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Practical synthesis of 4′-selenopyrimidine nucleosides using hypervalent iodine

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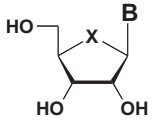
ABSTRACT

We report herein a synthesis of 4′-selenouridine **12** and 4′-selenocytidine **14**, substrates for the synthesis of 4′-selenoRNA. The Pummerer-like reaction between the selenoxide **9** and a silylated uracil afforded the desired 4′-selenouridine derivative **10**; however, the chemical yield of **10** was rather low. In addition, the reproducibility of this reaction was poor because of the instability of the selenoxide **9**. Improvement of this Pummerer-like reaction to give **10** was achieved when the 4-selenosugar **8** was treated with TMSOTf, 2,6-lutidine and the silylated uracil in the presence of iodosylbenzene.

1. Introduction

In recent years, synthetic oligonucleotides (ONs), especially RNA analogs, have become one of the most important molecular tools in the research field of nucleic acid-based therapeutics. Because of their potential utility especially in antisense and RNA interference (RNAi) applications, a variety of modified nucleoside units have now been synthesized and incorporated into ONs.[1,2] In our group, we have been working on developing a series of 4′-thionucleic acids, made up of 4′-thioribonucleosides, 2′-deoxy-4′-thioribonucleosides, and 2′-modified-4′-thionucleosides, for use as functional ONs.[3–5] These artificial nucleic acid analogs, for example, 4′-thioRNA and 4′-thioDNA, exhibited high hybridization ability and nuclease resistance[6–8] and thus were utilized in gene silencing via RNAi technology and selection of aptamers by SELEX.[9–12] These successful results prompted us to synthesize the novel nucleoside analogs, 4′-selenoribonucleosides, which have a seleno atom on the 4′-position instead of a sulfur, since the resulting RNA analog consisting of 4′-selenoribonucleosides (Fig. 1), that is 4′-selenoRNA,[13] is expected to act as a bioisostere of the promising 4′-thioRNA.

Thus far, successful syntheses of the 4′-selenoribonucleosides using a Pummerer-like reaction between the corresponding selenoxide and a silylated nucleobase have been reported by our group as well as others.[14–16] However, all the condensation yields were generally moderate and rather low compared with the Pummerer reaction between a sulfoxide and a silylated nucleobase. These unfavorable results are perhaps due to the instability of the starting selenoxide; however no experimental evidence has ever been presented. In this paper, we describe the experimental details of the 4′-selenopyrimidine nucleoside synthesis via the Pummerer-like reaction between a selenoxide and a silylated uracil. In addition, we also report a practical synthesis of the 4′-selenouridine derivative using hypervalent iodine.



X = O; ribonucleoside
X = S; 4'-thioribonucleoside
X = Se; 4'-selenoribonucleoside
B = nucleobase

Fig. 1. Structures of 4′-substituted nucleoside analogs.

2. Results and discussion

Our approach to 4′-selenoribonucleosides was based on our original chemistry of the 4′-thioribonucleoside synthesis. After conversion of D-ribose into the diol **1** in four steps,[17] **1** was treated with methanesulfonyl chloride (MsCl) in pyridine, followed by lithium bromide in methyl ethyl ketone to give **2** (Scheme 1). The resulting **2** was subsequently treated with selenium and sodium borohydride in EtOH[18] to provide the crystalline 4-selenosugar **3** in 44% yield from **1** after crystallization. Then the *p*-methoxybenzyl (PMB) groups of **3** were removed by treatment with a mixture of trifluoroacetic acid (TFA)–CH_2Cl_2 at room temperature. After co-evaporation of the reaction mixture with MeOH to remove the excess TFA, chromatographic purification of the residue afforded the desired compound **4** containing the inseparable compound **5** in 42%

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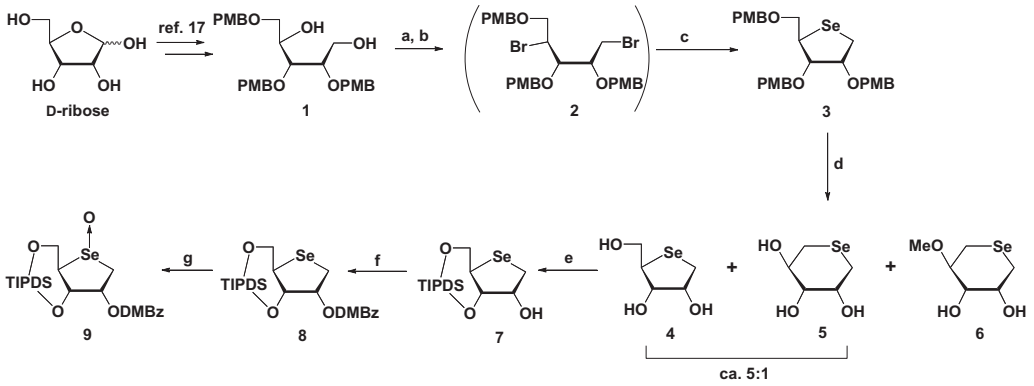


