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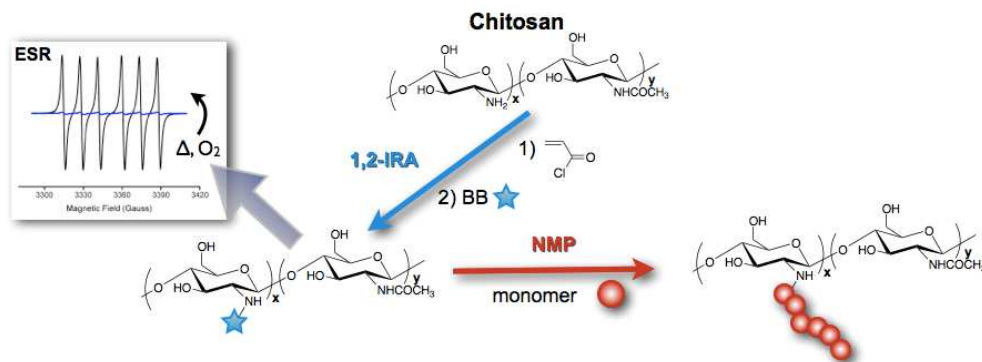
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TOC

Grafting of poly(methyl methacrylate) and poly(sodium 4-styrenesulfonate) chains onto chitosan backbone by SG1-based nitroxide-mediated polymerization under heterogeneous conditions.



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Heterogeneous modification of chitosan via nitroxide-mediated polymerization

Catherine Lefay,^{*a} Yohann Guillaneuf,^a Guillaume Moreira,^a Joel Thevarajah,^{b,c} Patrice Castignolles,^c Fabio Ziarelli,^d Emily Bloch,^e Mohamed Major,^a Laurence Charles,^a Marianne Gaborieau,^b Denis Bertin,^a Didier Gimes^{*a}

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Chitosan (CS) was modified by SG1-based nitroxide-mediated polymerization under heterogeneous conditions. After introduction of acrylamide and/or acrylate functions onto the CS backbone followed by intermolecular 1,2 radical addition of the BlocBuilder alkoxyamine (CS-BB), methyl methacrylate (MMA) in presence of a small amount of acrylonitrile (AN) or sodium 4-styrenesulfonate (SS) were polymerized by nitroxide-mediated polymerization (NMP). ESR and free-solution capillary electrophoresis confirmed the synthesis of CS-BB. The successful synthesis of CS-*g*-P(MMA-*co*-AN) and CS-*g*-PSS grafted copolymers was proved by TGA and solid-state NMR spectroscopy with ca. 20 to 30 wt% of grafted synthetic polymer in the final product.

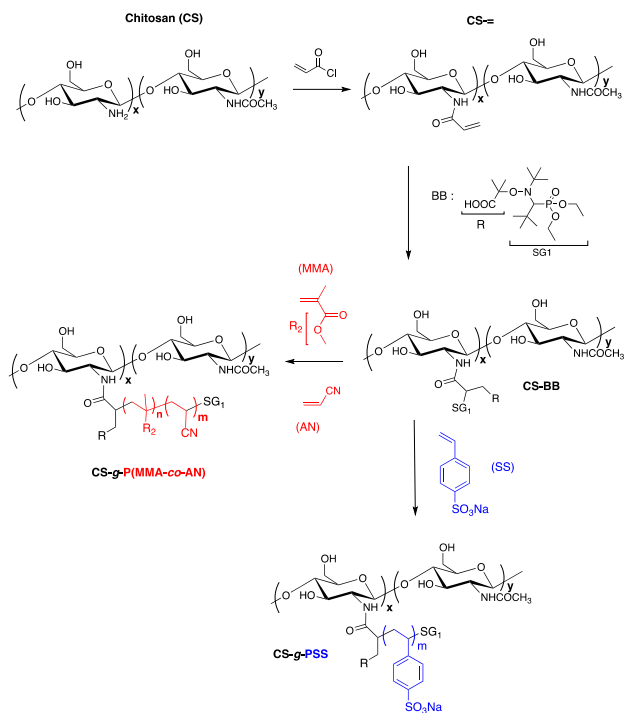
Introduction

Chitosan is a natural polysaccharide obtained by the partial deacetylation of chitin. It displays excellent biological properties such as biocompatibility, biodegradability, antibacterial and wound-healing activity.¹ Chitosan has thus promising applications in many fields such as biomedicine, wastewater treatment, functional membranes and flocculation. However, the main drawbacks of chitosan are its solubility only in acid solutions and its poor mechanical properties compared to synthetic polymers. To overcome these problems, chitosan has been modified by chemical treatments and more efficiently by graft copolymerization.² Graft copolymerization of synthetic monomers onto the chitosan backbone can indeed enhance the chemical or mechanical properties of chitosan and consequently widen its applications. Various grafting methods have been used in the last decades such as the grafting initiated by free radicals using ceric ammonium nitrate (CAN), ammonium persulfate (APS), 2,2-azobisisobutyronitrile (AIBN) or Fenton's reagent as radical initiator.³ The graft copolymerization onto chitosan was also reported by γ -irradiation, enzymes, ring-opening polymerization and polycondensation.² Besides these "grafting from" methods, several "grafting onto" methods were reported as well.⁴ However, these methods of grafting do not generally allow controlling the molecular weight and the number of the grafted chains. Recently, controlled/living radical polymerization (CLRP) techniques, such as nitroxide-mediated polymerization (NMP),⁵ Atom Transfer Radical Polymerization (ATRP)⁶ and Reversible Addition-Fragmentation chain Transfer (RAFT)⁷ have

opened new prospects in this domain allowing to tailor new polysaccharide-based hybrid materials by finely tuning the chemical composition and the topology of the grafted chains. Nevertheless, to the best of our knowledge and according to a recent review of Bernard et al.,⁸ CLRP techniques have not often been applied to chitosan with only 12 articles reporting the synthesis of grafted chitosan by ATRP^{9,10,11,12,13,14,15} (5 under heterogeneous conditions), 3 by RAFT^{16,17,18} (all under homogeneous conditions) and 2 by NMP^{19,20} (both under homogeneous conditions). Homogeneous conditions were attained only after chitosan was modified by heterogeneous modifications (i. e. phthaloylation or complexation with tosylic acid) to enable its solubilization in N,N-dimethylformamide (DMF). In the case of NMP, a chitosan-TEMPO macroinitiator was obtained from the ⁶⁰Co γ -ray irradiation of *N*-phthaloylchitosan in presence of 4-hydroxy-TEMPO. Styrene¹⁹ or sodium 4-styrenesulfonate²⁰ were then polymerized by NMP to yield the corresponding grafted copolymer. Note that ⁶⁰Co γ -ray irradiation is not easily accessible and that TEMPO nitroxide and its derivative are limited to styrenic monomers.

The aim of this work is thus to find a more versatile "grafting from" method to modify chitosan by NMP under heterogeneous conditions (Scheme 1). The growth of synthetic polymer chains from the polysaccharide backbone under heterogeneous conditions is indeed a powerful method to impart new properties to the polysaccharide without affecting its intrinsic biological properties. In the present study, chitosan-*g*-poly(methyl methacrylate-*co*-acrylonitrile), CS-*g*-P(MMA-*co*-AN), and chitosan-*g*-

poly(sodium 4-styrenesulfonate), CS-g-PSS, grafted copolymer chains have been synthesized for the first time by SG1-based NMP under heterogeneous conditions; i.e. without preliminary modification of the chitosan to increase its solubility in organic media. SG1 has been chosen as control agent for its broad range of polymerizable monomer types (styrenic, acrylic, methacrylic, etc).⁵



Scheme 1 General synthesis procedure of chitosan modification via nitroxide-mediated polymerization

Experimental part

Materials

Chitosan (CS, Aldrich, Low Molecular Weight, batch 06714DJ, average degree of acetylation of 9 %), triethylamine (Aldrich, 99+%), acryloyl chloride (Aldrich, 97+%), D-glucose (Aldrich, 99.5%), trityl chloride (Aldrich, ≥97%), acetic anhydride (Aldrich, ≥99%) and hydrogen bromide (Aldrich, 33% wt in acetic acid) were used as received. The 2-methyl-2-(*N*-*tert*-butyl-*N*-(1-diethoxyphosphoryl)-2,2-dimethylpropyl)aminoxy)-propionic acid alkoxyamine (BlocBuilder, BB) was kindly supplied by Arkema. Methyl methacrylate (MMA, Aldrich, 99%), acrylonitrile (AN, Aldrich, 99+%) and sodium 4-styrenesulfonate (SS, >99%, Aldrich) were used as received. Tetrahydrofuran (THF, SDS, purex for analysis) was distilled over sodium before use. *N,N*-dimethylformamide (DMF, SDS, Analytical grade), pentane (SDS, technical grade), methanol (SDS, technical grade), dichloromethane (Carlo Erba), ethyl acetate (Carlo Erba), anhydrous ethanol (Carlo Erba), glacial acetic acid (Carlo Erba), chloroform (Carlo Erba), diethyl ether (Aldrich) and pyridine (Alfa Aesar) were used as received. For the capillary electrophoresis experiments, all water was of MilliQ quality. Sodium borate, 200 mM, was prepared from boric acid

(BDH AnalaR) and sodium hydroxide (Sigma).²¹ Dimethylsulfoxide (DMSO) was used as received (Sigma).

Instrumentation

Electron spin resonance (ESR) experiments were performed on a Bruker EMX 300 spectrometer and *tert*-butylbenzene was used as solvent. The measurements were generally performed at 100 °C in a glass NMR tube under air.

The thermal degradation of chitosan, CS-g-P(MMA-co-AN) and CS-g-PSS was investigated by thermogravimetric analysis (TGA Q500, TA Instruments), performed between 25 and 600 °C with a maximum heating rate of 5 K/min under dynamic argon atmosphere (sample flow rate 60 mL/min) in platinum sample holders.

Free-solution capillary electrophoresis (CE) experiments were carried out using a HP3D (Hewlett Packard, now Agilent Technologies Australia) and an Agilent 7100 CE systems (Agilent Technologies, Waldbronn, Germany) equipped with diode array detectors. Capillaries were polyimide coated high-sensitivity fused silica capillaries (Agilent) with a 42.5 cm total length and a 35 cm effective length. Injections were carried out at 25 °C using sodium borate (200 mM, pH 9.2). The capillary was pre-treated with NaOH 1M for 40 min, NaOH 0.1M for 20 min, water for 5 min and sodium borate for 5 min. Before each injection, the capillary was treated with NaOH 1M for 20 min, NaOH 0.1 M for 10 min, MilliQ water for 5 min and sodium borate for 5 min. It is to be noted that so long a pre-injection treatment is unusual but was necessary to ensure repeatable separations (see ESI†). All injections were hydrodynamic, i.e., pressure (30 mbar) was applied during the injection time (10 s). Detection was set at 200 nm. Voltage was set at maximum (30 kV). DMSO was used to measure the electroosmotic flow (1 µL in each 450 µL sample). An oligoacrylate with a known separation²² and a broad range of mobilities was injected to validate the capillary and the instrument before each session.

