

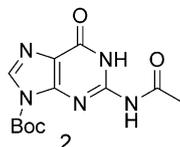
## Supporting Information

### Optochemical control of gene expression by photocaged guanine and riboswitches

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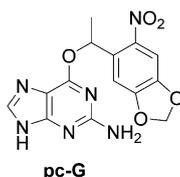
**General:** All chemicals and solvents (including dry DMF and dry dioxane) were purchased from commercial sources and were used without further purification. The starting compound (**1**) and the intermediate (**3**) shown in **Scheme 1** were prepared as previously reported.<sup>1,2</sup> Thin-layer chromatography (TLC) was performed on silica gel plates pre-coated with fluorescence indicator and visualized by UV light (254 nm). Silica gel (45-75  $\mu\text{m}$ ) was used for column chromatography. <sup>1</sup>H NMR spectra were recorded on 400 MHz Bruker or 600 MHz JEOL NMR instrument. The chemical shifts ( $\delta$ ) in parts per million were referenced to the residual proton signal of CD<sub>2</sub>Cl<sub>2</sub> (5.32 ppm) or DMSO-*d*<sub>6</sub> (2.50 ppm) for <sup>1</sup>H NMR spectra, and CD<sub>2</sub>Cl<sub>2</sub> (53.8 ppm) or DMSO-*d*<sub>6</sub> (39.5 ppm) for <sup>13</sup>C NMR spectra. Multiplicities of <sup>1</sup>H NMR spin couplings are reported as s (singlet), br s (broad singlet), d (doublet), t (triplet), and q (quartet). Values for apparent coupling constants (*J*) are reported in Hz. High-resolution mass spectrometry (HRMS) data were obtained using the positive ion electrospray ionization (ESI) mode.

#### *tert*-butyl 2-acetamido-6-oxo-1,6-dihydro-9H-purine-9-carboxylate (**2**)



Boc anhydride (0.14 mL, 0.62 mmol) was added to a stirred solution of *N*-2-acetyl guanine (100 mg, 0.52 mmol) in dry DMF (1 mL) and triethylamine (0.043 mL, 0.31 mmol). The reaction mixture was stirred at room temperature under N<sub>2</sub> atm for 17 h. The reaction mixture was passed through a silica gel column, and washed with a mixture of DCM and hexane (1:1, 120 mL) followed by DCM (50 mL) to remove DMF. Compound **2** was eluted with 3 % methanol in DCM and white solid (134 mg, 88 %) was obtained after removing the solvent. <sup>1</sup>H NMR (400 MHz, CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$  12.16 (br s), 10.17 (br, s), 8.13 (s, 1H), 2.26 (s, 3H), 1.62 (s, 9H); <sup>13</sup>C NMR (100 MHz, CD<sub>2</sub>Cl<sub>2</sub>)  $\delta$  173.1, 155.7, 149.3, 149.0, 146.1, 138.4, 122.8, 87.1, 28.1, 24.6; HRMS (ESI) Calcd for C<sub>12</sub>H<sub>16</sub>O<sub>4</sub>N<sub>5</sub> (M+H)<sup>+</sup> 294.1197, found 294.1202.

#### 6-(1-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethoxy)-9H-purin-2-amine (pc-G)



DIAD (90 %, 112  $\mu\text{l}$ , 0.51 mmol) was added to a stirred solution containing compound **2** (100 mg, 0.34 mmol), compound **3** (90 mg, 0.42 mmol), and PPh<sub>3</sub> (140 mg, 0.53 mmol) in dry dioxane (3 mL). The reaction mixture was stirred at room temperature under N<sub>2</sub> atm for 5 h. The

