

Supporting Information

Nitrous Oxide as a Primary Product in Base-Mediated β -Elimination Reactions of Diazeniumdiolated Benzylamine Derivatives

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Experimental Section

General. All reactions were performed under an inert atmosphere. All chemicals were purchased from Sigma-Aldrich and used without further purification.

Ultraviolet (UV) spectra were recorded on an Agilent Model 8453 or a Hewlett-Packard model 8451A diode array spectrophotometer in methanol solution, unless otherwise noted. Unless otherwise mentioned, nuclear magnetic resonance (NMR) spectra were collected with a 400-MHz Varian ^{UNITY} INOVA spectrometer using appropriate deuterated solvent with chemical shifts reported in parts per million (ppm) downfield from tetramethylsilane. The gas chromatography was performed on Shimadzu GC-2014 with ECD detector, equipped with ⁶³Ni 370MBq source. A Restek ShinCarbon 80/100 packed column (2 m x 2.0 mm ID) was used with helium as carrier gas. NO estimation was done with Sievers Nitric Oxide Analyzer (NOA), model 280i (Instruments Business Group, Boulder, CO). Elemental analyses were performed by Midwest Microlab, LLC (Indianapolis, IN).

General Procedure for Diazeniumdiolation Reactions. The equipment used for conducting reactions with NO gas under anaerobic conditions has been described previously.^{1,2} Nitric oxide was obtained in ultra-high purity grade from Matheson Tri-Gas (Montgomeryville, PA) and allowed to stand in a ballast tank at about five atm pressure over potassium hydroxide pellets for a minimum of several hours before use. The indicated solvent was placed in a standard Parr thick-walled glass hydrogenation bottle and to this was added the material to be treated with NO. Argon gas was passed through the apparatus and bubbled through the solution for 5-10 min, the NO supply line was clamped into the bottle neck, and the headspace was further deoxygenated by 20-25 repeat cycles of argon pressurization to 5 atm followed by venting. Magnetic stirring was begun, and NO gas was admitted to a pressure of ca. 5 atm. After the reaction was over, excess NO was vented and argon was bubbled through the resulting slurry for 5 min. The products were then isolated as described in the individual sections.

Sodium 1-[N-(2-Hydroxyethyl)-N-benzylamino]diazene-1-ium-1,2-diolate (3): To a Parr bottle, a solution of 2-benzylaminoethanol (5.0 g, 33.1 mmol) in ether (150 mL) was added following which a 25 wt. % solution of sodium methoxide in methanol (7.1 g, 33.1 mmol) was added to the bottle. The bottle was sealed, flushed with nitrogen (5 times) and pressurized with nitric oxide (60 psi). After 24 h, a white cake had formed which was washed with copious amounts of ether, filtered, and dried to produce **3** (5.6 g, 73 %) as a white solid: UV (0.1 M NaOH) λ_{max} (ϵ) 253 nm ($11.9 \text{ mM}^{-1} \text{ cm}^{-1}$); ^1H NMR (0.01 M NaOD in D_2O) δ 7.39-7.36 (m, 5H), 4.10 (s, 2H), 3.59 (t, $J=5.8$ Hz, 2H), 3.21 (t, $J=5.8$ Hz, 2H); ^{13}C NMR (0.01 M NaOD in D_2O) δ 135.2, 129.6, 128.3, 127.9, 109.9, 57.9, 54.9. The authenticity of **3** was further confirmed through O^2 -derivatizations and through analytical characterization of the derivatized products.

O^2 -Methyl 1-[N-(2-Hydroxyethyl)-N-benzylamino]diazene-1-ium-1,2-diolate (4): Compound **3** (2.0 g, 8.6 mmol) was dissolved in a suspension of 0.5 g of finely powdered potassium carbonate in 10 mL of anhydrous methanol and cooled to 0 °C with constant stirring. To this mixture, a solution of dimethyl sulfate (1.29 g, 10.3 mmol) in 3 mL of anhydrous methanol was slowly added. The reaction mixture was then allowed to warm to room temperature and stirred for an additional 8 h. The reaction mixture was then filtered, the methanol was removed on a rotary evaporator, and the residue was extracted with ethyl acetate (3x15 mL). The combined organic layers were washed with saturated brine solution and dried over anhydrous sodium sulfate. After concentration under reduced pressure, the crude product was purified on a silica gel column (hexanes:ethyl acetate 40:60) to afford **4** (1.5 g, 79% yield) as a pale yellow oil: UV (MeOH) λ_{max} (ϵ) 239 nm ($9.5 \text{ mM}^{-1} \text{ cm}^{-1}$); ^1H NMR (CDCl_3) δ 7.28-7.23 (m, 5H), 4.24 (s, 2H), 3.82 (s, 3H), 3.63-3.57 (m, 2H), 3.27 (t, $J=5.2$ Hz, 2H), 2.43 (br s, 1H); ^{13}C NMR (CDCl_3) δ 134.4, 129.2, 128.5, 128.2, 61.1, 59.0, 58.7, 55.5.

Anal. Calcd for $\text{C}_{10}\text{H}_{15}\text{N}_3\text{O}_3$: C, 53.32; H, 6.71; N, 18.66. Found: C, 52.94; H, 6.65; N, 18.34.

O^2 -Methyl 1-[N-Benzyl-N-ethylamino]diazene-1-ium-1,2-diolate (7a): Compound **6a** (1.5 g, 6.9 mmol) was dissolved in a suspension of 0.3 g of finely powdered potassium carbonate in 8 mL of anhydrous methanol and cooled to 0 °C with constant stirring. To this mixture, a solution of dimethyl sulfate (1.0 g, 8.3 mmol) in 1 mL of anhydrous methanol was slowly added. The

reaction mixture was then allowed to warm to room temperature and stirred for an additional 10 h. The reaction mixture was then filtered, the methanol was removed on a rotary evaporator, and the residue was extracted with ethyl acetate (3x15 mL). The combined organic layers were washed with saturated brine solution and dried over anhydrous sodium sulfate. After concentration under reduced pressure, the crude product was purified on a silica gel column (hexanes:ethyl acetate 90:10) to afford **7a** (0.9 g, 65% yield) as a colorless oil: UV (MeOH) λ_{\max} (ϵ) 240 nm (12.7 mM⁻¹cm⁻¹), 264 nm (10.7); ¹H NMR (CDCl₃) δ 7.30-7.24 (m, 5H), 4.16 (s, 2H), 3.83 (s, 3H), 3.14 (q, $J=7.1$ Hz, 2H), 1.08 (t, $J=7.1$ Hz, 3H); ¹³C NMR (CDCl₃) δ 134.8, 129.2, 128.4, 128.0, 61.0, 58.3, 48.3, 11.3.

Anal. Calcd. for C₁₀H₁₅N₃O₂ · (0.23 CDCl₃): C, 51.96; H, 6.39; N, 17.77. Found: C, 51.95; H, 6.48; N, 18.03.

O²-Ethyl 1-(Dibenzylamino)diazen-1-ium-1,2-diolate (7b): Compound **6b** (2.0 g, 7.2 mmol) was dissolved in a suspension of 0.5 g of finely powdered potassium carbonate in 10 mL of anhydrous methanol and cooled to 0 °C with constant stirring. To this mixture, a solution of diethyl sulfate (1.3 g, 8.6 mmol) in 3 mL of anhydrous methanol was slowly added. The reaction mixture was then allowed to warm to room temperature and stirred for an additional 10 h. The reaction mixture was then filtered, the methanol was removed on a rotary evaporator, and the residue was extracted with ethyl acetate (3x15 mL). The combined organic layers were washed with saturated brine solution and dried over anhydrous sodium sulfate. After concentration under reduced pressure, the crude product was purified on a silica gel column (hexanes:ethyl acetate 90:10) to afford **7b** (1.7 g, 82% yield) as a white crystalline solid: mp 39-41 °C; UV (MeOH) λ_{\max} (ϵ) 238 nm (9.3 mM⁻¹cm⁻¹); ¹H NMR (CDCl₃) δ 7.36-7.25 (m, 10H), 4.27 (s, 4H), 4.00 (q, $J=7.1$ Hz, 2H), 1.00 (t, $J=7.1$ Hz, 3H); ¹³C NMR (CDCl₃) δ 134.8, 129.2, 128.4, 128.0, 69.6, 57.7, 14.2.

