

## SUPPORTING INFORMATION

### Fast Click-Slow Release Strategy Towards HPLC-Free Synthesis of RNA.

*Edgar Agustin,<sup>‡</sup> Papa Nii Asare Okai,<sup>‡</sup> Irfan Khan, Michael Miller, Rui Wang, Jia Sheng  
and Maksim Royzen\**

University at Albany, SUNY, Department of Chemistry  
1400 Washington Avenue, Albany, NY 12222. Email: mroyzen@albany.edu

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## Materials and Methods

All chemicals were received from commercial sources and used without further purification. AG-1-X8 resin was purchased from Bio-Rad Laboratories, Inc. and was converted to bicarbonate form by elution with 1M NaHCO<sub>3</sub>. AG50W-X8 resin was also purchased from Bio-Rad Laboratories, Inc. and was used without any modifications. Chromatographic purifications were conducted using SiliaSphere™ spherical silica gel 5µm, 60 Å silica gel (Silicycle). Thin layer chromatography (TLC) was performed on SiliaPlate™ silica gel TLC plates (250 µm thickness) purchased from Silicycle. Preparative TLC was performed on SiliaPlate™ silica gel TLC plates (1000 µm thickness). HPLC purification was performed using Phenomenex Luna 5u C18(2) semi-preparative column (250 x 10 mm). <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy was performed on a Bruker NMR at 400 (<sup>1</sup>H), 100 (<sup>13</sup>C) MHz and 162 (<sup>31</sup>P) MHz.

### Solid phase synthesis of the RNA oligonucleotide SL3

The RNA oligonucleotide synthesis was carried out on a 1 µmol scale using MerMade 4 DNA synthesizer. All the natural nucleoside phosphoramidites (TBDMS as the 2'-OH protecting group) and the accessory reagents were purchased from ChemGenes. After synthesis, the RNA oligo was cleaved from the beads and deprotected by the treatment with AMA solution (a 1:1 aqueous solution of methylamine and concentrated ammonium hydroxide) at 65 °C for 2 h. After evaporating the resulting solution to dryness, the 2'-TBDMS deprotection was performed using NEt<sub>3</sub>•HF solution at 65 °C for 2.5 h. The RNA was finally precipitated by using ammonium acetate and ethanol, and re-dissolved in water.

### Immobilization of **1** - **5** on spin columns loaded with agarose beads

The NHS-activated agarose spin columns (product number 26198) were purchased from Pierce/Thermo Fisher Scientific (Rockford IL). Coupling of **1** - **5** to the agarose beads was performed using manufacturer's recommended protocol. In general, 33 mg of dry agarose was incubated with 4 µmol of a tetrazine in PBS buffer pH 7.4 for 3 h with gentle mixing. The agarose beads were washed three times with PBS. On average, 90% of the tetrazines were immobilized on the solid support, as was estimated by monitoring the absorbance at 520 nm of the supernatants obtained from the resin washes. The unreacted NHS groups on agarose were capped with 1 M Tris, pH 7.4 for 3h.

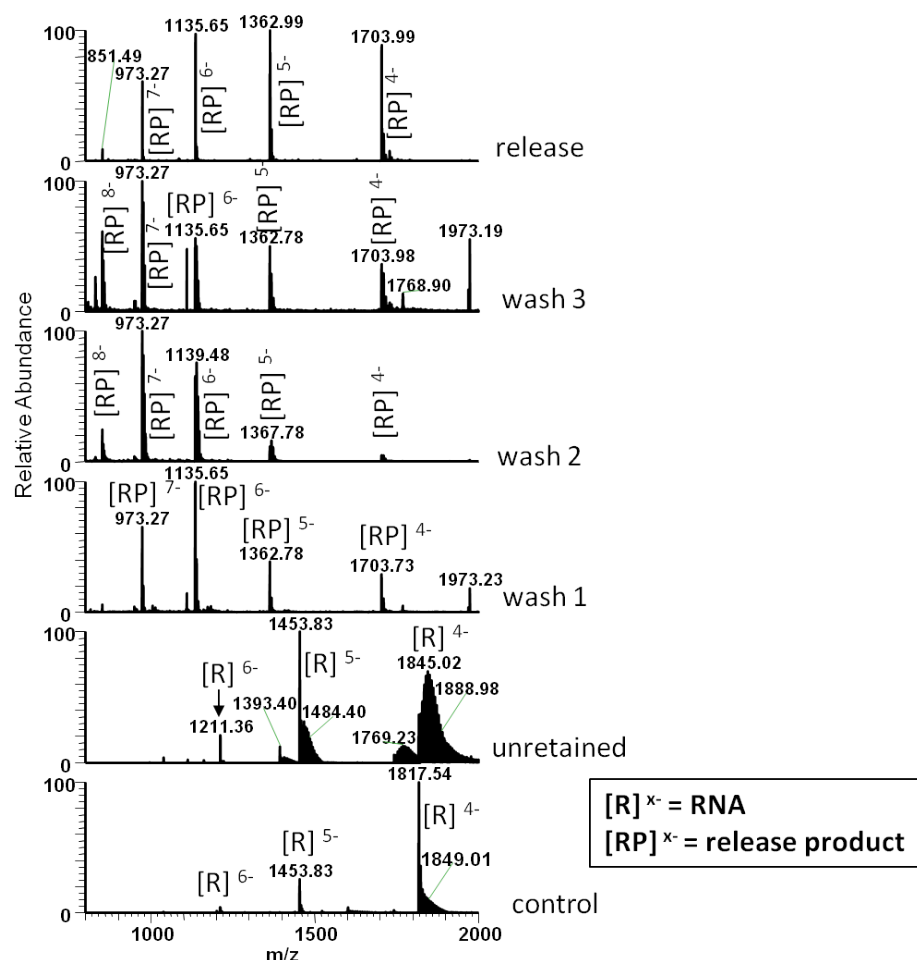
### Kinetic experiments of 'click-to-release'

