Supporting Information for

Understanding the Selectivity of a Multi-Channel Fluorescent Probe for Peroxynitrite Over Hypochlorite

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Preparation of stock solutions of various ROS/RNS.

Hypochlorite (ClO^-) stock solution (50 mM in H_2O) was prepared by diluting commercial NaClO solution in deionized water (pH = 10). The solution concentration was determined by UV-Vis spectroscopy ($\varepsilon_{293\ nm}$= 350 $cm^{-1}M^{-1}$).

Peroxynitrite ($OONO^-$) stock solution (37 mM in H_2O at pH > 10) was prepared following procedure by Pryor et al. (Anal. Biochem. 1996, 236, 242-249). The solution concentration was determined by UV-Vis spectroscopy ($\varepsilon_{302\ nm}$= 1,670 $cm^{-1}M^{-1}$).

Nitric Oxide (NO) stock solution (1.9 mM in H_2O) was generated by bubbling a stream of nitric oxide gas into deoxygenated deionized H_2O for 15 min. The nitric oxide gas was produced by dropping dilute H_2SO_4 (2 M) solution onto $NaNO_2$ solid and passed through 5 wt% NaOH solution to remove NO_2.

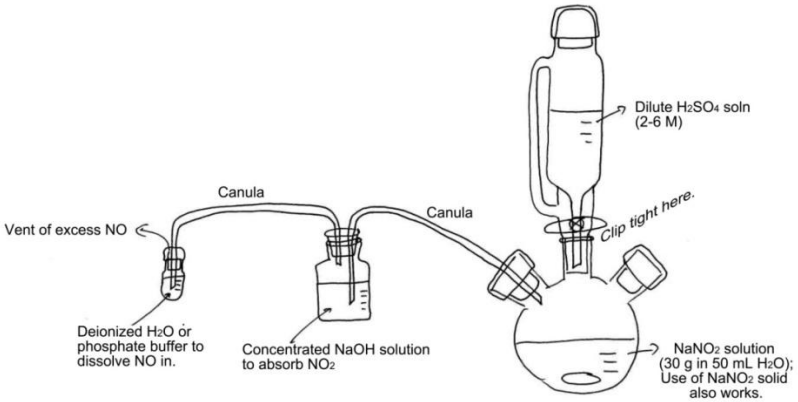

Figure S1. Setup for preparation of a nitric oxide stock solution. *Note: 1) the entire system should be thoroughly deoxygenated with inert gas, i.e. N_2 or Ar; 2) H_2SO_4 solution should be dropped into a vigorously stirring $NaNO_2$ solution (or onto $NaNO_2$ solid) very slowly at the very beginning to avoid sudden build-up of internal gas pressure; 3) NaOH solution to wash off NO_2 can be replaced by NaOH pellets; 4) the system should be set up in a fume hood with good ventilation.*

Superoxide($O_2^{\cdot-}$) stock solution (50 mM in DMF) was prepared by dissolving solid KO_2 in anhydrous DMF. Presence of H_2O in DMF leads to disproportionation of $O_2^{\cdot-}$.

Hydroxyl radical ($HO^{\cdot}$) was generated *in situ* by addition of an aliquot of $Fe(ClO)_4$ solution (200 mM in H_2O) into a solution containing excess H_2O_2.

Singlet oxygen ($^1O_2$) was generated *in situ* by addition of an aliquot of hypochlorite stock into a solution containing excess H_2O_2.

Hydrogen peroxide (H_2O_2) stock solution (50 mM in H_2O) was prepared by dilution of commercial 30% H_2O_2 solution in deionized H_2O.

Spectral studies

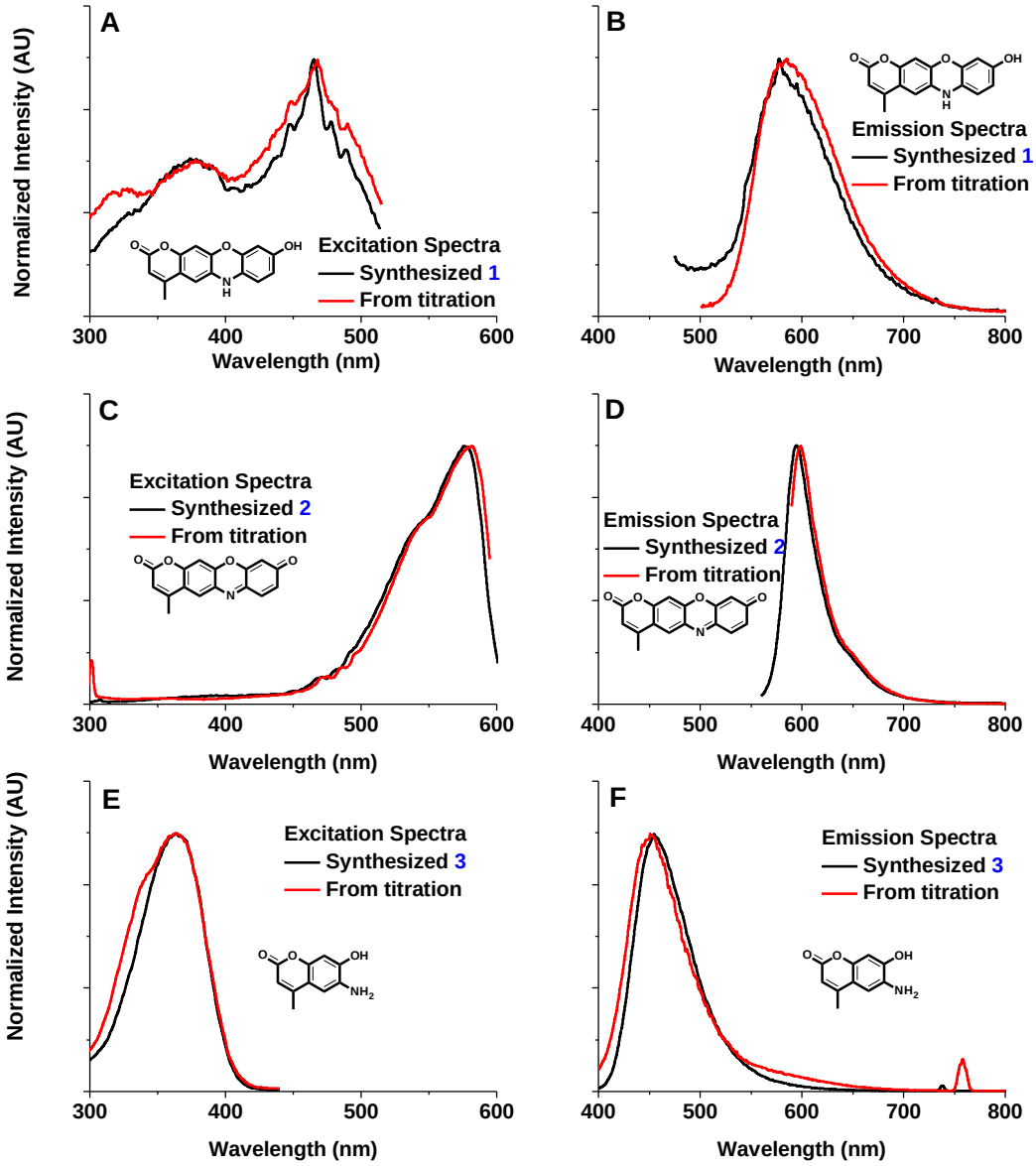

Figure S2. Comparison of fluorescence spectra of independently synthesized **1**, **2** and **3** with those extrapolated from fluorescence titration of probe **PN600** by hypochlorite or peroxynitrite, as a verification of their formation during titration.

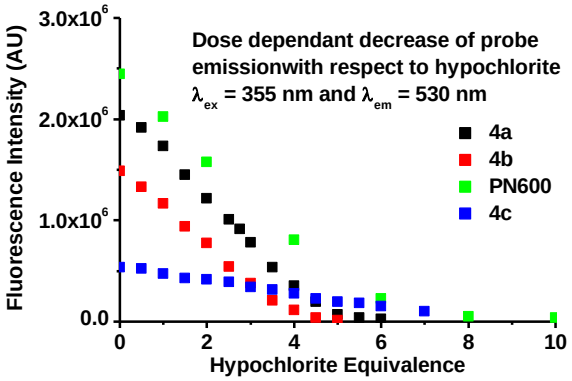

Figure S3. Dose dependant decrease of the probe emission upon addition of hypochlorite. *Note: it took similar amount of hypochlorite (ca. 5-7 equiv.) to consume probe **4a**, **4b** and **PN600**. In comparison, consumption of **4c** require significantly higher dose of hypochlorite. An explanation to this observation is that the oxidation product of probe **4a**, **4b** and **PN600** are not reactive toward hypochlorite and the oxidation production of **4c** is also reactive toward hypochlorite and therefore competes with **4c**.*

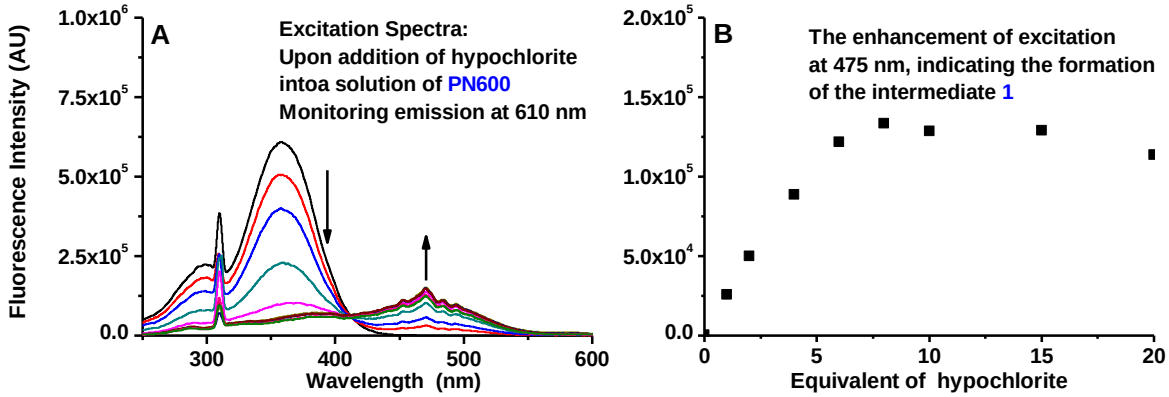

Figure S4. Excitation spectra monitoring emission at 610 nm, upon addition of an aliquot of hypochlorite into a solution of **PN600**, showing the decrease of excitation of **PN600** with a maximum at 350 nm and increase of excitation of **1** with a maximum at 467 nm as the dose of hypochlorite increases.

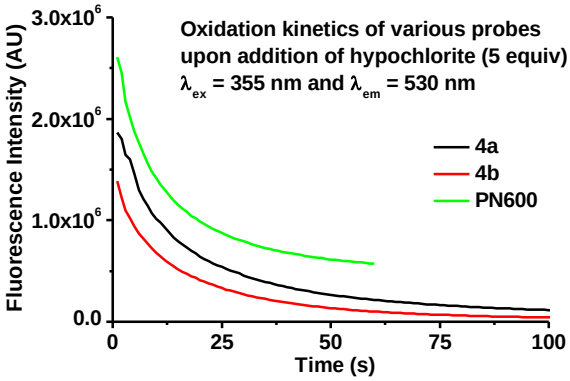

Figure S5. Oxidation kinetics of probe **4a**, **4b** and **PN600**, upon addition of an aliquot of hypochlorite (5 equiv.).

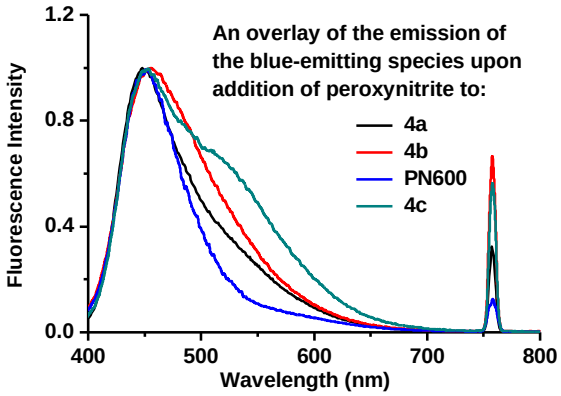

Figure S6. Overlay of the blue emissions (presumably from **3**) generated upon addition of an aliquot of peroxynitrite stock into a solution of probe **4a**, **4b**, **PN600** and **4c**, respectively. *Note: the presence of other species, which are also excitable by light of 355 nm and emit at longer wavelength than **3**, are responsible for the spectral difference in the range of 475 nm to 700 nm.*

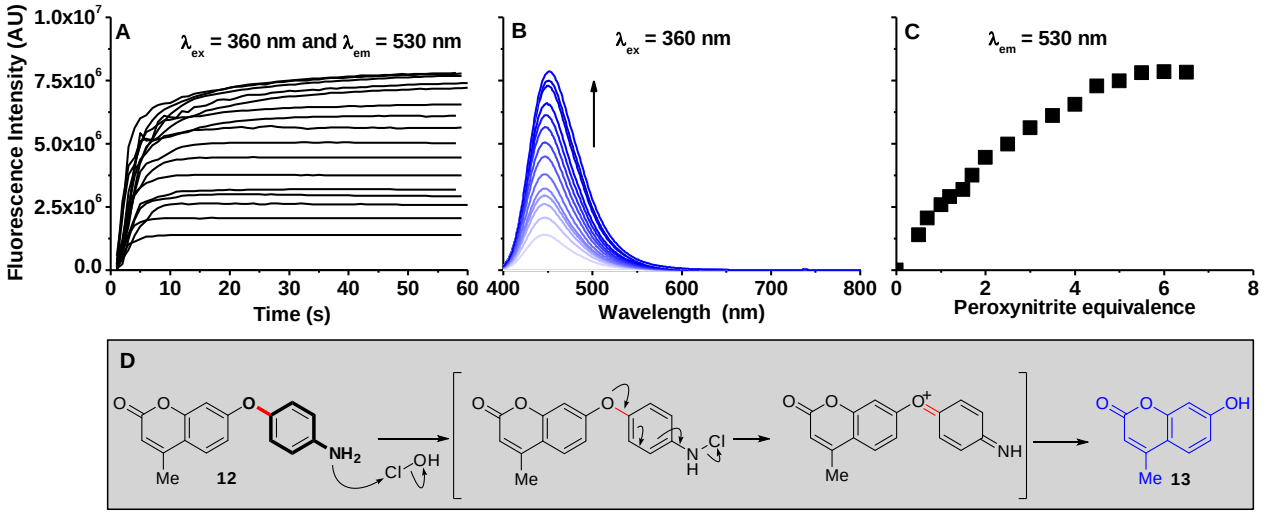

Figure S7. Oxidation of probe **12** by hypochlorite. A) Kinetics of the formation of **13**; B) emission spectra of a solution of probe **12**, upon addition of various equivalence of hypochlorite; C) dose dependent emission enhancement at 450 nm; D) a mechanistic explanation to the formation of **13** upon hypochlorite mediated oxidation of probe **12**.

