J. Braz. Chem. Soc., Vol. 25, No. 5, S1-S15, 2014.
Printed in Brazil - ©2014 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Supplementary Information

Microwave-Assisted Synthesis of *N*-Heterocycles and Their Evaluation Using an Acetylcholinesterase Immobilized Capillary Reactor

Diego P. Sangi,[a,b] Julia L. Monteiro,[a] Kenia L. Vanzolini,[a] Quezia B. Cass,[a] Marcio W. Paixão[a] and Arlene G. Corrêa*[*,a]

[a]*Departamento de Química, Universidade Federal de São Carlos, 13565-905 São Carlos-SP, Brazil*

[b]*Instituto de Ciências Exatas, Universidade Federal Fluminense, 27213-145 Volta Redonda-RJ, Brazil*

Synthesis

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker ARX-400 (400 and 100 MHz, respectively). Mass spectra were recorded on a Shimadzu GCMS-QP5000. Direct infusion ultrahigh resolution and accurate mass spectrometry (orbitrap ESI-FT-MS) were carried out with an LTQ Orbitrap Velos FT-MS instrument (Thermo Fischer Scientific, Bremen, Germany) equipped with an electrospray source (HESI-II) operating in full scan negative ionization mode. Elemental analyses were performed on a Fisons EA 1108 CHNS-O.

General procedure: nitroethene **4** (165 mg, 1.0 mmol), diamine or hydroxilamine (1.0 mmol) and ethanol (3 mL) were placed in a glass tube, sealed and irradiated during 20-60 min in a CEM Discovery® focused microwave oven at 110 ºC . *N*-heterocycles **5** and **7** were purified by flash chromatography employing hexanes:ethyl acetate (2:1 ratio) as eluent.

2-(nitromethylene)imidazolidine (**5a**)[1]: ^{1}H NMR (MeOD, 400 MHz) δ 3.75 (s, 4H), 6.57 (s, 1H); ^{13}C NMR (100 MHz, MeOD) δ 44.63, 98.13, 162.53.

2-(nitromethylene)oxazolidine (**5b**)[2]: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.14-3.56 (m, 4H), 6.53 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 44.71, 59.92, 97.69, 156.75.

2-(nitromethylene)hexahydropyrimidine (**5c**)[1]: ^{1}H NMR (MeOD, 400 MHz) δ 1.97 (quint, 2H, *J* 5.83 Hz), 3.41 (t, 4H, *J* 5.83 Hz), 6.48 (s, 1H); GC-MS (70 eV) *m/z* (%): 143 (M^+, 100), 116 (23), 91 (13), 64 (18).

*e-mail: agcorrea@ufscar.br

2-(nitromethylene)-1,3-oxazinane (**5d**)[2]: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.16-2.22 (m, 2H), 3.59 (t, 2H, *J* 6.02 Hz), 4.40 (t, 2H, *J* 5.40 Hz), 6.51 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.51, 37.50, 66.40, 101.07, 163.67.

(±)-2-(nitromethylene)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]oxazole (**5e**): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 3.25 (d, 1H, *J* 18.27 Hz), 3.44 (dd, 1H, *J* 18.27, 6.38 Hz), 5.54 (d, 1H, *J* 7.05 Hz), 5.63-5.67 (m, 1H), 6.74 (s, 1H); GC-MS (70 eV) *m/z* (%): 218 (18), 172 (75), 131 (85), 115 (100), 103(95); Anal. calcd. for $C_{11}H_{10}N_2O_3$: C 60.55%, H 4.62%, N 12.84%; found: C 59.47%, H 4.78%, N 12.57%.

(±)-2-(nitromethylene)octahydro-1H-benzo[d]imidazole (**5f**): ^{1}H NMR (MeOD, 400 MHz) δ 1.37-1.56 (m, 4H), 184-1.88 (m, 2H), 2.17-2.22 (m, 2H), 3.18-3.22 (m, 2H), 6.60 (s, 1H); ^{13}C NMR (MeOD, 100 MHz) δ 23.63, 28.66, 63.23, 97.64, 162.68; EI-HRMS ($[M–H]^–$) calcd. for $C_8H_{12}N_3O_2$: 182.0931; found: 182.0889.

2-(nitromethyl)benzo[d]oxazole (**7a**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.83 (s, 2H), 7.42-7.50 (m, 2H), 7.61-7.64 (m, 1H), 7.82-7.85 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 71.63, 111.21, 121.06, 125.28, 126.82, 140.62, 153.93; GC-MS (70 eV) *m/z* (%): 178 (M^+, 2), 132 (100), 104 (25), 77 (35); EI-HRMS ($[M–H]^–$) calcd. for $C_8H_5N_2O_3$: 177.0301; found: 177.0260.

4-methyl-2-(nitromethyl)benzo[d]oxazole (**7b**): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.64 (s, 3H), 5.80 (s, 2H), 7.21 (d, 1H, *J* 7.51 Hz), 7.38-7.34 (dd, 1H, *J* 7.51, 8.07 Hz), 7.41 (d, 1H, *J* 8.07 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.42, 71.69, 108.46, 125.74, 126.55, 131.71, 139.99, 151.19, 153.15; GC-MS (70 eV) *m/z* (%): 192 (M^+, 3), 146 (100), 117 (18), 91 (32); EI-HRMS ($[M–H]^–$) calcd. for $C_9H_7N_2O_3$: 191.0457; found: 191.0413.

5-methyl-2-(nitromethyl)benzo[d]oxazole (**7c**)[3]: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.50 (s, 3H), 5.78 (s, 2H), 7.25-7.29 (m, 1H), 7.47 (d, 1H, *J* 8.37 Hz), 7.57-7.60 (m, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.48, 71.70, 110.58, 120.82, 128.06, 135.32, 140.84, 153.97.

6-methyl-2-(nitromethyl)benzo[d]oxazole (**7d**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.52 (s, 3H), 5.78 (s, 2H), 7.23 (d, 1H, *J* 8.23 Hz), 7.38-7.42 (m, 1H), 7.67 (d, 1H, *J* 8.23 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 21.87, 71.69, 111.24, 120.36, 126.66, 137.60, 138.46, 151.69, 153.40; GC-MS (70 eV) *m/z* (%): 192 (M^+, 2), 146 (100), 118 (15), 78 (30). EI-HRMS ($[M–H]^-$) calcd. for $C_9H_7N_2O_3$: 191.0457; found: 191.0413.

5-chloro-2-(nitromethyl)benzo[d]oxazole (**7e**)[3]: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.81 (s, 2H), 7.44 (dd, 1H, *J* 8.49, 2.12 Hz), 7.54 (dd, 1H, *J* 8.49 Hz), 7.80 (d, 1H, *J* 2.12 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 71.48, 112.07, 125.74, 121.04, 127.34, 130.97, 141.67, 149.90, 155.26; GC-MS (70 eV) *m/z* (%): 212 (M^+, 5), 166 (100), 138 (25), 102 (45), 63 (40).

Bioassays

The biological assays were performed in an HPLC system (Kyoto, Japan) equipped with two LC-20AD pumps, auto sampler SIL-20A and CBM-20A interface at 20 °C. The system was coupled to a mass spectrometer Esquire 6000 IT (Bruker Daltonics, Germany) equipped with an ESI-type ionization source operating in positive mode. All buffers used were prepared using ultrapure water (Milli-Q system, Millipore, São Paulo, Brazil)

The acetylcholinesterase from *Electrophorus electricus* immobilized onto a fused silica capillary (*eel*AChE-ICER) was coupled with mass spectrometry and the enzyme reaction product, choline, was monitored in positive mode ($[M]^+$ 104 *m/z*). The mobile phase was ammonium acetate buffer (15 mmol L^{-1}, pH 8.0) at a flow rate of 0.05 mL min^{-1} (pump A) with injection sample volume at the *eel*AChE-ICER of 10 µL. Methanol was used to improve ionization, which was delivered by pump B, at the same flow rate of pump A. The analysis time was 20 minutes.

The preparation of *eel*AChE-ICER and the screening assay of *N*-heterocycles **5a**-**f** and **7a**-**e** (1.0 mg) were carried out following the procedure previously reported.[4] For each tested compound sample, a negative control (absence of ACh) and positive control samples (ACh and absence of *N*-heterocycles) were analyzed.

