Supplementary Information

Dual Functional Profluorescent Nitroxides for Detection of Reactive Oxygen Species and Inhibition of Collagen Degradation during Reassembly

Nattawut Decha[a], Jutakan Thonglam[b], Jirut Meesane[b], Soraya Pornsuwan[c], Chittreeya Tansakul[*a]

[a] Division of Physical Science and Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90110, Thailand

[b] Institute of Biomedical Engineering, Department of Biomedical Science and Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90100, Thailand

[c] Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

Corresponding Author's E-Mail: chittreeya.t@psu.ac.th

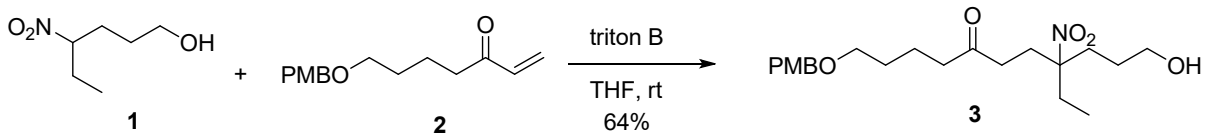
Contents

Experimental sections

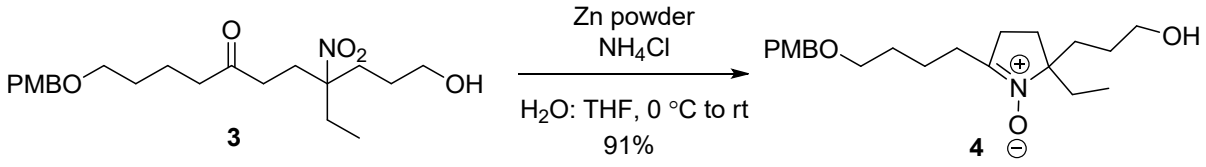
Chemicals and instruments

Chemical reagents were purchased from Tokyo Chemical Industry Co., Ltd. (TCI). Analytical grade solvents used for synthesis and analysis were used as received from suppliers. Solvents for extraction and column chromatography were distilled at their boiling point ranges prior to use. Analytical grade solvents for reactions were used as received from suppliers or distilled prior to use using standard procedures. Thin-layer chromatography (TLC) was performed on SiliaPlateTM R10011B-323 (Silicycle) or silica gel 60 GF254 (Merck) and were visualized by fluorescence quenching under UV light and *p*-anisaldehyde stain. Column chromatography was performed on SiliaFlash® G60 Silica (60-200 μm, Silicycle). ^{1}H NMR (300 and 500 MHz) and ^{13}C NMR (75 and 125 MHz) spectroscopic data were recorded on a 300 and 500 MHz Bruker FT-NMR Ultra Shield spectrometer using residual solvent as an internal standard. Chemical shifts are expressed in parts per million (ppm) downfield from TMS (δ 0.00) and coupling constants are reported as hertz (Hz). Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quatet; qn, quintet; m, multiplet. Infrared spectra (IR) were measured on a Perkin Elmer Spectrum GX FT-IR system and recorded on wavenumber (cm^{-1}). High-resolution ESI mass spectra were obtained on a liquid chromatograph-mass spectrometer (2690, LCT, Waters, Micromass). EPR spectra were collected using an EPR Bruker system, ELEXSYS E-500 model at X-band microwave frequencies (approximately 9.85 GHz) with the following parameters: a central field of 3517 G, a scanning field of 3552 G, 1024 data points, a modulation amplitude of 10 mT, a receiver gain of 60 dB, a time constant of 10.24 ms, a conversion time of 20.48 ms and a microwave power of 2 mW. Gel permeation chromatography (GPC) was performed to determine weight average molecular weight (Mw), number average molecular weight (Mn) and polydispersity index (PDI) using an Agilent Technologies (1260GPC/SEC MDS, USA) equipped with two PLgel 5 μm MIXED-C columns (Agilent) and a guard column (Agilent). Tetrahydrofuran (THF) was used as the eluent at a flow rate of 1 mL/min with injection volume as 20 μL. A refractive index detector was used and the molecular weights were calibrated against twelve linear polystyrene standards ranging from 162 to 364,000 (Mp[g/mol]).

Synthetic methods and characterization of probes PN1 and PN2-MMA copolymer

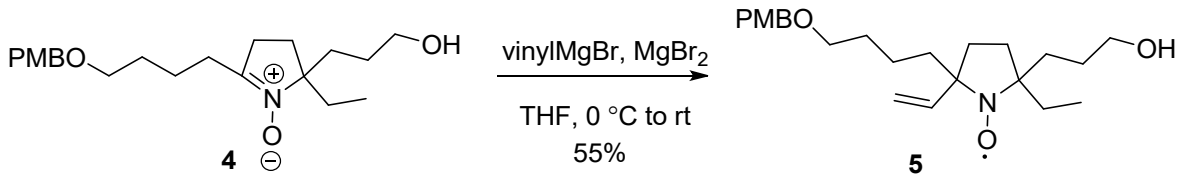


γ-Nitroketone 3: To a solution of 4-nitrohexan-1-ol[1] (**1,** 676 mg, 4.59 mmol) in THF (40 mL) was added triton B (40% in methanol, 192 μL, 0.461 mmol) and 7-((4-methoxybenzyl)oxy)hept-1-en-3-one[2] (**2**, 160 mg, 6.44 mmol) in THF (6 mL), respectively. After reaction mixture was stirred for 2 hours, it was quenched with saturated ammonium chloride, and extracted with ethyl acetate (3 x 50 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography with 20-40% ethyl acetate in hexane to give 1.24 g (64% yield) of **3** as a colorless viscous oil. R_f = 0.20 (30% EtOAc in hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 4.41 (s, 2H), 3.78 (s, 3H), 3.56 (t, *J* = 6.1 Hz, 2H), 3.44 (t, *J* = 6.1 Hz, 2H), 2.90 (brs, 1H), 2.42 (t, J = 6.7 Hz, 2H), 2.37-2.32 (m, 2H), 2.19-2.14 (m, 2H), 2.03-1.85 (m, 4H), 1.68-1.55 (m, 4H), 1.45-1.35 (m, 2H), 0.85 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR, DEPT (75 MHz, $CDCl_3$) δ 208.9 (C), 159.0 (C), 130.3 (C), 129.1 (2 x CH), 113.6 (2 x CH), 94.0 (C), 72.3 (CH_2), 69.4 (CH_2), 61.6 (CH_2), 55.1 (CH_3), 42.3 (CH_2), 36.5 (CH_2), 31.0 (CH_2), 28.8 (2 x CH_2), 28.4 (CH_2), 26.7 (CH_2), 20.5 (CH_2), 8.0 (CH_3) ppm; IR (thin film): 3447, 2940, 2867, 1715, 1537, 1247, 1098, 822 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{21}H_{33}NO_6$ $(M+Na)^+$ 418.2206, found 418.2201



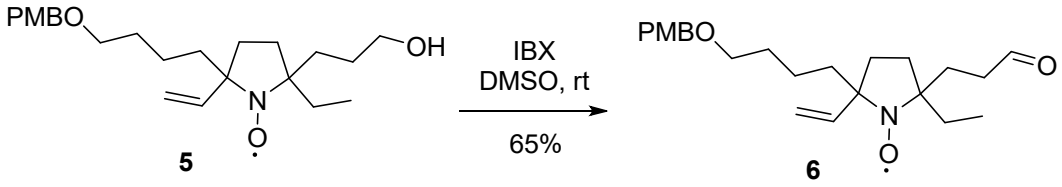
Nitrone 4: To a solution of γ-nitroketone **3** (1.24 g, 3.14 mmol) in THF:H_2O (22.5:7.5 mL) was added ammonium chloride (181 mg, 3.44 mmol) and zinc powder (818 mg, 12.5 mmol) at 0 °C, respectively. The reaction mixture was allowed to stirred overnight at room temperature. Zinc oxide was filtered through short path celite, and the filtrate was extracted with ethyl acetate (3 x 50 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography with 20% ethyl acetate in hexane, followed by 10% methanol in ethyl acetate to give 1.03 g

(91% yield) of nitrone **4** as a colorless viscous oil. R_f = 0.37 (10% methanol in EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 4.42 (s, 2H), 4.01 (brs, 1H), 3.78 (s, 3H), 3.57 (t, *J* = 6.1 Hz, 2H), 3.46 (t, *J* = 5.6 Hz, 2H), 2.57-2.42 (m, 4H), 1.99-1.83 (m, 4H), 1.64-1.49 (m, 7H), 1.40-1.33 (m, 1H), 0.82 (t, *J* = 7.3 Hz, 3H) ppm; ^{13}C NMR, DEPT (75 MHz, $CDCl_3$) δ 158.9 (C), 148.7 (C), 130.3 (C), 129.0 (2 x CH), 113.5 (2 x CH), 79.5 (C), 72.3 (CH_2), 69.1 (CH_2), 61.7 (CH_2), 55.0 (CH_3), 33.5 (CH_2), 30.5 (CH_2), 29.4 (CH_2), 28.3 (CH_2), 26.3 (2 x CH_2), 24.4 (CH_2), 21.6 (CH_2), 7.5 (CH_3) ppm; IR (thin film): 3366, 2936, 2863, 1611, 1513, 1247, 1174, 821 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{21}H_{33}NO_4$ $(M+Na)^+$ 386.2307, found 386.2310.



Nitroxide 5: To a solution of nitrone **4** (1.03 g, 2.83 mmol) in anhydrous THF (15 mL) was added magnesium bromide (260 mg, 1.41 mmol). The resulting solution was cooled at 0°C and a solution of vinyl magnesium bromide (1 M in THF, 16.9 mL, 16.9 mmol) was slowly added to the above solution. The reaction mixture was stirred for 18 hours. Excess vinyl magnesium bromide was quenched by slowly addition of saturated ammonium chloride until bubble disappeared, and white precipitate was observed. The white solid was filtered through short path celite. After THF was removed under reduced pressure, distilled water (50 mL) was added to crude product, which was extracted with EtOAc (3 x 50 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered, concentrated *in vacuo*. The crude residue was purified by column chromatography with 30% EtOAc in hexane to give 605 mg (55% yield) of nitroxide **5** as a yellow viscous oil. R_f = 0.40 (40% EtOAc in hexane); ^{1}H NMR (300 MHz, $CDCl_3$) of ethoxylamine derivatives as a mixture of diastereomers: δ 7.25 (d, *J* = 8.6 Hz, 4H), 6.87 (d, *J* = 8.6 Hz, 4H), 6.17 (dd, *J* = 10.5, 17.3 Hz, 2H), 5.12 (d, *J* = 10.5 Hz, 2H), 5.02 (d, *J* = 17.3 Hz, 2H), 4.41 (s, 4H), 3.85 (brs, 2H), 3.79 (s, 6H), 3.72-3.56 (m, 4H), 3.42 (t, *J* = 7.1 Hz, 4H), 1.92-1.78 (m, 8H), 1.71-1.49 (m, 18H), 1.34-1.22 (m, 10H), 1.32 (t, *J* = 6.6 Hz, 3H), 1.11 (t, *J* = 6.6 Hz, 3H), 0.91 (t, *J* = 7.1 Hz, 3H), 0.81 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR, DEPT (75 MHz, $CDCl_3$) of ethoxylamine derivatives as a mixture of diastereomers: δ 159.1 (2 x C), 140.6 (2 x CH), 130.8 (2 x C), 129.2 (4 x CH), 113.7 (4 x CH), 112.3 (CH_2), 112.2 (CH_2), 72.4 (2 x CH_2), 71.0 (2 x CH_2), 70.7 (2 x C), 70.1 (CH_2), 70.0 (CH_2), 67.8 (2 x C), 63.6 (2 x CH_2), 55.2 (2 x CH_3), 39.4 (2 x CH_2), 35.2 (2 x CH_2), 30.4 (CH_2), 30.3 (CH_2), 29.9 (2 x CH_2), 29.6

(2 x CH_2), 28.3 (2 x CH_2), 27.7 (2 x CH_2), 21.4 (2 x CH_2), 14.2 (2 x CH_3), 9.2 (CH_3), 8.6 (CH_3) ppm; IR (thin film): 3363, 2939, 2866, 1613, 1514, 1248, 1098, 1034, 916, 821 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{23}H_{36}NO_4$ $(M+Na)^+$ 413.2542, found 413.2547.

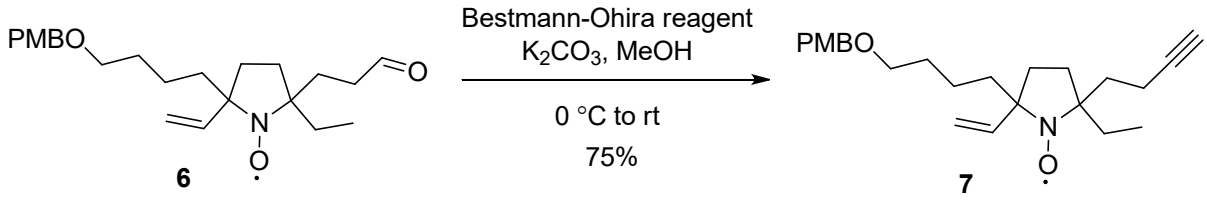


Aldehyde 6: To a solution of nitroxide **5** (251 mg, 0.641 mmol) in dimethyl sulfoxide (6 mL) was added IBX (221 mg, 0.961 mmol). Reaction mixture was stirred for 2 hours. After distilled water (20 mL) was added to above solution, white precipitate was observed. The solid was filtered under reduced pressure. Ethyl acetate (100 mL) was added to the filtrate, which was then washed with water (6 x 100 mL). Organic layer was washed with brine dried over anhydrous sodium sulphate, filtered, concentrated *in vacuo*. The crude residue was purified by column chromatography with 20% EtOAc in hexane to give 162 mg (65% yield) of aldehyde **6** as a yellow orange viscous oil.

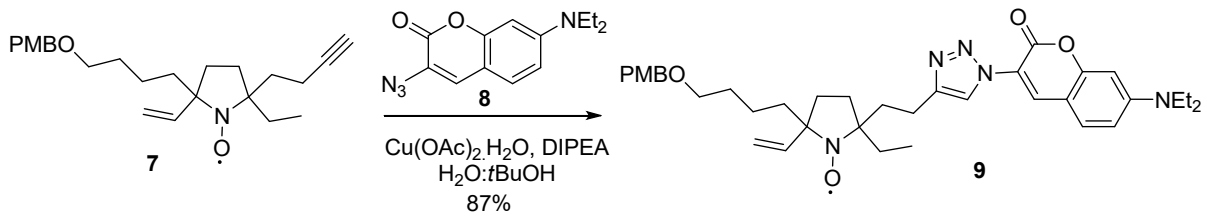
Major diastereomer (113 mg): R_f = 0.37 (15% EtOAc in hexane); ^{1}H NMR (300 MHz, $CDCl_3$) of ethoxylamine derivative: δ 9.83 (s, 1H), 7.25 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 6.19 (dd, *J* = 11.6, 17.9 Hz, 1H), 5.08 (d, 11.6 Hz, 1H), 4.98 (d, *J* = 17.9 Hz, 1H), 4.42 (s, 2H), 3.80 (s, 3H), 3.72 (q, *J* = 7.0 Hz, 2H), 3.42 (t, *J* = 6.6 Hz, 2H), 2.82-2.71 (m, 1H), 2.45-2.34 (m, 1H), 2.87-1.78 (m, 2H), 1.75-1.68 (m, 3H), 1.63-1.45 (m, 5H), 1.37-1.21 (m, 4H), 1.12 (t, *J* = 6.6 Hz, 3H), 0.82 (t, *J* = 7.5 Hz, 3H) ppm; ^{13}C NMR, DEPT (75 MHz, $CDCl_3$) of ethoxylamine derivative: δ 202.8 (CH), 158.9 (C), 140.1 (CH), 130.6 (C), 128.9 (2 x CH), 113.5 (2 x CH), 112.1 (CH_2), 72.2 (CH_2), 71.0 (CH_2), 70.4 (C), 69.7 (CH_2), 66.8 (C), 54.9 (CH_3), 39.5 (CH_2), 39.3 (2 x CH_2), 30.1 (CH_2), 29.7 (CH_2), 29.4 (CH_2), 28.1 (CH_2), 21.1 (CH_2), 14.0 (CH_3), 8.9 (CH_3) ppm; IR (thin film): 2936, 2870, 1724, 1513, 1459, 1247, 1100, 1040, 821 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{23}H_{34}NO_4$ $(M+Na)^+$ 411.2386, found 411.2387.

Minor diastereomer (50 mg): R_f = 0.44 (10% EtOAc in hexane); ^{1}H NMR (300 MHz, $CDCl_3$) of ethoxylamine derivative: δ 9.72 (s, 1H), 7.25 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 6.24 (brs, 1H), 5.09 (d, *J* = 11.0 Hz, 1H), 5.02 (d, *J* = 18.1 Hz, 1H), 4.42 (s, 2H), 3.79 (s, 3H), 3.74 (q, *J* = 7.1 Hz, 2H), 3.43 (t, *J* = 6.5 Hz, 2H), 2.49-2.44 (m, 1H), 2.37 (brs, 1H), 2.02 (brs, 1H), 1.80-1.70 (m, 2H), 1.63-1.52 (m, 7H), 1.39-1.27 (m, 4H), 1.11 (t, *J* = 7.3 Hz, 3H), 0.92 (t, *J* = 7.3 Hz, 3H) ppm; ^{13}C NMR, DEPT (75 MHz, $CDCl_3$) of ethoxylamine derivative: δ

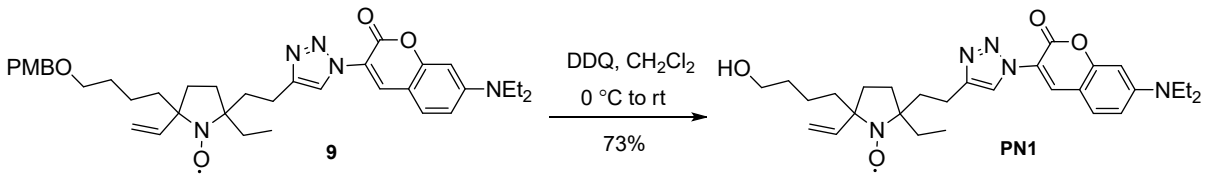
203.1 (CH), 159.1 (C), 140.9 (CH), 130.8 (C), 129.1 (2 x CH), 113.7 (2 x CH), 112.4 (CH_2), 72.4 (CH_2), 71.2 (CH_2), 70.5 (C), 70.0 (CH_2), 67.1 (C), 55.2 (CH_3), 40.2 (2 x CH_2), 31.5 (CH_2), 30.4 (2 x CH_2), 29.2 (CH_2) 28.3 (CH_2), 21.3 (CH_2), 14.2 (CH_3), 8.6 (CH_3) ppm.



Alkyne 7: To a solution of Bestmann-Ohira reagent (60 mg, 0.54 mmol) in methanol (1.5 mL) was added potassium carbonate (99 mg, 0.72 mmol) at 0˚C. Resulting mixture was stirred for 30 minutes before a solution of major diastereomer of the aldehyde **6** (141 mg, 0.363 mmol) in methanol (1.5 mL) was slowly added. The reaction mixture was stirred at room temperature for 6 hours. Methanol was removed under reduced pressure. Water (15 mL) was added to crude mixture, which was extracted with dichloromethane (3 x 15 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography with 20% ethyl acetate in hexane to give 105 mg (75% yield) of alkyne **7** as a yellow viscous oil. R_f = 0.50 (20% EtOAc in hexane); ^{1}H NMR (300 MHz, $CDCl_3$) of ethoxylamine derivative: δ 7.26 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 6.19 (dd, *J* = 11.1 Hz, 17.1, 1H), 5.07 (d, *J* = 11.1 Hz, 1H), 4.98 (d, *J* = 17.1 Hz, 1H), 4.42 (s, 2H), 3.80 (s, 3H), 3.75-3.73 (m, 2H), 3.42 (t, *J* = 7.1 Hz, 2H), 2.58 (brs, 1H), 2.17 (m, 1H), 1.93 (t, *J* = 2.6 Hz, 1H), 1.85-1.78 (m, 2H) 1.64-1.52 (m, 6H), 1.51-1.42 (m, 3H), 1.35-1.22 (m, 3H), 1.12 (t, *J* = 7.1 Hz, 3H), 0.81 (t, *J* = 7.5 Hz, 3H) ppm; ^{13}C NMR, DEPT (75 MHz, $CDCl_3$) of ethoxylamine derivative: δ 159.1 (C), 140.6 (CH), 130.8 (C), 129.1 (2 x CH), 113.7 (2 x CH), 112.2 (CH_2), 85.9 (C), 72.5 (CH_2), 71.2 (CH_2), 70.3 (C), 70.0 (CH_2), 67.6 (CH), 67.2 (C), 55.2 (CH_3), 39.9 (CH_2), 37.6 (CH_2), 30.4 (CH_2), 29.7 (CH_2), 29.3 (CH_2), 28.4 (CH_2), 21.3 (CH_2), 14.3 (CH_3), 13.6 (CH_2), 9.0 (CH_3) ppm; IR (thin film): 3306, 2935, 1614, 1514, 1463, 1247, 1040 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{24}H_{34}NO_3$ $(M+Na)^+$ 407.2436, found 407.2439.