The agarose beads modified with **1** - **5**, using the method described above, were treated with 300 µL of **6** using three different concentrations (5 mM, 1 mM, 0.5 mM) for 2 min. The supernatant was collected after a quick centrifugation and the agarose was resuspended in water (300 µL). The supernatants were collected at regular time intervals and analyzed by ESI-MS.

### ESI-MS experiments

The samples were analyzed on a Thermo Fisher Scientific (West Palm Beach, CA) LTQ Orbitrap Velos Mass spectrometer, using quartz capillary emitters. To facilitate spray optimization, 10 % isopropyl alcohol was added to each sample prior to MS analysis. The release product, **7**, was

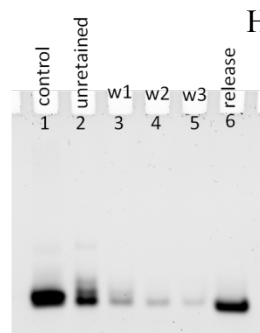
analyzed in the positive mode and the RNA was analyzed in a solution containing 150 mM ammonium acetate in the negative mode.



**Figure S1:** ESI-MS analysis of control and supernatant solutions obtained from the ‘click and release’ of HPLC purified SL3 treated with 1-modified agarose.

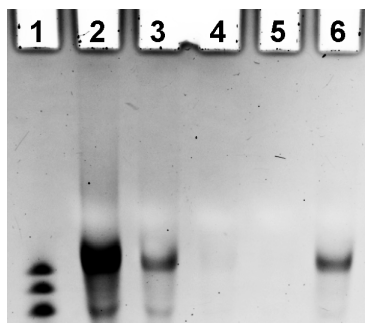
#### Calculation of the yield of the ‘click-to-release’ step

The RNA samples were denatured in 7M urea in the presence of 25 mM EDTA and resolved on a 20% denaturing polyacrylamide gel. The gel was stained with SYBR Green and imaged and analyzed on a Molecular Imager® Gel Doc™ XR System (Biorad). Pixel intensity was used to calculate the RNA concentration corresponding to each band. Lane 1 contains 80 pmol of the

