Differentiating positional isomers of nucleoside modifications by higher-energy collisional dissociation mass spectrometry (HCD MS)

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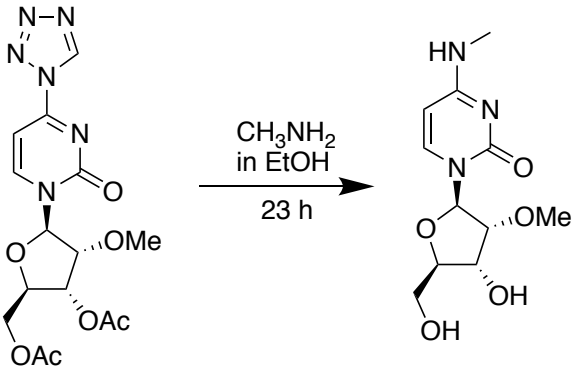
Supplemental Information

Experimental

N^4,2'-O-dimethylcytidine (m^4Cm) synthesis



1 **2**

1 - (4-(tetrazol-1-yl)-1-(3',5'-di-O-acetyl-2'-O-methyl-β-D-ribofuranosyl) pyrimidine-2-(1H)-one)
2 - N^4-,2'-O-dimethylcytidine (m^4Cm)

Compound **1** was prepared by previously described methods,[1] except that 0.45M tetrazole in anhydrous acetonitrile solution was used instead of solid tetrazole. All crude products were co-

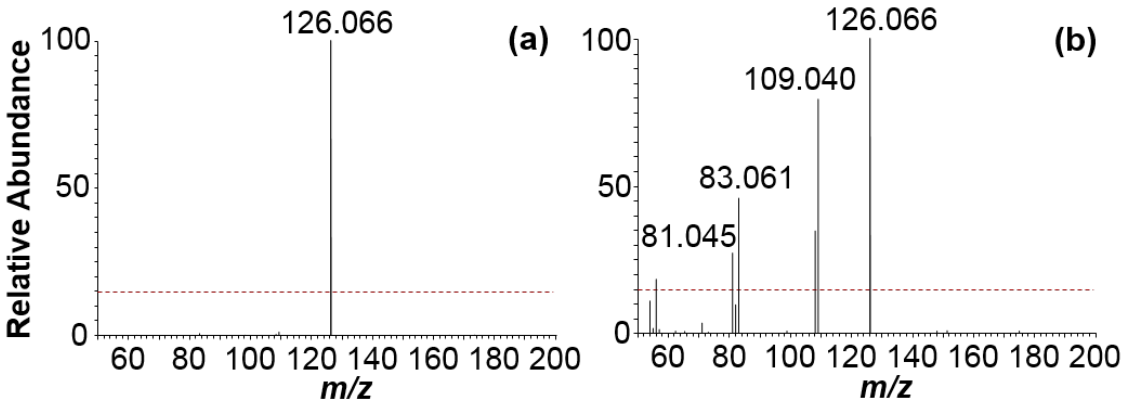
evaporated with dichloromethane prior to column chromatography. Products were formed in similar yields.

Compound **1** (370 mg, 0.93 mmol) was dried under high vacuum for 15 min and backfilled with Argon. Methylamine in ethanol (33%, 10 mL) was added via syringe and the mixture was stirred at room temperature for 23 h. Removal of all volatiles lead to formation of a brown syrup. The syrup was co-evaporated first with methanol (1 x 10 mL) followed by dichloromethane (2 x 10 mL). The crude product was further purified by silica gel column chromatography with 10% methanol in dichloromethane yielding 203 mg of m^4Cm (compound **2**, 80%) as a white solid. Spectroscopic data agreed with reported literature values.[1]

