Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Blue 'turn-on' fluorescent probes for the direct detection of free radicals and nitric oxide in *Pseudomonas aeruginosa* biofilms†

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Electronic Supplementary Information

General synthetic information; High resolution ESI/MS spectra for **16**, **16a** and **16b**; HPLC traces and EPR spectra for compounds **5**, **6**, **8** - **11**; ^{1}H and ^{13}C NMR spectra for compounds **5a**, **6a**, **8a** - **11a**; HPLC traces and ^{1}H and ^{13}C NMR spectra for compounds **13**, **14** and **16.**

General synthetic methods

Unless otherwise noted, reagents were obtained from commercial suppliers and were used without further purification. All anhydrous reactions were performed in oven-dried or flame-dried glassware under inert argon atmosphere. Low resolution ESI-MS were recorded on an Agilent 6220 ESI-TOF mass spectrometer. IR spectra were recorded neat on a Perkin-Elmer Spectrum One FT-IR spectrometer, equipped with a zinc selenide/diamond universal ATR sampling accessory. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. NMR spectra were recorded on an Agilent NMR400, Agilent DD2 or Bruker Advance IIIHD. Chemical shifts (δ) are reported in parts per million (ppm). EPR spectra were recorded on a Bruker Elexsys E-500 CW-EPR. High resolution mass spectroscopy (HRMS) was conducted on a Finnigan hybrid linear triple-quadrupole (LTQ) Fourier Transform ion cyclotron resonance (FTICR) mass spectrometer. Reverse phase preparatory HPLC was performed on an Agilent 1200 series HPLC system with a Phenomenex Luna C18(2) 100A packed (50 mm x 21.2 mm x 5 µm) Axia column using a 10-90% gradient of CH_3CN in H_2O.. Compound purity was assessed by analytical reverse phase HPLC on an Agilent 1100 series HPLC system with a Phenomenex Aeris peptide XB-C18 packed (250 mm x 4.6 mm x 3.6 µm) column using a 10-90% gradient of CH_3CN in H_2O. UV-vis absorbance spectra were collected using an Agilent 8453 UV-vis absorbance spectrophotometer system. Fluorescence spectroscopy was conducted on a Horiba Jobin Yvon Fluorolog-3.

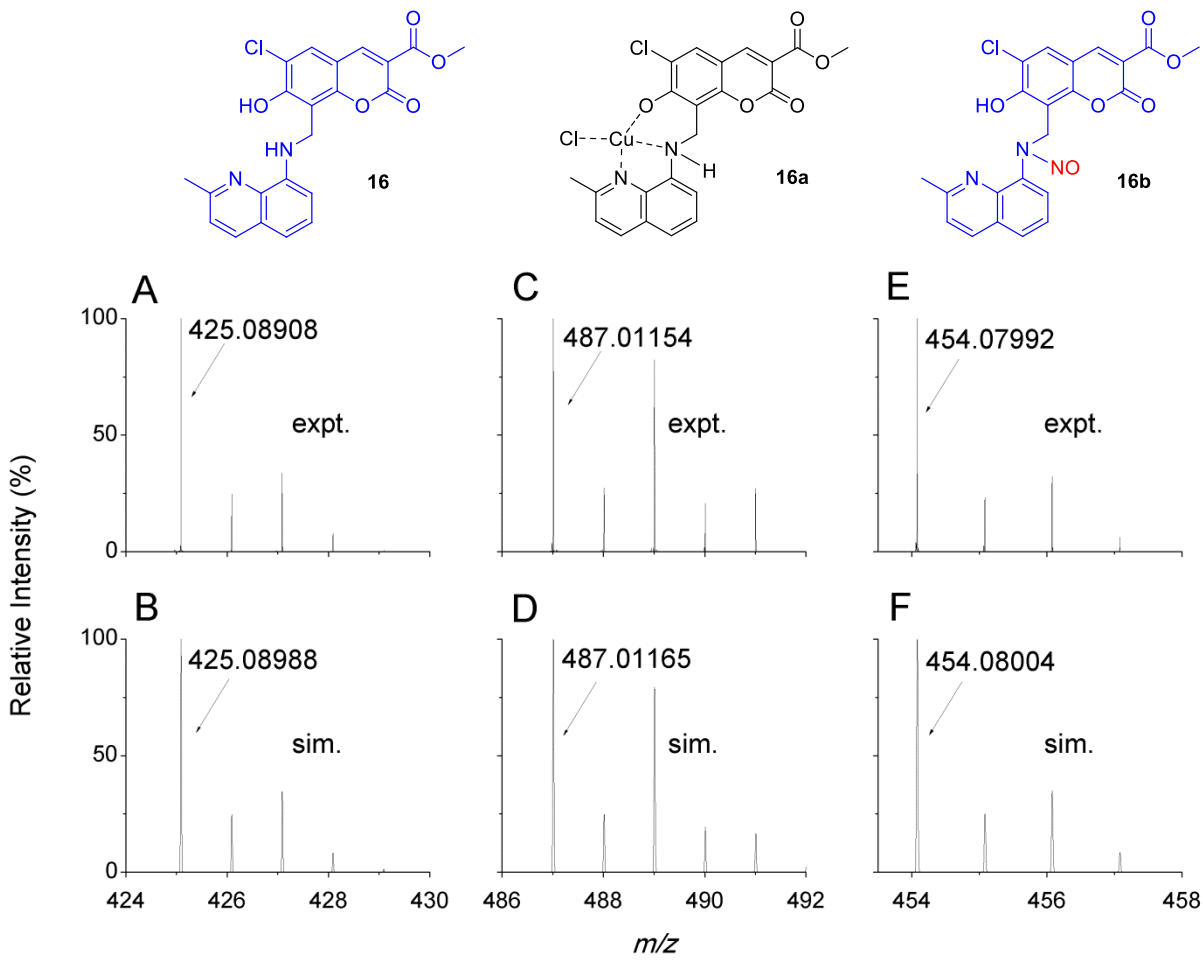
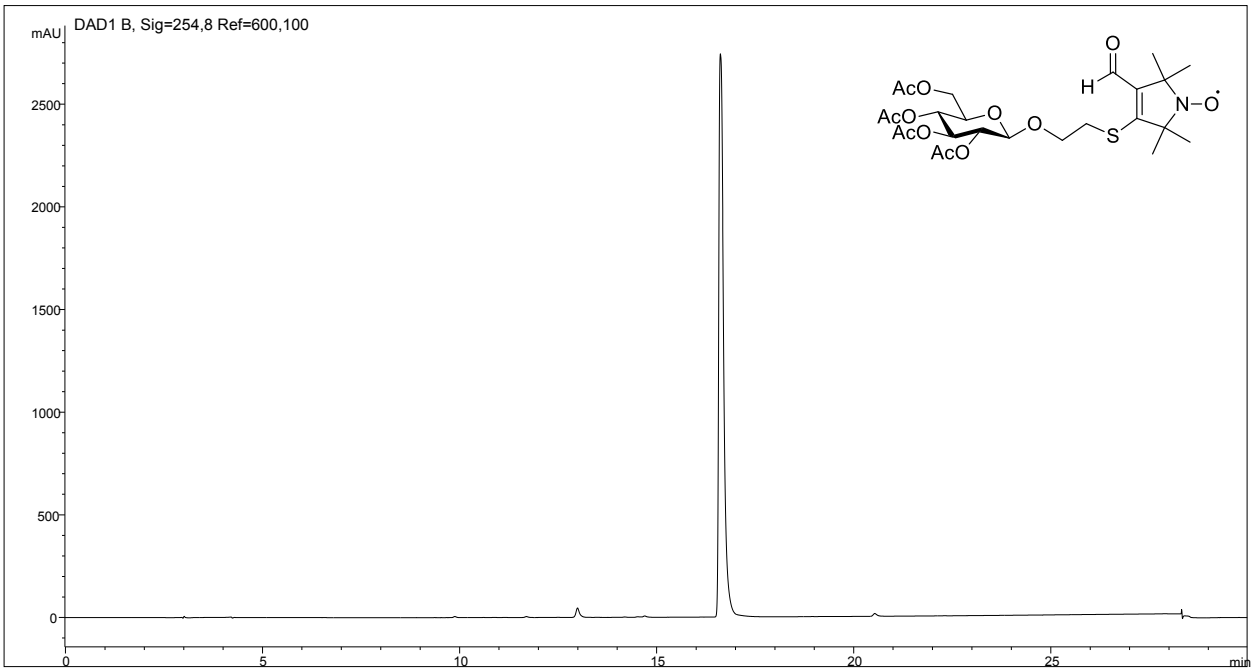


Fig. S1. Linear triple-quadrupole Fourier Transform ion cyclotron resonance (LTQ-FTICR) high resolution ESI/MS spectra. A comparison of measured (top panel - [**16** + H]$^+$ (**A**), [**16a** - Cl + H]$^+$ (**C**), and [**16b** + H]$^+$ (**E**)) vs. simulated (bottom panel - [**16** + H]$^+$ (**B**), [**16a** – Cl + H]$^+$ (**D**), and [**16b** + H]$^+$ (**F**)) isotope ratios and accurate mass determination. The most intense isotopic peak in the complex is shown by the *m/z* value.

