Supporting Information

Site-specific acetyl modification of 2'-OH of RNA by the oligonucleotide acetylating reagent

Hirotaka Murase*[a], Mio Eto[a], Jeongsu Lee[b], Yosuke Taniguchi[c], Shuhei Imoto[a], Shigeki Sasaki[b]

[a]Faculty of Pharmaceutical Sciences, Sojo University, 4-22-1 Ikeda, Nishi-ku, Kumamoto, Kumamoto 860-0082, Japan

[b]Graduate School of Pharmaceutical Sciences, Nagasaki International University, 2825-7 Huis Ten Bosch Machi, Sasebo 859-3298, Japan

[c]Faculty of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushimanaka, Kita-ku, Okayama 700-8530, Japan

*Corresponding Author Email: murase@ph.sojo-u.ac.jp; Tel: +81-326-3985

1. General

High performance liquid chromatography (HPLC) was performed on a JASCO LC-2000 PLUS series equipped with YMC-Triart Bio C18 column (4.6 x 250 mm). UPLC/QTOF-MS and MSMS measurement were performed on a Waters ACQUITY UPLC Xevo G2-XS QTof equipped with Oligonucleotide BEH C18 column (2.1 x 50 mm). The UV spectra measurement and the determination of the concentration of oligonucleotides were performed by Thermo Scientific NanoDrop One. The DNA and RNA oligomer were purchased from Japan Bio Services Co.,LTD. *S*-(bromomethyl) ethanethioate was purchased from Enamine Ltd.

2. General procedure of preparation of Ac-probe

To the 95 µL solution of 50 µM oligonucleotide probe containing a 6-thioguanine in 250 mM CAPS buffer pH10 was added 5 µL 10 % *S*-(bromomethyl) ethanethioate in ACN (v/v, final 0.5 %), and the reaction mixture was incubated at 0 °C for 1h. After filtration, HPLC purification was performed (YMC-Triart Bio C18 column (4.6 x 250 mm), A: 0.1 M TEAA buffer pH7 containing 5 % ACN, B: ACN, B % [0 to 30 % over 20 min], UV: 254 nm, flow rate: 1 mL/min, column oven: 40 °C). The separated solution was concentrated with (Amicon® Ultra 3K, 25 °C, 14,000xg, 25 min). After ultrapure water exchange with Amicon, pure Ac-probe was obtained. The determination of the concentration of oligonucleotide was performed by the measurement of the 260 nm absorption.

3. General procedure of acetyl modification of RNA

The following 10 µL solution was prepared on an ice bath and allowed to stand at respective temperatures. [Ac-probe (7.5 µM), target RNA (5 µM) and NaCl (100 mM) in buffer solution (50 mM, pH10: carbonate, pH8.5 and pH7.4: phosphate)]

4. UV spectra measurement

The following solutions were prepared and their UV absorption were measured by NanoDrop One (Thermo Scientific). [probe or Ac-probe (7.5 μM) in buffer solution (50 mM, pH5.2: sodium acetate, pH7.4: HEPES-NaOH, pH9.0: Tris-HCl, pH10: carbonate)

5. Analysis of acetyl modification of 131 mer RNA

131 mer RNA (5´-ggg ucu aga guu uaa cuu uaa gaa gga gau aua cau aug gcu agc aug acu ggu gga cag caa aug ggu acc gaa uuc aag acc gcg caa gac uac aag gac gac gac gau aag uag uga aua acu aau cc -3´) was prepared with general transcription method using T7-RNA polymerase from the purchased DNA template containing T7 promoter. Acetylation reaction of 131 mer RNA was analyzed by UPLC/MS and the MS numbers were obtained as ions adduct.

ACQUITY UPLC: Oligonucleotide BEH C18 Column, 130Å, 1.7μm, 2.1x50 mm, A: 15 mM TEA, 400 mM HFIP pH7.9, B: A/MeOH=1/1, D:35% to 70% /10min, Flow rate: 0.2 mL/min, Column oven: 40 °C.

MS conditions: Xevo G2-XS Qtof, Mass range: 450 – 3000 Da, Internal Standard: LE, Mode: ESI negative resolution, continuum, Cone voltage: 40 V, Capillary voltage: 4 kV, desolvation: 600 °C, desolvation gas flow: 700 L/hr.

The target MS numbers were set as below; 131 mer RNA [927.797 - 928.140 (46 valent ion)], Ac-RNA [928.703 – 929.021 (46 valent ion)].

6. Supplemental data

Name	Sequence	calcd. M.W.	found
Ac-probe-1	5'd-GTA GTC TTX' CGC GGT	4719	4719
regenerated probe-1	5'd-GTA GTC TTX CGC GGT	4631	4631
Ac-probe-2	5’d-CTT TX’T TCT CCT TTC T	4859	4859
RNA (N=a)	3'r-cau cag aaa gcg cca	4781	4781
Ac-RNA (N=a)		4823	4823
RNA (N=g)	3'r-cau cag aag gcg cca	4797	4797
Ac-RNA (N=g)		4839	4839
RNA (N=c)	3'r-cau cag aac gcg cca	4757	4757
Ac-RNA (N=c)		4799	4799
RNA (N=u)	3'r-cau cag aau gcg cca	4758	4758
Ac-RNA (N=u)		4800	4800

Table S1 Sequences and MS numbers.

