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Synthesis of novel di-Se-containing thymidine and Se-DNAs for structure and function studies

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Abstract

The selenium derivatization of nucleic acids and nucleic acid-protein complexes has provided a powerful tool to solve phase problem in X-ray crystallography. Selenium atoms in the nucleotides can serve as fine scattering centers in crystal diffraction. Towards the synthesis of multiple selenium atom-containing nucleotides, which offers strong phasing power to facilitate crystal structure determination, we report here the synthesis of the thymidine analogue containing two Se atoms in one nucleobase. The novel Se-containing nucleoside and oligonucleotide DNAs were synthesized and found with the red-shifted UV spectrum and yellow color. Their unique properties are useful in phase determination, nucleic acid-based detection as well as spectroscopic studies of nucleic acids and nucleic acid-protein complexes.

Keywords

selenium derivatization of nucleic acids; phase determination; color DNA; visualization

1 Introduction

Selenium element has been discovered in natural nucleic acids, such as tRNAs containing 2-selenouridine and 5-[(methylamino)methyl]-2-selenouridine [1]. The selenium functionality provides RNA with unique biochemical and biological functions owing to its unique steric and chemical properties. For example, the Se modification at the anticodon wobble position of tRNA is probably able to improve the translation accuracy and efficiency [2]. Furthermore, selenium is considered as an ideal anomalous scattering center in X-ray crystal diffraction due to its K edge (0.9795 Å). The Se anomalous signal for multiple-wavelength anomalous dispersion (MAD) and single-wavelength anomalous dispersion (SAD) has significantly facilitated crystallographic studies on biological macromolecules [3]. The successful seleno-methionine MAD technique and its application in structural determination of proteins have been developed for over 20 years [4]. Currently over two thirds of the novel protein structures are determined by the selenomethionine strategy and MAD phasing [5]. Similarly, in order to facilitate the crystal structure study, selenium atoms have also been stably introduced into nucleic acids. It has been found that the selenium modifications do not cause significant perturbations in structures and functions. Our laboratory has pioneered and developed the synthesis of the Se-derivatized nucleic acids (SeNA) for structure and function studies with great potentials [6–8]. The conventional strategy in nucleic acid phasing is the bromination or iodination, which are not very stable derivatizations [9]. This novel strategy with SeNA is rapidly revolutionizing the X-ray crystallography of nucleic acids and protein-nucleic acid complexes. Comparatively, Se-derivatization of nucleic acids

offers better stability and more diversity owing to the multiple oxygen atoms in different chemical environments. So far, many crystal structures, including DNAs, RNAs and nucleic acid-protein complexes have been successfully solved with the SeNA strategy, and their structure resolutions are high [10–16]. Besides the power in solving phase problem, the SeNA strategy has allowed the functional investigations, such as novel hydrogen bonding [17–20], nuclease resistance [21, 22] and crystallization facilitation [10, 15]. This single atom-specific substitution provides novel opportunities for nucleic acid functional studies. Moreover, by replacing single oxygen atom with selenium, the spectroscopic property of nucleic acid was successfully tailored [23]. For instance, we have managed to red-shift the UV absorption wavelength of DNA by over 100 nm, which is important for the DNA visualization, gene expression detection, and disease diagnosis.

In the past, we selectively replaced one oxygen atom with selenium in a single nucleotide. One single selenium atom is able to provide the phasing power for 30 nt. [8]. For the structure determination of longer oligonucleotides, more selenium atoms are necessary. Thus, instead of single selenium atom per one nucleotide unit, we decided to incorporate multiple Se atoms per one nucleotide unit. As the first step towards the multiple Se incorporation, we have synthesized the thymidine analog containing two selenium atoms. This multi-Se strategy will provide more selenium phasing power in structure determination. Moreover, as predicted, we have found that introducing the two selenium atoms into the nucleobase will generate further red-shift, which may have potential applications in spectroscopic investigation and DNA visualization.

