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New prospects for the synthesis of N-alkyl phosphonate/phosphonic acid-bearing oligo-chitosan

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N-phosphonomethylation reactions of oligo-chitosan were performed according to Moedritzer and Kabachnik-Fields conditions. The different Moedritzer reaction conditions used did not allowed the phosphonomethylation. On the contrary, the Kabachnik-Fields reactions led to oligo-chitosan methyl phosphonated derivatives. In addition, novel dialkyl phosphoryl oligo-chitosan were synthesized in water at room temperature via epoxy-amine reactions of oligo-chitosan with dialkyl (3-(oxiran-2-ylmethoxy)propyl) phosphonates. This simple and efficient synthetic method provides a new approach for the preparation of phosphonated oligo-chitosan derivative. Then, the hydrolysis of the phosphonated compounds to generate the phosphonic acid moities was investigated. The mildest conditions were determined in order to avoid the chitosan backbone degradation. All the products were characterized by ^{1}H and ^{31}P NMR analyses.

Introduction

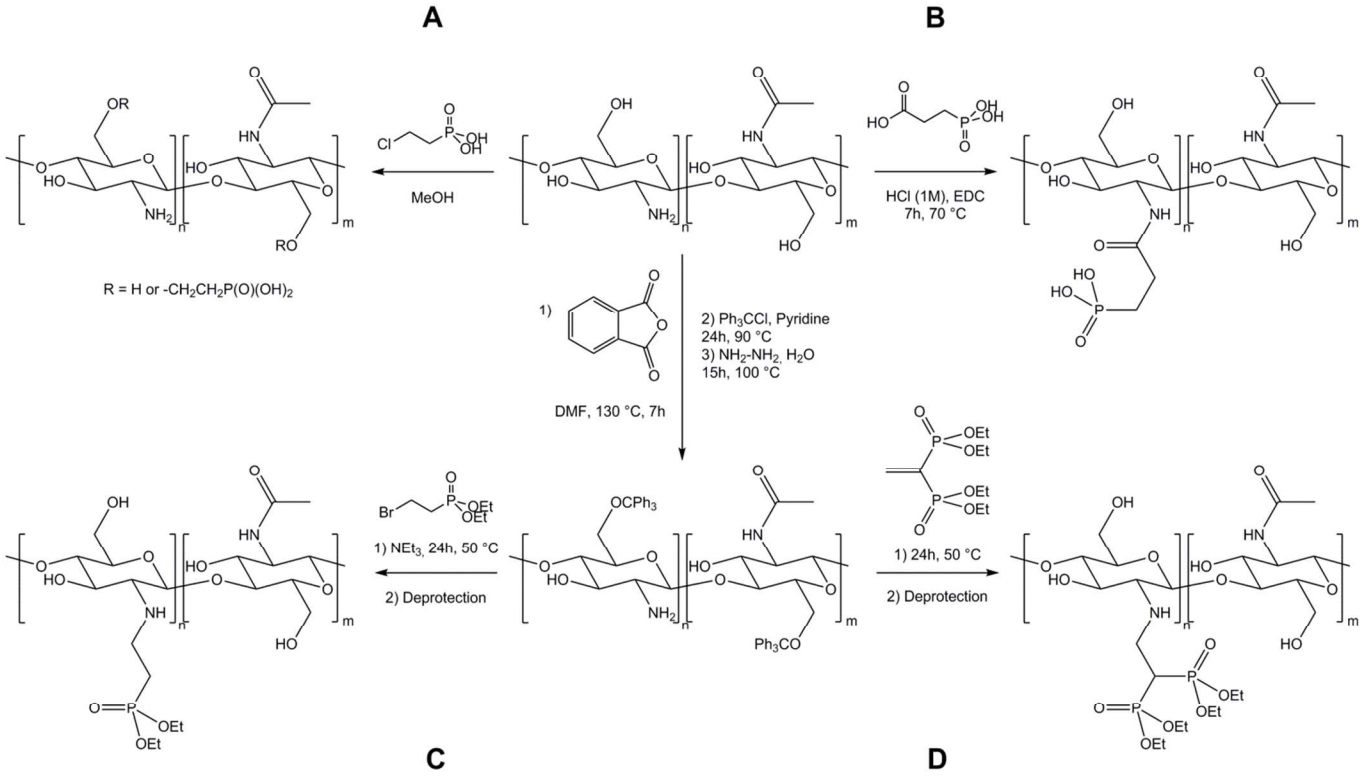
Chitosan is the fully or partially deacetylated form of chitin[1] the second most abundant natural polysaccharide derived from exoskeletons of crustaceans and also cell walls of fungi and insects[2]. With the poly(lysine), chitosan is one of the very few polymer from a natural origin which has primary amino groups along its backbone. This low-cost biopolymer possesses very interesting properties, for instance it is known to be biocompatible[3], biodegradable in the human body[4], non toxic[5] and antibacterial[6].

Interest in chitosan materials is quite recent compared to cellulose, which has an age-long exploitation history. Therefore, chitosan is one of the most promising materials derived from renewable resources and is currently explored very intensively[7]. In the last decades unmodified chitosan has been widely used in a variety of applications: for example as wound dressing[8], in tissue engineering[9], cosmetics[10], food[11] or textile industry[12], and in waste water treatment[13]. Specific groups can also be introduced to achieve original chitosan derivatives with new physicochemical properties and improved performances for selected applications. For instance, quaternized chitosan and PEGylated chitosan copolymers have been studied for anion exchange membrane synthesis[14] and for drug delivery applications[15], respectively. Similarly, carboxymethylated chitosan was used in tissue engineering[16].

Phosphorous containing polymers have numerous potential applications as they exhibit interesting properties in term of adhesion and thus are excellent anti-corrosion compounds[17, 18]. Ion-exchange resins[19], dental adhesives[20] and fire retardant[21] are some other common applications. Several techniques to synthesize phosphorous-containing chitosan derivatives have already been published due to their interesting biological and physico-chemical properties, for example bactericidal[22], flame retardant[23] and heavy metal-chelating properties[24]. Phosphorylation of the hydroxyl functions from chitosan to yield phosphate groups has been performed according to several ways: i/phosphorylated chitosan can be prepared by heating chitosan with orthophosphoric acid and urea in DMF[25, 26], ii/ by reacting chitosan with phosphorous pentoxide in the presence of methane sulfonic acid[27, 28], iii/ or in a H_3PO_4/Et_3PO_4/hexanol mixture[29]. Chitosan alkyl phosphates were also synthesized through the use of chlorophosphates: diethyl chlorophosphate[30] or 2-chloro-2-oxo-1,2,3-dioxaphospholane[31]. Less commonly phosphate-functionalized chitosan derivatives can be synthesized by grafting polymerization of mono (2-methacryloyl oxyethyl) acid phosphate initiated by ceric ammonium nitrate[32] or directly via Michael addition with mono-(2-acryloyloxyethyl) phosphonate[33]. Few papers deal with the synthesis of phosphate and amidophosphate chitosan derivatives by Atherton Todd reaction[34, 35].

