Supplementary Information

1. Materials & Methods

Synthesis of the thA and thI phosphoramidite and their oligonucleotides for GluR-B R/G site

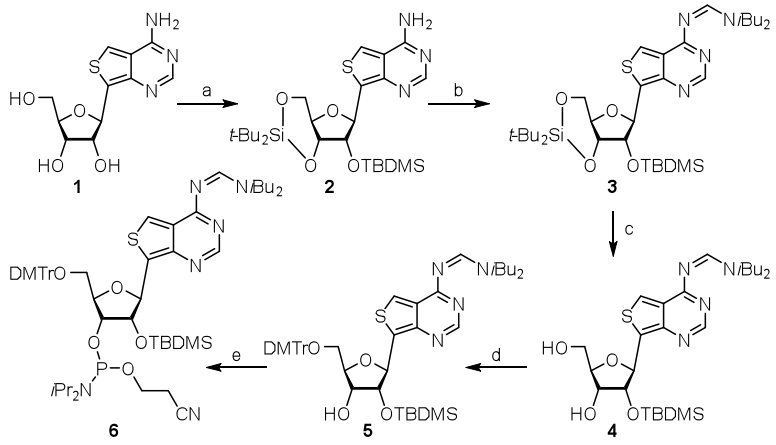
Reagents were purchased from Sigma-Aldrich, Fluka and Acros, and were used without further purification unless otherwise specified. Solvents were purchased from Sigma-Aldrich and Fisher Scientific, and dried by standard techniques. NMR solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). All reactions were monitored with analytical TLC (Merck Kieselgel 60 F254). All experiments involving air and/or moisture sensitive compounds were carried out under an argon atmosphere. Column chromatography was carried out with silica gel particle size 40-63 μm. NMR spectra were obtained on Varian Mercury 400 MHz, Varian VX 500 MHz and Jeol ECA 500 spectrometers. Mass spectra were obtained on an Agilent 6230 HR-ESI-TOF MS at the Molecular Mass Spectrometry Facility at the UCSD Chemistry and Biochemistry Department.

2. Synthesis, Analytical Data and Biochemical Methods

2.1. thA phosphoramidite synthesis

Regioselective protection of the 2'-hydroxyl group to give the corresponding 2'-O-TBDMS through 3',5'-O-di-tert-butylsily of the thA free nucleoside,[1] was followed by protection of the N^6 amine with *N,N*-diisobutylformamidine group (Scheme 1). Tritylation of the 5'-hydroxyl group in **4** and phosphitylation for 3'-hydroxyl group provided phosporamitide **6**, suitable for automated RNA synthesis. The phosphoramidite of thI **11** was synthesized in a similar way (Scheme 2). The free thI nucleoside synthesis was reported in our previous publication.[2]

Scheme S1. thA phosphoramidite synthesis[a]



[a] Reagents and conditions: (a) i) di-*tert*-BuSi(OTf)$_2$, DMF, 0 °C; ii) TBDMSCl, Im, 68 % for 2 steps; (b) D*i*BF-DMA, DMF, 87 %; (c) HF-Py, CH_2Cl_2, 0°C, 77 %; (d) DMTrCl, Py, 69 %; (e) 2-cyanoethyl N,N-diisopropylchloro phophoramidite, *i*Pr$_2$NEt, DCM, 0 °C – RT, 95 %.

Synthesis of $O^{3',5'}$-di-*tert*-butylsilyl-$O^{2'}$-TBDMS-thA (2)

To a suspension of nucleoside th**A** (**1**, 0.36 g, 1.27 mmol) in dry DMF (5 mL) *t*Bu$_2$Si(OTf)$_2$ (0.46 mL, 1.40 mmol) was added dropwise over 15 min at 0 °C, and the resulting solution was stirred at the same temperature for 2 hour. Imidazole (0.43 g, 6.35 mmol) was added to the mixture at 0 °C then stirred for 5 min followed by stirring at RT for 30 min. TBDMSCl (0.23 g, 1.52 mmol) was added

to the mixture and stirred at 60 °C for 2 h. All volatiles were evaporated and the residue was partitioned between DCM (100 mL) and saturated aq. $NaHCO_3$ (100 mL). The aq. layer was extracted with DCM (3 × 20mL), combined organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography with DCM:MeOH = 25:1 to 20:1 to afford a white solid (0.44 g, yield 68 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.73 (s, 1H), 6.11 (br, 2H), 5.68 (s, 1H), 4.51 (dd, *J* = 6.9, 2.6 Hz, 2H), 4.34 – 4.14 (m, 2H), 4.04 (dt, *J* = 15.3, 8.5 Hz, 1H), 1.02 (d, 18H), 0.92 (s, 9H), 0.17 (s, 3H), 0.12 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.99, 153.39, 146.12, 134.88, 121.06, 117.11, 84.05, 77.37, 77.28, 74.12, 68.70, 27.66, 27.48, 27.22, 26.14, 22.87, 20.48, 18.47, -4.14, -4.68; ESI-HRMS calculated for $C_{25}H_{44}N_3O_4SSi_2$ $[M+H]^+$ 538.2586, found 538.2584.

