Supporting Information

A Single-Label Phenylpyrrolocytidine Provides a Molecular Beacon-Like Response Reporting HIV-1 RT RNase H Activity

Alexander S. Wahba, Abbasali Esmaeili, Masad J. Damha, Robert H.E. Hudson*

robert.hudson@uwo.ca

Content

1. General Methods

Chemicals and solvents were ACS grade or higher and were purchased from Sigma-Aldrich or Thermo-Fisher. Anhydrous pyridine was obtained by distilling over calcium hydride. All reagents used for oligonucleotide synthesis, including 2'-deoxyribonucleotide and 2′-silylribonulceotide phosphoramidites, were purchased from Chemgenes Corp. Autoclaved Millipore water treated with diethyl pyrocarbonate (DEPC) was used to manipulate RNA and prepare aqueous buffers.

2. Synthesis and Gel purification of oligonucleotides

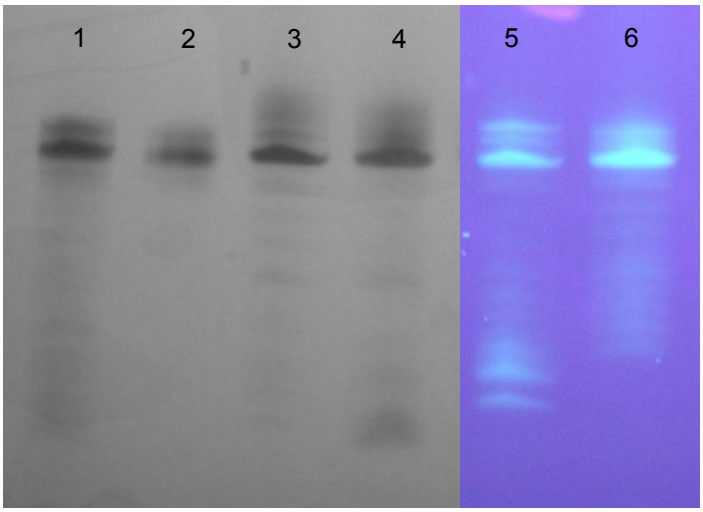


Figure S1. 24 % denaturing analytical PAGE of RNA strands used in the current study. Compound designations are the same as given in Table 1. Lane 1, crude RNA-1; Lane 2, purified RNA-1; Lanes 3 and 5 crude PhpC-1; Lanes 4 and 6 crude PhpC-2. Lanes 1 to 4 are visualized by UV shadowing with 260 nm light, lanes 5 and 6 were visualized using 365 nm light using the fluorescence of PhpC.

3. Mass Spectral Analysis of Oligonucleotides

The mass of oligonucleotides was determined by LC-MS ESI-TOF.

Entry	Name	Sequence (5' to 3')	Mass calculated	Mass measured
1	PhpC	***C**	343.2	366.2 (Na+)
2	5'-PO_4-PhpC	PO_4-***C**	423.2	423.1
3	PhpC-5	G***C**U	994.7	995.6 (H+)
4	PhpC-6	C***C**U	954.7	977.2 (Na+)
5	PhpC-7	U***C**U	955.7	956.07 (H+)
6	PhpC-1	GAU CUG AGC CUG GGA G***C**U	5887.6	5910.5 (Na+)
9	PhpC-2	GAU CUG AGC ***C**UG GGA GCU	5887.6	5887.4
12	RNA-1	GAU CUG AGC CUG GGA GCU	5787.4	5787.8
13	DNA-1	agc tcc cag gct cag atc	5444.6	5444.1
14	3'-Fluorescein RNA	GAU CUG AGC CUG GGA GCU-Fluorescein	6357.0	6352.9
15	5'-Dabcyl DNA	Dabcyl-agc tcc cag gct cag atc	5875.0	5874.9

Table S1. Sequences and MS data for PhpC and control oligonucleotides. Legend: RNA, dna, PhpC = ***C.**

4. Thermal Denaturation curves

1 µM samples were measured in 10 mM phosphate buffer (pH 7.0) and 50 mM NaCl. See Materials and Methods section for sample preparation.

