Supporting Information

A high-throughput quantitative approach reveals more small RNA modifications in mouse liver and their correlation with diabetes

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Supplementary Tables S1-S5

Table S1. Calibration curves of the nucleosides G, Am, Um, Gm, Cm, ac^4C, I, m^5U, m^5C, m^1A, $m^2{}_2G$, m^5Um, m^3U, Ψ, $m^{2,2,7}G$, m^3C, m^6A, i^6A and m^5Cm.

Nucleoside	Symbol	Regression equation	Correlation coefficient
guanosine	G	y = 6.410x - 0.1042	R^2 = 0.998
2'-*O*-methyladenosine	Am	y = 90.07x + 0.0276	R^2 = 0.998
2'-*O*-methyluridine	Um	y = 1.919x + 0.0012	R^2 = 0.996
2'-*O*-methylguanosine	Gm	y = 10.07x + 0.0069	R^2 = 0.991
2'-*O*-methylcytidine	Cm	y = 99.28x + 0.0227	R^2 = 0.994
N^4-acetylcytidine	ac^4C	y = 0.8666x + 0.0001	R^2 = 0.995
inosine	I	y = 4.053x - 0.0121	R^2 = 0.999
5-methyluridine	m^5U	y = 2.834x - 0.0001	R^2 = 0.984
5-methylcytidine	m^5C	y = 82.02x + 0.0014	R^2 = 0.997
1-methyladenosine	m^1A	y = 58.58x - 0.0677	R^2 = 0.997
N^2,N^2-dimethylguanosine	$m^2{}_2G$	y = 11.14x + 0.0039	R^2 = 0.996
5,2'-*O*-dimethyluridine	m^5Um	y = 4.814x + 0.0001	R^2 = 0.990
3-methyluridine	m^3U	y = 7.322x - 0.0003	R^2 = 0.997
pseudouridine	Ψ	y = 0.5297x - 0.0005	R^2 = 0.991
N^2,N^2,7-trimethylguanosine	$m^{2,2,7}G$	y = 123.8x + 0.0003	R^2 = 0.999
3-methylcytidine	m^3C	y = 29.99x - 0.0603	R^2 = 0.994
N^6-methyladenosine	m^6A	y = 80.32x - 0.1714	R^2 = 0.996
N^6-isopentenyladenosine	i^6A	y = 117.3x + 0.0041	R^2 = 0.980
5,2'-*O*-dimethylcytidine	m^5Cm	y = 137.4x - 0.0016	R^2 = 0.999

The stable isotope labeled guanosine (^{13}C, ^{15}N) was used as an internal standard.

The regression equation for the calibration curve was determined to be y = ax + b, where y is the area ratio of the indicated nucleoside and internal standard, and x is the concentration ratio of the indicated nucleoside and internal standard.

Table S2. Intra- and Inter-day test.

Nucleoside	Symbol	Intra-day		Inter-day	
		RSD[a] (%)	RE[b] (%)	RSD[a] (%)	RE[b] (%)
pseudouridine	Ψ	7.80	-10.92	11.78	-11.65
inosine	I	13.18	-1.14	12.36	-2.35
1-methyladenosine	m^1A	4.09	1.57	10.01	-2.29
2'-*O*-methyladenosine	Am	4.92	-8.14	10.32	-4.65

[a]RSD: relative standard deviation.

[b]RE: relative error. RE (%) = (O – T)/T x 100. O, observed value; T, true value.

Table S3. Comparison of nucleoside modifications in the indicated small RNAs.

Nucleoside	Symbol	tRNA			5.8S rRNA	5S rRNA	30-60nt				10-30nt		
		a	b	c	a	c	a	c	a	c	a	c	d
1-methyladenosine	m^1A	+	+	+	–	+	–	–	–	+	–	+	–
2'-*O*-methyladenosine	Am	+	–	+	+	+	–	+	–	+	–	+	+
N^6-isopentenyladenosine	i^6A	+	–	+	–	–	–	–	–	+	–	+	–
N^6-(*cis*-hydroxyisopentenyl)adenosine	io^6A	+	–	–	–	–	–	–	–	–	–	–	–
3-methylcytidine	m^3C	+	+	+	–	+	–	+	–	+	–	+	–
5-methylcytidine	m^5C	+	+	+	–	–	–	–	–	+	–	+	–
2'-*O*-methylcytidine	Cm	+	–	+	+	+	–	+	–	+	–	+	+
N^4-acetylcytidine	ac^4C	+	+	+	–	–	–	–	–	+	–	+	–
1-methylguanosine	m^1G	+	+	+	–	–	–	–	–	+	–	+	–
N^2-methylguanosine	m^2G	+	+	+	–	–	–	–	–	+	–	+	–
7-methylguanosine	m^7G	+	+	+	–	–	–	–	–	+	–	+	–
2'-*O*-methylguanosine	Gm	+	–	+	+	+	–	–	–	+	–	+	+
N^2,N^2-dimethylguanosine	m^2_2G	+	+	+	–	+	–	–	–	+	–	+	–
inosine	I	+	+	+	–	–	–	–	–	+	–	+	+
queuosine	Q	+	–	–	–	–	–	–	–	–	–	–	–
pseudouridine	Ψ	+	+	+	+	+	+	–	–	+	–	+	–
dihydrouridine	D	+	–	–	–	–	–	–	–	–	–	–	–
5-methyluridine	m^5U	+	+	+	–	–	–	–	–	+	–	+	–
2'-*O*-methyluridine	Um	+	–	+	+	+	–	–	–	+	–	+	+
5,2'-*O*-dimethyluridine	m^5Um	+	–	+	–	–	–	–	–	+	–	+	–
2-methylthio-N^6-(*cis*-hydroxyisopentenyl)adenosine	ms^2io^6A	+	–	–	–	–	–	–	–	–	–	–	–
N^6-methyladenosine	m^6A	–	–	+	–	+	–	+	–	+	–	+	–
3-methyluridine	m^3U	–	–	+	–	–	–	–	–	+	–	+	–
N^2,N^2,7-trimethylguanosine	$m^{2,2,7}G$	–	–	+	–	+	–	–	–	+	–	+	–
5-hydroxymethylcytidine	hm^5C	–	–	+	–	–	–	–	–	+	–	+	–
2'-*O*-methylinosine	Im	–	–	+	–	–	–	–	–	–	–	–	–
5,2'-*O*-dimethylcytidine	m^5Cm	–	–	+	–	–	–	–	–	+	–	+	–
5,6-dihydrouridine	hU	–	+	–	–	–	–	–	–	–	–	–	–
3-(3-Amino-3-carboxypropyl)-urindine	acp^3U	–	+	–	–	–	–	–	–	–	–	–	–
N-[9-(β-D-Ribofuranosyl)purin-6-yl-carbamoyl]threonine		–	+	–	–	–	–	–	–	–	–	–	–
2-methylthio-N^6-(cis-hydroxyisopentenyl)adenosine	g^6A	+	–	–	–	–	–	–	–	–	–	–	–
N^6-glycinylcarbamoyladenosine	t^6A	+	–	–	–	–	–	–	–	–	–	–	–
2-methylthio-N^6-threonylcarbamoyladenosine	ms^2t^6A	+	–	–	–	–	–	–	–	–	–	–	–
2'-*O*-ribosyladenosine(phosphate)	Ar(p)	+	–	–	–	–	–	–	–	–	–	–	–
1,2'-*O*-dimethyladenosine	m^1Am	+	–	–	–	–	–	–	–	–	–	–	–
5-formylcytidine	f^5C	+	–	–	–	–	–	–	–	–	–	–	–
5-formyl-2'-*O*-methylcytidine	f^5Cm	+	–	–	–	–	–	–	–	–	–	–	–
2'-*O*-ribosylguanosine(phosphate)	Gr(p)	+	–	–	–	–	–	–	–	–	–	–	–
wybutosine	yW	+	–	–	–	–	–	–	–	–	–	–	–
peroxywybutosine	o_2yW	+	–	–	–	–	–	–	–	–	–	–	–
hydroxywybutosine	OHyW	+	–	–	–	–	–	–	–	–	–	–	–
undermodified hydroxywybutosine	OHyW*	+	–	–	–	–	–	–	–	–	–	–	–
wyosine	imG	+	–	–	–	–	–	–	–	–	–	–	–
1-methylinosine	m^1I	+	–	–	–	–	–	–	–	–	–	–	–
galactosyl-queuosine	galQ	+	–	–	–	–	–	–	–	–	–	–	–
mannosyl-queuosine	manQ	+	–	–	–	–	–	–	–	–	–	–	–
2'-*O*-methylpseudouridine	Ψm	+	–	–	–	–	–	–	–	–	–	–	–
2-thiouridine	s^2U	+	–	–	–	–	–	–	–	–	–	–	–
5-methyl-2-thiouridine	m^5s^2U	+											
5-hydroxyuridine	ho^5U	+	–	–	–	–	–	–	–	–	–	–	–

