Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

In-source Fragmentation of Nucleosides at Electrospray Ionization: Towards More Sensitive and Accurate Nucleoside Analysis

‡Yu-Nan Chen[1], ‡Xu-Yang Shen[1], Yue Yu[2], Chen-Yu Xue[3], Ying-Lin Zhou[1]*, Xin-Xiang Zhang[1]

1 Beijing National Laboratory for Molecular Sciences (BNLMS), MOE Key Laboratory of Bioorganic Chemistry and Molecular Engineering, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China.

2 Institute of Biotechnology Development, Qilu Pharmaceutical, Jinan

3 Key Laboratory of Forensic Toxicology, Ministry of Public security, Beijing, China

‡ These authors contributed equally to this work.

* Tel: +86-10-62754112; Fax: +86-10-62754112; Email: zhouyl@pku.edu.cn.

Contents

Table 1. Calculated free energy (G_f) of unlabeled and labeled nucleosides protonated at different sites[a]

Species	G_f(N3) - G_f (O2) (kJ/mol)	Species	G_f - G_f (N1) (kJ/mol)	Species	G_f - G_f (N) (kJ/mol)
5cadC	-18.3785	5fdC- Et_2N -N1	0	5fdC-RBH-N[b]	0
5fdC	-22.00169	**5fdC- Et_2N -N2**	**-65.32244**	5fdC-RBH-O[b]	23.20942
5hmdC	-15.70049	5fdC- Et_2N -N3	-29.45811	5fdC-RBH-N1	-26.28126
5mdC	-10.39698	5fdC- Et_2N -N4	-27.90907	**5fdC-RBH-N-N1**	**-84.92967**
dC	-13.75762	5fdC- Et_2N -N5	44.42346		
rC	-15.621725	5fdC- Et_2N -N6	44.449715		
mrC	-12.366105	5fdC- Et_2N –O[b]	8.112795		
rCm	-15.411685	5fdC- Et_2N –N[b]	-18.82484		

[a] The protonation sites are shown in Figure S4

[b] –O and –N means protonation at O2 or N3 position of the nitrogenous bases

Table S2. Optimization of MS parameters for 5fdC analysis in MRM mode of LC-MS/MS

Analyte	Precursor ion (Q1)	Product ion (Q3)	Collision energy (CE)	Collision cell exit potential (CXP)	LOD (amol)
$[5fdC+H]^+$	256.1	140.1	18	10	1250
$[5fdC\text{-}i\text{-}Pr_2N+H]^+$	547.3	431.2	31	11	10
$[5fdC\text{-}RBH+H]^+$	694.3	578.3	25	10	3
$[5fdC\text{-}RBH+2H]^{2+}$	347.7	289.6	12	10	30

Table S3. Profiling of nucleosides in RNA of MCF-7 cells based on ion pairs resulted from ISF

Type	Original ion			Fragment ion		
	Mass	Peak height	RT	Mass	Peak height	RT
rA	268.104	2.02E+09	6.81	136.061	7.46E+07	6.77
mrA	282.118	8.05E+07	10.3	150.077	2.79E+06	10.3
$m^{6,6}rA$	296.135	2.76E+07	10.47	164.093	2.40E+06	10.46
rAm	282.118	1.84E+08	10.09	136.061	7.63E+06	10.09
$ct^{6}A$	395.132	1.49E+05	11.57	263.089	8.71E+04	11.59
rG	284.099	8.63E+08	5.53	152.057	2.04E+08	5.54
$m^{1}rG$	298.115	2.30E+06	9.21	166.072	3.00E+05	9.21
$m^{7}rG$	298.115	3.53E+06	3.00	166.072	3.76E+05	3.00
rGm	298.115	1.12E+07	8.29	152.057	2.55E+06	8.29
rC	244.093	1.05E+09	1.48	112.051	2.41E+08	1.48
$m^{5}rC$	258.109	5.33E+06	2.27	126.066	1.25E+06	2.27
rCm	258.109	4.46E+06	3.51	112.051	1.25E+06	3.53
$ac^{4}C$	286.104	1.01E+07	5.55	154.061	5.18E+05	5.55
U	245.077	1.16E+08	2.81	113.035	6.98E+07	2.81
$nm^{5}U$	274.103	2.93E+05	2.68	142.061	2.98E+04	2.66
I	269.088	1.22E+05	5.35	137.046	2.76E+05	5.35

