Supporting Information

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Independent Generation and Time-Resolved Detection of 2'-Deoxyguanosin-N2-yl Radical

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Abstract: Guanine radicals are important reactive intermediates in DNA damage. Hydroxyl radical (HO•) has long been believed to react with 2'-deoxyguanosine (dG) generating 2'-deoxyguanosin-*N1*-yl radical (dG(N1-H)•) via addition to the nucleobase π-system and subsequent dehydration. This basic tenet was challenged by an alternative mechanism, in which the major reaction of HO• with dG was proposed to involve hydrogen atom abstraction from the N2-amine. The 2'-deoxyguanosin-*N2*-yl radical (dG(N2-H)•) formed was proposed to rapidly tautomerize to dG(N1-H)•. We report the first independent generation of dG(N2-H)• in high yield via photolysis of **1**. dG(N2-H)• is directly observed upon nanosecond laser flash photolysis (LFP) of **1**. The absorption spectrum of dG(N2-H)• is corroborated by DFT studies, and *anti*- and *syn*-dG(N2-H)• are resolved for the first time. The LFP experiments showed no evidence for tautomerization of dG(N2-H)• to dG(N1-H)• within hundreds of microseconds. This observation suggests that the generation of dG(N1-H)• via dG(N2-H)• following hydrogen atom abstraction from dG is unlikely to be a major pathway when HO• reacts with dG.

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Experimental Procedures

Materials and methods.

Dichloromethane, TEA and DMF (under vacuum) were distilled from CaH_2. THF and dioxane were distilled from sodium. All other reagents were purchased from commercial sources and were used without further purification. All reactions were carried out under a positive pressure of argon atmosphere and monitored by TLC on Silica Gel G-25 UV254 (0.25 mm) plates. Spots were detected under UV light and/or by ethanolic solution p-anisaldehyde, or aqueous solution of ammonium molybdate. Column flash chromatography was performed with Silicycle® grade 70−230 mesh, 60−200 µm, 60 Å silica. UV absorbance was measured with a Thermo Scientific NanoDrop® 2000c spectrometer. HPLC was performed on a Phenomenex Luna C-18 column (250 × 4.6 mm).

Procedure for the photolysis of precursors and subsequent HPLC analysis.

Photolyses were carried out in Pyrex tubes using a Rayonet photochemical reactor (Southern New England Ultraviolet) equipped with a merry-go-round apparatus and 16 lamps having a maximum output at 350 nm. Photolyses were carried out at 25 °C. This temperature was maintained by using the fan at the bottom of the unit provided by the manufacturer and a Dayton® 239 CFM AC axial fan at the top facing such that air flowing through the unit is drawn out through the top. Reaction mixtures (50 µL each) containing precursor (100 µM), internal standard (thymidine, 100 µM), and additives (reducing agents, organic solvent, sensitizers) in buffer (10 mM phosphate, pH 7.2) were photolyzed at room temperature under aerobic or anaerobic conditions. Samples for anaerobic reactions were degassed by three freeze-pump-thaw cycles at 2 mTorr and flame sealed under vacuum. Samples containing acetone or a high percentage of acetonitrile (> 30%) were evaporated to dryness and resuspended in water. Samples using thiophenol as reducing agent were washed with hexanes (3 × 50 µL), evaporated to dryness and resuspended in water before HPLC analysis.

The reaction mixtures (including unphotolyzed controls) were analyzed by reversed-phase HPLC while being monitored at 260 nm and 284 nm. HPLC was performed on a Phenomenex Luna C-18 column (A, water; B, ACN; 3% B from t = 0 to t = 1 min; 3-28% B linearly over 9 min; 28-97% B linearly over 5 min; 97% B from t = 25 to t = 20 min; 97-3% B linearly over 2 min; 3% B from t = 22 to t = 42 min; flow rate, 1 mL/min). The retention times of the internal standard (thymidine), precursors, and products generated by photolysis, as well as their response factors are listed in Table S1.

Preparation of 6.

Hydroxylamine hydrochloride (345 mg, 5 mmol) was treated with TEA (1.01 g, 10 mmol) in dioxane (10 mL) for 30 min before **5**[1] (324 mg, 0.5 mmol) was added. The reaction was refluxed at 110 °C overnight. The reaction was cooled to room temperature, concentrated under vacuum, and purified by flash chromatography on a silica column. Elution with 5% MeOH and 1% Et_3N in DCM gave **6** as a white foam (186 mg, 62%).^{1}H NMR (400 MHz, $CDCl_3$) δ 9.70 (s, 1H), 8.09 (s, 1H), 7.58 (s, 1H), 7.41 (d, *J* = 6.6 Hz, 2H), 7.33 – 7.07 (m, 3H), 6.60 (t, *J* = 6.2 Hz, 1H), 5.48 (q, *J* = 12.3 Hz, 2H), 4.57 (dt, *J* = 7.4, 3.9 Hz, 1H), 4.00 (d, *J* = 3.1 Hz, 1H), 3.83 (dd, *J* = 11.3, 3.1 Hz, 1H), 3.79 (dd, *J* = 11.3, 3.1 Hz, 1H), 2.60 – 2.45 (m, 1H), 2.36 (dt, *J* = 12.7, 6.2 Hz, 1H), 0.91 (s, 9H), 0.90 (s, 9H), 0.11 (s, 6H), 0.08 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.3, 160.7, 152.4, 138.0, 136.0, 128.4, 128.3, 128.3, 128.27, 128.25, 128.23, 128.23, 128.22, 128.0, 117.0, 87.6, 84.0, 71.8, 68.4, 62.7, 42.2, 26.0, 25.8, 18.4, 18.0, -4.6, -4.8, -5.4, -5.5. HRMS (ESI-TOF) *m/z* calcd for $(C_{29}H_{47}N_5O_5Si_2)^+$ $(M + H)^+$ = 602.3194, found *m/z* = 602.3197.