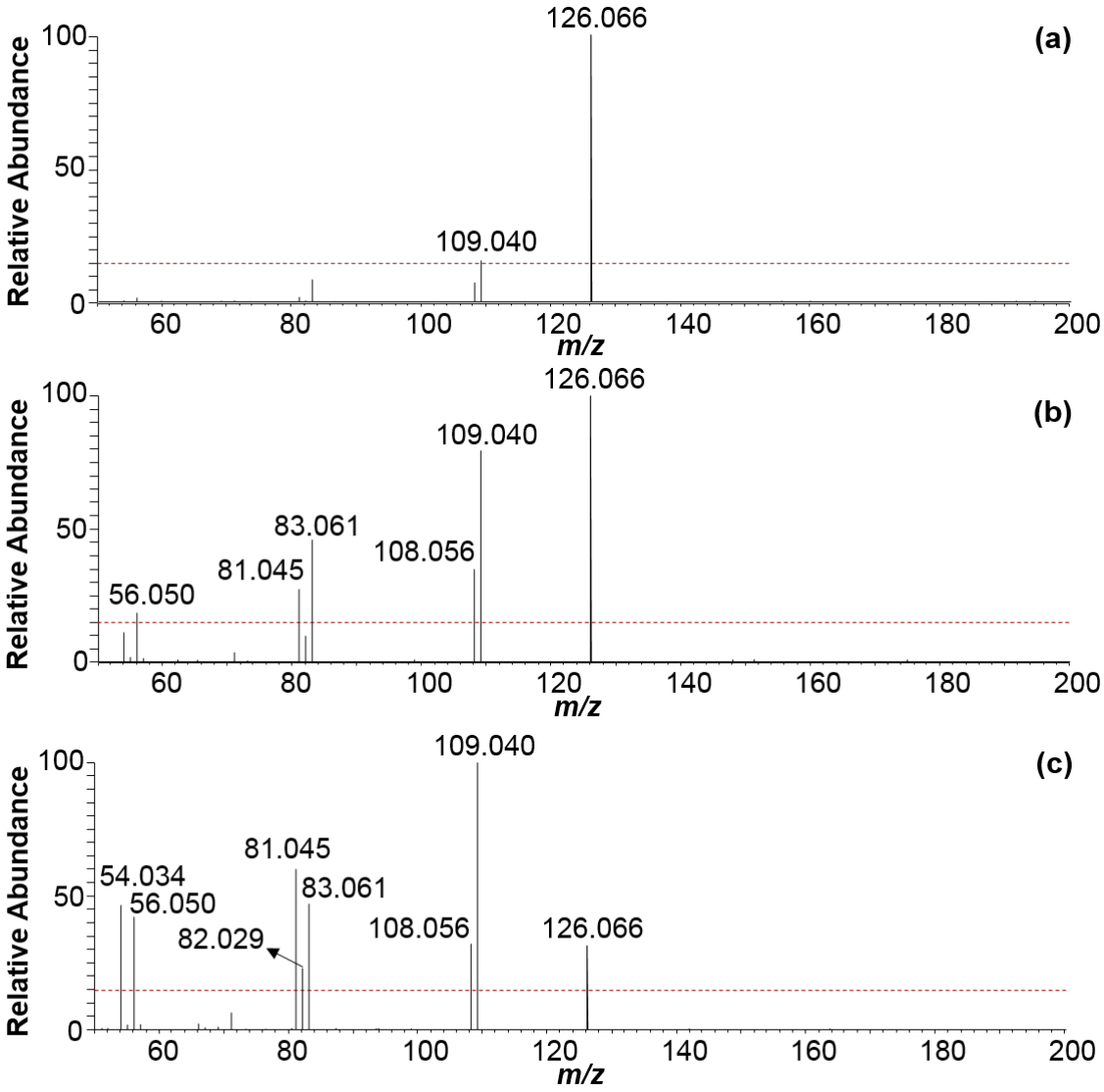
References

1. Mahto, S.K., Chow, C.S.: Synthesis and solution conformation studies of the modified nucleoside N(4), 2′-O-dimethylcytidine (m(4)Cm) and its analogues. Bioorganic Med. Chem. **16**, 8795-8800 (2008)

