Supporting Information:

Sensitive analysis of Multiple Low-molecular-weight Thiols in a Single Human Cervical Cancer Cell by Chemical Derivatization-Liquid Chromatography-Mass Spectrometry

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LWM thiols	Scan mode	Q1 Mass (Da)	Q3 Mass (Da)	Q1 Pre Bias (V)	Collision Energy (V)	Q3 Pre Bias (V)
N-isobutyryl-D-cysteine	(-)	190.1	112.1	12.0	12.0	18.0
2-mercaptosuccinic acid	(-)	149.2	115.1	25.0	14.0	24.0
ethyl 3-mercaptopropionate	(+)	135.0	103.1	-10.0	-8.0	-19.0
N-(2-mercaptoethyl)acetamide	(+)	120.0	61.0	-17.0	-21.0	-24.0
N-acetyl-L-cysteine	(-)	162.0	84.0	26.0	11.0	14.0
cysteinylglycine	(+)	179.0	76.1	-20.0	-16.0	-30.0
pantetheine	(+)	279.0	182.9	-13.0	-16.0	-18.0
glutathione	(+)	308.0	162.2	-11.0	-17.0	-30.0
homocysteine	(+)	136.1	56.1	-23.0	-18.0	-19.0
cysteine	(+)	122.1	59.0	-24.0	-23.0	-22.0

Table S2. Optimized MRM transitions and collision energies for AYBA derivatives of LWM thiols (all in positive ion mode).

Abbreviations	Analytes	Scan Mode	Q1 Mass (Da)	Q3 Mass (Da)	Q1 Pre Bias (V)	Collision Energy (V)	Q3 Pre Bias (V)
1	cysteinylaspartic acid	(+)	470.1	195.1	-17.0	-21.0	-20.0
2	cysteamine	(+)	312.1	195.1	-30.0	-46.0	-30.0
3	cysteinylglycine	(+)	413.1	195.1	-30.0	-55.0	-30.0
4	cysteine	(+)	356.1	195.1	-12.0	-22.0	-20.0
5	homocysteine	(+)	370.1	195.1	-23.0	-23.0	-19.0
6	methyl L-cysteinate	(+)	370.1	195.1	-13.0	-20.0	-20.0
7	glutathione	(+)	542.1	195.1	-20.0	-27.0	-20.0
8	2-mercaptoacetic acid	(+)	327.1	195.1	-30.0	-35.0	-30.0
9	3-mercaptopropan-1-ol	(+)	327.1	195.1	-30.0	-47.0	-30.0
10	pyridine-4-thiol	(+)	346.1	195.1	-30.0	-30.0	-20.0
11	2-mercaptosuccinic acid	(+)	385.1	195.1	-14.0	-28.0	-20.0
12	N-acetyl-L-cysteine	(+)	398.0	195.1	-30.0	-48.0	-30.0
13	N-(2-mercaptoethyl)acetamide	(+)	354.1	195.1	-30.0	-50.0	-30.0
14	hydrogen sulfide	(+)	503.1	195.1	-24.0	-19.0	-20.0
15	pantetheine	(+)	513.2	383.1	-40.0	-21.0	-30.0

16	3-mercaptotetrahydrothiophene 1,1-dioxide	(+)	387.1	195.1	-30.0	-55.0	-30.0
17	N-isobutyryl-D-cysteine	(+)	426.1	195.1	-15.0	-31.0	-20.0
18	ethyl 2-mercaptoacetate	(+)	355.1	195.1	-30.0	-44.0	-30.0
19	methyl 3-mercaptopropionate	(+)	355.1	195.2	-13.0	-26.0	-20.0
20	ethyl 3-mercaptopropionate	(+)	369.1	195.1	-30.0	-50.0	-30.0
21	ethyl 2-mercaptopropionate	(+)	369.1	195.1	-30.0	-55.0	-30.0
22	ethane-1,2-dithiol	(+)	563.1	195.1	-40.0	-43.0	-20.0
23	(*tert*-butoxycarbonyl)-L-cysteine	(+)	456.1	195.1	-16.0	-18.0	-25.0

Table S3. LOD values in MRM mode for the non-derivatized RSH and AYBA derivatives of RSH (AYBA-RSH) expressed in fmol.

Analytes	LODs of Non-derivatized RSH (fmol)	LODs of AYBA-RSH (fmol)
N-isobutyryl-D-cysteine	224	0.468
2-mercaptosuccinic acid	5.34	0.252
ethyl 3-mercaptopropionate	46.1	0.162
N-(2-mercaptoethyl)acetamide	5.70	0.0940
N-acetyl-L-cysteine	2.90	0.136
cysteinylglycine	4.10	0.0660
pantetheine	271	0.0285
glutathione	11.8	0.206
homocysteine	3.19	0.0675
cysteine	2.24	0.0120

Table S4. Linearities, LOD, and LOQ values of 23 LMW thiols derivatized by AYBA.

Abbreviations	Analytes	Linear range (nM)	y = ax + b			LODs(pM)	LOQs(pM)
			Slope a	Intercept b	*r* value		
1	cysteinylaspartic acid	0.1–100	0.2367	0.0308	0.9998	3.6	10.7
2	cysteamine	0.1–200	0.3261	−0.0539	0.9991	17.8	53.3
3	cysteinylglycine	0.1–200	0.2261	0.0164	0.9955	13.2	39.7
4	cysteine	0.1–100	0.2637	−0.0258	0.9998	2.4	7.3
5	homocysteine	0.1–100	0.2532	0.003	0.9981	13.5	40.4
6	methyl L-cysteinate	0.5–200	0.1287	0.0949	0.9971	55.7	167.0
7	glutathione	0.2–100	0.2570	0.0260	0.9989	41.1	146.3
8	2-mercaptoacetic acid	0.2–200	0.2580	−0.3070	0.9988	6.7	20.1
9	3-mercaptopropan-1-ol	0.1–100	0.2124	−0.0680	0.9966	34.4	103.3
10	pyridine-4-thiol	0.1–200	0.3119	0.1448	0.9996	5.0	15.0
11	2-mercaptosuccinic acid	0.1–100	0.1750	0.0071	0.9970	50.5	73.9
12	N-acetyl-L-cysteine	0.2–100	0.2157	−0.0299	0.9974	27.3	81.9
13	N-(2-mercaptoethyl)acetamide	1.0–200	1.0376	−0.2817	0.9982	18.8	56.4
14	hydrogen sulfide	2.0–400	0.4132	0.0757	0.9949	26.5	79.4
15	pantetheine	0.2–200	0.2503	0.0402	0.9989	5.7	17.2
16	3-mercaptotetrahydrothiophene 1,1-dioxide	0.2–200	0.3620	−0.0468	0.9997	5.8	17.3
17	N-isobutyryl-D-cysteine	0.5–200	0.2374	−0.0419	0.9960	93.7	281.2
18	ethyl 2-mercaptoacetate	0.1–200	0.2178	0.0726	0.9965	21.6	72.2
19	methyl 3-mercaptopropionate	0.1–200	0.2677	0.0754	0.9950	17.2	51.5
20	ethyl 3-mercaptopropionate	0.1–100	0.2557	0.0277	0.9982	32.5	97.6
21	ethyl 2-mercaptopropionate	0.1–100	0.3120	−0.1133	0.9982	22.0	66.1
22	ethane-1,2-dithiol	0.2–200	0.3137	−0.2024	0.9971	13.3	44.5
23	(*tert*-butoxycarbonyl)-L-cysteine	1.0–200	0.2787	−0.0125	0.9975	192.3	576.9

Table S5. Precisions (intra- and inter-day RSDs) and recoveries of the proposed method employing AYBA derivatization in ~1000 HeLa cells. LMW thiol standards were spiked in HeLa cells at three different concentrations: 100, 400, and 2000 nM for LWM thiols 4, 6, 7, 11, 13, 14, and 23; 50, 200, and 1000 nM for all other LWM thiols.

Abbreviations	Analytes	Intra-day precision (RSD, %; n=5)			Inter-day precision (RSD, %; n=3)			Recovery (%, n=3)		
		Low	Medium	High	Low	Medium	High	Low	Medium	High
1	cysteinylaspartic acid	10.2	9.3	9.4	12.5	12.2	9.0	85.1	99.9	97.7
2	cysteamine	2.4	5.6	5.2	5.0	6.4	6.9	97.7	107.3	113.0
3	cysteinylglycine	6.3	5.4	8.1	9.2	8.5	7.7	90.3	107.5	101.6
4	cysteine	10.0	6.7	4.9	9.3	6.3	5.0	99.7	98.8	104.1
5	homocysteine	10.8	8.7	9.3	12.1	10.7	8.8	100.4	104.2	99.8
6	methyl L-cysteinate	5.6	9.1	7.7	11.1	10.8	7.6	82.3	101.1	117.8
7	glutathione	10.9	8.3	7.3	9.6	9.6	9.0	97.6	93.7	88.9
8	2-mercaptoacetic acid	13.1	8.9	9.9	13.0	11.1	9.4	93.2	91.4	97.0
9	3-mercaptopropan-1-ol	18.9	12.1	17.6	18.4	12.3	15.0	87.7	96.5	95.9
10	pyridine-4-thiol	11.5	8.4	10.7	14.1	15.1	11.8	90.9	97.5	89.9
11	2-mercaptosuccinic acid	19.8	17.5	18.7	19.1	15.9	9.9	90.0	86.4	98.7
12	N-acetyl-L-cysteine	18.7	18.6	14.5	16.9	14.9	13.5	91.8	89.4	93.0
13	N-(2-mercaptoethyl)acetamide	15.7	14.1	13.7	13.1	13.7	12.5	91.8	92.7	89.6
14	hydrogen sulfide	19.4	17.5	11.6	11.4	10.3	8.6	76.6	81.6	92.2
15	pantetheine	13.1	9.0	9.4	9.9	11.3	10.6	84.1	108.7	108.4

16	3-mercaptotetrahydrothiophene 1,1-dioxide	6.1	6.3	7.3	6.0	7.5	6.8	101.2	103.8	104.1
17	N-isobutyryl-D-cysteine	17.2	15.2	15.4	16.8	14.1	9.7	82.2	94.1	91.6
18	ethyl 2-mercaptoacetate	16.7	11.3	9.3	15.0	9.4	11.5	93.8	99.9	96.7
19	methyl 3-mercaptopropionate	8.1	5.5	7.5	7.7	8.5	8.2	86.0	98.1	99.1
20	ethyl 3-mercaptopropionate	15.0	8.2	10.1	13.2	12.1	11.3	101.1	99.9	91.1
21	ethyl 2-mercaptopropionate	1.6	3.9	5.5	3.9	5.2	5.3	94.2	99.7	109.9
22	ethane-1,2-dithiol	15.0	14.6	6.7	19.0	11.6	8.1	93.8	99.6	97.0
23	(*tert*-butoxycarbonyl)-L-cysteine	15.6	16.8	16.2	18.6	18.8	13.5	83.6	97.8	108.0

Table S6. Matrix effects for the investigated LWM thiol derivatives of AYBA in sample extracts of ~1000 HeLa cells at three different concentrations: 100, 400, and 2000 nM for LWM thiols 4, 6, 7, 11, 13, 14, and 23; 50, 200, and 1000 nM for all other LWM thiols.

