Supporting Information

General.

All reagents were obtained from commercial sources and were used without further purification unless noted otherwise. Glassware for all reactions was oven dried at 125 ^{o}C overnight and cooled in a dessicator prior to use. Reactions were carried out under an atmosphere of dry nitrogen or argon. Liquid reagents were introduced by oven-dried microsyringes. To monitor the progress of reactions, thin layer chromatography (TLC) was performed with Merck silica gel 60 F254 precoated plates, eluting with the solvents indicated. Yields were calculated for material that appeared as a single spot by TLC and homogenous by ^{1}H NMR. Short and long wave visualization was performed with a Mineralight multiband ultraviolet lamp at 254 and 365 nanometers. Flash column chromatography was carried out using Mallinckrodt Baker silica gel 150 (60-200 mesh.) ^{1}H (300 MHz), ^{13}C (75 MHz), ^{19}F (282 MHz) and ^{31}P (121 MHz) Nuclear Magnetic Resonance spectra were acquired on a Varian VXR-300 spectrometer. Chemical shifts for proton and carbon NMR are reported in parts per million in reference to the solvent peak. Chemical shifts for phosphorus NMR are reported in parts per million using 85% phosphoric acid as an external standard. Chemical shifts for fluorine NMR are reported in parts per million using trifluoromethyltoluene (-63.7 relative to $CFCl_3$) as an external standard. The abbreviations s, d, dd, t, td, q, m, and brs stand for singlet, doublet, doublet of doublets, triplet, triplet of doublets, quartet, multiplet, and broad single, in that order. High resolution, FAB, and EI mass spectra were recorded on a Finnigan MAT 95 mass spectrometer, and in each case a $[M]^{+}$ or $[M+H]^{+}$ peak was found. Distilled, deionized water was used for all aqueous reactions and dilutions. Biochemical reagents were obtained from Sigma/Aldrich unless otherwise noted. Oligonucleotides were prepared on a Perkin-Elmer/ABI model 392 DNA/RNA synthesizer with β-cyanoethyl phosphoramidites. Protected adenosine, guanosine, cytidine and uridine phosphoramidites were purchased from Glen Research.

2',3',5'-Tri-*O*-acetyl-6-(trifluoromethyl)-9-β-D-ribofuranosyl)-purine (2).

Method A. A mixture of $FSO_2CF_2CO_2Me$ (MFSDA 393.9 mg, 0.2.05 mmol), CuI (374.8 mg, 0.1.97 mmol), HMPA (367.4 mg, 2.05 mmol) and 6-bromo purine derivative (**1**) (750 mg, 1.64 mmol) in anh. DMF (20 mL) was stirred for 13 h at 70 ^{o}C. The reaction mixture was then cooled to RT, 50 mL of EtOAc/Hexanes (7:3) were added and the mixture was successively washed with sat. aq. NH_4Cl (1 x 30 mL), sat. aq. $NaHCO_3$ (1 x 30 mL), water (1 x 30 mL) and brine (1 x 30 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Flash chromatography (2% MeOH/$CHCl_3$) afforded the title compound (664.4 mg, 91%) as a light yellow foam. Spectroscopic data agreed with reported values.[1]

Method B. A two-necked flask containing zinc powder (115.8 mg, 1.77 mmol), CF_3I (289.2 mg, 1.48 mmol) in anh. DMF (10 mL) and then equipped with a dry ice-acetone reflux condenser was stirred at 70 ^{o}C for 10 min (or until almost all zinc had reacted). Then, CuI (134.9 mg, 0.709 mmol) was added followed by the 6-bromo derivative (**1**)

(270 mg, 0.59 mmol) in DMF and the mixture was stirred at 70 ^{o}C for another 10 h. Work-up and purification was similar to above procedure. The title compound was obtained in 96% (253 mg).

9-(β-D-Ribofuranosyl)-6-(trifluoromethyl) purine (3).

A solution of 6-trifluoromethyl triacetate (**2**) (330 mg, 0.739 mmol) in methanolic ammonia (5 mL) was stored at 4 ^{o}C overnight. The mixture was concentrated under reduced pressure and purified by flash column chromatography (10% MeOH/$CHCl_3$) to afford the title compound (228.5 mg, 97%) as a white foam. Spectroscopic data agreed with reported values.[1]

5'-*O*-(4,4'-Dimethoxytrityl)- 9-(β-D-ribofuranosyl)-6-(trifluoromethyl) purine.