Solid-state nuclear magnetic resonance (NMR) experiments were performed on a Bruker DPX200 spectrometer operating at a 50 MHz ¹³C Larmor frequency with a 4 mm outer diameter rotor under magic-angle spinning (MAS) at a frequency of 12 kHz. ¹³C cross-polarization (CP-MAS) NMR spectra were recorded using a 2 ms contact time, a 5 s relaxation delay and 2,824 to 15,416 scans. The ¹³C chemical shift scale was externally referenced to tetramethylsilane (TMS) at 0.0 ppm using adamantane by setting the CH resonance to 38.5 ppm.²³

Synthesis of acrylamide-modified chitosan (CS=)

The synthesis of CS= under heterogeneous conditions was performed as followed. Typically, chitosan (2 g) was dispersed in THF (20 mL) with triethylamine (14 mL; 0.1 mol) and stirred for 1 h at 0 °C in an iced water bath. The suspension was then deoxygenated by argon bubbling for 15 min. Acryloyl chloride (7.4 mL; 0.09 mol; 0.9 mol eq. versus triethylamine) in solution in 10 mL of dried THF was added dropwise into the cool mixture under argon atmosphere. The mixture was then stirred at 40 °C for 19 h followed by 90 min at 55 °C. After 3 washes with

successively THF, acetone and dichloromethane, a light brown powder was recovered by filtration and then stirred overnight in acetone at room temperature. This wash with acetone was repeated 3 times for 2 h. The last acetone solution was analyzed by GC-MS and solution-state ^1H NMR. CS- was finally recovered by filtration and dried under vacuum at room temperature for 90 min before analysis by solid-state ^{13}C CP-MAS NMR spectroscopy.

Synthesis of BlocBuilder-modified chitosan (CS-BB)

1.5 g of CS- was previously described was suspended in a 15 mL solution of BlocBuilder (5.32 g; 0.014 mol) in DMF. After 15 min deoxygenation by argon bubbling, the mixture was heated at 90 °C for 2 h under stirring. The macromolecular multi-alkoxyamine CS-BB was recovered by filtration after 3 washes with THF (CS-BB particles stirred at room temperature for 30 min in THF) and pentane to eliminate residual BB alkoxyamine and SG1 nitroxide respectively. CS-BB was then dried under vacuum for 2 h before analysis by solid-state ^{13}C CP-MAS NMR spectroscopy, free-solution CE and ESR.

Synthesis of chitosan-g-poly(methyl methacrylate-co-acrylonitrile), CS-g-P(MMA-co-AN)

CS-BB (0.5 g), MMA (2 g; 20 mmol) and AN (0.1 g; 2 mmol) in 1 mL of DMF were deoxygenated by argon bubbling at room temperature for 15 min. After 17 h of polymerization at 90 °C, the obtained heterogeneous mixture was diluted with 30 mL THF and stirred overnight at room temperature to dissolve any free PMMA chains. After filtration, THF washes were repeated twice but only for 2 h each and CS-g-P(MMA-co-AN) was finally dried under vacuum for 2 h before analysis by solid-state ^{13}C CP-MAS NMR spectroscopy and TGA. Visual dissolution tests proved that CS-g-P(MMA-co-AN) was not soluble in various organic media such as DMSO, dichloromethane, THF, DMF, chloroform, *N,N*-dimethylacetamide and 1,4-dioxane.

Synthesis of chitosan-g-poly(sodium 4-styrenesulfonate), CS-g-PSS

CS-BB (0.3 g), sodium 4-styrenesulfonate (2 g) and water (4 mL) were poured into a two-necked round bottom flask equipped with a condenser. The mixture was heated at 50 °C (to dissolve the monomer) and deoxygenated by argon bubbling for 30 min. After 20 h of polymerization at 85 °C, 8 mL of water were added and the mixture precipitated in a water/acetone solution (10/90 % in vol). The brown powder isolated by filtration was stirred at room temperature for 2 days in water. A fine powder was isolated by centrifugation (15 min at 5000 rpm at 20 °C) and subsequently washed with acetone, then stirred 1 night at room temperature in water. The powder was finally recovered by filtration, washed with acetone and subsequently dried under vacuum for 5 h before analysis by TGA, solid-state ^{13}C CP-MAS NMR spectroscopy and ESR.

k_d measurement (ESR)

The time evolution of the doubly integrated ESR signal of the nitroxide radicals was followed by ESR spectroscopy at

85 °C for the molecular alkoxyamines 3, 4 and 7 (Schemes S1 & S2, see ESI†) and at 100 °C for the macromolecular multi-alkoxyamine (CS-BB). The appearance of the nitroxide was followed in *tert*-butylbenzene (0.5 mL) containing around 90 mg of modified chitosan (CS-BB or CS-g-PSS) or model alkoxyamines. It has to be noted that alkoxyamine 4 (see ESI†) and the modified chitosan chains not being soluble in organic media, measurements were performed in heterogeneous conditions. O₂ was used as radical scavenger. The activation energy E_a was calculated from k_d as $k_d = Ae^{-\frac{E_a}{RT}}$ with $A = 2.4 \times 10^{14} \text{ s}^{-1}$.²⁴

Results and Discussion

CS-g-(P(MMA-co-AN)/PSS) grafted copolymer chains were synthesized under heterogeneous conditions as depicted in scheme 1. More precisely, the macromolecular multi-alkoxyamine CS-BB was prepared by a two-step procedure including i) the modification of hydroxyl and/or primary amine functions of chitosan by acryloyl chloride to introduce acrylate/acrylamide functions onto the chitosan backbone (CS-), ii) Intermolecular 1,2 Radical Addition (1,2-IRA) of BlocBuilder alkoxyamine (BB) onto acrylate/acrylamide functions. MMA/AN and SS monomers were next polymerized from CS-BB to form the corresponding grafted copolymer, CS-g-P(MMA-co-AN) and CS-g-PSS respectively.

Synthesis of the macromolecular multi-alkoxyamines (CS-BB)

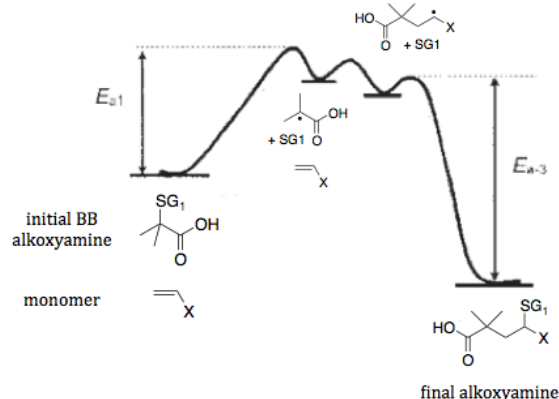
To introduce acrylate/acrylamide functions onto the chitosan backbone, 3 molar equivalents of acryloyl chloride per glucosamine unit were used. A sample of the last acetone washing solution of CS- was analyzed by GC-MS. No trace of acrylic acid (AA, coming from the hydrolysis of acryloyl chloride) and triethylamine was detected proving that no organic product was desorbed anymore. In parallel, a sample of the last washing acetone solution was slowly evaporated at room temperature to minimize the evaporation of acryloyl chloride (or acrylic acid) and then analyzed by solution-state ^1H NMR. No peak characteristic of either acryloyl chloride or acrylic acid was observed as well (see ESI†, Fig. S1).

The BlocBuilder alkoxyamine was then grafted onto chitosan by 1,2-IRA onto activated olefin, an efficient grafting method of BB that was recently reviewed.²⁵ Briefly, this method consists of decomposing the starting alkoxyamine to generate alkyl radicals that will then add onto an activated olefin to produce a new alkyl radical that is then trapped irreversibly by the *in situ* generated nitroxide to produce the final alkoxyamine.

To get a clean intramolecular 1,2 radical addition, the starting alkoxyamine structure has to be carefully designed to have a lower Bond Dissociation Energy BDE_{C-O} than the corresponding adduct (Scheme 2). It has to be noticed that in that case the BDE_{C-O} ($= E_{a,d} - E_{a,c}$) is roughly similar to the activation energy E_a of the dissociation rate constant $E_{a,d}$ since the one of the recombination $E_{a,c}$ is close to zero.²⁶

Consequently, at the reaction temperature (90 °C), the formed alkoxyamine is more stable than the initial

BlocBuilder alkoxyamine preventing undesired multiple additions.



Scheme 2 Mechanism of the intermolecular 1,2 radical addition (1,2-IRA) of the BlocBuilder alkoxyamine onto an activated olefin.

ESR analyses performed on CS-BB under heterogeneous conditions (*tert*-butylbenzene used to solubilize SG1 nitroxide) confirmed the presence of alkoxyamines onto the chitosan backbone. Indeed, upon heating at 100 °C in the presence of air as radical scavenger, the signal characteristic of SG1 was observed (Fig.1).

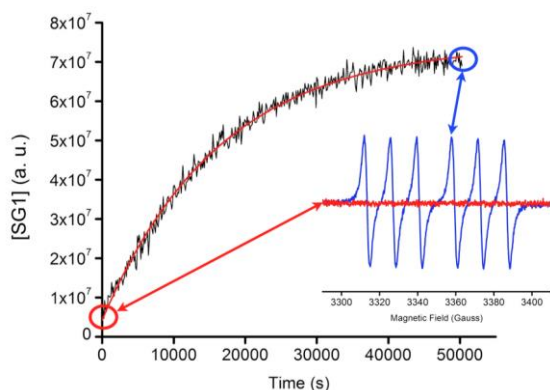


Fig. 1 Evolution of the SG1 concentration versus time upon heating of CS-BB at 100 °C under air in heterogeneous conditions. The inset illustrates the initial and final ESR spectra respectively.

The activation energy of the dissociation rate constant (k_d) of the macromolecular multi-alkoxyamine CS-BB was measured at 132 kJ.mol⁻¹. This $E_{a,d}$ value eliminates the hypothesis of BB only adsorbed onto CS since the $E_{a,d}$ of free BB is significantly lower, around 112 kJ.mol⁻¹.⁵ A model acrylamide glucosamine was also prepared to mimic the modified chitosan backbone. BB was then attached by 1,2-IRA onto its acrylamide function (acrylamide glucosamine-BB, see ESI†). The $E_{a,d}$ of this model acrylamide glucosamine-BB was measured at 127 kJ.mol⁻¹. This $E_{a,d}$ value being higher than the one measured in the case of the 1,2-IRA of BB onto acrylate (124.8 kJ.mol⁻¹ for the addition onto hydroxyethyl acrylate (HEA)²⁷ and 123 kJ.mol⁻¹ for the addition onto acrylate acetylated-glucose

(acryloylation of the primary OH of acetylated-glucose, acrylate acetylated-glucose-BB, see ESI†), we can postulate that in the case of chitosan, as expected, the acryloylation preferentially occurred onto amine functions and that subsequently the 1,2-IRA of BB preferentially occurred onto acrylamide functions rather than acrylate functions. The very high $E_{a,d}$ value measured in the case of CS-BB (132 kJ.mol⁻¹) could be explained by an entrapment of the alkoxyamine and thus of the SG1 in the chitosan matrix lowering the ability of SG1 to be liberated upon heating. The apparent C-ON bond of the CS-BB macromolecular multi-alkoxyamine may then be stronger than that of the model acrylamide glucosamine-BB alkoxyamine.

