A Dual Responsive "Turn-On" Fluorophore for Orthogonal Selective Sensing of Biological Thiols and Hydrogen Peroxide

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Electronic Supplementary Information (ESI)

Content **Page**

1. Time-dependent fluorescence spectra measurements

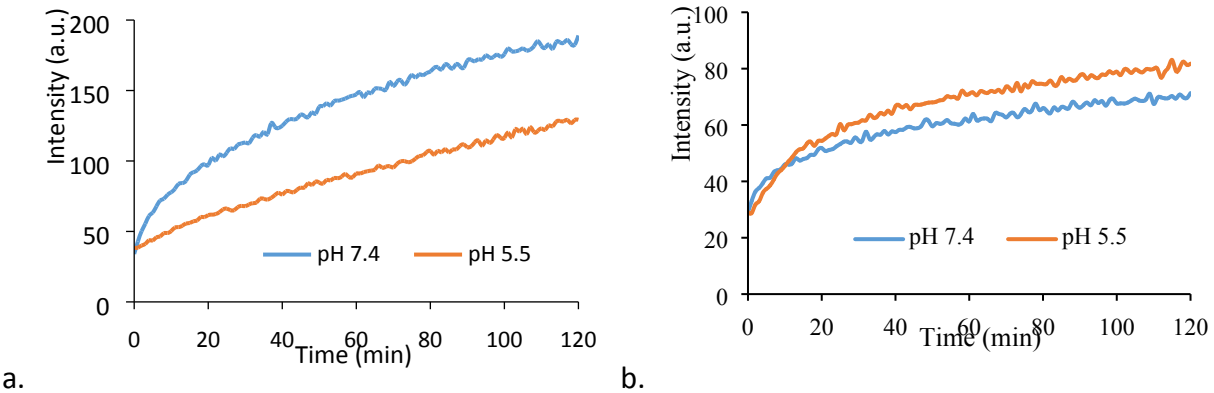
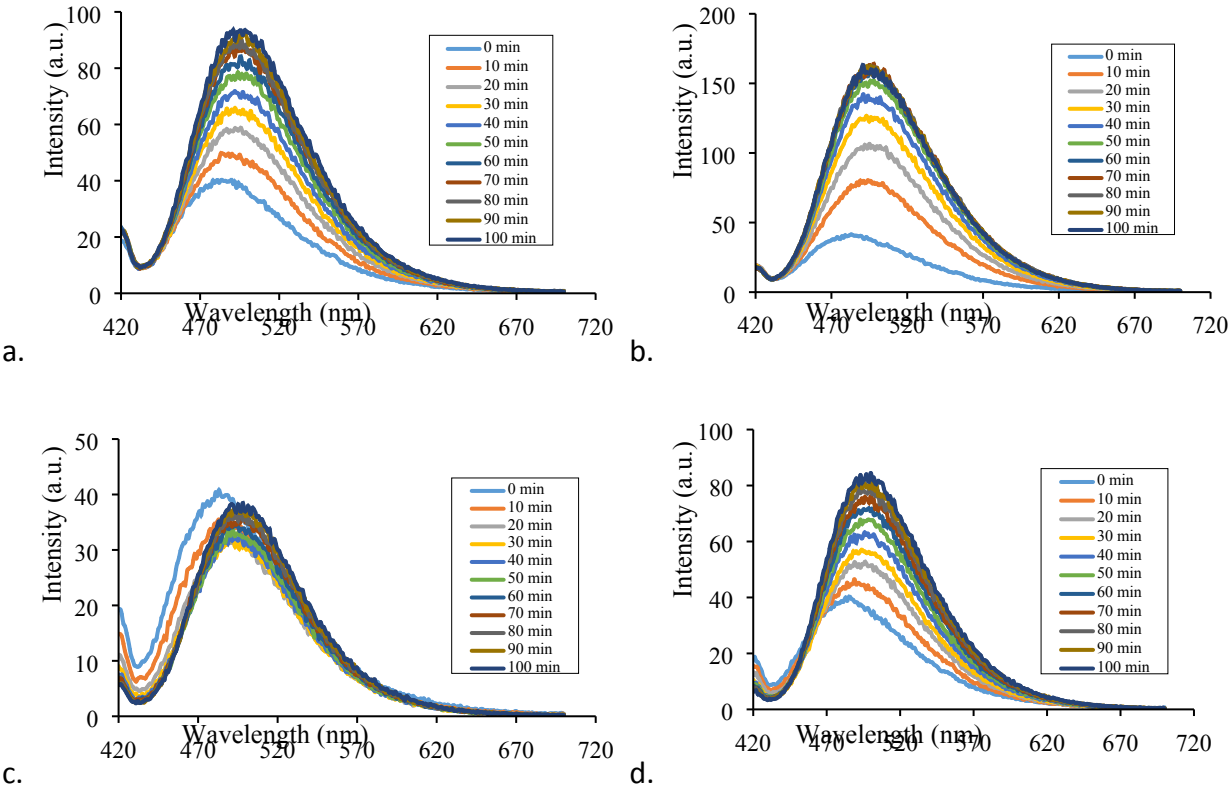
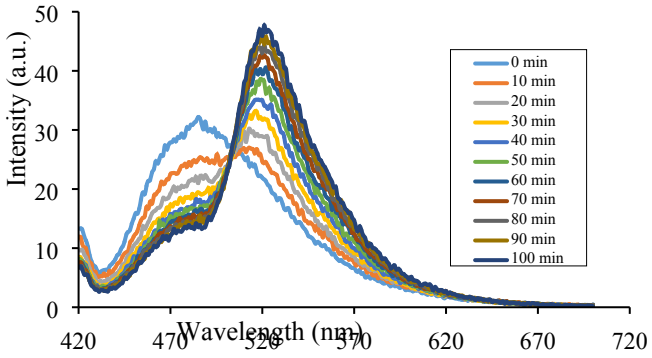


Figure S1. Time-dependent fluorescence response of FLB_2SSCou with (a) DTT (λ_{Ex} = 408 nm, λ_{Em} = 497 nm) and (b) H_2O_2 in pH 5.5 and pH 7.4.



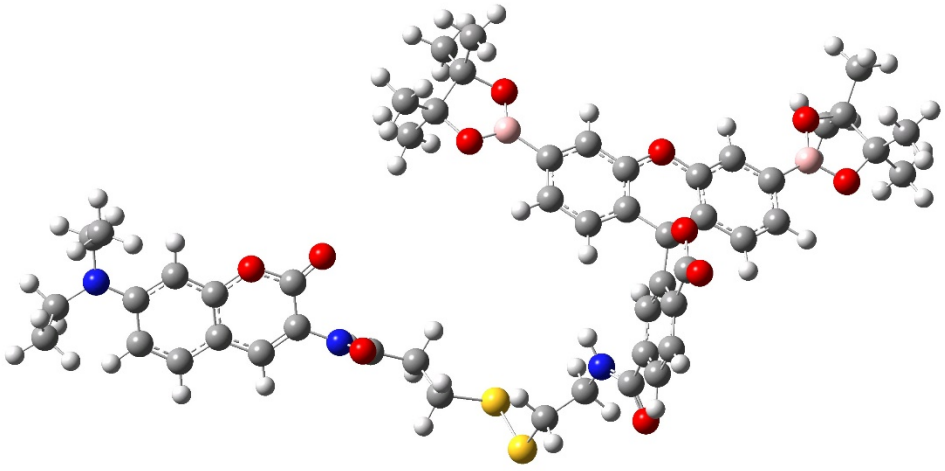


e.

Figure S2. Time-dependent fluorescence measurement (λ_{Ex} = 408 nm) of FLB_2SSCou (50 μM) in 0.1 X PBS upon the addition of (a) Cys (5.0 mM), (b) DTT (5.0 mM), (c) GSH (5.0 mM), (d) Hcy (5.0 mM) and (e) H_2O_2 (1.0 mM).

2. Computation details

Theoretical calculations of the various molecules were performed with the Gaussian 09 package.[1] DFT optimizations and TD-DFT calculations were performed using the basis set of B3LYP/6-31+G (d,p). The polarization continuum model using the integration formalism variant (IEFPCM) with water as solvent was included in the optimization and TD-DFT calculations process.



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6	10.866197	1.665032	-4.829017
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1	-3.333294	-3.705564	0.378717
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1	-15.818321	0.330158	1.129578
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Table S1. Coordinates of the optimized structure of FLB_2SSCou. Columns from left to right: atomic number, X coordinates, Y coordinates and Z coordinates.

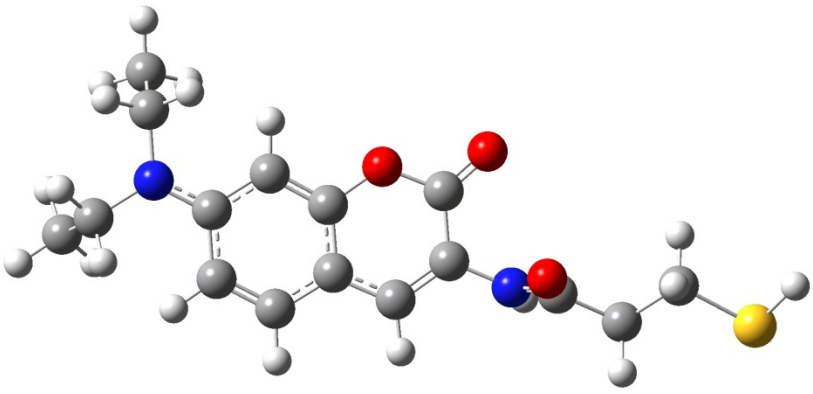

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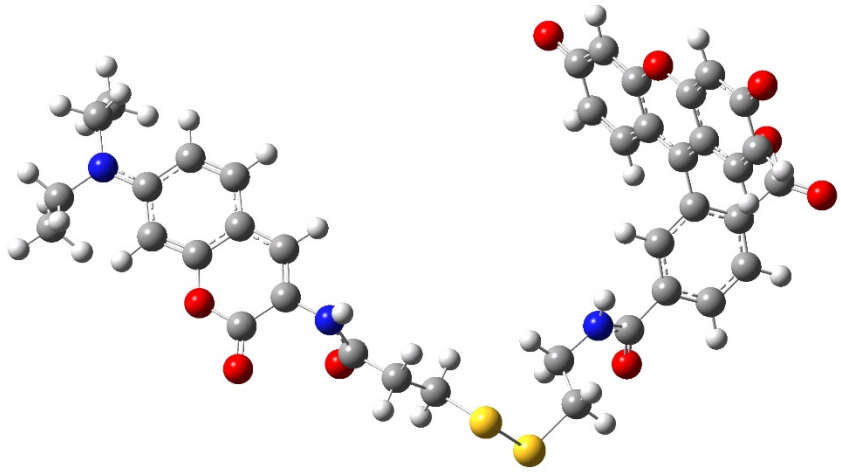
Table S2. Coordinates of the optimized structure of FLB_2SSCou after treatment with thiol. Columns from left to right: atomic number, X coordinates, Y coordinates and Z coordinates.



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16	0.773163	5.514725	1.081716
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1	2.337421	4.792096	-0.630418
1	1.409513	3.417057	0.008819
6	3.074904	4.026795	1.262149
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6	9.136525	-0.923664	0.191294
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1	7.705244	-4.032665	0.338600	1	11.132501	-4.927944	-0.100958
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1	12.252414	-3.188711	-0.265126	1	10.236737	-4.798983	-2.432875
1	11.637319	-1.631597	0.235839	1	9.555053	-6.188180	-1.569133
6	11.624595	-1.941491	-1.914078	1	8.578647	-4.729635	-1.812925
1	12.599400	-1.456203	-2.032784	8	-7.628843	-2.178255	5.155129
1	11.565814	-2.765598	-2.630830	8	-2.766770	-5.764679	-2.083163

Table S3. Coordinates of the optimized structure of FLB_2SSCou after treatment of H_2O_2. Columns from left to right: atomic number, X coordinates, Y coordinates and Z coordinates.

3. MTT cytotoxicity assay of FLB_2SSCou

HeLa cells were first seeded in a 96 well plate at a seeding density of 1×10^4 cells/well prior to overnight incubation. Thereafter, FLB_2SSCou and 2 equivalent of β-CD were added into the cells at different concentrations. The cells were then incubated overnight again before the medium was replaced with medium containing 0.5 mg/mL of MTT. The cells were allowed to incubate for another 4 h before removing the medium. The residual purple crystals were then dissolved in DMSO (100 µL/well). The cell viability was then calculated by taking the absorbance at 562 nm relative to the control well, where the cells were treated with water only. The viability data were then expressed in percentage. The MTT cytotoxicity results showed that the FLB_2SSCou exhibits insignificant toxicity to the HeLa cells, confirming its biocompatibility for biological applications.

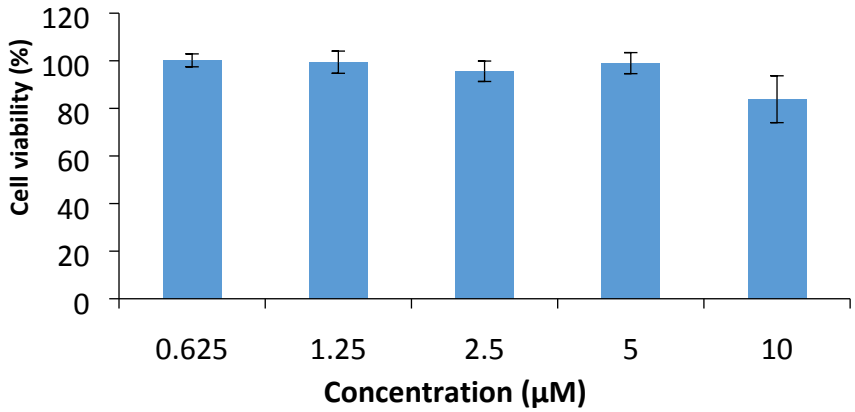

Figure S3. MTT cell viability profile of FLB2SSCou on HeLa cells.