2 Experimental section

2.1 Materials

Most reagent and chemicals were purchased from Sigma, Fluka or Aldrich. The THF, dichloromethane and acetonitrile solvents were distilled under argon. Before reaction, the solid starting materials were dried under high vacuum. All the reactions were performed under argon. Solvent mixtures are indicated as volume/volume ratios. Thin layer chromatography (TLC) was run on analytical Merck 60 F₂₅₄ plates (0.25 mm thick; *R_f* values in the text are for the title products) and visualized under UV-light. Flash column 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 ¹³C spectra were performed using Bruker-400 (400 MHz). Chemical shifts were in ppm relative to tetramethylsilane and coupling constants are in Hz. High resolution mass spectroms were performed at Georgia State University. The phosphoramidite used in solid-phase synthesis was from Glen Research.

2.2 Synthesis of 5-methylseleno-4-O-(2-nitrophenyl)-5'-O-(4,4-dimethoxytrityl)-thymidine (2)

After 5-methylseleno-5'-O-(4,4-dimethoxytrityl)-2'-deoxy uridine (624 mg, MW 624, 1 mmol) was dissolved in acetonitrile 10 mL, 1-methylpyrrolidine (0.95 mL, 10 mmol, 10 eq.) and trimethylsilyl chloride (3.1 mL, 3 mmol, 3 eq.) were added sequentially. The reaction mixture was stirred for 1 hour, and all the starting material was consumed by monitoring on TLC (5% methanol/dichloromethane) to get silylated intermediate. After completion, the reaction solution was directly transferred to vac-line to evacuate all the solvent and base. In this way, the further purification and the potential TMS group falling off were avoided.

After drying the synthesized intermediate for overnight, acetonitrile 10 mL was injected into reaction flask, before the diisopropylethyl amine (1.05 mL, 5 mmol, 5 eq.), triisopropylbenzene sulfonyl chloride (905 mg, 2.5 mmol, 2.5 eq.) and *p*-dimethylamino

pyridine (150 mg, 1 mmol, 1 eq.) were added sequentially. The reaction mixture was stirred for 16 hours and monitored on TLC (ethyl acetate/hexane, 3:7). Because the 4-TIPS-5-Se-thymidine is too unstable to decompose in silica gel column, we decided to do the one-pot reaction to synthesize 4-nitrophenyl substituted compound instead of isolating 4-TIPS substituted compound. Therefore, excessive 2-nitrophenol compound (1.37 g, 10 mmol, 10 eq.), triethylamine base (0.4 mL, 3 mmol, 3 eq.) and 1, 8-diazabicyclo[5.4.0]undec-7-ene (0.15 mL, 1 mmol, 1 eq.) were added drop-wise and the reaction was stirred under room temperature for 4 hours. After the reaction was completed (monitored on TLC, 50% ethyl acetate in dichloromethane, $R_f = 0.65$), the organic solvent was evaporated under reduced pressure. A silica gel column was equilibrated with CH_2Cl_2 before the crude product was loaded and purified on the column. The product was eluted by ethyl acetate in CH_2Cl_2 (10–50%). The fractions containing the product were combined, evaporated and dried on high vacuum overnight to yield a yellow solid **2** (588 mg, yield = 79%). ^1H NMR (400 MHz, DMSO) δ : 2.14 (s, 3H, SeCH_3), 2.24–2.28 and 2.65–2.66 (2x m, 2H, H-2'), 3.42–3.44 (2x m, 2H, H-5'), 3.81 (s, 6H, 2x OMe), 4.11–4.12 (m, 1H, H-3'), 4.12–4.13 (m, 1H, H-4'), 6.16–6.19 (t, $J = 5.6$ Hz, 1H, H-1'), 6.85–8.16 (m, 17H, arom-H), 8.40 (s, 1H, H-6). ^{13}C NMR (100 MHz, DMSO) δ : 8.94 (SeCH_3), 42.25 (C-2'), 55.32 (OMe), 63.49 (C-5'), 72.14 (C-3'), 87.74 (C-4'), 86.92 (C-1'), 113.34 (C-5), 125.52 (arom-C), 126.18 (arom-C), 127.02 (arom-C), 127.60 (arom-C), 128.10 (arom-C), 130.13 (arom-C), 135.27 (arom-C), 135.59 (arom-C), 145.46 (C-6), 149.13 (C-2), 154.83 (C-4). HR-MS (ESI-TOF, positive ion mode): molecular formula, $\text{C}_{37}\text{H}_{37}\text{N}_3\text{O}_9\text{Se}$: $[\text{M}+\text{Na}-2\text{H}]^+$: 768.1447 (calc. 768.1436).

