Supporting Information

Materials and Methods

Commercial chitosan (batch 244/020208; DA = 0%; Mw = 270 kg/mol; Mn = 115 kg/mol; D = 2.3) was furnished by Mahtani Chitosan Ltd (Veraval, India). Sodium nitrite (NaNO₂, 99%), deuterium oxide (D₂O, 99.96% atom D), sodium chlorite (NaClO₂, 80%) were provided by Sigma-Aldrich (Saint-Quentin Fallavier, France).

NMR spectroscopy: ¹H and ¹³C-NMR spectra were recorded on Bruker DRX300 and DRX500, respectively, using trimethylsilyl-3-propionic-2,2,3,3-D₄ acid sodium salt (99% atom D, TMSPA from Sigma-Aldrich, Saint-Quentin Fallavier, France) as the internal standard. All samples were dissolved at 10 mg/mL in D₂O with 5 µL HCl 12 N, and transferred to 5 mm NMR tubes. Chemical shifts are reported in ppm (δ units) downfield from TMSPA, coupling constants in Hz, and for signal multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet.

MALDI-TOF mass spectrometry: MALDI-TOF mass spectra were acquired with a Voyager-DE STR (AB Sciex, Framingham, MA, USA) equipped with a nitrogen laser emitting at 337 nm with a 3 ns pulse. The instrument was operated in the linear or reflectron mode. Ions were accelerated to a final potential of 20 kV. The positive ions were detected in all cases. Mass spectra were the sum of 300 shots and an external mass calibration of mass analyzer was used (mixture of peptides from SequazymeTM standards kit, AB Sciex). The matrix used for all experiments was 2,5-dihydroxybenzoic acid (DHB) purchased from Sigma-Aldrich and used directly without further purification. The solid matrix and samples were dissolved at 10 mg/mL and 1 mg/mL in water, respectively. A volume of 20 µL matrix solution was then mixed with 20 µL of sample solutions. An aliquot of 0.5 µL of each resulting solution was spotted onto the MALDI sample plate and air-dried at room temperature.

High Resolution ESI Mass Spectrometry: HRMS (ESI) was recorded in a positive ion mode on a hybrid quadrupole time-of-flight mass spectrometer (MicroTOFQ-II, Bruker Daltonics, Bremen, Germany) with an electrospray ionization (ESI) ion source. The gas flow of spray gas is 0.6 bar and the capillary voltage is +4.5 kV. The solution was infused at 180 µL/h. The mass range of the analysis is 50–2,000 m/z and the calibration was carried out with sodium formate. The solvent for HRMS is dichloromethane/MeOH/water/formic acid.

Size-exclusion chromatography (SEC): SEC was performed on a chromatographic equipment composed of a 1260 Infinity Agilent Technologies pump connected to two TSK gel G2500 and G6000 columns (Tosoh Bioscience) in series. A multi-angle laser light scattering (MALLS) detector Dawn EOS (Wyatt Technology) operating at 690 nm was coupled on line to a Wyatt Optilab T-Rex differential refractometer. Sample solutions at 2-5 mg/mL were prepared and eluted in a AcOH (0.2 M)/AcONH₄ (0.15 M) buffer (pH = 4.5). Solutions were previously filtered through 0.22 µm pore size membranes (Millipore) before injection. The eluent flow rate was 0.5 mL/min. The refractive index increment dn/dc used for molar mass calculations was equal to 0.198 cm³·g⁻¹.
Characterization of COSamf 1: $^1$H-NMR (300 MHz, D$_2$O, 300 °K): $\delta$ (ppm) 5.10 (d, $J = 5.3$ Hz, 1H, H-1 amf), 4.90–4.70 (m, 8H, H-1 GlcN), 4.42 (t, $J = 4.8$ Hz, 1H, H-3 amf), 4.18 (t, $J = 4.9$ Hz, 1H, H-4 amf), 4.10 (m, 1H, H-5 amf), 4.05–4.00 (m, 43H, H-2 and H-6 amf, H-3 to H-6 GlcN), 3.10-2.80 (m, 8H, H-2 GlcN). $^{13}$C-NMR (125 MHz, D$_2$O, 300 °K): $\delta$ (ppm) 99.5 (C-1' GlcN), 98.9 (C-1 GlcN), 89.8 (C-1 amf), 86.5 (C-4 amf), 85.6 (C-2 amf), 82.6 (C-5 amf), 77.2 (C-3 amf), 76.9 (C-4 GlcN), 76.9 (C-5' GlcN), 75.4 (C-5 GlcN), 72.8 (C-3' GlcN), 71.4 (C-3 GlcN), 70.2 (C-4' GlcN), 61.4 (C-6 amf), 61.0 (C-6' GlcN), 60.6 (C-6 GlcN), 56.5 (C-2 GlcN), 56.2 (C-2' GlcN). Note that C' represents carbon atoms of the GlcN unit linked to the amf unit. MALDI-TOF MS (positive reflectron mode): presence of a major peak in at $m/z$ 990.5 attributed to HO-(GlcN)$_5$-amf ($m/z$ monoisotopic calcd for [C$_{36}$H$_{65}$O$_{25}$N$_5$Na]$^+$ = 990.4 mass units ($\Delta = 0.01\%$)).