4. Flow cytometry assay (Histogram)

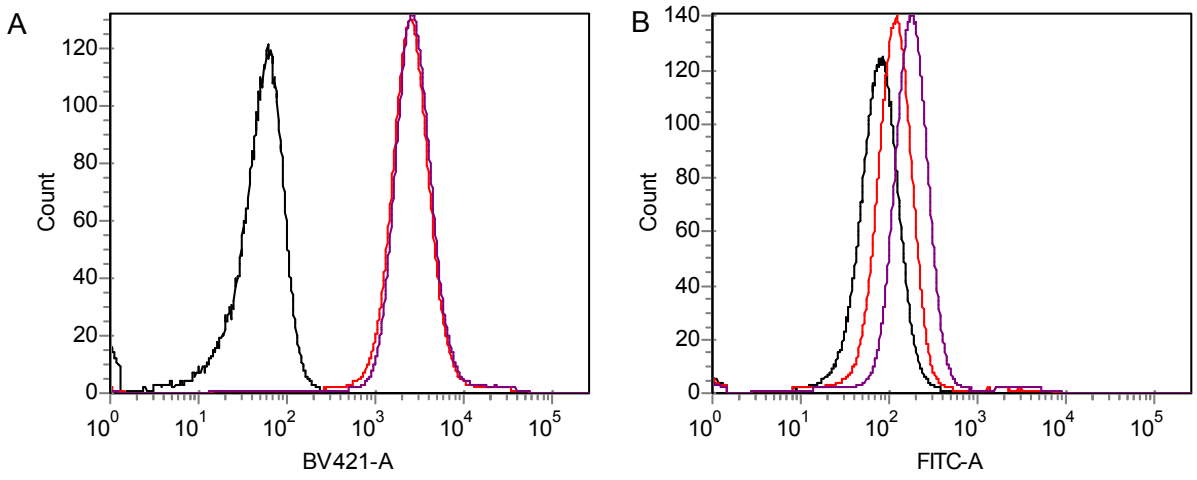

Figure S4. (A) BV421 channel and (B) FITC channel for 1D histogram plots of flow cytometry analysis with HeLa cells treated with blank control (black line), FLB_2SSCou (10 μM) with β-CD (20 μM) (red line), and FLB_2SSCou (10 μM) with β-CD (20 μM) followed by post-treatment with H_2O_2 (150 μM) (purple line). Note the shift in the BV421 signal upon treatment with the FLB_2SSCou probe (Figure S4A) and the shift in the FITC signal upon treatment with H_2O_2 (Figure S4B).

5. Spectroscopy data of the various synthesized compounds.

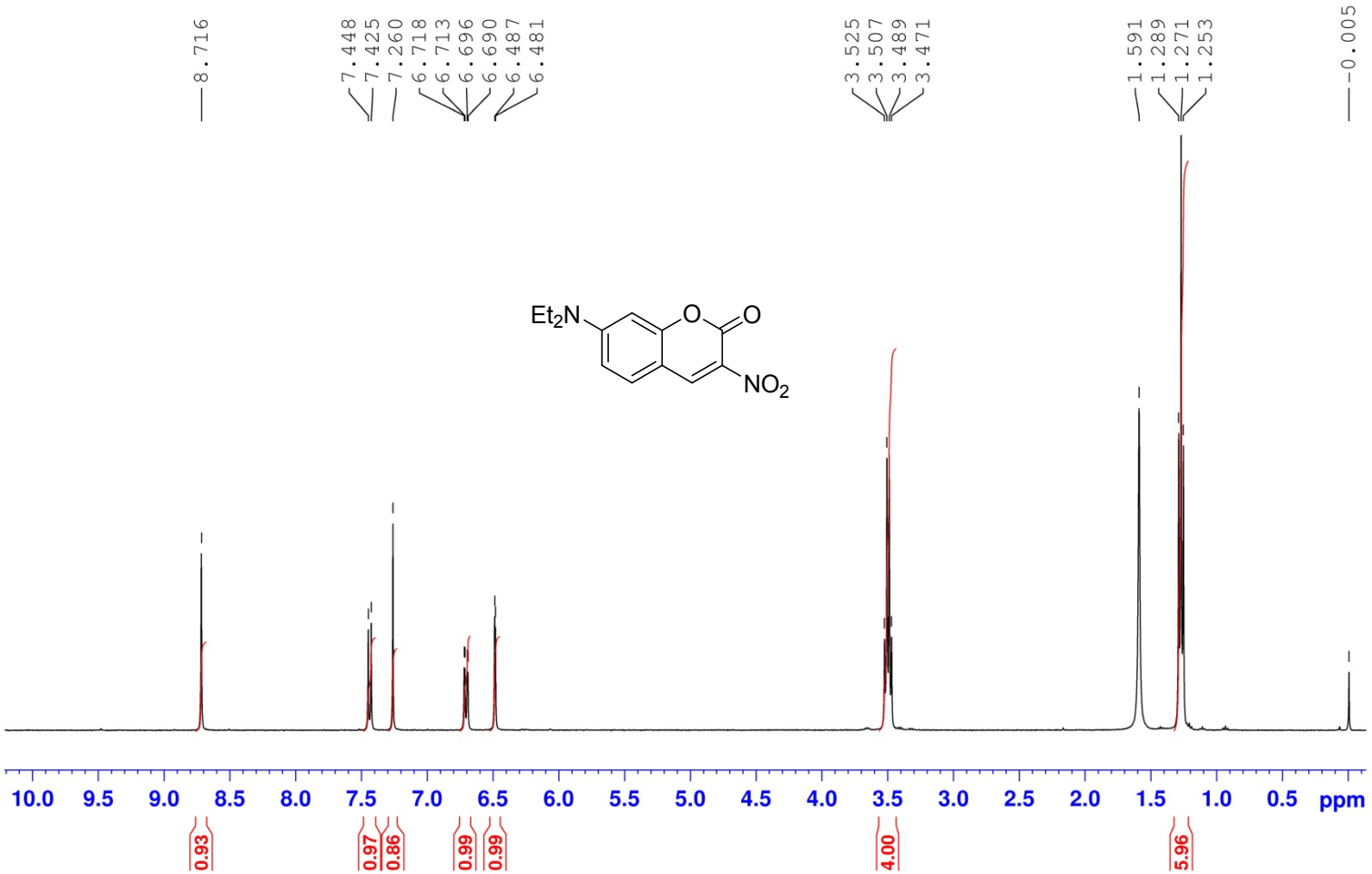

Figure S5. ^{1}H NMR spectrum of 7-(diethylamino)-3-nitro-2H-chromen-2-one **(1)**.

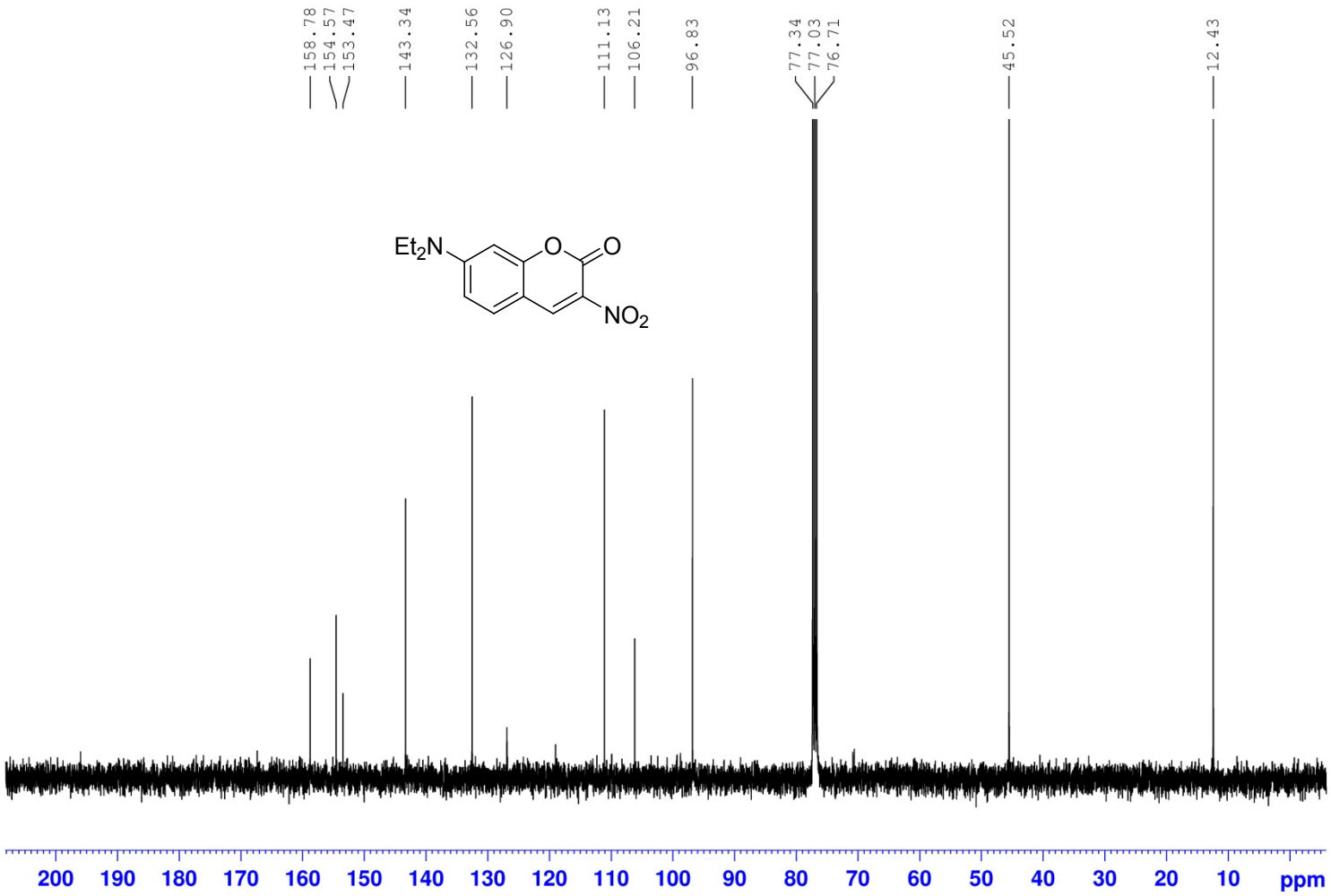

Figure S6. ^{13}C NMR spectrum of 7-(diethylamino)-3-nitro-2H-chromen-2-one **(1)**.

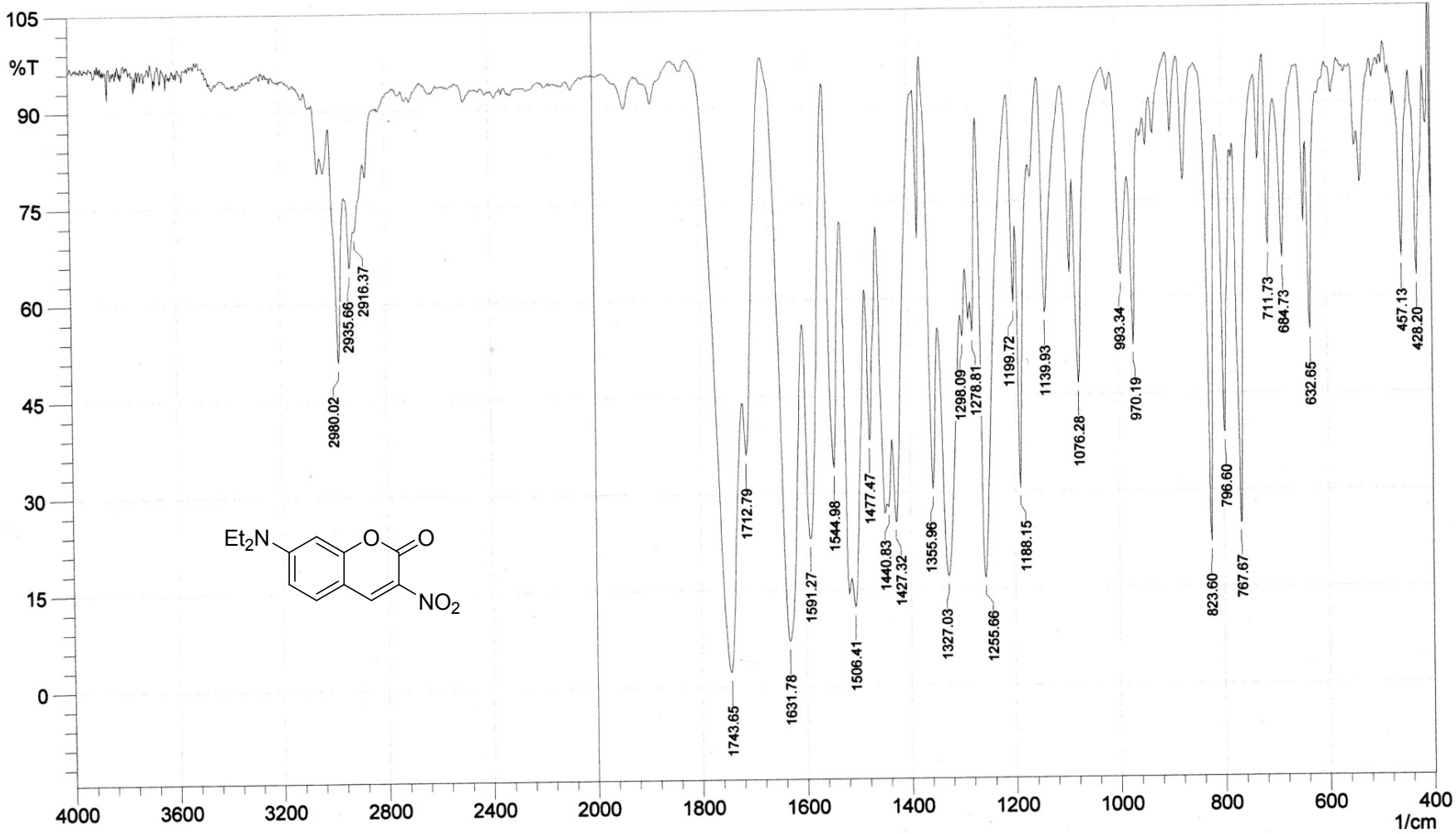

Figure S7. IR spectrum of 7-(diethylamino)-3-nitro-2H-chromen-2-one **(1)**.