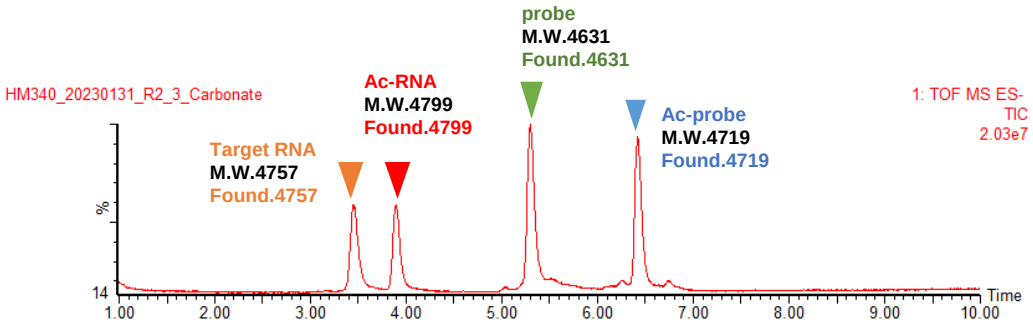
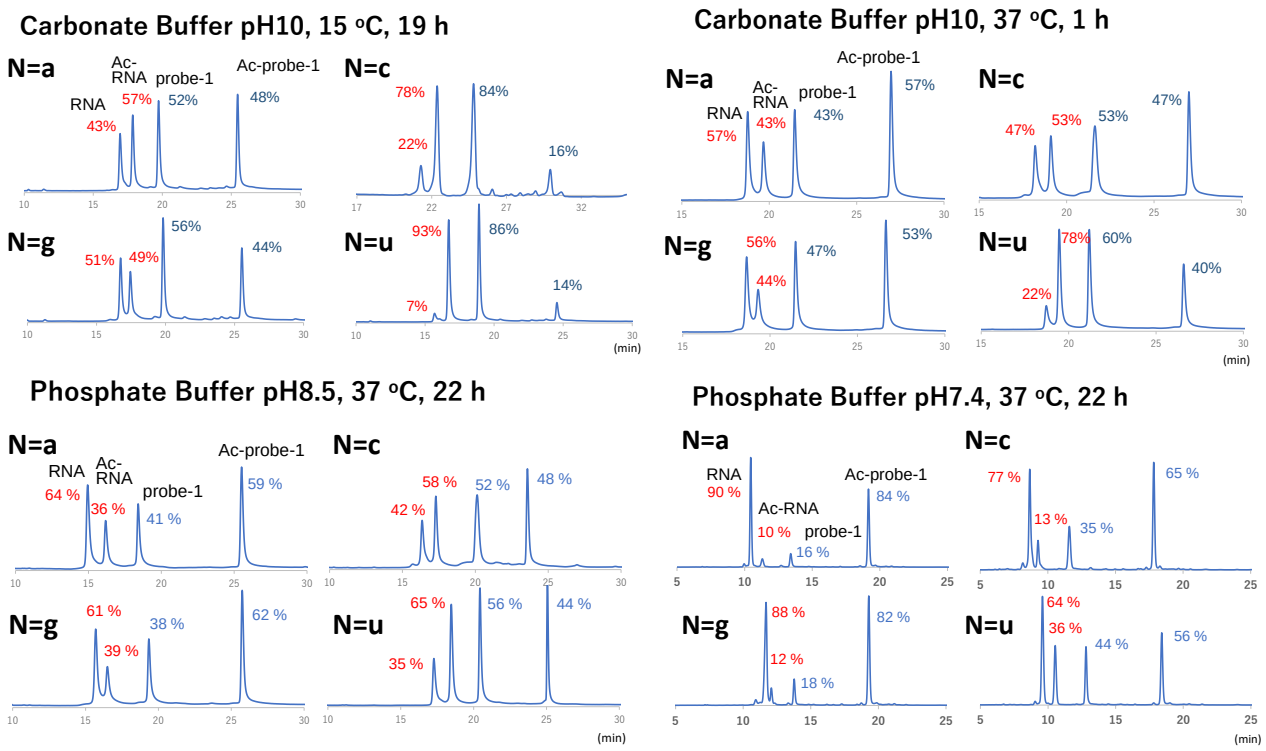


Fig. S1 TIC chart of the acetyl modification reaction under the condition of pH10 and 37 °C for 1h.

ACQUITY UPLC: Oligonucleotide BEH C18 Column, 130Å, 1.7µm, 2.1x50 mm, A: 15 mM TEA, 400 mM HFIP pH7.9, B: A/MeOH=1/1, D:35% to 70% /10min, Flow rate: 0.2 mL/min, Column oven: 40 °C.

MS conditions: Xevo G2-XS Qtof, Mass range: 450 – 3000 Da, Internal Standard: LE, Mode: ESI negative resolution, continuum, Cone voltage: 40 V, Capillary voltage: 4 kV, desolvation: 600 °C, desolvation gas flow: 700 L/hr.

a)



b)

