Supporting Information 2 (SI2)

Details of the Syntheses for

Improving the accuracy of Cu(II)-Nitroxide RIDME in the Presence of Orientation Correlation Evaluated with Water-soluble Cu(II)-Nitroxide Rulers

Irina Ritsch,[a‡] Henrik Hintz,[b‡] Gunnar Jeschke,[a] Adelheid Godt,*[b] and Maxim Yulikov*[b]

[a]Laboratory of Physical Chemistry, Department of Chemistry and Applied Bioscience, ETH Zurich, Vladimir-Prelogweg 2, 8093 Zurich, Switzerland. E-mail: maxim.yulikov@phys.chem.ethz.ch

[b]Faculty of Chemistry and Center for Molecular Materials (CM2), Bielefeld University, Universitätsstraße 25, 33615 Bielefeld, Germany. E-mail: godt@uni-bielefeld.de

*Corresponding authors

‡Authors contributed equally. I.R. performed the spectroscopic measurements and data processing. H.H. performed the synthetic work.

Nomenclature for the rulers PyMTA-nitroxide and TAHA-nitroxide

The nomenclature used in the following sections for the molecular rulers [Cu(II)-TAHA]-nitroxide **1** and [Cu(II)-PyMTA]-nitroxide **2** is explained on the example of ruler **1** (Figure S1).

The ruler consists of a complex ([Cu(II)-PyMTA]$^{2-}$), a nitroxide moiety (NO•), and phenylene (P) and ethynylene (E) units. With $(Na^+)_2$[Cu(II)-PyMTA]$^{2-}$-$(EP)_2$-NO• the constitution of the ruler is described. Information on the type of the side chains of the spacer is not contained. This information is given in the schemes and figures. "PyMTA" and "TAHA" refer to the fully deprotonated ligands.

Please, be aware that the naming of the rulers [Cu(II)-TAHA]-nitroxide and [Cu(II)-PyMTA]-nitroxide in the main manuscript is simplified and does not contain information on the protonation degree.

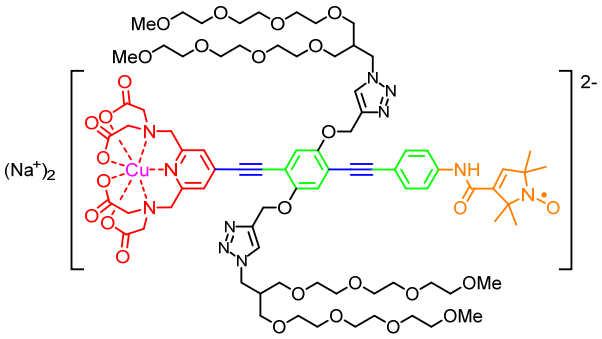
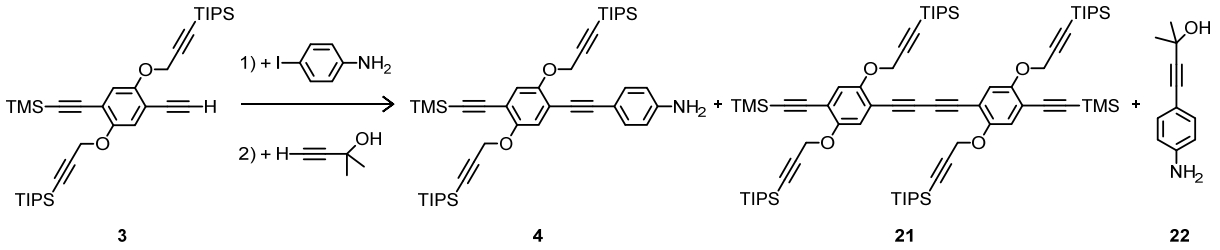


Figure S1. $(Na^+)_2$[Cu(II)-PyMTA]$^{2-}$-$(EP)_2$-NO• as an example for the nomenclature of PyMTA-nitroxide and TAHA-nitroxide rulers and their precursors.

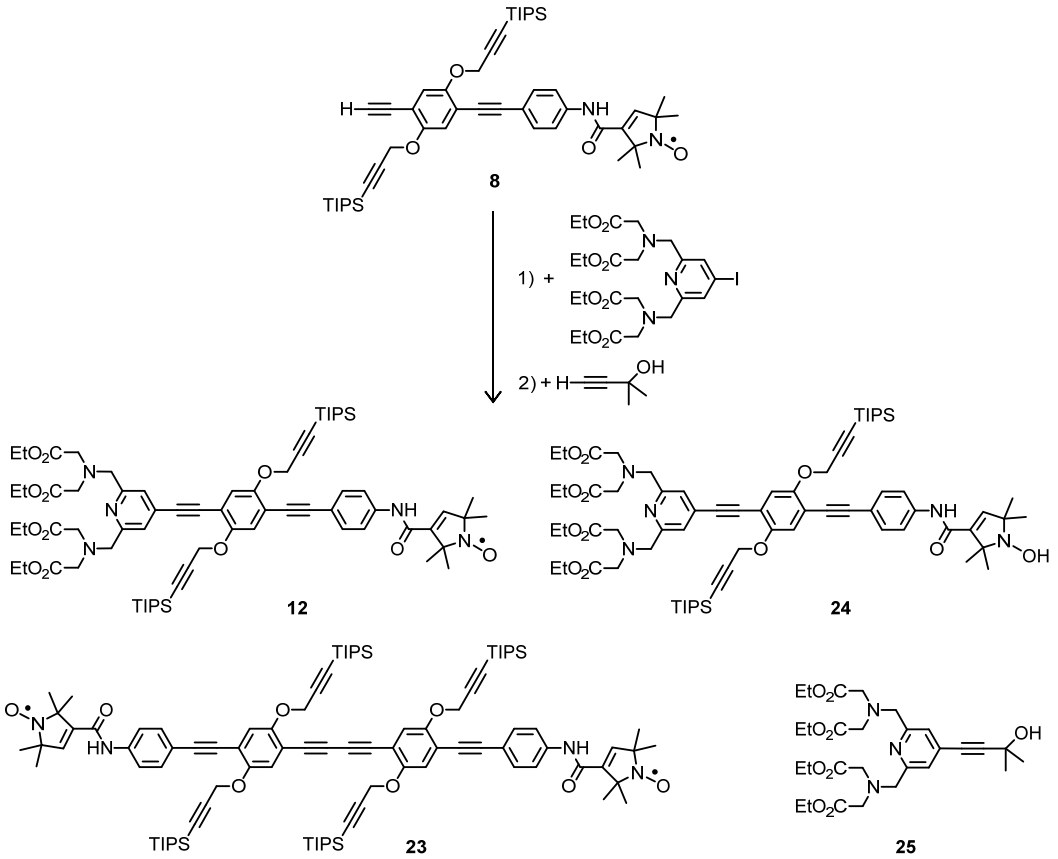
Identified byproducts and polar tagging for simple isolation

The reaction of alkyne **3** and 4-iodoaniline gave a mixture consisting mainly of the amino functionalized spacer (TMS-$(EP)_2$-NH_2) **4** and a small amount of alkyne dimer **21** and leftover 4-iodoaniline. TMS-$(EP)_2$-NH_2 **4** and alkyne dimer **21** are easy to separate because the amino group of TMS-$(EP)_2$-NH_2 **4** gave these compounds a significantly different chromatographic behavior. To ease the separation of TMS-$(EP)_2$-NH_2 **4** from 4-iodoaniline, residual 4-iodoaniline was trapped before work-up through coupling with 2-methylbut-3-yn-2-ol (Scheme S1). The resulting highly polar product **22** was easily separated from the main product TMS-$(EP)_2$-NH_2 **4** by column chromatography.



Scheme S1. Compounds isolated after Sonogashira-Hagihara coupling of alkyne **3** first with 4-iodoaniline and then with 2-methylbut-3-yn-2-ol.

A comparable situation was found in the case of Sonogashira-Hagihara coupling of alkyne **8** and 4-iodo-PyMTA ethyl ester (**10**) or 4-iodo-TAHA ethyl ester (**9**). Besides PyMTAester-$(EP)_2$-NO• **12** a small amount of dimer **23** was isolated (Scheme S2). This dimer was also identified in the product mixture of the Sonogashira-Hagihara coupling of alkyne **8** and 4-iodo-TAHA ethyl ester (**9**). Furthermore, 2-methylbut-3-yn-2-ol was used for polar tagging of remaining 4-iodo-PyMTA ethyl ester (**10**) giving the highly polar byproduct **25** and therefore facilitating the isolation of PyMTAester-$(EP)_2$-NO• **12**.
In addition to the expected compounds, hydroxylamine **24** was isolated. This compound was most probably generated by reduction of nitroxide **12** with copper(I).



Scheme S2. Compounds isolated after Sonogashira-Hagihara coupling of alkyne **8** first with 4-iodo-PyMTA ethyl ester (**10**) and then with 2-methylbut-3-yn-2-ol.

Syntheses of the Cu(II)-nitroxide rulers 1 and 2

Scheme 1 given in the main text provides the compound numbers of starting materials and products. Compound numbers of identified byproducts and side products can be found in the section above.

General

Unless otherwise stated, reactions were performed in dried glassware under argon. Argon was passed through anhydrous $CaCl_2$ prior to its use. Degassed solutions were prepared through applying three freeze-pump-thaw cycles. Solvents were removed at a bath temperature of ~40 °C and reduced pressure. The products were dried at room temperature at ~0.3 mbar. The pH/pD values of the solutions were determined using pH indicator strips (resolution: 0.5 pH).

Unless otherwise stated, commercial solvents and reagents were used. Since all commercial compounds used for syntheses had a purity of >95% their molar amount used in the syntheses were calculated with their compound mass and were not corrected by the manufacturer specified purities. Source, purity, and batch number of the reagents can be found in Table S1. THF and Et_2O (both HPLC grade) were dried with sodium/benzophenone prior to their use. For the preparation of aqueous solutions, deionized water was used. Solvents used for extraction and chromatography were of technical grade and were distilled prior to their use. $PdCl_2(PPh_3)_2$ was synthesized according to literature,[1] however using 2.1 times the given amount of methanol. The syntheses of alkyne **3**,[2] 4-iodo-PyMTA ethyl ester (**10**),[3] H_6TAHA • *n* TFA (**26**)[4] and PEG-N_3 **15**[2] have been reported elsewhere. The synthesis of 4-iodo-TAHA ethyl ester (**9**) will be published separately.

Column chromatography was carried out on silica gel 60 (0.035−0.070 mm) without applying pressure. In the procedures reported below, the size of the column is given as diameter x length. The material was loaded onto the column dissolved in a small quantity of the eluent. Thin layer chromatography (TLC) was performed on silica gel 60 containing fluorescent indicator F254. The solid support for the silica gel layer was aluminum foil. The spots were detected with UV light of λ = 254 nm and 366 nm. The compositions of solvent mixtures are given in volume ratios. For preparative HPLC a Phenomenex Luna® Silica(2) column (particle size: 5 µm, pore size 100 Å, column size 21.2 mm x 250 mm) was used.

The melting points were determined in open capillaries.

The content of a ligand in a batch was quantified by quantitative ^{1}H NMR spectroscopy[5] using a capillary filled with a solution of maleic acid in D_2O as the standard. Measurements were carried out in MeOD and with 90 s pulse delay.

NMR spectra were calibrated using the solvent signal as an internal standard [$CDCl_3$: δ (^{1}H) = 7.25, δ (^{13}C) = 77.0; CD_2Cl_2: δ (1H) = 5.32, δ (13C) = 53.8; CD_3OD: δ (1H) = 3.31, δ (^{13}C) = 49.0]. Signal assignments are supported by DEPT-135, COSY, HMBC, and HMQC experiments.

ESI MS spectra were recorded using an Esquire 3000 ion trap mass spectrometer (Bruker Daltonik) equipped with a standard ESI source. Accurate MS measurements were performed using an Agilent 6220 time-of-flight mass spectrometer (Agilent Technologies) equipped with a dual ESI source. The monoisotopic mass of the compounds is reported.

Table S1. Source, purity, and batch number of commercial compounds used for reactions and preparation of the solutions used for EPR spectroscopical experiments.

compound	manufacturer	purity [%]	batch number
Copper(II) chloride	Fluka	99	351228/1 396
$[Cu(phen)(PPh_3)_2]NO_3 \bullet 0.5\ CH_2Cl_2$	Aldrich	95	MKAA0733V
Deuterium oxide (for Cu(II)-TAHA **20**)	Roth	99.8	19750
Deuterium oxide (for the rulers **1** and **2**)	Deutero	99.9	B15529
Dichloromethane, dry, over molecular sieve	Acros	99.8	1417130
Dichloromethane, dry, over molecular sieve (degassed)	Acros	99.8	1277573
Dichloromethane	VWR	99.9	15D300512
Diisopropylamine	Merck	≥ 99.0	S4082816429
4-Dimethylaminopyridine	Janssen Chimica	99	44411/1
Ethanol	VWR	99.96	16E284008
Hydrochloric acid, 37%	Fisher Scientific	p.A.	1674520
4-Iodoaniline	Acros	99	A0340759
Methanol	VWR	100.0	16G074015
2-Methylbut-3-yn-2-ol	Aldrich	98	1349654 53307311
Piperidine	Alfa Aesar	99	10181422
Potassium carbonate	VWR	100.6	07E020030
Quadrapure BZA	Aldrich	---	BCBF4504V
Quadrapure TU	Aldrich	---	BCBL0423V
Sodium hydroxide	VWR	99.2	13K210004
Sodium deuteroxide, 40% in D_2O	Acros	---	A0333261
Tetrakis(triphenylphosphine)palladium(0)	Aldrich	99	SHBD8863VP
Tetra-*n*-butylammonium fluoride, 1 M solution in THF (containing 3.27% H_2O)	Alfa Aesar	---	10176007
2,2,5,5-Tetramethyl-3-pyrrolin-1-oxyl-3-carboxylic acid	Acros	99	A098034301
Thionyl chloride	Merck	≥ 99.0	S6968754534
Toluene, dry, over molecular sieve	Acros	99.85	1561013

Synthesis of Cu(II)-nitroxide ruler $(H^+)_x(Na^+)_{4-x}$[Cu(II)-TAHA]$^{4-}$-$(EP)_2$-NO• 1 and the corresponding Gd(III)-nitroxide ruler $(H^+)_x(Na^+)_{3-x}$[Gd(III)-TAHA]$^{3-}$-$(EP)_2$-NO•

TMS-$(EP)_2$-NH_2 4. To a degassed solution of alkyne **3** (500 mg, 808 µmol) and 4-iodoaniline (195 mg, 890 µmol) in THF (20 mL) and piperidine (4.0 mL, 40 mmol) was added $PdCl_2(PPh_3)_2$ (11.3 mg, 16.1 µmol) and CuI (6.2 mg, 32 µmol). Shortly after addition of the catalysts a colorless precipitate formed. The yellow/orange suspension was stirred at room temperature for 25 h. The reaction was monitored by TLC (pentane/CH_2Cl_2 3:1, R_f(**3**) = 0.38, R_f(**21**) = 0.28, R_f(**4**) = 0.15, R_f(4-iodoaniline) = 0.02). 2-Methylbut-3-yn-2-ol (39.5 µL, 404 µmol) was added and the suspension was stirred at room temperature for another 16 h. All volatiles were removed at room temperature and reduced pressure. The residual brown oily suspension was filtered through silica gel (1.5 cm x 2.5 cm, rinsing with Et_2O). The solvents were removed. The components of the residual brown oil were separated by column chromatography (3 cm x 29 cm). Eluting with pentane/Et_2O 3:1 gave alkyne dimer **21** (24 mg, 5%; R_f (pentane/Et_2O 3:1) = 0.79; R_f (pentane/Et_2O 1:1) = 0.85; R_f (Et_2O) = 0.88) as a yellow solid. Then, the eluent was changed to pentane/Et_2O 1:1 and TMS-$(EP)_2$-NH_2 **4** (482 mg, 84%, R_f (pentane/Et_2O 3:1) = 0.12; R_f (pentane/Et_2O 1:1) = 0.36; R_f (Et_2O) = 0.63; Mp: 39-41 °C) was obtained as a yellow solid. Finally, the eluent was changed to Et_2O and a yellow oil (28 mg, R_f (pentane/Et_2O 3:1) = 0; R_f (pentane/Et_2O 1:1) = 0.15; R_f (Et_2O) = 0.48) consisting of coupling product **22** and minor amounts of other compounds was obtained.
For ^{1}H NMR data of TMS-$(EP)_2$-NH_2 **4**, alkyne dimer **21** and coupling product **22** see Tables S2 and S3. For ^{13}C NMR data of TMS-$(EP)_2$-NH_2 **4** see Tables S4 and S5. Additional analytical data of TMS-$(EP)_2$-NH_2 **4**: MS (ESI), *m*/*z* = 710.5 $[M + H]^+$, 732.5 $[M + Na]^+$. Elemental analysis calcd (%) for $C_{43}H_{63}NO_2Si_3$: C, 72.72; H, 8.94; N, 1.97; found: C, 72.82; H, 9.08; N, 2.00.

TMS-$(EP)_2$-NO• 7. To a solution of 2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl-3-carboxylic acid (96 mg, 0.52 mmol) and 4-dimethylaminopyridine (146 mg, 1.30 mmol) in dry CH_2Cl_2 (10 mL) cooled with an ice water bath was added thionyl chloride (36.8 µL, 507 µmol). Immediately after the addition of thionyl chloride the color of the solution had changed from yellow to dark orange. After 5 min the ice water bath was removed and the solution was stirred for another 60 min at room temperature. A solution of TMS-$(EP)_2$-NH_2 **4** (181 mg, 255 µmol) in dry CH_2Cl_2 (10 mL) was added and the yellow/orange solution was stirred at room temperature for 75 min. The reaction was monitored by TLC (CH_2Cl_2/Et_2O 20:1, R_f(**4**) = 0.86, R_f(**7**) = 0.57). Subsequently, the solution was filtered through silica gel (2 cm x 3.5 cm, rinsing with 70 mL Et_2O). The resulting yellow solution was washed with 2 M aqueous solution of HCl (10 mL), saturated aqueous solution of $NaHCO_3$ (2 x 10 mL) and water (10 mL), dried over $MgSO_4 \cdot x\ H_2O$, and filtered. The solvents were removed. The components of the residual yellow solid were separated by column chromatography (3 cm x 24 cm). Elution with CH_2Cl_2/Et_2O 20:1 gave a mixture of unidentified compounds

(3 mg; R_f = 0.92, 0.86, 0.61) as a yellow solid and TMS-(EP)$_2$-NO• **7** (199 mg, 89%; R_f = 0.50) as a yellow solid.
For ^{1}H NMR data see Tables S2 and S3. For ^{13}C NMR data see Tables S4 and S5. MS (ESI): m/z = 898.4 $[M + Na]^+$.

H-(EP)$_2$-NO• 8. The reaction was performed in air. To a solution of TMS-(EP)$_2$-NO• **7** (100 mg, 114 µmol) in MeOH (10 mL) and CH_2Cl_2 (10 mL) was added K_2CO_3 (24 mg, 0.17 mmol). The suspension consisting of a yellowish solution and a colorless solid was stirred at room temperature for 20.5 h. Et_2O (40 mL) and H_2O (10 mL) were added. The organic phase was separated, and the aqueous phase was extracted with Et_2O (2 x 10 mL). The combined organic phases were washed with H_2O (10 mL), dried over $MgSO_4 \cdot x\ H_2O$, and filtered. The solvents were removed. The components of the residual yellow solid were separated by column chromatography (2 cm x 23 cm). Elution with CH_2Cl_2/Et_2O 20:1 gave H-(EP)$_2$-NO• **8** (91 mg, 99%; R_f = 0.31) as a yellow viscous oil.
For ^{1}H NMR data see Tables S2 and S3. For ^{13}C NMR data see Tables S4 and S5. MS (ESI): m/z = 826.5 $[M + Na]^+$.

TAHAester-(EP)$_2$-NO• 11. To a degassed solution of H-(EP)$_2$-NO• **8** (79 mg, 98 µmol) and 4-iodo-TAHA ethyl ester (**9**) (89 mg, 0.11 mmol) in THF (10 mL) and ^{i}Pr$_2$NH (690 µL, 4.91 mmol) was added $PdCl_2(PPh_3)_2$ (2.8 mg, 3.9 µmol) and CuI (1.5 mg, 7.8 µmol). The yellow solution was stirred at room temperature for 23 h. The reaction was monitored by TLC (Et_2O/pentane 10:1, R_f(**9**) = 0.67, R_f(**8**) = 0.52, R_f(**11**) = 0.40, R_f(**23**) = 0.21). All volatiles were evaporated, strictly keeping the reaction mixture under argon. The residue was dissolved in degassed anhydrous CH_2Cl_2 (10 mL) and metal scavenger QuadraPure TU (156 mg) was added. The yellow suspension was stirred at room temperature for 15 h. Metal scavenger QuadraPure BZA (15 mg) was added and the suspension was stirred for another 7 h at room temperature. The metal scavenger QuadraPure BZA did not change its color, which indicated that there had been no free Cu or Pd ions left in the solution. The suspension was filtered through a syringe filter (PTFE membrane, 13 mm diameter, 0.45 µm pore size) and the solvent was removed. The yellow/orange solid was suspended in Et_2O (5 mL). Again, the suspension was filtered through a syringe filter (PTFE membrane, 13 mm diameter, 0.45 µm pore diameter) and the solvent was removed. The components of the residual yellow/orange solid were separated by column chromatography (1.5 cm x 41 cm). Eluting with Et_2O/pentane 10:1 gave as a first fraction a mixture (35 mg; R_f = 0.70) of 4-iodo-TAHA ethyl ester (**9**) as the main component with triphenylphosphane oxide and a minor amount of unidentified compounds as a colorless oil. As the second fraction TAHAester-(EP)$_2$-NO• **11** (96 mg, 65%; R_f = 0.45) was obtained as a yellow solid. The third fraction was alkyne dimer **23** (27 mg, 34%; R_f = 0.30), a yellow solid.

For ^{1}H NMR data of TAHAester-(EP)$_2$-NO• **11** and alkyne dimer **23** see Tables **S2** and **S3**. For ^{13}C NMR data of TAHAester-(EP)$_2$-NO• **11** see Tables S4 and S5. MS (ESI) of TAHAester-(EP)$_2$-NO• **11**: *m*/*z* = 1497.8 $[M + H]^+$, 1519.8 $[M + Na]^+$.

Desilylated TAHAester-(EP)$_2$-NO• 13. TAHAester-(EP)$_2$-NO• **11** (95 mg, 63 µmol) was dissolved in THF (5 mL) and a 1.0 M solution of Bu_4NF in THF (190 µL, 190 µmol) was added. Immediately after addition of Bu_4NF the color of the reaction solution had changed from yellow to orange. The solution was stirred at room temperature for 15 min. It was filtered through silica gel (1.5 cm x 2.5 cm, rinsing with THF). Solvent removal gave a yellow oil (102 mg) consisting of desilylated TAHAester-(EP)$_2$-NO• **13** accompanied by TIPS-F and/or TIPS-OH, and a Bu_4N salt. For ^{1}H NMR data see Tables S2 and S3.

PEGylated TAHAester-(EP)$_2$-NO• 16. PEG-N_3 **15** (80 mg, 0.19 mmol) and material (102 mg; containing ~ 63 µmol desilylated TAHAester-(EP)$_2$-NO• **13**) that had been obtained by desilylation of TAHAester-(EP)$_2$-NO• **11** (see above) were dissolved in dry toluene (8 mL) and the solution was degassed. $[Cu(phen)(PPh_3)_2]NO_3$ • 0.5 CH_2Cl_2 (3.3 mg, 3.8 µmol) was added. The yellow solution was stirred at room temperature for 94 h. Metal scavenger QuadraPure TU (75 mg) was added. The yellow suspension was stirred at room temperature for 20.5 h. Metal scavenger QuadraPure BZA (15 mg) was added and the suspension was stirred for another 2 h at room temperature. The metal scavenger QuadraPure BZA did not change its color, which indicated that there had been no free Cu ions left in the solution. The solution was decanted off from the scavenger and the solvent was removed. The residual brown oil was filtered through silica gel (1 cm x 1.5 cm, rinsing with CH_2Cl_2/EtOH 9:1). After removal of the solvent the components of the residual brown oil were separated by preparative HPLC (two isocratic runs with CH_2Cl_2/EtOH 19:1, flow rate of 20 mL/min; 2 x 77 mg of the brown oil dissolved in CH_2Cl_2 (310 µL, 550 µL) were injected) This gave PEGylated TAHAester-(EP)$_2$-NO• **16** (95 mg, 74% over 2 steps; R_f = 0.20) at a retention time of 8.9 min.
For ^{1}H NMR data see Tables S2 and S3. For ^{13}C NMR data see Tables S4 and S5. MS (ESI): *m*/*z* = 1038.4 $[M + 2Na]^{2+}$, 2053.9 $[M + Na]^+$.

$(H^+)_x(Na^+)_{6-x}$[TAHA-(EP)$_2$-NO•]$^{6-}$ 18. The reaction was performed in air. To a solution of PEGylated TAHAester-(EP)$_2$-NO• **16** (28 mg, 14 µmol) in ethanol (2.0 mL) was added water (347 µL). A 0.10 M aqueous solution of NaOH (1653 µL, 165.2 µmol) was added which resulted in a color change of the solution from colorless to slightly yellowish within seconds. The solution was stirred at room temperature for 16.5 h. The pH was lowered to 7.0 by addition of a 0.10 M aqueous solution of HCl (1100 µL, 110.0 µmol). Removal of the solvents gave a slightly yellowish solid. The ^{1}H NMR spectrum revealed an incomplete saponification of the ester groups. The solid was dissolved in water (1173 µL) and a 0.10 M aqueous solution NaOH (827 µL, 82.7 µmol) was added. The solution was stirred at room temperature for 20.5 h. The pH was lowered to 7.0 by addition of a 0.10 M aqueous solution of HCl (525 µL, 52.5 µmol). After removal of the solvents a slightly

yellowish solid was obtained. Again, the ^{1}H NMR spectrum revealed an incomplete saponification of the ester groups. The solid was dissolved in water (1173 µL) and a 0.10 M aqueous NaOH (827 µL, 82.7 µmol) was added. The solution was stirred at room temperature for 17.5 h. The pH was lowered to 7.0 by addition of 0.10 M aqueous solution of HCl (800 µL, 80.0 µmol). ^{1}H NMR spectroscopy showed a complete ester hydrolysis. After removal of the solvents the slightly yellowish solid was suspended in CH_2Cl_2 (1.5 mL). The suspension was filtered through a syringe filter (PTFE membrane, 13 mm diameter, 0.2 µm pore size). The filter cake was washed with CH_2Cl_2 (1 x 1 mL, 1 x 0.5 mL) and the solvent of the combined filtrates was removed giving a slightly yellowish solid (23 mg) consisting of $(H^+)_x(Na^+)_{6-x}$[TAHA-$(EP)_2$-NO•]$^{6-}$ **18** and NaCl. The content of the structural motive [TAHA-$(EP)_2$-NO•]$^{6-}$ in this solid was determined by quantitative ^{1}H NMR spectroscopy to be 9.6 µmol (68% yield).
For ^{1}H NMR data see Tables S2 and S3. For ^{13}C NMR data see Tables S4 and S5. MS (ESI): *m*/*z* = 1861.7 [M - H]$^-$, 930.3 [M - 2H]$^{2-}$. Accurate MS (ESI): *m/z* calcd for [M + 2H]$^{2+}$, $C_{89}H_{130}N_{11}O_{32}^{2+}$, 932.4436; found, 932.4457; M = $(H^+)_6$[TAHA-$(EP)_2$-NO•]$^{6-}$

General procedure for syntheses of rulers with TAHA as the ligand. A solution (600 µL) of $(H^+)_x(Na^+)_{6-x}$[TAHA-$(EP)_2$-NO•]$^{6-}$ **18** (19.063 mg containing 7.9260 µmol of the structural motive [TAHA-$(EP)_2$-NO•]$^{6-}$) in D_2O was prepared. A part of the obtained solution (158.9 µL containing 2.099 µmol of the structural motive [TAHA-$(EP)_2$-NO•]$^{6-}$) was mixed with a 0.05 M solution of a metal salt in D_2O (39.9 µL, 2.00 µmol). A 0.10 M solution of NaOD in D_2O was added to raise the pH of the solution to pH 7. The solution was diluted with D_2O up to a total volume of 420 µL to obtain a 5.0 mM solution of the [metal ion-TAHA]-nitroxide ruler in D_2O containing NaCl.

Cu(II)-nitroxide ruler $(H^+)_x(Na^+)_{4-x}$[Cu(II)-TAHA]$^{4-}$-$(EP)_2$-NO• 1. Metal salt: $CuCl_2$. NaOD in D_2O: 15 µL, 1.5 µmol. A bluish solution of $(H^+)_x(Na^+)_{4-x}$[Cu(II)-TAHA]$^{4-}$-$(EP)_2$-NO• **1** in D_2O was obtained. MS (ESI): *m*/*z* = 1966.6 [M - 3H + 2Na]$^-$, 1944.6 [M - 2H + Na]$^-$, 1922.7 [M - H]$^-$, 960.7 [M - 2H]$^{2-}$; M = $(H^+)_4$[Cu(II)-TAHA]$^{4-}$-$(EP)_2$-NO•.

Gd(III)-nitroxide ruler $(H^+)_x(Na^+)_{3-x}$[Gd(III)-TAHA]$^{3-}$-$(EP)_2$-NO•. Metal salt: $GdCl_3 \cdot 6\ H_2O$. NaOD in D_2O: 20 µL, 2.0 µmol. A yellowish solution of $(H^+)_x(Na^+)_{3-x}$[Gd(III)-TAHA]$^{3-}$-$(EP)_2$-NO• **27** in D_2O was obtained. MS (ESI): *m*/*z* = 2016.7 [M - H]$^-$, 1007.7 [M - 2H]$^{2-}$; M = $(H^+)_3$[Gd(III)-TAHA]$^{3-}$-$(EP)_2$-NO•.

Synthesis of the Cu(II)-nitroxide ruler $(Na^+)_2$[Cu(II)-PyMTA]$^{2-}$-$(EP)_2$-NO• 2 and the corresponding metal ion-rulers $(Na^+)_{(4-n)}$[M^{n+}-PyMTA]$^{(4-n)-}$-$(EP)_2$-NO• with M = Mn^{2+}, Dy^{3+}, Gd^{3+})

PyMTAester-$(EP)_2$-NO• 12. To a degassed solution of H-$(EP)_2$-NO• **8** (43 mg, 53 µmol) and 4-iodo-PyMTA ethyl ester (**10**) (39 mg, 64 µmol) in THF (3.0 mL) and $^iPr_2NH$ (376 µL, 2.68 mmol) was added $Pd(PPh_3)_4$ (2.5 mg, 2.2 µmol) and CuI (1.0 mg, 5.3 µmol). The yellow suspension was stirred at room temperature for 65 h. The reaction was monitored by TLC (CH_2Cl_2/Et_2O/pentane 5:5:2, R_f(**8**) = 0.77, R_f(**23**) = 0.64, R_f(**10**) = 0.64, R_f(**12**) = 0.51). 2-Methylbut-3-yn-2-ol (5.0 µL, 51 µmol) was added and the suspension was stirred at room temperature for another 25 h. The reaction was monitored by TLC (Et_2O/pentane 1:1, R_f(**10**) = 0.30, R_f(**25**) = 0). All volatiles were evaporated, strictly keeping the reaction mixture in argon. The residue was dissolved in degassed anhydrous CH_2Cl_2 (3 mL) and metal scavenger QuadraPure TU (110 mg) was added. The yellow suspension was stirred at room temperature for 20 h. Metal scavenger QuadraPure BZA (15 mg) was added and the suspension was stirred for another 1.5 h at room temperature. The metal scavenger QuadraPure BZA did not change its color, which indicated that there had been no free Cu or Pd ions left in the solution. The suspension was filtered through a syringe filter (PTFE membrane, 13 mm diameter, 0.45 µm pore size) and the solvent was removed. The residual yellow oil was filtered through silica gel (1 cm x 1 cm, rinsing with Et_2O) and the solvents were removed. Preparative HPLC of the residual yellow oil (two gradient runs with CH_2Cl_2/EtOH, 1.3% EtOH at 0 min to 5.9% EtOH at 25 min, flow rate of 20 mL/min; each time 36 mg of the yellow oil dissolved in CH_2Cl_2 (284 µL) were injected) gave five fractions: (1) triphenylphosphane oxide (1 mg; R_f (CH_2Cl_2/Et_2O/pentane 5:5:2) = 0.74) at 3.5 min as a colorless solid; (2) alkyne dimer **23** (3 mg, 7%; R_f (CH_2Cl_2/Et_2O/pentane 5:5:2) = 0.56) at 7.2 min as a yellow oil; (3) a mixture (5 mg; R_f (CH_2Cl_2/Et_2O/pentane 5:5:2) = 0.56) of hydroxylamine **24** (about 7% yield) and small amounts of unidentified components at 9.3 min as a colorless oil; (4) PyMTAester-$(EP)_2$-NO• **12** (36 mg, 53%; R_f (CH_2Cl_2/Et_2O/pentane 5:5:2) = 0.44) at 10.4 min as a yellow oil, and (5) coupling product **25** (8 mg, R_f (CH_2Cl_2/Et_2O/pentane 5:5:2) = 0.26) at 13.2 min as a colorless oil.
For ^{1}H NMR data of the compounds **12**, **23, 24** and **25** see Tables **S2** and **S3**. For ^{13}C NMR data of PyMTAester-$(EP)_2$-NO• **12** see Tables S4 and S5. MS (ESI) of PyMTAester-$(EP)_2$-NO• **12**: *m*/*z* = 1283.8 $[M + H]^+$, 1305.7 $[M + Na]^+$. MS (ESI) of alkyne dimer **23**: *m*/*z* = 1627.8 $[M + Na]^+$.