A



Area Percent Report

Peak #	Retention Time (min)	Area	Height (mAu)	Width (min)	Area (%)	Symmetry
1	12.99	300.7	46.2	0.0959	1.262	0.712
2	16.616	23434.5	2740.4	0.1328	98.350	0.479
3	20.533	92.5	13.6	0.1014	0.388	0.664

B

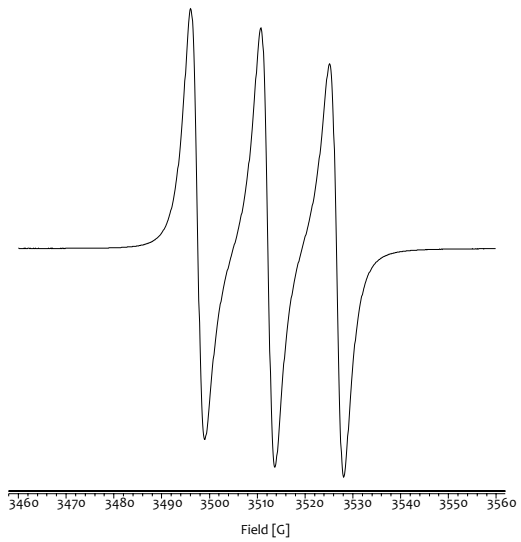
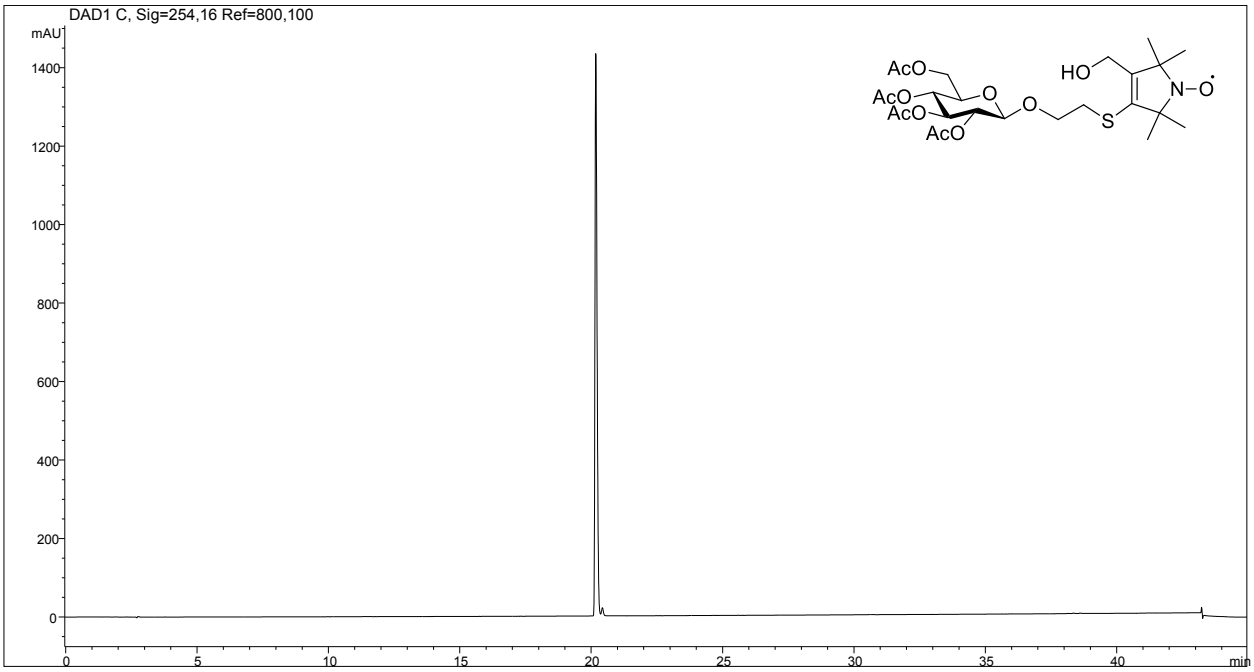


Fig S2. HPLC trace (**A**) and EPR spectrum (**B**) for nitroxide **5**.

A



Area Percent Report

Peak #	Retention Time (min)	Area	Height (mAu)	Width (min)	Area (%)	Symmetry
1	20.172	7825.7	1439.5	0.0906	99.071	0.623
2	20.424	73.4	17.4	0.0704	0.929	0.799

B

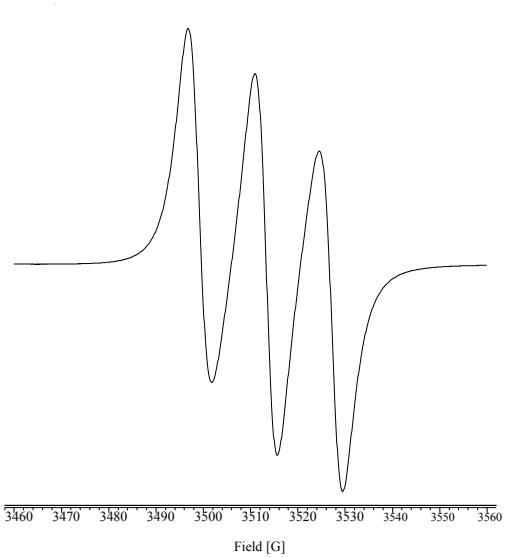
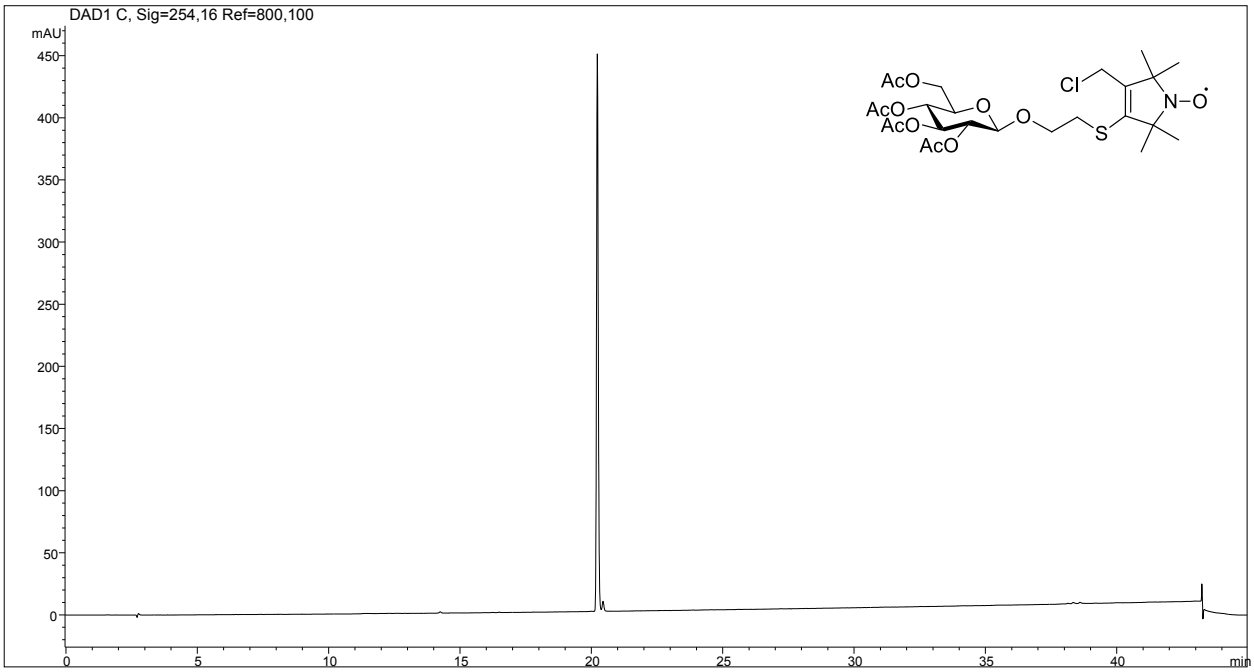


Fig S3. HPLC trace (**A**) and EPR spectrum (**B**) for nitroxide **6**.

A



Area Percent Report

Peak #	Retention Time (min)	Area	Height (mAu)	Width (min)	Area (%)	Symmetry
1	20.223	2055.1	449.4	0.0762	98.572	0.832
2	20.442	29.8	7.2	0.0693	1.428	0.857