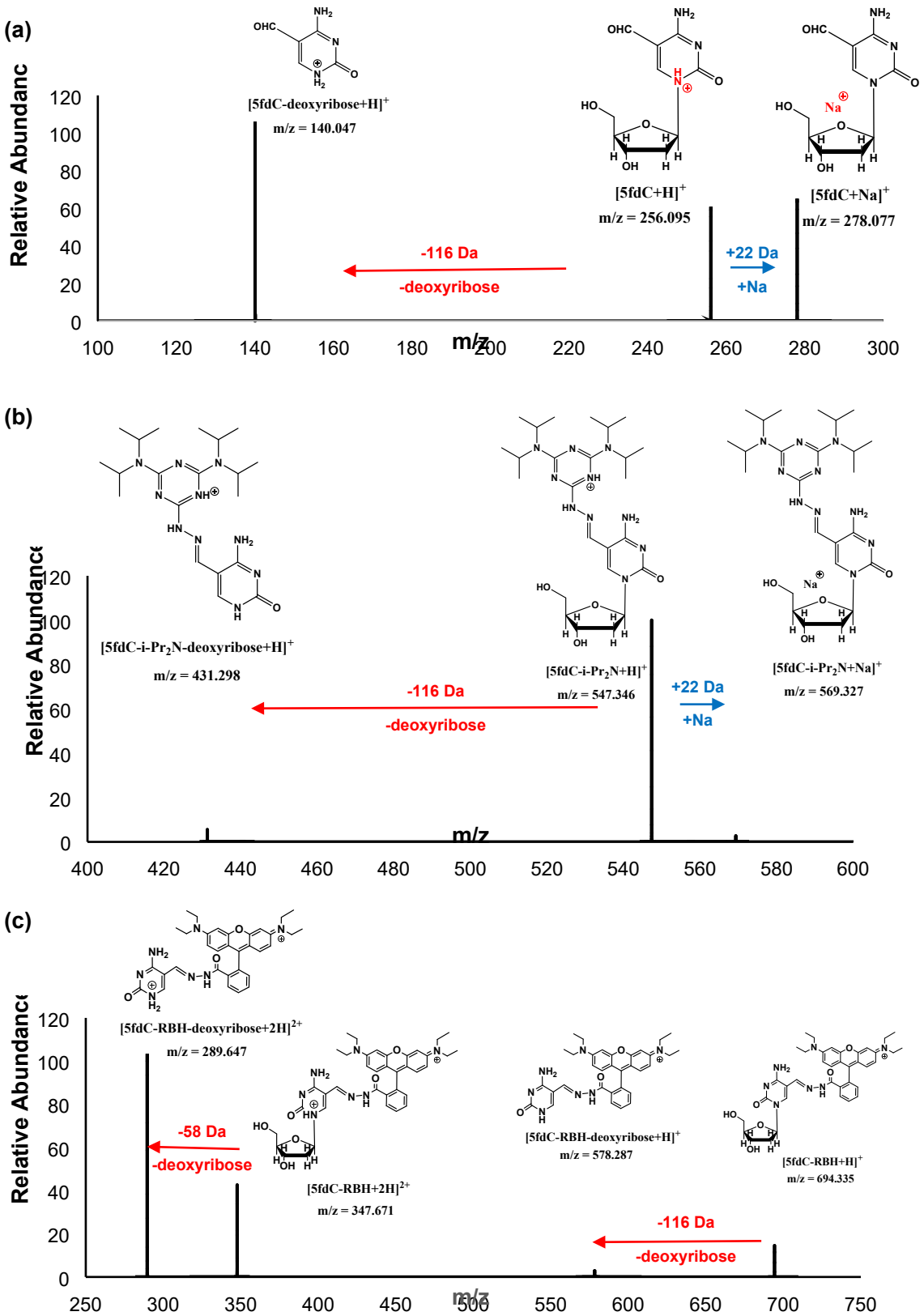


Figure S1. Extracted MS spectra of (a) 5fdC, (b) 5fdC-i-Pr_2N, (c) 5fdC-RBH in LC-MS analysis