2.3 Synthesis of 5-methylseleno-4-(2-cyanoethyl) seleno-5'-O-(4,4-dimethoxytrityl)-thymidine (3)

The NaBH_4 suspension (130 mg NaBH_4 in 4 mL of EtOH) was injected into a flask containing di(2-cyanoethyl) diselenide $[(\text{NCCH}_2\text{CH}_2\text{Se})_2]$, 0.35 mL, $d = 1.8 \text{ g mL}^{-1}$ and ethanol (8 mL) on an ice bath and under argon. After injection of the NaBH_4 suspension for 30 min, the ice bath was removed. The yellow color of the diselenide disappeared in approximately 15 min, giving an almost colorless solution of sodium selenide ($\text{NCCH}_2\text{CH}_2\text{SeNa}$). The solution of **2** (438 mg, 0.59 mmol) in THF (4 mL) was injected to the solution of sodium selenide. After the selenium incorporation was completed in 15 min, (monitored on TLC, 50% ethyl acetate in CH_2Cl_2 , product $R_f = 0.4$), water (100 mL) was added to the reaction flask. The solution was adjusted to pH 7–8 using CH_3COOH (10%), and was then extracted with ethyl acetate ($3 \times 100 \text{ mL}$). The organic phases were combined, washed with NaCl (sat., 100 mL), dried over MgSO_4 (s) for 30 min, and evaporated under reduced pressure. Deprotecting solution (5 mL, 10% triethylamine in methanol) was added to the crude product, and the reaction was stirred for 4 hours to completely remove the 3'-TMS group. After evaporating the deprotecting solution, the crude product was then dissolved in methylene chloride (5 mL) and purified on a silica gel column. The column was eluted with a gradient of ethyl acetate/methylene chloride (10–50%). After solvent evaporation and dry on high vacuum, the pure compound (**3**) was obtained as a light yellow foamy product (332 mg, 75% yield). ^1H NMR (400 MHz, DMSO) δ : 2.04 (s, 3H, SeCH_3), 2.30–2.31 and 2.95–2.97 (2x m, 2H, H-2'), 2.97–2.99 (t, 2H, $\text{SeCH}_2\text{CH}_2\text{CN}$), 3.25–3.28 (t, 2H, $\text{SeCH}_2\text{CH}_2\text{CN}$), 3.40–3.41 (2x m, 2H, H-5'), 3.80 (s, 6H, 2x OMe), 4.09–4.16 (m, 1H, H-3'), 4.51–4.52 (m, 1H, H-4'), 6.17–6.20 (t, $J = 6.4$ Hz, 1H, H-1'), 6.80–7.40 (m, 13H, arom-H), 8.19 (s, 1H, H-6). ^{13}C NMR (100 MHz, DMSO) δ : 10.69 (SeCH_3), 14.21 ($\text{SeCH}_2\text{CH}_2\text{CN}$), 22.11 ($\text{SeCH}_2\text{CH}_2\text{CN}$), 42.18 (C-2'), 55.28 (OMe), 63.24 (C-5'), 72.41 (C-3'), 86.85 (C-4'), 87.59 (C-1'), 113.32 (arom-C), 119.03 (C-5), 127.08 (arom-C), 128.03 (arom-C), 130.07 (arom-C), 135.38 (CN), 145.41 (C-6), 158.69 (C-2), 182.66 (C-4). HR-MS (ESI-TOF, negative ion mode): molecular formula, $\text{C}_{34}\text{H}_{37}\text{N}_3\text{O}_6\text{Se}_2$: $[\text{M}+\text{Na}-2\text{H}]^-$: 764.0764 (calc. 764.0754).

2.4 Synthesis of 3'-O-(2-cyanoethyl-N, N-diisopropyl-phosphoramidite)-4-(2-cyanoethyl)seleno-5-methylseleno-5'-O-(4,4-dimethoxytrityl)-thymidine (4)

