Supporting Information

A bifunctional nucleoside probe for the inhibition of the human immunodeficiency virus-type 1 reverse transcriptase

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Supplementary Tables

Table S1. Percentage coverage of HIV RT with chemically modified peptides by LC-MS/MS.

	p66			p51		
	Labelled	Peptide ID ctrl	Labelling ctrl	Labelled	Peptide ID ctrl	Labelling ctrl
Probe **1**	86.0	84.8	86.2	98.4	96.6	99.3
Probe **2**	87.1	87.1	85.2	92.4	91.7	91.7
Probe **3**	88.3	86.7	87.1	98.7	95.7	92.6

Table S2. LC-MS/MS hits of p66 subunit peptides labeled with azauracil probes.

Probe	Annotated Sequence	Modifications	Missed Cleavages	Charge	m/z [Da]	MH+ [Da]	DeltaM [ppm]	RT [min]	XCorr
	[R].GsELPIsPIETVPVK.[L]	S3	0	3	623.3219	1867.9511	6.769469224	39.71513	1.3253503
1	[K].yARMRGAHtNDVK.[Q]	Y354, T362	2	3	607.61139	1820.8196	0.115003425	43.6116	1.0448167
	[R].WGLTtPDKK.[H]	T216	1	2	605.80383	1210.6004	-6.015098489	35.29723	1.0266793
2	[K].HQKEPPFLWmGyELHPDKWTVQPIVLPEK.[D]	Y232	2	6	621.48138	3723.8519	-3.30851372	44.89825	1.5395731
	[-].mRGsELPIsPIETVPVKLKPGmDGPK.[V]	S3	2	5	650.93835	3250.6627	7.034926156	50.85798	1.0219656
	[-].mRGSELPIsPIEtVPVKLKPGmDGPK.[V]	S3, T7	2	5	650.93835	3250.6627	7.034926156	50.85798	1.0607207
3	[R].GTKALtEVIPLTEEAELELAENR.[E]	T290	1	3	916.46368	2747.3765	-8.458603087	56.65395	1.8054019

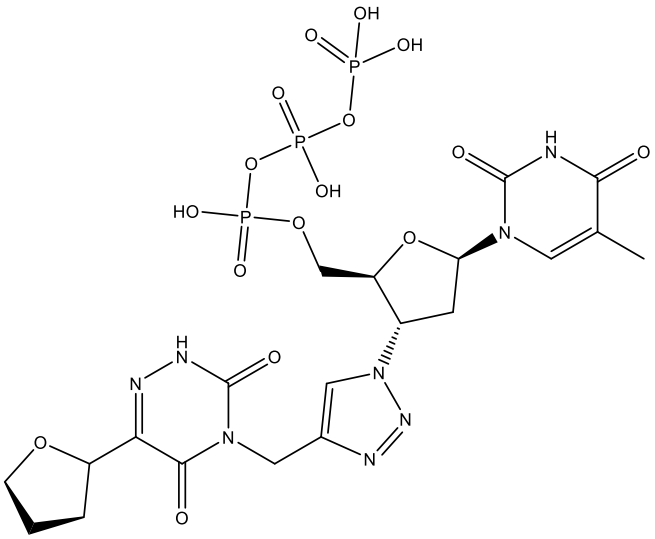
Table S3. LC-MS/MS hits of p51 subunit peptides labeled with azauracil probes.

Probe	Annotated Sequence	Modifications	Missed Cleavages	Charge	m/z [Da]	MH+ [Da]	DeltaM [ppm]	RT [min]	XCorr
2	[K].SVTVLDVGDAyFSVPLDEDFRK.[Y]	Y115	1	4	660.07355	2637.2724	1.161668674	52.10741	1.015239
	[K].IGPENPyNtPVFAIKKK.[D]	Y56, T58	2	4	590.30676	2358.2052	0.513781354	42.7129	1.1230226
3	[K].IGPENPyNtPVFAIKKK.[D]	Y56, T58	2	4	590.30713	2358.2067	1.134950576	41.95076	1.0856485

