Supporting Information

Magnetic Nanoparticles-Templated Assembly of Protein Subunits: A New Platform for Carbohydrate-Based MRI Nanoprobes

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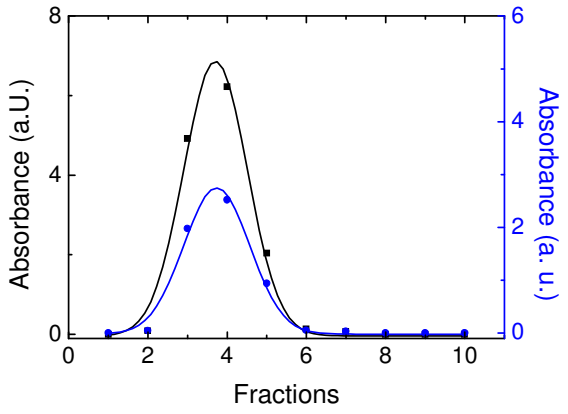
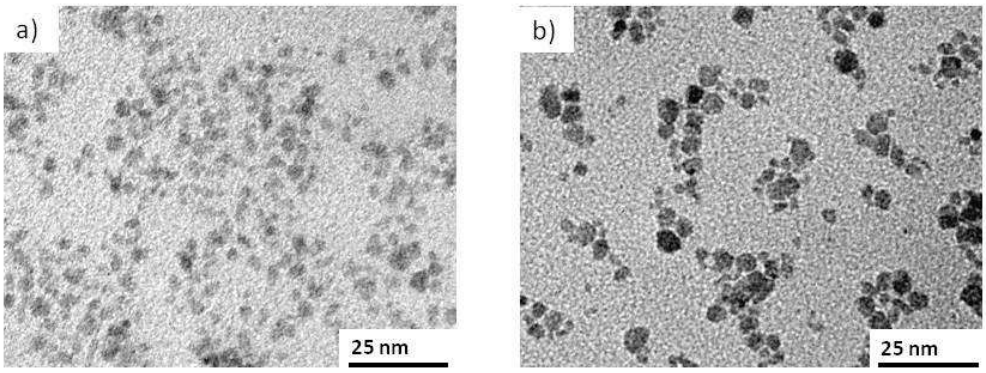
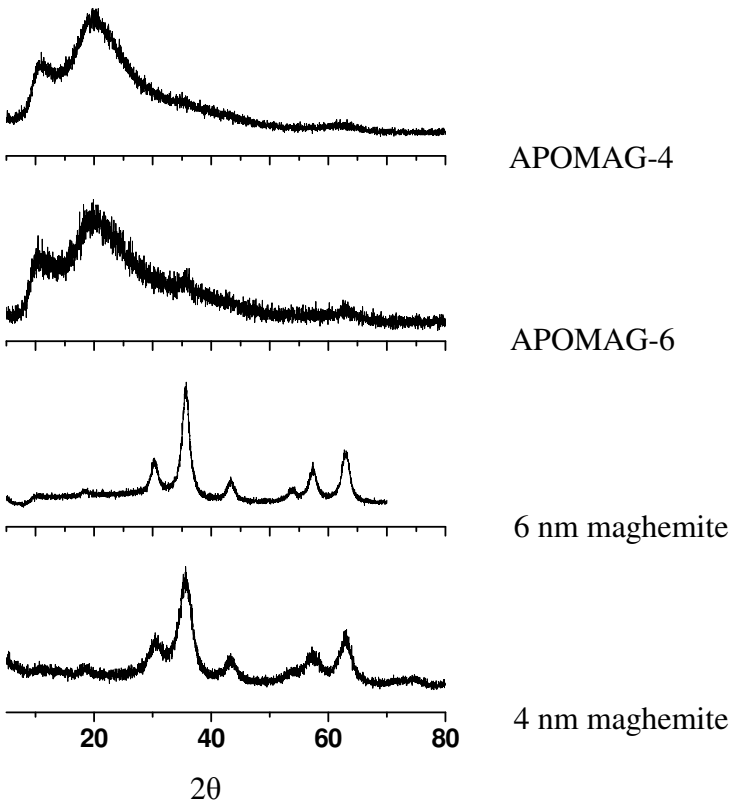
SI1. Size exclution chromatography coelution profile for the APOMAG-4 sample monitored at 280 (black) for the protein and at 330 nm (blue) for the iron oxide.



