Tetrahedron 68 (2012) 5655–5667

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Investigation of reactions postulated to occur during inhibition of ribonucleotide reductases by 2′-azido-2′-deoxynucleotides

Thao P. Dang [a,†], Adam J. Sobczak [a], Alexander M. Mebel [a], Chryssostomos Chatgilialoglu [b], Stanislaw F. Wnuk [a,*]

[a] Department of Chemistry and Biochemistry, Florida International University, Miami, Florida 33199, USA
[b] ISOF, Consiglio Nazionale delle Ricerche, 40129 Bologna, Italy

ARTICLE INFO

Article history:
Received 5 March 2012
Received in revised form 10 April 2012
Accepted 12 April 2012
Available online 21 April 2012

Keywords:
Azido nucleosides
Density functional calculations
Gamma irradiation
Ribonucleotide reductases
Thiyl radicals

ABSTRACT

Model 3′-azido-3′-deoxynucleosides with thiol or vicinal dithiol substituents at C2′ or C5′ were synthesized to study reactions postulated to occur during inhibition of ribonucleotide reductases by 2′-azido-2′-deoxynucleotides. Esterification of 5′-(*tert*-butyldiphenylsilyl)-3′-azido-3′-deoxyadenosine and 3′-azido-3′-deoxythymidine (AZT) with 2,3-*S*-isopropylidene-2,3-dimercaptopropanoic acid or *N*-Boc-*S*-trityl-L-cysteine and deprotection gave 3′-azido-3′-deoxy-2′-*O*-(2,3-dimercaptopropanoyl or cysteinyl) adenosine and the 3′-azido-3′-deoxy-5′-*O*-(2,3-dimercaptopropanoyl or cysteinyl)thymidine analogs. Density functional calculations predicted that intramolecular reactions between generated thiyl radicals and an azido group on such model compounds would be exothermic by 33.6–41.2 kcal/mol and have low energy barriers of 10.4–13.5 kcal/mol. Reduction of the azido group occurred to give 3′-amino-3′-deoxythymidine, which was postulated to occur with thiyl radicals generated by treatment of 3′-azido-3′-deoxy-5′-*O*-(2,3-dimercaptopropanoyl)thymidine with 2,2′-azobis-(2-methyl-2-propionamidine) dihydrochloride. Gamma radiolysis of N_2O-saturated aqueous solutions of AZT and cysteine produced 3′-amino-3′-deoxythymidine and thymine most likely by both radical and ionic processes.

1. Introduction

Ribonucleotide reductases (RNRs) catalyze the conversion of nucleotides to deoxynucleotides providing the monomeric precursors required for DNA replication and repair. Inhibition of RNRs disrupts this primary source of DNA components and therefore is an appealing concept for rational drug design. For example, gemcitabine (2′,2′-difluoro-2′-deoxycytidine), an anticancer pro-drug in clinical use, is enzymatically phosphorylated within cells to its 5′-di- and triphosphates. The diphosphate inactivates the R1 protein of ribonucleotide diphosphate reductase (RDPR), and the triphosphate undergoes incorporation and inhibits DNA polymerase.[1-5] The structure, function and mechanisms of action of RNRs have been studied in detail and the critical role of a tyrosine (Tyr122) free radical on the R2 subunit and cysteine residues (Cys439 and Cys225/462) on the R1 subunit have been established.[6–8]

Mechanism-based inhibitors of RDPR such as 2′-azido-2′-deoxyuridine-5′-diphosphate (**A**, N_3UDP) have provided insight into the mechanism(s) of reduction of natural NDPs into dNDPs.[9-12] Inhibition of RDPR by N_3UDP is accompanied by the appearance of new EPR signals for a nitrogen-centered radical and concomitant decay of peaks for the tyrosyl radical,[9] which was the first direct evidence for free radical chemistry with RDPR. A proposed mechanism postulated azide loss (as an anion) from the initial C3′ radical intermediate to give the ketyl radical **B** and subsequent reduction by proton-coupled electron transfer to generate the 2′-deoxy-3′-ketonucleotide **C** (Fig. 1). This process leaves a thiyl radical in the active site. Reaction of hydrazoic acid with the thiyl radical generates stoichiometric N_2 and a sulfinylimine radical **H**. The protonated azide (pK_a of 4.6) was hypothesized to be essential for that mechanism.[10,12] Conversion (**B** → **C**) is analogous to the proposed mechanism for the reduction of natural NDPs that proceeds by generation of the same 3′-keto-2′-deoxynucleotide intermediate, which makes investigation of the inhibition of RDPR by N_3UDP even more significant.[6,8,13]

The initial nitrogen-centered radical **H** reacts further with the oxygen or carbon atoms of a carbonyl group of the 3′-keto-2′-deoxynucleotide to generate radicals **D** or **G**, respectively.[11] Inactivation of the enzyme with 3′[^{17}O]-N_3UDP[15] **A** was consistent with formation of the R-S-N˙-C(3′)-OH radical **G** and provided the first evidence for trapping of a 3′-ketonucleotide in the reduction

* Corresponding author. Tel.: +1 305 348 6195; fax: +1 305 348 3772; e-mail address: wnuk@fiu.edu (S.F. Wnuk).
† Present Address: Georgia Bureau of Investigation, Atlanta, GA.

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2012.04.050

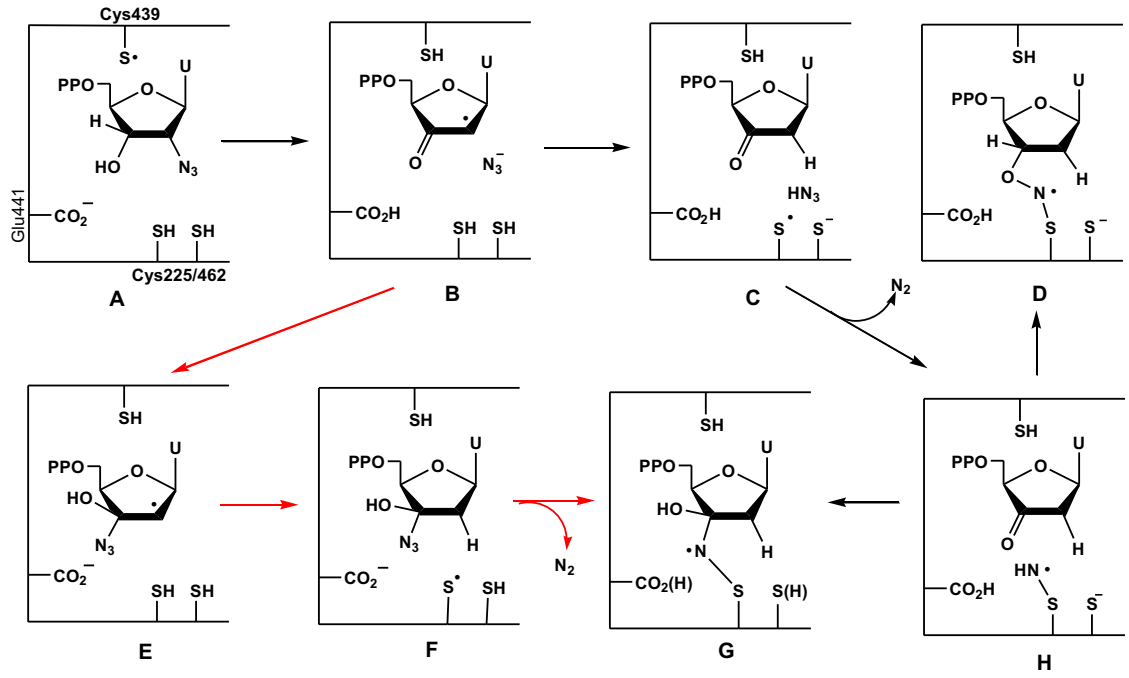


Fig. 1. Proposed structures for the nitrogen centered radicals and pathways for their generation during inactivation of RDPR by N_3UDP.[10-12,14]

process by a nitrogen-centered radical **H**.[12] Chemical requirements also favor formation of **G** (over **D**) and there is precedent in the literature for analogous addition of aminyl radicals to carbonyl[16] and imino groups.[17]

The theoretical modeling study of Pereira and coworkers generated an alternative mechanistic proposal.[14] In that hypothesis, the released azide ion was proposed to add to the 2′-ketyl radical **B** first with concomitant protonation of the ketone oxygen by E441. The resulting radical **E** is then reduced at the 2′-position by Cys225 to generate the Cys225 thiyl radical **F**. Subsequent attack of the thiyl radical on an alkyl azide (instead of HN_3 or azide anion) would lead to the same nitrogen center radical **G** that was detected experimentally.[12] Addition of azide to ketones has chemical precedent,[18] but there are no model systems where azide adds to ketyl radicals. Reduction of alkyl azides with tin,[16,19] alkoxy[20] and silyl (in the presence of thiols)[21-22] radicals have been reported[23] but information on reactions between alkyl thiyl radicals and alkyl azides is limited.[18,23-24] Herein, we report synthesis of model 3′-azido-3′-deoxynucleosides bearing thiol or vicinal dithiol substituents attached at C2′ or C5′ and treatment of these free radical precursors in the presence of 2,2′-azobis-(2-methyl-2-propionamidine) dihydrochloride as a radical initiator. Theoretical simulations of intramolecular reactions between thiyl radicals and azido groups and gamma-irradiation experiments with putative alkyl thiyl radicals and azido nucleosides also are described.

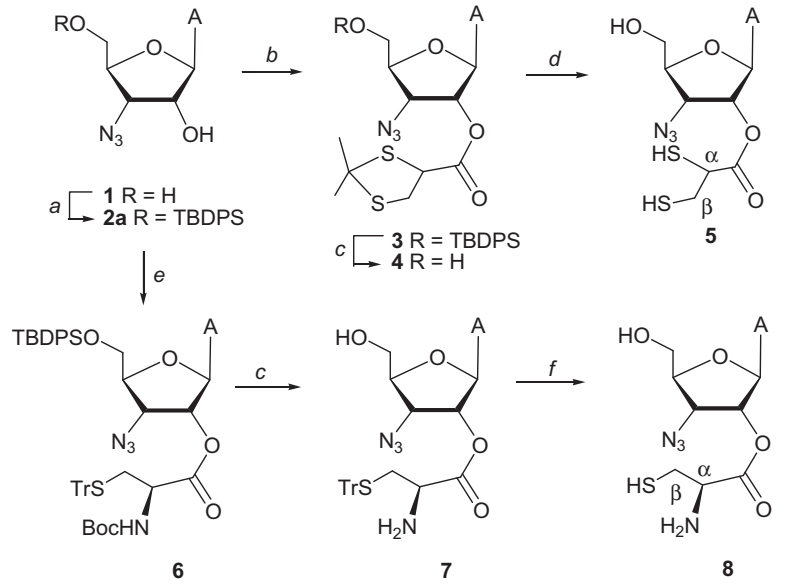
2. Results and discussion

2.1. Synthesis

Model substrates to study intramolecular interactions of thiyl radicals and azido groups[25] were prepared from 3′-azido-3′-deoxyadenosine (**1**) and 3′-azido-3′-deoxythymidine (**9**, AZT). Thus, silylation of **1**[26] with *tert*-butyldiphenylsilyl chloride (TBDPSCl) gave **2a** (89%) (Scheme 1). Condensation of **2a** and 2,3-*S*-isopropylidene-2,3-dimercaptopropanoic acid (synthesized from ethyl 2,3-dibromopropionate as described in SI section, Scheme S1) with EDCI as the activator in the presence of DMAP[27] afforded **3** (69%) as a 2:1 mixture of diastereomers. Removal of the isopropylidene group from **3** was ultimately achieved with a mercuric salt complex followed by treatment with hydrogen sulfide.[28] Thus, selective 5′-*O*-desilylation (TFA/H_2O)[29] of **3** and subsequent deacetonization ($HgCl_2$/H_2S/MeCN/H_2O)[28] of **4** afforded **5** (55%) as a single isomer. Thiyl radicals generated from **5** might interact with the azido group via seven- (S• at Cα) or eight-membered transition states (S• at Cβ). It is noteworthy that compound **5** does not possess a reactive α-amino C-H bond, in contrast with cysteine containing analogues (e.g., **8**), which were shown to be reactive with thiyl radicals.[30-31] Evidence for the addition of thiyl radicals to the adenine ring is lacking,[32] although addition of HO• radical at the C4 or C8 positions of the adenine ring is well documented.[33]

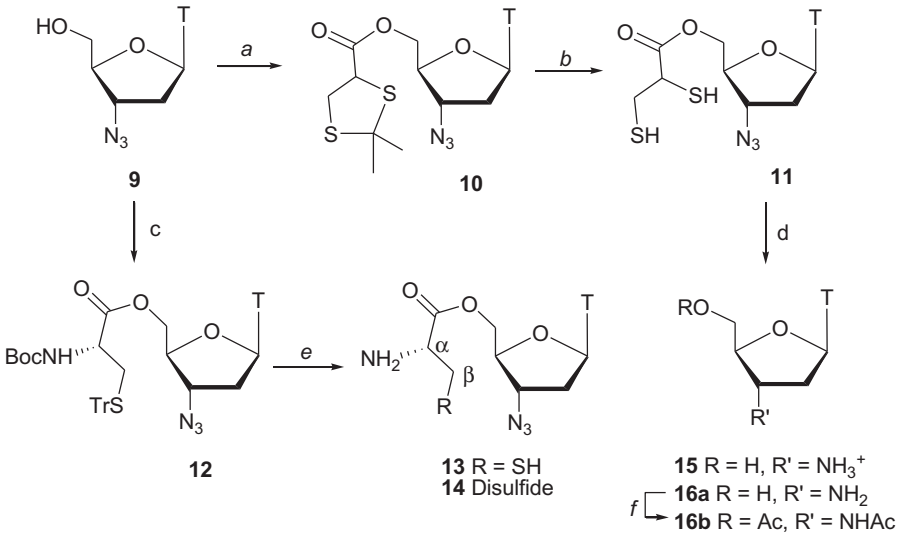
Condensation of **2a** with *N*-Boc-*S*-trityl-L-cysteine (EDCI/DMAP)[27] afforded **6** (79%). Treatment of **6** with TFA/H_2O[29] effectively removed the silyl and Boc protection groups to give **7** (77%) but protic acids were found to be ineffective for cleavage of a trityl thioether.[34] Treatment with Hg salts was also unsuccessful for *S*-detritylation,[34] but treatment of **7** with TFA in the presence of Et_3SiH[35] cleanly removed the *S*-trityl group affording **8** (79%). It is noteworthy that the azido group was not affected[36] by Et_3SiH employed in the *S*-detritylation step, although reduction of an azido group to a primary amino group by the more reactive radical-based reducing agent $(Me_3Si)_3SiH$ is known.[22] Compound **8** is prone to oxidation to the disulfide and/or cleavage of the cysteinate ester bond at the 2′ position during silica gel column purification. The thiol group in **8** could approach the azido group from the α face via an eight-membered ring, which might mimic the interaction between Cys225 of the enzyme and the azide group at C3′ (e.g., **F** → **G**, Fig. 1).