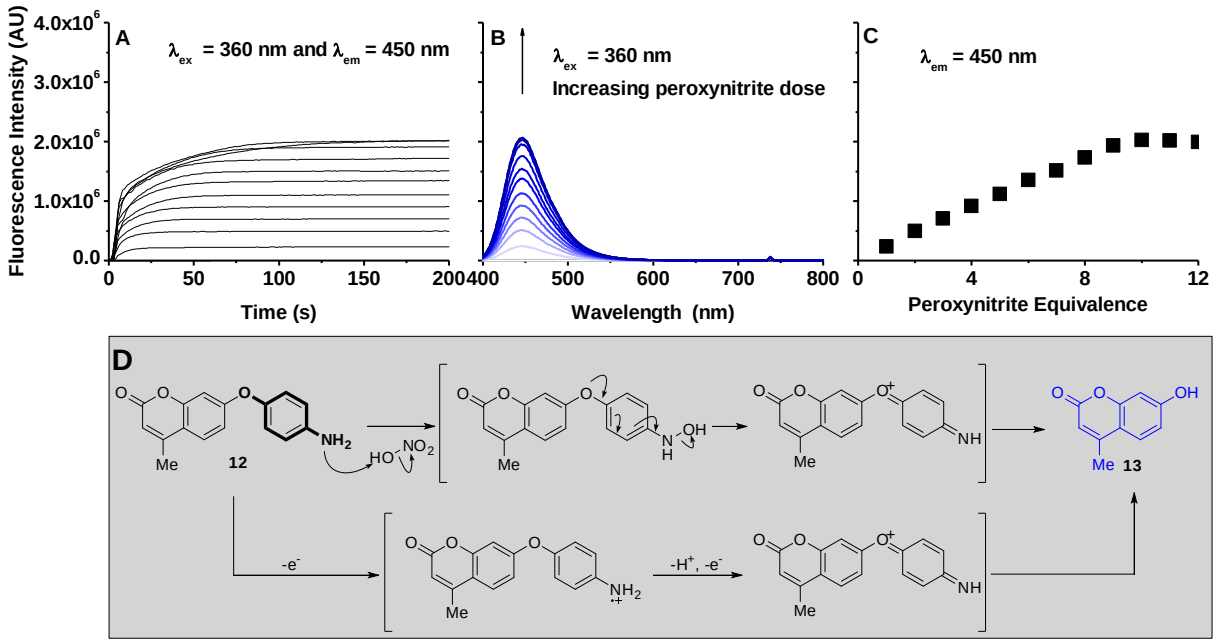

Figure S8. Oxidation of probe **12** by peroxynitrite. A) Kinetics of the formation of **13**; B) emission spectra of a solution of probe **12**, upon addition of various equivalence of peroxynitrite; C) dose dependent emission enhancement at 450 nm; D) a mechanistic explanation to the formation of **13** upon peroxynitrite mediated oxidation of probe **12**.

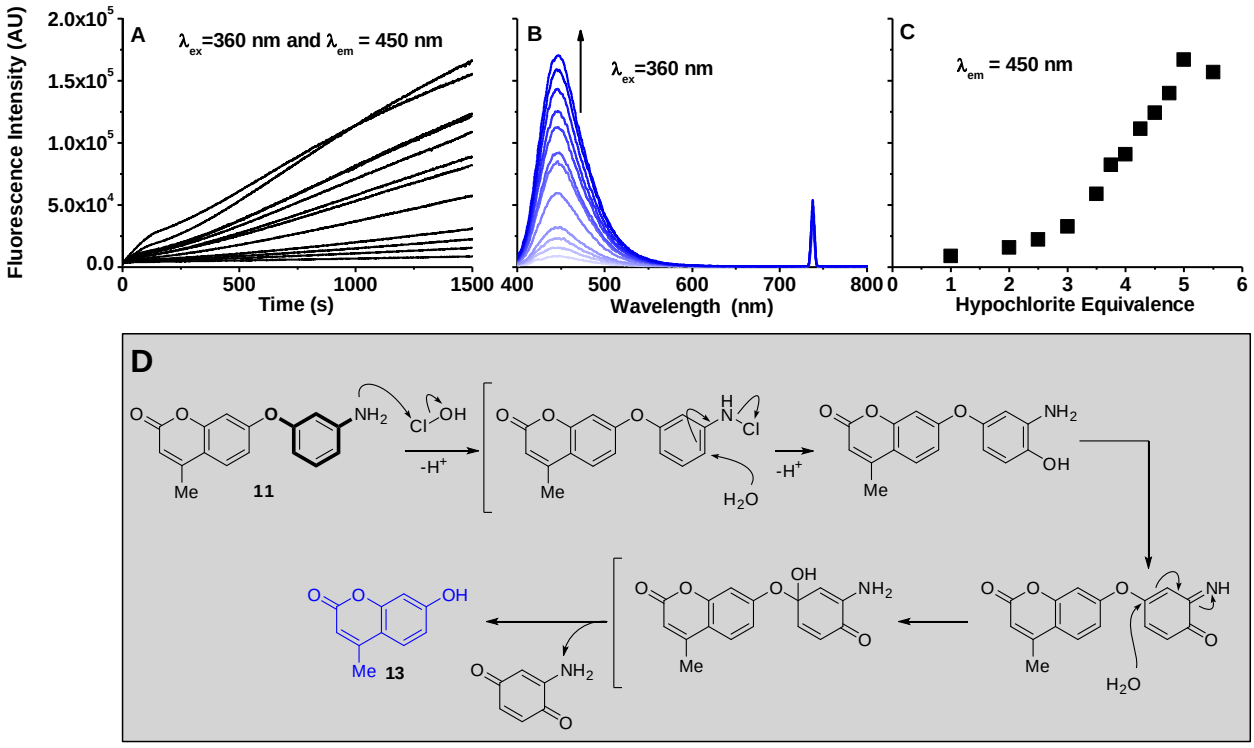

Figure S9. Oxidation of probe **11** by hypochlorite. A) Kinetics of the formation of **13**; B) emission spectra of a solution of probe **11**, upon addition of various equivalence of hypochlorite; C) dose dependent emission enhancement at 450 nm; D) a mechanistic explanation to the formation of **13** upon hypochlorite mediated oxidation of probe **11**. Note: probe **11** can be oxidized, but to a limited extend only and kinetics is much slower compared to peroxynitrite mediate oxidation of probe **11**.

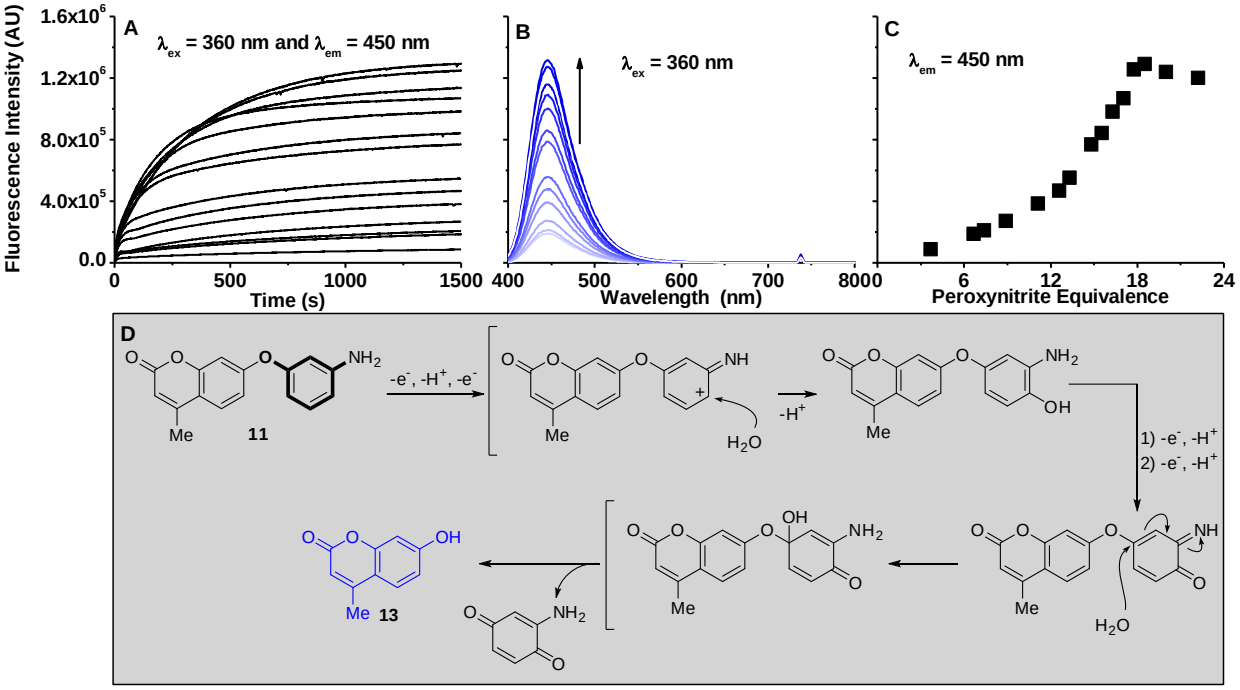

Figure S10. Oxidation of probe **11** by peroxynitrite. A) Kinetics of the formation of **13**; B) emission spectra of a solution of probe **11**, upon addition of various equivalence of peroxynitrite; C) dose dependent emission enhancement at 450 nm; D) a mechanistic explanation to the formation of **13** upon peroxynitrite mediated oxidation of probe **11**.

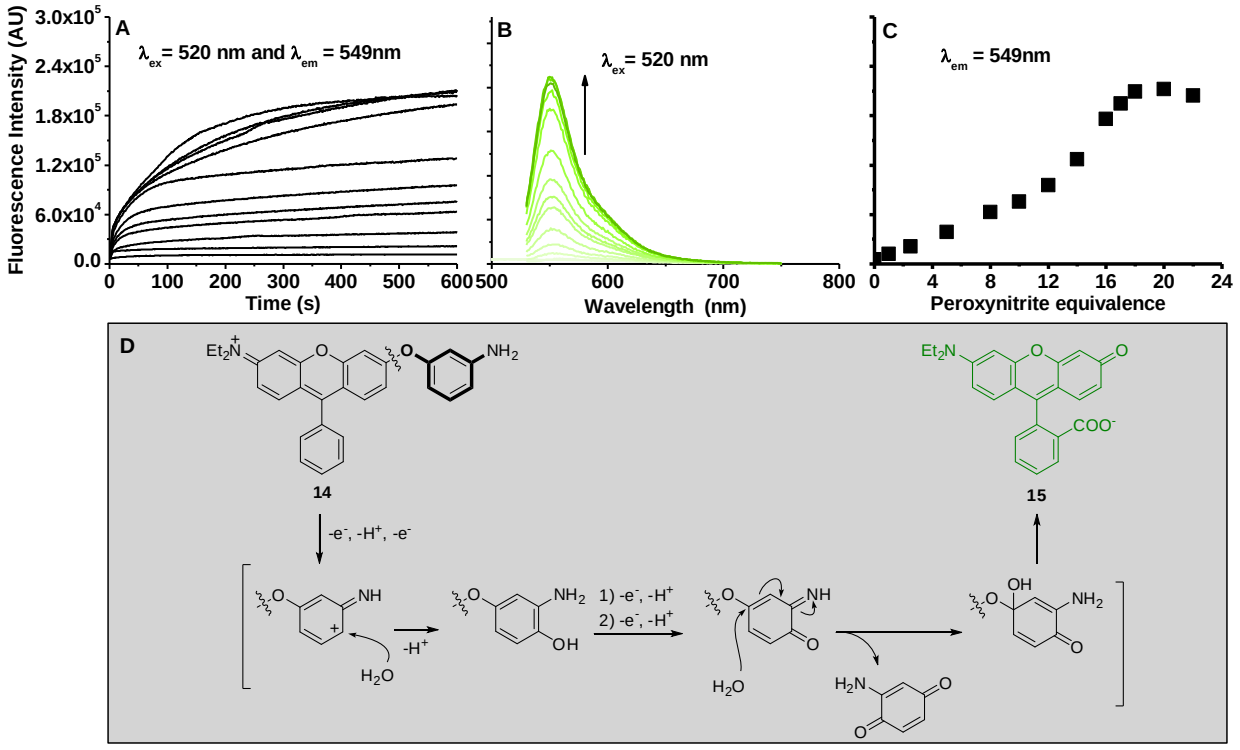

Figure S11. Oxidation of probe **14** by peroxynitrite. A) Kinetics of the formation of **15**; B) emission spectra of a solution of probe **14**, upon addition of various equivalence of peroxynitrite; C) dose dependent emission enhancement at 549 nm; D) a mechanistic explanation to the formation of **15** upon peroxynitrite mediated oxidation of probe **14**.

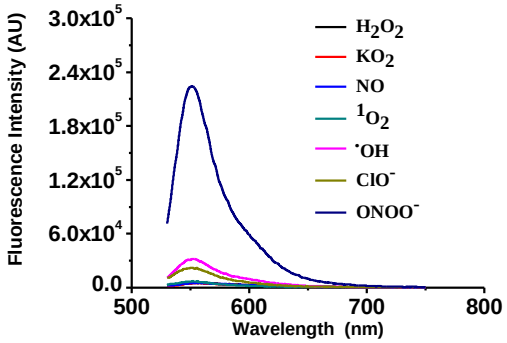

Figure S12. Selectivity study of probe **14** toward various reaction oxygen or nitrogen species.

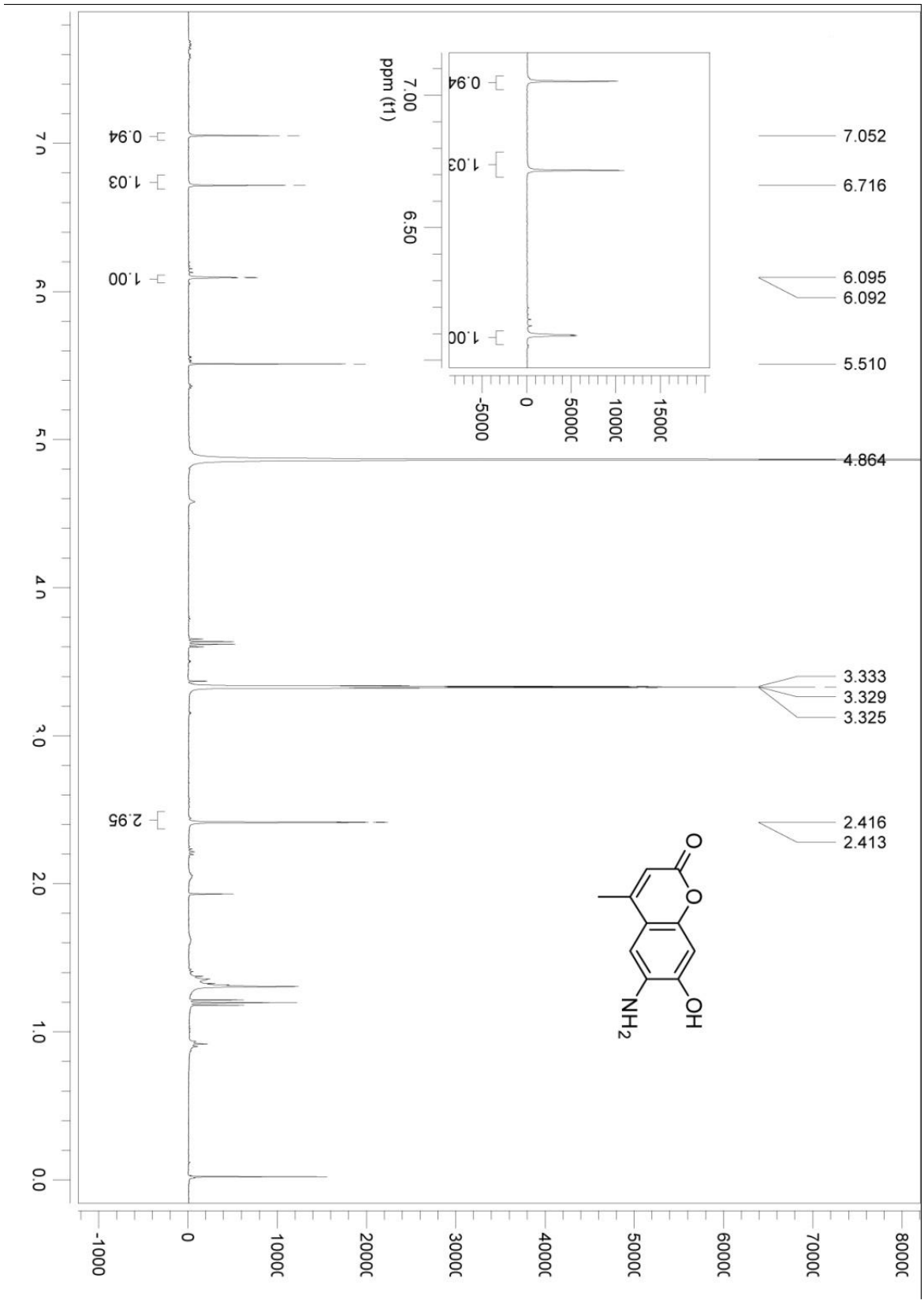

Figure S13. The ^{1}H-NMR spectrum of **3** in CD_3OD.

