Biomimetic Modeling of the First Substrate Reaction at the Active Site of Ribonucleotide Reductases. Abstraction of H3′ by a Thiyl Free Radical[1]

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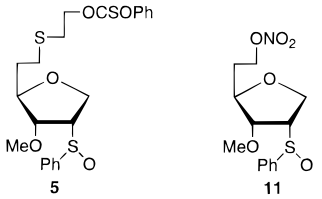
We have obtained the first *conclusive* chemical evidence consistent with the controversial first two steps in the mechanism postulated to occur during reductive deoxygenation at the active site of ribonucleotide reductases.[2,3] Generation of a proximal primary aliphatic thiyl radical in a tetrahydrofuran model substituted at C2 with a radical leaving group results in abstraction of H3 and elimination of phenylsulfinyl from C2.

Ribonucleotide reductases catalyze the deoxygenative reduction of ribonucleoside 5′-(di or tri)phosphates to 2′-deoxynucleotides and provide the only de novo source of DNA components. The ribonucleoside 5′-diphosphate reductase (RDPR) from *Escherichia coli* has been studied extensively.[4] Its R1 homodimer subunit contains substrate and allosteric binding sites and cysteine residues required for catalysis. The R2 homodimer contains a diiron chelate and an essential tyrosyl free radical.[2−5] Mammalian and certain viral-encoded RDPRs are similar. A postulated radical-cascade mechanism for substrate reduction invokes long-range electron transfer between •OTyr122 (in R2) and Cys439 (in R1) at the active site interface. The •SCys439 radical generated in proximity with the β face of the substrate is proposed[2,3] to abstract the 3′-hydrogen atom as the first substrate-activation step (Scheme 1[3]). Abstraction of H3′ from **1** by a primary aliphatic thiyl radical to generate **2** has aroused debate,[2e] owing to the absence of an appropriate chemical precedent.

We have shown that aminyl or oxyl radicals at C6 of hexofuranosyl models abstract H3 by a [1,5]-hydrogen shift.[1,6] Treatment (Bu_3SnD/AIBN/benzene/Δ) of 6-azido or 6-*O*-nitro precursors produces C3 radicals that undergo deuterium transfer from the stannane to give 3-[^{2}H] product(s). The absence of (H → D) exchange at C3 with a 6-S• radical in models that operate with 6-O• (60−80%) or 6-N• (∼20%) radicals was troubling.[1] However, RDPR executes abstraction of H3′ and a [1,2]-electron shift coupled with hydrogen transfer from O3′ to O2′ and "irreversible" loss of water from C2′ to give the stabilized oxallyl radical **3** (Scheme 1).[3] We now have synthesized thioether **5** (and control nitrate ester **11**) with a phenylsulfinyl radical leaving group[7] at C2 to more closely model the enzyme process.



Bu_3SnH (2 equiv)/AIBN (2 equiv)/benzene was added (24 h, syringe pump) to a refluxing solution of **5** (Scheme 2) in benzene. The concentrated mixture contained (^{1}H NMR) vinyl ether **9** and 3-*O*-methyl-2-(phenylsulfinyl)-containing products (∼2:3). Chromatography gave the somewhat unstable and volatile dihydrofuran **9** (21%) and 1,4-anhydro-2,5-dideoxy-3-*O*-methyl-2-(phenylsulfinyl)-6-thio-D-*ribo*-hexofuranitol (**10**, 52%). Formation of **9** and **10** is consistent with attack of a tributylstannyl radical on **5** to give **6**, followed by double homolytic β-elimination to generate thiyl radical **7** and ethylene. Intramolecular [1,5]-hydrogen transfer of H3 to the 6-thiyl radical and elimination of phenylsulfinyl radical from **8** would produce **9**. Coupling of **7** with tributylstannyl radical, and S−Sn bond cleavage upon chromatography, would give **10**. Conversion of **7** to **9** represents the first "relevant" biomimetic modeling of the proposed abstraction of H3′ from C3′ of ribonucleotides by •SCys439 of RDPR.

Formation of 6-S• radicals is assured by indirect Barton−Robins[8] generation via *S*-{2-[(phenoxythiocarbonyl)oxy]ethyl} group removal, and high dilution reduced rates of bimolecular coupling of tributylstannyl and thiyl radicals. Furan models minimize steric/stereoelectronic effects at C1 and preclude radical coupling with nucleobases (C8 of adenine, C6 of uracil).[9] The 6-*O*-nitro ester **11** served as a positive control with demonstrated ability to abstract H3 (via generation of a 6-O• radical),[1,6] and the sulfoxide **11** was thermally stable in refluxing benzene for 72 h.

Addition (5 h, syringe pump) of Bu_3SnH (2 equiv)/AIBN (2 equiv)/benzene to a refluxing solution of **11** in benzene resulted in exclusive formation of vinyl ether **14**. Benzoylation of the somewhat volatile **14** gave **15** (69% from **11**). Formation of **14** is consistent with abstraction of H3 (1,5-shift) to the 6-oxyl radical of **12** and β-elimination of phenylsulfinyl from **13**.[1,6] The stability of the phenylsulfinyl radical precludes its participation in chain reactions involving Bu_3SnH. Stoichiometric quantities of initiator were required, and only trace formation of **14** was observed with 10−15% molar ratios of AIBN.

