Supplementary Materials

HPLC Study of Product Formed in the Reaction of NBD-Derived Fluorescent Probe with Hydrogen Sulfide, Cysteine, *N*-acetylcysteine, and Glutathione

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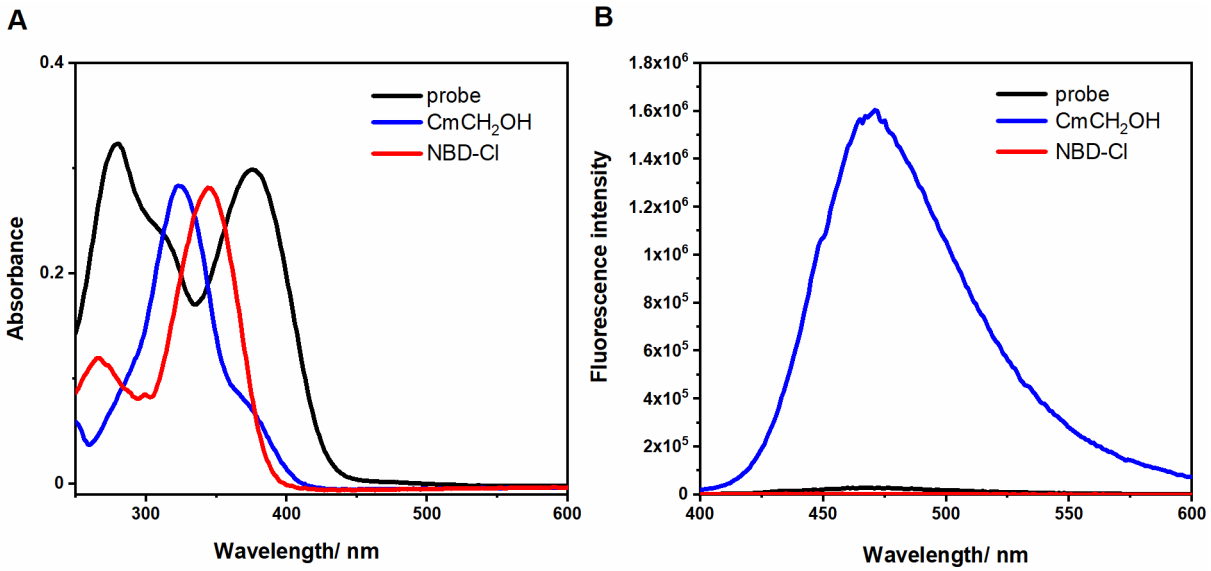


Figure S1. (A) UV-Vis absorption and **(B)** emission spectra of the **NBD-O-CmCH$_2$OH** probe, **CmCH$_2$OH,** and **NBD-Cl** recorded in phosphate buffer (0.1 M, pH 7.4) containing MeCN (10%). λ_{ex}=320 nm, slites 1.0/1.0 nm.

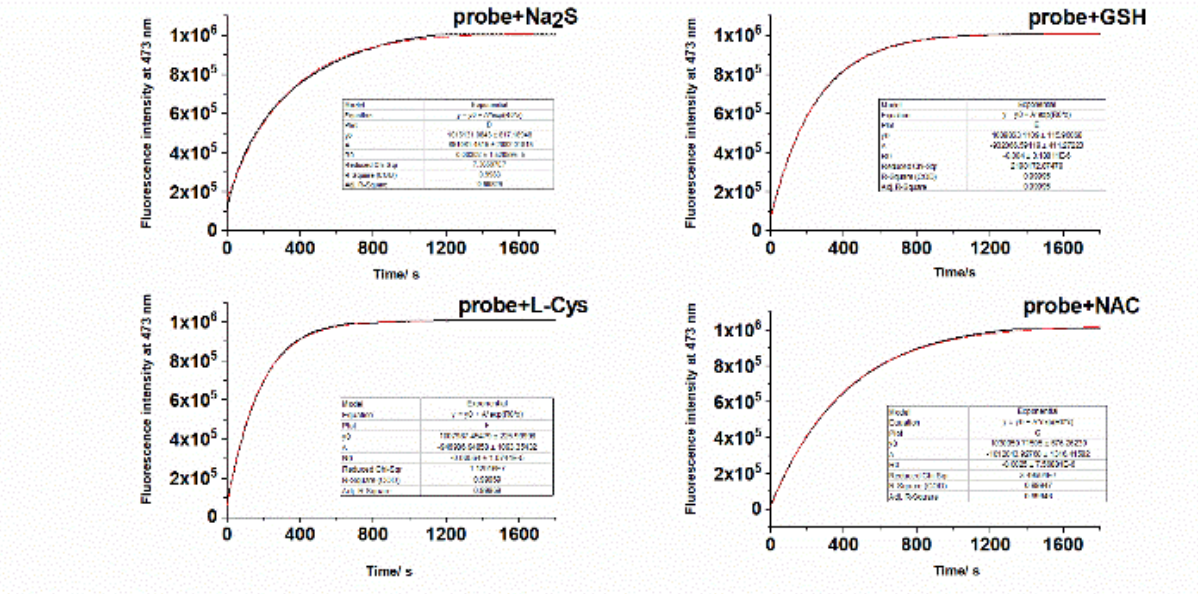


Figure S2. Time-dependent fluorescence intensity changes of **NBD-O-CmCH$_2$OH** (5 μM) at 473 nm when treated with various thiol species (50 μM) in PB buffer.

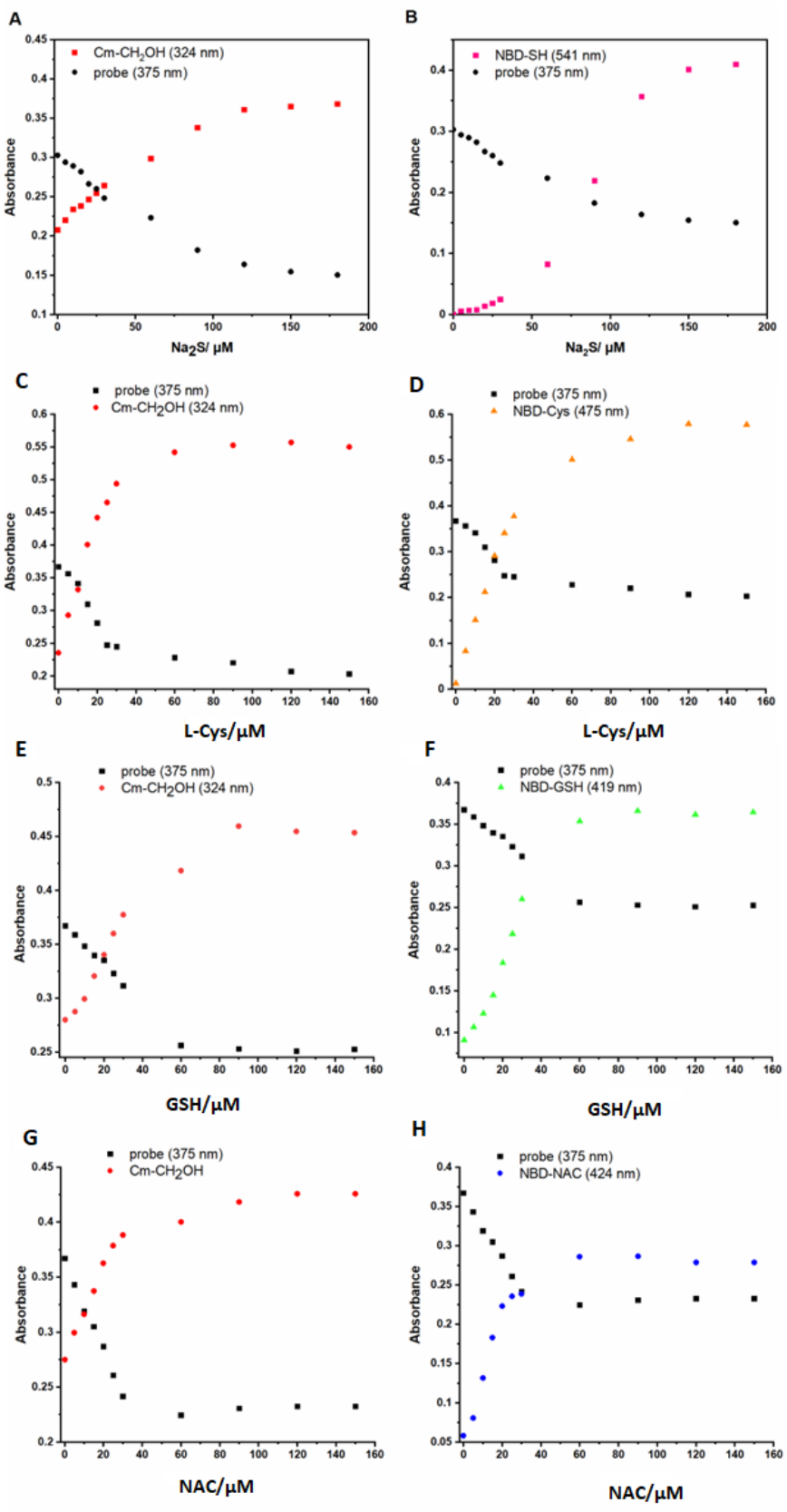


Figure S3. Changes in the characteristic absorption bands during the reaction between the **NBD-O-Cm CH_2OH** probe (30 μM) and (A,B) Na_2S, (C,D) L-Cys, (E,F) GSH (G,H) NAC.

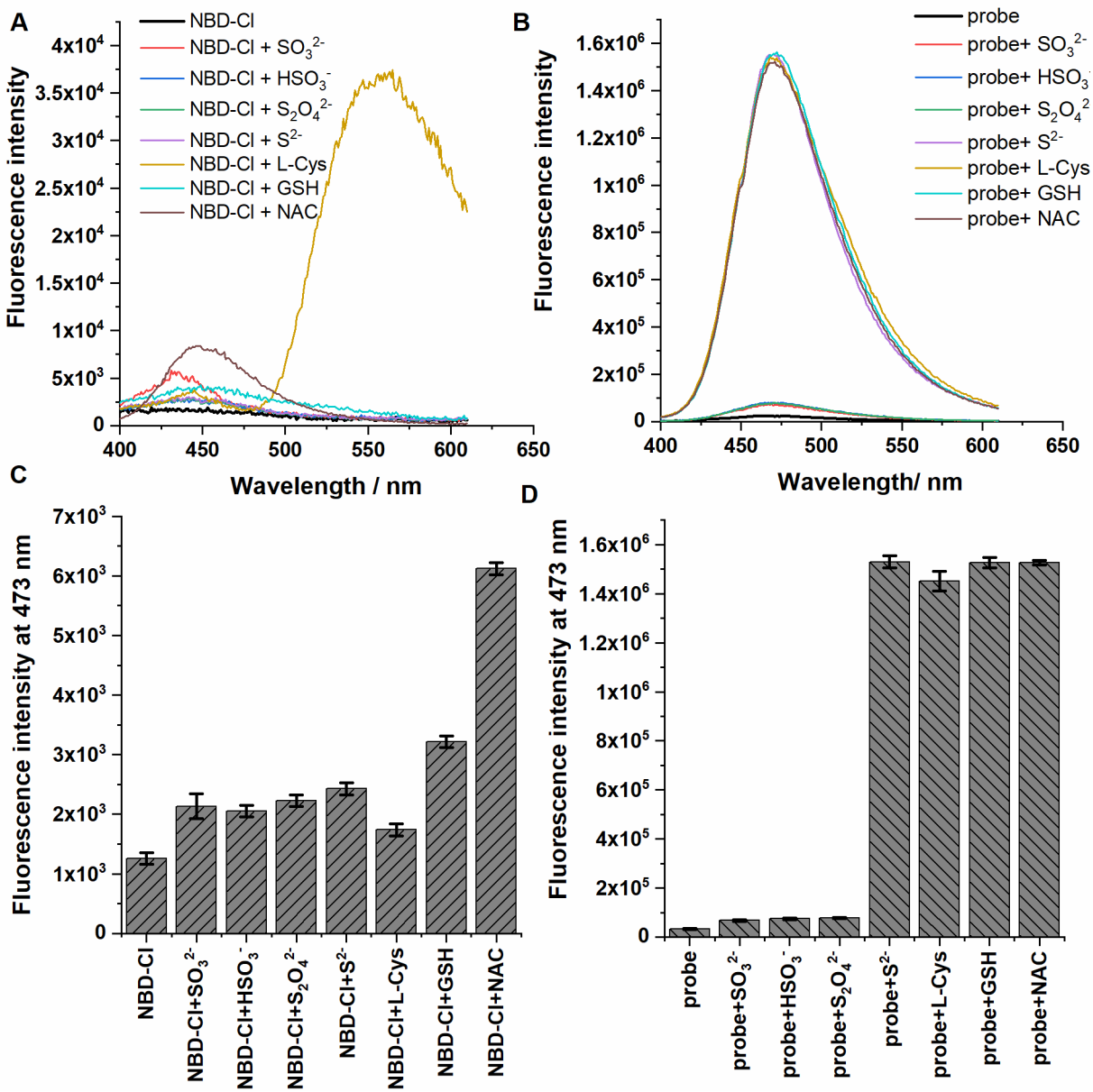


Figure S4. The fluorescence intensity changes of (**A,C**) NBD-Cl and (**B,D**) probe **NBD-O-CmCH$_2$OH** (7.5 μM) upon the addition of sulfur species (37.5 μM for each) in MeCN-PB (1:9. v/v, pH 7.4).