Analogous esterification of 3′-azido-3′-deoxythymidine (**9**; Scheme 2) with 2,3-*S*-isopropylidene-2,3-dimercaptopropanoic acid afforded **10** (90%) as a mixture of diastereomers (1.2:1), and the $HgCl_2$/H_2S-mediated deacetonization of **10** gave **11** (46%). Condensation of **9** with *N*-Boc-*S*-trityl-L-cysteine gave **12** (80%), which was treated with TFA/Et_3SiH to remove the Boc and trityl protection groups. The resulting **13** (84%) underwent slow oxidation to the more polar (TLC) disulfide **14** (^{1}H NMR: downfield shift of Δ 0.18 ppm for Hβ,β′ as compared to **13**) during purification. The 2′-deoxy compounds **11** and **13** provide a closer electronic analogy



Reagents and conditions: (*a*) TBDPSCl/pyridine/rt/48 h; (*b*) 2,3-S-isopropylidene-2,3-dimercaptopropanoic acid/EDCI/DMAP/CH_2Cl_2/0 °C/4 h; (*c*) TFA/H_2O/0 °C/2 h; (*d*) (i) $HgCl_2$/MeCN/H_2O/rt/3 h, (ii) H_2S/MeOH/rt/30 min; (*e*) *N*-Boc-*S*-trityl-Cys/EDCI/DMAP/CH_2Cl_2/0 °C/4 h; (*f*) TFA/Et_3SiH/CH_2Cl_2/0 °C/2 h.

Scheme 1.

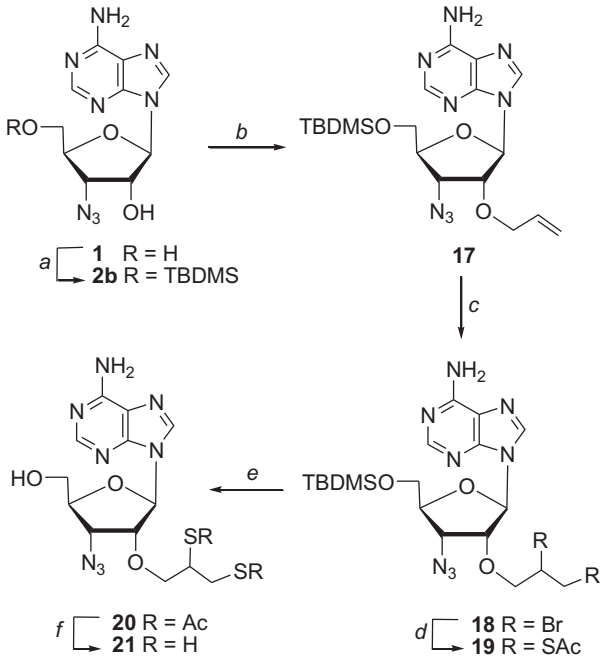


Reagents and conditions: (*a*) 2,3-*S*-isopropylidene-2,3-dimercaptopropanoic acid/EDCI/DMAP/CH_2Cl_2/0 °C/3 h; (*b*) (i) $HgCl_2$/MeCN/H_2O, (ii) H_2S/MeOH/rt/6 h; (*c*) *N*-*t*-butylcarboxyl-*S*-tritylcysteine/EDCI/DMAP/CH_2Cl_2/0 °C/4 h; (*d*) AAPH/D_2O/MeOH-d_4/50 °C/24 h; (*e*) TFA/Et_3SiH/CH_2Cl_2/0 °C/2 h; (f) Ac_2O/DMAP.

Scheme 2.

to intermediates generated during inhibition of RNR by N_3UDP (see Figure 1). Addition of thiyl radicals at C6 of pyrimidine nucleoside model substrates has been observed.[37]

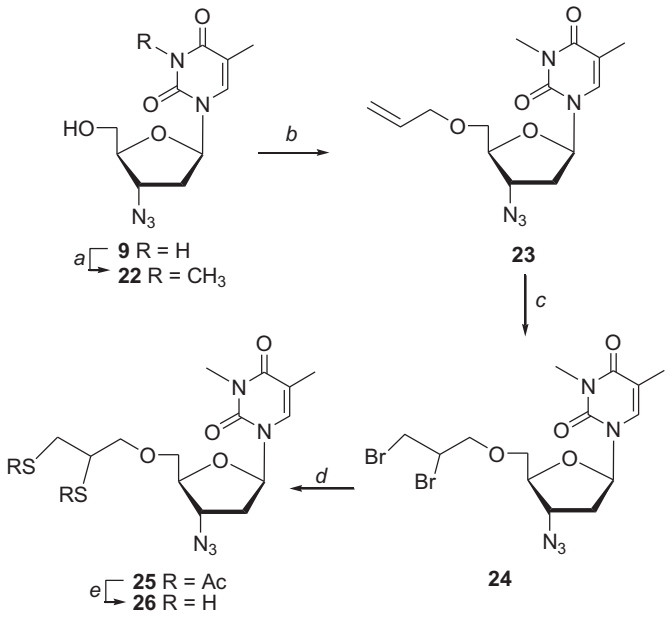
Analogues **21** and **26** with more stable ether linkages at C2′ and C5′ were also synthesized. Regioselective silylation of **1**[26] with *tert*-butyldimethylsilyl (TBDMS) chloride gave **2b** (76%), and careful treatment of **2b** with allyl bromide[38] and NaH gave the 2′-*O*-allylated product **17** (47%; Scheme 3). Allylation of **2b** with allyl ethyl carbonate in the presence of Pd(0) gave a mixture of 6-*N*- and 2′-*O*-allylated products.[27] Addition of bromine to **17** at −50 oC gave the dibromo compound **18** (44%) as a 1:1 mixture of diastereomers. Treatment of **18** with NaSH produced a mixture of thiooxiranes, but the bis-*S*-acetyl derivative **19** (81%) was formed cleanly with KSAc in DMF at ambient temperature. Desilylation of **19** (TFA/H_2O)[29] gave **20** (99%), and *S*-deacylation (NaOH/MeOH) afforded the desired vicinal dithiol **21** (99%). It is noteworthy that attempted deacylation of **20** with NH_3/MeOH produced a complex mixture.



Reagents and conditions: (*a*) TBDMSCl/pyridine/rt/12 h; (*b*) NaH/allyl bromide/DMF/rt/2 h; (*c*) $Br_2/CHCl_3$/-50 °C/30 min; (*d*) KSAc/DMF/rt/18 h; (*e*) TFA/H_2O/0 °C/1 h; (*f*) (i) NaOH/MeOH/-30 °C/30 min, (ii) HCl/H_2O/-50 °C/20 min

Scheme 3.

Treatment of **9** with allyl bromide/NaH resulted in predominant formation of the 3-*N*-allyl relative to the 5′-*O*-allyl[39] compound. Diazomethane converted **9** into its 3-*N*-methyl derivative **22** (99%; Scheme 4), which was treated with allyl bromide in the presence of 18-crown-6/KOH to give **23**. Bromination of **23** followed by thioacetylation and deacetylation provided the model vicinal dithiol **26** (36% overall from **22**) as a 3:2 mixture of diastereomers.



Reagents and conditions: (*a*) CH_2N_2/EtOH/0 °C/30 min; (*b*) allyl bromide/18-crown-6/KOH/THF/rt/2 h; (*c*) $Br_2/CHCl_3$/-50°C/40 min; (*d*) KSAc/DMF/rt/24 h; (*e*) (i) NaOH/MeOH/-30 °C/2 h, (ii) HCl/H_2O/-50°C/30 min.

Scheme 4.

Our generation of thiyl free radicals[30,40-41] employed the water-soluble initiator 2,2′-azobis-(2-methyl-2-propionamidine) dihydrochloride (AAPH) at 37 °C in N_2-saturated aqueous solutions at physiological pH. Control experiments demonstrated that the azido group in **9** was stable under conditions that generated radicals from AAPH, and that no abstraction/substitution of hydrogens in the thymine or sugar moieties was observed. Argon-saturated solutions of AZT (6.25 mM) and AAPH (37.5 mM) in MeOH-d_4:D_2O (2:1) or D_2O were heated (oil-bath) at 50 °C for 18 h with monitoring by ^{1}H NMR. Such heating of solutions of AZT/AAPH containing 2,3-dimercaptopropanoic acid or cysteine in MeOH-d_4:D_2O resulted in deuterium exchange for $H_\beta H_{\beta'}$ or H_α in the 2,3-dimercaptopropanoic acid or cysteine, respectively, but no exchange was observed in the recovered AZT. In contrast, thiols such as dithiolthreitol (DTT), glutathione, and 2-mercaptoethanol have been shown to reduce alkyl[42-43] and aryl azides.[44] Furthermore, the reduction of AZT to 3′-amino-3′-deoxythymidine with these thiols under physiological conditions (pH 7.2, 37 °C, H_2O) has been reported.[42,45] Second-order rate constants reported for those reductions were 2.77 x 10^7, 6.55 x 10^5, and 6.35 x 10^4 M^{-1} s^{-1}, respectively, with DTT, glutathione, and mercaptoethanol.[42]

Treatment of 3′-azido-3′-deoxy-5′-*O*-(2,3-dimercaptopropanoyl) thymidine (**11**) with AAPH in MeOH-d_4:D_2O (2:1) for 24 h resulted in formation of a polar product contaminated by AZT (~25%). Trituration of this material with $CHCl_3$ gave a residue containing a *single* thymidine-derived product (**15**, ~90% pure). The ^{1}H NMR spectrum of **15** differed from spectra of substrate **11,** AZT, 3′-NH_2T (**16a**) and 5′-*O*-Ac-3′-AcNHT (**16b**), as well as from reported spectra of 3′-*N*-acetyl-3′-amino-3′-deoxythymidine[46] and 3′-hydroxylamino-3′-deoxythymidine[47-48] (Figure S1 in the SI section). We then determined that the ^{1}H NMR spectrum of 3′-NH_2T (**16a**, synthesized as described,[49-50] see the SI section) in MeOH-d_4 (0.7 mL) after addition of 20% of DCl/D_2O (15 μL) was identical to the spectrum of product **15** (3′-amino-3′-deoxythymidine hydrochloride). Chromatography of **15** on silica gel or RP-HPLC resulted in isolation of **16a** (confirmed by ^{1}H and ^{13}C NMR and HRMS). Formation of **15** might involve radical or ionic pathways since ionized thiolates can reduce azido groups[24] and thiolates are present at pH ≈ 7 (~1–2%).[51] It is not possible to distinguish between those two pathways from our experiments, but analogous treatment of 3′-azido-5′-*O*-cysteinyl-3′-deoxythymidine (**13**) (lacking the vicinal dithiol moiety) produced AZT (~70-80%) without significant amounts of reduced-amino products.

Analogous treatment of 3′-azido nucleoside **21**, which has a vicinal dithiol moiety attached at C2′ with an ether lingkage, produced a mixture of products. Laborious purification yielded an adenosine-derived product that had ^{1}H NMR and MS spectra consistent with a 3′-amino-3′-deoxyadenosine core (see Experimental Part). Such treatment of dithiol **26** resulted in formation of a complex mixture with TLC and spectral propeties consistent with an intact azido group in the majority of products.

2.2. Gamma-irradiation

We also employed γ-radiolysis in N_2O-saturated aqueous solutions of cysteine (CySH) and AZT to investigate alternative generation of thiyl radicals in the bulk solution.[52] Radiolysis of neutral water produces hydrated electrons (e^-_{aq}), HO•, and H• as shown in Eq. 1, in which values in parentheses represent the chemical radiation yields (G) expressed in units of μmol J^{-1}. In N_2O-saturated solutions (~0.02 M), the e^-_{aq} react with N_2O and are transformed into HO• via Eq. 2 (k_2 = 9.1 x 10^9 M^{-1} s^{-1}). Thus, radiolysis of water affords G(HO•) = 0.55 μmol J^{-1}, whereas HO• and H• atoms represent 90% and 10%, respectively, of the reactive species.[53-54] The hydroxyl

radicals and hydrogen atoms can then react with thiols to generate thiyl radicals with rate constants for the reactions of $HO^{\bullet}$ and $H^{\bullet}$ with CySH of 4.7 x 10^{10} and 1.0 x 10^{9} M^{-1} s^{-1}, respectively (Eqs. 3 and 4).[55] The $H^{\bullet}$ atoms can react with CySH by two distinct pathways including hydrogen abstraction (Eq. 4) and homolytic substitution at sulfur (Eq. 5) with the preference being ~4:1 in favor of hydrogen abstraction.[56-57]

$$H_2O \xrightarrow{\gamma} e_{aq}^{-}\left(0.27\right), HO^{\bullet}\left(0.28\right), H^{\bullet}\left(0.06\right) \quad (1)$$

$$e_{aq}^{-} + N_2O + H_2O \rightarrow N_2 + HO^{\bullet} + HO^{-} \quad (2)$$

$$HO^{\bullet} + CySH \rightarrow CyS^{\bullet} + H_2O \quad (3)$$

$$H^{\bullet} + CySH \rightarrow CyS^{\bullet} + H_2 \quad (4)$$

$$H^{\bullet} + CySH \rightarrow Cy^{\bullet} + H_2S \quad (5)$$

$$e_{aq}^{-} + CySH \rightarrow Cy^{\bullet} + HS^{-} \quad (6)$$

Reaction of $HO^{\bullet}$ with AZT occurs[58] with a rate constant of 9.0 x 10^{9} M^{-1} s^{-1}, but the value for reaction of $H^{\bullet}$ with AZT is unknown. It is reasonable to assume a value of ~5 x 10^{8} M^{-1} s^{-1} based on the rate constants of 4.7 x 10^{9} and 3.2 x 10^{8} M^{-1} s^{-1} for the reactions of $HO^{\bullet}$ and $H^{\bullet}$, respectively, with thymidine.[59] On the basis of those kinetic values, hydroxyl and hydrogen radicals would react with cysteine to generate thiyl radicals at a faster rate than with AZT at concentrations of 1.0-mM AZT and 10-mM CySH. However, substantial amounts of HS^{-} can be formed by attack of e_{aq}^{-} upon CySH via Eq. 6 (k_6 = 1.0 x 10^{10} M^{-1} s^{-1}).[53-54]

Gamma irradiation of 1.0-mM AZT and 10-mM CySH in N_2O-saturated phosphate buffer (10 mM, pH 7.0) at doses of 2, 4, and 6 kGy with a dose rate of ca. 6.5 Gy/min produced (HPLC) 3′-amino-3′-deoxythymidine (3′-NH_2T), thymine (Th), H_2S, and cystine (CySSCy, Fig. 2). Concentration values derived from Fig. 2 allowed calculation of radiation chemical yields (G) (as described in the SI, Figure S2) of G(−AZT) = 0.05, G(CySSCy) = 0.46, G(3′-NH_2T) = 0.03 and G(Th) = 0.01 μmol J^{-1}, and the formation of H_2S was estimated to be ~0.10 μmol J^{-1}. The high G value for the formation of cystine indicated that the majority of $CyS^{\bullet}$ radicals recombined to form the disulfide in termination steps. Even though 3′-NH_2T and thymine were produced, it remained uncertain whether thiyl radicals were involved in their generation or if radical reactions caused decomposition of AZT to thymine, and ionic hydrosulfide reduced the azido group.[18,24]

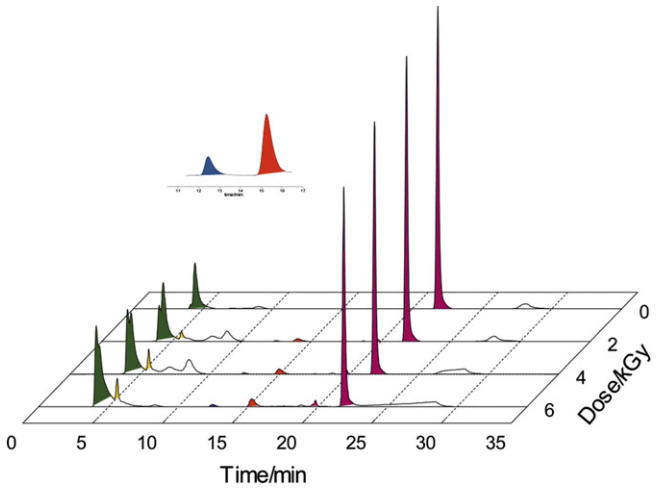


Fig. 2. HPLC analyses of γ-irradiation of N_2O-saturated aqueous solution containing AZT (1.0 mM) and cysteine (10 mM) at pH 7. Peaks correspond to cystine (t_R = 4.8 min), cysteine (t_R = 4.9 min), H_2S (t_R = 5 min), thymine (Th, t_R = 12 min), 3′-amino-3′-deoxythymidine (3′-NH_2T, t_R = 15 min), and AZT (t_R = 22 min).

