MODELING OF THE RIBONUCLEOTIDE REDUCTASES SUBSTRATE REACTION. HYDROGEN ATOM ABSTRACTION BY A THIYL FREE RADICAL AND DETECTION OF THE RIBOSYL-BASED CARBON RADICAL BY PULSE RADIOLYSIS

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Received April 21, 2011
Accepted May 24, 2011
Published online September 22, 2011

Dedicated to Professor Antonin Holý on the occasion of his 75th birthday in recognition of his outstanding contributions to the area of nucleic acid chemistry.

The 1,4-anhydro-5-deoxy-6-thio-D-*ribo*-hexofuranitol (**1**) was prepared from 1,2-*O*-isopropylidene-α-D-glucose in 10 steps. In a key step treatment of the 1,2-*O*-isopropylidenehexofuranose derivative with BF_3/Et_3SiH effected deacetonization and reductive deoxygenation at carbon 1. Pulse radiolysis experiments with 6-thiohexofuranitol **1** and its disulfide derivative demonstrated formation of the ribosyl-based carbon-centered radical upon generation of 6-thiyl radical in basic medium. The proposed [1,5]-hydrogen shift abstraction with generation of the C3 radical mimics the initial substrate reaction of RNRs. The reversible H-atom transfer has been quantified and was correlated with the computed rate constants for the internal H atom abstraction from C1, C2, C3 and C4 by the thiyl radical. The energy barrier for the H3 and H4 abstractions were calculated to be most favorable with the corresponding barriers of 11.1 and 11.2 kcal/mol, respectively.

Keywords: Radicals; Thiosugars; Pulse radiolysis; Ribonucleotide reductase; Carbohydrates; Bioorganic chemistry.

Ribonucleotide reductases (RNRs) catalyze the conversion of nucleotides to 2′-deoxynucleotides providing the monomeric precursors required for DNA replication and repair[1]. Inhibition of RNRs disrupts this primary source of

Collect. Czech. Chem. Commun. 2011, Vol. 76, No. 10, pp. 1223–1238

doi:10.1135/cccc2011085

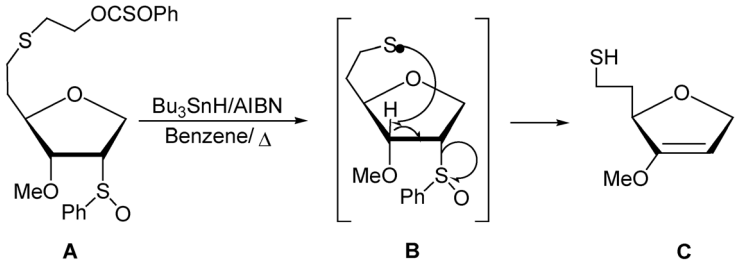
DNA components and therefore is an appealing concept for rational drug design[2]. In the first step of the mechanism of RNR a thiyl radical •SCys439 is generated in proximity to the β face of the nucleotide-substrate is proposed to abstract H3′ of the ribosyl, which promotes irreversible loss of water (C2′-H_2O) from the resulting C3′ radical[1,3,4]. Abstraction of H3′ by a primary aliphatic thiyl radical (•SCys439) to generate C3′ radical has aroused debate owing to the absence of appropriate chemical precedence. Stubbe based her original hypothesis[3,4] on the fact that thiyl radicals, generated via hydrogen atom abstraction by hydroxyl radical, were able to isomerize *cis*-2,5-dimethyl-tetrahydrofuran to its *trans* isomer[5]. Moreover, the rate constants for hydrogen atom abstraction from the activated C–H bonds (α-Hs of alcohols and ethers) by thiyl radical are approximately 10^4 times slower than the reverse donation of hydrogen to alkyl radicals by thiols[6–7].

$$R'H + RS^{\bullet} \underset{1 \times 10^7\ M^{-1}\ s^{-1}}{\overset{1–4 \times 10^4\ M^{-1}\ s^{-1}}{\rightleftharpoons}} R'^{\bullet} + RSH \qquad (1)$$

Chemical modeling by Giese[8] and Robins[9,10] as well as enzymatic studies with gemcitabine[11] and McCarthy's 2′-deoxy-2′-fluoromethylenecytidine[12–14] preserve the fundamental concepts of the Stubbe mechanism but detection of the ribosyl-based radical(s) during RNR-catalyzed deoxygenation of the natural substrates remains elusive. Nevertheless, kinetic studies of cytidine 5′-diphosphate with the E441Q mutant of *E. coli* class I RNR identified two radical species by EPR spectroscopy[15]. The first radical was established to be a disulfide radical anion[16] which gave rise to a second nucleotide-based radical of the semidione nature with a hyperfine coupling to two almost identical protons at C1′ and C4′ [17].

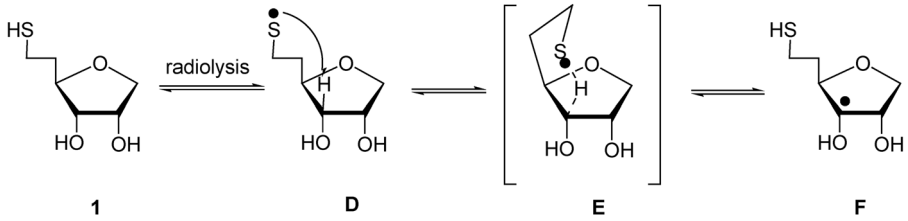
Robins and Ewing[18] demonstrated that thiyl free radical generated at C6 of hexofuranosyl model **A** abstracts H3 by a [1,5]-hydrogen shift (Scheme 1). (Analogous H-3 abstractions were also observed by an aminyl and oxyl radicals[19].) Thus, treatment of **A** with Bu_3SnH/AIBN/benzene/Δ generated thiyl radical by a radical fragmentation of *S*-2-[(phenoxythiocarbonyl)oxy]ethyl group, which initiated the abstraction of H3 via an intramolecular [1,5]-hydrogen transfer reaction with accompanying elimination of phenylsulfinyl radical from C2 (e.g. **B**) to generate **C**. The irreversible β-elimination was assumed to be a driving force for the equilibrium to shift towards product **C**, in spite of the poor ability of thiyl radicals to abstract the hydrogen from the activated carbons. In case of RNRs abstraction of H3′ and a

[1,2]-electron shift coupled with hydrogen transfer from O3′ to O2′ and irreversible loss of water from C2′ gives the stabilized oxyallyl radical[1,10].



