

## Supplementary Information

### Spatiotemporal Control of MicroRNA Function using Light-Activated Antagomirs

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#### Synthesis of NPOM-caged 2'OMe uridine

**1-((4*aR*,6*R*,7*R*,7*aS*)-2,2-di-*tert*-Butyl-7-hydroxytetrahydro-4H-furo[3,2-d][1,3,2]dioxasilin-6-yl)-3-((1-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethoxy)methyl)pyrimidine-2,4(1H,3H)-dione (3).** DBU (697  $\mu$ L, 4.5 mmol) was added to a solution of silyl protected uridine **1** (0.88 g, 2.3 mmol) in DMF (5 mL) stirred under inert atmosphere at room temperature. After 30 minutes, freshly prepared NPOM chloride (2.76 mmol) dissolved in DMF (0.5 mL) was added to the reaction mixture. The reaction mixture was stirred for 12 hours under an argon atmosphere and was then poured into a saturated solution of NaHCO<sub>3</sub> (20 mL). The product was extracted using EtOAc (2  $\times$  20 mL) and the combined organic layers were washed with a saturated aqueous NaHCO<sub>3</sub> (3  $\times$  20 mL), followed by brine (15 mL), and were dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated and the product was purified by silica gel column chromatography using EtOAc/hexane (1:1) with 1% TEA to obtain the NPOM-caged uridine **3** (0.95 g) as a yellow solid in 68% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 1.00-1.07 (m, 18H), 1.48 (d, *J* = 4.0 Hz, 3H), 3.93-4.15 (m, 4H), 4.27-4.45 (m, 2H), 5.09-5.12 (m, 1H), 5.21-5.33 (m, 3H), 5.42 (s, 0.5 H), 5.48 (s, 0.5 H), 5.66-5.71 (q, 1H), 6.06-6.07 (m, 2H), 7.08-7.13 (q, 1H), 7.15-7.17 (m, 1H), 7.41-7.43 (m, 1H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$  = 20.8, 23.2, 24.1, 27.7, 27.8, 67.6, 69.3, 73.5, 75.2, 76.2, 95.3, 96.0, 102.5, 102.4, 105.4, 106.8, 137.8, 139.8, 140.6, 142.8, 152.6, 162.6. HRMS: *m/z* calcd for C<sub>27</sub>H<sub>37</sub>N<sub>3</sub>O<sub>11</sub>Si [M+Na]<sup>+</sup>: 630.2090; found 630.2088.

**1-((2*R*,3*R*,4*R*,5*R*)-4-Hydroxy-5-(hydroxymethyl)-3-methoxytetrahydrofuran-2-yl)-3-((1-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethoxy)methyl)pyrimidine-2,4(1H,3H)-dione (4).** Methyl iodide (20 mL) was added to neat NPOM-caged uridine **3** (2.1 g, 3.4 mmol) under an argon atmosphere. Ag<sub>2</sub>O (2.3 g, 10.3 mmol) was added to the stirred solution and the reaction mixture was heated under reflux (50 °C) for 5 hours. The reaction mixture was cooled to room temperature and EtOAc (20 mL) was added. The mixture was filtered and the residue was washed with EtOAc. The combined organic layers were washed with saturated aqueous NaHCO<sub>3</sub> (3  $\times$  20 mL), followed by brine (20 mL), and were dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated and the product was purified by silica gel column chromatography using EtOAc/Hexane (2:3) with 1% TEA to obtain the 2'OMe uridine **4** (2.1 g) as a white solid in 98% yield. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  = 1.00-1.04 (m, 18H), 1.49 (d, *J* = 3.0 Hz, 3H) 3.63 (s, 3H), 3.80-3.97 (m, 3H), 4.03-4.12 (m, 2H), 4.42-4.47 (m, 1H), 5.11-5.17 (m, 2H), 5.27-5.34 (m, 2H), 5.54 (d, *J* = 1.6 Hz, 1H), 5.66-5.70 (m, 1H), 6.06 (m, 2H), 7.11-7.15 (m,

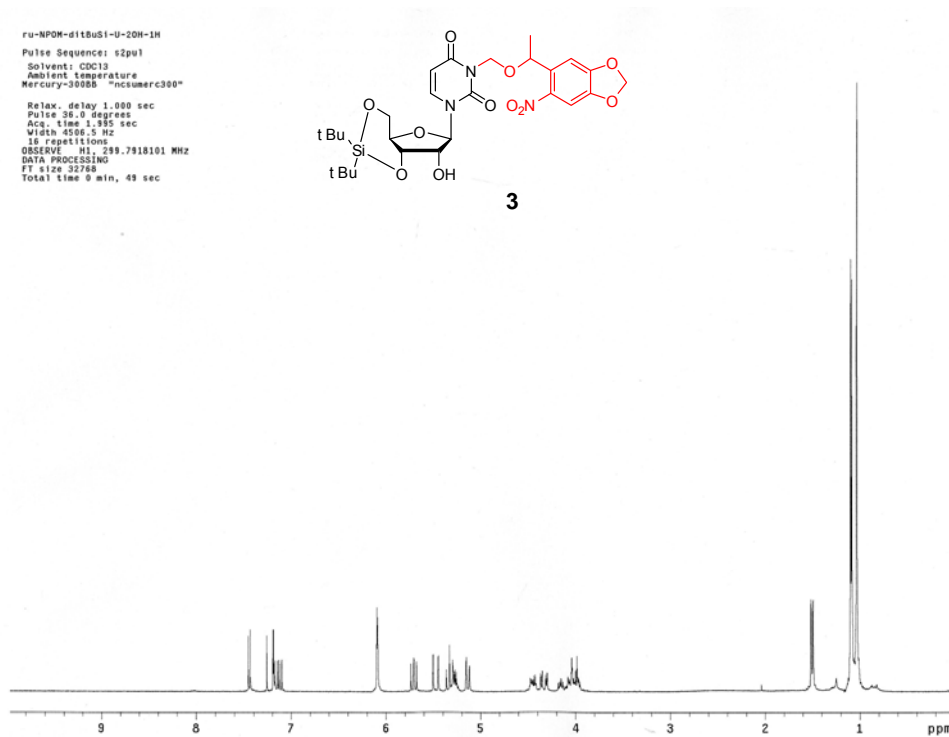
1H), 7.17 (d,  $J = 2.6$  Hz, 1H), 7.42 (d,  $J = 1.0$  Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz, acetone- $d_6$ ):  $\delta = 20.9, 27.7, 59.2, 67.8, 70.4, 74.2, 75.3, 77.6, 82.8, 92.2, 92.7, 102.1, 104.3, 105.2, 106.9, 138.7, 140.0, 140.4, 142.8, 148.0, 151.3, 153.1, 162.6, 162.7$ . HRMS:  $m/z$  calcd for  $\text{C}_{28}\text{H}_{39}\text{N}_3\text{O}_{11}\text{Si}$   $[\text{M}+\text{Na}]^+$  : 644.2246; found 644.2248.

