Supporting Information

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Novel Cytidine-Based Inhibitors of Orotidine-5'-Monophosphate Decarboxylase with an Unusual Twist

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Purity of the synthesized compounds:

Nucleoside purity was evaluated on a Water LC-MS system (Waters™ 2545 binary gradient module) equipped with a photodiode array detector using an X-Bridge C18 column (4.6 x 150 mm, 5 µm). The HPLC methods used for the purity assessment were Method A: (Isocratic conditions, 95% water with 0.05% TFA and 5% MeOH with 0.05% TFA, 10 min), Method B: (Isocratic conditions, 100% water with 0.05% TFA, 15 min). Nucleotide purity was evaluated on the same HPLC system using a Spherisorb C18 column (4.5 x 250 mm, 5µm). The HPLC methods used for the purity assessment were Method B: (Isocratic conditions, 100% water with 0.05% TFA, 10 min), Method C: (Gradient conditions, 100% water to 95% water and 5% AcCN with 0.05% TFA, 15 min). All HPLC solvents were filtered through membrane filters (47 mm GHP 0.45 µm, Pall Corporation). Injection samples were filtered using Pall Acrodisc ® Syringe filters 4 mm PTFE (0.2 µm).

Table S1. Purity Data.

Compound No	HPLC method	Retention time (min)	Purity
13	Method A	2.87	>99.9%
	Method B	2.68	>99.9%
15	Method A	3.93	>99.9%
	Method B	4.33	>99.9%
14	Method B	4.73	>99.9%
	Method C	4.77	>99.9%
27	Method B	4.76	>99.9%
	Method C	4.77	>99.9%

Semi preparatory Purification Method: Final compounds were purified on a Water HPLC using a semi-prep Spherisorb ODS2 C18 column (20 x150 mm, 4 ml/min). The HPLC method used for the purification was Method B: (Isocratic, 100% water with 0.05% TFA)

Table S2. UV spectra for compounds **14** and **27** after incubation in the crystallization buffer for 24 h at room temperature.

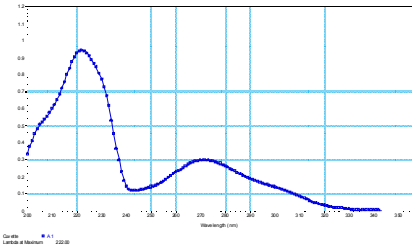
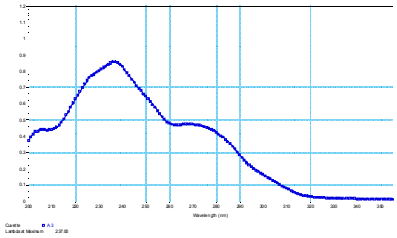
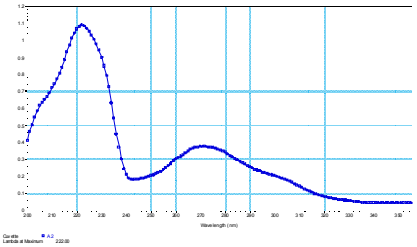
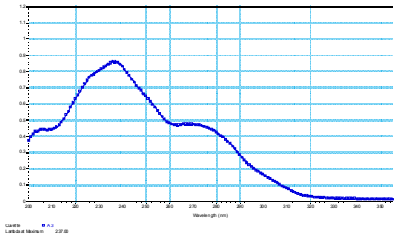
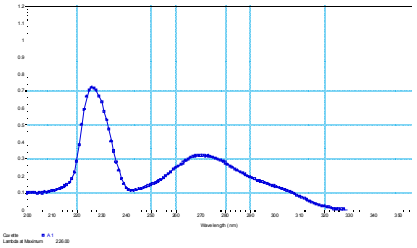
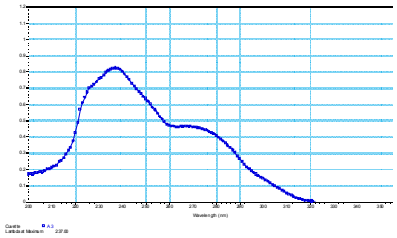
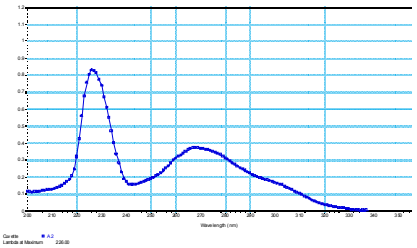
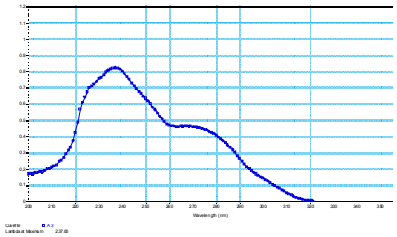
Time (h)	CMP -N^3-oxide (**14**)	CMP- N^4-OH (**27**)
Crystallization conditions: Tris Buffer (pH 7.0), Glycerol (12%), Ammonium Sulfate (1.8M), Inhibitor (396 μM). Incubated at room temperature 22 °C		
0		
24		
Crystallization conditions: HEPES (pH 7.75), glycerol (12%), ammonium sulfate (1.8 M), inhibitor (396 μM). Incubated at room temperature (22 °C).		
0		
24		

Table S3. Diffraction and refinement data for co-crystal complexes of compound **14** and **27** with *Hs* ODCase .