Anal. Calcd. for C₁₆H₁₉N₃O₂: C, 67.35; H, 6.71; N, 14.73. Found: C, 67.68; H, 6.73; N, 15.07.

O²-Methyl 1-[N-Benzyl-N-(2-methoxyethyl)amino]diazen-1-ium-1,2-diolate (9): Compound **3** (4.0 g, 17.2 mmol) was suspended in 20 mL of anhydrous THF and cooled to 0 °C. 15-Crown-5 ether (1.1 g, 5.2 mmol) was added to it and stirred at 0 °C for 20 min. Sodium

hydride (0.4 g, 17.2 mmol) suspended in 3 mL of anhydrous THF was added to the reaction mixture and stirred for 10 min. To this suspension, methyl iodide (6.1 g, 43.0 mmol) was slowly added and then it was allowed to warm to room temperature and stirred for an additional 24 h, following which the reaction mixture was quenched with saturated ammonium chloride solution at 0 °C and extracted with diethyl ether (3x20 mL). The combined organic layers were then washed with water and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified on a silica gel column (hexanes:ethyl acetate (90:10) to afford **9** (1.8 g, 44%) as a colorless oil: UV (MeOH) λ_{max} (ϵ) 239 nm (13.9 $\text{mM}^{-1}\text{cm}^{-1}$); ^1H NMR (CDCl_3) δ 7.26-7.21 (m, 5H), 4.26 (s, 2H), 3.81 (s, 3H), 3.46 (t, $J=5.4$ Hz, 2H), 3.32 (t, $J=5.4$ Hz, 2H), 3.25 (s, 3H); ^{13}C NMR (CDCl_3) δ 134.7, 129.1, 128.4, 128.0, 69.4, 61.0, 58.8, 58.7, 52.6.

Anal. Calcd. for $\text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_3$: C, 55.22; H, 7.16; N, 17.56. Found: C, 55.45; H, 7.11; N, 17.32.

General Procedure for Base-Mediated β -Elimination. A solution of compound **4** (0.2 g, 0.9 mmol) in 7 mL anhydrous THF was taken in a two-neck round bottom flask fitted with a reflux condenser. A 1 M solution of Li^iBuO (1.8 mL, 1.8 mmol) was added dropwise and the reaction mixture was heated at 50 °C for two hours with stirring. Subsequently, the reaction mixture was quenched with water at 0 °C and extracted with diethyl ether (3x10 mL). The combined organic layers were then washed with water and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the crude product was purified on a silica gel column (hexanes:ethyl acetate (97:3) to afford **5** (0.1 g, 84%) as a yellow oil: UV (MeOH) λ_{max} (ϵ) 246 nm (13.0 $\text{mM}^{-1}\text{cm}^{-1}$); ^1H NMR (CDCl_3) δ 8.32 (s, 1H), 7.73-7.70 (m, 2H), 7.43-7.38 (m, 3H), 3.91 (t, $J=5.1$ Hz, 2H), 3.75 (t, $J=5.1$ Hz, 2H); ^{13}C NMR (CDCl_3) δ 163.2, 135.8, 130.8, 128.6, 128.2, 63.3, 62.3; HRMS (ESI) m/z calculated for $\text{C}_9\text{H}_{12}\text{NO}$ $[\text{M}+\text{H}]^+$ 150.09134, found 150.09165.

Kinetic study:

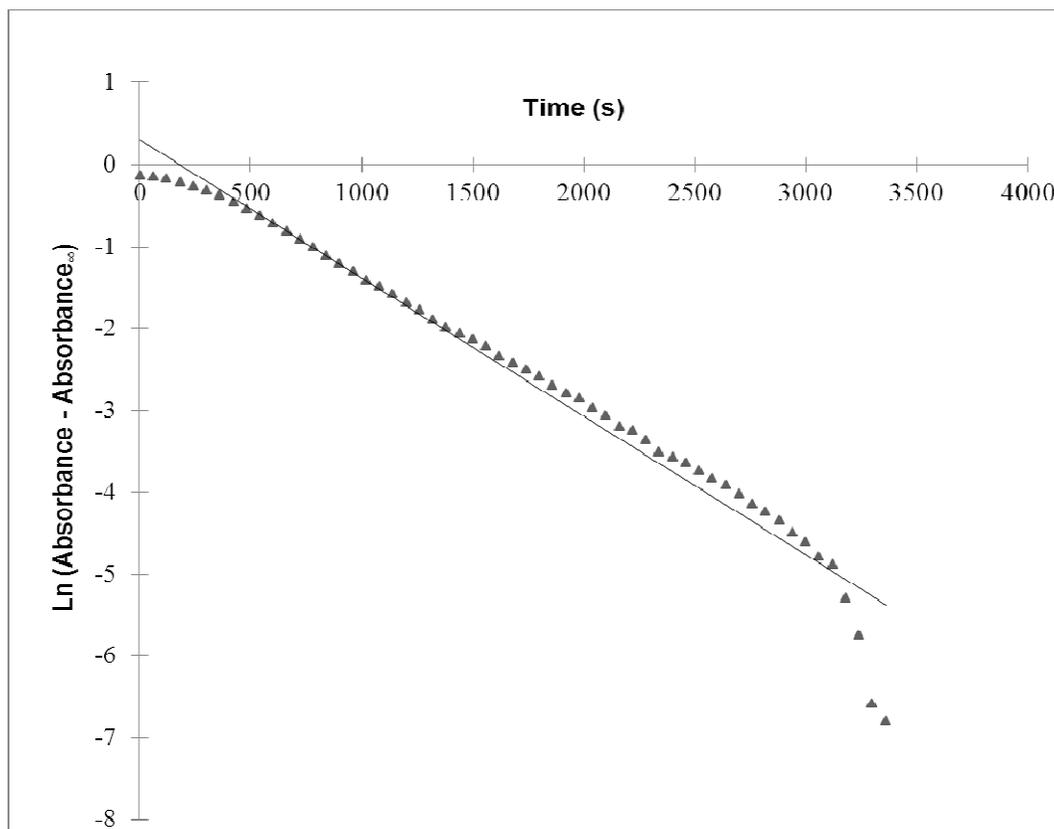
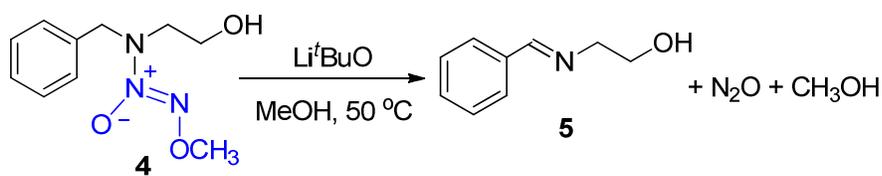


Figure S1. Semi-log kinetic rate curve for the Li^tBuO -mediated β -elimination reaction of **4** in MeOH with absorbance values adjusted for $t = \infty$.

The kinetics of Li^tBuO -mediated β -elimination reaction of **4** was studied spectrophotometrically by monitoring the decrease in absorbance of peaks at $\sim 240\text{-}250\text{ nm}$ characteristic of the diazeniumdiolate moiety. UV-visible spectroscopy was performed with a Hewlett-Packard 8453 diode-array spectrophotometer equipped with an Agilent 89090A thermostat set to $50\text{ }^\circ\text{C}$. The spectrophotometer was blanked after warming a quartz cuvette containing anhydrous methanol in the instrument heat block for 5 min. The kinetic analysis was achieved by the addition of $40\text{ }\mu\text{L}$ of Li^tBuO (1 M in THF) to a $2\text{ }\mu\text{M}$ stock solution (1 mL) of **4** in anhydrous methanol taken in a sealed quartz cuvette. Spectra were collected at 60-s intervals for a time period of 4000 s. Half-

life of the reaction ($t_{1/2}$) was found to be 14.3 min, calculated from the rate constant which was obtained by fitting the decomposition curve to the rate equation.

N₂O Estimation Assay: The gas chromatography was performed on a Shimadzu GC-2014 with an electron capture detector, equipped with ⁶³Ni 370MBq source. A Restek ShinCarbon 80/100 packed column (2 m x 2.0 mm ID) was used with helium as carrier gas. The GC operation conditions were as following: injector and detector temperature were at 250 °C, oven temperature programmed from 90 to 200 °C at 20 °C/min. Nitrogen was used as makeup gas at 2 mL/min. The reaction chamber was put in a 50 °C water bath and subsequently purged with helium gas for 5 min. A solution of **4** in anhydrous DMF (3 mL, 0.2 mM) was injected into the reaction chamber and the reaction was started by adding 20 μL of Li^tBuO (1 M in THF). N₂O generated was introduced into the GC sampling loop by continuously blowing helium through the reaction mixture (see resulting graph below). The final concentration of detected N₂O was calculated based on a calibration curve and was found to be 0.64 mol/mol of compound.