**1-((2*R*,3*R*,4*R*,5*R*)-4-Hydroxy-5-(hydroxymethyl)-3-methoxytetrahydrofuran-2-yl)-3-((1-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethoxy)methyl)pyrimidine-2,4(1*H*,3*H*)-dione (5).** HF-pyridine (330  $\mu\text{L}$ , 70% HF:pyridine) diluted in dry pyridine (2 mL) was added to a solution of 2'-OMe uridine **4** (2.0 g, 3.21 mmol) in DCM at 0 °C stirred under nitrogen atmosphere in a polyethylene reaction vessel. After stirring the reaction mixture for 2 hours at 0 °C, DCM (40 mL) was added followed by a saturated aqueous  $\text{NaHCO}_3$  (30 mL) to neutralize residual amounts of HF. The organic layer was separated and washed with a saturated aqueous  $\text{NaHCO}_3$  (30 mL), followed by 1M HCl (2  $\times$  20 mL), water (20 mL), and brine (15 mL), and was dried over anhydrous sodium sulfate. After filtration, the volume of filtrate was reduced and the crude product was purified by silica gel column chromatography using DCM/MeOH (92:8) with 1% TEA to obtain the caged 2'-OMe uridine **5** (1.4 g) as a yellow solid in 98% yield.  $^1\text{H}$  NMR (400 MHz, acetone- $d_6$ ):  $\delta = 1.45$  (d,  $J = 3.1$  Hz, 3H), 3.50 (s, 1.5H), 3.54 (s, 1.5H), 3.77-3.84 (m, 2H), 3.89-3.99 (m, 3H), 4.20-4.25 (m, 1H), 4.37 (t,  $J = 4.5$  Hz, 1H), 5.17-5.49 (m, 4H), 5.74 (d,  $J = 1.3$  Hz, 0.5H), 5.84 (d,  $J = 1.5$  Hz, 0.5H), 6.18-6.21 (m, 2H), 7.13 (d,  $J = 6.4$  Hz, 1H), 7.41 (s, 1H), 8.00-8.05 (q, 1H).  $^{13}\text{C}$  NMR (75 MHz, acetone- $d_6$ ):  $\delta = 24.1, 24.2, 58.6, 60.7, 61.2, 68.9, 69.3, 70.3, 70.5, 74.3, 74.7, 84.6, 84.8, 85.4, 85.7, 88.7, 101.3, 101.5, 104.3, 104.4, 105.2, 106.9, 138.8, 140.0, 140.1, 148.0, 148.1, 151.7, 152.9, 153.1, 162.8, 162.9$ . HRMS:  $m/z$  calcd for  $\text{C}_{20}\text{H}_{23}\text{N}_3\text{O}_{11}$   $[\text{M}+\text{Na}]^+$  : 504.1225; found 504.1226

**1-((2*R*,3*R*,4*R*,5*R*)-5-((bis(4-Methoxyphenyl)(phenyl)methoxy)methyl)-4-hydroxy-3-methoxytetrahydrofuran-2-yl)-3-((1-(6-nitrobenzo[d][1,3]dioxol-5-yl)ethoxy)methyl)pyrimidine-2,4(1*H*,3*H*)-dione (6).** DMTrCl (1.5 g, 4.6 mmol) was added to a solution of the alcohol **5** (1.4 g, 2.9 mmol) in dry pyridine (15 mL) stirred under an argon atmosphere at room temperature. The reaction mixture was stirred for 24 hours. MeOH (3 mL) was added into the reaction mixture in order to quench the unreacted DMTrCl and the mixture was stirred for 20 minutes. The solvent was removed and the residue was dissolved in EtOAc (40 mL) and was washed with a 5% aqueous citric acid solution (2  $\times$  20 mL), followed by saturated aqueous  $\text{NaHCO}_3$  (2  $\times$  20 mL), and brine (15 mL), and was dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated and the crude product was purified silica gel column chromatography using DCM/EtOAc (1:5) with 1% TEA to furnish the DMT protected caged 2'-OMe uridine **6** (1.9 g) as a white solid in 86% yield.  $^1\text{H}$  NMR (300 MHz, acetone- $d_6$ ):  $\delta = 1.44$ -1.47 (m, 3H), 3.46-3.52 (m, 2H), 3.56 (s, 1.5H), 3.60 (s, 1.5H), 3.77-3.81 (m, 6H), 4.02-4.11 (m, 1H), 4.36-4.43 (m, 1H), 5.05-5.32 (m, 2H), 5.40-5.47 (m, 1H), 5.70 (s, 0.5H), 5.82 (s, 0.5H), 6.12-6.22 (m, 2H), 6.90-6.94 (m, 4H), 7.12-7.20 (m, 2H), 7.26-7.38 (m, 6H), 7.43-7.48 (m, 2H), 7.80 (d,  $J = 4.2$  Hz, 0.5H), 7.92 (d,  $J = 3.9$  Hz, 0.5H).  $^{13}\text{C}$  NMR (75 MHz, acetone- $d_6$ ):  $\delta = 24.1, 24.3, 55.6, 58.8, 62.2, 62.9, 69.3, 69.4, 69.6, 69.7, 70.3, 70.6, 74.4, 74.8, 83.5, 83.8, 84.6, 84.8, 87.6, 88.9, 89.1, 101.4, 101.5, 104.4, 105.2, 106.9, 127.9, 128.3, 128.8, 129.1, 130.1, 131.0, 131.1, 136.3, 136.5, 139.09, 139.5, 139.6, 145.7, 245.8, 148.1, 151.4, 152.8, 159.8, 162.7$ . HRMS:  $m/z$  calcd for  $\text{C}_{41}\text{H}_{41}\text{N}_3\text{O}_{13}$   $[\text{M}+\text{Na}]^+$  : 806.2532; found 806.2526.

