

# Heterogeneous modification of chitosan via nitroxide-mediated polymerization

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## 1. Analytical Techniques

Gas Chromatography – Mass Spectrometry analyses were performed on a TRACE GC Chromatograph with a split/splitless injector (Thermo Scientific) and a POLARIS Q Ion Trap Mass Spectrometer (Thermo Scientific). Injections were done manually, and injection volume was 0.5  $\mu\text{L}$ . The column was a capillary column TR-1MS 30m $\times$ 0.25mm $\times$ 0.25 $\mu\text{m}$  from Thermo Scientific. Helium was the mobile phase; the flow rate was 1.0 mL $\cdot$ min $^{-1}$ . The oven temperature was 40  $^{\circ}\text{C}$  during 0.5 min and raised to 80  $^{\circ}\text{C}$  (2  $^{\circ}\text{C}\cdot$ min $^{-1}$ ) then to 200  $^{\circ}\text{C}$  (20  $^{\circ}\text{C}\cdot$ min $^{-1}$ ) hold for 2 min. Injector temperature was 150  $^{\circ}\text{C}$  with a split ratio of 50. The temperature of the MS transfer Line was 250  $^{\circ}\text{C}$ . Spectra were acquired following full scan mode with  $m/z$  between 35 and 650 amu, 5 min after injection. The source temperature was 200  $^{\circ}\text{C}$ . Ionization was achieved by electron impact with an ionization energy of 70 eV.

Capillary electrophoresis experimental conditions are as described in the manuscript except if stated otherwise in the following. Separations were carried out using sodium phosphate buffer (100 mM, pH 3.0). The capillary was pre-treated with NaOH 1M for 10 min, NaOH 0.1M for 5 min, MQ water for 5 min and sodium phosphate for 5 min. Before each injection, the capillary was treated with NaOH 1 M for 1 min and phosphate buffer for 10 min (100 mM).

Electrophoretic mobilities were calculated with the following equation:

$$m_{i,\text{app}} = v_i / E = (L_d / t_m) / (V / L_t) \text{ in } (\text{m}^2\cdot\text{s}^{-1}\cdot\text{V}^{-1})$$

where  $v_i$  is the velocity of the molecule  $i$  ( $\text{m}\cdot\text{s}^{-1}$ ),  $E$  is the electric field ( $\text{V}\cdot\text{m}^{-1}$ ),  $L_d$  is the length of capillary to the detector (m),  $t_m$  is the migration time (s),  $V$  is the voltage (V) and  $L_t$  is the total length of the capillary (m).

Data analysis was carried out using the software *Origin 8.5*.

High resolution MS experiments were performed using a QStar Elite mass spectrometer (Applied Biosystems SCIEX, Concord, ON, Canada) equipped with an electrospray ionization source operated in the positive mode. The capillary voltage was set at +5500 V and the cone voltage at +50 V. In this hybrid instrument, ions

were measured using an orthogonal acceleration time-of-flight (oa-TOF) mass analyzer. Accurate mass measurements were performed using two reference ions from a poly(ethylene glycol) internal standard. Air was used as the nebulizing gas (10 psi) while nitrogen was used as the curtain gas (20 psi). Instrument control, data acquisition and data processing of all experiments were achieved using Analyst software (QS 2.0 and 1.4.1) provided by Applied Biosystems. Sample solutions were introduced in the ionization source at a  $5 \mu\text{L}\cdot\text{min}^{-1}$  flow rate using a syringe pump.

Liquid  $^1\text{H}$  NMR experiments were performed in acetone- $\text{d}_6$  on a Bruker Avance 300 spectrometer at 300 MHz.

Solid-state  $^1\text{H}$  MAS spectra of samples CS, CS=, CS-BB, CS-*g*-PSS and CS-*g*-P(MMA-*co*-AN) were recorded on a Bruker DPX200 spectrometer operating at 200 MHz  $^1\text{H}$  Larmor frequency, with a with 4 mm outer diameter rotor at 12kHz MAS, with 64 scans and a 3 s relaxation delay. Solid-state  $^{13}\text{C}$  NMR experimental details are reported in the main text. The assignment of observed  $^{13}\text{C}$  NMR chemical shifts is given in Table S1.

Attenuation Total Reflectance (ATR) Fourier-Transform Infrared (IR) Spectroscopy was performed on a Perkin-Elmer Spectrum 100 FT-IR spectrometer equipped with a universal ATR sampling accessory. Spectra were acquired on the powders with 16 scans and a resolution of  $1 \text{ cm}^{-1}/\text{s}$  with the Spectrum software (Perkin Elmer) and processed with Origin 8.5 (smoothing by averaging over 20 points.)

**Table S1.** Assignment of observed  $^{13}\text{C}$  NMR chemical shifts done by comparison with literature spectra of CS,<sup>1</sup> PMMA,<sup>2</sup> PSS<sup>3</sup> and DMF.<sup>4</sup>

$\delta(\text{ppm})$	Assignment			
	CS	PMMA	PSS	DMF
17	CH <sub>3</sub>			
22		CH <sub>3</sub>		
32, 37				CH <sub>3</sub>
36-51			CH, CH <sub>2</sub>	
45		C		
52		OCH <sub>3</sub>		
55	C <sub>2</sub>	CH <sub>2</sub>		
61	C <sub>6</sub>			
73-75	C <sub>3</sub> +C <sub>5</sub>			
83	C <sub>4</sub>			
104	C <sub>1</sub>			
122-155			aromatics	
163				C=O
173	C=O			
178		C=O		

<sup>1</sup> C. Gartner, B. L. Lopez, L. Sierra, R. Graf, H. W. Spiess, M. Gaborieau, *Biomacromolecules* 2011, **12**, 1380

<sup>2</sup> M. Wilhelm, M. Neidhoefer, S. Spiegel, H. W. Spiess, *Macromolecular Chemistry and Physics*, 1999, **200**, 2205

<sup>3</sup> R. N. Smith, M McCormick, C. J. Barrett, L Reven, H. W. Spiess, *Macromolecules*, 2004, **37**, 4830

<sup>4</sup> H. E. Gottlieb, V. Kotlyar, A. Nudelman, *J. Org. Chem.* **1997**, *62*, 7512

## 2. Synthesis part

### 2.1 Dissolution tests

Visual observation of the dissolution of samples CS, CS=, CS-BB, CS-*g*-PSS and CS-*g*-P(MMA-*co*-AN) were carried out as follows. About 1 mg of each sample was weighed, and solvent was added to make exactly a 1 mg·mL<sup>-1</sup> dispersion. The dispersions were left to stand, manually shaken from time to time to enhance dissolution and were observed for various time intervals over several weeks (Table S2).

It was observed that CS, CS= and CS-BB all dissolved in 50 mM aqueous solutions of acetic acid (AcOH), hydrochloric acid (HCl) or trifluoroacetic acid (TFA) after a day or two. None of them dissolved in MilliQ water, sodium phosphate buffer (100 mM, pH 3.01, NP100), sodium borate buffer (200 mM, pH 9.2, NB200), and sodium hydroxide (1 M).

Dissolution tests were carried out for samples CS-*g*-PSS and CS-*g*-P(MMA-*co*-AN) in the same solvents as above. None of the samples dissolved in neutral or basic aqueous solutions, or in dimethyl sulfoxide (DMSO).

**Table S2.** Visual dissolution tests. A duration indicates how long it took to obtain a clear, transparent solution. 'Partial' indicates that the residual visible articles were in a quantity negligible compared to their initial amount. 'No' indicates that the sample was not visually dissolved. '-' indicates that the test was not conducted.

