

Supporting information

1,8-naphthalimide appended propiolate-based fluorescent sensor for selective detection of cysteine over glutathione and homocysteine in living cells

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Experimental Section

Synthesis of 4-Bromo-N-butyl-1,8-naphthalimide (2a). Compound **2a** (0.5 g, 1.80 mmol) and n-butylamine (0.69 g, 9.55 mmol) was refluxed for 6h in 20 mL glacial acetic acid. After cooling, the suspension was put into ice-water (250 mL), and then filtered to obtain a yellow solid. The precipitate was washed with excess H₂O and then re-crystallized from acetic acid gave a pale yellow crystal (yield: 79.3%).

¹H-NMR (600 MHz, CDCl₃): δ 8.61 (d, J = 7.2 Hz, 1H), 8.50(d, J = 8.4 Hz, 1H), 8.36 (d, J = 7.8 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H), 7.80 (t, J = 7.6 Hz, 1H), 4.17 (t, J = 7.6 Hz, 2H), 1.76–1.68 (m, 2H), 1.44 (dt, J = 7.4 Hz, 2H), 0.97 (t, J = 7.4 Hz, 3H). ¹³C-NMR (150 MHz, CDCl₃): δ 163.66, 133.23, 132.02, 131.22, 131.09, 130.64, 130.20, 129.02, 128.08, 123.16, 122.30, 40.38, 30.16, 20.37, 13.84.

Synthesis of 4-hydrazine hydrate N-butyl-1,8-naphthalic anhydride (3a). A mixture of compound **2a** (8.0 g, 24.08 mmol) and hydrazine hydrate (7.62 g, 85%, v/v) in ethylene glycol mono methyl ether (93 mL) was refluxed for 24 h. After cooling, the mixture was put into 180 mL of ice-H₂O, the precipitate was filtered and dried in vacuum to obtain a red precipitate (yield: 80.3%).

¹H-NMR (600 MHz, CDCl₃) δ 9.08 (s, 1H), 8.57 (d, J = 8.3 Hz, 1H), 8.37 (d, J = 7.2 Hz, 1H), 8.25 (d, J = 8.5 Hz, 1H), 7.59 (t, J = 7.8 Hz, 1H), 7.21 (d, J = 8.6 Hz, 1H), 4.64 (s, 2H), 3.98 (t, J = 7.3 Hz, 2H), 1.58–1.52 (m, 2H), 1.34–1.27 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H). ¹³C-NMR (150 MHz, CDCl₃) δ 164.17, 163.32, 153.60, 134.61, 130.97, 129.70, 128.64, 124.52, 122.15, 118.84, 107.78, 104.41, 39.46, 30.28, 20.30, 14.20.

Synthesis of 2-formylphenyl propiolate (4a). The propiolate derivative **4a** was prepared by the DCC/DMAP esterification reaction of salicyl aldehyde with propiolic acid. Briefly, to an

ice cooled and stirred solution of propiolic acid (315.4 mg, 4.50 mmol) and salicyl aldehyde (500 mg, 4.09 mmol) in DCM (dry) was added dropwise to the solution of dimethylaminopyridine (DMAP, 3.75 mg, 0.03 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC, 929 mg, 4.05 mmol) in DCM (dry, 12 mL) during 1 h under N_2 atmosphere. Afterward, the mixture was stirred and kept at rt for 20 h, and then filtered to get rid of the insoluble byproduct *N,N'*-dicyclohexylurea. The combined filtrate was washed with 1.0 N HCl followed by washing with brine and dried utilizing anhydrous Na_2SO_4 . The solvent was get rid of to give the crude product that was then purified by CC utilizing EtOAc: hexane (1:9, v/v) to give **4a** (90 %).

1H NMR (600 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.58–7.51 (m, 2H), 7.05–6.97 (m, 2H), 3.03 (s, 1H). ^{13}C -NMR (150 MHz, $CDCl_3$) δ 195.80, 160.56, 155.38, 136.15, 132.81, 119.56, 118.91, 116.60, 76.06, 72.96.

Preparation of 2-formylphenyl propiolate appended-N-butyl-1,8-naphthalimide (NASP).

An ethanolic solution of **4a** (0.23 g, 1.32 mmol) was carefully transferred to **3a** solution (0.37 g, 1.32 mmol) in EtOH (anhydrous, 3 mL). Acetic acid (2–3 drops) was transferred to the mixture, which was then refluxed at 80 °C for 24h. The orange solid formed was filtered and washed several times with cold EtOH. The product was dried under vacuum to give the probe **NASP** (71 %). Found (m/z): 437.810 and calculated (m/z): 439.15 for [**NASP**].

1H NMR (600 MHz, $DMSO-d_6$) δ 11.45 (s, 1H), 8.79 (s, 1H), 8.78 (s, 1H), 8.45 (d, $J = 7.2$ Hz, 1H), 8.35 (d, $J = 8.4$ Hz, 1H), 7.83 (d, $J = 7.7$ Hz, 1H), 7.75 (t, $J = 7.8$ Hz, 1H), 7.61 (d, $J = 8.4$ Hz, 1H), 7.24 (t, $J = 7.6$ Hz, 1H), 6.90 (dd, $J = 16.6, 8.1$ Hz, 2H), 4.35 (s, 1H), 4.00 (t, $J = 7.4$ Hz, 2H), 1.61–1.54 (m, 2H), 1.36–1.29 (m, 2H), 0.90 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (150 MHz, $DMSO-d_6$) δ 164.08, 163.37, 156.68, 154.02, 146.76, 142.31, 134.03, 131.30, 129.59,

128.72, 126.77, 125.40, 122.40, 120.98, 119.98, 119.01, 116.58, 111.16, 106.87, 77.76, 76.54,
30.23, 20.29, 14.20. Found (m/z): 437.810 and calculated (m/z): 439.15 for [NASP].

