SUPPORTING INFORMATION

Fast and solvent free polymerization of carbohydrates induced by non-thermal atmospheric plasma

Joakim Delaux,[a,b] Michaël Nigen,[b] Elodie Fourré,[a] Jean-Michel Tatibouët,[a] Abdellatif Barakat,[b] Loyda Atencio,[c] José M. García Fernández,[c] Karine De Oliveira Vigier[a] and François Jérôme*[a]

[a] Institut de Chimie des Milieux et Matériaux de Poitiers, CNRS/Université de Poitiers, ENSIP, 1 rue Marcel Doré, Bat 1, TSA 41105, 86073 Poitiers Cedex 9, France. E-mail: francois.jerome@univ-poitiers.fr

[b] INRA, UMR 1208 Ingénierie des Agropolymères et Technologies Emergentes (IATE), 2 Pierre Viala, Montpellier F-34060 France

[c] Instituto de Investigaciones Químicas (IIQ), CSIC - University of Sevilla, Avda. Americo Vespucio 49, E-41092 Sevilla, Spain

Chemicals: all mono- and disaccharides were purchased to Sigma-Aldrich and used as received.

FT/IR: An FTIR Spectrometer Frontier Perkin Elmer equipped with a Perkin ATR Sampling Accessory was used to record the spectra of polysaccharides (window: 900-4000 cm^{-1}).

RAMAN: Analyzes were performed at room temperature on a confocal microspectrometer HORIBA JOBIN YVON LabRAM HR800UV equipped with a CCD detector (1152 x 298 pixels) cooled by Peltier effect and a confocal microscope Olympus BXFM. The excitation wavelength is 514.5 nm and was produced by an extern A+ laser (Melles Griot). According to the sample, the

laser power was adjusted between 1 and 4 mW. Spectra were recorded between 50 and 4000 cm^{-1} with a resolution of 0.5cm^{-1}. The software LabSPEC V.5 was used for acquisition and treatment of data.

XPS: The XPS analyses were carried out with a Kratos Axis Ultra DLD spectrometer using a monochromatic Al Kα source (8mA, 15kV). The charge Neutraliser system was operated for all analyses to compensate the build-up of positive charge due to insulating samples. Instrument base pressure was 9 x 10-8 Pascal. High-resolution spectra were recorded using an analysis area of 300µm x 700 µm and a pass energy of 20 eV was used. These pass energies correspond to Ag 3d5/2 FWHM of 0.55 eV. Data were acquired with 0.1 eV steps. All the binding energies were calibrated with the C1s binding energy fixed at 284.6 eV as an internal reference. C1s and O 1s were recorded for the quantitative analysis. Spectra were fitted with CasaXPS software (version 2.3.17) and Gaussian-Lorentzian profiles.

^{13}C CP/MAS NMR: NMR investigations were performed on the spectrometer Bruker Avance III 400 WB equipped with a probe CPMAS 4mm H/X. Spectra were recorded at two rotations speed (5000 Hz and 9000 Hz) using glycin as an external reference (δ_{CO} = 176.03 ppm) to calibrate the chemical shift. Because spinning side bands may mask some peaks of interest, scans were collected at two different spinning rates (5 and 9 kHz). Spinning side bands are marked by a star on Fig. S6.

$^{1}H/^{13}C$ liquid NMR: Analyzes were performed on a spectrometer Bruker Avance 300 DPX at 300 MHz for the ^{1}H and 75 MHz for the ^{13}C. Liquid NMR was performed in D_2O.

MALDI/TOF (Matrix Assisted Laser Desorption Ionization/Time of Flight) analyses were recorded on Bruker AutoFlexIII using 2,5-dihydroxybenzoic acid (DHB) as matrix. Typically, 8 μL of a DHB solution (10mg/mL) was mixed with 2 μL of a carbohydrate solution (10 mg/mL). Then, 0.5 μL was deposed on a MTP 384 target plate ground. The MALDI/TOF was calibrated using tryptic digest of bovine serum albumin as external standard. MALDI/TOF spectra were recorded at a m/z > 500 Da to avoid adduct with the matrix.