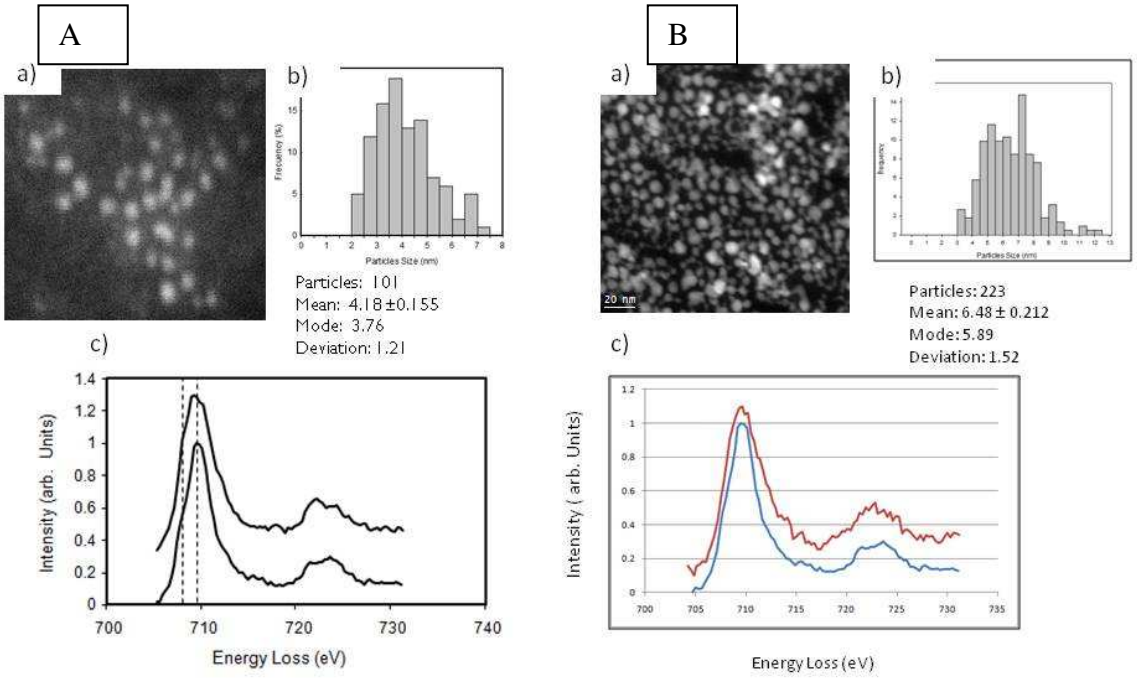
SI2. Transmission Electron Microscopy of magnetic colloids before encapsulation: a) 4 nm maghemite; b) 6 nm maghemite.



SI3. XRD measurements. Maghemite samples showed typical pattern of maghemite/magnetite iron oxide. Apomaghemites diffractogram showed 2 peaks at about 10º and 20º, typical of apoferritin, and the strongest peaks for maghemite/magnetite: the 311 at around 35º and the 440 at 65º 2θ.



SI4. HAADF-STEM images of the Apomaghemite samples. HAADF-STEM images of the Apomaghemite samples, showing that particles exhibit the characteristic spherical shape. The average particle size and standard deviation were estimated from the HAADF image analysis of 100 particles. The mean particle size is 4.2 ± 0.2 nm and 6.5 ± 0.2 nm for the APOMAG-4 and APOMAG-6, respectively. The EELS spectra of one particle for both samples, has one peak centred at 709.5 eV, with a shoulder at 708 eV. According to the literature, these two peaks correspond to transitions from the 2p level to t_{2g} (low energy) and e_g (high energy) orbitals of the 3d level.[1] These transitions are observed for both Fe^{2+} and Fe^{3+}, as can be seen from the EEL spectrum of ferrihydrite included in the graph as reference. However, the presence of Fe^{+2} is associated to an increase of the peak intensity corresponding to the t_{2g} transition.[2] The intensity of the peak at 708 eV for the Apomaghemite is higher than the one observed for ferrihydrite, so we cannot exclude the presence of small quantity of magnetite.



A. APOMAG-4: a) HAADF image of Apomaghemite sample; b) Particle size distribution; c) EELS spectra from a pure ferrihydrite (top spectrum) particle and from APOMAG-4 (bottom spectrum); **B.** APOMAG-6: the same as for A.

1 P. A. van Aken, S. Lauterbach Phys. Chem. Miner. 2003, 30, 469-477.

2 C. C. Calvert, A. Brown, R. Brydson J. Electron Spectrosc. Relat. Phenom. 2005, 143, 173-187.

SI5. Synthesis and characterization of the vinyl sulfone derivatized carbohydrates

General Experimental Procedures

TLC was performed on Merck Silica Gel 60 F254 aluminium sheets. Reagent used for developing plates include potassium permanganate (1% w/v), ninhydrin (0.3% w/v) in ethanol and UV light when applicable. Flash column chromatography was performed on Silica Gel Merck (230-400 mesh, ASTM). Optical rotations were recorded on a Perkin-Elmer 341 polarimeter at room temperature. ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Varian Direct Drive (500 MHz) spectrometer. Chemical shifts are given in ppm and referenced to internal DMSO. *J* values are given in Hz. NALDI-TOF mass spectra were recorded on an Autoflex Brucker spectrometer using HCCA and NaI, respectively, as matrix.