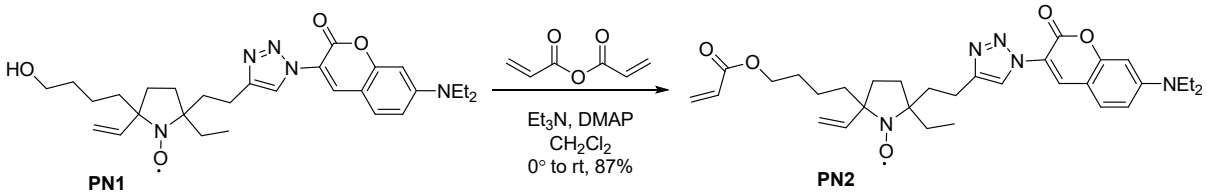


Triazole 9: To a solution of alkyne **7** (104 mg, 0.270 mmol) in ^{t}BuOH (39 mL) was added 3-azido-7-(diethylamino)-2*H*-chromen-2-one (**8**, 70 mg, 0.27 mmol), DIPEA (47 µL, 0.27 mmol) and cupric acetate hydrate (5 mg, 0.03 mmol) in distilled water (1 mL), respectively. The reaction mixture was stirred at room temperature for 6 hours. Extra distilled water (15 mL) was added to crude mixture, which was extracted with dichloromethane (3 x 15 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered, and concentrated *in vacuo*. The crude residue purified by column chromatography with 20% ethyl acetate in hexane, followed by 40% ethyl acetate in hexane to give 151.2 g (87% yield) of triazole **9** as a bright yellow viscous oil. R_f = 0.50 (40% EtOAc in hexane); ^{1}H NMR (300 MHz, $CDCl_3$) of ethoxylamine derivative: δ 8.29 (s, 1H), 8.28 (s, 1H), 7.33 (d, *J* = 8.9 Hz, 1H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 6.61 (dd, *J* = 2.1, 8.9 Hz, 1H), 6.48 (d, *J* = 2.1 Hz, 1H), 6.32-6.23 (m, 1H), 5.09 (d, *J* = 11.5 Hz, 1H), 5.03 (d, *J* = 17.7 Hz, 1H), 4.42 (s, 2H), 3.85-3.78 (m, 2H), 3.75 (s, 3H), 3.46 (t, *J* = 6.9 Hz, 2H), 3.39 (q, *J* = 7.1 Hz, 4H), 3.24 (brs, 1H), 2.76-2.68 (m, 1H), 2.02-1.96 (m, 1H), 1.66-1.53 (m, 8H), 1.85-1.75 (m, 3H), 1.41-1.31 (m, 2H), 1.20 (t, *J* = 7.1 Hz, 6H), 1.15 (t, *J* = 6.9 Hz, 3H), 0.87 (t, *J* = 7.1 Hz, 3H) ppm; ^{13}C NMR, DEPT (75 MHz, $CDCl_3$) of ethoxylamine derivative: δ 158.8 (C), 156.6 (C), 155.3 (C), 151.1 (C), 148.6 (C), 140.6 (CH), 133.9 (CH), 130.5 (C), 129.6 (CH), 128.8 (2 x CH) 121.1 (CH), 116.9 (C), 113.4 (2 x CH), 111.8 (CH_2), 109.7 (CH), 106.8 (C), 96.6 (CH), 72.1 (CH_2), 70.9 (CH_2), 70.2 (C), 69.8 (CH_2), 67.3 (C), 54.8 (CH_3), 44.6 (2 x CH_2), 39.6 (CH_2), 37.8 (CH_2), 30.1 (CH_2), 29.8 (CH_2), 29.2 (CH_2), 28.3 (CH_2), 21.0 (CH_2), 20.5 (CH_2), 14.3 (CH_3), 12.1 (2 x CH_3), 8.9 (CH_3) ppm; IR (thin film): 2950, 2846, 1721, 1628, 1615, 1524, 1454, 1248, 1096, 835 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{37}H_{48}N_5O_5$ $(M+Na)^+$ 665.3553, found 665.3558.



Probe PN1: To a solution of triazole **11** (743 mg, 1.16 mmol) in dichloromethane (10 mL) was added DDQ (350 mg, 1.73 mmol) at 0 °C. Distilled water (500 µL) was added to the above solution. The reaction mixture was stirred for 2 hrs. Extra distilled water (20 mL) was added to crude mixture, which was then extracted with dichloromethane (3 x 20 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered, and concentrated *in vacuo*. The crude residue was purified by column chromatography with 20% ethyl acetate in hexane, followed by 60% EtOAc in hexane to give 447 mg (73% yield) of probe **PN1** as a bright yellow

viscous oil. R_f = 0.50 (50% EtOAc in hexane); ^{1}H NMR (300 MHz, $CDCl_3$) of ethoxylamine derivative: δ 8.17 (s, 1H), 8.13 (s, 1H), 7.25 (d, *J* = 8.9 Hz, 1H), 6.52 (dd, *J* = 1.7, 8.9 Hz, 1H), 6.39 (s, 1H), 6.14 (brs, 1H), 4.97 (d, *J* = 10.6 Hz, 1H), 4.92 (d, *J* = 16.6 Hz, 1H), 3.72-3.64 (m, 2H), 3.53 (t, *J* = 6.5 Hz, 2H), 3.30 (q, J = 7.0 Hz, 4H), 2.85 (brs, 1H), 2.75-2.61 (m, 2H), 2.00-1.94 (m, 1H), 1.76-1.66 (m, 1H), 1.55-1.43 (m, 10H), 1.26-1.21 (m, 2H), 1.09 (t, *J* = 7.0 Hz, 6H), 1.01 (t, *J* = 6.5 Hz, 3H), 0.85 (t, *J* = 6.8 Hz, 3H), ppm; ^{13}C NMR, DEPT (75 MHz, $CDCl_3$) of ethoxylamine derivative: δ 156.9 C), 156.6 (C), 151.4 (C), 148.8 (C), 140.7 (CH), 134.4 (CH), 129.8 (CH), 121.4 (CH), 116.9 (C) 112.4 (CH_2), 110.0 (CH), 107.0 (C), 96.8 (CH), 71.2 (CH_2), 70.5 (C), 67.5 (C), 62.6 (CH_2), 44.9 (2 x CH_2), 39.9 (CH_2), 36.8 (CH_2), 34.4 (CH_2), 31.2 (CH_2), 30.3 (CH_2), 28.3 (CH_2), 21.4 (CH_2), 20.9 (CH_2), 14.3 (CH_3), 12.3 (2 x CH_3), 8.9 (CH_3) ppm; IR (thin film): 3393, 2972, 2934, 1723, 1624, 1607, 1430, 1353, 1132, 1042 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{29}H_{40}N_5O_5$ $(M+Na)^+$ 545.2978, found 545.2971.

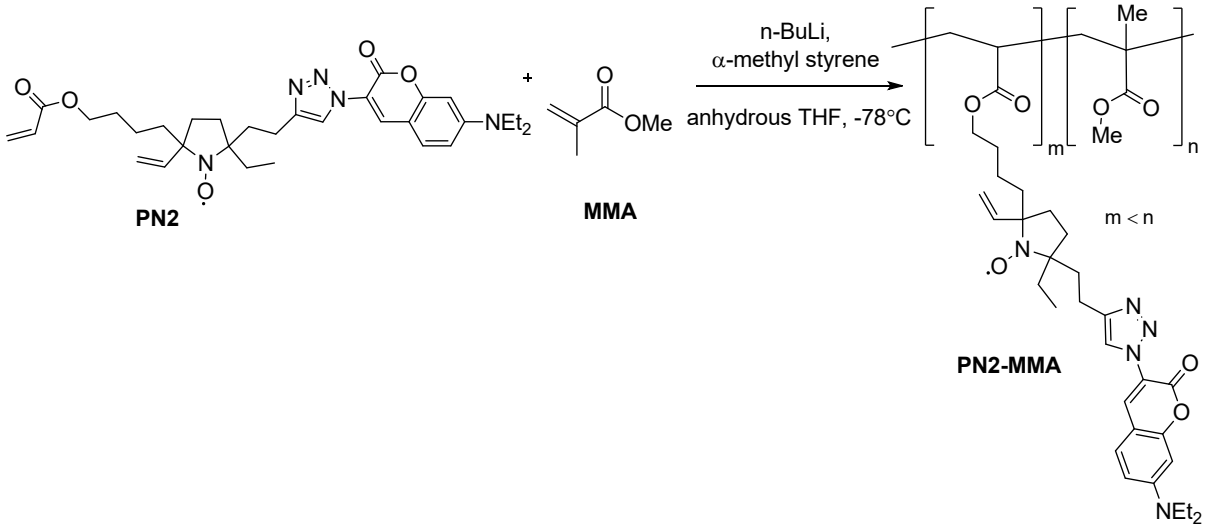


Monomer PN2: To a solution of probe **PN1** (179 mg, 0.342 mmol) in THF (3 mL) was added 4-dimethylaminopyridine (DMAP) (4 mg, 0.03 mmol) and triethylamine (187 µL, 1.34 mmol), respectively. Acrylic anhydride (86 µL, 0.75 mmol) was added to the above solution at 0 °C. The reaction mixture was allowed to stir at room temperature for 6 hours. After the mixture was quenched with saturated ammonium chloride (1 mL) at 0 °C, and the organic layer was separated. Aqueous layer was extracted with dichloromethane (3 x 10 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography with 20% EtOAc in hexane to give 296 mg (87% yield) of the corresponding acrylate **PN2** as a yellow viscous oil. R_f = 0.33 (30% EtOAc in hexane); ^{1}H NMR (300 MHz, $CDCl_3$) of ethoxylamine derivative: δ 8.33 (s, 1H), 8.27 (s, 1H), 7.38 (d, *J* = 8.9 Hz, 1H), 6.65 (d, *J* = 8.9 Hz, 1H), 6.54 (s, 1H), 6.37 (d, *J* = 17.2 Hz, 1H), 6.27-6.18 (m, 1H), 6.10 (dd, *J* = 10.5, 17.2 Hz, 1H), 5.79 (d, *J* = 10.5 Hz, 1H), 5.11 (d, *J* = 10.9 Hz, 1H), 5.03 (d, *J* = 18.0 Hz, 1H), 4.14 (t, *J* = 6.6 Hz, 2H), 3.86 (brs, 2H), 3.43 (q, *J* = 6.8 Hz, 1H), 3.17 (brs, 1H), 2.76-2.68 (m, 1H), 1.91-1.87 (m, 11H), 1.38-1.31 (m, 2H), 1.22 (t, *J* = 6.8 Hz, 6H), 1.14 (t, *J* = 6.6 Hz, 3H), 0.84 (t, *J* = 6.0 Hz, 3H) ppm; ^{13}C NMR, DEPT (75 MHz, $CDCl_3$) of ethoxylamine derivative: δ 166.3 (C), 157.1 C), 155.7 (C), 151.4 (C), 148.9 (C),

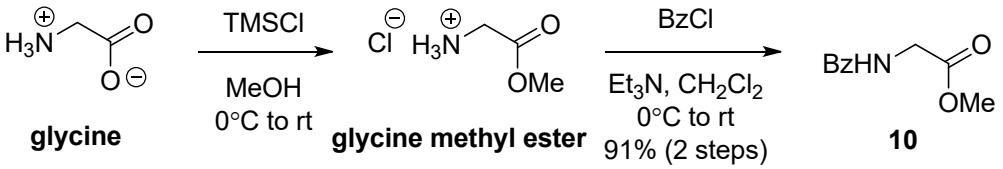
140.3 (CH), 134.5 (CH), 130.5 (CH_2), 129.9 (CH), 128.6 (CH), 121.4 (CH), 117.2 (C) 112.5 (CH_2), 110.0 (CH), 107.0 (C), 96.8 (CH), 71.4 (CH_2), 70.6 (C), 67.9 (C), 64.6 (CH_2), 45.0 (2 x CH_2), 39.6 (CH_2), 38.0 (CH_2), 30.2 (CH_2), 29.7 (CH_2), 29.2 (CH_2), 28.4 (CH_2), 21.1 .(CH_2), 20.8 (CH_2), 14.3 (CH_3), 12.7 (2 x CH_3), 9.1 (CH_3) ppm; IR (thin film): 2972, 1723, 1605, 1431, 1189, 1040 cm^{-1}; HRMS (ESI) *m/z* calcd for $C_{32}H_{42}N_5O_5$ $(M+Na)^+$ 599.3084, found 599.3080.

All of synthesized nitroxides were converted to diamagnetic ethoxylamine derivatives using the general procedure for nitroxide radical trapping, following Tansakul's protocol[2] for structural analysis by ^1H and ^{13}C NMR.

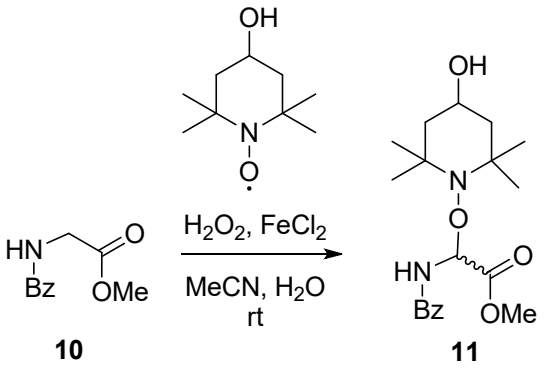
General procedure for nitroxide radical trapping: To a solution of nitroxide derivative (1.0 equiv) in toluene (0.1 M) was added triethylborane (1M in THF, 1.5 equiv) at 0 °C. The reaction mixture was allowed to stir at room temperature under open air for 1-2.5 hrs. Toluene was removed *in vacuo*. The crude residue was purified by column chromatography to give the corresponding ethoxylamine derivative.



PN2-MMA copolymer: To a solution of α-methylstyrene (1.2 μL, 9.3 μmol) in anhydrous THF (40 mL) was added n-BuLi (1M in hexane, 10 μL, 10 μmol). The resulting mixture was stirred for 5 min, and then a mixture of methyl methacrylate (MMA, 500 μL, 4.69 mmol) and probe **PN2** (120 mg, 0.208 mmol) were slowly added to the solution. After reaction mixture was stirred for 30 min at -78 °C, methanol was added to terminate the polymerization. Hexane was added to crude polymer until precipitate was observed. The precipitate was filtered and washed with hexane to give 525 mg of the corresponding copolymer as a yellow solid.



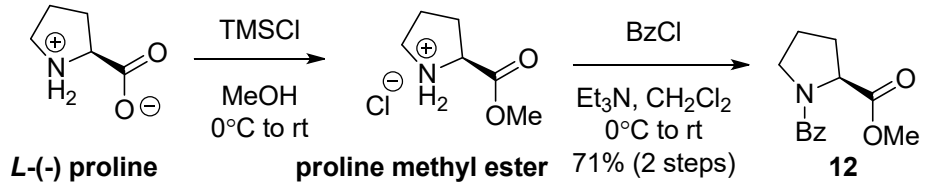
***N*-benzoyl glycine methyl ester**[3]: A suspension of glycine (500 mg, 6.65 mmol) in TMSCl (1.68 mL, 13.3 mmol) was stirred at 0 ◦C for 10 min. Methanol (7 mL) was then added to the suspension. The mixture was allowed to stir at rt overnight. Excess of TMSCl and methanol was evaporated under vacuum to give the corresponding glycine methyl ester as a slightly yellow oil, which was used for next step without further purification. ^{1}H NMR (300 MHz, D_2O): δ 3.95 (s, 2H), 3.86 (s, 3H) ppm; ^{13}C NMR, DEPT (75 MHz, D_2O): δ 168.6 (C), 53.4 (CH_3), 40.1 (CH_2) ppm. The methyl ester derivative (300 mg, 2.39 mmol) was dissolved in CH_2Cl_2, and was subsequently basified by Et_3N (990 µL, 7.14 mmol). Benzoyl chloride (415 µL, 3.58 mmol) was then added to the above solution at 0 ◦C. The mixture was allowed to warm to rt, and stirred overnight. The crude mixture was washed with sat. Na_2CO_3 (3 x 25 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography with 20-40% EtOAc in hexane to give 420 mg 91% (2 steps) of the corresponding benzamide **10** as a pale-yellow viscous oil. R_f = 0.17 (30% EtOAc in hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81 (d, J = 7.2 Hz, 2H), 7.52 (t, J = 7.2 Hz, 1H), 7.44 (t, J = 7.2 Hz, 2H), 6.70 (brs, 1H), 4.26 (d, J = 5.4 Hz, 2H), 3.80 (s, 3H) ppm; ^{13}C NMR, DEPT (75 MHz, $CDCl_3$): δ 170.6 (C), 167.8 (C), 133.6 (C), 131.8 (CH), 128.5 (2 x CH), 127.1 (2 x CH), 52.4 (CH_3), 41.7 (CH_2) ppm.



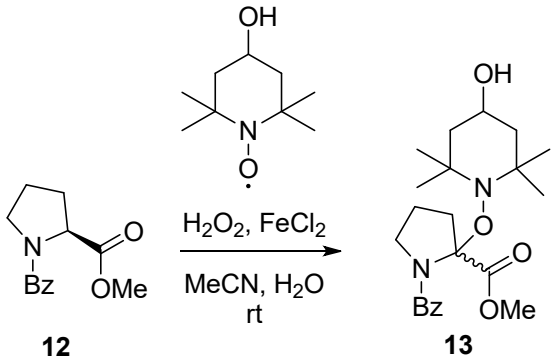
TEMPOL-glycine adduct: A solution of *N*-benzoyl glycine methyl ester (**10**, 163 mg, 0.85 mmol), TEMPOL (120 mg, 0.696 mmol) and $FeCl_2$ (10 mg, 0.085 mmol) in 1:1 H_2O: MeCN

(8 mL) was stirred under N_2 atmosphere for 3 h. The crude mixture was concentrated *in vacuo* to a crude product of **11**.

HRMS (ESI) *m*/*z* calcd for $C_{19}H_{28}N_2O_5Na^+$ $(M+Na)^+$ 387.1896, found 387.1899)



***N*-Benzoyl proline methyl ester**[4]: A suspension of *L*-(-)-proline (500 mg, 4.34 mmol) in TMSCl (1.10 mL, 6.68 mmol) was stirred at 0 ◦C for 10 min. Methanol (4.5 mL) was then added to the suspension. The mixture was allowed to stir at rt overnight. The crude product was concentration under vacuum to remove excess TMSCl and methanol. ^{1}H NMR (300 MHz, D_2O): δ 4.52-4.47 (m, 1H), 3.84 (s, 3H), 3.48-3.38 (m, 2H), 2.51-2.39 (m, 1H), 2.24-2.14 (m, 1H), 2.12-2.02 (m, 2H) ppm; ^{13}C NMR, DEPT (75 MHz, D_2O): δ 170.0 (C), 59.3 (CH), 53.8 (CH_3), 46.2 (CH_2), 27.9 (CH_2), 23.1 (CH_2) ppm. The product was used for next step without further purification. The proline methyl ester (718 mg, 4.34 mmol) was dissolved in CH_2Cl_2 (40 mL), and was subsequently basified with Et_3N (1.82 mL, 13.0 mmol) at 0 ◦C. Benzoyl chloride was then added to the above solution. The reaction mixture was allowed to stir at rt overnight. Crude product was washed with aq. Na_2CO_3 (3 x 40 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. Crude residue was purified by column chromatography with 20% EtOAc to give 719 mg (71%, 2 steps) of the corresponding benzamide **12** as a white solid. R_f = 0.30 (40% EtOAc in hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57 (d, *J* = 7.6 Hz, 2H), 7.47-7.45 (m, 1H), 7.41-7.36 (m, 1H), 4.67 (dd, *J* = 5.1, 7.6 Hz, 1H), 3.78 (s, 3H), 3.69-.3.61 (m, 1H), 3.56-3.49 (m, 1H), 2.37-2.28 (m, 1H), 2.08-1.95 (m, 2H), 1.93-1.81 (m, 1H) ppm; ^{13}C NMR, DEPT (75 MHz, $CDCl_3$): δ 172.8 (C), 169.8 (C), 136.2 (C), 130.3 (CH), 128.3 (2 x CH), 127.4 (2 x CH), 59.2 (CH), 52.3 (CH_3), 50.0 (CH_2), 29.5 (CH_2) 25.5 (CH_2) ppm.