Supplementary Materials, Methods and Figures

The [3+2] copper-catalyzed azide-alkyne cycloaddition (CuAAC/Click) reaction of AzTTP/AzT with probe 3. The click reaction was prepared in 1X PBS using 10 mM Ascorbate, 10 mM $CuSO_4$, 1mM TBTA, 11 mM probe **3**, and 10 mM AzTTP or AzT. The components were reacted for 1 hour and excess copper was removed using Nickel NTA resin. The resin was washed with PBS, stripped three times with Ni2+ stripping buffer (20 mM $NaHPO_4$ pH 7.4, 500 mM NaCl, 50 mM EDTA) and dried before adding the click reaction and collecting the reaction by centrifuging at 700 x g for 2 minutes. AzTTP (Cedarlane) and AzT (Sigma Aldrich) were purchased from commercial sources. Spectral data for probe **3** matched previously reported characterization data.[1] After quantitative coupling via [3+2] CuAAC, the product (approximately 0.8 mg of **4** and 1.2 mg of **5**) was characterized by ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR, and HMBC for **4**, and ESI mass spectrometry, ^{1}H-NMR, ^{13}C-NMR, and HSQC for **5** (ESI spectra were measured from D_2O samples), supporting structural assignment. Spectral data for the products was also consistent with similar previously reported AzT "clicked" analogues.[2]

Azauracil-AzTTP (4)



$C_{20}H_{27}N_8O_{16}P_3$

^{1}H-NMR (600 MHz, D_2O) δ 8.20 (s, 1H), 7.89 (s, 1H), 6.60 (t, *J* = 7.3 Hz, 1H), 5.64 (dt, *J* = 7.1, 3.2 Hz, 1H), 5.18 (s, 2H), 5.08 – 5.00 (m, 1H), 4.66 – 4.61 (m, 1H), 4.35 – 4.24 (m, 2H), 3.99 (q, *J* = 7.2 Hz, 1H), 3.92 – 3.87 (m, 1H), 2.83 – 2.75 (m, 2H), 2.33 – 2.26 (m, 1H), 2.09 – 2.03 (m, 1H), 2.03 – 1.97 (m, 2H), 1.95 (s, 3H). **^{13}C-NMR** (151 MHz, D_2O) δ 166.61, 156.35, 150.19, 141.59, 137.23, 111.98, 85.57, 75.55, 69.07, 65.83, 61.20, 59.65, 37.45, 34.75, 29.23, 24.86, 11.67. **HMBC ^{1}H-NMR** (600 MHz, D_2O) δ 8.15, 8.14, 7.85, 7.84, 7.84, 6.55, 6.55, 5.13, 5.13, 5.13, 5.13, 3.95, 3.94, 3.94, 3.85, 3.85, 3.84, 2.75, 2.74, 2.50, 2.25, 2.25, 2.01, 2.01, 2.01, 2.00, 2.00, 1.96, 1.95, 1.95, 1.90, 1.90, 1.90, 1.79. **HMBC ^{13}C-NMR**

(151 MHz, D_2O) δ 141.59, 141.64, 166.69, 11.68, 151.73, 137.15, 151.72, 141.61, 156.36, 125.10, 150.47, 75.44, 24.72, 29.28, 24.71, 75.53, 29.26, 85.23, 61.05, 61.22, 24.93, 145.02, 69.01, 145.02, 75.54, 11.67, 24.86, 69.04, 75.75, 29.25, 166.66, 137.26, 111.98, 11.68. **^{31}P NMR** (203 MHz, D_2O) δ -9.26(br, 1P), -11.73 (d, *J* = 20.1 Hz, 1P), -22.82 (t, *J* = 19.3 Hz, 1P).

