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First Synthesis of Fully Modified 4′-SelenoRNA and 2′-OMe-4′-selenoRNA Based on the Mechanistic Considerations of an Unexpected Strand Break

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Supporting Information

ABSTRACT: This study investigated oligonucleotide (ON) synthesis containing 4′-selenoribonucleoside(s) under standard phosphoramidite conditions. Careful operation using a manual ON synthetic system revealed that an unexpected strand break occurred to afford a C2-symmetric homodimer as a byproduct. In addition, this side reaction occurred during I_2 oxidation. On the basis of these findings, the first synthesis of fully modified 4′-selenoRNA and 2′-OMe-4′-selenoRNA was achieved using *tert*-butyl hydroperoxide (TBHP) as the alternative oxidant.

Development of the phosphoramidite method has made the synthesis of a huge number of oligonucleotides (ONs) possible, including chemically modified ONs.[1] Although slight modifications of the reaction conditions are sometimes required if the growing ON is labile,[2] this synthetic technology is fundamental for usual ON synthesis.[3]

We have been intensely studying a series of 4′-thionucleic acids in order to develop chemically modified ONs for application in medicinal chemistry.[4] Although oxidation of 4′-sulfur atom(s) into the corresponding sulfoxide(s) is a possible problem under standard oxidation conditions during ON synthesis, it has not been observed, and desired ONs have been obtained in good yields. Accordingly, we have prepared various 4′-thionucleic acid derivatives which exhibit biological activities.[5] As part of our continuous research project, we have explored the synthesis of 4′-selenonucleic acids,[6] which have selenium atoms on the 4′-position instead of sulfur atoms since these nucleic acid analogs can act as bioisosteres of the promising 4′-thionucleic acids.[7] Thus far, we and others have synthesized 4′-selenoribonucleosides, components of 4′-selenoRNA.[8,9] However, only two studies for the synthesis of ONs containing 4′-selenoribonucleosides have been reported.[6,10] As common results in these two studies, incorporation of only one 4′-selenoribonucleoside unit into the ONs under the standard phosphoramidite conditions can be achieved in very low yields, while that of more than two units was unsuccessful. Although a certain strand break has been suggested as a reason for the poor yields,[10] no obvious answers have been given so far. Since ONs containing only one 4′-selenoribonucleoside show a good ability for duplex formation with RNA,[10] development of a practical synthetic method for ONs containing 4′-selenoribonucleosides, namely fully and/or partially modified 4′-selenoRNA, will make several applications in nucleic acid based therapeutics and biotechnologies possible.

In this study, we carefully investigated ON synthesis containing 4′-selenonucleoside(s) under standard phosphoramidite conditions, which resulted in very poor yields. We found that an unusual strand break occurred during oxidation with I_2. On the basis of this finding, we succeeded in the first synthesis of a fully modified 4′-selenoRNA consisting of 4′-selenouridine (4′-selenoU) units and one consisting of 2′-OMe-4′-selenouridine (2′-OMe-4′-selenoU) units using *tert*-butyl hydroperoxide (TBHP) as the alternative oxidant.

We first prepared 2′-OMe-4′-selenoU phosphoramidite unit **1** and corresponding CPG resin **2** as a substrate for this investigation to avoid the tedious protection of the 2′-hydroxyl group with a silyl group and its deprotection after ON synthesis (Figure 1).[11] Prior to ON synthesis, we examined the stability of the 2′-OMe-4′-selenoU unit. Thus, CPG resin **2** was subjected to standard phosphoramidite conditions (3% TCA in CH_2Cl_2, 110 s; 0.45 M 1*H*-tetrazole in CH_3CN, 10 min; Ac_2O in THF/pyridine and 1-methylimidazole in THF, 18 s; and 0.02 M I_2 in THF/H_2O/pyridine, 40 s) without coupling to another phosphoramidite unit. After subjecting **2** to these conditions six times, it was treated with ammonium hydroxide to detach from the CPG resin. Then an aliquot of the reaction solution was analyzed by using HPLC. Only the peak corresponding to 2′-OMe-4′-selenoU was observed (Figure S1, Supporting Information), meaning that the nucleoside unit