<b>Sample</b> <b>Solvent</b>	<b>CS</b>	<b>CS=</b>	<b>CS-BB</b>	<b>CS-<i>g</i>- PSS</b>	<b>CS-<i>g</i>- P(MMA- <i>co</i>-AN)</b>
50 mM HCl	1 day	1 day	1 day	No	No
50 mM TFA	Partial	2 days	2 days	No	No
50 mM AcOH	1 day	2 days	2 days	No	No
NP100, pH 3.0	No	No	No	No	No

NB200, pH 9.2	No	No	No	No	No
MQ water	No	No	No	No	No
1 M NaOH	No	No	No	No	No
DMSO	-	-	No	No	No

## 2.2 Synthesis and analysis of acrylamide-modified chitosan (CS=)

### 2.2.1 Synthesis

Esterification and amidification are conventionally performed using acryloyl chloride at 0-5 °C during the addition followed by reaction overnight at 40 °C. Amidification on polymeric substrates is more difficult to perform due to steric hindrance and the reaction is conventionally performed at higher temperature. For example Cho and coworkers<sup>5</sup> end-functionalized a polycaprolactone with acryloyl chloride at 80 °C for 3 h. In our research group, we optimized the reaction between PEO and acryloyl chloride at 50 °C for 3 h leading to a 95 % yield.<sup>6</sup> Moreover, recently Charlot and coworkers<sup>7</sup> managed to functionalize polysaccharides (here a guar gum) with acryloyl chloride at 80 °C for 4 h. In this work, the reaction conditions (40 °C for 19 h followed by 90 min at 55 °C) are not expected to lead to polymerization of the acryloyl chloride and its derivatives and are in good agreement with the conditions reported in the literature to perform such reaction.

A sample of the last acetone washing solution of acrylamide-modified chitosan (CS=) was analyzed by GC-MS. No trace of acrylic acid (AA, coming from hydrolysis of acryloyl chloride) and triethylamine was detected proving that no organic products could be desorbed anymore. In parallel, a sample of the last washing acetone

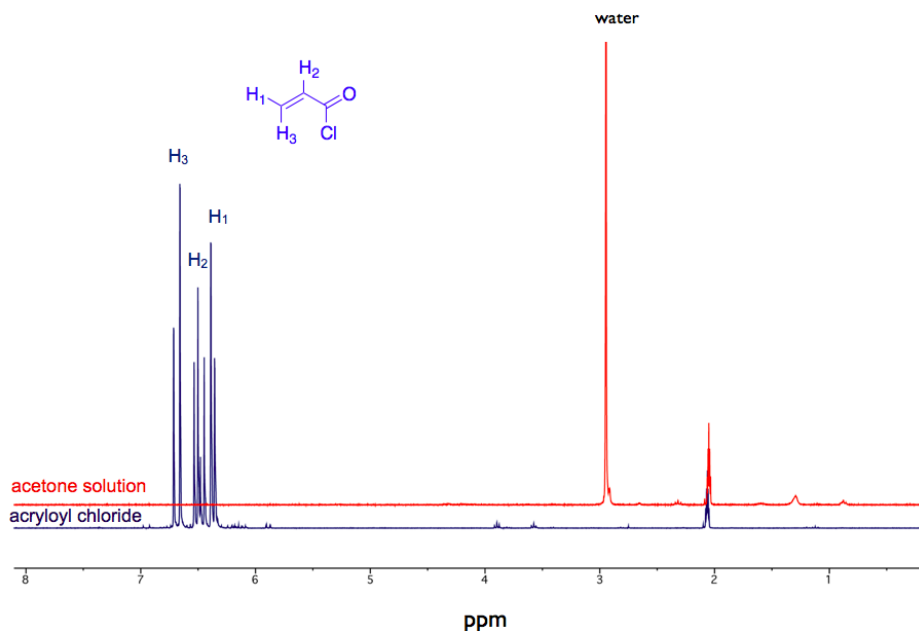
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<sup>5</sup> H. Kweon, M. K. Yoo, I. K. Park, T. H. Kim, H. C. Lee, H. S. Lee, J. S. Oh, T. Akaike and C. S. Cho, *Biomaterials*, 2003, **24**, 801-808.

<sup>6</sup> D. Quemener, G. Bonniol, T. N. T. Phan, D. Gignes, D. Bertin and A. Deratani, *Macromolecules*, 2010, **43**, 5060-5065.

<sup>7</sup> C. Lacroix, E. Sultan, E. Fleury and A. Charlot, *Polymer Chemistry*, 2012, **3**, 538-546.

solution was slowly evaporated at room temperature to avoid any evaporation of acryloyl chloride (or acrylic acid) and then analyzed by  $^1\text{H}$  liquid NMR (300 MHz, acetone- $d_6$ ) (Fig. S1). No acryloyl chloride characteristic peak was observed after 128 scans proving the absence of physical adsorption.

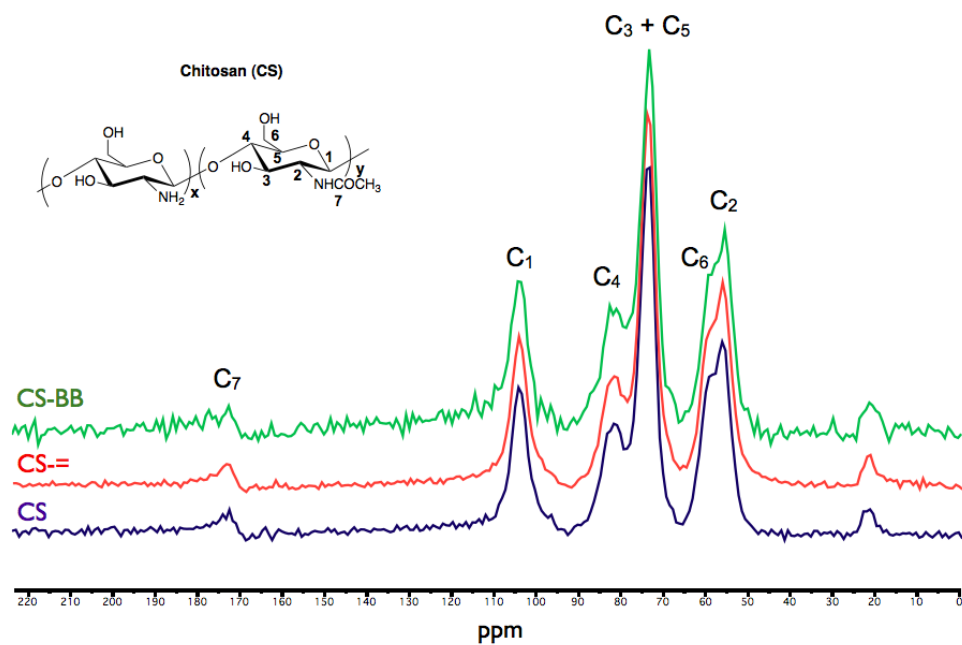


**Fig. S1.**  $^1\text{H}$  liquid NMR spectra of acryloyl chloride (blue) and acetone solution (red) used to extract possible acryloyl chloride adsorbed on CS= . The analyses were performed in acetone- $d_6$ .