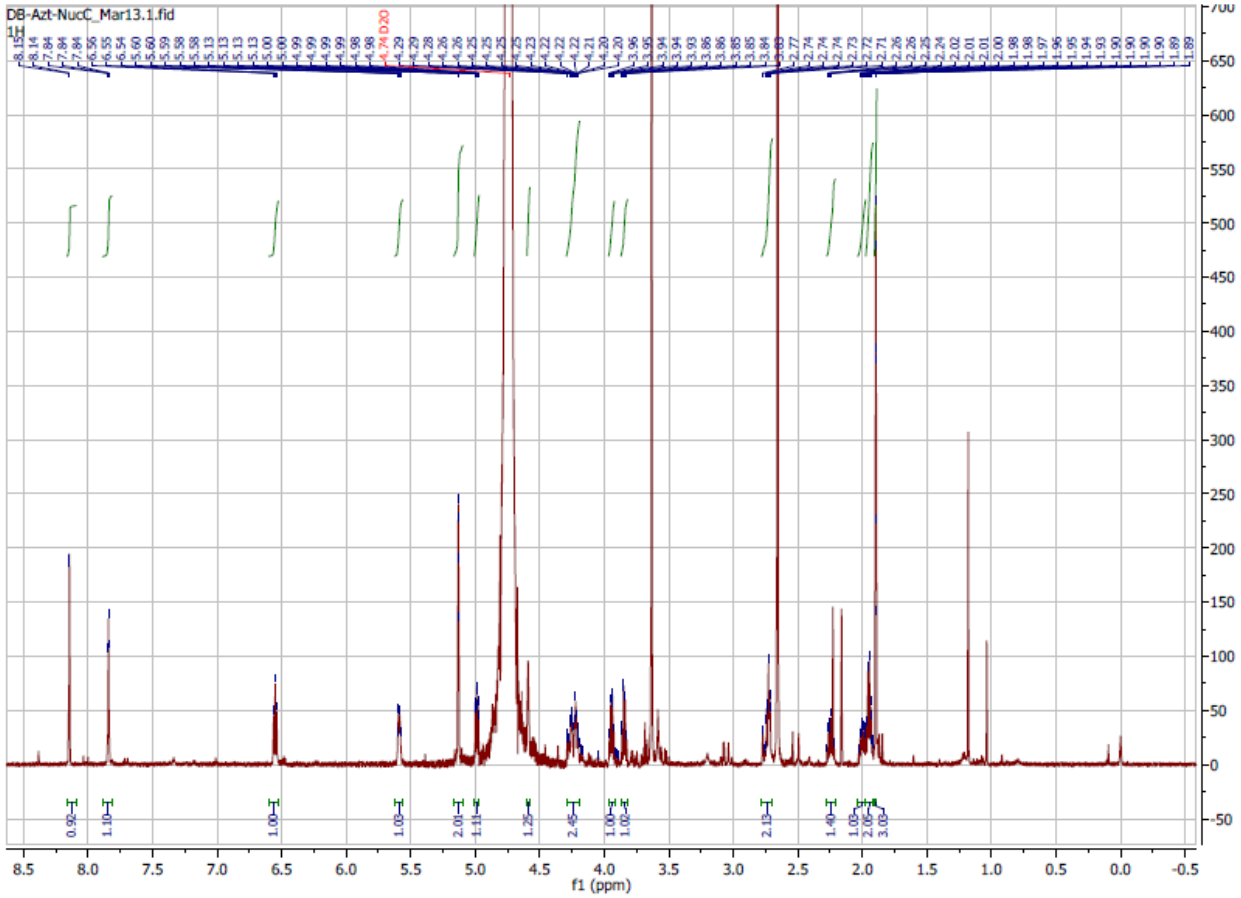


Figure S1. ^{1}H-NMR spectrum of Azauracil-AzTTP.