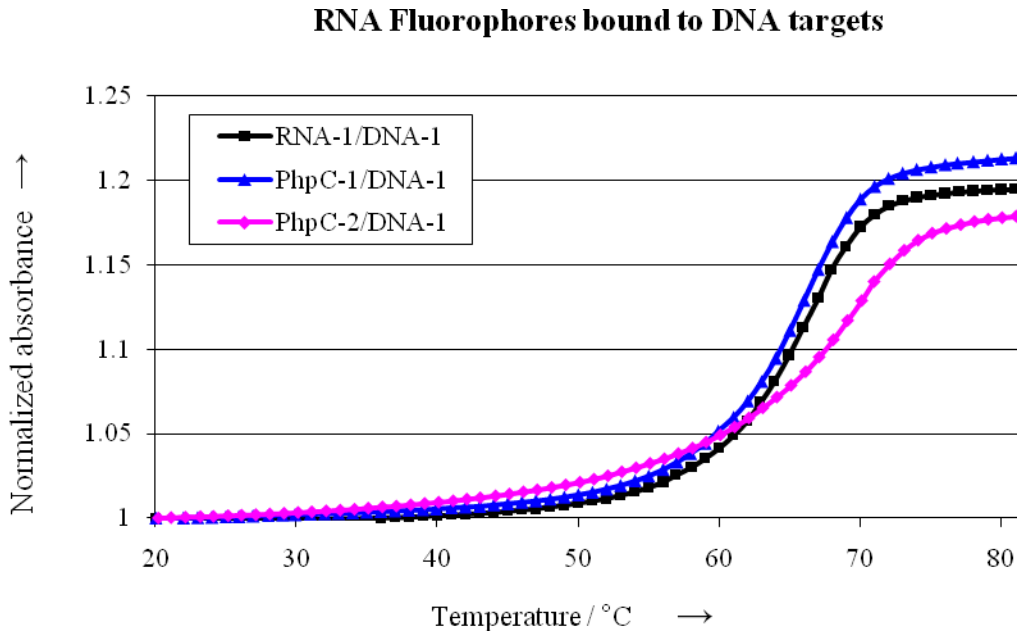


Figure S2. Thermal denaturation curves monitored by UV_{260} of RNA containing PhpC bound to their complementary DNA.

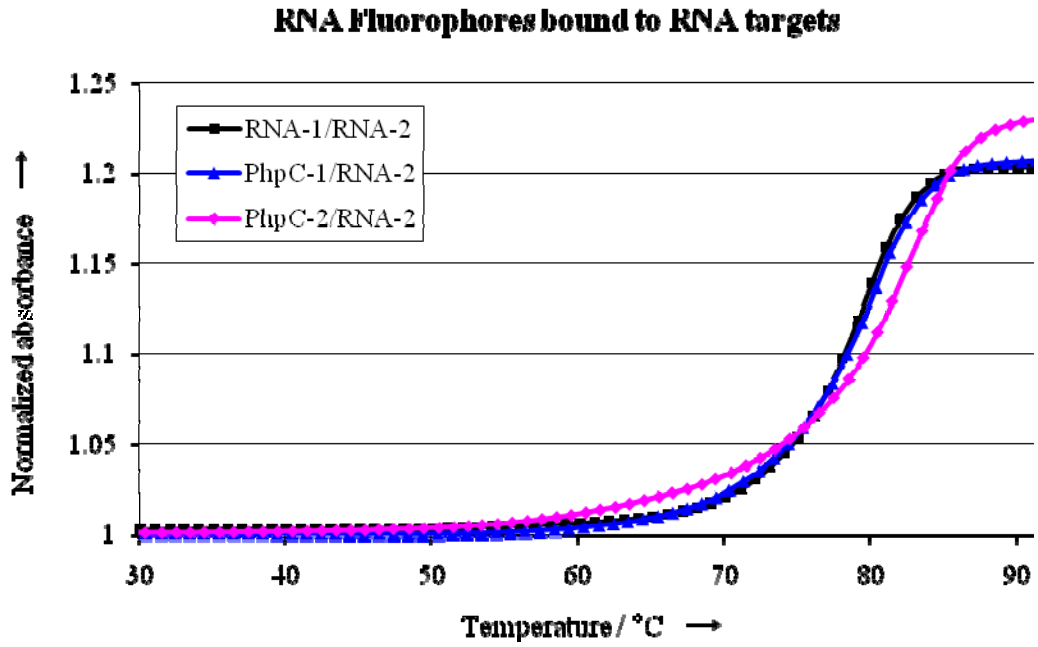


Figure S3. Thermal denaturation curves monitored by UV_{260} of RNA containing PhpC bound to their complementary RNA.

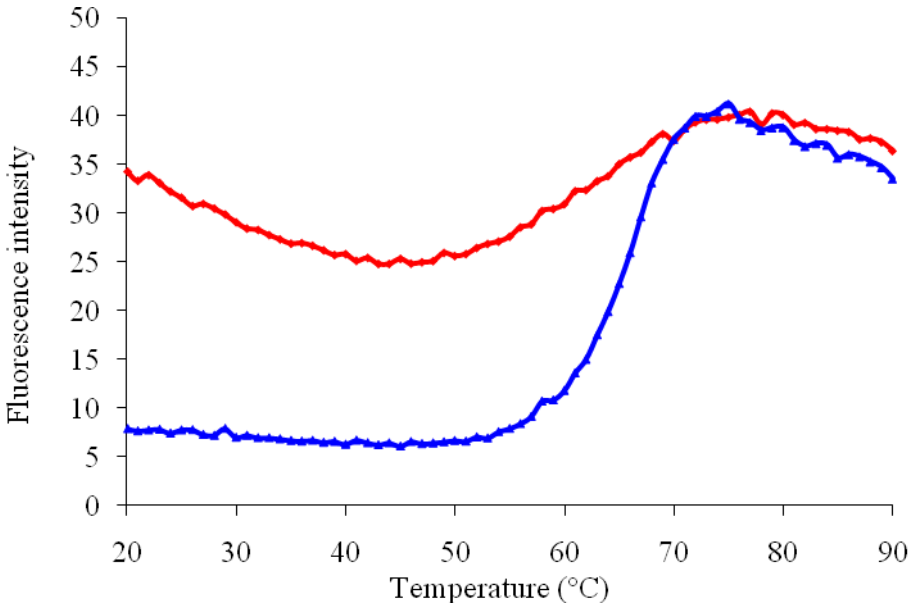


Figure S4. Thermal denaturation curves of PhpC-1single stranded (red diamonds) and PhpC-1 bound to its DNA complement (blue triangles) monitored by fluorescence. λ_{ex} = 360 nm, λ_{em} = 465 nm.

