Elimination of Chlorine (Radical) or Tosylate (Anion) from C2′ of Nucleoside C3′ Free Radicals as Model Reactions Postulated To Occur at the Active Site of Ribonucleotide Reductases[1]

Morris J. Robins,* Zhiqiang Guo, and Stanislaw F. Wnuk

Department of Chemistry and Biochemistry
Brigham Young University
Provo, Utah 84602-5700

Received January 21, 1997

Ribonucleotide reductases (RNRs) catalyze the conversion of ribonucleoside 5′-di- or -triphosphates to 2′-deoxynucleotides that are required for DNA biosynthesis.[2] The ribonucleoside diphosphate reductase (RDPR) from *Escherichia coli* (EC 1.17.4.1) has two nonidentical subunits (R1 and R2) whose structures have been determined by X-ray crystallography.[3] The R1 subunit contains allosteric control sites and five cysteine residues that participate in catalytic turnover and/or as redox dithiol/disulfide pairs. The R2 subunit contains a diiron chelate and a tyrosine-centered free radical that is responsible for generation of a proximate thiyl radical[4] on R1 via coupled electron and proton transfer reactions. The thiyl radical has been proposed to initiate nucleotide reduction by abstraction of H3′ from the substrate ribonucleotide.[2c] Water (O2′) is then lost from C2′ of the resulting C3′ radical.[2a,5] Interaction of a carboxylate group (glutamate) with OH3′ has recently been invoked to assist with the heterolytic release of water.[2c]

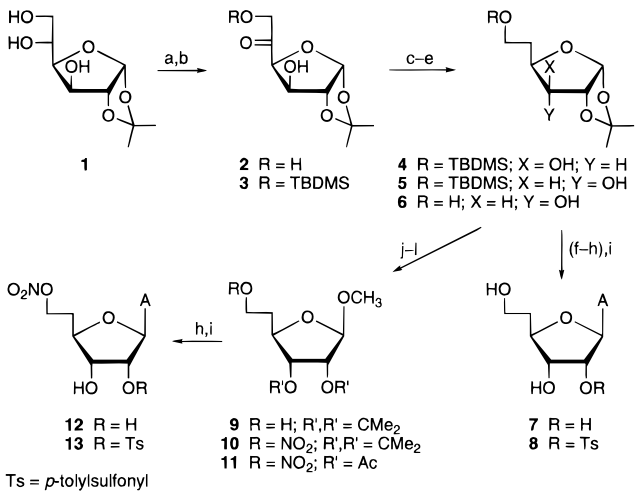
Abstraction of H3′ from 2′-chloro-2′-deoxynucleoside 5′-diphosphates to generate C3′ radicals was proposed[2a,c] to initiate reactions leading to inactivation of RDPR.[6] Spontaneous loss of chloride and transfer of the OH3′ proton to glutamate would give 2′-deoxy-3′-ketonucleotide intermediates without involvement of a cysteine pair on R1.[2a,c] Successive β-eliminations (H2′/base and H4′/pyrophosphate) would give the Michael acceptor 2-methylene-3(2*H*)-furanone, which could effect covalent inactivation of the enzyme.[7]

We recently demonstrated a mechanistic alternative for potential generation of the Michael acceptor that involved loss of a radical, rather than an anionic, species from C2′ of model 2′-substituted nucleosides.[8,9] Thus, treatment of 2′-(azido, bromo, chloro, iodo, or methylthio)nucleoside 3′-thionocarbonates with tributylstannane/AIBN resulted in loss of the 2′-substituents, as presumed radicals, to give 2′,3′-didehydro-2′,3′-dideoxy derivatives upon generation of C3′ radicals (without O3′); whereas 3′-thionocarbonates with 2′-fluoro or 2′-*O*-(mesyl or tosyl) substituents underwent radical-mediated hydrogen transfer to C3′ to give the 3′-deoxy-2′-[fluoro or *O*-(mesyl or tosyl)] products.[8] We also demonstrated that 6′-oxy radicals (*e.g.*, **20**, produced[10] from the 6′-*O*-nitro derivative **19**) generated OH3′-containing C3′ radicals that underwent chlorine loss and β-elimination (H/base) to provide the first model simulation of the initiation/elimination cascade that occurs during mechanism-based inactivation of RNRs with 2′-substituted nucleotides.[9] We now describe synthesis of 6′-*O*-nitro-2′-*O*-tosylhomoadenosine (**13**) and its treatment with Bu_3SnD/AIBN. Generation[10] of the 6′-oxy radical, relay abstraction of H3′ (by [1,5]-hydrogen shift via a six-membered transition state[11]) to produce the C3′ radical, loss of tosylate, and elimination (H/base) gave partially deuterated 2(*R*)-(2-hydroxyethyl)-3-(2*H*)-furanone (**18**).