5-(carboxyhydroxymethyl)uridine	chm^{5}U	+	–	–	–	–	–	–	–	–	–	–	–
5-(carboxyhydroxymethyl)uridine methyl ester	mchm^{5}U	+	–	–	–	–	–	–	–	–	–	–	–
5-methoxycarbonylmethyluridine	mcm^{5}U	+	–	–	–	–	–	–	–	–	–	–	–
5-methoxycarbonylmethyl-2'-*O*-methyluridine	mcm^{5}Um	+	–	–	–	–	–	–	–	–	–	–	–
5-methoxycarbonylmethyl-2-thiouridine	mcm^{5}s^{2}U	+	–	–	–	–	–	–	–	–	–	–	–
5-carbamoylmethyluridine	ncm^{5}U	+	–	–	–	–	–	–	–	–	–	–	–
5-carbamoylmethyl-2'-*O*-methyluridine	ncm^{5}Um	+	–	–	–	–	–	–	–	–	–	–	–
5-carboxymethylaminomethyluridine	cmnm^{5}U	+	–	–	–	–	–	–	–	–	–	–	–
5-taurinomethyluridine	τm^{5}U	+	–	–	–	–	–	–	–	–	–	–	–
5-taurinomethyl-2-thiouridine	τm^{5}s^{2}U	+	–	–	–	–	–	–	–	–	–	–	–

a: Data derived from a review of modified nucleosides in eukaryotic RNAs by Machnicka et al.[1]

b: Data derived from a report of modified nucleosides in female mouse liver by Tseng et al.[35]

c: Data derived from this study of modified nucleosides in male mouse liver.

d: Data derived from a review of modified nucleosides in small RNAs by Kim et al.[5]

+: indicates the detected modified nucleoside.

–: indicates the undetected modified nucleoside.

Table S4. Quantification of modified nucleosides in the indicated small RNAs and total RNA from mouse liver.

Symbol	tRNA	30–60nt	10–30nt	5.8S rRNA	5S-1 rRNA	5S-2 rRNA	total RNA
Ψ	18.30±3.66	14.04±2.98	13.37±1.38	0.06±0.00	ND[a]	ND[a]	9.20±0.91
m^1A	9.89±0.58	6.42±1.43	6.76±0.94	0.0012±0.0008	ND[a]	ND[a]	1.55±0.21
m^5C	6.02±0.91	1.77±0.79	0.78±0.24	ND[a]	ND[a]	ND[a]	0.28±0.03
m^5U	4.33±0.79	2.19±0.23	1.79±0.32	ND[a]	ND[a]	ND[a]	0.38±0.06
$m^2{}_2G$	3.56±0.46	3.14±0.37	2.14±0.52	0.0006±0.0002	ND[a]	ND[a]	0.40±0.07
I	2.94±0.69	3.85±0.81	3.42±0.57	ND[a]	ND[a]	ND[a]	2.56±0.50
Um	2.81±0.65	1.98±0.16	1.93±0.16	3.25±0.09	ND[a]	ND[a]	1.86±0.24
Gm	2.66±0.31	1.28±0.06	1.22±0.14	3.00±0.19	ND[a]	ND[a]	1.26±0.25
m^3C	2.19±0.24	5.33±2.30	3.46±0.73	0.0014±0.0004	0.0025±0.0001	0.042±0.003	1.77±0.65
m^6A	1.39±0.23	3.30±1.73	2.19±0.74	0.0001±0.0001	–[b]	0.002±0.000	0.51±0.10
Cm	1.34±0.17	0.79±0.11	0.70±0.19	0.16±0.08	0.0009±0.0001	0.024±0.002	1.28±0.11
ac^4C	1.16±0.34	3.69±0.89	2.91±0.78	ND[a]	ND[a]	ND[a]	0.94±0.32
i^6A	1.20±0.55	0.012±0.008	–[b]	ND[a]	ND[a]	ND[a]	ND[a]
m^5Um	0.79±0.19	0.53±0.09	0.42±0.10	ND[a]	ND[a]	ND[a]	0.070±0.028
Am	0.12±0.09	0.39±0.12	0.33±0.06	0.12±0.06	0.0001±0.0000	0.004±0.001	0.83±0.18
m^3U	0.07±0.01	0.27±0.06	0.11±0.07	ND[a]	ND[a]	ND[a]	0.074±0.010
$m^{2,2,7}G$	0.002±0.002	0.062±0.021	0.079±0.031	0.0005±0.0002	ND[a]	ND[a]	0.016±0.007
m^5Cm	0.003±0.001	–[b]	–[b]	ND[a]	ND[a]	ND[a]	ND[a]

The mole percentages (mol %) of each modified nucleoside to G in the indicated small RNAs or total RNA from mouse liver are listed. Data represent mean and standard deviation, n = 7 for tRNA, 30-60 nt, 10-30 nt, 5S-2 RNA and total RNA from normal mice, n = 6 for 5.8S and 5S-1 RNAs from normal mice.

[a]ND: indicates that the modified nucleoside is not detected.

[b]–: indicates the detected modified nucleoside, which is not suitable for quantification.

Table S5. The fold change of the indicated nucleoside modification in diabetic mouse liver to that in normal mouse liver.

Nucleoside	Symbol	tRNA	30-60nt	10-30nt	total RNA
pseudouridine	Ψ	0.853	1.324	0.956	1.091
1-methyladenosine	m^1A	1.015	1.313	1.144	1.141
5-methylcytidine	m^5C	1.037	0.796	0.766	0.979
5-methyluridine	m^5U	0.843	1.033	0.958	1.026
N^2,N^2-dimethylguanosine	$m^2{}_2G$	0.863	1.155	0.855	1.125
inosine	I	0.855	1.153	0.835	1.156
2'-*O*-methyluridine	Um	0.893	1.074	0.831	1.030
2'-*O*-methylguanosine	Gm	0.813	0.940	0.827	1.168
3-methylcytidine	m^3C	0.943	1.092	1.004	0.600
N^6-methyladenosine	m^6A	0.836	1.181	0.949	0.976
2'-*O*-methylcytidine	Cm	0.958	1.314	0.946	0.939
N^4-acetylcytidine	ac^4C	0.889	0.940	1.111	1.328
N^6-isopentenyladenosine	i^6A	1.130	0.491	–[b]	ND[a]
5,2'-*O*-dimethyluridine	m^5Um	0.878	1.011	1.076	1.275
2'-*O*-methyladenosine	Am	0.705	0.532	0.940	0.983
3-methyluridine	m^3U	0.922	1.087	0.554	1.147
N^2,N^2,7-trimethylguanosine	$m^{2,2,7}G$	0.810	1.087	0.984	1.108
5,2'-*O*-dimethylcytidine	m^5Cm	1.478	–[b]	–[b]	ND[a]

Shading color indicates significant difference between diabetic and normal mouse liver by Student's t-test: green, $p < 0.05$; yellow, $p < 0.1$. n = 7 for normal mice; n = 3 for 30-60 nt, 10-30 nt small RNA and total RNA from diabetic mice; n = 6 for tRNA from diabetic mice.

[a]ND: indicates that the modified nucleoside is not detected.

[b]–: indicates the detected modified nucleoside, which is not suitable for quantification.