12 **13**

TEMPOL-proline adduct: A solution of benzoyl protected proline methyl ester (**12**, 172 mg, 0.748 mmol), TEMPOL (108 mg, 0.627 mmol) and $FeCl_2$ (9.5 mg, 0.0748 mmol) in 1:1 H_2O:MeCN (7 mL) was stirred under N_2 atmosphere for 3 h. The crude mixture was concentrated *in vacuo* to a crude product of **13**.

HRMS (ESI) *m*/*z* calcd for $C_{22}H_{33}N_2O_5^+$ $(M+H)^+$ 405.2384, found 405.2382;

$C_{22}H_{32}N_2O_5Na^+$ $(M+Na)^+$ 427.2203, found 427.2205.

Cell viability assay of L-929 cell lines

PN1 was dissolved in sterile deionized water into different concentrations. L-929 fibroblasts was cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA). The medium was supplemented with 10% fetal bovine serum and 10,000U/mL penicillin-streptomycin. The cell lines were incubated at 37 °C, 5% CO_2, and 95% humidity to reach 80% cell confluent. 100 µL suspension with 30,000 cells/cm^2 cell density was inoculated into the 96-well plates. After incubation for 24 hours, culture medium in each well was replaced with 100 µL of fresh culture medium and 1 µL of **PN1** solution. The cells were incubated for 24 hours. MTT assays were performed following ISO 10993-5 (n=3). After blank subtraction, the percentage of living cells (% viability) was determined using the following equation. The tests were performed in triplicates. The toxicity concentration at 50% (TC_{50}) was determined from dose-response curve.

$$\%\ viability\ = \frac{mean\ OD\ from\ wells\ with\ PN1}{mean\ OD\ from\ wells\ without\ PN1} \times 100$$

Fluorescence titration of DCFH with ROS

As a control experiment for detection of ROS, a common dye dichlorodihydrofluorescein diacetate (DCFH-DA) was used as a reference for fluorescence titration.[5] To a suspension of DCFH-DA (1 mg) in 50 mL of 1:9 EtOH:H_2O (by volume) was added a solution of NaOH (0.02 M, 10 mL). The solvent was adjusted to 100 mL to produce a concentration of 20 μM. The mixture was then stirred for 30 min under dark condition to obtain deacetylated product (dichlorodihydrofluorescein, DCFH). 10 mL of DCFH stock solution was transferred into 100 mL volumetric flask, and the volume was adjusted to 100 mL with 1:9 EtOH:H_2O to give 2 μM of DCFH solution. To the corresponding DCFH solution (100 μL) was added various concentrations of H_2O_2 ranging from 0-18 μM in 1:9 EtOH:H_2O (100 μL) in the presence of Fe(II) (0.2 μM, 1 μL). The mixture was incubated for 30 min. Fluorescence emission spectra were recorded with excitation wavelength of 490 nm using excitation/emission slit widths of 10 nm to establish the fluorescence titration profile. Fluorescence intensities at 525 nm were plotted against final concentration of H_2O_2. Curve fitting of linear calibration profile between final concentration of hydrogen peroxide (0.5-3 μM,), and the corresponding fluorescence intensity was also performed.

Figures

^{1}H and ^{13}C NMR spectra

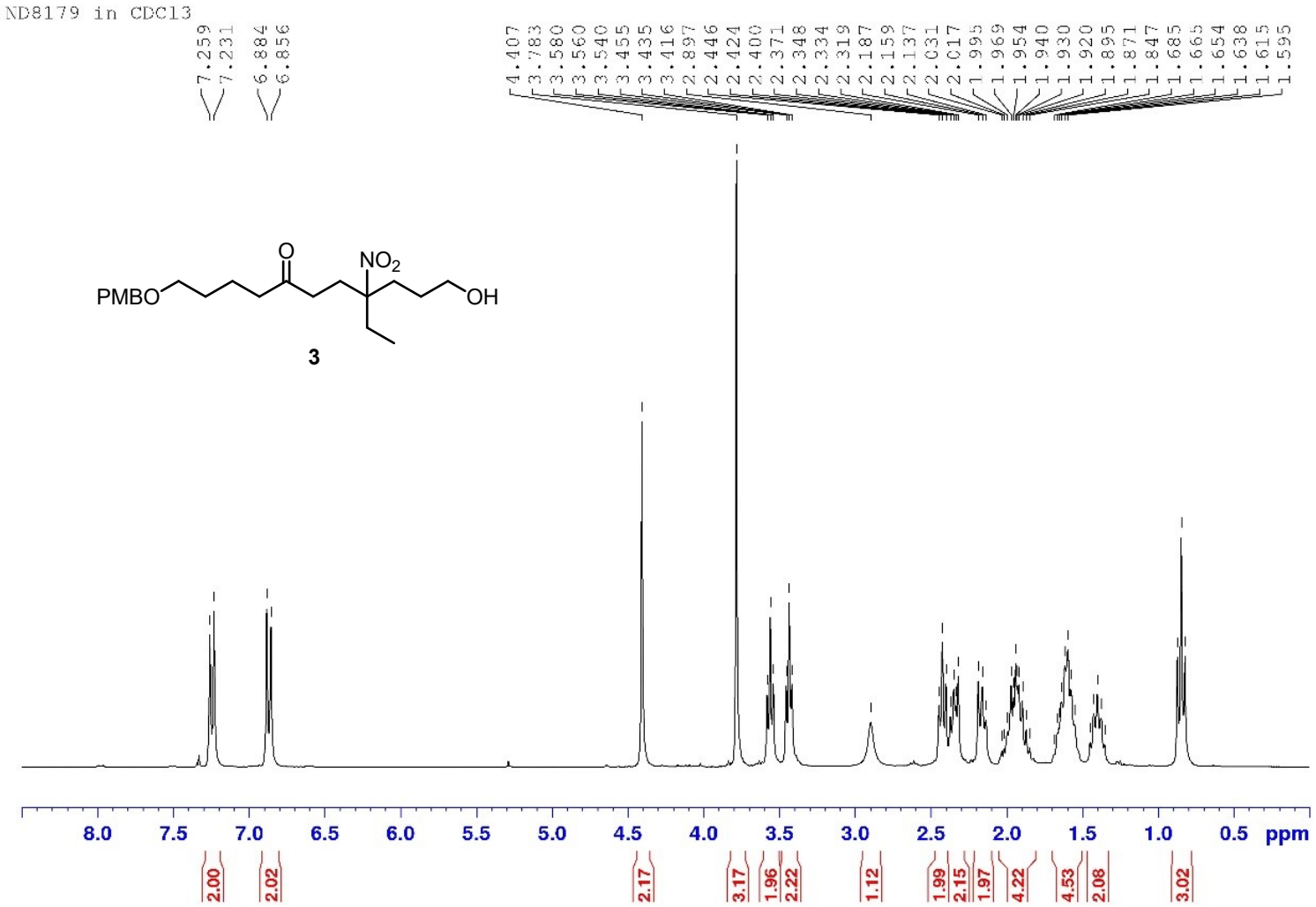


Figure S1. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of γ-nitro ketone **3**

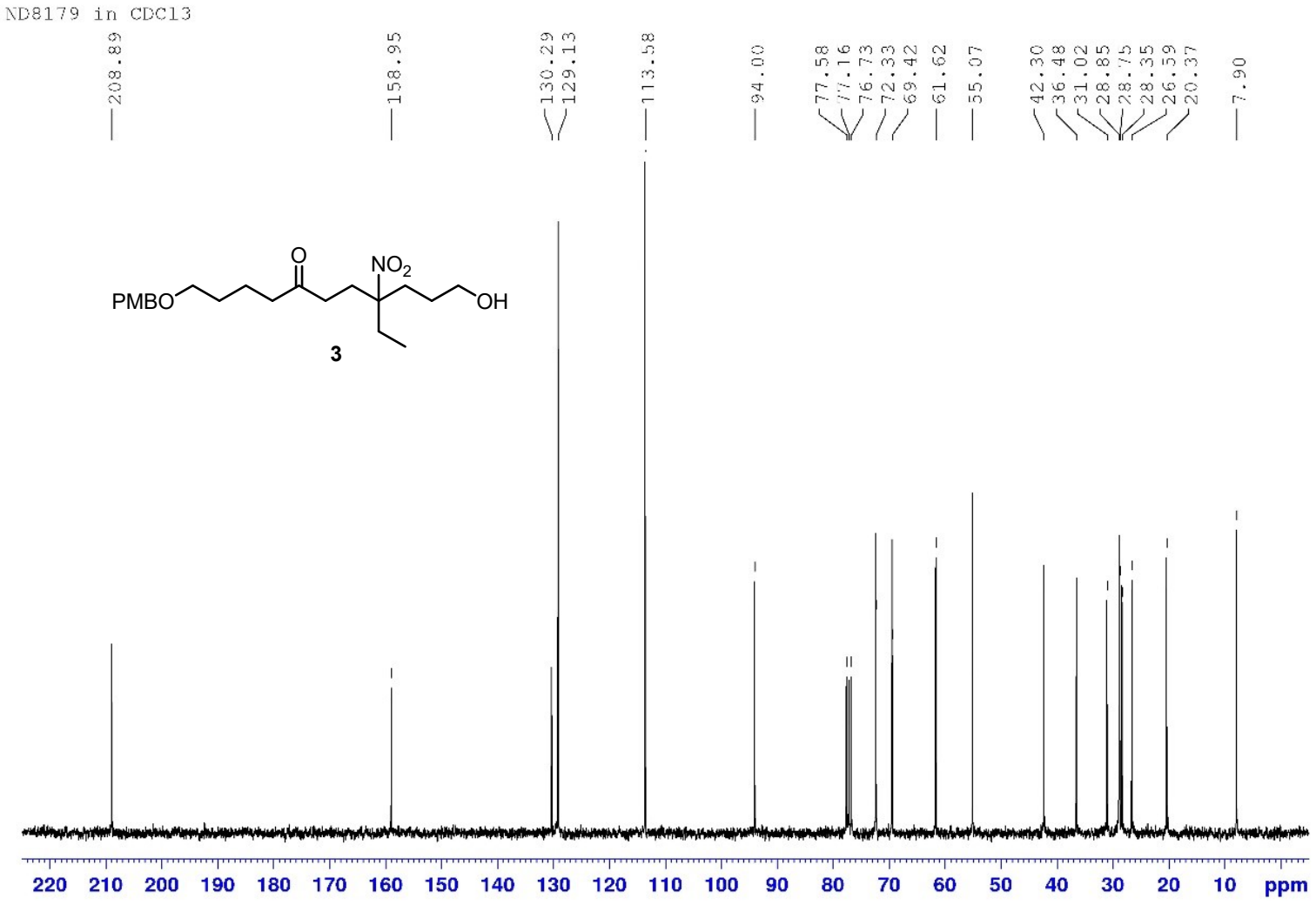


Figure S2. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of γ-nitro ketone **3**

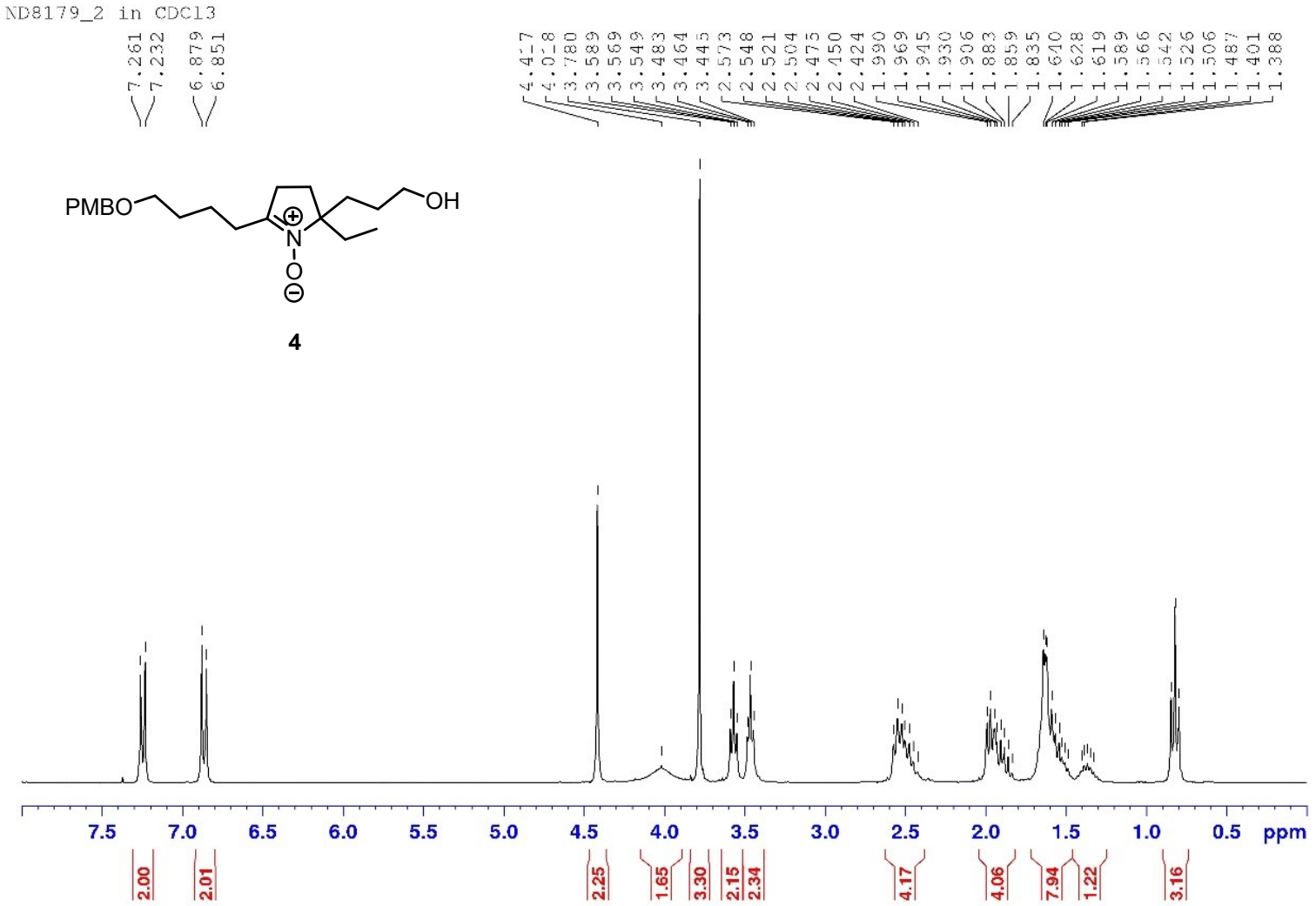


Figure S3. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of nitrone **4**

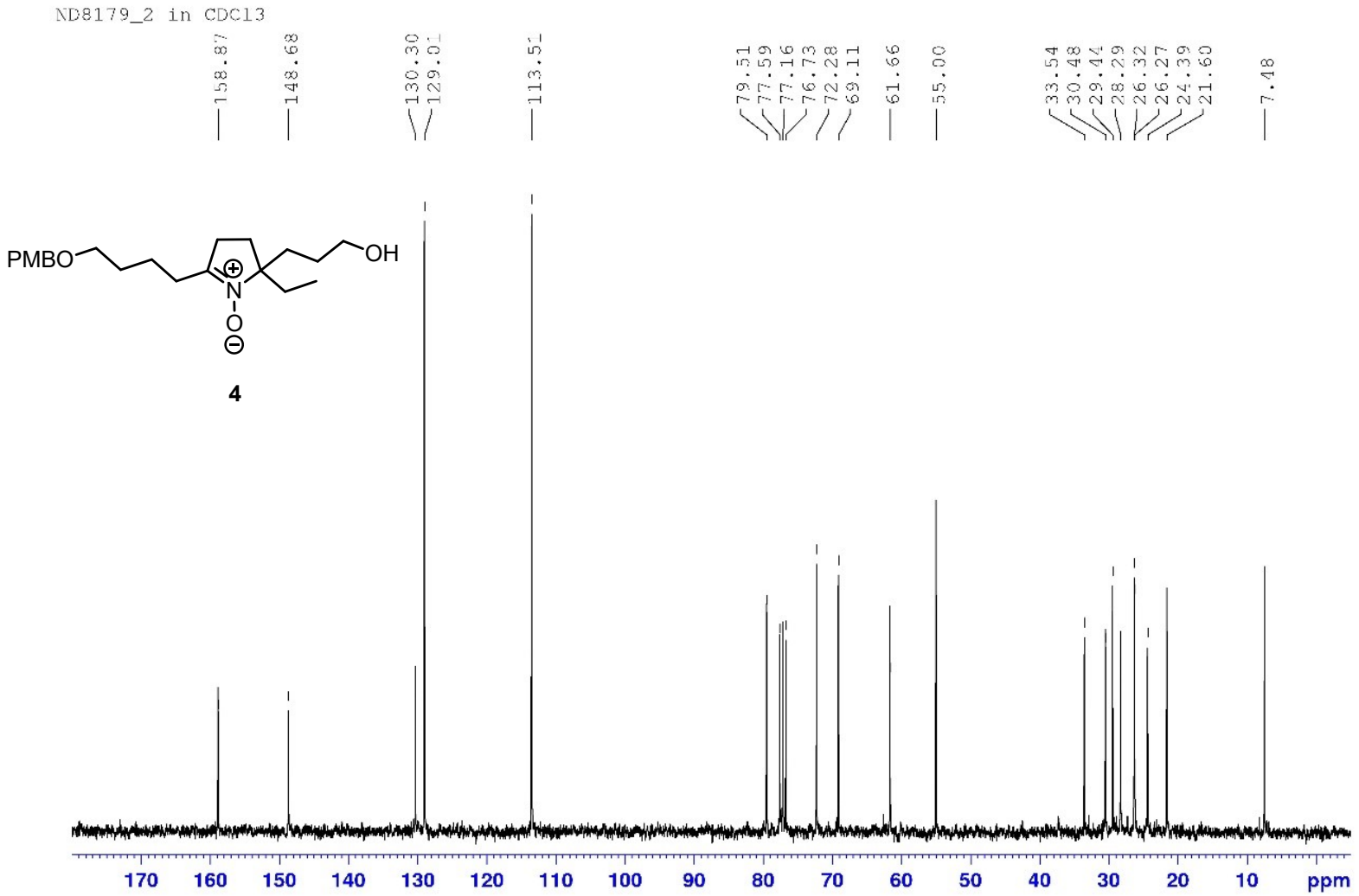


Figure S4. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of nitrone **4**

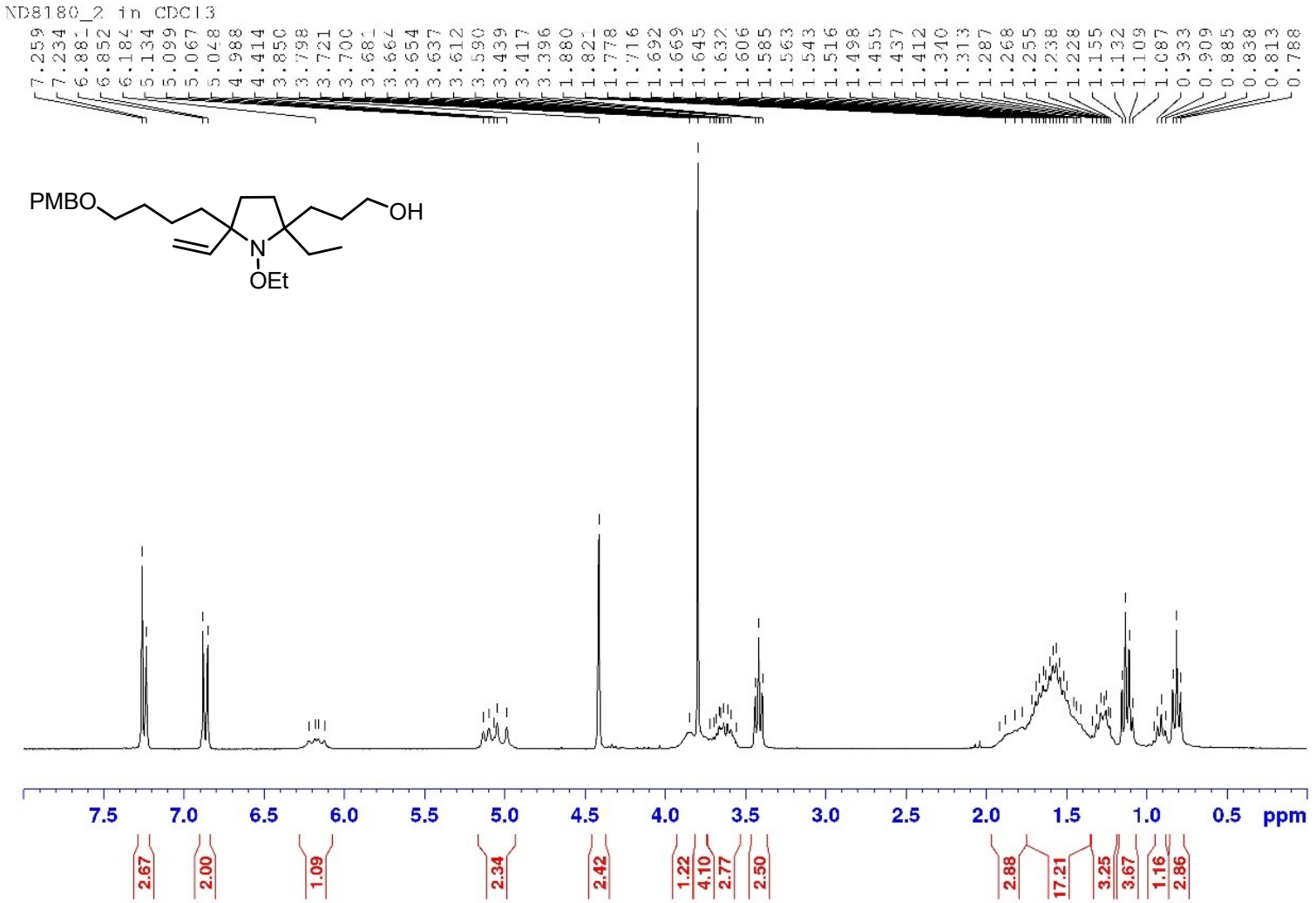


Figure S5. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of nitroxide **5**