Desilylated PyMTAester-$(EP)_2$-NO• 14. PyMTAester-$(EP)_2$-NO• **12** (31 mg, 24 µmol) was dissolved in THF (4 mL). Immediately after addition of a 1.0 M solution of Bu_4NF in THF (50.7 µL, 50.7 µmol) the color of the reaction solution had changed from faint yellow to deep orange. The solution was stirred at room temperature for 60 min. It was filtered through silica gel (1 cm x 1 cm, rinsing with THF). Solvent removal gave a yellow oil

(31 mg) consisting of desilylated PyMTAester-$(EP)_2$-NO• **14** accompanied by TIPS-F and/or TIPS-OH, and a Bu_4N-salt.
For ^{1}H NMR data see Tables S2 and S3.

PEGylated PyMTAester-$(EP)_2$-NO• 17. PEG-N_3 **15** (24.5 mg, 57.9 µmol) and material (31 mg; containing ~ 24 µmol desilylated PyMTAester-$(EP)_2$-NO• **14**) that had been obtained by desilylation of PyMTAester-$(EP)_2$-NO• **12** were dissolved in dry toluene (3 mL) and the solution was degassed. $[Cu(phen)(PPh_3)_2]NO_3$ • 0.5 CH_2Cl_2 (1.2 mg, 1.4 µmol) was added. The yellow solution was stirred at room temperature for 41 h. The reaction was monitored by TLC (CH_2Cl_2/EtOH 10:1, R_f(**14**) = 0.62, R_f(**17**) = 0.52). Metal scavenger QuadraPure TU (30 mg) was added. The yellow suspension was stirred at room temperature for 70 h. Metal scavenger QuadraPure BZA (14 mg) was added, and the suspension was stirred for another 7.5 h at room temperature. The metal scavenger QuadraPure BZA did not change its color, which indicated that there had been no free Cu ions left in the solution. The solution was decanted off from the scavenger and the solvent was removed. The residual yellow oil was filtered through silica gel (1 cm x 1 cm, rinsing with CH_2Cl_2/EtOH 10:1). After removal of the solvent the components of the residual yellow oil were separated by preparative HPLC (gradient run with CH_2Cl_2/EtOH, 5% EtOH at 0 min to 15% EtOH at 30 min, flow rate of 20 mL/min; 45 mg of the yellow oil dissolved in CH_2Cl_2 (464 µL) were injected) eluting PEGylated PyMTAester-$(EP)_2$-NO• **17** (33 mg, 76% over 2 steps; R_f (CH_2Cl_2/EtOH 10:1) = 0.50) at 9.5 min.
For ^{1}H NMR data see Tables S2 and S3. For ^{13}C NMR data see Tables S4 and S5. MS (ESI): *m*/*z* = 931.6 $[M + 2Na]^{2+}$, 936.6 $[M + Fe]^{2+}$, 1818.3 $[M + H]^+$, 1840.3 $[M + Na]^+$.

$(H^+)_2(Na^+)_2$[PyMTA-$(EP)_2$-NO•]$^{4-}$ 19. The reaction was performed in air. To a solution of PEGylated PyMTAester-$(EP)_2$-NO• **17** (24 mg, 13 µmol) in ethanol (2.0 mL) was added water (950 µL). After addition of 0.10 M aqueous solution of NaOH (1050 µL, 105.0 µmol) the yellow solution was stirred at room temperature for 45.5 h. The pH was lowered to 7.0 by addition of 0.10 M aqueous solution of HCl (500 µL, 50.0 µmol). After removal of the solvents the yellowish solid was suspended in CH_2Cl_2 (1.0 mL). The suspension was filtered through a syringe filter (PTFE membrane, 13 mm diameter, 0.2 µm pore size). The filter cake was washed with CH_2Cl_2 (2 x 1.0 mL) and the solvent of the combined filtrates was removed giving a mixture (24 mg) of $(H^+)_2(Na^+)_2$[PyMTA-$(EP)_2$-NO•]$^{4-}$ **19** and NaCl as a yellowish solid. The content of the structural motive [PyMTA-$(EP)_2$-NO•]$^{4-}$ in this solid was determined by quantitative ^{1}H NMR spectroscopy to be 11.7 µmol (90% yield).
For ^{1}H NMR data see Tables S2 and S3. For ^{13}C NMR data see Tables S4 and S5. MS (ESI): *m*/*z* = 878.2 $[M - 4H + Fe^{(II)}]^{2-}$. Accurate MS (ESI): *m/z* calcd for $[M - H + Fe^{(III)}]^{2+}$, $C_{82}H_{117}N_{11}O_{28}Fe^{2+}$, 879.8704; found, 879.8688; M = $(H^+)_4$[PyMTA-$(EP)_2$-NO•]$^{4-}$.

General procedure for synthesis of the rulers $(Na^+)_{(4-n)}[M^{n+}$-PyMTA$]^{(4-n)-}$-$(EP)_2$-NO• with M = Cu^{2+}, Mn^{2+}, Dy^{3+}. A solution (500 µL) of $(H^+)_2(Na^+)_2$[PyMTA-$(EP)_2$-NO•]$^{4-}$ **19** (21.7 mg containing 10.6 µmol of the structural motive [PyMTA-$(EP)_2$-NO•]$^{4-}$) in D_2O was prepared. A part of the obtained solution (51.8 µL containing 1.10 µmol of the structural motive [PyMTA-$(EP)_2$-NO•]$^{4-}$) was mixed with a 0.05 M solution of a metal salt in D_2O (20.9 µL, 1.05 µmol). A solution of NaOD in D_2O was added to raise the pH of the solution to pH 7. The solution was diluted with D_2O up to a total volume of 220 µL to obtain a 5.0 mM solution of $(Na^+)_{(4-n)}[M^{n+}$-PyMTA$]^{(4-n)-}$-$(EP)_2$-NO• in D_2O containing NaCl.

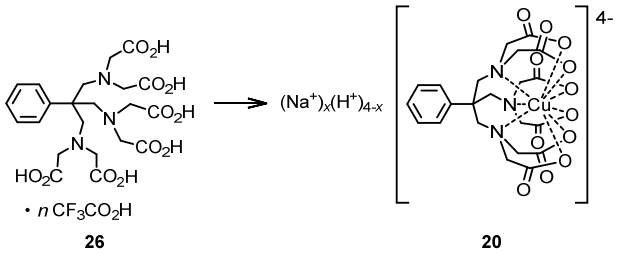
Cu(II)-nitroxide ruler $(Na^+)_2$[Cu(II)-PyMTA]$^{2-}$-$(EP)_2$-NO• 2. Metal salt: $CuCl_2$. 0.10 M NaOD in D_2O: 3 µL, 0.3 µmol. A bluish solution of $(Na^+)_2$[Cu(II)-PyMTA]$^{2-}$-$(EP)_2$-NO• **2** in D_2O was obtained. MS (ESI): *m*/*z* = 1764.6 [M - H]$^-$. M = $(H^+)_2$[Cu(II)-PyMTA]$^{2-}$-$(EP)_2$-NO•.

Mn(II)-nitroxide ruler $(Na^+)_2$[Mn(II)-PyMTA]$^{2-}$-$(EP)_2$-NO•. Metal salt: $MnCl_2 \cdot 4\ H_2O$. 0.01 M NaOD in D_2O: 10 µL, 0.1 µmol. A yellowish solution of $(Na^+)_2$[Mn(II)-PyMTA]$^{2-}$-$(EP)_2$-NO• **2** in D_2O was obtained. MS (ESI): *m*/*z* = 1756.8 [M - H]$^-$, 877.8 [M - H]$^{2-}$; M = $(H^+)_2$[Mn(II)-PyMTA]$^{2-}$-$(EP)_2$-NO•.

Dy(III)-nitroxide ruler (Na^+)[Dy(III)-PyMTA]$^-$-$(EP)_2$-NO•. Metal salt: $DyCl_3 \cdot 6\ H_2O$. 0.10 M NaOD in D_2O: 3 µL, 0.3 µmol. A yellowish solution of (Na^+)[Dy(III)-PyMTA]$^-$-$(EP)_2$-NO• **28** in D_2O was obtained. MS (ESI): *m*/*z* = 1864.5 [M - H]$^-$, 931.6 [M - 2H]$^{2-}$; M = (H^+)[Dy(III)-PyMTA]$^-$-$(EP)_2$-NO•.

Gd(III)-nitroxide ruler (Na^+)[Gd(III)-PyMTA]$^-$-$(EP)_2$-NO•. A solution (500 µL) of $(H^+)_2(Na^+)_2$[PyMTA-$(EP)_2$-NO•]$^{4-}$ **19** (21.7 mg containing 10.6 µmol of the structural motive [PyMTA-$(EP)_2$-NO•]$^{4-}$) in D_2O was prepared. A part of the obtained solution (75.3 µL containing 1.60 µmol of the structural motive [PyMTA-$(EP)_2$-NO•]$^{4-}$) was mixed with a 0.05 M solution of $GdCl_3 \cdot 6\ H_2O$ in D_2O (20.9 µL, 1.52 µmol). A solution of 0.10 M NaOD in D_2O (10 µL, 1.0 µmol) was added to raise the pH of the solution to pH 7. The solution was diluted with D_2O up to a total volume of 320 µL to obtain a 5.0 mM yellowish solution of (Na^+)[Gd(III)-PyMTA]$^-$-$(EP)_2$-NO• in D_2O containing NaCl. MS (ESI): *m*/*z* = 1858.7 [M - H]$^-$, 928.7 [M - 2H]$^{2-}$; M = (H^+)[Gd(III)-PyMTA]$^-$-$(EP)_2$-NO•.

Synthesis of $(H^+)_x(Na^+)_{4-x}[Cu(II)\text{-}TAHA]^{4-}$ (20)



$H_6TAHA \bullet n\ CF_3CO_2H$ (**26**) (10.053 mg containing 15.628 µmol of the structural motive H_6TAHA) was dissolved in D_2O (600 µL). A part of the obtained solution (100 µL containing 2.60 µmol of the structural motive H_6TAHA) was mixed with a 0.05 M solution of $CuCl_2$ in D_2O (49 µL, 2.5 µmol). A 0.10 M solution of NaOD in D_2O (135 µL, 13.5 µmol) was added to rise the pH of the solution to pH 7. The solution was diluted with D_2O up to a total volume of 520 µL to obtain a 5.0 mM bluish solution of $(H^+)_x(Na^+)_{4-x}[Cu(II)\text{-}TAHA]^{4-}$ (**20**) with an average protonation degree of 1.9 protons per molecule in D_2O containing $Na(O_2CCF_3)$. This means that the main species present in this solution were $(H^+)_2(Na^+)_2[Cu(II)\text{-}TAHA]^{4-}$ and $(H^+)_1(Na^+)_3[Cu(II)\text{-}TAHA]^{4-}$ in agreement with the reported pK_a values[6] of the ligand itself, H_6TAHA. MS (ESI): m/z = 292.8 $[M - 2H]^{2-}$. M = $(H^+)_4[Cu(II)\text{-}TAHA]^{4-}$.

NMR data

Influence of the nitroxide radical on the NMR spectra

The nitroxide influences the NMR signals of carbon and proton atoms in a distance dependent way. In Figure S2 the structure of the nitroxide unit of compounds **7**-**8**, **11**-**14** and **16**-**19** is highlighted with colors: The signals of the proton or carbon atoms in the area marked with red are not found in the NMR spectrum. The signals of the proton atoms in the yellow marked area are observed but are extremely broad and the signals of the carbon atoms of this area are only sometimes found. If observed the intensity of these signals is low. The signals of proton or carbon atoms of the green highlighted area are observed. Proton signals are still broad, the intensity of the carbon signals seems not to be reduced.

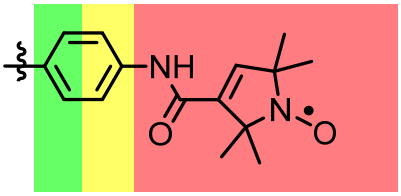


Figure S2. Nitroxide unit of compounds **7**-**8**, **11**-**14** and **16**-**19**. Hydrogen and carbon atoms, whose NMR signals are affected by the unpaired electron spin, are highlighted with colors.

Table S2. ^{1}H NMR (500 MHz) data: Signals with a chemical shift above 4 ppm.

compound, NMR figure solvent	$C_{triaz}H$ (s)	H_{benz} *ortho* to N	H_{benz} *meta* to N	NH (s)	$C_{Py}H$ (s)	H_{benz} *ortho* to $C(CH_2)_3$	H_{benz} *meta* to $C(CH_2)_3$	H_{benz} *ortho* to OCH_2 (s)	C=C<u>H</u>-CMe_2 (s)	$C_{benz}OCH_2$ (s)	$N_{triaz}CH_2$	$C\underline{H}_2CH_3$ (q)	$C_{Py}CH_2$ (s)
3 $CDCl_3$	**---**	---	---	**---**	**---**	---		7.22 (1H), 7.19 (1H)	---	4.77 (2H), 4.74 (2H)	---	---	---
4, S3 $CDCl_3$	**---**	6.61 (half of AA'XX' spin system, 2H)	7.32 (half of AA'XX' spin system, 2H)	**---**	**---**	---		7.20 (1H), 7.19 (1H)	---	4.77 (2H), 4.75 (2H)	---	---	---
21, S8 $CDCl_3$	**---**	**---**	**---**	**---**	**---**	**---**		7.21 (2H), 7.17 (2H)	---	4.76 (4H), 4.73 (4H)	---	---	---
22, S9 $CDCl_3$	**---**	6.57 (half of AA'XX' spin system, 2H)	7.20 (half of AA'XX' spin system, 2H)	**---**	**---**	---		---	---	---	---	---	---
7, S10 CD_2Cl_2	**---**	7.57 (br s, 3H)[#]		**---**	**---**	---		7.25 (1H), 7.23 (1H)	---	4.82 (2H), 4.79 (2H)	---	---	---
8, S15 CD_2Cl_2	**---**	7.55 (br s, 3H)[#]		**---**	**---**	---		7.29 (1H), 7.24 (1H)	---	4.81 (2H), 4.80 (2H)	---	---	---
11, S20 CD_2Cl_2	**---**	7.55 (br s, 3H)[#]		**---**	**---**	7.42 (AA'BB' spin system, 4H)*		7.30 (1H), 7.26 (1H)	---	4.83 (4H)	---	4.06 (12H, ^3J = 7.2 Hz)	---
12, S37 CD_2Cl_2	**---**	7.59 (br s, 3H)[#] overlap with $C_{Py}H$		**---**	7.56 (2H) overlap with H_{benz} *ortho* and *meta* to N	---		7.37 (1H), 7.31 (1H)	---	4.88 (2H), 4.85 (2H)	---	4.16 (8H, ^3J = 6.8 Hz)	4.02 (4H)
23, S42 CD_2Cl_2	**---**	7.57 (br s, 8H)[#]		**---**	**---**	---		7.32 (2H), 7.26 (2H)	---	---	---	---	---
24, S43 CD_2Cl_2	**---**	7.59 (half of AA'XX' spin system, 2H)	7.50 (half of AA'XX' spin system, 2H)	7.55 (1H)	7.54[##] (2H)	---		7.36 (2H), 7.28 (2H)	6.15 (1H)[%]	4.86 (2H), 4.84 (2H)	---	4.15 (8H, ^3J = 7.1 Hz)	4.00 (4H)
25, S44 CD_2Cl_2	**---**	**---**	**---**	**---**	7.45 (2H)	---		---	---	---	---	4.13 (8H, ^3J = 7.1 Hz)	3.95 (4H)
13, S25 CD_2Cl_2	**---**	7.59 (br s, 3H)[#]		**---**	**---**	7.45 (AA'BB' spin system, 4H)**		7.18 (2H)	---	4.82 (2H), 4.81 (2H)	---	4.07 (12H, ^3J = 7.1 Hz)	---
14, S45 CD_2Cl_2	**---**	7.61 (br s, 4H) overlap with $C_{Py}H$		**---**	7.60 (2H) overlap with H_{benz} *ortho* and *meta* to N	---		7.224 (1H), 7.219 (1H)	---	4.84 (2H), 4.83 (2H)	---	4.16 (8H, ^3J = 7.0 Hz)	4.01 (4H)
16, S26 CD_2Cl_2	7.89 (1H), 7.84 (1H)	7.65 (very br s, 1H)[###]	7.48 (br s, 2H)	**---**	**---**	7.42 (AA'BB' spin system, 4H)*		7.20 (1H), 7.18 (1H)	---	5.30 (2H), 5.23 (2H)	4.51 (2H, d ^3J = 6.1 Hz) 4.46 (2H, br d, ^3J = 5.5 Hz)	4.07 (12H, ^3J = 7.2 Hz)	---
17, S46 CD_2Cl_2	7.91 (1H), 7.85 (1H)	7.66 (very br s, 0.2H)[###]	7.48 (br s, 1.5H)[###]	**---**	7.56 (2H)	---		7.24 (1H), 7.22 (1H)	---	5.33 (2H), 5.23 (2H)	4.50 (2H, d ^3J = 6.3 Hz) 4.46 (br d, 2H, ^3J = 4.4 Hz)	4.14 (8H, ^3J = 7.1 Hz)	4.00 (4H)
18, S31 MeOD	8.17 (2H)	7.66 (very br s, 1H)[###]	7.48 (br s, 2H)	**---**	**---**	7.82 (half of AA'XX' spin system, 2H)	7.51 (half of AA'XX' spin system, 2H)	7.28 (1H), 7.16 (1H)	---	5.33 (2H), 5.29 (2H)	4.56 (br s with shoulder, 4H)	---	---
19, S51 MeOD	8.17 (2H)	7.66 (very br s, 2H)	7.47 (br s, 2H)	**---**	7.26*** (2H)	---		7.33 (1H), 7.26*** (1H)	---	5.33 (2H), 5.29 (2H)	4.56 (br s, 4H)	---	3.70 (4H)

benz = benzene; *Py* = pyridine; *triaz* = triazole. The data of alkyne **3**[2] are listed for the purpose of comparison. *The signal consists of a narrow line with high intensity (its chemical shift is given in the table) surrounded by two lines of low intensity at a distance of 8.6 Hz to the center. **The signal has a quartet-like appearance. The center of the signal has a chemical shift of 7.45 ppm. The distance between the center and the two outer lines is 8.3 Hz. ***The singlet at 7.26 ppm has an integral corresponding to 3H. [#]An integral of 4 H is expected. Precise integration is impossible because of broadness and sometimes overlap with other signals. [##]The singlet has a shoulder at 7.53 ppm which likely belongs to an unidentified component. [###]For the signal an integral of 2H is expected. Precise integration is impossible because of broadness and sometimes overlap with other signals. [%]The signal shift fits well to the 1H NMR data reported for the NOH signal of 1-hydroxy-2,2,5,5-tetramethylpyrroline-3-carboxamide.[7]

Table S3. ^{1}H NMR (500 MHz) data: Signals with a chemical shift below 4 ppm.

compound, NMR figure solvent	OCH_2CH_2 (several m)	CH_2CO (s)	$C(CH_2)_3$ (s)	$CHCH_2O$ (several m)	OMe (s)	NH_2 (s)	CMe_2OH (s)	C≡CH (s)	$CHCH_2O$	CMe_2OH (s)	CMe_2 (br s)	CH_2CH_3 (t)	$SiCHMe_2$ (s)	$SiMe_3$ (s)
3 $CDCl_3$	---	---	---	---	---	---	---	3.33 (1H)		---	---	---	1.03 (21H), 1.02 (21H)	0.24 (9H)
4, S3 $CDCl_3$	---	---	---	---	---	3.82 (2H)	---	---	---	---	---	---	1.04 (42H)	0.24 (9H)
21, S8 $CDCl_3$	---	---	---	---	---	---	---	---		---	---	---	1.04 (42H), 1.02 (42H)	0.24 (18H)
22, S9 $CDCl_3$	---	---	---	---	---	3.78 (2H)	2.16 (1H)	---	---	1.58 (6H)	---	---	---	---
7, S10 CD_2Cl_2	---	---	---	---	---	---	---	---	---	---	---	---	1.07 (42H)	0.27 (9H)
8, S15 CD_2Cl_2	---	---	---	---	---	---	---	3.39 (1H)	---	---	---	---	1.063 (21H), 1.055 (21H)	---
11, S20 CD_2Cl_2	---	3.37 (12H)	3.28 (6H)	---	---	---	---	---	---	---	---	1.22 (18H, ^3J = 7.2 Hz)	1.07 (21H), 1.06 (21H)	---
12, S37 CD_2Cl_2	---	3.60 (8H)	---	---	---	---	---	---	---	---	---	1.27 (12 H, ^3J = 6.8 Hz)	1.08 (21H), 1.07 (21H)	---
23, S42 CD_2Cl_2	---	---	---	---	---	---	---	---	---	---	---	---	1.08 (42H), 1.06 (42H)	---
24, S43 CD_2Cl_2	---	3.59 (8H)	---	---	---	---	---	---	---	---	1.45 (4H)##, 1.40 (4H)##	1.26 (18H, ^3J = 7.1 Hz)	1.06 (21H), 1.05 (21H)	---
25, S44 CD_2Cl_2	---	3.55 (8H)	---	---	---	---	---	---	---	1.58 (6H)	---	1.25 (12 H, ^3J = 7.1 Hz)	---	---
13, S25 CD_2Cl_2	---	3.37 (12H)	3.28 (6H)	---	---	---	---	2.64 (2H)	---	---	---	1.22 (18H, ^3J = 7.1 Hz)	---	---
14, S45 CD_2Cl_2	---	3.59 (8H)	---	---	---	---	---	2.70 (1H) 2.65 (1H)	---	---	---	1.27 (12H, ^3J = 7.0 Hz)	---	---
16, S26 CD_2Cl_2	3.9 – 3.4 (48H)	3.37 (12H) overlap with OCH_2CH_2	3.28 (overlap with s at 3.30 ppm, together 18H)	3.41 – 3.33 (8H)	3.30 (overlap with s at 3.28 ppm, together 18H	---	---	---	2.48 (sept like, 1H), 2.36 (br s, 1H)	---	---	1.22 (18H, ^3J = 7.2 Hz)	---	---
17, S46 CD_2Cl_2	3.8 – 3.4 (48H)	3.59 (8H), overlap with OCH_2CH_2	---	3.40 – 3.32 (8H)	3.29 (12H)	---	---	---	2.46 (sept like, 1H), 2.36 (br s, 1H)	---	---	1.25 (12H, ^3J = 7.1 Hz)	---	---
18, S31 MeOD	4.1 – 3.4 (66H), large overlap			3.40 – 3.21 (8H)	3.30 (12H)	---	---	---	2.46 (br s, 2H)	---	---	---	---	---
19, S51 MeOD	3.66 – 3.41 (48H), large overlap	3.02 (8H)	---	3.41 – 3.31 (8H)	3.29 (12H)	---	---	---	2.44 (br s, 2H)	---	---	---	---	---

benz = benzene; *Py* = pyridine; *triaz* = triazole. The data of alkyne **3**[2] are listed for the purpose of comparison. ##The integral is smaller than expected (6H). This may be a consequence of the signal broadness.

Table S4. ^{13}C NMR (125 MHz) data: Signals with a chemical shift above 90 ppm.

compound, NMR figure solvent	CO_2	$\underline{C}_{Py}CH_2$	$C_{benz}O$	$\underline{C}_{benz}C_q$	$C_{benz}N$	$\underline{C}$=CH of triazole	$C_{benz}H$ *meta* to $C_{benz}N$	C_{Py} *para* to N	$C_{benz}H$ *meta / ortho* to C_q	C=$\underline{C}$H of triazole	C_{Py} *meta* to N	C_{benz} *para* to C_q	C_{benz} *para* to $C_{benz}N$	$C_{benz}H$ *ortho* to $C_{benz}O$	$\underline{C}_{benz}C\equiv C$	$C_{benz}H$ *ortho* to $C_{benz}N$	$CH_2\underline{C}\equiv C$	$TMS\underline{C}\equiv\underline{C}$	$C_{benz}\underline{C}\equiv C$ *para* to C_q	$C_{benz}\underline{C}\equiv C$ *para* to $C_{benz}N$	$C_{Py}\underline{C}\equiv C$
3 $CDCl_3$	---	---	153.0, 152.7	---	---	---	---	---	---	---	---	---	---	120.0, 119.1	114.9, 113.0	---	101.5, 101.4	100.5, 100.2	---	---	---
4, S4 $CDCl_3$	---	---	153.0, 152.1	---	146.7	---	133.1	---	---	---	---	---	112.6	119.6, 118.9	115.4, 113.3	114.6	101.8, 101.7	100.7, 100.0	---	96.2	---
7, S11 CD_2Cl_2	---	---	152.5, 152.1	---	not found	---	132.0	---	---	---	---	---	119.4	119.0, 118.8	114.1, 113.8	120.8 (br)**	101.5*[2]	100.3, 100.2	---	94.3	---
8, S16 CD_2Cl_2	---	---	151.7, 150.8	---	133.5 (br)**	---	131.2	---	---	---	---	---	118.6	118.1, 117.4	113.4, 111.4	122.0 (br)**	100.22, 100.20	---	---	93.3	---
11, S21 CD_2Cl_2	171.0	---	151.90, 151.87	144.5	not found	---	131.8	---	130.9 / 126.5	---	---	120.4	119.2	118.4, 118.2	113.7, 113.5	121.1 (br)**	101.25, 101.23	---	94.4	94.0	---
12, S38 CD_2Cl_2	170.6	158.5	152.4, 151.9	---	not found	---	131.9	131.7	---	---	122.6	---	119.2	118.5, 118.4	114.7, 112.7	120.9 (br)**	101.32, 101.26	---	---	94.6	92.5
16, S27 CD_2Cl_2	171.4	---	153.5, 153.2	145.0	not found	143.6, 143.0	131.8	---	131.4 / 127.0	125.1, 124.4	---	120.8[#2]		118.3, 118.2	114.6, 114.5	not found	---	---	95.0	94.8	---
17, S47 CD_2Cl_2	170.9	158.8	153.44, 153.36	---	not found	143.5, 142.9	131.8	132.0	---	125.0, 124.3	123.0	---	118.7 *	118.3, 118.1	115.5, 113.3	120.5* (br)	---	---	---	95.2	92.8
18, S33 MeOD	176.5 (br)	---	154.7, 154.4	145.2 (br)	not found	144.43, 144.39	133.0 or 132.9	---	133.0 or 132.9 / 128.2	127.4, 127.3	---	123.0	121.6 or 120.3	120.1, 120.0	116.31, 116.27	121.6 (br) or 120.3 (br)	---	---	96.0, 95.9		
19, S52 MeOD	179.2	160.9	154.9, 154.6	---	not found	144.3, 144.2	132.9	134.0	---	127.4, 127.3	124.6	---	120.1 *	120.2, 119.8	117.6, 114.7	121.6* (br)	---	---	---	96.5	93.3

benz = benzene; *Py* = pyridine; *triaz* = triazole; C_q = quarternary C of $C(CH_2)_3$. The data of alkyne **3**[2] are listed for the purpose of comparison. [#2]means the signal has *2*-fold intensity compared to the signal of the carbon atom at the same position in TAHAester-$(EP)_2$-NO• **11** if normalized to the intensity of the signals of $C_{benz}H$ *ortho* to $C_{benz}O$. *Signal assignment based on signal broadness. The broader signal of the two signals was assigned to $C_{benz}H$ *ortho* to $C_{benz}N$. **Broad signals with very low intensity.

Table S5. ^{13}C NMR (125 MHz) data: Signals with a chemical shift below 90 ppm.

compound, NMR figure solvent	$CH_2C{\equiv}\underline{C}$	$C_{Py}C{\equiv}\underline{C}$	$C_{benz}C{\equiv}\underline{C}$ *para* to $C_{benz}N$	$C_{benz}C{\equiv}\underline{C}$ *para* to C_q	$\underline{C}{\equiv}\underline{C}H$	$O\underline{C}H_2\underline{C}H_2$	$C_q\underline{C}H_2$	$\underline{C}H_2CO$	$CH\underline{C}H_2O$	$C_{triaz}\underline{C}H_2$	$\underline{C}H_2CH_3$	$C_{Py}\underline{C}H_2$	OMe	$\underline{C}H_2C{\equiv}C$	C_q	$N_{triaz}\underline{C}H_2$	$\underline{C}HCH_2O$	$CH\underline{Me}_2$	$CH_2\underline{C}H_3$	$\underline{C}HMe_2$	$Si\underline{Me}_3$
3 $CDCl_3$	90.4, 89.9	---	---	---	82.6, 79.4	---	---	---	---	---	---	---	---	58.3, 58.0	---	---	---	18.5	---	11.6, 11.0	-0.1
4, S4 $CDCl_3$	89.9, 89.8	---	83.4	---	---	---	---	---	---	---	---	---	---	58.30, 58.29	---	---	---	18.5	---	11.10, 11.09	0.6
7, S11 CD_2Cl_2	89.9, 89.8	---	85.2	---	---	---	---	---	---	---	---	---	---	58.1, 58.0	---	---	---	18.2	---	11.0, 10.9	-0.6
8, S16 CD_2Cl_2	88.9, 88.7	---	83.9	---	82.4, 78.2	---	---	---	---	---	---	---	---	57.3, 57.2	---	---	---	17.0	---	9.8	---
11, S21 CD_2Cl_2	89.6, 89.5	---	85.00, 84.96		---	---	59.0	55.3	---	---	59.8	---	---	57.9, 57.8	48.3	---	---	17.9	13.6	10.6	---
12, S38 CD_2Cl_2	90.1, 89.7	88.8, 85.0		---	---	---	---	54.5	---	---	60.2	59.4	---	58.0, 57.9	---	---	---	18.0	13.8	10.8	---
16, S27 CD_2Cl_2	---	---	85.7, 85.4		---	71.93, 71.91*3, 70.6*4, 70.53*8, 70.46, 70.38*3, 70.33, 70.28*3	59.5	55.8	68.9	64.2, 63.7	60.2	---	58.8, 58.6	---	48.8	48.65, 48.56	40.7, 40.6	---	14.0	---	---
17, S47 CD_2Cl_2	---	89.2, 85.2		---	---	71.85, 71.82*3, 70.50*4, 70.43*8, 70.37, 70.29*3, 70.25, 70.17*3	---	54.7	68.8	64.1, 63.6	60.4	59.6	58.7, 58.6	---	---	48.6, 48.5	40.6, 40.5	---	14.0	---	---
18**, S31 MeOD	---	---	86.9, 86.4		---	(73.0, 72.9, 71.6, 71.5, 71.44, 71.42, 71.38, 71.35, 71.3, 71.2)[a]			70.02, 69.97	64.5, 64.3	---	---	59.3, 59.2	---	49.6	49.84, 49.78	41.8, 41.7	---	---	---	---
19, S52 MeOD	---	91.3, 86.3		---	---	73.0, 72.9, 71.6 – 71.3 large overlap	---	60.3	70.03, 69.99	64.5, 64.3	---	61.0	59.3, 59.1	---	---	49.92, 49.86	41.9, 41.8	---	---	---	---

benz = benzene; *Py* = pyridine; *triaz* = triazole; C_q = quaternary C of $C(CH_2)_3$. The data of alkyne **3**[2] are listed for the purpose of comparison. *^{n} means the signal has *n*-fold intensity compared to the other signals in the series. [a] It could not be determined how many carbon atoms of $(H^+)_x(Na^+)_{6-x}[TAHA\text{-}(EP)_2\text{-}NO\bullet]^{6-}$ **18** are represented by an individual signal. In total, the signals belong to 33 carbon atoms. **At 60.4 ppm a broad signal with low intensity was found which might belong to an unidentified component.

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NMR spectra

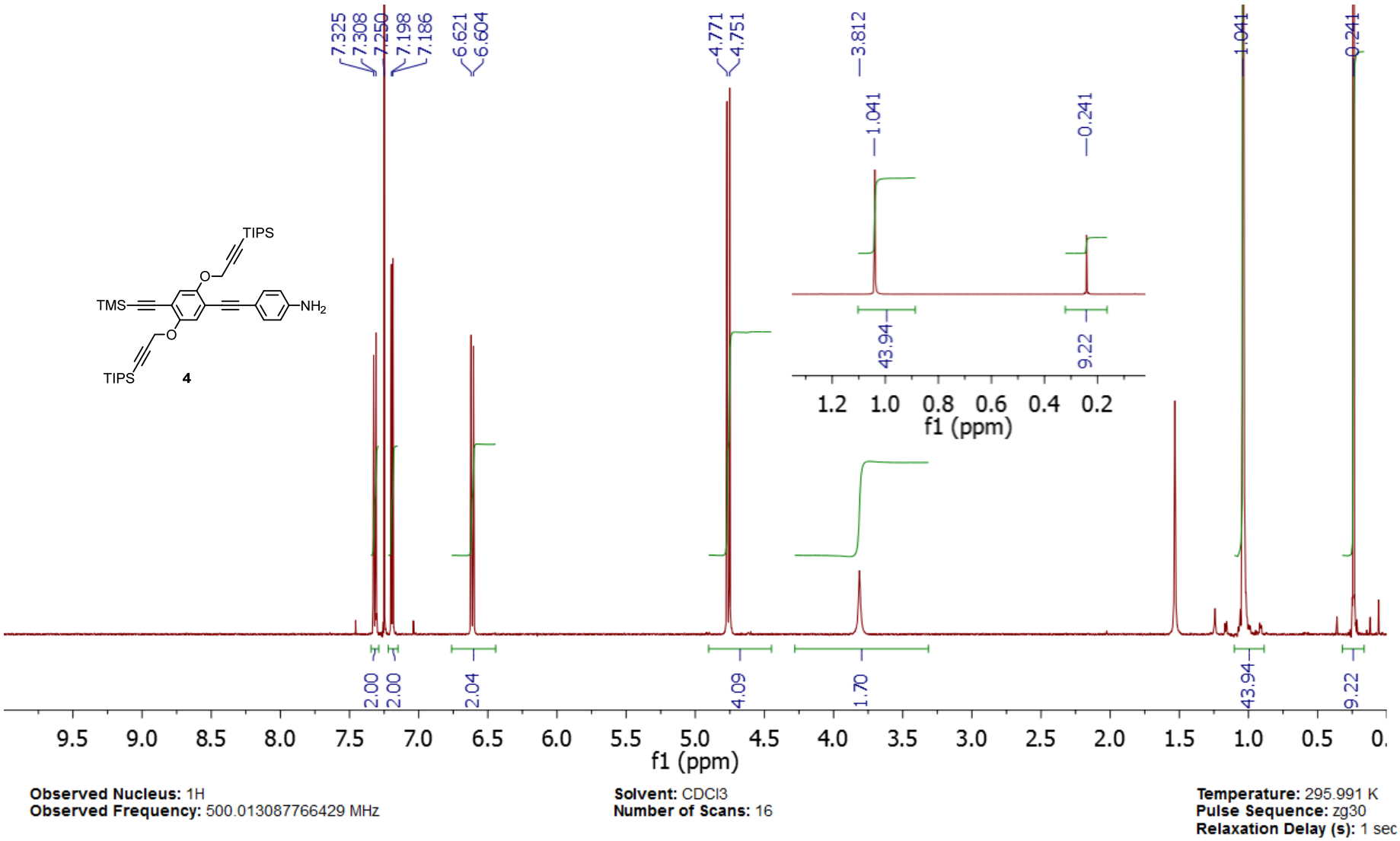

Figure S3. ^{1}H NMR spectrum of TMS-(EP)$_2$-NH$_2$ **4**.