Synthesis of *O*$^{3',5'}$-di-*tert*-butylsilyl-*O*$^{2'}$-TBDMS-*N*^{6}-diisobutylformamidine-thA (3)

To a solution of **2** (0.42 g, 0.78 mmol) in DMF (4 mL) was added *N*,*N*-diisobulylformamide dimethyl acetal[3] (0.32 g, 1.56 mmol), and the solution was stirred overnight. All volatiles were evaporated, and the residue was coevaporated with DMF (2 × 5 mL). The residue was purified by column chromatography with DCM:MeOH = 100:1 to afford a white foam. Yield 0.46 g, 87 %. ^{1}H NMR (500 MHz, CD_3Cl) δ 8.88 (d, *J* = 5.8 Hz, 1H), 8.43 – 8.39 (m, 1H), 8.12 – 8.07 (m, 1H), 5.73 (d, *J* = 4.9 Hz, 1H), 4.55 – 4.42 (m, 2H), 4.20 (dtd, *J* = 14.3, 9.6, 4.5 Hz, 2H), 4.12 – 3.97 (m, 1H), 3.50 (qd, *J* = 13.2, 7.6 Hz, 2H), 3.20 (d, *J* = 7.5 Hz, 2H), 2.32 – 2.14 (m, 1H), 2.09 – 1.95 (m, 1H), 1.04 (d, *J* = 3.2 Hz, 18H), 1.00 – 0.91 (m, 21H), 0.17 (s, 3H), 0.12 (s, 3H); ESI-MS calculated for $C_{34}H_{61}N_4O_4SSi_2$ $[M+H]^+$ 677.40, found 677.39; ESI-HRMS calculated for $C_{34}H_{61}N_4O_4SSi_2$ $[M+H]^+$ 677.3947, found 677.3943.

Synthesis of *O*$^{2'}$-TBDMS-*N*^{6}-diisobutylformamidine-thA (4)

To a solution **3** (0.45 g, 0.66 mmol) in dry DCM (5 mL) was added HF•Py (26 µL, 0.99 mmol, 5x diluted in Py) at 0 °C and the resultant was stirred for 4 hour at

the same temperature. The reaction was quenched by addition of water (50 mL). The mixture was diluted with DCM (50 mL). The separated organic layer was washed with saturated aq. $NaHCO_3$, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by column chromatography with DCM:MeOH = 50:1 to 40:1 to afford a yellow solid. Yield 0.27 g, 77 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.93 (s, 1H), 8.39 (d, *J* = 5.6 Hz, 1H), 8.10 – 8.00 (m, 1H), 5.03 (dt, *J* = 8.3, 6.3 Hz, 2H), 4.39 – 4.25 (m, 2H), 3.96 (dd, *J* = 12.5, 1.8 Hz, 1H), 3.73 (d, *J* = 12.4 Hz, 1H), 3.56 (dd, *J* = 13.2, 7.7 Hz, 1H), 3.47 (dd, *J* = 13.2, 7.4 Hz, 1H), 3.27 – 3.16 (m, 2H), 2.95 (d, *J* = 16.1 Hz, 1H), 2.22 (tt, *J* = 13.9, 6.9 Hz, 1H), 2.12 – 1.93 (m, 1H), 1.07 – 0.92 (m, 12H), 0.84 – 0.75 (m, 9H), -0.24 (s, 3H), -0.44 (s, 3H);^{13}C NMR (126 MHz, $CDCl_3$) δ 164.45, 158.08, 153.86, 147.42, 128.79, 128.40, 120.64, 87.11, 79.71, 77.49, 74.54, 64.04, 60.69, 53.85, 27.52, 26.71, 25.76, 20.51, 20.47, 20.03, 20.02, 18.00, -5.20, -5.26; ESI-MS calculated for $C_{26}H_{41}N_4O_4SSi$ $[M+H]^+$ 537.29, found 537.27; ESI-HRMS calculated for $C_{26}H_{41}N_4O_4SSi$ $[M+H]^+$ 537.2925, found 537.2922.

Synthesis of *O*$^{5'}$-Dimethoxytrityl-*O*$^{2'}$-TBDMS-*N*^{6}-diisobutylformamidine-thA (5)

4 (0.29 g, 0.54 mmol) was coevaporated with dry Py (3 × 2 mL), dried under high vacuum overnight, and dissolved in dry Py (3 mL). DMTrCl (0.22 g, 0.65 mmol) was added at one portion to the solution, and the solution was stirred at RT for 5 hour. After quenched by addition of MeOH (1 mL), all volatiles were evaporated, and the residue was purified by column chromatography with DCM:MeOH = 150:1 containing 1 % Py to afford a yellow solid. Yield 0.31 g, 69 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.99 – 8.91 (m, 1H), 8.47 (s, 1H), 8.18 (d, *J* = 7.6 Hz, 1H), 7.57 (d, *J* = 7.5 Hz, 2H), 7.51 – 7.40 (m, 4H), 7.28 (t, *J* = 7.6 Hz, 2H), 7.25 – 7.16 (m, 1H), 6.82 (t, *J* = 11.1 Hz, 4H), 5.83 (d, *J* = 6.9 Hz, 1H), 4.59 (dd, *J* = 6.6, 5.4 Hz, 1H), 4.24 (d, *J* = 2.9 Hz, 1H), 4.19 (d, *J* = 3.5 Hz, 1H), 3.77 (s, 6H), 3.69 – 3.43 (m, 4H), 3.31 – 3.16 (m, 2H), 2.92 (s, 1H), 2.31 – 2.17 (m, 1H), 2.04 (tt, *J* = 13.7, 6.9 Hz, 1H), 1.01 (d, *J* = 6.6 Hz, 6H), 0.97 (t, *J* = 6.8 Hz, 6H), 0.82 (d, *J* = 6.8 Hz, 9H), –0.10 (s, 3H), –0.16 (s, 3H);^{13}C NMR (126 MHz,