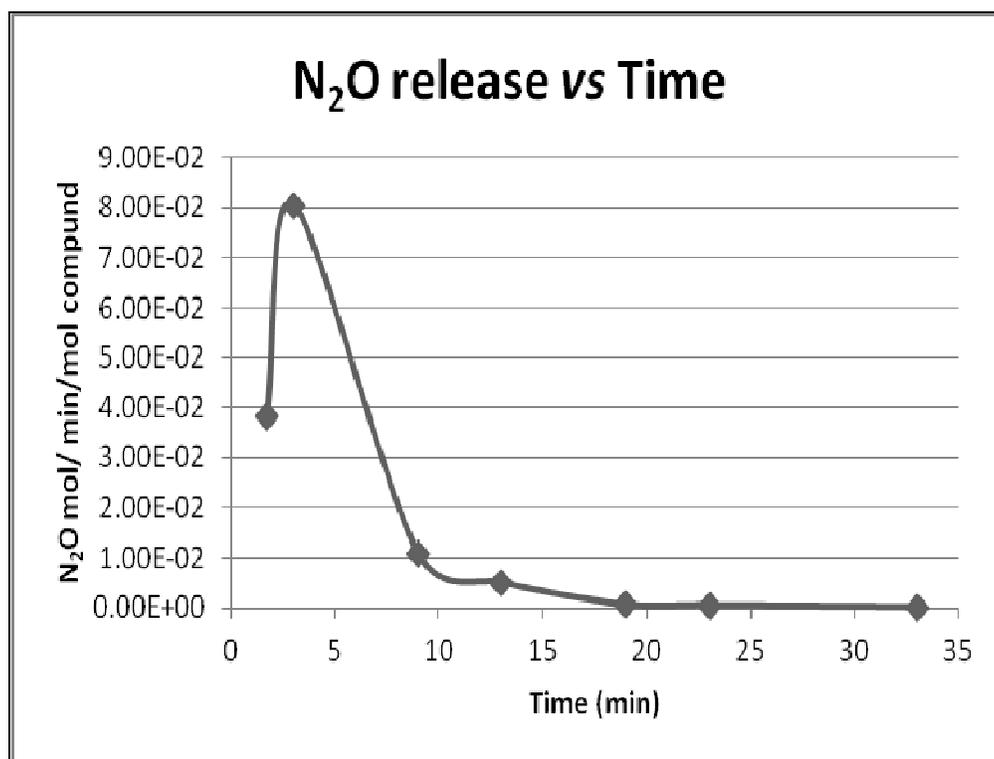


Figure S2. Gas chromatographic trace showing the time course of N₂O release from a solution of **4** in DMF in presence of Li^tBuO.

Estimation of NO-release by Chemiluminescence: Calibration of the Sievers Nitric Oxide Analyzer (NOA), model 280i (Instruments Business Group, Boulder, CO) was performed by injecting various volumes of known concentrations of NO in helium (50 ppm, 500 ppm and 5%) certified standards into the reaction chamber and recording the peak areas. Samples and reaction chambers were incubated at 37°C. Data were recorded using Agilent Chemstation software and processed using Microsoft Excel. 3 mL of pH 7.4 phosphate buffer was placed into the reaction chamber of the NOA and then purged for several minutes with argon. A 100 μ M solution of **3** in 0.1 M NaOH (50 μ L) was injected into the reaction chamber and nitric oxide release was recorded. Total amount of NO released was determined by integrating the area under the curve and applying a calibration curve. The NO release is an average of three independent experiments.

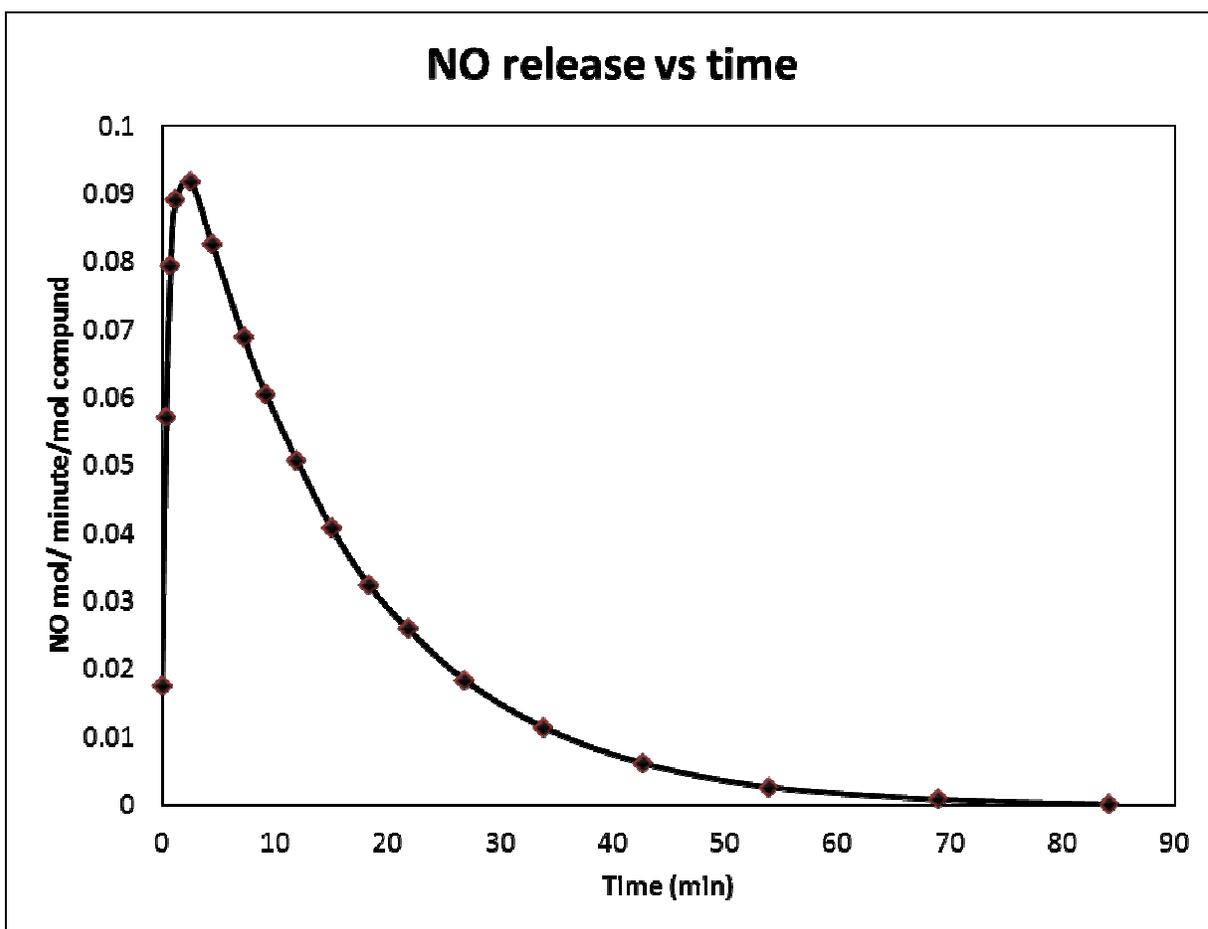


Figure S3. Time course of NO release from a solution of **3** in pH 7.4 phosphate buffer at 37 °C.

