Biomacromolecules

February 2006 Published by the American Chemical Society Volume 7, Number 2

Communications

Synthesis of Novel Quaternary Chitosan Derivatives via *N*-Chloroacyl-6-*O*-triphenylmethylchitosans

Jukka Holappa,*,† Tapio Nevalainen,† Pasi Soininen,‡ Már Másson,§ and Tomi Järvinen†

Departments of Pharmaceutical Chemistry and Chemistry, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland, and Faculty of Pharmacy, University of Iceland, Hofsvallagata 53, 107 Reykjavik, Iceland

Received September 23, 2005; Revised Manuscript Received December 3, 2005

Various quaternary chitosan derivative structures were synthesized by reacting *N*-chloroacyl-6-*O*-triphenylmethylchitosans with tertiary amines. Full substitutions were obtained from the quaternization reactions and the obtained water-soluble quaternary chitosan derivatives were thoroughly characterized with ^{1}H NMR, ^{13}C NMR, ^{1}H−^{13}C HSQC NMR, and FT-IR.

Introduction

Chitosan (poly-1,4-β-D-glucosamine) is a nontoxic and biodegradable biopolymer that has been studied recently in various fields. The cationic character of chitosan has been suggested to be the main cause of the unique properties and activity of chitosan. Chitosan derivatives with a quaternary ammonium moiety are thus interesting, since a permanent cationic charge is gained on the polysaccharide backbone. Also the poor water-solubility of chitosan can be enhanced by attaching a quaternary ammonium moiety into chitosan. Quaternary chitosan derivatives have shown potential in various pharmaceutical applications, such as in delivery of peptides,[1−3] vaccines[4] and genes,[5,6] and as antimicrobials.[7−10]

We recently reported the synthetic route for the preparation of *N*-chloroacyl-6-*O*-triphenylmethylchitosans, which are extremely useful intermediates for synthetic modifications of chitosan.[11] In the present study, we studied the usefulness of these organo-soluble intermediates for the preparation of various quaternary chitosan derivatives.

The chemical structures of the water-soluble quaternary end products were thoroughly characterized by ^{1}H NMR, ^{13}C NMR, ^{1}H−^{13}C HSQC NMR, and FT-IR. The degrees of substitution were determined from the ^{1}H NMR spectra.

* Corresponding author. Tel: +358-41-4361195. Fax: +358-17-162456. E-mail: Jukka.Holappa@uku.fi.

† Department of Pharmaceutical Chemistry, Kuopio.

‡ Department of Chemistry, Kuopio.

§ University of Iceland.

Experimental Section

Materials. Chitoclear chitosan donated by Primex Ltd (Reykjavik, Iceland) with M_w 201 kDa, M_n 89.8 kDa and degree of deacetylation of 85%, was used as a starting material for the synthesis. *N*-Chloroacetyl-6-*O*-triphenylmethylchitosan **(1)** with a degree of substitution (ds) of 0.85 and *N*-chlorobutyryl-6-*O*-triphenylmethylchitosan **(2)** with a ds of 0.67 were prepared as reported earlier.[11] Pyridine, *N*-methylpyrrolidone and triethylamine were purified by distillation and all other reagents were of high-purity grade and used as received.

Characterization. ^{1}H and ^{13}C NMR spectra were recorded at 300K or at 343 K on a Bruker AVANCE DRX 500, operating at 500.13 and 125.76 MHz, respectively. The concentration of the samples was 20 mg in 600 μL of D_2O. 3-(Trimethylsilyl)propionate-d_4 was used as an internal standard. For ^{1}H spectra, the recycling time was 6.2 s and 64 transients were collected. For {^{1}H}−^{13}C spectra, the recycling time was 4.7 s and 8192 transients were accumulated. ^{1}H−^{13}C gradient-enhanced heteronuclear single quantum correlation (ge-HSQC) experiments were carried out in the phase sensitive mode, using the Echo/Antiecho-TPPI gradient selection. FT-IR spectra were recorded on a Nicolet 510 P spectrometer from KBr pellets.

