# Supporting Information Part A

# Orthogonally spin-labeled rulers help to identify crosstalk signals and improve DEER signal fidelity

Markus Teucher[1], Mian Qi[2], Ninive Cati[2], Henrik Hintz[2], Adelheid Godt[2], and Enrica Bordignon[1]

[1]Faculty of Chemistry and Biochemistry, Ruhr University Bochum, Universitätsstraße 150, 44801 Bochum, Germany
[2]Faculty of Chemistry and Center for Molecular Materials ($CM_2$), Bielefeld University, Universitätsstraße 25, 33615 Bielefeld,Germany

Correspondence: Enrica Bordignon (enrica.bordignon@rub.de)

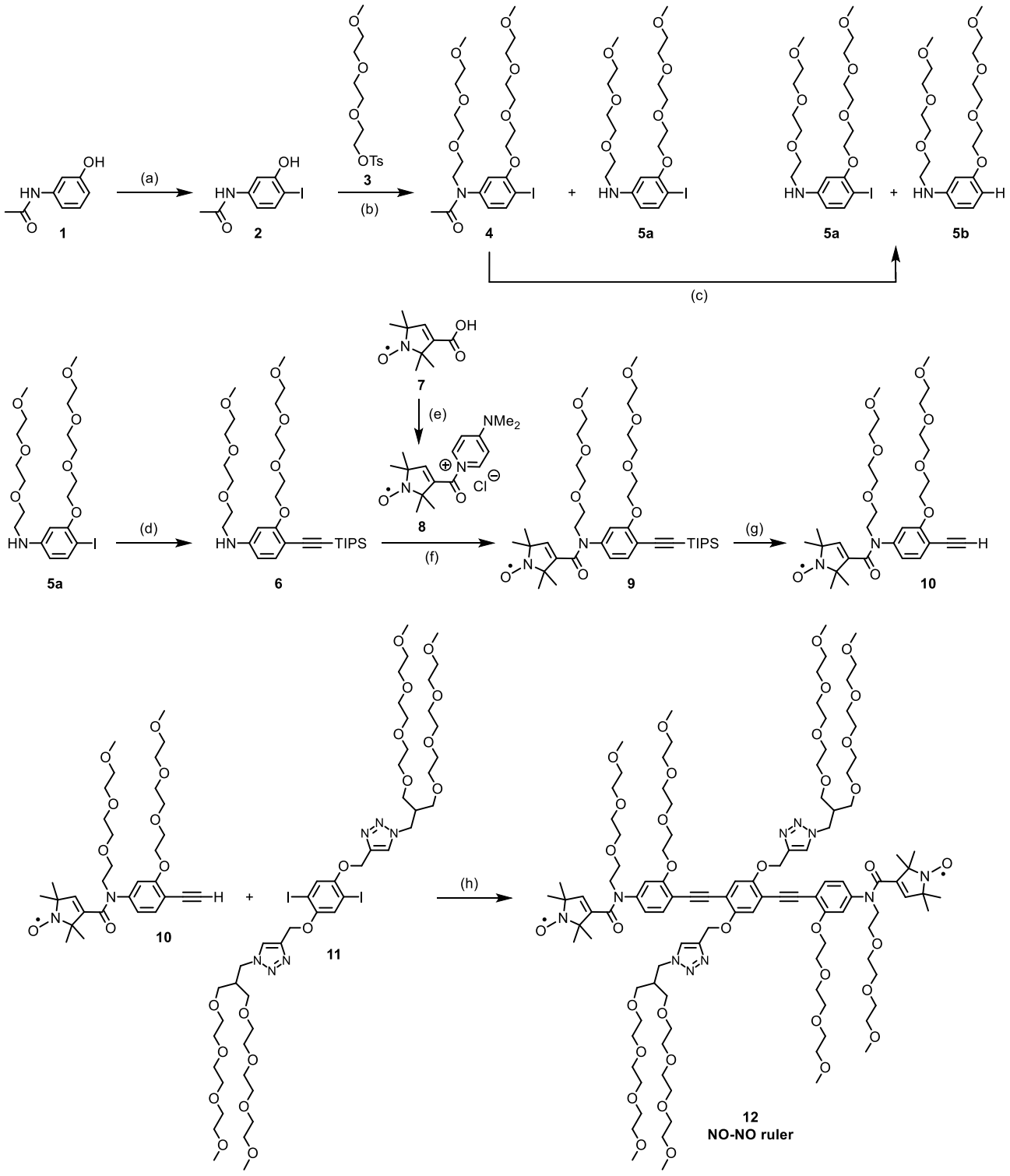
## Details of the syntheses

Contents

## Syntheses of NO-NO ruler 12

For this study, a water-soluble NO-NO ruler with a short and well-defined spin-spin distance was needed. In the past we had worked with NO-NO rulers fulfilling the requirement on the distance, but being insoluble in water.[1] To achieve water solubility, oligo(ethyleneglycol) (PEG) substituents were implemented as side chains. The same approach had been used for obtaining water soluble Gd-Gd and Gd-NO rulers.[2,3]

The synthetic route to NO-NO ruler **12** is depicted in Scheme S1. This ruler was assembled through Pd/Cu-catalyzed alkynyl aryl coupling of pegylated diiodobenzene **11**, a known building block,[2] with alkyne **10** contributing the nitroxide moiety. Both building blocks carry PEG substituents. Alkyne **10** was synthesized starting from acetaminophenol **1**. Firstly, acetaminophenol **1** was iodinated and the product furnished with PEG substituents. Unexpectedly, partial amide hydrolysis occurred during pegylation giving a mixture of amide **4** and amine **5a**. This hydrolysis is not of concern when amine **5a** is the next synthetic target. However, we had to separate the two products for their unambiguous identification. Isolated amide **4** was then converted into amine **5a**. Considering the partial amide hydrolysis during pegylation, we were surprised to find that heating to 85 $^{o}$C for more than 5 h was needed for amide hydrolysis through treatment with NaOH in aqueous methanol. The prolonged heating caused deiodination to a small extent (6%). The deiodinated amine **5b** was not removed. It did not interfere with the next synthesis step and was removed thereafter. Having amine **5a** in hand, the alkyne unit, which is relevant for the final ruler assembly, was installed via Pd/Cu-catalyzed alkynyl aryl coupling with (triisopropylsilyl)acetylene and the nitroxide moiety was attached through amide formation. Finally the triisopropyl group was removed to obtain the building block alkyne **10**.




**Scheme S1:** Synthesis of building blocks and assembly of NO-NO ruler **12**. (a) KI, $KIO_3$, $H_2SO_4$, $H_2O$, rt, 3 h, 84%; (b) NaH, THF, 60 °C, 5 d, yield of amide **4**: 59%, yield of amine **5a**: 17%; (c) NaOH, MeOH, $H_2O$, 85 °C, 46 h, yield of amine **5a**: 81%, yield of amine **5b**: 6%; (d) HC≡C-TIPS, piperidine, THF, CuI, $Pd(PPh_3)_2Cl_2$, rt, 4 d, 76%; (e) (1) DMAP, $CH_2Cl_2$, (2) thionyl chloride, 0 °C to rt, 3 h; nitroxide **8** was not isolated; (f) rt, 1 d, 95%; (g) $Bu_4NF$, THF; rt, 30 min, 100%; (h) THF, piperidine, CuI, $Pd(PPh_3)_2Cl_2$, 45 °C, 3 d, 35%. TIPS = triisopropylsilyl, DMAP = 4-*N*,*N*-dimethylaminopyridine, THF = tetrahydrofuran, rt = room temperature.

## Experimental part

### General

Unless otherwise stated, reactions were performed under ambient conditions using commercial solvents and reagents. The solvents used for extraction and chromatography were of technical grade and were distilled prior to their use. THF (HPLC grade) was dried with sodium/benzophenone.

The temperatures given for the reactions refer to the bath temperatures. Solvents were removed at a bath temperature of ca. 40 $^{o}$C and reduced pressure. The products were dried at room temperature at ca. 0.05 mbar. The pH values of the solutions were determined using pH indicator strips (resolution: 0.3 pH, Merck).

Column chromatography was carried out on silica gel 60 M (Macherey Nagel) applying slight pressure. In the procedures reported below the size of the column is given as diameter × 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 coated aluminum foil (Merck, 60 F254). The spots were detected with UV light of $\lambda$ = 254 and 366 nm. The compositions of solvent mixtures are given in volume ratios.

NMR spectra were calibrated using the solvent signal as an internal standard [$CDCl_3$: $\delta(^1H)$ = 7.26, $\delta(^{13}C\{^1H\})$ = 77.16; $CD_2Cl_2$: $\delta(^1H)$ = 5.32, $\delta(^{13}C\{^1H\})$ = 54.00; DMSO-$d_6$: $\delta(^1H)$ = 2.50, $\delta(^{13}C\{^1H\})$ = 39.52]. Signal assignments are supported by DEPT-135, COSY, HMBC and HMQC experiments.

EI mass spectra were recorded using an Autospec X magnetic sector mass spectrometer with EBE geometry (Vacuum Generators, Manchester, UK) equipped with a standard EI source. ESI mass spectra were recorded using an Esquire 3000 ion trap mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with a nano-ESI source. ESI accurate mass measurements were acquired using an Agilent 6220 time-of-flight mass spectrometer

(Agilent Technologies, Santa Clara, CA, USA) in extended dynamic range mode equipped with a Dual-ESI source or using a Q-IMS-TOF mass spectrometer Synapt G2Si (Waters GmbH, Manchester, UK) in resolution mode interfaced to a nano-ESI ion source.

The ratio of the components in a mixture was determined by $^{1}$H NMR spectroscopy and is given in a molar ratio.

## Syntheses

**2-Iodo-5-acetaminophenol (2).** The published procedure[4] was followed making small changes. A solution of KI (9.7 g, 58.6 mmol) in $H_2O$ (250 mL) and a solution of $KIO_3$ (6.1 g, 28.6 mmol) in $H_2SO_4$ (95%, 9.0 g) and $H_2O$ (250 mL) were separately but simultaneously added to a solution of 3-acetaminophenol (**1**) (13.0 g, 86.0 mmol) in aqueous $H_2SO_4$ (0.05 mol·L$^{-1}$, 1.5 L) within 2.5 h at such a rate that the addition rate of the solution of $KIO_3$ was slightly faster than the addition rate of the solution of KI. Pale yellow crystals precipitated during the addition. The suspension was stirred at room temperature for 1.5 h. The precipitate was collected by filtration, dried in a desiccator over $P_4O_{10}$ at reduced pressure for 2 d and recrystallized in acetone. 2-Iodo-5-acetaminophenol (**2**) (20.0 g, 84%) was obtained as colorless crystals. $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 10.28 (br s, 1 H, NH), 9.91 (s, 1 H, OH), 7.51 (d, $^{3}J$ = 8.5 Hz, 1 H, H *ortho* to I), 7.43 (d, $^{4}J$ = 2.3 Hz, 1 H, H *ortho* to OH), 6.75 (dd, $^{3}J$ = 8.5 Hz, $^{4}J$ = 2.3 Hz, 1 H, H *para* to OH), 2.01 (s, 3 H, $CH_3$). $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 168.4 (C=O), 156.6 ($C_{Ar}$O), 140.6 ($C_{Ar}$N), 138.3 (CH *ortho* to I), 112.0 (CH *para* to OH), 105.8 (CH *ortho* to OH), 76.4 ($C_{Ar}$I), 24.1 ($CH_3$). MS (EI, 70 eV) *m/z* (%) = 277.0 (66) $[M]^{+\bullet}$, 234.9 (100) $[M - Ac]^{+}$, 108.0 (9), 80.0 (21).

**PEG-tosylate 3.** Our procedure deviates slightly from the published one.[5] Under cooling with an ice-water-bath, tosylchloride (14.45 g, 75.8 mmol) was added to a solution of $CH_3(OCH_2CH_2)_3OH$ (10.8 g, 65.8 mmol) in pyridine (10.8 mL, 131 mmol). The pale-yellow

solution was stirred at 0 °C for 3.5 h and afterwards aqueous HCl (10%, 120 mL) was added. The solution was extracted with toluene (3 x 75 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. Column chromatography (6.5 cm x 43 cm $CH_2Cl_2$/$Et_2O$, 1:1) of the residual colorless oil (21.2 g) gave two fractions. The first fraction contained only PEG-tosylate **3** (0.53 g; $R_f$ = 0.40). The second fraction (18.0 g; $R_f$ = 0.40 and 0.42) was a mixture of PEG-tosylate **3** (17.68 g) and tosyl chloride (0.32 g). The tosyl chloride in this fraction was removed easily applying the reported procedure:[6] The second fraction was dissolved in $CH_2Cl_2$ (80 mL) and pyridine (2.80 mL, 34.5 mmol). Chopped filter paper (2.80 g) was added to the solution and the suspension was treated in an ultrasound-bath for 1 h. Then the suspension was filtered through filter paper, the filter cake was washed with $CH_2Cl_2$ (100 mL) and the combined filtrates were washed with aqueous HCl (1 M, 50 mL). The layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (25 mL). The combined organic layers were dried over magnesium sulfate. After removal of the solvents the residue was combined with the above mentioned first chromatographic fraction. PEG-tosylate **3** (18.2 g, 83 %) was obtained as a colourless oil. $^1$H NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.80 (AA'-part of a AA'XX' spin system, 2H, H *ortho* to $SO_3$), 7.34 (XX'-part of the AA'XX' spin system, 2 H, H *ortho* to Me), 4.16 (m, 2 H, TsO-C$\underline{H}_2$CH$_2$), 3.69 (m, 2 H, TsO-CH$_2$C$\underline{H}_2$), 3.60 (m, 6 H, TsO-CH$_2$CH$_2$O-C$\underline{H}_2$C$\underline{H}_2$O-C$\underline{H}_2$CH$_2$OCH$_3$), 3.53 (m, 2 H, TsO-(CH$_2$CH$_2$O)$_2$-CH$_2$C$\underline{H}_2$O-CH$_3$), 3.37 (s, 3 H, OCH$_3$), 2.45 (s, 3 H, ArC$\underline{H}_3$). The shifts of the aromatic protons is significantly different from those reported for this compound.[5] However, the shifts that we determined fit well to the shifts reported in the same reference for PEG-tosylates with other lengths of the PEG chain.