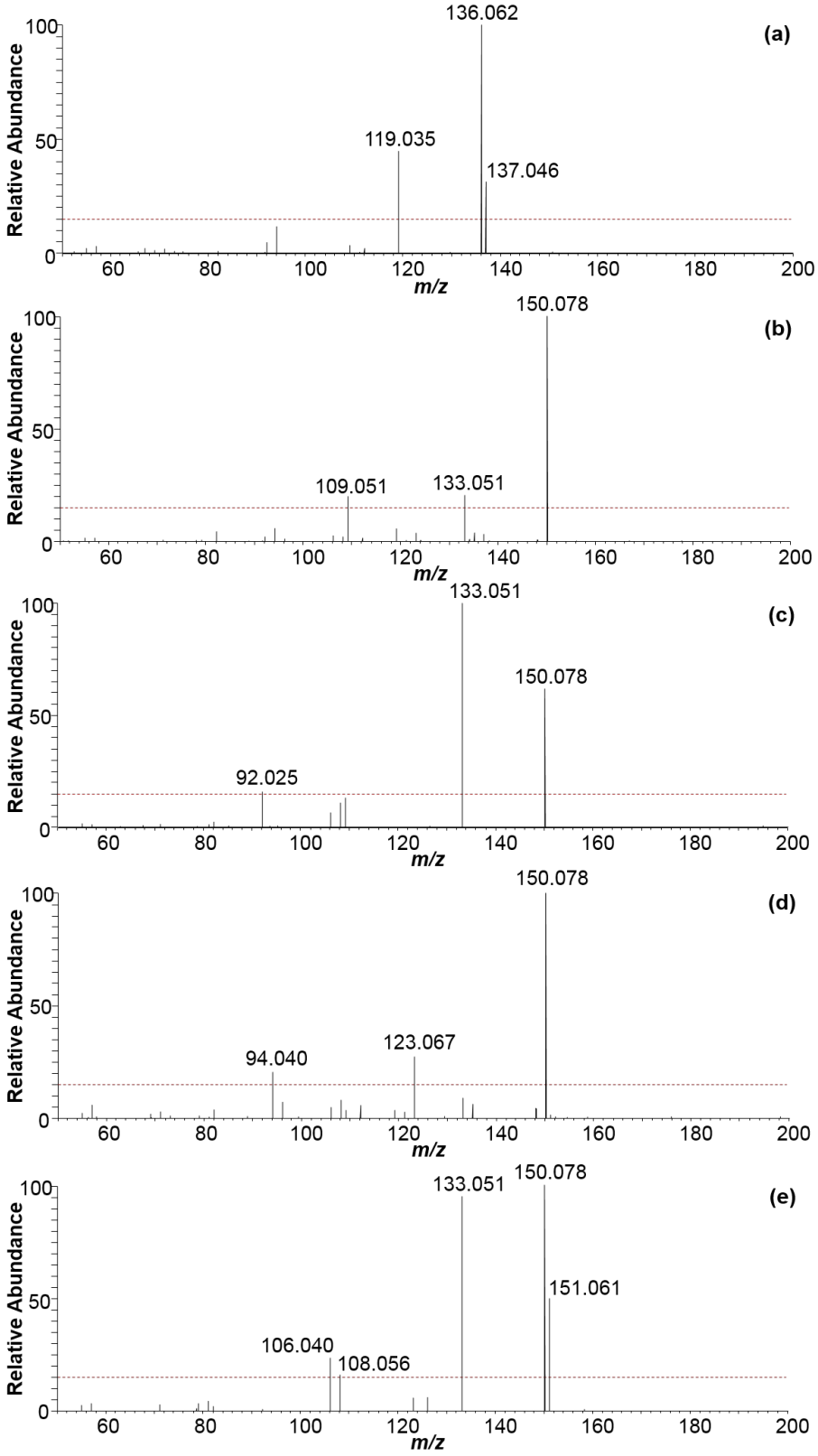
Supplemental Figures



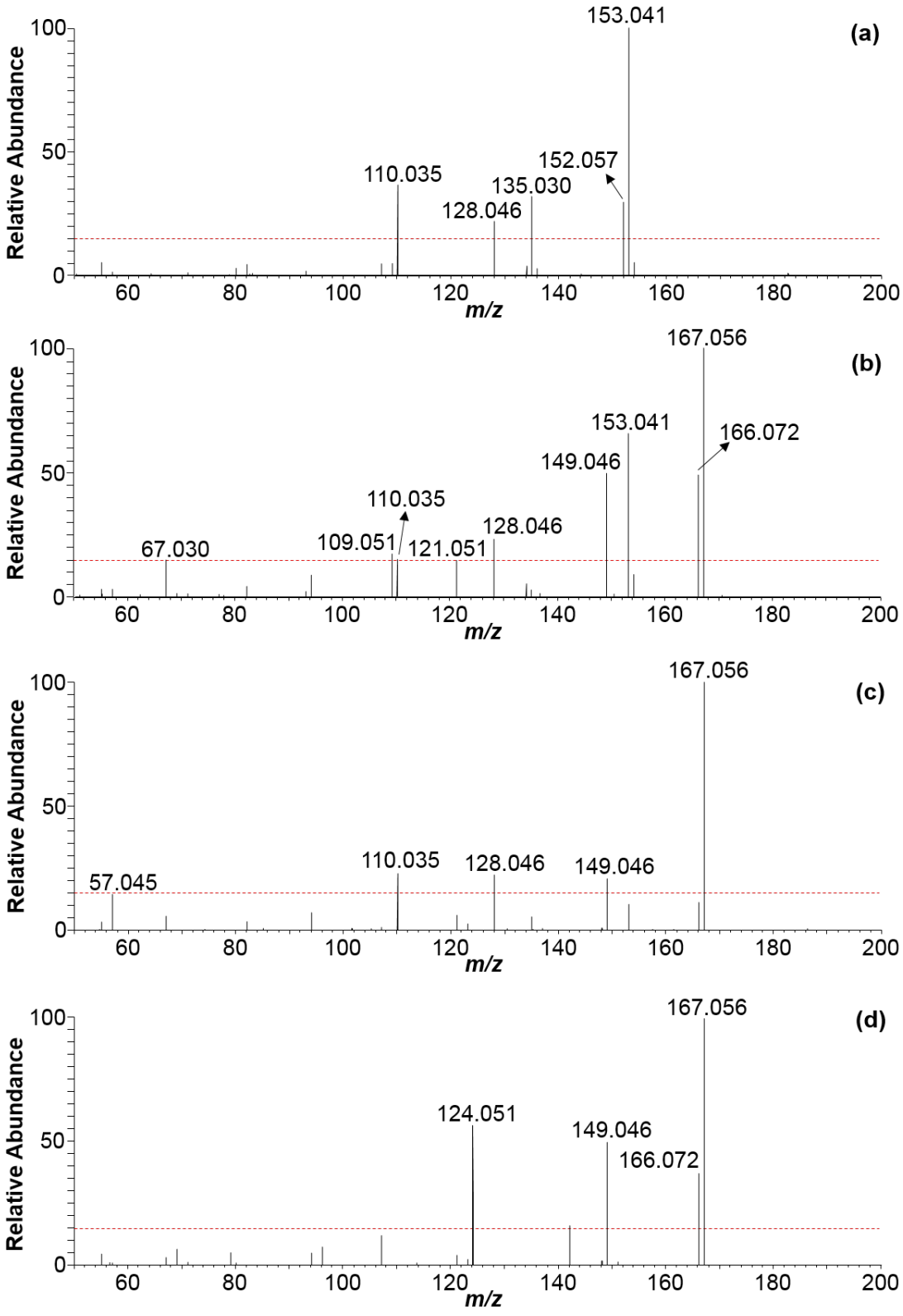
Supplemental Figure S1. MS/MS spectra for m^5C acquired by HCD at different CE. (a) CE 40 and (b) CE 80.