Both pH- and O_2-dependent experiments were then performed. An increase in pH from 7.0 to 9.65 (pK_a 8.3 for cysteine)[52] should decrease the concentration of thiyl radicals is in favor of the disulfide radical (Eq. 7). However, the formation of both 3′-NH_2T and thymine remained relatively constant indicating that the reduction of AZT to 3′-NH_2T did not result from reactions with thiyl radicals or disulfide radical anions.

$$CyS^{\bullet} + CyS^{-} \rightleftharpoons CySSCy^{\bullet -} \quad (7)$$

Irradiation of a 10% oxygenated solution of 1.0-mM AZT and 10-mM cysteine at 2 kGy dose in 10-mM phosphate buffer (pH 7.0; 9:1 N_2O:O_2-purged) yielded an amount of 3′-NH_2T similar to the irradiation in N_2O-saturated solution but a noticeably higher amount of thymine (See Figure S3 in SI section). The formation of thymine can be rationalized by hydrogen abstraction from the ribose ring of AZT by radicals to form sugar radicals (e.g., at C1′), which then can react with O_2 to form alkyl peroxyl radicals that are known to decompose to give thymine.[60]

Our findings indicated that alkyl thiyl radicals generated in bulk solution were unlikely to have reacted with AZT via intermolecular radical mechanisms under the described radiolysis conditions, and that hydrosulfide anions generated by γ-irradiation of cysteine most likely reduced[18] AZT to 3′-NH_2T by ionic processes. It remains possible that thiyl radicals might react (Eq. 8) with azide anion (or HN_3) present at the enzyme active site[12] because thiyl radicals are known[52] to form 3-electron bonds with nucleophiles.

$$RS^{\bullet} + N_3^{-} \rightarrow RSN_3^{\bullet -} \rightarrow RSN^{\bullet -} + N_2 \quad (8)$$

2.3. Theoretical feasibility studies

We performed density functional calculations (B3LYP/6-31G*)[61,62] with the GAUSSIAN 98 program package[63] for an intramolecular reaction between a thiyl radical and an azido group accompanied by N_2 elimination. The method is expected to provide a semiquantitative accuracy within 3-5 kcal/mol for the reaction energies and barrier heights. The B3LYP method has been shown to be qualitatively reliable for studies of similar thiyl radical reactions.[14,64] Models derived by replacement of the adenine or uracil bases with an NH_2 group were used to reduce calculation times (e.g., **8** → **8′**; Fig. 3). The strategy involved: (a) finding an appropriate conformation of the open structure with a minimal S...N distance, (b) calculation of the radical structures obtained by removal of H from the S-H group, (c) searching for transition states for the ring closure, and (d) calculation of the closed-ring structures formed after elimination of N_2.

Nucleoside **8** and **13**, with the cysteinate attached to C2′ and C5′, respectively, were assumed to undergo ring closure reactions between the cysteine-derived thiyl radical and the azido group via 8- and 9-membered transition states, respectively. Thus, intramolecular addition of the thiyl radical in **I** to the azido group via an eight-membered TS would produce the transient triazenyl radical **J**

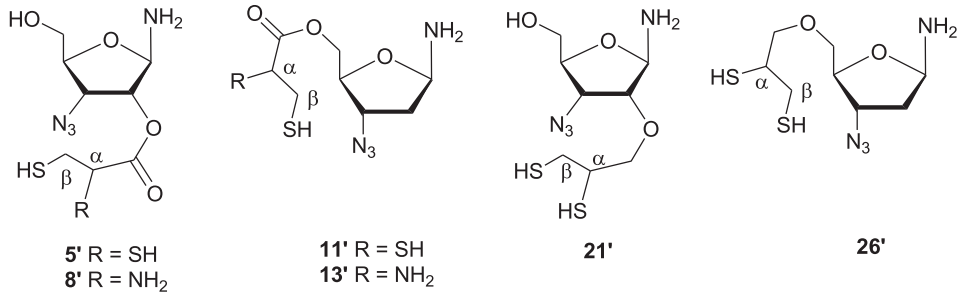


Fig. 3. Model compounds with NH_2 at C1 used for density functional calculations. For example compound 5′ is derived from 5 by replacing adenine base with amino group.

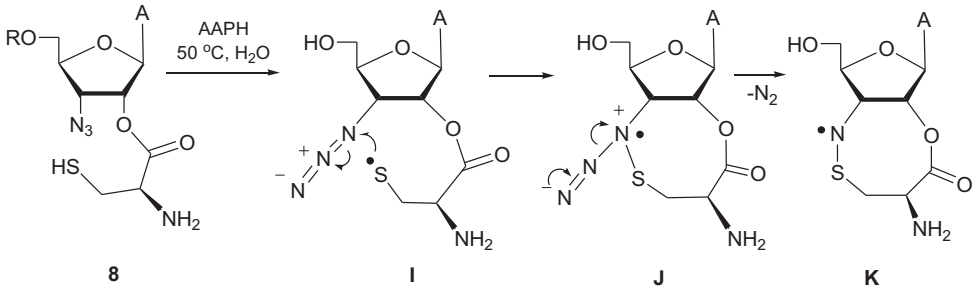


Fig. 4. Plausible intramolecular interactions of the thiyl radical derived from the cysteine with azido group at C3′ for adenosine-derived substrate 8. Figure S4 in SI section shows an intramolecular addition of the thiyl radical with azido group for thymidine-derived substrate 11.

(Fig. 4). Loss of N_2 would generate nitrogen-centered radical **K**, and abstraction of a hydrogen atom by **K** should give a cyclic sulfenamide, which might undergo ring opening to give 3′-amino-3′-deoxy products. Addition of thermally or photochemically generated organosilyl radicals to organic azides is known to produce 1,3- or 3,3-triazenyl species.[20]

Our calculations indicated that intramolecular reactions between the thiyl radical and azido group are favorable for substrates **8**′ and **13**′. The reactions were calculated to be exothermic by 33.6 to 40.6 kcal/mol and to have low energy barriers of 10.4 to 13.5 kcal/mol (Table 1). The energy barriers were calculated to be about 2 kcal/mol lower for isomers having the *R* stereochemistry for **8**′ and *S* for **13**′ at C_α of the cysteinyl fragment, but the reaction energies varied within 2-3 kcal/mol. Thus, such ring closure reactions involving a thiyl radical and an azide group in **8** and **13** were calculated to be feasible.

Table 1
Reaction energies and barrier heights for the ring closure reaction with substrates 8′ and 13′ bearing a cysteinyl moiety

Compounds	TS energy barrier $\Delta E^{\#}$, kcal/mol	Energy ΔE_r, kcal/mol
8′ (*R* at C_α)	10.4	−33.6
8′ (*S* at C_α)	12.1	−35.6
13′ (*R* at C_α)	13.5	−37.8
13′ (*S* at C_α)	11.7	−40.6

Fig. 5 shows optimized structures and relative energies along the path of ring closure and N_2 elimination reactions of **8**′ and **13**′. The calculations indicate that the reactions occur in two steps. First, the thiyl radical approaches the azide group via 8- and 9-membered transition states for **8**′ and **13**′, respectively, to form cyclic intermediates followed by molecular nitrogen loss in a second step. The first ring-closure step is rate-determining because it has a higher barrier. The cyclic intermediates are metastable with loss of N_2 calculated to have barriers in the 1.3-5.6 kcal/mol range.

Calculations for substrates **5**′ and **11**′ with 2,3-dimercaptopropanoate at C2′ and C5′, respectively, indicated that the ring-closure reactions involving thiyl radical $S^{\bullet}_{\beta}$ (at C_β) were exothermic (ΔE = -34.5 to -38.4 kcal/mol) with relatively low energy barriers of 9.1 to 17.8 kcal/mol (Table 2). Fig. 6 shows optimized geometries and relative energies for structures along the reaction path of the ring closure in **5**′ and **11**′ between the C_β thiyl radical from the vicinal disulfide and the azide. As in the case of the cysteine-derived thiyl radical, reactions proceed by a two-step mechanism, with ring closures occurring at the first step through 8- and 9-membered transition states for **5**′ and **11**′ followed by N_2 elimination in the second step. The ring closure steps exhibit the highest (rate-controlling) barriers and the cyclic intermediates are likely metastable [except for **11**′ (*S* at C_α; $S^{\bullet}_{\beta}$)] with respect to loss of N_2 (1.2-8.2 kcal/mol barriers). The position of the thiyl radical strongly affects the energy barrier for the ring-closure reaction. Thus, with the primary thiyl radical at the β position, closure between the thiyl radical and the azido group was feasible both for *S* and *R* diastereomers at C_α. The barriers heights for the two diastereomers of **5**′ did not differ significantly, but for **11**′ the closure was clearly favored for *S* at C_α (9.1 kcal/mol) versus that for *R* at C_α (17.8 kcal/mol). Calculated closures involving a secondary thiyl radical $S^{\bullet}_{\alpha}$ (at C_α) and the azido group, which required 7- and 8-membered transition states, were prohibitive with a barrier of ≥43.4 kcal/mol.

We also studied ring closure reactions in model substrates **21**′ and **26**′ in which the carbonyl moiety is replaced by a CH_2 group (Table 2 and Figure S7 in the SI section). There, we considered only transition states for the rate-determining ring closure and the final cyclic products after loss of molecular nitrogen. The results were similar to those described above for **5**′ and **11**′. The ring-closure barrier in **21**′ was computed to be 11.5 kcal/mol, very close to that for **5**′ (*R* at C_α), S_β, and the overall reaction exothermicity is 41.2 kcal/mol, 3-4 kcal/mol larger than values calculated for the diastereomers of **5**′. The ring closure barrier in **26** is ~3 kcal/mol higher than that for **11**′ (*S* at C_α), S_β, and the difference in the reaction energies is 1 kcal/mol. Thus, substitution of CH_2 for C=O does not significantly affect feasibility of the ring closure reaction.

Pereira et al.[14] had performed DFT B3LYP calculations on an analogous intermolecular reaction between a $CH_3S^{\bullet}$ radical and an azide-containing RNR-substrate model. Their Gibbs free energy

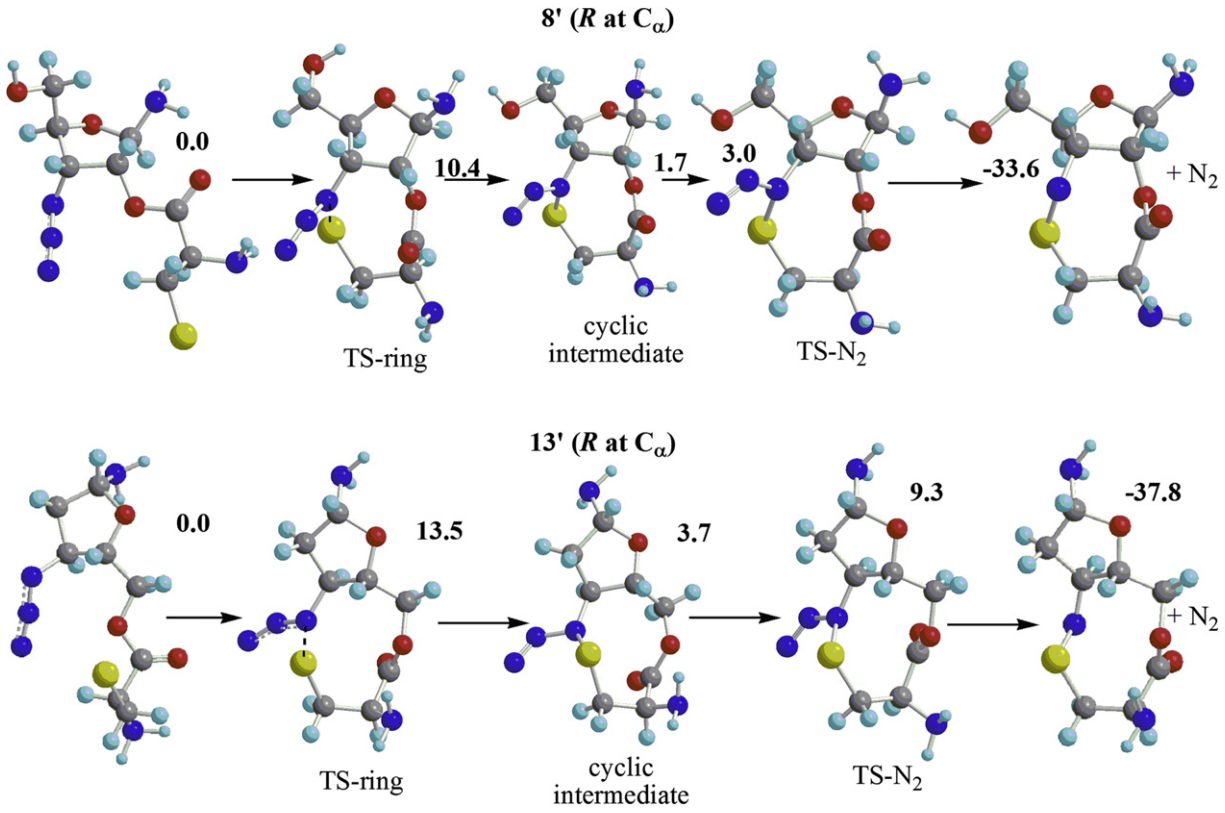


Fig. 5. Ring closure reactions between a thiyl radical from a cysteinyl moiety and azide in 8′ and 13′ through 8- and 9-membered TS. Bold numbers show relative energies in kcal/mol. Figure S5 in SI section shows the ring closure reactions with 8′ and 13′ having the unnatural *S* stereochemistry at C_α of cysteine.

Table 2
DFT B3LYP/6-31G*set calculated reaction energies and barrier heights for the ring closure in model substrates bearing a vicinal disulfide

Compounds	TS energy barrier $\Delta E^{\#}$, kcal/mol	Energy ΔE_r, kcal/mol
5′ (*R* at C_α), S_β	11.4	−38.1
5′ (*S* at C_α), S_β	12.6	−36.7
5′ (*R* at C_α), S_α	does not occur	-
5′ (*S* at C_α), S_α	43.4	−35.7
11′ (*R* at C_α), S_β	17.8	−34.5
11′ (*S* at C_α), S_β	9.1	−38.4
11′ (*R* at C_α), S_α	does not occur	-
11′ (*S* at C_α), S_α	does not occur	-
21′ (*R* at C_α), S_β	11.5	−41.2
26′ (*S* at C_α), S_β	12.1	−39.4

barrier ($\Delta G^{\#}$ 15.6 kcal/mol) and exoergicity (ΔG_r 53.8 kcal/mol) for an intermolecular thiyl radical addition to an azide moiety and loss of N_2 can be compared with our intramolecular model reactions. Our barriers for the intramolecular thiyl addition to the azido group (ring formation) are generally 3-6 kcal/mol lower (for favorable diastereomers) than those for the Pereira et al. intermolecular addition, whereas their intermolecular reaction for S-N bond formation and loss of N_2 is 12-18 kcal/mol more exothermic than our intramolecular processes. Computed values of $\Delta E^{\#}$ and ΔE_r do not differ by more than ~1 kcal/mol at physiological temperature from those for $\Delta G^{\#}$ and ΔG_r.