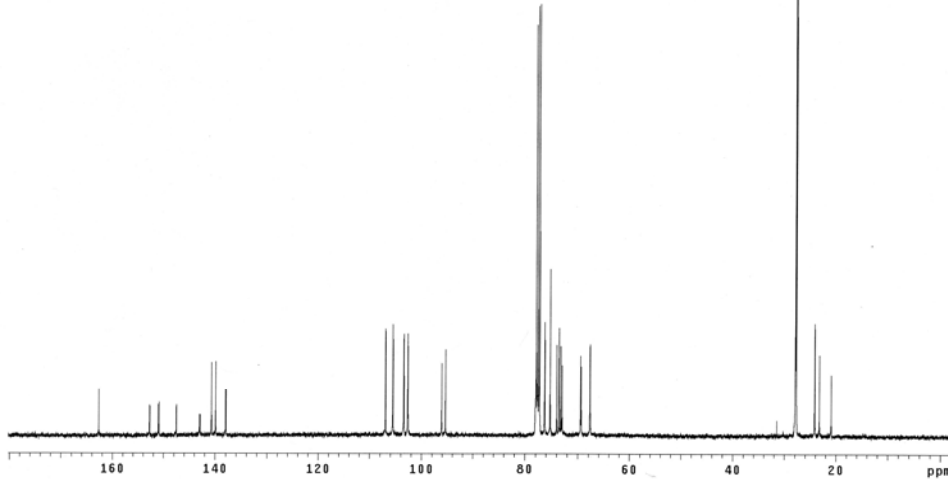
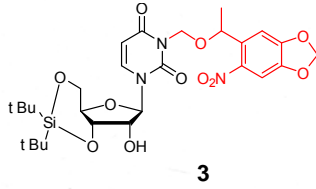
**(2*R*,3*R*,4*R*,5*R*)-2-((bis(4-Methoxyphenyl)(phenyl)methoxy)methyl)-4-methoxy-5-(3-((1-(6-nitrobenzo[*d*][1,3]dioxol-5-yl)ethoxy)methyl)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-3-yl (2-cyanoethyl) diisopropylphosphoramidite (**7**).** DIPEA (0.55 mL, 3.2 mmol) and 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.284 mL, 1.27 mmol) were added to a solution of the alcohol **6** (0.5 g, 0.64 mmol) in DCM (5.0 mL) stirred under an argon atmosphere at room temperature. After 2 hours, TLC showed the complete consumption of starting material. The mixture was then concentrated and the crude product was directly purified by silica gel chromatography using EtOAc/hexanes (4:6) with 1% TEA to obtain the caged phosphoramidite **7** (0.5 g) as a white solid in 80 % yield. <sup>1</sup>H NMR (300 MHz, acetone-*d*<sub>6</sub>): δ = 1.06-1.11 (m, 3H), 1.16-1.22 (m, 9H), 1.45 (d, *J* = 3.2 Hz, 3H), 2.607-2.65 (m, 1H), 3.48-3.58 (m, 5H), 3.61-3.74 (m, 3H), 3.80 (s, 6H), 3.91-4.01 (m, 2H), 4.20-4.27 (m, 1H), 4.46-4.54 (m, 1H), 4.57-4.66 (m, 1H), 5.04-5.31 (m, 4H), 5.44 (d, *J* = 5.0 Hz, 1H), 5.75-5.77 (m, 1H), 5.85-5.88 (m, 1H), 6.90-6.95 (m, 4H), 7.11-7.16 (m, 1H), 7.27-7.50 (m, 9H), 7.76-7.95 (m, 2H). <sup>13</sup>C NMR (100 MHz, acetone-*d*<sub>6</sub>): δ = 20.8, 20.9, 24.1, 24.8, 24.9, 25.1, 43.9, 44.1, 55.6, 58.5, 58.9, 59.0, 59.1, 59.4, 59.6, 59.8, 61.9, 62.2, 62.4, 62.7, 70.2, 70.8, 71.0, 71.1, 74.2, 74.4, 82.8, 83.15, 83.4, 84.2, 87.7, 89.3, 89.5, 89.7, 101.6, 101.7, 104.2, 104.4, 105.2, 105.3, 106.9, 107.0, 114.1, 127.9, 128.8, 129.2, 131.2, 136.1, 136.2, 136.4, 138.7, 138.9, 139.4, 139.5, 145.7, 148.0, 151.5, 151.8, 153.1, 159.8, 159.9, 162.6, 162.7. <sup>31</sup>P NMR (161 MHz, acetone-*d*<sub>6</sub>): δ = 150.19, 150.35, 150.74, 150.82.

### <sup>1</sup>H NMR, <sup>13</sup>C NMR, and <sup>31</sup>P NMR spectra of synthesized compounds



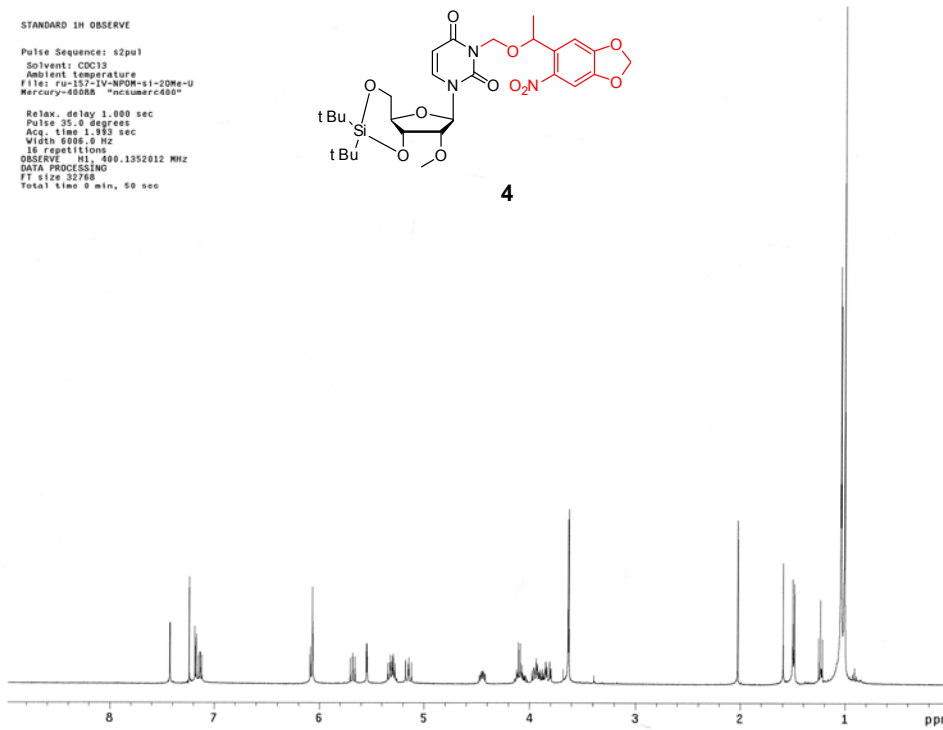
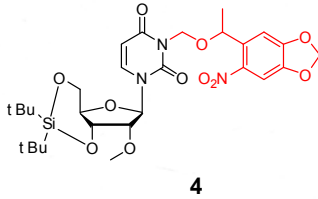
13C OBSERVE

