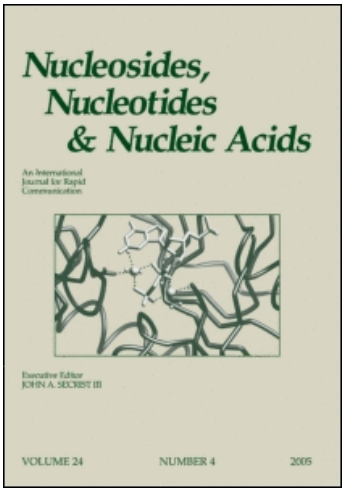

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Mechanism-Based Inhibition of Ribonucleotide Reductases: New Mechanistic Considerations and Promising Biological Applications

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MECHANISM-BASED INHIBITION OF RIBONUCLEOTIDE REDUCTASES: NEW MECHANISTIC CONSIDERATIONS AND PROMISING BIOLOGICAL APPLICATIONS

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ABSTRACT: Ribonucleotide reductases (RNRs) perform the *de novo* biosynthesis of 2'-deoxynucleoside 5'-(di or tri)phosphates. Inhibition of RNRs removes a crucial source of genetic components and enhances the probability of salvage incorporation of analogues into DNA. Several laboratories have clarified aspects of the reaction cascades initiated by generation of substrate nucleotide C3' free radicals by RNRs. New considerations for radical-mediated mechanism-based inhibition and biological applications are discussed.

The genetic material of most replicating systems is DNA, and replication cycles and cellular integration of genes to reproduce retroviruses also involve biosynthesis of DNA. Ribonucleotide reductases (RNRs) are ubiquitous enzymes that execute the only known conversion of 5'-(di or tri)phosphate esters of ribonucleosides into the requisite 2'-deoxy building blocks.[1,2] Inhibition of RNRs targets the primary source of DNA components, and depletion of pools of the natural substrates makes replicating systems more susceptible to incorporation of "fraudulent" nucleotide analogues.[3] Mechanism-based inhibition of RNRs is an appealing concept for the rational design of agents[4] against rapidly proliferating systems such as viruses and cancer cells. Recent experimental evidence is in harmony with promising biological applications of this concept.

RNRs are the common biological target of several structurally diverse compounds. Hydroxyurea (**1**) (Figure 1) has been employed as an anticancer drug for many years,[5] and has recently been used to impede the replication of the human immunodeficiency virus[3,6] (HIV). Anticancer activity has been demonstrated with two fluorine-containing nucleoside analogues, 2'-deoxy-2',2'-difluorocytidine[7,8] (gemcitabine, **2**) and (*E*)-2'-deoxy-2'-(fluoromethylene)cytidine[9] (**3b**), and also with 2'-deoxy-2'-methylenecytidine[10,11] (**3a**).

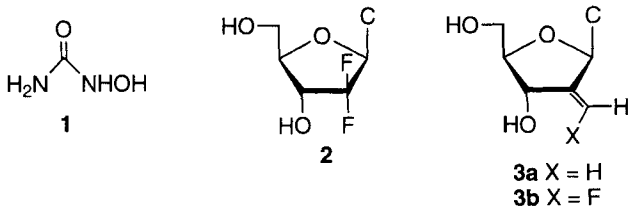


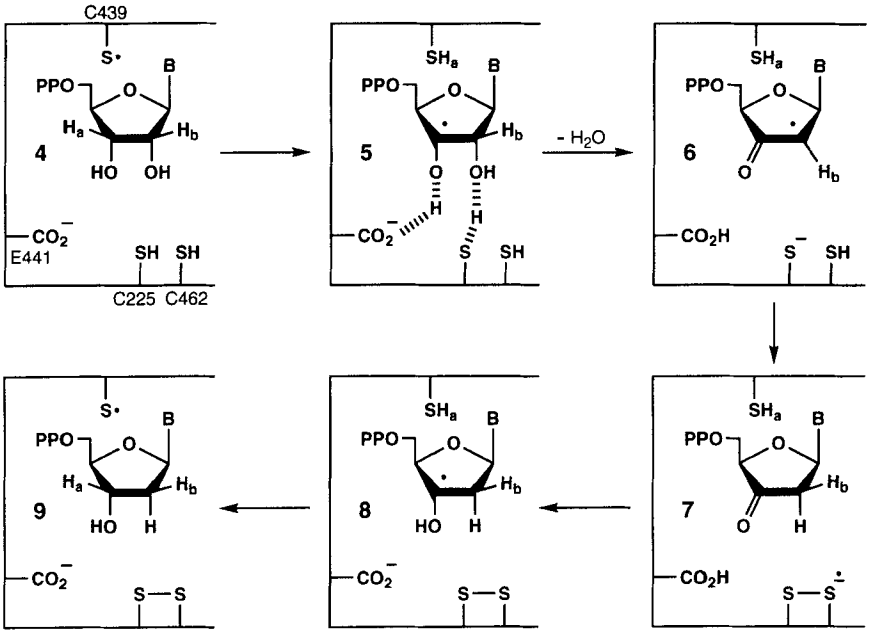
FIG. 1. Structures of some biologically active agents targeted to ribonucleotide reductases.