Abbreviations	Analytes	Low (%)	Medium (%)	High (%)
1	cysteinylaspartic acid	104.4±5.3	103.5±5.1	108.6±3.5
2	cysteamine	103.1±6.0	101.0±6.0	98.5±5.1
3	cysteinylglycine	101.6±6.6	102.7±5.5	107.0±4.9
4	cysteine	100.8±5.9	98.8±5.1	93.3±3.8
5	homocysteine	102.5±8.2	102.2±7.2	105.6±4.6
6	methyl L-cysteinate	105.6±5.4	96.1±9.6	103.8±2.4
7	glutathione	104.0±9.2	106.3±8.9	105.9±5.9
8	2-mercaptoacetic acid	102.5±9.8	102.0±4.7	102.3±7.9
9	3-mercaptopropan-1-ol	101.2±8.9	96.4±4.3	95.5±3.7
10	pyridine-4-thiol	93.5±5.1	90.0±4.5	92.9±4.6
11	2-mercaptosuccinic acid	107.4±2.1	98.8±3.5	100.0±6.4
12	N-acetyl-L-cysteine	104.3±9.8	101.2±9.3	102.5±4.9
13	N-(2-mercaptoethyl)acetamide	103.8±3.6	103.7±7.3	100.3±7.5
14	hydrogen sulfide	90.0±4.4	89.0±7.2	89.4±7.5
15	pantetheine	95.6±9.2	91.6±8.6	87.3±6.6
16	3-mercaptotetrahydrothiophene 1,1-dioxide	100.5±7.4	98.2±7.8	87.5±5.6

17	N-isobutyryl-D-cysteine	102.2±8.4	100.1±9.4	100.4±4.1
18	ethyl 2-mercaptoacetate	96.1±8.2	94.7±5.2	93.7±4.3
19	methyl 3-mercaptopropionate	99.3±8.6	98.7±8.8	96.3±8.6
20	ethyl 3-mercaptopropionate	106.4±7.3	101.9±10.1	103.7±8.8
21	ethyl 2-mercaptopropionate	100.1±5.5	100.2±8.6	99.9±6.1
22	ethane-1,2-dithiol	109.5±5.8	98.1±8.6	108.5±9.1
23	(*tert*-butoxycarbonyl)-L-cysteine	93.1±3.0	91.7±8.0	88.0±3.8

Table S7. Concentrations of the 23 LMW thiols detected in ~1000 HeLa cells (n.d., indicates no detection, while detected indicates that the detection was not quantitative).

Abbreviations	Analytes	Concentration of thiols in HeLa Cells (μM)
1	cysteinylaspartic acid	0.37±0.01
2	cysteamine	0.40±0.01
3	cysteinylglycine	0.61±0.02
4	cysteine	5.16±0.24
5	homocysteine	0.44±0.02
6	methyl L-cysteinate	0.84±0.10
7	glutathione	119.21±5.15
8	2-mercaptoacetic acid	1.80±0.14
9	3-mercaptopropan-1-ol	1.15±0.07
10	pyridine-4-thiol	n.d.
11	2-mercaptosuccinic acid	2.52±0.48
12	N-acetyl-L-cysteine	2.41±0.08
13	N-(2-mercaptoethyl)acetamide	1.20±0.15
14	hydrogen sulfide	62.90±7.65
15	pantetheine	0.73±0.09

16	3-mercaptotetrahydrothiophene 1,1-dioxide	0.28±0.01
17	N-isobutyryl-D-cysteine	1.14±0.12
18	ethyl 2-mercaptoacetate	0.59±0.02
19	methyl 3-mercaptopropionate	0.25±0.01
20	ethyl 3-mercaptopropionate	0.52±0.05
21	ethyl 2-mercaptopropionate	0.28±0.02
22	ethane-1,2-dithiol	7.28±0.27
23	(*tert*-butoxycarbonyl)-L-cysteine	n.d.

Table S8. Summary of the recent methods for the determination of LWM thiols with chemical derivatization assisted LC-MS.

Sample	Sample amounts	**Analytes**	**Derivatization reagents**	**LODs**	**LODs for GSH**	**Internal standards utilized**	**References**
Plasma	40 μL	6 thiols, including Cys, Cys-Gly, HCys, GSH	NTAM	6-10 nM	6 nM for GSH	Ala-Ala	(1)
Water	10 mL	16 thiols, including Cys, HCys, Cys-Gly , GSH	PHMB	0.06-0.5 nM (with SPE pre-concentration)	0.2 nM for GSH (with SPE pre-concentration)	-	(2)
Tree human erythrocyte cells	three cells	One thiol (GSH)	mBrB	2.5fmol/0.5 fmol	2.5fmol/0.5 fmol for GSH	S-Hexylglutathione	(3)
Plasma/Liver	30 μL	5 thiols, including Cys, Cys-Gly, HCys, GSH	mBrB	0.31-4.98 fmol	0.91 fmol for GSH	S-Hexylglutathione	(4)
HepG2 Cells	~5×10^5 cells	4 thiols, including Cys, HCys, GSH	2-IAN	~0.3-3 nM	~3 nM for GSH	isotopic reagents	(5)
BAEC Cells/ Heart tissue	One plate for cells/ 2 mg for heart tissue	5 thiols, including Cys, HCys, Cys-Gly, GSH	Alkyl maleimides	Not available	Not available	penicillamine	(6)
Whole blood	225 μL	7 thiols, including Cys, Cys-Gly, HCys, GSH	NEM	0.5-20 nM	0.2 nM for GSH	Commercial isotopic standards	(7)
Serum	100 μL	7 thiols, including Cys, Cys-Gly, HCys, GSH	BQB	0.7-6.0 nM(with SPE pre-concentration)	1.9 nM for GSH (with SPE pre-concentration)	isotopic reagents	(8)
Plasma	100 μL	4 thiols, including Cys, Cys-Gly, HCys, GSH	NPM	0.18-2.84 μM	590 nM for GSH	Commercial isotopic standards	(9)
A single HeLa cell	one cell	6 thiols were detected in a single HeLa cell, including Cys, Cys-Gly, HCys, GSH	AYBA	2.4-192.3 pM	41.1 pM for GSH	isotopic reagents	This work

BAEC—Bovine aortic endothelial cells; NTAM—*N*-[2-(Trimethylammonium)-ethylmaleimide chloride; PHMB—4-(hydroxymercuri) benzoate; mBrB—monobromobimane; 2-IAN—2-Iodoacetanilide; NEM—N-ethylmaleimide; BQB—*ω*-bromoacetonylquinolinium bromide; NPM—N-phenylmaleiamide; AYBA—N-(acridin-9-yl)-2-bromoacetamide. "-" represents for "not detected or not utilized in this research ".

Table S9. Summary of the recent methods for determination of LWM thiols by mass spectrometry in single cells.

Analytical method	Cell type	Name of detected thiols in single cells	LODs	Internal standards utilized	Reference
CE-μESI-MS	*Xenopus laevis* embryonic cell	GSH	-	-	(10)
CE-ESI-HRMS	*Xenopus laevis* embryonic cell	GSH	-	-	(11)
Home-built single-probe SCMS	HeLa cell	GSH	-	-	(12)
Droplet-based microextraction ESI-MS	Human breast cancer (MCF-7) cell	GSH	-	-	(13)
FluidFM-MALDI-MS	HeLa cell	GSH	-	-	(14)
Microprobe single-cell CE-ESI-MS	*Xenopus laevis* embryonic cell	Cys, GSH	-	-	(15)
Microprobe single-cell CE-ESI-MS	*Xenopus laevis* embryonic cell	GSH	-	-	(16)
Pico-ESI-MS	Human glioblastoma (A172) cell / Human astrocyte (HA) cell	GSH	-	-	(17)
Microprobe single-cell CE-ESI-MS	*Xenopus laevis* embryonic cell	GSH	-	-	(18)
Chemical derivatization assisted LC-MS	HeLa cell	CA, Cys, HCys, GSH, H_2S, PAN	2.4-192.3 pM	isotopic reagents	This work

CE-μESI-MS—*capillary electrophoresis-microflow electrospray ionization mass spectrometry*; CE-ESI-HRMS—*capillary electrophoresis-electrospray ionization-high resolution mass spectrometry*; SCMS—*single cell mass spectrometry*; FluidFM—*fluidic force microscopy*; FluidFM—*pulsed direct current electrospray ionization mass spectrometry*.

Figure S1. Chemical structures of the investigated 23 LWM thiols.

