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Preparation of 2′-Alkylselenouridine Derivatives via a 2-(Trimethylsilyl)ethylselenation Approach

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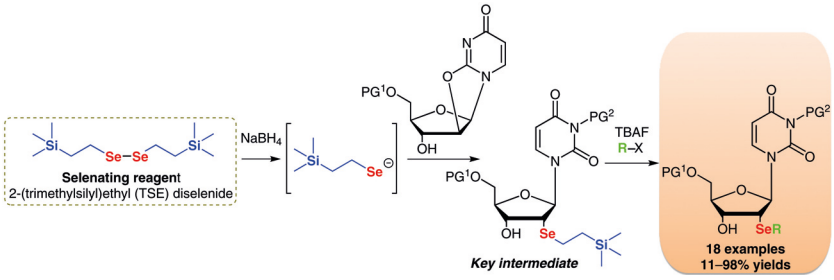

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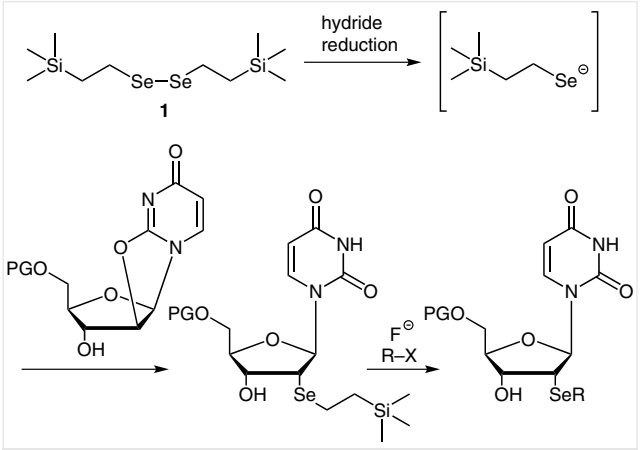
Abstract 2′-*O*-Methylation of nucleotides is well-known to increase siRNA stability against nuclease activities. Recently, selenium-containing biomolecules have been recognized as unique biological and medicinal agents for humans. In this study, 2′-alkylselenouridine derivatives were prepared through 2-(trimethylsilyl)ethylselenation at the C2′ position of 5′-DMT-2,2′-*O*-cyclouridine, followed by alkylation with various haloalkanes utilizing the characteristics of a Si atom. Overall, we demonstrated the versatility of a 2-(trimethylsilyl)ethylselenyl group for the synthesis of 2′-alkylselenouridines.

Key words selenium-containing biomolecule, 2-(trimethylsilyl)ethylselenyl group, selenation, 2′-alkylselenouridine derivative, phosphoramidite monomer

Small interfering RNAs (siRNAs) play an important role in the cellular RNA interference (RNAi) pathway to regulate gene expression.[1,2] RNA is unstable compared to DNA, because 2′-OH group in RNA promotes RNA hydrolysis under acidic and basic conditions.[3] Although this 2′-OH group in the pentose sugar is not very necessary for siRNAs activity,[4] the C2′ position holds the possibilities to strengthen nuclease resistance and to enhance duplex stability. Improvement of siRNAs stability is a primary factor direction for the therapeutic advantages.[5] Chemical modifications at the C2′ position have been intensively investigated;[6–10] especially, 2′-*O*-methylation stabilized siRNAs in serum with maintenance of RNA interference potency.[11]

The element selenium is essential in the nutrition of humans.[12,13] The glutathione peroxidase (GPx) having Se in its catalytic center[14] reduces hydroperoxides and participates in the antioxidant protection of cells.[15] In recent years, selenium-containing biomolecules have been documented as promising pharmacological agents.[16] Minor changes in molecular structures can cause extensive changes in biological activity. The replacement of an oxygen atom by homologous sulfur and selenium atoms affects the chemical properties and often leads to useful alterations of its efficacy. Within the scope of our ongoing program aimed at the synthetic study of selenium-containing nucleosides,[17,18] we herein describe the strategy for the synthesis of 2′-alkylselenouridine derivatives.