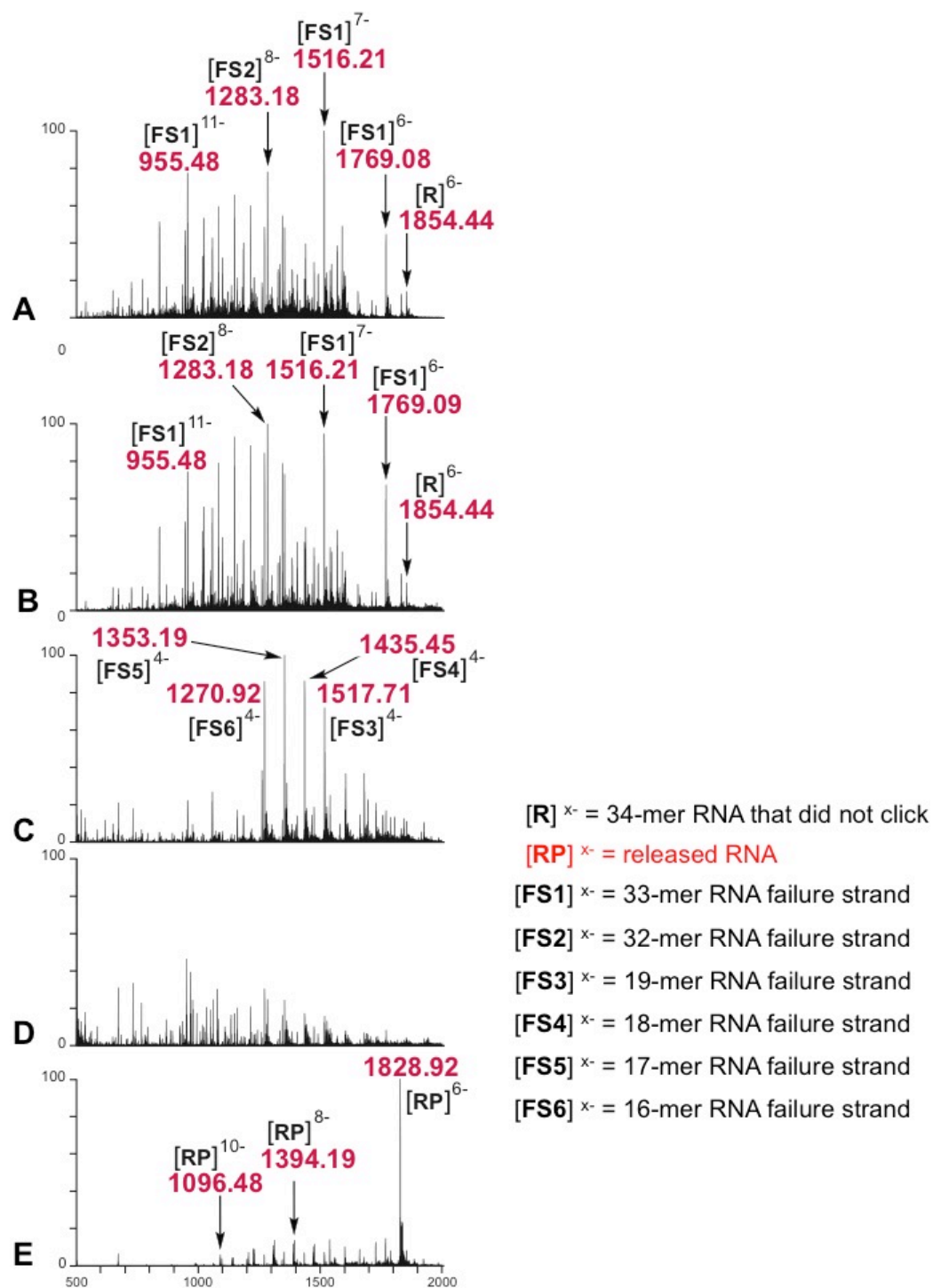


HPLC purified SL3 which was used as a reference to estimate RNA concentrations in lanes 2-6. Lane 6 corresponds to the **RNA released** from agarose beads. The amount of **RNA clicked** onto the agarose column was calculated by subtracting the amount calculated from the intensity of unretained band (well 2) from the amount of the **RNA loaded** onto the column. Based on the MS data, there is no release product in the well 2. The yield of the ‘click-to-release’ step was calculated using the formula:

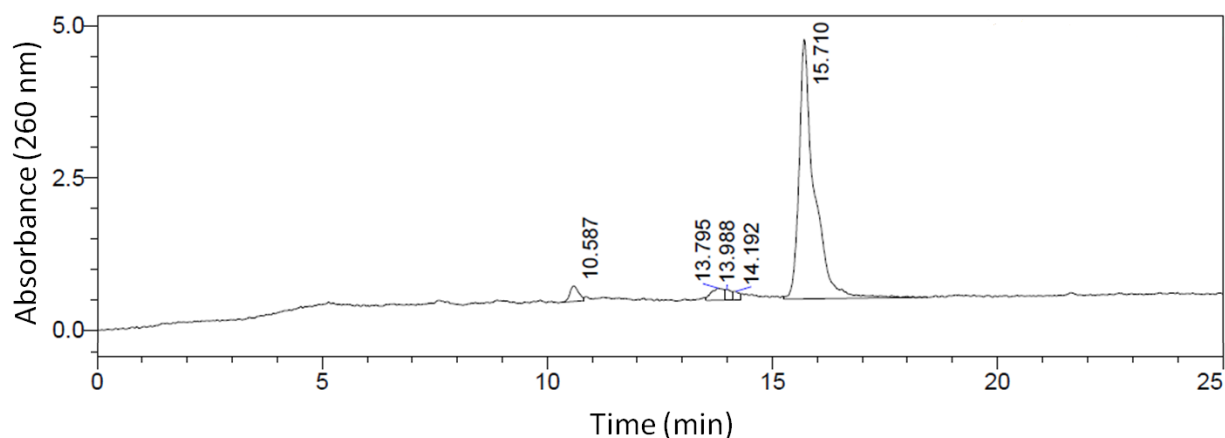
**RNA clicked / RNA released X 100%.** The amount of RNA lost during the wash steps (lanes 3-5) was calculated to be 5%. The experiments were repeated in triplicate and the obtained results were consistent within a 5% error.



**Figure S2.** PAGE analysis of purification of 34-nt long aptamer RNA using ‘click-to-release’ process. Lane 1 is a ladder showing 30-nt, 26-nt, and 22-nt long model RNA strand. Lane 2 is the supernatant collected after 2 min; lanes 3-5 are the three wash steps and lane 6 is the supernatant collected after the ‘release’ step.



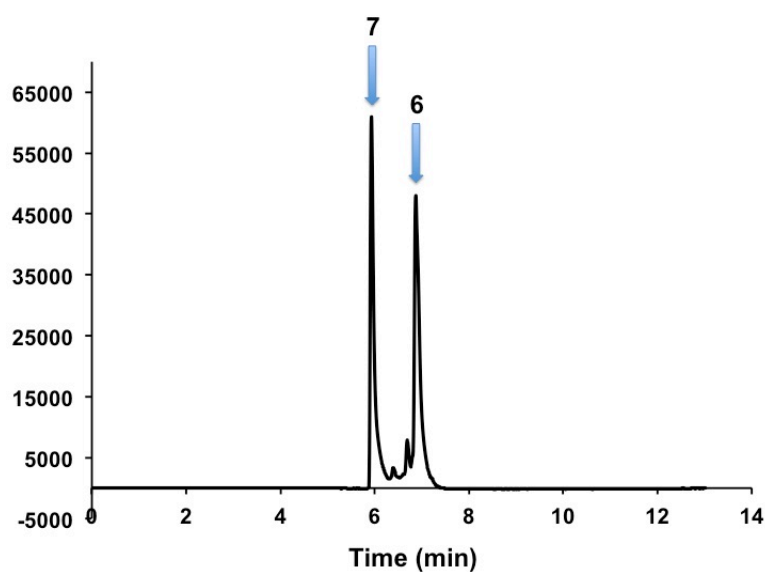
**Figure S3.** ESI-MS analysis of purification of 34-nt long aptamer RNA using ‘click-to-release’ process. Panel A represents the supernatant after 2 min. Panels B through D represent the three washes. Panel E is the supernatant collected after the ‘release’ step.