References

1. Kalisiak, J.; Ralph, E. C.; Cashman, J. R.; *J. Med. Chem.* **2012**, *55*, 465.
2. Schroeder, M. E.; Flattum, R. F.; *Pestic. Biochem. Physiol.* **1984**, *22*, 148.
3. Buevich, V. A.; Rudchenko, V. V.; Grineva, V. S.; Perekalin, V. V.; *Russ. J. Org. Chem.* **1978**, *14*, 2197.
4. Vanzolini, K. L.; Vieira, L. C. C.; Corrêa, A. G.; Cardoso, C. L.; Cass, Q. B.; *J. Med. Chem.* **2013**, *56*, 2038.

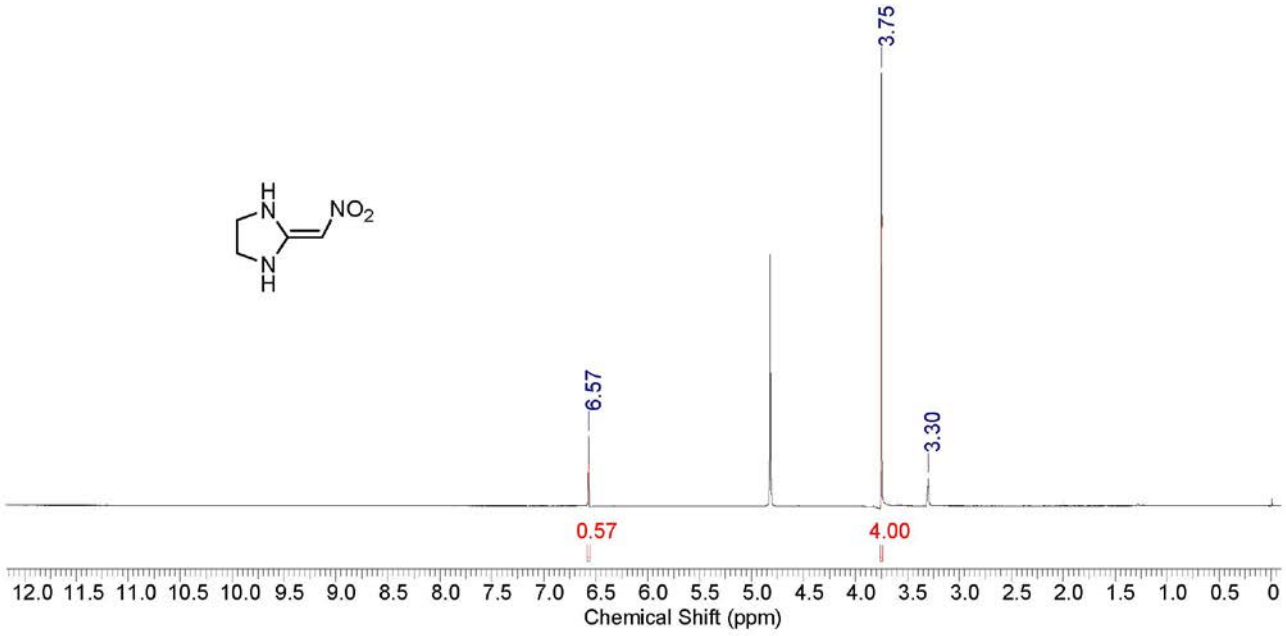

Figure S1. ^{1}H NMR (MeOD, 400 MHz) of 2-(nitromethylene)imidazolidine (**5a**).

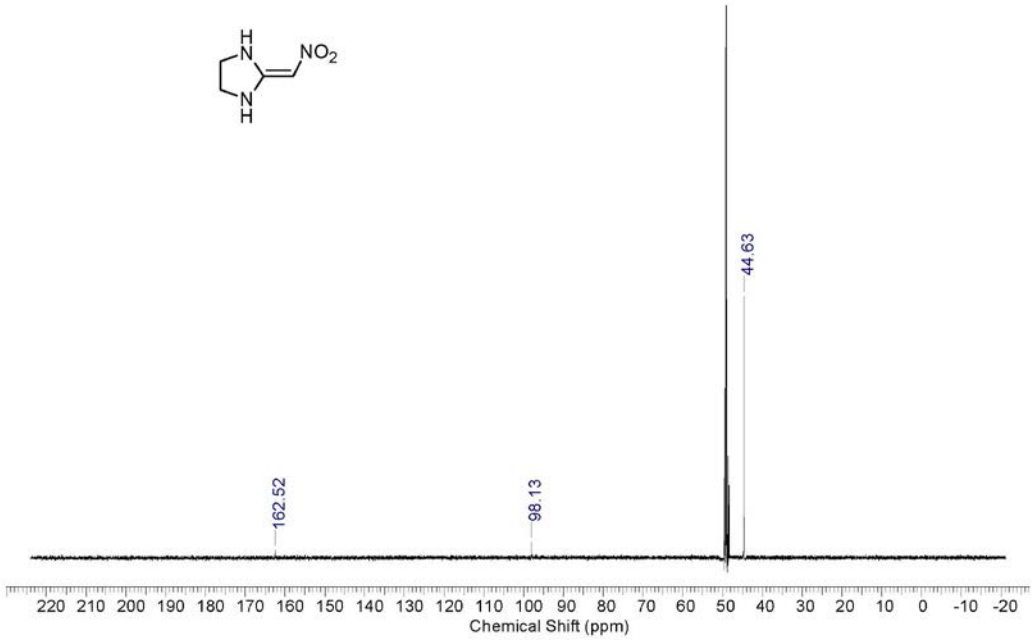

Figure S2. ^{13}C NMR (MeOD, 100 MHz) of 2-(nitromethylene)imidazolidine (**5a**).

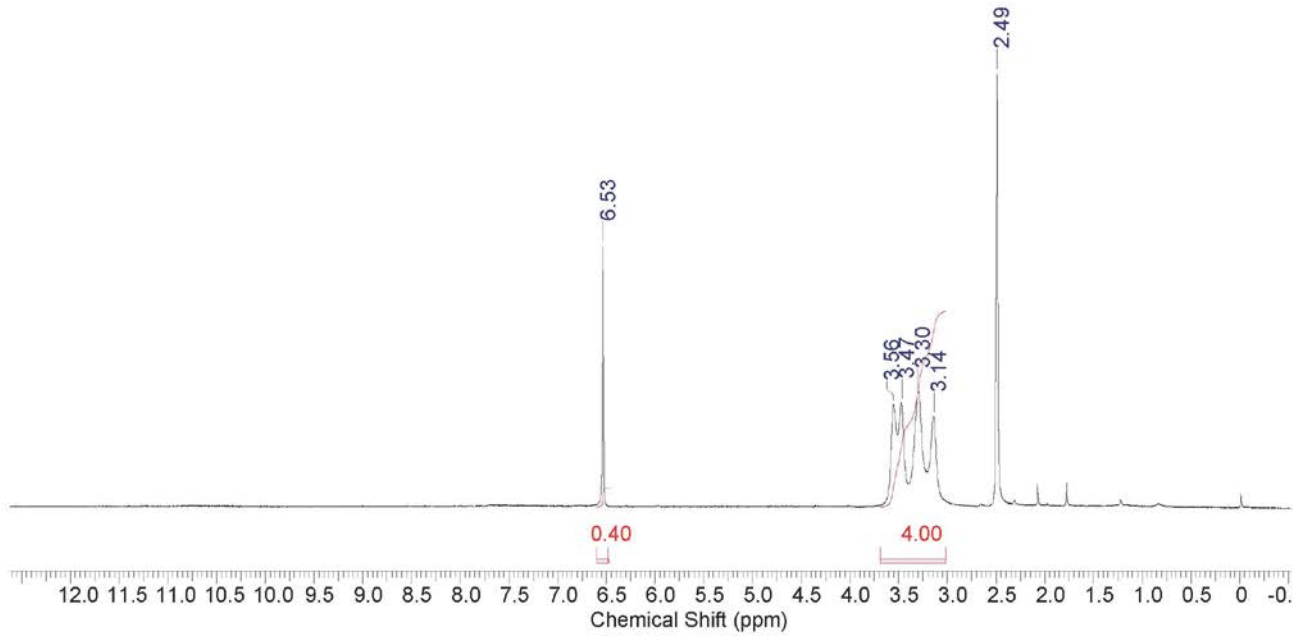

Figure S3. ^{1}H NMR (DMSO-d_6, 400 MHz) of 2-(nitromethylene)oxazolidine (**5b**).