For alkylselenation, most of previous reports employed alkyl diselenides (R–Se–Se–R).[19–21] However, in this methodology, alkyl diselenides must be prepared with respect to each alkyl functional group. Depending on the functionalities, it is difficult to prepare respective alkyl diselenides. We therefore planned to use 2-(trimethylsilyl)ethyl (TSE) diselenide (TSE–Se–Se–TSE, **1**) as a selenating reagent which has two latent sites of reactivity. Our strategy is outlined in Scheme 1. The initial step commences with in situ generation of a TSE selenolate anion (TSE–Se$^-$) from TSE diselenide (**1**) by hydride reduction. This anion is expected



Scheme 1 Synthetic strategy for the synthesis of 2′-alkylselenouridine derivatives

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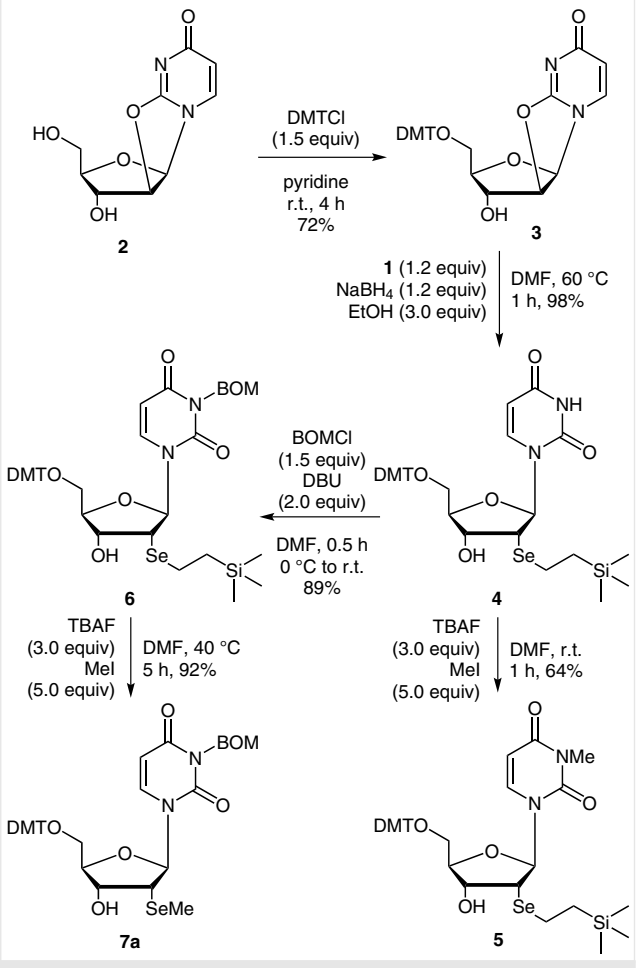
to attack to the C2′ position of 2,2′-*O*-cyclouridine, resulting in introduction of TSE selenyl moiety onto ribose in uridine skeleton. The second step involves *Se*-alkylation using high affinity of Si to F.

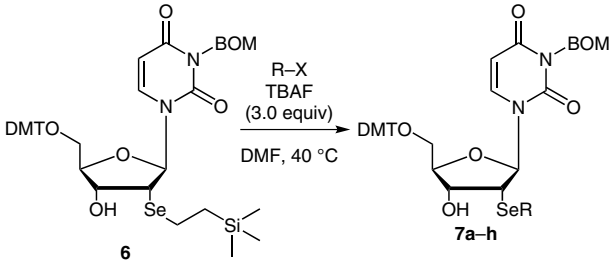
TSE diselenide (**1**) was prepared according to our procedure as reported previously;[17] following treatment of elemental selenium with sodium borohydride ($NaBH_4$), alkylation of the activated selenium with 2-(bromoethyl)trimethyl silane provided the desired selenating reagent **1** in 71% yield. Since 2,2′-*O*-cyclouridine (**2**) has a primary alcohol at the C5′ position, 4,4′-dimethoxytrityl (DMT) protection was initially conducted.[20] It is noteworthy that treatment of 5′-DMT-2,2′-*O*-cyclouridine (**3**) with TSE diselenide (**1**, 1.2 equiv) in the presence of $NaBH_4$ (1.2 equiv) at 60 °C for one hour proceeded smoothly to afford 2-(trimethylsilyl)ethylselenated uridine (**4**) in 98% yield.[22] The ^{77}Se NMR signal observed at δ = 150.3 ppm clearly revealed that a TSE selenolate anion attacked to the C2′ position rather than the C2 position. Methylation of **4** by means of iodomethane (MeI, 5.0 equiv) and tetrabutylammonium fluoride (TBAF, 3.0 equiv) at room temperature for one hour gave an unsolicited *N*-methylated uridine analogue **5** as a major product, indicating the need to protect an imide group. It was considered that basicity of TBAF accelerates *N*-methylation. Following N^3-protection using benzyloxymethyl chloride (BOMCl, 1.5 equiv) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 2.0 equiv), we tried *Se*-methylation again. Under slightly modified conditions (40 °C for 5 h), N^3-BOM-protected compound **6** favorably accepted a methyl group on the Se atom in 92% yield (**7a**, Scheme 2).[23]