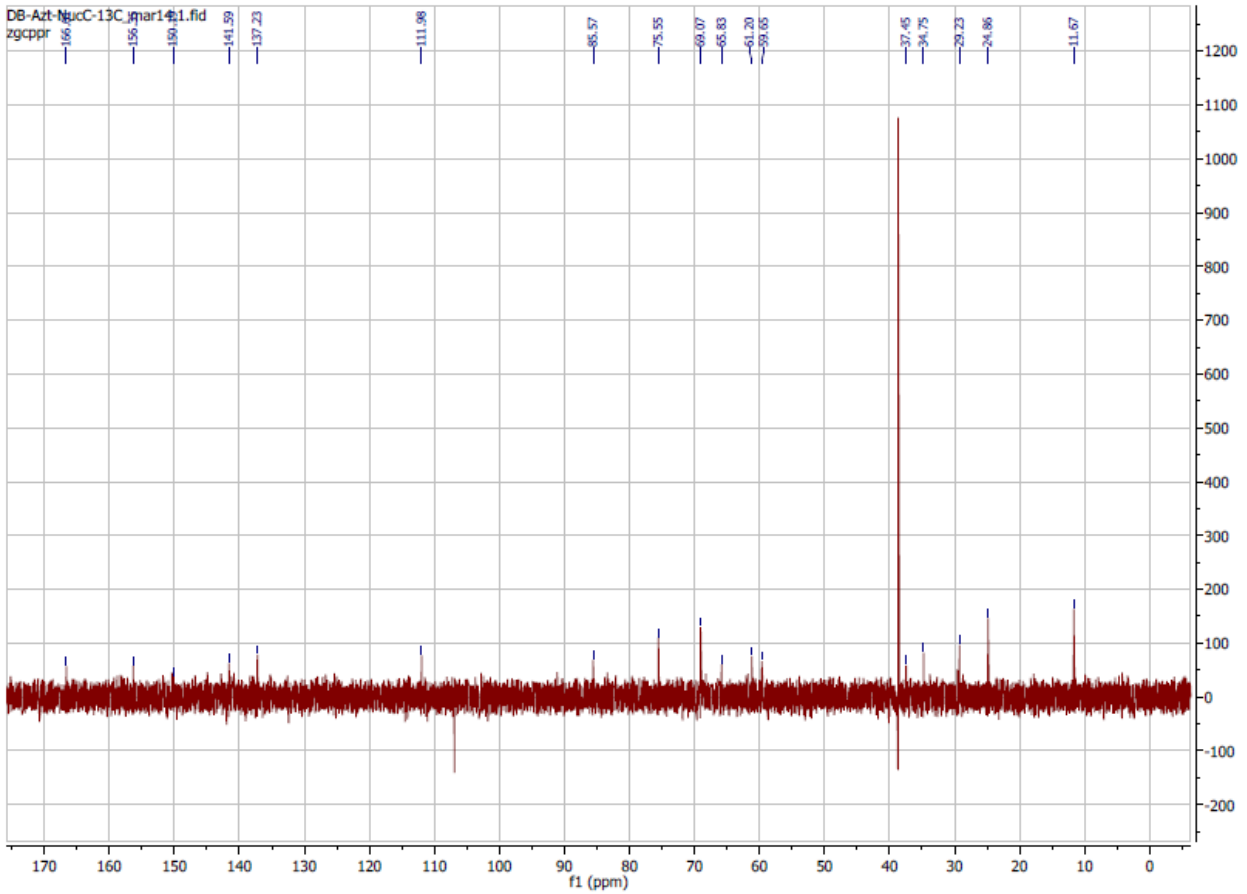


Figure S2. ^{13}C-NMR spectrum of Azauracil-AzTTP.

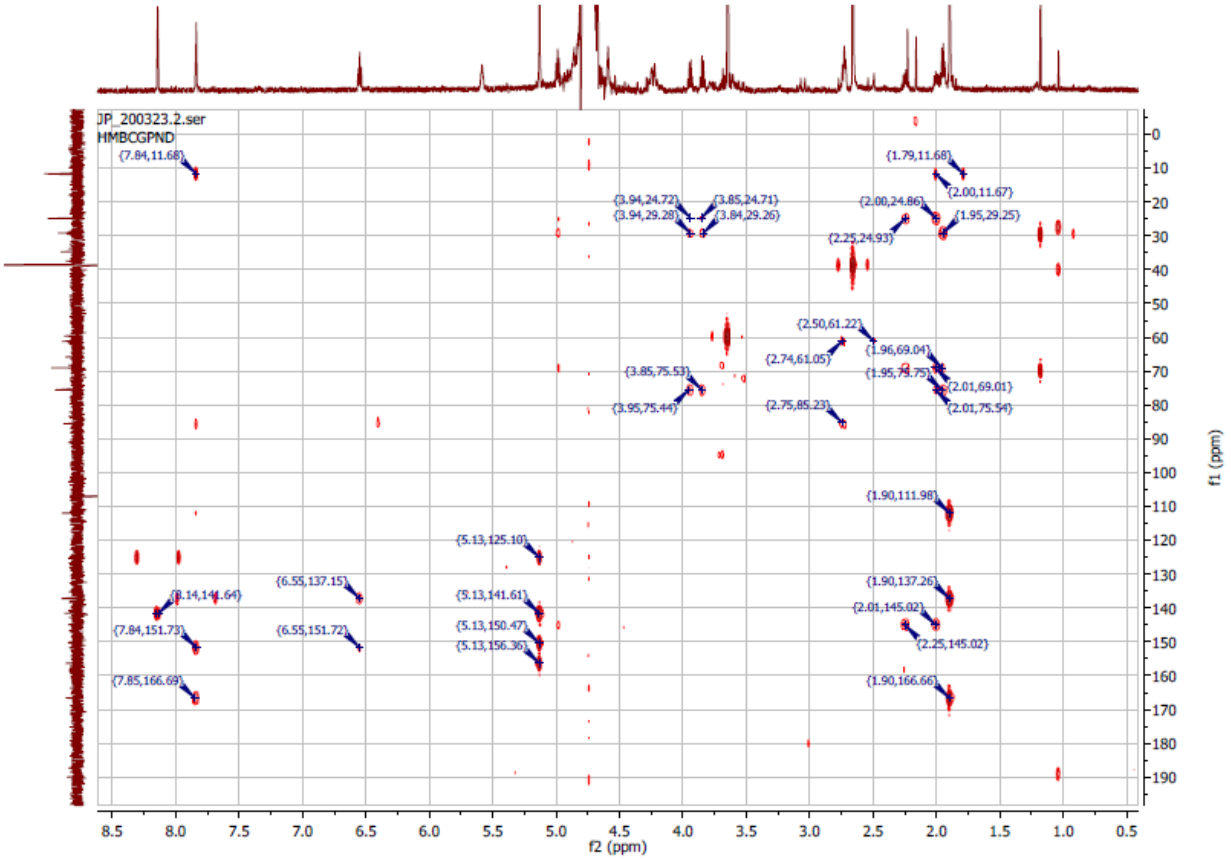


Figure S3. HMBC spectrum of Azauracil-AzTTP.