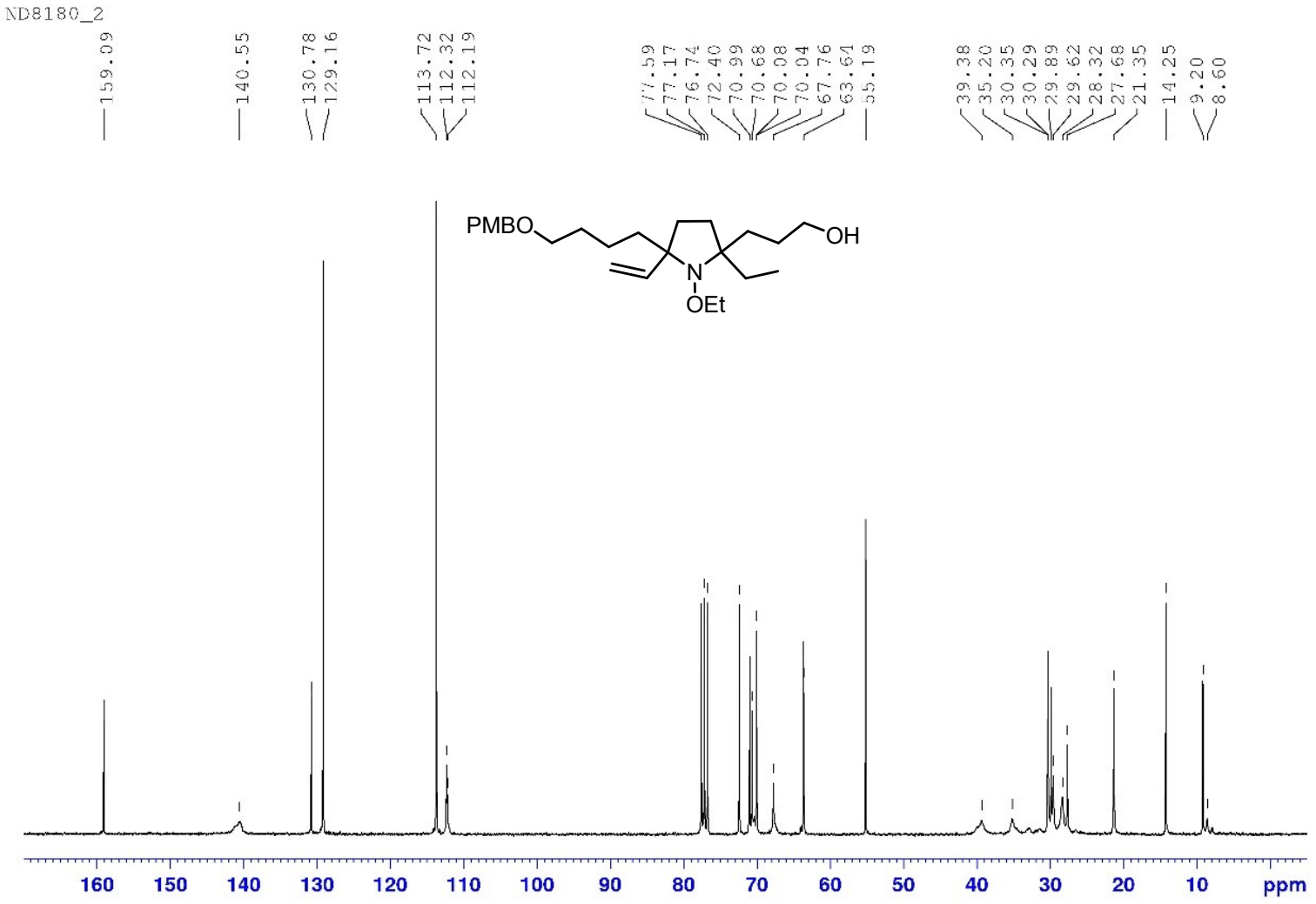


Figure S6. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of nitroxide **5**

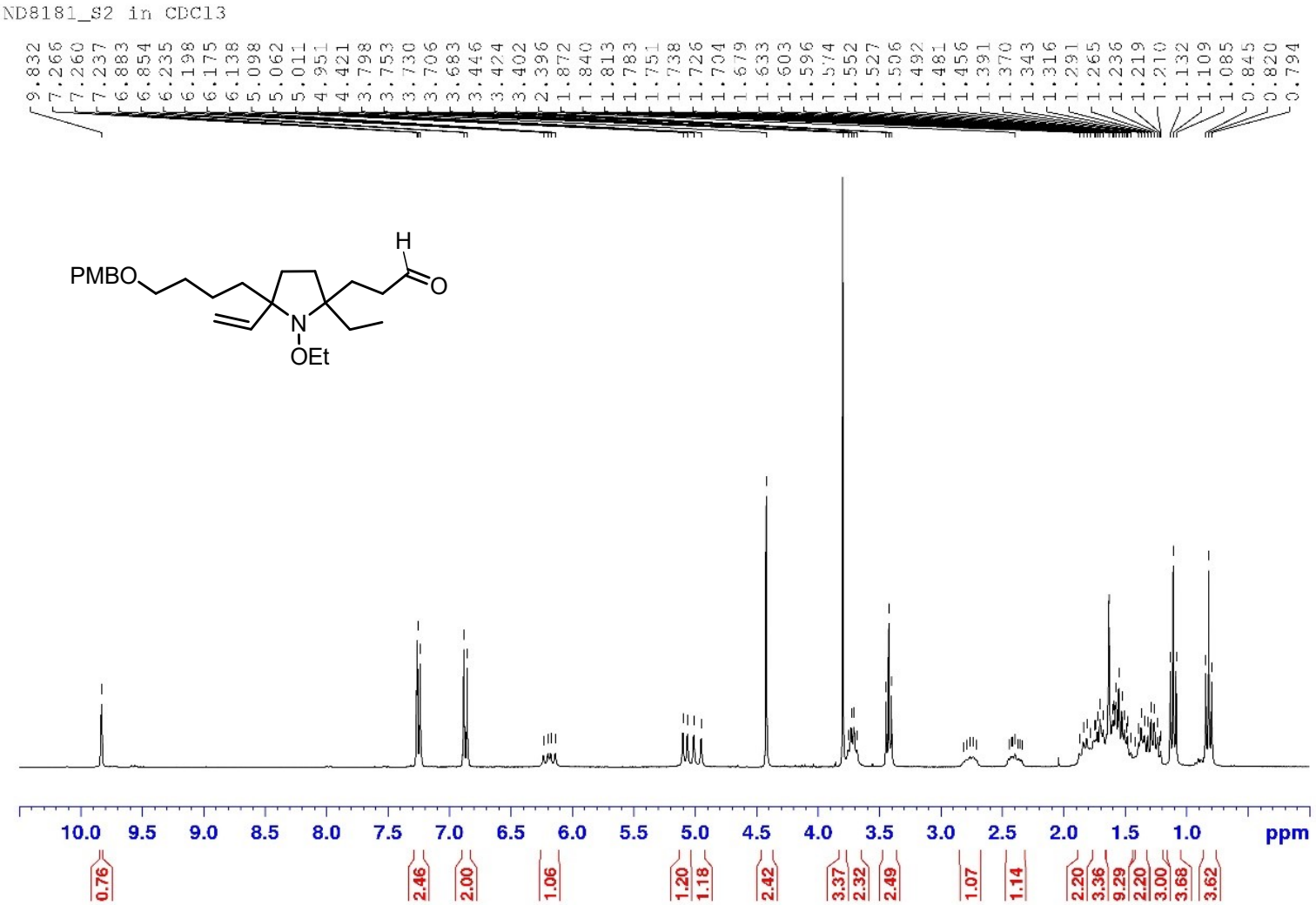


Figure S7. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of major diastereomer of aldehyde **6**

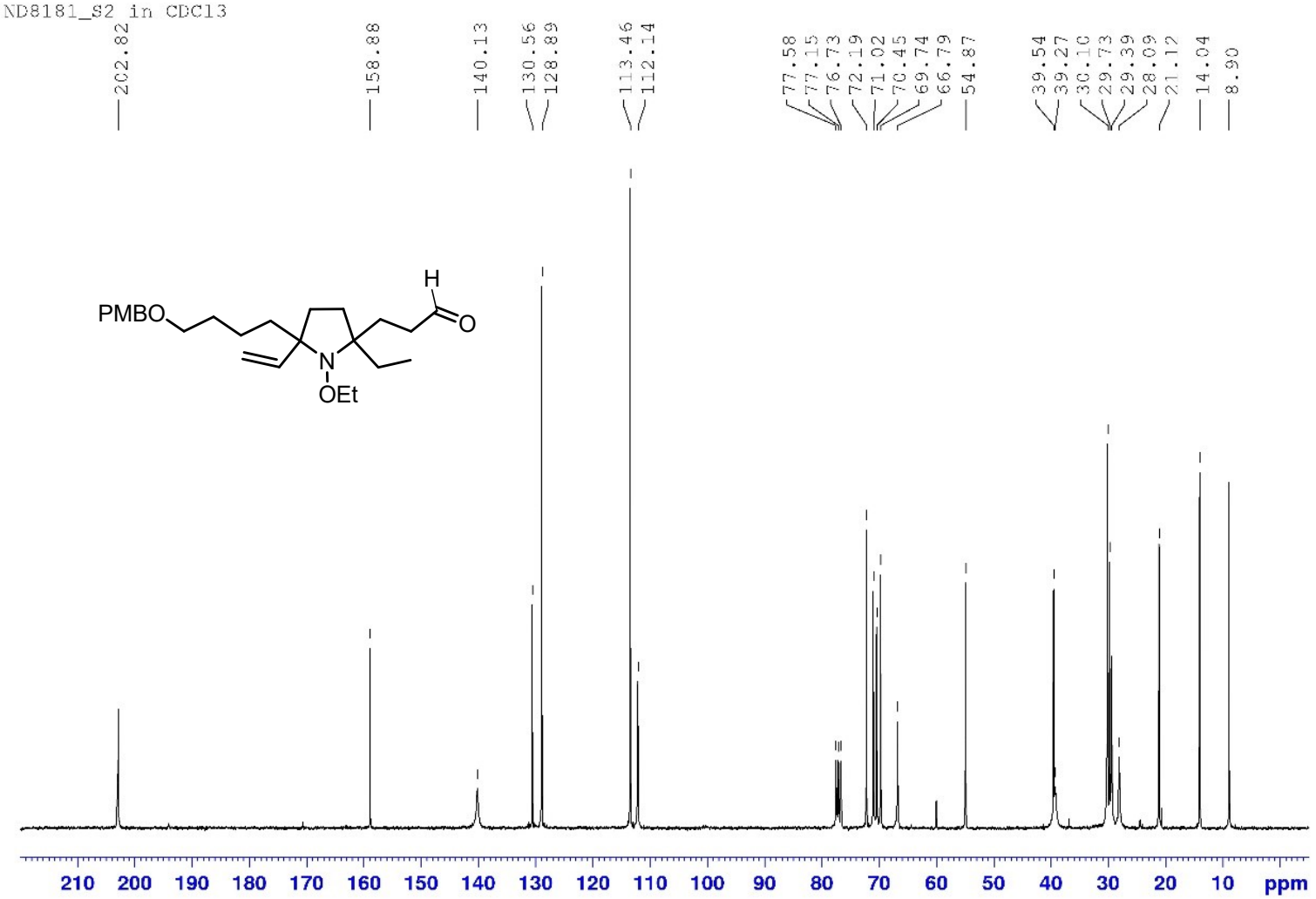


Figure S8. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of major diastereomer of aldehyde **6**

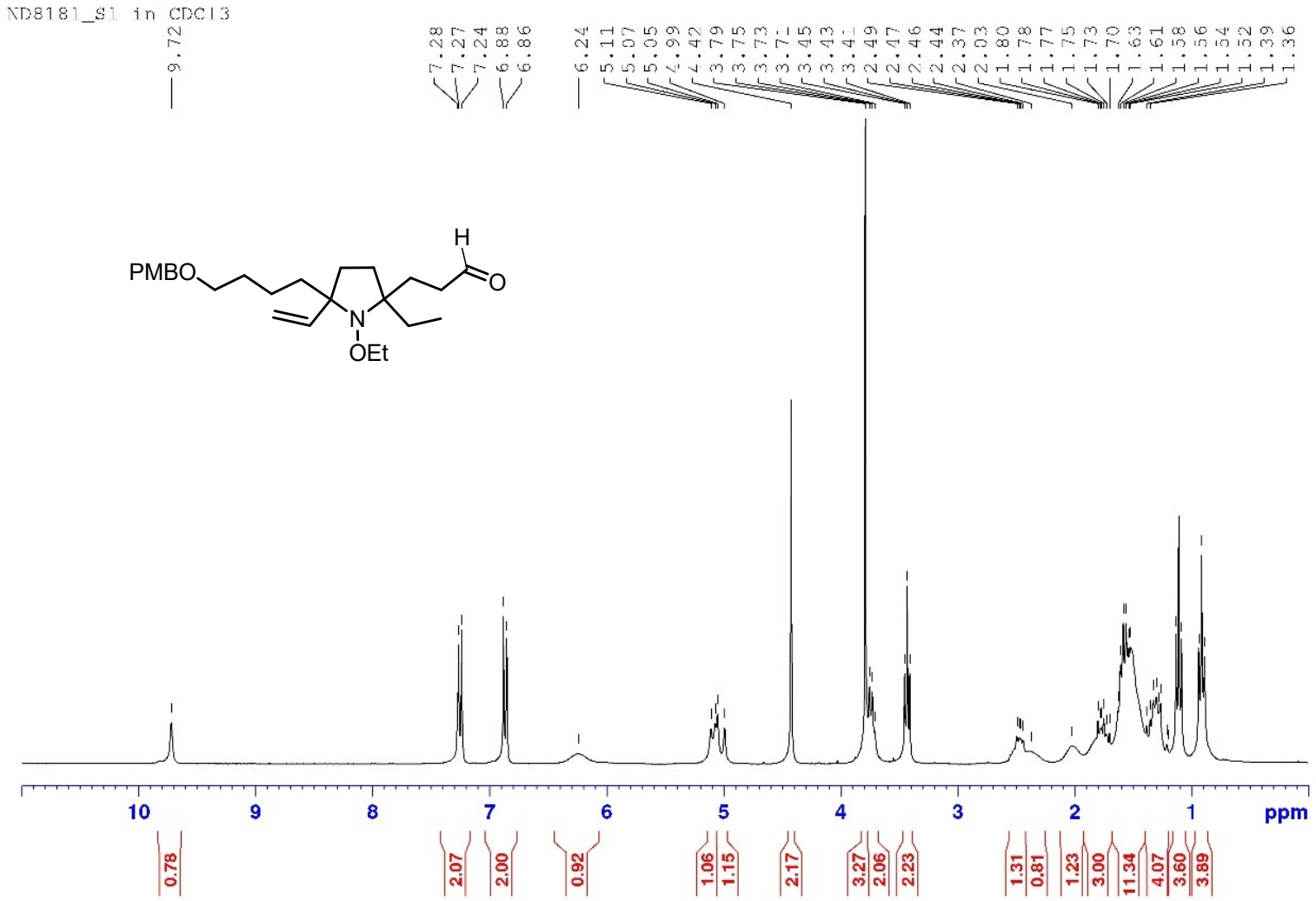


Figure S9. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of minor diastereomer of aldehyde **6**

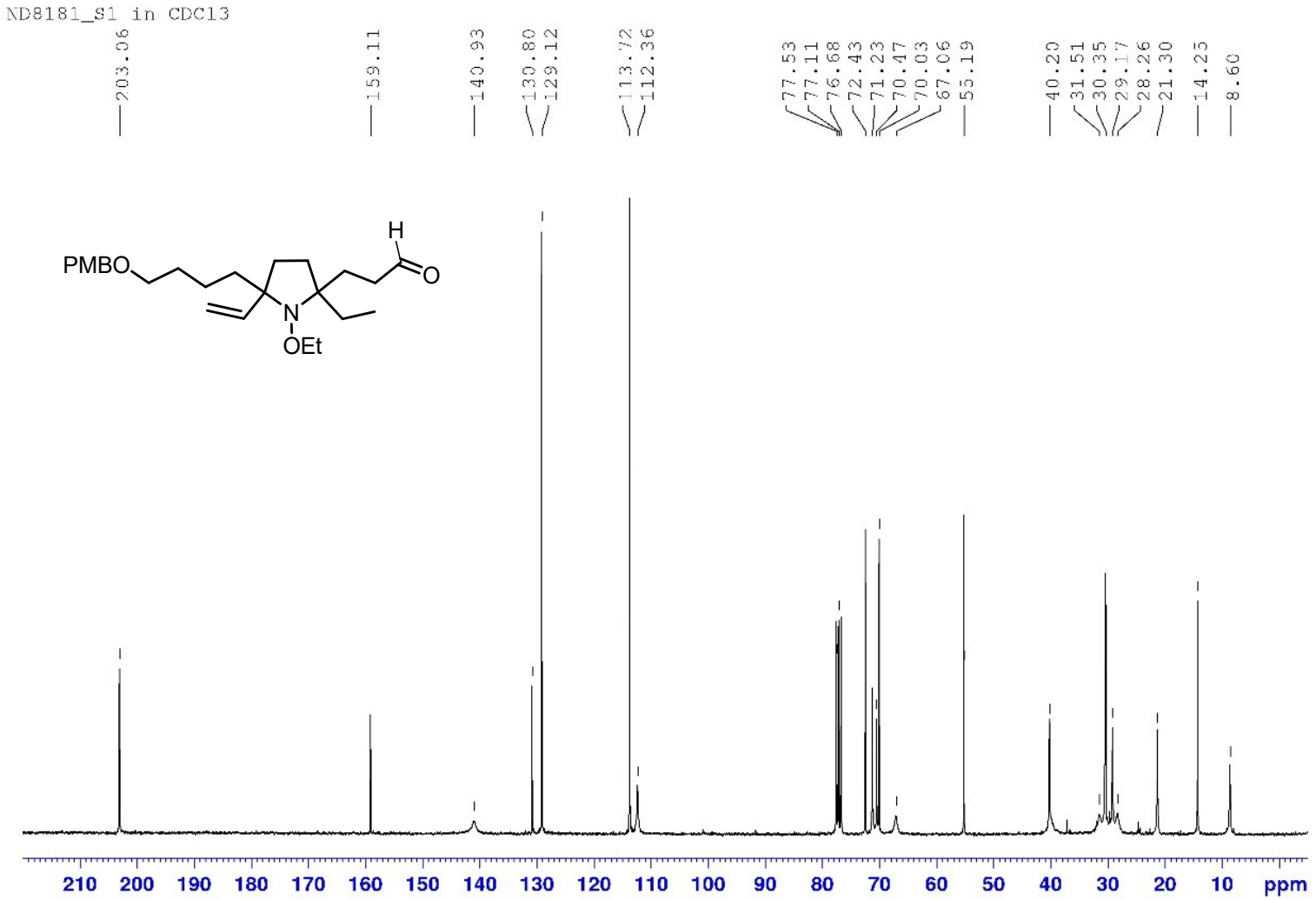


Figure S10. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of minor diastereomer of aldehyde **6**

