

**Supporting Information**

**Hydroxyl ion addition to one-electron oxidized thymine:  
Unimolecular interconversion of C5 to C6 OH-adducts**

by

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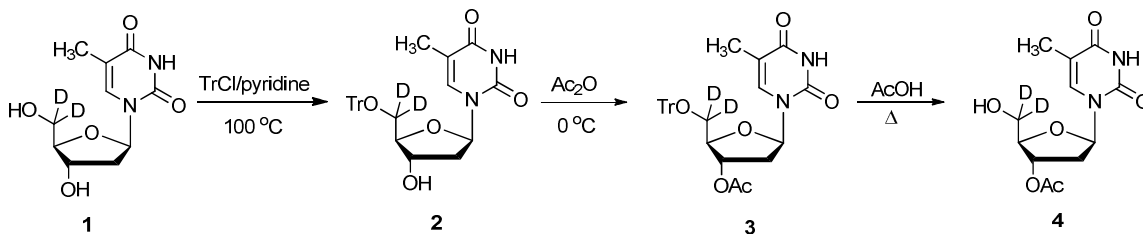
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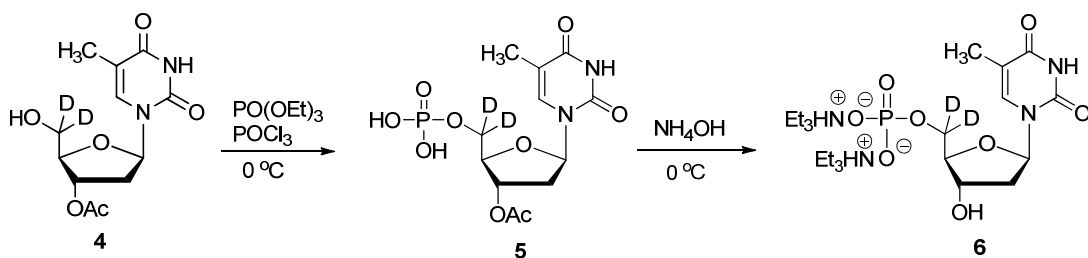
## Synthesis of [5',5''-D,D]-Thymidine 5'-monophosphate as triethylammonium salt



**[5',5''-D,D]-3'-O-Acetylthymidine (4).** [5',5''-D,D]-Thymidine [**1**, 50 mg, 0.20 mmol; dried in a pistol containing P<sub>2</sub>O<sub>5</sub> in vacuum at 110 °C (refluxed toluene) for 3 h] and trityl chloride (67 mg, 0.2 mmol) were dissolved in anhydrous pyridine (1 mL) and the resulting mixture was heated at 100 °C for 2 h. The mixture was then cooled down to ambient temperature and acetic anhydride (205 μL, 222 mg, 2.2 mmol) was added. After overnight stirring, the resulting colorless solution was slowly added to ice-water (5 mL) with stirring. The resulting cream-colored precipitate was filtered off and was washed with deionized water to remove the pyridine. The precipitate was dissolved in 80% acetic acid (1 mL) and the resulting solution was refluxed for 10 min., cooled down to ambient temperature and was added to ice-cold deionized water (5 mL) with stirring. The precipitated triphenylmethanol was filtered off and the mother liquor was evaporated to give crude **4**. Purification on silica gel column (EtOAc/hexane, 3:2 → 4:1) gave **4** (42 mg, 73% from **1**). The <sup>1</sup>H NMR spectra of this product in MeOH-*d*<sub>4</sub> corresponded to those of the unlabeled-**4**<sup>1(SI)</sup> except for the absence of the signal at δ 3.80 (H5',5'') and collapse of the multiplet δ 4.08 (H4') into a doublet (*J* = 2.0 Hz).

## Reference

- 1.(SI) Windhorst, A. D.; Klein, P. J.; Eisenbarth, J.; Oeser, T.; Kruijer, P. S.; Eisenhut, M. *Nucl. Med. Biol.* **2008**, *35*, 413-423.



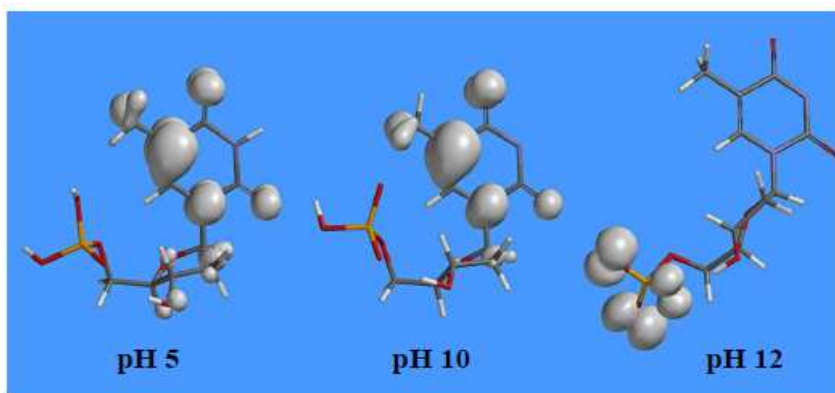
**[5',5''-D,D]-Thymidine-5'-monophosphate as triethylammonium salt (6).** Triethyl phosphate (380  $\mu\text{L}$ , 410 mg, 2.25 mmol) was added to [5',5''-D,D]-3'-*O*-acetylthymidine [4, 42 mg, 0.15 mmol; dried in a pistol containing  $\text{P}_2\text{O}_5$  in vacuum at 110  $^\circ\text{C}$  (refluxed toluene) for 3 h] at ambient temperature and the resulting mixture was cooled down to 5  $^\circ\text{C}$ . Freshly distilled  $\text{POCl}_3$  (28  $\mu\text{L}$ , 46 mg, 0.3 mmol) was added dropwise and the reaction mixture was stirred at 5  $^\circ\text{C}$  for 15 h. The mixture was then quenched with ice-water and neutralized with 1 N NaOH until the pH remained constant at 7.5. Concentrated  $\text{NH}_4\text{OH}$  (1 mL) was added and the resulting mixture was stirred at 0  $^\circ\text{C}$  (ice-bath) for 2 h. The reaction mixture was concentrated to approximately half of the volume ( $\sim 3$  mL) and loaded onto a DEAE Sephadex A-25 column and eluted with a linear gradient (0  $\rightarrow$  0.8 M) of triethylammonium bicarbonate (TEAB). The product, eluted at approximately 0.06 M TEAB, was concentrated in vacuum and repetitively coevaporated with water, until  $^1\text{H}$  NMR spectra showed no excess of peaks from triethylamine residue, to give **6** (37 mg, 47%):  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  1.18 (t,  $J = 7.2$  Hz, 18H, Et), 1.81 (d,  $J = 1.1$  Hz, 3H,  $\text{CH}_3$ ), 2.24-2.28 (m, 2H,  $\text{H}2',2''$ ), 3.10 (q,  $J = 7.2$  Hz, 12H, Et), 4.07 ("t",  $J = 2.5$  Hz, 1H,  $\text{H}4'$ ), 4.47 ("dt",  $J = 2.8, 4.7$  Hz, 1H,  $\text{H}3'$ ), 6.25 (t,  $J = 7.0$  Hz, 1H,  $\text{H}1'$ ), 7.67 ("q",  $J = 1.1$  Hz, 1H,  $\text{H}6$ );  $^{31}\text{P}$  NMR (161.9 MHz,  $\text{D}_2\text{O}$ )  $\delta$  - 0.02 (s).

#### Spin density calculations of different protonation states of one-electron oxidized 5'-TMP:

In order to gain a better understanding of the effect of pH on the radical site after one-electron oxidation of dThd and its derivatives, spin density distributions were calculated using DFT/B3LYP/6-31G(d) optimized geometries of one-electron oxidized 5'-TMP with protonation states appropriate to various pH values (see sub-section 1 of Section (A)). The effect of the full aqueous environment was included through the use of SM8 solvation model.<sup>2(S1)</sup> At pH ca. 11.5 and above, a structure with dideprotonated phosphate,  $-\text{OPO}_3^{2-}$  and a N3-deprotonated thymine is

expected (Figure S1 pH 12). The spin density calculations for this structure show the radical site is located on the phosphate group. Such a radical would likely result in C5'• formation in the sugar-phosphate group (see ref. 36 in the main manuscript). We do not observe C5'• formation instead oxidation of the thymine ring is found. Thus, this prediction is not in accord with experiment. This is likely explained by the fact that the counterions were not included. If included they would increase the IP at the phosphate substantially so that the base would be oxidized as is found experimentally and predicted in earlier calculations.<sup>3(SI), 4(SI)</sup>

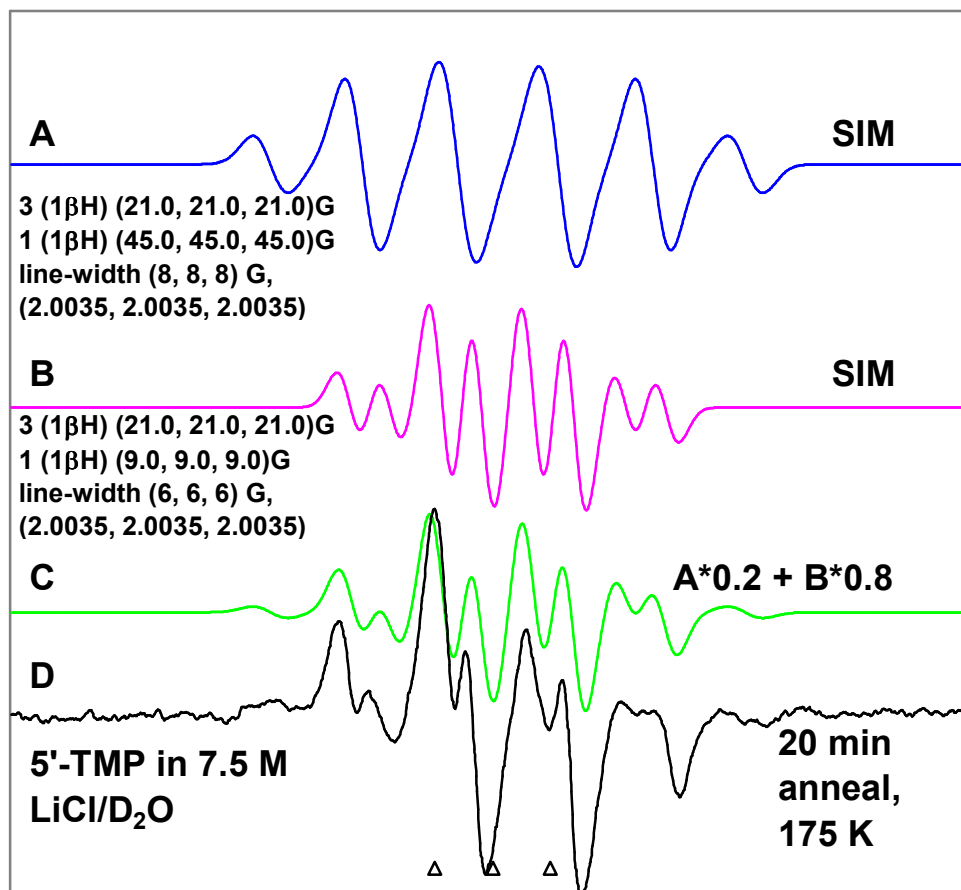
The spin density distribution found for one-electron oxidized 5'-TMP having either -OPO<sub>3</sub>H<sup>-1</sup> with N3-deprotonated thymine base (representative pH 10) or with -OPO<sub>3</sub>H<sub>2</sub> with thymine base having N3-proton (representative pH ca. 5) show that the radical site is located on thymine base (Figure S1). These theoretical predictions thus show that one-electron oxidation at high pH could result in formation of radicals on the sugar-phosphate and provide justification for employing the deuterated derivatives - [CD<sub>3</sub>]-dThd, [CD<sub>3,6D</sub>]-dThd, [5',5''-D,D]-dThd, and [5',5''-D,D]-5'-TMP (see Figure 3 in the main manuscript) for the unequivocal assignment of T(5OH)• and T(6OH)• where the radical sites are in the thymine ring.