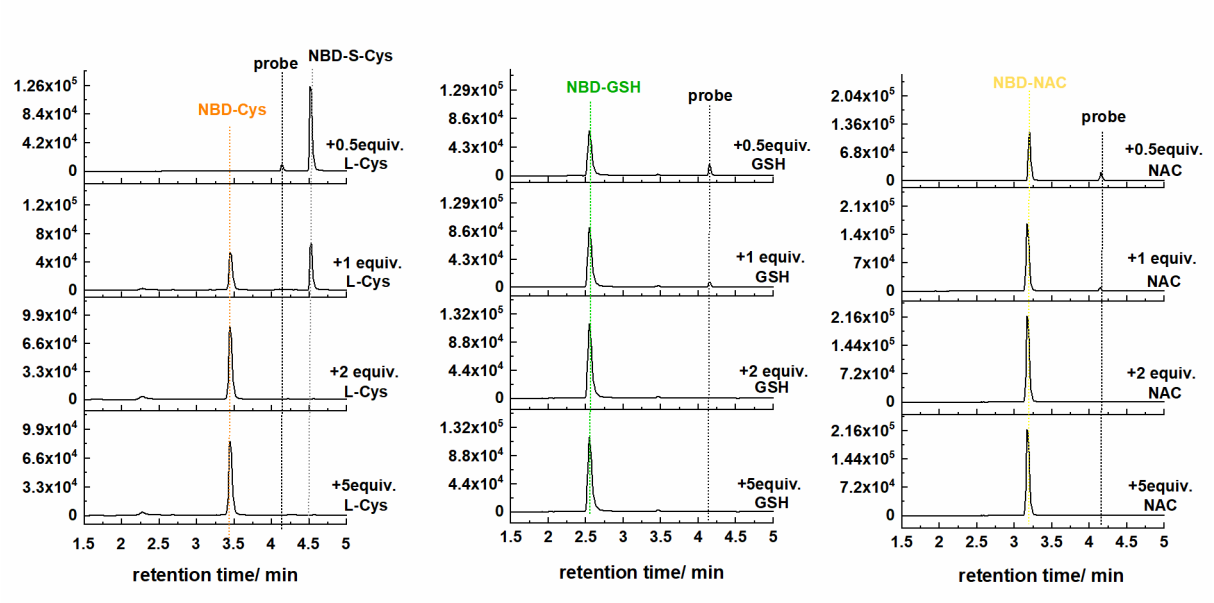
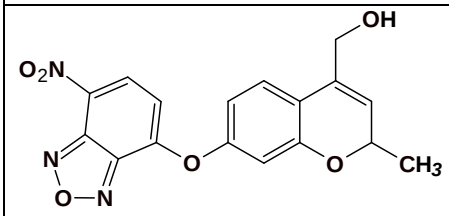
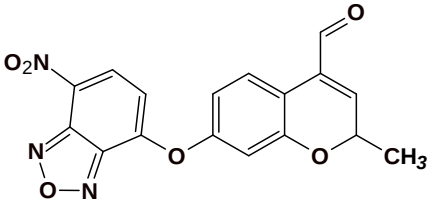
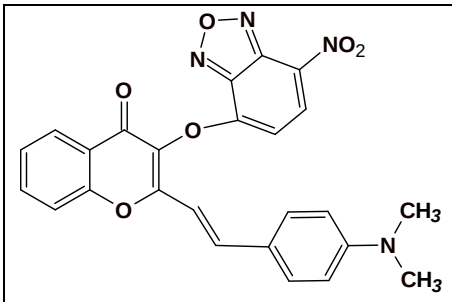
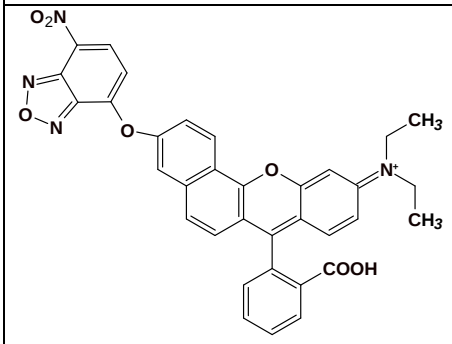
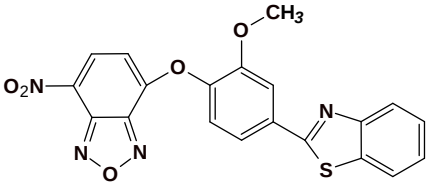
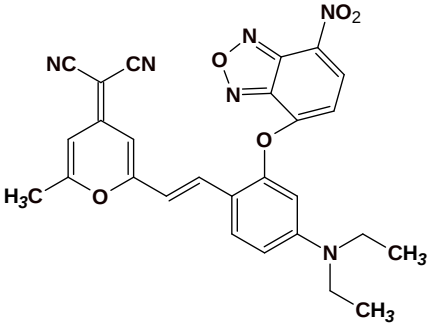
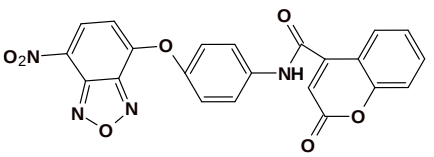
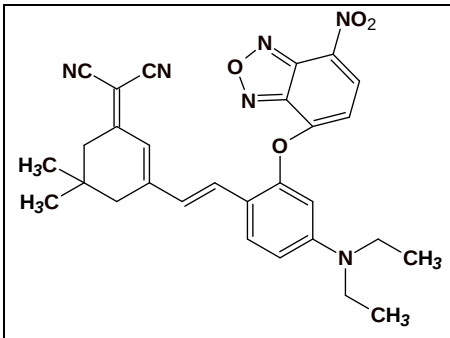
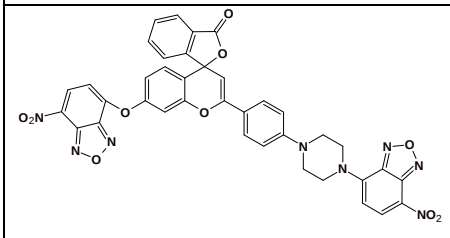


Figure S5. HPLC chromatogram of the reaction mixtures of **NBD-O-CmCH$_2$OH** (60 μM) with **(A)** L-Cys (30 – 300 μM), **(B)** GSH 30– 300 μM, **(C)** NAC 30 – 300 μM after 15 min incubation. The traces were collected using an absorption detector set at 420 nm.

Table 1. Comparison of **NBD-O-CmCH$_2$OH** with the fluorescent probes previously reported.

Structures	Medium	Detection targets	λ_{em} (nm)	λ_{ex} (nm)	Response time (min)	LOD (μM)	Ref.
 **NBD-O-CmCH$_2$OH**	MeCN:PB (1:9, v/v)	Cys GSH H_2S NAC	320	473	15	0.14 0.06 0.03 0.03	Our work
 **FHC-O-NBD**	MeCN:PBS (6:4, v/v)	Cys GSH H_2S NAC	340	486	20 120 ND* ND*	0.11 0.79 0.42 ND*	[S1]

 **NBD-OF**	MeCN:PBS (3:7, v/v)	Cys GSH H_2S NAC	488	545,621 621 ND* ND*	60 120 ND* ND*	2.1 6.4 ND* ND*	[S2]
 **SNARF-NBD**	MeCN:PBS (3:7, v/v)	Cys GSH H_2S NAC	435	543 543 624 ND*	10 15 ND* ND*	0.05 0.06 0.06 ND*	[S3]
 **BMNO**	MeCN:HEPES (1:1, v/v)	Cys GSH H_2S NAC	330	405	ND*	1.8 1.6 ND* ND*	[S4]
 **RED-NBD**	MeCN:PB (3:7, v/v)	Cys GSH H_2S NAC	450	560, 630 630 ND* ND*	10 10 ND* ND*	0.02 0.03 ND* ND*	[S5]
 **NC-NBD**	DMF:PBS (1:9, v/v)	Cys GSH H_2S NAC	420	520	2 ND* ND* ND*	0.43 0.36 ND* ND*	[S6]

 **NIR-NBD**	DMF:PBS (4:6, v/v)	Cys GSH H_2S NAC	588	756	15 8 ND* ND*	0.16 0.56 ND* ND*	[S7]
 **NJB**	MeCN:PBS (1:1, v/v)	Cys GSH H_2S NAC	478 ND* 546 ND*	553 ND* 604 ND*	30 ND* 180 ND*	0.06 ND* 0.08 ND*	[S8]