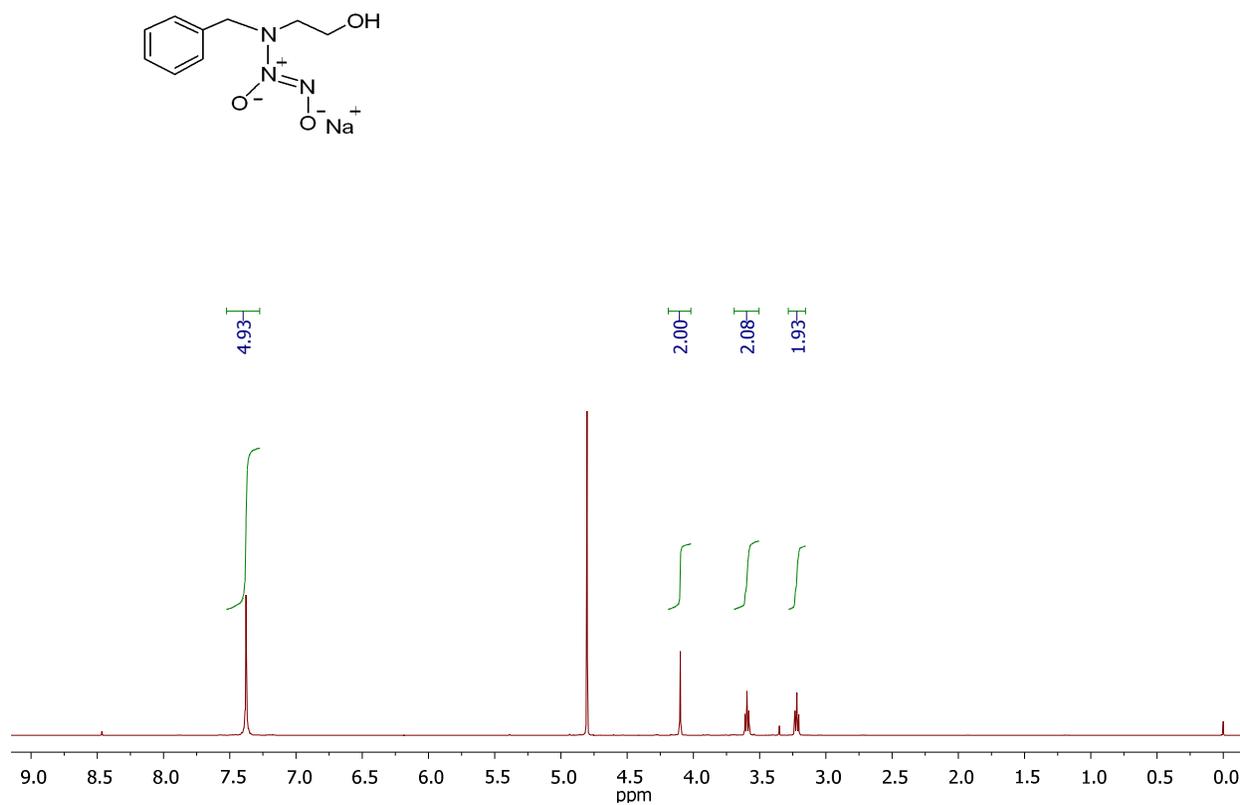


Figure S4. ¹H NMR spectrum of compound **3** in 0.01 M NaOD in D₂O.

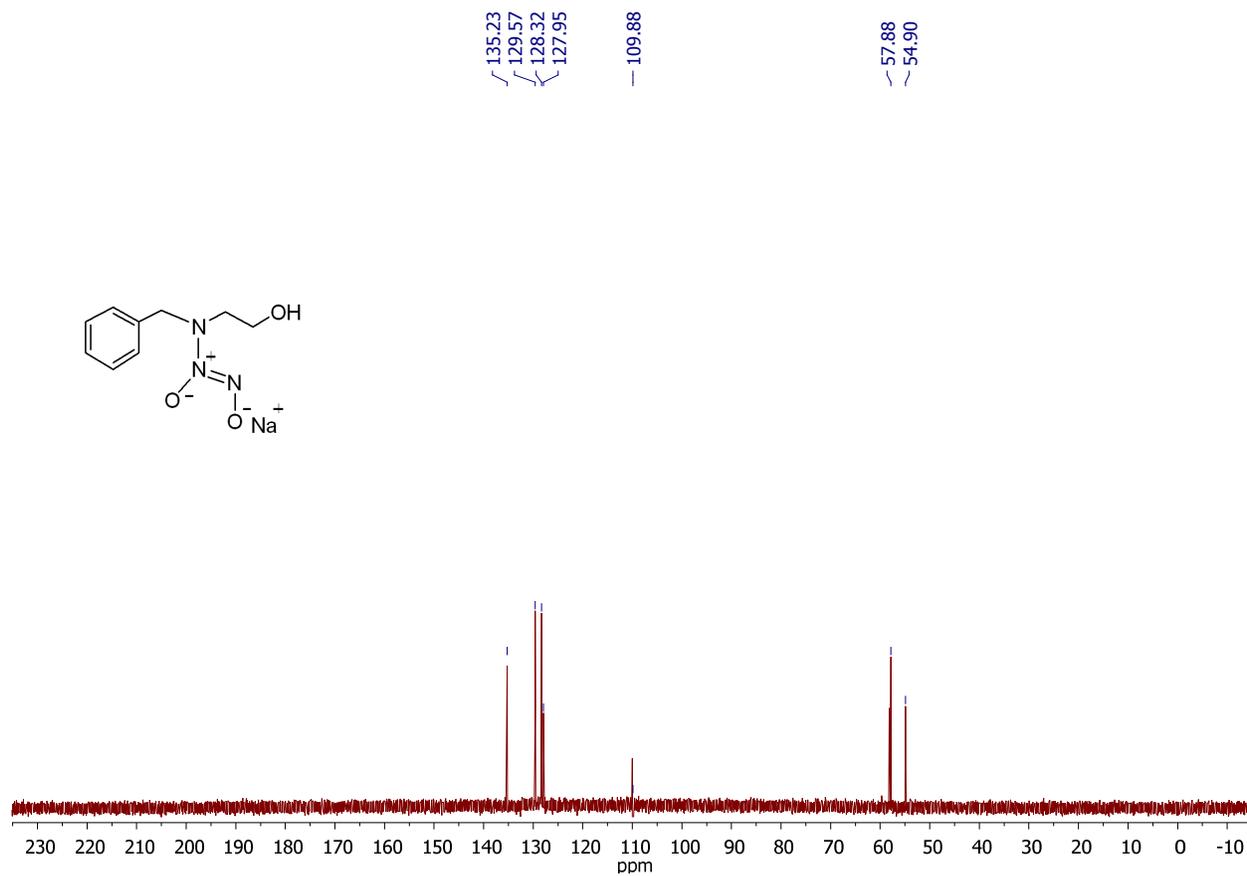


Figure S5. ^{13}C NMR spectrum of compound **3** in 0.01 M NaOD in D_2O .