5. Circular Dichroism Spectra

1 μM samples were measured in 10 mM phosphate buffer (pH 7.0) and 50 mM NaCl. See Materials and Methods section for sample preparation.

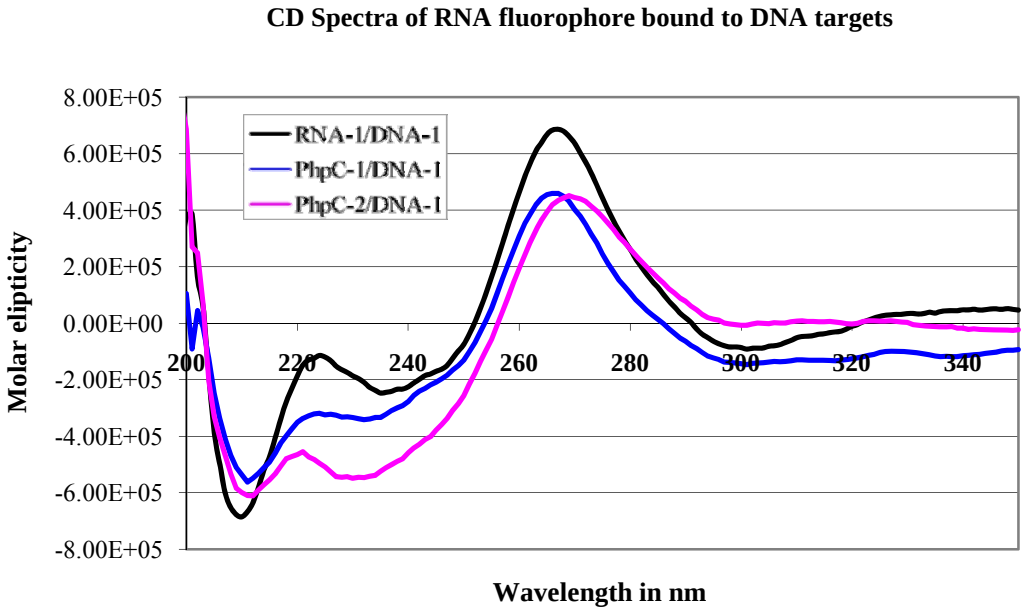


Figure S5. Circular dichroism spectra of RNA containing PhpC bound to their complementary DNA.

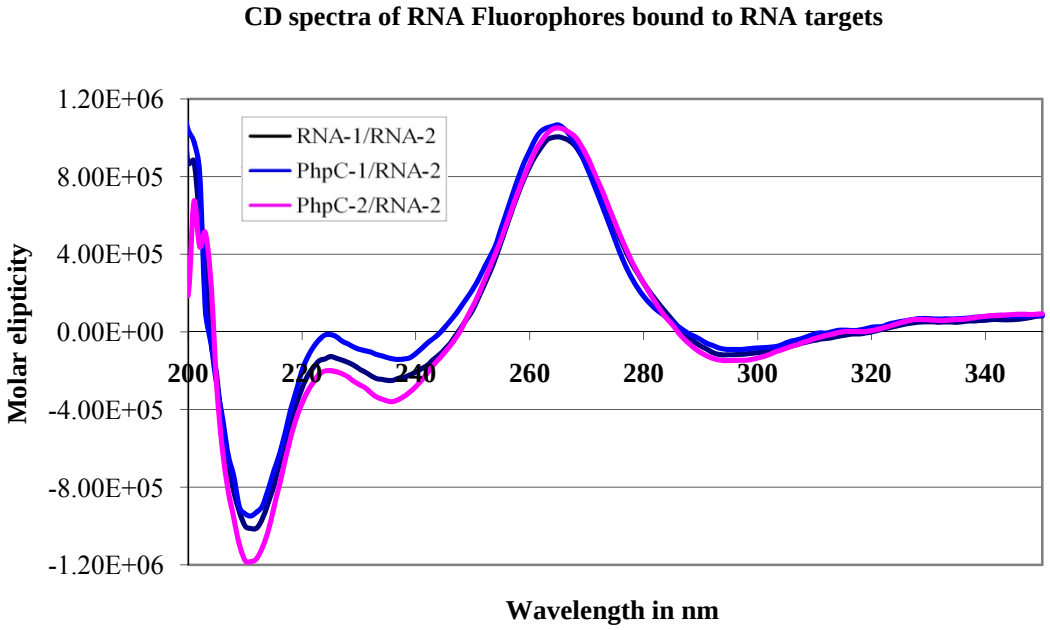


Figure S6. Circular dichroism spectra of RNA containing PhpC bound to their complementary RNA

6. Quantum Yield Determination

The measurement of fluorescence quantum yields (Φ_f) was determined using the relative method[1] using 9,10-diphenylanthracene in ethanol ($\Phi_f = 0.95$) as a reference standard. The quantum yield of the unknown $\Phi(x)$ can be calculated by the following equation:

$$\Phi_{(x)} = \Phi_{(ST)} (A_{ST}/A_X) (F_X/F_{ST}) (\eta^2_X/\eta^2_{ST})$$

Where $\Phi_{(ST)}$ is the quantum yield of the standard, A is the absorbance at the excitation wavelength, F is the integrated area in the emission curve, the subscripts X and ST refer to unknown and standard and η is the refractive index of the solvent. When measuring a series of diluted solutions with various absorbance readings the following equation may be used:

$$\Phi_{(x)} = \Phi_{(ST)} (Grad_X/Grad_{ST}) (\eta^2_X/\eta^2_{ST})$$

Wherein, Grad is the gradient from the plot of integrated area in the emission curve versus absorbance at the excitation wavelength.