3. Conclusion

We synthesized 3′-azido-3′-deoxy-2′-*O*-(cysteinyl or 2,3-dimercaptopropanoyl or 2,3-dimercaptopropyl)adenosine and 3′-azido-3′-deoxy-5′-*O*-(cysteinyl or 2,3-dimercaptopropanoyl or 2,3-dimercaptopropyl)thymidine derivatives as model structures for the study of reactions postulated to occur at the active sites of ribonucleotide reductases. Density functional calculations indicated that intramolecular reactions between the derived thiyl radicals and the proximal azido groups should have low energy barriers of 10.4–13.5 kcal/mol and be exothermic by 33.6–41.2 kcal/mol. We used 2,2′-azobis-(2-methyl-2-propionamidine) dihydrochloride (AAPH) as an initiator for the production of thiyl radicals in our 3′-azido-3′-deoxynucleoside derivatives bearing thiol or vicinal dithiol substituents. Such treatment of 3′-azido-3′-deoxy-5′-*O*-(2,3-dimercaptopropanoyl)thymidine gave 3′-amino-3′-deoxythymidine hydrochloride, whereas analogous treatment of 3′-azido-3′-deoxythymidine (AZT, a control substrate lacking a thiol substituent) resulted in isolation of major quantities of unchanged AZT. Subjection of N_2O-saturated aqueous solutions of AZT and cysteine to γ-radiolysis produced 3′-amino-3′-deoxythymidine and thymine. However, radical abstraction of hydrogen atoms from the sugar moiety of AZT could rationalize the formation of thymine, and ionic reduction of the azide group by hydrosulfide anions generated in situ is the most likely explanation for formation of 3′-amino-3′-deoxythymine. Our (1) DFT-calculated predictions, (2) results with radical-initiated intramolecular azide reduction with model structures bearing azido and thiol substituents, and (3) the lack of evidence for intermolecular reduction of the azido group in AZT by thiyl radicals generated in radiolysis experiments are in harmony with the enzymatic positioning of azido-containing substrates in close proximity with thiol functionalities that exist in the active sites of ribonucleotide reductases.

4. Experimental Part

4.1. General

UV spectra were measured with solutions in MeOH. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were determined with solutions in

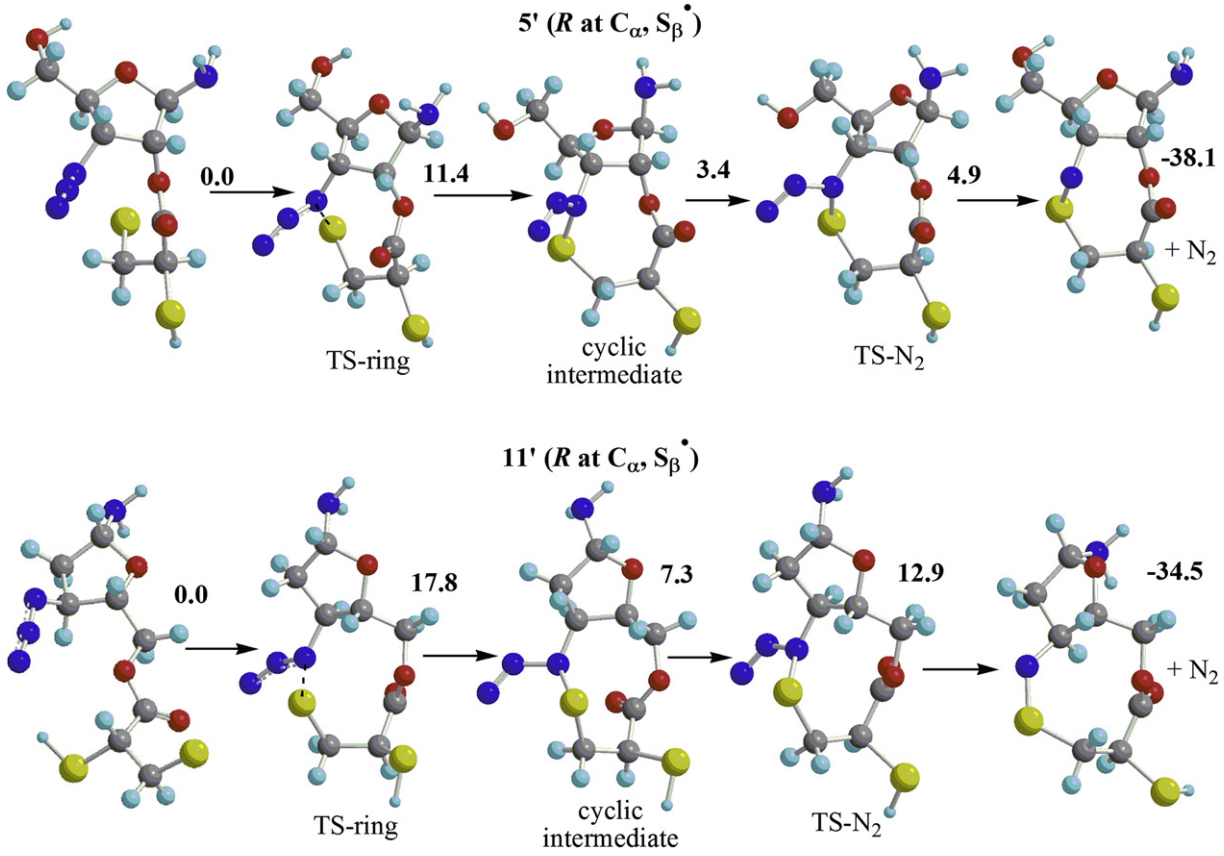


Fig. 6. Ring closure reactions between a thiyl radical from vicinal disulfide moiety and azide in 5′ and 11′ through 8- and 9-membered TS. Bold numbers show relative energies in kcal/mol. Figure S6 in SI section shows the ring closure reactions with *S* diastereomers (at C_α) of 5′ and 11′.

$CDCl_3$ unless otherwise noted. Mass spectra (MS) were obtained with atmospheric pressure chemical ionization (APCI) technique and HRMS in ESI mode unless otherwise noted. TLC was performed with Merck kieselgel 60-F_{254} sheets with products detected with 254 nm light or by development of color with I_2 or Ellman reagent. Merck kieselgel 60 (230-400 mesh) was used for column chromatography. 5% MeOH/EtOAc, 5% MeOH/$CHCl_3$ or SSE system (EtOAc/*i*-PrOH/H_2O 4:1:2, upper layer) was used as mobile phase for TLC or column chromatograpy. HPLC purifications were performed using XTerra® preparative RP_{18} OBD™ column (5μm 19 x 150 mm) with gradient program using CH_3CN/H_2O as a mobile phase. Reagent grade chemicals were used, and solvents were dried by reflux over and distillation from CaH_2 (except THF/potassium) under argon.

*4.1.1. 3′-Azido-5′-O-(tert-butyldiphenylsilyl)-3′-deoxyadenosine (**2a**).* TBDPSiCl (0.18 mL, 190 mg, 0.69 mmol) was added to a stirred solution of 3′-azido-3′-deoxyadenosine[26] (**1**; 100 mg, 0.34 mmol) in anhydrous pyridine (1.5 mL) under N_2 atmosphere at ambient temperature. After 48 h, volatiles were evaporated and the resulting residue was partitioned ($CHCl_3$//HCl/H_2O). The organic layer was washed (sat. $NaHCO_3/H_2O$, brine), dried ($MgSO_4$), and evaporated and the residue was column chromatographed (1 → 6% MeOH/$CHCl_3$) to give **2a** (161 mg, 89%): ^{1}H NMR δ 1.02 (s, 9, *t*-Bu), 3.82 (dd, *J* = 11.8, 3.0 Hz, 1, H5″), 3.98 (dd, *J* = 11.8, 3.4 Hz, 1, H5′), 4.28 ("q", *J* = 4.3 Hz, 1, H4′), 4.36 (t, *J* = 5.0 Hz, 1, H3′), 4.92 (t, *J* = 5.2 Hz, 1, H2′), 5.99 (d, *J* = 4.9 Hz, 1, H1′), 6.20 (br s, 2, NH_2), 7.37-7.64 (m, 10, Ar), 8.10 (s, 1, H2), 8.24 (s, 1, H8); HRMS *m/z* calcd for $C_{26}H_{31}N_8O_3Si$ $[M+H]^+$ 531.2283; found 531.2283.

*4.1.2. 3′-Azido-5′-O-(tert-butyldimethylsilyl)-3′-deoxyadenosine (**2b**).* TBDMSCl (128 mg, 0.85 mmol) was added to a stirred solution of **1**[26] (190 mg, 0.65 mmol) in anhydrous pyridine (3 mL) at ambient temperature. After 16 h, volatiles were evaporated and the oily residue was column chromatographed (hexane/EtOAc, 2:8) to give **2b** (201 mg, 76%): ^{1}H NMR δ 0.00 (s, 3, Me), 0.07 (s, 3, Me), 0.81 (s, 9, *t*-Bu), 3.81 (dd, *J* = 2.7, 11.5 Hz, 1, H5′), 3.89 (dd, *J* = 3.5, 11.5 Hz, 1, H5″), 4.29 ('q', *J* = 3.2 Hz, 1, H4′), 4.37 (dd, *J* = 3.4, 5.6 Hz, 1, H3′), 4.85 ('t', *J* = 5.3 Hz, 1, H2′), 5.56 (br., 1, OH), 5.62 (br., 2, NH_2), 5.94 (d, *J* = 5.3 Hz, 1, H1′), 8.07 (s, 1, H8), 8.33 (s, 1, H2); ^{13}C NMR δ -5.6 (Me), -5.5 (Me), 18.3 (*t*-Bu), 25.8 (*t*-Bu), 61.6 (C3′), 62.6 (C5′), 76.5 (C2′), 83.9 (C4′), 90.2 (C1′), 119.8 (C5), 138.8 (C8), 148.9 (C4), 152.5 (C2), 155.5 (C6); HRMS *m/z* calcd for $C_{16}H_{27}N_8O_3Si$ $[M+H]^+$ 407.1970; found 407.1968.

*4.1.3. 3′-Azido-3′-deoxy-5′-O-(tert-butyldiphenylsilyl)-2′-O-(2,3-S-isopropylidene-2,3-dimercaptopropanoyl)adenosine (**3**).* Compound **2a** (0.12 g, 0.23 mmol) was added to a stirred solution of 2,3-*S*-isopropylidene-2,3-dimercaptopropanoic acid (0.08 g, 0.45 mmol), 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDCI; 0.16 mL, 0.14 g, 0.90 mmol), and DMAP (0.084 g, 0.69 mmol) in CH_2Cl_2 (10 mL) under N_2 atmosphere at 0°C (ice bath). After 4 h, aqueous 5% HCl (30 mL) was added and the resulting mixture was extracted with CH_2Cl_2. The combined organic layer was washed with aqueous $NaHCO_3$, brine, dried ($MgSO_4$), and evaporated. Purification on silica gel column (EtOAc/hexane, 1:1) gave **3** (110 mg, 69%; 2:1): ^{1}H NMR δ 1.05 (s, 6, *t*-Bu), 1.07 (s, 3, *t*-Bu), 1.81 &1.82 (2 × s, 4, CH_3), 1.82 & 1.83 (2 × s, 2, CH_3), 3.51 (dd, *J* = 6.2, 12.6 Hz, 0.33, Hβ′), 3.55 (dd, *J* = 6.1, 12.6 Hz, 0.67, Hβ′), 3.76-3.78 (m, 0.33, Hβ″), 3.79 (dd, *J* = 5.9, 12.6 Hz, 0.67, Hβ″), 3.85 (dd, *J* = 3.4, 11.8 Hz, 1, H5′), 4.04 (dd, *J* = 3.2, 11.8 Hz, 0.67, H5″), 4.06 (dd, *J* = 3.2, 11.8 Hz, 0.33, H5″), 4.16 (dd, *J* = 3.4, 6.7 Hz, 1, H4′), 4.61 ("q", *J* = 4.0 Hz, 1, Hα), 4.87 (t, *J* = 6.5 Hz, 0.67, H3′), 4.78 (t, *J* = 6.5 Hz, 0.33, H3′), 6.03

("q", J = 2.7 Hz, 0.67, H2′), 5.97 ("q", J =2.7 Hz, 0.33, H2′), 6.10 (d, J = 3.8 Hz, 0.67, H1′), 6.13 (d, J = 3.8 Hz, 0.33, H1′), 6.29 (s, 2, NH_2), 7.35-7.66 (m, 10, Ar), 7.99 (s, 0.67, H8), 8.07 (s, 0.33, H8), 8.27 (s, 1, H2); ^{13}C NMR (major isomer) δ 19.2 (*t*-Bu), 26.8 (*t*-Bu), 33.2 (CH_3), 40.2 (Cβ), 56.6 (Cα), 59.9 (C3′), 62.6 (C5′), 63.5 (*C*Me$_2$), 76.4 (C1′), 82.6 (C4′), 86.9 (C2′), 120.1 (C5), 127.8, 127.9, 129.9, 130.0, 132.5, 132.5, 135.5, & 135.6 (Ar), 139.3 (C8), 149.5 (C6), 153.3 (C2), 155.6 (C4), 169.9 (CO); ^{13}C NMR (minor isomer) δ 19.2 (*t*-Bu), 26.8 (*t*-Bu), 33.3 (CH_3), 40.2 (Cβ), 56.5 (Cα), 60.0 (C3′), 62.6 (C5′), 63.5 (*C*Me$_2$), 76.3 (C1′), 82.8 (C4′), 86.7 (C2′), 120.1 (C5), 127.86, 127.91, 130.0, 130.1, 132.4, 132.5, 135.5 & 135.6 (Ar), 139.1 (C8), 149.5 (C6), 153.3 (C2), 155.6 (CNH_2), 170.0 (CO); HRMS *m/z* calcd for $C_{32}H_{39}N_8O_3S_2Si$ $[M + H]^+$ 691.2299; found 691.2281.

*4.1.4. 3′-Azido-3′-deoxy-2′-O-(2,3-S-isopropylidene-2,3-dimercaptopropanoyl)adenosine (**4**).* A solution of **3** (0.05 g, 0.072 mmol) in TFA/H_2O (9:1, 1.5 mL) was stirred at 0 °C for 2 h. The volatiles were evaporated and coevaporated with toluene to yield an oily residue which was purified on silica gel column (EtOAc) to give **4** (10 mg, 31%, 9:1): ^{1}H NMR δ 1.75 (s, 2.7, CH_3), 1.82 (s, 0.3, CH_3), 1.80 (s, 2.7, CH_3), 1.83 (s, 0.3, CH_3), 3.47 (dd, J = 6.2, 12.6, Hz, 0.9, Hβ′), 3.45 (dd, J = 6.2, 12.6, Hz, 0.1, Hβ′), 3.68 (dd, J = 5.8, 12.6 Hz, 0.9, Hβ″), 3.70 (dd, J = 5.8, 12.6 Hz, 0.1, Hβ″), 3.76 (dd, J = 0.9, 13.2 Hz, 1, H5′), 4.02 (dd, J = 1.3, 13.2 Hz, 1, H5″), 4.30 ("d", J = 1.5 Hz, 0.9, H4′), 4.33 ("d", J = 1.5 Hz, 0.1 H4′), 4.57 (t, J = 6.0 Hz, 0.9, Hα), 4.53 (t, J = 6.0 Hz, 0.1, Hα), 4.74 (dd, J = 1.8, 5.7 Hz, 0.9, H3′), 4.76 (dd, J = 1.8, 5.7 Hz, 0.1, H3′), 5.97-6.00 (m, 3, H2′, NH_2), 6.08 (dd, J = 1.3, 7.1 Hz, 1, H1′), 7.83 (s, 1, H8), 8.33 (s, 1, H2); ^{13}C NMR (major isomer) δ 32.9 & 33.2 (CH_3), 39.6 (Cβ), 55.9 (Cα), 61.7 (C3′), 63.0 (C5′), 63.5 (*C*Me$_2$), 75.4 (C1′), 86.1 (C4′), 88.6 (C2′), 121.1 (C5), 140.2 (C8), 148.5 (C6), 152.6 (C2), 156.0 (C4), 169.8 (CO); ^{13}C NMR (minor isomer) δ 33.0 & 33.3 (CH_3), 39.7 (Cβ), 56.3 (Cα), 62.0 (C3′), 63.0 (C5′), 63.5 (*C*Me$_2$), 75.4 (C1′), 86.3 (C4′), 88.4 (C2′), 121.1 (C5), 140.2 (C8), 148.5 (C6), 152.6 (C2), 156.0 (C4), 169.8 (CO); MS *m/z* 453 $[M + H]^+$.