Phosphate and phosphoramide groups have excellent chelating properties but they are not very stable toward hydrolysis. In general, phosphonate groups are much less sensitive towards hydrolysis than phosphates[36]. Therefore, even if they are slightly less efficient as chelating agent, it could be of great interest to introduce phosphonate or phosphonic acid groups onto chitosan. The phosphonation of chitosan has been studied according to several pathways (Scheme 1). Phosphonation of

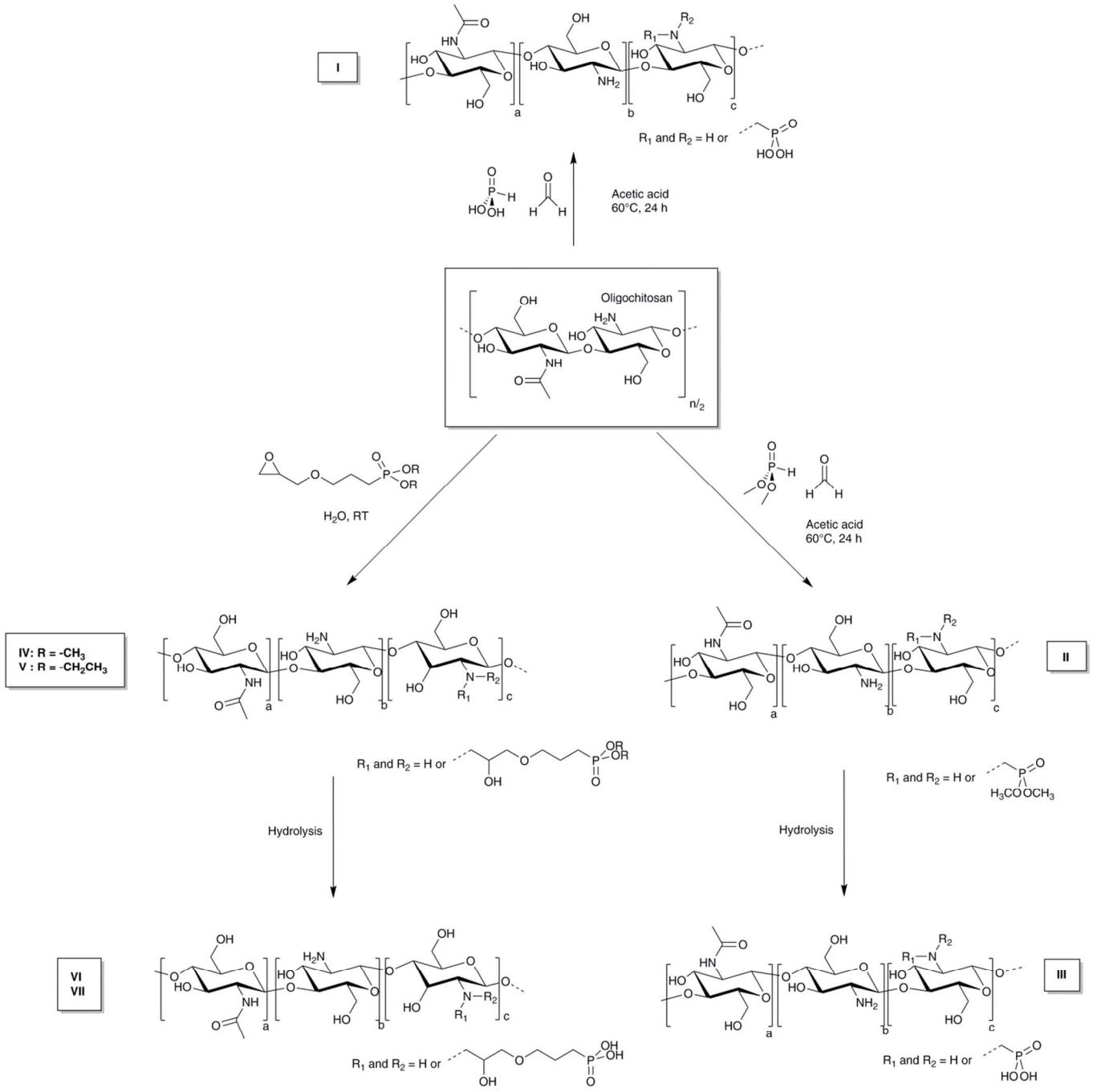
the hydroxyl functions was carried out with 2-chloro ethyl phosphonic acid (Scheme 1-A)[37]. Recently, Lebouc et al.[38] reported two different reactions with chitosan amino groups that led to alkyl-phosphonate-containing derivatives: alkylation with a halogeno-phosphonate compound (Scheme 1-C) and Michael addition using a tetraethyl vinylidenebisphosphonate (Scheme 1-D). These coupling reactions are characterized by high yields and soft conditions. However, they are performed in THF on 6-O-triphenylmethyl-chitosan and thus require additional protection and deprotection steps that appear to be not complete. Chitosan was also derivatized by the reaction of its primary amine groups with molecules containing carboxylic acid moieties. For instance by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) mediated coupling reaction, 2-carboxyethyl phosphonic acid was covalently grafted onto chitosan (Scheme 1-B)[39]. This reaction proceeds in very smooth condition and is almost quantitative. Nevertheless, the use of an expensive coupling reagent may not be suitable for large scale reactions.



Scheme 1: Preparation methods of phosphonated chitosan [37-39].

Currently, the most common chitosan phosphonation reaction is the Moedritzer reaction. Numerous papers report the introduction of α-aminomethylphosphonic acid groups onto chitosan using this reaction[40-51]. However, the results of these papers are sometimes contradictory. In fact, some authors claim a regioselective functionalization of the primary amines[40] in contradiction to others who also report the esterification reaction of the hydroxyl groups[41]. More recently, Lebouc et al.[46] noticed the predominance of a side reaction, the methylation of the amines leading to *N*-methyl and *N*,*N*-dimethyl chitosan. In contrast to Moedritzer reaction, the synthesis of α-aminomethylphosphonate by Kabachnik-Fields reaction was not much studied in spite of the interest of such groups[41].