Diffraction Data	**Human ODCase + Compound 14**	**Human ODCase + Compound 27**
Space Group	$P2_1$	$P2_1$
Unit cell axes [Å]	a=69.3	a=69.8
	b=61.8	b=61.6
	c=70.5	c=71.4
Unit cell angle [º]	α=90 β=112.9 γ=90	α=90 β=112.1 γ=90
Resolution [Å]	1.75 [1.80 – 1.75]	1.90 [1.95 – 1.90]
Measured reflections	216201 [1.75]	179171 [1.90]
Unique reflections	53542	50390
Completeness [%]	92.7	98.4
Rsym	0.029	0.038
Refinement Statistics		
Resolution [Å]	65.0 – 1.75	66.0 – 1.90
Protein atoms	3962	4048
Water molecules	304	190
Reflections used for	50805	42064
R_{cryst} [%]	16.1	19.0
R_{free} [%]	18.5	22.3
RMSD Bond Length [Å]	0.016	0.026
RMSD Bond Angle [º]	1.6	2.1
Average B-factor [Å]	17.9	28.5
Ramachandran plot		
Residues in allowed regions [%]	96.74	95.98
Residues in generously allowed regions [%]	2.65	3.59
Resiues in not allowed regions [%]	0.61	0.42

Figure S1. IR Spectrum for Cyd-N^3-oxide (**13**).

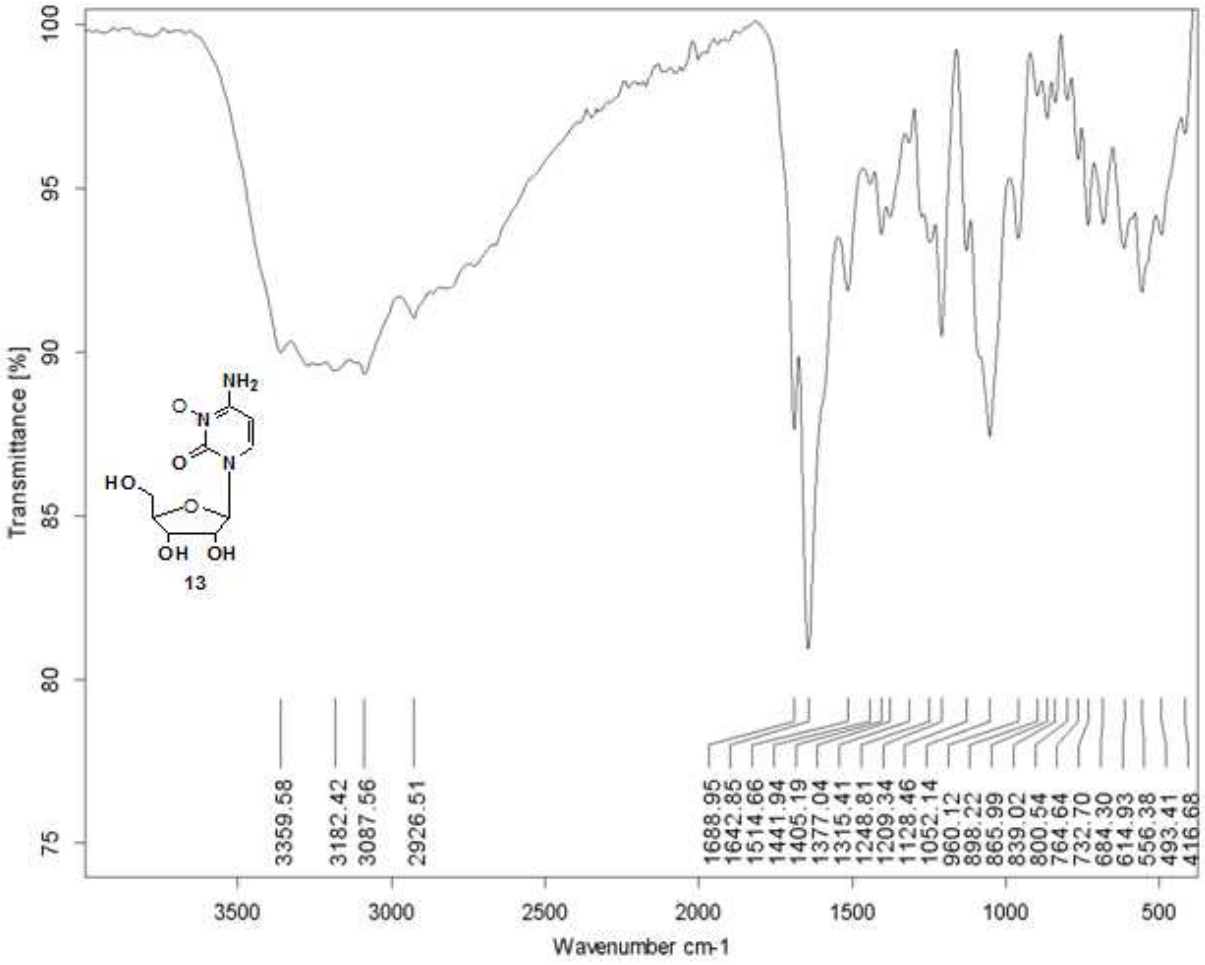


Figure S2. IR Spectrum for Cyd-N^4-OH (**15**).