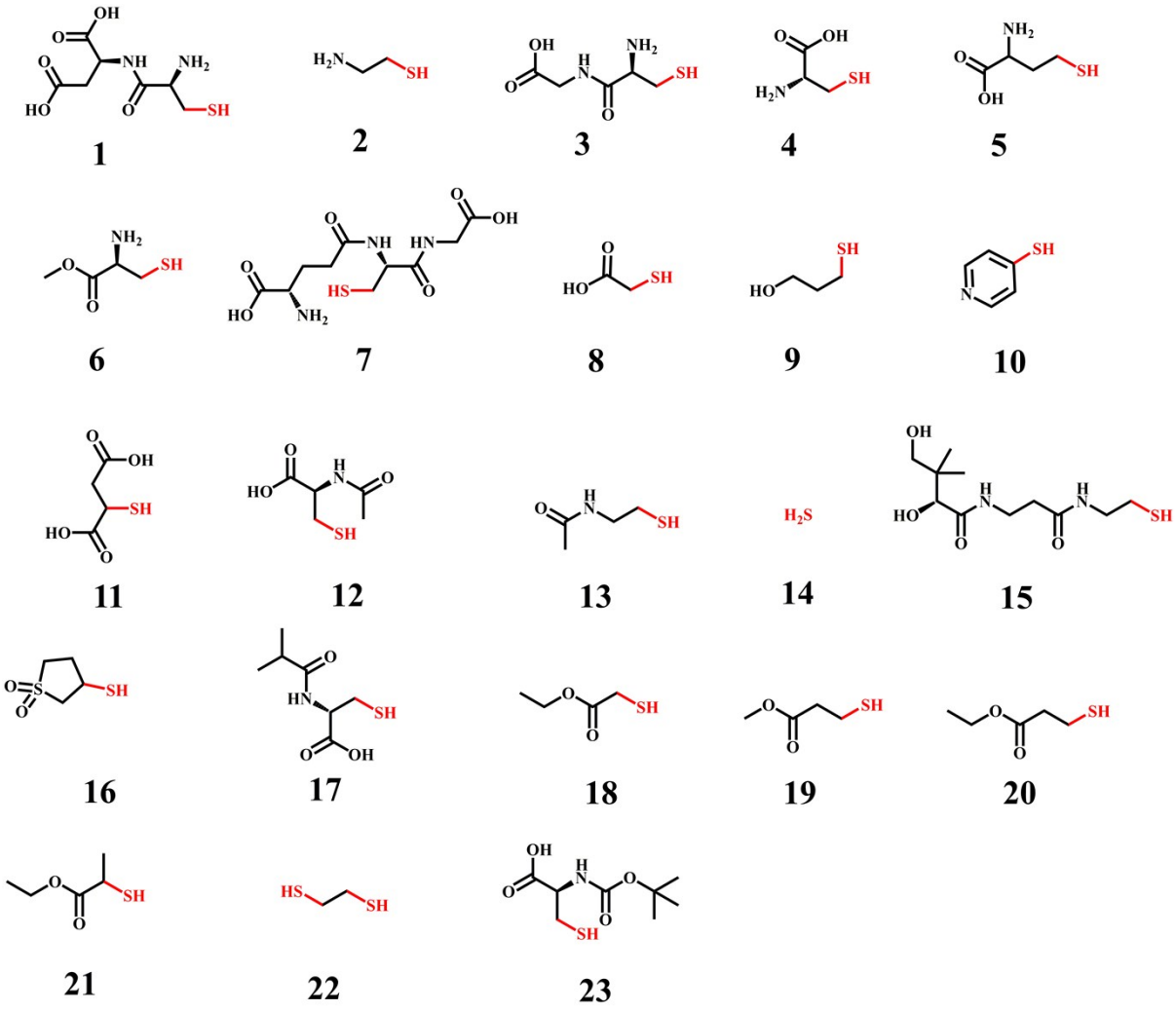


Figure S2. Sampling of a single Hela cell by means of a capillary microsampling method.

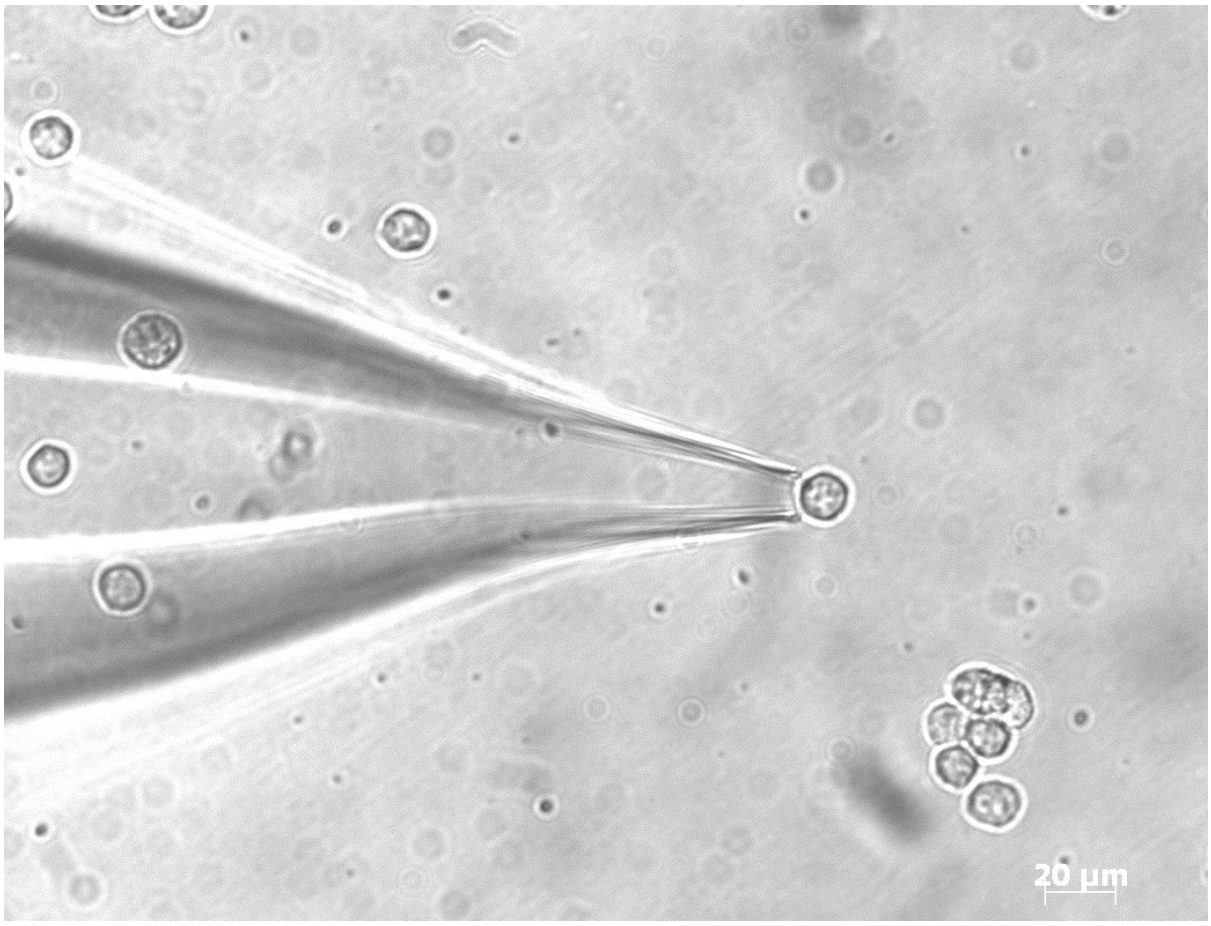


Figure S3. Heat map for derivatization reactions between AYBA and Cys, Cys-Gly, and HCys (10.0 ng mL^{-1} for each thiols) in different reaction media. Each lane represents for replicates of each conditions.

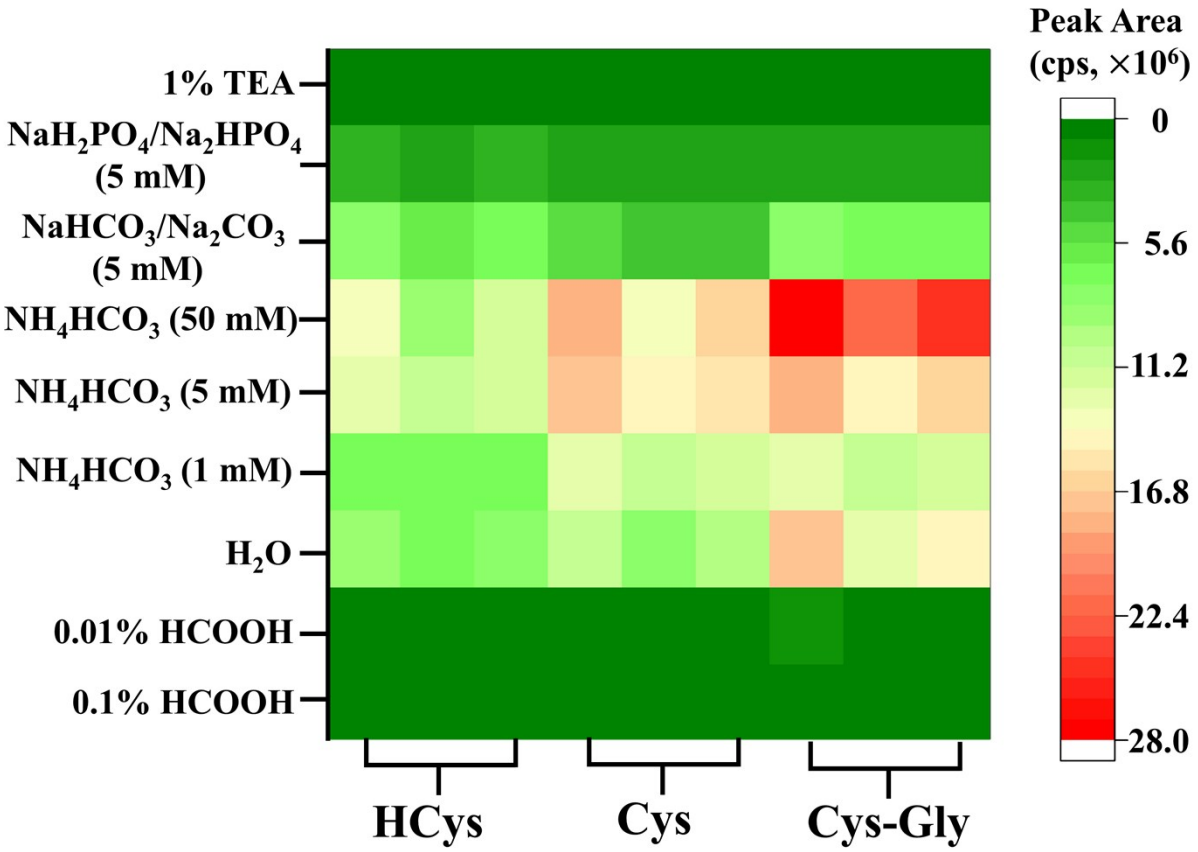


Figure S4. MRM chromatograms of thiol and carboxylic acid (1.0 ng mL^{-1}) derivatives that derivatized by AYBA under (a) TEA (1% (v/v) in acetonitrile) and (b) 50 mM NH_4HCO_3 buffer. (mobile phase: A, 0.1% (v/v 1:999) HCOOH (A) and MeOH (B); flow rate at 0.4 mL min^{-1}; the percentages (v/v) of solvent B was as following: 0−25 min at 20%).