In this paper, different strategies were used to synthesize aminoalkyl phosphonic acid (phosphonated) oligo-chitosans (Scheme 2). We first tried to introduce in one step phosphonic acid groups according to a Moedritzer-type reaction[40]. Then, we followed two-steps reactions pathways: i/during the first step, alkyl phosphonate groups were introduced either according to a Kabachnik-Fields reaction or according to epoxy-amine reactions of oligo-chitosan with dialkyl (3-(oxiran-2-ylmethoxy)propyl) phosphonates, ii/ then, the dealkylation of the phosphonic ester functions was investigated. Numerous papers show that chitosan primary amine groups react with epoxide compounds[52] but to our knowledge, it is the first time that the epoxy-amine reaction has been used to introduce phosphonate groups onto oligo-chitosan. All the products synthesized were fully characterized by ^{1}H and ^{31}P NMR and IR spectroscopies and their thermal properties were assessed.



Scheme 2: Syntheses of phosphonic acid-containing chitosan described in this paper: Moedritzer reaction (I), Kabachnik-Fields reaction (II) followed by hydrolysis (III) and epoxy-amine reaction (IV-V) followed by hydrolysis (VI-VII).

Experimental Part

Materials

Chitosan ("652", shrimp shell origin, degree of deacetylation = 90%, Mn ≈ 150 000 g.mol^{-1}, France Chitine), phosphorous acid (99%, Aldrich), acetic acid (> 99.7%, Aldrich), formaldehyde (37 w% aqueous solution, Aldrich), paraformaldehyde (95%, Aldrich), isopropanol (99%, Aldrich), dimethyl phosphite (98%, Aldrich), dimethyl (3-(oxiran-2-ylmethoxy)propyl) phosphonate (Specific Polymers), and diethyl (3-(oxiran-2-ylmethoxy)propyl) phosphonate (Specific Polymers) were used as received. Ultra-pure water was obtained from a Millipore Milli-Q purification system.

Oligo-chitosan (degree of deacetylation = 90%, Mn ≈ 2500 g.mol^{-1}) was synthesized according to Illy et al. [52].

Instrumentation

^{1}H, ^{13}C, ^{31}P, and COSY 2D NMR were recorded in D_2O and dimethyl sulfoxide-d6 (between 20 and 75 mg of the polymeric materials were dissolved in 0.6 mL of solvent) using a Bruker Advance 400 MHz NMR spectrometer at a temperature of 25 °C. FTIR spectra were recorded on Nicolet 6700 FTIR spectrometer coupled with thermo spectra tech. The thermal stability of the different samples was evaluated by thermogravimetric analyses (TGA) on a TA instruments TGA 51. The data were collected under air from 25 to 600 °C at a heating rate of 10 °C.min^{-1}. Differential scanning calorimetry (DSC) measurements were carried out using a DSC Q200 TA instrument. Scans were recorded at heating/cooling rate of 10 °C.min^{-1} from 80 to 250 °C.

Depolymerization of chitosan

Oligo-chitosan was synthesized according to Illy et al.[52, 53]. In an 80 mL vial, 1.25 g of chitosan were dissolved in 37.0 mL of a 0.93 mol.L^{-1} aqueous acetic acid solution. 1.25 g of a 35% hydrogen peroxide solution in water were added to the mixture and the vial was sealed by a screw cap. The solution was irradiated at 100 W constant power during around 6 min. The reaction vessel was cooled with compressed air during the whole microwave irradiation. After irradiation had been stopped, the fully translucent yellow solution was cooled to room temperature. Then the solution was adjusted to pH = 9.0 and centrifuged at 10,000 rpm for 10 min. The precipitate and the supernatant were separated. The precipitate was washed several times with methanol and dried under vacuum for 3 days. The water-soluble oligochitosan was recovered by precipitation in isopropanol, washed with methanol, and dried overnight under vacuum at 40 °C.

Synthesis of *N*-methylene phosphonic oligo-chitosan (I) by Moedritzer reaction

N-methylene phosphonic oligo-chitosan was synthesized according to Heras and al. [40]. In a 250 mL three-neck round bottom flask, 2.5 g of oligo-chitosan (15.2 mmol of NH_2 groups, 1 NH_2 eq.) were dissolved in 122.5 g of a 1 w% acetic acid aqueous solution. After complete dissolution of oligo-chitosan, 2.5 g of phosphorous acid H_3PO_3 (30.5 mmol, 2 eq.) dissolved in 4.0 mL of milliQ water were added drop-wise with continuous stirring. Then the temperature was raised to 70 or 90 °C and 4.5 mL of a 37 w% formaldehyde solution (60.4 mmol, 4 eq.) were added dropwise. Reaction mixtures were stirred at 60 or 70 °C for 72 hours. A small portion of the reaction mixture was sampled through a septum at different times for ^{1}H and ^{31}P NMR analysis. Deviations from this general procedure (reagent ratios and reaction temperatures) are summarized in Table 1. The polymer was recovered and purified by successive precipitations in isopropanol.

Synthesis of *N*-methylene phosphonate oligo-chitosan (II) by Kabachnik-Fields reaction

In a 250 mL three-neck round bottom flask, 2.5 g of oligo-chitosan (15.2 mmol of NH_2 groups, 1 NH_2 eq.) were dissolved at 60 °C in 122.5 g of a 1 w% acetic acid aqueous solution. After complete dissolution of oligo-chitosan at 60 °C, 3.33 g of dimethyl phosphite (30.2 mmol, 2.0 eq.) and 3.54 g of a 37 w% formaldehyde solution (44 mmol, 2.9 eq.) were added drop-wise with continuous stirring. Reaction mixtures were stirred at 60 °C for 24 h. A small portion of the reaction mixture was sampled through a septum at different times for ^{1}H and ^{31}P NMR analysis. Deviations from this general procedure (reagent ratios and reaction temperatures) are summarized in Table 2. The polymer was recovered and purified by successive precipitations in isopropanol. The degree of substitution is determined *via* ^{1}H NMR, by comparing the >C*H*-NH_2 and >C*H*-NR_2 signals of non-functionnalized and functionnalized chitosan.

Reaction between oligo-chitosan and diethyl (3-(oxiran-2-ylmethoxy) propyl) phosphonate (IV)

0.148 g of oligo-chitosan (0.896 mmol of NH2 groups, 1 NH2 eq.) was dissolved in 6.0 mL of water under vigorous stirring for 2 h. 0.45 g of diethyl (3-(oxiran-2-ylmethoxy)propyl) phosphonate (1.79 mmol, 2.0 eq.) was added dropwise to the solution. The mixture was stirred at room temperature for 72 hours. The polymer was recovered after precipitation in acetone and centrifugation. The white powder was dried under vacuum at 30 °C for 96 h.