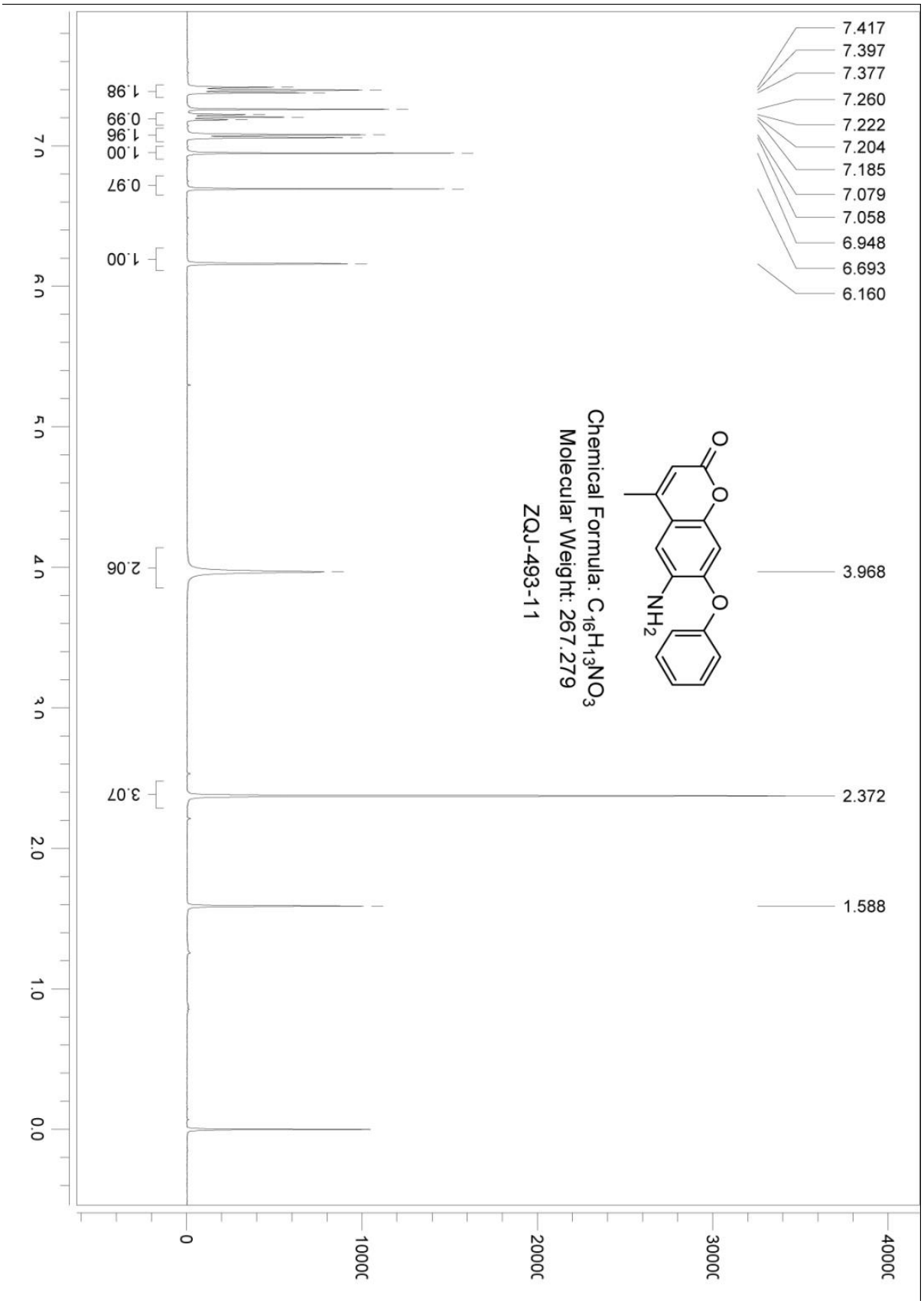

Figure S14. The ^{1}H-NMR spectrum of **4a** in $CDCl_3$.

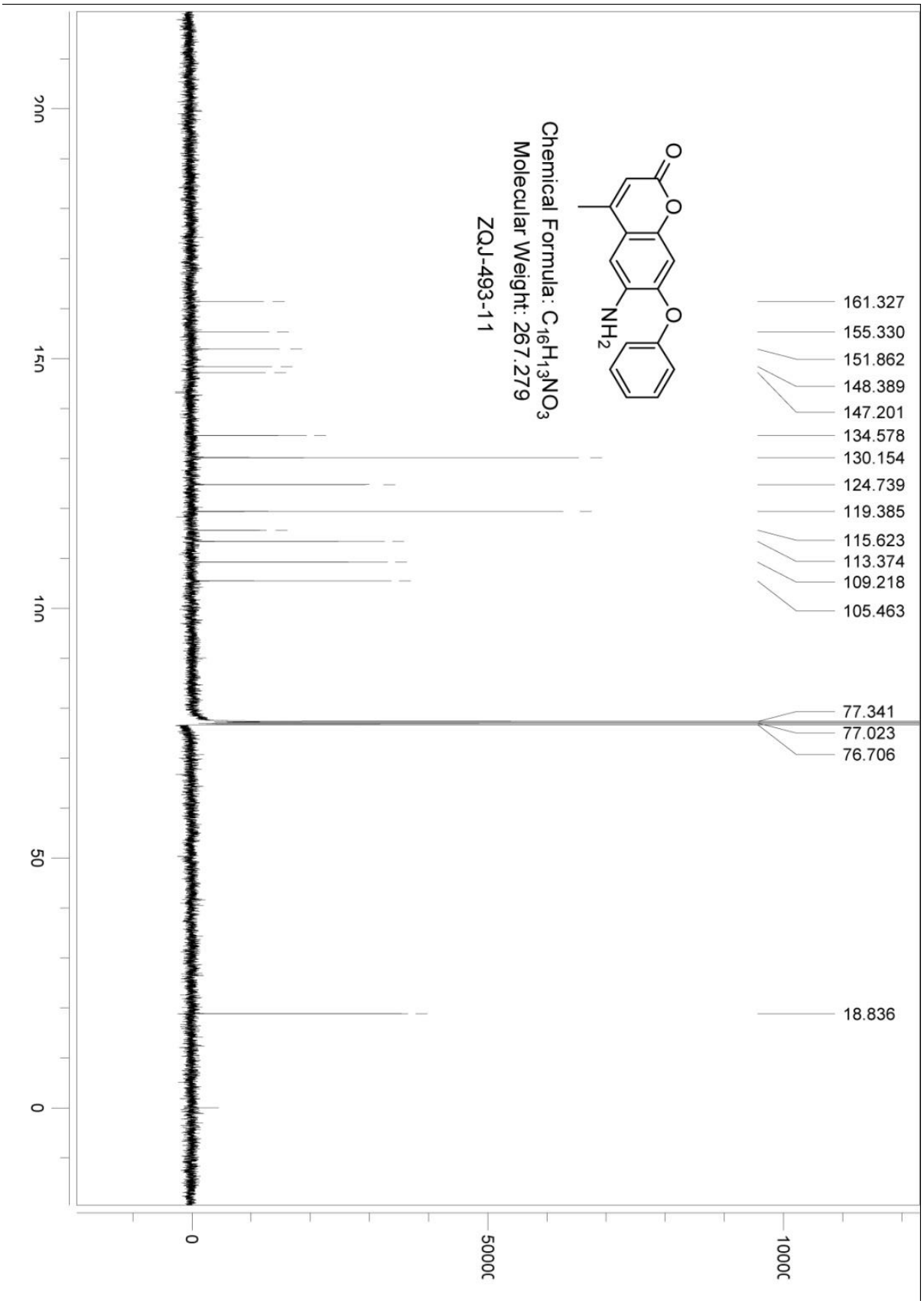

Figure S15. The ^{13}C-NMR spectrum of **4a** in $CDCl_3$.

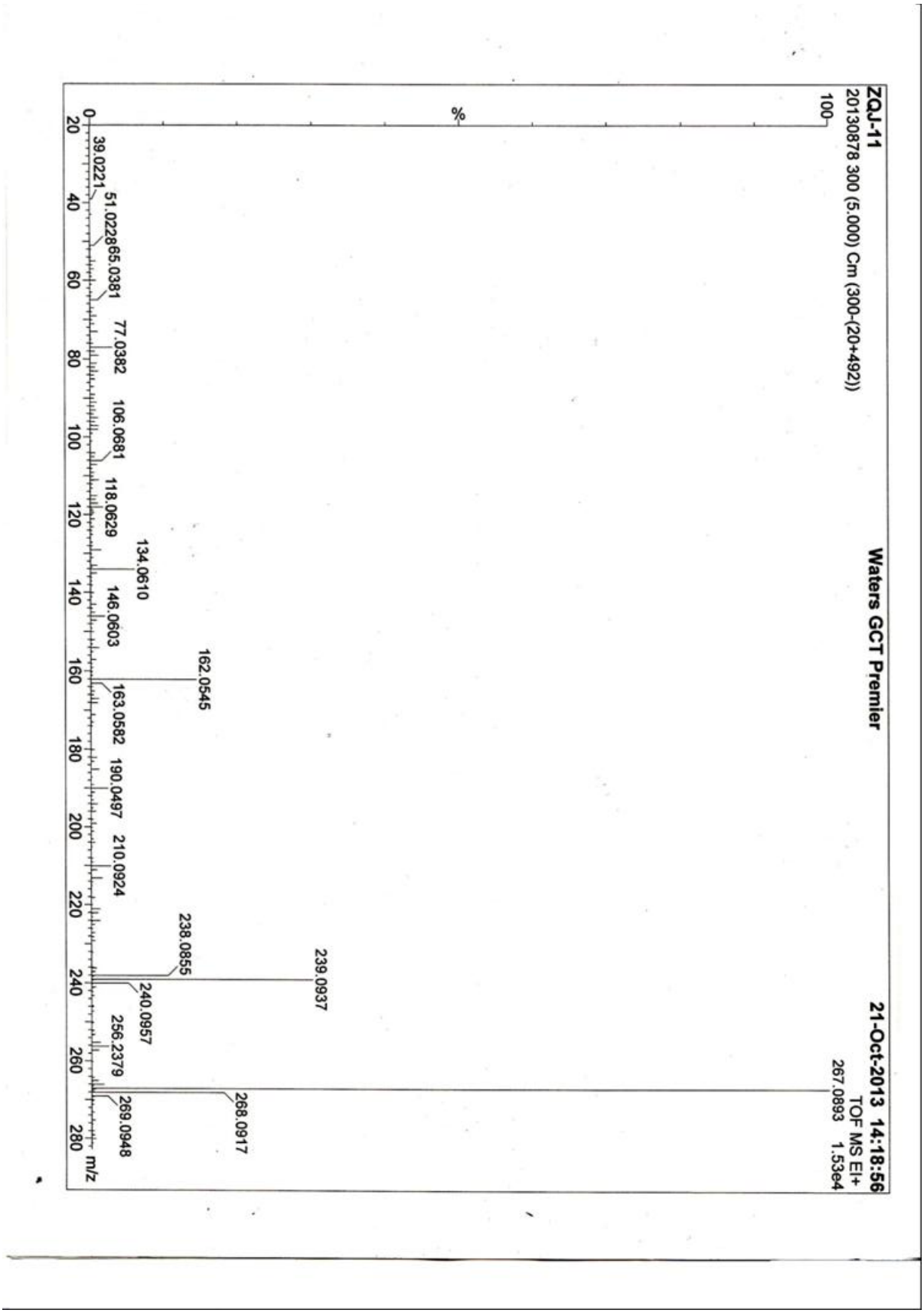

Figure S16. The HRMS spectrum of **4a**.

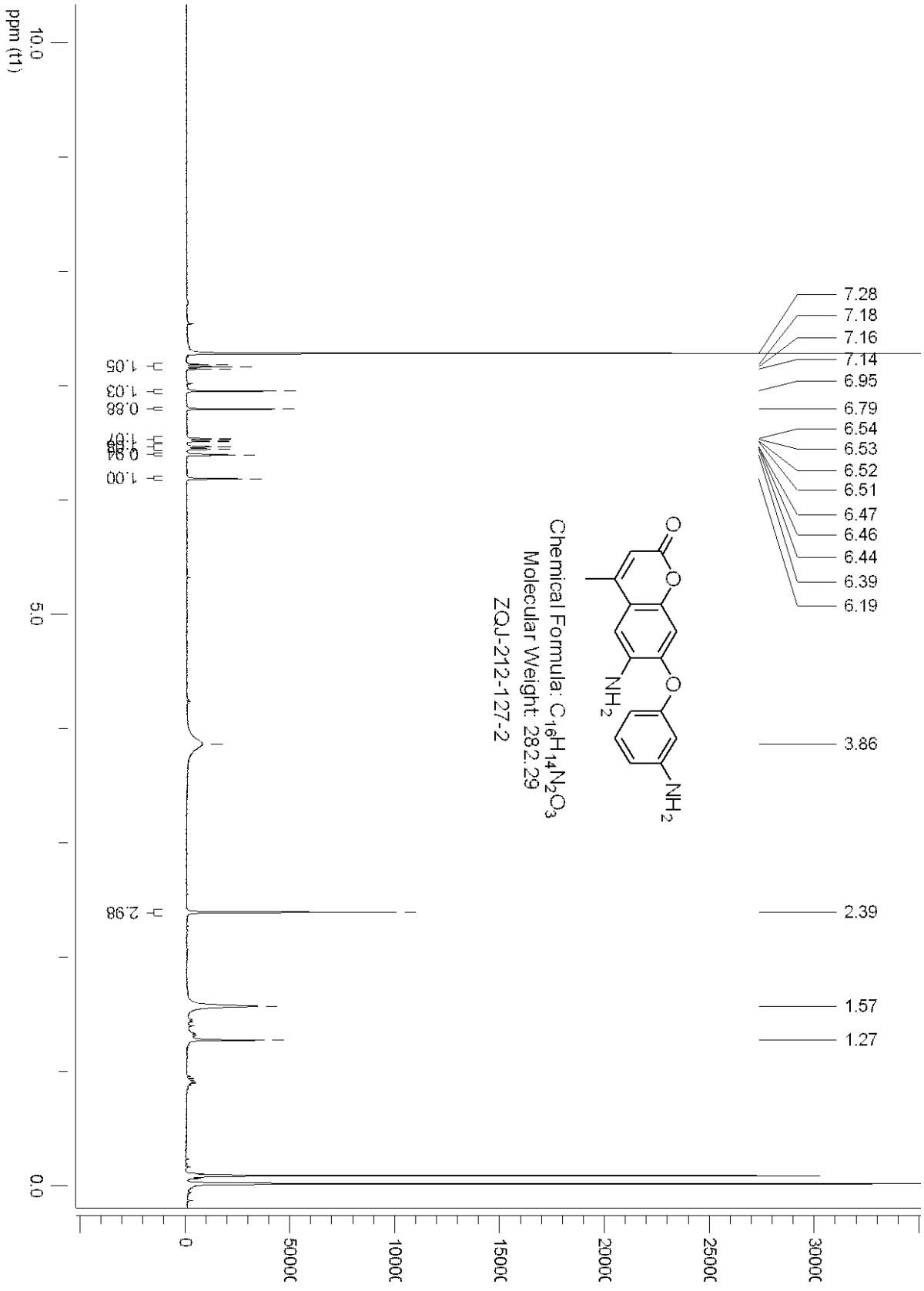

Figure S17. The ^{1}H-NMR spectrum of **4c** in $CDCl_3$.