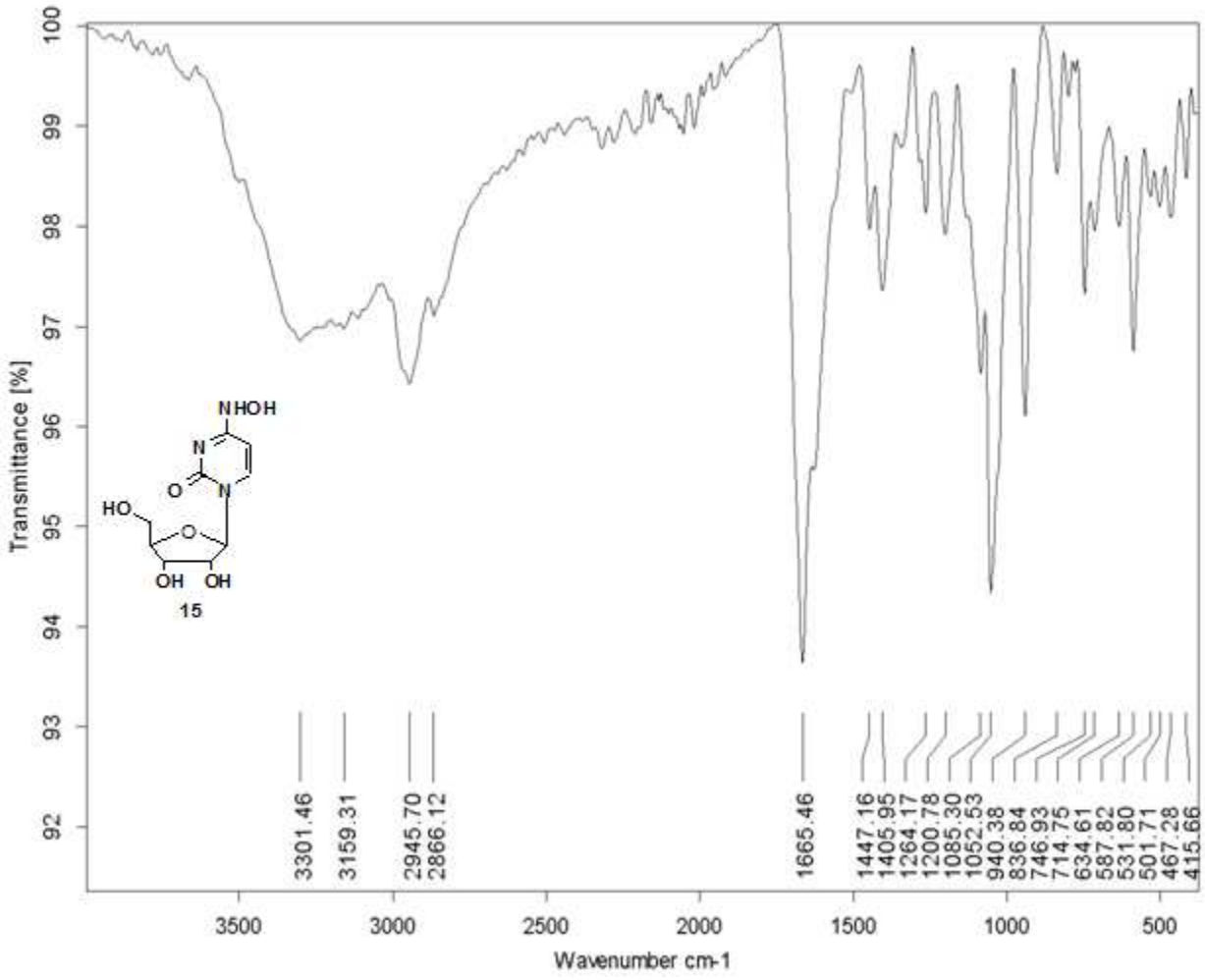


Figure S3. IR Spectrum for CMP- N^3-oxide (**15**).