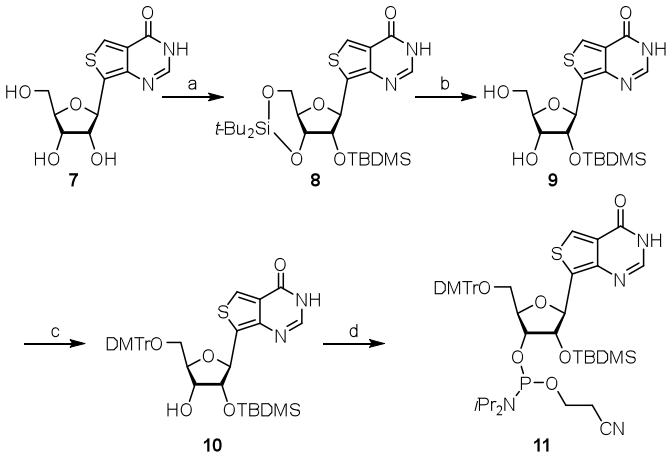
$CDCl_3$) δ 163.63, 158.42, 158.40, 157.61, 154.01, 149.03, 145.12, 136.29, 136.09, 132.10, 130.24, 130.21, 128.32, 127.80, 126.67, 121.20, 113.13, 113.10, 86.12, 84.21, 79.87, 77.51, 72.93, 64.16, 60.50, 55.20, 53.67, 27.42, 26.59, 25.72, 20.43, 20.42, 19.96, 19.95, 18.02, –4.86, –5.39; ESI-MS calculated for $C_{47}H_{63}N_4O_6SSi$ $[M+H]^+$ 839.42 and $C_{47}H_{62}N_4NaO_6SSi$ $[M+Na]^+$ 861.41, found 838.97 and 861.98, respectively; ESI-HRMS calculated for $C_{47}H_{63}N_4O_6SSi$ $[M+H]^+$ 839.4232, found 839.4235.

Synthesis of *O*$^{3'}$-(2-Cyanoethyldiisopropylphosphoramidite)-*O*$^{5'}$-dimethoxytrityl-*O*$^{2'}$-TBDMS-*N*^{6}-diisobutylformamidine-thA (6)

5 (0.30 g, 0.36 mmol) was coevaporated with dry Py (2 × 2 mL) and dissolved in dry DCM (4 mL). *N*,*N*-Diisopropylethylamine (0.31 mL, 1.80 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.24 mL, 1.08 mmol) were successively added to the solution at 0 °C, and the solution was stirred overnight at RT. All volatiles were evaporated and the residue was purified by column chromatography with Hex:EtOAc = 1:1 with 1 % Py to afford a yellow foam. Yield 0.35 g, 95 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.91 (s, 2H), 8.43 (s, 1H), 8.41 (s, 1H), 8.13 (s, 2H), 7.61 – 7.50 (m, 4H), 7.50 – 7.35 (m, *J* = 10.2, 2.4 Hz, 9H), 7.32 – 7.24 (m, *J* = 12.4, 3.8 Hz, 7H), 7.24 – 7.11 (m, 4H), 6.86 – 6.75 (m, 9H), 5.84 (d, *J* = 8.2 Hz, 1H), 5.79 (d, *J* = 6.7 Hz, 1H), 4.69 – 4.58 (m, 2H), 4.39 – 4.33 (m, *J* = 2.6 Hz, 1H), 4.30 – 4.18 (m, 4H), 4.09 – 3.97 (m, *J* = 10.6, 6.5 Hz, 1H), 3.94 – 3.83 (m, 1H), 3.77 (dd, *J* = 3.5, 1.5 Hz, 13H), 3.64 – 3.41 (m, 13H), 3.21 (d, *J* = 7.5 Hz, 4H), 3.17 – 3.06 (m, 2H), 2.74 – 2.64 (m, 3H), 2.30 – 2.16 (m, 4H), 2.02 (dp, *J* = 13.7, 6.8 Hz, 2H), 1.35 – 1.19 (m, 4H), 1.19 – 1.09 (m, 16H), 1.05 – 0.89 (m, 30H), 0.77 (s, 9H), 0.72 (s, 9H); ^{31}P NMR (202 MHz, $CDCl_3$) δ 150.55, 148.84; ESI-MS calculated for $C_{56}H_{80}N_6O_7PSSi$ $[M+H]^+$ 1039.53, found 1039.00; ESI-HRMS calculated for $C_{56}H_{80}N_6O_7PSSi$ $[M+H]^+$ 1039.5311, found 1039.5319.

2.2. thI phosphoramidite synthesis

Scheme S2. thI phosphoramidite synthesis[a]



[a] Reagents and conditions: (a) i) di-*tert*-BuSi(OTf)$_2$, DMF, 0 °C; ii) TBDMSCl, Im, 81 % for 2 steps; (b) HF-Py, CH_2Cl_2, 0°C, 89 %; (c) DMTrCl, Py, 86 %; (d) 2-cyanoethyl N,N-diisopropylchloro phosphoramidite, *i*Pr$_2$NEt, DCM, 0 °C – RT, 79 %.

Synthesis of *O*$^{3',5'}$-di-*tert*-butylsilyl-*O*$^{2'}$-TBDMS-thI (8)