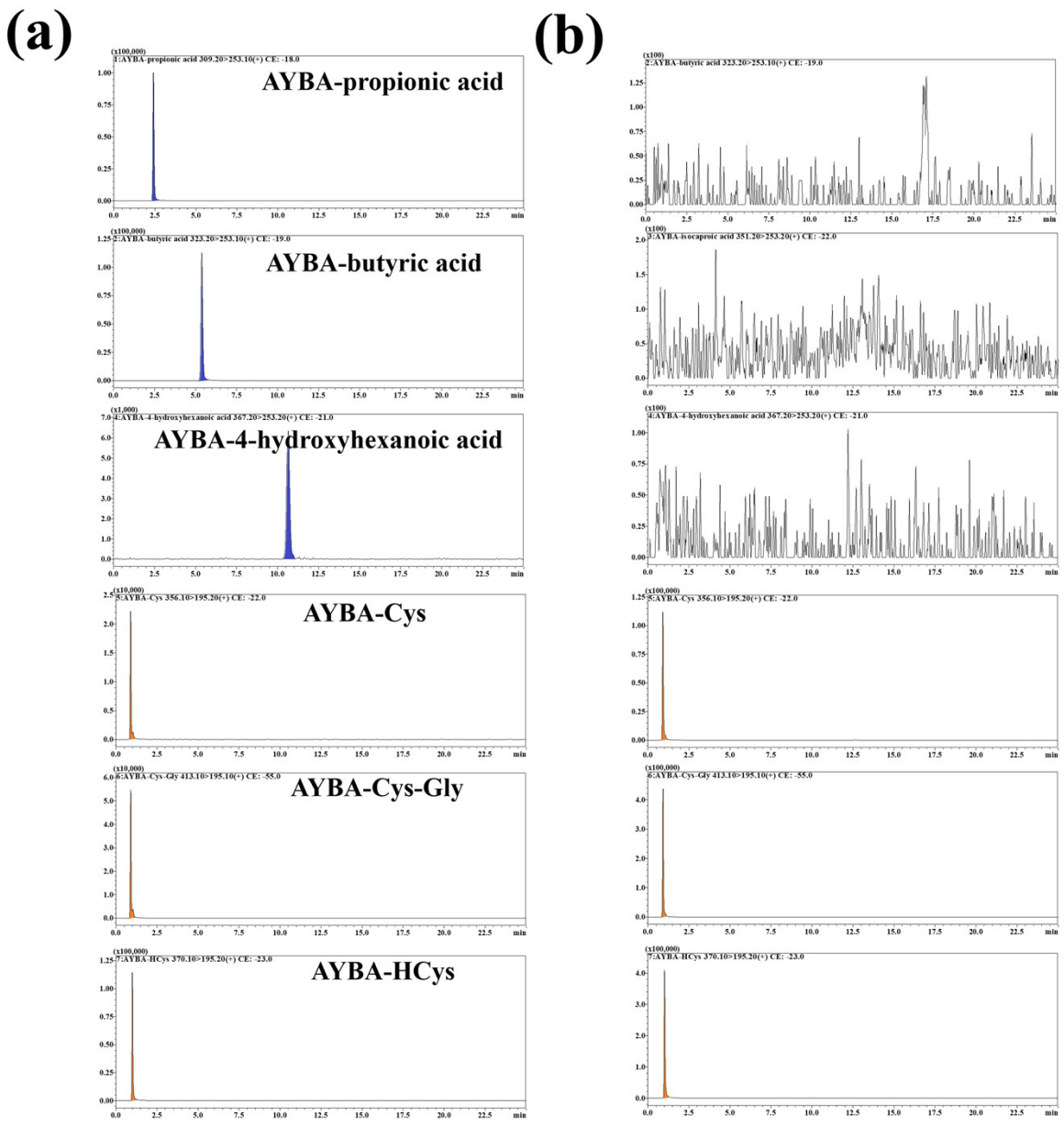


Figure S5. MRM chromatograms of thiol (1.0 ng mL^{-1}) and carboxylic acid (0.2 μg mL^{-1}) derivatives that derivatized by AYBA under (a) TEA (1% (v/v) in acetonitrile) and (b) 50 mM NH_4HCO_3 buffer. (mobile phase: A, 0.1% (v/v 1:999) HCOOH (A) and MeOH (B); flow rate at 0.4 mL min^{-1}; the percentages (v/v) of solvent B was as following: 0−25 min at 20%).

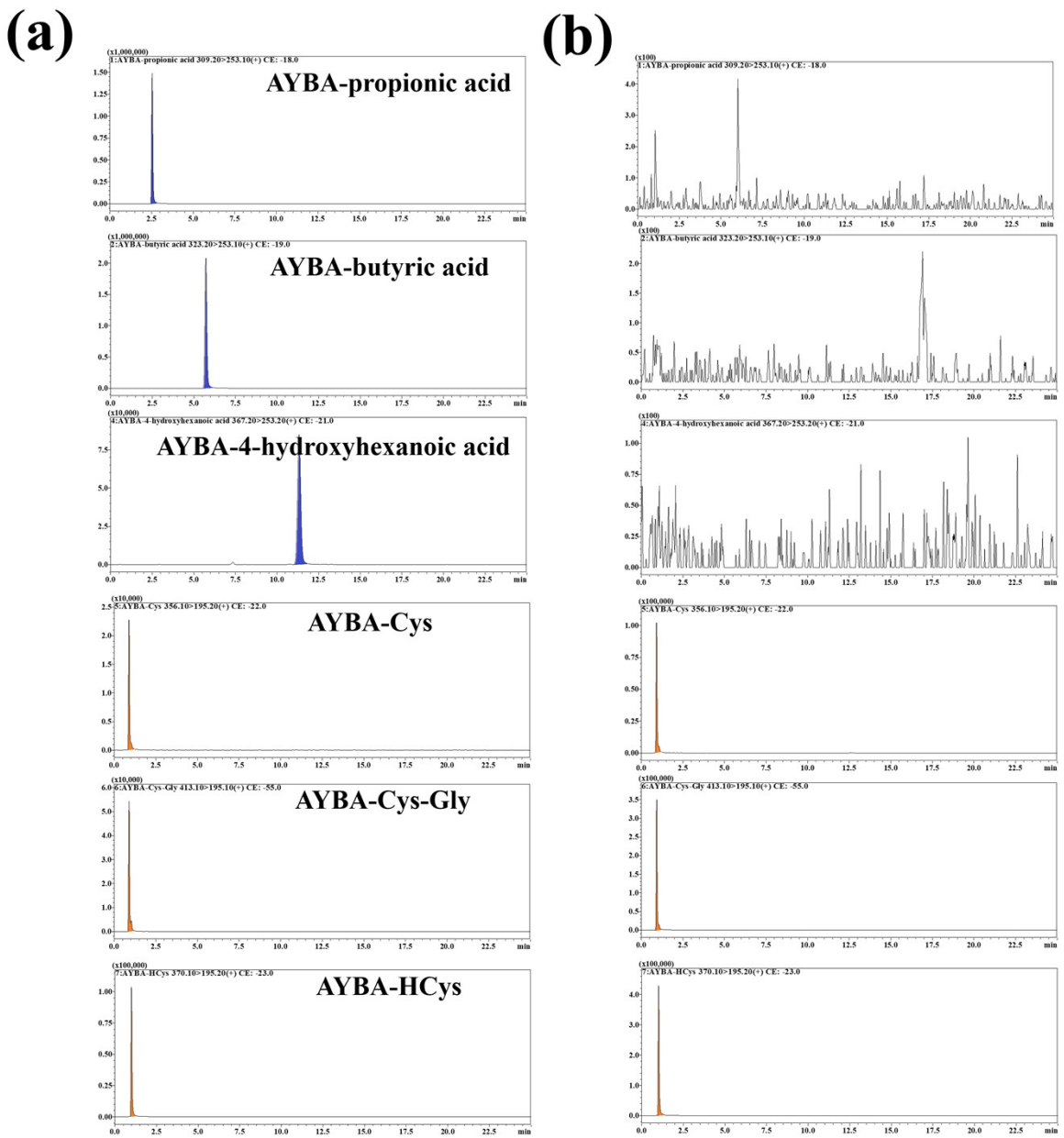


Figure S6. Effect of the concentration of (a) TCEP and (b) AYBA on peak areas in ~1000 HeLa cells, respectively.

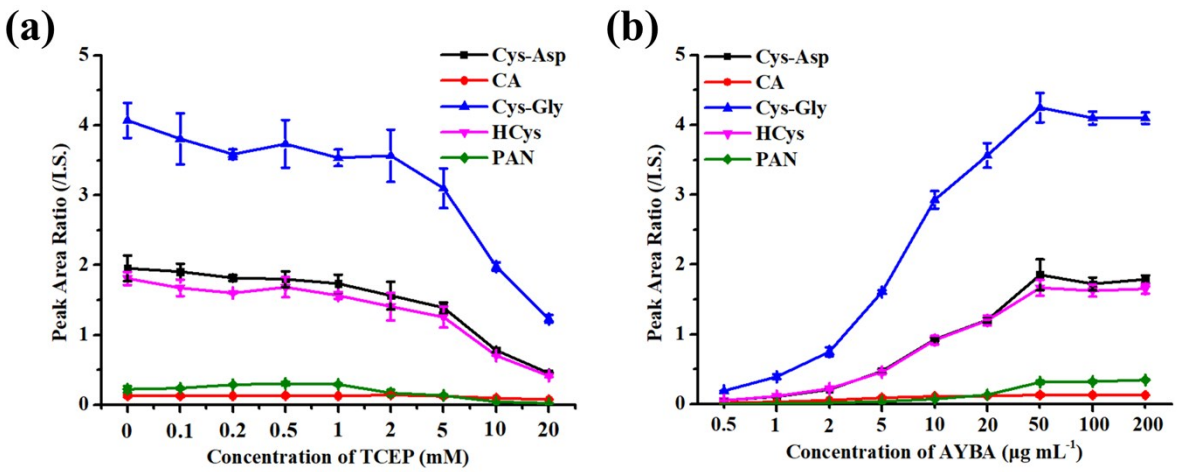


Figure S7. Short term stability of AYBA derivatives of Cys-Gly, Cys, and HCys (RSD<7.9%).