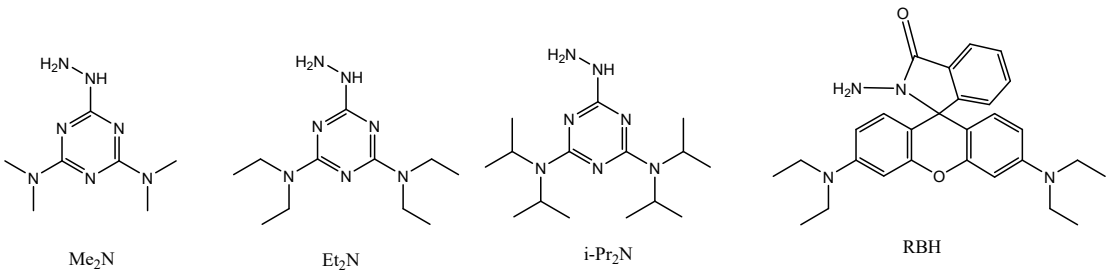


Figure S2. Structures of labeling reagents for 5fdC

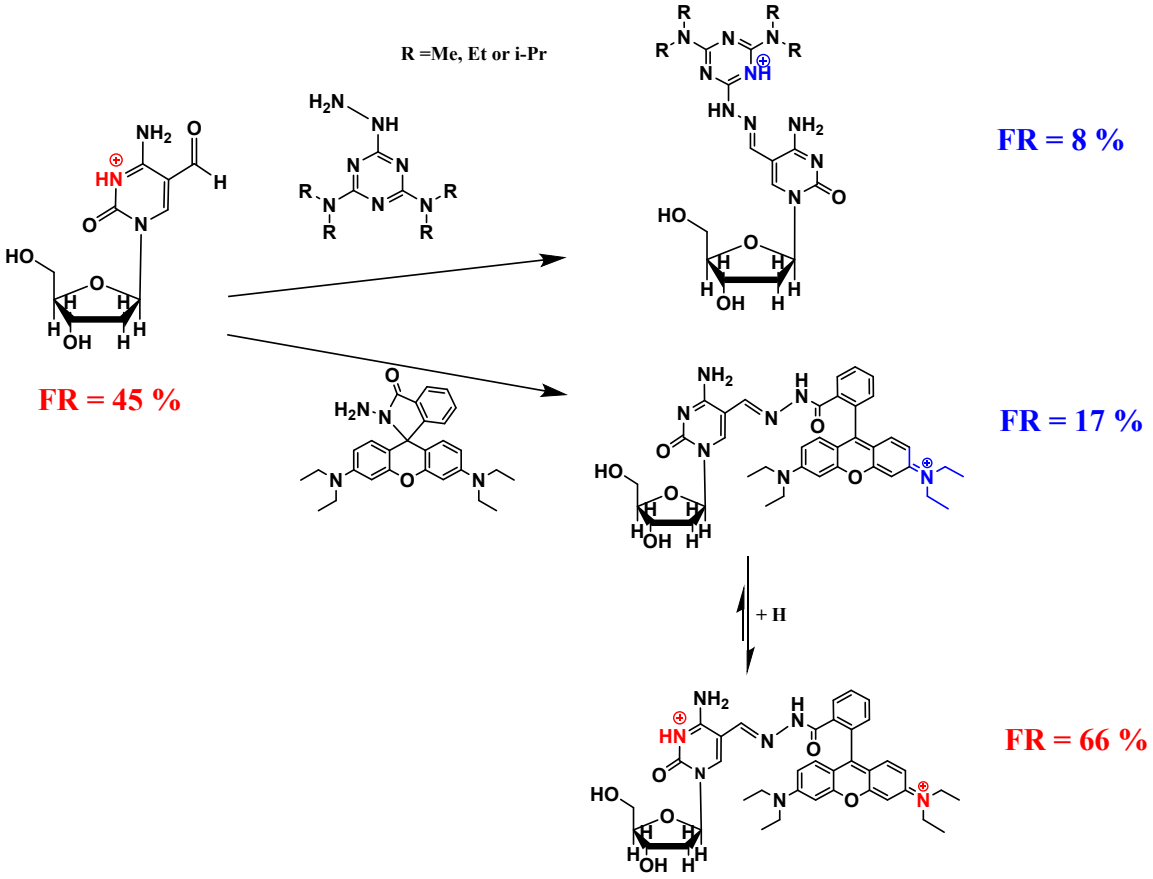
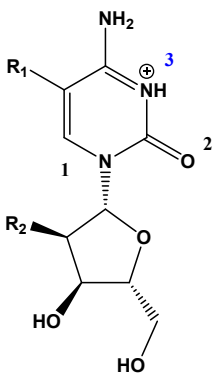
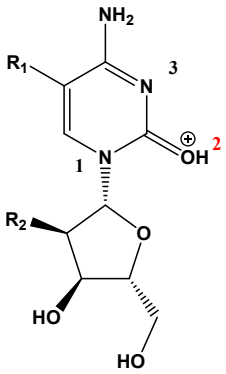