**Pegylated amide 4.** This reaction was performed in dried glassware under argon using the Schlenk technique. NaH (60 wt% dispersion in mineral oil, 735 mg, 18.4 mmol) was suspended in dry THF (20 mL). 2-Iod-5-acetaminophenol (**2**) (2.00 g, 7.23 mmol) was added slowly in portions (caution: strong gas development and foaming). The milky viscous

suspension was stirred at room temperature for 5 min. PEG-tosylate **3** (4.96 g, 15.57 mmol) was added, whereupon the color of the suspension turned from white to slight yellow. Dry THF (10 mL) was added to reduce the viscosity of the suspension, whereupon the suspension took on a beige color. The suspension was stirred at 60 °C for 70 h. Under cooling with an ice-water bath, aqueous HCl (1 M, 1 mL) and $CH_2Cl_2$ (20 mL) were added. Additional aqueous HCl (1 M, 2.5 mL) was added to dissolve the precipitate. Water (15 mL) was added, the organic phase was separated, and the aqueous phase was extracted with $CH_2Cl_2$ (3 x 20 mL). The combined organic phases were washed with saturated aqueous $Na_2CO_3$ solution (20 mL) and then with water (20 mL), dried over $Na_2SO_4$, and filtered. The solvents of the filtrate were removed. Chromatography (5 cm x 52 cm, $CH_2Cl_2$/EtOH, 20:1) of the residual brown oil gave pegylated amide **4** (2.42 g, 59%; $R_f$ = 0.30) as a pale yellow oil and pegylated amine **5a** (0.65 g, 17%; $R_f$ = 0.42) as a green oil.

Analytical data of pegylated amide **4**: $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ [ppm] = 7.77 (d, $^3J$ = 8.2 Hz, 1H, H *ortho* to I), 6.74 (d, $^4J$ = 1.5 Hz, 1H, H *ortho* to O), 6.64 (dd, $^3J$ = 8.2 Hz, $^4J$ = 1.5 Hz, 1H, H *para* to O), 4.15 and 3.93 (2 m, 2 H each, $CH_2$), 3.82 and 3.69 (2 m, 4 H each, $CH_2$ of PEG), 3.59 (m, 10 H, $CH_2$ of PEG), 3.52 (m, 2 H, $CH_2$ of PEG), 3.38 and 3.36 (2 s, 3 H each, $OCH_3$), 1.86 (s, 3 H, C(=O)$CH_3$). $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ [ppm] = 170.2 (C=O), 158.3 ($C_{Ar}$O), 144.8 ($C_{Ar}$N), 139.8 (CH *ortho* to I), 122.4 (CH *para* to O), 112.4 (CH *ortho* to O), 85.5 ($C_{Ar}$-I), 71.88, 71.86, 71.2, 70.7, 70.5, 70.43, 70.41, 70.0, 69.4, 69.3 and 68.1 ($CH_2$ of PEG-chain), 58.94 and 58.05 ($OCH_3$), 22.7 (C(=O)$\underline{C}H_3$). MS (ESI): *m/z* = 592.2 $[M + Na]^+$.

Analytical data of pegylated amine **5a**: $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ [ppm] = 7.44 (d, $^3J$ = 8.5 Hz, 1 H, H *ortho* to I), 6.16 (d, $^4J$ = 2.3 Hz, 1 H, H *ortho* to O), 6.05 (dd, $^3J$ = 8.5 Hz, $^4J$ = 2.3 Hz, 1 H, H *para* to O), 4.25 (br s, 1 H, NH), 4.11, 3.90 und 3.82 (3 m, 2 H each, $CH_2$ of

PEG), 3.66 (m, 12 H, $CH_2$ of PEG), 3.55 (m, 4 H, $CH_2$ of PEG), 3.381 and 3.376 (2 s, 3 H each, $OCH_3$), 3.25 (m, 2 H, $NCH_2$). MS (ESI): *m/z* = 550.2 $[M + Na]^+$.

**Pegylated amine 5a.** Pegylated amide **4** (2.42 g, 4.26 mmol) was dissolved in methanol (15 mL), water (15 mL) and aqueous NaOH solution (2 M, 10 mL). The solution was stirred at 85 °C for 5 h. TLC showed an incomplete conversion. NaOH (0.669 g, 16.73 mmol) and methanol (8 mL) were added. The solution was stirred at 85 °C for 41 h. The brown solution was cooled to room temperature and extracted with $CH_2Cl_2$ (3 x 15 mL), the organic phase was dried over $Na_2SO_4$, and the suspension was filtered. The solvents of the filtrate were removed. Chromatography (3.5 cm x 53 cm, $CH_2Cl_2$/EtOH, 20:1) of the residual orange-brown oil (2.08 g) gave a mixture (1.88 g) of pegylated amine **5a** (1.80 g, yield: 81%) and the corresponding deiodinated amine **5b** (83 mg, yield: 6%) as a green oil. This material was used for the next reaction without further treatment. Analytical data of amine **5a** are given above.

**TIPS-protected alkyne 6.** This reaction was performed under argon using the Schlenk technique. Pegylated amine **5a** (194 mg, 367 µmol), piperidine (0.38 mL, 3.84 mmol), and TIPS-acetylene (123 µL, 550 µmol) was dissolved in THF (6 mL). The solution was degassed through three freeze-pump-thaw cycles. CuI (4.41 mg, 21.2 µmol) and $Pd(PPh_3)_2Cl_2$ (2.13 mg, 3.04 µmol) were added and the pale yellow reaction solution was stirred at room temperature for 96 h, during which a precipitate formed. Water (5 mL) and $CH_2Cl_2$ (10 mL) were added. Water (10 mL) was added and the phases were separated. The aqueous phase was extracted with $CH_2Cl_2$ (3 x 10 mL). The combined organic phases were washed with water (2 x 10 mL), dried over $Na_2SO_4$ and filtered. The solvents were removed. Chromatography (3 cm x 17 cm, $CH_2Cl_2$/EtOH, 15:1, a few drops of $Et_3N$ were added to the eluent at the beginning of the elution) of the residual yellow oil gave TIPS-protected alkyne **6** (163 mg, 76%; $R_f$ = 0.41) as a brown oil. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$

[ppm] = 7.22 (d, $^{3}J$ = 8.3 Hz, 1 H, H *ortho* to C≡CTIPS), 6.14 (dd, $^{3}J$ = 8.6 Hz, $^{4}J$ = 1.7 Hz, 1 H, H *para* to O), 6.09 (d, $^{4}J$ = 1.5 Hz, 1 H, H *ortho* to O), 4.34 (s, 1 H, NH), 4.13, 3.85, 3.75, and 3.69 (4 m, 2 H each, $CH_2$ of PEG), 3.66 (m, 10 H, $CH_2$ of PEG), 3.56 (m, 4 H, $CH_2$ of PEG), 3.38 and 3.37 (2 s, 3 H each, $OCH_3$), 3.28 (m, 2 H, $CH_2N$), 1.11 (s, 21 H, $CH(CH_3)_2$).

**Nitroxide 9.** This reaction was performed in dried glassware under argon using the Schlenk technique. Under cooling with an ice-water bath, thionyl chloride (40.8 µL, 561 µmol) was added to a yellow solution of 2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl-3-carboxylic acid (**7**) (112 mg, 606 µmol) and DMAP (178 mg, 1.46 mmol) in dry $CH_2Cl_2$ (7 mL), which made the color of the solution to change from yellow to red. The ice-water bath was removed and the red solution was stirred at room temperature for 3 h. A solution of TIPS-protected alkyne **6** (149.5 mg, 0.257 mmol) in $CH_2Cl_2$ (3 mL) was added. The solution was stirred at room temperature for 1 d. During this time the color of the solution changed to yellow. The solution was filtered through silica gel (1.7 cm x 2 cm, rinsing with $CH_2Cl_2$/EtOH, 15:1). The filtrate was washed with aqueous HCl (1 M, 8 mL), saturated aqueous $Na_2CO_3$ solution (10 mL), and water (10 mL), dried over $Na_2SO_4$, and filtered. The solvents of the filtrate were removed. Chromatography (3 cm x 10 cm, $CH_2Cl_2$/EtOH 20:1) of the residue gave nitroxide **9** (183 mg, 95%; $R_f$ = 0.22) as a pale-yellow oil. $^{1}$H NMR (500 MHz, $CD_2Cl_2$): All signals are broad and featureless and integrals in the low field region come with a large error. $\delta$ [ppm] = 7.43 (very br s, 1 H, H *ortho* to C≡C-TIPS), 6.85 (very br s, 1.7 H, both H *meta* to C≡C-TIPS), 4.18 (br s, 3.6 H, 2 $CH_2$ of PEG), 3.88 (br s, 2 H, $CH_2$ of PEG), 3.71 - 3.53 (m, 18 H, $CH_2$ of PEG), 3.37 (br s, 6 H, $OCH_3$), 1.17 (s, 21 H, $C\underline{H}(C\underline{H}_3)_2$). $^{13}$C NMR (126 MHz, $CD_2Cl_2$) $\delta$ [ppm] = 159.1 ($C_{Ar}O$), 143.8 (br, $C_{Ar}N$), 132.4 ($C_{Ar}H$ *ortho* to C≡C-TIPS), 119.7 ($C_{Ar}H$ *para* to O), 111.3 and 110.8 ($C_{Ar}H$ *ortho* to O and $\underline{C}_{Ar}$-C≡C-TIPS), 100.8 ($\underline{C}$≡C-TIPS), 94.7 (C≡$\underline{C}$-TIPS), 71.12, 71.06, 69.94, 69.62, 69.60, 69.48, 69.45, 69.23, 68.49, 68.09, and 67.55 ($CH_2$ of PEG), 57.82 and 57.78 ($OCH_3$), 46.7 ($NCH_2$), 17.4 ($CH(\underline{C}H_3)_2$), 10.2 ($\underline{C}H(CH_3)_2$). MS (ESI)

*m/z* = 770.6 $[M + Na]^+$, 748.6 $[M + H]^+$. Accurate MS (ESI) calcd. for $[M + Na]^+$ $C_{40}H_{67}N_2O_9SiNa^+$: 770.4508; found: 770.4498.

**Alkyne 10.** A solution of $Bu_4NF$ in THF (1 M, 0.3 mL, 0.3 mmol) was added to a solution of nitroxide **9** (185.6 mg, 0.248 mmol) in THF (6 mL). The solution was stirred at room temperature for 30 min. The solution was filtered through silica gel (1.7 cm x 2 cm, rinsing with $CH_2Cl_2$/EtOH, 15:1). After removal of the solvents, a mixture (167 mg) of alkyne **10** and TIPS-X (X most probably being F) and $Bu_4NY$ (Y = F, OH) was obtained. This mixture was used for the next reaction without further purification. $^1$H NMR (500 MHz, $CD_2Cl_2$): All signals are broad and featureless and integrals in the low field region come with a large error. *δ* [ppm] = 7.43 (br s, 0.9 H, H *ortho* to C≡C-H), 6.83 (br s, 1.8 H, both H *meta* to C≡C-H), 4.14 (br s, 3.6 H, 2 $CH_2$ of PEG), 3.9 (br s, 2 H, $CH_2$ of PEG), 3.72 - 3.50 (m, 16 H, $CH_2$ of PEG), 3.34 and 3.32 (2 br s, 5.5 H, 2 $OCH_3$ and C≡C-H), 3.26 (m, 0.8 H, $NCH_2$).

**NO-NO ruler 12.** This reaction was performed under argon using the Schlenk technique. The material obtained through desilylation of nitroxide **9** (109.4 mg, ca. 0.185 mmol of alkyne **10**) and diiodo benzene **11**[2] (101.2 mg, 78.79 μmol) were dissolved in piperidine (90 μL, 0.9 mmol) and THF (6 mL). The solution was degassed through three freeze-pump-thaw cycles. CuI (1.29 mg, 6.77 μmol) and $Pd(PPh_3)_2Cl_2$ (1.98 mg, 2.82 mol) were added and the pale yellow solution was stirred at 45 °C for 41.5 h. TLC ($CH_2Cl_2$/EtOH, 15:1; $R_f$ of nitroxide **10** = 0.55; $R_f$ of diiodo benzene **11** = 0.33; $R_f$ of NO-NO ruler **12** = 0.10) of the reaction mixture (a brown suspension) indicated an incomplete conversion. Piperidine (500 μL, 5 mmol), CuI (3.15 mg, 16.51 μmol) and $Pd(PPh_3)_4$ (3.64 mg, 3.15 μmol) were added. The solution was stirred at 45 °C for 1 d. Water (15 mL) and $CH_2Cl_2$ (15 mL) were added and the phases were separated. The aqueous phase was extracted with $CH_2Cl_2$ (3 x 15 mL). The combined organic phases were washed with aqueous HCl (1 M, 2 x 10 mL) and water (10 mL), dried over $Na_2SO_4$, and filtered. The solvents of the filtrate were removed. Preparative

HPLC on a Silica(2) column (5 µm, 100 Å, 21.2 mm × 250 mm, Luna® Phenomenex) applying an isocratic elution ($CH_2Cl_2$/EtOH, 93:7) with a flow rate of 15 mL/min at room temperature and UV detection at 254 nm gave NO-NO ruler **12** (61.7 mg, 35%; retention time: 7.8 – 9.5 min) as a yellow oil. $^1$H NMR (500 MHz, $CD_2Cl_2$): All signals are broad and featureless and integrals in the low field region come with a large error. δ [ppm] = 7.90 (br s, 1.8 H, 2 triazole-H), 7.49 (very br s, 2 H, H *meta* to N), 7.23 (br s, 2 H, H *ortho* to $OCH_2$-triazole), 6.96 (very br s, 4 H, H *ortho* to N), 5.31 (br s, 4 H, $C_{triazole}$-$CH_2$), 4.49 (br s, 4 H, $N_{triazole}$-$CH_2$), 4.20 (br s, 6.2 H, 4 $CH_2$ of PEG), 3.90 bis 3.28 (br s, 102 H, 42 $CH_2$ and 8 $CH_3$ of PEG), 2.50 (br s, 2 H, $N_{triazole}$-$CH_2C\underline{H}$). $^{13}$C NMR (126 MHz, $CD_2Cl_2$) *δ* [ppm] = 158.9 ($C_{benzene}O$ *ortho* to N), 152.4 ($\underline{C}_{benzene}OCH_2$-triazole), 144.5 (br, $C_{benzene}N$), 142.2 ($\underline{C}_{triazole}CH_2$), 132.4 (br, $C_{benzene}H$ *meta* to O), 124.2 ($C_{triazole}H$), 120.3 (br, $C_{benzene}H$ *para* to O), 117.9 ($C_{benzene}H$ *para* to $OCH_2$-triazole), 113.8 ($\underline{C}_{benzene}C\equiv C$-benzene-N), 111.9 and 111.5 ($C_{benzene}$ *para* to N and $C_{benzene}H$ *ortho* to N and *ortho* to O), 89.97 and 89.63 ($\underline{C}\equiv\underline{C}$), 71.45, 71.37, 71.35, 70.38, 70.19, 70.07, 70.02, 69.85, 69.79, 69.76, 69.54, 68.77, 68.52, and 67.81 ($CH_2$ of PEG), 62.8 ($C_{triazole}$-$\underline{C}H_2$), 58.21, 58.16, and 58.09 ($OCH_3$ of PEG), 48.6 ($N_{triazole}$-$\underline{C}H_2$), 47.0 (br, $\underline{C}H_2NC=O$), 39.97 ($N_{triazole}$-$CH_2\underline{C}H$). MS (ESI) *m/z* = 2235.4 [M + Na + H]$^{2+}$. Accurate MS (ESI) calcd. for [M + 2Na]$^{2+}$ $C_{110}H_{174}N_{10}O_{36}Na_2^{2+}$: 1128.59384; found: 1128.5950.