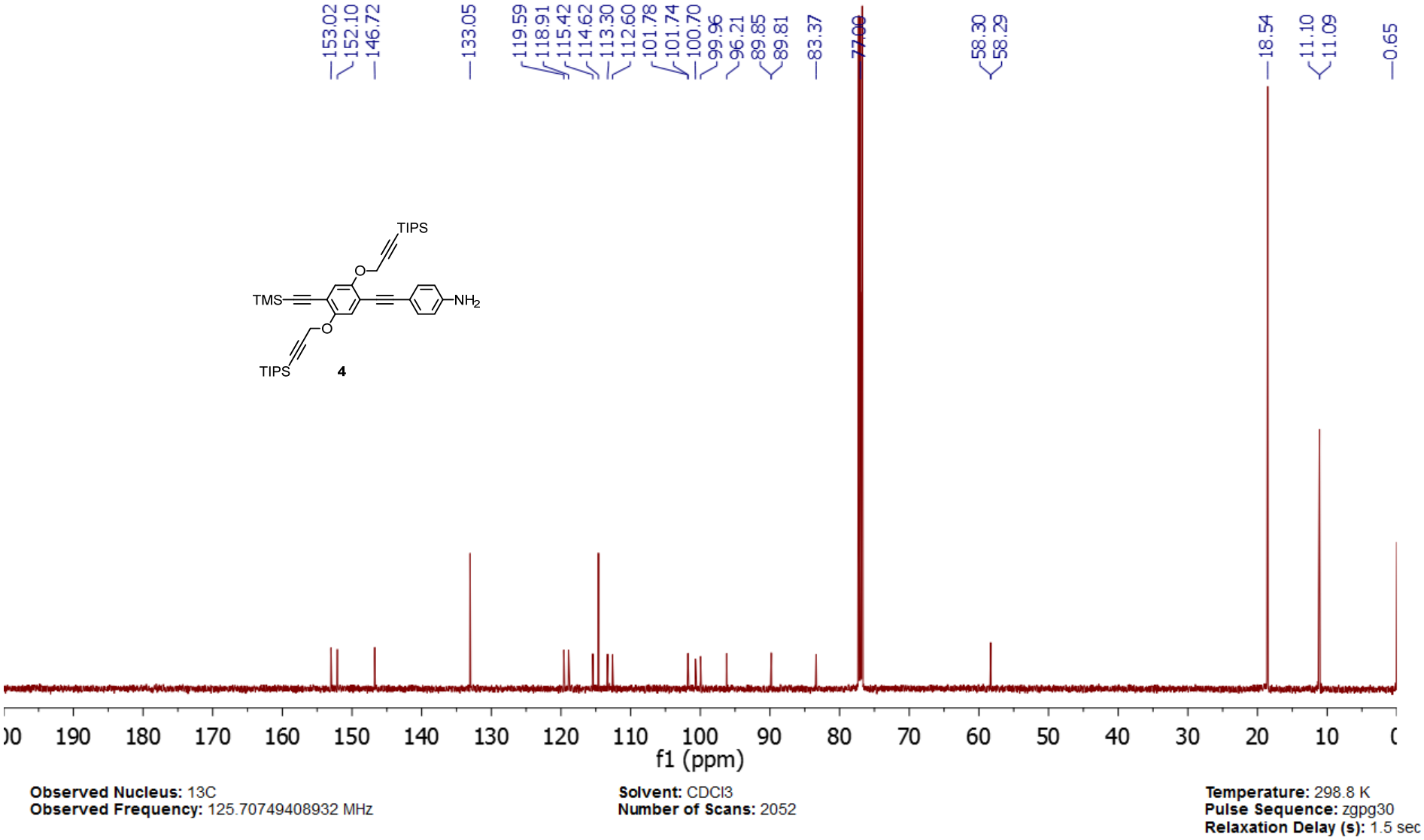

Figure S4. ^{13}C NMR spectrum of TMS-$(EP)_2$-NH_2 **4**.

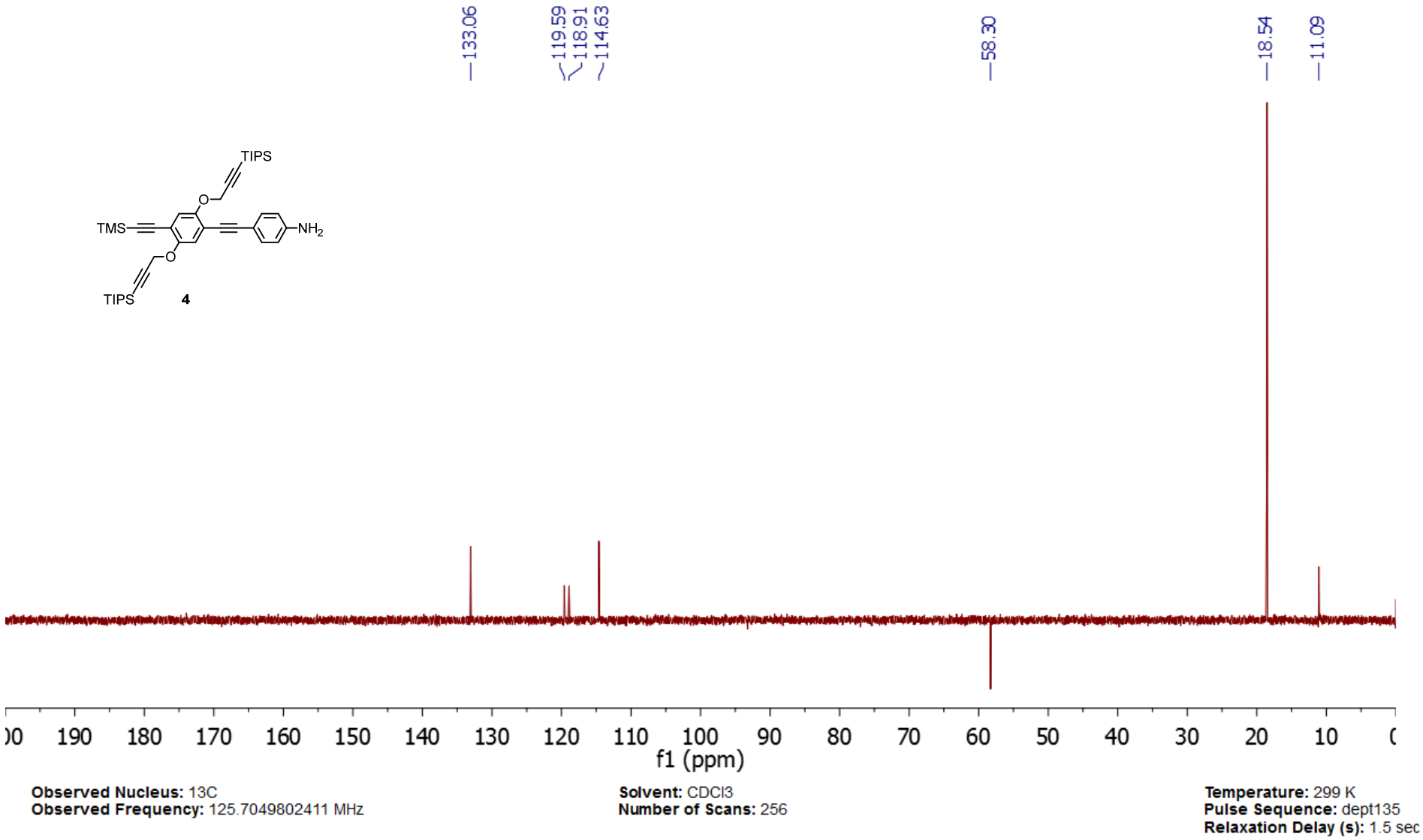

Figure S5. ^{13}C DEPT-135 NMR spectrum of TMS-$(EP)_2$-NH_2 **4**.

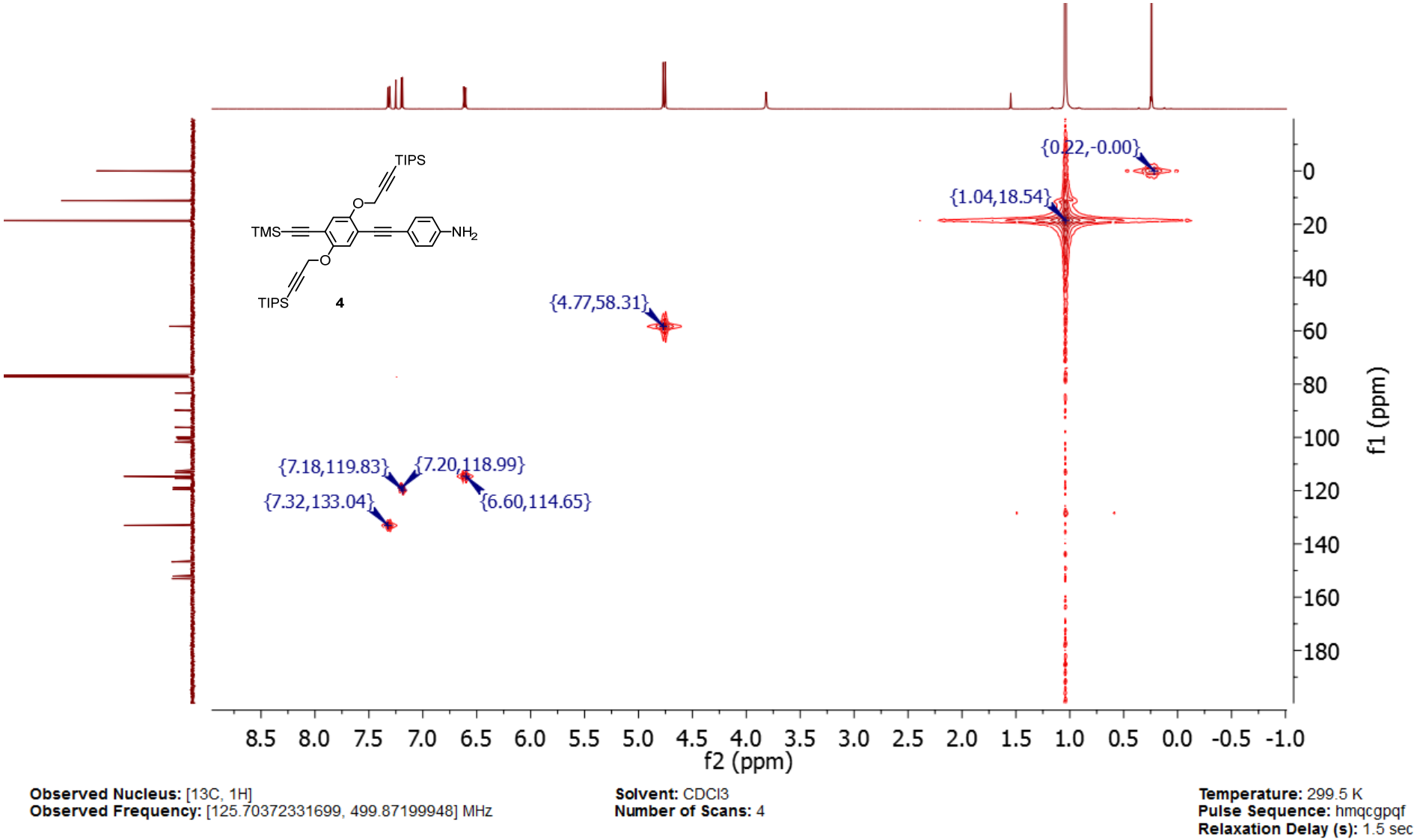

Figure S6. HMQC NMR spectrum of TMS-$(EP)_2$-NH_2 **4**.

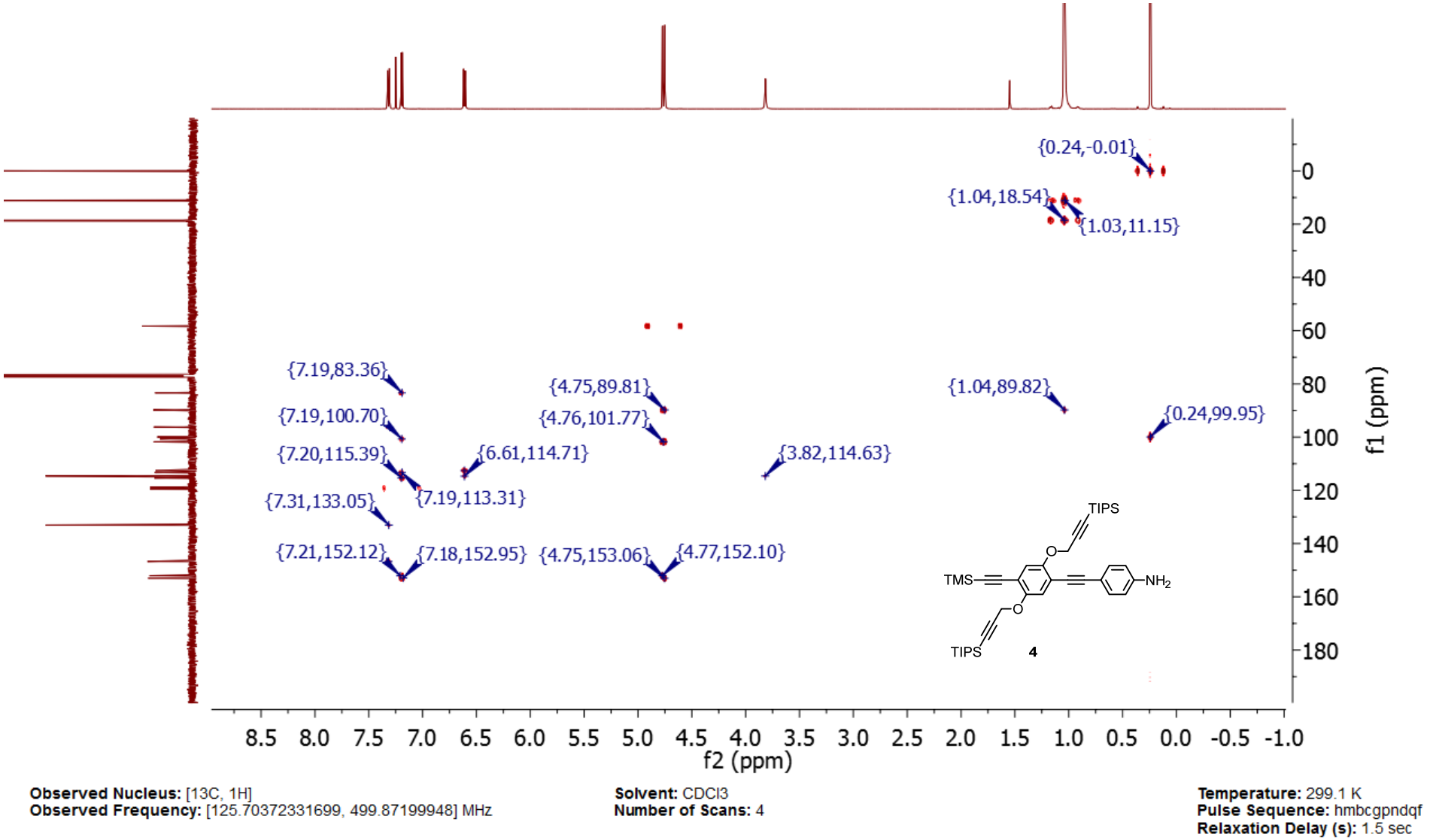

Figure S7. HMBC NMR spectrum of TMS-$(EP)_2$-NH_2 **4**.

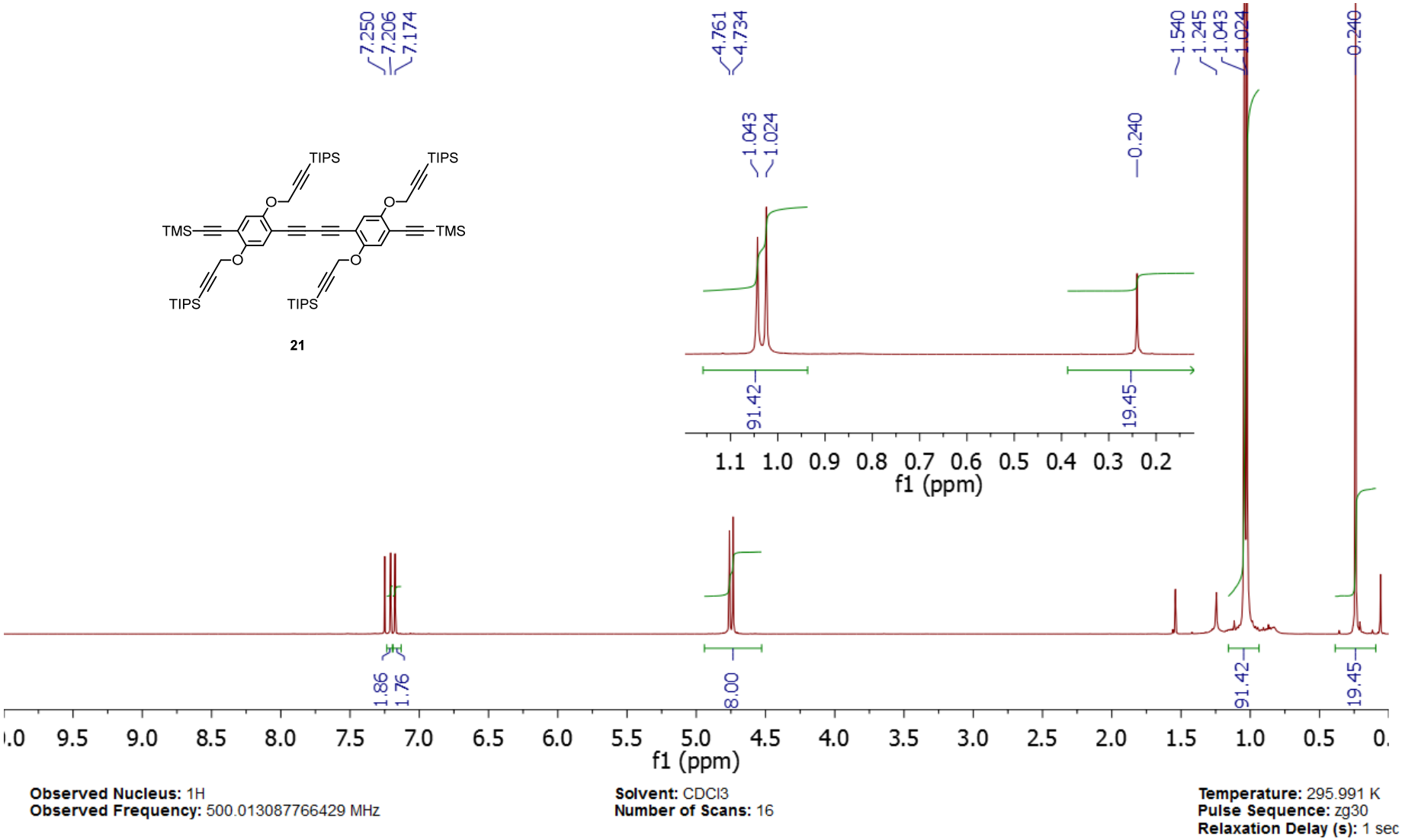

Figure S8: ^{1}H NMR spectrum of dimer alkyne **21**.

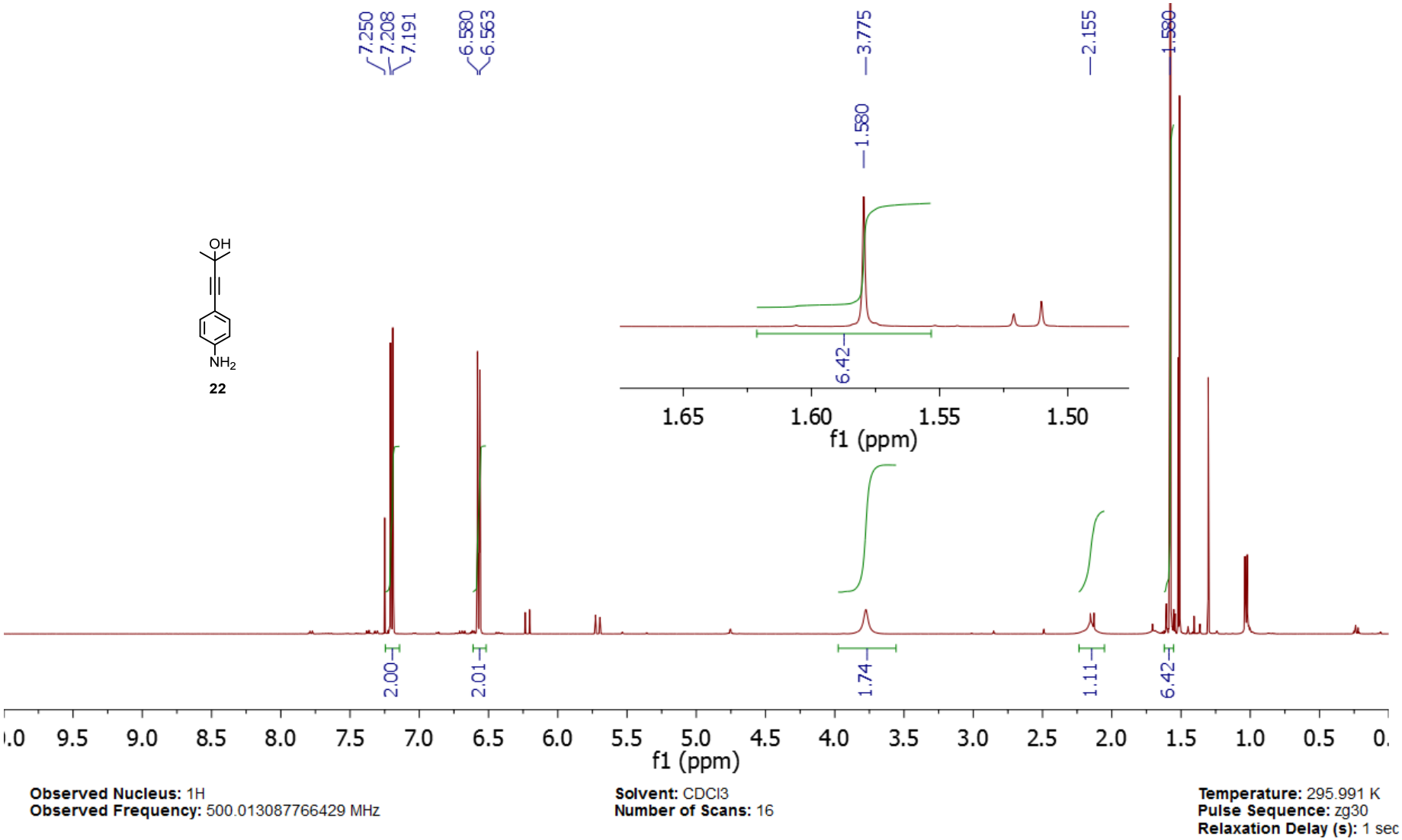

Figure S9: ^{1}H NMR spectrum of the mixture of compound **22** and of minor amounts of unidentified components.

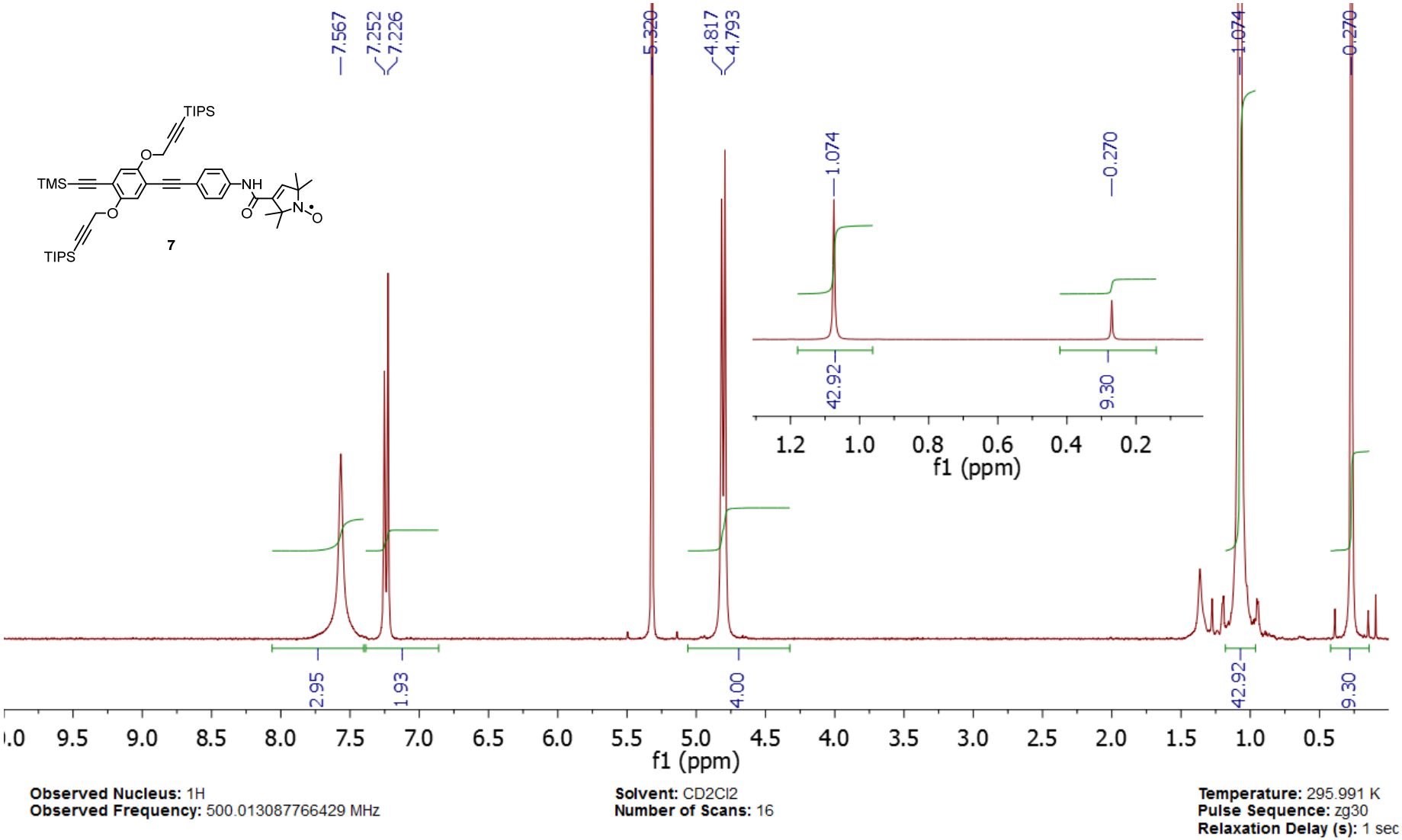

Figure S10. ^{1}H NMR spectrum of TMS-(EP)$_2$-NO• **7**.

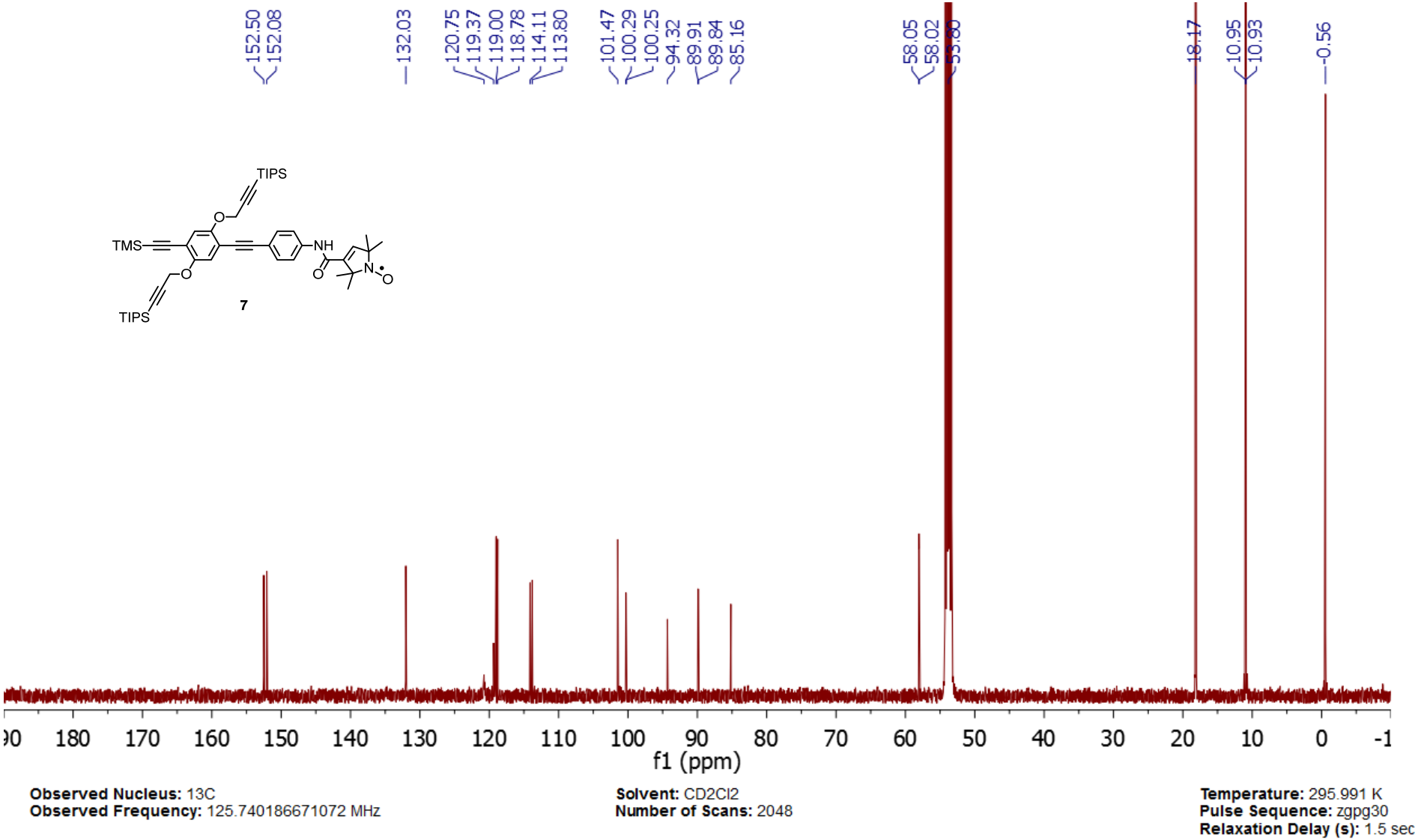

Figure S11. ^{13}C NMR spectrum of TMS-$(EP)_2$-NO• **7**.

