

Synthesis and biological activity of thia-Michael-type adducts between naphthoquinones and *N*-acetyl-*L*-cysteine

Gabriele Micheletti^{1*}, Carla Boga¹, Chiara Zalambani², Giovanna Farruggia², Erika Esposito³, Jessica Fiori³, Nicola Rizzardi², Paola Taddei⁴, Michele Di Foggia⁴, and Natalia Calonghi^{2*}

¹ Department of Industrial Chemistry 'Toso Montanari', Alma Mater Studiorum - Università di Bologna Viale Del Risorgimento 4, 40136 Bologna Italy; gabriele.micheletti@unibo.it (GM), carla.boga@unibo.it (CB).

² Department of Pharmacy and Biotechnology, University of Bologna, Bologna (Italy); natalia.calonghi@unibo.it (NC), zalachiara@hotmail.it (CZ), nicola.rizzardi2@unibo.it (NR).

³ Department of Chemistry 'G. Ciamician', Alma Mater Studiorum - Università di Bologna, via Selmi 2, 40126 Bologna (Italy); erika.esposito8@unibo.it (EE), jessica.fiori@unibo.it (JF).

⁴ Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum - Università di Bologna, via Irnerio 48, 40126 Bologna (Italy); paola.taddei@unibo.it (PT), michele.difoggia.2@unibo.it (MF).

¹ HNMR spectrum of compound 8	Page 1
¹³ CNMR spectrum of compound 8	Page 2
ESI-MS spectrum of compound 8	Page 3
¹ HNMR spectrum of compound 9	Page 4
¹³ CNMR spectrum of compound 9	Page 5
ESI-MS spectrum of compound 9	Page 6
¹ HNMR spectrum of compound 10	Page 7
¹³ CNMR spectrum of compound 10	Page 8
ESI-MS spectrum of compound 10	Page 9
¹ HNMR spectrum of compound 11	Page 10
¹³ CNMR spectrum of compound 11	Page 11
ESI-MS spectrum of compound 11	Page 12
¹ HNMR spectrum of compound 12	Page 13
¹³ CNMR spectrum of compound 12	Page 14
ESI-MS spectrum of compound 12	Page 15
Direct infusion mass spectra of the reaction mixtures with GSH of compound 8	Page 16
Direct infusion mass spectra of the reaction mixtures with GSH of compound 9	Page 17
Direct infusion mass spectra of the reaction mixtures with GSH of compound 11	Page 18

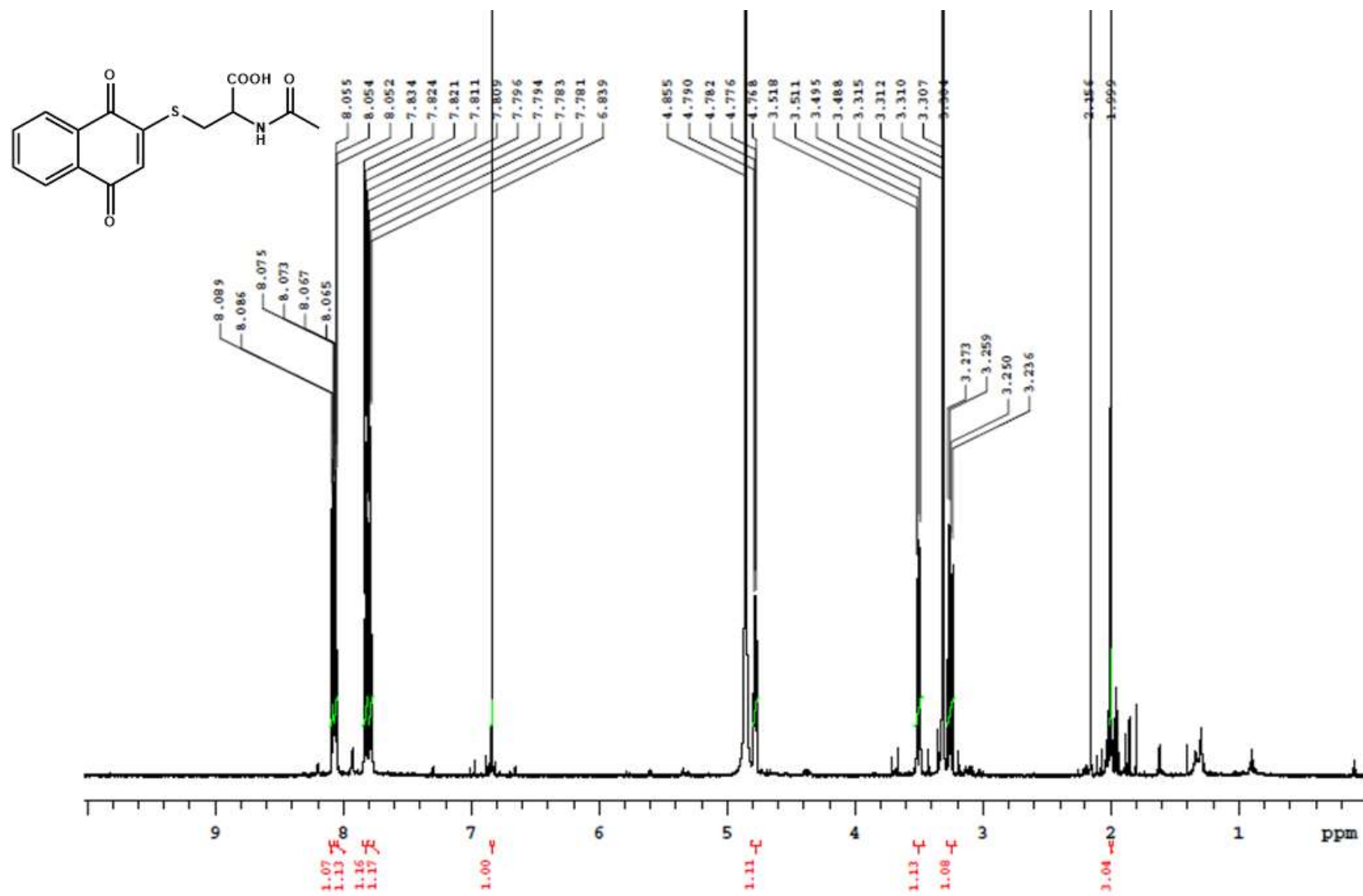


Figure S1. ¹H NMR spectrum in CD₃OD of compound 8.

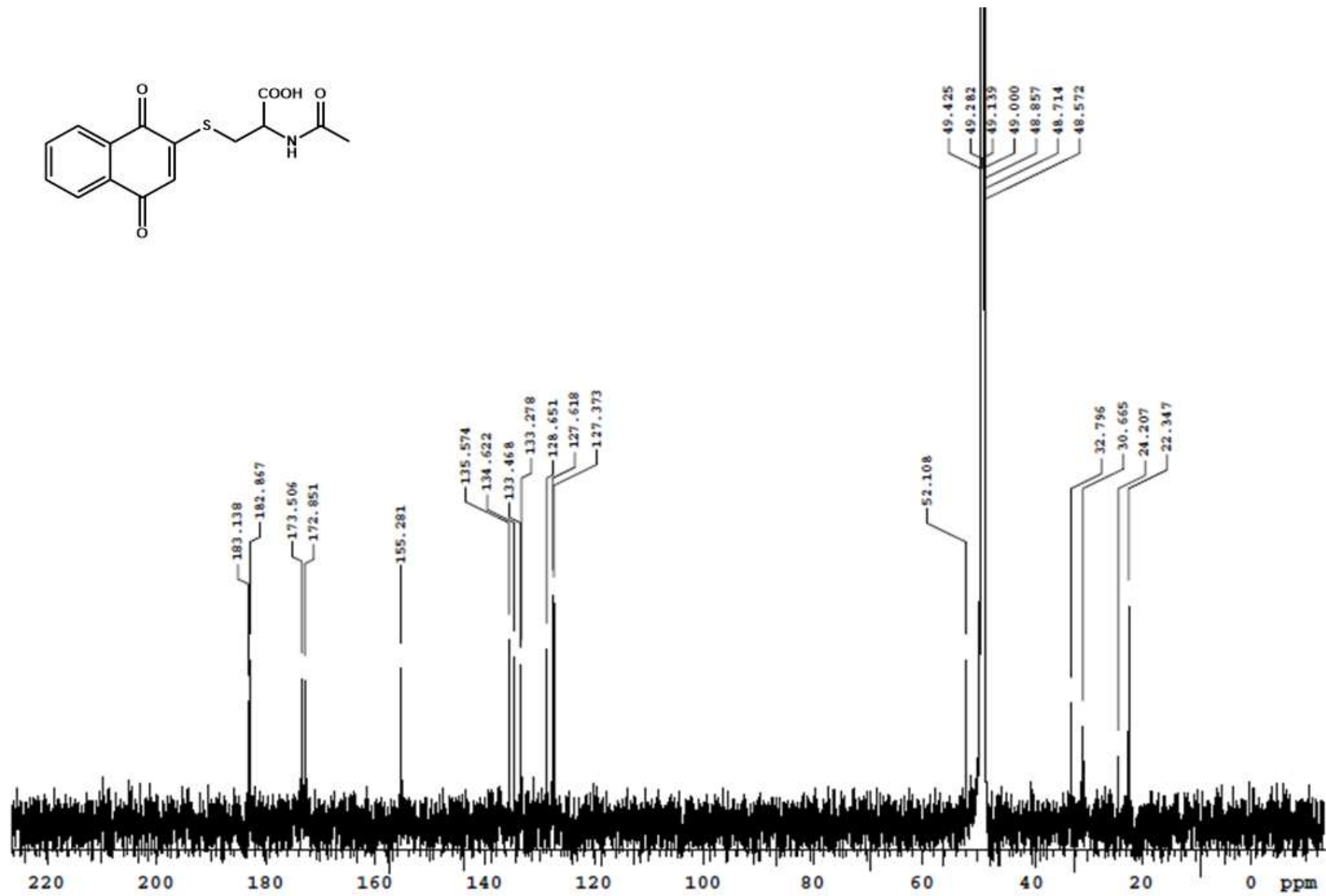


Figure S2. ¹³C NMR spectrum in CD₃OD of compound 8.

