Supporting Information for

Site-specified Unnatural Base Excision via Visible Light

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Experimental Procedures

Materials. Primers and DNA sequences were synthesized in Shanghai Sangon Biotech. dNTPs and NTPs were purchased from New England Biolabs, dNaMTP and dTPT3TP were synthesized and purified in the laboratory, Deep Vent polymerase were purchased from New England Biolabs, One Taq DNA polymerase were purchased from New England Biolabs, $MgSO_4$ were purchased from Sigma, DTT were purchased from New England Biolabs, T7 RNA polymerase were purchased from New England Biolabs, RNase Inhibitor were purchased from Thermo Scientific.Chemical reagents were purchased from Sigma-Aldrich, Macklin Lab, and Aladdin Bio-Chem Technology. Cell free protein expression kit was purchased from GZL Bioscience.

Analytic methods. HRMS of all compounds were performed on Bruker compact Ultra-high-resolution electro-spray time-of-flight mass spectrometry. MS of nucleoside triphosphates were performed on Bruker Autoflex speed MALDLTOF/TOF spectrometry. MS analyses of ssDNA were customer performed by Shanghai Sangon Biotech. NMR spectra were performed on Bruker AVANCE III HD (600MHZ) or AVANCE NanoBay (400MHz) .

Photoreaction of natural and unnatural deoxynucleosides. Nucleosides were dissolved in 200 μL pH 7.4 PBS buffer to a final concentration of 1 mM**.** Then the solutions were subjected to light irradiation with or without RB in a RAYONET PRP200 photo reactor, and the light intensity was controlled at $26.4*10^{16}$ photons/sec/cm^3(or 16 light rods, the power of each rod is 14 watt). Then 1 μL sample solution was adsorbed and referred to spectral analysis.

DNA photoreaction. The DNA photoreactions were carried in solution and conditions described in main-manuscript, note that DNA reactions were carried out with 2 light rods at 520 nm (the power of each rod is 14 watt).

Spectral Analysis of the nucleoside concentrations. 1 μL solution was absorbed from the photo reaction mixture, and referred to 210-450 nm absorption spectra on Pultton P200+ Micro Volume Spectrophotometer. The concentrations of dTPT3 and dNAM were calculated

with the molar absorption coeffcient of 8659 $L*mol^{-1}*cm^{-1}$ and 1554 $L*mol^{-1}*cm^{-1}$ at 356 nm and 325 nm respectively. And the concentrations of dA, dT, dG, dC were calculated with Lambert-Beer Law Equation. (The molar absorption coeffcient of 15400 $L*mol^{-1}*cm^{-1}$, 8700 $L*mol^{-1}*cm^{-1}$, 11500 $L*mol^{-1}*cm^{-1}$ and 7400 $L*mol^{-1}*cm^{-1}$, respectively, at 260 nm.)

HPLC analysis. HPLC analyses of TPT3 deoxynucluoside were carried out on Thermol Ultimate 3000 system with a VWD detector at 356 nm. A thermol hypersil gold C18 column was used. Mobile phase A was 0.1 M TEAB buffer (pH 7.5), and mobile phase B was acetonitrile. During the analysis procedure, the flow rate was 1 mL min^{-1}, and mobile phase B was raised from 5% to 50% in 15 min.

Unnatural DNA PCR Amplification. OneTaq 2X Master Mix with standard buffer was purchased from New England Biolabs. Single strand unnatural base containing DNA templates and DNA primers were customer synthesized by Sangon biotechnology. During PCR amplification, equal volume of water was added to the Master Mix, then 100 μM dNAMTP and dTPT3PT together with the primers and templates were added to the mixture. 20 PCR cycles was used to control the fidelity.

Dansy probe labelling of DNA. 100 μg of UN63 and N63 were dissolved in 0.5 mL pH 7.4 PBS buffer respectively, followed by the addition of 1 μM RB and the exposure to 520 nm green light for 15 min. After that, 1 mM dansyl probe was added to the mixtures and further incubated at 37 ˚C for 60 min in dark. As treated reaction mixtures were extracted with 5 volumes of n-butanol to remove the unbonded fluorescence probe, then equal volume of 2-propanol was added to the mixtures in order to precipitate the DNA, which was washed with 2 volumes of ethanol, and finally dissolved in 100 μL pH 7.4 PBS buffer and referred to fluorescent imaging and gel electrophoresis.

Post electrophoresis double image experiment: 40 ng DNA samples were dissolved in 10 μL pH 7.4 PBS and treated at respectively conditions in the legend of Figure 3. Then samples were loaded onto 1 mm thick PAGE gel and electrophoresed at 200 V for 2 h. After that the gel was peeled out and directed imaged on 550 fluorescent emission. Then the same gel was stained in gelred solution (10 mg/L in 100 mM NaCl water solution) for 10 min, and referred

to normal DNA image.

DNA LC-MS analysis. DNA LC-MS analysis were custom performed in Shanghai Sangon Biotech.

Cell free protein expression of GFP. A GZL CF-EC-1000B cell free protein expression kit was employed. For GFP expression, 7.4 μL *E.coli* cell extract, 17.2 μL reaction mix, 1.5 mM NAMTP and TPT3TP were added to the 96 well white plate, then DNA samples were added and incubated at 30 ℃ for 6 h .

GFP expression sequence construction: Natural GFP coding sequence was PCR amplified from pRSETB-SFGFP plasmid with primers GFP-F and GFP-R. Then the resulting DNA was retrieved and PCR amplified with primers GYY-1 and GFP-R following the Unnatural DNA PCR Amplification procedure to construct the dTPT/dNAM containing GFP expression sequence.

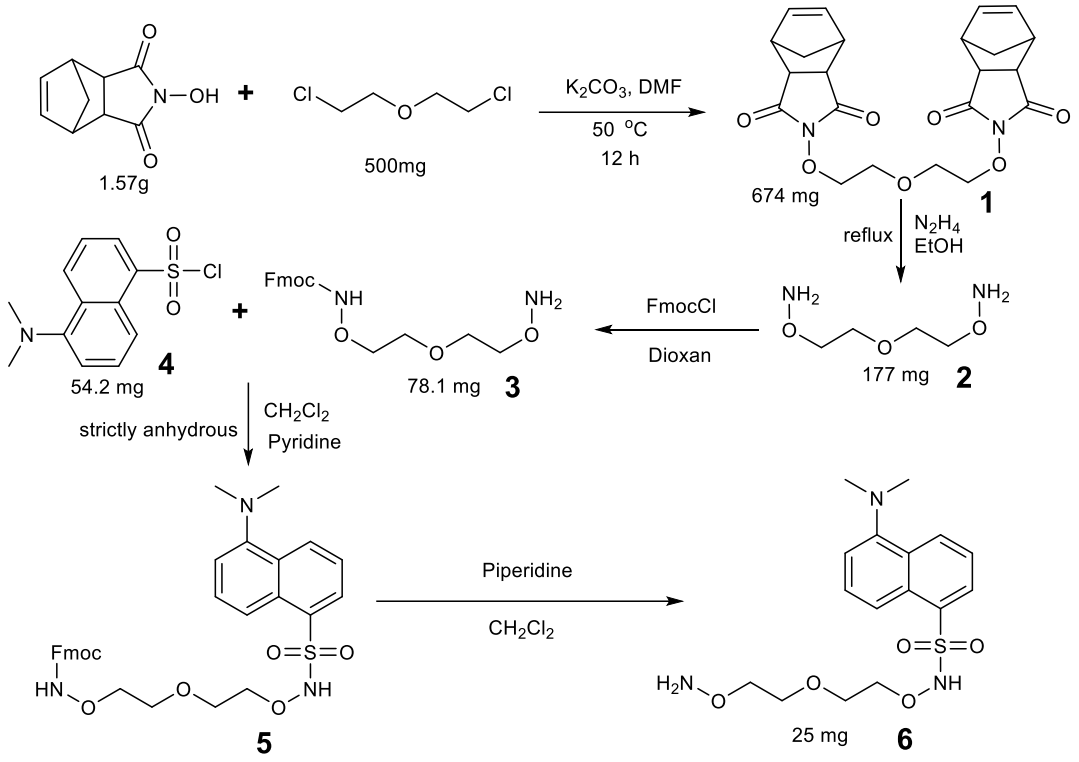
GFP fluorescence measurement. Fluorescence measurements were carried out on a PerkinElmer EnSpire Multimode Plate Reader. The sample temperature was maintained at 24 ˚C. All samples were excited at 395/488 nm wavelength, and fluorescence emission were detected at 509 nm wavelength.

Preparation of DNA templates containing UBP dTPT3-dNaM for transcription. To prepare dsDNA templates containing UBP dTPT3-dNaM for transcription experiment by PCR, 1 μM ssDNA template T1 containing an unnatural nucleobase dNaM (Table S1) was mixed with 0.3 mM each of dNTPs, 0.5 μM forward primers (Int20-F or Int40-F or Int60-F, Table S1), 0.5 μM lower primers (T1-R, Table S1), 1.2 mM $MgSO_4$, 5 μM dTPT3TP, 0.1 mM dNaMTP, 2.42 U/ml Deep Vent polymerase and 0.2 U/μl OneTaq DNA polymerase in 1× OneTaq DNA polymerase buffer. The PCR reaction was carried out with the following thermocycling program: 98 °C 2 min; 15 cycles of (91 °C, 30 s; 60 °C, 1 min; 68 °C, 4 min); 68 °C, 10 min.

T7 Transcription of templates containing UBP dTPT3-dNaM. For each T7 transcription reaction, 150 nM PCR product was mixed with 2 mM each of NTPs, 0.5 mM NaMTP, 10 mM

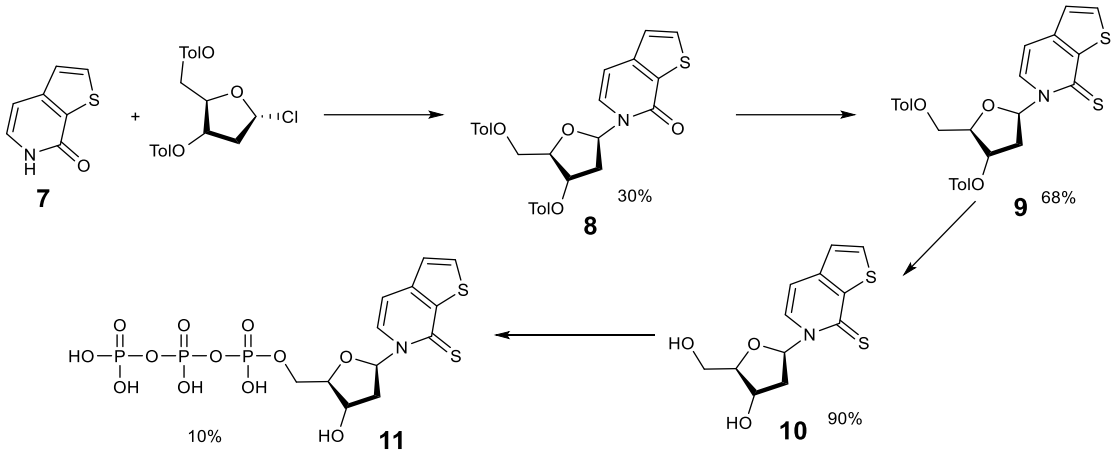
$MgCl_2$, 5 mM DTT, 1 U/μL RNase Inhibitor and 2.5 U/μL T7 RNA polymerase in 1× RNA Reaction buffer. The reaction was incubated at 37 °C for 1 h, and then incubated at 65 °C for 10 min to inactivate T7 RNA polymerase. The product was mixed with 0.2 U/μl DNase I in 1× DNase I Reaction buffer, and incubated at 37 °C for 4 hours to degrade the transcription template. To inactivate DNase I, the product was supplemented with 5 mM EDTA, and incubated at 65 °C for 10 min. The products were analyzed with 15% urea PAGE, stained with SYBR gold, and imaged using a gel imager.

Optical regulation of transcription mediated by photo-excision of unnatural nucleobase dTPT3. To confirm that the optical regulation of the transcription was mediated by the cleavage of dTPT3, rather than the affect of photo-excision reaction conditions on other parts of the DNA template, natural DNA template without any UBP in the sequence was also treated with the same photo-excision treatment, and subjected to a transcription reaction. Unnatural DNA T1 (with 60 bp interval between T7 promoter and UBP dTPT3-dNaM) or natural DNA T1-N were used as templates, and the transcription templates were first exposed to green light with a wavelength of 520 nm in the presence of 1 μM RB for 15 min to cleave the dTPT3 nucleobase off the template, purified with columns, and then subjected to T7 transcription reaction.



Scheme S1. Synthetic scheme of dansyl probe: *N*-(2-(2-(aminooxy)ethoxy)ethoxy)-5-(dimethylamino)naphthalene-1-sulfonamide.

