SUPLEMMENTARY DATA FOR:

Nucleotide Sugar Pucker Preference Mitigates Excision by HIV-1 RT

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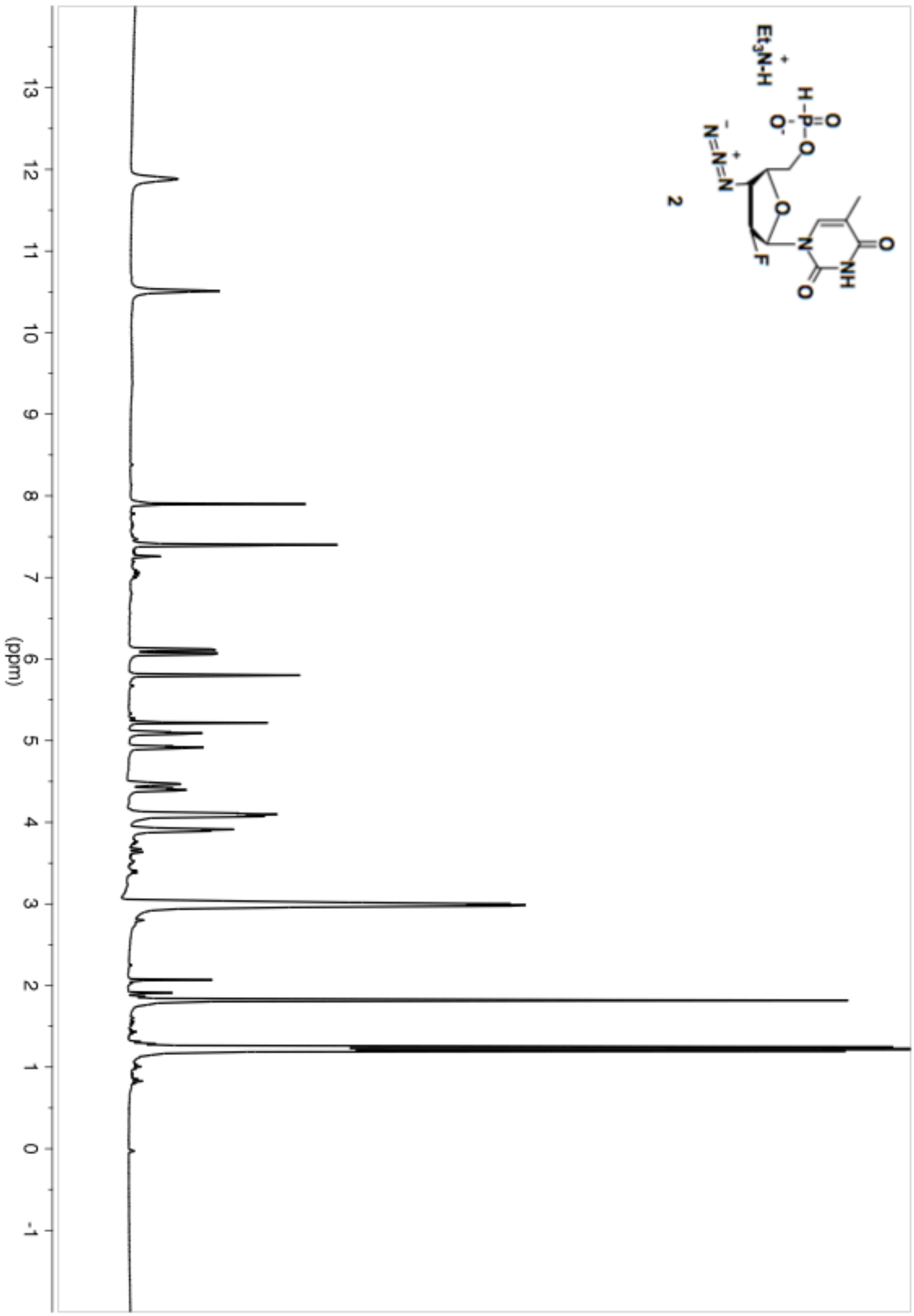
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(ppm)
