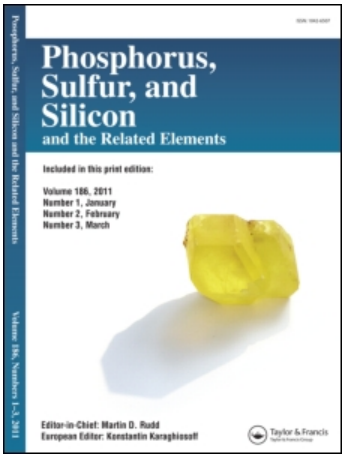


## Phosphorus, Sulfur, and Silicon and the Related Elements

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# Synthesis of Phosphorus-Containing Chitosan Derivatives

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# Synthesis of Phosphorus-Containing Chitosan Derivatives

**Fanny Lebouc,[1] Isabelle Dez,[1] Mihaela Gulea,[1] Pierre-Jean Madec,[1] and Paul-Alain Jaffrès[2]**
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*Three new phosphorus-containing chitosan derivatives were prepared in good yield under mild conditions from 6-O-triphenylmethyl-chitosan and native chitosan. The three reactions used are thioacylation by a phosphonodithioester, alkylation by a halogeno-phosphonate, and Michael addition using a tetraethyl vinylidenebisphosphonate. The modified chitosan derivatives were fully characterized; their solubilities and thermal properties were evaluated.*

**Keywords** Chitosan; NMR analyses; phosphonate; thermal analyses



## INTRODUCTION

Chitosan is a cationic natural biopolymer obtained by alkaline *N*-deacetylation of chitin, the most abundant natural polymer after cellulose. It ideally consists of 2-amino-2-deoxy-(1–4)-$\beta$-D-glucopyranose residues (D-glucosamine units) and can include or

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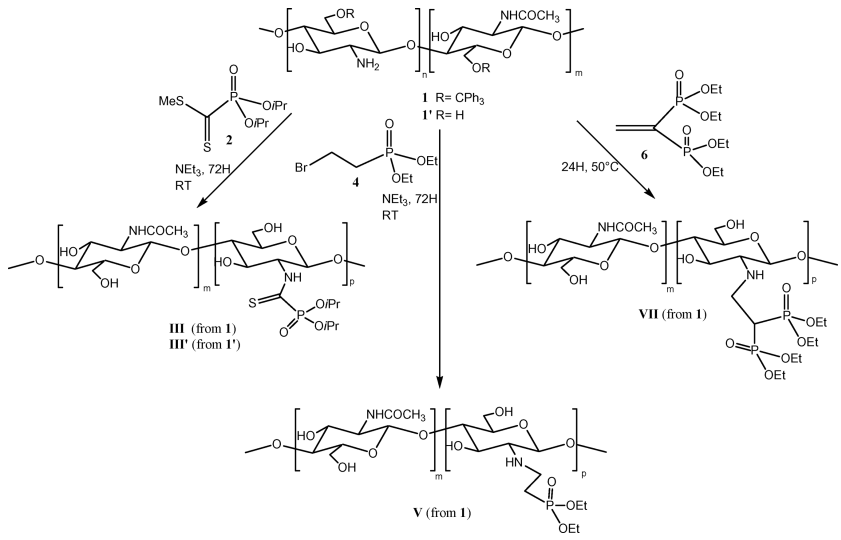
not a small amount of *N*-acetyl-D-glucosamine units. Chitosan is a remarkable biomaterial because of its numerous biological and immunological activities.[1] Moreover, chitosan is a nontoxic and biodegradable biopolymer, and therefore it has received considerable attention for its applications in biomedical, food, and chemical industries.[2]

The degree of acetylation (DA) and the molar mass of chitosan strongly influence its physical and chemical properties, including emulsification and aggregation capacity, rheology, and physicochemical properties.[3–7] A limiting factor in the application of chitosan is its low solubility in neutral and alkaline aqueous media and in most of the organic solvents. Therefore, various studies have focused on the improvement in the solubility of chitosan in an aqueous solution by employing water-soluble moieties that were introduced through the hydroxy[8,9] or the amino[10–16] groups of chitosan. The introduction of phosphoryl groups in the chitosan structure is known to increase its chelating properties[17–19] and to modify its solubility.[20–24] The functionalization of chitosan can be performed by a Mannich-type reaction involving the free amino group of chitosan, formaldehyde, and phosphorous acid leading to $\alpha$-aminophosphonic acid.[10] In a previous study, we showed that such functionalization can lead to the formation of side products such as *N*-methyl and *N*, *N*-dimethyl chitosan.[11] These problems could be avoided if the phosphonate function is introduced on amino groups of chitosan by coupling reactions characterized by high yield and soft conditions. We describe in this article the synthesis of new phosphonate derivatives of chitosan using three selected reactions, which are in accordance with the former criteria (Scheme 1).

The first one involved phosphonodithioformate **2**, which is an efficient thioacylating agent of amines,[26] to form phosphonothioamides-functionalized chitosans **III** and **III′**. The second reaction used 2-bromoethylphosphonate **4** as an electrophile,[27,28] leading to *N*-(2-diethylphosphono-ethyl)-chitosan **V**. The last reaction involved the vinylidenediphosphonate **6**, which is an excellent Michael acceptor,[29] to give the *N*-(2,2-diethylbisphosphono-ethyl)-chitosan **VII**. Both alkylation and Michael addition reactions were performed using the 6-*O*-triphenylmethyl-chitosan (6-*O*-trityl-chitosan) **1** that is workable in homogeneous conditions giving respectively **V** and **VII**. The thioacylation was performed using either the native or protected chitosan **1′** or **1** to give products **III′** and **III**, respectively. The new phosphorus-containing chitosans were fully characterized by IR and NMR spectroscopy; their solubilities in various solvents and their thermal properties were evaluated.