***N*-(2-(2-(aminooxy)ethoxy)ethoxy)-5-(dimethylamino)naphthalene-1-sulfonamide**: The compound was synthesized based on literature as shown in Scheme 1.[5] Briefly, compound **2** was prepared from the commercially available 2,2-dichlorodiethylether and *N*-hydroxy-5-norbornene-2,3-dicarboximide. Compound **2** was then protected selectively with a fluorenylmethoxycarbonyl group (fmoc) by reacting with the chloroformate in excess in dioxan to afford the monoprotected compound **3**. Dansyl label was then introduced by reacting compound **3** with dansyl sulfonyl chlorides (compound **4**) in strictly anhydrous conditions to give compound **5**. Finally, the fmoc protection was removed by basic hydrolysis using piperidine in dichloromethane to afford the resulting probe **6** (yellow oil). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.60 (d, *J* = 8.5 Hz, 1H), 8.34 (d, *J* = 7.3 Hz, 1H), 8.27 (d, *J* = 8.6 Hz, 1H), 7.74 (br s, 1H), 7.62 – 7.48 (m, 2H), 7.19 (d, *J* = 7.5 Hz, 1H), 5.45 (br s, 2H), 4.09 – 4.02 (m, 2H), 3.85 – 3.71 (m, 2H), 3.66 – 3.61 (m, 2H), 3.60 – 3.53 (m, 2H), 2.89 (s, 6H). HRMS of compound **6,** $[M+H]^+$ calculated for 370.1437, found as 370.1431.

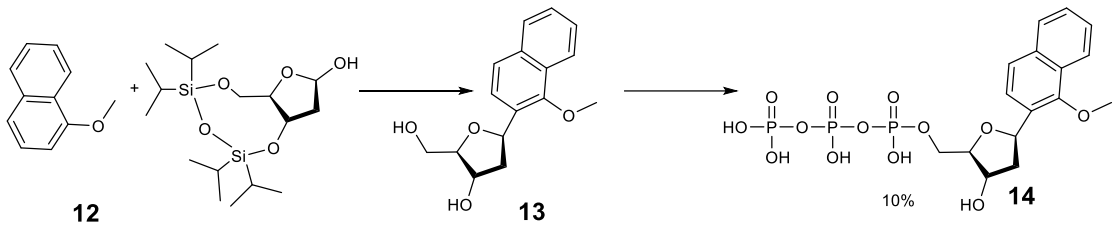


Scheme S2. Synthetic scheme of dTPT3 and dTPT3TP.

Synthesis of dTPT3 (10). dTPT3 and dTPT3TP were synthesized according to literature.[2] 400 mg of **7** was dissolved in 16 mL CH_2Cl_2 under nitrogen atmosphere, followed with the addition of 596mg bis(trimethylsilyl)acetamide. After stirring for 40 min, 1126 mg of 3,5-bis(toluoyl)-2-deoxyribosyl chloride was added and cooled to 0 °C, then 1320 μL of 1.0 M $SnCl_4$'s CH_2Cl_2 solution was added dropwise and stirred for 2 h at room temperature. 400 mg pure product of **8** was obtained after silica gel column chromatography (Hexane/EtOAc). 100 mg **8** was dissolved in 2 mL anhydrous toluene, followed by the addition of 96 mg Lawesson's reagent and heated overnight at reflux. 71 mg compound **9** was obtained after silica gel column chromatography (Hexane/EtOAc) as a yellow foam. 30 mg **9** was dissolved in 1.5 mL methanol and added with 30% NaOMe dropwise, the mixture was stirred for 1 h at room temperature. 15 mg compound **10** was obtained after silica gel column chromatography (MeOH/CH_2Cl_2). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.46 (d, J = 7.2 Hz, 1H), 8.01 (d, J = 5.3 Hz, 1H), 7.46 – 7.35 (m, 2H), 7.31 (d, J = 7.2 Hz, 1H), 4.49 – 4.45 (m, 1H), 4.10 (m, 1H), 3.95 (dd, J = 12.0, 3.4 Hz, 1H), 3.87 (dd, J = 12.2, 3.9 Hz, 1H), 2.77 (m, 1H), 2.24 – 2.10 (m, 1H). $[M+H]^+$ calculated for 284.0415, found as 284.0413.

Synthesis of dTPT3TP (11). Proton sponge (1.3 equiv) and **10** (1.0 equiv) were dissolved in dry trimethyl phosphate (40 equiv) and cooled to -15 °C under nitrogen atmosphere. Freshly distilled $POCl_3$ (1.3 equiv) was added dropwise and the resulting mixture was stirred at -10 °C for 2 h. Tributylamine (6.0 equiv) and a solution of tributylammonium pyrophosphate (5.0 eq.)

in dimethylformamide (0.5 M) were added. Over 30 min, the reaction was allowed to warm slowly to 0 °C and then was quenched by addition of 0.5 M aqueous $Et_3NH_2CO_3$ (TEAB) pH 7.5 (2 vol-equiv.). The mixture was diluted two-fold with H_2O and the product was isolated on a DEAE Sephadex column (GE Healthcare) with an elution gradient of 0 to 1.2 M TEAB, evaporated, and co-distilled with H_2O (3×). Additional purification by reverse-phase (C18) HPLC (0 - 35% CH_3CN in 0.1 M TEAB, pH 7.5) was performed, (10% – 31% yield). ^{31}P NMR (243 MHz, D_2O) δ -10.74 (d, *J* = 20.9 Hz), -11.23 (d, *J* = 20.1 Hz), -23.21 (t, *J* = 17.9 Hz). HRMS $[M-H]^-$ calculated for 521.9249, found as 521.9246.

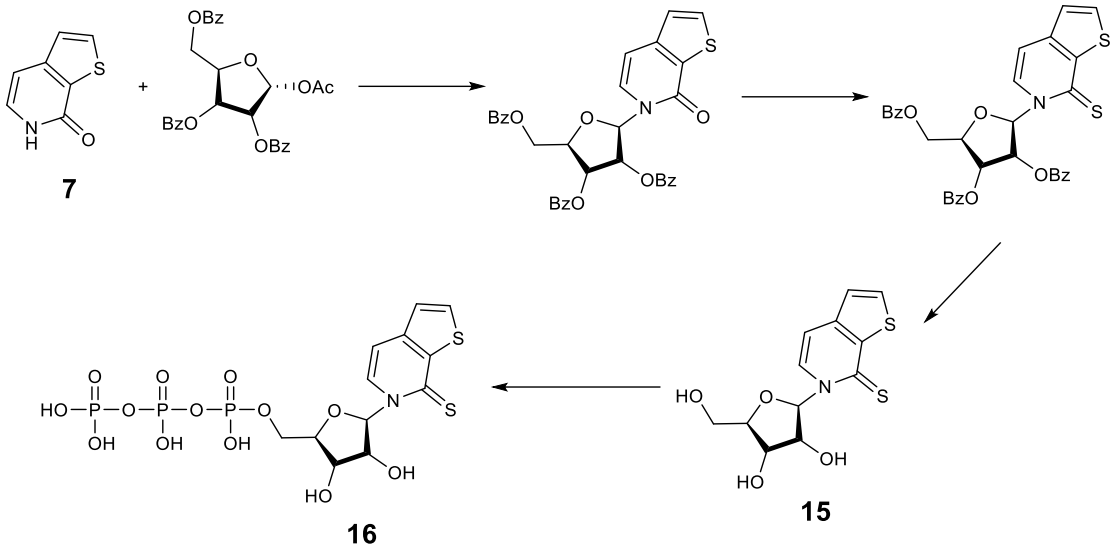


Scheme S3. Synthetic scheme of dNAM and dNAMTP.

Synthesis of dNAM (13). dNAM and dNAMTP were synthesized according to literature.[3] 700mg of 2-methoxy naphatalene was dissolved in 10 mL THF and stirred under nitrogen atmosphere at room temperature for 30 minutes, after which 2.7 mL of 1.6 M *n*-BuLi was added dropwise and stirred for 2 h. After cooling to -10 °C, 3,5-O-[1,1,3,3-tetrakis(1-methylethyl)-1,3-disiloxanediyl] 2-deoxy-D-erythro-pent-1-enitol (537 mg, 1.43 mmol) was added and the temperature was increased slowly to room temperature and further stirred for 30 min. The reaction mixture was added with 50 mL saturated NH_4Cl aq, and extracted with 150 mL EtOAc, the residue was purified by silica gel column chromatography (EtOAc/hexane). The eluted product was dissolved in 3 mL THF and added with 87 μL tri-n-butyl phosphin. The resulting solution was cooled to 0 °C, and added with 60 mg *N*,*N*,*N*',*N*'-tetramethylazodicarboxamide, The solution was stirred overnight at room temperature. 0.45 mL tetrabutylammoniumfluoride was added to this mixture and stirred for 3 h at room temperature. The reaction mixture was added with 30 mL saturated $NaHCO_3$ aq and extracted with 100 mL EtOAc. 86 mg **13** was obtained after silica gel column chromatography (EtOAc/hexane) as a white solid. ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.00 (s, 1H), 7.77 (m, 2H), 7.39 (m, 1H), 7.36 – 7.26 (m, 1H), 7.22 (s, 1H), 5.51 (m, 1H), 4.38 – 4.31 (m, 1H), 4.13 – 4.00 (m, 1H), 3.95 (s, 3H), 3.84 – 3.67 (m, 2H), 2.55 – 2.42 (m, 1H), 1.87 (m, 1H). $[M+H]^+$ calculated for 275.1283, found as 275.1279.

Synthesis of dNAMTP (14). Proton sponge (1.3 equiv) and **13** (1.0 equiv) were dissolved in dry trimethyl phosphate (40 equiv) and cooled to -15 °C under nitrogen atmosphere. Freshly distilled $POCl_3$ (1.3 equiv) was added dropwise and the resulting mixture was stirred at -10 °C for 2 h. Tributylamine (6.0 equiv) and a solution of tributylammonium pyrophosphate (5.0 eq.) in dimethylformamide (0.5 M) were added. Over 30 min, the reaction was allowed to

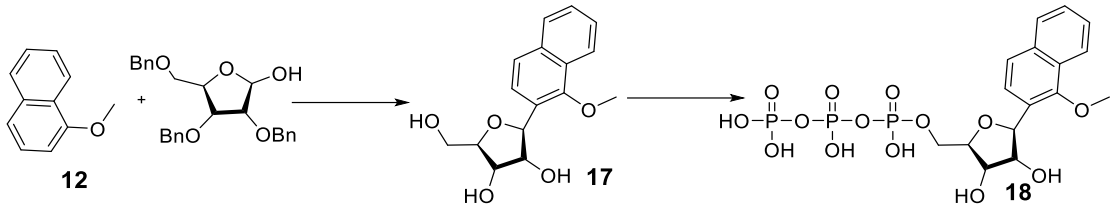
warm slowly to 0 °C and then was quenched by addition of 0.5 M aqueous $Et_3NH_2CO_3$ (TEAB) pH 7.5 (2 vol-equiv.). The mixture was diluted two-fold with H_2O and the product was isolated on a DEAE Sephadex column (GE Healthcare) with an elution gradient of 0 to 1.2 M TEAB, evaporated, and co-distilled with H_2O (3×). Additional purification by reverse-phase (C18) HPLC (0 - 35% CH_3CN in 0.1 M TEAB, pH 7.5) was performed, (10% – 31% yield). ^{31}P NMR (243 MHz, D_2O) δ -10.90 (s), -11.21 (s), -23.21 (s). HRMS $[M-H]^-$ calculated for 513.0117, found as 513.0003.



Scheme S4. Synthetic scheme of TPT3TP.

