

## Supporting Information

### A mitochondria-targeted nitric oxide donor triggered by superoxide radical to alleviate myocardial ischemia/reperfusion injury

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#### List of Contents

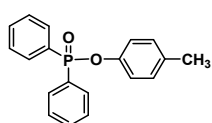
1. General information .....	2
2. Synthetic procedure and characterization.....	2
3. Preparation of the reactive oxygen species (ROS).....	4
4. Detection of superoxide radical by EPR .....	4
5. Detection of nitric oxide .....	4
6. Confocal visualization of NO release in H9c2 cells.....	5
7. Effect of I/R treatment on cell viabilities .....	5
8. Measurement of $\Delta\psi_m$ .....	6
9. Perfusion of isolated rat hearts .....	6
10. HPLC analysis of Mito-SNOD and SNOD.....	6
11. NMR spectra of the compounds 1-3, TPP-alkyne, MitoSNOD and SNOD .....	10

## 1. General information

UV-Vis absorption spectra were taken on Hitachi U-3900 spectrophotometer. The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on a BrukerAvance-400 FT nuclear magnetic resonance spectrometer. HPLC analysis was performed with an Agilent 1100 HPLC system or Shimadzu LC-20AT system using a Cromasil 5  $\mu\text{m}$  C18 column (250 mm x 4.6 mm). High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer. Confocal images were scanned by Laser Confocal Scanning Biological Microscope Olympus FV1200. EPR spectra were recorded at room temperature using a Bruker X-band EPR spectrometer.

## 2. Synthetic procedure and characterization

### p-tolyldiphenylphosphinate (1)



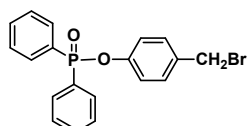
1

To the solution of diphenylphosphinyl chloride (1.067 g, 0.84 mL, 4.51 mmol) in dry THF (100 mL) was added  $\text{Et}_3\text{N}$  (0.96 mL, 6.76 mmol) and p-Cresol (0.49 g, 0.47 mL, 4.51 mmol).

The mixture was refluxed for 3 h and the reaction was monitored by TLC. After completion, the reaction was quenched by water and extracted with ethyl acetate three times. The

organic phase was washed with saturated brine, dried over  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure to give the crude product which was purified by chromatography, eluting with petroleum/EtOAc (6:1), to afford the desirable compound (0.8 g) as a white solid in 64% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.91-7.85 (m, 4H), 7.55-7.51 (m, 2H), 7.48-7.43 (m, 4H), 7.07 (d,  $J = 8.4$  Hz, 2H), 7.02 (d,  $J = 8.4$  Hz, 2H), 2.25 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  148.6 (d,  $J = 8.0$  Hz), 134.1 (s), 132.4 (d,  $J = 2.7$  Hz), 131.8 (d,  $J = 10.1$  Hz), 131.1 (d,  $J = 136.7$  Hz), 130.4 (s), 128.6 (d,  $J = 13.2$  Hz), 120.5 (d,  $J = 4.7$  Hz), 20.7 (s)

### 4-(bromomethyl)phenyldiphenylphosphinate (2)



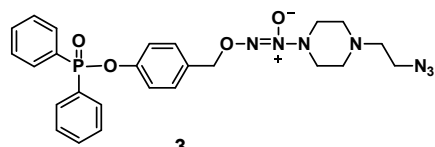
2

To the solution of compound 1 (480 mg, 1.56 mmol) in  $\text{CCl}_4$  (30 mL) was added benzoyl peroxide BPO (42 mg, 0.16 mmol) and N-Bromo Succinimide NBS (335 mg, 1.56 mmol).

The mixture was stirred for 6 h at  $80^\circ\text{C}$  at argon atmosphere. After the reaction mixture was cooled to room temperature, the suspension was filtered and the filtrate was

concentrated under reduced pressure. The resulting residue was purified by chromatography eluting with petroleum/EtOAc (10:1) to afford the desirable compound as a pale solid in 30% yield.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.91-7.85 (m, 4H), 7.55-7.51 (m, 2H), 7.48-7.45 (m, 4H), 7.25 (d,  $J = 8.0$  Hz, 2H), 7.17 (d,  $J = 8.0$  Hz, 2H), 4.38 (s, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  150.8 (d,  $J = 8.3$  Hz), 134.1 (s), 132.7 (d,  $J = 2.6$  Hz), 131.7 (d,  $J = 10.2$  Hz), 130.7 (d,  $J = 137.4$  Hz), 130.5 (s), 128.7 (d,  $J = 13.6$  Hz), 120.5 (d,  $J = 4.7$  Hz), 32.8.

### 1-(4-(2-azidoethyl)piperazin-1-yl)-2-((4-((diphenylphosphoryl)oxy)benzyl)oxy)diazene oxide (3)

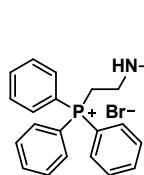


3

To the solution of the compound 2 (220 mg, 0.568 mmol) and diazeniumdiolate sodium (174 mg, 1.136 mmol) in dry DMF (6 mL) was added KI powder (20 mg, 0.120 mmol) on ice bath. The resulting mixture

was stirred under argon atmosphere at 0°C for 24 h. Then, water was added to quench the reaction. The reaction mixture was extracted with EtOAc three times, and the combined organic phase was washed with water twice, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The resulting residue was purified by chromatography eluting with petroleum/EtOAc (10:1) to afford the desirable compound as a pale oil (200 mg, 67.5%). <sup>1</sup>H NMR (400 MHz, MeOD) δ 7.92-7.87 (m, 4H), 7.64-7.60 (m, 2H), 7.56-7.51 (m, 4H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.23 (dd, *J* = 8.8 Hz, 1.2 Hz, 2H), 5.14 (s, 2H), 3.93-3.36 (m, 6H), 2.68-2.61 (m, 6H); <sup>13</sup>C NMR (100 MHz, MeOD) δ 150.9 (d, *J* = 8 Hz), 132.9 (s), 132.8 (s), 131.5 (d, *J* = 10.5 Hz), 130.6 (d, *J* = 138.3 Hz), 130.1 (s), 128.7 (d, *J* = 13.5 Hz), 120.6 (d, *J* = 3.7 Hz), 74.4, 56.0, 51.1, 50.6 (one less due to overlap with solvent)

### (2-(hex-5-ynamido)ethyl)triphenylphosphonium bromide (TPP-alkyne)

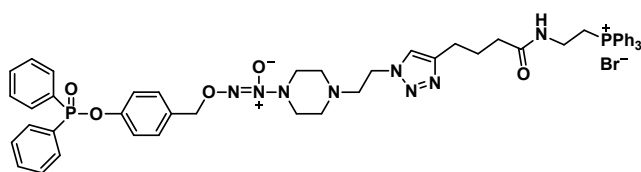


TPP-alkyne

To the solution of (2-aminoethyl)triphenylphosphoniumbromide (200 mg, 0.518 mmol) and hex-5-ynoic acid (58 mg, 0.518 mmol) in dry DMF (3 mL) was added HATU (296 mg, 0.776 mmol) and DIPEA (200 mg, 0.27 mL, 1.550 mmol). The resulting mixture was stirred at room temperature for 24 h. Then, water was added to quench the reaction.

The reaction mixture was extracted with EtOAc three times. The combined organic phase was washed with water twice, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The resulting residue was purified by column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (v/v, 95:5) to give TPP-alkyne as a waxy solid (210 mg, 84%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.86-7.81 (m, 3H), 7.74 - 7.67 (m, 11H), 7.45 (apparent s, 1H), 5.75 (s, br, 1H), 3.65-3.58 (m, 2H), 3.51 - 3.44 (m, 2H), 2.27 (t, *J* = 7.5 Hz, 2H), 2.19 (td, *J* = 7.0, 2.5 Hz, 2H), 1.96 (t, *J* = 2.6 Hz, 1H), 1.81 - 1.72 (m, 2H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 174.0, 135.5 (d, *J* = 2.9 Hz), 133.3 (d, *J* = 10.2 Hz), 130.7 (d, *J* = 12.7 Hz), 117.4 (d, *J* = 86 Hz), 83.3, 69.3, 34.4, 33.7, 23.9, 22.2 (d, *J* = 49.7 Hz), 17.9

### 2-((4-((diphenylphosphoryl)oxy)benzyl)oxy)-1-(4-(2-(4-(4-oxo-4-((2-(triphenylphosphonio)ethyl)amino)butyl)-1H-1,2,3-triazol-1-yl)ethyl)piperazin-1-yl)diazene oxide bromide (MitoSNOD)



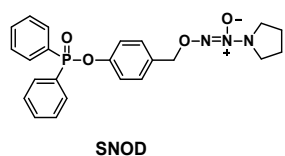
MitoSNOD

To the solution of TPP-alkyne (119 mg, 0.297 mmol) and the compound 3 (186 mg, 0.357 mmol) in the mixed solvents (5 mL, *t*-BuOH:DCM:water = 1:1:1) was added sodium ascorbate (8.2 mg, 0.042 mmol) and CuSO<sub>4</sub>·5H<sub>2</sub>O (14.8 mg, 0.059 mmol). The resulting

mixture was stirred at room temperature for 4 h. Once the reaction was completed, the solvent was removed under reduced pressure. The crude product was purified by chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>/MeOH (v/v, from 99:1 to 96:4) to give MitoSNOD as a white solid (187 mg, 63%). <sup>1</sup>H NMR (400 MHz, MeOD) δ 7.94-7.83 (m, 14H), 7.79-7.75 (m, 6H), 7.65-7.53 (m, 6H), 7.32 (d, *J* = 8.3 Hz, 2H), 7.24 (d, *J* = 8.1 Hz, 2H), 5.13 (s, 2H), 4.51 (t, *J* = 5.9 Hz, 2H), 3.68 - 3.61 (m, 2H), 3.58-3.52 (m, 2H), 2.88 (t, *J* = 6.0 Hz, 2H), 2.71 (t, *J* = 7.4 Hz, 2H), 2.65-2.61 (m, 4H), 2.16 (t, *J* = 7.4 Hz, 2H), 2.0 (s, 0.5H), 1.91 (dd, *J* = 14.9, 7.5 Hz, 2H). (Four proton less due to overlap with MeOD); <sup>13</sup>C NMR (100 MHz, MeOD) δ 174.5, 151.1 (d, *J* = 8.0 Hz), 135.1 (d, *J* = 2.4 Hz), 133.5 (d, *J* = 10.3 Hz), 132.9 (d, *J* =

