

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

Eric Besson,*^a Stéphane Gastaldi,^a Emily Bloch,^b Jacek Zielonka,^{c,d} Monika Zielonka,^{c,d} Balaraman Kalyanaraman,^{c,d} Selma Aslan,^a Hakim Karoui,^a Antal Rockenbauer,^{e,f} Olivier Ouari^a and Micael Hardy*^a

^a*Aix Marseille Univ, CNRS, ICR, Marseille, France.*

^b*Aix Marseille Univ, CNRS, MADIREL, Marseille, France.*

^c*Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI 53226, USA.*

^d*Free Radical Research Center, Medical College of Wisconsin, Milwaukee, WI 53226, USA.*

^e*Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, 1117 Budapest, Hungary.*

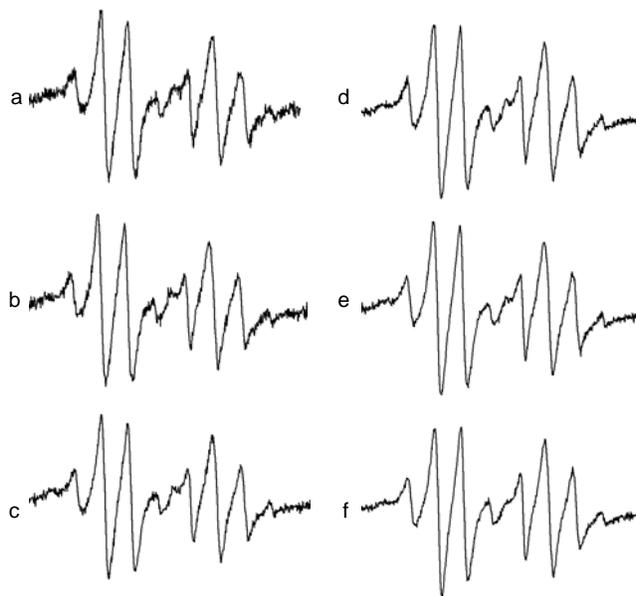
^f*Department of Physics, Budapest University of Technology and Economics, Budafoki ut 8, 1111 Budapest, Hungary.*

Electronic supplementary information

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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 60F₂₅₄ and by ³¹P nuclear magnetic resonance (NMR). ³¹P NMR and ¹³C NMR spectra were recorded with Bruker DPX 400 and Bruker Avance III WB 400 spectrometers. Chemical shifts (δ) are reported in ppm and coupling constant J values in Hertz. DIPPMPPO was obtained from Radical Vision (Marseille, France). 3-aminopropyltriethoxysilane is commercially available. The amino-propyl-SBA₁₅ (SBA₁₅-NH₂)¹ and NHS-DIPPMPPO² 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- α]) and a CCD camera as a detection system. The N₂ 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 2×10^{-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 ¹³C, ²⁹Si, and ³¹P 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

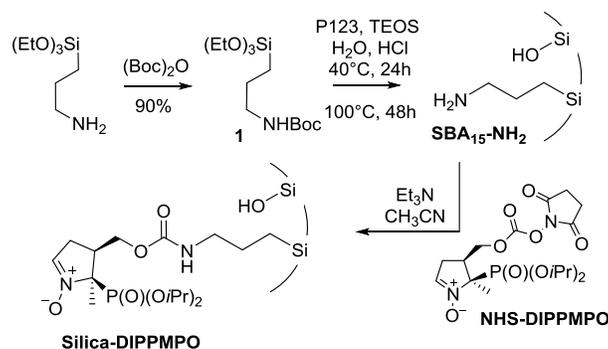
¹ A. Mehdi, C. Reye, S. Brandes, R. Guillard and R. J. P. Corriu, *New J. Chem.*, 2005, **29**, 965–968.

² M. Hardy, F. Poulhes, E. Rizzato, A. Rockenbauer, K. Banaszak, H. Karoui, M. Lopez, J. Zielonka, J. Vasquez-Vivar, S. Sethumadhavan, B. Kalyanaraman, P. Tordo and O. Ouari, *Chem. Res. Toxicol.*, 2014, **27**, 1155–1165.

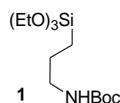
³ F. Rouquerol, J. Rouquerol, P. Llewellyn, G. Maurin, K. S. W. Sing, *Adsorption by Powders and Porous Solids: Principles, Methodology and Applications 2nd edition*; Academic Press: London, 2013.