Reaction between oligo-chitosan and dimethyl (3-(oxiran-2-ylmethoxy) propyl) phosphonate (V)

0.148 g of oligo-chitosan (0.896 mmol of NH_2 groups, 1 NH_2 eq.) was dissolved in 6.0 mL of water under vigorous stirring for 2 h. 0.41 g of dimethyl (3-(oxiran-2-ylmethoxy)propyl)phosphonate (1.83 mmol, 2.04 eq.) was added dropwise to the solution. The mixture was stirred at room temperature for 24 hours. The polymer was recovered after precipitation in acetone and centrifugation. The white powder was dried under vacuum at 30 °C for 96 h.

General procedure for the hydrolysis of phosphonate diester

The starting phosphonate diester was dissolved in 6 mL of an HCl solution (0.001, 0.1 or 1.0 mol.L^{-1}). The reaction mixture was stirred at 25-70 °C. The hydrolysis of phosphonate groups to phosphonic acid groups was monitored by taking aliquots of the reaction mixture at different time points and identifying the products by ^{31}P-NMR. The products were precipitated in acetone, filtered off and dried under vacuum.

The hydrolysis of **(II)**, **(IV)** and **(V)** phosphonate diester containing compounds gave **(III)**, **(VI)** and **(VII)** phosphonic acid containing compounds respectively.

Results and Discussions

We recently published a depolymerisation method of chitosan under microwave irradiation which enables to obtain oligo-chitosan[52, 53]. Due to their low polymerization degree (DP = 10-15), these oligomers exhibit water solubility (even at high pH). Thus, they are easier to process and can be used for reactions where an acidic medium is detrimental like epoxy-amine reaction. We first carried out Moedritzer reactions under different conditions in order to introduce in one step phosphonic acid moieties (Scheme 2, I). The phosphonated polymers were characterized by ^{1}H and ^{31}P NMR along with ATR-IR.

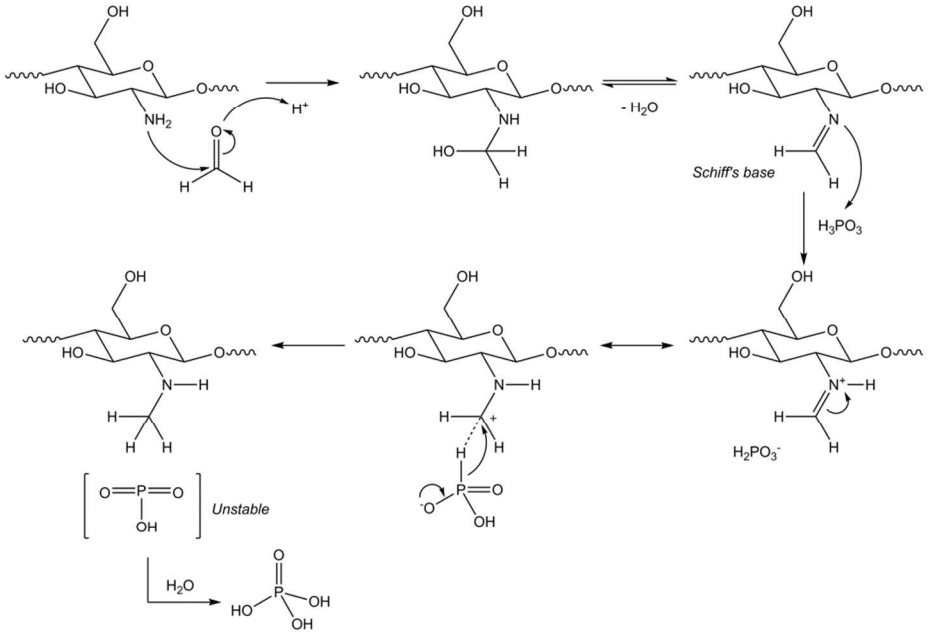
Direct Phosphonation *via* the Moedritzer Reaction

The reactions are conducted according to the work of Heras et al.[40, 42, 43] on oligo-chitosan (Mn ≈ 1700 g.mol^{-1}). Experimental conditions are summarized in Table 1.

Table 1: Summary of the Experimental Conditions Used for the Moedritzer Reaction.

Run	T (°C)	$NH_2 : H_3PO_3 : CH_2O$
1	60	1 : 2 : 4
2	70	1 : 2 : 4
3	70	1 : 2 : 2

For runs 1 and 2, a 50 µL aliquot of the reaction mixture was taken at different sampling time, diluted with 0.5 mL of D_2O and analyzed by ^{31}P-NMR spectroscopy. Figure 1 shows the evolution over time of the ^{31}P-NMR spectrum for the reaction at 60 °C (run 1). At t = 0, the spectrum exhibited only one peak at 3.08 ppm that corresponds to phosphorous acid H_3PO_3. Then, when the reaction is carried out a second peak appeared at 0.08 ppm and was attributed to phosphoric acid H_3PO_4. Concomitantly, the signal intensity of H_3PO_3 decreased. No other signal appeared on the spectrum meaning that the reaction of phosphonomethylation did not occur. Similar results were obtained for the reaction performed at 70 °C (run 2). For all experiments, the products have been purified by successive precipitations in isopropanol and characterized by NMR spectroscopy. In each case, the signal corresponding to the methylphosphonate groups is missing on the ^{31}P-NMR spectrum and only two peaks due to phosphoric and phosphorous acids can be seen (see Figure S1 in Supporting Information).



Scheme 3: Mechanism for the formation of H_3PO_4 during the Moedritzer reaction on chitosan, proposed by Lebouc et al.[46]

The presence of phosphorous acid shows that strong interactions occur between oligo-chitosan and H_3PO_3 making the purification difficult. Lebouc et al.[46] proposed a mechanism to explain the formation of H_3PO_4, arising from a side-reaction (Scheme 3). In a first step, the amino groups react with formaldehyde to give a Schiff base, which is reduced by phosphorous acid resulting in *N*-methyl and *N*,*N*-dimethyl oligo-chitosan and HPO_3. HPO_3 is unstable and reacts with water to give H_3PO_4. The signals corresponding to the methyl- and the dimethyl- amines are actually observed at 2.82 and 3.02 ppm in the ^{1}H-NMR spectrum (Figure S2 in Supporting Information).

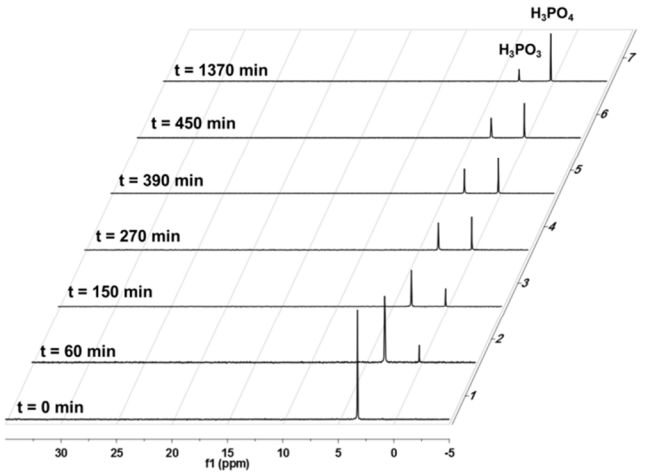


Figure 1: ^{31}P-NMR spectra at different times of the reaction mixture of the Moedritzer reaction performed at 60 °C.