Scheme 1. (a) MsCl, pyridine; (b) LiBr, methyl ethyl ketone, reflux; (c) Se, $NaBH_4$, EtOH, 60 °C; (d) TFA, CH_2Cl_2; (e) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, pyridine; (f) 2,4-dimethoxybenzoyl chloride, pyridine; (g) O_3, CH_2Cl_2, −78 °C.

yield (ca. 5:1) and the separable compound **6** in 51% yield.[19] Since the formation of these unfavorable products has never been observed in the case of the corresponding 4-thiosugar derivative,[17] one proposed reaction mechanism to give **5** and **6**, illustrated in Fig. 2, would be likely for a more nucleophilic selenium atom in its structure. The requisite deprotection reaction has been achieved by neutralizing the reaction mixture with sodium hydrogen carbonate prior to evaporation to give **4** without any formation of **5** and **6**. In order to convert **4** into the substrate for the Pummerer-like reaction, the hydroxyl groups at the 3- and 5-positions of **4** were protected with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane. The resulting **7** was then treated with 2,4-dimethoxybenzoyl chloride (DMBzCl) to give the protected 4-selenosugar **8**. Oxidation of **8** was next carried out by treatment with ozone in CH_2Cl_2 at −78 °C to give the selenoxide **9** quantitatively as a diastereomeric mixture (3:2 ratio from ^{1}H NMR spectrum).[20] In our previous study using 4-thiosugar derivatives,[3] we showed that the two diastereomeric sulfoxides, namely the *R*- and *S*-sulfoxides, afforded quite different results in the Pummerer reaction. Therefore, we examined several oxidation conditions to vary the diastereomeric ratio of the resulting selenoxide. However, all oxidation conditions, such as *m*-chloroperbenzoic acid (*m*CPBA) and sodium periodate treatment produced the same results as the ozone oxidation. Since the selenoxide is known to isomerize easily in the presence of H_2O,[21] the diastereomeric ratio of **9** appeared to reach its equilibrium state in all cases. We therefore decided to use the diastereomeric mixtures of the selenoxide (3:2 ratio) for the Pummerer-like reaction.

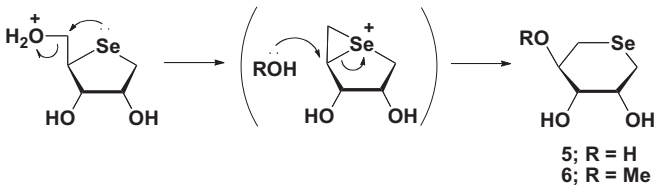


Fig. 2. Proposed mechanism of formation of **5** and **6**.

The research groups, which had previously prepared the 4′-selenoribonucleosides reported that the starting selenoxide was unstable.[15,16] We also encountered the same problem with our selenoxide **9**. As shown in Fig. 3, the ^{1}H NMR spectrum of **9** in $CDCl_3$ immediately after chromatographic purification gave reasonable proton signals corresponding to the diastereomeric mixture (Fig. 3A). However, after 1 day at room temperature, its spectrum changed to collapsed signals, which indicated the instability of **9** (Fig. 3B). However, to our surprise, after 1 week, its spectrum started giving varied signals corresponding to a single compound (Fig. 3C), which was identical with the 4-selenosugar **8** (Fig. 3D).

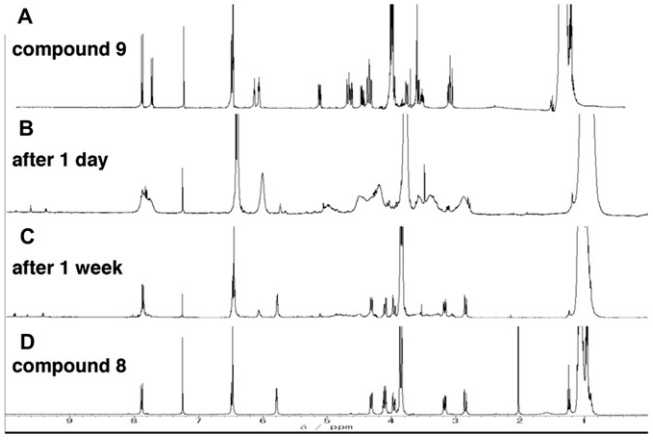
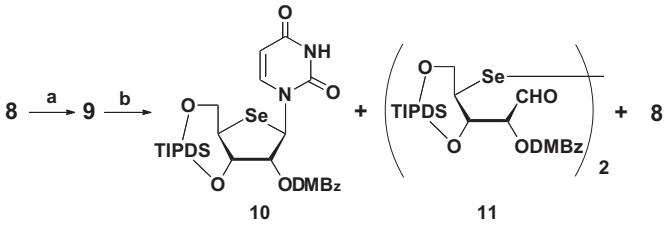


Fig. 3. ^{1}H NMR studies on time-course of **9** in $CDCl_3$.