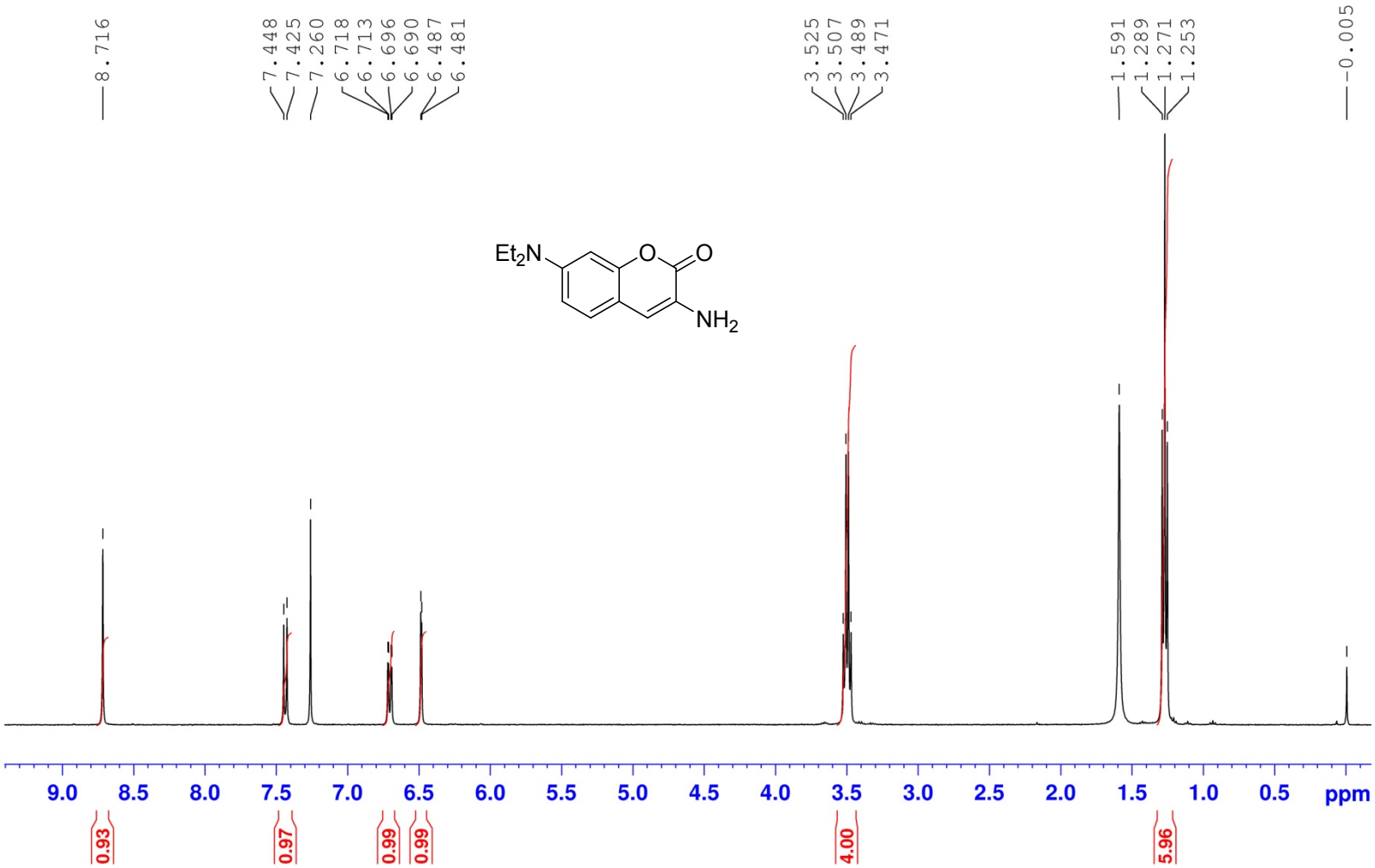

Figure S8. ^{1}H NMR spectrum of 3-amino-7-(diethylamino)-2H-chromen-2-one **(2).**

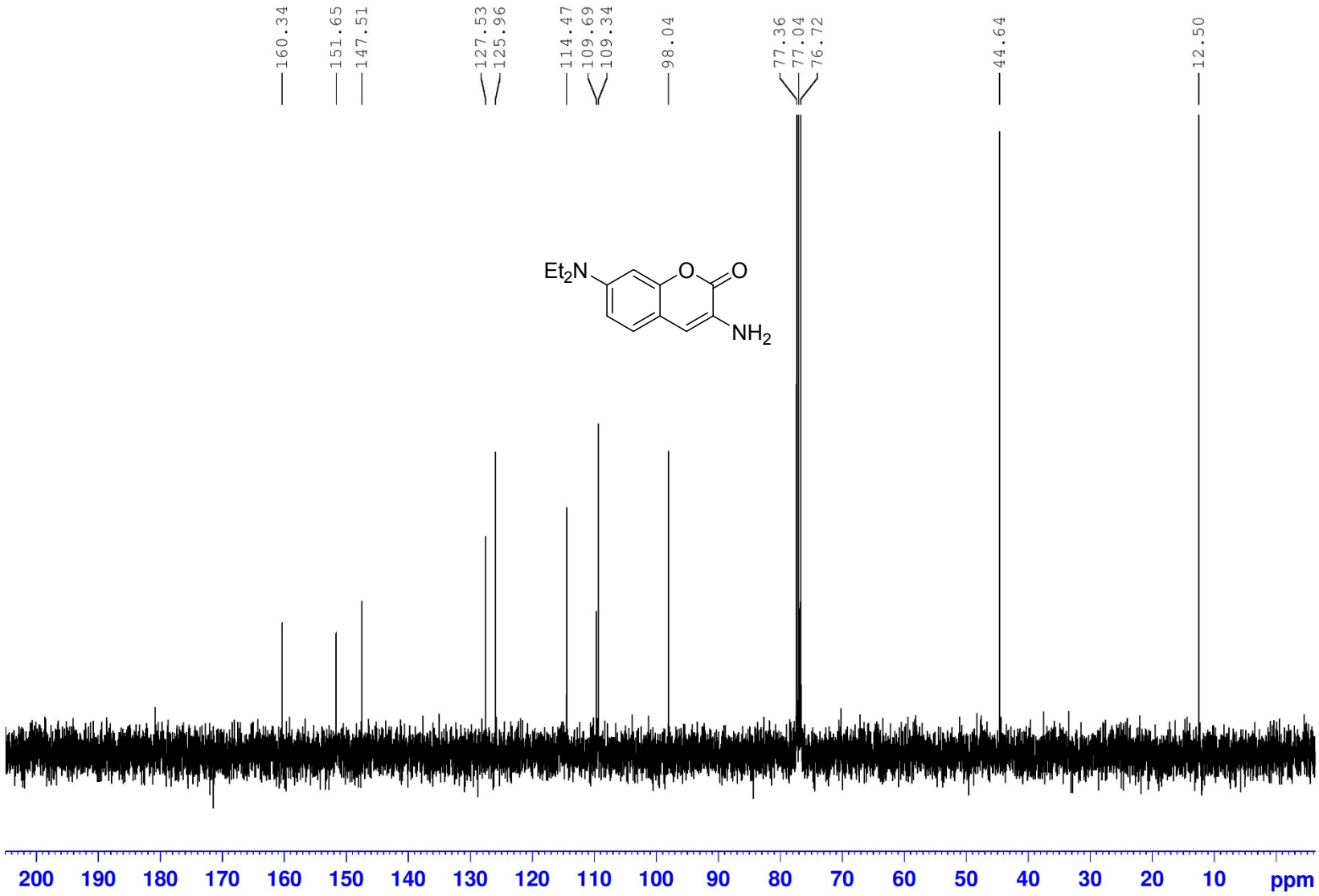

Figure S9. ^{13}C NMR spectrum of 3-amino-7-(diethylamino)-2H-chromen-2-one **(2)**.

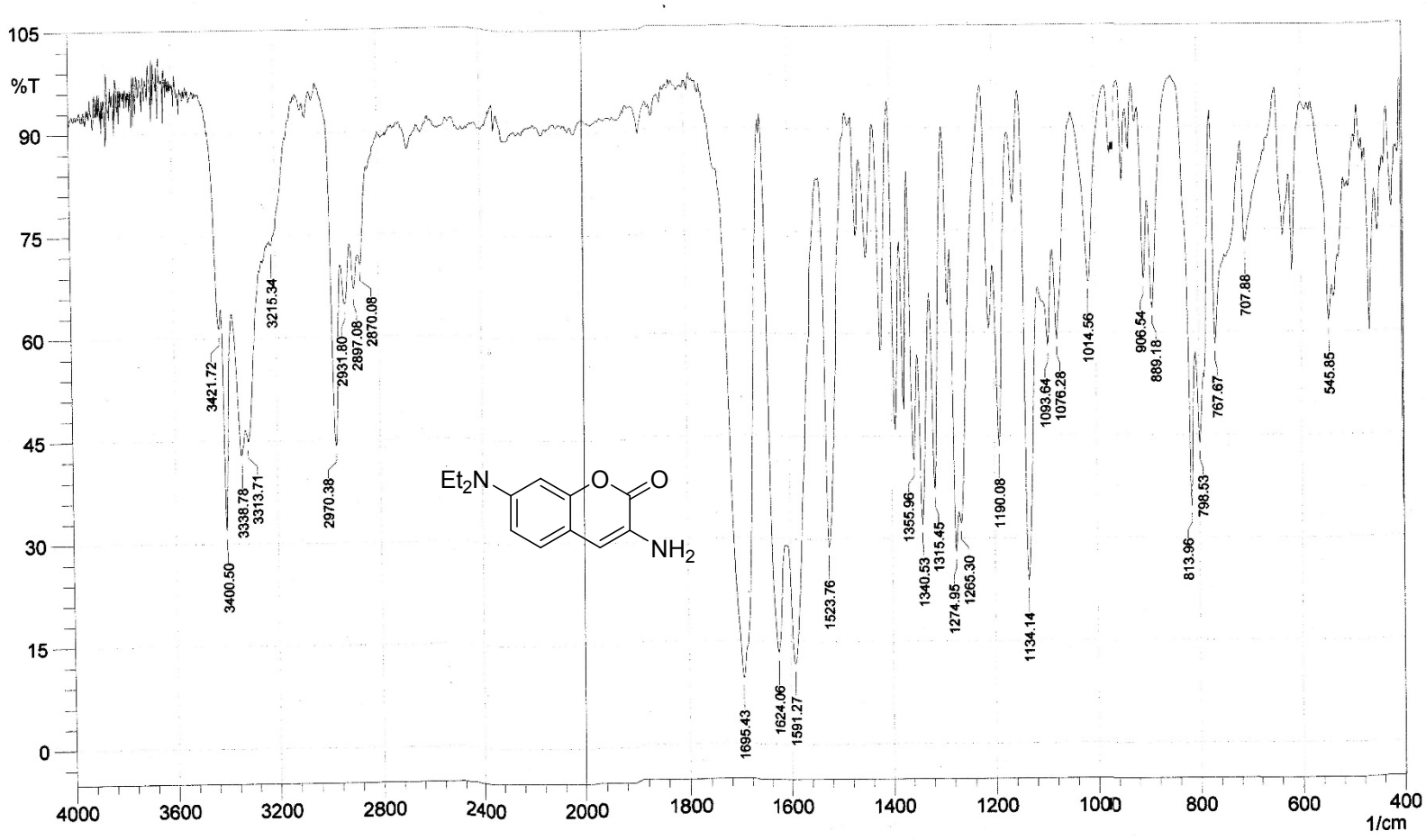

Figure S10. IR spectrum of 3-amino-7-(diethylamino)-2H-chromen-2-one **(2)**.

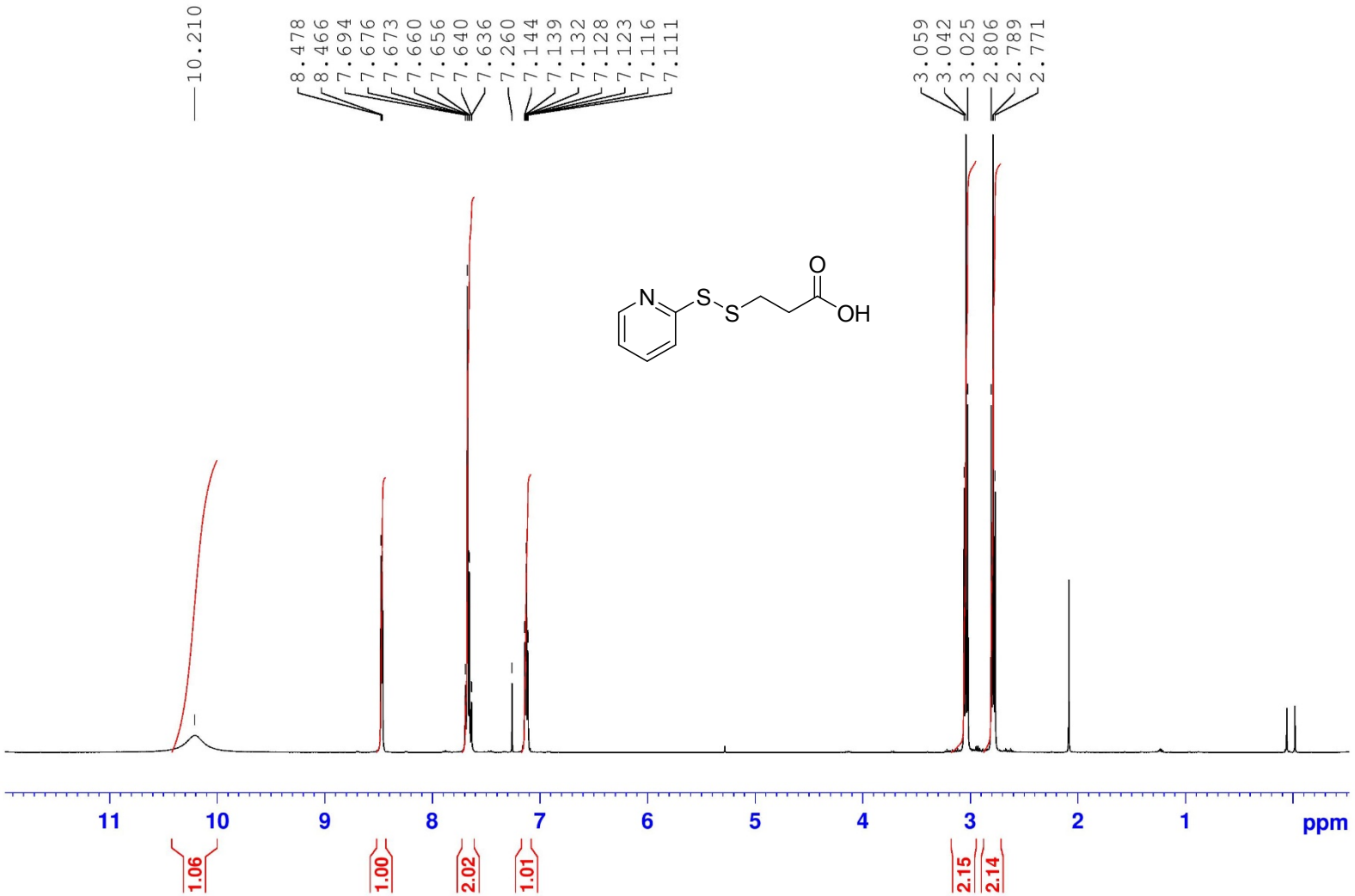

Figure S11. ^{1}H NMR spectrum of 3-(pyridin-2-yldisulfanyl)propanoic acid **(3).**