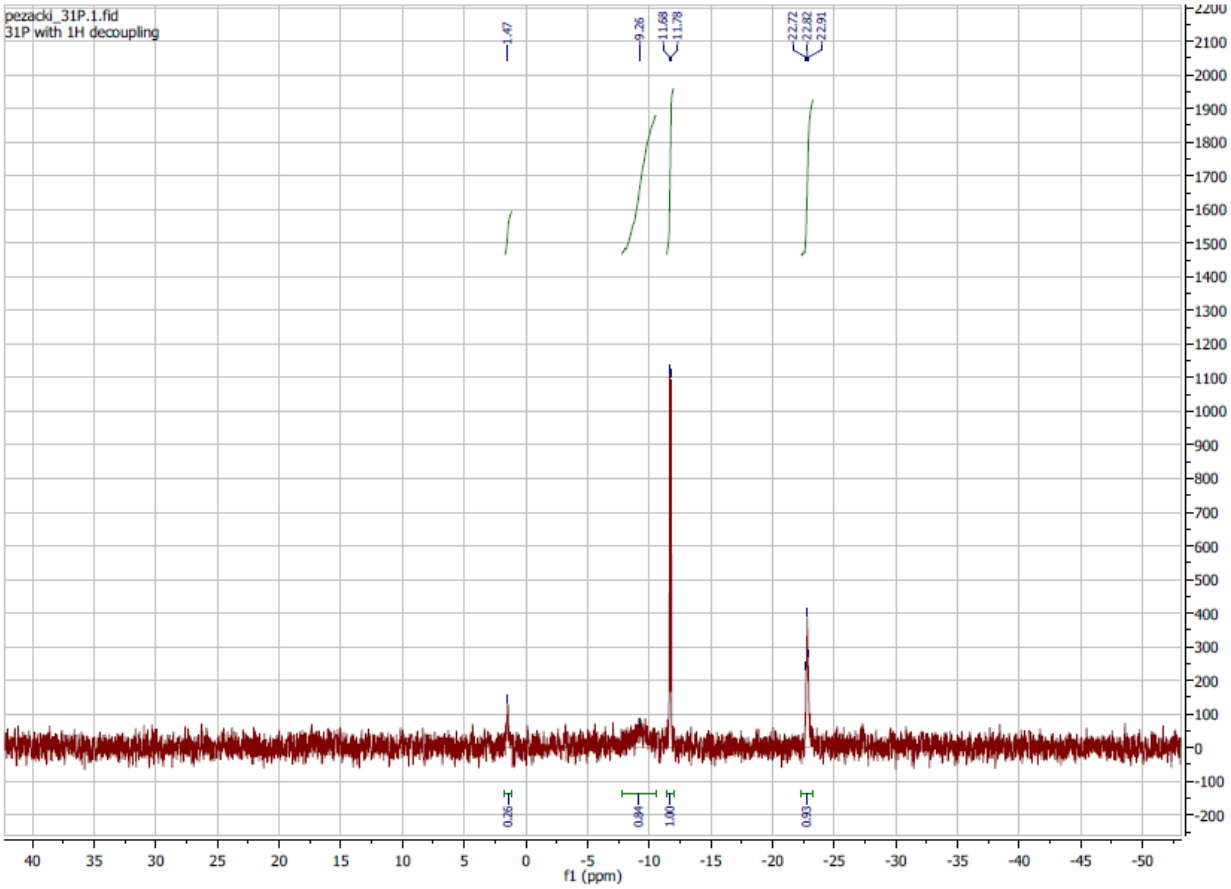
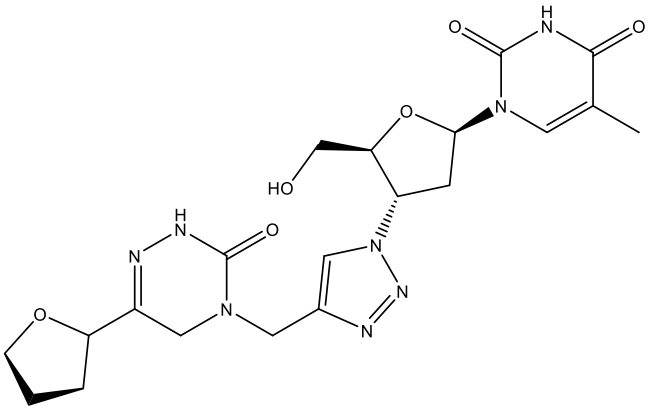


Figure S4. ^{31}P (^{1}H decoupled) spectrum of Azauracil-AzTTP.