7[2] (0.17 g, 0.60 mmol) was coevaporated with dry Py (2 × 3 mL) and dissolved in dry DMF (2.5 mL). *t*Bu$_2$Si(OTf)$_2$ (0.21 mL, 0.66 mmol) was added over 10 min at 0 °C to the solution and the solution was stirred for 1 hour. Imidazole (0.20 g, 2.99 mmol) was added to the reaction mixture at 0 °C, stirred for 5 min followed by stirring at RT for 30 min. TBDMSCl (0.11 g, 0.72 mmol) was added to the mixture, which was then heated at 60 0 °C for 1 hour. All volatiles were evaporated, and the residue was partitioned between DCM (50 mL) and saturated aq. $NaHCO_3$ (50 mL). The aq. layer was extracted with DCM (3 × 20mL), combined organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography with 0–20 % gradient MeOH in DCM to afford a white solid. Yield 0.26 g, 81 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.57 (s, 1H), 8.20 (s, 1H), 7.71 (d, *J* = 3.1 Hz, 1H), 5.59 (s, 1H), 4.50 (dd, *J* = 9.1, 4.9 Hz, 1H), 4.43 (d, *J* = 4.2 Hz, 1H), 4.18 (tt, *J* = 8.7, 4.4 Hz, 1H), 4.14 – 4.07 (m, 1H), 4.01 (t, *J* = 9.6 Hz, 1H), 1.07 – 0.99 (m, *J* = 7.6 Hz, 18H), 0.94 (s, 9H), 0.17 (s, 3H), 0.14 (s, 3H);ESI-MS calculated for $C_{25}H_{43}N_2O_5SSi_2$ $[M+H]^+$ 539.53,

found 538.93; ESI-HRMS calculated for $C_{25}H_{43}N_2O_5SSi_2$ $[M+H]^+$ 539.2426, found 539.2428.

Synthesis of *O*$^{2'}$-TBDMS-thI (9)

To a solution of **8** (0.25 g, 0.46 mmol) in dry DCM (2.5 mL) was added HF•Py (18 µL, 0.69 mmol, 5 × diluted in Py) at 0 °C and stirred 3 hour at the same temperature. The reaction was quenched by addition of water (10 mL) and diluted with DCM (30 mL). The separated organic layer washed with saturated aq. $NaHCO_3$ (30 mL), dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography with 0 – 5 % gradient of MeOH in DCM to afford a white solid. Yield 0.16 g, 89 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.28 (s, 1H), 8.22 (s, 1H), 7.90 (s, 1H), 6.00 (d, *J* = 10.8 Hz, 1H), 5.03 (d, *J* = 8.2 Hz, 1H), 4.74 (dd, *J* = 8.1, 4.8 Hz, 1H), 4.35 – 4.19 (m, 2H), 3.92 (d, *J* = 12.1 Hz, 1H), 3.86 – 3.65 (m, 1H), 2.99 (s, 1H), 0.76 (s, 9H), –0.20 (s, 3H), –0.36 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.28, 144.44, 142.90, 132.59, 127.23, 125.76, 86.70, 78.97, 77.91, 73.92, 63.60, 25.63, 17.94, –5.27, –5.30; ESI-MS calculated for $C_{17}H_{27}N_2O_5SSi$ $[M+H]^+$ 399.14, found 398.97; ESI-HRMS calculated for for $C_{17}H_{26}N_2O_5SSiNa$ $[M+Na]^+$ 421.1224, found 421.1225.

Synthesis of *O*$^{5'}$-Dimethoxytrityl-*O*$^{2'}$-TBDMS-thI (10)

9 (0.54 g, 1.35 mmol) was coevaporated with dry Py (2 × 3 mL) and dissolved in dry Py (7 mL). DMTrCl (0.55 g, 1.62 mmol) was added to the solution and stirred overnight at RT. The reaction was quenched by addition of MeOH (5 mL) and all volatiles were evaporated. The residue was coevaporated with EtOAc (10 mL) to remove residual pyridine and purified by column chromatography with 0 – 1 % gradient MeOH in DCM with 1 % Py to afford an off-white solid. Yield 0.82 g, 86 %.^{1}H NMR (500 MHz, $CDCl_3$) δ 9.80 (br, 1H), 8.28 (s, 1H), 7.76 (d, *J* = 3.2 Hz, 1H), 7.56 – 7.49 (m, 2H), 7.45 – 7.36 (m, 4H), 7.31 – 7.25 (m, 3H), 6.87 – 6.79 (m, 4H), 5.65 (d, *J* = 7.0 Hz, 1H), 4.54 – 4.46 (m, 1H), 4.24 – 4.17 (m, 1H), 4.17 – 4.10 (m, 1H), 3.79 (d, *J* = 0.9 Hz, 6H), 3.57 – 3.52 (m, 1H), 3.25 – 3.14 (m, 1H), 2.83 (d, *J* = 3.2 Hz, 1H), 0.81 (d, *J* = 2.9 Hz, 9H), –0.08 (s, 3H), –0.15 (d, *J* =

3.1 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.80, 158.52, 158.51, 145.85, 145.08, 136.23, 136.18, 135.96, 130.26, 128.30, 127.93, 126.85, 126.49, 126.29, 113.23, 113.20, 86.29, 84.54, 80.09, 73.03, 64.02, 55.32, 25.72, 18.10, –4.88, –5.26; ESI-MS calculated for $C_{38}H_{44}N_2O_7SSiNa$ $[M+Na]^+$ 723.25, found 723.13; ESI-HRMS calculated for $C_{38}H_{44}N_2O_7SSiNa$ $[M+Na]^+$ 723.2531, found 723.2527.

