Electronic Supplementary Information (ESI)

Mito-DEPMPO synthesized from a novel NH_2-reactive DEPMPO spin trap: A new and improved trap for detection of superoxide

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Preparation of Mitochondria.

Mitochondria were isolated from RAW 264.7 cells myocardial tissue as described (*1*). Briefly, the tissue was homogenized in H-medium (220 mM mannitol, 70 mM sucrose, 10 mM HEPES pH 7.0, 2 mM EDTA) supplemented with protease and phosphatase inhibitors. The homogenate was centrifuged at 2000 X g for 10 minutes to remove cellular debris and nuclear pellet. This step was repeated twice to remove any contaminating nuclear fraction. The post nuclear supernatant was then centrifuged at 10,000 X g for 20 minutes to pellet mitochondria. The pellet was washed twice with H-medium and resuspended in the same buffer. The mitochondrial suspension was layered on 0.8 M sucrose and centrifuged at 10,000 X g for 20 minutes to remove contaminating cytosol and microsomes. The pellet was resuspended in H-medium and protein content was estimated by Lowry's method.

When succinate (100 μM) was added to a solution of Mito-DEPMPO (50 mM) as control experiment, no EPR signal was observed.

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Spectrometers settings: microwave power 10 mW (Fig 1a-b) modulation amplitude, 0.7G (Fig 1a), 0.5G (Fig 1b); time constant, 0.32 ms (Fig 1a), 0.64 ms (Fig 1b); gain 10^5 (Fig 1a-b); sweep time, 84 s (Fig 1a), 335.5 s (Fig 1b, Fig 2a-b); conversion time, 82 ms (Fig 1a), 163 ms (Fig 1b, Fig 2a-b), microwave power 20 mW (Fig 2a-b); modulation amplitude, 1 G (Fig 2a-b); time constant, 1.28 ms (Fig 2a-b); gain 10^6 (Fig 2a-b).

Synthesis :

Nitrofuranone 2 was obtained in 80% yield[1] by reaction of the nitrophosphonate 1 with 2(5H)-furanone in the presence of a catalytic amount of tributylphosphine in a mixture of cyclohexane and CH_2Cl_2. Reduction of 2 to form the hemiacetal 3 was performed using 2.5 equivalents of DIBAL-H at -78ºC in CH_2Cl_2 (55% yield).[2] Reductive cyclization of 3 with Zn dust and ammonium chloride in a mixture of THF / H_2O led to nitrone 4 as a mixture of cis-trans (67 / 33) diastereoisomers, separated by silicagel column chromatography, in 55% yield.[3] Reaction of the cis diastereoisomer, cis-4, with N,N'-disuccinimidylcarbonate (DSC),[4] an unsymmetrical reactive linker, in the presence of 1.2 equivalent of Et_3N in CH_3CN afforded NHS-DEPMPO 5 in 95% yield.

Coupling of NHS-DEPMPO with either (2-aminoethyl) triphenylphosphonium bromide or biotinylamidopropylammonium trifluoroacetate led to Biotin-DEPMPO 6 and Mito-DEPMPO 7 in almost quantitative yields. All these compounds have been characterized by ^1H, ^{13}C, ^{31}P NMR and MS spectroscopies and by elemental analysis.

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