B

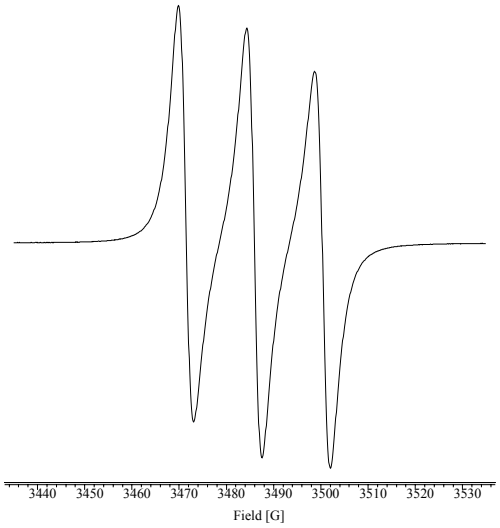
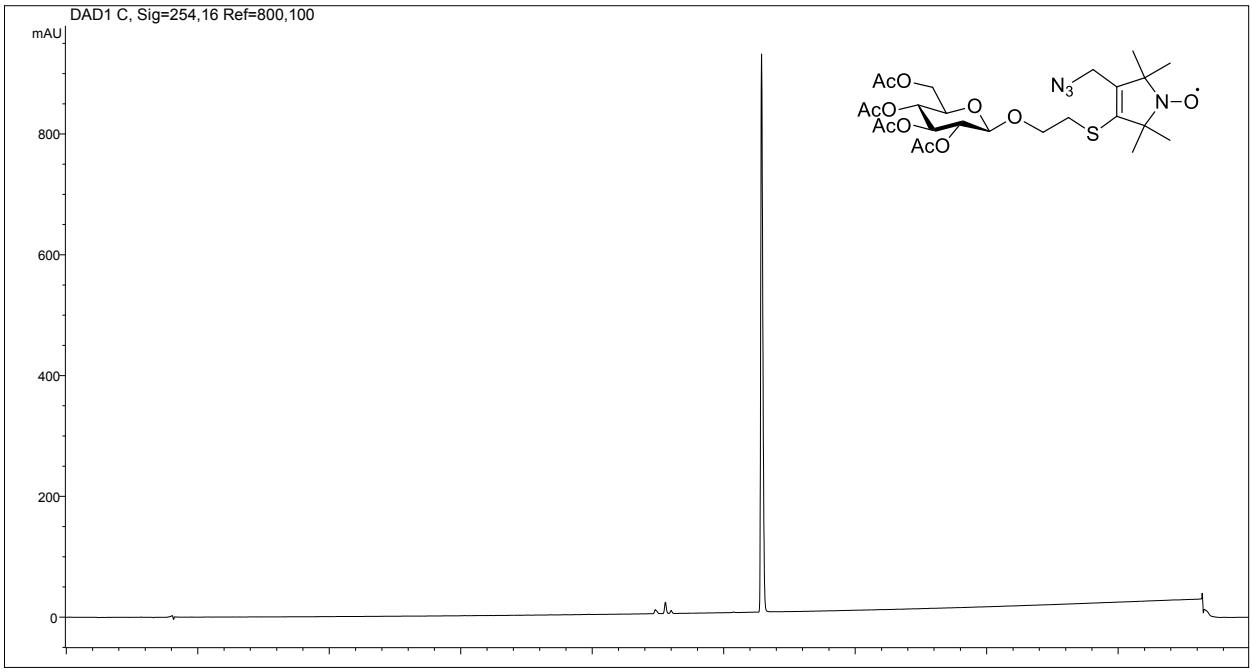


Fig S4. HPLC trace (**A**) and EPR spectrum (**B**) for nitroxide **8**.

A



Area Percent Report

Peak #	Retention Time (min)	Area	Height (mAu)	Width (min)	Area (%)	Symmetry
1	22.396	41.1	6.5	0.1054	0.791	0.518
2	22.778	86	18.9	0.076	1.657	0.928
3	22.997	20.7	4.9	0.0711	0.399	0.958
4	26.435	5045.6	925.6	0.0909	97.153	0.648

B

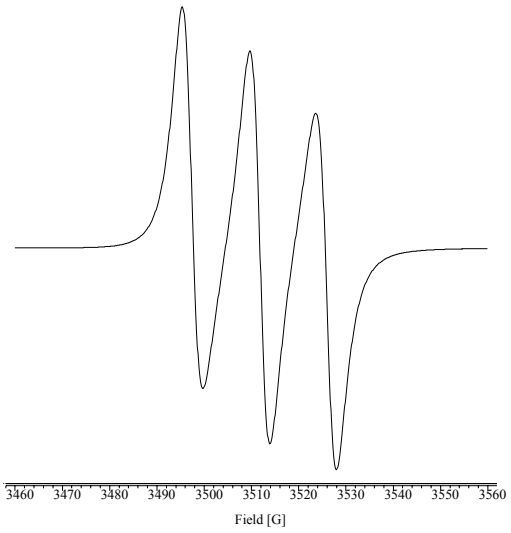
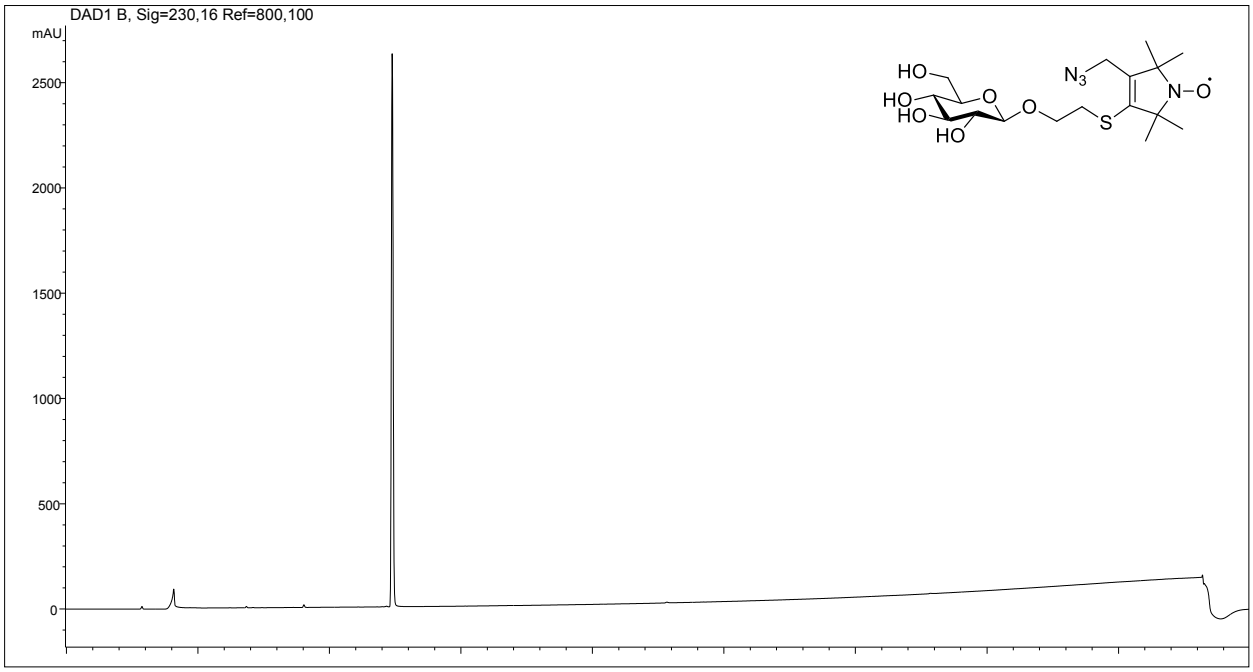


Fig S5. HPLC trace (**A**) and EPR spectrum (**B**) for nitroxide **9**.

A



Area Percent Report

Peak #	Retention Time (min)	Area	Height (mAu)	Width (min)	Area (%)	Symmetry
1	12.382	12334.6	2634.3	0.078	100.000	0.892

B

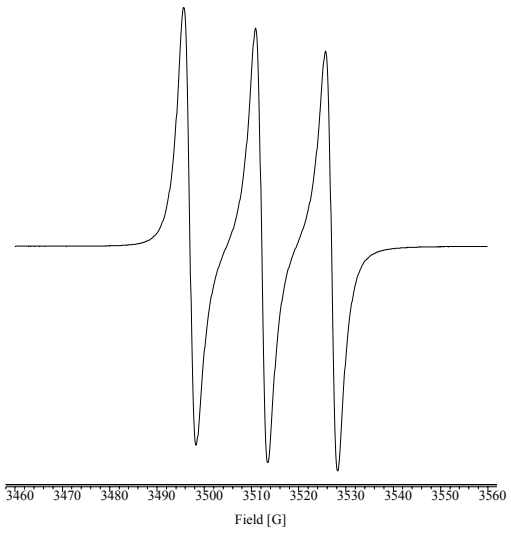
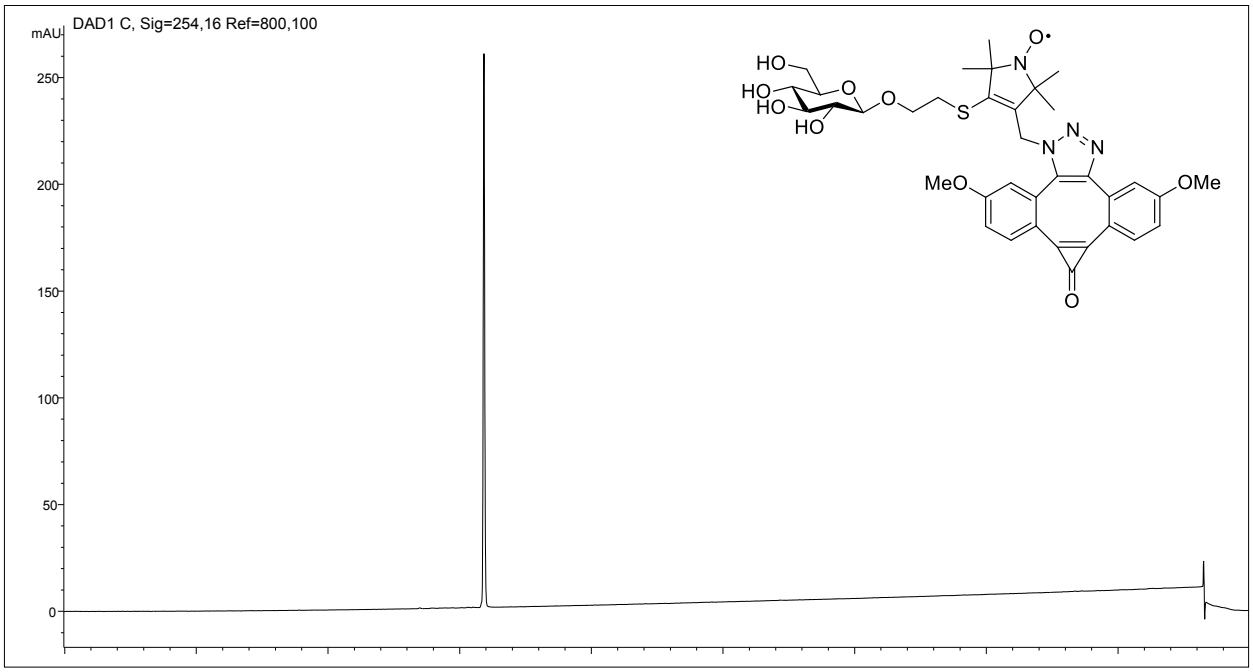


Fig S6. HPLC trace (**A**) and EPR spectrum (**B**) for nitroxide **10**.