Synthesis of *O*$^{3'}$-(2-Cyanoethyldiisopropylphosphoramidite)-*O*$^{5'}$-dimethoxytrityl-*O*$^{2'}$-TBDMS-thI (11)

10 (0.82 g, 2.34 mmol) was coevaporated with dry Py (2 × 3 mL) and dissolved in dry DCM (11 mL). *N*,*N*-Diisopropylethylamine (1.0 mL, 5.85 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.52 mL, 2.34 mmol) were added successively to the solution at 0 °C followed by stirring at RT for 16 hour. All volatiles were evaporated, oily residue was treated with EtOAc (10 mL) and the resulting white precipitate, DIPEA•HCl salt, was filtered off. The filtrate was evaporated and the residue was purified by column chromatography with 0 – 60 % gradient EtOAc in Hexanes in presence of 1 % Py to afford a white solid. Yield 0.83 g, 79 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.17 (br, 2H), 8.27 (s, 1H), 8.26 (s, 1H), 7.75 – 7.67 (m, 2H), 7.56 – 7.50 (m, 2H), 7.46 – 7.36 (m, 6H), 7.33 – 7.25 (m, 8H), 7.25 – 7.15 (m, 2H), 6.87 – 6.76 (m, 8H), 5.71 (d, *J* = 8.0 Hz, 1H), 5.62 (d, *J* = 7.1 Hz, 1H), 4.53 – 4.46 (m, 2H), 4.36 (d, *J* = 2.7 Hz, 1H), 4.22 (d, *J* = 2.1 Hz, 1H), 4.18 (ddd, *J* = 6.3, 5.1, 3.1 Hz, 2H), 4.04 (dtd, *J* = 10.4, 7.3, 5.8 Hz, 1H), 3.97 – 3.88 (m, 1H), 3.81 – 3.76 (m, 12H), 3.62 – 3.45 (m, 6H), 3.11 (dt, *J* = 10.4, 3.5 Hz, 2H), 2.85 – 2.65 (m, 3H), 2.28 – 2.14 (m, 1H), 1.61 (d, *J* = 5.3 Hz, 6H), 1.14 (dt, *J* = 6.7, 4.2 Hz, 12H), 0.93 (d, *J* = 6.8 Hz, 6H), 0.77 (s, 9H), 0.74 (s, 9H), –0.04 (s, 3H), –0.12 (s, 3H), –0.19 (s, 3H), –0.22 (s, 3H); ^{31}P NMR (202 MHz, $CDCl_3$) δ 150.71, 149.07; ESI-MS calculated for $C_{47}H_{61}N_4O_8PSSiNa$ $[M+Na]^+$ 923.36, found 923.21; ESI-HRMS calculated for $C_{47}H_{61}N_4O_8PSSiNa$ $[M+Na]^+$ 923.3609, found 923.3611.

2.3. Oligonucleotide synthesis

Two 27-mer GluR-B R/G site RNAs were prepared: oligo **12** as an ADAR2 substrate and oligo **13** as an authentic product of the ARAR2 reaction.

12 5'-r-CAU UAth**A** GGU GGG UGG AAU AGU AUA ACA-3'

13 5'-r-CAU UAth**I** GGU GGG UGG AAU AGU AUA ACA-3'

Solid-phase oligonucleotide synthesis was performed on an Expedite 8909 synthesizer using commercially available reagents and phosphoramidites (2'-O-TOM, Glen Research). The modified phosphoramidite was chemically synthesized as described above and incorporated into oligonucleotide with coupling efficiency comparable to the commercially available phosphoramidites. The solution of the modified phosphoramidite was dried overnight over molecular sieve 3A (dried for 2 days at 300 ^{o}C under high vacuum) and was filtered using syringe filter right before use. Oligodeoxynucleotides were synthesized (with trityl-off) on a 500 Å CPG solid support column (1 µmol scale). Cleavage from the solid support and deprotection were accomplished with AMA (1.5 mL, 50:50 of $MeNH_2$ in 40 wt. % in water and con. NH_4OH at 65^{o}C for 1 h. The 2'-O-TOM group was removed by TEA·3HF at 65 ^{o}C for 3 h and the residue was desalted by precipitation (Glen Report 19-20, Glen Research). All oligonucleotides were purified by preparative polyacrylamide gel electrophoresis (PAGE) using the crush and soak method; the desired band was cut out, pulverized, extracted with 50 mM TEAA (pH 7.0) for a minimum of 12 h (while shaking) and decanted. The buffer containing the purified oligonucleotide was lyophilized and the residue was taken up in 0.2 M TEAB (pH 7.0) buffer and desalted on a Sep-pek C-18 (Waters). The oligonucleotides were eluted with 40 v% acetonitrile in water. The purified oligonucleotides were quantified by UV absorbance at 260 nm at 70 °C and their identity confirmed by MALDI-TOF mass spectrometry. Oligonucleotide concentration was then determined using Beer's Law with the following extinction coefficients: rCMP, 7200; rUMP, 9900; rGMP, 11500; rAMP, 15400; th**A**, 7106, and th**I** 2068 and then th**A**-oligonucleotide (**12**) = 321,406 L·mol^{-1}·cm^{-1} and th**I**-oligonucleotide (**13**) = 316,368 L·mol^{-1}·cm^{-1}

2.3.1. Oligonucleotides; MALDI-TOF MS

The MW of the modified oligonucleotides was determined via MALDI-TOF MS. An aliquot of 400 pmol of the oligonucleotide was lyophilized and then dissolved in 1 uL of water and combined with 1 µL of 100 mM ammonium citrate buffer (PE Biosystems), and 4 µL of saturated 3-hydroxypicolinic acid. The sample was spotted onto a gold-coated plate and air dried. MALDI-TOF spectra were recorded on a PE Biosystems Voyager-DE STR MALDI-TOF spectrometer in negative-ion, delayed-extraction mode.

Table S1. Oligonucleotides MALDI-TOF-MS Data

Oligonucleotide	MS calculated	MS found
12	8761.1	8764.4
13	8762.1	8763.1

2.4. Biochemical Methods

2.4.1. General

Unless otherwise stated, reagents were purchased from Fisher Scientific, Sigma Aldrich or Life Technologies. T4 polynucleotide kinase and RNase inhibitor were purchased from New England Biolabs. γ-[^{32}P]ATP was purchased from Perkin-Elmer Life Sciences. AMV RT and dNTP mix were purchased from Promega. GluR B R/G 27 nt DNA and Primer were purchased from Integrated DNA Technologies. The human ADAR2 – RD protein was purified as previously described.[4] Storage phosphor imaging plates from Molecular Dynamics were imaged using Molecular Dynamics 9400 Typhoon phosphorimager. Fluorescent assays were performed on the Horiba Scientific, Fluorolog-3. Data was analyzed using Molecular Dynamics ImageQuant 5.2 software.