## References


[1] G. Jeschke, M. Sajid, M. Schulte, N. Ramezanian, A. Volkov, H. Zimmermann, A. Godt, *J. Am. Chem. Soc.* **2010**, *132*, 10107.

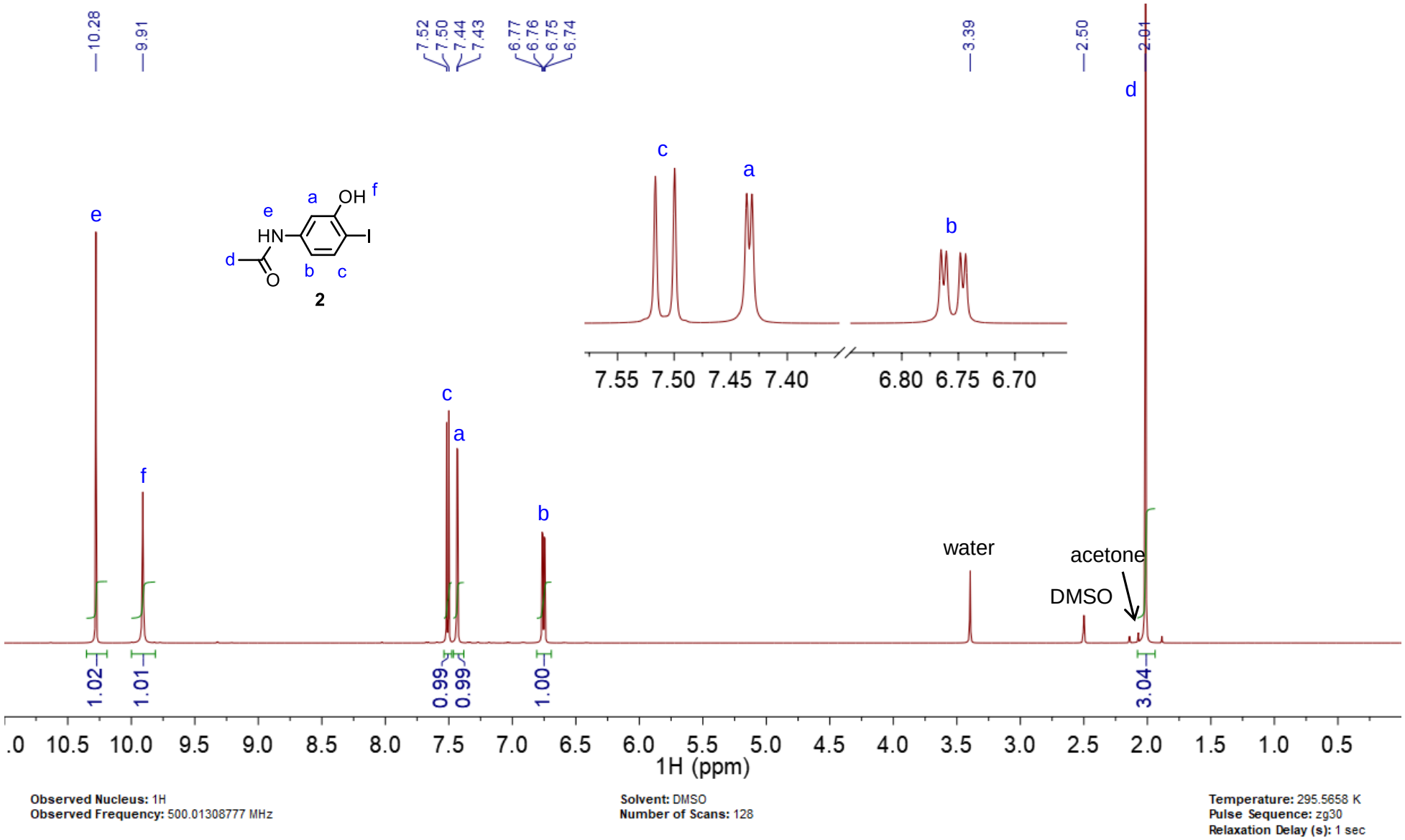
[2] M. Qi, M. Hülsmann, A. Godt, *J. Org. Chem.* **2016**, *81*, 2549.

[3] I. Ritsch, H. Hintz, G. Jeschke, A. Godt, M. Yulikov, *Phys. Chem. Chem. Phys.* **2019**, *21*, 9810.

[4] H. Wunderer, *Arch. Pharm. Pharm. Med. Chem.* **1973**, *306*, 371.

[5] R. Heathcote, J. A. S. Howell, N. Jennings, D. Cartlidge, L. Cobden, S. Coles, M. Hursthouse, *Dalton Trans.* **2007**, 1309.

[6] D. V. Schoonover, H. W. Gibson, *Tetrahedron Lett.* **2017**, *58*, 242.