Synthesis. *N-(1-Carboxymethyl-2-pyridinium)chitosan Chloride (3).* A total of 300 mg of *N*-chloroacetyl-6-*O*-triphenylmethylchitosan **(1)** was stirred in 10 mL of pyridine under argon at 60 °C for 72 h. The solvent was evaporated and the product was washed with methanol and diethyl ether. The product yield was 176 mg (51%).

The 6-*O*-triphenylmethyl protecting group was removed during a 3-h reaction by stirring 170 mg of the product from the previous reaction with 20 mL of 1 M HCl at room temperature. The reaction mixture was evaporated to dryness, and the product was washed with methanol and diethyl ether. The degree of substitution was calculated from the

10.1021/bm050707j CCC: $33.50
Published on Web 12/27/2005

^{1}H NMR spectrum by comparing the aromatic proton signals (H-9, H-10, and H-11) to the pyranose proton signals (H-2, H-3, H-4, H-5, and H-6), and it was 0.85. The product **(3)** yield was 63 mg (65%). IR (KBr): ν 3600−3100 (O−H), 3100−3000 (C−H, pyridinium), 2950−2700 (C−H), 1687 (amide I), 1559 (amide II), 1490 (C═C, pyridinium), 1374 (C═C, pyridinium), 1150−950 (C−O, pyranose), 783 (arom, pyridinium), 725 (arom, pyridinium), 677 cm^{-1} (arom, pyridinium). ^{1}H NMR at 300K (D_2O): δ 2.0 (CH_3, *N*-acetyl), 3.5−3.6 (H-5), 3.7−4.0 (H-6), 3.7−3.8 (H-4), 3.8−4.0 (H-3), 3.85−4.0 (H-2), 4.7−4.8 (H-1), 5.5−5.7 (H-8), 8.1−8.2 (H-10), 8.65−8.75 (H-11), 8.75−8.9 ppm (H-9). ^{13}C NMR at 300K (D_2O): δ 25.0 (CH_3, *N*-acetyl), 58.8 (C-2), 63.0 (C-6), 64.6 (C-8), 74.7 (C-3), 77.6 (C-5), 81.1 (C-4), 103.3 (C-1), 131.0 (C-10), 148.6 (C-9), 149.8 (C-11), 169.4 ppm (C-7).

*N-[1-Carboxymethyl-2-(1-methylimidazolium)]chitosan Chloride (***4**). A total of 295 mg of *N*-chloroacetyl-6-*O*-triphenylmethylchitosan **(1)** was stirred in 10 mL of 1-methylimidazole under argon at 60 °C for 72 h. The reaction mixture was evaporated to dryness, and the product was washed with methanol and diethyl ether. The product yield was 116 mg (34%).

The 6-*O*-triphenylmethyl protecting group was removed during a 3-h reaction by stirring 105 mg of the product from the previous reaction with 15 mL of 1 M HCl at room temperature. The reaction mixture was evaporated to dryness, and the product was washed with methanol and diethyl ether. The degree of substitution was calculated from the ^{1}H NMR spectrum by comparing the H-9, H-10, and H-11 proton signals to the pyranose proton signals (H-2, H-3, H-4, H-5, and H-6) and it was 0.85. The product **(4)** yield was 45 mg (77%). IR (KBr): ν 3600−3100 (O−H), 3100−3000 (C−H, imidazolium), 2950−2700 (C−H), 1685 (amide I), 1560 (amide II), 1375 (C═C, imidazolium), 1150−950 (C−O, pyranose). ^{1}H NMR at 300K (D_2O): δ 2.1 (CH_3, *N*-acetyl), 3.5−3.6 (H-5), 3.6−3.9 (H-6), 3.6−3.8 (H-4), 3.7−3.9 (H-3), 3.8−3.9 (H-2), 3.9−4.0 (H-12), 4.6−4.8 (H-1), 5.1−5.3 (H-8), 7.50 (H-11), 7.52 (H-10), 8.75−8.85 ppm (H-9). ^{13}C NMR at 300K (D_2O): δ 25.0 (CH_3, *N*-acetyl), 38.8 (C-12), 53.6 (C-8), 58.6 (C-2), 63.0 (C-6), 74.7 (C-3), 77.6 (C-5), 81.3 (C-4), 103.4 (C-1), 126.3 (C-11), 126.5 (C-10), 140.3 (C-9), 170.5 ppm (C-7).