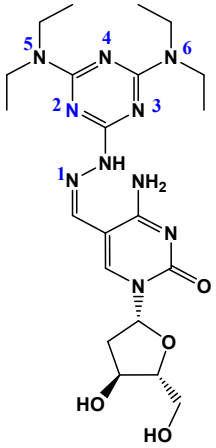
Figure S3. The labeling reaction of 5fdC labeled with different labeling reagents and the fragmentation ratio



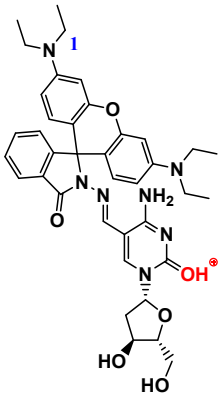
modified C-N3



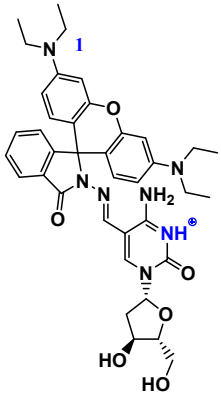
modified C-O2



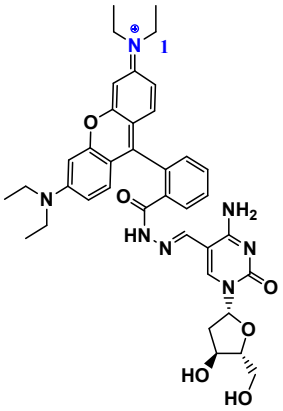
5fdC-Et$_2$N-N2



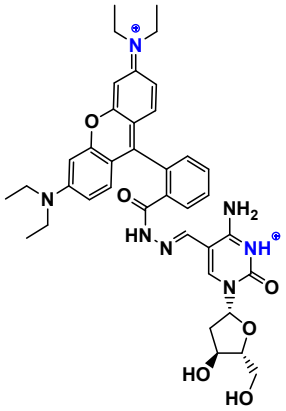
5fdC-RBH-O



5fdC-RBH-N



5fdC-RBH-N1



5fdC-RBH-N-N1

Figure S4. Structures of different 5fdC species for theoretical calculation

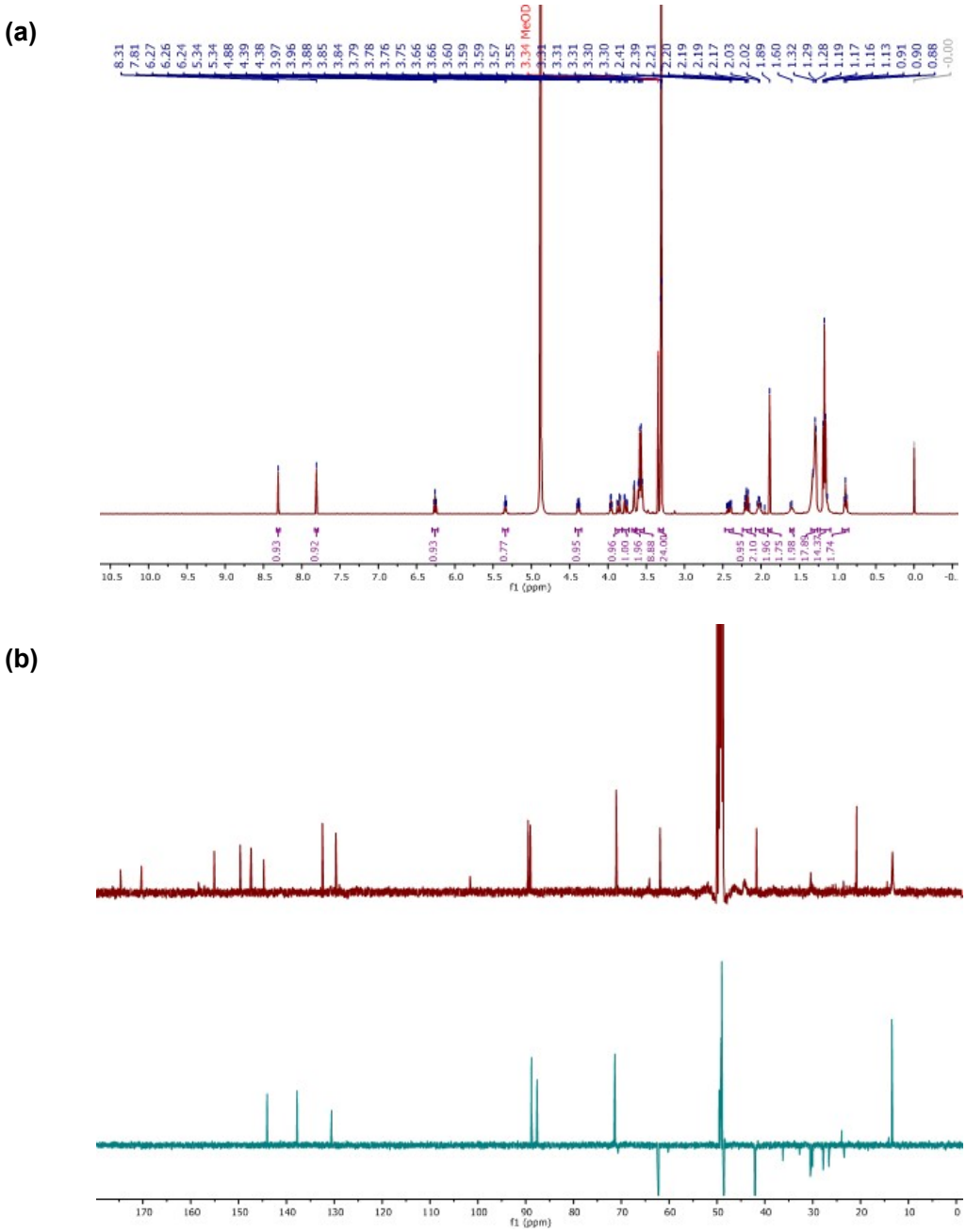


Figure S5. (a) 1-H NMR (400 M) spectrum of 5fdC-Et_2N (in CD_3OD). δ 8.31 (s, 1H), 7.81 (s, 1H), 6.26 (t, J = 6.3 Hz, 1H), 5.34 (dd, J = 5.4, 4.2 Hz, 1H), 4.39 (dt, J = 6.4, 4.1 Hz, 1H), 3.86 (dd, J = 12.1, 3.1 Hz, 1H), 3.77 (dd, J = 12.2, 3.7 Hz, 1H), 3.66 (d, J = 2.0 Hz, 2H), 3.58 (q, J = 7.0 Hz, 9H), 3.31 (p, J = 1.6 Hz, 24H), 2.42 (ddd, J = 13.7, 6.2, 4.2 Hz, 1H), 2.24 – 2.13 (m, 2H), 2.02 (dt, J = 10.7, 5.7 Hz, 2H), 1.89 (s, 2H), 1.60 (s, 2H), 1.30 (t, J = 7.9 Hz, 18H), 1.17 (t, J = 7.0 Hz, 14H), 0.94 – 0.86 (m, 2H). **(b)** 13-C NMR (600 M) spectra with DEPT of 5fdC-Et_2N