Synthesis of TPT3TP (16). TPT3TP was synthesized according to literature.[4] A mixture of **7** (1.0 eq) and N,O-bis(trimethylsilyl)acetamide (1.2 eq) in acetonitrile to 0.1 M was stirred under argon atmosphere at room temperature for 30 min. At this time, 1-O-acetyl-2,3,5-tri-O-benzoyl-ß-D-ribofuranose (1.2 eq) was added, and the resulting reaction mixture as cooled to 0 °C. $SnCl_4$ (1.0 eq) was then added to the reaction mixture and the solution was stirred overnight at room temperature. The reaction was extracted with ethyl acetate and saturated $NaHCO_3$ aq, and combined organic layers were dried over anhydrous Na_2SO_4. After filtration and evaporation, the residue was purified by flash chromatography on silica gel. Pyridone (1.0 eq) was dried by repeated coevaporation with dry toluene. Lawesson's reagent (3.0 eq) was added under argon, and the mixture was heated overnight at 120 °C. After filtration on cotton, the filtrate was concentrated, and the crude product was purified by flash chromatography on silica gel. The product dissolved in CH_2Cl_2 and added with 30% NaOMe dropwise, the mixture was stirred for 1 h at room temperature. Compound **15** was obtained after silica gel column chromatography (MeOH/CH_2Cl_2). Proton sponge (1.3 equiv) and **13** (1.0 equiv) were dissolved in dry trimethyl phosphate (40 equiv) and cooled to -15 °C under nitrogen atmosphere. Freshly distilled $POCl_3$ (1.3 equiv) was added dropwise and the resulting mixture was stirred at -10 °C for 2 h. Tributylamine (6.0 equiv) and a solution of tributylammonium pyrophosphate (5.0 eq.) in dimethylformamide (0.5 M) were

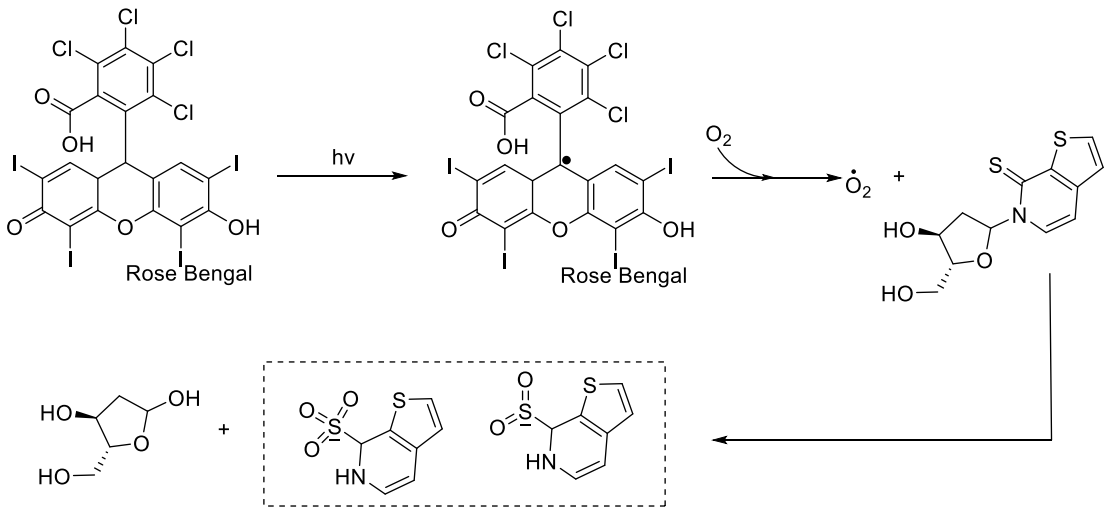
added. Over 30 min, the reaction was allowed to warm slowly to 0 °C and then was quenched by addition of 0.5 M aqueous $Et_3NH_2CO_3$ (TEAB) pH 7.5 (2 vol-equiv.). The mixture was diluted two-fold with H_2O and the product was isolated on a DEAE Sephadex column (GE Healthcare) with an elution gradient of 0 to 1.2 M TEAB, evaporated, and co-distilled with H_2O (3×). Additional purification by reverse-phase (C18) HPLC (0 - 35% CH_3CN in 0.1 M TEAB, pH 7.5) was performed, (10% – 31% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.59 (d, *J* = 7.3 Hz, 1H), 7.99 (d, *J* = 5.2 Hz, 1H), 7.38 (d, *J* = 5.2 Hz, 1H), 7.26 (d, *J* = 7.2 Hz, 1H), 7.03 (s, 1H), 4.36 – 4.15 (m, 3H), 4.05 (d, *J* = 12.5 Hz, 1H), 3.88 (d, *J* = 12.6 Hz, 1H). ^{31}P NMR (243 MHz, Deuterium Oxide) δ -3.74 – -6.98 (m), -8.48 – -11.95 (m), -18.32 – -23.60 (m). HRMS $[M-H]^-$ calculated for 537.9198, found as 537.9193.



Scheme S5. Synthetic scheme of NAMTP.

Synthesis of TPT3TP (18). NAMTP was synthesized according to literature.[4] 151 mg **12** was dissolved in 1 mL THF and cooled to -40 °C. Then, 0.6 mL of 1.6 M *n*-BuLi was added dropwise under nitrogen purge. The solution was heated to room temperature and stirred for 3 h. After cooling to -10 °C, 200 mg of 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone was added, heated to room temperature, and stirred for 1 h. After adding 50 mL saturated NH_4Cl aq to this solution, the mixture was extracted with 150 mL EtOAc. The crude product was dissolved in acetonitrile (10 mL) under argon and cooled to -40 °C, followed by the addition of 110 mg triethylsilane and 74 mg boron trifluoride diethyl etherate dropwise. After stirring 1 h at -40 °C, the temperature was increased to 0 °C. 50 mL saturated $NaHCO_3$ aq was added to this solution and extracted with 100 mL diethyl ether. Crude residue was dissolved in 10 mL CH_2Cl_2 and cooled to -78 °C under argon. 0.08 mL BBr_3 was added to this solution and stirred for 5 h, and then the reaction was quenched with the addition of 1 mL pyridine and evaporated dried. 20.7 mg **17** was obtained after silica gel column chromatography (EtOH/MC) as white foam. Proton sponge (1.3 equiv) and **17** (1.0 equiv) were dissolved in dry trimethyl phosphate (40 equiv) and cooled to -15 °C under nitrogen atmosphere. Freshly distilled $POCl_3$ (1.3 equiv) was added dropwise and the resulting mixture was stirred at -10 °C for 2 h. Tributylamine (6.0 equiv) and a solution of tributylammonium pyrophosphate (5.0 eq.) in dimethylformamide (0.5 M) were added. Over 30 min, the reaction was allowed to warm slowly to 0 °C and then was quenched by addition of 0.5 M aqueous $Et_3NH_2CO_3$ (TEAB) pH 7.5 (2 vol-equiv.). The mixture was diluted two-fold with H_2O and the product was isolated on a DEAE Sephadex column (GE Healthcare) with an elution gradient of 0 to 1.2 M TEAB, evaporated, and co-distilled with H_2O (3×). Additional purification by reverse-phase (C18) HPLC (0 - 35% CH_3CN in 0.1 M TEAB, pH 7.5) was performed, (10% – 31% yield). ^1H

NMR (400 MHz, Methanol-d_4) δ 7.97 (s, 1H), 7.80 – 7.70 (m, 2H), 7.38 (m, 1H), 7.29 (m, 1H), 7.21 (s, 1H), 5.41 (d, J = 3.0 Hz,1H), 4.43 (m,1H), 4.33 (m, 1H), 4.08 (m, 1H), 3.95 (s, 3H), 3.72 – 3.82 (m, 2H). ^{31}P NMR (243 MHz, Deuterium Oxide) δ -11.24 (d, J = 19.3 Hz), -23.18 – -23.63 (m), -23.73 – -23.94 (m). HRMS $[M-H]^-$ calculated for 529.0066, found as 529.0058.



Scheme S6. Photo reaction mechanism of dTPT3.

Isolation and characterization of photo reaction product. 20 mg dTPT3 were dissolved in 3.5 mL pH 7.4 PBS buffer, then the solutions were subjected to light irradiation with 0.1 mM RB in a RAYONET PRP200 photo reactor for 60 min. Then the sample solution was vacuum dried and referred to TLC (MeOH/CH_2Cl_2, stained with phosphomolybdic acid). The resulting product was vacuum dried and dissolved in D_2O for NMR and HRMS analysis. TLC retention, ^1H NMR spectrum and MS of the photoreaction product complied with deoxyribose. HRMS $[M+H]^+$ calculated for 135.0657, found as 135.0649.

Table S1 Primers

Name	Sequence (5' to 3')	Remarks
UN63 A strand	CTCGAGTACAACTTTAACTCACACAATGTAA/TPT3/CATCACGGCAGACAAACAAAAGAATGGAATC	Double strand sequence of the 63 bp unnatural DNA
UN63 B strand	GATTCCATTCTTTTGTTTGTCTGCCGTGATG/NAM/TTACATTGTGTGAGTTAAAGTTGTACTCGAG	
N63 A strand	CTCGAGTACAACTTTAACTCACACAATGTAAACATCACGGCAGACAAACAAAAGAATGGAATC	Double strand sequence of the 63 bp natural DNA
N63 B strand	GATTCCATTCTTTTGTTTGTCTGCCGTGATGTTTACATTGTGTGAGTTAAAGTTGTACTCGAG	
YZ351	CTCGAGTACAACTTTAACTCACAC	Primers used for PCR of N63 and UN63
YZ352	GATTCCATTCTTTTGTTTGTCTGC	
GYY-1	AATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGT/NAM/TAACTTTAAGAAGGAGATATACATATGGTGAGCAAGGGCGAGGAGCTGTTC	Primer used for the construction of GFP expression sequence
GFP-R	AGCAAAAAACCCCTCAAGACCCGTT	GFP coding sequence amplification
GFP-F	ATGGTGAGCAAGGGCGAGGAGCTGTTC	

Table S2. Oligonucleotides used for transcription

Name	Sequence
T1	5’-AAACAGCTATGACGAATTCAGTGTGGAGAGAXGTAGTTAAA CAGGAAACAGGGATCGGGCGGA-3’
Int20-F	5'-ATAATACGACTCACTATAGGGCTATGACGAATTCAGTGTGG-3'
Int40-F	5'-ATAATACGACTCACTATAGGGGTTATCCACACAGGAAACAGC TATGACGAATTCAGTGTGG-3'
Int60-F	5'-ATAATACGACTCACTATAGGGACTGTTTCCTGTGTGAAATTG TTATCCACACAGGAAACAGCTATGACGAATTCAGTGTGG-3'
T1-R	5'-TCCGCCCGATCCCTGTTTCCTG-3'
T1-N	5'-CTATGACGAATTCAGTGTGGAGAGATGTAGTTAAACAGGAA ACAGGGATCGGGCGGA-3'

X=dNaM

GFP expression DNA sequence:

AATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGT/NAM/TAACTTT

AAGAAGGAGATATACATATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT

CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGAGAGGCGAGGGCGAGGGCGATGCCAC

CAATGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCG

TGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCGCCACGACTTC

TTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCAGTTTCAAGGACGACGGCACAT

ACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCA

TCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACGT

CTATATCACGGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACGTGGAG

GACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGC

TGCCCGACAACCACTACCTGAGCACCCAGTCCGTGCTGAGCAAAGACCCCAACGAGAAGCGCGATCA

CATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCACGGCATGGACGAGCTGTACAAGTAAG

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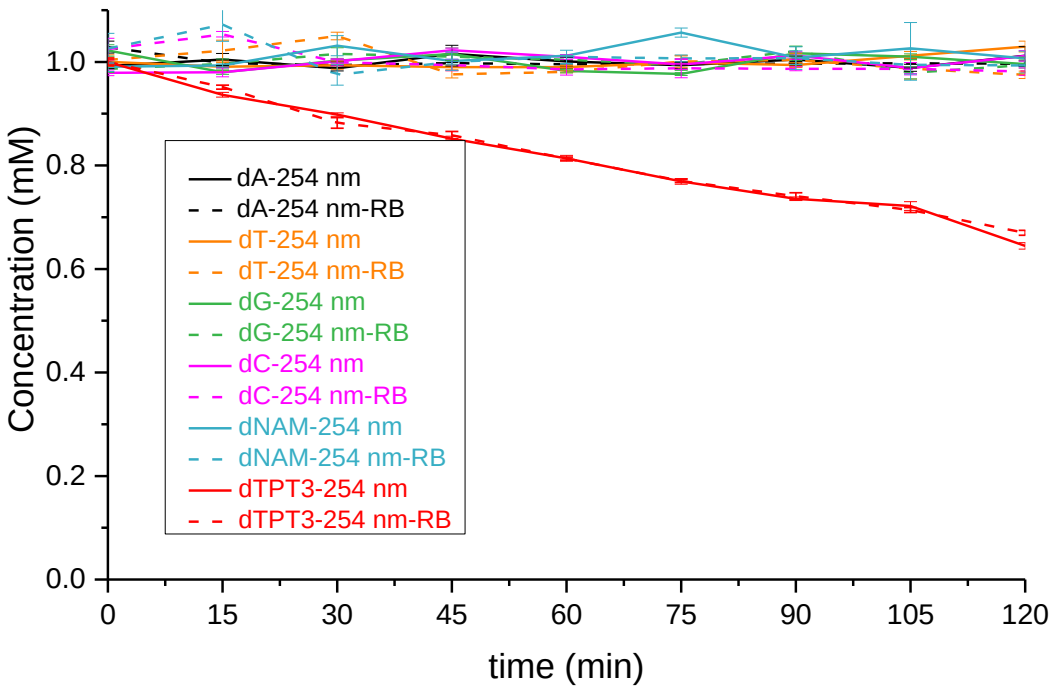

Figure S1. Photo activity of natural and unnatural deoxynecluosides at 254 nm. The concentration changing dynamic of dA, dT, dG, dC, dNAM and dTPT3 upon 254 nm light with or without RB. Nucleoside concentration were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