The abstraction of H3′ from C3′ by •SCys439 has remained controversial,[2e] and it is often assumed that thiyl radicals are poor hydrogen abstractors because thiols are excellent hydrogen donors. However, the chemistry of sulfur radicals is complex.[10] Bond dissociation energies for certain RS−H and RR′(HO)C−H systems are similar,[2e,11] but rates of hydrogen abstraction by thiyl radicals (RH + •SR → •R + HSR) are generally ∼10^4 slower than the reverse donation of hydrogen to alkyl radicals by thiols.[11,12]

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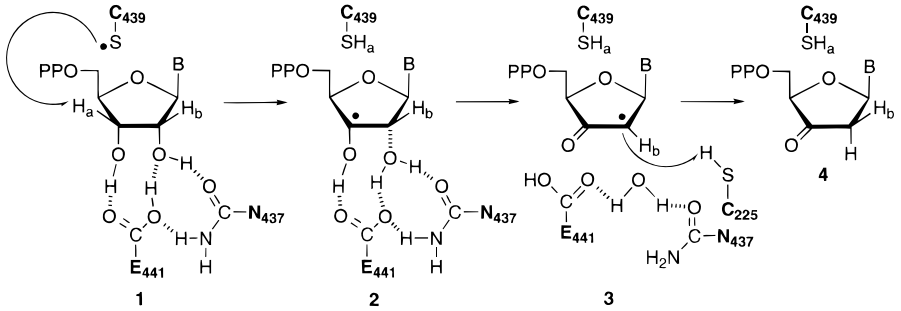
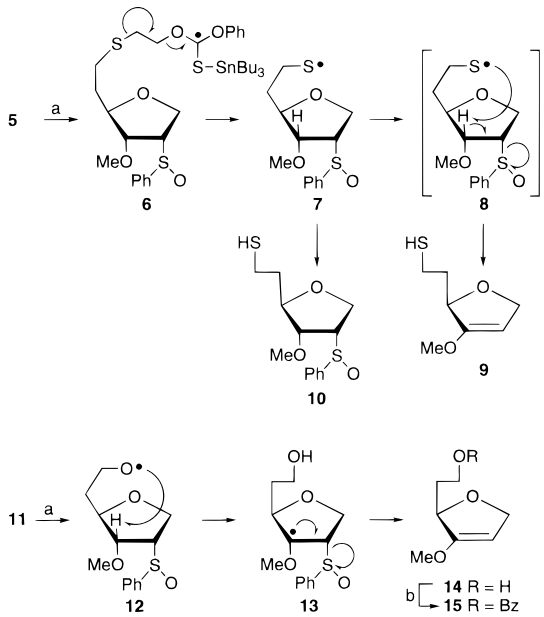
Scheme 1[a]



[a] Early steps of the Stubbe−Siegbahn mechanism for substrate reduction with RDPR.[2,3]

Scheme 2[a]



[a] (a) Bu_3SnH/AIBN/benzene/Δ. (b) BzCl/DMAP/pyridine/CH_2Cl_2.

It was shown in 1966 that treatment of *β*-hydroxy thioethers with thiyl radicals gave thiols and ketones,[13] and evidence for hydrogen abstraction by thiyl radicals has been noted.[2e,10−14] However, Roberts recently demonstrated that highly electrophilic thiyl radicals such as •$SSiPh_3$ efficiently catalyze the racemization of (*R*)-2-(acetoxymethyl)tetrahydrofuran (via α-hydrogen abstraction), whereas a •SCys(OMe) model was "totally ineffective" as a catalyst.[14b] Thus, hydrogen abstraction by thiols in isolated models, taken out of context of the aliphatic cysteinyl radical abstraction of a proximal secondary carbinol hydrogen on a furanosyl ring,[2e] does not provide compelling evidence for such a process at the enzyme active site.

In summary, we have prepared **5** and demonstrated hydrogen abstraction from C3 by an aliphatic thiyl radical with accompanying elimination of phenylsulfinyl radical from C2 to generate **9** (and a parallel positive control with oxyl radical generation from **11** to produce **14**). This biomimetic modeling of abstraction of H3′ by •SCys439 *conclusively* demonstrates the feasibility of step 1 in Scheme 1 for the first time. It is compatible with Siegbahn's calculations that indicate a "concerted" [1,2]-electron shift from C3′ to C2′, hydrogen shift from O3′ to O2′, and elimination of hydrogen-bonded water from C2′ with minimal charge separation.[3] Hydrogen transfer from Cys225 to C2′ at the α-face of **3** produces the 2′-deoxy-3′-ketonucleotide intermediate **4** in the reductive deoxygenation sequence executed by the remarkable RDPR.

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Supporting Information Available: Synthesis (Scheme 3) and experimental details with characterization and spectral data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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