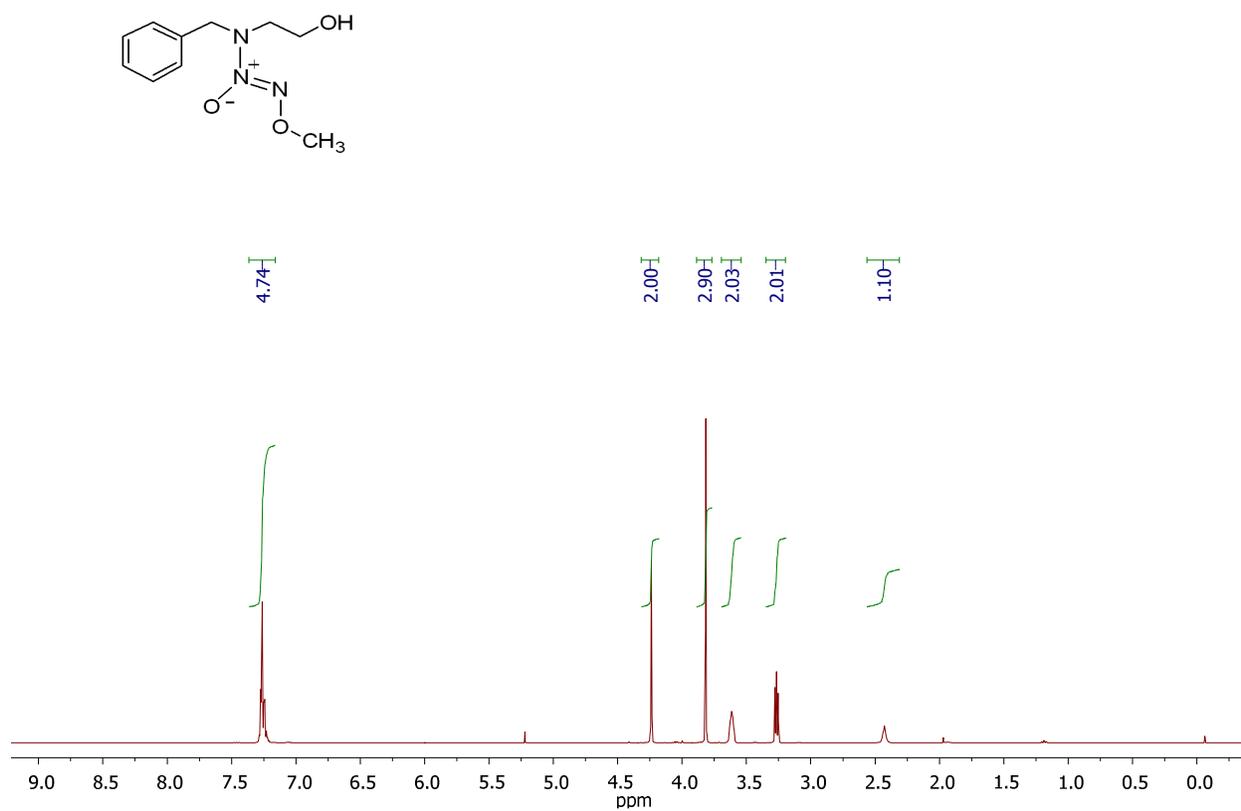


Figure S6. ¹H NMR spectrum of compound **4** in CDCl₃.

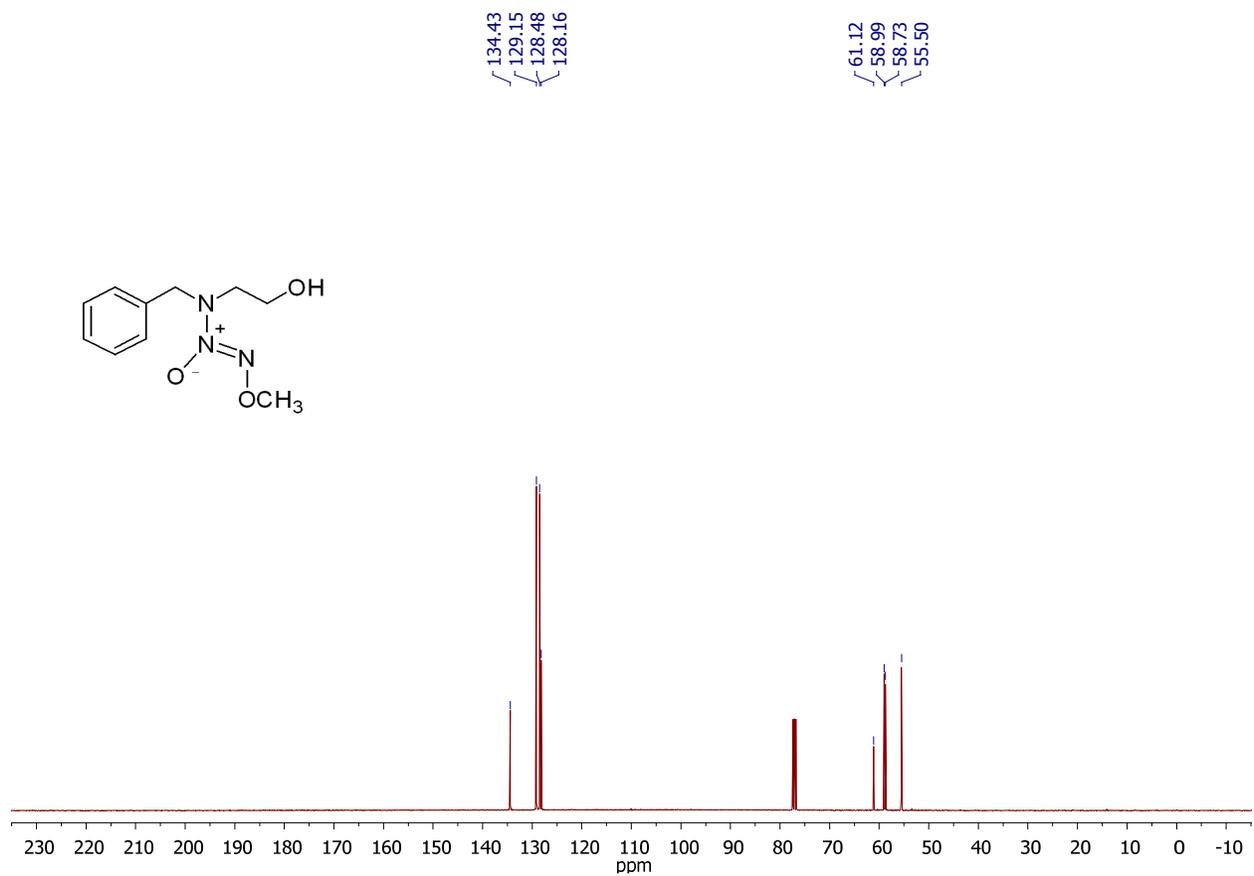


Figure S7. ¹³C NMR spectrum of compound **4** in CDCl₃.

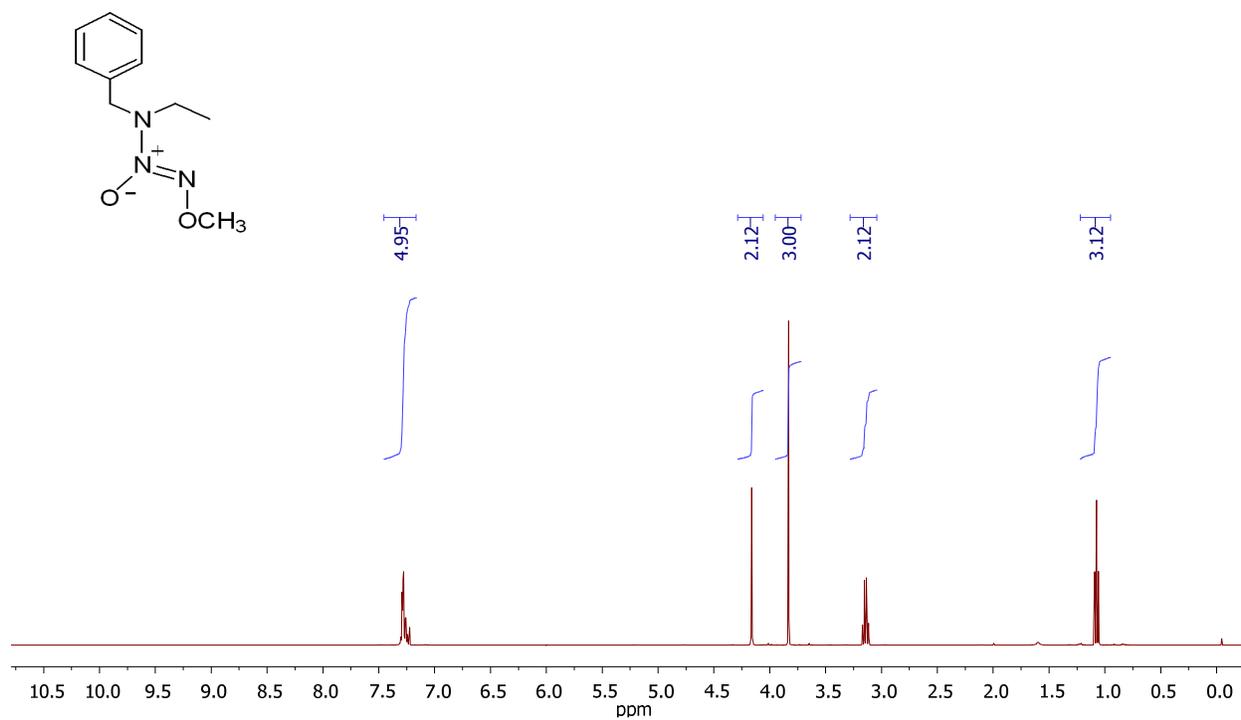


Figure S8. ¹H NMR spectrum of compound **7a** in CDCl₃.