SCHEME 1
Biomimetic modeling of the first step of substrate reaction at active site of RNR [18]

We now report synthesis and pulse radiolysis experiments with a 1,4-anhydro-5-deoxy-6-thio-D-*ribo*-hexofuranitol **1** which allowed us to detect the ribosyl-based carbon-centered radical(s) and to measure rate constant for the H abstraction(s). The computed rate constants for the internal H atom abstractions from C1, C2, C3 and C4 by the thiyl radical are correlated with the experimental results. The conversion of thiyl free radical **D** into carbon radical **F** through an intramolecular [1,5]-hydrogen shift (via an obligated six-membered transition state **E**) mimics the initial substrate reaction of RNRs (Scheme 2).



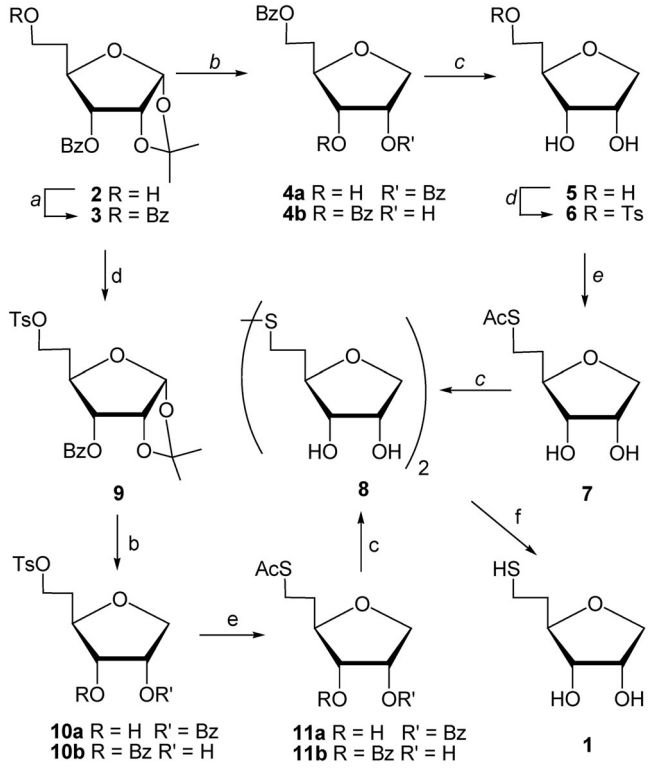
SCHEME 2
Proposed biomimetic modeling for the H3 abstraction by 6-thiyl radical

RESULTS AND DISCUSSION

Synthesis

Synthesis of the 6-thiohexofuranitol **1** started from readily available 1,2-*O*-isopropylidene-α-D-glucose which was converted to 3-*O*-benzoyl-5-deoxy-1,2-

O-isopropylidene-α-D-*ribo*-hexofuranose **2** in five steps (55%, Scheme 3)[9,20]. Attempted selective deoxygenation of **2** at the anomeric carbon with the excess of boron trifluouride etherate and triethylsilane[18,21–23] produced a complex mixture. However, treatment of the 3,6-*O*-dibenzoyl hexofuranose **3** with BF_3/Et_3SiH (5 equiv.) effected deacetonization and reductive deoxygenation (at C1) to give a mixture of the 2,6- and 3,6-di-*O*-benzoyl hexofuranitols **4a** and **4b** due to the partial migration[23] of the benzoyl group from 3-OH to 2-OH group. Debenzoylation of **4a**/**4b** mixture with NH_3/MeOH afforded a single 1,4-anhydroalditol **5**. Selective tosylation of **5** gave 6-*O*-tosylate **6** and subsequent S_N2 displacement with AcSK/DMF produced 6-*S*-acetyl hexofuranitol **7**. Deacylation with NH_3/MeOH and column chromatography yielded disulfide **8** instead of the expected free thiol **1**.



SCHEME 3

(a) BzCl/pyridine. (b) $Et_3SiH/BF_3{\cdot}Et/CH_2CH_2$. (c) NH_3/MeOH. (d) TsCl/pyridine. (e) AcSK/DMF. (f) $PPh_3/MeOH/H_2O$

Alternatively, tosylation of **2** produced **9**, which upon treatment with three equivalents each of BF_3OEt_2 and Et_3SiH gave a product profile very similar to the deoxygenation of 3,6-*O*-dibenzoyl sugar **3**. Two closely migrating on TLC 2-*O*-benzoyl and 3-*O*-benzoyl sugars **10a**/**10b** were obtained after column chromatography. Subsequent nucleophilic displacement with AcSK/DMF afforded 6-*S*-thioacetate mixture **11a**/**11b**. Deacylation with NH_3/MeOH (without exclusion of oxygen) resulted in the formation of disulfide **8**.

Reduction of disulfide **8** with PPh_3/MeOH/H_2O produced a more rapidly migrating (TLC) thiol **1**, in agreement with the migratory properties of other carbohydrate alkanethiol-disulfide pairs[19]. The presence of the thiol function was also qualitatively confirmed by the colorization upon treatment with Ellman's reagent. In the ^{1}H NMR spectra, an upfield shift for the H6,6′ signals of thiol **1** (δ 2.56–2.69) as compared to disulfide **8** (δ 2.78–2.94) was observed.

Treatment of 1,4-anhydro-5-deoxy-6-thio-D-*ribo*-hexofuranitol **1** with 2,2′-azobis-2-methylpropanimidamide dihydrochloride (AAPH) as a radical initiator in D_2O (pD = 5.5) at 35, 45 or 55 °C for up to 72 h under argon[24] resulted in the recovery of unchanged **1** and/or formation of its disulfide **8** with no deuterium incorporation at any of the ribosyl carbons (^{1}H NMR, MS). Presumably very fast reverse reaction (Eq. (*1*)) and/or water elimination at the acidic pD might be responsible for the lack of deuterium incorporation. Also, treatment of **1** with Bu_3SnD/AIBN in refluxed MeOH-d_4 or benzene resulted in neither deuterium exchange nor epimerization on C3 in agreement with the results reported for the analogouos thiosugar models[19].