Supplementary Figures S1-S8

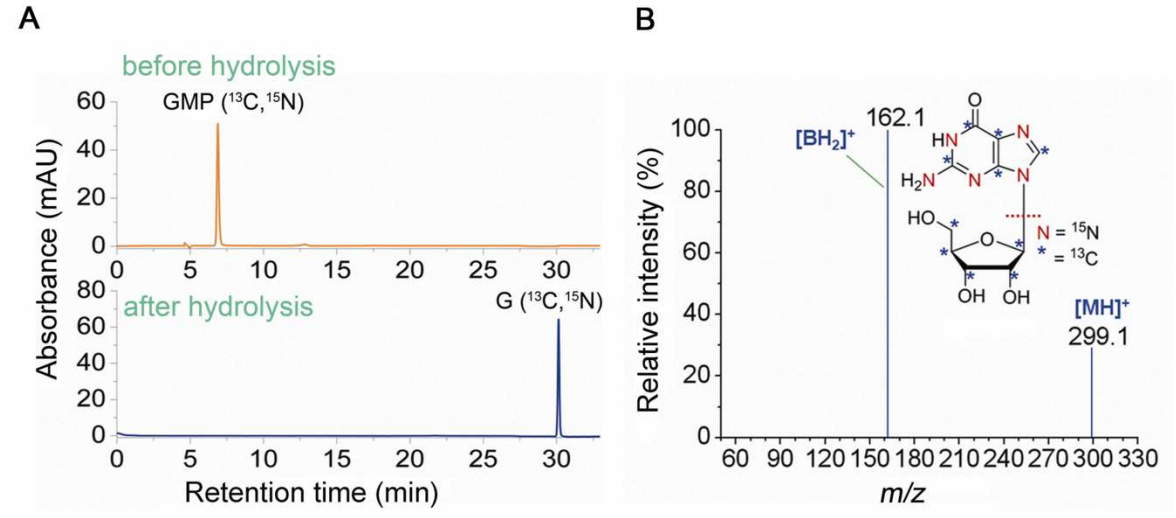
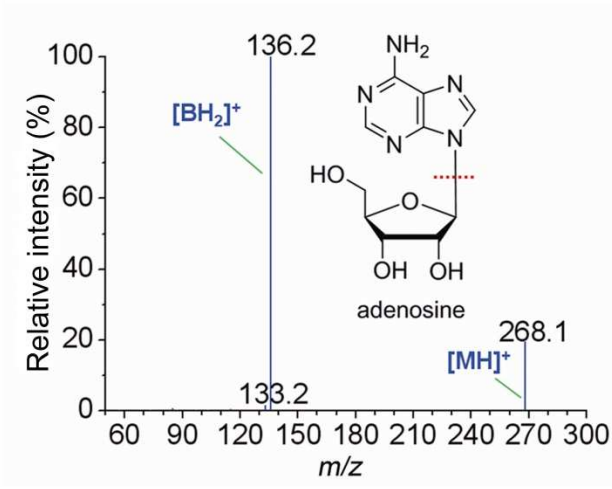
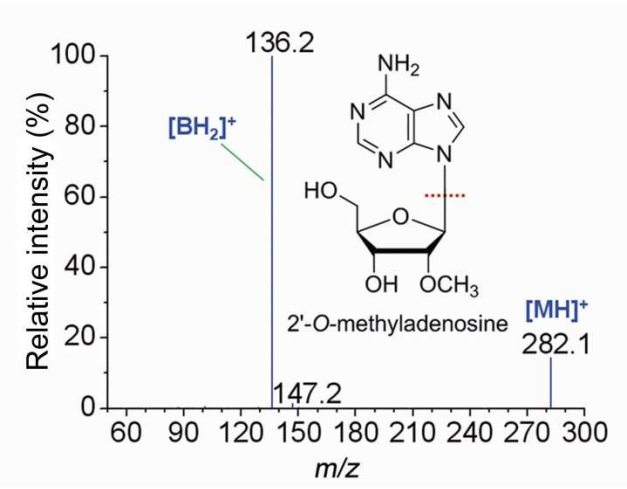
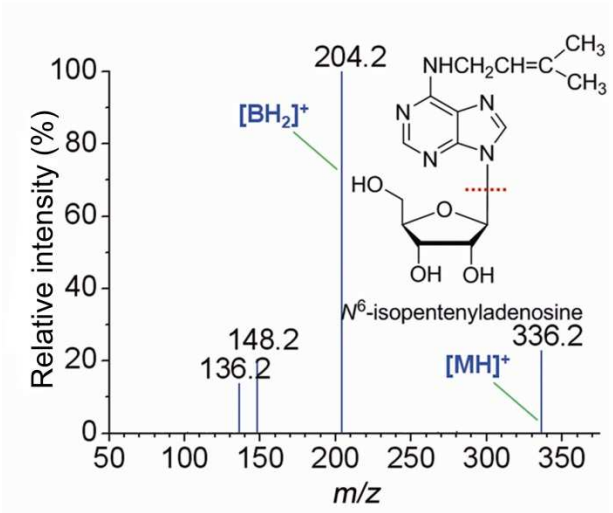
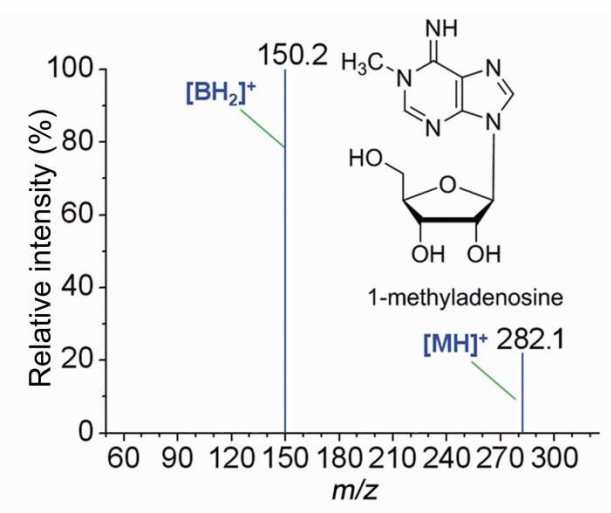


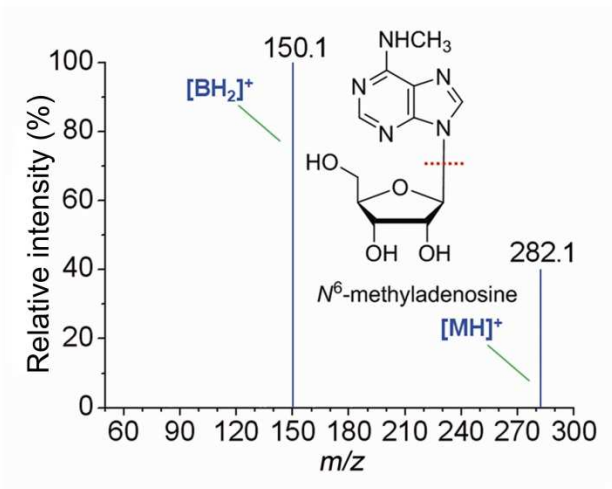
Figure S1 HPLC chromatogram and mass spectrum of isotope labeled guanosine (^{13}C, ^{15}N). (A) HPLC analysis of isotope-labeled guanosine 5'-monophosphate (^{13}C, ^{15}N) (before hydrolysis) and isotope labeled guanosine (^{13}C, ^{15}N) (after hydrolysis with calf intestine alkaline phosphatase) monitored by UV at 254 nm. (B) ESI mass spectrum of isotope labeled guanosine (^{13}C, ^{15}N). The quasimolecular ion peak $[MH]^+$ and product ion peak $[BH_2]^+$ of guanosine (^{13}C, ^{15}N) are indicated.

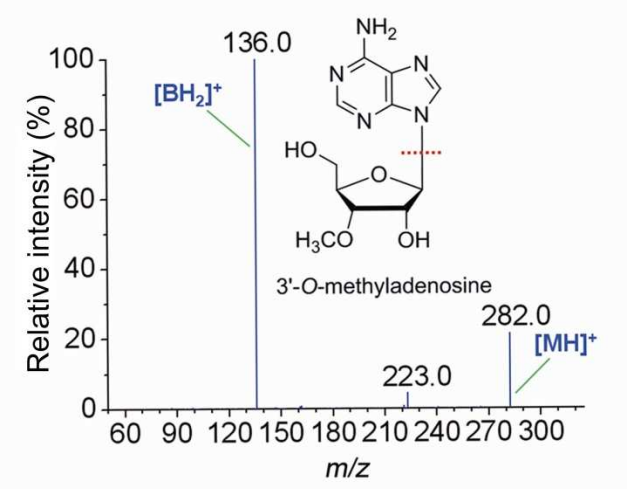

136.2
[BH2]+
adenosine
268.1
[MH]+
133.2
Relative intensity (%)
m/z

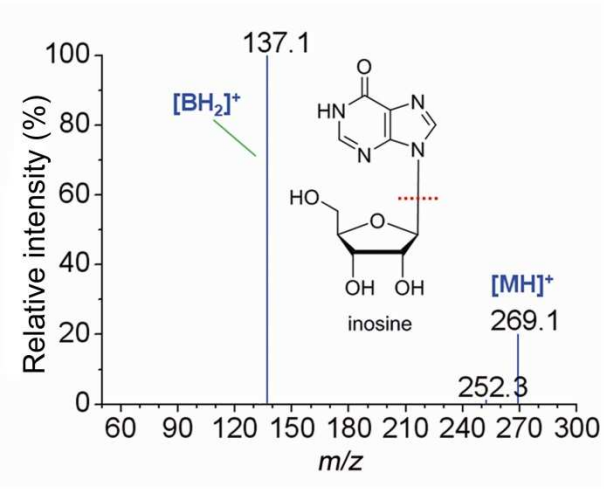

136.2
[BH2]+
2'-O-methyladenosine
[MH]+
282.1
147.2
Relative intensity (%)
m/z

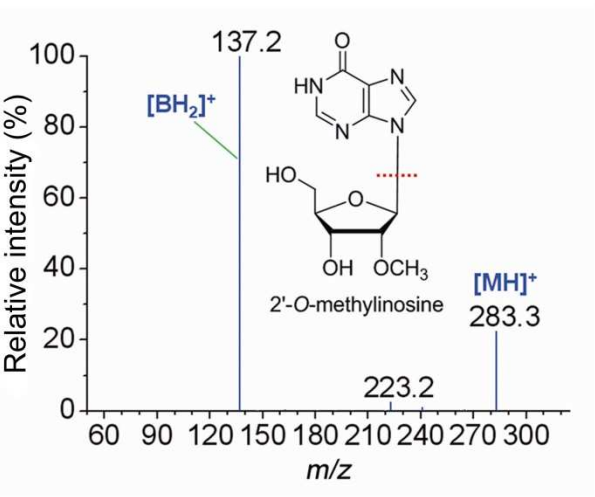

204.2
[BH2]+
N6-isopentenyladenosine
148.2
136.2
336.2
[MH]+
Relative intensity (%)
m/z