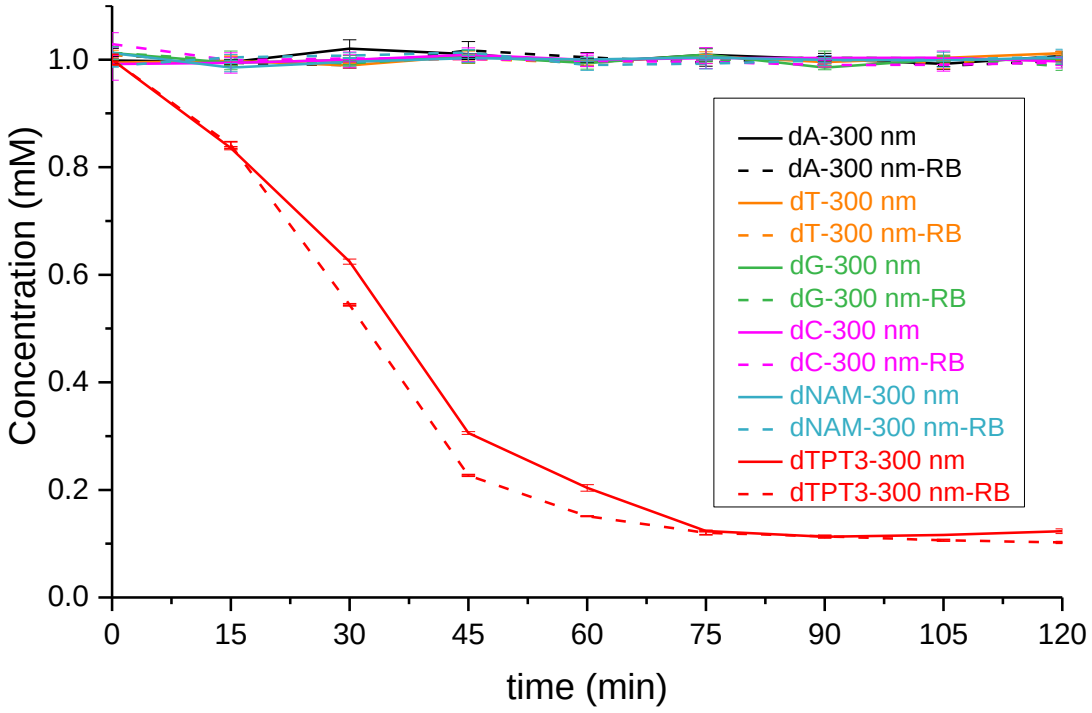

Figure S2. Photo activity of natural and unnatural deoxynecluosides at 300 nm. The concentration changing dynamic of dA, dT, dG, dC, dNAM and dTPT3 upon 300 nm light with or without RB. Nucleoside concentration were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

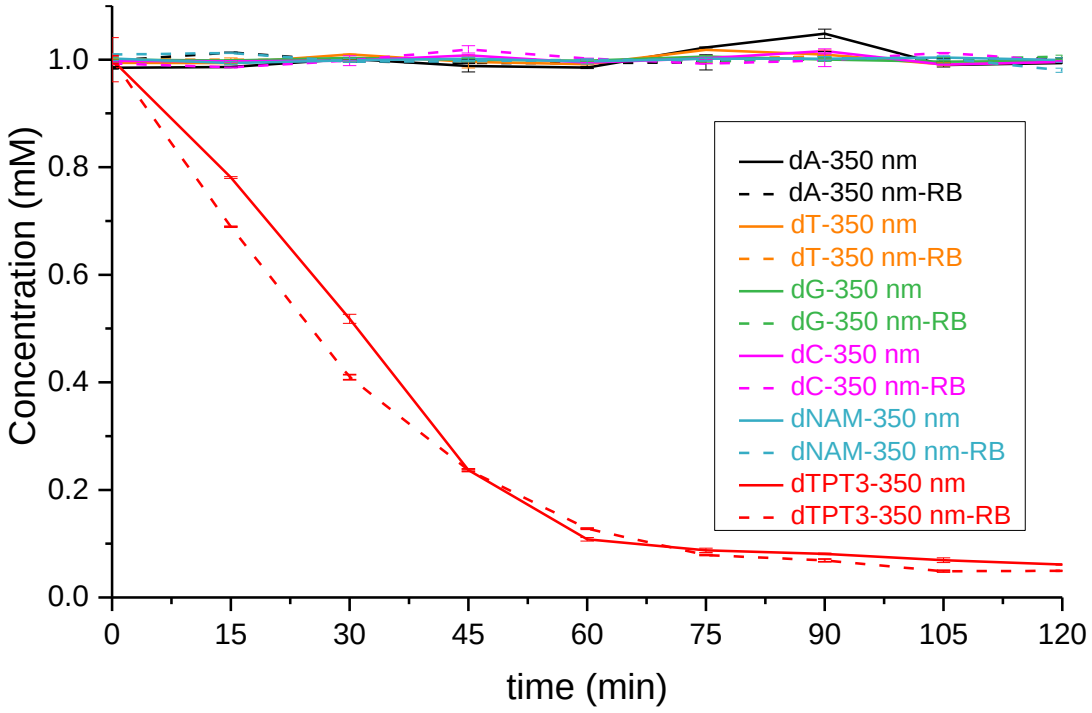

Figure S3. Photo activity of natural and unnatural deoxynecluosides at 350 nm. The concentration changing dynamic of dA, dT, dG, dC, dNAM and dTPT3 upon 350 nm light with or without RB. Nucleoside concentration were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

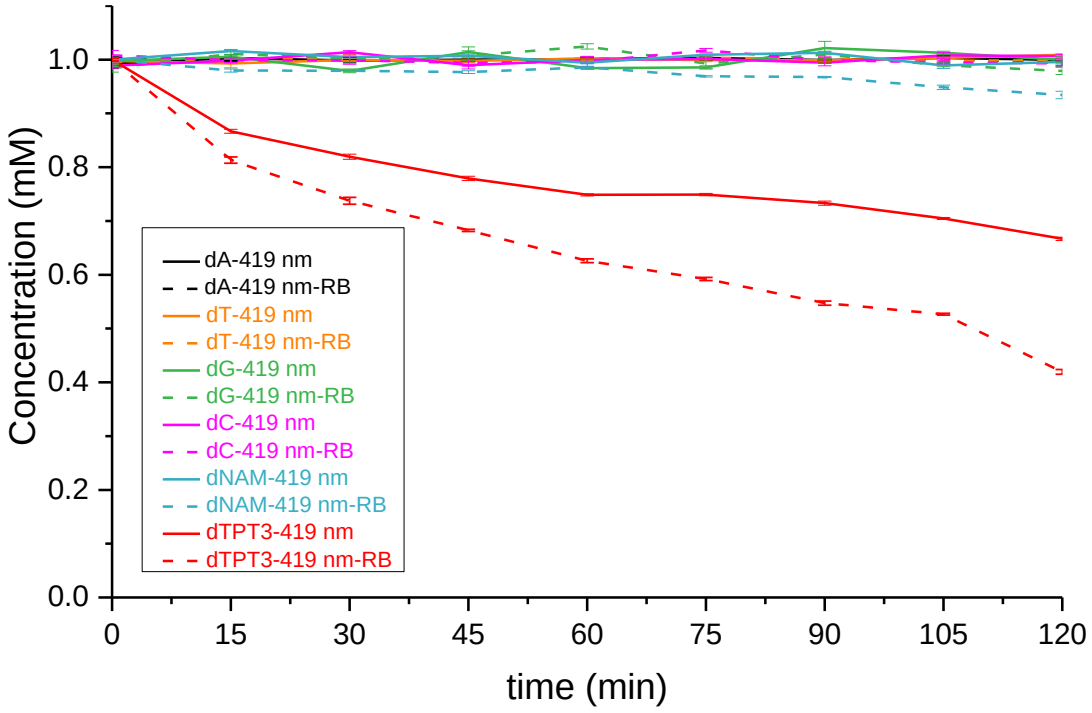

Figure S4. Photo activity of natural and unnatural deoxynecluosides at 419 nm. The concentration changing dynamic of dA, dT, dG, dC, dNAM and dTPT3 upon 419 nm light with or without RB. Nucleoside concentration were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

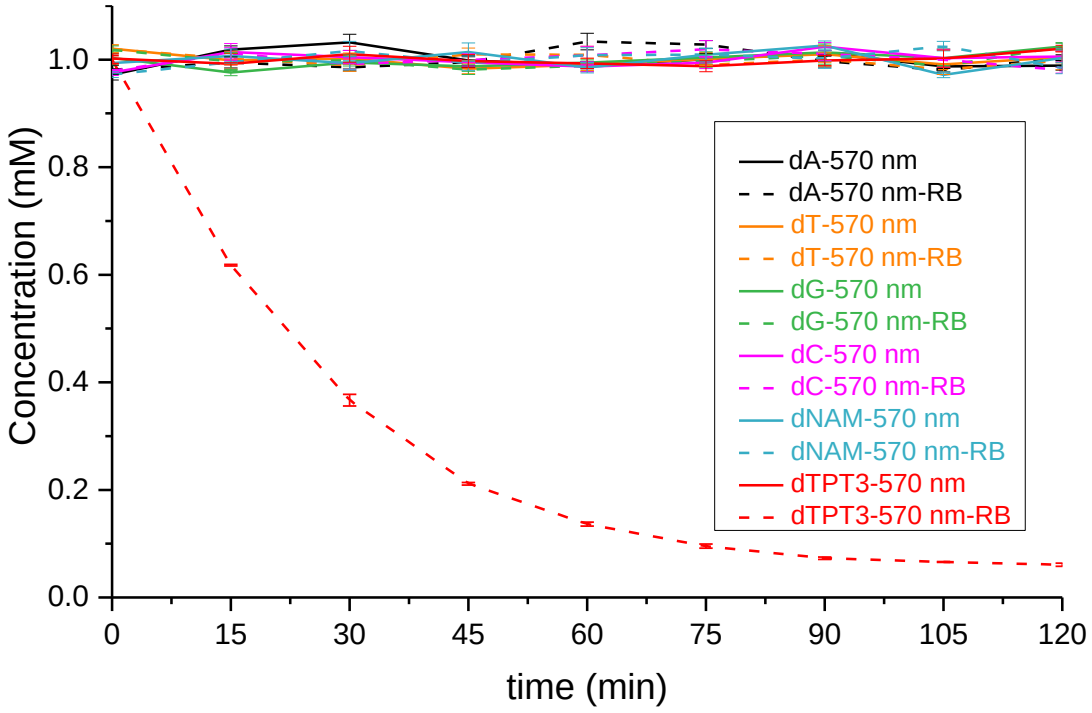

Figure S5. Photo activity of natural and unnatural deoxynecluosides at 570 nm. The concentration changing dynamic of dA, dT, dG, dC, dNAM and dTPT3 upon 570 nm light with or without RB. Nucleoside concentration were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

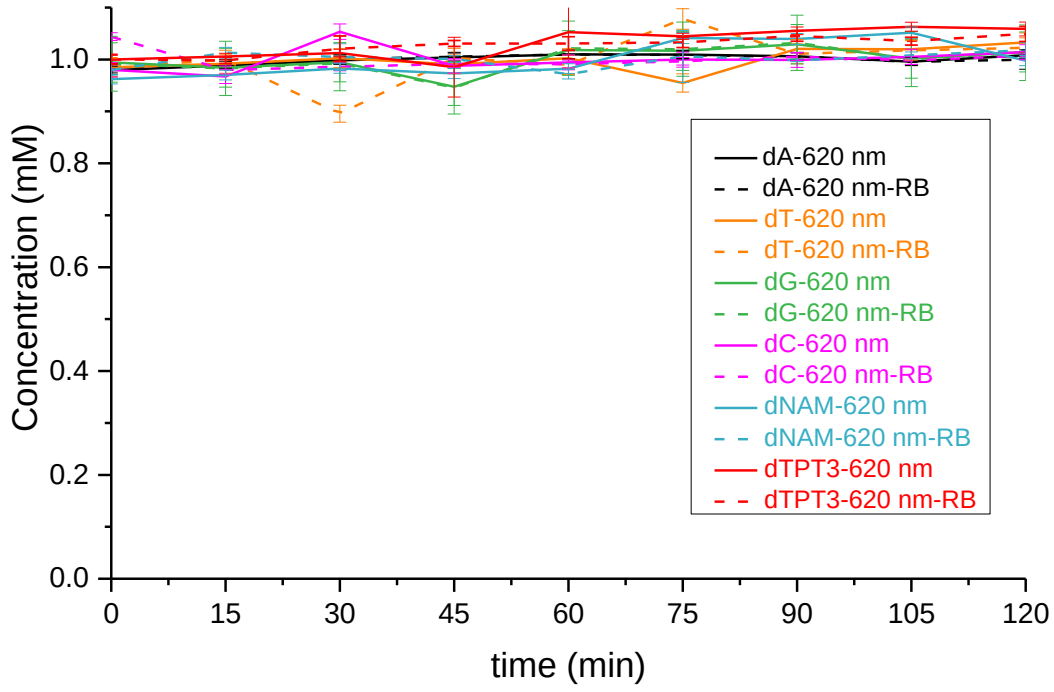

Figure S6. Photo activity of natural and unnatural deoxynecluosides at 620 nm. The concentration changing dynamic of dA, dT, dG, dC, dNAM and dTPT3 upon 620 nm light with or without RB. Nucleoside concentration were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