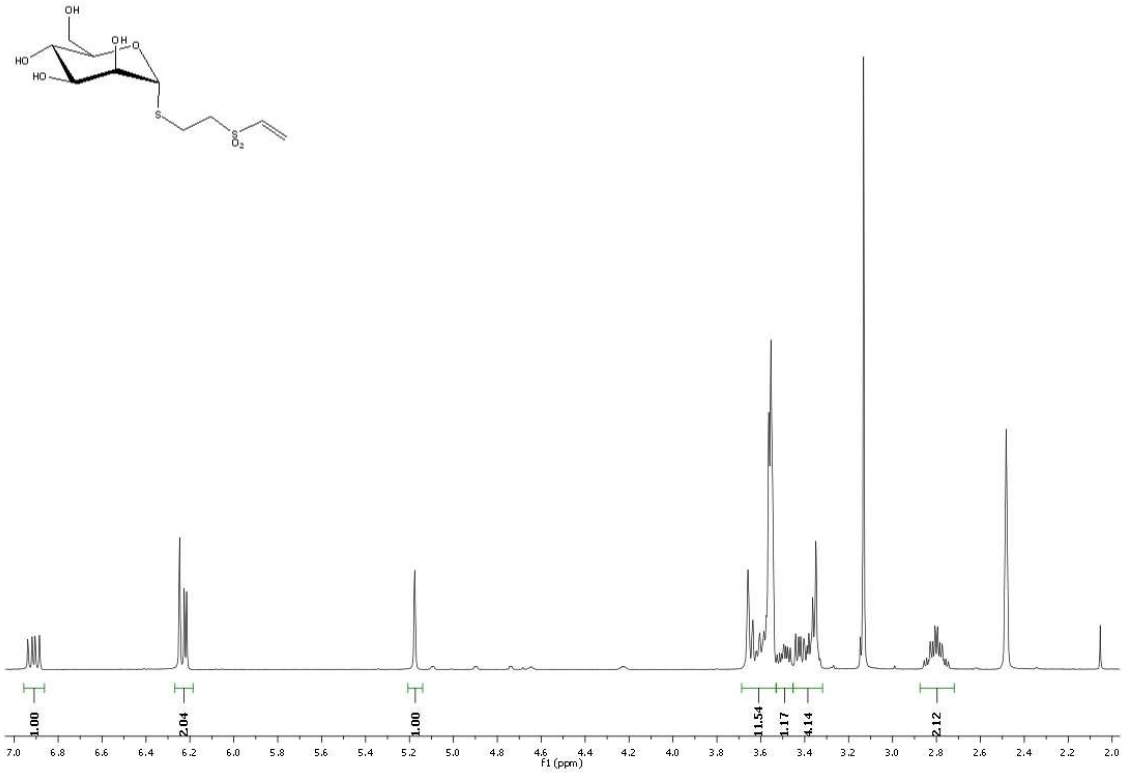
Synthesis of 2-ethenylsulfinyl ethyl 1-thio-□-D-mannopyranoside: To a solution of 2,3,4,6-tetra-*O*-acetyl-1-thio-□-D-mannopyranose[1] (2.2 g, 6.04 mmol) in anhydrous methanol (20 mL) was added sodium methoxide (360 mg, 6.65 mmol) and the reaction mixture was stirred a rt for 1 h. The precipitated material obtained was dissolved in H_2O and the solution neutralized with Amberlite IR-120H. The resin was filtered and the resulting solution used directly without any further treatment. SiO_2 (5 g) and divinylsulfone (3.54 g, 30 mmol) were added and the new reaction mixture stirred at rt for 30 min. Evaporation of the solvent gave a crude that was purified by chromatography on silica gel (CH_2Cl_2:methanol, 3:1) to give the title compound (564 mg, 30%) as a syrup. □□□$_D$ +90º (*c* 1, H_2O); ^{1}H-RMN (DMSO-d_6 x D_2O, 500 MHz): δ 6.91 (dd, 1H, *J* = 16.6 and 10.0 Hz, C*H*=CH_2), 6.24-6.21 (m, 2H, CH=CH_2), 4.37 (d, 1H, *J* = 10.3 Hz, H-1) 3.65 (dd, 1H, *J* = 13.0 and 1.9 Hz, H-6), 3.50-3.32 (m, 4H, H-2,H-6, CH_2SO_2), 3.23 (t, 1H, *J* = 9.3 Hz, H-4), 3.13 (m, 1H, H-5), 3.05 (t, 1H, *J* = 9.0 Hz, H-3), 2.90-2.85 (m, 1H,CH_2S), 2.75-2.70 (m, 1H,CH_2S), 1.77 (s, 3H, *Me*CO); ^{13}C-RMN (DMSO-d_6 x D_2O,

1 Matta, L. L.; Girotra, R. N.; Barlow, J. J.; Carbohydr. Res. 1975, 43, 101.

125 MHz): δ 169.8 (CO), 136.9 ($SO_2CH=CH_2$), 130.8 ($SO_2CH=CH_2$), 84.9 (C-1), 81.4 (C-5), 75.5 (C-4), 70.7 (C-3), 61.4 (C-6), 54.5 (C-2), 54.4 (CH_2SO_2), 29.3 (CH_3CO), 22.7 (CH_2S). HRMS (m/z) (NALDI-TOF) calcd. for $C_{10}H_{18}O_7S_2Na$ [M+Na]+: 337.0386; found: 337.0387.

