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First Synthesis of 4′-Selenonucleosides Showing Unusual Southern Conformation

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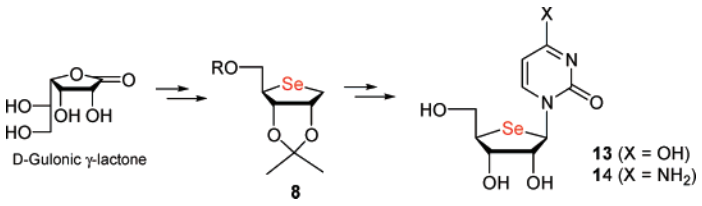
ABSTRACT



The first synthesis of 4′-selenonucleosides was achieved using a Pummerer-type condensation as a key step. All stereoelectronic effects shown in 4′-oxonucleosides were overwhelmed by the size of selenium and steric interactions, driving the conformation to the *C2′-endol* C3′-*exo* twist (Southern) conformation.

The nucleoside structure has proven to be an effective template for the development of therapeutically useful agents with antiviral and antitumor activity. It can also serve as a precursor to oligonucleotides, which may be useful in antisense and gene therapy and as biochemical probes.[1] For example, the modification of uridine to 2′-deoxy-2′-fluoro-β-L-arabinofuranosyl-5-methyluracil (L-FMAU)[2] provides a compound with potent anti-hepatitis B virus (HBV) activity that can also serve as a building block of antisense oligonucleotides or small interfering RNAs.

The 4′-oxonucleoside, a first-generation nucleoside (Figure 1), has played a key role in the development of clinical or biochemical probes. However, the emergence of adverse effects such as resistance and toxicity, and chemical and enzymatic degradation, has stimulated the search for new templates and has led to the discovery of 4′-thionucleosides[3] and 4′-carbonucleosides,[4] which are second-generation nucleosides. The 4′-thionucleoside and the 4′-oxonucleoside are bioisosterically related and are known to adopt the same conformation.[5]

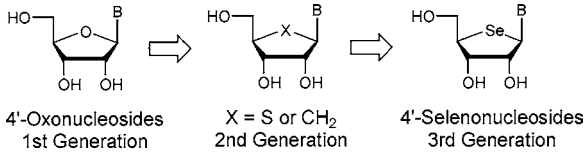


Figure 1. Rationale for the design of the target nucleosides.

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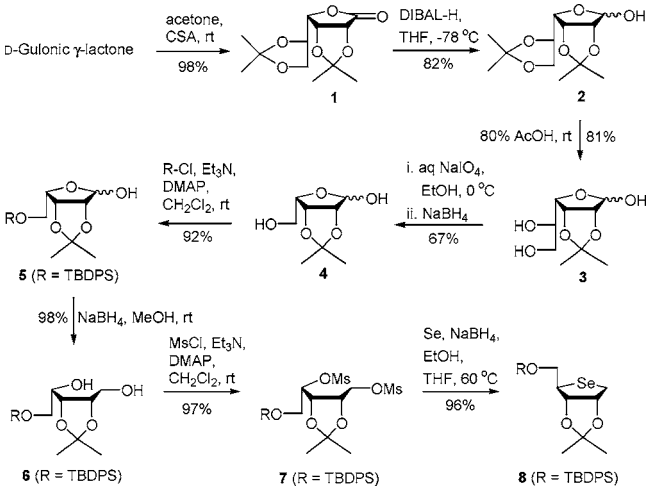
Notwithstanding the potent biological activity and metabolic stability of 4′-thionucleosides, only a few compounds have entered clinical development because the compounds are generally highly cytotoxic. The 4′-carbonucleoside is also bioisosterically related to the 4′-oxonucleoside and exhibits excellent chemical and metabolic stability about the glycosidic bond. However, it has been shown to have a conformation that is very different from that of the 4′-oxonucleoside, which results in the loss of biological activity.[6] Thus, as a part of our continuing efforts to search for a novel template for the development of new therapeutic or biochemical probes, we turned our attention to 4′-selenonucleosides, third-generation nucleosides which are also bioisosterically related to the 4′-oxo- or 4′-thionucleosides. Although selenonucleosides[7] have been reported as precursors to 2′,3′- and 4′,5′-unsaturated nucleosides or oligonucleotides containing 2′-selenonucleosides,[8] no examples of 4′-selenonucleosides have so far been reported in the literature, due to the difficulties of their synthesis.