Mechanism-based inactivation of RNRs has been demonstrated with **1**,[5] as well as with the 5'-phosphorylated metabolites of **2**[7b,12] **3a**,[12a] and **3b**.[13]

Swedish investigators have determined the basic structural and functional features of RNRs.[1,14] The ribonucleoside 5'-diphosphate reductase (RDPR) from *Escherichia coli* has been studied most extensively, and RDPRs of mammalian cells and some viruses are similar. The *E. coli* enzyme is composed of two homodimeric subunits designated R1 and R2. The larger R1 subunit contains allosteric binding sites for regulation of substrate reactivity, and cysteine residues that participate in redox processes. R2 contains a diferric complex in proximity with the key tyrosyl free radical. Stubbe[2] has pursued extensive mechanism studies, with substrates and analogues, that are in general harmony with recent X-ray structures[14] of the enzyme subunits and analogue-bound complexes.

Scheme 1 illustrates Stubbe's update[2c] of the mechanism proposed for the reduction of substrates. The ultimate initiator for enzyme turnover is a "stable" tyrosyl free radical species (•OTyr122) that is "buried" within the R2 subunit. It is proposed[2c] that long-range electron and proton transfers occur to generate the Cys439 free radical (•SCys439) in close proximity with the β-face of the substrate ribonucleoside 5'-diphosphate (**4**). Abstraction of the 3'-hydrogen atom by •SCys439 gives HSCys439 and generates the C3' radical **5**. Base-promoted (Glu441) removal of the 3'-hydroxyl proton facilitates rapid loss of the hydrogen-bonded 2'-hydroxyl group (as a water molecule) to produce the α-keto radical **6**. Hydrogen atom transfer from the cysteine thiols (Cys225/462) in proximity with the α-face of **6** gives the 3-keto intermediate **7** with complete stereoretention of the 2'-hydrogen atom (H_b). Electron and proton transfers produce **8** plus the cystine disulfide and glutamate. The original 3'-hydrogen atom (H_a) is regained from HSCys439 by •C3' to complete the synthesis of the 2'-deoxynucleoside 5'-diphosphate (**9**) and regenerate •SCys439 for the next catalytic cycle.[2c] Electron/proton transfers, with NADPH as the ultimate reductant,[1a] convert the disulfide into the Cys225/462 dithiol pair for the next catalytic turnover with a new **4**. Amino acid residues invoked in Scheme 1 have been identified in X-ray crystal structures[14] of the R1 and R2 subunits of *E. coli* RDPR. A recent theoretical analysis[14g] supports the basic concepts of Scheme 1, but adds important new features.

Scheme 1[a]

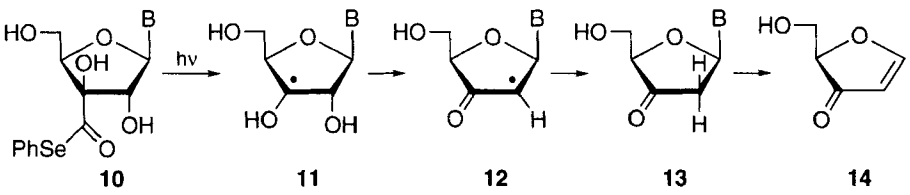


[a] Proposed substrate mechanism for ribonucleoside diphosphate reductase.[2c]

We recently demonstrated the first radical relay systems which generate 3'-radical models that undergo reaction cascades in biomimetic harmony with processes postulated to occur at the active sites of RNRs.[15] Lenz and Giese studied photolysis of *xylo*-nucleoside selenoester models.[16] Generation of 3'-radicals by cleavage of the C–Se bond and loss of CO (**10** → **11**) (Scheme 2) resulted in loss of the 2'-hydroxyl group to give **12**. Hydrogen transfer to **12** produced the 2'-deoxy-3'-ketonucleoside intermediate **13**, and β-elimination of H2'/base gave enone **14**.[16] These eliminations occur spontaneously, and are enhanced by mildly basic conditions.[17] The photolytic reaction cascade was subject to general base catalysis,[16] consistent with known leaving group reactivities at C2'[18] as well as the close proximity of Glu441 with the 3'-hydroxyl group in X-ray structures.[14] The base-promoted loss of water from C2' required an adjustment to the prior mechanism, in which acid-catalyzed loss of water from C2' to generate a cation radical was proposed.[2a,b]