Potential solvents for CS- and CS-BB were screened by visual observation (see Table S2 in ESI†). It was observed that CS, CS- and CS-BB all dissolved in 50 mM aqueous solutions of acetic acid, hydrochloric acid or trifluoroacetic acid after a day or two, but none of them dissolved in neutral or basic aqueous solutions, or in dimethyl sulfoxide (DMSO). This can be explained by the protection of the residual amine function in the corresponding ammonium promoting dissolution at low pH. This feature allows the separation and characterization of the prepared sample in solution. The most commonly used characterization method, Size-Exclusion Chromatography (SEC/GPC), is tedious due to potential adsorption onto stationary phases and aggregation.²⁸ The heterogeneity of the chitosan in terms of degree of acetylation²⁹ and grafting also makes the separation by hydrodynamic volume meaningless with potential high local dispersities.³⁰ Free-solution capillary electrophoresis (CE) involves the separation of ionized species through an electric field within the capillary filled only with buffer. The separation of various compounds depends therefore on the electrophoretic mobility and on the electric field applied. Contrary to SEC, CE is consequently not limited by aggregation³¹ and has been successfully applied once to grafted chitosan³² and to synthetic polymers.³³ CS, CS- and CS-BB were injected in sodium phosphate buffer at pH 3, the chitosan bearing thus ammonium phosphate groups and not amines (see ESI†).

No significant differences were observed in terms of the apparent mobilities of the different samples. These mobilities can not vary by more than 5 % but this could correspond to up to 25 % of the amine groups being converted into amide (see ESI†). Indeed, the charge density is too high on this CS to prove or disprove grafting at pH 3. CS and CS-BB were also injected in sodium borate buffer at pH 9.2. CS is not charged at this pH and its injection led to no peak corresponding to a non-zero mobility (see ESI†). The carboxylic group of the BB is however negatively charged at this pH and then the CS-BB should exhibit a non-zero mobility if grafted since oligoacrylates have been successfully separated in these conditions.^{21,22} Indeed CS-BB exhibited two peaks with electrophoretic mobilities of 0.85-0.96×10⁻⁸ m².V⁻¹.s⁻¹ and 1.06×10⁻⁸ m².V⁻¹.s⁻¹ (Fig. 2).

Injection of BB solution in 10 mM NaOH aqueous solution leads to two peaks with electrophoretic mobility 1.06×10⁻⁸ m².V⁻¹.s⁻¹, also observed in CS-BB and 1.82-1.83×10⁻⁸ m².V⁻¹.s⁻¹.

$1.s^{-1}$. The peak at $1.06 \times 10^{-8} m^2.V^{-1}.s^{-1}$ corresponds to BB degradation products expected from a BB solution with 10 mM NaOH. The peak at $1.82-1.83 \times 10^{-8} m^2.V^{-1}.s^{-1}$ corresponds to the free BB and interestingly it is not observed in CS-BB.

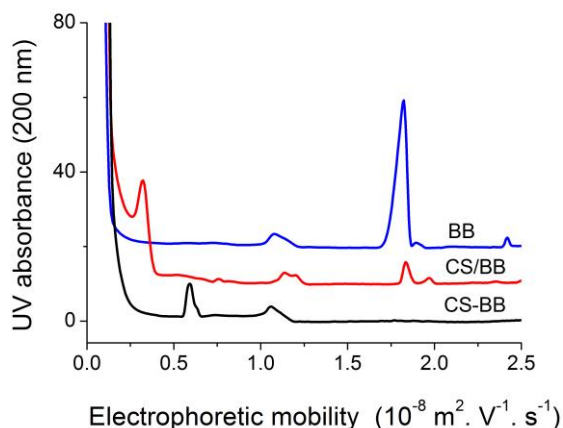


Fig. 2 Free-Solution Capillary Electrophoresis (CE) of BB and CS-BB as well as BB adsorbed onto CS (CS/BB) in sodium borate buffer (pH 9.2)

Moreover, this peak is detected when BB adsorbed onto chitosan is injected, confirming that BB in CS-BB is covalently grafted and not adsorbed. The peak at $0.85-0.96 \times 10^{-8} m^2.V^{-1}.s^{-1}$ can thus only correspond to BB grafted to chitosan (CS-BB). A peak at a significantly lower mobility, $0.59 \times 10^{-8} m^2.V^{-1}.s^{-1}$, is observed with the BB adsorbed onto CS and might correspond to CS with some BB or BB decomposition products (bearing the carboxylic acid moiety) strongly adsorbed onto CS. The success of the 1,2-IRA of BB onto chitosan was thus confirmed by free-solution CE.

It is here worth noticing that the acryloylation reactions being performed under heterogeneous conditions and almost exclusively onto amine functions as proved by ESR, the degrees of substitution (DS) of chitosan by both acryloyl chloride and BB are consequently expected to be low and very difficult to quantify. Because of this low modification ratio, solid-state ^{13}C CP-MAS and ^{31}P NMR spectroscopy could not detect the appearance of alkene groups or an increase of the carbonyl peak by solid-state ^{13}C CP-MAS NMR spectroscopy in CS- and CS-BB, contrary to Tsai et al.,³⁴ (see ESI†, Fig. S2). This is in agreement with the work of Hennig and coworkers showing that solid-state NMR may not be sensitive enough for low amounts of surface functional groups.³⁵ Nevertheless, ESR and CE, two very sensitive analytical techniques, clearly prove the presence of alkoxyamines functionalities grafted onto chitosan i. e. CS-BB. CS, CS- and CS-BB lead to identical infrared spectra because of limited resolution (see ESI, Fig. S3).

Synthesis of CS-g-P(MMA-co-AN) and CS-g-PSS grafted copolymers

As CS-BB was not soluble in organic media such as DMF, 1,4-dioxane or DMSO, the polymerizations of MMA and SS were performed under heterogeneous conditions.

The polymerization of MMA was performed in DMF at 90 °C for 17 h with 10 mol% of acrylonitrile (AN) versus MMA. Note that AN has been used as comonomer of MMA to allow the control of MMA by NMP as already reported.^{36,37,38} To eliminate possible ungrafted PMMA chains, the obtained heterogeneous mixture was diluted in 30 mL of THF and stirred overnight at room temperature. After filtration, the washes with THF were repeated twice for 2 h and CS-g-P(MMA-co-AN) was finally dried under vacuum for 2 h. Note that contrary to PMMA, CS-g-P(MMA-co-AN) was not soluble in THF and DMF, and that contrary to chitosan, it was not soluble in acidic aqueous solvents (see ESI†). The obtained dried light yellow powder was thus analyzed by solid-state ^{13}C CP-MAS NMR spectroscopy, which is an appropriate method to characterize the composition of grafted polysaccharides.³⁹ It clearly proved the presence of both chitosan⁴⁰ and PMMA,⁴¹ as well as some residual DMF (Fig 3a). Note that AN being used only in minimal amount to control the NMP of MMA, its incorporation in the copolymer is too low to be detected by ^{13}C solid-state NMR spectroscopy.

Since free-solution CE showed that BB was covalently attached to the chitosan, the synthetic polymer chains were expected to be covalently grafted as well. Nevertheless, solid-state NMR cannot distinguish CS-g-P(MMA-co-AN) from a mixture of CS and P(MMA-co-AN) homopolymer chains.

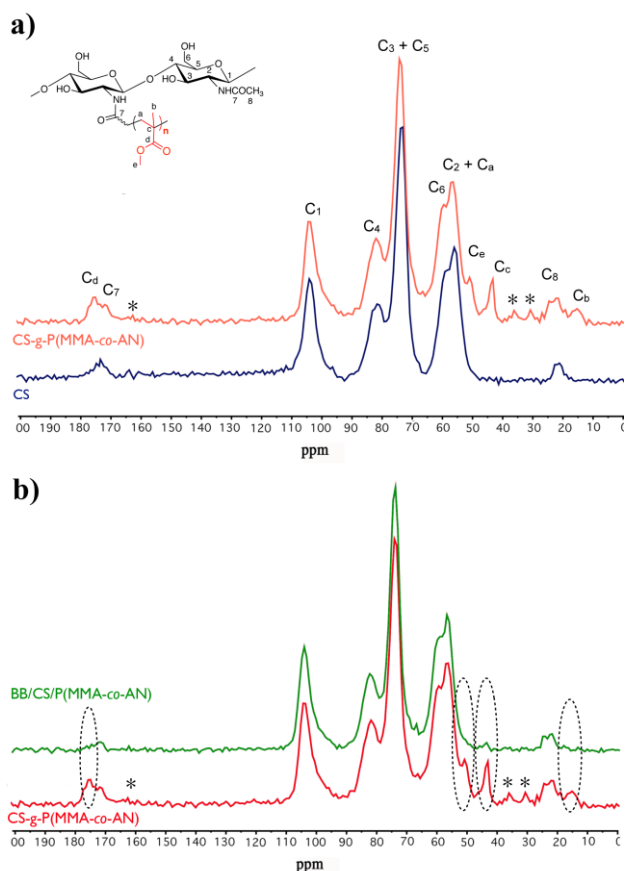


Fig. 3 Solid-state ^{13}C CP-MAS NMR spectra of a) chitosan (CS) and chitosan-*g*-poly(methyl methacrylate-*co*-acrylonitrile), CS-*g*-P(MMA-*co*-AN). b) BB-adsorbed chitosan/P(MMA-*co*-AN), BB/CS/P(MMA-*co*-AN) and CS-*g*-P(MMA-*co*-AN). * indicate the residual DMF signals. Note that all spectra were normalized with respect to the C_1 carbon of chitosan. Chemical shifts are listed in Table S1 (See ESI†).

To prove the actual synthesis of a grafted copolymer, the polymerization of MMA 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 (see ESI†). After the MMA polymerization step, the obtained BB/CS/P(MMA-*co*-AN) (co)polymer was analyzed by solid-state ^{13}C CP-MAS NMR spectroscopy. This analysis showed that the fraction of PMMA in BB/CS/P(MMA-*co*-AN) was negligible (strong decrease of the PMMA characteristic signal at 45 ppm by at least a factor 4 and other characteristic signals at the limit of detection (Fig 3b). This is confirmed by ATR-FTIR spectra showing a significantly lower PMMA fraction in BB/CS/P(MMA-*co*-AN) than in CS-*g*-P(MMA-*co*-AN) (see ESI, Fig. S6).