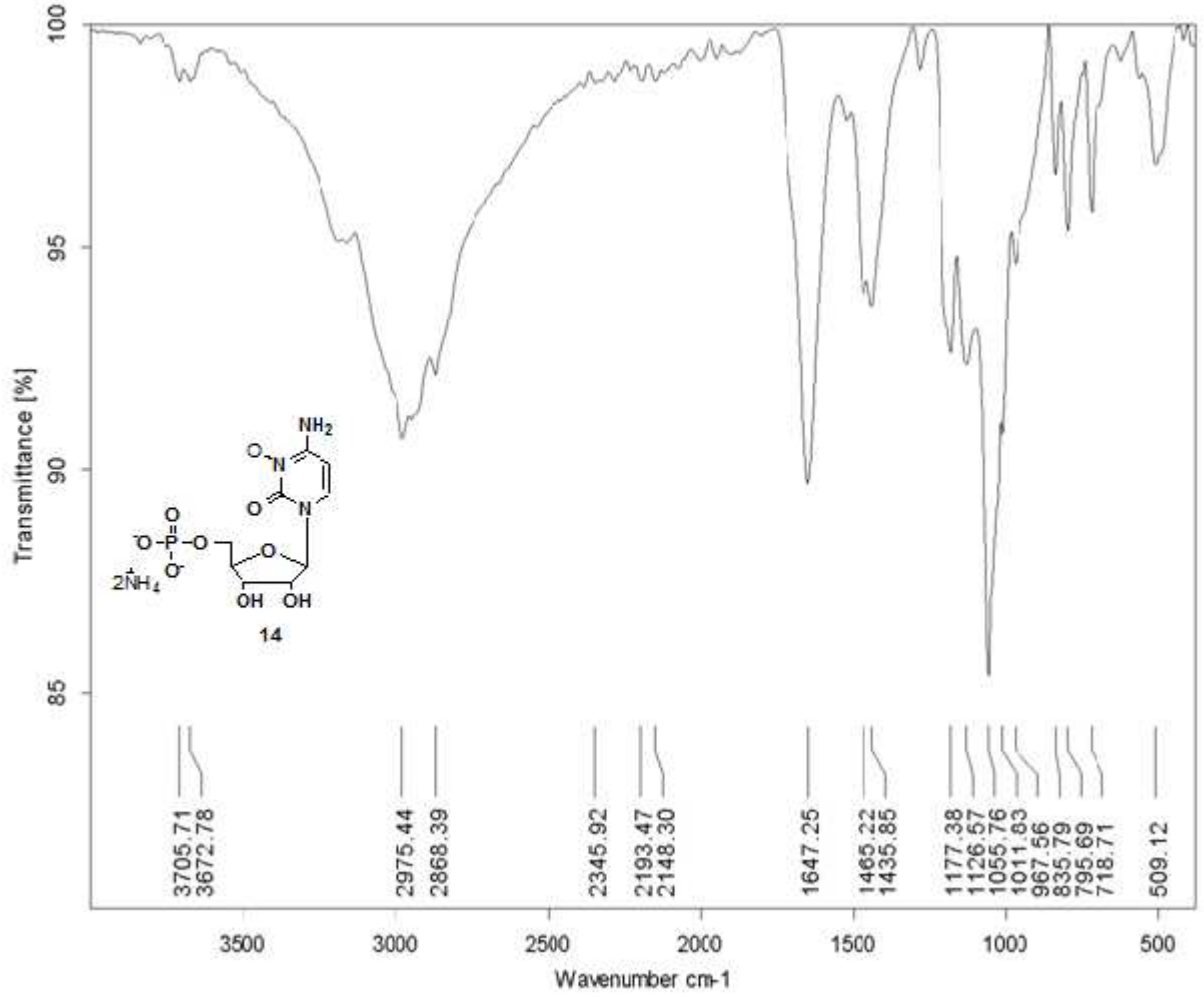


Figure S4. IR Spectrum for CMP- N^4-OH (**27**).