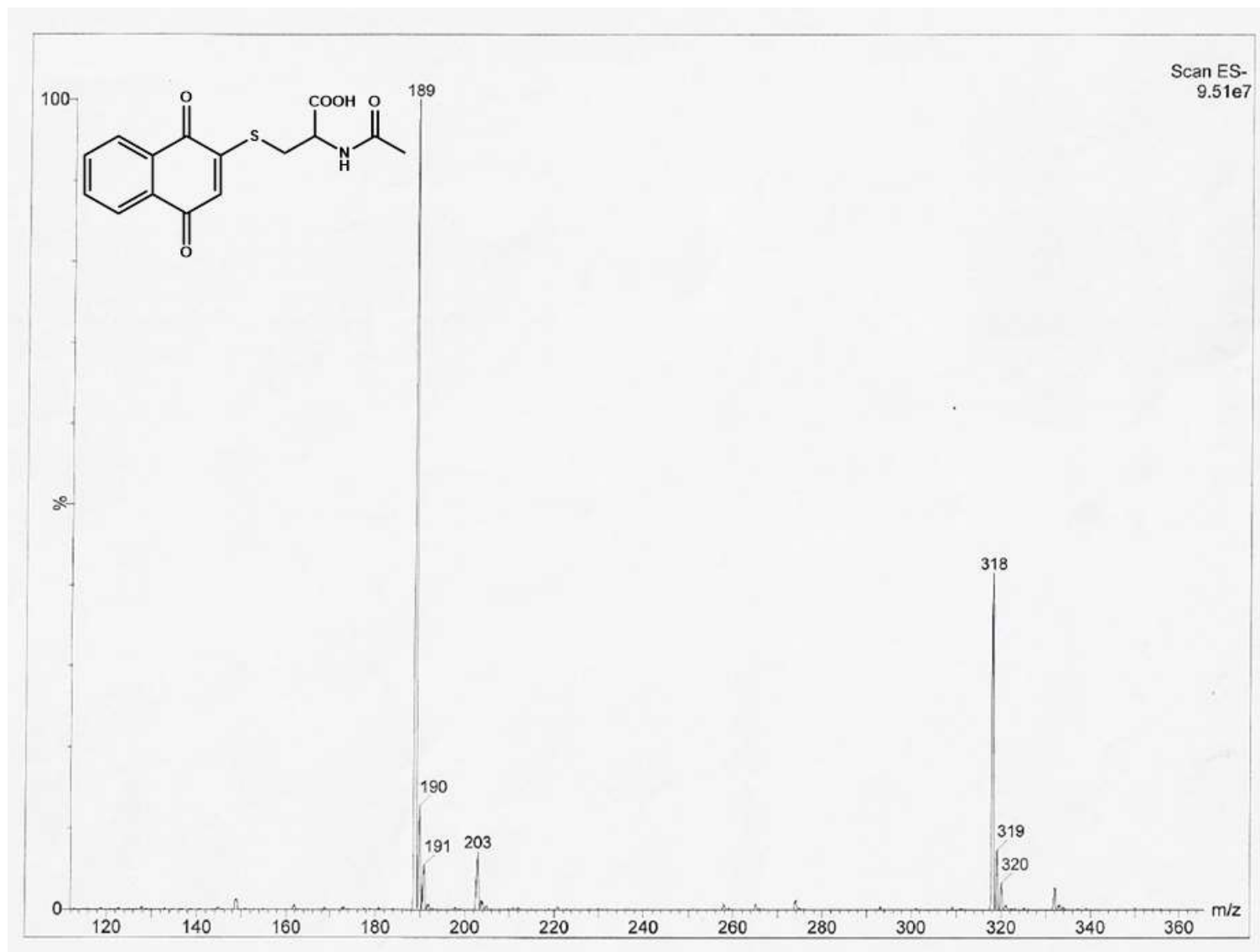


Figure S3. ESI-MS⁻ spectrum of compound 8.

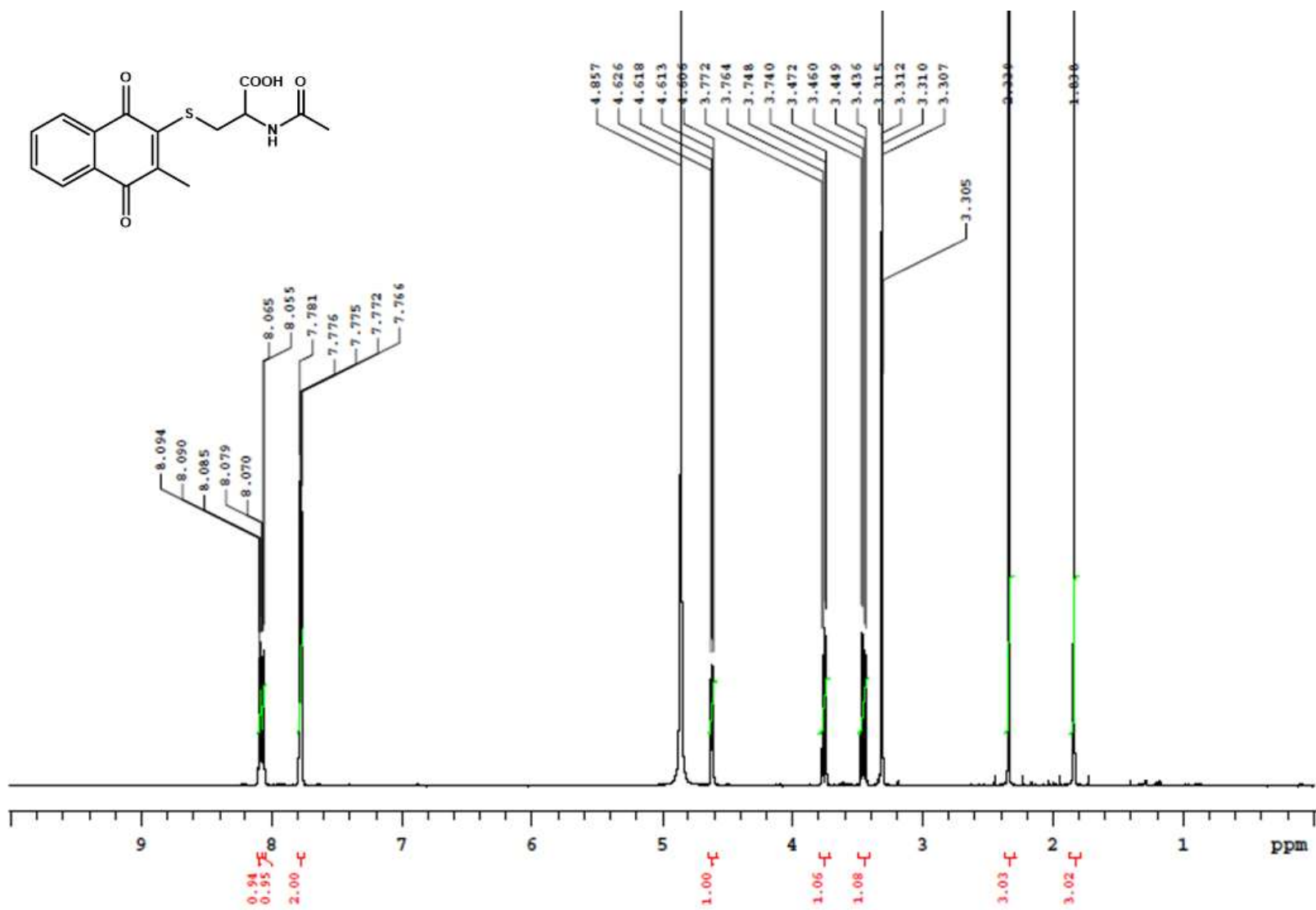


Figure S4. ¹H NMR spectrum in CD₃OD of compound 9.