Theoretical Calculations

Using density functional calculations at the B3LYP/6-311G** level of theory[25–27] we computed reaction energies and barrier heights for internal H atom abstractions by the thiyl radical from C1, C2, C3 and C4 of 6-thiohexofuranitol **1** (Fig. 1 and Table I). The results show that all considered H shift reactions are endothermic and the least endothermic of them are H4 abstraction occurring by the 1,4-H shift (5.2 kcal/mol) and H1 abstraction taking place by the 1,6-H shift (5.4 kcal/mol). These are followed by H3 abstraction (1,5-H shift, 7.7 kcal/mol) and H2 abstraction (1,6-H shift, 10.5 kcal/mol). In terms of the calculated barrier height, the H3 and H4 abstractions are most favorable, with the corresponding barriers of 11.1 and 11.2 kcal/mol, respectively. These calculations are in agreement with the

TABLE I
Reaction energies, barrier heights and rate constants for the internal H atom abstractions by the thiyl radical from C1, C2, C3 and C4 of 6-thiohexofuranitol **1**

Reaction channel	H1 abstraction	H2 abstraction	H3 abstraction	H4 abstraction
ΔE, kcal/mol[a]	5.4	10.5	7.7	5.2
ΔG, kcal/mol[b]	5.7	10.4	7.9	5.3
$\Delta E^{\#}$, kcal/mol[c]	13.4	17.7	11.1	11.2
$\Delta G^{\#}$, kcal/mol[d]	14.6	19.1	12.2	12.1
k_f, s^{-1} [e]	1.18×10^2	6.30×10^{-2}	6.77×10^3	7.80×10^3
k_r, s^{-1} [f]	1.74×10^6	2.61×10^6	4.04×10^9	6.04×10^7

[a] Reaction energy. [b] Reaction Gibbs free energy at T = 298.15 K. [c] Barrier height. [d] Gibbs free energy barrier height at T = 298.15 K. [e] Reaction rate constants in the forward direction. [f] Reaction rate constants in the reverse direction.

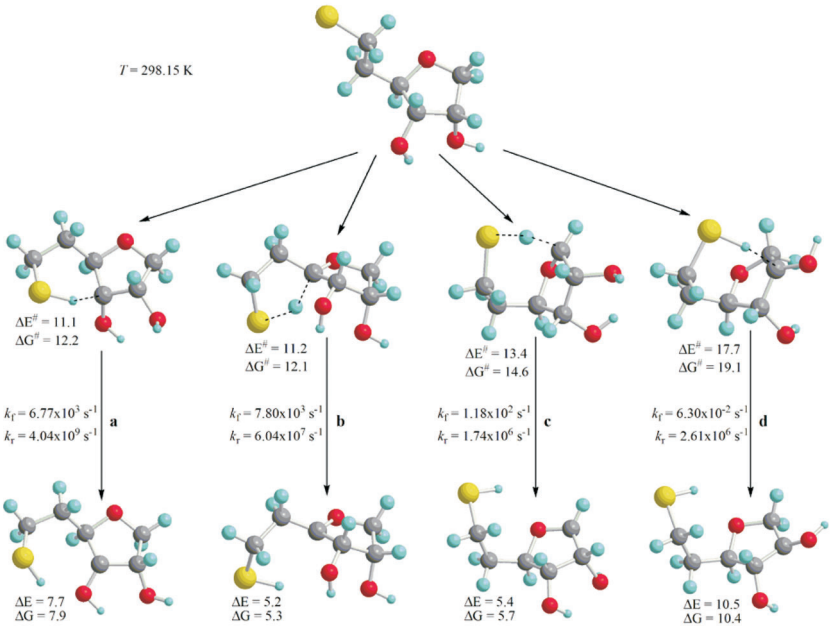


FIG. 1
Optimized geometries of the 6-thiyl radical and products and transition states of its internal H abstraction (H shift) reactions from C3 (path **a**), C4 (path **b**), C1 (path **c**) and C2 (path **d**). Calculated reaction energies ΔE, reaction Gibbs free energies ΔG at 298.15 K, barrier heights $\Delta E^{\#}$, and Gibbs free energy barrier heights $\Delta G^{\#}$ at 298.15 K (all in kcal/mol), as well reaction rate constants in forward (k_f) and reverse (k_r) directions are also shown

theoretical values for reaction energy (5.9 kcal/mol) and energy barrier (9.2 kcal/mol)[28] for H3′ abstraction by thiyl radicals in the enzymatic model systems for natural substrates[28–30]. The barriers for the H1 and H2 abstractions are higher by 2.2 and 6.5 kcal/mol.

Rate constant calculations at room temperature using transition state theory (TST) confirm the kinetic preference of the H3 and H4 abstraction channels given similar values of about 7–8 × 10^3 s^{-1}. The computed rate constant for the H1 abstraction is about 60 times slower and that for the H2 abstraction is negligibly small. It should be also noted that coupled clusters[31] CCSD(T)/6-311G** calculations confirmed the B3LYP results for the barrier heights giving very close barriers for the H3 and H4 abstractions, with the barriers for the H1 and H2 abstractions being 2.5 and 6.6 kcal/mol higher, respectively. In summary, according to the theoretical calculations, the H3 and H4 abstractions are expected to give comparable product yields, whereas the H1 and H2 abstractions would not contribute significantly.