Regioselective oxidation of 1,2-*O*-isopropylidene-α-D-glucofuranose (**1**) gave the 5-ulose **2**[12,13] (91%, Scheme 1). Silylation (O6) and deoxygenation (C5, via its tosylhydrazone[14]) of **3** gave the 5-deoxy sugar **4** (∼60% from **1**). Oxidation (C3) of **4** and stereoselective reduction[15] gave **5** which was desilylated to give ribohexofuranose **6** (77%). Homoadenosine[16] (**7**) was obtained by benzoylation (O3 and O6) of **6**, acetal hydrolysis, acetylation, coupling[17] of the anomeric acetates (adenine, $SnCl_4$), and deacylation. However, glycosyl cleavage occurred upon attempted nitration[18] of derivatives of **7**.

Methanolysis[19] of **5** and one-pot treatment with acetone gave **9**, which was nitrated[18] to give **10**. Acetal hydrolysis [Amberlite (H^+)] and acetylation gave **11** (81%) which was coupled

Scheme 1[a]



[a] (a) (i) $(Bu_3Sn)_2O/CHCl_3/\Delta$; (ii) Br_2. (b) TBDMSCl/pyridine. (c) (i) $TsNHNH_2$/MeOH; (ii) $NaBH_4$/MeOH/Δ. (d) (i) CrO_3/pyridine/Ac_2O; (ii) $NaBH_4$/EtOH. (e) TBAF/THF. (f) BzCl/pyridine. (g) (i) TFA/H_2O; (ii) Ac_2O/pyridine. (h) (i) Adenine/$SnCl_4$/CH_3CN; (ii) NH_3/MeOH. (i) (i) Bu_2SnO/MeOH; (ii) TsCl/Et_3N. (j) (i) HCl/MeOH; (ii) Me_2CO/$Me_2C(OMe)_2$/Δ. (k) HNO_3/Ac_2O/−60 °C. (l) (i) Amberlite IR-120 (H^+)/MeOH; (ii) Ac_2O/DMAP.

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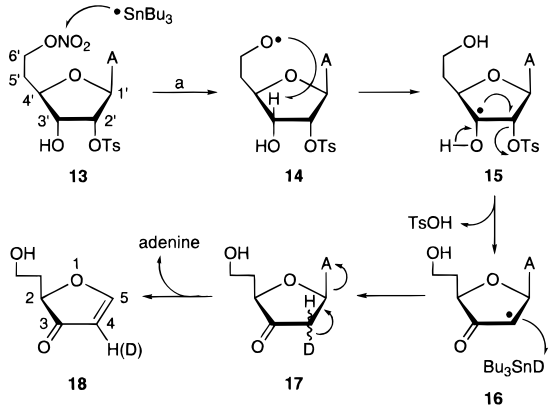
S0002-7863(97)00171-6 CCC: $14.00

Scheme 2[a]



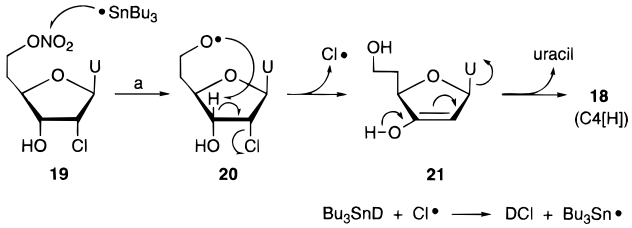
[a] (a) Bu_3SnD/AIBN/benzene/Δ.

(adenine, $SnCl_4$)[17] and deprotected to give 6′-*O*-nitrohomoadenosine (**12**). Regioselective 2′-*O*-tosylation[20] of **7** and **12** gave **8** (62%) and **13** (63%), respectively.

