Supporting Information

Materials and Methods

Commercial chitosan (Batch 244/020208; DA = 0%; Mw = 270 kg/mol; Mn = 115 kg/mol; Đ = 2.3) was furnished by Mahtani Chitosan Ldt (Veraval, India). $NaNO_2$ (99%), D_2O (99.96% atom D), 4-(hexyloxy)aniline (99%) and $NaBH_3CN$ (99%) from Sigma Aldrich (Saint-Quentin Fallavier, France).

NMR spectroscopy: ^1H and ^{13}C-NMR spectra were recorded on Bruker DRX300 and DRX500, respectively, using trimethylsilyl-3-propionic-2,2,3,3-D_4 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 D_2O, and transferred to 5 mm NMR tubes. The signal of HOD (δ 4.80 ppm at 298 °K) was also used as internal reference. 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 dichlomethane/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 1–2 mg/mL were prepared and eluted in a AcOH (0.2 M)/$AcONH_4$ (0.15 M) buffer (pH = 4.5). Solutions were previously filtered through 0.22 µm pore size membranes (Millipore, Molsheim, France) 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 $cm^3\ g^{-1}$.

Synthesis of COSamf 1: A fully *N*-deacetylated chitosan (2.1 g, 13 mmol GlcN unit) was solubilized in 1 L of water by addition of 11.5 mL HCl (37% w/w). A freshly prepared solution of $NaNO_2$ (1.3 mmol) was added and the reaction was allowed to proceed for 24 h at room temperature. The product was precipitated by addition of conc. NH_4OH, centrifuged (15 min, 11,200 rpm), washed with distilled water until neutral pH, then freeze-dried leading to COSamf **1** (1.4 g, 67% mass yield) as a light yellow powder. ^{1}H-NMR (300 MHz, D_2O, 300 °K): δ (ppm) 5.10 (d, *J* = 5.4 Hz, 1H, H-1 amf), 4.90–4.70 (m, 23H, H-1 GlcN), 4.44 (t, *J* = 4.9 Hz, 1H, H-3 amf), 4.24 (t, *J* = 4.9 Hz, 1H, H-4 amf), 4.15 (m, 1H, H-5 amf), 4.05–3.45 (m, 118H, H-2 and H-6 amf, H-3 to H-6 GlcN), 3.18 (t, *J* = 9.1 Hz, 23H, H-2 GlcN). ^{13}C-NMR (125 MHz, D_2O, 300 °K): δ (ppm) 99.2 (C-1' GlcN), 98.6 (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), 77.0 (C-4 GlcN), 76.9 (C-5' GlcN), 75.3 (C-5 GlcN), 72.5 (C-3' GlcN), 71.0 (C-3 GlcN), 70.2 (C-4' GlcN), 61.4 (C-6 amf), 60.9 (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* 1473.9 attributed to HO-$(GlcN)_8$-amf (*m/z* monoisotopic calcd for $[C_{54}H_{98}O_{37}N_8Na]^+$ = 1473.6 mass units (Δ = 0.02%)).

Figure S1. ^{1}H-NMR spectrum (D_2O, 300 MHz) of the commercial fully *N*-deacetylated chitosan.

