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Syntheses of Silatranyl- and Germatranyluridines

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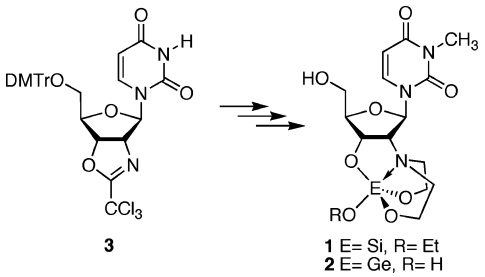
ABSTRACT



Silatranyluridine 1 and germatranyluridine 2 have been prepared in five steps from oxazolinouridine 3 in 27 and 29% yields, respectively. These compounds are novel transition-state analogues (TSAs) for RNA hydrolysis and offer a number of advantages over traditional vanadium- or rhenium-based TSAs. Germatrane 2 is completely stable in D_2O at room temperature, and the half-life of silatrane 1 in D_2O was found to be >7 days by ^1H NMR spectroscopy.

Transition-state analogues[1] (TSAs) have proven to be exceptionally useful compounds with wide-ranging applications. For example, TSAs can be used for the analysis of enzyme mechanisms and conformational changes by X-ray crystallography[2] or NMR spectroscopy.[3] They can also be used to prepare novel catalysts via catalytic antibody[4] or imprinted polymer methodologies.[5] Furthermore, information about the transition state can be used to design TSAs that are potent enzyme inhibitors.[6] However, there are relatively few examples of TSAs for RNA hydrolysis, in which nucleophilic attack of the ribose 2′-oxygen atom on the adjacent phosphorus atom leads to a trigonal bipyramidal oxyphosphorane transition state, Figure 1a. Existing TSAs for RNA hydrolysis include ribonucleoside derivatives that contain a vanadium, technetium, or rhenium atom attached via the 2′- and 3′-positions of the ribose, Figures 1b and 1c. These compounds have been utilized in a number of

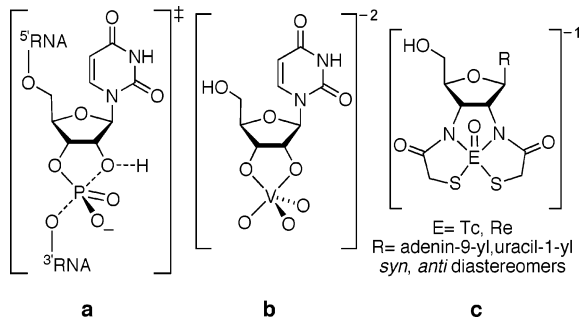


Figure 1. (a) Putative transition state for RNA hydrolysis. (b) Structure of the vanadate−uridine complex bound in the active site of RNase A as determined by X-ray crystallography. (c) Janda's technetium- and rhenium-based TSAs for RNA hydrolysis.

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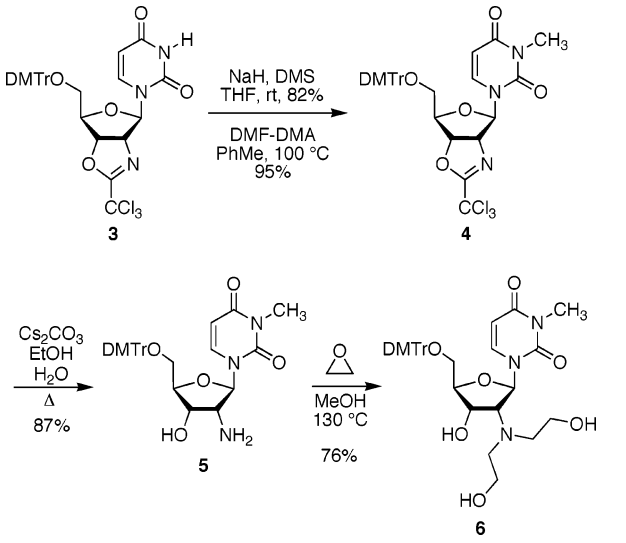
experiments. For example, the vanadate−uridine complex has been shown to be an inhibitor[7,8] of ribonuclease A (RNase A), and numerous X-ray crystal structures of the enzyme−TSA complex have been determined.[2,9] Furthermore, a similar vanadate−RNA complex was recently used to determine the TSA structure of the hairpin ribozyme by X-ray crystallography.[10] It is interesting to note that a similar approach with the hammerhead ribozyme[11] has not worked despite repeated attempts;[12] thus, vanadium TSAs may be limited in scope. Meanwhile, Janda and co-workers have shown that the rhenium compounds (Figure 1c) can inhibit ribonuclease U_2 and be used to elicit antibodies capable of catalyzing the cleavage of the phosphodiester bond in uridine 3′-(*p*-nitrophenyl phosphate).[13]