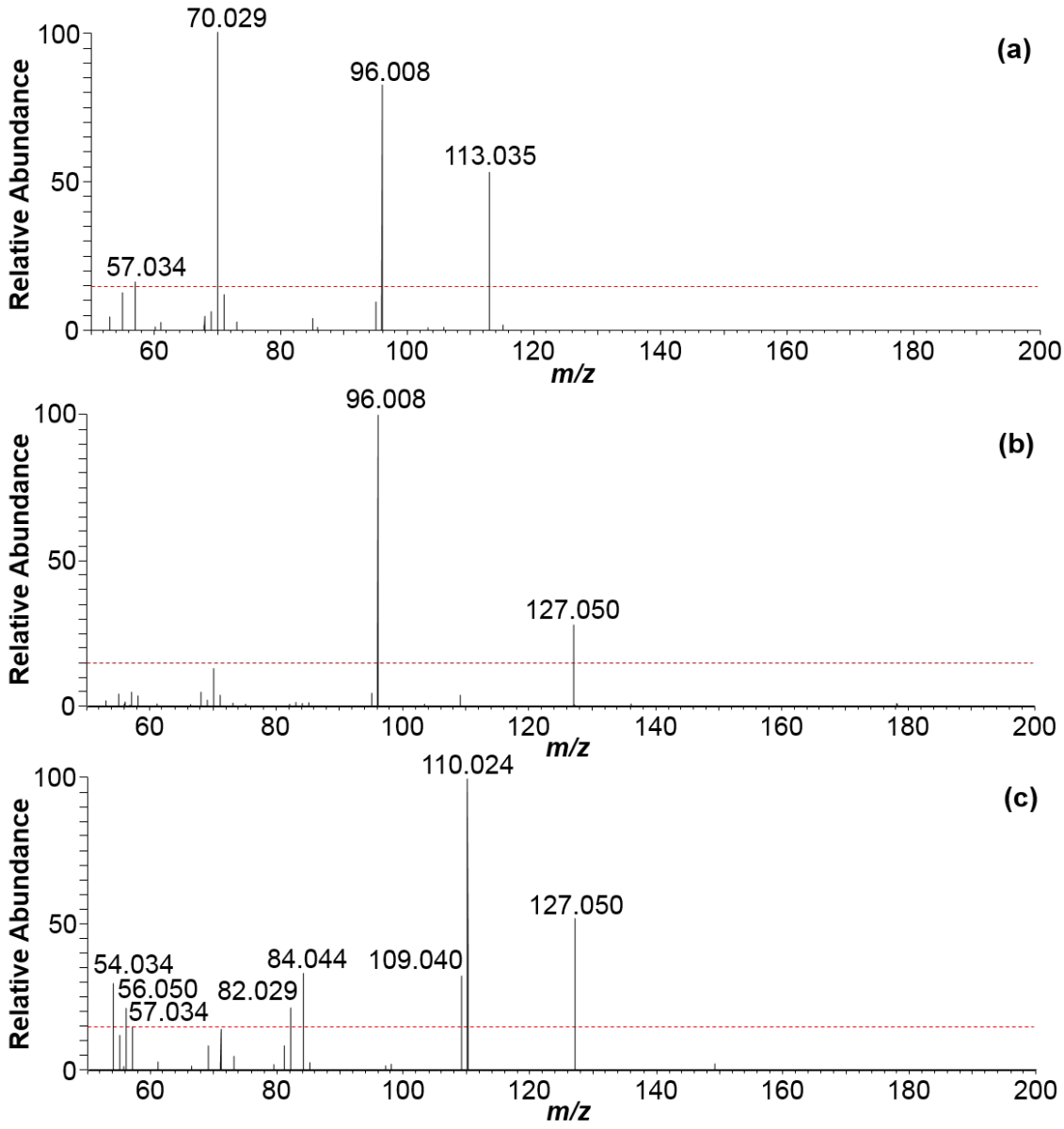
Supplemental Figure S2. Fingerprint MS/MS of m^5C acquired at different HCD CE. (a) 60, (b) 80, and (c) 100.