**2**

**Figure S1.** $^{1}$H NMR spectrum (500 MHz, DMSO-$d_6$) of 2-iodo-5-acetaminophenol (**2**).

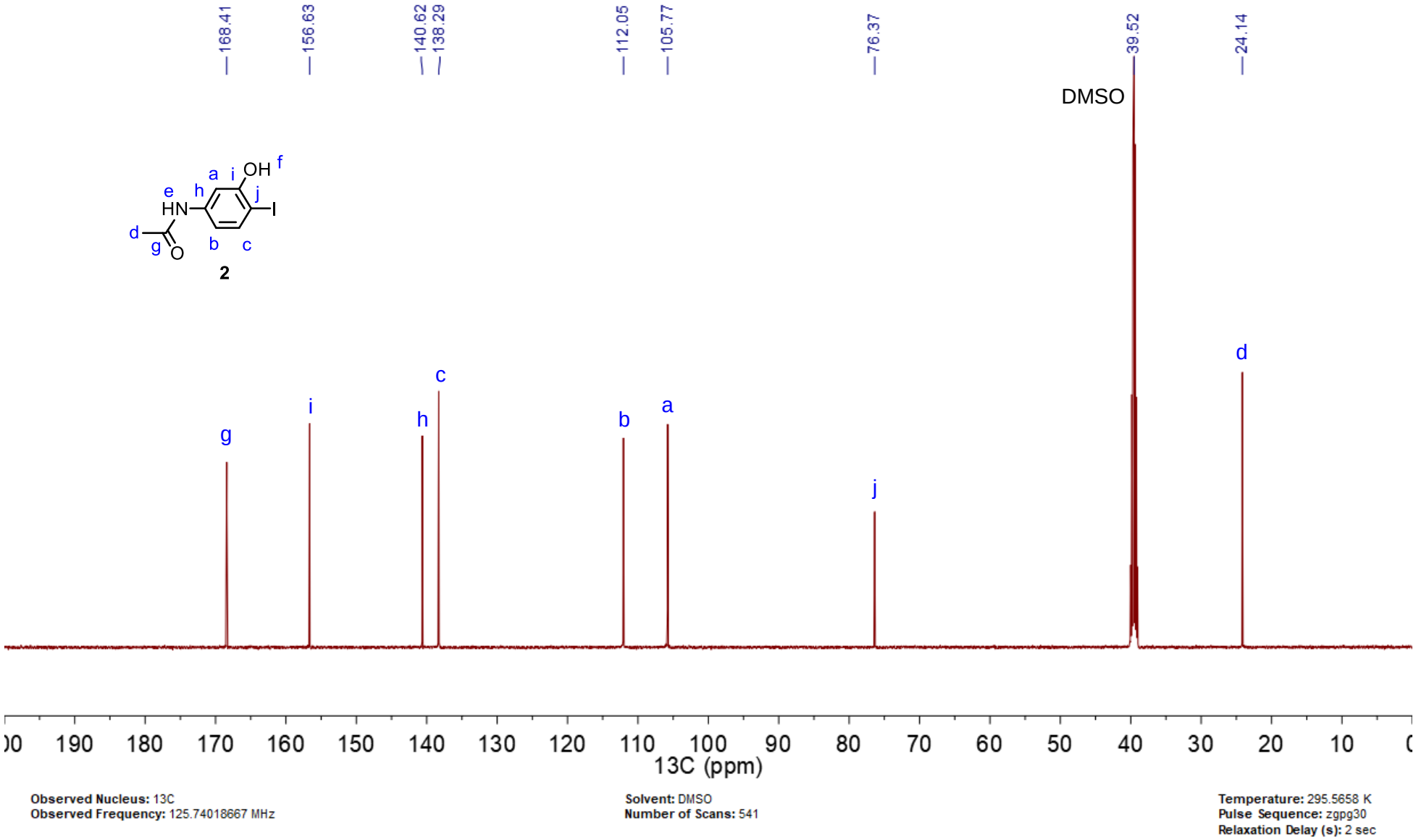



**Figure S2.** $^{13}C$ NMR spectrum (126 MHz, DMSO-$d_6$) of 2-iodo-5-acetaminophenol (**2**).

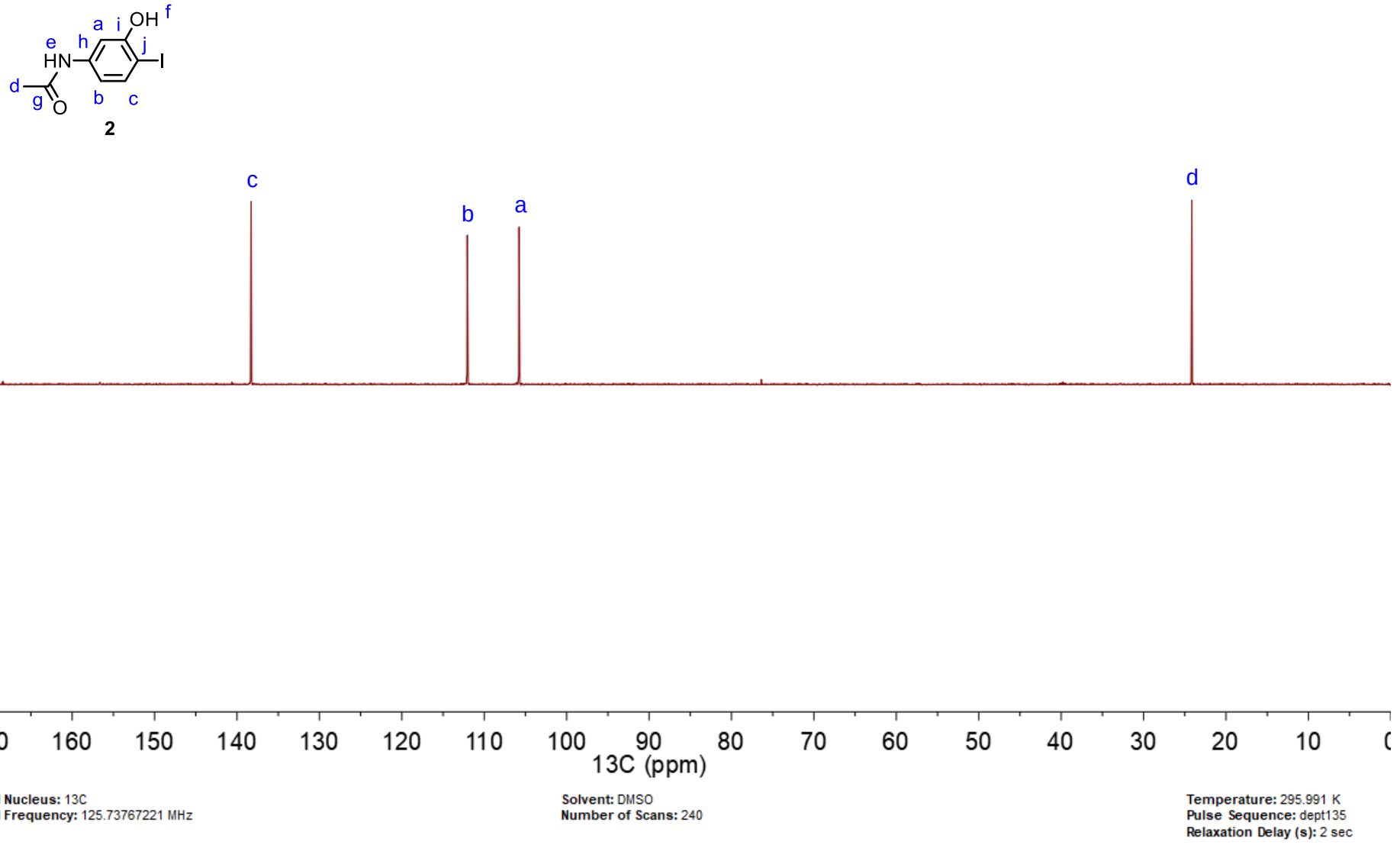



**Figure S3.** $^{13}C$ DEPT 135 NMR spectrum (126 MHz, DMSO-$d_6$) of 2-iodo-5-acetaminophenol (**2**).

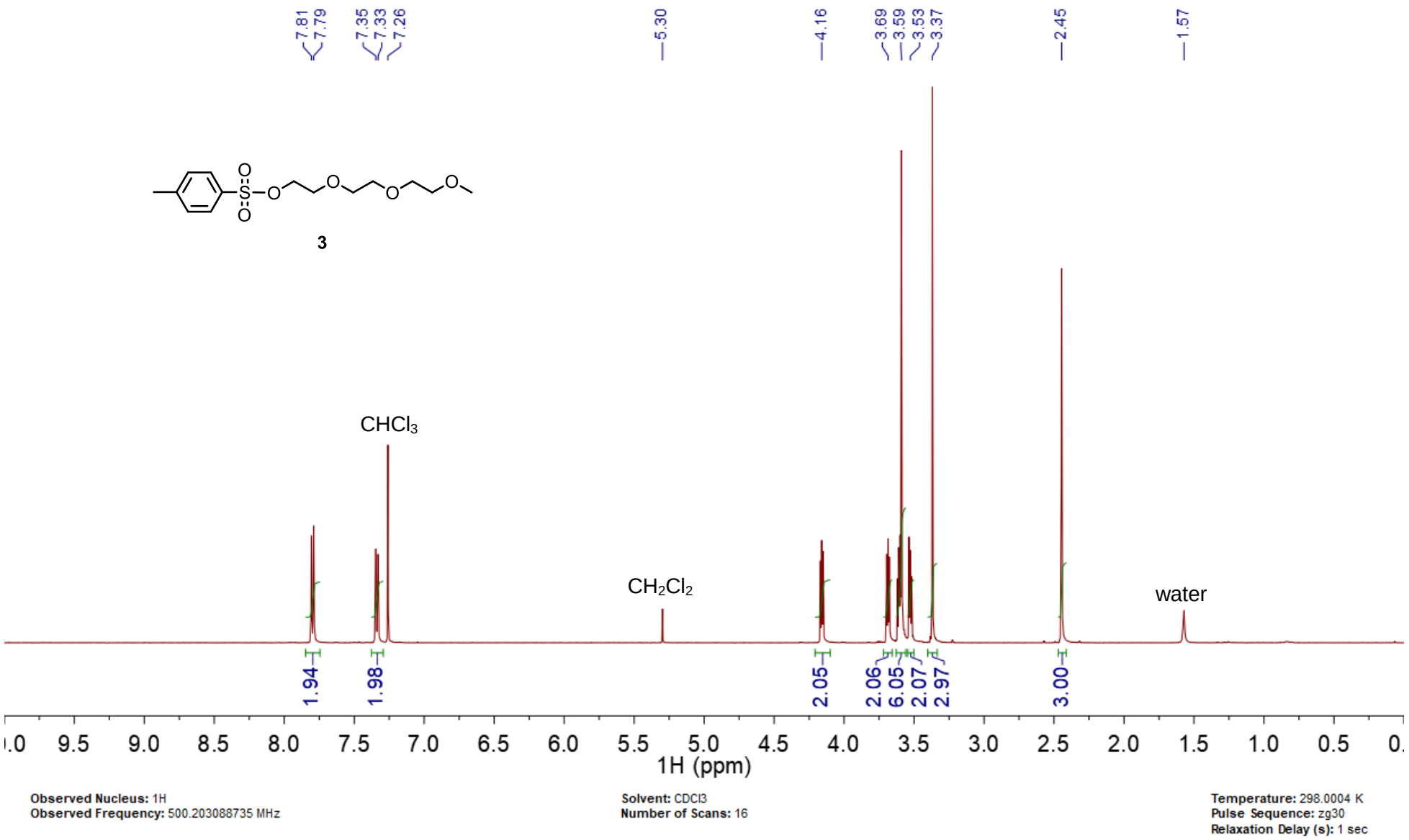



**Figure S4.** $^{1}H$ NMR spectrum (500 MHz, $CDCl_3$) of PEG-tosylate **3**.

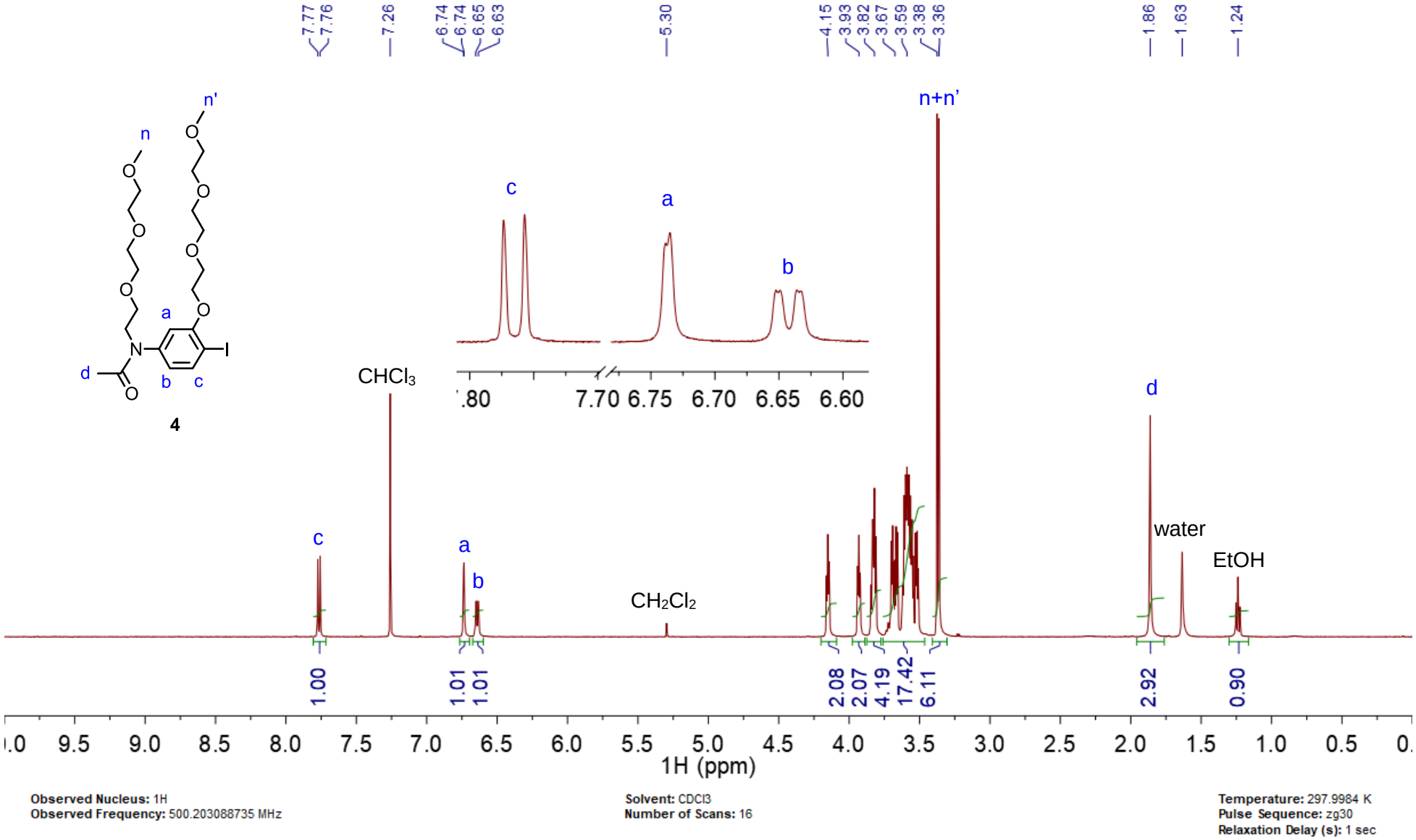