3.1 Hz), 132.8, 131.5 (d,  $J = 10.1$  Hz), 130.2 (d,  $J = 12.8$  Hz), 130.1, 129.8 (d,  $J = 138.6$  Hz), 128.7 (d,  $J = 13.3$  Hz), 120.6 (d,  $J = 4.5$  Hz), 118.5, 117.6, 74.4, 56.2, 50.9, 50.7, 34.4, 33.2, 25.0, 24.2, 21.4 (d,  $J = 50.0$  Hz);  $^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )  $\delta$  30.79 (1P, s), 20.44 (1P, s); HRMS (ESI) calcd for  $\text{C}_{51}\text{H}_{55}\text{N}_8\text{O}_5\text{P}_2$   $[\text{M}-\text{Br}]^+$ : 921.3765 found: 921.3779; HPLC analysis: retention time = 9.53 min, peak area 97.8%, eluted with  $\text{CH}_3\text{CN}/\text{CH}_3\text{COONH}_4$  solution (from 45% to 95%, 20min)

### 2-((4-((diphenylphosphoryl)oxy)benzyl)oxy)-1-(pyrrolidin-1-yl)diazene oxide (SNOD)



SNOD was obtained as a white solid in 67% yield according to the synthetic procedure of the compound 3.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.91-7.86 (m, 4H), 7.57-7.53(m, 2H), 7.49-7.46 (m, 4H), 7.27 (d,  $J = 7.3$  Hz, 2H), 7.19 (d,  $J = 8.1$  Hz, 2H), 5.07 (s, 2H), 3.49-3.45 (m, 4H), 1.92-1.89 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  151.0 (d,  $J = 8.4$  Hz), 132.5 (d,  $J = 2.7$  Hz), 132.3 (s), 131.8 (d,  $J = 10.6$  Hz), 130.8 (d,  $J = 137.5$  Hz), 130.2 (s), 128.7 (d,  $J = 13.3$  Hz), 120.7 (d,  $J = 4.6$  Hz), 74.5, 50.9, 22.8;  $^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )  $\delta$  30.71 (1P, s); HPLC analysis: retention time = 12.24 min, peak area 97.6%, eluted with  $\text{CH}_3\text{CN}/\text{CH}_3\text{COONH}_4$  solution (from 45% to 95%, 20min)

### 3. Preparation of the reactive oxygen species (ROS)

3-Morpholinosydnonimine (SIN-1) was used as the source of peroxynitrite. The solution of  $\text{KO}_2$  in dry DMSO containing 18-crown-6 (3.5 eq.) was used and the concentration of  $\text{O}_2^{\cdot-}$  was determined by UV-vis spectroscopy ( $\epsilon_{260} = 1350 \text{ M}^{-1} \text{ cm}^{-1}$ ). Enzymatic source of  $\text{O}_2^{\cdot-}$  was produced from xanthine (100  $\mu\text{M}$ ) and xanthine oxidase (20 mU/mL) in PB (20 mM, pH = 7.4). The aqueous solution of  $\text{H}_2\text{O}_2$  was freshly prepared. Hydroxyl radical ( $\text{OH}^{\cdot}$ ) was generated *in situ* by adding  $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$  into 10 eq. of  $\text{H}_2\text{O}_2$ . Alkylperoxyl radical was generated by thermolysis of 2, 2'-azobis-2- methylpropanimidamide dihydrochloride (AAPH) at 37  $^\circ\text{C}$ .

### 4. Detection of superoxide radical by EPR

The amount of superoxide anion was detected by EPR technique. The following instrumental setting was used: microwave power, 10 mW; modulation frequency, 100 kHz; modulation amplitude, 1.0 G; scan time, 30 s. 5-Dimethyl-1-pyrroline *N*-oxide (DMPO) was used as a spin trap. The testing solution contains xanthine (X, 100  $\mu\text{M}$ ), xanthine oxidase (XO, 20 mU/mL), DMPO (10 mM) and MitoSNOD (0, 150 or 300  $\mu\text{M}$ ). EPR spectral simulation was conducted by the WINSIM program.

### 5. Detection of nitric oxide

**Griess Reagent:** To investigate the time-dependent NO release of MitoSNOD and SNOD triggered by  $\text{O}_2^{\cdot-}$ , the pre-incubated solution of  $\text{KO}_2$  (200  $\mu\text{M}$ ) with MitoSNOD or SNOD (66.7  $\mu\text{M}$ ) in DMSO was quenched by PB (20 mM, 7.4) at the designated time (20, 40, 60 or 120 s). Then the resulting solution (100  $\mu\text{L}$ ) was mixed with Griess Reagent I (100  $\mu\text{L}$ ) and Griess Reagent II (100  $\mu\text{L}$ ). After 30-min incubation with periodic stirring, the UV-vis absorbance of the solution at 541 nm was measured. Similarly, the NO release from MitoSNOD and SNOD induced by various concentrations of  $\text{KO}_2$  (200, 66.7, 33.3 and 13.3  $\mu\text{M}$ ) was also investigated.

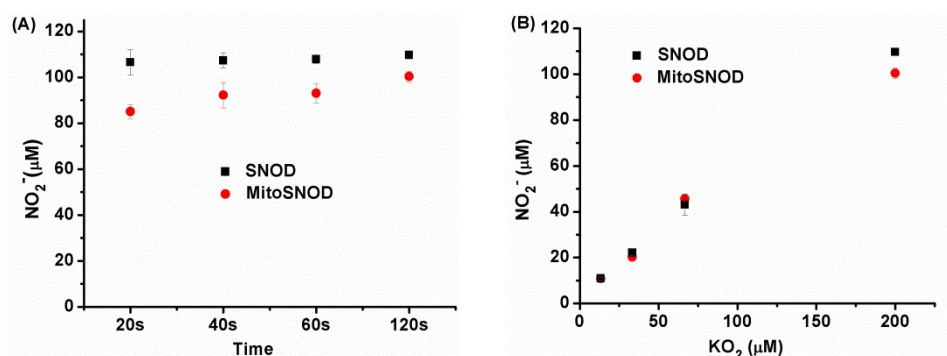


Fig. S1 (A) Time-dependent NO release detected by Griess Reagent after incubation of MitoSNOD (66.7  $\mu\text{M}$ ) with  $\text{KO}_2$  (200  $\mu\text{M}$ ) for various time (20s, 40s, 60s and 120s); (B) Dose-dependent NO release after incubation of MitoSNOD (66.7  $\mu\text{M}$ ) with different concentrations of  $\text{KO}_2$  (200, 66.7, 33.3 and 13.3  $\mu\text{M}$ ) for 2 min.

**Nitric Oxide ( $\text{NO}_2^-/\text{NO}_3^-$ ) detection kit:** MitoSNOD (80  $\mu\text{M}$ ), xanthine (100  $\mu\text{M}$ ) and xanthine oxidase (20 mU/mL) were mixed in PB (20 mM, pH = 7.4). The reaction mixture was incubated at 37  $^\circ\text{C}$  for 1 h. According to the assay kit, NADPH, FAD and nitrate reductase were added into the reaction mixture subsequently and incubated at 37 $^\circ\text{C}$  for 30 min. Then LDH buffer and LDH were added and incubated at 37 $^\circ\text{C}$  for another 30 min. Finally, Griess Reagent I and Griess Reagent II were added to the reaction mixture. The resulting mixture was incubated for 30 min with periodic stirring and the absorbance of the solution was measured at 548 nm.

**EPR spectroscopy:** The following acquisition parameters were used: microwave power, 10 mW; modulation frequency, 100 kHz; modulation amplitude, 1.0 G; scan time, 30 s. *N*-methyl-*D*-glucaminedithiocarbamate (MGD) sodium salt was synthesized as described previously<sup>2</sup>. Fresh stock solution of (5 mM) was prepared by dissolving MGD sodium salt (7.3 mg, 10  $\mu\text{mol}$ ) and ammonium ferrous sulphate hexahydrate (7.3 mg, 50  $\mu\text{mol}$ ) in argon-purged DDI water (2 mL). The solution of MitoSNOD (80  $\mu\text{M}$ ), X (100  $\mu\text{M}$ ), XO (20 mU/mL) and  $\text{Fe}^{2+}$ -(MGD)<sub>2</sub> (1 mM) was incubated at room temperature for 12 h and then measured by EPR spectroscopy.

## 6. Confocal visualization of NO release in H9c2 cells

H9c2 cells were seeded onto 35-mm confocal dishes at a density of  $10^4$  cells in 2 ml MEM medium with fetal bovine serum (10%) at 37  $^\circ\text{C}$  in a humidified incubator with 5%  $\text{CO}_2$ . After incubation for 24 h, medium was removed and the cells were re-incubated with fresh medium containing Mito-SOX (2  $\mu\text{M}$ ) and DAF-FM-DA (2  $\mu\text{M}$ ) at 37  $^\circ\text{C}$  for 30 min, respectively. After removing medium and washing with PBS, the cells were incubated in medium with MitoSNOD (20  $\mu\text{M}$ , final concentration) for 1 h. Subsequently, the medium was removed and the cells were washed with PBS and re-incubated in the medium containing  $\text{CoCl}_2$  (800  $\mu\text{M}$ ) for 30 min. Confocal fluorescence imaging studies were carried out using Laser Confocal Scanning Biological Microscope OlympusFV1200 with excitation wavelengths at 488 nm and 559 nm, respectively.