*N-(1-Carboxymethyl-2-triethylammonium)chitosan Chloride (***5**). A total of 400 mg of *N*-chloroacetyl-6-*O*-triphenylmethylchitosan **(1)**, 5.05 mL of triethylamine, and 239 mg of KI were stirred in 20 mL of *N*-methylpyrrolidone under argon at 60 °C for 72 h. The reaction mixture was cooled in ice−water and the product was precipitated with diethyl ether and washed with methanol and diethyl ether. The product yield was 180 mg (33%).

The 6-*O*-triphenylmethyl protecting group was removed during a 3-h reaction by stirring 180 mg of the product from the previous reaction with 18 mL of 1 M HCl at room temperature. The reaction mixture was evaporated to dryness, and the product was washed with methanol and diethyl ether. The degree of substitution was calculated from the ^{1}H NMR spectrum by comparing the H-10 proton signal to the hemiacetal proton signal (H-1), and it was 0.85. The product **(5)** yield was 40 mg (45%). ^{1}H NMR at 343K (D_2O): δ 1.3 (H-10), 2.1 (CH_3, *N*-acetyl), 3.45−3.6 (H-5), 3.5−3.7 (H-9), 3.5−3.9 (H-6), 3.65−3.8 (H-4), 3.7−3.85 (H-3), 3.8−3.9 (H-2), 3.95−4.05 (H-8), 4.6−4.7 ppm (H-1). ^{13}C NMR at 343K (D_2O): δ 9.8 (C-10), 25.0 (CH_3, *N*-acetyl), 57.8 (C-9), 58.3 (C-2), 59.2 (C-8), 63.1 (C-6), 74.8 (C-3), 77.6 (C-5), 81.2 (C-4), 102.9 (C-1), 167.5 ppm (C-7).

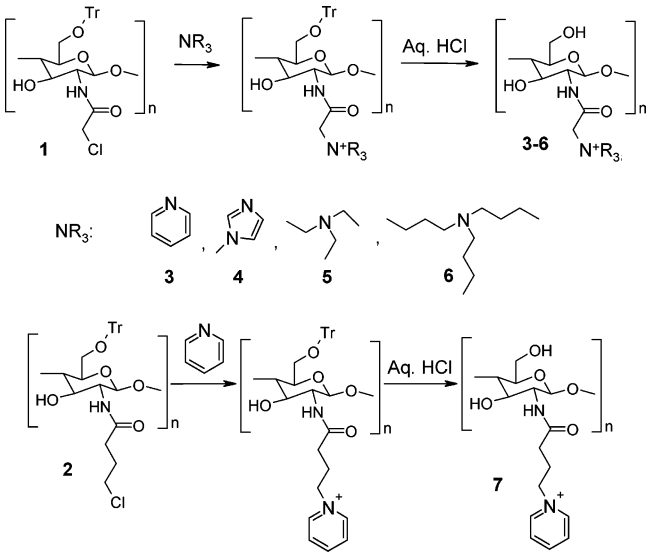
*N-(1-Carboxymethyl-2-tributylammonium)chitosan Chloride (***6**). A total of 400 mg of *N*-chloroacetyl-6-*O*-triphenylmethylchitosan **(1)**, 8.58 mL of tributylamine, and 239 mg of KI were stirred in 20 mL of *N*-methylpyrrolidone under argon at 60 °C for 72 h. The reaction mixture was cooled in ice−water and the product was precipitated with diethyl ether and washed with methanol and diethyl ether. The product yield was 120 mg (20%).