Azauracil-AzT (5)



HRMS (ESI+) [M+Na]: calcd. for $C_{20}H_{23}D_3N_8O_6Na^+$: 514.1848, found: 514.2145

^{1}H-NMR (600 MHz, D_2O) δ 8.10 (s, 1H), 7.68 (s, 1H), 6.39 (t, *J* = 6.5 Hz, 1H), 5.36 (dt, *J* = 8.9, 6.1 Hz, 1H), 5.13 (s, 2H), 4.97-5.00 (m, 1H), 4.40 (dt, *J* = 6.7, 3.8 Hz, 1H), 3.94 (q, *J* = 7.0 Hz, 1H), 3.83-3.87 (m, 2H), 3.74 (dd, *J* = 12.7, 4.4 Hz, 1H), 2.87-2.93 (m, 1H), 2.82 – 2.73 (m, 1H), 2.29 – 2.20 (m, 1H), 2.04 – 1.90 (m, 3H), 1.86 (s, 3H). **^{13}C-NMR** (151 MHz, D_2O) δ 166.57, 156.36, 151.60, 150.39, 144.92, 137.78, 124.39, 111.49, 85.51, 84.18, 75.55, 69.08, 60.47, 59.55, 38.70, 36.84, 34.73, 29.16, 24.88, 11.53. **^{13}C/HSQC NMR** (151 MHz, D_2O) δ 124.51, 137.77, 85.54, 59.56, 34.72, 75.56, 84.21, 69.05, 69.08, 60.47, 60.47, 36.85, 36.83, 29.09, 29.08, 24.87, 11.47 **^{1}H/HSQC NMR** (600 MHz, D_2O) δ 8.09, 7.68, 6.39, 5.36, 5.13, 4.98, 4.40, 3.94, 3.85, 3.84, 3.74, 2.90, 2.78, 2.25, 2.01, 1.95, 1.86.