SEC: A Shimadzu SEC consisting of a degasser (DGU-20A3), a pump (LC-20AD), a thermostated autosampler (SIL-20AC), an oven at 40 ° C (CTO-20AC) and a refractive index detector (RID-10A) was used. Water was used as eluent at a flow rate of 1 mL/min. A column Shodex Sugar KX-802 was used (at 40°C) for separation. Typically, samples were dissolved in water at a concentration of 10 mg/mL prior to be analyzing by SEC using external calibration.

General procedure for the treatment of mannose by NTAP

Typically, 0.2 g of saccharide was charged into the DBD reactor. The air flow was adjusted to 100 mL/min prior to starting the NTAP treatment (bipolar pulse signal was used at a maximum voltage of 9.5 kV at 2.2 kHz frequency (*i.e.* 15 W)). The reaction progress was monitored by size exclusion chromatography by taking samples from the DBD reactor at the desired time. The temperature of the reactor was measured on the external surface of the upper electrode using an

optical infrared probe. Note that this temperature does not represent the real temperature inside the DBD reactor which is technically more complex to determine. After the NTAP treatment, polysaccharides were recovered from the DBD reactor as a white powder with a reaction yield of 93%.

Identification and quantification of mannodisaccharides in polymannosides by GC-FID.

GC-FID was carried out using an Agilent 6890 Series Plus chromatograph with an EPC injector fitted with a cross-linked 5% phenyl-dimethylsiloxane column (HP-5; 30 m x 320 μm x 0.25 μm). Operating conditions were: injection port temperature 310 ºC; splitting ratio 25:1; injection volume 1 μL of derivatized samples; column oven temperature programmed from 180 to 310 ºC at 5 ºC min^{-1}, with a 5 min hold at 310 ºC; carrier gas helium (constant flow at 1.2 mL min^{-1}); detector port temperature 325 ºC. Total acquisition time was 31 min.

The identity of mannodisaccharides in the samples was confirmed by comparison of the GC chromatograms with that of authentic samples. Response factors (RFs) for 2-*O*-(α-D-mannopyranosyl)-D-mannose (0.41), 3-*O*-α-D-mannopyranosyl)-D-mannose (0.51), 4-*O*-(α-D-mannopyranosyl)-D-mannose (0.48), 6-*O*-(α-D-mannopyranosyl)-D-mannose (0.51), 2-*O*-(β-D-mannopyranosyl)-D-mannose (0.48), 3-*O*-(β-D-mannopyranosyl)-D-mannose (0.48), 4-*O*-(β-D-mannopyranosyl)-D-mannose (0.47), 6-*O*-(β-D-mannopyranosyl)-D-mannose (0.48), at concentrations similar to those encountered in the experiments, were determined relative to the internal standard phenyl β-D-glucopyranoside.

For GC-FID analysis of the mannodisaccharide fraction in polymannosides, the sugar components were first transformed into the corresponding aldononitrile peracetates. To 100 μL of a 16 mg mL^{-1} solution of the polymannoside sample in water, 100 μL of a solution of phenyl β-D-glucopyranoside (internal standard) in acetone-water agua (1:9 v/v, 4 mg mL^{-1}) was added and lyophilized. Derivatization was next conducted according to the procedure of Zhang and Amelung.[1] The derivation reagent (0.3 mL), which contained 32 $mg.mL^{-1}$ hydroxylamine hydrochloride and 40 $mg.mL^{-1}$ of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) in pyridine–methanol (4:1, v/v), was added to a vial containing the dry sample and the internal standard. The capped vial was shaken and heated for 50 min at 60 ºC. Then, the vial was cooled to room temperature and 1 mL of Ac_2O was added. The vial was closed, shaken again, kept at room temperature for 40 min and concentrated under vacuum at 40 ºC. The residue was dissolved in 0.1 mL CH_2Cl_2 and centrifuged (8.5 rpm, 2.5 min) before injection in the GC apparatus.

Determination of the branching pattern of polymannosides by GC-MS analysis of the methylation-hydrolysis-reduction-acetylation products.