Synthesis of 2-ethenylsulfinyl ethyl 2-acetamido-2-deoxy-1-thio-□-D-glucopyranoside: A solution of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-□-D-glucopyranosyl chloride[2] and thiourea (2.52 g, 33.1 mmol) in anhydrous acetone (30 mL) was heated under reflux for 4 h. After this time, the volume of the reaction mixture was reduced approximately up to half by evaporation under vacuum with gentle heating. A solution of Na_2SO_3 (2.5 g, 19.8 mmol) in H_2O (50 mL) was then added. After stirring for 30 min, aqueous HCl (5%, 16 mL) and H_2O (50 mL) were added. Extraction with CH_2Cl_2 (2 x 50 mL) gave a combined organic phase that was washed with H_2O (50 mL), dried (Na_2SO_4), filtered and evaporated. The crude product obtained was de-*O*-acetylated by addition of sodium methoxide (360 mg, 6.65 mmol) in methanol (20 mL) at rt for 1 h. The solution was neutralized by addition of Amberlite IR-120H. The resin was filtered and the resulting solution used directly without any further treatment. SiO_2 (5 g) and divinylsulfone (3.54 g, 30 mmol) were added and the reaction mixture stirred at rt for 30 min. Evaporation of the solvent gave a crude that was purified by chromatography on silica gel (CH_2Cl_2:methanol, 4:1) to give the title compound (930 mg, 40%) as a solid: m.p. 159-160 ºC; □□□$_D$ -26.7º (*c* 1, H_2O); ^{1}H-RMN (DMSO-d6 x D_2O, 500 MHz): δ 6.90 (dd, 1H, *J* = 16.6 and 9.9 Hz, C*H*=CH_2), 6.24-6.21 (m, 2H, CH=C*H*$_2$), 5.17 (s, 1H, H-1), 3.66-3.35 (several m, 8H, H-2, H-3, H-4, H-5, H-6, H-6', C*H*$_2SO_2$), 2.80-2.77 (m, 2H, C*H*$_2$S); ^{13}C-RMN (DMSO-d_6 x D_2O, 125 MHz): δ 136.9 ($SO_2CH=CH_2$), 130.8 ($SO_2CH=CH_2$), 86.0 (C-1), 74.9, 71.7, 67.5 (C-2, C-3, C-4, C-5), 61.3 (C-6), 53.9 (CH_2SO_2), 23.6 (CH_2S). HRMS (m/z) (NALDI-TOF) calcd. for $C_{12}H_{21}O_7NS_2Na$ [M+Na]+: 378.0651; found: 378.0666.