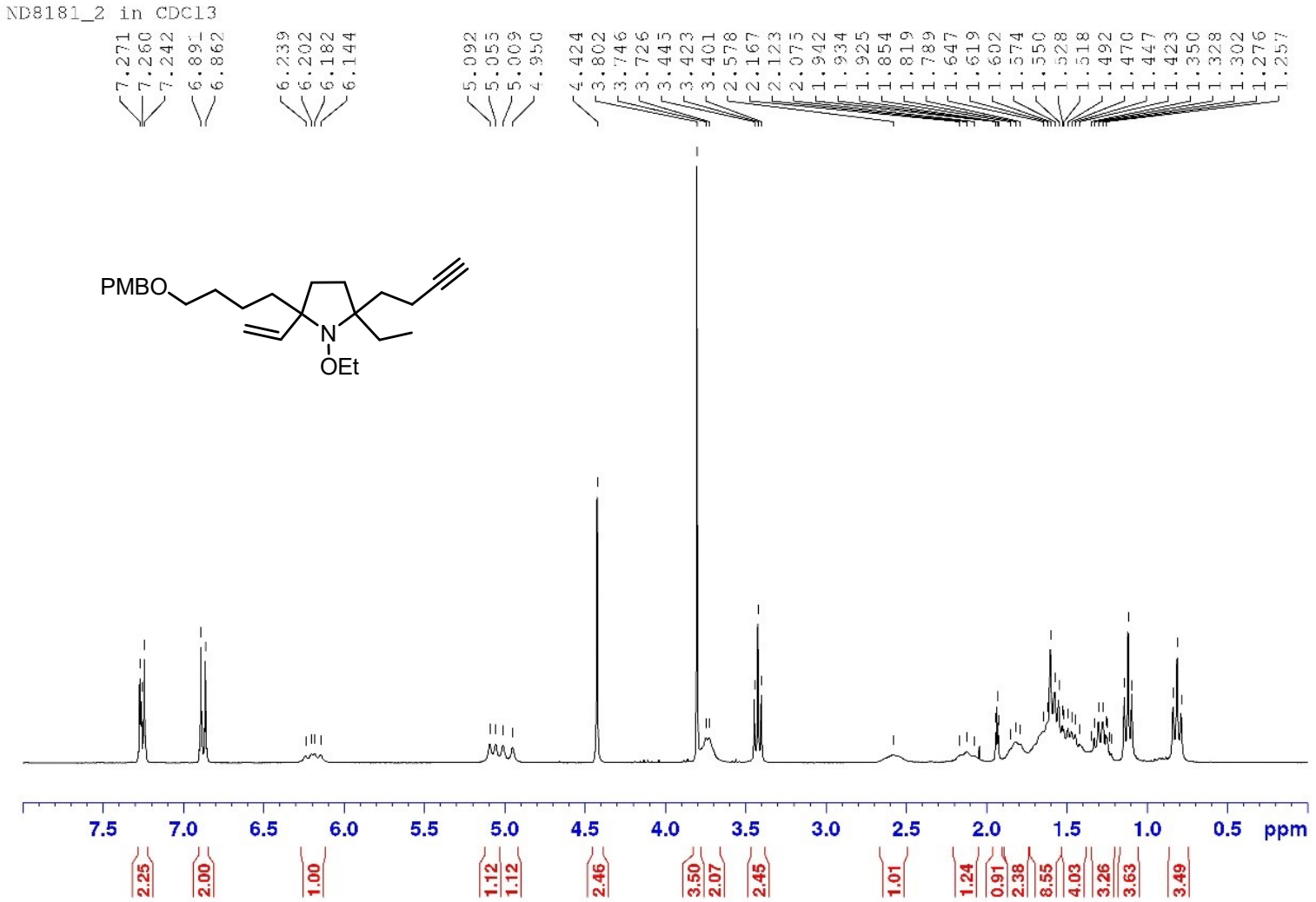


Figure S11. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of alkyne **7**

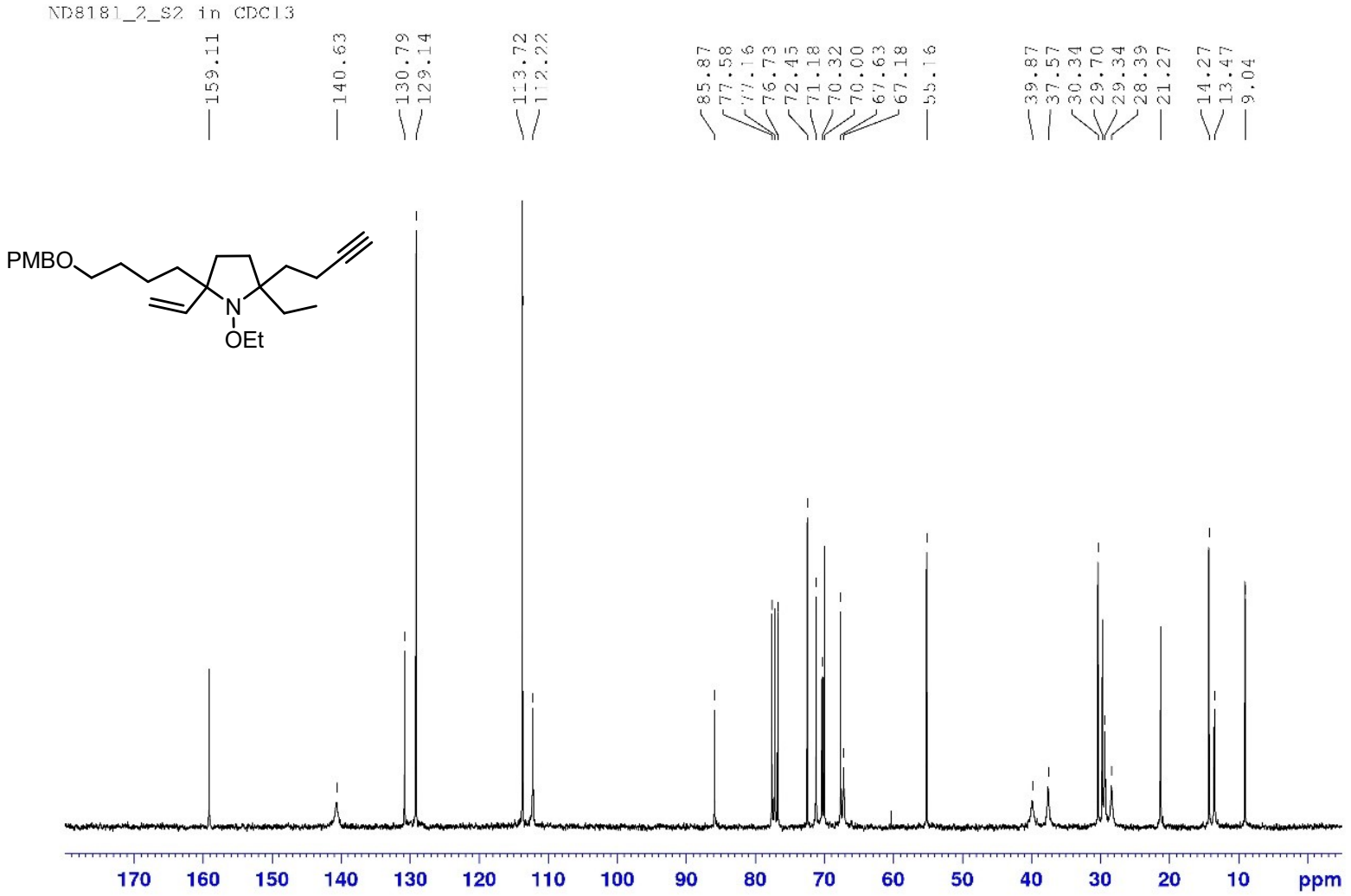


Figure S12. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of alkyne **7**

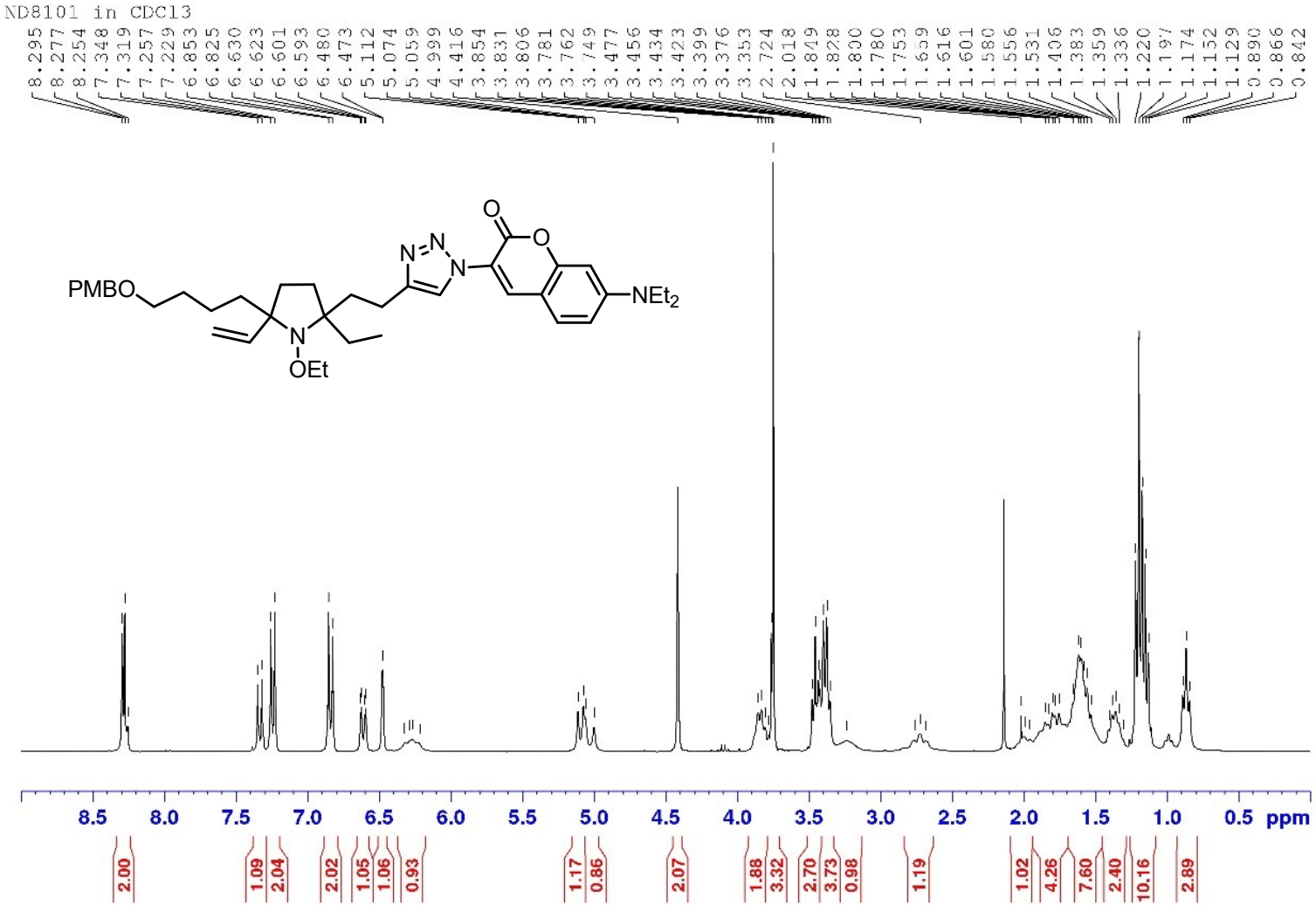


Figure S13. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of triazole **9**

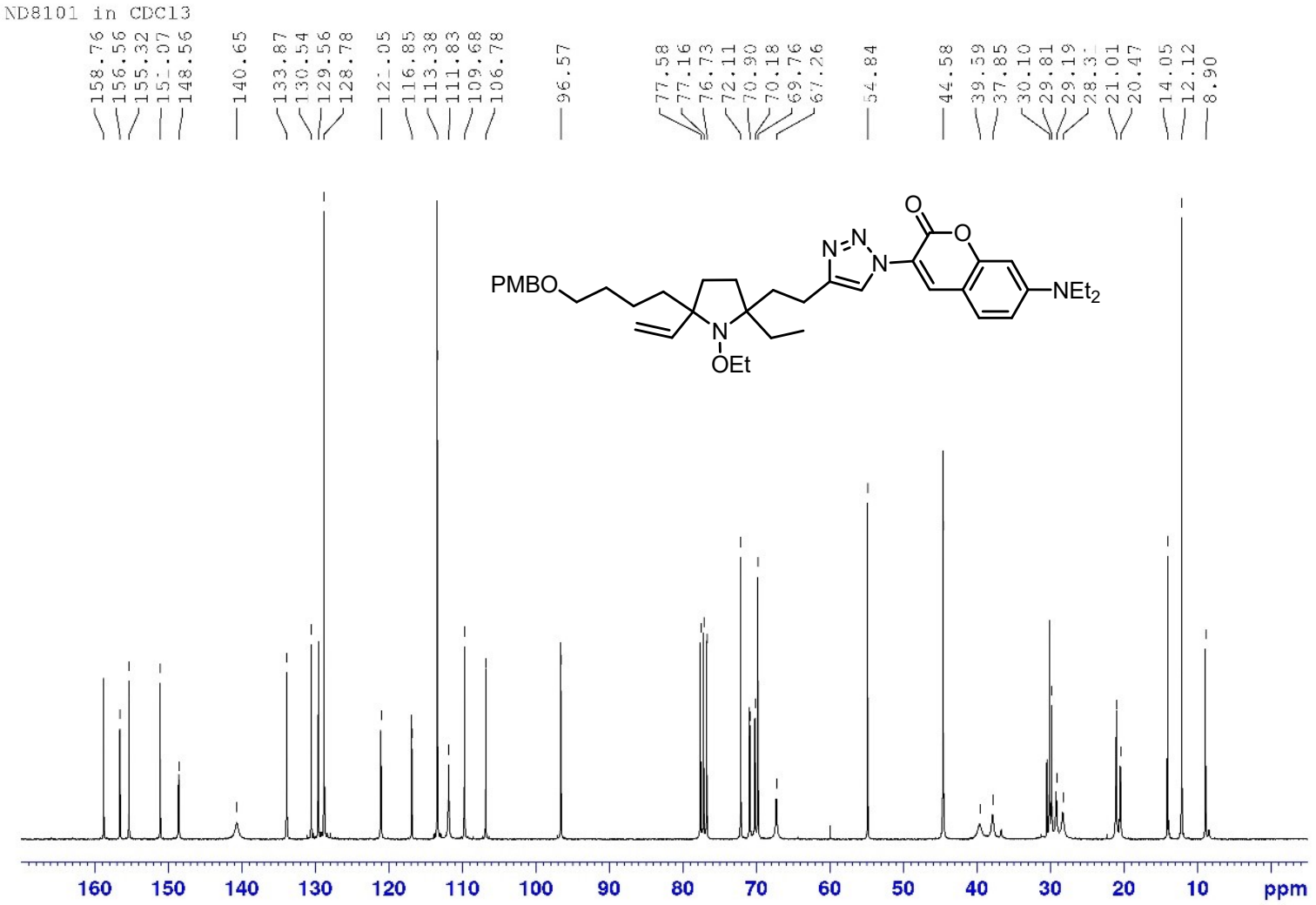


Figure S14. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of triazole **9**