In conclusion, we were not able to reproduce the synthesis of alpha-methylphosphonate oligo-chitosan described by Heras et al.[40]. However, our results are in good agreement with the work of Lebouc et al.[46] claiming that a side reaction occurs according to a mechanism based on the Leuckart-Wallart reaction, leading to the *N*-methyl and *N,N*-dimethyl oligo-chitosan. This group was still able to perform the partial phosphonomethylation of the amino groups but only in a very large excess of phosphorous acid and formaldehyde (20 eq.). Nevertheless, the latter has to be avoided in regard to its high toxicity[54].

The synthesis of oligo-chitosan carrying phosphonic acid groups according to one step Moedritzer conditions was unsuccessfull; therefore two different two-step pathways were investigated. In the first one, phosphorous acid was replaced by dimethyl phosphite according to the Kabachnik-Fields reaction conditions. This reaction may allow the introduction of bisphosphonate groups. Compounds with bisphosphonate ligands have very high stability constants with metal ions [55] and thus might offer future advantages over their monodentate analogues. The second pathway involves the epoxy-amine reaction of oligo-chitosan with commercial epoxy dialkyl phosphonates. In both case, the last step is the conversion of the dimethylphosphonate groups onto the corresponding diphosphonic acid groups (Scheme 2).

Functionalization through the Kabachnik-Fields Reaction

PHOSPHONATATION STEP

To our knowledge, only one paper deals with the Kabachnik-Fields reaction of chitosan with dialkyl phosphite[41]. However, in this paper the phosphonated copolymers synthesized were only characterized by infrared spectroscopy. Our syntheses were performed at various reaction temperatures and with various reagents ratios in order to determine the optimal reaction parameters. The experimental conditions are summarized in Table 2. The phosphonated copolymers obtained were characterized by ^{1}H and ^{31}P NMR along with ATR-IR.

Table 2: Summary of the experimental conditions used for the Kabachnik-Fields reactions.

Run	Temperature (°C)	NH_2:H_3PO_3:$(CH_2O)_x$	Time (h)	Degree of substitution
1[a]	30	1 : 2 : 3	24	< 0.05
2[a,c]	40	1 : 2 : 3	18	0.30
3[b,c]	40	1 : 2 : 3	24	0.14
4[a,c]	50	1 : 2 : 3	24	0.40
5[a]	50	1 : 4 : 3	24	0.15
6[a]	50	1 : 8 : 3	24	0.20
7[a]	60	1 : 2 : 3	18	0.45
8[b,c]	70	1 : 2 : 3	19	0.30

[a] Reagent = formaldehyde solution

[b] Reagent = paraformaldehyde

[c] Reaction under nitrogen atmosphere

For all experiments, the products have been recovered and purified by successive precipitations in isopropanol. They have been characterized by NMR spectroscopy (Figure 2, Figure 3 & Figure S3). ^{31}P NMR with proton decoupling spectra of purified products show two main peaks at 8.53 and 30.90 ppm (Figure 2). The peak around 30 ppm corresponds to phosphonate diester groups showing that the reaction of phosphonomethylation did occur. The peak at 8.53 ppm was attributed to phosphonic acid mono methyl ester (CH_5O_3P). This attribution is confirmed by gated decoupled ^{31}P-NMR (Figure 2) where a doublet of quadruplet is observed. The coupling constant of this doublet is 634 Hz and corresponds to the one-bond coupling between phosphorous and an hydrogen atom directly bonded to it. The quadruplet coupling constant has a value of 12 Hz corresponding to the three-bond coupling constant between the phosphorous atom and the protons of the methoxy group.

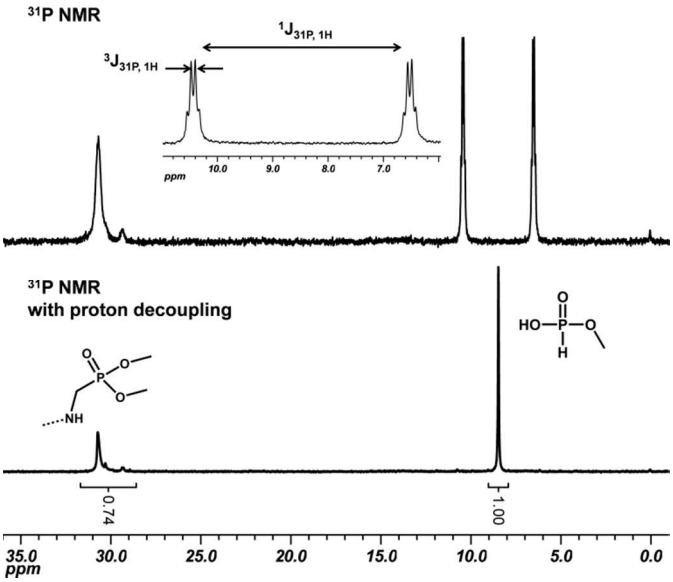
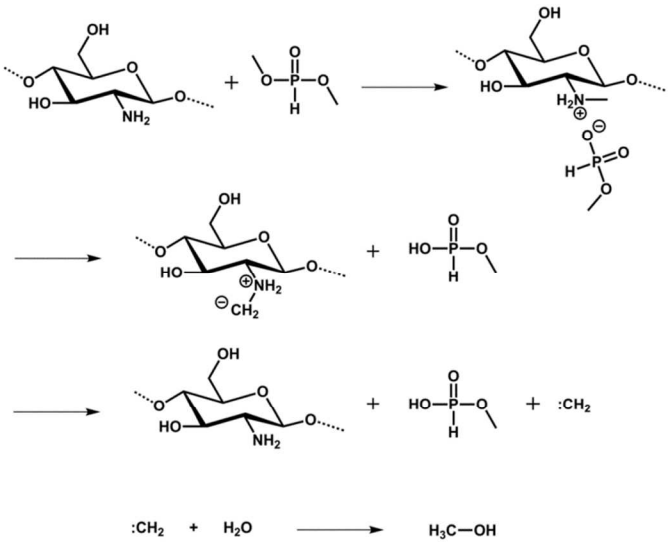


Figure 2: ^{31}P-NMR spectra of compound (II) (run 4) in D_2O at 25 °C.