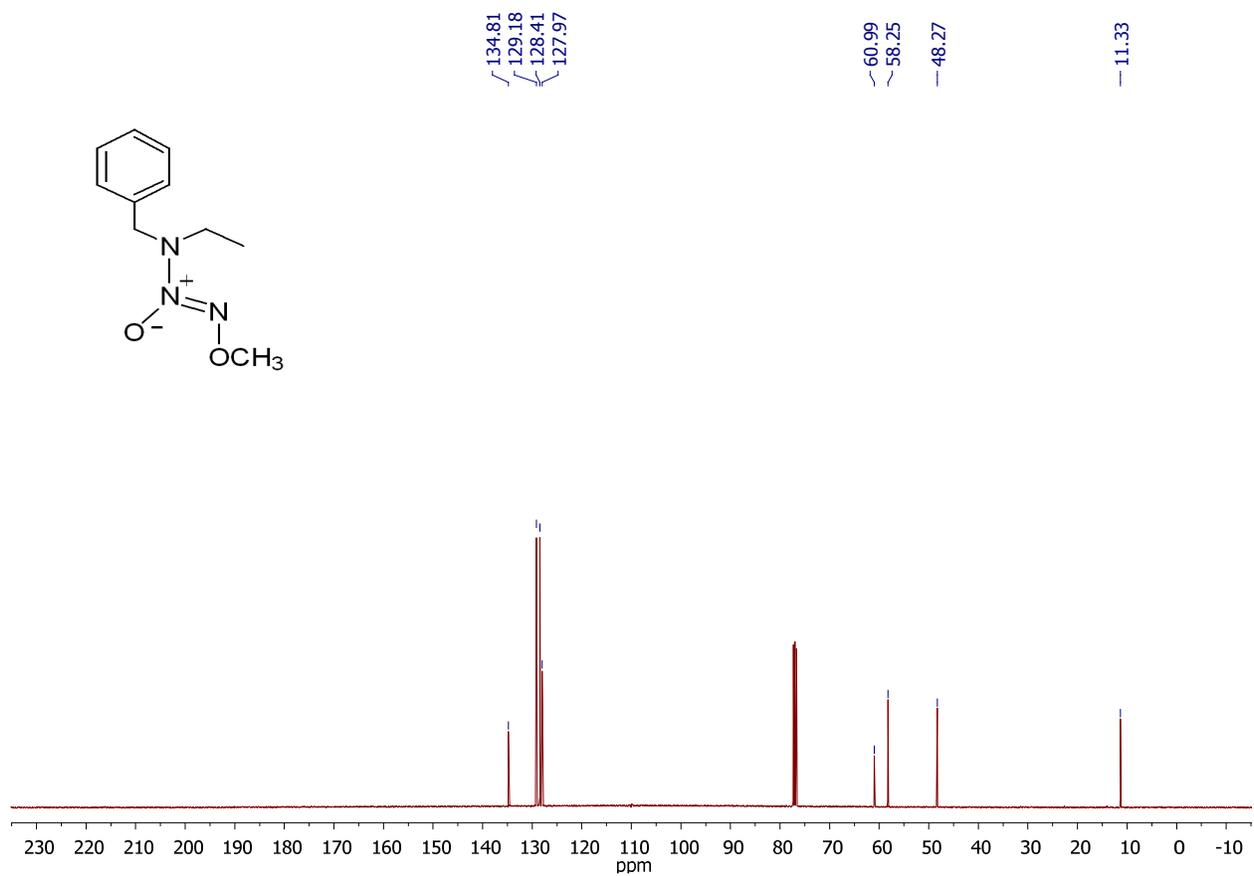


Figure S9. ¹³C NMR spectrum of compound **7a** in CDCl₃.

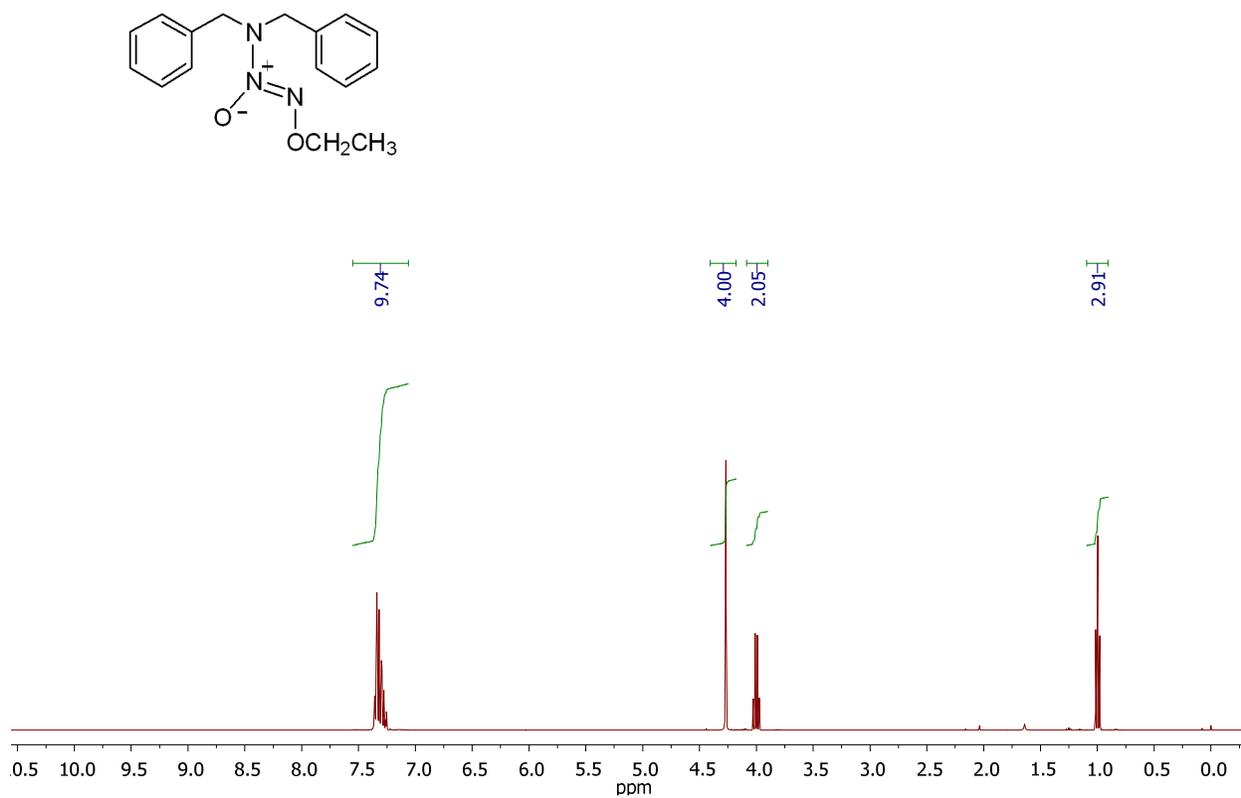


Figure S10. ¹H NMR spectrum of compound **7b** in CDCl₃.