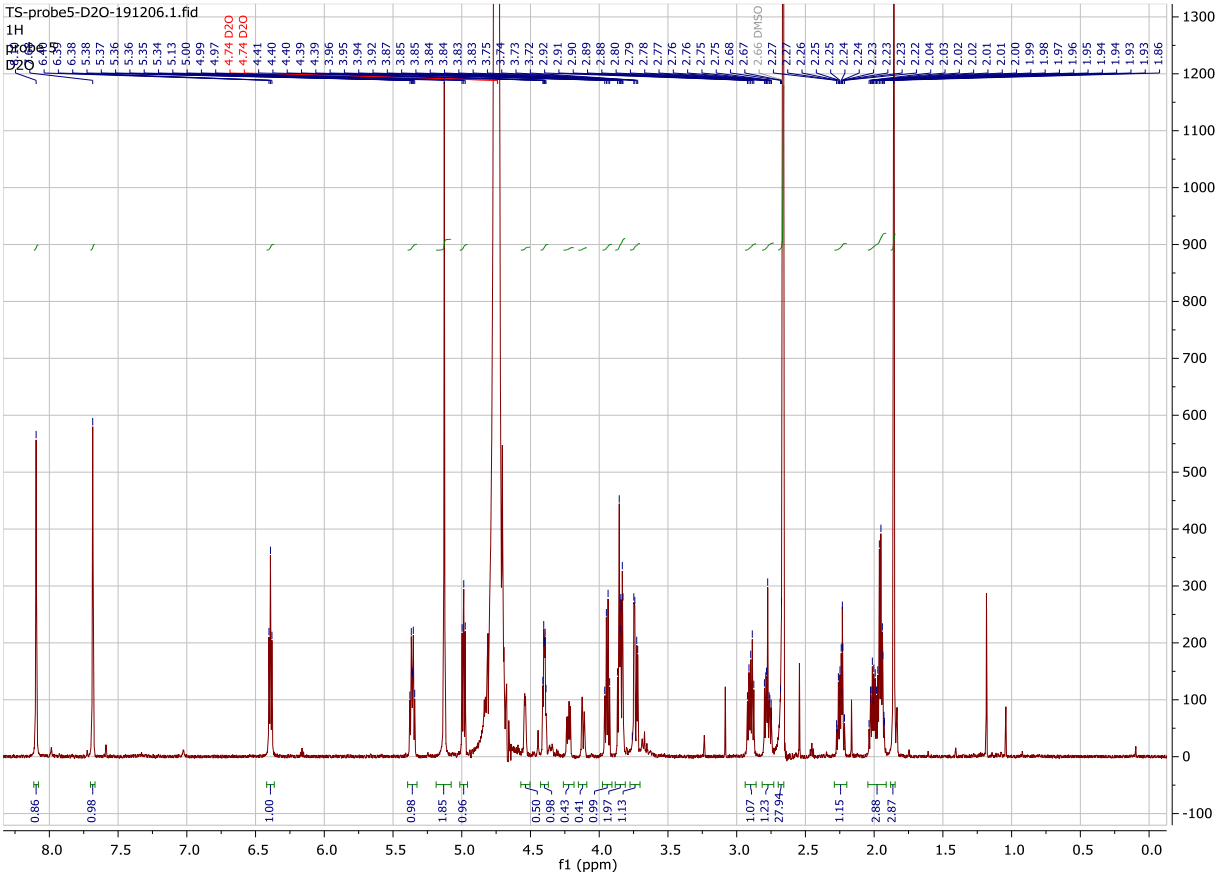


Figure S5. ^{1}H-NMR spectrum of **A**zauracil-AzT.

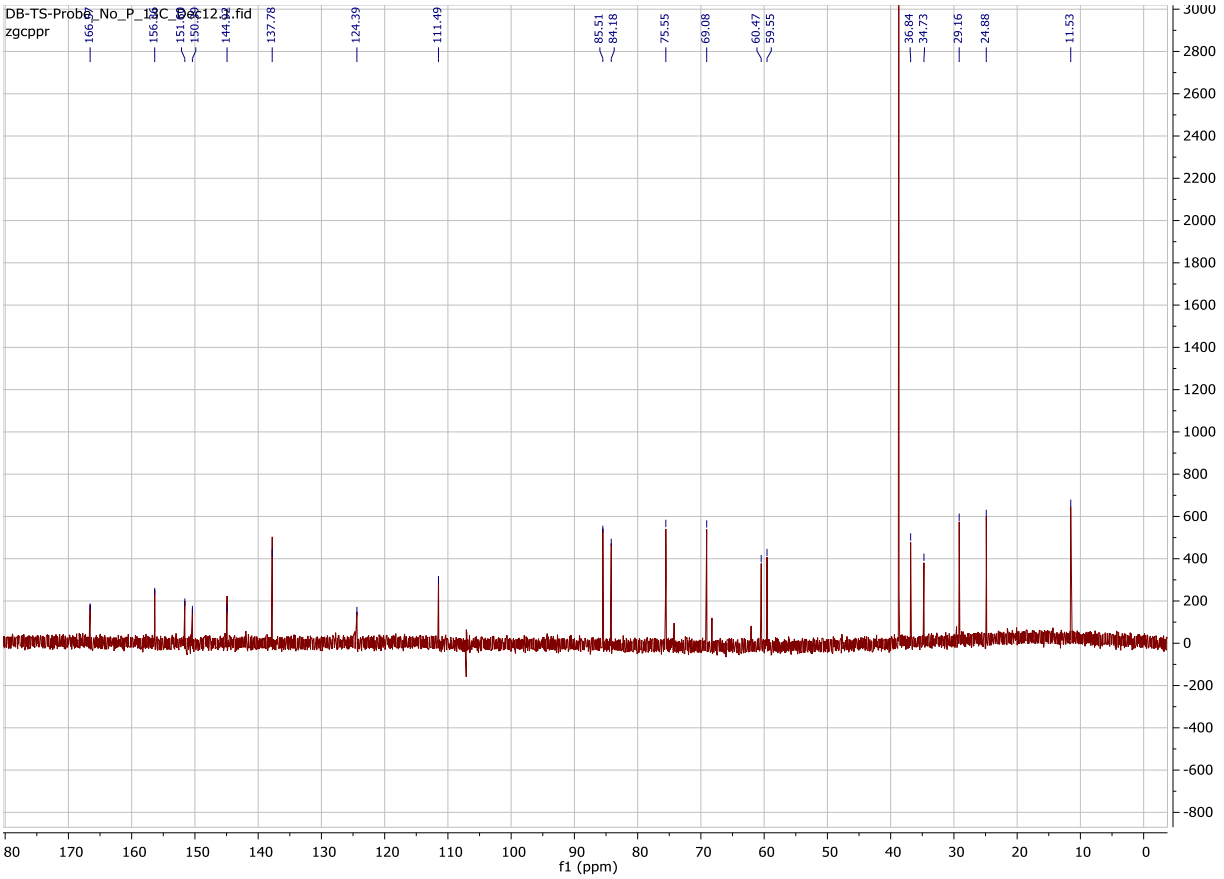


Figure S6. ^{13}C-NMR spectrum of Azauracil-AzT.