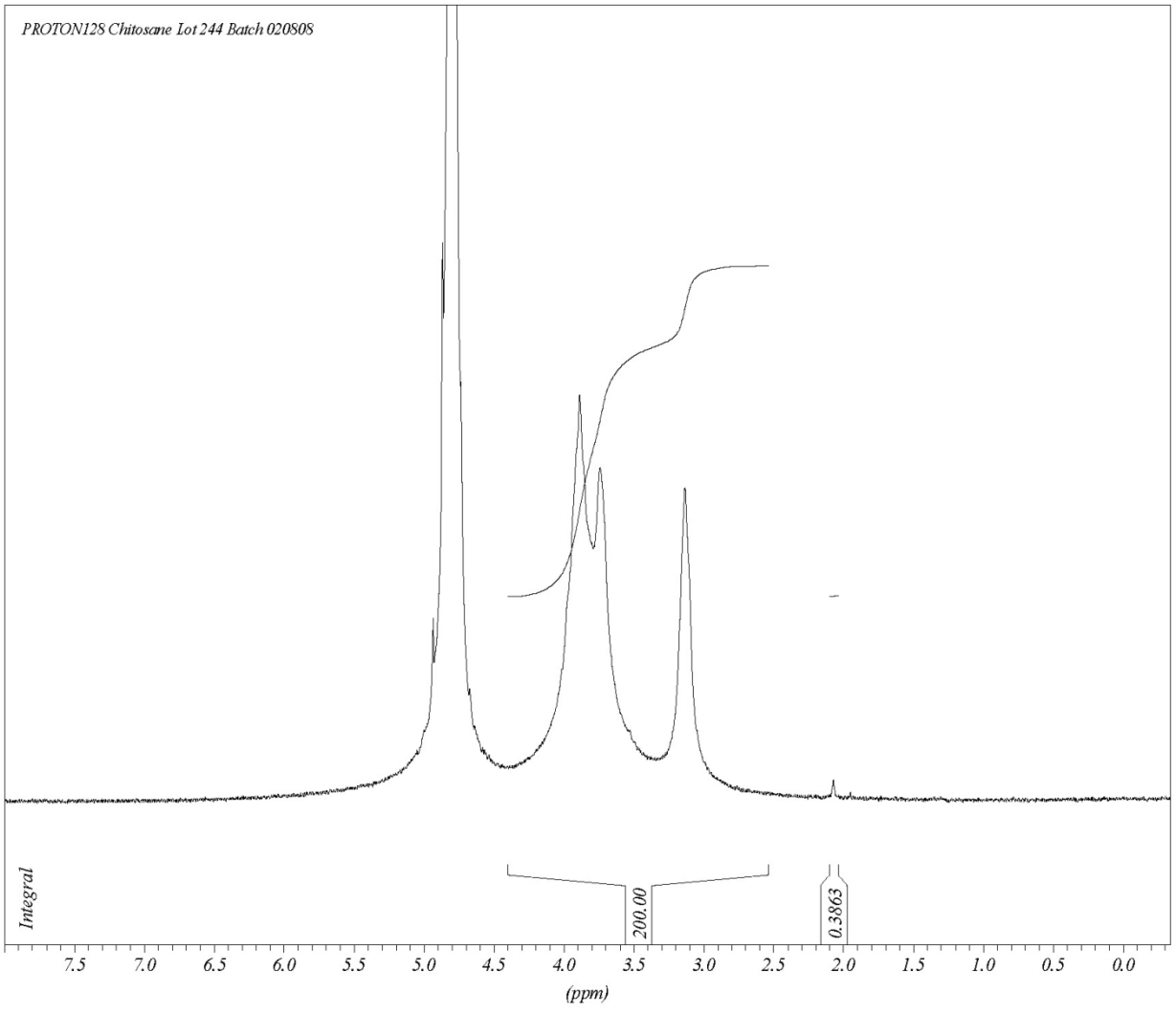
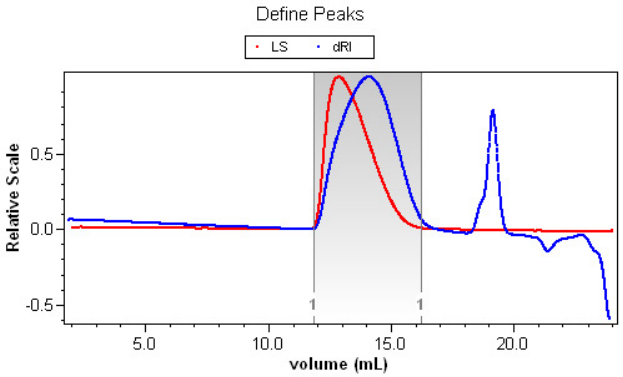


Figure S2. Size-exclusion chromatogram of the commercial fully *N*-deacetylated chitosan.

WYATT TECHNOLOGY

File Name: F:244-2[10oct2012].afe6
Collection Operator: LMPB-AR2000\Aqueux (LMPB-AR2000\Aqueux (Aqueux))
Processing Operator: UNIV-LYON1\stephane.trombotto (TROMBOTTO STEPHANE)

Sample: 244-2
Concentration: 1.010 mg/mL
Injected Volume: 100.0 µL



Configuration

Notes:
Colonnes : TSK6000 et TSK2500, Solvant filtré sur CME 0,1 et échantillon filtré sur CME 0,45

Concentration Source: RI
Flow Rate: 0.500 mL/min

Light Scattering Instrument: DAWN EOS
Cell Type: K5
Wavelength: 690.0 nm
Calibration Constant: 7.4800×10^{-6} 1/(V cm)