A formation mechanism of phosphonic acid mono methyl ester is proposed in Scheme 4, according to the work of Georgiev et al.[56] and Vassileva et al.[57] about the dealkylation of

phosphonate esters in presence of amine. Dimethyl phosphite reacts with the chitosan primary amine groups to yield methyl the ammonium salt of the monomethyl ester of the phosphonic acid. This alkylated salt is not stable and decomposes to the corresponding ammonium salt, presumably by a carbene cleavage reaction. The carbene can subsequently react with water to yield methanol. ^{1}H NMR spectra of the reaction mixture (Figure 3) confirmed the formation of methanol over time with the apparition of a peak at 3.22 ppm. The strong interaction that occur between oligo-chitosan and CH_5PO_3 made the purification difficult and the signal of phosphonic acid mono methyl ester remained visible on the ^{31}P NMR spectra even after several precipitations (Figure 2). On the contrary dimethyl phosphite was fully removed by precipitation and is not visible on the ^{31}P NMR spectra.



Scheme 4: Proposed mechanism for the formation of phosphonic acid mono methyl ester according to Georgiev et al.[56] and Vassileva et al.[57]

No ^{31}P-NMR signals corresponding to the phosphorylation of the hydroxyl groups is detected. This result is contradictory with the observations of Matevosyan et al.[41] who claimed simultaneous phosphorylation of the OH groups in position 6 and N-phosphonomethylation during the reaction of chitosan with diethyl phosphite in presence of formaldehyde.

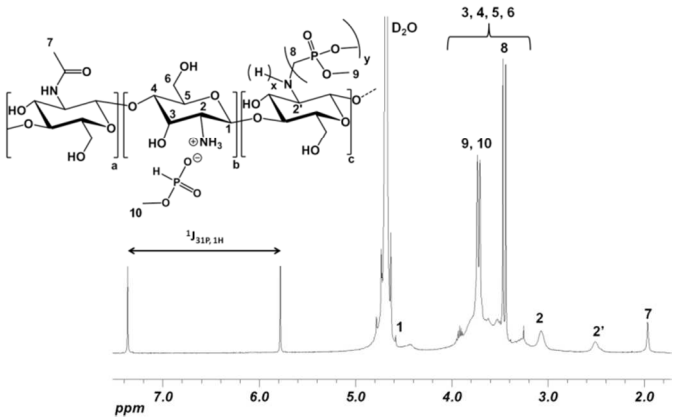


Figure 3: ^{1}H-NMR spectra of compound (II) (run 4) in D_2O at 25 °C.

The FT-IR spectrum of oligo-chitosan (Figure 4-a) shows the stretching of the carbonyl group due to NH-$COCH_3$ at 1650 cm^{-1} and a band at 1590 cm^{-1} corresponding to the axial stretching of the amino groups. In the spectrum of compound **(II4)** (Figure 4-b), the band corresponding to acetylated amino groups remains stable but the broad absorption bands in the 3600-3000 cm^{-1} range, corresponding to OH and NH stretching vibrations, decrease upon the reaction. The primary amino signal at 1590 cm^{-1} disappears and a band corresponding to N-H bending vibrations in secondary amines[58] appears at 1540 cm^{-1}. Compound **(II4)** also exhibits three main bands characteristic of the phosphonate groups: at 1190 cm^{-1} due to the stretching of the P=O double-bond, at 960 cm^{-1} due to the stretching of the P-O bonds and at 780 cm^{-1} due to the deformation of the P-O-C bonds.

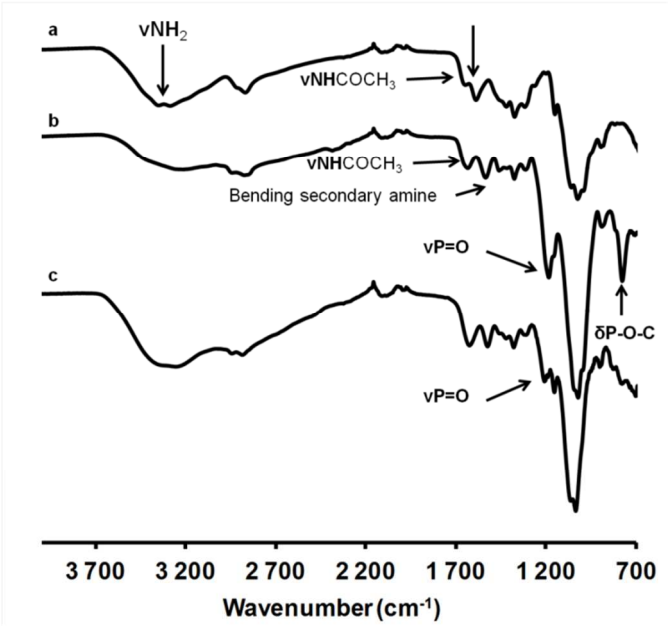


Figure 4: IR spectra of oligo-chitosan (a), phosphonate-containing oligo-chitosan (II4) (b) and phosphonic acid containing oligo-chitosan (c).

For each run of Table 2, a partial phosphonomethylation of the oligo-chitosan primary amine groups took place.

HYDROLYSIS OF PHOSPHONATED COMPOUND (II)

The next step in the synthesis is hydrolysis of the alkyl esters groups to obtain the corresponding free phosphonic acids. The mildest conditions have been determined in order to avoid the degradation of the oligo-chitosan backbone. All the products were characterized by ^{1}H and ^{31}P NMR analyses.

Acidic hydrolysis of alkyl diester phosphonate compounds is widely used for the preparation of phosphonic acids[59]. The hydrolysis of **(II)** was investigated at different temperatures in the presence of aqueous hydrochloric acid solutions at different concentrations leading to the corresponding **(III)** compound (Table 3). The ^{1}H NMR spectral analysis does not give insight on the structure, apart from the signal intensity of methyl ester protons, which decreased during the hydrolysis (Figure 5). The dealkylation of **(II)** was monitored by ^{31}P-NMR spectroscopy. The starting dimethyl phosphonate groups produce a ^{31}P-NMR signal at around 30 ppm, whereas the ^{31}P NMR signals of phosphonic acid monomethyl ester and phosphonic groups have high-field shifted to 22 and 12 ppm, respectively. The hydrolysis efficiency increases with the acid concentration and the temperature.

Table 3: Experimental conditions of the hydrolysis of phosphonated oligo-chitosan **(II)** (run 4, Table 2) with HCl.