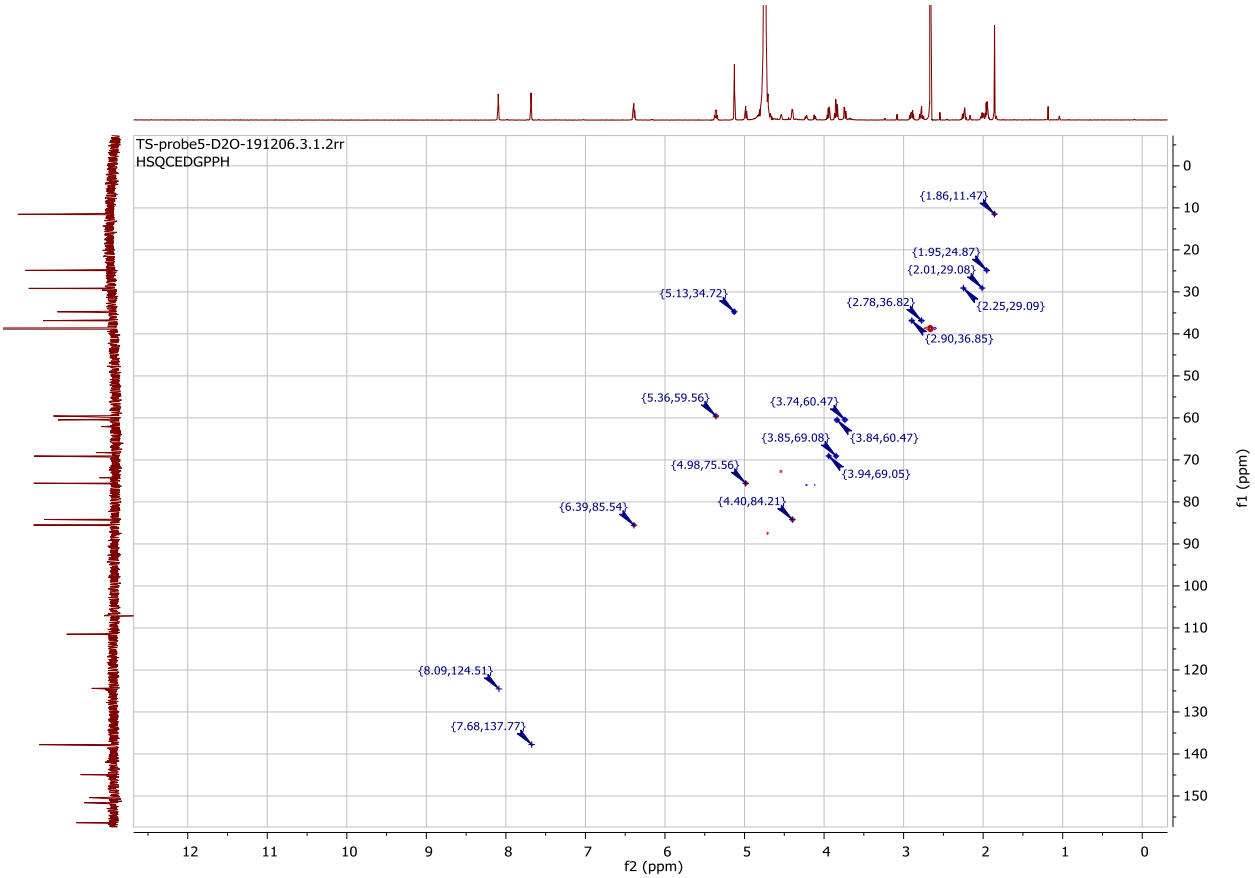


Figure S7. HSQC NMR spectrum of Azauracil-AzT.

Supplementary References

1. J.-F. Vincent-Rocan, R. A. Ivanovich, C. Clavette, K. Leckett, J. Bejjani and A. M. Beauchemin, *Chem. Sci.*, 2016, **7**, 315–328.
2. L. Zhou, A. Amer, M. Korn, R. Burda, J. Balzarini, E. De Clercq, E. R. Kern and P. F. Torrence, *Antivir. Chem. Chemother.*, 2005, **16**, 375–383.