Pulse Radiolysis

The radiolysis of disulfide **8** in an argon saturated aqueous solution containing *tert*-butanol under alkaline conditions (pH 12) generates the absorption of disulfide radical anion ($\varepsilon_{420} \approx 2 \times 10^3$ M^{-1} cm^{-1}; Fig. 2, panel a). This absorption bleaches quickly ($k_{decay} = 2 \times 10^5$ s^{-1}) and the equilibrium is irreversibly shifted to a carbon centered radical ($\varepsilon_{260} \approx 4.2 \times 10^3$ M^{-1} cm^{-1}; Fig. 2, panel a) with $k_{build\text{-}up}$ of 2 × 10^5 s^{-1}. The absorption of the carbon radical(s) at 260 nm turned out to be astonishingly stable at pH 12. At this pH the $HSR^{\bullet}$ radical (R = ribose fragment) is deprotonated to the thiolate-containing radical $^{-}SR^{\bullet}$. Because the reverse reaction relies on protonated thiol groups, the equilibrium is shifted fully to the carbon centered radical(s). Experiments with methyl viologen (MV^{2+}) proved the reducing nature of our radical product(s) (Fig. 2, panel b and Fig. 3). At 600 nm, the absorption-time profile is dependent on the concentration of methyl viologen radicals ($MV^{\bullet+}$) only. There are three processes governing this profile

$$RSSR^{\bullet-} + MV^{2+} \rightarrow RSSR + MV^{\bullet+} \qquad (2)$$

$$RR'C^{\bullet}OH + MV^{2+} \rightarrow RR'CO + H^{+} + MV^{\bullet+} \qquad (3)$$

$$MV^{\bullet+} + (CH_2^{\bullet})(CH_3)_2COH + H^{+} \rightarrow MV^{2+} + (CH_3)_3COH \qquad (4)$$

Reactions (*2*) and (*3*) contribute to the build up of the absorptivity, reaction (*4*) causes its decay. Because these reactions are concurrent, a direct determination of reaction (*3*) and its yield is not possible. Quantification of

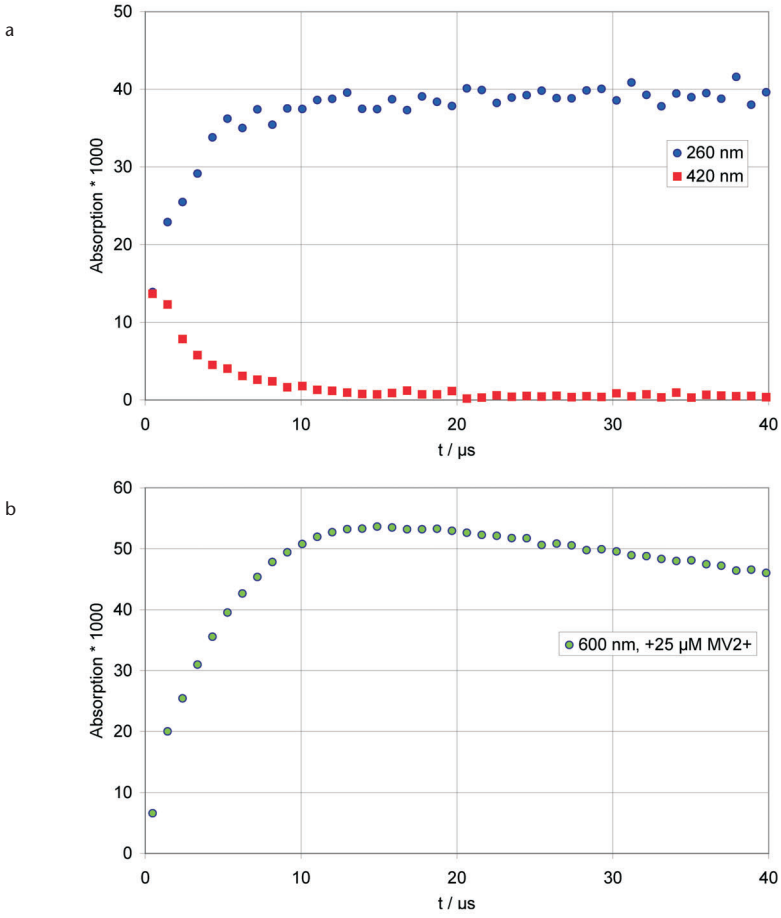


FIG. 2

Pulse radiolysis of 0.9 mM of thiosugar disulfide **8** in 1 M *tert*-butanol solution saturated with Ar in basic medium (pH 12): Panel a shows both the decay of the disulfide radical ion monitored at 420 nm and the build-up of a carbon-centered radical monitored at 260 nm after a pulse of 30 Gy. Panel b demonstrates the reducing nature of the radical product(s) in experiments with methyl viologen (MV^{2+}, 25 µM) detected at 600 nm. The build up is caused by reduction of MV^{2+} to $MV^{\bullet+}$ by both the disulfide radical anion and an additional reducing species, most probably a ribosyl based radical (Dose 16 Gy). The decay visible is caused by recombination of $^{\bullet}CH_2(CH_3)_2COH$ radicals with $MV^{\bullet+}$ ($k_4 = 2.5 \times 10^6$ M^{-1} s^{-1})

the yield of reaction (*3*) however is necessary for the carbon-centered radical to be identified as reducing (located at an alcoholic carbon). We therefore analyzed the kinetics traces in more detail. Because of the low concentration of MV^{2+} (25 μM), the kinetics of reaction (*2*) is governed by the competing intramolecular hydrogen abstraction from the ribose by thiyl radicals and therefore by k_1 (Eq. (*1*)). The rate constant of reaction (*3*) will be comparable with the known rate constants of 1,2-dihydroxycyclohexanyl radicals with MV^{2+} [32]. The rate constant $k_4 \approx 2.5 \times 10^6$ M^{-1} s^{-1} can easily be derived in the time range 30–500 μs after the electron pulse (not shown), when reactions (*2*) and (*3*) are completed. We then corrected our experimental trace for reaction (*4*) and analyzed if the corrected trace could be explained by reaction (*2*) only or if reaction (*3*) must be invoked to explain experimental results. Figure 3 shows residuals for least squares fits with the known rate constants k_2 and k_2 plus k_3, respectively. Without invoking reaction (*3*) with a contribution of at least 15% of the total reducing equivalents, there is a systematic and significant deviation between experiment and fit. The total yield of $MV^{\bullet+}$ with respect to initially formed sulfur radicals is >95%. Thus, we confirmed intramolecular abstraction of a H from a carbon adjacent to either an ether (C1 or C4) or an hydroxyl group (C2 or