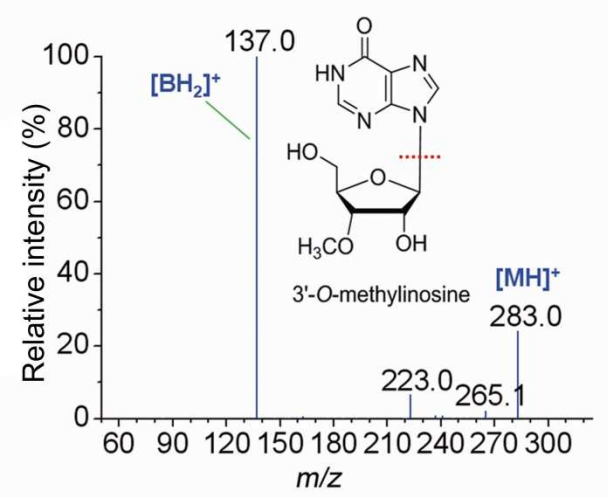

150.2
[BH2]+
1-methyladenosine
[MH]+ 282.1
Relative intensity (%)
m/z

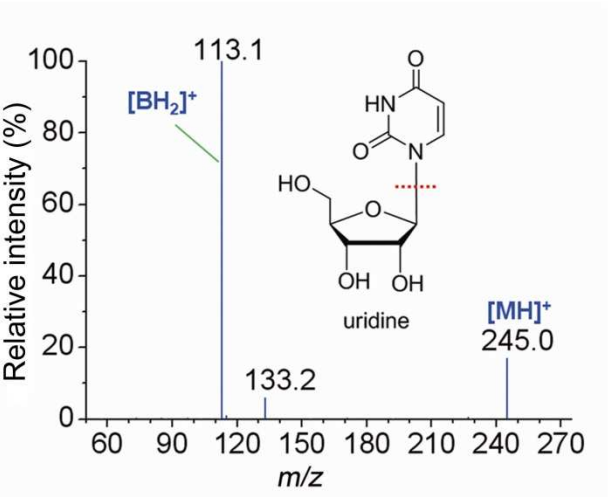

150.1
[BH2]+
N6-methyladenosine
282.1
[MH]+
Relative intensity (%)
m/z

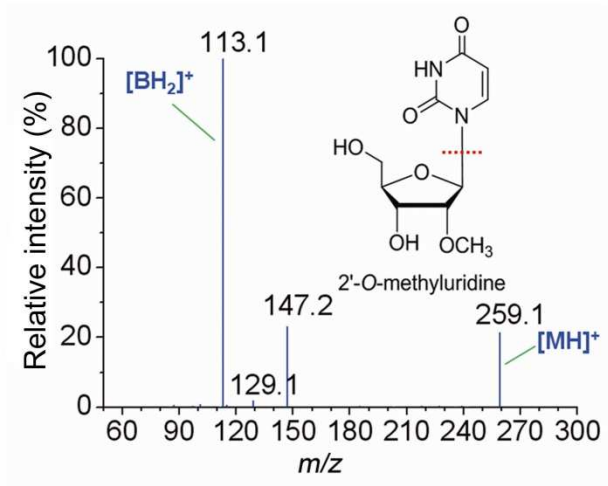

136.0
[BH2]+
3'-O-methyladenosine
282.0
[MH]+
223.0
Relative intensity (%)
m/z

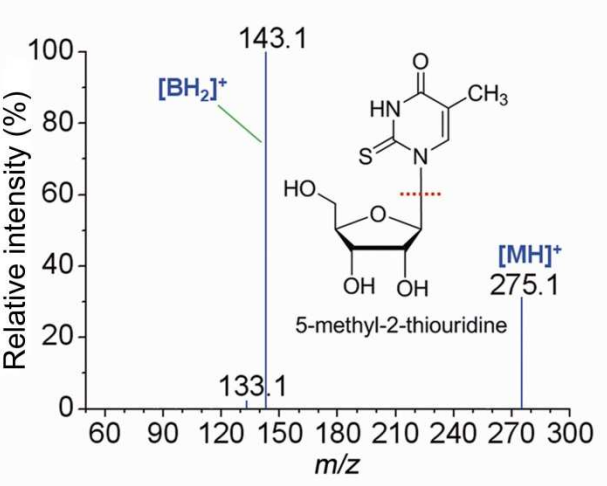

137.1
[BH2]+
[MH]+
269.1
252.3
inosine
Relative intensity (%)
m/z

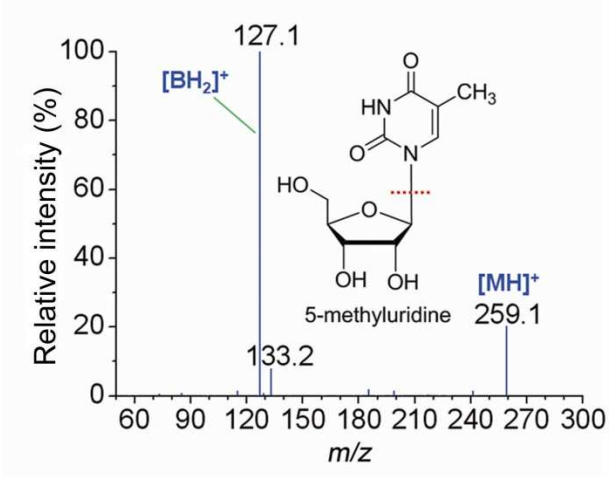

137.2
[BH2]+
[MH]+
283.3
223.2
2'-O-methylinosine
Relative intensity (%)
m/z

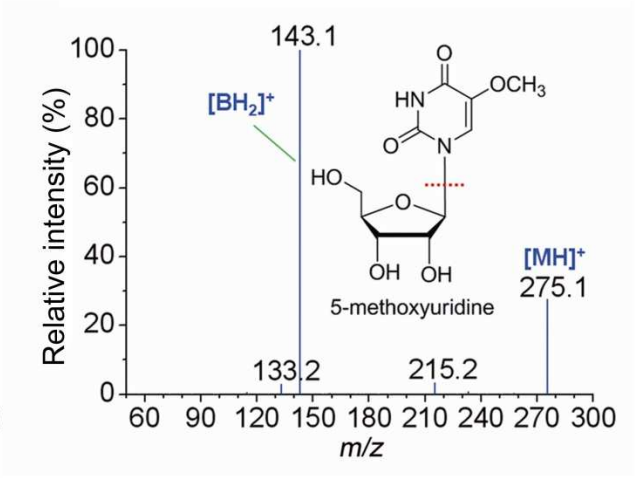

137.0
[BH2]+
[MH]+
283.0
223.0
265.1
3'-O-methylinosine
Relative intensity (%)
m/z

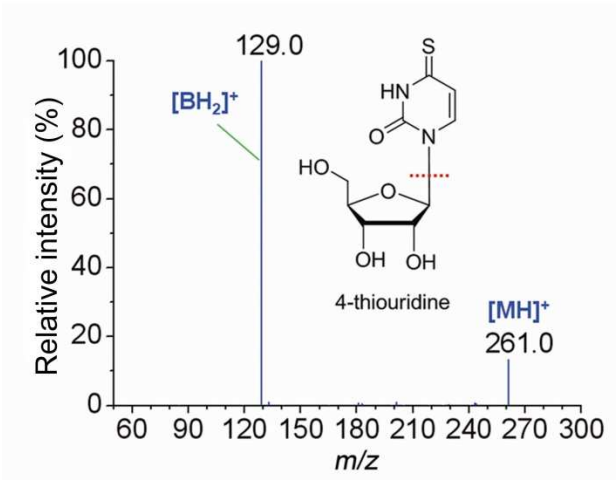

113.1
[BH2]+
[MH]+
245.0
133.2
uridine
Relative intensity (%)
m/z

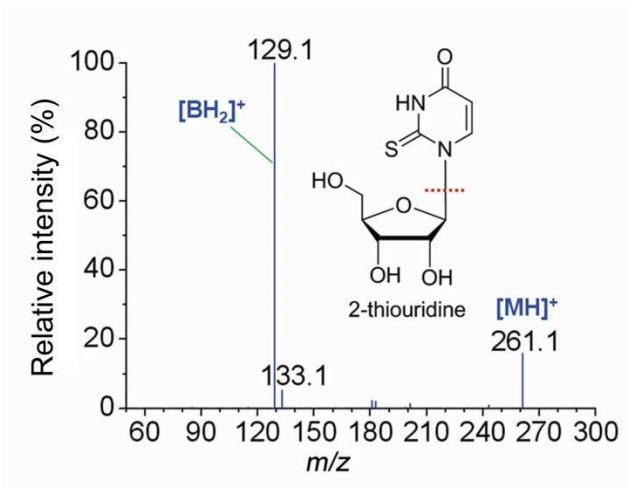

113.1
[BH2]+
147.2
259.1
[MH]+
129.1
2'-O-methyluridine
Relative intensity (%)
m/z