### 2.2.2 Analysis by solid-state NMR spectroscopy

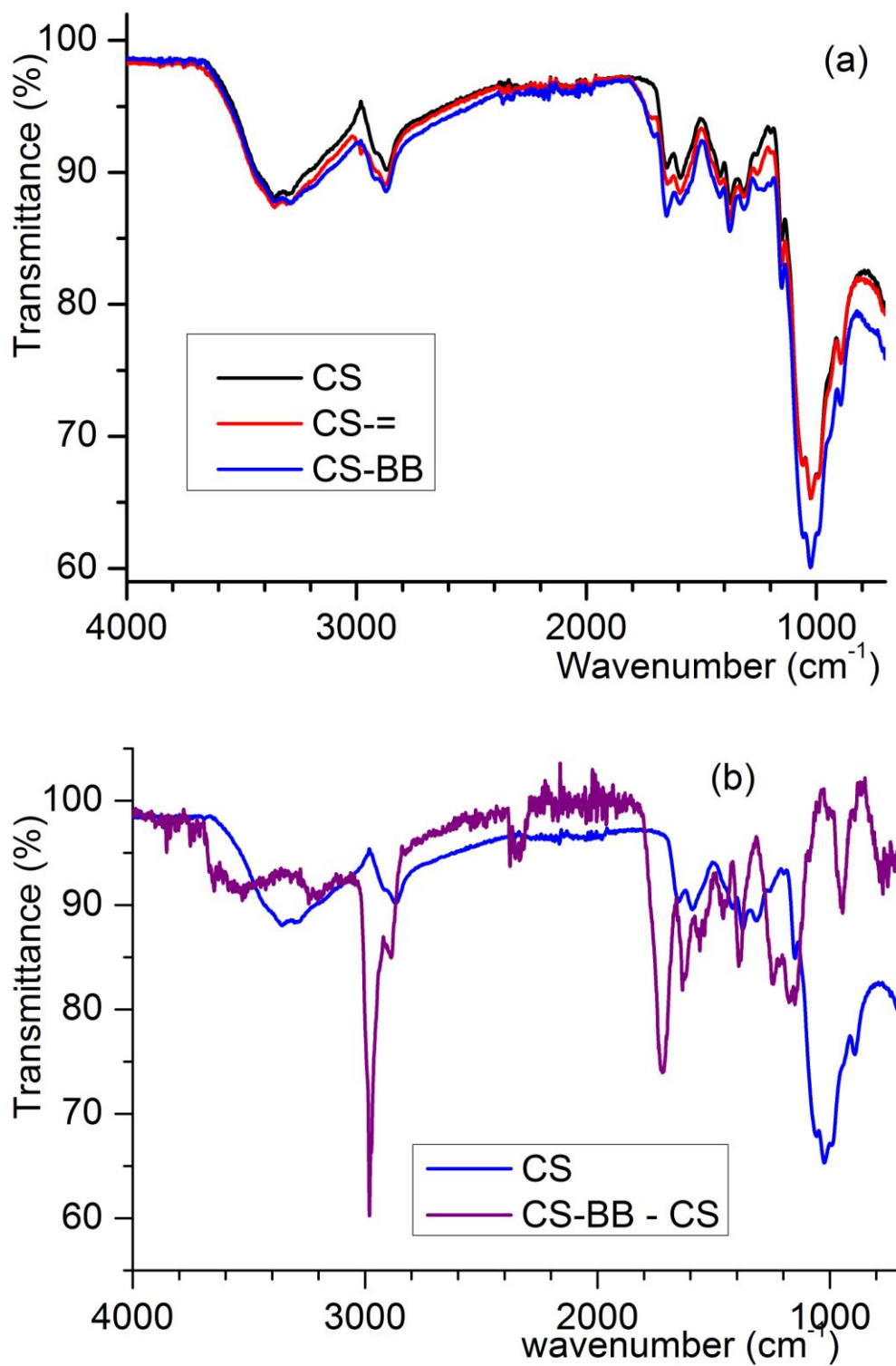
Since insoluble in organic media (THF, acetone, chloroform, DMF, DMSO ...) CS= was analyzed by solid state  $^{13}\text{C}$  CPMAS NMR and IR spectroscopy. Nevertheless, no significant difference was observed between CS and CS= by this technique likely due to a lack of sensitivity even after 16,384 scans (Fig S2).





**Fig. S2.** Solid-state  $^{13}\text{C}$  CPMAS NMR spectra of CS, CS= and CS-BB.

### 2.2.3 Analysis by IR Spectroscopy



**Fig. S3.** ATR FT-IR spectra: (a) CS, CS= and CS-BB; (b) CS and the subtraction of CS from CS-BB.

The FT-IR spectra of CS, CS- and CS-BB are similar (Fig. S3a). The only different band is a shoulder at 1722 cm<sup>-1</sup> on CS- and CS-BB and to a lesser extent at narrow band at 2982 cm<sup>-1</sup> (Fig. S3b). These bands correspond to the ones of acetone (used for washes) but to no known potential grafting product (see Table S3). The resolution of the spectra is too low to be able to detect bands related to the grafted moieties if they form amide bonds. The formation of ester bond should lead to one separate band that is not observed.

Wavenumber (cm <sup>-1</sup> ) in this work (Fig. S3)	Assignment of band	Wavenumber in <sup>8</sup>	Wavenumber in <sup>9</sup>
3321	Hydroxyl stretching	3400	
2871	C-H stretching	2878	
Not observed	C=O stretching in ester		1731
Not resolved	C=O stretching in conjugated secondary amide		1680
1650	C=O stretching in secondary amide (amide I)	1654	
Not resolved	C=C vibration (from acrylamide on chitosan)		1634
1589	C=O stretching in secondary amide (amide I)	1625	
1420	C-H deformations	1420	
Not resolved	C=C (from acrylamide on chitosan)		1410

<sup>8</sup> Z. J. Liu, G. D. Zhao, J. G. Yu, J. Q. Zhang, X. F. Ma and G. Han, *Journal of Applied Polymer Science* 2011, **120**, 2707-2715.

<sup>9</sup> Y. Dong, W. Mao, H. Wang, Y. Zhao, D. Bi, L. Yang, Q. Ge, Z. Ou, *Carbohydrate Polymers*, 2006, **65**, 42-48.

1303	C-N stretching in secondary amide (amide III)	1325	
1058	C-O stretching	1078	
1022	C-O stretching	1022	
893	Saccharide structure	897	
Not resolved	C=C deformation		806

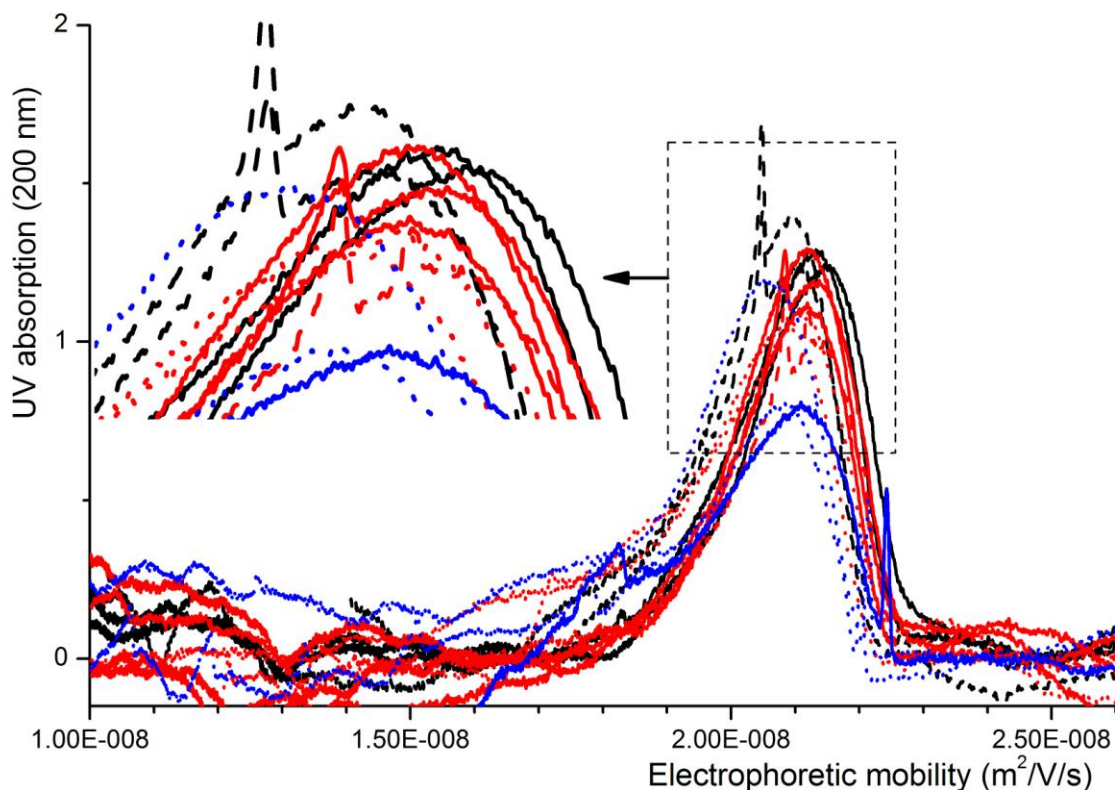
**Table S3:** Band assignment for the IR spectra on Figure S3 and comparison with literature.

