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Preparation and NMR characterization of highly substituted *N*-trimethyl chitosan chloride

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Abstract

N,N,N-Trimethyl chitosan chloride (TMC) is a chemically modified chitosan with improved aqueous solubility, compared with the native chitosan. It is essential to follow a synthesis procedure in which the degree of substitution of the final product can be controlled by means of the number of reaction steps, the duration of each reaction step and the amount of methyl iodide as reagent. A two-step reaction yields products with high degrees of substitution (40–80%). Comparison of the NMR spectra of the product TMC, after a two-step reaction, indicates that there is a peak assigned to the substituted amino group that shifts from 2.5 to 3.1 ppm upon acidification. This peak must be assigned to $N(CH_3)_2$ and not to $N(CH_3)_3^+$. A three-step reaction procedure yields products with a degree of substitution $> 80\%$, but with substantially decreased water-solubility.

Keywords: *N,N,N*-Trimethyl chitosan chloride; NMR; Synthesis; Chitosan; Absorption enhancers

1. Introduction

Chitosan is a polymer which attracts high interest in the pharmaceutical research area as a polymeric carrier of drugs. Recently, it has been shown that chitosan enhances the absorption of peptide and protein drug across nasal (Illum et al., 1994) and intestinal epithelia (Artursson et al., 1994). Chitosan glutamate does not only enhance the in vitro transport of small hydrophilic compounds like ^{14}C-mannitol (Borchard et al., 1996), but also the transport of high-molecular-weight peptide drugs like 9-desglycinamide, 8-arginine vasopressin (Lueßen et al., 1997). Chitosan hydrochloride has been validated as an intestinal absorption enhancer in vivo of the peptide drug buserelin when it was coadministered in rats (Lueßen et al., 1996).

However, chitosan has an apparent pK_a of 5.6 (as measured by potentiometric titration) and is only soluble in acidic solutions with pH values lower than 6.0. This interferes with the biomedical application of this polymer, especially at the physiological pH value (7.4) where chitosan is insoluble and consequently less effective. In past years, several derivatives of chitosan have been synthesized which are water-soluble over a wider pH range. Among these are *N*-carboxymethylated chitosan (Muzzarelli et al., 1982; Le Dung et al., 1994) and *N*-trimethyl chitosan chloride (Le Dung et al., 1994; Domard et al., 1986; Muzzarelli & Tanfani, 1985). *N*-Trimethyl chitosan chloride (TMC) is of particular interest because of its well-defined structure, improved solubility and easy preparation as described by Le Dung et al. (1994). They obtained 53% of quaternization of the amino group in just one reaction step, without any observable O-methylation. This method was used as a basis to prepare TMC with different degrees of substitution from chitosan, in order to study the potential of these derivatives as absorption enhancers for hydrophilic and macromolecular drugs such as peptides and proteins.

2. Experimental

Chitosan (93% deacetylated) was a gift from Pronova Biopolymer A.S. (Drammen, Norway). The material was crushed in a mortar with a pestle and sieved to obtain the

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fraction less than 500 μm. Only material of small particle size showed sufficient solubility. Methyl iodide and 1-methyl-2-pyrrolidinone were obtained from Acros (Geel, Belgium). All other chemicals are commercially available and were used as received.

One-step synthesis. To prepare TMC, 2 g of chitosan (sieved, < 500 μm) and 4.8 g of sodium iodide were dissolved in 80 ml of 1-methyl-2-pyrrolidinone on a water bath at 60°C with stirring. After the chitosan was dissolved, 11 ml of a 15% aqueous sodium hydroxide solution were added, and then 11.5 ml of methyl iodide, both with stirring. The mixture was stirred for 1 h (Le Dung et al., 1994). The product was precipitated using ethanol and subsequently isolated by centrifugation. After washing with ethanol and centrifugation, the material was dissolved in 40 ml of water, to which 250 ml of 1 M HCl in ethanol (96%) were added carefully, thus exchanging the iodide for chloride. Centrifugation and washing with ethanol and subsequently with ether yielded a white, water-soluble powder, which was dried in vacuo at 40°C.