4-cyanoethylselenenyl-5-methylseleno-5'-O-(4,4-dimethoxytrityl)-2'-deoxyuridine (3, 200 mg, MW 741.2, 0.27 mmol) was placed in a 25 mL round flask and dried on high vacuum. Dry CH_2Cl_2 3 mL, *N, N*-diisopropylethylamine 0.06 mL (0.4 mmol, 1.5 eq.) and 2-cyanoethyl-*N, N*-diisopropyl chlorophosphoramidite 80 mg (0.4 mmol, 1.5 eq.) were then added sequentially. The reaction mixture was stirred at room temperature for 2 h, and the reaction completion was indicated by TLC (4% MeOH/ CH_2Cl_2 , $R_f = 0.3$ and 0.32, two diastereomers). The reaction was then quenched with NaHCO_3 (sat.) and extracted with CH_2Cl_2 three times, and dried over anhydrous MgSO_4 , followed by filtration and solvent evaporation. The crude product was re-dissolved in CH_2Cl_2 (3 mL) and this solution was added dropwise to pentane (200 mL) under vigorous stirring to yield yellow precipitate. The pentane solution was decanted carefully (sometimes filtration was necessary) and the crude product was loaded into a silica gel column that was equilibrated with CH_2Cl_2 containing 1% triethylamine. The product was eluted with an increasing stepwise gradient of MeOH/ CH_2Cl_2 in the presence of 1% triethylamine (1–5%). The fractions containing target compound were combined evaporated under reduced pressure to afford a yellow solid (216 mg, yield = 85%).

2.5 Synthesis and purification of Se-functionalized DNA oligonucleotides

All the DNA oligonucleotides were chemically synthesized in a 1.0 μmol scale using an ABI392 or ABI3400 DNA/RNA Synthesizer. The ultramild nucleoside phosphoramidite reagents were used in this work (Glen Research). The concentration of the double-Se-T phosphoramidite was identical to that of the conventional ones (0.1 M in acetonitrile). Coupling was carried out using a 5-(benzyl-mercaptop)-1H-tetrazole (5-BTT) solution (0.25 M) in acetonitrile. The coupling time was 25 seconds for both native and modified samples. 3% trichloroacetic acid in methylene chloride was used for the 5'-detritylation. Synthesis were performed on control pore glass (CPG-500) immobilized with the appropriate nucleoside through a succinate linker. All the oligonucleotides were prepared with DMTr-on form. After synthesis, the DNA oligonucleotides were cleaved from the solid support and fully deprotected by the treatment of 0.05 M K_2CO_3 solution in methanol for 12 h at room temperature. The 5'-DMTr deprotection was performed in a 3% trichloroacetic acid solution for 2 min, followed by neutralization to pH 7.0 with a freshly made aqueous solution of triethylamine (1.1 M) and extraction by petroleum ether to remove the by-product DMTr-OH. The DMTr-off oligonucleotides were purified again by HPLC. The typical HPLC profiles and MS analysis of the Se-oligonucleotide are shown in Supporting Information.

2.6 UV-melting temperature experiments

The experiments were performed using the samples (2 μM DNA duplexes) dissolved in the buffer of 50 mM NaCl, 10 mM $\text{NaH}_2\text{PO}_4\text{-Na}_2\text{HPO}_4$ (pH 6.5), 0.1 mM EDTA and 10 mM MgCl_2 . The samples were heated to 60 $^\circ\text{C}$ and allowed to cool down to room temperature slowly. These experiments were carried out by Cary 300 UV-Visible Spectrophotometer with a temperature controller at a heating rate of 0.5 $^\circ\text{C min}^{-1}$. Typical melting curves are shown in Supporting Information.

3 Results and discussion

The synthesis (Scheme 1) of the thymidine analogue containing two selenium atoms started from the 5'-DMTr-5-methylselenenyl-deoxyuridine (**1**) [24]. To avoid the possible competition between 3'-hydroxy and 4-keto group, the protection of 3'-OH was performed. Considering the fact that the introduced 4-Se functionality is sensitive to strong acid or base treatment, this 3'-protection group needs to be removed under mild conditions. Therefore, we selected