*4.1.5. 3′-Azido-3′-deoxy-2′-O-(2,3-dimercaptopropanoyl)adenosine (**5**).* $HgCl_2$ (0.078 g, 0.29 mmol) was added to a stirred solution of **4** (10 mg, 0.022 mmol) in MeCN/H_2O (3:1, 2 mL) at room temperature. After 3 h, the resulting mixture was concentrated to dryness and the residue solid was washed with water, and dried. Hydrogen sulfide, generated from HCl/NaSH, was bubbled through a capillary into the stirring suspension of the dried residue in MeOH (8 mL). After 30 min, the resulting black solid was filtered, and the filtrate was concentrated. Purification on a silica gel column gave **5** (5 mg, 55%): ^{1}H NMR (MeOH-d_4) δ 2.88 (dd, J = 9.6, 13.7 Hz, 1, Hβ′), 2.96 (dd, J = 5.2, 13.7 Hz, 1, Hβ″), 3.65 ("q", J = 4.8 Hz, 1, Hα), 3.83 (dd, J = 3.0, 12.6 Hz, 1, H5′), 3.96 (dd, J = 2.8, 12.6 Hz, 1, H5″), 4.23 ("p", J = 2.8 Hz, 1, H4′), 4.78 (t, J = 7.3 Hz, 1, H3′), 5.99 (dd, J = 4.3, 5.6 Hz, 1, H2′), 6.35 (d, J = 4.3 Hz, 1, H1′), 8.45 (s, 1, H8), 8.70 (s, 1, H2); ^{13}C NMR (MeOH-d_4) 29.8 (Cβ), 44.3 (Cα), 61.8 (C3′), 61.8 (C5′), 78.1 (C1′), 85.5 (C4′), 88.4 (C2′), 120.5 (C5), 143.9 (C8), 145.7 (C6), 149.7 (C2), 151.9 (C4), 172.3 (CO); HRMS *m/z* calcd for $C_{13}H_{17}N_8O_4S_2$ $[M + H]^+$ 413.0809; found 413.0816.

*4.1.6. 3′-Azido-3′-deoxy-5′-O-(tert-butyldiphenylsilyl)-2′-O-(N-tert-butoxycarbonyl-S-tritylcysteinyl)adenosine (**6**).* Compound **2a** (30 mg, 0.057 mmol) was added to a stirred solution of *N*-*t*-butylcarboxyl-*S*-trityl-L-cysteine (52 mg, 0.112 mmol), 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDCI; 30 μL, 26.3 mg, 0.170 mmol), and DMAP (0.014 g, 0.115 mmol) in CH_2Cl_2 (5 mL) under N_2 atmosphere at 0o C (ice bath). After 4 h, aqueous 5% HCl (20 mL) was added and the resulting mixture was extracted with CH_2Cl_2. The combined organic layer was washed with $NaHCO_3/H_2O$, brine, dried ($MgSO_4$), and evaporated. Purification on silica gel column (EtOAc/hexane, 1:1) gave **6** (44 mg, 79%): ^{1}H NMR δ 0.94 (s, 9, *t*-Bu), 1.34 (s, 9, *t*-Bu), 2.54 (dd, J = 4.5, 12.6 Hz, 1, Hβ′), 2.63 (dd, J = 7.5, 12.4 Hz, 1, Hβ″), 3.71 (dd, J = 3.6, 11.4 Hz, 1, H5′), 3.89-3.95 (m, 2, H4′,5″), 4.09 ("q", J = 6.5 Hz, 1, Hα), 4.83 (t, J = 6.3 Hz, 1, H3′), 5.05 (br. d, J = 8.0 Hz, 1, NH), 5.88-5.92 (m, 2, H1′,2′), 5.97 (br. s, 2, NH_2), (7.10-7.54 (m, 25, Ar), 7.80 (s, 1, H8), 8.11 (s, 1, H2); HRMS *m/z* calcd for $C_{53}H_{58}N_9O_4S_2Si$ $[M + H]^+$ 976.3995; found 976.3986.

*4.1.7. 3′-Azido-3′-deoxy-2′-O-(S-tritylcysteinyl)adenosine (**7**).* A solution of **6** (90 mg, 0.092 mmol) in TFA/H_2O (9:1, 3 mL) was stirred at 0 °C for 2 h. Volatiles were evaporated and coevaporated to with toluene to yield an oily residue which was purified on silica gel column ($CHCl_3$/MeOH, 9:1) to give **7** (45 mg, 77%): ^{1}H NMR (MeOH-d_4) δ 2.91 (dd, J = 5.9, 13.9 Hz, 1, Hβ′), 2.96 (dd, J = 8.5, 13.9 Hz, 1, Hβ″), 3.23 (dd, J = 5.3, 8.4 Hz, 1, Hα), 3.76 (dd, J = 2.8, 12.7 Hz, 1, H5′), 3.92 (dd, J = 2.6, 12.7 Hz, 1, H5″), 4.03 (dt, J = 2.8, 6.6 Hz, 1, H4′), 4.80 (t, J = 5.9 Hz, 1, H3′), 5.93 (dd, J = 4.1, 5.5 Hz, 1, H2′), 6.16 (d, J = 3.9 Hz, 1, H1′), 7.27-7.49 (m, 15, Tr), 8.21 (s, 1, H8), 8.39 (s, 1, H2); ^{13}C NMR (MeOH-d_4): δ 33.4 (Cβ), 53.1 (Cα), 61.6 (C3′), 69.1 (C5′), 78.3 (C2′), 85.2 (C4′), 88.1 (C1′), 120.6 (C5), 128.4, 129.4, 130.7, 145.2 (Tr), 141.4 (C8), 150.0 (C6), 153.4 (C2), 157.1 (C4), 168.4 (CO); MS *m/z* 638 $[M + H]^+$.

*4.1.8. 3′-Azido-3′-deoxy-2′-O-cysteinyladenosine (**8**).* TFA (1.0 mL) and Et_3SiH (40 μL, 29 mg, 0.25 mmol) were added to the solution of **7** (10 mg, 0.016 mmol) in anhydrous CH_2Cl_2 (1.5 mL) at ambient temperature. After 2 h, the volatiles were evaporated and coevaporated with toluene under high vacuum. The residual solid was washed with ethyl ether to give **8** (5 mg, 79%): ^{1}H NMR (MeOH-d_4) δ 3.19 (dd, J = 4.5, 15.1 Hz, 1, Hβ′), 3.25 (dd, J = 5.4, 15.2 Hz, 1, Hβ″), 3.83 (dd, J = 2.8, 12.7 Hz, 1, H5′), 3.99 (dd, J = 2.8, 12.7 Hz, 1, H5″), 4.22 (dt, J = 2.8, 6.6 Hz, 1, H4′), 4.54 (dd, J = 4.5, 5.3 Hz, 1, Hα), 4.92 (t, J = 6.5 Hz, 1, H3′), 6.09 (dd, J = 3.6, 5.5 Hz, 1, H2′), 6.32 (d, J = 3.5 Hz, 1, H1′), 8.32 (s, 1, H8), 8.52 (s, 1, H2); ^{13}C NMR (MeOH-d_4) δ 25.3 (Cβ), 55.7 (Cα), 61.6 (C3′), 66.9 (C5′), 78.7 (C1′), 85.2 (C4′), 88.5 (C2′), 120.5 (C5), 142.3 (C8), 145.7 (C6), 150.4 (C2), 151.9 (C4), 172.3 (CO); HRMS *m/z* calcd for $C_{13}H_{18}N_9O_4S$ $[M + H]^+$ 396.1197, found 396.1195.

*4.1.9. 3′-Azido-3′-deoxy-5′-O-(2,3-S-isopropylidene-2,3-dimercaptopropanoyl)thymidine (**10**).* DMAP (69 mg, 0.57 mmol) and EDC (131 μl, 115 mg, 0.74 mmol) were added to a stirred solution of 2,3-*S*-isopropylidene-2,3-dimercaptopropanoic acid (67 mg, 0.38 mmol) in CH_2Cl_2 (4 mL) at 0 °C under argon atmosphere. After 5 min AZT (**9**; 50 mg, 0.187 mmol) was added and stirring was continued for 3 h. The resulting mixture was diluted with CH_2Cl_2 and was successively washed with saturated $NaHCO_3/H_2O$ and 0.05 M HCl. Acidic water layer was extracted with CH_2Cl_2 and the combined organic layer was washed with saturated $NaHCO_3/H_2O$, brine and was dried (Na_2SO_4) to give 105 mg of the yellow oily residue which was column chromatographed (EtOAc/hexane, 6:4) to give **10** (72 mg, 90%; 0.45:0.55 mixture of diastereomers): ^{1}H NMR δ 1.81 (s, 1.35, CH_3), 1.82 (s, 1.65, CH_3), 1.83 (s, 1.35, CH_3), 1.84 (s, 1.65, CH_3), 1.97 (s, 3, CH_3), 2.31-2.52 (m, 2, H2′,2″), 3.512 (dd, J = 6.1, 12.6 Hz, 0.45, C*H*HS), 3.515 (dd, J = 6.1, 12.6 Hz, 0.55, C*H*HS), 3.76 (dd, J = 6.5, 12.6 Hz, 0.45, CH*H*S), 3.80 (dd, J = 6.0, 12.6 Hz, 0.55, CH*H*S), 4.06-4.11 (m, 1, H4′), 4.23-4.29 (m, 1, H3′), 4.31 (dd, J = 3.1, 12.4 Hz, 0.55, H5′), 4.39 (dd, J = 4.2, 12.2 Hz, 0.45, H5′), 4.45 (dd, J = 3.5, 12.2 Hz, 0.45, H5″), 4.556 (t, J = 6.0 Hz, 0.55, CHS), 4.560 (t, J = 6.3 Hz, 0.45, CHS), 4.59 (dd, J = 3.4, 12.4 Hz, 0.55, H5″), 6.12 (t, J = 6.6 Hz, 0.45, H1′), 6.20 (t, J = 6.6 Hz, 0.55, H1′) 7.21 ('q', J = 1.1 Hz, 0.45, H6), 7.27 ('q', J = 1.1 Hz, 0.55, H6), 9.54 (br., 1, NH); ^{13}C NMR δ 12.6 (0.45, C5-*C*H$_3$), 12.7 (0.55, C5-*C*H$_3$), 33.19 (0.45, C*Me*$_2$), 33.20 (0.55, C*Me*$_2$), 33.22 (0.55, C*Me*$_2$), 33.24 (0.45, C*Me*$_2$), 37.4 (0.45, C2′), 37.5 (0.55, C2′), 39.8 (0.55, CH_2S), 39.9 (0.45, CH_2S), 56.5 (0.45, CHS), 56.6 (0.55, CHS), 60.3 (0.55, C3′), 60.6 (0.45, C3′), 63.6 (0.45, *C*Me$_2$), 63.7 (0.55, *C*Me$_2$), 64.1 (0.55, C5′), 64.4 (0.45, C5′), 81.4 (0.45, C4′), 81.7 (0.55, C4′), 85.0 (0.55, C1′), 85.4 (0.45, C1′), 111.5 (1, C5), 135.27 (0.55, C6), 135.31 (0.45, C6), 150.2 (0.45, C2) 150.3 (0.55, C2), 163.7 (0.55, C4), 163.8 (0.45, C4), 170.30 (0.45, CO),

170.32 (0.55, CO); HRMS *m/z* calcd for $C_{16}H_{22}N_5O_5S_2$ $[M + H]^+$ 428.1057, found 428.1039.

*4.1.10. 3′-Azido-3′-deoxy-5′-O-(2,3-dimercaptopropanoyl)thymidine (**11**).* $HgCl_2$ (760 mg, 2.8 mmol) was added in one portion to a stirred solution of **10** (80 mg, 0.187 mmol) in MeCN/H_2O (3:1, 8 mL) at rt. The resulting white suspension continued to stir for 60 min. until only trace of substrate **10** was detected (TLC). The reaction mixture was decanted, the white solid was washed with water (2x), and dried. Mercury complex was suspended in MeOH (25 mL), and H_2S was bubbled through the stirring suspension. Almost immediately, white solid was dissolved, and black HgS precipitated. After 30 min reaction mixture was evaporated to dryness, and the black residue was treated with $CHCl_3$ giving a slurry, which was directly chromatographed (60 → 70 %, EtOAc/hexane) to give **11** (33.5 mg, 46%; 0.45:0.55 mixture of diasteromers) as an colorless oil: ^{1}H NMR δ 1.86 (t, *J* = 8.9 Hz, 0.45, SH), 1.88 (t, *J* = 8.9 Hz, 0.55, SH), 1.969 (d, *J* = 1.2 Hz, 1.35, CH_3), 1.973 (d, *J* = 1.2 Hz, 1.65, CH_3), 2.32 (d, *J* = 10.4 Hz, 0.45, SH), 2.33 (d, *J* = 10.5 Hz, 0.55, SH), 2.38-2.54 (m, 2, H2′,2″), 2.92-3.04 (m, 2, CH_2S), 3.51 (ddd, *J* = 5.7, 8.7, 10.5 Hz, 0.45, CHS), 3.53 (ddd, *J* = 5.7, 8.7, 10.5 Hz, 0.55, CHS), 4.12 ("q", *J* = 4.3 Hz, 1, H4′), 4.27-4.33 (m, 1, H3′), 4.41 (dd, *J* = 3.6, 12.2 Hz, 0.55, H5′), 4.46 (dd, *J* = 3.6, 12.2 Hz, 0.45, H5′), 4.52 (dd, *J* = 4.3, 12.2 Hz, 0.45, H5″) 4.58 (dd, *J* = 4.2, 12.2 Hz, 0.55, H5″), 6.12 (t, *J* = 6.6 Hz, 0.45, H1′), 6.14 (t, *J* = 6.6 Hz, 0.55, H1′), 7.24 (q, *J* = 1.2 Hz, 0.45, H6), 7.25 (q, *J* = 1.2 Hz, 0.55, H6), 8.81 (br., 1, NH); ^{13}C NMR δ 12.67 (0.45, CH_3), 12.71 (0.55, CH_3), 29.4 (1, CH_2S), 37.4 (1, C2′), 43.56 (0.45, CHS), 43.62 (0.55, CHS), 60.4 (0.55, C3′), 60.5 (0.45, C3′), 64.1 (0.55, C5′), 64.3 (0.45, C5′), 81.5 (0.45, C4′), 81.7 (0.55, C4′), 85.56 (0.55, C1′), 85.62 (0.45, C1′), 111.5 (1, C5), 135.4 (1, C6), 150.0 (1, C2), 163.4 (1, C4), 171.5 (0.55, CO), 171.6 (0.45, CO); HRMS *m/z* calcd for $C_{13}H_{18}N_5O_5S_2$ $[M + H]^+$ 388.0744, found 388.0748.