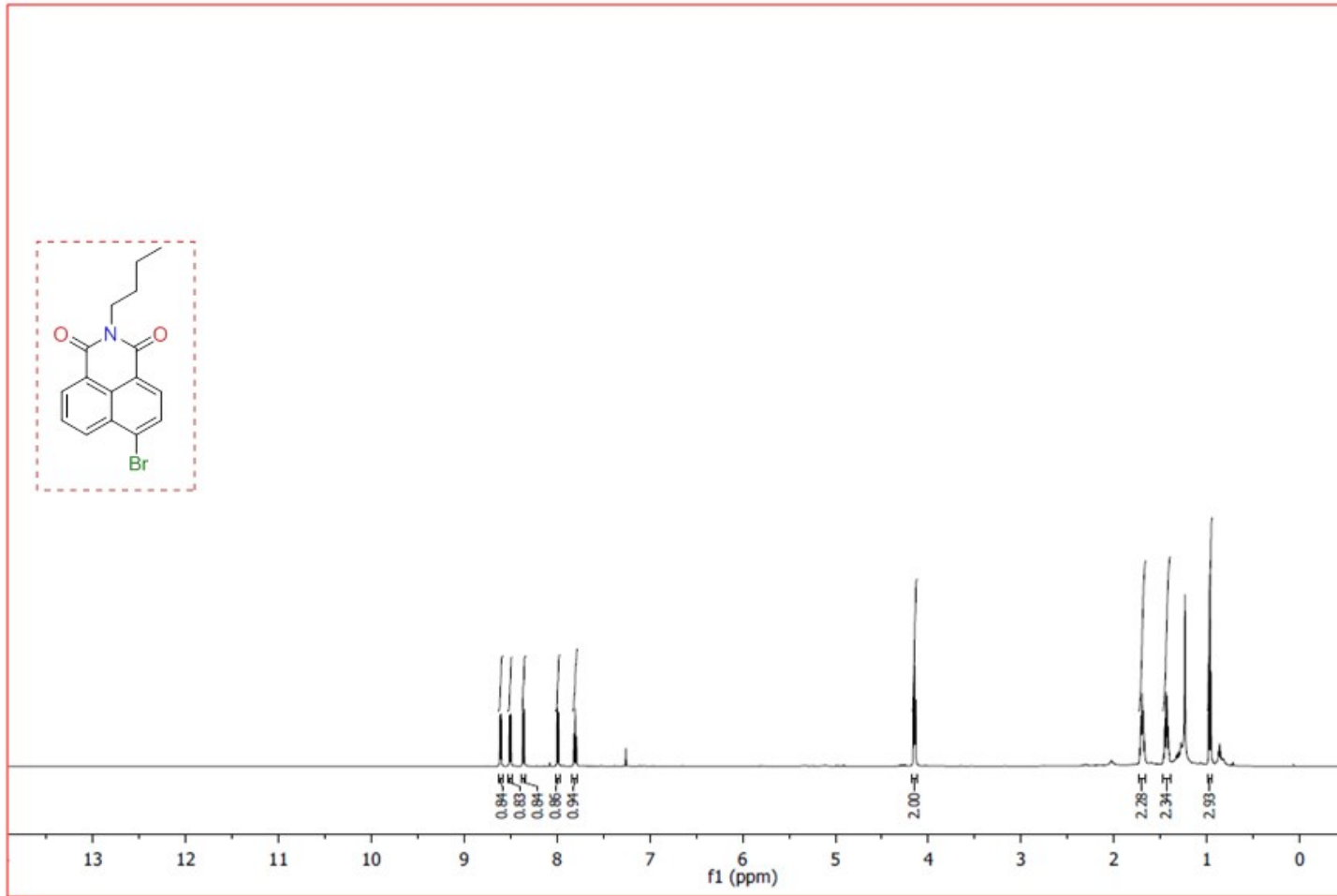


Fig S1. $^1\text{H-NMR}$ spectrum of the compound **2a** in CDCl_3-d_6

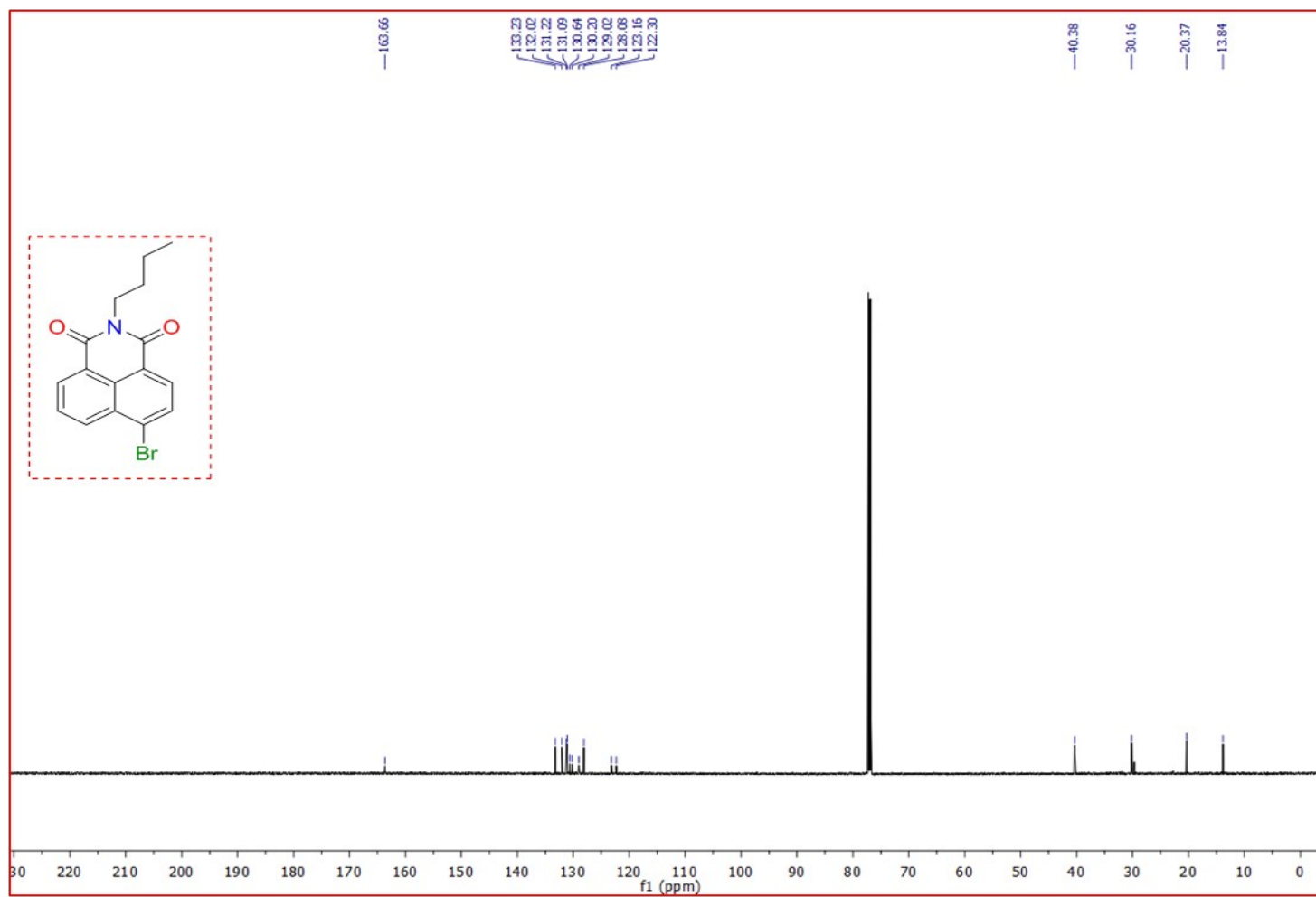


Fig S2. ^{13}C -NMR spectrum of the compound **2a** in CDCl_3-d_6

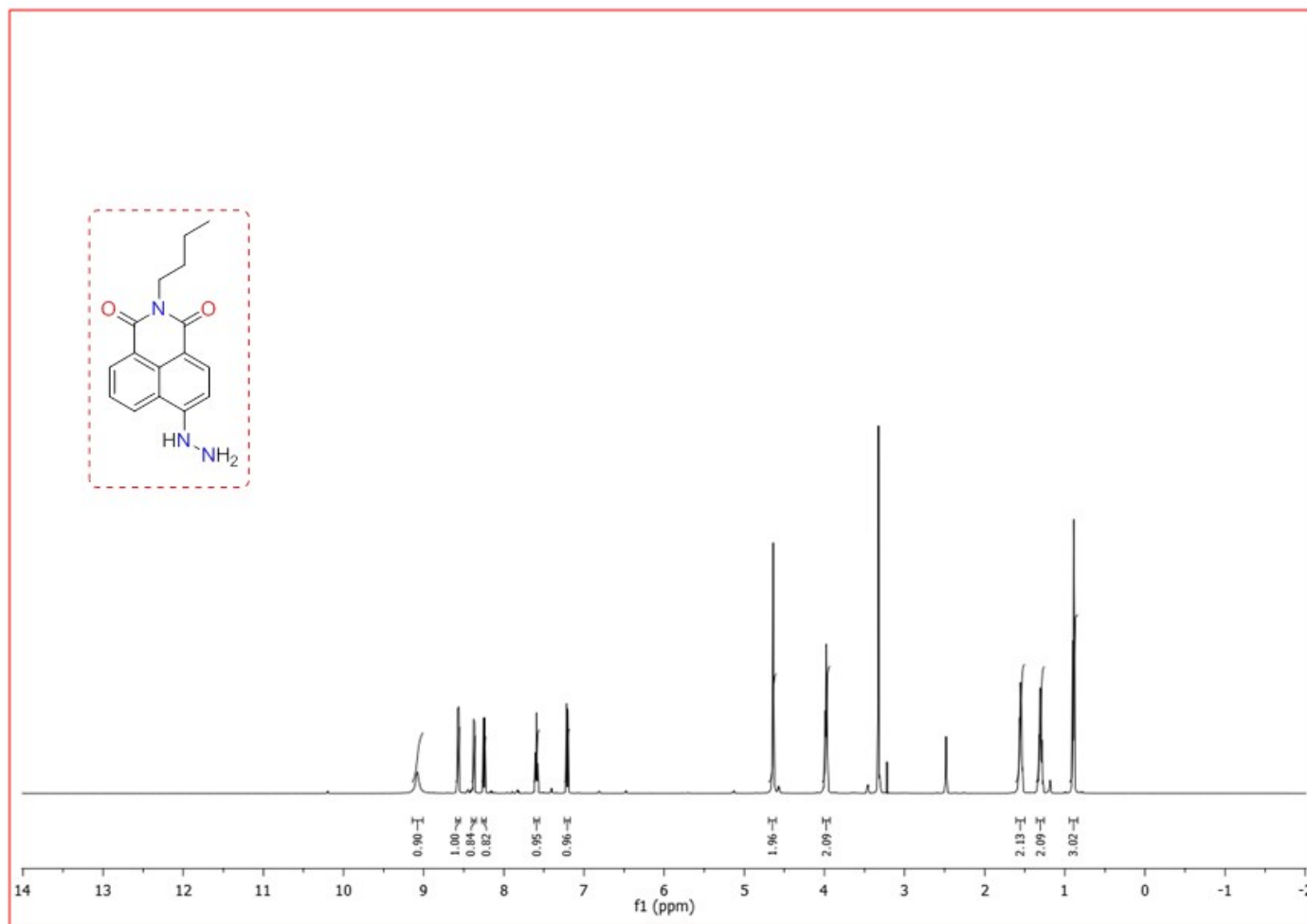


Fig S3. $^1\text{H-NMR}$ spectrum of the compound **3a** in CDCl_3-d_6

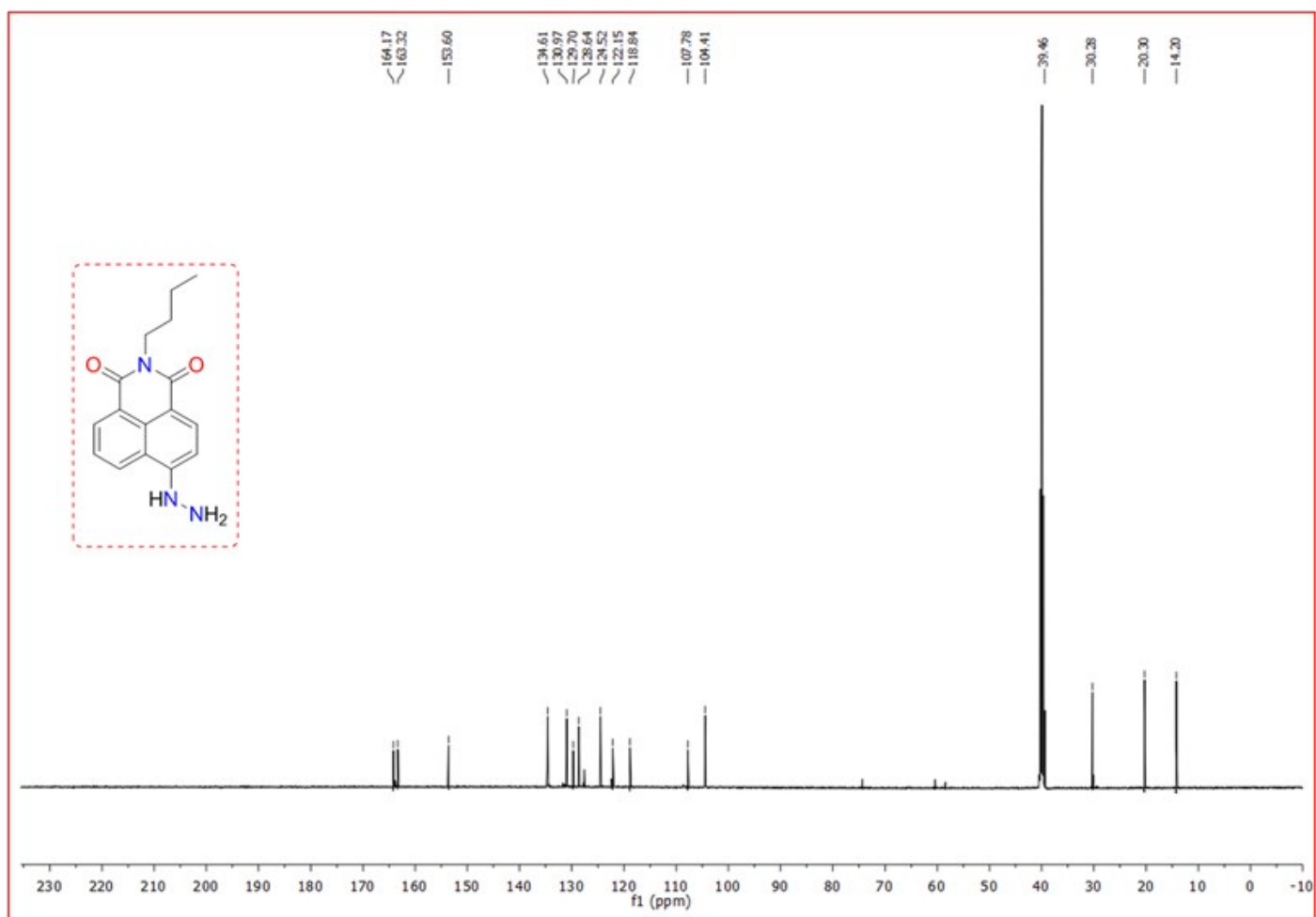