2 Horton, D.; Methods in Carbohydrate Chemistry, 1972, 6, 282.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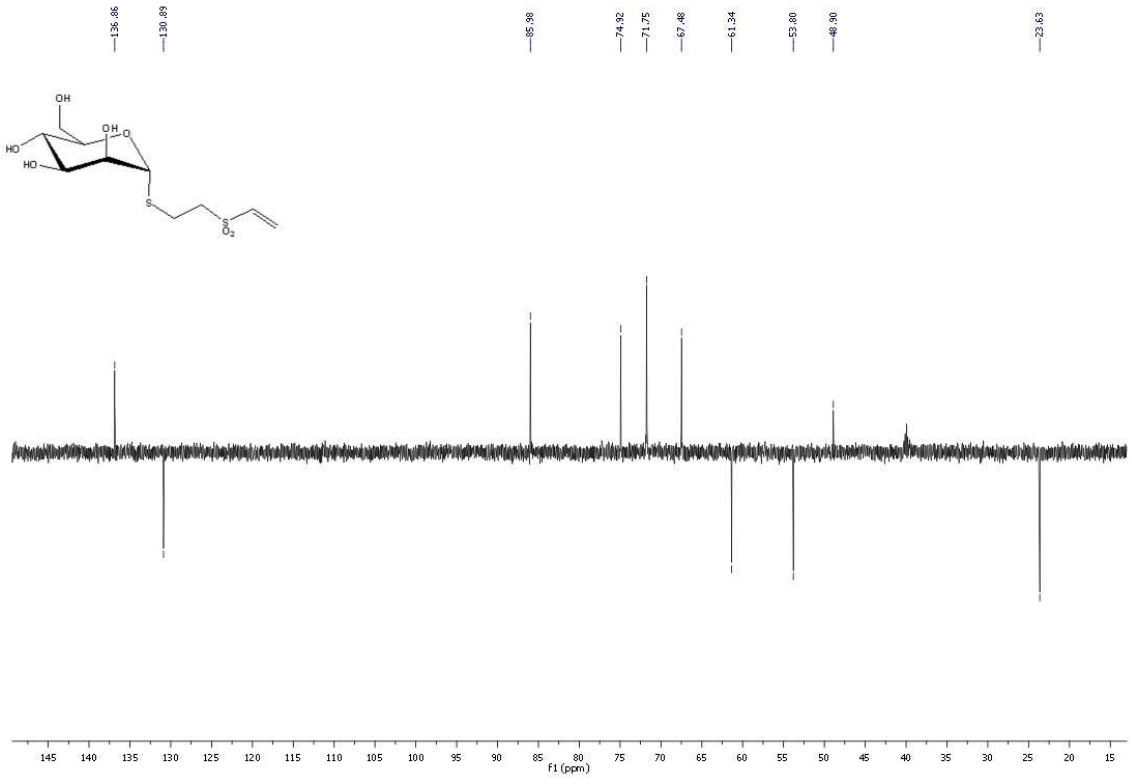
f1 (ppm)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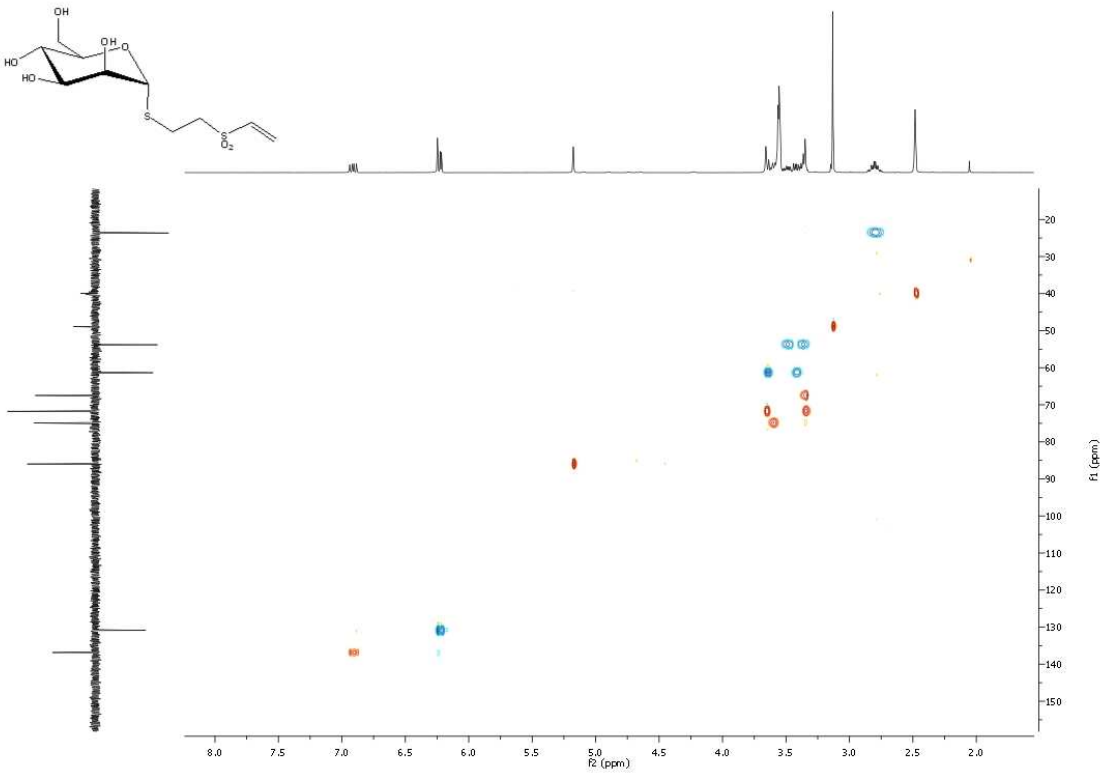
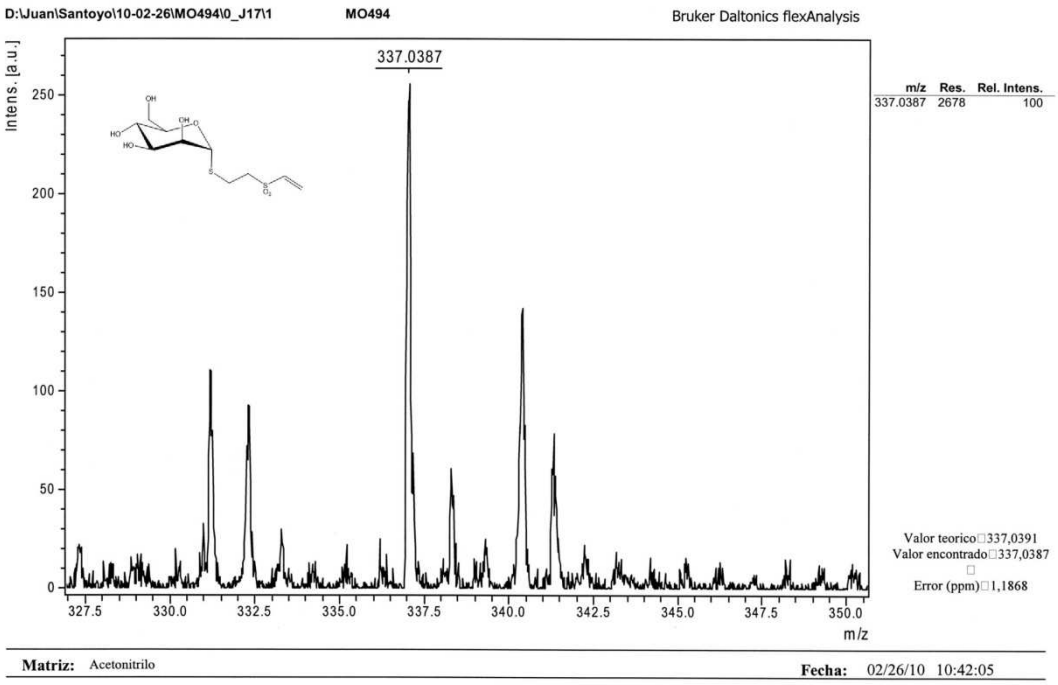
HO
HO
S
S
O2
f1 (ppm)