Two-step synthesis. A mixture of 2 g of sieved chitosan (93% deacetylated), 4.8 g of sodium iodide, 11 ml of a 15% aqueous sodium hydroxide solution and 11.5 ml of methyl iodide in 80 ml of 1-methyl-2-pyrrolidinone was stirred on a water bath of 60°C for 1 h (Le Dung et al., 1994). Special care was taken to keep the methyl iodide in the reaction mixture by using a Liebig condenser. The product was precipitated using ethanol and thereafter isolated by centrifugation. The *N*-trimethyl chitosan iodide obtained after this first step was washed twice with ether on a glass filter to remove the ethanol. It was dissolved in 80 ml of 1-methyl-2-pyrrolidinone and heated to 60°C, thus removing most of the absorbed ether. Subsequently, 4.8 g of NaI, 11 ml of 15% NaOH solution and 7 ml of methyl iodide were added with rapid stirring and the mixture was heated on a water bath at 60°C for 30 min. An additional 2 ml of methyl iodide and 0.6 g of NaOH pellets were added and the stirring was continued for 1 h.

The product, prepared as described above, was dissolved in 40 ml of a 10% NaCl aqueous solution, instead of HCl, to exchange the iodide. The polymer was precipitated with ethanol, isolated by centrifugation and thoroughly washed with ethanol and ether. In vacuo drying yielded a white, water-soluble powder.

^{1}H NMR spectra were measured in D_2O at 80°C, using a 300 or 600 MHz spectrometer (Bruker, Switzerland). ^{13}C NMR spectra were measured in D_2O at 80°C at 150 MHz. No attempts were made to remove the residual water from the NMR sample, because at 80°C this peak does not interfere with the spectrum of the polymer.

3. Results and discussion

The ^{1}H NMR spectrum of the TMC obtained after one-step synthesis is depicted in Fig. 1. It is similar to the spectrum reported by Le Dung et al. (1994), except for the smaller acetyl peak at 2.0 ppm. It clearly shows a peak at 3.1 ppm assigned to $N(CH_3)_3^+$, together with a smaller peak at 3.4 ppm assigned to $N(CH_3)_2$. According to the peak assignment by Le Dung et al. (1994), we calculated primarily the degree of quaternization as 35%.

To investigate the effect of degree of substitution for pharmaceutical experiments, we tried to prepare TMC with different and preferably higher substitution degrees. After several attempts, varying the reaction times (45 to 360 min) and the amount of sodium hydroxide (aqueous solutions from 15 to 25%), we concluded that the optimal way to synthesize highly substituted *N*-trimethyl chitosan is the two-step synthesis as described above. After the first step complete removal of the ether turned out to be problematic, but the residual amount could be used to our advantage, because it refluxes together with the methyl iodide, thus increasing the amount of methyl iodide in solution. The addition of 2 ml of methyl iodide and 0.6 g of NaOH pellets, at the end of the second step of the reaction, was necessary since without this lower degrees of quaternization were obtained.

A ^{1}H NMR spectrum of the product obtained via the two-step synthesis showed unexpected results when compared to the intermediate material. The peak at 3.0 ppm assigned by Le Dung et al. (1994) to the quaternized amino group has disappeared, the peak at 3.35 ppm assigned to the tertiary amino group has increased substantially, and a new peak appeared at 2.5 ppm (Fig. 2). Upon addition of a drop of DCl, this new peak shifted to the quaternized position at 3.1 ppm (Fig. 3). It is very unlikely that the NMR signal of a quaternized amino group will shift upon acidification of the solution. Therefore, the signal at 2.5 ppm in the neutral solution must be from the dimethylated amino group, which, upon acidification, will shift to a lower field. This leaves the peak at 3.4 ppm, which must be assigned to the quaternary amino group. The results described above indicate that Le Dung et al. (1994) misinterpreted their spectra and did not prepare *N*-trimethyl chitosan, but a mainly dimethylated polymer with only 10–15% of quaternization. Using the two-step method, degrees of quaternization of at least 60% were obtained. This also explains our observation that a poorly water-soluble polymer was obtained after the first step (15 min for the dissolution of a 2% (w/v) product upon stirring), when the iodide was exchanged for chloride using NaCl instead of hydrochloric acid. Upon acidification, this material instantly dissolved in water. The product after the two-step reaction rapidly dissolves in water in concentrations up to 5% (w/v).