## 7. Effect of I/R treatment on cell viabilities

H9c2 cells were washed twice with PBS and then incubated in Tyrode solution (NaCl 140 mM, KCl 6 mM,  $\text{MgCl}_2$  1

mM, CaCl<sub>2</sub> 1 mM, HEPES 5 mM, Glucose 5.8 mM) for 2 h prior to experiments. To induce I/R injury, cells were exposed to a simulated ischemia solution (glucose-free Tyrode solution containing 10 mM 2-deoxy-D-glucose and 10 mM sodium dithionite) for 40 min, followed by 30 min of reperfusion with the normal Tyrode solution. MitoSNOD, SNAP or intermediate 1 (20 μM), was given at the onset of reperfusion for 30 min, respectively. The cell viability was detected by CCK-8 kit.

## 8. Measurement of $\Delta\Psi_m$

$\Delta\Psi_m$  was measured by incubation of H9c2 cells with JC-1 (100 nM) in Tyrode solution for 40 min. The fluorescence changes were detected with an laser scanning confocal microscope (Olympus FV 1200). The green fluorescence was excited at 488 nm and imaged through a 525-nm-long path filter. The red fluorescence was excited at 543 nm and imaged through a 590-nm-long path filter.

## 9. Perfusion of isolated rat hearts

Male Wistar rats (250-350 g) were anesthetized with sodium pentobarbital (100 mgkg<sup>-1</sup>, i.p.). The hearts were removed rapidly and mounted on a Langendorff apparatus. The hearts were perfused with Krebs-Henseleit buffer containing NaCl (118.5 mM), KCl (4.7 mM), MgSO<sub>4</sub> (1.2 mM), CaCl<sub>2</sub> (1.8 mM), NaHCO<sub>3</sub> (24.8 mM), KH<sub>2</sub>PO<sub>4</sub> (1.2 mM) and glucose (10 mM) which was heated to 37°C and gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub>. A 5-0 silk suture was placed around the left coronary artery, and the ends of the suture were passed through a small piece of soft vinyl tubing to form a snare. All hearts were allowed to stabilize for at least 20 min. Ischemia was induced by pulling the snare and then fixing it by clamping the tubing with a small hemostat. All hearts were subjected to 30 min of regional ischemia followed by 1 h of reperfusion. MitoSNOD (2 μM) was given before ischemia for 1.5 h. Finally, the hearts were stained with *p*-nitro-blue tetrazolium (NBT, 0.5 mg/mL, 20 minutes at 37°C) to distinguish between ischemia and infarcted tissue.

## 10. HPLC analysis of Mito-SNOD and SNOD

The purities of MitoSNOD and SNOD were determined to be >95% by HPLC on an Agilent 1100 HPLC system with a Cromasil C18 column (250 mm x 4.6 mm, 5 μm), UV-Vis wavelength = 254 nm, eluted at 1.0 mL/min with CH<sub>3</sub>COONH<sub>4</sub> solution/CH<sub>3</sub>CN, gradient 45% to 95% in 20 min.

The solutions of SNOD in PBS (50% CH<sub>3</sub>CN) and MitoSNOD in PBS (20% CH<sub>3</sub>CN) were incubated at 37°C for 24 h and their stability were analyzed by HPLC. The HPLC experiments were carried out on Shimadzu LC-20 AT system with a Cromasil C18 column (250 mm x 4.6 mm, 5 μm), UV-Vis wavelength = 254 nm, eluted at 1.0 mL/min with CH<sub>3</sub>COONH<sub>4</sub> solution/CH<sub>3</sub>CN, gradient 55% to 95% in 20 min. Potassium superoxide was used as a chemical source of O<sub>2</sub><sup>•-</sup> (3. eq.). To test the selectivity of MitoSNOD and SNOD against other ROS, they were incubated mixed with the individual ROS (e.g., H<sub>2</sub>O<sub>2</sub>, ONOO<sup>-</sup>, <sup>•</sup>OH and ROO<sup>•</sup>) at room temperature for 10 min and then HPLC analysis was conducted.

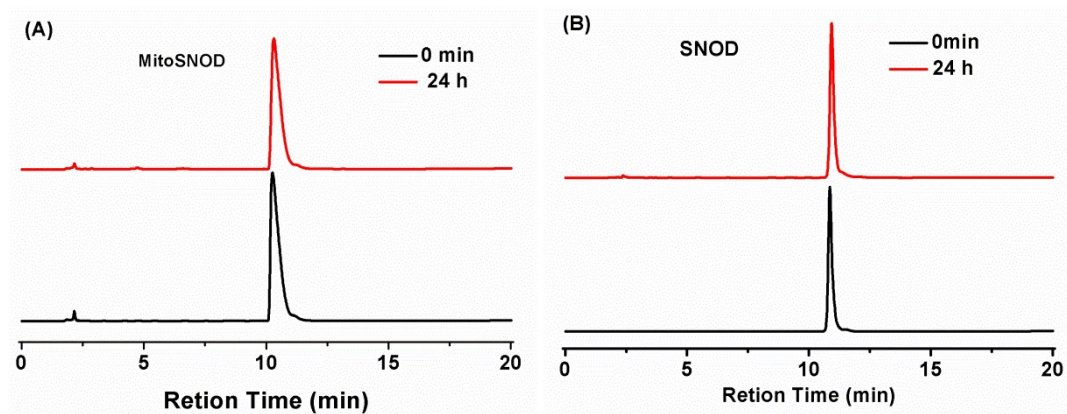


Fig. S2 (A) The stability of MitoSNOD in PBS containing 20% CH<sub>3</sub>CN; (B) The stability of MitoSNOD in PBS containing 50% CH<sub>3</sub>CN.

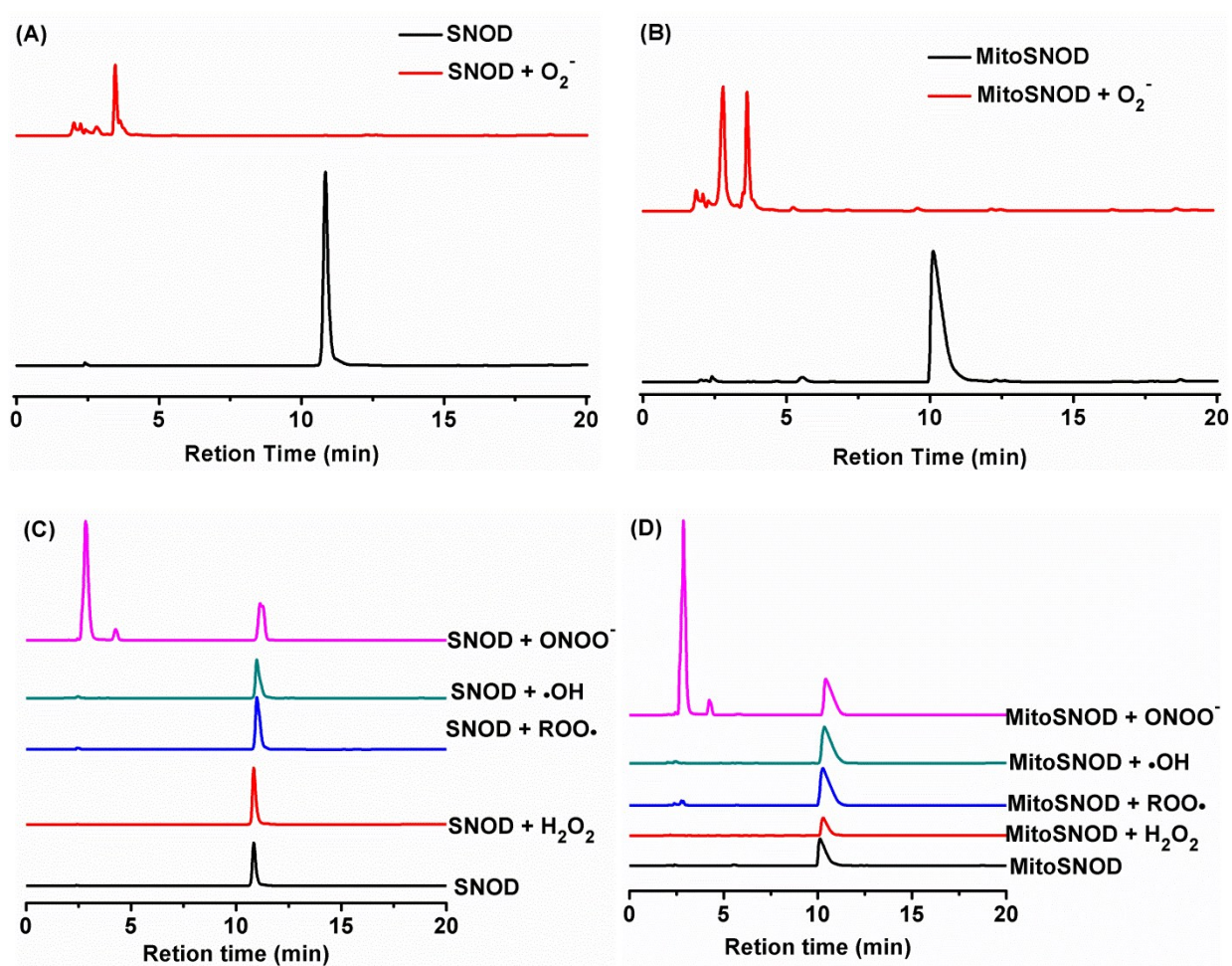
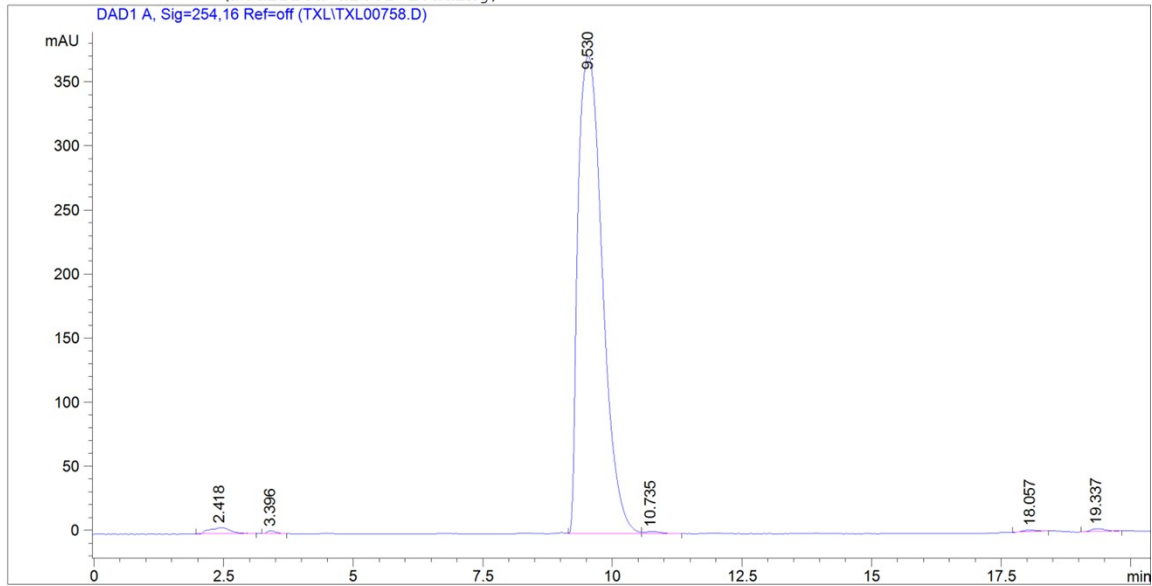