OH
HO
HO
S
S
O2
f1 (ppm)
f2 (ppm)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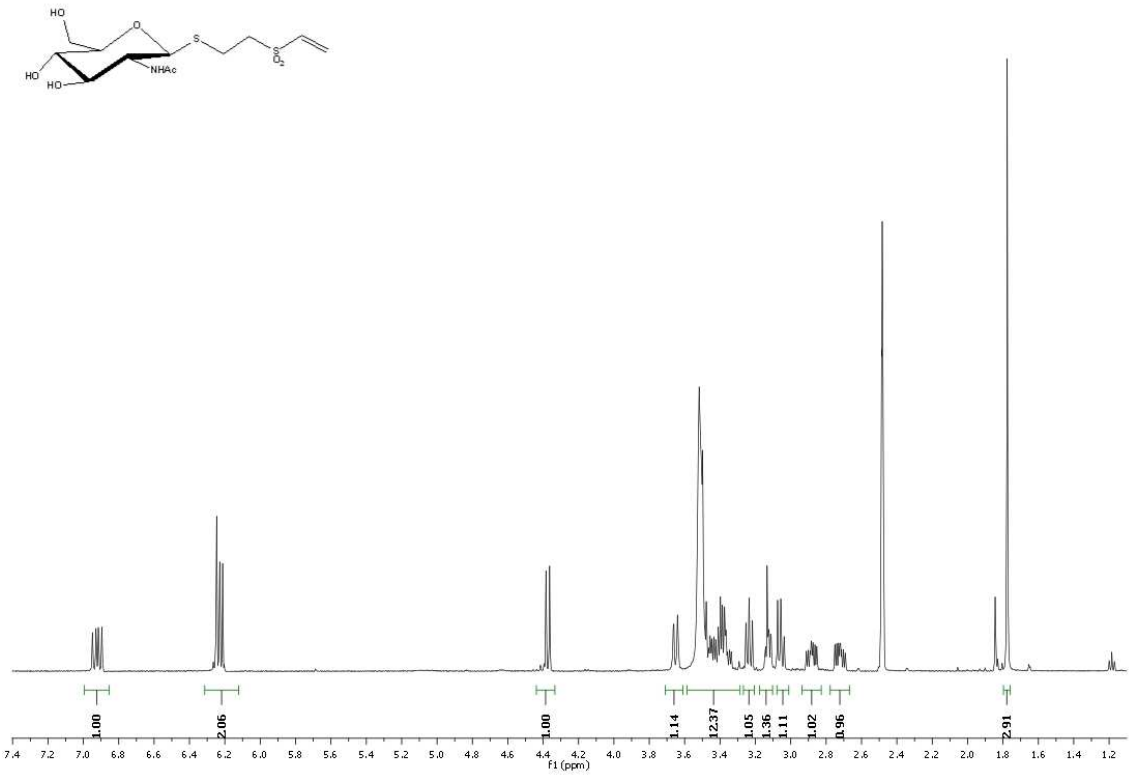
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HO
HO
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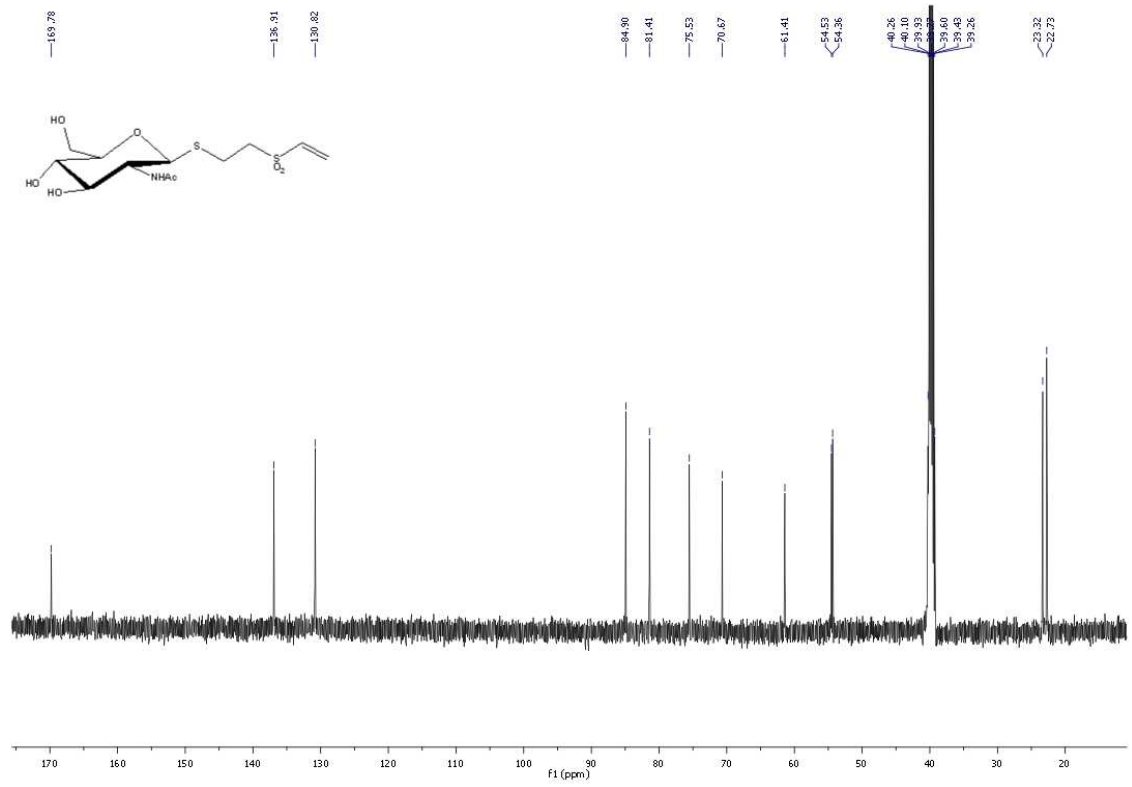