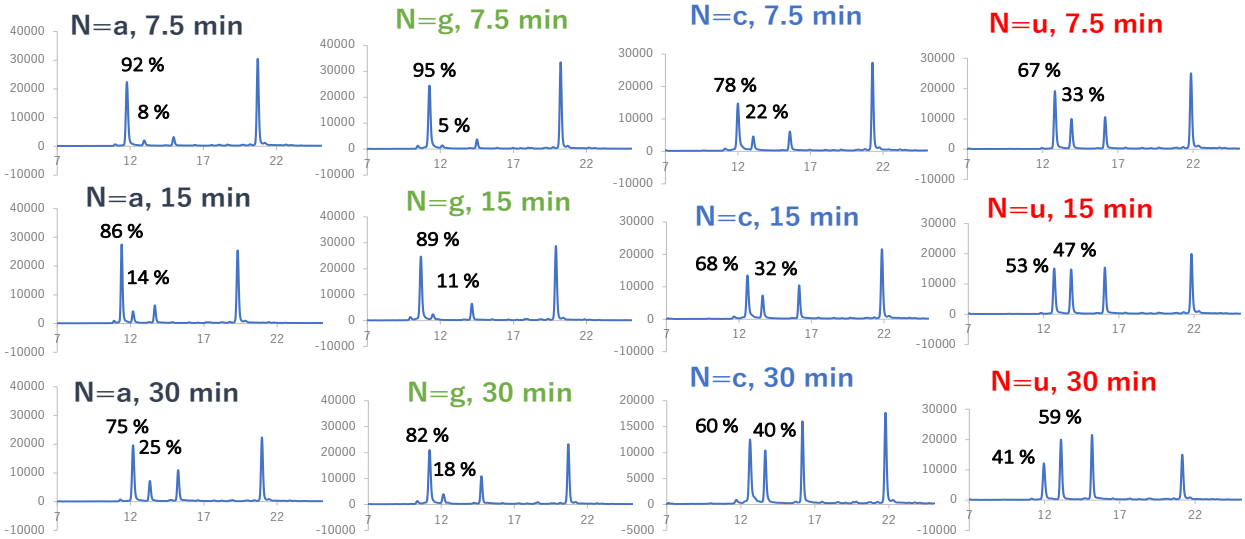
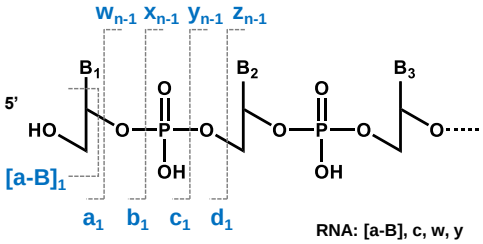


Fig. S2 HPLC charts of the acetyl modification. a) under various conditions, b) for time-course plots under pH10 at 37 °C.

YMC-Triart Bio C18 column (4.6 x 250 mm), A: 0.1 M TEAA buffer pH7 containing 5 % ACN, B: ACN, B % [0 to 10 % over 30 min], UV: 254 nm, flow rate: 1 mL/min, column oven: 40 °C.

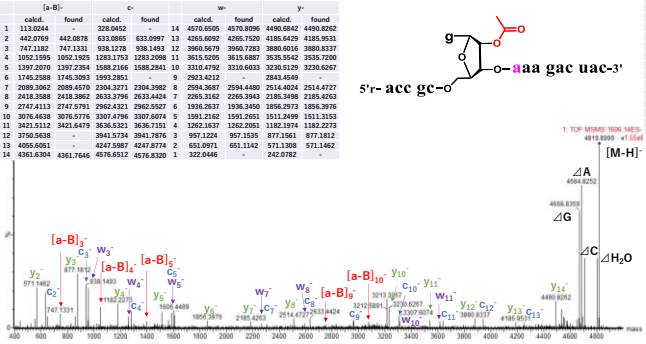


	[M-H]$^-$	precursor ion [M-3H]$^{3-}$
Ac-RNA (N=a)	4819.74	1605.91
Ac-RNA (N=g)	4835.73	1611.24
Ac-RNA (N=c)	4795.73	1597.90
Ac-RNA (N=u)	4796.71	1598.23
RNA (N=a)	4777.73	1591.90
RNA (N=g)	4793.72	1597.24
RNA (N=c)	4753.71	1583.90
RNA (N=u)	4754.70	1584.23

Ac-modified RNA

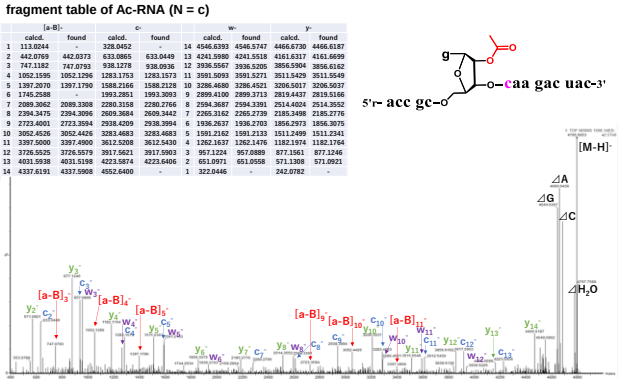
fragment table of Ac-RNA (N = a)

	[a-B]-		c-		w-		y-	
	calcd.	found	calcd.	found	calcd.	found	calcd.	found
1	113.0244	-	328.0452	-				
2	442.0769	442.0878	633.0865	633.0997	13	4265.6092	4265.7509	4185.6429	4185.9531
3	747.1182	747.1331	938.1278	938.1493	12	3960.5679	3960.7283	3880.6016	3880.8337
4	1052.1595	1052.1925	1243.1753	1283.2098	11	3615.5205	3615.6887	3535.5542	3535.7200
5	1397.2070	1397.2354	1588.2166	1588.2841	10	3310.4792	3310.6033	3230.5129	3230.6267
6	1745.2588	1745.3093	1993.2851	-	9	2923.4212	-	2843.4549	-
7	2089.3062	2089.4570	2304.3271	2304.3982	8	2594.3687	2594.4480	2514.4024	2514.4727
8	2418.3588	2418.3862	2633.3796	2633.4424	7	2265.3162	2265.3943	2185.3498	2185.4263
9	2747.4113	2747.5791	2962.4321	2962.5527	6	1936.2637	1936.3450	1856.2973	1856.3976
10	3076.4638	3076.5776	3307.4796	3307.6074	5	1591.2162	1591.2651	1511.2499	1511.3153
11	3421.5112	3421.6479	3636.5321	3636.7151	4	1262.1637	1262.2051	1182.1974	1182.2273
12	3750.5638	-	3941.5734	3941.7876	3	957.1224	957.1535	877.1561	877.1812
13	4055.6051	-	4247.5987	4247.8774	2	651.0971	651.1142	571.1308	571.1462
14	4361.6304	4361.7646	4576.6512	4576.8320	1	322.0446	-	242.0782	-