The 6-*O*-triphenylmethyl protecting group was removed during a 3-h reaction by stirring 120 mg of the product from the previous reaction with 12 mL of 1 M HCl at room temperature. The reaction mixture was evaporated to dryness, and the product was washed with methanol and diethyl ether. The degree of substitution was calculated from the ^{1}H NMR spectrum by comparing the H-10, H-11, and H-12 proton signals to the hemiacetal proton signal (H-1), and it was 0.85. The product **(6)** yield was 55 mg (83%). IR (KBr): ν 3600−3100 (O−H), 3100−2700 (C−H, pyranose and alkyl chains), 1685 (amide I), 1568 (amide II), 1150−950 cm^{-1} (C−O, pyranose). ^{1}H NMR at 343K (D_2O): δ 0.9−1.1 (H-12), 1.3−1.5 (H-11), 1.6−1.8 (H-10), 2.1 (CH_3, *N*-acetyl), 3.2−3.3 (H-9), 3.35−3.5 (H-9), 3.4−3.6 (H-5), 3.6−3.9 (H-6), 3.6−3.75 (H-4), 3.65−3.8 (H-3), 3.7−3.8 (H-2), 3.95−4.05 (H-8), 4.6−4.7 ppm (H-1). ^{13}C NMR at 343K (D_2O): δ 15.5 (C-12), 21.9 (C-11), 25.0 (CH_3, *N*-acetyl), 26.3 (C-10), 28.2 (C-10), 58.2 (C-9), 58.7 (C-2), 60.3 (C-8), 63.3 (C-6, C-9), 74.8 (C-3), 77.7 (C-5), 81.4 (C-4), 103.1 (C-1), 167.6 (C-7).

*N-(1-Carboxybutyl-4-pyridinium)chitosan Chloride (***7**). A total of 220 mg of *N*-chlorobutyryl-6-*O*-triphenylmethylchitosan (2) was stirred in 8 mL of pyridine under argon at 60 °C for 72 h. The solvent was evaporated, and the product was washed with methanol and diethyl ether. The product yield was 140 mg (57%).

The 6-*O*-triphenylmethyl protecting group was removed during a 3-h reaction by stirring 140 mg of the product from the previous reaction with 14 mL of 1 M HCl at room temperature. The reaction mixture was evaporated to dryness and the product was washed with methanol and diethyl ether. The degree of substitution was calculated from the ^{1}H NMR spectrum by comparing the aromatic proton signals (H-9, H-10, and H-11) to the pyranose proton signals (H-2, H-3, H-4, H-5, and H-6) and it was 0.67. The product **(7)** yield was 44 mg (56%). IR (KBr): ν 3600−3100 (O−H), 3100−3000 (C−H, pyridinium), 3000−2700 (C−H), 1655 (amide I), 1554 (amide II), 1489 (C═C, pyridinium), 1150−950 cm^{-1} (C−O, pyranose), 775 (arom, pyridinium), 682 (arom, pyridinium). ^{1}H NMR at 343K (D_2O): δ 2.0 (CH_3, *N*-acetyl), 2.3−2.4 (H-9), 2.4−2.5 (H-8), 3.1−3.2 (H-2, when amino group unsubstituted), 3.4−3.9 (H-6, H-5, H-4, H-3, H-2 substituted), 4.5−4.6 (H-1, substituted), 4.6−4.7 (H-10), 4.8−4.9 (H-1, unsubstituted), 8.1−8.2 (H-12), 8.5−8.6 (H-13), 8.8−8.9 ppm (H-11). ^{13}C NMR at 343K (D_2O): δ 25.1 (CH_3, *N*-acetyl), 29.1 (C-9), 34.8 (C-8), 58.2 (C-2, substituted), 58.8 (C-2, unsubstituted), 63.2 (C-6, substituted), 63.4 (C-6, unsubstituted), 63.7 (C-10), 73.5 (C-3, unsubstituted), 74.9 (C-3, substituted), 77.6 (C-5, substituted), 78.1 (C-5, unsubstituted), 80.2 (C-4 unsubstituted), 82.2 (C-4, substituted), 100.5 (C-1, unsubstituted), 103.8 (C-1, substituted), 131.3 (C-12), 147.0 (C-13), 148.8 (C-11), 177.3 (C-7).