2.4.2 Sequences of oligonucleotides

Unmodified GluR B R/G 27 nt top strand: 5’ - CAU UAA GGU GGG UGG AAU AGU AUA ACA - 3’; GluR B R/G 27 nt bottom strand: 5’ - UGU UAU AGU AUC CCA CCU ACC CUG AUG - 3’; GluR B R/G 27 nt DNA: 5’ – CAT CAG GGT

AGG TGG GAT ACT ATA ACA – 3'; Primer used for primer extension: 5' - TGT TAT ACT ATT CCA CCC ACC – 3'.

2.4.3 Oligonucleotide synthesis and purification

GluR B R/G 27 nt DNA was desalted by the manufacturer.

2.4.4 ^{32}P labeling of oligonucleotides

The primer was labeled as previously described previously.[5]

2.4.5 ADAR-catalyzed deamination kinetics with modified RNA

The GluR B R/G 27 nt top strand was hybridized to the GluR B R/G 27 nt bottom strand by dissolving the RNAs in buffer containing 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA and 100 mM NaCl at a concentration of 20 μM for the top strand and 30 μM for the bottom strand. The samples were then heated at 95 °C for 5 min and allowed to slowly cool to room temperature. Deamination reactions were carried out as described previously.[4] RNA was incubated at 30 °C for 30 min prior to the addition of hADAR2 - RD. hADAR2 - RD was added and the reaction was stopped at various time points (3, 5, 10, 30, 60, and 120 min for the fluorescent assay and 3.33, 5.33, 10.33, 30.33, 60.33, and 120.33 min for the poisoned primer extension assay) at 30 °C. For the poisoned primer extension assay, reactions were stopped by diluting the reaction tenfold and then incubating at 95 °C for 5 min. Samples were phenol-chloroform extracted and ethanol precipitated. A 70% ethanol wash was carried out and the samples were lyophilized to dryness. Each RNA (≤ 0.1 pmol) was resuspended in 1x Promega AMV RT buffer and ≤ 2 pmol primer was added. The resulting solution was incubated at 62 °C for 15 min. Extent of editing was evaluated using AMV RT-catalyzed ddNTP incorporation (see below). For the fluorescence assay, reactions were stopped by the addition of an equal volume of 20 μM GluR B R/G 27 nt DNA. The solution was incubated at 95 °C for 5 min and then placed on ice.

2.4.6 Poisoned primer extension assay by AMV RT-catalyzed ddNTP incorporation

Assay was performed as previously described[5] except concentrations in the reaction were 10 nM RNA, 200 nM ^{32}P labeled primer, 10 μM of the 3 dNTPs, 10 μM of the appropriate ddNTP, 1X Promega AMV RT buffer and 5 units AMV RT.

2.5 Fluorescence Assay

Samples for fluorescence analysis were prepared in 10 mM Tris-HCl, pH 7, 8 mM NaCl, 2% glycerol, 30 μM 2-mercaptoethanol, 0.3 mM DTT, 30 mM KCl, 0.75 mM EDTA, 0.0015% NP-40, 80 units/mL RNase inhibitor, 0.5 ug/mL tRNA, 1 μM RNA, 10 μM DNA, and 1 μM hADAR2-RD. A Horiba Fluoromax-3 was set with excitation at 380 nm with a slit width of 5 nm and emission from 395 nm – 600 nm with a slit width of 5 nm at 30 °C. Samples were normalized to the fluorescence intensity of the thI-containing duplex. Fraction Intensity (FI) at 417 nm was graphed vs deamination reaction time where FI = ($FI_{417\ nm}$ 0 min - $FI_{417\ nm}$) / ($FI_{417\ nm}$ 0 min). Data were fitted to the equation: $[P]_t = \alpha[1-\exp(-k_{obs} \bullet t)]$, where $[P]_t$ is the fraction edited at time t, α is the fitted reaction endpoint and k_{obs} is the fitted rate constant using KaleidaGraph. Each experiment was carried out in triplicate and the rate constants reported in the text are average values ± standard deviations.

2.6. Fluorescence Lifetime Measurements

Nucleosides samples for the fluorescence lifetime analysis were prepared in bidistilled water at pH 6.9 for thA to a final concentration of 9.5 and 0.95 μM (Sol-1 and Sol-2) and for thI 11.3 and 1.13 μM (Sol-3 and Sol-4). GluR B R/G site single stranded RNA containing thA solutions were prepared at 1 μM concentration in ADAR2 buffer (Sol-5), with addition of 0.3 mM of DTT (Sol-6) and in bidistilled water at pH 6.9 (Sol-7). The same buffered solutions without (Sol-8) and with 0.3 mM DTT (Sol-9) were prepared with GluR B R/G site single stranded RNA containing thI at 1 μM concentration. The fluorescence decay traces were recorded at 30°C on a PTI luminescence upon excitation with a LED

light source at 340 nm using slits-widths of 8 mm (equal 32 nm) and an acquisition delay time at 50 ns. The thA and thI nucleosides and containing oligonucleotides were monitored at 417 and 390 nm respectively. IRF scatter curves were measured using the same instrumental settings. The decay curves were fitted to a mono-exponential decay function with deconvolution of the IRF using PTI's Felix32 software from which the fluorescence lifetimes were extracted. The sample temperature was controlled with a Quantum Northwest TLC50 fluorescence cuvette holder in conjunction with a software controllable TC 125 temperature controller. Steady state fluorescence experiment was also carried out with the same nucleosides and oligonucleotides solutions on the same instrument with the excitation wavelength set at 340 nm and using slits-widths of 1 mm (equal to 4 nm) for the excitation and 0.5 mm (equal to 2 nm) for the emission.