**Figure S4:** RP-HPLC chromatogram of purified DMT-on synthetic SL3 RNA strand.

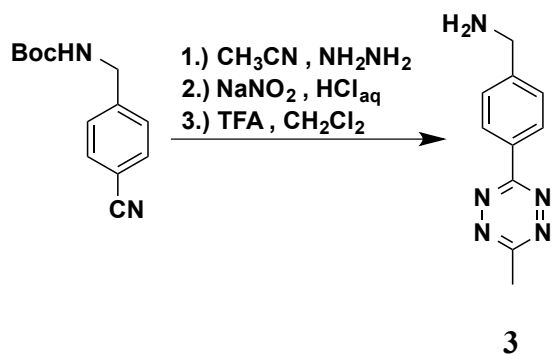
#### RP-HPLC Purification of SL3 RNA Strand

The synthetic SL3 RNA strand was purified by RP-HPLC using a C18 column (Phenomenex, Torrance CA). Buffer A (100 mM TEAA and 5 % acetonitrile); Buffer B (100 mM TEAA 50 % acetonitrile). The purification was achieved using a 15 min gradient of 0-80 % Buffer B, followed by a 7 min gradient of 80-100% Buffer B. After purification the solvents were lyophilized and the sample was desalted, re-suspended in water and quantified using nano-drop.



**Figure S5:** RP-HPLC chromatogram of the compounds **6** and **7**.

The supernatant fractions in Fig. 1 were analyzed by RP-HPLC using a Kinetex 2.6u XB-C18 100A column (Phenomenex, Torrance CA). Buffer A (H<sub>2</sub>O, 0.1% formic acid); Buffer B (CH<sub>3</sub>CN, 0.1% formic acid). The separation was achieved using a 9 min gradient of 0-90 % Buffer B.



Compound **3** was synthesized by following the procedure described in:

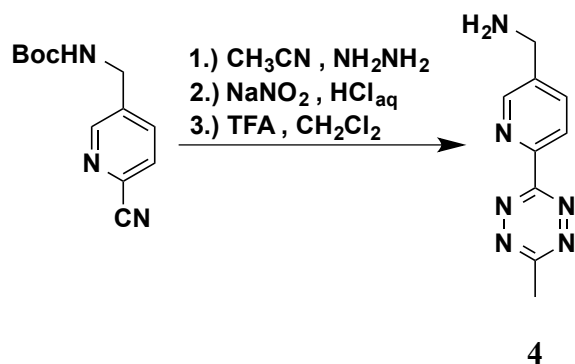
J. Yang, M. R. Karver, W. Li, S. Sahu, N. K. Devaraj, *Angew. Chem. Int. Ed.* 2012, **51**, 5222-5225.

$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  8.62 (d,  $J = 9.6$  Hz, 2H), 7.72 (d,  $J = 8.2$  Hz, 2H), 4.27 (s, 2H), 3.05 (s, 3H).

$^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  169.20, 165.06, 138.93, 134.47, 130.96, 129.58, 44.07, 21.25.

HRMS (ESI)  $m/z$ : calcd. for  $\text{C}_{10}\text{H}_{12}\text{N}_5$   $[\text{M}+1]^+$  202.1093; found 202.1112.

The spectra are consistent with the ones reported previously.



Compound **4** was synthesized by following the procedure described in:

J. Yang, M. R. Karver, W. Li, S. Sahu, N. K. Devaraj, *Angew. Chem. Int. Ed.* 2012, **51**, 5222-5225.

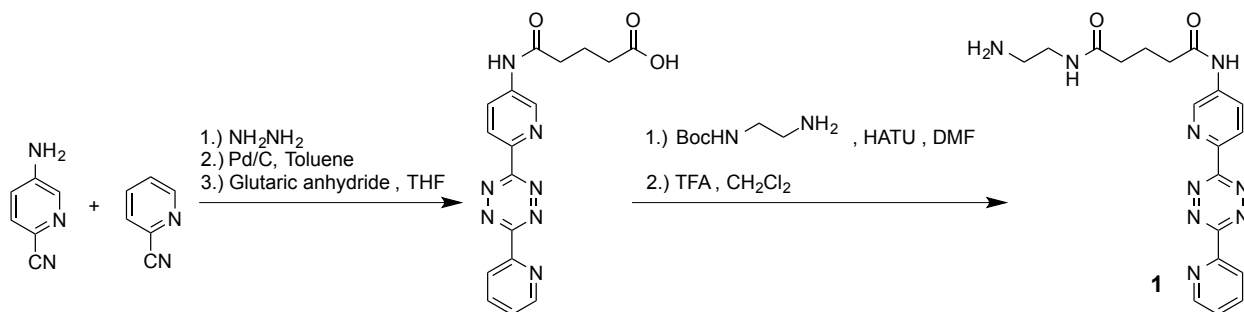
$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  8.93 (s, 1H),

8.73 (d,  $J = 8.2$  Hz, 1H), 8.25 (dd,  $J_1 = 5.5$  Hz,  $J_2 = 8.2$  Hz, 1H), 4.36 (s, 2H), 3.12 (s, 3H).