Uridine assumes the C2′-exo/C3′-endo twist (Northern) conformation.[9] Among the stereoelectronic effects driving the conformation of uridine are the [O4′−C1′−C2′−O2′] and [O4′−C4′−C3′−O3′] gauche interactions which cancel one another.[10] The other gauche effect, involving [O2′−C2′−C1′−N1], tends to drive the *N*/*S* equilibrium to the *S* conformation, but this effect is weaker with π-deficient pyrimidines; the dominant driving force leading to the *N* conformation in uridine is the anomeric effect, which is much stronger in pyrimidines than in purines. The same forces described for uridine, pertain with 4′-thiouridine, but are weaker in this case. The result is the same; the *N* conformation is still preferred. However, in 4′-selenouridine, all stereoelectronic effects are expected to be overwhelmed by the size of selenium and steric interactions driving the conformation to the C2′-*endo*/C3′-*exo* twist (Southern) conformation. This unusual conformation of 4′-selenouridine could play important roles in developing new therapeutic agents or biochemical probes for studying antisense oligonucleotide or small interfering RNA (siRNA) interactions with potential therapeutic targets. This unusual conformation could also prove very useful for studying the substrate properties of 4′-selenonucleoside-5′-triphosphate (4′-seleno-NTP) and 2′-deoxy-4′-selenonucleoside-5′-triphosphate (2′-deoxy-4′-seleno-NTP) toward RNA and DNA polymerases, respectively. Thus, in addition to the first synthesis of 4′-selenonucleosides, it is also of great interest to compare the conformation of a 4′-selenonucleoside with that of the 4′-oxonucleoside. We wish to report here the first synthesis and the unusual conformation of 4′-selenonucleosides. Our strategy for the synthesis of 4′-selenonucleosides was to condense the 4-selenoxide with uracil or cytosine, using a Pummerer-type condensation. The 4-selenoxide was easily synthesized from D-gulonic-γ-lactone.

Based on this synthetic strategy, we prepared the 4-selenosugar **8** from D-gulonic-γ-lactone, as shown in Scheme 1.

Scheme 1. Syntheis of the Key Selenosugar **8**



2,3;5,6-di-*O*-Isopropylidene-D-gulonic-γ-lactone (**1**),[11] prepared from D-gulonic-γ-lactone, was converted to the L-lyxose derivative **4**,[12] using modifications of the reported procedures.[12] Treatment of **1** with DIBAL-H at −78 °C afforded the lactol **2** in 82% yield. Selective hydrolysis of **2** with 80% aqueous acetic acid gave **3** in 81% yield. Oxidative cleavage of diol **3** with sodium metaperiodate followed by reduction of the resulting aldehyde with sodium borohydride afforded the L-lyxose derivative **4** in 67% yield. Selective protection of the primary hydroxyl group of **4** with TBDPSCl gave the silyl ether **5**, which upon treatment with sodium borohydride provided the diol **6** in excellent yield. Mesylation of diol **6** with mesyl chloride in the presence of triethyl amine afforded the dimesylate **7**. Treatment of **7** with selenium in the presence of sodium borohydride in EtOH−THF at 60 °C furnished the 4-seleno sugar **8** in 96% yield.[13] The presence of two doublets of doublets, at δ 2.96 and 3.14 ppm, and disappearance of two methyl peaks of dimesylates, at δ 3.00 and 3.07 ppm, in the 400 MHz ^{1}H NMR spectrum of compound **8** in $CDCl_3$ clearly indicated that cyclization