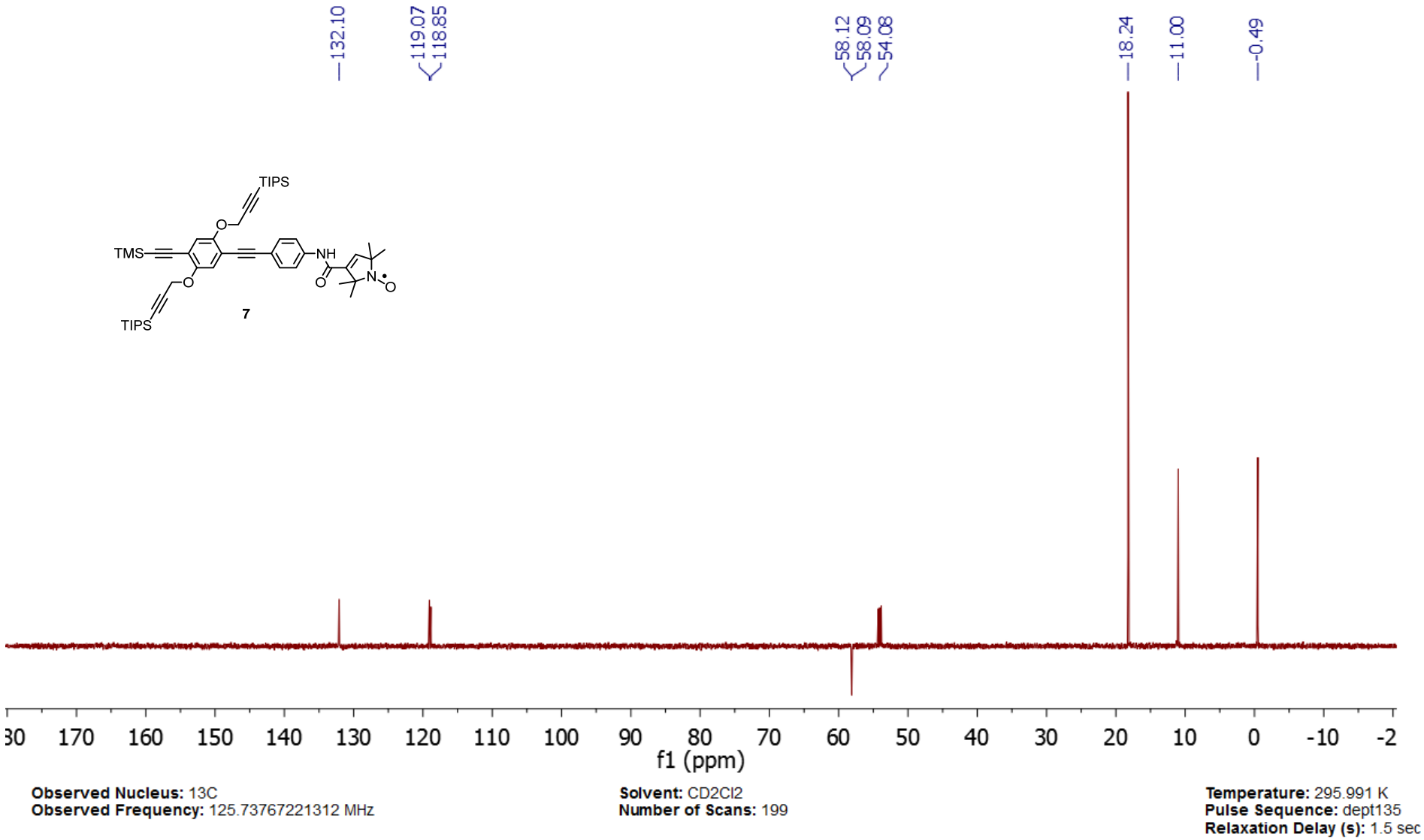

Figure S12. ^{13}C DEPT-135 NMR spectrum of TMS-$(EP)_2$-NO• **7**.

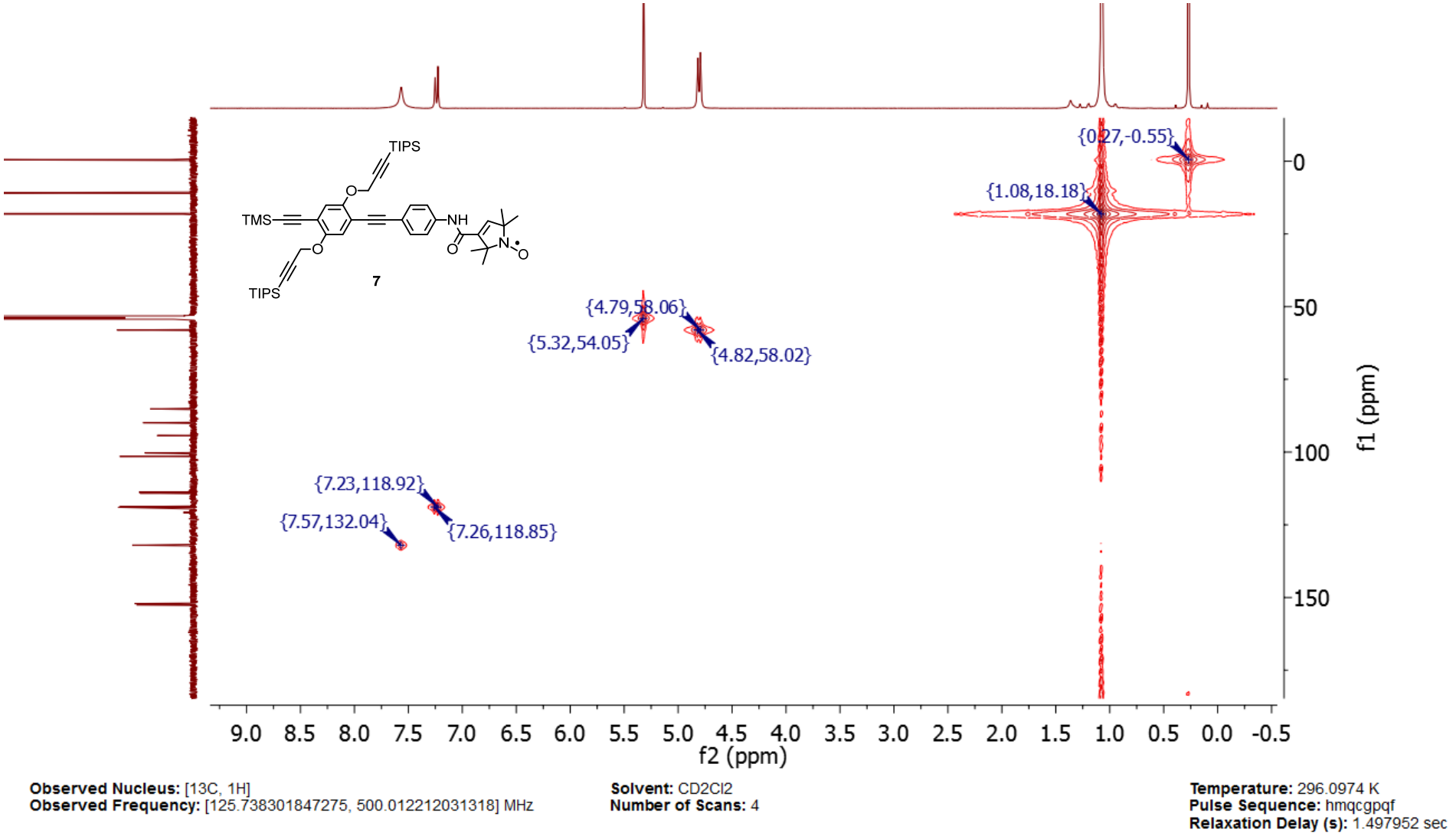

Figure S13. HMQC NMR spectrum of TMS-(EP)$_2$-NO• **7**.

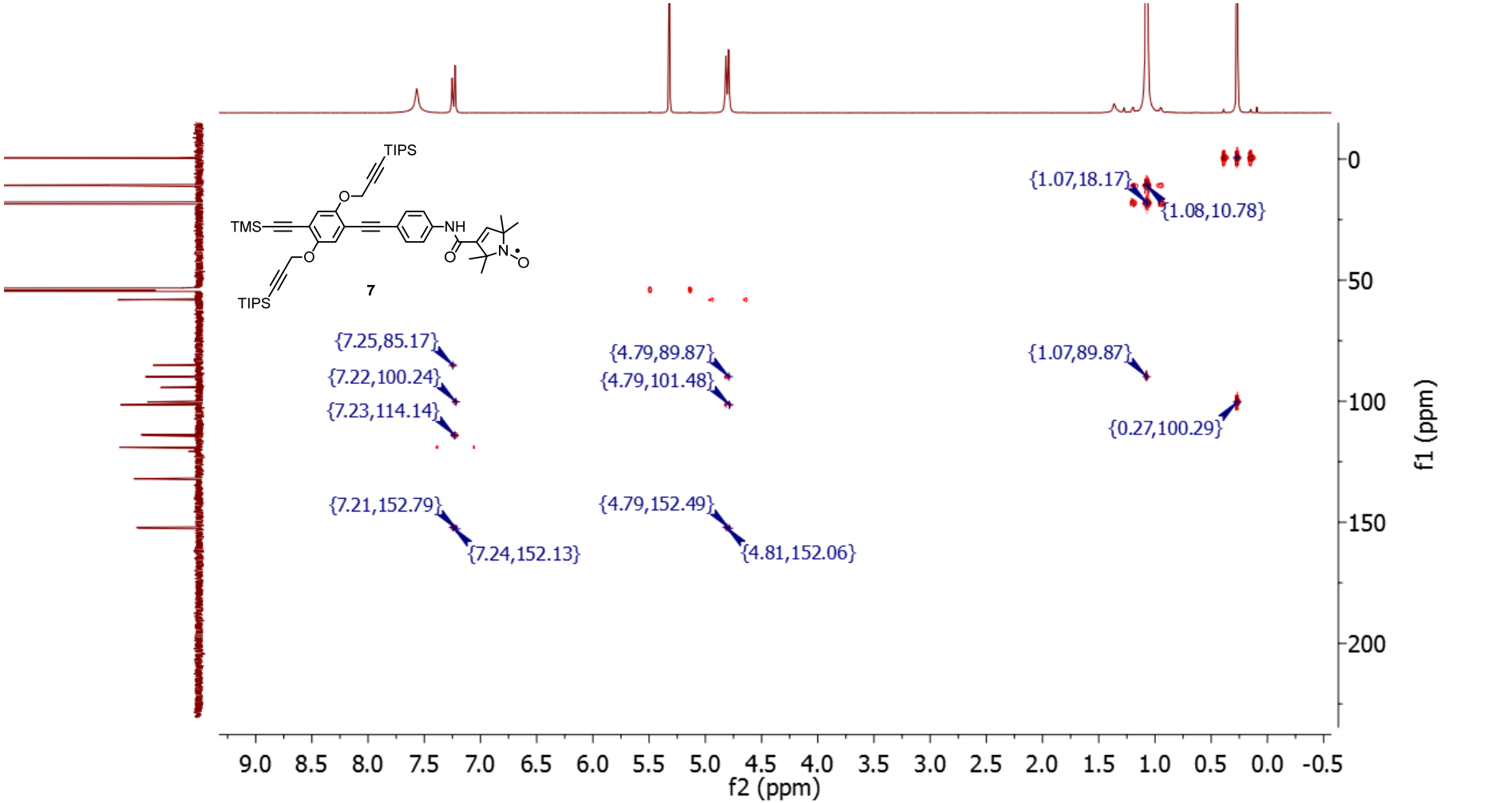

Figure S14. HMBC NMR spectrum of TMS-(EP)$_2$-NO• **7**.

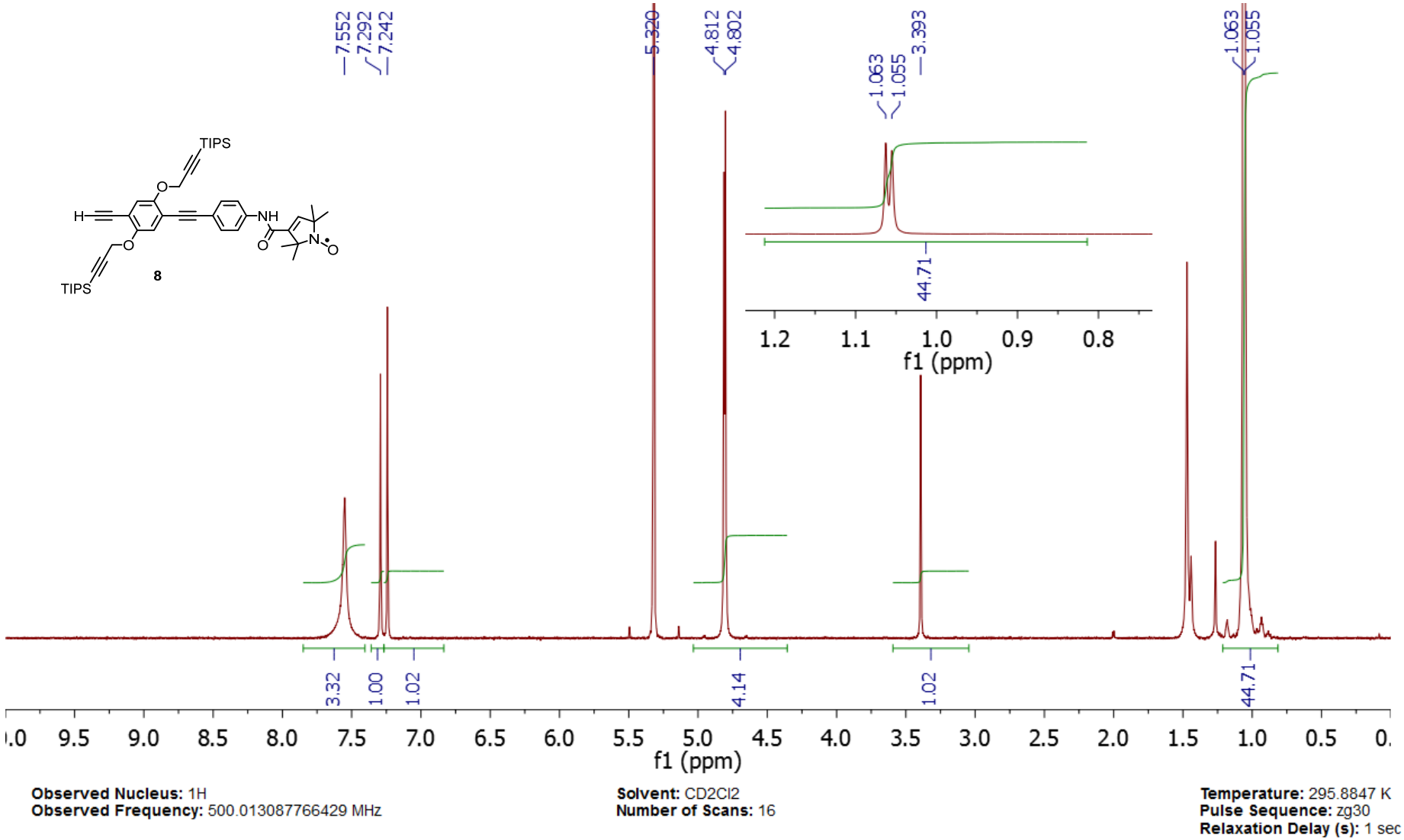

Figure S15. ^{1}H NMR spectrum of H-$(EP)_2$-NO• **8**.

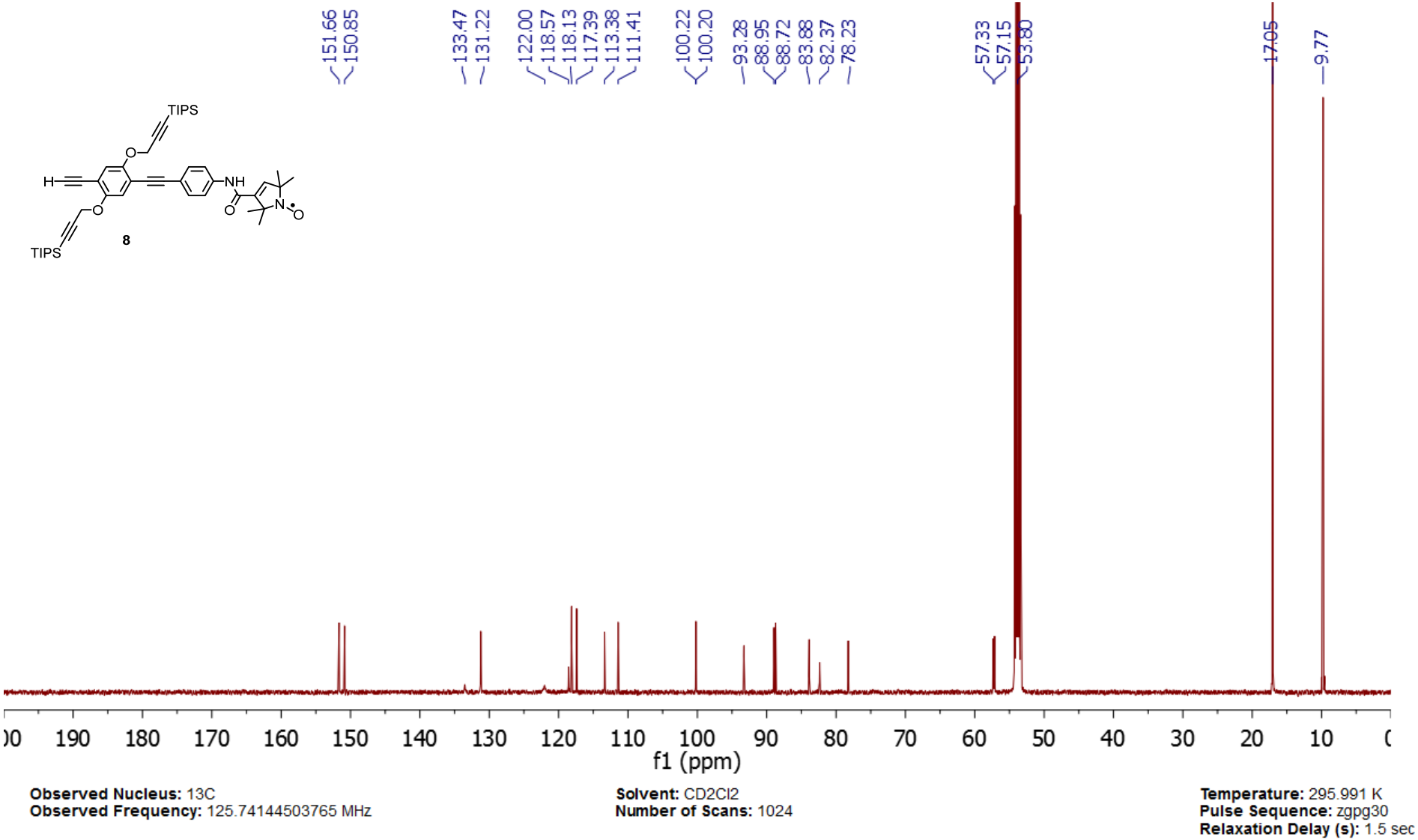

Figure S16. ^{13}C NMR spectrum of H-$(EP)_2$-NO• **8**.

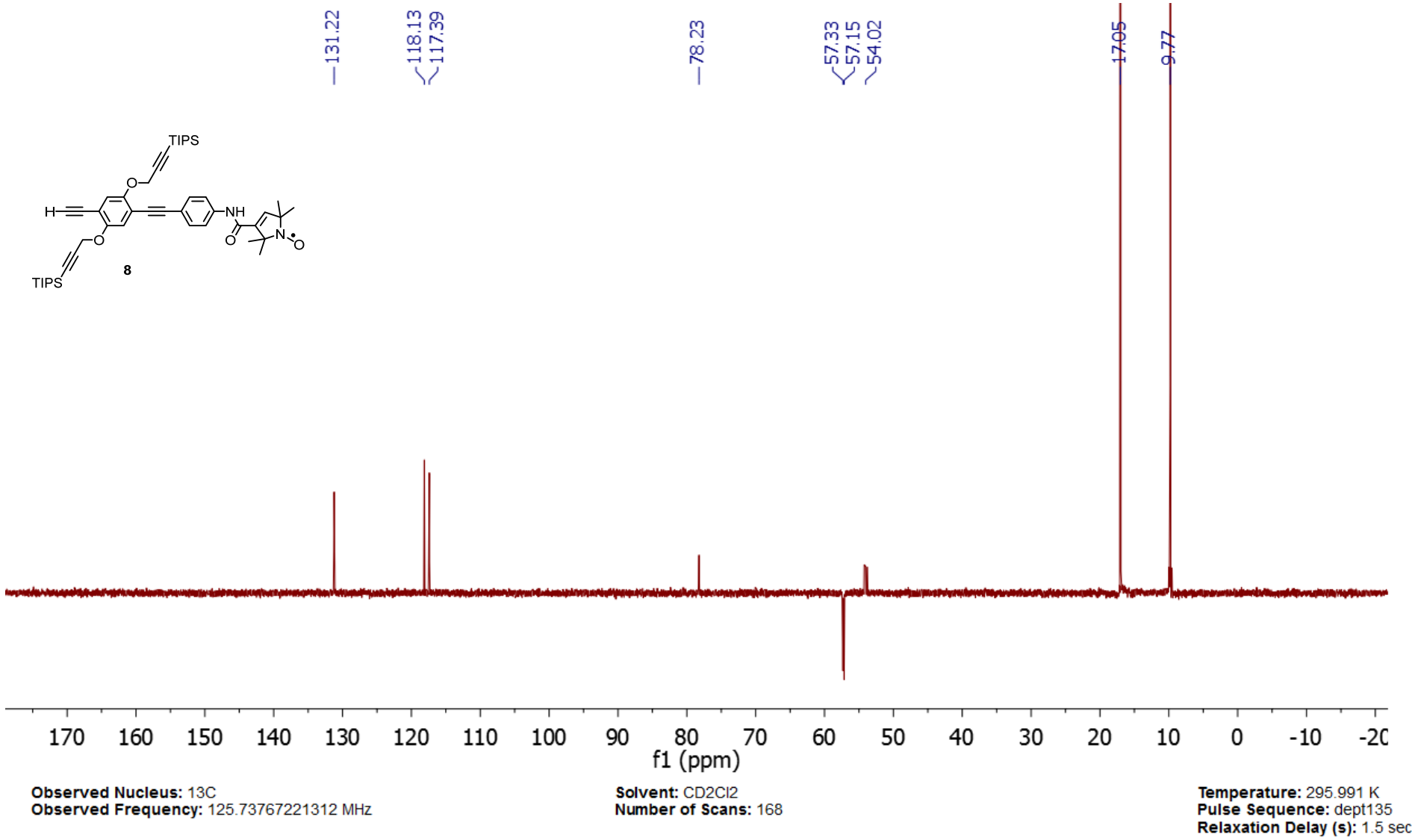

Figure S17. ^{13}C DEPT-135 NMR spectrum of H-(EP)$_2$-NO• **8**.

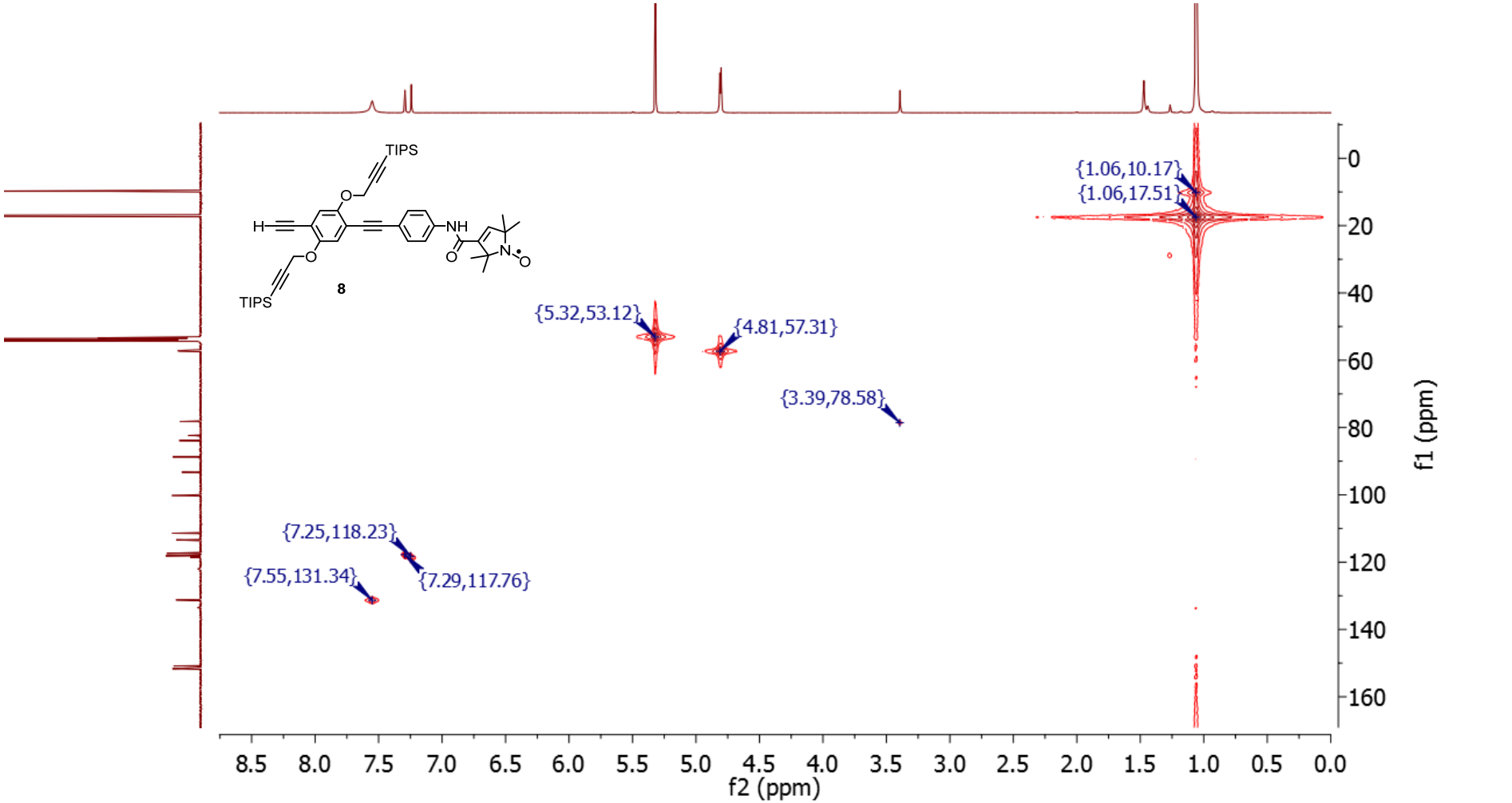

Figure S18. HMQC NMR spectrum of H-(EP)$_2$-NO• **8**.

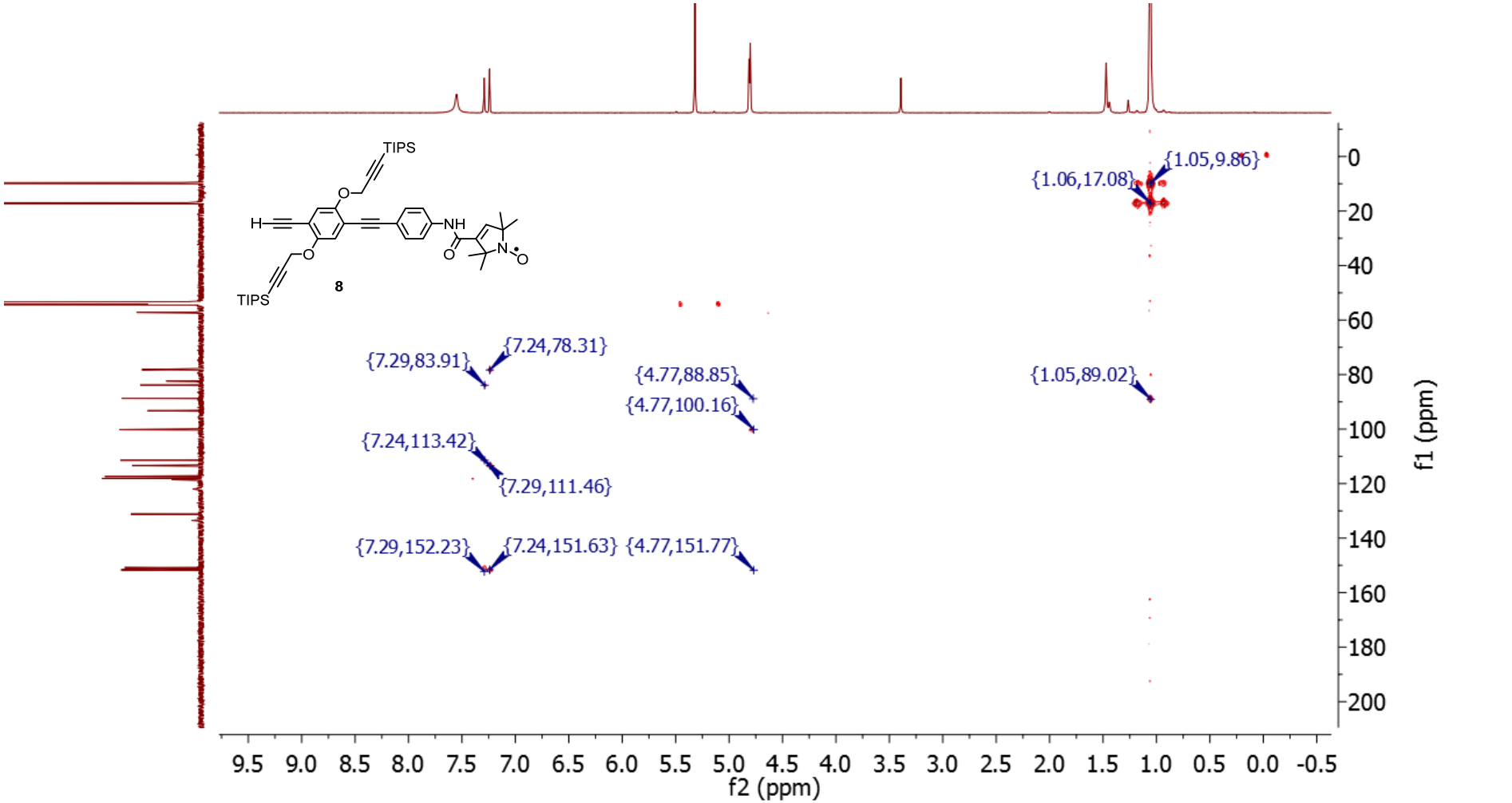

Figure S19. HMBC NMR spectrum of H-(EP)$_2$-NO• **8**.

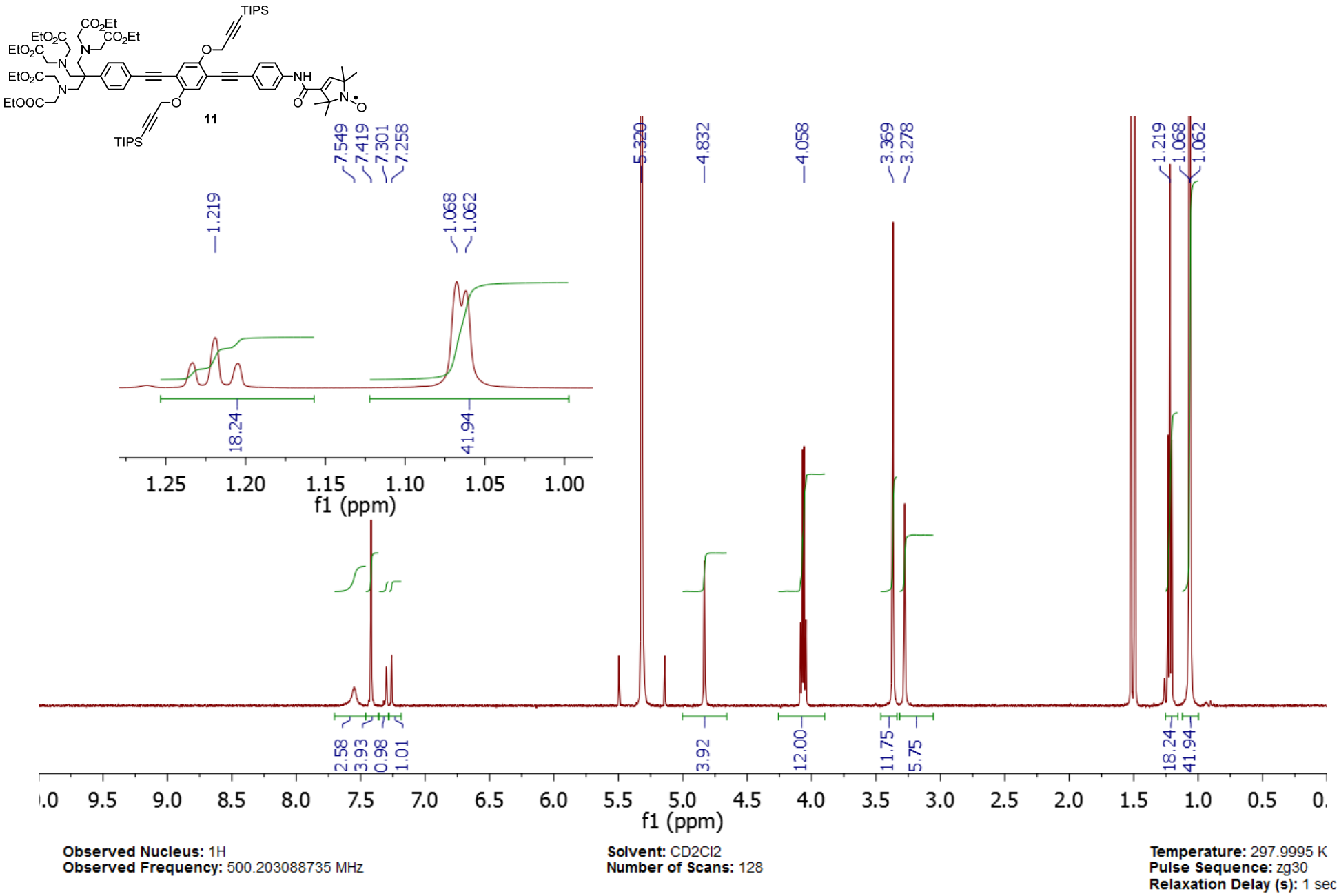

Figure S20. ^{1}H NMR spectrum of TAHAester-(EP)$_2$-NO• **11**.