Pulse Sequence: s2pu1  
Solvent: CDCl3  
Ambient temperature  
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Mercury-40085 "ncsmrc400"  
Relax. delay 2.000 sec  
Pulse 90.0 degrees  
Acq. time 1.199 sec  
Width 25000.0 Hz  
8000 repetitions  
OBSERVE: C13, 100.6140225 MHz  
DECOUPLE: H1, 400.1371641 MHz  
Power: 46 dB  
Continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 65536  
Total time 8 hr, 40 min, 0 sec

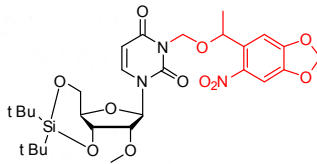


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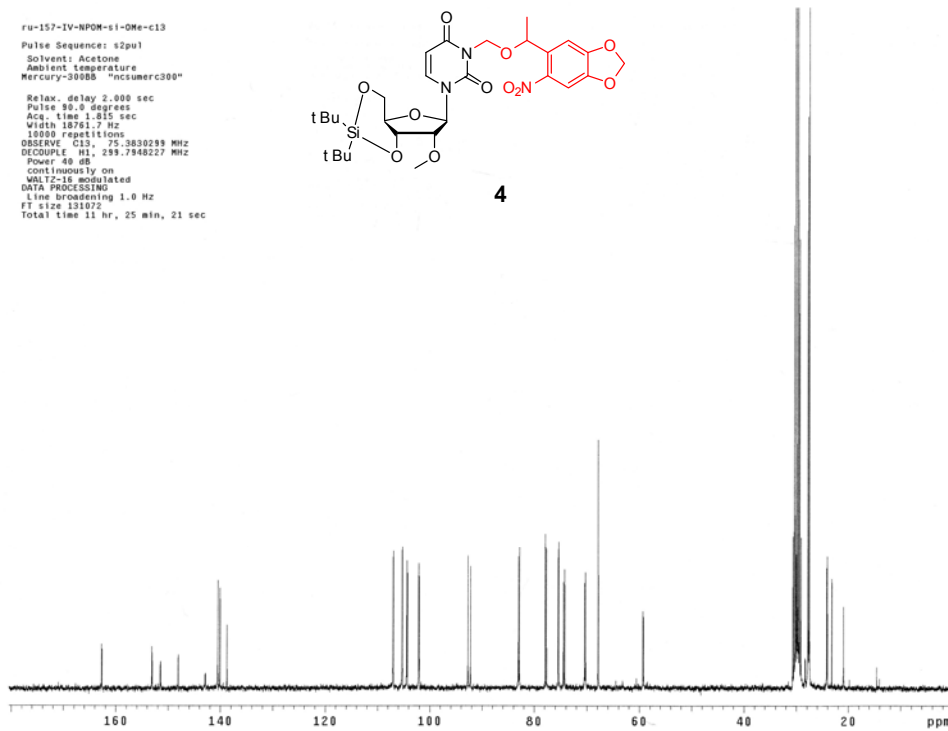
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Ambient temperature  
File: ru-157-IV-NPOM-si-20Me-U  
Mercury-40085 "ncsmrc400"  
Relax. delay 1.000 sec  
Pulse 35.0 degrees  
Acq. time 1.993 sec  
Width 6006.0 Hz  
16 repetitions  
OBSERVE: H1, 400.1352012 MHz  
FT size 32768  
Total time 0 min, 50 sec



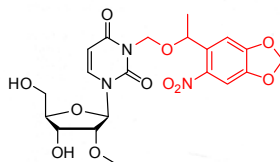
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Ambient temperature  
Mercury-30000 "ncnumerc300"  
Relax. delay 2.000 sec  
Pulse 30.0 degrees  
Acq. time 1.515 sec  
Width 19761.7 Hz  
10000 repetitions  
OBSERVE C13, 75.2830239 MHz  
DECOUPLE H1, 299.7948227 MHz  
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continuously on  
MULTI-16 modulated  
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Line broadening 1.0 Hz  
FT size 131072  
Total time 11 hr, 25 min, 21 sec



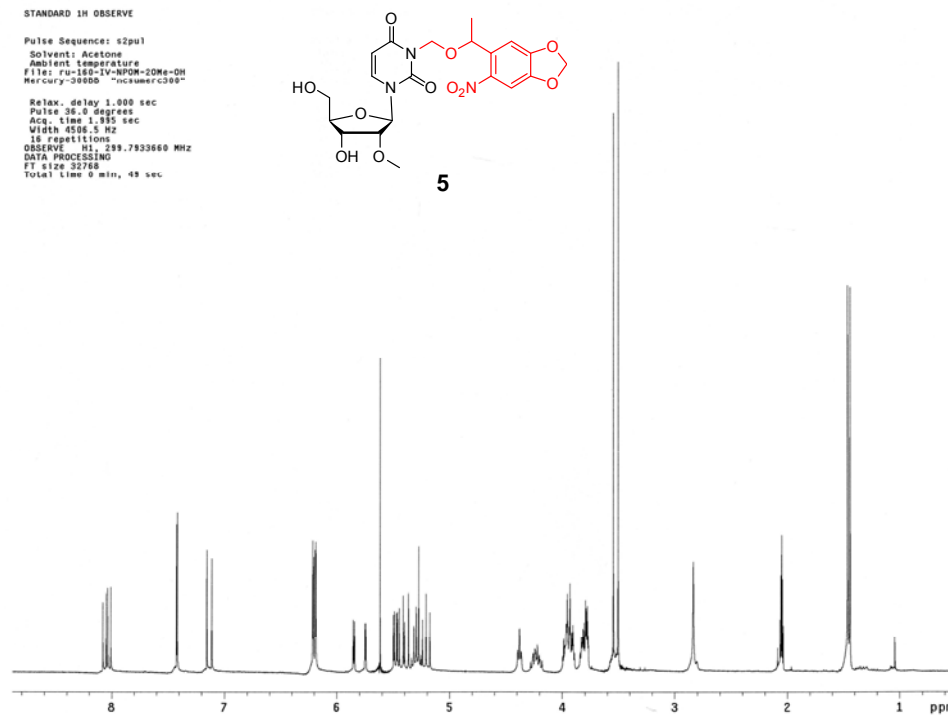
4



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Solvent: Acetone  
Ambient temperature  
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Mercury-30000 "ncnumerc300"  
Relax. delay 1.000 sec  
Pulse 30.0 degrees  
Acq. time 1.985 sec  
Width 4506.5 Hz  
18 repetitions  
OBSERVE H1, 299.7933660 MHz  
DATA PROCESSING  
FT size 32768  
Total time 0 min, 48 sec