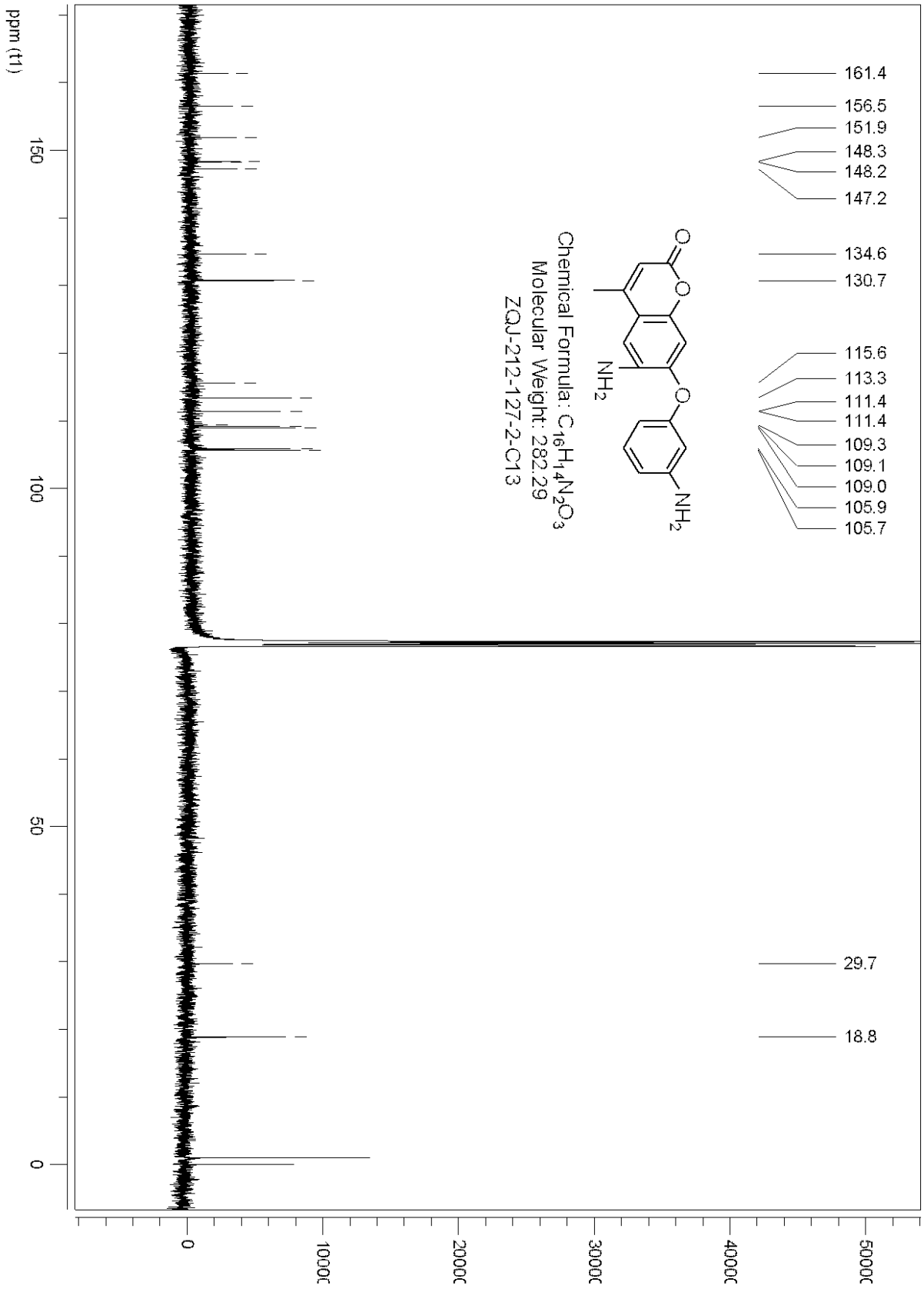

Figure S18. The ^{13}C-NMR spectrum of **4c** in $CDCl_3$.

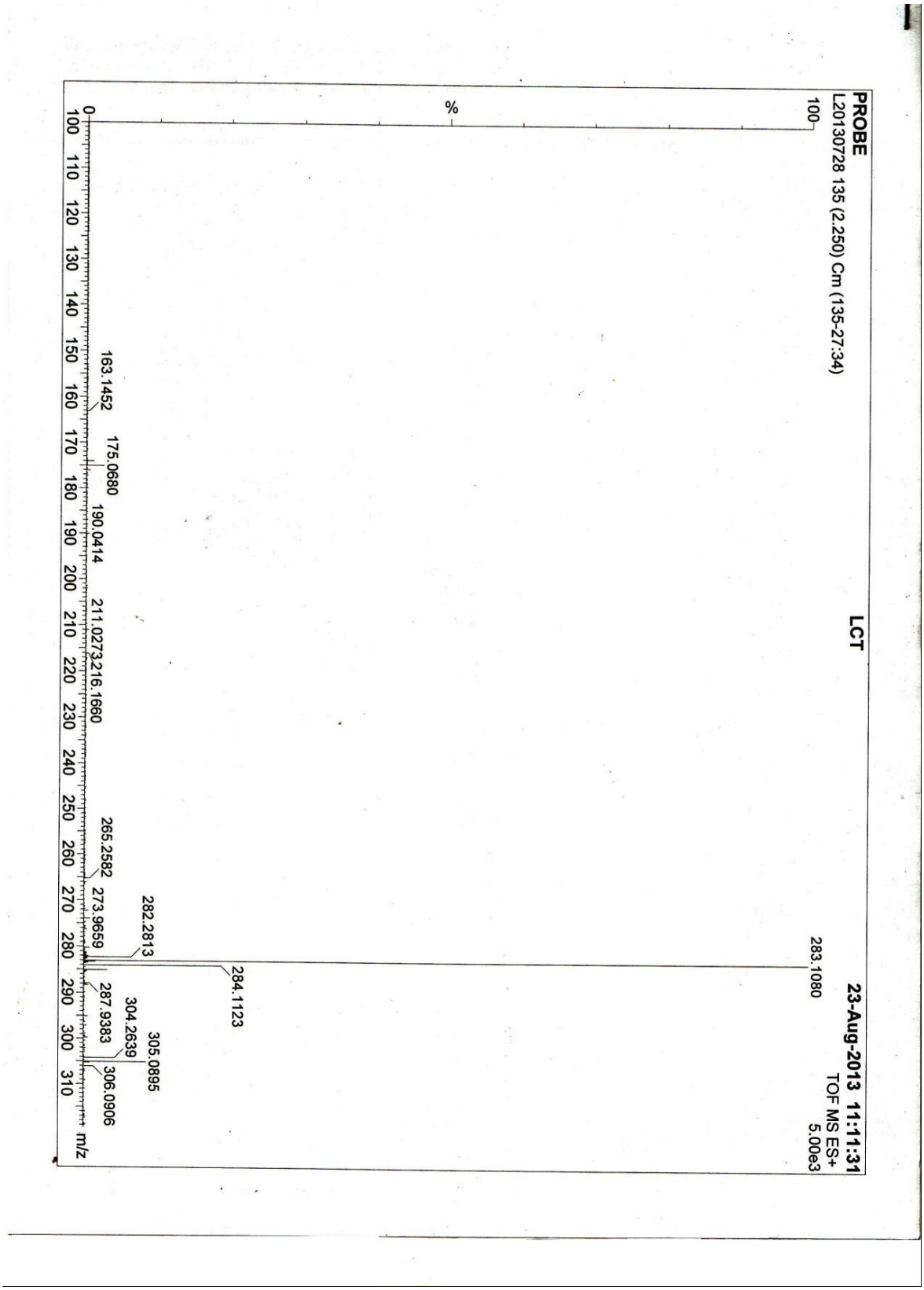

Figure S19. The HRMS spectrum of **4c**.

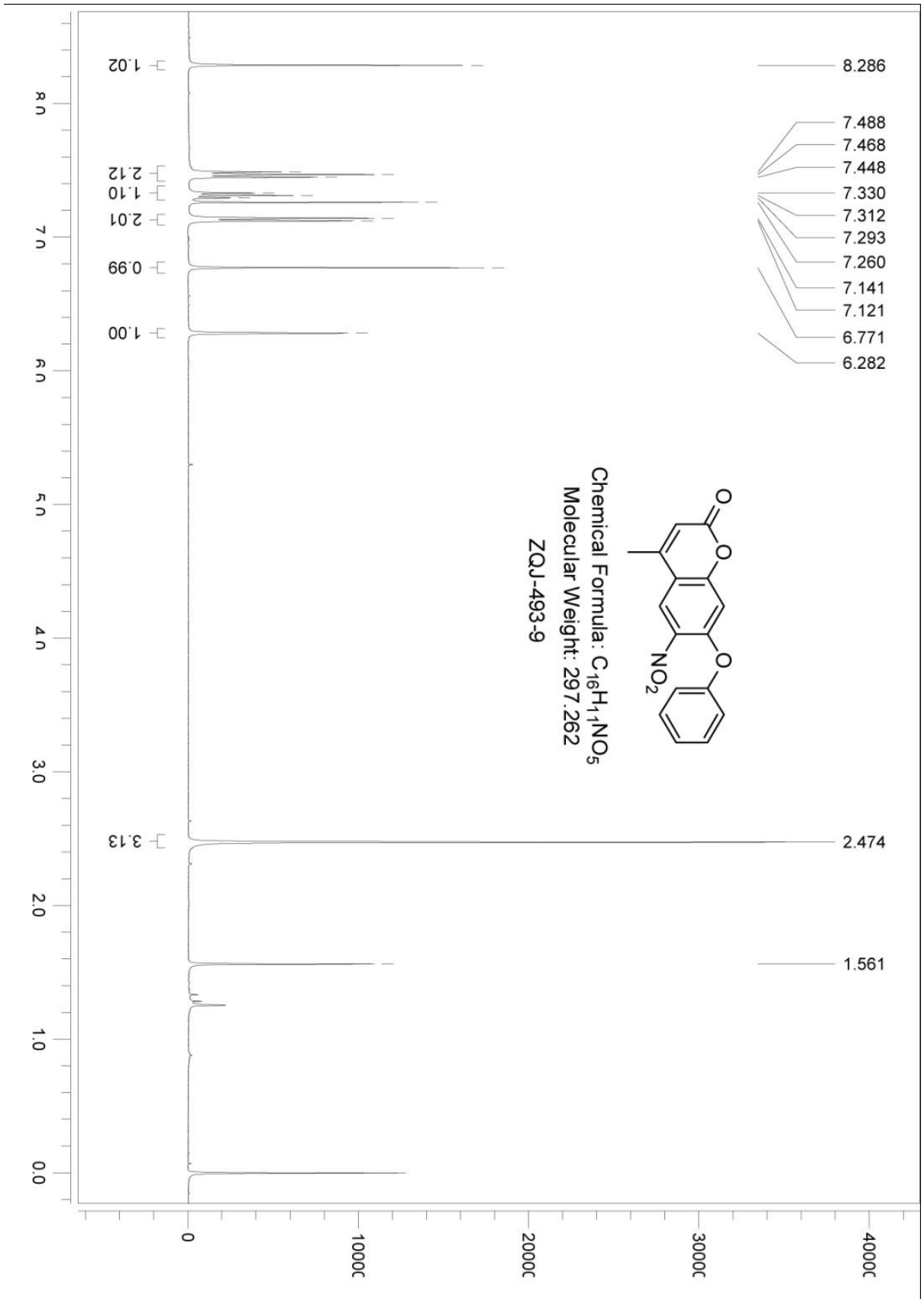

Figure S20. The ^{1}H-NMR spectrum of **7a** in $CDCl_3$.

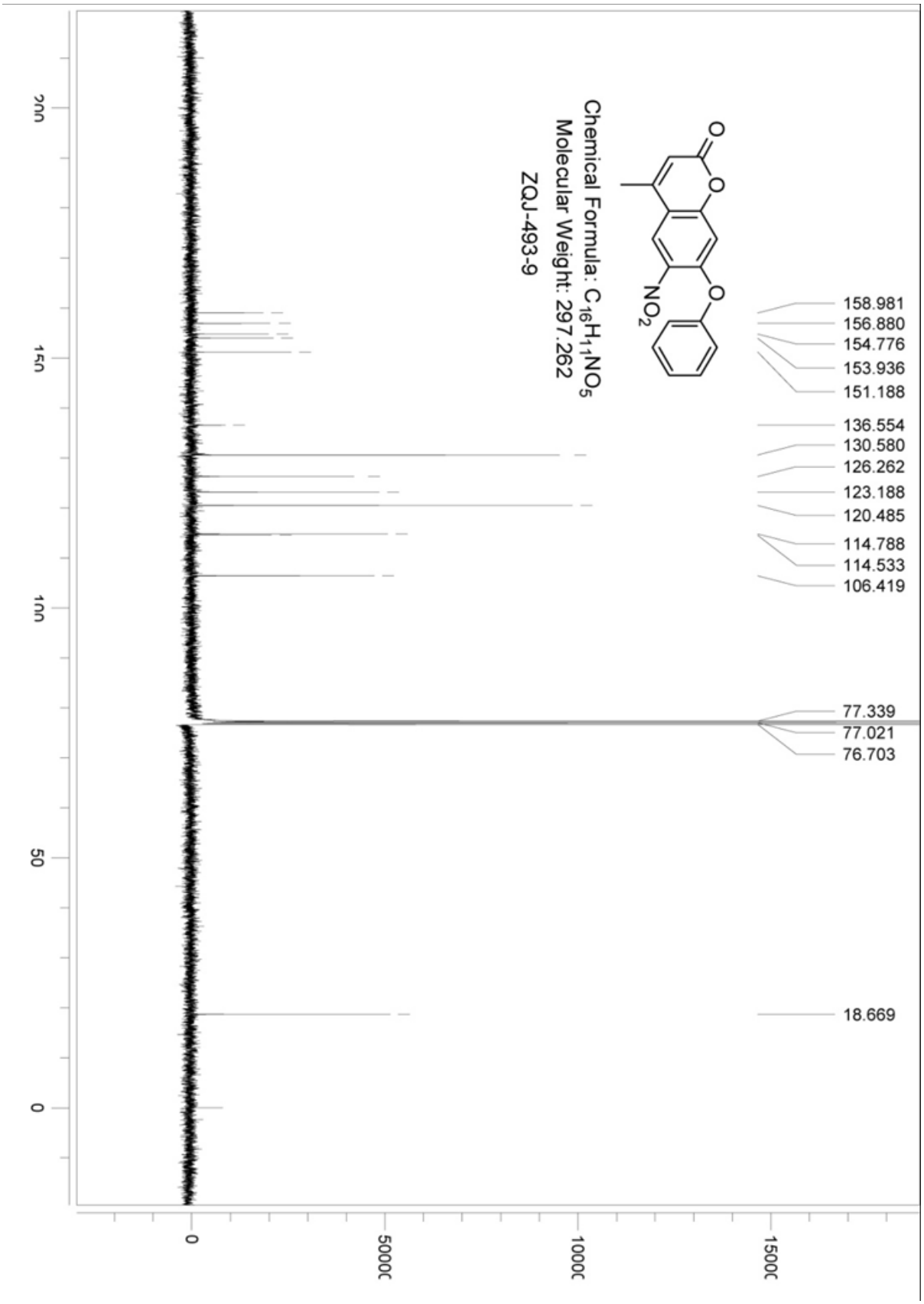

Figure S21. The ^{13}C-NMR spectrum of **7a** in $CDCl_3$.

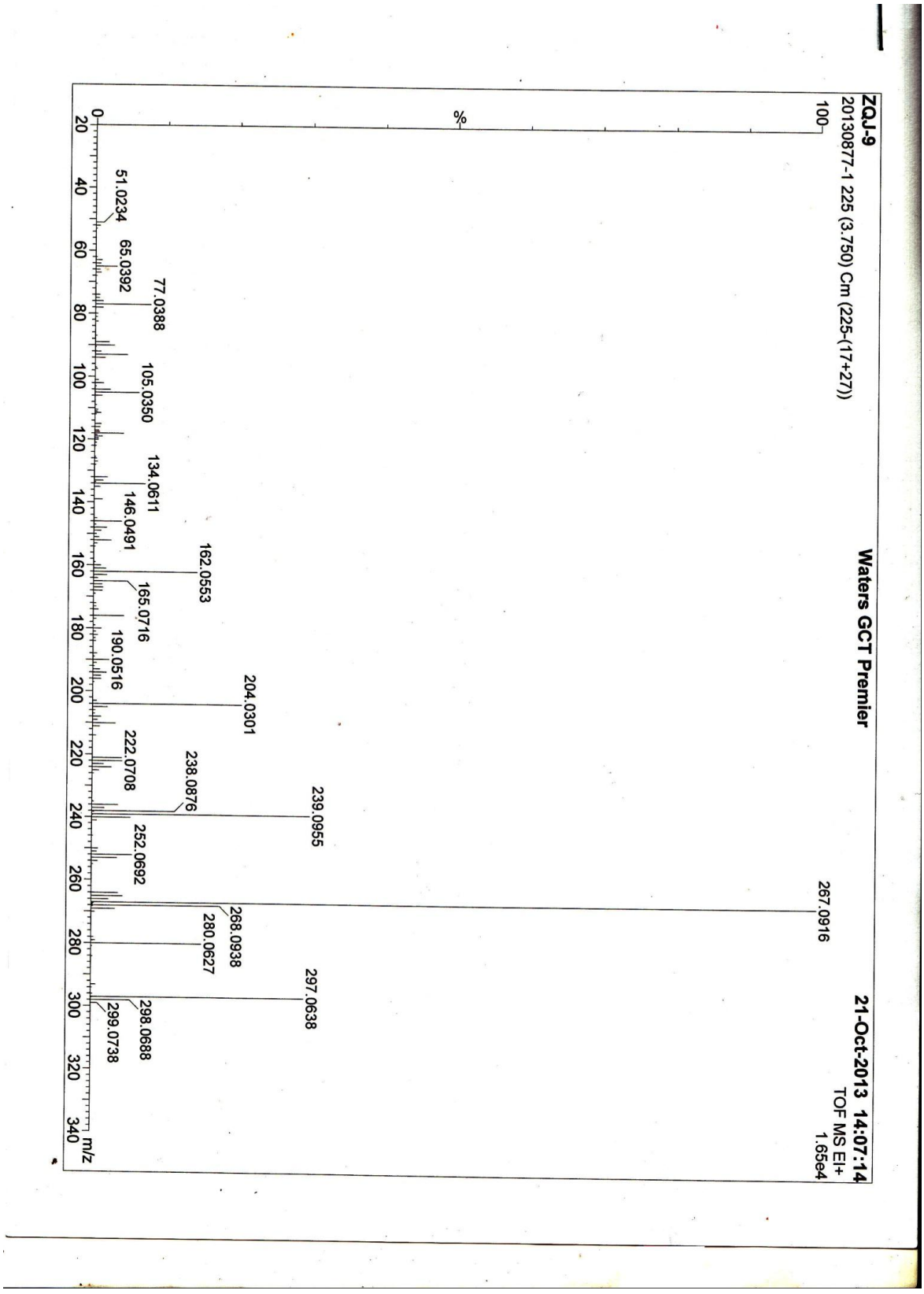

Figure S22. The HRMS spectrum of **7a**.