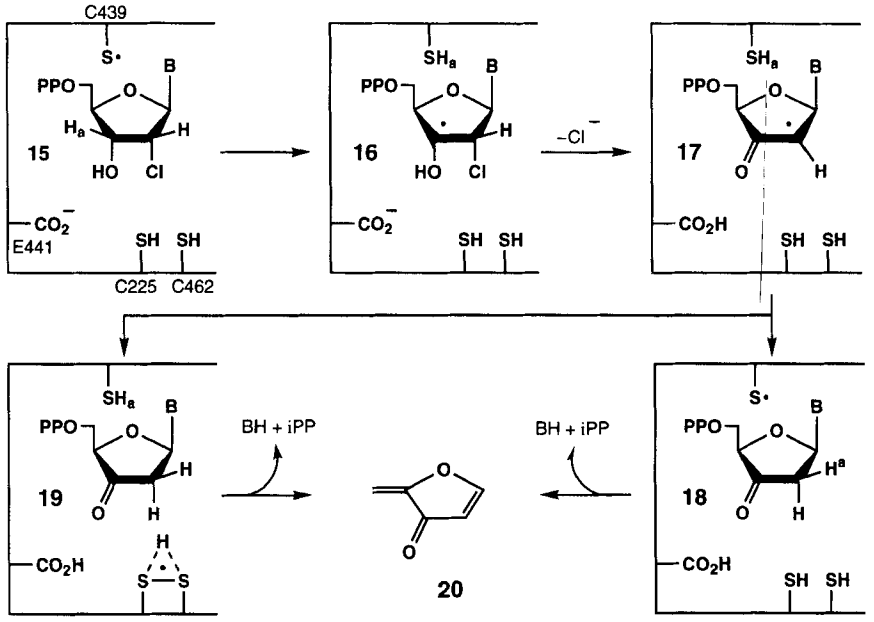
Mechanism-based inactivation of *E. coli* RDPR with 2'-azido or 2'-chloro analogues of 2'-deoxycytidine 5'-diphosphate was first reported by Thelander and coworkers.[19] The nucleotide analogues underwent fragmentation with concomitant irreversible inactivation of the enzyme, but different processes were involved. EPR revealed that inactivation with the 2'-azido analogues generated a nitrogen-centered free radical with accompanying loss of the

Scheme 2[a]



[a] Photolysis of nucleoside selenoester models in the presence of Bu_3SnH.[16]

Scheme 3[a]

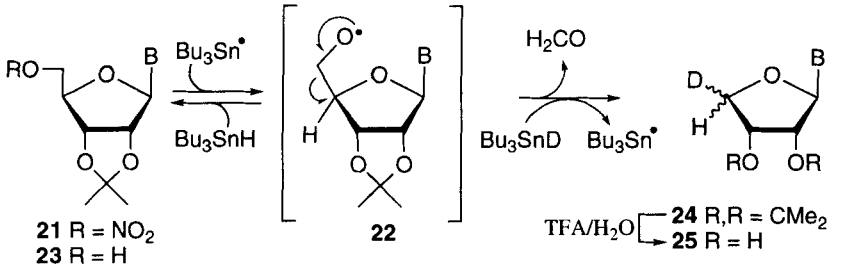


[a] Proposed anionic mechanism for inactivation of RDPR by 2'-chloro-2'-deoxyNDPs.[2c]

signal for •OTyr122.[20] This was the first experimental evidence in support of free radical chemistry associated with RNRs. Stubbe and coworkers pursued mechanistic studies with labeled substrates and analogues, spectroscopic techniques, and site-directed mutagenesis that have clarified many aspects of the reaction cascades initiated by C3' radical generation with RNRs.[2]

Scheme 3 illustrates Stubbe's update of mechanism-based inactivation of RDPR with 2'-chloro-2'-deoxyNDPs.[2c] Initiation of the process occurs by abstraction of H3' from **15** by •SCys439, the same as with substrate reduction (Scheme 1). Loss of chloride anion

Scheme 4

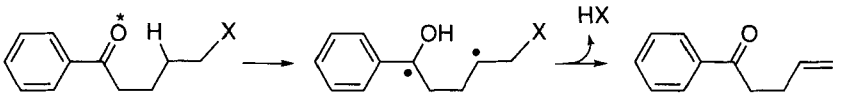


Series a: B = adenin-1-yl
b: B = uracil-1-yl

and the 3'-hydroxyl proton from the C3' radical **16** gives α-keto radical **17**, but with no involvement of the dithiol pair (Cys225/462).[2c] Abstraction of H_a from Cys439 by •C2', dissociation of the 2'-deoxy-3'-ketoNDP **18**, and successive β-eliminations (H2'/B and H4'/iPP) produce bis(en)one **20** [2-methylene-3(2*H*)-furanone]. Alternative abstraction of a hydrogen atom from the dithiol pair at the α-face of **17** gives the same 2'-deoxy-3'-ketoNDP **19** (except when H_a is isotopically labeled), but without regeneration of •SCys439 for initiation of another catalytic cycle. Dissociation of **19** and β-eliminations generate the Michael acceptor **20**, which alkylates nucleophiles on the enzyme and causes time-dependent inactivation. This mechanism[2c] and the earlier hypothesis for reduction of substrates involving cation radical intermediacy[2a,b] are based on Fenton chemistry[21] under acidic conditions.[22]