**Figure S5.** $^{1}H$ NMR spectrum (500 MHz, $CDCl_3$) of pegylated amide **4**.

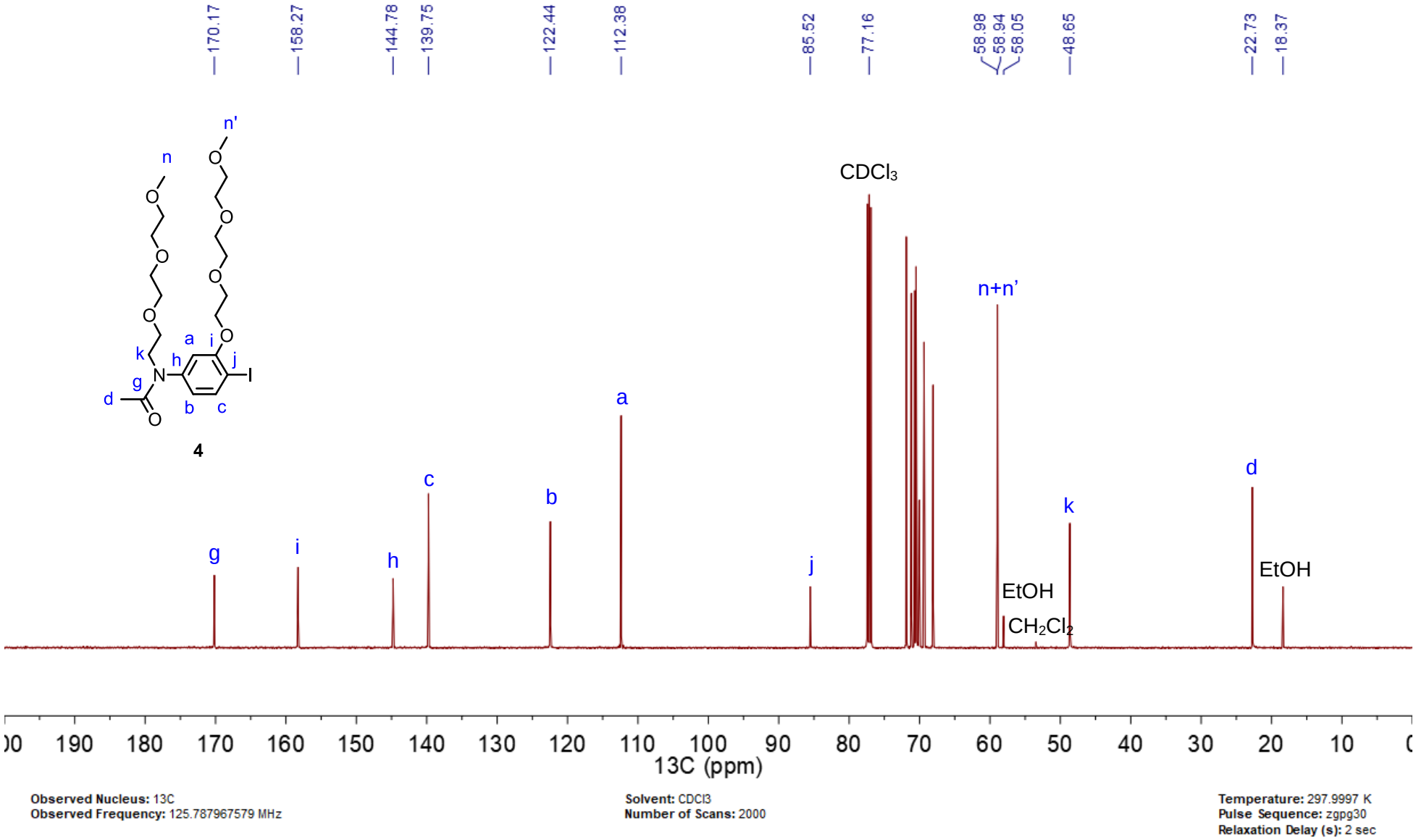



**Figure S6.** $^{13}C$ NMR spectrum (126 MHz, $CDCl_3$) of pegylated amide **4**.

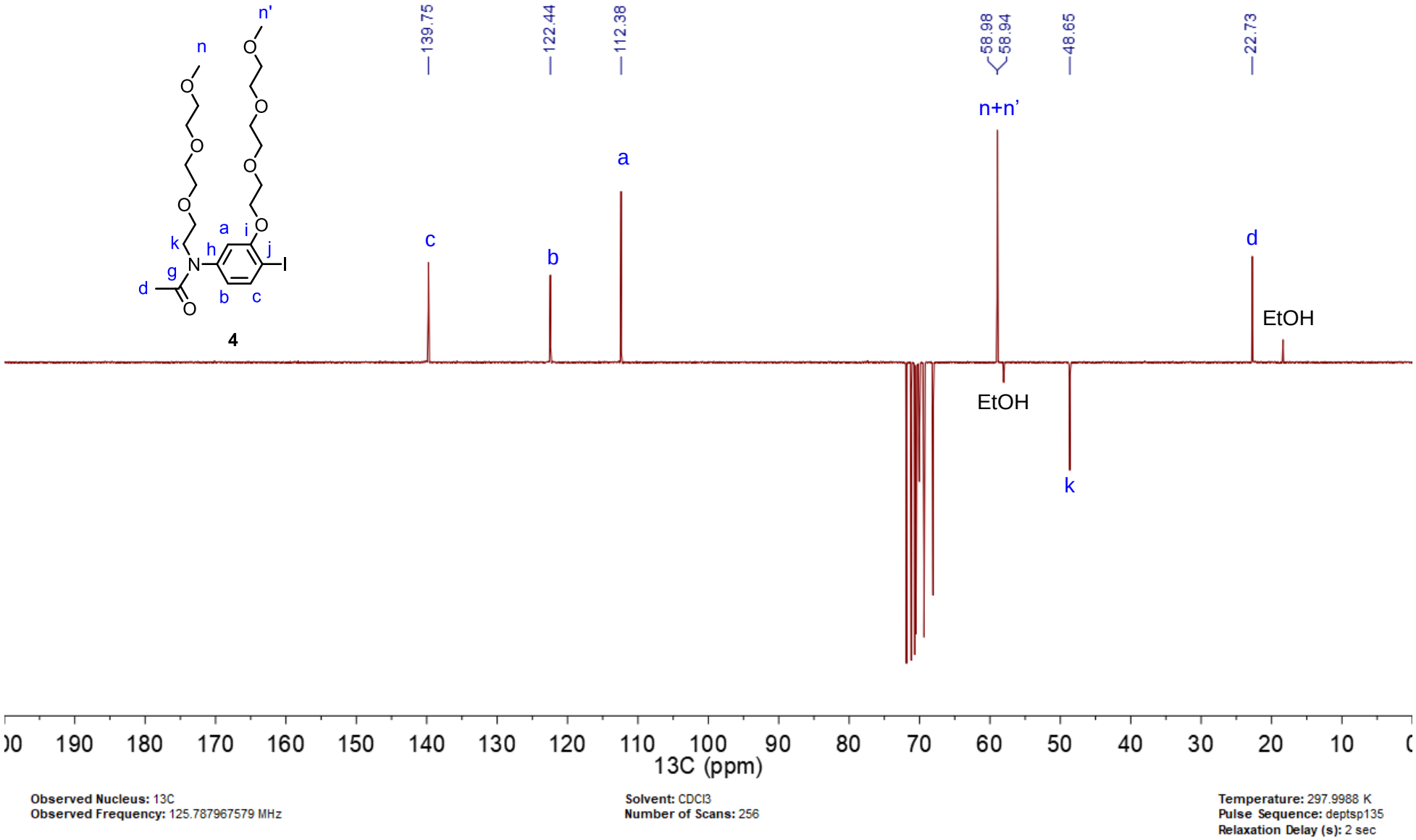



**Figure S7.** $^{13}C$ DEPT 135 NMR spectrum (126 MHz, $CDCl_3$) of pegylated amide **4**.

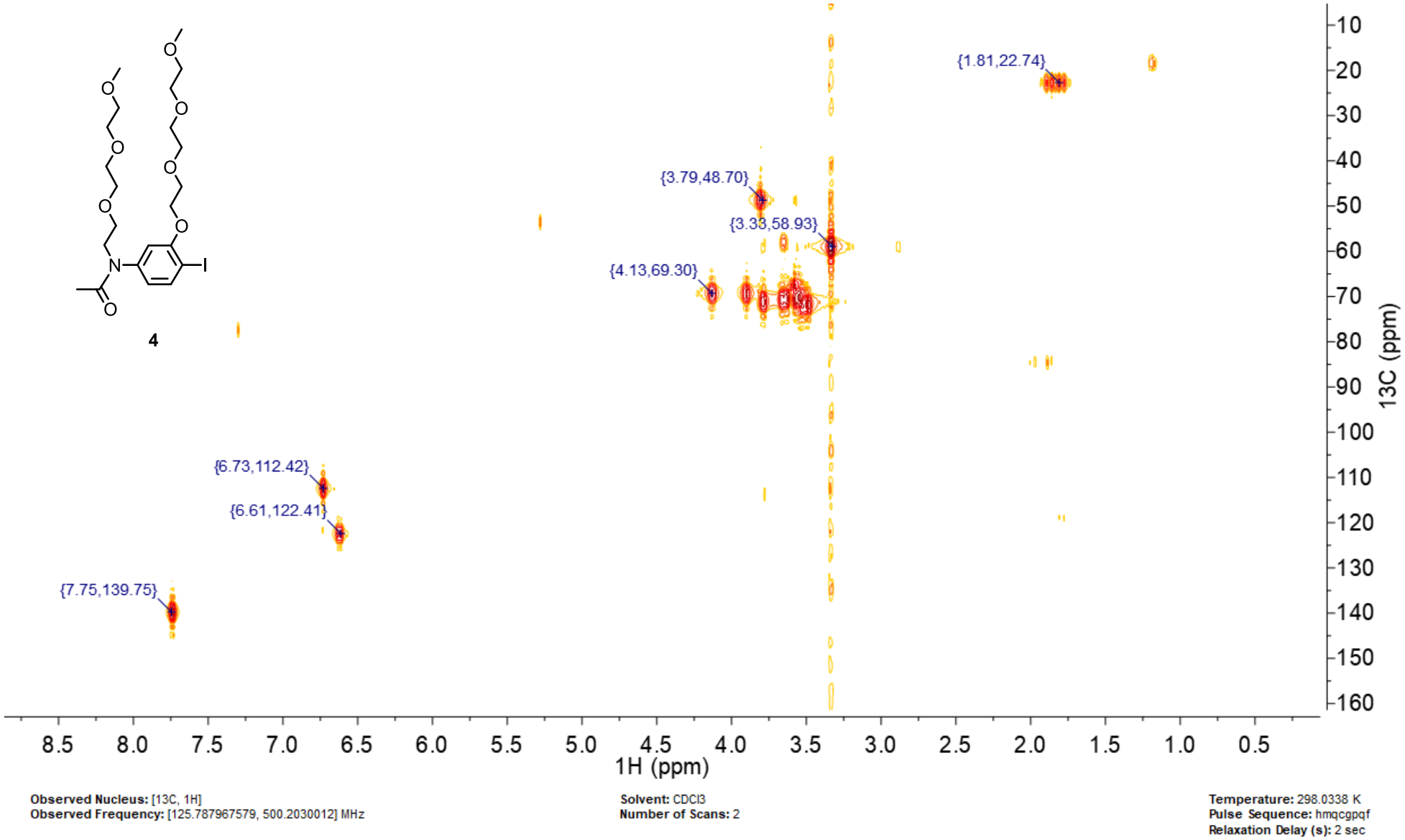



**Figure S8.** HMQC NMR spectrum (126 MHz, 500 MHz, $CDCl_3$) of pegylated amide **4**.

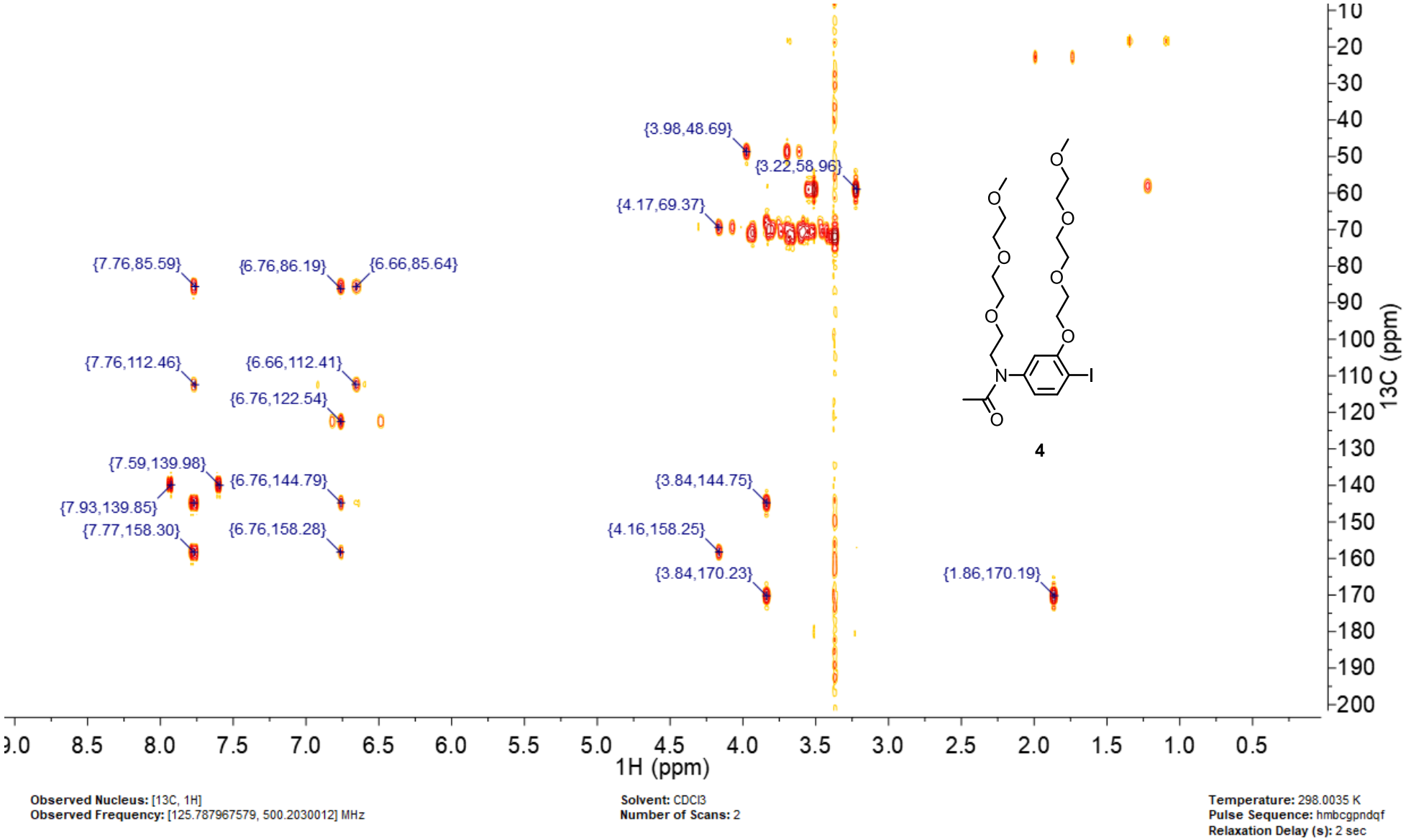