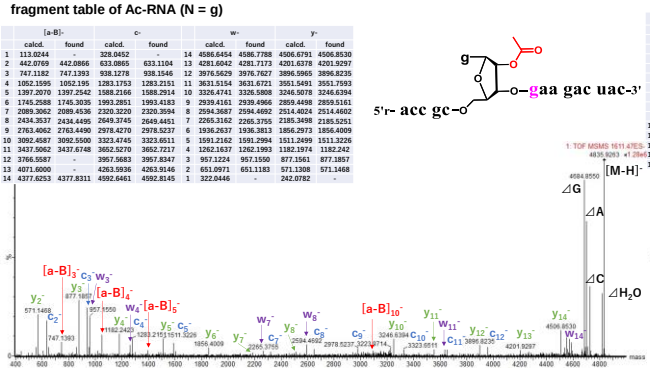
fragment table of Ac-RNA (N = c)

	[a-B]-		c-		w-		y-	
	calcd.	found	calcd.	found	calcd.	found	calcd.	found
1	113.0244	-	328.0452	-	14	4546.6393	4546.5747	4466.6730	4466.6187
2	442.0769	442.0373	633.0865	633.0449	13	4241.5980	4241.5518	4161.6317	4161.6699
3	747.1182	747.0793	938.1278	938.0936	12	3936.5567	3936.5205	3856.5904	3856.6162
4	1052.1595	1052.1296	1283.1753	1283.1573	11	3591.5093	3591.5271	3511.5429	3511.5549
5	1397.2070	1397.1790	1588.2166	1588.2128	10	3286.4680	3286.4521	3206.5017	3206.5037
6	1745.2588	-	1993.2851	1993.3093	9	2899.4100	2899.3713	2819.4437	2819.5166
7	2089.3062	2088.3308	2280.3158	2280.2766	8	2594.3687	2594.3391	2514.4024	2514.3552
8	2394.3475	2394.3096	2609.3684	2609.3442	7	2265.3162	2265.2739	2185.3498	2185.2776
9	2723.4001	2723.3594	2938.4209	2938.3994	6	1936.2637	1936.2703	1856.2973	1856.3075
10	3052.4526	3052.4426	3263.4683	3263.4683	5	1591.2162	1591.2133	1511.2499	1511.2341
11	3397.5000	3397.4900	3612.5208	3612.5430	4	1262.1637	1262.1476	1182.1974	1182.1764
12	3726.5525	3726.5579	3917.5621	3917.5903	3	957.1224	957.0889	877.1561	877.1246
13	4031.5938	4031.5198	4223.5874	4223.6406	2	651.0971	651.0558	571.1308	571.0921
14	4337.6191	4337.5908	4552.6400	-	1	322.0446	-	242.0782	-



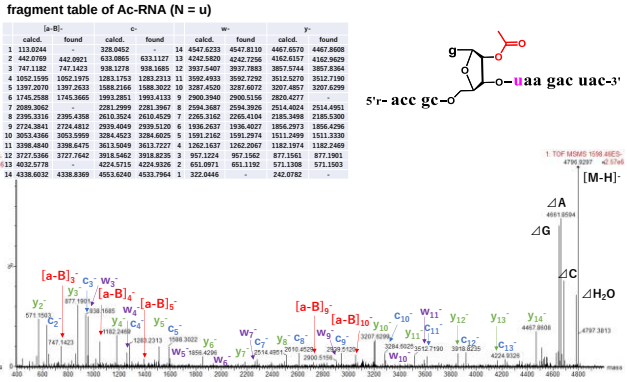
fragment table of Ac-RNA (N = g)

	[a-B]-		c-		w-		y-	
	calcd.	found	calcd.	found	calcd.	found	calcd.	found
1	113.0244	-	328.0452	-	14	4586.6454	4586.7788	4506.6791	4506.8530
2	442.0769	442.0866	633.0865	633.1104	13	4281.6042	4281.7173	4201.6378	4201.9297
3	747.1182	747.1393	938.1278	938.1546	12	3976.5629	3976.7627	3896.5965	3896.8235
4	1052.1595	1052.195	1283.1753	1283.2151	11	3631.5154	3631.6721	3551.5491	3551.7593
5	1397.2070	1397.2542	1588.2166	1588.2914	10	3326.4741	3326.5808	3246.5078	3246.6394
6	1745.2588	1745.3035	1993.2851	1993.4183	9	2939.4161	2939.4966	2859.4498	2859.5161
7	2089.3062	2089.4536	2320.3220	2320.3594	8	2594.3687	2594.4692	2514.4024	2514.4602
8	2434.3537	2434.4495	2649.3745	2649.4451	7	2265.3162	2265.3755	2185.3498	2185.4251
9	2763.4062	2763.4490	2978.4270	2978.5237	6	1936.2637	1936.3813	1856.2973	1856.4009
10	3092.4587	3092.5500	3323.4745	3323.6511	5	1591.2162	1591.2994	1511.2499	1511.3226
11	3437.5062	3437.6748	3652.5270	3652.7217	4	1262.1637	1262.1993	1182.1974	1182.242
12	3766.5587	-	3957.5683	3957.8347	3	957.1224	957.1550	877.1561	877.1857
13	4071.6000	-	4263.5936	4263.9146	2	651.0971	651.1183	571.1308	571.1468
14	4377.6253	4377.8311	4592.6461	4592.8145	1	322.0446	-	242.0782	-



fragment table of Ac-RNA (N = u)