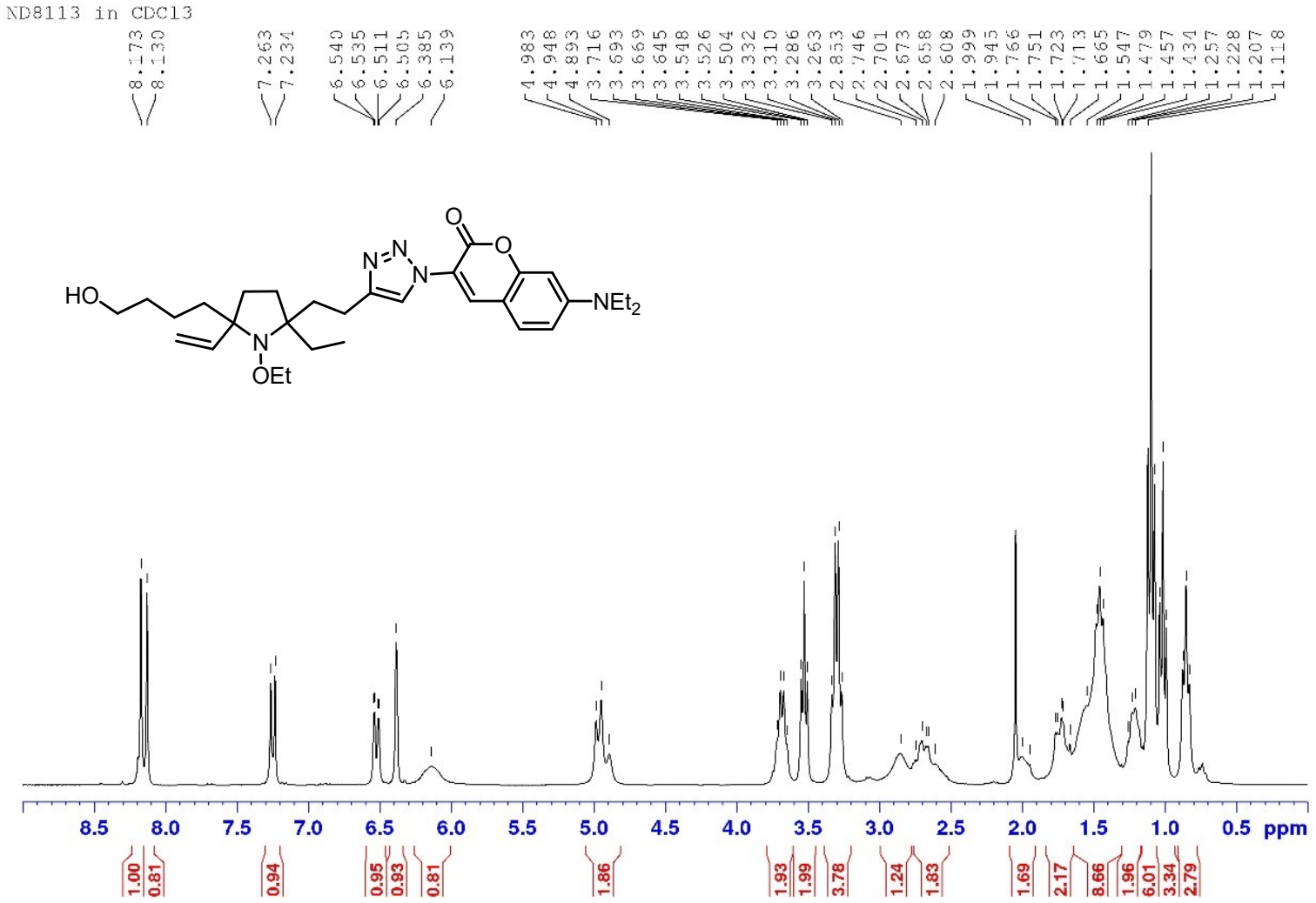


Figure S15. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of probe **PN1**

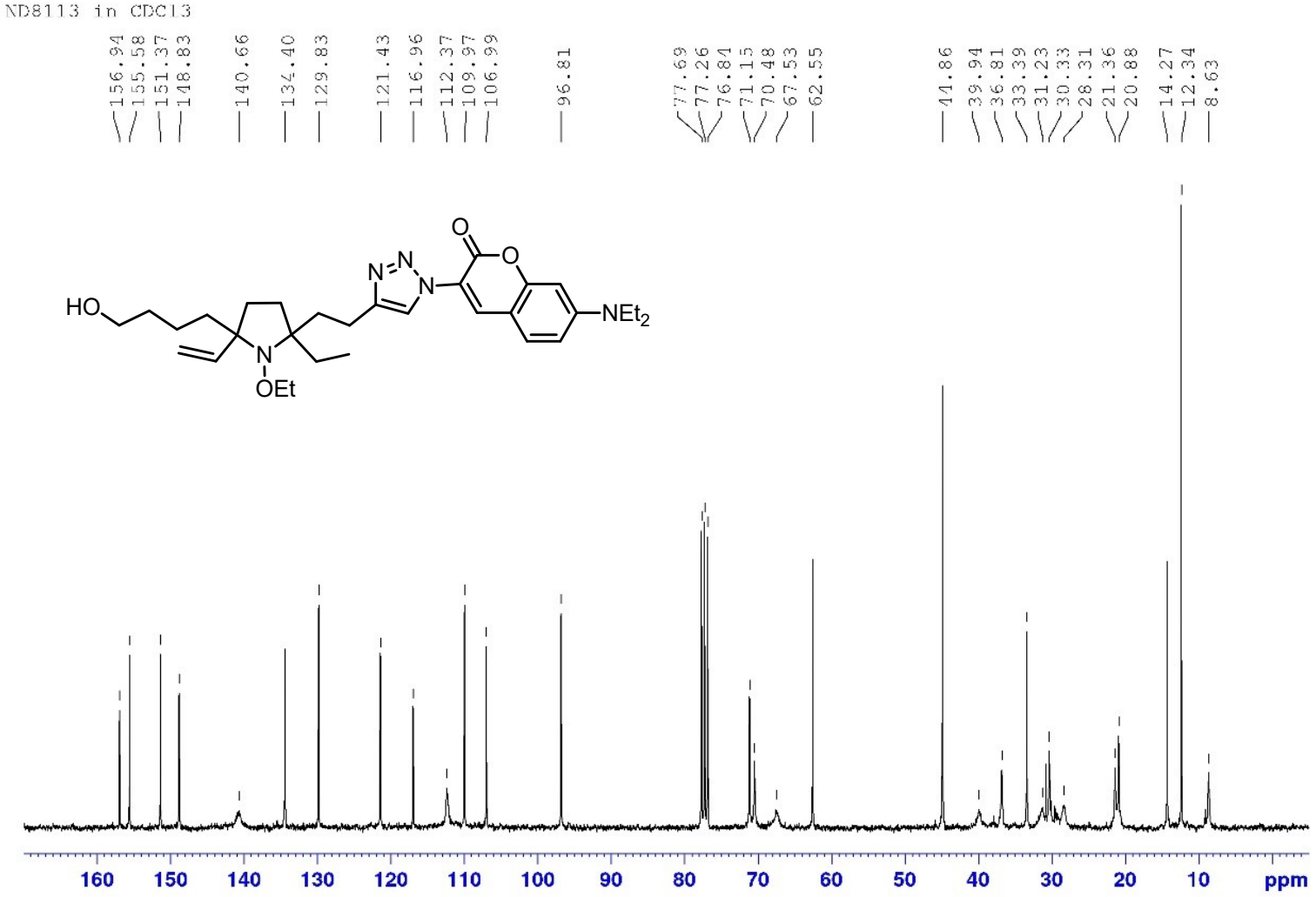


Figure S16. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of probe **PN1**

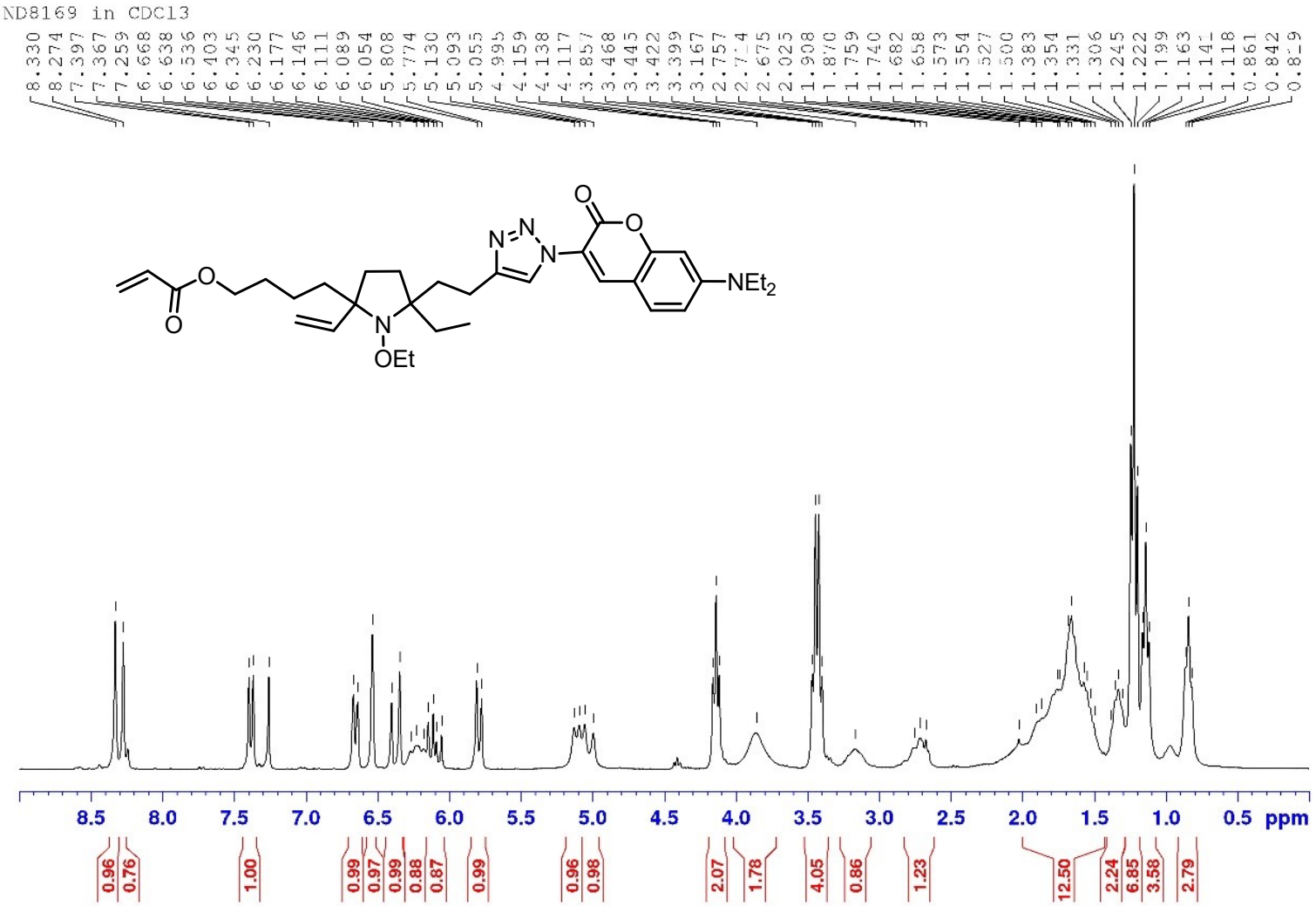


Figure S17. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of **PN2**

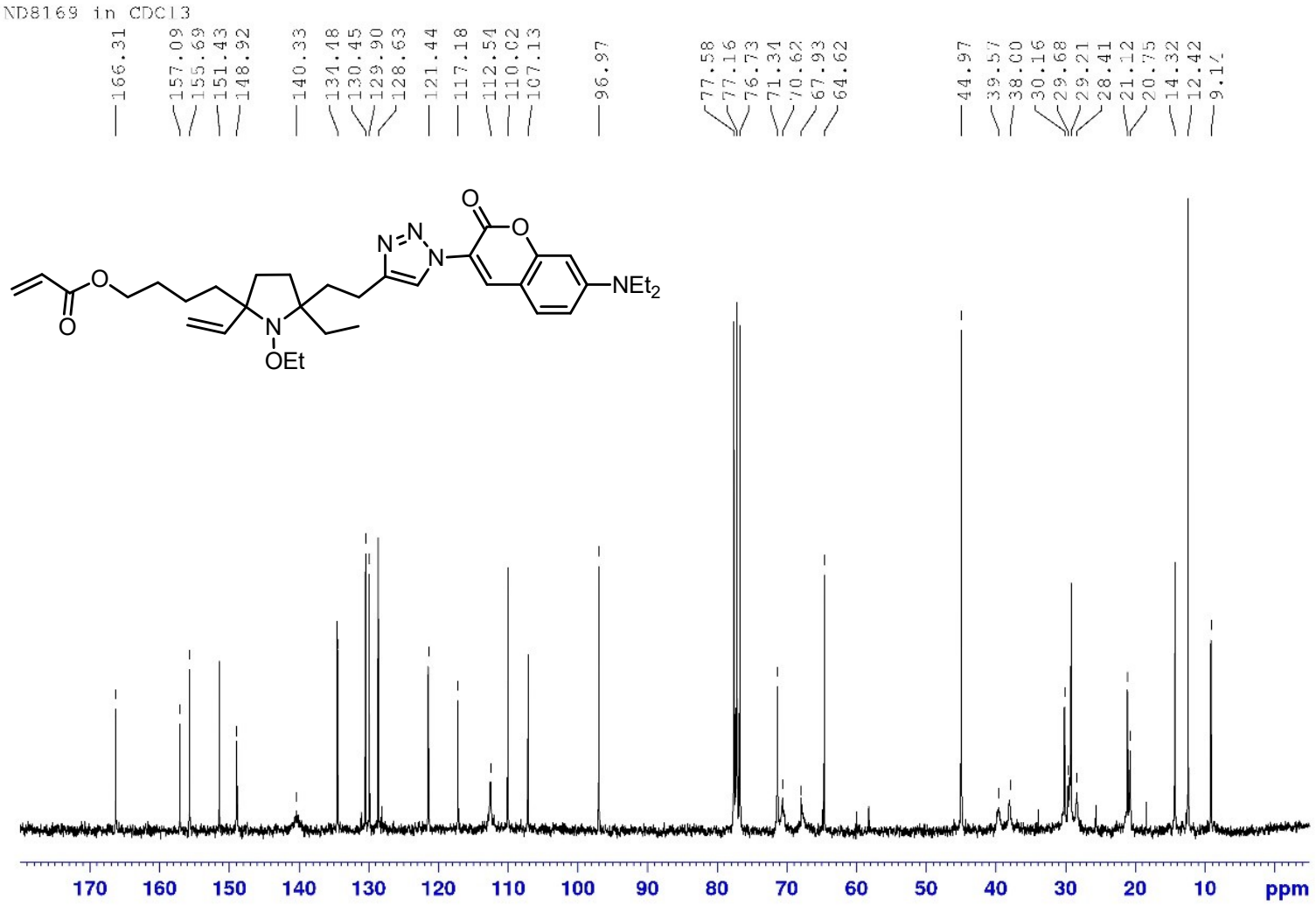


Figure S18. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of ethoxylamine derivative of **PN2**

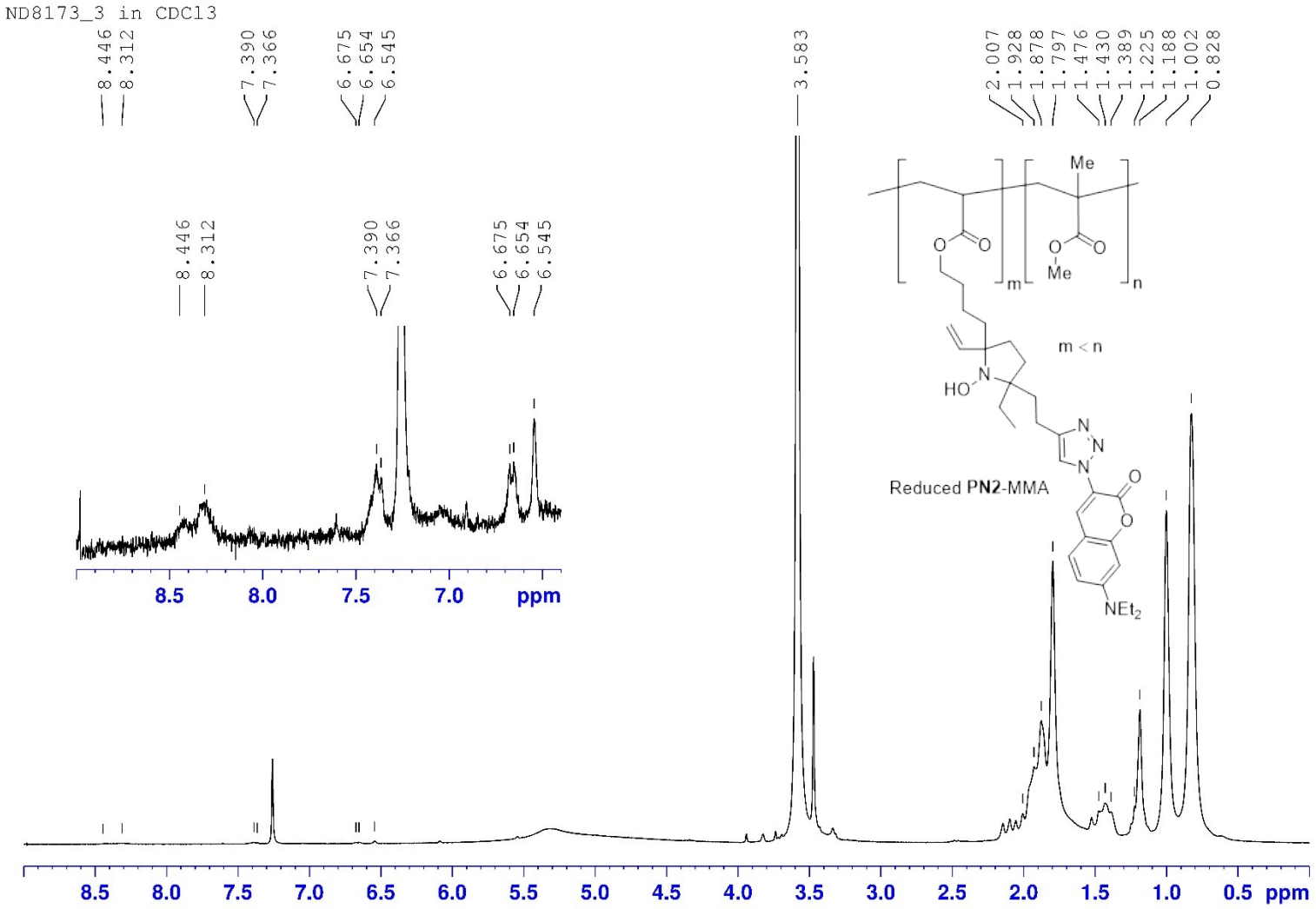


Figure S19. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of reduced **PN2**-MMA copolymer