Fig. S3 The stability of MitoSNOD and SNOD toward various ROS. (A) SNOD / O<sub>2</sub><sup>-</sup>; (B) MitoSNOD / O<sub>2</sub><sup>-</sup>; (C) SNOD/ROS; (D) MitoSNOD / ROS.

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Area Percent Report  
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Use Multiplier & Dilution Factor with ISTDs
    
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3	9.530	VB	0.5295	1.22020e4	373.12885	97.8111
4	10.735	BB	0.2766	40.10925	1.80923	0.3215
5	18.057	PB	0.2429	26.77786	1.31852	0.2147
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Results obtained with enhanced integrator!

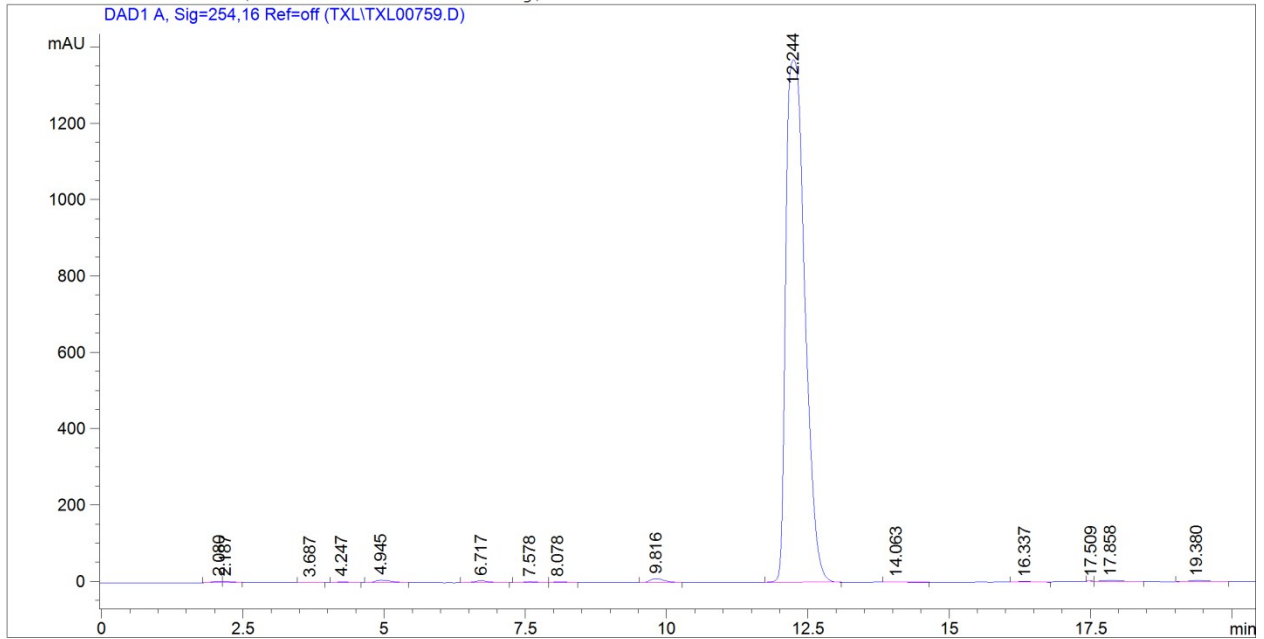
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\*\*\* End of Report \*\*\*

Fig. S4 Analysis of the purity of MitoSNOD



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Injection Date   : 6/4/2018 4:59:46 PM
Sample Name     : TXL
Acq. Operator  : TXL
Acq. Instrument : Instrument 1
Acq. Method    : D:\HPCHEM2\1\METHODS\HCC.M
Last changed   : 6/4/2018 4:09:12 PM by TXL
                (modified after loading)
Analysis Method : D:\HPCHEM2\1\METHODS\HCC.M
Last changed   : 6/4/2018 5:24:23 PM by SFQ
                (modified after loading)
    
```



Area Percent Report

```

=====
Sorted By      : Signal
Multiplier    : 1.0000
Dilution      : 1.0000
Sample Amount  : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: DAD1 A, Sig=254,16 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.080	PV	0.1584	40.12126	3.33294	0.1226
2	2.187	VB	0.1572	36.16662	3.21482	0.1106
3	3.687	BB	0.1562	12.82792	1.03758	0.0392
4	4.247	PB	0.2113	34.12393	2.29741	0.1043
5	4.945	BB	0.2827	126.48660	6.50962	0.3867
6	6.717	BB	0.2579	88.65140	4.83532	0.2710
7	7.578	BV	0.2178	40.79569	2.50805	0.1247
8	8.078	VV	0.2080	34.64922	2.08327	0.1059
9	9.816	BB	0.2676	178.84302	10.15047	0.5467
10	12.244	BB	0.3641	3.19178e4	1368.76501	97.5684
11	14.063	BB	0.2736	34.47754	1.50290	0.1054
12	16.337	VP	0.2352	40.17028	2.06285	0.1228
13	17.509	BV	0.0767	7.06505	1.31362	0.0216

Fig. S5 Analysis of the purity of SNOD by HPLC

# 11. NMR spectra of the compounds 1-3, TPP-alkyne, MitoSNOD and SNOD

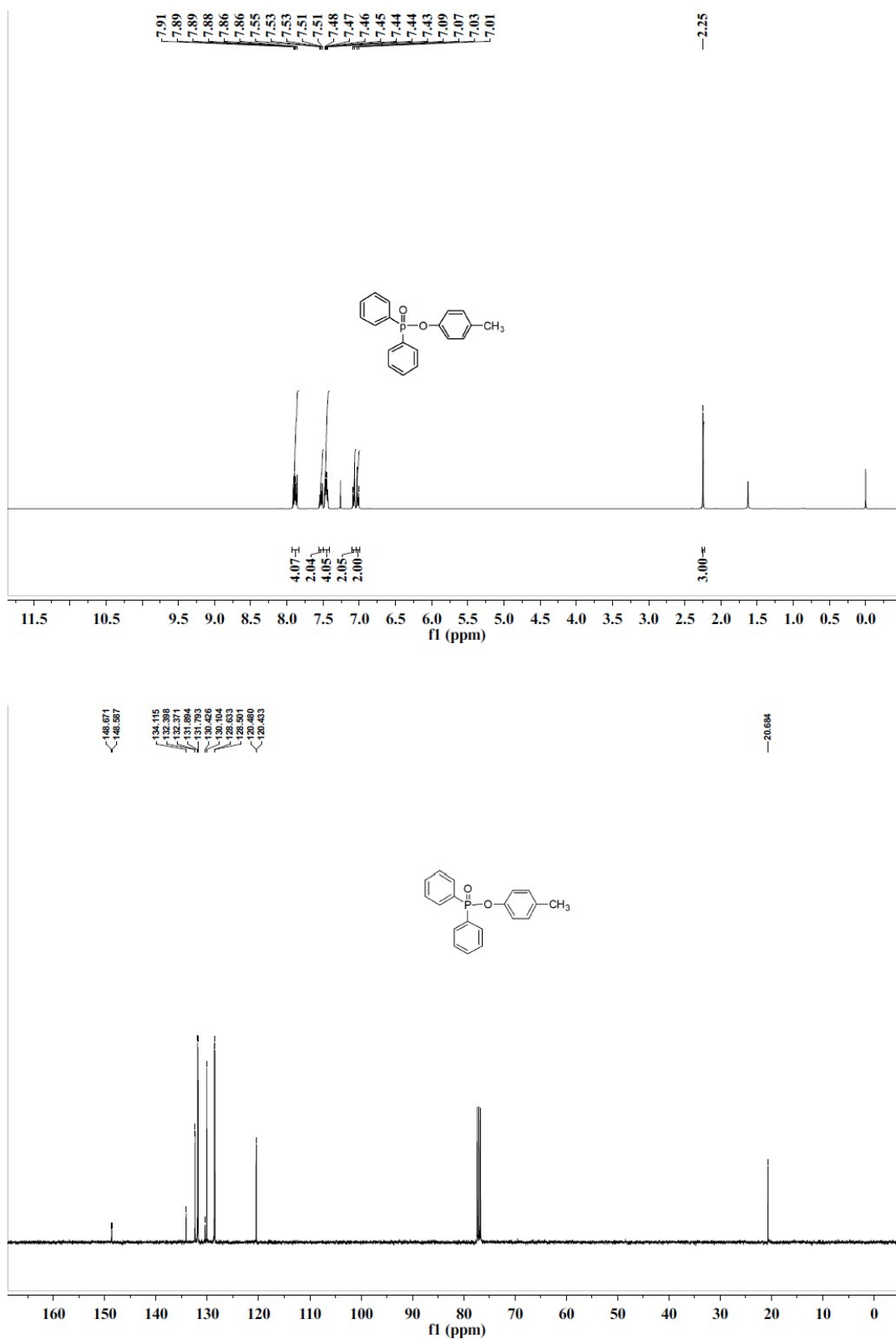


Fig. S6 <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra for the compound 1