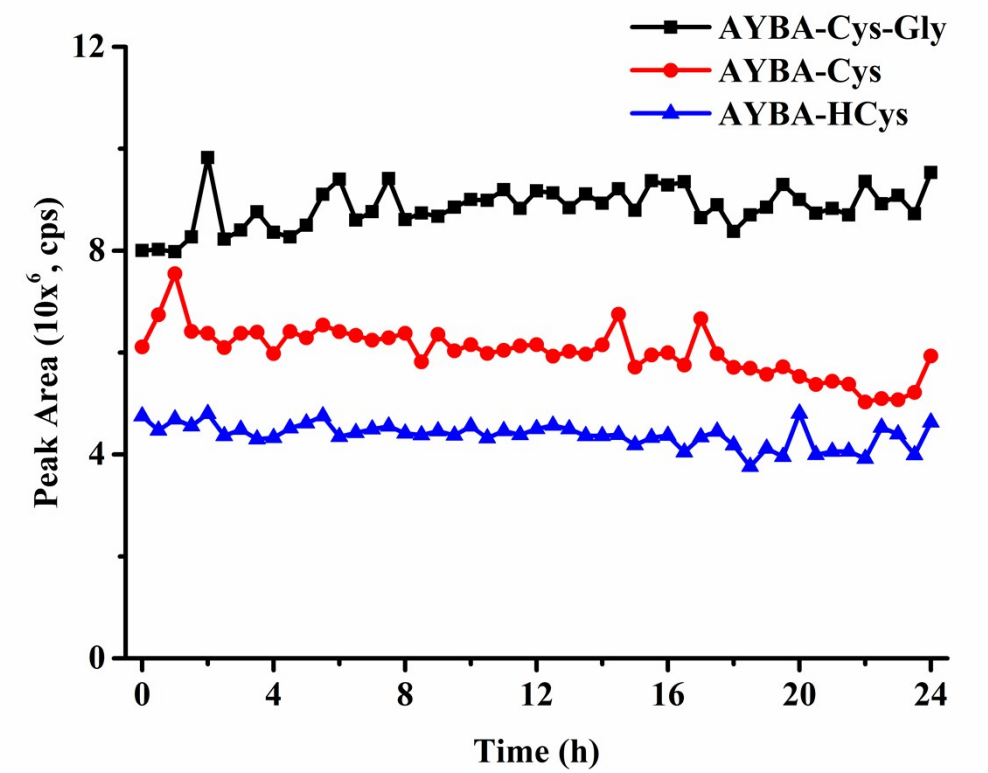


Figure S8. Typical MRM chromatograms of Cys, HCys, and Cys-Gly (10 ng mL^{-1} for each thiols) (a) before derivatization and (b) after derivatization.

The residual thiols after derivatization were detected and their concentrations were calculated by the calibration curves of the free carboxylic acids. Then, derivatization efficiency could be expressed by the following:

$$Derivatization\ efficiency\ (\%) = 100 - \frac{C_{after\ derivatization}}{C_{before\ derivatization}} \times 100\%$$

where $C_{before\ derivatization}$ denotes the concentration of thiols before derivatization, while $C_{after\ derivatization}$ denotes the concentration of thiols after derivatization.

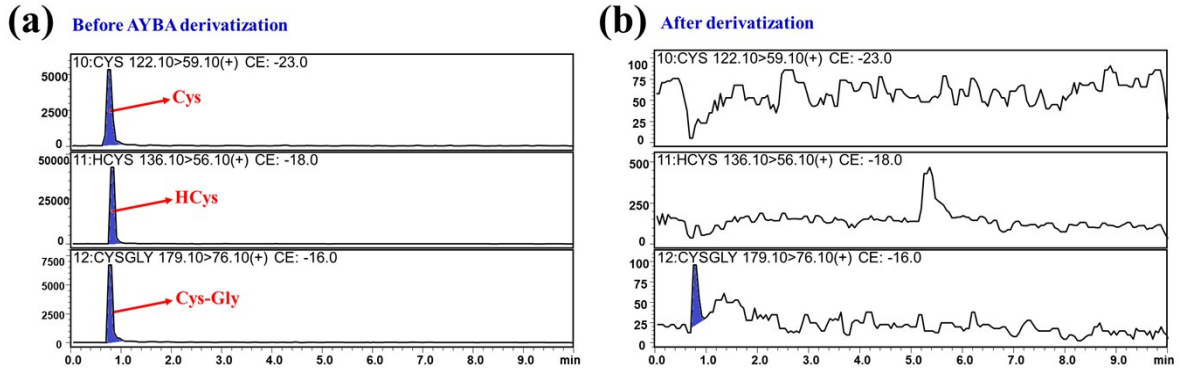


Figure S9. Lowest derivatization concentration of AYBA for Cys at 0.1 pM; (a) 1 pM Cys derivatized by AYBA (100 µL); (b) 0.1 pM Cys derivatized by AYBA (1 mL condensed to 100 µL prior to LC-MS analysis).

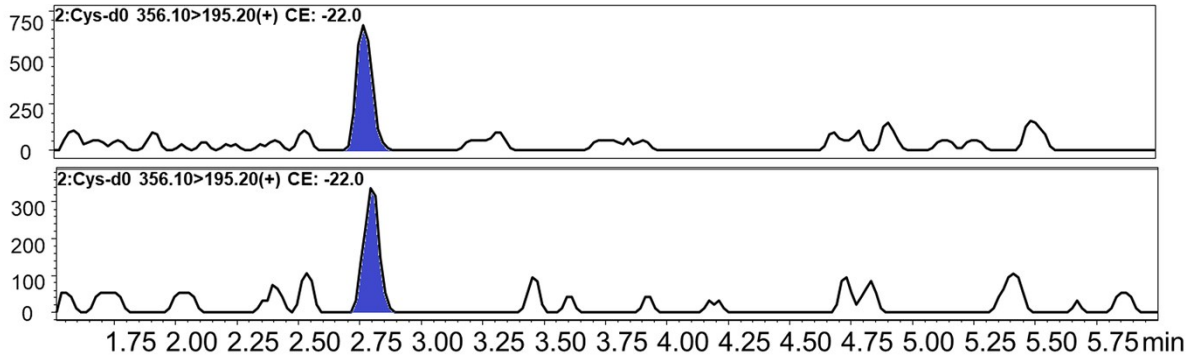


Figure S10. Typical MRM chromatograms of non-derivatized thiols on C18 column, including (a) N-isobutyryl-D-cysteine, (b) 2-mercaptosuccinic acid, (c) ethyl 3-mercaptopropanoate, (d) N-(2-mercaptoethyl)acetamide, (e) N-acetyl-L-cysteine, (f) pantetheine, (g) glutathione. (1 μM for N-isobutyryl-D-Cysteine, ethyl 3-mercaptopropanoate, and pantetheine, 200 nM for the other thiols) (mobile phase: A, 0.1% (v/v 1:999) HCOOH (A) and MeOH (B); flow rate at 0.4 mL min^{-1}; the percentages (v/v) of solvent B was as following: 0−5 min at 5%, 5−10 min from 5% to 50%, 10−12 min from 50% to 90%, 12−15 min at 90%, 15−16 min from 90% to 5%).

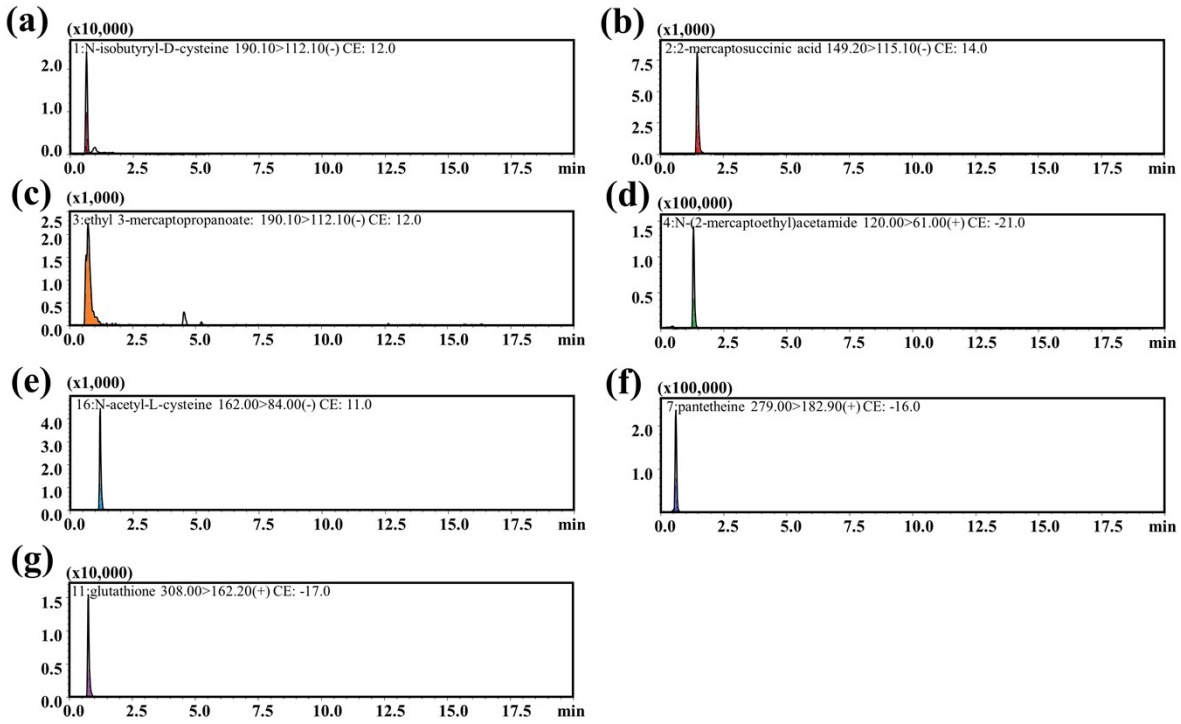


Figure S11. Isotopic effects of AYBA-RSH with [$^2H_4$]AYBA-RSH under the same elution condition were revealed from typical MRM chromatograms of them in ~1000 Hela cells.