Fig S4. ^{13}C -NMR spectrum of the compound **3a** in CDCl_3-d_6

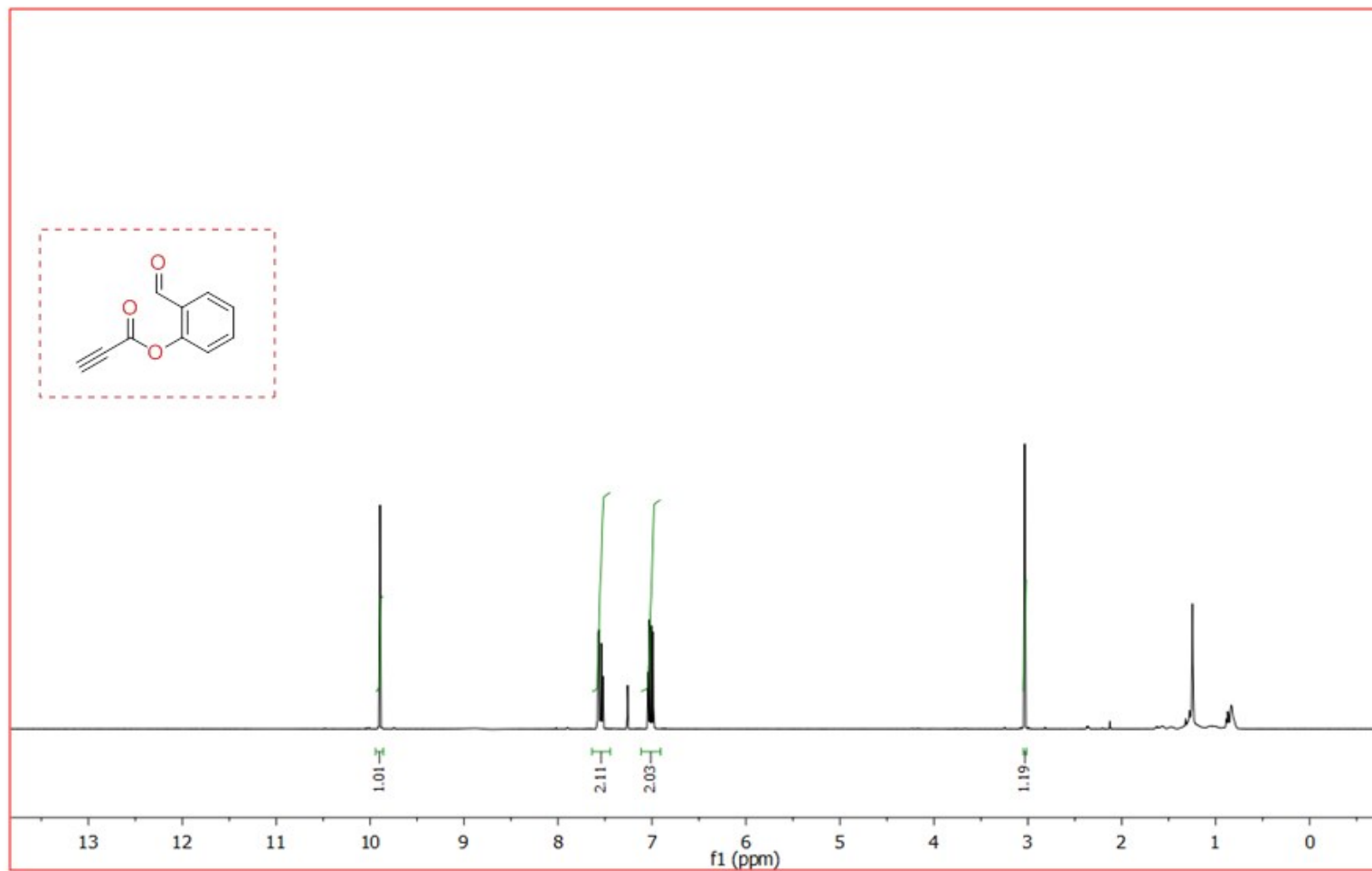


Fig S5. $^1\text{H-NMR}$ spectrum of the compound **4a** in CDCl_3-d_6

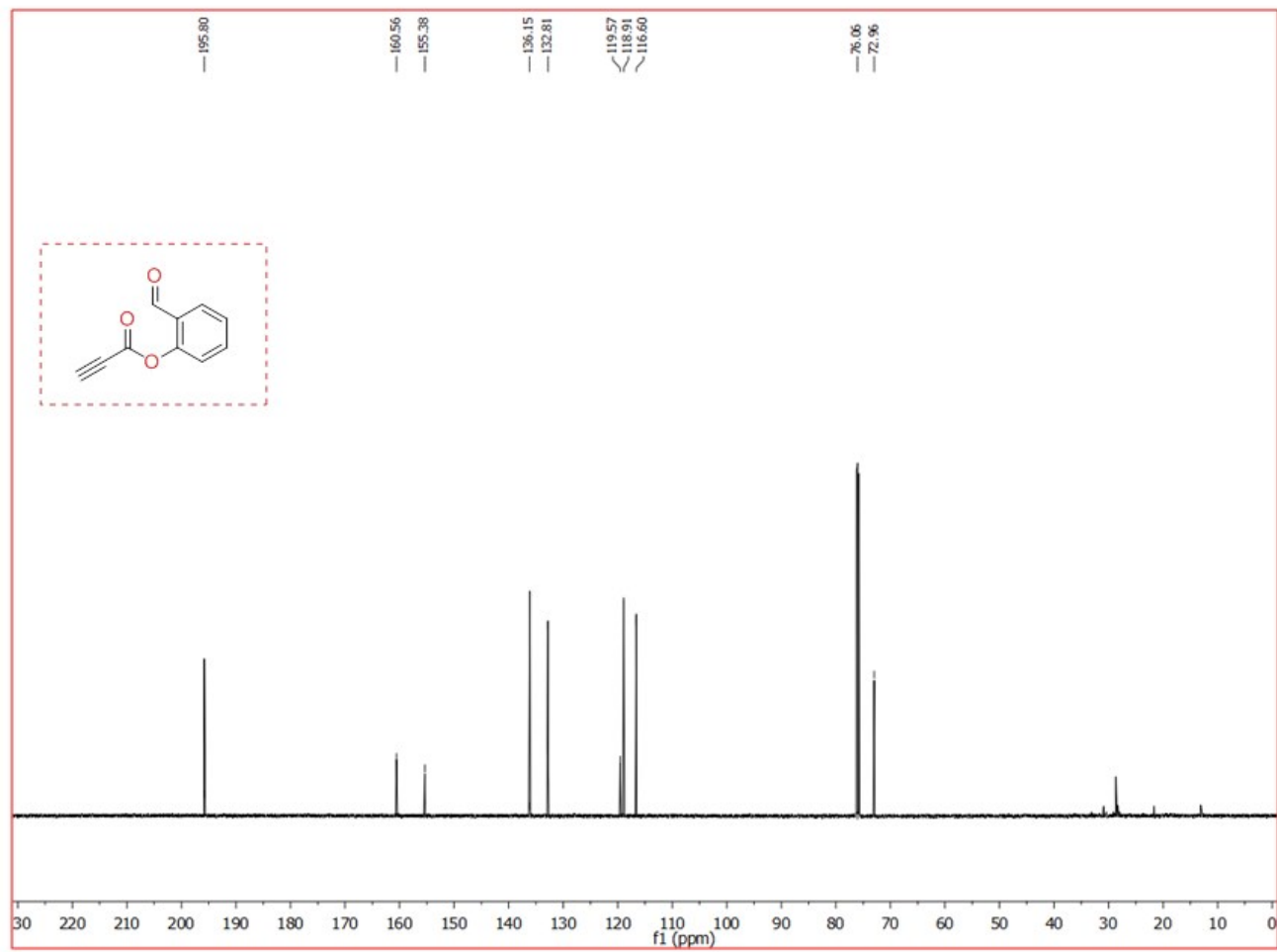


Fig S6. ^{13}C -NMR spectrum of the compound **4a** in CDCl_3-d_6

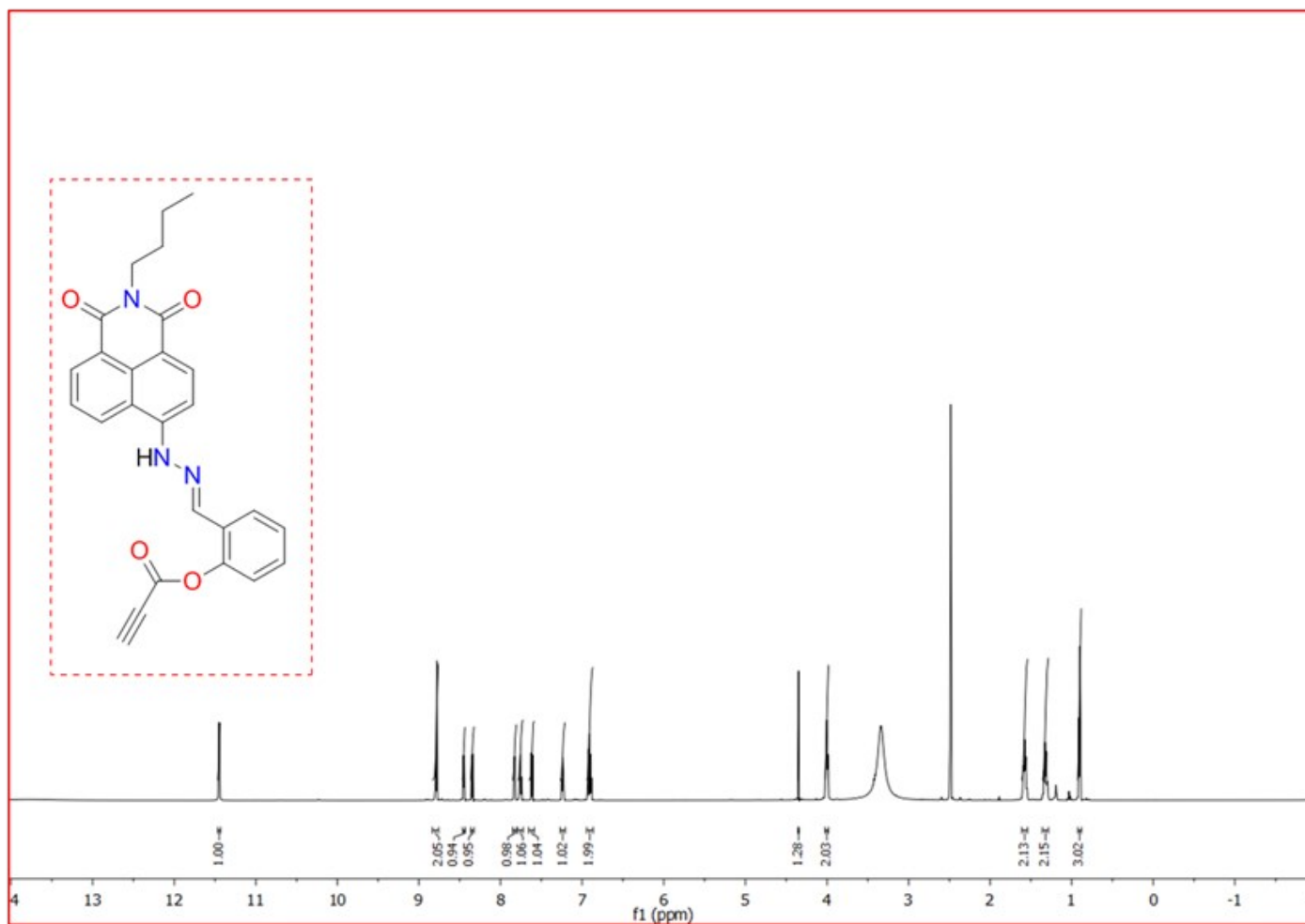


Fig S7. ¹H-NMR spectrum of the NASP in DMSO-*d*₆

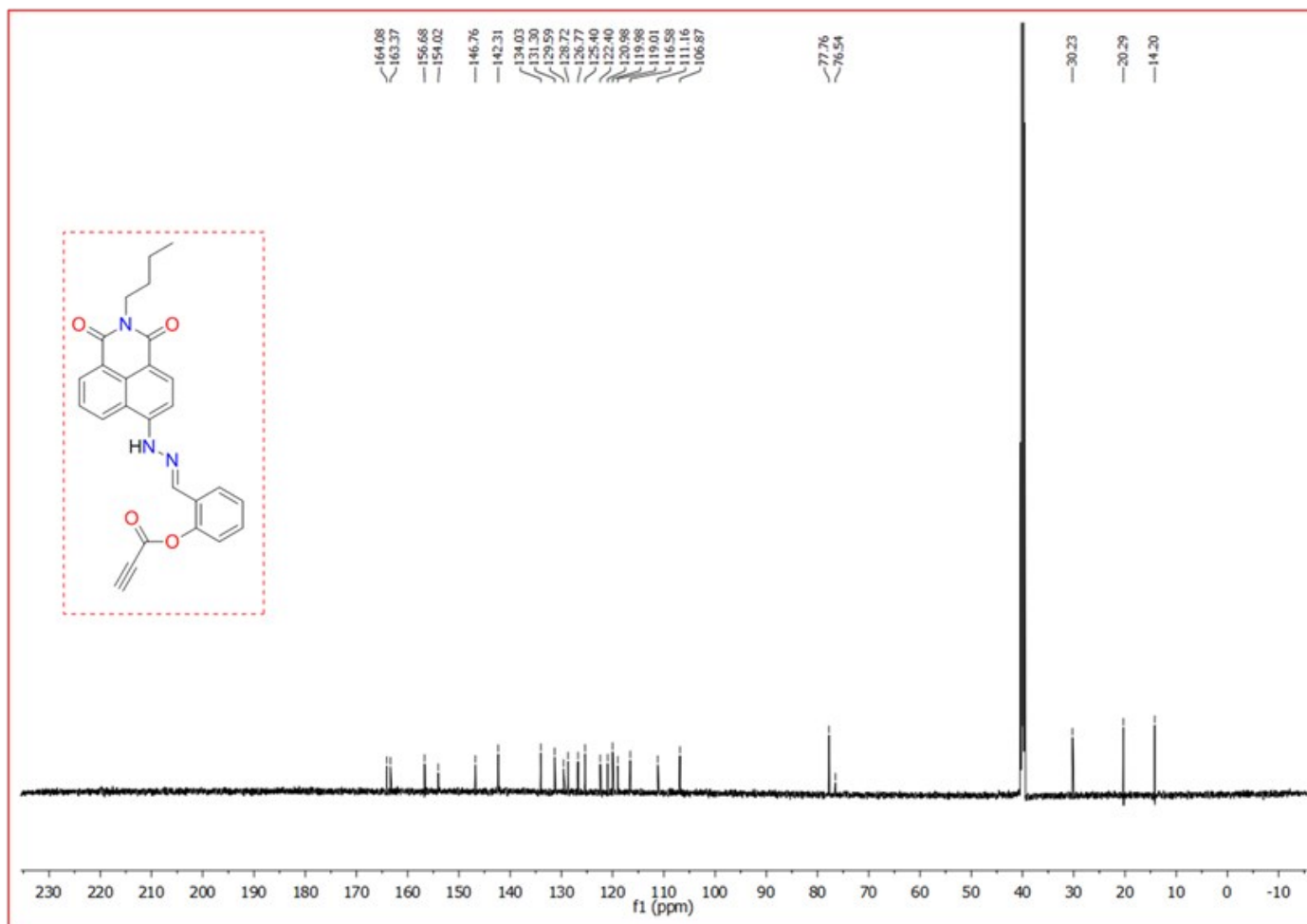