RI Instrument: Optilab rEX

Solvent: Tampon AcAc/AcNH pH 4.5
Refractive Index: 1.330

Processing

Collection Time: Thursday October 11, 2012 03:35:55 AM Paris, Madrid (heure d'été)
Processing time: Thursday October 11, 2012 11:05:24.828 AM Paris, Madrid (heure d'été)

Peak settings:

Peak Name	Peak 1
Light Scattering Model	Zimm
Fit Degree	1
dn/dc (mL/g)	0.1980
A2 (mol mL/g²)	0.000

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

Results

Peak Results

	Peak 1
Masses	
Injected Mass (µg)	101.00
Calculated Mass (µg)	77.56
Molar mass moments (g/mol)	
Mn	1.146×10^{5} (±1.632%)
Mp	1.528×10^{5} (±0.797%)
Mv	n/a
Mw	2.702×10^{5} (±0.713%)
Mz	6.120×10^{5} (±1.768%)
Polydispersity	
Mw/Mn	2.357 (±1.781%)
Mz/Mn	5.338 (±2.406%)
rms radius moments (nm)	
Rn	42.6 (±4.5%)
Rw	59.6 (±1.6%)
Rz	86.9 (±0.7%)

Figure S3. ^{1}H-NMR spectrum (D_2O, 300 MHz) of COSamf **1**.