*4.1.11. 3′-Azido-5′-O-(N-tert-butoxycarbonyl-S-tritylcysteinyl)-3′-deoxythymidine (**12**).* 3′-Azido-3′-deoxythymine (**9**; 100 mg, 0.37 mmol) was added to a stirred solution of *N*-*t*-butoxycarbonyl-*S*-trityl-L-cysteine (0.35 g, 0.75 mmol), EDCI (195 μL, 171 mg, 1.1 mmol), and DMAP (90 mg, 0.74 mmol) in CH_2Cl_2 (7.0 mL) under N_2 atmosphere at 0 °C. After 4 h, the resulting mixture was added to aqueous 5% HCl (30 mL) and was extracted with CH_2Cl_2. The organic layer was washed with sat. $NaHCO_3$/H_2O, brine, dried ($MgSO_4$), and evaporated. The residue was purified on a silica gel column (EtOAc/hexane, 1:1) to give **12** (211 mg, 80%): ^{1}H NMR δ 1.55 (s, 9, *t*-Bu), 2.02 (s, 3, CH_3), 2.33 (dt, *J* = 6.9, 13.9 Hz, 1, H2"), 2.50 (dt, *J* = 6.0, 13.9 Hz, 1, H2′), 2.71 (dd, *J* = 5.5, 12.3 Hz, 1, Hβ′), 2.76 (dd, *J* = 6.2, 12.3 Hz, 1, Hβ″), 4.14 ("q", *J* = 3.7 Hz, 1, H4′), 4.28 (dt, *J* = 4.8, 7.4 Hz, 1, H3′), 4.38 ("q", *J* = 7.0 Hz, 1, Hα), 4.44 (dd, *J* = 3.3, 12.3 Hz, 1, H5′), 4.52 (dd, *J* = 3.6, 12.3 Hz, 1, H5″), 5.31 (d, *J* = 7.7 Hz, 1, NH), 6.23 (t, *J* = 6.3 Hz, 1, H1′), 7.31-7.50 (m, 15, Tr); ^{13}C NMR δ 12.7 (CH_3), 28.3 (*t*-Bu), 33.9 (CH_2S), 37.4 (C2′), 52.7 (C3′), 60.3 (CHNH), 64.2 (C5′), 67.1 (Tr), 80.4 (*t*-Bu), 81.5 (C4′), 85.2 (C1′), 111.5 (C5), 127.0, 128.1, 129.4 & 144.0 (Tr), 135.1 (C6), 150.3 (C2), 155.0 (Boc), 164.0 (C4), 170.7 (CO); HRMS *m/z* calcd for $C_{37}H_{41}N_6O_7S$ $[M + H]^+$ 713.2752, found 713.2759.

*4.1.12. 3′-Azido-5′-O-cysteinyl-3′-deoxythymidine (**13**).* TFA (9 mL) and Et_3SiH (62 μL, 45 mg, 0.39 mmol) were added to a stirring solution of **12** (80 mg, 0.112 mmol) in anhydrous CH_2Cl_2 (9 mL) at 0 °C. After 2 h, volatiles were evaporated and the residue was purified on silica gel column (EtOAc → SSE) to give **13** (35 mg, 84%): ^{1}H NMR (MeOH-d_4) δ 1.80 (s, 3, CH_3), 2.34 (ddd, *J* = 6.1, 7.7, 13.9 Hz, 1, H2′), 2.48 (ddd, *J* = 5.6, 6.9, 12.8 Hz, 1, H2″), 3.05 (dd, *J* = 6.7, 14.2 Hz, 1, Hβ′), 3.11 (dd, *J* = 5.4, 14.3 Hz, 1, Hβ″), 3.94-3.99 (m, 2, Hα,4′), 4.30-4.38 (m, 3, H3′, 5′,5"), 6.0 (t, *J* = 6.5 Hz, 1, H1′), 7.36 (s, 1, H6); ^{13}C NMR (MeOH-d_4) δ 12.6 (CH_3), 37.4 (Cβ), 38.3 (C2′), 61.7 (C5′), 54.2 (Cα), 65.9 (C3′), 82.7 (C4′), 86.0 (C1′), 111.9 (C5), 138.1 (C6), 152.1 (C2), 166.3 (C4); HRMS *m/z* calcd for $C_{13}H_{19}N_6O_5S$ $[M + H]^+$ 371.1132, found 371.1134.

During purification and/or manipulation in open air, thiol **13** (R_f 0.45 in SSE) slowly oxidized to the corresponding disulfide **14** (R_f 0.40 in SSE): ^{1}H NMR (MeOH-d_4) δ 1.80 (s, 1, CH_3), 2.34 (ddd, *J* = 5.8, 7.3, 13.8 Hz, 1, H2′), 2.54 (ddd, *J* = 5.8, 8.0, 13.9 Hz, 1, H2″), 3.22 (dd, *J* = 7.0, 14.9 Hz, 1, Hβ′), 3.29 (dd, *J* = 4.9, 14.9 Hz, 1, Hβ″), 3.98 ("q", *J* = 5.3 Hz, 1, H4′), 4.34-4.44 (m, 4, Hα, H3′,5′, 5"), 5.97 (t, *J* = 5.9 Hz, 1, H1′), 7.36 (s, 1, H6); ^{13}C NMR (MeOH-d_4) δ 12.5 (CH_3), 36.9 (Cβ), 38.3 (C2′), 53.0 (Cα), 64.4 (C3′), 66.8 (C5′), 82.5 (C4′), 87.9 (C1′), 112.0 (C5), 139.1 (C6), 152.2 (C2), 166.3 (C4), 169.2 (CO). MS *m/z* 371 $[M + H]^+$.

*4.1.13. 2′-O-Allyl-3′-azido-5′-O-(tert-butyldimethylsilyl)-3′-deoxyadenosine (**17**).* NaH (20 mg, 0.5 mmol, 60% dispersion in oil) was added to a stirred solution of **2b** (100 mg, 0.246 mmol, vacuum dried at 110 °C for 3h) in DMF (2.5 mL) at room temperature and after 15 min allyl bromide (64 μL, 90 mg, 0.74 mmol) was added. After 2 h, volatiles were evaporated and the residue was partitioned ($NaHCO_3$/H_2O//$CHCl_3$), organic layer was washed (brine), dried (Na_2SO_4), evaporated, and column chromatographed (EtOAc/hexane, 4:1 → EtOAc → MeOH/EtOAc, 1:39) to give **17** (52 mg, 47%) as a transparent oil: ^{1}H NMR δ 0.14 (s, 3, Me), 0.15 (s, 3, Me), 0.95 (s, 9, *t*-Bu), 3.85 (dd, *J* = 2.5, 11.7 Hz, 1, H5′), 4.10 (dd, *J* = 2.8, 11.7 Hz, 1, H5″), 4.16 (dd, *J* = 5.2, 6.9 Hz, 1, H3′), 4.26 (tdd, *J* = 1.3, 6.0, 12.8 Hz, 1, OC*H*H), 4.26- 4.29 (m, 1, H4′), 4.33 (tdd, *J* = 1.3, 5.5, 12.8 Hz, 1, OCH*H*), 4.58 (dd, *J* = 3.1, 5.1 Hz, 1, H2′), 5.21 (dq, *J* = 1.3, 10.4 Hz, 1, CH=C*H*H), 5.31 (dq, *J* = 1.5, 17.2 Hz, 1, CH=CH*H*), 5.90 (ddt, *J* = 5.7, 10.4, 17.2 Hz, 1, C*H*=CH_2), 6.15 (br., 2, NH_2), 6.18 (d, *J* = 3.1 Hz, 1, H1′), 8.21 (s, 1, H8), 8.35 (s, 1, H2); ^{13}C NMR δ -5.5 (Me), -5.3 (Me), 18.5 (*t*-Bu), 26.0 (*t*-Bu), 58.7 (C3′), 61.9 (C5′), 71.9 (OCH_2), 81.8 (C2′), 82.2 (C4′), 87.4 (C1′), 118.6 (CH=CH_2), 120.1 (C5), 133.3 (*C*H=CH_2), 138.8 (C8), 149.4 (C4), 153.1 (C2), 155.6 (C6); HRMS *m/z* calcd for $C_{19}H_{31}N_8O_3Si$ $[M + H]^+$ 447.2283, found 447.2276.

*4.1.14. 3′-Azido-5′-O-(tert-butyldimethylsilyl)-2′-O-(2,3-dibromopropyl)-3′-deoxyadenosine (**18**).* A solution of bromine (6 μL, 19 mg, 0.117 mmol) in $CHCl_3$ (1.0 mL) was added dropwise to a stirred solution of **17** (52 mg, 0.117 mmol) in $CHCl_3$ (1.5 mL) at -50 °C under Ar atmosphere. The resulting mixture was stirred at -50 °C for 30 min and then was allowed to warm to 0 °C. Volatiles were evaporated under reduced pressure and the oily residue was directly applied onto a silica gel column (EtOAc/hexane, 4:1 → EtOAc → MeOH/EtOAc, 1:39) to give **18** (31.5 mg, 44%) as a 1:1 mixture of diastereomers: ^{1}H NMR δ 0.16 (s, 3, Me), 0.17 (s, 3, Me) 0.96 (s, 9, *t*-Bu), 3.83-3.86 (m, 2, CH_2Br), 3.879 & 3.885 (2×d, *J* = 11.8 Hz, 1, H5′), 4.13 & 4.14 (2×d, *J* = 11.8 Hz, 1, H5″), 4.14-4.25 (m, 3, H3′, OCH_2), 4.27-4.40 (m, 2, H4′, CHBr), 4.66 (dd, *J* = 2.7, 5.1 Hz, 0.5, H2′), 4.67 (dd, *J* = 2.7, 5.1 Hz, 0.5, H2′), 5.76 (br. s, 2, NH_2), 6.21 (d, *J* = 2.7 Hz, 0.5, H1′), 6.22 (d, *J* = 2.7 Hz, 0.5, H1′), 8.22 (s, 1, H8), 8.36 (s, 0.5, H2), 8.37 (s, 0.5, H2); ^{13}C NMR δ -5.5 (Me), -5.3 (Me), 18.5 (*t*-Bu), 26.0 (*t*-Bu), 32.2, 32.4 (CH_2Br), 48.0, 48.1 (CHBr), 58.7, 58.8 (C3′), 61.7, 61.7 (C5′), 72.0, 72.2 (OCH_2), 81.9, 82.0 (C4′), 84.0 (C2′), 87.4, 87.5 (C1′), 120.2 (C5), 138.8 (C8), 149.3 (C4), 153.17, 153.20 (C2), 155.4 (C6); MS *m/z* 605 (48, [$^{79}Br_2$]), 607 (100, [$^{79/81}Br_2$]), 609 (50, [$^{81}Br_2$]). HRMS *m/z* calcd for $C_{19}H_{31}{}^{79}Br_2N_8O_3Si$ $[M + H]^+$ 605.0650, found 605.0662.

*4.1.15. 3′-Azido-5′-O-(tert-butyldimethylsilyl)-3′-deoxy-2′-O-[(2,3-S-diacetyl-2,3-dimercapto)propyl]adenosine (**19**).* KSAc (106 mg, 0.93 mmol) was added to a stirred solution of **18** (47 mg, 0.078 mmol) in DMF (3 mL) at ambient temperature under Ar atmosphere. After 18 h, when no starting material was observed by TLC and ^{1}H NMR, the volatiles were evaporated. The residue was partitioned ($NaHCO_3$/H_2O//CH_2Cl_2), the organic layer was washed ($NaHCO_3$/H_2O, brine), dried (Na_2SO_4), and evaporated to give 48 mg of colorless oil. This crude product was column chromatographed (EtOAc/hexane, 4:1

→ EtOAc → MeOH/EtOAc, 1:39) to give **19** (37.5 mg, 81%) as a 1:1 mixture of diastereomers: ^{1}H NMR δ 0.14 (s, 3, Me), 0.15 (s, 3, Me), 0.94 (s, 9, *t*-Bu), 2.31 & 2.33 (2 × s, 3, Ac), 2.34 (s, 3, Ac), 3.17 & 3.20 (2 × dd, J = 6.6, 13.9 Hz, 1, CH_2S), 3.36 (dd, J = 7.1, 13.9 Hz, 0.5, CH_2S), 3.37 (dd, J = 6.6, 13.9 Hz, 0.5, CH_2S), 3.81 (dd, J = 5.4, 10.0 Hz, 0.5, OCH_2), 3.858 & 3.864 (2 × d, J = 11.8 Hz, 1, H5′), 3.86-3.91 (m, 1.5, OCH_2, CHS), 3.92-3.97 (m, 0.5, OCH_2) 4.01 (dd, J = 4.2, 10.0 Hz, 0.5, OCH_2), 4.10 & 4.11 (2 × br. d, J = 11.8 Hz, 1, H5″), 4.17 & 4.19 (2 × dd, J = 2.5, 5.1 Hz, 1, H3′), 4.27 & 4.29 (2 × "t", J = 2.7 Hz, 1, H4′), 4.57 & 4.58 (2 × dd, J = 2.7, 5.5 Hz, 1, H2′), 5.99 (br. s, 2, NH_2), 6.15 & 6.16 (2 × d, J = 2.7 Hz, 1, H1′), 8.20 & 8.21 (2 × s, 1, H8), 8.33 & 8.34 (2 × s, 1, H2); ^{13}C NMR δ -5.4 (Me), -5.1 (Me), 18.5 (*t*-Bu), 26.0 (*t*-Bu), 30.2, 30.4 (CH_2S), 30.5, 30.6, 30.60 (Ac), 43.6, 43.7 (CHS), 58.7, 58.8 (C3′), 61.75, 61.76 (C5′), 71.79, 71.82 (OCH_2), 81.9 (C4′), 83.4, 83.6 (C2′), 87.3, 87.4 (C1′), 120.1, 120.1 (C5), 138.8, 138.9 (C8), 149.3, 149.3 (C4), 153.11, 153.12 (C2), 155.5 (C6), 194.1, 194.4, 194.52, 194.55 (SAc); HRMS *m/z* calcd for $C_{23}H_{37}N_8O_5S_2Si$ $[M + H]^+$ 597.2092, found 597.2107.

*4.1.16. 3′-Azido-3′-deoxy-2′-O-[(2,3-S-diacetyl-2,3-dimercapto)propyl]adenosine (**20**).* A solution of **19** (37.5 mg, 0.063 mmol) in TFA/H_2O (9:1, 1.0 mL) was stirred at 0 °C for 1 h. Volatiles were evaporated and the residue was coevaporated with toluene to give **20** (30 mg, 99%) as a 1:1 mixture of diastereomers: ^{1}H NMR δ 2.291 (s, 1.5, Ac), 2.293 (s, 1.5, Ac), 2.32 (s, 1.5, Ac), 2.33 (s, 1.5, Ac), 2.97 (dd, J = 6.6, 14.1 Hz, 0.5, C*H*HS), 3.12 (dd, J = 6.4, 14.1 Hz, 0.5, CH*H*S), 3.29 (dd, J = 6.4, 14.1 Hz, 0.5, CH*H*S), 3.34 (dd, J = 6.6, 14.1 Hz, 0.5, C*H*HS), 3.65 (dd, J = 5.6, 9.6 Hz, 0.5, OCH_2), 3.68-3.78 (m, 1.5, CHS, OCH_2), 3.79-3.88 (m, 1, CHS, OCH_2), 3.88 (d, J = 12.6 Hz, 1, H5′), 4.12 (d, J = 12.6 Hz, 1, H5″), 4.34-4.37 (m, 1, H4′), 4.37-4.40 (m, 1, H3′), 4.68-4.72 (m, 1, H2′), 6.12 & 6.17 (2 × d, J = 4.8 Hz, 1, H1′), 8.41 (s, 1, H2), 8.72 (s, 0.5, H8), 8.74 (s, 0.5, H8), 8.89 (br. s, 1, OH), 9.43 (br. s, 2, NH_2); ^{13}C NMR δ 29.9, 30.2 (CH_2S), 30.3, 30.4, 30.5, 30.6 (Ac), 43.5, 43.6 (CHS), 59.9, 60.0 (C3′), 61.6 (C5′), 71.9, 72.2 (OCH_2), 83.3, 83.4 (C2′), 84.31, 84.33 (C4′), 88.7, 88.8 (C1′), 119.4 (C5), 137.9 (C4), 142.9, 142.9 (C8), 145.0, 145.1 (C2), 151.7 (C6), 194.2, 194.7, 194.93, 194.94 (SAc); MS *m/z* 483 $[M + H]^+$).