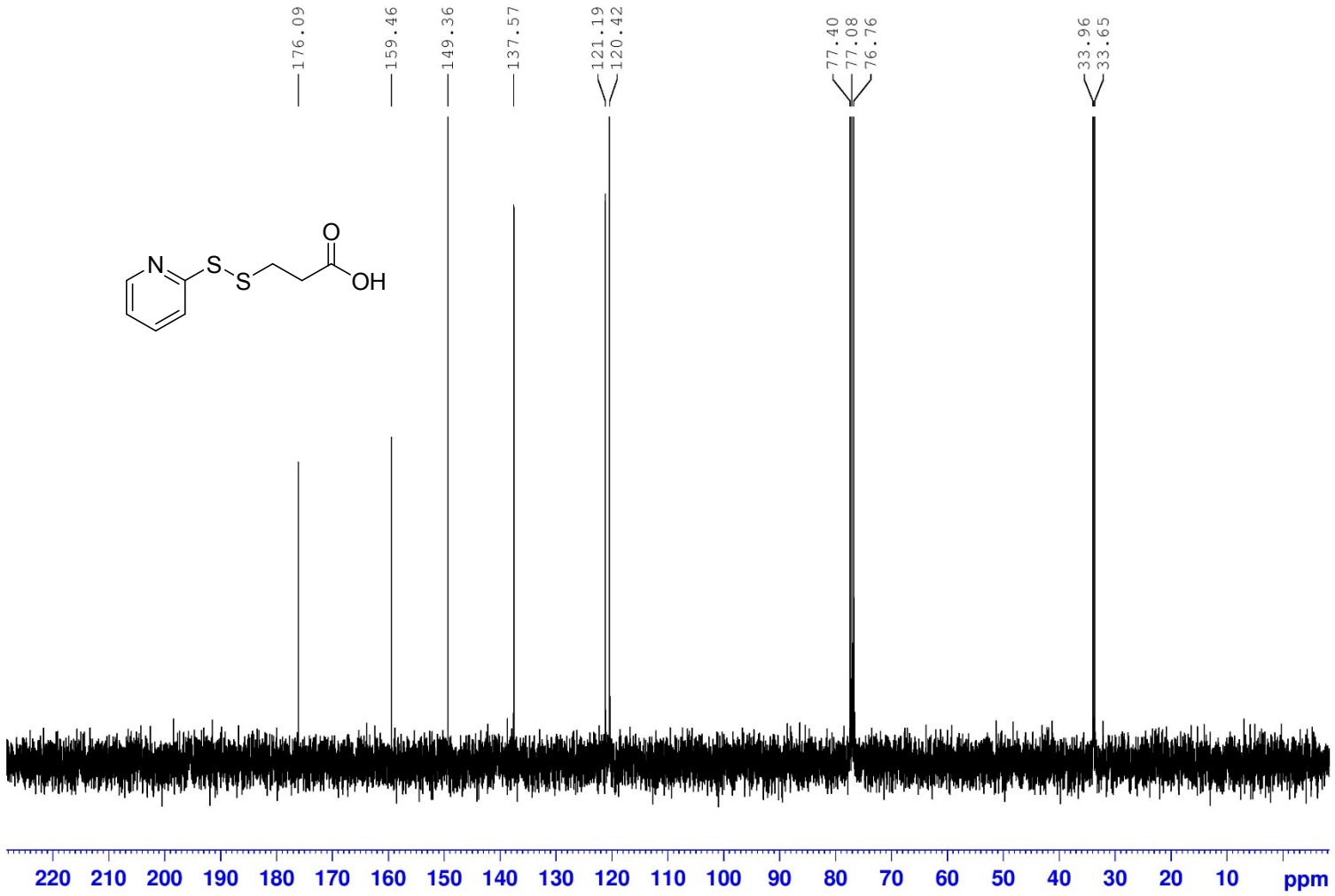

Figure S12. ^{13}C NMR spectrum of 3-(pyridin-2-yldisulfanyl)propanoic acid **(3).**

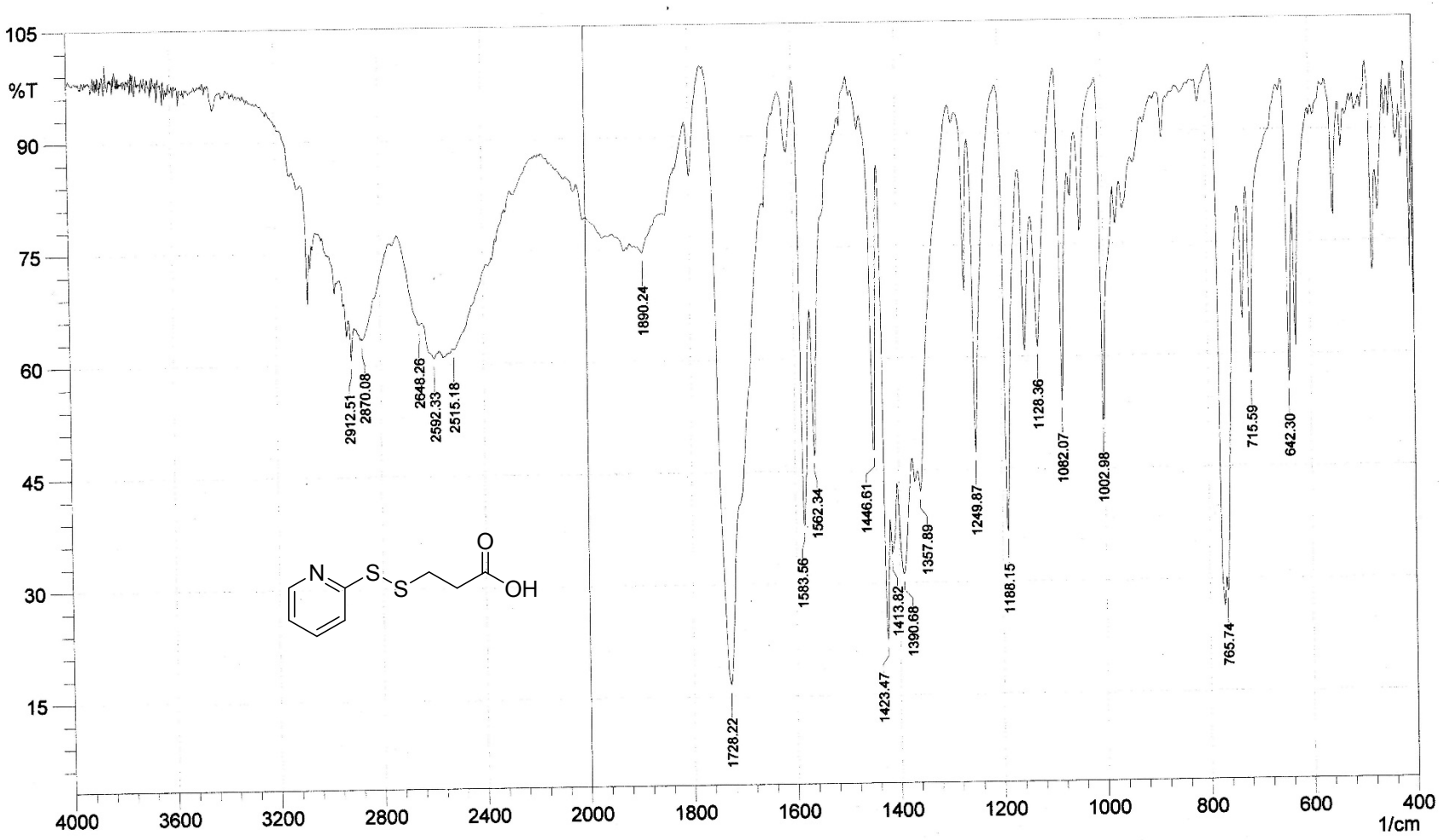

Figure S13. IR spectrum of 3-(pyridin-2-yldisulfanyl)propanoic acid **(3).**

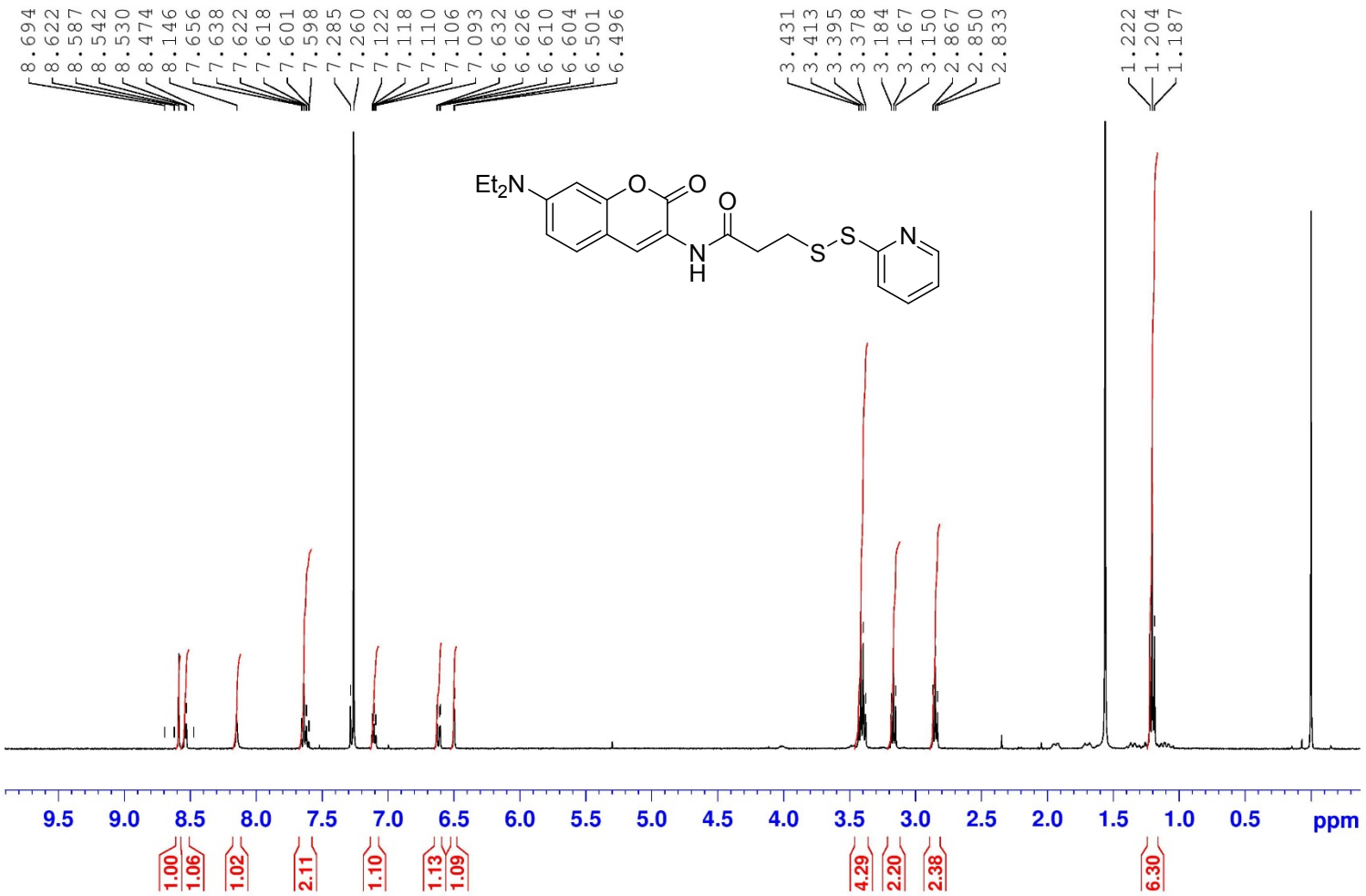

Figure S14. ^{1}H NMR spectrum of N-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-(pyridin-2-yldisulfanyl)propanamide **(4)**.

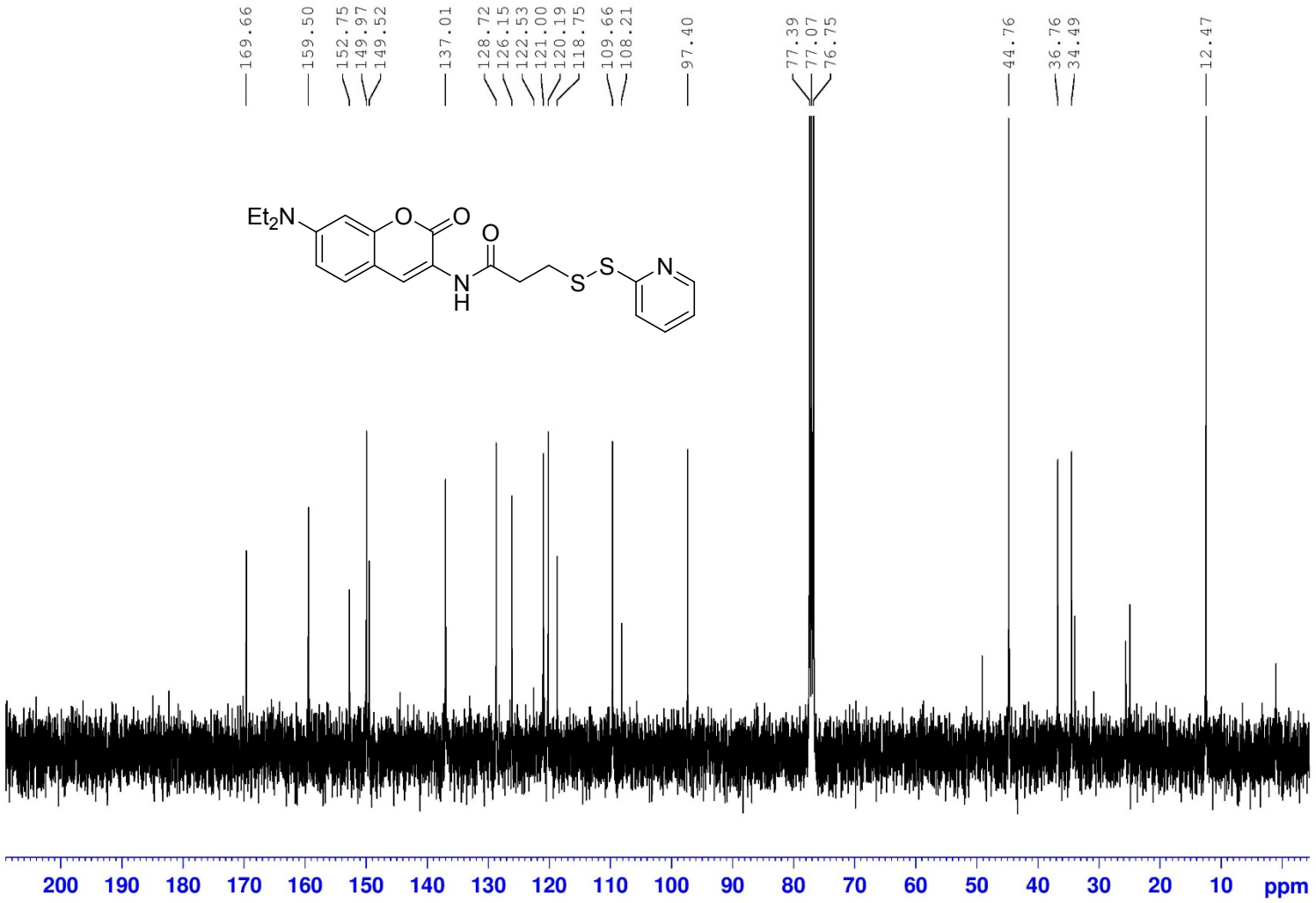

Figure S15. ^{13}C NMR spectrum of N-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-(pyridin-2-yldisulfanyl)propanamide **(4)**.