Further evidence for our hypothesis was obtained from ^{13}C NMR spectroscopy. The spectra for the low- and high-substituted material are presented in Fig. 4 and Fig. 5, respectively. Also here the disappearance of the dimethylated signal, at 43.7 ppm (Muzzarelli & Tanfani, 1985), and the appearance of the trimethylated signal, at 55.1 ppm, are clearly visible. As expected, the highly substituted polymer

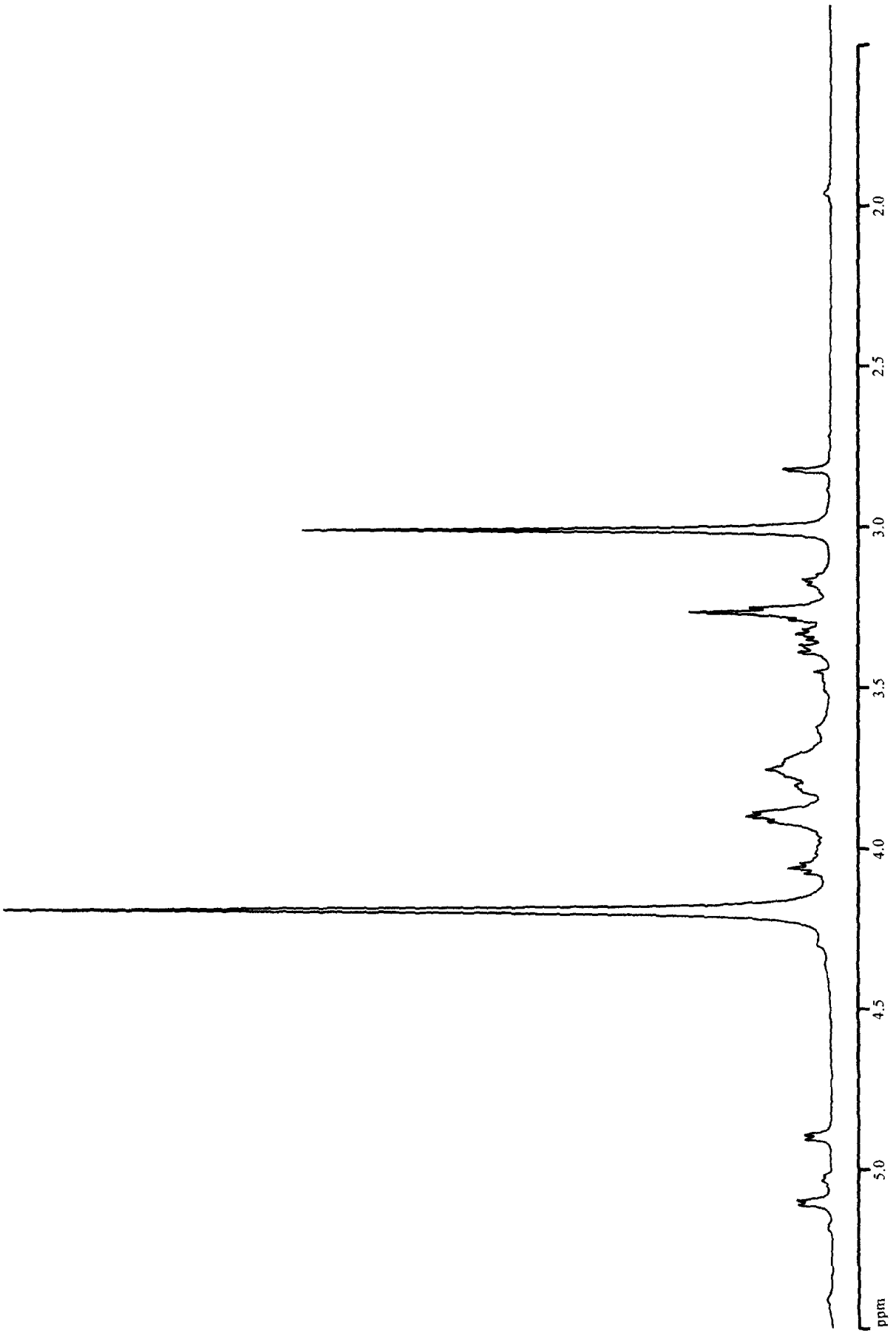

Fig. 1. ^{1}H NMR spectrum of *N*-trimethyl chitosan chloride after the one-step reaction, according to the proposed peak assignment; degree of substitution 35%.