*ND-no determined

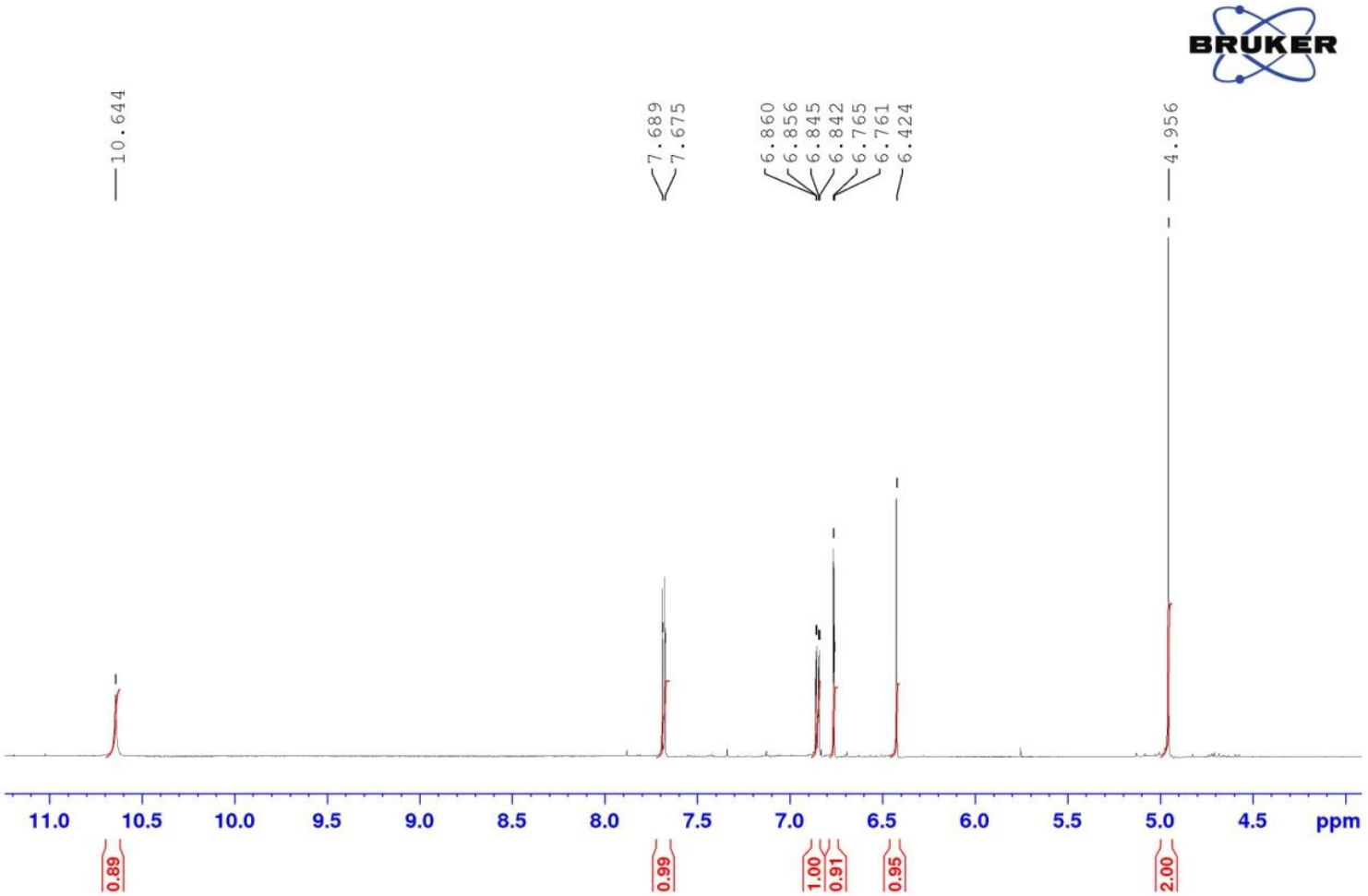


Figure S6. ^{1}H NMR spectrum of **CmCH_2Cl**

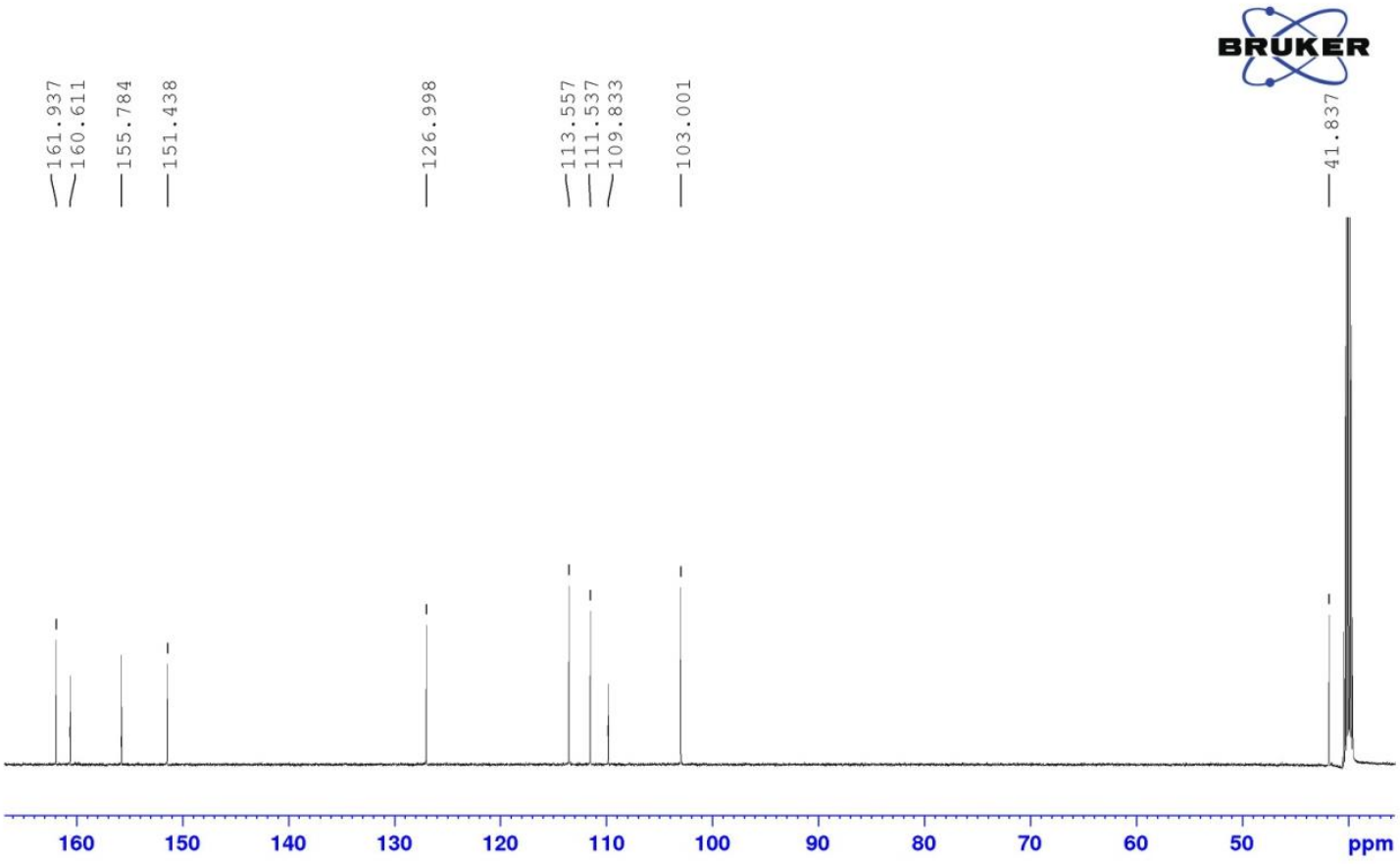


Figure S7. ^{13}C NMR spectrum of **$CmCH_2Cl$**

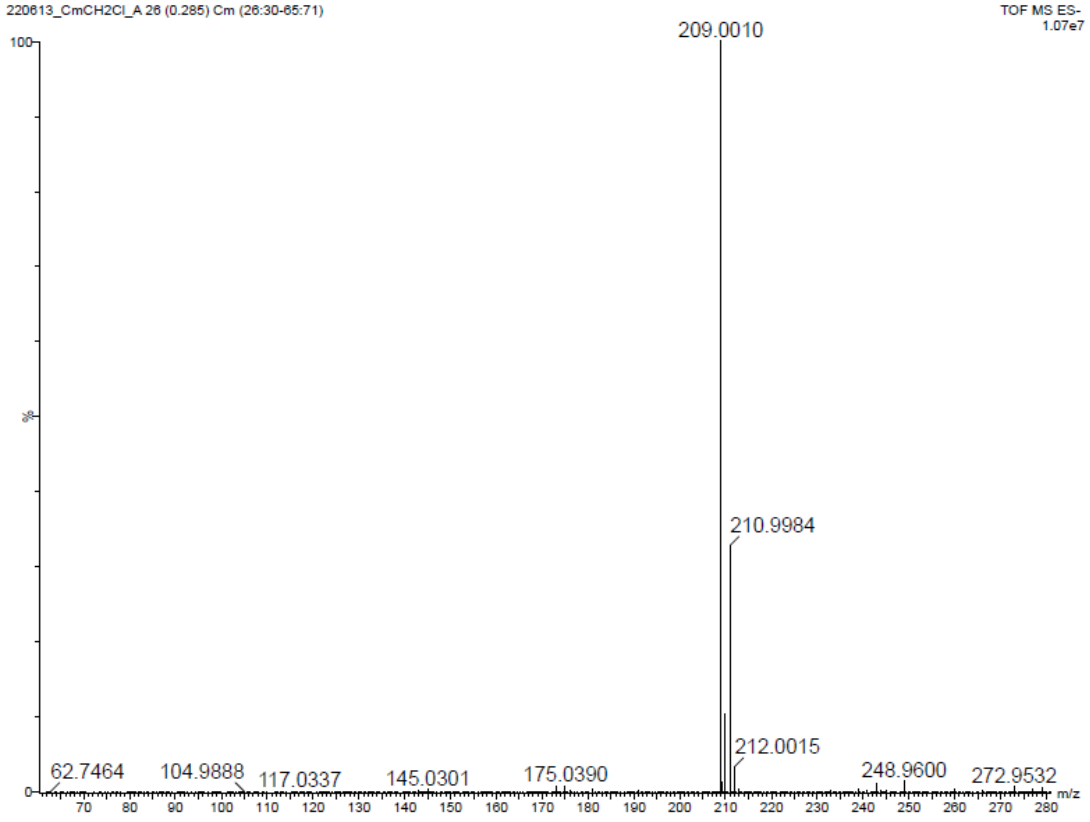


Figure S8. HRMS spectrum of **CmCH$_2$Cl**

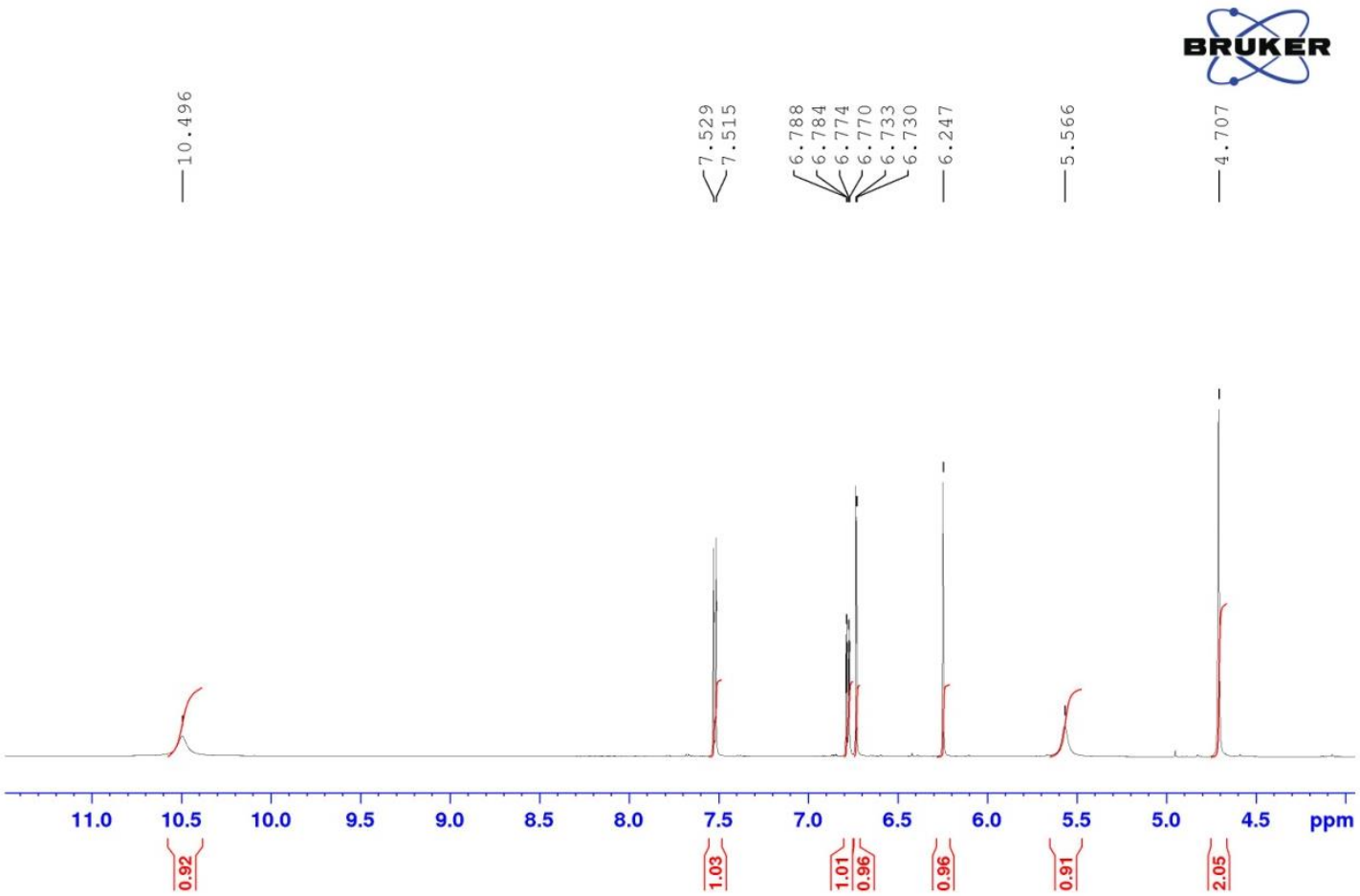


Figure S9. ^{1}H NMR spectrum of **CmCH_2OH**

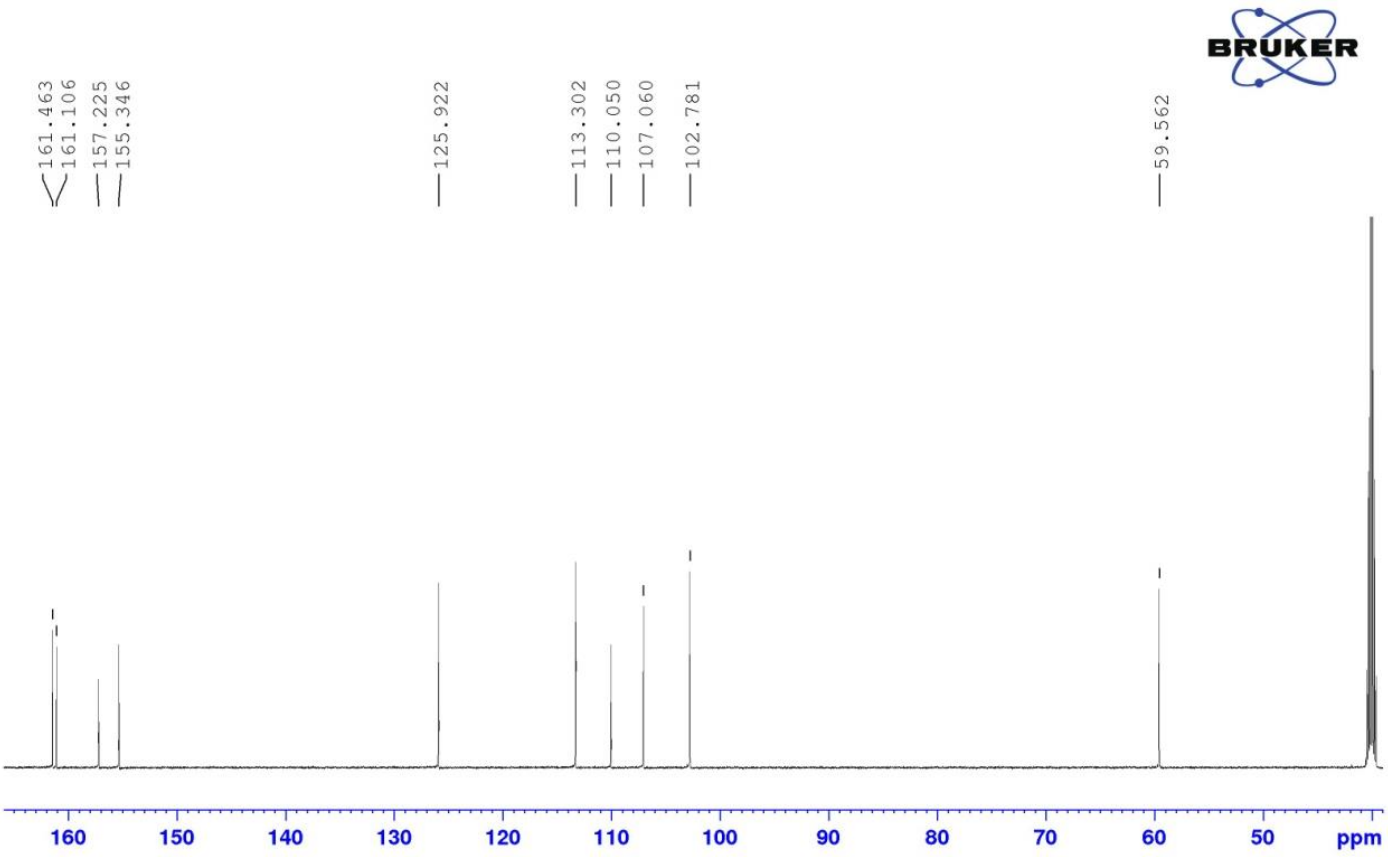


Figure S10. ^{13}C NMR spectrum of **CmCH_2OH**

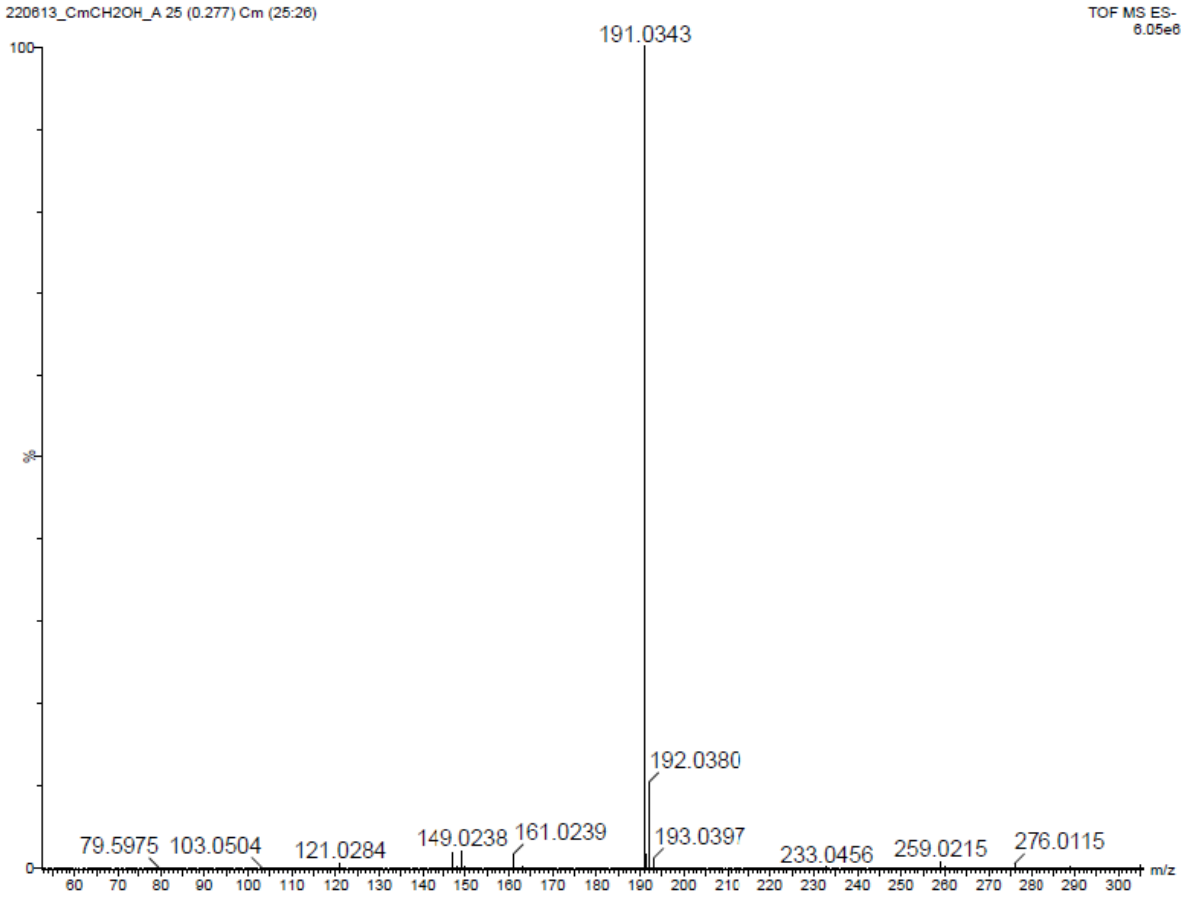


Figure S11. HRMS spectrum of **CmCH_2OH**

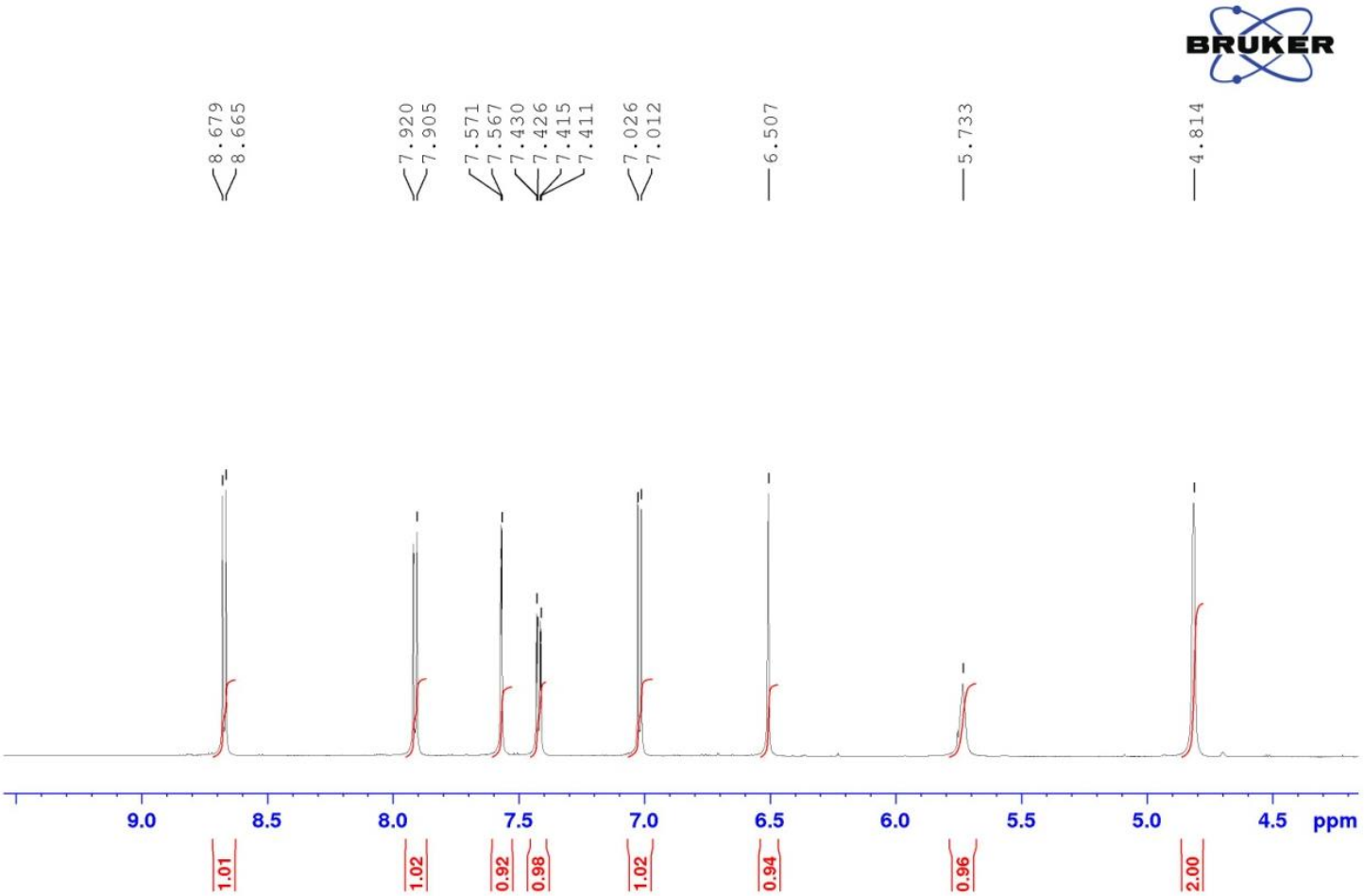


Figure S12. ^{1}H NMR spectrum of **NBD-O-CmCH$_2$OH**