**SCHEME 1** Synthesis of phosphorus-containing chitosans.

## RESULTS AND DISCUSSION

### Synthesis and Characterization

The first new phosphorylated chitosan derivative was obtained by a thioacylation reaction from the 6-*O*-trityl-chitosan **1**, which is soluble in THF, applying the classical reaction conditions reported by Bulpin et al.[37] by reacting methyl diisopropylphosphono-dithioformate **2** in the presence of triethylamine. After deprotection of the primary alcohol function, the polymer was precipitated in acetone, and the excess of reagent was eliminated by soxhlet with acetone. After drying, the pale yellow solid obtained was soluble in neutral and acidic water. No absorption bands corresponding to the trityl groups at 764, 748, and 702 $cm^{-1}$ were observed, indicating a complete deprotection of the primary OH group. Moreover, IR spectrum showed an absorption band at 1246 $cm^{-1}$, which was assigned to the P=O stretching vibration of the phosphonate group. Residual absorption bands at 1716 and 1776 $cm^{-1}$, attributed to the phthalimido groups coming from the protection of the amino functions before the reaction of the alcohol with trityl chloride, were still observed (Figure 1). As described previously, the $NH_2$-deprotection reaction was not complete, and 20% of $NH_2$ functions remained protected by phtalimido groups.[35]

The introduction of the phosphorus moiety was also confirmed by the $^{31}P$ NMR spectrum with a signal at −2.9 ppm attributed to the expected

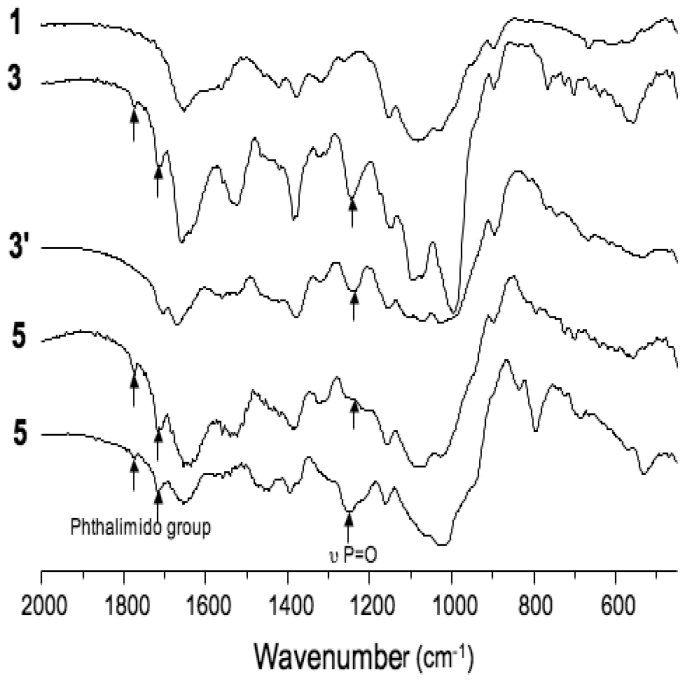
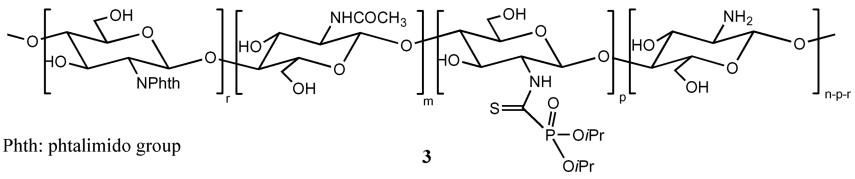



**FIGURE 1** IR spectra of chitosan **1** and of the phosphorus-containing chitosans **III**, **III′**, **V**, and **VII**.



phosphorylated chitosan. No signal belonging to **2** was detected (−0.8 ppm). The thioacylation of chitosan was also proved by the $^{13}C$ NMR spectrum showing a signal at 197.5 ppm (d, $J_{PC} = 186$ Hz), which was assigned to the N**C**=S carbon atom (Figure 2). Because of the long relaxation time of the N**C**=S carbon nucleus, the corresponding peak was only detected when using a relaxation agent such as chromium acetylacetonate.

These results associated with the positive nihydrine test, indicating that free $NH_2$ functions are still present, are in agreement with the structure **3** represented in Scheme 2.




Phth: phtalimido group

**3**

**SCHEME 2**

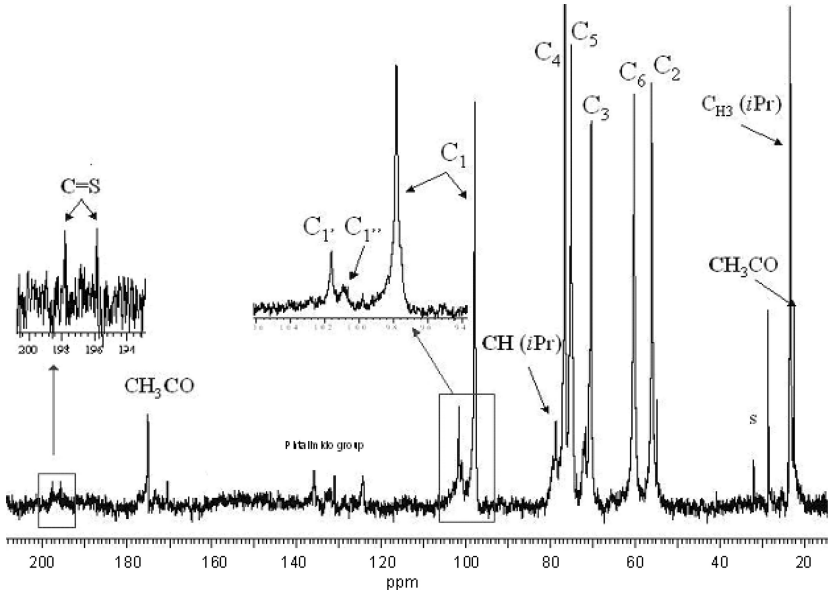
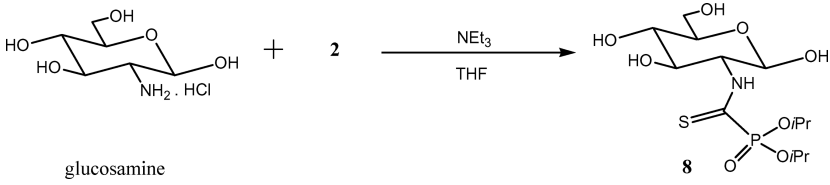



**FIGURE 2** $^{13}$C NMR spectrum of **III** (relaxation delay: 10 sec; 20000 scans).

The second thioacylation reaction was performed on the native chitosan **1**′. When compound **2** in THF was added to the chitosan solution in the presence of triethylamine, a pink gel was formed. The reaction was carried out under strong stirring and followed by $^{31}$P NMR spectroscopy. The expected phosphorus derivative was obtained as a pale yellow solid after purification by soxhlet. This new compound was soluble in acidic solution and swelled in water. The $^{31}$P NMR spectrum showed a broad signal at −2.9 ppm attributed to the phosphonate derivative **III**′. To improve the resolution of the spectrum, it was scanned at 70°C. In addition to the expected peak at −2.9 ppm, the spectrum showed another peak at −1.4 ppm with an intensity ratio 80:20 (Figure 3).

The same reaction was performed using the D-glucosamine, a structural unit of chitosan, leading to **8** (Scheme 3).