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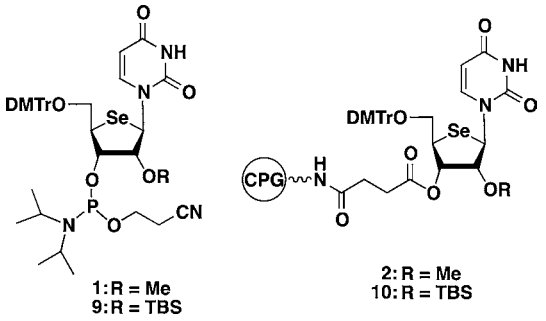

Figure 1. Structures of 4′-selenoribonucleoside derivatives prepared in this study.

was stable. These results strongly suggested that the low yield of ON synthesis containing 4′-selenoribonucleosides was due to a strand-break during the formation of a phosphite(III) and/or phosphate(V) linkage in the growing ON.

To clarify which conditions induced the strand break, manual ON synthesis using **1** and **2** was performed in a reservoir tube, but not using a DNA/RNA synthesizer.[12] Starting with the CPG resin **2** in the reservoir tube, the resin was first treated with 3% TCA in CH_2Cl_2. Then phosphoramidite **1** (0.2 M in CH_3CN) and the coupling reagent (0.45 M 1*H*-tetrazole in CH_3CN) were added to the reservoir tube, and the mixture was allowed to stand for 1 h. After the capping procedure, the CPG resin was treated with 0.02 M I_2 in THF/H_2O/pyridine to give a CPG resin-supported dimer. Repeating these procedures once and twice more afforded a CPG resin-supported trimer and tetramer, respectively. After treatment with ammonium hydroxide, the resulting solutions were analyzed by using HPLC. As a result, insufficient yields of the dimer, trimer, and tetramer synthesis consisting of 2′-OMe-4′-selenoU units (DMTrO-UU−OH, DMTrO-UUU−OH, and DMTrO-UUUU−OH, respectively; U = 2′-OMe-4′-selenoU) were suggested (Figure 2A, each vertical axis was scaled up to 600% for comparison). In addition, their HPLC profiles were rather complicated, and multiple peaks were observed between 10 to 15 min in the HPLC profile when the coupling reactions were repeated. Since the formation of byproducts, to which the multiple peaks correspond, was thought to be one of problems in 4′-selenoON synthesis, we planed to determine the structure of the byproduct (marked with asterisk) at 14.5 min for the dimer synthesis. In the HRMS of this byproduct, a peak was observed at m/z = 611.0146 (MH^+), which corresponds to a chemical formula of $C_{20}H_{26}N_4O_8Se_2$ (Figure S3, Supporting Information). This indicates that the byproduct in the dimer synthesis has already lost a phosphorus atom despite having two selenium atoms. To better understand the structure, the CPG resin after dimer synthesis was treated with 3% TCA in CH_2Cl_2, and the resulting products were analyzed by using HPLC. As a result, the peak corresponding to DMTrO-UU−OH disappeared, and a new peak for HO-UU−OH appeared at 8.5 min in the HPLC profile (Figure S4, Supporting Information). On the other hand, the peak for the byproduct at 14.5 min remained even after treatment with TCA. Therefore, we concluded that the byproduct was no longer the tritylated product. To determine the structure of the byproduct in detail, we isolated it by using HPLC and analyzed it by using ^{1}H NMR spectroscopy (Figure 3). As presumed, no signals corresponding to a DMTr group were observed. In addition, unexpected vinyl protons (marked with arrows) were observed in the spectrum (full spectrum is shown in Figure S5,