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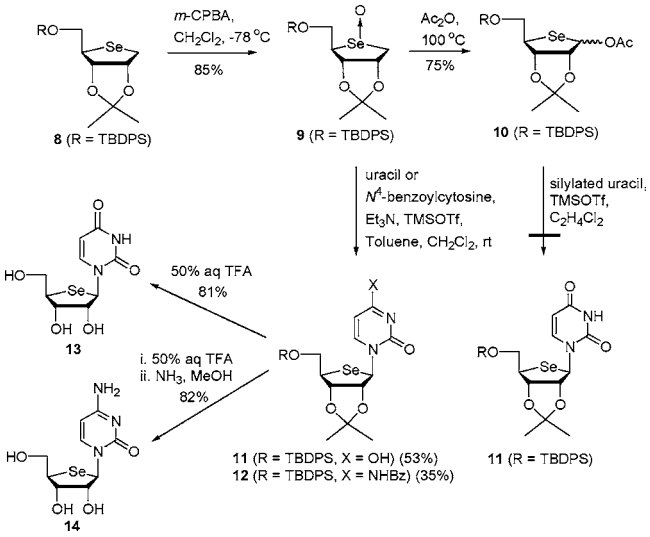
had occurred, and this was further confirmed by spectral and analytical data.

With the 4-selenosugar **8** in hand, the next goal was to synthesize the 4′-selenouridine **13** and 4′-selenocytidine **14** from **8** (Scheme 2). Thus, oxidation of **8** with *m*-CPBA gave

Scheme 2. Synthesis of the Final Nucleosides **13** and **14**



the unstable selenoxide **9** as a diastereomeric mixture, which was immediately subjected to a Pummerer rearrangement[14] in the presence of Ac_2O at 100 °C to give the acetate **10**. However, the conventional condensation method which reacted **10** with silylated uracil in the presence of TMSOTf failed to give the desired product **11**, and it was therefore decided to attempt a direct Pummerer-type base condensation[15] with the selenoxide **9**. This condensation method afforded the desired uracil derivative **11** (53%) and the corresponding cytosine derivative **12** (35%) without formation of their corresponding α-isomers. It is hypothesized that as in the case of 4′-thionucleosides,[15] the condensation reaction of selenoxide **9** with uracil proceeded via the α-selenocarbocation intermediate formed by an E2 anti elimination under the reaction conditions, in which the sole formation of the *β*-isomer is attributable to a steric effect[11] from 2′,3′-*O*-isopropylidene group. The presence of two doublets at *δ* 6.35 (H-5) and 7.52 (H-6) ppm and a doublet of doublets at *δ* 5.56 (1′-H) ppm in the 400 MHz ^{1}H NMR of **11** in $CDCl_3$ clearly indicated that condensation had occurred as expected. The anomeric assignment of **11** could not be confirmed by ^{1}H NMR NOE but was established by X-ray crystallography (vide infra). Removal of the protecting groups from **11** with aqueous 50% trifluoroacetic acid furnished the desired final nucleoside **13**, whose structure was confirmed by X-ray crystallography.[16]

Compound **12** was similarly converted to the cytosine derivative **14**. X-ray crystallographic analysis showed that 4′-selenouridine **13** assumes an unusual C2′-*endo*/C3′-*exo* twist (Southern) conformation unlike uridine[9] which takes the C2′-*exo*/C3′-*endo* twist (Northern) conformation (Figure 2), indicating that in 4′-selenonucleoside, the *anti* orientation

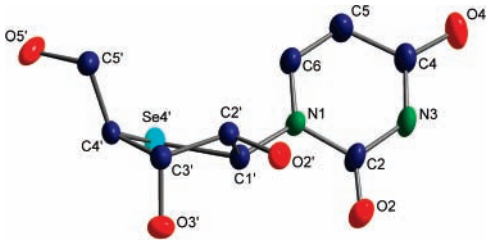


Figure 2. X-ray crystal structure of **13**.

was more favorable than the *gauche* orientation, presumably due to the steric effects of selenium. This unusual conformation may confer resistance to RNA cleaving enzymes such as RNAse H and nuclease. Furthermore, the O5′-hydroxyl group in **13** is also in the unusual *ap* conformation, distinct from uridine in which this hydroxyl group adopts the +*sc* orientation. The unusual *ap* orientation of the 5′-hydroxyl group of 4′-selenonucleosides may affect phosphorylation by the cellular kinases, but it is important to note that the conformation of compounds in the crystal and the solution forms can be very different.