**SCHEME 3**

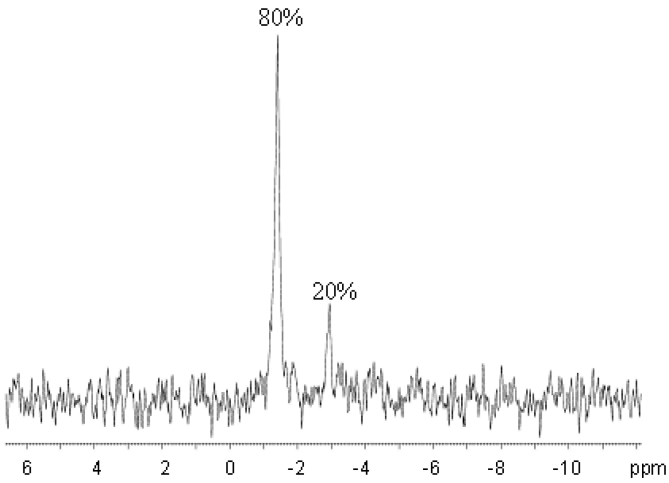



**FIGURE 3** $^{31}$P NMR spectrum of **III′** obtained at 70°C.



The $^{31}$P NMR spectrum of **8** showed two peaks at −1.8 and −2.4 ppm with an intensity ratio of 25:75 (Figure 4).

The chemical shift at −1.8 ppm was attributed to compound **8**, and the one at −2.4 ppm was attributed to the compound having the phosphoryl group linked to the $C_3$—OH group by the hydrogen bond. Based

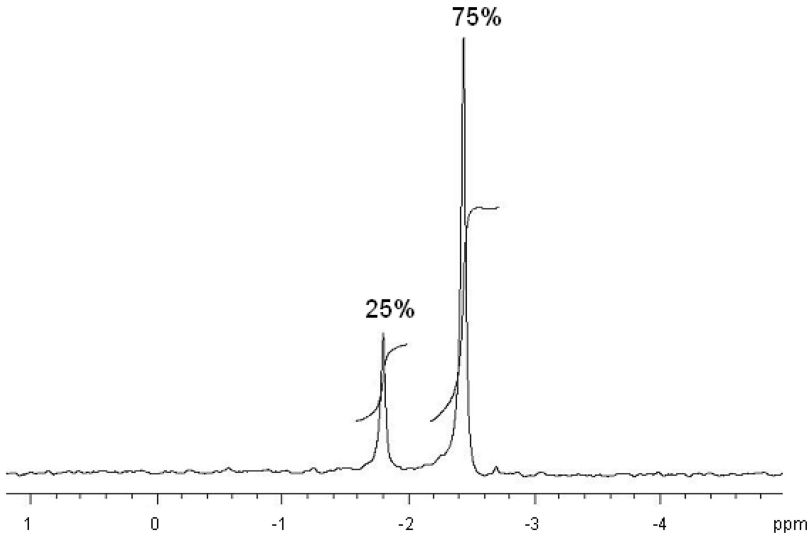



**FIGURE 4** $^{31}$P NMR spectrum of **8**.

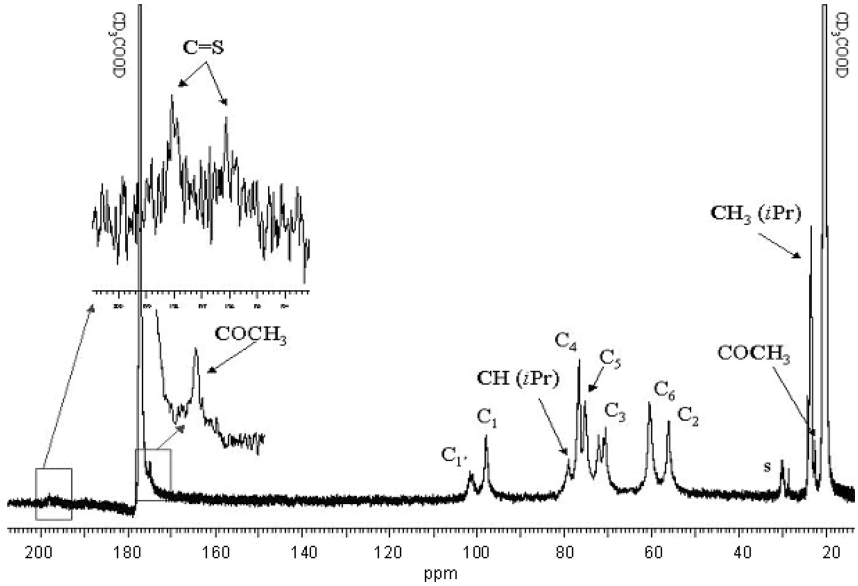


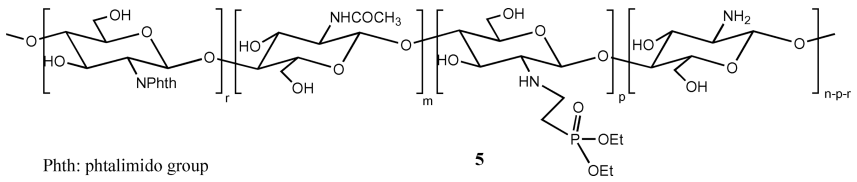

**FIGURE 5** $^{13}$C NMR spectrum of **III′** (relaxation delay: 10 sec; 20000 scans).



on this test reaction, we propose that in the case of the modified chitosan, most of the phosphoryl groups are linked via intramolecular bonds with the $C_3$-OH giving a strong signal at −2.9 ppm. This proposal was confirmed by studying the effect of temperature on the yield of the modified chitosan. When the polymer was heated at 70°C (Figure 5), the higher mobility of the chain allowed the breaking of the intramolecular bonds, giving an additional peak in the $^{31}$P spectrum at −1.4 ppm as expected.

The $^{13}$C spectrum confirmed unequivocally the structure of **III′**. The peak corresponding to the thiocarbonyl group was observed at 197 ppm (d, $J_{PC} = 198$ Hz).

The IR spectrum showed a characteristic band for $\upsilon_{P=O}$ at 1242 cm$^{-1}$ (Figure 1). Compound **V** was synthesized by the reaction of the 6-*O*-trityl protected chitosan **1** with diethyl (2-bromo-ethyl)phosphonate **4** in THF. After deprotection of the primary alcohol function, a brown solid soluble in acidic solution or in water was obtained. The product was characterized by $^{31}$P NMR spectroscopy and showed one signal at 28.2 ppm corresponding to the alkylphosphonate moiety.[38,39] $^{13}$C and $^{1}$H NMR analyses confirmed the structure of the product. The competitive elimination reaction leading to the synthesis of the corresponding vinylphosphonate was never observed. The band of the phosphonate




Phth: phtalimido group

**5**

**SCHEME 4**

group was also observed in the IR spectrum (1218 $cm^{-1}$) (Figure 1). These results together with the positive nihydrine test confirmed the following structure **5** (Scheme 4).