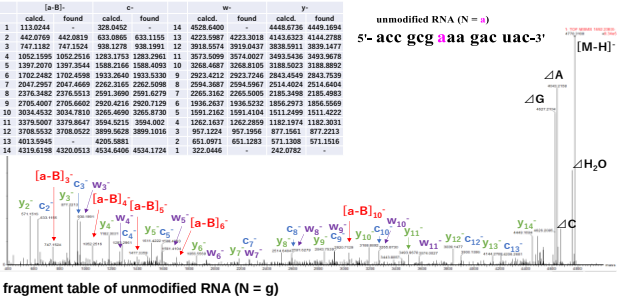
	[a-B]-		c-		w-		y-	
	calcd.	found	calcd.	found	calcd.	found	calcd.	found
1	113.0244	-	328.0452	-	14	4547.6233	4547.8110	4467.6570	4467.8608
2	442.0769	442.0921	633.0865	633.1127	13	4242.5820	4242.7256	4162.6157	4162.9629
3	747.1182	747.1423	938.1278	938.1685	12	3937.5407	3937.7883	3857.5744	3857.8364
4	1052.1595	1052.1975	1283.1753	1283.2313	11	3592.4933	3592.7292	3512.5270	3512.7190
5	1397.2070	1397.2633	1588.2166	1588.3022	10	3287.4520	3287.6072	3207.4857	3207.6299
6	1745.2588	1745.3665	1993.2851	1993.4133	9	2900.3940	2900.5156	2820.4277	-
7	2089.3062	-	2281.2959	2281.3967	8	2594.3687	2594.3926	2514.4024	2514.4951
8	2395.3316	2395.4358	2610.3524	2610.4529	7	2265.3162	2265.4104	2185.3498	2185.5300
9	2724.3841	2724.4812	2939.4049	2939.5120	6	1936.2637	1936.4027	1856.2973	1856.4296
10	3053.4366	3053.5959	3264.4023	3264.6025	5	1591.2162	1591.2974	1511.2499	1511.3330
11	3398.4840	3398.6475	3613.5049	3613.7227	4	1262.1637	1262.2067	1182.1974	1182.2469
12	3727.5366	3727.7642	3918.5462	3918.8235	3	957.1224	957.1562	877.1561	877.1901
13	4032.5778	-	4224.5715	4224.9326	2	651.0971	651.1192	571.1308	571.1503
14	4338.6032	4338.8369	4553.6240	4533.7964	1	322.0446	-	242.0782	-



unmodified RNA

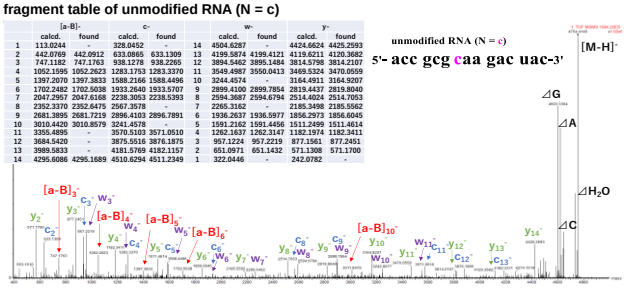
fragment table of unmodified RNA (N = a)

	[a-B]-		c-		w-		y-	
	calcd.	found	calcd.	found	calcd.	found	calcd.	found
1	113.0244	-	328.0452	-	14	4528.6400	-	4448.6736	4449.1694
2	442.0769	442.0819	633.0865	633.1155	13	4223.5987	4223.3018	4143.6323	4144.2788
3	747.1182	747.1524	938.1278	938.1991	12	3918.5574	3919.0437	3838.5911	3839.1477
4	1052.1595	1052.2516	1283.1753	1283.2961	11	3573.5099	3574.0027	3493.5436	3493.9678
5	1397.2070	1397.3544	1588.2166	1588.4093	10	3268.4687	3268.8105	3188.5023	3188.8892
6	1702.2482	1702.4598	1933.2640	1933.5330	9	2923.4212	2923.7246	2843.4549	2843.7539
7	2047.2957	2047.4669	2262.3165	2262.5098	8	2594.3687	2594.5967	2514.4024	2514.6404
8	2376.3482	2376.5513	2591.3690	2591.6279	7	2265.3162	2265.5005	2185.3498	2185.4983
9	2705.4007	2705.6602	2920.4216	2920.7129	6	1936.2637	1936.5232	1856.2973	1856.5569
10	3034.4532	3034.7810	3265.4690	3265.8730	5	1591.2162	1591.4104	1511.2499	1511.4222
11	3379.5007	3379.8647	3594.5215	3594.002	4	1262.1637	1262.2859	1182.1974	1182.3031
12	3708.5532	3708.0522	3899.5628	3899.1016	3	957.1224	957.1956	877.1561	877.2213
13	4013.5945	-	4205.5881	-	2	651.0971	651.1283	571.1308	571.1516
14	4319.6198	4320.0513	4534.6406	4534.1724	1	322.0446	-	242.0782	-



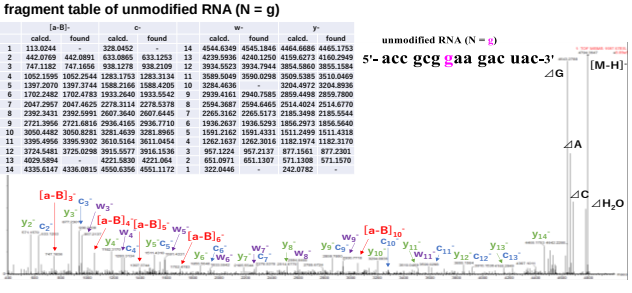
fragment table of unmodified RNA (N = c)