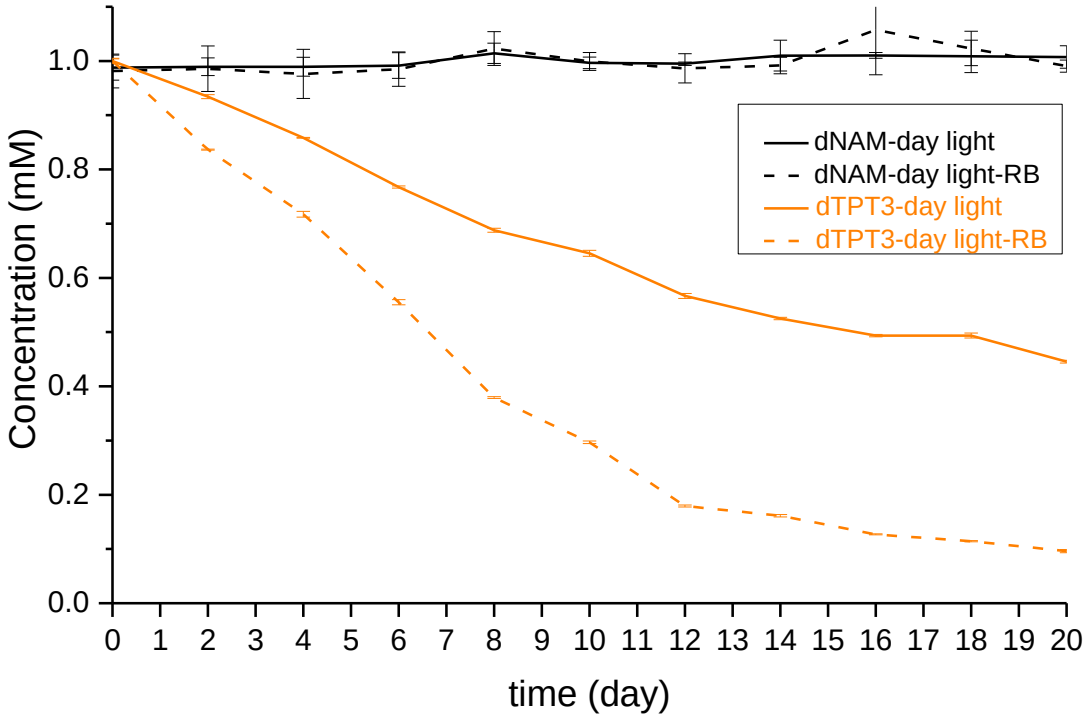

Figure S7. Photo activity of unnatural deoxynecluosides at day light. The concentration changing dynamic of dTPT3 upon daylight with or without RB. Nucleoside concentration were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

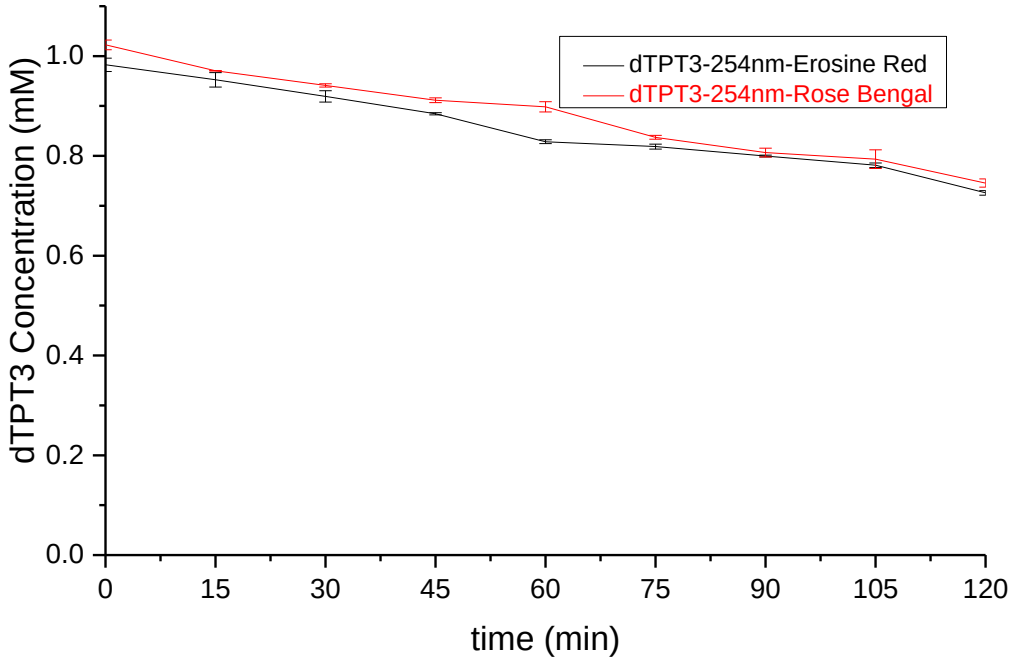

Figure S8. Photosensitizers comparison at 254 nm. dTPT3 photo degradation at 254 nm with 10 μM Rose Bengal and Erosine Red respectively as photosensitizers. The concentration of dTPT3 were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

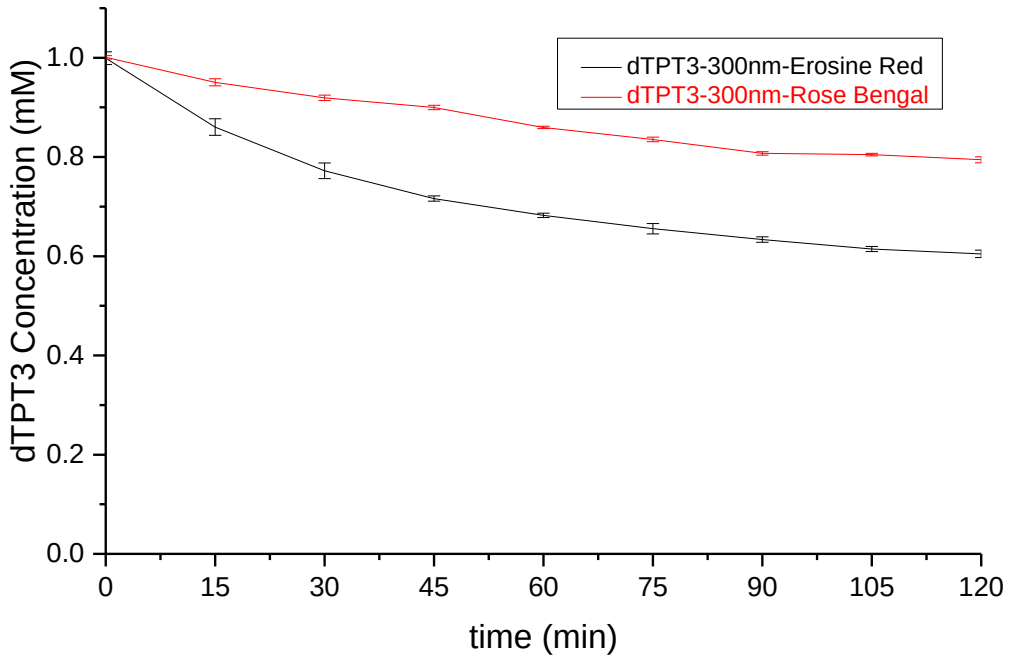

Figure S9. Photosensitizers comparison at 300 nm. dTPT3 photo degradation at 254 nm with 10 μM Rose Bengal and Erosine Red respectively as photosensitizers. The concentration of dTPT3 were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

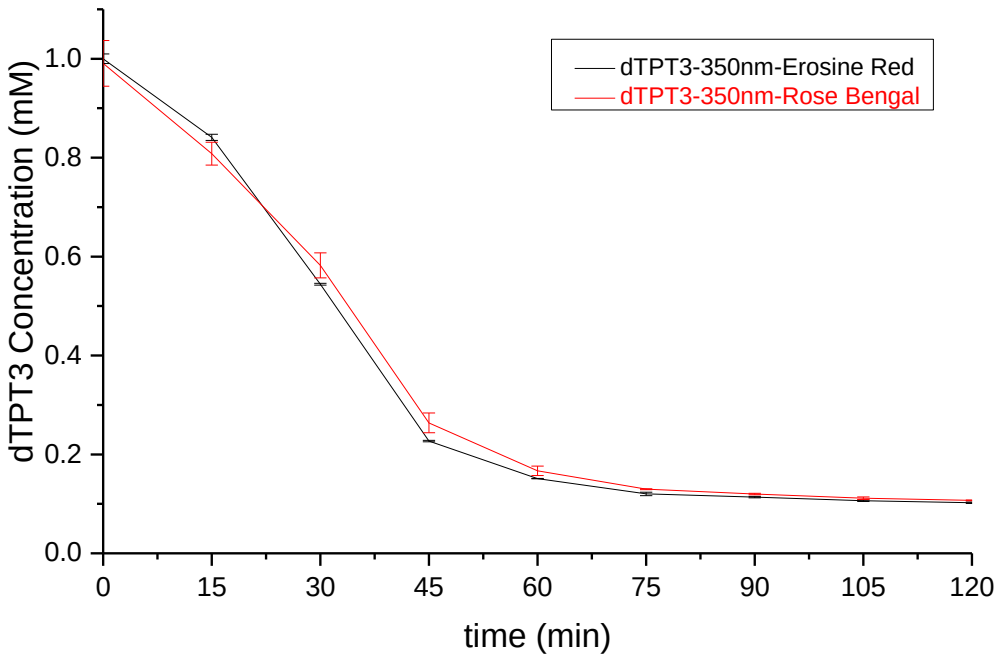

Figure S10. Photosensitizers comparison at 350 nm. dTPT3 photo degradation at 254 nm with 10 μM Rose Bengal and Erosine Red respectively as photosensitizers. The concentration of dTPT3 were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

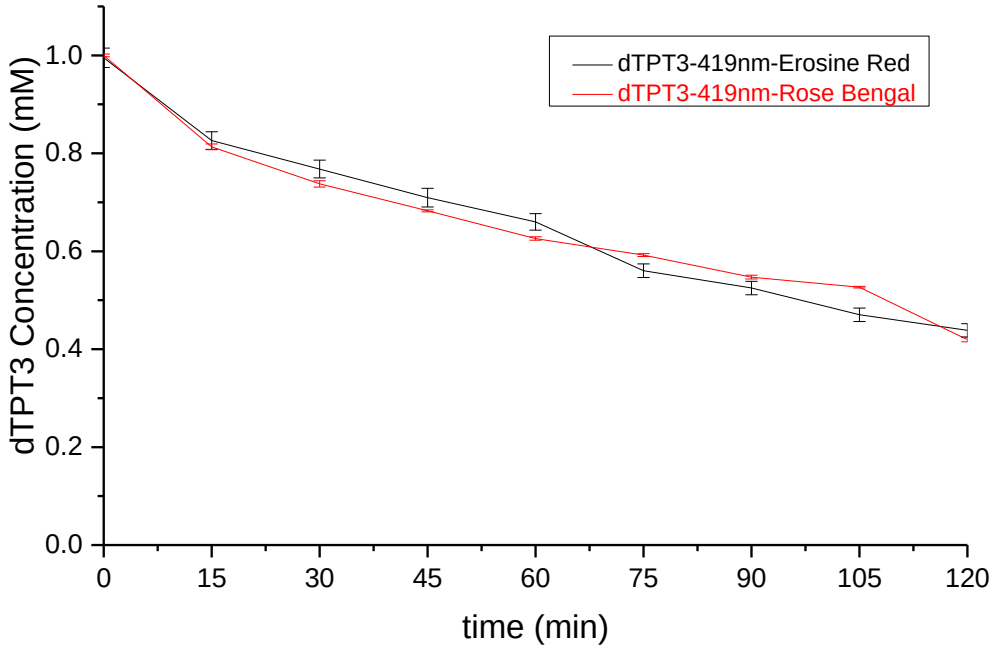

Figure S11. Photosensitizers comparison at 419 nm. dTPT3 photo degradation at 254 nm with 10 μM Rose Bengal and Erosine Red respectively as photosensitizers. The concentration of dTPT3 were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