UV_{370} was measured on a Cary-300 UV-Vis spectrophotometer (Varian Inc.) from 0.1 to 0.03 absorbance units in 0.01 increments. The fluorescence emission curves were immediately measured afterwards in a Cary Eclipse (Varian Inc.) fluorescent spectrophotometer with an excitation wavelength of 370 nm. Prior to measuring the quantum yield of the unknown samples, the validity of the methodology was confirmed by measuring the quantum yield of anthracene in ethanol which gave a value of 0.27, which is in good agreement with the literature value ($\Phi_f = 0.29$).

[1] (a) Williams A. T. R.; Winfield S. A. *Analyst*, **1983**, 108, 1067-1071. (b) Lavabre, D.; Fery-Forgues, S. *J Chem Ed,* **1999**, *76*, 1260-1264. (c) Morris, J. V.; Mahaney, M. A.; Huber, J. R. *J Phys Chem* **1976**, *80*, 969-974.

.7. Ribonuclease H (RNase H) assays of 3'-fluorescein RNA and 5'-dabcyl DNA

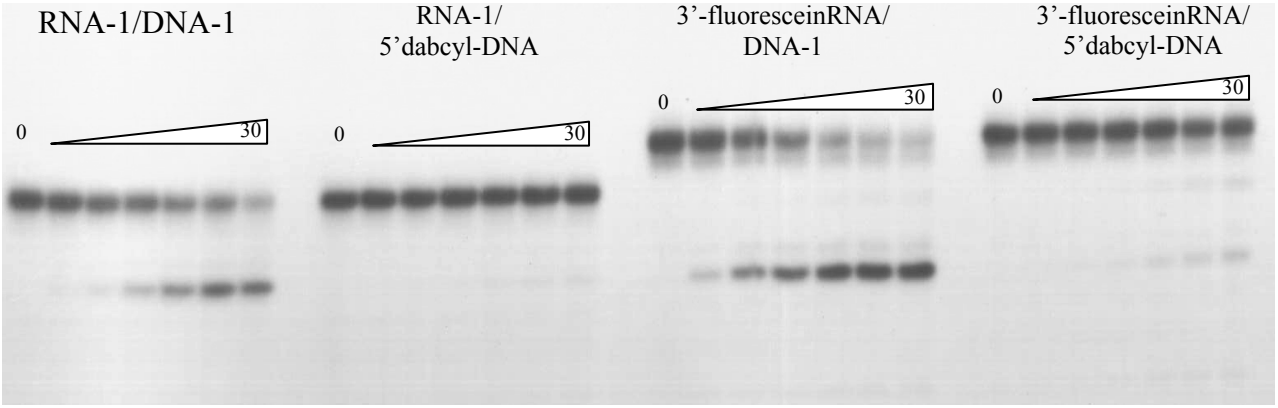


Figure S7. Comparison of the RNase H mediated cleavage of RNA-1/DNA-1 hybrid with a 3’-terminated fluorescein on the RNA and a 5’-terminated dabcyl on the DNA. Reaction progress was monitored over time (0, 2, 5, 10, 15, 20 and 30 minutes) as indicated.

8. Fluorescent polarization RNase H assay on 96-well microplates

The equation to calculate fluorescence polarization (P) as described by the Cary Eclipse follows:

$P = (I_{vv} – GI_{vh}) / (I_{vv} + GI_{vh})$

where

I_{vv} equals the intensity of fluorescence with the excitation polarizer = vertical and emission polarizer = vertical

I_{vh} equals the intensity of fluorescence with the excitation polarizaer = vertical and emission polarizer = horizontal.

G is a factor that accounts for the polarization bias of the instrument and is calculated automatically before the experiment.

The G factor is given by:

$G = I_{hv}/I_{hh}$

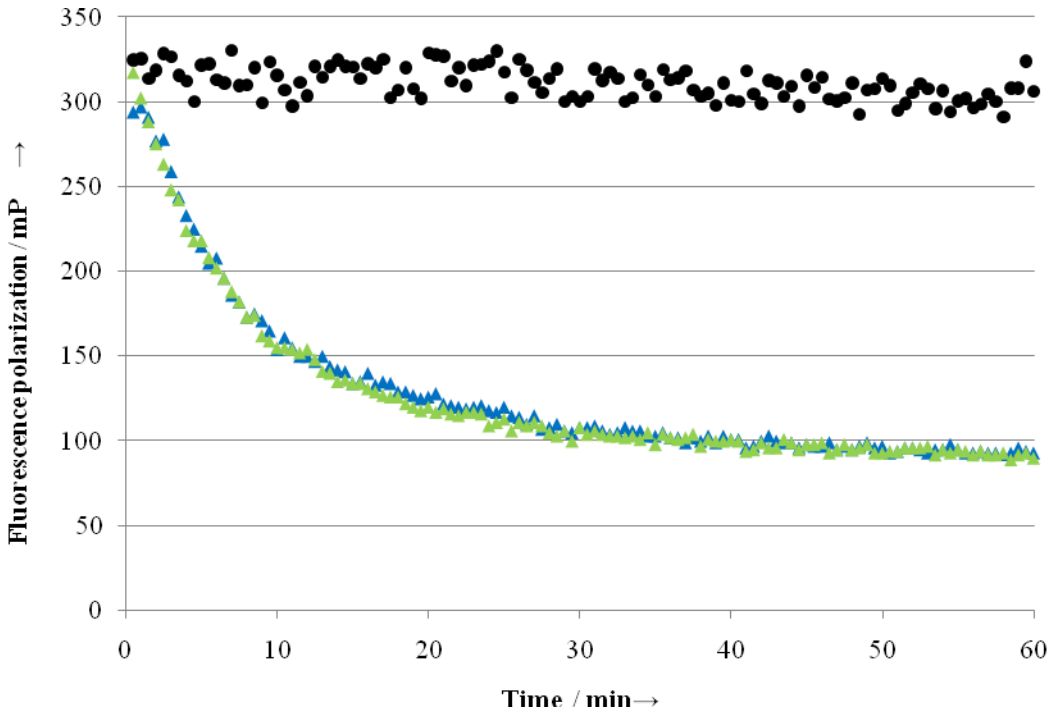


Figure S8. Fluorescence polarization HIV-1 RT RNase H assay of 0.5 μM PhpC-1:DNA-1 monitored in a 96-well plate spectrofluorometer. Colored triangles represent reactions in two individual wells. The average of two controls without enzyme is represented by the black circles. Conditions are identical to those described in the Materials and Methods section.

9. Michealis-Menten plot from fluorescent PhpC RNase H assay

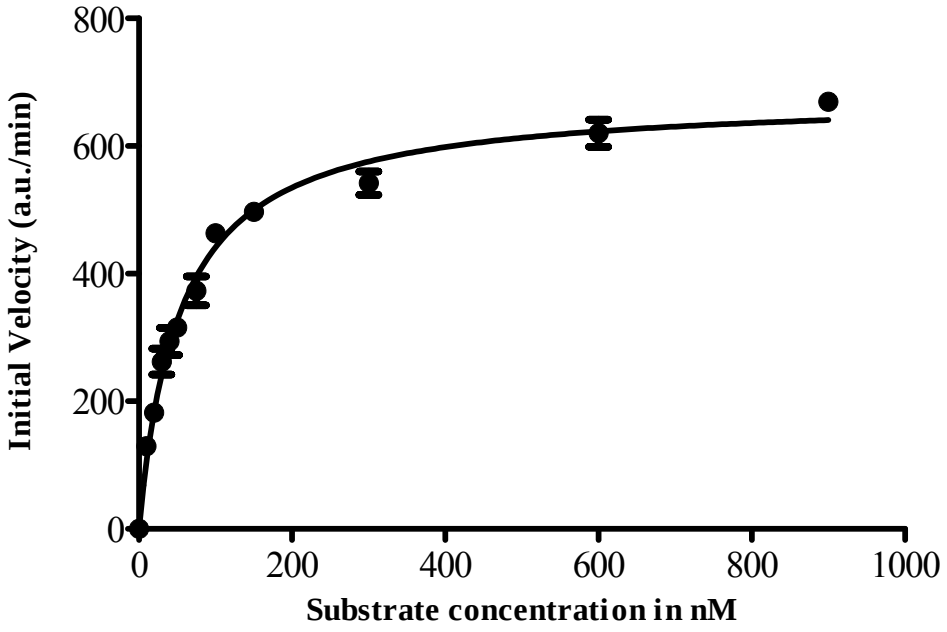


Figure S9. Michealis-Menten plot of initial velocity versus substrate concentration. Initial velocities were measured in triplicates and the data fitted in Prism 5.0.