	[a-B]-		c-		w-		y-	
	calcd.	found	calcd.	found	calcd.	found	calcd.	found
1	113.0244	-	328.0452	-	14	4504.6287	-	4424.6624	4425.2593
2	442.0769	442.0912	633.0865	633.1309	13	4199.5874	4199.4121	4119.6211	4120.3682
3	747.1182	747.1763	938.1278	938.2265	12	3894.5462	3895.1484	3814.5798	3814.2107
4	1052.1595	1052.2623	1283.1753	1283.3370	11	3549.4987	3550.0413	3469.5324	3470.0559
5	1397.2070	1397.3833	1588.2166	1588.4496	10	3244.4574	-	3164.4911	3164.9207
6	1702.2482	1702.5038	1933.2640	1933.5707	9	2899.4100	2899.7854	2819.4437	2819.8040
7	2047.2957	2047.6168	2238.3053	2238.5393	8	2594.3687	2594.6794	2514.4024	2514.7063
8	2352.3370	2352.6475	2567.3578	-	7	2265.3162	-	2185.3498	2185.5562
9	2681.3895	2681.7219	2896.4103	2896.7891	6	1936.2637	1936.5977	1856.2973	1856.6045
10	3010.4420	3010.8579	3241.4578	-	5	1591.2162	1591.4456	1511.2499	1511.4614
11	3355.4895	-	3570.5103	3571.0510	4	1262.1637	1262.3147	1182.1974	1182.3411
12	3684.5420	-	3875.5516	3876.1875	3	957.1224	957.2219	877.1561	877.2451
13	3989.5833	-	4181.5769	4182.1157	2	651.0971	651.1432	571.1308	571.1700
14	4295.6086	4295.1689	4510.6294	4511.2349	1	322.0446	-	242.0782	-



fragment table of unmodified RNA (N = g)

	[a-B]-		c-		w-		y-	
	calcd.	found	calcd.	found	calcd.	found	calcd.	found
1	113.0244	-	328.0452	-	14	4544.6349	4545.1846	4464.6686	4465.1753
2	442.0769	442.0891	633.0865	633.1253	13	4239.5936	4239.6273	4159.6211	4160.2949
3	747.1182	747.1656	938.1278	938.2109	12	3934.5523	3934.7944	3854.5860	3855.1584
4	1052.1595	1052.2544	1283.1753	1283.3134	11	3589.5049	3590.0298	3509.5385	3510.0469
5	1397.2070	1397.3744	1588.2166	1588.4205	10	3284.4636	-	3204.4972	3204.8936
6	1702.2482	1702.4783	1933.2640	1933.5542	9	2939.4161	2940.7585	2859.4498	2859.7800
7	2047.2957	2047.4625	2278.3114	2278.5378	8	2594.3687	2594.6465	2514.4024	2514.6770
8	2392.3431	2392.5991	2607.3640	2607.6445	7	2265.3162	2265.5173	2185.3498	2185.5544
9	2721.3956	2721.6816	2936.4165	2936.7710	6	1936.2637	1936.5293	1856.2973	1856.5640
10	3050.4482	3050.8281	3281.4639	3281.8965	5	1591.2162	1591.4331	1511.2499	1511.4318
11	3395.4956	3395.9302	3610.5164	3611.0454	4	1262.1637	1262.3016	1182.1974	1182.3170
12	3724.5481	3725.0298	3915.5577	3916.1536	3	957.1224	957.2137	877.1561	877.2301
13	4029.5894	-	4221.5830	4221.064	2	651.0971	651.1307	571.1308	571.1570
14	4335.6147	4336.0815	4550.6356	4551.1172	1	322.0446	-	242.0782	-



fragment table of unmodified RNA (N = u)

	[a-B]-		c-		w-		y-	
	calcd.	found	calcd.	found	calcd.	found	calcd.	found
1	113.0244	-	328.0452	-	14	4505.6128	4506.2339	4425.6464	4426.2598
2	442.0769	442.0935	633.0865	633.1327	13	4200.5715	4201.1172	4120.6051	4121.3711
3	747.1182	747.1787	938.1278	938.2300	12	3895.5302	3896.1272	3815.5638	3816.218
4	1052.1595	1052.2792	1283.1753	1283.3420	11	3550.4827	3551.0557	3470.5164	3471.0554
5	1397.2070	1397.3959	1588.2166	1588.4539	10	3245.4414	3245.8374	3165.4751	3165.9209
6	1702.2482	1702.5078	1933.2640	1933.6002	9	2900.3940	2900.7712	2820.4277	2820.7996
7	2047.2957	-	2239.2893	2239.5508	8	2594.3687	2594.6506	2514.4024	2514.7253
8	2353.3210	2353.6052	2568.3418	2568.6560	7	2265.3162	2265.5623	2185.3498	2185.5811
9	2682.3735	2682.7390	2897.3943	-	6	1936.2637	1936.5837	1856.2973	1856.6072
10	3011.4260	3011.8542	3242.4418	3242.9277	5	1591.2162	1591.4559	1511.2499	1511.4727
11	3356.4735	3356.9421	3571.4943	3572.0620	4	1262.1637	1262.3291	1182.1974	1182.3448
12	3685.5260	3686.1382	3876.5356	3878.1860	3	957.1224	957.2286	877.1561	877.2471
13	3990.5673	-	4182.5609	4183.0176	2	651.0971	651.1478	571.1308	571.1735
14	4296.5926	4296.0869	4511.6134	-	1	322.0446	-	242.0782	-

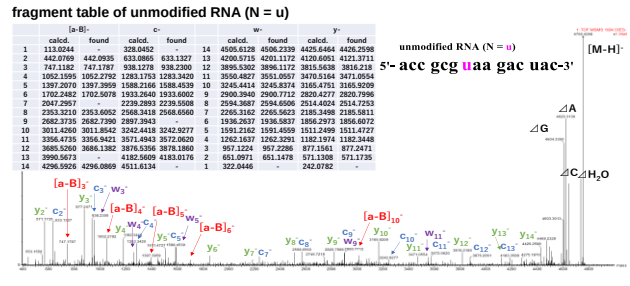


Fig. S3 MS/MS fragment analysis of the acetylated RNA and unmodified RNA.