**Figure S1.** $^1$H-NMR spectrum (D$_2$O, 300 MHz) of commercial fully N-deacetylated chitosan (from Mahtani Chitosan).
Figure S2. Size-exclusion chromatogram of commercial fully N-deacetylated chitosan (from Mahtani Chitosan).

**Configurations**

Notes:
- Columns: TSK6000 et TSK2500, Solvant filtré sur CNE 0,1 et échantillon filtré sur CNE 0,45
- Concentration Source: SI
- Flow Rate: 0.500 mL/min

**Light Scattering Instrument**
- Cell Type: X5
- Wavelength: 690.0 nm
- Calibration Constant: 5.6000 x 10^-6 1/(V cm)
- RI Instrument: Optilab SDL
- Solvent: Tampons acide/AcONH pH 4.5
- Refractive Index: 1.330

**Processing**


**Peak settings:**
- Peak Name: Peak 1
- Light Scattering Modul: Time
- Fit Degree: 1
- dI/dC (mL/mg): 0.1990

**Results Fitting Procedure:**
- Data Fit Model Degree $R^2$ Extrapolation

**Results**

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<th>Peak Results</th>
<th>Peak 1</th>
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<tbody>
<tr>
<td>Injected Mass (mg)</td>
<td>101.00</td>
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<tr>
<td>Calculated Mass (mg)</td>
<td>77.56</td>
</tr>
<tr>
<td>Molar mass moments (g/mol)</td>
<td></td>
</tr>
<tr>
<td>$M_n$</td>
<td>$1.146 x 10^6$ (±1.632%)</td>
</tr>
<tr>
<td>$M_p$</td>
<td>$1.528 x 10^6$ (±7.70%)</td>
</tr>
<tr>
<td>$M_w$</td>
<td>0/0</td>
</tr>
<tr>
<td>$M_w$</td>
<td>$2.702 x 10^6$ (±0.713%)</td>
</tr>
<tr>
<td>$M_z$</td>
<td>$6.120 x 10^6$ (±3.706%)</td>
</tr>
<tr>
<td>Polydispersity</td>
<td></td>
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<tr>
<td>$M_w/M_n$</td>
<td>2.357 (±1.781%)</td>
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<tr>
<td>$M_z/M_n$</td>
<td>5.938 (±3.404%)</td>
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<tr>
<td>RMS radius moments (nm)</td>
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<tr>
<td>$R_h$</td>
<td>42.6 (±2.5%)</td>
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<tr>
<td>$R_w$</td>
<td>59.6 (±1.6%)</td>
</tr>
<tr>
<td>$R_z$</td>
<td>86.9 (±0.7%)</td>
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</tbody>
</table>
Figure S3. $^1$H-NMR spectrum (D$_2$O, 300 MHz) of COSamf 1 (DP ~10).
Figure S4. $^{13}$C-NMR spectrum ($D_2O$, 125 MHz) of COSamf 1 (DP ~10).

Figure S5. MALDI-TOF mass spectrum of COSamf 1 (DP ~10).

Note that for each oligomer peak, the number of GlcN unit into the chain is given in green.
Figure S6. Size-exclusion chromatogram of COSamf 1 (DP ~10).

Configuration

Notes:
Columns: TSK4000 et TSK4000, Solvant filtré sur CHE 0,1 et échangeur filtré sur CHE 0,45
Concentration Source: SI
Flow Rate: 0.500 mL/min
Light Scattering Instrument: DAWN EOS
Cell Type: ES
Wavelength: 690.0 nm
Calibration Constant: 7.4000x10^-7 l/ (V cm)
RI Instrument: Optilab rEX
Solvent: Tetrahydrofuran (THF) pH 4.5
Reflective Index: 1.330

Processing

Collection Time: Thursday January 24, 2013 09:26:40 PM Paris, Madrid (heure d’été)
Peak settings:

Results Fitting Procedure:
Data Fit Model Degree $R^2$ Extrapolation

Results

Peak Results

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<td>316.58</td>
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Molar mass moments (g/mol)