A



Area Percent Report

Peak #	Retention Time (min)	Area	Height (mAu)	Width (min)	Area (%)	Symmetry
1	15.918	1021.7	261.7	0.0651	100.000	0.922

B

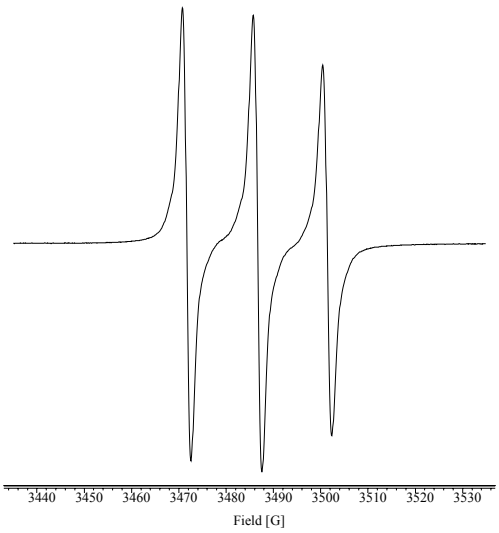


Fig S7. HPLC trace (**A**) and EPR spectrum (**B**) for nitroxide **11**.

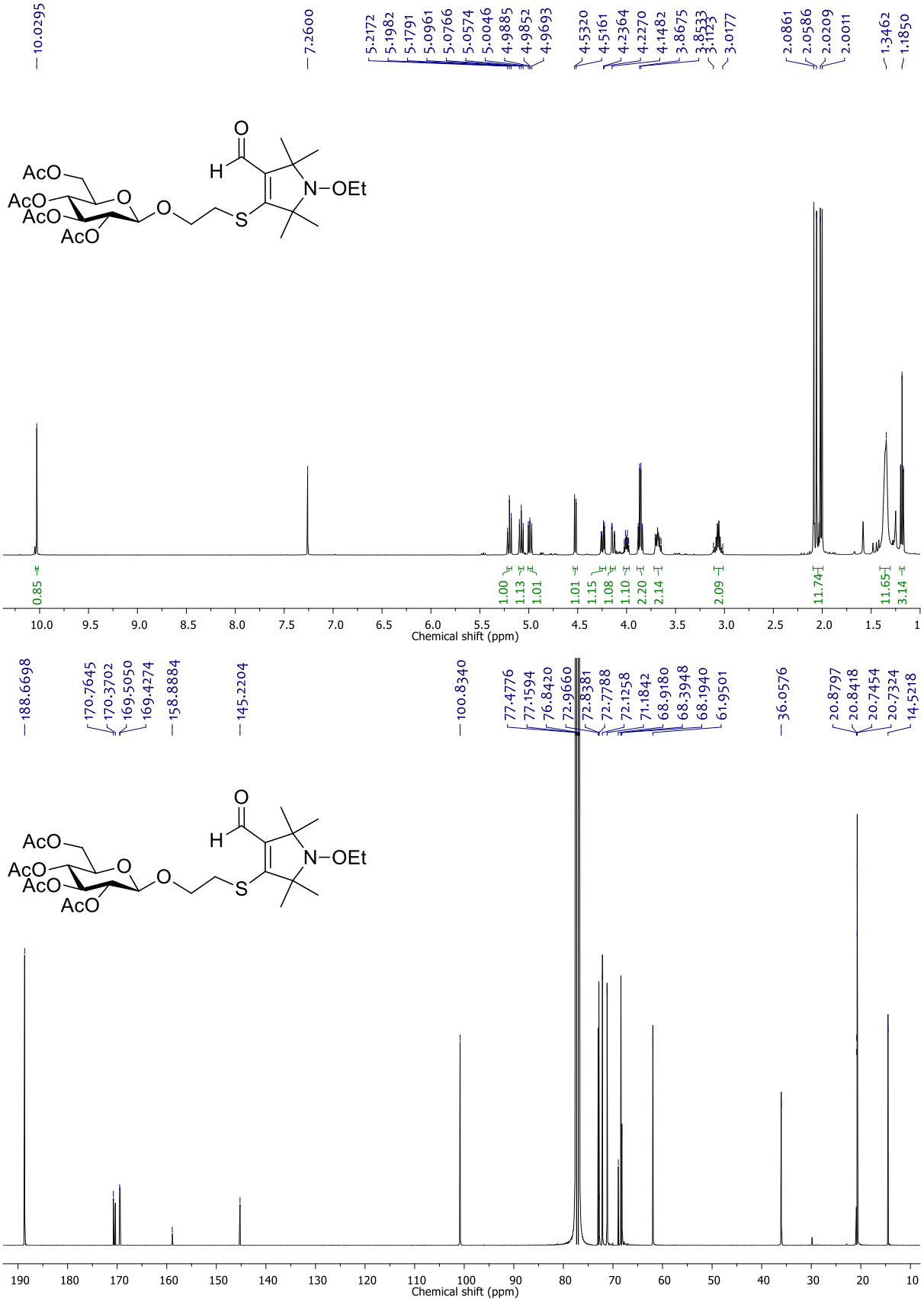


Fig S8. ^{1}H (top) and ^{13}C (bottom) NMR spectra for ethoxyamine **5a**.

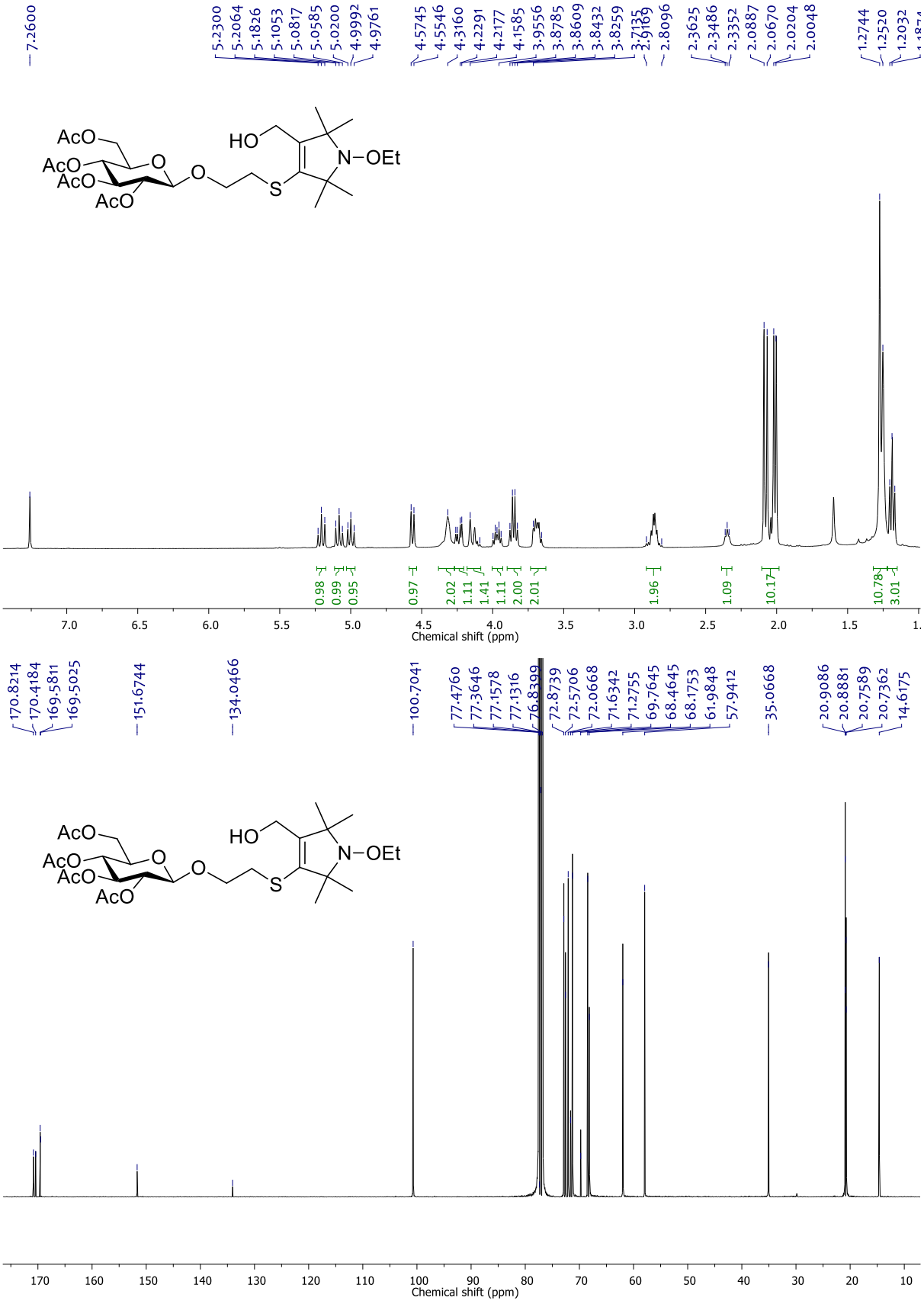


Fig S9. ^{1}H (top) and ^{13}C (bottom) NMR spectra for ethoxyamine **6a**.