Scheme 2 Synthesis of 2′-methylselenouridine derivatives

After successful methylation of **6**, the stage was now set for the comparative screening of the reactivity of **6** with various alkyl halides. Results are summarized in Table 1. Reactions with MeI, EtI, *i*-PrI, allyl bromide, and propargyl bromide afforded the corresponding *Se*-alkylated products (**7a–e**) in good to excellent yields (Table 1, entries 1–5). Comparing with these results, treatment with bromoacetonitrile furnished the respective product **7f** in 19% yield, while the yield was decreased considerably (Table 1, entry 6). With 3-bromopropionitrile (an additional one carbon of bromoacetonitrile), **6** did not react at all (Table 1, entry 7). When 2-bromoethyl methyl ether was used, the good yield of 72% (**7h**) was achieved (Table 1, entry 8).

Table 1 Reactivity of **6** with Various Alkyl Halides



Entry	Electrophile (equiv)	Time (h)	Yield (%)
1	MeI (5.0)	1.0	**7a** 92
2	EtI (5.0)	0.5	**7b** 98
3	*i*-PrI (3.0)	5.0	**7c** 70
4	CH_2=$CHCH_2Br$ (3.0)	1.5	**7d** 73
5	CH≡CCH_2Br (3.0)	0.5	**7e** 65
6	N≡CCH_2Br (3.0)	4.0	**7f** 19
7	N≡CCH_2CH_2Br (3.0)	4.0	**7g** 0
8	$MeOCH_2CH_2Br$ (3.0)	4.0	**7h** 72

In order to verify the practical effectiveness of a TSE group, we next examined the reactivity of **6** with benzyl bromides bearing a variety of functional groups on the benzene ring. Upon treatment of benzyl and 4-methylbenzyl bromides, the *Se*-benzylated products (**7i** and **7j**) were obtained in 81% and 69% yields, respectively (Table 2, entries 1 and 2). With 2-, 3-, and 4-nitro-substituted benzyl bromides, the yields were reduced (Table 2, entries 3–5). The electron-withdrawing properties of nitro substitutions

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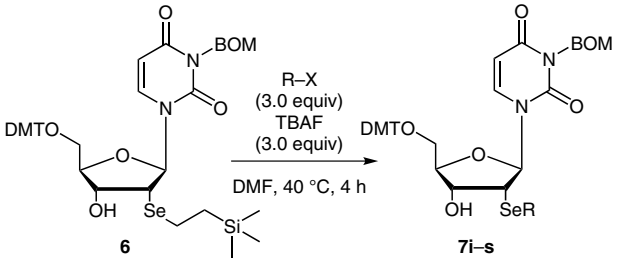
have a negative effect on the reaction progress. Chloro substitutions on the benzene ring slightly increased the yields (Table 2, entries 6–8). When 2-(bromomethyl)naphthalene was used, the reaction could easily access to the product (**7q**) in 76% yield (Table 2, entry 9). Furthermore, by using α-haloketone (phenacyl bromide) and ester (methyl bromoacetate), the yields were poor in both cases (Table 2, entries 10 and 11).

In addition, we attempted *Se*-acetylation of **6** to expand the feasibility of the approach, however, the reaction with acetyl chloride using TBAF under the same conditions with Table 2 produced the 3′-OAc product instead of 2′-*Se*-acetylated compound. Even when silver(I) tetrafluoroborate ($AgBF_4$)[24] was employed as a promoter, 2′-*Se*-acetylation did not progress.

Interest in oligonucleotide phosphoramidite synthetic chemistry has grown over last thirty years.[25–27] Nucleosidic phosphoramidites are powerful tools for the automated solid-phase synthesis of oligonucleotide-based antisense drugs. With **4** in hand, we turned our attention to synthesize a fully protected phosphoramidite monomer of 2′-methylselenouridine for the assembly of its oligonucleotide. (Pivaloyloxy)methyl (POM) as a suitable protective group for an imide group was employed.[28] From **4** as the starting point, treatment with POMCl (1.5 equiv) in the presence of K_2CO_3 (2.0 equiv) for five hours afforded the N^3-POM product **8** in 31% yield. Subsequent *Se*-methylation offered easy access to the N^3-POM-2′-*Se*-methylated uridine (**9**, 83% yield), which was phosphitylated by the standard procedure,[11,29] to yield the corresponding phosphoramidite (**10**, 56% yield) as a diastereomeric mixture (Scheme 3).[30]