Note that even though CP-MAS spectra are not intrinsically quantitative, all ^{13}C CP-MAS spectra in this work were recorded at the same magnetic field, with the same contact time and the same repetition delay, allowing semi-quantitative comparisons. This control experiment proves that extensive washes is an efficient method to eliminate possible free synthetic polymer chains adsorbed onto CS and that consequently the PMMA chains observed by solid-state ^{13}C CP-MAS NMR spectroscopy in CS-*g*-P(MMA-*co*-AN) are covalently grafted onto chitosan.

TGA confirmed the presence of both chitosan and PMMA in the CS-*g*-P(MMA-*co*-AN) copolymer with a proportion of PMMA close to 20% (Fig 5). The temperatures of decomposition under argon (T_d) were clearly separated and measured at 250 °C and 360 °C for CS and P(MMA-*co*-AN) respectively.

Note that enzymes such as lysozyme or chitosanase are commonly used to digest chitosan, and could be considered to release the P(MMA-*co*-AN) chains. However, the presence of synthetic polymers is known to affect enzymes, and it is expected that such an experiment would release copolymers of P(MMA-*co*-AN) and of oligosaccharides.

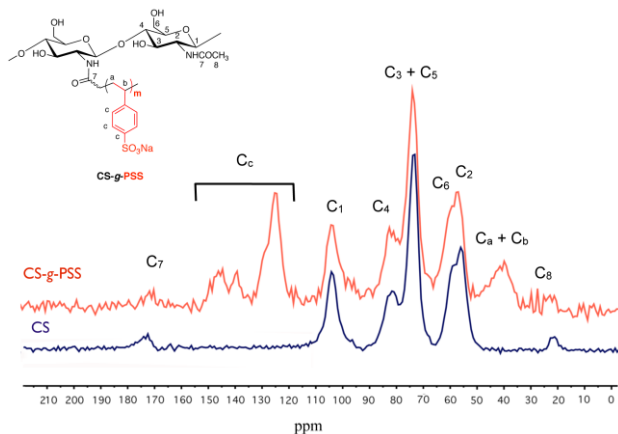


Fig.4 Solid-state ^{13}C CP-MAS NMR spectra of chitosan (CS) and chitosan-*g*-poly(sodium 4-styrenesulfonate), CS-*g*-PSS. Note that both spectra were normalized with respect to the C_1 carbon of chitosan. Chemical shifts are listed in Table S1 (See ESI†)

The same “grafting from” method was applied to sodium 4-styrenesulfonate to synthesize CS-*g*-PSS grafted copolymer. The polymerization was performed under heterogeneous conditions in water at 85 °C for 20 h. The presence of both chitosan and PSS in the sample was confirmed by solid-state ^{13}C CP-MAS NMR spectroscopy^{40,42} (Fig 4) and TGA (Fig 5) that gives a proportion of PSS in the CS-*g*-PSS grafted copolymer close to 30 %.

In addition, the release of the SG1 nitroxide upon heating of CS-*g*-PSS in heterogeneous conditions (ESR experiment in *tert*-butylbenzene) at 120 °C for 2 h proved the partial living character of the CS-*g*-PSS grafted copolymer chains (see ESI†, Fig. S7). However, the precise evaluation of the living fraction was not possible in this study as it not possible to measure the molecular weight of the copolymer (insoluble in organic and aqueous solvents).

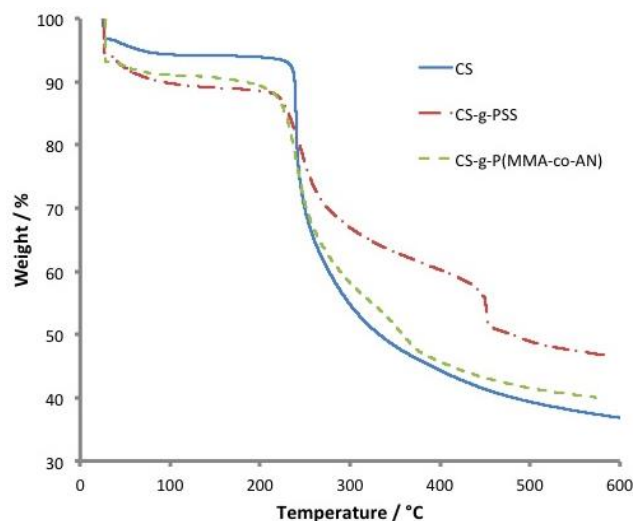


Fig. 5 TGA thermograms of CS, CS-*g*-PSS and CS-*g*-P(MMA-*co*-AN) and at a heating rate of 5 °C·min⁻¹ under argon. T_d (CS) = 250 °C, T_d (PMMA) = 360 °C and T_d (PSS) = 450 °C.

Conclusions

This work proved that 1,2-IRA could be used to introduce BlocBuilder alkoxyamine onto chitosan under heterogeneous conditions to subsequently polymerize methacrylic monomers (MMA in this work) or styrenics (sodium 4-styrenesulfonate here) and obtain the corresponding grafted copolymers (CS-*g*-P(MMA-*co*-AN) and CS-*g*-PSS respectively). The successful synthesis of the aforementioned grafted copolymers was demonstrated by solid-state ^{13}C CP-MAS NMR spectroscopy and TGA. Very interestingly, the ESR analyses of the macromolecular multi-alkoxyamine CS-BB proved that the BlocBuilder alkoxyamine was coupled onto chitosan via 1,2-IRA onto

acrylamide functions meaning that only amine functions were modified by acryloyl chloride. Free-solution CE confirmed the presence of CS-BB (and not free or adsorbed BB onto CS). Because of the heterogeneous conditions chosen to keep intact the intrinsic biological properties of the chitosan backbone, the number of amine functions that can really be modified is largely smaller than the total number of amine functions present on the chitosan backbone. This low availability degree, inherent to the grafting method under heterogeneous conditions, explains the difficulty to analyze CS= and CS-BB by solid-state NMR or Infrared spectroscopy. Nevertheless, according to TGA results, between 20 and 30 wt% of synthetic polymers were grafted onto chitosan backbone yielding a hybrid material with potential applications such as biocompatibilizer.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Complete experimental informations analytical techniques, synthetic methods, ESR, solid-state NMR, and ATR-FTIR complementary figures) See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

¹ M. Dash, F. Chiellini, R. M. Ottenbrite, E. Chiellini, *Progress in Polymer Science*, 2011, **36**, 981-1014.

² R. Jayakumar, M. Prabakaran, R. L. Reis, J. F. Mano, *Carbohydrate Polymers*, 2005, **62**, 142-158.

³ D. W. Jenkins, S. M. Hudson, *Chemical Reviews*, 2001, **101**, 3245-3273.

⁴ V. K. Mourya, N. N. Inamdar, *React. Funct. Polym.*, 2008, **68**, 1013-1051.

⁵ J. Nicolas, Y. Guillauneuf, C. Lefay, D. Bertin, D. Gigmes, B. Charleux. *Prog. Polym. Sci.* 2012, <http://dx.doi.org/10.1016/j.progpolymsci.2012.06.002>

⁶ K. Matyjaszewski, J. H. Xia, *Chemical Reviews*, 2001, **101**, 2921-2990.

⁷ J. Chiefari, Y. K. Chong, F. Ercole, J. Krstina, J. Jeffery, T. P. T. Le, R. T. A. Mayadunne, G. F. Meijs, C. L. Moad, G. Moad, E. Rizzardo, S. H. Thang, *Macromolecules*, 1998, **31**, 5559-5562.

⁸ M. Tizzotti, A. Charlot, E. Fleury, M. Stenzel, J. Bernard, *Macromolecular Rapid Communications*, 2010, **31**, 1751-1772.

⁹ J. Lindqvist, E. Malmstrom, *J. Appl. Polym. Sci.*, 2006, **100**, 4155-4162.

¹⁰ K. El Tahlawy, S. M. Hudson, *J. Appl. Polym. Sci.*, 2003, **89**, 901-912.

¹¹ N. Li, R. B. Bai, C. K. Liu, *Langmuir*, 2005, **21**, 11780-11787.

¹² P. Liu, Z. X. Su, *Materials Letters*, 2006, **60**, 1137-1139.

¹³ T. Fujie, J. Y. Park, A. Murata, N. C. Estillore, M. C. R. Tria, S. Takeoka, R. C. Advincula, *Acs Applied Materials & Interfaces*, 2009, **1**, 1404-1413.

¹⁴ N. H. Munro, L. R. Hanton, S. C. Moratti, B. H. Robinson, *Carbohydrate Polymers*, 2009, **77**, 496-505.

¹⁵ H. Q. Bao, J. H. Hu, L. H. Gan, L. Li, *J. Polym. Sci. Pol. Chem.*, 2009, **47**, 6682-6692.

¹⁶ D. B. Hua, J. Tang, J. X. Cheng, W. C. Deng, M. L. Zhu, *Carbohydrate Polymers*, 2008, **73**, 98-104.

¹⁷ J. Tang, D. B. Hua, J. X. Cheng, J. Jiang, X. L. Zhu, *Int. J. Biol. Macromol.*, 2008, **43**, 383-389.

¹⁸ J. Jiang, X. Pan, J. Cao, J. Jiang, D. Hua, X. Zhu, *Int. J. Biol. Macromol.*, 2012, **50**, 586-590.

¹⁹ D. B. Hua, W. C. Deng, J. Tang, J. X. Cheng, X. L. Zhu, *Int. J. Biol. Macromol.*, 2008, **43**, 43-47.

²⁰ J. Jiang, D. Hua, J. Jiang, J. Tang, X. Zhu, *Carbohydrate Polymers*, 2010, **81**, 358-364.

²¹ P. Castignolles, M. Gaborieau, E. F. Hilder, E. Sprong, C. J. Ferguson, R. G. Gilbert, *Macromolecular Rapid Communications*, 2006, **27**, 42-46.

²² M. Gaborieau, T. J. Causon, Y. Guillauneuf, E. F. Hilder, P. Castignolles, *Australian Journal of Chemistry*, 2010, **63**, 1219-1226.

²³ C. R. Morcombe, K. W. Zilm, *Journal of Magnetic Resonance*, 2003, **162**, 479-486.

²⁴ D. Bertin, D. Gigmes, S. R. A. Marque, P. Tordo, *Chem. Soc. Rev.*, 2011, **40**, 2189-2198.

²⁵ D. Gigmes, P. E. Dufils, D. Gle, D. Bertin, C. Lefay, Y. Guillauneuf, *Polym. Chem.*, 2011, **2**, 1624-1631.

²⁶ J. Sobek, R. Martschke and H. Fischer, *J. Am. Chem. Soc.*, 2001, **123**, 2849-2857.