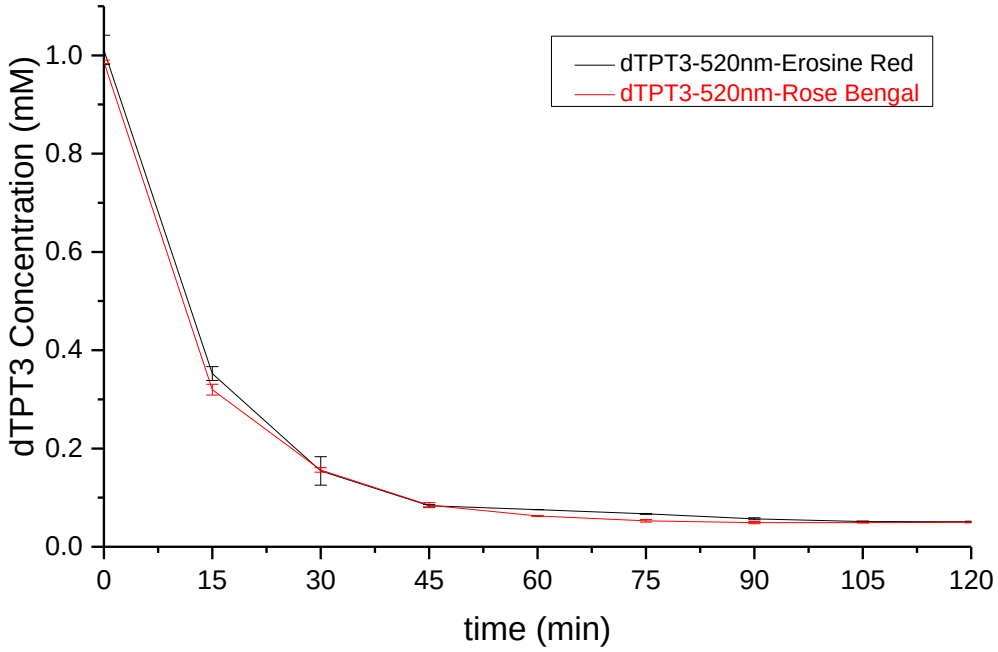

Figure S12. Photosensitizers comparison at 520 nm. dTPT3 photo degradation at 254 nm with 10 μM Rose Bengal and Erosine Red respectively as photosensitizers. The concentration of dTPT3 were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

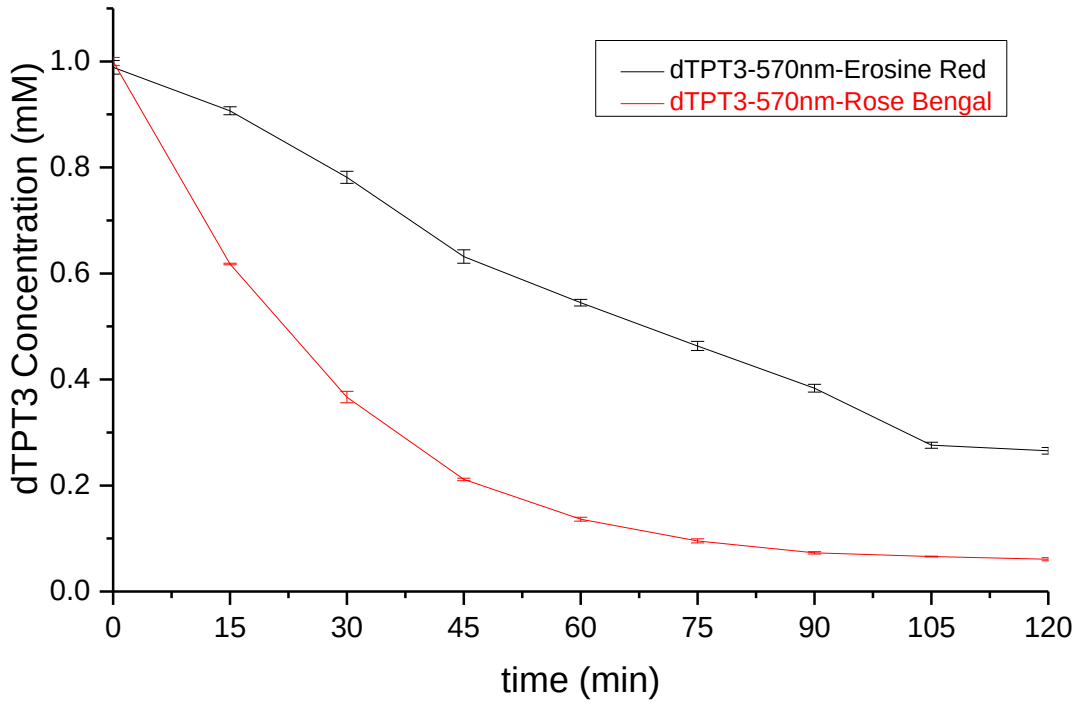

Figure S13. Photosensitizers comparison at 570 nm. dTPT3 photo degradation at 254 nm with 10 μM Rose Bengal and Erosine Red respectively as photosensitizers. The concentration of dTPT3 were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

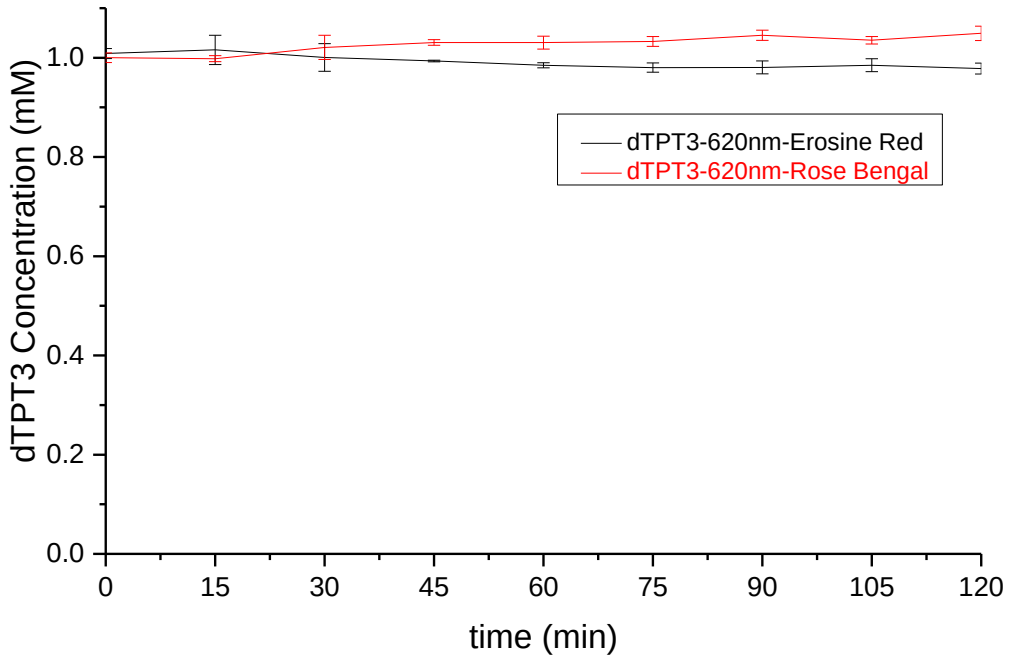

Figure S14. Photosensitizers comparison at 620 nm. dTPT3 photo degradation at 254 nm with 10 μM Rose Bengal and Erosine Red respectively as photosensitizers. The concentration of dTPT3 were measured with Micro Volume Spectrophotometer and calculated with Lambert-Beer Law Equation.

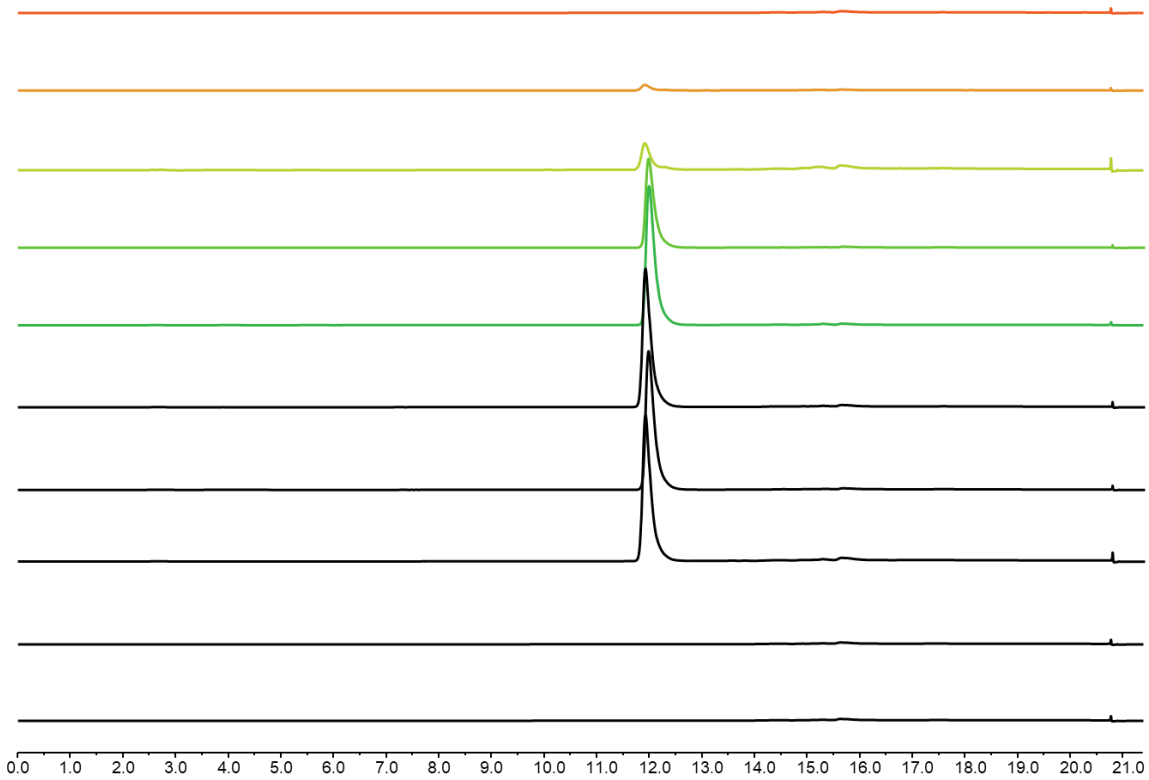

Figure S15. HPLC analysis of dTPT3 photo cleavage at 325 nm.

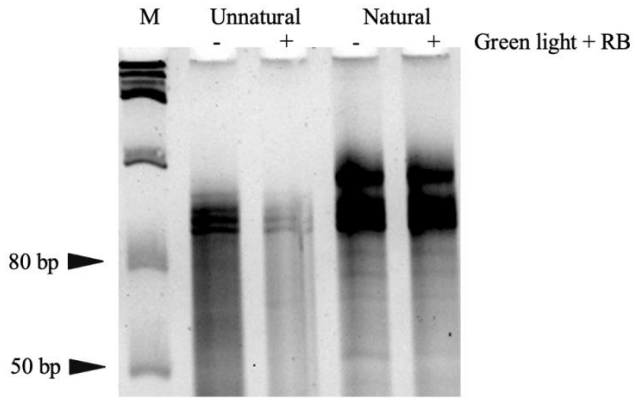

Figure S16. Optical regulation of T7 transcription by photo-excision of dTPT3. Unnatural: the UBP-containing transcription products; Natural: transcription products without UBP; RB: Rose Bengal; Green light: light with a wavelength of 520 nm.

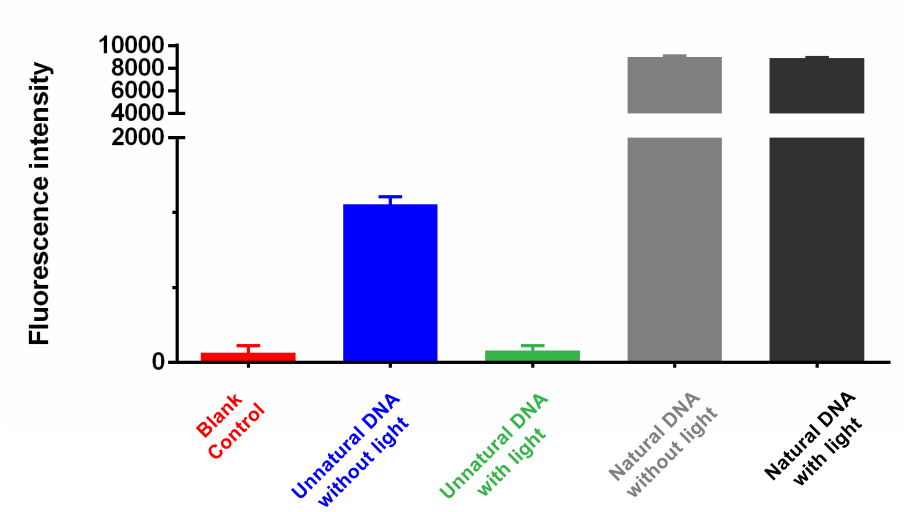

Figure S17. Fluorescence measurement of GFP, 395 nm excitation wavelength and 509 nm emission wavelength were employed. All samples were repeated for three times.