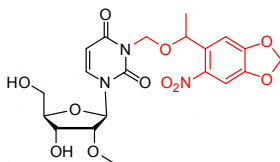
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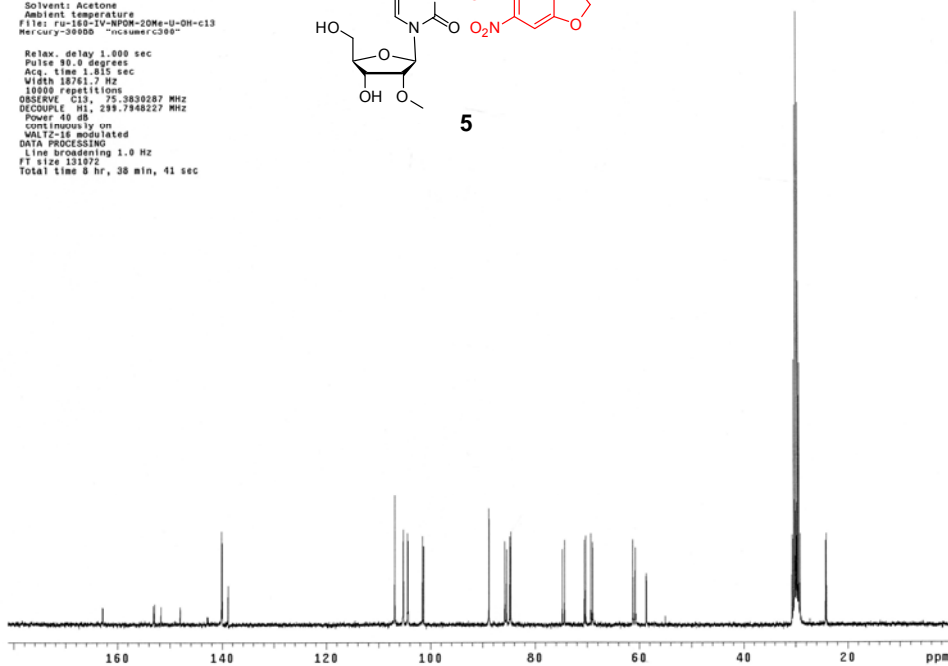
13C OBSERVE

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Ambient temperature  
File: ru-169-IV-NPOM-2DMs-U-OH-c13  
Mercury-30000 "insumerc300"

Relax. delay 1.000 sec  
Pulse 30.0 degrees  
Acq. time 1.815 sec  
Width 18761.7 Hz  
19000 repetitions  
OBSERVE C13, 75.3838227 MHz  
DECDUPLE H1, 299.7548227 MHz  
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continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 1.0 Hz  
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Total time 8 hr, 38 min, 41 sec



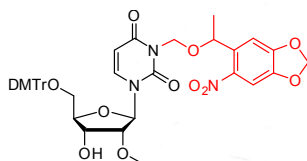
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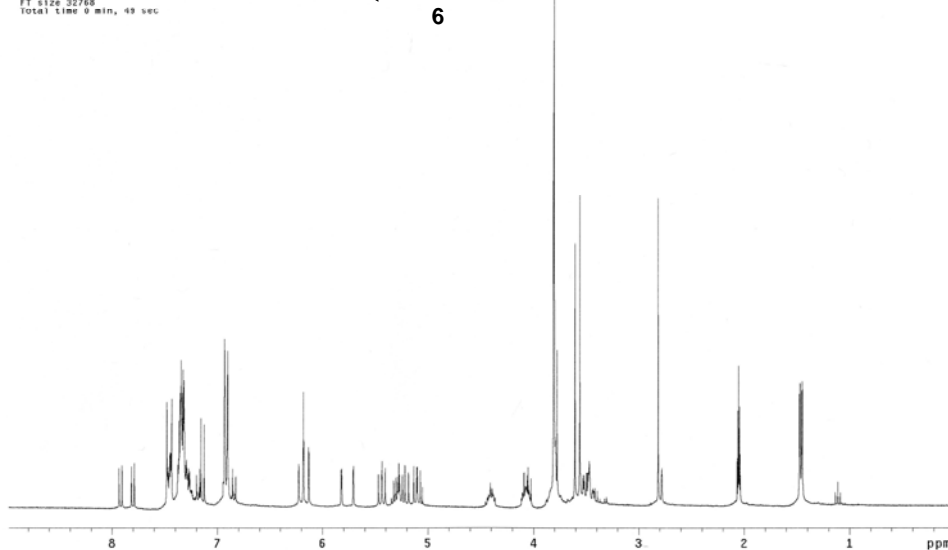
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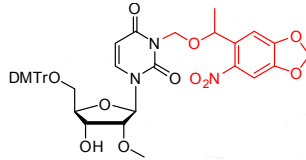
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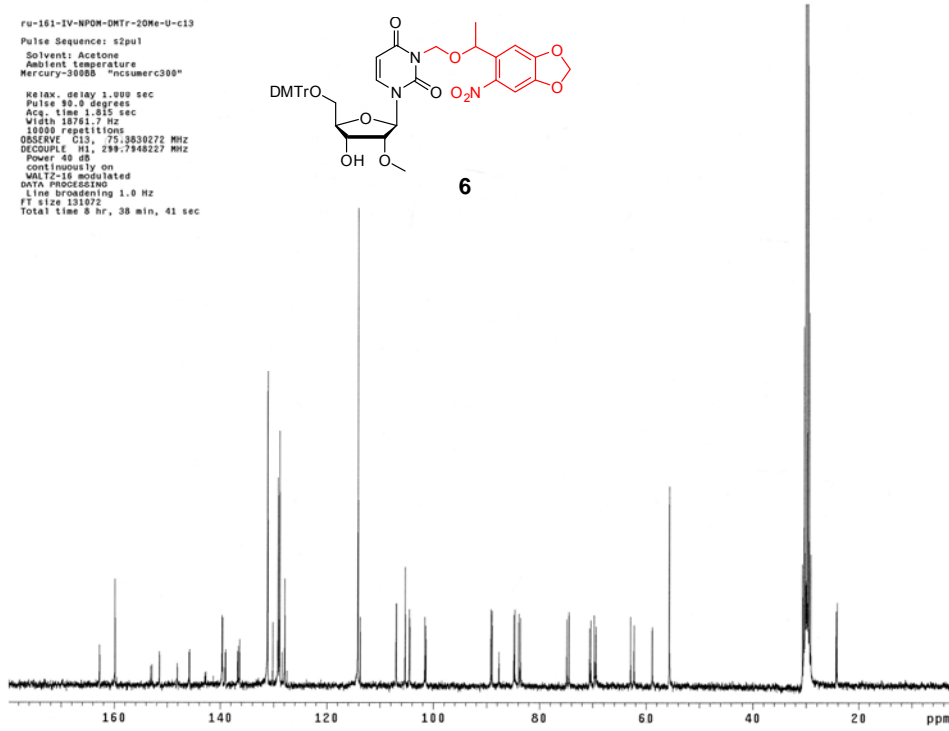
6