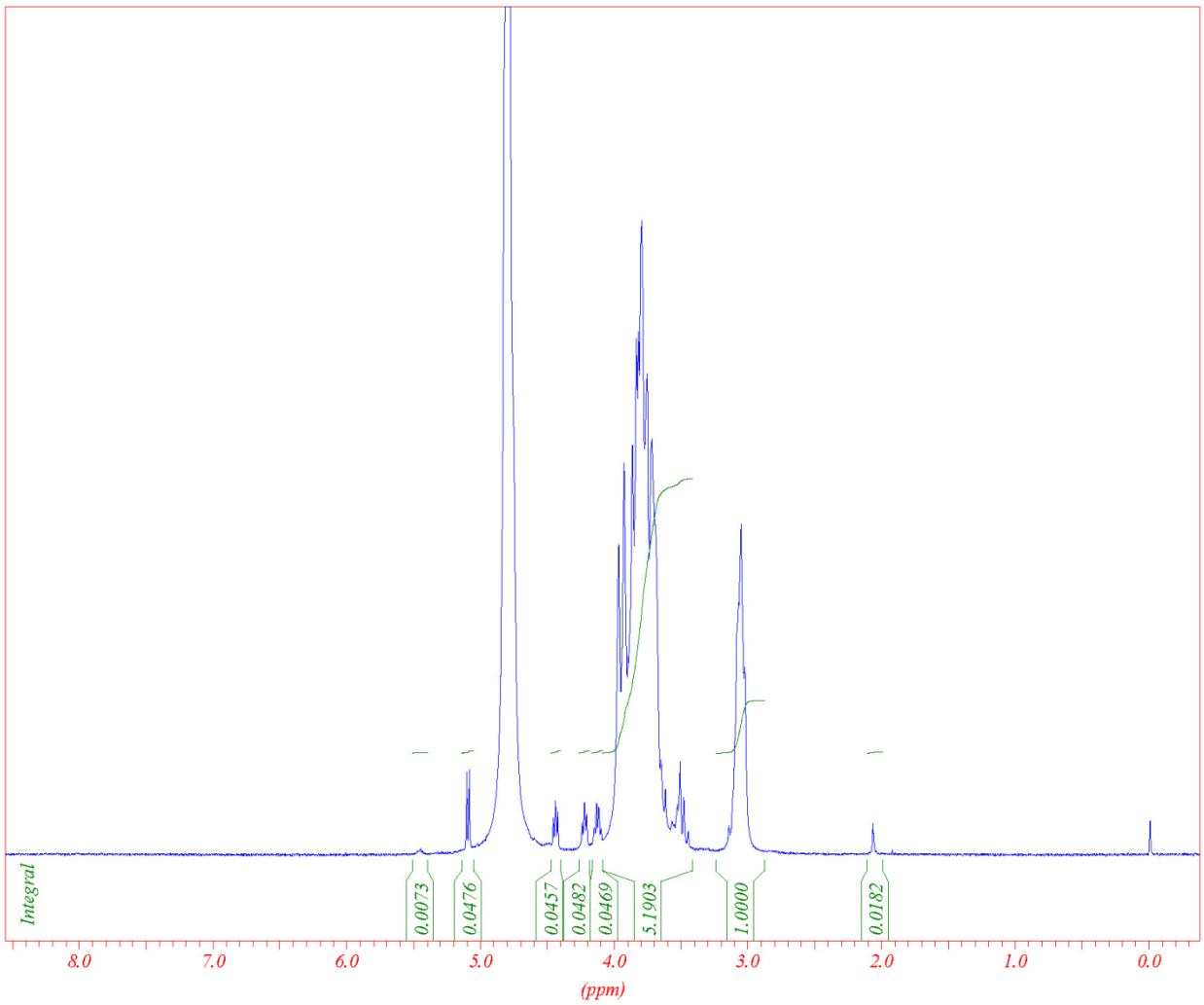


Figure S4. ^{13}C-NMR spectrum (D_2O, 125 MHz) of COSamf **1**.

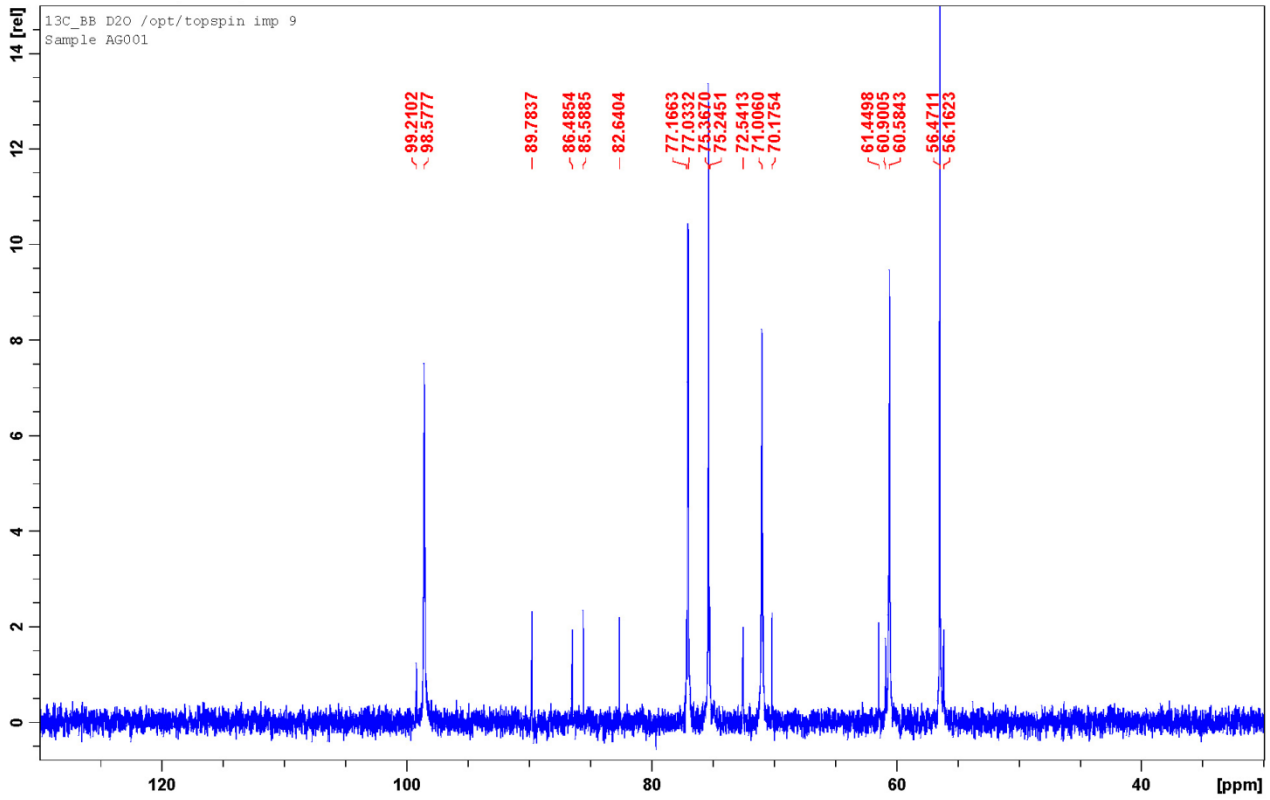
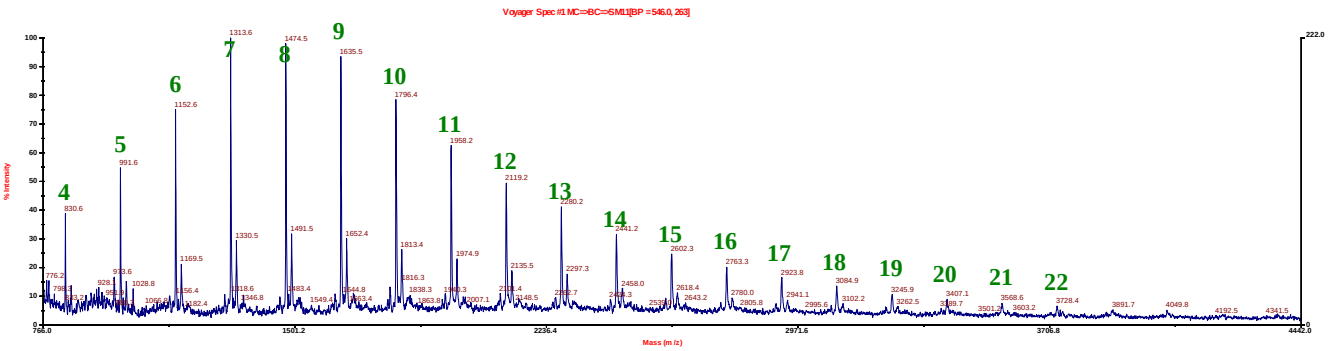


