

# Light Activation of Gene Function in Mammalian Cells Via Ribozymes

Douglas D. Young<sup>a</sup>, R. Aaron Garner<sup>b</sup>, Jeffrey A. Yoder<sup>b,c</sup>, and Alexander Deiters<sup>a,c\*</sup>

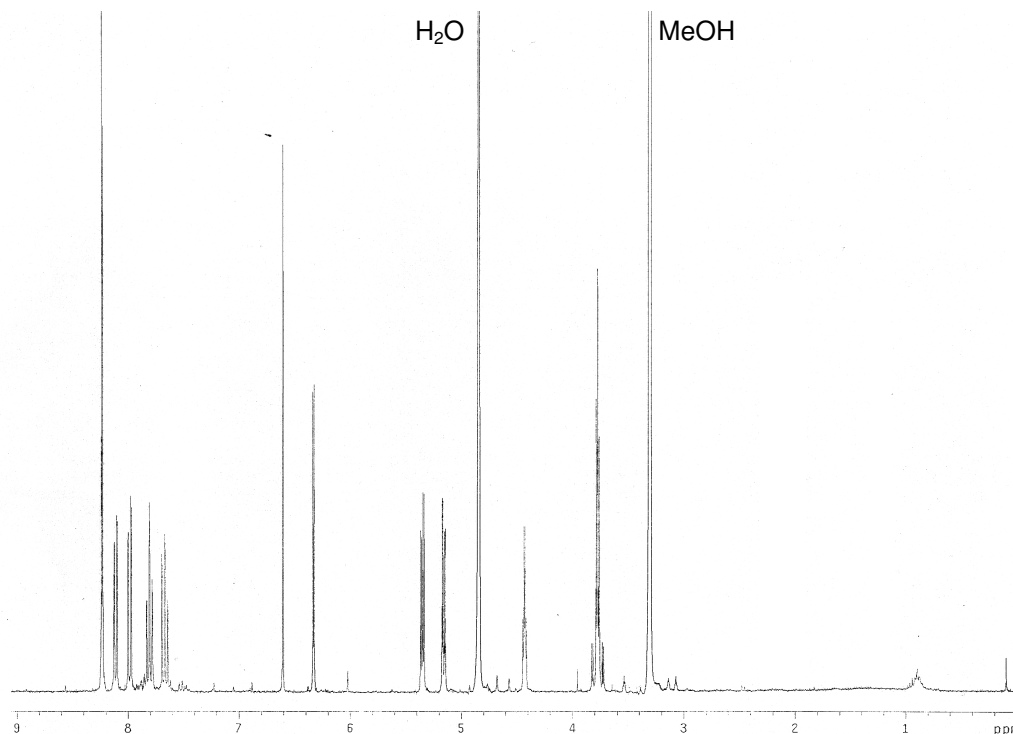
<sup>a</sup>Department of Chemistry, North Carolina State University, Raleigh, NC 27695, <sup>b</sup>Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606, and <sup>c</sup>Center for Comparative Medicine and Translational Research, North Carolina State University, Raleigh, NC 27606