From these results, it was determined that the selenoxide **9** was being gradually deoxygenated,[22] which may be one reason for the instability of the selenoxides reported by other groups. We therefore examined the Pummerer-like reaction without chromatographic purification of **9**. Thus, compound **8** was first treated with ozone at −78 °C in CH_2Cl_2, and the solvent was removed in vacuo after removing excess ozone by bubbling N_2 gas. The resulting residue was immediately subjected to the Pummerer-like reaction by treatment with trimethylsilyl trifluoromethanesulfonate (TMSOTf) and Et_3N in the presence of the silylated uracil (Scheme 2). As a result, the desired 4′-selenouridine derivative **10** was obtained in 36% yield stereoselectively along with the diselenide **11** and **8** in 4% and 19% yields, respectively. In addition to the low chemical yield of **10**, the reproducibility of this reaction was poor probably because of the instability of **9**. In order to improve the chemical yield of **10**, reaction conditions including temperature and order of addition of chemicals were examined. However, all attempts resulted in decreased formation of **10** and increased production of **8** (data not shown). When **9** was treated with TMSOTf and Et_3N in the absence of the silylated uracil, **11** and **8** were afforded in 21% and 31% yields, respectively. Considering this result, it was concluded that the deoxygenation of **9** could not be avoided, and thus a drastic improvement would be difficult in this reaction system.

Nishizono et al. reported a novel method for the synthesis of 4′-thioribonucleosides using hypervalent iodine.[23] In their method, a 4-thiosugar was condensed with a silylated nucleobase to give the desired 4′-thioribonucleoside derivative without preparation of the corresponding sulfoxide. If their method could be applied to the



Scheme 2. (a) O_3, CH_2Cl_2, −78 °C; (b) uracil, TMSOTf, Et_3N, toluene, CH_2Cl_2.

synthesis of the 4′-selenoribonucleoside, this would be advantageous because isolation of the unstable selenoxide would then not be needed. According to their method, the 4-selenosugar **8** was treated with iodosylbenzene, TMSOTf and Et_3N in the presence of the silylated uracil. However, none of the desired **10** was obtained under their conditions (Table 1, entry 1). Since tertiary aliphatic amines are known to react with iodosylbenzene,[24] 2,6-lutidine was next employed instead of Et_3N. As a result, the desired **10** was obtained in 48% yield together with the selenoxide **9** (20%) and the starting **8** (8%) (entry 2). Using more reactive hypervalent iodine sources resulted in a decreased chemical yield of **10** (entries 3–5). The best result was obtained when **8** was treated with iodosylbenzene, TMSOTf, 2,6-lutidine and the silylated uracil in dichloroethane at 50 °C to give **10** in 64% yield together with a small amount of **8** (entry 6). Unlike the aforementioned Pummerer-like reaction, this reaction afforded **10** with good reproducibility.

The proposed reaction mechanism is illustrated in Fig. 4. First, iodosylbenzene activated by TMSOTf in the presence of 2,6-lutidine, that is, $PhI(L^+)_2(TfO^-)_2$ [L=2,6-lutidine],[25] reacts with **8** to give the intermediate **A**. If the resulting TfO^- (represented as B^-) removes H-1 involving elimination of iodobenzene as shown in **B** (path a), formation of the selenium intermediate **C** is expected. The resulting intermediate **C** will immediately react with the silylated uracil via neighboring group participation of the DMBz group on the 2-position to give the desired **10** stereoselectively. However, formation of the intermediate **C** from **B** appears to be rather slow (it takes 17 h for completion of the reaction). Therefore, nucleophilic attack of the contaminant H_2O (path b) would compete to give the selenoxide **9**, followed by deoxygenation to give **8**.[26] When the reaction was carried out in dichloroethane at 50 °C (Table 1, entry 6), the rate determining elimination reaction to give the intermediate **C** should be accelerated. Accordingly, formation of the desired **10** was increased and that of **9** and **8** was decreased compared with the conditions in entry 2. In the TLC analysis of this reaction, the starting material **8** disappeared immediately after addition of iodosylbenzene activated by TMSOTf, and the selenoxide **9** was first observed as a major product. This compound is thought to come from intermediate **A** after hydrolysis on the TLC plate. Compound **9** gradually disappeared and the formation of **10** increased as the reaction proceeded. These observations appear to support the proposed mechanism (Fig. 4).

Finally, the resulting **10** was converted into 4′-selenouridine **12** and 4′-selenocytidine **14** (Scheme 3). Thus, treatment of **10** with tetrabutylammonium fluoride (TBAF), followed by methylamine afforded 4′-selenouridine **12**.[15] For the synthesis of 4′-selenocytidine **14**, **10** was first treated with triisopropylbenzenesulfonyl chloride (TPSCl), followed by ammonium hydroxide to give the 4′-selenocytidine derivative **13**. Then, **13** was subjected to similar conditions as described above for **12** to give **14**.[15]