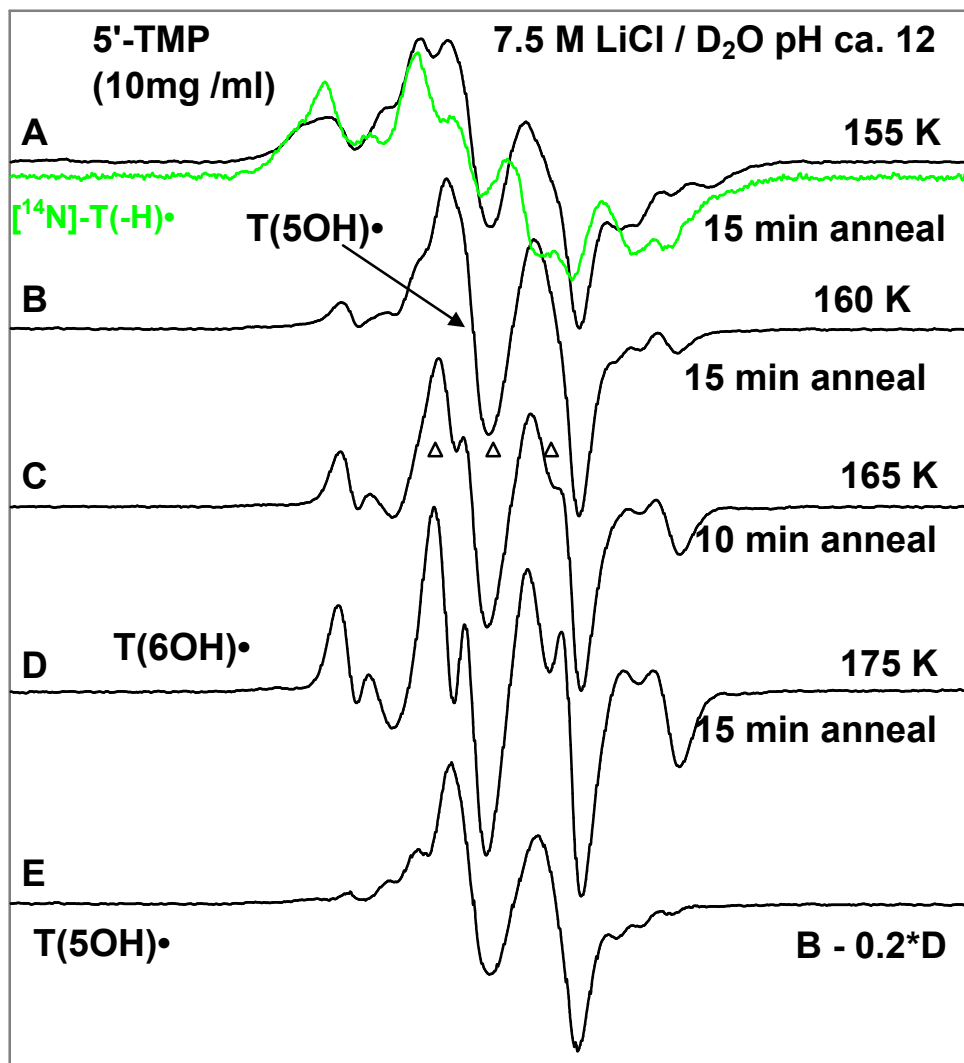
**Figure S1.** Spin density distributions calculated by the DFT/B3LYP/6-31G(d) method for the optimized geometries of one-electron oxidized 5'-TMP for protonation states expected at pHs 5, 10, and 12. The SM8 method for solvation was employed. The distribution for pH 12 does not reflect the spin density from experiment or theory when counterions are included. The spin distribution at pH 12 when properly calculated is similar to that found at pH 10.

## Reference

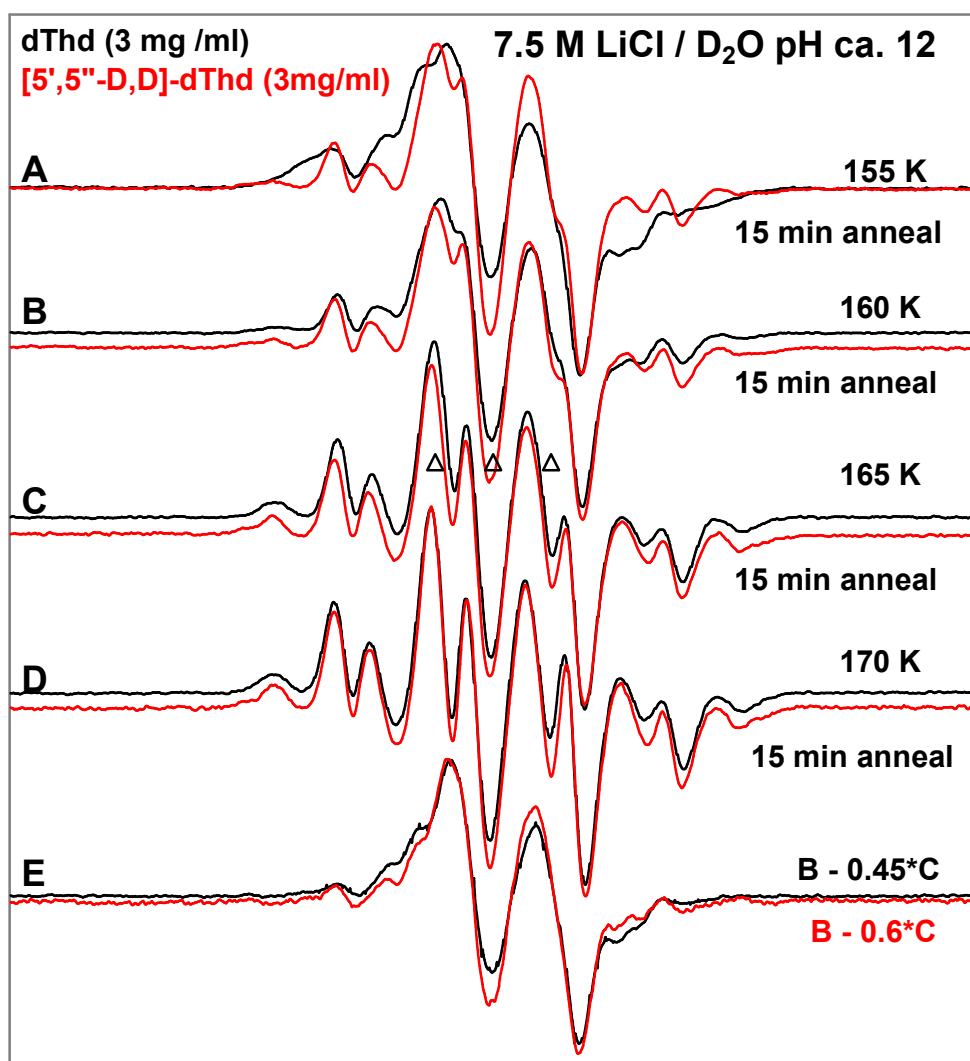
- 2.(SI) Chamberlin, A. C.; Cramer, C. J.; Truhlar, D. G. *J. Phys. Chem. B*, **2008**, *112*, 8651 – 8655.
3. (SI) Fernando, H.; Papadantonakis, G. A.; Kim, N. S.; LeBreton, P. R. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 5550 – 5555.
4. (SI) Colson, A. O.; Besler, B.; Sevilla, M. D. *J. Phys. Chem.*, **1993**, *97*, 8092 – 8097.



**Figure S2.** (A) Simulated T(6OH)• spectrum (blue) using the parameters: HFCC values of 3 methyl protons (21, 21, 21) G and of C6-H (45, 45, 45) G,  $g = 2.0035$  along with a mixed Lorentzian/Gaussian (1/1) linewidth of 8 G. (B) Simulated T(6OH)• spectrum (pink) using the parameters: HFCC values of 3 methyl protons (21, 21, 21) G and of C6-H (9, 9, 9) G,  $g = 2.0035$  along with a mixed Lorentzian/Gaussian (1/1) linewidth of 6 G. (C) Spectrum T(6OH)• (green) due to addition of 80% of spectrum (A) to 20% of spectrum (B). The green spectrum (C) matches well with the experimentally recorded (77 K) T(6OH)• spectrum (D, black) glassy (7.5 M LiCl/D<sub>2</sub>O) sample of 5'-TMP (0.5 mg/ml) in the presence of the electron scavenger K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> at pH ca. 12,  $\gamma$ -irradiated to a dose of 1.4 kGy and progressively annealed to 175 K for 20 min.

