

Supporting Information

A Benzophenone-Based Photocaging Strategy for the N7 Position of Guanosine

*Lea Anhäuser, Nils Klöcker, Fabian Muttach, Florian Mäsing, Petr Špaček, Armido Studer, and Andrea Rentmeister**

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A benzophenone-based photocaging strategy for the *N7* position of guanosine

Lea Anhäuser, Nils Klöcker, Fabian Muttach, Florian Mäsing, Petr Špaček, Armido Studer and Andrea Rentmeister*

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Experimental Procedures

General Information

All chemicals were purchased from *Alfa Aesar*, *Applichem*, *Fluorochem*, *Sigma Aldrich* or *TCI* unless otherwise noted. The HPLC grade acetonitrile was purchased from *VWR*. All components were used without further purification.

G(5')ppp(5')A (GpppA) cap analogue and G(5')ppp(5')G (GpppG) cap analogue were purchased from *New England Biolabs* or *Jena Bioscience*.

The short RNA with the sequence 5' UUGUU 3' (purification: HPLC) was purchased from *Biologio*, the short RNAs with the sequences 5' UUUCUUUUGUAUAUCUUUC 3', 5' UUUCUUUGGUAUAUCUUUC 3' and 5' CACACGAACUUAUAGGUAGGC 3' (all purification: HPLC) were purchased from *biomers.net*.

The short single stranded DNAs (with a M. TaqI recognition site, underlined) with the sequence 5'CTTGGAGCCACTATCGACTACG CGATCATGG3' and 3'GAACCTCGGTGATAGCTGATGCGCTAGTACC5' (purification: desalination) were purchased from *Biologio*.

Phusion High-Fidelity DNA Polymerase, FastAP Thermosensitive Alkaline Phosphatase, RiboLock RNA Inhibitor, T7 RNA polymerase, pyrophosphatase, DNase I were purchased from *Thermo Scientific*, nuclease P1 from *Sigma Aldrich*, Snake Venom Phosphodiesterase from *MP Biomedicals*, TaqI Methyltransferase (M. TaqI) and restriction enzyme Taq^qI (R. TaqI) were purchased from *New England Biolabs*, Ecm1, MTAN and LuxS were recombinantly expressed and purified as previously described.^[1]

The plasmid pBR322 was purchased from *New England Biolabs*. NTP bundle was purchased from *Jena Bioscience*.

¹H and ¹³C NMR spectra were measured at 299 K on a *Bruker Avance II 300*, an *Agilent DD2 500* or *Agilent DD2 600* spectrometer. The chemical shifts (δ) were reported in ppm relative to deuterated solvents as internal standard (δ_{H} : CDCl₃ = 7.26, DMSO-*d*₆ = 62.50 ppm, D₂O = 4.79 and δ_{C} : CDCl₃ = 77.16, DMSO-*d*₆ = 39.52 ppm). Multiplicities are indicated as s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dt (doublet of triplet) t (triplet), q (quartet) and m (multiplet).

MALDI spectra were recorded on a *Bruker Daltonics Autoflex Speed MALDI-TOF* instrument.

High-resolution mass spectra were recorded on a *Thermo Scientific Orbitrap LTQ XL* or on a *Bruker MicroTof* instrument.

High-resolution mass spectra of UHPLC-MS measurements were performed on a *Bruker maXis II* ultra-high resolution QTOF coupled to a *Thermo Scientific UltiMate 3000*[®] UHPLC using a *Nucleodur*[®] C18 Pyramid reversed-phase column (5 μ m, 125 x 2 mm) from *Macherey-Nagel*. Elution was performed at a flow rate of 0.6 mL/min applying a linear gradient for buffer A (7 mM ammonium formate and 12 mM formic acid, pH = 3.5) and buffer B (MeOH).

HPLC analysis and purification of cap analogues were performed on an *Agilent 1260 Infinity HPLC* equipped with a diode array detector (DAD) (190-640 nm) using a *Nucleodur*[®] C18 Pyramid reversed-phase column (5 μ m, 125 x 4 mm) from *Macherey-Nagel*. Elution was performed at a flow rate of 1 mL/min applying a linear gradient for buffer A (50 mM ammonium acetate, pH = 6.0) and buffer B (1:1 buffer A : acetonitrile).

Semi-preparative HPLC purification of AdoMet analogues was performed on an *Agilent 1260 Infinity HPLC* using a *Nucleodur*[®] C18 Pyramid reversed-phase column (5 μ m, 125 x 10 mm) from *Macherey-Nagel*. Elution was performed at a flow rate of 5 mL/min applying a linear gradient for buffer A (ddH₂O with 0.01% trifluoroacetic acid) and buffer B (acetonitrile with 0.01% trifluoroacetic acid).

Preparative HPLC purification of guanosine derivatives was performed on a *Büchi PrepChrom C-700 V5* using a *Varioprep* reverse-phase column (5 μ m, 250 x 21 mm) from *Macherey-Nagel*. Elution was performed at a flow rate of 12 mL/min applying a linear gradient for buffer A (50 mM ammonium acetate, pH = 6.0) and buffer B (acetonitrile).

RNA sizing was performed using the ladder 20/100 from *Integrated DNA Technologies*. DNA sizing was performed using the ladder *GeneRuler*[™] 1kb DNA Ladder from *Thermo Fisher Scientific*.

IR spectra were recorded on a *Digilab FTS 3100* spectrometer combined with a *Specac MKII Golden Gate Single Reflection ATR System*. Signals were given in wavenumbers $\tilde{\nu}$ (cm⁻¹). Intensities were abbreviated with (vs) very strong, (s) strong, (m) medium, (w) weak, (vw) very weak and (br) broad.

For preparation of HeLa cell lysate HeLa cells were cultured in MEM Earle's media (*Merck*) supplemented with L-glutamine (2 mM), non-essential amino acids (1%), penicillin and streptomycin (1%), and fetal calf serum (FCS, 10%) under standard conditions (5% CO₂, 37 °C). 24 h prior to cell lysis, 3 x 10⁶ cells were seeded on a Petri dish (90 mm). For cell lysis, the cell supernatant was discarded and

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the cells were washed with 1× PBS, lysed with CellLytic™-M reagent (1.5 mL, *Sigma Aldrich*) according to the manufacturer's instructions and stored at -80 °C.

UV-A LED (3 W, $\lambda_{\text{max}} = 365$ nm) was used for the irradiation of cap analogues (see **Figure S1A**). The LED was purchased by *LED Engin* and installed in a custom made LED box (see **Figure S2A**). The temperature inside the box was regulated to 23°C using a fan.

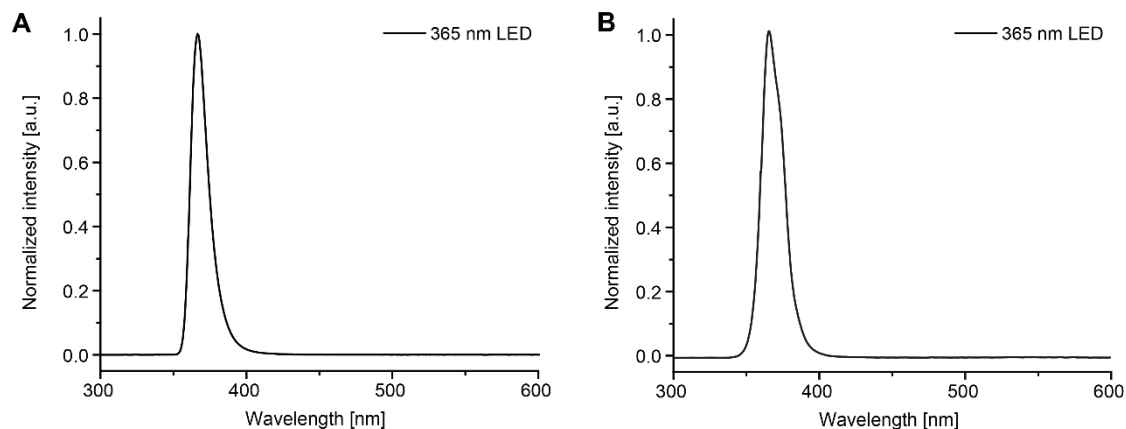


Figure S1. Emission spectra of the light sources used, recorded with the fluorescence spectrometer *Jasco FP-8300*.

UV-A LED (3W, $\lambda_{\text{max}} = 365$ nm) was used for the irradiation of nucleoside derivatives (see **Figure S1B**). The LEDs were purchased from *Avonec* and installed in a custom made LED box (see **Figure S2B**). The fan regulated the temperature to no more than 9 °C above room temperature inside the box. A stirring plate installed under the LED box allowed mixing of the reaction components during irradiation. The Glorius working group (WWU Münster) provided this LED box.

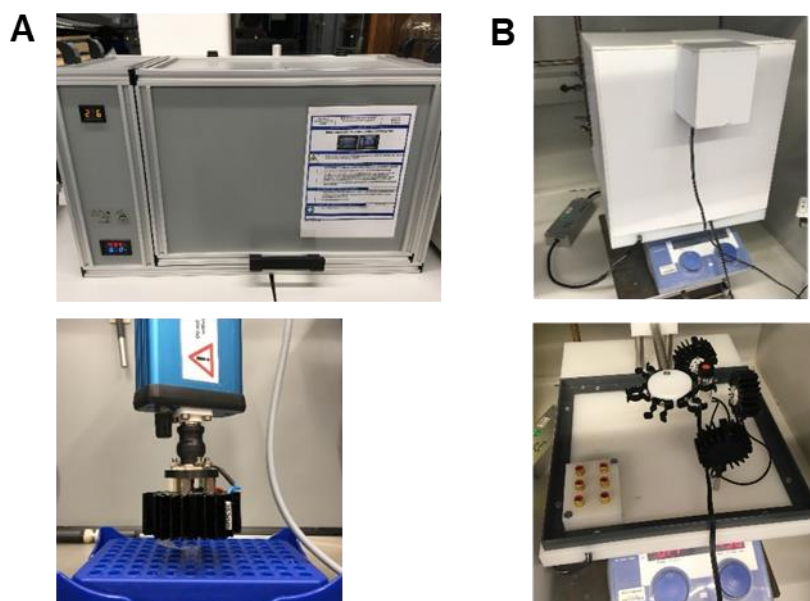
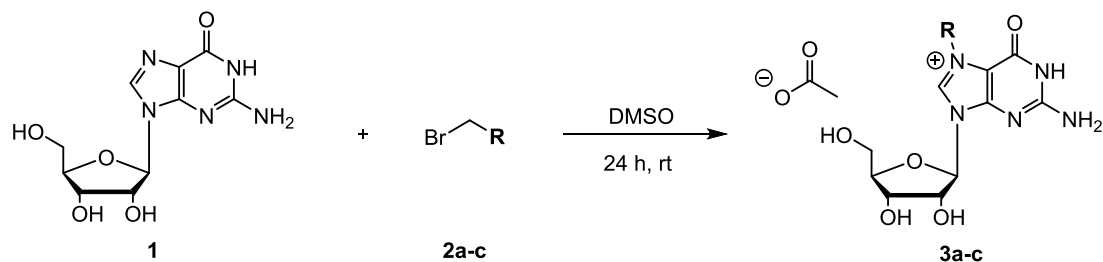


Figure S2. Custom made LED boxes used for the irradiation experiments of cap analogues (A) or nucleoside derivatives (B).

An UV-transilluminator (312 nm, *Intas GDS*) and a polystyrene plate to remove short wavelength UV light (<290 nm) was used for the irradiation of cap analogues.

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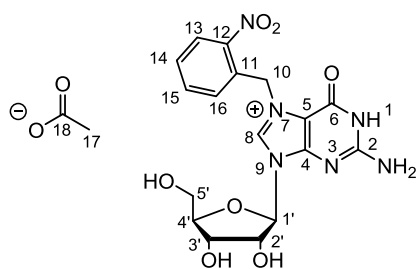
General synthesis of *N*7-modified guanosines (GS1)

The products **3a-c** were synthesized following a modified literature procedure by Pautus and coworkers.^[2]

To a 4-mL reaction vessel equipped with a magnetic stirring bar guanosine **1** (142 mg, 0.5 mmol, 1.0 equiv.) and the corresponding bromide **2a-c** (0.6 mmol, 1.2 equiv.) were added. DMSO (2.5 mL) were added and the solution was vigorously stirred for 24 h at room temperature. The reaction mixture was lyophilized, and the raw product was taken up in 800-1200 μL of a mixture of ddH₂O and acetonitrile and isolated by preparative HPLC. The collected product fractions were lyophilized.

2-Amino-9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-7-(2-nitrobenzyl)-6-oxo-6,9-dihydro-1*H*-purin-7-ium (*N*7-ONB-guanosine, **3a**)

Prepared from guanosine **1** and 1-(1-bromomethyl)-2-nitrobenzene **2a** (130 mg) following the GS1 to obtain the desired product **3a** (165 mg, 0.266 mmol, 53%) as white solid.



¹H-NMR (600 MHz, DMSO-*d*₆, 299 K): δ [ppm] = 9.29 (s, 1H, H-8), 8.16 (dd, 1H, H-13, ³*J*_{13,14} = 8.2 Hz, ⁴*J*_{13,15} = 1.3 Hz), 7.73 (ddd, 1H, H-15, ³*J*_{15,14} = ³*J*_{15,16} = 7.6 Hz, ⁴*J*_{15,13} = 1.3 Hz), 7.63 (ddd, 1H, H-14, ³*J*_{14,13} = ³*J*_{14,15} = 7.6 Hz, ⁴*J*_{14,16} = 1.3 Hz), 7.22 (dd, 1H, H-16, ³*J*_{16,15} = 7.8 Hz, ⁴*J*_{16,14} = 1.3 Hz), 5.97 (s, 2H, H-10), 5.85 (d, 1H, H-1', ³*J*_{1',2'} = 3.7 Hz), 4.44 (dd, 1H, H-2', ³*J*_{2',1'} = 3.7 Hz, ³*J*_{2',3'} = 4.6 Hz), 4.13 (dd, 1H, H-3', ³*J*_{3',2'} = 4.7 Hz, ³*J*_{3',4'} = 5.5 Hz), 3.99 (dt, 1H, H-4', ³*J*_{4',3'} = 5.7 Hz, ³*J*_{4',5'} = 3.2 Hz), 3.68 (dd, 1H, H-5'a, ³*J*_{5'a,4'} = 3.2 Hz, ²*J*_{5'a,5'b} = 12.3 Hz), 3.57 (dd, 1H, H-5'b, ³*J*_{5'b,4'} = 3.2 Hz, ²*J*_{5'b,5'a} = 12.3 Hz), 1.78 (s, 10H, H-17)*.

* Due to the preparative purification in 50 mM ammonium acetate buffer, the integral of H-17 does not correspond to 3 protons, the yield was adjusted accordingly.

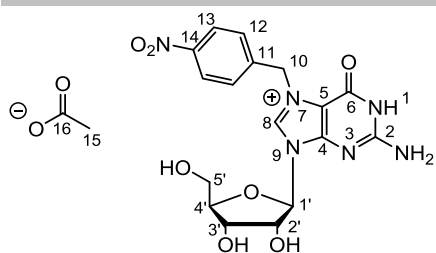
¹³C-NMR (151 MHz, DMSO-*d*₆, 299 K): δ [ppm] = 173.6 (C-18), 161.2 (C-2), 159.6 (C-6), 149.6 (C-4), 147.3 (C-12), 134.6 (C-15), 134.2 (C-8), 130.4 (C-11), 129.6 (C-14), 129.1 (C-16), 125.1 (C-13), 107.6 (C-5), 89.4 (C-1'), 85.7 (C-4'), 74.2 (C-2'), 69.3 (C-3'), 60.4 (C-5'), 48.5 (C-10), 22.9 (C-17).

HR-MS (ESI): *m/z* calculated for [(C₁₇H₁₉N₆O₇)⁺]: 419.1310, found: 419.1324.

2-Amino-9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-7-(4-nitrobenzyl)-6-oxo-6,9-dihydro-1*H*-purin-7-ium (*N*7-PNB-guanosine, **3b**)

Prepared from guanosine **1** and 1-(1-bromomethyl)-4-nitrobenzene **2b** (130 mg) following the GS1 to obtain the desired product **3b** (130 mg, 0.28 mmol, 56%) as white solid.

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¹H-NMR (600 MHz, D₂O, 299 K): δ [ppm] = 8.15 (d, 1H, H-13, ³J_{13,12} = 8.9 Hz), 7.49 (d, H-12, ³J_{12,13} = 9.0 Hz), 5.96 (d, 1H, H-1', ³J_{1',2'} = 3.4 Hz), 5.68 (s, 1H, H-10), 4.59 (dd, 1H, H-2', ³J_{2',3'} = 5.0 Hz, ³J_{2',1'} = 3.5 Hz), 4.26 (dd, 1H, H-3', ³J_{3',4'} = 6.0 Hz, ³J_{3',2'} = 5.0 Hz), 4.16 (ddd, 1H, H-4', ³J_{4',3'} = 6.1 Hz, ³J_{4',5'a} = 3.1 Hz, ³J_{4',5'b} = 3.1 Hz), 3.85 (dd, 1H, H-5'a, ³J_{5'a,5'b} = 12.9 Hz, ³J_{5'a,4'} = 2.8 Hz), 3.72 (dd, 1H, H-5'b, ³J_{5'b,5'a} = 12.9 Hz, ³J_{5'b,4'} = 3.5 Hz), 1.82 (s, 2H, H-15)*.

* Due to the preparative purification in 50 mM ammonium acetate buffer, the integral of H-15 does not correspond to 3 protons, the yield was adjusted accordingly.

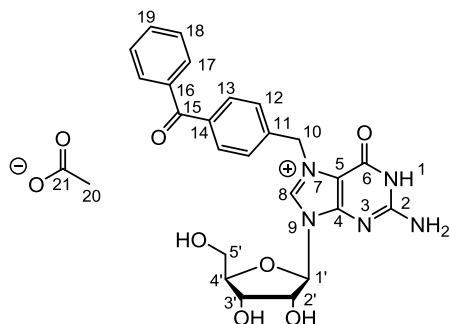
¹³C-NMR (151 MHz, D₂O, 299 K): δ [ppm] = 181.3 (C-16), 157.0 (C-6)*, 156.3 (C-2)*, 149.81 (C-4)*, 147.8 (C-14), 140.53 (C-11), 124.2 (C-12), 128.9 (C-13), 108.2 (C-5), 90.3 (C-1'), 85.3 (C-4'), 74.1 (C-2'), 69.0 (C-3'), 60.0 (C-5'), 51.6 (C-10), 23.1 (C-15).

*Signals could not be unambiguously assigned.

HR-MS (ESI): *m/z* calculated for [(C₁₇H₁₉N₆O₇)⁺]: 419.1310, found: 419.1323.

2-Amino-7-(4-benzoylbenzyl)-9-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-6-oxo-6,9-dihydro-1H-purin-7-ium (N7-BP-guanosine, 3c)

Prepared from guanosine **1** and 4-(bromomethyl)benzophenone **2c** (165 mg) following the GS1 to obtain the desired product **3c** (137 mg, 0.266 mmol, 53%) as white solid.



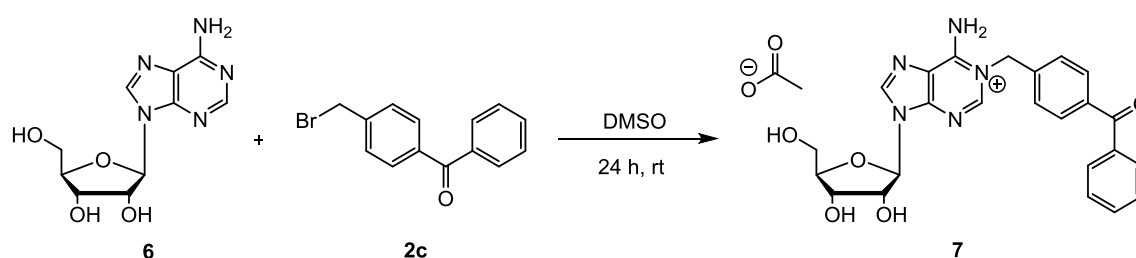
¹H-NMR (500 MHz, DMSO-*d*₆, 299 K): δ [ppm] = 9.30 (s, 1H, H-8), 7.75-7.71 (m, 4H, H-17, H-18), 7.70-7.65 (m, 1H, H-19), 7.65-7.62 (m, 2H, H-12), 7.57-7.53 (m, 2H, H-13), 5.82 (d, 1H, H-1', ³J_{1',2'} = 4.6 Hz), 5.77 (s, 2H, H-10), 4.50 (dd, 1H, H-2', ³J_{2',1'} = 4.7 Hz, ³J_{2',3'} = 4.7 Hz), 4.13 (dd, 1H, H-3', ³J_{3',2'} = 4.6 Hz, ³J_{3',4'} = 4.6 Hz), 4.00 (m, 1H, H-4'), 3.70 (dd, 1H, H-5'a, ³J_{5'a,4'} = 3.3 Hz, ²J_{5'a,5'b} = 12.3 Hz), 3.58 (dd, 1H, H-5'b, ³J_{5'b,4'} = 3.3 Hz, ²J_{5'b,5'a} = 12.3 Hz), 1.84 (s, 2H, H-20)*.

* Due to the preparative purification in 50 mM ammonium acetate buffer, the integral of H-20 does not correspond to 3 protons, the yield was adjusted accordingly.

¹³C-NMR (126 MHz, DMSO-*d*₆, 299 K): δ [ppm] = 195.3 (C-15), 173.0 (C-21), 162.6 (C-6), 161.7 (C-2), 149.5 (C-4), 140.1 (C-16), 136.9 (C-11), 136.8 (C-14), 132.8 (C-19), 132.5 (C-8), 130.6 (C-17), 129.7 (C-18), 128.6 (C-12), 128.3 (C-13), 107.9 (C-5), 89.1 (C-1'), 86.6 (C-4'), 73.7 (C-2'), 69.7 (C-3'), 60.7 (C-5'), 50.2 (C-10), 22.2 (C-20).

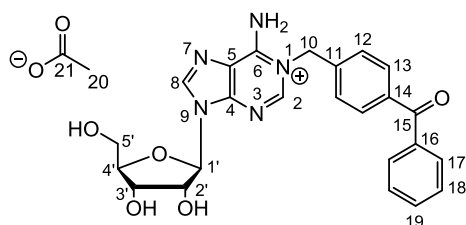
HR-MS (ESI): *m/z* calculated for [(C₂₄H₂₄N₅O₆)⁺]: 478.1721, found: 478.1737.

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6-Amino-1-(4-benzoylbenzyl)-9-((2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-9H-purin-1-ium (N1-BP-adenosine, **7)**

The product **7** was synthesized following a modified literature procedure by Pautus and coworkers.^[2]

To a 4-mL reaction vessel equipped with a magnetic stirring bar adenosine **6** (134 mg, 0.5 mmol, 1.0 equiv.) and 4-(bromomethyl)benzophenone **2c** (165 mg, 0.6 mmol, 1.2 equiv.) were added. DMSO (2.5 mL) were added and the solution was vigorously stirred for 24 h at room temperature. The reaction mixture was lyophilized, and the raw product was taken up in 4 mL of a mixture of ddH₂O, acetonitrile and methanol and isolated by preparative HPLC. The collected product fractions were lyophilized and the desired product **7** (108 mg, 0.225 mmol, 45%) was obtained as white solid.



¹H NMR (600 MHz, DMSO-*d*₆, 299 K): δ [ppm] 8.32 (s, 1H, H-2), 8.19 (s, 1H, H-8), 7.73-7.70 (m, 4H, H-17, H-18), 7.68-7.65 (m, 1H, H-19), 7.56-7.53 (m, 2H, H-13), 7.52-7.50 (m, 2H, H-12), 5.78 (d, 1H, H-1', ³*J*_{1',2'} = 5.9 Hz), 5.35 (s, 1H, H-10), 4.48 (dd, 1H, H-2', ³*J*_{2',1'} = 5.9 Hz, ³*J*_{2',3'} = 5.0 Hz), 4.12 (dd, 1H, H-3', ³*J*_{3',2'} = 5.0 Hz, ³*J*_{3',4'} = 3.4 Hz), 3.93 (dt, 1H, H-4', ³*J*_{4',3'} = ³*J*_{4',5'} = 3.8 Hz), 3.64 (dd, 1H, H-5'a, ³*J*_{5'a,4'} = 4.0 Hz, ²*J*_{5'a,5'b} = 12.0 Hz), 3.54 (dd, 1H, H-5'b, ³*J*_{5'b,4'} = 4.0 Hz, ²*J*_{5'b,5'a} = 12.0 Hz), 1.84 (s, 0.8H, H-20)*.

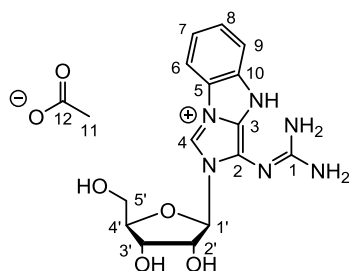
* Due to the preparative purification in 50 mM ammonium acetate buffer, the integral of H-20 does not correspond to 3 protons, the yield was adjusted accordingly.

¹³C NMR (151 MHz, DMSO-*d*₆, 299 K): δ [ppm] 195.4 (C-15), 172.5 (C-21), 153.6 (C-6), 148.5 (C-2), 142.3 (C-16), 141.3 (C-4), 138.0 (C-8), 137.0 (C-14), 136.0 (C-11), 132.7 (C-19), 129.9 (C-18), 129.6 (C-17), 128.6 (C-13), 127.5 (C-12), 123.0 (C-5), 87.6 (C-1'), 85.7 (C-4'), 74.0 (C-2'), 70.4 (C-3'), 61.4 (C-5'), 48.8 (C-10), 21.9 (C-20).

HR-MS (ESI): *m/z* calculated for [(C₂₄H₂₄N₅O₅)⁺]: 462.1772, found: 462.1768.

Irradiation of N7-ONB-guanosine (3a) and isolation of photo product (4)

N7-ONB-guanosine **3a** (10 mg, 16 μmol) was dissolved in 2 mL ddH₂O and irradiated using a LED (λ_{max} = 365 nm) for 3 h under vigorously stirring. The formation of photo product **4** was followed by analytical HPLC and then the reaction mixture was lyophilized. The raw product was taken up in 800-1000 μL of a mixture of ddH₂O and acetonitrile and then isolated by preparative HPLC. The collected product fractions were lyophilized and the photo product **4** (4.0 mg, 7.3 μmol, 46%) was obtained as yellow-orange oil.



¹H-NMR (600 MHz, DMSO-*d*₆, 299 K): δ [ppm] = 9.80 (s, 1H, H-4), 8.33-8.30 (m, 1H, H-6), 8.19 (ddd, 1H, H-8, ³*J*_{8,9} = 8.3 Hz, ³*J*_{8,7} = 6.9 Hz, ⁴*J*_{8,6} = 1.4 Hz), 8.13-8.10 (m, 1H, H-9) 7.94 (ddd, 1H, H-7, ³*J*_{7,6} = 8.9 Hz, ³*J*_{7,8} = 6.9 Hz, ⁴*J*_{7,9} = 1.2 Hz), 5.83 (d, 1H, H-1',

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$^3J_{1',2'} = 3.6$ Hz), 4.13-4.08 (m, 2H, H-2', H-3'), 3.85-3.82 (m, 1H, H-4'), 3.59 (dd, 1H, H-5'a, $^3J_{5'a,4'} = 3.8$ Hz, $^2J_{5'a,5'b} = 11.7$ Hz), 3.57 (dd, 1H, H-5'b, $^3J_{5'b,4'} = 3.7$ Hz, $^2J_{5'b,5'a} = 11.7$ Hz), 1.78 (s, 10 H, H-11)*.

* Due to the preparative purification in 50 mM ammonium acetate buffer, the integral of H-11 does not correspond to 3 protons, the yield was adjusted accordingly.

$^{13}\text{C-NMR}$ (151 MHz, DMSO- d_6 , 299 K): δ [ppm] = 172.9 (C-12), 165.2 (C-1)*, 161.3 (C-4), 154.2 (C-3)*, 152.0 (C-2)*, 148.7 (C-10), 135.8 (C-8), 130.0 (C-7), 128.2 (C-9), 128.1 (C-6), 124.3 (C-5), 86.5 (C-1'), 83.9 (C-4'), 73.9 (C-2'), 70.1 (C-3'), 60.9 (C-5'), 22.5 (C-11).
*Signals could not be unambiguously assigned.

HR-MS (ESI): m/z calculated for $[(\text{C}_{15}\text{H}_{19}\text{N}_6\text{O}_5)]^+$: 347.1462, found: 347.1458.

Irradiation of *N7*-PNB-guanosine (**3b**)

N7-PNB-guanosine **3b** (5 mg, 10 μmol) was dissolved in 2 mL ddH₂O, irradiated using a LED ($\lambda_{\text{max}} = 365$ nm) for 1 h under vigorously stirring and further analyzed by HPLC.

Irradiation of *N7*-BP-guanosine (**3c**)

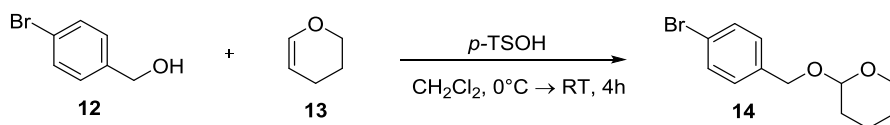
N7-BP-guanosine **3c** (1.5 mg, 2.9 μmol) was dissolved in 200 μL acetonitrile and 800 μL aqueous solution (ddH₂O, buffer (50 mM Tris-HCl (pH 8), 200 mM NaCl, 1 mM EDTA (pH 8), 20% glycerol), 50 mM Tris-HCl (pH 8), 1 mM EDTA (pH 8), 200 mM NaCl or 20% glycerol) or 800 μL cell lysate, irradiated using a LED ($\lambda_{\text{max}} = 365$ nm) for 30 min under vigorously stirring and further analyzed by HPLC.

Irradiation of *N1*-BP-adenosine (**7**)

N1-BP-adenosine **7** (1.5 mg, 3.13 μmol) was dissolved in 200 μL acetonitrile and 800 μL ddH₂O, irradiated using a LED ($\lambda_{\text{max}} = 365$ nm) for 30 min under vigorously stirring and further analyzed by HPLC.

Synthesis and purification of AdoMet analogues

AdoMet analogues **9a-d** and corresponding precursors were synthesized as previously described.^[3]

2-((4-Bromobenzyl)oxy)tetrahydro-2H-pyran (**14**)

(4-bromophenyl)methanol **12** (3.00 g, 16.0 mmol, 1.0 equiv.) was dissolved at 0°C into dichloromethane (20 mL) and 3,4-dihydro-2H-pyran **13** (2.69 g, 32.0 mmol, 2.0 equiv.). After addition of *p*-toluenesulfonic acid (4.00 mg, 23.2 μmol , 0.15 mol%) the reaction mixture was warmed up to room temperature and then stirred for further 4 h. The reaction was stopped by adding water (30 mL). The reaction mixture was extracted with dichloromethane (3 x 20 mL), the combined organic phases dried over MgSO_4 and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography using silica gel (*n*-pentane/methyl *tert*-butyl ether 10 : 1) to obtain the desired product **14** (4.23 g, 15.6 mmol, 98%) as colorless oil.

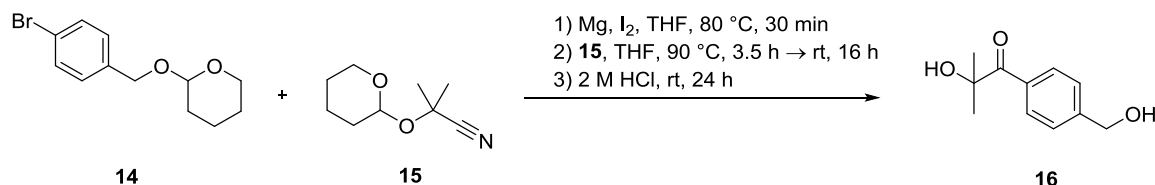
$^1\text{H-NMR}$ (300 MHz, CDCl_3 , 299 K): δ (ppm) 7.50 (d, $J = 8.4$ Hz, 2H), 7.27 (d, $J = 8.4$ Hz, 2H), 4.81- 4.68 (m, 2H), 4.49 (d, $J = 12.3$ Hz, 1H), 3.99-3.86 (m, 1H), 3.63-3.51 (m, 1H), 1.98-1.50 (m, 6H).

$^{13}\text{C-NMR}$ (75 MHz, CDCl_3 , 299 K): δ (ppm) 137.6 (C_q), 131.6 (CH_{Aryl}), 129.6 (CH_{Aryl}), 121.5 (C_q), 98.0 (CH), 68.2 (CH_2), 62.3 (CH_2), 30.7 (CH_2), 25.6 (CH_2), 19.5 (CH_2).