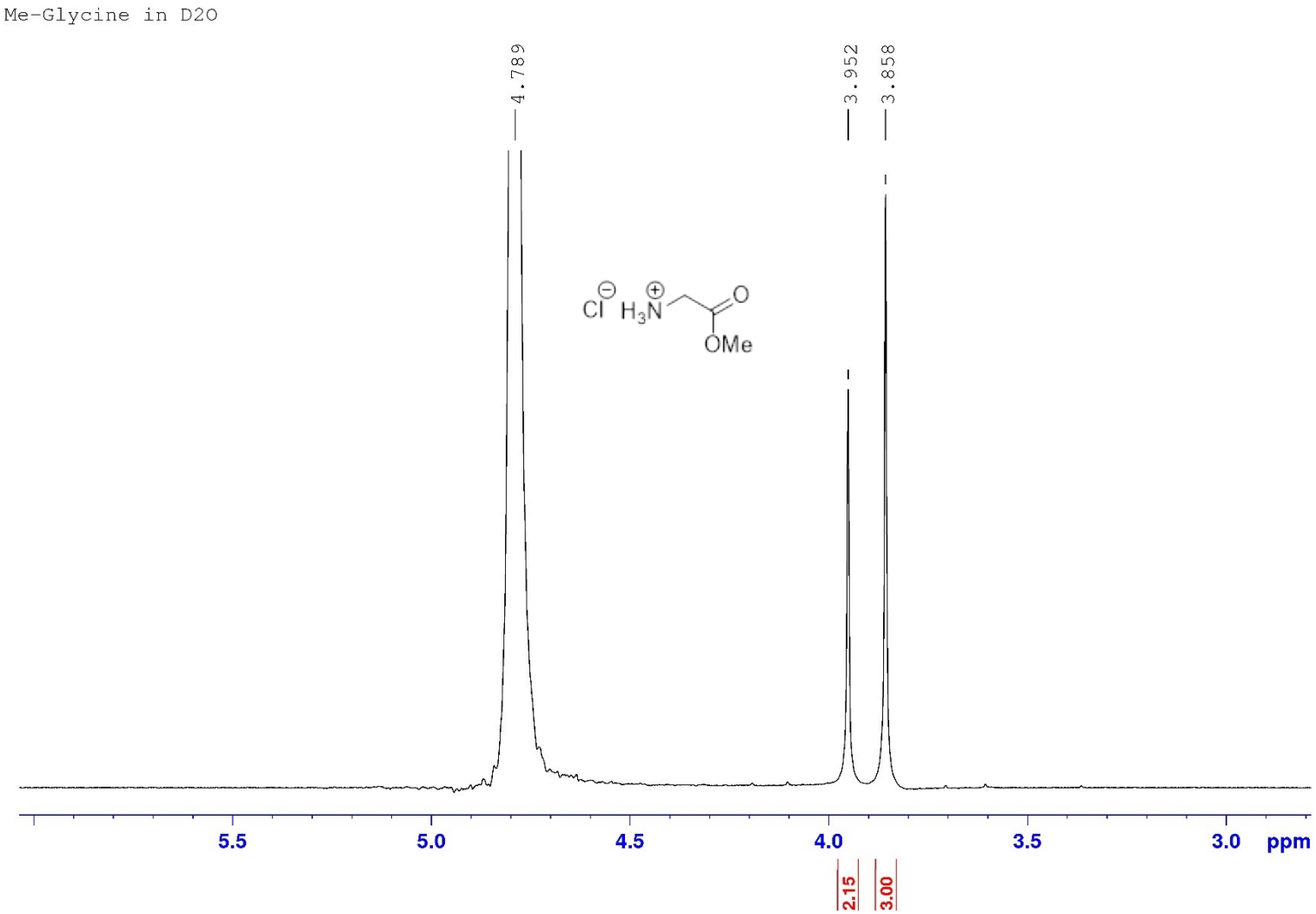


Figure S20. ^{1}H NMR (300 MHz, D_2O) spectrum of glycine methyl ester

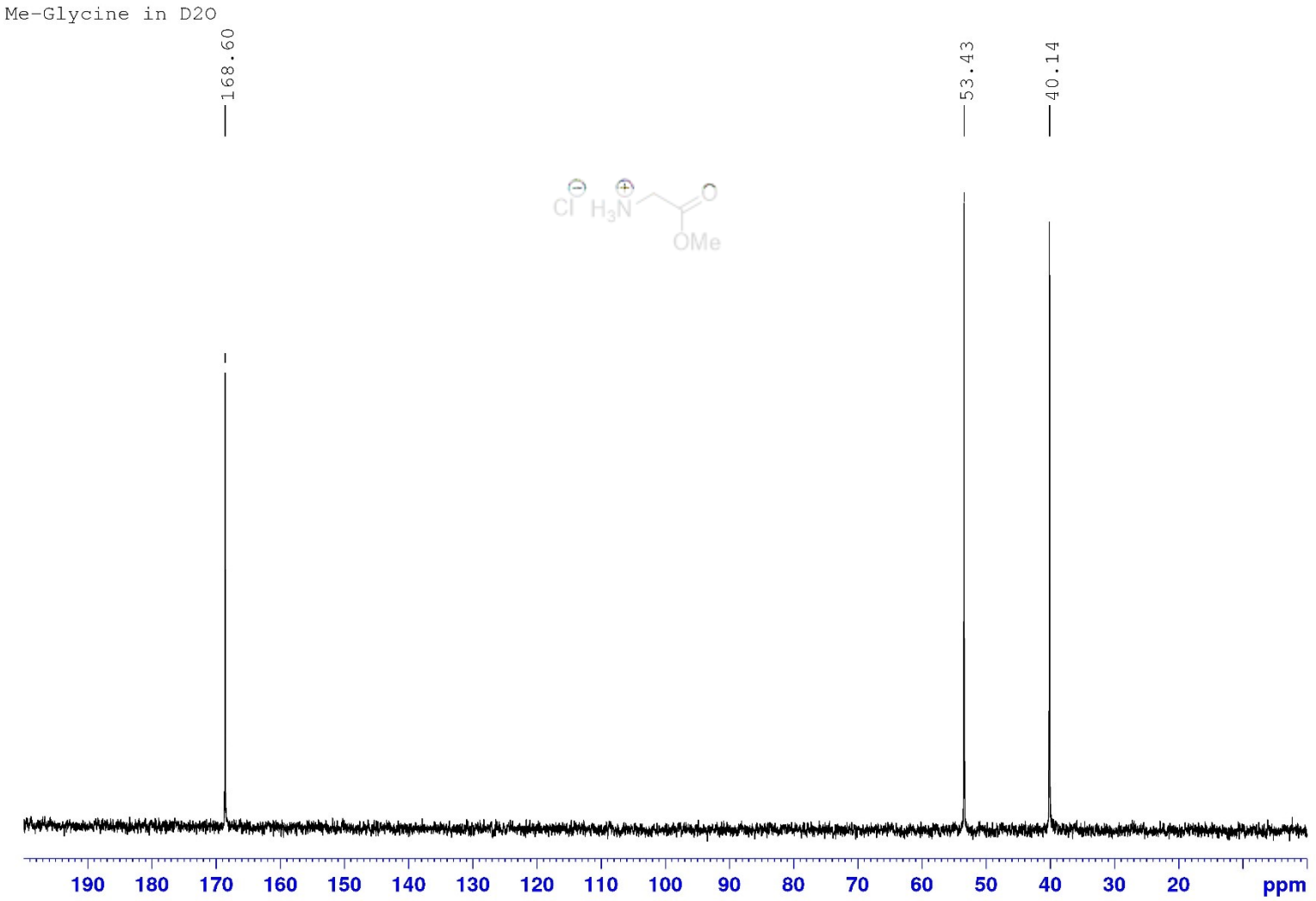


Figure S21. ^{13}C NMR (75 MHz, D_2O) spectrum of glycine methyl ester

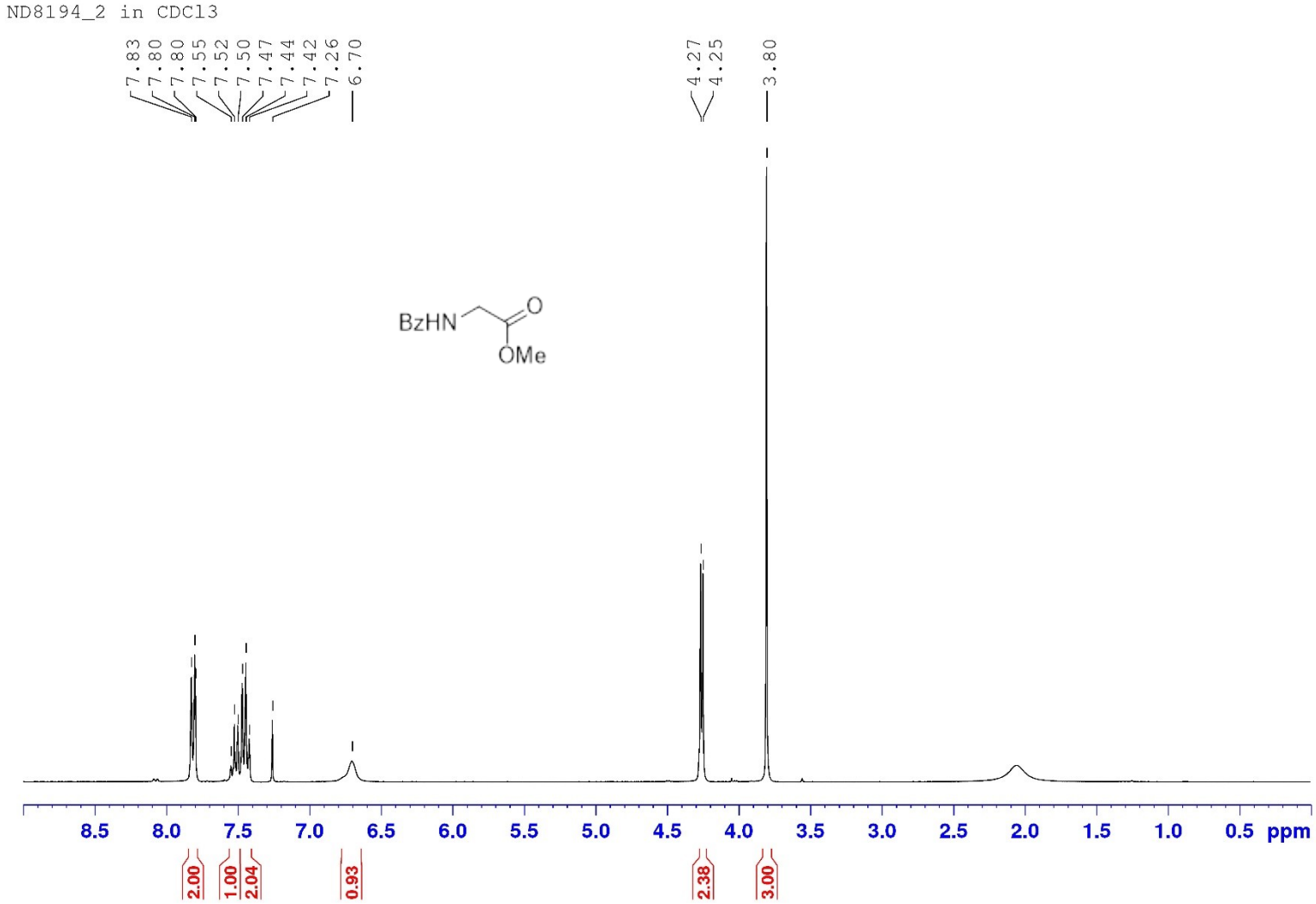


Figure S22. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of *N*-benzoyl glycine methyl ester (**10**)

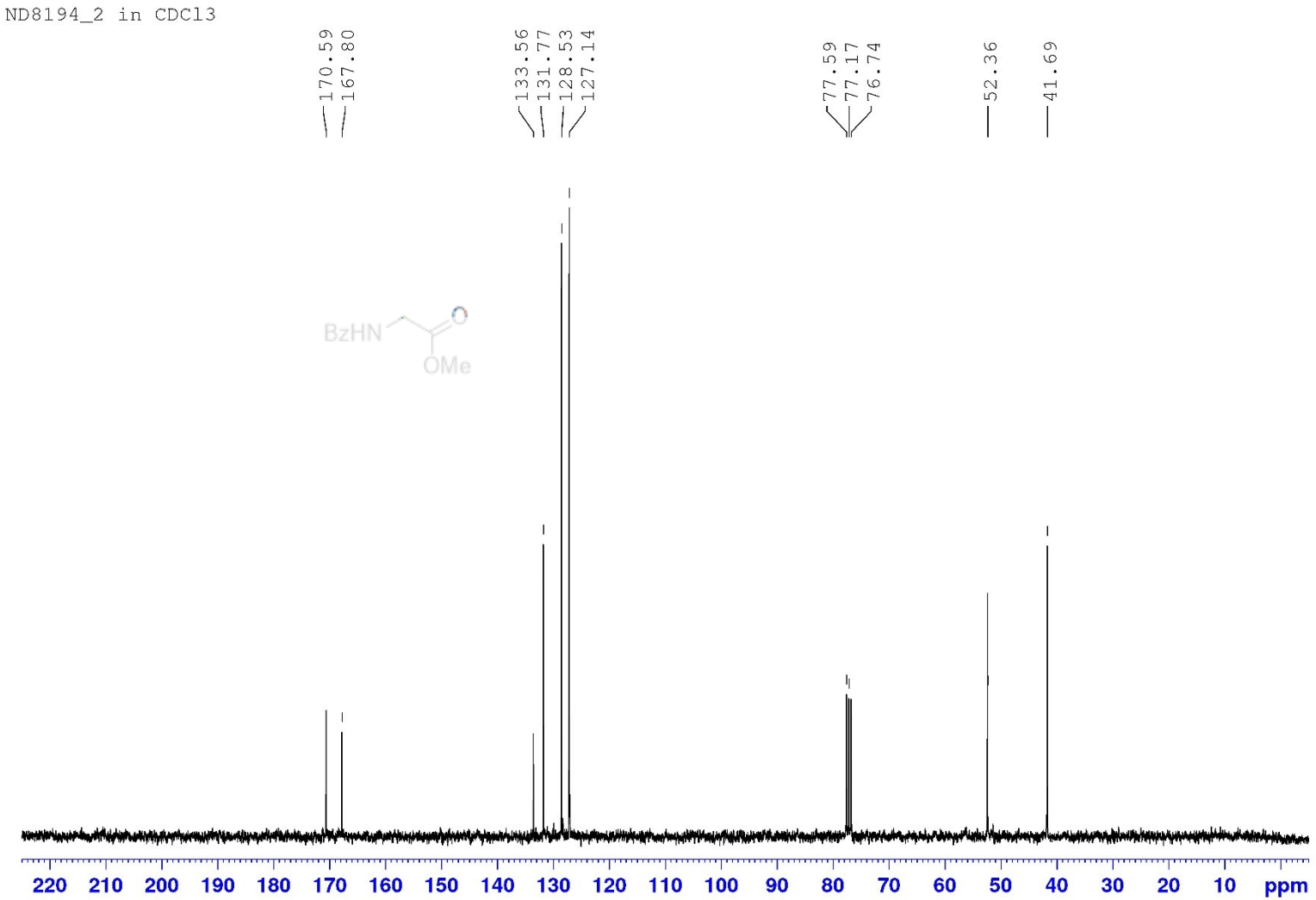


Figure S23. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of *N*-benzoyl glycine methyl ester (**10**)

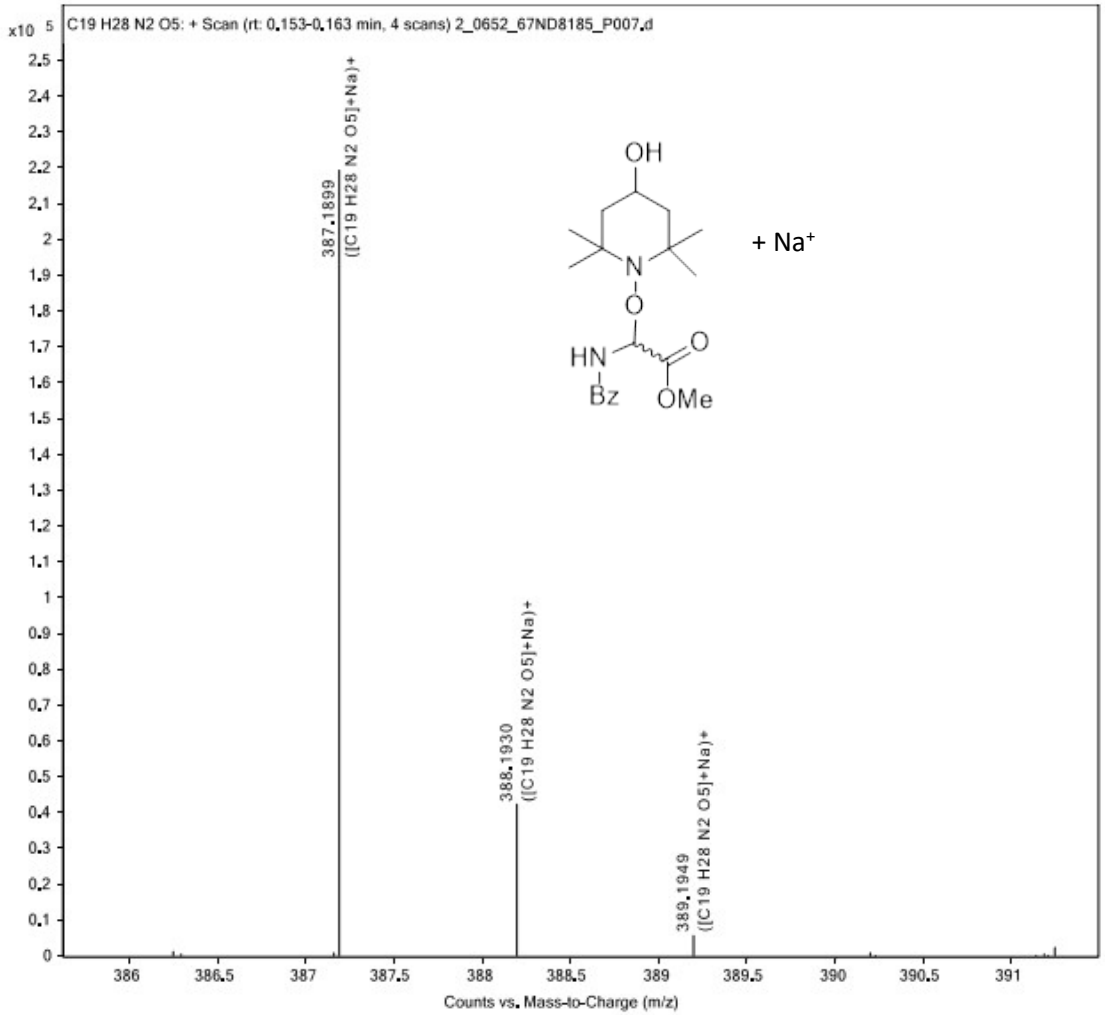


Figure S24. Mass spectrometry data of TEMPOL-glycine adduct **11** and its isotopes