4 mm outer diameter and spun at a Magic Angle Spinning rate of 10 kHz. The CP technique⁴ was applied with a ramped ¹H-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 ¹³C CPMAS and 5 ms for ²⁹Si CPMAS. To improve the resolution, a dipolar decoupling GT8 pulse sequence⁷ 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 ¹³C CPMAS experiment, and 4096 scans with a delay of 5 s in ²⁹Si CPMAS experiment. The ¹³C and ²⁹Si chemical shifts were referenced to tetramethylsilane and H₃PO₄ for ³¹P. Commercially available tetraethylorthosilicate (TEOS) was distilled before use.

Synthesis of the nitron **Silica-DIPPMPO**



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.

⁴ J. Schaefer, E. O. R. Stejskal, *J. Am. Chem. Soc.*, 1976, **98**, 1031-1032.

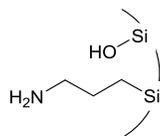
⁵ O. B. Peersen, X. Wu, I. Kustanovich, S. O. Smith, *J. Magn. Reson.* 1993, **104**, 334-339.

⁶ R. L. Cook, C. H. Langford, R. Yamdagni, C. M. A. Preston, *Anal. Chem.* 1996, **68**, 3979-3986.

⁷ G. Gerbaud, F. Ziarelli, S. Caldarelli, *Chem. Phys. Lett.* 2003, **377**, 1-5.

^1H NMR (δ ppm, 400 MHz, CDCl_3): 0.60 (m, 2H), 1.20 (t, 9H, $J_{\text{HH}} = 6.9$), 1.41 (s, 9H, C), 1.54 (m, 2H), 3.09 (m, 2H), 3.79 (q, 6H, $J_{\text{HH}} = 7.0$), 4.76 (s, 1H).⁸

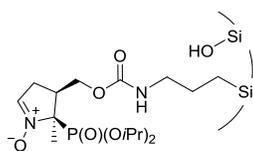
SBA₁₅-NH₂



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) $\delta = 57.9$ (P123), 41.1, 19.7, 14.4 (P123), 7.1; ^{29}Si CP MAS NMR (79 MHz) $\delta = -69.5$ (T³), -94.7 (Q²), -103.4 (Q³), -112.6 (Q⁴); $S_{\text{BET}} = 708.3 \text{ m}^2/\text{g}$; $D_{\text{pore}} = 9.1 \text{ nm}$; $V_{\text{poreux}} = 1.96 \text{ cm}^3/\text{g}$; DRX, $d_{100} = 11.3 \text{ nm}$.