Finally,**VII** was synthesized by the reaction of the 6-*O*-trityl-chitosan **1** with tetraethyl ethylidenediphosphonate **6**, followed by the deprotection of the primary hydroxyl group. The obtained pale yellow solid was soluble in acetic acid solution and water. The $^{31}P$ NMR spectrum of the purified product showed a large peak at 23 ppm corresponding to **VII** and a peak corresponding to the residual starting ethenylidene diphoshonate **6** reagent at 14.6 ppm with an intensity ratio 80:20 (Figure 6).

These results reveal that the reactivity of the chitosan amino group towards the ethenylidenediphoshonate is as good as that of simple amines.[29] The presence of a residual amount of the starting material could be explained by either an incomplete reaction or as the result of a retro-Michael reaction, which is also observed with smaller

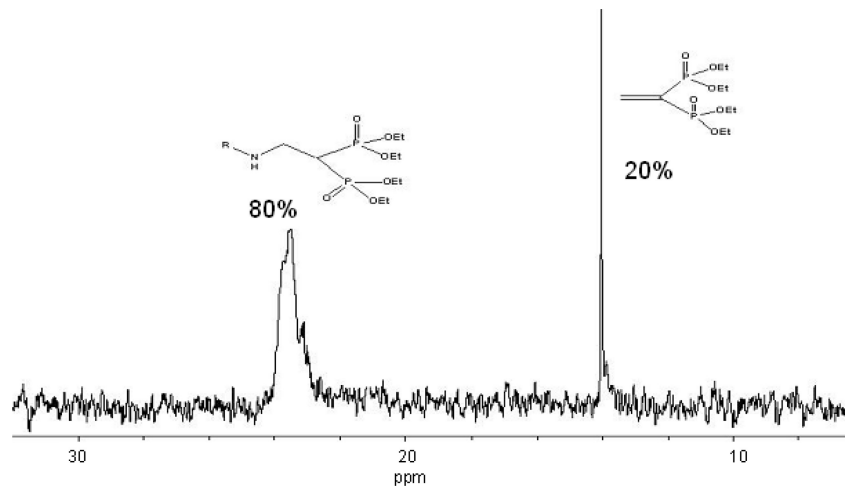
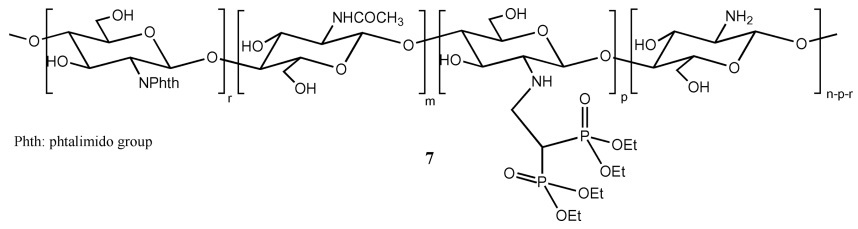



**FIGURE 6** $^{31}P$ NMR spectrum of **VII**.

molecules.[29,40] It is well-known that the retro-Michael reaction is favored by higher temperatures. Indeed, after heating **VII** at 70°C, the $^{31}P$ NMR spectrum only showed the peak at 14.6 ppm, corresponding to the starting reagent. A complete disappearance of the peak related to **VII** at 23 ppm was observed indicating the low thermal stability of the Michael adduct in water solution. **VII** was fully characterized. The bands corresponding to the residual phthaloyl groups were observed in the IR spectrum at 1776 and 1716 $cm^{-1}$ and $\upsilon_{P=O}$ was also detected at 1252 $cm^{-1}$ (Figure 1), confirming the introduction of the phosphonate group onto chitosan.

These analyses, together with the positive nihydrine test, are in agreement with the structure **7** given in Scheme 5.




**SCHEME 5**

## Degrees of Substitution and Solubility Properties

The degrees of substitution (DS) were estimated by $^1H$ NMR spectroscopy or by a colloidal titration method[41]: A negative colloid solution of phosphorus chitosan derivative was titrated with 1/400 N polyanionic solution of potassium poly(vinylsulfate) using toluidine blue as indicator (Table I).

The obtained DS of the different phosphorus reagents were moderate in the case of **3′** and low in the other cases. For **3** and **5**, the low DS values could result from the steric hindrance of the residual phthalimido groups (20%), which limit the DS. Similar DS values for compounds **3** and **5** indicate that the chitosan amino group has similar

**TABLE I Degree of Substitution of the Phosphorus-Containing Chitosans**

| | **3** | **3′** | **5** | **7** |
|---|---|---|---|---|
| DS (%) | 20 | 35 | 21 | 14 |

**TABLE II Solubility Properties of the Phosphorus-Containing Chitosans**

| | **3** | **3′** | **5** | **7** |
|---|---|---|---|---|
| Acidic water | Soluble | Soluble | Soluble | Soluble |
| Water | Soluble | Swelled | Soluble | Soluble |
| Methanol | Insoluble | Insoluble | Insoluble | Soluble |
| DMSO | Soluble | Swelled | Soluble | Soluble |

reactivities towards **2** and **4**. The DS value for **7** is lower because of the retro-Michael reaction that could occur in water.

The solubility properties of the phosphorylated chitosan derivatives **3**, **3′**, **5**, and **7** were also studied (Table II). The samples were soaked in each solvent at the concentration of 5 mg/mL, and the solubility after 24 h was determined.



Compounds **3**, **5**, and **7** are soluble in water and in other polar solvents such as methanol and DMSO in spite of the low grafting extent of the phosphorylated groups. Our previous works showed that the protection–deprotection reactions of OH and $NH_2$ groups of chitosan lead to an important degradation of the original chitosan to give low molar masses.[35] This degradation has been shown to improve the solubility properties of chitosan,[22] which could explain the new solubility properties of **3**, **5**, and **7**. For compound **3′**, the higher DS did not improve its solubility: **3′** was soluble in acetic solution and swelled in water and DMSO. The $\bar{M}_v$ of **3′** with a DS of 35% was 345,000 g.mol$^{-1}$ while the $\bar{M}_v$ of chitosan precursor was 330,000 g.mol$^{-1}$. Considering the experimental errors and calculation inaccuracy due to the difficulty in determining the equation parameters, it could be stated that unlike other functionalizations, negligible hydrolysis of the chitosan backbones occurred after the thioacylation reaction. The hydrolysis of the ester function in **3′** to yield the phosphonic acid chitosan derivative should improve the water solubility properties.