**Figure S9.** HMBC NMR spectrum (126 MHz, 500 MHz, $CDCl_3$) of pegylated amide **4**.

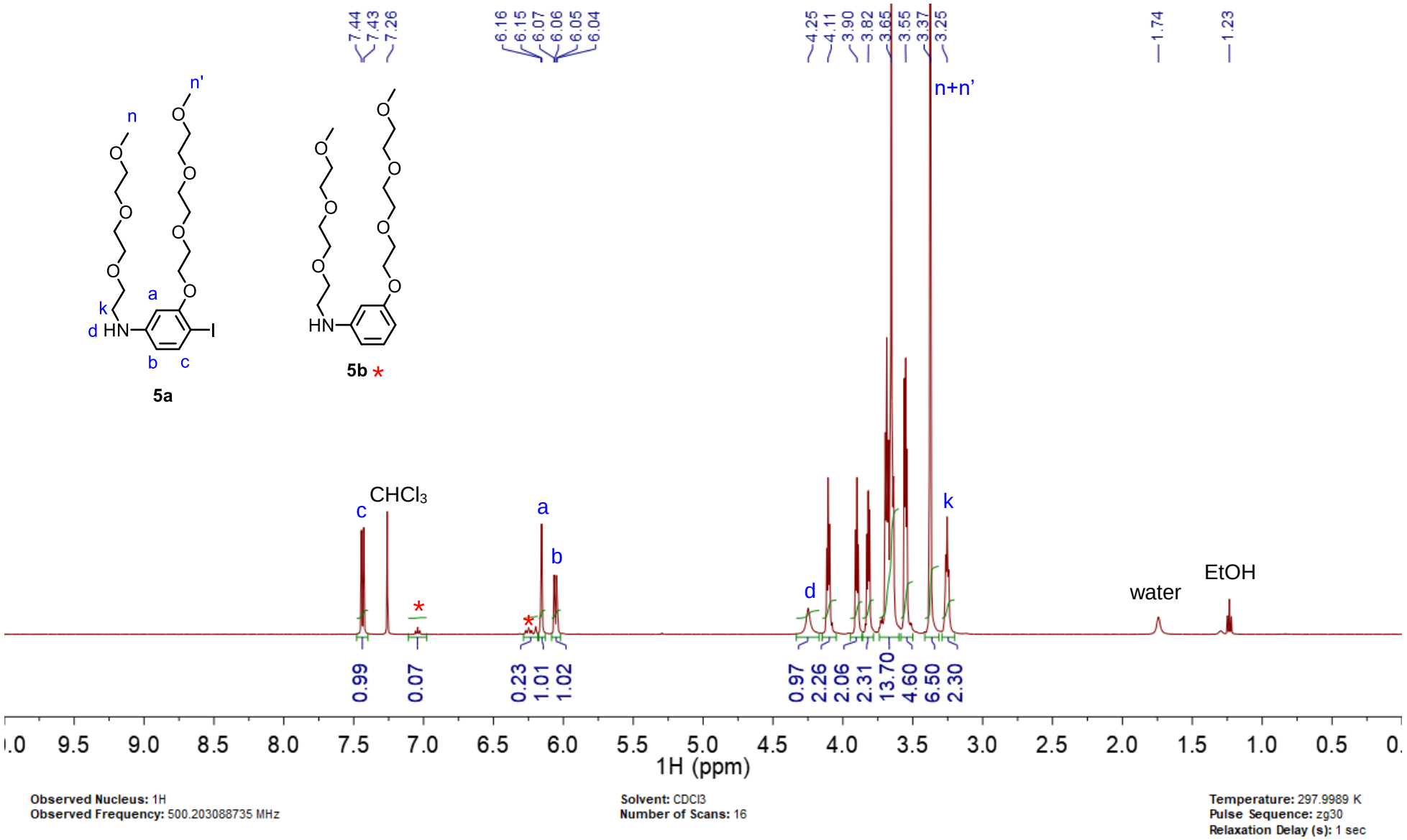



**Figure S10.** $^{1}H$ NMR spectrum (500 MHz, $CDCl_3$) of pegylated amine **5a**.

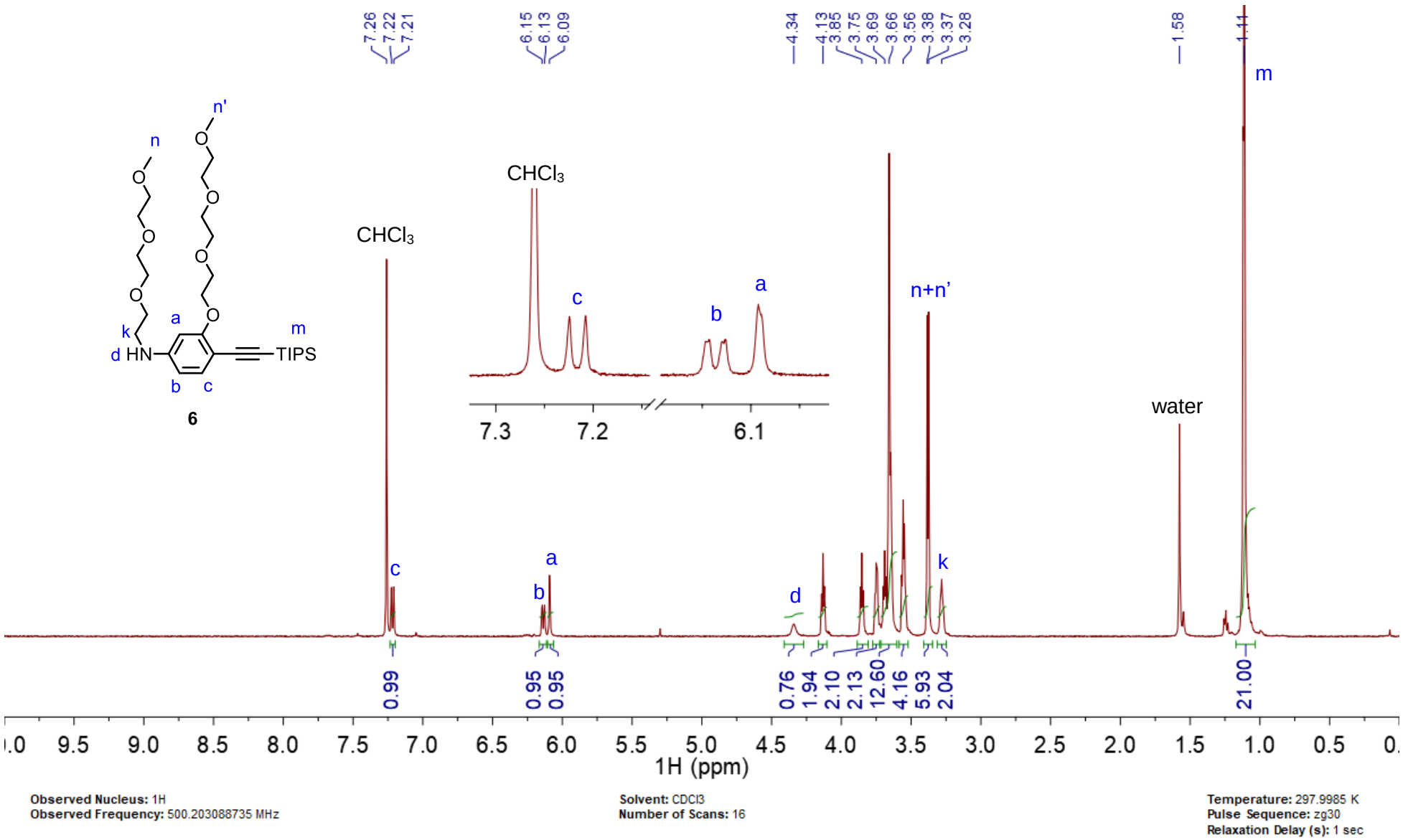



**Figure S11.** $^{1}$H NMR spectrum (500 MHz, $CDCl_3$) of TIPS-protected acetylene **6**.

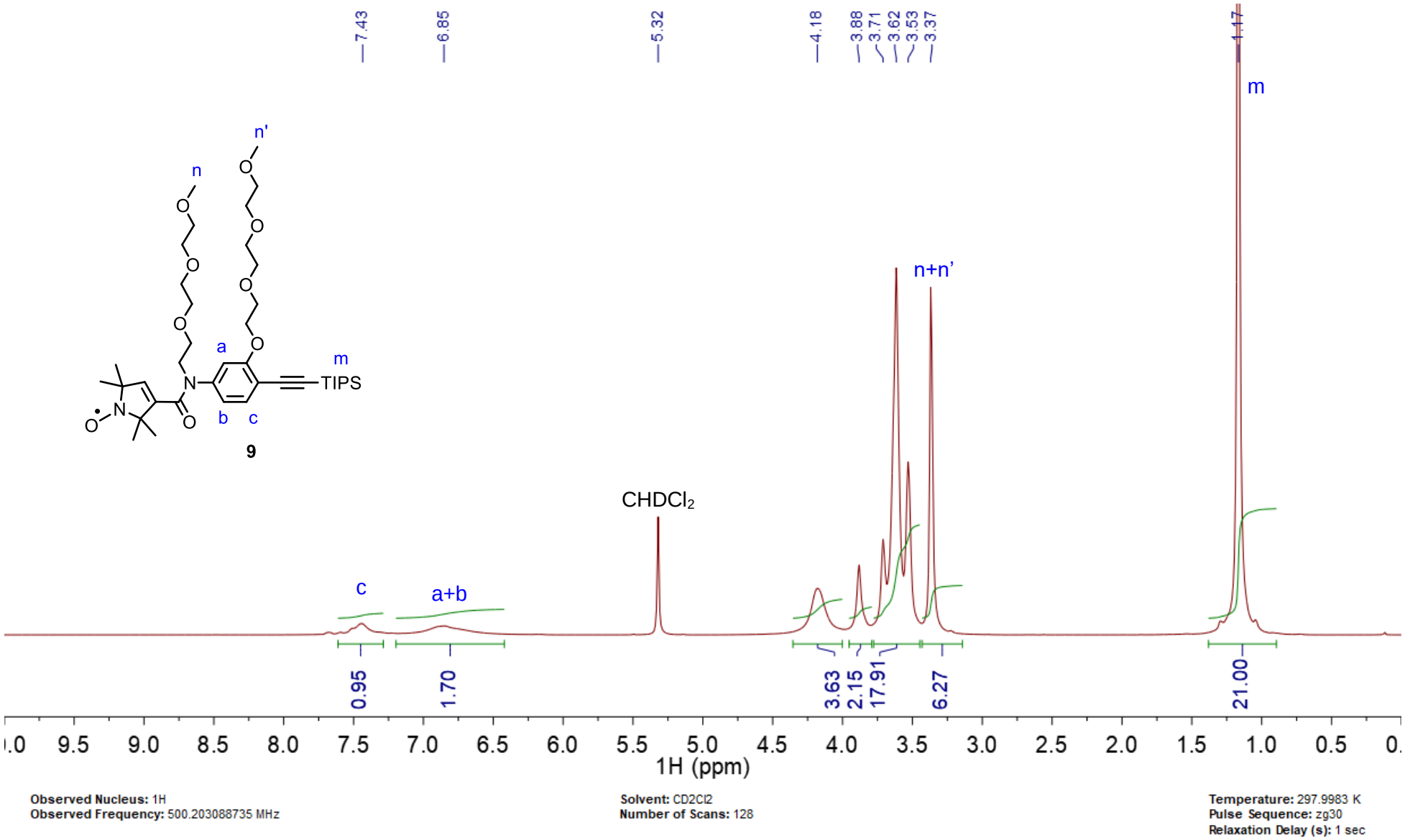



**Figure S12.** $^{1}H$ NMR spectrum (500 MHz, $CDCl_3$) of nitroxide **9**.

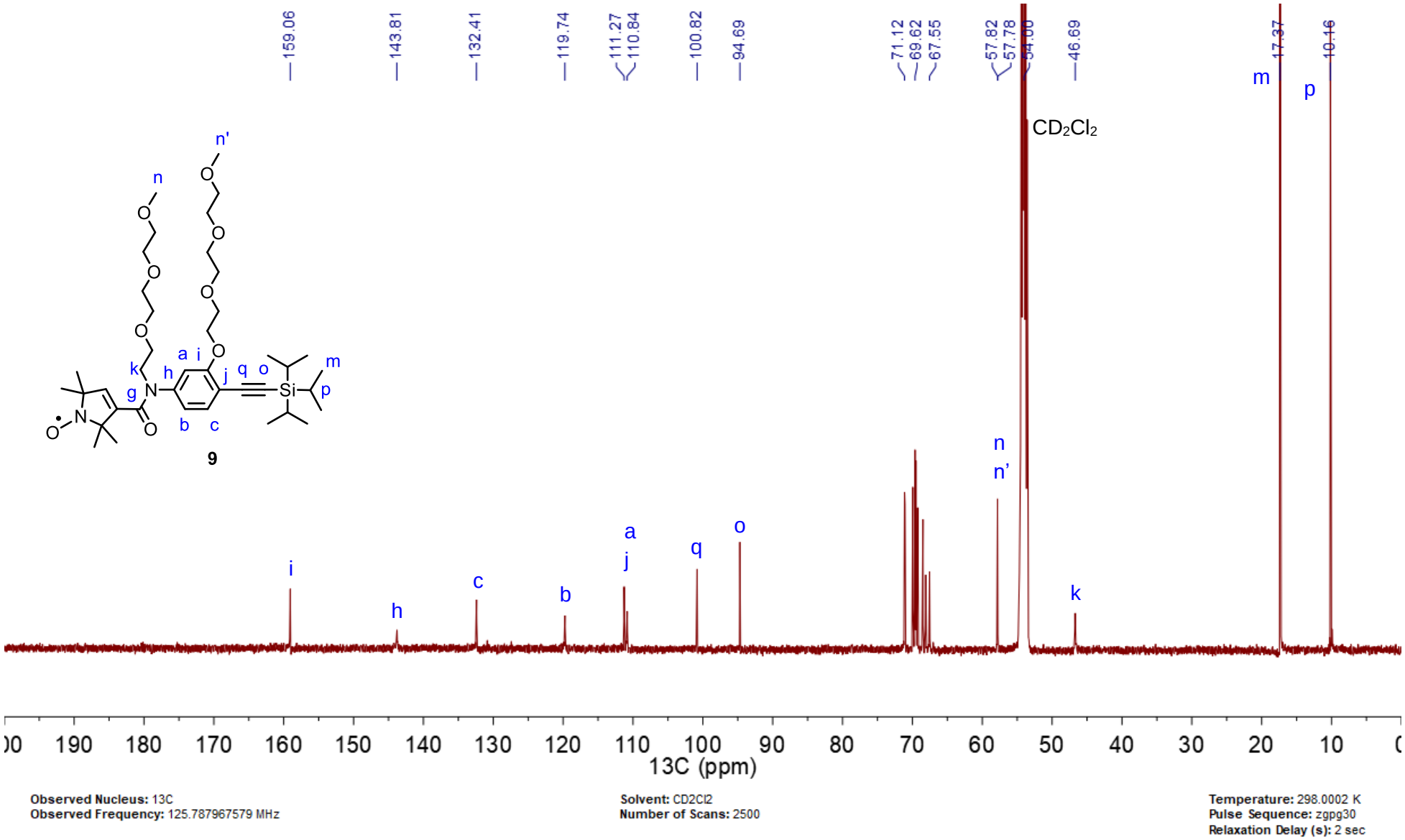



**Figure S13.** $^{13}C$ NMR spectrum (126 MHz, $CDCl_3$) of nitroxide **9**.

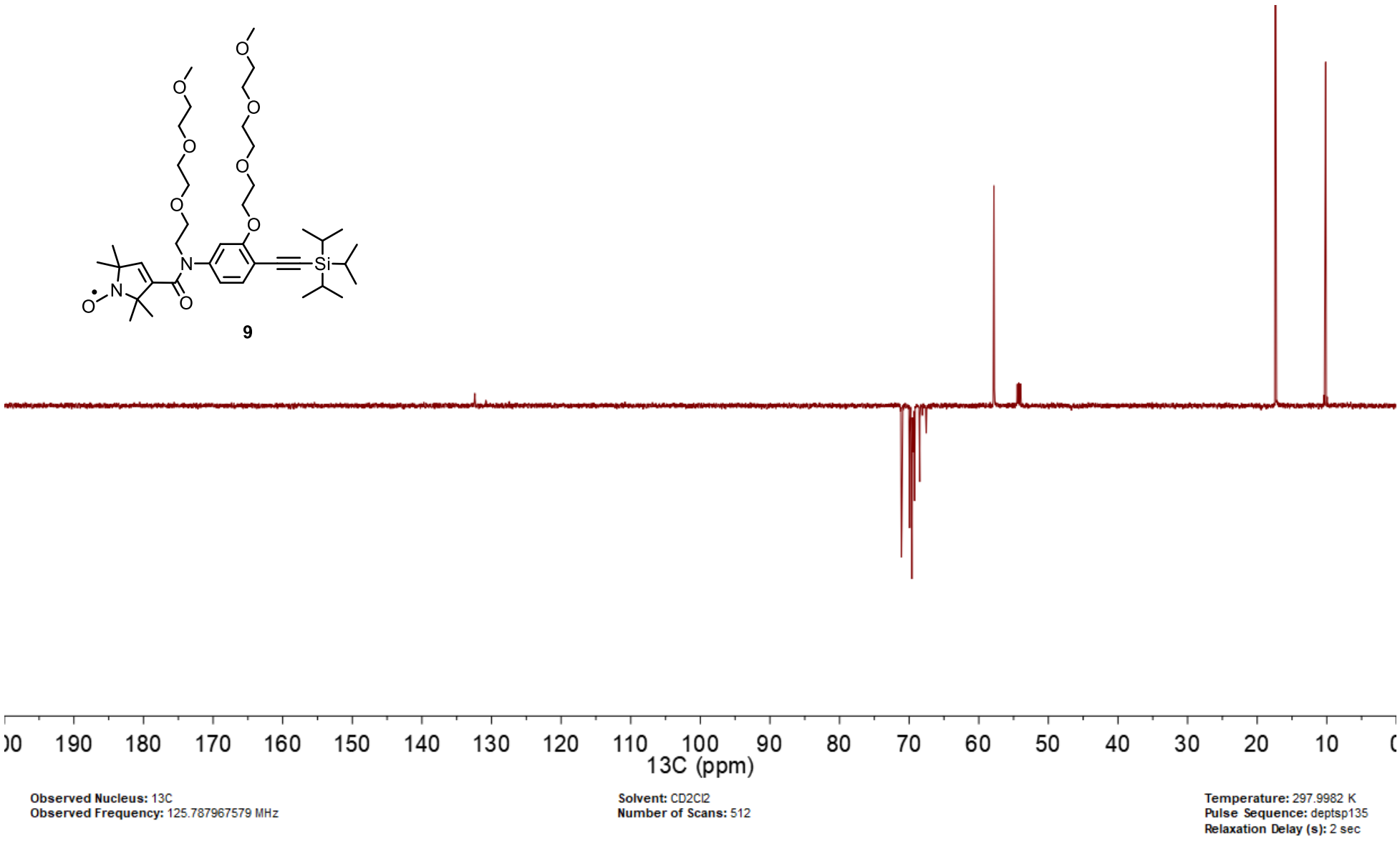