Silica-DIPPMPO

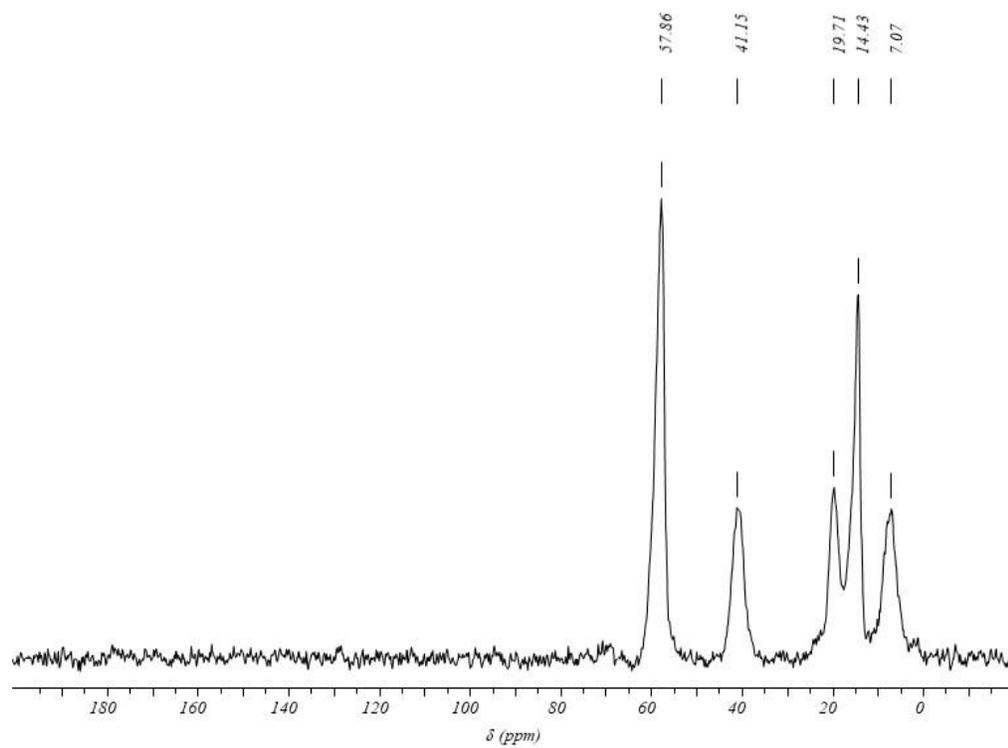


To a mixture of NHS-DIPPMPO (0.072 g, 0.6 mmol) and SBA₁₅-NH₂ (0.2 g, 0.25 mmol) in acetonitrile (10 mL), triethylamine (53 μL , 0.52 mmol) was added at room temperature under argon. The reaction mixture was stirred for 12 h. Then, the reaction mixture was filtered and washed successively with CH₂Cl₂,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

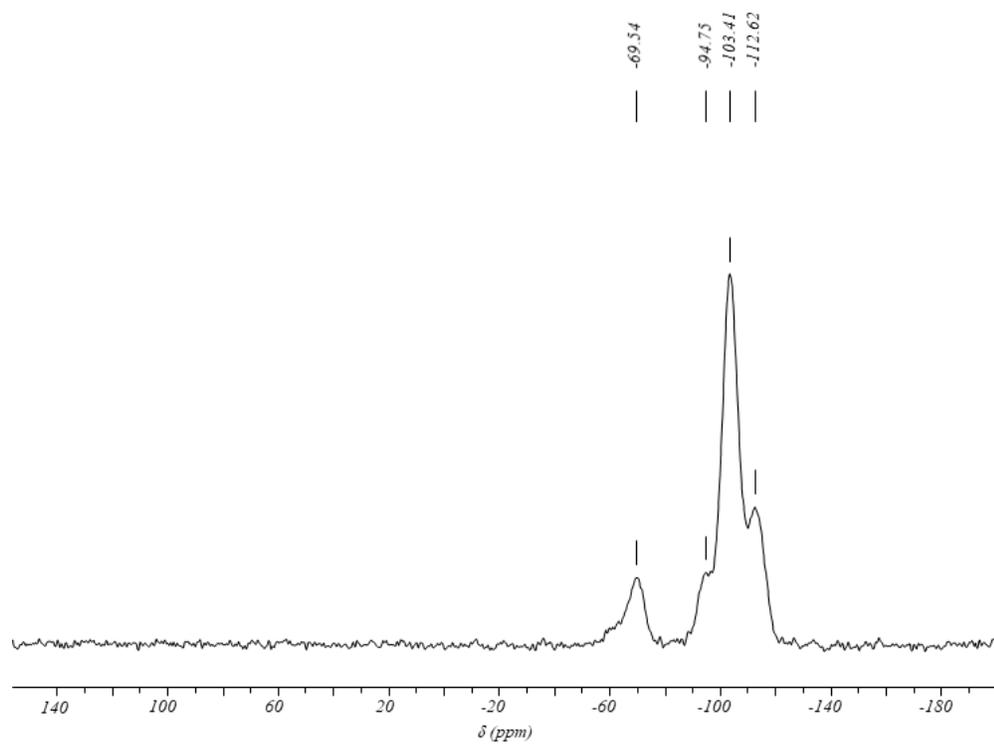
⁸ G. Subra, A. Mehdi, C. Enjalbal, M. Amblard, L. Brunel, R. Corriu, J. Martinez, *J. Mater. Chem.* 2011, **21**, 6321-6326.

^{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.