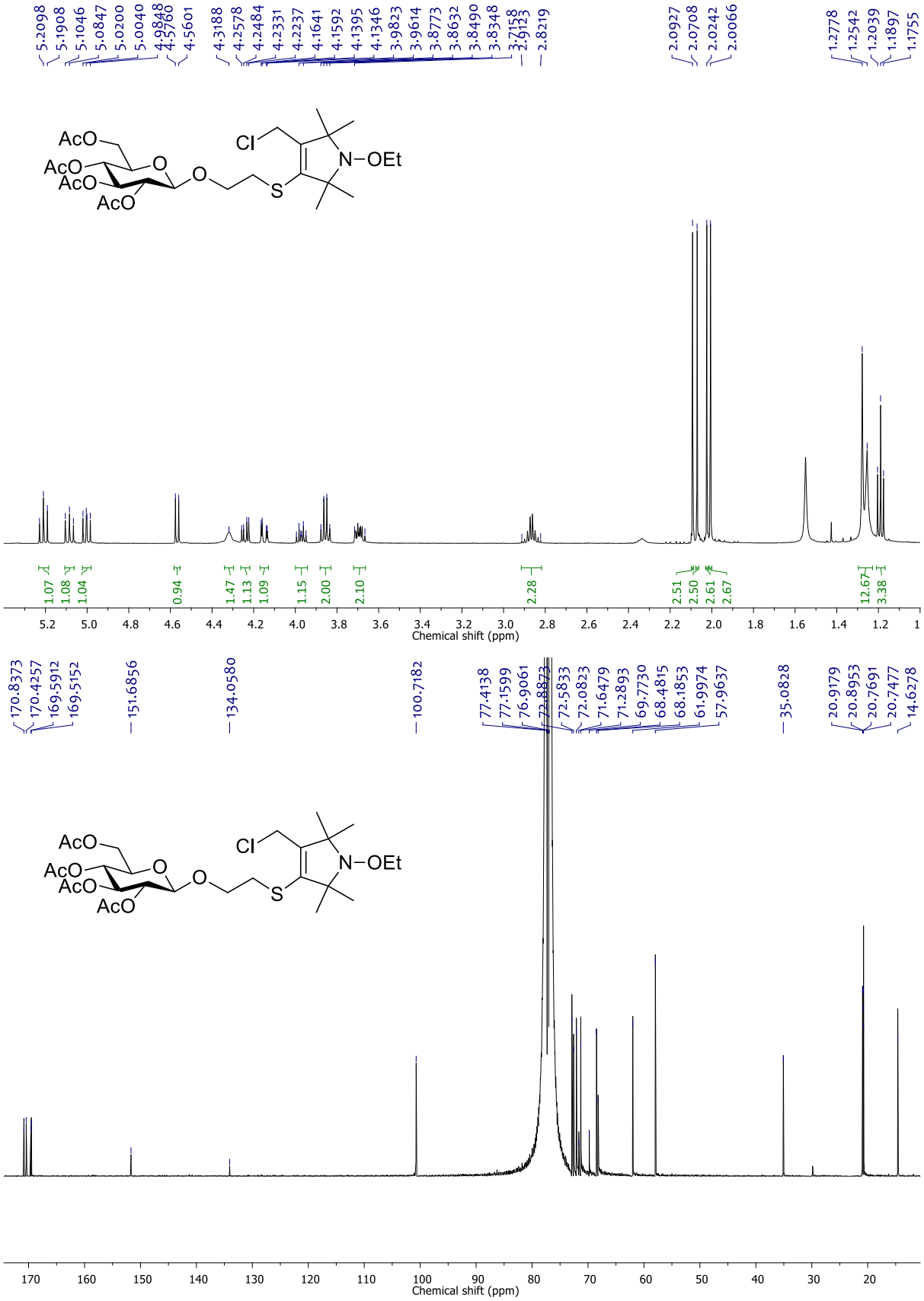


Fig S10. ^{1}H (top) and ^{13}C (bottom) NMR spectra for ethoxyamine **8a**.

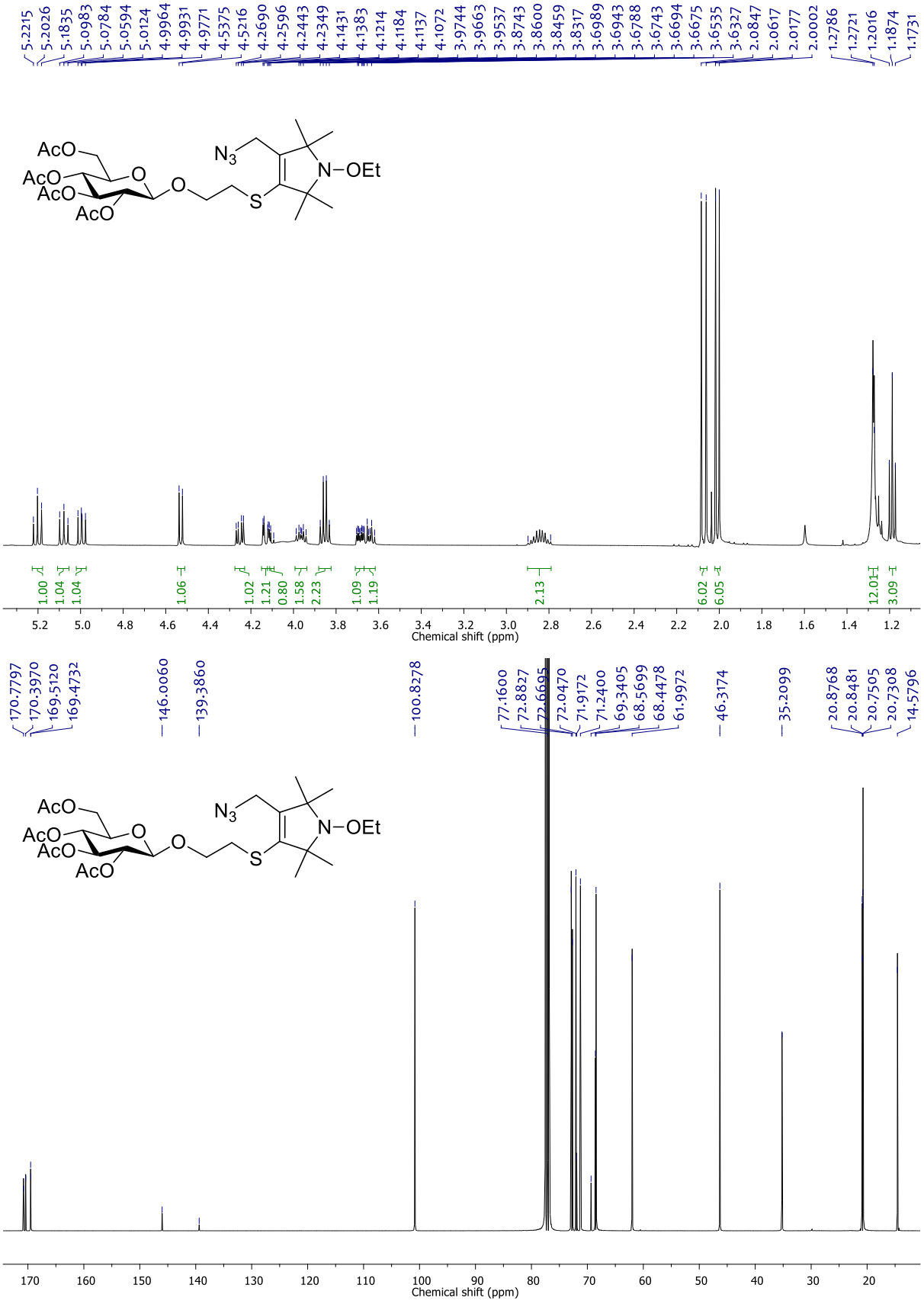


Fig S11. ^{1}H (top) and ^{13}C (bottom) NMR spectra for ethoxyamine **9a**.