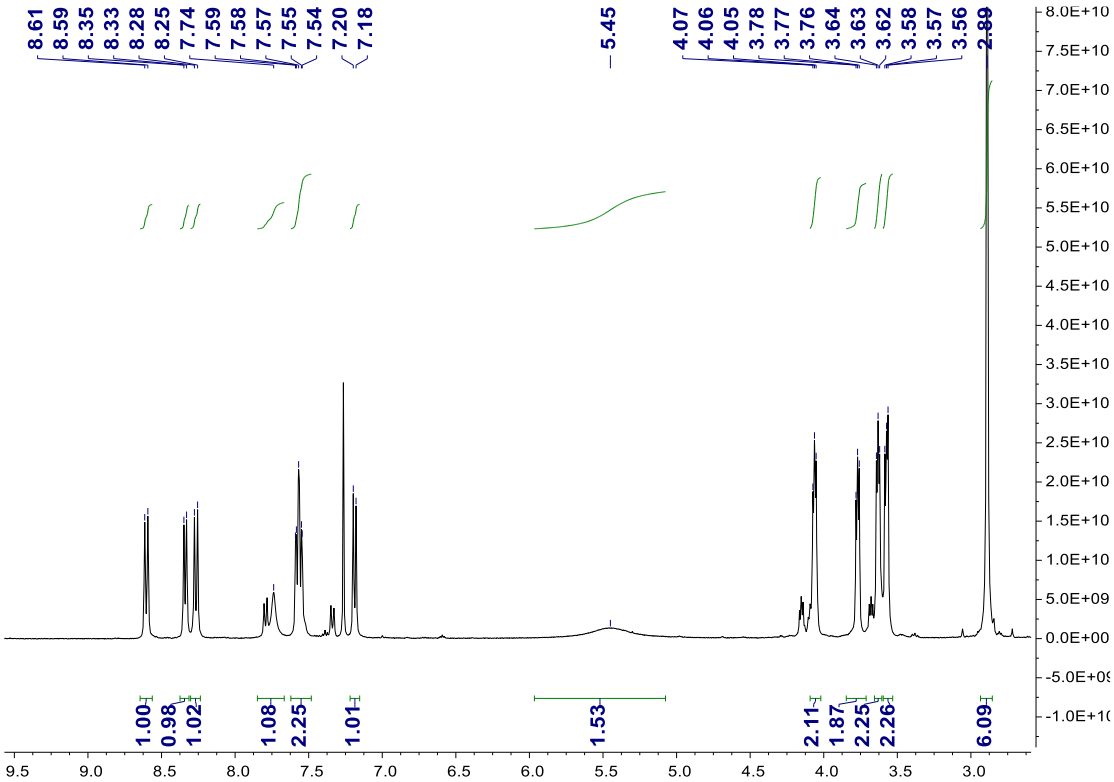

Figure S18. 1H-NMR of dansyl probe (**6**):
N-(2-(2-(aminooxy)ethoxy)ethoxy)-5-(dimethylamino)naphthalene-1-sulfonamide.

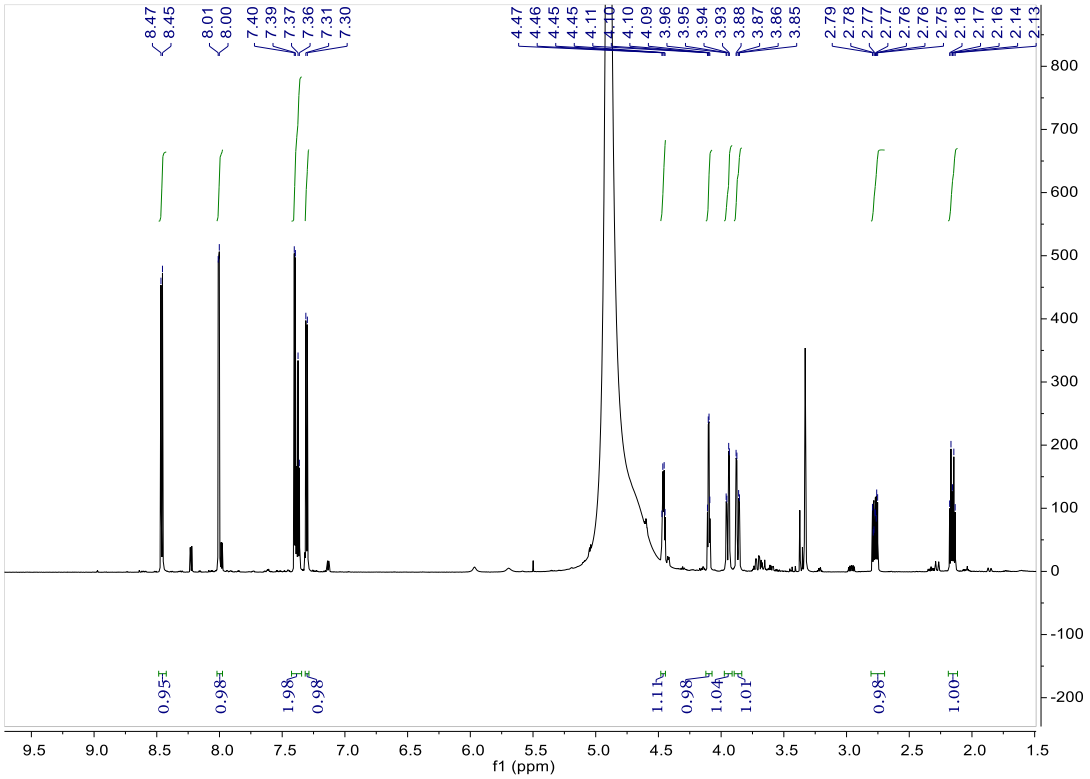

Figure S19 1H-NMR of dTPT3 (**10**).

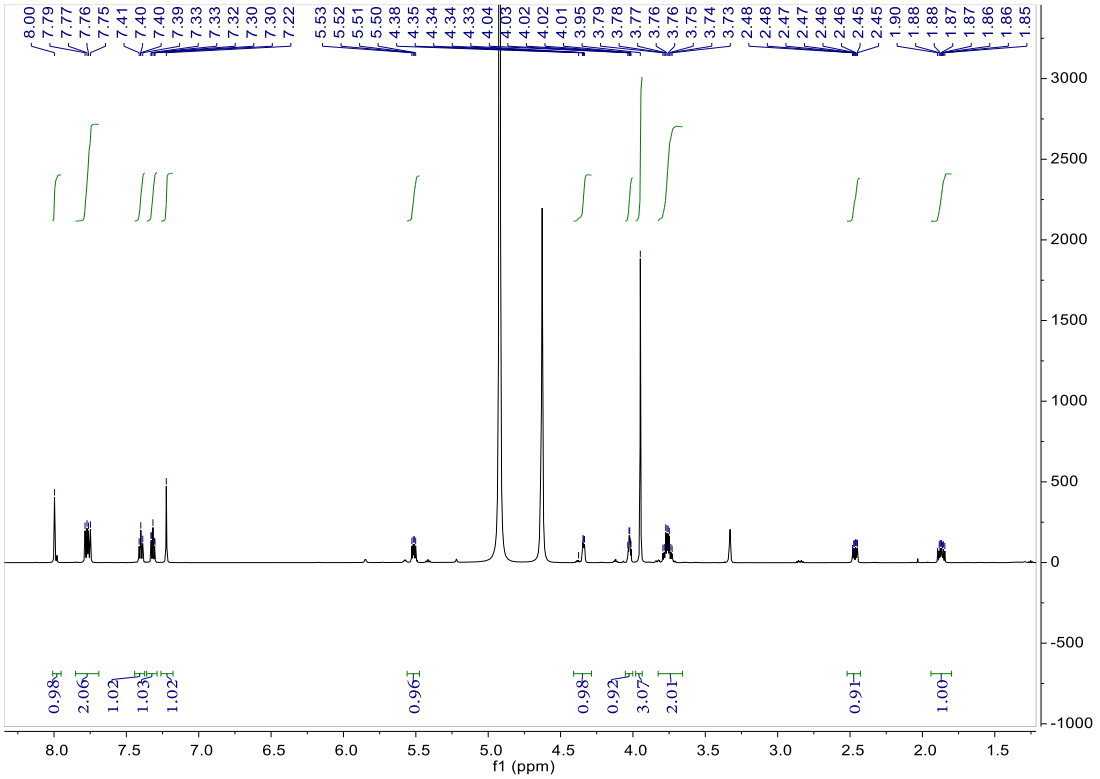

Figure S20. 1H-NMR of dNAM (**13**).

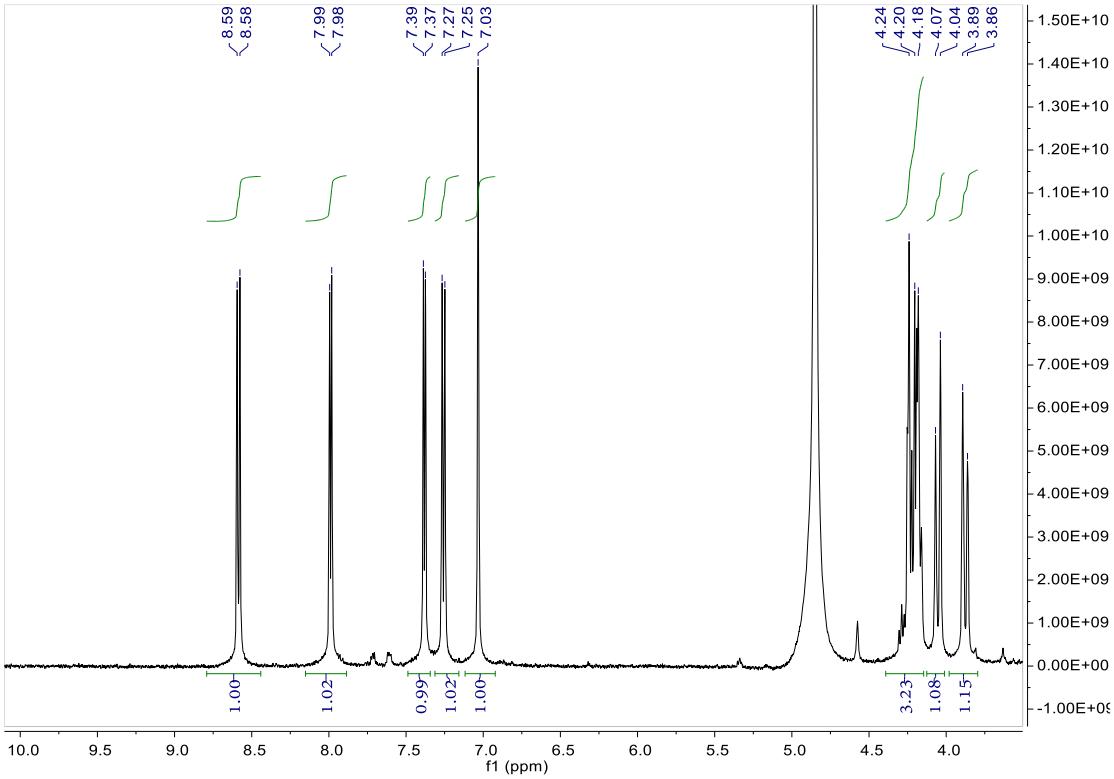

Figure S21. 1H-NMR of TPT3TP (**16**).

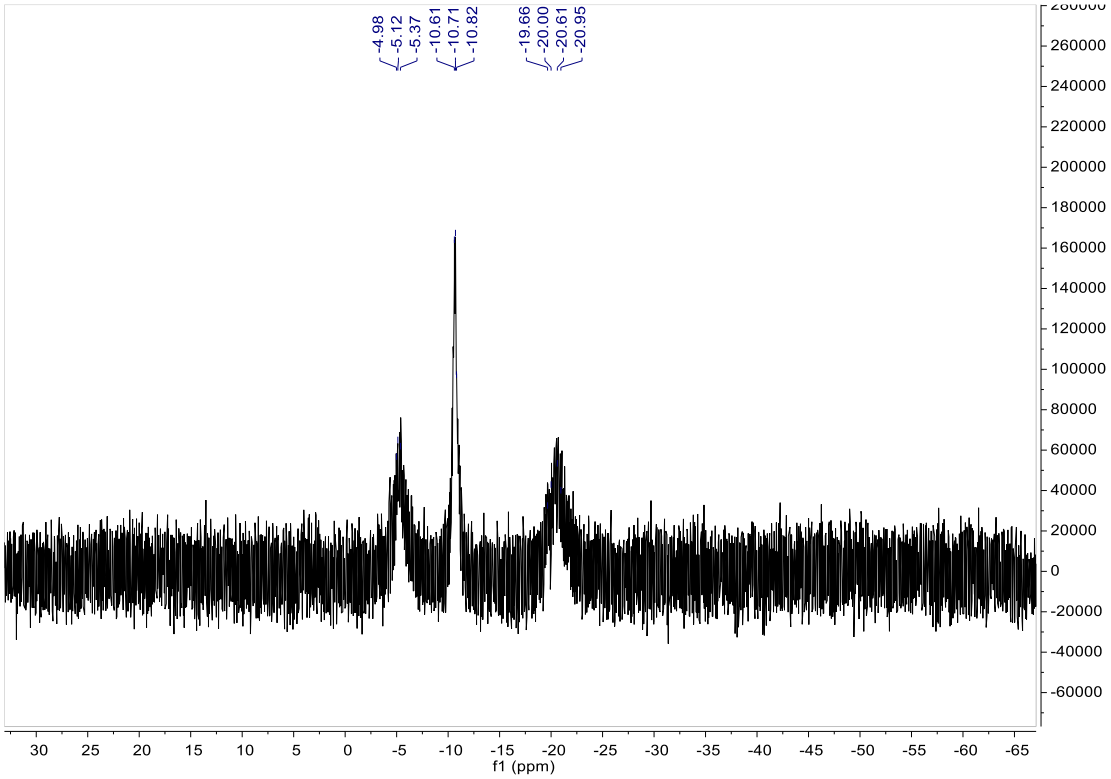

Figure S22. 31P-NMR of TPT3TP (**16**).