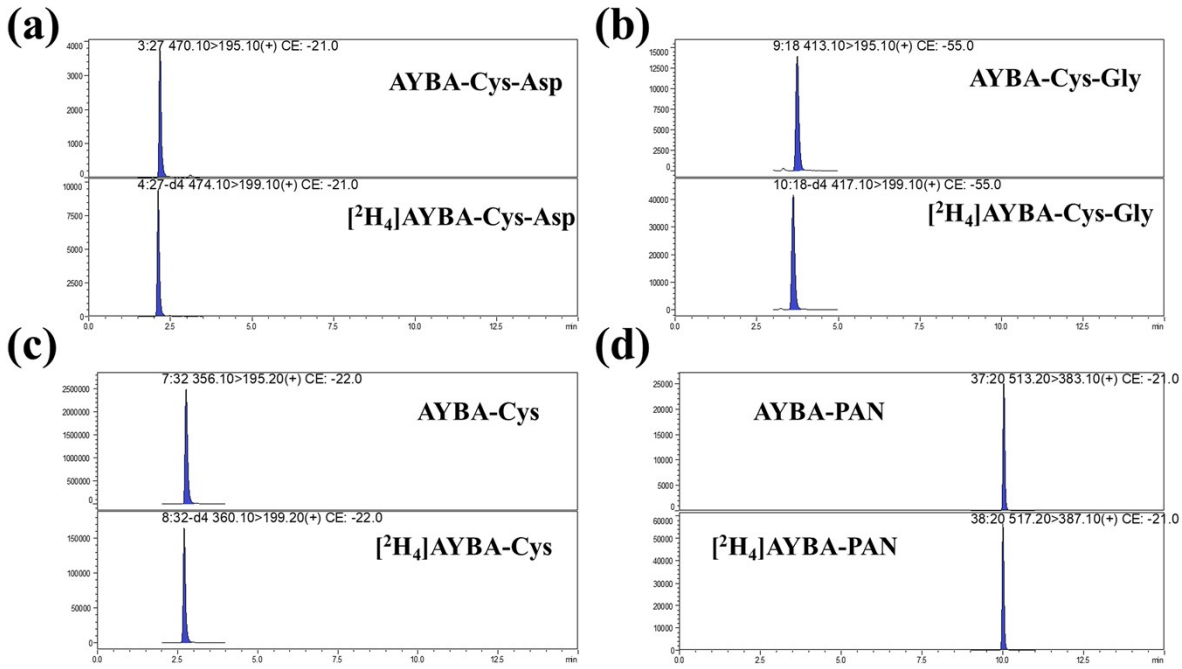


Figure S12. CID-MS spectra of (a) Cys, (b) HCys, (c) Cys-Gly and (d) AYBA-Cys, (e) AYBA-HCys, (f) AYBA-Cys-Gly at collision energies at 15 V by LC-MS.

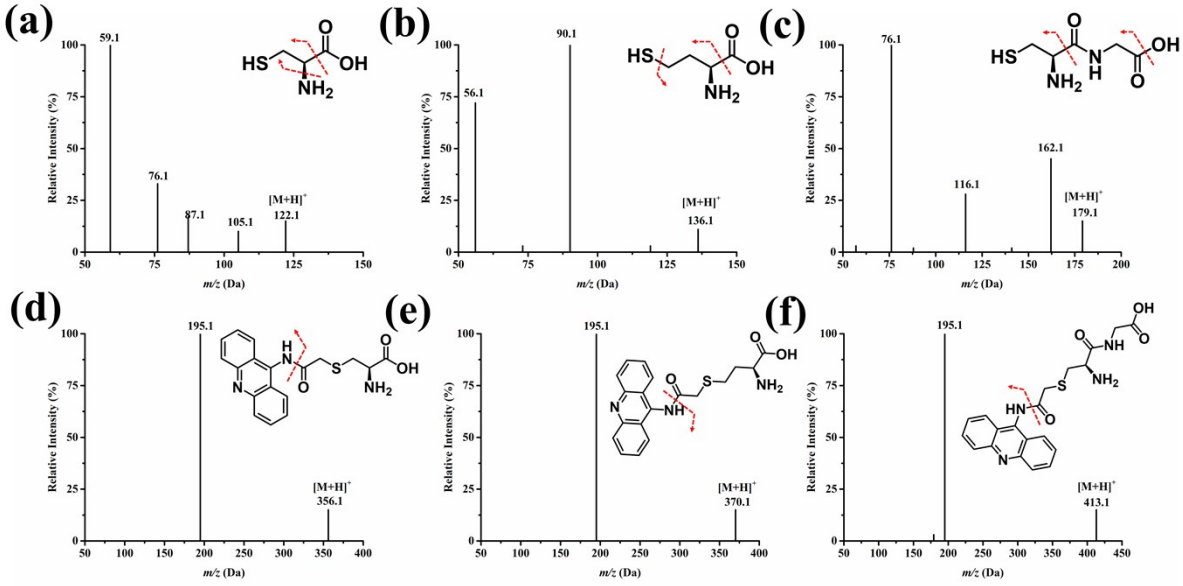


Figure S13. MRM chromatograms of AYBA derivatives of 21 detected LWM thiols from extracts of ~1000 Hela cells.

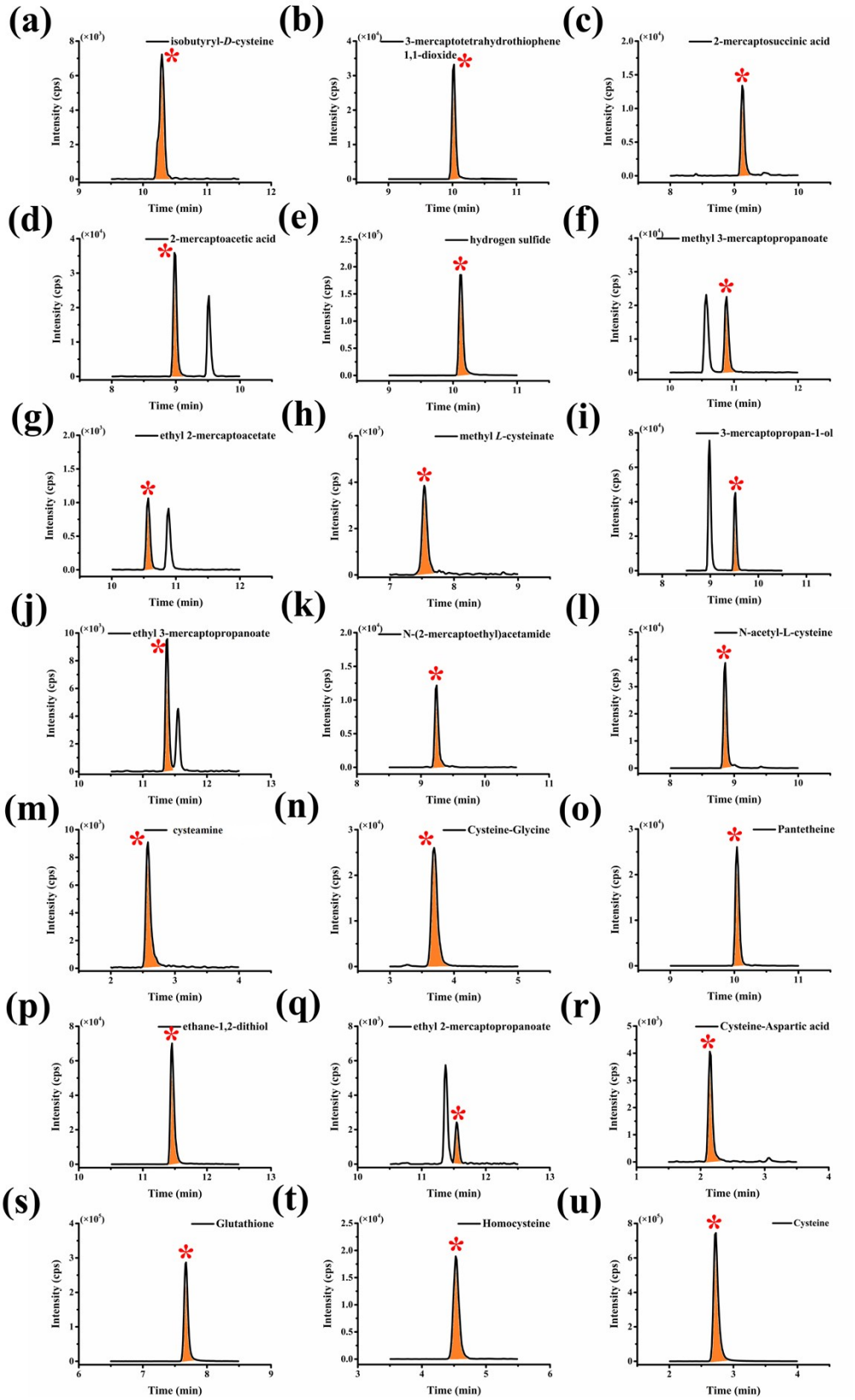


Figure S14. Possible metabolic pathway of the investigated LWM thiols involved in this study from KEGG pathway database (https://www.genome.jp/kegg/pathway.html) and Metacyc pahway database (https://metacyc.org/). The dotted lines represent for the indirect metabolic pathway; CA represents for cysteamine; PAN represents for pantetheine; PPAN represents for phosphate-pantetheine; MC represents for methyl L-cysteinate; TA represents for thiolmalic acid (2-mercaptosuccinic acid); Cys represents for cysteine; HCys represents for homocysteine; Cys-Gly represents for cysteine-glycine; GSH represents for glutathione.