IR (ATR): $\tilde{\nu}$ (cm^{-1}) 2942 (m), 2870 (w), 1488 (w), 1387 (vw), 1349 (vw), 1201 (w), 1123 (m), 1069 (m), 1034 (s), 974 (w), 906 (w), 871 (w), 802 (w).

HR-MS (ESI): m/z calculated for $[\text{C}_{12}\text{H}_{15}\text{BrO}_2\text{Na}]^+$ 293.0148, found: 293.0152.

SUPPORTING INFORMATION

2-Hydroxy-1-(4-(hydroxymethyl)phenyl)-2-methylpropan-1-one (**16**)

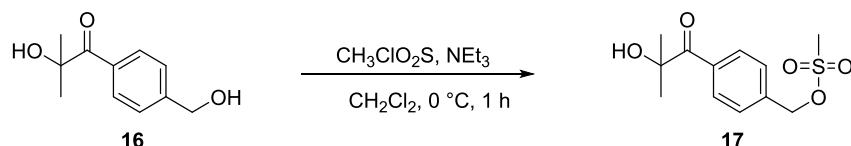
2-((4-Bromobenzyl)oxy)tetrahydro-2H-pyran **14** (4.00 g, 14.8 mmol, 1.3 equiv.) was dissolved in THF (10 mL) and added to magnesium (1.43 g, 59.0 mmol, 5.2 equiv.). After the addition of I₂ (some grains) the reaction mixture was heated to 80 °C for 30 min and the Grignard reagent was formed. After cooling down to room temperature, the Grignard reagent was added to a solution of 2-methyl-2-((tetrahydro-2H-pyran-2-yl)oxy)propanitrile **15** (1.92 g, 11.3 mmol, 1.0 equiv.) in THF (40 mL). The reaction mixture was further treated for 3.5 h at 90 °C, cooled down to room temperature and stirred overnight. HCl (2 M in water, 30 mL) was added to the reaction mixture and then again stirred over night. The reaction mixture was extracted with dichloromethane (3 x 20 mL), the organic phases were dried over MgSO₄ and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography using silica gel (*n*-pentane/ ethylacetate 1 : 1) to obtain the desired product **16** (720 mg, 3.71 mmol, 33%) as colorless oil.

¹H-NMR (300 MHz, CDCl₃, 299 K): δ (ppm) 7.99 (d, *J* = 8.4 Hz, 2H, 2 x CH_{Aryl}), 7.43 (d, *J* = 8.4 Hz, 2H, 2 x CH_{Aryl}), 4.75 (s, 2H, CH₂), 4.11 (s, 1H, OH), 2.31 (s, 1H, OH), 1.61 (s, 6H, 2 x CH₃).

¹³C-NMR (75 MHz, CDCl₃, 299 K): δ (ppm) 204.4 (CO), 146.3 (C_q), 133.0 (C_q), 130.1 (CH_{Aryl}), 126.6 (CH_{Aryl}), 76.5 (C_q), 64.6 (CH₂), 28.5 (CH₃).

IR (ATR): $\tilde{\nu}$ (cm⁻¹) 3375 (br), 2979 (w), 2934 (w), 2873 (w), 1670 (vs), 1608 (m), 1569 (vw), 1460 (w), 1413 (w), 1372 (m), 1265 (m), 1168 (vs), 1045 (m), 958 (s), 830 (w), 755 (w).

HR-MS (ESI): *m/z* calculated for [C₁₁H₁₄O₃Na]⁺ 217.0835, found: 217.0834.

4-(2-Hydroxy-2-methylpropanoyl)benzyl sulfonate (**17**)

2-Hydroxy-1-(4-(hydroxymethyl)phenyl)-2-methylpropan-1-one **16** (480 mg, 2.47 mmol, 1.0 equiv.) was dissolved in CH₂Cl₂ (10 mL) and methanesulfonyl chloride (283 mg, 2.47 mmol, 1.0 equiv.) and NEt₃ (250 mg, 2.47 mmol, 1.0 equiv.) were added at 0 °C. The reaction mixture was stirred for 1 h and the reaction was stopped by adding 20 mL water. The reaction mixture was extracted with dichloromethane (3 x 20 mL), the combined organic phases dried over MgSO₄ and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography using silica gel (*n*-pentane/ ethylacetate 3 : 1) to obtain the desired product **17** (346 mg, 1.27 mmol, 51%) as colorless solid.

¹H-NMR (300 MHz, CDCl₃, 299 K): δ (ppm) 8.06 (d, *J* = 8.4 Hz, 2H, 2 x CH_{Aryl}), 7.50 (d, *J* = 8.4 Hz, 2H, 2 x CH_{Aryl}), 5.28 (s, 2H, CH₂), 3.80 (s, 1H, OH), 3.01 (s, 3H, CH₃), 1.62 (s, 6H, 2 x CH₃).

¹³C-NMR (75 MHz, CDCl₃, 299 K): δ (ppm) 204.2 (CO), 138.4 (C_q), 134.8 (C_q), 130.3 (CH_{Aryl}), 128.3 (CH_{Aryl}), 76.7 (C_q), 70.1 (CH₂), 38.4 (CH₃), 28.5 (CH₃).

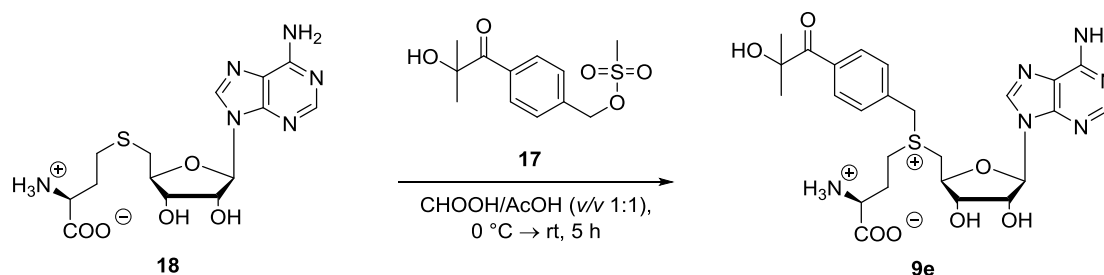
IR (ATR): $\tilde{\nu}$ (cm⁻¹) 3513 (br), 2981 (vw), 2939 (vw), 1677 (m), 1611 (w), 1462 (vw), 1352 (s), 1262 (vw), 1172 (vs), 950 (m), 829 (m), 753 (w).

Melting point: 58 °C

HR-MS (ESI): *m/z* calculated for [C₁₂H₁₆O₅SN_a]⁺ 295.0611, found: 295.0601.

SUPPORTING INFORMATION

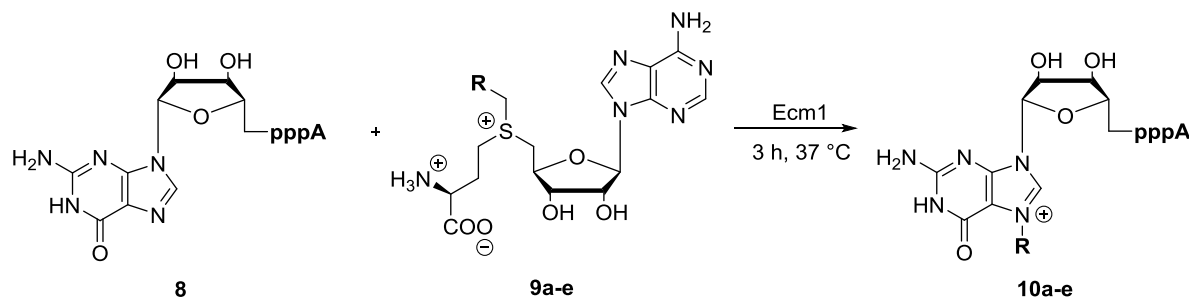
5'-[(R/S)-[(3S)-3-Amino-3-carboxypropyl] 4-(2-hydroxy-2-methyl-propanoyl)-benzylsulfonio]-5'-deoxyadenosine (AdoHAK, **9e**)



4-(2-Hydroxy-2-methylpropanoyl)benzylsulfonate **17** (100 mg, 0.29 mmol, 22 equiv.) was dissolved in a 1:1 mixture of formic acid (0.75 mL) and acetic acid (0.75 mL) at 0 °C. *S*-adenosyl-L-homocysteine **18** (5 mg, 0.013 mmol, 1 equiv.) was added and the reaction was stirred for 5 h at room temperature. Afterwards, ddH₂O (5 mL) was added and the aqueous phase was extracted with diethyl ether. The aqueous phase was lyophilized, redissolved in ddH₂O (1 mL) with 0.01 % trifluoroacetic acid and purified by semi-preparative HPLC. The collected product fractions were lyophilized, the product **9e** was taken up in 40 µL ddH₂O and respective aliquots were stored at -20 °C.

HR-MS (ESI): m/z calculated for C₂₅H₃₃N₆O₇S⁺ = 561.21 [M]⁺, found: 561.28 (MALDI-TOF analysis).

Enzymatic modification of the GpppA cap analog using Ecm1



For enzymatic modification, GpppA **8** (0.4 mM), AdoMet analogue **9a-e** (1.2 mM), AMP (1 mM, optional addition), MTAN (4 µM), LuxS (4 µM) were incubated with Ecm1 (50-70 µM) for 3 h at 37 °C in a final volume of 25 µL. The reaction was stopped by heating to 65 °C for 10 min. The denatured enzymes were removed by centrifugation for 10 min at 21130 g and 4 °C and the reaction mixture was analyzed by analytical HPLC or the modified cap analogue was isolated by analytical HPLC. The collected product fractions were lyophilized, the product **10a-d** was taken up in 10 µL ddH₂O (by a turnover of 100% GpppA) and further analyzed by HPLC or UHPLC-MS.

Irradiation of *N*7-PC-GpppA cap analogue

When irradiating non-isolated *N*7-modified cap analogue, 1/6 of the reaction mixture was diluted to a final volume of 10 µL with ddH₂O (for irradiation of **10a-c**) or with buffer (for irradiation of **10d**), irradiated using a LED ($\lambda_{\text{max}} = 365$ nm) and then analyzed by HPLC or UHPLC-MS. Non-isolated *N*7-modified cap analogue **10e** (10 µL) was irradiated using the UV-transilluminator with a polystyrene filter ($\lambda = 312$ nm) and then analyzed by HPLC or UHPLC-MS.

When irradiating purified *N*7-modified cap analogue, 1.5-2.0 µL of isolated approach was diluted to a final volume of 10 µL with ddH₂O (for irradiation of **10a-c**) or buffer (for irradiation of **10d**), irradiated using a LED ($\lambda_{\text{max}} = 365$ nm) and then analyzed by HPLC or UHPLC-MS. For further digestion of the photo product of irradiated *N*7-ONB-GpppA **10a**, a complete approach of purified *N*7-ONB-GpppA **10a** was irradiated as described above and the photo product was isolated by analytical HPLC. The collected product fractions were lyophilized, the photo product **11a** was taken up in 10 µL ddH₂O and then analyzed by UHPLC-MS.

Enzymatic digestion of cap analogue

SUPPORTING INFORMATION

For enzymatic digestion, GpppA **8** (0.5 mM), purified *N7*-ONB-GpppA **9a** (10 μ L) or isolated photo product **11a** (10 μ L) were digested in 7.5 μ L digestion buffer (0.11 M Tris-HCl (pH 8.9), 0.11 M NaCl, 15 mM MgCl₂) with Snake Venom phosphodiesterase (0.05 U) for 2 h at 37 °C in a final volume of 20 μ L. Then, FastAP (1 U) was added and the mixture was incubated for additional 30 min at 37 °C. Enzymes were precipitated with HClO₄ (200 mM) by incubation for 10 min at room temperature and further analyzed by UHPLC-MS.

Chemical modification of short RNA

For the chemical modification, short RNA (**RNA1**: 40 μ M, **RNA2**: 200 μ M, **RNA3**: 200 μ M and **RNA4**: 20 μ M) was incubated with bromide **2c** (1-2.5 mM) in a ddH₂O-DMSO mixture for 24 h at 37 °C in a final volume of 5 μ L. Then, the sample was dialyzed for 10 min, the sample volume was adjusted with ddH₂O to 20 μ L and 20 μ L buffer (100 mM Tris-HCl (pH 7.4) and 2 mM EDTA) was added. For irradiation, half of the sample (20 μ L) was irradiated using a LED ($\lambda_{\text{max}} = 365$ nm) for 10 min. The modified RNA was analyzed by 15% denatured PAGE. For UHPLC-MS analysis the modified RNA was digested with nuclease P1 (0.13U) and 5.1 μ L 10x P1 buffer (0.2 M NH₄OAc, 1 mM ZnCl₂) for 1 h at 50 °C and then with FastAP (1 U) for 1 h at 37°C. Enzymes were denatured by incubation for 10 min at 65 °C.

Enzymatic plasmid DNA modification and subsequent UV irradiation

The plasmid pBR322 (0.5 μ g) was incubated with AdoBP **9d** (4 mM), M. TaqI (20 U), MTAN (4 μ M), LuxS (4 μ M) in 1x CutSmart buffer (NEB, 50 mM KOAc, 20 mM Tris-OAc, 10 mM Mg₂OAc, 100 μ g/mL BSA, pH 7.9) in a total volume of 10 μ L for 2 h at 60 °C. For photocleavage, the respective samples were irradiated with UV light ($\lambda_{\text{max}} = 365$ nm) for 10 min. Or 5 μ L buffer (same as for irradiation of cap analogues) were added to the respective sample and irradiated as described. Afterwards, 15 μ L of 1x CutSmart buffer or 10 μ L of 1.5x CutSmart buffer for irradiated samples with buffer and R. TaqI (5 U) were added to the reactions and the mixture was incubated for 45 min at 65 °C. Plasmid DNA was linearized by incubation with BamHI for 45 min at 37 °C and deactivated for 5 min at 80 °C. Samples were directly loaded onto a 1% agarose gel (100 V, 1 h). The gel was stained with ethidium bromide and scanned using a Typhoon FLA9500 laser scanner (GE healthcare).

Enzymatic modification of short DNA and subsequent digestion for mass analysis

For the production of short double stranded DNA (dsDNA), the two complementary sDNAs were hybridized in a 1:1 (v/v) mixture at 95 °C for 5 min and cooled to room temperature. DsDNA (500 pmol) was incubated with AdoBP **9d** (2.5 mM), M. TaqI (30 U), MTAN (4 μ M) and LuxS (4 μ M) in 1x CutSmart buffer (NEB) in a total volume of 20 μ L for 3 h at 37 °C. Proteinase K (0.6 μ U) was added to the reactions followed by incubation for 1 h at 55 °C. Then, nuclease P1 (0.33 U) and FastAP (1 U) were added to the reaction followed by incubation over night at 37 °C. Samples were directly used for UHPLC-MS measurements.

Expression of DcpS

The plasmid pET28b hDcpS H277N was transformed into the *Escherichia coli* strain BL21 (DE3) and used directly for the inoculation of the 30 ml LB-kanamycin culture. The culture was grown overnight at 37°C with constant shaking at 200 rpm. Five 400 ml LB-cultures were each inoculated with 5 ml overnight culture and grown at 37°C, 200 rpm until the OD₆₀₀ reached 1.6. The cultures were cooled to room temperature for 30 min and then IPTG added to 1 mM and grown further at 25°C, 200 rpm for 4h. The cells were harvested by centrifugation, the cell pellets frozen in liquid nitrogen and stored at -20°C until use.

Purification of DcpS

The thawed cell pellet was resuspended in 10 ml lysis buffer (50 mM Tris-HCl pH 8, 350 mM NaCl, 20 mM imidazole, 20% sucrose and 1 mM mercaptoethanol) and PMSF added to 300 μ M. The cells were lysed by sonication, 0.5 sec on, 0.5 off at 30% for 3 min (repeated 3 times). The supernatant was obtained by centrifugation at 12000 rpm for 30 min at 4°C. This was then filtered through 0.4 μ m membrane and loaded on 1 ml HisTrap column. The column was washed with 5 % buffer B (50 mM Tris-HCl pH 8, 350 mM NaCl, 250 mM imidazole, 20% sucrose and 1 mM mercaptoethanol) before elution with 5-100% buffer B gradient in 10 c.v. The eluate fractions were pooled and dialyzed against dialysis buffer (10 mM Tris-HCl pH 8, 25 mM NaCl and 1 mM mercaptoethanol.) overnight and then concentrated to 0.3 ml. 10 μ l aliquots were frozen in liquid nitrogen and stored at -80°C. The protein concentration was determined by comparison with BSA standard on SDS-PAGE gel.

Expression of eIF4E

SUPPORTING INFORMATION

The plasmid pET16b-eIF4E was transformed into the *Escherichia coli* strain BL21 (DE3) and used directly for the inoculation of the 30 ml LB-ampicillin culture. The culture was grown overnight at 37°C with constant shaking at 200 rpm. Five 400 ml LB-ampicillin cultures were each inoculated with 5 ml overnight culture and grown at 37°C, 200 rpm until the OD₆₀₀ reached 0.6. IPTG added to 1 mM and the cells grown further at 37°C, 200 rpm for 3h. The cells were harvested by centrifugation, the cell pellets frozen in liquid nitrogen and stored at -20°C until use.

Purification of eIF4E

The thawed cell pellet was resuspended in 10 ml lysis buffer (50 mM sodium phosphate pH 7.5, 300 mM NaCl, 10 mM imidazole) and PMSF added to 300 μ M. The cells were lysed by sonication, 0.5 sec on, 0.5 off at 30% for 3 min (repeated 3 times). The supernatant was obtained by centrifugation at 12000 rpm for 30 min at 4°C. This was then filtered through 0.4 μ m membrane and loaded on 1 ml HisTrap column. The column was washed with 5 % buffer B (50 mM sodium phosphate pH 7.5, 300 mM NaCl, 250 mM imidazole) before elution with 5-100% buffer B gradient in 10 c.v. The eluate fractions were pooled and dialyzed against dialysis buffer (20 mM Hepes pH 7.5, 50 mM KCl, 0.2 mM EDTA, 7 mM mercaptoethanol and 0.01% Triton X-100) overnight and then concentrated to 0.3 ml. 10 μ l aliquots were frozen in liquid nitrogen and stored at -80°C. The protein concentration was determined by comparison with BSA standard on SDS-PAGE gel.

Microscale thermophoresis measurements

Microscale thermophoresis (MST) measurements were performed on a Monolith NT.115 series instrument (NanoTemper). Prior to thermophoresis measurements proteins were labeled by incubation with Cy5-NHS (Lumiprobe) for 30 min at room temperature. Unreacted dye was separated from the protein using PD SpinTrap™ G-25 gel filtration columns (GE Healthcare) according to the manufacturer protocol. Serial dilutions of the cap analogues (starting from 300 μ M of cap analog) in reaction buffer pH 8 containing 0.025% Tween-20 were prepared and mixed with an equal volume of the labeled protein (~50 nM). The mixture was filled into premium coated capillaries (4 μ L) and directly measured. MST power was set to 30-40%, LED power was set to 20% Red (Exc.: 625 nm, Em.: 680 nm). Thermophoresis measurements were performed with the following settings: fluorescence before (5 s), MST on (30 s), fluorescence after (3 s). The capillaries were measured three times in direct succession. MST data were normalized to baseline differences and K_d values were calculated using nonlinear regression assuming a Hill coefficient of 1.0 (GraphPad Prism).

The remethylated cap analogs were produced by irradiation of compound **10d** with subsequent enzymatic modification with Ecm1 and the natural AdoMet. The MST measurements of the unmethylated GpppA cap analogue, the commercially available m⁷GpppA cap analogue and the enzymatically synthesized m⁷GpppA cap analogue (Figure S39) served as controls and resulted in values corresponding to the ranges of previous reported data (Table S1).^[3a, 4]

Table S1. Approximate binding affinities between mRNA cap analogues and the cap-binding proteins eIF4E from *S. cerevisiae* and DcpS H227N from *humans*.

	eIF4E <i>Saccharomyces cerevisiae</i>		DcpS H227N <i>human</i>	
	K_d [μ M]	Times measured	K_d [μ M]	Times measured
GpppA	No binding observed	1	No binding observed	1
m ⁷ GpppA (commercially)	~ 0.60	1	~ 0.70	2
m ⁷ GpppA (enzymatically)	~ 0.47	2	~ 0.78	4
N7-BP-GpppA	No binding observed	2	No binding observed	1
remethylated m ⁷ GpppA	~ 0.85	4	~ 1.98	2

Enzymatic synthesis of capped Firefly mRNA

For the production of the Firefly-mRNAs the respective Firefly pMRNA[®] vector was used.^[1] The respective DNA template was amplified in 1 \times HF puffer using plasmid (70 ng), dNTP mix (0.5 mM), forward primer (0.5 μ M), reverse primer (0.5 μ M) and Phusion High-Fidelity DNA Polymerase (1 U). The N7-BP-GpppG cap analogue was enzymatically prepared and purified as described above, GpppG was used instead of GpppA. Then, *in vitro* T7 transcription was performed in 1 \times transcription buffer using DNA template (100 ng), A/C/UTP mix (0.5 mM), GTP (0.1 mM), cap analog (1 mM GpppG, ~ 0.6 mM N7-BP-GpppG), RiboLock RNase Inhibitor (30 U), T7 RNA polymerase (50 U) and pyrophosphatase (0.1 U) for 3 h at 37 °C. Remaining DNA template was digested by incubation with 2 U DNase I for 1 h at 37°C and then, mRNAs were purified using the RNA Clean & Concentrator™-5 Kit (Zymo Research). Then, the modified mRNA was irradiated in ddH₂O using a LED (λ_{max} = 365 nm) for 10 min and subsequently analyzed by 7.5% denat. PAGE.

SUPPORTING INFORMATION

Supplementary Figures

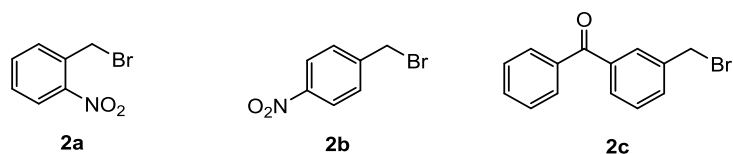


Figure S3. Chemical structure of bromides 1-(1-bromomethyl)-2-nitrobenzene **2a**, 1-(1-bromomethyl)-4-nitrobenzene **2b** and 4-bromomethylbenzophenone **2c**.

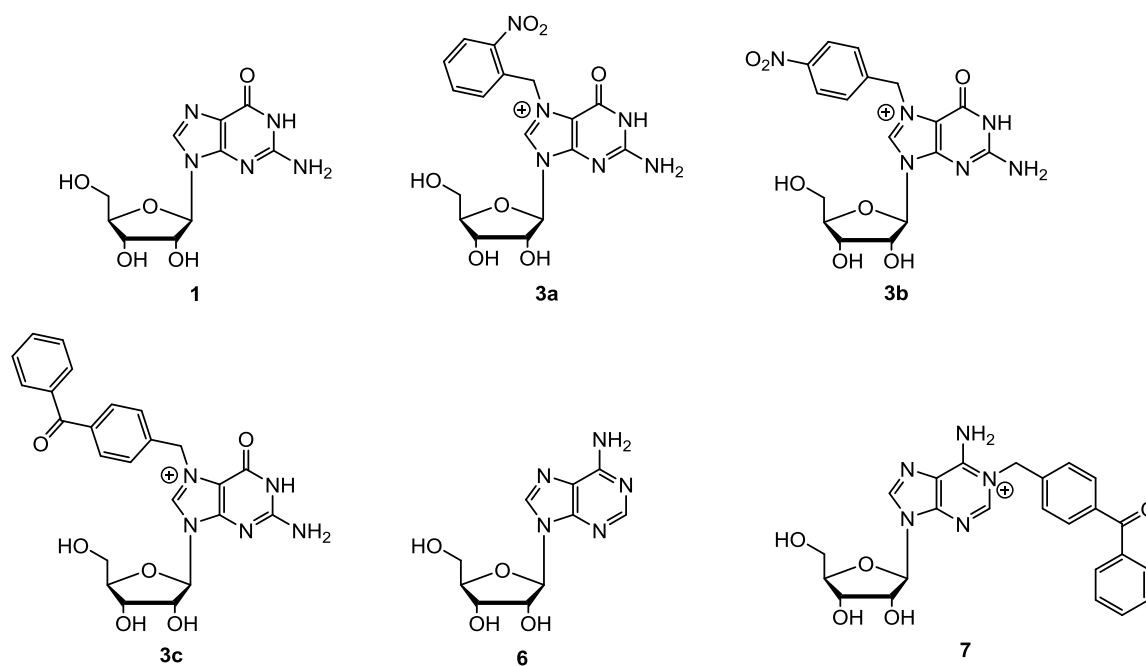


Figure S4. Chemical structures of guanosine **1**, *N*7-photocaged guanosine derivatives *N*7-ONB-guanosine **3a**, *N*7-PNB-guanosine **3b** and *N*7-BP-guanosine **3c**, adenosine **6** and *N*1-benzophenone-modified adenosine **7**.

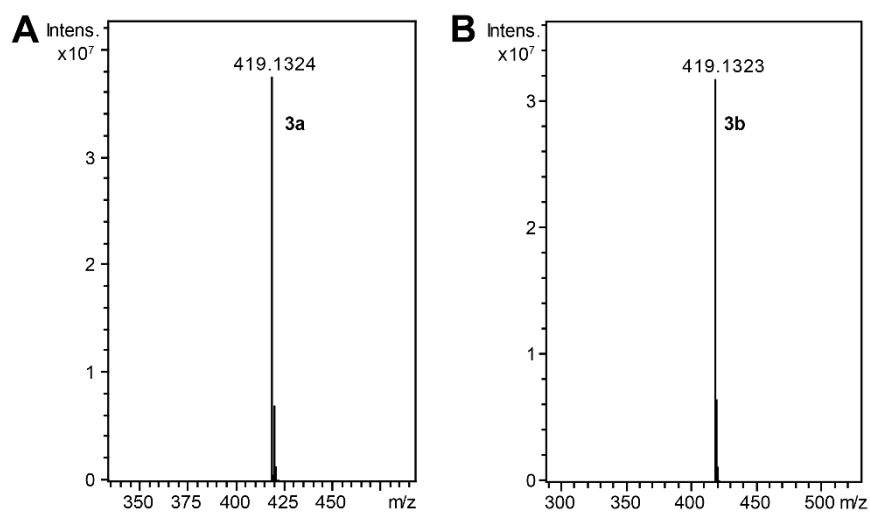


Figure S5. Mass spectrometric analysis of purified *N*7-photocaged guanosine derivatives **3a-b** (A-B). **(A) 3a**: Calculated mass of $[C_{17}H_{19}N_6O_7]^+$ = 419.1310 [M]⁺, found: 419.1324. **(B) 3b**: Calculated mass of $[C_{17}H_{19}N_6O_7]^+$ = 419.1310 [M]⁺, found: 419.1323.

SUPPORTING INFORMATION

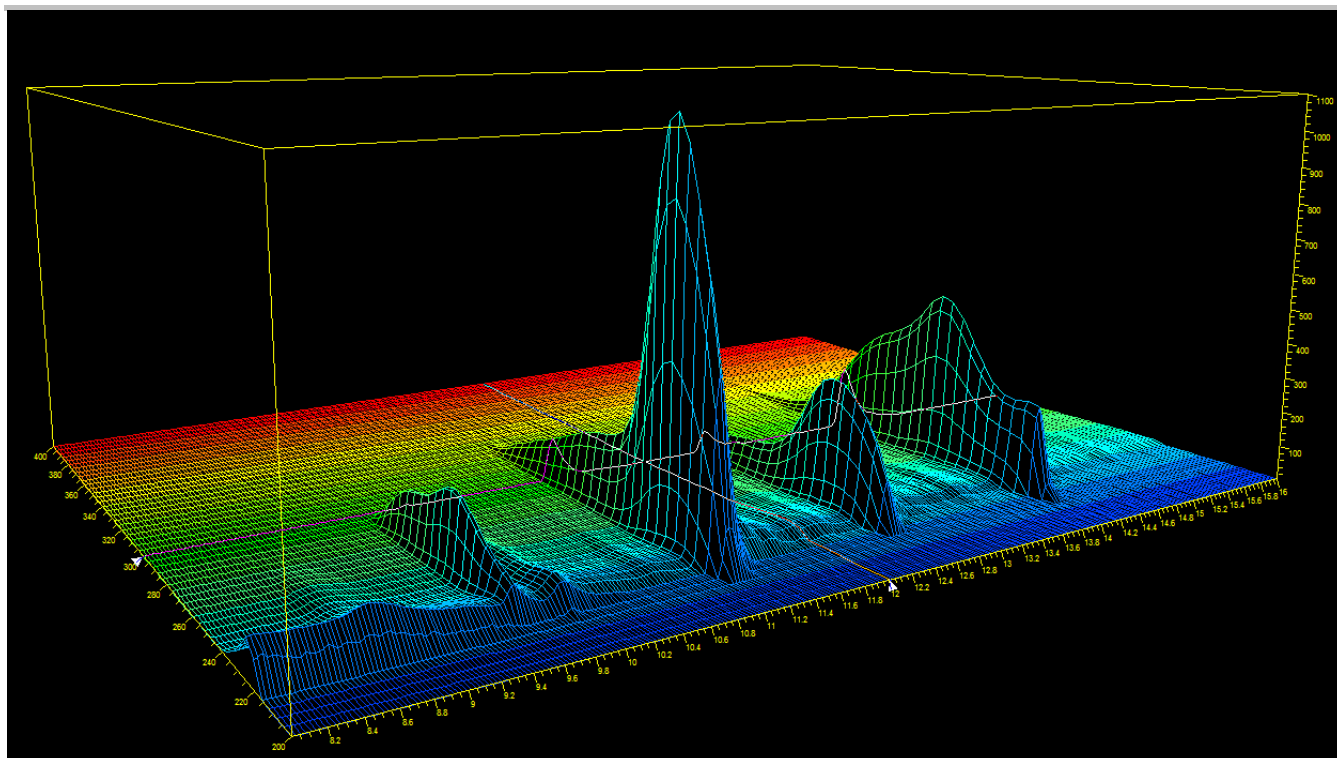


Figure S6. Section of the three-dimensional spectrum of irradiated *N7*-ONB-guanosine **3a** and formation of photo product **4**. 3D-plot shows section from 8-16 min and $\lambda = 200$ -400 nm.

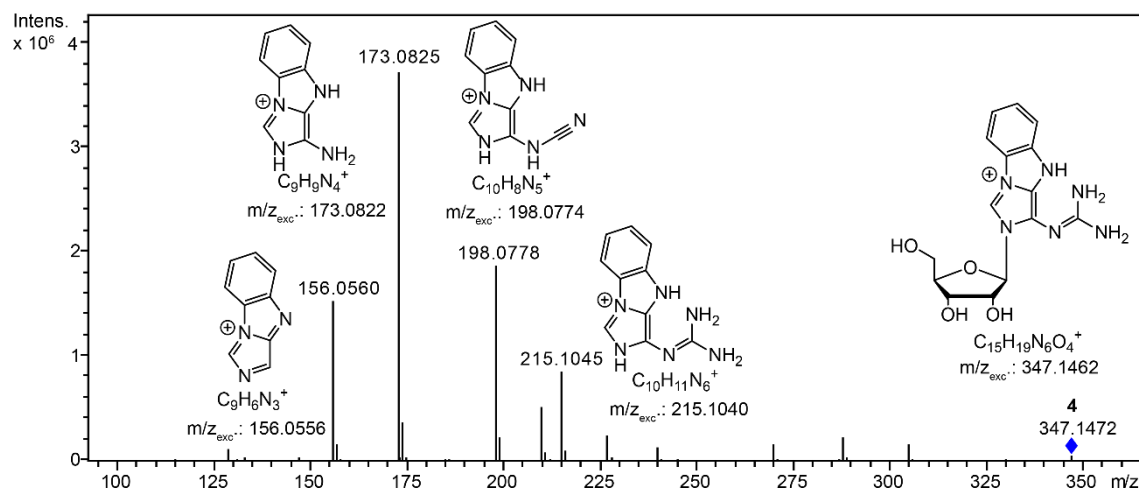


Figure S7. MS/MS analysis of isolated photo product **4**. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond. **4**: Calculated mass of $[C_{15}H_{19}N_6O_4]^+ = 347.1462$ [M]⁺, found: 347.1472.