²⁷ D. Bertin, P. E. Dufils, I. Durand, D. Gigmes, B. Giovanetti, Y. Guillauneuf, S. R. A. Marque, T. Phan, P. Tordo, *Macromol. Chem. Phys.*, 2008, **209**, 220-224.

²⁸ M. Rinaudo, *Progress in Polymer Science*, 2006, **31**, 603-632.

²⁹ S. Nguyen, S. Hisiger, M. Jolicoeur, F. M. Winnik, M. D. Buschmann, *Carbohydrate Polymers*, 2009, **75**, 636-645.

³⁰ M. Gaborieau, P. Castignolles, *Analytical and Bioanalytical Chemistry* 2011, **399**, 1413-1423.

³¹ D. L. Taylor, C. J. Ferris, A. R. Maniego, P. Castignolles, M. in het Panhuis, M. Gaborieau, *Australian Journal of Chemistry*, 2012, in press, <http://dx.doi.org/10.1007/CH12211>

³² X. Fu, L. Huang, M. Zhai, W. Li, H. Liu, *Carbohydrate Polymers*, 2007, **68**, 511-516.

³³ H. Cottet, P. Gareil, *Methods Mol. Biol. (Totowa, NJ, U. S.)* 2008, **384**, 541-567.

³⁴ B. H. Tsai, C. H. Lin, J. C. Lin, *J. Appl. Polym. Sci.*, 2006, **100**, 1794-1801.

³⁵ A. Hennig, H. Borchering, C. Jaeger, S. Hatami, C. Wurth, A. Hoffmann, K. Hoffmann, T. Thiele, U. Schedler, U. Resch-Genger *J. Am. Chem. Soc.* 2012, **134**, 8268-8276.

³⁶ J. Nicolas, S. Brusseau, B. Charleux, *J. Polym. Sci. Pol. Chem.*, 2010, **48**, 34-47.

³⁷ B. Charleux, J. Nicolas, O. Guerret, *Macromolecules*, 2005, **38**, 5485-5492

-
- ³⁸ J. Nicolas, C. Dire, L. Mueller, J. Belleney, B. Charleux, S. R. A. Marque, D. Bertin, S. Magnet, L. Couvreur, *Macromolecules*, 2006, **39**, 8274–8282.
- ³⁹ M. Gaborieau, H. DeBruyn, S. Mange, P. Castignolles, A. Brockmeyer, R. G. Gilbert, *Journal of Polymer Science Part A Polymer Chemistry*, 2009, **47**, 1836-1852.
- ⁴⁰ C. Gartner, B. L. Lopez, L. Sierra, R. Graf, H. W. Spiess, M. Gaborieau, *Biomacromolecules*, 2011, **12**, 1380-1386.
- ⁴¹ M. Wilhelm, M. Neidhoefer, S. Spiegel, H. W. Spiess, *Macromolecular Chemistry and Physics*, 1999, **200**, 2205-2207.

Heterogeneous modification of chitosan via nitroxide-mediated polymerization

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Electronic Supporting Information

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2.7 Synthesis of acrylate acetylated-glucose-BB

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 μL . The column was a capillary column TR-1MS 30m \times 0.25mm \times 0.25 μm 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 ^1H NMR experiments were performed in acetone- d_6 on a Bruker Avance 300 spectrometer at 300 MHz.

Solid-state ^1H 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 ^1H 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}C NMR experimental details are reported in the main text. The assignment of observed ^{13}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 cm^{-1}/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}C NMR chemical shifts done by comparison with literature spectra of CS,¹ PMMA,² PSS³ and DMF.⁴

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

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

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

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

⁴ 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⁻¹ 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.

| Sample Solvent | CS | CS= | CS-BB | CS-<i>g</i>- PSS | CS-<i>g</i>- P(MMA- <i>co</i>-AN) |
|---------------------------------|-----------|------------|--------------|-----------------------------|--|
| 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 coworkers⁵ 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.⁶ Moreover, recently Charlot and coworkers⁷ 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

⁵ 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.

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

⁷ 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 ^1H 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.

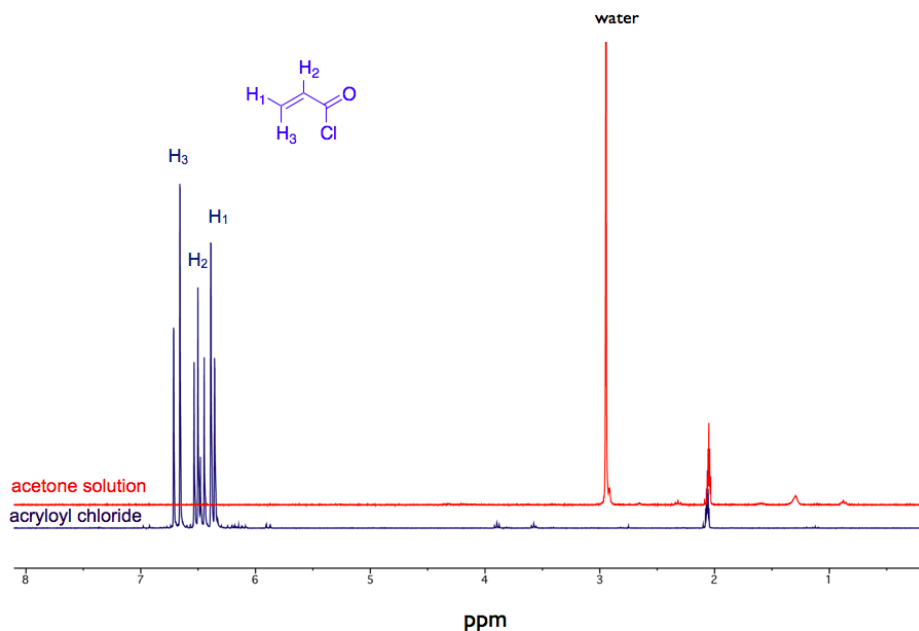


Fig. S1. ^1H liquid NMR spectra of acryloyl chloride (blue) and acetone solution (red) used to extract possible acryloyl chloride adsorbed on CS= . The analyses were performed in acetone- d_6 .

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}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).

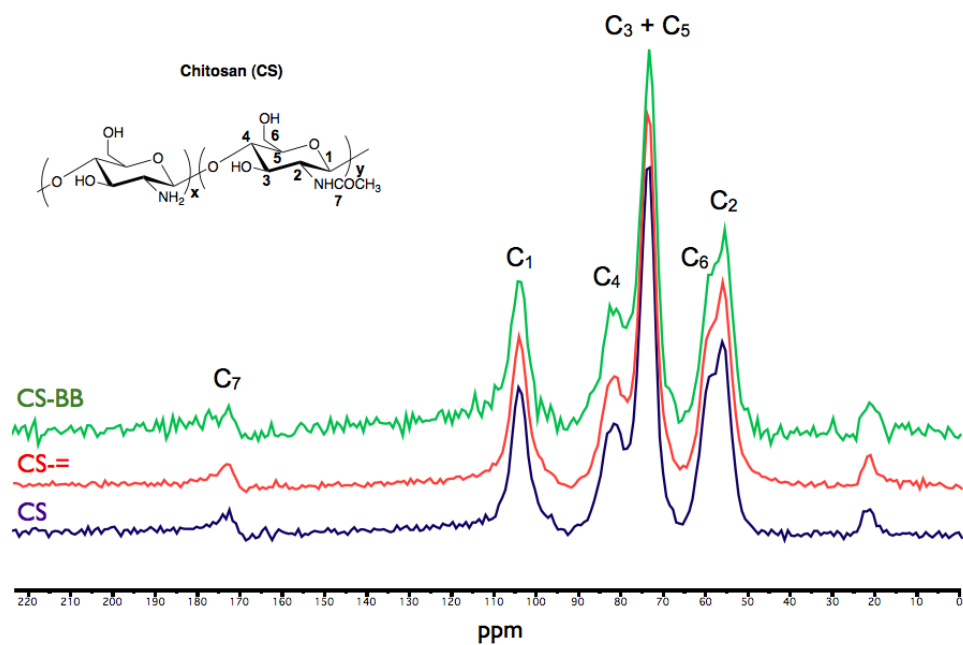


Fig. S2. Solid-state ^{13}C CPMAS NMR spectra of CS, CS-=- and CS-BB.

2.2.3 Analysis by IR Spectroscopy

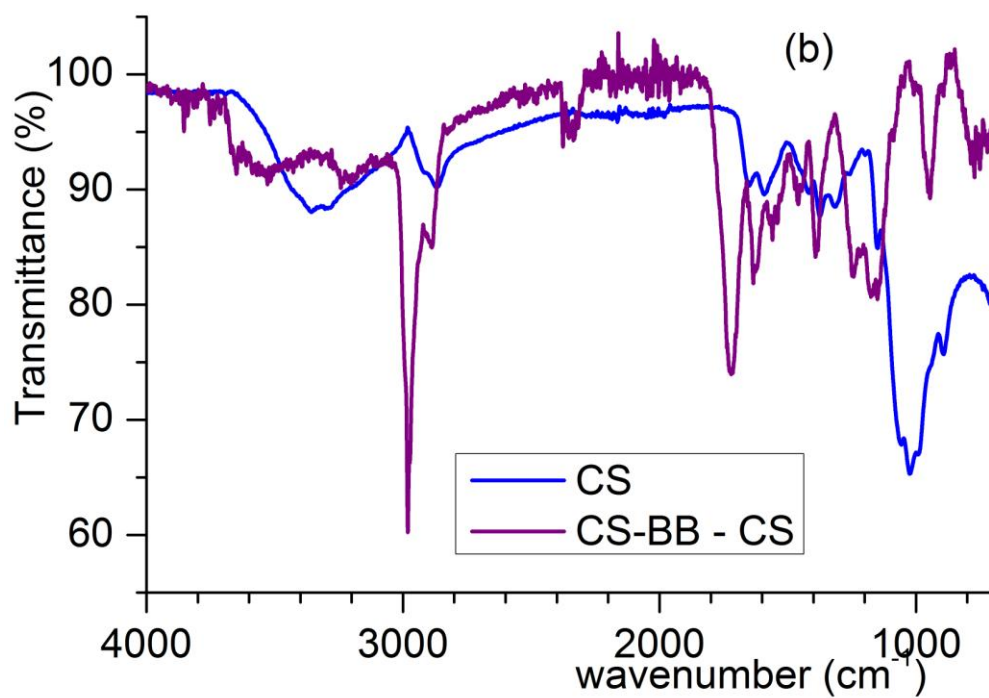
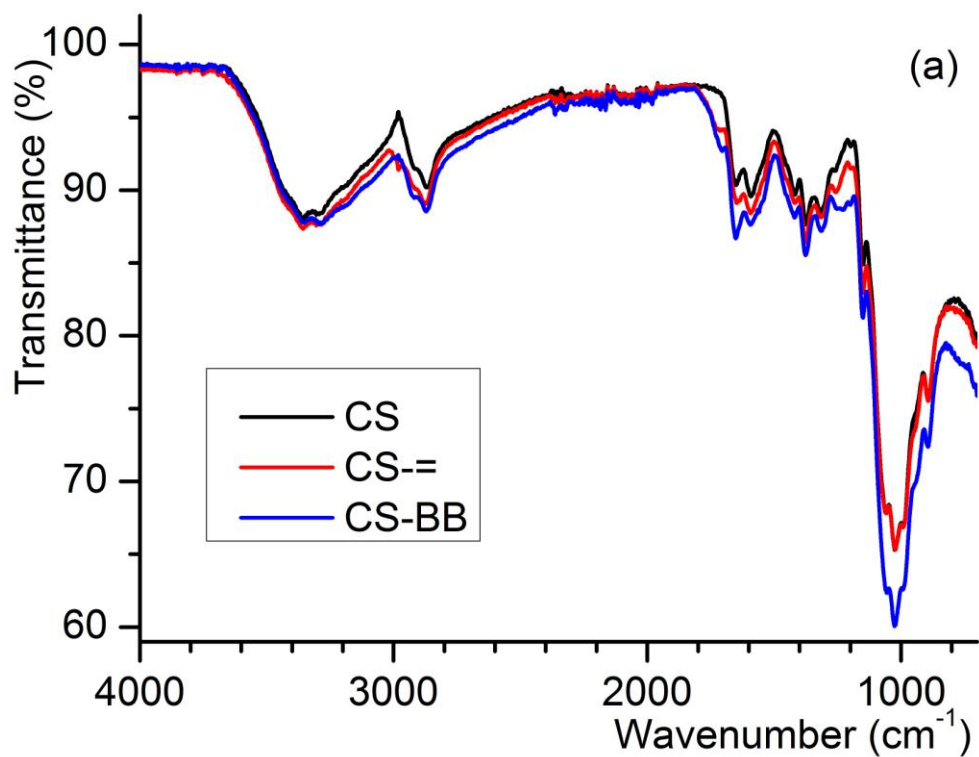