Scheme 1. Synthetic Route for the Preparation of Various Quaternary Chitosan Derivatives (**3**-**7**) via *N*-Chloroacyl-6-*O*-triphenylmethylchitosans (**1** and **2**)



Results and Discussion

N-Chloroacyl-*6*-*O*-triphenylmethylchitosans (**1** and **2**), used as starting materials for the quaternization reactions, were

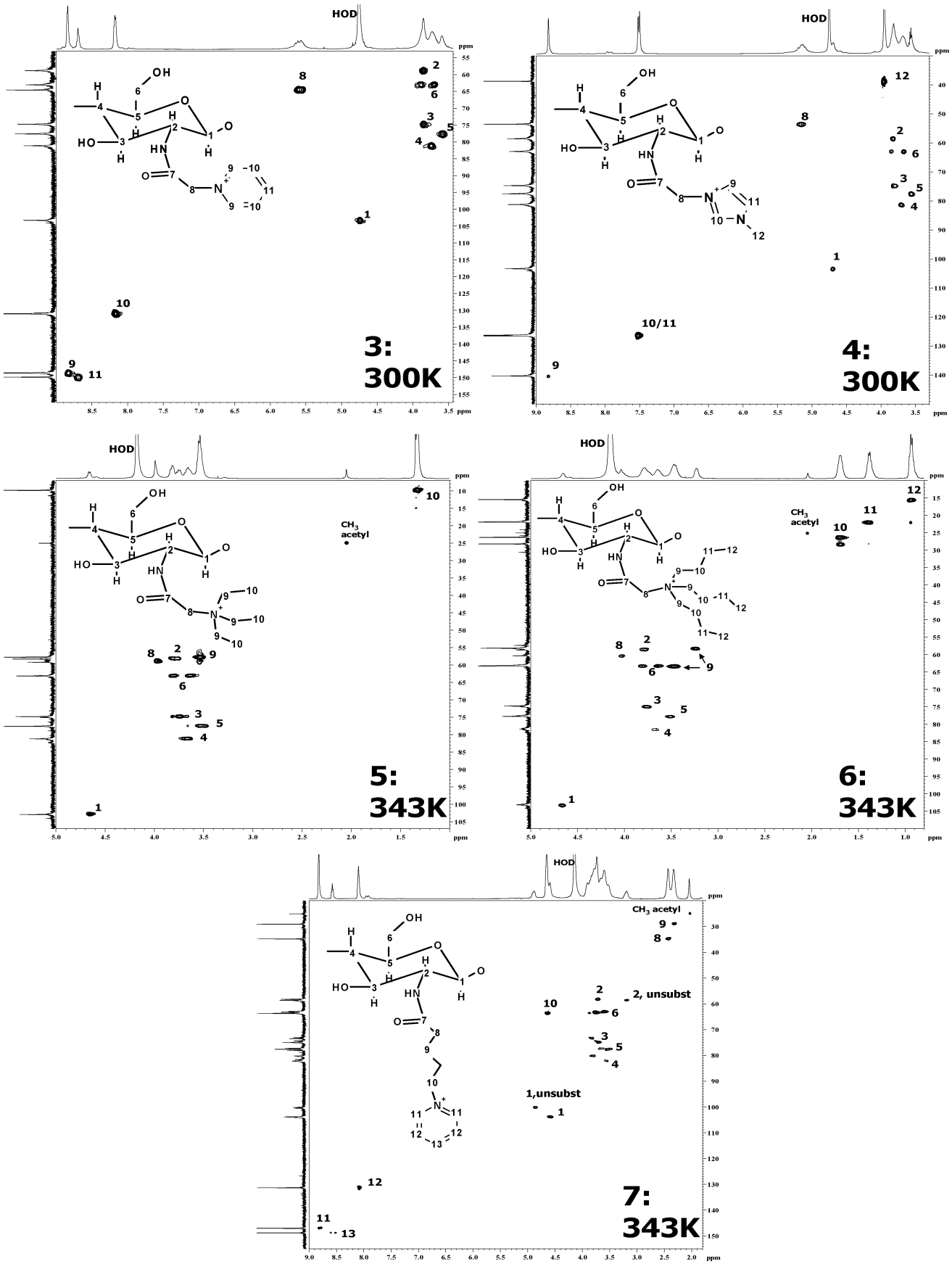


Figure 1. $^{1}H-^{13}C$ HSQC NMR spectra of quaternary chitosan derivatives measured in D_2O.

prepared as reported earlier via *N*-phthaloylchitosan, *N*-phthaloyl-6-*O*-triphenylmethylchitosan, and 6-*O*-triphenylmethylchitosan.[11] *N*-Chloroacetyl-6-*O*-triphenylmethylchitosan (**1**) had a full degree of *N*-chloroacetylation (0.85), whereas *N*-chlorobutyryl-6-*O*-triphenylmethylchitosan had a degree of *N*-chlorobutyrylation of 0.67. The ds value of 0.85 can be considered as

full substitution, since there were 15% of the *N*-acetyl groups remaining in the starting material. Various tertiary amines (**3**−**6**) were reacted with *N*-chloroacetyl-6-*O*-triphenylmethylchitosan (**1**), and a pyridine derivative (**7**) was also prepared from *N*-chlorobutyryl-6-*O*-triphenylmethylchitosan (**2**) (Scheme 1).

N-Chloroacyl-6-*O*-triphenylmethylchitosans are soluble in organic solvents, which enable the synthetic modifications in homogeneous reaction mixtures. Solubilization of chitosan is crucial in order to obtain high degrees of substitution and also to have control over the modification reaction.[12] It has also been suggested that heterogeneous reaction conditions can result in block structures in chitosan that can result in, for example, solubility problems.