SUPPORTING INFORMATION

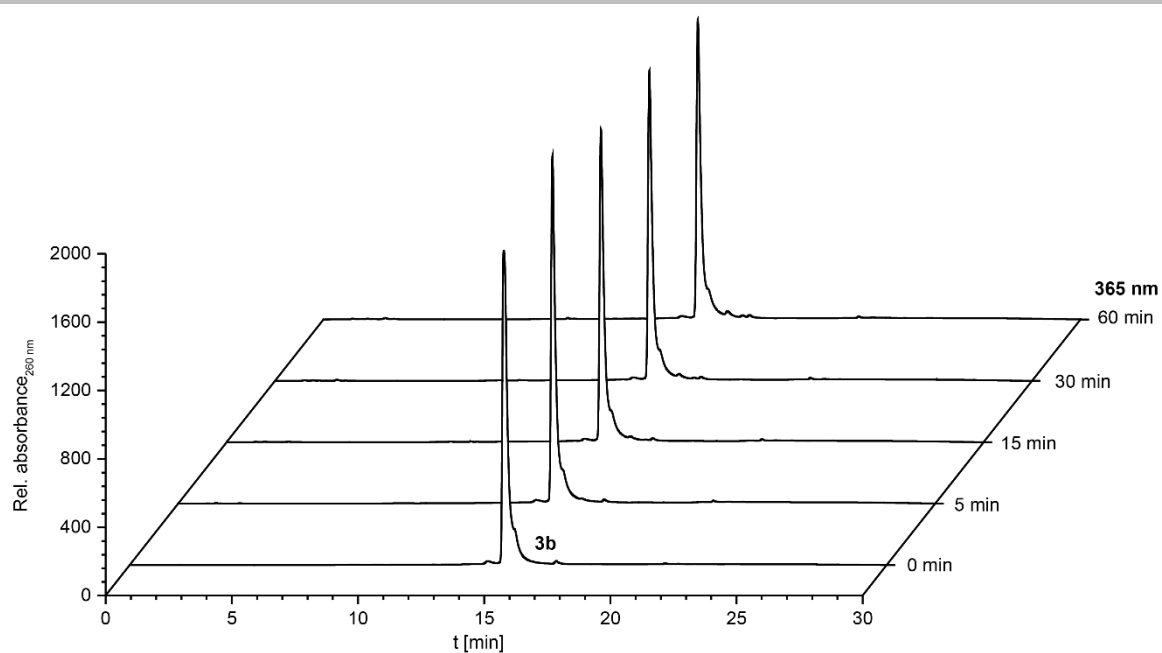


Figure S8. Irradiation of *N7*-PNB-guanosine **3b**. *N7*-PNB-guanosine **3b** was irradiated in ddH₂O using a LED ($\lambda_{\text{max}} = 365 \text{ nm}$) for different times. HPLC analysis showed no cleavage of the **3b**.

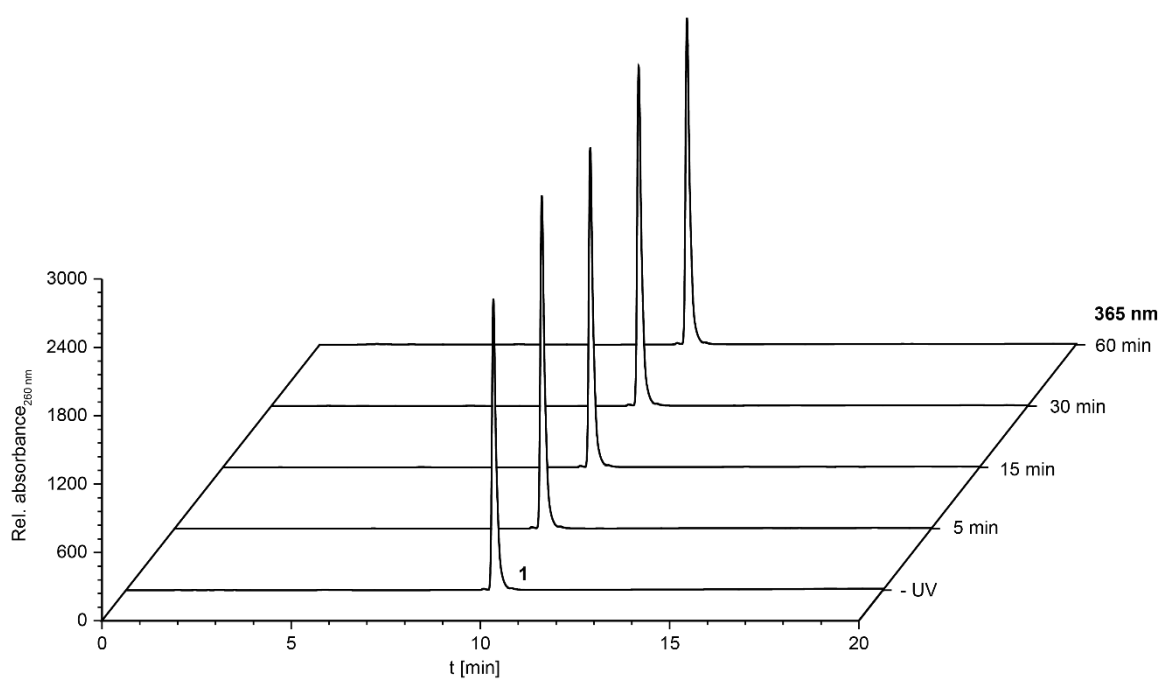


Figure S9. Photo stability of guanosine **1**. Guanosine **1** was irradiated in ddH₂O using an LED ($\lambda_{\text{max}} = 365 \text{ nm}$) for different times and analyzed by HPLC.

SUPPORTING INFORMATION

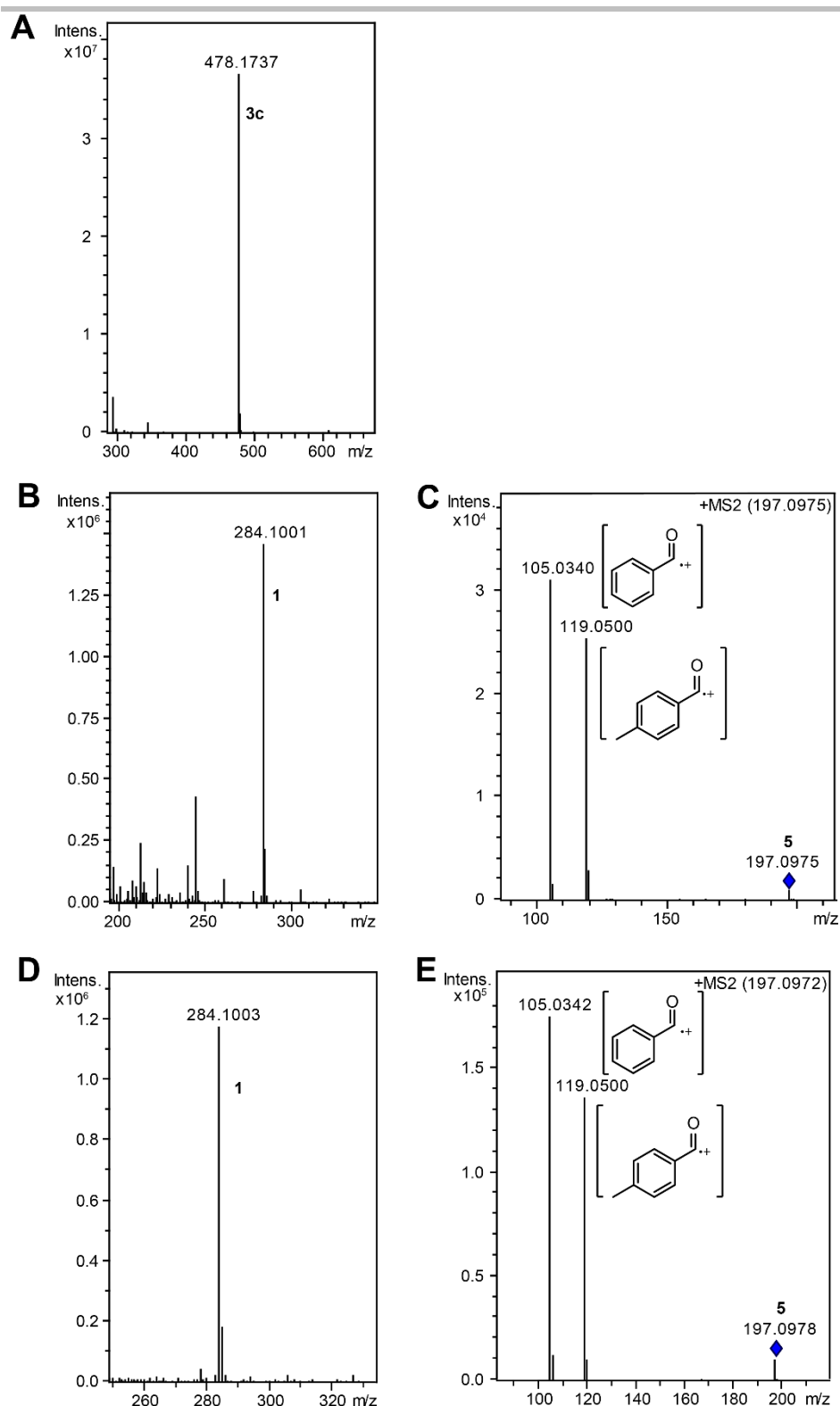


Figure S10. Mass spectrometric analysis of purified *N7*-BP-guanosine **3c** and of photocleavage products **1** and **5** after irradiation of **3c**. **(A)** **3c**: Calculated mass of $[\text{C}_{24}\text{H}_{24}\text{N}_5\text{O}_6]^+ = 478.1721$ $[\text{M}]^+$, found: 478.1737. **(B,C)** Irradiation in buffer. **(B)** **1**: Calculated mass of $[\text{C}_{10}\text{H}_{14}\text{N}_5\text{O}_5]^+ = 284.0989$ $[\text{M}+\text{H}]^+$, found: 284.1001. **(C)** MS/MS analysis of **5**. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond. **5**: Calculated mass of $[\text{C}_{14}\text{H}_{13}\text{O}]^+ = 197.0961$ $[\text{M}+\text{H}]^+$, found: 197.0968. **(D,E)** Irradiation in buffer used for irradiation of RNA. **(D)** **1**: Calculated mass of $[\text{C}_{10}\text{H}_{14}\text{N}_5\text{O}_5]^+ = 284.0989$ $[\text{M}+\text{H}]^+$, found: 284.1003. **(E)** MS/MS analysis of **5**. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond. **5**: Calculated mass of $[\text{C}_{14}\text{H}_{13}\text{O}]^+ = 197.0975$ $[\text{M}+\text{H}]^+$, found: 197.0978.

SUPPORTING INFORMATION

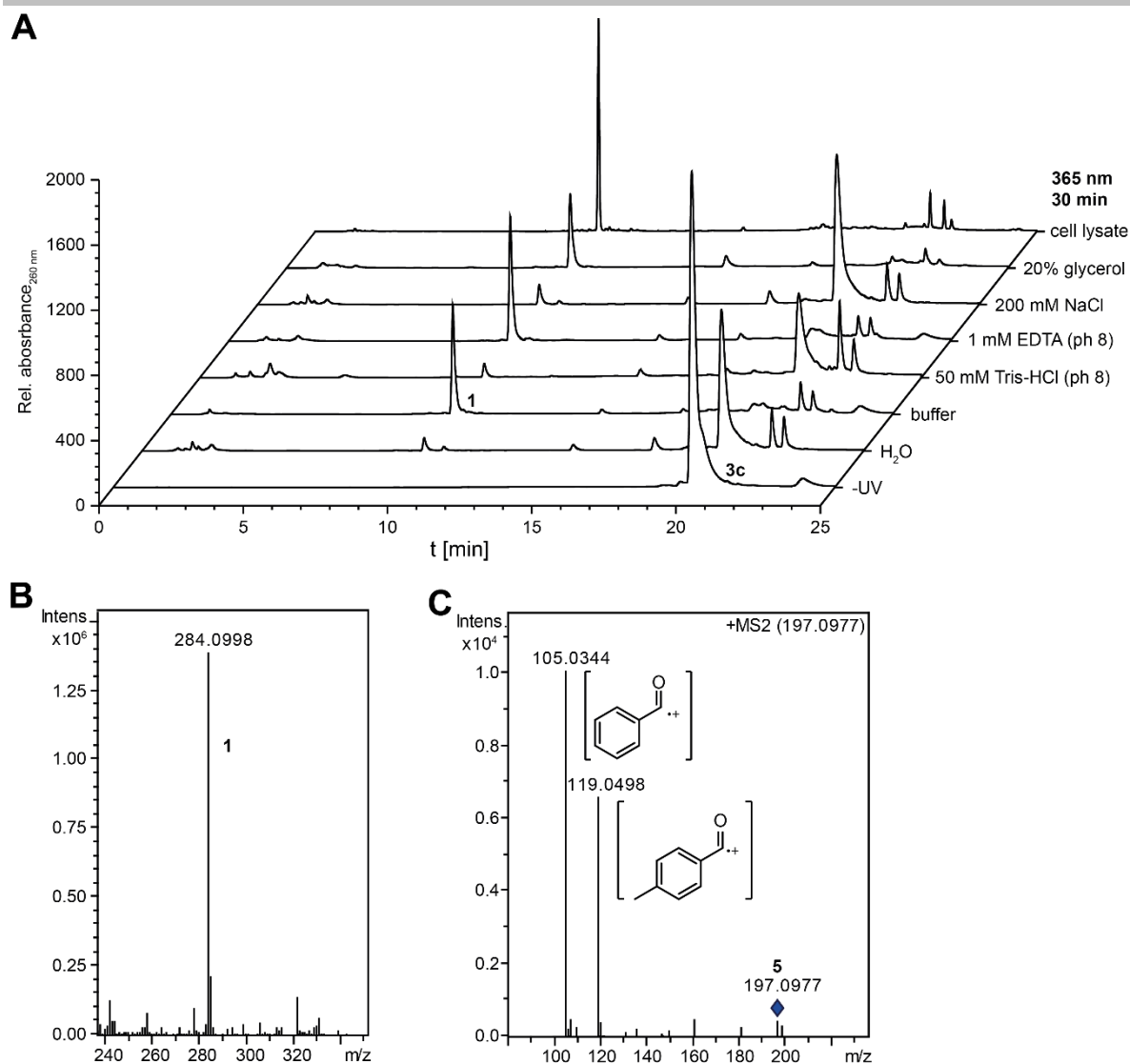


Figure S11. Irradiation of purified *N7*-BP-guanosine **3c**. **(A)** Purified *N7*-BP-guanosine **3c** was irradiated in ddH₂O, in buffer, in aqueous solution under addition of individual buffer components or in cell lysate using a LED ($\lambda_{\text{max}} = 365$ nm) for 30 min. HPLC analysis showed no recovery of guanosine **1** in ddH₂O, 50 mM Tris-HCl and 200 mM NaCl, however recovery of guanosine **1** in buffer, 1 mM EDTA, 20% glycerol and cell lysate. **(B,C)** Mass spectrometric analysis of photocleavage products after irradiation of *N7*-BP-guanosine **3c**. **(B)** **1**: Calculated mass of $[\text{C}_{10}\text{H}_{14}\text{N}_5\text{O}_5]^+ = 284.0989$ $[\text{M}+\text{H}]^+$, found: 284.0998. **(C)** MS/MS analysis of **5**. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond. **5**: Calculated mass of $[\text{C}_{14}\text{H}_{13}\text{O}]^+ = 197.0961$ $[\text{M}+\text{H}]^+$, found: 197.0977.

SUPPORTING INFORMATION

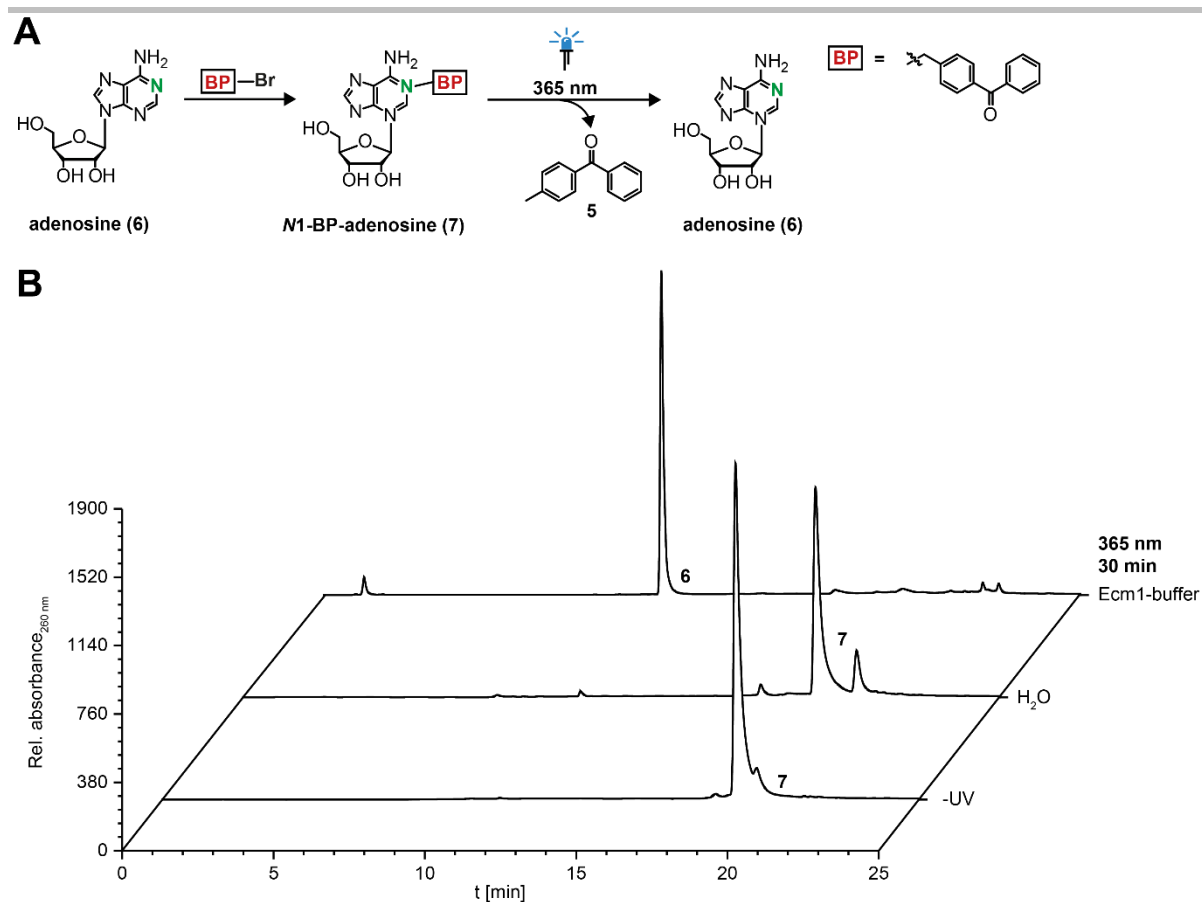


Figure S12. Irradiation of purified *N*1-BP-adenosine **7** in H₂O or in buffer. **(A)** Concept scheme. **(B)** Purified *N*1-BP-adenosine **7** was irradiated in ddH₂O or in buffer using a LED ($\lambda_{\text{max}} = 365 \text{ nm}$) for 30 min. HPLC analysis showed no recovery of adenosine **6** in ddH₂O, however recovery of adenosine **6** in buffer.

SUPPORTING INFORMATION

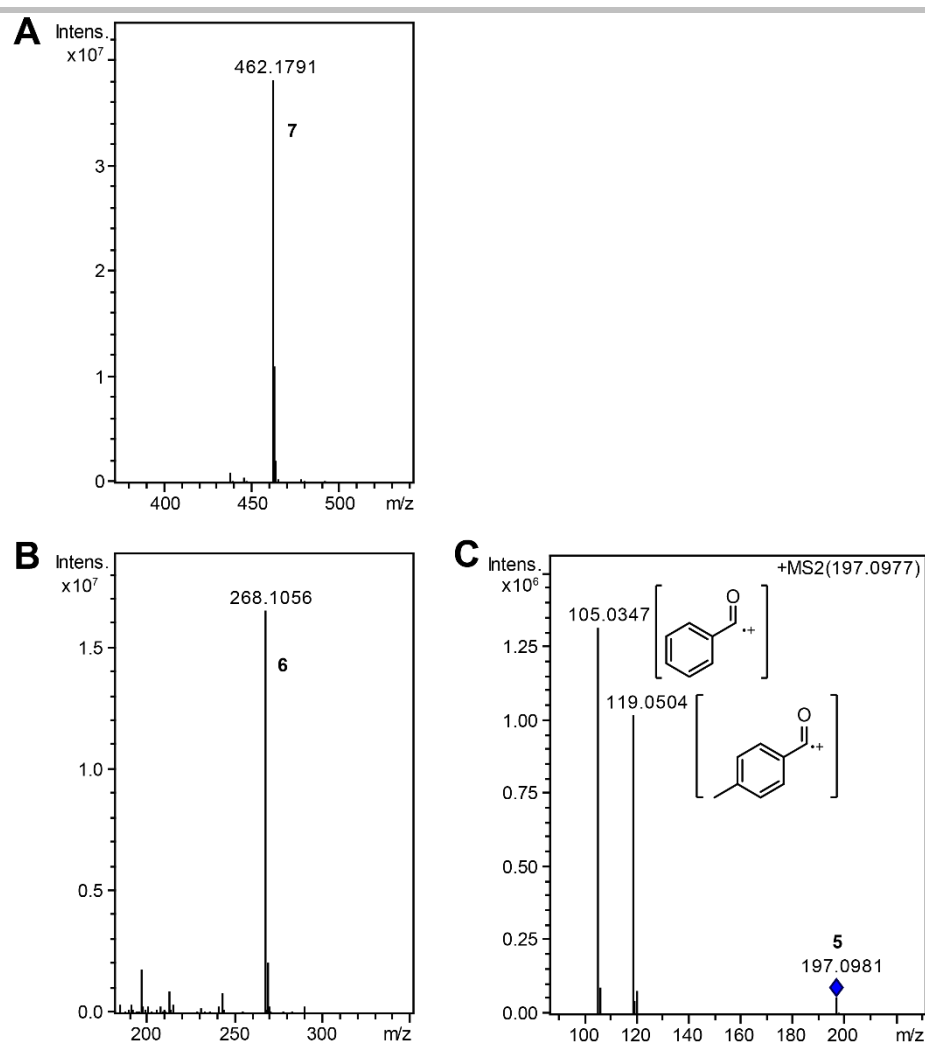


Figure S13. Mass spectrometric analysis of purified M1-BP-adenosine **7** and of photocleavage products **6** and **5** after irradiation of **7**. **(A)** **7**: Calculated mass of [C₂₄H₂₄N₅O₅]⁺ = 462.1772 [M]⁺, found: 462.1791. **(B,C)** Irradiation in buffer. **(B)** **6**: Calculated mass of [C₁₀H₁₄N₅O₄]⁺ = 268.1040 [M+H]⁺, found: 268.1056. **(C)** MS/MS analysis of **5**. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond. **5**: Calculated mass of [C₁₄H₁₃O]⁺ = 197.0961 [M+H]⁺, found: 197.0981.

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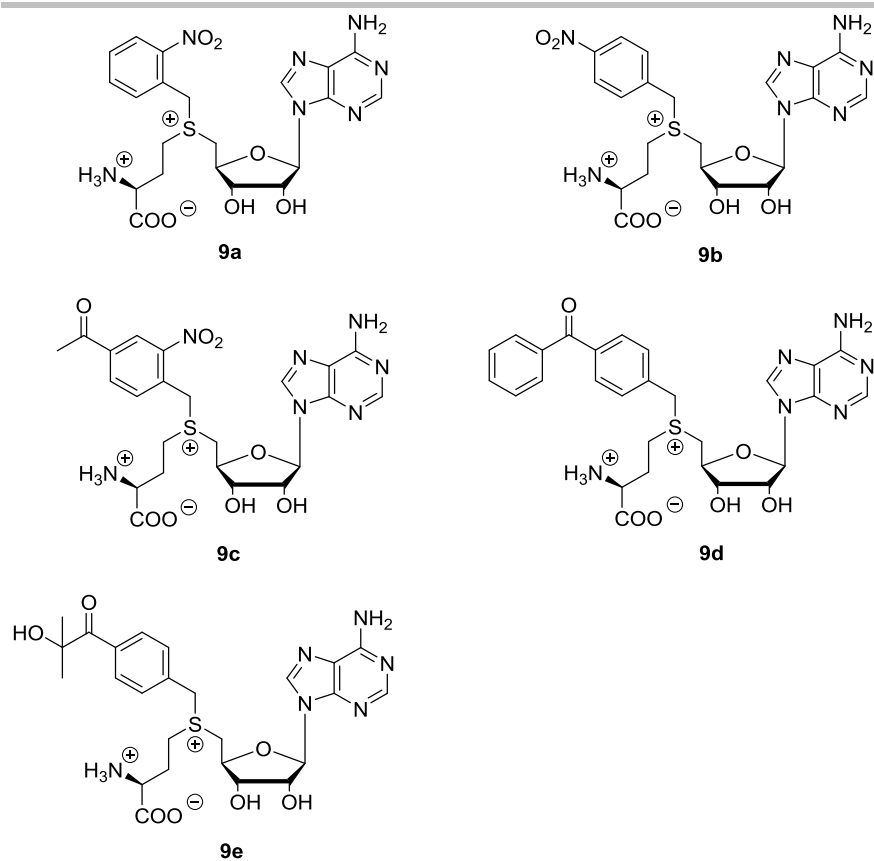


Figure S14. Chemical structure of AdoMet analogues AdoONB **9a**, AdoPNB **9b**, AdoANB **9c**, AdoBP **9d** and AdoHAK **9e**.

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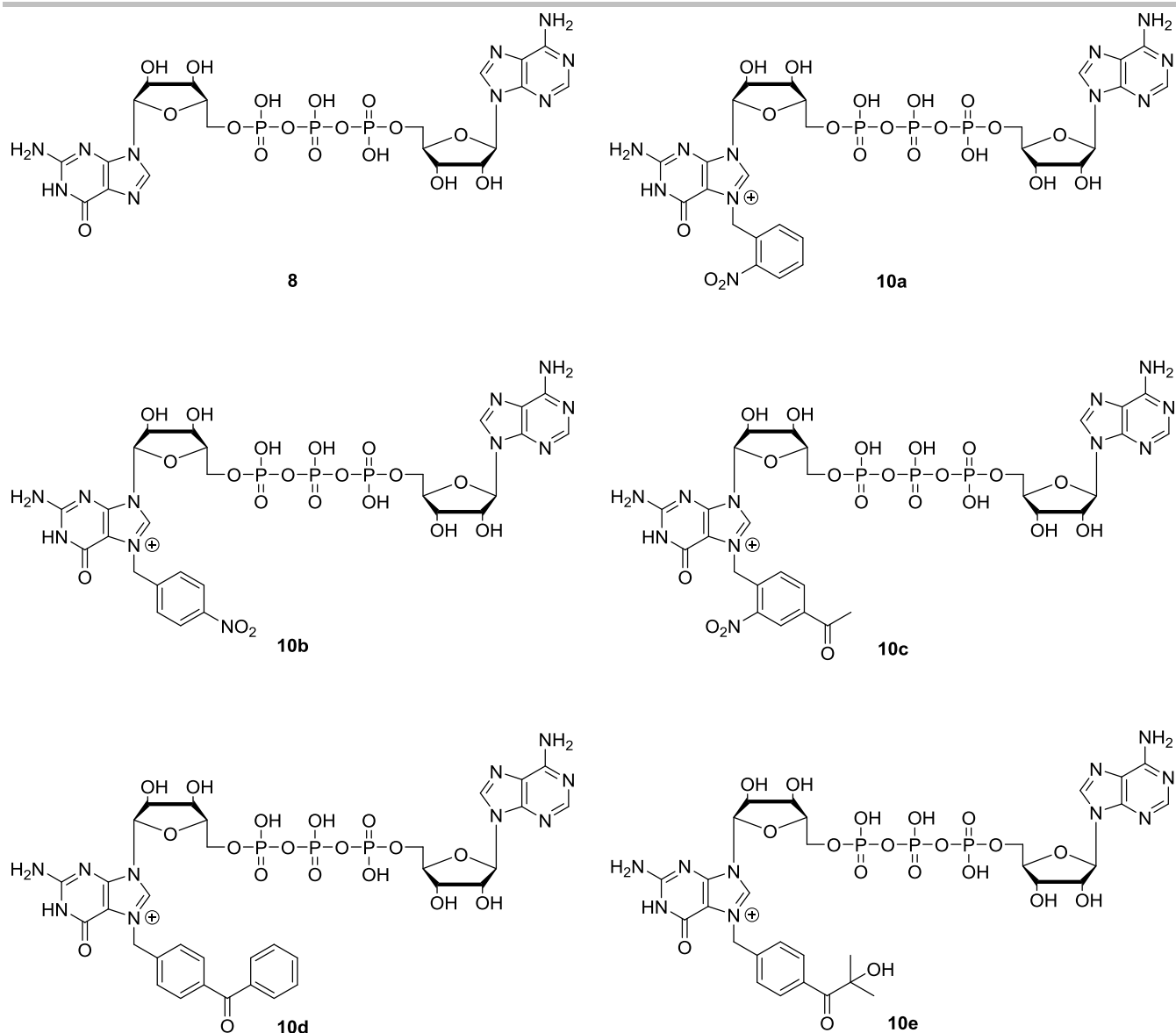


Figure S15. Chemical structures of G(5')ppp(5')A cap analogue **8** and N7-photocaged cap analogues N7-ONB-GpppA **10a**, N7-PNB-GpppA **10b**, N7-ANB-GpppA **10c**, N7-BP-GpppA **10d** and N7-HAK-GpppA **10e**.

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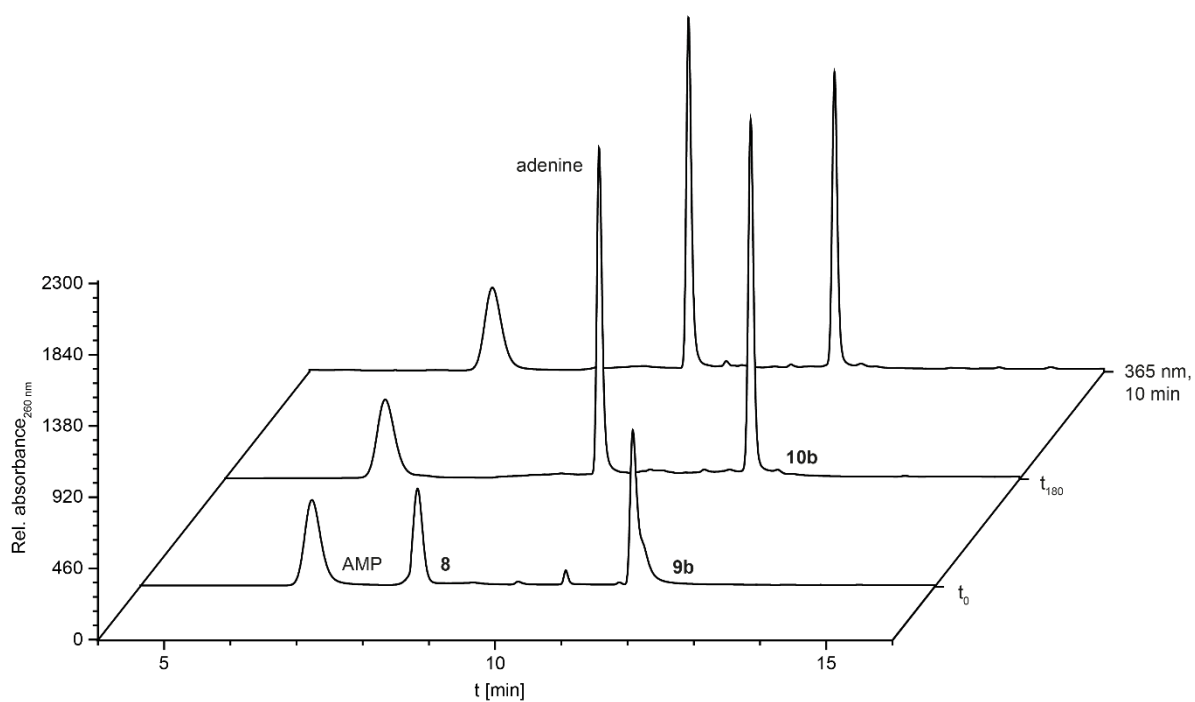


Figure S16. Enzymatic installation of *N*7-PNB-GpppA **10b** and subsequent irradiation. GpppA **8** was enzymatically modified using Ecm1 and AdoPNB **9b** and then the unpurified *N*7-PNB-GpppA **10b** was irradiated in ddH₂O using a LED ($\lambda_{\text{max}} = 365 \text{ nm}$) for 10 min. HPLC analysis showed no cleavage of the **10b**.

