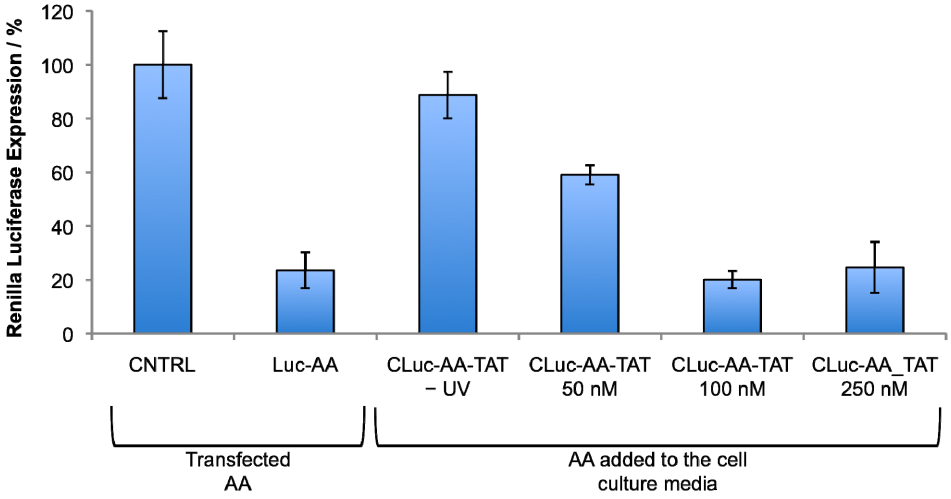
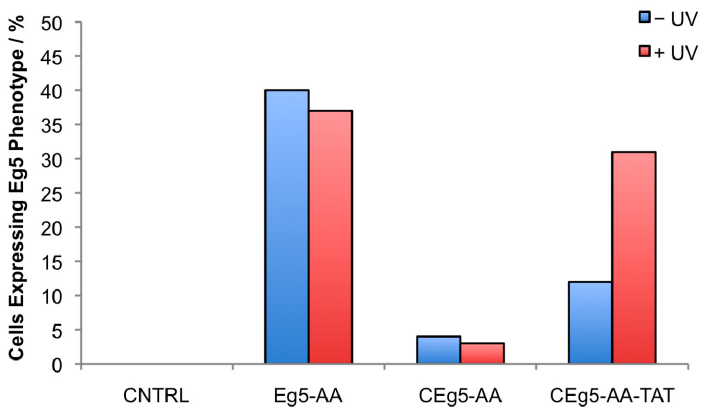
# Cellular Delivery and Photochemical Activation of Antisense Agents through a Nucleobase Caging Strategy

Jeane M. Govan[1], Rajendra Uprety[1], Meryl Thomas[1], Hrvoje Lusic[1], Mark O. Lively[2], and Alexander Deiters[1,*]

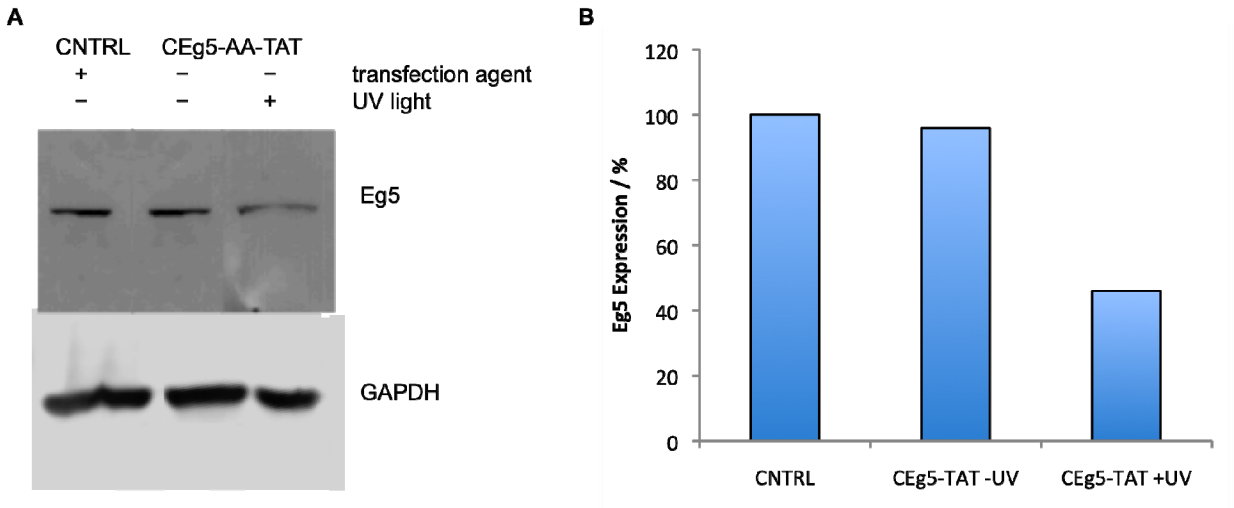
*[1]North Carolina State University, Department of Chemistry, Raleigh, NC 27695, and [2]Wake Forest University School of Medicine, Center for Structural Biology, Winston-Salem, NC 27157*
*alex_deiters@ncsu.edu*