We have pursued biomimetic studies for over a decade[23] that are designed to simulate radical-initiated cascade reactions postulated to occur at active sites of RNRs. We sought a radical relay system in which a functional group would react to generate a free radical that would abstract H3' (analogous to this step involving •SCys439 with *E. coli* RDPR). Our first approach[23a] involved treatment of 5'-*O*-nitro esters[24] of two readily available 2',3'-*O*-isopropylidenenucleosides with tributylstannane/AIBN/benzene/Δ. Such conditions were known to effect cleavage of nitrate esters to generate oxyl radicals.[25] A nucleoside 5'-oxyl radical is in a 1,4-relationship with C3', whereas 1,5-hydrogen atom shifts[26,27] with oxyl radicals (6-membered ring transition state) were known to be required. Indeed, treatment of 2',3'-*O*-isopropylidene-5'-*O*-nitro[adenosine (**21a**) or uridine (**21b**)] (Scheme 4) with Bu_3SnH/AIBN/toluene/Δ resulted in formation of the 5'-oxyl radicals (**22**). Hydrogen transfer from the stannane gave 2',3'-*O*-isopropylidene[adenosine (**23a**) or uridine (**23b**)]. Minor product (40–50%) 9-(2,3-*O*-isopropylidene-β-D-erythrofuranosyl)adenine (**24a**) or the uracil analogue **24b** were formed by β-scission of the 5'-oxyl radicals. Formaldehyde was released to generate C4' radicals that underwent hydrogen transfer from the stannane

Scheme 5[a]



[a] Substituents included: X = Cl, Br, I, SR, SOR, SO_2R.[27]

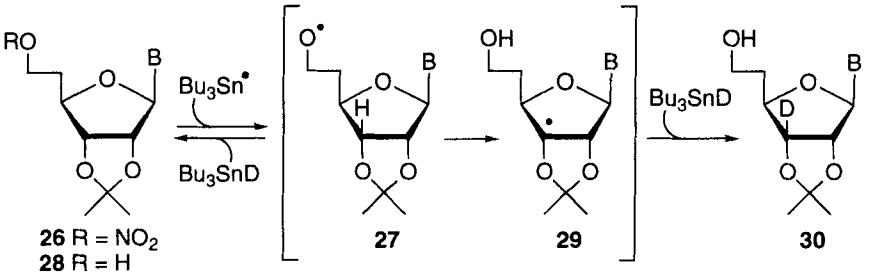
to give the dehomologated products **24**. No exchange of deuterium for H3' was detected in the major or minor products when Bu_3SnD was substituted for Bu_3SnH, and deuterium was incorporated only at C4' (preferentially at the β-face) of **24**. Deprotection of **24** completed a new conversion of ribonucleosides to their erythrofuranosyl counterparts **25**.

A 1,5-hydrogen atom shift had been observed[25b] with an oxyl radical generated from a nitrate ester. The Barton nitrite ester photolysis[26] as well as photolytic elimination studies with aryl ketones[27] had been demonstrated to require 1,5-transposition termini for efficient reactions. Wagner and coworkers had shown that aryl ketones with δ-(halo or sulfur) substituents underwent photolytic elimination to give the alkenyl ketone products expected from a 1,5-hydrogen shift from the γ-carbon to the excited carbonyl oxygen followed by loss of the δ-substituent radical[27] (Scheme 5). Therefore, we constructed homonucleoside analogues that could provide the requisite six-membered transition states. Carbohydrate transformations with glucose and coupling reactions with derivatives of adenine and uracil furnished the biomimetic substrates.[15]

Efficient ^2H for H3' exchange occurred upon treatment of 2',3'-*O*-isopropylidene-6'-*O*-nitro[homoadenosine (**26a**) and homouridine (**26b**)] with Bu_3SnD/AIBN/benzene/Δ, and 3'-deuterio-2',3'-*O*-isopropylidene[homoadenosine (**30a**) and homouridine (**30b**)] were obtained as major products in high overall yields[15] (Scheme 6). This is consistent with generation of the 6'-oxyl radical **27**, which is quenched in two competing pathways. Intermolecular transfer of deuterium from stannane to oxygen gives **28** [after aqueous (exchange) workup], and an intramolecular 1,5-hydrogen shift converts **27** into the C3' radical **29** that undergoes deuterium transfer from the stannane to give **30**. These and other model studies clearly demonstrated the viability of generation of C3' radicals via homolytic cleavage of 6'-*O*-nitro esters followed by the 1,5-hydrogen shift relay.