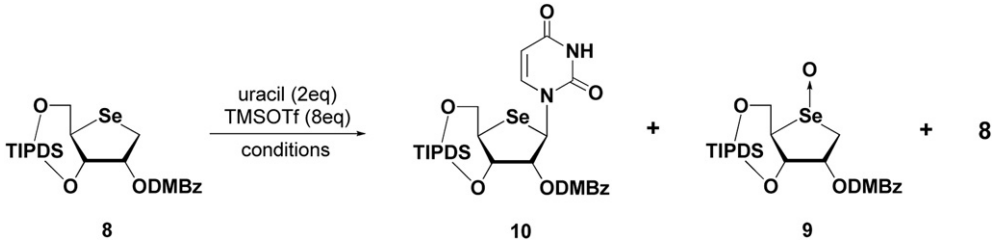
In conclusion, we have investigated the practical synthesis of 4′-selenoribonucleosides. The Pummerer-like reaction between the selenoxide **9** and the silylated uracil afforded **10**, however, the chemical yield of **10** was rather low. We showed what happens in this reaction system, namely deoxygenation of **9** and diselenide formation to give **8** and **11**, respectively. The desired Pummerer-like reaction successfully occurred when the 4-selenosugar **8** was treated with iodosylbenzene, TMSOTf, 2,6-lutidine and the silylated uracil in dichloroethane at 50 °C to give **10** in 64% yield. Since the 4′-selenouridine **12** and the 4′-selenocytidine **14** are now available, investigations of the synthesis and properties of 4′-selenoRNA containing these nucleoside units are in progress.

3. Experimental section

3.1. General methods

Physical data were measured as follows: melting points are uncorrected. ^1H and ^{13}C NMR spectra were recorded at 400 or 500 MHz and 100 or 125 MHz instruments (JEOL-ECX400, JEOL-ECA500, or Bruker AV400N) in $CDCl_3$ or DMSO-d_6 as the solvent with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. TLC was done on Merck Kieselgel F_{254} precoated plates. Silica

Table 1
The Pummerer-lile reaction using hypervalent iodine



Entry	Conditions					Yield		
	Hypervalent iodine (1.2 equiv)	Base (8 equiv)	Solvent	Temp (°C)	Time (h)	**10** (%)	**9** (%)	**8** (%)
1	PhIO	Et_3N	CH_2Cl_2	0	4.5	0	0	33
2	PhIO	2,6-lutidine	CH_2Cl_2	rt	17	48	20	8
3	$PhI(OCOCF_3)_2$	2,6-lutidine	CH_2Cl_2	rt	3	38	0	40
4	$PhI(OAc)_2$	2,6-lutidine	CH_2Cl_2	rt	5	25	0	20
5	PhI(OH)(OMs)	2,6-lutidine	CH_2Cl_2	rt	1.5	0	83	12
6	PhIO	2,6-lutidine	$ClCH_2CH_2Cl$	50	1.5	64	0	13

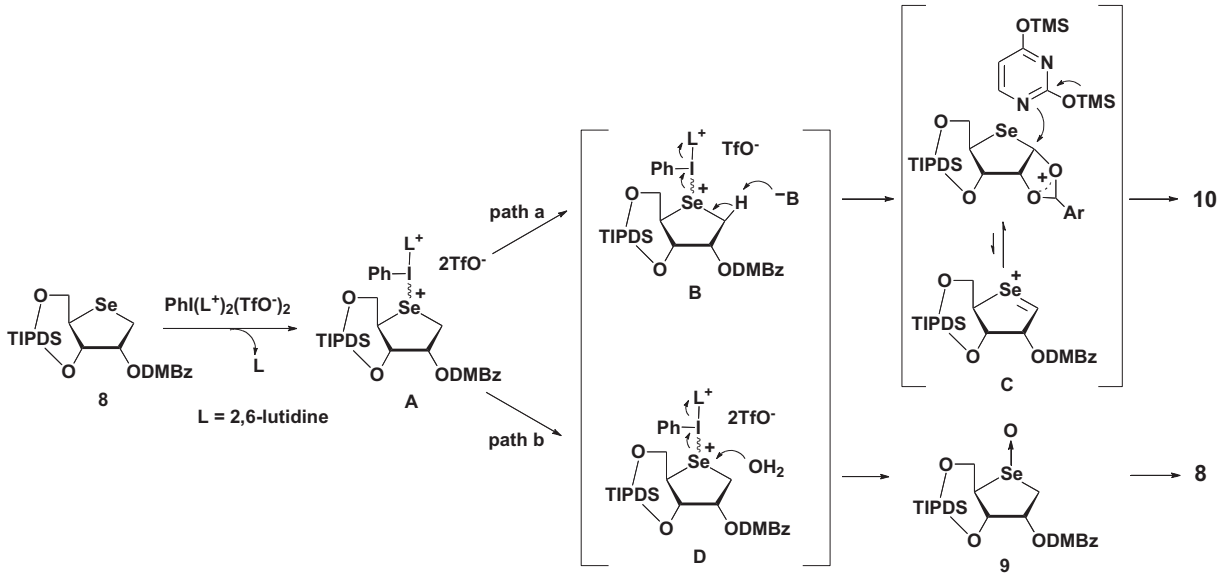
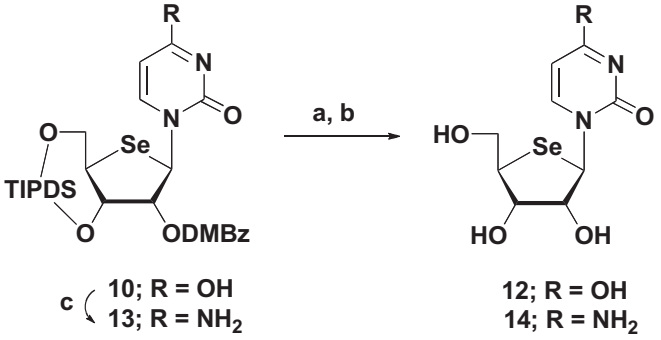


Fig. 4. Proposed mechanism of the Pummerer-like reaction using hypervalent iodine.