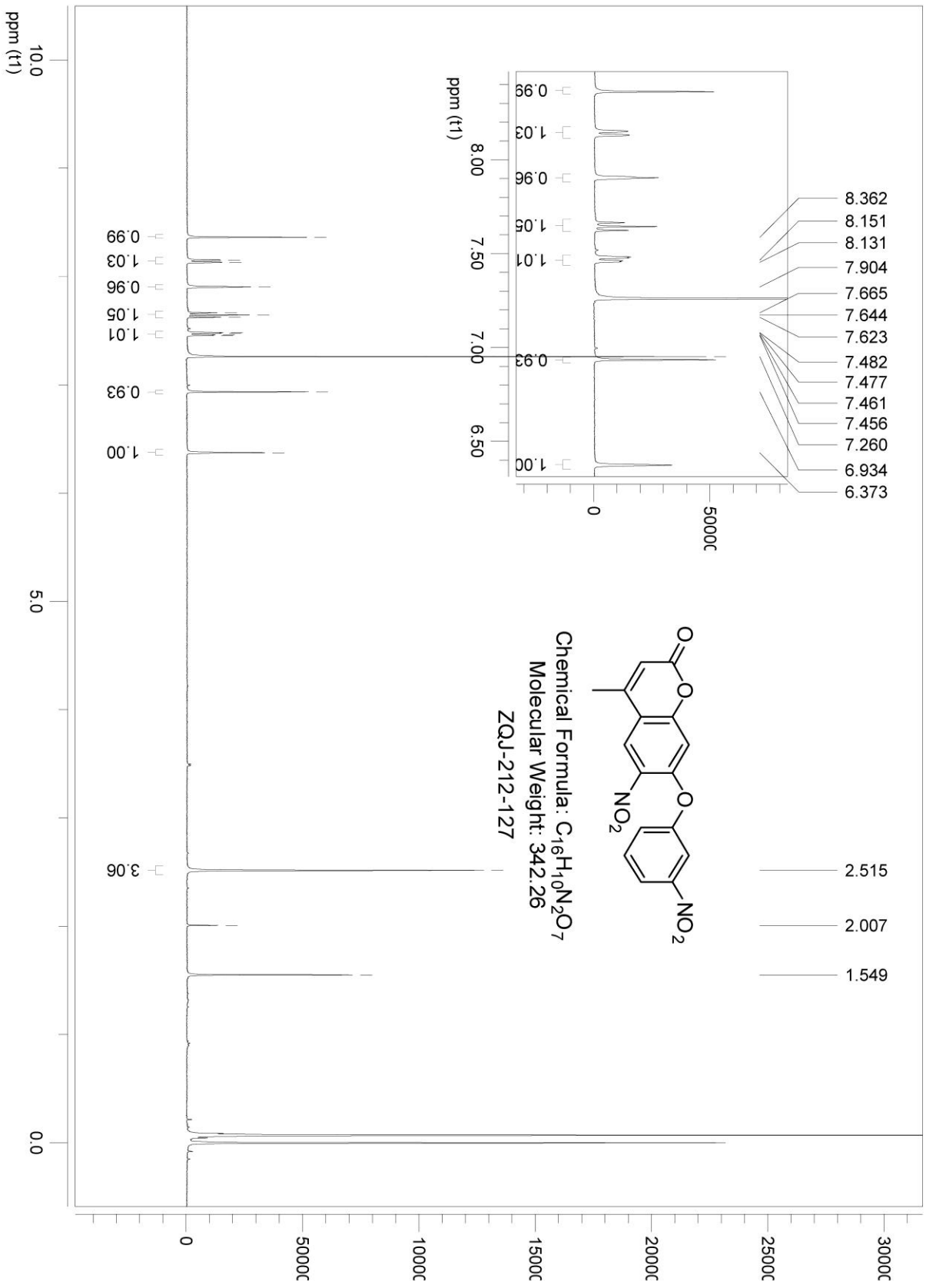

Figure S23. The ^{1}H-NMR spectrum of **7c**in $CDCl_3$.

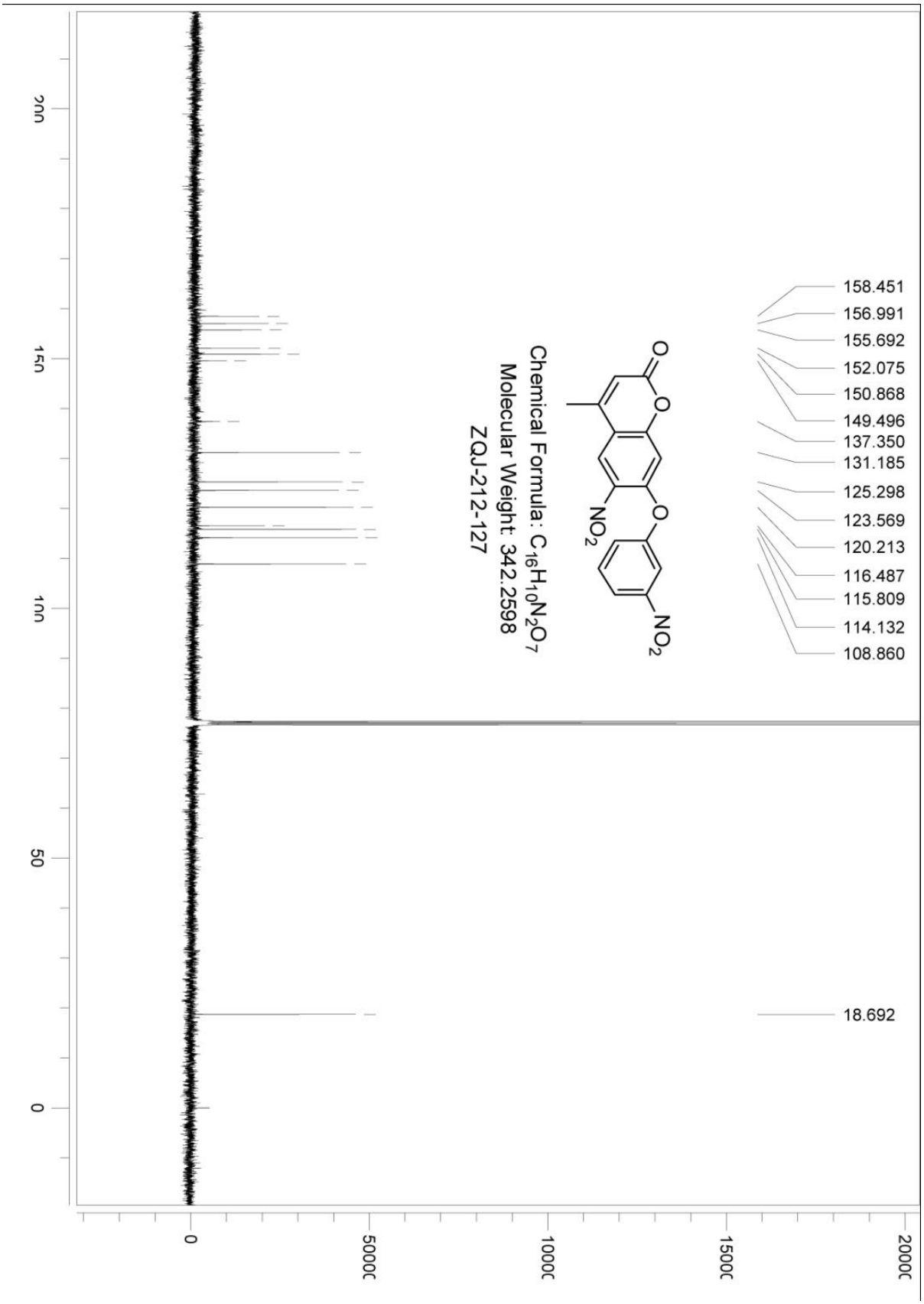

Figure S24. The ^{13}C-NMR spectrum of **7c** in $CDCl_3$.

Elemental Composition Report

Single Mass Analysis

Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
19 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-16 H: 0-11 N: 0-2 O: 0-7

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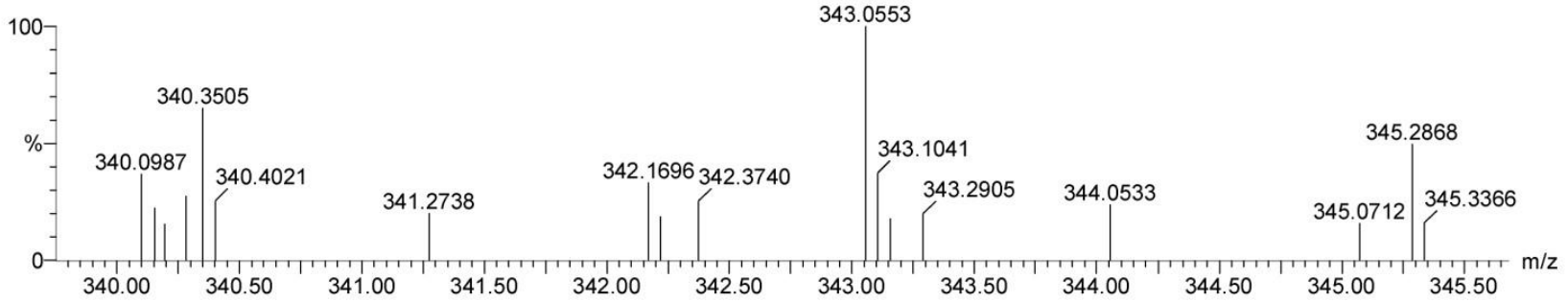

Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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Figure S25. The HRMS spectrum of **7c**.

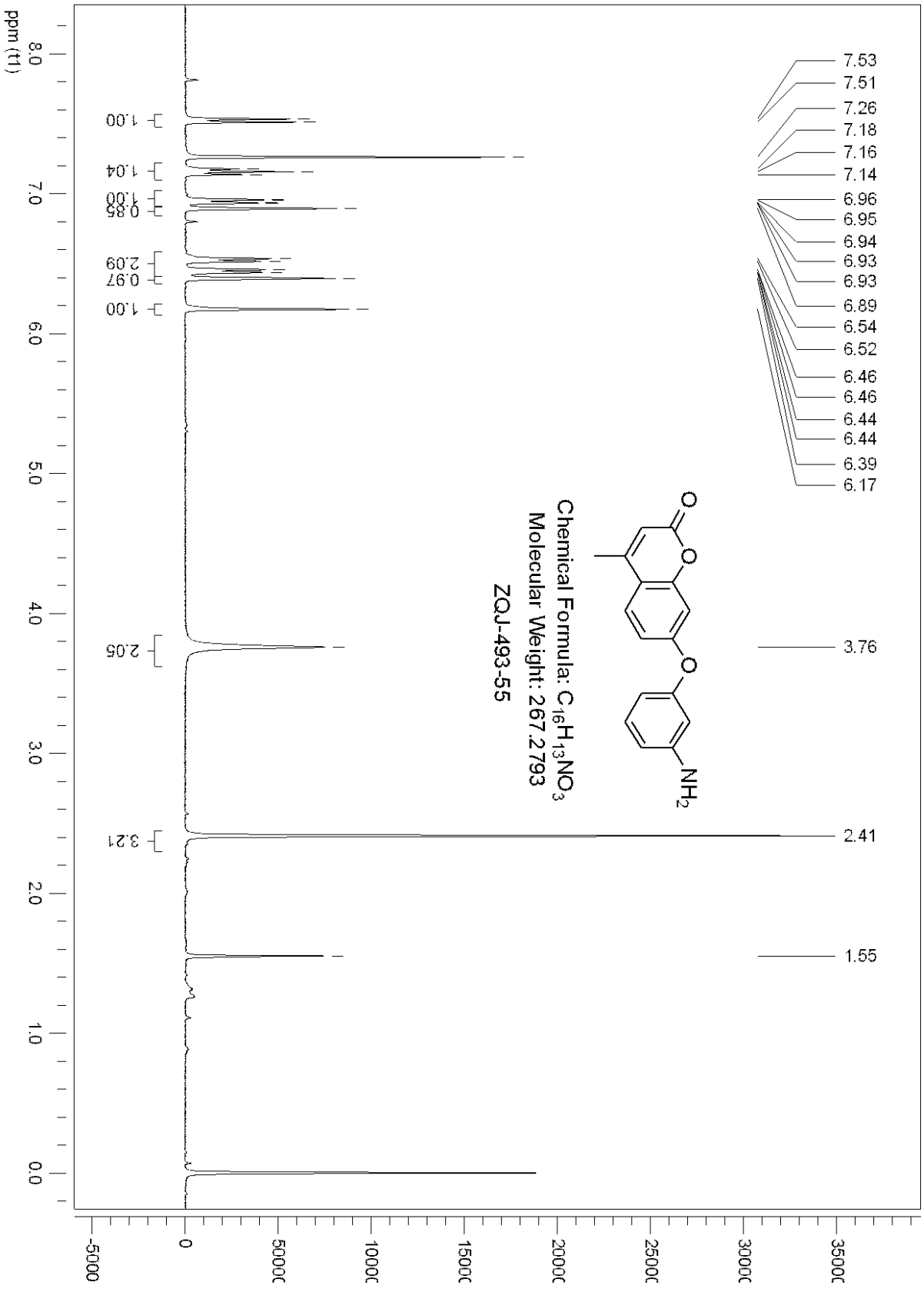

Figure S26. The ^{1}H-NMR spectrum of **11** in $CDCl_3$.