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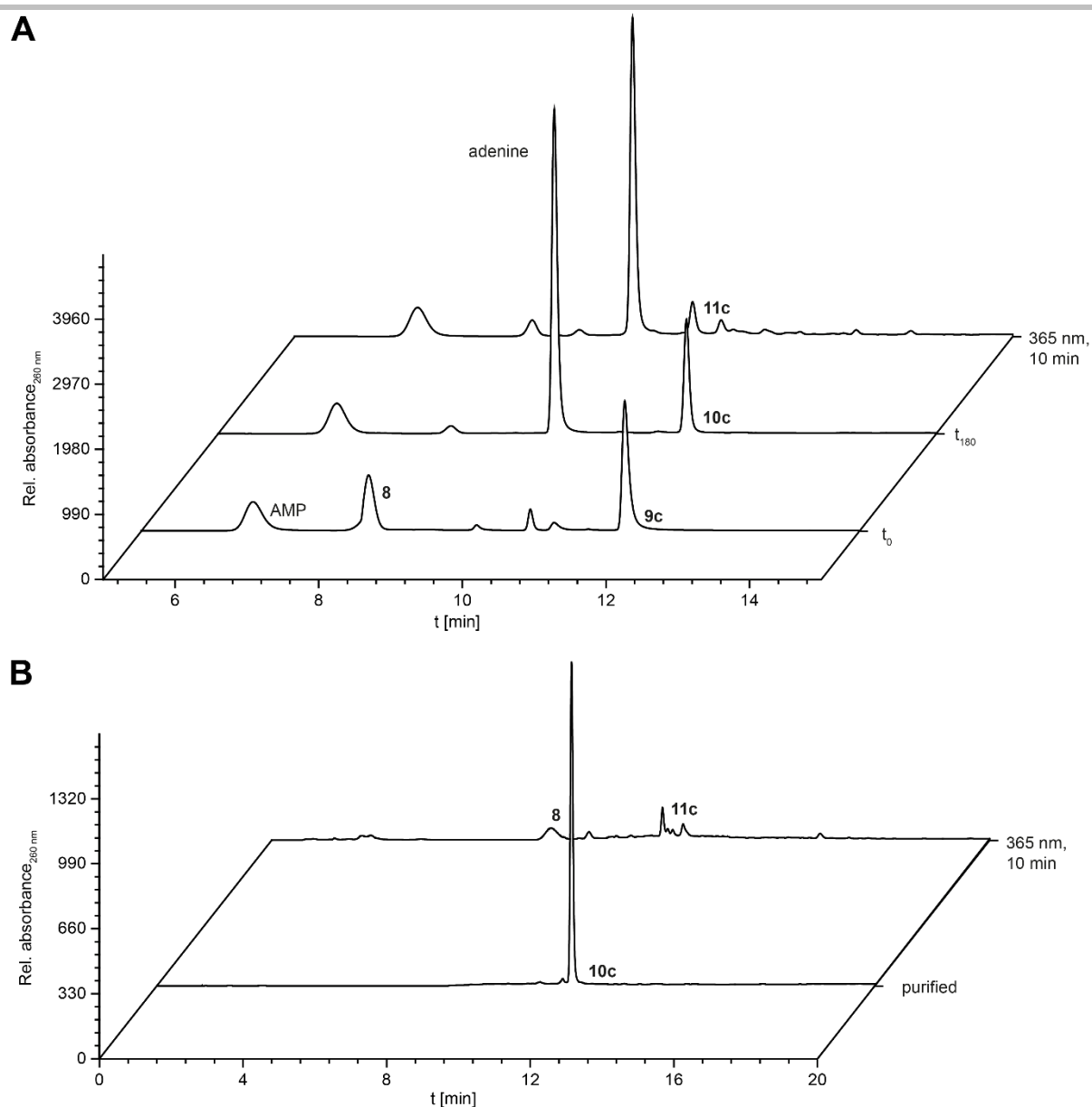


Figure S17. Enzymatic installation of *N7*-ANB-GpppA **10c** and subsequent irradiation. (A) GpppA **8** was enzymatically modified using Ecm1 and AdoANB **9c** and then the unpurified *N7*-ANB-GpppA **10c** was irradiated in ddH₂O using a LED ($\lambda_{\text{max}} = 365$ nm) for 10 min. HPLC analysis showed no recovery of GpppA **8** instead the formation of a new photo product **11c**. (B) Purified *N7*-ANB-GpppA **10c** was irradiated in ddH₂O using a LED ($\lambda_{\text{max}} = 365$ nm) for 10 min. HPLC analysis showed no recovery of GpppA **8** instead the formation of a new photo product **11c**.

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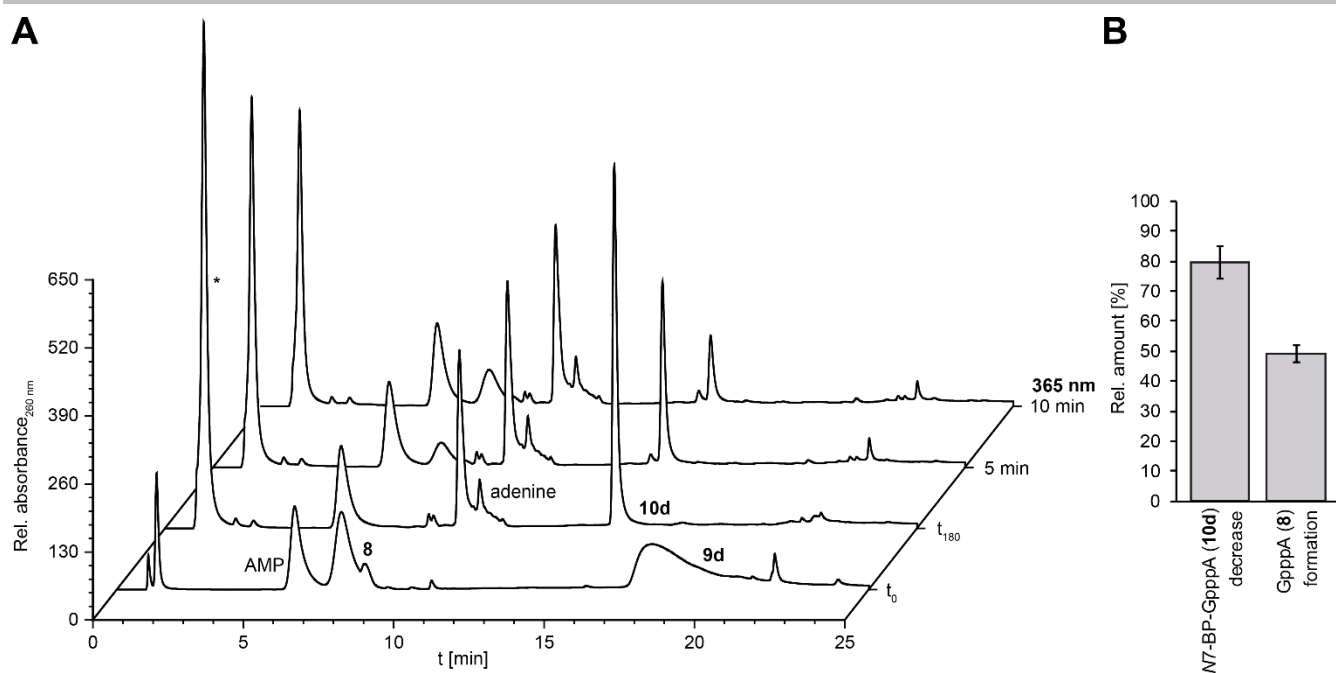


Figure S18. Enzymatic modification to and irradiation of *N7*-BP-GpppA **10d**. (A) GpppA **8** was enzymatically modified using Ecm1 and AdoBP **9d** and then the unpurified *N7*-BP-GpppA **10d** was irradiated in buffer using a LED ($\lambda_{\text{max}} = 365$ nm) for different times. HPLC analysis showed recovery of GpppA **8** and decrease of *N7*-BP-GpppA **10d** (Signal marked with an * refer to compounds of the buffer). (B) Decrease of **10d** and recovery of **8** after irradiation (365 nm, 10 min), analyzed by HPLC. Data and error bars show averages and standard deviations of three independent experiments.

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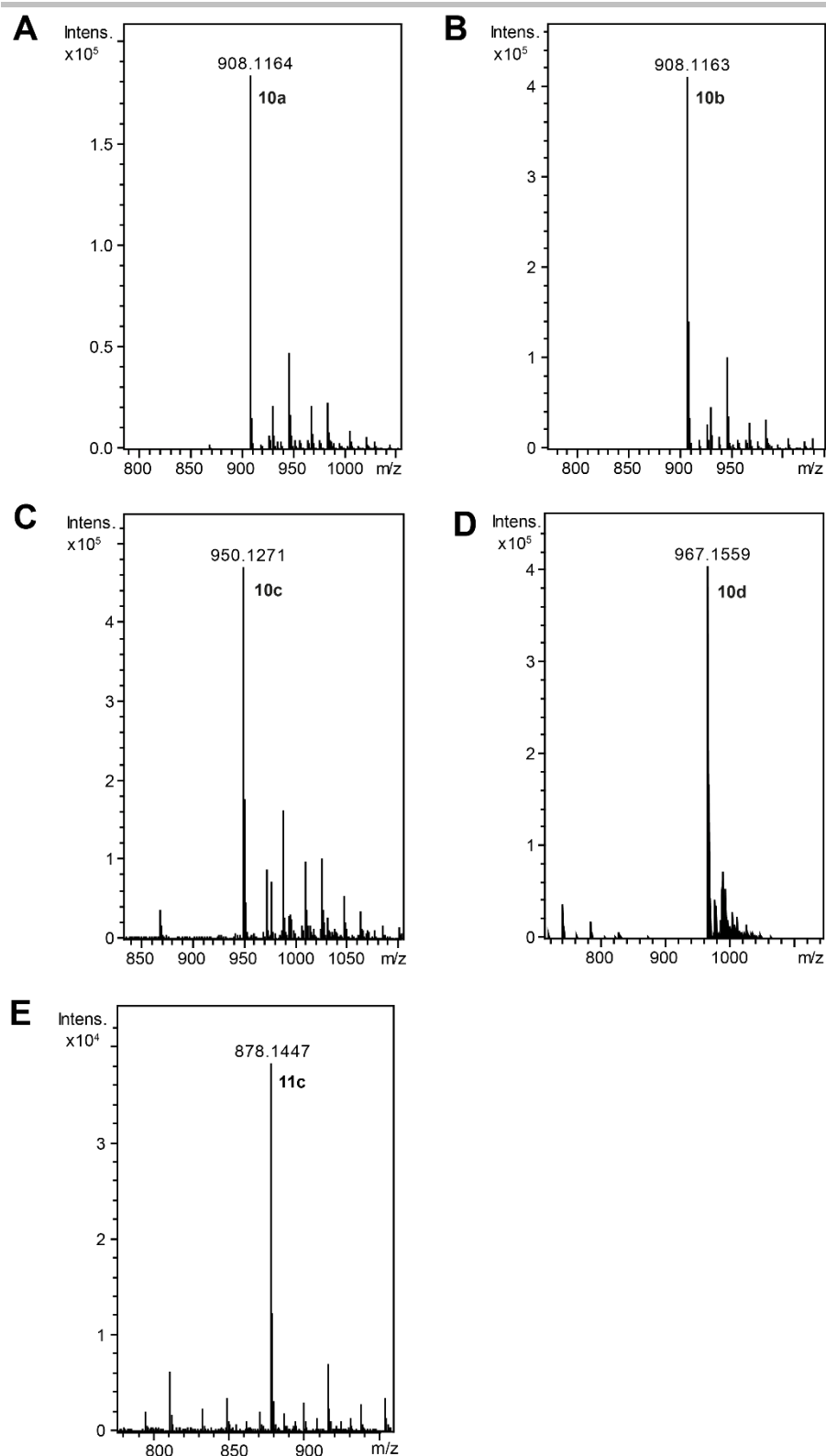


Figure S19. Mass spectrometric analysis of purified *N7*-photocaged GpppA cap analogues **10a-d** (A-D) and photo product **11c** of irradiated **10c** (E). (A) **10a**: Calculated mass of $[\text{C}_{27}\text{H}_{33}\text{N}_{11}\text{O}_{19}\text{P}_3]^+$ = 908.1162 [M]⁺, found: 908.1164. (B) **10b**: Calculated mass of $[\text{C}_{27}\text{H}_{33}\text{N}_{11}\text{O}_{19}\text{P}_3]^+$, found: 908.1163. (C) **10c**: Calculated mass of $[\text{C}_{29}\text{H}_{35}\text{N}_{11}\text{O}_{20}\text{P}_3]^+$ = 950.1267 [M]⁺, found: 950.1271. (D) **10d**: Calculated mass of $[\text{C}_{34}\text{H}_{38}\text{N}_{10}\text{O}_{18}\text{P}_3]^+$ = 967.1573, found: 967.1559. (E) **11c**: Calculated mass of $[\text{C}_{27}\text{H}_{35}\text{N}_{11}\text{O}_{17}\text{P}_3]^+$ = 878.1420 [M]⁺, found: 878.1447.

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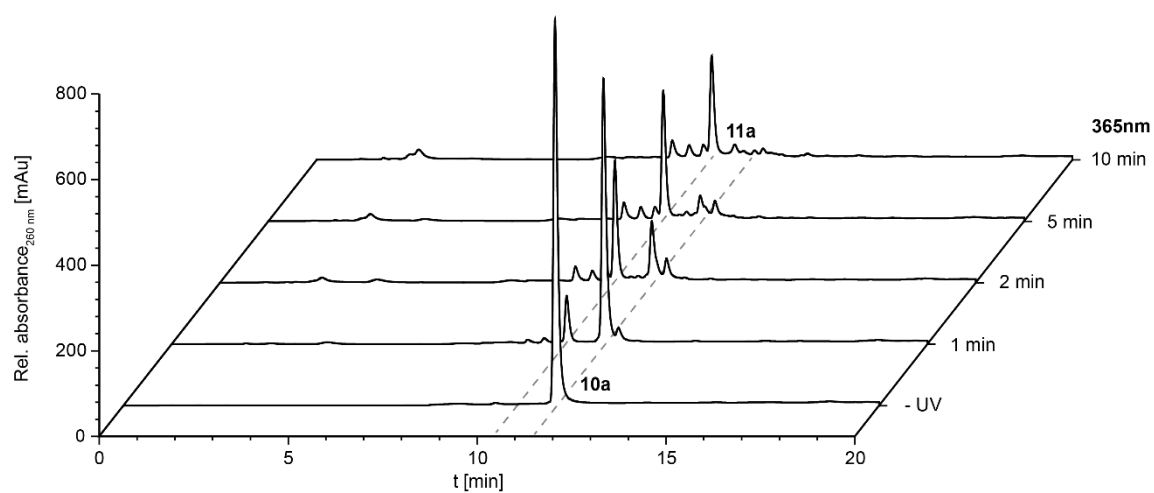


Figure S20. Irradiation of purified *N7*-ONBGpppA **10a**. Irradiation of **10a** in ddH₂O using a LED ($\lambda_{\text{max}} = 365 \text{ nm}$) for different times showed no recovery of GpppA **8** instead the formation of a new photo product **11a**, which was analyzed by HPLC.

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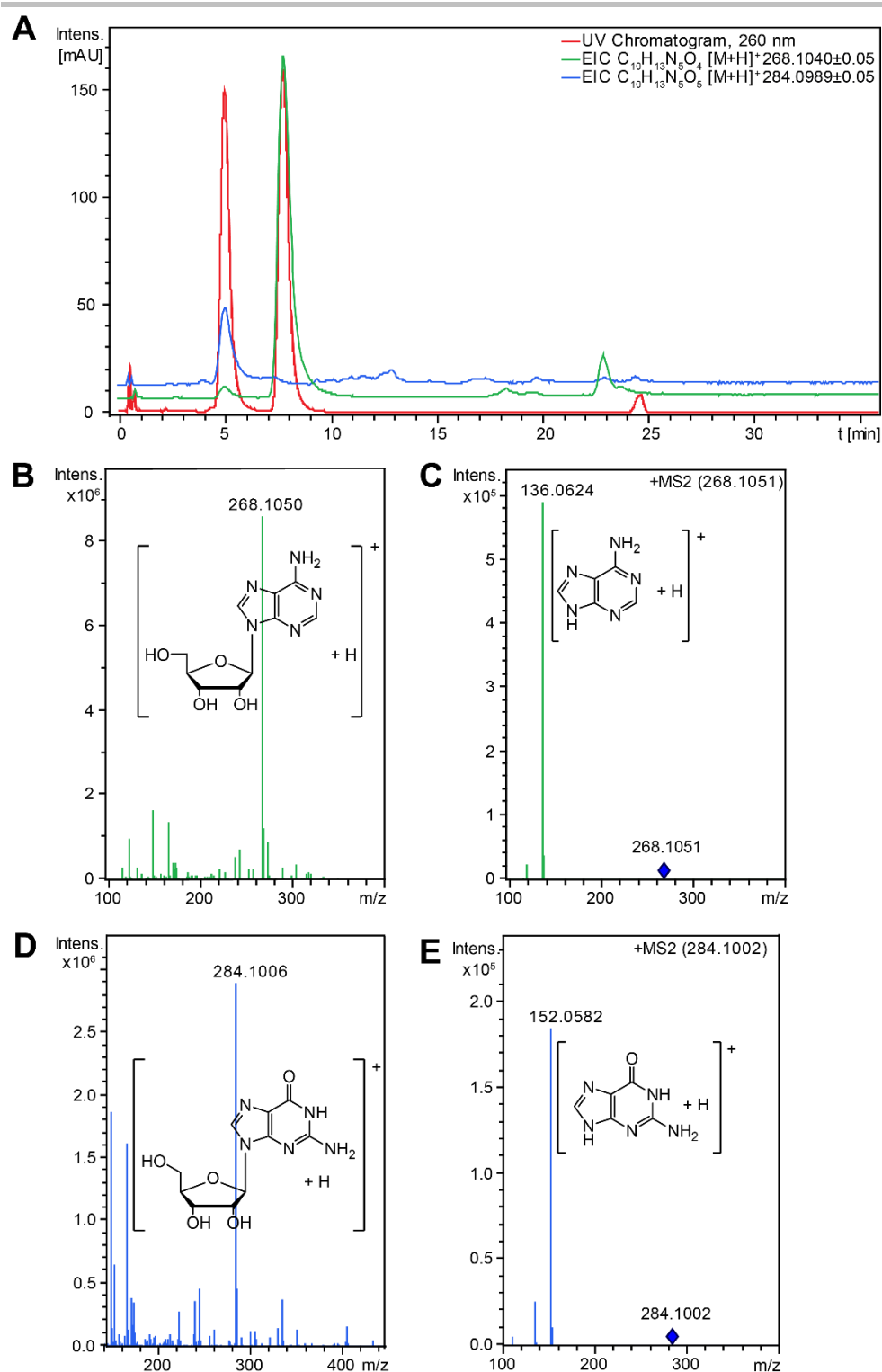


Figure S21. Mass spectrometric analysis of GpppA **8** after enzymatic digestion. **(A)** UV chromatogram of digested sample and extracted ion count (EIC) chromatograms of adenosine and guanosine. **(B)** Adenosine: Calculated mass of $[C_{10}H_{14}N_5O_4]^+$ = 268.1040 $[M+H]^+$, found: 268.1050. **(D)** Guanosine: Calculated mass of $[C_{10}H_{14}N_5O_5]^+$ = 284.0989 $[M+H]^+$, found: 284.1006. **(C+E)** MS/MS analysis of adenosine and guanosine. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond.

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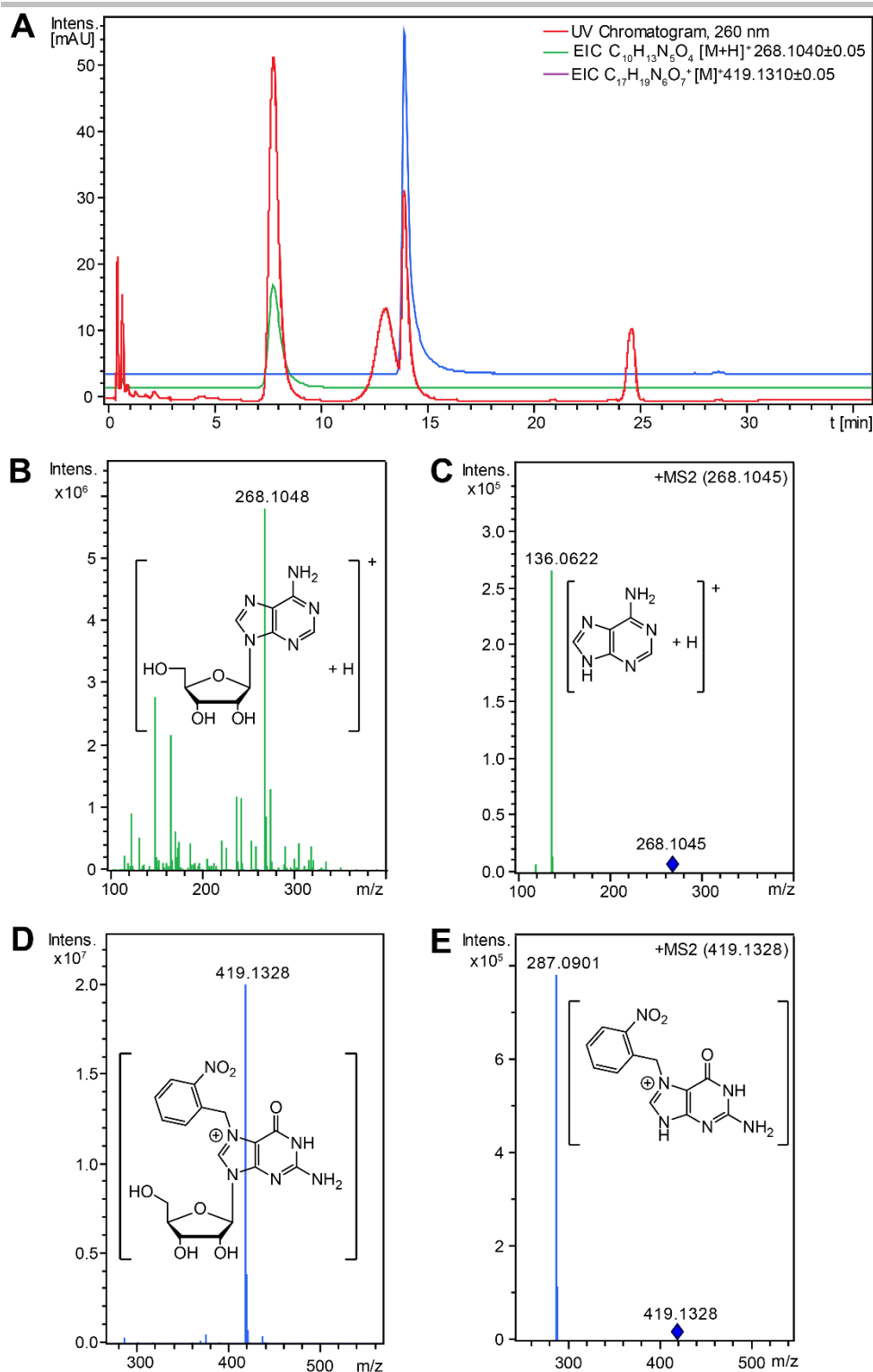


Figure S22. Mass spectrometric analysis of *N7*-ONB-GpppA **10a** after enzymatic digestion. **(A)** UV chromatogram of digested sample and extracted ion count (EIC) chromatograms of adenosine and *N7*-ONB-guanosine. **(B)** Adenosine: Calculated mass of $[C_{10}H_{14}N_5O_4]^+$ = 268.1040 $[M+H]^+$, found: 268.1048. **(D)** *N7*-ONB-guanosine: Calculated mass of $[C_{17}H_{19}N_6O_7]^+$ = 419.1310 $[M]^+$, found: 419.1328. **(C+E)** MS/MS analysis of adenosine and *N7*-ONB-guanosine. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond.

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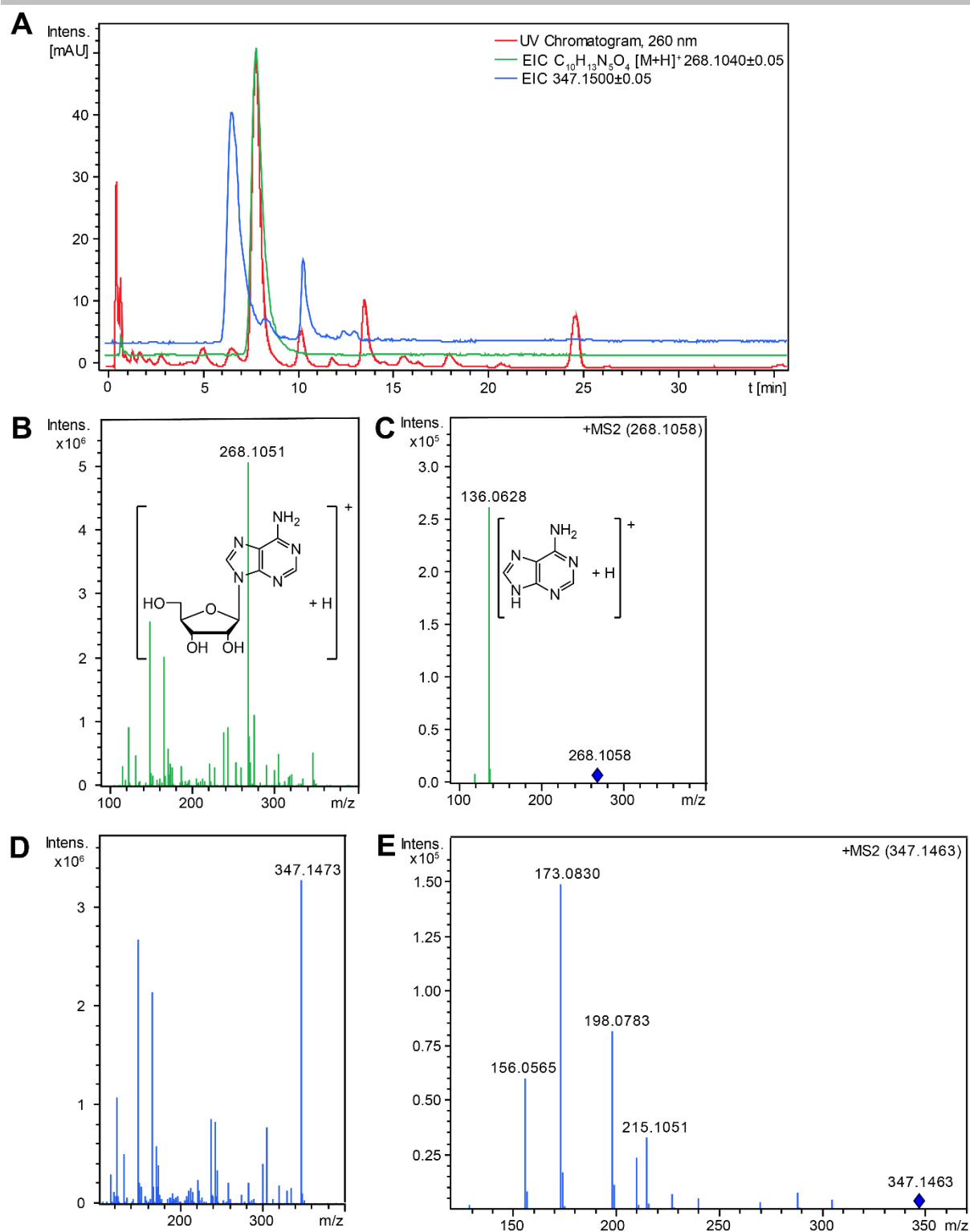


Figure S23. Mass spectrometric analysis of photo product **11a** after enzymatic digestion. (A) UV chromatogram of digested sample and extracted ion count (EIC) chromatograms of adenosine and of an unknown guanosine derivative. (B) Adenosine: Calculated mass of $[C_{10}H_{13}N_5O_4]^+ = 268.1040$ $[M+H]^+$, found: 268.1050. (D) Unknown guanosine derivative: Calculated mass of $[C_{15}H_{19}N_6O_4]^+ = 347.1462$ $[M]^+$, found: 347.1473. (C+E) MS/MS analysis of adenosine and unknown guanosine derivative. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond.

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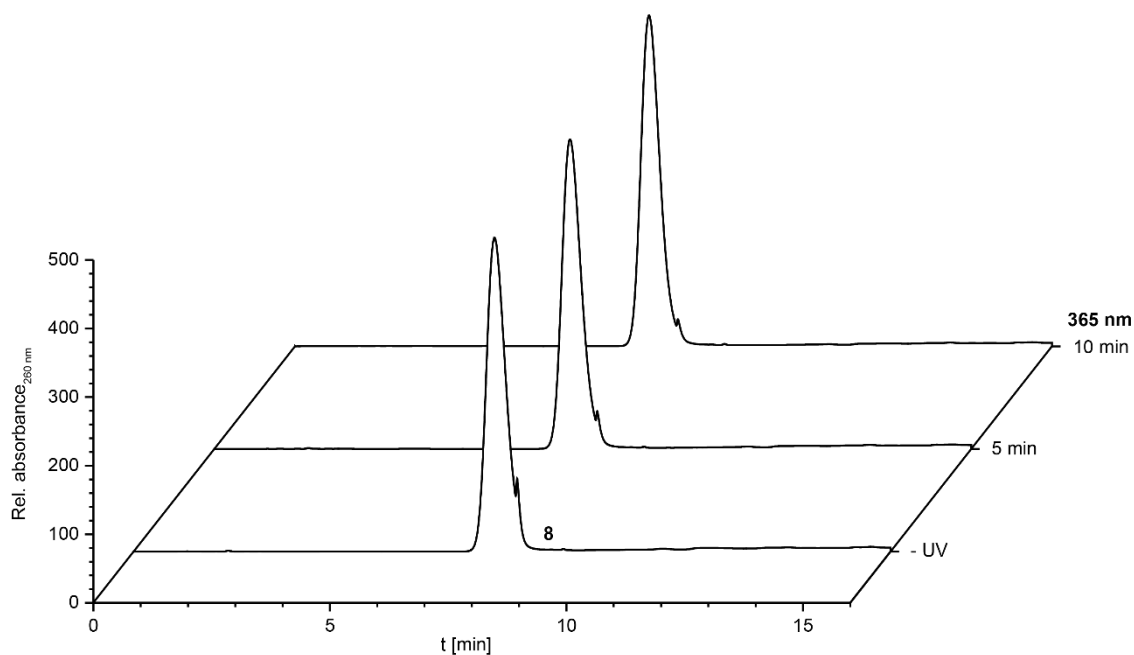


Figure S24. Photo stability of GpppA **8**. Cap analogue GpppA **8** was irradiated in ddH₂O using an LED ($\lambda_{\text{max}} = 365$ nm) for different times and analyzed by HPLC.

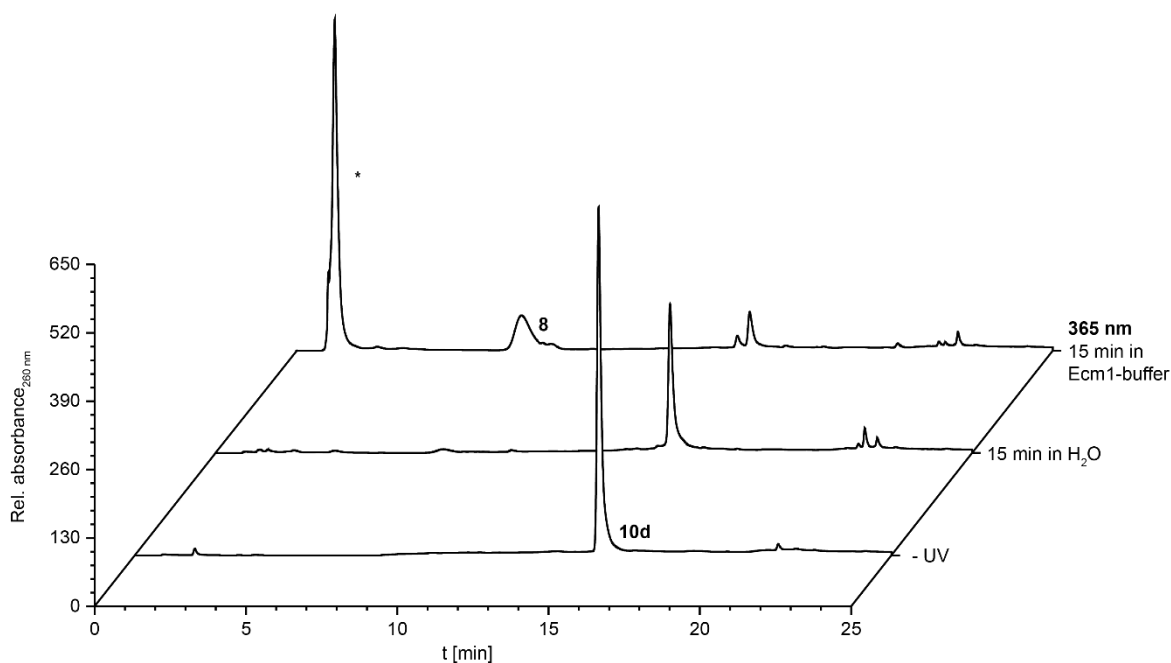


Figure S25. Irradiation of purified *N7*-BP-GpppA **10d** in H₂O or in buffer. Purified *N7*-BP-GpppA **10d** was irradiated in ddH₂O or in buffer using a LED ($\lambda_{\text{max}} = 365$ nm) for 15 min. HPLC analysis showed no recovery of GpppA **8** in ddH₂O, however recovery of GpppA **8** in buffer.