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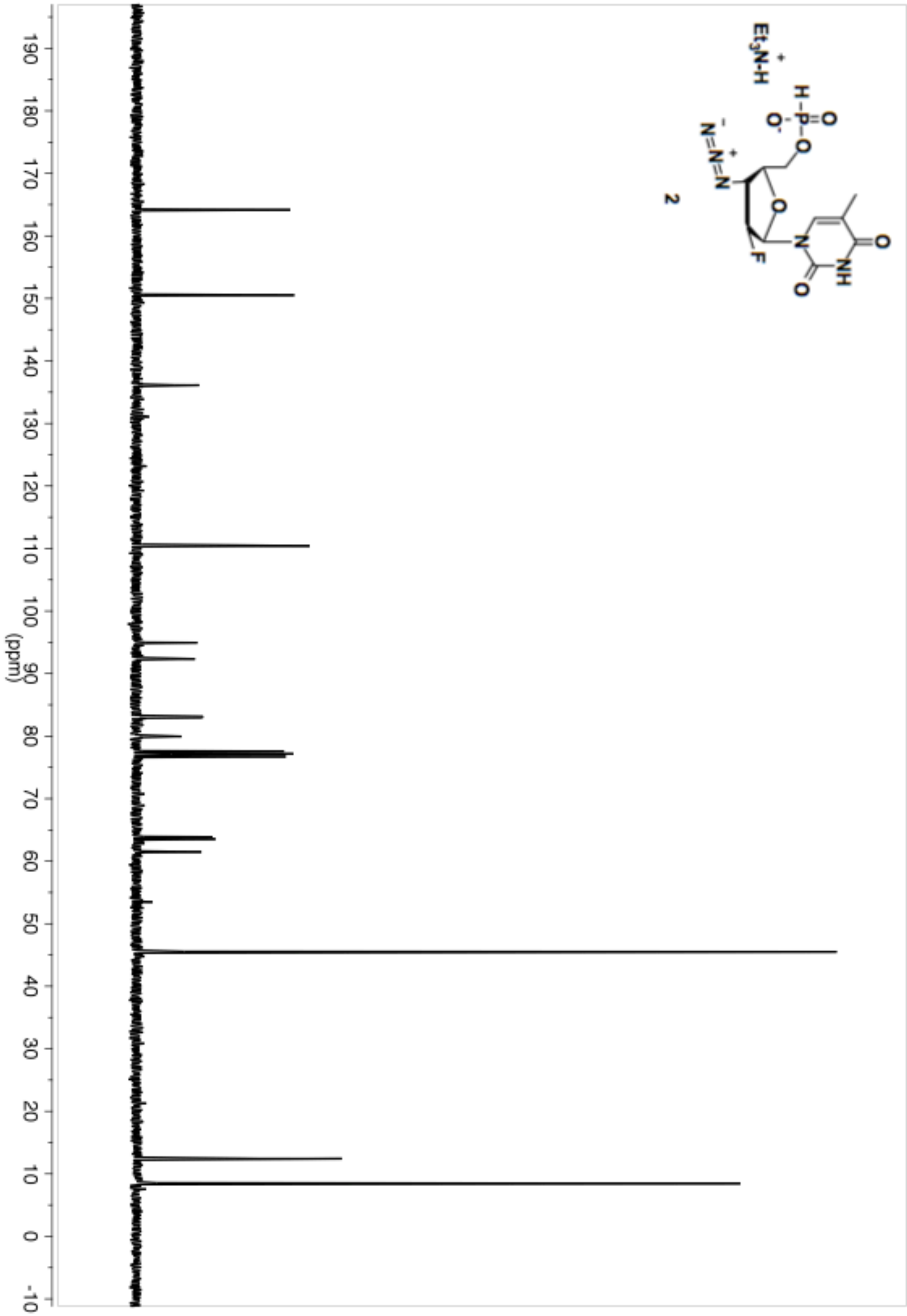
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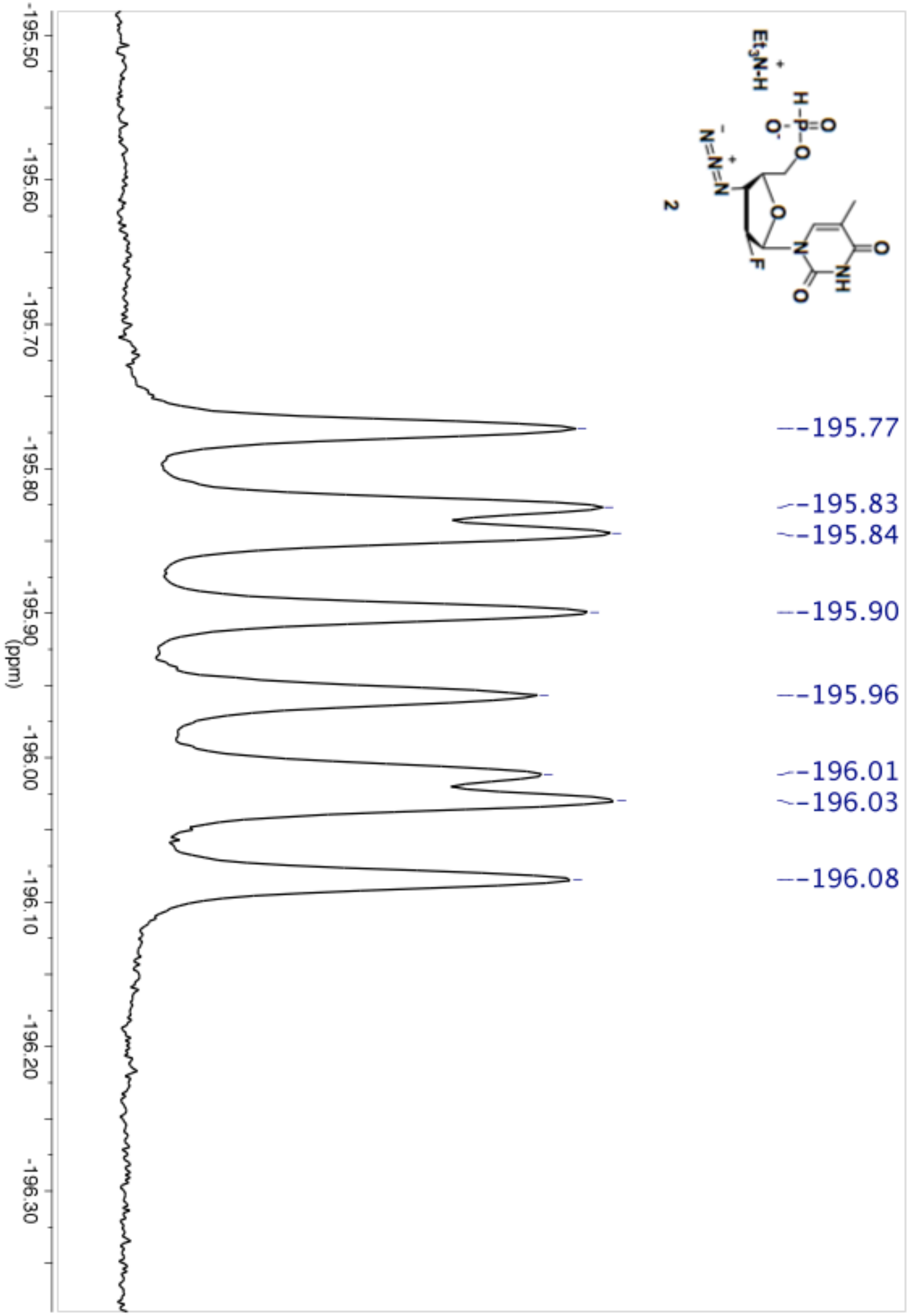
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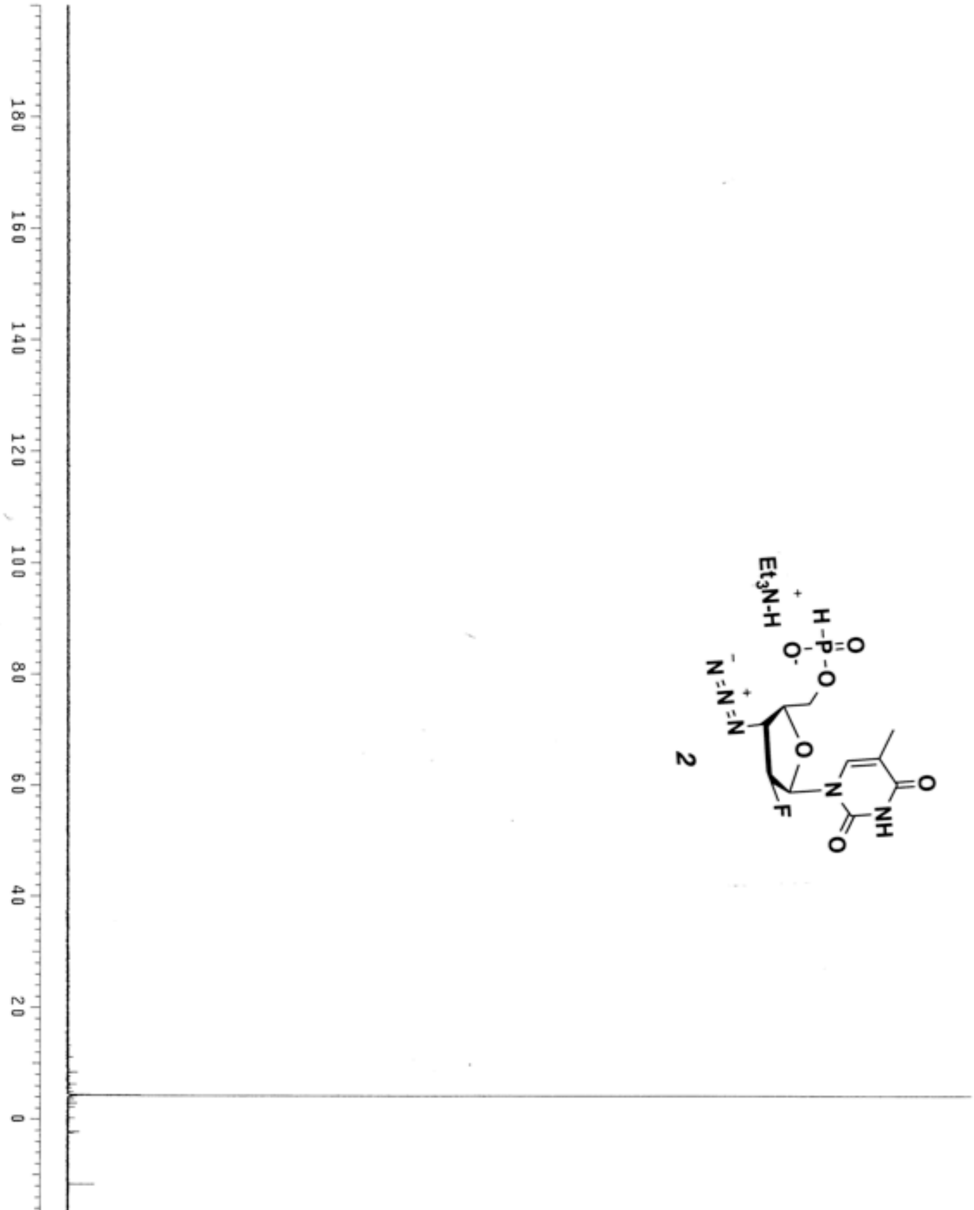