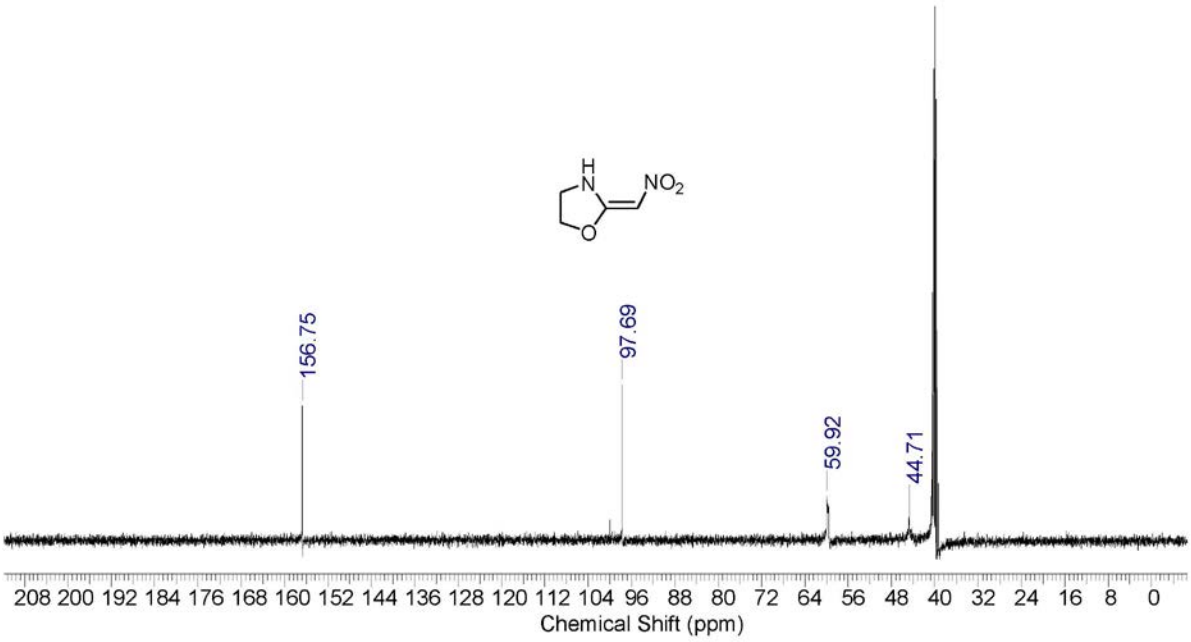

Figure S4. ^{13}C NMR (DMSO-d_6, 100 MHz) of 2-(nitromethylene)oxazolidine (**5b**).

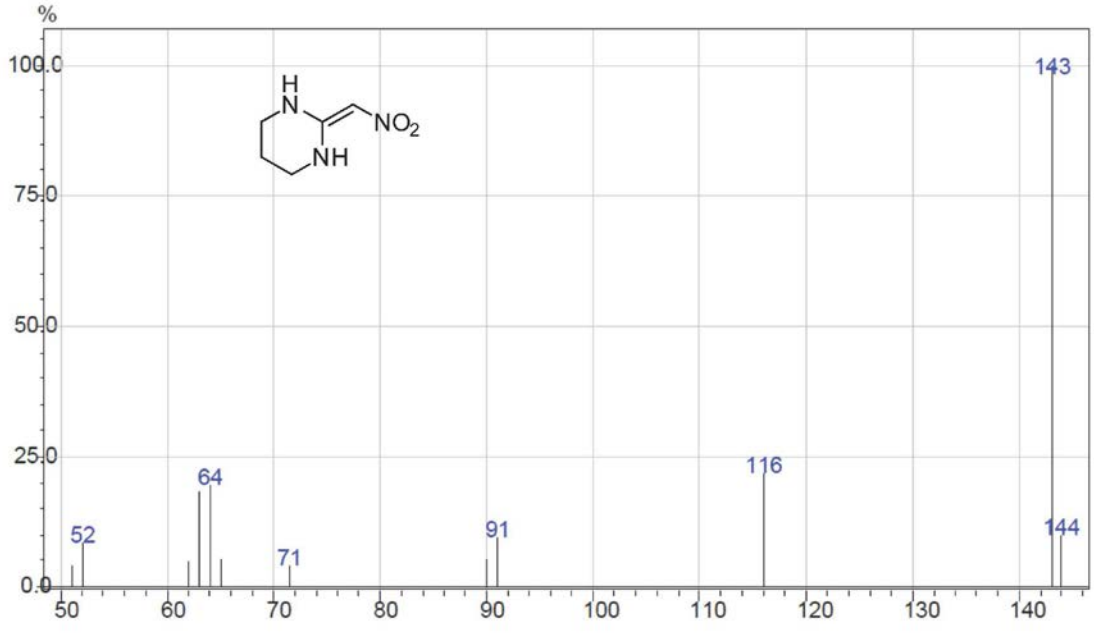

Figure S5. Mass spectrum of 2-(nitromethylene)hexahydropyrimidine (**5c**).

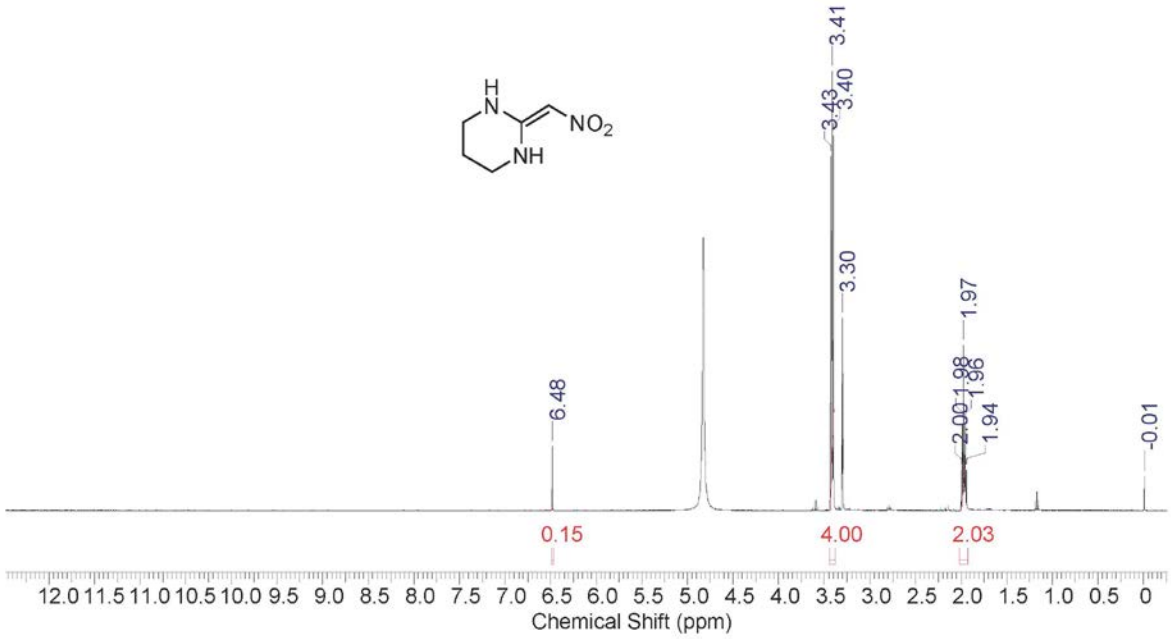

Figure S6. [1]H NMR spectrum (MeOD, 400 MHz) of 2-(nitromethylene)hexahydropyrimidine (**5c**).