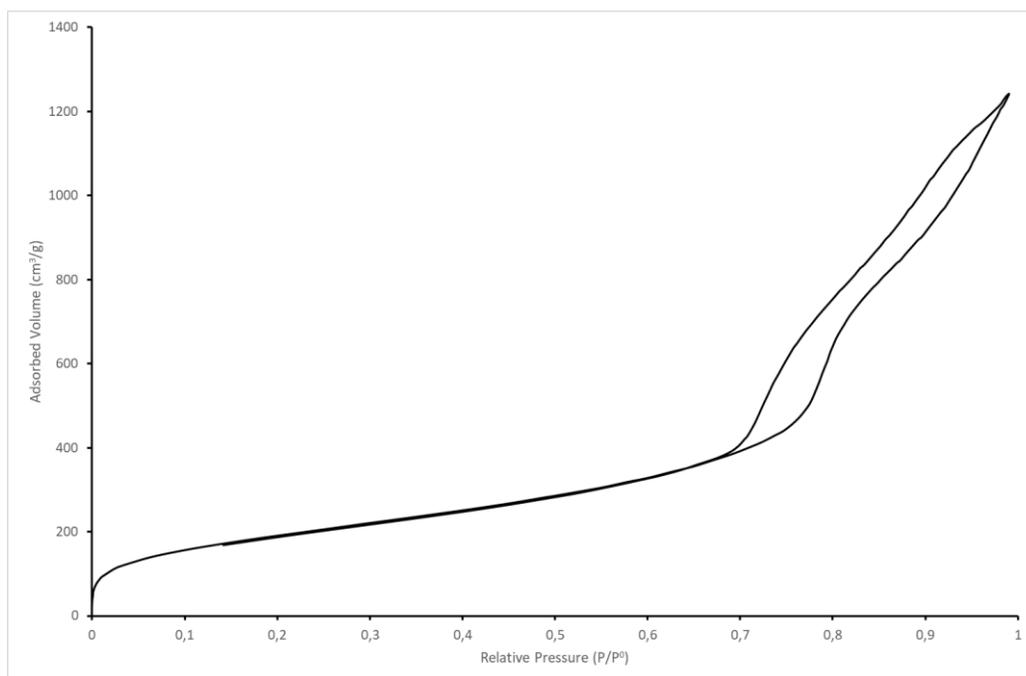
^{13}C solid-state NMR of SBA₁₅-NH₂



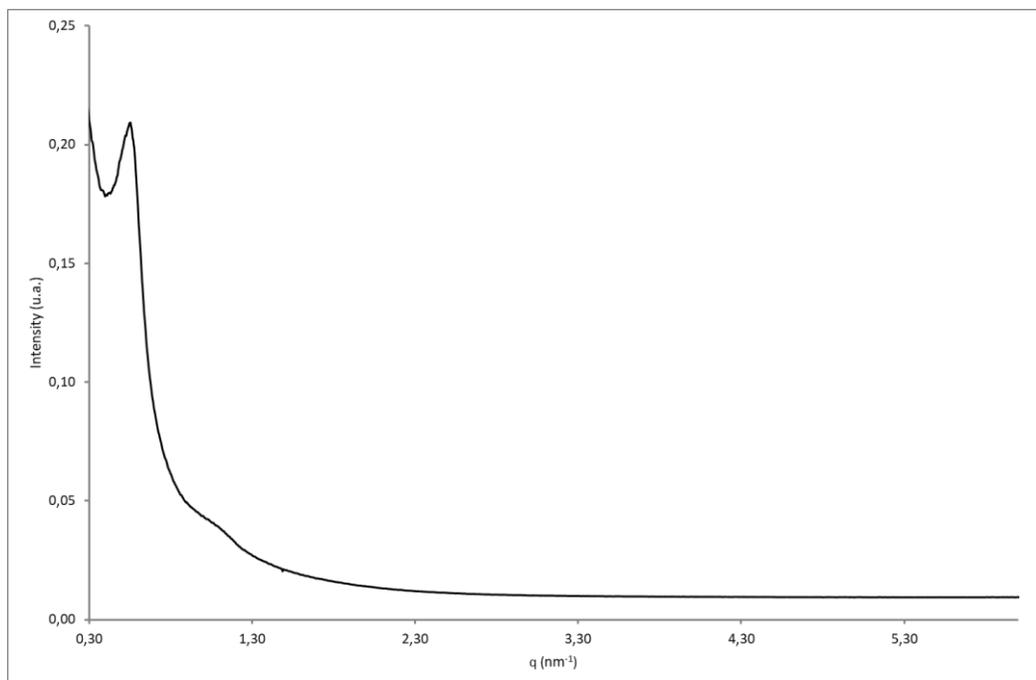
^{29}Si CP-MAS solid-state NMR of SBA₁₅-NH₂