GC-FID was carried out using a Shimadzu GC-2010 chromatograph fitted with a cross-linked 5% phenyl-dimethylsiloxane column (ZB-5MS; 10 m × 0,18 mm × 0,18 mm) and connected to a Shimadzu GCMS-QP2010 Plus mass spectrometer. The ionization potential was 70 eV, and spectra were recorded in low-resolution mode. Operating conditions were: injection port temperature 275 ºC; splitting ratio 5:1; injection volume 1 μL of derivatized samples; the temperature programme was set as follows: the initial column temperature of 120 ºC was held for 1 min and then the temperature was increased at 20 ºC min^{-1} to 250 ºC, with a 2.67 min hold at

250 ºC; carrier gas helium (constant flow at 0.7 mL min^{-1}). Total acquisition time was 30 min. Sugar identification was based on their retention times and mass spectra compared to those of authentic standards[2]

For derivatization, vacuum-desiccated samples of polymannosides were methylated by using the method of Ciucanu and Costello.[3] The product arising from NTAP treatment of mannose (1 mg) was dissolved in 10 μL of water and 0.5 mL of DMSO was added under stirring. Methyl iodide (50 μL) and finely powdered sodium hydroxide (5 mg) were added to the solution and stirred vigorously for 1 min to get a suspension. After this, a larger amount of sodium hydroxide (15 mg) was added to the contents of the vial and the mixture was stirred at room temperature for 10 min. The samples were partitioned with 2 mL of water and 2 mL of CH_2Cl_2 and the organic phase was washed with 3-4 mL of water and dried under a stream of nitrogen. Further hydrolysis, reduction with NaBD4 and acetylation were performed according to Kim et al.[4] Briefly, the permethylated polysaccharide was hydrolyzed into the monosaccharide constituents by treatment with 2 M TFA (250 μl) at 121 ºC for 2 h. TFA was then removed under vacuum by co-evaporation with drops of methanol. The dry residue was dissolved in aqueous $NaBD_4$ (10 mg mL^{-1}, 100 μL) and the solution was magnetically stirred ta room temperature for 1 h. The reaction was then stopped by addition of a drop of glacial AcOH and dried by coevaporation wilt methanol (3 × 100 μL). The mixture of partially methylated alditols thus obtained was acetylated by reaction with Ac_2O (100 μL) and TFA (80 μL) at 50 ºC for 10 min, then dried by co-evaporation with acetone, partitioned between CH_2Cl_2 (2 mL) and water (4 × 2 mL), the organic phase was dried (Na_2SO_4) and concentrated to dryness. Finally, the residue was re-dissolved in CH_2Cl_2 (50 μL) before injection in the GC-MS.

Multi-detector high performance size exclusion chromatography (HPSEC)

HPSEC experiments were performed using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) composed of one Shodex OHpak SB-G pre-column followed by four Shodex OHpak SB columns in series (OHpak SB 806 HQ, OHpak SB 805 HQ, OHpak SB 804 HQ and OHpak SB 803 HQ). 40 µl of sample at 17.5 $mg.ml^{-1}$ in MilliQ water were injected. The macromolecules were eluted with 0.1 $mol.L^{-1}$ of a solution of $LiNO_3$ containing 0.02% NaN_3 at a constant flow rate of 1 $ml.min^{-1}$ and 30 °C.

The HPSEC system was fitted in series to a UV-Visible detector measuring the absorbance at 280 nm (Shimadzu, Kyoto, Japan), a Dawn Heleos II multi-angle laser light scattering (Wyatt Technology Corp., Santa Barbara, Ca, USA), a VISCOSTAR II viscosimeter (Wyatt Technology Corp., Santa Barbara, Ca, USA) and an Optilab T-rEX refractometer (Wyatt Technology Corp., Santa Barbara, Ca, USA). The weight-average molar mass (Mw), the polydispersity index (Mw/Mn), the intrinsic viscosity (η) and the hydrodynamic radius (Rh) were calculated using the ASTRA software 6.1.1.17 (Wyatt Technologies, Santa Barbara, CA). The data were analyzed using a refractive index increment (dn/dc) of 0.145 $mL \cdot g^{-1}$.