Preparation of 7.

Compound **6** (186 mg, 0.31 mmol) was dissolved in DMF (3 mL). Cs_2CO_3 (101 mg, 0.31 mmol) was slowly added while stirring. The reaction was stirred at 0 °C for 30 min before **8**[2] (81 mg, 0.39 mmol) was added at 0 °C. The reaction was stirred at room temperature overnight. The reaction was quenched by water and extracted with DCM (3 × 10 mL). The organic phase was dried over Na_2SO_4. Na_2SO_4 was removed by filtration, and the mixture was concentrated under vacuum. The residue was purified by flash chromatography on a silica column. Elution with 50% EtOAc in hexanes gave **7** as a yellow foam (158 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.60 (s, 1H), 7.38 (d, *J* = 6.7 Hz, 2H), 7.22 (ddd, *J* = 8.5, 7.7, 2.3 Hz, 3H), 6.33 (t, *J* = 6.5 Hz, 1H), 5.57 – 5.40 (m, 2H), 4.60 – 4.41 (m, 1H), 3.95 (dd, *J* = 6.3, 3.1 Hz, 1H), 3.75 (qd, *J* = 11.2, 3.5 Hz, 2H), 2.48 – 2.36 (m, 1H), 2.30 (ddd, *J* = 12.9, 5.8, 3.5 Hz, 1H), 1.48 (s, 7H), 1.32 (s, 10H), 0.86 (s, 18H), 0.04 (d, *J* = 2.3 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 215.6, 161.1, 160.6, 153.0, 138.4, 136.1, 128.3, 128.0, 127.9, 117.4, 91.2, 87.8, 84.1, 72.0, 68.1, 62.9, 45.2, 41.7, 26.8, 26.0, 25.9, 25.7, 24.4, 24.3, 18.4, 17.9, -4.7, -4.8, -5.4, -5.5. HRMS (ESI-TOF) *m/z* calcd for $(C_{37}H_{62}N_5O_6Si_2)^+$ $(M + H)^+$ = 728.4239, found *m/z* = 728.4245.

Preparation of debenzylated 7.

Compound **7** (158 mg, 0.22 mmol) was dissolved in MeOH (4 mL), and 20 mg 10% Pd/C was added to the solution. The flask was sparged with hydrogen using a balloon. The reaction was monitored by TLC (3% MeOH in DCM) and was complete after 30 min. The Pd/C was removed by passing the reaction through a silica plug. The reaction was concentrated under vacuum and purified by flash chromatography on a silica column. Elution with 1% to 3% MeOH in DCM gave debenzylated **7** as a white solid (126 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 6.23 (t, *J* = 6.5 Hz, 1H), 4.51 (br, 1H), 3.96 (br, 1H), 3.72 (d, *J* = 12.1 Hz, 2H), 2.55 – 2.21 (m, 2H), 1.54 (s, 3H), 1.52 (s, 3H), 1.33 (s, 9H), 0.89 (s, 9H), 0.88 (s, 9H), 0.08 (s, 6H), 0.06 (s, 3H), 0.05 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 214.9, 157.7, 154.7, 149.6, 136.0, 119.4, 90.9, 87.9, 83.9, 72.2, 62.9, 44.9, 41.4, 27.2, 25.9, 25.7, 24.1, 18.4, 18.0, -4.7, -4.8, -5.4, -5.5. HRMS (ESI-TOF) *m/z* calcd for $(C_{30}H_{56}N_5O_6Si_2)^+$ $(M + H)^+$ = 638.3769, found *m/z* = 638.3742.

Preparation of 1.

Debenzylated **7** (126 mg, 0.2 mmol) was dissolved in THF (2 mL). Et_3N•3HF (322 mg, 2.0 mmol) was slowly added while stirring. The reaction was stirred at 25 ºC overnight. The reaction was concentrated under vacuum and purified by flash chromatography on a silica column. Elution with 7% MeOH in DCM gave **1** as a light pink foam (63.8 mg, 78%). ^{1}H NMR (400 MHz, MeOH-d4) δ 8.14 (s, 1H), 6.31 (t, *J* = 6.7 Hz, 1H), 4.56 – 4.42 (m, 1H), 3.98 (dd, *J* = 7.3, 3.8 Hz, 1H), 3.75 (dd, *J* = 7.3, 4.0 Hz, 1H), 2.64 (ddd, *J* = 13.4, 7.1, 6.2 Hz, 1H), 2.39 (ddd, *J* = 13.4, 6.2, 3.6 Hz, 1H), 1.57 (d, *J* = 1.1 Hz, 6H), 1.31 (s, 9H). ^{13}C NMR (101 MHz, MeOH-d4) δ 215.5, 157.2, 155.3, 149.8, 137.4, 118.4, 90.3, 87.8, 84.2, 71.1, 61.7, 44.4, 40.3, 26.4, 23.3. HRMS (ESI-TOF) *m/z* calcd for $(C_{18}H_{28}N_5O_6)^+$ $(M + H)^+$ = 410.2040, found *m/z* = 410.2038.