## Thermal Analyses

The thermal stability and degradation behavior of the phosphorylated chitosan derivatives were investigated by thermogravimetric analysis in $N_2$ (Figure 7).

The first stage of degradation for both **3** and **5** started at 80°C with a weight loss of 5% for **3** and 10% for **5**. The second stage started at 251°C for **3** and 230°C for **5** and reached a maximum at 305°C for **3** and

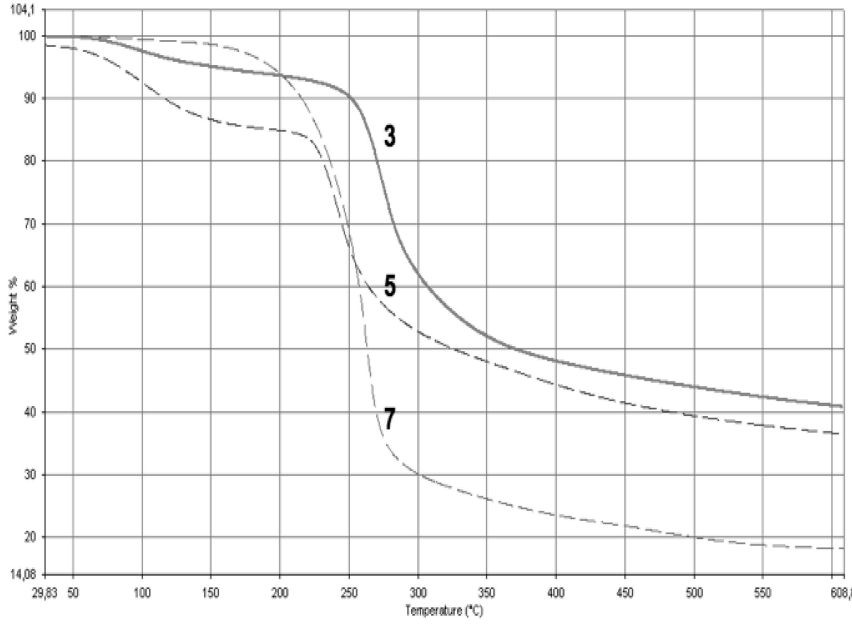



**FIGURE 7** ATG thermograms of the phosphorus-containing chitosans.



258°C for **5** with a weight loss of 45% and 43%, respectively. In comparison, the main thermal process of chitosan occurs at the temperature of 270°C with a maximum at 334°C and a weight loss of 49%, which could be attributed to a complex process including dehydratation of the saccharide rings, depolymerization, and decomposition of the acetylated and deacetylated units of the polymers.[42,43] These thermal analyses showed that the introduction of phosphorus moieties decreased the thermal stability of chitosan. That could be explained by the lower ratio of the free amino groups in modified chitosan, which are known to stabilize the chitosan against the thermal depolymerization process.[40] In the case of **7**, the low thermal stability of the Michael adduct led to a degradation starting at 200°C and reaching a maximum at 270°C with a weight loss of 70%.

The DSC thermograms of phosphorus chitosan derivatives are shown in Figure 8.

The DSC curves of **3** and **5** show an endothermal peak at 80°C. This peak is due to the moisture absorbed by the chitosan. The other endothermal peaks are assigned to a degradation of the polymer at 238°C and 210°C for **3** and **5**, respectively. In the case of **7**, the DSC curve is characteristic of a Michael adduct.[45] The first transition from 131°C

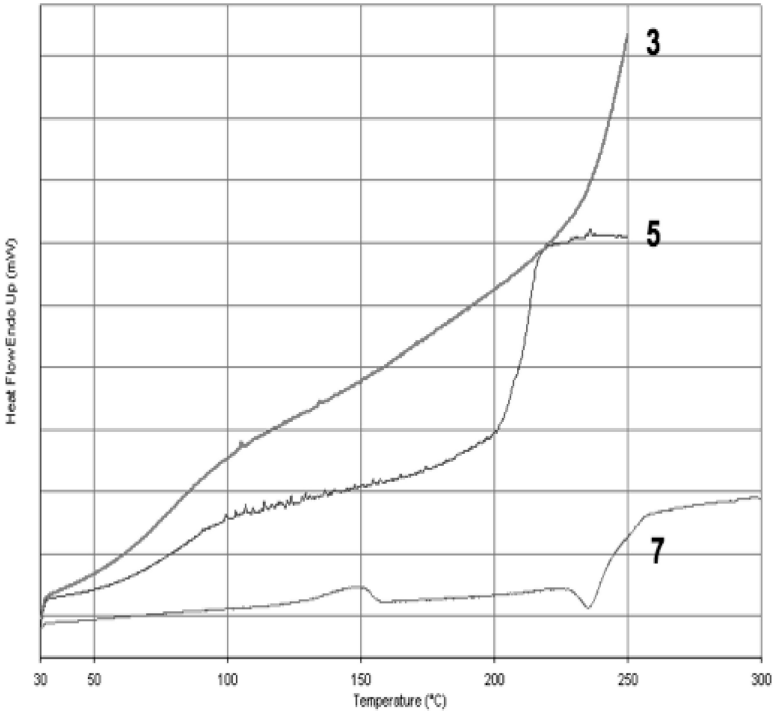



**FIGURE 8** DSC thermograms of the phosphorus-containing chitosans.



to 160°C is attributed to the retro-Michael reaction and reveals that **7** starts to be damaged at 130°C. The second transition from 230°C to 260°C is attributed to the degradation of both the ethylidenediphosphonate and the chitosan backbone.

## CONCLUSION

The modification of the chitosan described in this article is based on the reactivity of the amino function toward three different phosphorylating reagents leading to three new phosphorus-containing chitosan derivatives:

- The *N*-(diisopropylphosphono-thiooxomethyl) chitosan **3** and **3′**
- The *N*-(2-diethylphosphono-ethyl)-chitosan **5**
- The *N*-(2,2-diethylbisphosphono-ethyl)-chitosan **7**

The new chitosan derivatives were fully characterized by NMR and IR spectroscopy. Introduction of the phosphonate moieties modified their solubility properties, allowing most of them to be soluble in water, unlike the native chitosan. The introduction of a phosphonate group leads to a decrease of the thermal stability of the phosphorylated chitosan compared to the native chitosan, which is presumably due to the breaking of the crystalline structure of the chitosan. In the case of **7**, the thermal analyses confirmed its low thermal stability due to the retro-Michael addition reaction. Futher studies on the chelating properties of these new chitosan derivatives are currently under process.