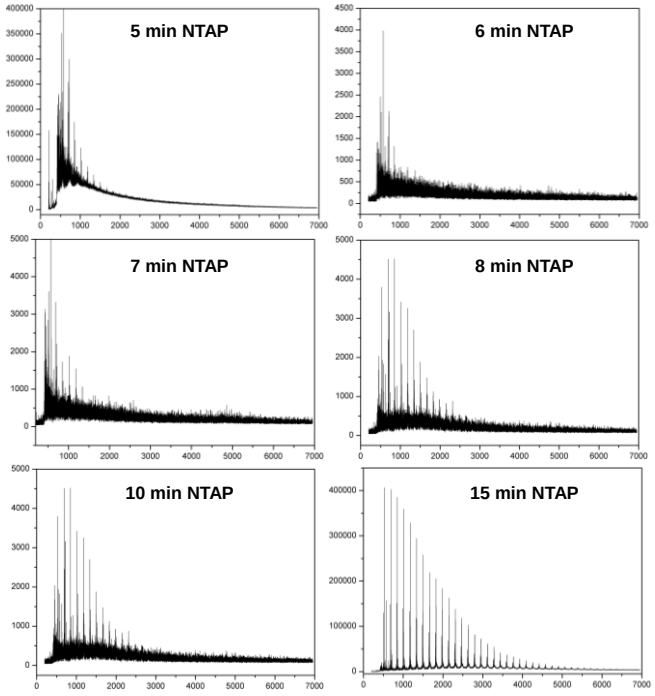

Fig. S1. Polymerization of mannose monitored by MALDI-TOF

Note: The polymerization reaction was clearly observable by MALDI-TOF. However information on the mean molecular weight of polysaccharides with this technic is difficult due to the signal intensity discrimination against high mass (poly)oligomers. For this reason, produced polymannosides were also analyzed in this work by size exclusion chromatography coupled to multi-angle light scattering (SEC-MALS) to get more insight on the molar mass distribution and the conformation of the macromolecules.