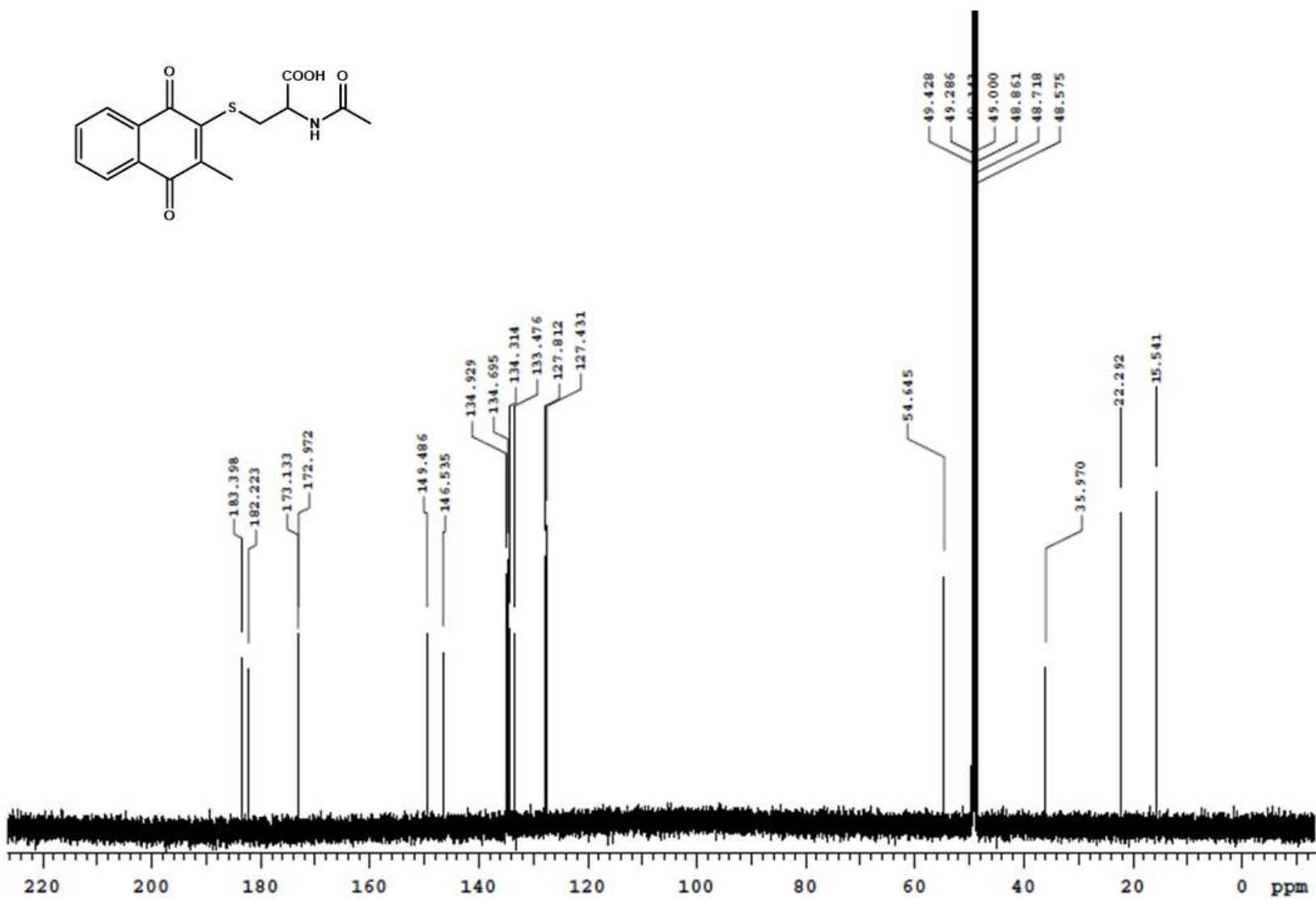


Figure S5. ¹³C NMR spectrum in CD₃OD of compound 9.

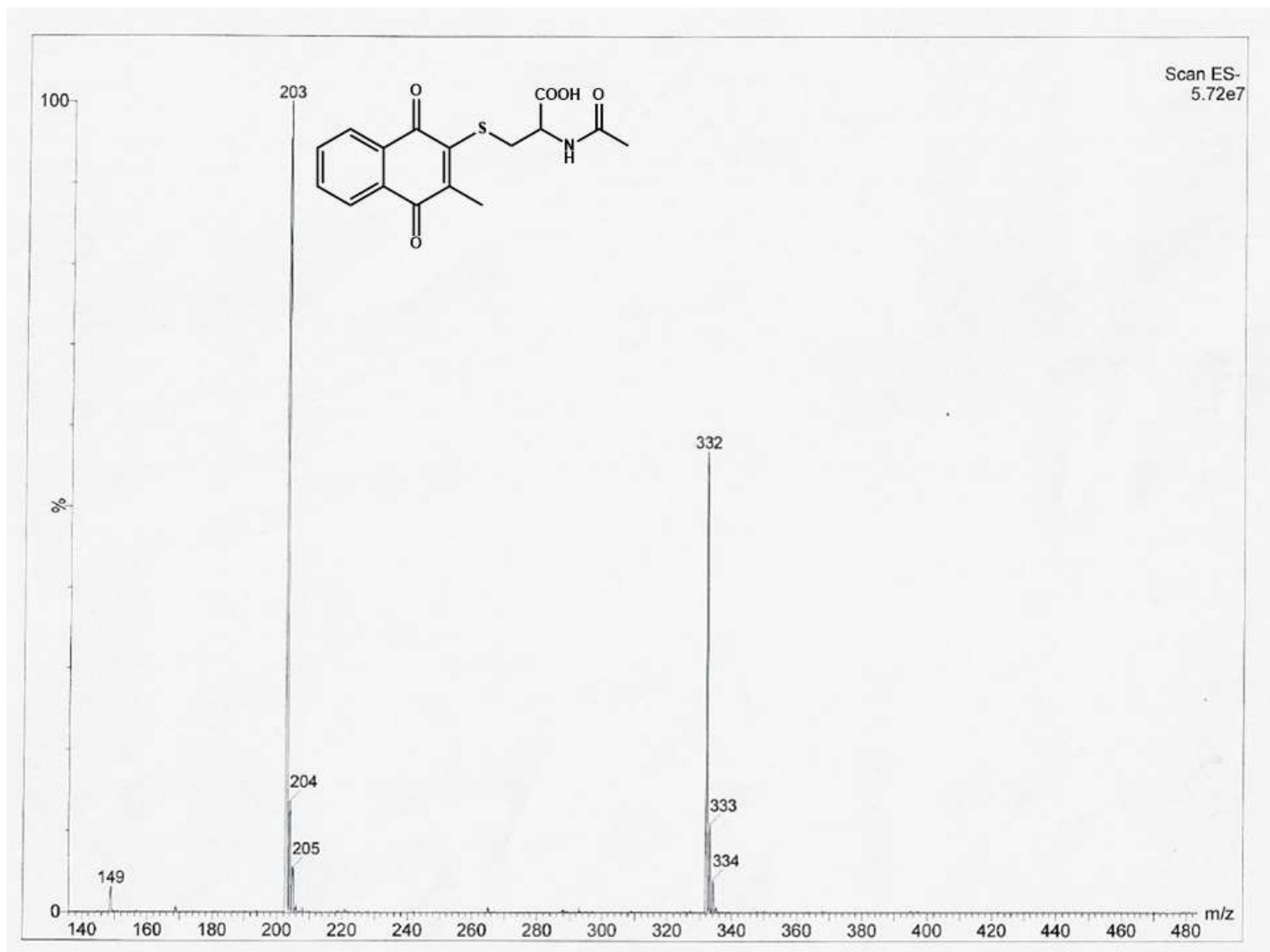


Figure S6. ESI-MS⁻ spectrum of compound 9.