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<tr>
<th>Peak</th>
<th>Mn</th>
<th>1.773x10^5 (28.496%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mw</td>
<td>1.486x10^5 (22.695%)</td>
</tr>
<tr>
<td></td>
<td>Mv</td>
<td>8.793x10^3 (16.101%)</td>
</tr>
<tr>
<td></td>
<td>Mz</td>
<td>2.672x10^3 (57.879%)</td>
</tr>
</tbody>
</table>

Polydispersity

<table>
<thead>
<tr>
<th></th>
<th>Mw/Mn</th>
<th>1.095 (±0.400%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mz/Mn</td>
<td>1.139 (±0.223%)</td>
</tr>
</tbody>
</table>

rms radius moments (nm)

<table>
<thead>
<tr>
<th></th>
<th>Rn</th>
<th>27.3 (±0.4%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rw</td>
<td>29.2 (±0.5%)</td>
</tr>
<tr>
<td></td>
<td>Rz</td>
<td>30.5 (±0.2%)</td>
</tr>
</tbody>
</table>
Figure S7. $^1$H-NMR spectrum (D$_2$O, 300 MHz) of chitooligosaccharide-2,5-anhydro-D-mannonic acid 2 (acidic form, DP ~10).
**Figure S8.** $^{13}$C-NMR spectrum (D$_2$O, 125 MHz) of chitooligosaccharide-2,5-anhydro-d-mannonic acid 2 (acidic form, DP ~10).
Figure S9. MALDI-TOF mass spectrum of chitooligosaccharide-2,5-anhydro-D-mannonic acid 2 (DP ~10).

Positive linear mode:

* corresponds to matrix peaks
Note that for each oligomer peak, the number of GlcN units into the chain is given in green

Positive reflectron mode:

* corresponds to matrix peaks
Note that for each oligomer peak, the number of GlcN units into the chain is given in green
Figure S10. Size-exclusion chromatogram of chitooligosaccharide-2,5-anhydro-D-mannonic acid 2 (DP ~10).

File Name: fit5115.1fma10133.mce6
Collection Operator: LNF9-20000Aqua (LNF9-20000Aqua)
Processing Operator: UNIV-17001stephe.stephe (UNIV-17001stephe.stephe)
Sample: ZHS
Concentration: 2.000 mg/mL
Injected Volume: 200.0 μL

Configuration

Notes:
Colonnes : TSK6000 et TSK5500, Solvant filtré sur CHE 0,1 et échantillon filtré sur CHE 0,45
Concentration Source: RI
Flow Rate: 0.500 mL/min
Light Scattering Instrument: DAWN EOS
Cell Type: XS
Wavelength: 690.0 nm
Calibration Constant: 7.4600×10⁻⁶ l/(g cm)
RI Instrument Optilab TDX
Solvent: Tous les Acs/ACN pH 4.5
Refractive Index: 1.330

Processing


Peak settings:
Peak Name: Peak 1
Light Scattering Model: 1
Fit Degree: 1
dn/dc (mg/mL): 0.2980
A2 (mol mL/g): 0.000

Results

Peak Results

Masses
Calculated Mass (μg): 145.52
Molar mass moments (g/mol)
Mn: 1.805×10⁷ (±7.53%)  
Mw: 1.767×10⁷ (±4.39%)  
Mv: n/a  
Mw/Mn: 1.042×10⁷ (±7.62%)  
Mz: 2.424×10⁷ (±21.225%)  
Polydispersity
Mw/Mn: 1.076 (±30.567%)  
Mw/Mv: 1.043 (±22.62%)  
RMS radius moments (nm)
Rg: 20.3 (±50.1%)  
Rg: 16.4 (±120.8%)  
Rg: 10.6 (±30.4%)
Figure S11. $^1$H-NMR spectrum (D$_2$O, 300 MHz) of chitooligosaccharide-2,5-anhydro-D-mannonic acid (acidic form, DP ~20).
Figure S12. $^1$H-NMR spectrum (D$_2$O, 300 MHz) of chitooligosaccharide-2,5-anhydro-D-mannonic acid (basic form, DP ~20).
Figure S13. $^{13}$C-NMR spectrum (D$_2$O, 125 MHz) of chitooligosaccharide-2,5-anhydro-D-mannonic acid (basic form, DP ~20).
Figure S14. MALDI-TOF mass spectrum of chitooligosaccharide-2,5-anhydro-D-mannonic acid (DP ~20).

Positive linear mode:

Note that for each oligomer peak, the number of GlcN units into the chain is given in green.

