Carbohydrate Research 343 (2008) 2576–2582

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

tert-Butyldimethylsilyl O-protected chitosan and chitooligosaccharides: useful precursors for N-modifications in common organic solvents

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ARTICLE INFO

Article history:
Received 25 June 2008
Received in revised form 7 August 2008
Accepted 11 August 2008
Available online 15 August 2008

Keywords:
Chitosan
Chitin
Protection groups
tert-Butyldimethylsilyl chloride
3,6-Di-*O*-TBDMS-chitosan
Silylation

ABSTRACT

An efficient and chemoselective procedure for preparing highly organosoluble 3,6-di-*O*-*tert*-butyldimethylsilyl (TBDMS)-chitosan and chitooligosaccharides is reported. The selective modification of the chitooligosaccharides with 0.50 degree of N-acetylation was achieved by using TBDMSCl as the reagent in combination with DMF/imidazole. These protocols yielded partly TBDMS-substituted chitooligosaccharides that were subsequently reacted with TBDMSOTf in dichloromethane in order to silylate the remaining, more sterically hindered hydroxyl groups. In the case of the chitosan polymer, a mesylate salt of chitosan was silylated using TBDMSCl in DMSO, yielding full silylation of the hydroxyl groups without using N-protection groups. The silyl-protected polymers displayed excellent solubility in a number of common organic solvents. The 3,6-di-*O*-TBDMS-chitosan and chitooligosaccharides were reacted with acetic anhydride, and deprotected to obtain the corresponding *N*-acetyl derivatives (chitin and chitinoligosaccharide). Our results show that the readily prepared 3,6-di-*O*-TBDMS-chitosan and chitooligosaccharides are useful precursors for selective N-modifications in common organic solvents.

1. Introduction

Chitin is a structural polysaccharide and is related to cellulose in structure.[1] Chitin can be produced from the exoskeleton of crustaceans or from fungi. Chitin is a polymer of *N*-acetyl glucosamine units that are linked by β-(1→4)-bonds, like cellulose. Chitosan (poly-β-(1→4)-glucosamine) is produced from chitin by deacetylation, generally performed by treatment with a strong base.

Chitosan has many promising applications in water treatment,[2] food science,[3] and for pharmaceutical use.[4] In pharmaceutical science chitosan as a biomaterial has shown many interesting properties such as fat binding,[5] mucoadhesion,[6] increased drug absorption,[7] and antibacterial activity.[8] However, the main obstacle for many applications is its low solubility in aqueous solution at physiological pH, which limits its biological activity. Therefore, there is a growing interest in chemically modifying chitosan to enhance its good bioactive properties and to increase its solubility at physiological pH. However, chitosan has low solubility in most common organic solvents, which is a major limitation for chemical synthesis of novel bioactive derivatives.

The organic solvents normally used when modifying chitosan are *N*-methylpyrrolidone (NMP),[9–11] pyridine, or DMF.[12,13] These solvents can also be used in combination with water in order to aid in the solubilization of chitosan.[14] However, these reactions are often performed under heterogeneous conditions, in which the chitosan is only partially solubilized and thus in low concentration.[11,13,14] This leads to long reaction times, and extensive heating is often required. The material can degrade during heating, and the products are often reported to be darkly colored, whereas the starting material is white or light-brown in color.

In an attempt to increase the solubility of chitosan derivatives, various protecting groups have been introduced into the polymers. In particular, the hydroxyl groups have been protected, which enables N-selective modifications. The phthaloyl group is usually used for protection of the amino group, but other protection groups have been used for the hydroxyl groups, for example, acetyl,[15] tosyl,[16] and recently silyl groups, such as trimethylsilyl (TMS).[17,18] One of the most commonly used and probably the most useful chitosan protecting group for the purpose of N-selective modification, is the trityl group, which is used for protection of the primary 6-O alcohol groups.[11] The trityl group can be introduced in a three-step procedure.[12] First, the amine is protected with the phthaloyl group, which increases the organosolubility and thereby enables introduction of a trityl group at the primary alcohol through reaction with triphenylmethyl chloride. Finally, the phthaloyl group is removed by treating the material with hydrazine to obtain 6-O-tritylated chitosan. The main advantage

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doi:10.1016/j.carres.2008.08.014

of 6-*O*-trityl protected materials, is the protection of the more reactive primary hydroxyl group and its relatively good organosolubility. This justifies this somewhat elaborate procedure as it allows various synthetic reactions to be performed for modification of the amino group. However, N-selective modification is not guaranteed as the 3-O hydroxyl group is left unprotected. Furthermore, although 6-O-tritylated chitosan has greater solubility than chitosan, it is only partially soluble in many common organic solvents.[16] Characterization of the protected material by NMR spectroscopy is also difficult because of its limited solubility in NMR solvents. This is a general problem in the characterization of chitosan derivatives.[19] The degree of substitution (DS) therefore has to be determined by elemental analysis,[12] but traces of solvent or other impurities can significantly affect these results.