Fig. S3. ATR FT-IR spectra: (a) CS, CS= and CS-BB; (b) CS and the subtraction of CS from CS-BB.

The FT-IR spectra of CS, CS= and CS-BB are similar (Fig. S3a). The only different band is a shoulder at 1722 cm⁻¹ on CS= and CS-BB and to a lesser extent at narrow band at 2982 cm⁻¹ (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 ⁻¹) in this work (Fig. S3) | Assignment of band | Wavenumber in ⁸ | Wavenumber in ⁹ |
|---|--|----------------------------|----------------------------|
| 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 |

⁸ 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.

⁹ 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- α , 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 %.¹⁰ 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.

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

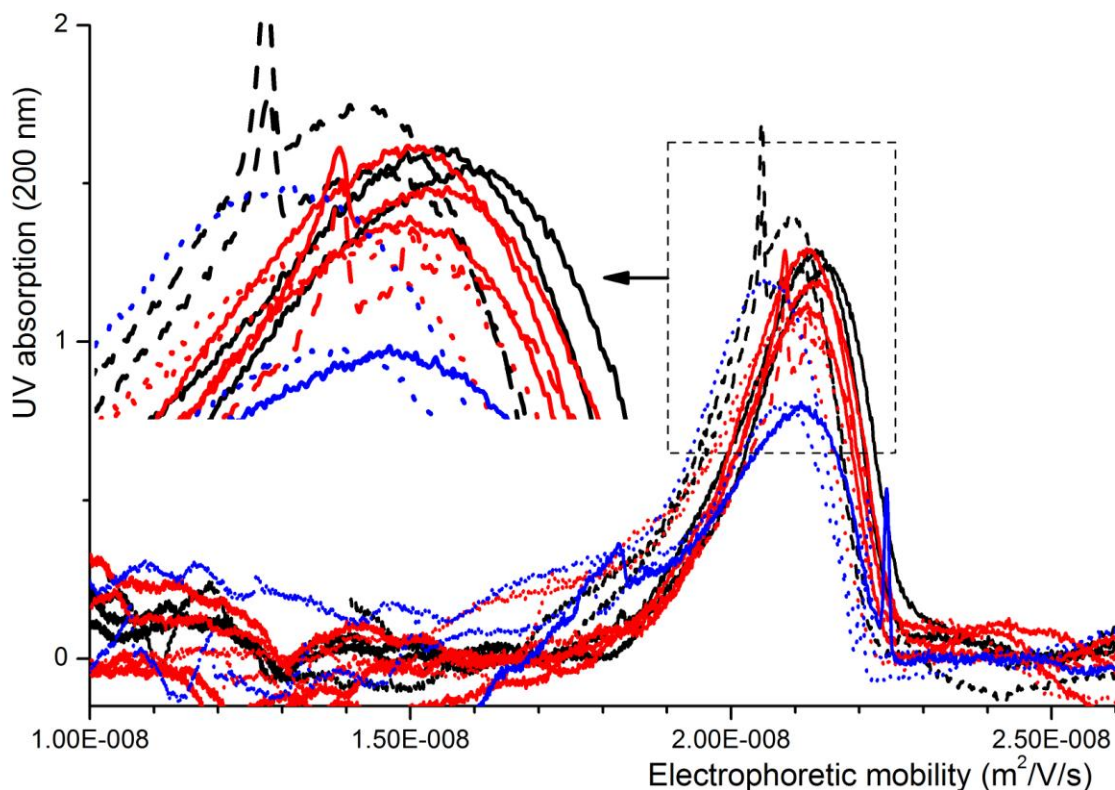


Fig S4. Free-solution Capillary Electrophoresis (CE) of CS, CS-= and CS-BB in sodium borate buffer at pH 3: CS dissolved in acetic acid (solid black line), CS dissolved in HCl (dashed black line), CS-= dissolved in acetic acid (solid red line), CS-= dissolved in HCl (dashed red line), CS-= dissolved 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.¹¹ In term of composition, a degree of sulfonation in polystyrene corresponds to the degree of deacetylation of chitosan.

¹¹ 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,¹² 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 ¹³C 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 m².V⁻¹.s⁻¹). 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

¹² 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.¹³ 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).

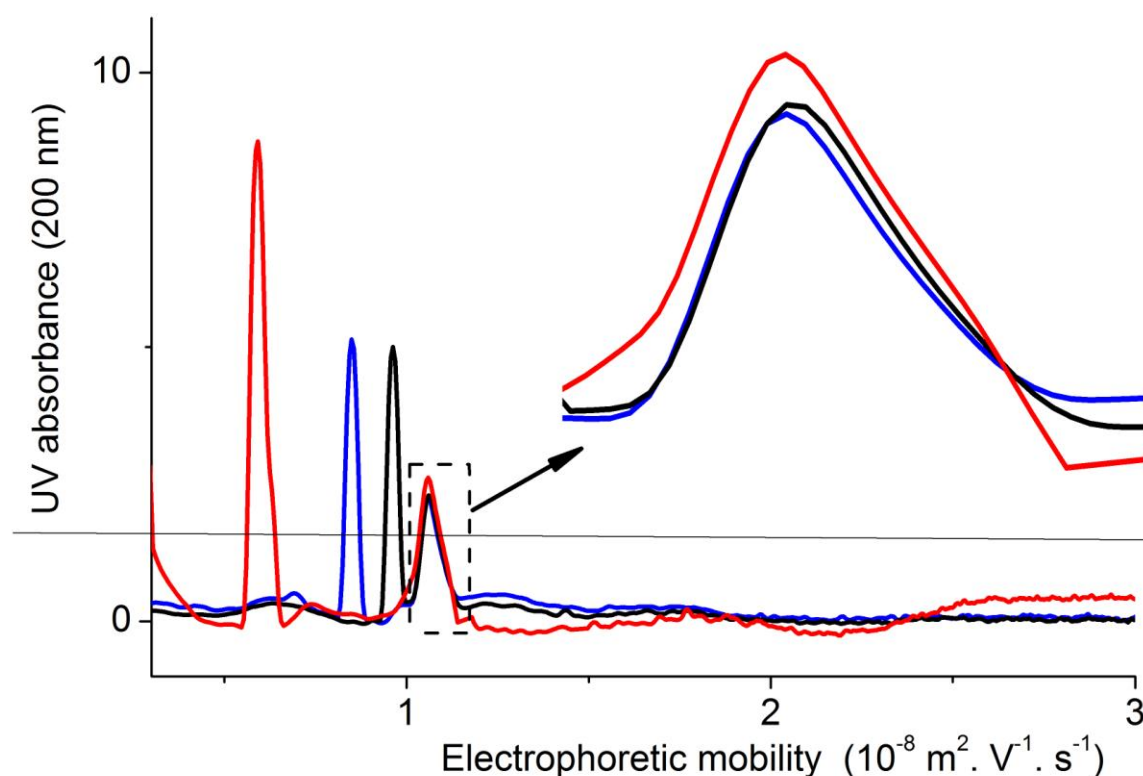


Fig S5. Repeatability plot for the injection of CS-BB in sodium borate

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

¹³ N. L. Hoang, A. Landolfi, A. Kravchuk, E. Girard, J. Peate, J. M. Hernandez, M. Gaborieau, O. Kravchuk, R. G. Gilbert, Y. Guillaueuf, 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 ¹³C 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.