Figure S5. MALDI-TOF mass spectrum (positive linear mode) of COSamf **1**.

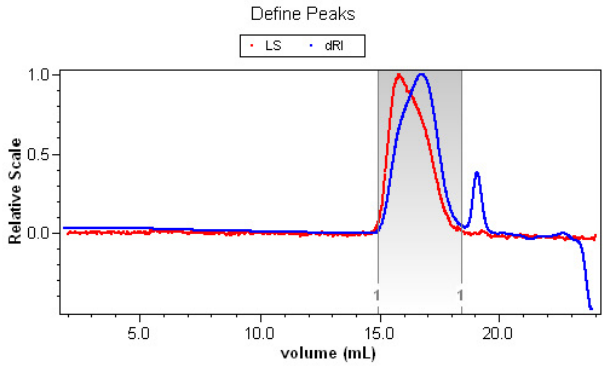


(Note that for each oligomer peak, the number of GlcN unit into the chain is given in green).

Figure S6. Size-exclusion chromatogram of COSamf **1**.

File Name: F:ES01[16mai2013].afe6
Collection Operator: LMPB-AR2000\Aqueux (LMPB-AR2000\Aqueux (Aqueux))
Processing Operator: UNIV-LYON1\stephane.trombotto (TROMBOTTO STEPHANE)

Sample: ES01
Concentration: 2.000 mg/mL
Injected Volume: 100.0 µL



Configuration

Notes:
Colonnes : TSK6000 et TSK2500, Solvant filtré sur CME 0,1 et échantillon filtré sur CME 0,45

Concentration Source: RI
Flow Rate: 0.500 mL/min

Light Scattering Instrument: DAWN EOS
Cell Type: K5
Wavelength: 690.0 nm
Calibration Constant: 7.4800×10^{-6} 1/(V cm)

RI Instrument: Optilab rEX

Solvent: Tampon AcAc/AcNH pH 4.5
Refractive Index: 1.330

Processing

Collection Time: Thursday May 16, 2013 08:15:17 PM Paris, Madrid (heure d'été)
Processing time: Friday May 17, 2013 10:04:49.460 AM Paris, Madrid (heure d'été)

Peak settings:

Peak Name	Peak 1
Light Scattering Model	Zimm
Fit Degree	1
dn/dc (mL/g)	0.1980
A2 (mol mL/g^2)	0.000

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

Results

Peak Results

	Peak 1
Masses	
Calculated Mass (µg)	192.73
Molar mass moments (g/mol)	
Mn	3.858×10^3 (±4.384%)
Mp	3.157×10^3 (±1.989%)
Mv	n/a
Mw	4.445×10^3 (±3.913%)
Mz	5.362×10^3 (±8.419%)
Polydispersity	
Mw/Mn	1.152 (±5.877%)
Mz/Mn	1.390 (±9.492%)
rms radius moments (nm)	
Rn	22.9 (±39.7%)
Rw	22.3 (±39.7%)
Rz	21.5 (±40.5%)

Figure S7. ^{1}H-NMR spectrum (D_2O, 300 MHz) of 4-(hexyloxy)aniline-linked COSamf **2**.

