

Biomimetic Modeling of the Decomposition of 2'-Chloro-2'-deoxynucleotides by Ribonucleotide Reductases To Give 3(2H)-Furanones Which Can Effect Mechanism-Based Inactivation by Michael-Type Alkylation¹

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Ribonucleotide reductases are crucial biosynthetic enzymes that catalyze the conversion of ribonucleotides to 2'-deoxy-nucleotide monomers for DNA synthesis. The ribonucleoside diphosphate reductase (RDPR) from *Escherichia coli* (EC 1.17.4.1) is composed of two nonidentical subunits R1 and R2. R1 subunits contain allosteric control sites and redox dithiol/disulfide pairs, and R2 subunits contain a diiron chelate and a tyrosine-centered free radical. Mammalian RDPRs have a similar composition, whereas RTPR from *Lactobacillus leichmannii* requires ribonucleoside triphosphate substrates and employs adenosylcobalamin as the radical initiator.² Stubbe and co-workers³ have proposed generic mechanisms for RDPRs in which the tyrosyl radical^{4a,b} in R2 (via long-range electron transfer with a cysteine in R1 to give a proximate thiyl radical^{4c}) initiates the reduction cascade by abstraction of the 3'-hydrogen atom from nucleotide substrates. The resulting C3' radical is proposed to lose O₂' as water in a heterolytic cleavage step followed by hydrogen/electron transfers via redox-active cysteine residues in R1 to give overall replacement of the 2'-hydroxyl group by hydrogen with complete stereoretention. X-ray crystal structure determinations of R2^{5a} and R1^{5b} are in harmony with this model.

In 1976, Thelander and co-workers reported that 2'-azido- and 2'-chloro-2'-deoxynucleoside 5'-diphosphates were potent inactivators of RDPR.⁶ Sjöberg and co-workers found that inactivation of RDPR by 2'-azido-2'-deoxynucleotides was accompanied by appearance of new EPR signals for a nitrogen-centered radical and concomitant decay of peaks for the tyrosyl radical,^{7a} which was the first direct evidence for free radical chemistry. The structure of the nitrogen-centered radical has been studied extensively and shown to be derived from the azide moiety.^{7b-e} Stubbe and co-workers⁸ demonstrated that EPR

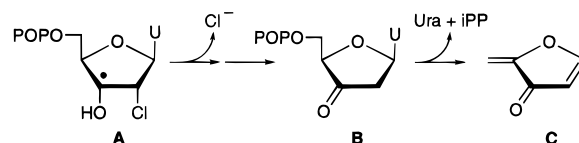


Figure 1.

signals for the tyrosyl radical in R2 were not diminished during inactivation of RDPR by 2'-chloro-2'-deoxynucleotides.⁶ However, the presence of the tyrosyl radical was necessary to trigger elimination of chloride from the substrate via A (Figure 1). Decomposition proceeded via the 2'-deoxy-3'-keto intermediate B with β-elimination of H2'/uracil and H4'/inorganic pyrophosphate to produce the Michael acceptor 2-methylene-3(2H)-furanone (C) which effected covalent alkylation/inactivation of the enzyme.

We now describe synthesis of precursors and generation of C3' free radicals containing O3'. This provides the first chemical models for simulation of radical initiation (at C3') and radical elimination from C2', which resulted in the cascade proposed^{1b,c} to occur during inactivation of ribonucleotide reductases by 2'-chloro-2'-deoxynucleotides. Since a radical center generated in a 1,5-relationship with H3' on the sugar moiety of a nucleoside should abstract H3', this process would mimic the initiation step in the proposed enzyme mechanism.³ We were gratified to observe that 6'-alkoxyl radicals generated in situ by treatment of 6'-nitrate ester⁹⁻¹² derivatives of homouridine (e.g., **8** or **11**, Scheme 1) with tributylstannane/AIBN participated in relay abstraction of H3' to generate C3' radicals.¹³