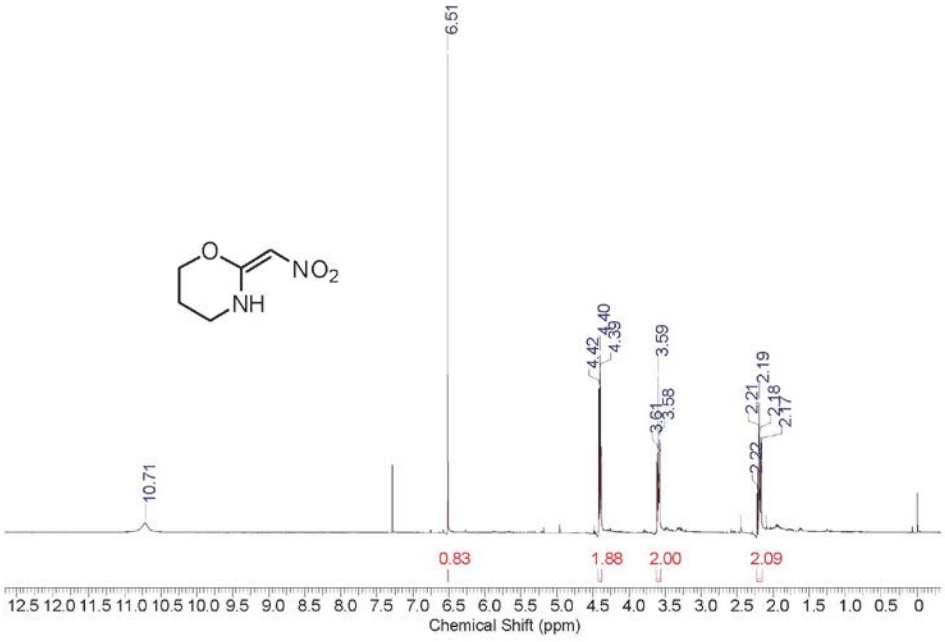

Figure S7. [1]H NMR spectrum ($CDCl_3$, 400 MHz) of 2-(nitromethylene)-1,3-oxazinane (**5d**).

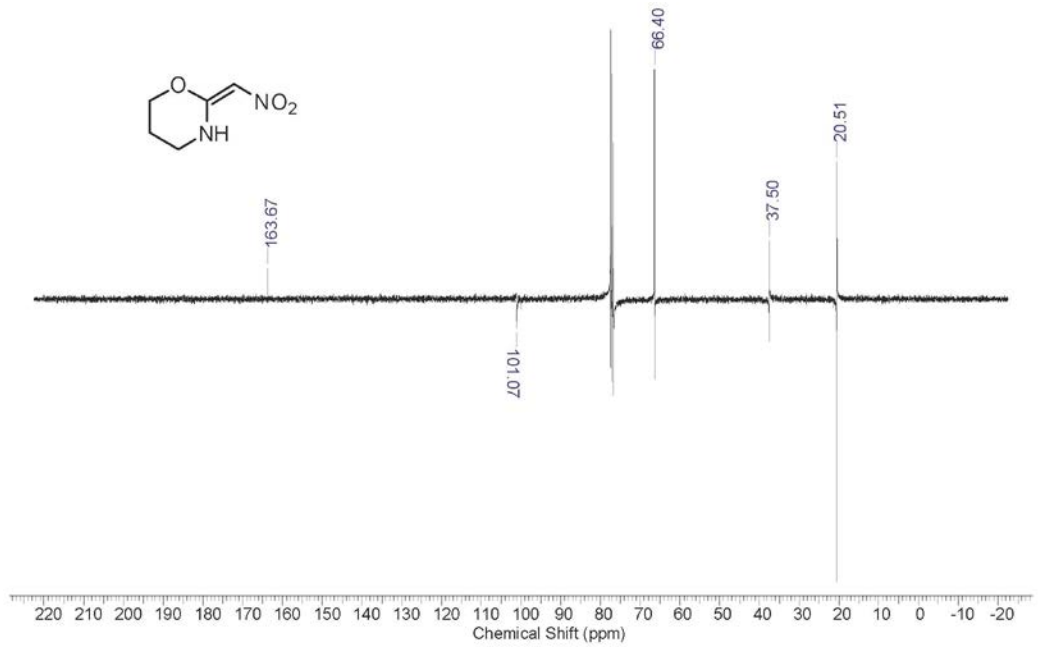

Figure S8. ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of 2-(nitromethylene)-1,3-oxazinane (**5d**).

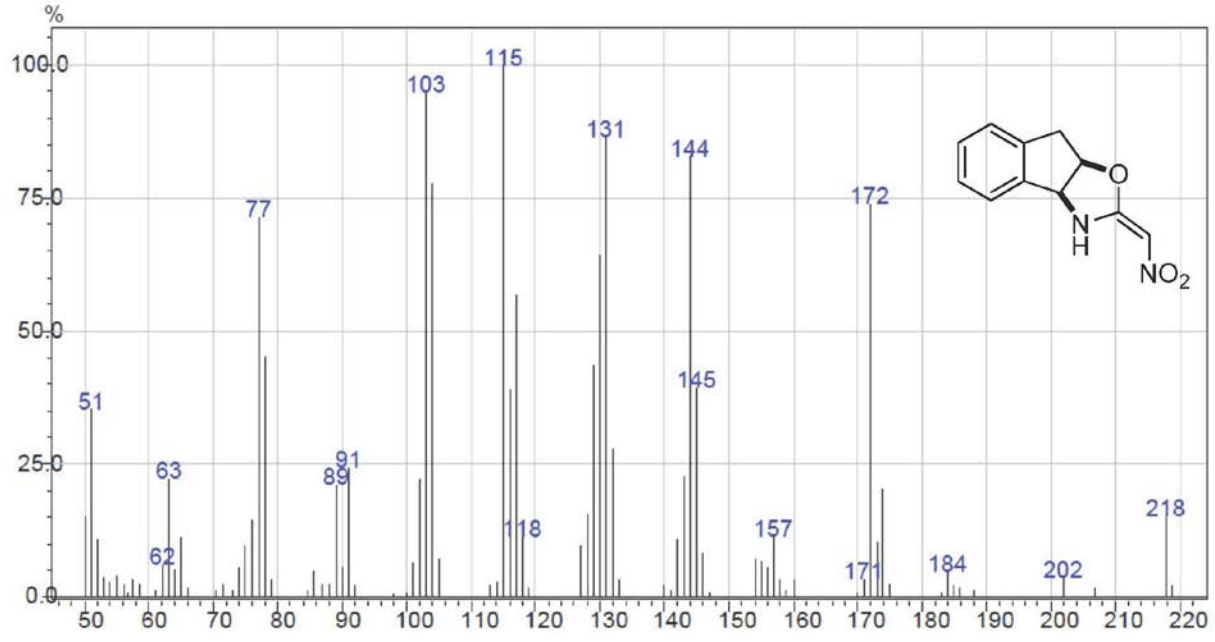

Figure S9. Mass spectrum of 2-(nitromethylene)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]oxazole (**5e**).

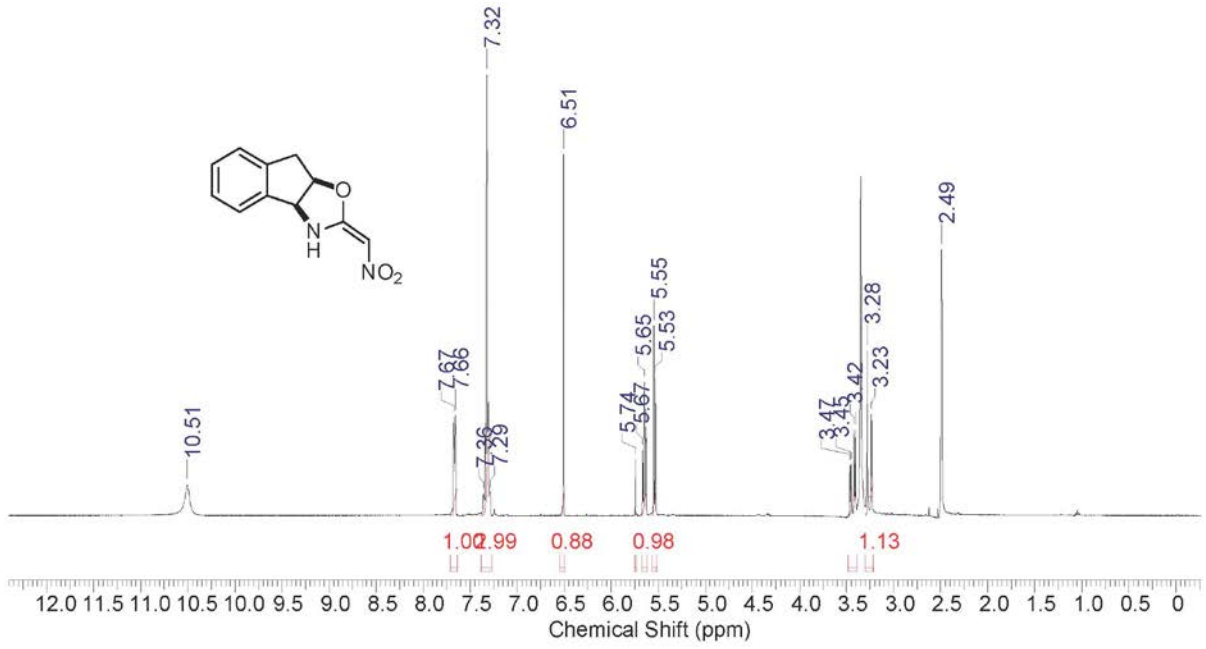

Figure S10. [1]H NMR spectrum (DMSO-d_6, 400 MHz) of 2-(nitromethylene)-3,3a,8,8a-tetrahydro-2H-indeno[1,2-d]oxazole (**5e**).