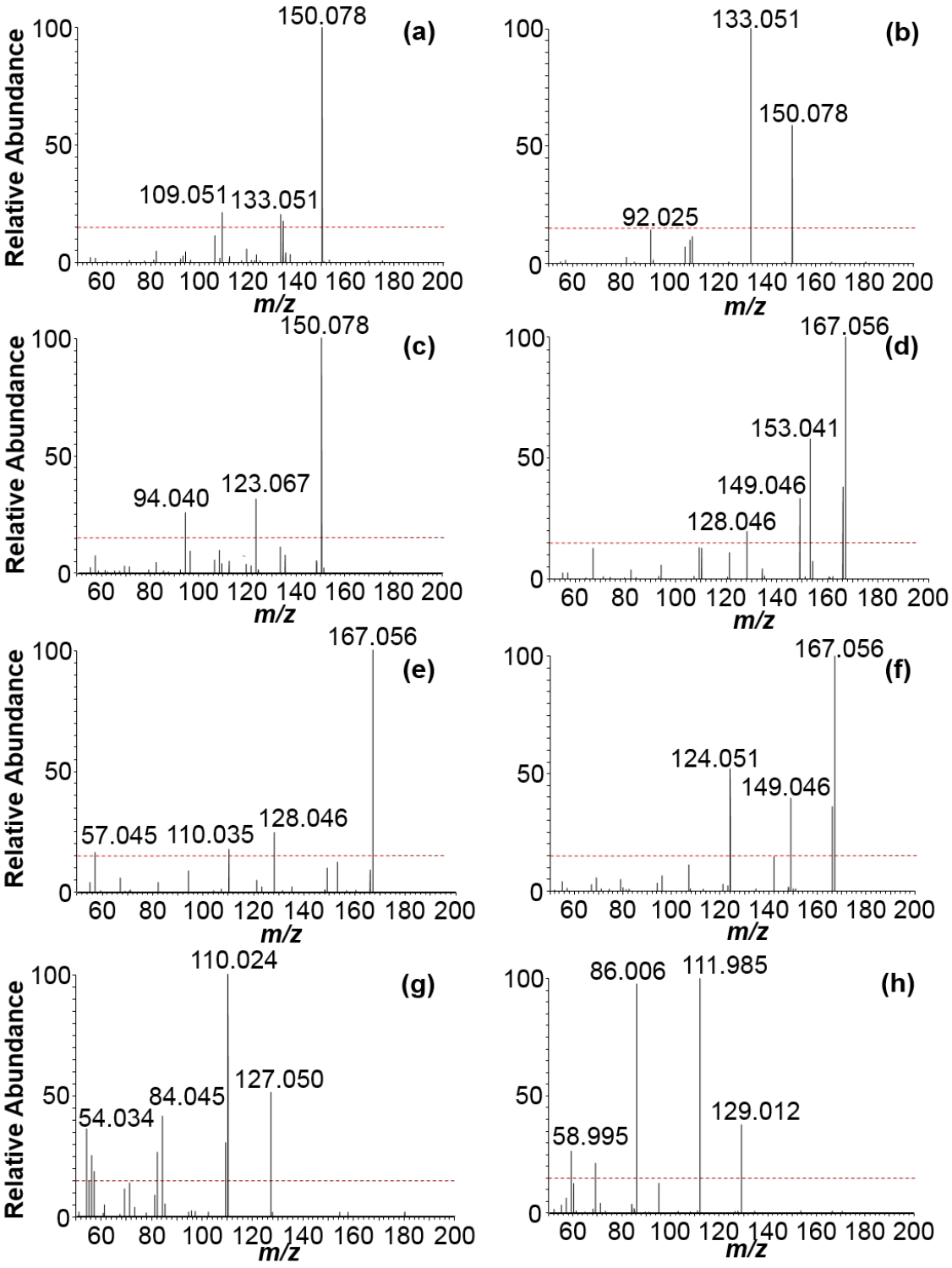
Supplemental Figure S3. Fingerprint MS/MS at HCD CE 80 of adenosine (a) and positional isomers of methyl adenosine standards: (b) m^1A, (c) m^2A, (d) m^6A, and (e) m^8A. See Supplemental Table S1 for more information on the peaks labeled.