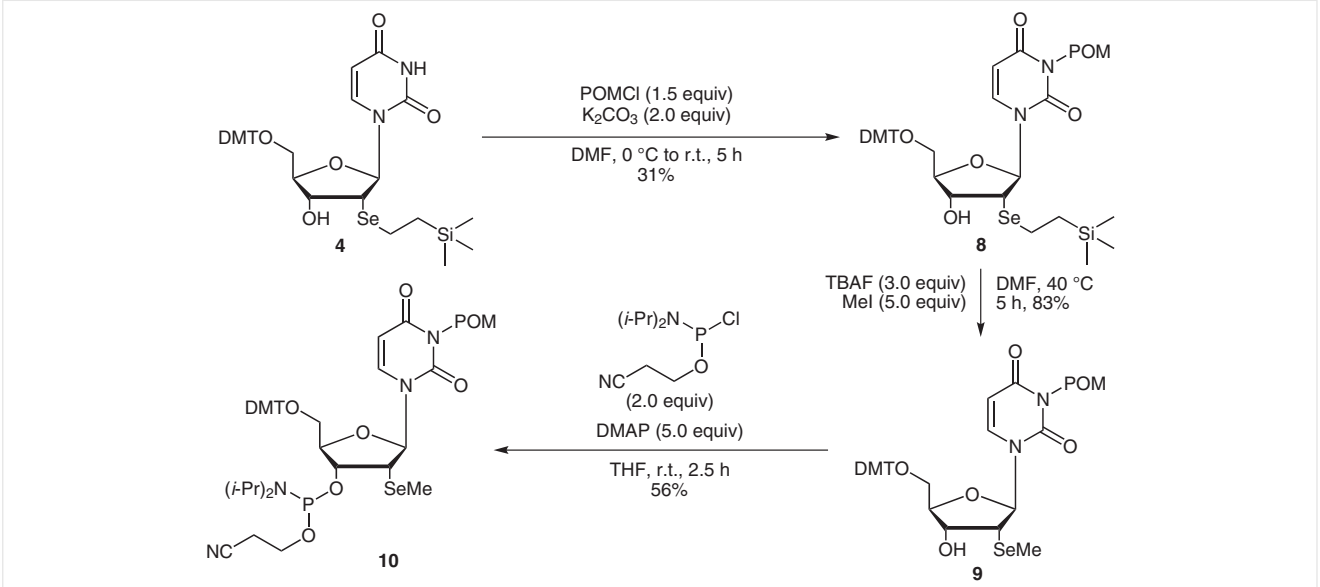
Table 2 Reactivity of **6** with Various Benzyl Bromides, α-Bromoketone, and α-Bromoester



Entry	Electrophile	Yield (%)
1	BnBr	**7i** 81
2	4-$MeC_6H_4CH_2Br$	**7j** 69
3	2-$O_2NC_6H_4CH_2Br$	**7k** 39
4	3-$O_2NC_6H_4CH_2Br$	**7l** 11
5	4-$O_2NC_6H_4CH_2Br$	**7m** 45
6	2-$ClC_6H_4CH_2Br$	**7n** 82
7	3-$ClC_6H_4CH_2Br$	**7o** 72
8	4-$ClC_6H_4CH_2Br$	**7p** 55
9	$NaphCH_2Br$	**7q** 76
10[a]	$Ph(C{=}O)CH_2Br$	**7r** 30
11[a]	$MeO(C{=}O)CH_2Br$	**7s** 22

[a] 10 equiv of electrophiles were used for 0.5 h.

In conclusion, we have demonstrated a valuable route for the synthesis of 2′-alkylselenouridine derivatives. The simplified methodology in this article has a wide applica-



Scheme 3 Synthesis of a 2′-methylselenouridine phosphoramidite

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tion for incorporation of an alkylselenyl moiety into biomolecule frameworks. Our findings highlight the great versatility and limitations of a 2-(trimethylsilyl)ethylselenyl group.

Acknowledgment

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Supporting Information

Supporting information for this article is available online at http://dx.doi.org/10.1055/s-0036-1588937.