$^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  170.13, 164.32, 152.14, 151.97, 140.26, 133.91, 125.14, 41.60, 21.45.

HRMS (ESI)  $m/z$ : calcd. for  $\text{C}_9\text{H}_{11}\text{N}_6$   $[\text{M}+1]^+$  203.1045; found 203.1047.

The spectra are consistent with the ones reported previously.



Compound **1** was synthesized by following the procedure described in:

R. Selvaraj, S. Liu, M. Hassink, C. Huang, L. Yap, R. Park, J. M. Fox, Z. Li, P. S. Conti, *Bioorg. Med. Chem. Lett.* 2011, **21**, 5011-5014.

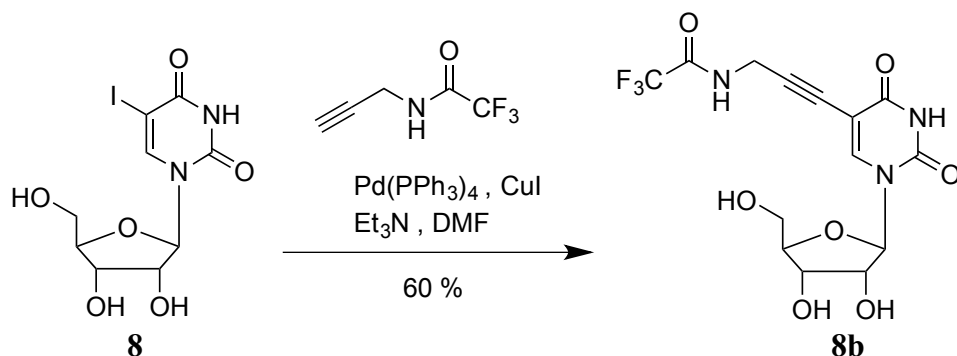
$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  9.03 (d,  $J$  = 2.7 Hz, 1H), 8.88 (d,  $J$  = 4.2 Hz, 1H), 8.77 (d,  $J$  = 8.2 Hz, 1H), 8.71 (d,  $J$  = 9.6 Hz, 1H), 8.38 (d,  $J$  = 10.9 Hz, 1H), 8.20 (t,  $J$  = 6.8 Hz, 1H), 7.77-7.72 (m, 1H), 3.49 (t,  $J$  = 5.5 Hz, 2H), 3.11 (t,  $J$  = 5.4 Hz, 2H), 2.53 (t,  $J$  = 6.9 Hz, 2H), 2.36 (t,  $J$  = 6.8 Hz, 2H), 2.02 (quint,  $J$  = 6.8 Hz, 2H).

$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  176.62, 174.43, 164.41, 151.20, 150.82, 144.97, 142.48, 140.41, 140.08, 128.57, 128.47, 128.28, 125.73, 118.57, 111.49, 40.91, 38.19, 36.94, 35.89, 22.15.

HRMS (ESI)  $m/z$ : calcd. for  $\text{C}_{19}\text{H}_{22}\text{N}_9\text{O}_2$   $[\text{M}+1]^+$  408.1696; found 408.1893.

The spectra are consistent with the ones reported previously.





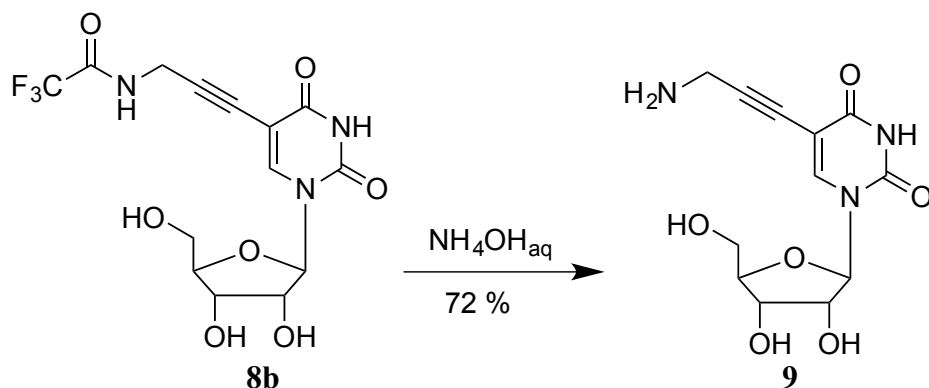
### 5-[3-(2,2,2-trifluoroacetamido)propyl]uridine (**8b**)

5-Iodouridine (1.078 g, 2.92 mmol), *N*-propargyl trifluoroacetamide (1.323 g, 8.76 mmol),  $\text{CuI}$  (111 mg, 0.584 mmol), and  $\text{Pd(PPh}_3)_4$  (337 mg, 0.292 mmol) were placed in a 100 mL round bottom flask and dissolved in anhydrous and thoroughly degassed  $\text{DMF}$  (25 mL). The round bottom flask was placed under vacuum and refilled with  $\text{N}_2$ . Triethylamine (0.812 mL, 2.156 mmol) was added to the reaction mixture which was stirred at room temperature under nitrogen for 18 h. The reaction mixture was diluted with a 1:1 solution of  $\text{MeOH}:\text{CH}_2\text{Cl}_2$  (18 mL) and AG-1-X8 resin (bicarbonate form, 3 g) was added. After stirring for 30 min, the crude product was filtered and the resin was once rinsed with a 1:1 solution of  $\text{MeOH}:\text{CH}_2\text{Cl}_2$  (18 mL). The solvents were removed under high vacuum and the title product was obtained as a light yellow-brown solid by flash chromatography (5%  $\text{MeOH}$  in  $\text{CH}_2\text{Cl}_2$ ). Yield = 0.680 g (60%).