**Supporting Figure 1.** Titration of HIV TAT conjugated antisense agents. Antisense agents (CNTRL and Luc-AA) were actively transfected into HEK293T cells. Increasing concentrations of CLuc-AA-TAT (0, 50, 100, 250 nM) were simply added to HEK293T cell culture media. Cells were either kept in the dark or irradiated (365 nm, 2 min, 25 W). The cells were expressing firefly and *Renilla* luciferase from pGL3 and pRL-TK plasmids, respectively, and a dual-luciferase assay was performed after 48 h. *Renilla* luciferase expression was normalized to firefly luciferase expression and the negative control antisense agent (CNTRL) was set to 100%. All experiments were performed in triplicate and error bars represent standard deviations.

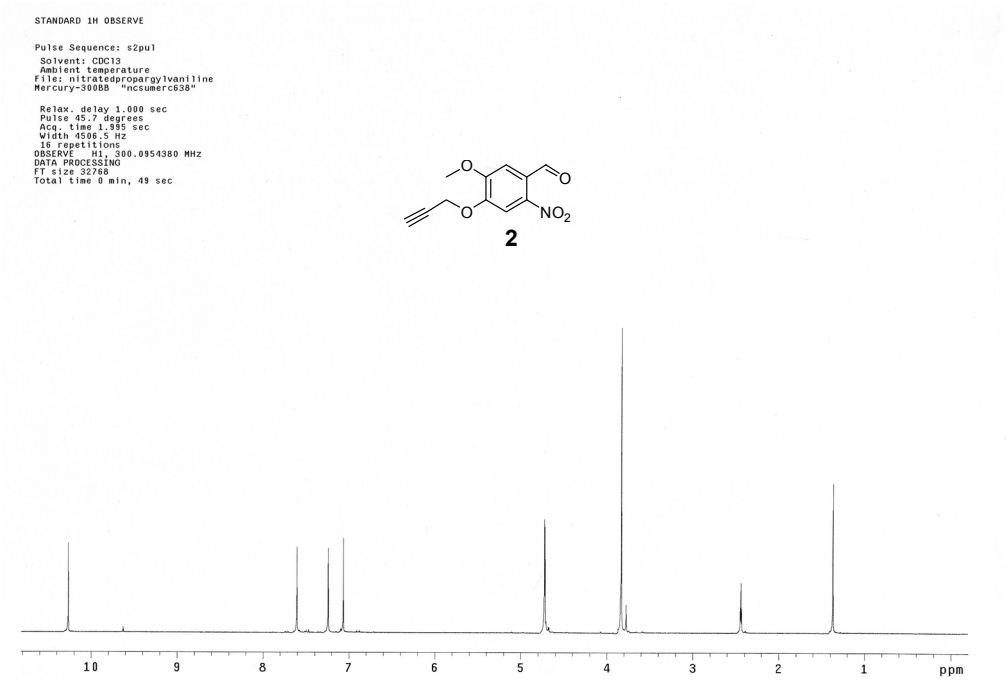


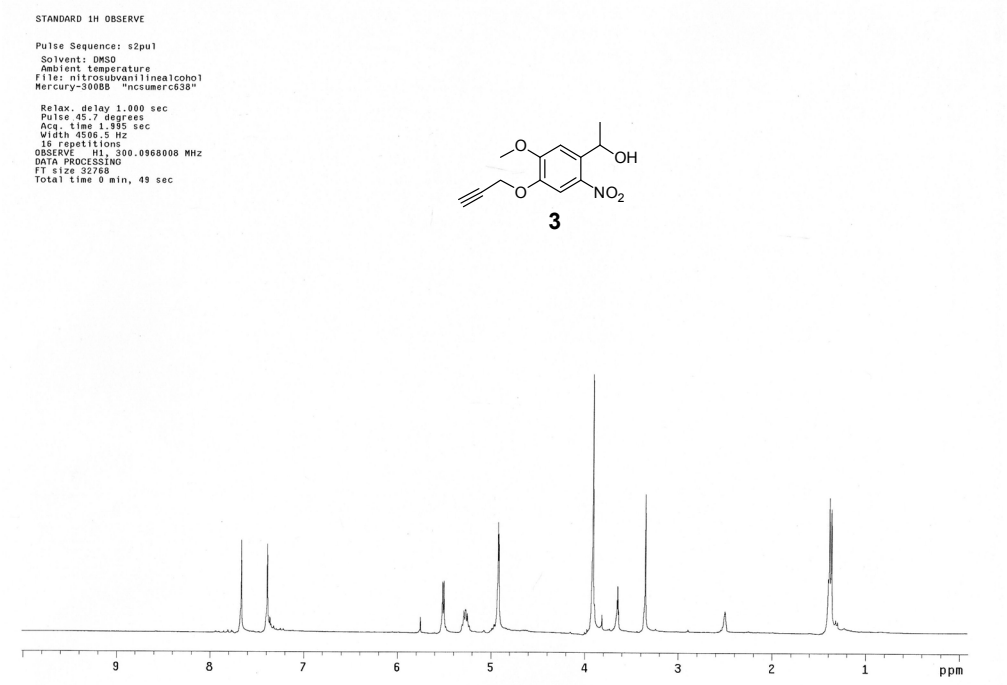

**Supporting Figure 2.** HeLa cells were passaged into 4-well chamber slide (1 mL per well, ~4 × $10^4$ cells per well) and grown to ~70% confluence within 24 h. Conjugated antisense agents (100 nM) were added directly to the cell culture media and incubated overnight at 37 °C, 5% $CO_2$. The media was removed and the cells were irradiated for 2 min on a UV transilluminator (365 nm, 25 W). Control antisense agents were transfected into the cells with X-tremeGENE siRNA reagent (Roche). DMEM media was added and the cells were incubated at 37 °C, 5% $CO_2$ for 48 h. The cells were fixed with formaldehyde (3.75%) and permeabilized with Triton-X100, strained with Alexa Fluor 488 Phalloidin (ex/em 488 nm/495-630 nm, Invitrogen) and DAPI (ex/em 405 nm/410-495 nm, Invitrogen). 100 total cells were counted for the binucleated phenotype and plotted against the antisense agent treatment.