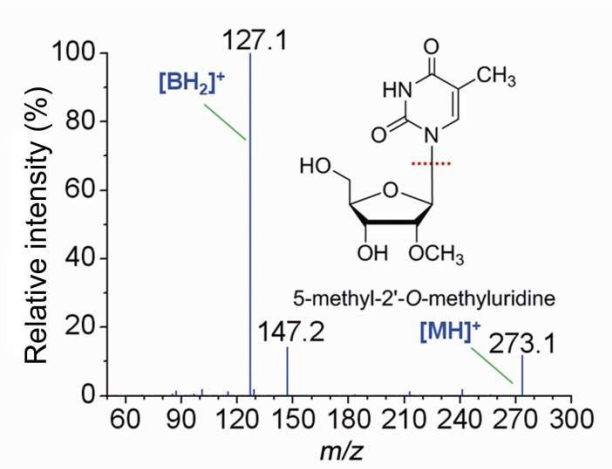

143.1
[BH2]+
[MH]+
275.1
133.1
5-methyl-2-thiouridine
Relative intensity (%)
m/z

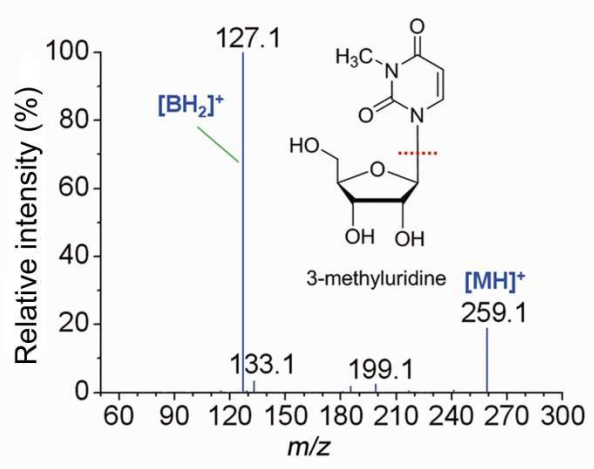

127.1
[BH2]+
[MH]+
259.1
133.2
5-methyluridine
Relative intensity (%)
m/z

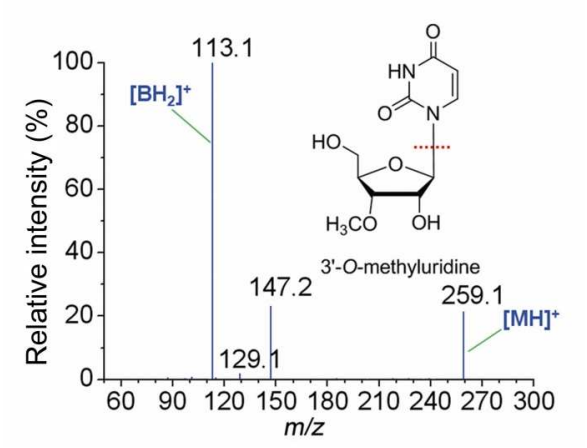

143.1
[BH2]+
[MH]+
275.1
133.2
215.2
5-methoxyuridine
Relative intensity (%)
m/z

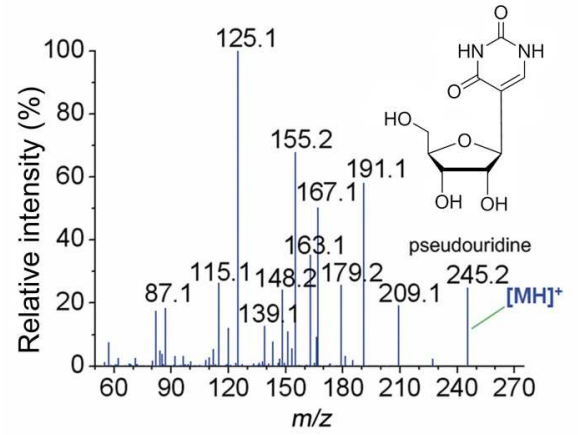

129.0
[BH2]+
[MH]+
261.0
4-thiouridine
Relative intensity (%)
m/z

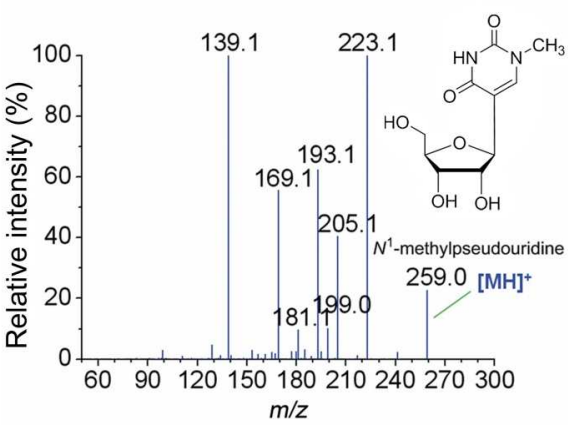

129.1
[BH2]+
[MH]+
261.1
133.1
2-thiouridine
Relative intensity (%)
m/z

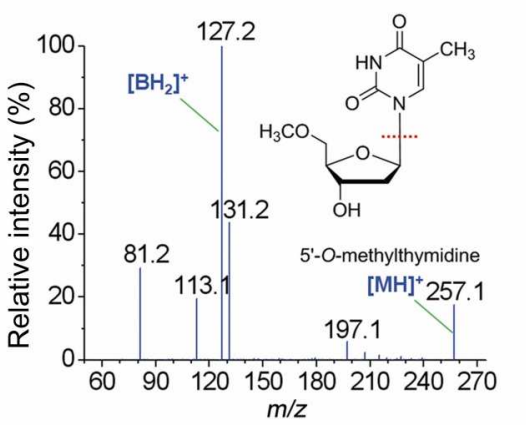

127.1
[BH2]+
147.2
[MH]+
273.1
5-methyl-2'-O-methyluridine
Relative intensity (%)
m/z

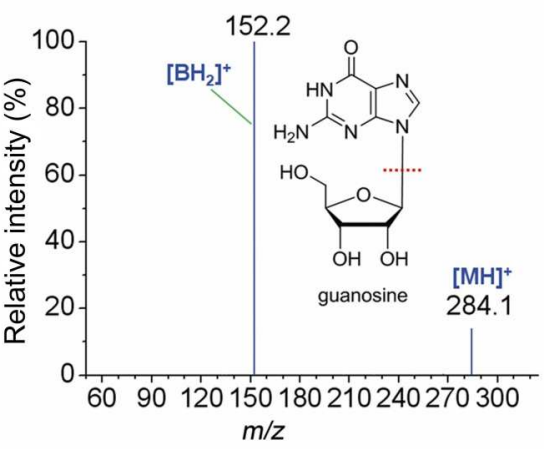

127.1
[BH2]+
[MH]+
259.1
133.1
199.1
3-methyluridine
Relative intensity (%)
m/z

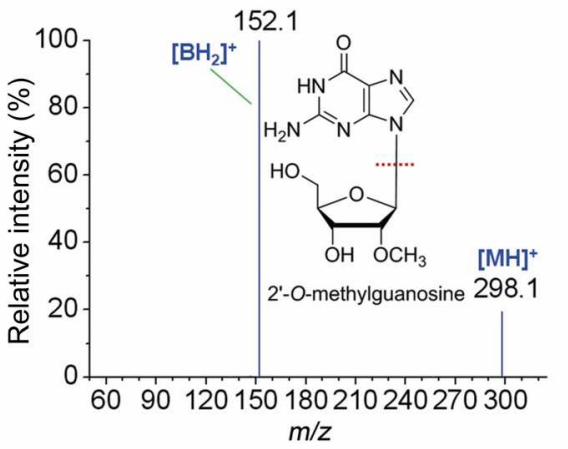

113.1
[BH2]+
147.2
129.1
259.1
[MH]+
3'-O-methyluridine
Relative intensity (%)
m/z