Time-resolved laser flash photolysis.

Nanosecond time-resolved transient absorption spectra were measured using a laser flash photolysis setup Edinburgh LP980 spectrometer (Edinburgh Instrument Ltd.), combined with a Nd:YAG laser (Quanta-Ray, Spectral Physics Inc.). The sample was excited by a 355 nm laser pulse (1 Hz, 10 mJ/pulse, fwhm≈ 7 ns). The analyzing light was from a 150 W pulsed xenon lamp. A monochromator equipped with a photomultiplier for collecting the spectral range from 350 to 700 nm was used to analyze transient absorption spectra. Data were analyzed by the online software of the LP980 spectrophotometer. Quartz cuvettes of 1 cm path length were used for all LFP measurements. All LFP experiments were performed in anaerobic solution.

Theoretical calculations.

All the geometries and energies of the reactants and intermediates were calculated using the hybrid density functional theory (B3LYP) with the standard basis sets of 6-311++G(d,p). Bulk solvation effects were simulated by using the polarized continuum model (PCM). And their time-dependent (TD) version was employed to characterize the excited electronic states and simulate the absorption spectra at the same level. All the calculations were carried out using the Gaussian 09 program package.[3] In order to enable easier comparison with the experimental spectra, each transition was convoluted with a Gaussian half width of 0.33 eV, after being red-shifted by 60 nm. To reduce the calculation costs, all the calculations were performed with guanine instead of 2'-deoxyguanosine since the deoxyribose (sugar) ring has little effect on the electronic transitions at long wavelength.

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Supplementary Figures

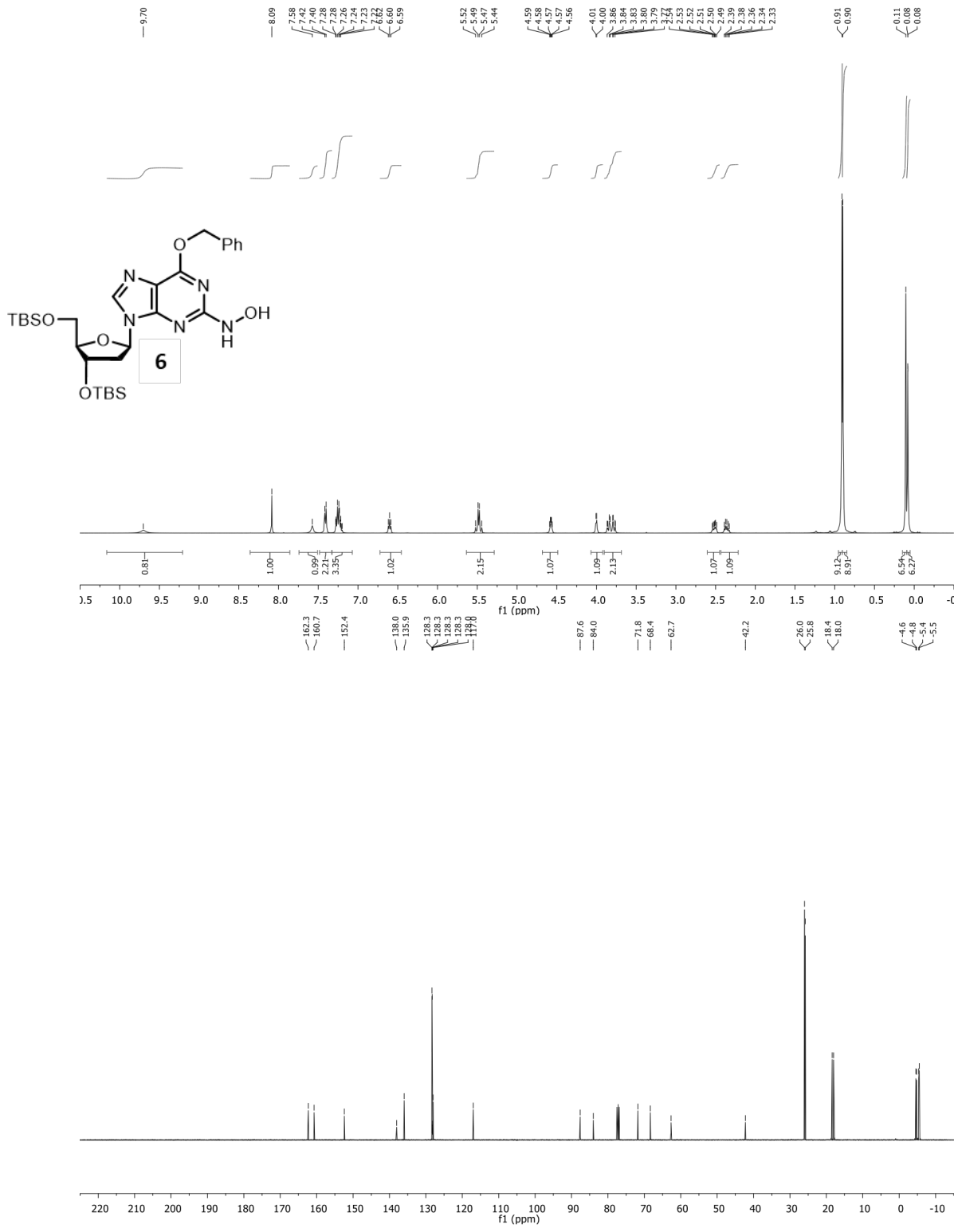


Figure S1. ^{1}H NMR and ^{13}C NMR spectra of **6**.