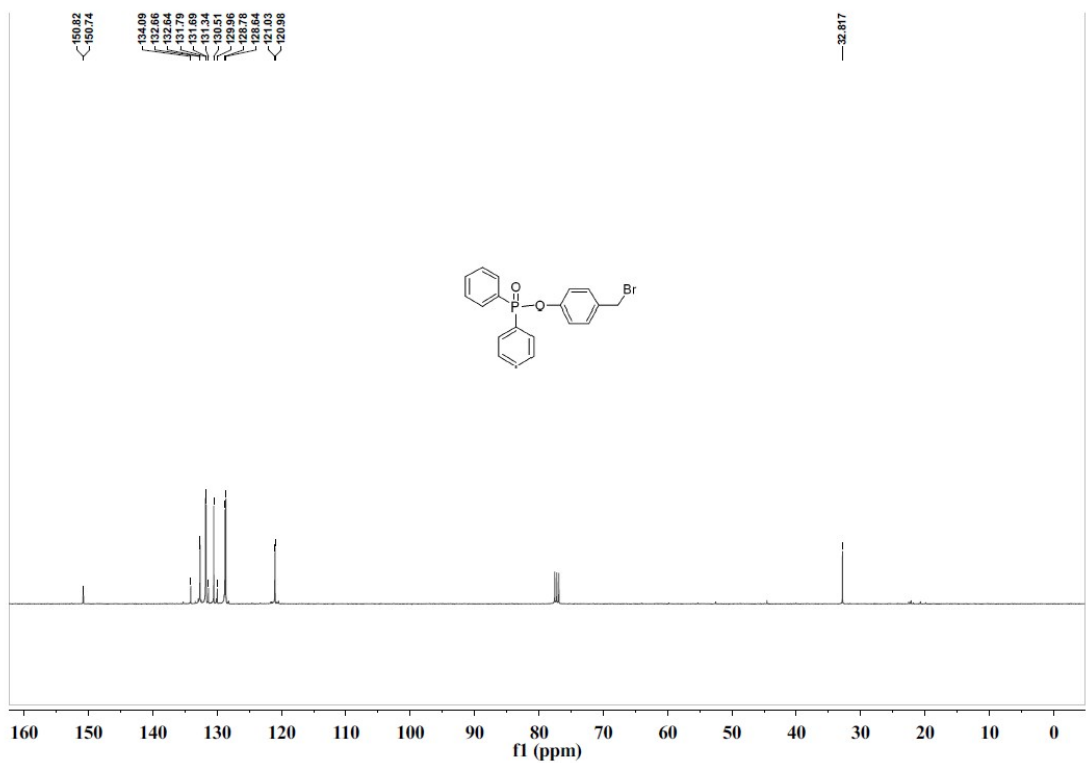
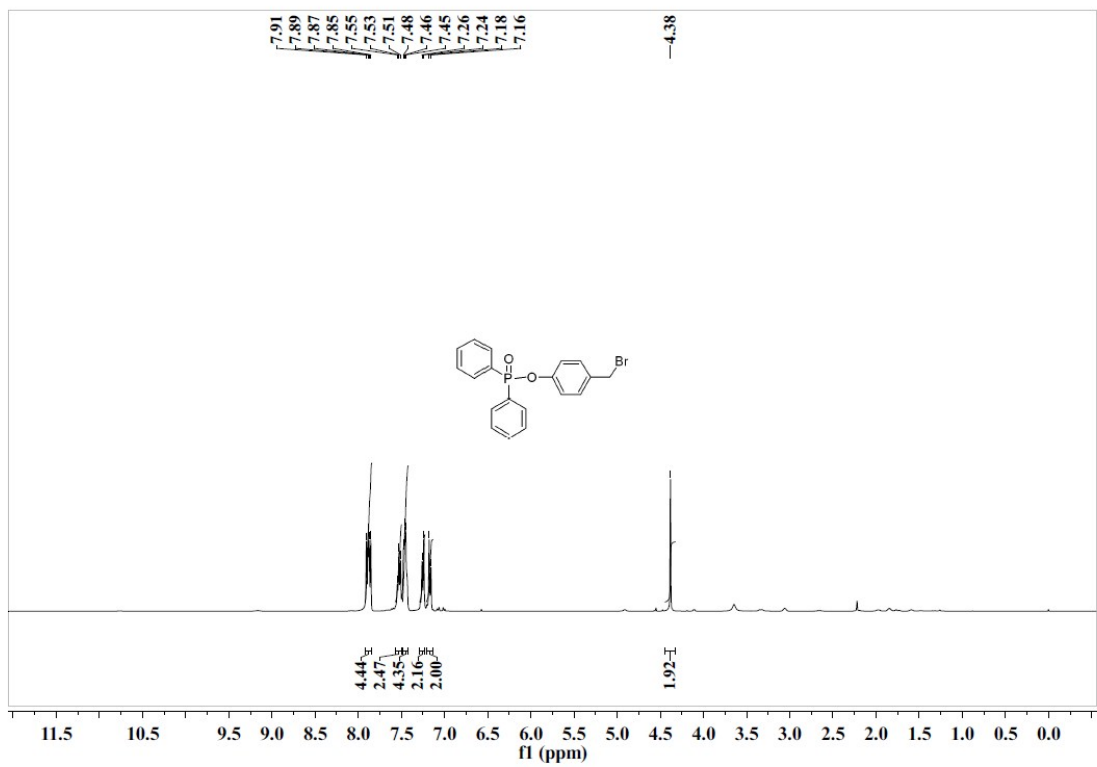


Fig. S7 <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra for the compound 2

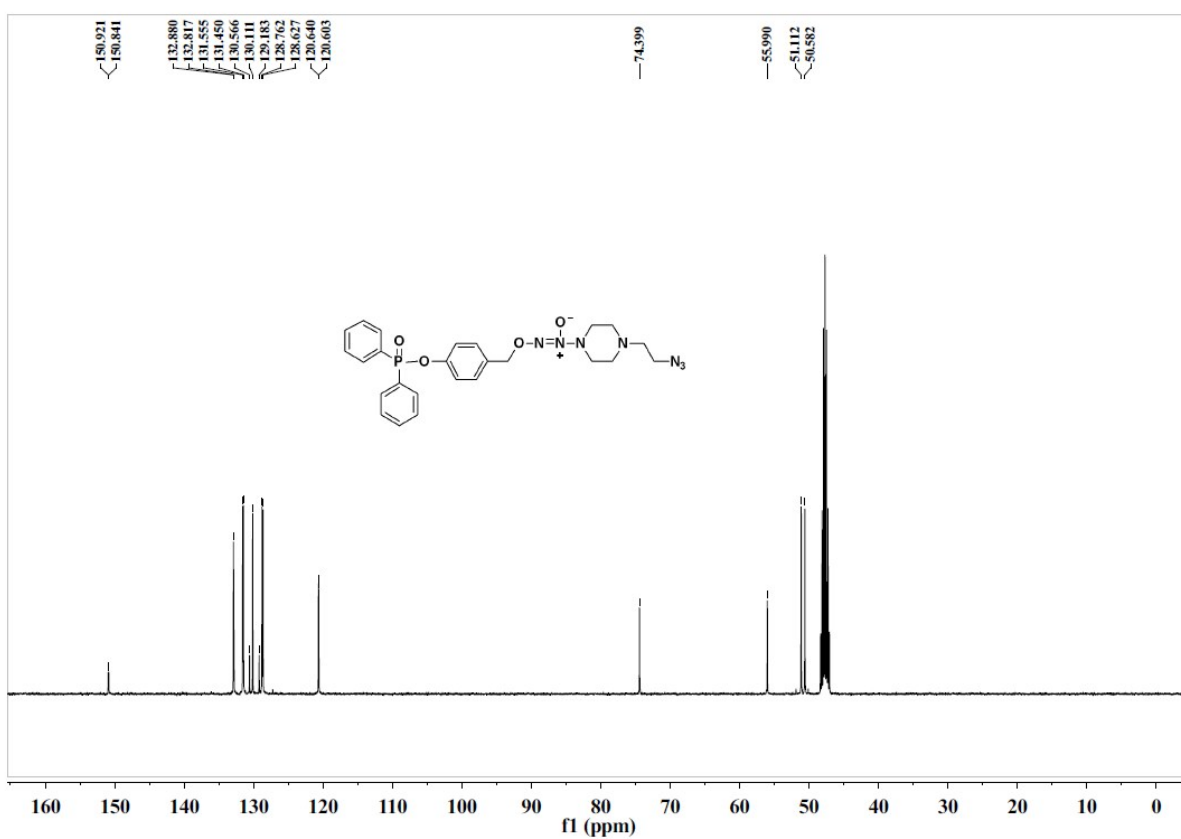
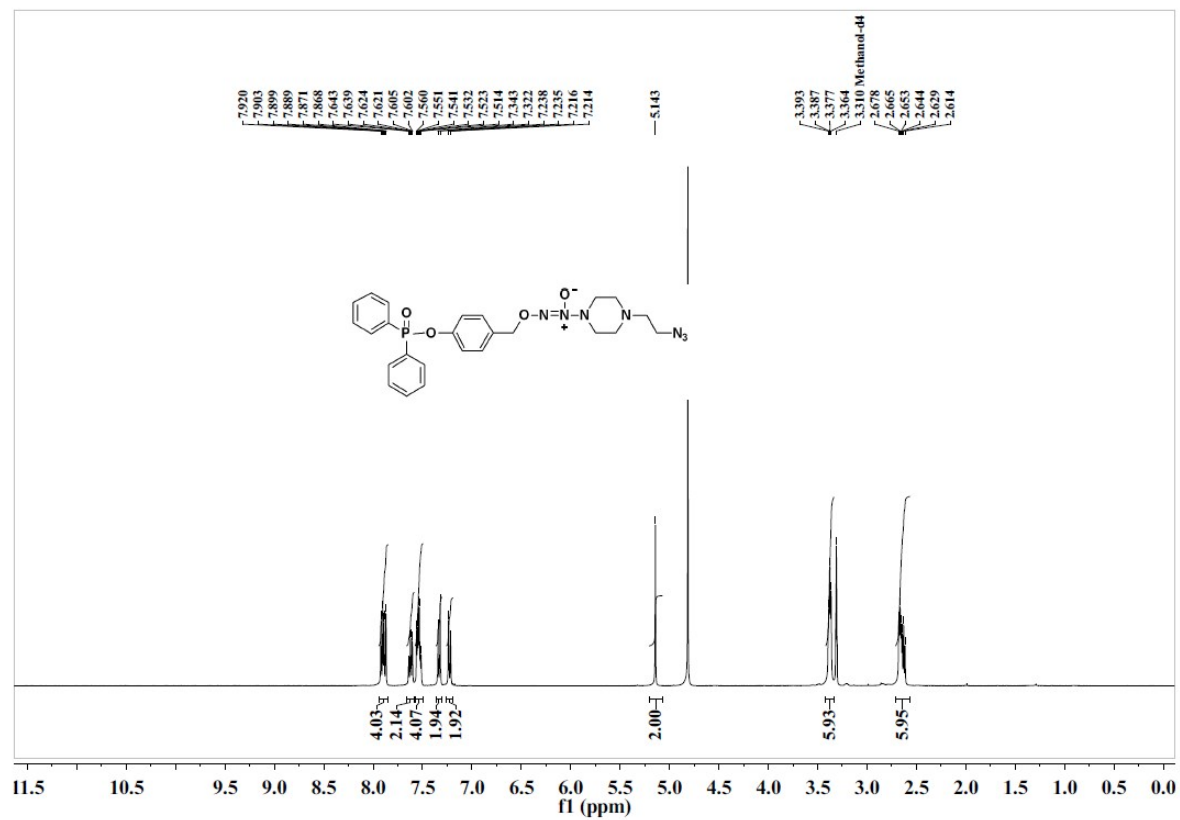