**Figure S14.** $^{13}C$ DEPT 135 NMR spectrum (126 MHz, $CDCl_3$) of nitroxide **9**.

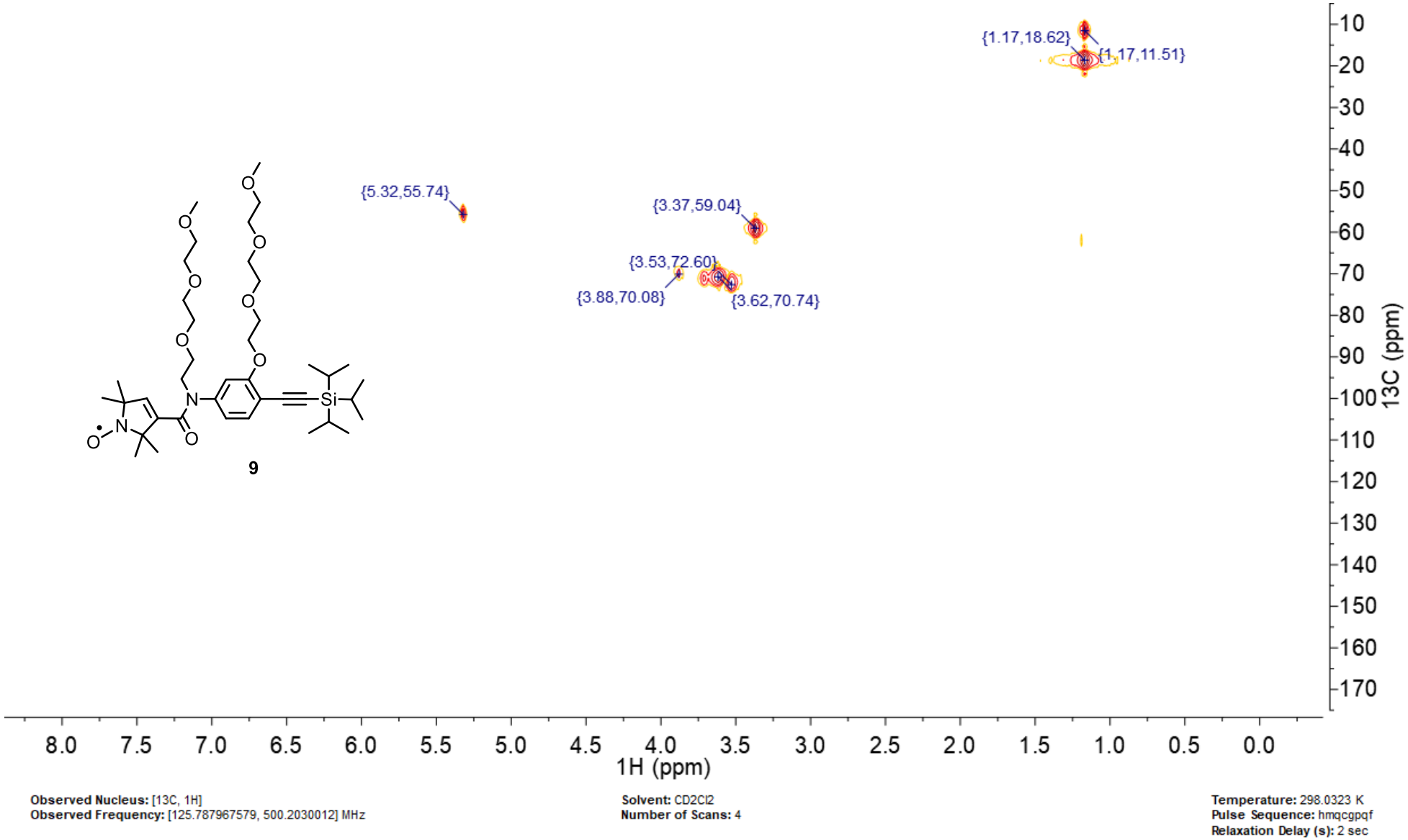



**Figure S15.** HMQC NMR spectrum (126 MHz, 500 MHz, $CD_2Cl_2$) of nitroxide **9**.

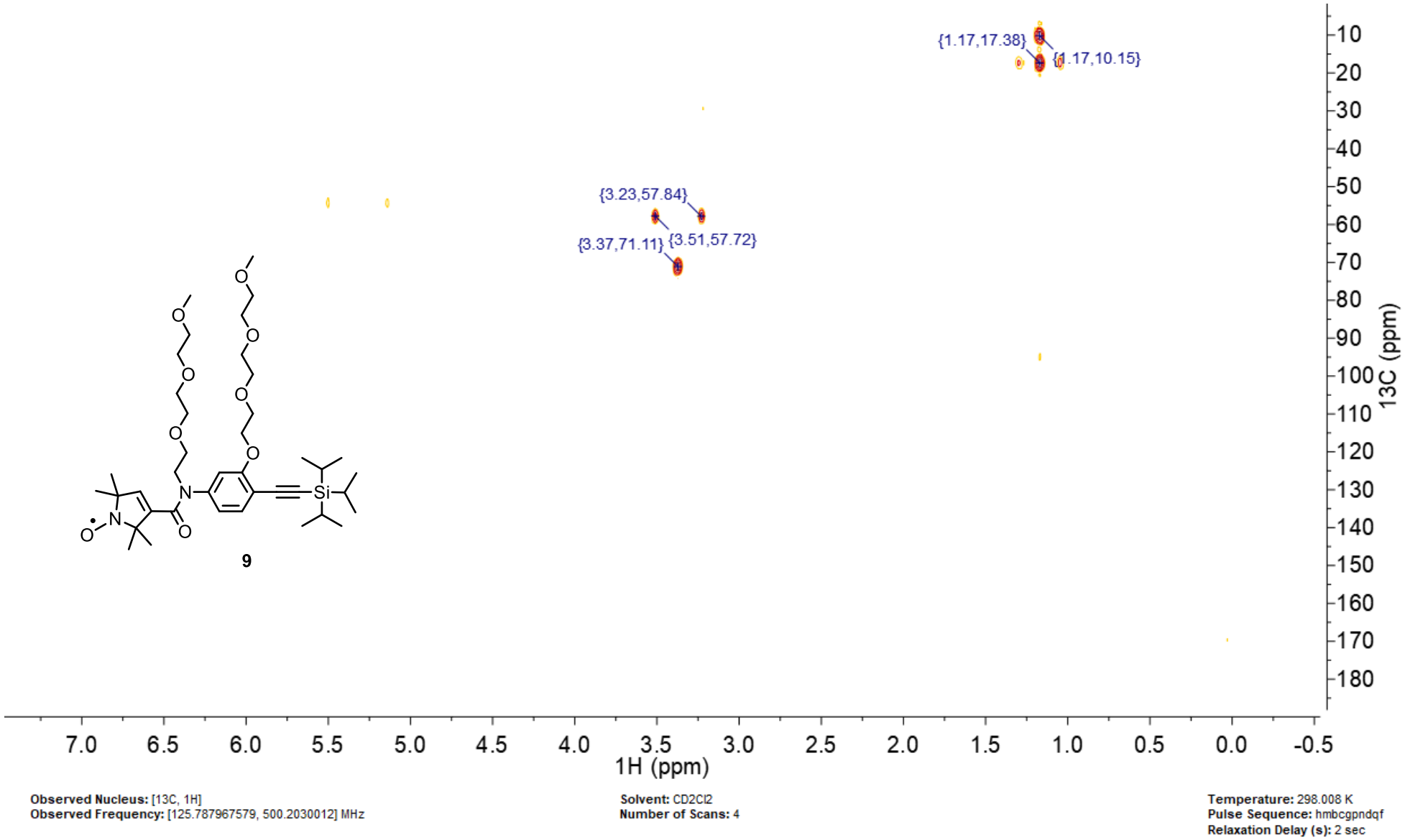



**Figure S16.** HMBC NMR spectrum (126 MHz, 500 MHz, $CD_2Cl_2$) of nitroxide **9**.

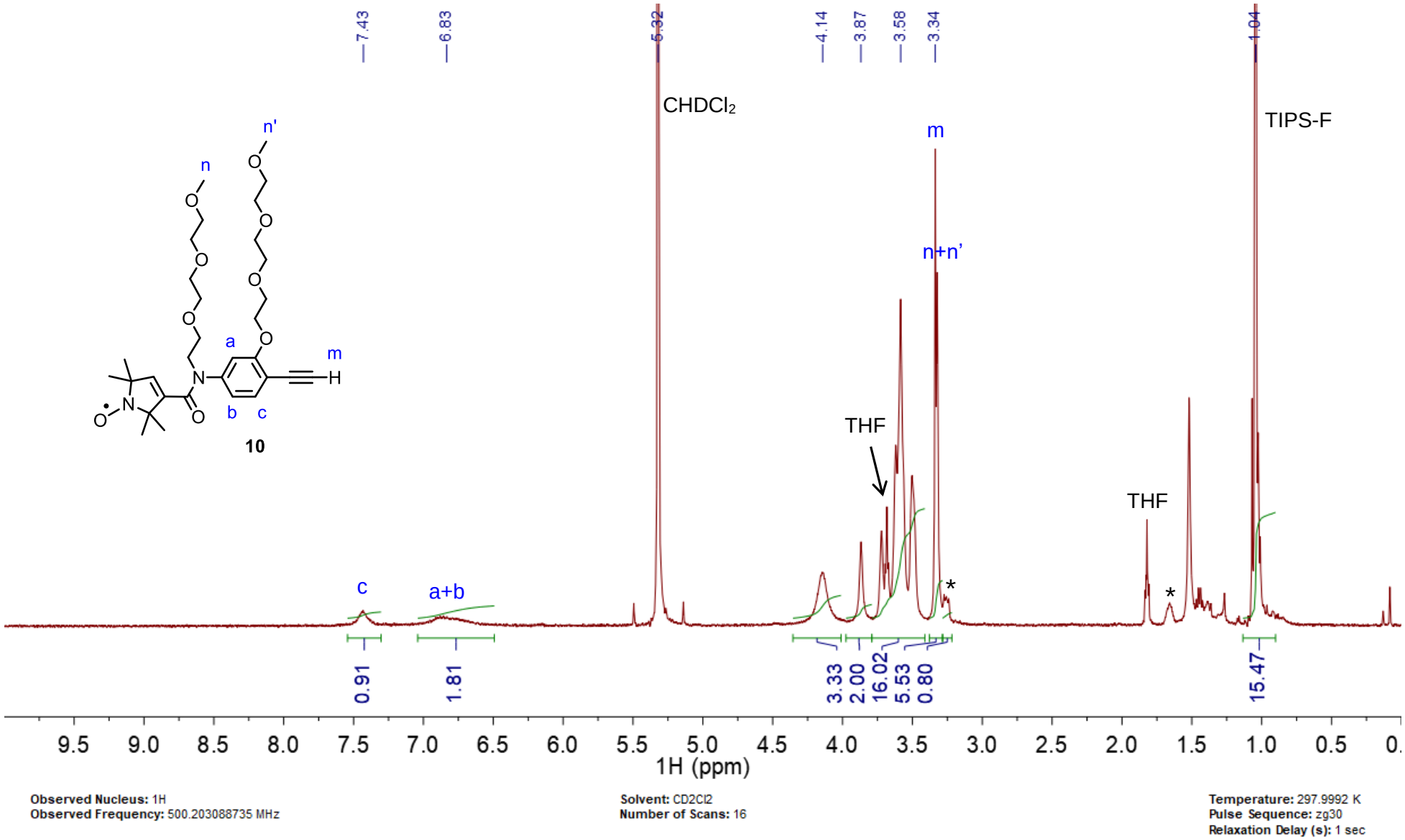



**Figure S17.** $^{1}H$ NMR spectrum (500 MHz, $CD_2Cl_2$) of alkyne **10**. * $Bu_4NY$ (Y = F, OH).

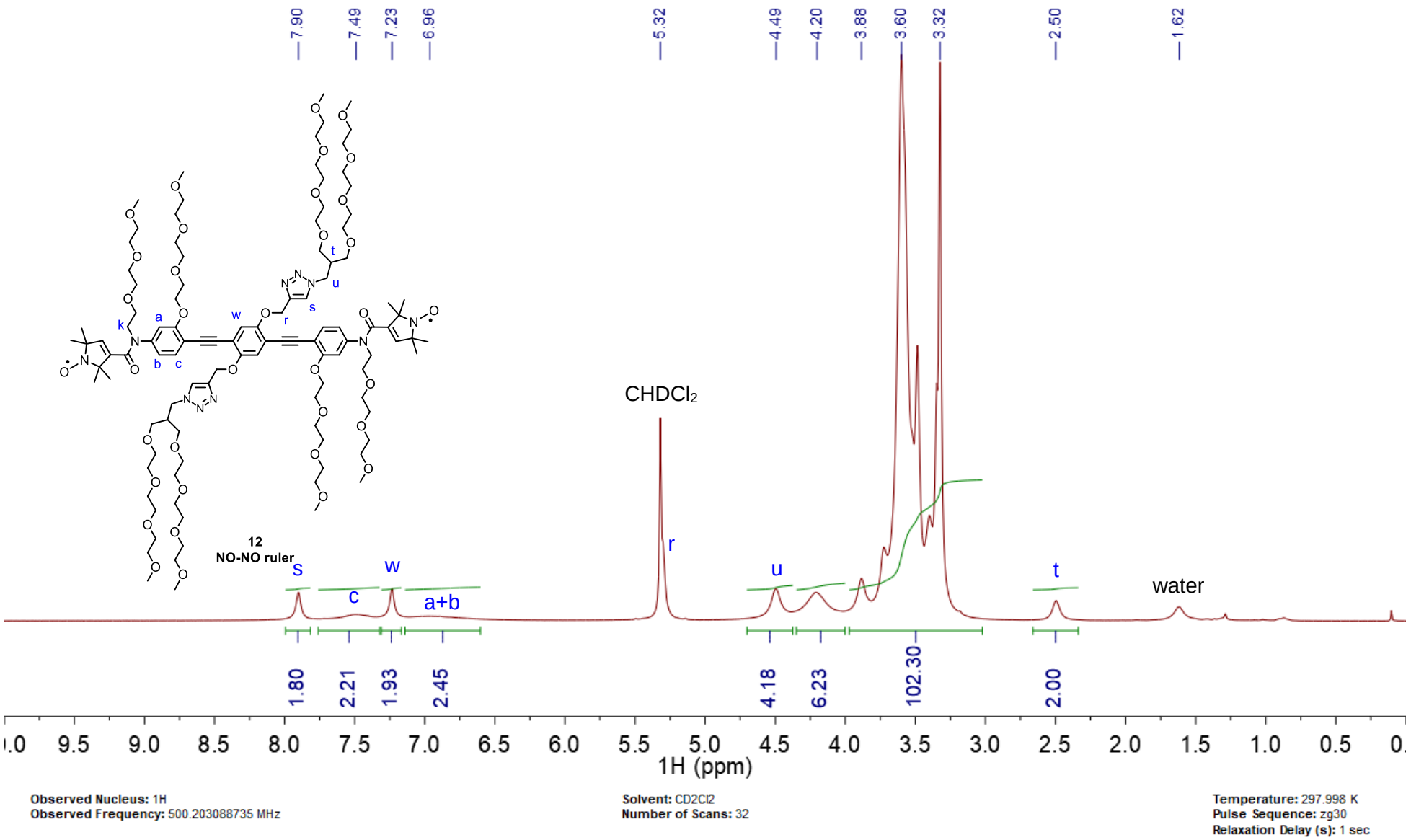