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 Solvent: Acetone  
 Ambient temperature  
 Mercury-30005 "ncsumerc300"  
 Relax. delay 1.000 sec  
 Pulse 90.0 degrees  
 Acq. time 1.815 sec  
 Width 18761.7 Hz  
 10000 repetitions  
 OBSERVE C13 75.3830272 MHz  
 DECOUPLE H1 299.7948227 MHz  
 Power 00  
 continuously on  
 WALTZ-16 modulated  
 DATA PROCESSING  
 Line Broadening 1.0 Hz  
 FT size 131072  
 Total time 8 hr, 38 min, 41 sec

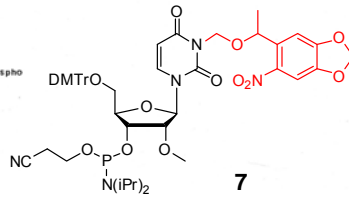


6

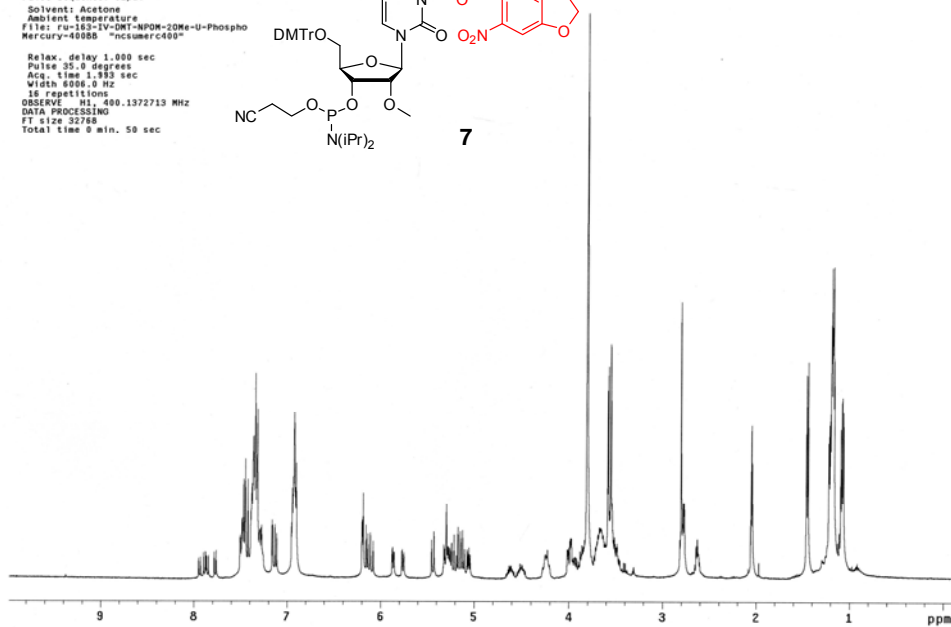


STANDARD 1H OBSERVE

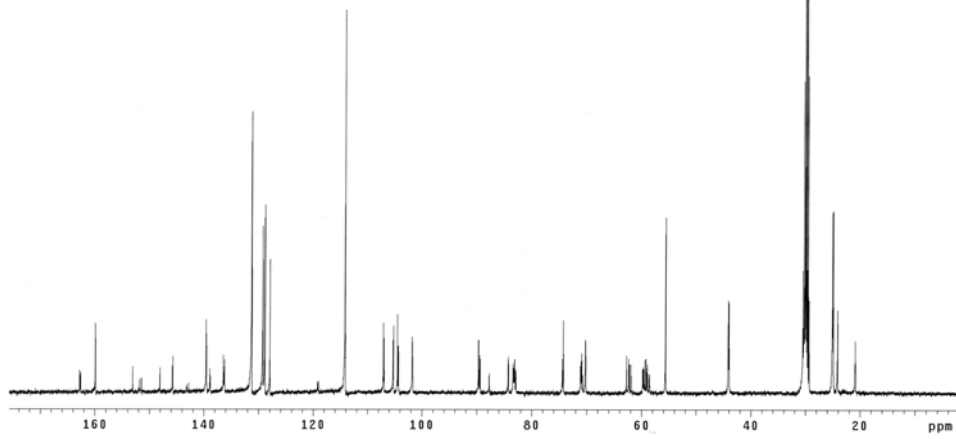
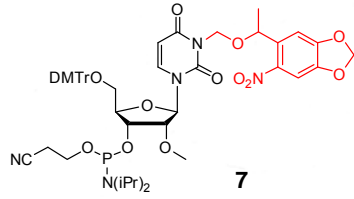
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 Solvent: Acetone  
 Ambient temperature  
 File: ru-163-IV-DMTr-NPOM-2OMe-U-Phospho  
 Mercury-60005 "ncsumerc400"  
 Relax. delay 1.000 sec  
 Pulse 35.0 degrees  
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 DATA PROCESSING  
 FT size 32768  
 Total time 0 min, 50 sec