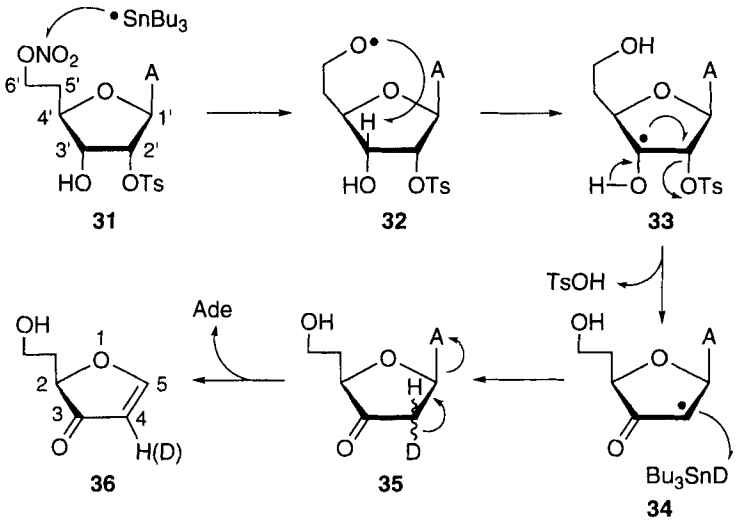
The 6'-*O*-nitro-2'-*O*-tosylhomoadenosine (**31**) (Scheme 7) substrate was prepared by selective functionalization of homoadenosine, and also by coupling of a carbohydrate derivative and adenine.[15b] Treatment of **31** with Bu_3SnD/AIBN/benzene/Δ resulted in formation of (*R*)-2-(2-hydroxyethyl)-3(2*H*)-furanone (**36**) [a homologue of **14** (Scheme 2), obtained by generation of •C3' in the selenoester photolysis studies of Lenz and Giese,[16] and an analogue of **20** (Scheme 3), the Michael alkylating agent that is proposed[2]

Scheme 6



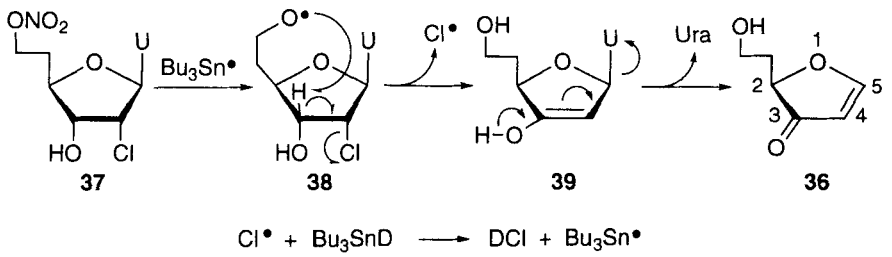
Series a: B = adenin-1-yl
b: B = uracil-1-yl

Scheme 7



to effect mechanism-based inhibition of RNRs]. Homolysis of the nitrate ester of **31** to give **32**, and 1,5-shift of H3' to the 6'-oxyl radical would generate **33**. Loss of the 3'-hydroxyl proton, with a [1,2]-electron shift[15b] from C3' to C2', would promote loss of the tosylate group from C2' and create the α-keto radical **34**. Transfer of deuterium from the stannane to C2' (preferentially at the less-hindered α-face) to give **35** followed by anti β-elimination ([^{2}H/H]2'/adenine) would give the observed enone **36**. Incorporation of deuterium (~30%) at C4 (furan numbering) of **36** was observed (^{1}H NMR and HRMS) in harmony with this mechanism, which involves known stereoselectivities for deuterium transfer to the α-face of C2' radicals[28] and anti-elimination of H2'/base. The structure of

Scheme 8



the unstable enone **36** was supported by independent synthesis of its *tert*-butyldimethylsilyl ether derivative, and a model anti β-elimination of [^{2}H/H]2'/adenine was demonstrated.[15]

We have succeeded in chemically modeling key steps in the proposed mechanism for reduction of substrate ribonucleotides by RNRs (Scheme 1). Homolytic cleavage of our nitrate ester generates a proximal 6'-oxyl radical, analogous to generation of the proximal •SCys439 by long-range electron transfer from the "buried" tyrosyl radical in RDPRs. The 1,5-shift of H3' to O6' models the abstraction of H3' by •SCys439. Loss of the 3'-hydroxyl proton and tosylate from C2' models abstraction of the 3'-hydroxyl proton by Glu441 and loss of water (hydrogen-bonded 2'-hydroxyl group). Transfer of deuterium from the stannane to C2', with ~70% stereoselection at the α-face, models the completely stereoselective transfer of hydrogen from the dithiol pair (Cys225/462) at the α-face of C2' by RDPRs. Our chemical system lacks the ability for electron transfer to the 3'-keto group and stereoselective delivery of H3' to the β-face of C3'. However, it is noteworthy that all steps prior to this function of RNRs have been modeled. It is remarkable that two altered RDPRs, obtained by site-directed mutagenesis (Cys225→Ser)[29a] and (Glu441→Gln),[29b] are modeled by our system. Those mutants also were unable to perform the final reduction steps, and the 3'-ketonucleotides (analogous to our **35**) were enzymatic end products.[29] Those 3'-ketonucleotides underwent successive β-eliminations to give the bis(en)one **20** (Scheme 3) Michael alkylation inactivator,[29] analogous to our enone **36** (Scheme 7).