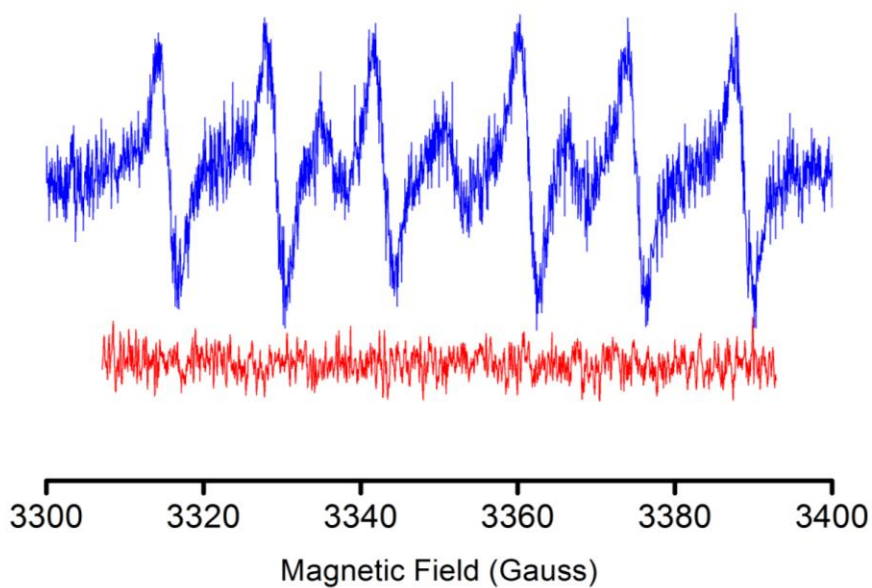
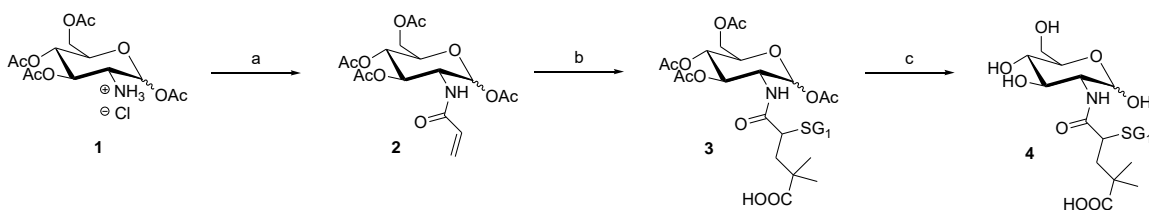


Fig S6. ESR measurement of CS-*g*-PSS heated at 120 °C for 2 h in the cavity in presence of *tert*-butylbenzene (heterogeneous conditions)

2.6 Synthesis of acrylamide glucosamine-BB (4)



Scheme S1. Reagents and conditions (a) $\text{CH}_2=\text{CHC}(\text{O})\text{Cl}$, Et_3N , $\text{CH}_2\text{Cl}_2/\text{THF}$, (b) BB, EtOH , (c) KOH , MeOH

2.6.1. 1,3,4,6-tetra-*O*-acetyl-*N*-acryloyl- β -glucosamine (2)

To a solution of **1**¹⁴ (2 g, 5.74 mmol) in CH₂Cl₂/anhydrous THF (17 mL/30 mL) was added Et₃N (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 CH₂Cl₂. The organic media was washed twice with successively saturated NaHCO₃ and 1M HCl. After drying over MgSO₄, CH₂Cl₂ 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%). ¹³C NMR (300 MHz, CDCl₃, 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=CH₂)), 129.9 (CH=CH₂), 128.1 (CH=CH₂), 90.8 (C₁), 70.8, 69.9, 67.6 (C₃, C₄, C₅), 61.7 (C₆), 51.3 (C₂), 21.0 (CH₃), 20.8 (2 CH₃), 20.7(CH₃).

ESI(+)-TOF HR-MS calculated for [C₁₇H₂₃NO₁₀ + H]⁺, m/z 402.1395; found m/z 402.1404 (error: +2.2 ppm)

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

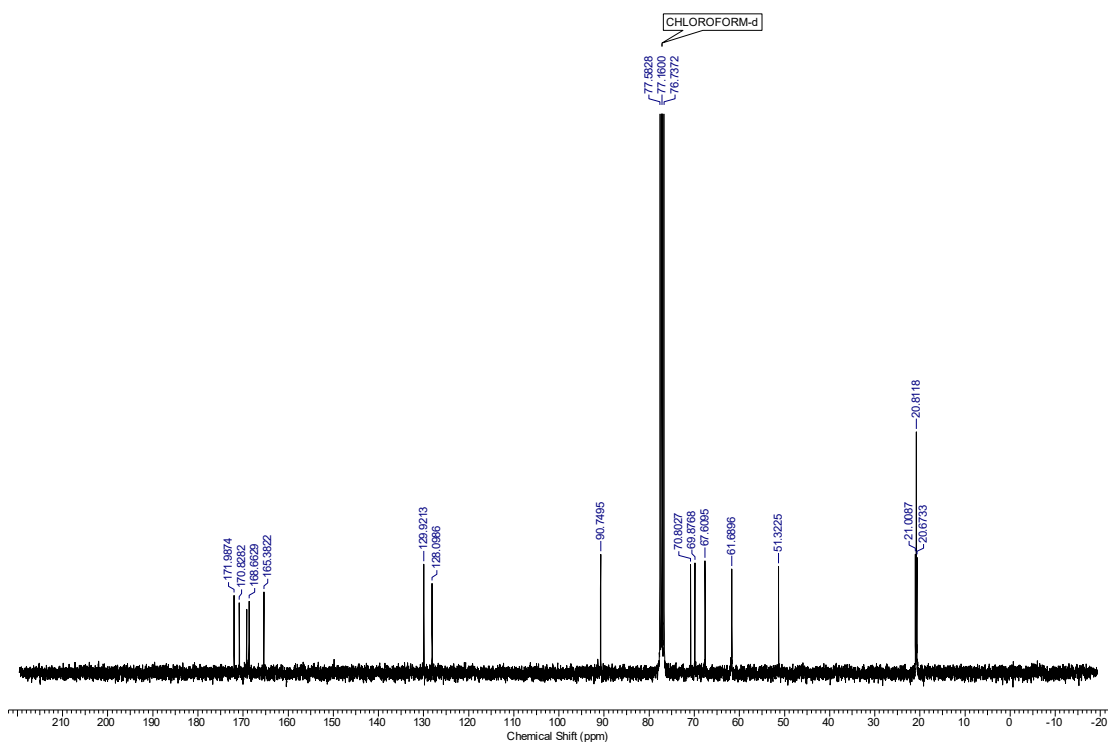


Figure S7. ^{13}C liquid NMR spectra of **2** performed in CDCl_3 .

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}P NMR (300 MHz, 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)

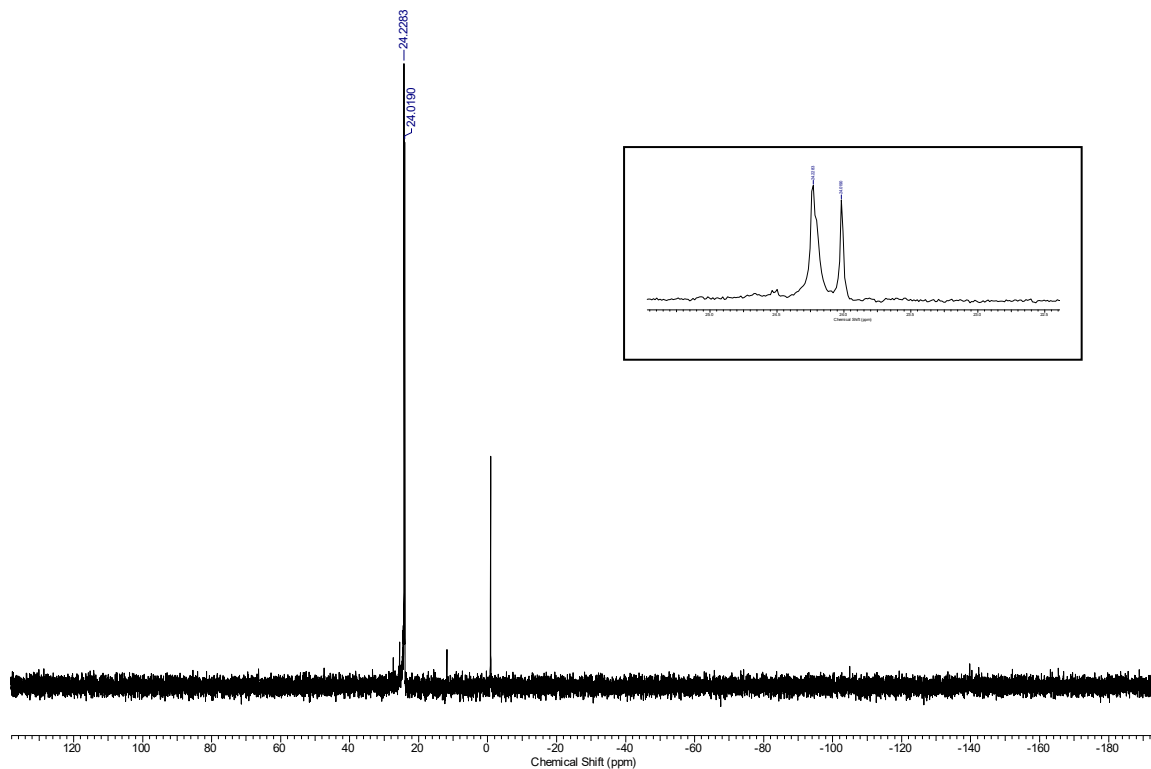


Figure S8. ^{31}P liquid NMR spectra of **3** performed in $CDCl_3$.

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) δ (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).

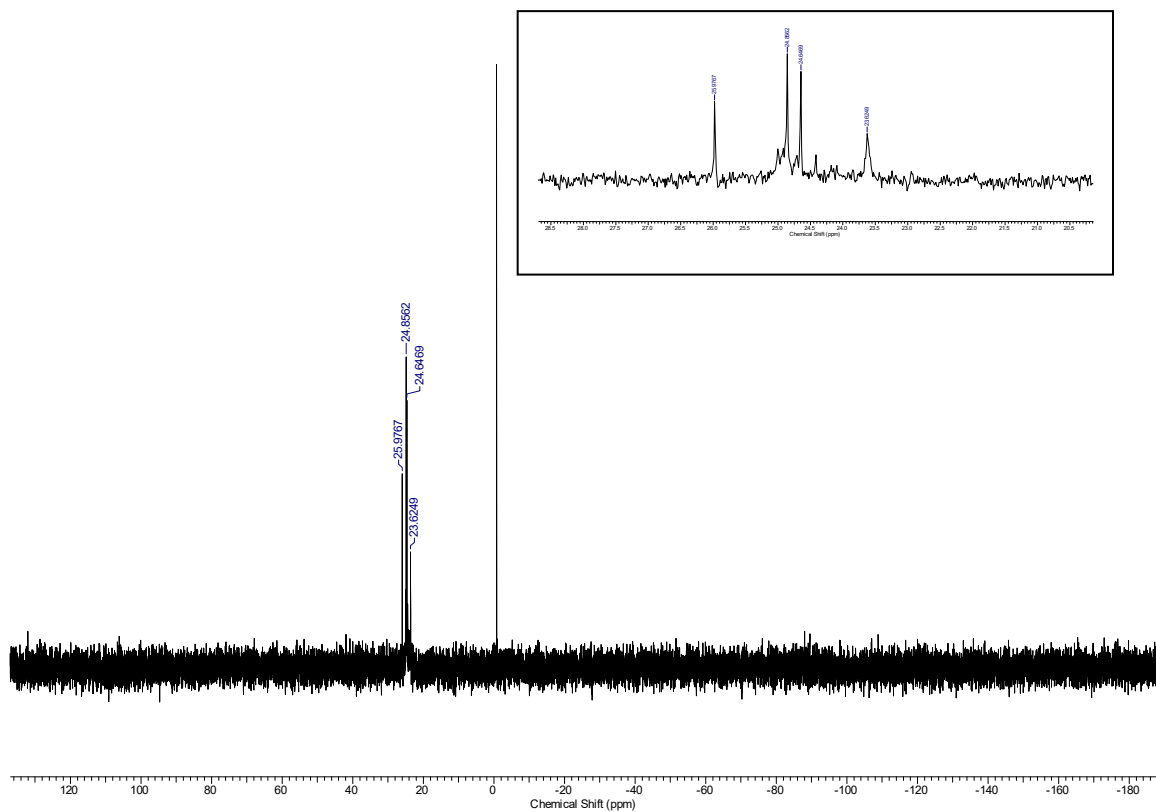
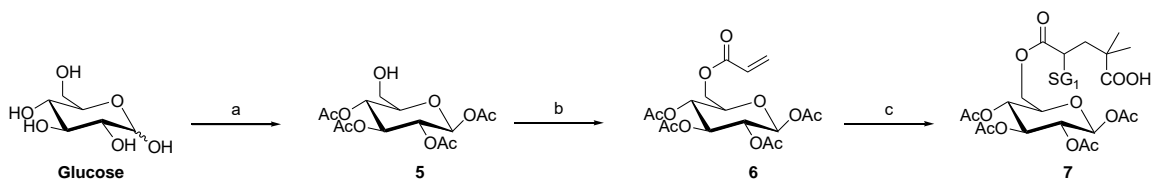


Figure S9. ^{31}P liquid NMR spectra of 4 performed in $DMSO-d_6$.