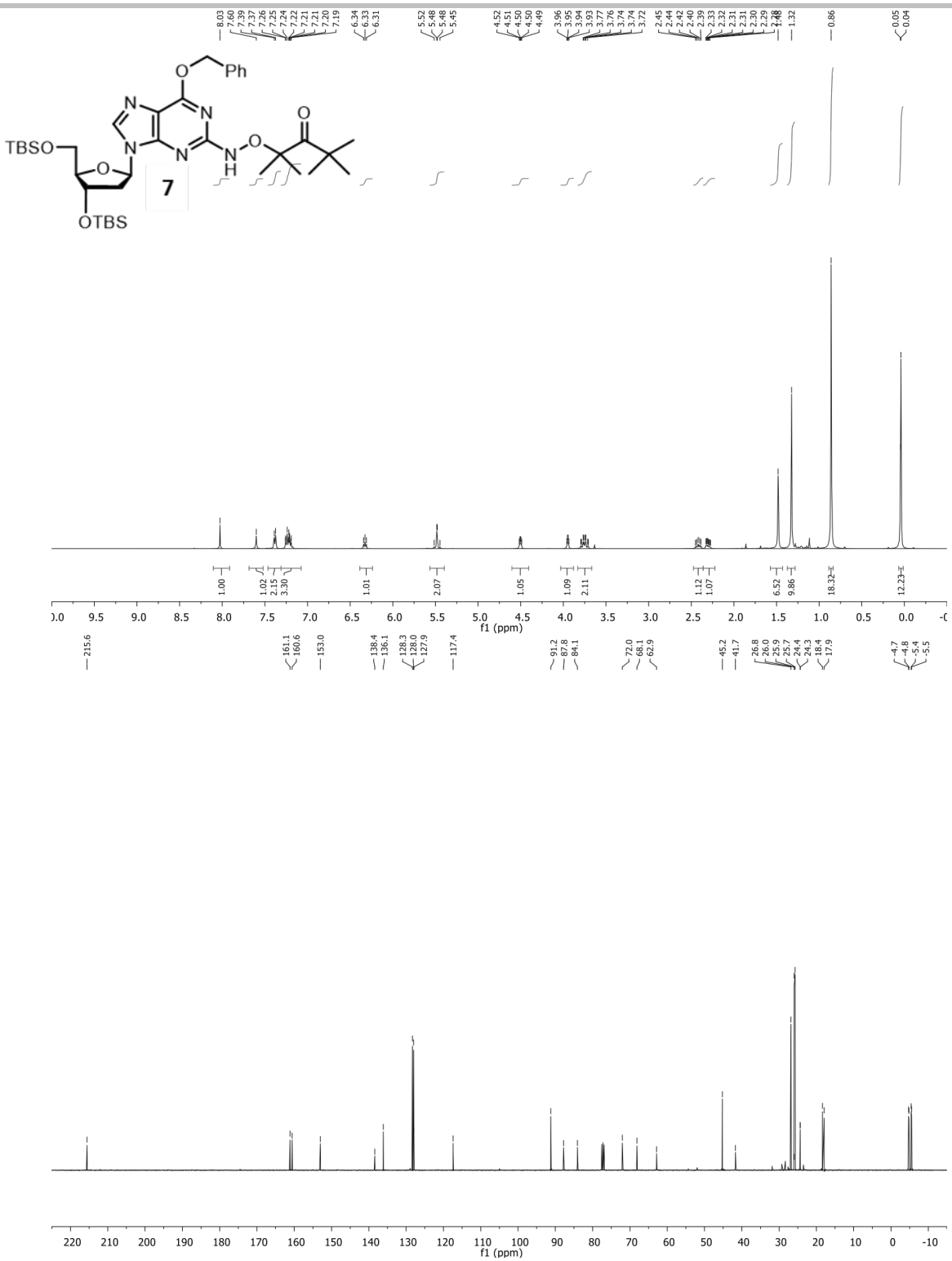


Figure S2. ^{1}H NMR and ^{13}C NMR spectra of **7**.