#### 2.2.4 Analysis by capillary electrophoresis

The apparent mobility of CS- $\alpha$ , as well as CS-BB, was measured by capillary electrophoresis and compared to the one of CS in sodium phosphate buffer, pH 3. Carboxymethyl-chitosan and chitosan have been shown to be separated in these conditions, although the carboxymethyl-chitosan has a high average degree of substitution of 91 %.<sup>10</sup> The electropherogram (Figure S4) exhibits a broad band for the three samples without any systematic variation. The apparent mobility of chitosan in the literature in conditions similar to ours (migration time around 8 min) corresponds to a mobility around  $2.7 \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$  similar to our values (considering the buffer concentrations are different, 50 mM against 100 mM, and the types of chitosan are differing e.g in terms of degree of acetylation). It is to note that we used 30 kV without significant current instabilities contrary to the literature.

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<sup>10</sup> X. F. Fu, L. Huang, M. L. Zhai, W. Li, H. W. Liu, *Carbohydrate Polymers*, 2007, **68**, 511



**Fig S4.** Free-solution Capillary Electrophoresis (CE) of CS, CS- and CS-BB in sodium borate buffer at pH 3: CS dissolved in acetic acid (solid black line), CS dissolved in HCl (dashed black line), CS- dissolved in acetic acid (solid red line), CS- dissolved in HCl (dashed red line), CS- dissolved in trifluoroacetic acid (dotted red line), CS-BB dissolved in acetic acid (solid blue line) and CS-BB dissolved in trifluoroacetic acid (dotted blue line).

Grafting should lead to a decrease in apparent mobility. No such trend is observed. The mobilities at the maximum of the peaks vary by 4.2 %. This value is consistent with the relative standard deviation of 0.6 % obtained in the literature for chitosan migration time. This difference of mobility was however observed in the literature for polystyrene of 88 and 62 % sulfonation rate.<sup>11</sup> In term of composition, a degree of sulfonation in polystyrene corresponds to the degree of deacetylation of chitosan.

<sup>11</sup> H. Cottet, P. Gareil, O. Theodoly, C.E. Williams *Electrophoresis*, 2000, **21**, 3529

The average degree of deacetylation being 90 % in the starting material, the remaining free amines could go from 90 % to ca 65 % with a 4.2 % change in mobility. In the case of copolymeric galacturonans,<sup>12</sup> the fraction of the sugar rings charged can vary from 90 % to 70 % with a 4.2 % decrease of mobility. Around 25 % of the amine groups could thus have undergone grafting without significant change of apparent mobility.

### 2.3. Analysis of BlocBuilder-modified chitosan (CS-BB)

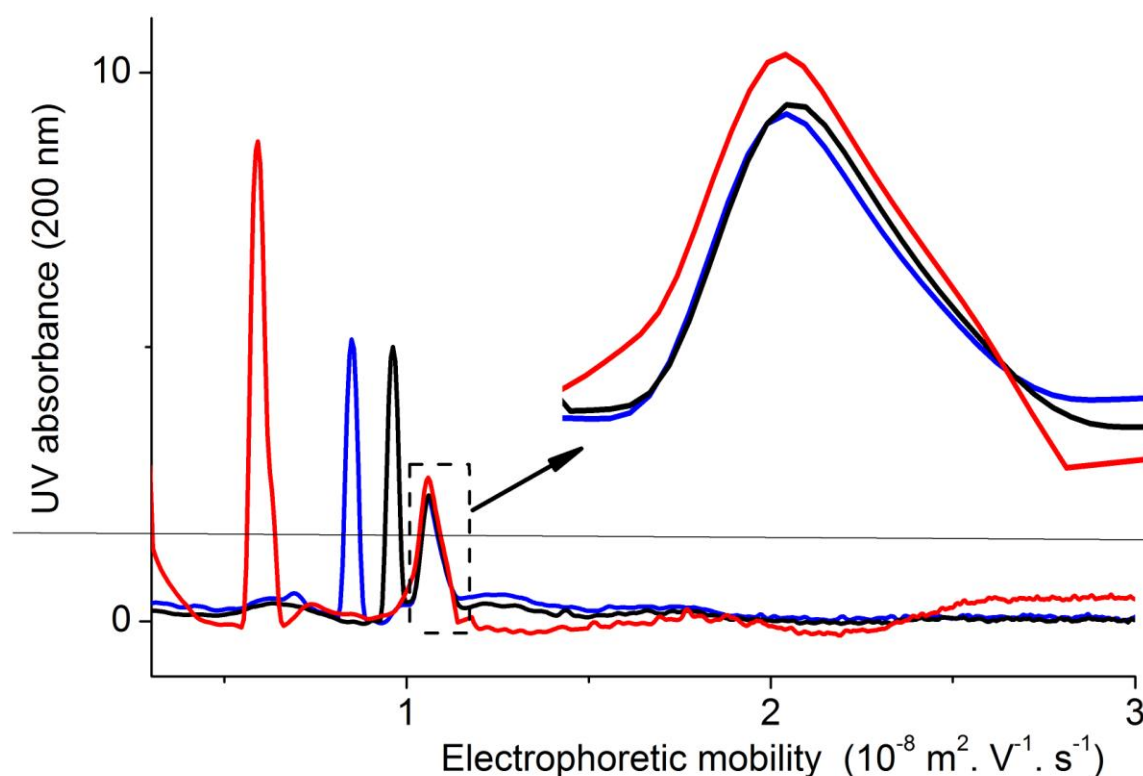
Intermolecular 1,2 radical addition (1,2-IRA) of a high dissociation rate constant alkoxyamine (BlocBuilder) was performed in DMF at 90 °C for 2 h under heterogeneous conditions (CS= not soluble in organic media). Visual observation showed that CS-BB was not soluble in DMSO, DMF and 1,4-dioxane. Like previously in the case of CS=, no significant difference was observed between CS and CS-BB by solid state <sup>13</sup>C CPMAS NMR spectroscopy (Fig S2).

Free-solution capillary electrophoresis in sodium borate (pH 9.2) confirmed the grafting of the BlocBuilder alkoxyamine through an observed mobility for CS-BB at this pH (Fig. 2). The absence of mobility of CS at pH 9.2 is expected (no charge) and was already observed in the literature. The injection of trifluoroacetic acid used for the sample dissolution also led to no detected peaks (with a mobility lower than 3 m<sup>2</sup>.V<sup>-1</sup>.s<sup>-1</sup>). The injection of CS-BB in sodium borate has been in triplicate (Fig. S5), The BlocBuilder degradation products (mobility above unity) have a highly repeatable mobility. It is not the case of the BB-grafted chitosan (CS-BB). The peak is always completely separated from the degradation product: this low mobility peak can only be explained by covalent binding of some BlocBuilder moieties onto the chitosan. The peak shifts significantly with the dissolution time of the sample: the red line corresponds to one sample dissolved and injected within hours while the

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<sup>12</sup> M. A. K. Williams, A. Cuheval, A. Strom, M. C. Ralet, *Biomacromolecules*, 2009, **10**, 1523

blue and black lines correspond to another dissolution with injection performed first for the blue one and then the black one. The low repeatability is first explained by low repeatability of the dissolution process, which is not unexpected for polysaccharide.<sup>13</sup> It might also be due to the low solubility of chitosan at the high pH (pH 9.2) necessary for this separation and which could also explain the necessity for unusually long preconditioning (see main text).



