Embedding cyclic nitrone in mesoporous silica particles for EPR spin trapping of superoxide and other radicals

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Electronic supplementary information

Table of contents:

ESR spectra: Variable-temperature experiments and decay kinetics S2
Experimental procedures for organic precursors and material S3
13C and 29Si CP-MAS solid-state NMR for SBA15-NH2 S7
Nitrogen adsorption/desorption analysis for SBA15-NH2 S8
Small Angle X-Ray Scattering (SAXS) for SBA15-NH2 S8
31P solid-state NMR for Silica-DIPPMPMO S9
13C solid-state NMR for Silica-DIPPMPMO S9
Thermogravimetric analysis for SBA15-NH2 and Silica-DIPPMPMO S10
ESR spectra: Variable-temperature experiments

Variable-temperature experiments conducted for the Silica-DIPPMPO-OOH. (a) ESR spectrum obtained after 10 min incubation of a mixture containing hypoxanthine (0.4 mM), xanthine oxidase (0.4 U/mL), dtpa (1 mM), and Silica-DIPPMPO (30 mg/mL) in oxygenated phosphate buffer (0.1 M, pH 7.3) at 10°C. (b) As in (a) but at 20°C. (c) As in (a) but at 30°C. (d) As in (a) but at 37°C. (e) As in (a) but at 40°C. (f) As in (a) but at 50°C. Spectrometer settings: microwave power, 10 mW; modulation amplitude, 1 G; time constant, 1.28 ms; gain, 90; sweep time, 30 s; conversion time, 29.3 ms, 3 scans.

Decay kinetics of Silica-DIPPMPO-OOH

The decay curves of the superoxide adducts were calculated using the ROKI-EPR program. Using this program, it was feasible to determine the concomitant evolution of different radical adducts. The kinetics of decay of the superoxide adducts were tested in parallel under the same set of conditions. Solutions containing hypoxanthine (0.4 mM), xanthine oxidase (0.4 U/mL), dtpa (1 mM), and DIPPMPO (20 mM) or Silica-DIPPMPO (30 mg/mL) in a phosphate buffer (0.1 M, pH 7.3) were incubated at room temperature. Once the adduct concentration had reached steady-state (10–15 min), superoxide dismutase enzymes (600 U/mL) was added to inhibit the generation of superoxide adduct, and each spectrum was subsequently recorded within 150 min at a rate of 1 full scan/41 s.
Experimental procedures for organic precursors and material

All reagents were used as received without further purification. The reactions were monitored by TLC on silica gel Merck 60F_{254} and by $^{31}$P nuclear magnetic resonance (NMR). $^{31}$P NMR and $^{13}$C NMR spectra were recorded with Bruker DPX 400 and Bruker Avance III WB 400 spectrometers. Chemical shifts ($\delta$) are reported in ppm and coupling constant $J$ values in Hertz. DIPPMPO was obtained from Radical Vision (Marseille, France). 3-aminopropyltrioethoxysilane is commercially available. The amino-propyl-SBA_{15} ($\text{SBA}_{15}$-$\text{NH}_2$)$^1$ and NHS-DIPPMPO$^2$ were prepared according to literature procedures.

Thermogravimetric (TGA) measurements were carried out with a TGA Q500 apparatus (TA Instruments) under dynamic air atmosphere (sample flow rate 40 ml/min). SAXS experiments were performed on SAXSess-MC2 (Anton-Paar, GmbH, Austria) with a sealed copper tube as an X-ray source (wavelength is 0.15417 nm [Cu K-$\alpha$]) and a CCD camera as a detection system. The N$_2$ adsorption/desorption isotherms were obtained at 77 K on a Micrometrics ASAP2010. The specific surface area was determined according to the Brunauer, Emmett, and Teller (BET) method, and the pore size distribution was calculated from the desorption isotherms using the Barrett Joyner Halenda (BJH) method.$^3$ Prior to adsorption, the samples were degassed at 373 K overnight under a vacuum pressure of $2 \times 10^{-3}$ mbar. All solid-state Cross Polarization Magic Angle Spinning (CPMAS) NMR spectra were obtained on a Bruker Avance 400 MHz NMR spectrometer operating at $^{13}$C, $^{29}$Si, and $^{31}$P resonance frequencies of 101.6 MHz, 79.5 MHz, and 162.1 MHz, respectively. About 100 mg of samples were placed in zirconium dioxide rotors of

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4 mm outer diameter and spun at a Magic Angle Spinning rate of 10 kHz. The CP technique\(^4\) was applied with a ramped \(^1\)H-pulse starting at 100% power and decreasing to 50% during the contact time in order to circumvent Hartmann-Hahn mismatches.\(^5,6\) The contact times were 2 ms for \(^{13}\)C CPMAS and 5 ms for \(^{29}\)Si CPMAS. To improve the resolution, a dipolar decoupling GT8 pulse sequence\(^7\) was applied during the acquisition time. In order to obtain a good signal-to-noise ratio, 6144 scans were accumulated using a delay of 2 s in \(^{13}\)C CPMAS experiment, and 4096 scans with a delay of 5 s in \(^{29}\)Si CPMAS experiment. The \(^{13}\)C and \(^{29}\)Si chemical shifts were referenced to tetramethylsilane and \(\text{H}_3\text{PO}_4\) for \(^{31}\)P. Commercially available tetraethylorthosilicate (TEOS) was distilled before use.