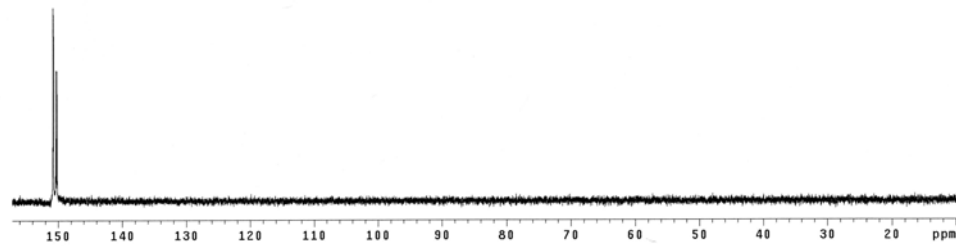
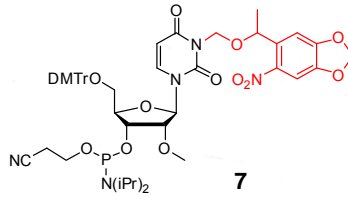
7



ru-163-IV-DMTrO-NPOM-20Me-U-Phos-c13  
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Pulse 90.0 degrees  
Acq. time 1.199 sec  
Width 25000.0 Hz  
13000 repetitions  
OBSERVE C13, 100.6144975 MHz  
DECOUPLE H1, 400.1392400 MHz  
Power 44 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 65536  
Total time 8 hr, 54 min, 25 sec



ru-163-IV-DMTr-NPOM-20Me-U-phos-P31  
Pulse Sequence: s2pu1  
Solvent: Acetone  
Ambient temperature  
Mercury-400BB "nsumerc400"  
Pulse 90.0 degrees  
Acq. time 0.800 sec  
Width 50251.0 Hz  
120 repetitions  
OBSERVE P31, 163.8783951 MHz  
DECOUPLE H1, 400.1392400 MHz  
Power 44 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 1.0 Hz  
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Total time 1 min, 56 sec

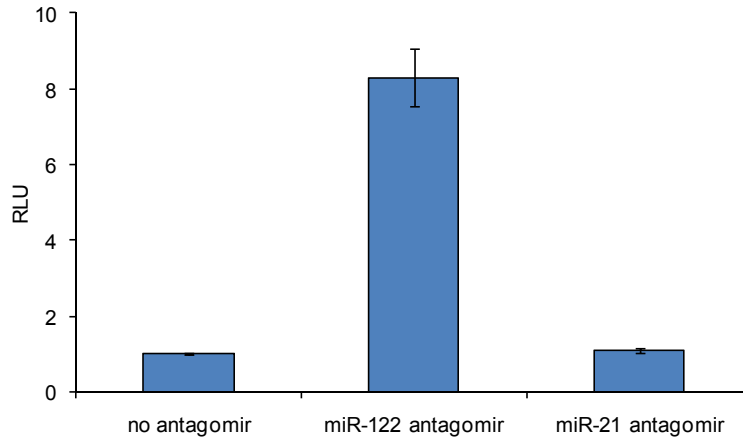




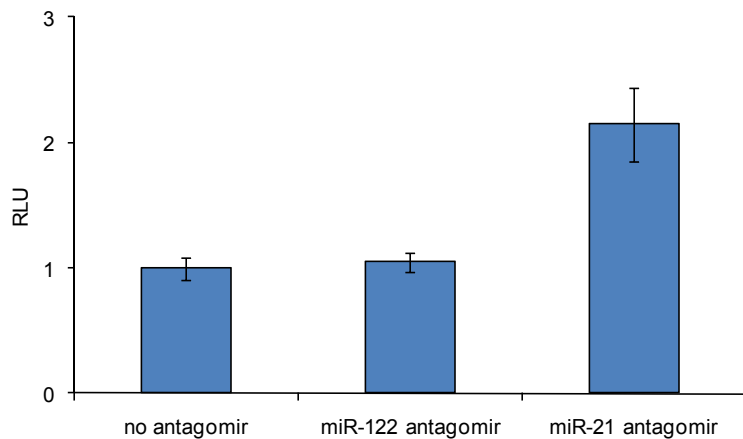
**Cloning of psiCHECK-miR21 and pEGFP-C2-miR21.** The psiCHECK-2 plasmid (1 µg; Promega) was digested with Sgfl (10 units, 50 µL reaction; Promega) followed by PmeI (10 units; New England Biolabs) at 37 °C for 2 h. The pEGFP-C2 plasmid (5 µg; Clontech) was digested with XhoI (20 units, 100 µL reaction; New England Biolabs) and EcoRI-HF (20 units; New England Biolabs) at 37 °C for 2 h followed by Antarctic phosphatase (New England Biolabs) treatment at 37 °C for 1 h. The digested plasmid backbones were separated on a 1% agarose gel in TBE buffer at 90 V for 30 min and were excised and purified with the Qiagen Gel Extraction Kit. Insert DNA containing the miR-21 binding site was purchased from IDT DNA (psiCHECK-miR21 insert: 5' CGCAGTAGAGCTCTAGTTCAACATCAGTCTGATAAGCTAGTTT 3' and 5' AAAGTAGCTTATCAGACTGATGTTGAACTAGAGCTCTACTGCGAT 3', pEGFP-C2-miR21 insert: 5' TCGACTCAACATCAGTCTGATAAGCTACTCGAGTAG 3' and 5' AATTCTACTCGAGTAGCTTATCAGACTGATGTTGAG 3') and were hybridized (72 °C for 15 min, cooled to 20 °C over 5 min) and ligated at 4 °C overnight with T4 DNA ligase (200 units, 10 µL reaction, 1:10 vector/insert ratio; New England Biolabs) into either the digested psiCHECK-2 or pEGFP-C2 vector. The ligation reactions were transformed into Nova Blue competent cells and clones were sequenced to verify the construction of the psiCHECK-miR21 and pEGFP-C2-miR21 vectors (psiCHECK-miR21 sequencing primer: 5' GCTAAGAAGTTCCCT 3'; IDT DNA, pEGFP-C2-miR21 sequencing primer: 5' TTCATCTGCACCACCGGCAAG 3'; IDT DNA).

**Cell culture.** Experiments were performed using the Huh7 human hepatoma cell line cultured in Dulbecco's Modified Eagle Medium (DMEM; Hyclone) supplemented with 10% fetal bovine serum (FBS; Hyclone) and 2% penicillin/streptomycin (Mediatech) and maintained at 37 °C in a 5% CO<sub>2</sub> atmosphere. Huh7-psiCHECK-miR122 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Hyclone) supplemented with 10% fetal bovine serum (FBS; Hyclone), 500 µg/mL of G418 (Sigma Aldrich), and 2% penicillin/streptomycin (Mediatech) and maintained at 37 °C in a 5% CO<sub>2</sub> atmosphere.