solvent was evaporated under reduced pressure, and the product, as judged by TLC, was purified from the mixtures by column chromatography (30 % ethyl acetate in hexane). This compound was dissolved in methanol (8 mL) and was transferred to a tube containing aq. ammonia (28 %, 10 mL) and was sealed by screw cap. It was stirred at 80 °C for 2 days. After cooled to room temperature, the solvents were removed under reduced pressure. The resulting residue was purified by column chromatography (4 % Methanol in DCM and 0.1% Et<sub>3</sub>N) to furnish the desired compound as yellow solid (57.6 mg, 4 % after 2 steps). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 12.46 (br s, 1H, NH), 7.82 (s, 1H), 7.59 (s, 1H), 7.22 (s, 1H), 6.68 (q, J = 4.0 Hz, 1H), 6.18 (d, J = 20.0 Hz, 2H, O-CH<sub>2</sub>-O-), 5.94 (br s, 2H, NH<sub>2</sub>), 1.70 (d, J = 4.0 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (150 MHz, DMSO-d<sub>6</sub>) δ 159.3, 158.8, 155.4, 152.0, 147.0, 142.1, 138.1, 134.9, 113.5, 105.7, 104.5, 103.4, 68.7, 21.7; HRMS (ESI) Calcd for C<sub>14</sub>H<sub>13</sub>O<sub>5</sub>N<sub>6</sub> (M+H)<sup>+</sup> 345.0942, found 345.0942.

### Absorption and photolysis analysis

Absorption spectra were recorded on Nanodrop 2000c using a quartz cuvette with 10 mm path length. Analytical HPLC (Shimadzu) was performed using a reversed-phase column (Phenomenex Clarity 3 μm oligo-RP) (50 x 4.6 mm) under an isocratic condition (30 % acetonitrile in water) over a period of 10 min followed by a washing step with 100 % acetonitrile at 35 °C. Compounds (3-6 nmoles in 30 % acetonitrile in water) were injected using an autosampler and detected at 254 nm. For decaging analysis, 1 mM solution of pc-G (30 % acetonitrile in water, in a PCR tube) was irradiated by UV light (365 nm) from the bottom through a UV transilluminator (8 W).

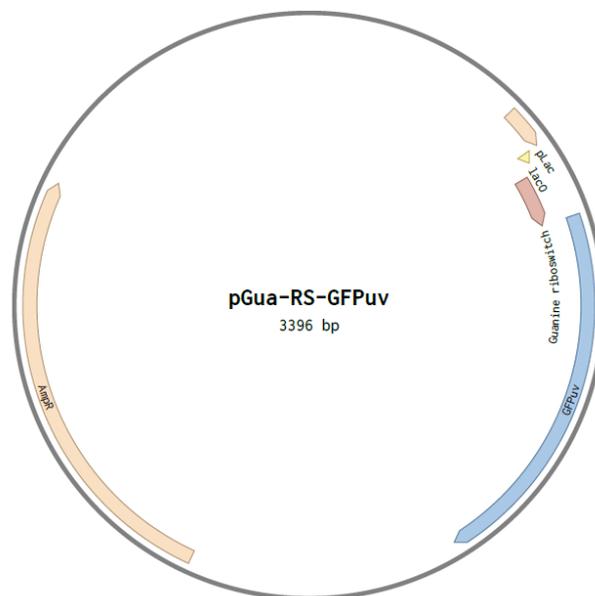
### Mammalian cell culture, fluorescence imaging and EGFP measurement

HEK293 cells (RIKEN BioResource Research Center) were maintained in a 5 % CO<sub>2</sub> humidified incubator at 37 °C in Dulbecco's modified Eagle's medium (DMEM) (Wako) supplemented with 10 % fetal bovine serum (FBS) (Gibco) and 1× antibiotics (Wako 161-2318). A day before transfection, HEK293 cells were trypsinized and centrifuged, and the resulting supernatant was discarded. The cells were diluted with fresh complete medium to 3.8 × 10<sup>5</sup> cells/mL, and were dispensed 100 μL per well in two 96-well tissue culture plates. Fifty nanograms of the EGFP-GuaM8HDV plasmid (pEGFP-GuaM8HDVRz),<sup>3</sup> 10 ng of pCMV-mCherry plasmid (constitutively expresses mCherry) and 50 ng of pUC19 plasmid (to adjust the total plasmid amount to 110 ng) were cotransfected using 1 μL of PolyFect reagent (QIAGEN) per well according to the manufacturer's instruction. After 3.5 h of incubation, the medium was removed and replaced with 100 μL of fresh complete medium containing either pc-G (100, 200, or 300 μM) or guanine (300 μM). Guanine (Sigma Aldrich) stock solution was prepared at 30 mM in 0.2 M NaOH and was diluted to the required concentration (300 μM) in the complete medium before use. Stock solutions of pc-G were prepared in DMSO (CultureSure grade, Wako). The cells were incubated in the presence of the compounds for 1 h. Then, one of the plates was exposed to UV light (365 nm, 8 W) from the bottom using a transilluminator for 16 min, and the cells were further incubated for 2 days. Fluorescence images were generated using EVOS FL cell imaging system. Cellular fluorescence was measured and normalized as reported previously.<sup>3</sup> Briefly, the cell culture medium was removed and was washed with PBS (100 μL × 3 times). Finally, PBS (150 μL per well) was added and incubated at 37 °C until measurement (10 min). Fluorescence intensities were measured for EGFP (484 nm excitation/510 nm