We have studied other model reactions related to the mechanism-based inactivation of RNRs with 2'-chloro-2'-deoxynucleotides. Parallel treatment of 2'-chloro-2'-deoxy-6'-*O*-nitrohomouridine[15a] (**37**) (Scheme 8) with Bu_3SnD/AIBN/benzene/Δ resulted in formation of enone **36**. However, no deuterium incorporation at C4 (furan numbering) of **36** was detected.[15a,c] These results are consistent with homolytic cleavage of the nitrate ester of **37** followed by a 1,5-shift of H3' to O6' of **38**. Concomitant loss of a chlorine atom from **38** to give enol **39** is directly analogous to the photochemical elimination studies of Wagner[27] (Scheme 5). Loss of the 3'-hydroxyl proton from **39** and conjugate elimination of uracil,

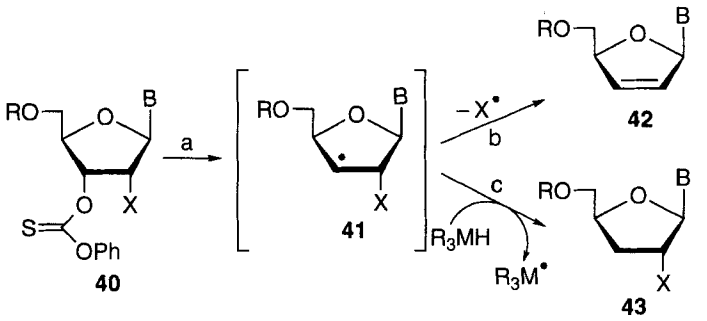
or conversion of the enol to the 3'-keto tautomer of **39** and β-elimination (H2'/uracil), would give enone **36**. In this case (Scheme 8), radical elimination of chlorine followed by deuterium transfer from stannane to chlorine would regenerate stannyl radicals to propagate chain reactions. No involvement of nucleoside radicals, beyond the initial relay generation of •C3', is required. In contrast, our substrate model reactions of **31** (Scheme 7) require free radical chain propagation by deuterium transfer from the stannane to •C2' of **34** (generated by loss of tosylate from **33**, rather than loss of a chlorine atom from **38**).

Our mechanistic hypothesis of Scheme 8 differs from that of Stubbe (Scheme 3) in which loss of a chloride anion (and a proton) from **16** is postulated to give α-keto radical **17**. Her rationalization[2c,30] of the experimental observations invokes abstraction of a hydrogen atom from Cys439 by •C2' (β-face), which results in overall transfer of isotope H_a from C3' to C2'. Dissociation of **18** from the enzyme leaves the active site available for catalytic turnover. Alternatively, abstraction of a hydrogen atom from the Cys225/462 pair (at the α-face of **17**) leaves $H_aSCys439$ quenched from further catalytic turnover. The hypothesized[2c] abstraction of H_a from Cys439 followed by dissociation of **18** from the enzyme, anti β-eliminations (H2'/B and H4'/iPP), and alkylation by [^3H_a]**20** are consistent with detection of enzyme-bound ^3H_a after inactivation of RDPR with 3'[^{3}H]-**15**.

The experimental observations also are compatible with interpretations derived from our Scheme 8. Loss of a chlorine radical (from **38**) followed by abstraction of a hydrogen atom from the proximal Cys225/462 pair by chlorine would give hydrogen chloride and a thiyl/disulfide radical. Attraction of the 3'-hydroxyl proton by Glu441 would promote the flow of negative charge to the C2' terminus of enol **39**. The resulting incipient enolate could abstract H_a from Cys439 at the β-face, or a proton from the Cys225/462 radical pair (or HCl) at the α-face, to give **18** or **19**. Thus, predicted observations from both Schemes 3 and 8 would be parallel subsequent to the rapid hydrogen atom abstraction and proton equilibration processes. The immediate oxidation state of the Cys225/462 pair would differ by one electron [Scheme 3 (anion) versus Scheme 8 (radical) leaving group], but electron transfer via thioredoxin/R1-cysteines and/or other pathways could blur any experimental distinctions. The hypothesis of Scheme 8 extends possibilities for the rational design of mechanism-based inactivators of RNRs. Substituents at C2' that have a propensity for participation in free radical cascades are viable candidates, as well as those which function preferentially in anion-generating processes.