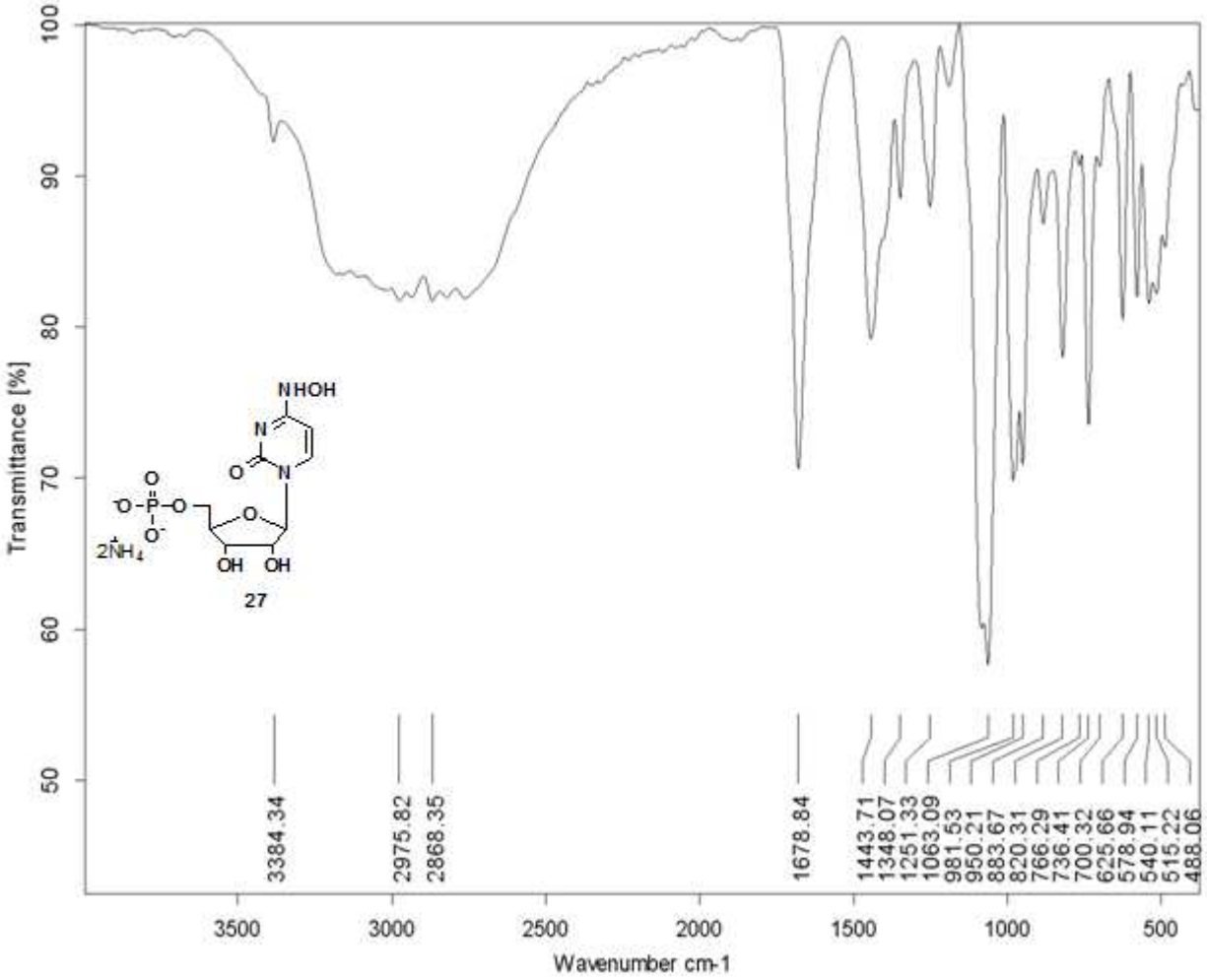


Figure S5. ^{1}H NMR spectrum for compound **13.**

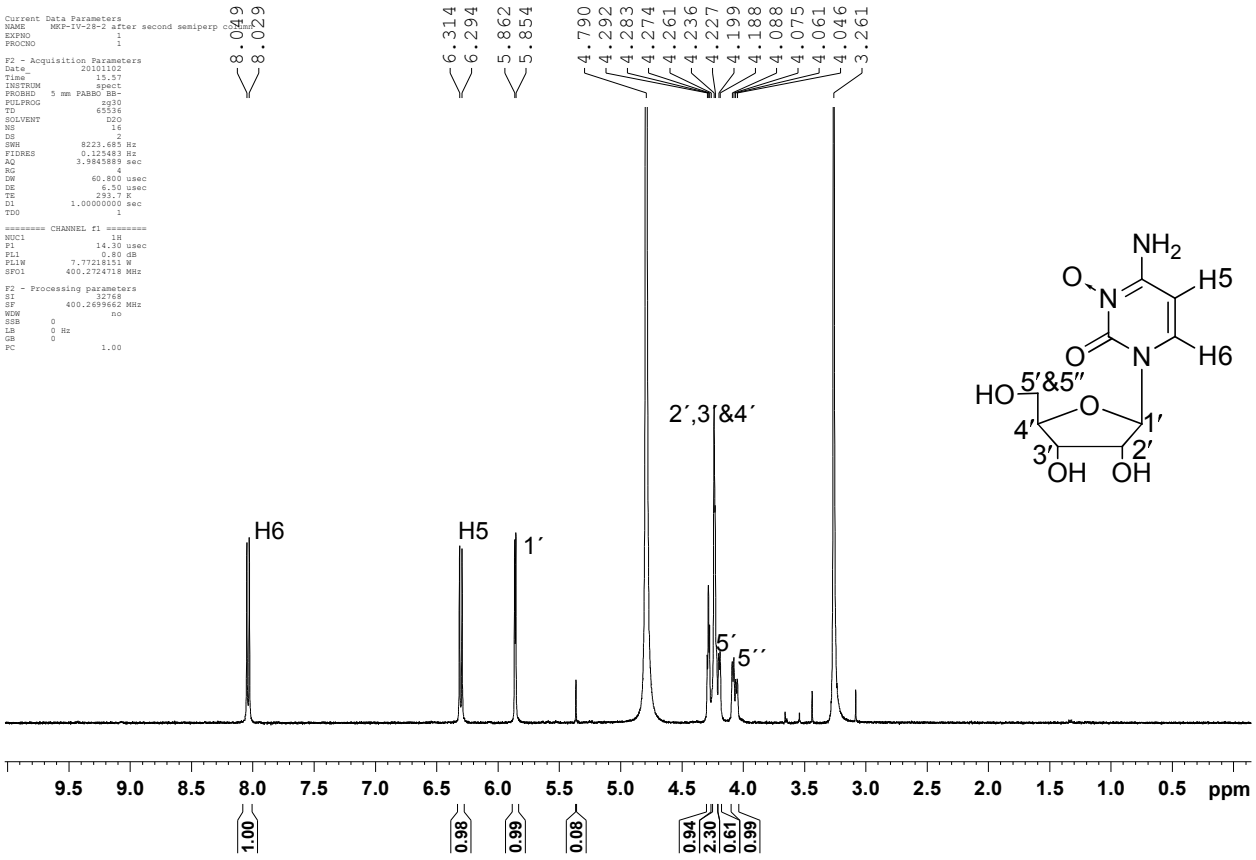