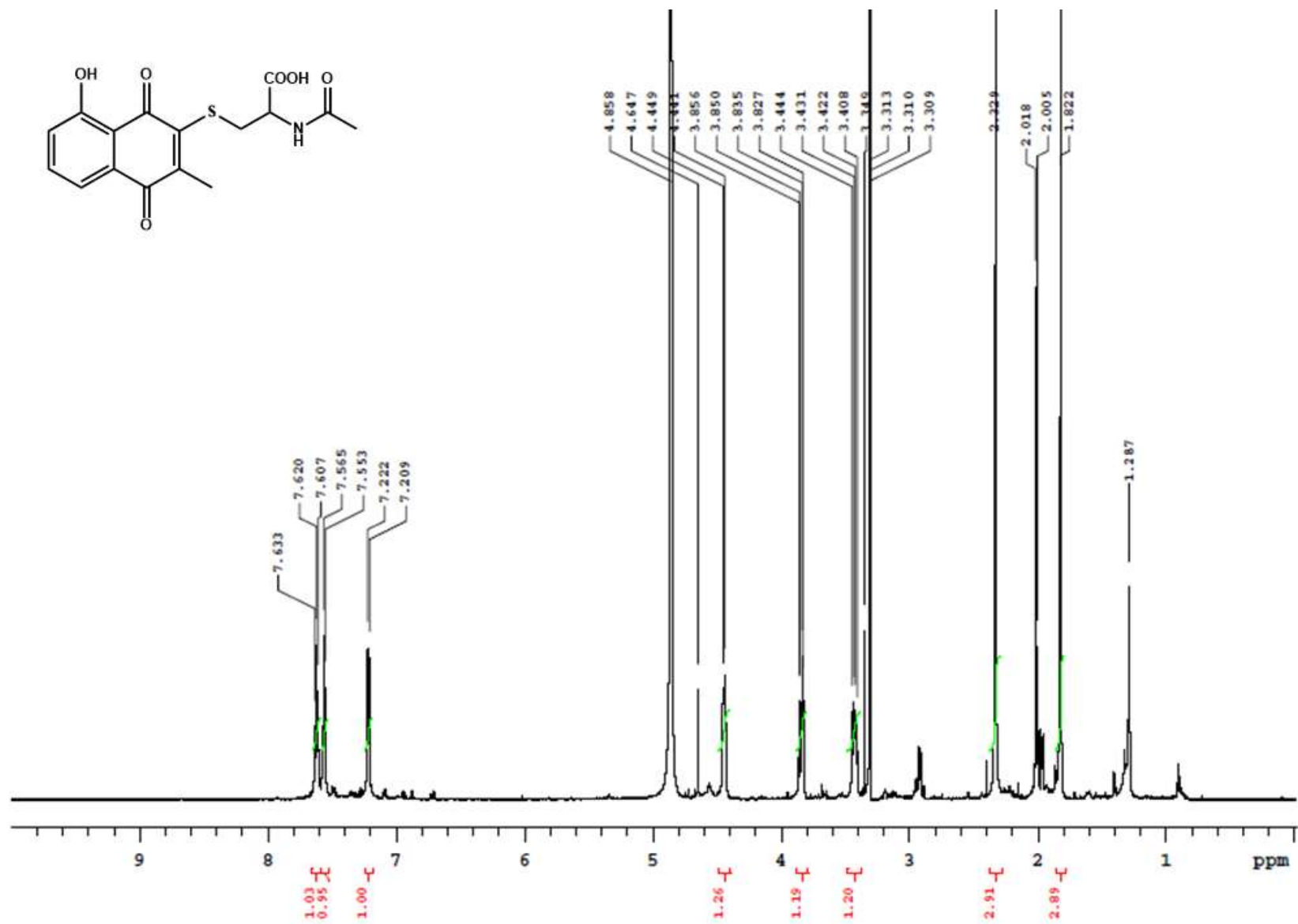


Figure S7. ¹H NMR spectrum in CD₃OD of compound 10.

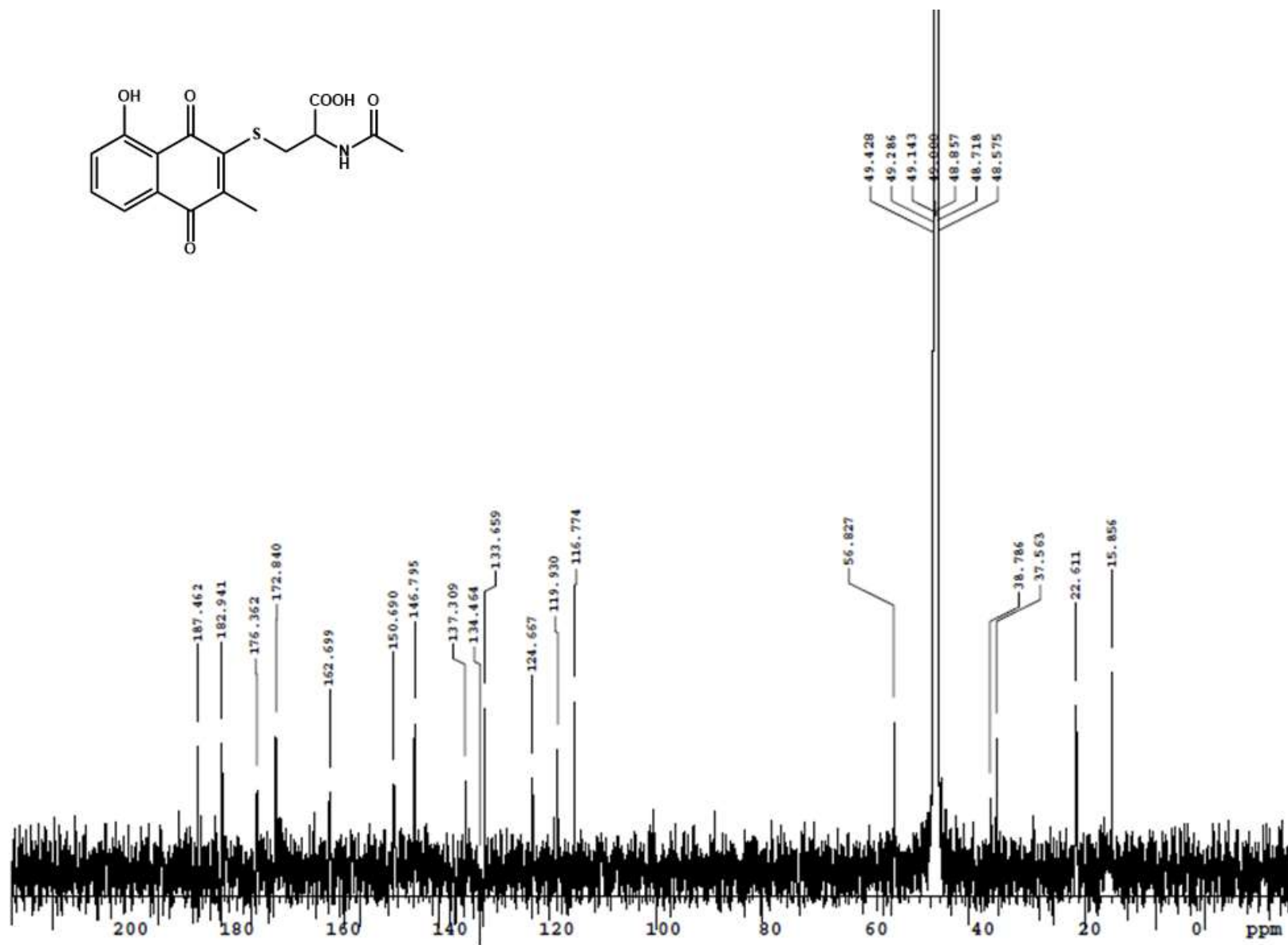


Figure S8. ¹³C NMR spectrum in CD₃OD of compound 10.

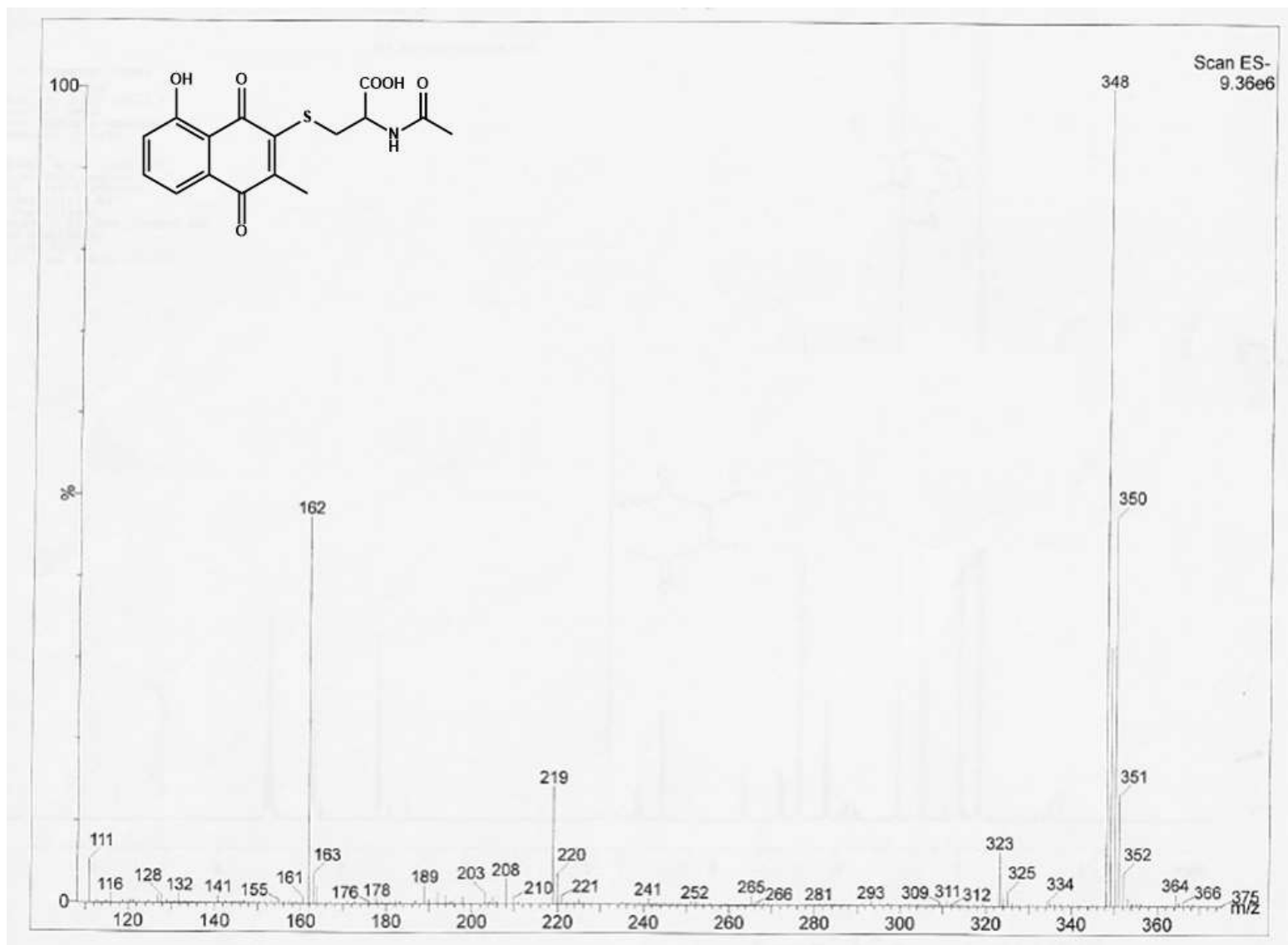


Figure S9. ESI-MS⁻ spectrum of compound 10.

