Biomimetic Modeling of the Decomposition of 2′-Chloro-2′-deoxynucleotides by Ribonucleotide Reductases To Give 3(2*H*)-Furanones Which Can Effect Mechanism-Based Inactivation by Michael-Type Alkylation[1]

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Ribonucleotide reductases are crucial biosynthetic enzymes that catalyze the conversion of ribonucleotides to 2′-deoxynucleotide 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/dilsulfide 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-workers[3] 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-workers[8] demonstrated that EPR 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 aceptor 2-methylene-3(2*H*)-furanone (**C**) which effected covalent alkylation/inactivation of the enzyme.

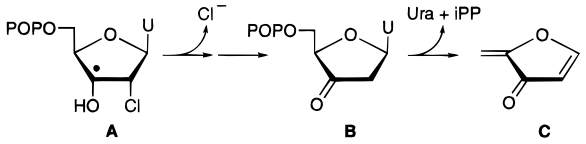

Figure 1.

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 ester[9−12] 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 deoxygenation[16] 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 Bu_3SnH/AIBN. Significant 6-deoxy isomer and other byproducts[13] were produced, and similar results for analogous deoxygenations have been noted.[17] Regioselective acetylation[18] of **1** gave primary acetate **2** (93%)

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

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(1) (a) Nucleic Acid Related Compounds. 92. (b) Part 91: Robins, M. J.; Wnuk, S. F.; Hernández-Thirring, A. E.; Samano, M. C. *J. Am. Chem. Soc.* In press. (c) Chlorine atoms could be reduced to chloride anions by proximal thiol groups in the enzyme.

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(9) Alkoxyl radicals are generated by treatment of nitrate esters with Bu_3SnH[10] 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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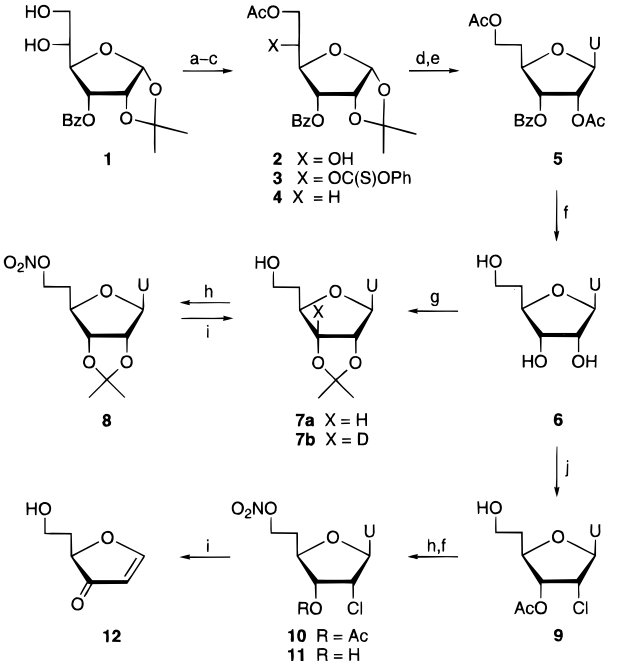
Scheme 1[a]



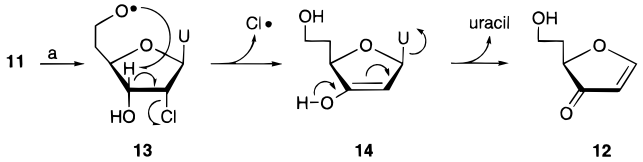
[a] (a) AcCl/*sym*-collidine/CH_2Cl_2/−78 °C. (b) PTCCl/DMAP/CH_3CN. (c) Bu_3SnH/AIBN/toluene/Δ. (d) (i) TFA/H_2O; (ii) Ac_2O/pyridine. (e) Persilylated uracil/$SnCl_4$/CH_3CN. (f) NH_3/MeOH. (g) Me_2CO/HC$(OEt)_3$/*p*-TsOH. (h) HNO_3/Ac_2O/−60 °C. (i) Bu_3SnD/AIBN/benzene/Δ. (j) (i) $(CH_3)_2C(OAc)COCl/CH_3CN$/Δ; (ii) 0.05 M HCl/MeOH.

which was converted into its 5-*O*-phenoxythiocarbonyl derivative **3**. Deoxygenation[19] of **3** (Bu_3SnH/AIBN) gave α-D-*ribo*-hexofuranose **4** (∼78% overall). Removal of the isopropylidene group (TFA/H_2O), acetylation, and coupling[20] of the anomeric acetates with silylated uracil gave **5**. Deacylation of **5** gave 1-(5-deoxy-β-D-*ribo*-hexofuranosyl)uracil[21] (**6**, homouridine; 63% from **4**). Homouridine (**6**) was converted into its 2′,3′-*O*-isopropylidene derivative **7a** (82%) and nitrated[22] to give **8** (92%).

Treatment of 6′-*O*-nitro ester **8** with Bu_3SnD/AIBN/benzene at reflux for 1 h (conditions used for generation of alkoxyl radicals from nitrates[10]) gave mixtures of **7a**/**7b** (86%, ∼1:4) [∼80% reduction in the integrated ^{1}H NMR signal at δ 4.76 (H3′) and simplification of the doublet of doublets at δ 4.99 ($J_{2'-1'} = 2.0$ Hz, $J_{2'-3'} = 6.0$ Hz, H2′) to a doublet ($J_{2'-1'} = 2.0$ Hz) for **7a**/**7b**; MS (CI, CH_4) *m/z* 300 (MH^+, 100; **7b**), 299 (MH^+, 22; **7a**)]. These results are in harmony with generation of an alkoxyl radical at O6′, 1,5-abstraction of H3′ via the obligate six-membered transition state,[11,12] and quenching of the C3′ radical by deuterium transfer from the stannane.

Treatment of homouridine (**6**) with α-acetoxyisobutyryl chloride gave the expected[23] 2′-chloro-3′-*O*-acetyl derivative **9** (31%). Nitration[22] of **9** and deacetylation of **10** gave 1-(2-chloro-2-deoxy-6-*O*-nitro-β-D-*ribo*-hexofuranosyl)uracil (**11**, 75%).

Scheme 2[a]



[a] (a) Bu_3SnD/AIBN/benzene/Δ.

Treatment of **11** with Bu_3SnD/AIBN/benzene/Δ 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 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 Bu_3SnD/AIBN/benzene/Δ should generate 6′-alkoxyl radical **13**, which should abstract H3′ by a 1,5-hydrogen atom transfer. Departure of the chlorine atom[1b,c] (rather than chloride[8]) 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 D3′ for H3′ under free radical conditions. Generation of a 6′-hydroxyl radical, 1,5-hydrogen atom transfer of H3′, and deuterium transfer from the stannane to the resulting 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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