Run	[HCl] (mol.L-1)	T (°C)	Reaction Time (h)	Deprotection Yield (%)	
				Mono Phosphonic	Di Phosphonic
1	0.001	50	96	0	26
2	0.1	50	96	30	70
3	0.1	70	17	11	85.5
4	0.1	70	24	0	93
5	1.0	25	24	76	17.5
6	1.0	50	26	20	78
7	1.0	70	18	0	100

The mildest efficient conditions were obtained for a 0.1 $mol.L^{-1}$ hydrochloric acid solution, a reaction temperature of 70 °C and a reaction time of 24 h. The ^{31}P NMR spectrum of compound **(III4)** is given in Figure 6, showing a main signal at $\delta = 12$ ppm, characteristic of the diphosphonic acid groups. The ester cleavage was carried out almost quantitatively as the signal of the dimethylphosphonate group totally disappeared and the signal of phosphonic acid monomethyl ester is absent. Minor impurities are visible at 9 and 10.5 ppm but could not be attributed. This result is confirmed by the infrared spectrum (Figure 4-c) where the band at 780 cm^{-1} due to the deformation of the P-O-C bonds is not visible.

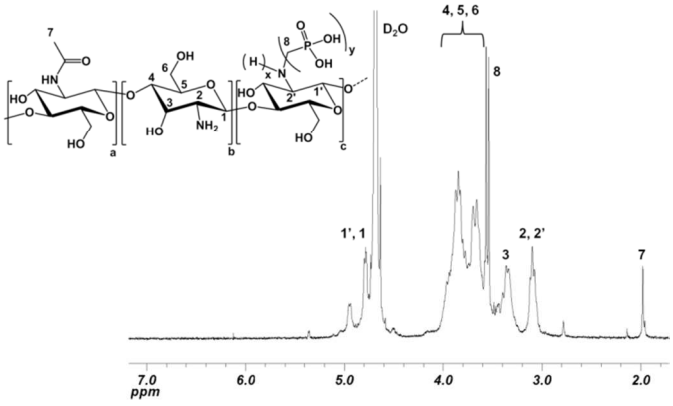


Figure 5: ^{1}H-NMR spectra of compound (III) (run 4) in D_2O at 25 °C after hydrolysis.

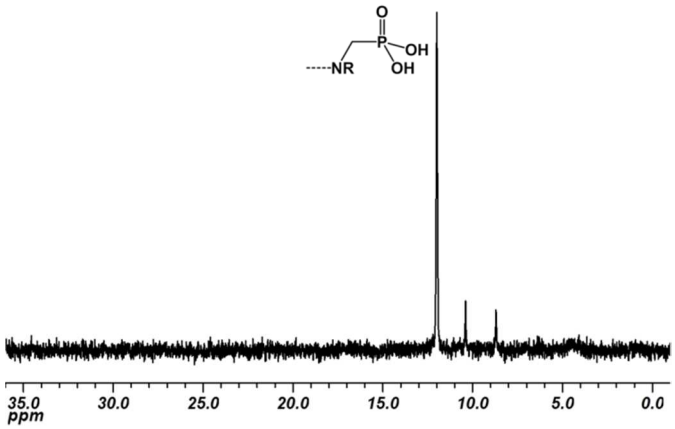


Figure 6: ^{31}P-NMR spectra of compound (III) (run 4) in D_2O at 25 °C after hydrolysis.

To conclude, the phosphonomethylation of oligo-chitosan amino groups by the Kabachnik-Fields reaction was successfully carried out, and for the first time, the structure of the copolymers was investigated through ^{1}H and ^{31}P NMR spectroscopy. However, phosphonic acid mono methyl ester was formed as a side-product during the phosphonatation step, and the purification of the product was made difficult by the strong interaction of oligo-chitosan and CH_5O_3P. Thus, we aim to avoid such drawbacks by using a new methodology, which will also be environmentally-friendly, efficient and soft. We report in the next section the preparation of new *N*-alkyl phosphonate/phosphonic oligo-chitosan derivatives in water using the very efficient epoxy-amine reaction. In fact, the reaction between amino and epoxide groups is known to proceed quantitatively and in soft conditions[60].

Synthesis of phosphonic acid-bearing compounds *via* an epoxy-amine reaction

SYNTHESIS OF PHOSPHONATED COMPOUNDS (IV) AND (V) VIA AN EPOXY-AMINE REACTION

Oligo-chitosan was reacted in an easy one-step process at room temperature with commercial epoxy dialkylphosphonate

(Scheme 2). Compound **(IV)** was synthesized by the reaction of oligo-chitosan with diethyl (3-(oxiran-2-ylmethoxy) propyl) phosphonate. After purification by precipitation in acetone and drying for 96 h under vacuum, a white powder was obtained. The product was characterized by ^{31}P-NMR in D_2O and shows two peaks at 35.1 and 36.1 ppm corresponding to the alkoxyphosphonate moiety (Figure 7). ^{1}H-NMR spectrum did not show any evidence for side reactions and confirmed the molecular structure of the product (Figure 8 and Figure S4). The ethyl groups of the phosphonate groups are clearly visible, with signals at 1.32 ppm (C**H$_3$**-CH$_2$-P) and 4.13 ppm (CH$_3$-C**H$_2$**-P).

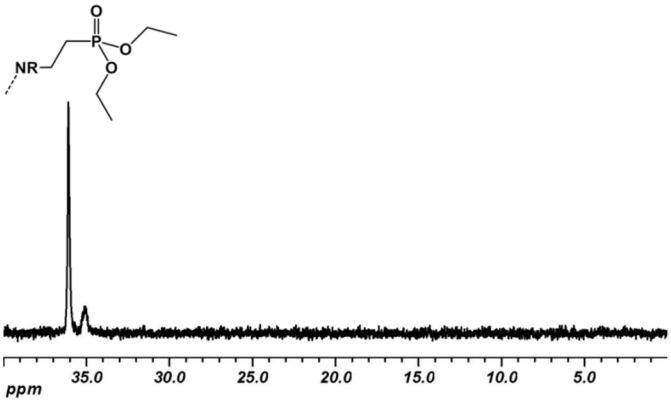


Figure 7: ^{31}P-NMR spectrum of (IV) in D_2O at 25 °C.

The substitution degree was calculated from the signal intensity of ethyl groups and the signal of the proton in position 1 on the glycosic ring and was found to be 0.6.

Complementary 2D COSY experiments allowed the full attribution of the signals (Figure 8). The proton on the tertiary carbon of the diethyl (3-(oxiran-2-ylmethoxy) propyl) phosphonate shifts from 2.85 ppm to 3.80 ppm during the reaction which proves the opening of the oxiran ring. The correlation between this proton and the proton of the -CH$_2$- group in alpha position of the amine is clearly visible.

The FT-IR spectrum (Figure 9) shows the stretching of the carbonyl group due to NH-COCH$_3$ at 1650 cm^{-1}. As previously, the band at 1590 cm^{-1} corresponding to the axial stretching of the amino groups disappears on the FT-IR spectrum of **(IV)**. Compound **(IV)** also presents phosphonate groups characteristic bands: at 1200 cm^{-1}, due to the P=O double-bond stretching, at 960 cm^{-1} due to the stretching of the P-O bonds and at 791 cm^{-1} due to the deformation of the P-O-C bonds.