emission/10 nm band width) and mCherry (587 nm excitation/610 nm emission/10 nm bandwidth) using M1000PRO microplate reader (Tecan). The raw fluorescence values were first subtracted with that of the untransfected cells (background). For each well, EGFP fluorescence was normalized by mCherry fluorescence ([EGFP fluorescence]/[mCherry fluorescence]) to account for variations in transfection efficiency. The values were further normalized by the cells transfected with pEGFP-N1-BspEI (= 1.0). The reported values are mean  $\pm$  s.d. from 5 replicate samples.

### ***E. coli* guanine riboswitch design and plasmid construction**

The synthetic guanine riboswitch was based on the adenine riboswitch design previously reported by Dixon et al.,<sup>4</sup> with only a single nucleotide change in the core aptamer region (“T” to “C”) to change the specificity of the aptamer from adenine to guanine (see sequence below). The plasmid encoding the riboswitch (pGuaRS-GFPuv) was constructed based on pGFPuv (Clontech), and the sequence was confirmed by Sanger sequencing.



Plasmid map of pGuaRS-GFPuv

pLac-GuaRS-GFPuv sequence (pLac: blue, lacO: brown, guanine riboswitch: orange, guanine aptamer: orange/underlined, T-to-C mutation: red, and GFPuv: green)

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CGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGC
TCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACACAGGAAACAGCTGGATCCTCAACGCTT
CATATAATCCTAATGATATGGTTTTGGGAGTTTCTACCAAGAGCCTTAAACTCTTGACTATGAAGTCTG
TCGCTTTATCCGAAATTTTATAAAGAGAAGACTATGAATGCCATGATCATGAGTAAAGGAGAAGAAC
TTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCA
GTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACCTACCCTTAAATTTATTTGCACTACTGGAAA
ACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCTCTTATGGTGTTCATGCTTTTCCCGTTATCCG
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ATCTTTCAAAGATGACGGGAACACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTA
ATCGTATCGAGTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTCGGACACAAACTCGAGTA
CAACTATAACTCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTC
AAAATTCGCCACAACATTGAAGATGGATCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAA
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TTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCTGCCCTTTCGAAAGAT  
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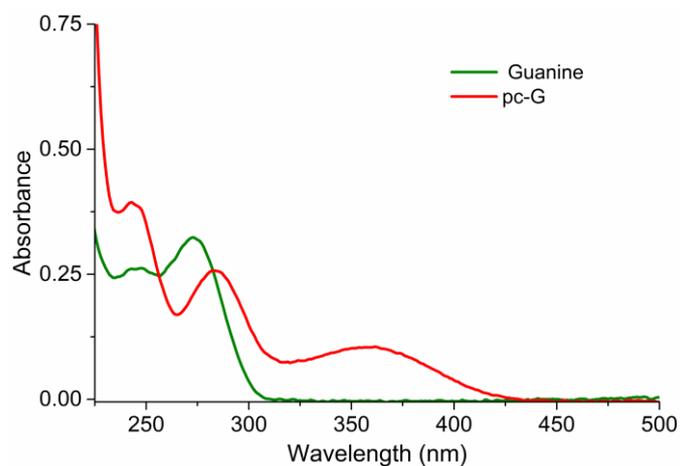
### **Guanine riboswitch assay in *E. coli***