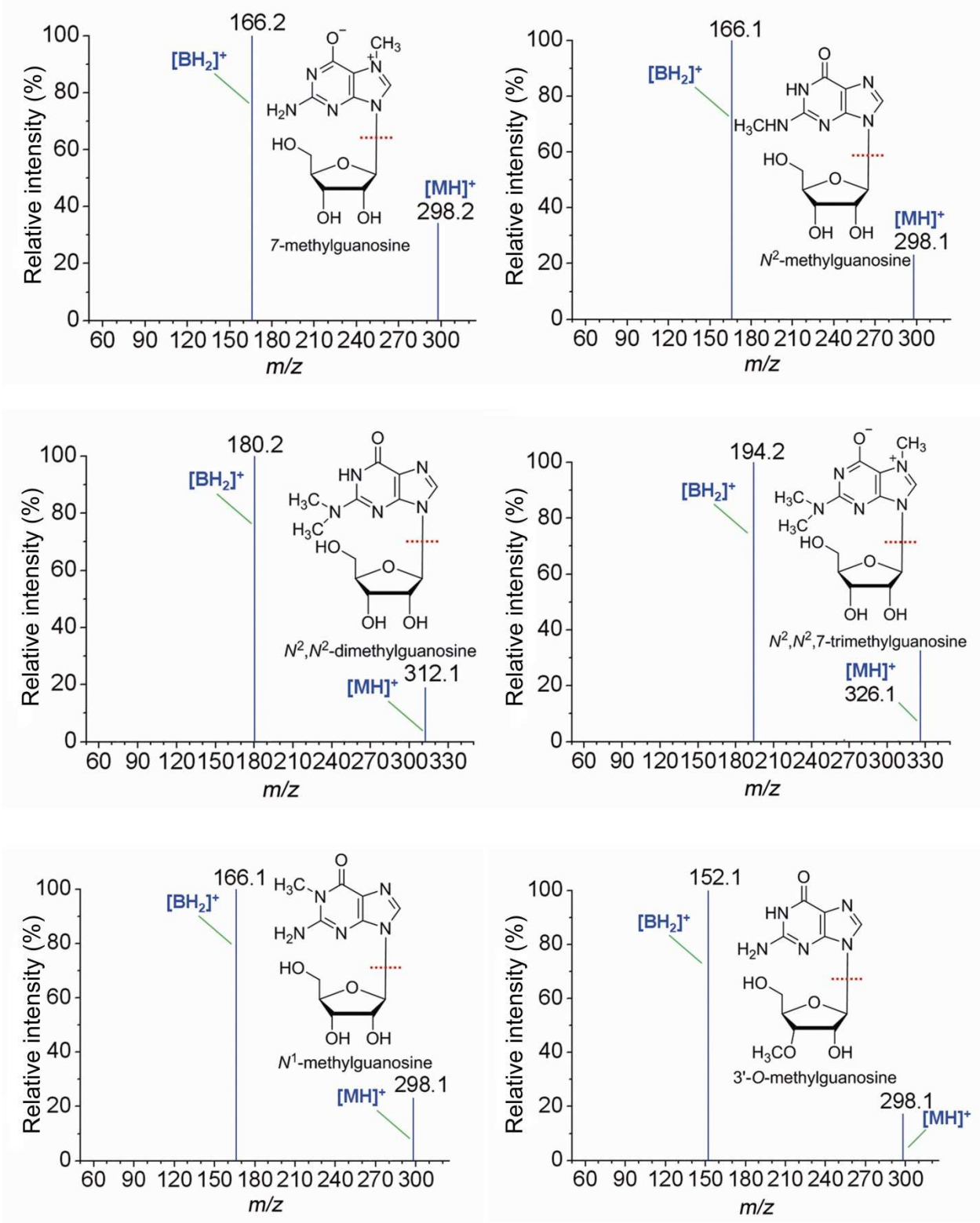

125.1
155.2
191.1
167.1
163.1
115.1
148.2
179.2
87.1
139.1
209.1
245.2
[MH]+
pseudouridine
Relative intensity (%)
m/z

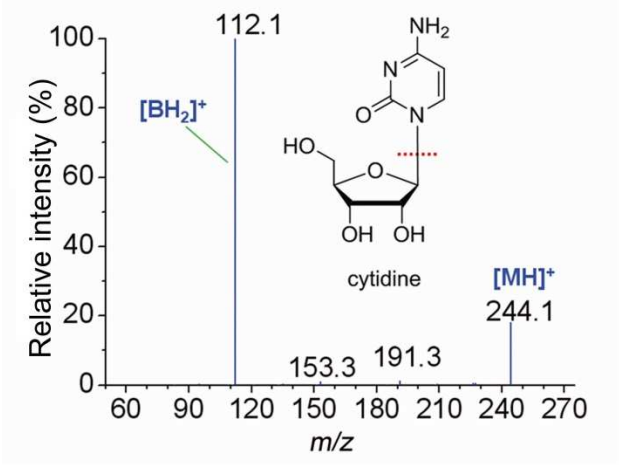

139.1
223.1
193.1
169.1
205.1
181.1
199.0
259.0
[MH]+
N1-methylpseudouridine
Relative intensity (%)
m/z

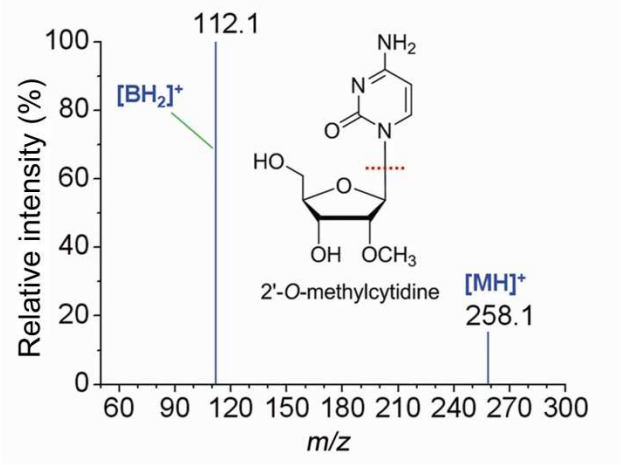

127.2
[BH2]+
131.2
81.2
113.1
197.1
[MH]+
257.1
5'-O-methylthymidine
Relative intensity (%)
m/z

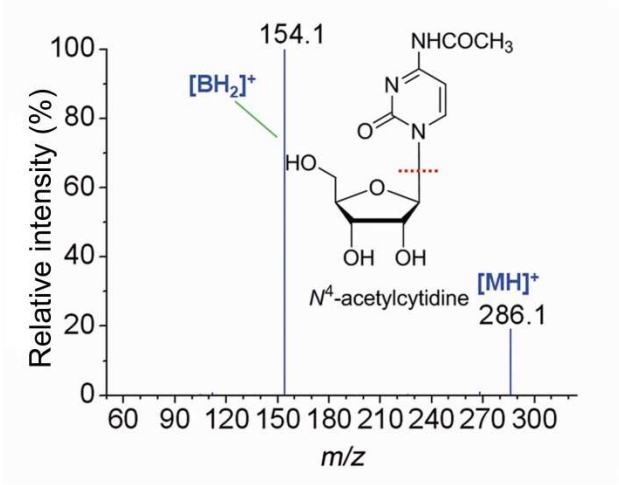

152.2
[BH2]+
[MH]+
284.1
guanosine
Relative intensity (%)
m/z

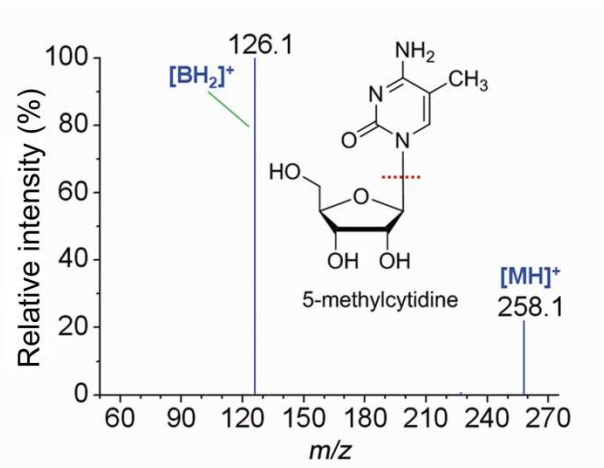

152.1
[BH2]+
[MH]+
298.1
2'-O-methylguanosine
Relative intensity (%)
m/z

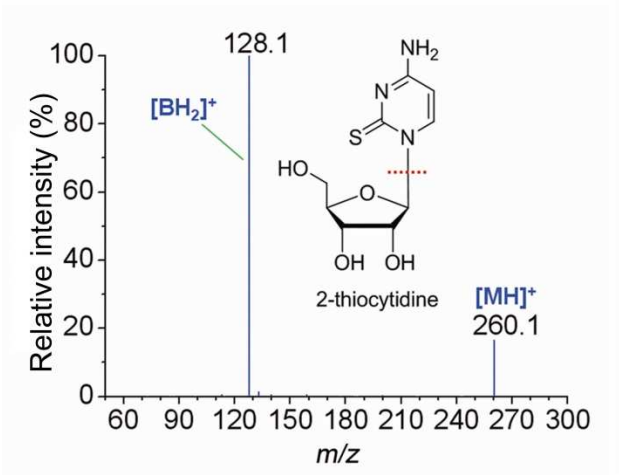

166.2
[BH2]+
[MH]+
298.2
7-methylguanosine
Relative intensity (%)
m/z
166.1
[BH2]+
[MH]+
298.1
N2-methylguanosine
180.2
[BH2]+
[MH]+
312.1
N2,N2-dimethylguanosine
194.2
[BH2]+
[MH]+
326.1
N2,N2,7-trimethylguanosine
166.1
[BH2]+
[MH]+
298.1
N1-methylguanosine
152.1
[BH2]+
[MH]+
298.1
3'-O-methylguanosine