Figure S6. ^{1}H NMR spectrum for compound **15.**

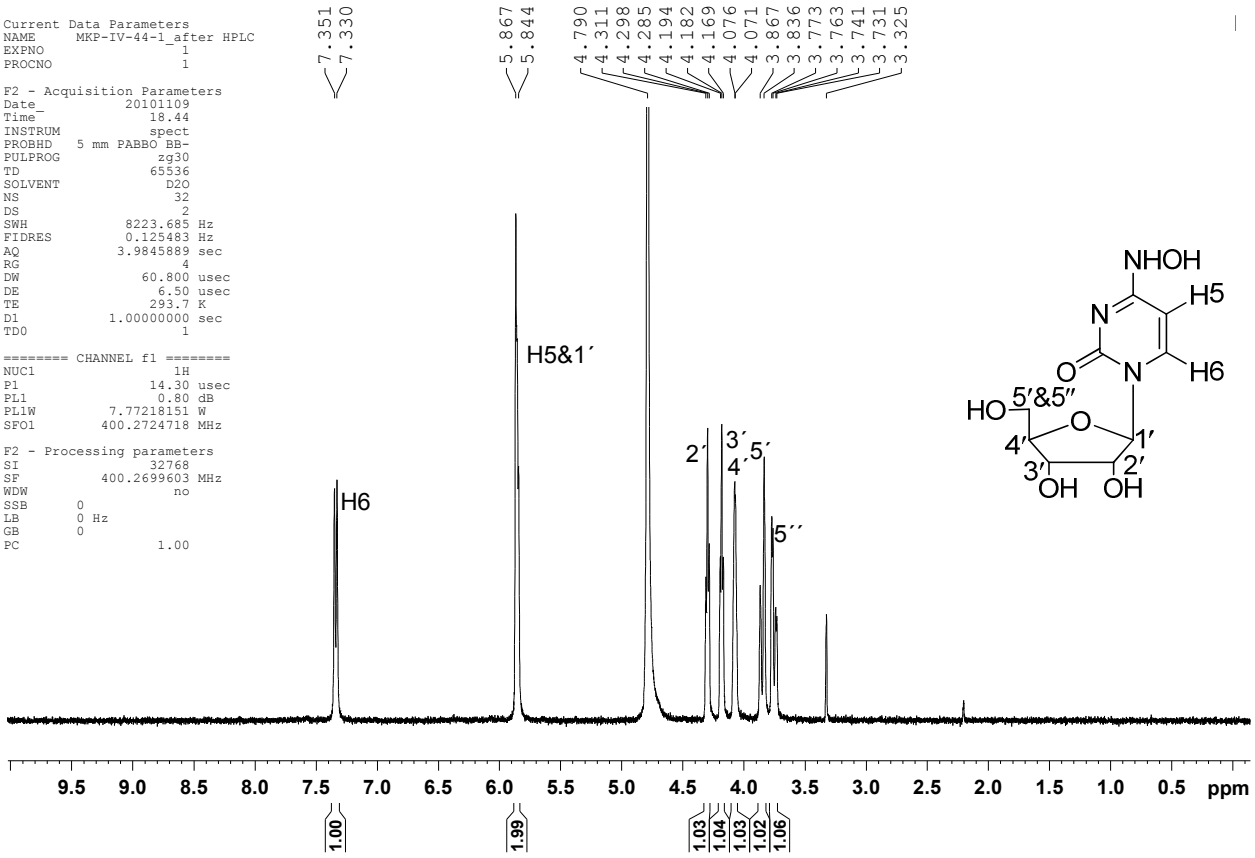


Figure S7. ^{1}H NMR spectrum for compound **14.**