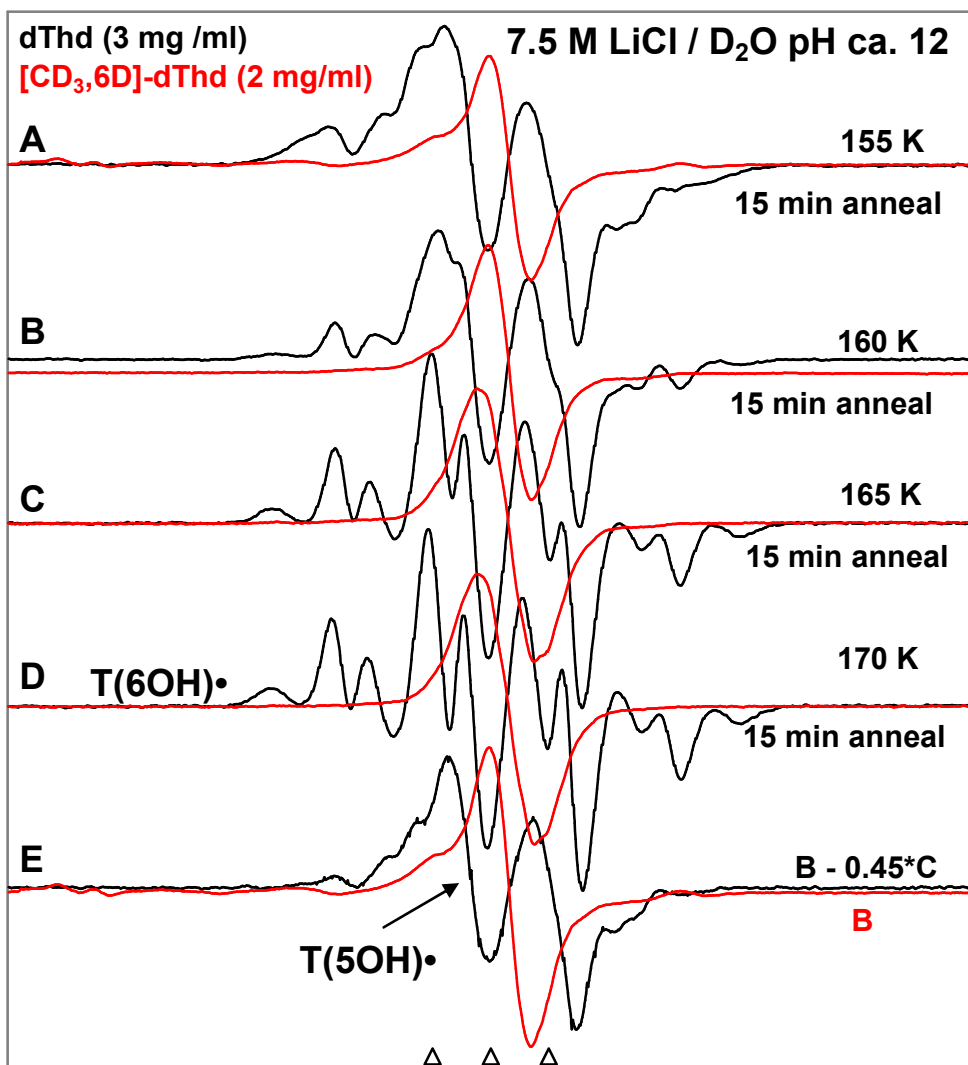


**Figure S3.** ESR spectra of  $\gamma$ -irradiated glassy sample of 5'-TMP (10 mg/ml in 7.5 M LiCl/D<sub>2</sub>O) in the presence of electron scavenger K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> at pH ca. 12 (black). (A-D) Spectra (black) found after annealing to (A) 155 K for 15 min, (B) 160 K for 15 min, (C) 165 K for 15 min, (D) 175 K for 15 min. The spectrum of T(-H)• (green, Figure 1A in the manuscript) is overlapped on spectrum (A). The spectrum in (D) is assigned to spectrum of T(6OH)• with beta C6-H HFCC of 9 G. See text and supporting information Figure S2 for details of simulation. (E) The spectrum is obtained by 20% subtraction of spectrum (D) from spectrum (B) and is assigned to T(5OH)•. All spectra are recorded at 77 K.



**Figure S4.** The ESR spectrum of  $\gamma$ -irradiated glassy sample of [5',5''-D,D]-dThd (3 mg/ml) in the presence of the electron scavenger  $K_2S_2O_8$  at pH ca. 12 recorded immediately after irradiation at 77 K show only the line components due to  $SO_4^{\bullet-}$  and  $Cl_2^{\bullet-}$  similar to spectra 3A in the manuscript. Therefore, this spectrum is not shown.

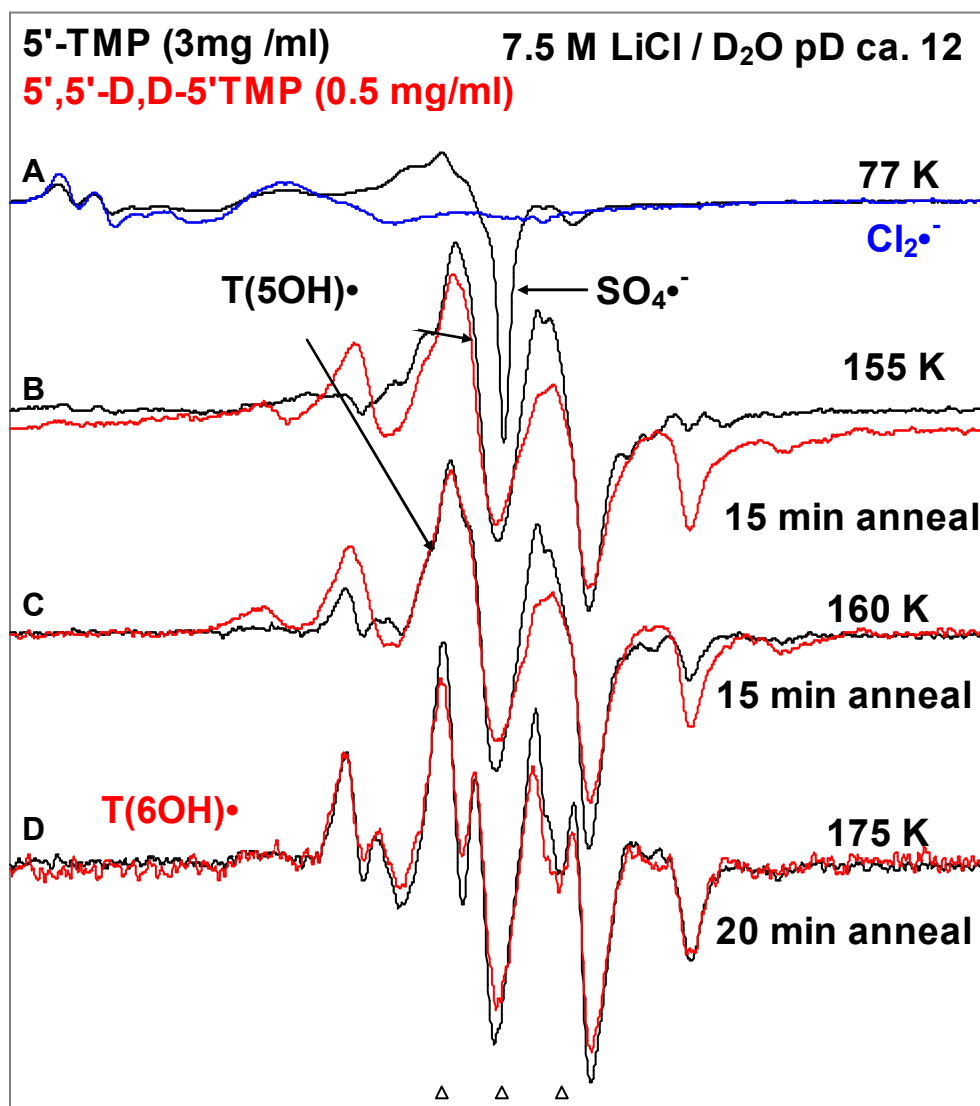
All ESR spectra (red) of [5',5''-D,D]-dThd (A) annealed to 155 K for 15 min, (B) annealed to 160 K for 15 min, (C) annealed to 165 K for 15 min, (D) annealed to 170 K for 15 min and this spectrum, similar to spectra S2D and S3D and is assigned to  $T(6OH)^{\bullet}$ . (E) The black spectrum  $T(5OH)^{\bullet}$  (the central anisotropic doublet) and remaining line components of  $T(-H)^{\bullet}$  (indicated by arrows) due to residual one-electron oxidized [5',5''-D,D]-dThd is obtained by 60% subtraction of spectrum (C). The ESR spectra (black) of the matched dThd sample (see supporting information Figure S7) are superimposed for comparison.



**Figure S5.** The ESR spectrum of  $\gamma$ -irradiated glassy sample of  $[\text{CD}_3,6\text{D}]\text{-dTd}$  (2 mg/ml) in the presence of the electron scavenger  $\text{K}_2\text{S}_2\text{O}_8$  at pH ca. 12 recorded immediately after irradiation at 77 K show only the line components due to  $\text{SO}_4^{\bullet-}$  and  $\text{Cl}_2^{\bullet-}$  similar to spectra 3A in the manuscript. Therefore, this spectrum is not shown.

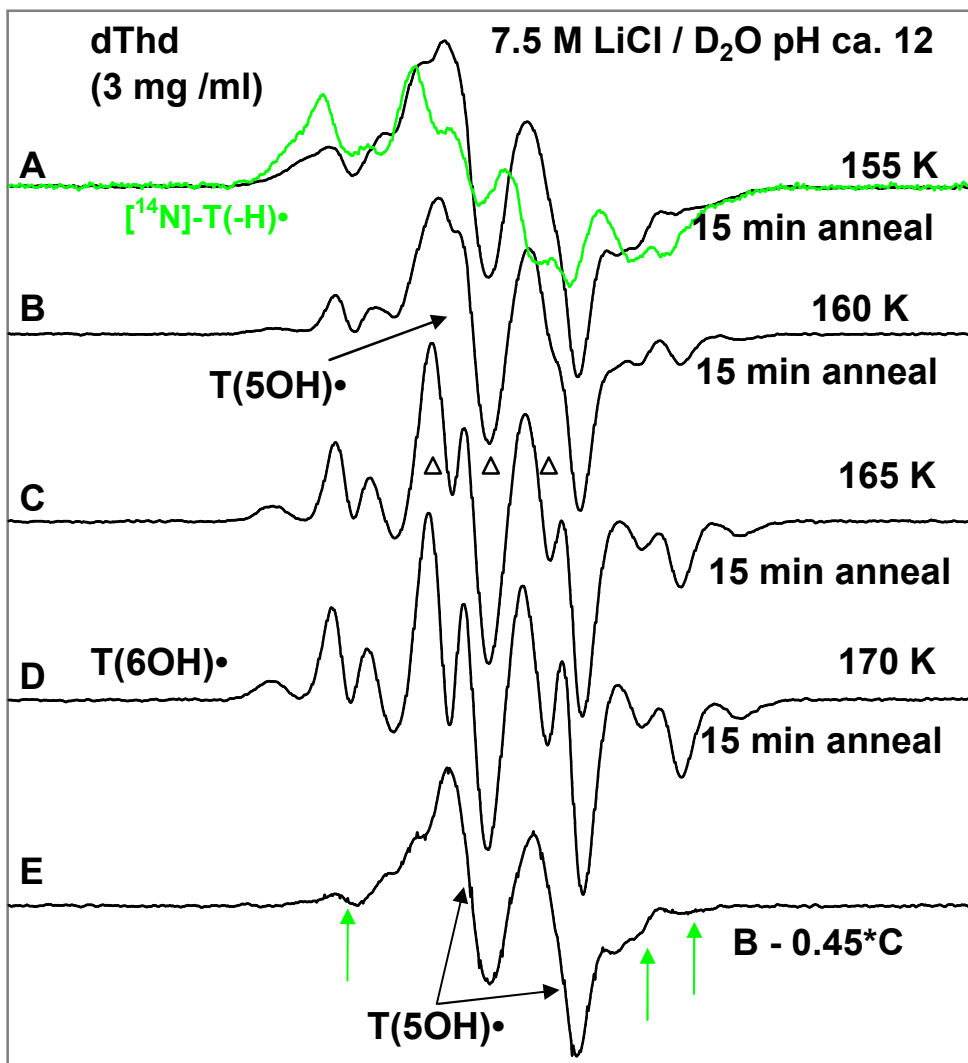
All ESR spectra (red) of  $[\text{CD}_3,6\text{D}]\text{-dTd}$  (2 mg/ml) (A) annealed to 155 K for 15 min, (B) annealed to 160 K for 15 min, (C) annealed to 165 K for 15 min, (D) annealed to 170 K for 15 min and this spectrum, similar to spectra S2D, S3D, and S4D assigned to  $\text{T}(6\text{OH})\bullet$ . The ESR spectra (black) of the matched dTd sample (see supporting information Figure S7) are superimposed for comparison.