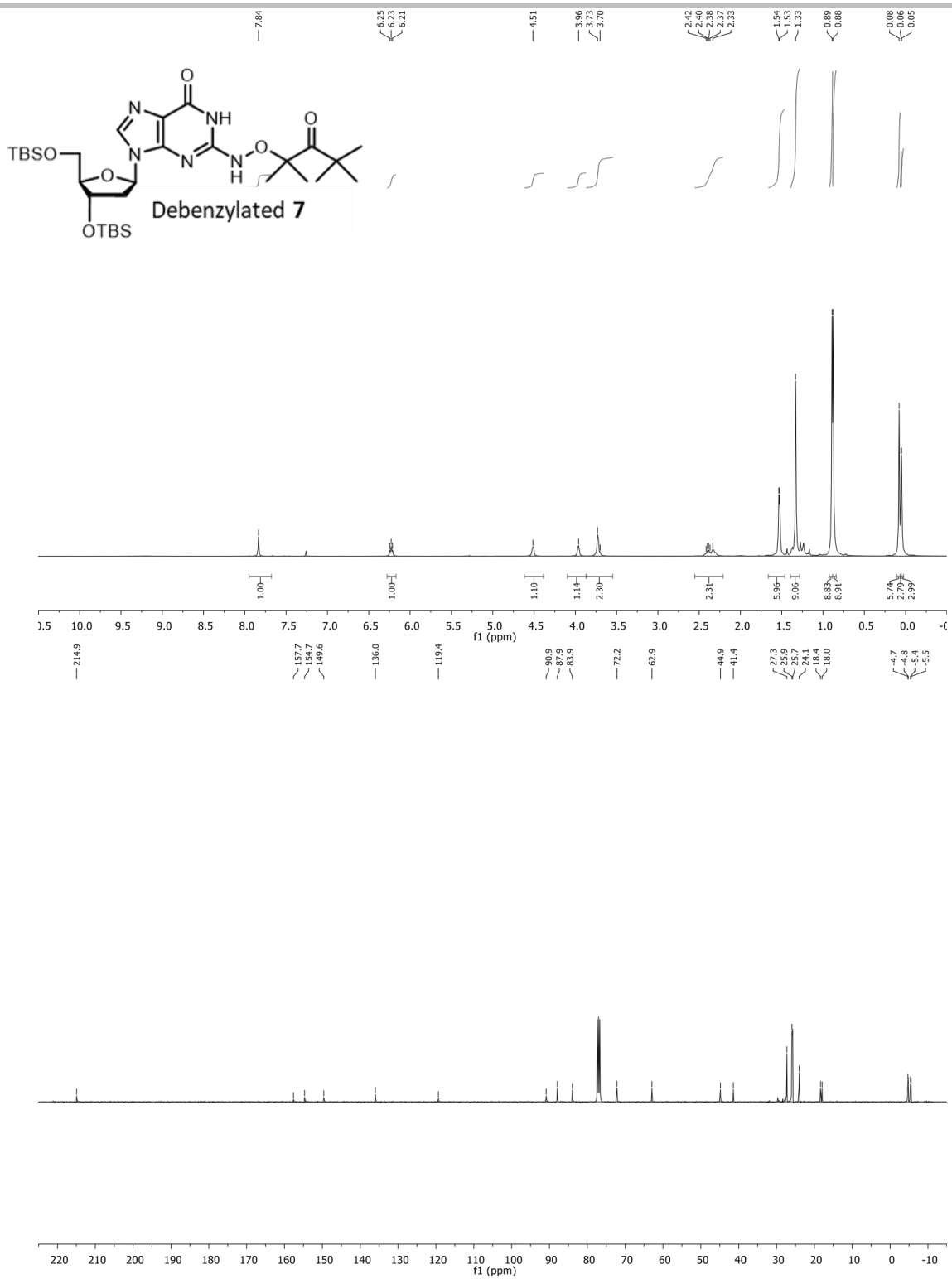


Figure S3. ^{1}H NMR and ^{13}C NMR spectra of debenzylated **7**.