We also studied radical elimination of groups at C2' upon generation of •C3' with no 3'-hydroxyl group. Treatment of 3'-*O*-(phenoxythiocarbonyl)-2'-(substituted)nucleosides (**40**) (Scheme 9) with Bu_3SnH/AIBN or $Ph_3SiH/(BzO)_2$ caused β-scission of the O3'–C3' bond to give 3'-radical intermediate **41**.[31] Elimination of chloro, bromo, iodo, azido, and methylthio radicals from **41** occurred spontaneously to produce the 2',3'-olefin **42**, but

Scheme 9[a]



[a] (a) [Bu_3SnH/AIBN or $Ph_3SiH/(BzO)_2$]/(benzene or toluene)/Δ.
(b) When **X** = Cl, Br, I, N_3, SMe. (c) When **X** = F, OSO_2Me, OTs.

only hydrogen transfer from the stannane or silane to C3' occurred to give **43** with the 2'-fluoro, 2'-*O*-methanesulfonyl, and 2'-*O*-tosyl compounds.[31] With no oxygen atom on •C3' of **41**, there are no α-nonbonding electrons available to promote a 1,2-electron shift from C3' to C2' and eliminate an anion. Therefore, even such good anionic leaving groups as methanesulfonate and tosylate remain bonded to C2' during intramolecular hydrogen transfer from the stannane. Also, no loss of fluoride from C2' was detected by careful analysis of the crude product from that reaction. Tosylates and bromides usually have similar rates in comparable leaving group reactions, and mesylates and tosylates are more reactive than chlorides. There is a greater propensity for elimination of free radicals than anions from C2' of the 3'-deoxy radical species, **41**, since all halogens but fluorine underwent elimination to give **42** (homolytic bond dissociation energies: R–F >> R–Cl > R–Br > R–I). Azido and alkylthio radicals can be generated readily, whereas fluorine atoms and sulfonyloxyl radicals are high energy species. RNRs can execute expulsion of a hydrogen-bonded 2'-hydroxyl group, and fluoride anions are detected upon incubation of 2'-deoxy-2'-fluoronucleotides.[2] However, recent theoretical studies[14g] indicate minimal charge separation in the transition state for cleavage of the C2'–O2' bond, and a concerted process is plausible[15c] for loss of toluenesulfonic acid from **33** (Scheme 7). Our present results and additional biomimetic studies indicate that radical elimination from C2' of •C3' species is favorable.

Recent mechanistic investigations with RNRs and promising biological applications are in harmony with free radical-mediated reaction cascades. Subsequent to the observation of mechanism-based inactivation of *E. coli* RDPR with 2'-azido-2'-deoxynucleotides,[19] EPR spectra revealed formation of a nitrogen-centered radical,[20] and extensive studies with isotope-labeled alternative substrates as well as labeled enzyme proteins were pursued.[32]

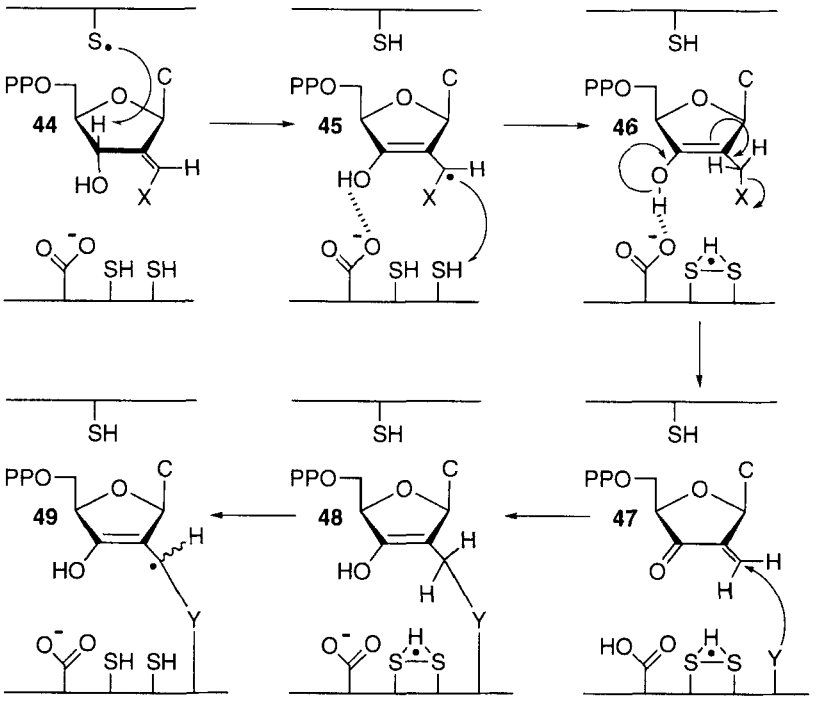
Identity of the spectra of the nitrogen-centered radical generated upon treatment of *E. coli* RDPR with 2'-[$^{15}N_3$]azido-2'-dUDP or our doubly labeled isotopomer, 2'-[$^{15}N_3$]azido-2'-[^{13}C]deoxyuridine 5'-diphosphate, provided compelling evidence for elimination of the azido group ([^{15}N]–[^{13}C] bond cleavage) prior to generation of the EPR-active species.[32] Product and EPR studies are consistent with loss of an azido radical from C2' of the initial C3' radical followed by reduction involving the proximal thiols to give dinitrogen ($^{15}N_2$) and a sulfur-linked nitrogen radical.[33]