**Fig S5.** Repeatability plot for the injection of CS-BB in sodium borate

#### 2.4. Synthesis of PMMA from BB-adsorbed chitosan (BB/CS/P(MMA-co-AN))

The aim of this experiment was to check if BlocBuilder could be adsorbed onto chitosan and then initiate the polymerization of MMA. The polymerization of MMA

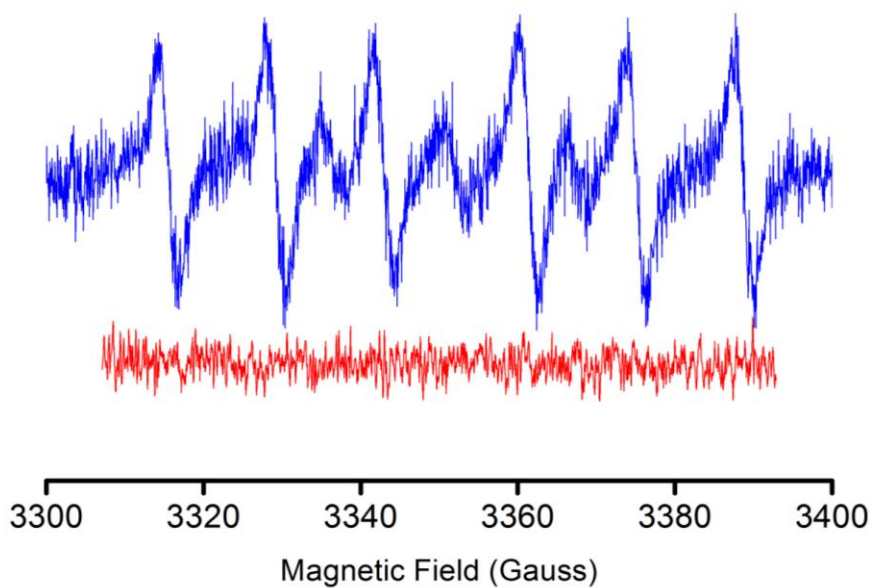
<sup>13</sup> N. L. Hoang, A. Landolfi, A. Kravchuk, E. Girard, J. Peate, J. M. Hernandez, M. Gaborieau, O. Kravchuk, R. G. Gilbert, Y. Guillaneuf, P. Castignolles, *Journal of Chromatography A*, 2008, **1205**, 60

with 10 mol% of AN versus MMA was performed in the same conditions than previously described but by using BB adsorbed onto CS (BB/CS) as macroinitiator. (BB/CS) was prepared by adding 2 g of CS and 8.51 g of BB to 20 mL of DMF. After 10 min deoxygenation under argon bubbling at room temperature, the heterogeneous solution was stirred over night at room temperature (particles of CS suspended in DMF). The suspended particles were recovered by filtration and washed 3 times carefully with THF (BB/CS particles stirred at room temperature for 30 min in THF) and pentane to eliminate residual free BB alkoxyamine and SG1 nitroxide respectively. After drying under vacuum for 3 h, the (BB/CS) particles (1.5 g) were suspended in DMF (6 mL) in presence of MMA (6 g) and AN (0.3 g). After 15 min deoxygenation by argon bubbling, the mixture was heated at 90 °C under stirring for 15 h. A part of the mixture was then poured in THF and stirred over night at room temperature. After filtration, the THF washes were repeated twice but only for 2 h each and BB/CS/P(MMA-*co*-AN) was finally dried under vacuum for 2 h. BB/CS/ P(MMA-*co*-AN) was finally analyzed by solid-state <sup>13</sup>C CP-MAS NMR spectroscopy (Fig. 3b).

#### 2.5. Analysis of chitosan-*g*-poly(sodium 4-styrenesulfonate) (CS-*g*-PSS)

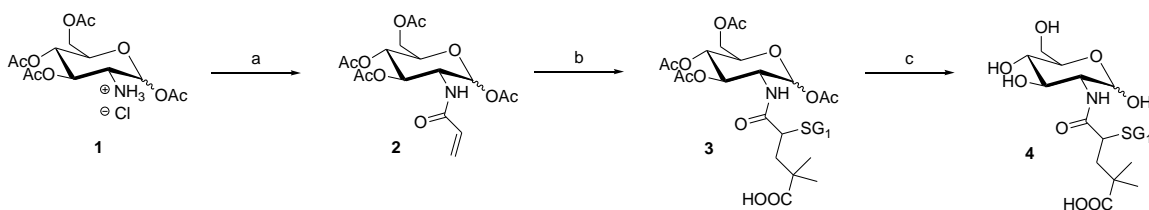
The isolated CS-*g*-PSS polymer was analyzed by heterogeneous ESR to check the release of the SG1 nitroxide upon heating.





**Fig S6.** ESR measurement of CS-*g*-PSS heated at 120 °C for 2 h in the cavity in presence of *tert*-butylbenzene (heterogeneous conditions)

## 2.6 Synthesis of acrylamide glucosamine-BB (**4**)



**Scheme S1.** Reagents and conditions (a)  $\text{CH}_2=\text{CHC}(\text{O})\text{Cl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2/\text{THF}$ , (b) BB,  $\text{EtOH}$ , (c)  $\text{KOH}$ ,  $\text{MeOH}$

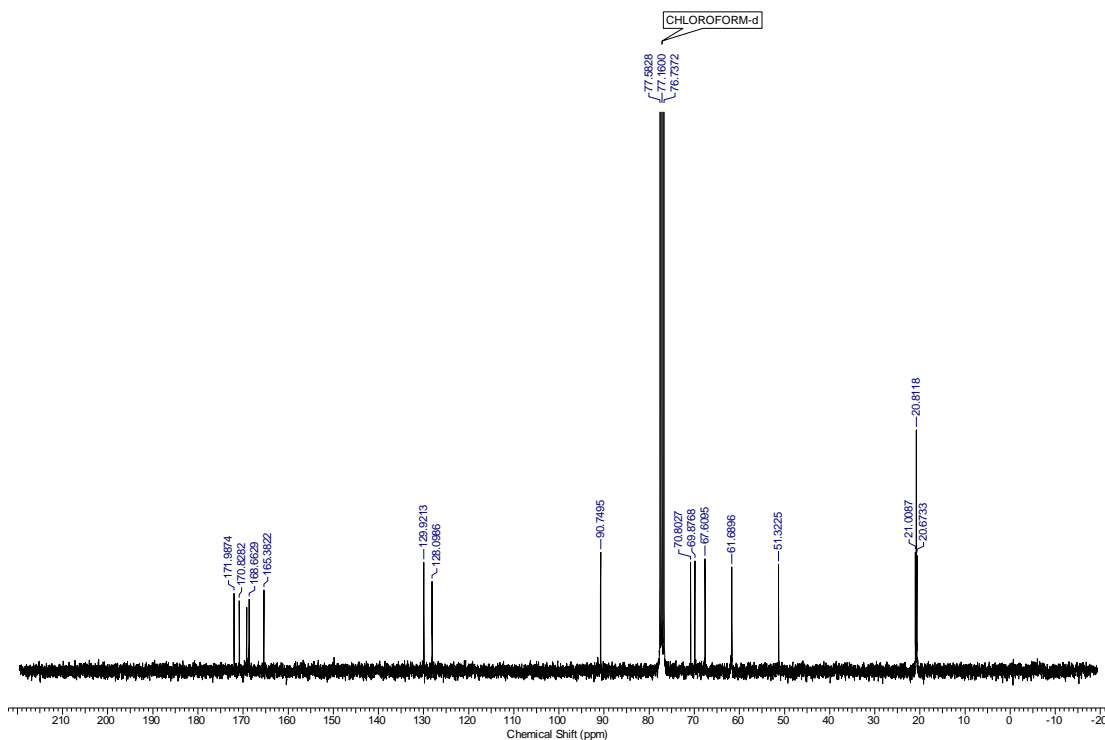
### 2.6.1. 1,3,4,6-tetra-*O*-acetyl-*N*-acryloyl- $\beta$ -glucosamine (**2**)

To a solution of **1**<sup>14</sup> (2 g, 5.74 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/anhydrous THF (17 mL/30 mL) was added Et<sub>3</sub>N (7.2 mL, 51.7 mmol) at room temperature under argon. At 0 °C, acryloyl chloride (1.4 mL, 17.2 mmol) was introduced dropwise. The mixture was stirred for 90 min until TLC (EtOAc) indicated the complete conversion of **1**. Solvents were evaporated under reduced pressure and the resulting residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub>. The organic media was washed twice with successively saturated NaHCO<sub>3</sub> and 1M HCl. After drying over MgSO<sub>4</sub>, CH<sub>2</sub>Cl<sub>2</sub> was evaporated and the obtained solid was dissolved in EtOAc before filtration over silica gel. Finally, EtOAc was evaporated to give a yellow powder **2** (1.5 g, 65%). <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>, major α-anomer shown): δ(ppm) 172.0 (C=O), 170.8 (C=O), 169.2(C=O), 168.7 (C=O), 165.4 (-CO-(CH=CH<sub>2</sub>)), 129.9 (CH=CH<sub>2</sub>), 128.1 (CH=CH<sub>2</sub>), 90.8 (C<sub>1</sub>), 70.8, 69.9, 67.6 (C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub>), 61.7 (C<sub>6</sub>), 51.3 (C<sub>2</sub>), 21.0 (CH<sub>3</sub>), 20.8 (2 CH<sub>3</sub>), 20.7(CH<sub>3</sub>).