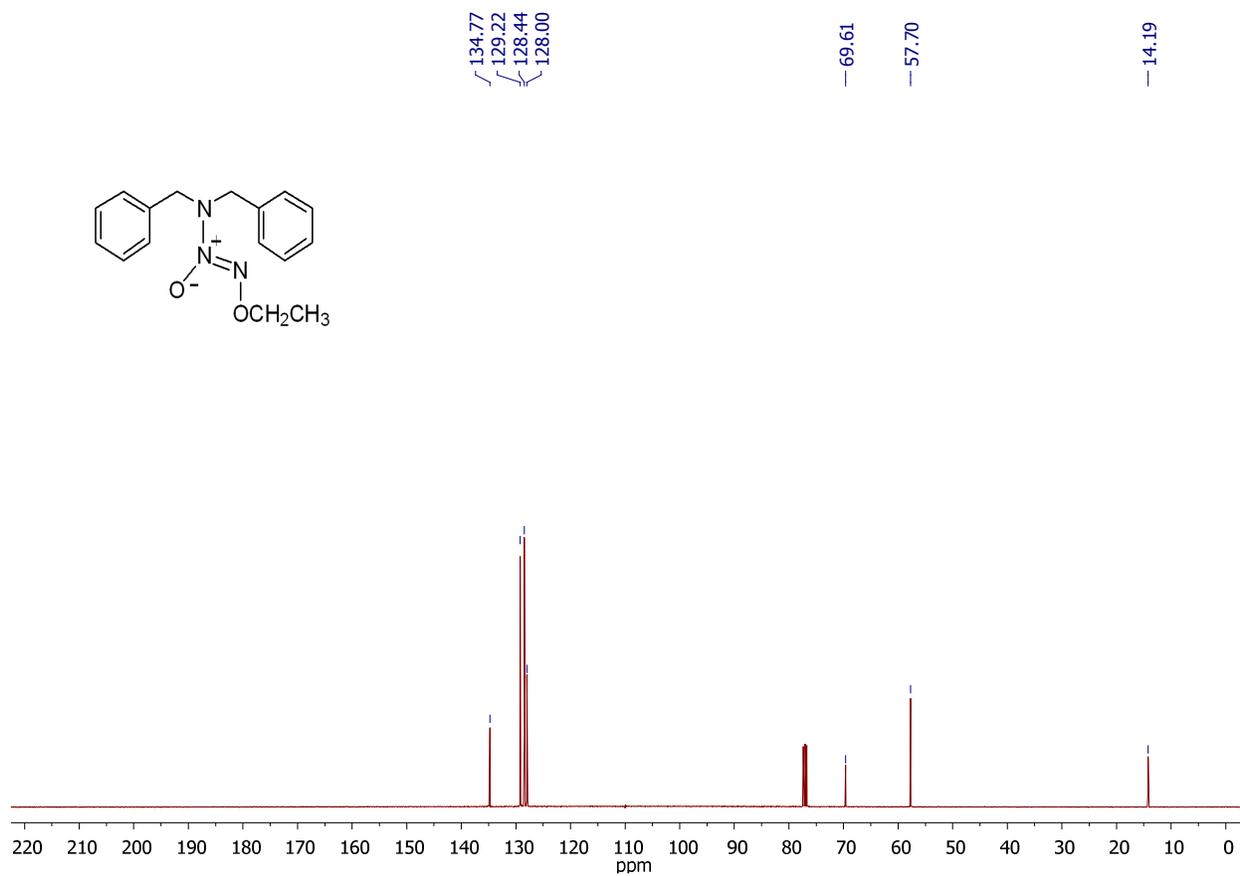


Figure S11. ^{13}C NMR spectrum of compound **7b** in CDCl_3 .

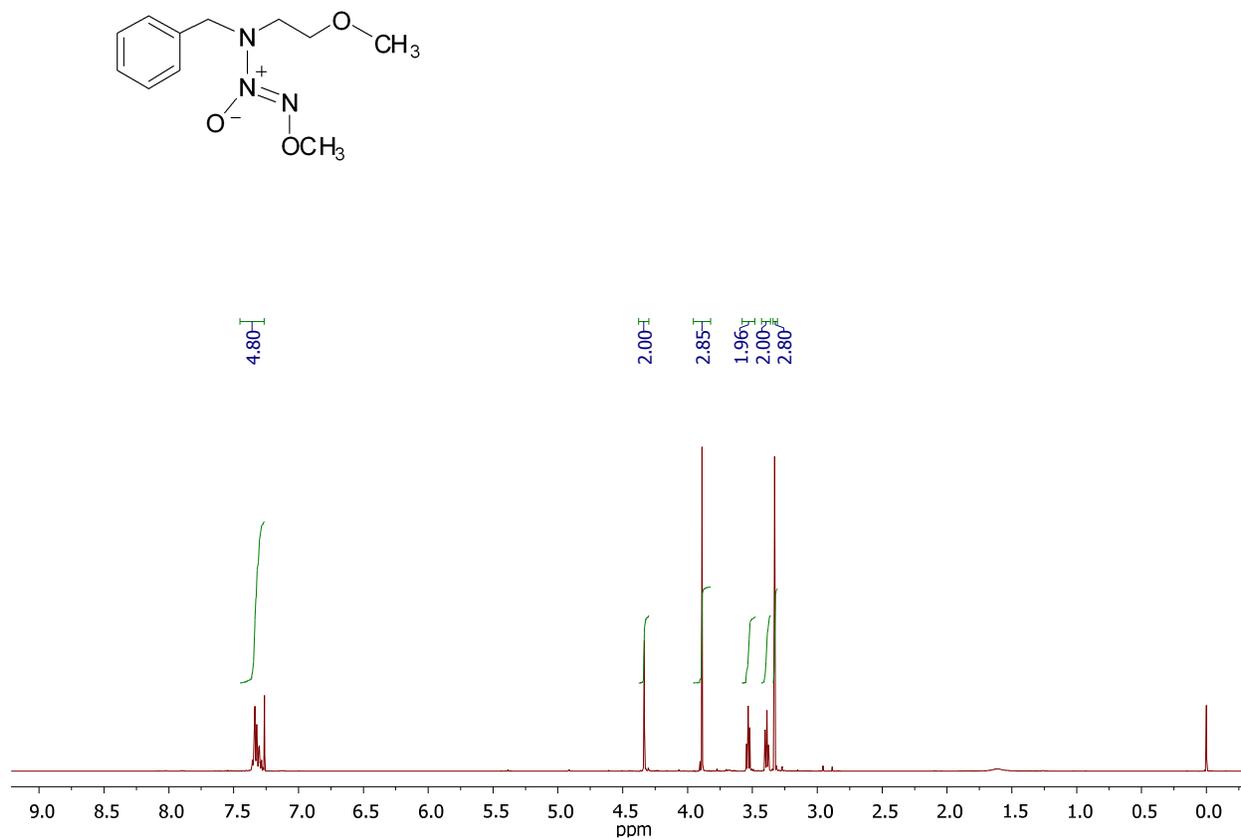


Figure S12. ¹H NMR spectrum of compound **9** in CDCl₃.

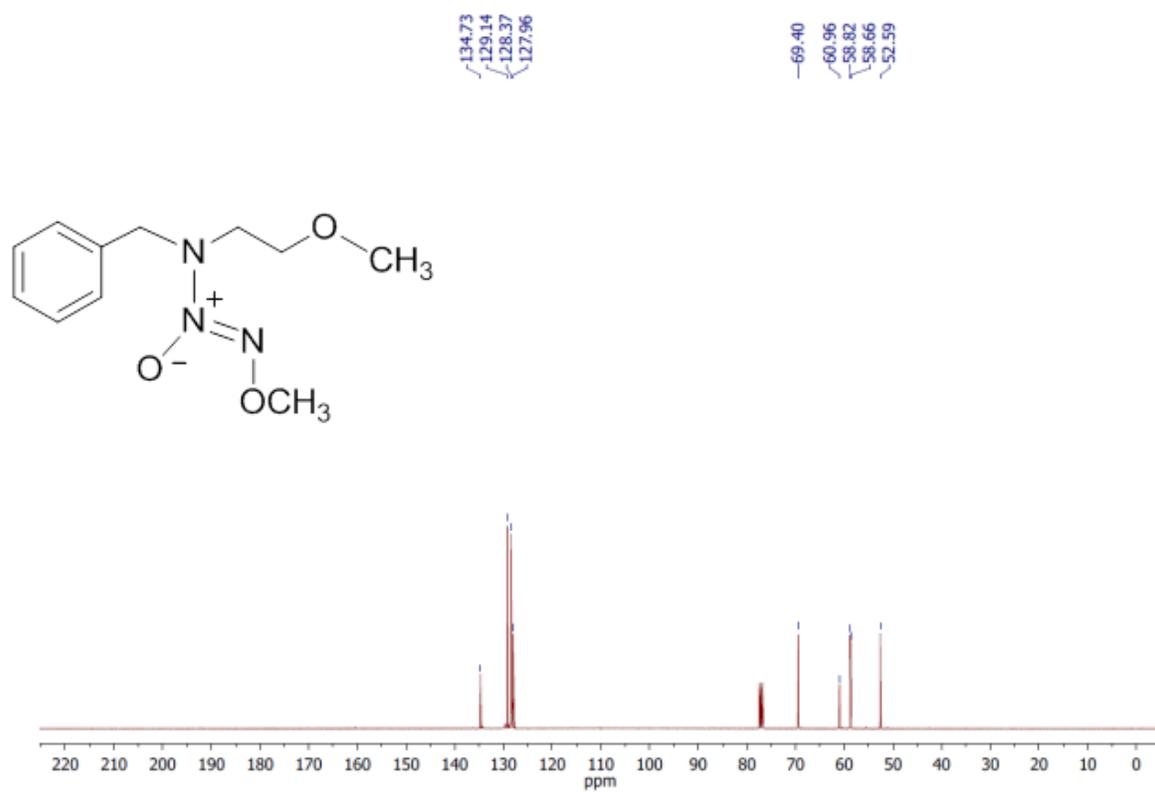


Figure S13. ^{13}C NMR spectrum of compound **9** in CDCl_3 .