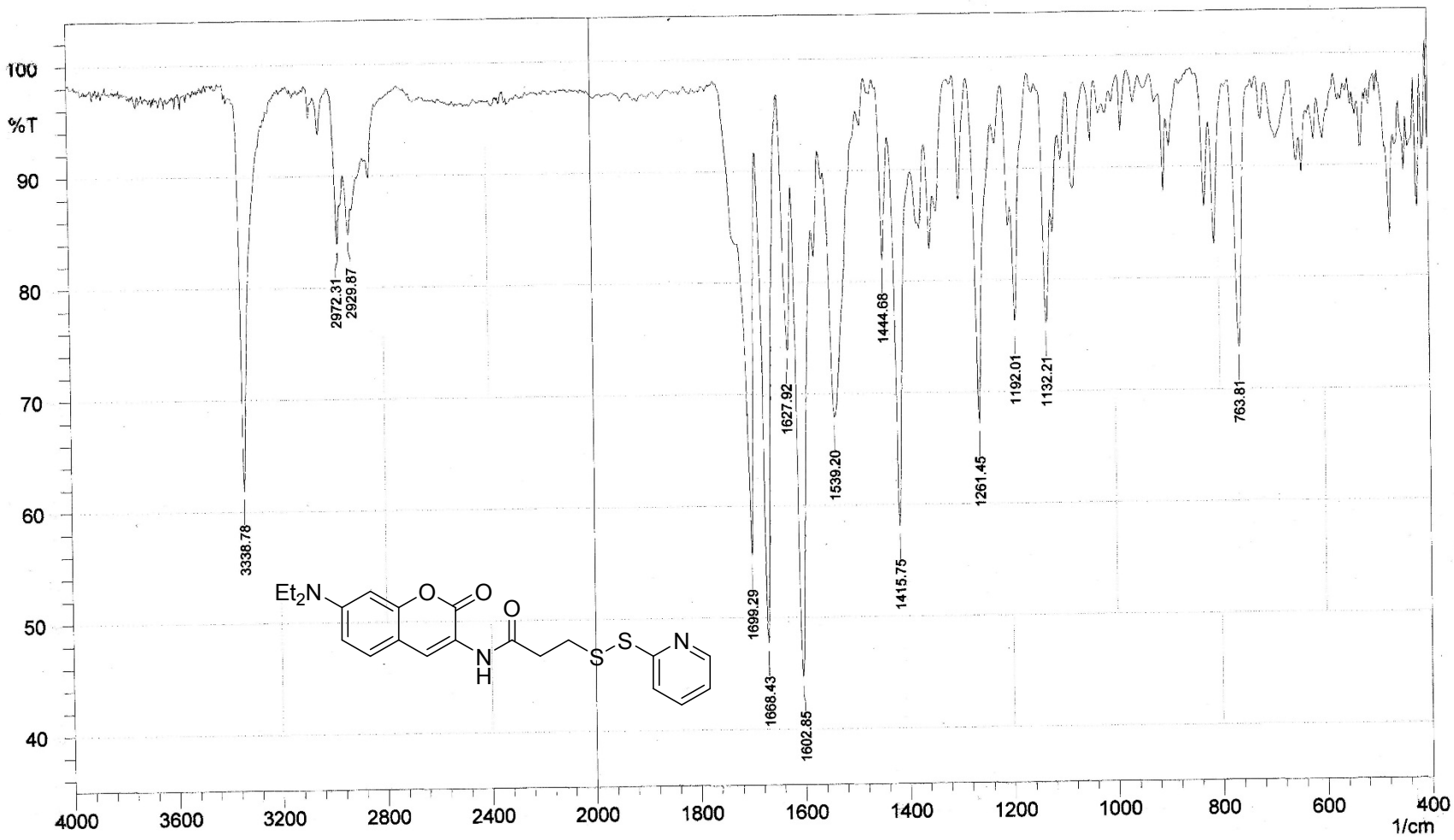

Figure S16. IR spectrum of N-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-(pyridin-2-yldisulfanyl)propanamide **(4)**.

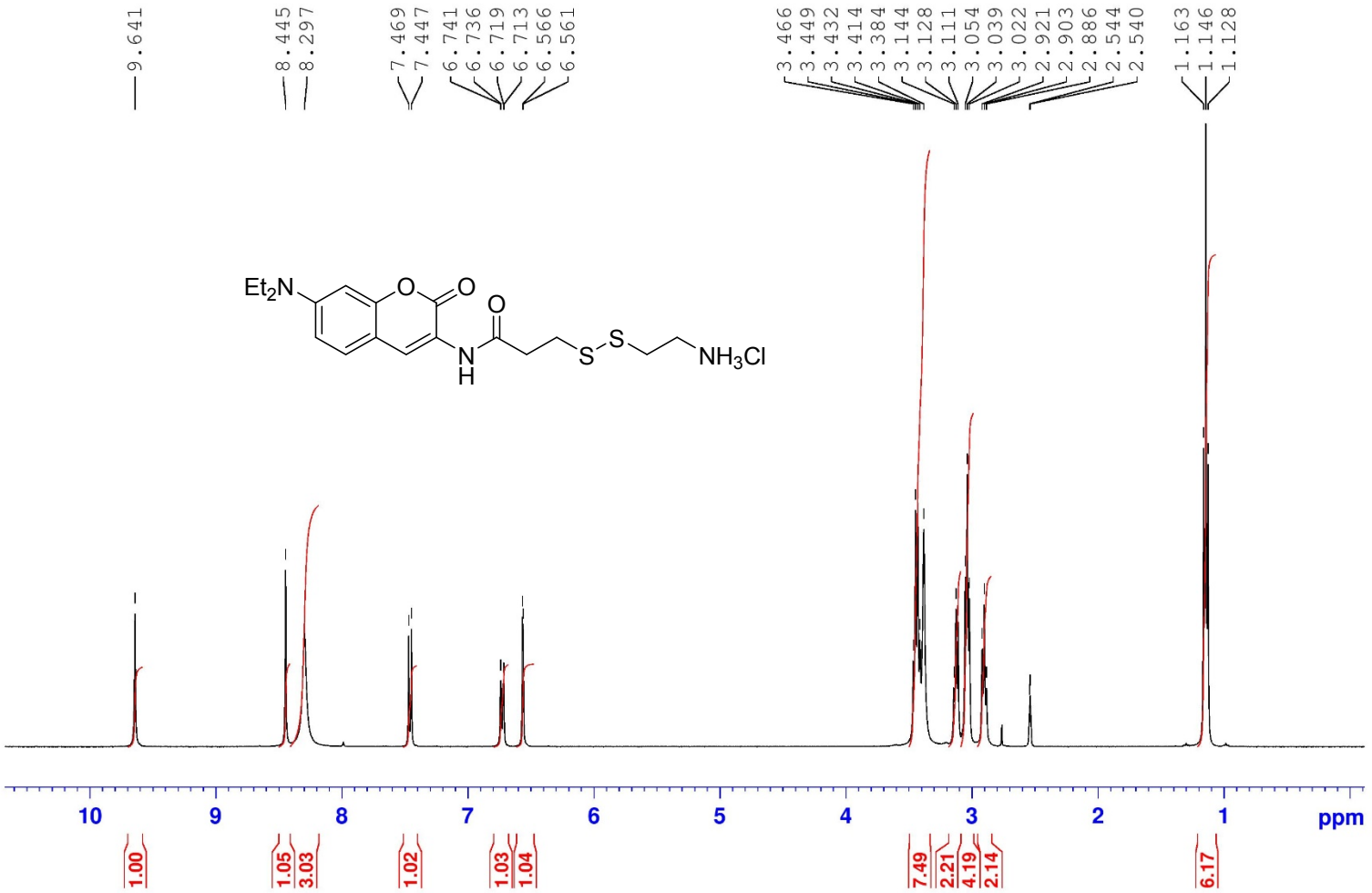

Figure S17. ^{1}H NMR spectrum of 3-((2-aminoethyl)disulfanyl)-N-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)propanamide.HCl salt **(5).**

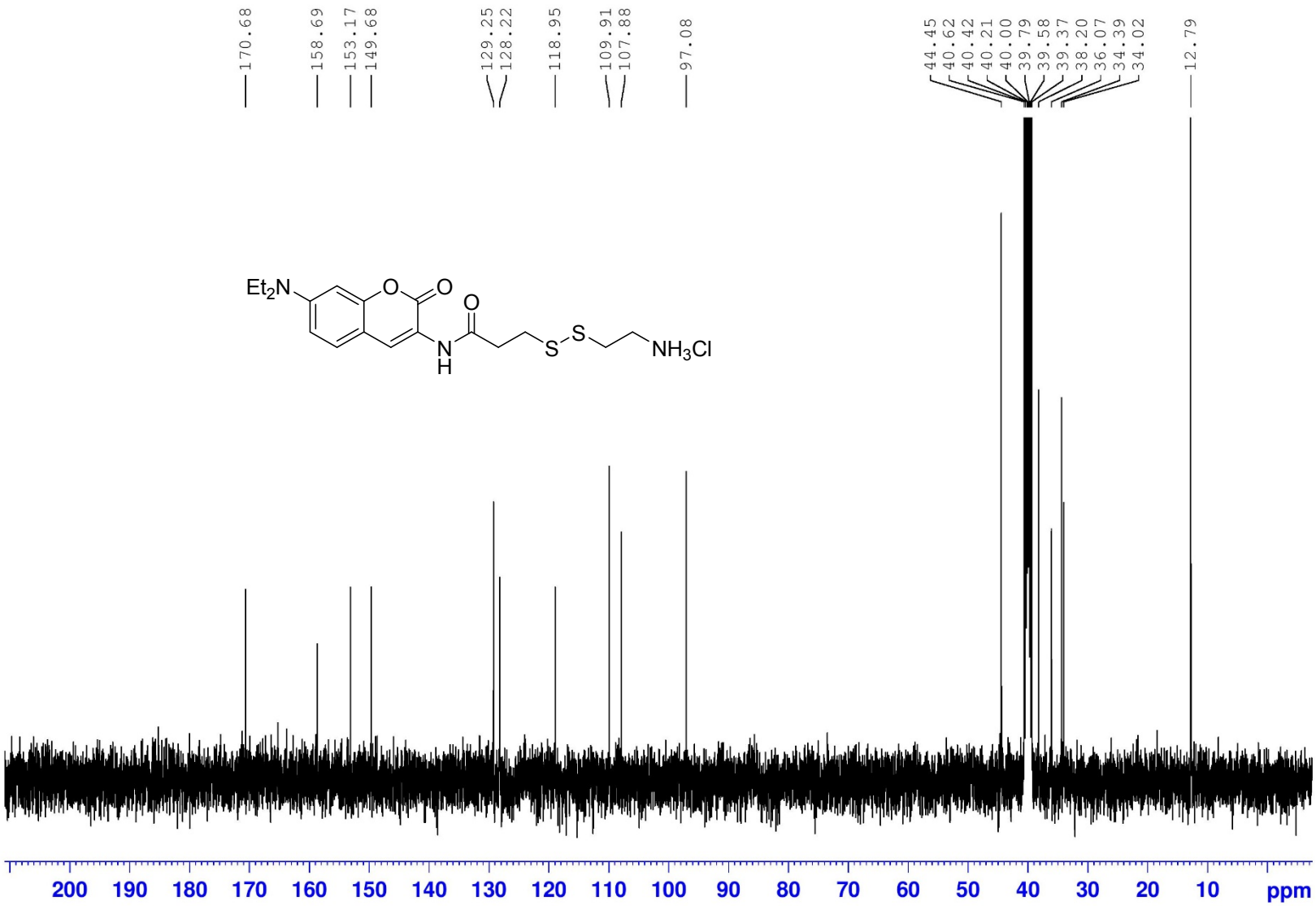

Figure S18. ^{13}C NMR spectrum of 3-((2-aminoethyl)disulfanyl)-N-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)propanamide.HCl salt **(5).**