Fig S8. ^{13}C -NMR spectrum of the NASP in $\text{DMSO-}d_6$

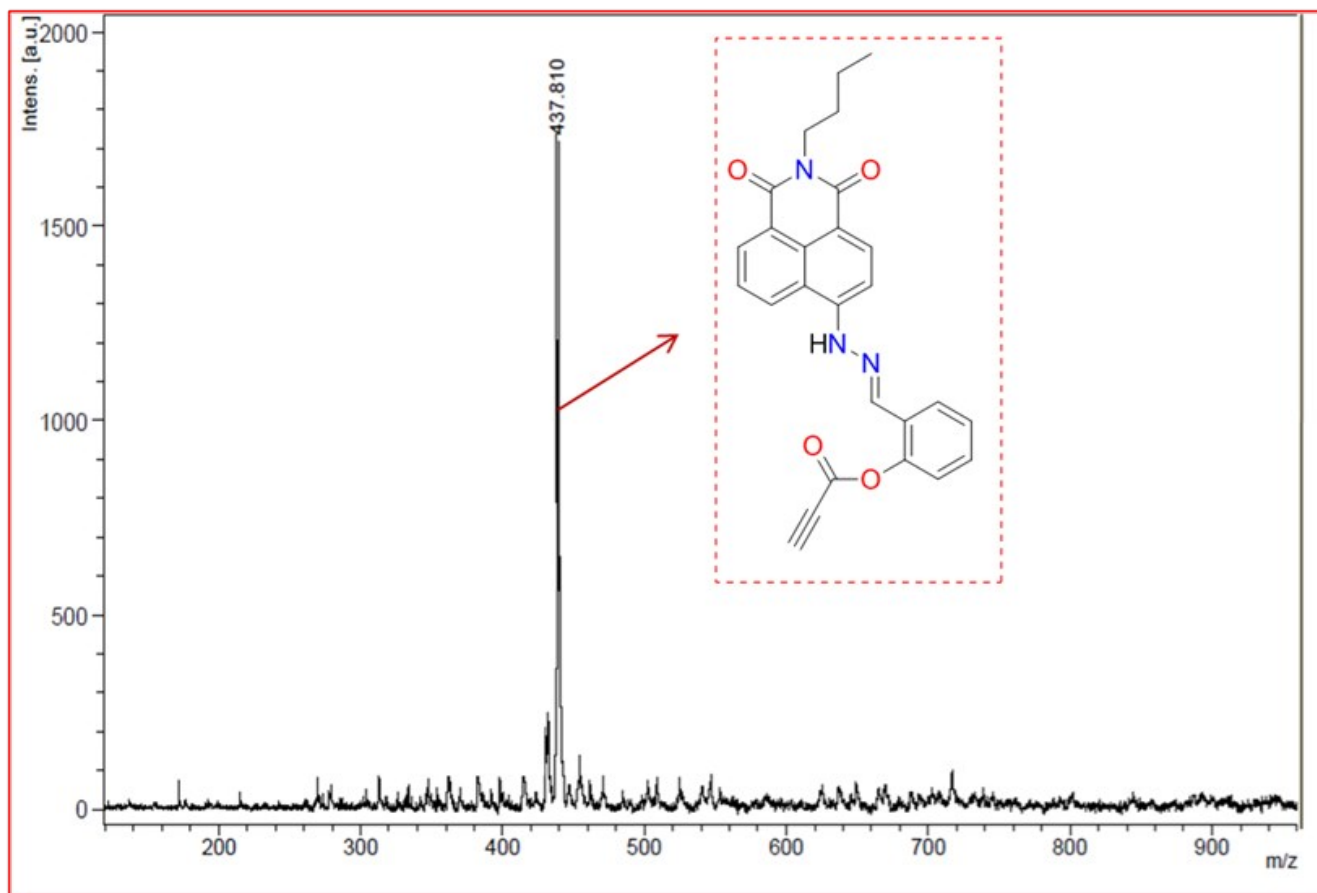


Fig S9. MALDI-TOF MS spectra of the NASP

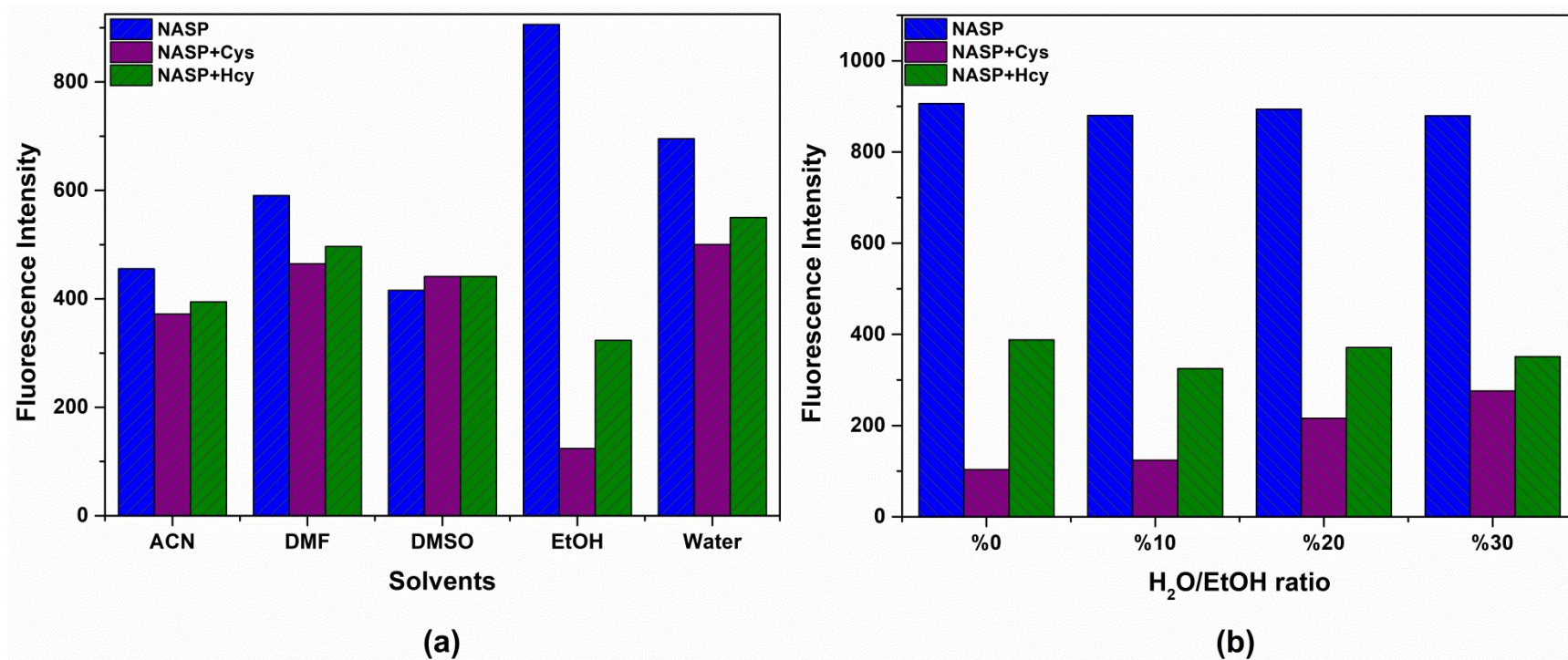


Fig S10. The fluorescence emission spectra of the **NASP**, **NASP–Cys** and **NASP–Hcy** **(a)** in different solvents and **(b)** EtOH–water mixture study in different ratios for the **NASP** in the presence and absence of Cys or Hcy