LC conditions, ACQUITY UPLC: Oligonucleotide BEH C18 Column, 130Å, 1.7µm, 2.1x50 mm, A: 15 mM TEA, 400 mM HFIP pH7.9, B: A/MeOH=1/1, D:35% to 70% /10min, Flow rate: 0.2 mL/min, Column oven: 40 °C.

MS/MS conditions, MS system: Xevo G2-XS Qtof, Mass range: 450 – 2500 Da, Mode: ESI negative resolution, continuum, Capillary voltage: 2.7 kV, Cone voltage: 31 V, source: 120 °C, desolvation: 300 °C, desolvation gas flow: 500 L/hr, precursor ion: $[M-3H]^{3-}$, LockMass: 1685.765 ($Cs_6I_7^-$), scan time: 0.2sec, collision energy ramp: 25 to 55 V.

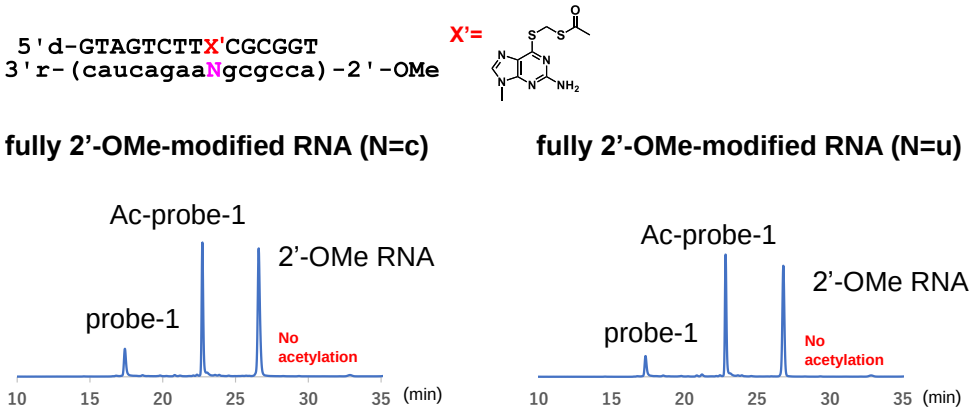


Fig. S4 The results of Ac-probe-1 vs. fully 2'-OMe-modified RNAs under the condition of pH10, 37°C for 1 h. YMC-Triart Bio C18 column (4.6 x 250 mm), A: 0.1 M TEAA buffer pH7 containing 5 % ACN, B: ACN, B % [0 to 10 % over 30 min], UV: 254 nm, flow rate: 1 mL/min, column oven: 40 °C.

a)

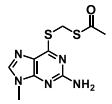
Ac-probe-S1

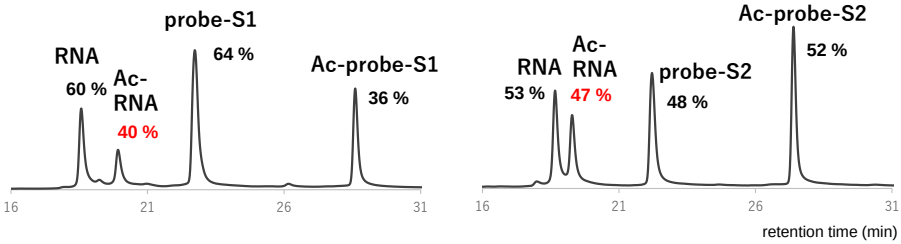
5'd-GTA**X**'TCTTGCGCGGT
3'r-cau**c** agaacgcgcca

Ac-probe-S2

5'd-GTAGTCTTGC**X**'CGGT
3'r-caucagaacg**c** gcca

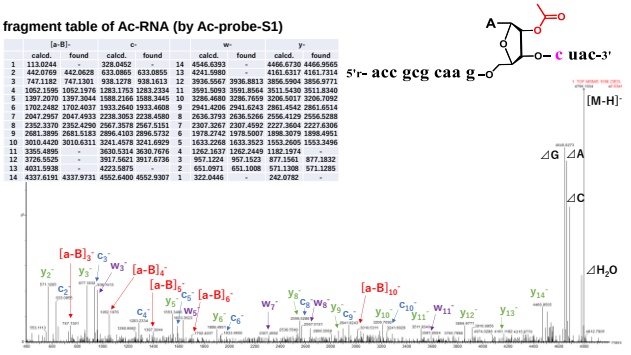
X'=





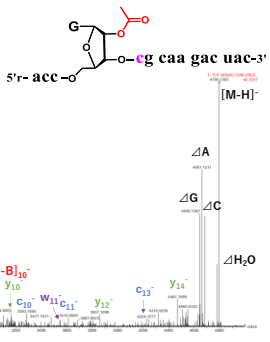
fragment table of Ac-RNA (by Ac-probe-S1)