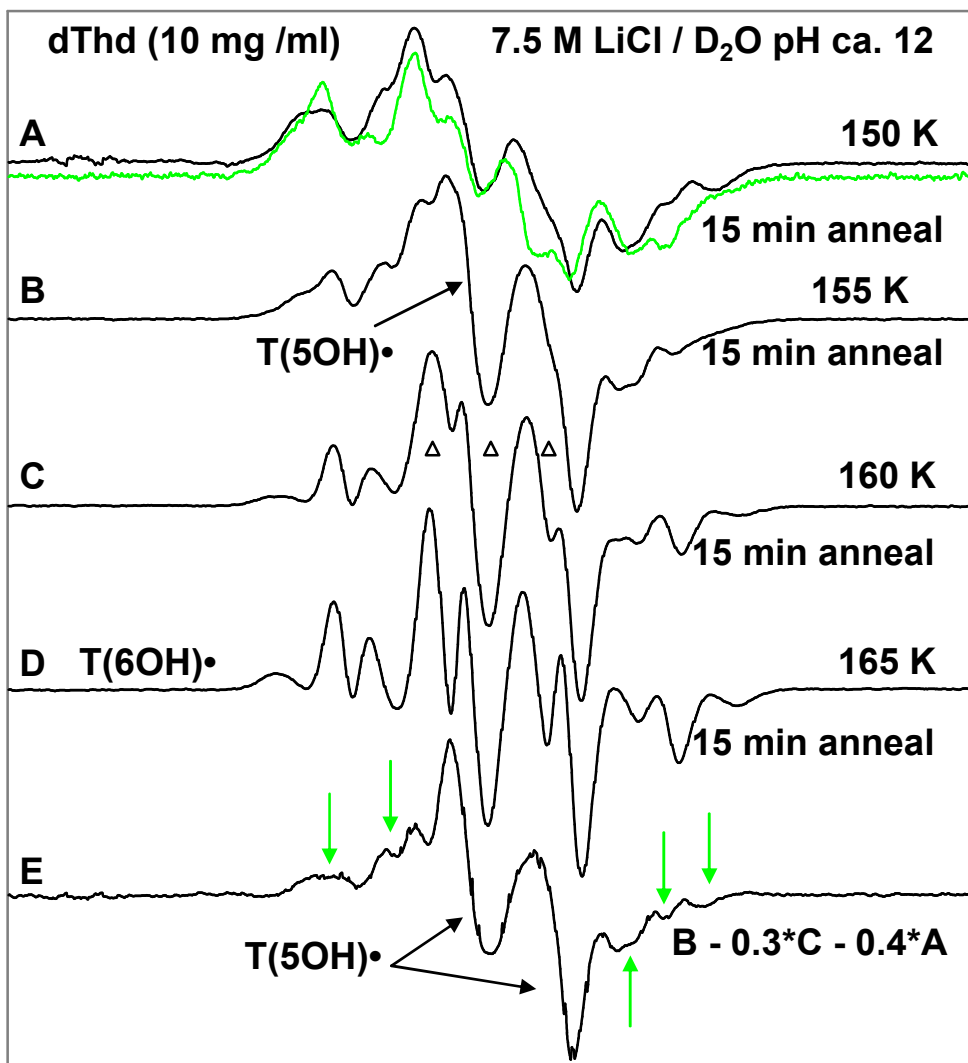
**Figure S6.** ESR spectra (red) of (A)  $\gamma$ -irradiated glassy sample of [5',5''-D,D]-5'-TMP (0.5 mg/ml in 7.5 M LiCl/D<sub>2</sub>O) in the presence of electron scavenger K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> at pH ca. 12 (black); partial spectrum (brown, 220 G scan) of a pure Cl<sub>2</sub>•<sup>-</sup> spectrum is overlapped on the black spectrum in A to show the Cl<sub>2</sub>•<sup>-</sup> line components in the black spectrum.

All ESR spectra (red) of [5',5''-D,D]- 5'-TMP (0.5 mg/ml) (B) annealed to 155 K for 15 min, (C) annealed to 160 K for 15 min, (D) annealed to 175 K for 15 to 20 min, and this spectrum, similar to spectra S2D is assigned to T(6OH)•. The central doublet in spectra (B) and (C) is assigned to T(5OH)•. The ESR spectra (black) of 5'-TMP sample (3 mg/ml) are superimposed for comparison.



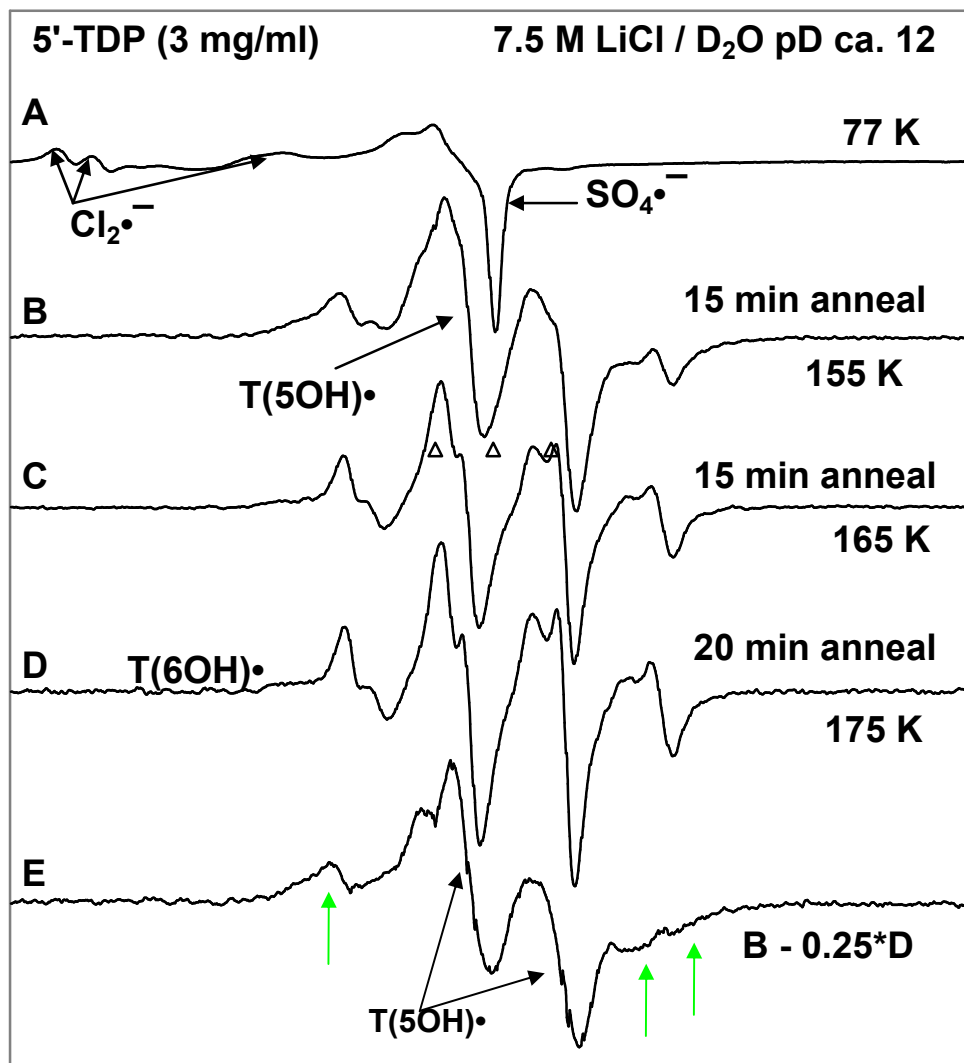
**Figure S7.** The ESR spectrum of  $\gamma$ -irradiated glassy sample of dThd (3 mg/ml) in the presence of the electron scavenger  $\text{K}_2\text{S}_2\text{O}_8$  at pH ca. 12 recorded immediately after irradiation at 77 K show only the line components due to  $\text{SO}_4^{\bullet-}$  and  $\text{Cl}_2^{\bullet-}$  similar to spectra 3A in the manuscript. Therefore, this spectrum is not shown.

ESR spectra of (A) annealed to 155 K for 15 min and the spectrum of  $\text{T}(-\text{H})\bullet$  (green, Figure 1A in the manuscript) is overlapped on spectrum (A), (B) annealed to 160 K for 15 min, (C) annealed to 165 K for 15 min, (D) annealed to 170 K for 15 min and this spectrum, similar to spectra S2D, S3D, and S4D is assigned to  $\text{T}(6\text{OH})\bullet$ . (E) The black spectrum  $\text{T}(5\text{OH})\bullet$  (the central anisotropic doublet, black arrows) and line components of  $\text{T}(-\text{H})\bullet$  (indicated by green arrows) due to residual one-electron oxidized dThd) is obtained by 45% subtraction of spectrum (C) from spectrum (B).



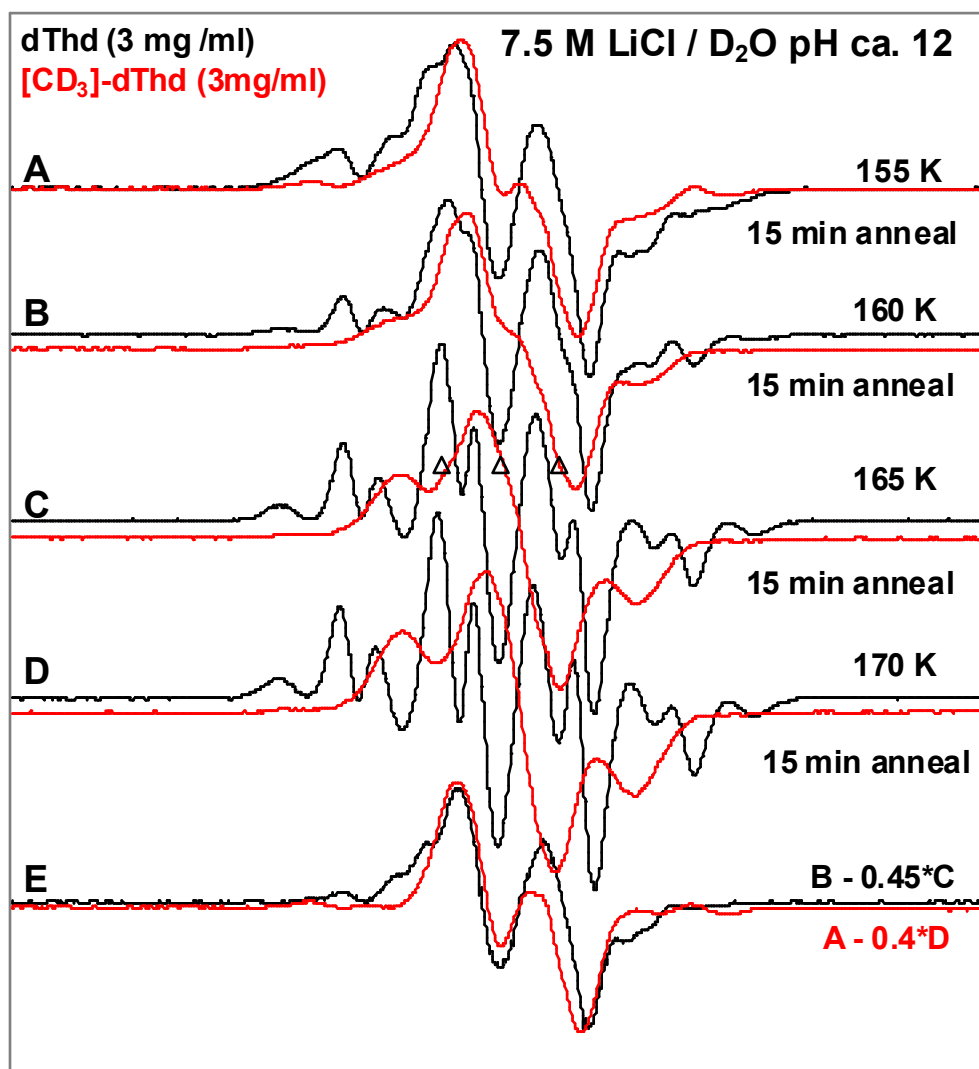
**Figure S8.** The ESR spectrum of  $\gamma$ -irradiated glassy sample of dThd (10 mg/ml) in the presence of the electron scavenger  $\text{K}_2\text{S}_2\text{O}_8$  at pH ca. 12 recorded immediately after irradiation at 77 K show only the line components due to  $\text{SO}_4^{\bullet-}$  and  $\text{Cl}_2^{\bullet-}$  similar to spectra 3A in the manuscript. Therefore, this spectrum is not shown.