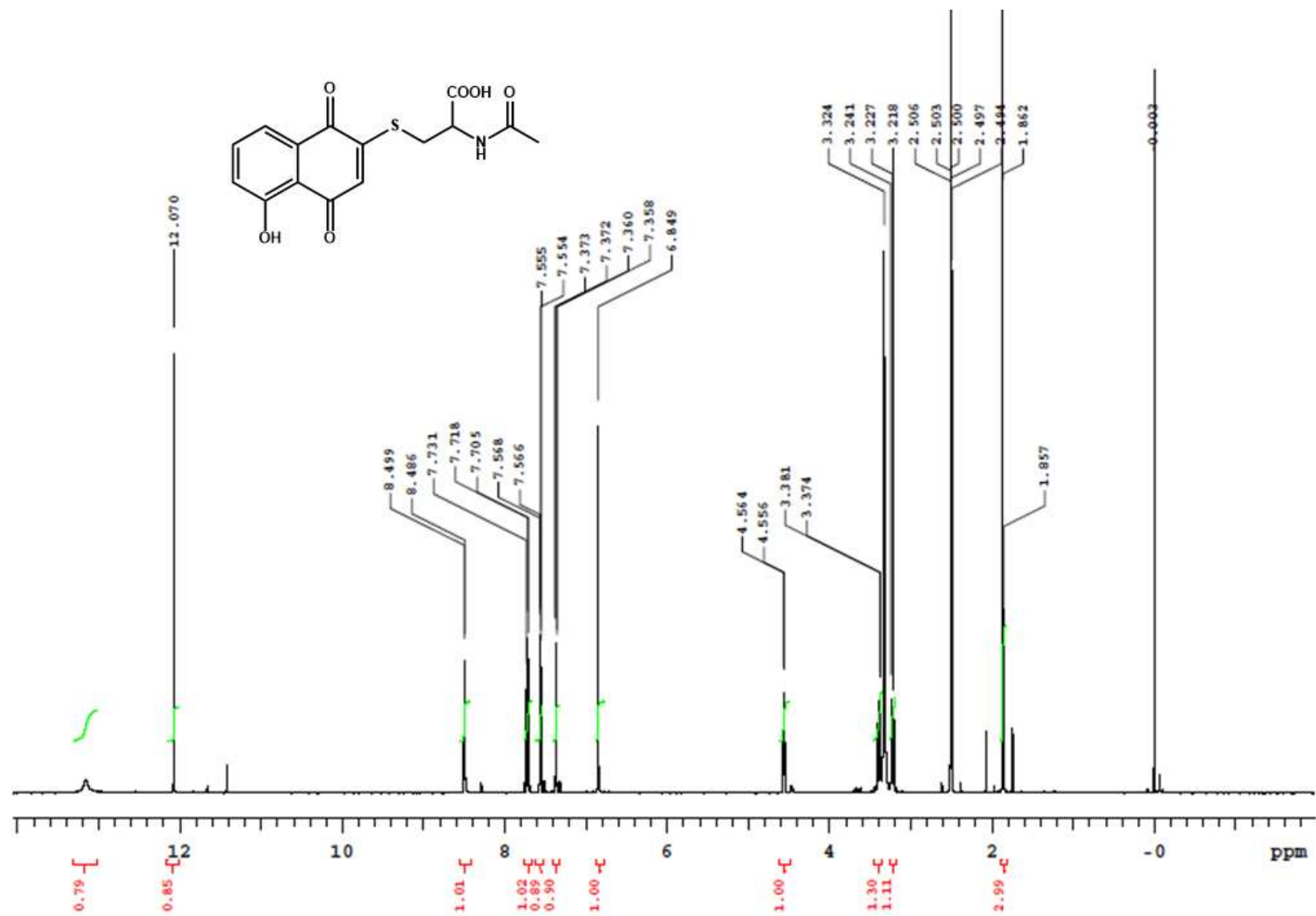


Figure S10. ¹H NMR spectrum in DMSO-d₆ of compound 11.

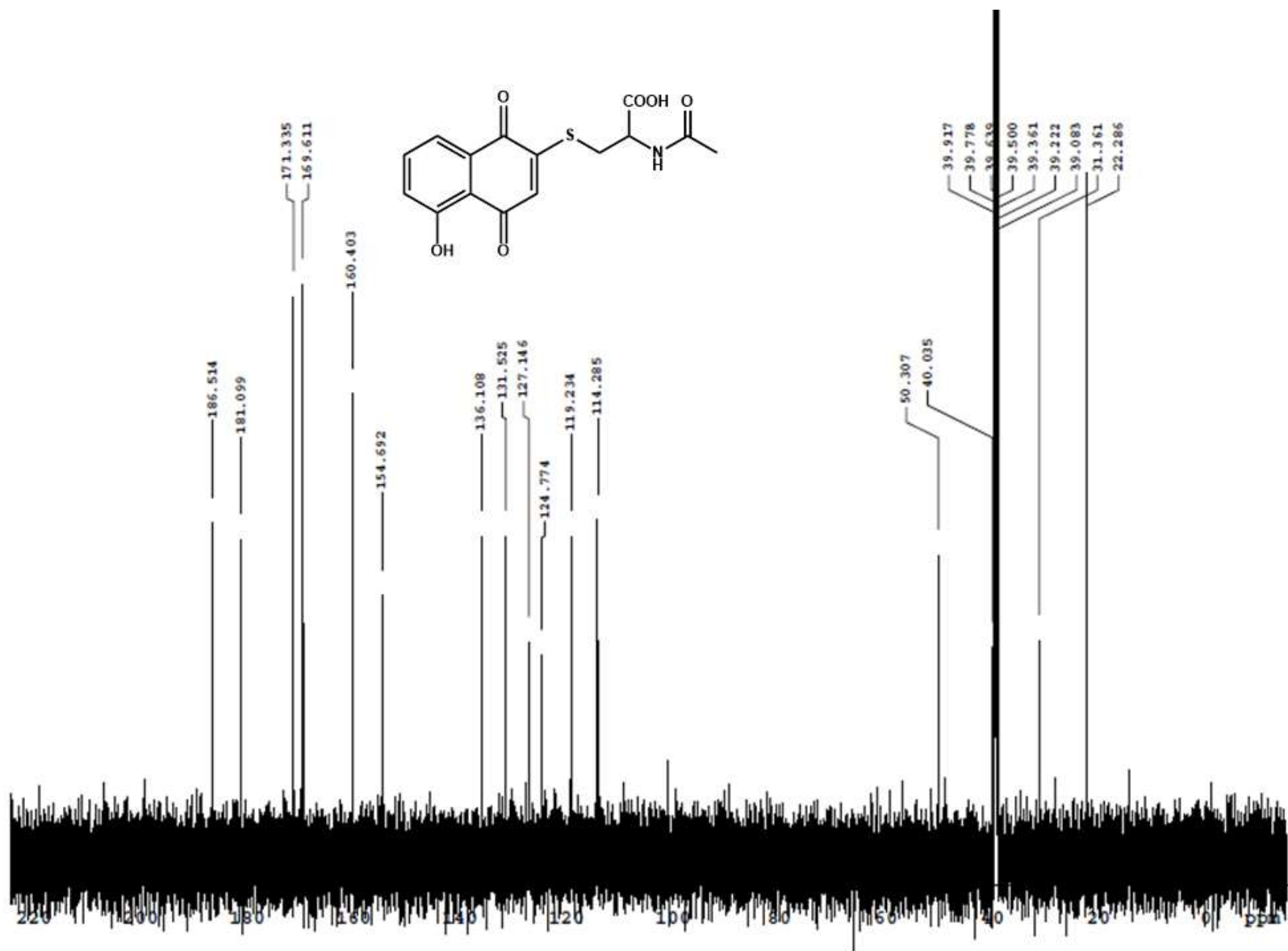


Figure S11 ¹³C NMR spectrum in DMSO-d₆ of compound 11.