Supplemental Figure S4. Fingerprint MS/MS at HCD CE 80 of guanosine (a) and positional isomers of methyl guanosine standards: (b) m^1G, (c) m^2G, and (d) m^7G. See Supplemental Table S1 for more information on the peaks labeled.



Supplemental Figure S5. Fingerprint MS/MS at HCD CE 80 of uridine (a) and positional isomers of methyl uridine standards: (b) m^3U, and (c) m^5U. See Supplemental Table S1 for more information on the peaks labeled.



Supplemental Figure S6. Fingerprint MS/MS at HCD CE 80 of m^1A (a), m^2A (b), m^6A (c), m^1G (d), m^2G (e), m^7G (f), m^5U (g), and s^4U (h), detected in *E. coli* tRNA.

Supplemental Table S1. Theoretical *m/z*, experimental *m/z*, *m/z* measurement error (parts per million, PPM), as well as molecular formula of ions with RIA ≥15% present in the HCD fingerprint of the different positional isomers discussed in the main text. Theoretical *m/z* values were computed by ChemCalc (http://www.chemcalc.org).

Nucleoside(s)	Experimental *m/z*	Theoretical *m/z*	Error (PPM)	Molecular formula
m^3C	95.0241	95.0245	4.2	$C_4H_3N_2O^+$
m^3C	109.0397	109.0402	4.6	$C_5H_5N_2O^+$
m^3C	126.0662	126.0667	4.0	$C_5H_8N_3O^+$
m^4C/m^4Cm	56.0497	56.0500	5.4	$C_3H_6N^+$
m^4C/m^4Cm	66.0340	66.0344	6.1	$C_4H_4N^+$
m^4C/m^4Cm	83.0606	83.0609	3.6	$C_4H_7N_2^+$
m^4C/m^4Cm	95.0243	95.0245	2.1	$C_4H_3N_2O^+$
m^4C/m^4Cm	108.0558	108.0562	3.7	$C_5H_6N_3^+$
m^4C/m^4Cm	109.0398	109.0402	3.7	$C_5H_5N_2O^+$
m^4C/m^4Cm	126.0663	126.0667	3.2	$C_5H_8N_3O^+$
m^5C/m^5Cm	56.0497	56.0500	5.4	$C_3H_6N^+$
m^5C/m^5Cm	81.0450	81.0453	3.7	$C_4H_5N_2^+$
m^5C/m^5Cm	83.0606	83.0609	3.6	$C_4H_7N_2^+$
m^5C/m^5Cm	108.0558	108.0562	3.7	$C_5H_6N_3^+$
m^5C/m^5Cm	109.0398	109.0402	3.7	$C_5H_5N_2O^+$
m^5C/m^5Cm	126.0663	126.0667	3.2	$C_5H_8N_3O^+$
m^1A	109.0510	109.0514	3.7	$C_4H_5N_4^+$
m^1A	133.0510	133.0514	3.0	$C_6H_5N_4^+$
m^1A	150.0775	150.0780	3.3	$C_6H_8N_5^+$
m^2A	92.0246	92.0249	3.3	$C_4H_2N_3^+$
m^2A	133.0509	133.0514	3.8	$C_6H_5N_4^+$
m^2A	150.0774	150.0780	4.0	$C_6H_8N_5^+$
m^6A	94.0402	94.0405	3.2	$C_4H_4N_3^+$
m^6A	123.0666	123.0671	4.1	$C_5H_7N_4^+$
m^6A	150.0775	150.0780	3.3	$C_6H_8N_5^+$
m^8A	106.0401	106.0405	3.8	$C_5H_4N_3^+$
m^8A	108.0558	108.0562	3.7	$C_5H_6N_3^+$
m^8A	133.0509	133.0514	3.8	$C_6H_5N_4^+$
m^8A	150.0774	150.0780	4.0	$C_6H_8N_5^+$
m^8A	151.0614	151.0620	4.0	$C_6H_7N_4O^+$
m^1G	67.0293	67.0296	4.5	$C_3H_3N_2^+$
m^1G	109.0510	109.0514	3.7	$C_4H_5N_4^+$
m^1G	110.0350	110.0354	3.6	$C_4H_4N_3O^+$
m^1G	121.0510	121.0514	3.3	$C_5H_5N_4^+$
m^1G	128.0455	128.0460	3.9	$C_4H_6N_3O_2^+$
m^1G	149.0459	149.0463	2.7	$C_6H_5N_4O^+$
m^1G	153.0409	153.0412	2.0	$C_5H_5N_4O_2^+$
m^1G	166.0725	166.0729	2.4	$C_6H_8N_5O^+$
m^1G	167.0564	167.0569	3.0	$C_6H_7N_4O_2^+$
m^2G	57.0449	57.0453	7.0	$C_2H_5N_2^+$