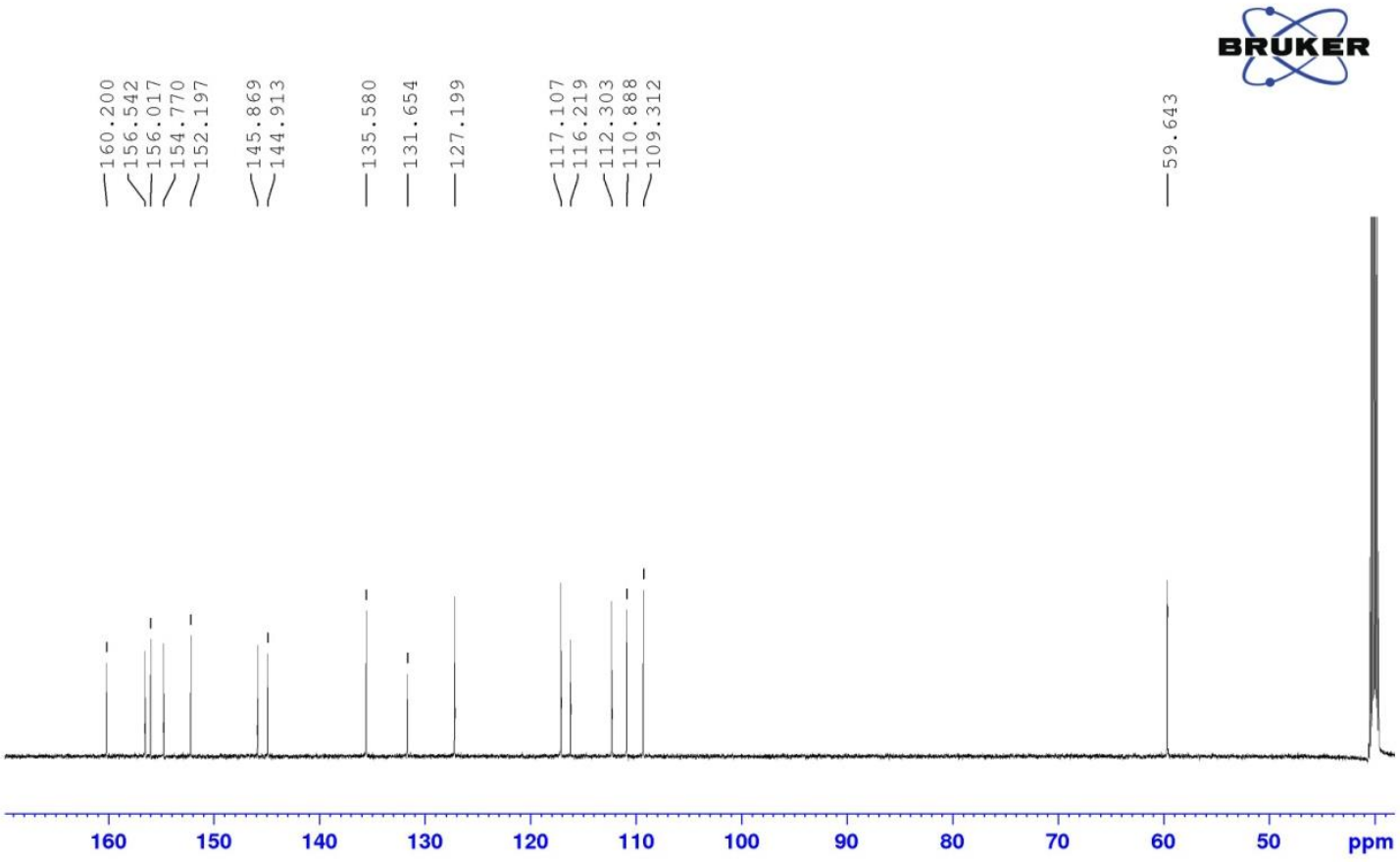


Figure S13. ^{13}C NMR spectrum of **NBD-O-CmCH$_2$OH**

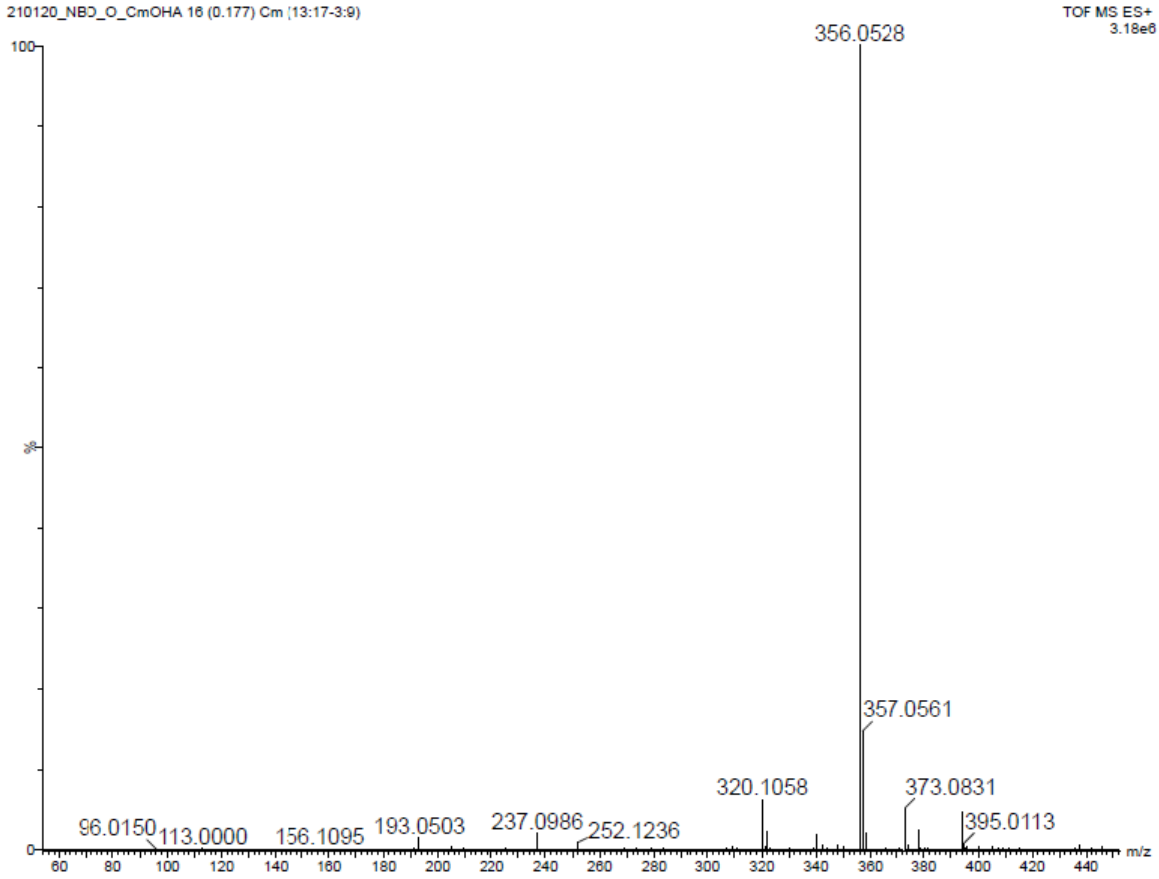


Figure S14. HRMS spectrum of **NBD-O-CmCH$_2$OH**

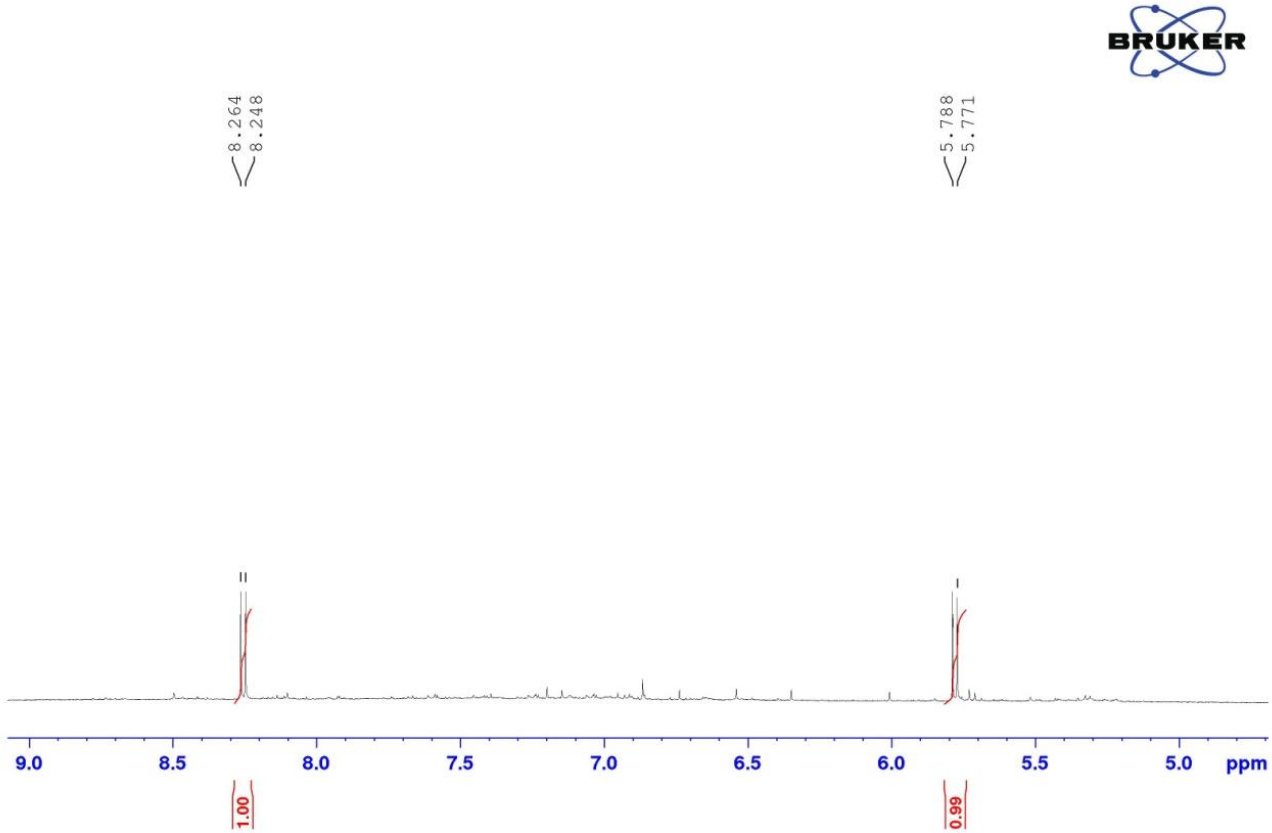


Figure S15. ^{1}H NMR spectrum of **NBD-SH**

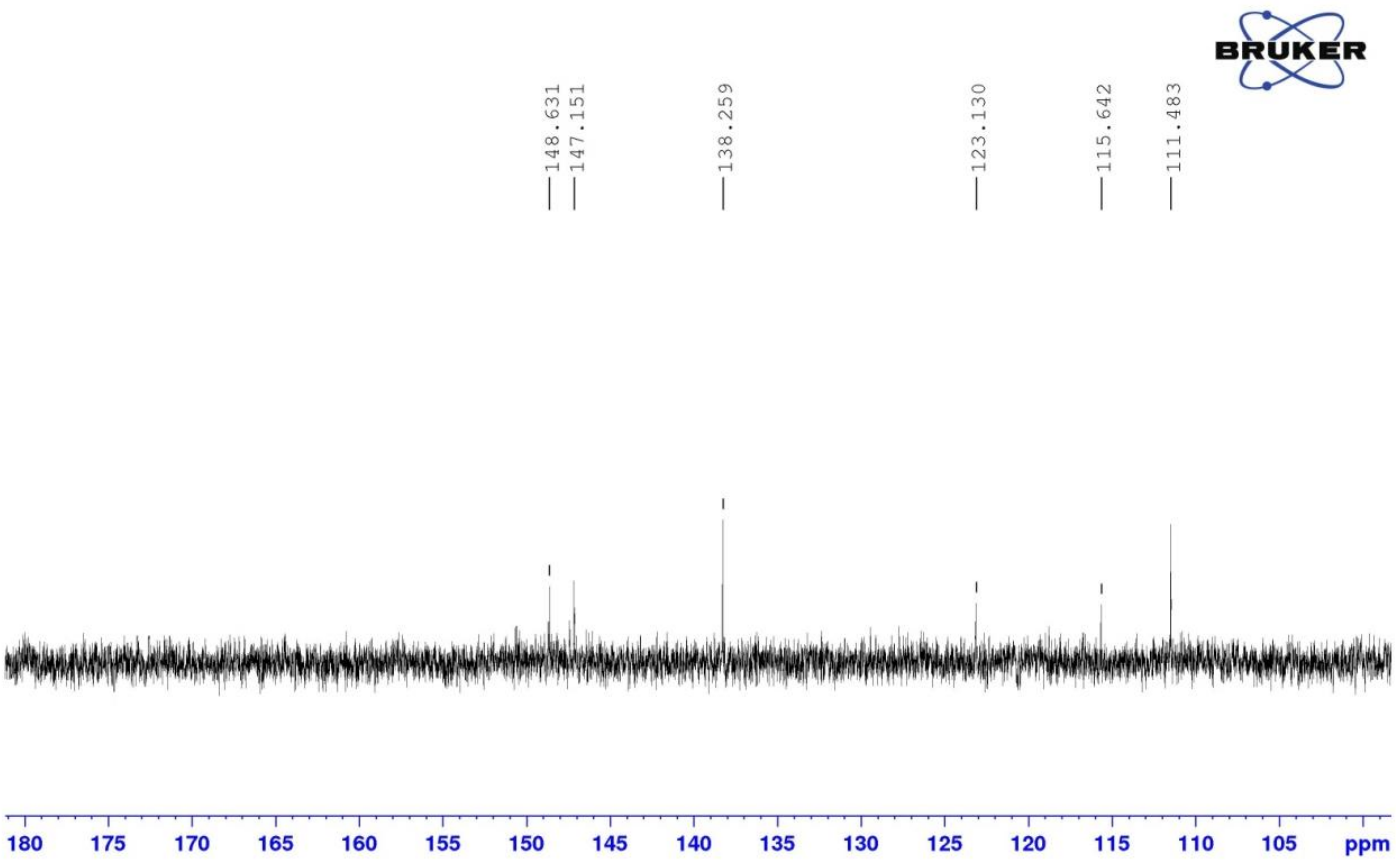


Figure S16. ^{13}C NMR spectrum of **NBD-SH**

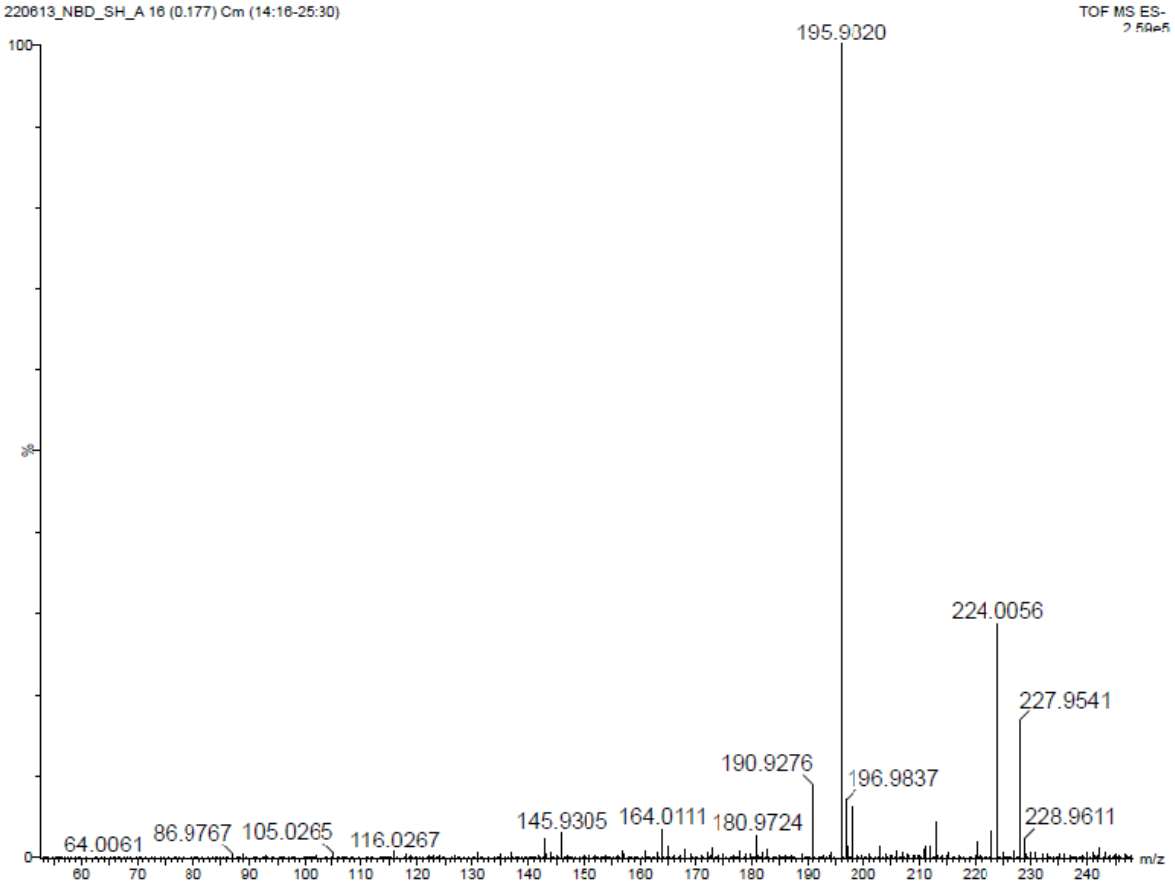


Figure S17. HRMS spectrum of **NBD-SH**

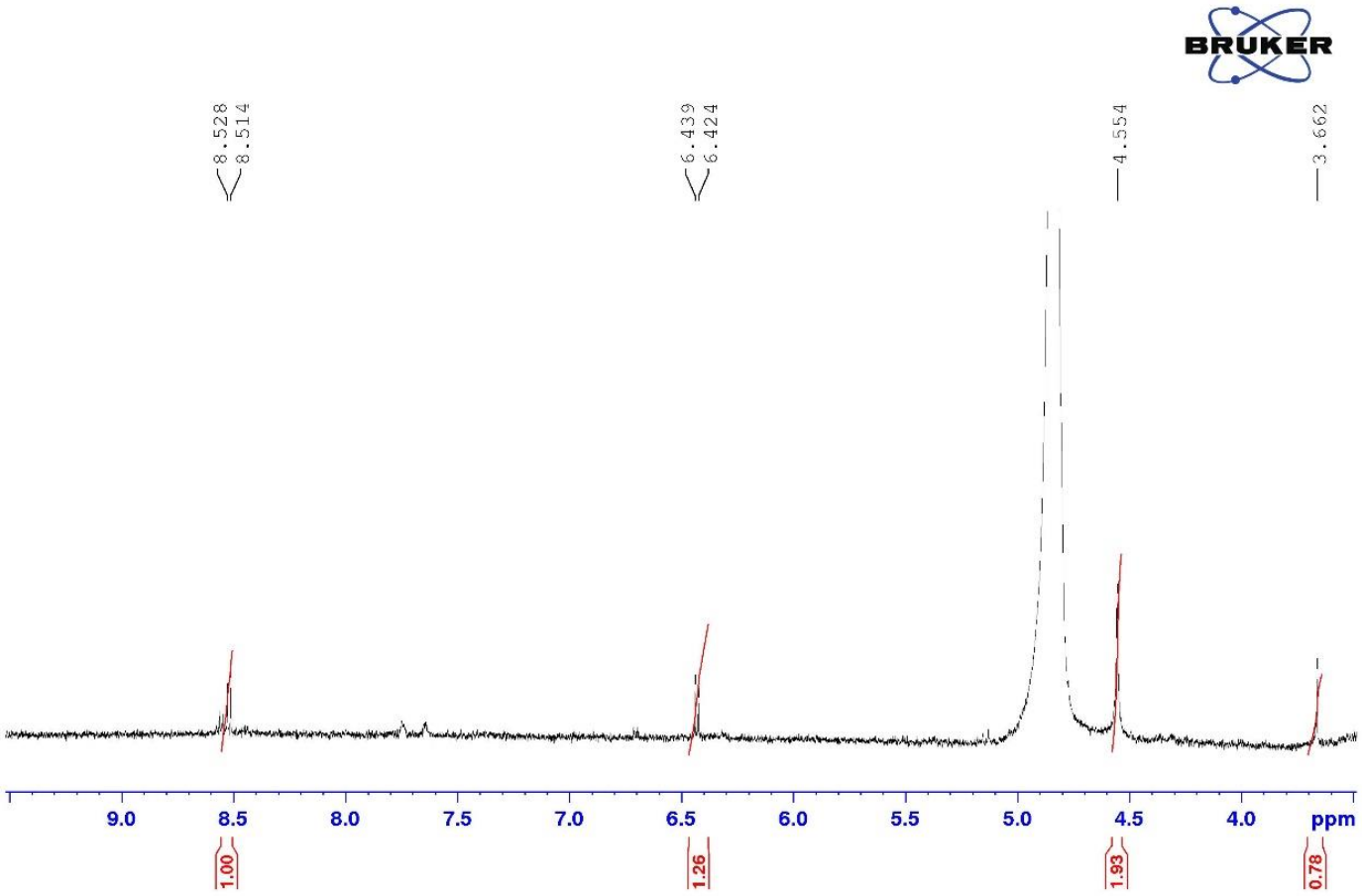


Figure S18. ^{1}H NMR spectrum of **NBD-Cys**

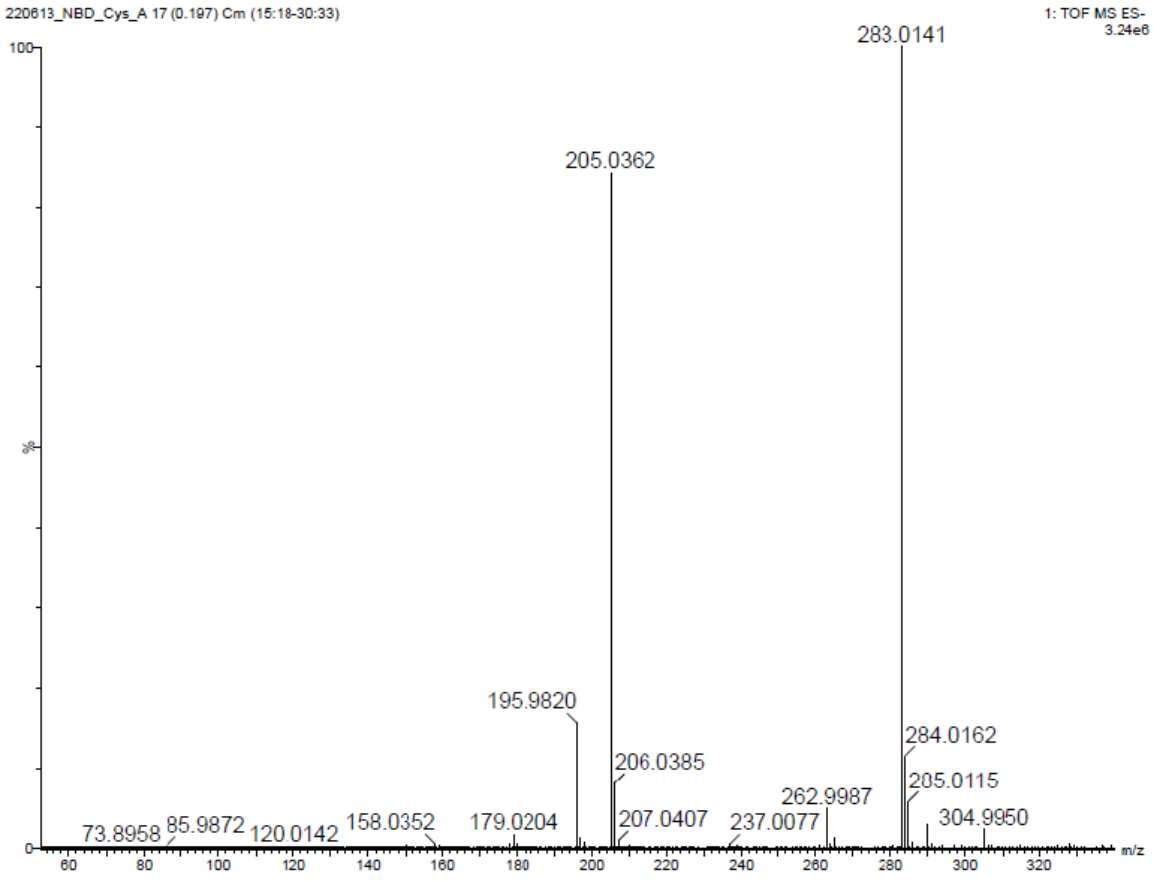


Figure S19. HRMS spectrum of **NBD-Cys**