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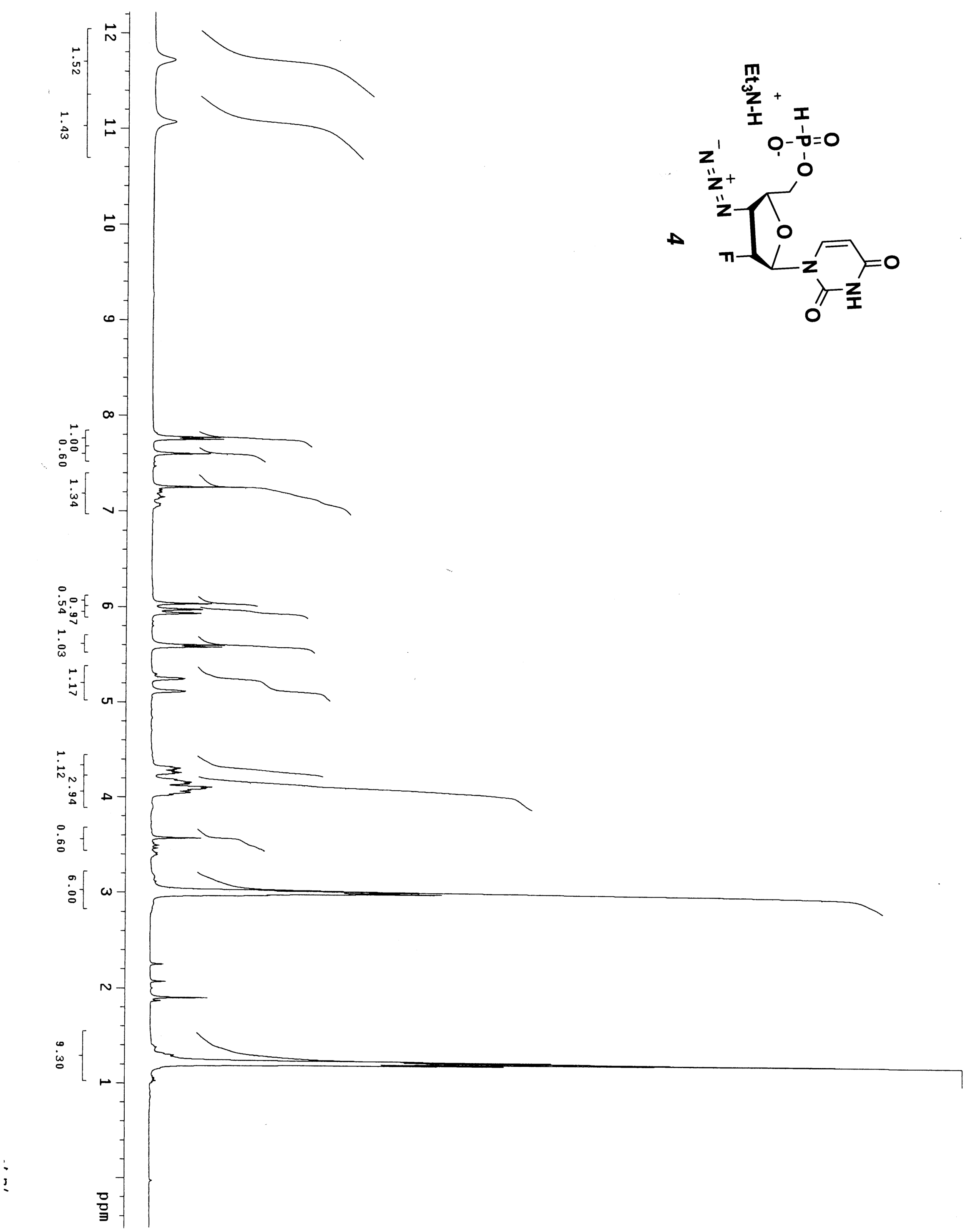
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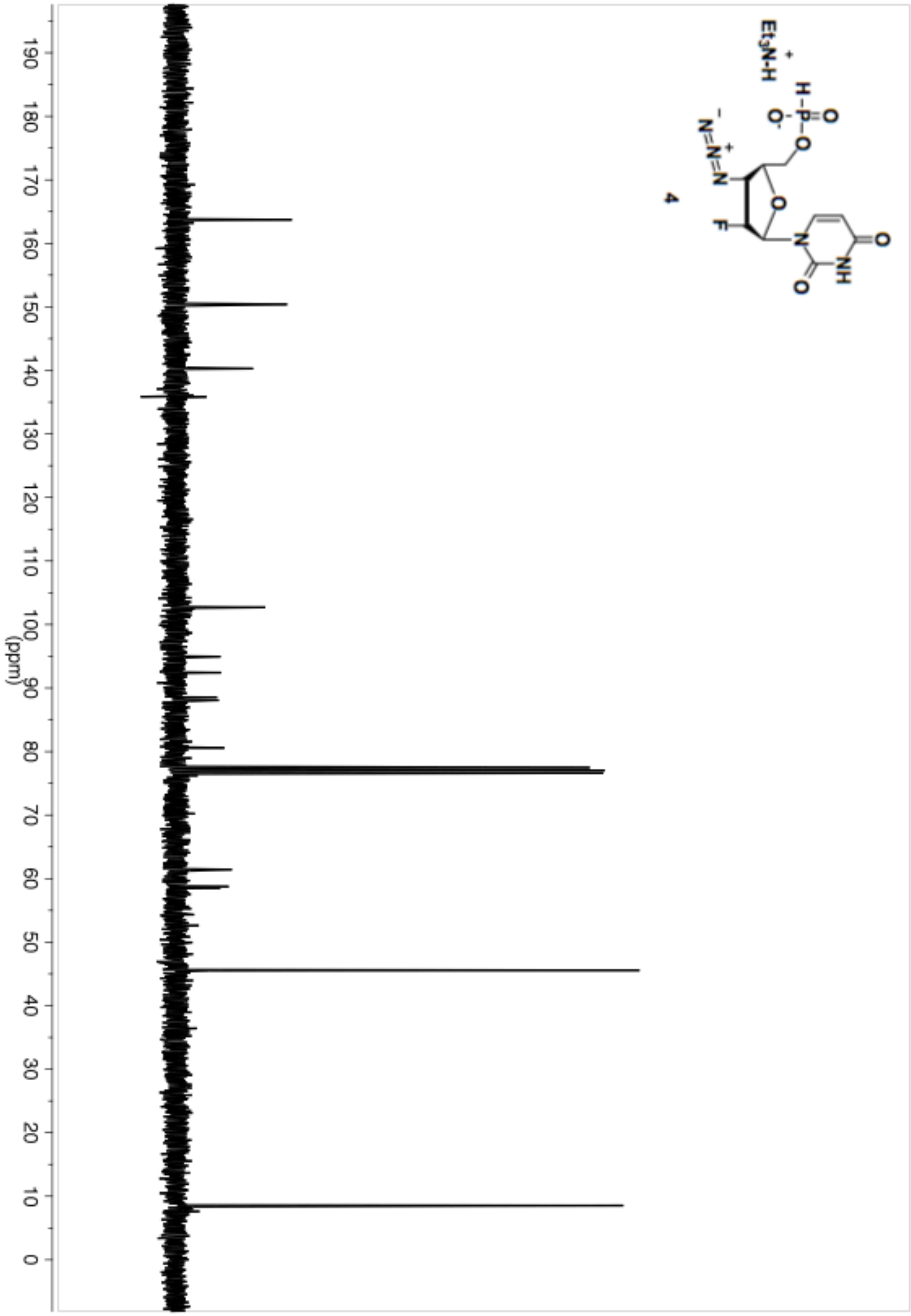