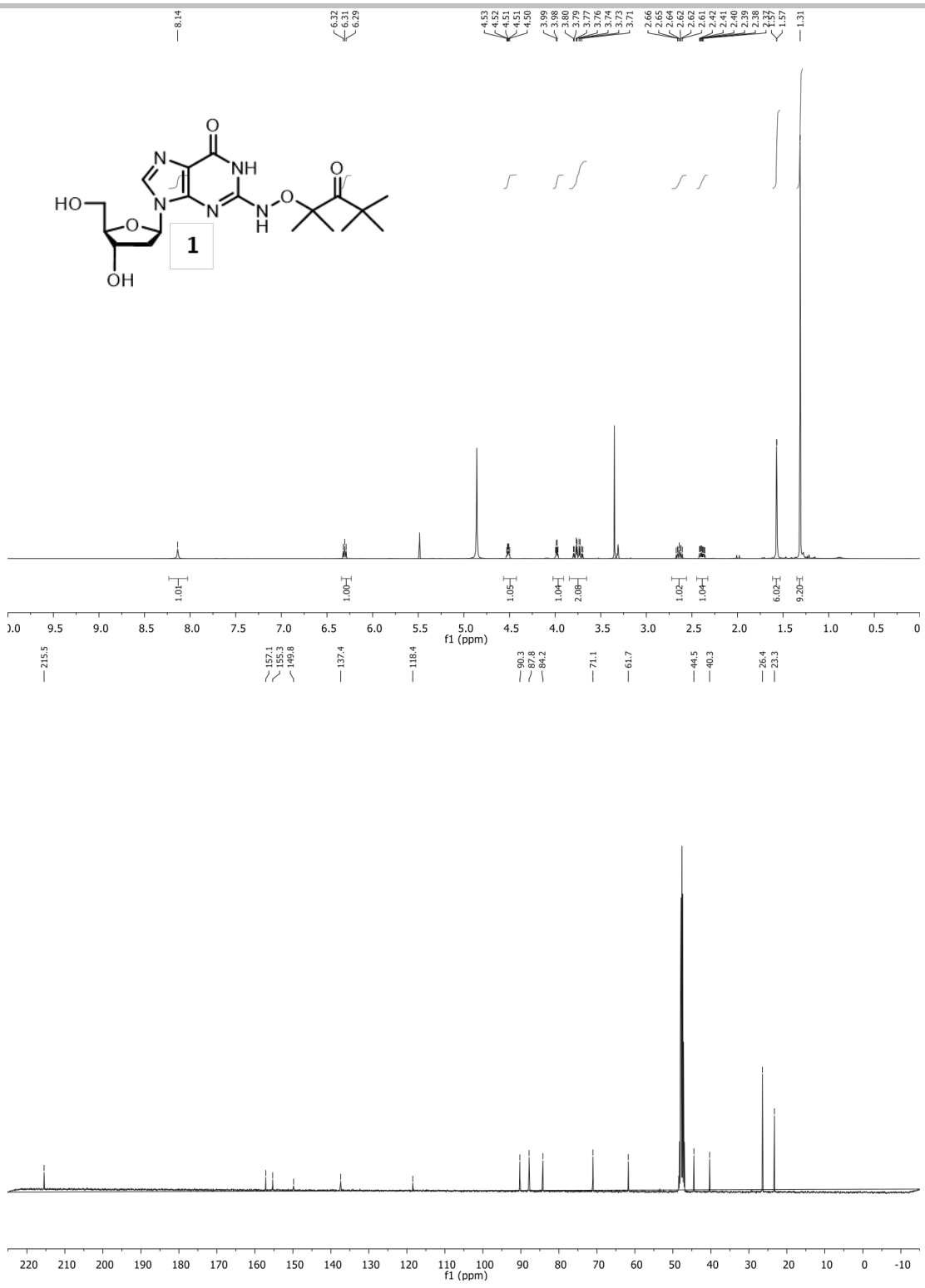


Figure S4. ^{1}H NMR and ^{13}C NMR spectra of **7**.

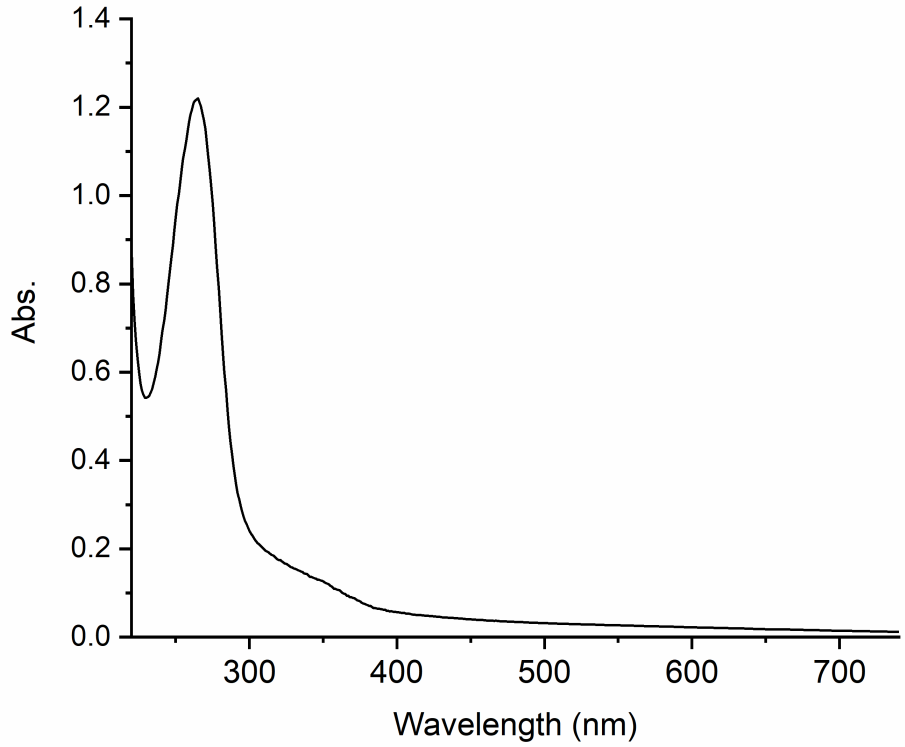


Figure S5. UV-Vis spectrum of **1** (100 µM) in water.

Table S1. Retention times and response factors for HPLC analysis.

Compound	Retention time (min)	Response factor (260 nm)
T	8.2	-
dG	7.4	0.84
1	15.1	0.78