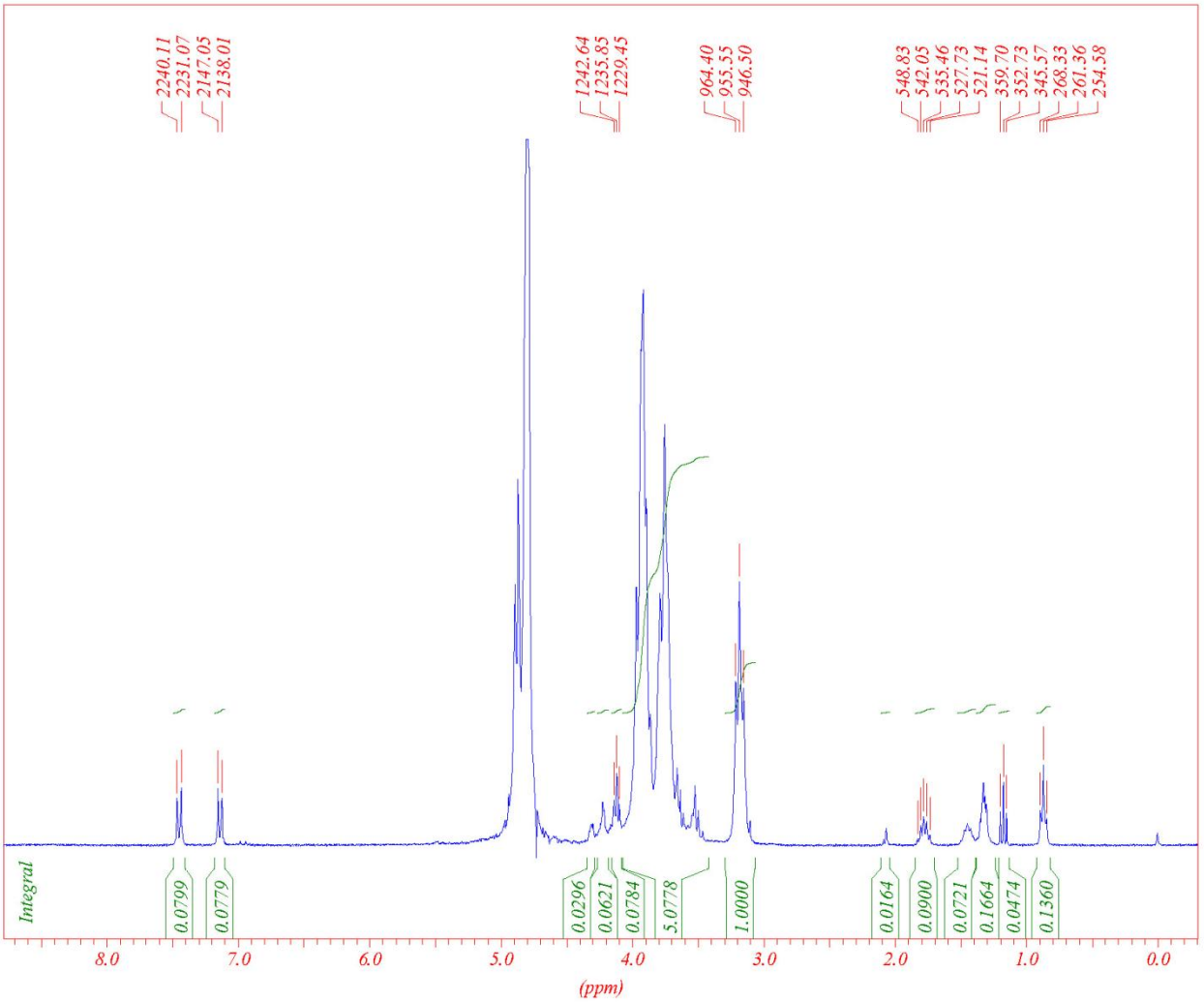


Figure S8. ^{13}C-NMR spectrum (D_2O, 300 MHz) of 4-(hexyloxy)aniline-linked COSamf **2**.