	[a-B]-		c-		w-		y-	
	calcd.	found	calcd.	found	calcd.	found	calcd.	found
1	113.0244	-	328.0452	-	4546.6393	-	4466.6730	4466.9565
2	442.0769	442.0628	633.0865	633.0855	4241.5980	-	4161.6317	4161.7314
3	747.1182	747.1301	938.1278	938.1613	3936.5567	3936.8813	3856.5904	3856.9771
4	1052.1595	1052.1976	1283.1753	1283.2334	3591.5093	3591.8564	3511.5430	3511.8340
5	1397.2070	1397.3044	1588.2166	1588.3445	3286.4680	3286.7659	3206.5017	3206.7092
6	1702.2482	1702.4037	1933.2640	1933.4608	2941.4206	2941.6243	2861.4542	2861.6514
7	2047.2957	2047.4933	2238.3053	2238.4580	2636.3793	2636.5266	2556.4129	2556.5288
8	2352.3370	2352.4290	2567.3578	2567.5151	2307.3267	2307.4592	2227.3604	2227.8306
9	2681.3895	2681.5183	2896.4103	2896.5732	1978.2742	1978.5007	1898.3079	1898.4951
10	3010.4420	3010.6311	3241.4578	3241.6929	1633.2268	1633.3523	1553.2606	1553.3496
11	3355.4895	-	3630.5314	3630.7676	1262.1637	1262.2449	1182.1974	-
12	3726.5525	-	3917.5621	3917.6736	957.1224	957.1523	877.1561	877.1832
13	4031.5938	-	4223.5875	-	651.0971	651.1008	571.1308	571.1285
14	4337.6191	4337.9731	4552.6400	4552.9307	322.0446	-	242.0782	-



fragment table of Ac-RNA (by Ac-probe-S2)

	[a-B]-		c-		w-		y-	
	calcd.	found	calcd.	found	calcd.	found	calcd.	found
1	113.0244	-	328.0452	-	14 4546.6393		4466.6730	4467.1099
2	442.0769	442.0746	633.0865	633.1063	13 4241.5980	-	4161.6317	4162.2246
3	747.1182	747.1495	938.1278	938.1761	12 3936.5567	-	3856.5904	3857.1099
4	1094.1700	1094.2314	1343.1964	1344.2343	11 3549.4987	3549.8145	3469.5324	-
5	1439.2175	-	1630.2271	1630.3905	10 3244.4574	3244.7202	3164.4911	3164.8003
6	1744.2588	1744.4773	1975.2746	1975.3708	9 2899.4100	2899.6907	2819.4437	2819.6672
7	2089.3062	-	2280.3158	2280.6545	8 2594.3687	2594.5627	2514.4024	2514.6077
8	2394.3475	-	2609.3684	2609.5317	7 2265.3162	2265.4346	2185.3498	2185.4956
9	2723.4001	2723.6514	2938.4209	2938.7043	6 1936.2637	1936.4332	1856.2973	1856.5389
10	3052.4526	3052.7192	3283.4683	3283.7800	5 1591.2162	1591.3635	1511.2499	1511.3916
11	3397.5000	3397.7891	3612.5208	3612.9006	4 1262.1637	1262.2505	1182.1974	1182.2855
12	3726.5525	-	3917.5621	3918.0588	3 957.1224	957.1861	877.1561	877.2006
13	4031.5938	4031.7395	4223.5874	4224.1577	2 651.0971	651.1103	571.1308	571.1416
14	4337.6191	4337.9272	4552.6400	-	1 322.0446	-	242.0782	-

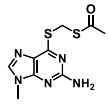


b)

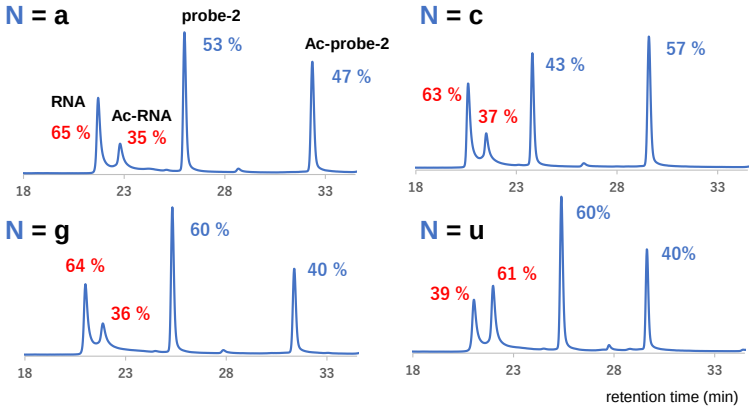
Ac-probe-2

5'd-CTTT**X**'TTCTCCTTTCT
3'r-gaaa**N** aagaggaaaga

X'=



N = a, g, c or u



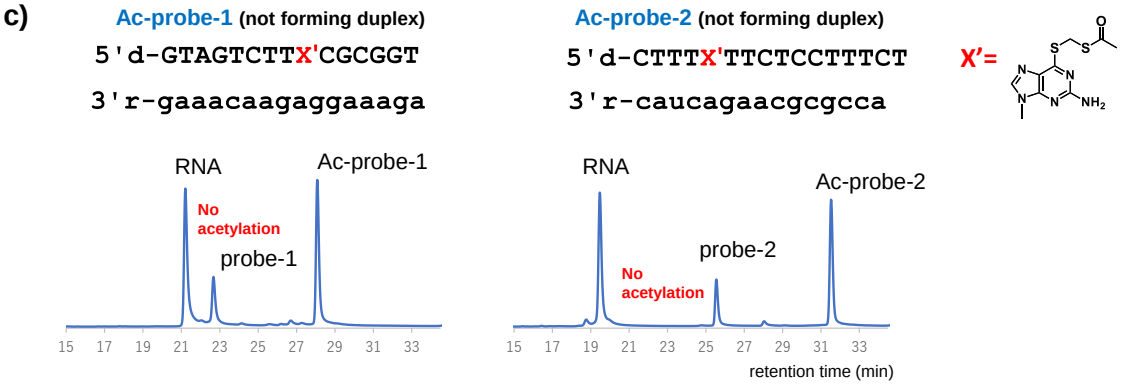


Fig. S5 The variation of modification reactions under the condition of pH10 and 37°C for 1 h. a) using Ac-probe-S with a 6-thioguanine reactive site (X') at different position (upper part), MS/MS analysis of the product RNAs (lower part), b) using Ac-probe-2 and its complementary target RNAs, c) using the pairs of Ac-probe and target RNA not forming a duplex.