## EXPERIMENTAL

### Materials



Chitosan obtained from Fluka is purified by dissolution in aqueous hydrochloric acid (0.2%) to give a solution with a polymer concentration of 1% (w/v) and is precipitated in aqueous NaOH solution (pH >7). After filtration, the residue is washed several times with deionized water to attain the water conductivity and finally freeze-dried. The viscosity-average molar mass ($\bar{M}_v$) is 330,000 g.mol$^{-1}$ (determined by viscosimetry at 25°C, $CH_3COOH$ 0.3 M / $CH_3COONa$ 0.2 M using an Ubbelohde viscometer according to the literature[30]) and the degree of acetylation determined by $^1$H NMR spectroscopy is close to 20%.[31]

The methyl diisopropylphosphonodithioformate was synthesized according to the method reported by Grisley[32] and the tetraethyl ethylidenediphosphonate according to the method reported by Degenhardt and Burdsall.[33]

6-*O*-triphenylmethyl-chitosan (6-*O*-trityl-chitosan) was prepared by the method reported by Nishimura et al.[34] As described in previous work,[35] the deprotection reaction of amino groups is not complete, and 20% of the $NH_2$ functions remained protected by the phthalimido groups.

Reactions were carried out under nitrogen with magnetic stirring, unless otherwise specified. Solvents were dried by distillation, prior to use.

### NMR Spectroscopy

The NMR spectra were recorded in $D_2O$ / $CD_3COOD$ for **3′** and $D_2O$ for the other phosphorylated chitosan derivatives using a Bruker DRX400

spectrometer. Chemical shifts ($\delta$) are expressed in ppm relative to $Me_4Si$ for $^1H$ and $^{13}C$ and to 85% $H_3PO_4$ for $^{31}P$; the coupling constants ($J$) are given in Hz; conventional abbreviations are used. For the description of the NMR spectra, the protons and carbon atoms of the glucosamine units are symbolized by H and C; for the *N*-acetyl-D-glucosamine units, they are symbolized by H′ and C′; for the residual *N*-phtalimido-glucosamine units,[35] by H″ and C″; and finally for the phosphorylated glucosamine units, by H‴ and C‴.

## FTIR Spectroscopy

The infrared spectra were recorded with a Perkin-Elmer 16 PC spectrophotometer as KBr pellets and absorbances are given in $\upsilon$ ($cm^{-1}$).



## Thermal Analyses

Differential scanning calorimetry (DSC) measurements were performed on a Perkin Elmer DSC7 instrument. DSC curves were obtained from the heating run at a rate of 10 K/min under nitrogen conditions. Thermal gravimetric analyses (TGA) were performed on a Perkin Elmer TGA7 at a rate of 20 K/min under nitrogen conditions.

## Synthesis of *N*-(Diisopropylphosphono-thiooxomethyl) Chitosan (3) from 6-*O*-Triphenylmethyl Chitosan

Triethylamine (0.13 mL, 1 equiv) and **2** (0.25 g, 1 equiv) (red color) were added to a solution of 6-*O*-trityl-chitosan **1** (0.4 g, 1 $NH_2$ equiv) in THF under $N_2$. The solution was stirred for 3 days at 20°C, until the disappearance of the red color. Then the polymer was precipitated in acetone, washed three times with acetone, and dried under vacuum. To deprotect the primary hydroxy groups, the obtained solid was dissolved in a mixture of chloroform (10 mL) and methanol (0.32 mL) and a solution of acetyl chloride (0.06 mL, 0.84 mmol) in methanol (0.71 mL) was added dropwise. The mixture was stirred at 20°C for 5 h.[36] **3** was precipitated in acetone, washed by soxhlet in acetone, and dried under vacuum. A yellow solid was obtained (0.26 g, 66%). $^{31}P\{^1H\}$ NMR ($D_2O$): $\delta = -2.9$; $^1H$ NMR ($D_2O/CD_3CO_2D$): $\delta = 1.36$ (m, 12H, $CH_3$ (*i*Pr)) , 2.03 (s, 3H, $CH_3CO$), 3.15 (s, 1H, $H_2$), 3.47–4.03 (m, 23H, $H_{2'}$, $H_{2''}$, $H_{2'''}$, $H_3$, $H_{3'}$, $H_{3''}$, $H_{3'''}$, $H_4$, $H_{4'}$, $H_{4''}$, $H_{4'''}$, $H_5$, $H_{5'}$, $H_{5''}$, $H_{5'''}$, $H_6$, $H_{6'}$, $H_{6''}$, $H_{6'''}$), 4.55 (s, 1H, $H_{1'}$, $H_{1''}$), 4.60–5.00 (m, 3H, $H_1$,CH (*i*Pr)), 7.65–7.92 (m, 5H, CH phtalimide, NHCS); $^{13}C$ NMR ($D_2O$): $\delta = 23.1$ ($CH_3CO$), 24.0 ($CH_3$ (*i*Pr)), 55.8–57.8 ($C_2$, $C_{2'}$, $C_{2''}$, $C_{2'''}$), 61.67–61.71 ($C_6$, $C_{6'}$, $C_{6''}$,

$C_{6''}$), 70.7–71.5 ($C_3$, $C_{3'}$, $C_{3''}$, $C_{3'''}$), 75.7 ($C_5$, $C_{5'}$, $C_{5''}$, $C_{5'''}$), 76.4–77.2 ($C_4$, $C_{4'}$, $C_{4''}$, $C_{4'''}$), 77.2–77.7 (CH (*i*Pr)), 98.5 ($C_1$), 101.4 ($C_{1'''}$), 103.0 ($C_{1'}$, $C_{1''}$), 124.7–137.8 (C and CH phtalimide), 171.0 (CO phtalimide), 175.7 ($COCH_3$), 197.6 (d, $^1J_{PC} = 186.7$ Hz, C=S); IR: 3442 ($\nu_{N-H}$, $\nu_{O-H}$), 2982 ($\nu_{C-H}$), 1776 ($\nu_{C=O}$ phtalimide), 1716 ($\nu_{C=O}$ phtalimide), 1656 ($\delta_{N-H}$, $\nu_{C=O}$), 1246 ($\nu_{P=O}$), 1096 ($\nu$ pyranose unit, $\nu_{C=S}$), 996 ($\nu_{C-O}$ alcohol).