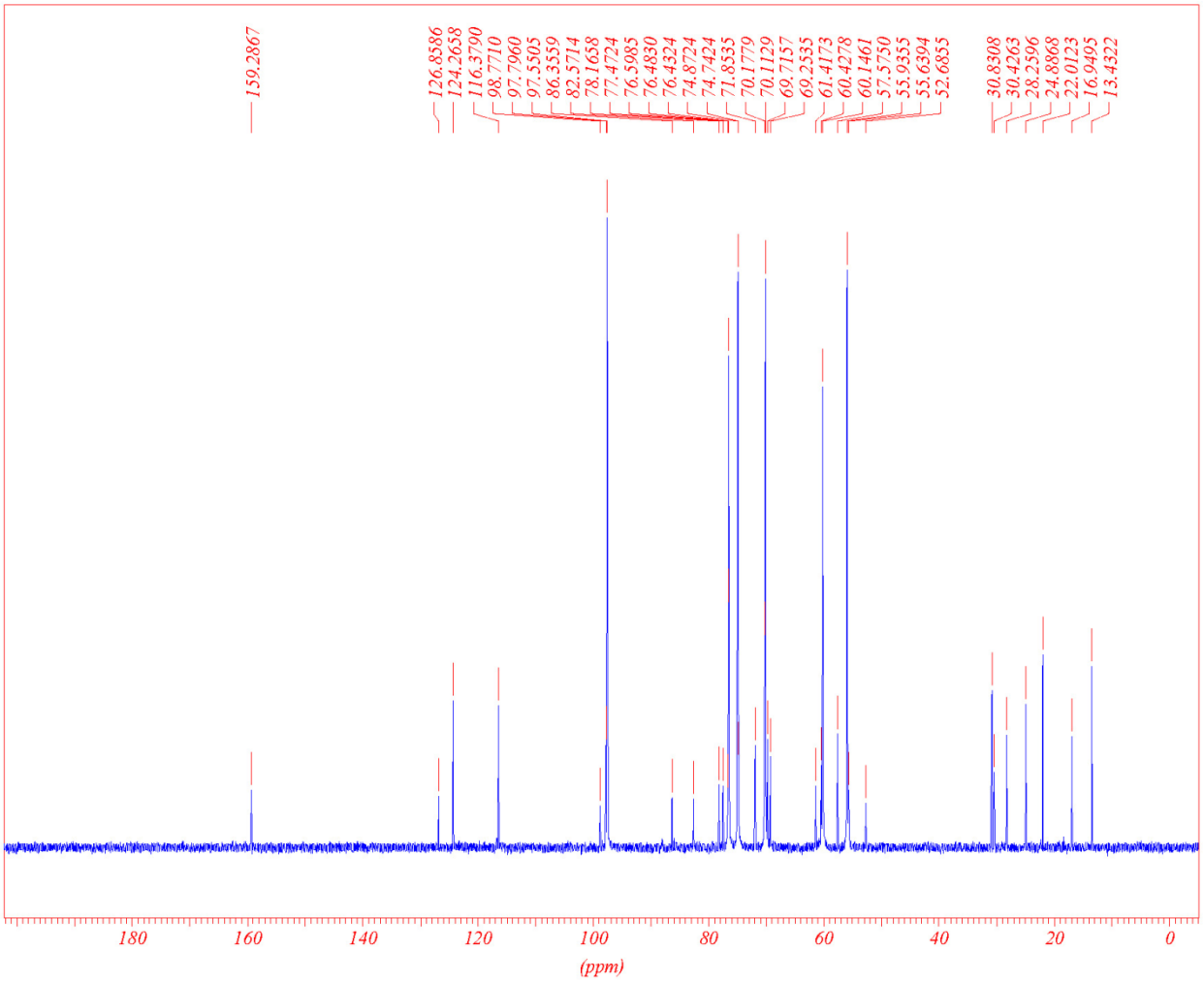
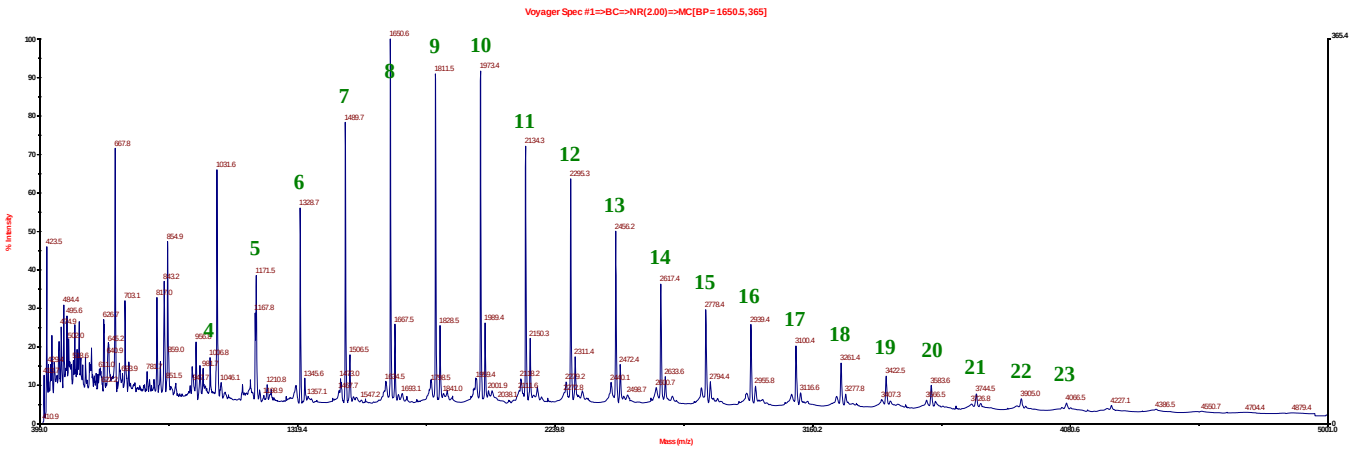