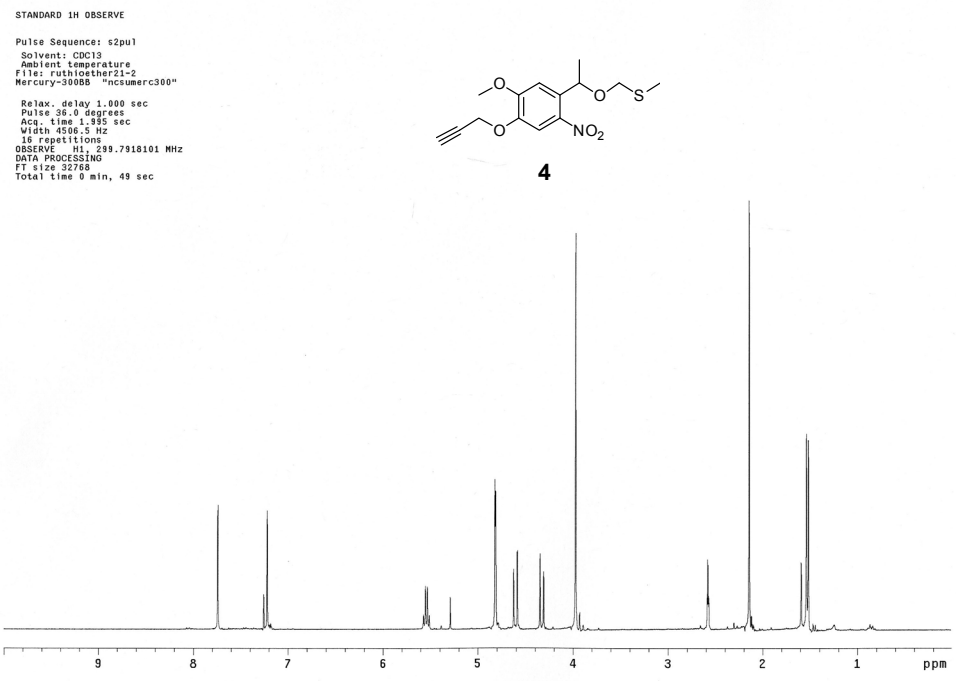


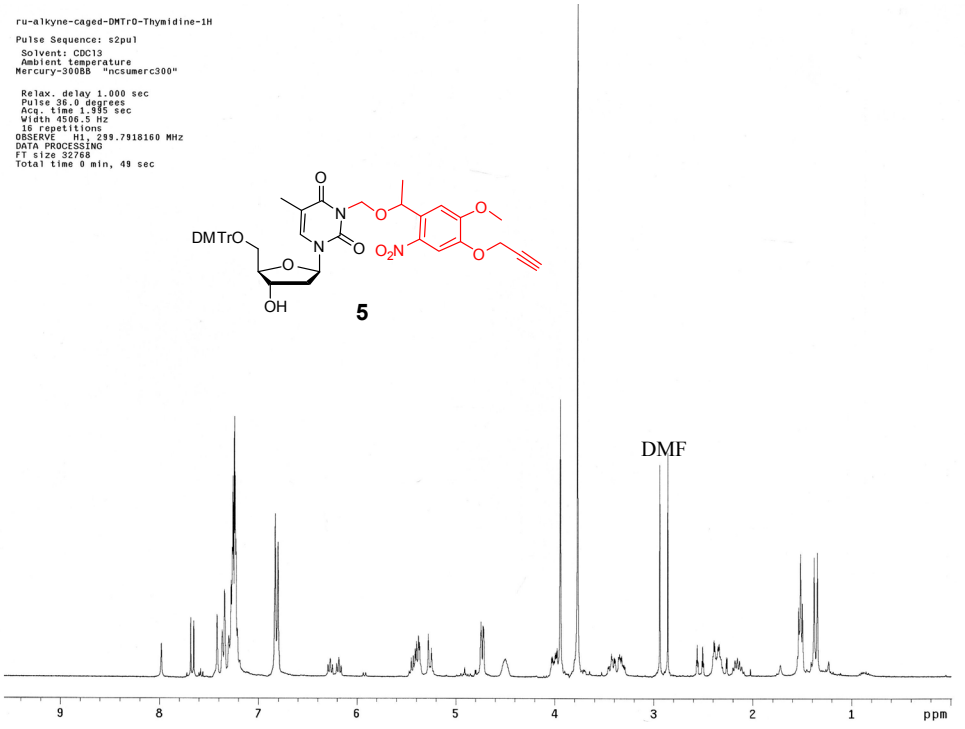

**Supporting Figure 3.** Western Blot of photochemical Eg5 inhibition. HeLa cells were passaged into 6-well plates (2 mL per well, ~2 × $10^5$ cells per well) and grown to ~70% confluence within 24 h. Conjugated antisense agents (100 nM) were added directly to the cell culture media and incubated overnight at 37 °C, 5% $CO_2$. The media was removed and the cells were irradiated for 2 min on a UV transilluminator (365 nm, 25 W). Control antisense agents were transfected into the cells with X-tremeGENE siRNA reagent (Roche). DMEM media was added and the cells were incubated at 37 °C, 5% $CO_2$ for 48 h. The cells were lysed with mammalian protein extraction buffer (GE Healthcare) and the lysate was analyzed by 12 % SDS-PAGE. A Western blot was performed using Eg5 (1:500 dilution, Santa Cruz Biotechnology) and GAPDH (1:1,000 dilution, Santa Cruz Biotechnology) primary antibodies, and a goat-anti-mouse-HRP (1:1,000 dilution, Santa Cruz Biotechnology) secondary antibody. The Western blot was developed by HRP colorimetric staining (Bio-Rad) and (**A**) imaged on a Typhoon 7000 phosphorimager (GE Healthcare). (**B**) The bands were quantified with *ImageQuant TL* software and the bands corresponding to Eg5 expression were normalized to GAPDH expression bands. For normalization purposes, the CNTRL antisense agent was set to 100% Eg5 expression.