## SUPPLEMENTARY INFORMATION

### 1. Synthesis and Analytical Data of Caged Toyocamycin 2

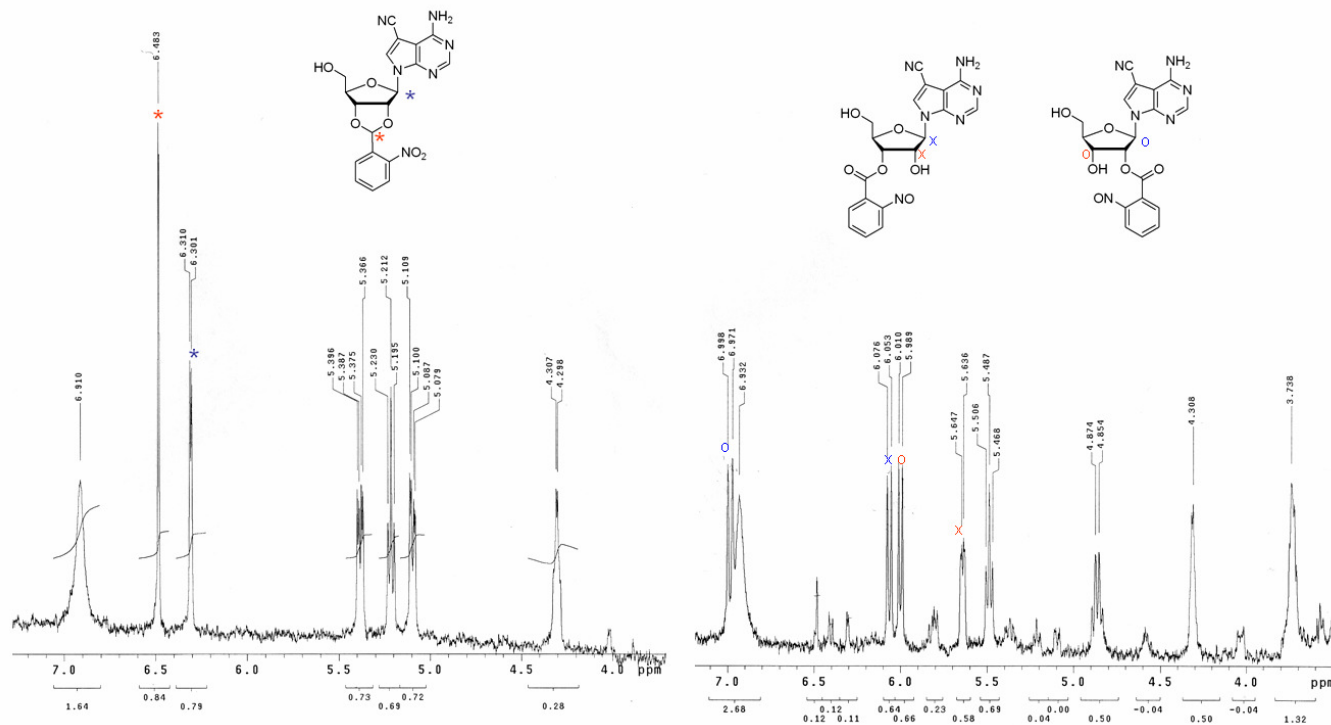
A flame dried vial under a nitrogen atmosphere was charged with toyocamycin (**1**) (14 mg, 0.05 mmol), 2-nitrobenzaldehyde (151 mg, 1.00 mmol) and ZnBr<sub>2</sub> (45 mg, 0.20 mmol). The reaction mixture was heated to 60 °C for 12 hours and directly purified via flash chromatography (100% EtOAc) on SiO<sub>2</sub> to yield an off-white solid (14 mg, 0.03 mmol, 68%). <sup>1</sup>H NMR (300 MHz; CD<sub>3</sub>OD) δ 8.24 (s, 1H), 8.22 (s, 1H), 8.11 (dd, *J*<sub>1</sub> = 7.8 Hz, *J*<sub>2</sub> = 1.5 Hz, 1H), 7.98 (dd, *J*<sub>1</sub> = 7.8 Hz, *J*<sub>2</sub> = 1.5 Hz, 1H), 7.80 (dt, *J*<sub>1</sub> = 7.8 Hz, *J*<sub>2</sub> = 1.5 Hz, 1H), 7.66 (dt, *J*<sub>1</sub> = 7.8 Hz, *J*<sub>2</sub> = 1.5 Hz, 1H), 6.60 (s, 1H), 6.33 (d, *J* = 3.3 Hz, 1H), 5.35 (dd, *J*<sub>1</sub> = 6.3 Hz, *J*<sub>2</sub> = 3.3 Hz, 1H), 5.16 (dd, *J*<sub>1</sub> = 6.3 Hz, *J*<sub>2</sub> = 2.1 Hz, 1H), 4.42 (dd, *J*<sub>1</sub> = 3.9 Hz, *J*<sub>2</sub> = 2.1 Hz, 1H), 3.77 (m, 2H); <sup>13</sup>C NMR (75 MHz; CD<sub>3</sub>OD) δ 157.5, 153.5, 149.9, 133.3, 132.5, 131.0, 130.5, 127.9, 124.3, 114.7, 102.9, 91.2, 86.4, 85.3, 84.3, 83.1, 62.1; HRMS (MALDI TOF): *m/z* calculated for C<sub>19</sub>H<sub>17</sub>N<sub>6</sub>O<sub>6</sub>: 425.1137, found: 425.1235; UV-VIS λ<sub>max</sub> 290nm (ε = 931 cm<sup>-1</sup>M<sup>-1</sup>); λ 365nm (ε = 174 cm<sup>-1</sup>M<sup>-1</sup>).