10. Inhibition of HIV-1 RT by DHBNH

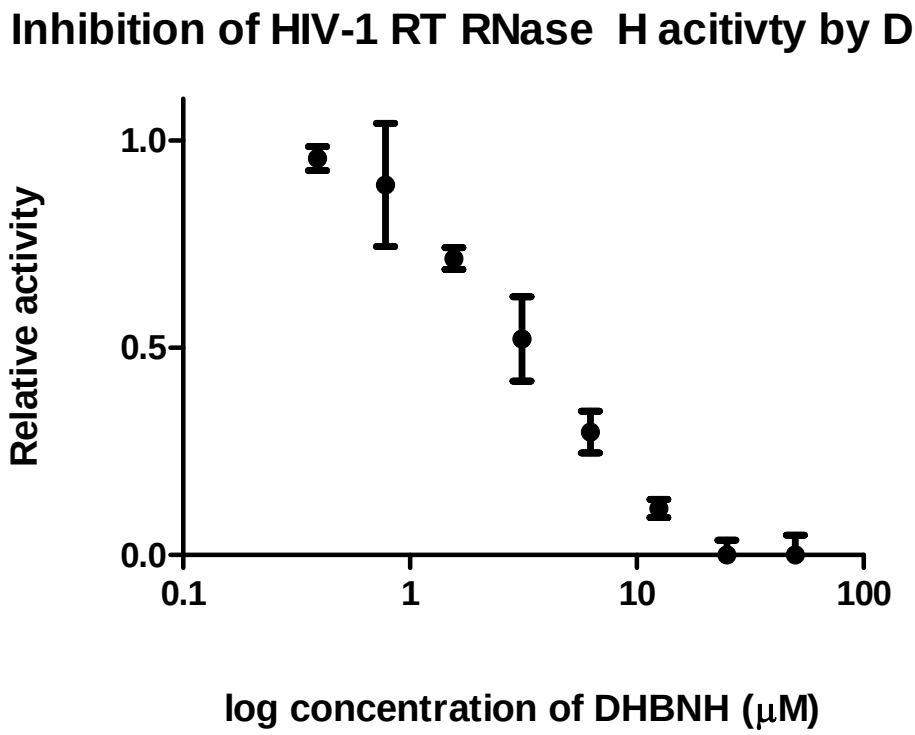
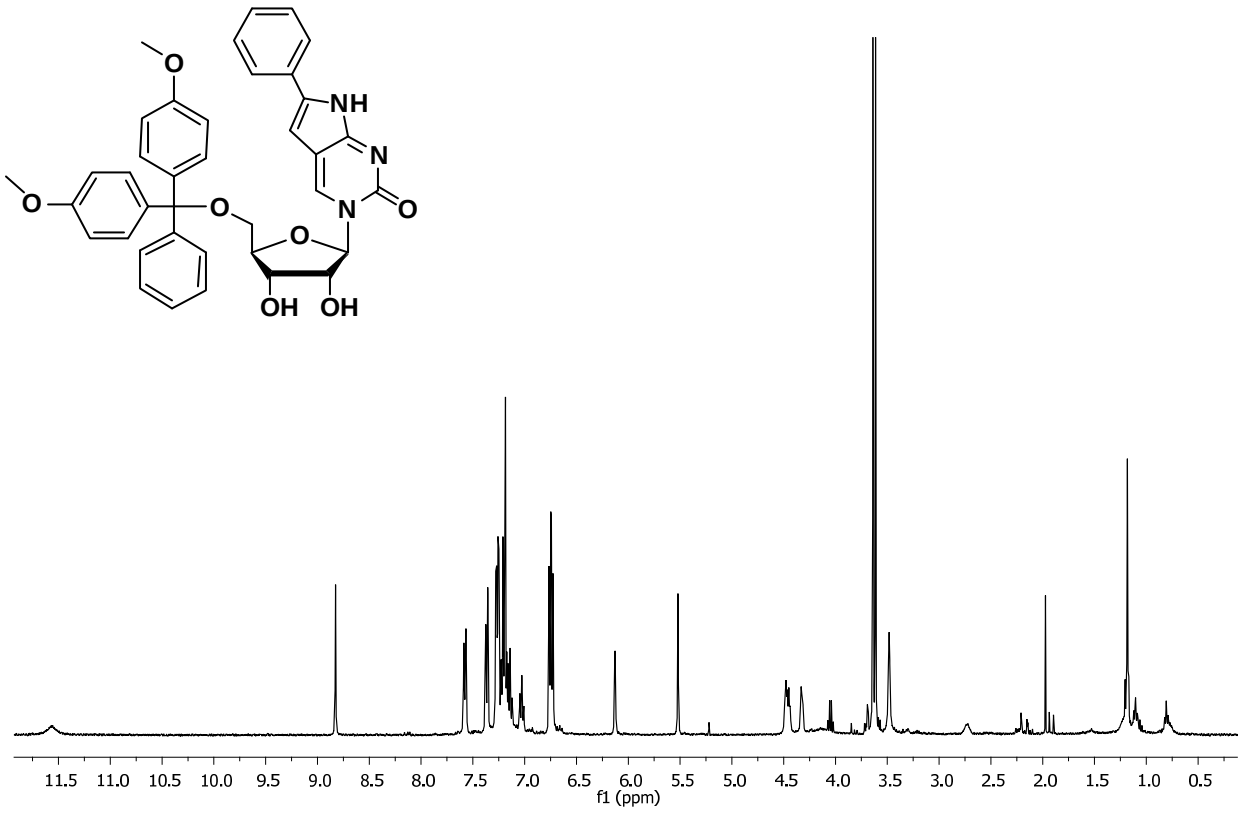