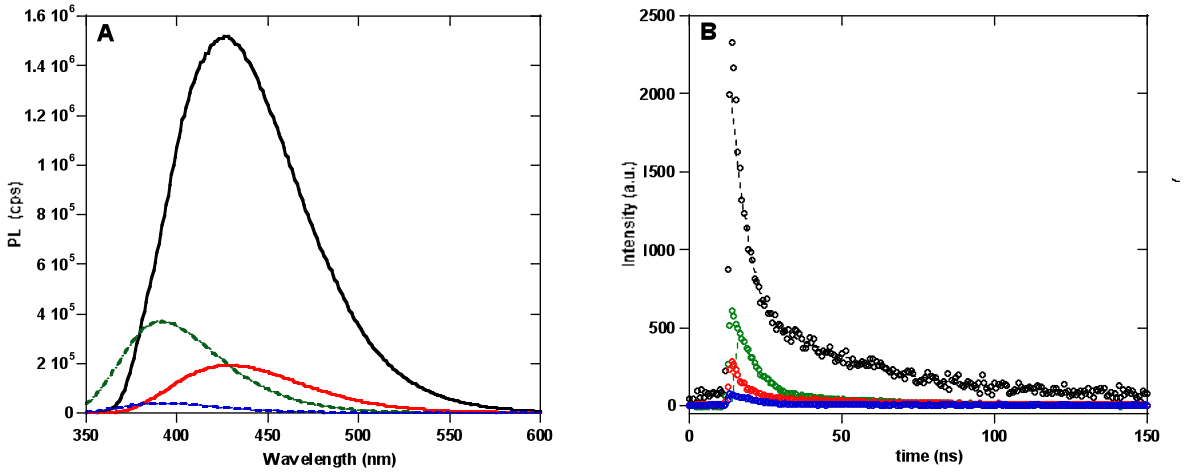


Figure S1. Emission spectra (A) and fluorescence lifetime traces (B) of Sol-1 (black), Sol-2 (red) Sol-3 (green), and Sol-4 (blue).

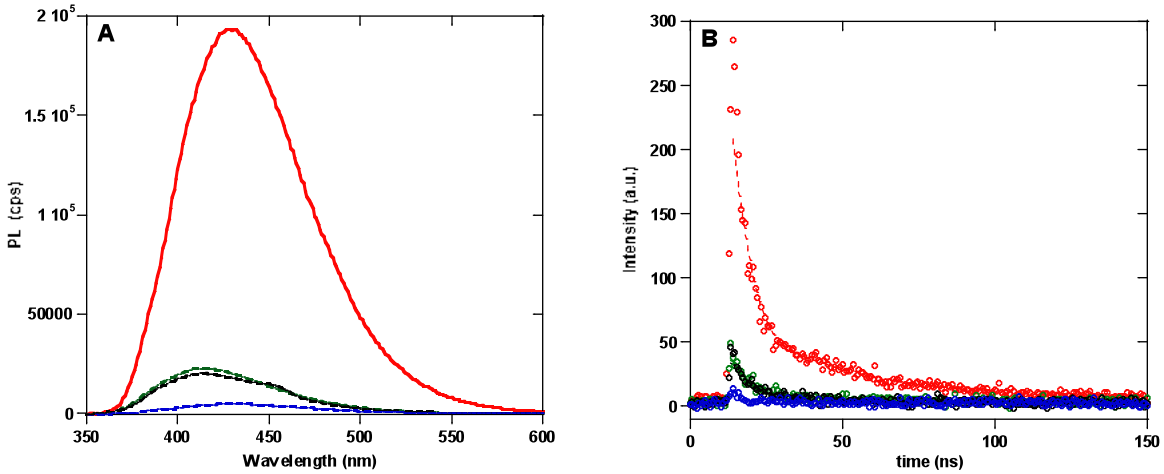


Figure S2. Emission spectra (A) and fluorescence lifetime traces (B) of Sol-2 (red), Sol-5 (green), Sol-6 (black) and Sol-7 (blue).

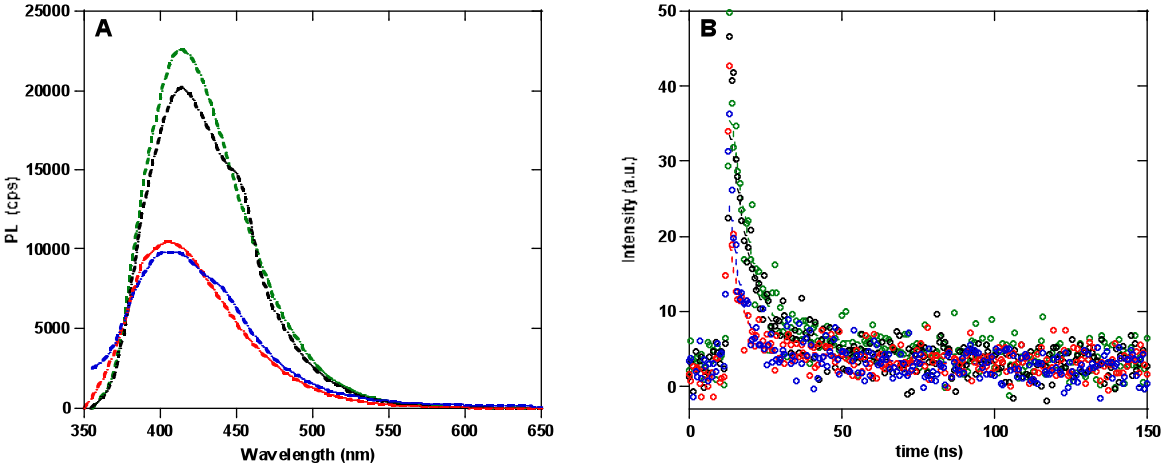


Figure S3. Emission spectra (A) and fluorescence lifetime traces (B) of Sol-5 (green), Sol-6 (black) Sol-8 (red) and Sol-9 (blue).