<sup>1</sup>H NMR (300 MHz; CD<sub>3</sub>OD)



## 2. UV Irradiation of Caged Toyocamycin

A solution of **2** (0.1 mM in MeOH) was irradiated for 5 minutes at 365 nm with a transilluminator (25 W). After irradiation the product was concentrated and analyzed by NMR and LC/MS. Based on the results of these two experiments the ratio of **3/4** was determined to be 1:1. Proton assignments were made based on literature reports for adenosine esters.<sup>1</sup>



## 3. Luciferase Assay in Mammalian Cell Culture

Human Embryonic Kidney (HEK-293T) cells were passaged into two 96-well culture plates, and grown to 60% confluence. The cells were then transfected with the N117-luc construct (0.5  $\mu$ g) and a pRL-CMV (Promega) transfection control construct (0.5  $\mu$ g) using Fugene HD (3:2 Fugene/DNA; Roche Biomedicals) in OptiMEM media (Invitrogen). The transfection was incubated at 37 °C for 4 hours, followed by replacement of transfection media with standard growth media (Dulbecco's modified Eagle's media (Hyclone) with 10% Fetal Bovine serum (Hyclone) and 10% streptomycin/ampicillin (MP Biomedicals). The cell media was then supplemented with either 1% DMSO, 10  $\mu$ M Toyocamycin (1% DMSO), or 10  $\mu$ M caged toyocamycin (1% DMSO) and incubated 48 hours at 37 °C (5% CO<sub>2</sub>) to afford compound diffusion into cells. All incubation conditions were repeated in triplicate on each plate. After 48 hours the media was removed and replaced with standard growth media not containing toyocamycin or caged toyocamycin. One of the two plates was irradiated for 5 minutes with a hand-held UV Lamp (25W), and the cells were incubated for another 24h at 37 °C to afford luciferase expression. Following a visual inspection, the media was removed and the cells were assayed with the Dual-Luciferase Reporter Assay system (Promega) using a Wallac VICTOR<sup>3</sup>V luminometer with a measurement time of 1 s and a delay time of 2 s. The ratio of *Renilla* to Firefly luciferase expression was calculated for each of the triplicates, the data was averaged, and standard deviations were calculated using Microsoft Excel.

<sup>1</sup> Limmer, S. Vogtherr, M. Nawrot, B. Hillenbrand, R. Sprinzl, M.; *Angew. Chem. Int. Ed.* **1997**, *36*, 2485-2489.

#### 4. GFP Reporter System Construction

In order to construct a N117-EGFP plasmid, the luciferase open reading frame (ORF) was removed from N117-luc (*NotI/BamHI*) and replaced with a PCR amplified ORF of EGFP (derived from EGFP-N1; Clontech). In brief, the ORF of EGFP was amplified with primers that introduced a 5' *NotI* site and a 3' *BglII* site and ligated into the *NotI/BamHI* sites of N117-luc. PCR employed *Pfu* DNA polymerase (Stratagene), a forward primer 5'-GATCCGCGGCCGCGGTACCGCGGGCCCGGGATCCACC-3' and reverse primer 5'-GATCCAGATCTTACTTGTACAGCTCGTCCATGCC-3' (restriction sites are underlined).

#### 5. Spatial Control of GFP Expression in Mammalian Cell Culture

Human Embryonic Kidney (HEK-293T) cells were passaged into a 6-well culture plate, and grown to 40% confluence. The cells were then transfected with the N117-GFP construct (1 µg) and a pIRES2-DsRed Express transfection control construct (1 µg; Clontech) using Fugene HD (3:2 Fugene/DNA; Roche Biomedicals) in OptiMEM media (Invitrogen). The transfection was incubated at 37 °C for 4 hours, followed by replacement of transfection media with standard growth media (Dulbecco's modified Eagle's media (Hyclone) with 10% Fetal Bovine serum (Hyclone) and 10% streptomycin/ampicillin (MP Biomedicals) supplemented with caged toyocamycin (10 µM final concentration; 1% DMSO). The cells were then incubated at 37 °C (5% CO<sub>2</sub>) for 48 hours to afford compound diffusion into cells, followed by the removal of the media and spot irradiation with a Jenco Epi-fluorescence inverted microscope equipped with a 100W mercury lamp and a DANSA filter cube (330-400 nm excitation) for 30 seconds. The media was replaced and the cells were incubated for another 24 hours at 37 °C to allow for protein expression and folding. Cells were then imaged on a Leica DM5000B compound microscope to assess the spatial control of GFP expression.