Cont. Supplemental Table S1.

Nucleoside(s)	Experimental *m/z*	Theoretical *m/z*	Error (PPM)	Molecular formula
m^2G	110.0351	110.0354	2.7	$C_4H_4N_3O^+$
m^2G	128.0455	128.0460	3.9	$C_4H_6N_3O_2^+$
m^2G	149.0459	149.0463	2.7	$C_6H_5N_4O^+$
m^2G	167.0564	167.0569	3.0	$C_6H_7N_4O_2^+$
m^7G	124.0507	124.0511	3.2	$C_5H_6N_3O^+$
m^7G	142.0611	142.0616	3.5	$C_5H_8N_3O_2^+$
m^7G	149.0458	149.0463	3.4	$C_6H_5N_4O^+$
m^7G	166.0724	166.0729	3.0	$C_6H_8N_5O^+$
m^7G	167.0564	167.0569	3.0	$C_6H_7N_4O_2^+$
m^3U	96.0081	96.0086	5.2	$C_4H_2NO_2^+$
m^3U	127.0502	127.0508	4.7	$C_5H_7N_2O_2^+$
m^5U	54.0340	54.0344	7.4	$C_3H_4N^+$
m^5U	56.0497	56.0500	5.4	$C_3H_6N^+$
m^5U	57.0337	57.0340	5.3	$C_3H_5O^+$
m^5U	82.0289	82.0293	4.9	$C_4H_4NO^+$
m^5U	84.0445	84.0449	4.8	$C_4H_6NO^+$
m^5U	109.0398	109.0402	3.7	$C_5H_5N_2O^+$
m^5U	110.0237	110.0242	4.5	$C_5H_4NO_2^+$
m^5U	127.0503	127.0508	3.9	$C_5H_7N_2O_2^+$
s^2U	59.9905	59.9908	5.0	CH_2NS^+
s^2U	70.0290	70.0293	4.3	$C_3H_4NO^+$
s^2U	83.9905	83.9908	3.6	$C_3H_2NS^+$
s^2U	111.9853	111.9857	3.6	$C_4H_2NOS^+$
s^4U	58.9952	58.9955	5.1	$C_2H_3S^+$
s^4U	68.9796	68.9799	4.3	C_3HS^+
s^4U	86.0061	86.0064	3.5	$C_3H_4NS^+$
s^4U	111.9853	111.9857	3.6	$C_4H_2NOS^+$
s^4U	129.0119	129.0122	2.3	$C_4H_5N_2OS^+$