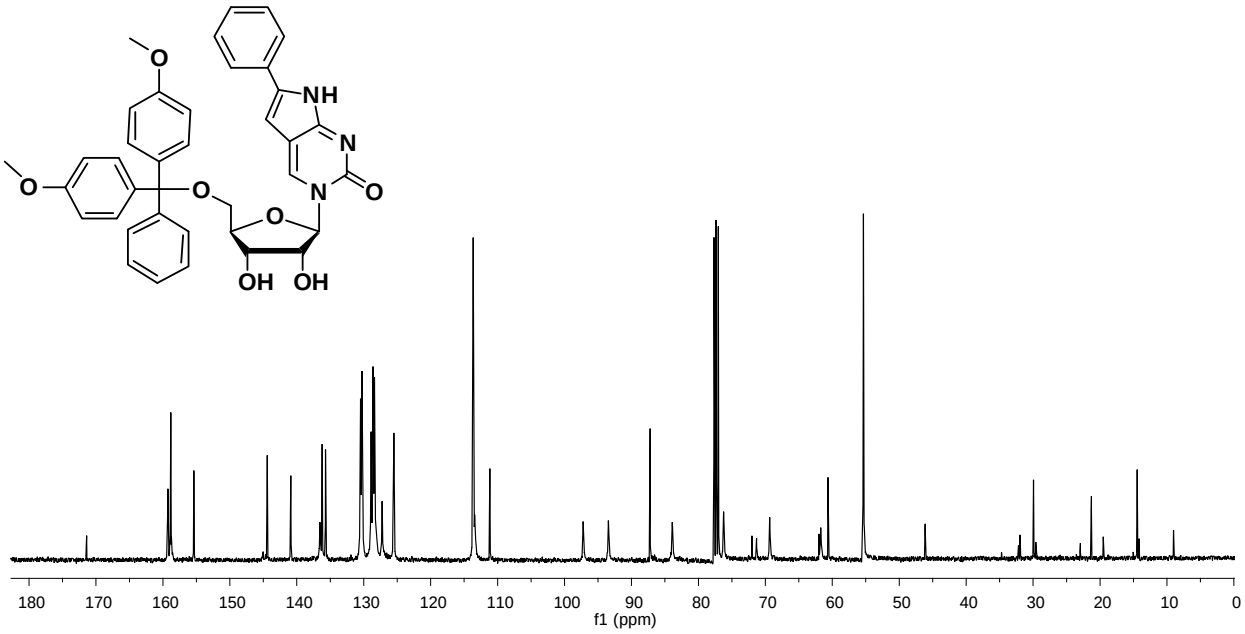
Figure S10. Dose-dependent inhibition curve for the RNase H ihibitor DHBNH. The IC_{50} plot was generated using Prism 5.0.

11. Selected NMR Spectra

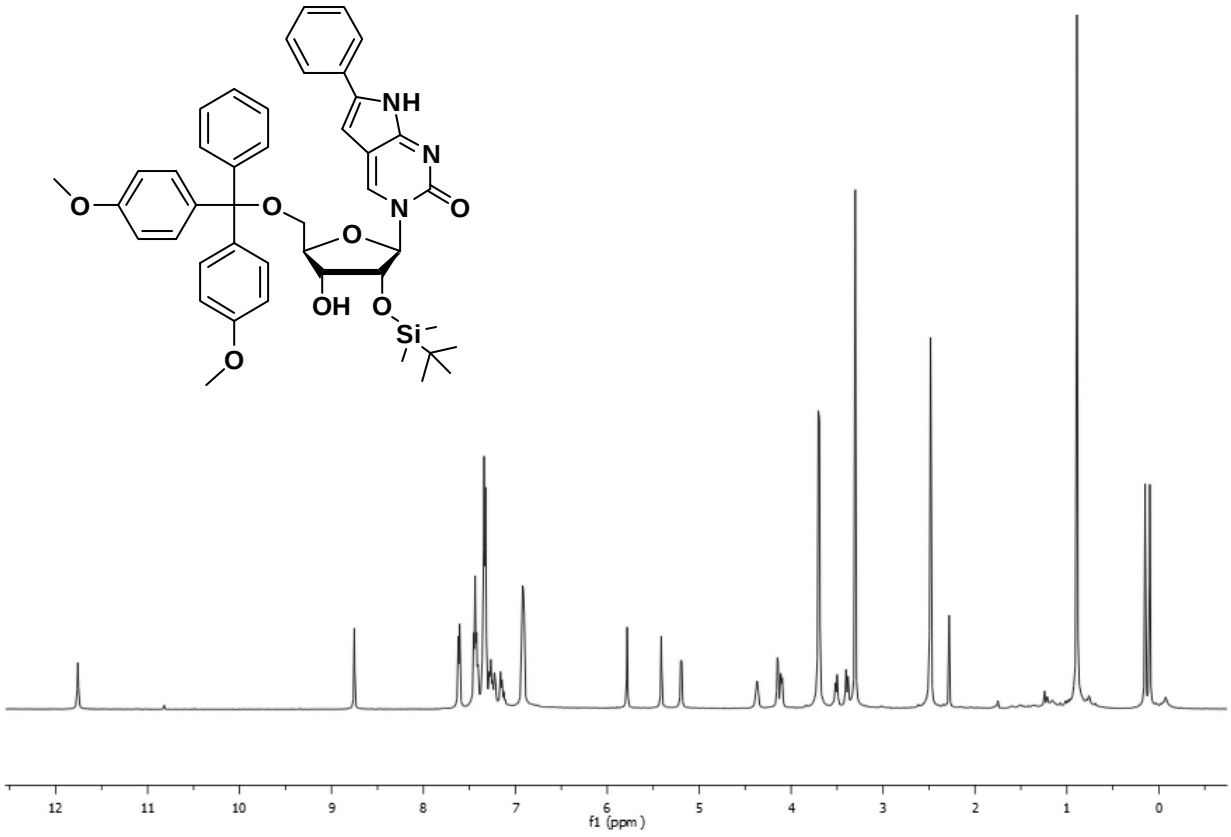
^{1}H (400 MHz, CD_3Cl) of **5**: 5'-*O*-(4',4'-Dimethoxytrityl)-6-phenylpyrrolocytidine