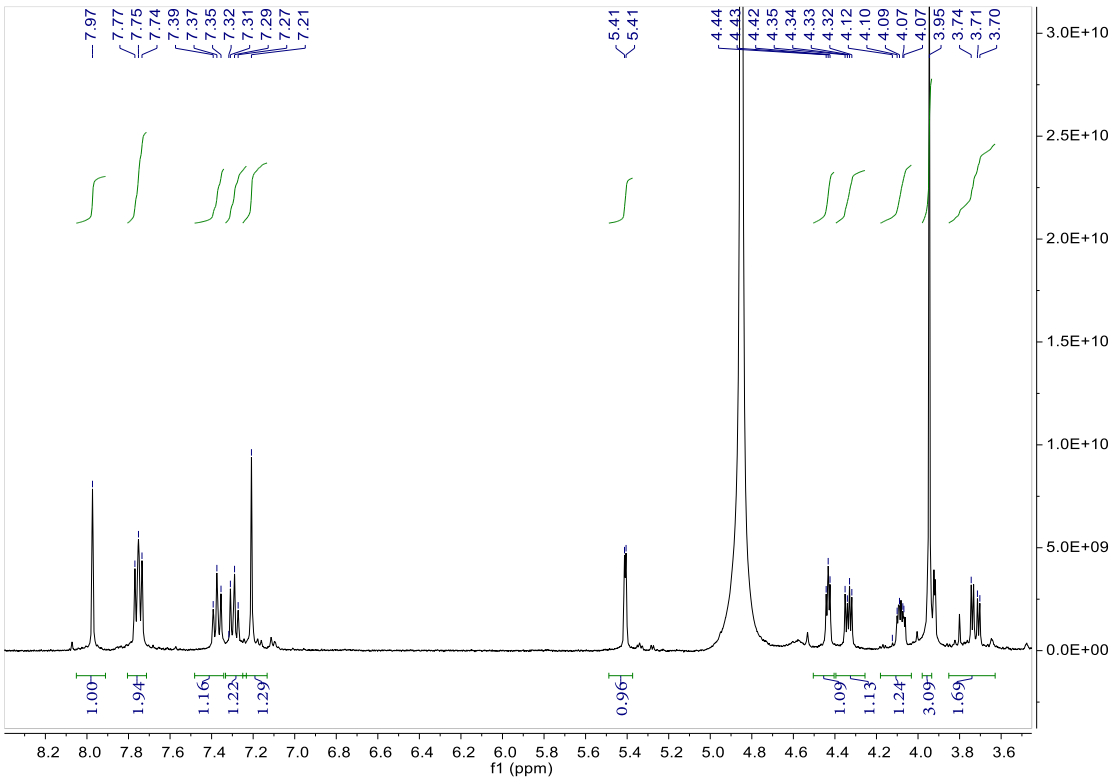

Figure S23. 1H-NMR of NAMTP (**18**).

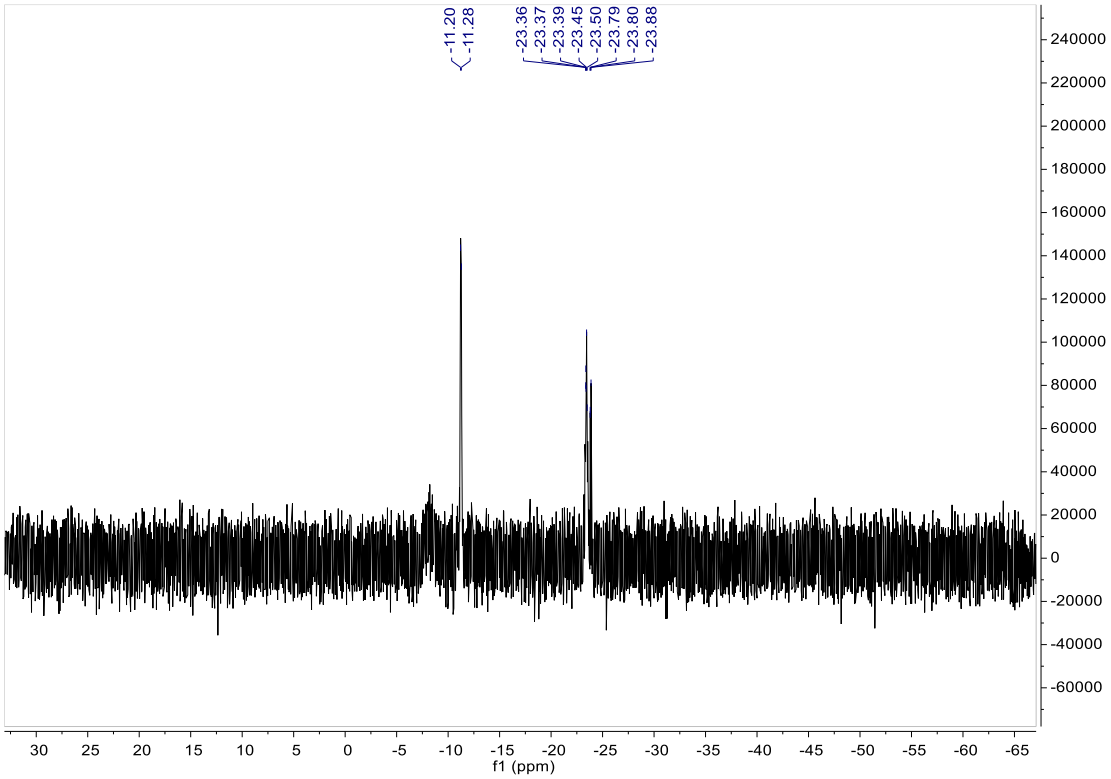

Figure S24. 31P-NMR of NAMTP (**18**).

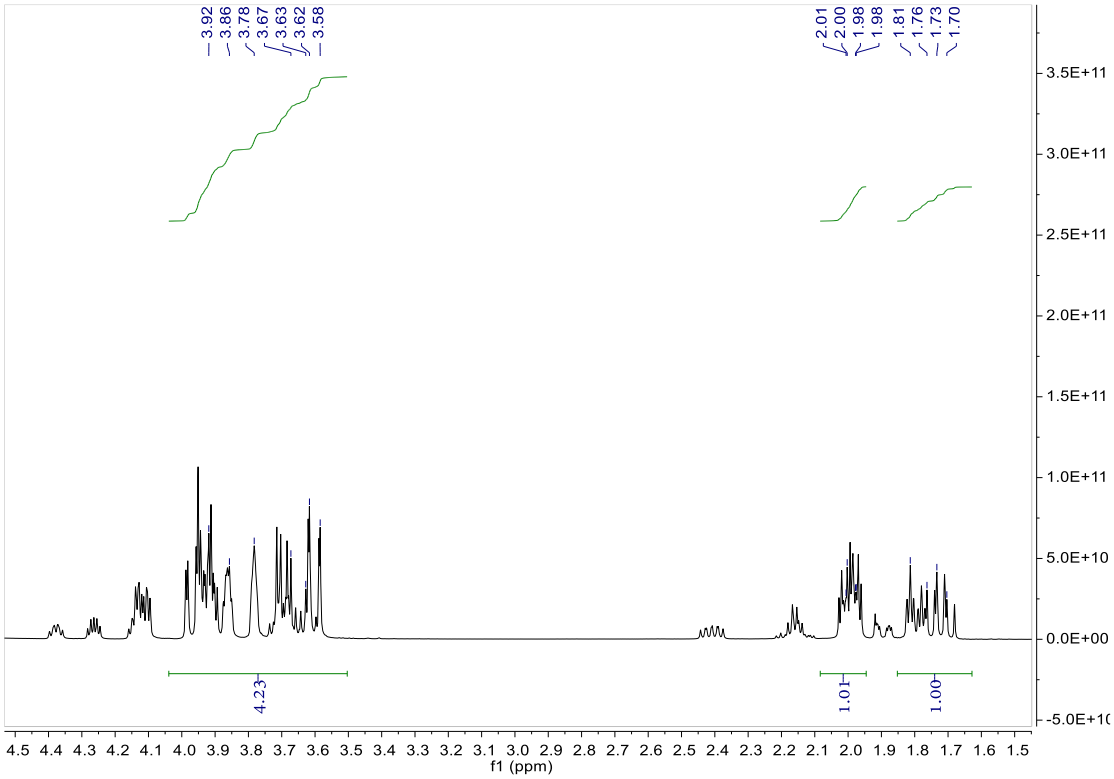

Figure S25. 1H -NMR of deoxyribose.

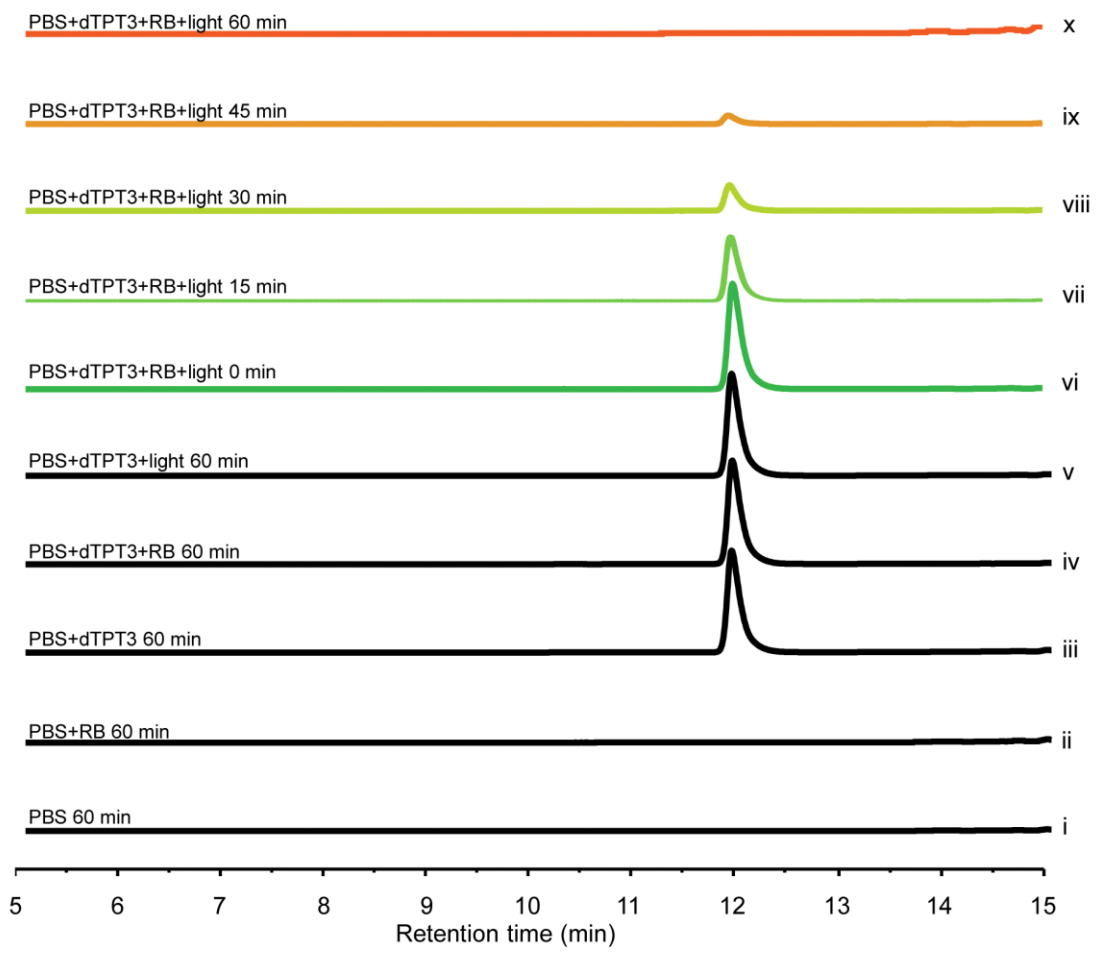

Figure S26. HPLC analysis of dTPT3 photocleavage at 325 nm. 1.0 mM dTPT3 and 10 μM RB were employed, and samples were irradiated for the corresponding time scope and subjected to HPLC analysis.

References

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[3] Y. J. Seo, G. T. Hwang, P. Ordoukhanian, F. E. Romesberg, *J. Am. Chem. Soc.* **2009,** *131* (9), 3246-3252.

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