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N
O
H-P-O
O⁻
Et3N•H
N=N=N
F
4
(ppm)
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10
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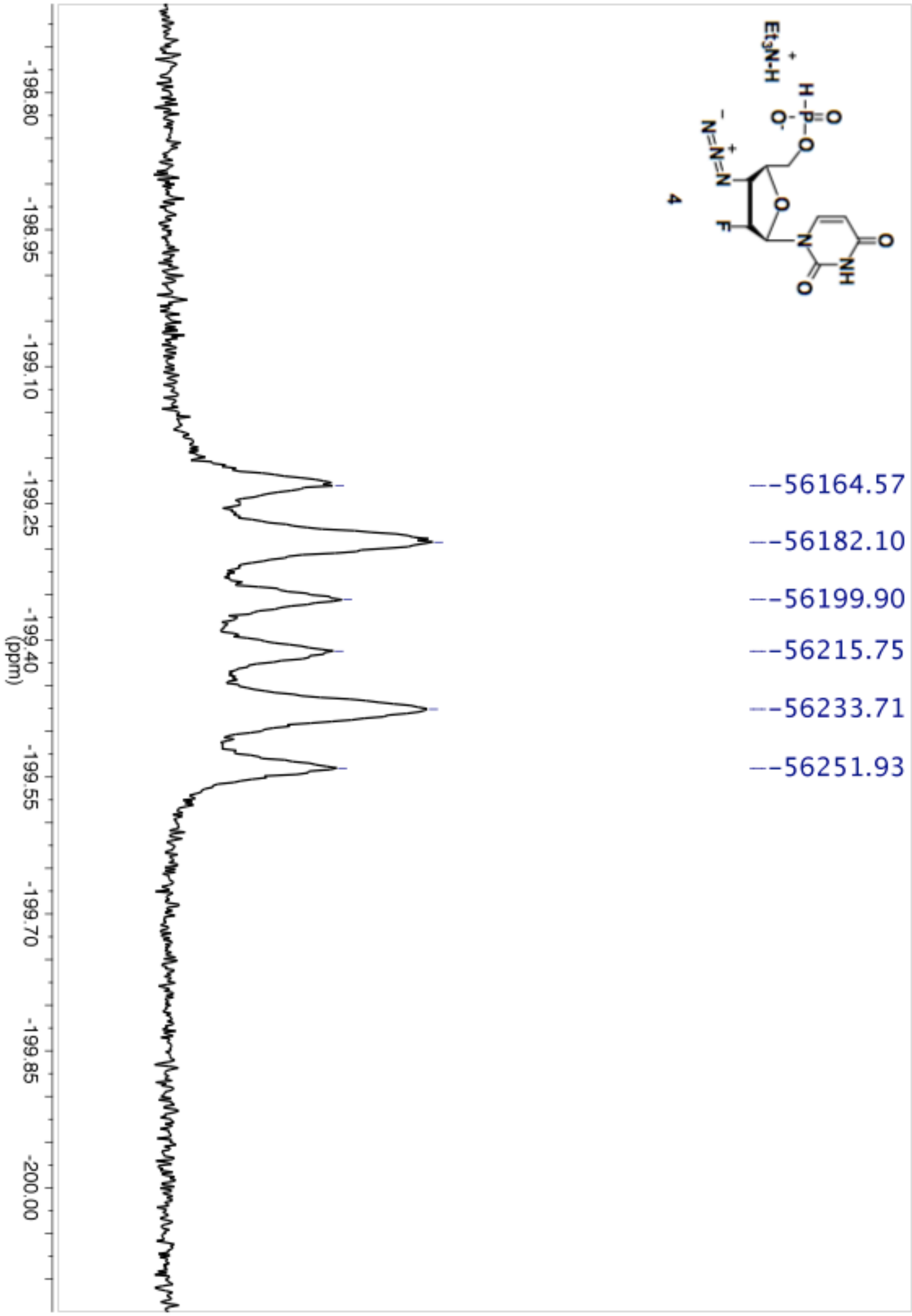

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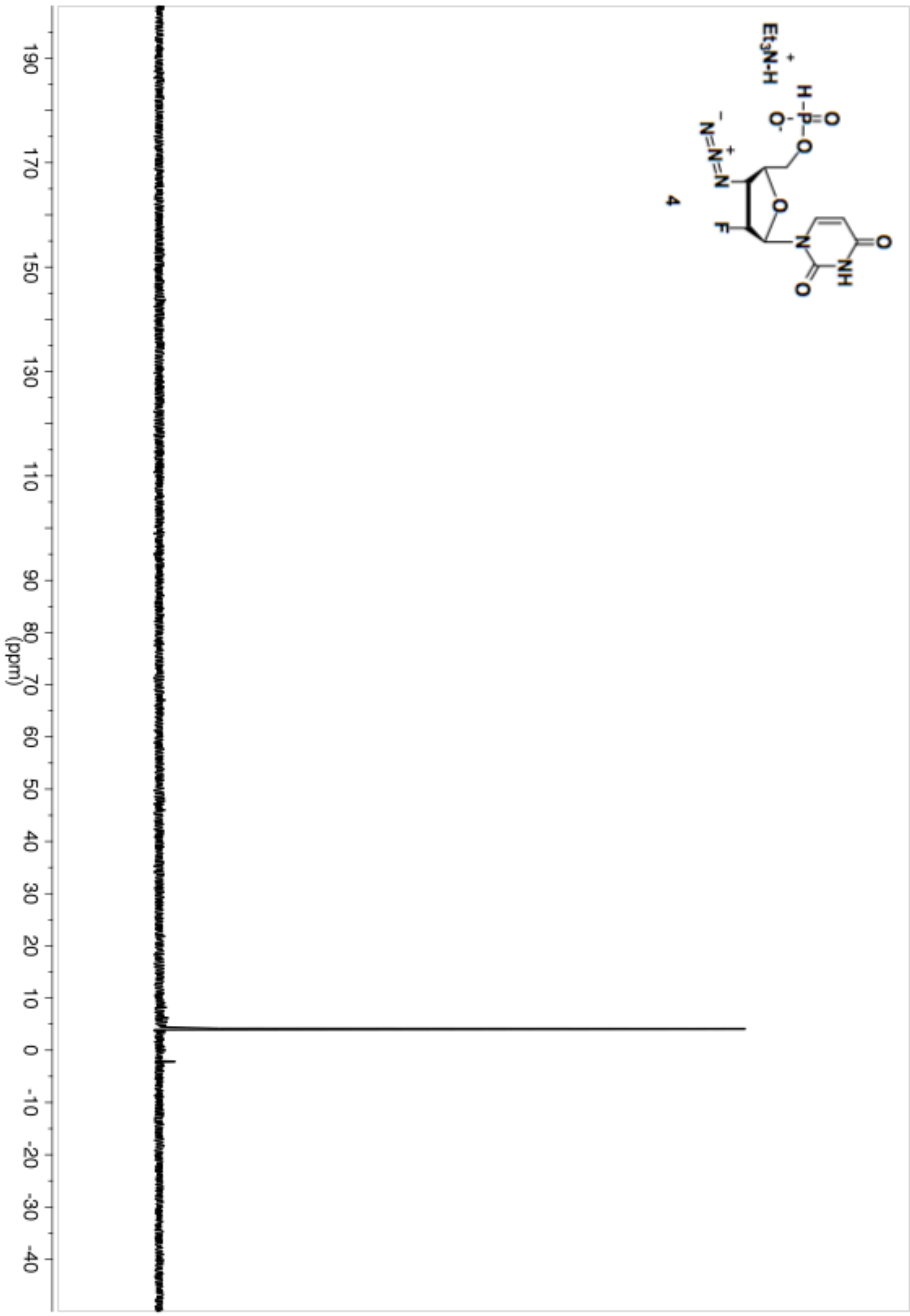
(ppm)

Table S1. Sequences and Mass data of synthetic primers used in assays.