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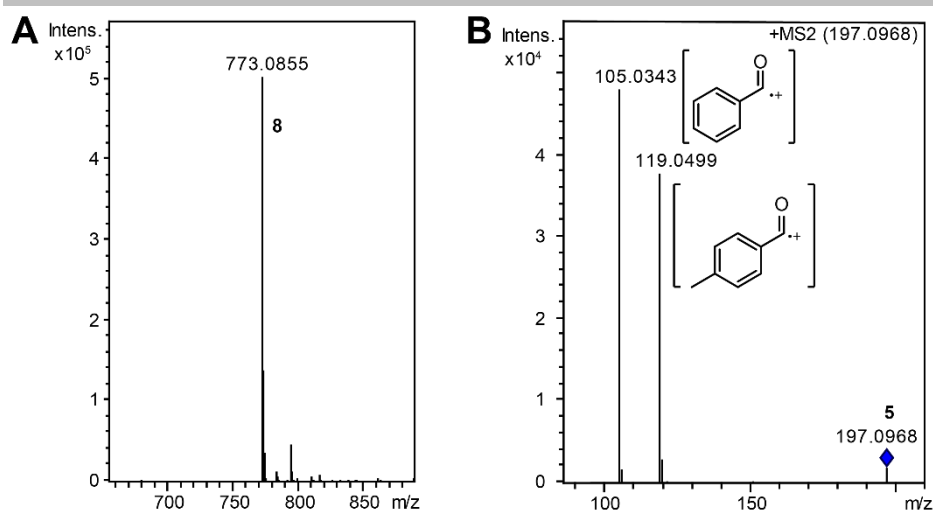


Figure S26. Mass spectrometric analysis of photocleavage products **8** and **5** after irradiation of **10d** in buffer solution. (A) **8**: Calculated mass of $[\text{C}_{20}\text{H}_{28}\text{N}_{10}\text{O}_{17}\text{P}_3]^+ = 773.0841$ $[\text{M}+\text{H}]^+$, found: 773.0855. (B) MS/MS analysis of **5**. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond. **5**: Calculated mass of $[\text{C}_{14}\text{H}_{13}\text{O}]^+ = 197.0961$ $[\text{M}+\text{H}]^+$, found: 197.0968.

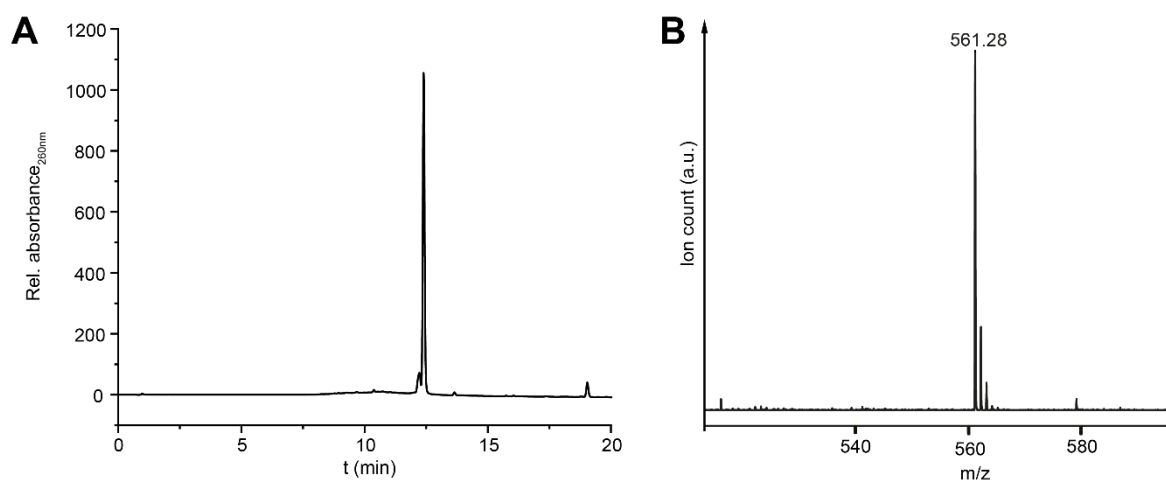


Figure S27. HPLC trace and mass spectrometric analysis of AdoHAK **9e** (A-B). (B) **9e**: Calculated of $[\text{C}_{25}\text{H}_{33}\text{N}_6\text{O}_7\text{S}]^+ = 561.21$ $[\text{M}]^+$, found: 561.28 (MALDI analysis).

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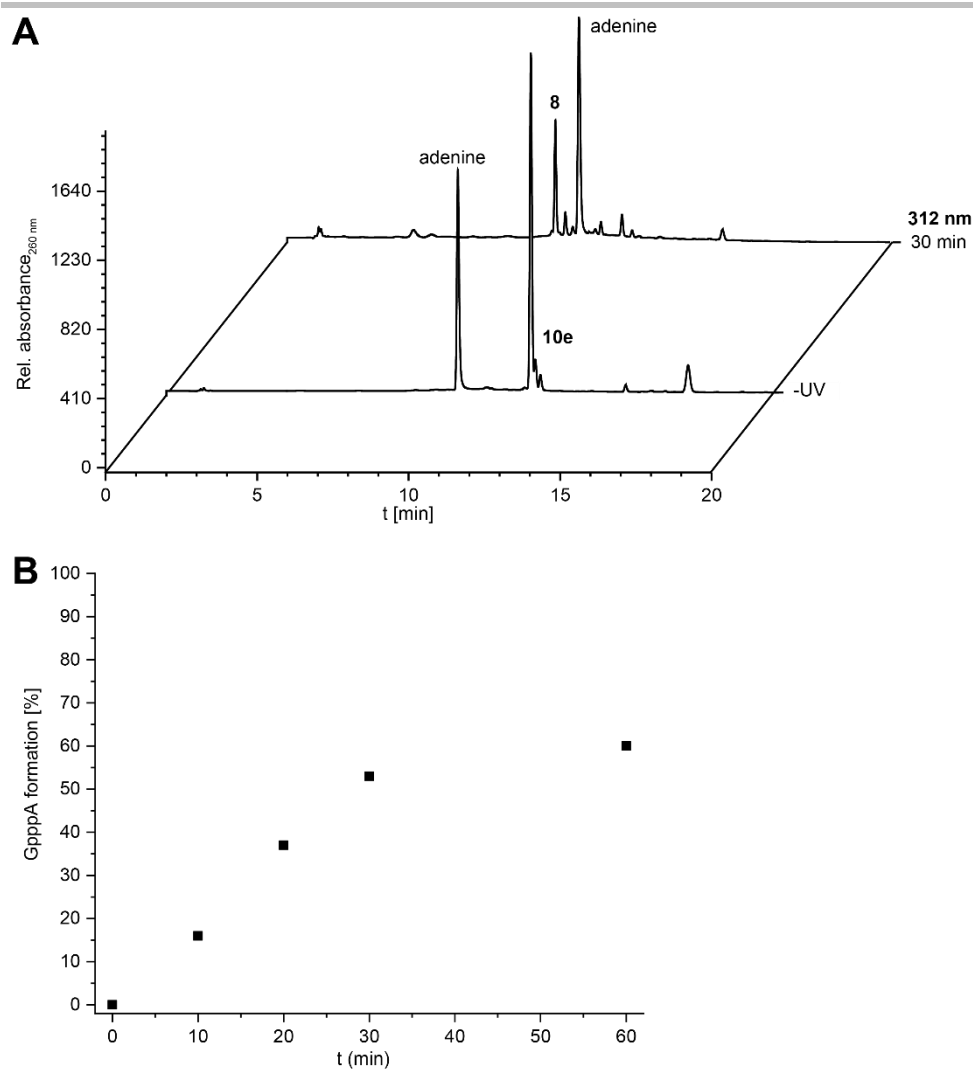


Figure S28. Enzymatic modification of *N7*-HAK-GpppA **10e** and subsequent irradiation with recovery of GpppA **8**. **(A)** GpppA **8** was enzymatically modified using Ecm1 and AdoHAK **9e** and then the unpurified *N7*-HAK-GpppA **10e** was irradiated using a UV-transilluminator with a polystyrene filter ($\lambda_{\text{max}} = 312 \text{ nm}$) for 30 min. HPLC analysis showed recovery of GpppA **8** and decrease of *N7*-HAK-GpppA **10e**. **(C)** Time series for the recovery of GpppA **8**.

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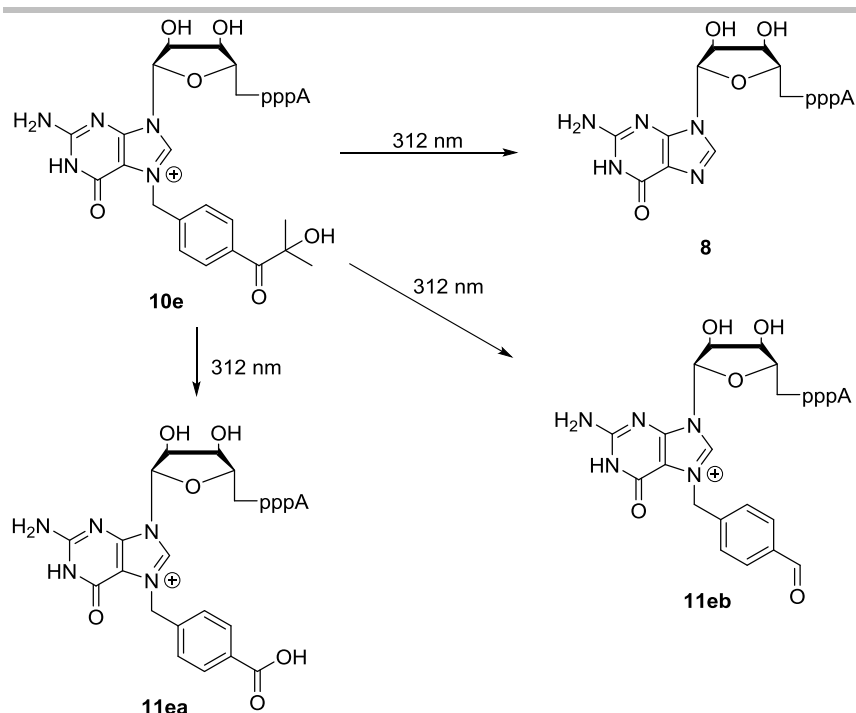


Figure S29. Structure of recovered GpppA **8** and side-products **11ea-eb** upon irradiation of *N7*-HAK-GpppA **10e**.

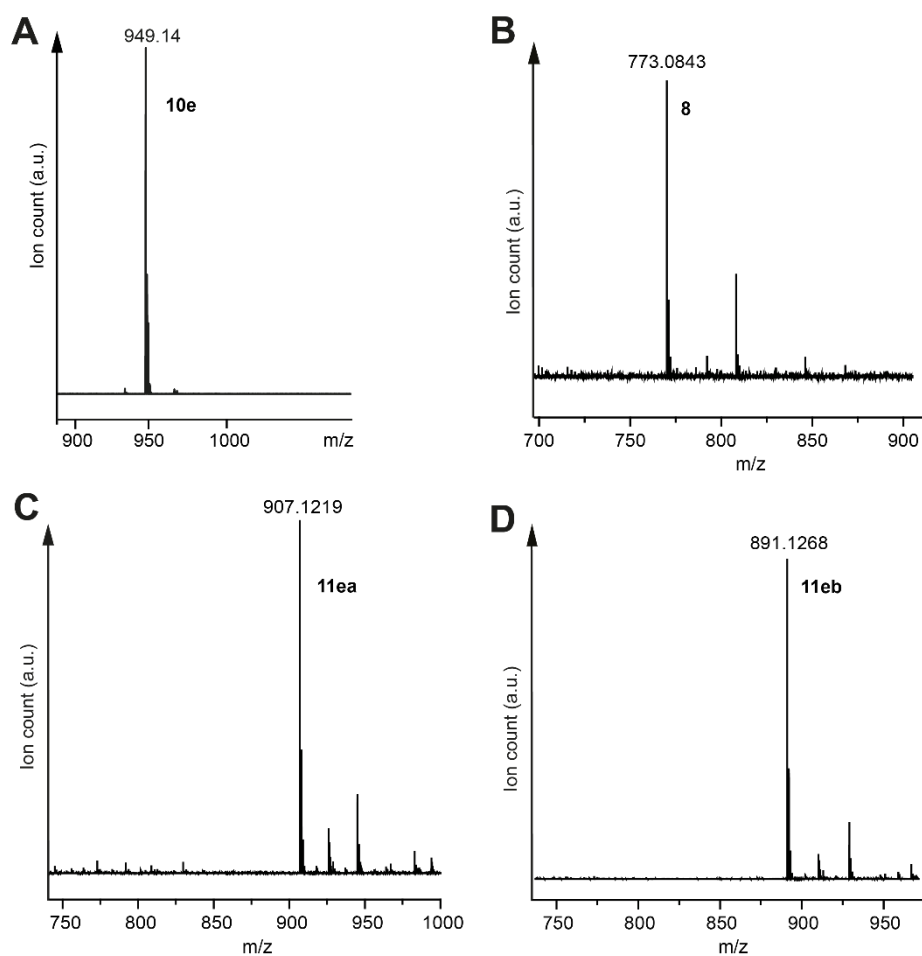


Figure S30. Mass spectrometric analysis of *N7*-HAK-GpppA **10e** and photocleavage products **8** and **11ea-11eb**. (A) **10e**: Calculated mass of $[C_{31}H_{40}N_{10}O_{19}P_3]^+ = 949.17$ [M]⁺, found: 949.14 (MALDI analysis). (B) **8**: Calculated mass of $[C_{20}H_{28}N_{10}O_{17}P_3]^+ = 773.0841$ [M+H]⁺, found: 773.0843. (C) **11ea**: Calculated mass of $[C_{28}H_{34}N_{10}O_{19}P_3]^+ = 907.1209$ [M]⁺, found: 907.1219. (D) **11eb**: Calculated mass of $[C_{28}H_{34}N_{10}O_{18}P_3]^+ = 891.1260$, found: 891.1268.

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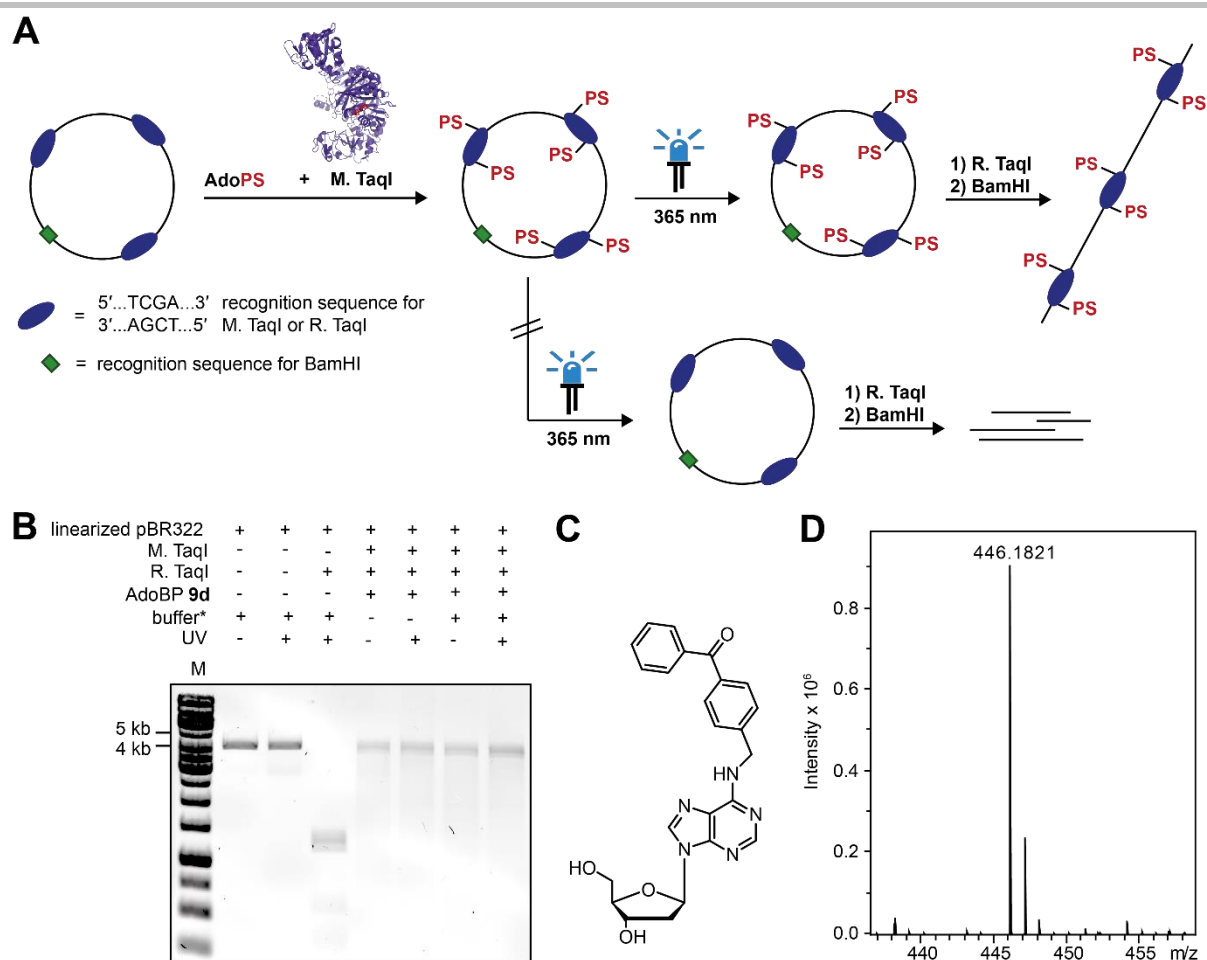


Figure S31: Enzymatic modification of DNA plasmid pBR322 using MTase TaqI and subsequent photocleavage. (A) Concept for the transfer of AdoPC to the N^6 position of 2'-deoxyadenosine using MTase TaqI followed by irradiation with UV light ($\lambda_{max} = 365$ nm) (For clarity, only three of the seven M. TaqI recognition sites in pBR322 are shown). (B) Analysis of enzymatic modification and irradiation using a LED ($\lambda_{max} = 365$ nm, 10 min) of plasmid DNA using AdoBP and M. TaqI, which was analyzed by 1% agarose gel (100 V, 1 h) and visualized by staining with ethidium bromide. (C) Structure of N^6 -BP-modified 2'-deoxyadenosine after enzymatic digestion of enzymatically modified short dsDNA. (D) Mass spectrometric analysis of N^6 -BP-modified 2'-deoxyadenosine: Calculated mass of $[C_{24}H_{24}N_5O_4]^+ = 446.1823$ $[M+H]^+$, found: 446.1821.

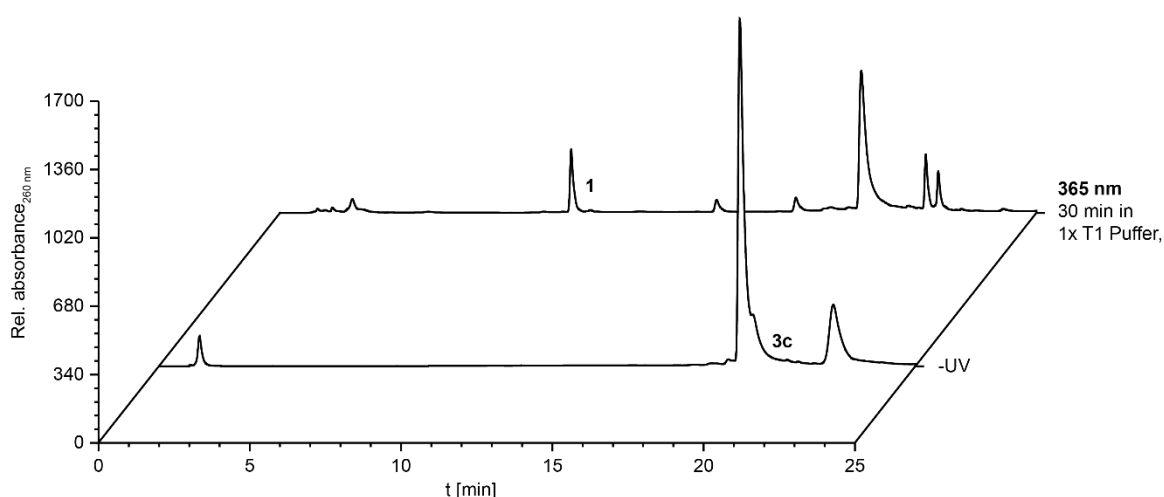


Figure S32. Irradiation of purified N^7 -BP-guanosine **3c** in buffer used for irradiation of RNA. Purified **3c** was irradiated in 1x T1 buffer using an LED ($\lambda_{max} = 365$ nm) for 30 min. HPLC analysis showed recovery of guanosine **5**.

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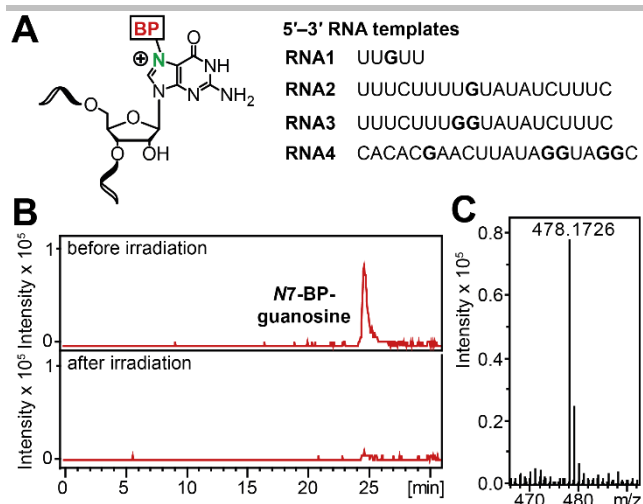


Figure S33. Photocaging of internal *N7*-BP-modified guanosines in short RNA. (A) Sequences of short RNAs used in this study. (B) Extracted ion count (EIC) chromatogram of *N7*-BP-guanosine ($[C_{24}H_{24}N_5O_6]^+ [M]^+$ 478.1721 \pm 0.005 of chemically modified and then digested RNA1 before and after irradiation. (C) Mass analysis of *N7*-BP-guanosine (calculated mass of $[C_{24}H_{24}N_5O_6]^+ = 478.1721 [M]^+$, found 478.1726.

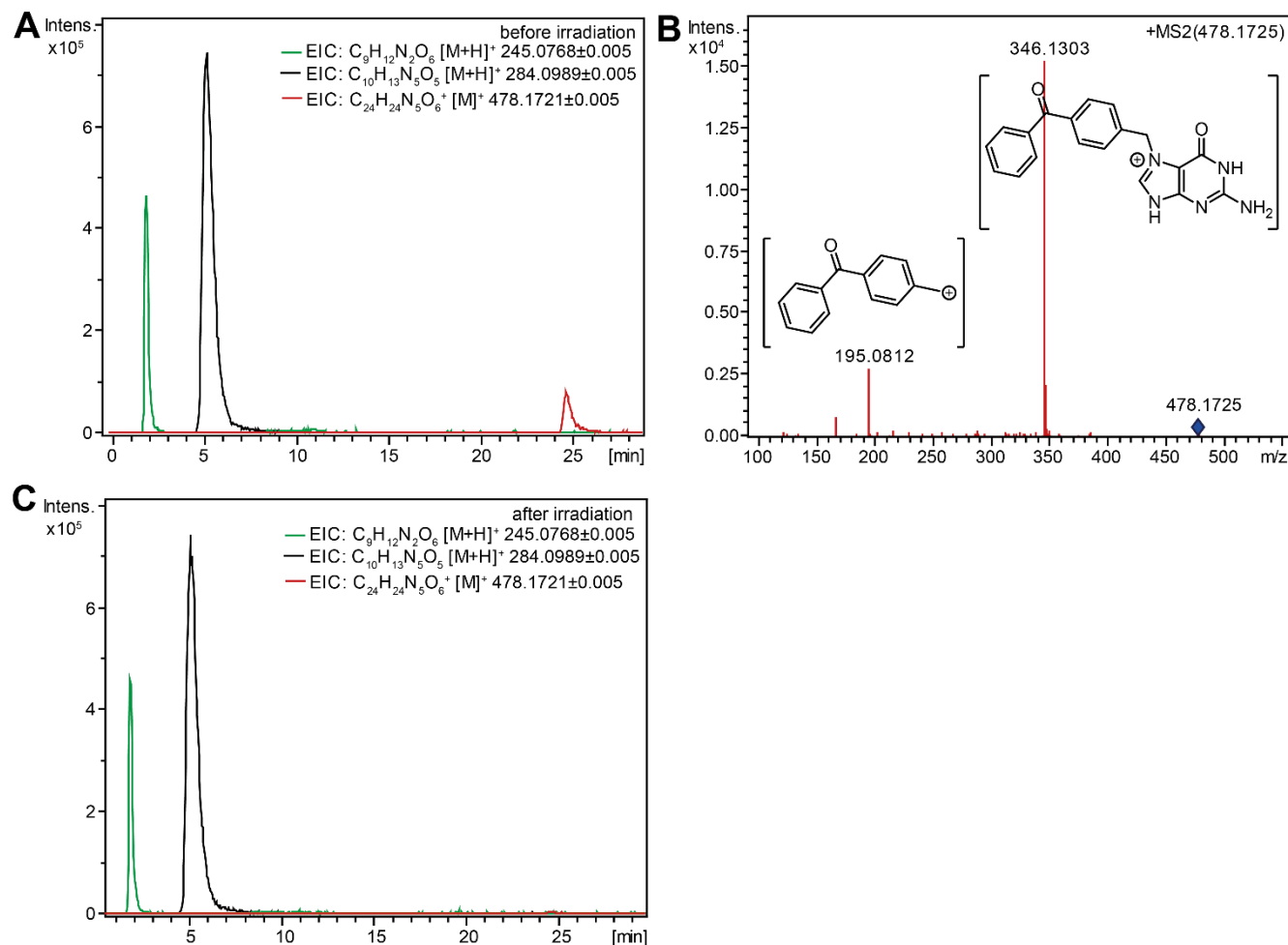


Figure S34. Mass spectrometric analysis of chemically modified RNA1 UUGUU before and after irradiation. (A) Extracted ion count (EIC) chromatograms of uridine, guanosine and *N7*-BP-guanosine before irradiation. (B) MS/MS analysis of *N7*-BP-guanosine. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond. Calculated mass of $[C_{24}H_{24}N_5O_6]^+ = 478.1721 [M]^+$, found: 478.1725. (C) Extracted ion count (EIC) chromatograms of uridine, guanosine and *N7*-BP-guanosine after irradiation.

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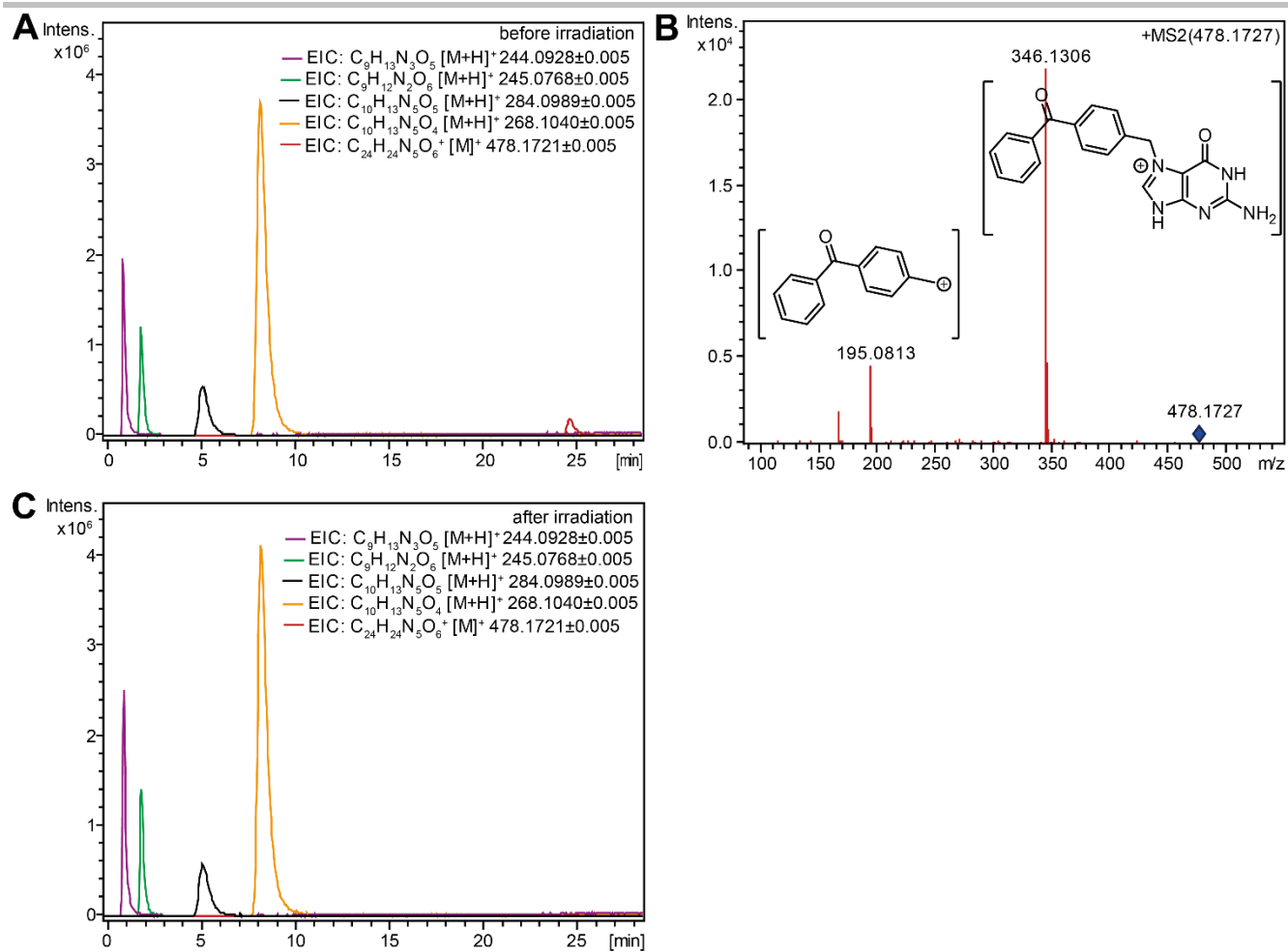


Figure S35. Mass spectrometric analysis of chemically modified RNA2 UUUCUUUGUAUAUCUUUC before and after irradiation. **(A)** Extracted ion count (EIC) chromatograms of cytidine, uridine, guanosine, adenosine and *N7*-BP-guanosine before irradiation. **(B)** MS/MS analysis of *N7*-BP-guanosine. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond. Calculated mass of $[C_{24}H_{24}N_5O_6]^+ = 478.1721$ $[M]^+$, found: 478.1727. **(C)** Extracted ion count (EIC) chromatograms of cytidine, uridine, guanosine, adenosine and *N7*-BP-guanosine after irradiation.