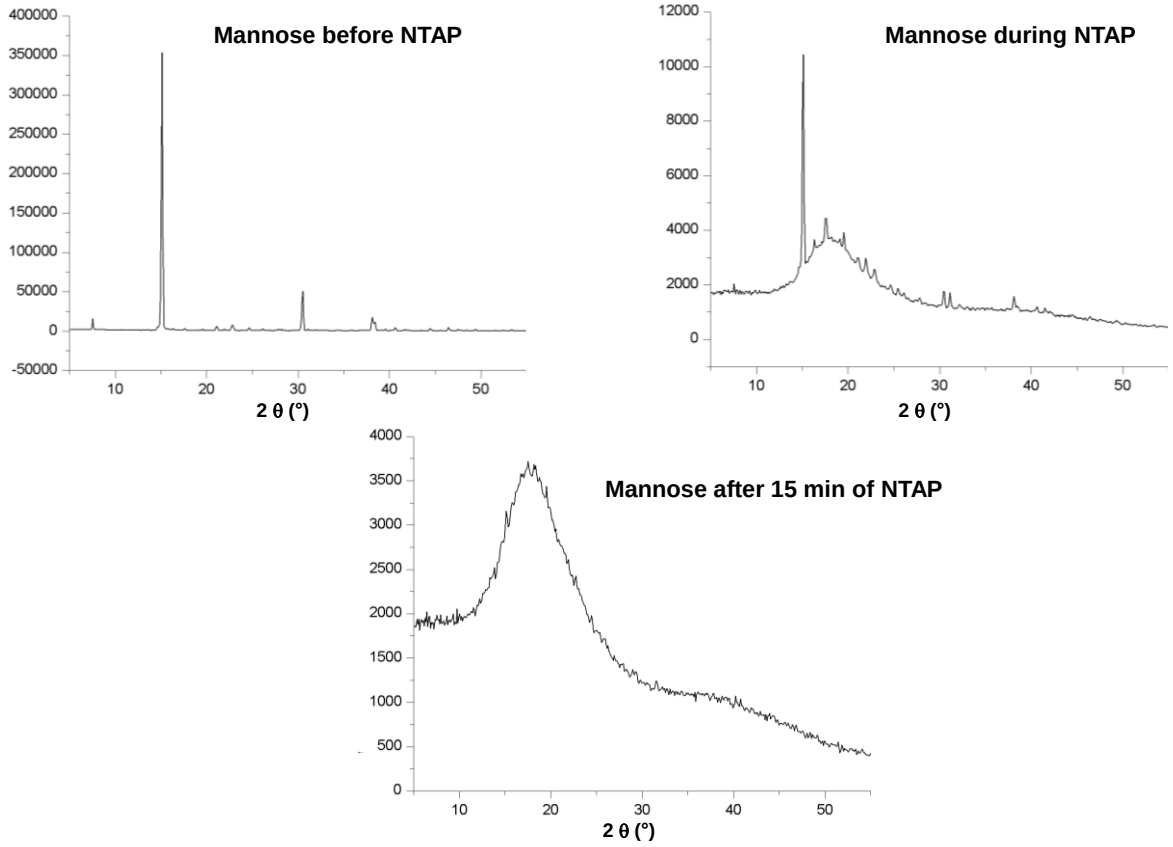

Fig. S2. XRD of mannose and polymannosides (after 15 min of NTAP treatment)

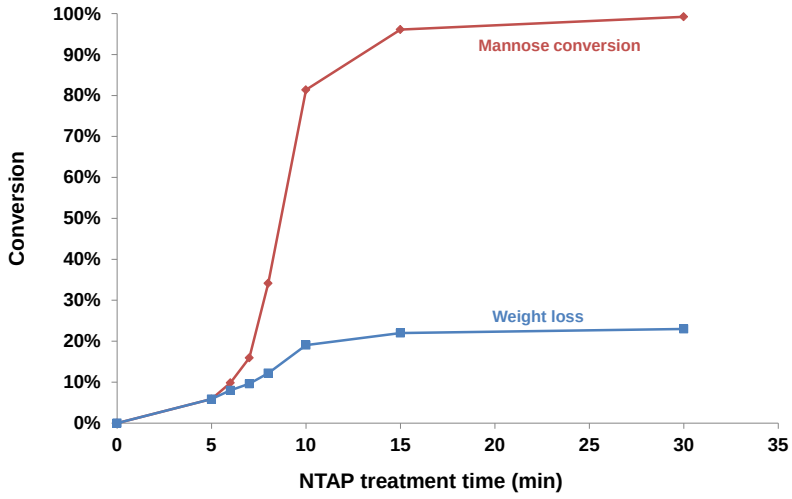

Fig. S3. Mass loss as a function of the NTAP treatment time

Note: It is noteworthy that after the complete polymerization of mannose (15 min), no weight loss was observed up to 30 min of NTAP supporting the stability of polymannosides under our working conditions. A white powder was observed after 15 min of NTAP which further reinforces the stability of polymannosides (consistent with analytical methods). However, with prolonged NTAP treatment time up to 30 min, one should mention that the color of the powder starts turning from white to yellow-wish suggesting in this case the slight production of side products.