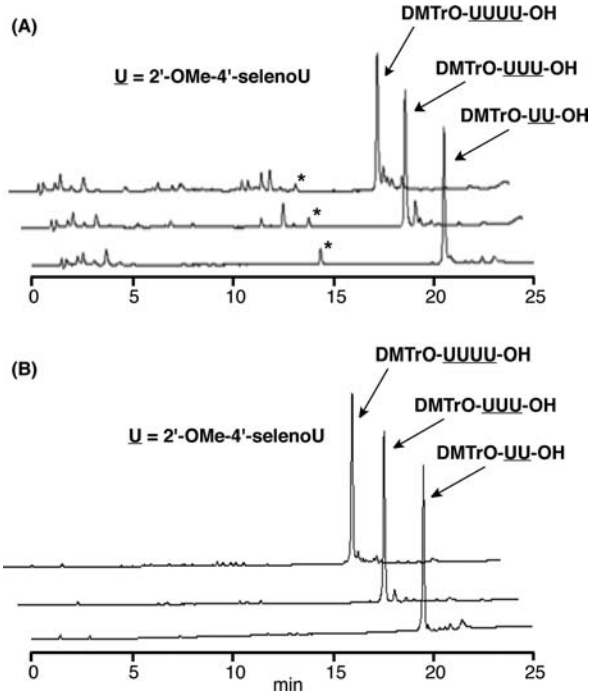

Figure 2. HPLC profiles for dimer, trimer, and tetramer synthesis consisting of 2′-OMe-4′-selenoU (U) using (A) 0.02 M I_2 in THF/H_2O/pyridine (B) 1.0 M TBHP in toluene as an oxidant. The arrows indicate the desired sequences. After treatment with ammonium hydroxide, each sample was analyzed by using HPLC (conditions were described in the Supporting Information).

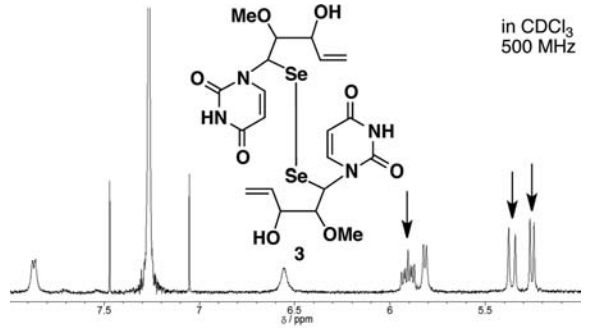

Figure 3. Partial ^{1}H NMR spectrum and structure of the byproduct **3**.

Supporting Information). Because of the rather simple ^{1}H NMR spectrum despite the higher molecular weight, the byproduct appears to be a C2-symmetric homodimer. The structure of byproduct was determined to be compound **3** (Figure 3), which was also supported by MS/MS analysis (Figure S6, Supporting Information).

To determine under which reaction conditions homodimer **3** formed, the CPG resin after dimer synthesis (in this case, the DMTr group had already been removed) was exposed again to (A) 3% TCA in CH_2Cl_2, (B) 0.45 M 1*H*-tetrazole in CH_3CN, and (C) 0.02 M I_2 in THF/H_2O/pyridine for 1 h, respectively. As a result, there were no obvious changes in any of the HPLC profiles compared with that of the CPG resin-supported dimer treated with ammonium hydroxide without any other exposure (Figure S7, Supporting Information). These results indicate that the internucleotide phosphate(V) linkage is quite stable under the standard phosphoramidite conditions once it has formed. In other words, this means that the strand break occurs

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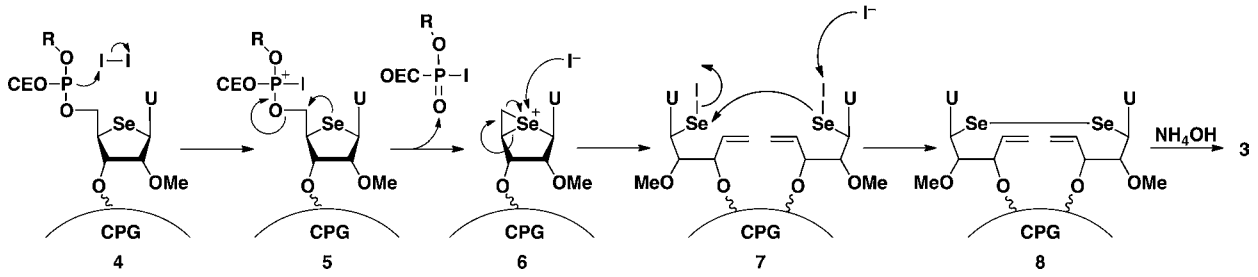