Table S2. Fluorescence lifetime values of nucleoside and oligonucleotides solutions

Solutions	τ [1] /(ns)	χ^2 [1]	τ_{Sol}/τ_{Sol} [2]	F_{Sol}/F_{Sol} [2]
Sol-1	5.74±0.28	0.858	-	-
Sol-2	6.05±0.32	1.005	-	-
Sol-3	7.07±0.10	1.207	(1/3) 0.81	(1/3) 4.12
Sol-4	6.85±0.47	0.760	(2/4) 0.88	(2/4) 4.69
Sol-5	6.71±0.02	0.643	(2/5) 0.91	(2/5) 8.48
Sol-6	6.34±0.76	0.750	(2/6) 0.95 (5/6) 1.06	(2/6) 9.54 (5/6) 1.12
Sol-7	2.33±0.33	1.100	(2/7) 2.60	(2/7) 39.94
Sol-8	3.79±0.02	0.726	(4/8) 1.81 (5/8) 1.77	(4/8) 3.77 (5/8) 2.14
Sol-9	3.71±0.87	0.785	(4/9) 1.84 (6/9) 1.71	(4/9) 4.03 (6/9) 2.05

[1] Values from the mono-exponential fit of the fluorescence decay traces. [2] Fluorescence lifetime and intensity ratio.

2.7 Computations

Computations were carried out as described in [4]: “Hydration free energies (purine analogue + water → C6-hydrated purine analogue) in water were calculated using the CPCM-B3LYP/6-31+G(d,p) method. Geometries of all structures were fully optimized in solvent, and UA0 radii and a dielectric constant of 78.39 were used in all calculations. The GAUSSIAN program suite was used for all calculations.” Data for the systems examined herein:

thA.log

After PCM corrections, the SCF energy is -778.067425897 a.u.

Zero-point correction= 0.117019 (Hartree/Particle)
Thermal correction to Energy= 0.125213
Thermal correction to Enthalpy= 0.126158
Thermal correction to Gibbs Free Energy= 0.083316
Sum of electronic and zero-point Energies= -777.950407
Sum of electronic and thermal Energies= -777.942213
Sum of electronic and thermal Enthalpies= -777.941268
Sum of electronic and thermal Free Energies= -777.984110

thAh2o.log

After PCM corrections, the SCF energy is -854.520791717 a.u.

Zero-point correction= 0.143248 (Hartree/Particle)
Thermal correction to Energy= 0.153330
Thermal correction to Enthalpy= 0.154274
Thermal correction to Gibbs Free Energy= 0.107415
Sum of electronic and zero-point Energies= -854.377544
Sum of electronic and thermal Energies= -854.367462
Sum of electronic and thermal Enthalpies= -854.366517
Sum of electronic and thermal Free Energies= -854.413376

difference = 76.429266

parent 9-methylpurine (**8**) from energies in JCC SI...

8... Sum of electronic and thermal Free Energies= -451.212731
8 hydrate... Sum of electronic and thermal Free Energies= -527.628442

difference = 76.415711

ΔΔ = 0.013555 * 627.51 = 8.5 kcal/mol more likely to hydrate than 8

Coordinates:

thA

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	6	0	-0.138311	0.475586	-0.000049
2	6	0	-0.528122	-0.919316	0.000012
3	6	0	-1.927022	-1.186981	0.000029
4	6	0	-2.333078	1.082048	0.000003
5	6	0	1.239002	0.662138	-0.000033
6	6	0	0.555220	-1.781253	0.000019
7	1	0	-2.297952	-2.215104	0.000019
8	1	0	-3.101965	1.855402	0.000032
9	1	0	0.568333	-2.867418	-0.000047
10	6	0	2.001701	1.950861	0.000054
11	1	0	2.641282	2.047690	-0.884788
12	1	0	1.291474	2.781559	-0.000063
13	1	0	2.641062	2.047730	0.885047
14	16	0	2.020311	-0.886242	-0.000011
15	7	0	-1.084070	1.475865	-0.000055
16	7	0	-2.813580	-0.214221	0.000021

thAh20

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z

1	6	0	0.274985	0.609046	0.013191
2	6	0	-0.385852	-0.654325	-0.209850
3	6	0	-1.885629	-0.720018	-0.248720
4	6	0	-1.689061	1.751931	-0.170058
5	6	0	1.649876	0.494522	0.097256
6	6	0	0.481225	-1.704761	-0.307427
7	1	0	-2.279731	2.670290	-0.180173
8	1	0	0.261862	-2.757578	-0.458671
9	6	0	2.659418	1.581022	0.309922
10	1	0	3.339001	1.683869	-0.544841
11	1	0	2.136059	2.531296	0.442981
12	1	0	3.274478	1.401411	1.199743
13	16	0	2.124611	-1.174411	-0.115787
14	7	0	-0.417300	1.825022	0.091539
15	7	0	-2.384736	0.624476	-0.491598
16	1	0	-2.252937	-1.374506	-1.050178
17	8	0	-2.348657	-1.241282	1.009550
18	1	0	-3.308066	-1.450108	0.921924
19	1	0	-3.390705	0.725164	-0.660057

3. Supporting References

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