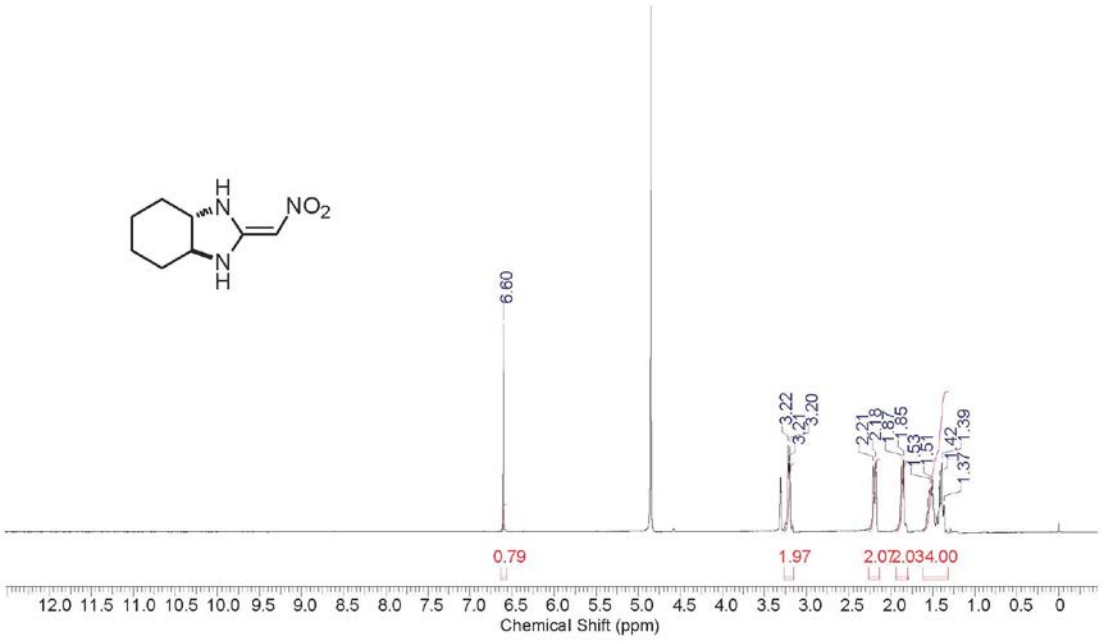

Figure S11. [1]H NMR spectrum (MeOD, 400 MHz) of 2-(nitromethylene)octahydro-1H-benzo[d]imidazole (**5f**).

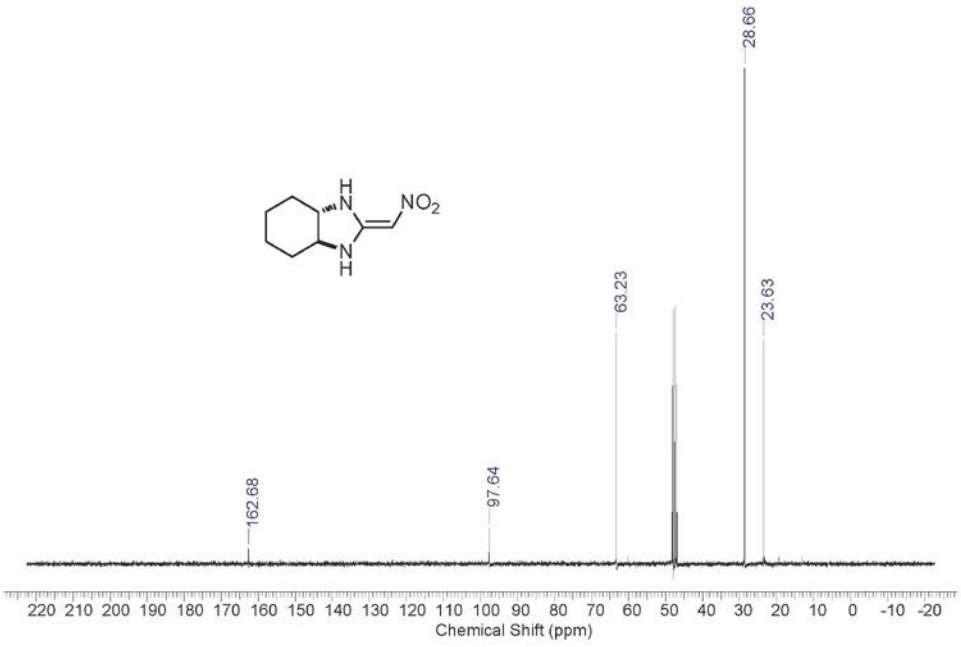

Figure S12. ^{13}C NMR spectrum (MeOD, 100 MHz) of 2-(nitromethylene)octahydro-1H-benzo[d]imidazole (**5f**).

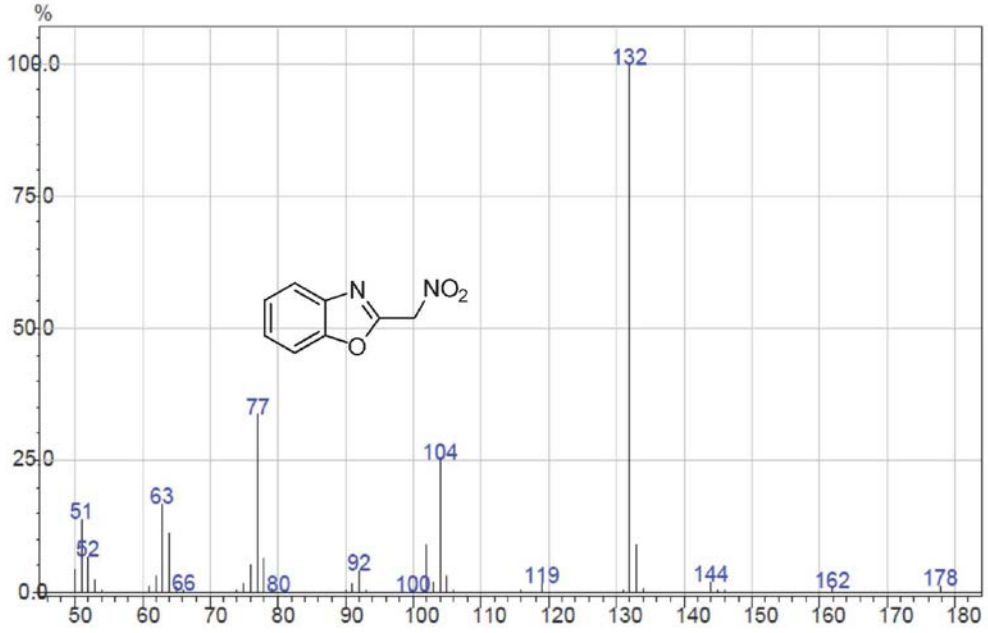

Figure S13. Mass spectrum of 2-(nitromethyl)benzo[d]oxazole (**7a**).