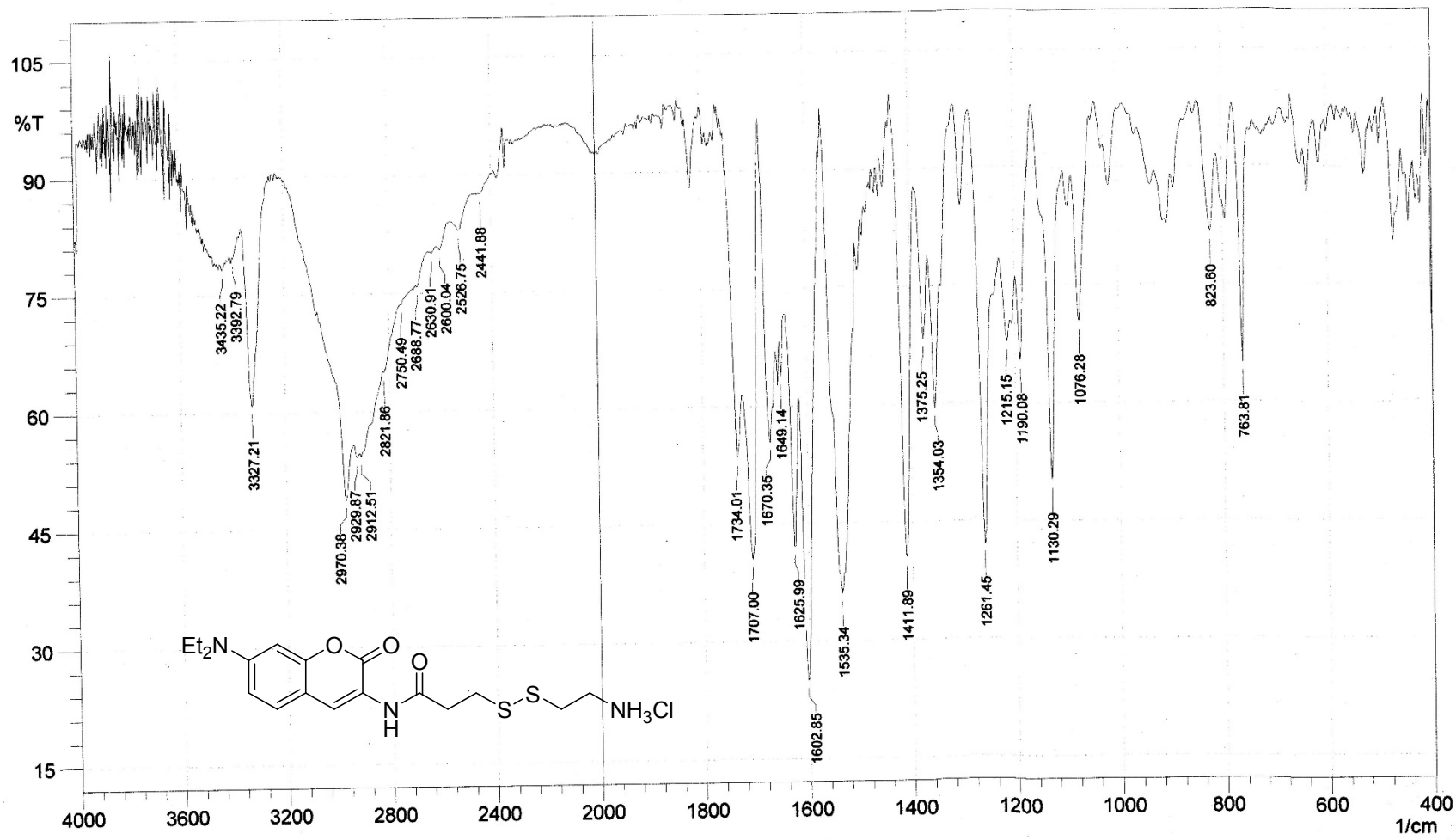

Figure S19. IR spectrum of 3-((2-aminoethyl)disulfanyl)-N-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)propanamide.HCl salt **(5).**

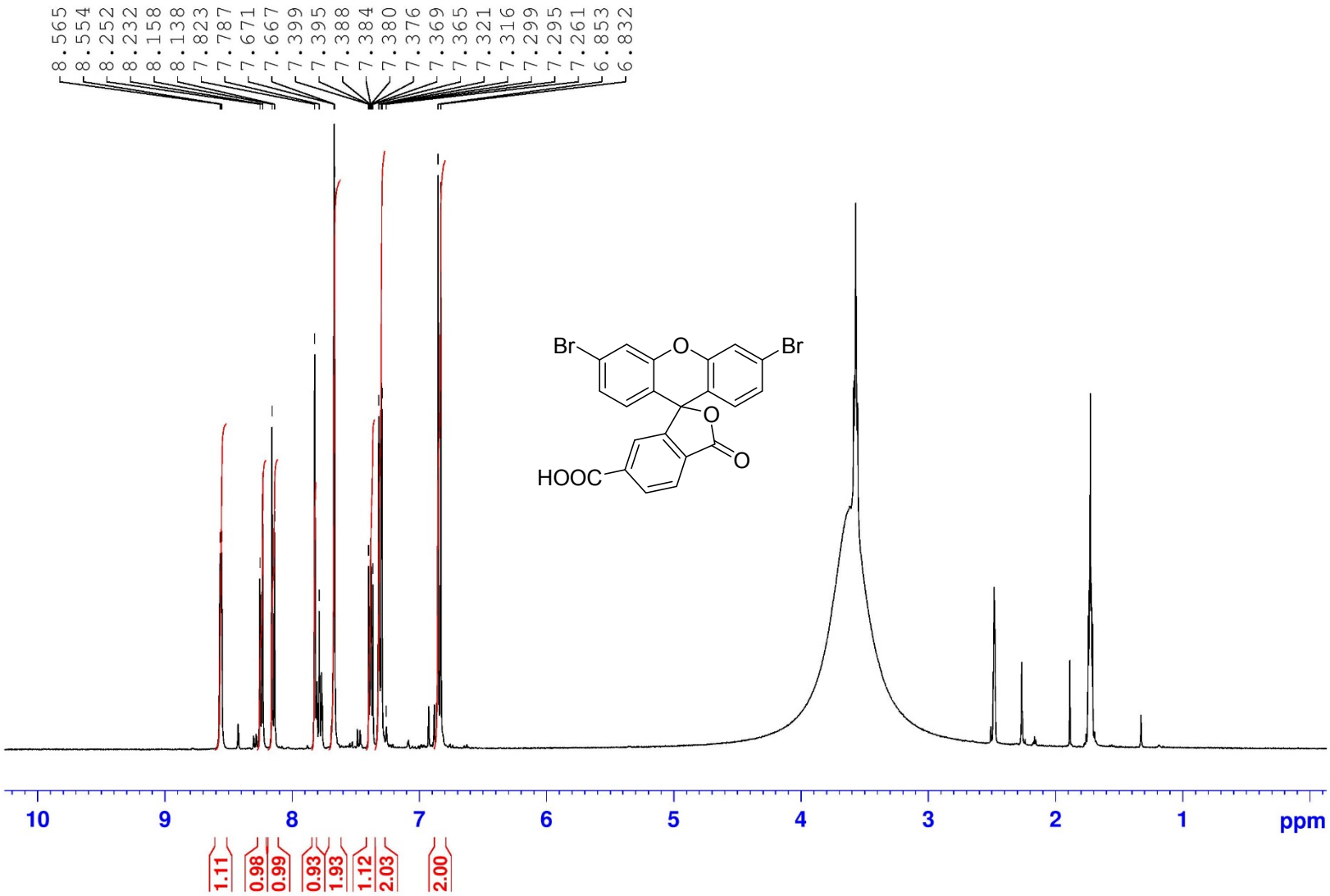

Figure S20. ^{1}H NMR spectrum of 3',6'-dibromo-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxylic acid **(6)**.

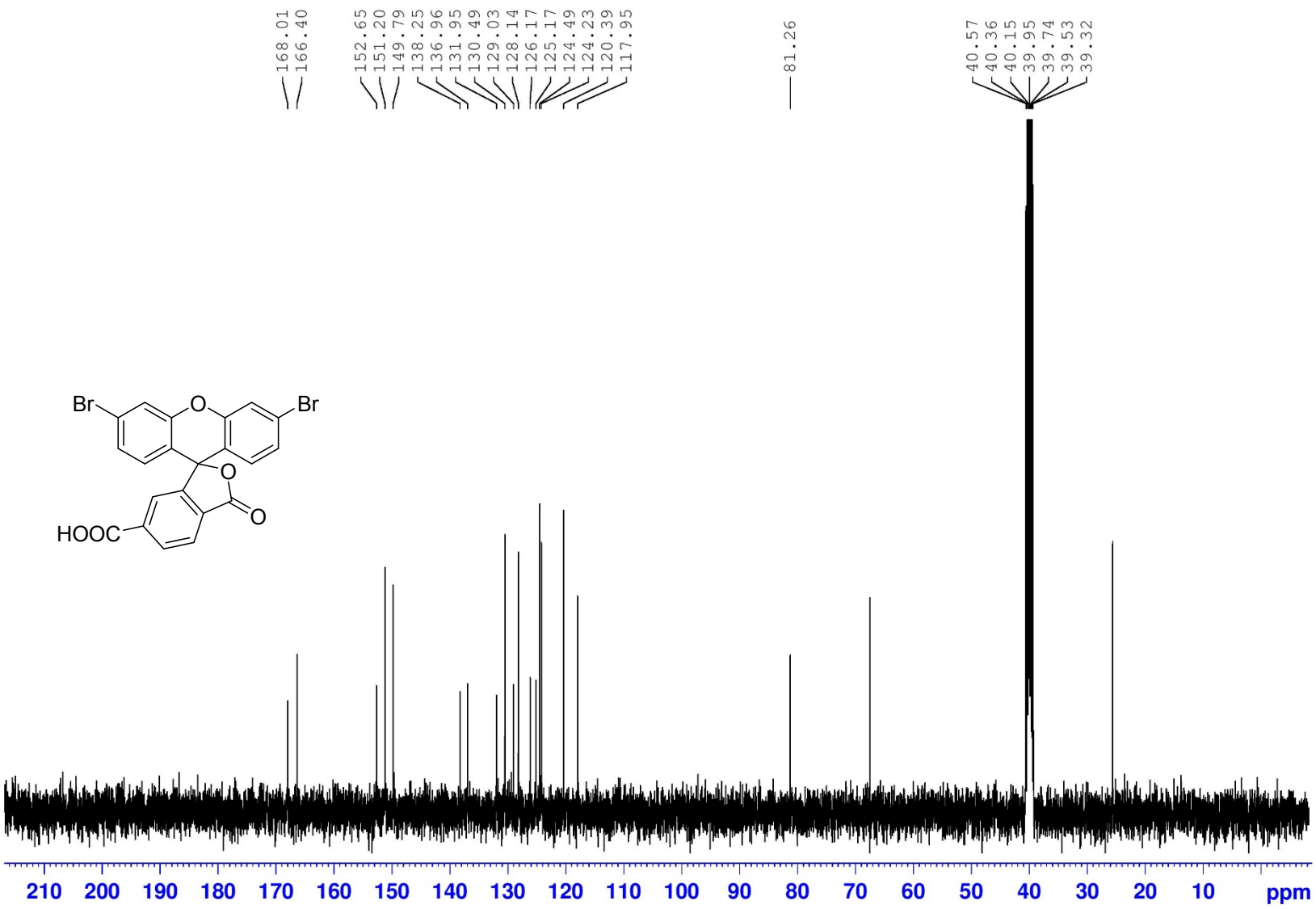

Figure S21. ^{13}C NMR spectrum of 3',6'-dibromo-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxylic acid **(6)**.

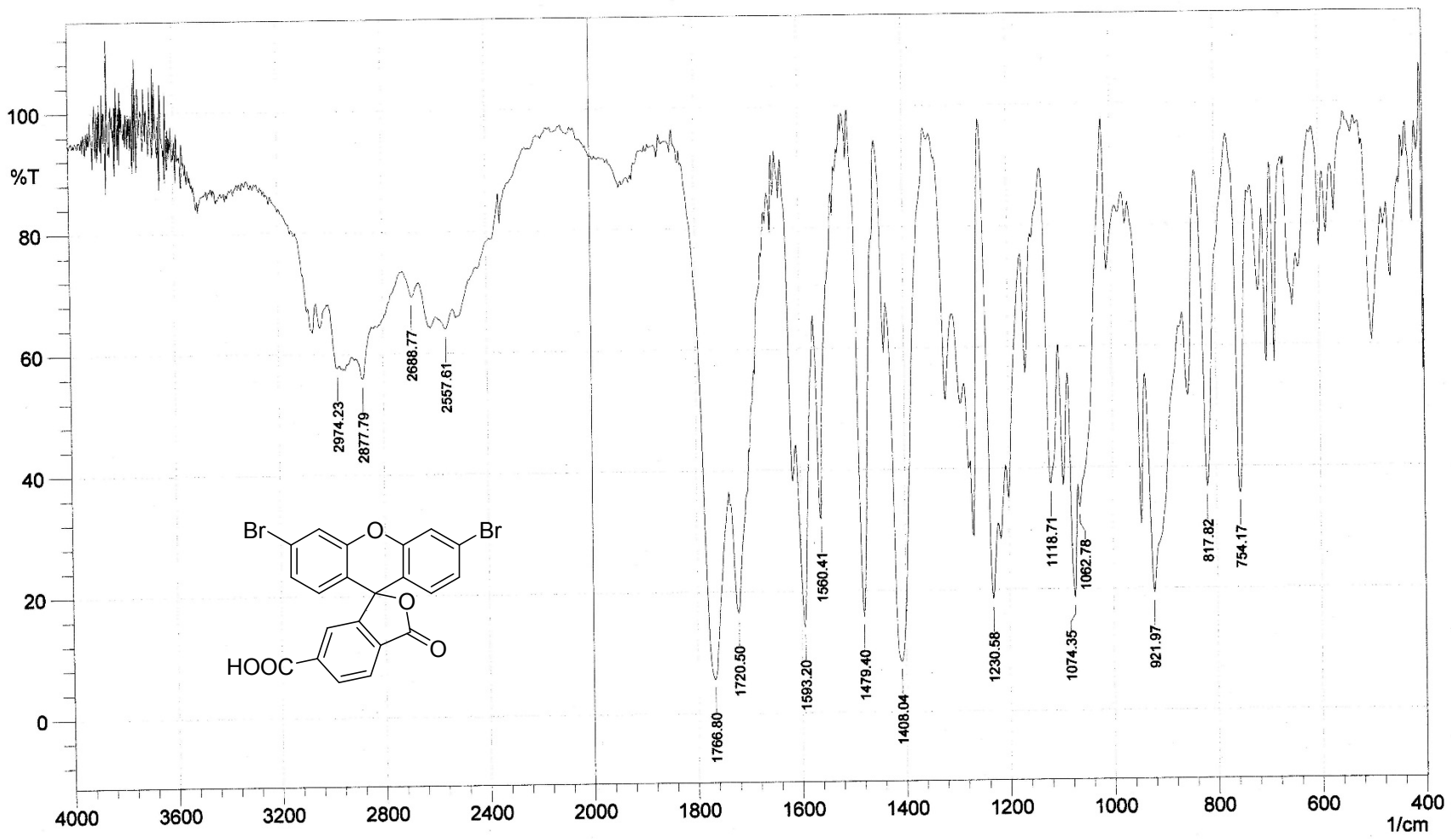

Figure S22. IR spectrum of 3',6'-dibromo-3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxylic acid **(6)**.