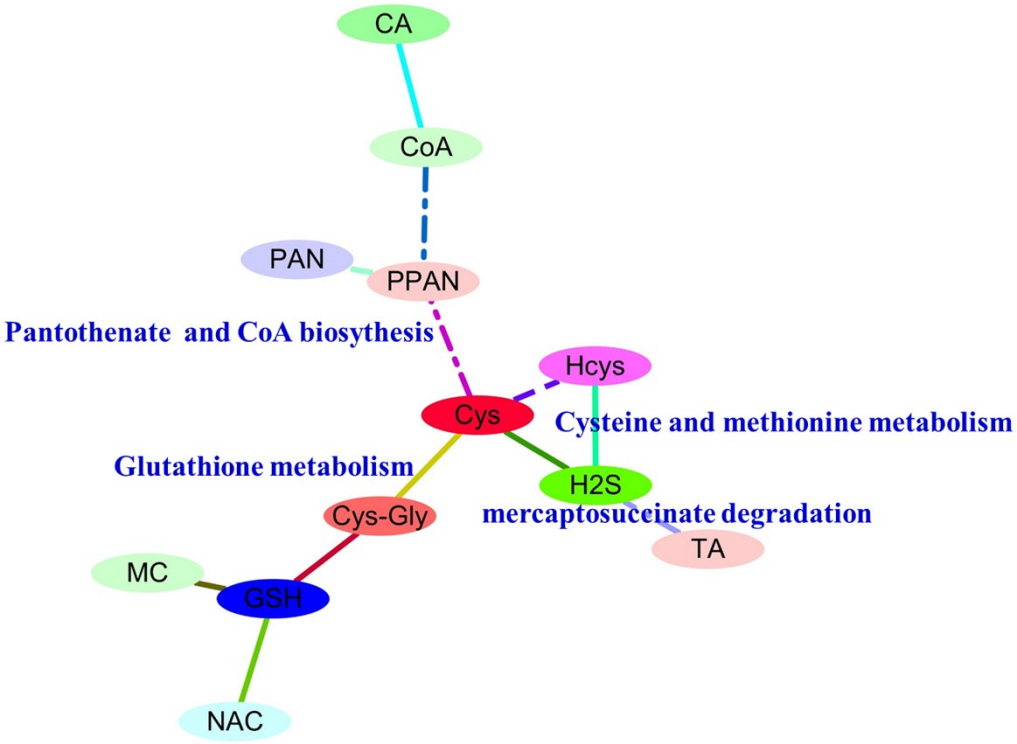
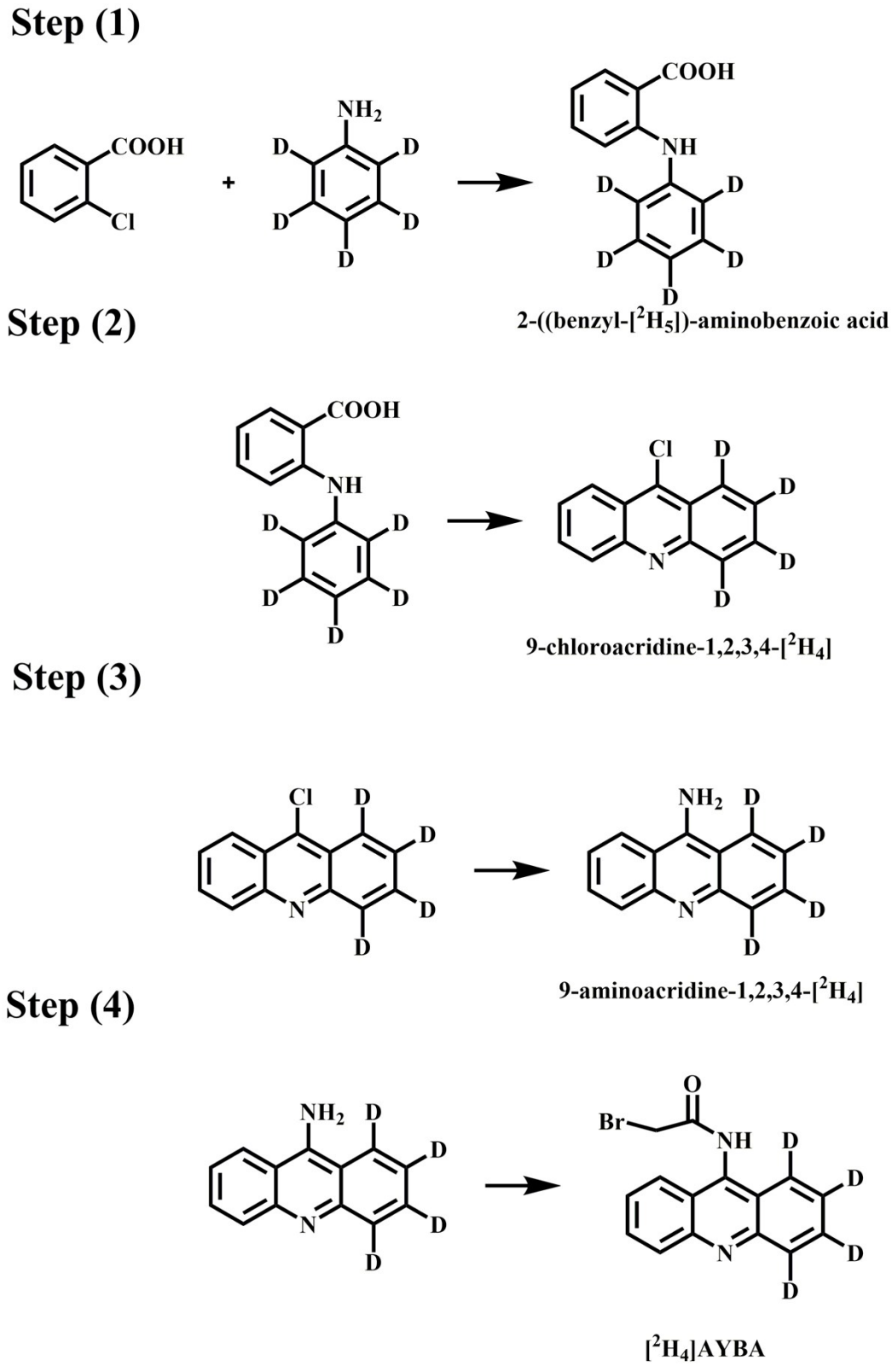
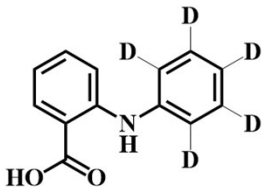
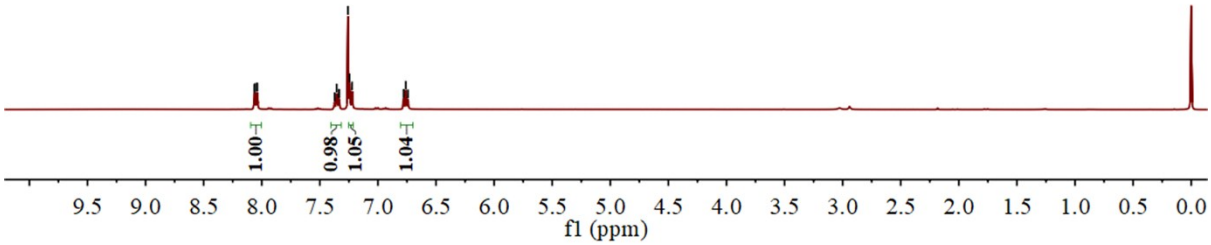


Figure S15. Synthesis route for [$^2H_4$]AYBA and ^{1}H NMR spectra data of compounds involved in synthesis of [$^2H_4$]AYBA.

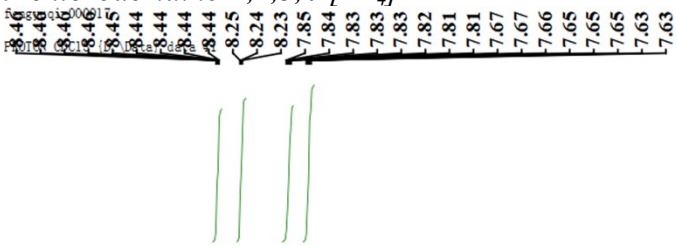


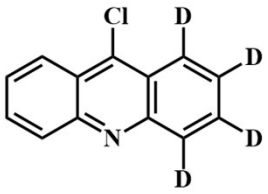
2-((benzyl-[$^2H_5$])-aminobenzoic acid

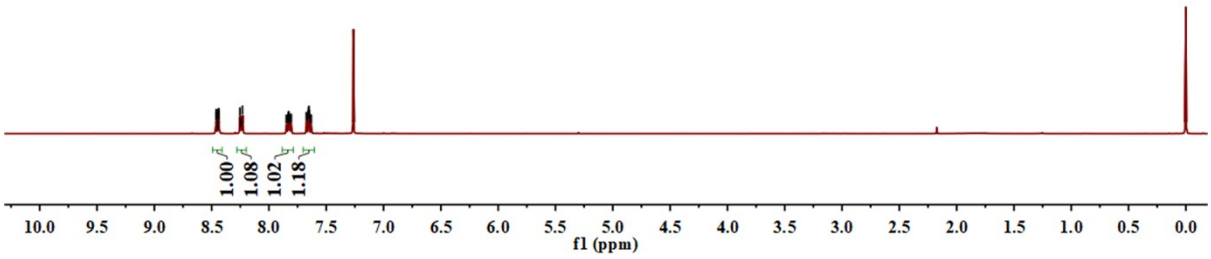




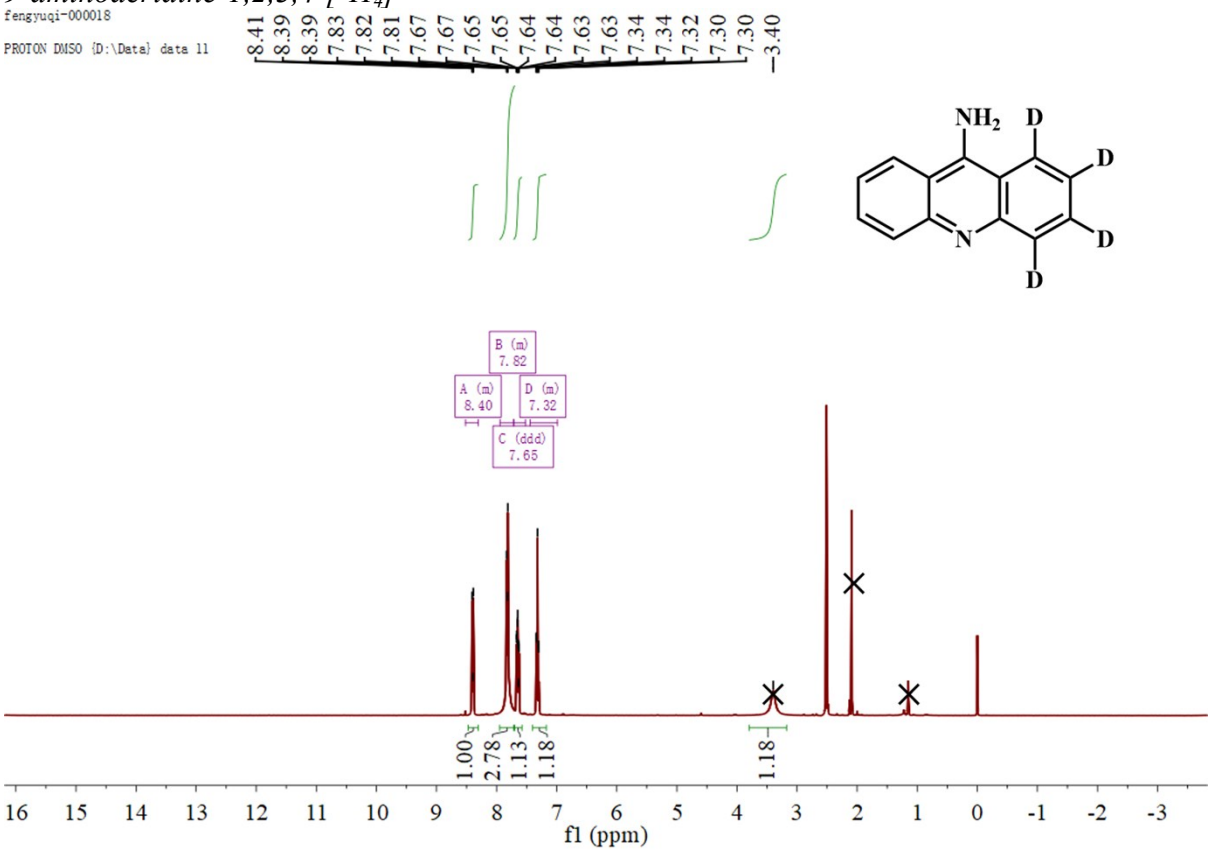
9-chloroacridine-1,2,3,4-[$^2H_4$]