the trimethylsilyl protecting group, which is removed during the silica gel flash column chromatography. To introduce the selenium functionality into 4-position, the 3'-silylated intermediate was first activated by 2, 4, 6-triisopropylbenzenesulfonyl (TIPS) group. Similar to the synthetic strategy for 5-Se-dC [14] and 4-Se-T [17], the isolation of this TIPS-activated thymidine analogue was attempted. Unfortunately, the column chromatography purification did not work due to the instability of the intermediate. Finally, we decided to replace this TIPS group with 2-nitrophenyl group, which could improve the nucleoside stability significantly. After the silylation, compound **1** was directly converted to **2**. After isolating the 5'-DMTr-5-methylselenyl-4-(2'-nitrophenyl)-deoxyuridine (**2**), we did the substitution reaction using cyanoethylselenide to achieve **3**. In this way, the seleno-functionality was introduced with the protection group, avoiding the potential oxidation, which may lead to deselenization. The next step was the conversion of **3** to the corresponding phosphoramidite (**4**) in high yield. In this step, the base (diisopropylethylamine) was used to facilitate the reaction by neutralizing the formed acid. This phosphoramidite (**4**) serves as a building block for DNA synthesis. Double-selenium modified thymidine phosphoramidite **4** was found compatible with the solid-phase synthesis, including capping reaction, I₂ oxidation, and acid treatments. Stability of the Se-moieties allowed us to successfully synthesize the Se-oligonucleotides using the ultramild protecting groups.

To demonstrate the stability of these two Se-functionalities, we studied the deprotection treatment of this di-Se-T under the base deprotection conditions (50 mM K₂CO₃/MeOH solution). Compound **3** was used for the test. **3** dissolved in methanol shows the maximum absorbance at 305 nm in UV (Figure 1). When **3** was dissolved in 50 mM K₂CO₃/MeOH solution, the 4-Se-protecting group was removed gradually, and the reaction was completed in 3 hours, indicated by the disappearance of the 305 nm peak. Under the basic condition, the NH at 3-position was deprotonated to generate intermediate **3a**, which has maximum absorbance at 360 nm. After neutralization of the basic condition, **3a** was protonated to generate **3b**. UV maximum absorbance of **3b** is 390 nm. The di-Se functionalized thymidine is stable under both the deprotecting condition and aqueous solution.

According to our previous work, the 4-Se-thymidine generates the UV red-shift and yellow color [23]. It is a promising strategy for the nucleic acid-based detection. We have hypothesized that due to the electron-donating effect, introduction of two Se-atoms into the same nucleobase will further red-shift the UV absorption. Our hypothesis was confirmed by our experiment. We still applied compound **3** for the study. After the full deprotection and neutralization, the maximum UV absorption of the di-Se-thymidine is 390 nm (Figure 1), red-shifted by approximately 30 nm compared with 4-Se-thymidine [23]. This phenomenon also confirms the double-selenium modification.

In the solid-phase synthesis, 5-BTT was applied as the coupling reagent, and ultramild phosphoramidites were used [16, 17, 20]. The synthetic DNAs were cleaved off the solid support and fully deprotected by K₂CO₃/methanol solution at room temperature overnight. The Se-oligonucleotides are yellow and were then purified with DMTr-on and DMTr-off twice by reversed-phase HPLC. For the analytical HPLC, the di-Se-T-DNAs (^{Se}T-DNAs) were monitored under both 260 and 390 nm. The typical HPLC profiles of the synthesized di-Se-T-oligonucleotides are shown in Figure 2 and S8 in the Supporting Information. The MS characterization is listed in Table 1.

The UV-melting experiment was then carried out to compare the thermostability of the ^{Se}T-DNA duplexes with the corresponding native ones. After dissolving the DNA samples in the melting buffers (50 mM NaCl, 10 mM NaH₂PO₄-Na₂HPO₄, 10 mM MgCl₂, 0.1 mM EDTA, pH 6.8; the final duplex concentration of the Se-DNA and complementary native DNA: 1

μM), we heated the solution to 60 °C and allowed it to cool to room temperature slowly. After keeping the DNA samples at 4 °C overnight, the UV melting measurements were performed using Cary 300 UV-Visible spectrophotometer with a temperature controller at a heating rate of 0.5 °C min⁻¹. We successfully determined the melting temperatures of two oligonucleotides. The original profiles are provided in the Supporting Information (Figure S13 and S14), and results are listed in Table 1. It demonstrated that the ^{Se}T-DNA duplexes had almost identical thermostability as the native ones, indicating that these Se-functionalities caused no obvious structural perturbation. Since the melting temperatures (*T_m*) of the native and Se-modified sequences (1–4) are very similar, suggesting the Se-moiety does not cause the significant perturbation. Thus, *T_m* of sequence 5 and 6 was not measured. These incorporated Se-functionalities in thymidine may contribute significantly to the base stacking interaction, which helps stabilizing the Se-DNA duplexes.