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



Scheme S2. Reagents and conditions (a) 1. $\text{ClC}(\text{Ph})_3$, Ac_2O , Pyridine ; 2. HBr , AcOH , (b) $\text{CH}_2=\text{CHC}(\text{O})\text{Cl}$, Et_3N , THF, (c) BB, EtOH

2.7.1. 1,2,3,4-tetra-O-acetyl- β -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 CHCl_3 and after separation, the organic layer was washed three times with iced water, dried over MgSO_4 and evaporated under reduced pressure to give a viscous oil. Et_2O (250 mL) and pentane (250 mL) were successively added under stirring to give a white solid after filtration (2.15 g, 19 %). ^1H NMR (300 MHz, CDCl_3) δ (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, CH_3), 2.04 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.99 (s, 3H, CH_3).

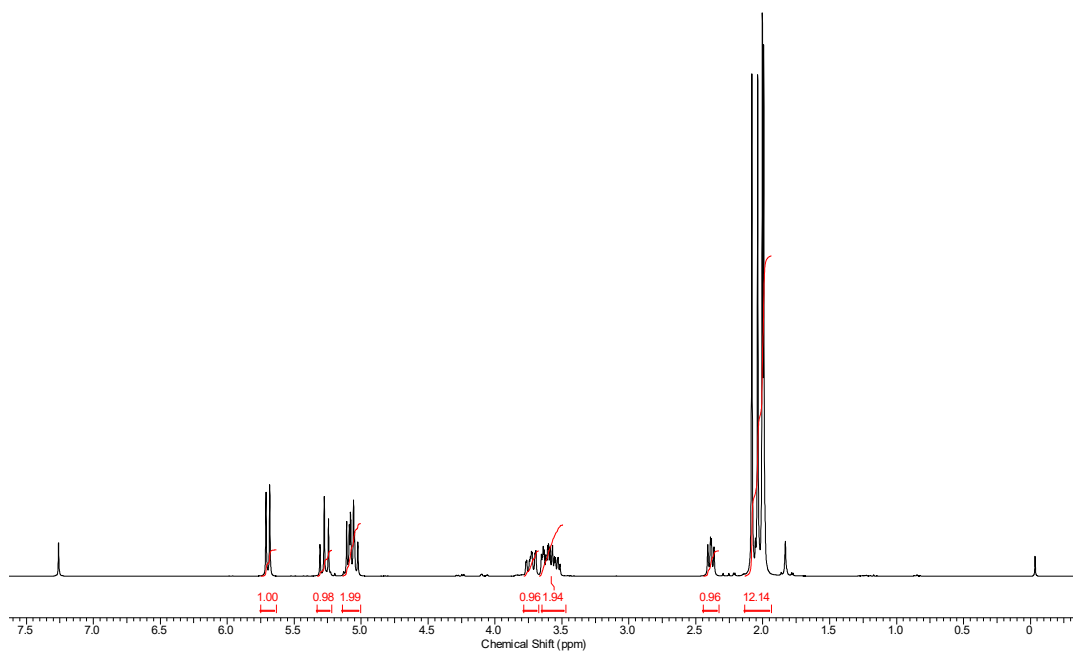


Figure S10. ^1H liquid NMR spectra of **5** performed in CDCl_3

^{13}C NMR (300 MHz, CDCl_3) δ (ppm) 170.3 (C=O), 170.2 (C=O), 169.4(C=O), 169.2 (C=O), 91.8 (C_1), 75.0, 72.7, 70.5, 68.3 (4C), 60.9 (C_6), 20.9 (2 CH_3), 20.7 (2 CH_3).

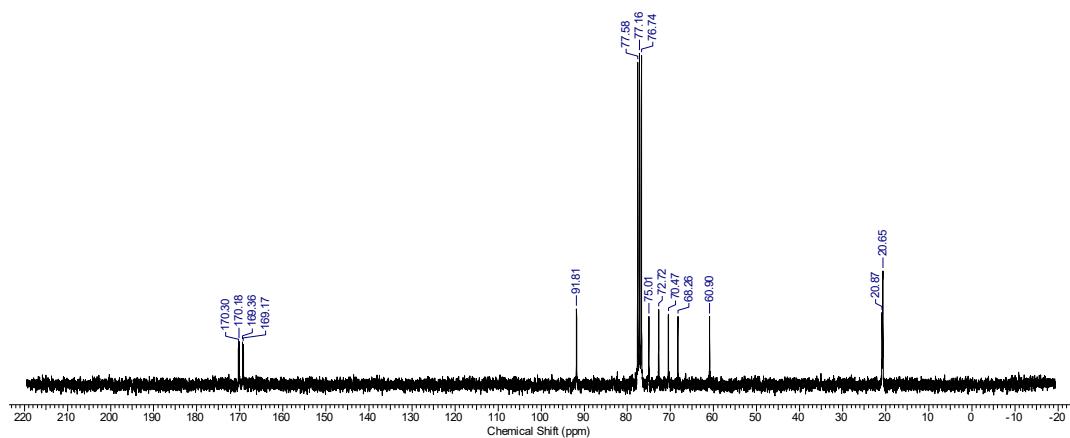


Figure S11. ^{13}C liquid NMR spectra of **5** performed in CDCl_3

2.7.2. 1,2,3,4-tetra-*O*-acetyl-6-*O*-acryloyl- β -D-glucose (**6**)

Et₃N (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 %). ¹³C NMR (300 MHz, CDCl₃) δ (ppm) 170.3 (C=O), 169.5 (C=O), 169.4(C=O), 169.1 (C=O), 165.7 ((-CO-(CH=CH₂))), 132.0 (CH=CH₂), 127.7 (CH=CH₂), 91.7 (C₁), 72.9, 72.7, 70.3, 68.0 (4C), 61.8 (C₆), 20.8 (CH₃), 20.6 (3CH₃).

ESI(+)-TOF HR-MS calculated for [C₁₇H₂₂O₁₁ + NH₄]⁺, m/z 420.1500; found m/z 420.1510 (error: +2.4 ppm) .

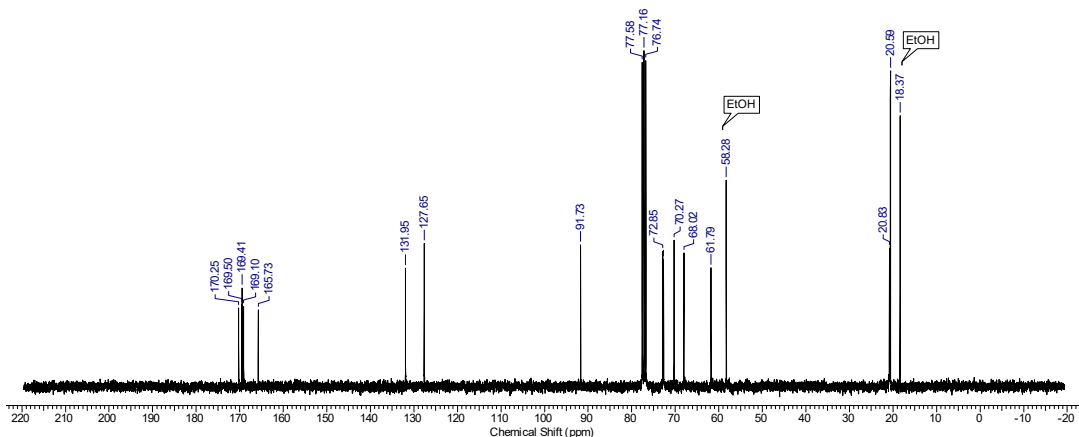


Figure S12. ¹³C liquid NMR spectra of **6** performed in CDCl₃

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- β -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}P NMR (400 MHz, CDCl_3 , major diastereoisomers) δ (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).

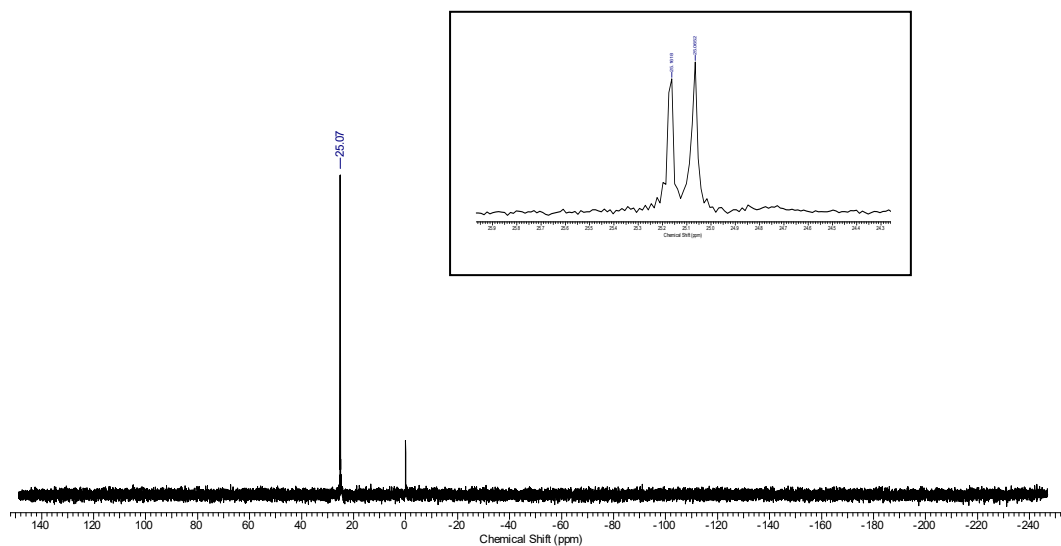


Figure S13. ^{31}P liquid NMR spectra of **7** performed in CDCl_3