Scheme 3. (a) TBAF, THF; (b) $MeNH_2$ in MeOH; (c) TPSCl, Et_3N, DMAP, CH_3CN, then NH_4OH.

gel used for column chromatography was YMC gel 60A (70–230 mesh).

*3.1.1. 1,4-Anhydro-2,3,5-tri-O-p-methoxybenzyl-4-seleno-D-ribitol (**3**).* To a solution of **1** (83.9 g, 164 mmol) in dry pyridine (450 mL) was added MsCl (65.0 mL, 840 mmol) at 0 °C, and the mixture was stirred at room temperature for 30 min. The reaction was quenched by addition of ice, and the reaction mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo and coevaporated with toluene (three times). The resulting dimesylate was dissolved in dry methyl ethyl ketone (500 mL), and LiBr (139 g, 1.6 mmol) was added to the solution. The mixture was heated for 9.5 h under reflux. The reaction mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo to give dibromide **2** as brown oil. To a suspension of selenium powder (20.7 g, 262 mmol) in ethanol (640 mL) was added sodium borohydride (15 g, 567 mmol) portionwise. The resulting dibromide **2** in THF (160 mL) was then added to the above mixture and the whole was heated at 60 °C for 1.5 h. The reaction mixture was partitioned between AcOEt and H_2O. The separated organic layer was further washed with H_2O, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (6:1 to 1:1), to give **3**, which was further purified by crystallization form hexane–AcOEt to give white crystals (40.3 g, 44% in three steps): mp 82–83 °C; ^1H NMR ($CDCl_3$) δ 7.22 (m, 6H), 6.84 (m, 6H), 4.52–4.42 (m, 6H), 4.03 (m, 1H), 3.94 (dd, 1H, J=3.3 and 4.6 Hz), 3.80 (m, 10H), 3.58 (dd, 1H, J=7.3 and 10.2 Hz), 3.44 (dd, 1H, J=6.6 and 10.2 Hz), 3.00 (dd, 1H, J=6.9 and 9.9 Hz), 2.89 (dd, 1H, J=5.3 and 9.9 Hz); ^{13}C NMR ($CDCl_3$) δ 159.28, 130.33, 130.24, 129.64, 129.37, 129.33, 113.83, 113.76, 81.79, 80.64, 72.65, 72.33, 71.51, 71.47, 55.36, 41.10, 22.24; IR (neat) 1612, 1513 (Ar) cm^{-1}; EIMS-LR m/z=559 (MH^+). Anal. Calcd for $C_{29}H_{34}O_6Se$: C, 62.47; H, 6.15. Found: C, 62.47; H, 6.26.

*3.1.2. 1,4-Anhydro-4-seleno-D-ribitol (**4**).* To a solution of **3** (10.0 g, 17.9 mmol) in dry CH_2Cl_2 (85 mL) was added a solution of TFA in CH_2Cl_2 (17 mL in 150 mL) dropwise at room temperature. The reaction mixture was neutralized with $NaHCO_3$, and insoluble salts were filtered through a Celite pad, and washed with MeOH. The solvent was removed in vacuo, and the residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (5–12%), to give **4** (2.6 g, 73% as a white oil): ^1H NMR (DMSO-d_6) δ 4.88 (m, 2H, exchangeable with D_2O), 4.80 (d, 1H, J=5.4 Hz, exchangeable with D_2O), 4.10 (m, 1H), 3.80 (ddd, 1H, J=2.3, 3.2, and 5.4 Hz), 3.72 (ddd, 1H, J=3.6, 5.4, and 10.1 Hz), 3.34 (m, 2H), 2.80 (dd, 1H, J=5.5 and 10.0 Hz), 2.59 (dd, 1H, J=5.8 and 10.0 Hz); ^{13}C NMR (DMSO-d_6) δ 77.15, 75.57, 64.90, 47.23, 24.15; IR (neat) 3334 (OH) cm^{-1}; EIMS-LR m/z=221 (MNa^+); ESIMS-HR calcd for $C_5H_{10}O_3SeNa$ 220.9693, found 220.9686.