Fig. S8 <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra for the compound 3

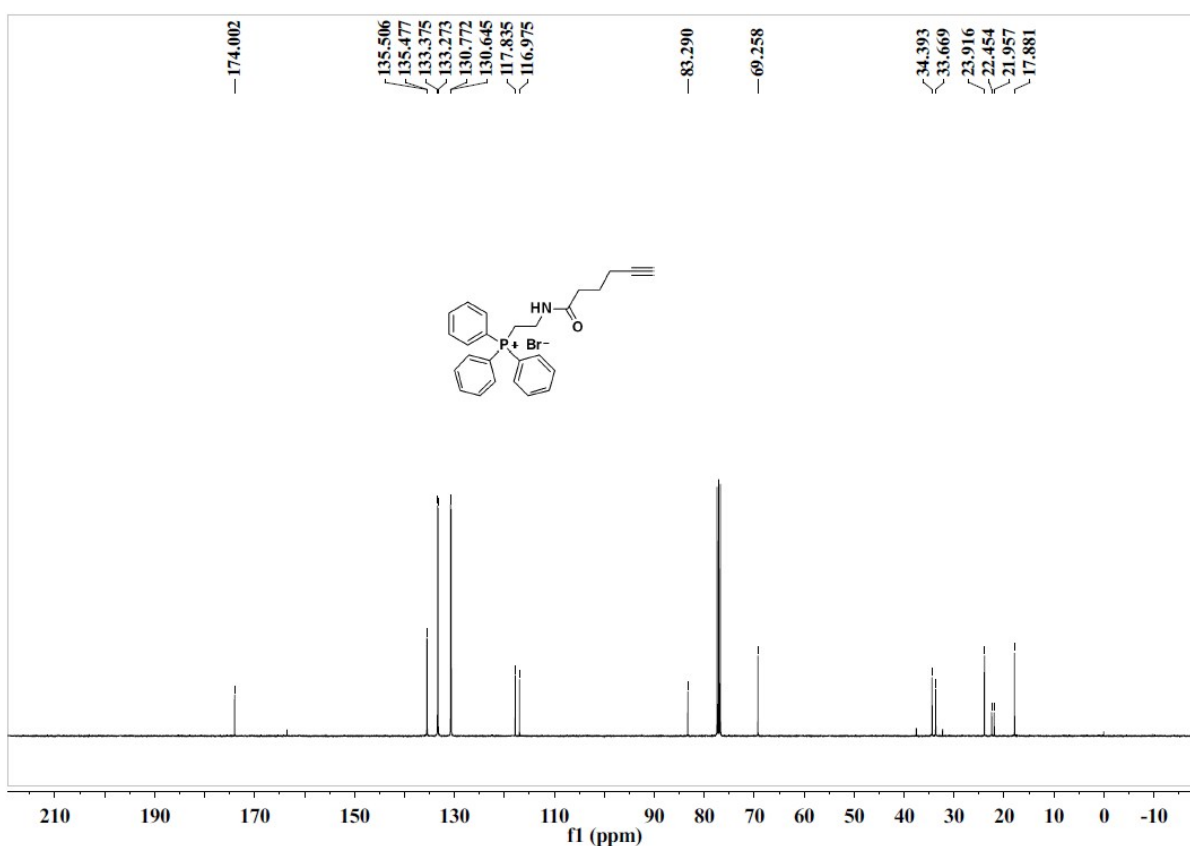
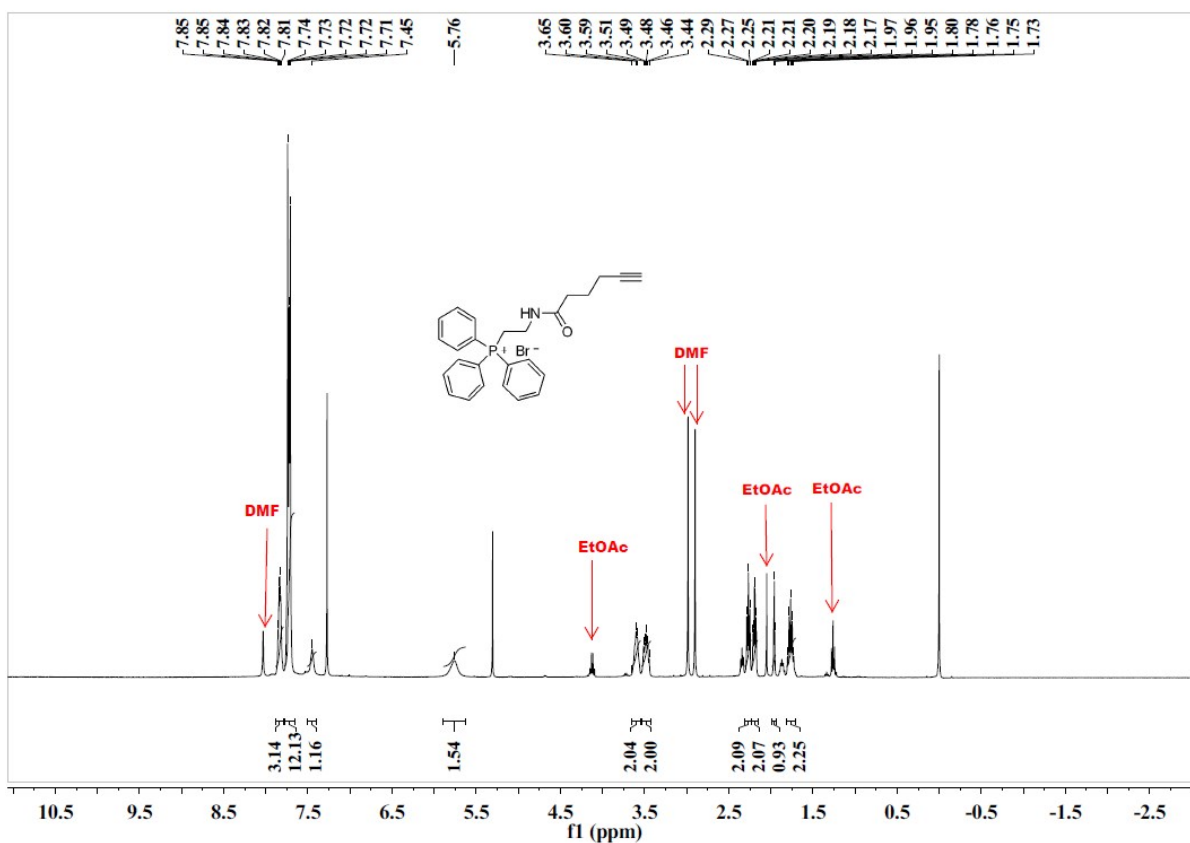
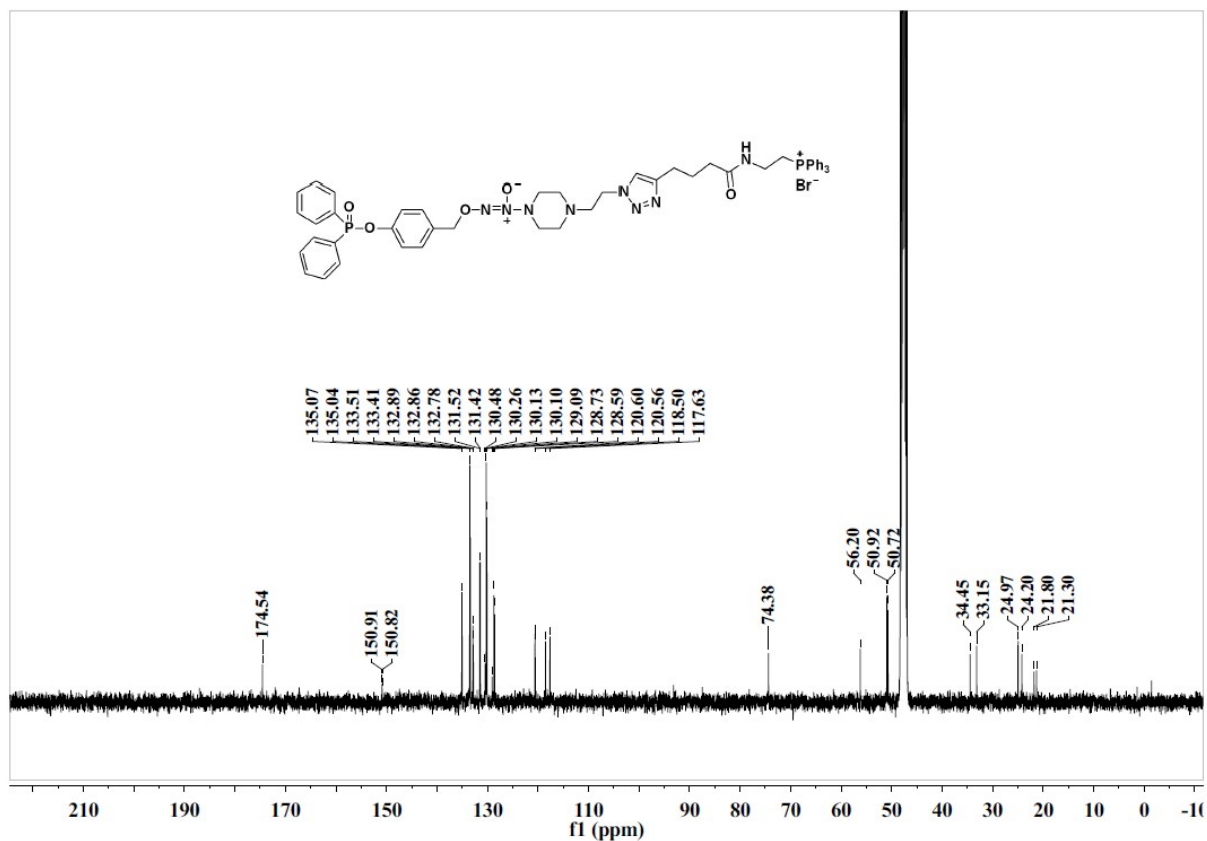
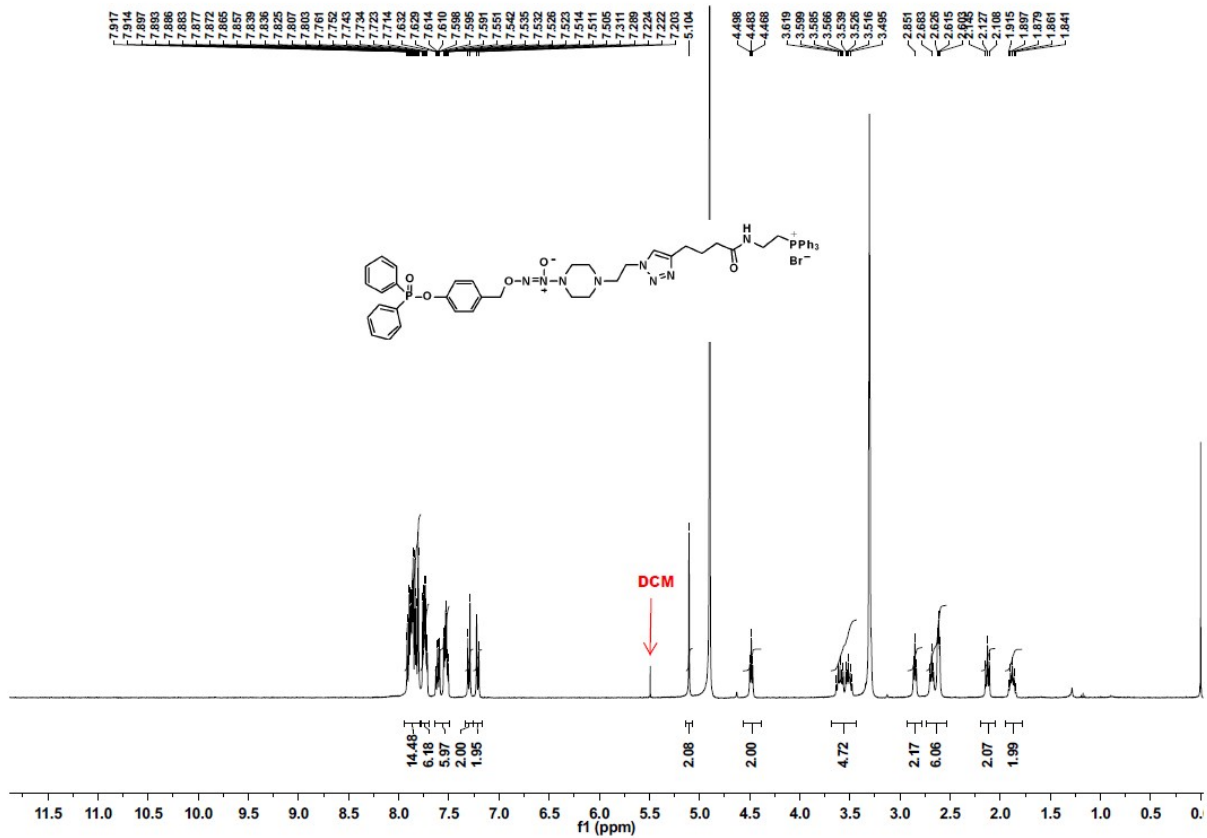