Oxidation^{14a} (at C3) of 1,2,5,6-di-O-isopropylidene-α-D-glucofuranose, stereoselective reduction,^{14b} benzoylation of O3, and hydrolytic removal of the terminal isopropylidene group^{14c} gave α-D-allofuranose derivative **1**^{14c,15} (~65% overall). The Barton deoxygenation¹⁶ of cyclic 5,6-O-thionocarbonates appeared to be a straightforward route to the 5-deoxy sugar, but the 5,6-O-thionocarbonate of **1** gave moderate yields of the desired product upon treatment with Bu₃SnH/AIBN. Significant 6-deoxy isomer and other byproducts¹³ were produced, and similar results for analogous deoxygenations have been noted.¹⁷ Regioselective acetylation¹⁸ of **1** gave primary acetate **2** (93%)

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(9) Alkoxyl radicals are generated by treatment of nitrate esters with Bu₃SnH¹⁰ and by photolysis of nitrites.^{11,12} Such alkoxyl radicals undergo a 1,5-hydrogen shift^{10b} via the well-known six-membered transition state.^{11,12}

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[†] Equivalent contributions to the success of this work were made by these coauthors.

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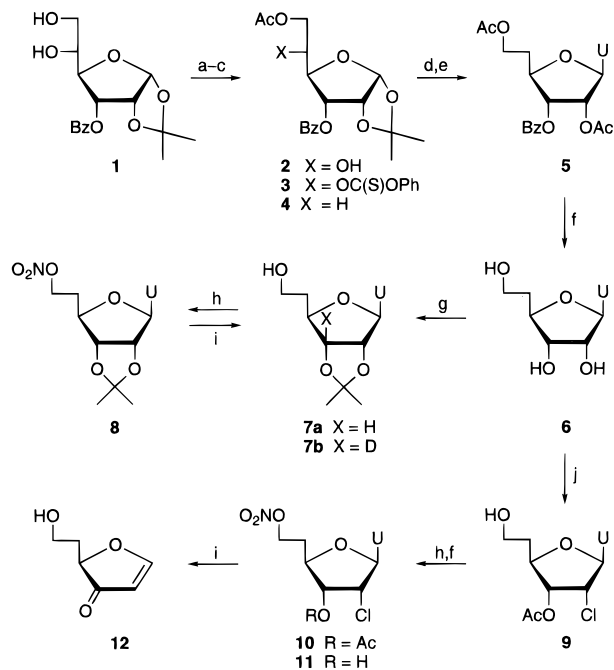
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Scheme 1^a

which was converted into its 5-*O*-phenoxythiocarbonyl derivative **3**. Deoxygenation¹⁹ of **3** ($\text{Bu}_3\text{SnH}/\text{AIBN}$) gave α -D-ribo-hexofuranose **4** (~78% overall). Removal of the isopropylidene group ($\text{TFA}/\text{H}_2\text{O}$), acetylation, and coupling²⁰ of the anomeric acetates with silylated uracil gave **5**. Deacylation of **5** gave 1-(5-deoxy- β -D-ribo-hexofuranosyl)uracil (**6**, homouridine; 63% from **4**). Homouridine (**6**) was converted into its 2',3'-*O*-isopropylidene derivative **7a** (82%) and nitrated²² to give **8** (92%).

Treatment of 6'-*O*-nitro ester **8** with $\text{Bu}_3\text{SnD}/\text{AIBN}/\text{benzene}$ at reflux for 1 h (conditions used for generation of alkoxy radicals from nitrates¹⁰) gave mixtures of **7a/7b** (86%, ~1:4) [$\sim 80\%$ reduction in the integrated ^1H NMR signal at δ 4.76 ($\text{H3}'$) and simplification of the doublet of doublets at δ 4.99 ($J_{2'-1'} = 2.0$ Hz, $J_{2'-3'} = 6.0$ Hz, $\text{H2}'$) to a doublet ($J_{2'-1'} = 2.0$ Hz) for **7a/7b**; MS (Cl , CH_4) m/z 300 (MH^+ , 100; **7b**), 299 (MH^+ , 22; **7a**)]. These results are in harmony with generation of an alkoxy radical at $\text{O6}'$, 1,5-abstraction of $\text{H3}'$ via the obligate six-membered transition state,^{11,12} and quenching of the $\text{C3}'$ radical by deuterium transfer from the stannane.