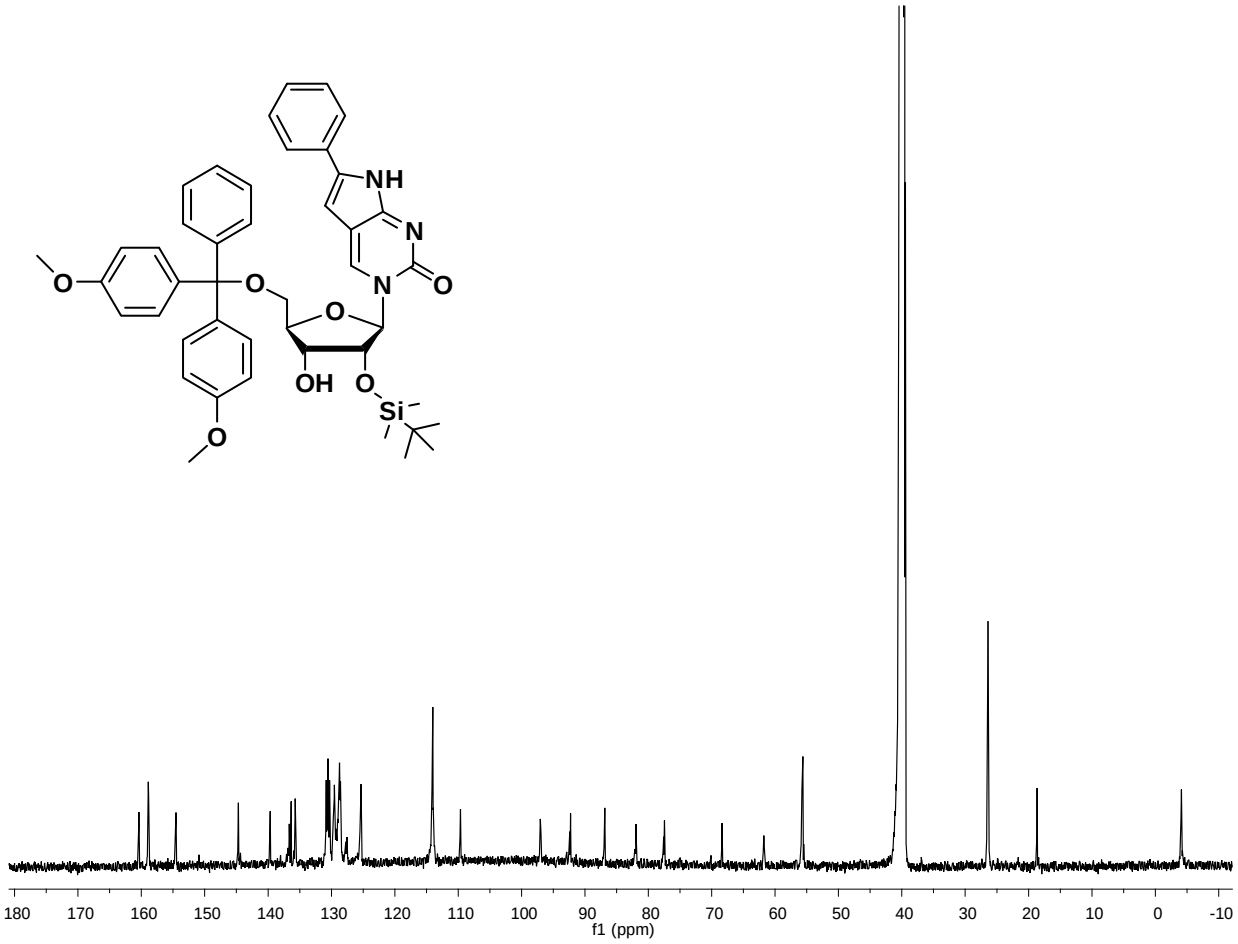
^{13}C (101 MHz, CD_3Cl) of **5**: 5'-*O*-(4',4'-Dimethoxytrityl)-6-phenylpyrrolocytidine



^{1}H (500 MHz, DMSO) of **6**: 5'-*O*-(4',4'-Dimethoxytrityl)-2'-*O*-tert-butyldimethylsilyl-6-phenylpyrrolocytidine



^{13}C (126 MHz, DMSO) of **6**: 5'-*O*-(4',4'-Dimethoxytrityl)-2'-*O*-tert-butyldimethylsilyl-6-phenylpyrrolocytidine



^{31}P (200 MHz, CD_3CN) of **7**: 5'-*O*-(4',4'-Dimethoxytrityl)-2'-*O*-tert-butyldimethylsilyl-3'-*O*-(2-cyanoethyldiisopropylphosphoramidite)-6-phenylpyrrolocytidine.