*4.1.17. 3′-Azido-3′-deoxy-2′-O-(2,3-dimercaptopropyl)adenosine (**21**).* Saturated solution of NaOH in MeOH (2.0 mL) was added to a stirred solution of the crude **20** (30 mg, 0.062 mmol) in MeOH (1.0 mL) at -30 oC under Ar atmosphere. The stirring was continued for 30 min until all starting material was consumed as judged by TLC. The reaction mixture was cooled down to -50 oC, acidified with 1 M HCl and was stirred for an additional 20 min. The resulting mixture was extracted with CH_2Cl_2 (2x) and the combined organic extract was washed with $NaHCO_3/H_2O$, brine, and was dried (Na_2SO_4) to give **21** (24.5 mg, 99%; 1:1 mixture of diastereomers) as a white foam. ^{1}H NMR δ 1.44 (t, J = 8.7 Hz, 0.5, CH_2SH), 1.50 (dd, J = 7.9, 9.5 Hz, 0.5, CH_2SH), 1.67 (d, J = 9.2 Hz, 0.5, CHS*H*), 1.76 (d, J = 8.9 Hz, 0.5, CHS*H*), 2.64-2.80 (m, 1, CH_2S), 2.77 (dd, J = 5.7, 8.7 Hz, 1, CH_2S), 2.87-2.95 (m, 0.5, CHS), 2.97-3.05 (m, 0.5, CHS), 3.41 (dd, J = 6.9, 9.5 Hz, 0.5, OC*H*H), 3.55 (dd, J = 5.2, 9.6 Hz, 0.5, OCH*H*), 3.70 (dd, J = 6.7, 9.7 Hz, 0.5, OCH*H*), 3.73 (br. "d", J =13 Hz, 1, H5′), 3.82 (dd, J = 4.7, 9.5 Hz, 0.5, OC*H*H), 3.98 (br. d, J =13.2 Hz, 1, H5″), 4.24 & 4.26 (2 × "q", J = 1.0 Hz, 1, H4′), 4.51 (d, J = 5.4 Hz, 1, H3′), 5.08 & 5.09 (2 × dd, J = 5.4, 7.6 Hz, 1, H2′) 5.87 & 5.89 (2 × d, J = 7.6 Hz, 1, H1′), 6.23 (br. s, 2, NH_2), 6.77 (br. s, 1, OH), 7.90 & 7.91 (2 × s, 1, C8), 8.33 (s, 1, C2); ^{13}C NMR δ 29.4, 29.7 (CH_2S), 41.5, 41.7 (CHS), 62.10, 62.12 (C3′), 63.36, 63.38 (C5′), 73.5, 73.7 (OCH_2), 81.3, 81.7 (C2′), 85.67, 85.70 (C4′), 89.29, 89.32 (C1′), 121.2 (C5), 140.6 (C8), 148.4 (C4), 152.64, 152.66 (C2), 156.2 (C6); MS *m/z* 399 $[M + H]^+$; HRMS *m/z* calcd for $C_{13}H_{18}N_8NaO_3S_2$ $[M + Na]^+$ 421.0835, found 421.0850.

*4.1.18. 3′-Azido-3′-deoxy-3-N-methylthymidine (**22**).* Freshly distilled diazomethane solution in ether (10 mL), generated from Diazald (3.0 g, 14.0 mmol), was added dropwise to a stirred solution of AZT (**9**; 200 mg, 0.75 mmol) in ethanol (15 mL) at 0 °C. After 30 min, the volatiles were evaporated to give **22**[65] (208 mg, 99%): ^{1}H NMR δ 1.95 (s, 3, CH_3), 2.38 (ddd, J = 5.4, 6.4, 13.8 Hz, 1, H2′), 2.45 (dt, J = 6.8, 13.5 Hz, 1, H2"), 3.35 (s, 1, NCH_3), 3.83 (dd, J = 1.3, 11.0 Hz, 1, H5″), 3.97-4.04 (m, 2, H4′,5′), 4.43 (dt, J = 5.0, 7.2 Hz, H3′), 6.06 (t, J = 6.5 Hz, H1′), 7.37 (s, 1, H6); ^{13}C NMR δ 13.2 (CH_3), 27.8 (NCH_3), 37.6 (C2′), 59.9 (C3′), 61.8 (C5′), 84.6 (C4′), 86.8 (C1′), 110.0 (C5), 134.6 (C6), 150.9 (C2), 163.7 (C4); HRMS *m/z* calcd for $C_{11}H_{16}N_5O_5$ $[M + H]^+$ 282.1197, found 282.1186.

*4.1.19. 5′-O-Allyl-3′-azido-3′-deoxy-3-N-methylthymidine (**23**).* KOH (78 mg, 1.39 mmol), 18-crown-6 (5 mg, 0.019 mmol) and allyl bromide (0.12 mL, 168 mg, 1.39 mmol) were added to a stirred solution of **22** (130 mg, 0.46 mmol) in dry THF (3 mL) at ambient temperature. After 2 h, the volatiles were evaporated and the residue was partitioned between H_2O and $CHCl_3$. The organic layer was concentrated and purified on silica gel column (EtOAc/hexane, 3:7) to afford **23** (120 mg, 81%): ^{1}H NMR δ 1.80 (s, 3, CH_3), 2.31 (dt, J = 6.6, 13.6 Hz, 1, H2′), 2.41 (ddd, J = 5.1, 6.3, 13.8 Hz, 1, H2"), 3.31 (s, 3, NCH_3), 3.63 (dd, J = 2.5, 10.8 Hz, 1, H5′), 3.78 (dd, J = 2.5, 10.8 Hz, 1, H5″), 4.02 ("quint", J = 2.5 Hz, 1, H4′), 4.06 ("q", J = 1.4 Hz, 1, OC*H*H), 4.08 ("q", J = 1.4 Hz, 1, OCH*H*), 4.31 (dt, J = 4.8, 6.9 Hz, 1, H3′), 5.23 (dq, J = 1.2, 10.4 Hz, 1, CH=C*H*H), 5.29 (dq, J = 1.6, 17.2 Hz, 1, CH=CH*H*), 5.90 (ddt, J = 5.6, 10.4, 17.2 Hz, 1, C*H*=CH_2), 6.27 (t, J = 6.2 Hz, 1, H1′), 7.60 (s, 1, H6); ^{13}C NMR δ 13.3 (CH_3), 27.7 (NCH_3), 38.1 (C2′), 60.5 (C3′), 69.4 (C5′), 72.4 (OCH_2), 83.4 (C4′), 85.5 (C1′), 109.9 (C5), 117.9 (CH=CH_2), 133.4 (*C*H=CH_2), 133.7 (C6), 150.9 (C2), 163.6 (C4); HRMS *m/z* calcd for $C_{14}H_{20}N_5O_4$ $[M + H]^+$ 322.1510, found 322.1501.

*4.1.20. 3′-Azido-5′-O-(2,3-dibromopropyl)-3′-deoxy-3-N-methylthymidine (**24**).* A solution of bromine (14 μL, 44 mg, 0.28 mmol) in $CHCl_3$ (1 mL) was added dropwise to a stirred solution of **23** (90 mg, 0.28 mmol) in $CHCl_3$ (2 mL) at -50 oC. After 40 min, the reaction mixture was concentrated and column chromatographed (EtOAc/hexane, 3:7) to give **24** (81 mg, 60%) as a 1:1 mixture of diastereomers: ^{1}H NMR δ 1.95 (s, 3, CH_3), 2.31 (ddd, J = 0.9, 7.8, 13.8 Hz, 1, H2′), 2.40-2.47 (m, 1, H2″), 3.32 (s, 3, NCH_3), 3.69-3.76 (m, 2, H5′, C*H*HBr), 3.80 (dd, J = 4.5, 10.4 Hz, 1, CH*H*Br), 3.90-3.95 (m, 2, H5", OC*H*H), 4.01-4.06 (m, 2, H4′, OCH*H*), 4.26-4.32 (m, 1, CHBr), 4.36 ("dt", J = 4.5, 11.5 Hz, 1, H3′), 6.25 ("dt", J = 6.5, 10.3 Hz, 1, H1′), 7.38 (s, 0.5, H6), 7.42 (s, 0.5, H6); ^{13}C NMR δ 13.5 (CH_3), 27.8 (NCH_3), 31.8 & 32.0 (CH_2Br), 37.8 (C2′), 48.5 & 48.6 (CHBr), 60.5 & 60.7 (C3′), 70.8 (C5′), 72.6 & 72.7 (OCH_2), 82.97 & 82.98 (C4′), 85.48 & 85.50 (C1′), 110.21 & 110.24 (C5), 133.0 & 133.1 (C6), 150.91 & 150.93 (C2), 163.47 & 163.50 (C4); MS *m/z* 480 (48, $[^{79}Br_2]$), 482 (100, $[^{79/81}Br_2]$), 484 (52, $[^{81}Br_2]$). HRMS *m/z* calcd for $C_{14}H_{20}{}^{79}Br_2N_5O_4$ $[M + H]^+$ 479.9877, found 479.9891.

*4.1.21. 3′-Azido-5′-O-[(2,3-S-diacetyl-2,3-dimercapto)propyl]-3′-deoxy-3-N-methylthymidine (**25**).* KSAc (0.18 g, 1.58 mmol) was added to a stirred solution of **24** (75 mg, 0.156 mmol) in DMF (5 mL) at ambient temperature under N_2 atmosphere. After 24 h, the volatiles were evaporated and the residue was partitioned between H_2O and $CHCl_3$. The organic layer was concentrated and column chromatographed (EtOAc/hexane, 4:6) to give **25** (55 mg, 75%) as a 1:1 mixture of diastereomers: ^{1}H NMR δ 1.96 (d, J = 1.1 Hz, 1.5, CH_3), 1.98 (d, J = 1.1 Hz, 1.5, CH_3), 2.27-2.38 (m, 7, 2 × Ac, H2′), 2.41-2.48 (m, 1, H2"), 3.03 (dd, J = 6.6, 13.9 Hz, 0.5, SC*H*H), 3.11 (dd, J = 7.1, 13.9 Hz, 0.5, SC*H*H), 3.34-3.43 (m, 4, NCH_3, SCH*H*), 3.53-3.75 (m, 3, H5′, OCH_2), 3.79-3.87 (m, 2, H5", CHS), 4.03 ("dt", J = 2.6, 7.0 Hz, 1, H4′), 4.37 (dt, J = 4.4, 7.0 Hz, 0.5, H3′), 4.43 (dt, J = 4.1, 7.0 Hz, 0.5, H3′), 6.28 ("q", J = 7.0 Hz, 1, H1′), 7.44 & 7.45 (2 × d, J = 1.2 Hz, 1, H6); ^{13}C NMR δ 13.3 & 13.4 (CH_3), 27.83 & 27.85 (NCH_3), 30.4, 30.5, 30.51, & 30.54 (Ac), 30.67 & 30.68 (SCH_2), 37.9 (C2′), 43.7 & 43.8 (SCH), 60.6

& 60.7 (C3′), 70.5 & 70.6 (C5′), 71.9 & 72.2 (OCH_2), 83.05 & 83.08 (C4′), 85.4 & 85.5 (C1′), 110.09 & 110.14 (C5), 133.1 & 133.2 (C6), 151.0 (C2), 163.57 & 163.58 (C4), 194.0, 194.1, 194.4, & 194.5 (SAc); HRMS *m/z* calcd for $C_{18}H_{26}N_5O_6S_2$ $[M+H]^+$ 472.1319, found 472.1311.

*4.1.22. 3′-Azido-5′-O-(2,3-dimercaptopropyl)-3′-deoxy-3-N-methylthymidine (**26**).* A saturated solution of NaOH in MeOH (4 mL) was added to a stirred solution of **25** (55 mg, 0.12 mmol) in MeOH (2 mL) at -30 oC under N_2 atmosphere. After 2 h, the resulting mixture was cooled down to -50 oC, acidified with 1 M HCl and was stirred for an additional 30 min. The reaction mixture was extracted with $CHCl_3$ (2x), and the combined organic layer was washed with $NaHCO_3/H_2O$, brine, and was dried ($MgSO_4$) to give crude **26** (45 mg, 99%) as a 3:2 mixture of diastereomers: ^{1}H NMR δ 1.63 (ddd, *J* = 4.0, 8.6, 12.7 Hz, 1, CH_2SH), 1.84 (dd, *J* = 2.0, 8.1 Hz, 1, CHS*H*), 1.96 ("t", *J* = 1.1 Hz, 3, CH_3), 2.28-2.36 (m, 1, H2′), 2.43 (dd, *J* = 5.2, 6.4 Hz, 0.6, H2″), 2.46 (dd, *J* = 5.2, 6.4 Hz, 0.4, H2″), 2.84 (ddd, *J* = 2.3, 5.8, 8.6 Hz, 2, CH_2S), 3.12-3.21 (m, 1, CHS), 3.34 (s, 3, NCH_3), 3.65-3.72 (m, 2, OCH_2), 3.75 (dd, *J* = 1.6, 5.6 Hz, 0.6, H5′), 3.77 (dd, *J* = 2.1, 6.2 Hz, 0.4, H5′), 3.83 (dd, *J* = 2.9, 4.9 Hz, 0.6, H5″), 3.86 (dd, *J* = 2.9, 5.0 Hz, 0.4, H5″), 4.00-4.03 (m, 1, H4′), 6.22 ("dt", *J* = 3.8, 8.9 Hz, 1, H1′), 7.38 (dd, *J* = 1.2, 4.0 Hz, 1, H6); ^{13}C NMR δ 13.4 & 13.5 (CH_3), 27.8 (NCH_3), 29.91 & 29.93 (CH_2S), 37.8 & 37.9 (C2′), 41.9 & 42.1 (CHS), 60.4 & 60.5 (C3′), 70.3 & 70.4 (C5′), 74.1 & 74.2 (OCH_2), 82.9 (C4′), 85.6 & 85.7 (C1′), 110.18 & 110.22 (C5), 133.1 & 133.2 (C6), 150.9 (C2), 163.47 & 163.48 (C4); MS *m/z* 388 $[M+H]^+$; HRMS *m/z* calcd for disulfide is $C_{28}H_{38}N_{10}NaO_8S_4$ $[M+Na]^+$ 793.1655, found 793.1657.

4.1.23. Reactions of 3′-azido nucleosides with AAPH. AAPH (40 mg, 0.15 mmol) was added to a solution of 3′-azido nucleosides (**5**, **8**, **11**, **13**, **21**, **26**, or AZT; 0.025 mmol) in Ar-saturated MeOH-d_4:D_2O (2:1, 4 mL) solution. The reaction mixture was then heated at 50 °C. Aliquots were taken at 6 h, 12 h, 18 h, and 24 h and the progress of the reaction was monitored by ^{1}H NMR.