Fig. S9 <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra for the compound TPP-alkyne



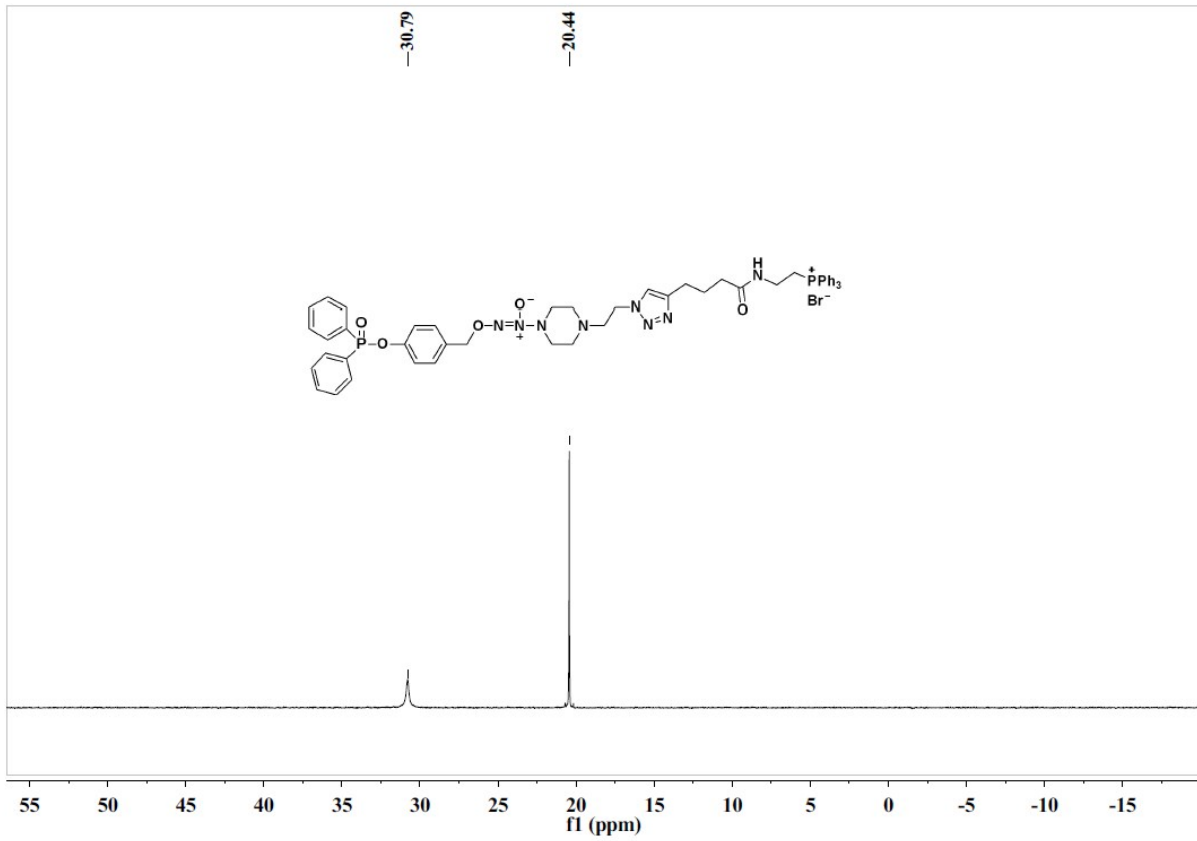
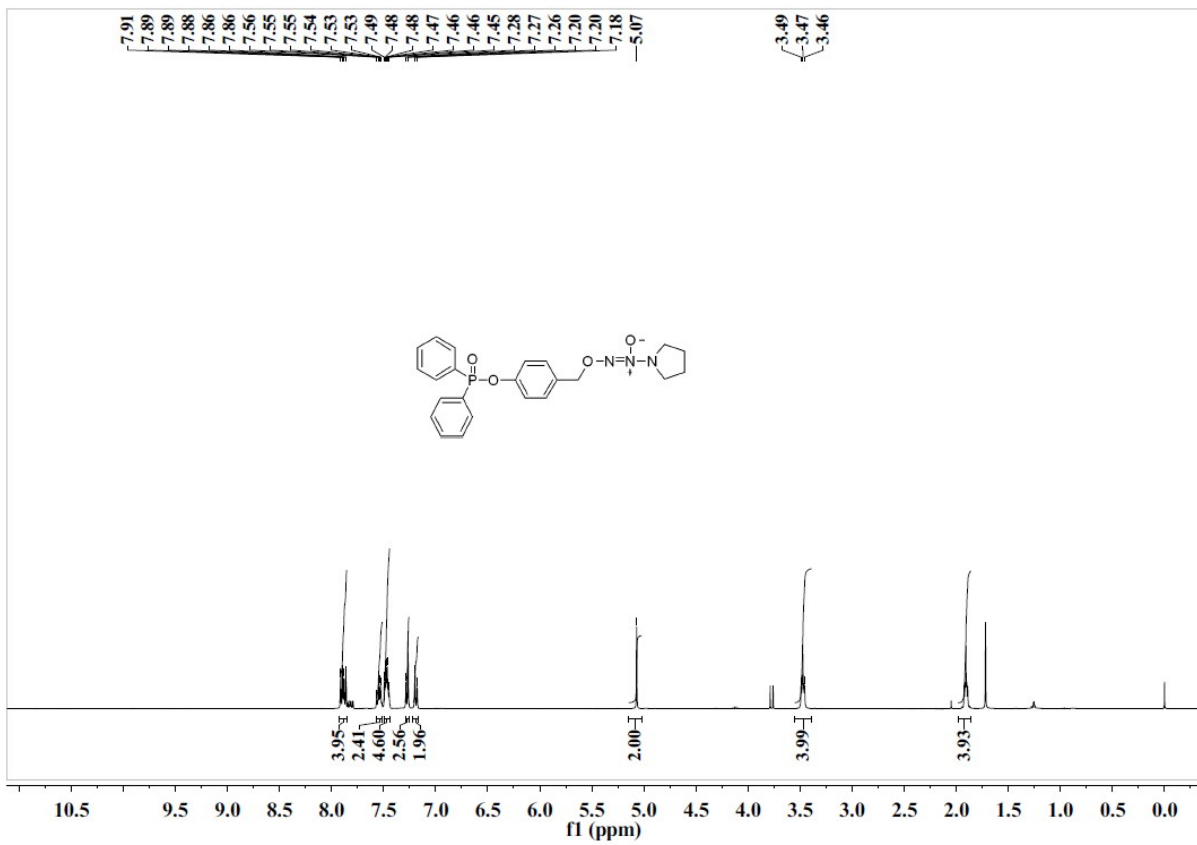