Figure 4. Proposed mechanism of the byproduct **3** formation during I_2 oxidation.

during I_2 oxidation of an internucleotide phosphite(III) linkage formed right after the coupling with another phosphoramidite unit. In Figure 4, a proposed mechanism of the homodimer **3** formation was illustrated. Thus, the internucleotide phosphite-(III) linkage of **4** reacts with I_2 as an initial oxidation step to give phosphonium intermediate **5**. This intermediate is usually hydrolyzed to give the internucleotide phosphate(V) linkage. In the case of 4′-selenoON synthesis, however, nucleophilic attack of the selenium atom on the 5′-position can occur to afford seleniranium intermediate **6** because of high nucleophilicity of the selenium atom and good leaving ability of the phosphonium skeleton. Then intermediate **6** spontaneously cleaves to produce selenyl iodide **7**. Because of this strand break, the byproduct has a vinyl group and no DMTr group in its structure. Since the resulting nucleoside unit is loaded on a CPG resin, some of nucleoside unit forms homodimer **8** with a neighboring nucleoside unit via Se−Se bond formation on the CPG resin.[13] Subsequent treatment with ammonium hydroxide to detach from the CPG resin afforded **3**. This proposed mechanism is strongly supported by a report from Goto and co-workers, in which they show that an iodide ion promotes deselenylative alkene formation from *β*-chloro- and *β*-oxy-selenides via a seleniranium intermediate.[14] On the basis of these considerations, the structures corresponding to the other peaks appearing between 10 and 15 min in the HPLC profile of the trimer and tetramer synthesis (Figure 2A) were all determined (Figure S8, Supporting Information).

As described above, oxidation using I_2 induced an unexpected strand break in the growing ON, which resulted in an insufficient yield of the desired ON. Thus, we examined alternative oxidation conditions, such as the use of 1*S*-(+)-(10-camphorsulfonyl)oxaziridine (CSO)[15,16] and TBHP.[16−18] To fix the apporopriate oxidation conditions, the stability of 4′-selenonucleoside unit against these oxidants was examined. A solution of 0.5 M CSO in CH_3CN or 1.0 M TBHP in toluene was added to CPG resin **2** in a reservoir tube, and the mixture was allowed to stand for 1, 5, 15, and 30 min. As a result, a new peak was observed at 2.5 min in the HPLC profile within 1 min of treatment with CSO, and the peak for 2′-OMe-4′-selenoU, which appeared at 12.5 min, was almost completely converted into the new peak within 15 min of treatment (Figure S9A, Supporting Information). Since this new peak was thought to be the corresponding 4′-selenoxide derivative, the resin was treated with dithiothreitol.[2b] As expected, the new peak at 2.5 min disappeared and was completely converted into that for a initial one. On the other hand, oxidation with TBHP seemed to be milder than that with CSO (Figure S9B, Supporting Information). Accordingly, the synthesis of a dimer, trimer, and tetramer consisting of 2′-OMe-4′-selenoU unit was examined again according to the general manual synthetic method, except for the oxidation conditions. To our delight, only the peaks corresponding to the desired ONs were observed in the HPLC profiles (Figure 2B), unlike those for the ON synthesis using I_2 (Figure 2A).