ESR spectra of (A) annealed to 150 K for 15 min and the spectrum of  $\text{T}(-\text{H})^{\bullet}$  (green, Figure 1A in the manuscript) is overlapped on spectrum (A), (B) annealed to 155 K for 15 min, (C) annealed to 160 K for 15 min, (D) annealed to 165 K for 15 min and this spectrum, similar to spectra S2D and S7D is assigned to  $\text{T}(6\text{OH})^{\bullet}$ . (E) The black spectrum  $\text{T}(5\text{OH})^{\bullet}$  (the central anisotropic doublet, black arrows) and remaining line components of  $\text{T}(-\text{H})^{\bullet}$  (indicated by green arrows) due to residual one-electron oxidized dThd is obtained by 30% subtraction of spectrum (C) and 40% subtraction of black spectrum A from spectrum (B).



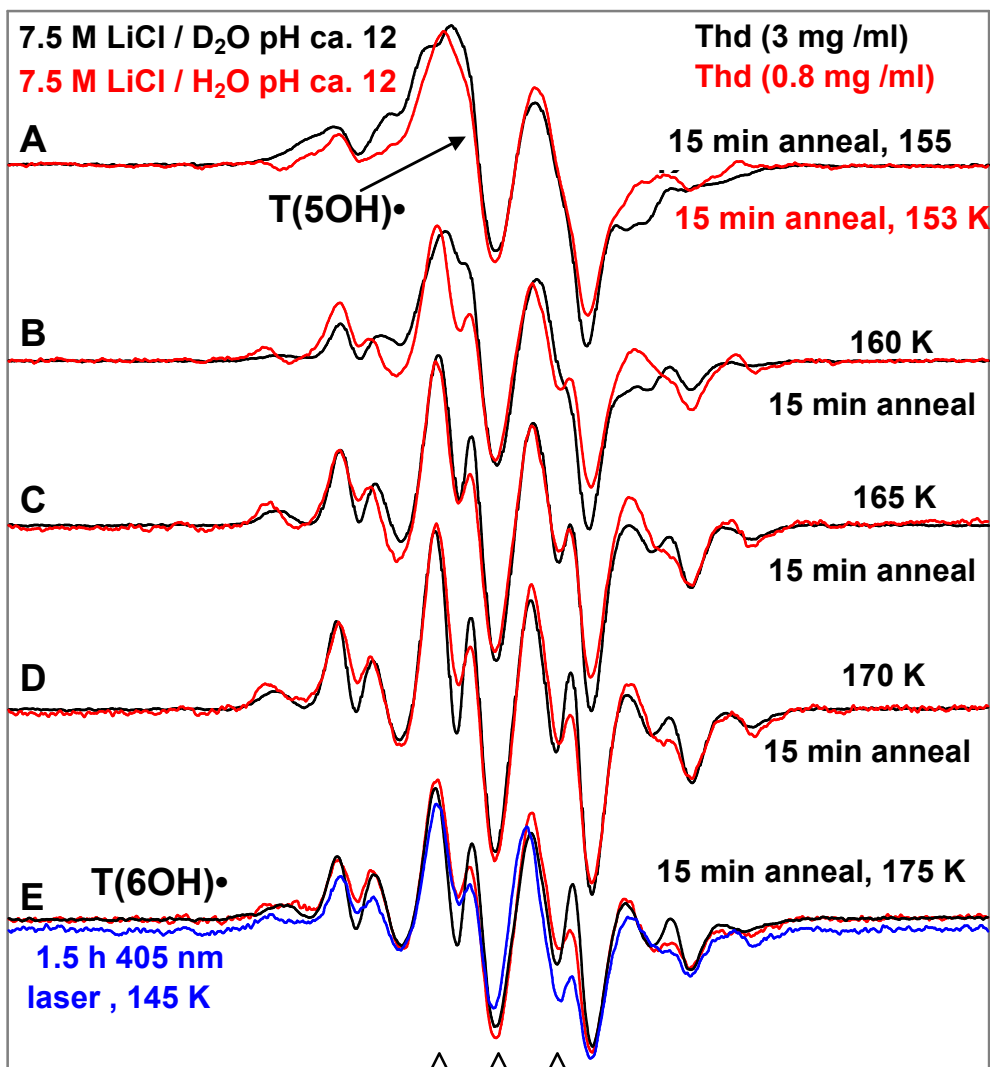
**Figure S9.** ESR spectra of (A) glassy (7.5 M LiCl/D<sub>2</sub>O) sample of 5'-TDP (3 mg/ml) in the presence of the electron scavenger K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> at pH ca. 12,  $\gamma$ -irradiated to a dose of 1.4 kGy having the line components owing to  $\text{Cl}_2\cdot^-$  and  $\text{SO}_4\cdot^-$  in the spectrum, (B) annealed to 155 K for 15 min, (C) annealed to 165 K for 15 min, (D) annealed to 175 K for 20 min ( $\text{T}(6\text{OH})\cdot$ ), (E) The  $\text{T}(5\text{OH})\cdot$  spectrum (the central anisotropic doublet, black arrows) and line components of  $\text{T}(-\text{H})\cdot$  (indicated by green arrows) due to residual one-electron oxidized 5'-TDP) is obtained by 25% subtraction of black spectrum in (D) from spectrum (B)

The  $\gamma$ -irradiated glassy sample of 5'-TTP (3 mg/ml) in the presence of the electron scavenger K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> at pH ca.12 (exactly identically prepared and handled as the 5'-TDP sample) produced spectra which were found to be very similar to the spectra obtained in 5'-TDP samples. Hence only the spectra of 5'-TDP samples are presented here.



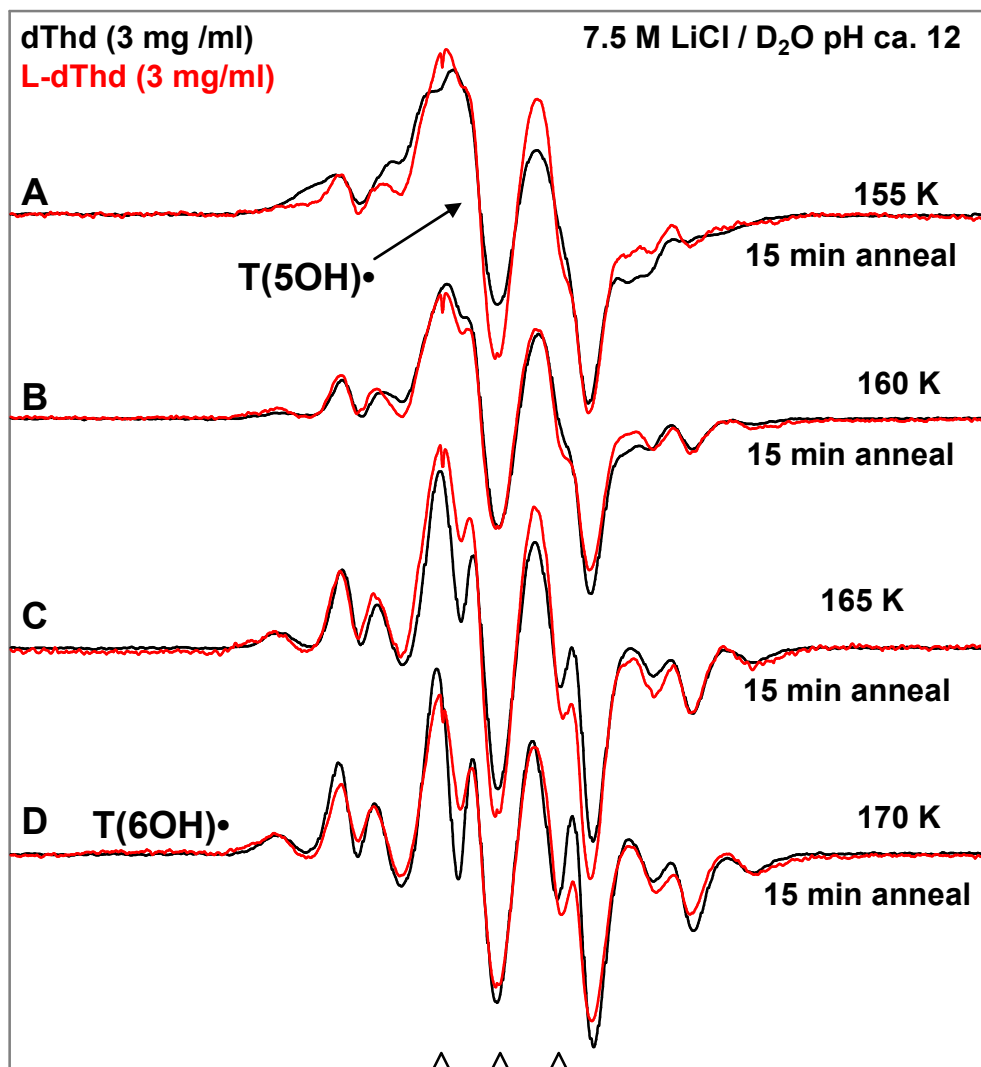
**Figure S10.** The ESR spectrum of  $\gamma$ -irradiated glassy sample of  $[\text{CD}_3]\text{-dThd}$  (3 mg/ml) in the presence of the electron scavenger  $\text{K}_2\text{S}_2\text{O}_8$  at pH ca. 12 recorded immediately after irradiation at 77 K show only the line components due to  $\text{SO}_4^{\bullet-}$  and  $\text{Cl}_2^{\bullet-}$  similar to spectra 3A in the manuscript. Therefore, this spectrum is not shown.

All ESR spectra (red) of  $[\text{CD}_3]\text{-dThd}$  (3 mg/ml) (A) annealed to 155 K for 15 min, (B) annealed to 160 K for 15 min, (C) annealed to 165 K for 15 min, (D) annealed to 170 K for 15 min and this spectrum, similar to spectra S2D, S3D, and S4D assigned to  $\text{T(6OH)}^{\bullet}$ . The ESR spectra (black) of the matched dThd sample (see supporting information Figure S7) are superimposed for comparison.