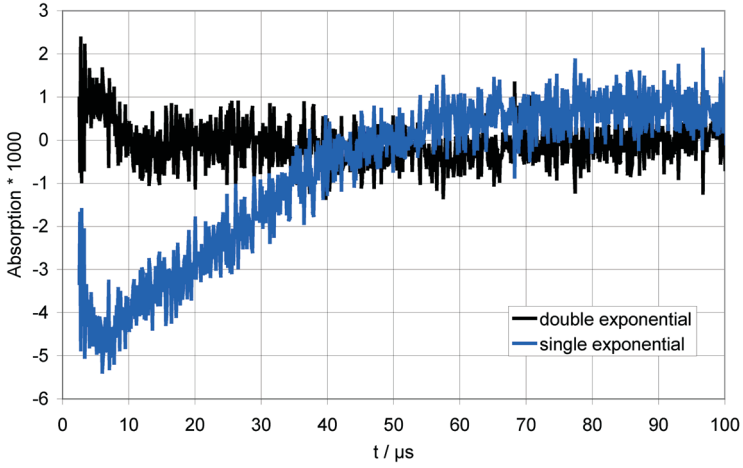


FIG. 3
Residuals of least square fits to the build-up of methyl viologen radicals. The rate constants k_2 (reduction of MV^{2+} by $RSSR^{\bullet-}$) and k_3 (reduction by ribosyl radicals) are taken from independent measurements. The single exponential fit is based on reaction (*2*) only. Clearly part of the reduction is caused by ribosyl radicals

C3) by a thiyl radical which produces carbon radical species that are substantially reducing. Steenken et al.[32] showed, that such radicals indeed undergo rapid dehydration reactions.

The pulse radiolysis experiments in acidic medium (pH 3) increased the decay rate of disulfide radical, $k_{obs} \geq 5 \times 10^5$ s^{-1}, and decreased formation of absorbing species at 260 nm (25% compared to pH 12). If we take this information to be indicative of equilibrium (*1*), we derive an equilibrium constant of $K \approx 0.3$, which implies $\Delta G \approx 1$ kcal/mol. This is comparable, but lower, than the known value for the equilibrium between thiyl radicals and tetrahydrofuran, $\Delta G = 4$ kcal/mol [6].

CONCLUSION

We have synthesized 1,4-anhydro-5-deoxy-6-thio-D-*ribo*-hexofuranitol disulfide (**8**) and demonstrated efficient formation of the ribosyl-based carbon-centered radical upon generation of 6-thiyl radical using pulse radiolysis experiments. One electron reduction of **8** by hydrogen atoms and solvated electrons under alkaline conditions (pH 12) generated the disulfide radical anion which bleached quickly and equilibrium was irreversibly shifted to the more *stable* carbon centered radical ($k \approx 2 \times 10^5$ s^{-1}). Experiments with methyl viologen (MV^{2+}) proved the reducing nature of our radical product(s). The reversible H-atom transfer has been quantified and was correlated with the computed rate constants for the internal H atom abstraction from the C1, C2, C3 and C4 of the sugar ring by the thiyl radical. The energy barrier for the H3 abstraction by 6-thiyl radical was calculated to be about 11.1 kcal/mol.

EXPERIMENTAL

^{1}H (400 MHz), ^{1}H (600 MHz) and ^{13}C (100.6 MHz) NMR spectra (δ, ppm; *J*, Hz) were recorded on a Bruker NMR spectrometer with solutions in $CDCl_3$, unless otherwise specified. Mass spectra were obtained by atmospheric pressure chemical ionization (APCI) technique. Merck Kieselgel 60-F_{254} sheets were used for TLC, and products were detected by visualization with $Ce(SO_4)_2/(NH_4)_6Mo_7O_{24}{\cdot}4H_2O/H_2SO_4/H_2O$ reagent or under 254 nm light. Merck Kieselgel 60 (230–400 mesh) was used for column chromatography. Reagent grade chemicals were used, and solvents were dried by reflux over distillation from calcium hydride under argon atmosphere or by passing through the activated cartridges using solvent purifying system.

Pulse radiolysis experiments were conducted using the facility at ETH Zürich as described before[33]. Curve fitting was carried out with Excel software and its Solver plugin which allowed for determination of yields and rate constants.

3,6-*O*-Dibenzoyl-5-deoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hexofuranose (**3**)

BzCl (508 μl, 2 mmol) was added to a solution of **2**[20] (290 mg, 0.94 mmol) in pyridine (5 ml) at ambient temperature and stirring was continued for 3 h. The volatiles were evaporated and the residue was partitioned ($CHCl_3/H_2O$). The organic phase was washed (1 M HCl/H_2O, $NaHCO_3/H_2O$, brine) and dried (Na_2SO_4). Volatiles were evaporated and the residue was column chromatographed (25% EtOAc/hexanes) to give **3** as a white solid (342 mg, 88%). ^{1}H NMR: 1.32, 1.54 (2 × s, 2 × Me), 2.10–2.25 (m, 2, H5), 4.44–4.53 (m, 3, H4,6,6′), 4.76 (dd, *J* = 9.2, 4.8, 1, H3), 4.97 (t, *J* = 4.5, 1, H2), 5.89 (d, *J* = 3.9, 1, H1), 7.38–7.43 (m, 4, Ar) 7.52–7.58 (m, 2, Ar), 7.98–8.02 (m, 4, Ar). ^{13}C NMR: 21.77, 26.66, 26.76, 31.98, 67.04, 73.70, 76.50, 77.37, 104.27, 113.26, 128.08, 128.65, 129.33, 130.02, 130.10, 133.45, 133.65, 144.91, 165.98. MS *m/z*: 413 (100, MH^+).

1,4-Anhydro-2,6-*O*-dibenzoyl-5-deoxy-D-*ribo*-hexofuranitol (**4a**) and
1,4-Anhydro-3,6-*O*-dibenzoyl-5-deoxy-D-*ribo*-hexofuranitol (**4b**)

Et_3SiH (659 μl, 4.13 mmol) and $BF_3{\cdot}Et_2O$ (314 μl, 2.48 mmol) were added to a solution of **3** (340 mg, 0.59 mmol) in CH_2Cl_2 (5 ml) at 0 °C. The reaction mixture was stirred for 3 h at ambient temperature, and then carefully partitioned between saturated $NaHCO_3/H_2O$ (15 ml) and CH_2Cl_2 (25 ml). The aqueous layer was washed with CH_2Cl_2 (3 × 10 ml), and the combined organic phase was washed (brine), dried (Na_2SO_4) and column chromatographed (30% EtOAc/hexanes) to give partially separated mixtures of **4a**/**4b** (~2:3; 148 mg, 52% total).