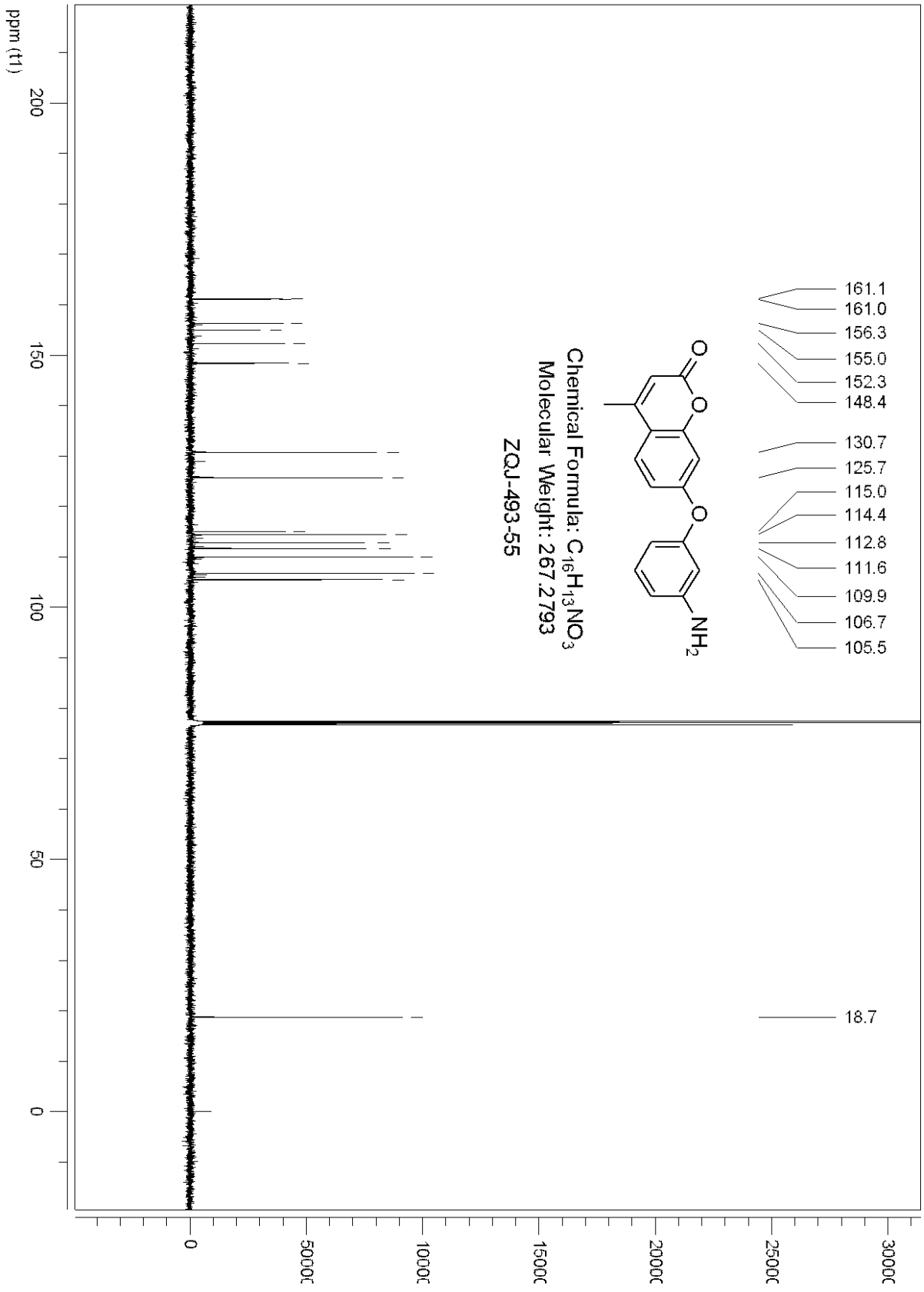

Figure S27. The ^{13}C-NMR spectrum of **11** in $CDCl_3$.

Elemental Composition Report

Single Mass Analysis

Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
41 formula(e) evaluated with 5 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-20 H: 0-21 N: 0-2 O: 0-7

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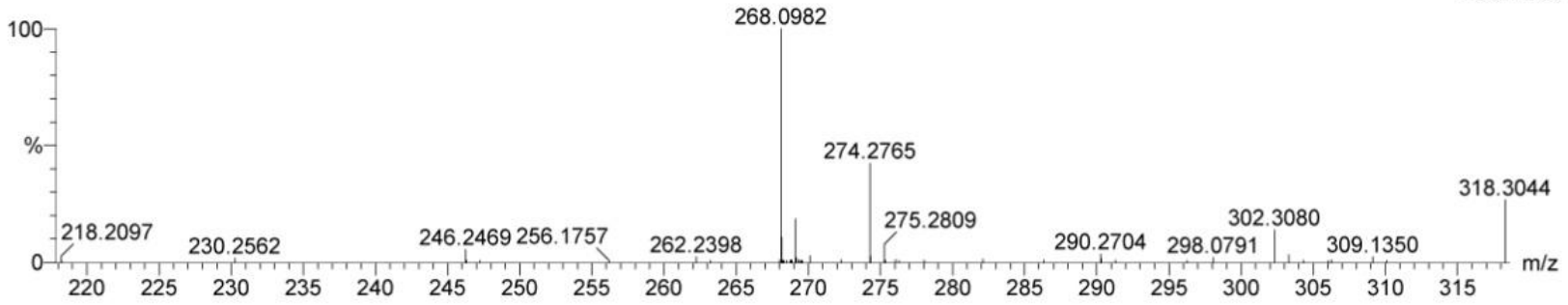

Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
268.0982	268.0974	0.8	3.0	10.5	68.3	0.0	C16 H14 N O3

Figure S28. The HRMS spectrum of **11**.

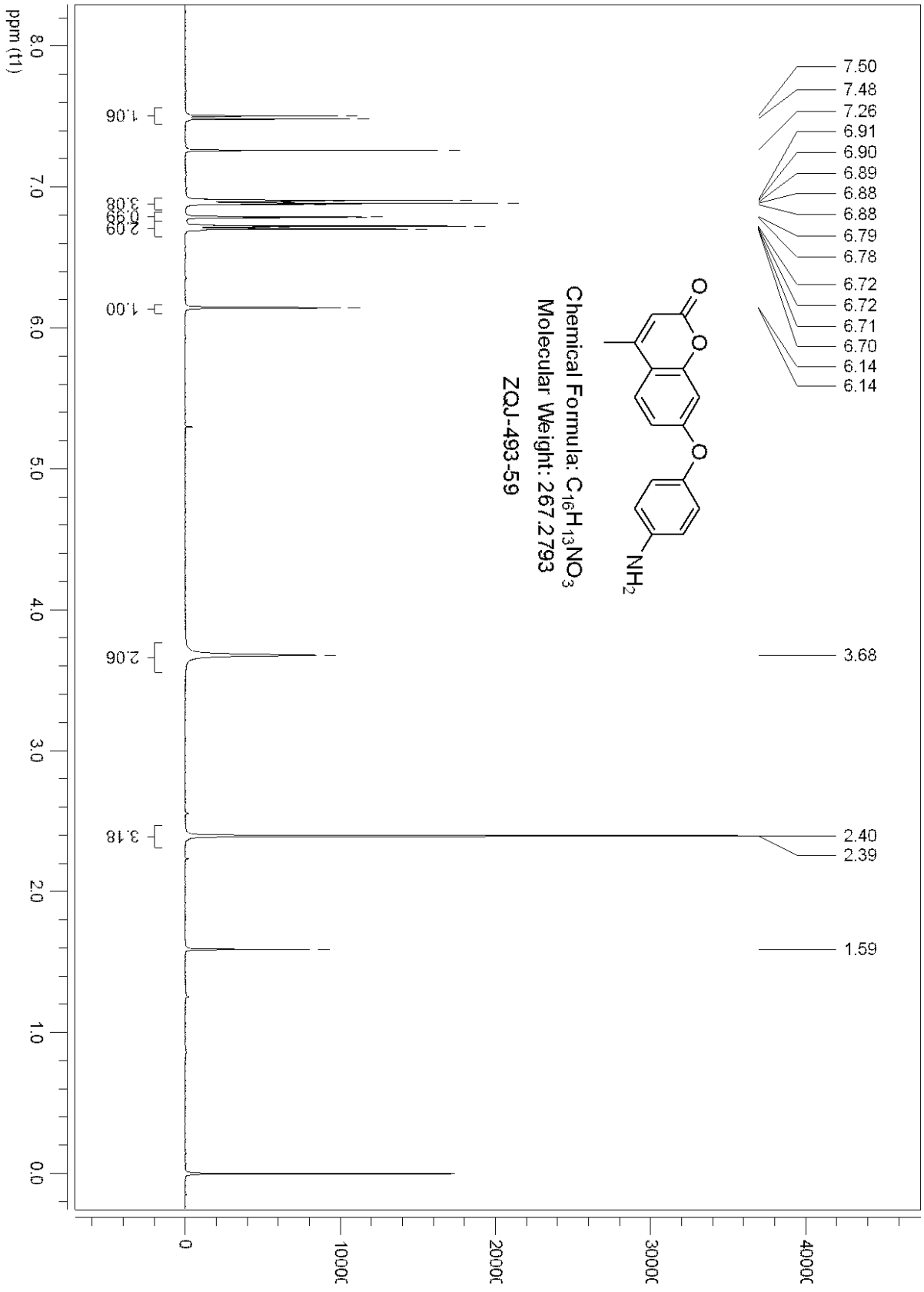

Figure S29. The ^{1}H-NMR spectrum of **12** in $CDCl_3$.

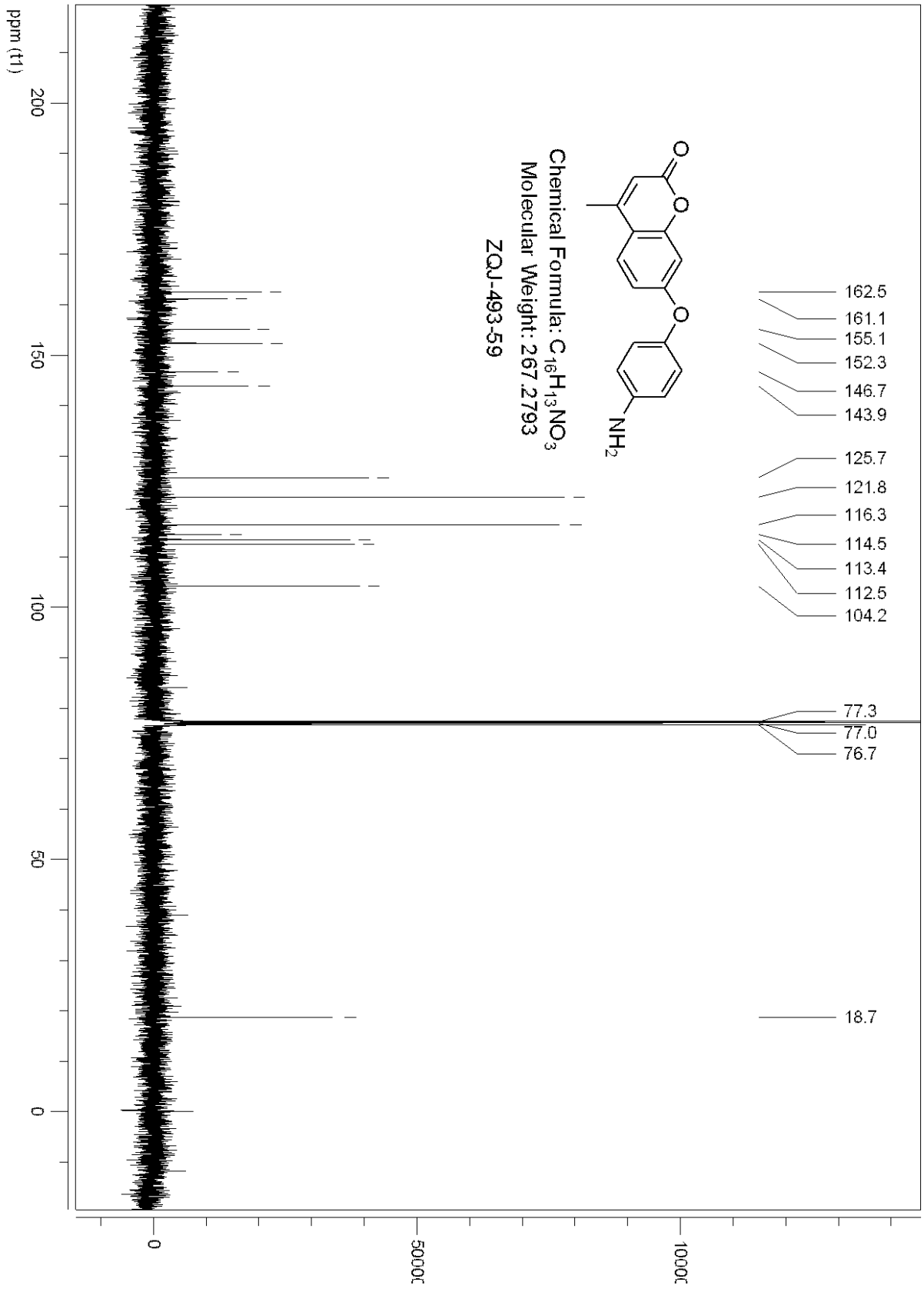

Figure S30. The ^{13}C-NMR spectrum of **12** in $CDCl_3$.

Elemental Composition Report

Single Mass Analysis

Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
9 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-16 H: 0-15 N: 0-1 O: 0-5

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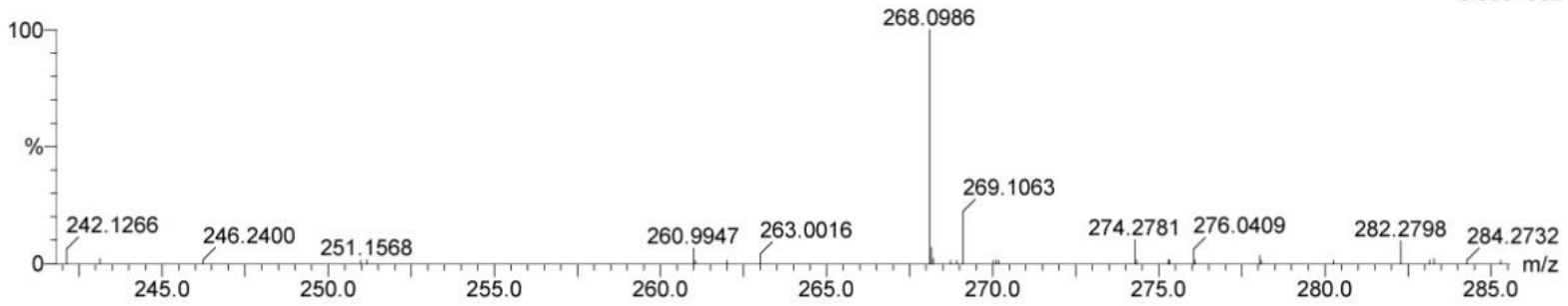

Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
268.0986	268.0974	1.2	4.5	10.5	29.2	0.0	C16 H14 N O3

Figure S31. The HRMS spectrum of **12**.

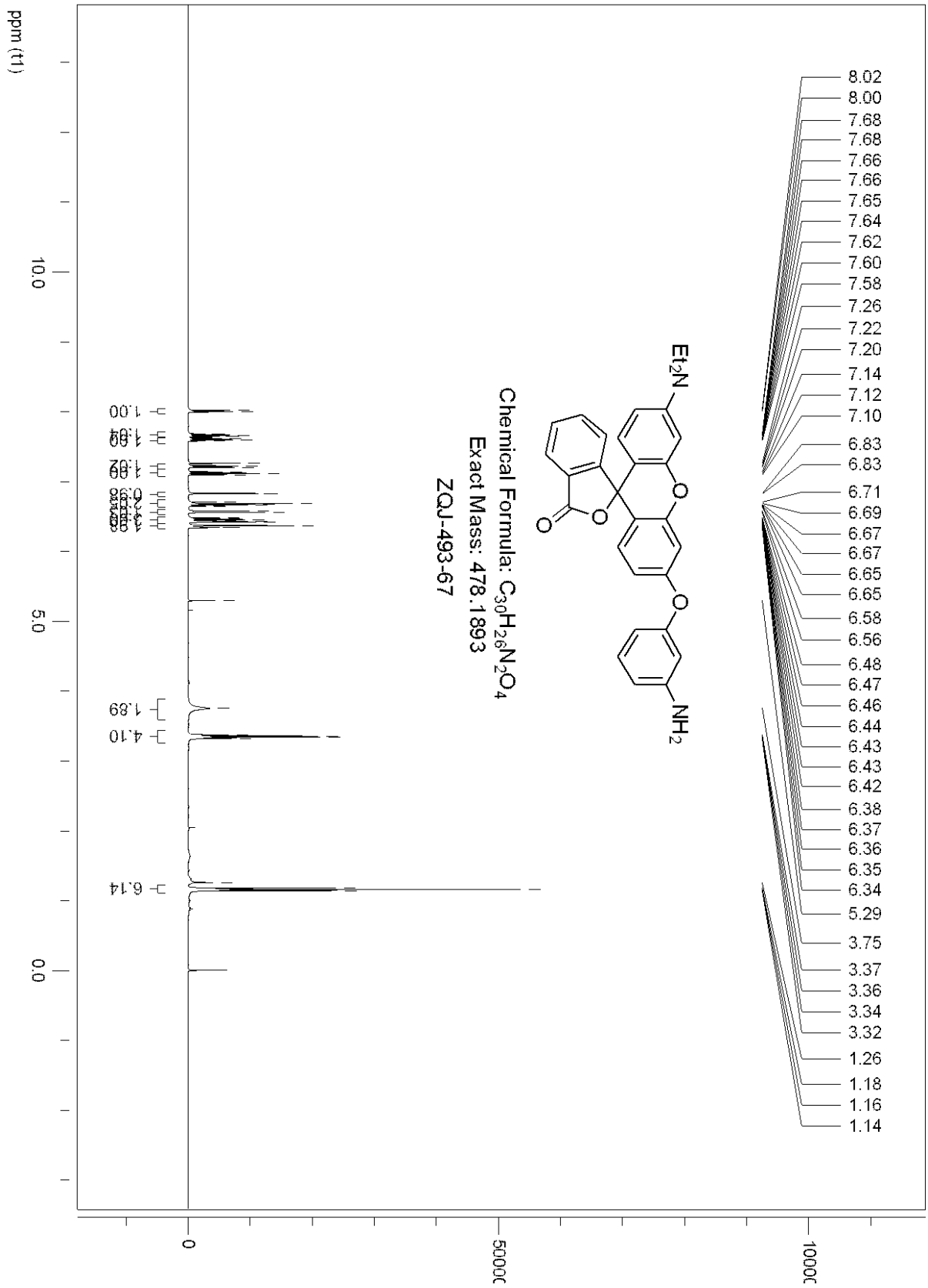

Figure S32. The ^{1}H-NMR spectrum of **14** in $CDCl_3$.