*3.1.3. 1,4-Anhydro-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-seleno-D-ribitol (**7**).* To a solution of **4** (2.5 g, 12.6 mmol) in pyridine (40 mL) was added a solution of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (4.2 mL, 13.2 mmol) in pyridine (20 mL) dropwise, and the reaction mixture was stirred for 3 h at room temperature. The reaction was quenched by addition of ice, and the reaction mixture was partitioned between AcOEt and H_2O. The separated organic layer was further washed with H_2O, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (40:1–20:1), to give **7** (4.5 g, 81% as a colorless oil): ^1H NMR ($CDCl_3$) δ 4.45 (m, 1H), 4.23 (dd, 1H, J=3.3 and 9.2 Hz), 4.05 (dd, 1H, J=3.3 and 12.5 Hz), 3.94 (dd, 1H, J=4.0 and 12.5 Hz), 3.70

(ddd, 1H, *J*=3.3, 4.0, and 9.2 Hz), 3.04 (dd, 1H, *J*=4.6 and 11.2 Hz), 2.84 (dd, 1H, *J*=1.3 and 11.2 Hz), 2.04 (br s, 1H, exchangeable with D_2O), 1.09–0.82 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 79.42, 75.92, 61.05, 43.72, 22.92, 17.56, 17.50, 17.47, 17.38, 17.34, 17.30, 17.24, 13.60, 13.52, 13.29, 12.83, 12.79; IR (neat) 3567 (OH) cm^{-1}; FABMS-LR *m*/*z*=441 (MH^+).; FABMS-HR calcd for $C_{17}H_{37}O_4Si_2Se$ 441.1395, found 441.1391.

*3.1.4. 1,4-Anhydro-2-O-(2,4-dimethoxybenzoyl)-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-seleno-D-ribitol (**8**).* To a solution of **7** (4.4 g, 10.0 mmol) in dry pyridine (100 mL) was added 2,4-dimethoxybenzoyl chloride (4.4 g, 22.0 mmol) at 0 °C, and the mixture was stirred at room temperature for 30 h. The reaction was quenched by addition of ice, and the mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$ (three times), followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the residue was purified by a silica gel column, eluted with hexane/AcOEt (20:1 to 6:1), to give **8** (5.9 g, 98% as a colorless oil): ^1H NMR ($CDCl_3$) δ 7.90 (d, 1H, *J*=9.2 Hz), 6.50 (m, 2H), 5.80 (m, 1H), 4.33 (dd, 1H, *J*=3.3 and 9.9 Hz), 4.12 (dd, 1H, *J*=2.9 and 12.8 Hz), 3.97 (dd, 1H, *J*=3.0 and 12.8 Hz), 3.87 (m, 7H,), 3.19 (dd, 1H, *J*=4.3 and 11.5 Hz), 2.87 (m, 1H), 1.11–0.91 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 171.18, 164.70, 164.34, 161.60, 133.96, 112.52, 104.51, 98.93, 76.11, 60.43, 59.79, 55.98, 55.51, 44.49, 21.56, 21.08, 17.47, 17.43, 17.35, 17.33, 17.15, 17.12, 14.24, 13.50, 13.13, 12.76; IR (neat) 1728 [C(=O)–O], 1608, 1505 (Ar) cm^{-1}; FABMS-LR *m*/*z*=605 (MH^+).; FABMS-HR calcd for $C_{26}H_{45}O_7Si_2Se$ 605.1870, found 605.1873.

*3.1.5. 1-[2-O-(2,4-Dimethoxybenzoyl)-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-seleno-β-D-ribofuranosyl]uracil (**10**).* The Pummerer-like reaction via **9**: ozone was bubbled through a solution of **8** (120 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) at −78 °C. After 10 min, N_2 gas was bubbled through the solution to remove excess ozone. The reaction mixture was allowed to warm to room temperature and concentrated in vacuo. The reaction mixture was dried (Na_2SO_4) and concentrated in vacuo to give **9** as a colorless oil. To a suspension of uracil (44 mg, 0.4 mmol) in toluene (1.3 mL) were added Et_3N (110 μL, 0.8 mmol) and TMSOTf (290 μL, 1.6 mmol), and the mixture was stirred at room temperature until giving a clear solution. Additional CH_2Cl_2 (0.7 mL) was added to the above solution, which gave a one-phase clear solution, and the whole was added to a solution of **9** in dry CH_2Cl_2 (1.3 mL) dropwise. An additional Et_3N (110 μL, 0.8 mmol) in toluene (0.7 mL) was added dropwise. After being stirred for 15 min at room temperature, the reaction was quenched by addition of ice, and the reaction mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (9:1 to 1:1), to give **10** (53 mg, 36% as a white foam), **11** (10 mg, 4% as a yellow oil), and **8** (23 mg, 19%).

Physical data for **10**: ^1H NMR ($CDCl_3$) δ 8.10 (br s, 1H, exchangeable with D_2O), 8.04 (d, 1H, *J*=8.6 Hz), 7.85 (d, 1H, *J*=8.6 Hz), 6.49 (m, 2H), 6.15 (d, 1H, *J*=1.1 Hz), 5.73 (dd, 1H, *J*=2.7 and 8.6 Hz), 5.67 (dd, 1H, *J*=1.1 and 4.0 Hz), 4.55 (m, 1H), 4.19 (dd, 1H, *J*=2.9 and 12.5 Hz), 4.05 (m, 1H), 3.97 (m, 1H), 3.86 (s, 6H), 1.15–0.87 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 164.70, 163.63, 162.58, 161.80, 149.73, 142.23, 134.13, 111.80, 104.75, 102.60, 99.08, 78.65, 73.44, 58.46, 56.09, 55.66, 55.30, 47.57, 17.58, 17.51, 17.46, 17.26, 17.23, 17.04, 17.02, 13.49, 13.39, 13.11, 12.71; IR (neat) 3202 (NH), 1694 [C(=O)–O, C=O], 1608, 1508 (Ar) cm^{-1}; FABMS-LR *m*/*z*=715 (MH^+).; FABMS-HR calcd for $C_{30}H_{47}N_2O_9Si_2Se$ 715.1985, found 715.1982.