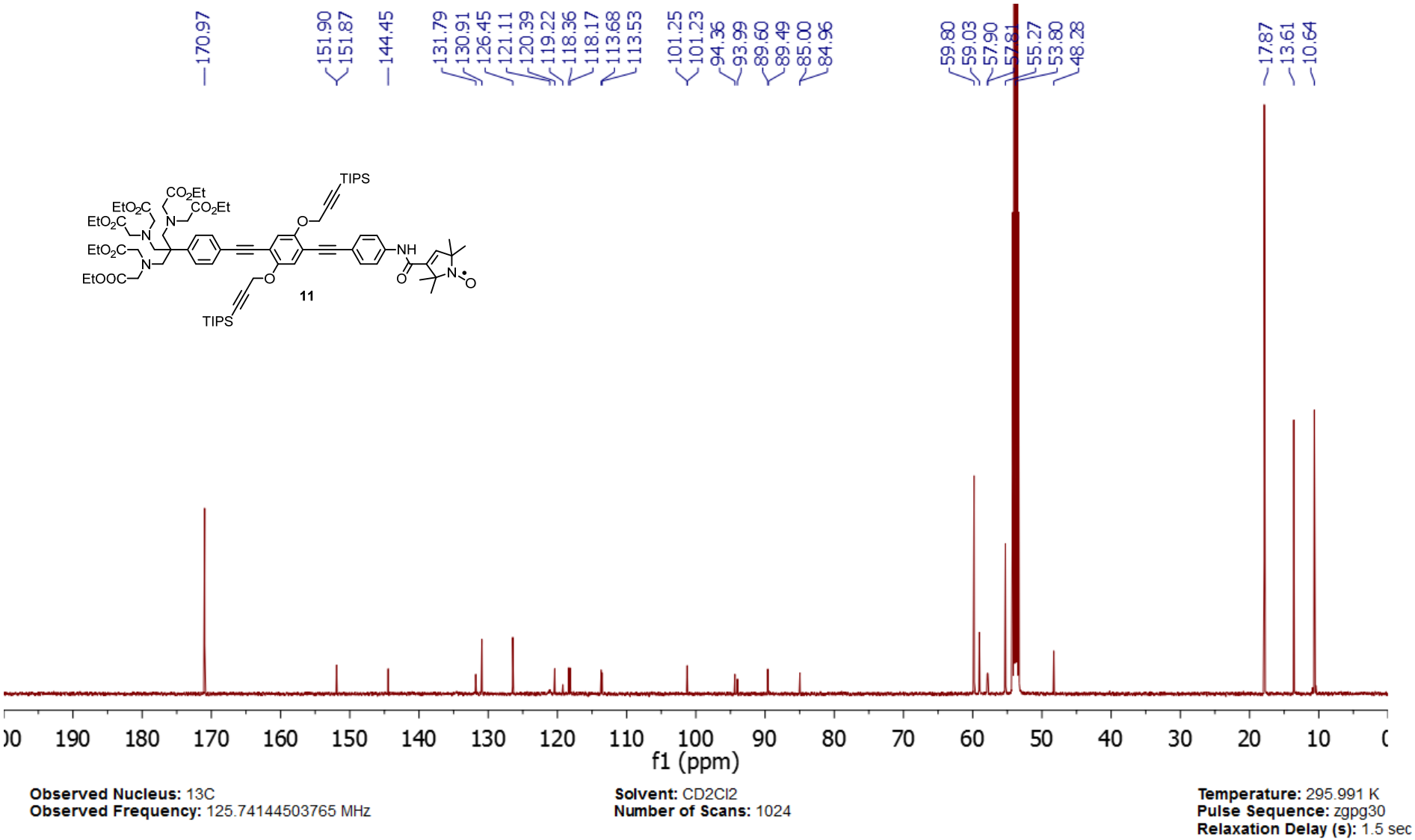

Figure S21. ^{13}C NMR spectrum of TAHAester-$(EP)_2$-NO• **11.**

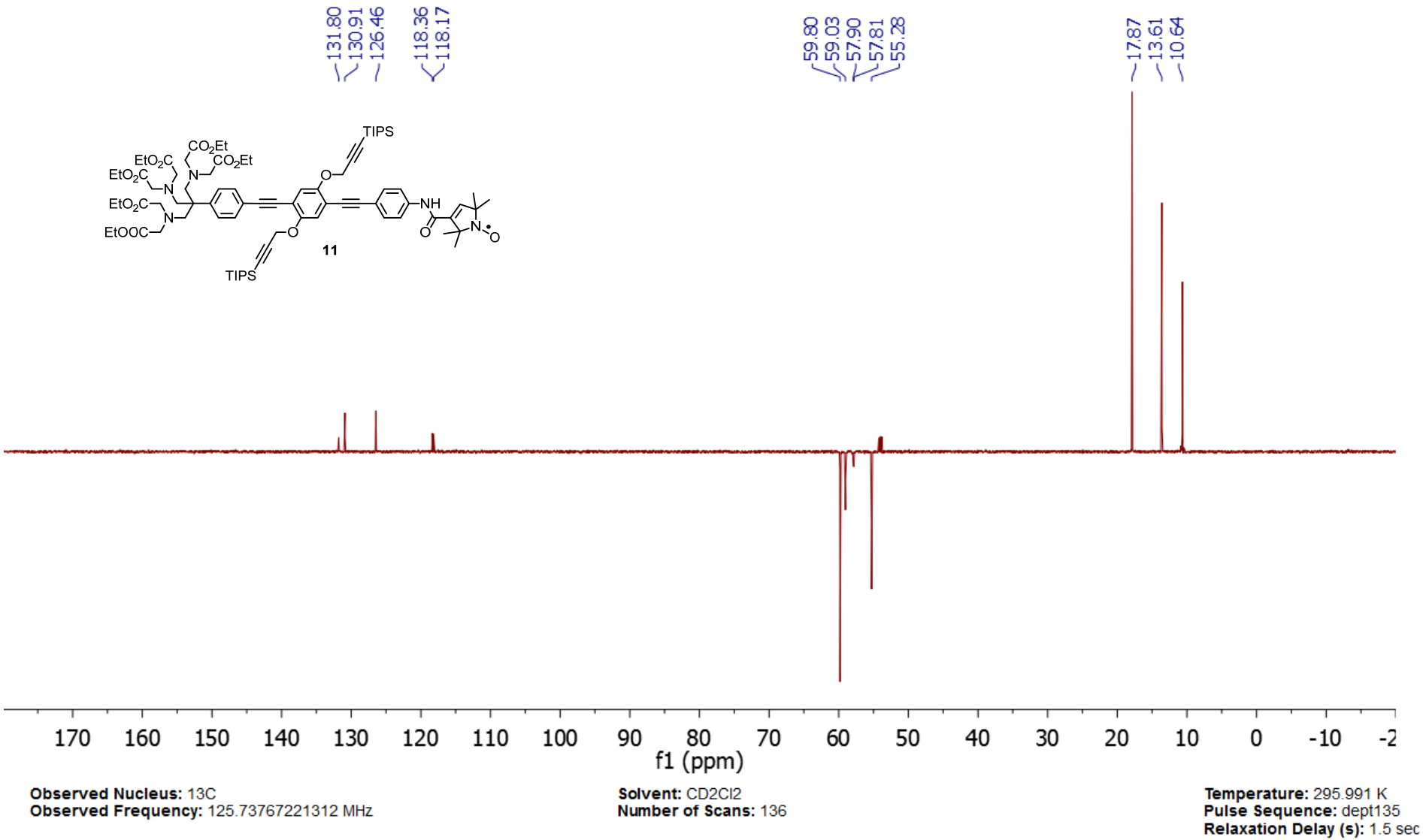

Figure S22. ^{13}C DEPT-135 NMR spectrum of TAHAester-$(EP)_2$-NO• **11.**

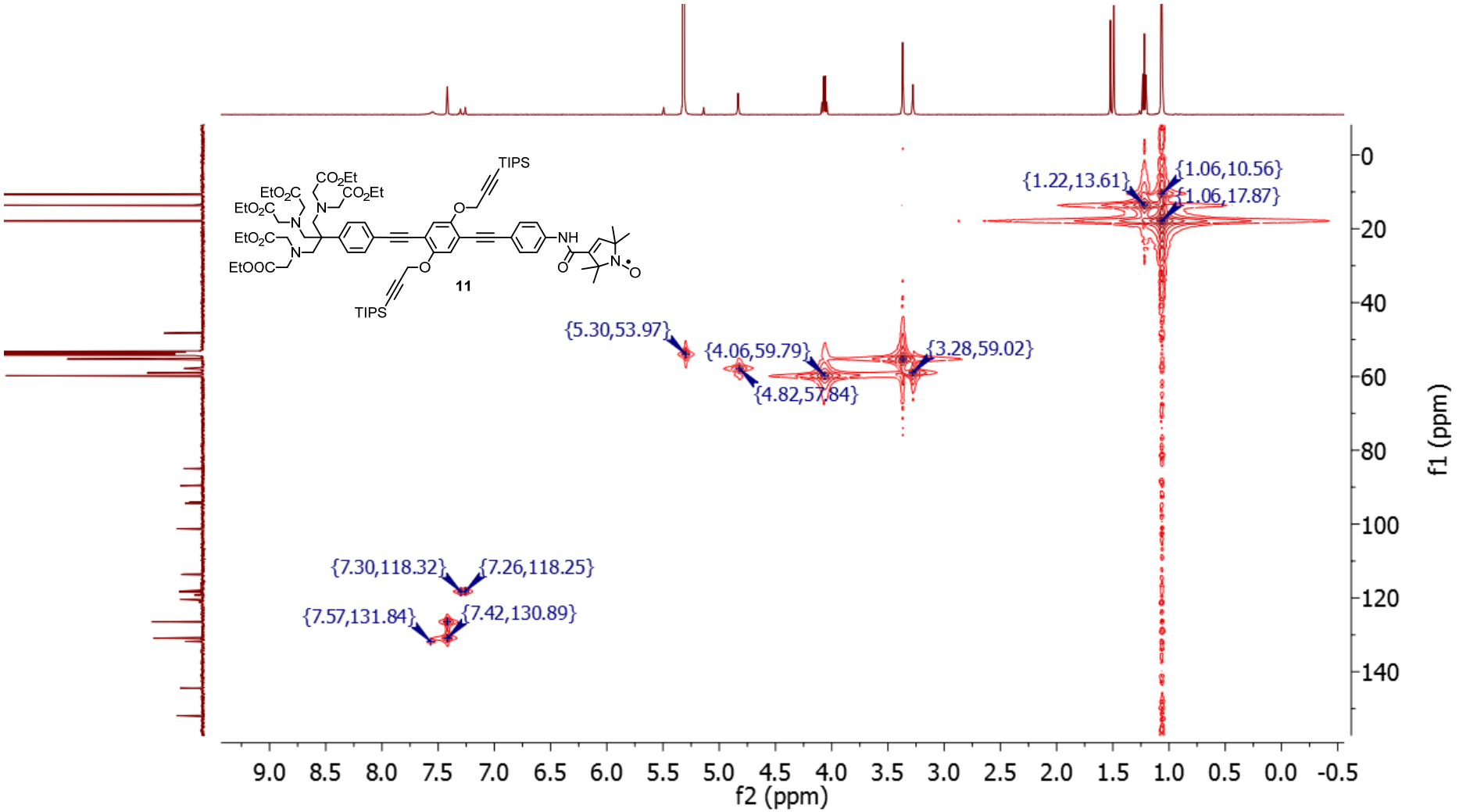

Figure S23. HMQC NMR spectrum of TAHAester-$(EP)_2$-NO• **11.**

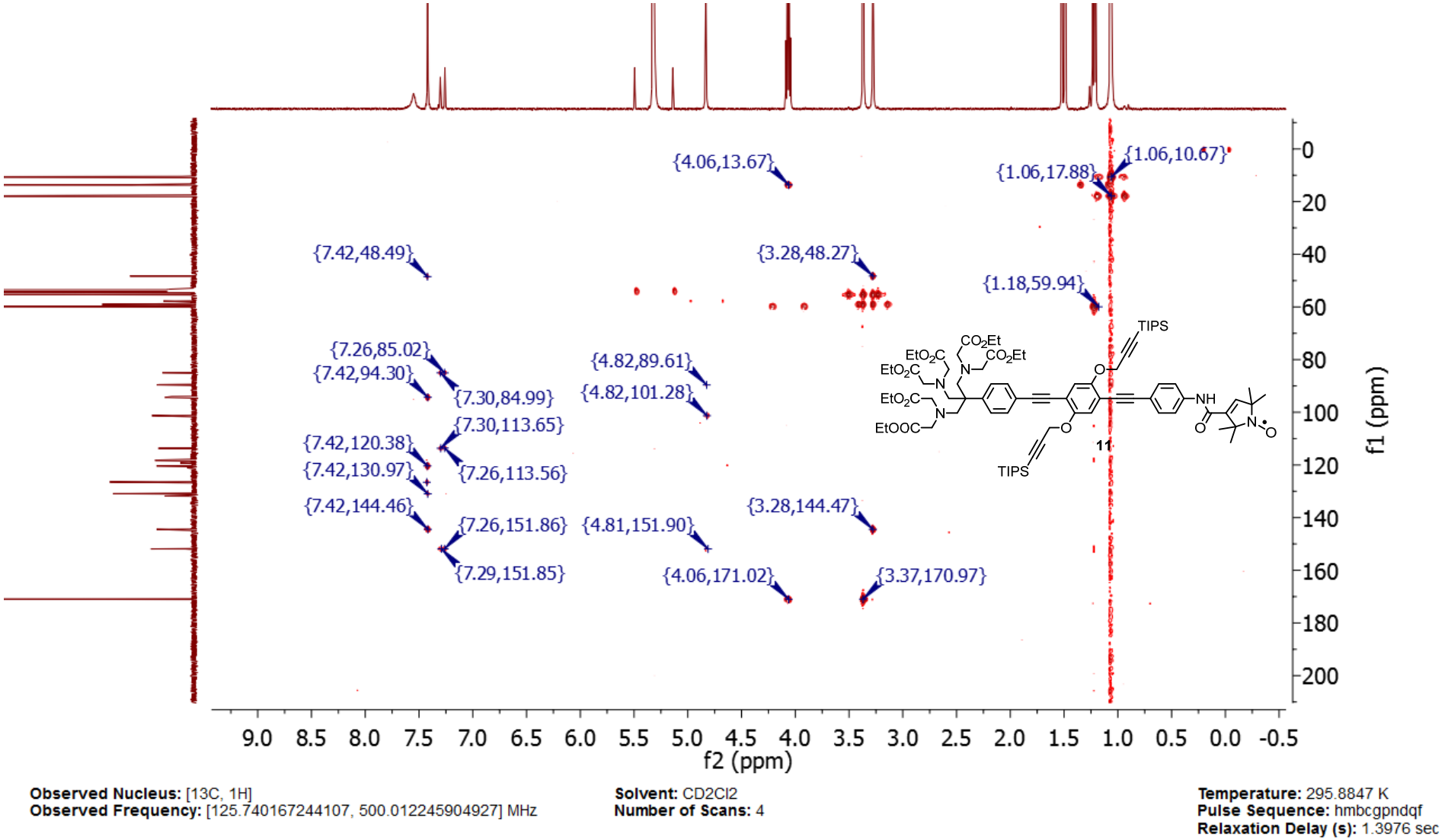

Figure S24. HMBC NMR spectrum of TAHAester-$(EP)_2$-NO• **11.**

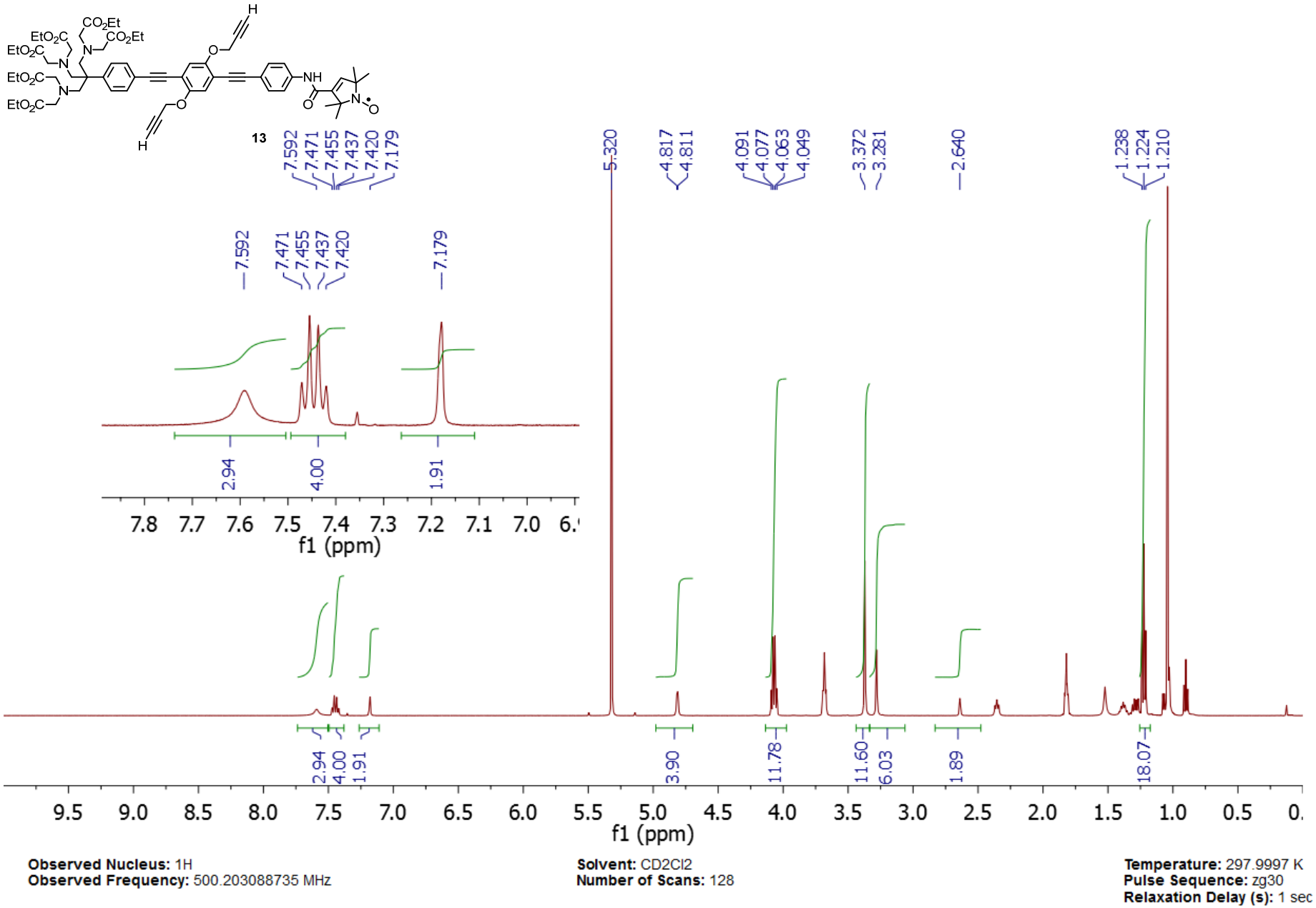

Figure S25: ^{1}H NMR spectrum of the mixture of desilylated TAHAester-(EP)$_2$-NO• **13**, TIPS-F and/or TIPS-OH, a Bu$_4$N-salt, and THF.

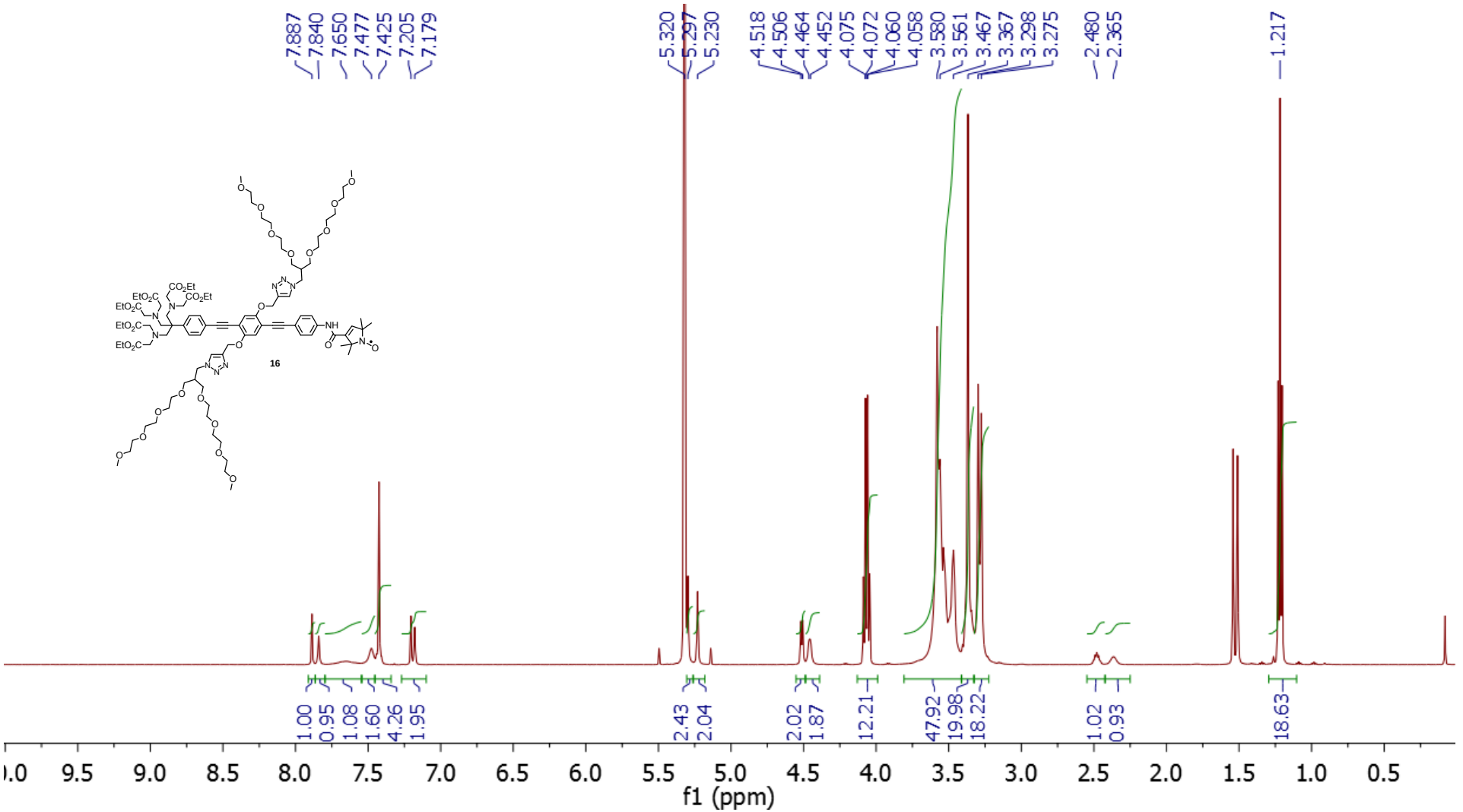

Figure S26. ^{1}H NMR spectrum of PEGylated TAHA-$(EP)_2$-NO• **16**.

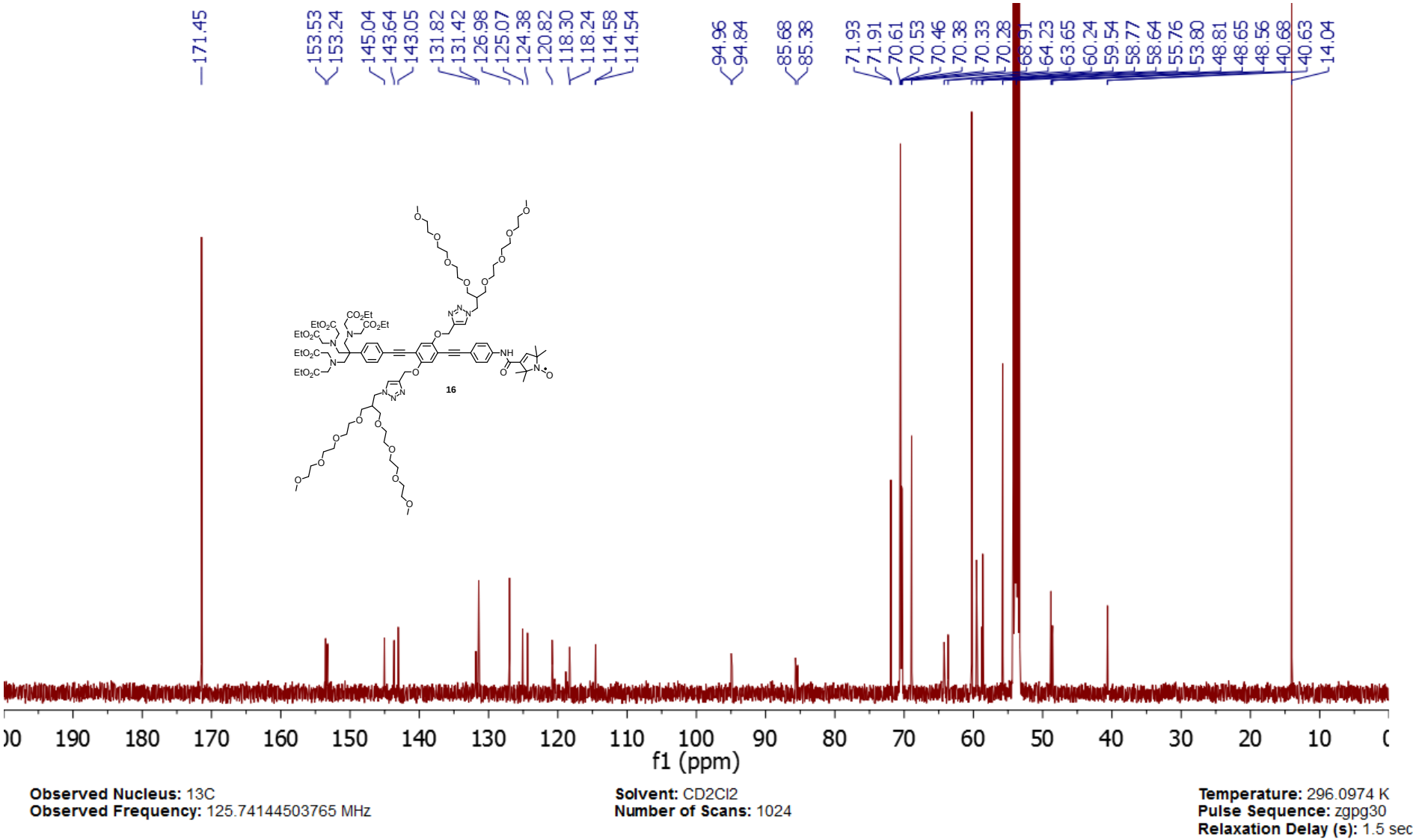

Figure S27. ^{13}C NMR spectrum of PEGylated TAHAester-$(EP)_2$-NO• **16**.

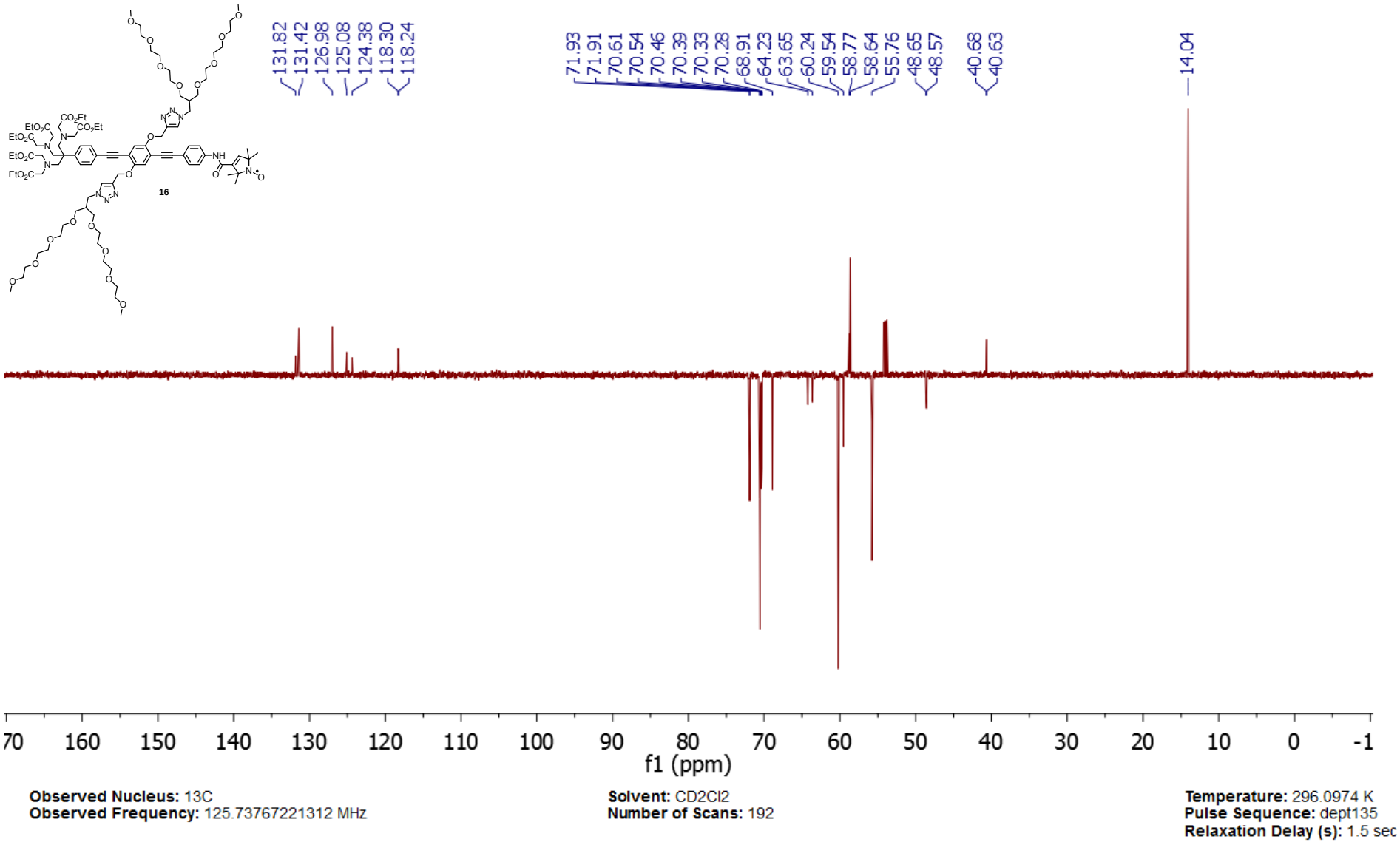

Figure S28. ^{13}C DEPT-135 NMR spectrum of PEGylated TAHAester-$(EP)_2$-NO• **16**.

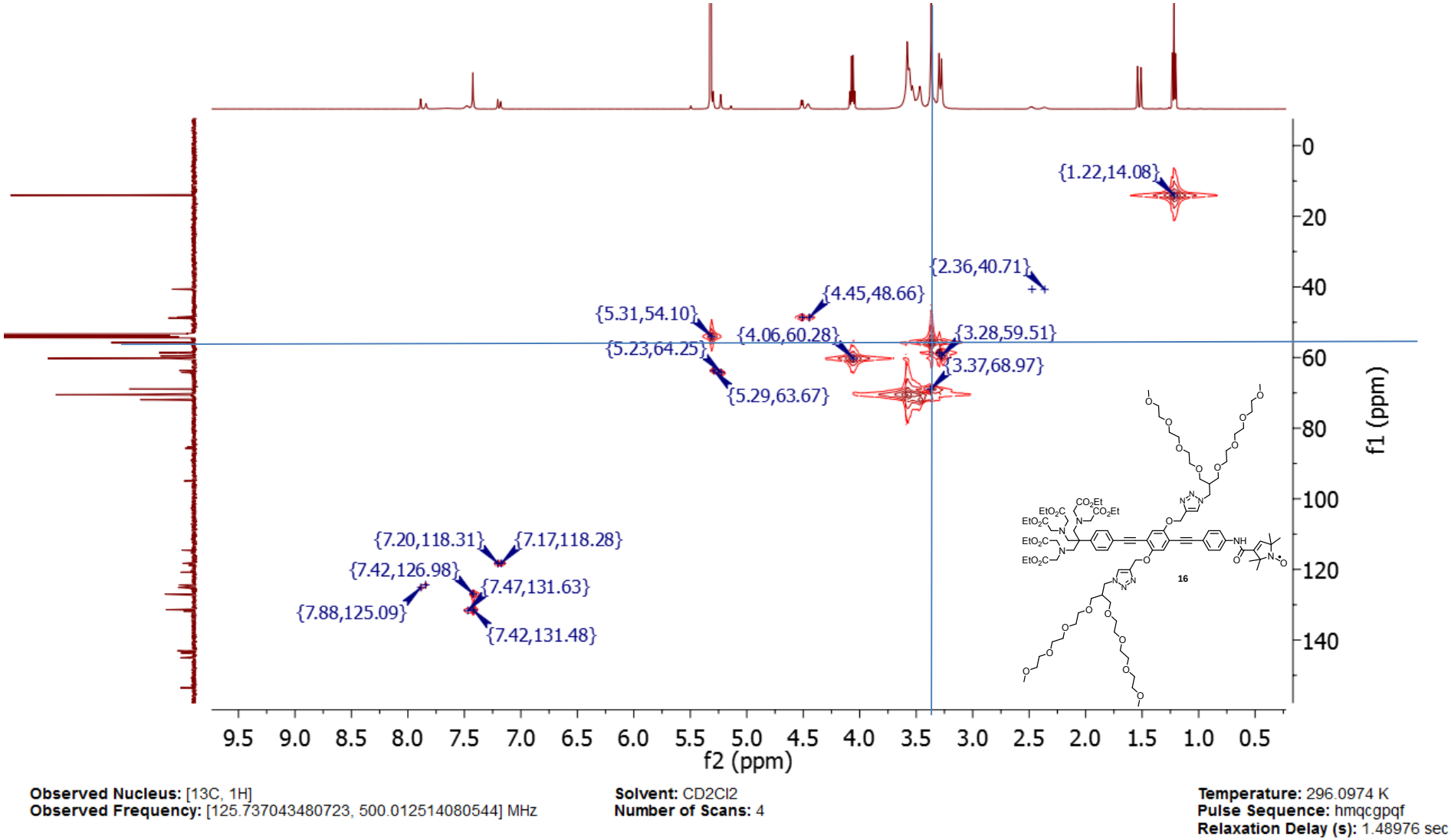

Figure S29. HMQC NMR spectrum of PEGylated TAHAester-$(EP)_2$-NO• **16**.