We next examined the synthesis of fully modified 4′-seleoRNA with phosphoramidite unit **9** and CPG resin **10** (Figure 1)[11] using a DNA/RNA synthesizer. Prior to ON synthesis, CPG resin **10** was subjected to the modified phosphoramidite conditions (3% TCA in CH_2Cl_2, 40 s; 0.25 M 5-benzylthio-1*H*-tetrazole in CH_3CN, 60 s; Ac_2O in THF/pyridine and 1-methylimidazole in THF, 60 s; and 1.0 M THBP in toluene, 50 s) without coupling to another phosphoramidite unit. After repeating the above treatment 12 times, CPG resin **10** was treated with ammonium hydroxide. Then the 2′-silyl group was removed by treatment with 3HF·Et_3N in DMSO at 65 °C, and an aliquot of the reaction mixture was analyzed by using HPLC. As a result, it was shown that the 4′-selenium atom was tolerated under the oxidation conditions (Figure S10, Supporting Information). Since the conditions for ON synthesis have been optimized, we attempted to synthesize fully modified 4′-selenoRNA consisting of 4′-selenoU (12 mer) (see the Supporting Information for experimental details). The coupling yields of each cycle was estimated to be >99% on the basis of trityl cation assays, which was much higher than the results reported by Damha and co-workers (10%−40%).[10] After completion of the synthesis, the resulting ON was detached from the CPG resin, and the 2′-silyl groups were removed to give 5′-DMTr-ON products (Figure S11A, Supporting Information). Finally, we removed the 5′-DMTr group to give the desired 4′-selenoRNA (Figure S11B, Supporting Information). From the HPLC analyses of the resulting products, whose profiles were as clean as that of natural RNA (12 mer) consisting of uridine units prepared under the same conditions, no byproducts formed (Figures S11C and D, Supporting Information). Similarly, we also prepared a fully modified 2′-OMe-4′-selenoRNA (8 mer) (Figure S12, Supporting Information). To the best of our knowledge, these syntheses are the first examples of fully modified 4′-selenoRNA and 2′-OMe-4′-selenoRNA.

In conclusion, we developed a pratical synthetic method for ON synthesis containing 4′-selenonucleosides. Careful operation of a manual ON synthetic system and mechanistic considerations revealed that a C2-symmetric homodimer byproduct formed due to a strand break during oxidation with I_2. By changing the oxidation conditions (1.0 M TBHP in toluene), we succeeded in the first synthesis of fully modified 4′-selenoRNA and 2′-OMe-4′-RNA in good yields. We believe that the results presented in this paper should open a new gate for nucleic acid derivative.

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■ ASSOCIATED CONTENT

Supporting Information

Experimental procedure for the synthesis of **1**, **2**, **9**, and **10**, mass analysis of ONs, two schemes, and 12 figures as described in the text. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) For examples, see: (a) Verma, S.; Eckstein, F. *Annu. Rev. Biochem.* **1998**, *67*, 99−134. (b) Hocek, M.; Fojta, M. *Chem. Soc. Rev.* **2011**, *40*, 5802−5814.

(2) For examples, see: (a) Kohli, V.; Wintzerith, M.; Sauerwald, R.; Staub, A.; Lecocq, J. P. *Nucleic Acids Res.* **1982**, *10*, 7439−7448. (b) Hobartner, C.; Rieder, R.; Kreutz, C.; Puffer, B.; Lang, K.; Polonskaia, A.; Serganov, A.; Micura, R. *J. Am. Chem. Soc.* **2005**, *127*, 12035−12045.

(3) Beaucage, S. L.; Iyer, R. *Tetrahedron* **1992**, *48*, 2223−2311.

(4) (a) Hoshika, S.; Minakawa, N.; Matsuda, A. *Nucleic Acids Res.* **2004**, *32*, 3815−3825. (b) Inoue, N.; Minakawa, N.; Matsuda, A. *Nucleic Acids Res.* **2006**, *34*, 3476−3483. (c) Takahashi, M.; Minakawa, N.; Matsuda, A. *Nucleic Acids Res.* **2009**, *37*, 1353−1362.