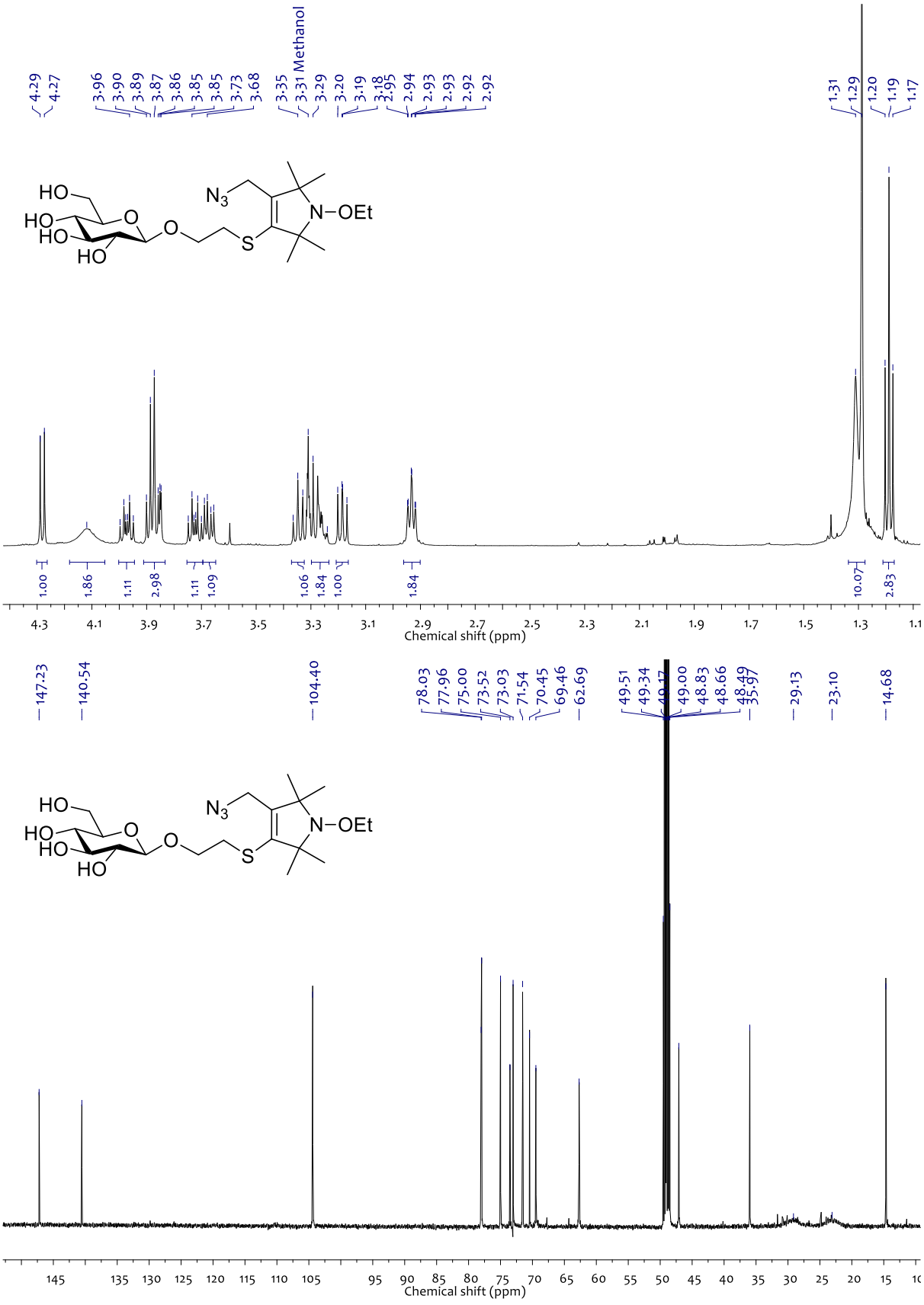


Fig S12. ^{1}H (top) and ^{13}C (bottom) NMR spectra for ethoxyamine **10a**.

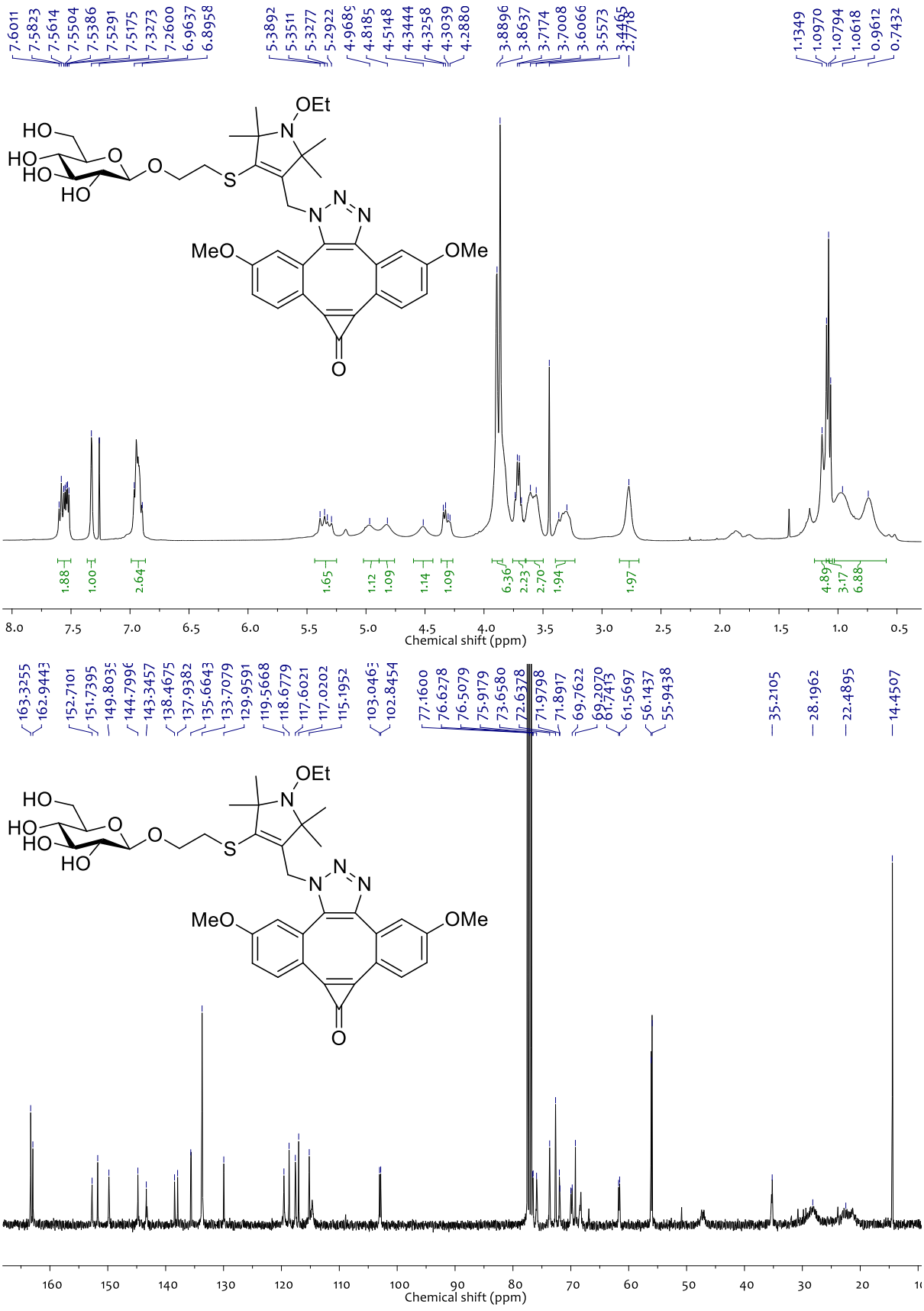
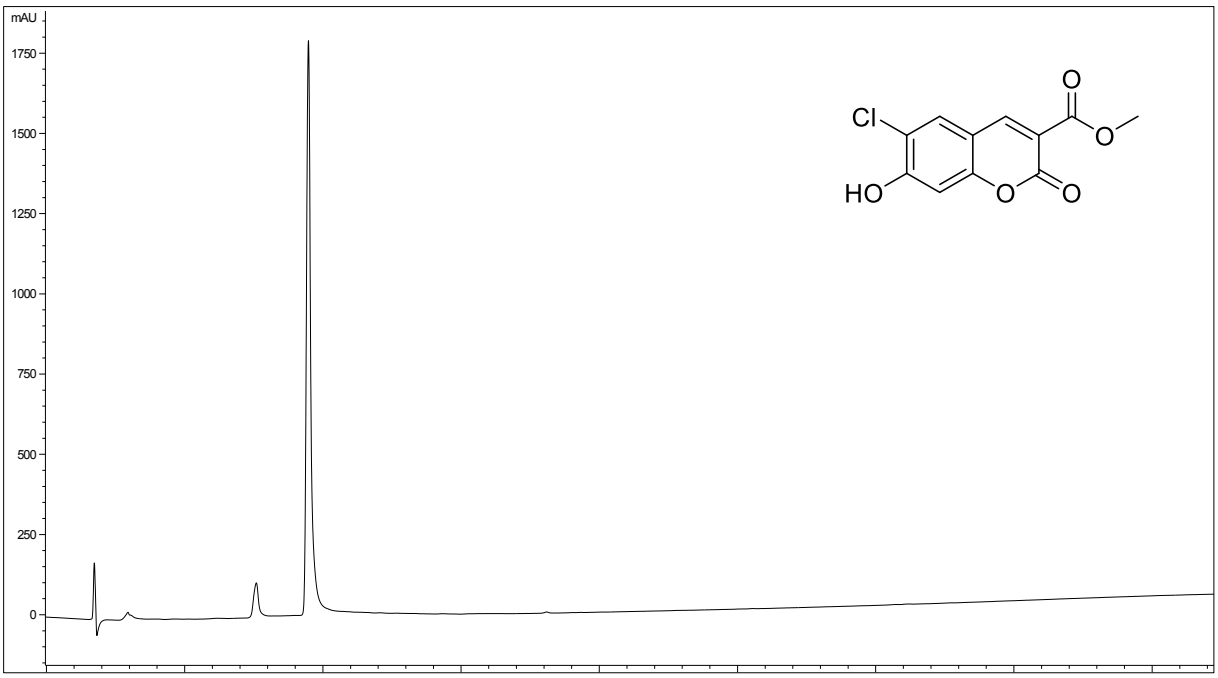


Fig S13. ^{1}H (top) and ^{13}C (bottom) NMR spectra for ethoxyamine **11a**.