To a solution of (**3**) (440 mg, 1.37 mmol) in freshly distilled THF (25 mL) was added sequentially anh. pyridine (1.30 g, 16.5 mmol) 4,4'-dimethoxytrityl chloride (512.1 mg, 1.51 mmol) and $AgNO_3$ (256.7 mg, 1.51 mmol). The reaction mixture was stirred at RT overnight. The mixture was filtered and concentrated under reduced pressure to yield a light amber oil. The oil was redissolved in EtOAc (20 mL) and washed with 5% aq. $NaHCO_3$ (1 x 40 mL) and brine (1 x 30 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification by flash column chromatography (MeOH/TEA/CH_2Cl_2 1:1:98) afforded the 5'-*O*-DMT protected purine derivative as a pale yellow foam (842.2 mg, 98%). ^{1}H-NMR (CD_2Cl_2, 300 MHz): δ (ppm) 9.02 (s, 1H), 8.58 (s, 1H), 7.40 (dd, *J* = 7.5, 1.2 Hz, 2H), 7.30 (d, *J* = 9 Hz, 4H), 7.27-7.21 (m, 3H), 6.80 (d, *J* = 9 Hz, 4H), 6.20 (d, *J* = 4.5 Hz, 1H), 4.92 (t, *J* = 5.1 Hz, 1H), 4.59 (t, *J* = 4.8 Hz, 1H), 4.41 (q, *J* = 3.6 Hz, 1H), 3.78 (s, 6H), 3.51 (dd, *J* = 10.8, 3.6 Hz, 1H), 3.44 (dd, *J* = 10.8, 3.9 Hz). ^{13}C NMR (CD_2Cl_2, 75 MHz): δ (ppm) 159.2, 153.7, 152.2, 147.0, 145.4 (q, ^{2}J = 37 Hz), 145.1, 136.0. 135.9, 131.3, 130.5, 128.4, 128.4, 127.4, 121.2 (q, *J* = 273.8 Hz), 113.6, 90.8, 87.2, 85.7, 75.7, 72.1, 63.8, 55.7. FAB-HRMS: calcd for $C_{32}H_{30}F_3N_4O_6$ 623.2117, obsd 623.2103.

5'-*O*-(4,4'-Dimethoxytrityl)-2'-*O*-(*t*-butyldimethylsilyl)-9-(β-D-ribofuranosyl)-6-(trifluoromethyl) purine.

The 5'-O-DMT protected purine derivative (250 mg, 0.402 mmol) was silylated using the method of Olgilvie.[2] Purification by flash column chromatography (EtOAc/hexanes 1:4) afforded 164.3 mg (56%) of the 2'-silylated product as a white foam and 76.4 mg of the 3'-silylated derivative. Total yield of the reaction: 81%. ^{1}H-NMR (CD_2Cl_2, 300 MHz): δ (ppm) 9.01 (s, 1H), 8.51 (s, 1H), 7.47 (dd, *J* = 7.8, 1.2 Hz, 2H), 7.36 (d, *J* = 9 Hz, 4H), 7.33-7.25 (m, 3H), 6.84 (d, *J* = 9 Hz, 4H), 6.18 (d, *J* = 4.8 Hz, 1H), 5.33 (t, *J* = 1.2 Hz, 1H), 5.00 (t, *J* = 4.8 Hz, 1H), 4.42 (q, *J* = 4.5 Hz, 1H), 4.3 (q, *J* = 3.6 Hz, 1H), 5.51 (dd, *J* = 10.8, 3 Hz, 1H), 3.79 (s, 6H), 3.45 (dd, *J* = 10.8, 3.9 Hz, 1H), 2.66 (d, *J* = 4.8 Hz, 1H), 0.87 (s, 9H), 0.04 (s, 3H), -0.09 (s, 3H). ^{13}C NMR (CD_2Cl_2, 75 MHz): δ (ppm) 159.3,

[1] Kobayashi, Y.; Yamamoto, K.; Asai, T.; Nakano, M.; Kumadaki, I. *J. Chem. Soc., Perkin Trans. 1* **1980**, 2755-2761.
[2] Hakimelahi, G.H.; Proba, Z.A.; Ogilvie, K.K. *Can. J. Chem.* **1982**, *60*, 1106-1113.

154.0, 152.5, 146.8, 145.2, 136.1, 136.0, 130.6, 128.6, 128.5, 127.5, 113.7, 89.4, 87.3, 84.9, 76.3, 71.9, 63.8, 55.7, 25.8, 18.3, -4.7, -4.8. FAB-HRMS: calcd for $C_{38}H_{44}F_3N_4O_6Si$ 737.2982, obsd 737.3003.

5'-*O*-(4,4'-Dimethoxytrityl)-3'-*O*-[(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphino]-2'-*O*-(*t*-butyldimethylsilyl)-9-(β-D-ribofuranosyl)-6-(trifluoromethyl) purine (4).

The phosphoramidite formation was performed according to standard procedure with the 5'-DMT-2'-TBDMS protected purine derivative (195.0 mg, 0.265 mmol in 3 mL of anhydrous THF) and 2-cyanoethyldiisopropylphosphoramidochloridite to afford, after flash column purification (EtOAc/hexanes 1:4), 146.6 mg (80%) of the titled compound.[3] ^{31}P-NMR (121 MHz, CD_2Cl_2, 85% H_3PO_4 as external standard): δ (ppm) 150.7, 149.4. FAB-HRMS: calcd for $C_{47}H_{61}F_3N_6O_7SiP$ 937.40607, obsd 937.40882.