[13−15] Pyridine (**3** and **7**) and 1-methylimidazole (**4**) were attached into *N*-chloroacetyl-6-*O*-triphenylmethylchitosan (**1**) and pyridine also into *N*-chlorobutyryl-6-*O*-triphenylmethylchitosan (**2**) just by stirring the compounds **1** and **2** solely in dry pyridine and 1-methylimidazole, respectively. This strategy was attempted for triethylamine (**5**) and tributylamine (**6**) also, but this resulted in poor degrees of substitution. However, triethylamine and tributylamine were successfully attached to *N*-chloroacetyl-6-*O*-triphenylmethylchitosan (**1**) by reaction in *N*-methylpyrrolidone with addition of KI.

Reactions proceeded smoothly, and water-soluble end products were obtained after removal of the *O*-triphenylmethyl moiety. For the modification of *N*-chloroacetyl-6-*O*-triphenylmethylchitosan (**1**), the end products (**3**−**6**) showed a degree of substitution of 0.85; that is, all of the *N*-chloroacetyl moieties were substituted with tertiary amines. This was also the case with the modification of *N*-chlorobutyryl-6-*O*-triphenylmethylchitosan (**2**); that is, all of the chlorines were exchanged with pyridine moieties, so that the end product had a similar ds value (0.67) to that of the starting material. NMR spectra of the end products measured at 33 mg/mL in D_2O were well-defined and all the signals could be assigned (Figure 1). For the *N*-(1-carboxymethyl-2-tributylammonium)chitosan chloride (**6**), the NMR signals from the methylene-9 and methylene-10 are divided (Figure 1) due to the steric hindrance of the tributylamine side chains. For the *N*-(1-carboxybutyl-4-pyridinium)-chitosan chloride (**7**), the signals of H-1 and H-2 from the unsubstituted pyranose moiety can be clearly detected from the NMR spectra (Figure 1).