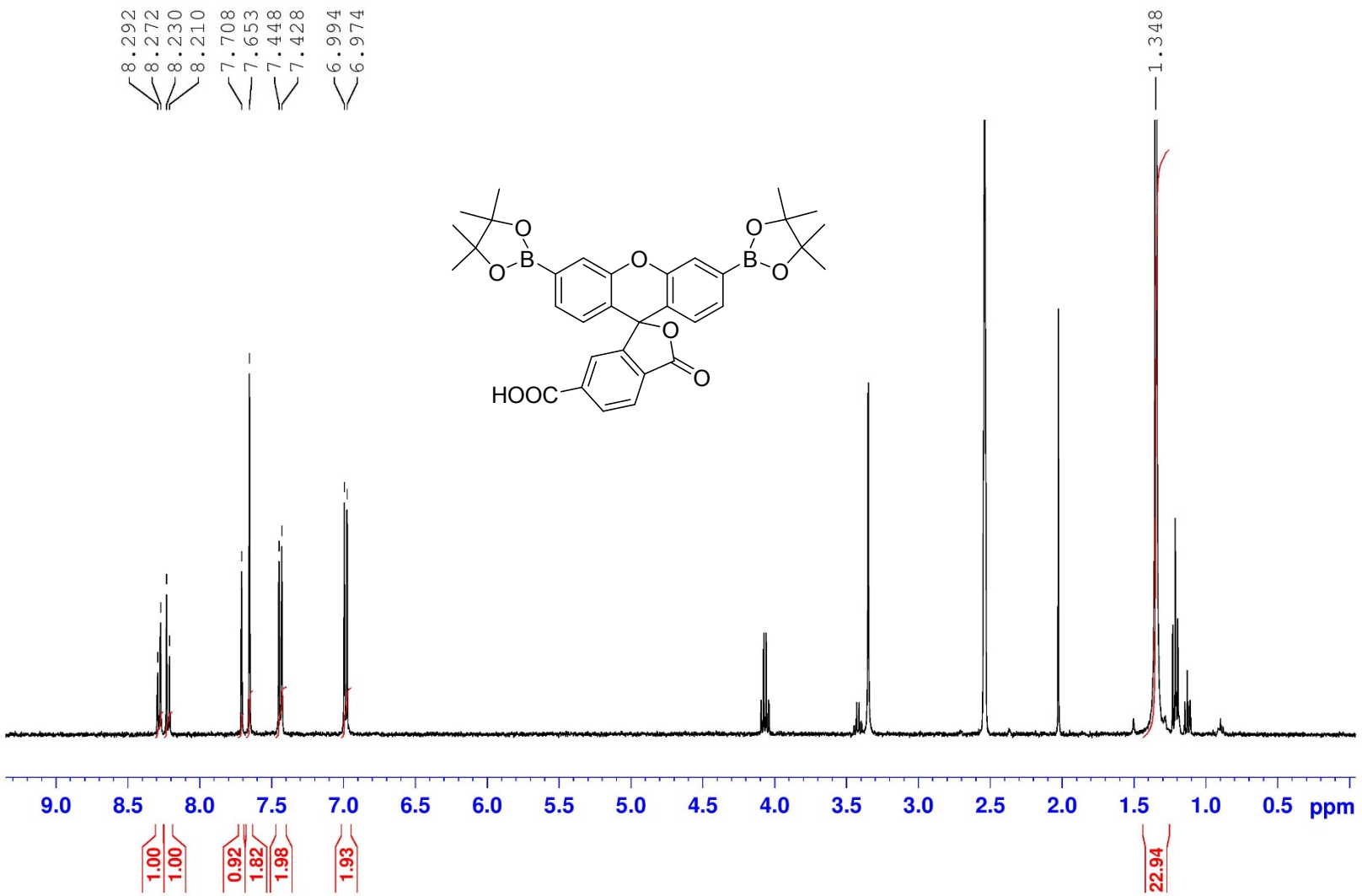

Figure S23. ^{1}H NMR spectrum of 3-oxo-3',6'-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxylic acid **(7)**.

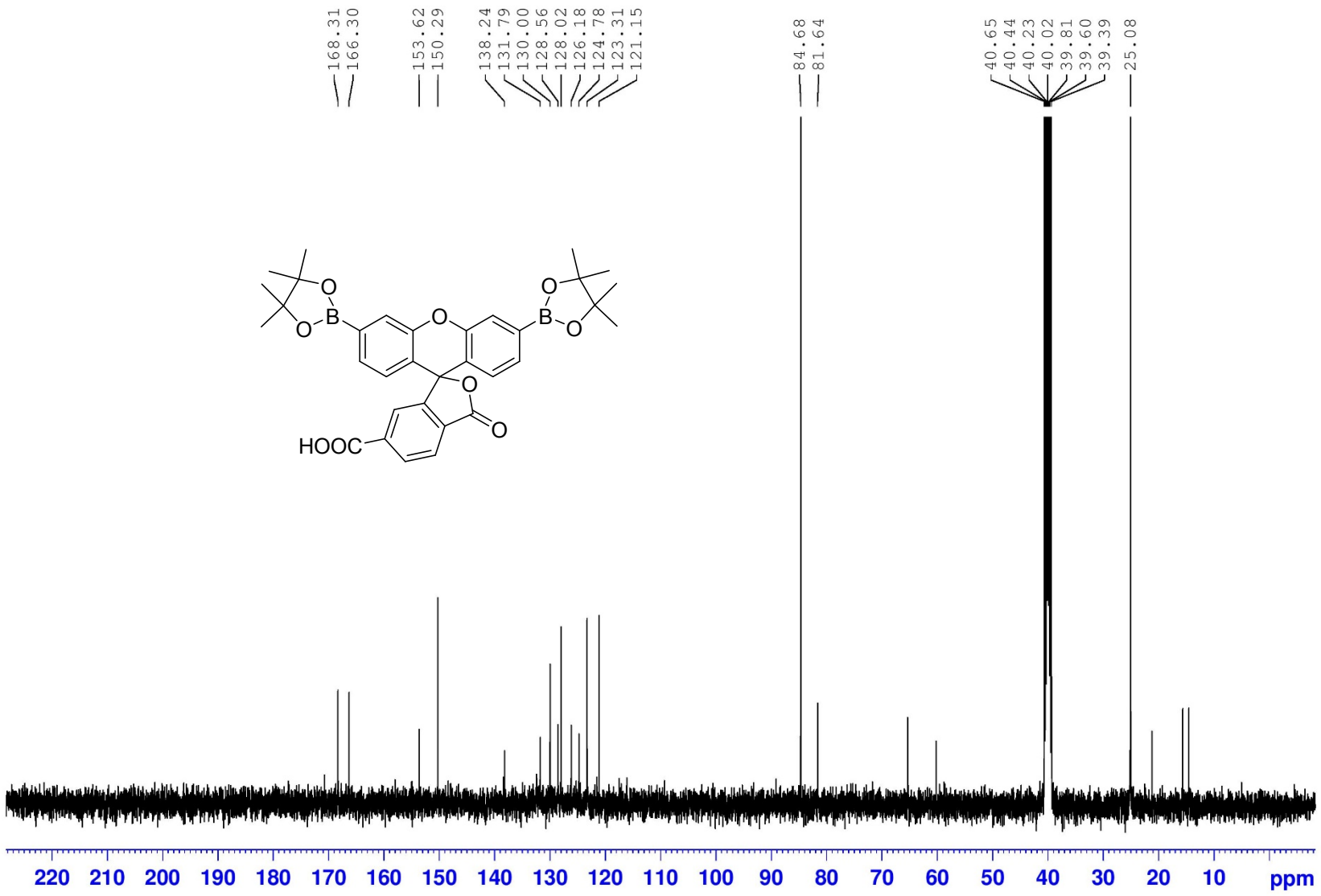

Figure S24. ^{13}C NMR spectrum of 3-oxo-3',6'-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxylic acid **(7)**.

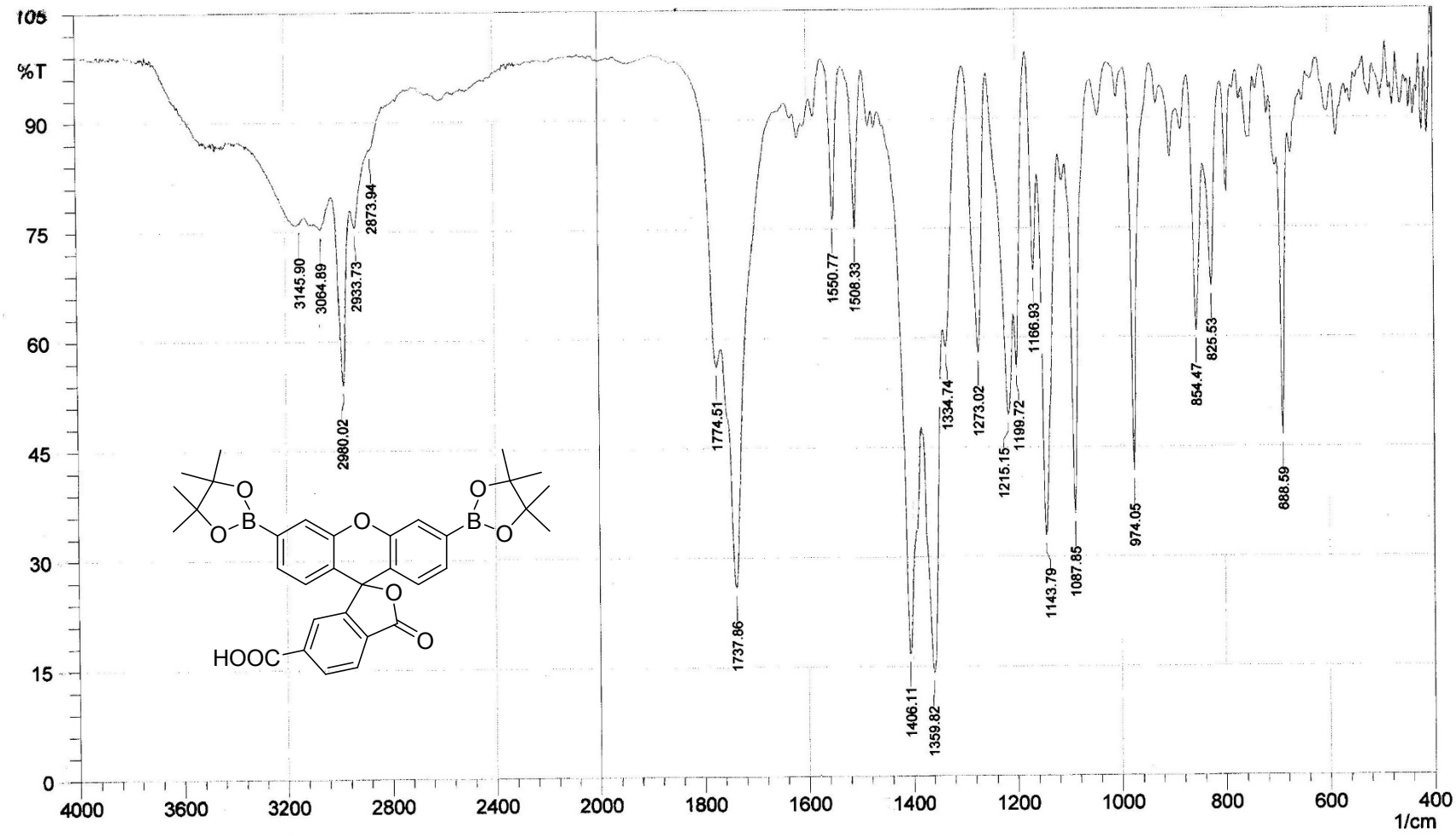

Figure S25. IR spectrum of 3-oxo-3',6'-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxylic acid **(7)**.

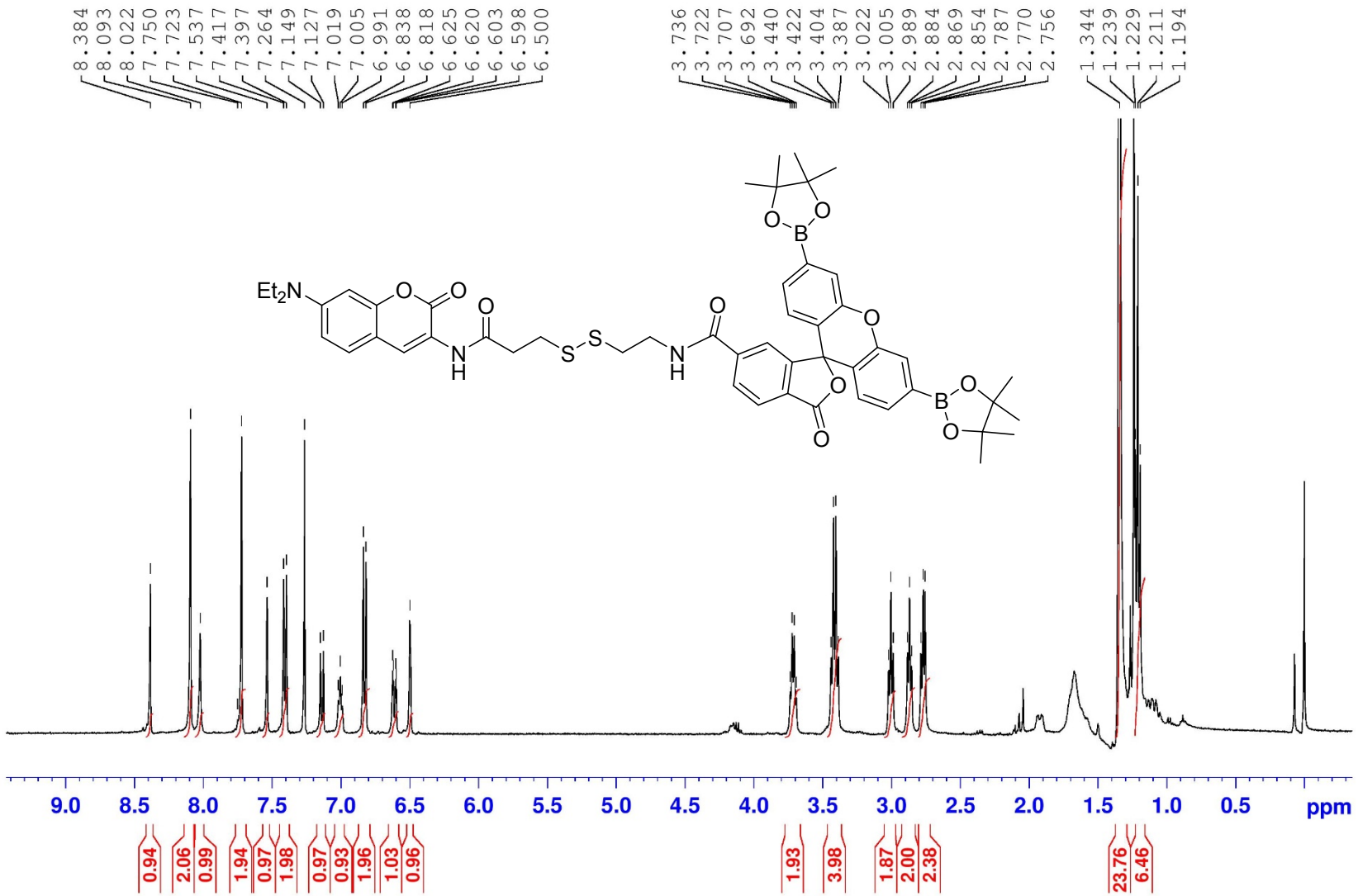

Figure S26. ^{1}H NMR spectrum of N-(2-((3-((7-(diethylamino)-2-oxo-2H-chromen-3-yl)amino)-3-oxopropyl)disulfanyl)ethyl)-3-oxo-3',6'-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxamide **(8)**.