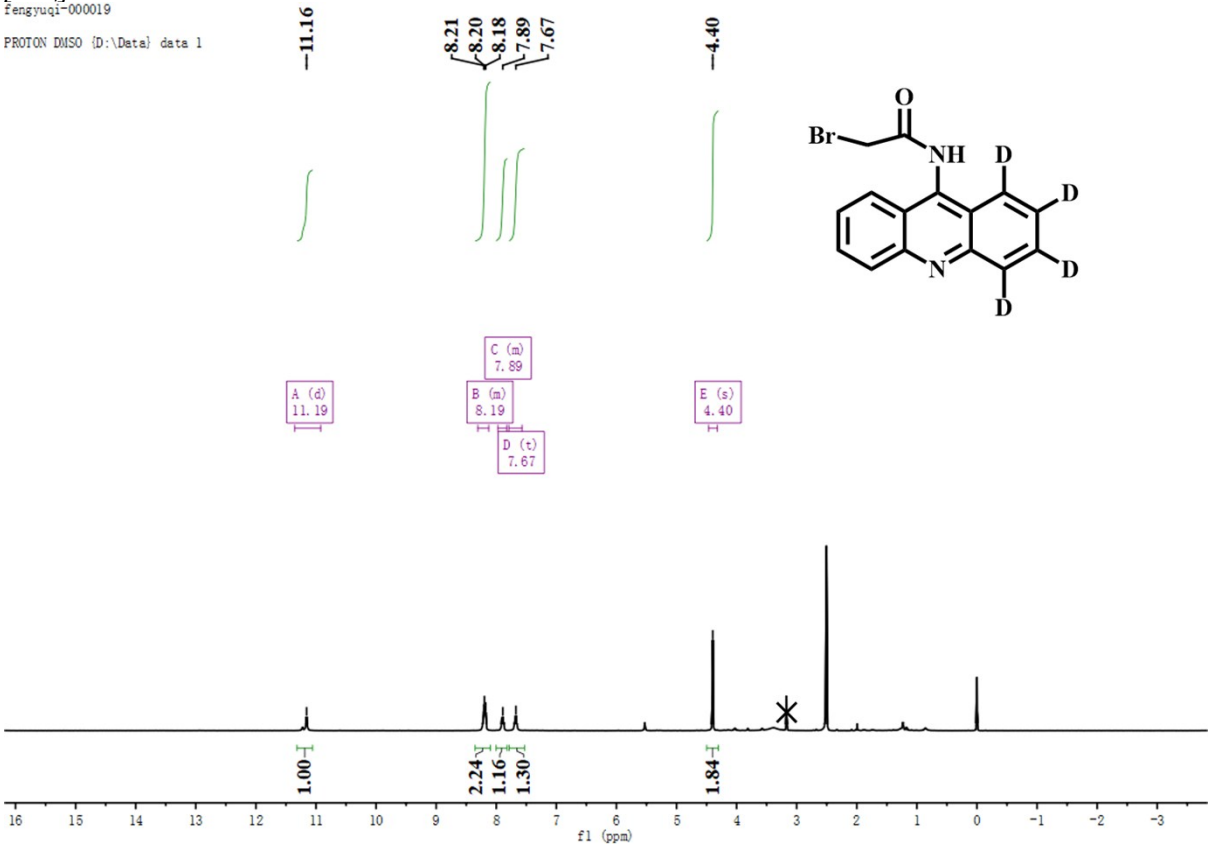




9-aminoacridine-1,2,3,4-[$^{2}H_4$]



[$^2H_4$]-AYBA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B (m)
8.19
C (m)
7.89
D (t)
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E (s)
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1.30
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16
15
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-1
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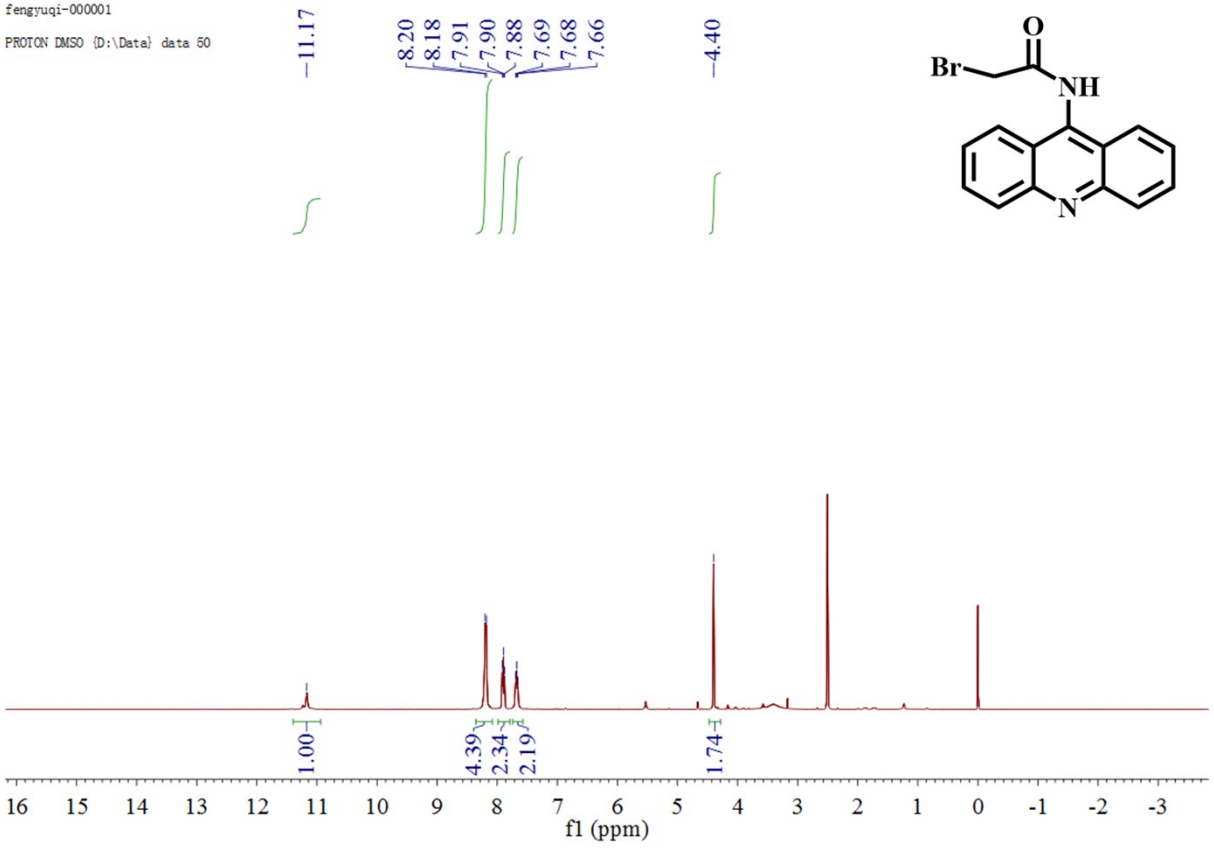
f1 (ppm)

AYBA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2.34
2.19
1.74
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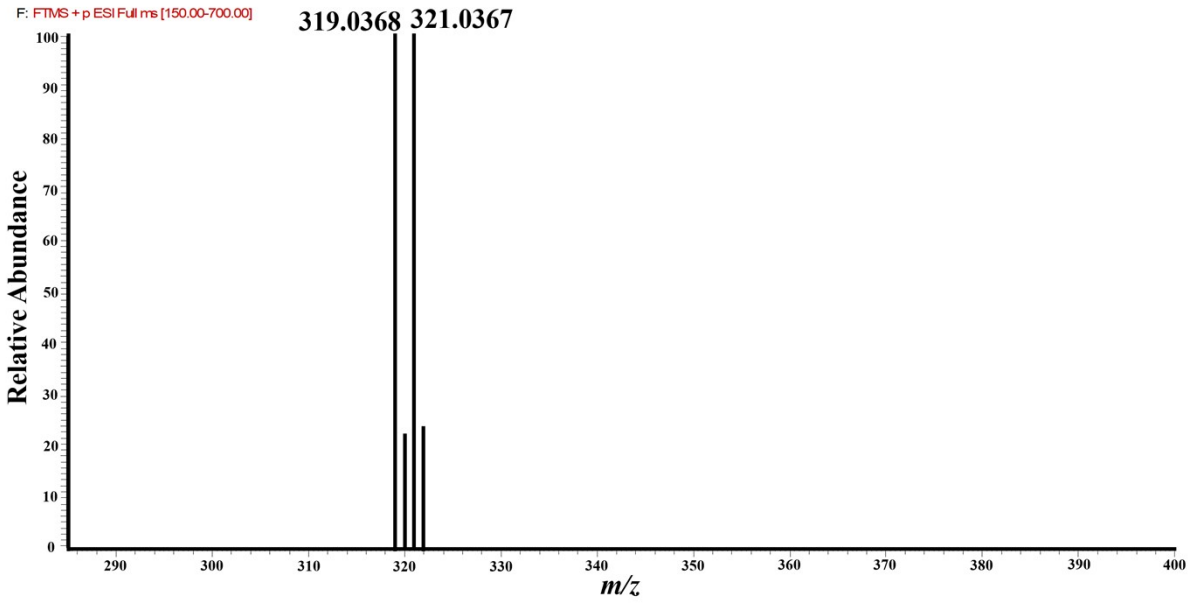
f1 (ppm)

Figure S16. Full scan MS spectra of [$^2H_4$]AYBA in the positive ion mode with the LTQ-Orbitrap Elite mass spectrometer (ThermoFisher Scientific Inc., USA).



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