Figure S9. MALDI-TOF mass spectrum (positive linear mode) of 4-(hexyloxy)aniline-linked COSamf **2**.

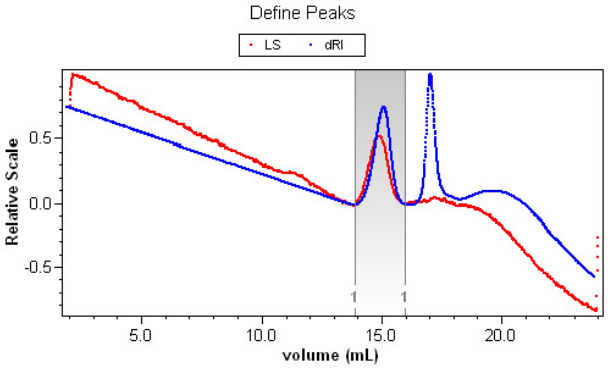


(Note that for each oligomer peak, the number of GlcN unit into the chain is given in green).

Figure S10. Size-exclusion chromatogram of 4-(hexyloxy)aniline-linked COSamf **2**.

File Name: G:ES19[5juillet2013].afe6
Collection Operator: LMPB-AR2000\Aqueux (LMPB-AR2000\Aqueux (Aqueux))
Processing Operator: UNIV-LYON1\stephane.trombotto (TROMBOTTO STEPHANE)

Sample: ES19
Concentration: 5.000 mg/mL
Injected Volume: 100.0 µL



Configuration

Notes:
Colonnes : TSK6000 et TSK1000, Solvant filtré sur CME 0,1 et échantillon filtré sur CME 0,45

Concentration Source: RI
Flow Rate: 0.500 mL/min

Light Scattering Instrument: DAWN EOS
Cell Type: K5
Wavelength: 690.0 nm
Calibration Constant: 7.4800×10^{-6} 1/(V cm)

RI Instrument: Optilab rEX

Solvent: Tampon AcAc/AcNH pH 4.5
Refractive Index: 1.330

Processing

Collection Time: Friday July 05, 2013 12:44:36 PM Paris, Madrid (heure d'été)
Processing time: Friday July 05, 2013 04:49:41.354 PM Paris, Madrid (heure d'été)

Peak settings:

Peak Name	Peak 1
Light Scattering Model	Zimm
Fit Degree	1
dn/dc (mL/g)	0.1980
A2 (mol mL/g^2)	0.000

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

Results

Peak Results

	Peak 1
Masses	
Calculated Mass (µg)	78.90
Molar mass moments (g/mol)	
Mn	3.941×10^3 (±4.600%)
Mp	3.421×10^3 (±1.788%)
Mv	n/a
Mw	4.666×10^3 (±4.753%)
Mz	6.180×10^3 (±14.444%)
Polydispersity	
Mw/Mn	1.184 (±6.615%)
Mz/Mn	1.568 (±15.158%)
rms radius moments (nm)	
Rn	n/a
Rw	n/a
Rz	11.3 (±151.8%)