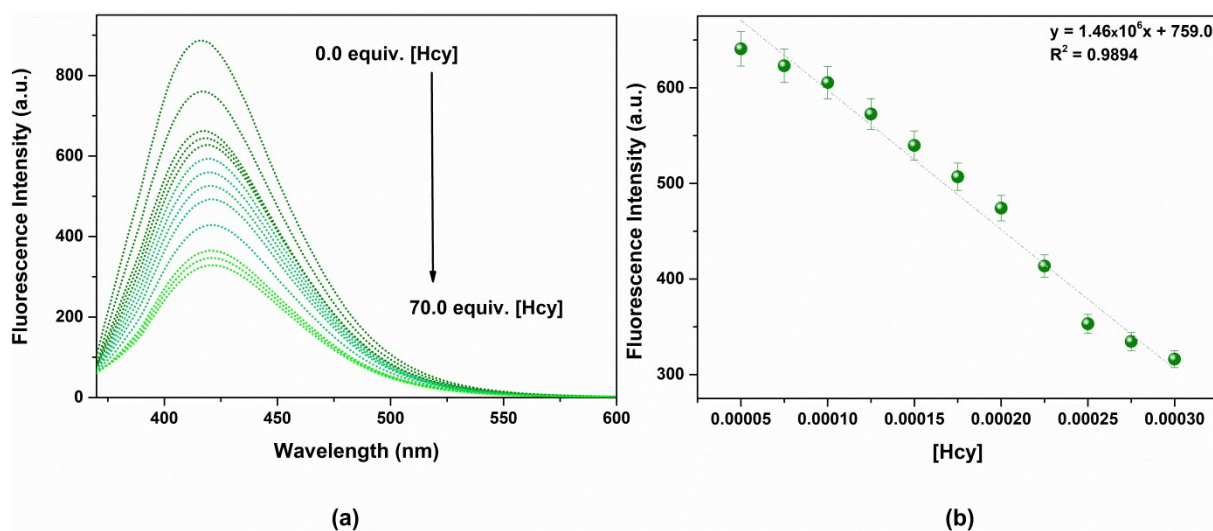


Fig S11. Spectra of the **NASP** (5.0 μM) in the presence of Hcy (0.0–70.0 equiv) in EtOH:H₂O (90:10, v:v, 0.0670 M PBS buffer pH=7.0) media, LOD plot of the **NASP** versus Hcy concentrations.

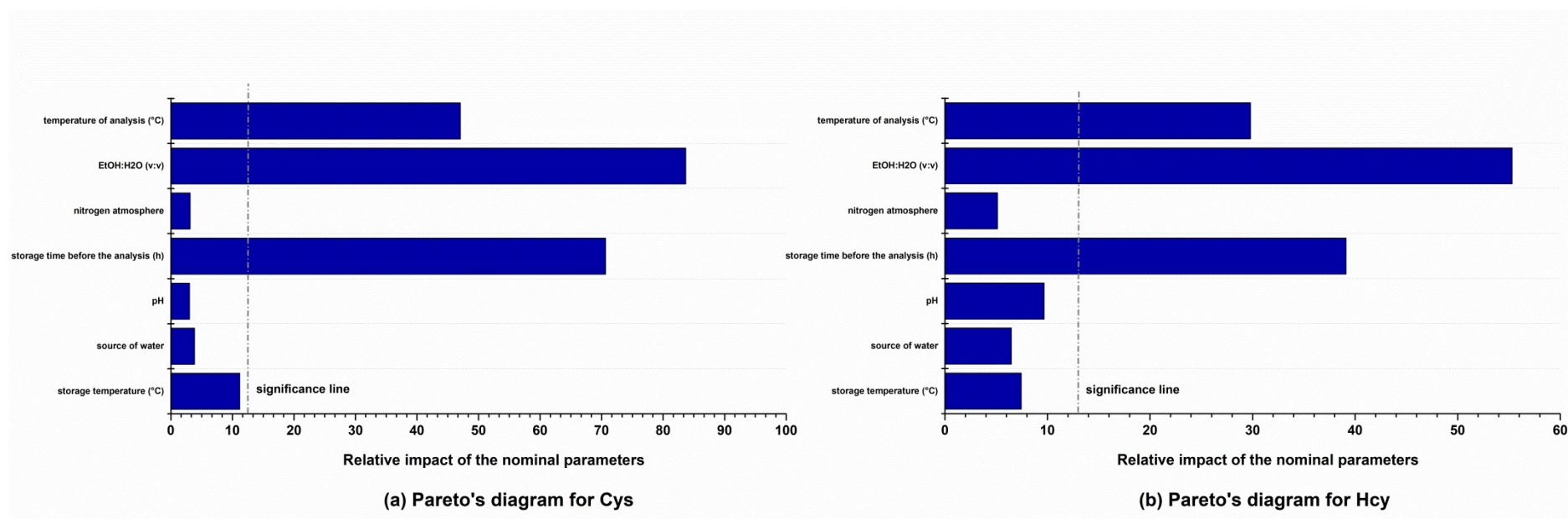


Fig S12. Pareto's diagram of the proposed method for Cys and Hcy

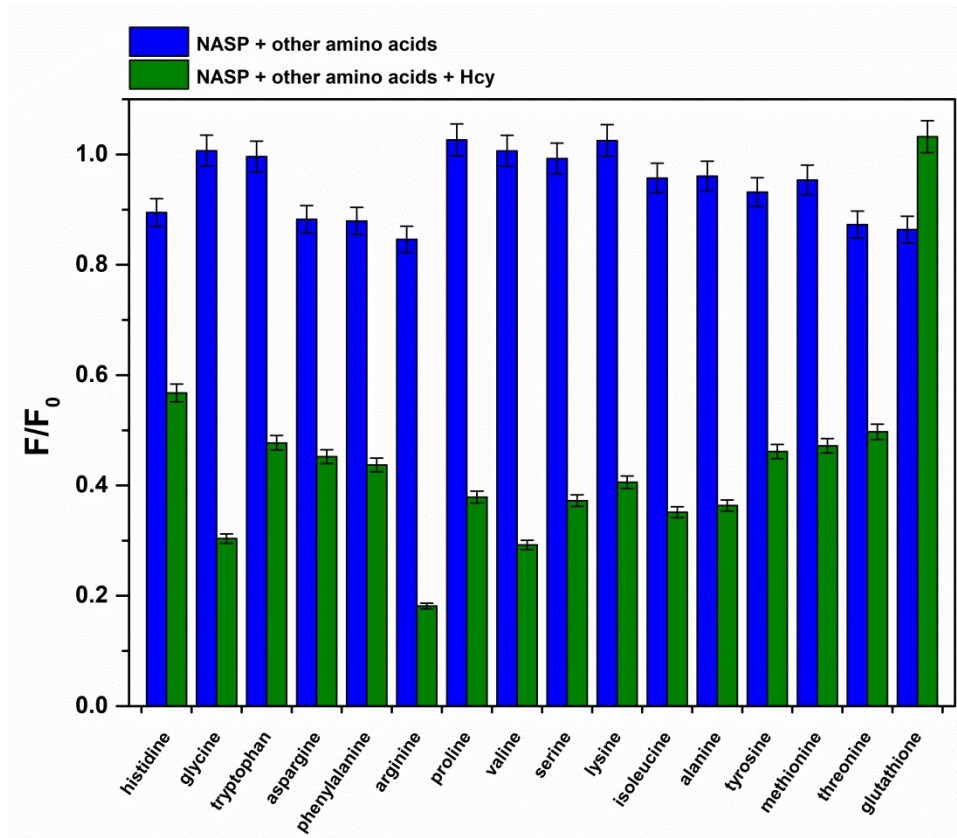


Fig S13. The emission intensity of the chemoprobe **NASP** (**5.0 μM**) with competitive amino acids (*Asn, Trp, Phe, Ser, Thr, Met, Pro, Gly, Tyr, Val, Ala, Lys, Arg, His, Ile, GSH*)(**70 equiv., 0.350 mM**) (blue bar) and the emission intensity of **NASP** (**5.0 μM**) in the presence of and **70 equiv. other competitive analytes** and **70 equiv. Hcy** (**0.350 mM**) in EtOH:H₂O (90:10, v:v, 0.0670 M PBS buffer pH=7.0) media