*E. coli* TOP10 (Thermo Fisher) cells harbouring the plasmid pGuaRS-GFPuv was streaked from the -80 °C glycerol stock on an LB (Luria-Bertani) agar plate. Colonies were then picked to inoculate LB liquid cultures. After overnight incubation at 37 °C in a shaking incubator (250 rpm), the cells were diluted 60-fold in 3 mL M9 medium supplemented with casamino acids (1 g/L), glucose (4 g/L), and ampicillin (200 µg/mL). The composition of the M9 medium was as described previously.<sup>5</sup> Guanine and pc-G were added to the cultures at 500 µM final concentration as follows: 50 µL of guanine stock solution (30 mM in 0.2 M NaOH), 15 µL of pc-G stock solution (100 mM in DMSO) and 35 µL of 0.2 M NaOH, 50 µL of 0.2 M NaOH for “no compound” culture. All cultures were then incubated for 24 h at 37 °C in a shaking incubator. UV treated pc-G cultures were grown for 1h after dilution, exposed to UV (365 nm) for 16 min, and then returned to the incubator shaker. TOP10 cells harboring the empty pUC19 vector were grown under the same conditions as a negative control.

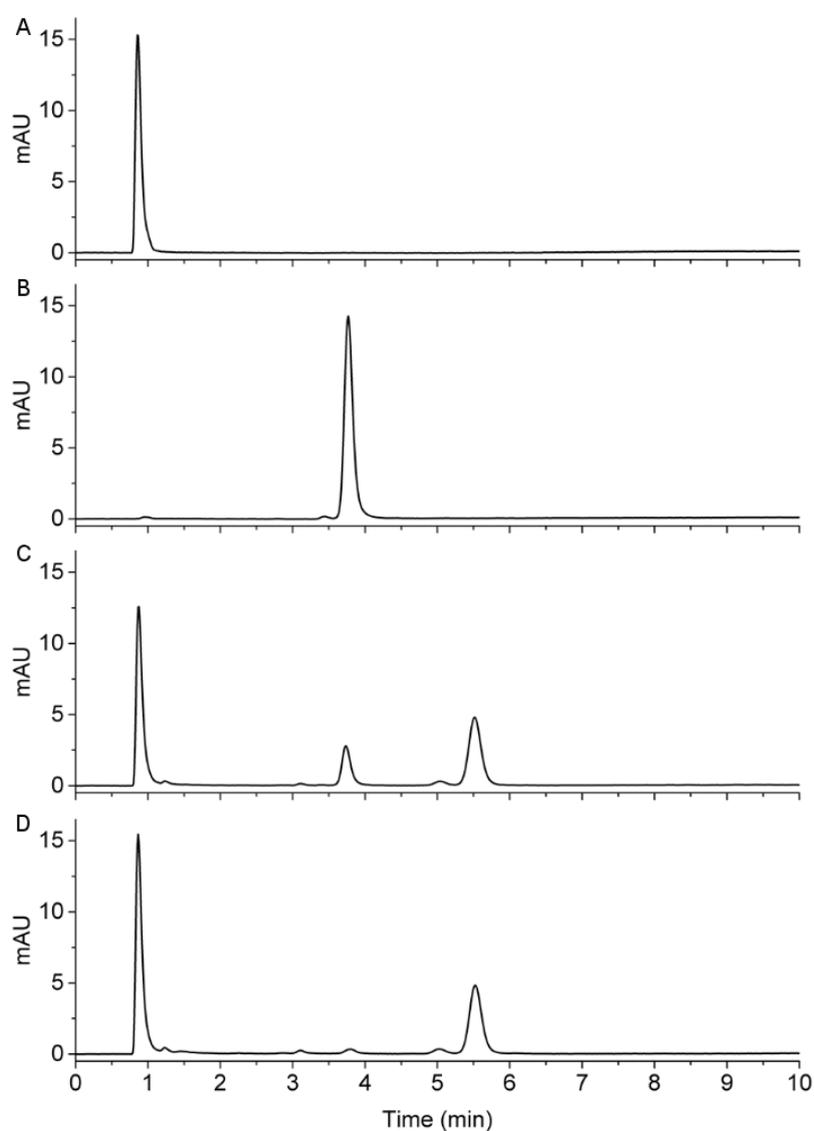
After 24 h, 1 mL from each culture was sampled and centrifuged. The medium was removed and the cell pellets were suspended in 250 µL PBS. The cells in PBS (200 µL) were transferred to a 96-well microplate to measure GFP fluorescence (excitation 484 nm / emission 510 nm) and OD600 using a microplate reader (Tecan M1000PRO). The reported normalized GFP levels ( $FL_{norm}$ ) were calculated as follows:

$$FL_{norm} = [(FL_{sample} - FL_{PBS}) / (OD600_{sample} - OD600_{PBS})] - [(FL_{pUC19} - FL_{PBS}) / (OD600_{pUC19} - OD600_{PBS})]$$

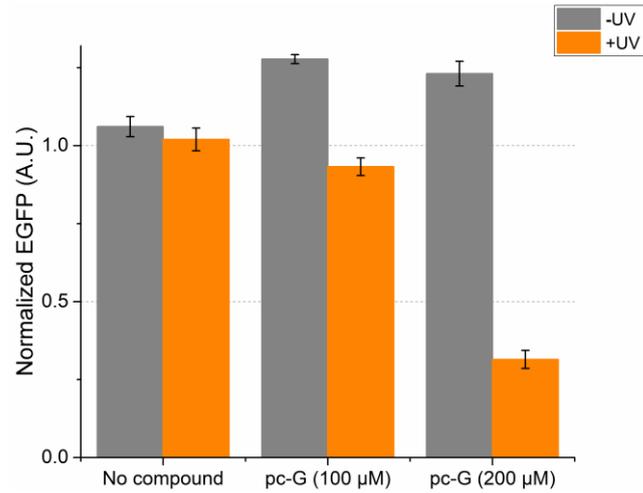
Where  $FL_{sample}$ ,  $FL_{PBS}$ , and  $FL_{pUC19}$  are the raw fluorescence values of sample cells, PBS, and pUC19 cells, respectively, and  $OD600_{sample}$ ,  $OD600_{PBS}$ , and  $OD600_{pUC19}$  are the raw OD600 values of sample cells, PBS, and pUC19 cells, respectively.



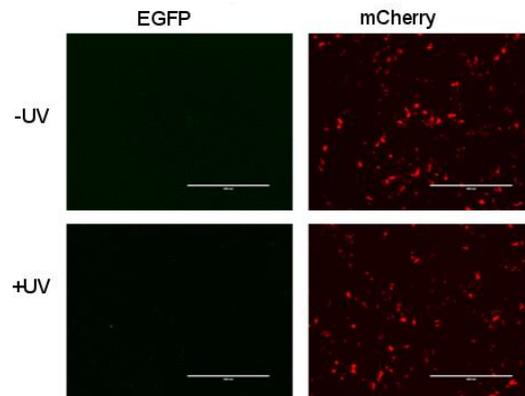
**Figure S1.** Absorbance spectra of guanine (40  $\mu$ M in water) and pc-G (40  $\mu$ M in water).



**Figure S2.** HPLC traces of (A) guanine (3 nmol), (B) pc-G (6 nmol), (C) pc-G (6 nmol) after UV irradiation (6 min) and (D) pc-G (6 nmol) after UV irradiation (16 min). The samples were analysed on an RP column under isocratic condition (30 % acetonitrile in water) at 254 nm and 35  $^{\circ}$ C.