## Synthesis of *N*-(Diisopropylphosphono-thiooxomethyl) Chitosan (3′) from Unprotected Chitosan

To a stirred solution of chitosan (0.2 g, 1 $NH_2$ equiv) in 10 mL of 0.2 N acetic acid, 5 mL of THF was added followed by the addition of triethylamine (0.19 mL, 1.2 equiv) leading to precipitation of chitosan. Then **2** (0.3 g, 1 equiv) was added to the suspension. A pink gel was formed. Stirring at 20°C was continued until the disappearance of the color (3 d). The gel was then washed by soxhlet in acetone and dried under vacuum. A pale yellow solid was obtained (0.18 g, 90%). $^{31}P\{^1H\}$ NMR ($D_2O/CD_3CO_2D$): $\delta = -2.9$ ; $^1H$ NMR ($D_2O/CD_3CO_2D$): $\delta = 1.37$ (s, 12H, $CH_3$ (*i*Pr)), 2.21 (s, 3H, $CH_3CO$), 3.13 (s, 1H, $H_2$), 3.40–4.10 (m, 17H, $H_{2'}$, $H_{2'''}$, $H_3$, $H_{3'}$, $H_{3'''}$, $H_4$, $H_{4'}$, $H_{4'''}$, $H_5$, $H_{5'}$, $H_{5'''}$, $H_6$, $H_{6'}$, $H_{6'''}$), 4.60–5.10 (5H, $H_{1'}$, $H_{1'''}$, $H_1$, CH (*i*Pr)), 7.64 (m, 1H, NHCS); $^{13}C$ NMR ($D_2O/CD_3CO_2D$): $\delta = 23.5$ ($CH_3CO$), 30.1 ($CH_3$ (O*i*Pr)), 56.2 ($C_2$,$C_{2'}$, $C_{2'''}$), 60.4 ($C_6$, $C_{6'}$, $C_{6'''}$), 70.5–72.1 ($C_3$, $C_{3'}$, $C_{3'''}$), 75.2 ($C_5$, $C_{5'}$, $C_{5'''}$), 76.5 ($C_4$, $C_{4'}$, $C_{4'''}$), 78.9 (CH (O*i*Pr)), 97.9 ($C_1$), 101.4 ($C_{1'}$, $C_{1'''}$), 174.9 ($COCH_3$), 197.1 (d, $^1J_{PC} = 187.3$ Hz, PC=S); IR: 3448 ($\nu_{N-H}$, $\nu_{O-H}$), 2924 ($\nu_{C-H}$), 1672 ($\delta_{N-H}$, $\nu_{C=O}$), 1242 ($\nu_{P=O}$), 1152 ($\nu_{C-O}$ether, $\delta_{OH}$ alcohol), 1072 ($\nu$ pyranose unit, $\nu_{C=S}$), 1028 ($\nu_{C-O}$alcohol).

## Synthesis of *N*-(2-Diethylphosphono-ethyl)-chitosan (5)

Compound **1** (0.4 g, 1 equiv) was dissolved in THF (6 mL); distilled triethylamine (0.12 mL, 0.9 equiv) and **4** (0.23 g, 1 equiv) were then added. The solution was stirred for 3 days at 20°C and the polymer was precipitated in acetone. The solid was washed with acetone and dried under vacuum. For the deprotection, the obtained solid was dissolved in a mixture of chloroform (10 mL) and methanol (0.32 mL), and a solution of acetyl chloride (0.06 mL, 0.84 mmol) in methanol (0.7 mL) was added dropwise. Then the mixture was stirred at 20°C for 5 h.[36] Compound **5** was precipitated in acetone, washed by soxhlet in acetone, and dried under vacuum. A brown solid was obtained (0.35 g, 88%); $^{31}P\{^1H\}$ NMR ($D_2O$): $\delta = 28.2$; $^{31}P$ NMR ($D_2O$): $\delta = 29.2$ (t, $^2J_{PH} = 10.3$ Hz); $^1H$ NMR ($D_2O$): $\delta = 1.33$ (t, $^3J_{HH} = 6.9$ Hz, 6H,

P(OCH$_2$C$\underline{H}_3$)$_2$), 2.04 (s, 3H, C$\underline{H}_3$CO), 2.22–2.37 (m, 2H, NCH$_2$C$\underline{H}_2$P), 3.09–3.18 (m, 1H, H$_2$), 3.44–3.59 (m, 2H, NC$\underline{H}_2$CH$_2$P), 3.59–4.10 (m, 23H, H$_{2'}$, H$_{2''}$, H$_{2'''}$, H$_3$, H$_{3'}$, H$_{3''}$, H$_{3'''}$, H$_4$, H$_{4'}$, H$_{4''}$, H$_{4'''}$, H$_5$, H$_{5'}$, H$_{5''}$, H$_{5'''}$, H$_6$, H$_{6'}$, H$_{6''}$, H$_{6'''}$), 4.18 (m, 4H, P(OC$\underline{H}_2$CH$_3$)$_2$), 4.56 (s, 3H, H$_{1'}$, H$_{1''}$, H$_{1'''}$), 4.80 (s, 1H, H$_1$), 7.62–7.89 (m, 4H, CH phtalimide); $^{13}$C NMR (D$_2$O): $\delta = 15.9$ (d, $^3J_{PC} = 5.2$ Hz, P(OCH$_2$C$\underline{H}_3$)$_2$), 22.1 (d, $^1J_{PC} = 139.1$ Hz, NCH$_2$C$\underline{H}_2$P), 22.4 (s, C$\underline{H}_3$CO), 41.7 (s, NC$\underline{H}_2$CH$_2$P), 55.8–56.0 (C$_{2'}$, C$_{2''}$, C$_{2'''}$), 56.1 (s, C$_2$), 60.2 (s, C$_6$), 60.5–61.4 (C$_{6'}$, C$_{6''}$, C$_{6'''}$), 64.4 (d, $^2J_{PC} = 6.4$ Hz, P(OC$\underline{H}_2$CH$_3$)$_2$), 70.3–71.6 (C$_3$, C$_{3'}$, C$_{3''}$, C$_{3'''}$), 74.6–75.0 (C$_5$, C$_{5'}$, C$_{5''}$, C$_{5'''}$), 76.5–76.9 (C$_4$, C$_{4'}$, C$_{4''}$, C$_{4'''}$), 97.8 (s, C$_1$) 101.56–101.59 (C$_{1'}$, C$_{1''}$, C$_{1'''}$), 127.2–133.3 (C and CH phtalimide), 170.1 (CO phtalimide), 174.9 (COCH$_3$); IR: 3450 ($\nu_{N-H}$, $\nu_{O-H}$), 2882 ($\nu_{C-H}$), 1775 ($\nu_{C=O}$ phtalimide), 1715 ($\nu_{C=O}$ phtalimide), 1638 ($\delta_{N-H}$, $\nu_{C=O}$), 1539 ($\delta_{N-H}$), 1218 ($\nu_{P=O}$), 1162 ($\nu_{C-O}$ether, $\delta_{OH}$ alcool), 1074 ($\nu_{C-O}$alcool, $\delta_{OH}$ alcool).