Since each *N* or *S* conformation identified in the solid structures of the 4′-oxonucleoside coexist in a dynamic *N*/*S* equilibrium in solution,[17] we have also determined the conformation of 4′-selenouridine in the solution state and compared it with that of uridine. All protons in 4′-selenouridine except for 4′-H were shifted downfield from those in uridine (Tables 1 and 2 in the Supporting Information). The $^3J_{1'-H,2'-H}$ value of 4′-selenouridine was 8.70 Hz, while the same coupling in uridine was 4.62 Hz, indicating a different ribose ring puckering. This coupling is generally small for the C3′-*endo* ribose ring puckering found in A-form RNA and larger for the *C2′-endo* ribose ring puckering commonly found in B-form DNA.[18] In addition to the comparison of coupling constants, NOE experiments were also performed in order to confirm the conformation of 4′-

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(16) Crystal structure data for $C_{10}H_{12}N_2O_5Se$: $M_r = 319.18$, orthorhombic, space group $P2_12_12_1$ (no. 19), $a = 4.9520(1)$ Å, $b = 13.4320(3)$ Å, $c = 16.9083(5)$ Å, $V = 1124.65(5)$ Å^3, $T = 293(1)$ K, $Z = 4$, $\rho_{calc} = 1.885$ gcm^{-3}, $F(000) = 640$, crystal dimension $0.5 \times 0.3 \times 0.3$ mm^3, μ(Mo Kα) = 3.355mm^{-1}, Mo Kα radiation($\lambda = 0.7107$ Å). Of 11026 reflections collected in the 2θ range 3.0∠55.0° using an ω scans on a Rigaku Rapid R-axis diffractometer, 2565 were unique reflections ($R_{int} = 0.052$). The structure was solved and refined against F^2 using SHELXS97 and SHELXL97, 202 variables, wR2 = 0.064, R1 = 0.027 (the 2434 reflections having $F_o^2 > 2\sigma(F_o^2)$), GOF = 1.041, and max/min residual electron density 0.59/−0.49 eÅ^{-3}. Flack *x* parameter = 0.009(9). Further details of the crystal structure investigation(s) may be obtained from the Cambridge Crystallographic Data Centre (CCDC, 12 Union Road, Cambridge, CB2 1EZ (UK); Tel: (+44)1223-336-408, Fax: (+44)1223-336-033, e-mail: deposit@ccdc.cam.ac.uk) on quoting the depository no. CSD-652601.

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selenouridine. A strong NOE between H-6 and the 2′-H was detected in 4′-selenouridine, while no NOE effect was observed in case of uridine, indicating that 4′-selenouridine exists in the 3′-*exo* (*S*) conformation and uridine exists in the 3′-*endo* (*N*) conformation. The base can adopt two relative orientations, *anti* and *syn*, with respect to the sugar moiety, and these can be described by glycosidic torsion angle χ (O4′−C1′−N1−C2 in uridine, Se4′−C1′−N1−C2 in 4′-selenouridine). In the *anti* conformation, the six-membered uracil ring is pointing away from the sugar, thus providing a shorter distance between H-6 and the 2′-H in the 2′-*endo* or between H-6 and the 3′-H in the 3′-*endo* sugar ring than that between H-6 and the 1′-H.[19] These data are all consistent with the observation that replacement of the ribose ring oxygen by selenium causes a conformational change from a C3′-*endo* to a *C*2′-*endo* puckered ribose ring in the solution state, while the same type of uracil link exists with the ribose sugar ring since the *anti*-glycosidic torsion angle is unchanged.

In summary, using a Pummerer-type base condensation of selenoxide **9** as a key step, we have accomplished the first synthesis of 4′-selenonucleosides. These third-generation 4′-selenonucleosides exhibit an unusual *C*2′-*endo*/C3′-*exo* twist (Southern) conformation. It was revealed that all electronic effects found in uridine were overwhelmed by the steric effects from the bulky selenium, which force 4′-selenonucleosides to adopt the unusual Sourthern conformation. This unnatural conformation may provide valuable information for the study of the conformational preferences of metabolic enzymes such as kinases and nucleases. We are sure that the results reported here will open up a new era in nucleoside, nucleotide, and nucleic acids chemistry.

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Supporting Information Available: Complete experimental procedure for all compounds described herein and ^{1}H and ^{13}C NMR copies of **13** and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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