We also determined the extinction coefficient of the ^{Se}T-moiety. For this purpose, the oligonucleotide trimer (T^{Se}TT) was synthesized and purified; its UV spectrum is presented in Figure 3. On the basis of the native thymidine ($\epsilon_{260-N} = 7.9 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$) and Eq. (1) and (2), we calculated the extinction coefficient of the ^{Se}T nucleobase at 390 nm (ϵ_{390-Se}) as $6.2 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$.

$$A_{260} = \epsilon_{260-N} \times c \times d \quad (1)$$

$$A_{390} = \epsilon_{390-Se} \times c \times d \quad (2)$$

4 Conclusion

In conclusion, we have synthesized the novel Se-nucleoside containing two Se atoms in thymine nucleobase (^{Se}T) and corresponding Se-phosphoramidite. These di-Se-functionalities have been efficiently incorporated into DNAs through solid-phase synthesis. Our experimental results indicate that the ^{Se}T-functionality is relatively stable and causes no obvious change on the DNA stability. Moreover, the ^{Se}T-DNA duplex has almost the same stability and structure as the native. Furthermore, these two selenium atoms in the same nucleobase can generate a significant UV red-shift (to 390 nm) and make DNA colored (yellow). The extinction coefficient of this ^{Se}T-nucleotide was determined as $6.2 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$. This Se-double modification will offer strong phasing power in the structure determination of nucleic acids and protein-nucleic acid complexes. The ^{Se}T-DNAs with the unique color and spectroscopic properties will have great potentials in DNA detection, nucleic acid visualization, gene expression profiling, and investigation of protein-nucleic acid complexes.

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Figure 1. Deprotection, UV absorption and stability of the di-Se-functionalized thymidine.

Figure 2.

Reversed-phase HPLC analysis of DMTr-off Se-DNA (5'-CTCCCG^{Se}TCC-3'). The HPLC analysis was performed on a Zorbax SB-C18 column (4.6 × 250 mm) with a linear gradient from buffer A (20 mM triethylammonium acetate, pH 7.1) to 40% buffer B (50% acetonitrile, 20 mM triethylammonium acetate, pH 7.1) in 15 min; the Se-DNA retention time was 11.3 min. Black line: monitored at 260 nm; red line: monitored at 390 nm.

Figure 3.
UV spectrum of TSeTT.

Scheme 1.

Reaction scheme of synthesis of 4,5-seleno-derivatized thymidine DNA. a) TMS-Cl, 1-methylpyrrolidine, acetonitrile; b) TIPS-Cl, DIEA, DMAP, acetonitrile; c) 2-nitrophenol, TEA, DBU, acetonitrile; d) di-2-cyanoethyl diselenide, NaBH₄, ethanol, THF; e) 2-cyanoethyl *N,N*-diisopropyl-chlorophosphoramidite, DIEA, CH₂Cl₂; f) solid-phase synthesis.

Table 1MS data and UV-melting temperatures of the 4,5-di-Se-T-DNAs (^{Se}T-DNAs)

Entry	Oligonucleotide	measured (calcd.) <i>m/z</i>	<i>T_m</i> (±1.0 °C)
1	5'-ATGG ^{Se} TGCTC-3' C ₈₈ H ₁₀₄ N ₃₂ O ₅₃ P ₈ Se ₂	[M+H] ⁺ : 2873.2 (2872.8)	39.9 ^{a)}
2	5'-ATGGTGCTC-3'		40.3 ^{a)}
3	5'-CTTCT ^{Se} TGTC CG-3' C ₁₀₆ H ₁₂₈ N ₃₂ O ₆₈ P ₁₀ Se ₂	[M+H] ⁺ : 3417.3 (3417.2)	42.9 ^{a)}
4	5'-CTTCTTGTC CG-3'		44.9 ^{a)}
5	5'-CTCCCA ^{Se} TCC-3' C ₈₄ H ₁₀₃ N ₂₇ O ₅₂ P ₈ Se ₂	[M+H] ⁺ : 2738.3 (2737.7)	
6	5'-GdU ₂ ^{Se} G ^{Se} TACAC-3' C ₇₈ H ₉₉ N ₃₀ O ₄₅ P ₇ Se ₃	[M+H] ⁺ : 2632.1 (2631.6)	

^{a)} The *T_m* was measured in the presence of its complementary sequence.