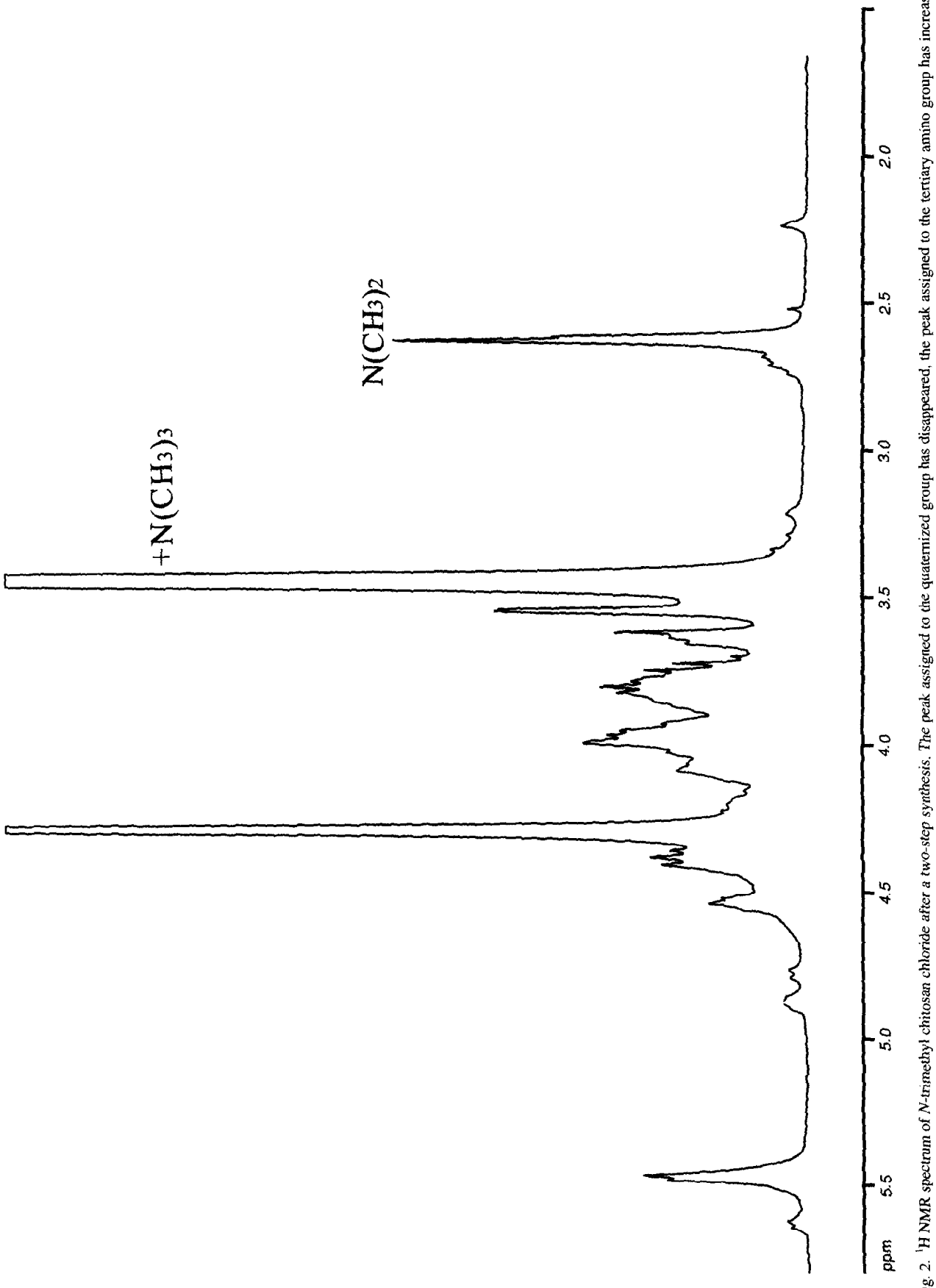

Fig. 2. ^{1}H NMR spectrum of *N*-trimethyl chitosan chloride after a two-step synthesis. The peak assigned to the quaternized group has disappeared, the peak assigned to the tertiary amino group has increased substantially and a new peak appeared at 2.5 ppm.