## Synthesis of *N*-[(2,2-bis-(diethylphosphono)-ethyl]-chitosan (7)

To a solution of **1** (0.4 g, 1 equiv) in THF (10 mL) was added a solution of **6** (0.24 g, 1 equiv) in THF (2 mL). The solution was stirred for 24 h at 50°C, and then THF was partially evaporated. To deprotect the *O*-Tr, the obtained red gel was dissolved in a mixture of chloroform (10 mL) and methanol (0.32 mL), and a solution of acetyl chloride (0.06 mL, 0.84 mmol) in methanol (0.7 mL) was added dropwise, then the mixture was stirred at 20°C for 5 h.[36] Compound **7** was precipitated with acetone, washed in soxhlet in acetone, and dried under vacuum. A brown solid was obtained (0.37 g, 92%). $^{31}$P{$^1$H} NMR (D$_2$O): $\delta = 22.7$–24.4. $^1$H NMR (D$_2$O): $\delta = 1.22$ (t, $^3J_{HH} = 6.8$ Hz, 12H, P(OCH$_2$C$\underline{H}_3$)$_2$), 1.93 (s, 3H, CH$_3$CO), 3.06–3.18 (m, 1H, H$_2$), 3.50–3.95 (m, 23H, H$_{2'}$, H$_{2''}$, H$_{2'''}$, H$_3$, H$_{3'}$, H$_{3''}$, H$_{3'''}$, H$_4$, H$_{4'}$, H$_{4''}$, H$_{4'''}$, H$_5$, H$_{5'}$, H$_{5''}$, H$_{5'''}$, H$_6$, H$_{6'}$, H$_{6''}$, H$_{6'''}$), 4.12–4.20 (m, 8H, P(OC$\underline{H}_2$CH$_3$)$_2$), 4.45 (s, 3H, H$_{1'}$, H$_{1''}$, H$_{1'''}$), 4.78 (s, 1H, H$_1$), 7.65–7.90 (m, 4H, CH phtalimide). $^{13}$C NMR (D$_2$O): $\delta = 15.9$ (d, $^3J_{PC} = 6.0$ Hz, P(OCH$_2$C$\underline{H}_3$)$_2$), 22.5 (s, C$\underline{H}_3$CO), 55.4–55.9 (C$_{2'}$, C$_{2''}$, C$_{2'''}$), 56.1 (s, C$_2$), 60.3 (s, C$_6$), 60.5–61.2 (C$_{6'}$, C$_{6''}$, C$_{6'''}$), 64.6 (m, P(OC$\underline{H}_2$CH$_3$)$_2$), 69.9–70.8 (C$_3$, C$_{3'}$, C$_{3''}$, C$_{3'''}$), 75.1–75.4 (C$_5$, C$_{5'}$, C$_{5''}$, C$_{5'''}$), 76.6–78.7 (C$_4$, C$_{4'}$, C$_{4''}$, C$_{4'''}$), 97.9 (s, C$_1$), 101.5–101.6 (C$_{1'}$, C$_{1''}$, C$_{1'''}$), 123.7–135.7 (C and CH phtalimide), 170.4 (s, CO phtalimide), 175.0 (s, COCH$_3$). IR: 3448 ($\nu_{N-H}$; $\nu_{O-H}$), 2932 ($\nu_{C-H}$), 1778 ($\nu_{C=O}$ phtalimide), 1716 ($\nu_{C=O}$ phtalimide), 1654 ($\delta_{N-H}$; $\nu_{C=O}$), 1600 ($\delta_{N-H}$), 1252 ($\nu_{P=O}$), 1162 ($\nu_{C-O}$ ether), 1022 ($\nu_{C-O}$ alcohol).

## Synthesis of *N*-(Diisopropylphosphono-thiooxomethyl)-D-glucosamine (8)

To a stirred solution of D-glucosamine (0.15 g, 0.714 mmol, 1 equiv) in water (5 mL), 5 mL of THF was added, then triethylamine (0.15 mL, 1.071 mmol, 1.5 equiv). Compound **2** (0.18 g, 0.714 mmol, 1 equiv) was added. The red solution was stirred until the disappearance of the color. After precipitation of the residual D-glucosamine with acetone, filtration, and evaporation of the solvent, a yellow oil was obtained (0.251 g, 95%). $^{31}P\{^{1}H\}$ NMR ($D_2O$): $\delta = -1.8$ (s, 25%, NC(S)P(O)(*i*Pr)$_2$, form 1), $-2.4$ (s, 75%, NC(S)P(O)(*i*Pr)$_2$, form 2). $^{1}H$ NMR ($D_2O$): $\delta = 1.36$ (d, $^{3}J_{HH} = 6.4$ Hz, 6H, $CH_3$ (O*i*Pr)), 1.38 (d, $^{3}J_{HH} = 6.0$ Hz, 6H, (O*i*Pr)), 3.28 (dd, $^{3}J_{HH} = 7.2$ Hz, 1H, $H_2$), 3.45–3.54 (m, 1H, $H_4$), 3.65–4.09 (m, 4H, $H_3$, $H_5$, $H_6$), 4.93 (m, 2H, CH (O*i*Pr)), 5.43 (d, $^{3}J_{HH} = 3.6$ Hz, 1H, $H_1$), 8.70 (s, 1H, NHC(S), form 1), 8.75 (s, 1H, NHC(S), form 2). $^{13}C$ NMR ($D_2O$): form 1: $\delta = 22.8$ (d, $^{3}J_{PC} = 4.6$ Hz, $CH_3$ (O*i*Pr)), 23.0 (d, $^{3}J_{PC} = 3.9$ Hz, $CH_3$ (O*i*Pr)), 58.9 (d, $^{3}J_{PC} = 8.0$ Hz, $C_2$), 60.3 (s, $C_6$), 69.5 (s, $C_4$), 71.5 (s, $C_3$), 75.9 (s, $C_5$), 76.1 (d, $^{2}J_{PC} = 6.4$ Hz, CH (O*i*Pr)), 88.6 (s, $C_1$), 195.0 (d, $^{1}J_{PC} = 185.2$ Hz, C=S); form 2: $\delta = 22.8$ (d, $^{3}J_{PC} = 4.6$ Hz, $CH_3$ (*i*Pr)), 23.0 (d, $^{3}J_{PC} = 3.9$ Hz, $CH_3$ (O*i*Pr)), 60.5 (s, $C_6$), 60.9 (d, $^{3}J_{PC} = 8.1$ Hz, $C_2$), 69.6 (s, $C_4$), 71.6 (s, $C_3$), 75.9 (s, $C_5$), 76.1 (d, $^{2}J_{PC} = 6.4$ Hz, CH (O*i*Pr)), 94.4 (s, $C_1$), 195.8 (d, $^{1}J_{PC} = 185.0$ Hz, C=S). HRMS ($[M+H]^+ = [C_{13}H_{27}NO_8SP]$): 387.9401 (calcd: 387.9406).



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