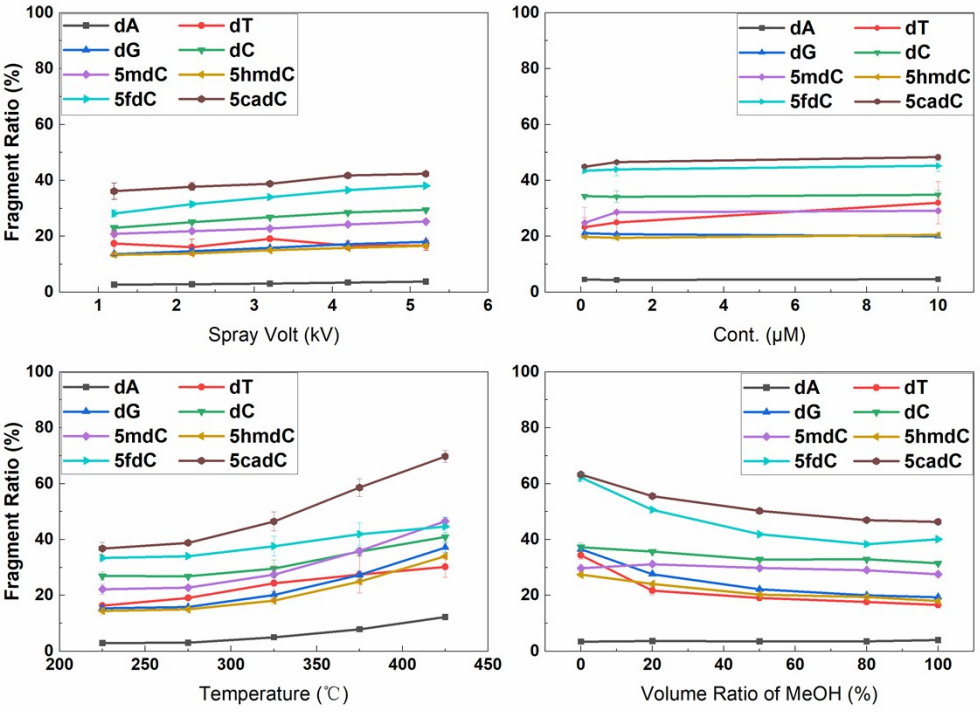


Figure S6. The fragmentation ratio of deoxyribonucleosides under (a) different spray voltage, (b) different analyte concentration, (c) different temperature of ion transfer capillary, and (d) different background buffer composition