Compound **4a**. ^{1}H NMR: 2.10–2.00 (m, 1, H5), 2.28–2.20 (m, 1, H5′), 3.97 (td, *J* = 8.0, 4.3, 1, H4), 3.99 (dd, *J* = 10.8, 3.2, 1, H1), 4.14 (dd, *J* = 7.7, 5.6, 1, H3), 4.34 (dd, *J* = 10.8, 5.4, 1, H1), 4.58–4.45 (m, 2, H6,6′), 5.46 (td, *J* = 5.4, 3.1, 1, H2), 7.47–7.42 (m, 4, Ar), 7.61–7.54 (m, 2, Ar), 8.07–8.04 (m, 4, Ar). ^{13}C NMR: 32.57, 61.90, 70.79, 74.30, 75.91, 79.13, 128.51, 128.68, 129.53, 129.76, 129.92, 130.43, 133.07, 133.67, 166.54, 166.72. MS *m/z*: 357 (100, MH^+).

Compound **4b**. ^{1}H NMR: 2.14–2.00 (m, 1, H5), 2.24–2.16 (m, 1, H5′), 3.81 (dd, *J* = 9.8, 5.0, 1, H1), 4.24 (dd, *J* = 9.8, 5.7, 1, H1′), 4.28 (ddd, *J* = 8.1, 6.4, 4.5, 1, H4), 4.56–4.44 (m, 2, H6,6′), 4.62 (“q”, *J* = 5.4, 1, H2), 5.03 (t, *J* = 5.9, 1, H3), 7.39–7.47 (m, 4, Ar), 7.61–7.52 (m, 2, Ar), 8.07–7.99 (m, 4, Ar). ^{13}C NMR: 32.71, 61.71, 70.43, 72.73, 77.75, 78.11, 128.47, 128.67, 129.31, 129.71, 129.91, 130.28, 133.05, 133.70, 166.33, 166.59. MS *m/z*: 357 (100, MH^+).

1,4-Anhydro-5-deoxy-D-*ribo*-hexofuranitol (**5**)

A solution of **4a**/**4b** (142 mg, 0.4 mmol) in NH_3/MeOH was stirred overnight at ambient temperature in a sealed flask. Volatiles were evaporated, and the residue was partitioned between H_2O/Et_2O. The aqueous layer was evaporated to give **5** (53 mg, 86%). ^{1}H NMR (D_2O): 1.71–1.80 (m, 1, H5), 1.86–1.94 (m, 1, H5′), 3.65–3.76 (m, 2, H6,6′), 3.73 (dd, *J* = 10.5, 2.8, 1, H1), 3.84 (td, *J* = 8.2, 4.1, 1, H4), 3.91 (dd, *J* = 7.4, 4.9, 1, H3), 4.08 (dd, *J* = 10.2, 4.7, 1, H1′), 4.26 (td, *J* = 4.7, 2.9, 1, H2). ^{13}C NMR: 35.04, 58.66, 70.66, 71.93, 75.50, 78.45. MS *m/z*: 149 (100, MH^+).

1,4-Anhydro-5-deoxy-6-*O*-tosyl-D-*ribo*-hexofuranitol (**6**)

TsCl (58 mg, 0.3 mmol) was added to a solution of **5** (30 mg, 0.2 mmol) in pyridine (3 ml) at ambient temperature and stirring was continued for 12 h. The volatiles were evaporated and the residue was partitioned ($CHCl_3/H_2O$). The organic phase was washed (1 M HCl/H_2O, $NaHCO_3/H_2O$, brine) and dried (Na_2SO_4). Volatiles were evaporated and the residue was column chromatographed (80% EtOAc/hexanes) to give **6** as a syrup (37 mg, 61%). ^{1}H NMR: 1.81–1.90 (m, 1, H5), 2.04–2.12 (m, 1, H5′), 2.46 (s, 3, Me), 3.69 (dd, *J* = 10.2, 3.3, 1, H1′), 3.73 (dd, *J* = 8.0, 4.2, 1, H3), 3.79 (t, *J* = 7.1, 1, H4), 4.05 (dd, *J* = 10.2, 5.2, 1, H1), 4.13–4.24 (m, 3, H2,6,6′), 7.36 (d, *J* = 8.3, 2, Ar), 7.80 (d, *J* = 8.1, 2, Ar). ^{13}C NMR: 21.79, 32.74, 67.61, 70.97, 73.16, 75.85, 78.59, 128.10, 130.03, 133.14, 133.70, 145.01. MS *m/z*: 303 (100, MH^+).

6-*S*-Acetyl-1,4-anhydro-5-deoxy-6-thio-D-*ribo*-hexofuranitol (**7**)

AcSK (21 mg, 0.18 mmol) was added to a stirred solution of **6** (35 mg, 0.12 mmol) in a dried DMF (4 ml) at ambient temperature. After 2 h, volatiles were evaporated and the residue was column chromatographed (5% MeOH/$CHCl_3$) to give **7** (19 mg, 72%). ^{1}H NMR: 1.79 (dddd, *J* = 13.0, 8.3, 8.1, 6.8, 1, H5), 1.91 (dddd, *J* = 13.0, 8.5, 6.5, 5.5 1, H5′), 2.32 (s, 3, Me), 2.92 (ddd, *J* = 13.7, 8.3, 6.8, 1, H6), 3.01 (ddd, *J* = 13.7, 8.5, 6.1, 1, H6′), 3.21 (br, 1, OH), 3.32 (br, 1, OH), 3.67–3.73 (m, 2, H1,4), 3.80 (t, *J* = 6.0, 1, H3), 4.08 (dd, *J* = 10.6, 5.2, 1, H1), 4.22 (dd, *J* = 9.0, 5.1, 1, H2). ^{13}C NMR: 25.67, 30.74, 33.44, 71.11, 72.92, 75.78, 81.07, 196.69. MS *m/z*: 207 (100, MH^+).