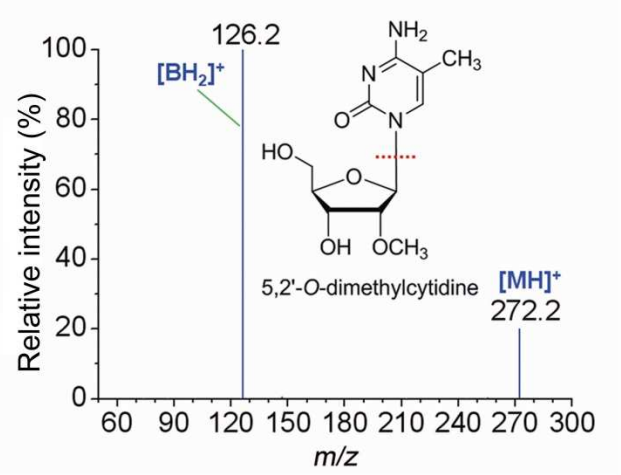

112.1
[BH2]+
cytidine
[MH]+
244.1
153.3
191.3
Relative intensity (%)
m/z


112.1
[BH2]+
2'-O-methylcytidine
[MH]+
258.1
Relative intensity (%)
m/z


154.1
[BH2]+
N4-acetylcytidine
[MH]+
286.1
Relative intensity (%)
m/z


126.1
[BH2]+
5-methylcytidine
[MH]+
258.1
Relative intensity (%)
m/z


128.1
[BH2]+
2-thiocytidine
[MH]+
260.1
Relative intensity (%)
m/z


126.2
[BH2]+
5,2'-O-dimethylcytidine
[MH]+
272.2
Relative intensity (%)
m/z

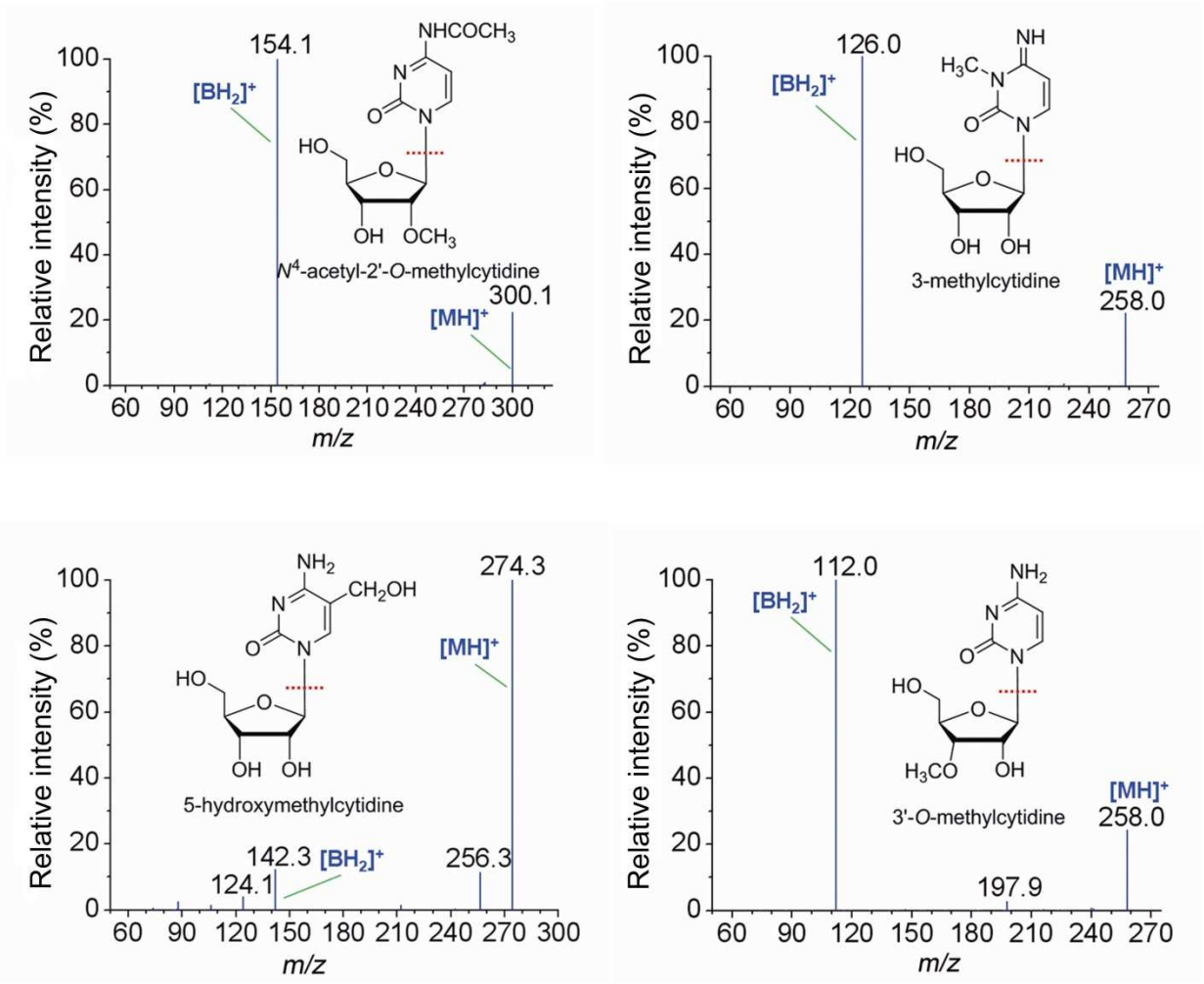


Figure S2 ESI mass spectra of the indicated 40 nucleosides. They are adenosine (A), 2'-*O*-methyladenosine (Am), N^6-isopentenyladenosine (i^6A), 1-methyladenosine (m^1A), N^6-methyladenosine (m^6A), 3'-*O*-methyladenosine (3'-*O*MeA), inosine (I), 2'-*O*-methylinosine (Im), 3'-*O*-methylinosine (3'-*O*MeI), uridine (U), 2'-*O*-methyluridine (Um), 5-methyl-2-thiouridine (m^5s^2U), 5-methyluridine (m^5U), 5-methoxyuridine (mo^5U), 4-thiouridine (s^4U), 2-thiouridine (s^2U), 5,2'-*O*-dimethyluridine (m^5Um), 3-methyluridine (m^3U), 3'-*O*-methyluridine (3'-*O*MeU), pseudouridine (Ψ), 1-methylpseudouridine ($m^1Ψ$), 5'-*O*-methylthymidine (5'-*O*MeT), guanosine (G), 2'-*O*-methylguanosine (Gm), 7-methylguanosine (m^7G), N^2-methylguanosine (m^2G), N^2,N^2-dimethylguanosine ($m^2{}_2G$), N^2,N^2,7-trimethylguanosine ($m^{2,2,7}G$), 1-methylguanosine (m^1G), 3'-*O*-methylguanosine (3'-*O*MeG), cytosine (C), 2'-*O*-methylcytidine (Cm), N^4-acetylcytidine (ac^4C), 5-methylcytidine (m^5C), 2-thiocytidine (s^2C), 5,2'-*O*-dimethylcytidine (m^5Cm), N^4-acetyl-2'-*O*-methylcytidine (ac^4Cm), 3-methylcytidine (m^3C), 5-hydroxymethylcytidine (hm^5C) and 3'-*O*-methylcytidine (3'-*O*MeC). The quasimolecular ion peak $[MH]^+$ and/or product ion peak $[BH_2]^+$ of each nucleoside are indicated.

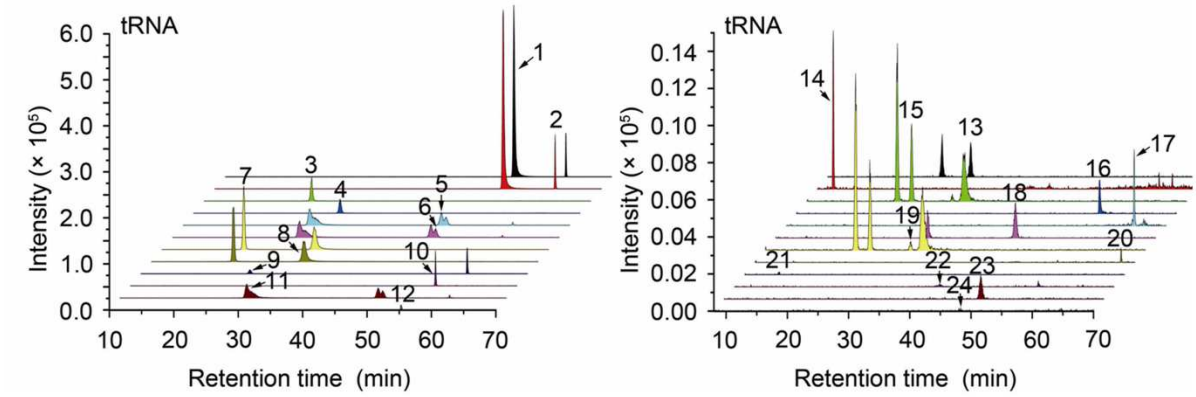


Figure S3 LC-MS/MS chromatogram of modified nucleosides from an enzymatic digestion of tRNA from C57BL/6 mouse liver. Numbered peaks are: 1, m^1A; 2, m^6A; 3, Cm; 4, G (^{13}C, ^{15}N); 5, m^1G; 6, m^2G; 7, m^3C; 8, m^5C; 9, I; 10, $m^2{}_2G$; 11, m^7G; 12, Gm; 13, Um; 14, Ψ; 15, m^5U; 16, $m^{2,2,7}G$; 17, Am; 18, ac^4C; 19, m^3U; 20, i^6A; 21, hm^5C; 22, m^5Cm; 23, m^5Um, 24, Im.