Positive reflectron mode:

Note that for each oligomer peak, the number of GlcN units into the chain is given in green.
Figure S15. Size-exclusion chromatogram of chitooligosaccharide-2,5-anhydro-D-mannonic acid (DP ~20).

**Configuration**

**Notes:**
- Column: TSK8000 and TSK2500, Solvent filtré sur CME 0,1 et échantillon filtré sur CME 0,46
- Concentration Source: FI
- Flow Rate: 0.400 mL/min
- Light Scattering Instrument: DAWN EOS
  - Cell Type: ES
  - Wavelength: 690.0 nm
  - Calibration Constant: 7.4800×10^{-5} l/(V cm)
- RI Instrument: Optilab rEX
- Solvent: Tris/acetate/NaCl pH 4.5
- Refractive Index: 1.330

**Processing**

**Collection Time:** Thursday May 16, 2013 09:11:30 PM Paris, Madrid (heure d'été)
**Processing time:** Friday May 17, 2013 10:08:14 AM Paris, Madrid (heure d'été)

**Peak settings:**
- Peak Name: Peak 1
- Light Scattering Model: Zimm
- Fit Degree: 1
- dn/dc (mL/g): 0.1080
- A2 (mol mL/g): 0.000

**Results Fitting Procedure:**
- Data Fit Model Degree R² Extrapolation

**Peaks Results**

<table>
<thead>
<tr>
<th>Peak 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masses</td>
</tr>
<tr>
<td>Calculated Mass (g/mol): 175.02</td>
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<tr>
<td>Molar mass moments (g/mol)</td>
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<tr>
<td>Mn: 3.56×10^3 (±4.02%)</td>
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<tr>
<td>Mp: 3.05×10^3 (±1.65%)</td>
</tr>
<tr>
<td>Mv: n/a</td>
</tr>
<tr>
<td>Mw: 4.06×10^3 (±5.37%)</td>
</tr>
<tr>
<td>Zr: 4.87×10^2 (±7.31%)</td>
</tr>
<tr>
<td>Polydispersity</td>
</tr>
<tr>
<td>Mw/Mn: 1.123 (±5.24%)</td>
</tr>
<tr>
<td>Mz/Mn: 1.264 (±8.30%)</td>
</tr>
<tr>
<td>rms radius moments (nm)</td>
</tr>
<tr>
<td>Rg: 23.3 (±35.4%)</td>
</tr>
<tr>
<td>Rw: 21.7 (±36.7%)</td>
</tr>
<tr>
<td>Rz: 16.9 (±43.7%)</td>
</tr>
</tbody>
</table>
Figure S16. MALDI-TOF mass spectrum of chitooligosaccharide-2,5-anhydro-D-mannonic acid (acidic form, DP ~30).
Figure S17. $^{13}$C-NMR spectrum (D$_2$O, 125 MHz) of chitooligosaccharide-2,5-anhydro-D-mannonic acid (acidic form, DP ~30).
**Figure S18.** Size-exclusion chromatogram of chitooligosaccharide-2,5-anhydro-D-mannonic acid (DP ~30).

**Configuration**

Notes:
- Columns: TSK4000 and TSK2000, Solvent filtré sur CMK 0.1 et échantillon filtré sur CMK 0.45
- Concentration Source: DI
- Flow Rate: 0.500 mL/min
- Light Scattering Instrument: DAWN EOS
  - Cell Type: KS
  - Wavelength: 690 nm
  - Calibration Constant: 7.4800 × 10⁻¹⁰ l/(g cm)
- RI Instrument: Optilab dex
- Solvent: Tampon Acé/ACE pH 4.5
  - Refractive Index: 1.330

**Processing**

Collection Time: Friday May 17, 2013 22:01:30 AM Paris, Madrid (heure d’été)

**Peak settings:**
- Peak Name: Peak 1
- Light Scattering Model: Zimm
- Fitted Degree: 1
- de/dc (M/L/g): 0.1280
- AU (mol mL/g): 0.000

**Results Fitting Procedure:**
- Data Fit Model Degree R² Extrapolation

**Peak Results**

- Masses
  - Calculated Mass (µg): 184.88
  - Mn: 5.151 × 10⁵ ± 3.650%
  - Mp: 5.744 × 10⁵ ± 1.358%
  - Mr: n/a
  - Mw: 6.698 × 10⁵ ± 1.289%
  - Mz: 8.353 × 10⁵ ± 1.408%
- Polydispersity
  - Mw/Mn: 1.254 (±2.039%)
  - Mz/Mn: 1.622 (±5.723%)
- RMS radius moments (nm)
  - R1: n/a
  - Rw: n/a
  - Rz: 1.1 (±454.8%)