**Figure S18.** $^{1}$H NMR spectrum (500 MHz, $CD_2Cl_2$) of NO-NO ruler **12**.

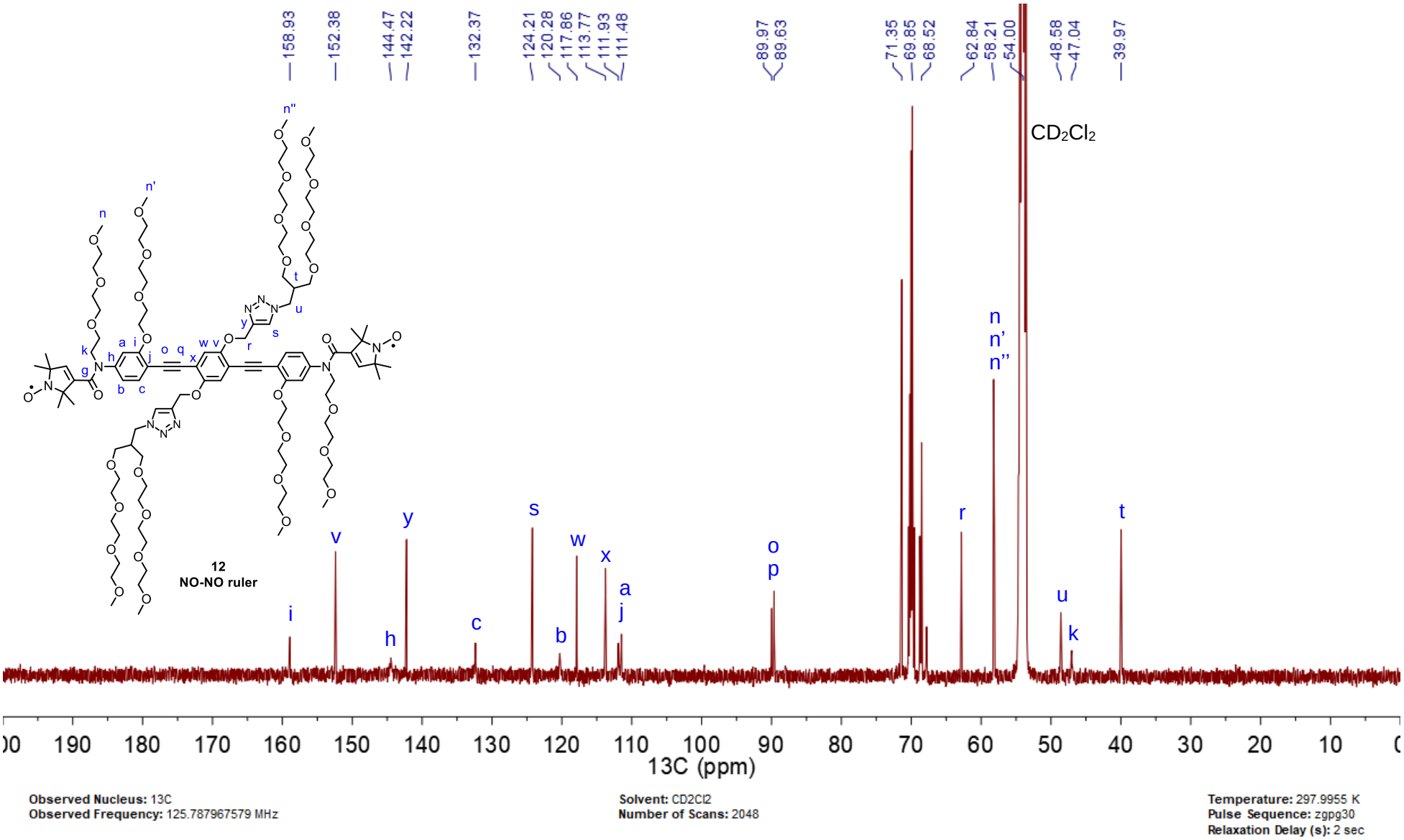



**Figure S19.** $^{13}C$ NMR spectrum (126 MHz, $CD_2Cl_2$) of NO-NO ruler **12**.

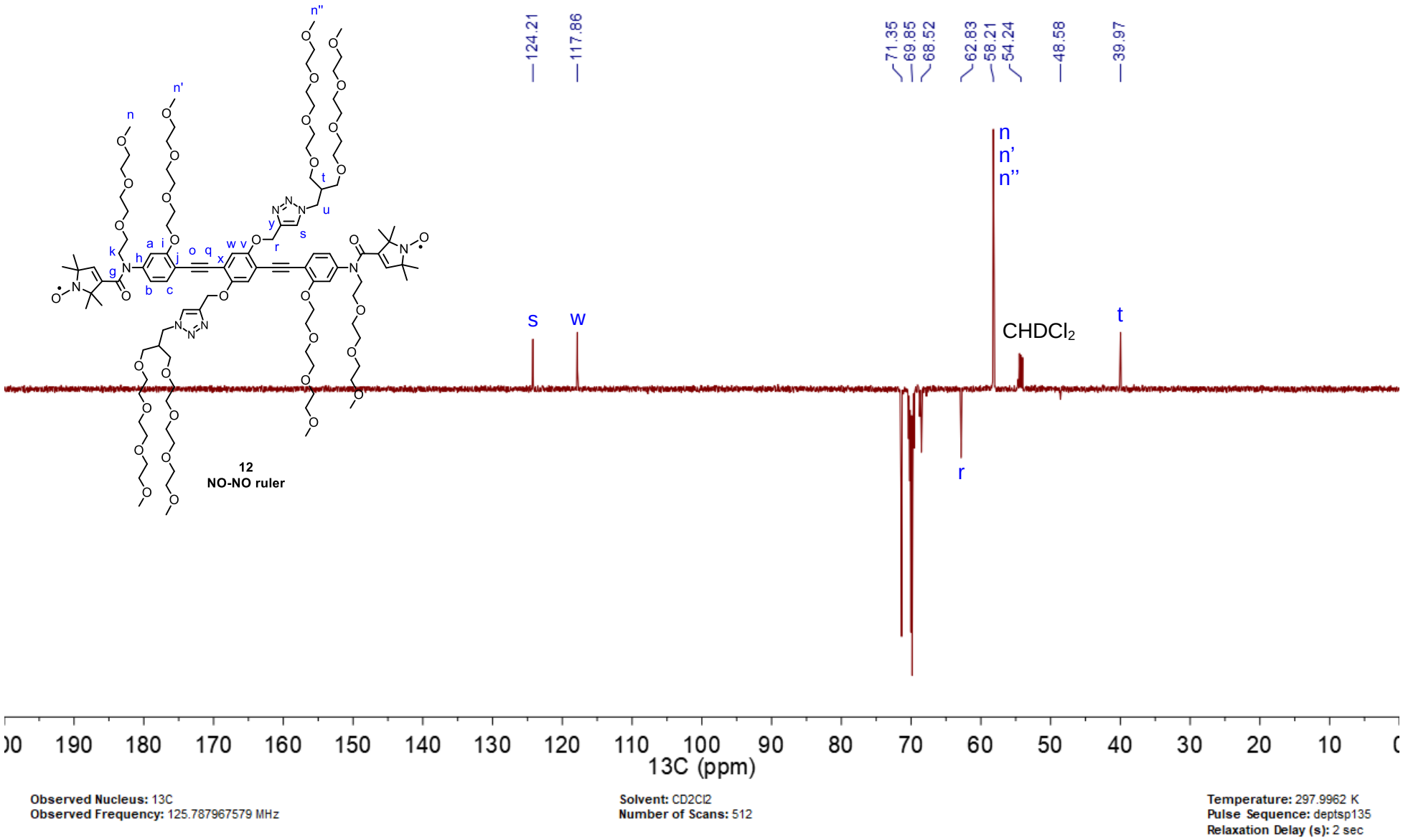



**Figure S20.** $^{13}C$ DEPT 135 NMR spectrum (126 MHz, $CD_2Cl_2$) of NO-NO ruler **12**.

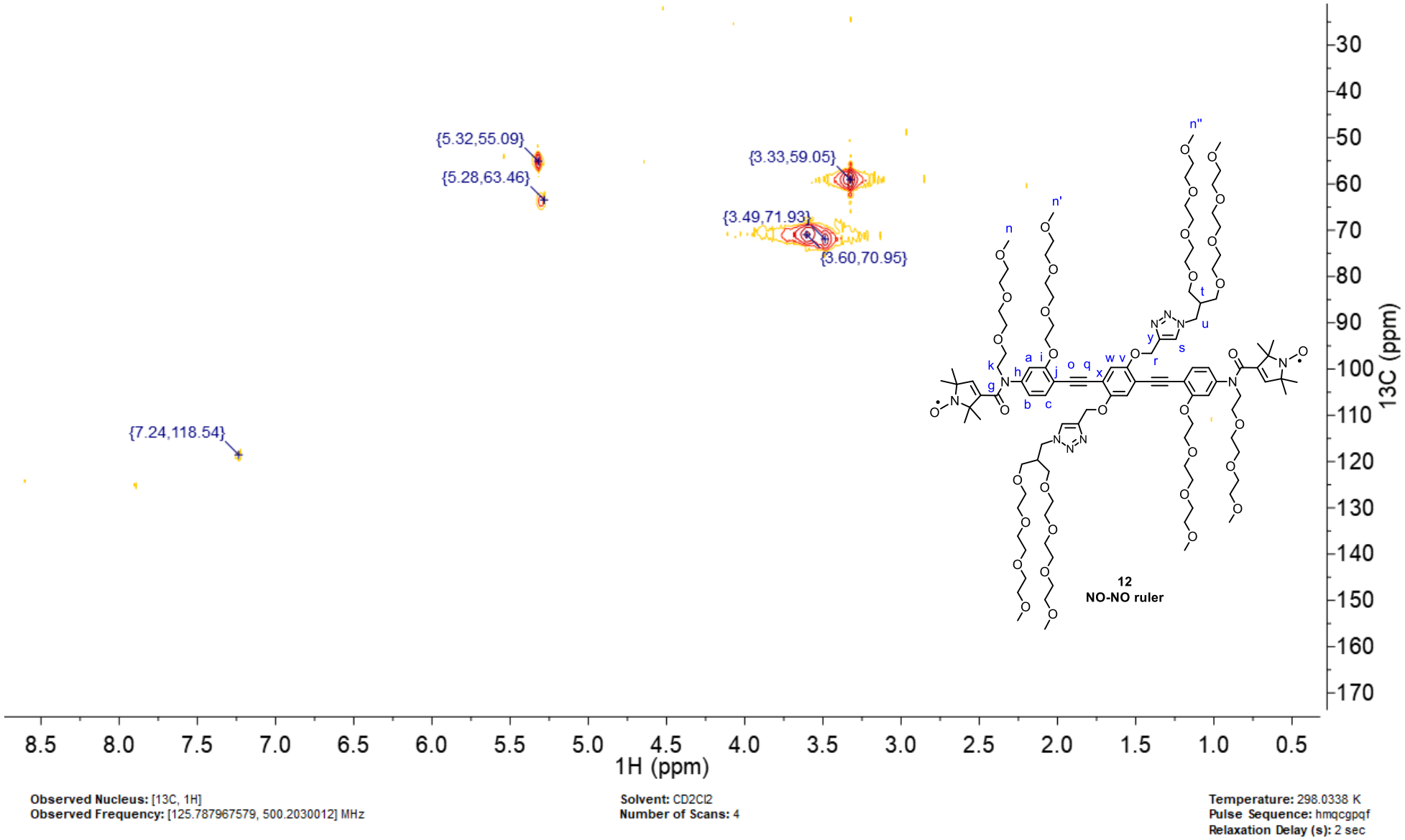



**Figure S21.** HMQC NMR spectrum (126 MHz, 500 MHz, $CD_2Cl_2$) of NO-NO ruler **12**.

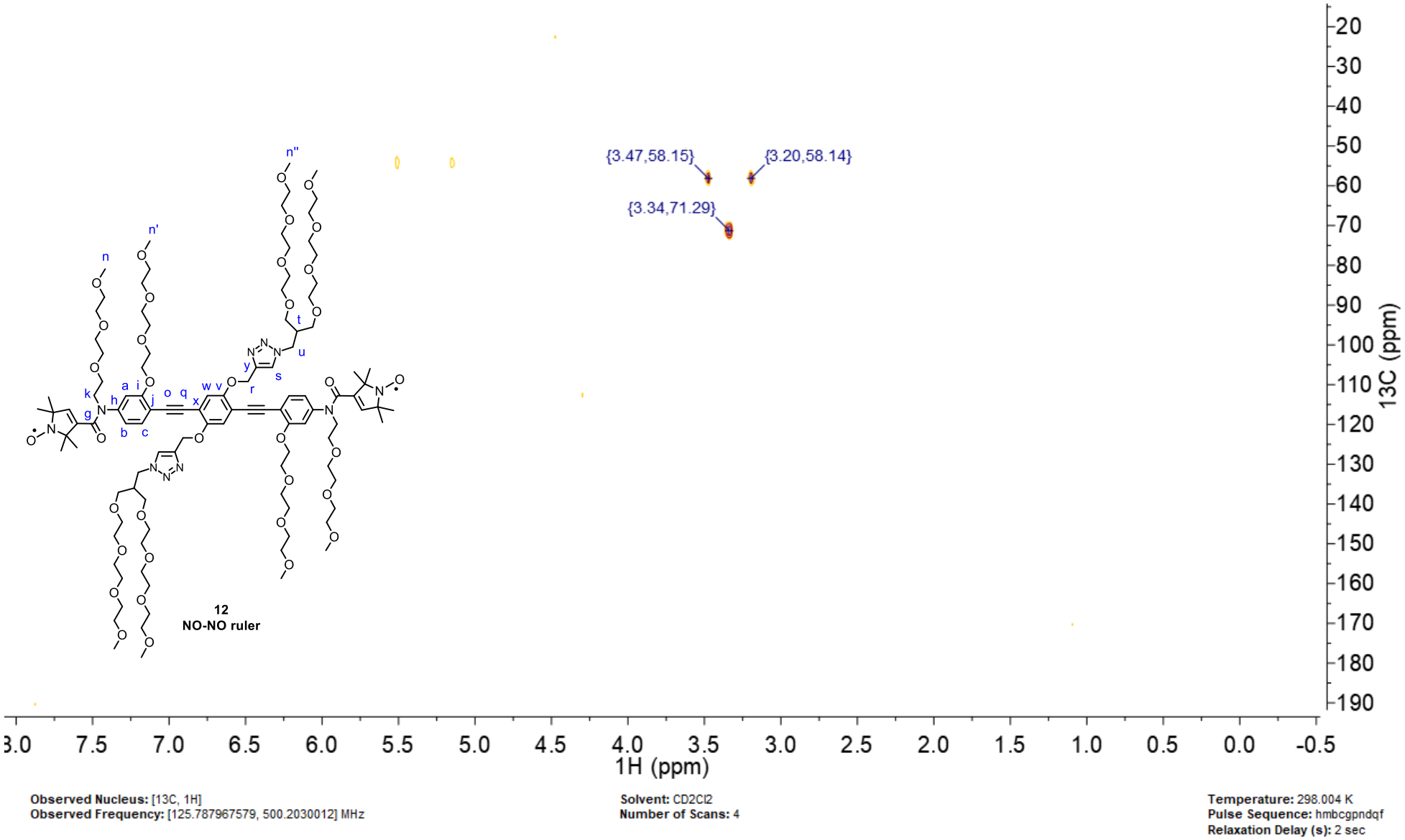



**Figure S22.** HMBC NMR spectrum (126 MHz, 500 MHz, $CD_2Cl_2$) of NO-NO ruler **12**.