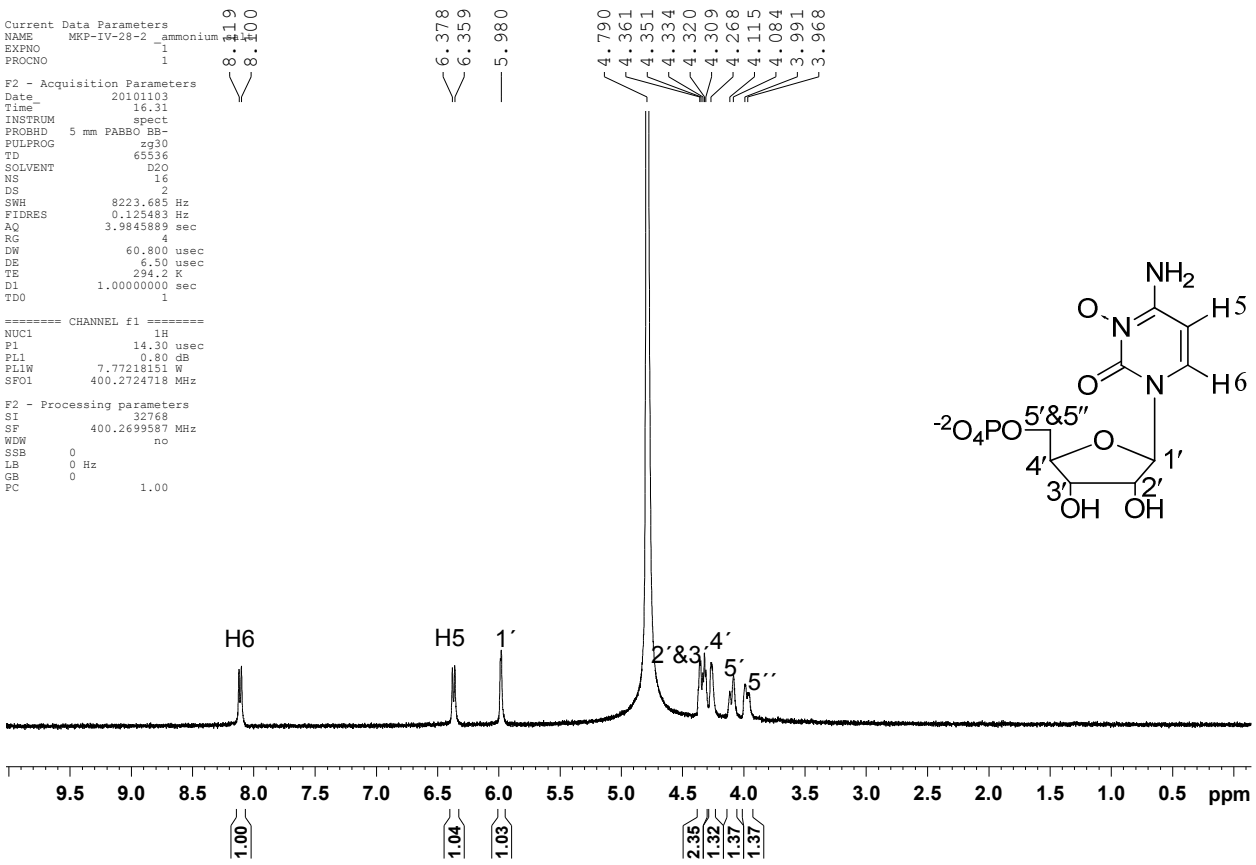


Figure S8. ^{1}H NMR spectrum for compound **27.**

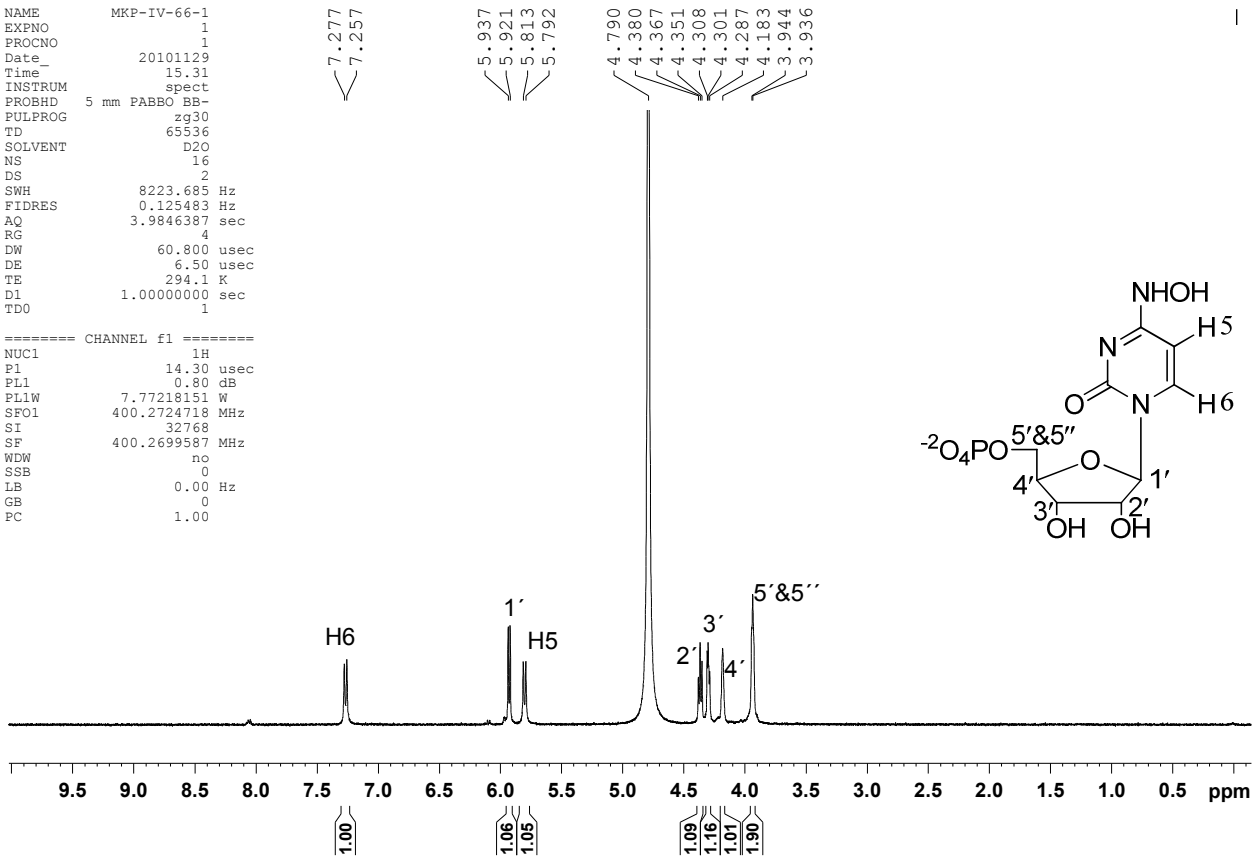