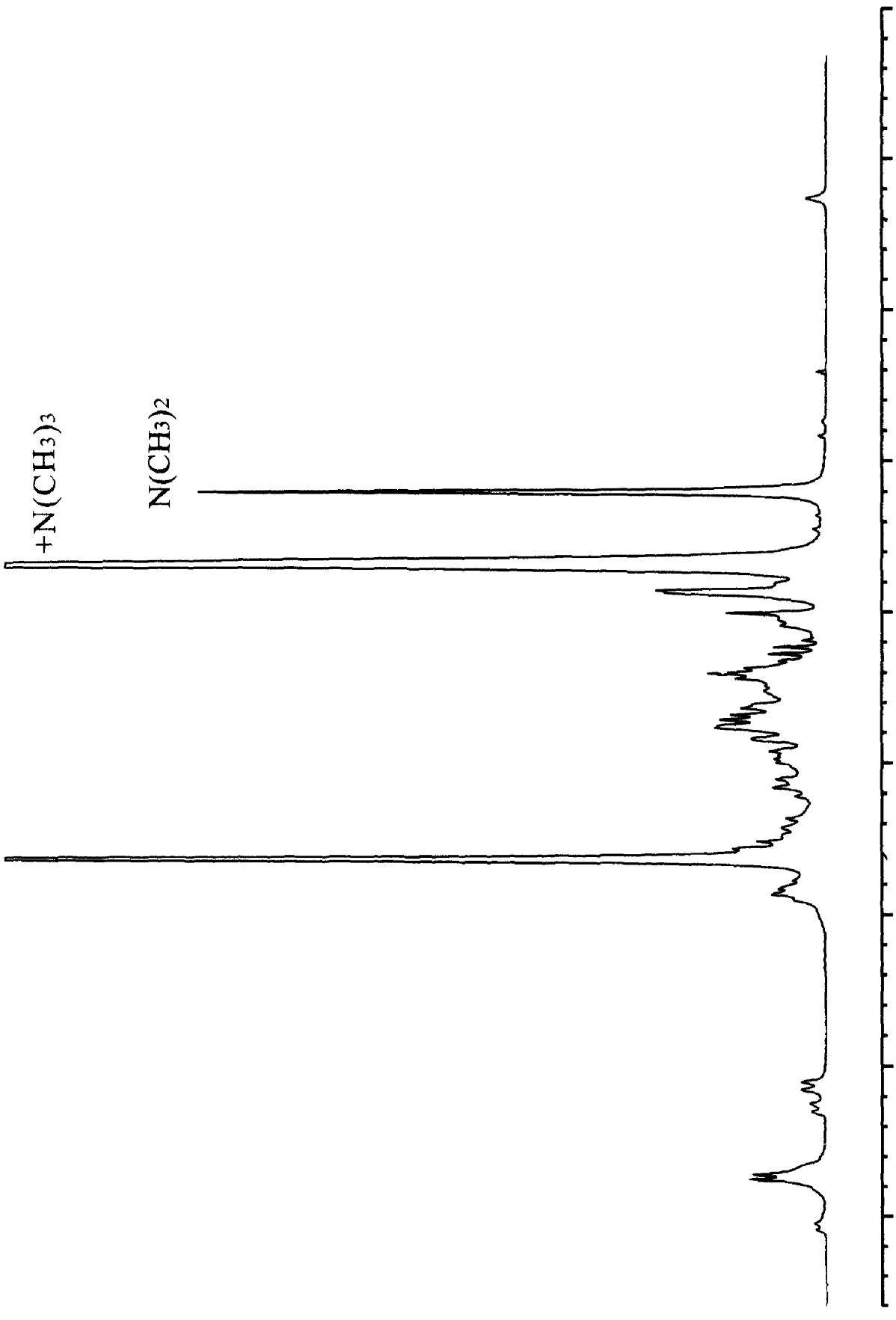

Fig. 3. ^{1}H NMR spectrum of *N*-trimethyl chitosan chloride after a two-step synthesis upon the addition of DCl (one drop). The peak which had appeared at 2.5 ppm shifted after acidification to the referred quaternized position 3.1 ppm. Therefore, the signal at 2.5 ppm in Fig. 2 must be from the dimethylated group.