Physical data for **11**: ^1H NMR ($CDCl_3$) δ 9.62 (s, 2H), 7.97 (d, 2H, *J*=8.6 Hz), 6.50 (m, 4H, *J*=8.6 Hz), 5.71 (s, 2H), 4.57 (d, 2H, *J*=11.3 Hz), 4.43 (d, 2H, *J*=11.8 Hz), 4.21 (d, 2H, *J*=11.8 Hz), 3.87 (s, 12H), 3.70 (d, 2H, *J*=11.3 Hz), 1.10–0.99 (m, 56H); ^{13}C NMR ($CDCl_3$) δ 197.77, 164.99, 162.08, 134.37, 111.03, 104.61, 98.94, 80.60, 73.74, 62.72, 55.94, 55.67, 50.54, 17.71, 17.51, 17.45, 17.40, 17.34, 17.30, 13.57, 13.52, 12.79, 12.75; IR (neat) 1725 [C(=O)-O, C(=O)-H], 1608, 1465 (Ar) cm^{-1}; FBMS-LR *m*/*z*=1261 (MNa^+); FABMS-HR calcd for $C_{52}H_{86}O_{16}Si_4Se_2Na$ 1261.3221, found 1261.3228.

Physical data for **9**: ^1H NMR ($CDCl_3$) δ 7.94 (d, 0.6H, *J*=9.4 Hz), 7.78 (d, 0.4H, *J*=9.3 Hz), 6.48–6.42 (m, 2H), 6.10 (m, 0.4H), 6.02 (m, 0.6H), 5.02 (dd, 0.6H, *J*=3.2 and 10.0 Hz), 4.58 (m, 1H), 4.56 (dd, 0.4H, *J*=3.1 and 13.1 Hz), 4.31 (dd, 0.4H, *J*=3.3 and 13.3 Hz), 4.22 (m, 0.6H), 4.20 (m, 0.4H), 3.88–3.81 (m, 6.6H), 3.58 (m, 0.6H), 3.41 (dd, 0.4H, *J*=5.0 and 14.3 Hz), 3.34 (m, 0.4H), 2.89 (m, 0.4H), 2.86 (m, 0.6H), 1.19–0.91 (m, 28H); ESIMS-LR *m*/*z*=621 (MH^+).

The Pummerer-like reaction using iodosylbenzene: to a suspension of uracil (44 mg, 0.4 mmol) in dichloroethane (2 mL) were added 2,6-lutidine (90 μL, 0.8 mmol) and TMSOTf (290 μL, 1.6 mmol), and the mixture was stirred at room temperature until giving a clear solution. The resulting solution containing silylated uracil was added to a solution of **8** (120 mg, 0.2 mmol) in dichloroethane (2 mL) containing iodosylbenzene (52 mg, 0.24 mmol) dropwise. Then, an additional 2,6-lutidine (90 μL, 0.8 mmol) in dichloroethane (1 mL) was added dropwise to the reaction mixture. The whole was heated at 50 °C for 1.5 h, and the reaction was quenched by addition of ice. The reaction mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a silica gel column, eluted with hexane/AcOEt (3:1 to 1:1), to give **10** (91 mg, 64% as a white foam) together with **8** (16 mg, 13%).

*3.1.6. 1-(4-Seleno-β-D-ribofuranosyl)uracil (**12**)*[15]. To a solution of **10** (360 mg, 0.5 mmol) in THF (4 mL) was added TBAF (1 M in THF, 1.5 mL, 1.5 mmol) at 0 °C. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo. After short column purification, the resulting product was dissolved in methylamine in MeOH (40%, 4 mL), and the mixture was kept for 3 h at room temperature. The solvent was removed in vacuo, and the residue was coevaporated with MeOH. The residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (5–10%), to give **12** (108 mg, 66% in two steps as a pale yellow foam). An analytical sample was crystallized from H_2O to give pale yellow crystals: mp 199 °C (lit.[15] mp 198–200 °C); ^1H NMR (DMSO-d_6) δ 11.32 (br s, 1H, exchangeable with D_2O), 7.95 (d, 1H, *J*=8.0 Hz), 6.05 (d, 1H, *J*=8.6 Hz), 5.69 (d, 1H, *J*=8.0 Hz), 5.41 (d, 1H, *J*=6.3 Hz, exchangeable with D_2O), 5.27 (d, 1H, *J*=4.0 Hz, exchangeable with D_2O), 5.16 (t, 1H, *J*=5.2 Hz, exchangeable with D_2O), 4.23 (ddd, 1H, *J*=3.4, 6.3, and 8.6 Hz), 4.13 (ddd, 1H, *J*=1.7, 3.4, and 4.0 Hz), 3.74 (ddd, 1H, *J*=5.2, 8.0, and 11.5 Hz), 3.58 (ddd, 1H, *J*=5.2, 6.3, and 11.5 Hz), 3.32 (ddd, 1H, *J*=1.7, 6.3, and 8.0 Hz); ^{13}C NMR (DMSO-d_6) δ 163.01, 151.11, 142.24, 102.24, 77.28, 73.70, 63.91, 55.46, 49.13; IR (KBr) 3184 (NH, OH), 1671 (C=O), 1605 (C=C) cm^{-1}; FABMS-LR *m*/*z*=309 (MH^+). Anal. Calcd for $C_9H_{12}N_2O_5Se$: C, 35.19; H, 3.94; N, 9.12. Found: C, 35.11; H, 3.87; N, 9.00.