Figure S9. Mass spectrum for compound **27.**

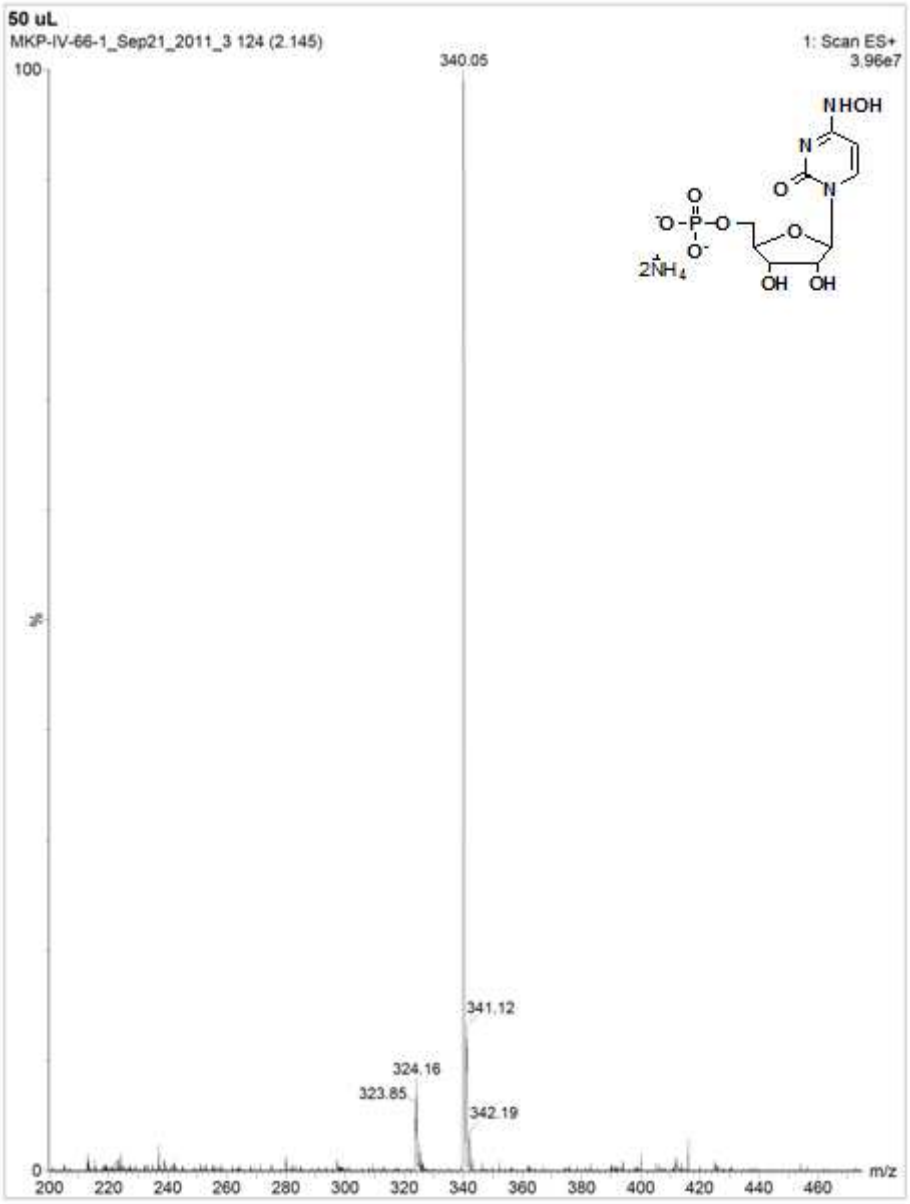


Figure S10. Mass spectrum for compound **15.**