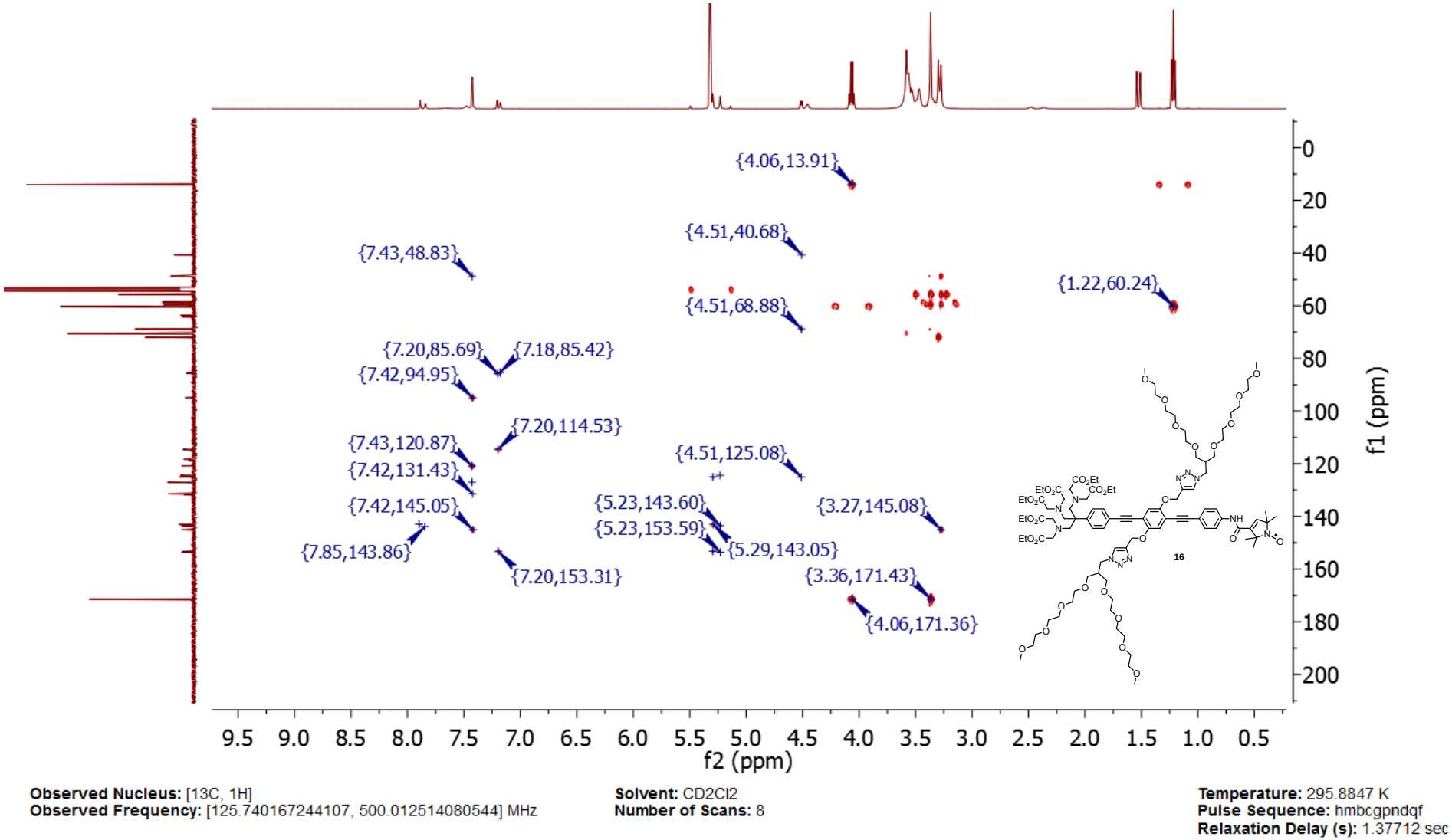

Figure S30. HMBC NMR spectrum of PEGylated TAHAester-$(EP)_2$-NO• **16**.

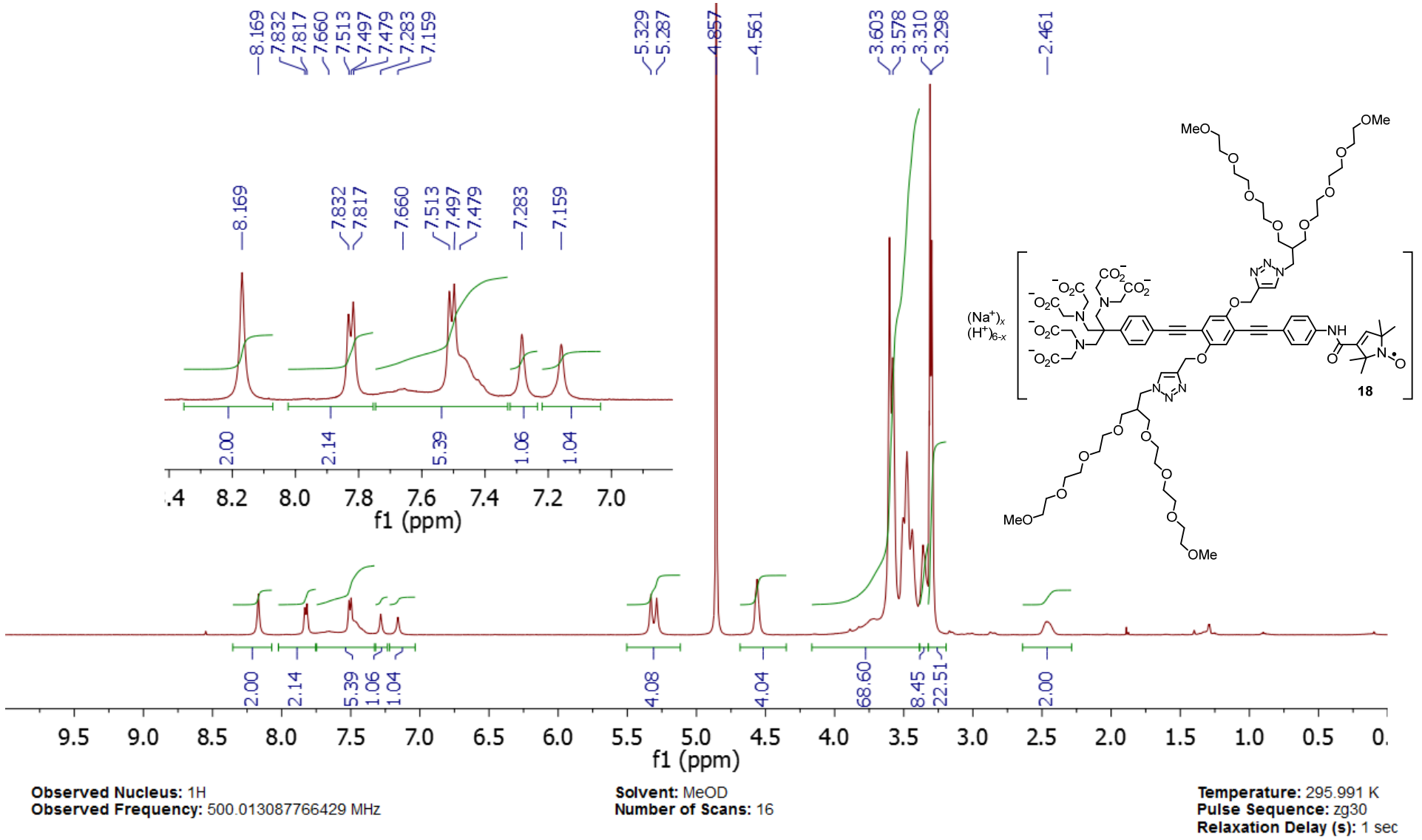

Figure S31. ^{1}H NMR spectrum of $(H^{+})_{x}(Na^{+})_{6-x}[TAHA\text{-}(EP)_{2}\text{-}NO\bullet]^{6-}$ **18**.

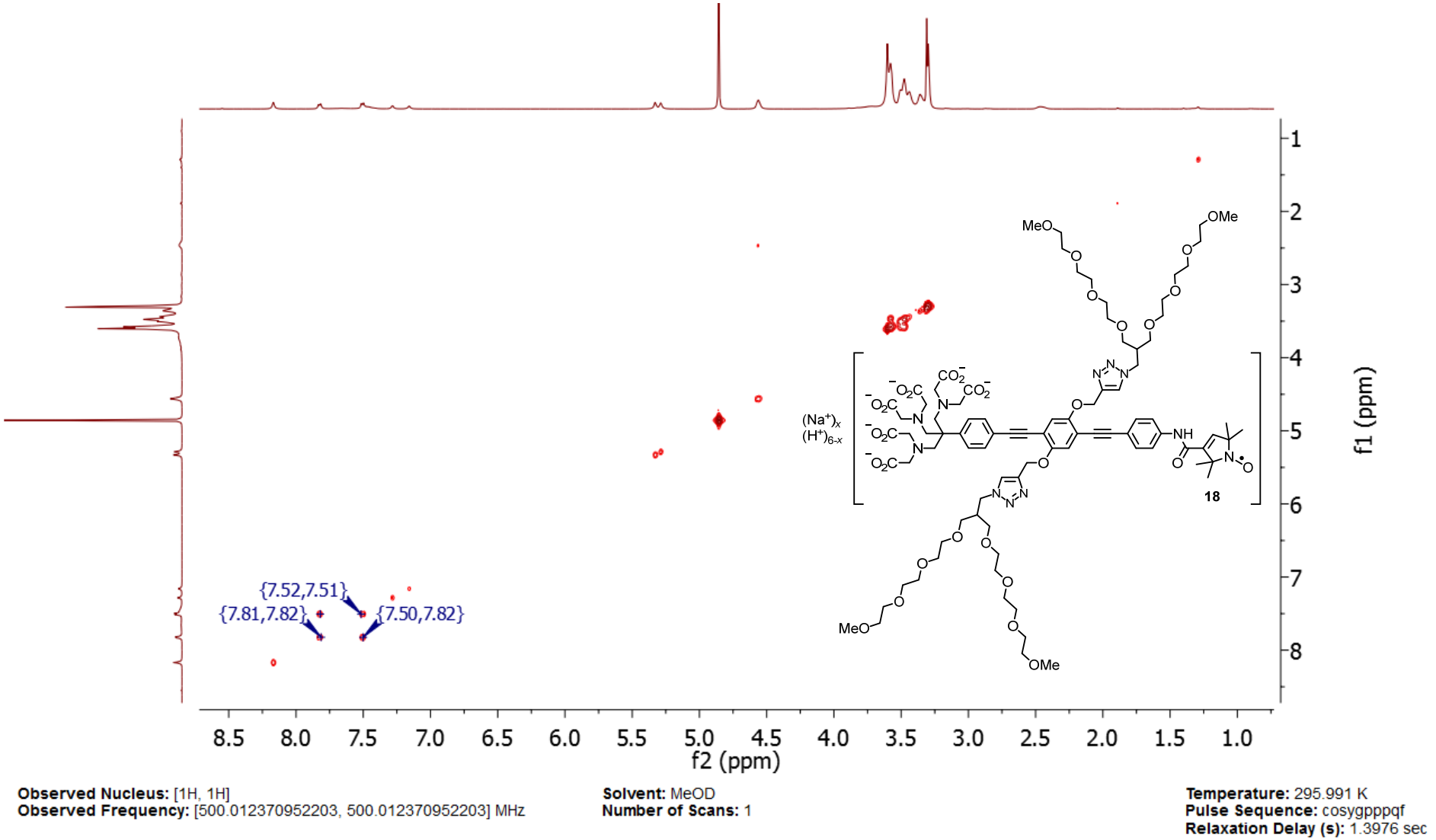

Figure S32. COSY NMR spectrum of $(H^+)_x(Na^+)_{6-x}$[TAHA-$(EP)_2$-NO•]$^{6-}$ **18**.

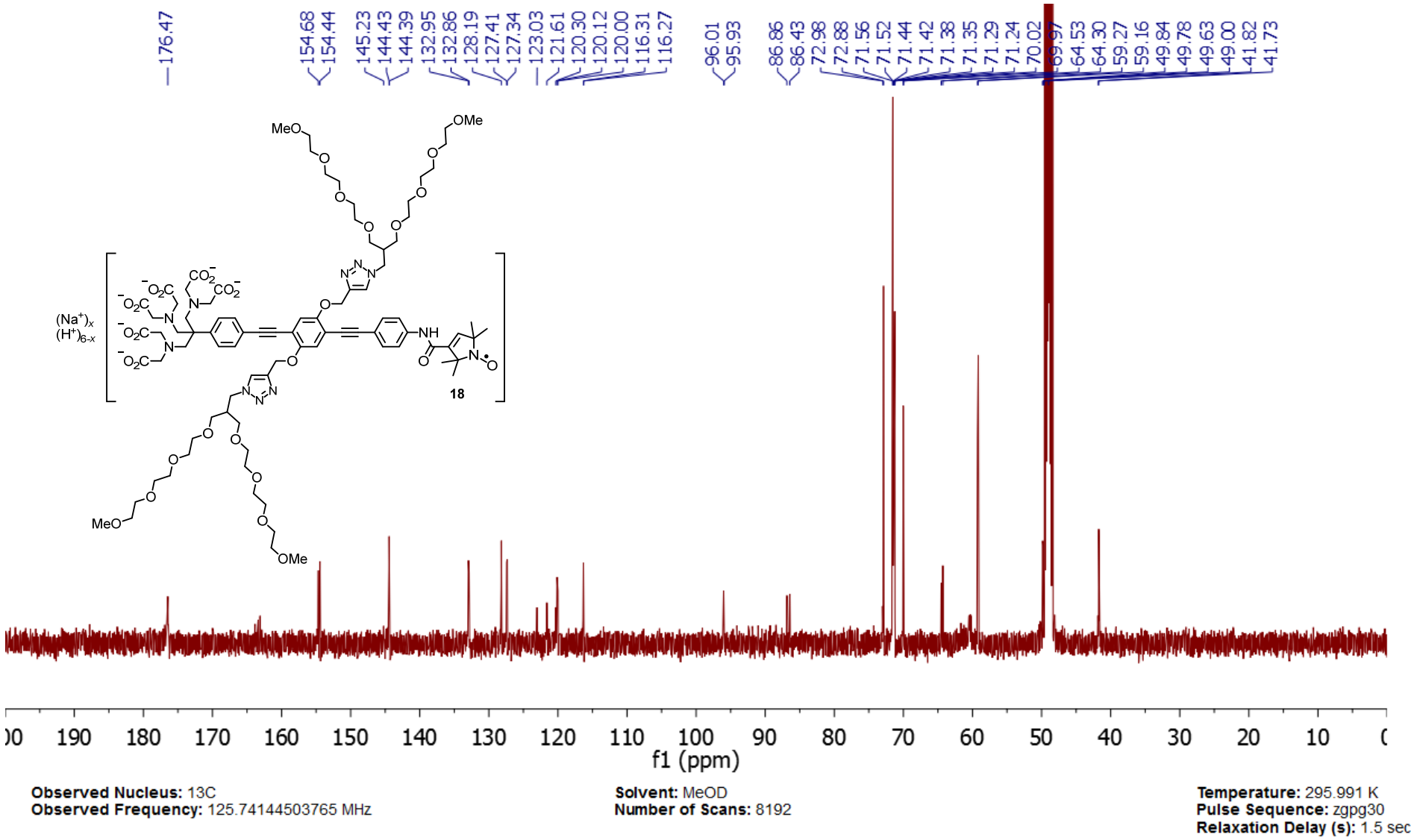

Figure S33. ^{13}C NMR spectrum of $(H^+)_x(Na^+)_{6-x}[TAHA\text{-}(EP)_2\text{-}NO\bullet]^{6-}$ **18**.

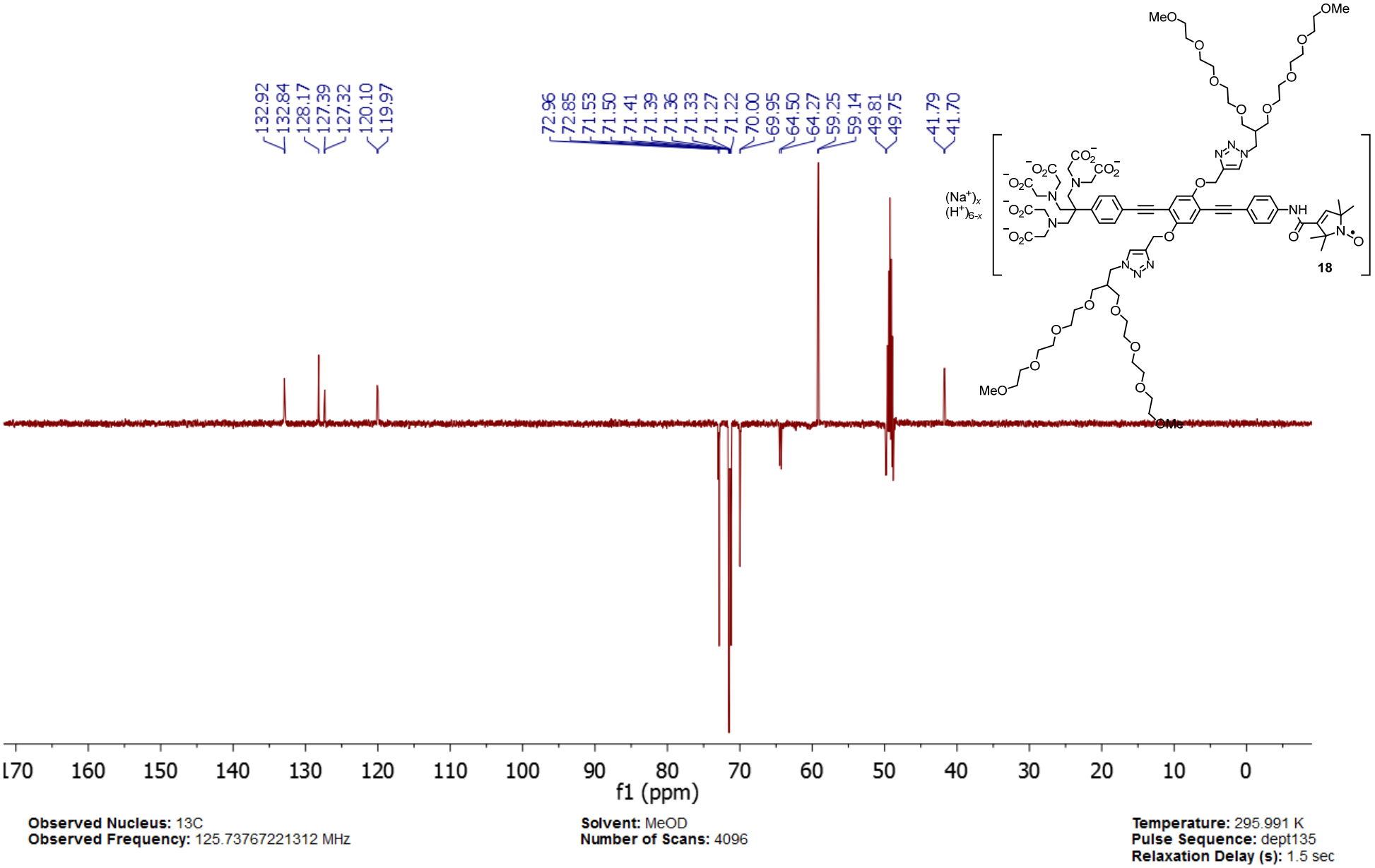

Figure S34. ^{13}C DEPT-135 NMR spectrum of $(H^+)_x(Na^+)_{6-x}[TAHA\text{-}(EP)_2\text{-}NO\bullet]^{6-}$ **18**.

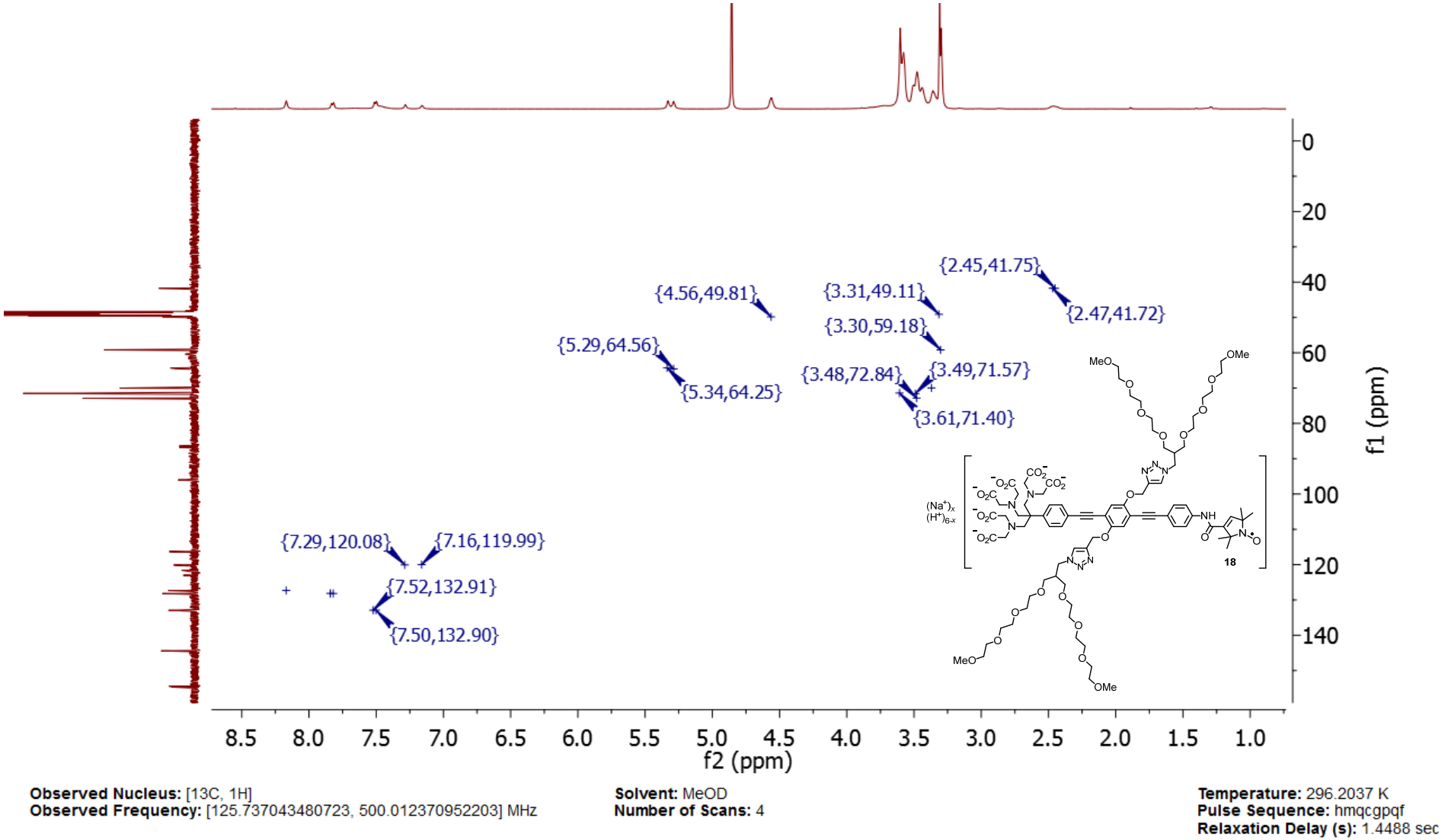

Figure S35. HMQC NMR spectrum of $(H^+)_x(Na^+)_{6-x}$[TAHA-$(EP)_2$-NO•]$^{6-}$ **18**.

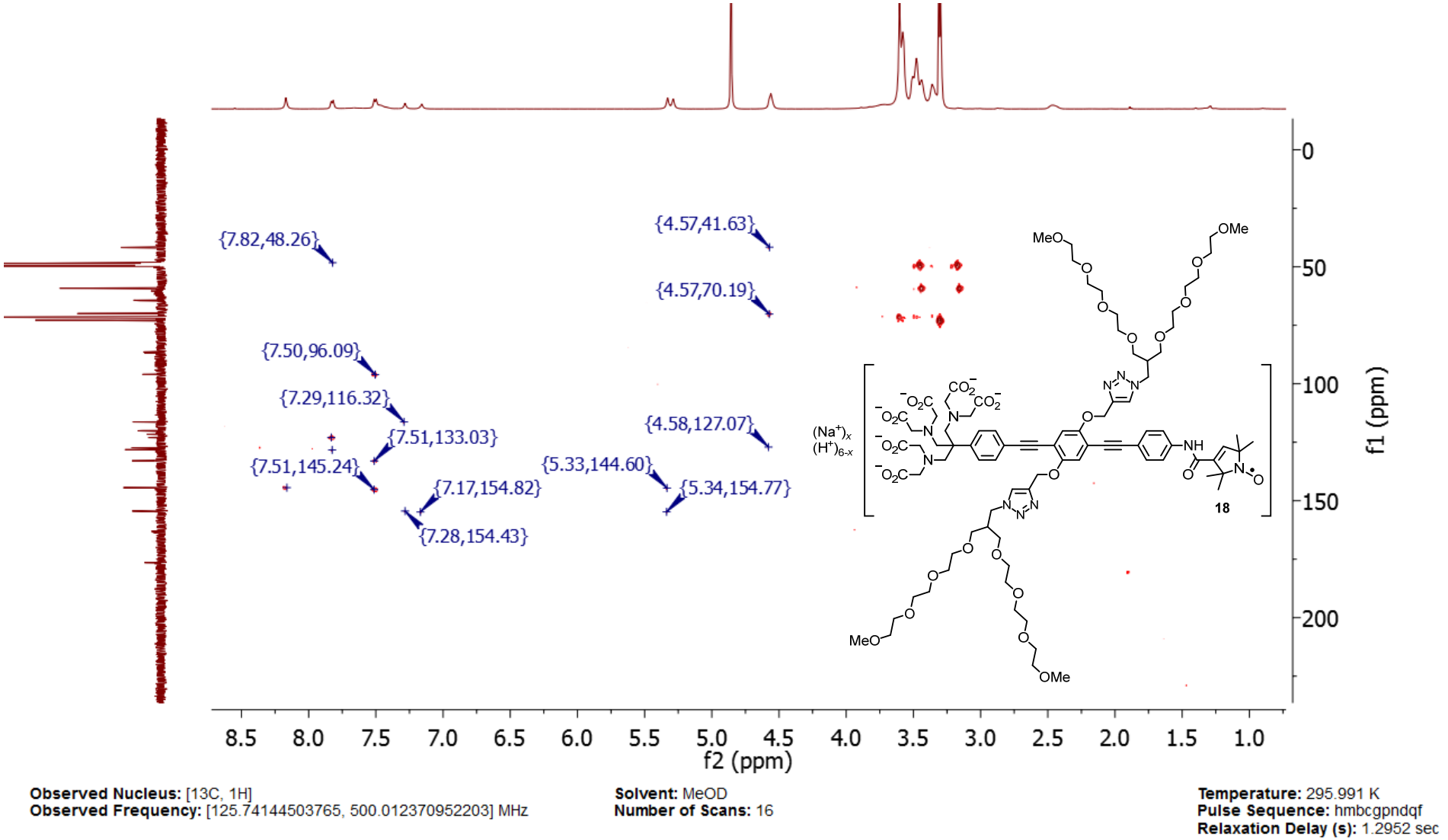

Figure S36. HMBC NMR spectrum of $(H^+)_x(Na^+)_{6-x}$[TAHA-$(EP)_2$-NO•]$^{6-}$ **18**.

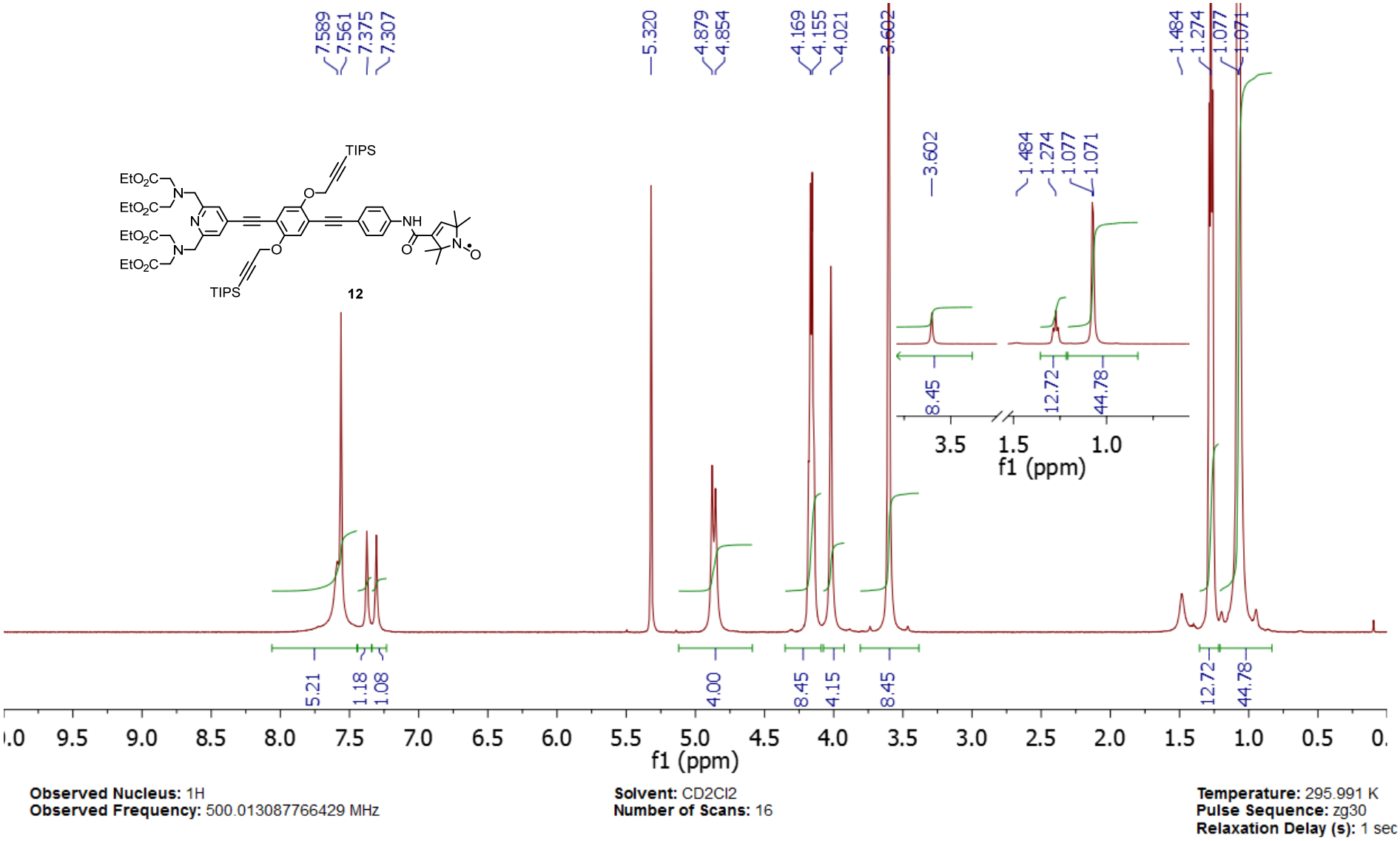

Figure S37. ^{1}H NMR spectrum of PyMTAester-(EP)$_2$-NO• **12**.

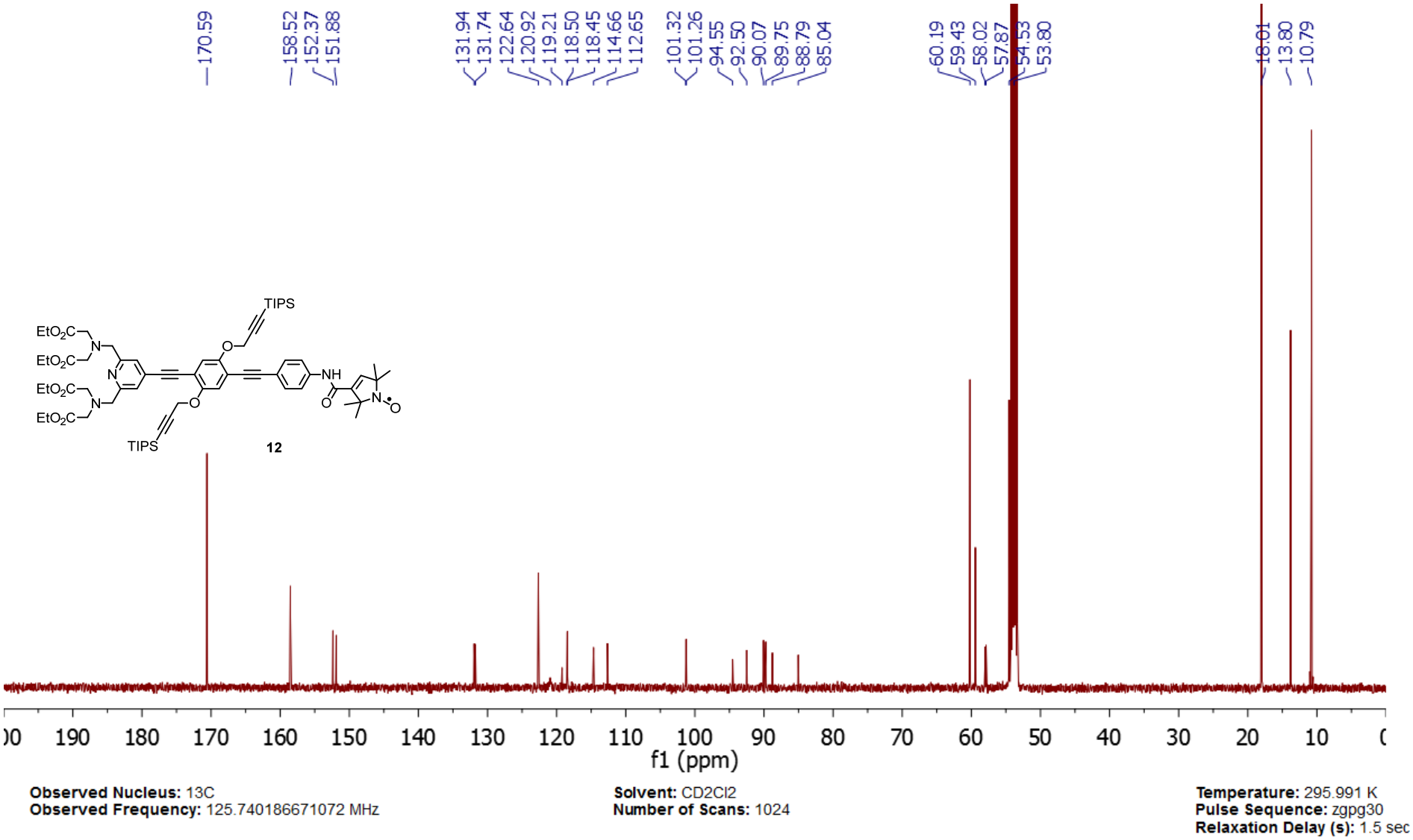

Figure S38. ^{13}C NMR spectrum of PyMTAester-$(EP)_2$-NO• **12**.