Bis(1,4-anhydro-5-deoxy-6-thio-D-*ribo*-hexofuranitol) Disulfide (**8**)

Method A. Solution of **7** (18 mg, 0.08 mmol) in NH_3/MeOH was stirred overnight at ambient temperature in a sealed flask. Volatiles were evaporated and the residue was partitioned between H_2O/Et_2O. The aqueous layer was evaporated to give **8** (11 mg, 67%). ^{1}H NMR (D_2O): 1.84–1.98 (m, 1, H5), 2.07–2.16 (m, 1, H5′), 2.78–2.94 (m, 2, H6,6′), 3.75 (dd, *J* = 10.2, 2.9, 1, H1), 3.90 (td, *J* = 7.9, 4.0, 1, H4), 3.97 (dd, *J* = 7.3, 4.8, 1, H3), 4.10 (dd, *J* = 10.2, 4.7, 1, H1′), 4.29 (td, *J* = 4.7, 3.0, 1, H2). ^{13}C NMR: 32.13 (C5), 34.16 (C6), 70.79 (C2), 71.92 (C1), 75.25 (C3), 79.74 (C4). MS *m/z*: 327 (100, MH^+). HRMS (CI) *m/z*: calculated for $C_{12}H_{23}O_6S_2$ $[M + H]^+$ 327.0936, found 327.0941.

Method B. A solution of **11a**/**11b** (98 mg, 0.31 mmol) in NH_3/MeOH (4 ml) was stirred overnight at ambient temperature in a sealed flask. Volatiles were evaporated and the residue was partitioned (H_2O/Et_2O). The aqueous layer was evaporated to give **8** (48 mg, 96%) with spectroscopic data as in method A.

3-*O*-Benzoyl-5-deoxy-1,2-*O*-isopropylidene-6-*O*-tosyl-α-D-*ribo*-hexofuranose (**9**)

TsCl (441 mg, 2.31 mmol) was added to a solution of **2** [20] (283 mg, 0.96 mmol) in pyridine (3 ml) at ambient temperature and stirring was continued for 2 h. The volatiles were evaporated and the residue was partitioned ($CHCl_3/H_2O$). The organic phase was washed (1 M HCl/H_2O, $NaHCO_3/H_2O$, brine) and dried (Na_2SO_4). Volatiles were evaporated and the residue was column chromatographed (40% EtOAc/hexanes) to give **9** as a syrup (306 mg, 72%). ^{1}H NMR: 1.30, 1.52 (2 × s, 2 × Me), 1.88–1.97 (m, 1, H5), 2.09–2.31 (m, 1, H5′), 2.42 (s, 3, Me), 4.09–4.26 (m, 2, H6,6′), 4.31 (td, *J* = 8.9, 3.6, 1, H4), 4.63 (dd, *J* = 9.2, 4.8, 1, H3), 4.89 (t, *J* = 4.4, 1, H2), 5.79 (d, *J* = 3.8, 1, H1), 7.30 (d, *J* = 8.0, 2, Ar), 7.48 (t, *J* = 7.5, 2, Ar), 7.61

(tt, *J* = 7.4, 1.3, 1, Ar), 7.78 (d, *J* = 8.2, 2, Ar), 8.04–8.06 (m, 2, Ar). ^{13}C NMR: 21.77, 26.66, 26.76, 31.98, 67.04, 73.70, 76.50, 77.37, 104.27, 113.26, 128.08, 128.65, 129.33, 130.02, 130.10, 133.45, 133.65, 144.91, 165.98. MS *m/z*: 463 (100, MH^+).

1,4-Anhydro-2-*O*-benzoyl-5-deoxy-6-*O*-tosyl-D-*ribo*-hexofuranitol (**10a**) and
1,4-Anhydro-3-*O*-benzoyl-5-deoxy-6-*O*-tosyl-D-*ribo*-hexofuranitol (**10b**)

Et_3SiH (477 µl, 2.90 mmol) and $BF_3{\cdot}Et_2O$ (227 µl, 1.79 mmol) were added to a solution of **47** (275 mg, 0.595 mmol) in CH_2Cl_2 (5 ml) at 0 °C. The solution was stirred for 3 h at ambient temperature, and then carefully partitioned between saturated $NaHCO_3/H_2O$ (15 ml) and CH_2Cl_2 (30 ml). The aqueous layer was washed with CH_2Cl_2 (3 × 10 ml), and the combined organic phase was washed (brine), dried (Na_2SO_4) and column chromatographed (50% EtOAc/hexanes) to give partially separated mixtures of **10a**/**10b** (~2:3; 68 mg, 28% total).

Compound **10a**. ^{1}H NMR: 1.83–1.91 (m, 1, H5), 2.07–2.15 (m, 1, H5′), 3.79 (td, *J* = 8.3, 4.0, 1, H4), 3.87 (dd, *J* = 10.9, 2.9, 1, H1), 3.97–4.02 (m,1, H3), 4.15–4.25 (m, 3, H1′,6,6′), 5.37 (td, *J* = 5.4, 2.9, 1, H2), 7.30–7.32 (m, 2, Ar), 7.43–7.47 (m, 2, Ar) 7.57–7.60 (m, 1, Ar), 7.78–7.80 (m, 2, Ar), 8.02–8.04 (m, 2, Ar). ^{13}C NMR: 21.74, 32.63, 67.16, 70.32, 72.67, 75.75, 77.78, 128.10, 128.74, 129.24, 129.97, 133.13, 133.81, 144.92, 166.23. MS *m/z*: 407 (100, MH^+).

Compound **10b**. ^{1}H NMR: 1.84–1.93 (m, 1, H5), 2.03–2.15 (m, 1, H5′), 3.71 (dd, *J* = 9.8, 4.9, 1, H1), 4.09–4.24 (m, 4, H1,2,6,6′), 4.53 (dt, *J* = 10.1, 4.9, 1, H4), 4.89 (t, *J* = 6.0, 1, H3), 7.30–7.32 (m, 2, Ar), 7.45–7.49 (m, 2, Ar), 7.58–7.63 (m, 1, Ar) 7.75–7.81 (m, 2, Ar), 8.03–8.05 (m, 2, Ar). ^{13}C NMR: 21.74, 32.88, 67.43, 70.92, 74.09, 76.70, 77.92, 128.07, 128.71, 129.90, 129.46, 133.18, 133.73, 144.92, 166.44. MS *m/z*: 407 (100, MH^+).