Preparation of ^{TFM}P-substituted RNA samples for Mass Spectral Analysis.

Deprotection of synthetic oligoribonucleotides was carried out in a 3:1 solution of ammonium hydroxide in ethanol for 24 h at RT followed by triethyl amine 3●HF for 24 h at RT. Deprotected oligonucleotides were desalted with a Sep Pac column and redissolved in 50:50 isopropanol:0.1 M ammonium acetate. ESI MS samples were directly infused into the mass spectrometer. MALDI samples were added to 6-aza-2-thiothymine (ATT matrix) and ammonium citrate co-matrix. ATT matrix was prepared by mixing 0.04 g 6-aza-2-thiothymine to 500 µl 50 % acetonitrile. Ammonium citrate co-matrix solution was prepared by dissolving 0.023 g of ammonium citrate in 1 mL water. 0.5 µl of a ~10 pmol/L RNA sample was spotted onto a gold plated sample well and allowed to air dry. First a 0.5 µl aliquot of co-matrix solution was added to the dry sample and allowed to air dry. A 0.5 µl of matrix solution was then added to the sample/co-matrix in the sample well. Once the sample was dry, the MALDI probe was introduced into the mass spectrometer.

ESI Mass Spectrometery.

ESI spectra were obtained on a Micromass Quatro II triple quadrapole mass spectrometer. Samples were directly infused at a rate of 0.3 ml/min. Capillary voltage was set at 3 kV; cone voltage was set at 45 V; extractor voltage was set at 5 V, and desolvation temperature was set at 120 ^{o}C. Data was collected from a range of 400-2000 m/z. Data was analyzed by Mass Lynx software version 3.4.

MALDI Mass Spectrometry.

MALDI spectra were obtained on a reflectron MALDI/TOF mass spectrometer (Model: Perseptive Voyager-DE STR) equipped with a time lag focusing (delayed extraction) ion source, and a pulsed linear detector. Nitrogen laser at 337 nm (3 ns pulse width) was

[3] Easterwood, L.M.; Véliz, E.A.; Beal, P.A. *J. Am. Chem. Soc.* **2000**, 122, 11537-11538.

used to desorb the ions in the source region. Each laser shot produced a mass spectrum. Experiments were carried out under linear mode with delayed extraction at an acceleration voltage of 25 kV. Ion detection and signal amplification was achieved through a hybrid conversion microchannel plate-discrete dynode electron multiplier assembly. The amplified signal was visually monitored with a four channel digital oscilloscope (Model: Tektronix TDS 540C) and stored as time-of-flight data on a Micron datastation. The time of flight data were either externally calibrated or mass converted using ion peaks of known masses. Background pressure within the instrument was less than 1 x 10^{-7} Torr as measured by a Bayard-Alpert ion gauge below the source.

Preparation of Duplex RNAs.

Six 12mer RNAs were synthesized with the following sequences, 5'-CAUUAXGGUGGG-3' where X = A or ^{TFM}P and 5'-CCUACCYUGAUG-3' where Y = A, G, C, or U. The oligonucleotides were deprotected and purified by PAGE as previously described.[4] The 12mers were visualized on the gel by UV shadowing and extracted by the crush and soak method with 0.5 M NH_4OAc, 0.1% SDS, 0.1 mM EDTA. The oligonucleotides were ethanol precipitated and redissolved in deionized water. Concentrations were determined by the UV absorbance at 260 nm using extinction coefficients calculated based on the nearest-neighbor approximation. The identity of the ^{TFM}P-containing 12 mer was confirmed by mass spectrometry. Duplexes were formed by hybridizing 325 pmoles of complementary strands in TE buffer (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA) with 50 mM NaCl. The mixture was heated at 95 $^{\circ}$C for 5 min and allowed to slow-cool overnight to RT. These duplexes were directly used in Tm analyses.

Tm analysis.

Tm experiments were performed on a Beckmann DU 7400 Spectrophotometer with a temperature controller appendage. Duplexes were denatured and annealed from a temperature range from 10 ^{o}C to 75 ^{o}C and the absorbance at 260 nm was recorded for both the forward and reverse experiment. The fraction of oligonucleotides in a duplex (*f*) was determined by fitting the data to the equation:

$$f = \frac{A - Ass}{Ads - Ass}$$

A = Absorption of sample at each temperature
A_{ds}= Absorption of double stranded oligo
A_{ss}= Absorption of single stranded oligo

The *f* vs. temperature was graphed for a range of values near the Tm (or where the *f* is approximately 0.5). A linear regression was performed and the Tm is determined from the point on the line where *f* =0.5. The values reported represent the average of three forward and reverse experiments ± standard deviation.

[4] Stephens, O.M.; Yi-Brunozzi, H.-Y.; Beal, P.A. *Biochemistry*, **2000**, 39, 12243-12251.