F1 (ppm)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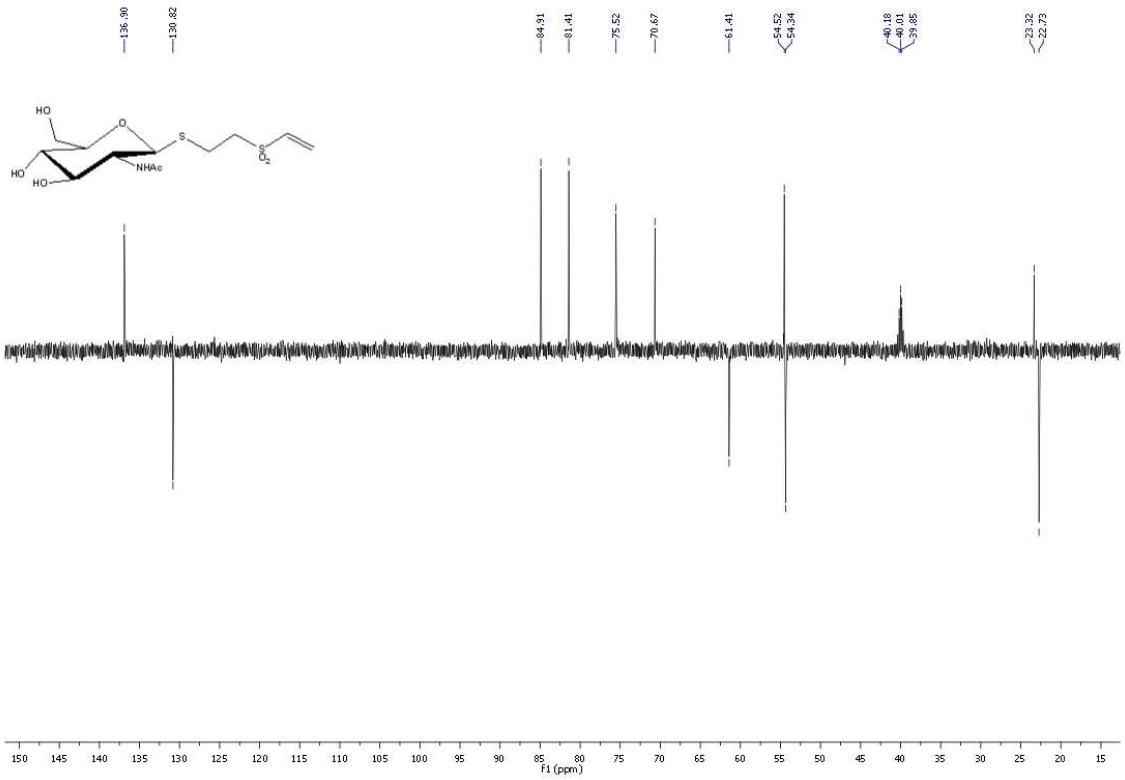
F1 (ppm)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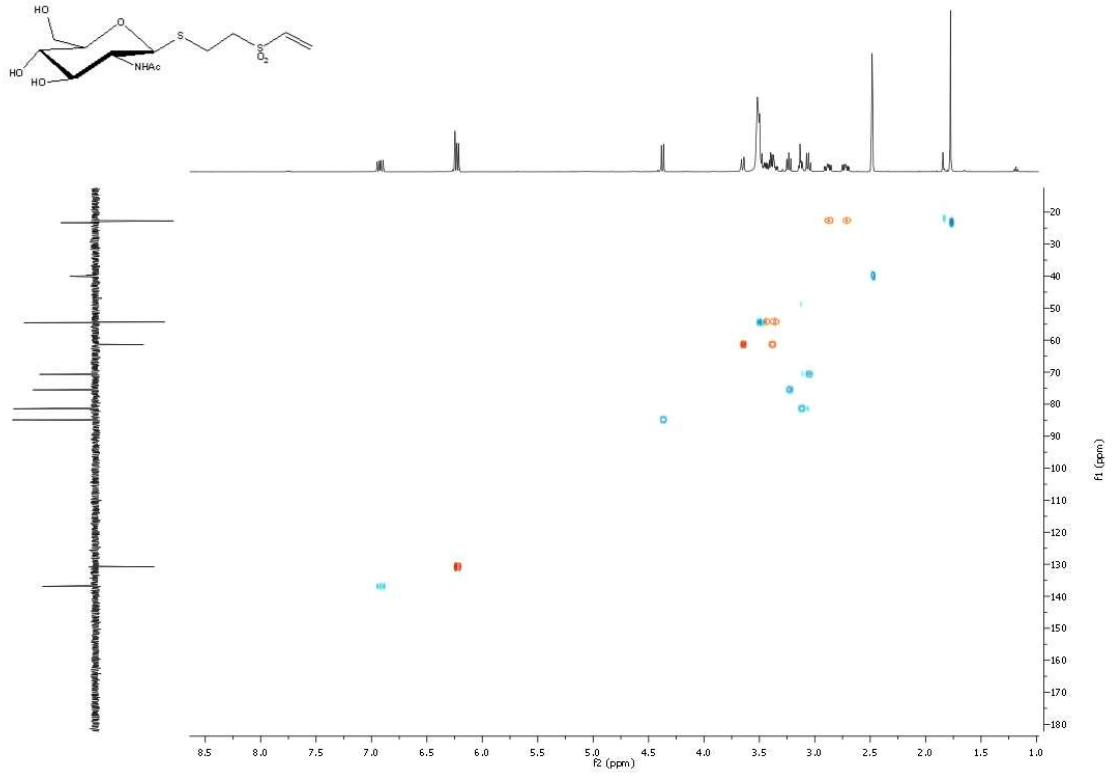