**Analysis of reporter assay specificity for the miR-122 or miR-21 antagomir.** Huh7-psiCHECK-miR122 or Huh7 cells were seeded at 10,000 cells per well in white clear-bottom 96-well plates (BD Falcon). After an overnight incubation, the Huh7-psiCHECK-miR122 cells were transfected with either the miR-122 or miR-21 antagomir (10 pmol; 50 nM) using X-tremeGENE siRNA transfection reagent (1.5 µL/well; Roche) in Opti-Mem media (Invitrogen) at 37 °C for 4 h. The Huh7 cells were transfected with the psiCHECK-miR21 plasmid (250 ng/well) and either the miR-21 or miR-122 antagomir (10 pmol; 50 nM) using X-tremeGENE siRNA transfection reagent (1.0 µL/well) in Opti-Mem media at 37 °C for 4 h. The media was removed and replaced with standard DMEM growth media (supplemented with 10% FBS and 2% penicillin/streptomycin) and the cells were incubated at 37 °C for 48 h. The media was then removed and the cells were lysed and assayed with a Dual Luciferase Assay Kit (Promega). The ratio of *Renilla* to firefly luciferase expression was calculated for each of the triplicates, the data was averaged, and standard deviations were determined (see Supporting Figures S1 & S2).



**Supporting Figure S1.** Validation of the Huh7-psiCHECK-miR122 reporter assay specificity for the miR-122 antagomir. A significant increase in *Renilla* luciferase expression is observed when Huh7-psiCHECK-miR122 cells are transfected with the miR-122 antagomir, whereas no change is observed upon transfection with the miR-21 antagomir.

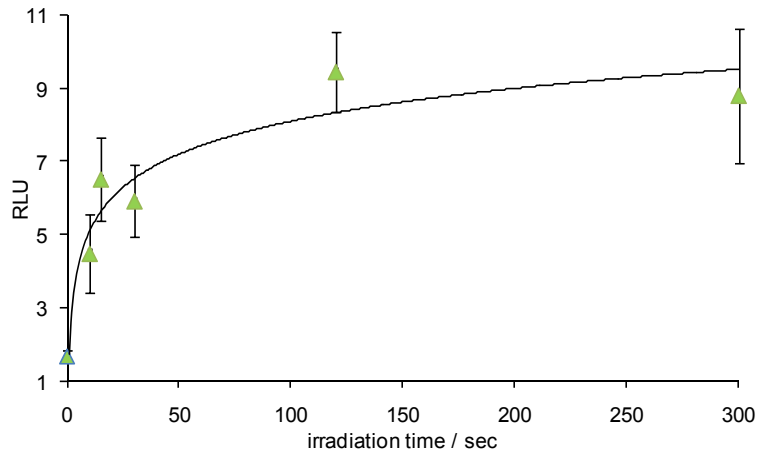


**Supporting Figure S2.** Validation of the psiCHECK-miR21 reporter assay specificity for the miR-21 antagomir. An increase in *Renilla* luciferase expression is observed when Huh7 cells are transfected with psiCHECK-miR21 and the miR-21 antagomir, whereas no change is observed upon transfection with the miR-122 antagomir.

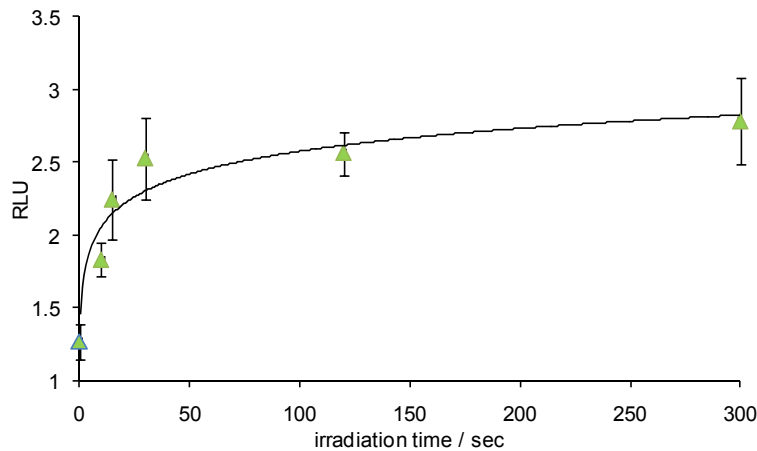
**Irradiation time course of caged miR-122 and miR-21 antagomirs in mammalian cells.**

Huh7-psiCHECK-miR122 or Huh7 cells were seeded at 10,000 cells per well in white clear-bottom 96-well plates (BD Falcon). After an overnight incubation, the Huh7-psiCHECK-miR122 cells were transfected with the caged miR-122 antagomir (50 pmol; 250 nM) using X-tremeGENE siRNA transfection reagent (1.5  $\mu$ L/well; Roche) in Opti-Mem media (Invitrogen) at 37 °C for 4 h. The Huh7 cells were transfected with the psiCHECK-miR21 plasmid (250 ng/well) and the caged miR-21 antagomir (10 pmol; 50 nM) using X-tremeGENE siRNA transfection reagent (1.0  $\mu$ L/well) in Opti-Mem media at 37 °C for 4 h. The media was removed and replaced with PBS (50  $\mu$ L/well) and the cells were irradiated on a transilluminator for 0, 10, 15, 30, 120, or 300 sec (365 nm, 25 W) in triplicate. The PBS was replaced with standard DMEM growth media

(supplemented with 10% FBS and 2% penicillin/streptomycin) and the cells were incubated at 37 °C for 48 h. The media was then removed and the cells were lysed and assayed with a Dual Luciferase Assay Kit (Promega). The ratio of *Renilla* to firefly luciferase expression was calculated for each of the triplicates, the data was averaged, and standard deviations were determined (see Supporting Figures S3 & S4).



**Supporting Figure S3.** UV irradiation time course of the caged miR-122 antagomir in Huh7-psiCHECK-miR122 cells. Irradiation (365 nm, 25 W) for 2 or 5 min efficiently activated antagomir function. The error bars represent standard deviations from three independent experiments.



**Supporting Figure S4.** UV irradiation time course of the caged miR-21 antagomir in Huh7 cells transfected with a luciferase reporter for miR-21 function. Irradiation (365 nm, 25 W) for 2 or 5 min efficiently activated antagomir function. The error bars represent standard deviations from three independent experiments.