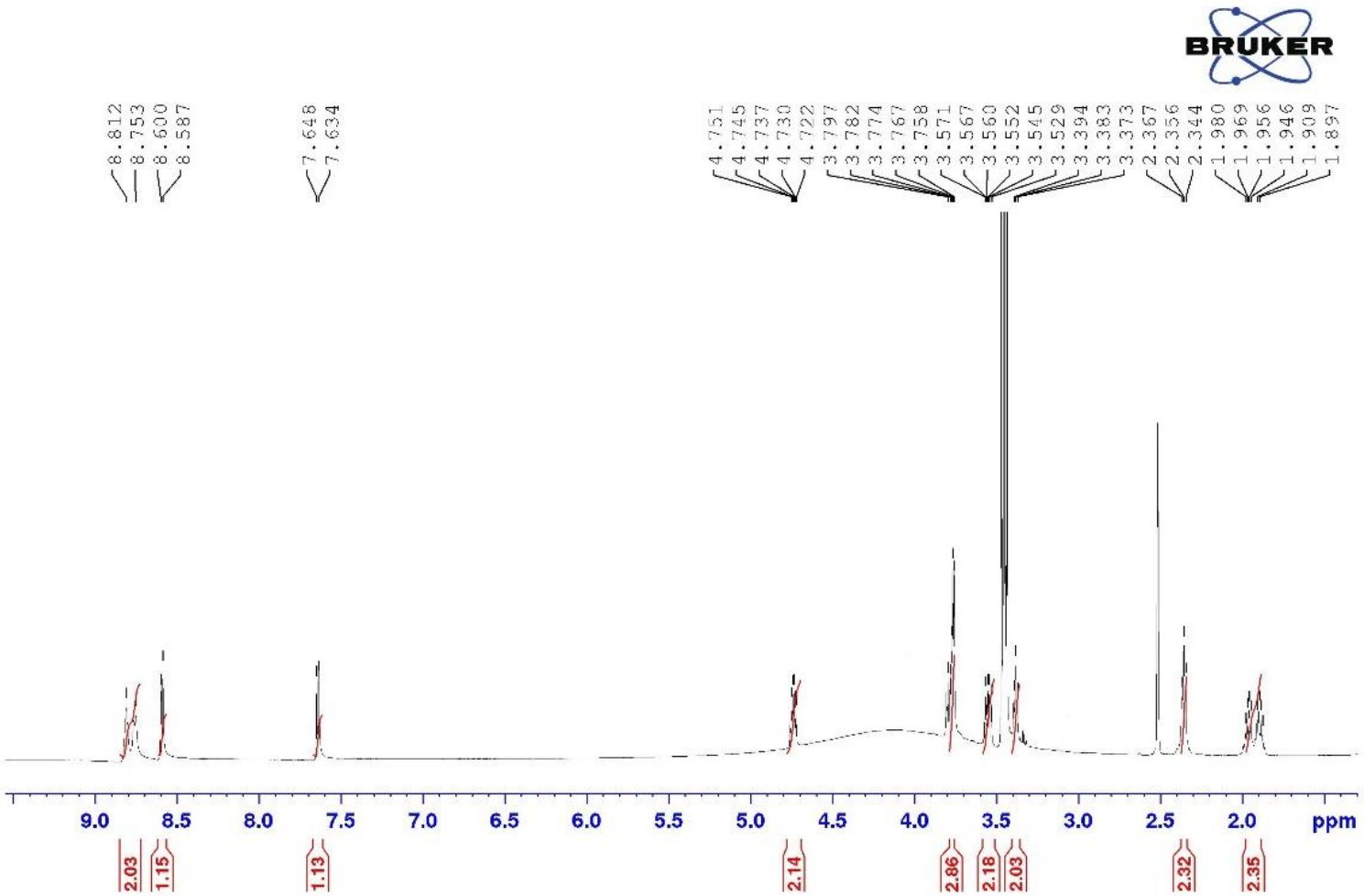


Figure S20. ^{1}H NMR spectrum of **NBD-GSH**

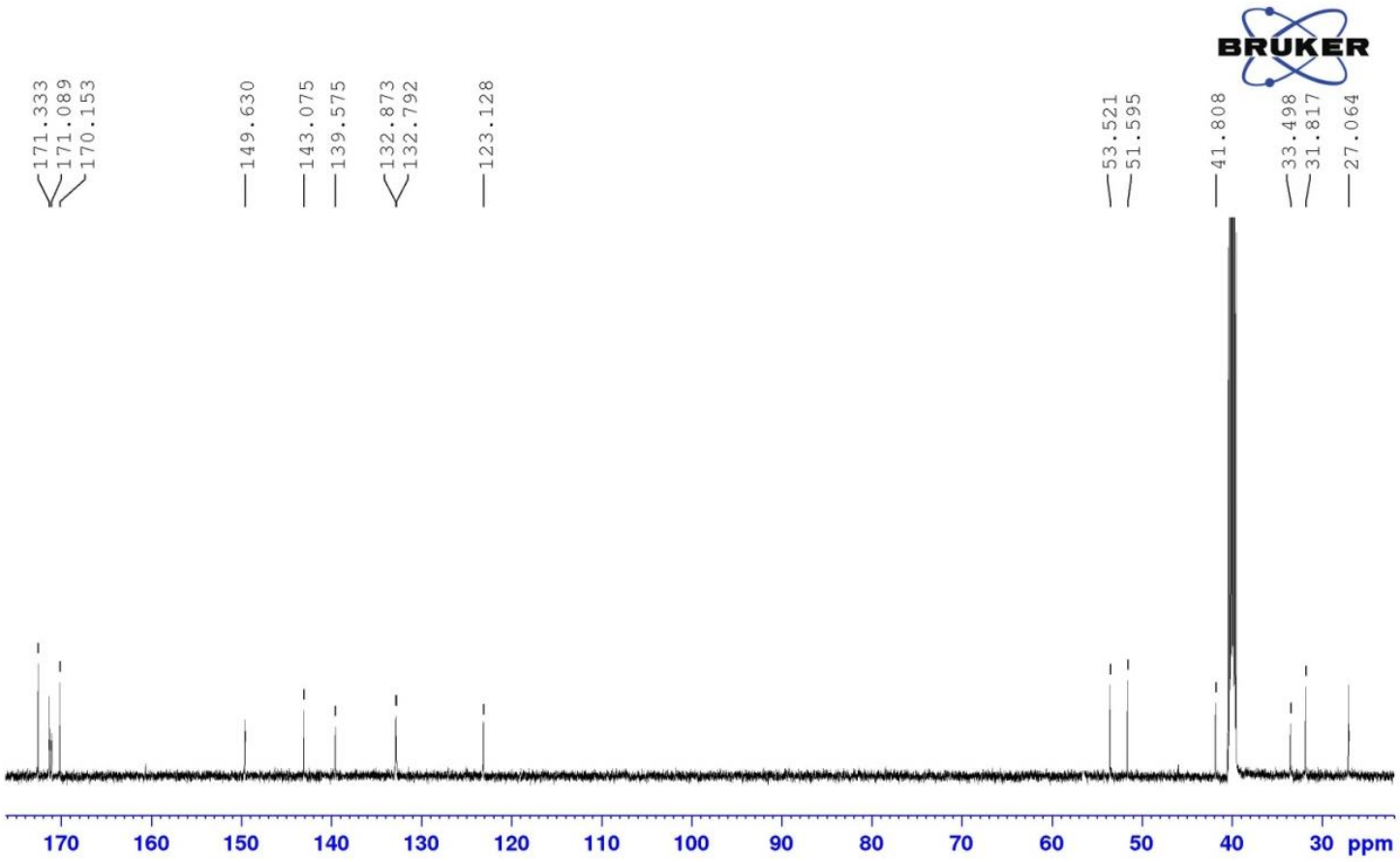


Figure S21. ^{13}C NMR spectrum of **NBD-GSH**

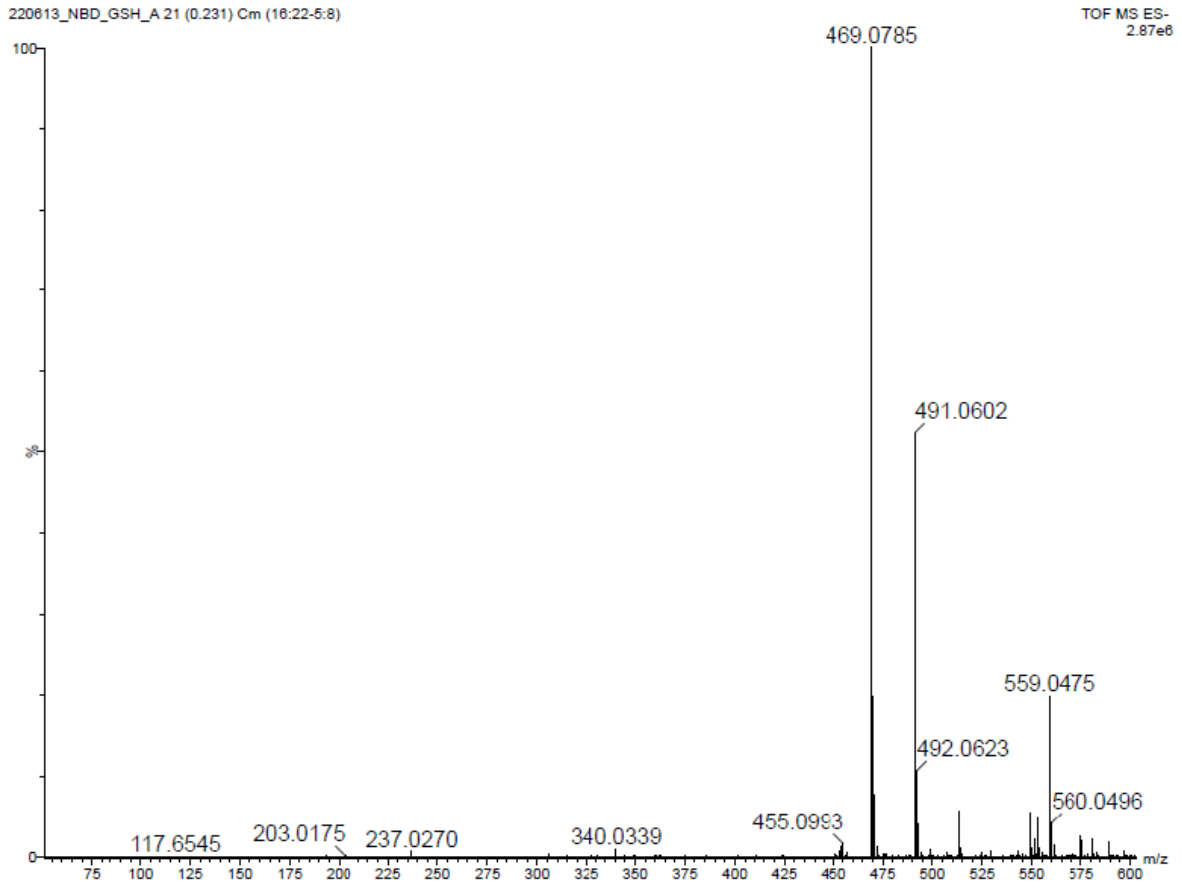


Figure S22. HRMS spectra of **NBD-GSH**

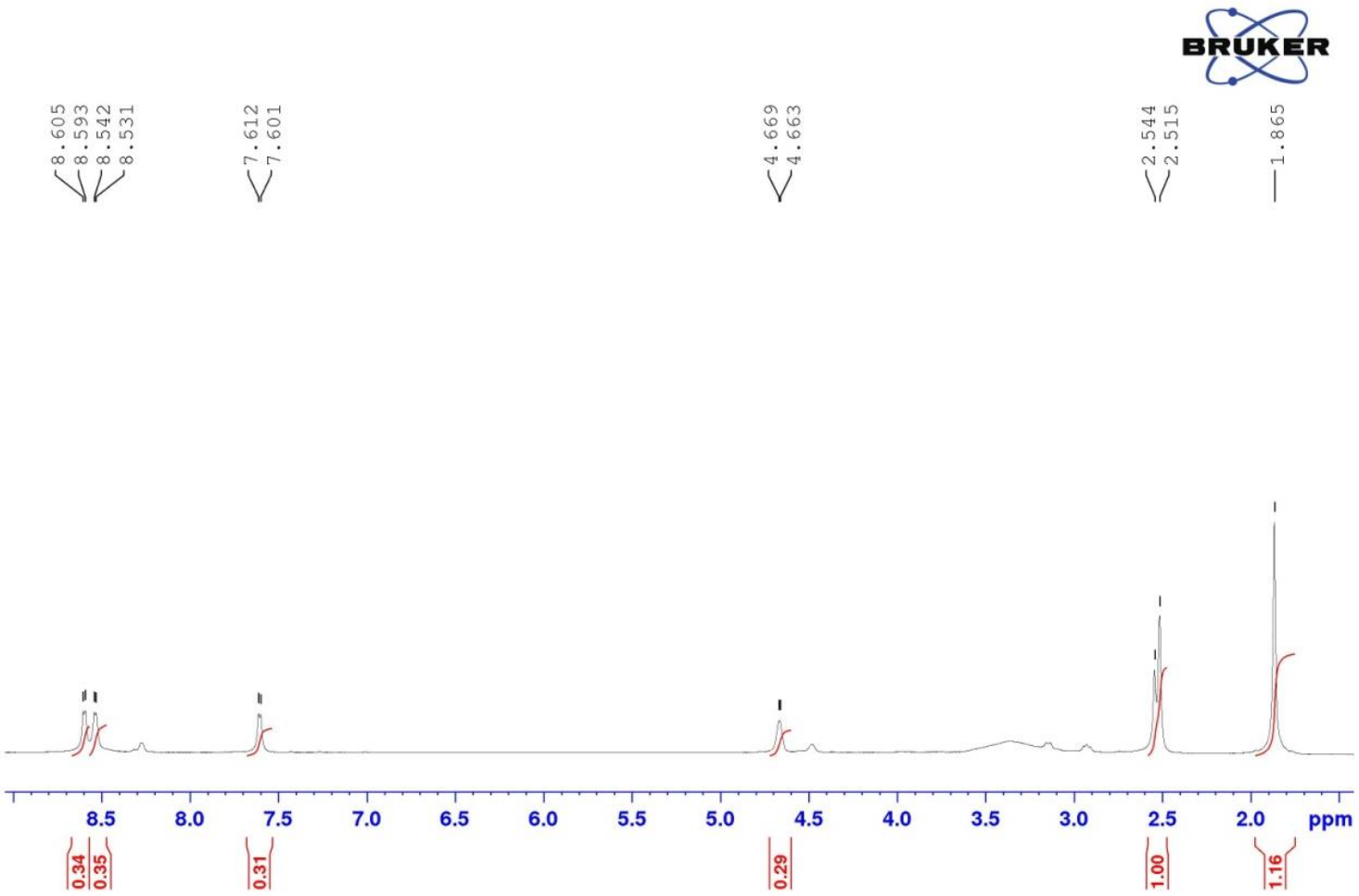


Figure S23. [1]HNMR spectrum of **NBD-NAC**

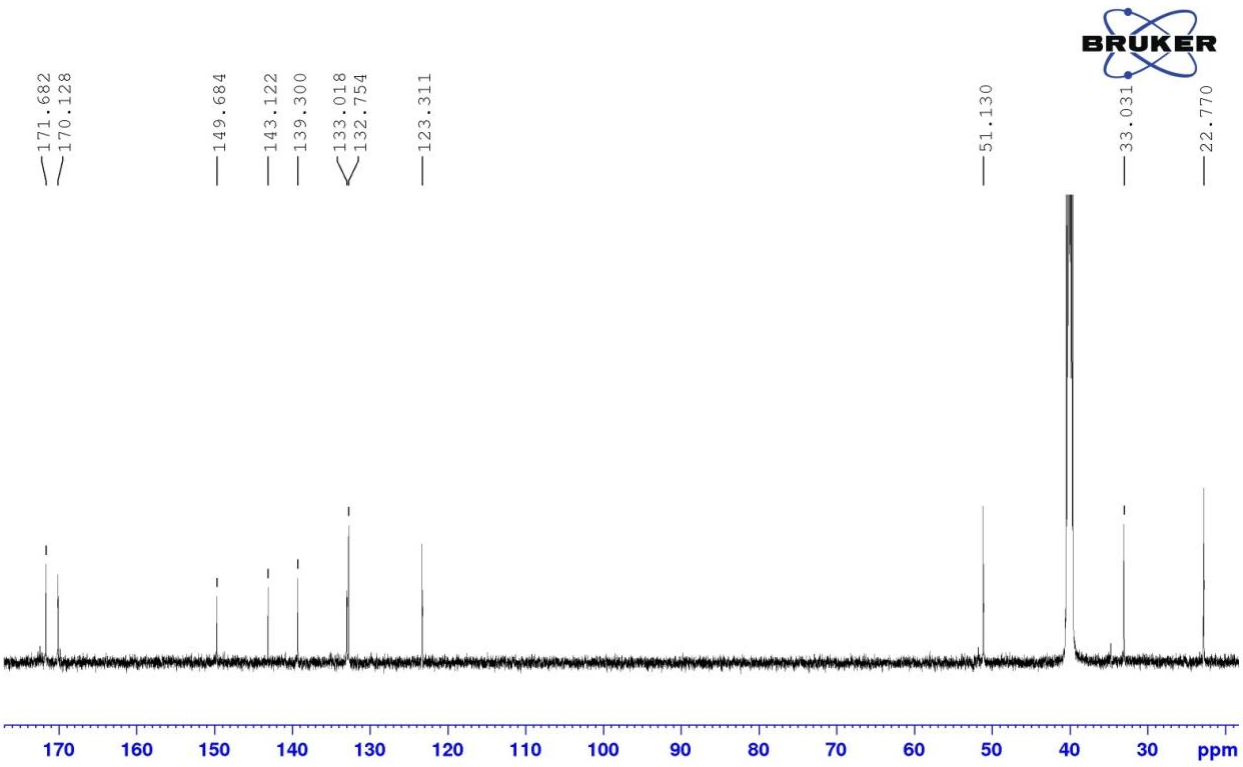


Figure S24. ^{13}C NMR spectrum of **NBD-NAC**

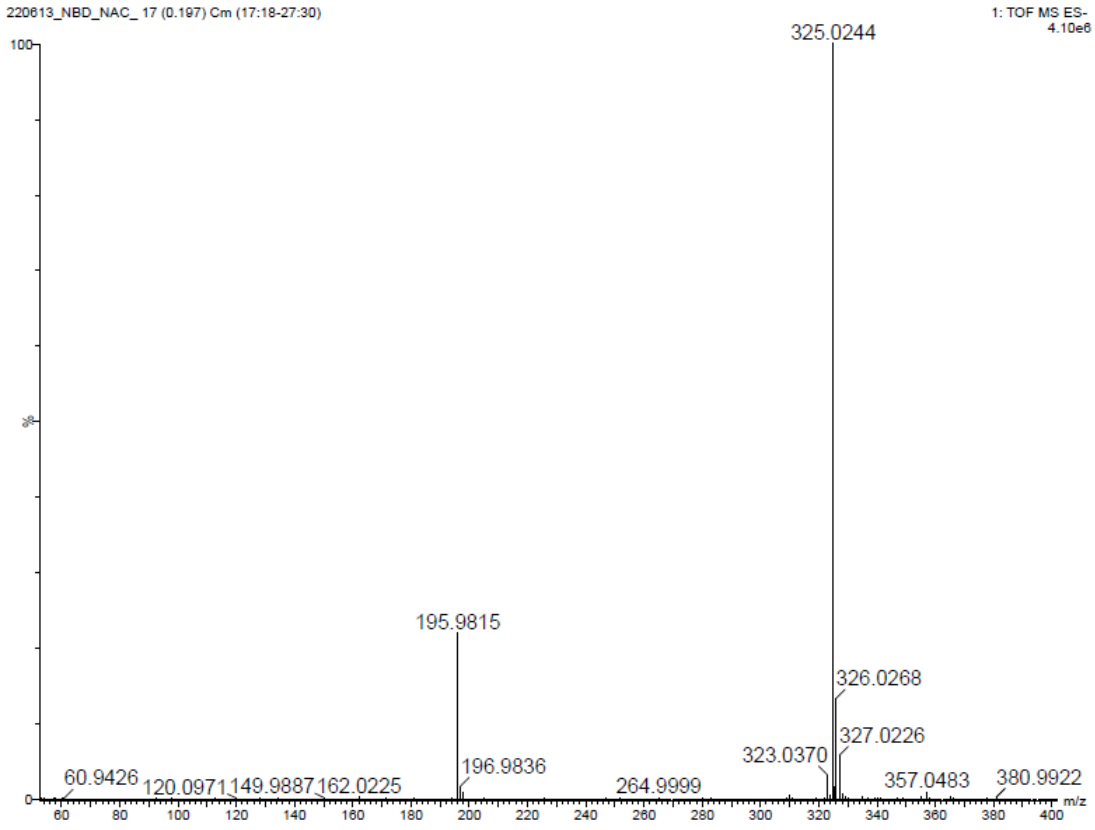


Figure S25. HRMS spectrum of **NBD-NAC**

[S1] Zhang, Y.; Wang, J.; Yue, J.; Chao, J.; Huo, F.; Yin, C. A new strategy for the fluorescence discrimination of Cys/Hcy and GSH/H_2S simultaneously colorimetric detection for H_2S. *Spectrochim. Acta - A: Mol. Biomol.