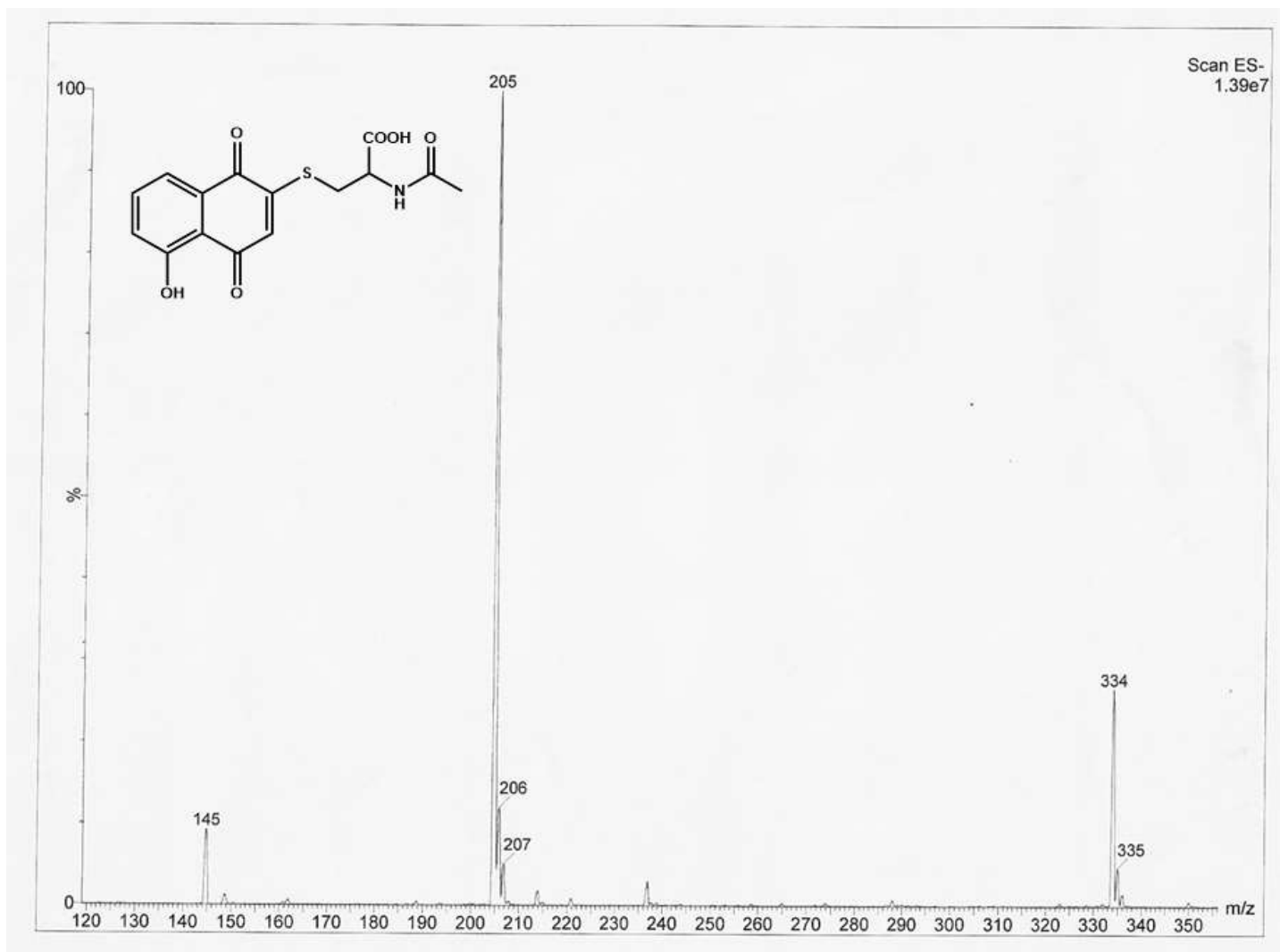


Figure S12. ESI-MS⁻ spectrum of compound 11.

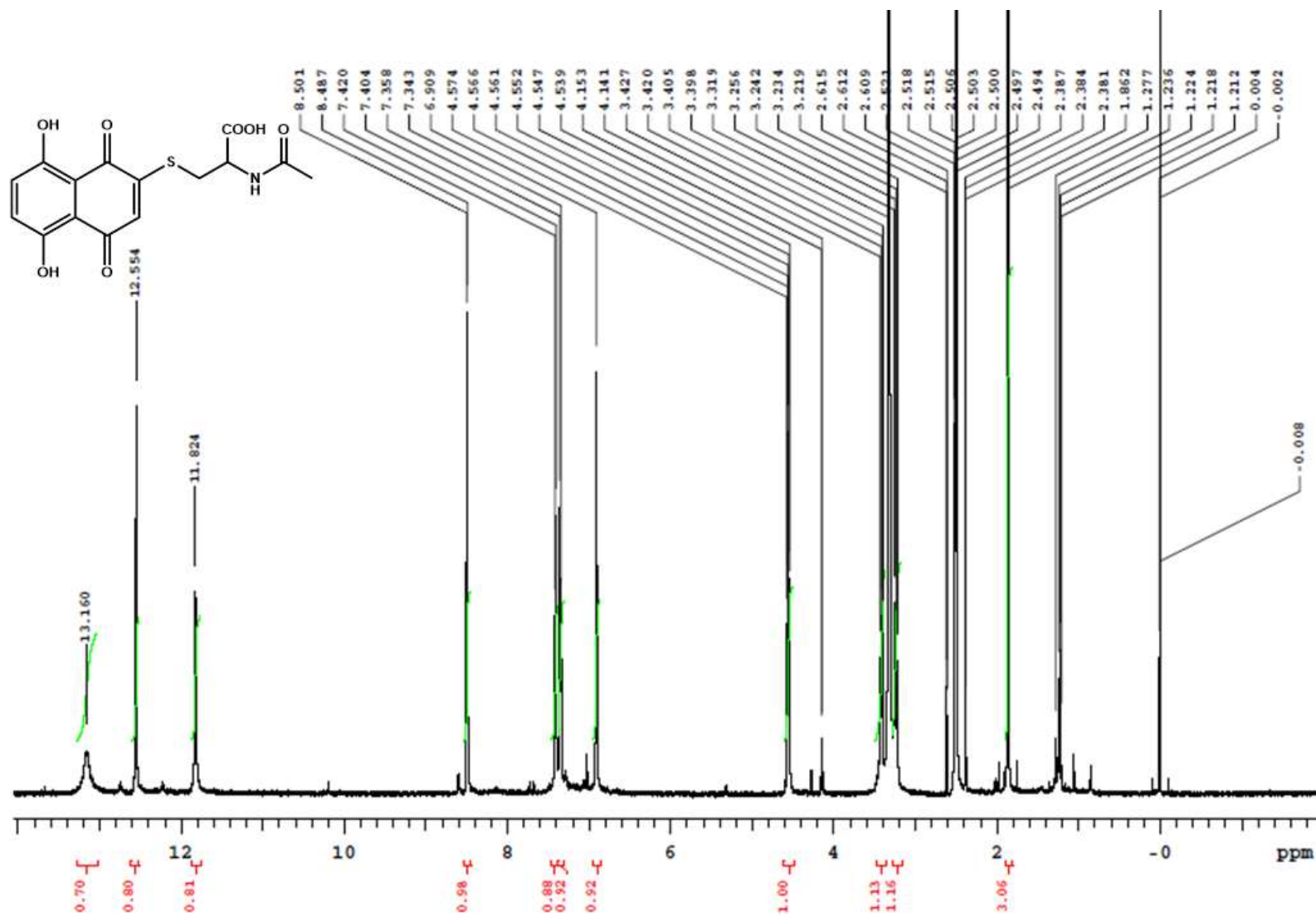


Figure S13. ¹H NMR spectrum in DMSO-d₆ of compound 12.