Name	3'-terminal nucleotide (**X**)	Sequence (5' to 3')	Mass calcd	Mass found
G1	dT	5'- TTA AAA GAA AAG GGG GGA C**X**	6263.1	6263.3
G2	2′-araF-T	5'- TTA AAA GAA AAG GGG GGA C**X**	6281.1	6281.6
G3	dUr	5'- TTA AAA GAA AAG GGG GGA C**X**	6249.1	6248.1
G4	ara-Ur	5'- TTA AAA GAA AAG GGG GGA C**X**	6265.1	6264.3
G5	AZT	5'- TTA AAA GAA AAG GGG GGA C**X**	6285.1	6285.1
G6	2′-araF-AZT	5'- TTA AAA GAA AAG GGG GGA C**X**	6303.1	6303.1
G7	ddT	5'- TTA AAA GAA AAG GGG GGA C**X**	6247.1	6247.5
G8	Ur	5'- TTA AAA GAA AAG GGG GGA C**X**	6265.1	6264.9
G9	2′-F-Ur	5'- TTA AAA GAA AAG GGG GGA C**X**	6267.1	6267.0
G10	2′-F-3′-azido-Ur	5'- TTA AAA GAA AAG GGG GGA C**X**	6292.1	6292.1
G11	2′,4′-diF-Ur	5'- TTA AAA GAA AAG GGG GGA C**X**	6285.1	6284.8
G12	LNA-T	5'- TTA AAA GAA AAG GGG GGA C**X**	n.a.*	
G13	seco-Ur	5'- TTA AAA GAA AAG GGG GGA C**X**	6267.1	6266.2
G14	Oxepane-T	5'- TTA AAA GAA AAG GGG GGA C**X**	6278.1	6278.1

Primer G12 was purchased from Exiqon.

Table S2. Correlation between sugar conformation, excision, and translocation

Primers	Nucleoside[a]	Northern P[b]	Southern P[b]	% North[c]	Excision by AZTr-RT	Translocation % Pre (AZTr-RT) [d]
G12	LNA-T	17	n.a.	100	-	43
G11	2',4'-diF-Ur	18	n.a.	100	-	30
G9	2'-F-Ur	21	159	87	-	25
G10	2'-F-3'-N_3-Ur	17	155	85	-	30
G7	ddT	11	154	75	++	58
G8	Ur	18	162	58	-	21
G5	AZT	22	160	50	+++	75
G4	ara-Ur	22	151	46	+	38
G6	2'-araF-AZT	11	120	40	++	75
G3	dUr	18	162	39	++++	71
G1	dT	n.a.	n.a.	37	++++	66
G2	2’-araF-T	-6	126	31	+++	72
G13	seco-Ur	n.a.	n.a.	n.a.	-	26
G14	oxepane-T	n.a.	n.a.	n.a.	-	30

Legend: - no excision, + moderate excision, ++ fast excision, +++ very fast excision, ++++ ultra fast excision. [a] Nucleoside sugar conformational preferences were obtained from indicated references shown in the main manuscript. [b] Northern (Southern) P is indicative of the pseudorotational angle of the nucleoside in the most stable form of the north (south) conformation. [c] %N = 100 - %S; for ribonucleosides, 2'-(α)-fluoro-2'-deoxyribonucleosides and ddT: %S = 10 $J_{1'2'}$; for deoxyribonucleosides, 2'-(β)-fluoro-2'-deoxyribonucleosides and 2'-ara-Ur: %S = 100 [$J_{1'2'}$ *(cis)* + $J_{1'2'}$ *(trans)* – 7.1] / 9. [d] for South conformers and AZT: % Pre = Pre/[Pre + Post] x100 for North conformers, seco-Ur and oxepane-T :%Pre = Pre/[ex-Pre + Pre + Post] x100.

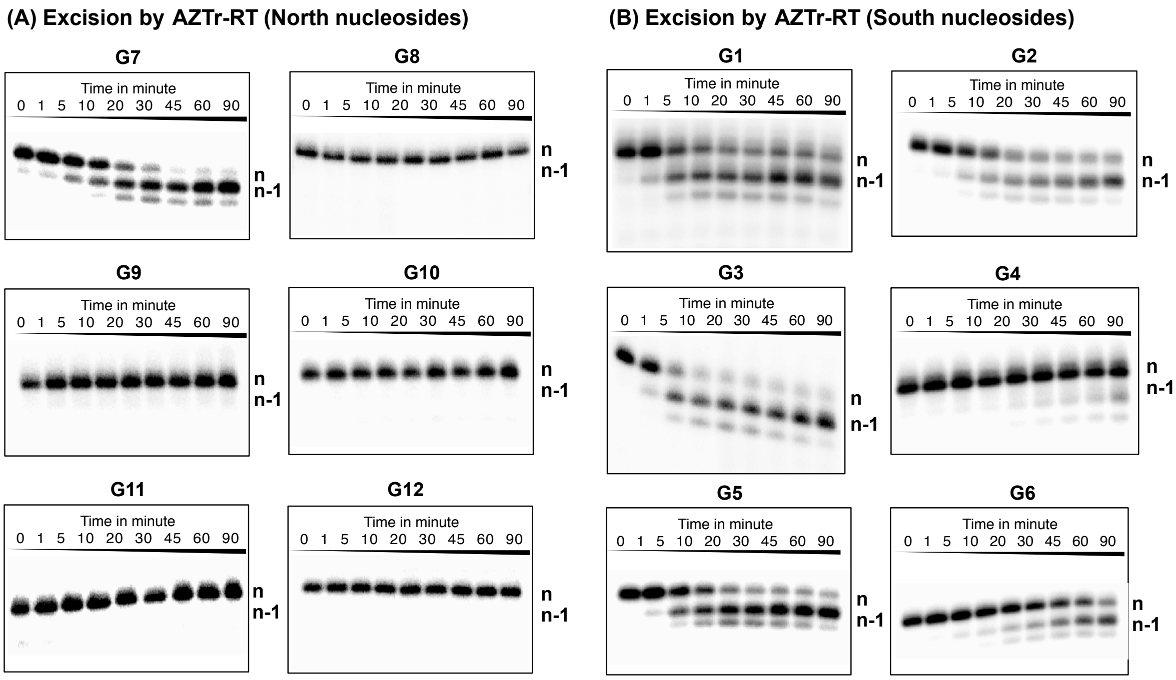

Figure S1. Nucleotide Excision by AZTr-RT. Aliquots were taken at each time points (1, 5, 10, 20, 30, 45, 60 and 90 min, respectively). For the control experiment, aliquots were taken before mixing "ATP-mix" solution, which were used as aliquot at 0 min.

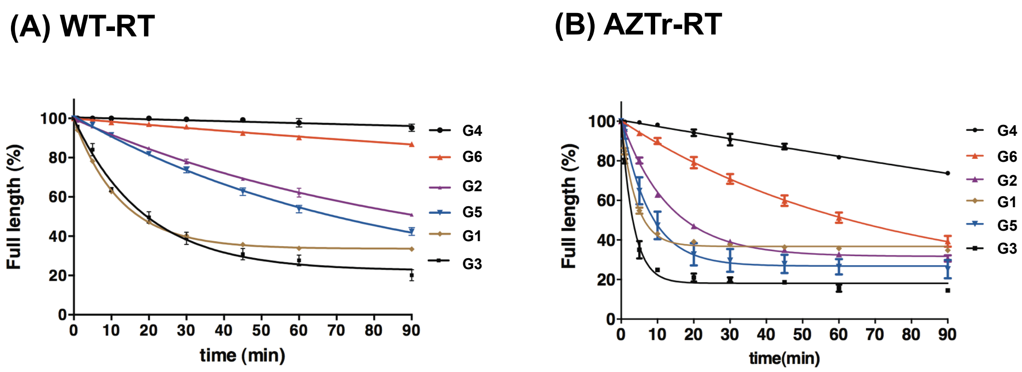

Figure. S2 Quantitative analysis of nucleotide excision of predominantly South-biased conformers.