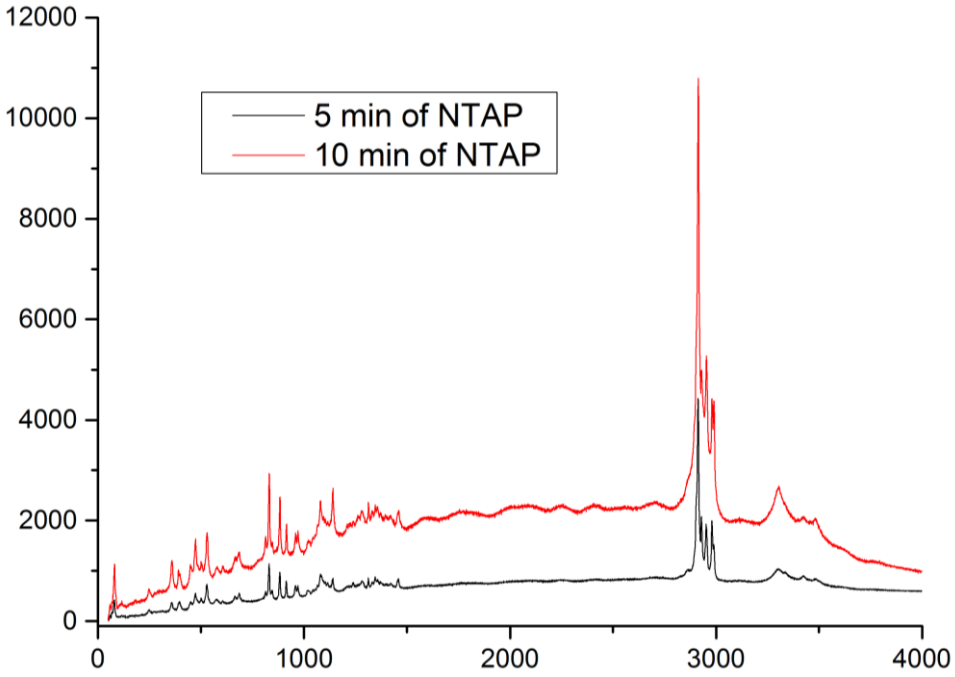

Fig. S4. RAMAN spectra of mannose and polymannosides (after 15 min of NTAP treatment)

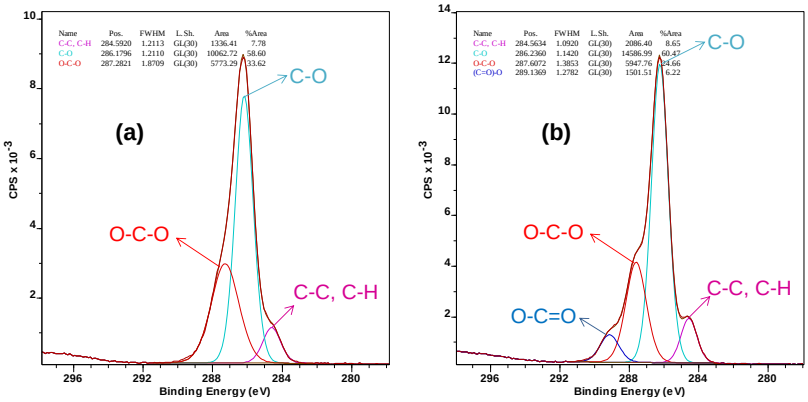

Fig. S5. XPS analysis of the polymannoside surface (a) mannose and (b) after 15 min of a NTAP treatment time

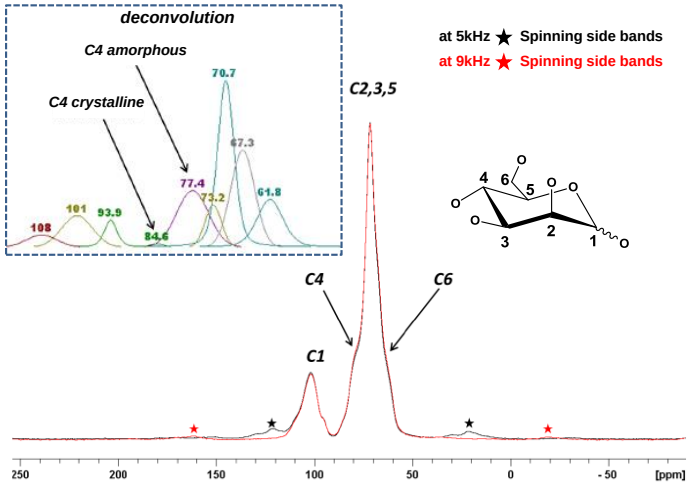

Fig. S6. ^{13}C CP/MAS NMR spectrum of polymannoside (the deconvolution of the ^{13}C CP/MAS NMR spectrum is provided in the rectangle).