$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  8.38 (s, 1H), 5.88 (d,  $J = 4.0$  Hz, 1H), 4.27 (s, 2H), 4.17 (qi,  $J = 5.5$  Hz, 2H), 4.02 (t,  $J = 2.5$  Hz, 1H), 3.87 (dd,  $J_1 = 12.3$  Hz,  $J_2 = 2.3$  Hz, 1H), 3.75 (dd,  $J_1 = 12.3$  Hz,  $J_2 = 2.5$  Hz, 1H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  166.54, 159.11, 158.73, 157.02, 146.71, 121.75, 118.90, 116.05, 113.20, 92.56, 91.03, 85.95, 76.51, 75.68, 70.51, 61.70, 31.15.

The spectra is consistent with the one previously reported by:

N. K. Carg, C. C. Woodroffe, C. J. Lacenere, S. R. Quake and B. M. Stoltz, *Chem. Commun.* **2005**, 4551-4553.



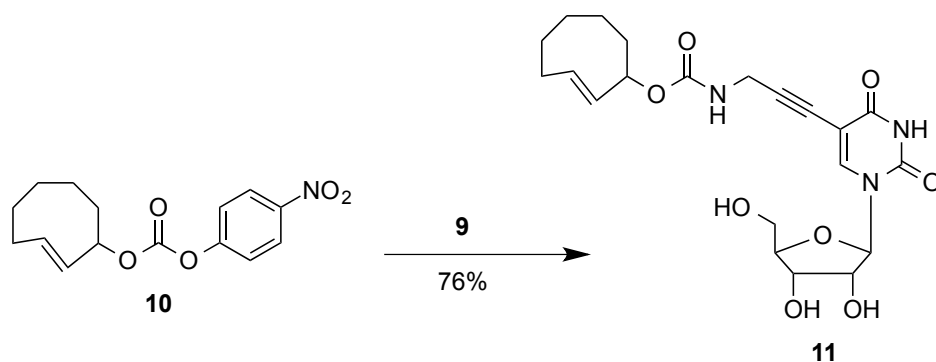
### 5-(3-aminopropyl)uridine (**9**)

Compound **8b** (0.372 g, 0.946 mmol) was dissolved in DI H<sub>2</sub>O (8.5 mL). After complete dissolution, aqueous ammonium hydroxide (NH<sub>4</sub>OH) (8.5 mL) was added. The reaction mixture was stirred for 3 h at room temperature and then concentrated under reduced pressure to give the crude product as a yellow-brown residue. The crude product was redissolved in DI H<sub>2</sub>O (10 mL) and AG50W-X8 resin (2.5 g) was added. The mixture was stirred for 15 min and filtered over a bed of AG50W-X8 resin (2.5 g). The resin was washed once with DI H<sub>2</sub>O and the product was eluted with 4:1 mixture of DI H<sub>2</sub>O:NH<sub>4</sub>OH, (100 mL). The title product was obtained as a light tan solid after lyophilization. Yield = 0.203 g (72%).

<sup>1</sup>H NMR (D<sub>2</sub>O, 400 MHz) 8.06 (s, 1H), 5.81 (d, *J* = 3.9 Hz, 1H), 4.24 (t, *J* = 4.3 Hz, 1H), 4.15 (t, *J* = 5.5 Hz, 1H), 4.07-4.04 (m, 1H), 3.88 (dd, *J*<sub>1</sub> = 12.4 Hz, *J*<sub>2</sub> = 2.6 Hz, 1H), 3.77-3.74 (m, 3H).  
<sup>13</sup>C NMR (D<sub>2</sub>O, 100 MHz) δ 168.07, 153.20, 144.55, 141.53, 98.11, 89.80, 83.71, 77.11, 73.86, 68.78, 60.18, 29.82.

The spectra is consistent with the one previously reported by:

H. Bjelosevic, C. Spegel, A. S. Snygg, L. Gorton, S. K. C. Elmroth, T. Persson, *Tetrahedron*, **2006**, 62, 4519-4527.

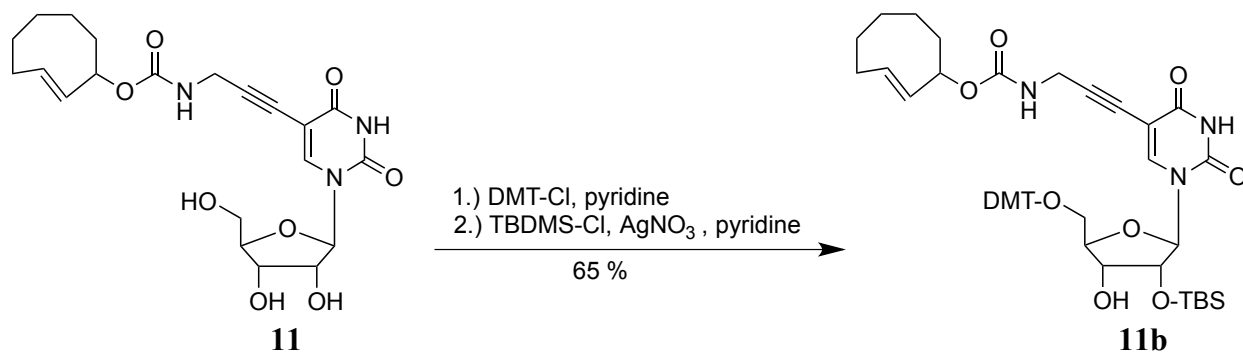


**5-[(*E*)-(cyclooct-2-enyl-ethynyl)cabamoyl]uridine (**11**)**

Compounds **9** (0.352 g, 1.20 mmol) and **10** (0.359 g, 1.20 mmol) were dissolved in anhydrous DMF (7 mL). Triethyl amine (336  $\mu$ L, 2.41 mmol) was added and the reaction mixture was stirred at 30 °C for 18 h. The solvent was removed under high vacuum and the title product was obtained as a yellow foam after preparative column chromatography using 5%MeOH in CH<sub>2</sub>Cl<sub>2</sub> as a mobile phase. Yield = 414 mg (76%).

<sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  8.34 (s, 1H), 5.88 (d,  $J$  = 4.07 Hz, 1H), 5.54 (d,  $J$  = 16.47 Hz, 1H), 5.26 (bs, 1H), 4.21-4.14 (m, 2H), 4.08 (s, 2H), 4.02 (d,  $J$  = 4.14 Hz, 1H), 3.87 (dd,  $J_1$  = 12.3 Hz,  $J_2$  = 2.4 Hz, 1H), 3.74 (dd,  $J_1$  = 12.2 Hz,  $J_2$  = 2.4 Hz, 1H), 2.44 (d,  $J$  = 10.96 Hz, 1H), 2.07-1.91 (m, 3H), 1.90-1.80 (m, 1H), 1.77-1.57 (m, 2H), 1.56-1.42 (m, 2H), 1.19-1.08 (m, 1H), 0.91-0.79 (m, 1H).

<sup>13</sup>C NMR (CD<sub>3</sub>OD, 100 MHz)  $\delta$  165.00, 158.42, 151.89, 146.01, 133.24, 133.11, 100.60, 91.44, 86.89, 76.44, 75.98, 75.41, 71.51, 62.46, 42.13, 37.50, 37.28, 32.37, 30.57, 25.66. HRMS (ESI)  $m/z$ : calcd. for C<sub>21</sub>H<sub>28</sub>N<sub>3</sub>O<sub>8</sub> [M+1]<sup>+</sup> 450.1871; found 450.1863



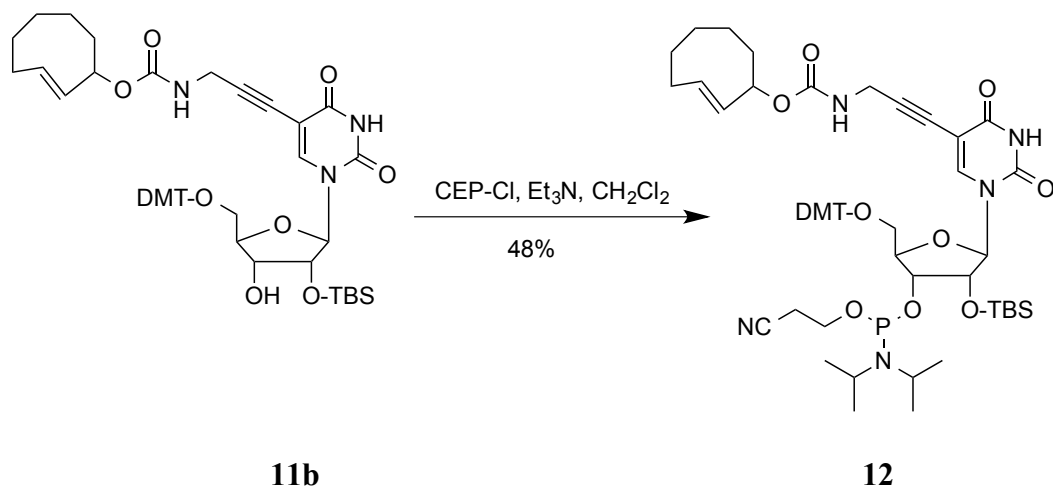
**5-(*E*)-(cyclooct-2-enyl-ethynyl)cabamoyl-2'-O-(*tert*-butyldimethylsilyl)-5'-O-(4,4'-dimethoxytrityl)uridine (**11b**)**

Compound **11** (100 mg, 0.222 mmol) was coevaporated three times with anhydrous pyridine (3x3 mL) and dried on high vacuum overnight. It was dissolved in anhydrous pyridine (3 mL). A solution of 4,4'-dimethoxytrityl chloride (83 mg, 0.244 mmol) in anhydrous pyridine (3 mL) was added dropwise over 40 min at room temperature. The reaction mixture was stirred for 4.5 h at room temperature and quenched with methanol (1 mL). The solvent was evaporated under reduced pressure and the product mixture was redissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL). The organic phase was washed with 5% citric acid (10 mL), water (10 mL) and saturated sodium bicarbonate solution (10 mL). The organic layer was dried with sodium sulfate, and evaporated to dryness. The crude product was carried over to the next step without further purification.