Treatment of **13** (Scheme 2) with Bu_3SnD/AIBN/benzene/Δ/2 h[10] resulted in its total conversion to adenine, 2(*R*)-(2-hydroxyethyl)-3(2*H*)-furanone[9] (**18**, 62%), and **8** [28%, deuterium transfer to the 6′-oxy radical; no observed ^2H exchange at C3′ (^1H NMR)]. ^1H NMR spectra of our homologated[9] furanone[7] **18** had ∼30% integrated reduction in the signal at δ 5.71 (H4), and HRMS peaks at *m/z* 129.0545 (100, MH^+ [$C_6H_9O_3$] = 129.0552) and 130.0619 (41, MH^+ [$C_6H_8DO_3$] = 130.0614) confirmed the incorporation of deuterium. A mechanism for conversion of **13** into **18** is in harmony with generation of 6′-oxy radical **14** followed by a [1,5]-shift of H3′ to give the C3′ radical **15**. Departure of the 2′-tosylate anion, induced by a [1,2]-electron shift, would produce the C2′-radical intermediate **16**. Deuterium transfer (Bu_3SnD to **16**) should occur selectively from the less-hindered α-face[21] to give the unstable 2′-deoxy-2′-deuterio-3′-ketohomoadenosines **17** [C2′ (*R*/*S*) ≈ 30:70] which would undergo β-elimination to give **18** (with ∼30% deuterium incorporation at C4). Loss of tosylate from **15** to give **16** is analogous to the [1,2]-hydride shift rearrangement[22,23] observed during conversion of 2′-*O*-tosyladenosine into 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine ($LiEt_3BH$/DMSO).[22] In that case, a 2′-deoxy-3′-keto intermediate is formed by a [1,2]-hydride shift (H3′ from C3′ to C2′) with tosylate expulsion. The present [1,2]-electron shift with generation of a carbonyl group (at C3′) and electron charge repulsion at C2′ would provide the driving force for expulsion of the 2′-tosylate (**15** → **16**).

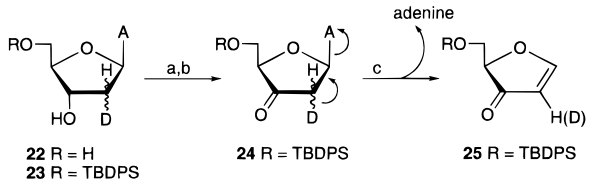
Deuterium incorporation into **18** [C4 (D/H) ≈ 30:70] occurred in contrast with our parallel treatment of 2′-chloro-2′-deoxy-6′-*O*-nitrohomouridine (**19**) (no ^2H in **18**).[9,24] Departure of a chlorine atom, rather than a chloride anion,[7] from **20** followed by elimination (H/base) gives enol **21**.[9] Deuterium transfer from Bu_3SnD to chlorine would propagate radical chains (Scheme 3, no ^2H incorporation into **18**); whereas tosylate loss from **15** produces radical **16** which would undergo deuterium transfer from Bu_3SnD to propagate the **13** to **16** radical chains (Scheme 2).

Scheme 3[a]



[a] (a) Bu_3SnD/AIBN/benzene/Δ.

Scheme 4[a]



[a] (a) TBDPSCl/pyridine. (b) Dess−Martin periodinane/CH_2Cl_2. (c) Bu_3SnD/AIBN/benzene/Δ.

Support for the latter mechanism was provided by parallel treatment of the 2′-deoxy-3′-keto[25] derivatives **24** (Scheme 4). Silylation of 2′-deoxy-2′-deuterioadenosine[21] [**22**; C2′ (*R*/*S*) ≈ 85:15) gave **23**, and Dess−Martin oxidation[26] (C3′) gave **24**. Downfield shifts of the H2′,2″ signals, reduction in their intensities, and simplification in splittings in ^1H NMR spectra were in harmony with **24**. Subjection of **24** to Bu_3SnD/AIBN/benzene/Δ gave 2(*R*)-{[(*tert*-butyldiphenylsilyl)oxy]methyl}-3(2*H*)-furanone (**25**), a dehomologated analogue of **18**. Integrated reduction (∼15%) of the ^1H NMR signal at δ 5.75 (H4) was in harmony with trans stereoselective β-elimination (D/adenine) from **24** (∼85% *S*-[^2H]). Spontaneous decomposition of such 2′-deoxy-3′-ketonucleosides with elimination of the base is well-known.[23a,27]

In summary, we prepared 6′-*O*-nitro-2′-*O*-tosylhomoadenosine (**13**) and demonstrated its radical-induced decomposition (Bu_3SnD/AIBN) to adenine and 2(*R*)-(2-hydroxyethyl)-3-(2*H*)-furanone (**18**) with ∼30% deuterium at C4. Parallel treatment of 2′-chloro-2′-deoxy-6′-*O*-nitrohomouridine (**19**) gave **18** without deuterium. This provides the first biomimetic models for differentiation between radical (no ^2H in **18**) and anionic (∼30% ^2H in **18**) departure of 2′-substitutents upon generation of O3′-containing C3′ radicals at active sites of RNRs.

Acknowledgment. We thank the American Cancer Society (DHP-34) and Brigham Young University development funds for support and Mrs. Jeanny K. Gordon for assistance with the manuscript.

Supporting Information Available: Experimental details and characterization/spectral data for compounds **2**−**13**, **18**, and **23**−**25** (10 pages). See any current masthead page for ordering and Internet access instruction.

JA970171C

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(24) Careful repetition of the **19**/Bu_3SnD/AIBN/benzene/Δ experiment[9] showed <2% ^2H incorporation at C4 of **18** (^1H NMR integration limit; no [^2H]-containing ion peaks with HRMS).
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