Note: Because spinning side bands may mask some peaks of interest, scans were collected at two different spinning rates (5 and 9 kHz). Spinning side bands are marked by a star on the figure S6. The anomeric carbon (C1) is clearly distinguished from the C2, C3, C4, C5, and C6 atoms. Deconvolution of the spectrum indicated the presence of three environments for carbons in the 90-110 ppm zone which correspond to anomeric carbons of the polymannoside. These peaks represent 15.8% of the total surface indicating that only anomeric carbons are present in this area. The signal located at 93.9 ppm probably corresponds to reducing end of the mannosyl residue. The chemical shift and the fact that this peak was 2.5-fold thinner than the two other peaks is consistent with a higher mobility, characteristic of a reducing end. The two other environments located at 101 and 108 ppm must correspond to the C1 positions involved in polymer chain growing.

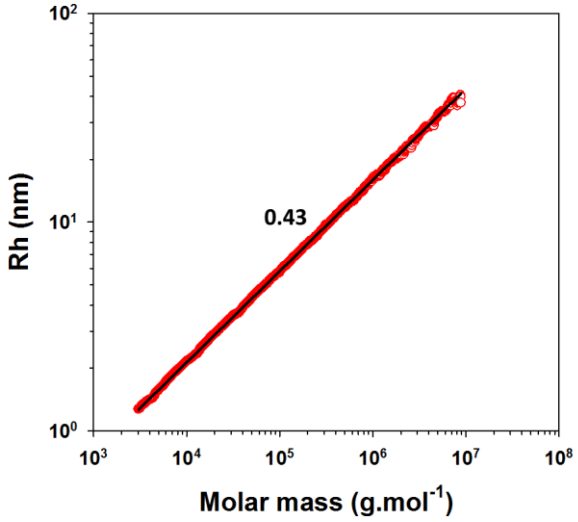

Fig. S7. Rh conformation plot for polymannoside sample.

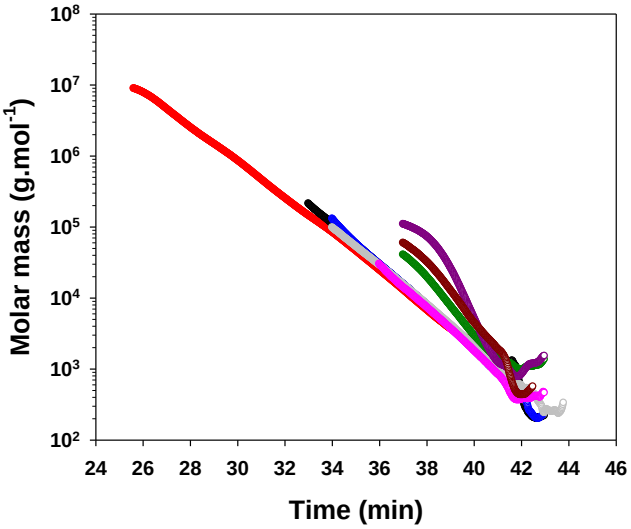

Fig. S8. Molar mass distribution of mannose (red), galactose (black), glucose (blue), xylose (grey), maltose (pink), maltulose (green), isomaltulose (mauve) and turanose (brown) samples submitted to NTAP treatment. Macromolecules were eluted using 0.1 M $LiNO_3$ containing 0.02% NaN_3.

References

(1) W. Zhang, H. He, X. Zhang, *Soil Biol. Biochem.*, **2007**, *39*, 2665-2669.

(2) G. Sassaki, P. Gorin, L. Souza, P. Czelusniak, M. Iacomini, M. *Carbohydr. Res.* **2005**, *340*, 731-739.

(3) I. Ciucanu, C. E. Costello, *J. Am. Chem. Soc.* **2003**, *125*, 16213-16219.

(4) J. S. Kim, B. L. Reuhs, F. Michon, R. E. Kaiser, R. G. Arumugham, *Carbohydr. Res.* **2006**, *341*, 1061-1064.