ESI(+)-TOF HR-MS calculated for [C<sub>17</sub>H<sub>23</sub>NO<sub>10</sub> + H]<sup>+</sup>, *m/z* 402.1395; found *m/z* 402.1404 (error: +2.2 ppm)

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<sup>14</sup> A. Kenani, C. Bailly, N. Helbecque, R. Houssin, J-L. Bernier, J-P. Henichart, *Eur. J. Med. Chem.*, 2010, **24**, 371.

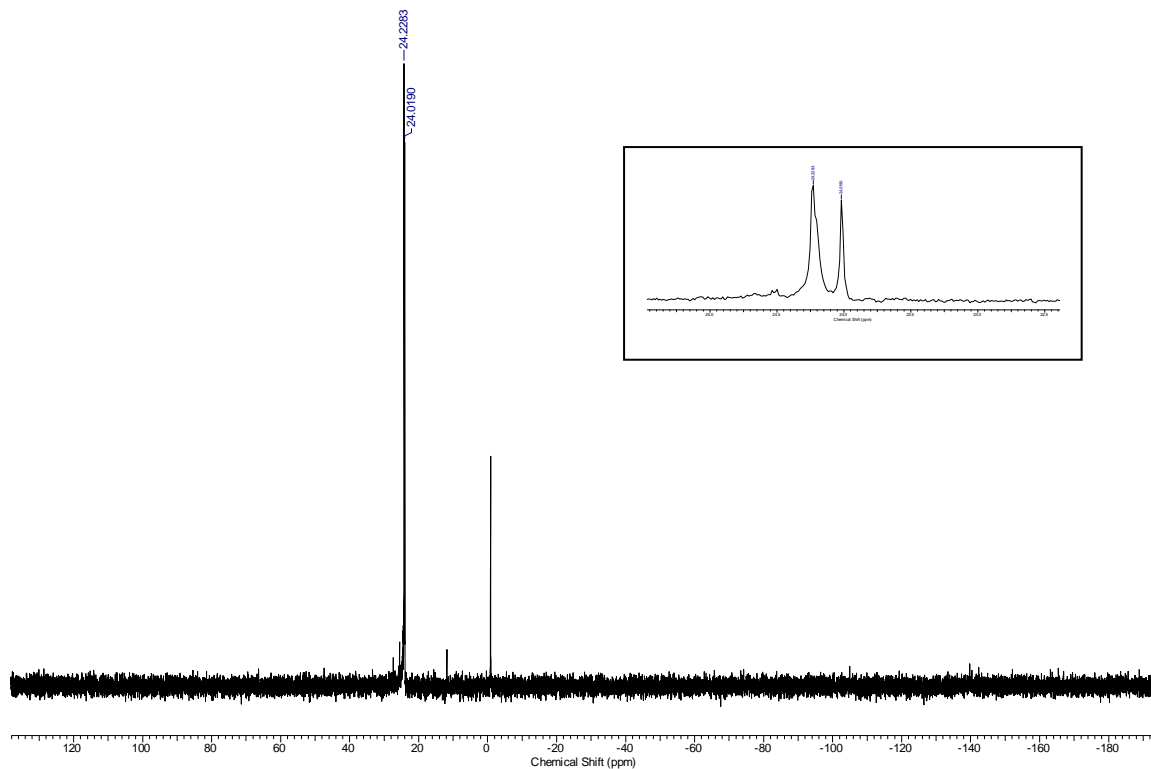


**Figure S7.**  $^{13}\text{C}$  liquid NMR spectra of **2** performed in  $\text{CDCl}_3$ .

2.6.2. 2,2-Dimethyl-4-[*N*-tertio-butyl-*N*-(1-diethoxyphosphoryl)-2,2-dimethylpropyl]aminoxy]-4-[*N*-carbamoyl-(1,3,4,6-tetra-*O*-acetyl)-*D*-glucosamine] butanoic acid (**3**)

**2** (1 g, 2.49 mmol) and BB (1.1 g, 2.87 mmol) in solution in absolute EtOH (14 mL) was degassed by argon bubbling for 15 min at room temperature. After heating under reflux for 285 min, the solvent was evaporated and the solid residue stirred in pentane at room temperature. After filtration and several washes with pentane, a yellow solid **3** was obtained (0.86 g, 44%).  $^{31}\text{P}$  NMR (300 MHz,  $\text{CDCl}_3$ , major diastereoisomers)  $\delta(\text{ppm})$  24.23, 24.02.

ESI(+)-TOF HR-MS calculated for  $[C_{34}H_{59}N_2O_{16}P + H]^+$ ,  $m/z$  783.3675; found  $m/z$  783.3674 (error: -0.1 ppm)



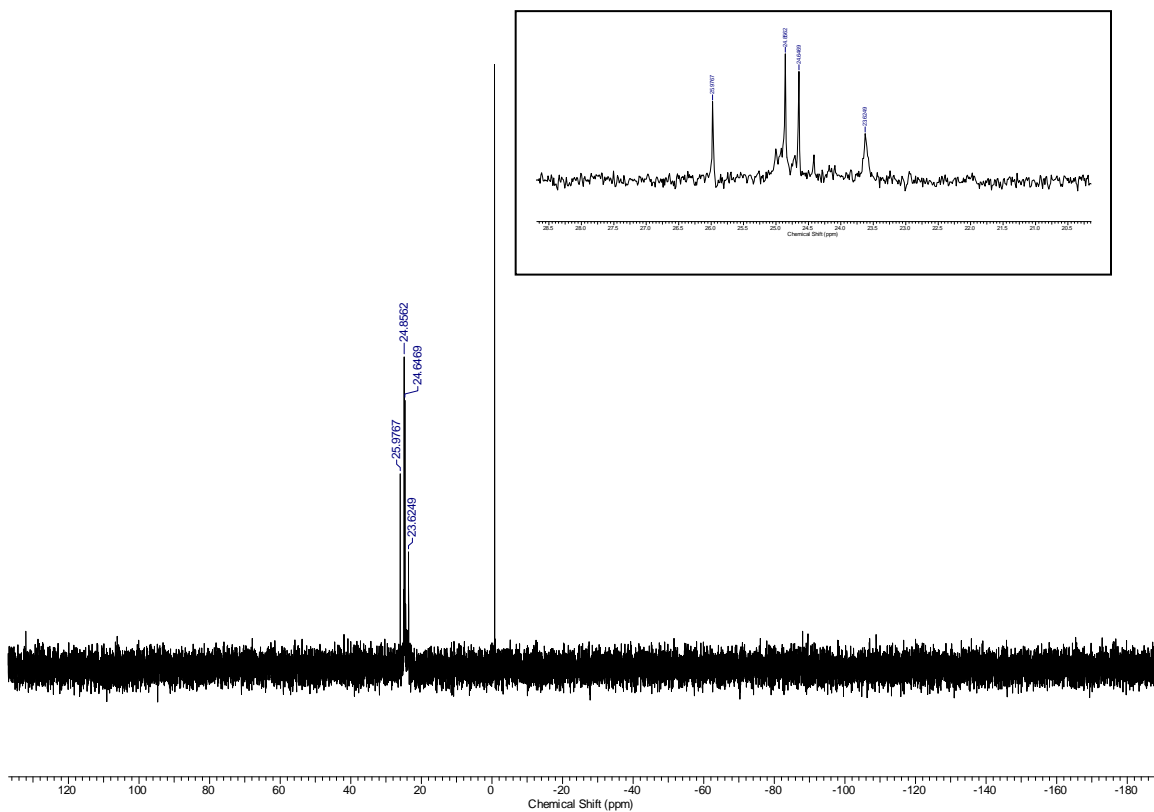
**Figure S8.**  $^{31}P$  liquid NMR spectra of **3** performed in  $CDCl_3$ .