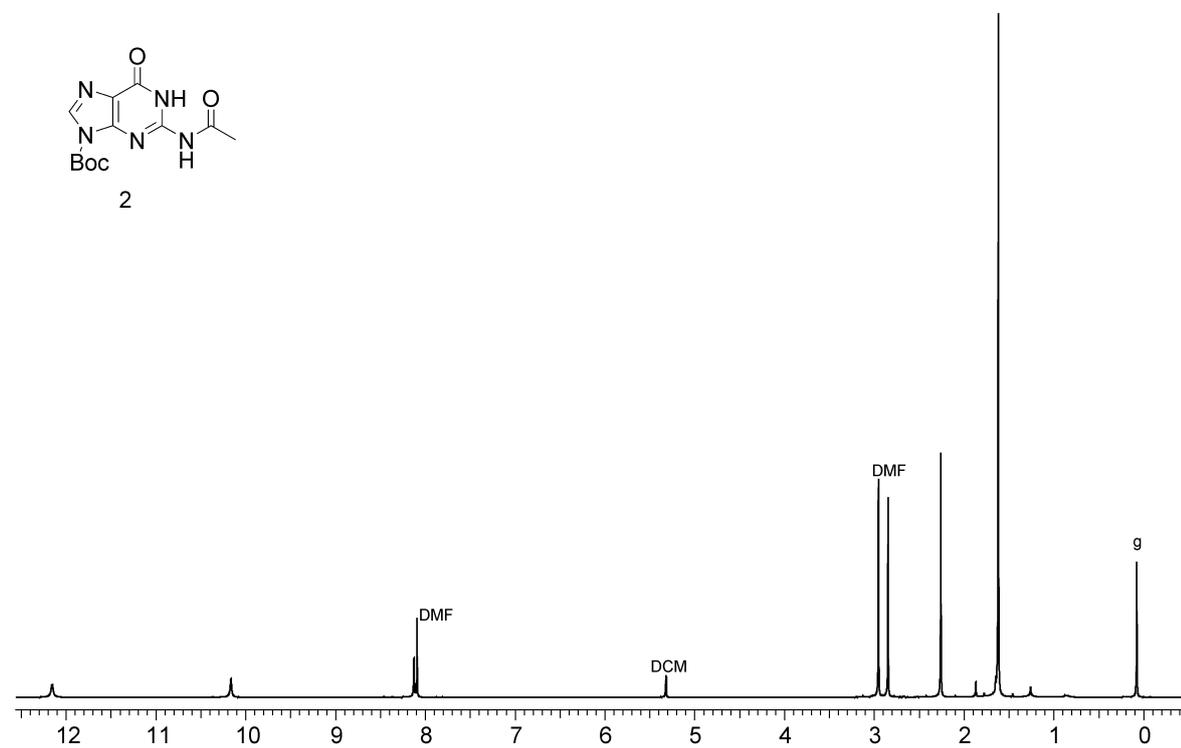
**Figure S3.** EGFP expression levels of HEK 293 cells transfected with the guanine-responsive riboswitch plasmid measured by a microplate reader. The cells were cultured in the presence or absence of pc-G (100  $\mu$ M and 200  $\mu$ M), with or without UV irradiation (8 W, 16 min). Error bars represent s.d. of five samples.