Note: In separate experiments it was demonstrated that heating of AAPH (18 mM) in D_2O at 37 °C, 47 °C, or 56 °C for 16 h resulted in 5.5%, 20.5%, or 73.0% fragmentation of AAPH, respectively (^{1}H NMR). Also, using MeOH-d_4/D_2O (2:1) as solvent instead of D_2O did not affect the rate of AAPH fragmentation.

Compound **11** (9.7 mg, 0.025 mmol) was treated with AAPH for 24 h as described above. The resulting mixture was evaporated and the residue was triturated with $CHCl_3$. The resulting white solid was filtered off and was dried to give a 3′-amino-3′-deoxythymidine hydrochloride **15**(~5 mg) with the following spectroscopic data: ^{1}H NMR (D_2O) δ 1.80 (s, 3, Me), 2.62-2.67 (m, 2, H2′,2″), 3.83 (dd, *J* = 4.8, 12.7 Hz, 1, H5′), 3.92 (dd, *J* = 3.4, 12.6 Hz, 1, H5″), 4.08 ("td", *J* = 4.1, 8.3 Hz, 1, H3′), 4.25 (q, *J* = 4.5 Hz, 1, H4′), 6.31 (t, *J* = 6.7 Hz, 1, H1′), 7.65 (s, 1, H6); ^{1}H NMR (MeOH-d_4) δ 1.92 (s, 3, Me), 2.49-2.57 (m, 2, H2′,2″), 3.82 (dd, *J* = 3.5, 12.0 Hz, 1, H5′), 3.88 (dd, *J* = 3.2, 12.0 Hz, 1, H5″), 4.05 (td, *J* = 4.8, 7.9 Hz, 1, H3′), 4.12 (“q”, *J* = 3.8 Hz, 1, H4′), 6.32 (t, *J* = 6.8 Hz, 1, H1′), 7.84 (s, 1, H6); MS *m/z* 258 (100), 254 (12), 242 (5, $[3'\text{-}NH_2T+H]^+$); HRMS *m/z* calcd for $C_{10}H_{16}N_3O_4$ $[M+H]^+$ 242.1135, found 242.1134.

Note: The ^{1}H NMR spectrum of **15** in MeOH-d_4 was identical to the spectrum of 3′-amino-3′-deoxythymidine **16a** recorded in MeOH-d_4 (0.7 mL) with addition of 15 μL of 20% of DCl/D_2O.

Compound **21** (10 mg, 0.025 mmol) was treated with AAPH for 24 h as described above. Volatiles were evaporated and the residue was partitioned between $H_2O/CHCl_3$. The aqueous layer was evaporated and was column chromatographed ($CHCl_3$/MeOH, 8:2) and purified further on reverse phase HPLC (H_2O/MeCN, 85:15) to give adenosine-derived product (~3 mg) with the following spectroscopic data: ^{1}H NMR (MeOH-d_4) δ 2.73-2.84 (m, 1, CHS), 3.01-3.13 (m, 2, CH_2S), 3.64-3.72 (m, 2, OCH_2), 3.77 (dd, *J* = 2.1, 12.7 Hz, 1, H5′), 3.90 (dd, *J* = 2.0, 12.6 Hz, 0.8, H5″), 4.17-4.22 (m, 1, H4′), 4.52-4.56 (m, 1, H3′), 4.80-4.85 (m, 1, H2′; in the envelope of signals from MeOH), 6.09-6.10 (m, 1, H1′), 8.23 (s, 1, H2), 8.38 (s, 1, H6); MS (ESI) *m/z* 369 $[M+H]^+$.

4.2. General method for gamma irradiation

AZT (13.4 mg, 0.05 mmol; 1.0 mM) and cysteine (60.7 mg, 0.5 mmol; 10 mM) were dissolved in 50 mL of phosphate buffer solution (pH 7.08; made from 1.56 g of $NaH_2PO_4 \bullet 2H_2O$ and 3.58 g of $Na_2HPO_4 \bullet 12\ H_2O$ in 1 L of Milli-Q water). The solution was divided into 5 vials of 9 mL each. The 5 vials of solution were saturated with N_2O (or 9:1 N_2O: O_2) for 30 min before irradiated at 2 kGy, 4 kGy, and 6 kGy, which were equivalents to 313.3, 622.7, and 919.5 min in the gamma cell, respectively. At the equivalent time of the dosage, the sample was taken for HPLC and LCMS analysis.

4.3. Theoretical density functional calculations

Theoretical density functional calculations of the structure and relative energies of model species simulating reactants, intermediates, transition states, and products along the considered reaction pathways were performed using the hybrid B3LYP functional[61,62] with the 6-31G* basis set and GAUSSIAN 98 program package.[63] This theoretical method is normally expected to provide a semiquantitative accuracy within 3-5 kcal/mol for the reaction energies and barrier heights and within 0.01-0.03 Å and 1-3° for geometric parameters.[66] In particular, a similar theoretical approach was employed by Pereira et al.[14] to compute the barriers and reaction energetics of the thiyl radical attack toward the azido moiety in an analogous intermolecular reaction between the $CH_3S^\bullet$ radical and a model RNR substrate. The B3LYP method has been also proven to be qualitatively reliable for the studies of related radical systems similar to the ones considered in the present work.[64,67-69]

Acknowledgements

This investigation was supported by award SC1CA138176 from NIGMS and NCI. TPD is grateful to FIU University Graduate School for her Doctoral Evidence Acquisition Fellowship and Kauffman Doctoral Student Assistantship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2012.04.050

References and notes

1. Cerqueira, N. M. F. S. A.; Fernandes, P. A.; Ramos, M. J. *Recent Pat. Anti-Cancer Drug Discovery* **2007**, *2*, 11.
2. Manegold, C. *Exp. Rev. Anticancer Ther.* **2004**, *4*, 345.
3. Shao, J.; Zhou, B.; Chu, B.; Yen, Y. *Curr. Cancer Drug Targets* **2006**, *6*, 409.
4. Bonate, P. L.; Arthaud, L.; Cantrell, W. R., Jr.; Stephenson, K.; Secrist, J. A., III; Weitman, S. *Nat. Rev. Drug Discov.* **2006**, *5*, 855.
5. Jeha, S.; Kantarjian, H. *Exp. Rev. Anticancer Ther.* **2007**, *7*, 113.
6. Stubbe, J.; van der Donk, W. A. *Chem. Biol.* **1995**, *2*, 793.
7. Stubbe, J.; Nocera, D. G.; Yee, C. S.; Chang, M. C. Y. *Chem. Rev.* **2003**, *103*, 2167.
8. Nordlund, P.; Reichard, P. *Annu. Rev. Biochem.* **2006**, *75*, 681.
9. Sjoberg, B. M.; Graslund, A.; Eckstein, F. *J. Biol. Chem.* **1983**, *258*, 8060.
10. Salowe, S.; Bollinger, J. M., Jr.; Ator, M.; Stubbe, J.; McCracken, J.; Peisach, J.; Samano, M. C.; Robins, M. J. *Biochemistry* **1993**, *32*, 12749.
11. van der Donk, W. A.; Stubbe, J.; Gerfen, G. J.; Bellew, B. F.; Griffin, R. G. *J. Am. Chem. Soc.* **1995**, *117*, 8908.
12. Fritscher, J.; Artin, E.; Wnuk, S.; Bar, G.; Robblee, J. H.; Kacprzak, S.; Kaupp, M.; Griffin, R. G.; Bennati, M.; Stubbe, J. *J. Am. Chem. Soc.* **2005**, *127*, 7729.
13. Zipse, H.; Artin, E.; Wnuk, S.; Lohman, G. J. S.; Martino, D.; Griffin, R. G.; Kacprzak, S.; Kaupp, M.; Hoffman, B.; Bennati, M.; Stubbe, J.; Lees, N. *J. Am. Chem. Soc.* **2009**, *131*, 200.
14. Pereira, S.; Fernandes, P. A.; Ramos, M. J. *J. Comput. Chem.* **2004**, *25*, 227.
15. Wnuk, S. F.; Chowdhury, S. M.; Garcia, P. I., Jr.; Robins, M. J. *J. Org. Chem.* **2002**, *67*, 1816.

16. Kim, S.; Joe, G. H.; Do, J. Y. *J. Am. Chem. Soc.* **1993**, *115*, 3328.
17. Kim, S.; Joe, G. H.; Do, J. Y. *J. Am. Chem. Soc.* **1994**, *116*, 5521.
18. Bräse, S.; Gil, C.; Knepper, K.; Zimmermann, V. *Angew. Chem., Int. Ed.* **2005**, *44*, 5188.
19. Benati, L.; Bencivenni, G.; Leardini, R.; Minozzi, M.; Nanni, D.; Scialpi, R.; Spagnolo, P.; Zanardi, G. *J. Org. Chem.* **2005**, *70*, 3046.
20. Roberts, B. P.; Winter, J. N. *J. Chem. Soc., Perkin Trans. 2* **1979**, 1353.
21. Benati, L.; Bencivenni, G.; Leardini, R.; Minozzi, M.; Nanni, D.; Scialpi, R.; Spagnolo, P.; Zanardi, G. *J. Org. Chem.* **2006**, *71*, 5822.
22. Postigo, A.; Kopsov, S.; Ferreri, C.; Chatgilialoglu, C. *Org. Lett.* **2007**, *9*, 5159.
23. Minozzi, M.; Nanni, D.; Spagnolo, P. *Chem.—Eur. J.* **2009**, *15*, 7830.
24. Amantini, D.; Fringuelli, F.; Pizza, F.; Vaccaro, L. *Org. Prep. Proced. Int.* **2002**, *34*, 109.
25. Pathak, T. *Chem. Rev.* **2002**, *102*, 1623.
26. Robins, M. J.; Hawrelak, S. D.; Hernandez, A. E.; Wnuk, S. F. *Nucleosides Nucleotides* **1992**, *11*, 821.
27. Busca, P.; Etheve-Quelquejeu, M.; Valery, J.-M. *Tetrahedron Lett.* **2003**, *44*, 9131.
28. Heinrich, T. K.; Kraus, W.; Pietzsch, H.-J.; Smuda, C.; Spies, H. *Inorg. Chem.* **2005**, *44*, 9930.
29. Robins, M. J.; Samano, V.; Johnson, M. D. *J. Org. Chem.* **1990**, *55*, 410.
30. Nauser, T.; Schoneich, C. *J. Am. Chem. Soc.* **2003**, *125*, 2042.
31. Zhao, R.; Lind, J.; Merenyi, G.; Eriksen, T. E. *J. Am. Chem. Soc.* **1994**, *116*, 12010.
32. Breen, A. P.; Murphy, J. A. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2979.
33. Vieira, A. J. S. C.; Steenken, S. *J. Am. Chem. Soc.* **1990**, *112*, 6986.
34. Maltese, M. *J. Org. Chem.* **2001**, *66*, 7615.
35. Rudolph, J.; Theis, H.; Hanke, R.; Endermann, R.; Johannsen, L.; Geschke, F. U. *J. Med. Chem.* **2001**, *44*, 619.
36. Robins, M. J.; Wnuk, S. F.; Hernandez-Thirring, A. E.; Samano, M. C. *J. Am. Chem. Soc.* **1996**, *118*, 11341.
37. Carter, K. N.; Taverner, T.; Schiesser, C. H.; Greenberg, M. M. *J. Org. Chem.* **2000**, *65*, 8375.
38. Manfredini, S.; Baraldi, P. G.; Bazzanini, R.; Simoni, D.; Balzarini, J.; De Clercq, E. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 473.
39. Roy, V.; Colombeau, L.; Zerrouki, R.; Krausz, P. *Carbohydr. Res.* **2004**, *339*, 1829.
40. Pogocki, D.; Schoneich, C. *Free Radical Biol. Med.* **2001**, *31*, 98.
41. Nauser, T.; Schoneich, C. *Chem. Res. Toxicol.* **2003**, *16*, 1056.
42. Handlon, A. L.; Oppenheimer, N. J. *Pharm. Res.* **1988**, *5*.
43. Bayley, H.; Standring, D. N.; Knowles, J. R. *Tetrahedron Lett.* **1978**, *19*, 3633.
44. Staros, J. V.; Bayley, H.; Standring, D. N.; Knowles, J. R. *Biochem. Biophys. Res. Commun.* **1978**, *80*, 568.
45. Reardon, J. E.; Crouch, R. C.; St John-Williams, L. *J. Biol. Chem.* **1994**, *269*, 15999.
46. Hampton, A.; Kappler, F.; Chawla, R. R. *J. Med. Chem.* **1979**, *22*, 621.
47. Schreiber, S.; Ikemoto, N. *Tetrahedron Lett.* **1988**, *29*, 3211.
48. Iwamoto, T.; Hiraku, Y.; Oikawa, S.; Mizutani, H.; Kojima, M.; Kawanishi, S. *Arch. Biochem. Biophys.* **2003**, *416*, 155.
49. Poopeiko, N. E.; Pricota, T. I.; Mikhailopulo, I. A. *Synlett* **1991**, 342.
50. Samano, M. C.; Robins, M. J. *Tetrahedron Lett.* **1991**, *32*, 6293.
51. Halliwell, B.; Gutteridge, J. *Free Radicals in Biology and Medicine*, 4th ed.; Oxford University: New York, NY, 2007.
52. Asmus, K.-D.; Bonifačić, M. In *S-Centered Radicals*; Alfassi, Z. B., Ed.; John Wiley: New York, NY, 1999; p 141.
53. Buxton, G. V.; Greenstock, C. L.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513.
54. Ross, A. B.; Mallard, W. G.; Helman, W. P.; Buxton, G. V.; Huie, R. E.; Neta, P. *NDRL-NIST Solution Kinetic Database, Version 3*; Notre Dame Radiation Laboratory: Notre Dame, 1998; IN and NIST Standard Reference Data, Gaithersburg, MD.
55. Mezyk, S. *J. Phys. Chem.* **1996**, *100*, 8295.
56. Ferreri, C.; Pierotti, S.; Barbieri, A.; Zambonin, L.; Landi, L.; Rasi, S.; Luisi, P. L.; Barigelletti, F.; Chatgilialoglu, C. *Photochem. Photobiol.* **2006**, *82*, 274.
57. Markakis, P.; Tappel, L. *J. Am. Chem. Soc.* **1960**, *82*, 1613.
58. Joshi, R.; Adhikari, S.; Mukherjee, T. *Res. Chem. Intermed.* **2001**, *27*, 623.
59. Shinohara, H.; Masuda, T.; Kondo, M. *J. Radiat. Res.* **1976**, *17*, 230.
60. Emanuel, C. J.; Newcomb, M.; Ferreri, C.; Chatgilialoglu, C. *J. Am. Chem. Soc.* **1999**, *121*, 2927.
61. Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
62. Lee, C.; Yang, W.; Parr, R. G. *Physiol. Rev.* **1988**, *B37*, 785.
63. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, R. E.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98, Revision A.11*; Gaussian: Pittsburgh, PA, 2001.
64. Siegbahn, P. E. M. *J. Am. Chem. Soc.* **1998**, *120*, 8417.
65. Adams, D. R.; Perez, C.; Maillard, M.; Florent, J.-C.; Evers, M.; Hénin, Y.; Litvak, S.; Litvak, L.; Monneret, C.; Grierson, D. S. *J. Med. Chem.* **1997**, *40*, 1550.
66. Cramer, C. J. *Essentials of Computational Chemistry*; Wiley: Chichester, England, 2004.
67. Siegbahn, P. E. M.; Eriksson, L.; Himo, F.; Pavlov, M. *J. Phys. Chem. B* **1988**, *102*, 10622.
68. Fernandes, P. A.; Eriksson, L. A.; Ramos, M. J. *Theor. Chem. Acc.* **2002**, *108*, 352.
69. Fernandes, P. A.; Ramos, M. J. *J. Am. Chem. Soc.* **2003**, *125*, 6311.