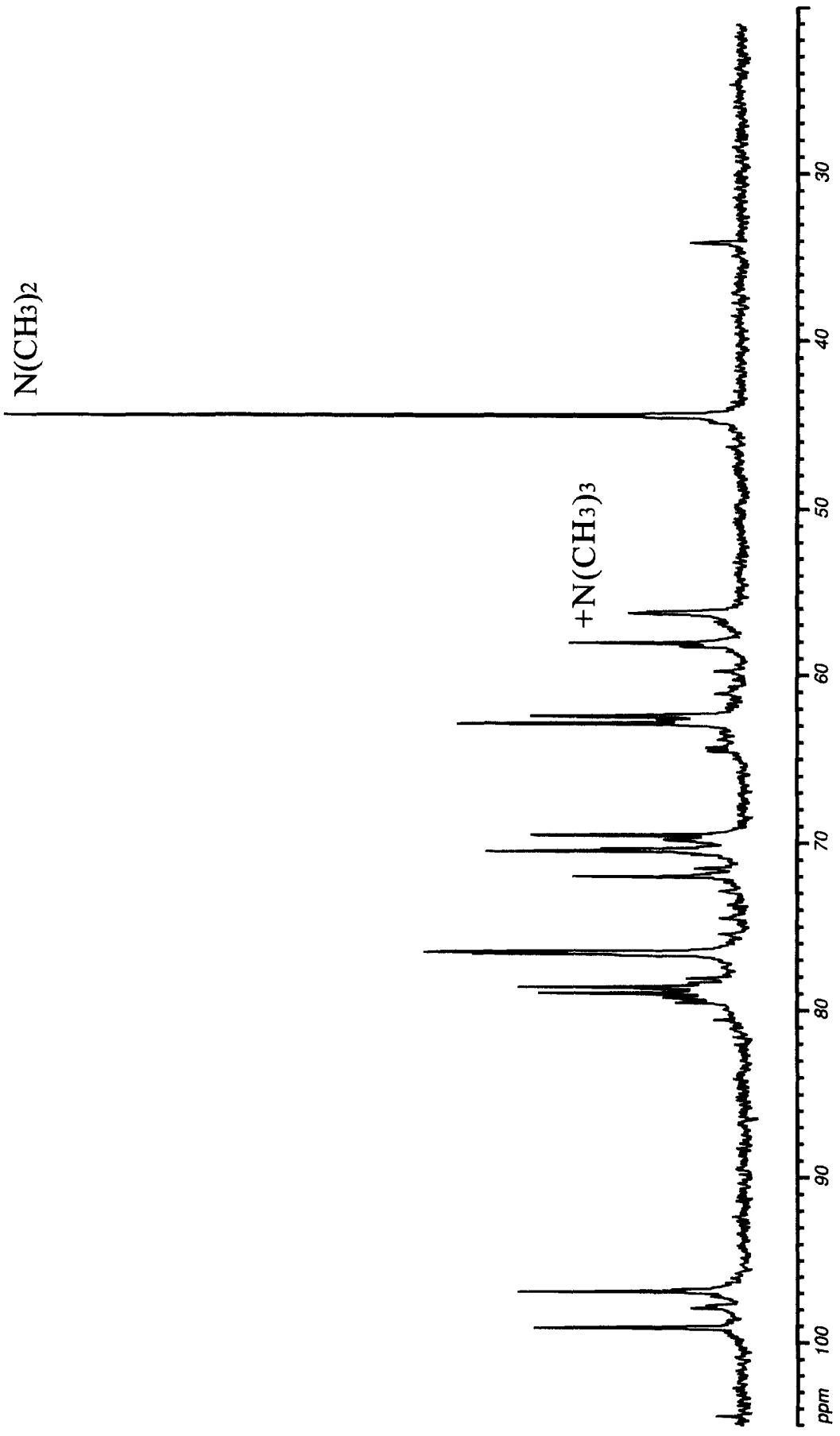

Fig. 4. ^{13}C NMR spectrum of *N*-trimethyl chitosan chloride after the one-step synthesis.