However, despite these successes, or maybe because of them, more and better RNA hydrolysis TSAs are needed. This is due to the unfortunate fact that the vanadium and rhenium complexes have significant limitations. For example, it is well established that oxovanadium alkoxides are especially unstable in aqueous solution and rapidly undergo ligand exchange.[14] In fact, vanadate tends to form dimers and trimers even at millimolar concentrations, complicating the structural analysis of the vanadate-nucleosides and their precise binding constants with RNase.[8] Thus, the vanadium TSA compounds used in the X-ray diffraction studies of RNase A[2,9] and the hairpin ribozyme[10] were prepared in situ. In fact, 1:1 complexes such as the one shown in Figure 1b have never been directly detected in solution[15] and may, in fact, only exist in enzyme active sites. The exact nature of the products formed from vanadate and a nucleoside in aqueous solution (i.e., outside an enzyme active site) has been the subject of considerable debate.[16] Detailed NMR and X-ray crystallographic studies[15,17] have led to the understanding that the major product is a 2:2 complex containing two trigonal bipyramidal vanadium atoms and two nucleoside ligands. On the other hand, the major limitations of the rhenium complexes (Figure 1c) are that they have relatively poor water solubility (a DMSO cosolvent is required in some experiments) and exist in a distorted square planar geometry rather than in a true trigonal bipyramid.[13]

The limitations inherent with existing RNA hydrolysis TSAs led us to consider atranyl-nucleosides[18] as alternatives. Atranes are well-characterized compounds in which triethanol amine, or a derivative thereof, complexes a central trigonal bipyramidal atom.[19,20] Specifically, we envisioned silatranyluridine **1** and germatranyluridine **2** as novel TSAs.

The syntheses of targets **1** and **2** first required the preparation of a key triethanol amine derivative, uridine-triol **6**, Scheme 1. Thus, uridine was converted to oxazolin-

Scheme 1



ouridine **3** using McGee's intramolecular cyclization method.[21] The uracil N-3 was then methylated, either by deprotonation/alkylation (NaH/dimethyl sulfate) or, more conveniently, by heating with dimethylformamide-dimethylacetal. Methylation of N-3 was necessary to prevent reactivity and to ensure the stability of the atranes.[22] Removal of the 2′,3′-oxazoline from **4** by cesium carbonate treatment[21] provided amino-alcohol **5** in 87% yield. Double alkylation of the 2′-amino group with ethylene oxide in a sealed tube provided 76% yield of the desired uridine-triol **6**.

With triol **6** in hand, preparation of the atrane moiety proceeded smoothly. Thus, heating **6** with tetraethyl ortho-

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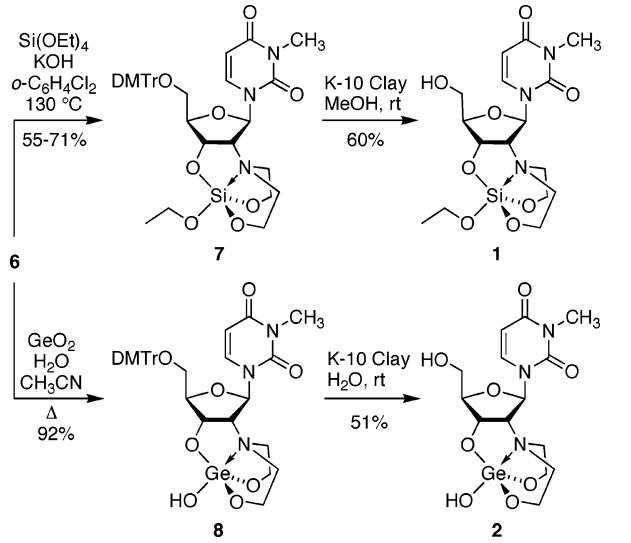
(22) If unmethylated, N-3 is subsequently alkylated by ethylene oxide further into the synthesis (see Supporting Information). The N3-methyl is also believed to provide stability to all of the atranyl-nucleosides (**1**, **2**, **7**, and **8**). This is due to the fact the 2′-nitrogen is a decent leaving group (it has a formal positive charge) and it is well-known that uridines with leaving groups positioned on the 2′-position are subject to anhydrouridine formation. See Supporting Information and the following reference: Yung, N. C.; Fox, J. J. *J. Am. Chem. Soc.* **1961**, *83*, 3060−3066.