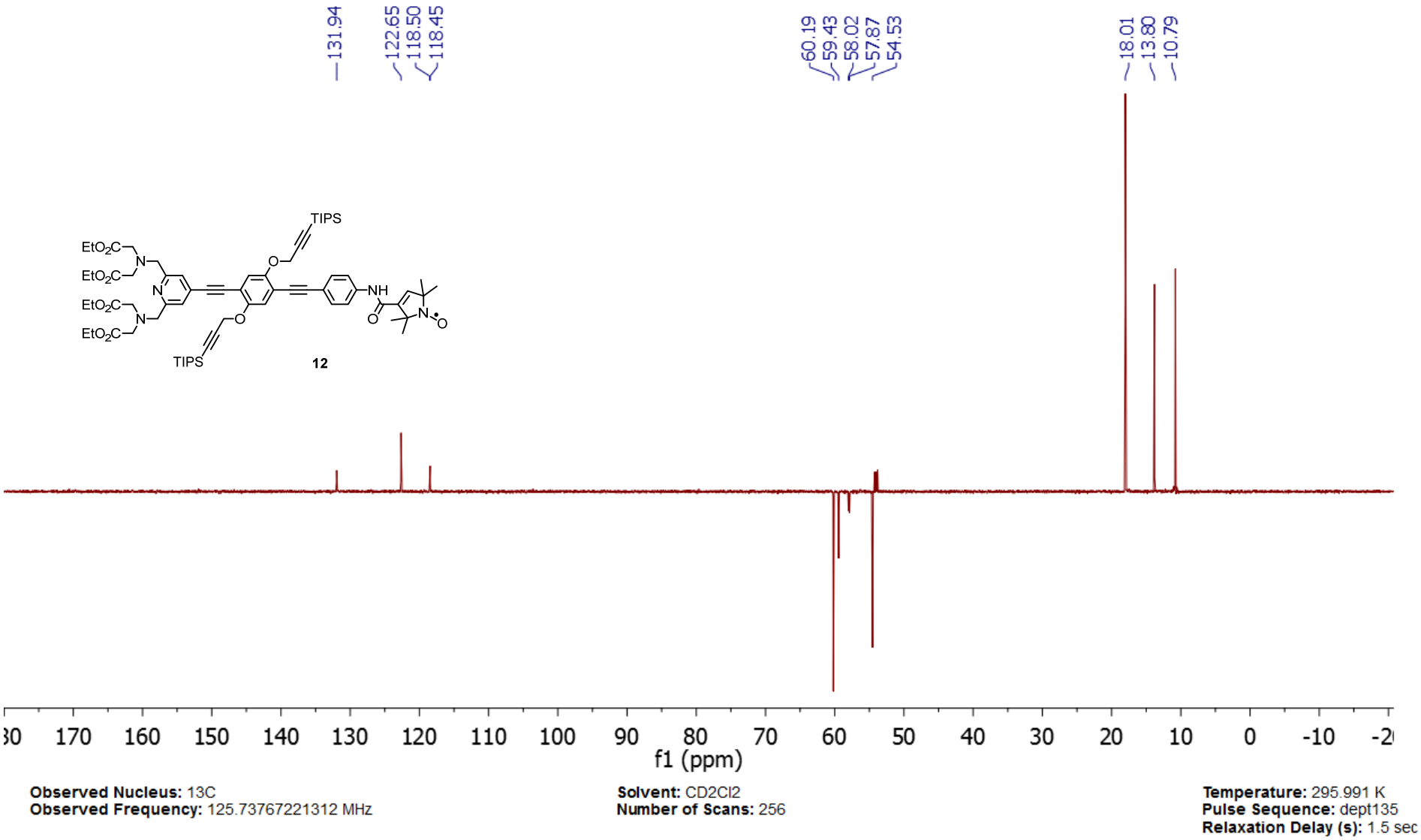

Figure S39. ^{13}C DEPT-135 NMR spectrum of PyMTAester-$(EP)_2$-NO• **12**.

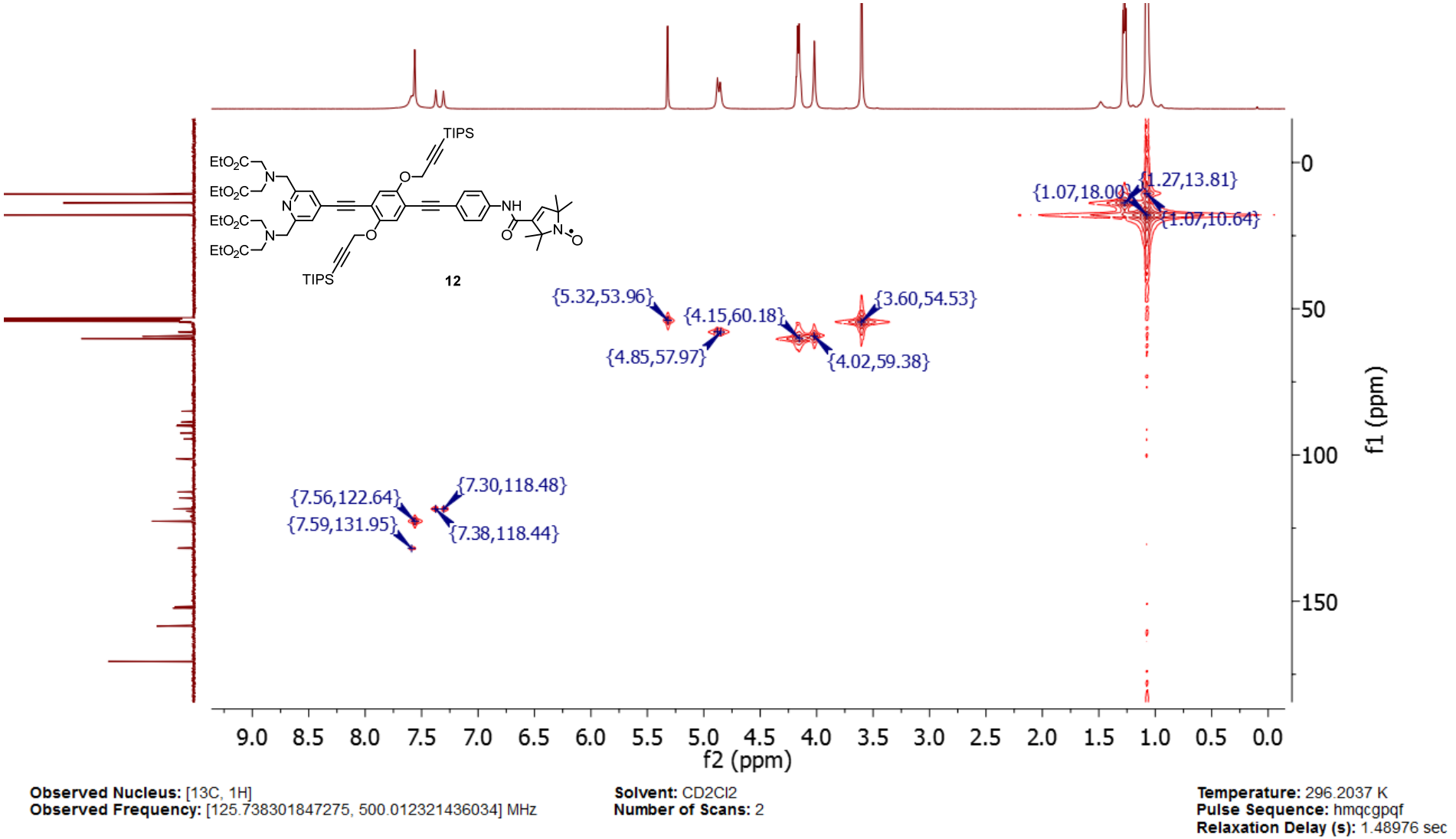

Figure S40. HMQC NMR spectrum of PyMTAester-$(EP)_2$-NO• **12**.

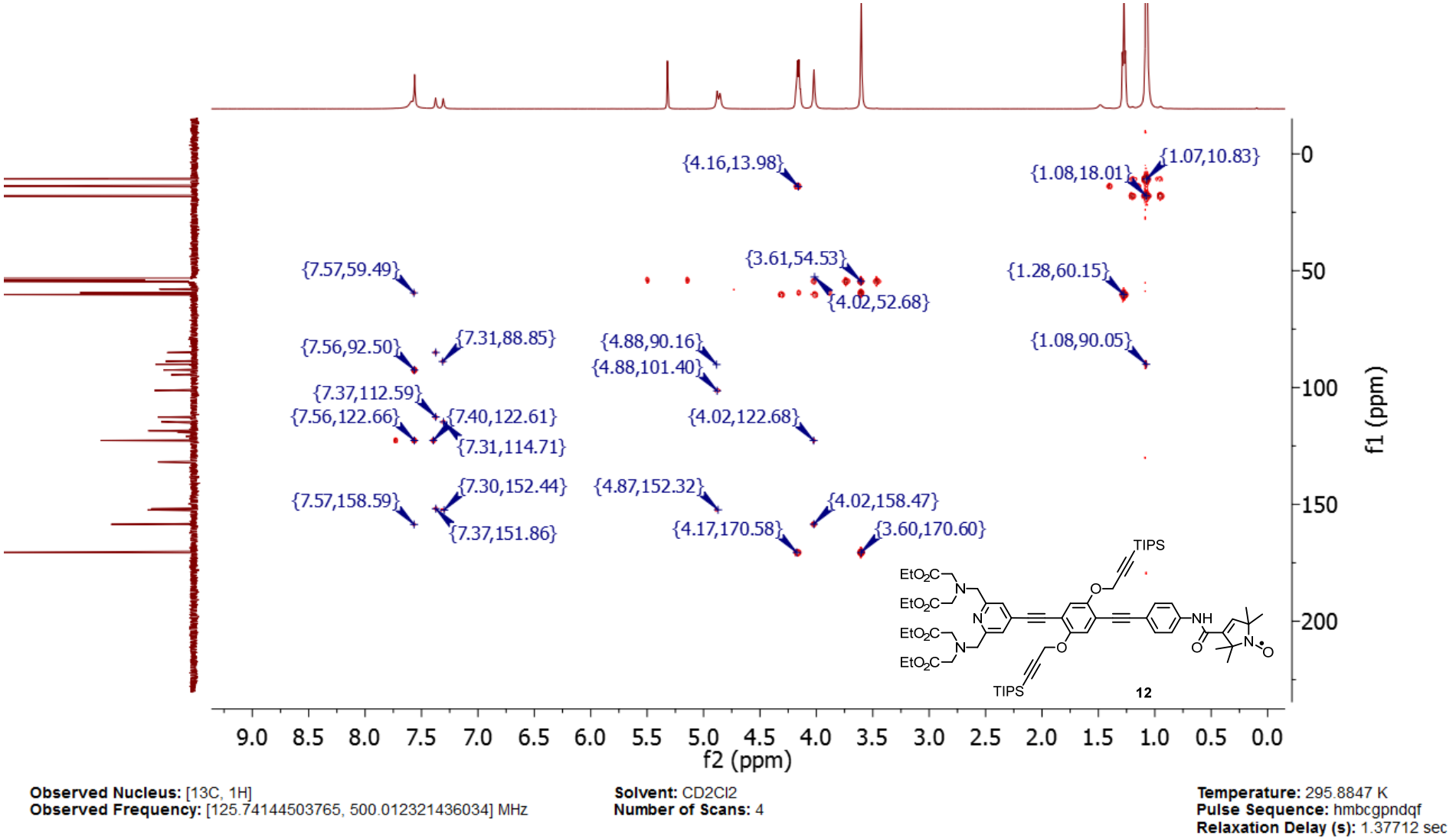

Figure S41. HMBC NMR spectrum of PyMTAester-$(EP)_2$-NO• **12**.

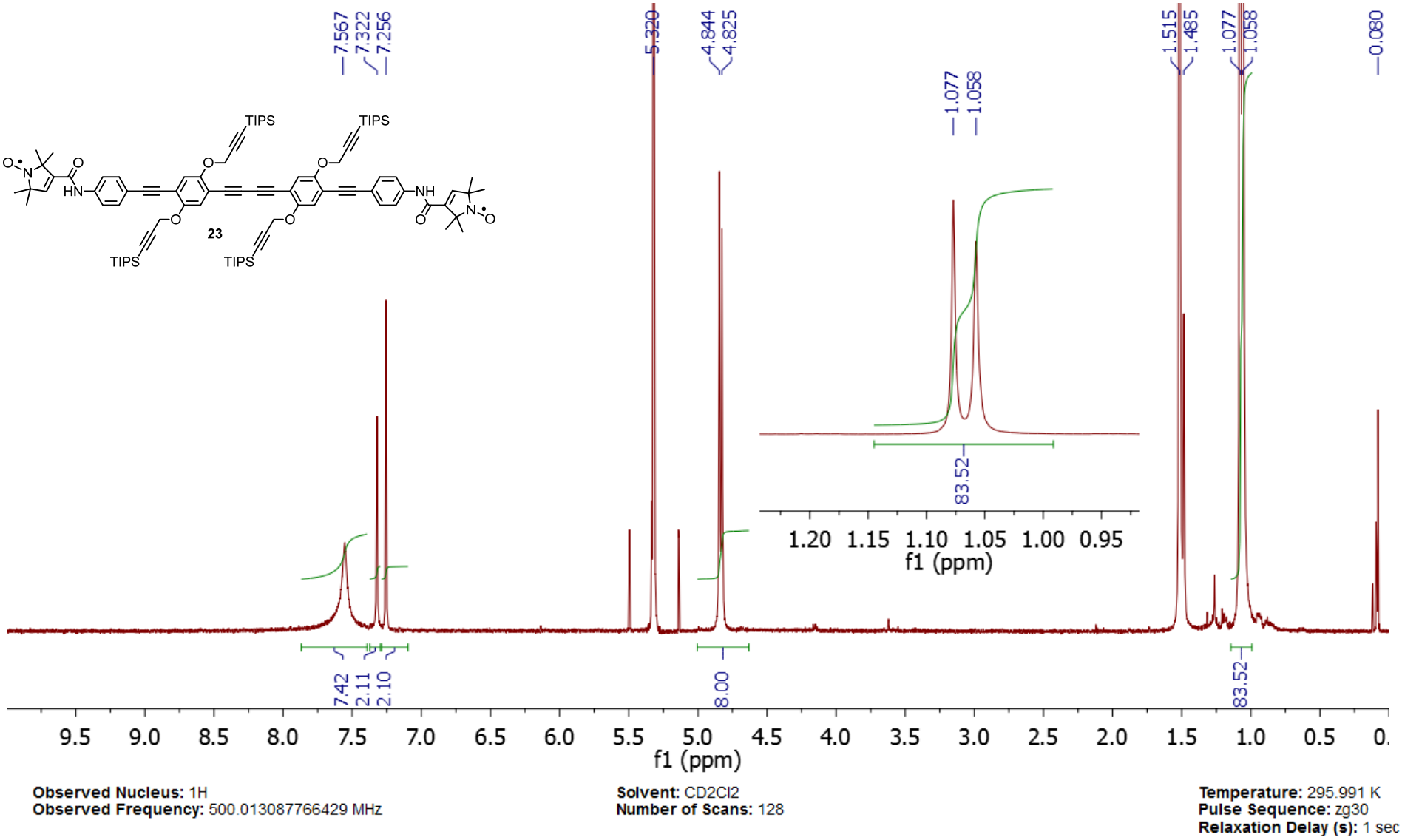

Figure S42. ^{1}H NMR spectrum of alkyne dimer **23**.

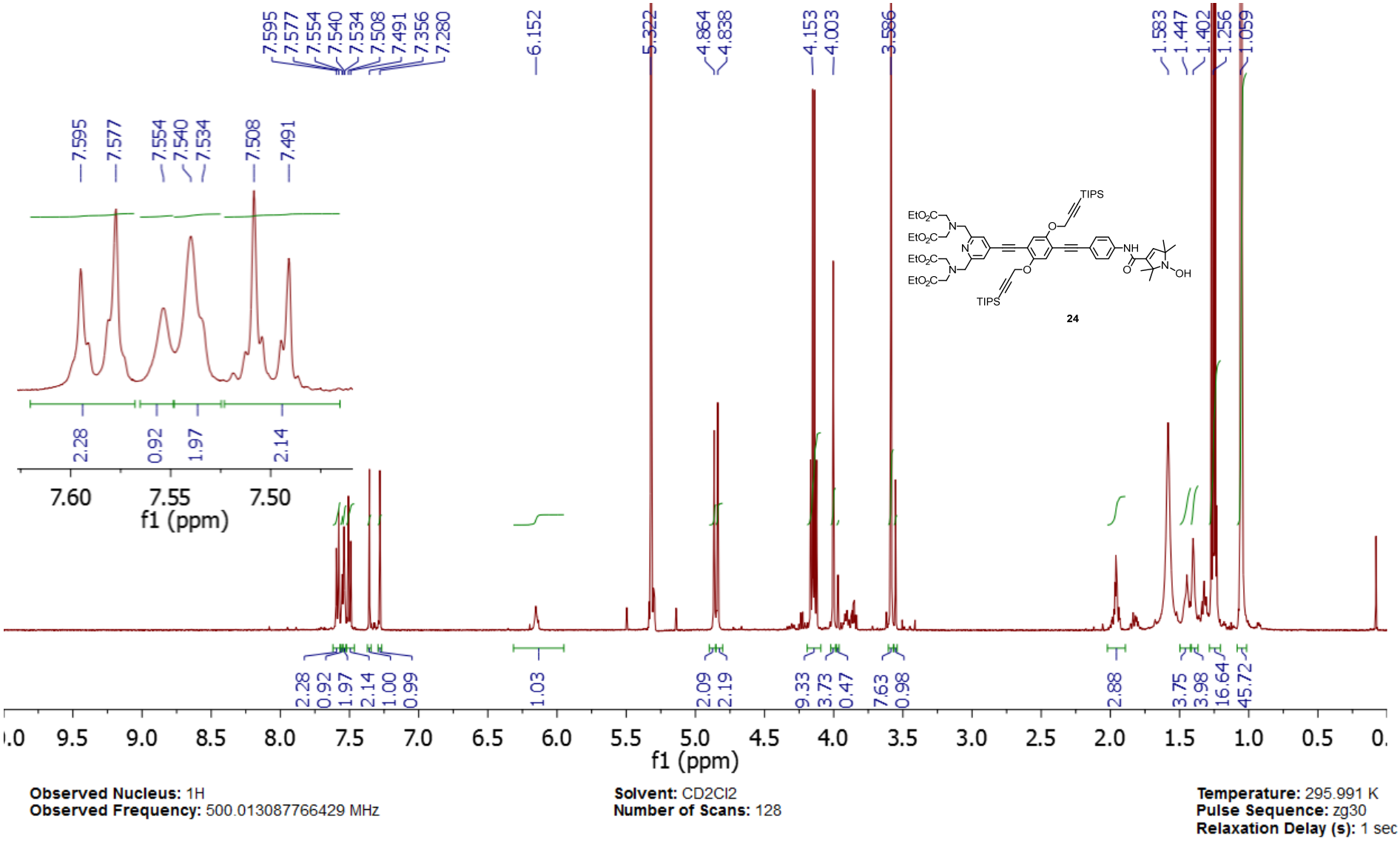

Figure S43. ^{1}H NMR spectrum of a mixture consisting of hydroxylamine **24** and minor amounts of unidentified components.

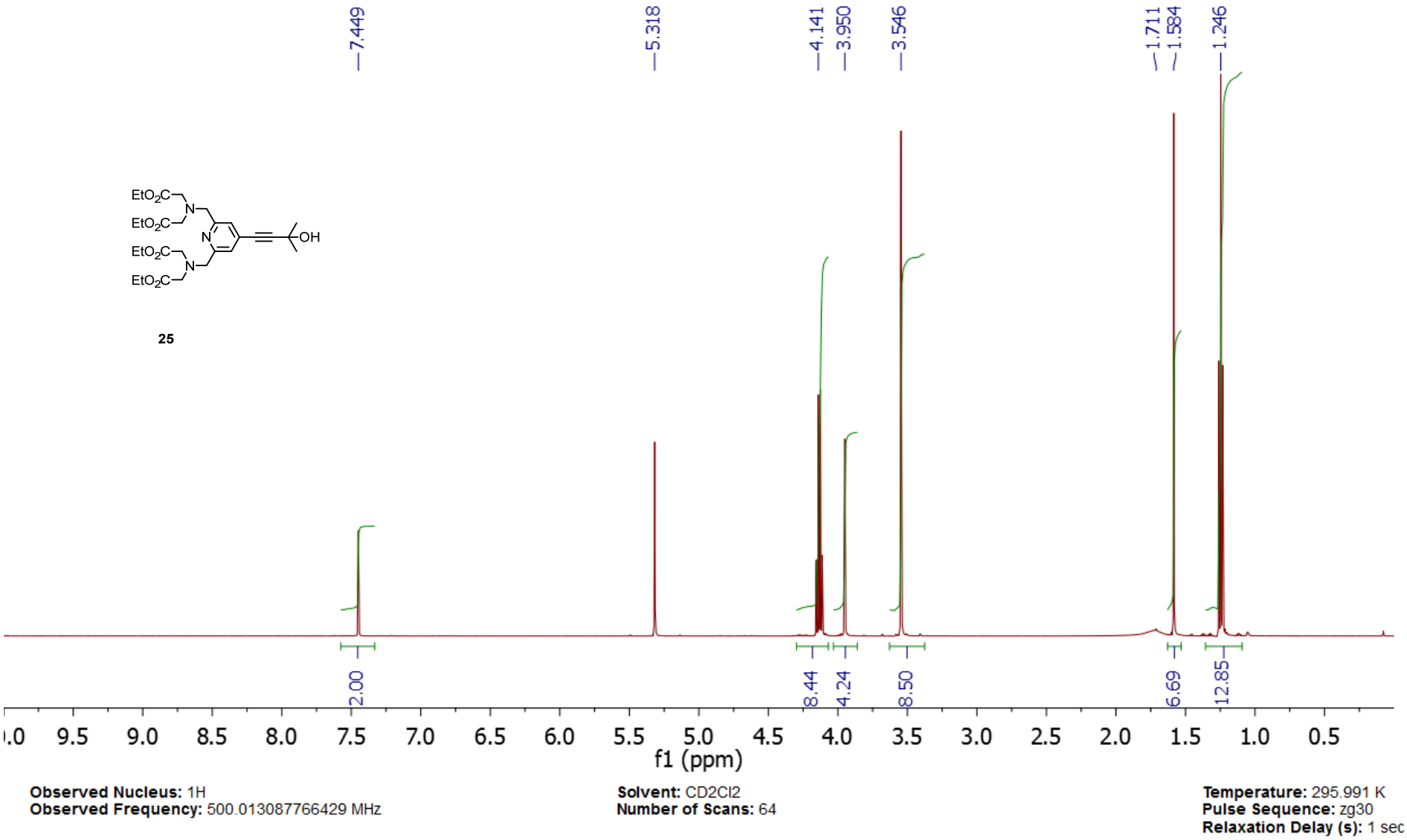

Figure S44. ^{1}H NMR spectrum of byproduct **25**.

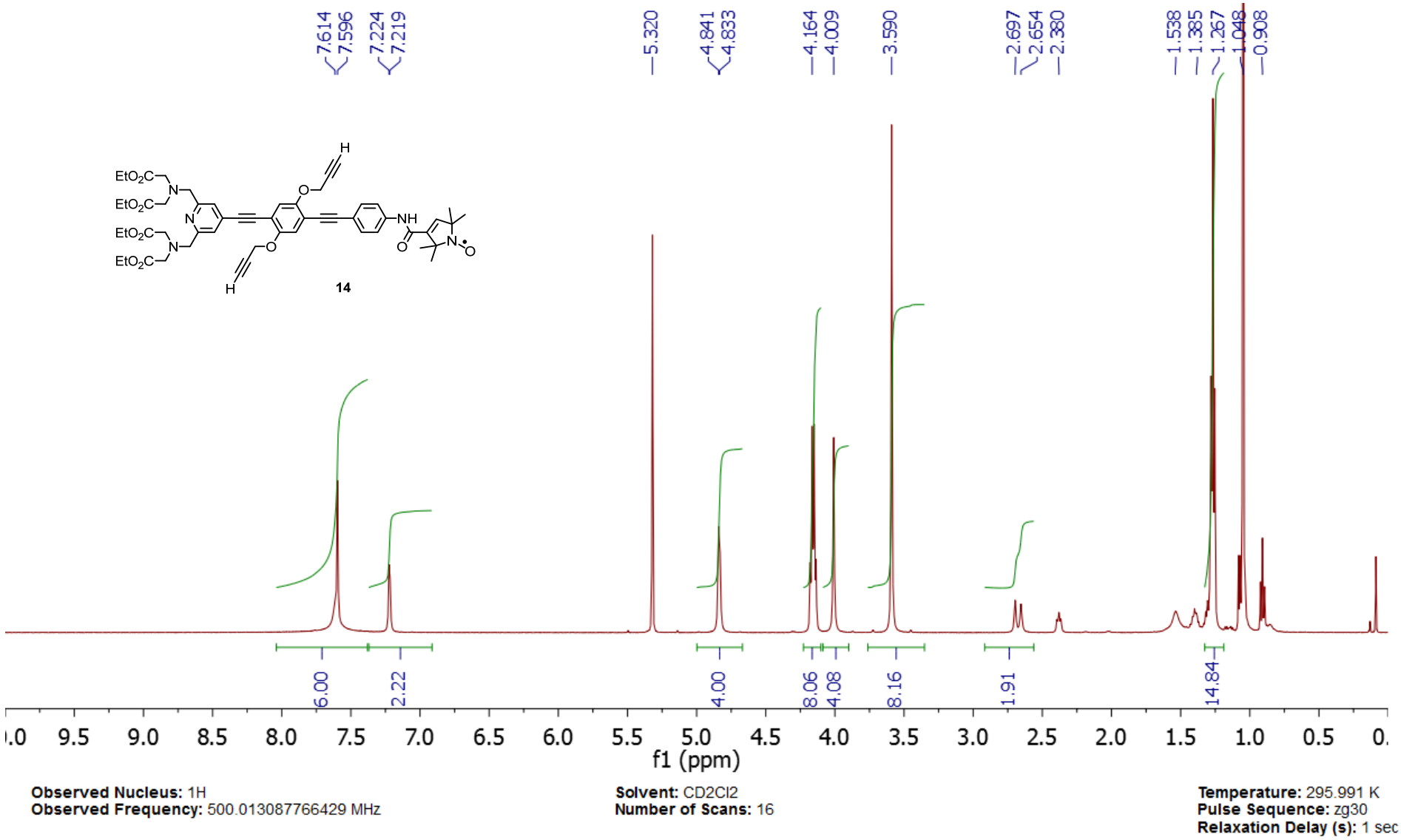

Figure S45. ^{1}H NMR spectrum of desilylated PyMTAester-$(EP)_2$-NO• **14**, TIPS-F and/or TIPS-OH, and a Bu_4N-salt.

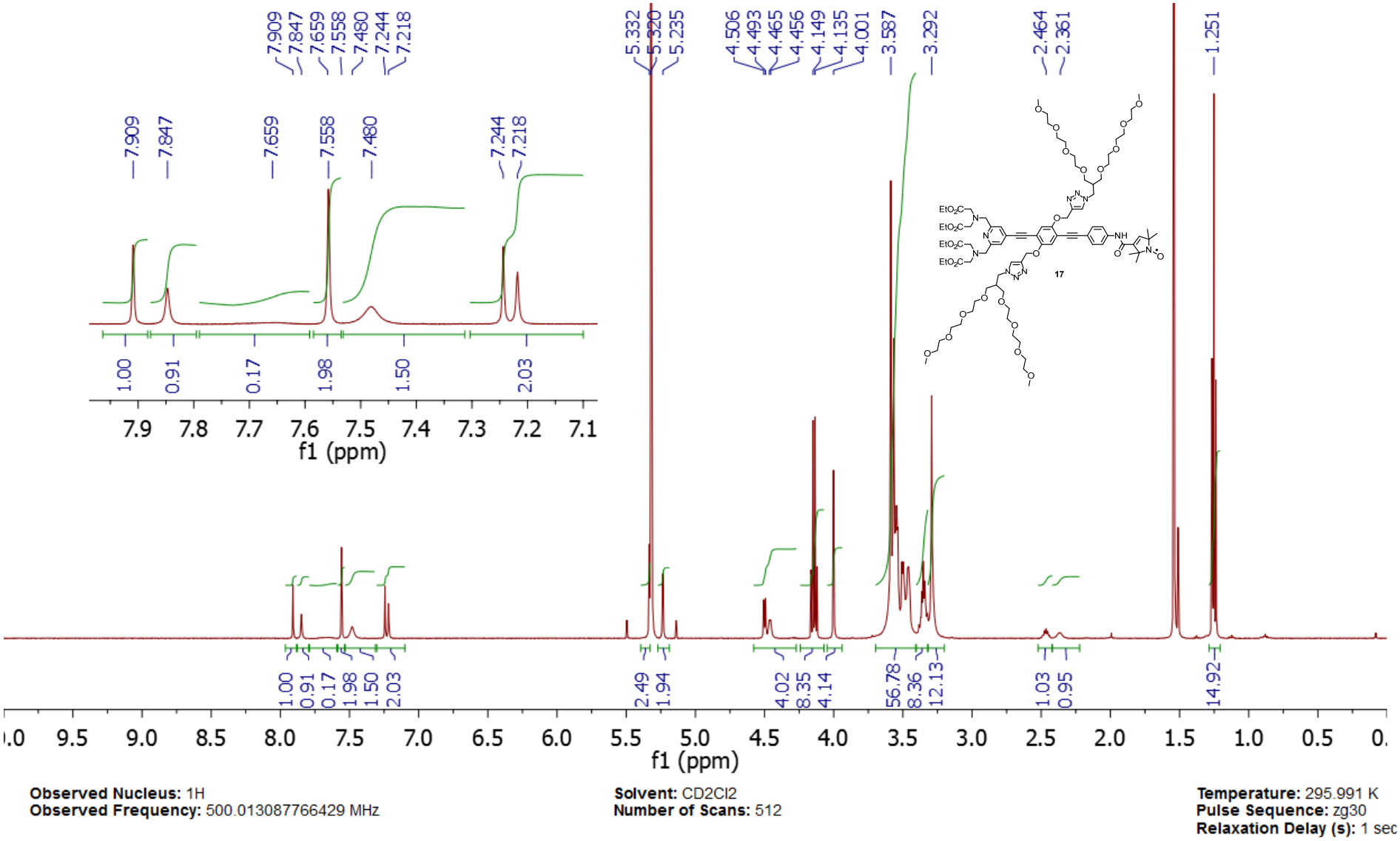

Figure S46. ^{1}H NMR spectrum of PEGylated PyMTAester-$(EP)_2$-NO• **17**.