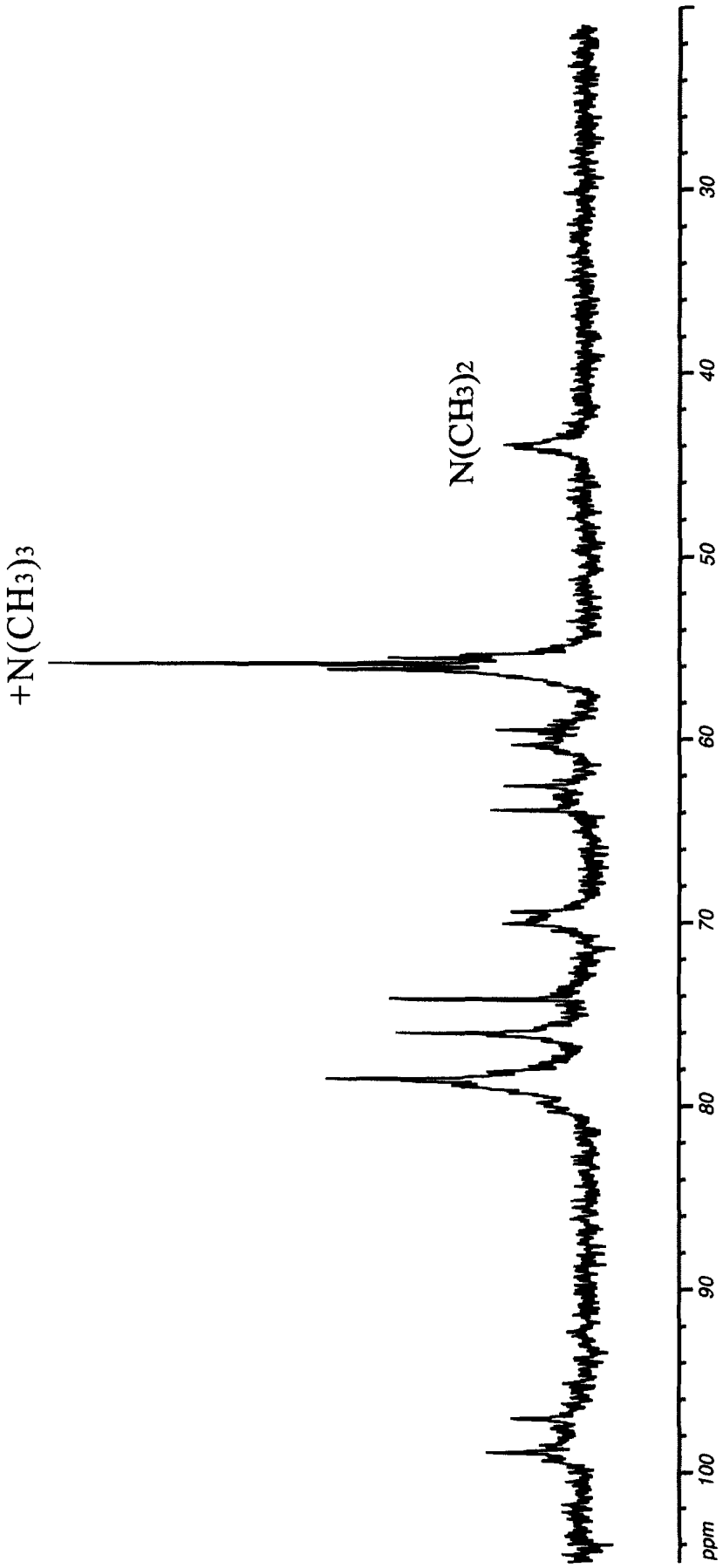

Fig. 5. ^{13}C NMR spectrum of *N*-trimethylchitosan chloride after a two-step synthesis.