f1 (ppm)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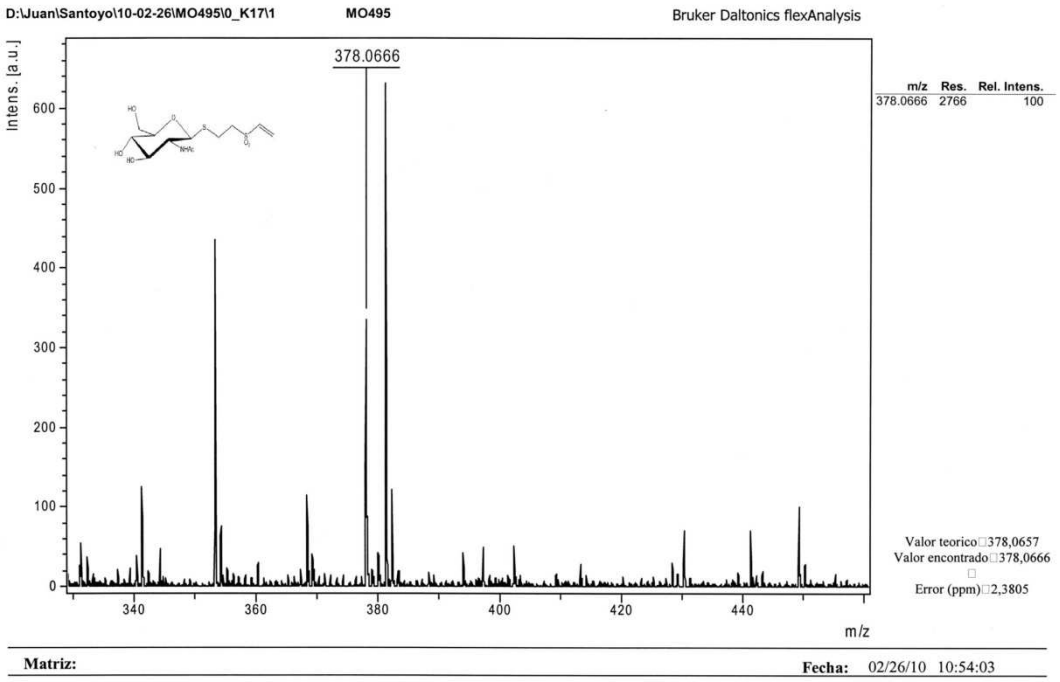
f1 (ppm)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MO495
Bruker Daltonics flexAnalysis
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