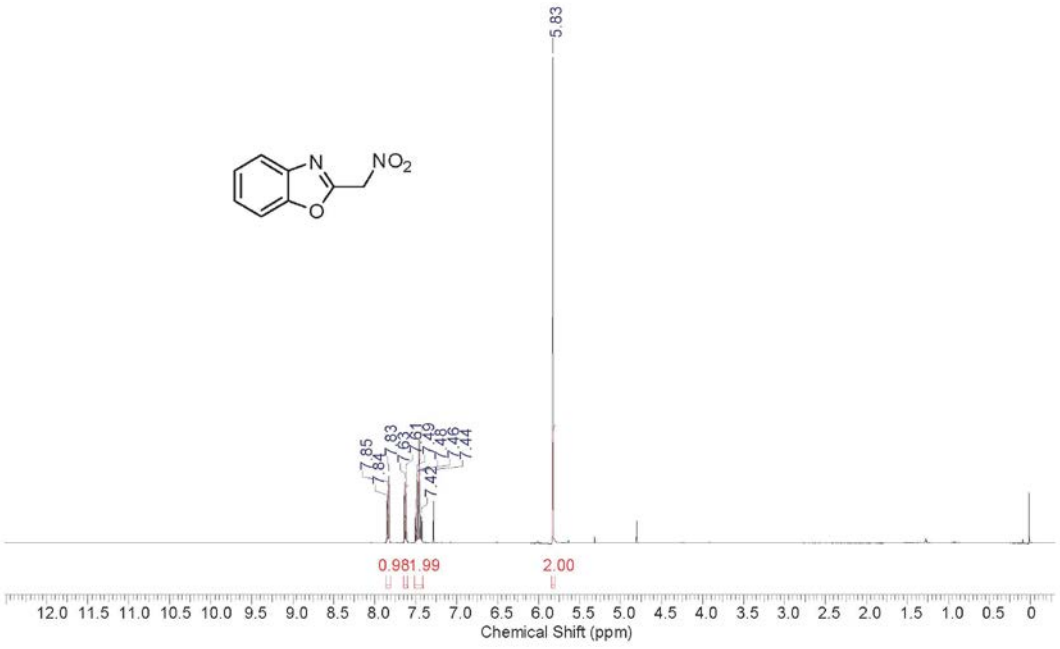

Figure S14. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of 2-(nitromethyl)benzo[d]oxazole (**7a**).

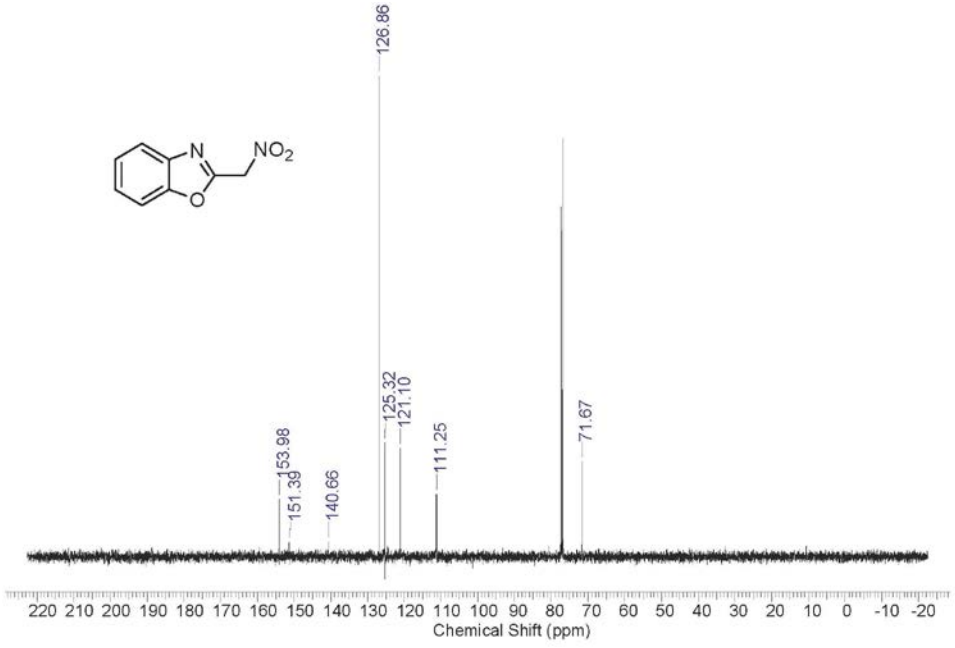

Figure S15. ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of 2-(nitromethyl)benzo[d]oxazole (**7a**).

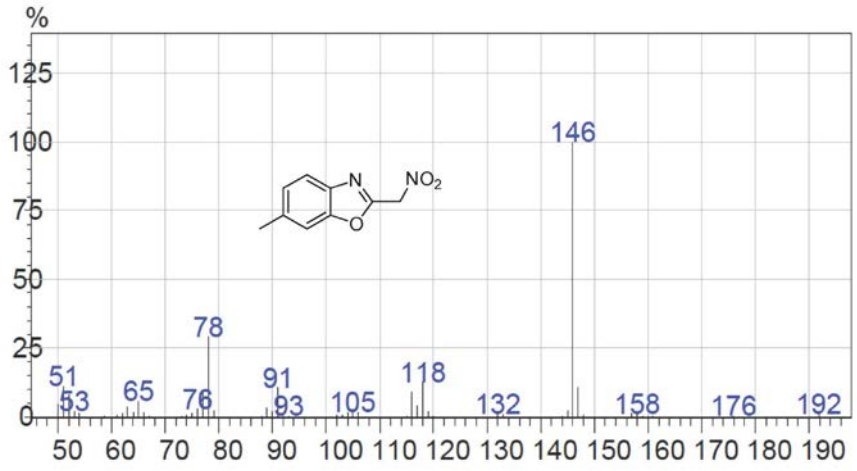

Figure S16. Mass spectrum of 6-methyl-2-(nitromethyl)benzo[d]oxazole (**7b**).

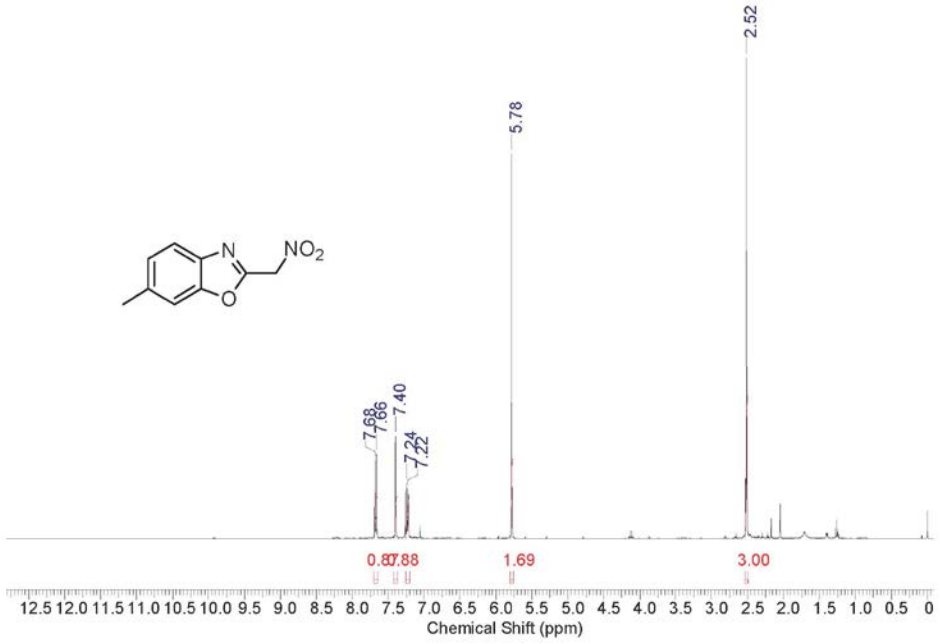

Figure S17. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of 6-methyl-2-(nitromethyl)benzo[d]oxazole (**7b**).

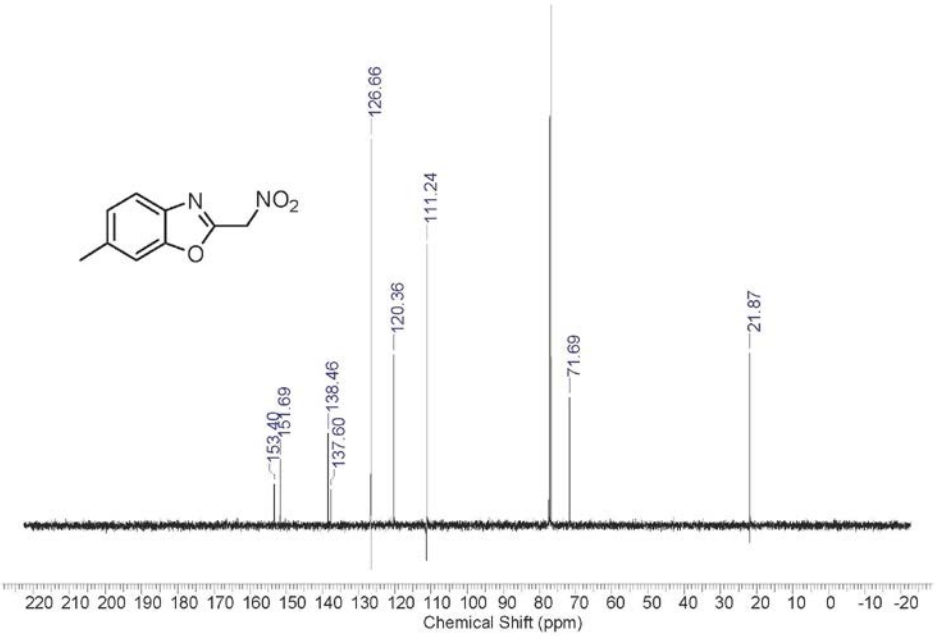

Figure S18. ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of 6-methyl-2-(nitromethyl)benzo[d]oxazole (**7b**).