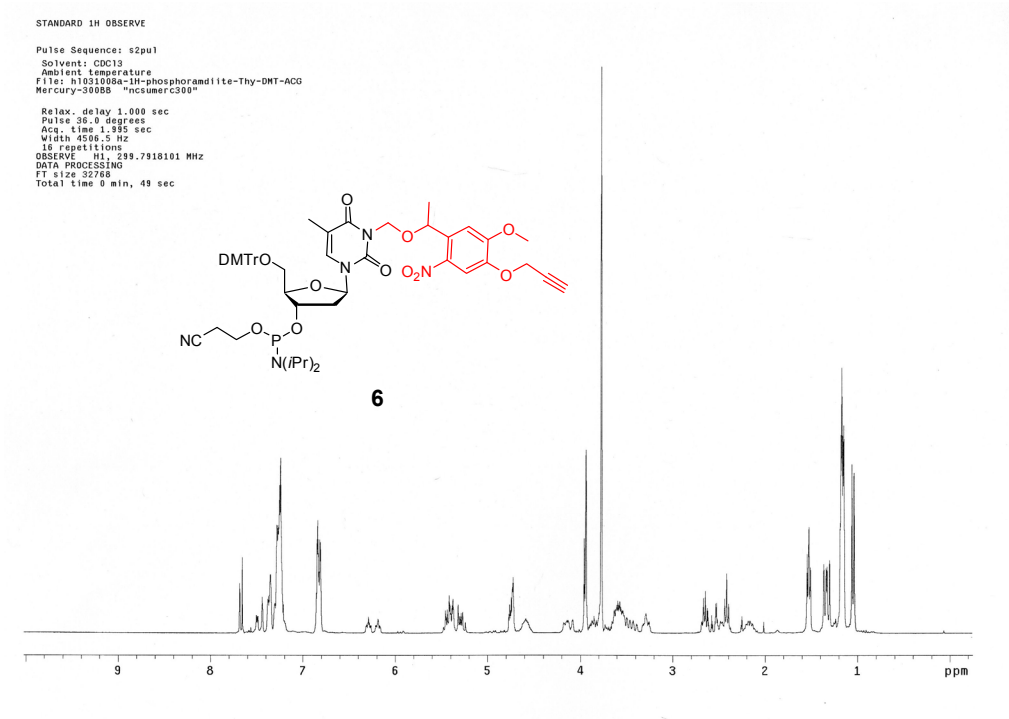
*$^{1}H$ NMRs of synthesized compounds:*



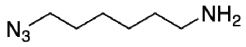

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: ruthioether21-2
Mercury-300BB "ncsumerc300"
Relax. delay 1.000 sec
Pulse 36.0 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
16 repetitions
OBSERVE H1, 299.7918101 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 49 sec
O
O
S
O
NO2
4
9
8
7
6
5
4
3
2
1
ppm


ru-alkyne-caged-DMTrO-Thymidine-1H
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-300BB "ncsumerc300"
Relax. delay 1.000 sec
Pulse 36.0 degrees
Acq. time 1.995 sec
Width 4506.5 Hz
16 repetitions
OBSERVE H1, 299.7918160 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 49 sec
DMTrO
O
N
O
O
O2N
O
O
OH
5
DMF
9
8
7
6
5
4
3
2
1
ppm

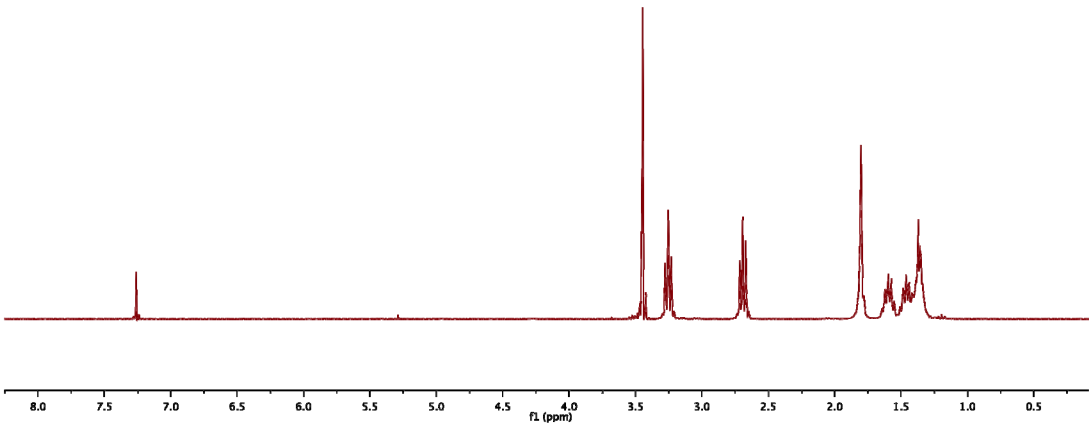



**6**

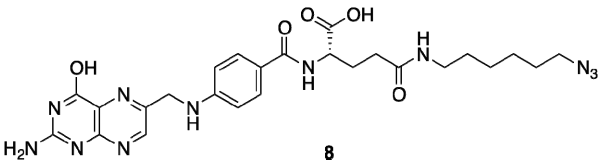
MT03-169
MT03-169




**7**



MT04-110-ON
MT04-110-HPLC




8