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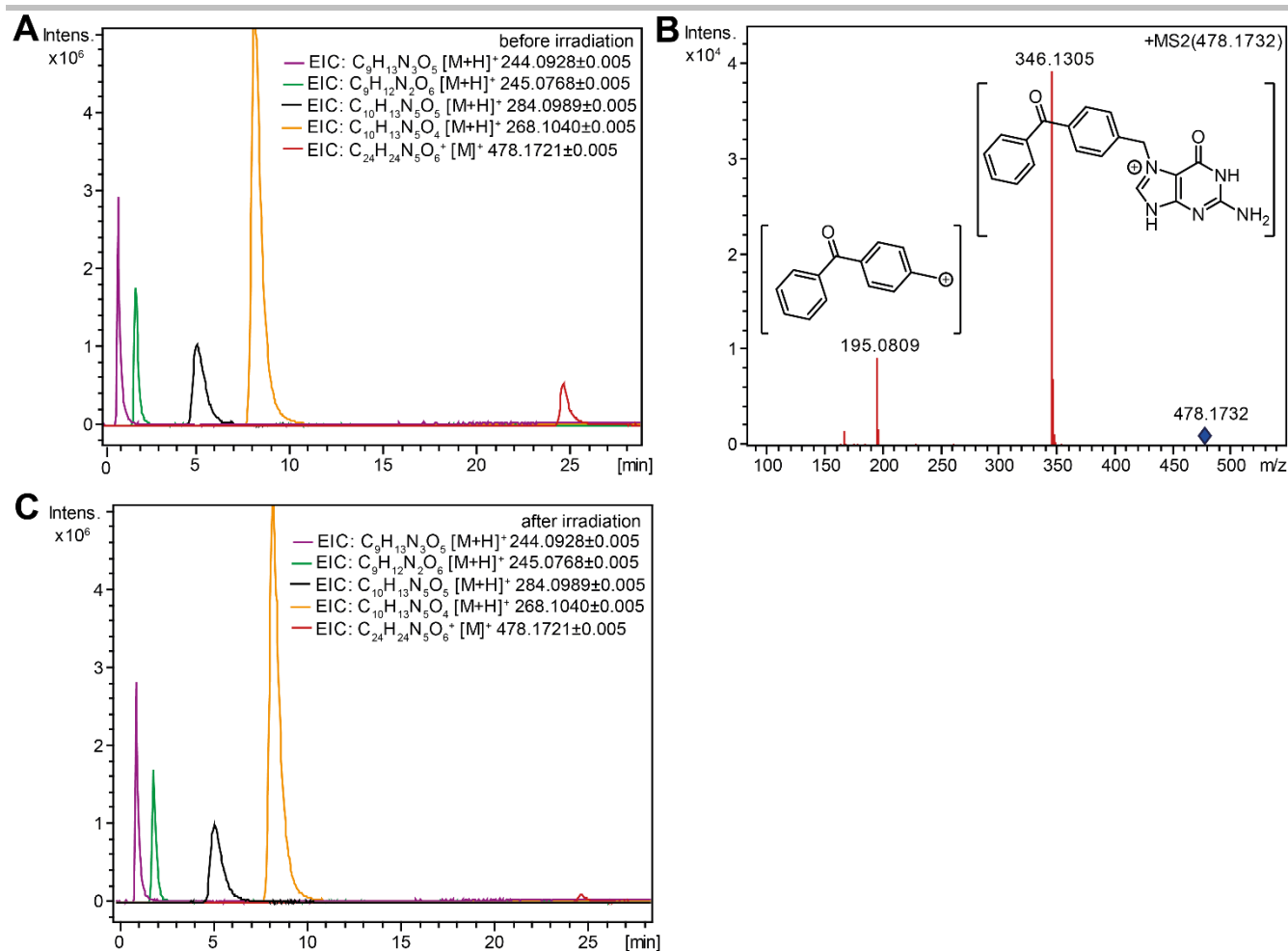


Figure S36. Mass spectrometric analysis of chemically modified RNA3 UUUCUUUGGUAUAUCUUUC before and after irradiation. **(A)** Extracted ion count (EIC) chromatograms of cytidine, uridine, guanosine, adenosine and *N7*-BP-guanosine before irradiation. **(B)** MS/MS analysis of *N7*-BP-guanosine. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond. Calculated mass of $[C_{24}H_{24}N_5O_6]^+ = 478.1721$ $[M]^+$, found: 478.1732. **(C)** Extracted ion count (EIC) chromatograms of cytidine, uridine, guanosine, adenosine and *N7*-BP-guanosine after irradiation.

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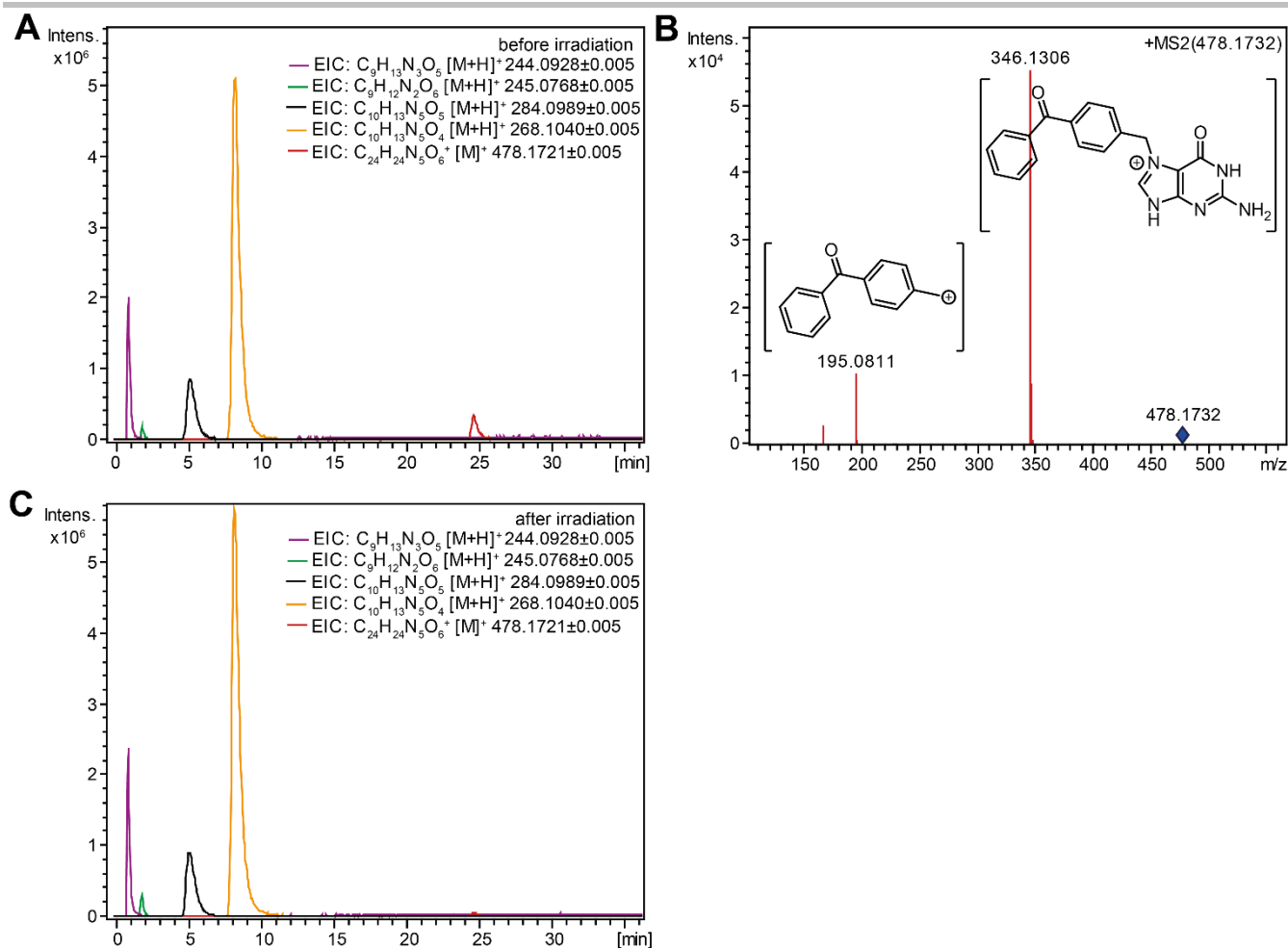


Figure S37. Mass spectrometric analysis of chemically modified RNA4 CACACGAACUUAUAGGUAGGC before and after irradiation. (A) Extracted ion count (EIC) chromatograms of cytidine, uridine, guanosine, adenosine and *N7*-BP-guanosine before irradiation. (B) MS/MS analysis of *N7*-BP-guanosine. Fragmentation patterns were obtained by low energy CID at 25 eV. Mass for the precursor ion is marked with a blue diamond. Calculated mass of $[C_{24}H_{24}N_5O_6]^+$ = 478.1721 $[M]^+$, found: 478.1732. (C) Extracted ion count (EIC) chromatograms of cytidine, uridine, guanosine, adenosine and *N7*-BP-guanosine after irradiation.

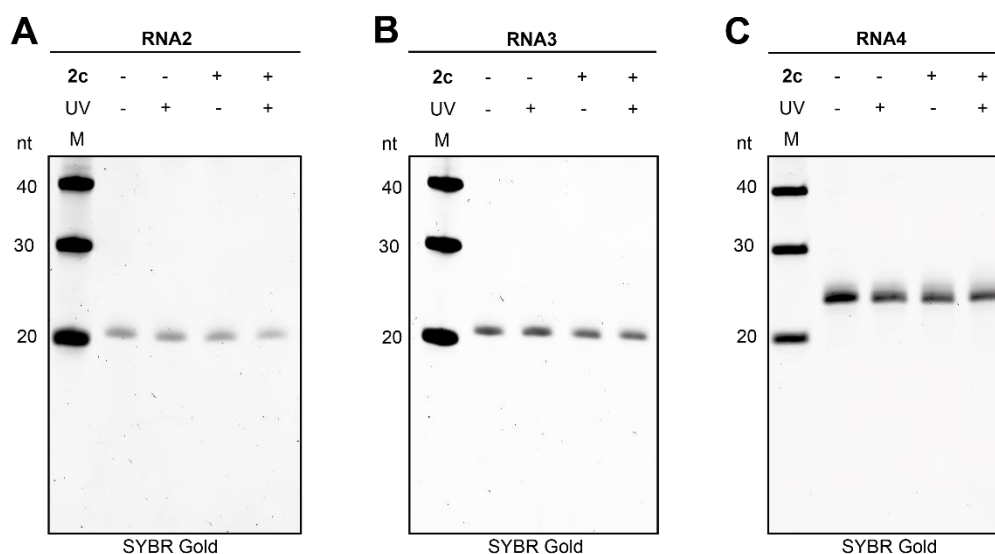


Figure S38. Chemical modification of short RNA. (A, B, C) RNA2, RNA3 or RNA4 was incubated with bromide **2c** for 24 h at 37 °C and then irradiated using a LED (λ_{max} = 365 nm) for 10 min. RNAs were analyzed by PAGE (15% PAGE, 15 W, 2 h, rt) and visualized using SYBRTM Gold (*Invitrogen*) staining.

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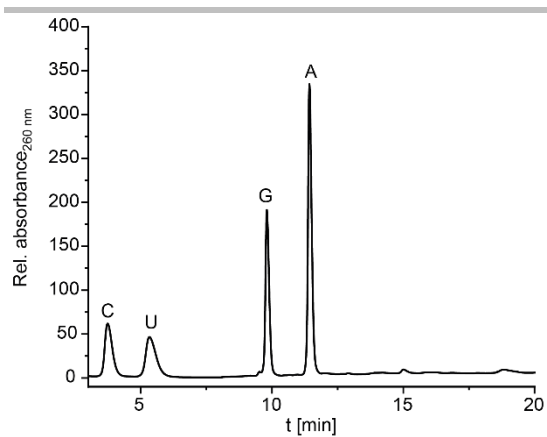


Figure S39. UV chromatogram of chemically modified RNA4 CACACGAACUUAUAGGUAGGC after irradiation using a LED ($\lambda_{\text{max}} = 365 \text{ nm}$) for 10 min. Then RNA4 was digested and dephosphorylated to single nucleosides and analyzed by HPLC.

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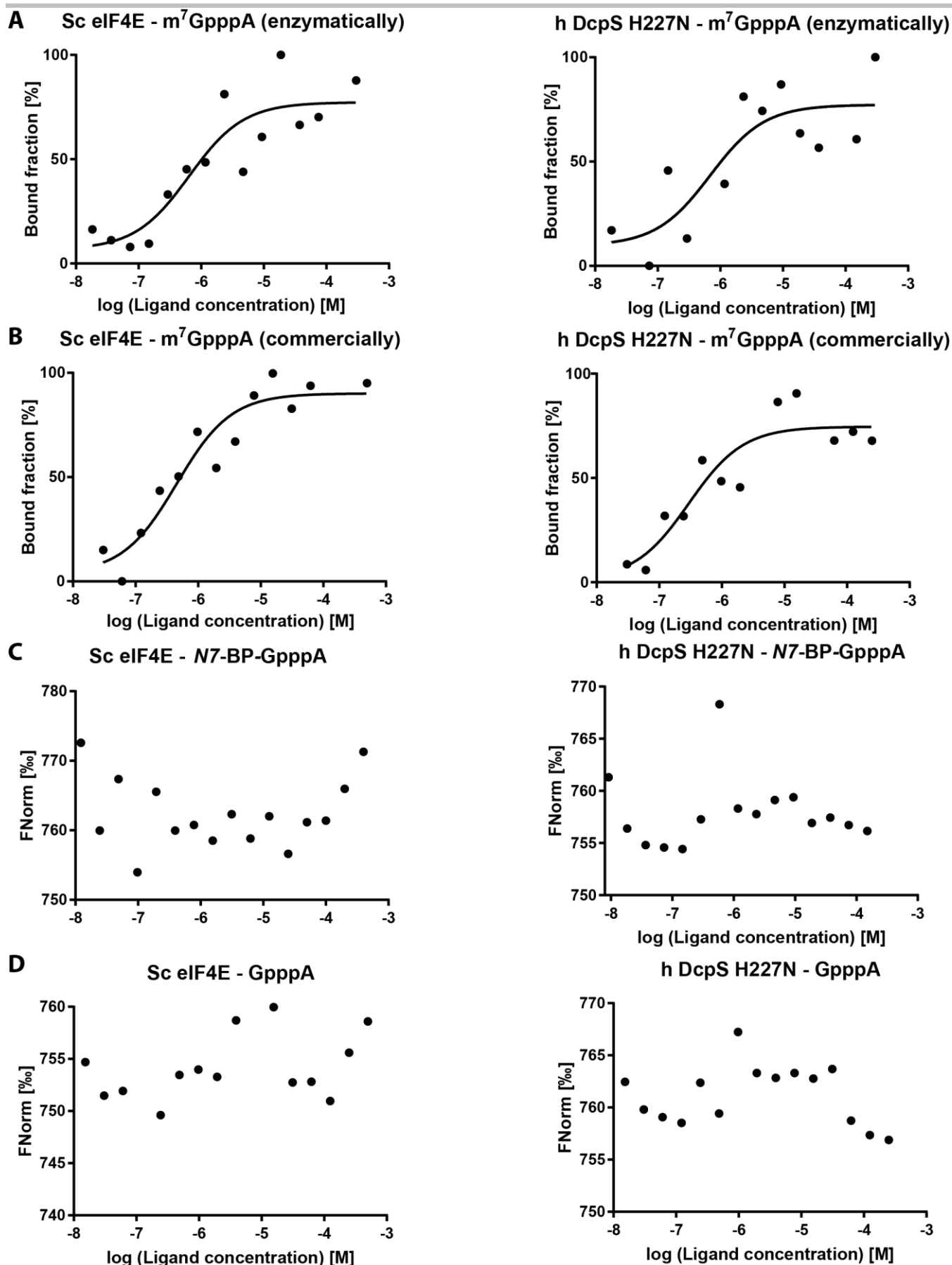


Figure S40. Representative microscale thermophoresis measurements to determine K_d values of different RNA cap analogues and eIF4E from *S. cerevisiae* as well as *human* DcpS H227N. The proteins were fluorescently labelled using Cy5-NHS. Both enzymes were used in a concentration of 50 nM. (A) enzymatically

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synthesized m⁷GpppA. (B) Commercially available m⁷GpppA. (C) N⁷-BP-GpppA showed no binding or fitting curve. (D) unmethylated GpppA showed no binding or fitting curve either. The K_d values shown in **Table S1** were calculated using nonlinear regression assuming a Hill coefficient of 1.0 (GraphPad Prism).

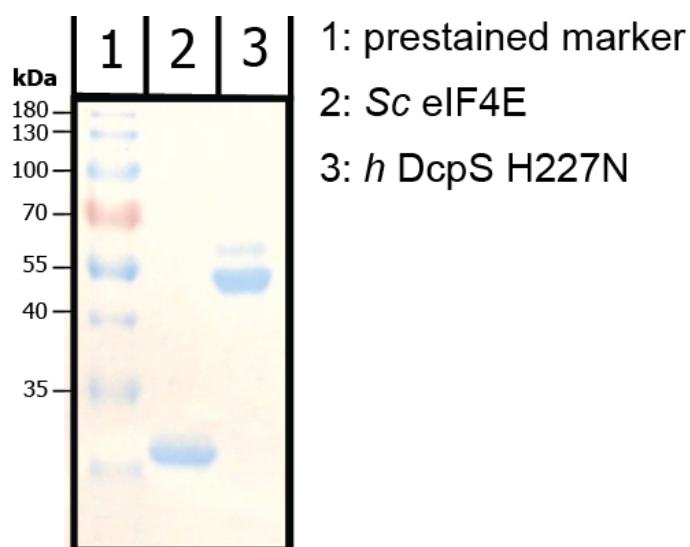


Figure S41: Denatured 12 % PAA-gel loaded with a *prestained protein ladder*, *h* DcpS H227N (53 kDa) and *Sc* eIF4E (30 kDa). Gel electrophoresis was performed with 160 V for 60 min.

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NMR Spectra

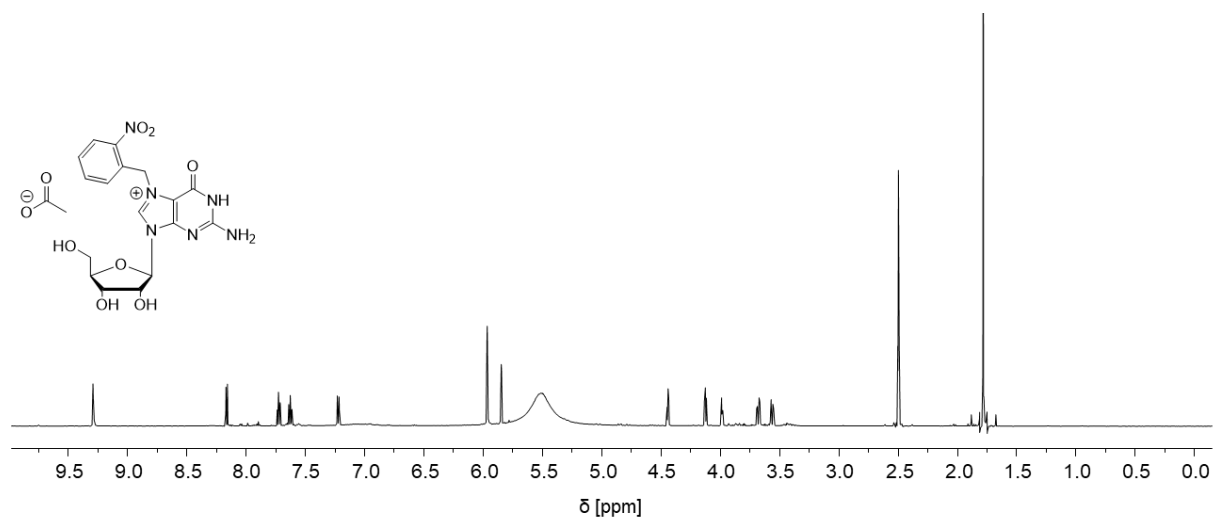


Figure S42. ¹H NMR spectrum (600 MHz, DMSO-*d*₆, 299 K) of 3a.

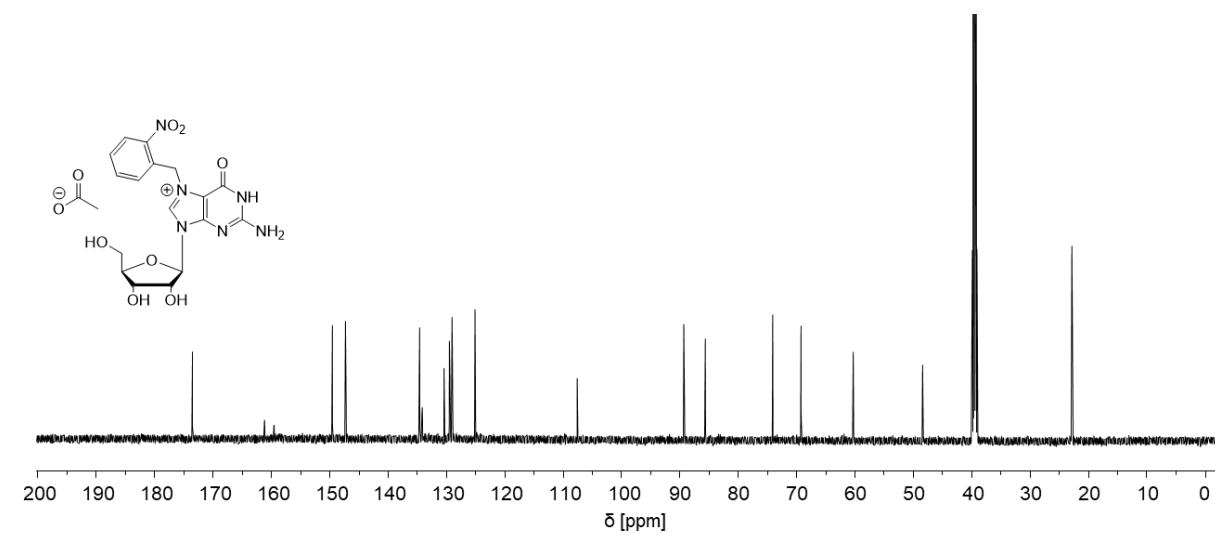


Figure S43. ¹³C NMR spectrum (151 MHz, DMSO-*d*₆, 299 K) of 3a.

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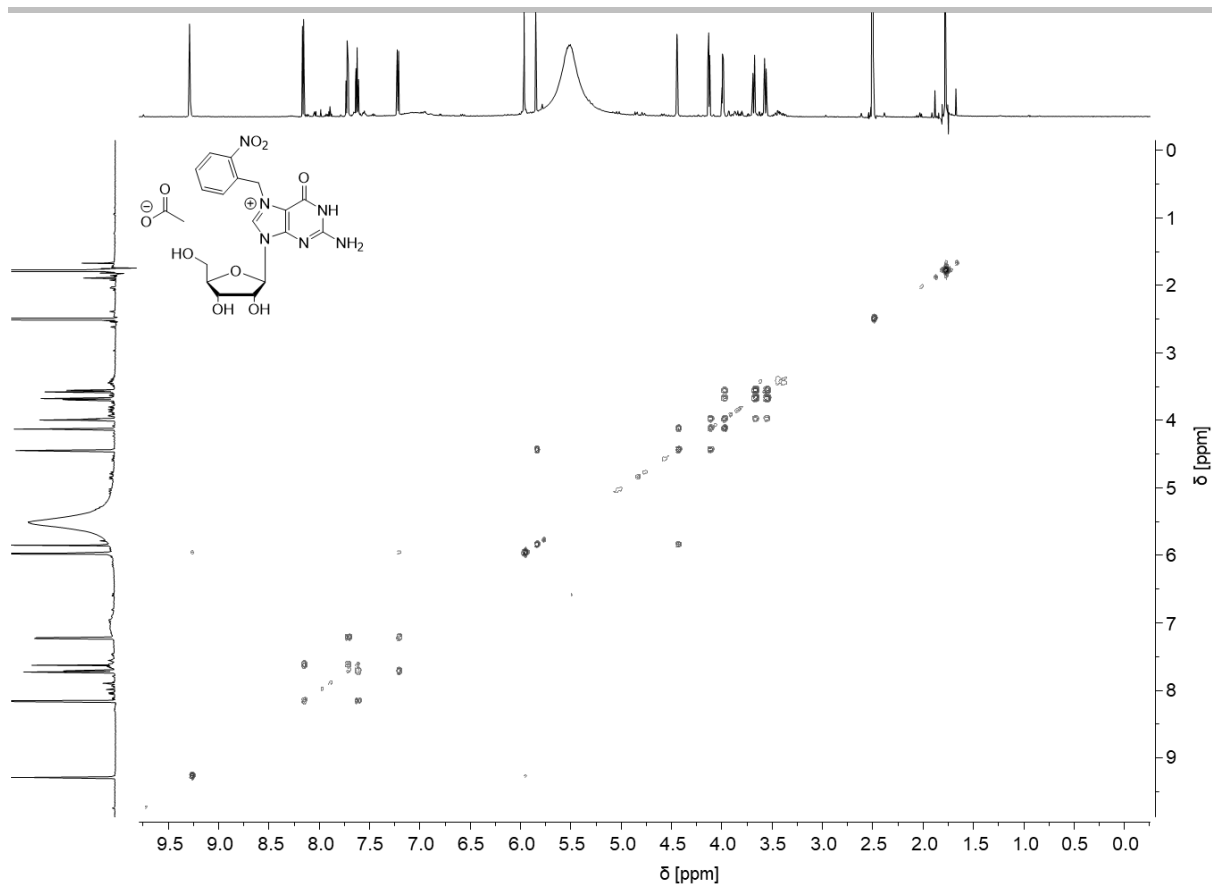


Figure S44. ¹H, ¹H-COSY NMR spectrum (600 MHz, DMSO-*d*₆, 299 K) of 3a.

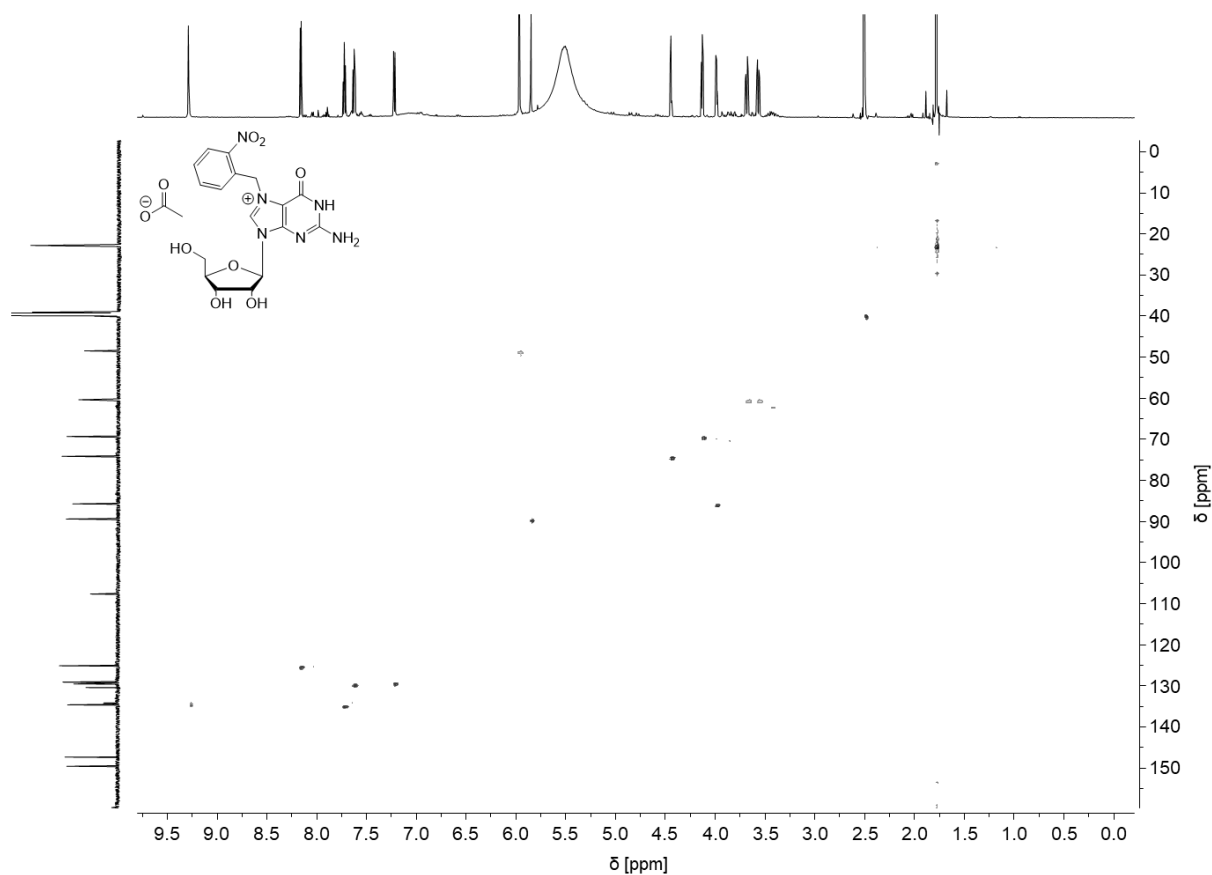


Figure S45. ¹H, ¹³C-HSQC NMR spectrum (600 MHz, 151 MHz, DMSO-*d*₆, 299 K) of 3a.

SUPPORTING INFORMATION

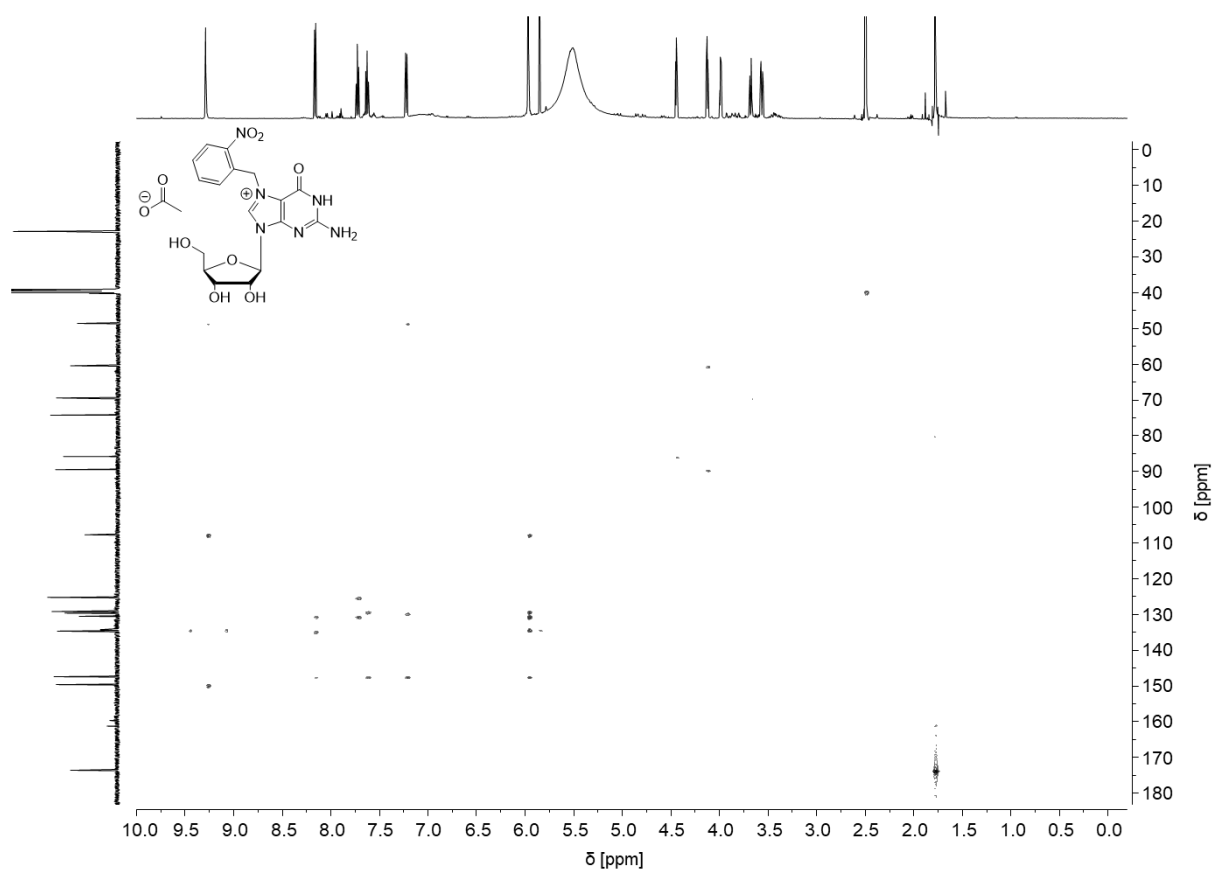


Figure S46. ^1H , ^{13}C -HMBC NMR spectrum (600 MHz, 151 MHz, $\text{DMSO-}d_6$, 299 K) of **3a**.

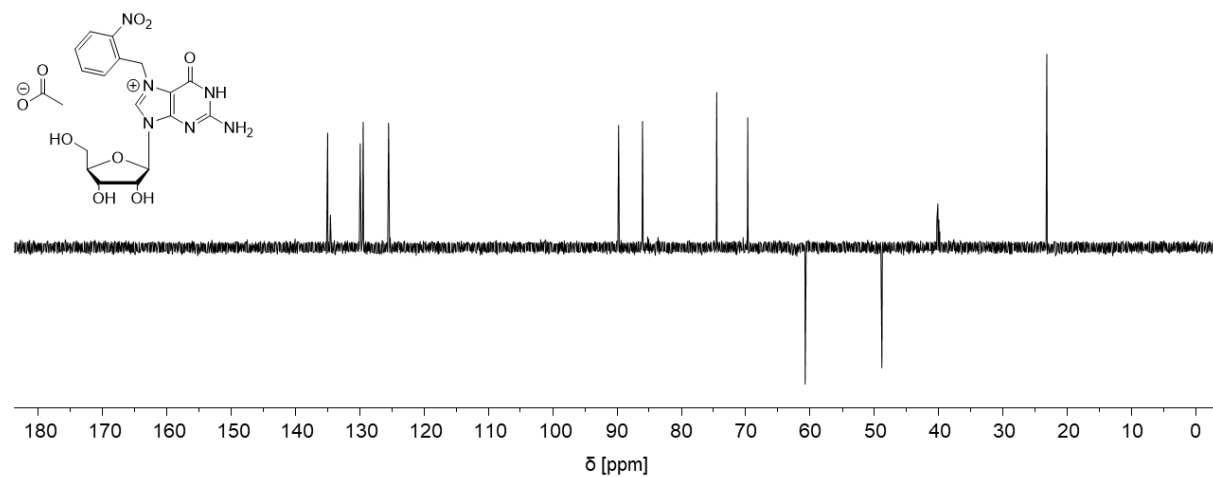


Figure S47. ^{13}C -DEPT NMR spectrum (151 MHz, $\text{DMSO-}d_6$, 299 K) of **3a**.