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(22) **Synthesis of 5′-(4,4′-Dimethoxytrityl)-2′-trimethylsilylethyluridine (4)**
After stirring of 2-(trimethylsilyl)ethyl diselenide (**1**, 1.14 mmol), $NaBH_4$ (1.14 mmol), and EtOH (2.85 mmol) in DMF (5 mL) at 0 °C for 30 min, **3** (0.95 mmol) was added to the solution, and the reaction was continued at 60 °C for an additional 1 h. The resultant solution was quenched by 5% NH_3Cl aq, and then was poured into distilled water, partitioned with EtOAc, and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified using silica gel column chromatography eluted with *n*-hexane–EtOAc (3:1 to 1:1) to afford **4** (98% yield) as a white amorphous powder. $[\alpha]_D$ +79.39 (*c* 0.64, $CHCl_3$). IR (film): ν_{max} = 3407, 2924, 1685 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 8.79 (br s, 1 H, NH), 7.89 (d, 1 H, *J* = 7.6 Hz, H5), 7.36 (d, 2 H, *J* = 6.8 Hz, Ph), 7.30 (t, 2 H, *J* = 7.6 Hz, Ph), 7.26–7.23 (m, 5 H, Ph), 6.84 (d, 4 H, *J* = 8.9 Hz, Ph), 6.19 (d, 1 H, *J* = 8.3 Hz, H1′), 5.36 (d, 1 H, *J* = 8.2 Hz, H6), 4.37 (dd, 1 H, *J* = 2.8, 4.8 Hz, H3′), 4.18 (d, 1 H, *J* = 2.7 Hz, H4′), 3.79 (s, 6 H, 2 OMe), 3.66 (dd, 2 H, *J* = 4.8, 7.6 Hz, H2′), 3.51 (dd, 1 H, *J* = 2.7, 11.0 Hz, H5′a), 3.47 (dd, 2 H, *J* = 2.7, 11.0 Hz, H5′b), 2.85 (d, 1 H, *J* = 3.5 Hz, OH), 2.78–2.71 (m, 2 H, $SeCH_2CH_2TMS$), 1.03–0.95 (m, 2 H, $SeCH_2CH_2TMS$), 0.008 (s, 9 H, $SiMe_3$). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 163.1, 158.9, 150.4, 144.4, 140.0, 135.2, 135.0, 130.21, 130.16, 128.2, 127.4, 113.5, 102.7, 88.1, 87.4, 84.7, 71.6, 63.5, 55.4, 50.4, 20.8, 19.2, –1.8. ^{77}Se NMR (115 MHz, $CDCl_3$): δ = 150.3. HRMS (ESI, TOF): *m/z* calcd for $C_{35}H_{42}N_2O_7SeNa$, 733.1824; found: 733.1816 $[M + Na]^+$.
(23) **Alkylation of 6**
TBAF (1.0 M in THF, 0.15 mmol) was added to a solution of **6** (0.05 mmol) and MeI (0.25 mmol) in DMF (0.5 ml). After stirring at 40 °C for 5 h, the resultant solution was poured into distilled water, partitioned with EtOAc twice, and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified using silica gel column chromatography eluted with *n*-hexane–EtOAc (3:1 to 2:1) to afford **7a** (92% yield) as a white amorphous powder. Reaction parameters are given in Tables 1 and 2. The operation procedures for **7b–s** are exactly the same with **7a**. $[\alpha]_D$ +52.18 (*c* 0.62, $CHCl_3$). IR (film): ν_{max} = 3398, 2925, 1713, 1661 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.78 (d, 1 H, *J* = 8.0 Hz, H5), 7.38–7.25 (m, 14 H, Ph), 6.84 (d, 4 H, *J* = 8.9 Hz, Ph), 6.21 (d, 1 H, *J* = 6.7 Hz, H1′), 5.47 (s, 2 H, CH_2OCH_2Ph), 5.40 (d, 1 H, *J* = 8.1 Hz, H6), 4.69 (s, 2 H, CH_2OCH_2Ph), 4.38 (d, 1 H, *J* = 5.4 Hz, H3′), 4.15 (d, 1 H, *J* = 3.5 Hz, H4′), 3.79 (s, 6 H, 2 OMe), 3.55–3.46 (m, 3 H, H2′ and H5′), 2.75 (d, 1 H, *J* = 4.0 Hz, OH), 2.14 (s, 3 H, SeMe). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.7, 158.9, 151.3, 144.3, 138.5, 138.0, 135.3, 135.1, 130.2, 128.4, 128.2, 127.8, 127.4, 113.4, 102.4, 102.3, 88.8, 87.4, 84.5, 72.3, 71.3, 70.5, 63.0, 55.4, 51.0, 4.9. ^{77}Se NMR (75 MHz, $CDCl_3$): δ = 27.9. HRMS (ESI, TOF): *m/z* calcd for $C_{39}H_{40}N_8O_7SeNa$: 767.1848; found: 767.1860 $[M + Na]^+$.
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