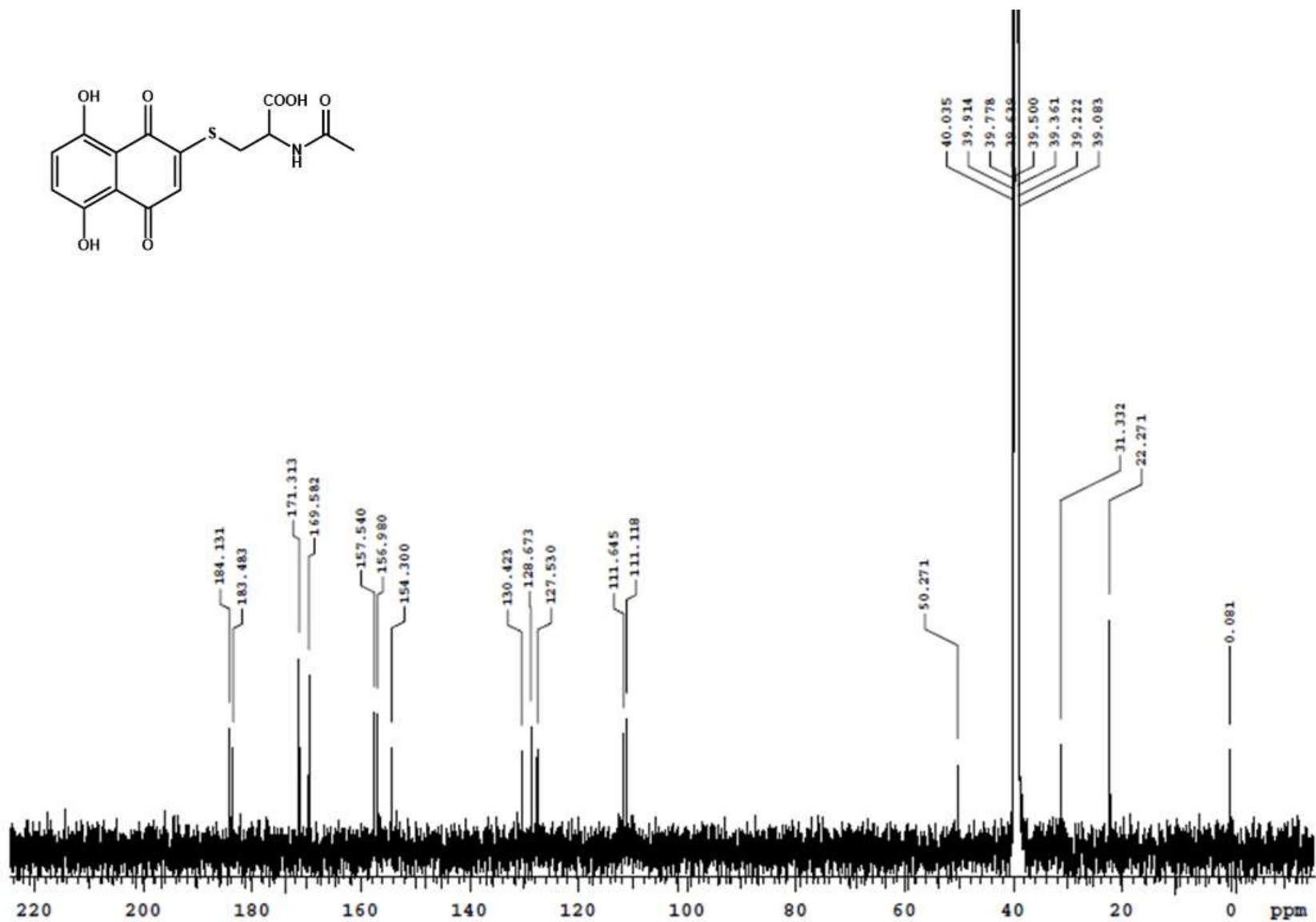


Figure S14. ¹³C NMR spectrum in DMSO-d₆ of compound 12.

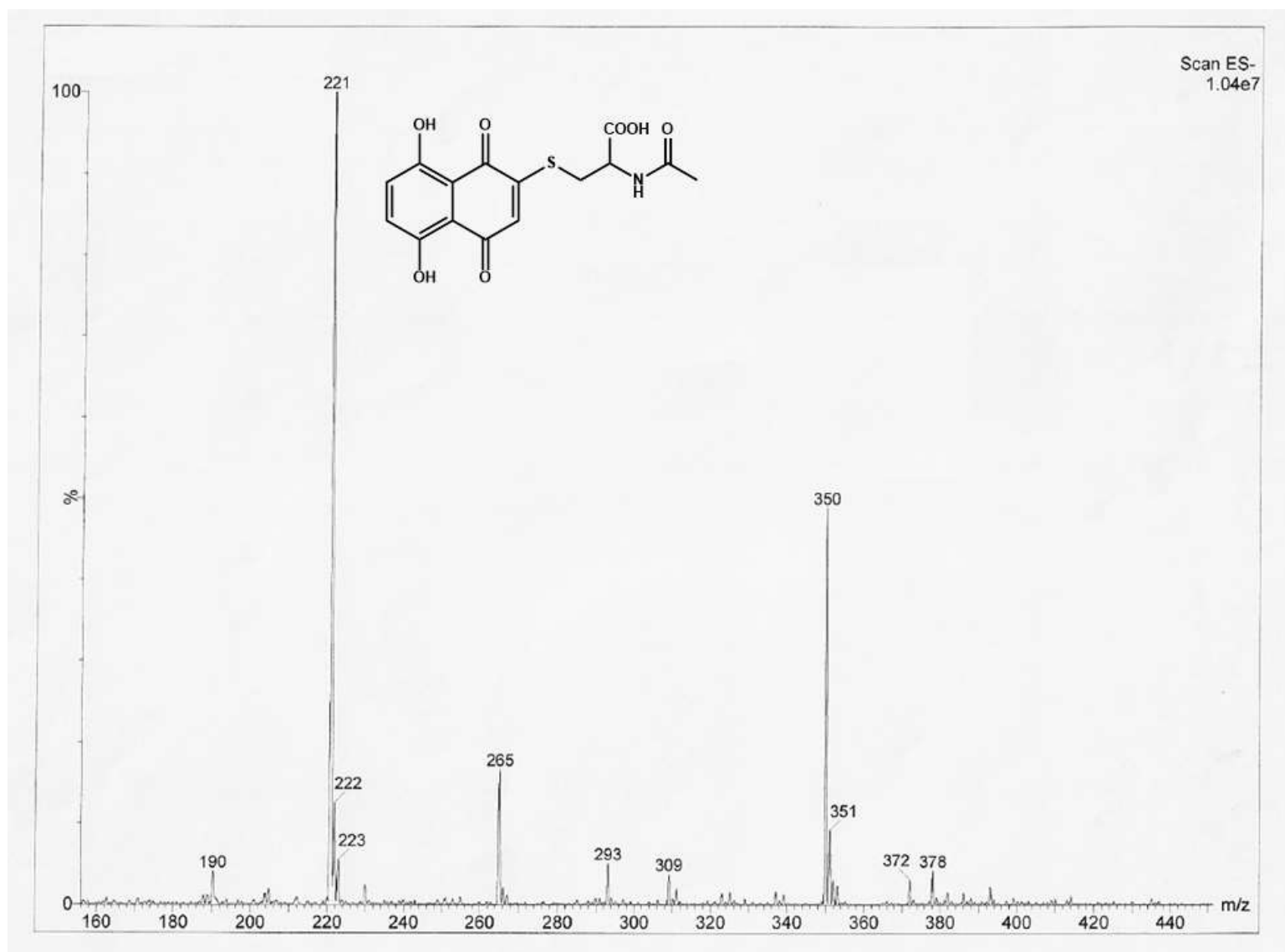


Figure S15. ESI-MS- spectrum of compound 12.

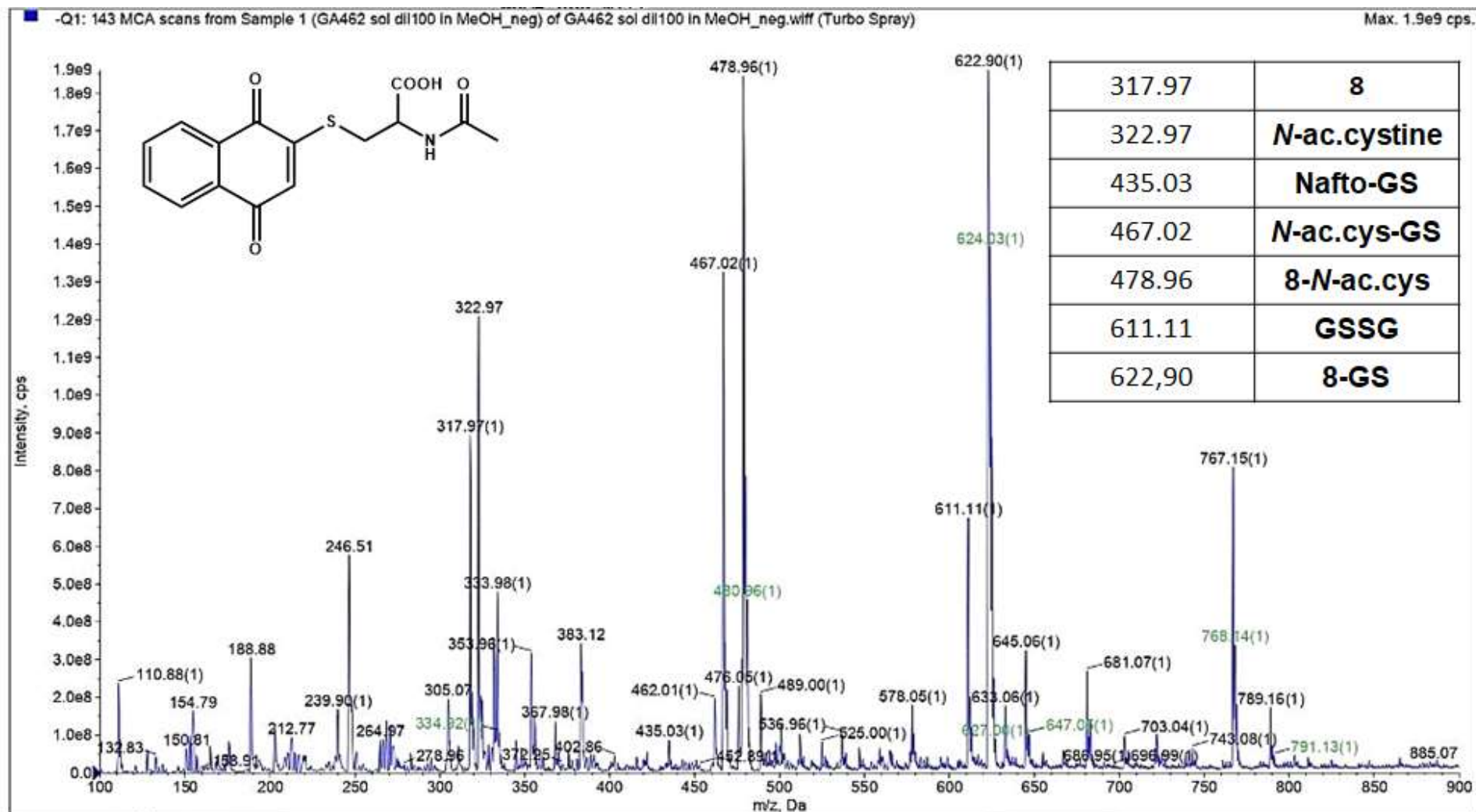


Figure S16. Direct infusion mass spectra of the reaction mixtures with GSH of compound **8**. All the detected ions are in the deprotonated form $[M-H]^-$. Abbreviations: 8-GS is the adduct of **8** with glutathione, 8-*N*-ac.cys is the adduct of **8** with *N*-acetyl-*L*-cysteine, Nafto-GS is the adduct of naphthoquinone (**1**) and glutathione, GSSG: glutathione dimer; *N*-ac.cystine is the *N*-acetyl-*L*-cysteine dimer; *N*-ac.cys-GS is the *N*-acetyl-*L*-cysteine – glutathione heterodimer.