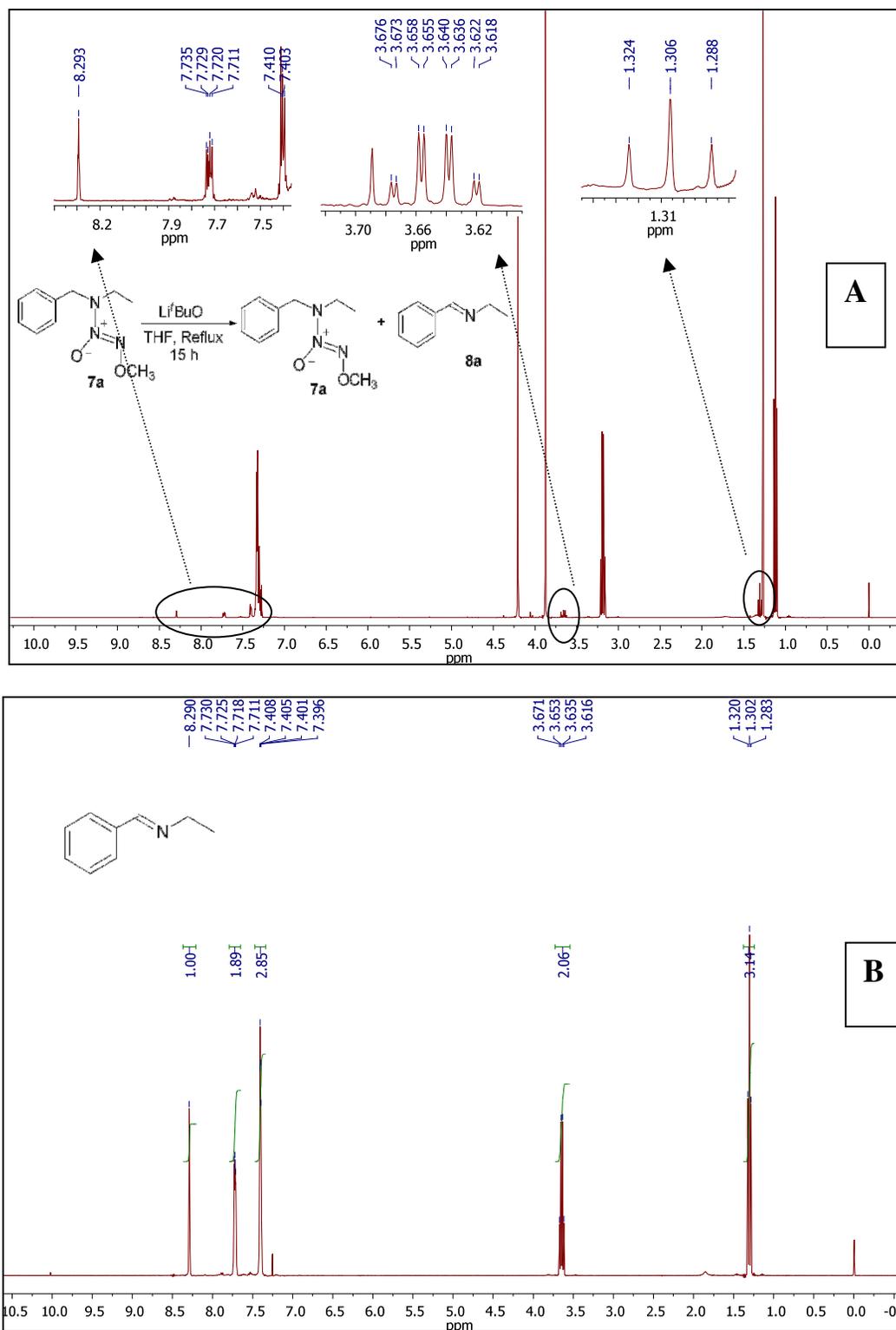


Figure S14. ¹H NMR spectra (CDCl₃) of **A**) the crude reaction mixture obtained by the treatment of 7a with Li^tBuO in THF under reflux followed by aqueous workup, **B**) an authentic sample of 8b.

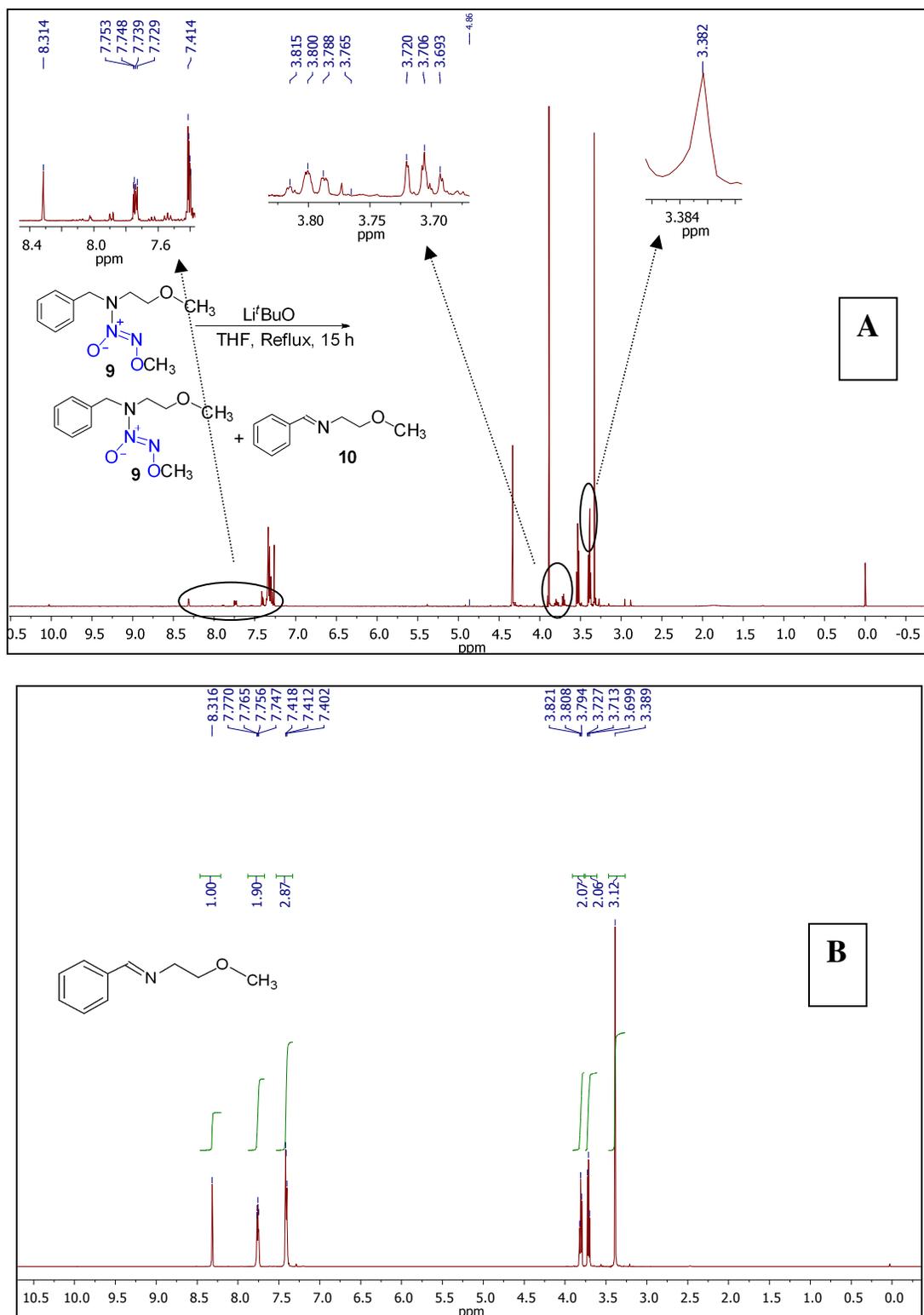


Figure S15. ¹H NMR spectra (CDCl₃) of **A**) the crude reaction mixture obtained by the treatment of **9** with Li^tBuO in THF under reflux followed by aqueous workup, **B**) an authentic sample of **10**.

References

1. Hrabie, J. A.; Klose, J. R.; Wink, D. A.; Keefer, L. K. *J. Org. Chem.* **1993**, *58*, 1472-1478.
2. Keefer, L. K.; Nims, R. W.; Davies, K. M.; Wink, D. A. *Methods Enzymol.* **1996**, *268*, 281.