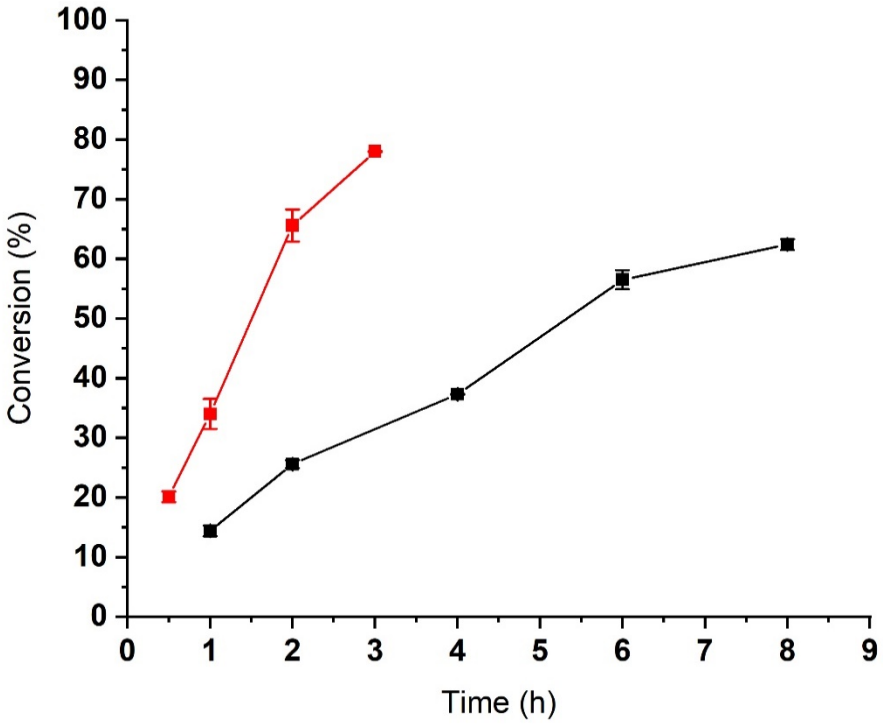


Figure S6. Time course experiment for the conversion of **1** (100 μM) during anaerobic photolysis in the absence of reducing agents (red square), or in the presence of PhSH (10 mM, black square).

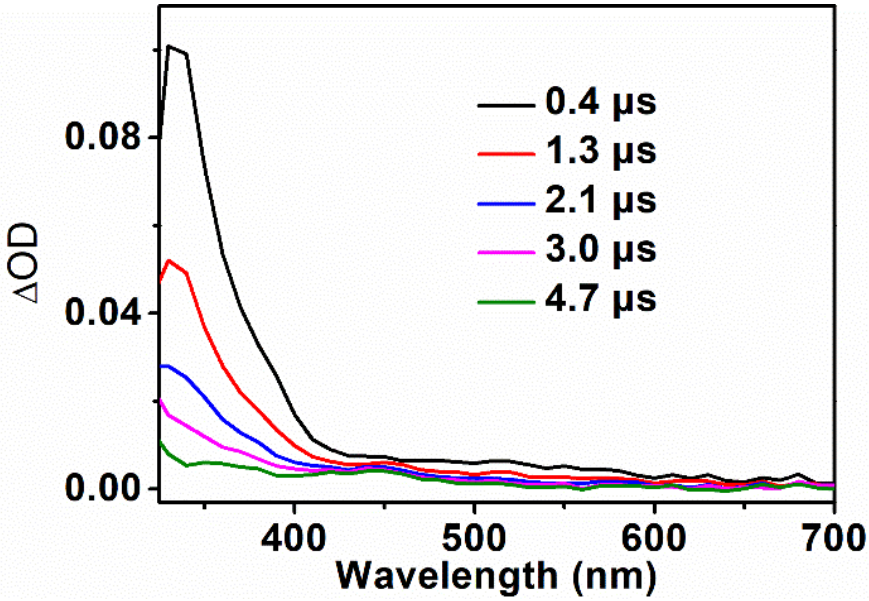


Figure S7. The transient UV-vis absorption spectra of pure acetophenone (30 mM) in aqueous buffer (pH 7.0) /acetonitrile (1:1, v:v) upon 355 nm laser flash photolysis in anaerobic conditions

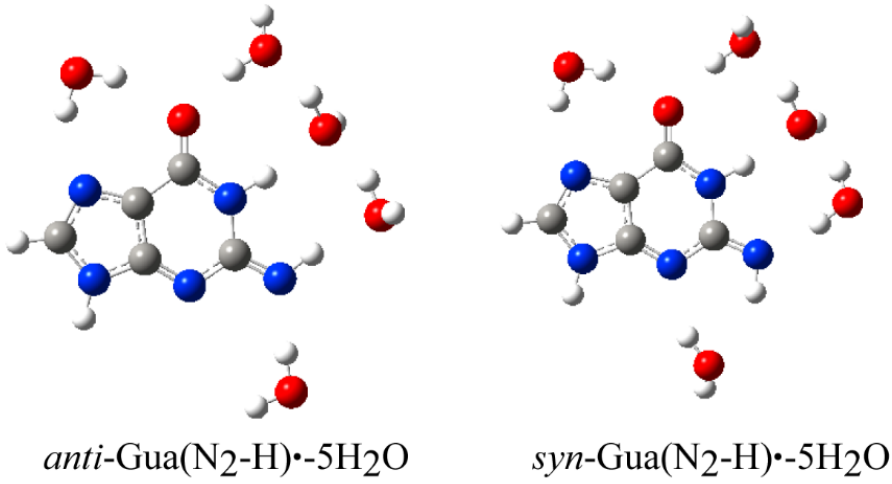


Figure S8. The B3LYP/6-311++G(d,p)//PCM optimized geometries of *anti*-Gua(N_2-H)• and *syn*-Gua(N_2-H)•, including five explicit water molecules.