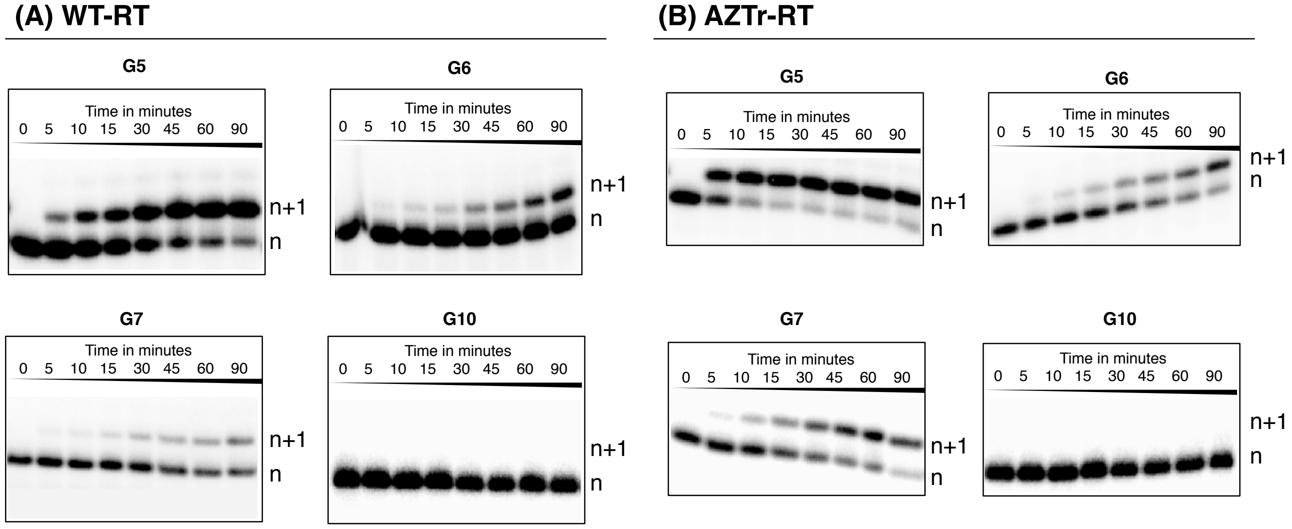

Figure S3. PAGE of rescue assay of primers G5, G6, G7, and G10.

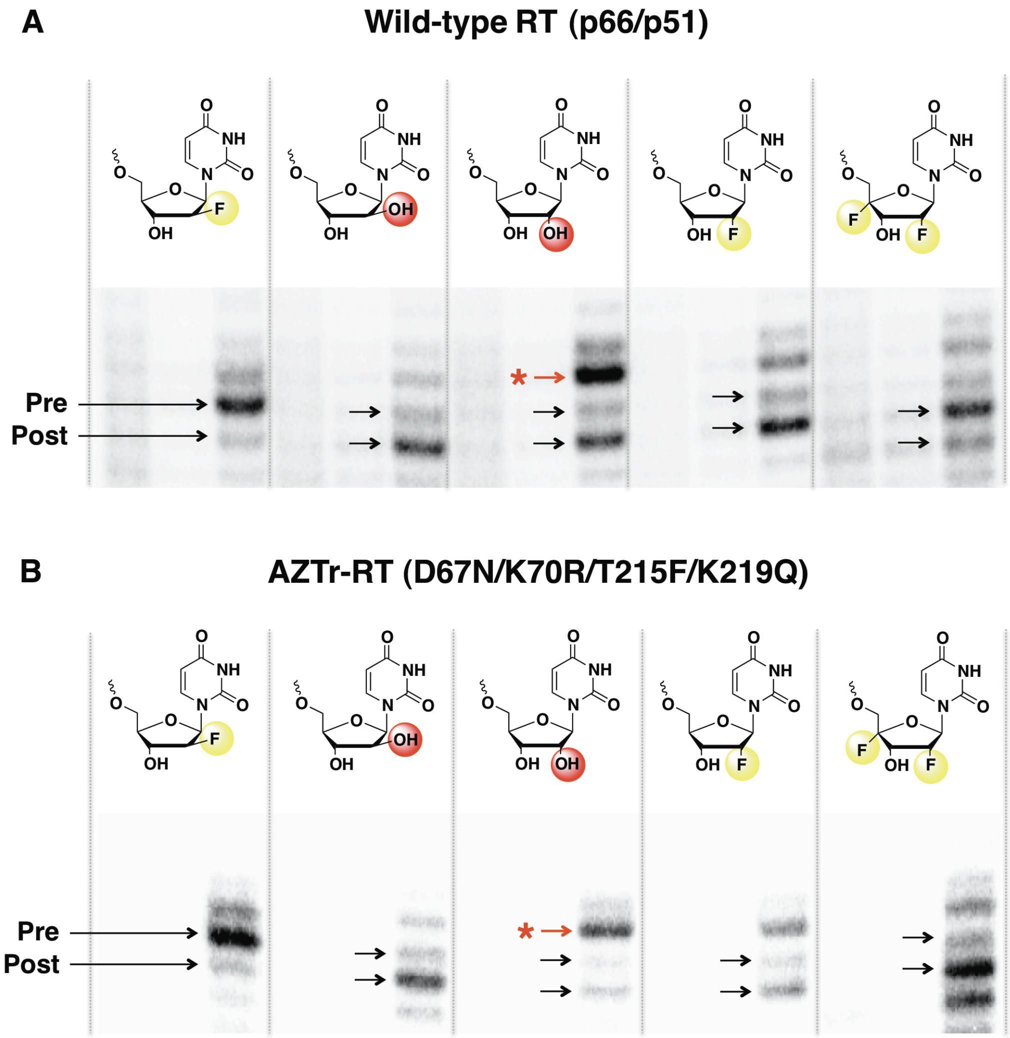

Figure S4. KOONO-mediated site-specific footprinting assay of Primer G2, G4, G8, G9, and G11. Primer G8 containing Ur induced significant ex-Pre translocation in both case (A) WT-RT and (B) AZTr-RT.