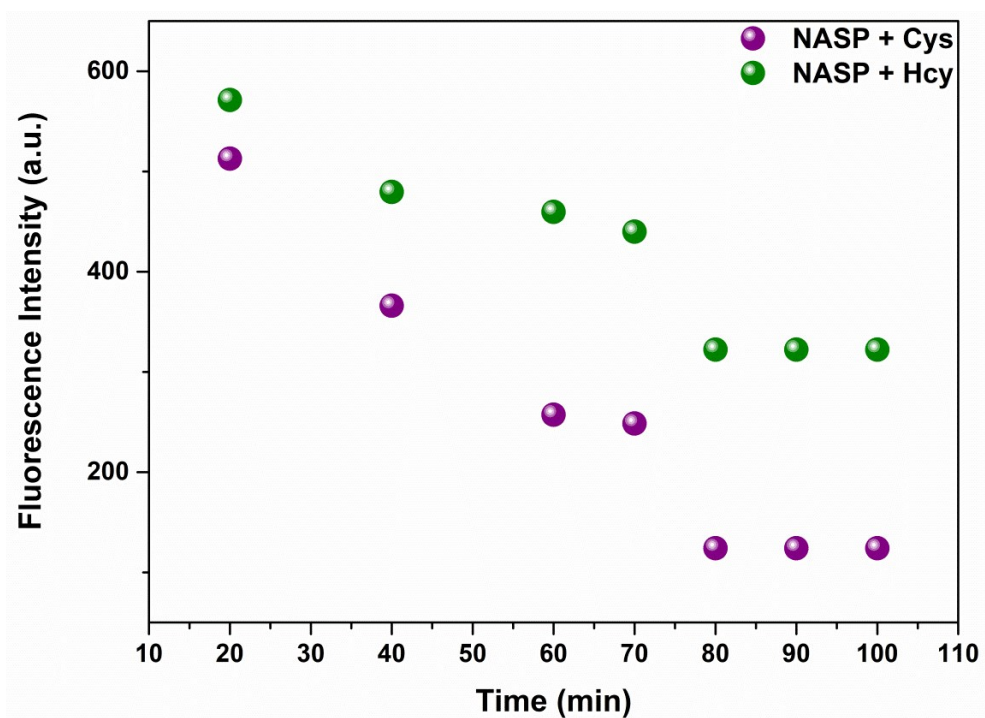


Fig S14. Response time experiments of the chemoprobe **NASP** (5.0 μM) in the presence of Cys, and Hcy in EtOH:H₂O (90:10, v:v, 0.0670 M PBS buffer pH=7.0) media

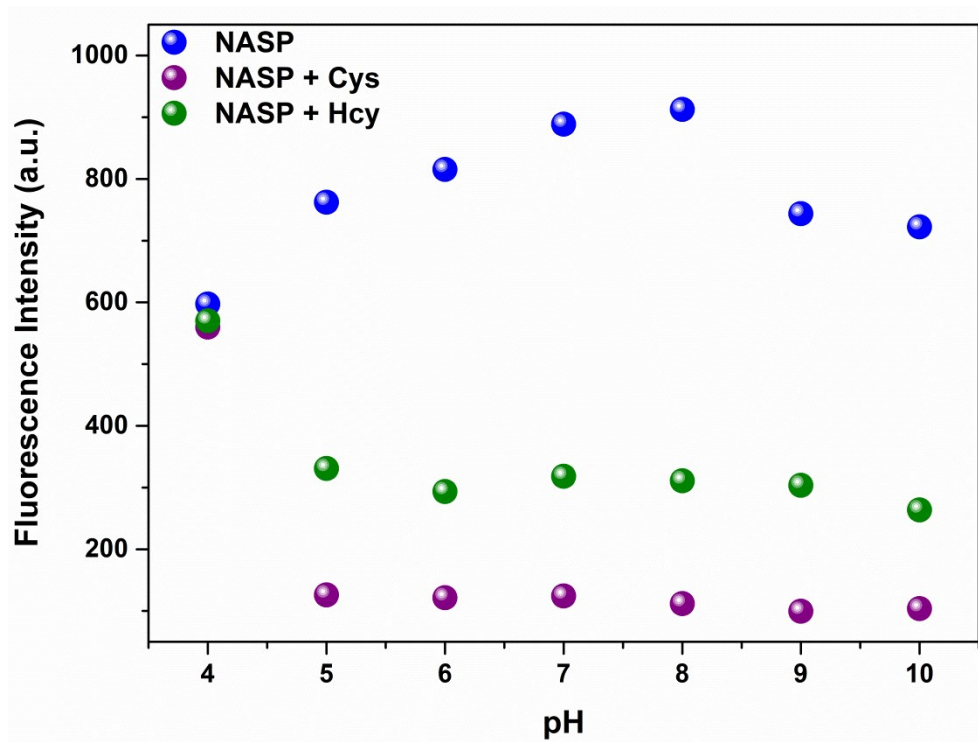


Fig S15. pH study of the **NASP** with or without Cys/Hcy in EtOH:H₂O (90:10, v:v, 0.0670 M PBS buffer pH=7.0) media