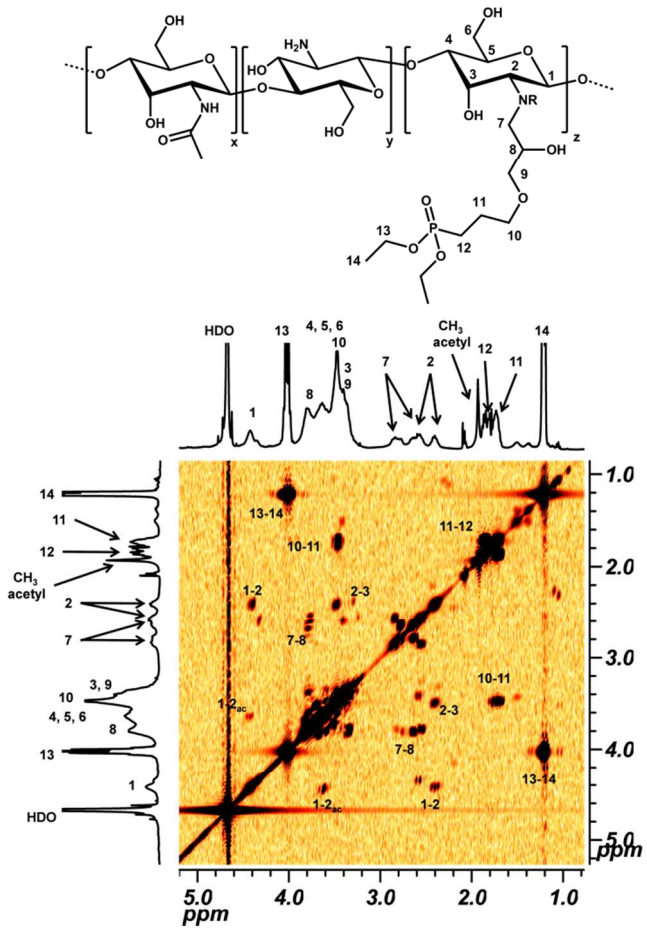


Figure 8: COSY 2D NMR of (IV) in D_2O at 25 °C.

The epoxide-amine reaction was extended to dimethyl (3-(oxiran-2-ylmethoxy) propyl) phosphonate (4) to give compound **(V)**. ^{1}H NMR, ^{31}P NMR and FT-IR spectra can be found as supplementary materials and confirm the chemical structure of **(V)** (Figure S5 and Figure S6).

Hydrolysis of the dialkyl ester phosphonate groups of (IV) and (V)

Attempts to hydrolyse the dialkyl ester phosphonate groups of (IV) and (V) were carried out in hydrochloric acid solutions (Table 4). When a 0.1 mol.L^{-1} hydrochloric acid solution was used, no dealkylation of the phosphonate groups was observed for both compounds (IV) and (V). This acid concentration was sufficient to deprotect the aminomethyl dimethyl phosphonate groups generated by the Kabachnik-Fields reaction which suggest that the nitrogen atom in β-position of the phosphorus atom favors the dealkylation process. The use of a 1.0 mol.L^{-1} hydrochloric acid solution allowed the partial deprotection of the methyl phosphonate group of compound **(V)**. Nevertheless, in the case of compound **(V)**, the diethyl phosphonate groups remain intact during the reaction. This result is not surprising because the reactivity of HCl is low and higher reaction temperatures and longer reaction times (tens to hundreds of hours) are usually required[61]. However, in the case of chitosan, a temperature elevation, a higher acid concentration or longer

reaction times are not worth considering because of the fragility of the polysaccharide backbone.

Table 4: Experimental conditions of the hydrolysis of phosphonated oligo-chitosan (IV) and (V) with HCl.

Run	P oligo-chitosan	[HCl] ($mol.l^{-1}$)	T (°C)	Reaction time (h)	Deprotection Yield (%)	
					Mono Phosphonic	Di Phosphonic
1	IV	0.1	70	18	0	0
2	V	0.1	70	18	0	0
3	IV	1.0	70	22	4	0
4	V	1.0	70	22	20	14

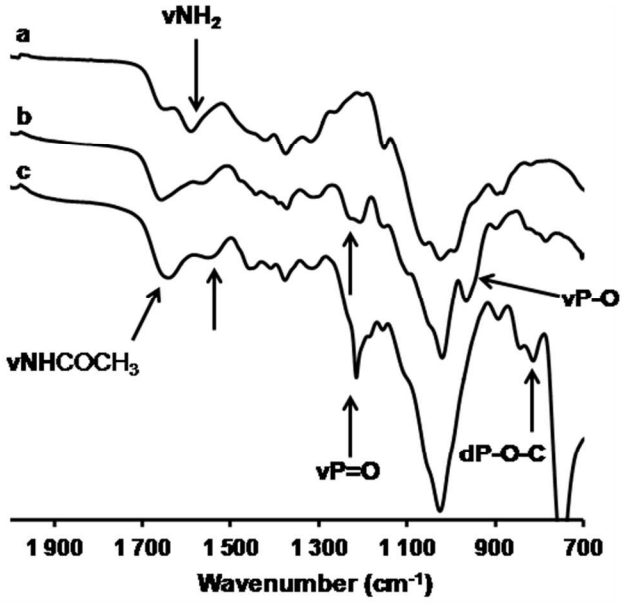


Figure 9: IR spectra of oligo-chitosan (a) and of phosphonate-containing oligo-chitosan (V) (c) and (IV) (d).

Conclusions

The efficiencies of different phosphonation methods have been evaluated on oligo-chitosan. On the one hand, phosphonated oligo-chitosan was not obtained via the Moedritzer reaction, yielding only *N*-methyl-, *N*,*N*-dimethyl-oligo-chitosan and phosphoric acid through a side reaction. On the other hand, the successful functionalization of oligo-chitosan has been achieved via the Kabachnik-Fields reaction with degree of substitution up to 0.45, and via an epoxy-amine reaction exhibiting a degree of substitution of 0.6. The hydrolysis of the phosphonate diester to free phosphonic acid was only possible in the case of phosphonated oligo-chitosan prepared by the Kabachnik-Fields pathway, indicating a possible role of the nitrogen atom in β-position of the phosphorus atom during this step. Nevertheless, the epoxy-amine reactions were carried out at room temperature in aqueous solution, therefore providing an interesting alternative route for the synthesis of phosphorous-containing oligo-chitosan derivatives.

Acknowledgements

Notes and references

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