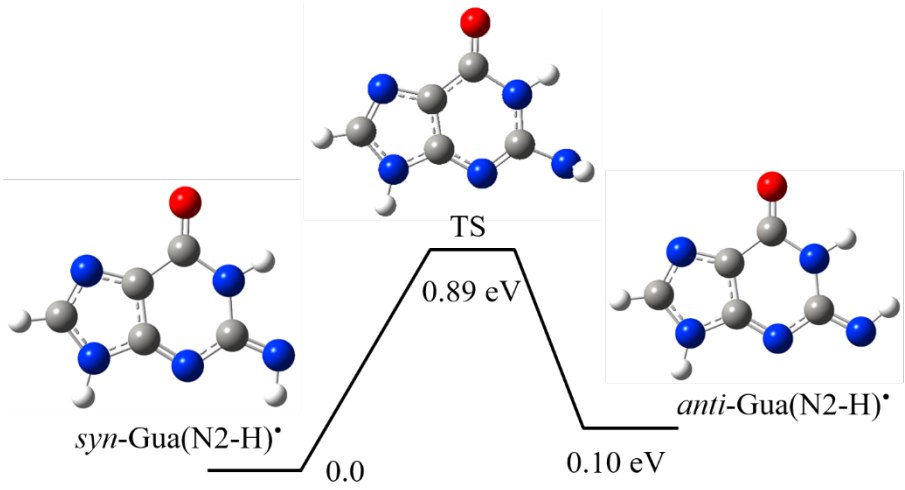


Figure S9. Potential energy profiles for the interconversion between *syn*-Gua(N2-H)•and *anti*-Gua(N2-H)• through the transition state(TS). The geometries were optimized and energies calculated using the DFT/B3LYP/6-311++G(d,p)//PCM level of calculation.

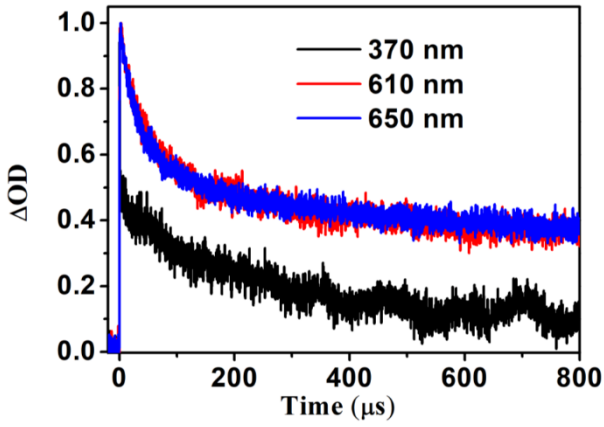


Figure S10. Normalized decay kinetics traces for the 370 nm, 610 nm and 650 nm bands obtained from the photosensitized photolysis of 1 (1mM).

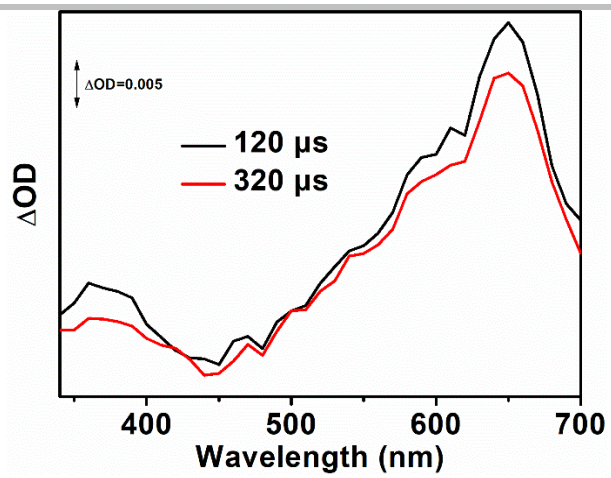
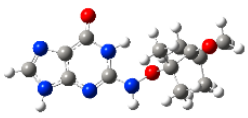


Figure S11. Transient UV-vis absorption spectra of 1 (1 mM) and acetophenone (30 mM) in aqueous buffer (pH 7.0) /acetonitrile (1:1, v:v) upon 355 nm laser flash photolysis in anaerobic conditions at 120 μs and 320 μs.

Table S2. Calculated absorption maxima and oscillator strengths (in parentheses[a]) of the transient species possibly involved, using the TD/B3LYP/6-311++G(d,p) method in water (SCRF=PCM) and in vacuum.

Radical	λ_{max}/nm in water	λ_{max} /nm in vacuum
 anti-Gua(N2-H).	556 (0.0938) 418 (0.0139) 344 (0.0513)	572 (0.0629) 402 (0.0146) 339 (0.0284)
 syn-Gua(N2-H).	572 (0.0901) 419 (0.0167) 345 (0.0532)	612 (0.0580) 403 (0.0188) 341 (0.0313)
 Gua(N1-H).	736 (0.0158) 454 (0.0291) 358 (0.0887)	818 (0.0103) 437 (0.0187) 349 (0.0515)
 triplet-9	380 (0.0192) 309 (0.0134)	
 10	364 (0.0105) 292 (0.0139) 267 (0.0212) 258 (0.1604) 254 (0.0193) 247 (0.0335)	

[a] Only oscillator strengths >0.01 are listed here

References

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[2] L. Zheng, M. Griesser, D. A. Pratt, M. M. Greenberg, *J. Org. Chem.* **2017**, *82*, 3571-3580.

[3] M. J. T. Frisch, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A. Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. .

Author Contributions

L. Zheng, M. M. Greenberg and H. Su concieved the project. L. Zheng designed and syntheiszed the photochemical precursor and performed product analyses. X. Dai carried out LFP studies and computational experiments. All authors wrote the original manuscript. All authors discussed results and revised the manuscript. L.Zheng and X. Dai contributed to the manuscript equally.