Scheme 2

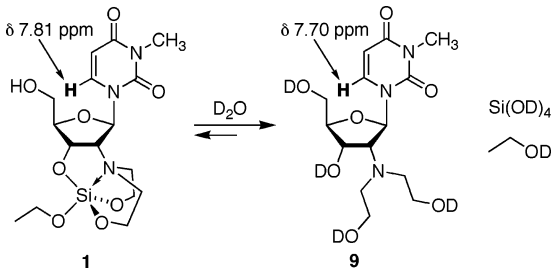


silicate provided 55−71% yield of silatrane **7**, Scheme 2. Alternatively, **6** was heated with germanium dioxide[23] in acetonitrile−water to provide 92% yield of germatrane **8**. Detritylation of **7** and **8** was best accomplished by treatment with montmorillonite K 10 (K-10 clay)[24] to produce silatranyluridine **1** and germatranyluridine **2** in 60 and 51% yields, respectively.

The structures of **1** and **2** are supported by NMR spectroscopy and mass spectrometry. Of particular note are the ^{29}Si NMR signal of **7** at −90.1 ppm, which is indicative of silatranes,[25] and the germanium isotope pattern in the mass spectrum of **2**.[26] One indicator of the successful formation of an atrane in these systems was found to be the coupling constant for the 1′- and 2′-protons in the ribose ring, $^3J_{H1'-H2'}$. Thus, in amine **5** and triol **6**, the ribose is in an S-type (C2′-endo) conformation with a relatively large $^3J_{H1'-H2'}$ (8−9 Hz), whereas in compounds that contain the atrane moiety (i.e., **1**, **2**, **7**, and **8**), the ribose conformation changes to an N-type (C3′-endo) conformation with a smaller $^3J_{H1'-H2'}$ (<2 Hz).[27] (Oxazolinouridines **3** and **4** also exist in an N-type conformation.)

In order for these TSAs to be employed as probes of enzymes or ribozymes, they must have adequate aqueous solubility and stability. Thus, the aqueous stability of the TSAs was determined by monitoring a D_2O solution by ^{1}H NMR spectroscopy.[18a] In the case of silatranyluridine **1**, the decrease in integration of the doublet for the H^6-proton in **1** and the corresponding increase in the integration of the doublet for the same proton in uridine **9** were monitored, Scheme 3. This experiment revealed that the half-life of **1**

Scheme 3



in the D_2O solution at room temperature is greater than 7 days.[26] As expected,[23] germatranyluridine **2** proved to be completely stable in water. The ^{1}H NMR spectrum of **2** in D_2O remained unchanged after 3 weeks at room temperature.[26]

In summary, silatranyluridine **1** and germatranyluridine **2** have been prepared in 27 and 29% yields, respectively, in five steps from oxazolinouridine **3**.[21] These compounds represent a new class of TSAs for RNA hydrolysis and possess several advantages over existing TSAs. Namely, unlike the vanadate TSAs, **1** and **2** can be isolated and purified. Furthermore, they have sufficient aqueous solubility and stability to be employed as probes of RNA hydrolysis enzymes. For example, insertion of TSAs **1** or **2** at the cleavage site (replacing C17) of the hammerhead ribozyme should allow a TSA X-ray crystal structure to be obtained.[12,28] Such a structure will aid in the elucidation of this ribozyme's catalytic mechanism[29] and may address the contested issue regarding the magnitude of conformational rearrangement during catalysis.[30] Compounds **1** and **2** should also prove to be useful as TSAs for the generation of new catalysts.[4,5,13c] Finally, just as silicon- and germanium-containing amino acids have been prepared and studied,[31] one can view compounds **1** and **2** as novel examples of silicon- and gemanium-containing nucleosides.[32]

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Supporting Information Available: Experimental procedures for the preparation of compounds **1**, **2**, and **4**−**8**; spectral data (^{1}H and ^{13}C NMR and mass spectra for compounds **2** and **4**−**8** and ^{1}H NMR and mass spectra for **1**); ^{1}H NMR spectra for compounds **1**, **2**, and **5**−**8**; ^{13}C NMR spectrum for compound **4**; ^{29}Si NMR spectrum of compound **7**; the mass spectral germanium isotope pattern of **2**; ^{1}H NMR spectra for the aqueous stability studies of **1** and **2**; and a discussion of the N-3 methyl group's role in the stability of the atranyluridines. This material is available free of charge via the Internet at http://pubs.acs.org.

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