*3.1.7. 1-[2-O-(2,4-Dimethoxybenzoyl)-3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-4-seleno-β-D-ribofuranosyl]cytosine (**13**).* To a solution of **10** (573 mg, 0.8 mmol) in CH_3CN (8 mL) including Et_3N (336 μL, 2.41 mmol) and DMAP (292 mg, 2.39 mmol) was added TPSCl (724 mg, 2.39 mmol), and the reaction mixture was stirred for 30 min at room temperature. Then, ammonium hydroxide (10 mL) was added to the reaction mixture, and the whole was kept for 3 h. The reaction mixture was partitioned between AcOEt and H_2O. The separated organic layer was washed with 1 N HCl, saturated aqueous $NaHCO_3$, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The

residue was purified by a silica gel column, eluted with MeOH in $CHCl_3$ (0–1%), to give **13** (519 mg, 91% as a yellow foam): ^1H NMR ($CDCl_3$+D_2O) δ 8.10 (d, 1H, J=7.4 Hz), 7.83 (d, 1H, J=8.6 Hz), 6.47 (m, 2H), 6.26 (s, 1H), 5.77 (d, 1H, J=7.4 Hz), 5.66 (d, 1H, J=2.9 Hz), 4.48 (dd, 1H, J=2.9 and 6.9 Hz), 4.16 (dd, 1H, J=2.3 and 12.6 Hz), 4.03 (m, 1H), 3.93 (m, 1H), 3.85 (s, 6H), 1.25–0.93 (m, 28H); ^{13}C NMR ($CDCl_3$) δ 165.54, 164.36, 163.58, 161.52, 155.58, 143.09, 133.91, 112.27, 104.62, 99.04, 95.16, 78.15, 73.66, 58.87, 56.11, 56.00, 55.49, 46.69, 17.46, 17.40, 17.38, 17.15, 16.95, 13.41, 13.30, 12.92, 12.65; IR (neat) 3177 (NH_2), 1716 [C(=O)–O], 1646 (C=O), 1605, 1470 (Ar) cm^{-1}; FABMS-LR m/z=714 (MH^+).; FABMS-HR calcd for $C_{30}H_{48}N_3O_8Si_2Se$ 714.2145, found 714.2149.

*3.1.8. 1-(4-Seleno-β-D-ribofuranosyl)cytosine (**14**)*[15]. In the similar manner as described for **12**, **13** (140 mg, 0.2 mmol) was treated with TBAF in THF, followed by methylamine in MeOH to give **14** (39 mg, 64% in two steps as a yellow foam). An analytical sample was crystallized from EtOH/hexane to give white crystals: mp 220–221 °C (lit.[15] mp 150–153 °C); ^1H NMR (DMSO-d_6) δ 7.87 (d, 1H, J=7.5 Hz), 7.11 (br s, 2H, exchangeable with D_2O), 6.05 (d, 1H, J=8.0 Hz), 5.75 (d, 1H, J=7.5 Hz), 5.22 (d, 1H, J=6.3 Hz, exchangeable with D_2O), 5.16 (d, 1H, J=4.0 Hz, exchangeable with D_2O), 5.08 (t, 1H, J=5.7 Hz, exchangeable with D_2O), 4.17 (ddd, 1H, J=2.9, 6.3, and 8.0 Hz), 4.10 (ddd, 1H, J=2.3, 2.9, and 4.0 Hz), 3.73 (ddd, 1H, J=5.7, 8.0, and 11.5 Hz), 3.53 (ddd, 1H, J=5.7, 6.3, and 12.5 Hz), 3.34 (ddd, 1H, J=2.3, 6.3, and 8.0 Hz); ^{13}C NMR (DMSO-d_6) δ 165.18, 155.73, 142.61, 94.58, 77.25, 74.01, 64.09, 56.46, 48.36; IR (KBr) 3423 (NH_2, NH, OH), 1712 (C=O), 1607 (C=C) cm^{-1}; FABMS-LR m/z=308 (MH^+). Anal. Calcd for $C_9H_{13}N_3O_4Se \cdot 1/16EtOH$: C, 35.23; H, 4.33; N, 13.51. Found: C, 35.21; H, 4.28; N, 13.25.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2011.08.020.

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