SUPPORTING INFORMATION

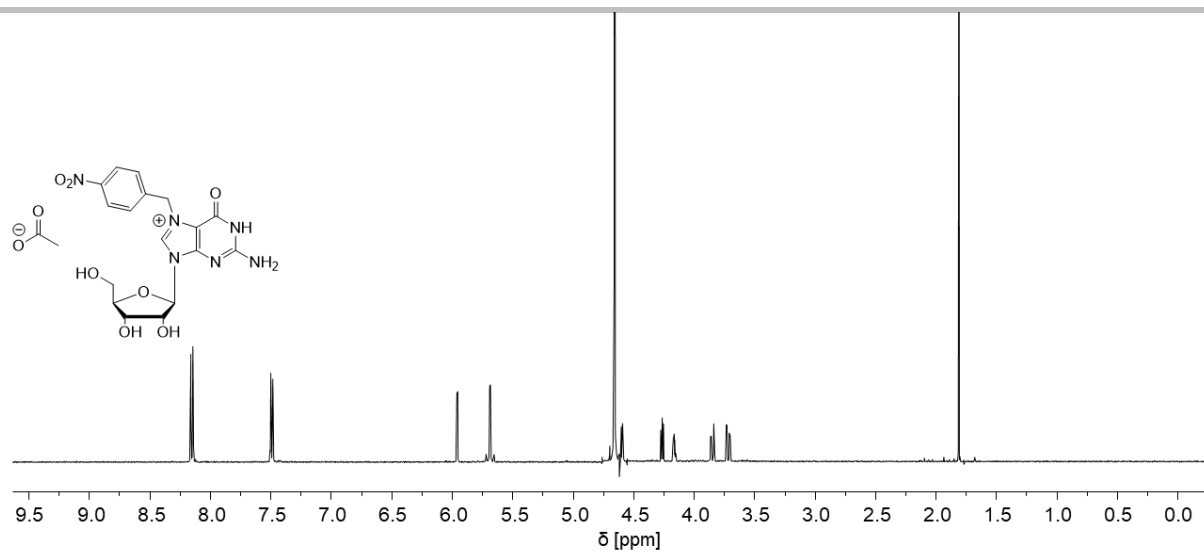


Figure S48. ^1H NMR spectrum (600 MHz, D_2O , 299 K) of **3b**.

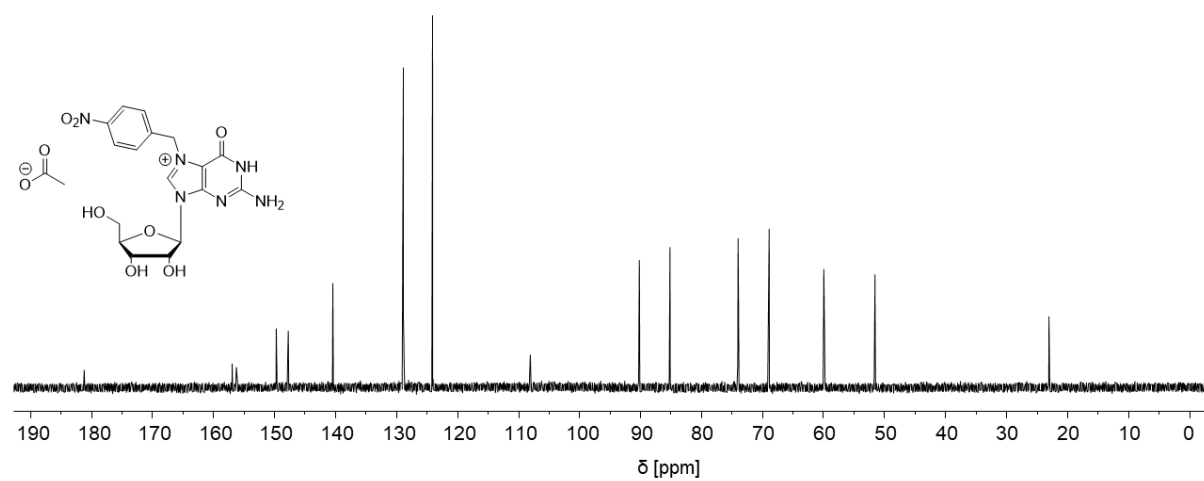


Figure S49. ^{13}C NMR spectrum (151 MHz, D_2O , 299 K) of **3b**.

SUPPORTING INFORMATION

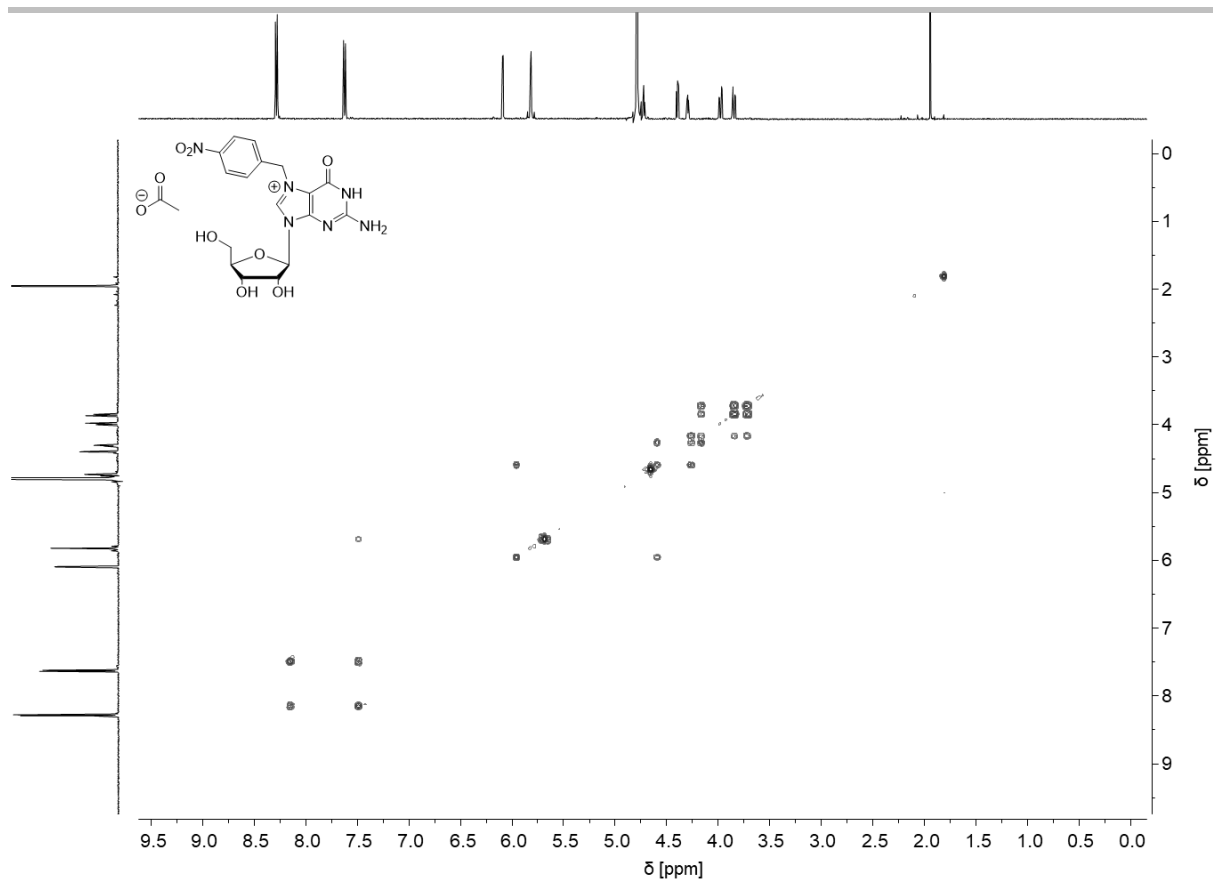


Figure S50. ^1H , ^1H -COSY NMR spectrum (600 MHz, D_2O , 299 K) of **3b**.

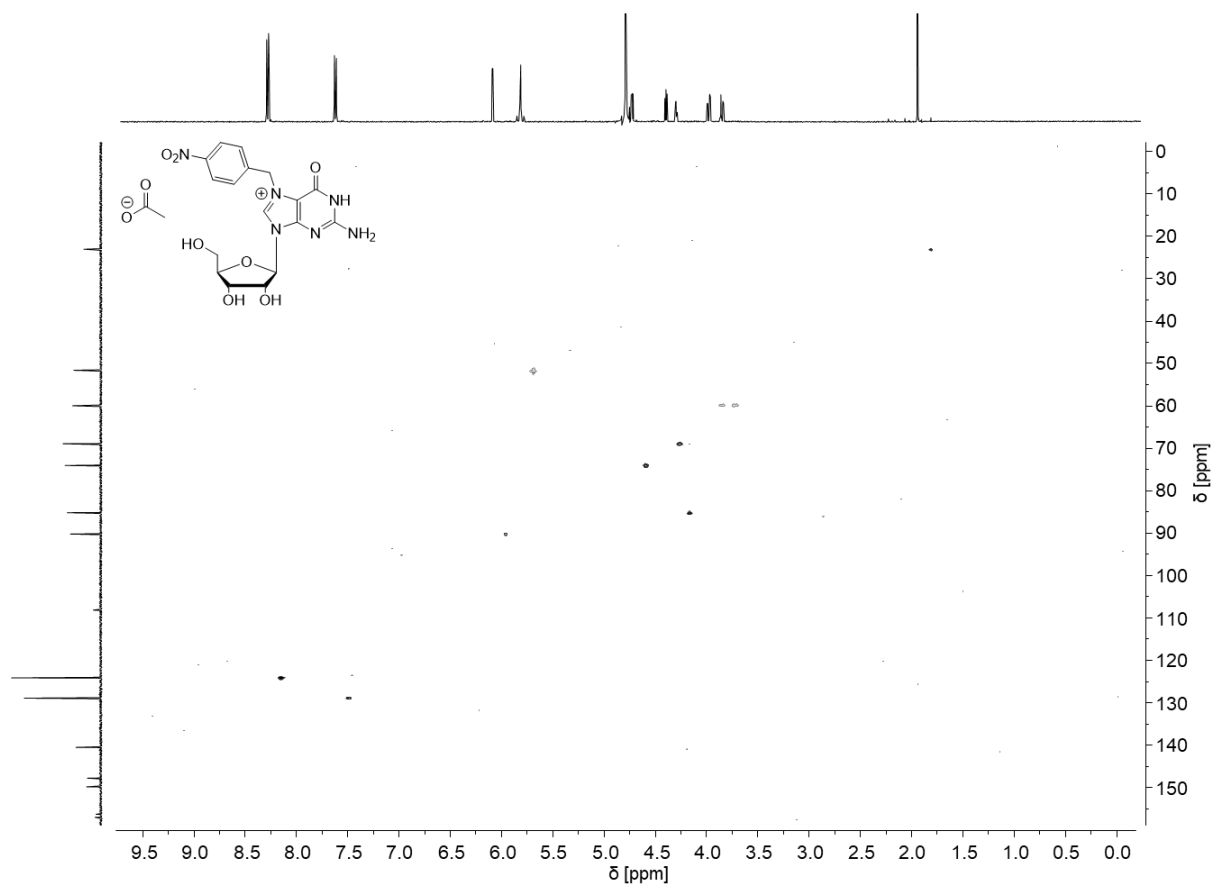


Figure S51. ^1H , ^{13}C -HSQC NMR spectrum (600 MHz, 151 MHz, D_2O , 299 K) of **3b**.

SUPPORTING INFORMATION

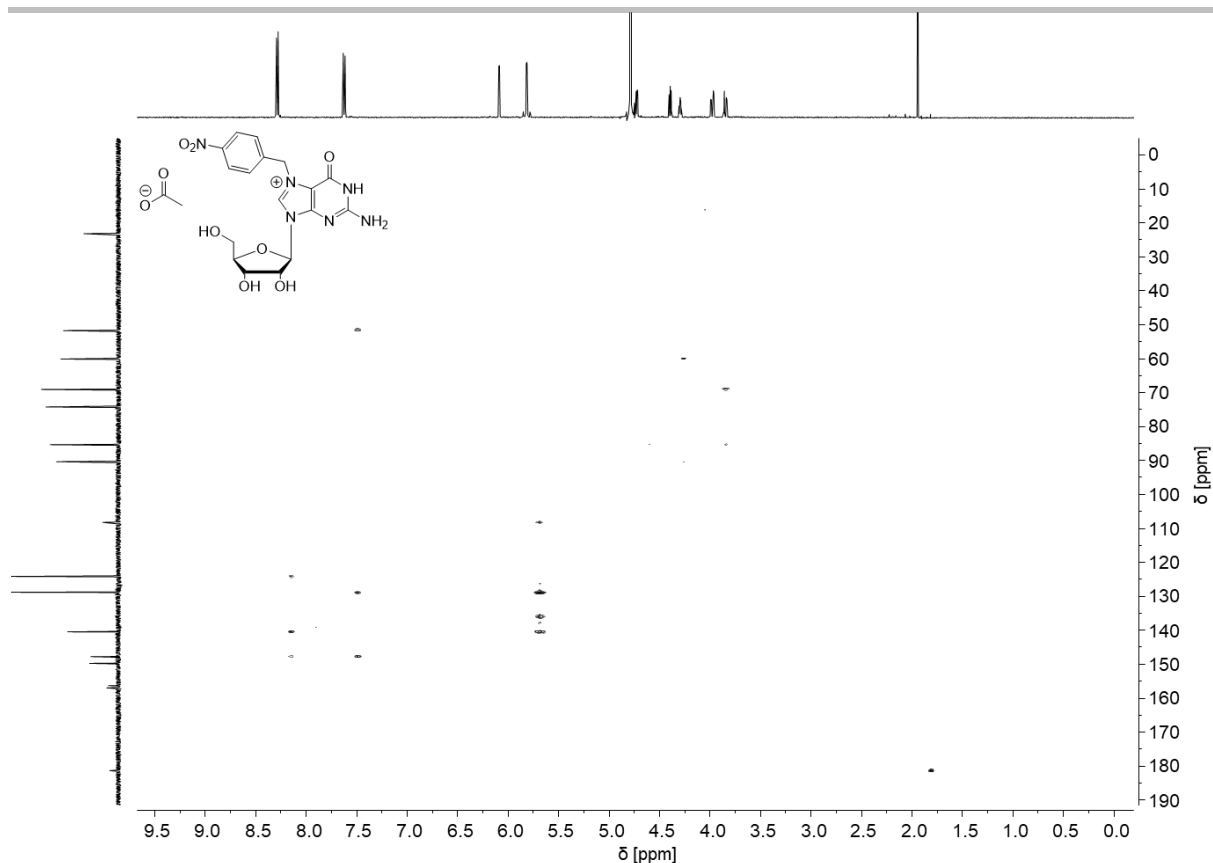


Figure S52. ^1H , ^{13}C -HMBC NMR spectrum (600 MHz, 151 MHz, D_2O , 299 K) of **3b**.

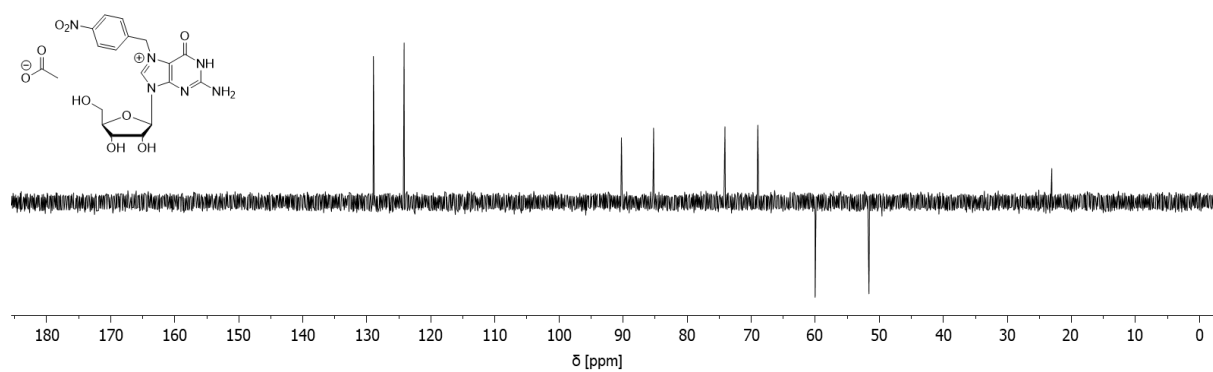


Figure S53. ^{13}C -DEPT NMR spectrum (151 MHz, D_2O , 299 K) of **3b**.

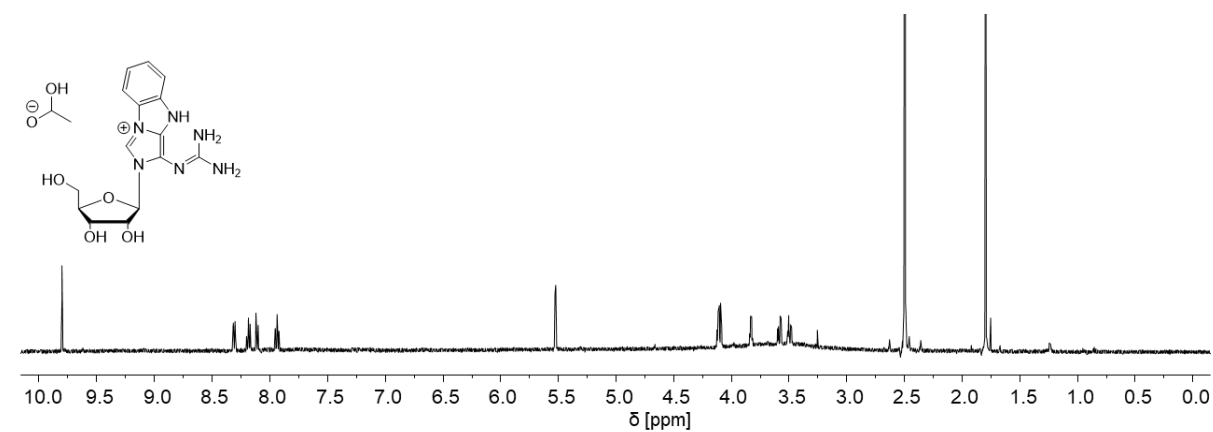


Figure S54. ^1H NMR spectrum (600 MHz, $\text{DMSO}-d_6$, 299 K) of **4**.

SUPPORTING INFORMATION

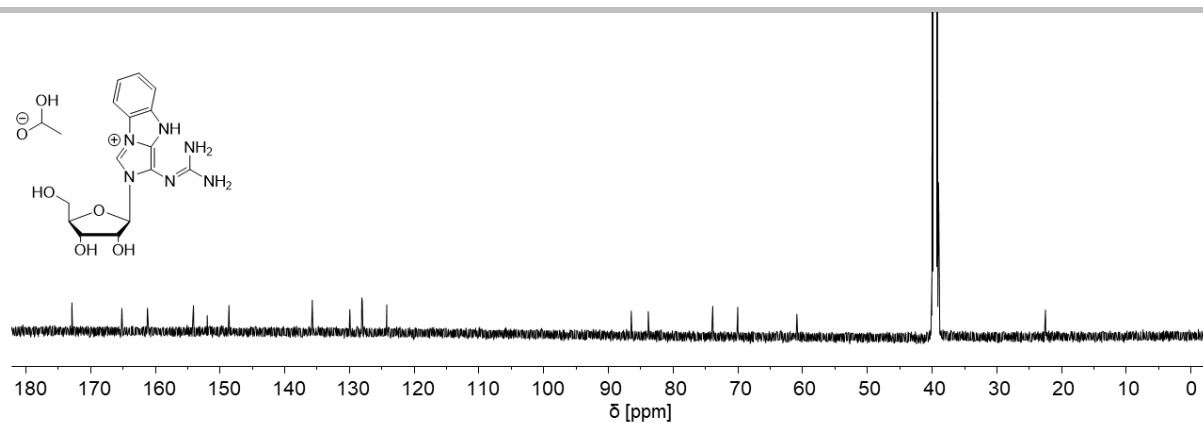


Figure S55. ^{13}C NMR spectrum (151 MHz, $\text{DMSO-}d_6$, 299 K) of 4.

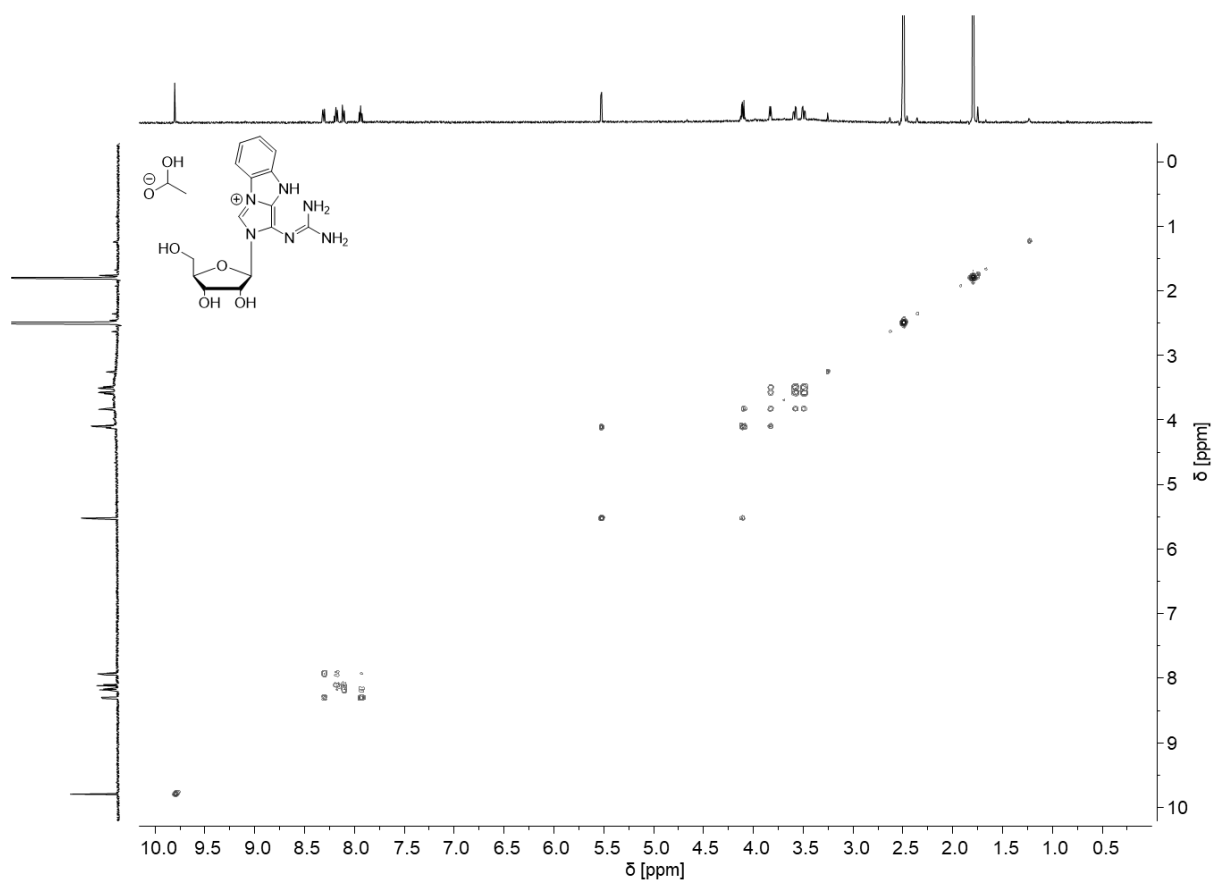


Figure S56. ^1H , ^1H -COSY NMR spectrum (600 MHz, $\text{DMSO-}d_6$, 299 K) of 4.

SUPPORTING INFORMATION

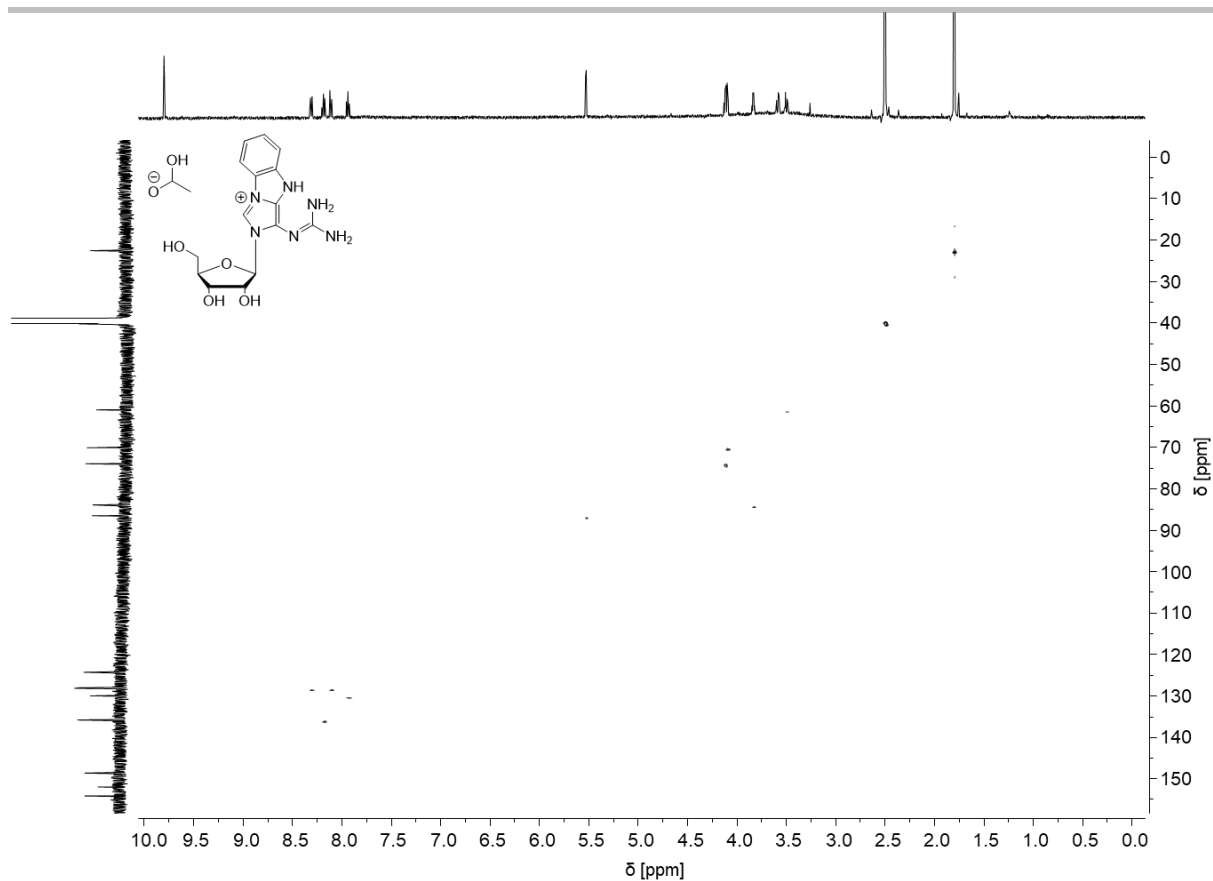


Figure S57. ¹H, ¹³C-HSQC NMR spectrum (600 MHz, 151 MHz, DMSO-*d*₆, 299 K) of 4.

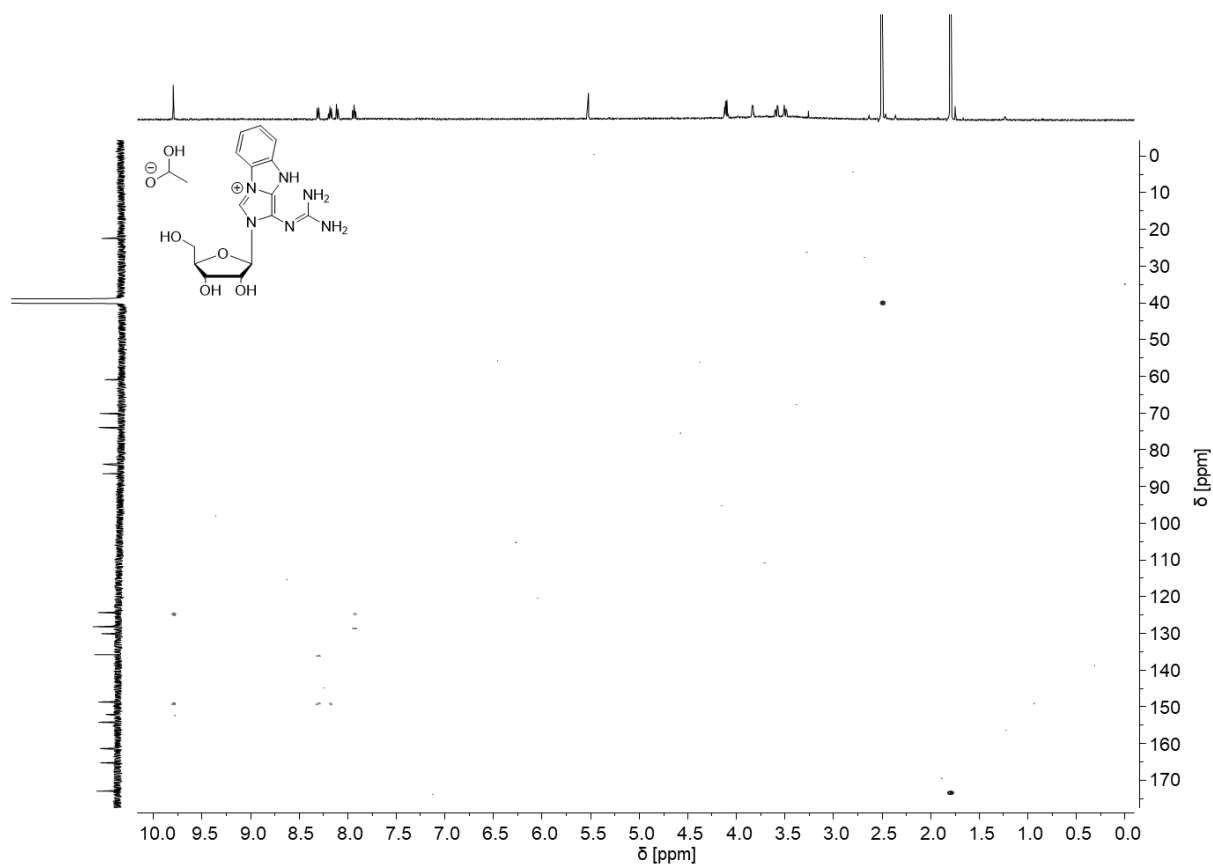


Figure S58. ¹H, ¹³C-HMBC NMR spectrum (600 MHz, 151 MHz, DMSO-*d*₆, 299 K) of 4.

SUPPORTING INFORMATION

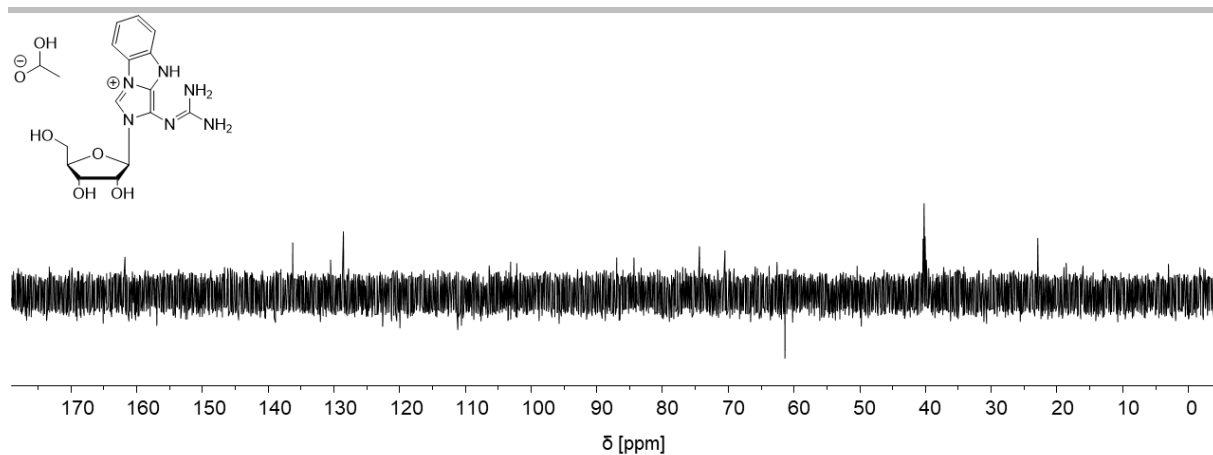


Figure S59. ¹³C-DEPT NMR spectrum (126 MHz, DMSO-*d*₆, 299 K) of **4**.