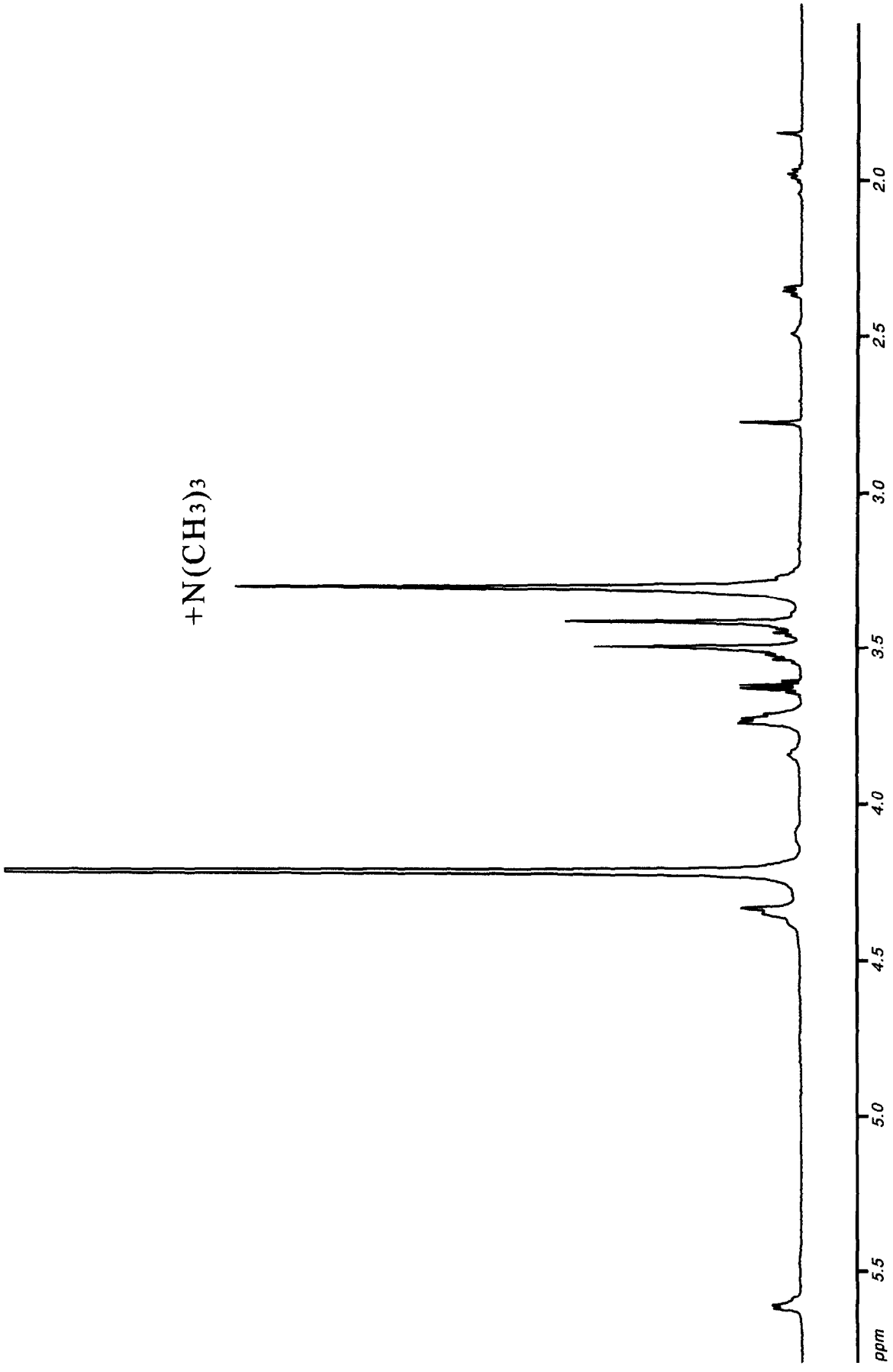

Fig. 6. ^{1}H NMR spectrum of *N*-trimethyl chitosan chloride after a three-step synthesis. The product has reduced solubility because of the O-permethylation.

still shows a residual signal from the dimethylated part at 43.1 ppm.

A three-step reaction procedure resulted in an even higher substituted polymer (degree of quaternization > 85%). However, it also resulted in complete O-methylation, which strongly decreases the solubility. The ^{1}H NMR spectrum of this *N,O*-permethyl chitosan in D_2O is depicted in Fig. 6. It shows the same $N(CH_3)_3^+$ peak as in Fig. 3, together with those for 3-OCH_3 and 6-OCH_3 at 3.55 and 3.45 ppm, respectively. This spectrum is similar to the one previously published (Domard et al., 1987).

4. Conclusions

Our results show that *N*-trimethyl chitosan chloride cannot be prepared in high degrees of substitution by the one-step method as described by Le Dung et al. (1994). It results mainly in the formation of the HCl salt of *N*-dimethyl chitosan. The synthesis of *N*-trimethyl chitosan chloride with higher degrees of quaternization requires a two-step reaction. With this procedure high degrees of substitution up to 70% can be obtained.

Furthermore, our results show that great care has to be taken when preparing derivatives of chitosan. The presence of even a small amount of acid, e.g. when used to exchange the counterion or for the dissolution of chitosan, can give rise to misleading results.

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