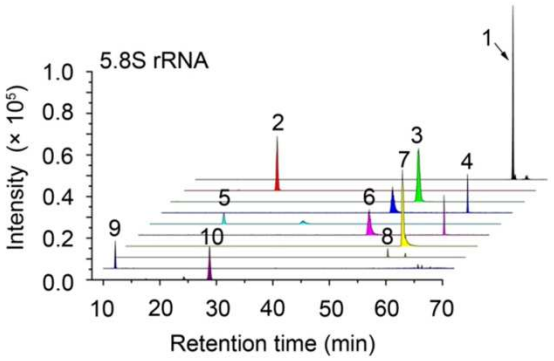


Figure S4 LC-MS/MS chromatogram of modified nucleosides from an enzymatic digestion of 5.8S rRNA from mouse liver. Numbered peaks are: 1, Am; 2, Cm; 3, Gm; 4, m^6A; 5, m^3C; 6, m^1A; 7, $m^{2,2,7}G$; 8, $m^2{}_2G$; 9, Ψ; 10, Um.

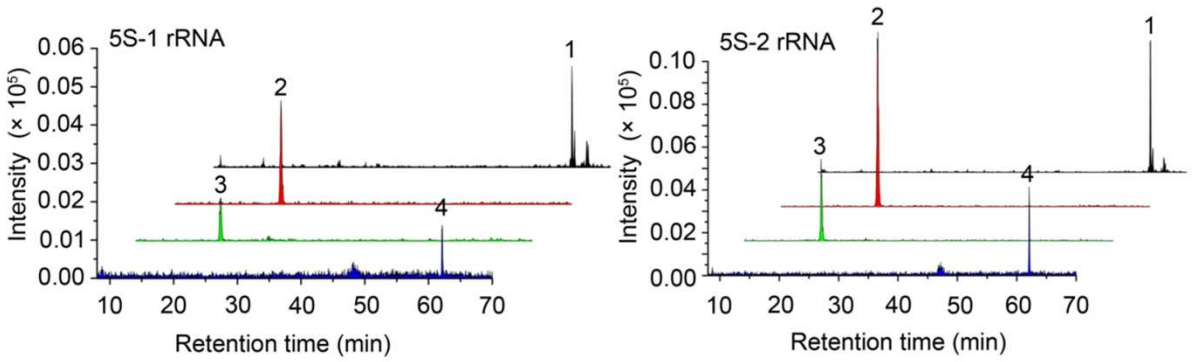


Figure S5 LC-MS/MS chromatogram of modified nucleosides from an enzymatic digestion of 5S rRNA from mouse liver. Numbered peaks are: 1, Am; 2, Cm; 3, m^3C; 4, m^6A.

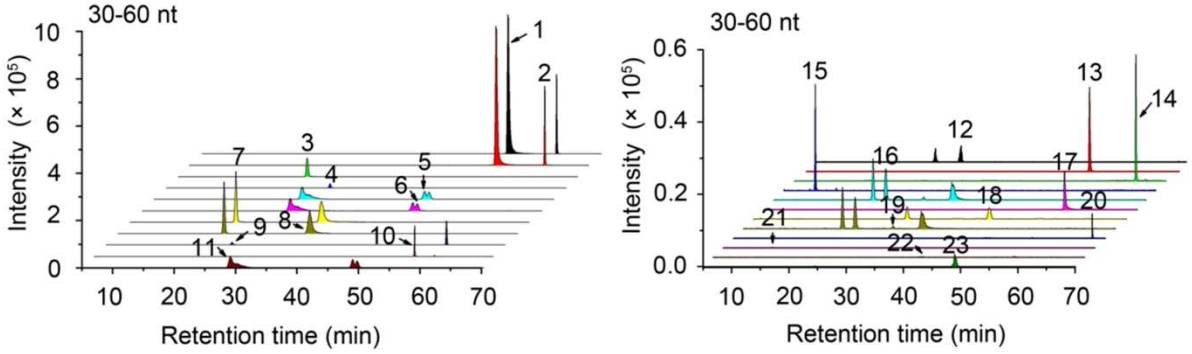


Figure S6 LC-MS/MS chromatogram of modified nucleosides from an enzymatic digestion of small RNAs sized at 30-60 nt from mouse liver. Numbered peaks are: 1, m^1A; 2, m^6A; 3, Cm; 4, G (^{13}C, ^{15}N); 5, m^1G; 6, m^2G; 7, m^3C; 8, m^5C; 9, I; 10, $m^2{}_2G$; 11, m^7G; 12, Um; 13, Gm; 14, Am; 15, Ψ; 16, m^5U; 17, $m^{2,2,7}G$; 18, ac^4C; 19, m^3U; 20, i^6A; 21, hm^5C; 22, m^5Cm; 23, m^5Um.

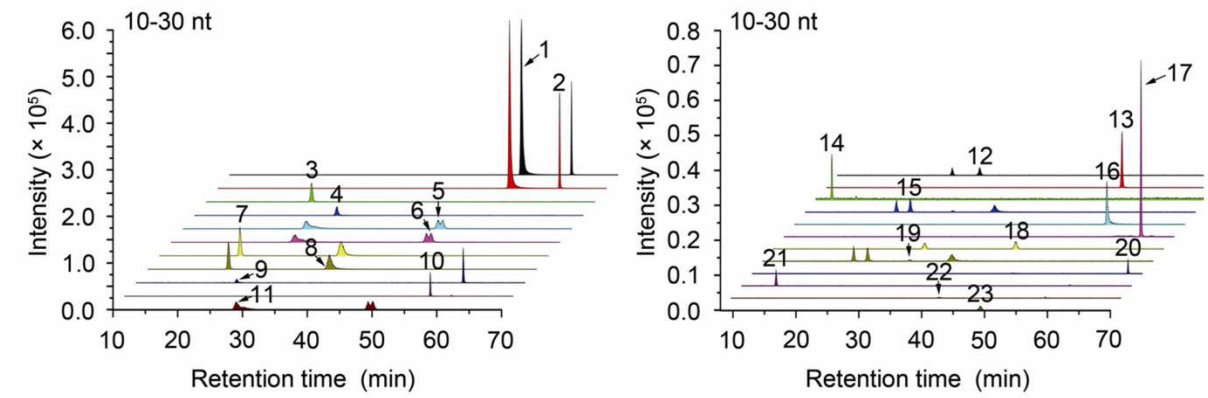


Figure S7 LC-MS/MS chromatogram of modified nucleosides from an enzymatic digestion of small RNAs sized at 10-30 nt from mouse liver. Numbered peaks are: 1, m^1A; 2, m^6A; 3, Cm; 4, G (^{13}C, ^{15}N); 5, m^1G; 6, m^2G; 7, m^3C; 8, m^5C; 9, I; 10, $m^2{}_2G$; 11, m^7G; 12, Um; 13, Gm; 14, Ψ; 15, m^5U; 16, $m^{2,2,7}G$; 17, Am; 18, ac^4C; 19, m^3U; 20, i^6A; 21, hm^5C; 22, m^5Cm; 23, m^5Um.

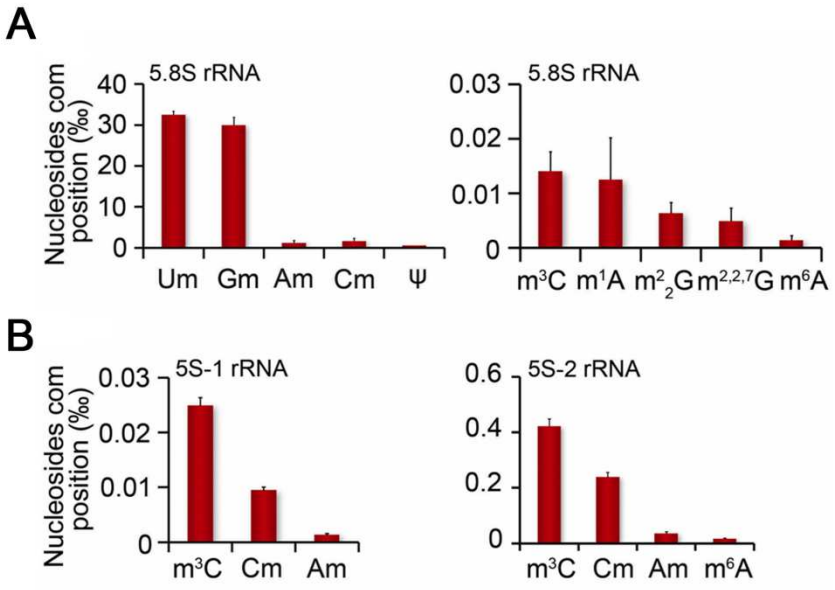


Figure S8 Modified nucleoside levels in 5.8S, 5S-1 and 5S-2 RNA from mouse liver. The modified nucleoside compositions (mol ‰) were normalized to guanosine. Error bars represent standard deviations. n = 6 for 5.8S and 5S-1 RNAs from normal mice, n = 7 for 5S-2 RNA from normal mice.