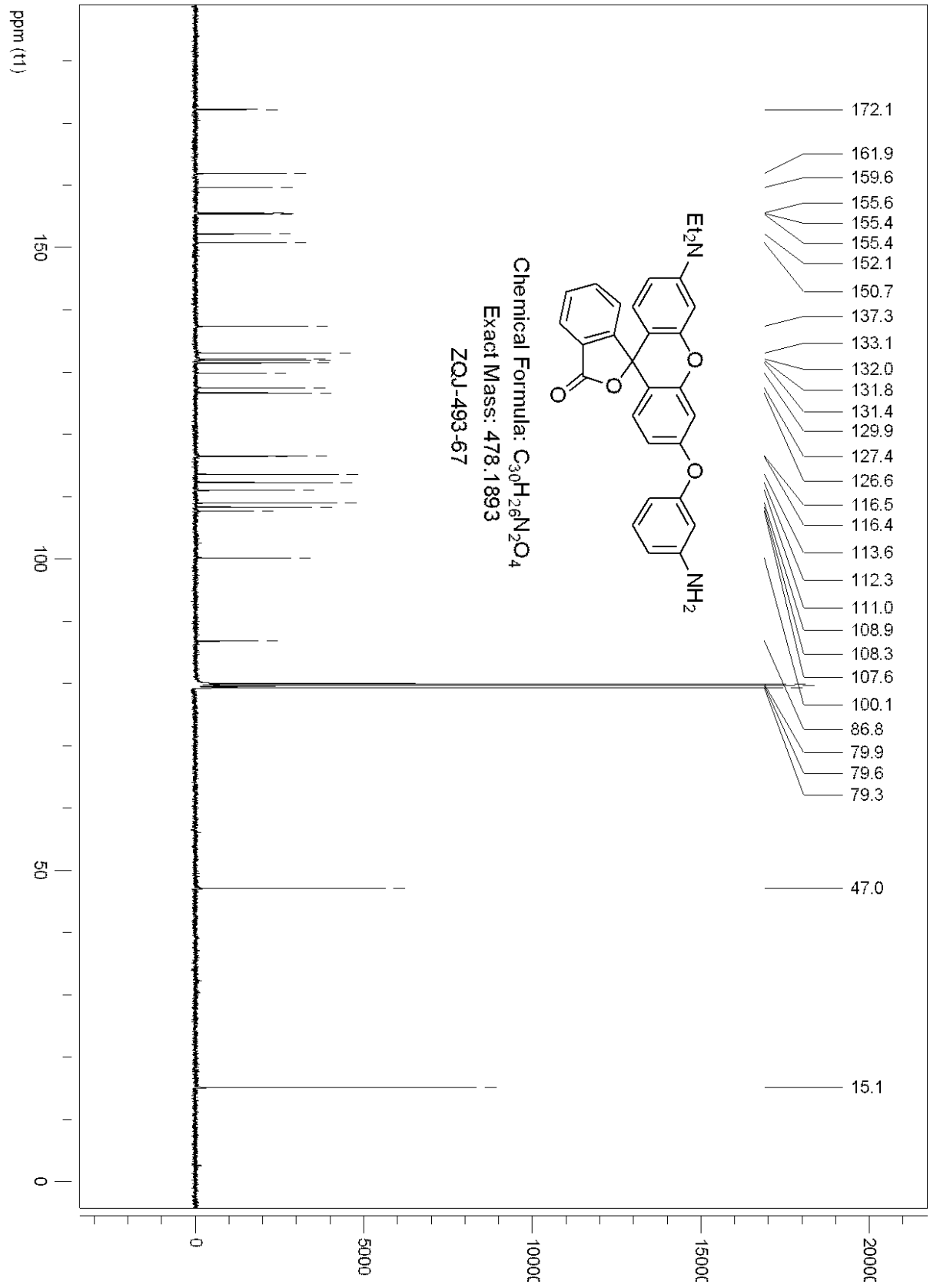

Figure S33. The ^{13}C-NMR spectrum of **14** in $CDCl_3$.

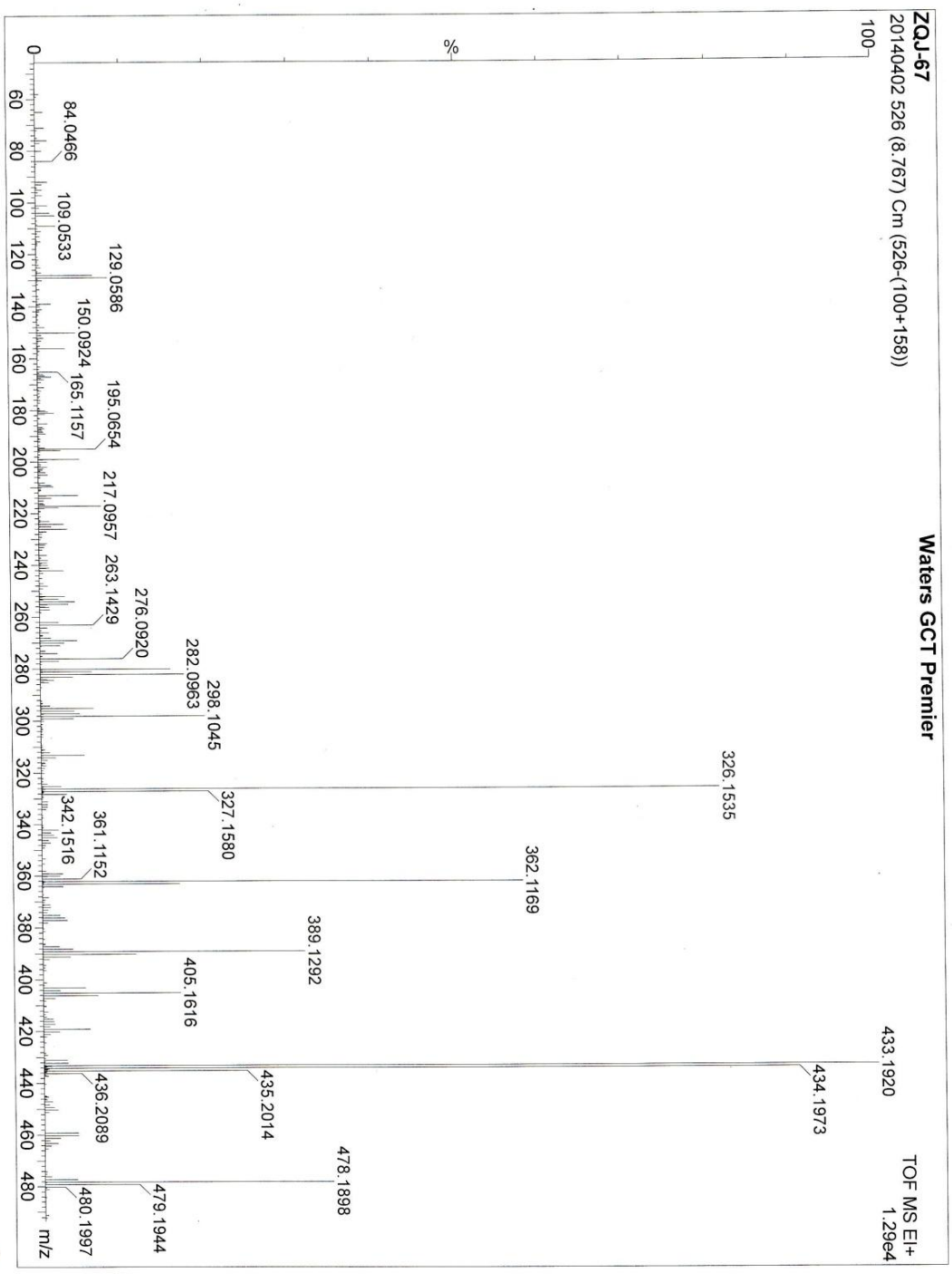

Figure S34. The HRMS spectrum of **14**.

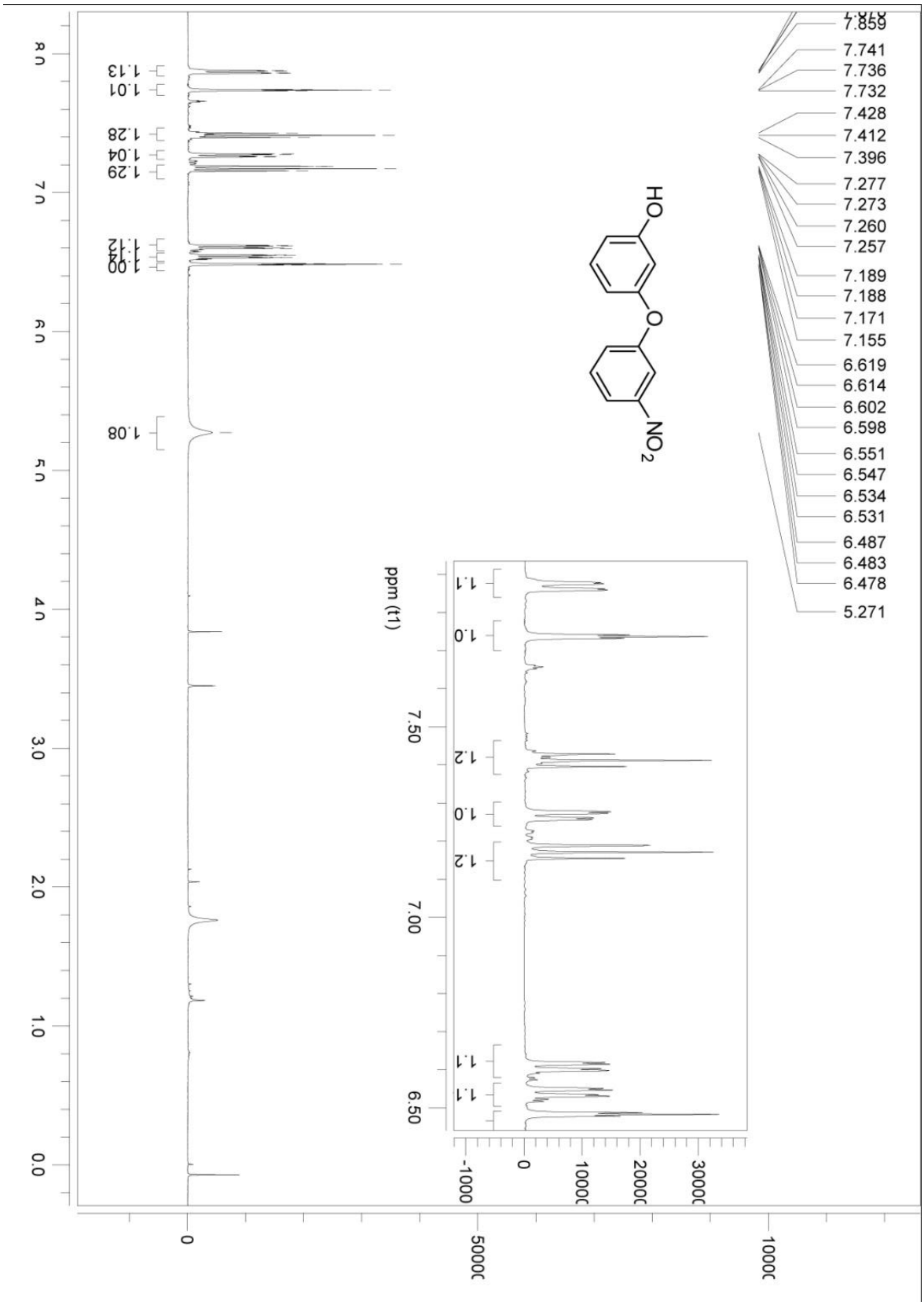

Figure S35. The ^{1}H-NMR spectrum of **19** in $CDCl_3$.

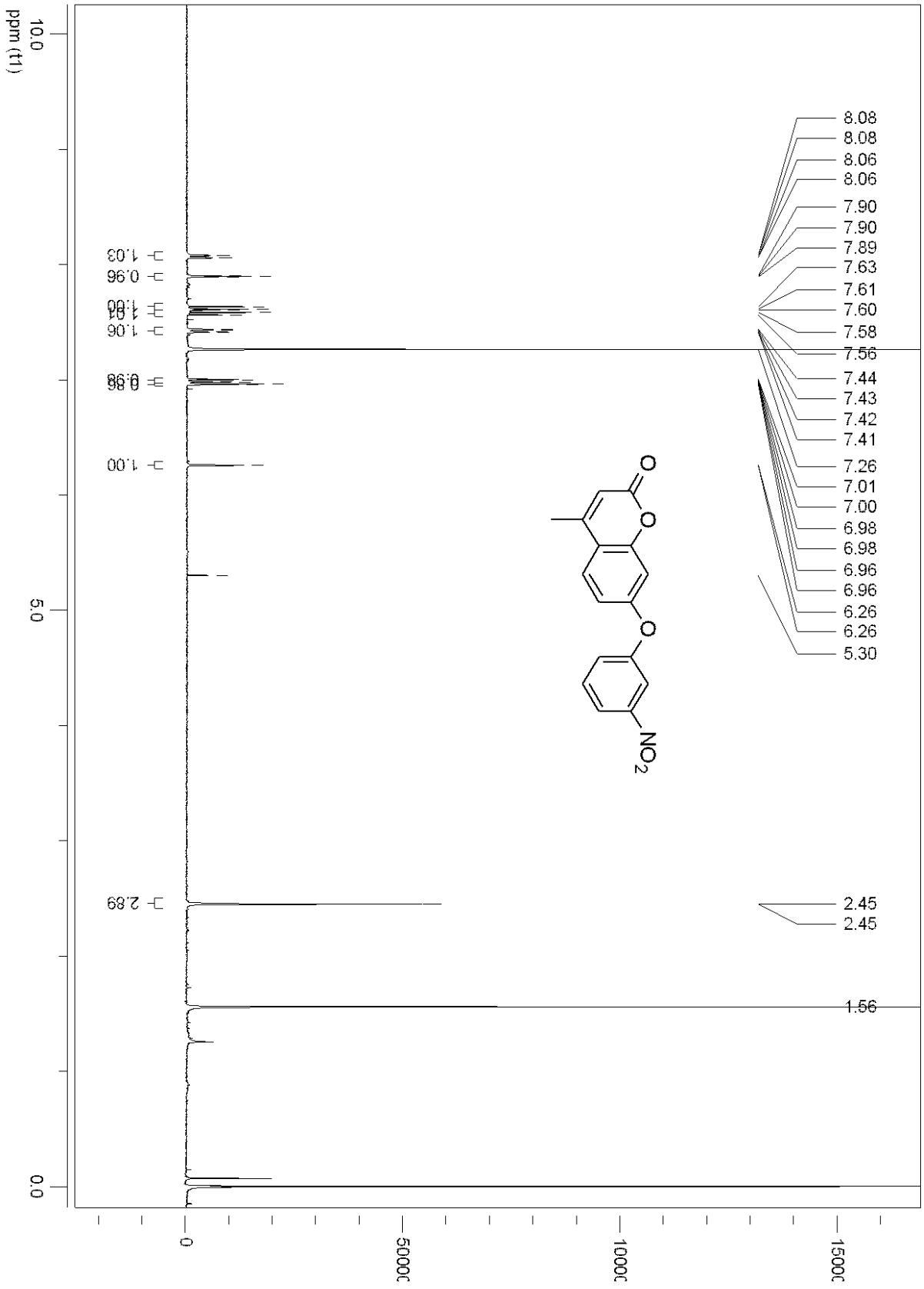

Figure S36. The ^{1}H-NMR spectrum of **21** in $CDCl_3$.