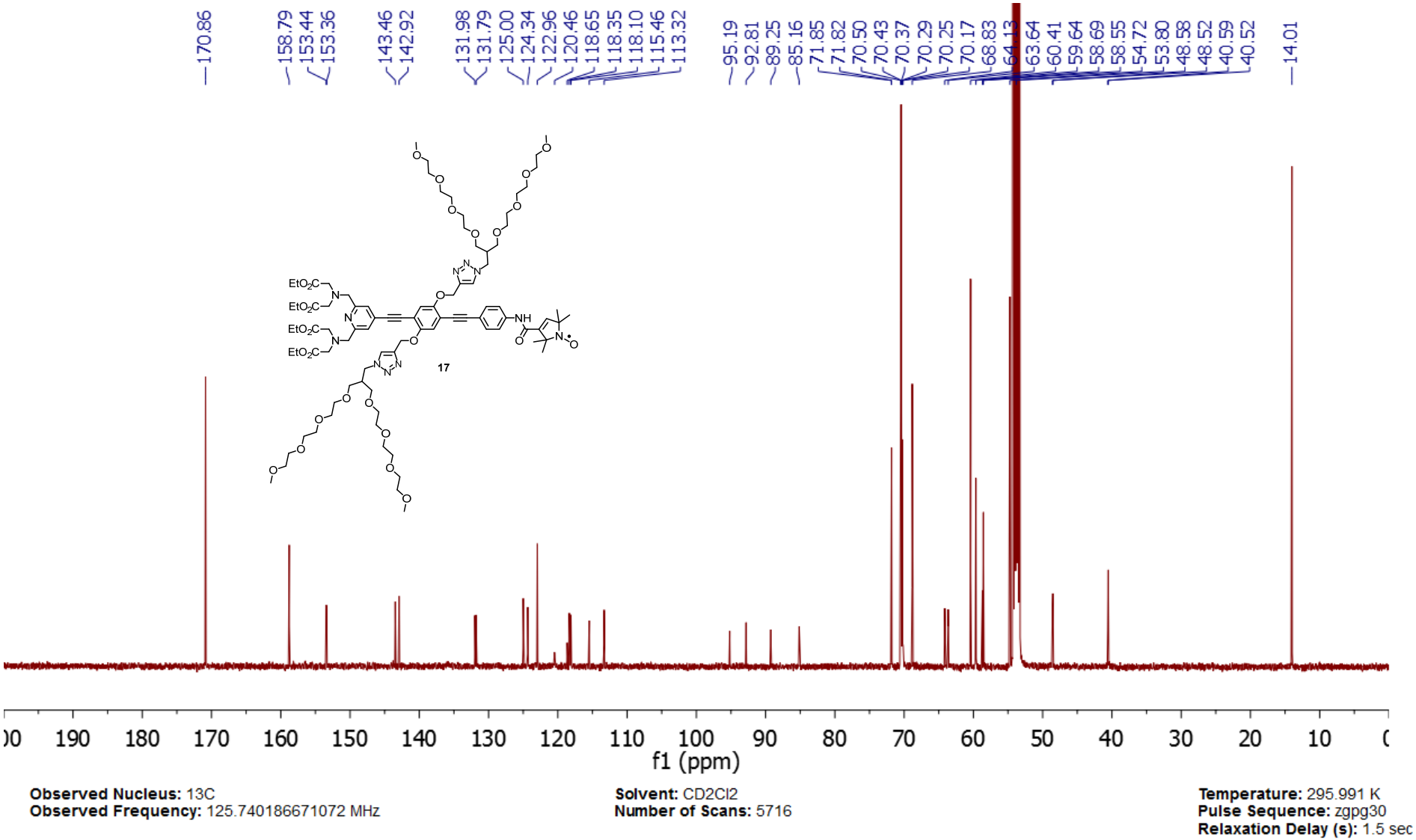

Figure S47. ^{13}C NMR spectrum of PEGylated PyMTAester-$(EP)_2$-NO• **17**.

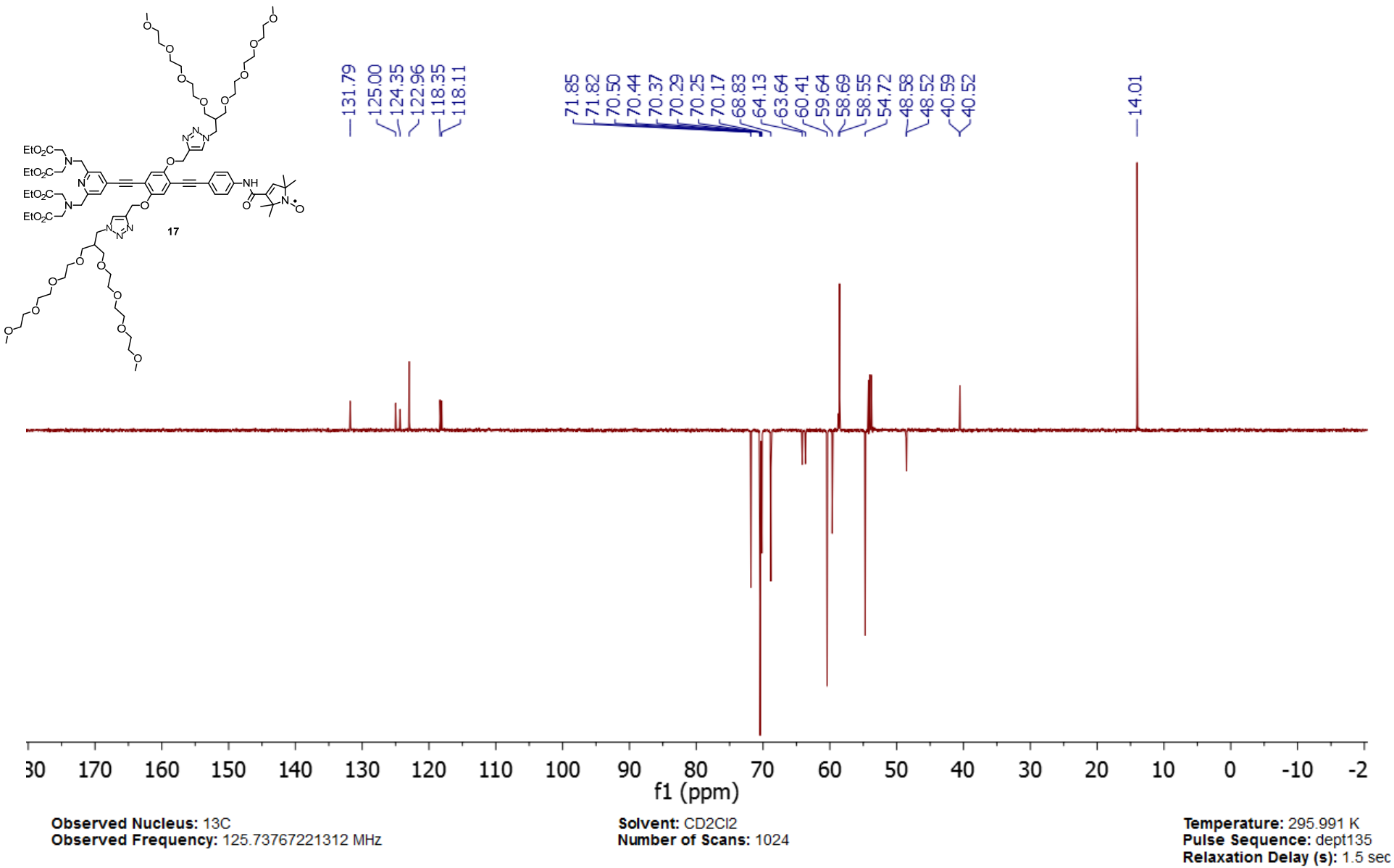

Figure S48. ^{13}C DEPT-135 NMR spectrum of PEGylated PyMTAester-(EP)$_2$-NO• **17**.

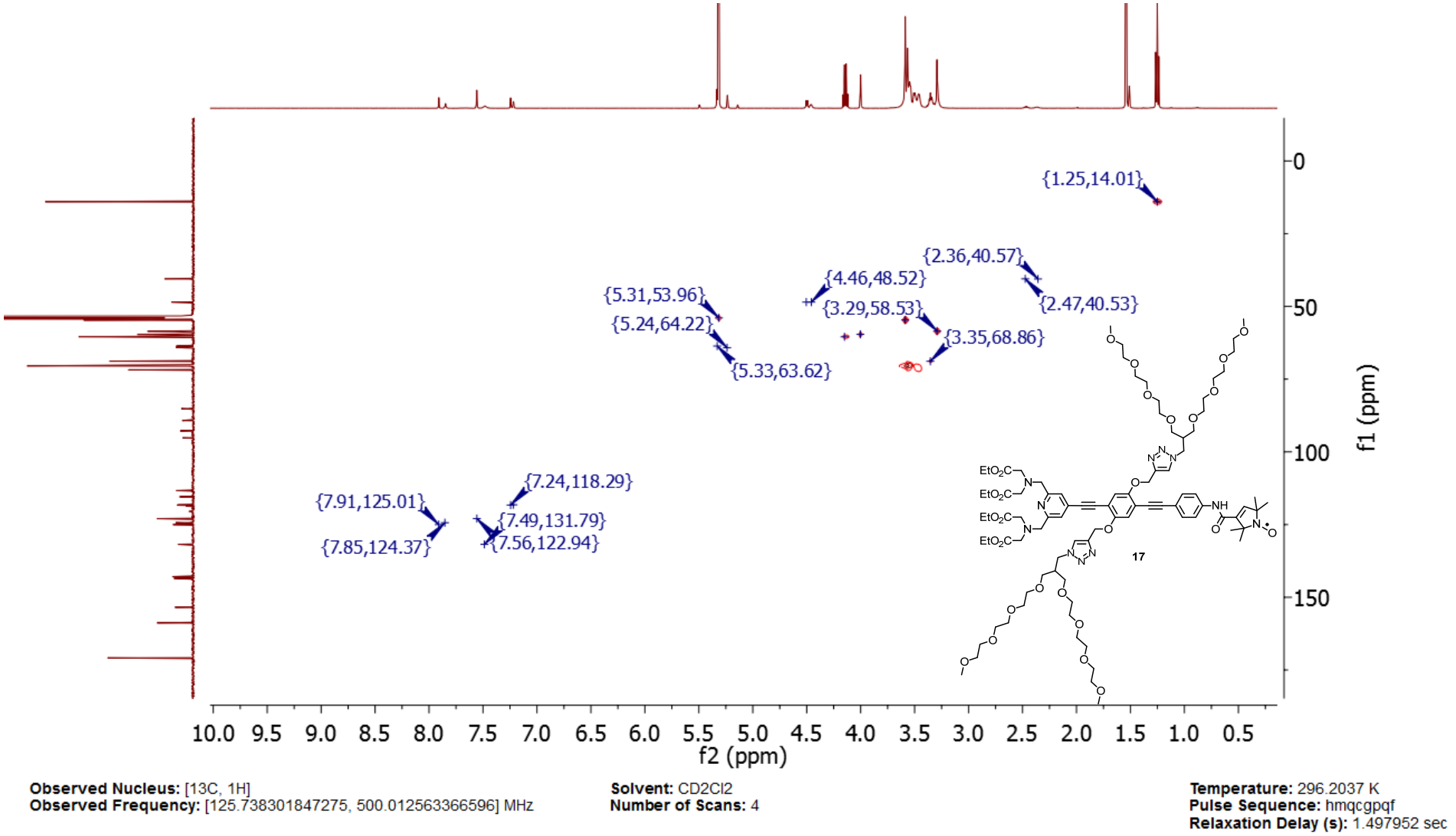

Figure S49. HMQC NMR spectrum of PEGylated PyMTAester-$(EP)_2$-NO• **17**.

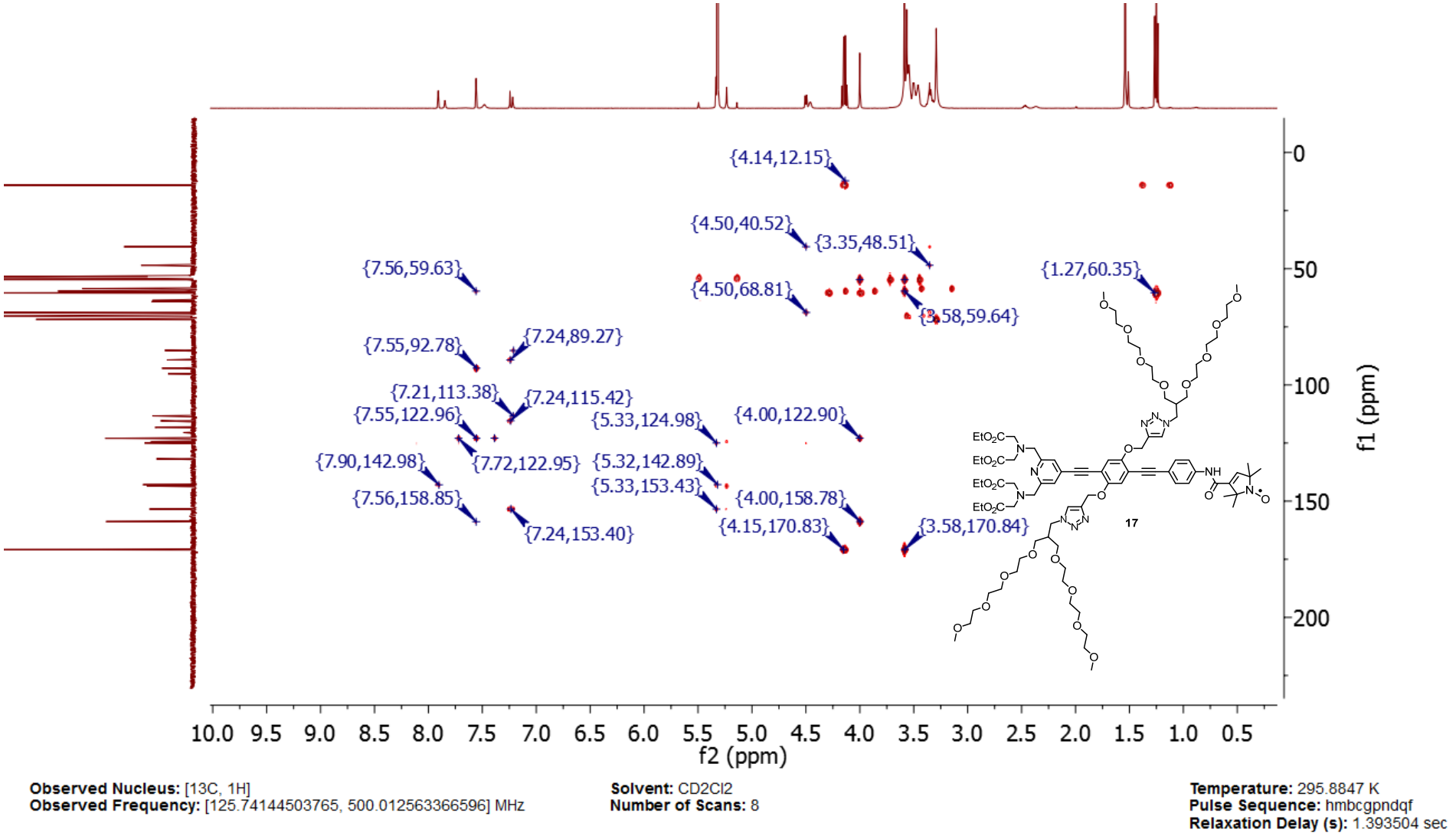

Figure S50. HMBC NMR spectrum of PEGylated PyMTAester-$(EP)_2$-NO• **17**.

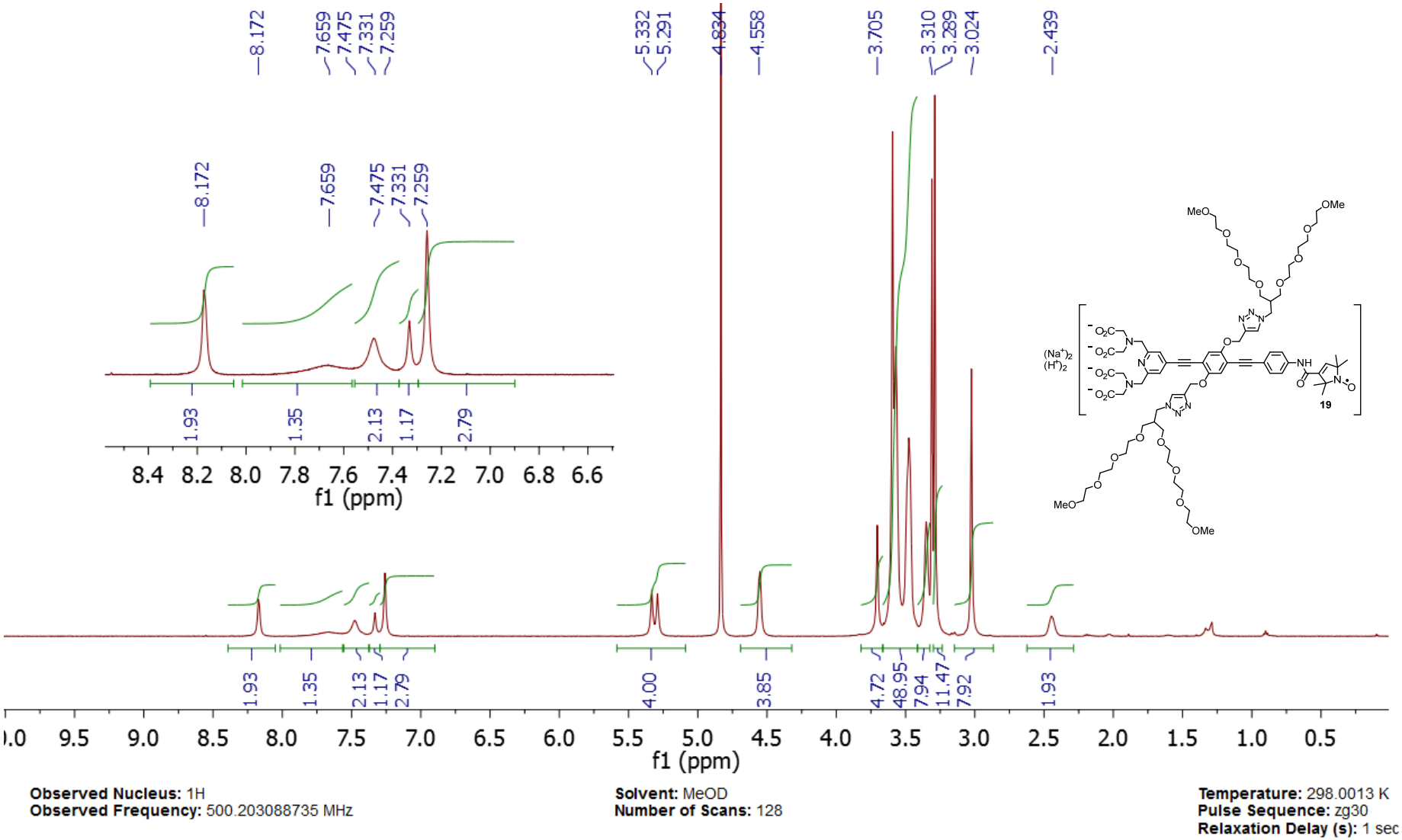

Figure S51. ^{1}H NMR spectrum of $(H^{+})_2(Na^{+})_2[PyMTA\text{-}(EP)_2\text{-}NO\bullet]^{4-}$ **19**.

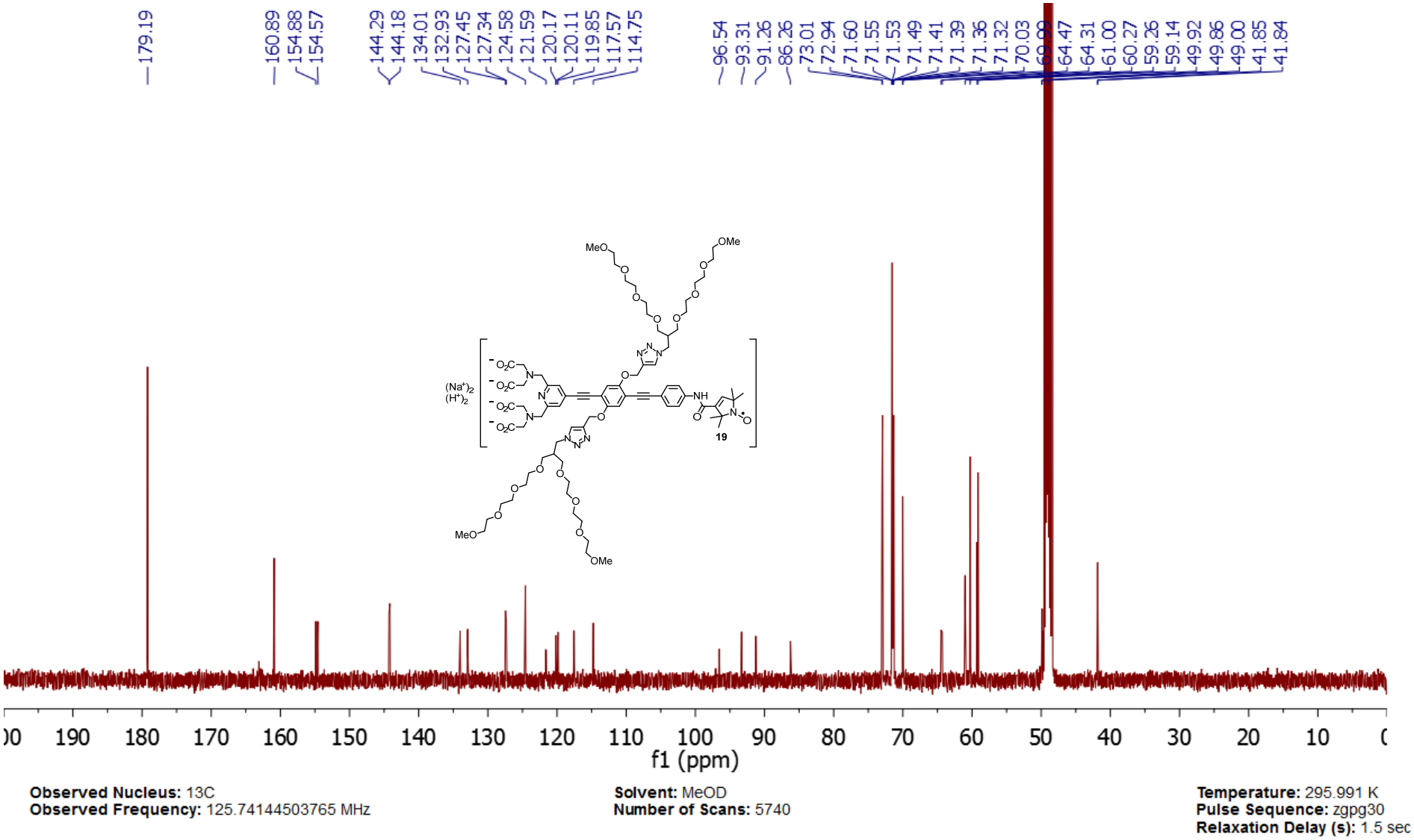

Figure S52. ^{13}C NMR spectrum of $(H^+)_2(Na^+)_2$[PyMTA-$(EP)_2$-NO•]$^{4-}$ **19**.

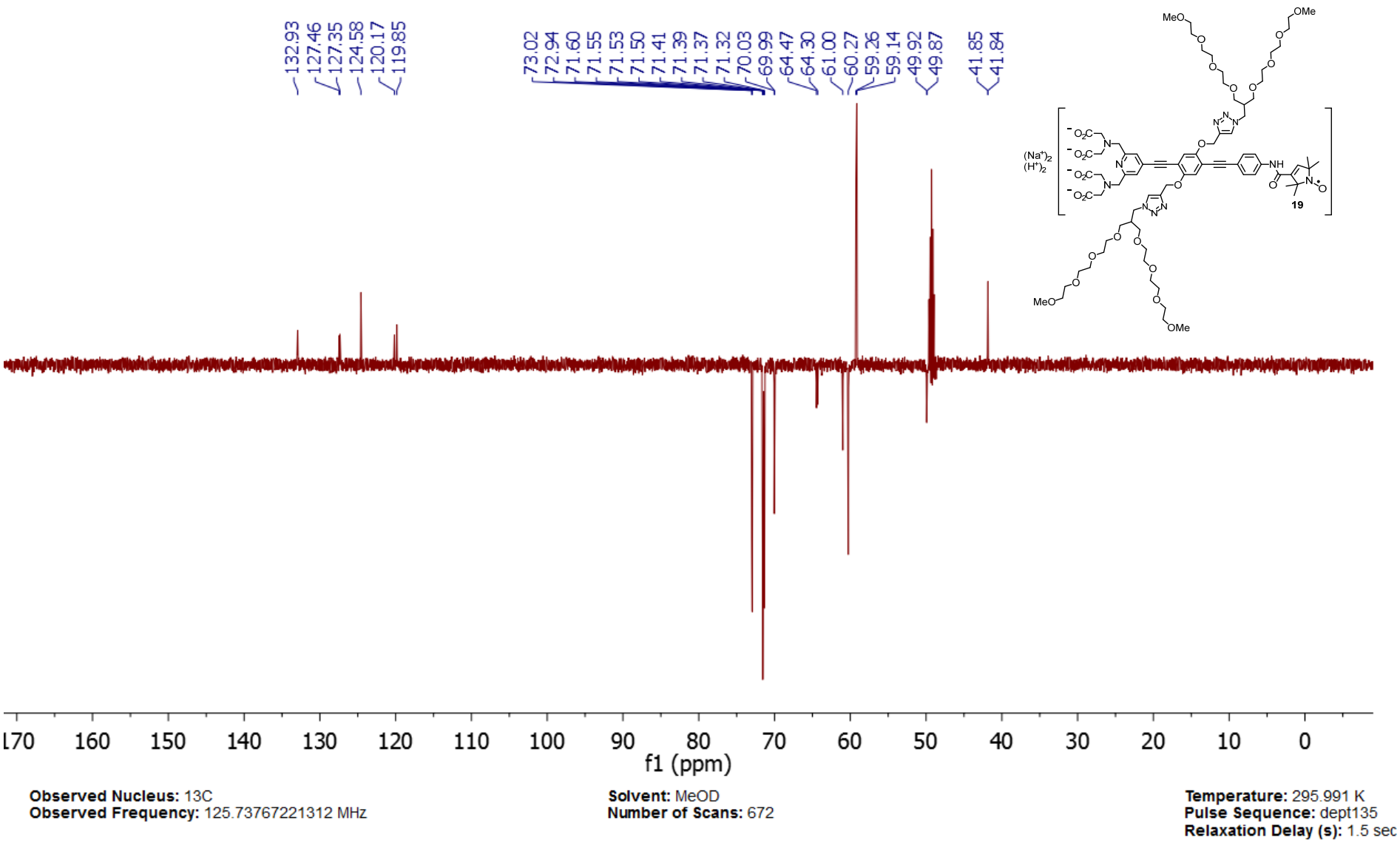

Figure S53. ^{13}C DEPT-135 NMR spectrum of $(H^+)_2(Na^+)_2$[PyMTA-$(EP)_2$-NO•]$^{4-}$ **19**.

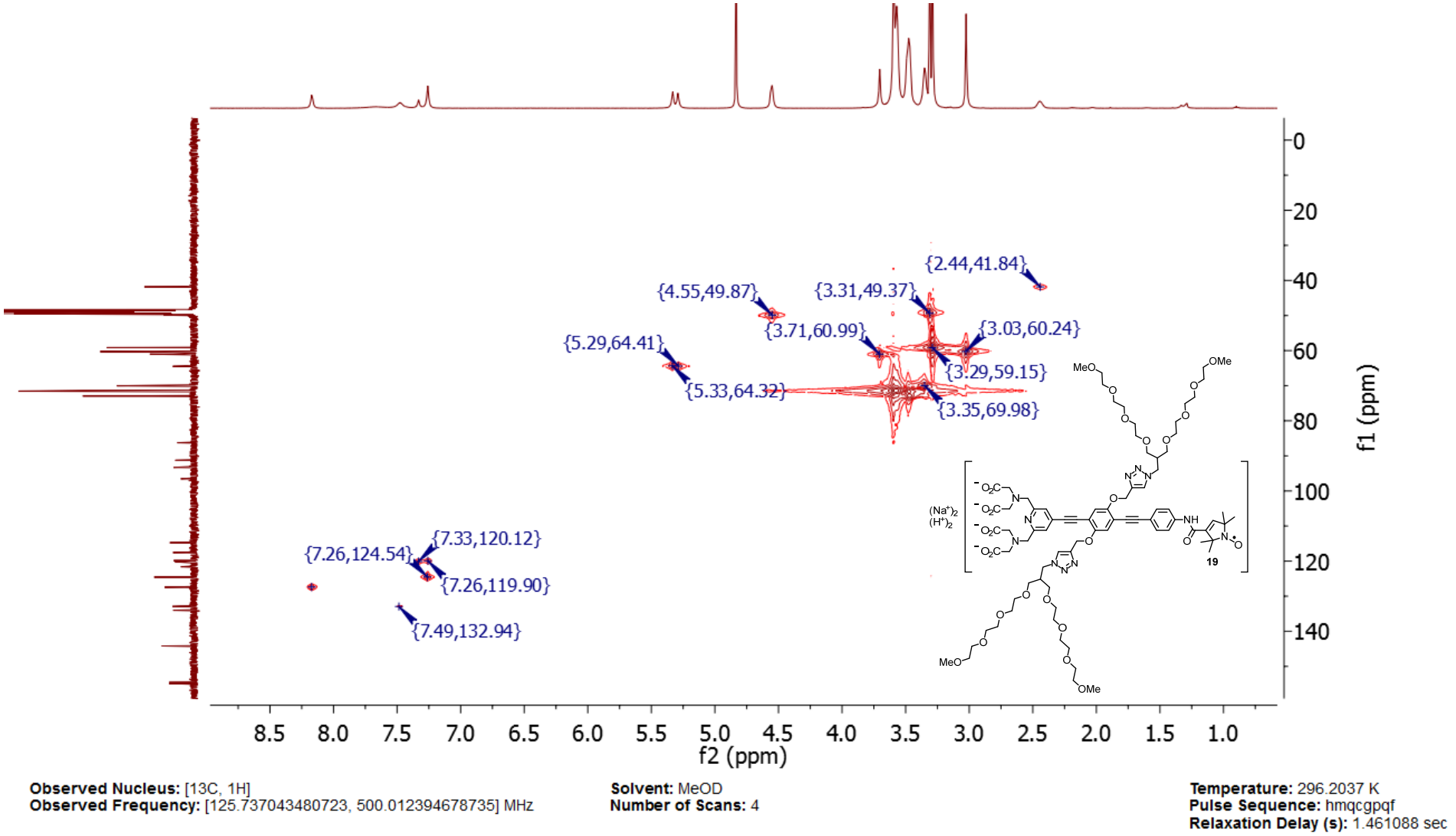

Figure S54. HMQC NMR spectrum of $(H^+)_2(Na^+)_2$[PyMTA-$(EP)_2$-NO•]$^{4-}$ **19**.

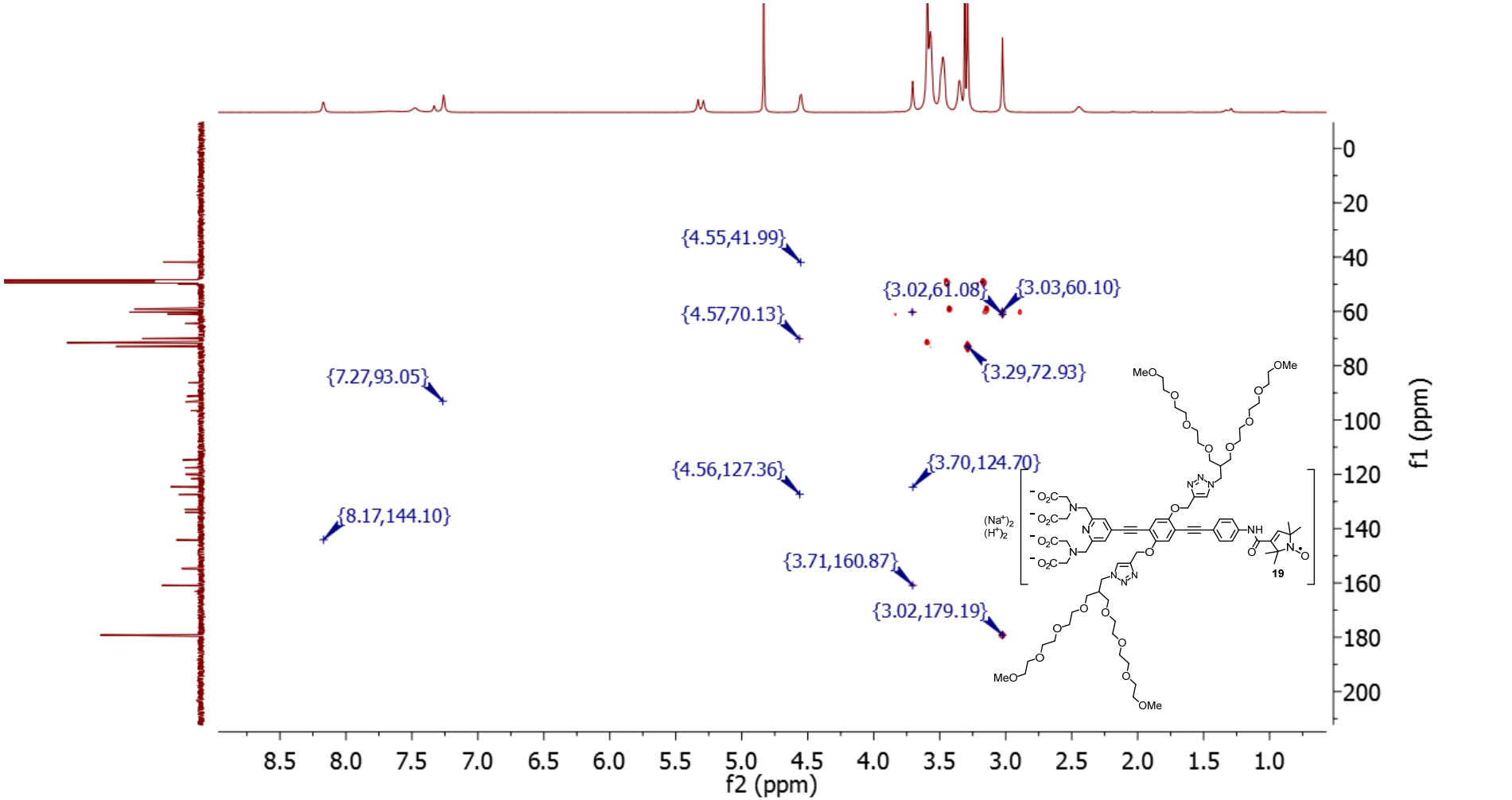

Figure S55. HMBC NMR spectrum of $(H^+)_2(Na^+)_2$[PyMTA-$(EP)_2$-NO•]$^{4-}$ **19**.