Treatment of homouridine (**6**) with α -acetoxyisobutyryl chloride gave the expected²³ 2'-chloro-3'-*O*-acetyl derivative **9** (31%). Nitration²² of **9** and deacetylation of **10** gave 1-(2-chloro-2-deoxy-6-*O*-nitro- β -D-ribo-hexofuranosyl)uracil (**11**, 75%).

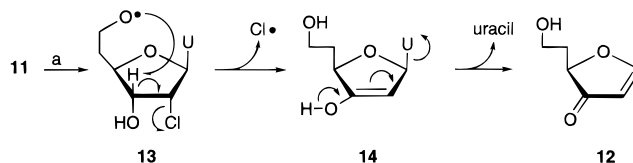
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Scheme 2^a

^a (a) $\text{Bu}_3\text{SnD}/\text{AIBN}/\text{benzene}/\Delta$.

Treatment of **11** with $\text{Bu}_3\text{SnD}/\text{AIBN}/\text{benzene}/\Delta$ resulted in total decomposition of **11** with formation of uracil and 2-(2-hydroxyethyl)-3(2*H*)-furanone (**12**), a homologated analogue of the 2-methylene-3(2*H*)-furanone formed by incubation of 2'-chloro-2'-deoxynucleoside 5'-diphosphates with RDPR.^{8a}

The structure of the somewhat unstable enone **12** was indicated by NMR and HRMS spectra and confirmed by synthesis of 2-[2-((*tert*-butyldimethylsilyl)oxy)ethyl]-3(2*H*)-furanone (the TBDMS derivative of **12**) from 2-deoxyglucose.²⁴ The formation of **12** is in harmony with results on the $\text{C3}'$ oxidation of 5'-*O*-tritylthymidine. The resulting 2'-deoxy-3'-keto derivative undergoes β -elimination under mild conditions to give 2-[(trityloxy)methyl]-3(2*H*)-furanone.²⁵

A plausible mechanism for the conversion of **11** into **12** is illustrated in Scheme 2. Treatment of **11** with $\text{Bu}_3\text{SnD}/\text{AIBN}/\text{benzene}/\Delta$ should generate 6'-alkoxy radical **13**, which should abstract $\text{H3}'$ by a 1,5-hydrogen atom transfer. Departure of the chlorine atom^{1b,c} (rather than chloride⁸) would produce enol **14**. Conjugate elimination (or tautomerization of **14** to the 3'-ketone and β -elimination) of uracil would give **12**.

In summary, we have constructed 6'-*O*-nitrohomouridine esters and demonstrated exchange of $\text{D3}'$ for $\text{H3}'$ under free radical conditions. Generation of a 6'-hydroxyl radical, 1,5-hydrogen atom transfer of $\text{H3}'$, and deuterium transfer from the stannane to the resulting $\text{C3}'$ radical follow established precedents. Treatment of the 6'-*O*-nitro ester of 2'-chloro-2'-deoxyhomouridine under analogous conditions resulted in decomposition to give uracil and 2-(2-hydroxyethyl)-3(2*H*)-furanone. This provides direct chemical evidence for a radical-induced cascade that mimics the postulated process for mechanism-based inhibition of ribonucleotide reductases by 2'-chloro-2'-deoxynucleotides. We propose that departure of a chlorine atom^{1b,c} is a plausible pathway for this process.

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Supporting Information Available: Experimental details and characterization/spectral data for compounds **2–12** (6 pages). See any current masthead page for ordering and Internet access instruction.

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(24) (a) Methyl 2-deoxy- α -D-arabino-hexofuranoside was prepared^{24b} from 2-deoxyglucose and was converted^{15,24c} into methyl 2,5-dideoxy-6-*O*-TBDMS- α -D-glycero-hexofuranosid-3-ulose. Treatment of this ketone with TEA/MeOH effected β -elimination to give the 6-*O*-TBDMS derivative of **12**. An analogous β -elimination occurs with methyl 2-deoxy-5-*O*-trityl- α -D-glycero-pentofuranosid-3-ulose to give 2-[(trityloxy)methyl]-3(2*H*)-furanone.^{25a} (b) Walker, T. E.; Ehler, D. S.; Unkefer, C. J. *Carbohydr. Res.* **1988**, *181*, 125–134. (c) Robins, M. J.; Guo, Z. Unpublished results.

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