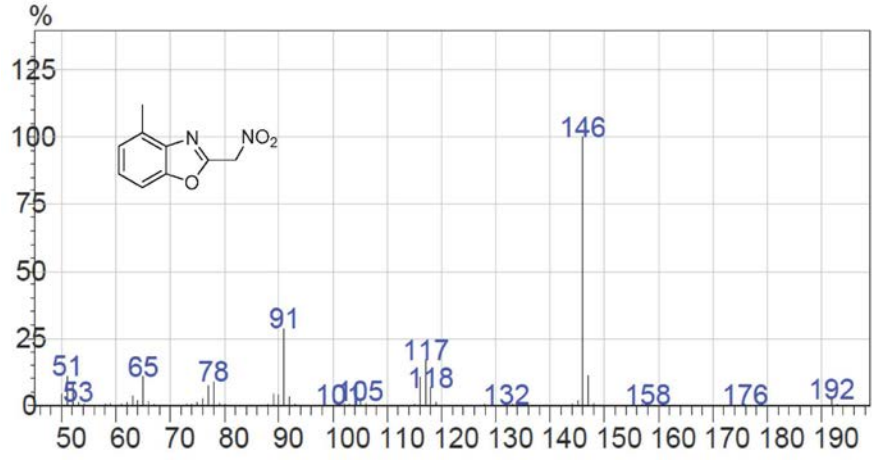

Figure S19. Mass spectrum of 4-methyl-2-(nitromethyl)benzo[d]oxazole (**7c**).

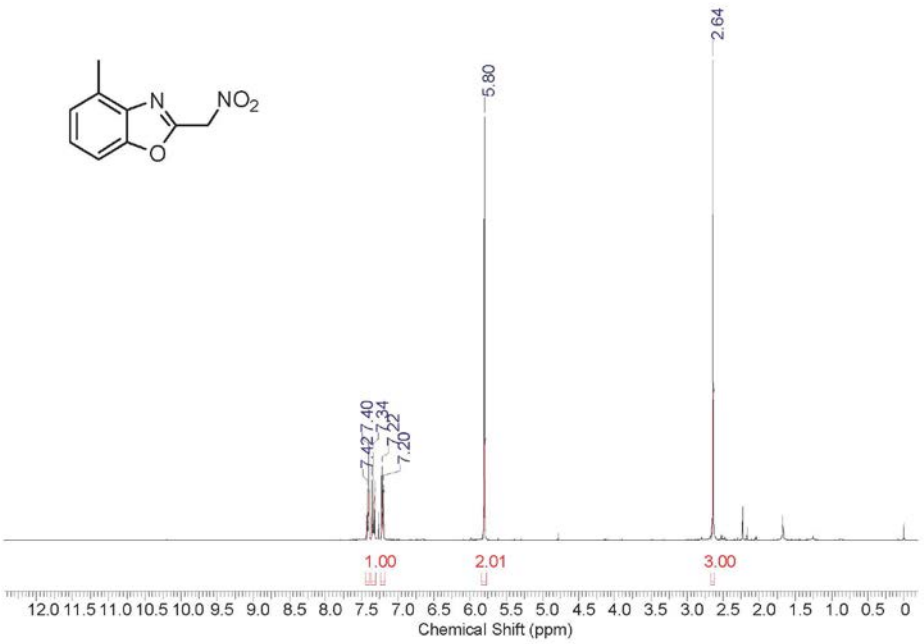

Figure S20. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of 4-methyl-2-(nitromethyl)benzo[d]oxazole (**7c**).

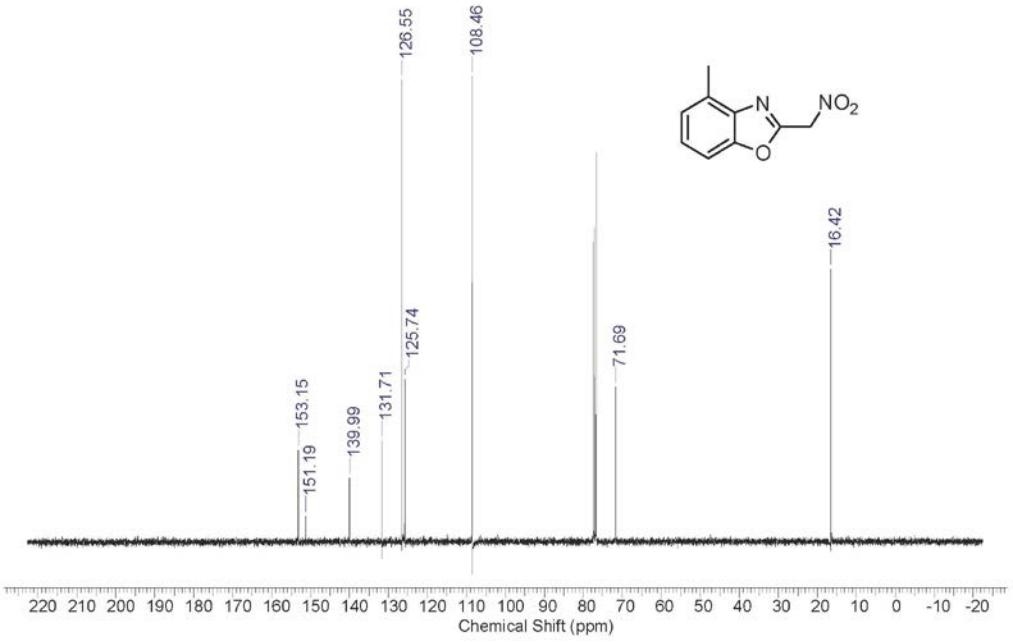

Figure S21. ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of 4-methyl-2-(nitromethyl)benzo[d]oxazole (**7c**).

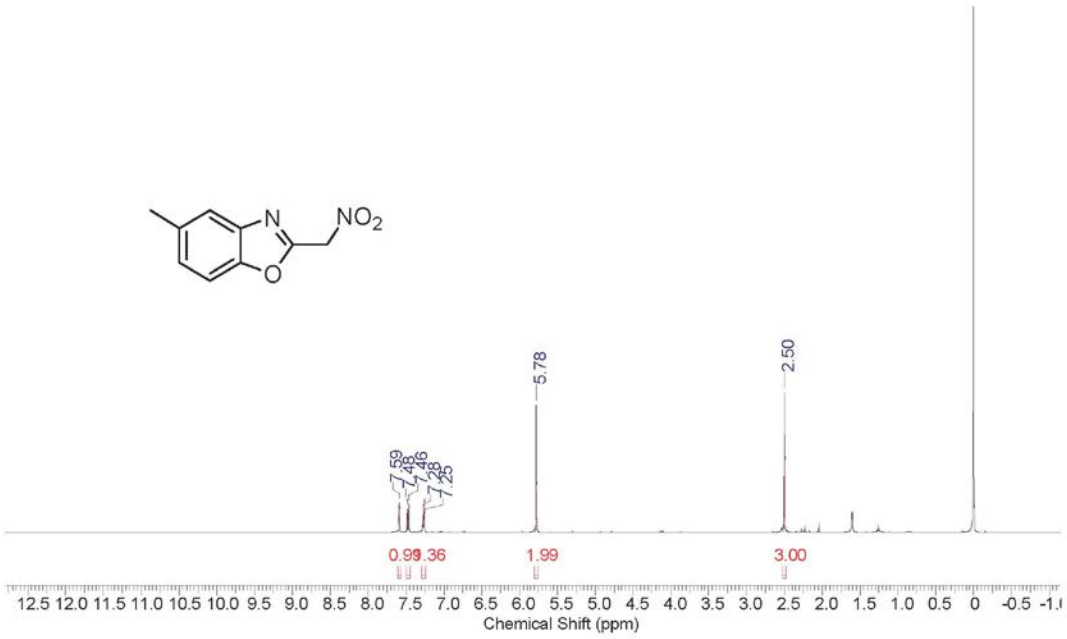

Figure S22. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of 5-methyl-2-(nitromethyl)benzo[d]oxazole (**7d**).