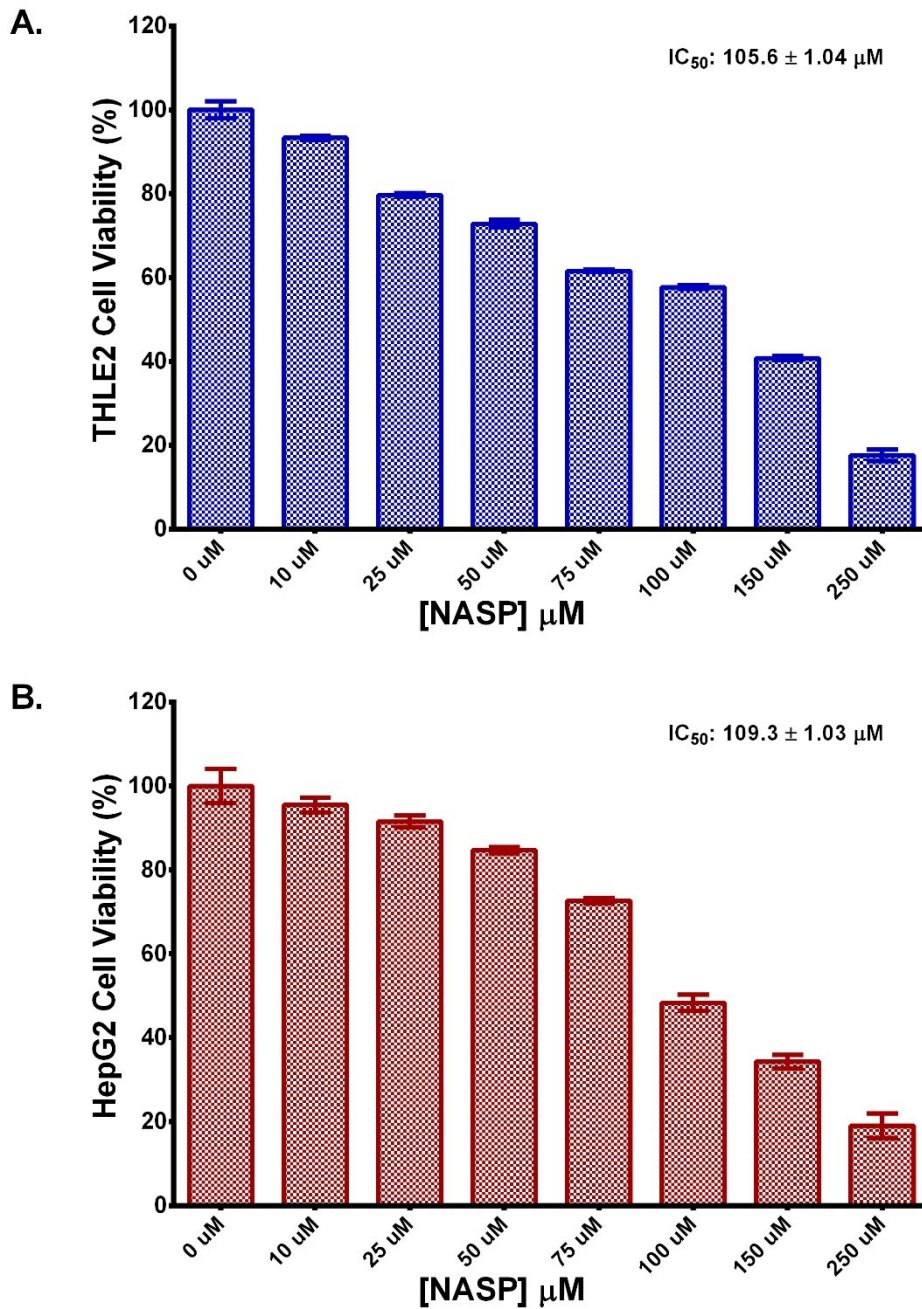
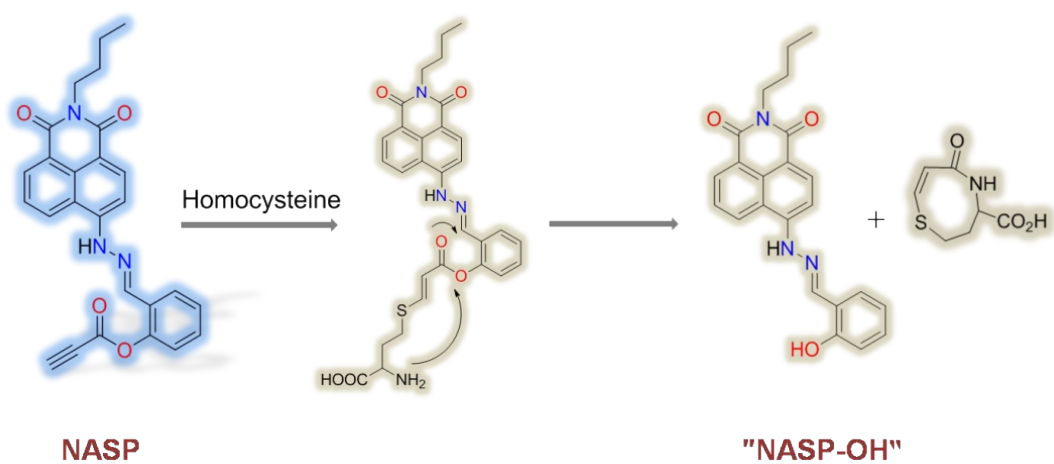


Fig S16. In vitro cytotoxic effects of NASP on THLE2 (**a**) and HepG2 (**b**) cells for 24-h incubation. Data presented the mean of at least triplicate measurements and given as mean \pm standard error of the mean. The IC_{50} value for the 24-h incubation was calculated as $105.6 \pm 1.04 \mu\text{M}$ for THLE2 cells and $109.3 \pm 1.03 \mu\text{M}$ for HepG2 cells.



Scheme S1. Proposed mechanism for the recognition of homocysteine

Table S1. Nominal parameters specified during the assessment of the proposed method for Cys and Hcy

slit of excitation	10 nm
slit of emission	10 nm
monitored wavelength	$\lambda_{\text{ex}}=360$ nm, $\lambda_{\text{em}}=416$ nm
photomultiplier tube (PMT) voltage	600 Volt
temperature	rt
pH	0.0670 M PBS buffer pH=7.0 (EtOH:H ₂ O (90:10, v:v))

Table S2. Parameters used in the robustness analysis of the proposed method for Cys and Hcy

	parameters	situation	
		nominal (+)	Changed (-)
1	storage temperature (°C)	37.5	25
2	source of water	ultrapure	distilled
3	pH	7.0	5.0
4	storage time before the analysis (h)	16	24
5	nitrogen atmosphere	no	yes
6	EtOH:H ₂ O (v:v)	90 / 10	80 / 20
7	temperature of analysis (°C)	25	15

Table S3. Factorial combinations used in the Youden test of robustness analysis of the proposed method for Cys and Hcy

Parameter \ C_i	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8
1	+	+	+	+	-	-	-	-
2	+	+	-	-	+	+	-	-
3	+	-	+	-	+	-	+	-
4	+	+	-	-	-	-	+	+
5	+	-	+	-	-	+	-	+
6	+	-	-	+	+	-	-	+
7	+	-	-	+	-	+	+	-

Table S4. Dixon's test utilized to the repeatability of the proposed method for Cys and Hcy

	concentration	average values (\bar{x})			highest value of emission	Q_{value}	lowest value of emission	Q_{value}
		16h	20h	24h				
Cys	1×10^{-5} M	91.60	99.40	111.24	111.24	0.60	91.60	0.40
	2×10^{-5} M	75.25	77.67	87.07	87.07	0.80	75.25	0.20
Hcy	1×10^{-5} M	75.29	75.31	78.11	78.11	0.07	75.29	0.93
	2×10^{-5} M	179.69	172.43	175.21	179.69	0.80	172.43	0.20

[†] $Q_{critical} = 0.970$

Table S5. Intermediate precision analysis of the proposed method for Cys and Hcy verified by the HorRat ratio

		Cys			Hcy		
		analyst (1)	analyst (2)	$F_{calculated}$	analyst (1)	analyst (2)	$F_{calculated}$
16 h 1×10^{-5} M	Average (\bar{x})	91.60	92.49		75.30	78.86	
	SD	0.13	0.12		0.15	0.12	
	RSD (%)	0.14	0.13	1.29	0.2	0.15	1.76
	RSD Horwitz (%)	7.58	7.58	$F_{cal} < F_{critical}$	7.58	7.58	$F_{cal} < F_{critical}$
	HorRat ratio	0.02	0.02		0.03	0.02	
16 h 2×10^{-5} M	Average (\bar{x})	75.25	79.14		179.69	176.10	
	SD	0.14	0.12		0.13	0.19	
	RSD (%)	0.19	0.15	1.56	0.07	0.11	2.34
	RSD Horwitz (%)	6.83	6.83	$F_{cal} < F_{critical}$	6.83	6.83	$F_{cal} < F_{critical}$
	HorRat ratio	0.03	0.02		0.01	0.02	
20 h 1×10^{-5} M	Average (\bar{x})	99.40	91.94		75.30	79.20	
	SD	0.19	0.17		0.13	0.11	
	RSD (%)	0.19	0.18	1.08	0.18	0.14	1.56
	RSD Horwitz (%)	7.58	7.58	$F_{cal} < F_{critical}$	7.58	7.58	$F_{cal} < F_{critical}$
	HorRat ratio	0.03	0.02		0.02	0.02	
20 h 2×10^{-5} M	Average (\bar{x})	77.67	76.53		172.43	176.43	
	SD	0.21	0.20		0.11	0.16	
	RSD (%)	0.27	0.27	1.02	0.07	0.09	1.89
	RSD Horwitz (%)	6.83	6.83	$F_{cal} < F_{critical}$	6.83	6.83	$F_{cal} < F_{critical}$
	HorRat ratio	0.04	0.04		0.01	0.01	
24 h 1×10^{-5} M	Average (\bar{x})	111.24	117.77		78.11	78.18	
	SD	0.15	0.10		0.09	0.07	
	RSD (%)	0.14	0.08	2.83	0.12	0.1	1.46
	RSD Horwitz (%)	7.58	7.58	$F_{cal} < F_{critical}$	7.58	7.58	$F_{cal} < F_{critical}$
	HorRat ratio	0.02	0.01		0.02	0.01	
24 h 2×10^{-5} M	Average (\bar{x})	108.4356	103.62		175.21	173.04	
	SD	0.13	0.14		0.14	0.14	
	RSD (%)	0.12	0.14	1.28	0.08	0.08	1.03
	RSD Horwitz (%)	6.83	6.83	$F_{cal} < F_{critical}$	6.83	6.83	$F_{cal} < F_{critical}$
	HorRat ratio	0.02	0.02		0.01	0.01	

[†] $F_{critical}=2.98$