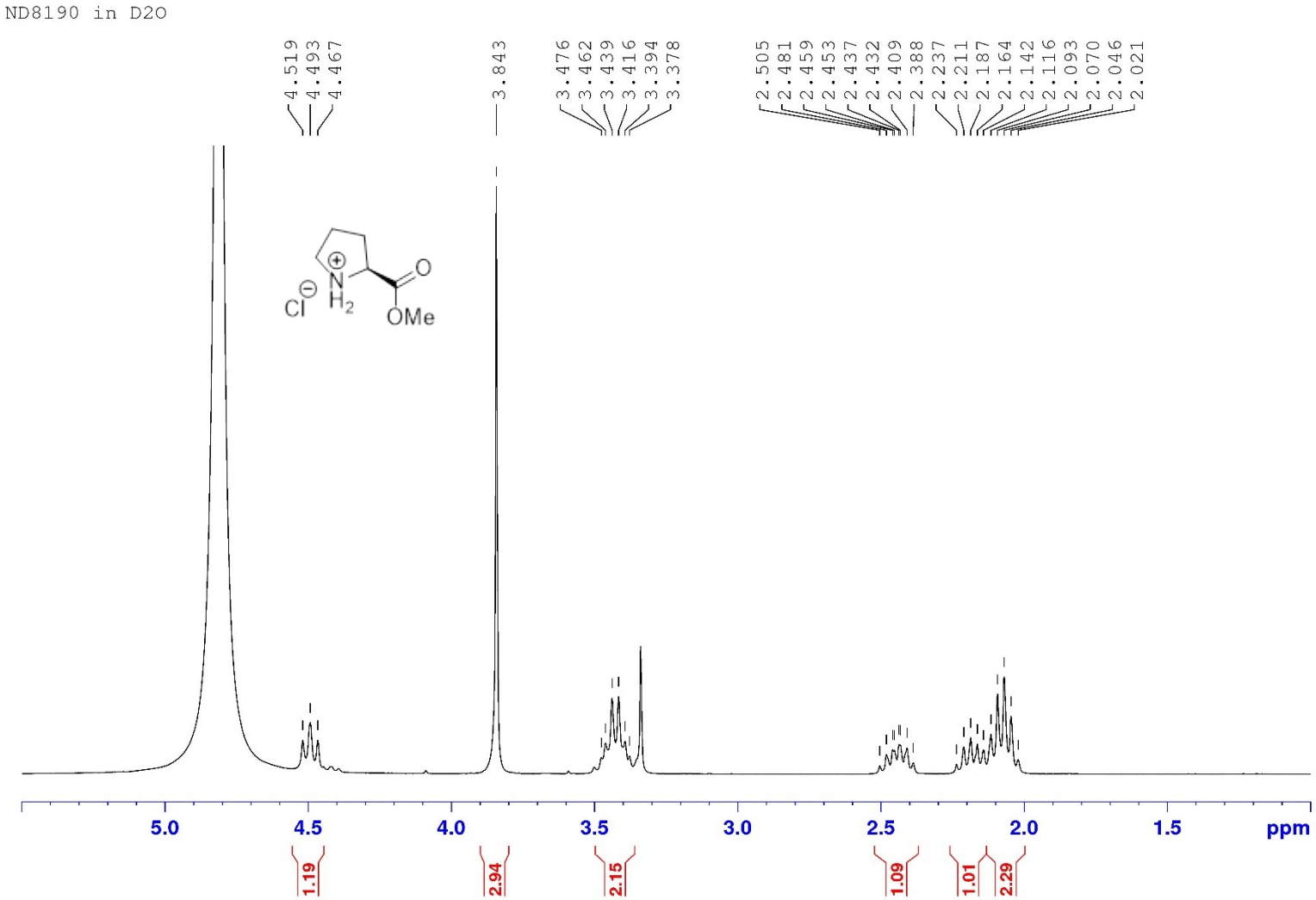


Figure S25. ^{1}H NMR (300 MHz, D_2O) spectrum of proline methyl ester

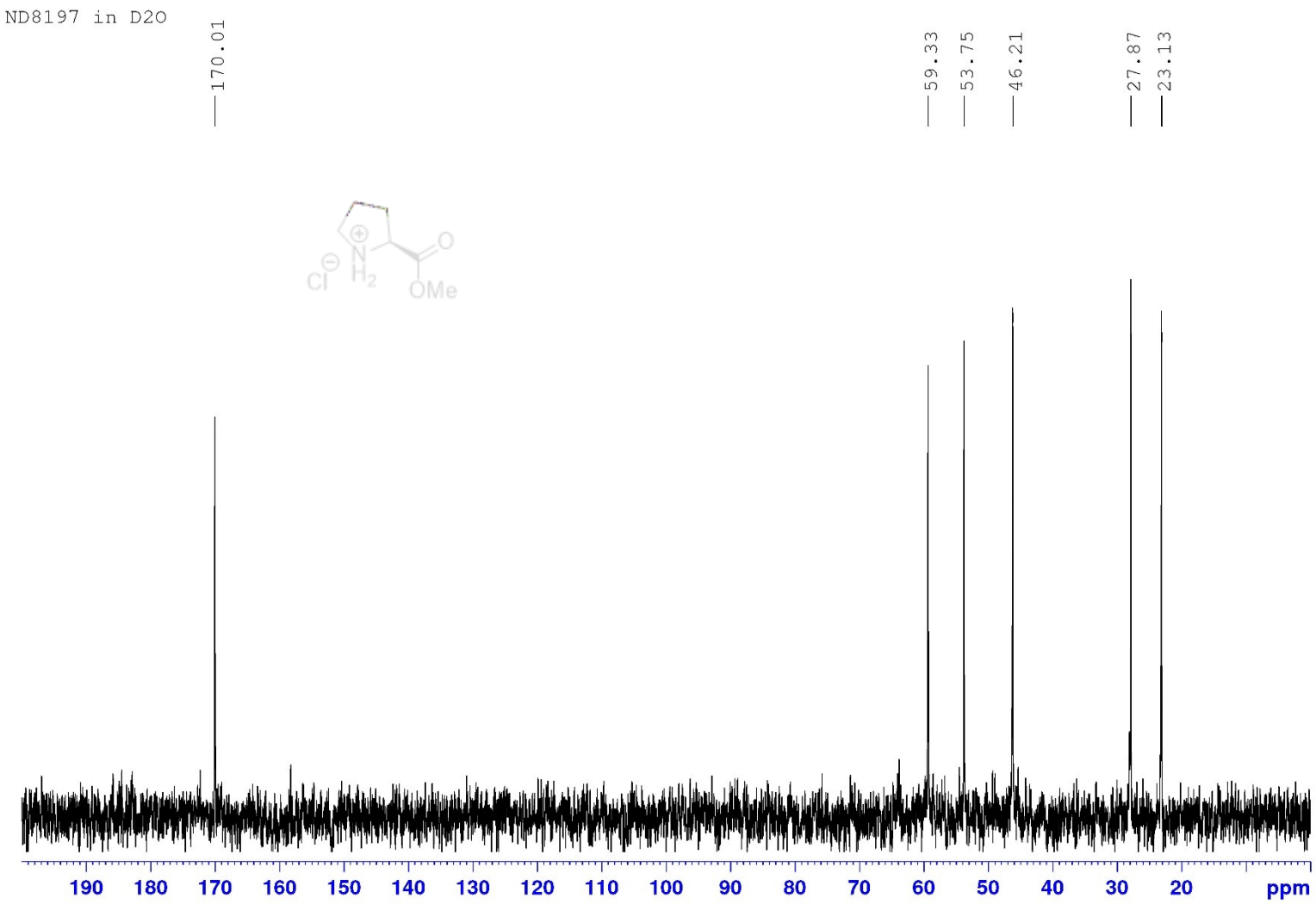


Figure S26. ^{13}C NMR (75 MHz, D_2O) spectrum of proline methyl ester

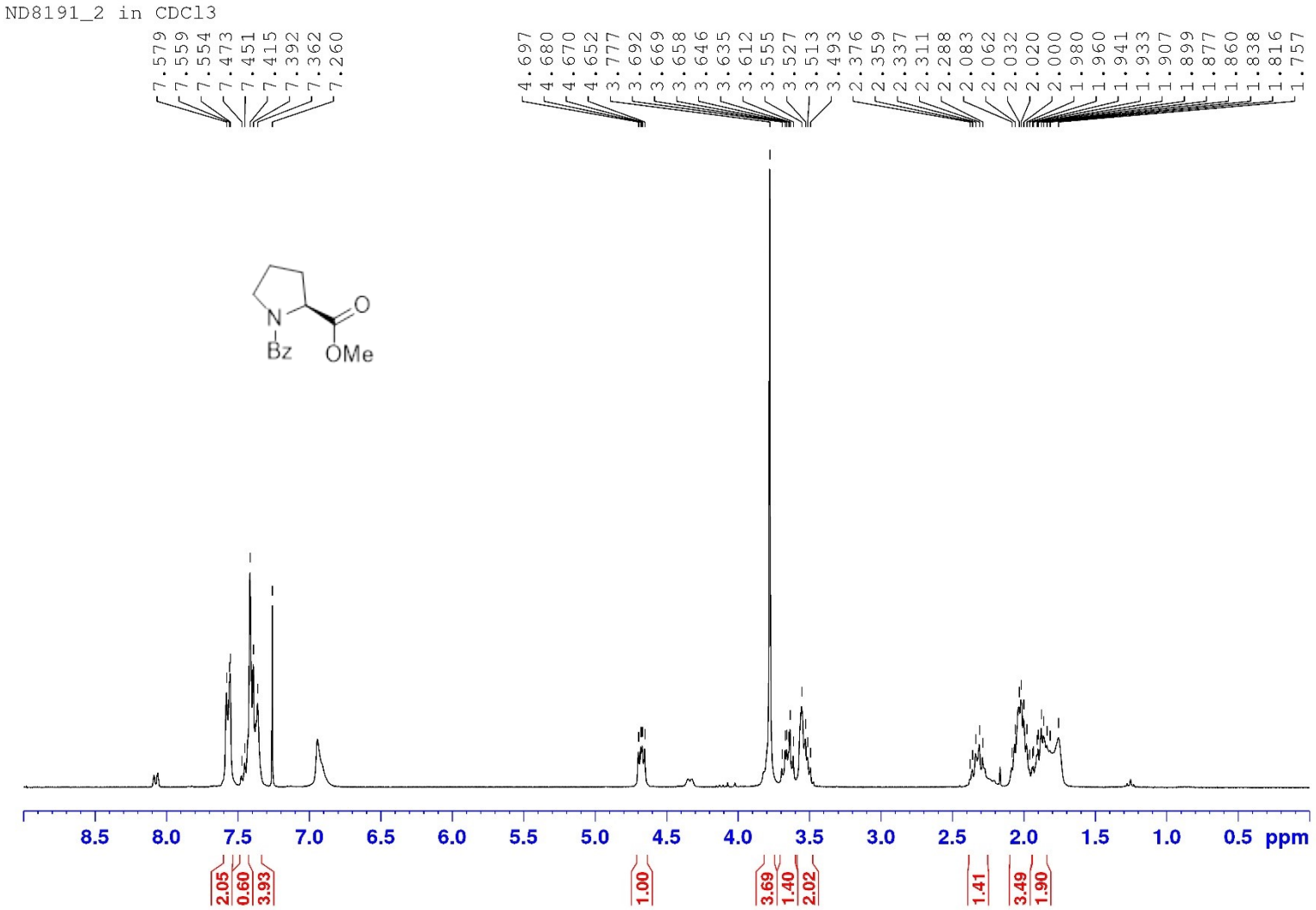


Figure S27. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of *N*-benzoyl proline methyl ester (**12**)

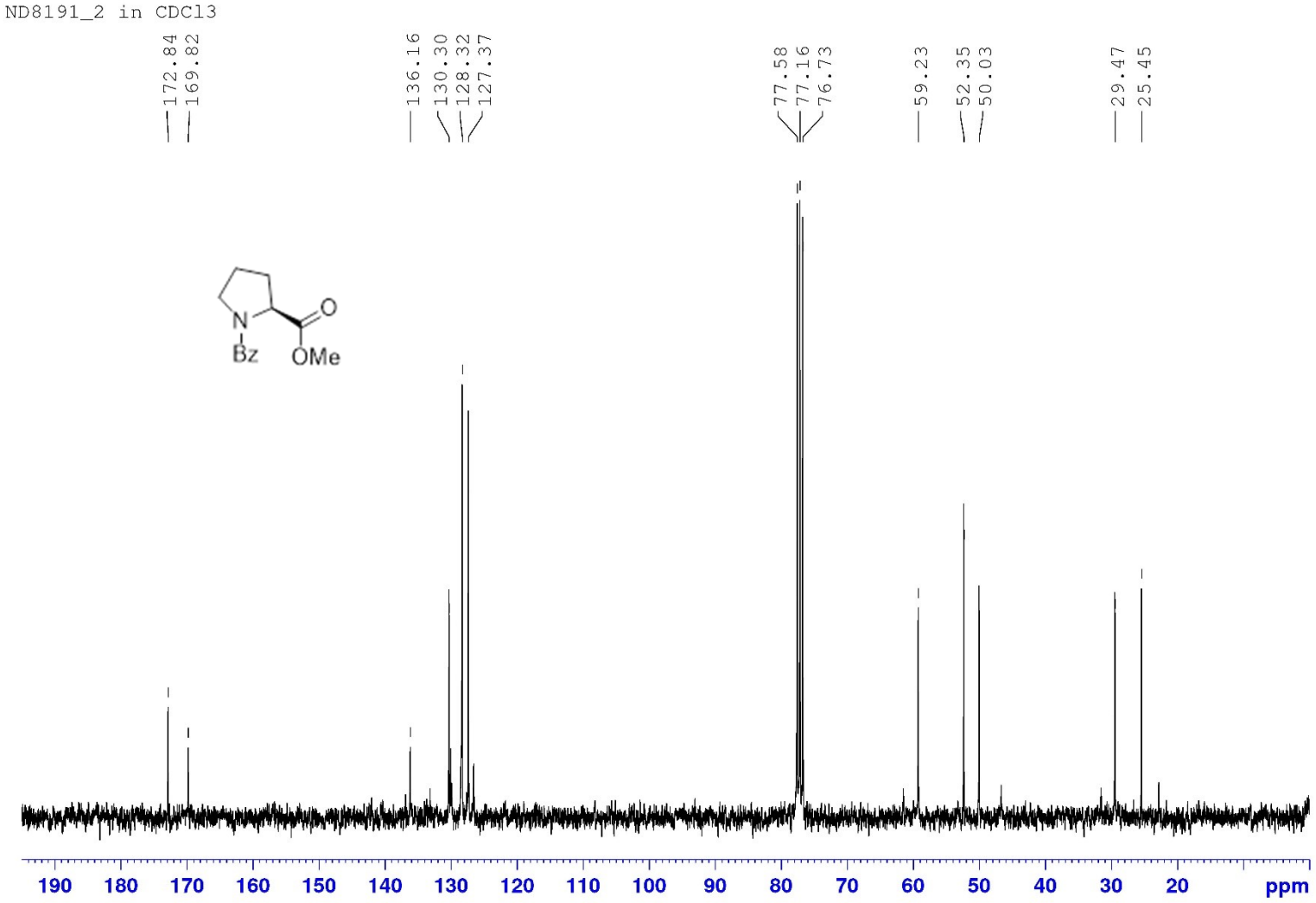


Figure S28. ^{13}C NMR (75 MHz, $CDCl_3$) spectrum of *N*-benzoyl proline methyl ester (**12**)

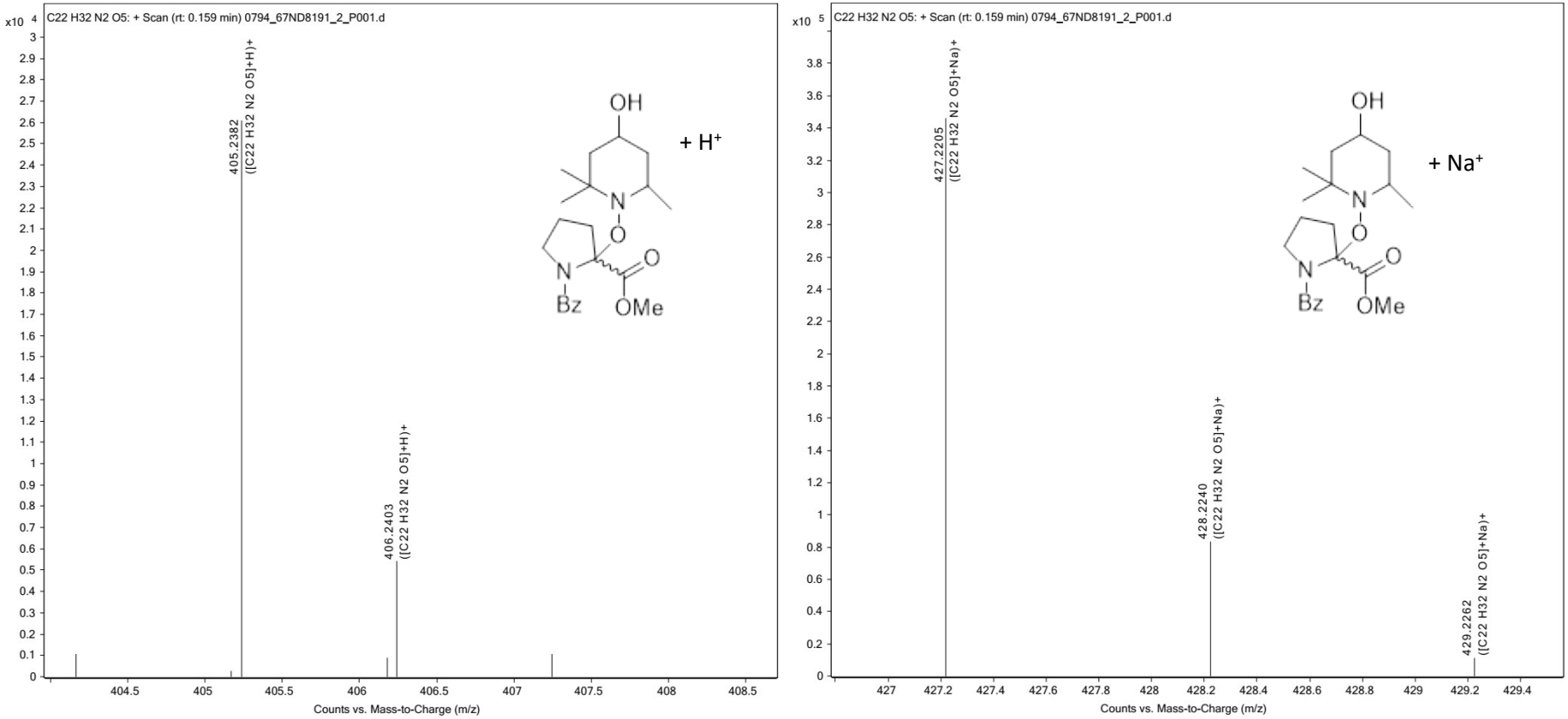


Figure S29. Mass spectrometry data of TEMPOL-proline adduct **13** and their isotopes

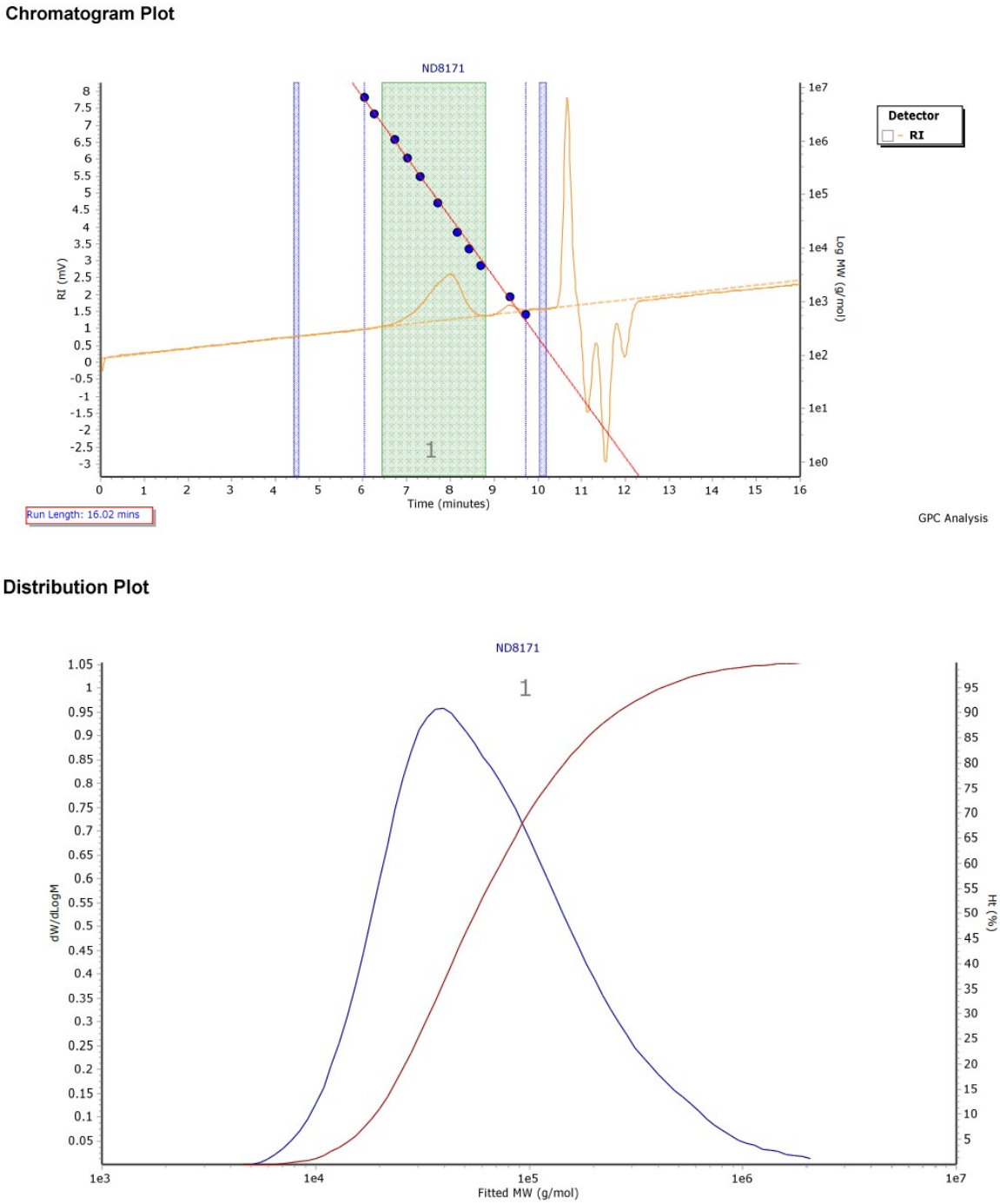


Figure S30. GPC traces of **PN2**-MMA copolymer

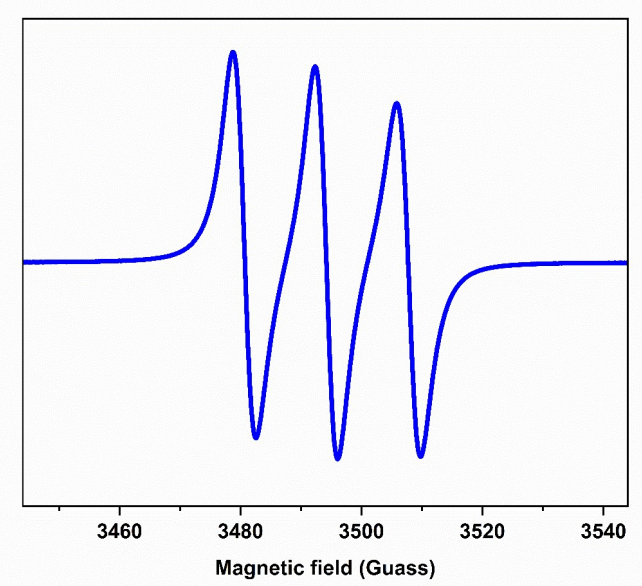


Fig. S31 EPR spectrum of **PN1** (10 mM) in deaerated toluene

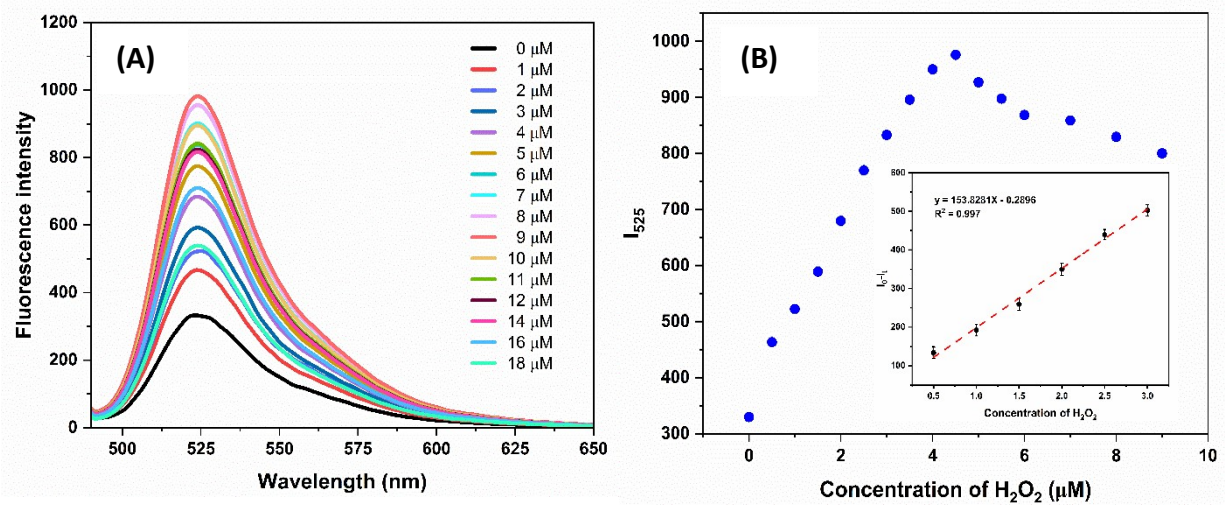


Fig. S32 (A) Fluorescence titration spectra of DCFH (2 µM) in 1:9 EtOH:H_2O after mixing with various concentrations of hydrogen peroxide (0-18 µM) and catalytic amount of $FeCl_2$ in 1:9 EtOH:H_2O. The excitation wavelength was 490 nm.; (**B**) plot of fluorescence intensity of DCFH at 525 nm (I_t-I_o) against various final concentrations of hydrogen peroxide (0-9 µM). Inset: selected range of a linear relationship between fluorescence intensity at 525 nm (I_t-I_0) and concentration of hydrogen peroxide (µM). Note: I_0 = fluorescence intensity of DCFH, and I_t = fluorescence intensity of DCFH in the presence of ROS.

Table

Table 1. Raw data of average pore sizes of collagen by ImageJ

Sample	Count	Total Area (μm²)	Average Pore Size (μm)	%Area
Collagen with ROS	155	32743.016	211.245	53.823
	225	49400.433	219.557	69.791
	146	50345.254	344.831	78.941
			258.544	
		SD	**74.842**	
Collagen with ROS/**PN1**	210	26071.192	124.149	62.811
	147	19993.819	136.012	59.401
	124	20389.464	164.431	58.939
			141.531	
		SD	**20.700**	
Reassembled Collagen	115	11924.446	103.691	39.908
	183	16736.151	91.454	55.357
	100	13710.75	137.108	44.251
			110.751	
		SD	**23.632**	

References

1 Y. Moussaoui and R. B. Salem, *J. Soc. Chim. Tunisie*, 2009, **11**, 37–43.

2 N. Decha, J. Sirirak, D. Sooksawat, A. Phonchai, S. Pornsuwan and C. Tansakul, *RSC Adv.*, 2023, **13**, 27663-27671.

3 J. Y.L. Chung, et al., US Pat., US20140242645A1, 2014.

4 B. Rosanne, et al., WO Pat., WO0146199A1, 2001.

5 B. Miljevic, F. Hedayat, S. Stevanovic, K. E. Fairfull-Smith, S. E. Bottle and Z. D. Ristovski, *Aerosol Sci. Technol.,* 2014, **48**, 1276-1284.