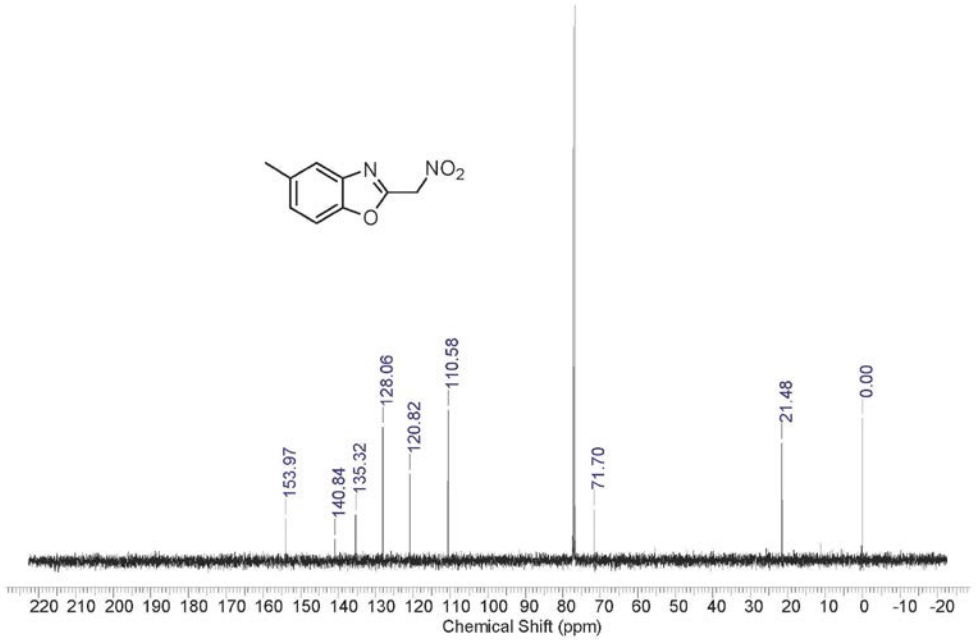

Figure S23. ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of 5-methyl-2-(nitromethyl)benzo[d]oxazole (**7d**).

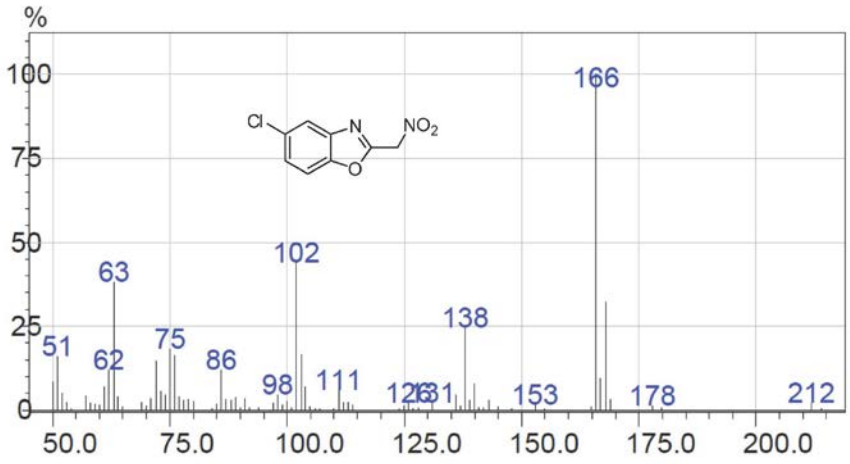

Figure S24. Mass spectrum of 5-chloro-2-(nitromethyl)benzo[d]oxazole (**7e**).

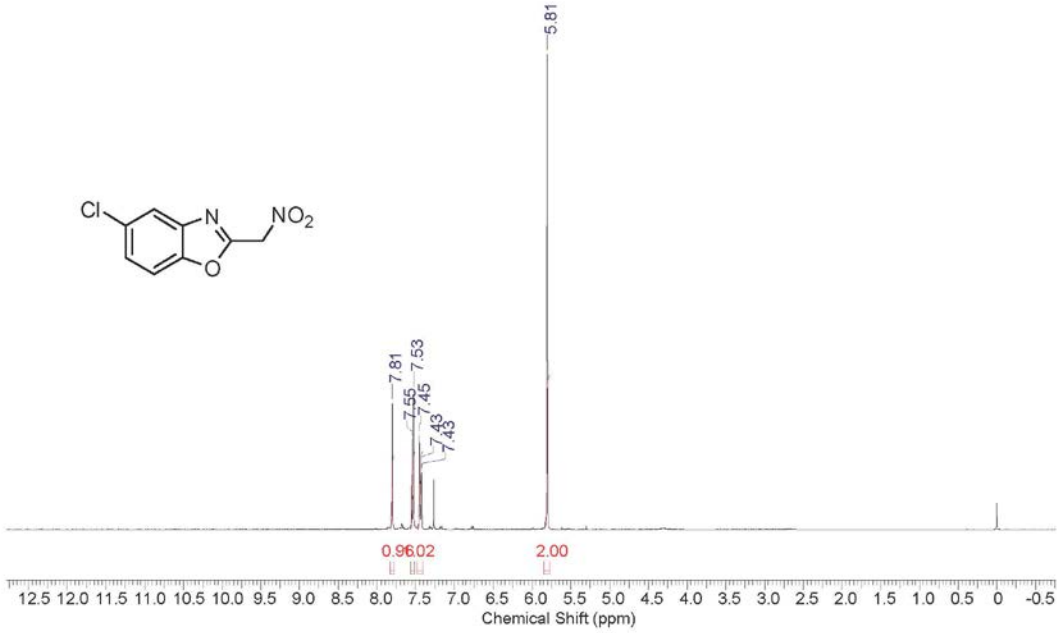

Figure S25. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of 5-chloro-2-(nitromethyl)benzo[d]oxazole (**7e**).

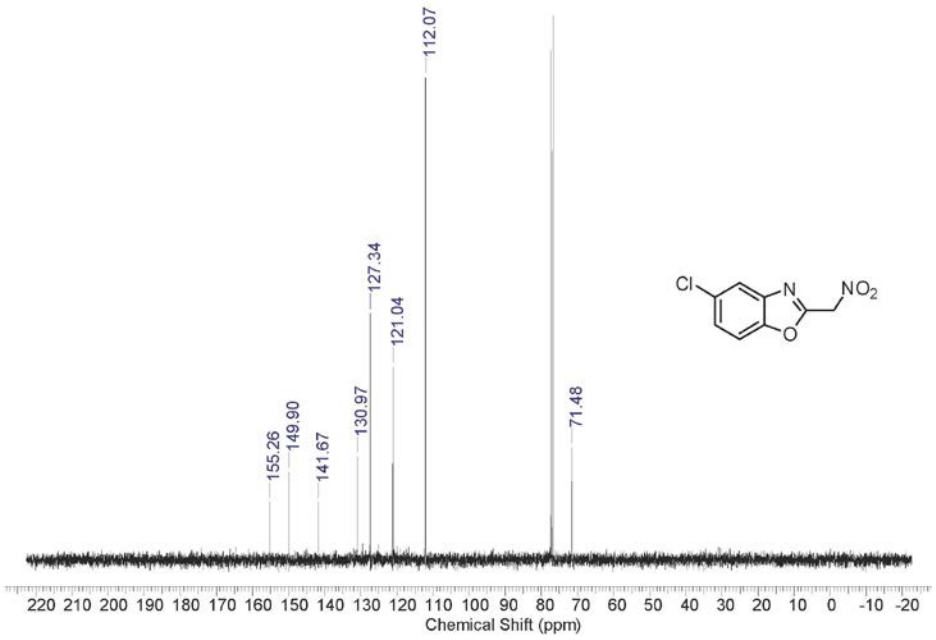

Figure S26. ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of 5-chloro-2-(nitromethyl)benzo[d]oxazole (**7e**).