The crude product of the previous step (150 mg, 0.199 mmol) was coevaporated three times with anhydrous pyridine (3x3 mL) and dried on high vacuum overnight. It was dissolved in anhydrous tetrahydrofuran (6 mL). Silver nitrate (40 mg, 0.239 mmol) and pyridine (80  $\mu$ L, 0.997 mmol) were added, and the suspension was stirred for 0.5 h in the dark. Then, *tert*-butyldimethylsilyl chloride (39 mg, 0.259 mmol) was added, and the mixture was stirred for 4 h at room temperature in the dark. The suspension was filtered over Celite, and the filtrate was evaporated to dryness. The residue was redissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL). The organic phase was washed with saturated sodium bicarbonate solution (10 mL), dried over sodium sulfate, and evaporated to dryness. The product was purified by gravity SiO<sub>2</sub> column using Et<sub>2</sub>O as a mobile phase. Compound **11b** was isolated as a yellow foam. Yield over two steps: 97 mg (65%).

<sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz)  $\delta$  9.18 (s, 1H), 8.24 (bs, 1H), 7.75 (d,  $J$  = 7.08 Hz, 2H), 7.62 (d,  $J$  = 8.18 Hz, 4H), 7.51 (d,  $J$  = 8.27 Hz, 1H), 7.40-7.33 (m, 3H), 6.99 (t,  $J$  = 9.51 Hz, 5H), 6.86-6.78 (m, 2H), 5.93 (t,  $J$  = 2.71 Hz, 2H), 5.53 (bs, 1H), 5.33 (d,  $J$  = 16.14 Hz, 1H), 4.56 (bs, 1H), 4.48-4.40 (m, 1H), 4.35 (bs, 1H), 4.16-3.64 (m, 5H), 3.56 (s, 2H), 3.51-3.45 (m, 7H), 2.55-2.40 (m, 2H), 2.17-1.81 (m, 4H), 1.8-1.65 (m, 2H), 1.62-1.27 (m, 11H), 0.98 (s, 9H), 0.75 (s, 4H), 0.32 (s, 4H), 0.22 (s, 4H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  158.72, 158.67, 148.91, 144.58, 144.55, 142.86, 135.20, 131.80, 130.05, 129.90, 128.14, 127.76, 127.02, 113.73, 113.43, 113.41, 113.15, 99.97, 89.73, 88.56, 84.13, 74.18, 73.68, 71.30, 63.01, 55.29, 40.63, 35.94, 35.88, 31.57, 29.05, 25.60, 24.07, 22.64, 17.99, 14.11.

HRMS (ESI)  $m/z$ : calcd. for C<sub>48</sub>H<sub>59</sub>N<sub>3</sub>NaO<sub>10</sub>Si [M+Na]<sup>+</sup> 888.3867; found 888.3851



**5-(*E*)-(cyclooct-2-enyl-ethynyl)cabamoyl-2'-O-(*tert*-butyldimethylsilyl)-3'-O-[(2-cyanoethyl)(diisopropylamino)]phosphino-5'-O-(4,4'-dimethoxytrityl)uridine (**12**)**

Compound **11b** (67 mg, 0.077 mmol) was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (3 mL) followed by addition of a catalytic amount of DMAP (~ 2 mg), anhydrous triethylamine (43 μL, 0.309 mmol), and 2-cyanoethyl chlorodiisopropylamino phosphoramidite (35 μL, 0.154 mmol). After 2 h, the crude product was purified by preparative column chromatography using a 1:2 mixture of CH<sub>2</sub>Cl<sub>2</sub> : EtOAc (with 0.2% TEA) as a mobile phase. Yield = 40 mg (48%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ 8.30-8.22 (m, 1H), 7.45 (t, *J* = 8.27 Hz, 2H), 7.40-7.21 (m, 7H), 6.89-6.81 (m, 4H), 6.07 (d, *J* = 6.77 Hz, 1H), 5.74-5.60 (m, 1H), 5.47-5.17 (m, 2H), 4.52-4.42 (m, 2H), 4.37-4.21 (m, 2H), 3.99-3.82 (m, 1H), 3.79 (s, 6H), 3.75-3.50 (m, 6H), 3.44-3.26 (m, 2H), 2.68-2.62 (m, 1H), 2.47-2.32 (m, 2H), 2.03-1.90 (m, 2H), 1.89-1.79 (m, 1H), 1.69-1.54 (m, 2H), 1.38 (t, *J* = 6.79 Hz, 1H), 1.33-1.20 (m, 4H), 1.18-1.12 (m, 10H), 1.05-0.98 (m, 3H), 0.92-0.85 (m, 11H), 0.15-0.05 (m, 7H).

<sup>31</sup>P NMR (CDCl<sub>3</sub>, 162 MHz) δ 150.41, 149.21.

HRMS (ESI) *m/z*: calcd. for C<sub>57</sub>H<sub>77</sub>N<sub>5</sub>O<sub>11</sub>PSi [M+1]<sup>+</sup> 1088.6946; found 1088.6986.