6-*S*-Acetyl-1,4-anhydro-2-*O*-benzoyl-5-deoxy-6-thio-D-*ribo*-hexofuranitol (**11a**) and
6-*S*-Acetyl-1,4-anhydro-3-*O*-benzoyl-5-deoxy-6-thio-D-*ribo*-hexofuranitol (**11b**)

AcSK (20.9 mg, 0.18 mmol) was added to a stirred solution of compound **10a**/**10b** (68 mg, 0.17 mmol) in a dried DMF (6 ml) at ambient temperature. After 2 h, volatiles were evaporated and the residue was partitioned between H_2O/CH_2Cl_2. The organic phase was washed ($NaHCO_3/H_2O$ and brine), dried (Na_2SO_4) and column chromatographed (1% $MeOH/CHCl_3$) to give partially separated mixtures of **11a**/**11b** (2:3; 37 mg, 72% total).

Compound **11a**. ^{1}H NMR: 1.82–1.92 (m, 1, H5), 1.98–2.06 (m, 1, H5′), 2.34 (s, 3, Me), 2.96–3.12 (m, 2, H6,6′), 3.83 (td, *J* = 7.9, 4.4, 1, H4), 3.97 (dd, *J* = 10.8, 3.2, 1, H1′), 4.07 (dd, *J* = 7.5, 6.3, 1, H3), 4.31 (dd, *J* = 10.8, 5.3, 1, H1), 5.43 (td, *J* = 5.5, 3.2, 1, H2), 7.45–7.49 (m, 1, Ar), 7.58–7.62 (m, 2, Ar), 8.05–8.08 (m, 2, Ar). ^{13}C NMR: 25.66, 30.76, 33.39, 70.76, 74.36, 75.75, 80.78, 128.71, 129.57, 129.93, 133.70, 166.51, 195.93. MS *m/z*: 311 (100, MH^+).

Compound **11b**. ^{1}H NMR: 1.82–1.92 (m, 1, H5), 1.98–2.06 (m, 1, H5′), 2.34 (s, 3, Me), 2.94–3.06 (m, 2, H6,6′), 3.78 (dd, *J* = 14.8, 5.1, 1, H1), 4.14 (dt, *J* = 8.4, 5.1, 1, H4), 4.21 (dd, *J* = 9.7, 5.7, 1, H1′), 4.58 (td, *J* = 5.3, 10.6, 1, H2), 4.97 (t, *J* = 5.8, 1, H3), 7.46–7.50 (m, 1, Ar), 7.59–7.63 (m, 2, Ar), 8.06–8.08 (m, 2, Ar). ^{13}C NMR: 25.57, 30.73, 33.55, 70.61, 72.60, 77.36, 77.88, 79.56, 128.74, 129.38, 129.97, 133.77, 166.30, 195.64. MS *m/z*: 311 (100, MH^+).

1,4-Anhydro-5-deoxy-6-thio-D-*ribo*-hexofuranitol (**1**)

Ph_3P (16 mg, 0.06 mmol) and water (40 µl) were added to a stirred solution of disulfide **8** (9.6 mg, 0.03 mmol) in MeOH (3 ml). After 72 h, the volatiles were evaporated, and the residue was dissolved in 3 ml of deoxygenated water and quickly extracted with dichloromethane. The aqueous layer was evaporated to give **1** (6.6 mg, 69%). ^{1}H NMR (D_2O): 1.82–1.97 (m, 2, H5,5′), 2.69–2.56 (m, 2, H6,6′), 3.73 (dd, *J* = 10.2, 2.8, 1, H1), 3.86–3.93 (m, 2, H3,4), 4.07 (dd, *J* = 10.2, 4.6, 1, H1′), 4.26 (dd, *J* = 4.5, 3.0, 1, H2). ^{13}C NMR: 20.21, 36.71, 70.77, 71.88, 75.21, 79.60. HRMS (CI) *m/z*: calculated for $C_6H_{13}O_3S$ $[M + H]^+$ 165.0585, found 165.0592.

Chemical Biomimetic Studies with 6-Thiohexafuranitol **1**

The 2,2′-azobis-2-methylpropanimidamide dihydrochloride (AAPH; 49 mg, 0.18 mmol) dissolved in deoxygenated D_2O (0.5 ml) was added under argon atmosphere to a solution of **1** (5 mg, 0.03 mmol) in deoxygenated D_2O (0.5 ml) in an NMR tube at ambient temperature. The solution was flushed with argon for 15 min, and then the NMR tube was tightly sealed and heated at 55 °C, and the reaction progress was followed by ^{1}H NMR. After 17 h, about 80% of AAPH decomposed as judged by the disappearance of a singlet at 1.53 ppm in ^{1}H NMR. After 72 h, the reaction mixture was concentrated to the 50% of the volume and was stirred in the open air for 48 h. Volatiles were evaporated and the residue was column chromatographed (3 → 8% MeOH/$CHCl_3$) to give a product with spectroscopic properties identical to disulfide **8**.

Bu_3SnD (32 µl, 0.12 mmol) and AIBN (~1 mg) were added to a suspension of **1** (6 mg, 0.03 mmol) in deoxygenated benzene (0.5 ml) and *N*,*N*-dimethylacetamide (0.3 ml). The reaction mixture was deoxygenated (Ar, 20 min) and refluxed for 5 h. Volatiles were evaporated and the residue was dissolved in H_2O. The aqueous solution was washed (CH_2Cl_2) and evaporated. The residue was column chromatographed (1 → 8% MeOH/$CHCl_3$), and the appropriate fractions were analyzed by ^{1}H NMR and MS but no incorporation of deuterium was detected for the isolated thiol **1** and disulfide **8**.

Pulse Radiolysis Experiments with **8**

Aqueous solutions of 900 µM of thiosugar disulfide **8** were freshly prepared in 1 M *tert*-butanol solution at pH 12 (10 mM KOH) and pH 3 (0.5 mM H_2SO_4). The solutions were degassed in Schlenk tubes by evacuation to boiling and subsequently filled with Ar twice. The solutions were then anaerobically transferred to a gas-tight syringe (Hamilton, Bonaduz, Switzerland) and then with a syringe pump transferred batchwise through standard HPLC tubing to a quartz flow cell with 1 cm optical pathlength. Irradiations were always carried out on fresh samples.

This investigation was supported by grants from the NIGMS/NCI (SC1CA138176). T. D. is grateful to FIU University Graduate School for her Doctoral Evidence Acquisition Fellowship.

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