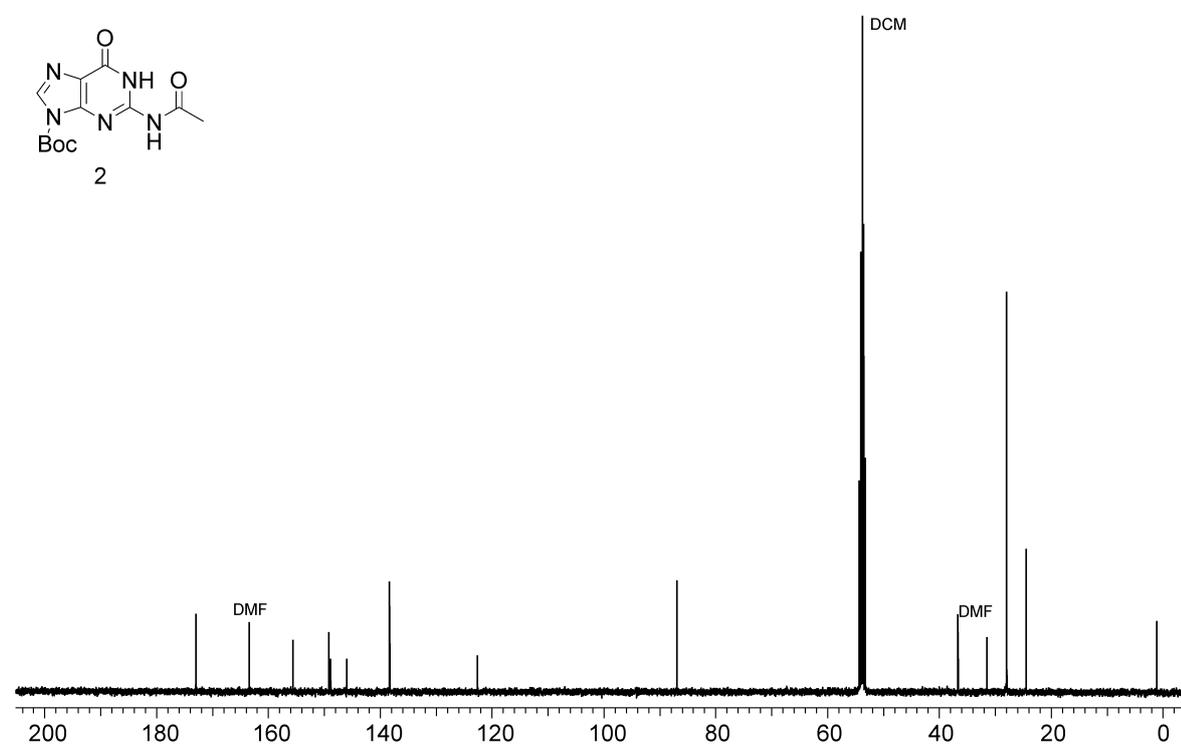
**Figure S4.** Fluorescence micrographs showing the expression of levels of EGFP (left) and mCherry (right) of the cells transfected with EGFP-GuaM8HDV and mCherry expression plasmids in the presence of guanine (300  $\mu$ M). The cells shown on the bottom were exposed to UV (8 W, 16 min).

## NMR spectra ( $^1\text{H}$ and $^{13}\text{C}$ )

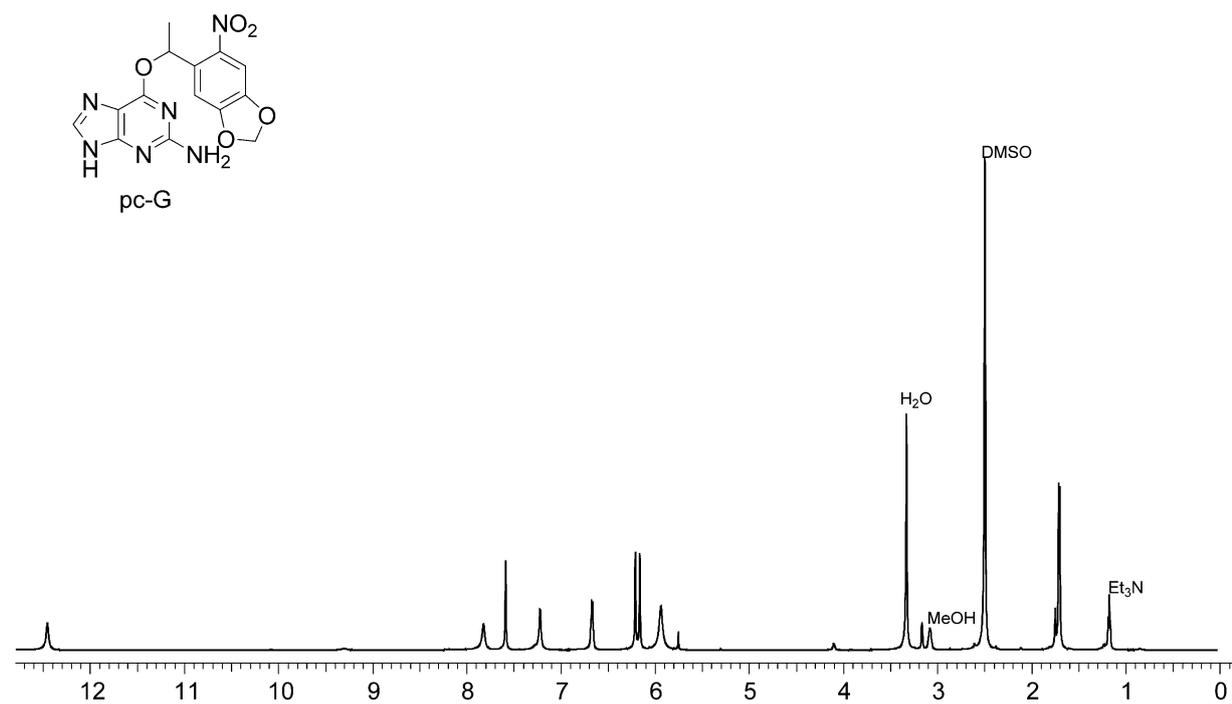
$^1\text{H}$  NMR spectrum of compound **2**