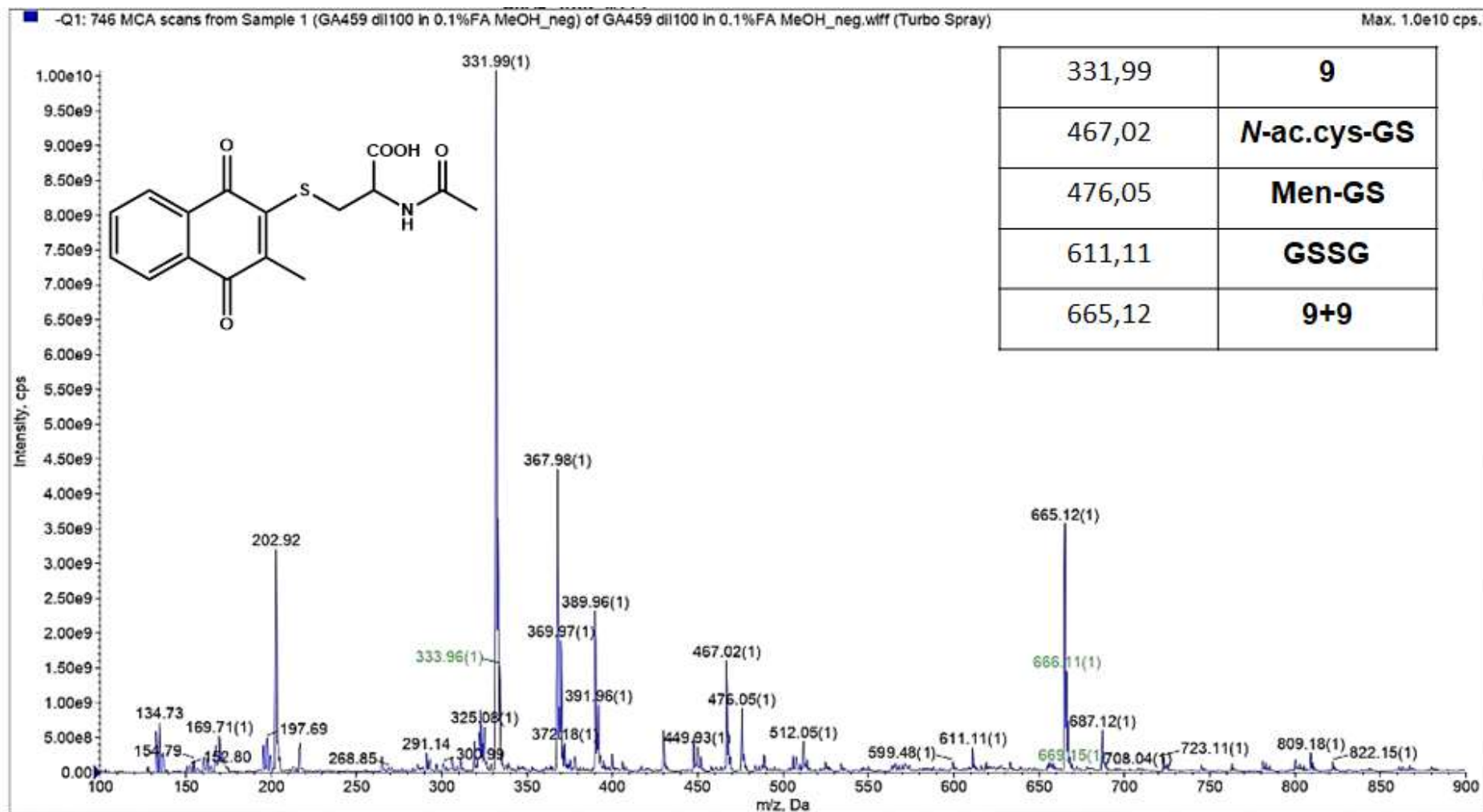


Figure S17. Direct infusion mass spectra of the reaction mixtures with GSH of compound **9**. All the detected ions are in the deprotonated form [M-H]. Abbreviations: Men-GS is the adduct of Menadione (**2**) and glutathione, GSSG: glutathione dimer, *N*-ac.cys-GS is the *N*-acetyl-*L*-cysteine – glutathione heterodimer. 9+9 is the cluster of compound **9**.

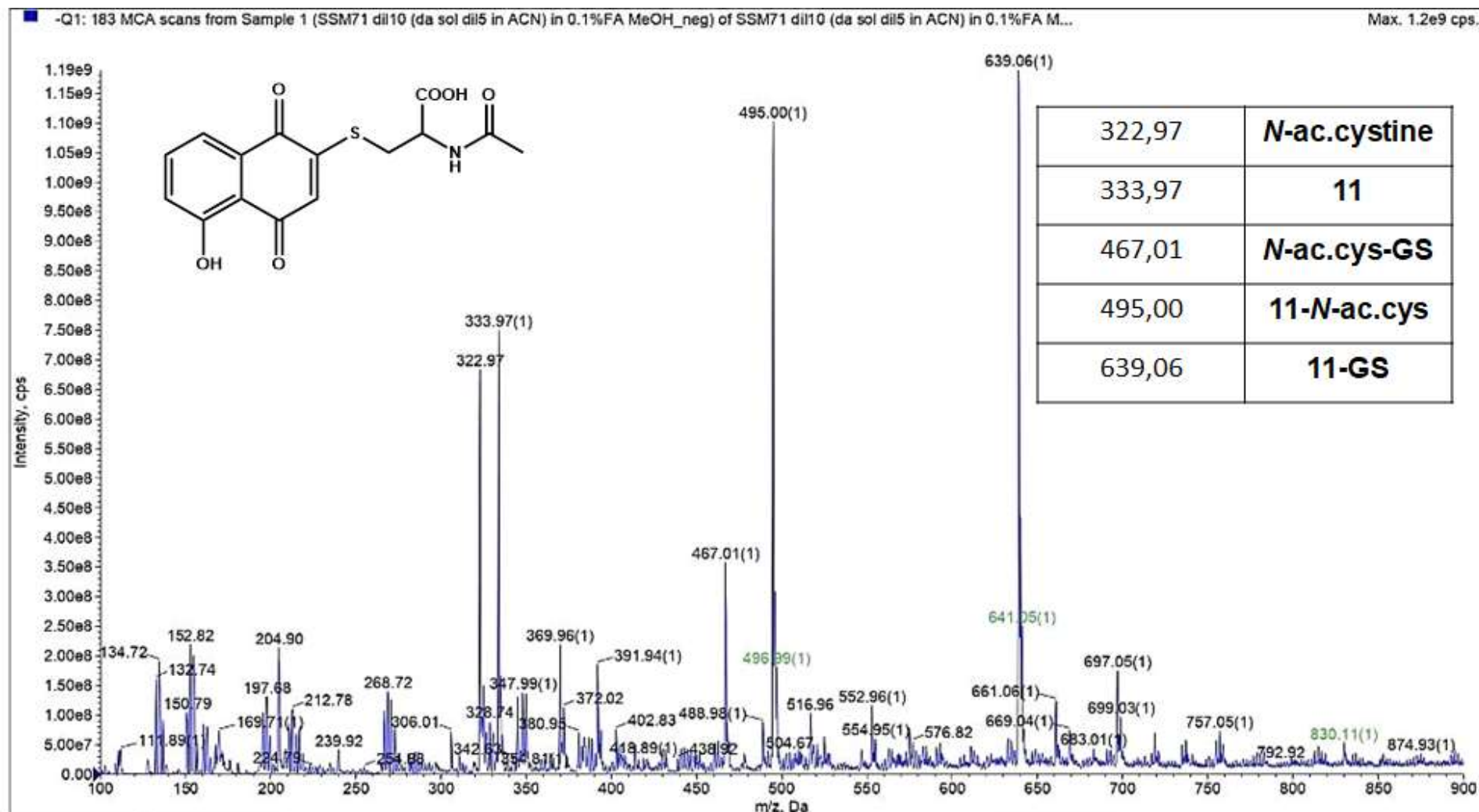


Figure S18. Direct infusion mass spectra of the reaction mixtures with GSH of compound **11**. All the detected ions are in the deprotonated form [M-H]⁻. Abbreviations: 11-GS is the adduct of **11** with glutathione, 11-*N*-ac.cys is the adduct of **11** with *N*-acetyl-*L*-cysteine, *N*-ac.cystine is the *N*-acetyl-*L*-cysteine dimer; *N*-ac.cys-GS is the *N*-acetyl-*L*-cysteine – glutathione heterodimer.