(5) (a) Inoue, N.; Shionoya, A.; Minakawa, N.; Kawakami, A.; Ogawa, N.; Matsuda, A. *J. Am. Chem. Soc.* **2007**, *129*, 15424−15425. (b) Takahashi, M.; Nagai, C.; Hatakeyama, H.; Minakawa, N.; Harashima, H.; Matsuda, A. *Nucleic Acids Res.* **2012**, *40*, 5787−5793. (c) Kikuchi, Y.; Yamazaki, N.; Tarashima, N.; Furukawa, K.; Takiguchi, Y.; Itoh, K.; Minakawa, N. *Bioorg. Med. Chem.* **2013**, *21*, 5292−5296. (d) Takahashi, M.; Yamada, N.; Hatakeyama, H.; Murata, M.; Sato, Y.; Minakawa, N.; Harashima, H.; Matsuda, A. *Nucleic Acids Res.* **2013**, *41*, 10659−10667.

(6) Inagaki, Y.; Minakawa, N.; Matsuda, A. *Nucleic Acids Symp. Ser.* **2008**, *52*, 329−330.

(7) For example, see: (a) Johnston, B. D.; Ghavami, A.; Jensen, M. T.; Svensson, B.; Pinto, B. *J. Am. Chem. Soc.* **2002**, *124*, 8245−8250. (b) Wilds, C. J.; Pattanayek, R.; Pan, C.; Wawrzak, Z.; Egli, M. *J. Am. Chem. Soc.* **2002**, *124*, 14910−14916. (c) Lin, L.; Sheng, J.; Huang, Z. *Chem. Soc. Rev.* **2011**, *40*, 4591−4602. (d) Abdur, R.; Gerlits, O. O.; Gan, J.; Jiang, J.; Salon, J.; Kovalevsky, A. Y.; Chumanevich, A. A.; Weber, I. T.; Huang, Z. *Acta Crystallogr., Sect. D* **2014**, *D70*, 354−361.

(8) Taniike, H.; Inagaki, Y.; Matsuda, A.; Minakawa, N. *Tetrahedron* **2011**, *67*, 7977−7982.

(9) (a) Jeong, L. S.; Tosh, D. K.; Kim, H. O.; Wang, T.; Hou, X.; Yun, H. S.; Kwon, Y.; Lee, S. K.; Choi, J.; Zhao, L. X. *Org. Lett.* **2008**, *10*, 209−212. (b) J Yu, J.; Kim, J.-H.; Lee, H. W.; Alexander, V.; Ahn, H.-C.; Choi, W. J.; Choi, J.; Jeong, L. S. *Chem.—Eur. J.* **2013**, *19*, 5528−5532.

(10) Watts, J. K.; Johnston, B. D.; Jayakanthan, K.; Wahba, A. S.; Pinto, B. M.; Damha, M. J. *J. Am. Chem. Soc.* **2008**, *130*, 8578−8579.

(11) Experimental details for compounds **1**, **2**, **9**, and **10** are given in the Supporting Information (Schemes S1 and S2).

(12) Experimental details are given in the Supporting Information (Figure S2).

(13) Similar dimer formation between on-column substrates was observed in our previous study, see; Minakawa, N.; Ono, Y.; Matsuda, A. *J. Am. Chem. Soc.* **2003**, *125*, 11545−11552.

(14) Sase, S.; Ebisawa, K.; Goto, K. *Chem. Lett.* **2012**, *41*, 766−768.

(15) Dellinger, D. J.; Sheehan, D. M.; Christensen, N. K.; Lindberg, J. G.; Caruthers, M. H. *J. Am. Chem. Soc.* **2003**, *125*, 940−950.

(16) Prakash, T. P.; Johnston, J. F.; Graham, M. K.; Condon, T. P.; Manoharan, M. *Nucleic Acids Res.* **2004**, *32*, 828−833.

(17) Scaringe, S. A. *Methods* **2001**, *23*, 206−217.

(18) Hayakawa, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1986**, *27*, 4191−4194.