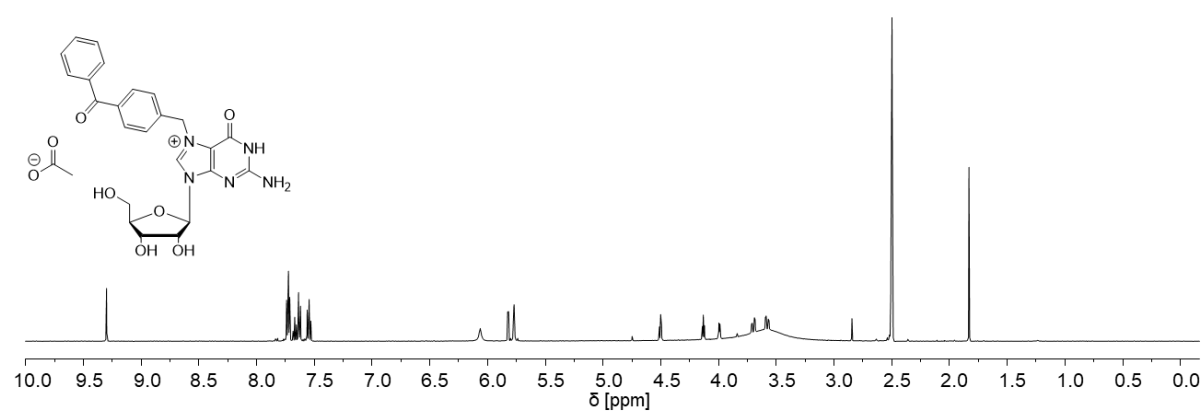


Figure S60. ¹H NMR spectrum (500 MHz, DMSO-*d*₆, 299 K) of **3c**.

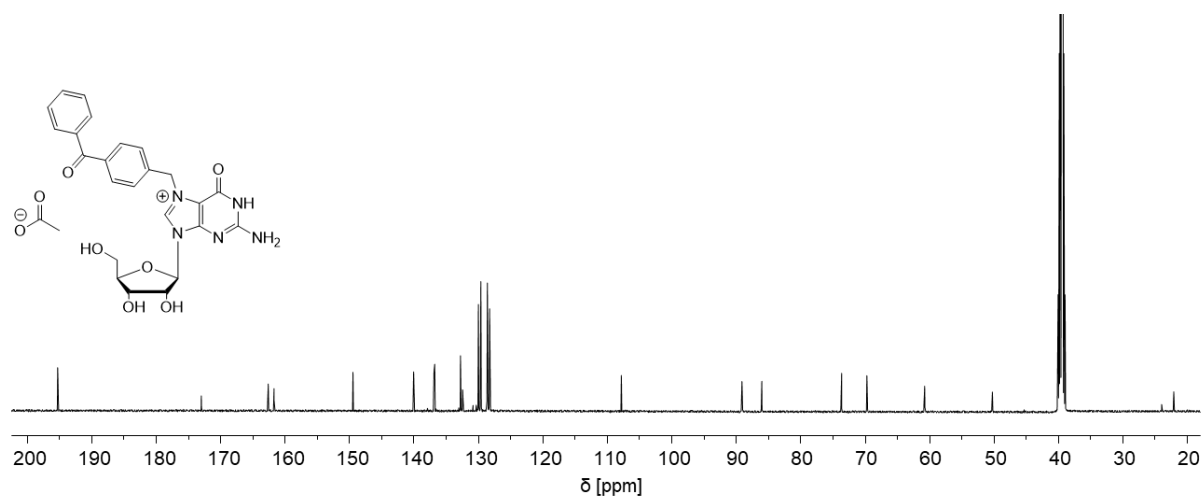


Figure S61. ¹³C NMR spectrum (126 MHz, DMSO-*d*₆, 299 K) of **3c**.

SUPPORTING INFORMATION

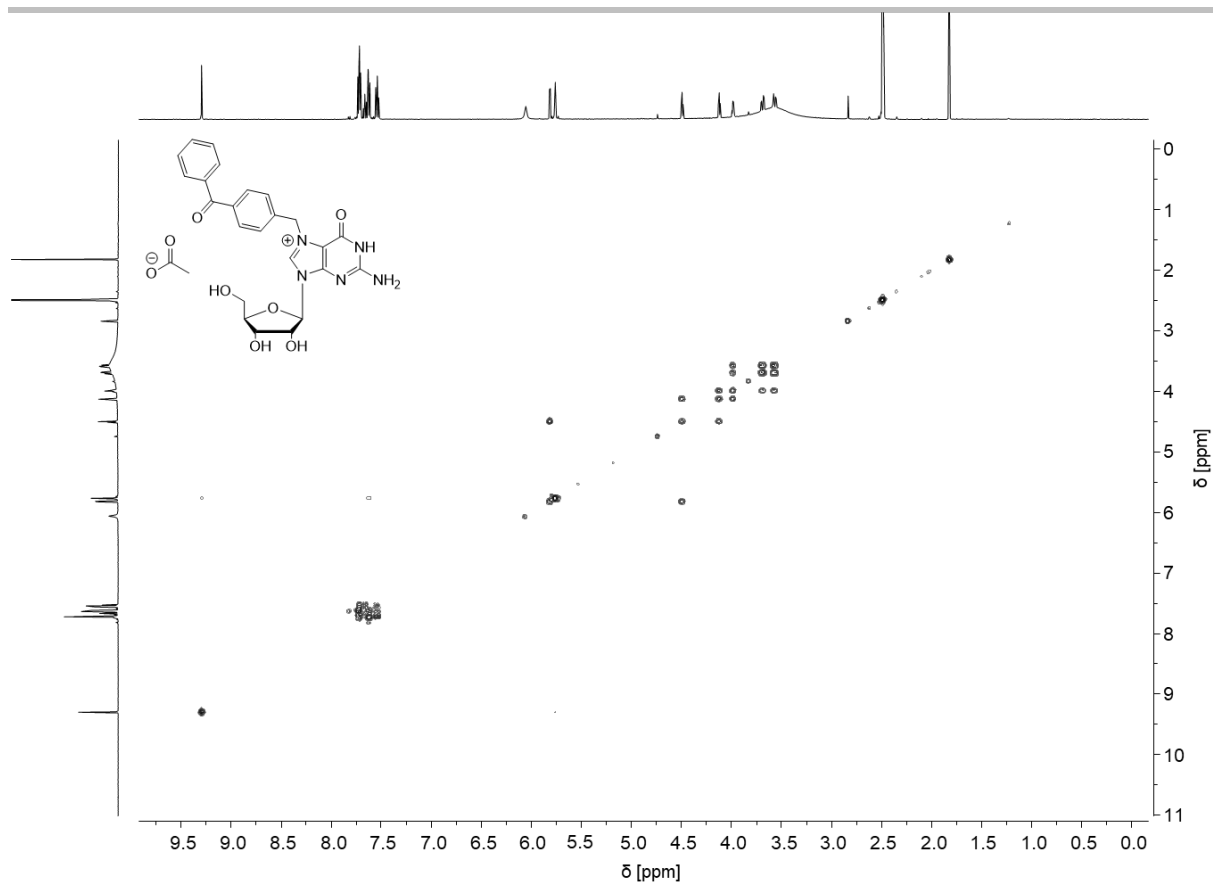


Figure S62 ^1H , ^1H -COSY NMR spectrum (500 MHz, $\text{DMSO}-d_6$, 299 K) of **3c**.

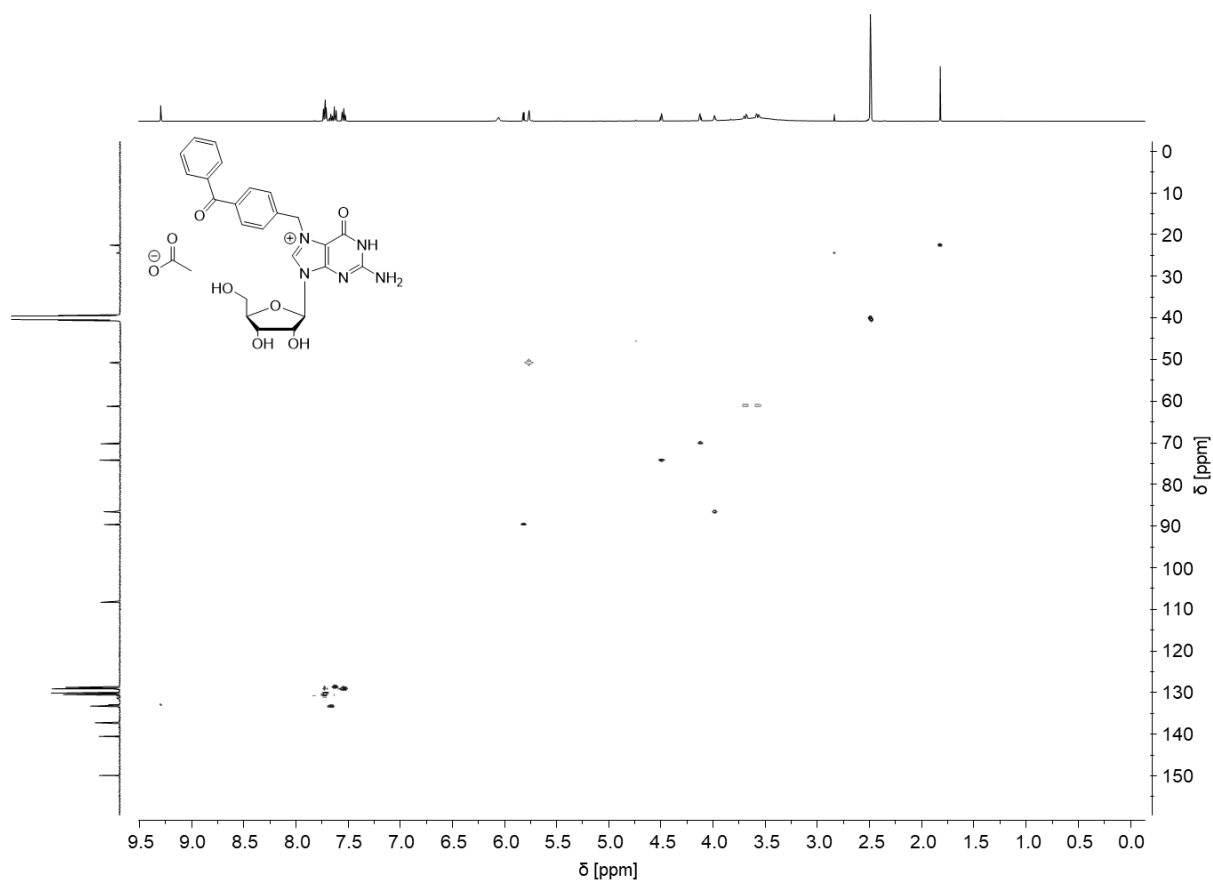


Figure S63. ^1H , ^{13}C -HSQC NMR spectrum (500 MHz, 126 MHz, $\text{DMSO}-d_6$, 299 K) of **3c**.

SUPPORTING INFORMATION

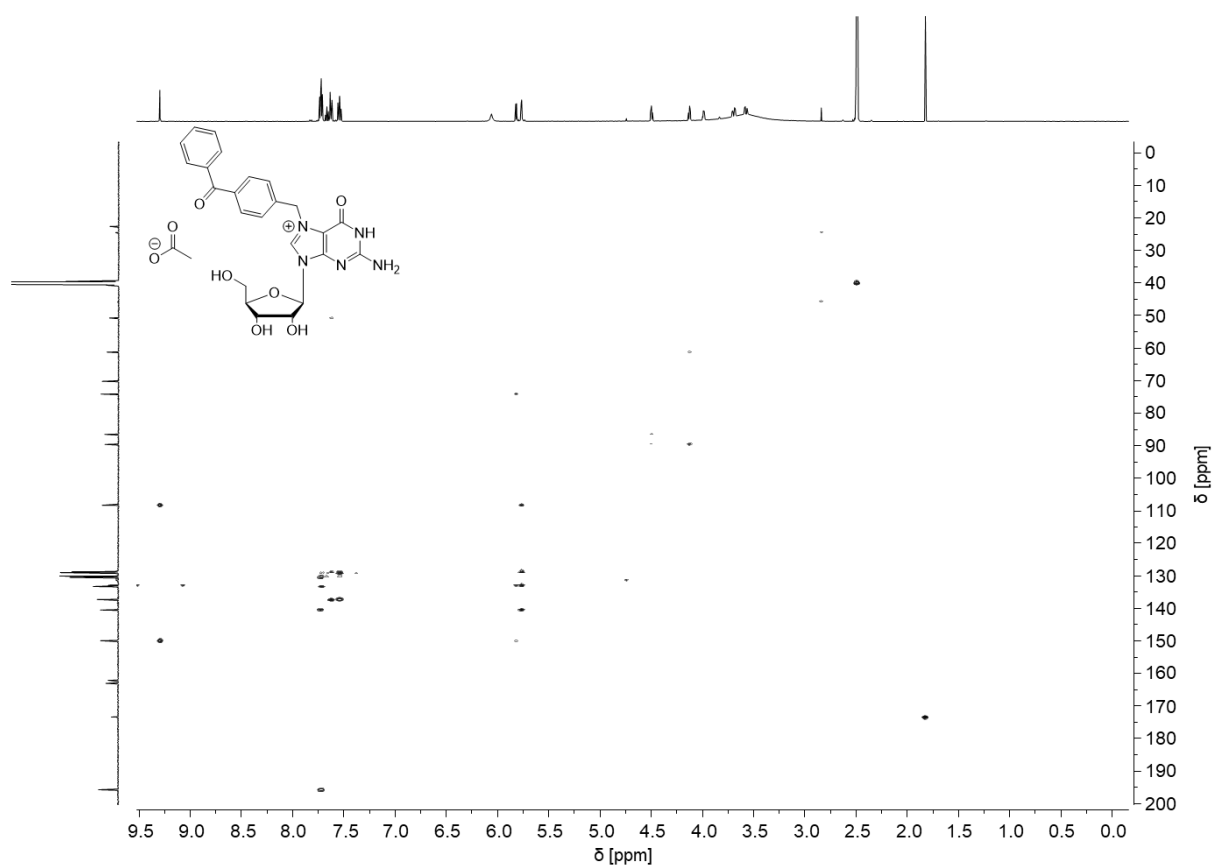


Figure S64. ^1H , ^{13}C -HMBC NMR spectrum (600 MHz, 126 MHz, $\text{DMSO}-d_6$, 299 K) of **3c**.

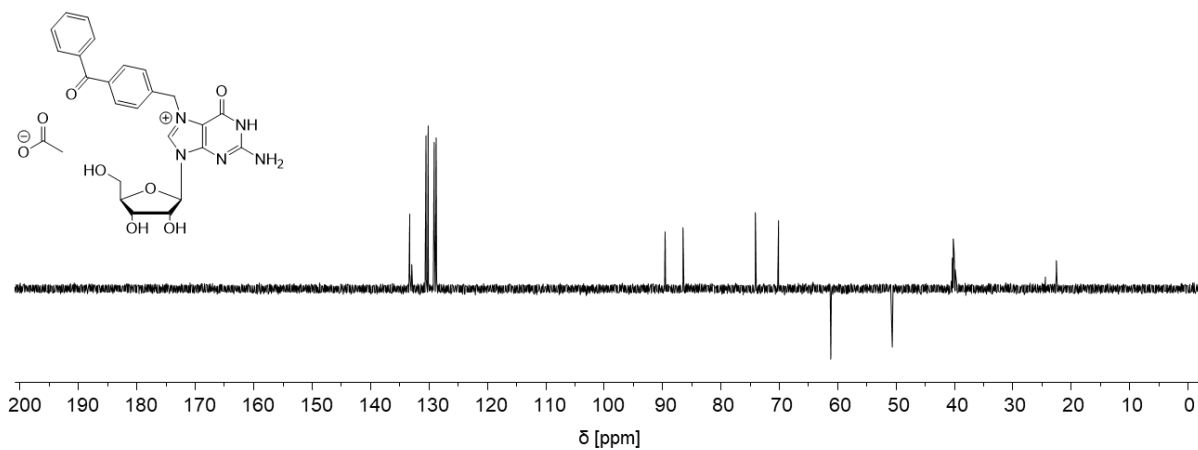


Figure S65. ^{13}C -DEPT NMR spectrum (126 MHz, $\text{DMSO}-d_6$, 299 K) of **3c**.

SUPPORTING INFORMATION

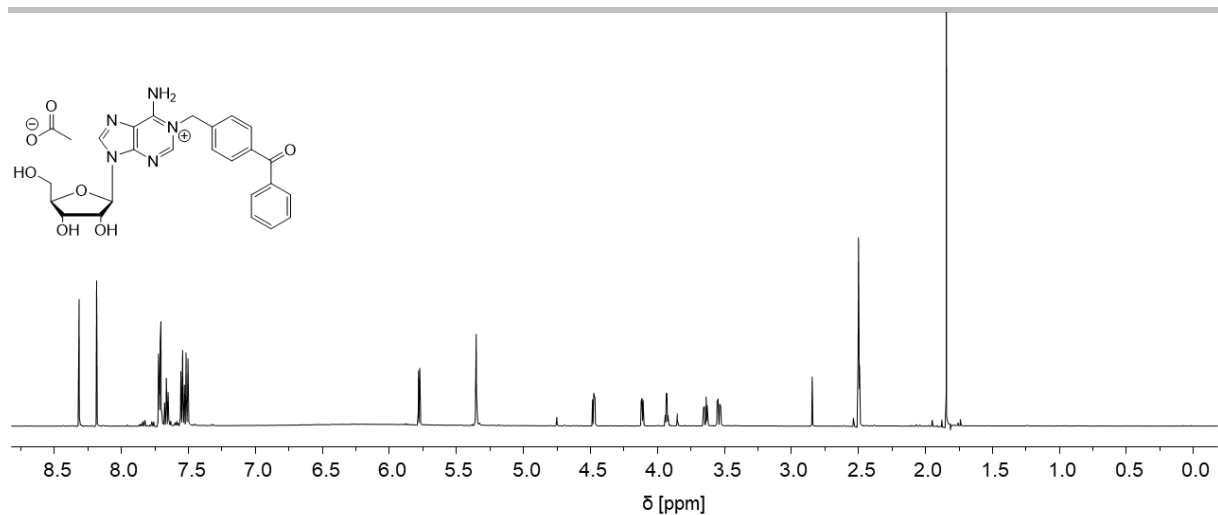


Figure S66. ¹H NMR spectrum (600 MHz, DMSO-d₆, 299 K) of 7.

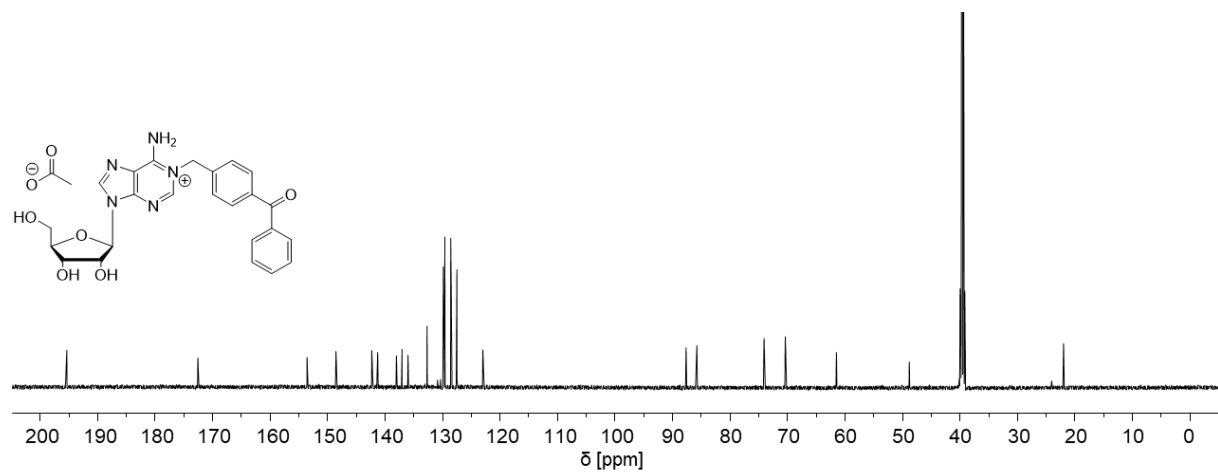


Figure S67. ¹³C NMR spectrum (151 MHz, DMSO-d₆, 299 K) of 7.

SUPPORTING INFORMATION

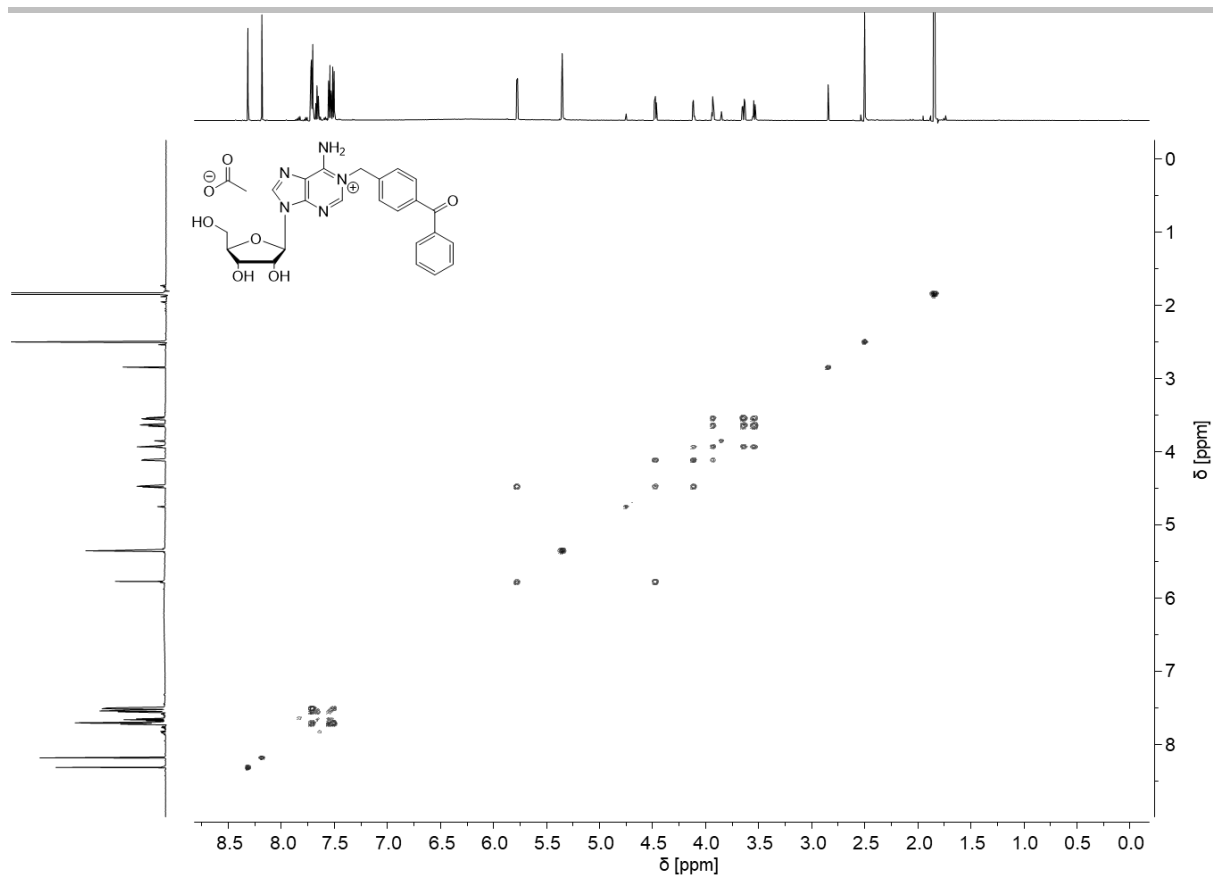


Figure S68. ^1H , ^1H -COSY NMR spectrum (600 MHz, $\text{DMSO-}d_6$, 299 K) of 7.

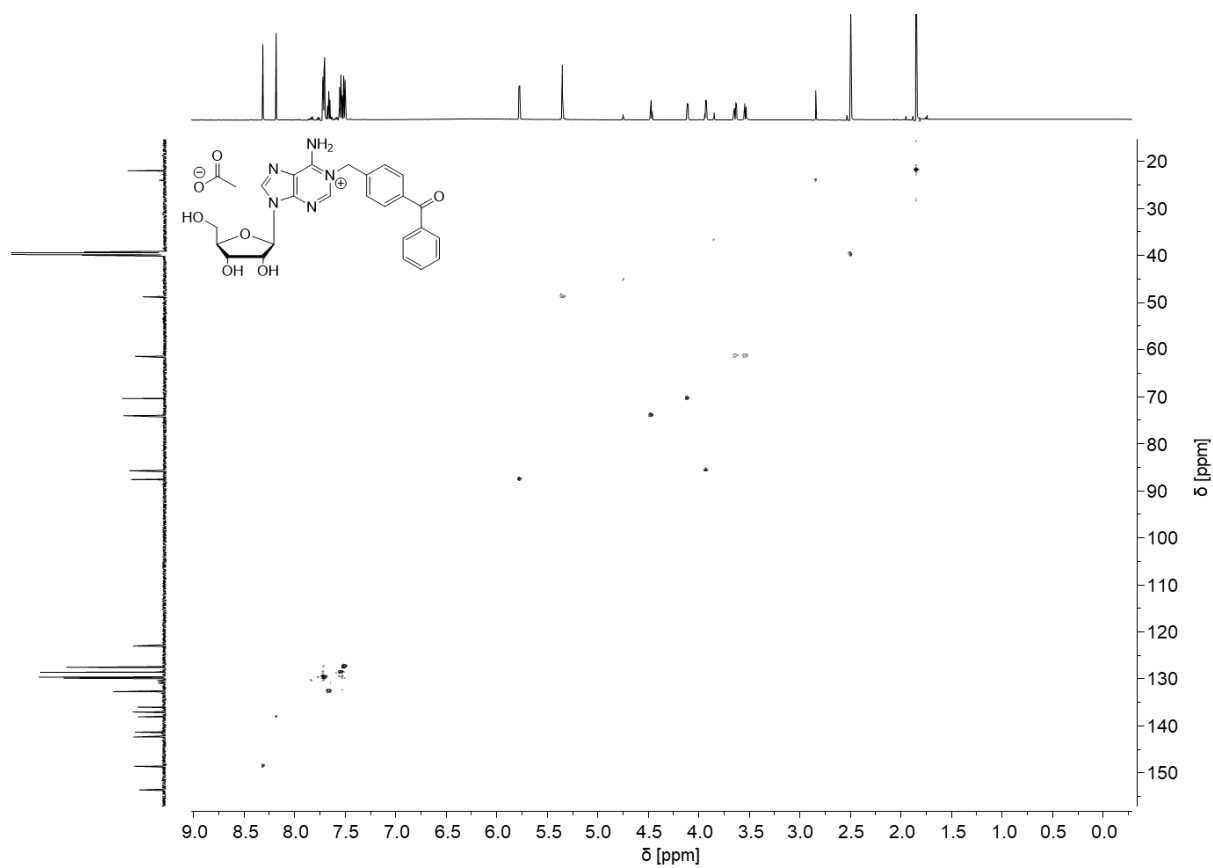


Figure S69. ^1H , ^{13}C -HSQC NMR spectrum (600 MHz, 151 MHz, $\text{DMSO-}d_6$, 299 K) of 7.

SUPPORTING INFORMATION

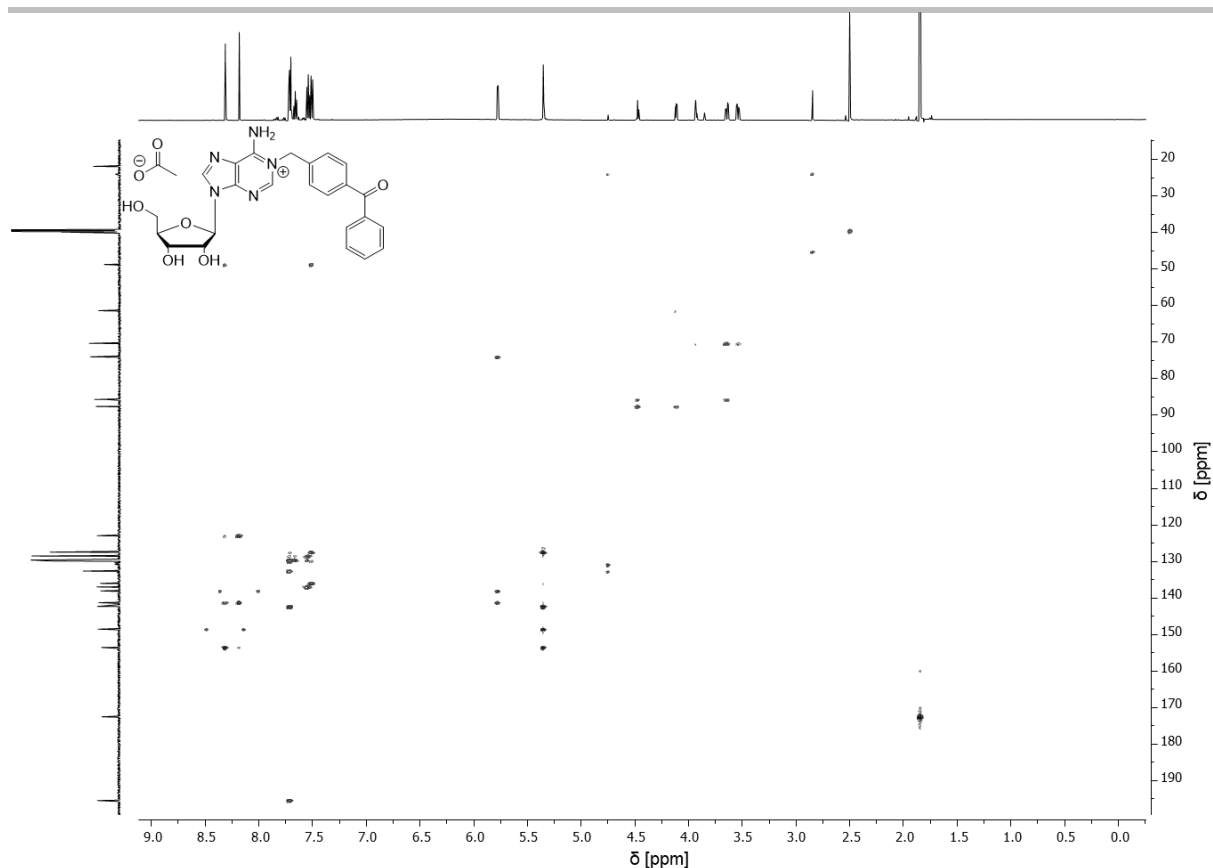


Figure S70. ^1H , ^{13}C -HMBC NMR spectrum (600 MHz, 151 MHz, $\text{DMSO}-d_6$, 299 K) of **7**.

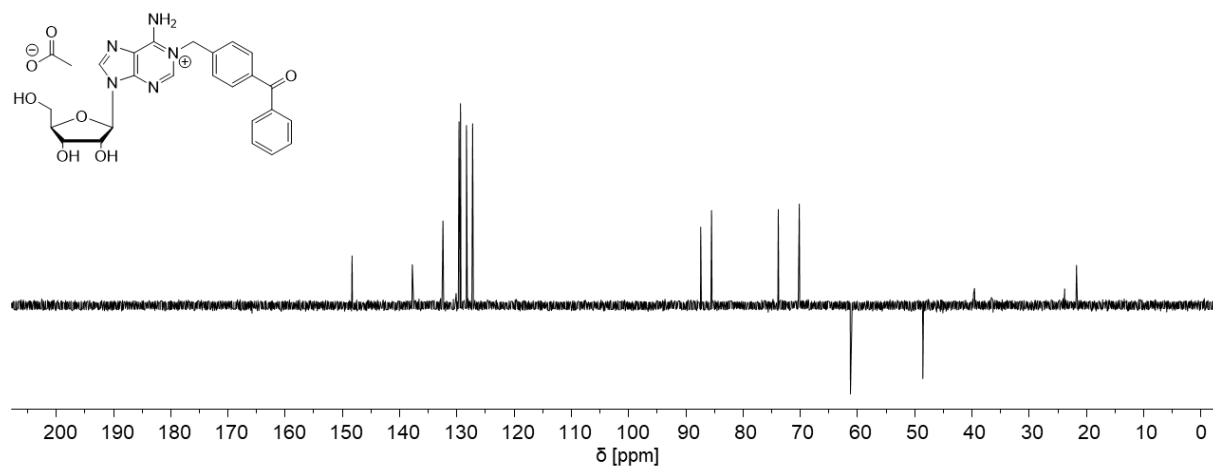


Figure S71. ^{13}C -DEPT NMR spectrum (151 MHz, $\text{DMSO}-d_6$, 299 K) of **7**.

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