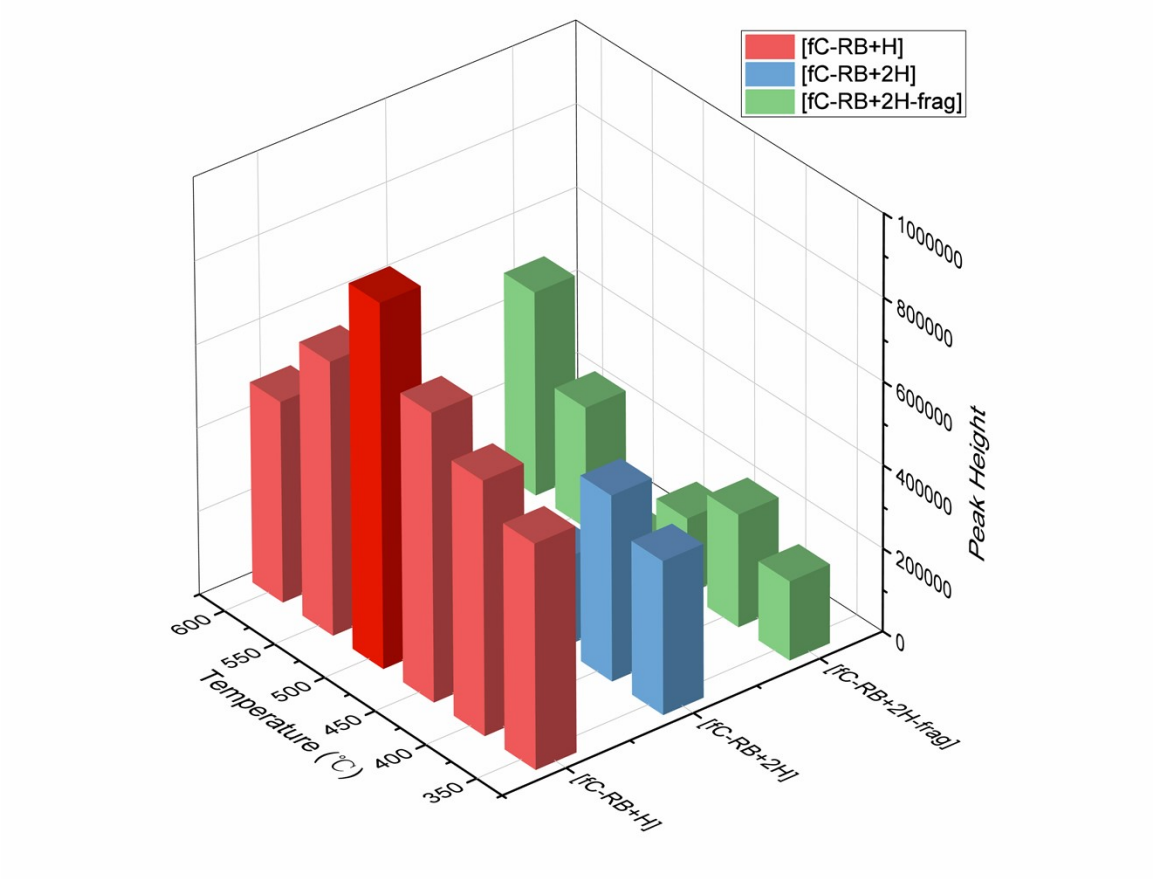


Figure S7. Optimization of LC-MS conditions for 5fdC-RBH analysis

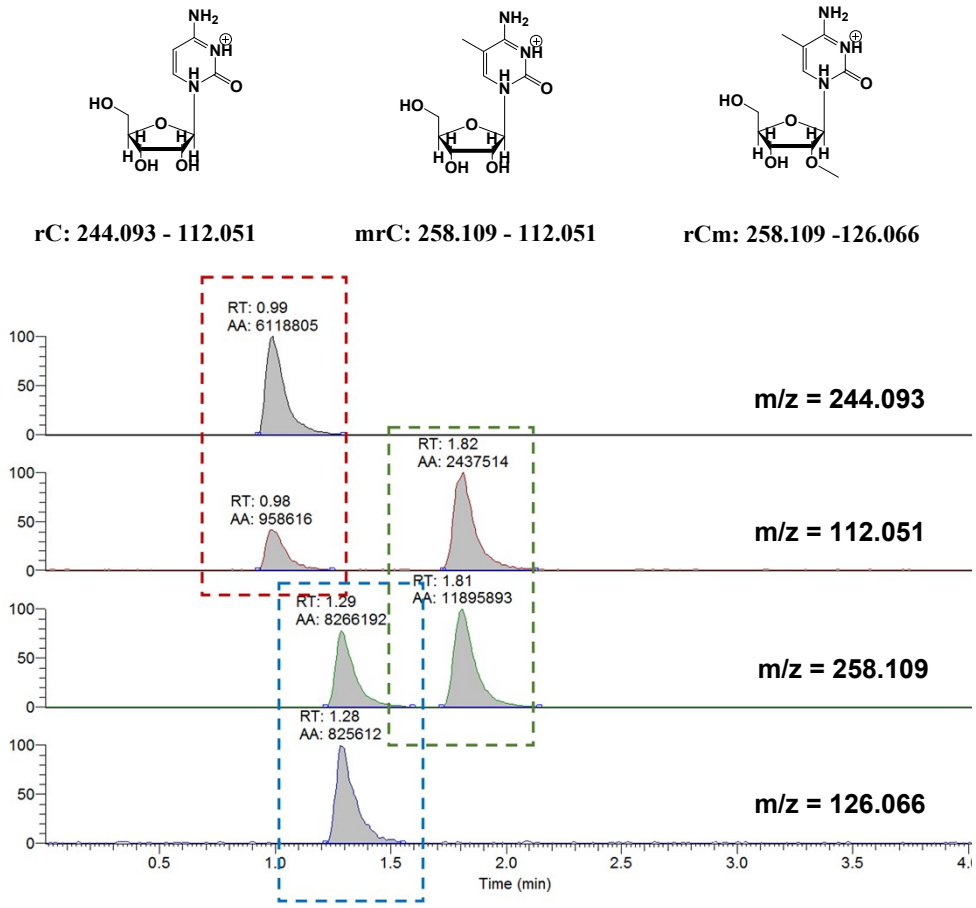


Figure S8. Identification of rC, mrC and rCm based on different original and fragment ions