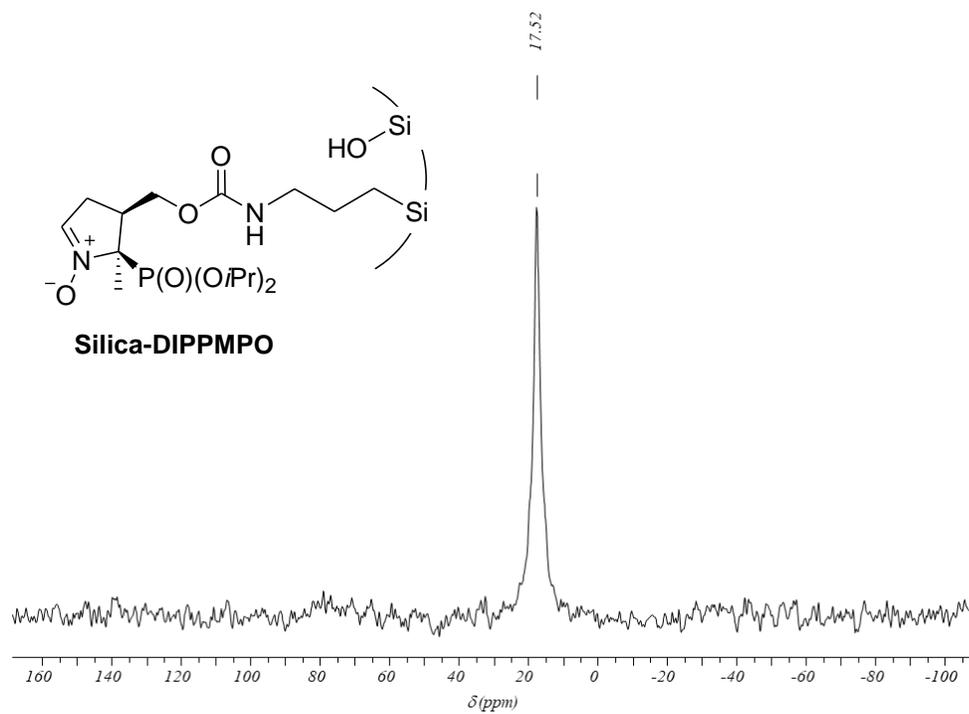
Nitrogen adsorption/desorption analysis for SBA₁₅-NH₂



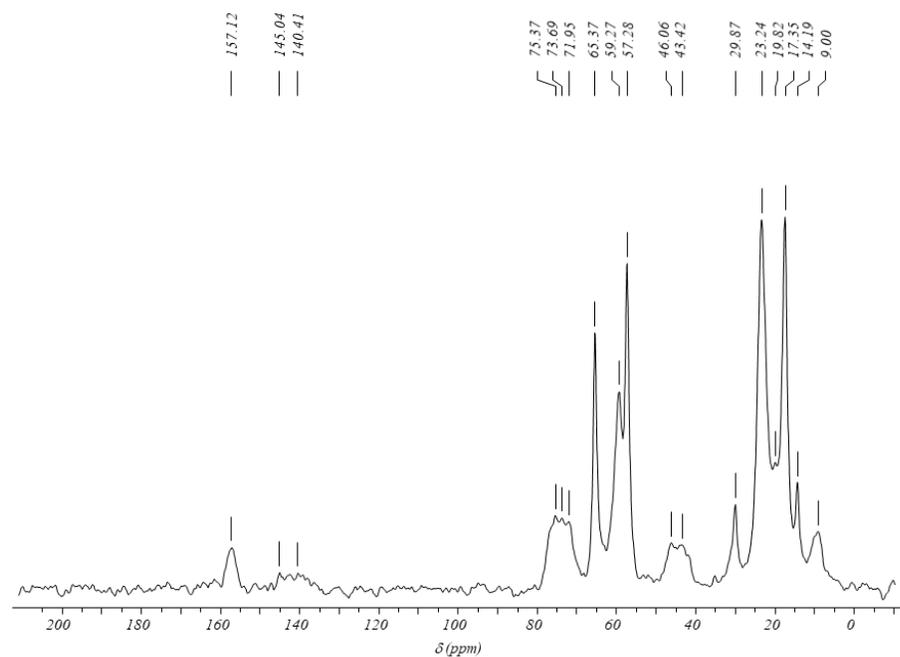
Small Angle X-Ray Scattering (SAXS) for SBA₁₅-NH₂



³¹P solid-state NMR for Silica-DIPPMPO



¹³C solid-state NMR for Silica-DIPPMPO



Thermogravimetric analysis for SBA₁₅-NH₂ and Silica-DIPPMPO