Synthesis of the nitron \textbf{Silica-DIPPMPO}

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\begin{align*}
\text{(EtO)}_3\text{Si} & \quad \text{(EtO)}_3\text{Si} \\
\text{NH}_2 & \quad \text{NH}_2 \\
\text{Boc} & \quad \text{Boc} \\
\text{H}_2\text{O}, \text{HCl} & \quad \text{H}_2\text{O}, \text{HCl} \\
40^\circ\text{C}, 24\text{h} & \quad 100^\circ\text{C}, 48\text{h} \\
\text{H}_3\text{N} & \quad \text{H}_3\text{N} \\
\text{SBA}_{12}\text{-NH}_2 & \quad \text{SBA}_{12}\text{-NH}_2 \\
\text{Silica-DIPPMPO} & \quad \text{Silica-DIPPMPO} \\
\text{Et}_3\text{N} & \quad \text{Et}_3\text{N} \\
\text{CH}_3\text{CN} & \quad \text{CH}_3\text{CN} \\
\text{NHS-DIPPMPO} & \quad \text{NHS-DIPPMPO}
\end{align*}
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\textbf{3-TertioButyl-oxycarbonyl-aminopropyltriethoxysilane} (1)

A mixture of 3-aminopropyltriethoxysilane (1.30 g, 5.11 mmol), and di-tert-butyl dicarbonate (1.25 g, 5.73 mmol), in 5 ml ethanol was stirred at room temperature overnight. The solvent was distilled under reduced pressure. Then, the product 1 was distilled (95°C under 0.05 T) to obtain 1.21 g (3.73 mmol, 73%) of a colorless liquid.

$^1$H NMR (δ ppm, 400 MHz, CDCl₃): 0.60 (m, 2H), 1.20 (t, 9H, $J_{HH} = 6.9$), 1.41 (s, 9H, C), 1.54 (m, 2H), 3.09 (m, 2H), 3.79 (q, 6H, $J_{HH} = 7.0$), 4.76 (s, 1H).

SBA₁₅-NH₂

Pluronic P-123 (PEG-PPG-PEG) (4 g) was dissolved in deionized water (28 mL) and HCl (2 M, 120 mL) and stirred for 3 h at 40°C. Then, tetraethoxysilane (8.10 g, 38.9 mmol, 19 eq.) and 1 (0.658 g, 2 mmol, 1 equiv) were added. The reaction mixture was stirred for 24 h at 40°C and heated at 100°C for 2 days without stirring. The silica was filtered and washed successively with H₂O and EtOH and extracted using a Soxhlet device (EtOH) for 24 h. The resulting powder was filtered and washed with EtOH, acetone, and Et₂O. The SBA₁₅-NH₂ was dried overnight at 80°C under vacuum.

$^{13}$C CP MAS NMR (100.7 MHz) δ = 57.9 (P123), 41.1, 19.7, 14.4 (P123), 7.1; $^{29}$Si CP MAS NMR (79 MHz) δ = −69.5 (T³), −94.7 (Q²), −103.4 (Q³), −112.6 (Q⁴); $S_{BET} = 708.3$ m²/g; $D_{pore} = 9.1$ nm; $V_{poreux} = 1.96$ cm³/g; DRX, $d_{100} = 11.3$ nm.

Silica-DIPPMPO

To a mixture of NHS-DIPPMPO (0.072 g, 0.6 mmol) and SBA₁₅-NH₂ (0.2 g, 0.25 mmol) in acetonitrile (10 mL), triethylamine (53 µL, 0.52 mmol) was added at room temperature under argon. The reaction mixture was stirred for 12 h. Then, the reaction mixture was filtered and washed successively with CH₂Cl₂, EtOH, acetone, and Et₂O. A white powder was obtained, corresponding to Silica-DIPPMPO (0.220 g).

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$^{31}$P HPDEC MAS NMR (162 MHz) $\delta = 18.0$; $^{13}$C CP MAS NMR (100.7 MHz) $\delta = 157.1, 145.0, 140.4, 75.4, 73.7, 71.9, 65.4, 59.2, 57.3$ (P123), 46.1, 43.4, 29.9, 23.2, 19.8, 17.4, 14.2 (P123), 9.0.
$^{13}$C solid-state NMR of SBA$_{15}$-NH$_2$

$^{29}$Si CP-MAS solid-state NMR of SBA$_{15}$-NH$_2$
Nitrogen adsorption/desorption analysis for SBA_{15}-NH_{2}

Small Angle X-Ray Scattering (SAXS) for SBA_{15}-NH_{2}
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Silica-DIPPMPO

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