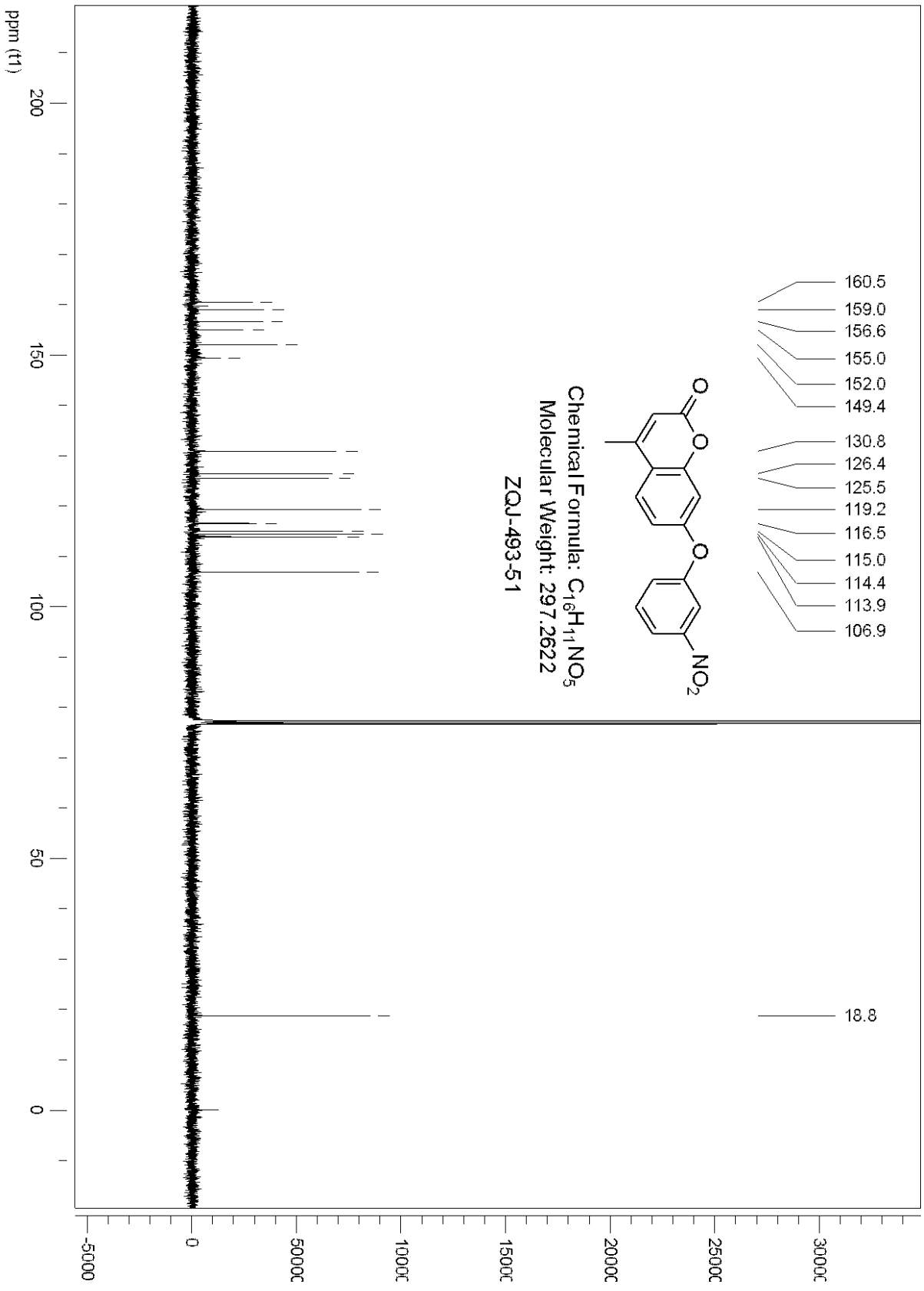

Figure S37. The ^{13}C-NMR spectrum of **21** in $CDCl_3$.

Elemental Composition Report

Single Mass Analysis

Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
31 formula(e) evaluated with 4 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-20 H: 0-21 N: 0-2 O: 0-7

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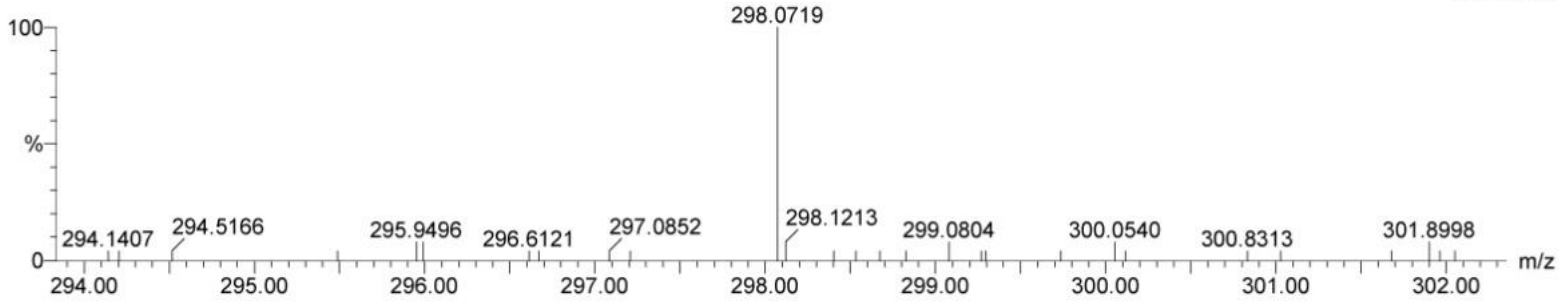
ZWB-THY-51 47 (1.528) Cm (45:47) 1: TOF MS ES+ 2.63e+001



Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
298.0719	298.0715	0.4	1.3	11.5	17.4	0.0	C16 H12 N O5

Figure S38. The HRMS spectrum of **21**.

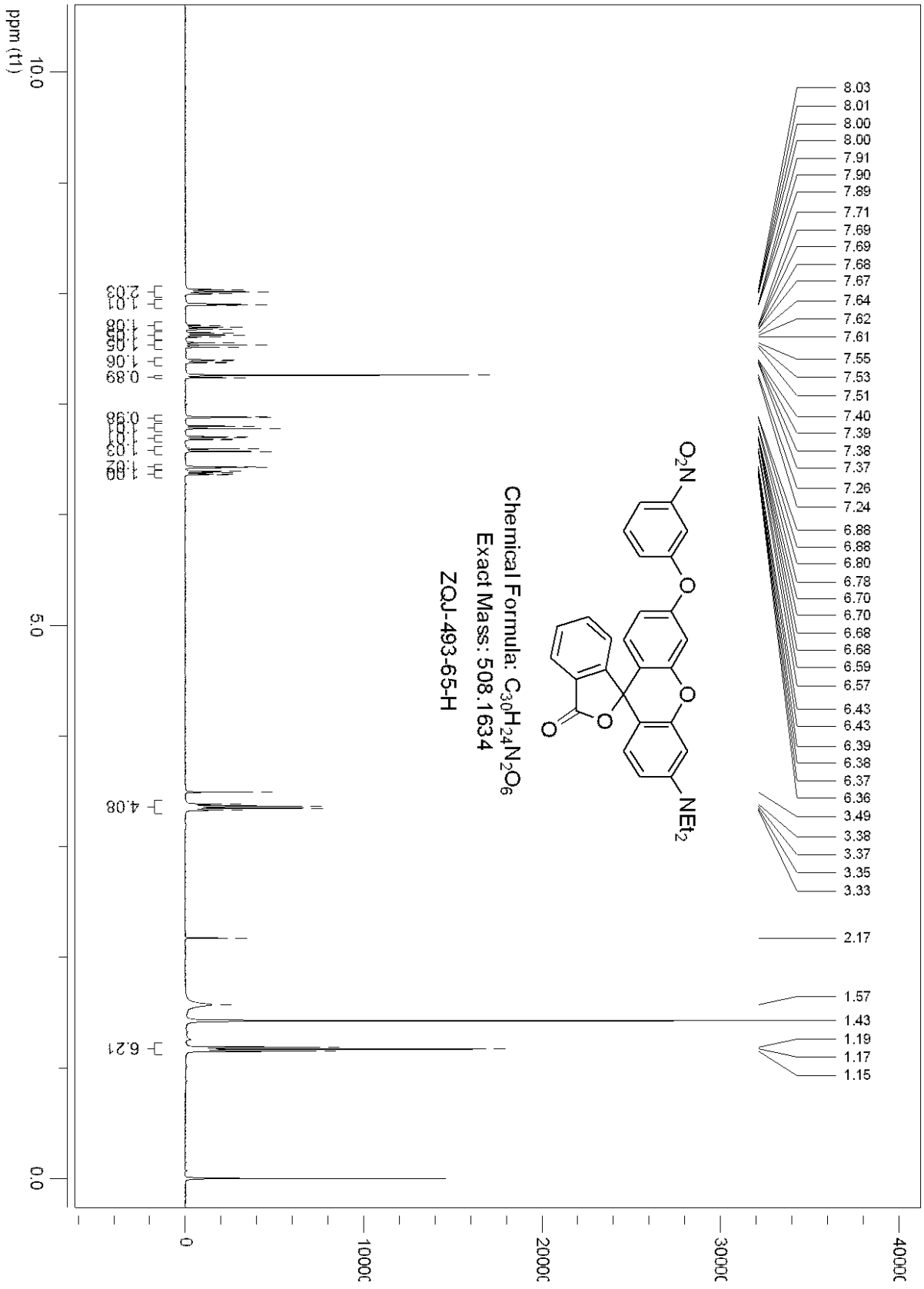


Figure S39. The ^{1}H-NMR spectrum of **23** in $CDCl_3$.

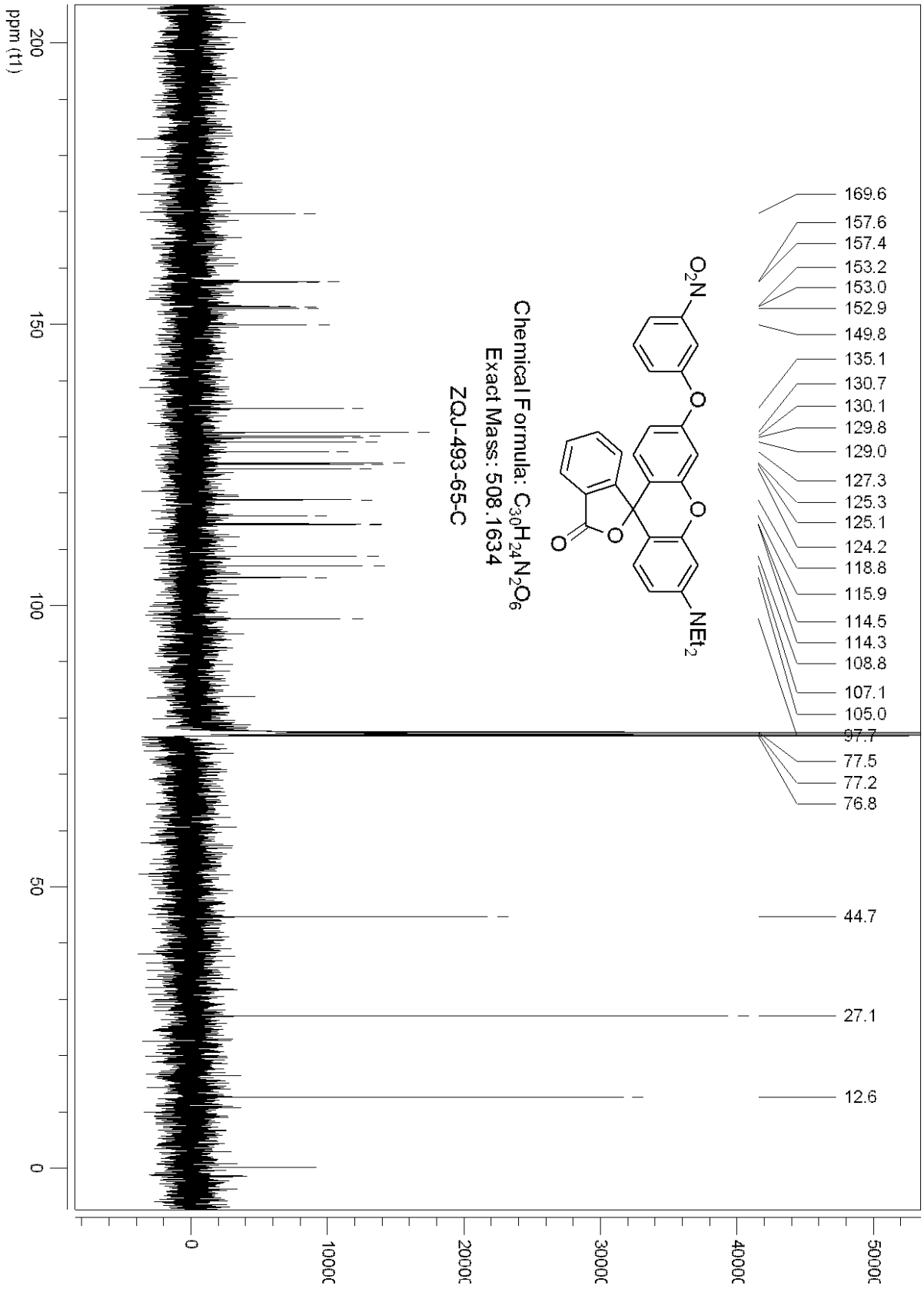


Figure S40. The ^{13}C-NMR spectrum of **23** in $CDCl_3$.

Elemental Composition Report

Single Mass Analysis

Tolerance = 30.0 mDa / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
13 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-30 H: 0-25 N: 0-2 O: 0-6

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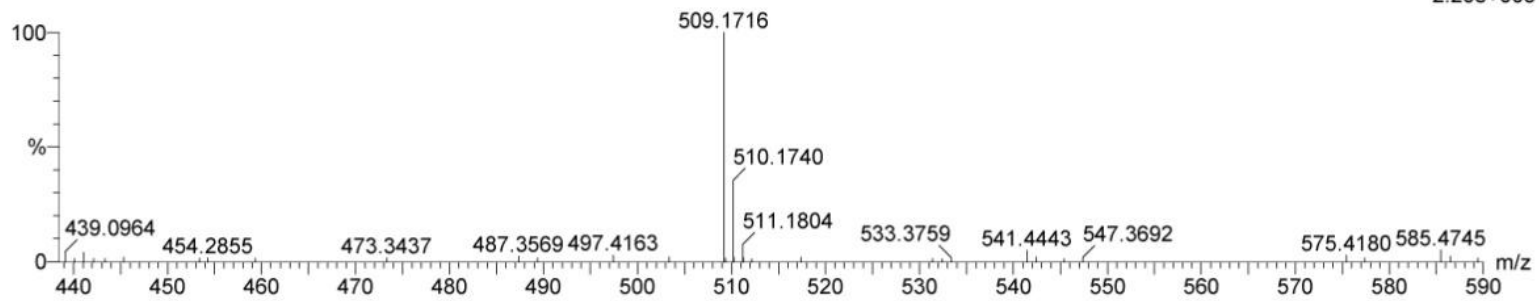
ZWB-THY-65 39 (1.282) Cm (39:42) 1: TOF MS ES+ 2.20e+003



Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		30.0	50.0	100.0			
509.1716	509.1713	0.3	0.6	19.5	21.3	0.0	C30 H25 N2 O6

Figure S41. The HRMS spectrum of **23**.