Area Percent Report

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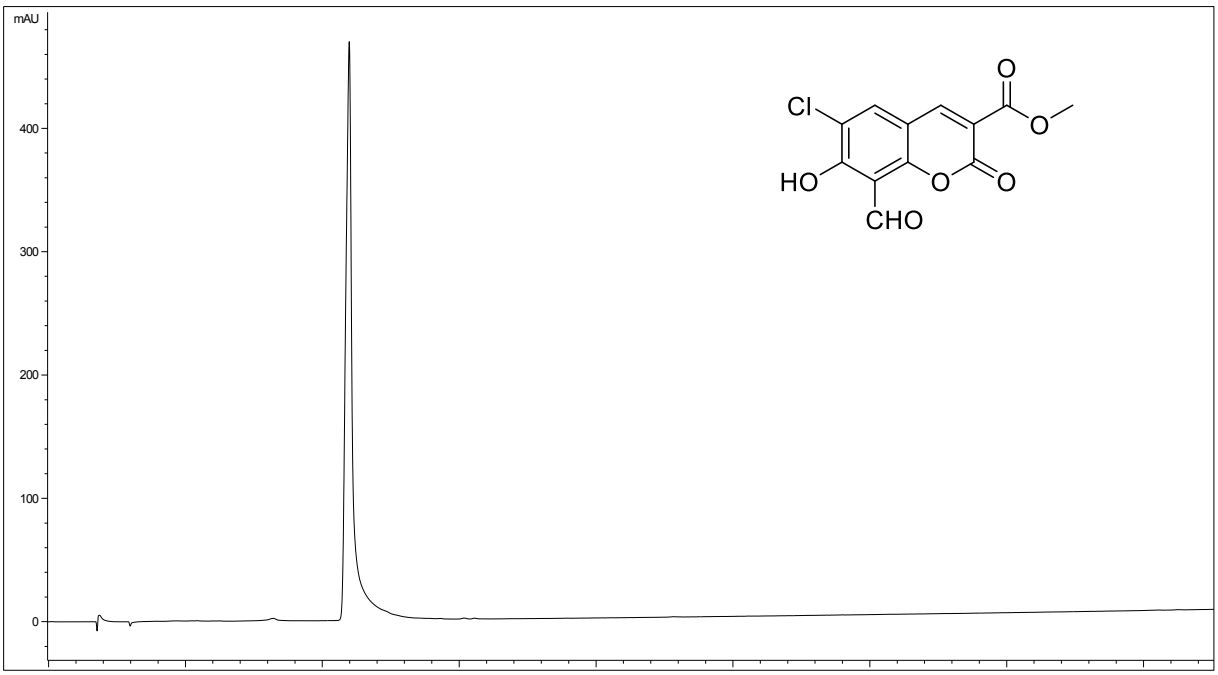
1	7.578	2798.2	121.1	0.3216	11.698	1.53
2	9.743	21123	1797.7	0.1771	88.302	0.718

Fig S14. HPLC trace for Coumarin **13**



Area Percent Report

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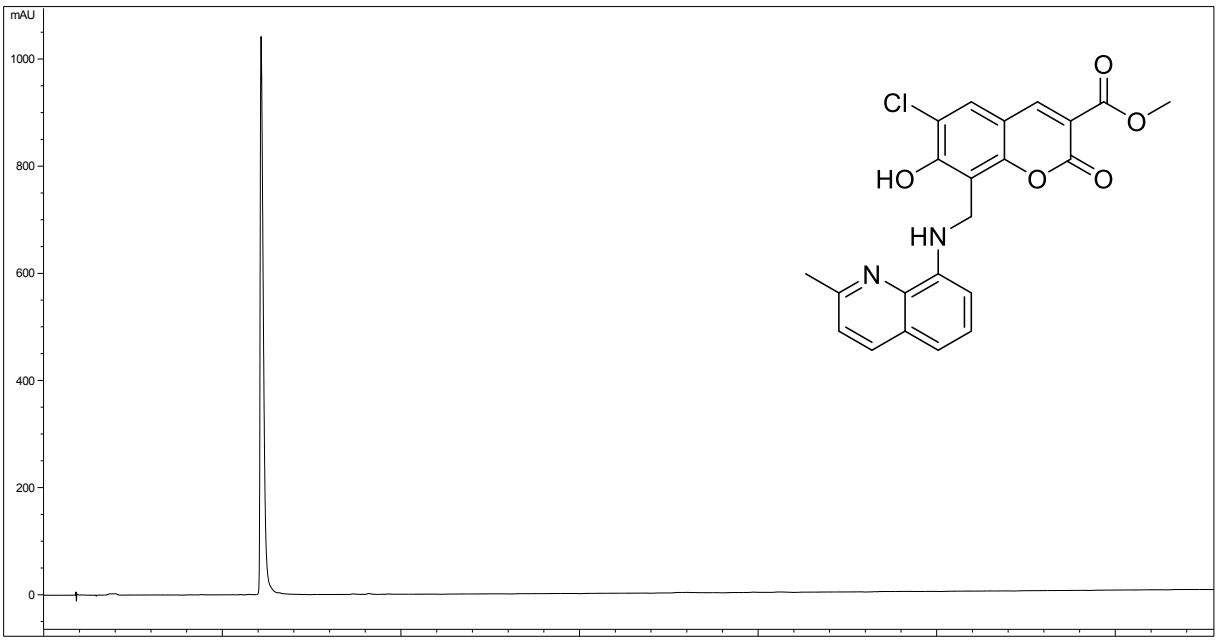
1	10.98	7952.1	468.5	0.234	100.000	0.882

Fig S15. HPLC trace for Coumarin **14**.



Area Percent Report

Peak #	Retention Time (min)	Area	Height (mAu)	Width (min)	Area (%)	Symmetry
1	12.162	12956.2	1041.2	0.1902	100.000	0.381

Fig S16. HPLC trace for Coumarin **16**.