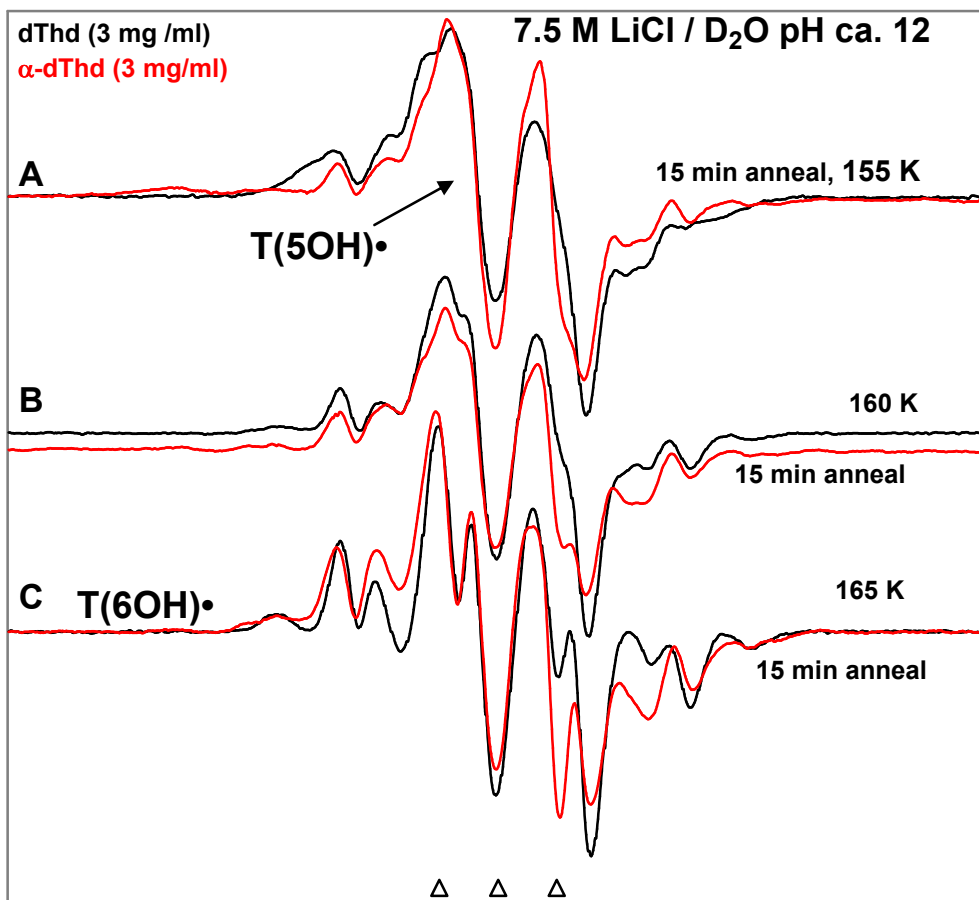
**Figure S11.** The ESR spectrum of  $\gamma$ -irradiated glassy sample of dThd (0.8 mg/ml) in the presence of the electron scavenger  $\text{K}_2\text{S}_2\text{O}_8$  at pH ca. 12 recorded immediately after irradiation at 77 K show only the line components due to  $\text{SO}_4^{\bullet-}$  and  $\text{Cl}_2^{\bullet-}$  similar to spectra 3A in the manuscript. Therefore, this spectrum is not shown.

All ESR spectra (red) of dThd (3 mg/ml) (A) annealed to 155 K for 15 min, (B) annealed to 160 K for 15 min, (C) annealed to 165 K for 15 min, (D) annealed to 170 K for 15 min, (E) annealed to 175 K for 15 min. The central doublet in spectrum A is assigned to  $\text{T}(5\text{OH})^{\bullet}$ . The spectra D and E are assigned to  $\text{T}(6\text{OH})^{\bullet}$ . The ESR spectra (black) of the matched dThd sample (see supporting information Figure S7) are superimposed for comparison. The blue spectrum in Figure E is the spectrum recorded at 77 K after photo-exciting the sample at ca. 145 K for 1.5 h at 405 nm.



**Figure S12.** The ESR spectrum of  $\gamma$ -irradiated glassy sample of L-dThd (3 mg/ml) in the presence of the electron scavenger  $\text{K}_2\text{S}_2\text{O}_8$  at pH ca. 12 recorded immediately after irradiation at 77 K show only the line components due to  $\text{SO}_4^{\bullet-}$  and  $\text{Cl}_2^{\bullet-}$  similar to spectra 3A in the manuscript. Therefore, this spectrum is not shown.

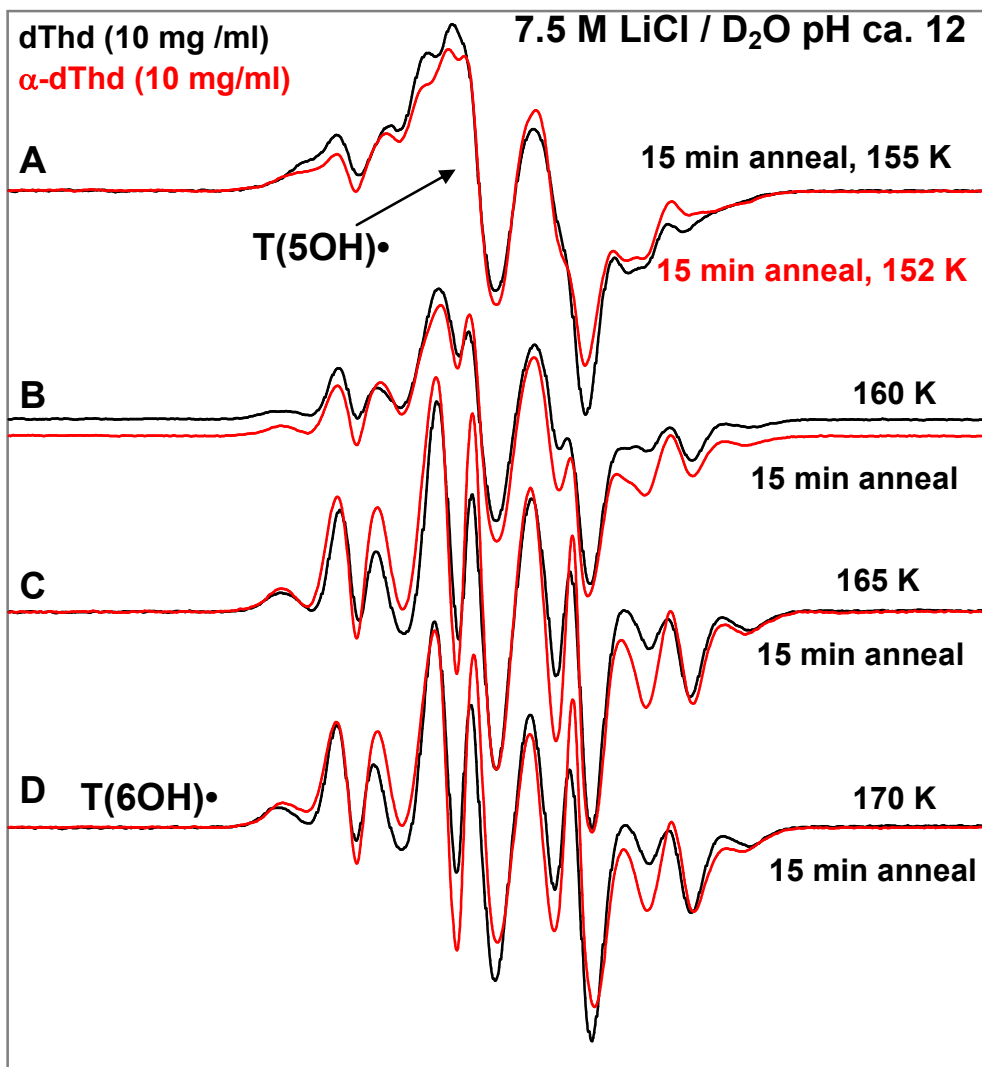
All ESR spectra (red) of dThd (3 mg/ml) (A) annealed to 155 K for 15 min, (B) annealed to 160 K for 15 min, (C) annealed to 165 K for 15 min, (D) annealed to 170 K for 15 min. The central doublet in spectrum A is assigned to  $\text{T}(5\text{OH})^{\bullet}$ . The spectrum D is assigned to  $\text{T}(6\text{OH})^{\bullet}$ . The ESR spectra (black) of the matched dThd sample (see supporting information Figure S7) are superimposed for comparison.



**Figure S13.** The ESR spectrum of  $\gamma$ -irradiated glassy sample of  $\alpha$ -dThd (3 mg/ml) in the presence of the electron scavenger  $\text{K}_2\text{S}_2\text{O}_8$  at pH ca. 12 recorded immediately after irradiation at 77 K show only the line components due to  $\text{SO}_4^{\bullet-}$  and  $\text{Cl}_2^{\bullet-}$  similar to spectra 3A in the manuscript. Therefore, this spectrum is not shown.

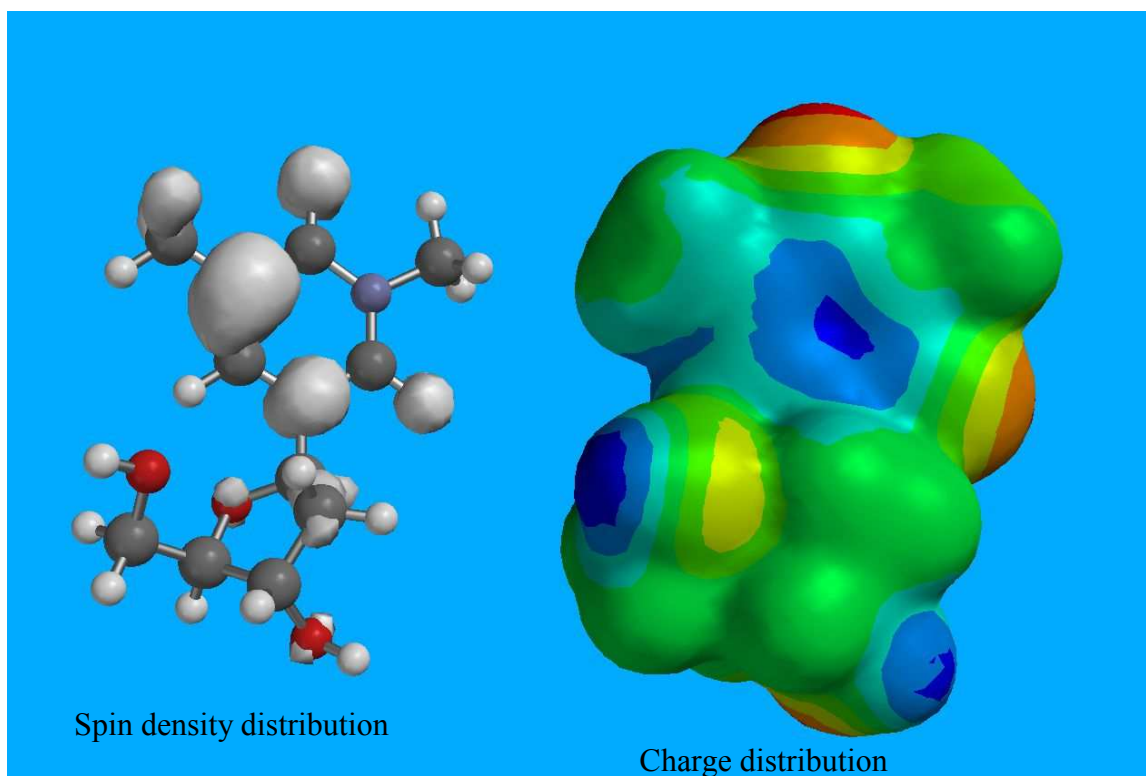
All ESR spectra (red) of dThd (3 mg/ml) (A) annealed to 155 K for 15 min, (B) annealed to 160 K for 15 min, (C) annealed to 165 K for 15 min. The central doublet in spectrum A is assigned to  $\text{T}(5\text{OH})\bullet$ . The spectrum D is assigned to  $\text{T}(6\text{OH})\bullet$ . The ESR spectra (black) of the matched dThd sample (see supporting information Figure S7) are superimposed for comparison.