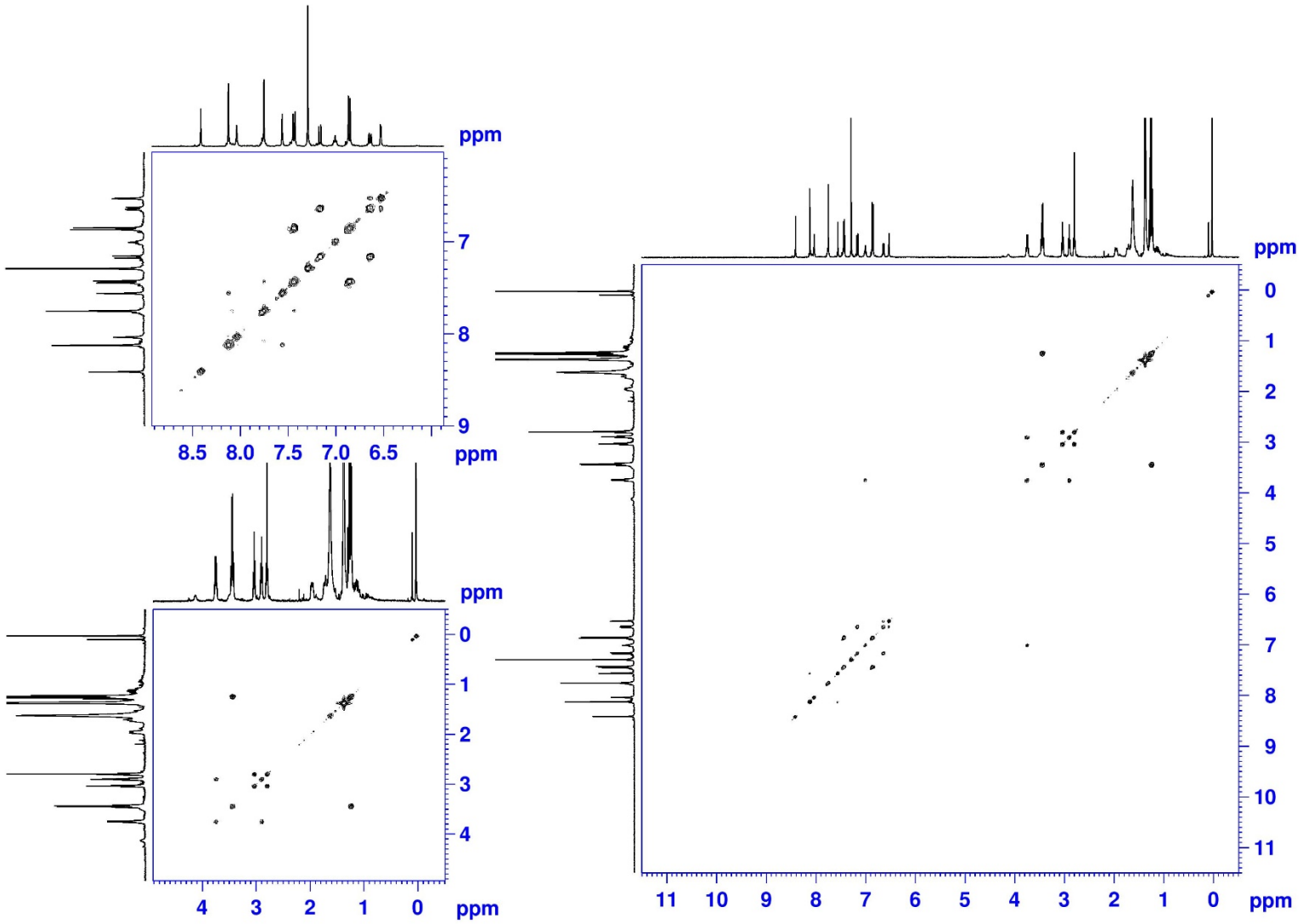

Figure S27. ^{1}H NMR 2-dimension correlation spectroscopy (COSY) spectrum of N-(2-((3-((7-(diethylamino)-2-oxo-2H-chromen-3-yl)amino)-3-oxopropyl)disulfanyl)ethyl)-3-oxo-3',6'-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxamide **(8)**.

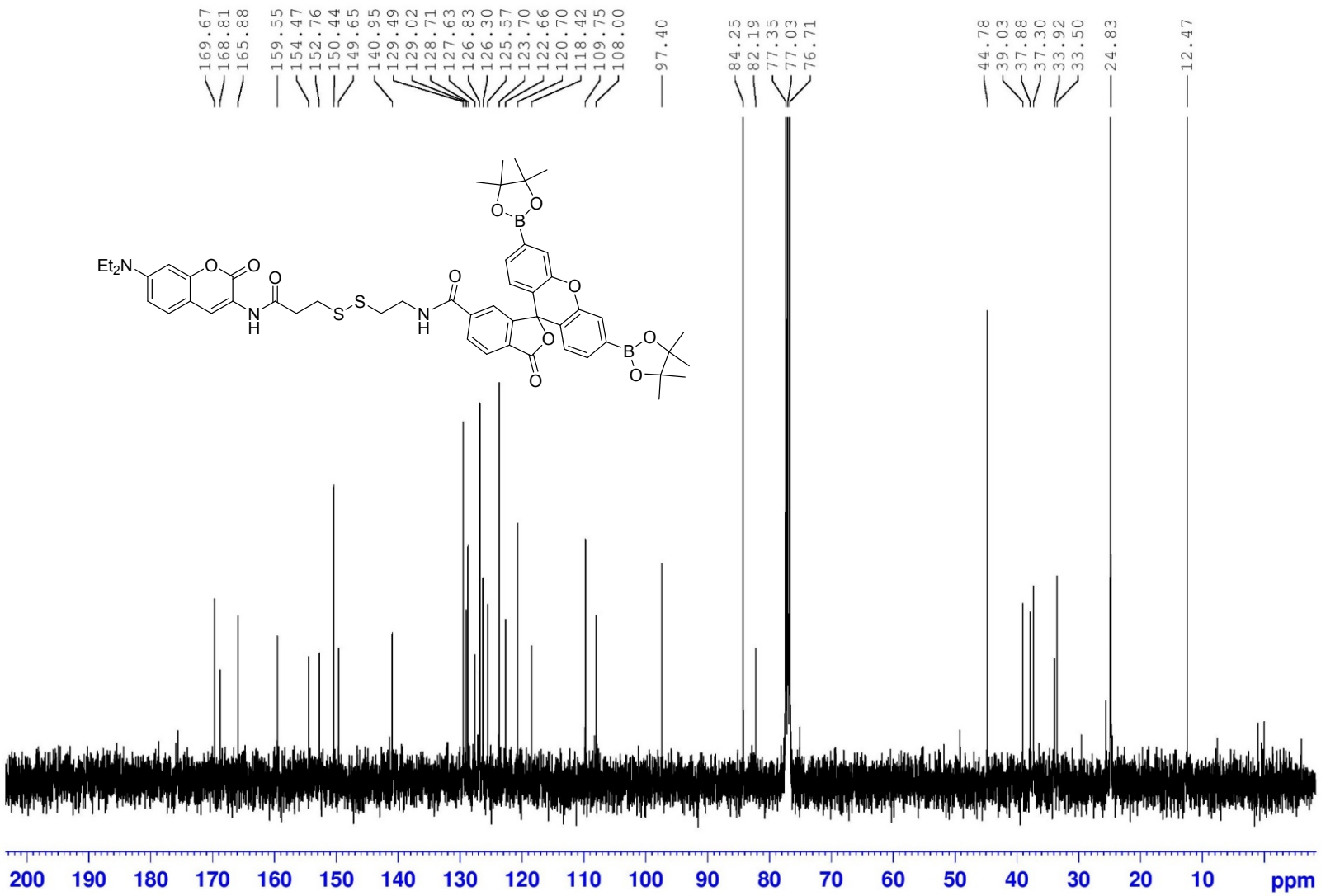

Figure S28. ^{13}C NMR spectrum of N-(2-((3-((7-(diethylamino)-2-oxo-2H-chromen-3-yl)amino)-3-oxopropyl)disulfanyl)ethyl)-3-oxo-3',6'-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxamide **(8)**.

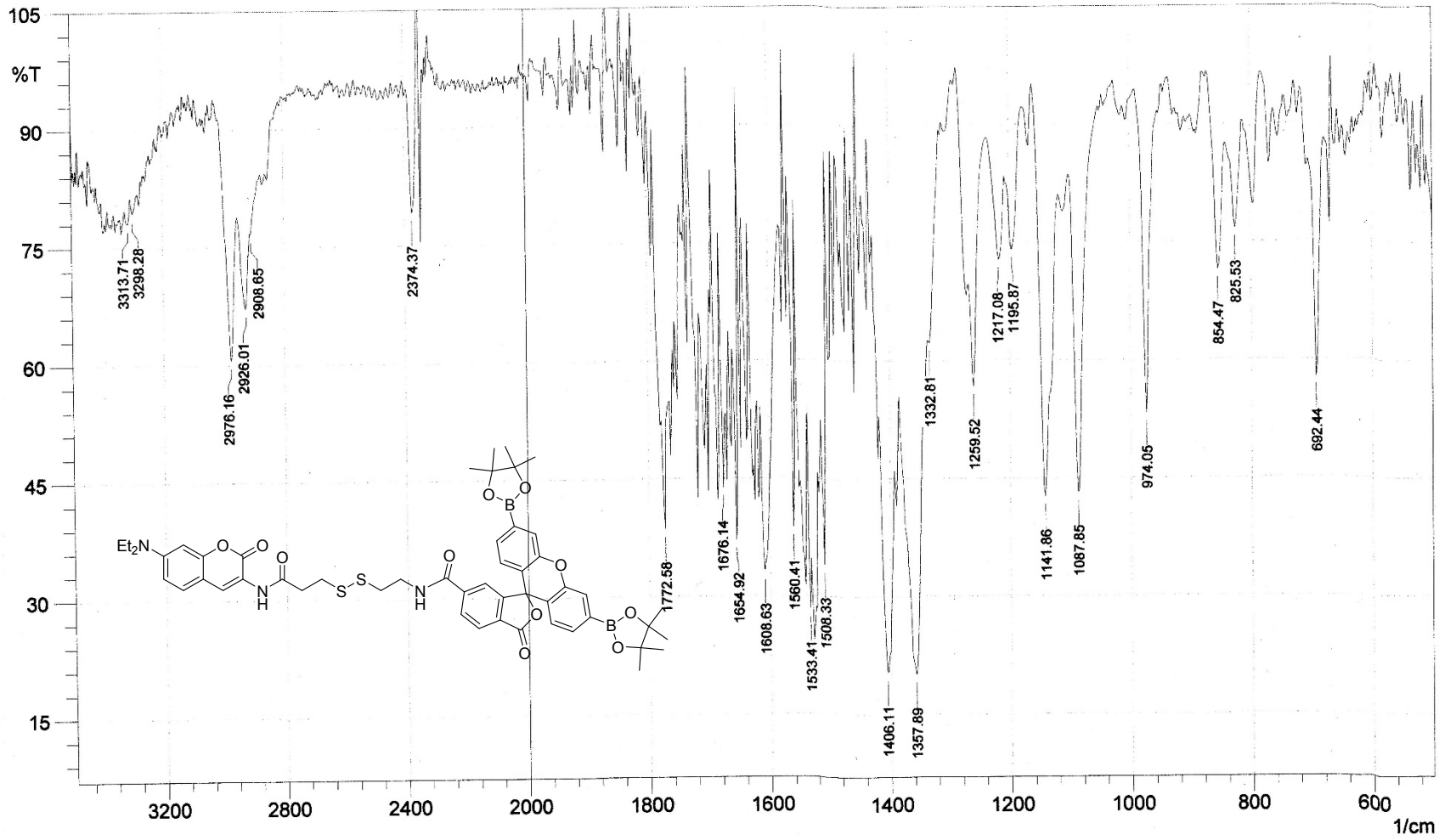

Figure S29. IR spectrum of N-(2-((3-((7-(diethylamino)-2-oxo-2H-chromen-3-yl)amino)-3-oxopropyl)disulfanyl)ethyl)-3-oxo-3',6'-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3H-spiro[isobenzofuran-1,9'-xanthene]-6-carboxamide **(8)**.

6. Reference

(1) M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A., Jr. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, Gaussian 09, Revision A.02, Gaussian, Inc., Wallingford CT, 2009.