Attempts to exploit biological activity with 2'-azido analogues have been noted, including inhibition of HIV replication in lymphoid cells with 2'-azido-2'-deoxycytidine.[34] Phosphorylation and biological activity of 2'-azido-(dUrd and dCyd),[35a] and studies with the bis(pivaloyloxymethyl) ester prodrug of 2'-azido-2'-dUMP have been reported.[35b] Hydroxyurea (**1**), the radical-quenching RDPR inactivator, has been shown to function effectively against HIV in combination with 2',3'-dideoxynucleosides, and clinical trials with AIDS patients have been promising.[3,6] Fontecave and coworkers prepared[36a] 2'-thio derivatives of uridine and cytidine, and demonstrated that 2'-thiouridine 5'-diphosphate is an effective inactivator of RDPR.[36b] Such 2'-thionucleotides might be subject to a variety of radical processes.

Promising biological results have been noted with three nucleoside analogues whose 5'-diphosphates are potent inactivators of RDPRs. The 5'-diphosphate ester of 2'-deoxy-2'-methylenecytidine (**3a**) (Figure 1) is a mechanism-based inactivator of RDPR,[12a] and **3a** has a wide spectrum of anticancer activity.[10,11] The 5'-diphosphate of gemcitabine[7a] (**2**) (Figure 1) is a potent mechanism-based inactivator of RDPR,[12] and its triphosphate is incorporated into DNA.[7b] McCarthy and coworkers have synthesized (*E*)-2'-deoxy-2'-(fluoromethylene)cytidine (**3b**), and this fluoro derivative of **3a** has a broad range of potent anticancer activity.[9] Scheme 10 illustrates Stubbe's rationalization[13] of the stoichiometric inactivation of RDPR with the diphosphate ester (**44b**) of **3b** and the accompanying EPR spectral data. Abstraction of H3' from **44b** by •SCys439 gives allylic radical **45b**, which removes a hydrogen atom from the Cys225/462 pair to give **46b**. Conjugate elimination via deprotonation of the 3'-hydroxyl group by Glu441 and loss of the fluoride anion generates enone **47**. Michael addition of a "Y" nucleophile gives **48**. Transfer of an allylic proton from **48** to the dithiol radical system produces allylic radical **49**, which is covalently bound to protein via the "Y" linker. An initially parallel sequence with the 2'-methylene analogue (**44a** → **46a**) followed by conjugate elimination of the 3'-hydroxyl proton and cytosine from C1' (rather than the illustrated loss of fluoride from the 2'-fluoromethyl group) would give the 4-methyl-2-methylene-3(2*H*)-furanone analogue of **20**.[12a]

It appears that the mechanism for reduction of ribonucleoside 5'-diphosphates by the RDPR of *E. coli* and related enzymes is approximated quite well by reactions illustrated[2c]

Scheme 10[a]



[a] Modified scheme to rationalize EPR spectra with isotopomers of **44b**[13] (X = F); (**44a**, X = H).

in Scheme 1, with recent modifications.[14g] Our biomimetic sequence (Scheme 7) models all but the final ketone reduction steps of this process. Mechanism-based decomposition of the 5'-diphosphate ester of the clinical anticancer drug[8] 2'-deoxy-2',2'-difluorocytidine (**2**) results in loss of fluoride,[12] and mechanism-based inactivations of RNRs are initiated by enzymatic removal of H3'. The mechanism in Scheme 3 invokes loss of chloride anion from the 3'-radical intermediate **16** to give **17**.[2c] However, our mechanism in Scheme 8 provides an alternative rationalization for loss of a free radical (chlorine atom) rather than an anion from C2'.

Research during the past decade has clarified a number of mechanistic questions. Our biomimetic studies provide supporting models for chemical steps involved in substrate reduction and mechanism-based inactivation of RNRs. *Both* heterolytic and homolytic cleavage pathways are available for elimination of groups from C2' of 3'-radical species. This invites design and biological evaluation of new putative inhibitors of ribonucleotide reductases constructed with substituents at C2' that could undergo activation by either one

or two-electron processes to generate active species that cause the mechanism-based inactivation of ribonucleotide reductases.

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