we have discovered a novel C−H···O hydrogen bond (or CH_3···O^-−PO_3 interaction) *via* the biophysical and structural studies and the synthesis of the 5-Se-thymidine and the 5-Se-derivatized DNAs. Our experimental results have revealed that the Se-modification does not cause significant perturbation of the native DNA structure. This unique CH_3···O^-−PO_3 interaction can be used to advantage in designing inhibitors for the backbone digestion. Our research results have suggested a new research direction to design and develop nuclease-resistant DNAs and RNAs functioning as antisense DNAs, siRNAs, or microRNAs. Moreover, our exciting discovery of the C−H···phosphate hydrogen bond may provide new insight into atomic mechanisms of phosphorylation and dephosphorylation, which are common strategies in cellular signal transduction regulations.[5] Furthermore, this selenium-derivatization has great potential in the determination of nucleic acid crystal structures *via* multiwavelength anomalous dispersion (MAD) or single-wavelength anomalous (SAD) phasing.

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Supporting Information Available: Detailed experimental procedures, ^1H, ^{13}C NMR, and MALDI-MS spectra, UV-melting, HPLC, and crystal diffraction data. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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