### 2.6.3. 2,2-Dimethyl-4-[*N*-tertio-butyl-*N*-(1-diethoxyphosphoryl-

#### 2,2-dimethylpropyl)aminoxy]-4-[*N*-carbamoyl-*D*-glucosamine] butanoic acid (**4**)

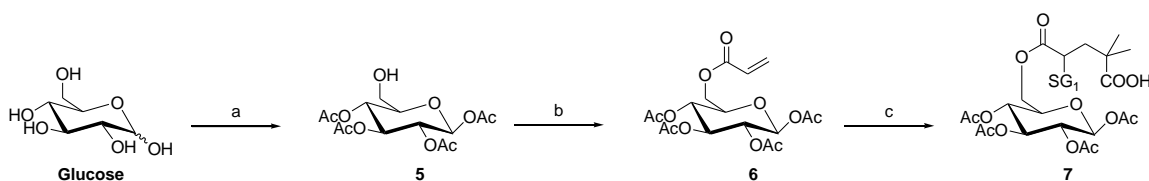
**3** (0.97 g, 1.24 mmol) and KOH (0.21 g, 3.74 mmol) in solution in anhydrous MeOH (8 mL) was stirred at room temperature under argon for 270 min. After neutralization with resin [Amberlite IR-120 ( $H^+$ )], the solvent was evaporated. The solid residue was washed with THF and  $Et_2O$  to give after filtration a yellow powder **4** (0.61 g, 80%).  $^{31}P$  NMR (300 MHz,  $DMSO-d_6$ , major diastereoisomers)  $\delta$ (ppm) 25.98, 24.86, 24.65, 23.62.

ESI(+)-TOF HR-MS calculated for  $[C_{26}H_{51}N_2O_{12}P + H]^+$ ,  $m/z$  615.3252; found  $m/z$  615.3262 (error: +1.6 ppm).



**Figure S9.**  $^{31}P$  liquid NMR spectra of 4 performed in  $DMSO-d_6$ .

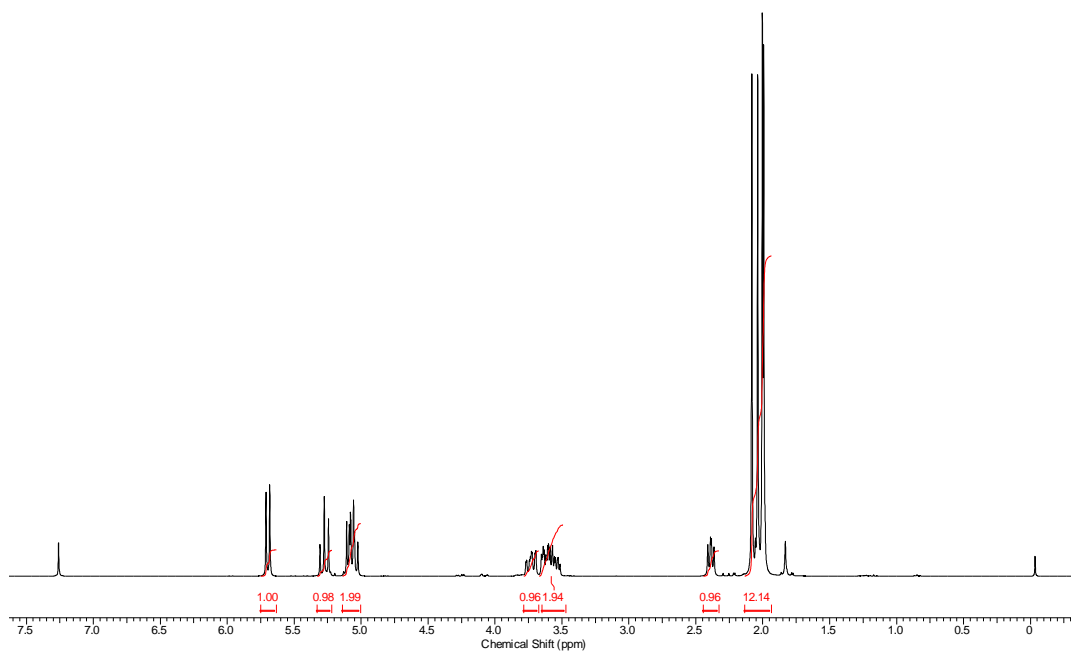
## 2.7 Synthesis of acrylate acetylated -glucose-BB (7)



**Scheme S2.** Reagents and conditions (a) 1.  $\text{ClC}(\text{Ph})_3$ ,  $\text{Ac}_2\text{O}$ , Pyridine ; 2.  $\text{HBr}$ ,  $\text{AcOH}$ , (b)  $\text{CH}_2=\text{CHC}(\text{O})\text{Cl}$ ,  $\text{Et}_3\text{N}$ , THF, (c) BB, EtOH

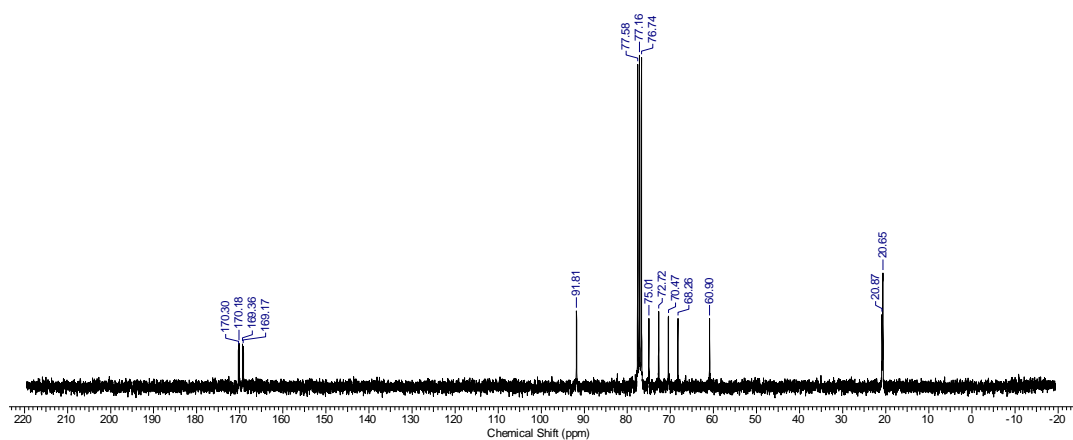
### 2.7.1. 1,2,3,4-tetra-O-acetyl- $\beta$ -D-glucose (5)

Once D-glucose (6 g, 33.3 mmol) and trityl chloride (9.7g, 34.8 mmol) dissolved in pyridine (25 mL) at 95 °C, acetic anhydride (159 mmol, 15 mL) was added in one shot and the solution stirred overnight at room temperature. After precipitation in an iced water/acetic acid solution (475mL/25 mL), the precipitate was isolated by filtration. To remove residual pyridine, the white solid was poured into iced water (500 mL) under stirring for 20 min. The solid was then filtrated and washed carefully with water. After dissolution at 70 °C in glacial acetic acid (100 mL), the mixture was cooled down to 10 °C and a solution of hydrogen bromide in glacial acetic acid solution (33%, 41.1 mmol, 7.2 mL) was added. After 3 min, trityl bromide was removed by filtration and the filtrate was poured in iced water (400 mL). The aqueous layer was extracted three times with  $\text{CHCl}_3$  and after separation, the organic layer was washed three times with iced water, dried over  $\text{MgSO}_4$  and evaporated under reduced pressure to give a viscous oil.  $\text{Et}_2\text{O}$  (250 mL) and pentane (250 mL) were successively added under stirring to give a white solid after filtration (2.15 g, 19 %).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ (ppm) 5.70 (d,  $J = 8.3$  Hz, 1H,  $\text{C}_1\text{-H}$ ), 5.28 (t,  $J = 9.4$  Hz, 1H), 5.00-5.15 (m, 2H), 3.68-3.79 (m, 1H), 3.49-3.67 (m, 2H), 2.34-2.44 (m, 1H), 2.08 (s, 3H,  $\text{CH}_3$ ), 2.04 (s, 3H,  $\text{CH}_3$ ), 2.00 (s, 3H,  $\text{CH}_3$ ), 1.99 (s, 3H,  $\text{CH}_3$ ).