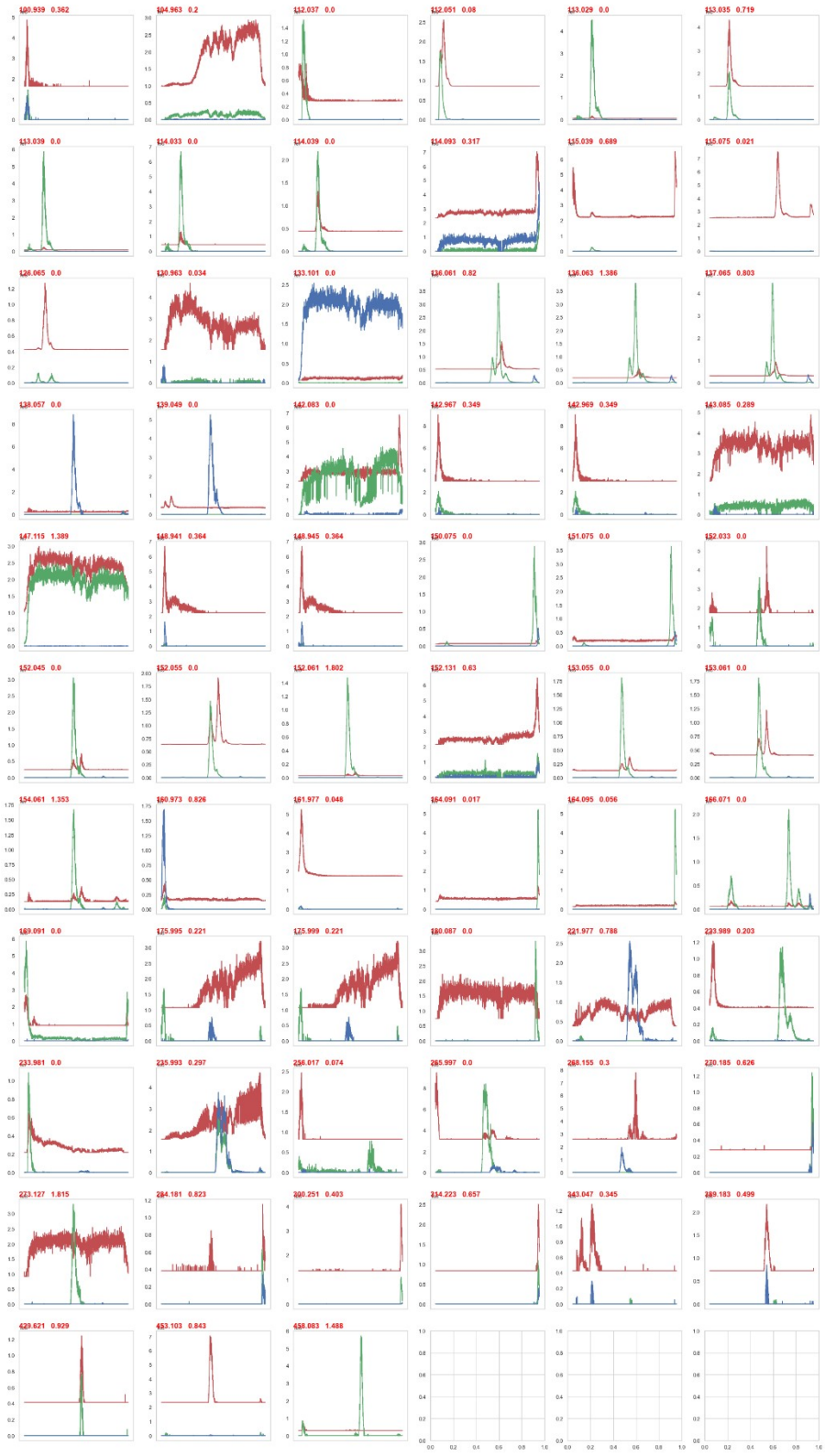


Figure S9. Determination of nucleosides and modified nucleosides in total RNAs of MCF-7 cells based on different original and fragment ions with Python