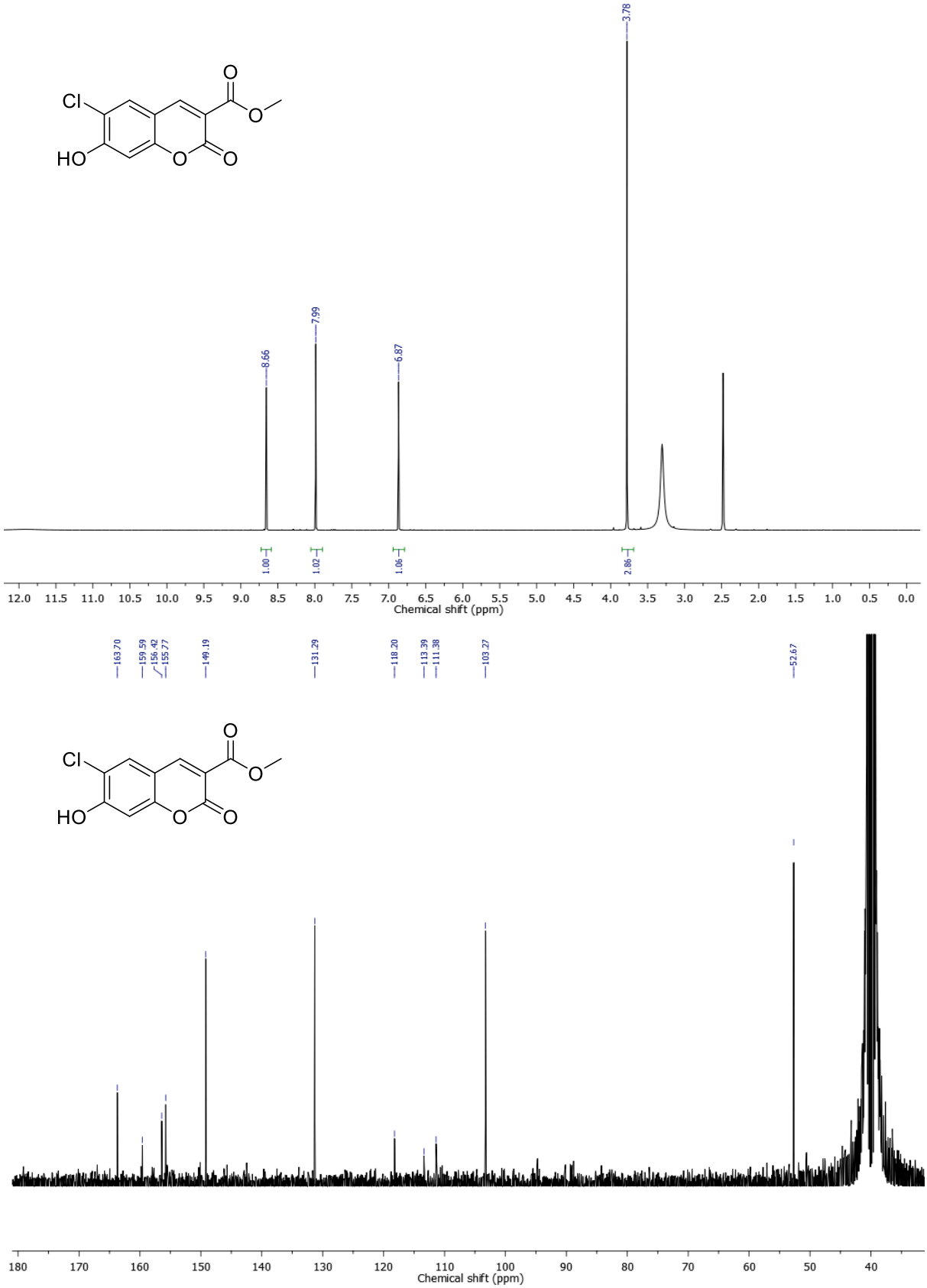


Fig S17. ^{1}H (top) and ^{13}C (bottom) NMR spectra for Coumarin **13**.

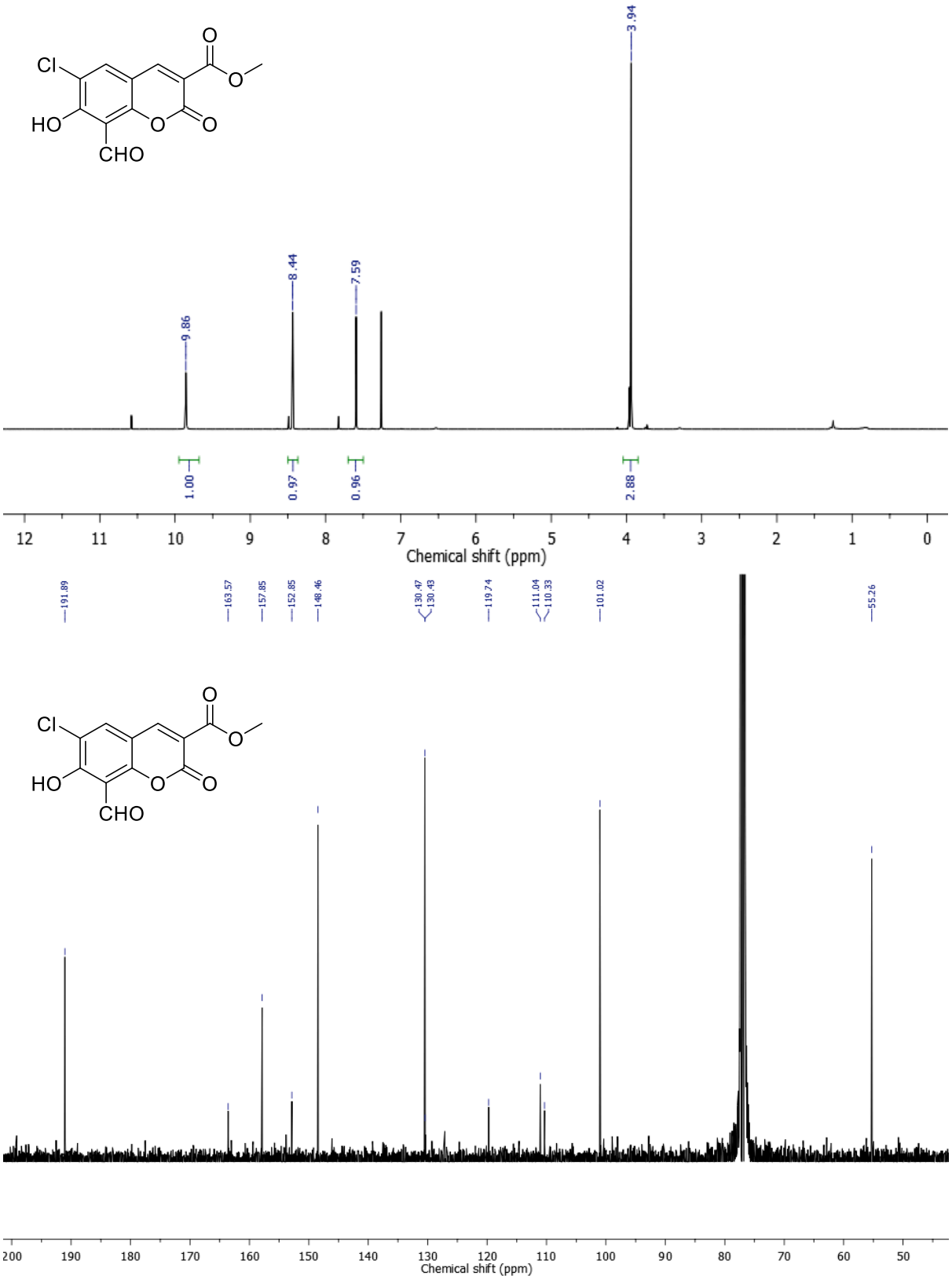


Fig S18. ^{1}H (top) and ^{13}C (bottom) NMR spectra for Coumarin **14**.

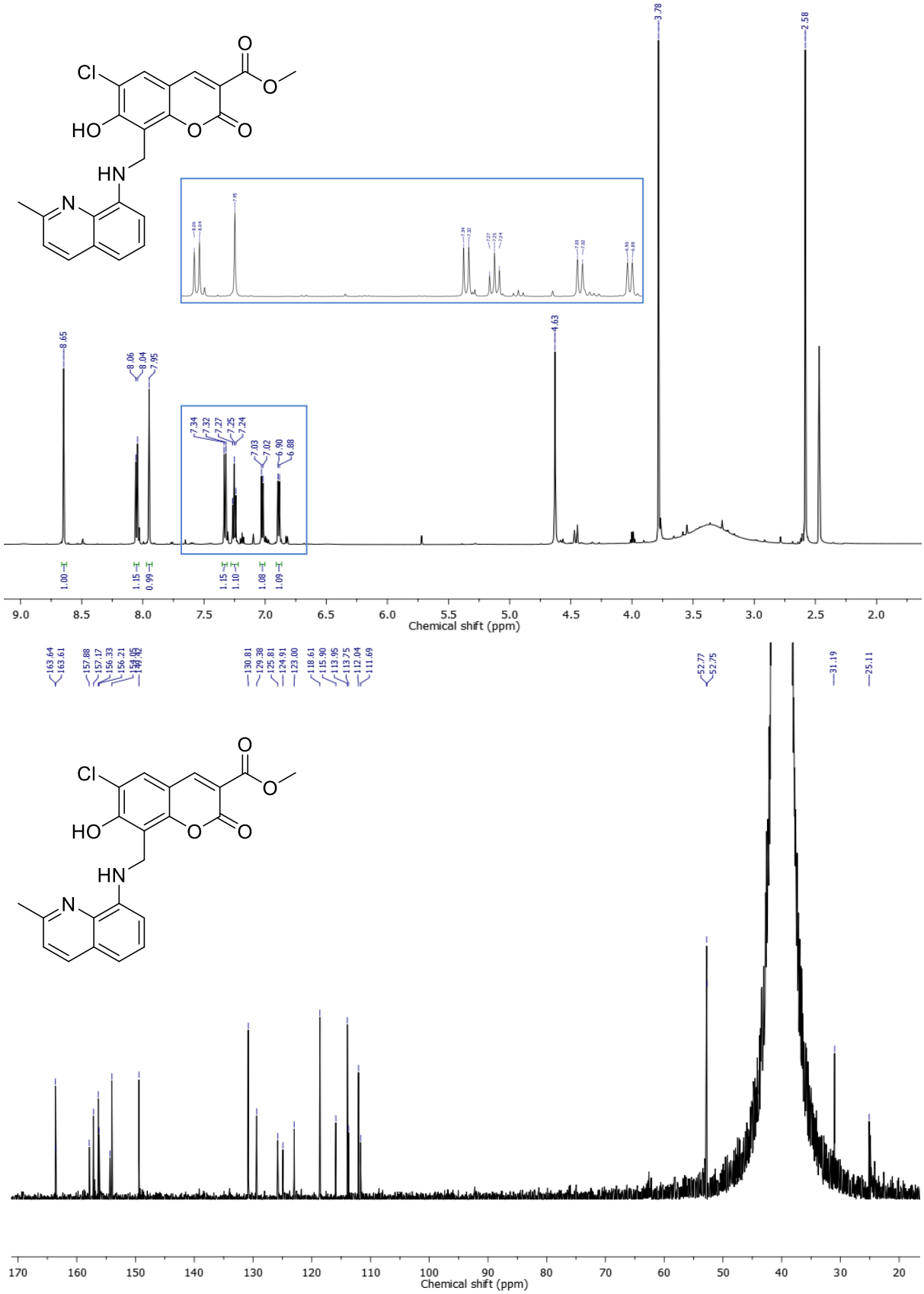


Fig S19. ^{1}H (top) and ^{13}C (bottom) NMR spectra for Coumarin **16**.