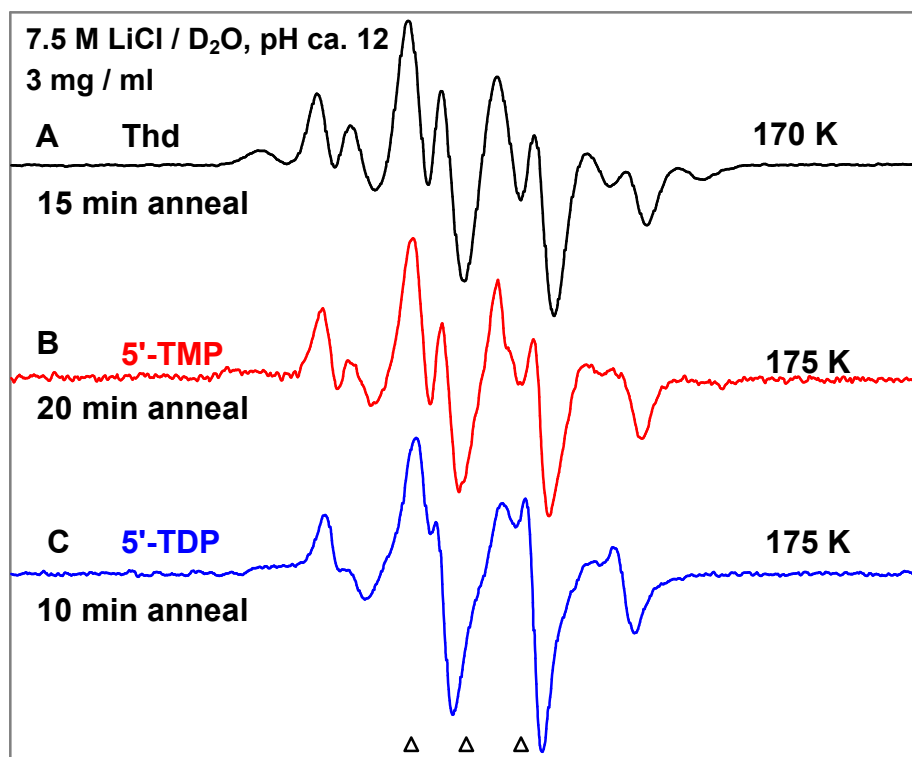


**Figure S14.** The ESR spectrum of  $\gamma$ -irradiated glassy sample of  $\alpha$ -dThd (10 mg/ml) in the presence of the electron scavenger  $\text{K}_2\text{S}_2\text{O}_8$  at pH ca. 12 recorded immediately after irradiation at 77 K show only the line components due to  $\text{SO}_4^{\bullet-}$  and  $\text{Cl}_2^{\bullet-}$  similar to spectra 3A in the manuscript. Therefore, this spectrum is not shown.

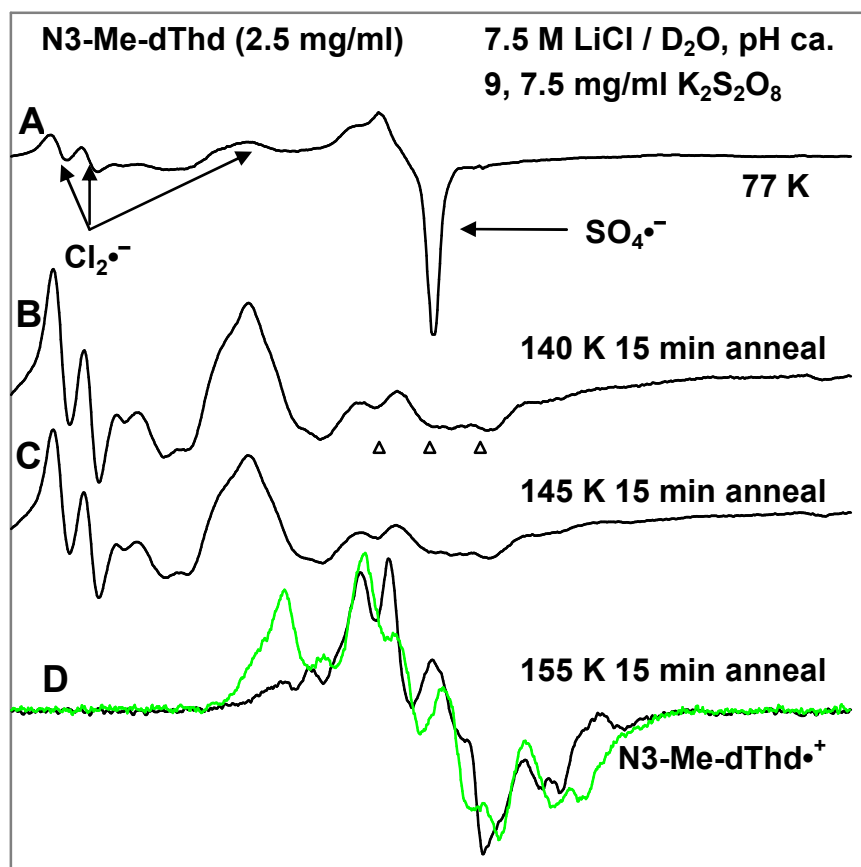
All ESR spectra (red) of dThd (3 mg/ml) (A) annealed to 155 K for 15 min, (B) annealed to 160 K for 15 min, (C) annealed to 165 K for 15 min, (D) annealed to 170 K for 15 min. The central doublet in spectrum A is assigned to  $\text{T}(5\text{OH})\bullet$ . The spectrum D is assigned to  $\text{T}(6\text{OH})\bullet$ . The ESR spectra (black) of the matched dThd sample (see supporting information Figure S8) are superimposed for comparison.



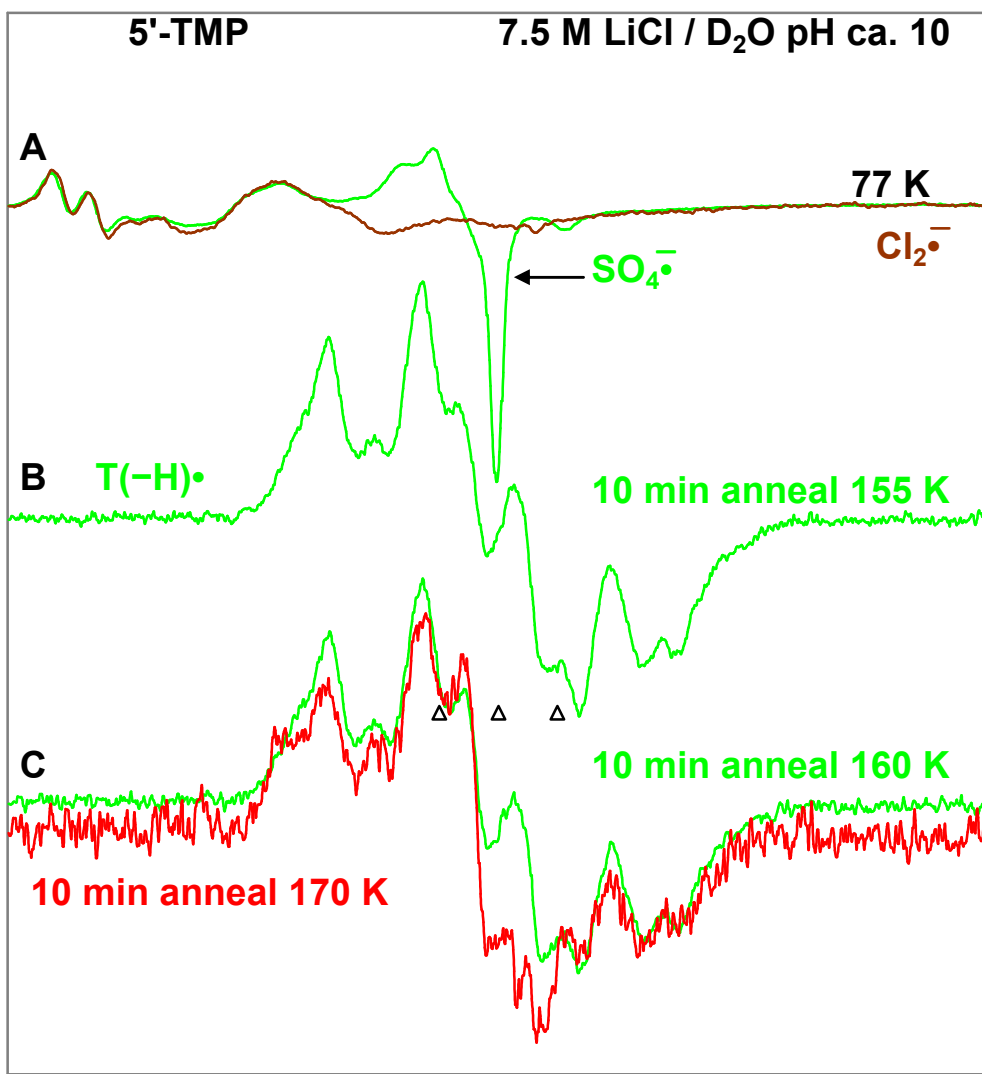
**Figure S15.** The optimized structure with spin densities and distribution of charge in N3-Me-dThd<sup>•+</sup> calculated at the B3LYP/6-31G\* level in the gas phase. Red color region represents negative charge whereas the blue color region represents positive charge distributions.



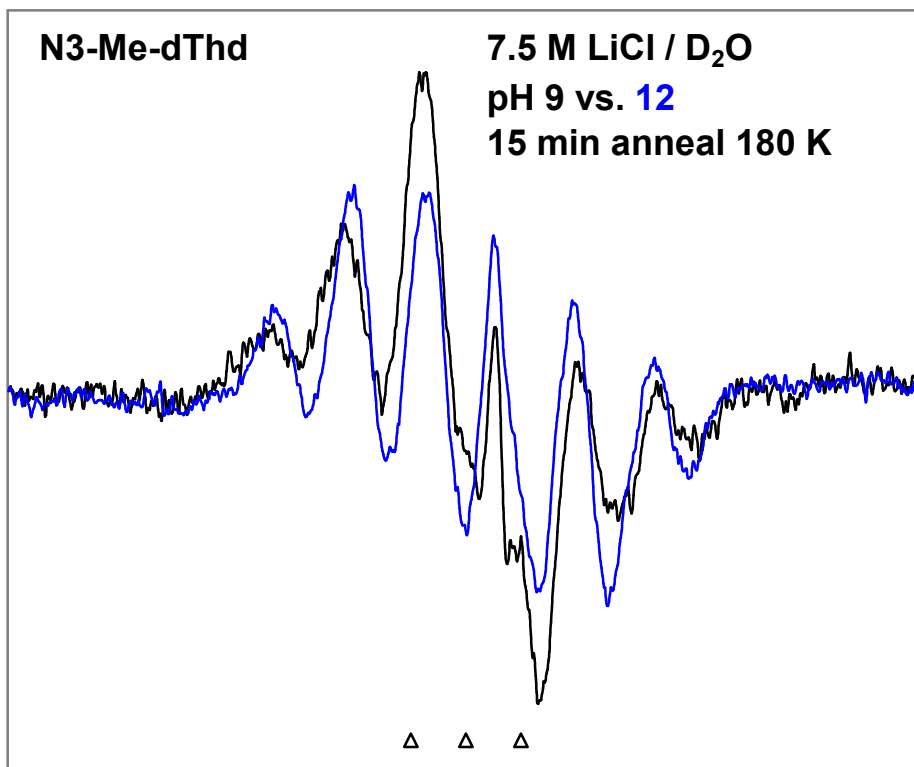
**Figure S16.** Dependence of the number of phosphate groups at 5'-site on the type of T(6OH)• formation via annealing at 170 to 175 K. ESR spectra of one-electron oxidized (A) Black, dThd (3mg/ml) with no phosphate at 5'-, in Figure S7D, (B) Red, 5'-TMP (3 mg/ml) from Figure 6B, (C) Blue, 5'-TDP (3mg/ml), Figure S9D.