The molecular weights of the end products were not measured in this preliminary study. We have earlier reported that the protection−deprotection strategy applied here degrades the chitosan polymer.[11,16]

Conclusions

The present study shows that various water-soluble quaternary chitosan derivative structures can be prepared by reacting *N*-chloroacyl-6-*O*-triphenylmethylchitosans with tertiary amines. Some tertiary amines could be attached just by mixing them solely with *N*-chloroacyl-6-*O*-triphenylmethylchitosans, whereas the modification reaction in *N*-methylpyrrolidone, with the addition of KI, is required for other tertiary amines.

Full degree of substitution of the quaternization step was obtained, so chitosan derivatives with various degrees of quaternization can be prepared by using *N*-chloroacyl-6-*O*-triphenylmethylchitosans having various degrees of *N*-chloroacylation as starting materials.

Acknowledgment. This research was supported by the Academy of Finland (Grant #75268) and by the Graduate School of Pharmacy (University of Kuopio). Primex ehf is gratefully acknowledged for the donation of chitosan.

References and Notes

(1) Kotzé, A. F.; de Leeuw, B. J.; Lueben, H. L.; de Boer, A. G.; Verhoef, J. C.; Junginger, H. E. *Int. J. Pharm.* **1997**, *159*, 243−253.
(2) Thanou, M. M.; Verhoef, J. C.; Junginger, H. E. *S. T. P. Pharma Sci.* **2000**, *10*, 315−319.
(3) Thanou, M. M.; Verhoef, J. C.; Verheijden, J. H. M.; Junginger, H. E. *Pharm. Res.* **2001**, *18*, 823−828.
(4) Van der Lubben, I. M.; Verhoef, J. C.; Fretz, M. M.; Van Opdorf, F. A. C.; Mesu, I.; Kersten, G.; Junginger, H. E. *S. T. P. Pharma Sci.* **2002**, *12*, 235−242.
(5) Thanou, M. M.; Florea, B. I.; Geldof, M.; Jungigner, H. E.; Borchard, G. *Biomaterials* **2002**, *23*, 153−159.
(6) Kean, T.; Roth, S.; Thanou, M. *J. Controlled Release* **2005**, *103*, 643−653.
(7) Kim, C. H.; Choi, J. W.; Chun, H. J.; Choi, K. S. *Polym. Bull.* **1997**, *38*, 387−393.
(8) Daly, W. H.; Manuszak-Guerrini M. *Polym. Mater. Eng.* **1998**, *79*, 220−221.
(9) Jia, Z.; Shen, D.; Xu, W. *Carbohydr. Res.* **2001**, *333*, 1−6.
(10) Avadi, M. R.; Sadeghi, A. M. M.; Tahzibi, A.; Bayati, K. H.; Pouladzadeh, M.; Zohuriaan-Mehr, M. J.; Rafiee-Tehrani, M. *Eur. J. Polym. Sci.* **2004**, *40*, 1355−1361.
(11) Holappa, J.; Nevalainen, T.; Soininen, P.; Elomaa, M.; Safin, R.; Másson, M.; Järvinen, T. *Biomacromolecules* **2005**, *6*, 858−863.
(12) Kurita, K. *Prog. Polym. Sci.* **2001**, *26*, 1921−1971.
(13) Sannan, T.; Kurita, K.; Iwakura, Y. *Makromol. Chem.* **1976**, *177*, 3589−3600.
(14) Kurita, K.; Sannan, T.; Iwakura, Y. *Macromol. Chem.* **1977**, *178*, 3197−3202.
(15) Aiba, S.-I. *Int. J. Biol. Macromol.* **1991**, *13*, 40−44.
(16) Holappa, J.; Nevalainen, T.; Savolainen, J.; Soininen, P.; Elomaa, M.; Safin, R.; Suvanto, S.; Pakkanen, T.; Másson, M.; Loftsson, T.; Järvinen, T. *Macromolecules* **2004**, *37*, 2784−2789.

BM050707J