**Figure S10.**  $^1\text{H}$  liquid NMR spectra of **5** performed in  $\text{CDCl}_3$

$^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta(\text{ppm})$  170.3 (C=O), 170.2 (C=O), 169.4(C=O), 169.2 (C=O), 91.8 ( $\text{C}_1$ ), 75.0, 72.7, 70.5, 68.3 (4C), 60.9 ( $\text{C}_6$ ), 20.9 (2  $\text{CH}_3$ ), 20.7 (2  $\text{CH}_3$ ).

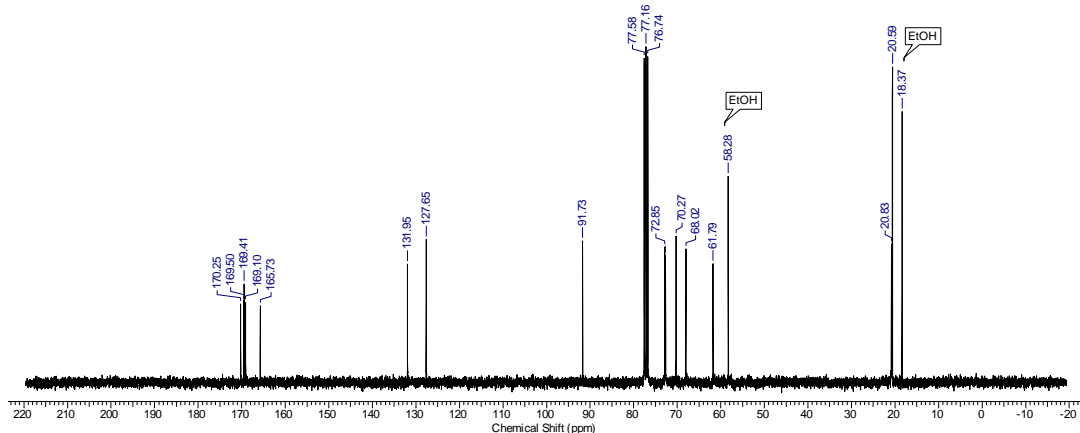


**Figure S11.**  $^{13}\text{C}$  liquid NMR spectra of **5** performed in  $\text{CDCl}_3$

### 2.7.2. 1,2,3,4-tetra-*O*-acetyl-6-*O*-acryloyl- $\beta$ -D-glucose (**6**)

Et<sub>3</sub>N (1.9 mL, 13.3 mmol) was added to **5** (1.5 g, 4.3 mmol) in solution in anhydrous THF (30 mL) at room temperature under argon. At 0 °C, acryloyl chloride (1.05 mL, 12.9 mmol) in solution in anhydrous THF (10 mL) was introduced dropwise. The mixture was then stirred overnight under argon at room temperature. Triethylammonium chloride was removed by filtration and the solvent evaporated under reduced pressure. The yellow residue was recrystallized in EtOH to give a white powder **6** (0.8 g, 46 %). <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ (ppm) 170.3 (C=O), 169.5 (C=O), 169.4(C=O), 169.1 (C=O), 165.7 ((-CO-(CH=CH<sub>2</sub>))), 132.0 (CH=CH<sub>2</sub>), 127.7 (CH=CH<sub>2</sub>), 91.7 (C<sub>1</sub>), 72.9, 72.7, 70.3, 68.0 (4C), 61.8 (C<sub>6</sub>), 20.8 (CH<sub>3</sub>), 20.6 (3CH<sub>3</sub>).

ESI(+)-TOF HR-MS calculated for [C<sub>17</sub>H<sub>22</sub>O<sub>11</sub> + NH<sub>4</sub>]<sup>+</sup>, *m/z* 420.1500; found *m/z* 420.1510 (error: +2.4 ppm).



**Figure S12.** <sup>13</sup>C liquid NMR spectra of **6** performed in CDCl<sub>3</sub>

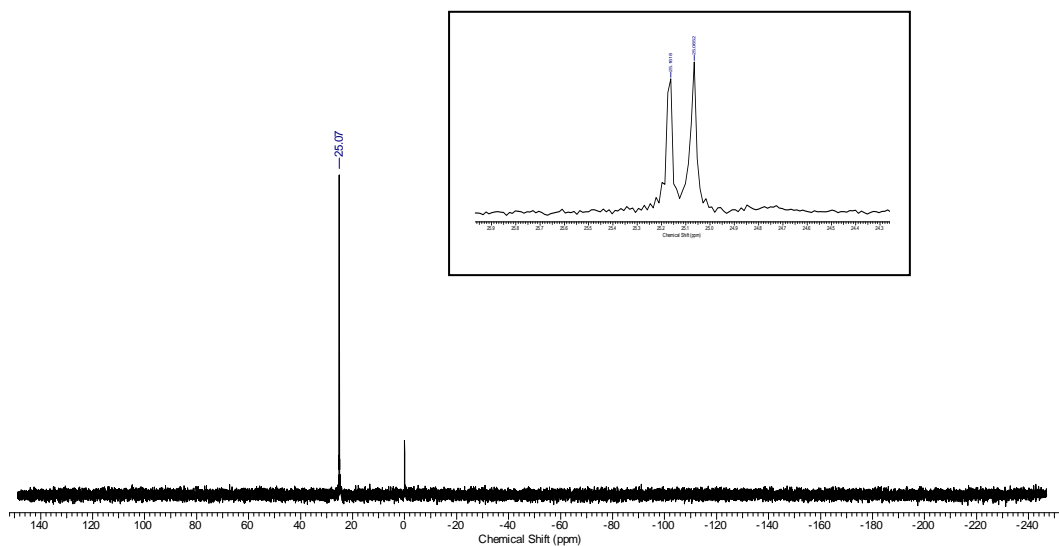


2.7.3. 2,2-Dimethyl-4-[N-tertio-butyl-N-(1-diethoxyphosphoryl-  
2,2-dimethylpropyl)aminoxy]-4-[6-O-(1,2,3,4-tetra-*O*-acetyl- $\beta$ -D-  
glucose)carbonyl] butanoic acid (**7**)

**6** (0.22 g, 0.55 mmol) and **BB** (0.29 g, 0.77 mmol) in solution in absolute EtOH (3 mL) was degassed by argon bubbling for 30 min at room temperature. The solution was then heated under reflux for 5 h. After cooling, the solvent was evaporated under reduced pressure and the solid residue was poured in a large excess of pentane. After filtration, a yellow solid **7** was obtained (0.24 g, 56%).

$^{31}\text{P}$  NMR (400 MHz,  $\text{CDCl}_3$ , major diastereoisomers)  $\delta$ (ppm) 25.16, 25.07.

ESI(+)-TOF HR-MS calculated for  $[\text{C}_{34}\text{H}_{58}\text{NO}_{17}\text{P} + \text{H}]^+$ ,  $m/z$  784.3515; found  $m/z$  784.3502 (error: -1.7 ppm).



**Figure S13.**  $^{31}\text{P}$  liquid NMR spectra of **7** performed in  $\text{CDCl}_3$