* **2020**, *227*, 117537.

[S2] Chen, W.; Luo, H.; Liu, X.; Foley J. W.; Song, X. Broadly applicable Strategy for the fluorescence based detection and differentiation of glutathione and cysteine/homocysteine: demonstration in vitro and in vivo. *Anal. Chem.* **2016**, *88*, 3638-3646.

[S3] Zhu, H.; Liu, C.; Yuan, R.; Wang, R.; Zhang, H.; Li, Z.; Jia, P.; Zhu, B.; Sheng, W. A simple highly specific fluorescent probe for simultaneous discrimination of cysteine/ homocysteine and glutathione/hydrogen sulfide in living cells and zebrafish using two separated fluorescence channels under single wavelength excitation. *Analyst* **2019,** *144*, 4258

[S4] Liu, T.; Li, S.; Zhang, X.; Wang, J.; Deng, Y.; Sun, X.; Xing, Z.; Wu, R. A facile probe for fluorescence turn-on and simultaneous naked-eyes discrimination of H_2S and biothiols (Cys and GSH) and its application. *J Fluoresc.* **2022**, *32*, 175-188.

[S5] Hao,Y., Zhang, Y.; Zhu, D.; Luo, L.; Chen, L.; Tang, Z.; Zeng, R.; Xu, M.; Chen, S. Dual-emission fluorescent probe for discriminative sensing of biothiols. *Chinese J. Anal. Chem.* **2022**, *50*, 100153.

[S6] Yang, Y.; Xu, Z.; Han, L.; Fan, Y.; Qing, M.; Li, N; Luo, H. A simple fluorescent probe with two different fluorescence signals for rapid sequence distinguishing of Cys/Hcy/GSH and intracellular imaging. *Dyes and Pigments* **2021**, *184*, 108722.

[S7] Xu, S.; Zhou, J.; Dong, X.; Zhao, W.; Zhu, Q. Fluorescent probe for sensitive discrimination of Hcy and Cys/GSH in living cells via dual-emission. *Anal. Chim. Acta.* **2019**, *1074*, 123-130.

[S8] Niu, H.; Duan, Y.; Zhang ,Y.; Hua, X.; Xu, C.; Li, Z.; Ma, J.; Qin, F.; Zhai, Y.; Ye, Y.; Zhao, Y. A bifunctional fluorescent probe based on PET & ICT for simultaneously recognizing Cys and H_2S in living cells. *J. Photochem. Photobiol. B, Biol.* **2022**, *230*, 112441.