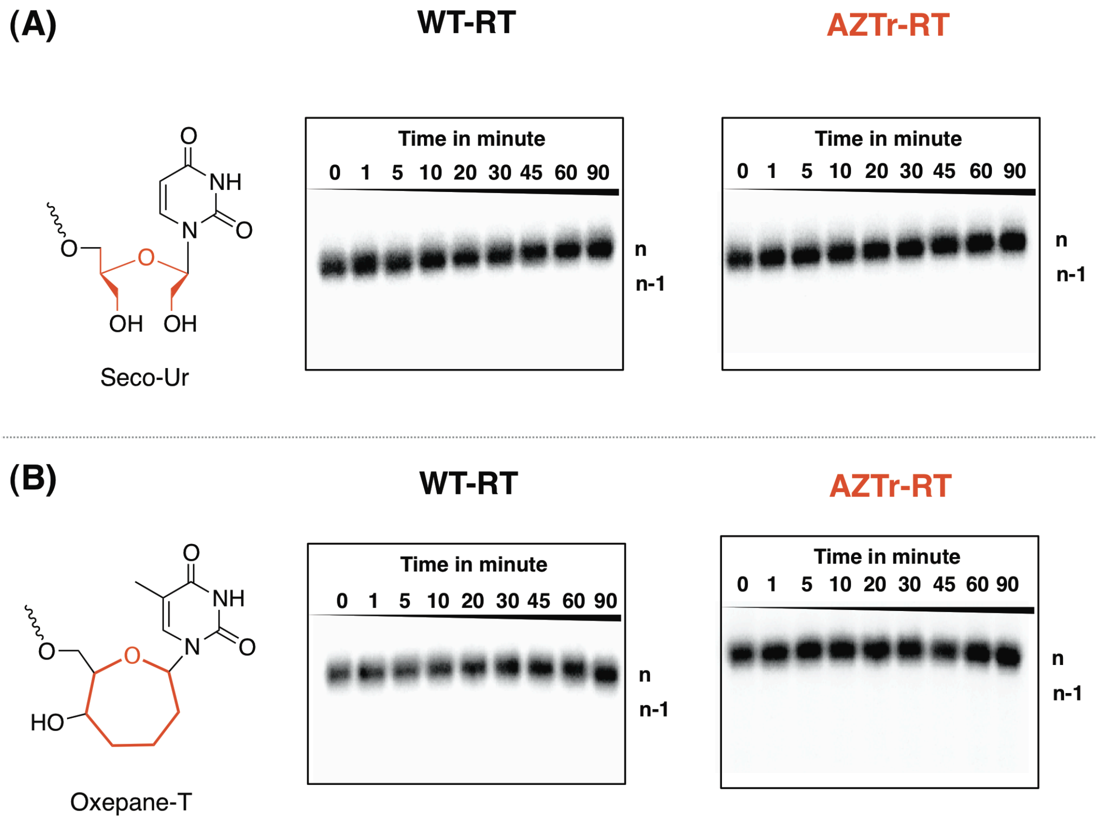

Figure S5. Nucleotide excision of seco-Ur and Oxepane-T by WT-RT (A) or AZTr-RT (B). No significant excised products were observed on PAGEs.

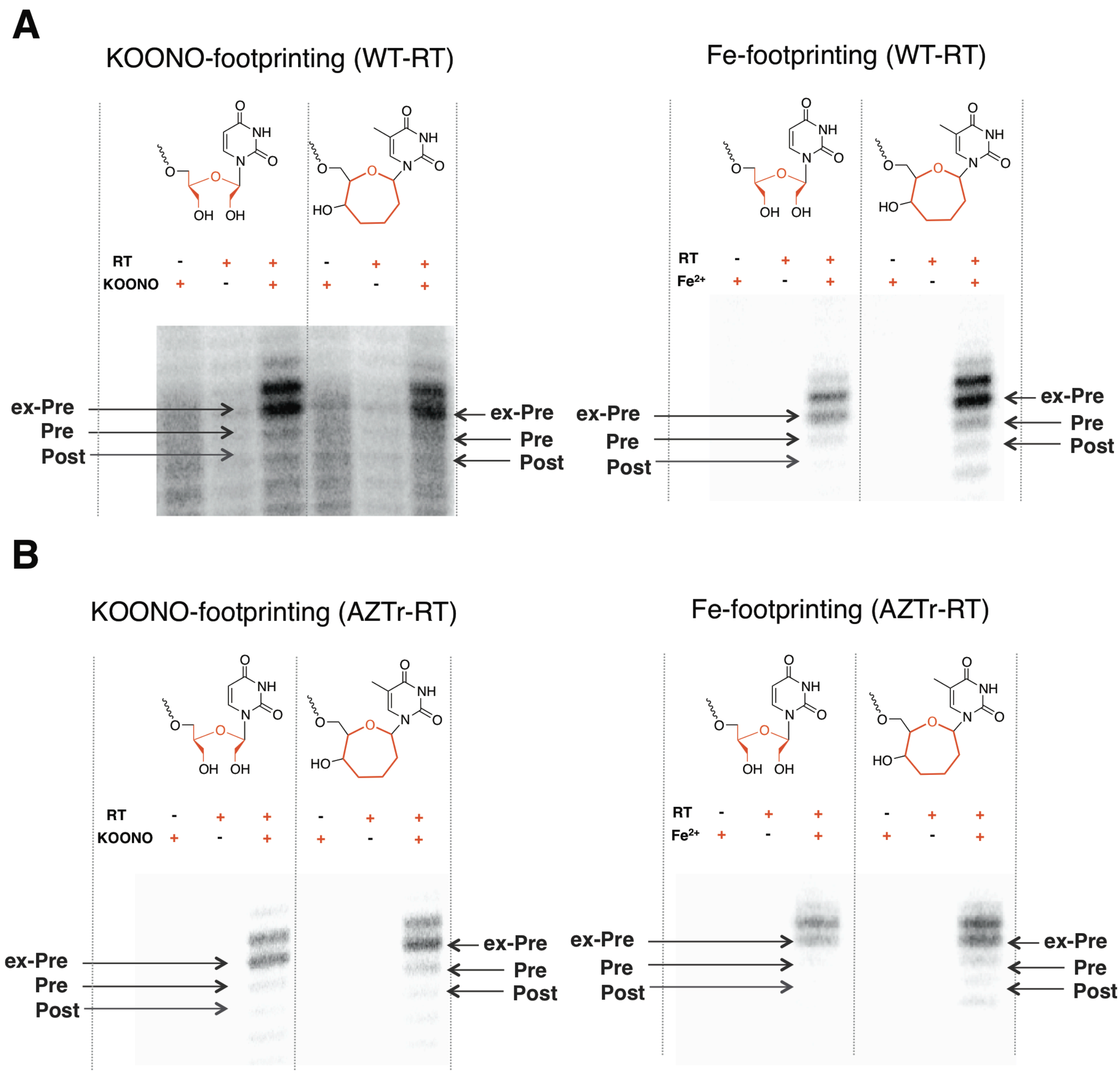

Figure S6. KOONO or Fe-mediated site-specific footprinting assays for seco-Ur (G13) and Oxepane-T (G14). (A) WT-RT, (B) AZTr-RT.

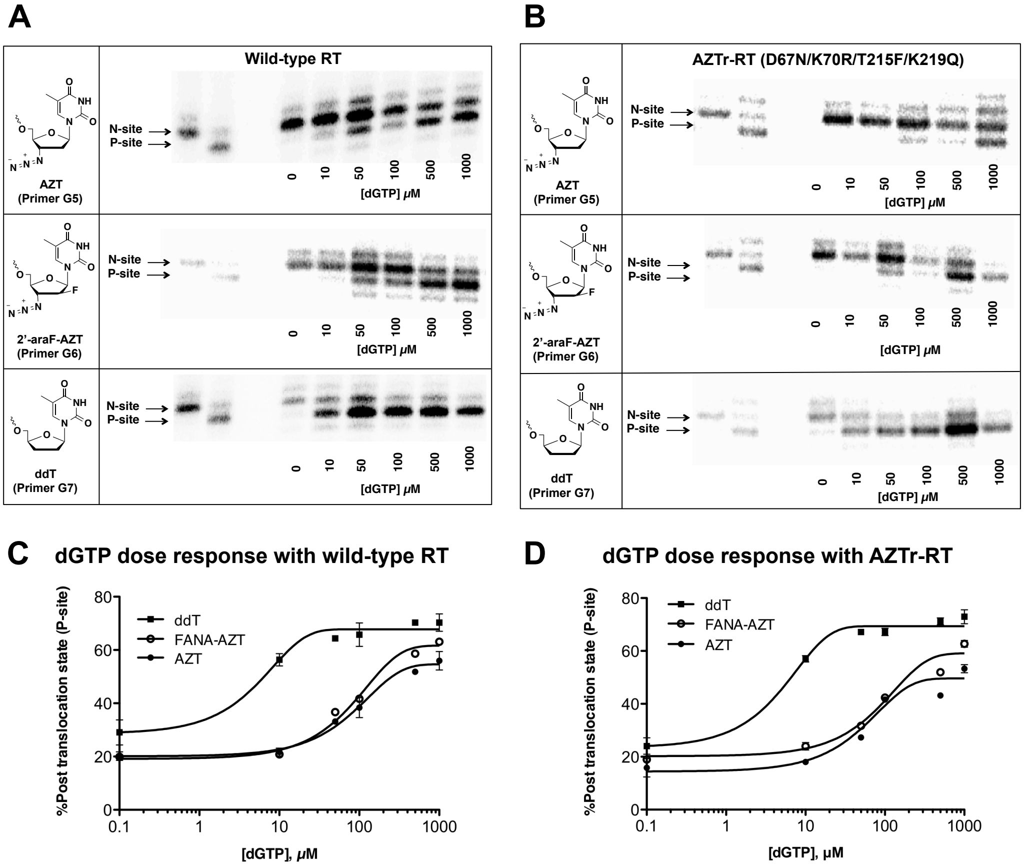

Figure S7. (A, B) Fe-mediated footprintings performed in a different range of concentration of next-incoming dGTP. PAGEs of footprintings of complexes containing AZT, 2'-araF-AZT, or ddT. (C, D) Graphical representation data of data shown in (A) and (B).