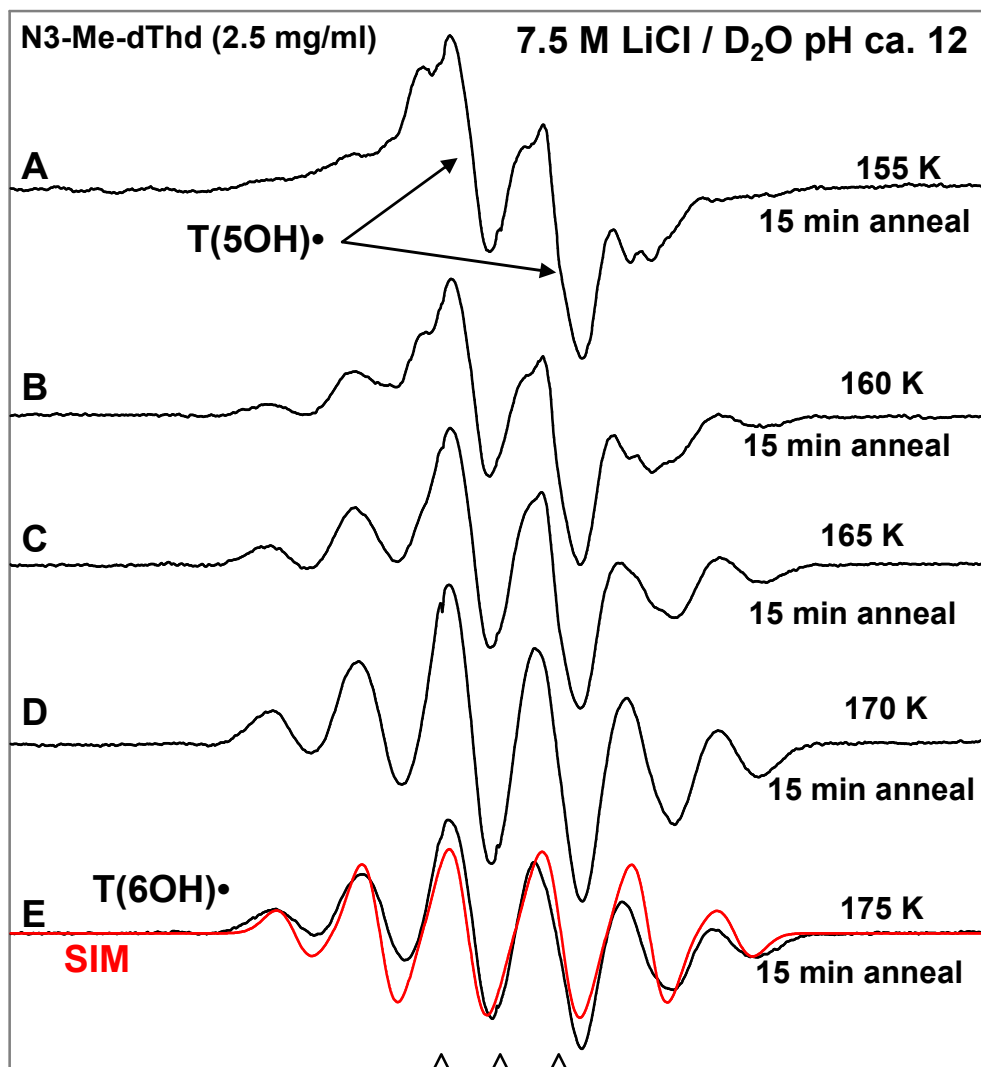
**Figure S17.** (A) Spectrum (black) showing  $\text{SO}_4\bullet^-$  and  $\text{Cl}_2\bullet^-$  formation in a  $\gamma$ -irradiated (1.4 kGy) 7.5 M  $\text{N}_2$ -saturated LiCl/D<sub>2</sub>O glass containing N3-Me-dThd (2.5 mg/ml) and  $\text{K}_2\text{S}_2\text{O}_8$  (7.5 mg/ml as an electron scavenger). (B) Spectrum (black) of the sample in (A) after annealing to 140 K for 15 min.  $\text{SO}_4\bullet^-$  reacts with  $\text{Cl}^-$  at this temperature to form  $\text{Cl}_2\bullet^-$ . (C) Spectrum (black) after further annealing at 145 K for 15 min. (D) Black spectrum after annealing for 15 min at 155 K and this spectrum is assigned to  $\text{N3-Me-dThd}\bullet^+$ . Here,  $\text{Cl}_2\bullet^-$  reacts with N3-Me-dThd to form  $\text{N3-Me-dThd}\bullet^+$  via one-electron oxidation. ESR spectrum of T(-H) $\bullet$  (green) formed in unlabeled 5'-TMP is superimposed for comparison and assigning the black spectrum in (D) as  $\text{N3-Me-dThd}\bullet^+$ .



**Figure S18.** (A) ESR spectrum (green) of a  $\gamma$ -irradiated glassy sample of 5'-TMP (2 mg/ml in 7.5 M LiCl/D<sub>2</sub>O) in the presence of electron scavenger K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> at pH ca. 12 (black) showing only  $\text{SO}_4^{\bullet-}$  and  $\text{Cl}_2^{\bullet-}$  formation. Partial spectrum (brown, 220 G scan) of a pure  $\text{Cl}_2^{\bullet-}$  spectrum is overlapped on the green spectrum in A to show the  $\text{Cl}_2^{\bullet-}$  line components in the black spectrum. (B) Spectrum (green) found after annealing to 155 K for 10 min and is assigned to  $\text{T}(-\text{H})^{\bullet}$  (see Figure 1 in the main manuscript); (C) Spectrum (green) found after annealing the same 5'-TMP at 160 K for 10 min. The superimposed spectrum (red) is obtained after annealing the same sample for 10 min at 170 K.



**Figure S19.** Black spectrum from glassy (7.5 M LiCl/D<sub>2</sub>O) sample of N3-Me-dThd (2.5 mg/ml) in the presence of the electron scavenger K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> at pH ca. 9,  $\gamma$ -irradiated to a dose of 1.4 kGy and progressively annealed to 180 K for 15 min. Blue spectrum from a matched glassy (7.5 M LiCl/D<sub>2</sub>O) sample of N3-Me-dThd (2.5 mg/ml) in the presence of the electron scavenger K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> at pH ca. 12,  $\gamma$ -irradiated to a dose of 1.4 kGy and progressively annealed to 180 K for 15 min.



**Figure S20.** ESR spectra of (A)  $\gamma$ -irradiated glassy sample of N3-Me-dThd (2.5 mg/ml in 7.5 M LiCl/D<sub>2</sub>O) in the presence of electron scavenger K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> at pH ca. 12 and annealed to 155 K for 15 min and the central doublet is assigned to T(5OH)•. (B) 160 K for 15 min, (C) 165 K for 15 min, (D) 170 K for 15 min. (E) 180 K for 15 min. This spectrum is assigned to T(6OH)• with C6-H axial conformation (scheme 4). The red spectrum in (E) is the simulated spectrum of T(6OH)• using HFCC values of each of the 3  $\beta$  methyl protons (19 G) with  $\beta$  C6-H HFCC of 41 G, linewidth 8 G, and  $g = 2.0035$ .

**Table T1.** B3LYP/6-31G\*\* calculated Mulliken charge and spin density distribution (in brackets) in T(-H)• + <sup>-</sup>OH and Thymine + •OH in C5- and C6- adduct radicals and at different dihedral angles.<sup>a</sup>

Structure No.	T(-H)• + <sup>-</sup> OH			Thy + •OH		
	Mulliken Charge (spin)			Mulliken Charge (spin)		
	Thymine	OH <sup>b</sup>	Water	Thy	OH <sup>c</sup>	Water
1 (C5-adduct)	-0.56238 (0.92644)	-0.28685 (0.06514)	-0.15077 (0.008419)	0.466490 (0.935680)	-0.435132 (0.062404)	-0.031358 (0.001918)
2 ( $\Phi = -25^\circ$ )	-0.54764 (0.907902)	-0.29578 (0.083358)	-0.15659 (0.008741)	0.494315 (0.917259)	-0.462853 (0.079906)	-0.031460 (0.002834)
3 ( $\Phi = -20^\circ$ )	-0.52715 (0.87251)	-0.30892 (0.118427)	-0.16393 (0.009064)	0.520730 (0.893502)	-0.490055 (0.102984)	-0.030676 (0.003513)
4 ( $\Phi = -15^\circ$ )	-0.45107 (0.658353)	-0.34282 (0.338296)	-0.20611 (0.00335)	0.550480 (0.847106)	-0.521867 (0.148742)	-0.028613 (0.004151)
5 ( $\Phi = -10^\circ$ )	-0.44511 (0.597853)	-0.34312 (0.401121)	-0.21177 (0.001027)	0.359527 (0.509837)	-0.331358 (0.490861)	-0.028167 (-0.000698)
6 ( $\Phi = -5^\circ$ )	-0.44663 (0.576262)	-0.34174 (0.423571)	-0.21163 (0.000165)	0.240624 (0.371124)	-0.205029 (0.632701)	-0.035598 (-0.003824)
7 ( $\Phi = 0^\circ$ )	-0.41005 (0.608855)	-0.36294 (0.391029)	-0.22701 (0.000117)	0.209859 (0.329192)	-0.168363 (0.675588)	-0.041495 (-0.004782)
8 ( $\Phi = 5^\circ$ )	-0.41122 (0.617658)	-0.36695 (0.38307)	-0.22184 (-0.00073)	0.199459 (0.318107)	-0.153499 (0.686494)	-0.045960 (-0.004600)
9 ( $\Phi = 10^\circ$ )	-0.56697 (0.9508)	-0.27279 (0.046467)	-0.16024 (0.002733)	0.490318 (0.963071)	-0.436163 (0.035081)	-0.054153 (0.001850)
10 (C6-adduct)	-0.56672 (0.950692)	-0.27205 (0.046114)	-0.16123 (0.003197)	0.531440 (0.954564)	-0.490746 (0.045008)	-0.040692 (0.000430)

<sup>a</sup>See Figures 11 and 12.

<sup>b</sup>Charge on oxygen of OH varies from ca. -0.6 to -0.68e.

<sup>c</sup>Charge on oxygen of OH varies from ca. -0.58 -0.60e.