Fig. S10  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and  $^{31}\text{P}$  NMR spectra for the compound MitoSNOD



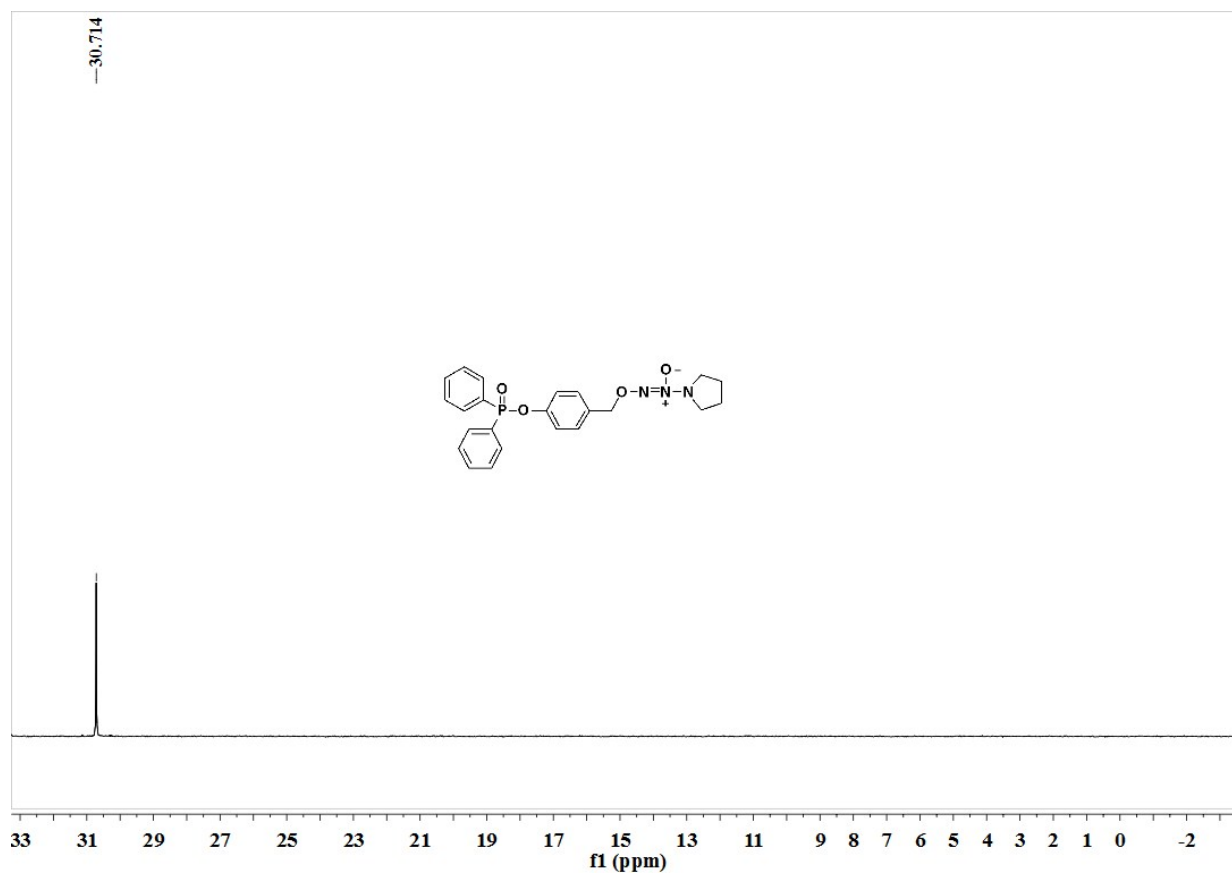
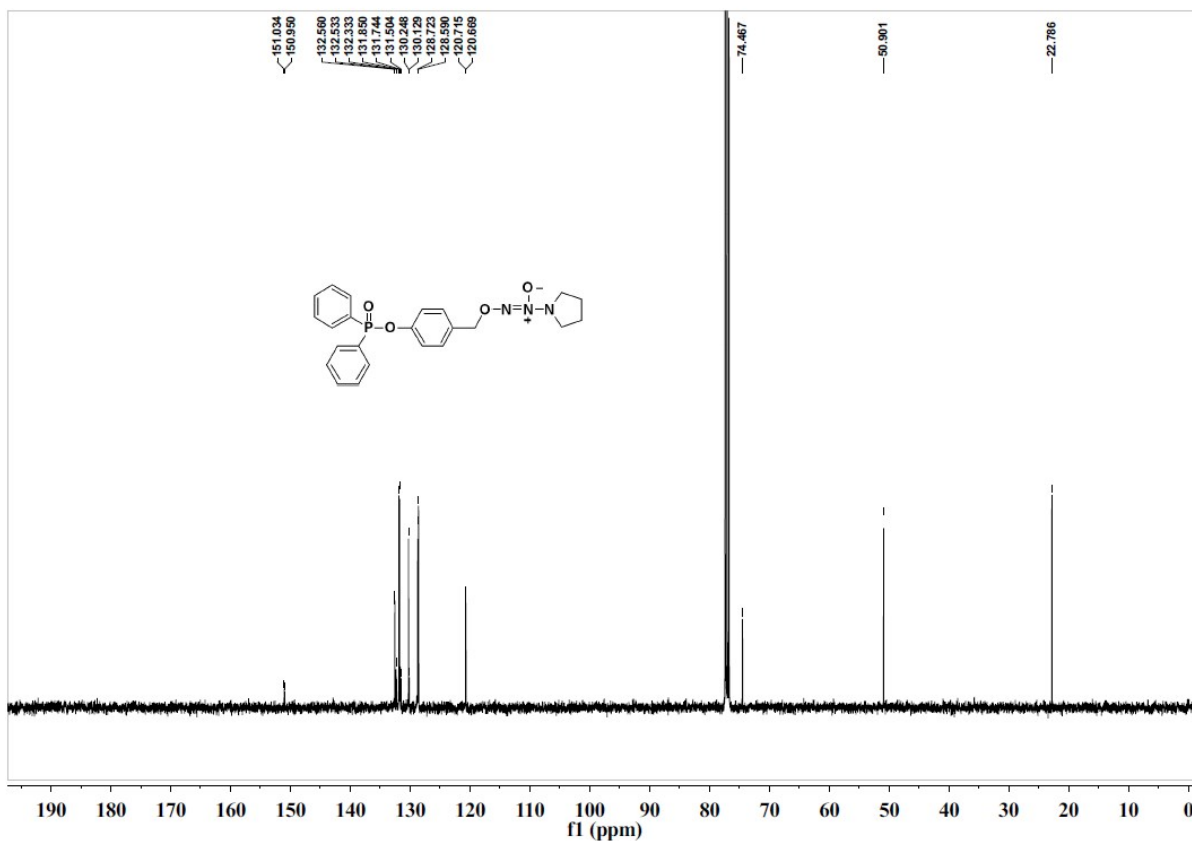


Fig. S11  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and  $^{31}\text{P}$  NMR spectra for the compound SNOD



**Reference:**

1. M.A. Packer and M.P. Murphy, *FEBS Lett.*, 1994, **345**, 237-240.
2. L. A. Shinobu, S. G. Jones and M. M. Jones. *Acta.Pharmacol. Toxicol.*, 1984, **54**, 189-194.