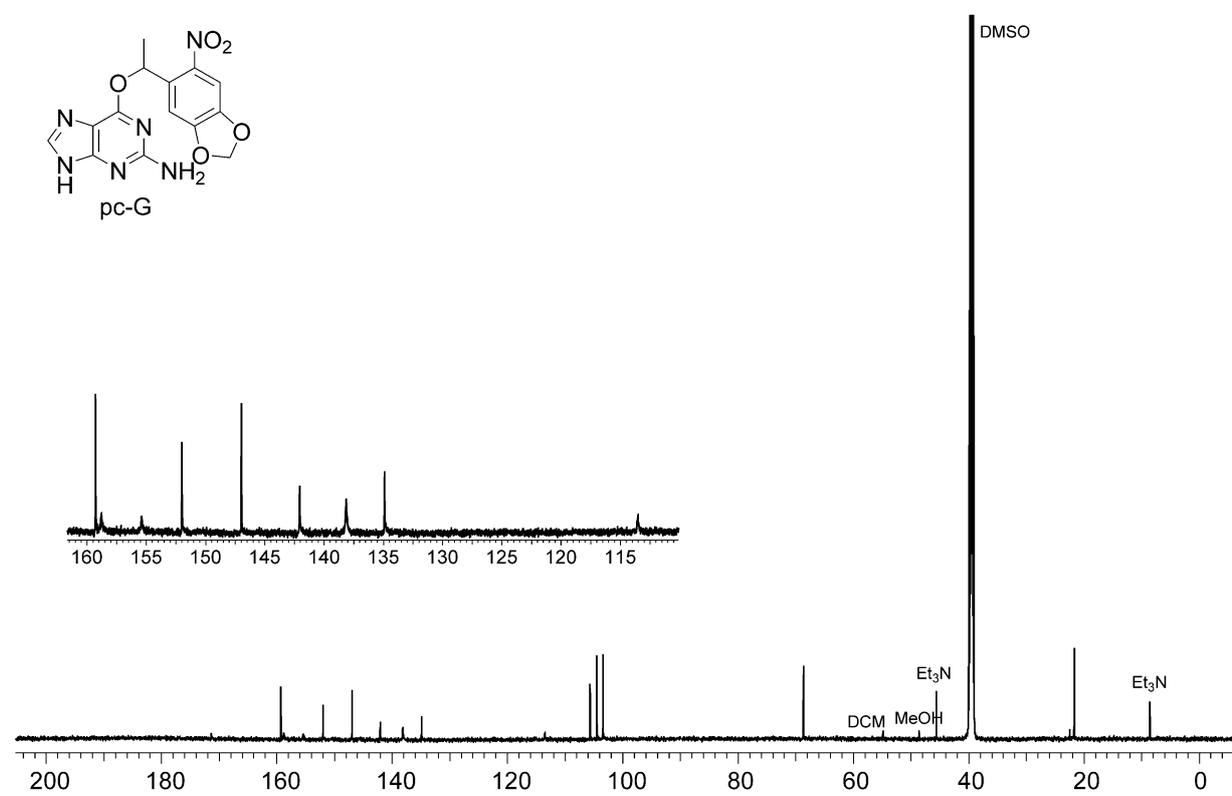
$^{13}\text{C}$  NMR spectrum of compound **2**



### $^1\text{H}$ NMR spectrum of pc-G



### $^{13}\text{C}$ NMR spectrum of pc-G



### References

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