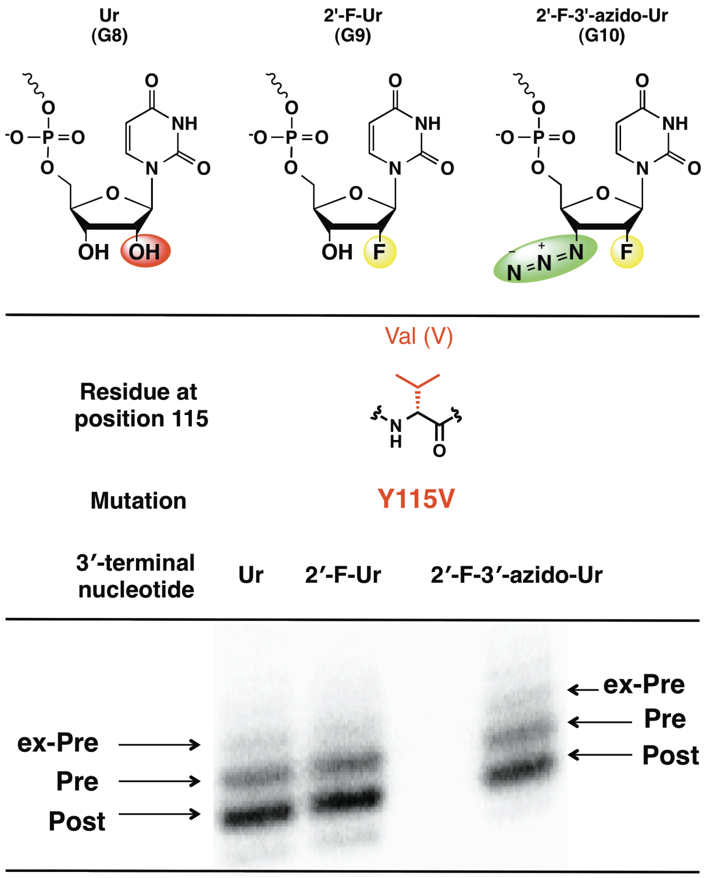

Figure S8. Translocation studies of primer G8, G9, and G10 using mutant RT (Y115V). KOONO-mediated site-specific footprintings were carried out in the same condition as the footprinting assay for wild-type RT or AZTr-RT.

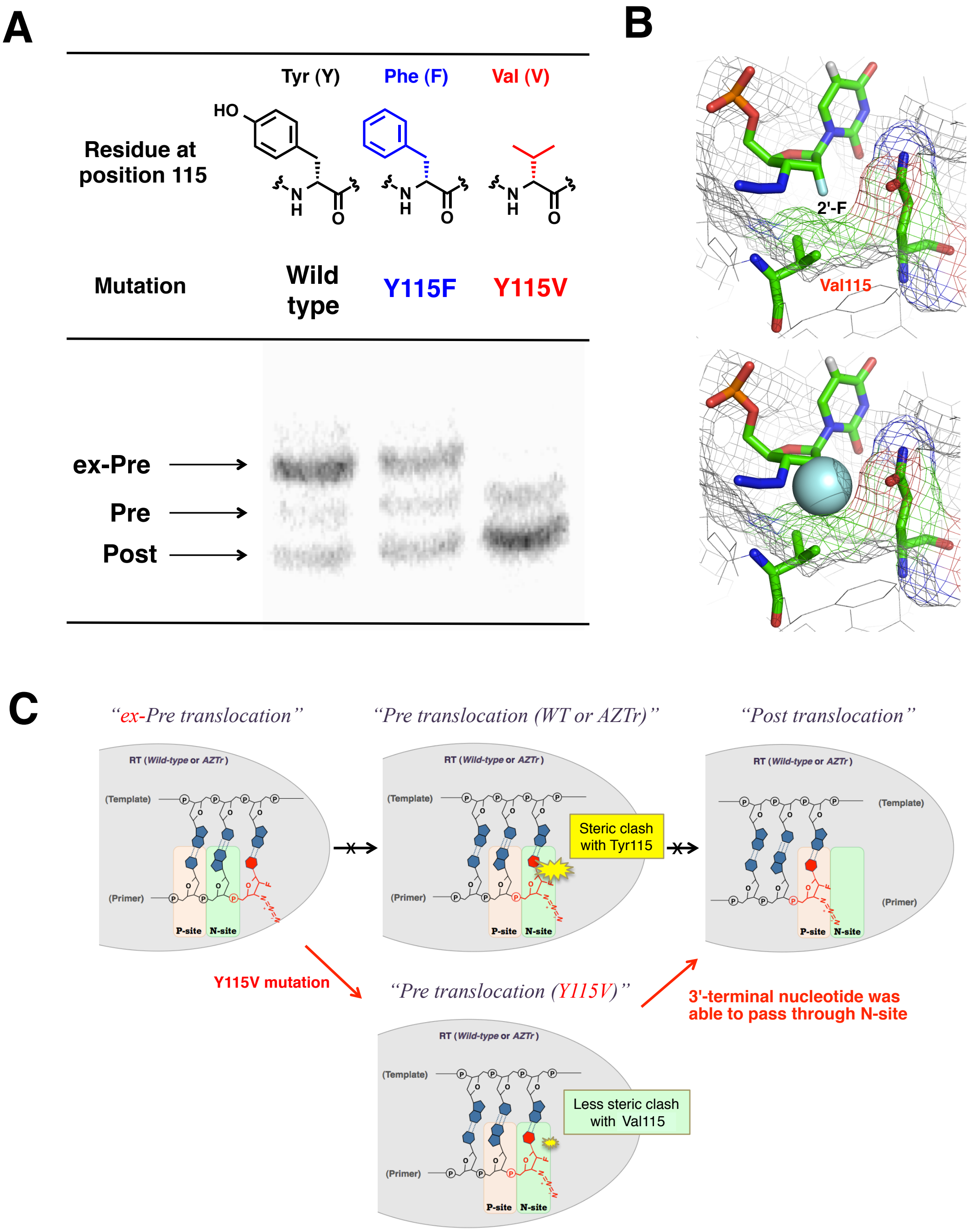

Figure S9. Comparative studies of translocation of 2'-F-3'-azido-Ur (G10) among wild-type-RT, Y115F, and Y115V. (A) Only the case of Y115V, significant amount of Post-translocation was detected on the gel. (B) Modeling of complexes of AZT-terminated DNA primer/DNA template/RT (*wild-type*) in the structure of the pre-translocation complex where the AZT and Tyr115 were transformed to 2'-F-3'-azido-Ur and valine, respectively. In contrast to the modeling in Figure 6, the modeling indicated the less steric clashes between 2'-fluorine and the isopropyl group of valine residue. (C) Proposed mechanism of forming Post-translocation of G10 with Y115F-mutant R