Using silyl groups to protect hydroxyl groups of chitomaterials is relatively new in chitochemistry, although it has been used in cellulose chemistry for over 50 years to produce materials with good organosolubility.[20–22] Cellulose and chitosan solubility properties are similar to the degree that they are poorly soluble in organic solvents and water. Kurita et al. have developed synthetic strategies to introduce trimethylsilyl (TMS) to the hydroxyl groups of chitomaterials with interesting results.[17,18,23] TMS-protected materials produce chitoderivatives with increased solubility in polar aprotic solvents. However, the TMS group is rather labile and is removed with ease. Thus, it is of interest to use silyl protecting groups that tolerate a wider range of reaction conditions. The *tert*-butyldimethylsilyl (TBDMS) group is a popular alternative to TMS for protection of hydroxyl groups, especially since TBDMS is reported to be 10^4 times more stable against hydrolysis than TMS.[24,25]

The first report of the TBDMS group for protection of hydroxyl groups dates back to 1972,[25] where the reaction of *tert*-butyldimethylsilyl chloride (TBDMSCl) in DMF was reported to take place very slowly with alcohols and gave unsatisfactory yields. However, the addition of 2 equiv of imidazole was found to lead to a smooth reaction with high yields.[24] It has also been demonstrated that this reagent can be used to selectively introduce the TBDMS group on hydroxyl groups in the presence of amino groups.[26,27] Furthermore, highly sterically hindered hydroxyl groups can also be modified by using *tert*-butyldimethylsilyl triflate (TBDMSOTf) as the reagent and 2,6-lutidine as the base.[28] The main hindrance thus far in using this method on chitosan is that the solvent used for the latter reaction is CH_2Cl_2, in which chitosan is insoluble. TBDMS derivatives of *N*-phthaloyl chitosan have recently been reported. In this case *N*-phthaloyl chitosan was used as starting material for the reaction, presumably due to low solubility of chitosan under the silylaton conditions, and TBDMS modification was limited to the primary 6-hydroxyl group.[19]

Here we report a chemoselective TBDMS O-protection on chitooligomers and chitosan, which does not require prior N-protection, yielding material that has excellent solubility in several common organic solvents. Acetylation was used to demonstrate the usefulness of the silylated material as a precursor for N-selective modification.

2. Experimental

2.1. Materials

Two chitosaccharides provided by Genis EHF, Iceland, were used for the silylation reactions: chitooligosaccharide HCl G061023 (average MW 951 Da, determined from the weight average of the MALDI TOF MS signals,[29] N-acetylation of 0.50 and polymerization between 1 and 15 sugar units) and chitosan polymer HCl G020102-1 (0.05 degree of N-acetylation and an average MW of 8.1 kDa as determined by end reducing-assay[30] and 8.5 kDa determined with viscometric methods[31]). All other chemicals used were commercially available and used as received except DMF, pyridine, and DMSO, which were distilled in a usual manner[32] and stored over molecular sieves and under nitrogen atmosphere.

2.2. Characterization

2.2.1. NMR analysis

^{1}H NMR and ^{13}C NMR samples were measured with a Bruker AVANCE 400 instrument (Bruker Biospin GmbH, Karlsruhe, Germany) operating at 400.13 and 100.61 MHz, respectively, at 298 K. The *N*-acetyl peak was used as the internal reference with $CDCl_3$, DMSO, or D_2O as solvents. The integral values from ^{1}H NMR spectroscopy were used to evaluate the degree of substitution of the 3,6-di-*O*-TBDMS- and *3*,6-di-*O*-TBDMS-*N*-acetyl derivatives. The integral of protons H-2, H-3, H-4, H-5, H-6, and H-6′ on the sugar backbone of the chitosaccharide derivatives representing 6 Hs were compared to the integral for the *tert*-butyl peak of TBDMS at δ 0.83 or the acetyl peak at δ 2.1 to obtain the degree of substitution (DS), which is the average number of substituents per sugar unit. Our previously described method was used to determine the *N*-acetyl DS of the chitosaccharide/chitin materials.[14] The following equations were used to estimate the DS:

(1) DS for TBDMS = $([(CH_3)_3-]/[\text{H-2} - \text{H-6}'] \times (6/9))$.

(2) DS for acetylation = $([(CH_3)C{=}O]/[\text{H-2} - \text{H-6}'] \times (6/3))$.

2.2.2. FTIR analysis

FTIR-measurements were performed with an AVATAR 370 FTIR instrument (Thermo Nicolet Corporation, Madison, USA). Samples were kneaded thoroughly with KBr. The sample was pressed into pellets with a Specac compressor (Specac Inc., Smyrna, USA).

2.3. The Synthesis

2.3.1. Chitosan polymer mesylate (1)

The chitosan polymer HCl (G020102-1, 376 mg) was stirred in methanesulfonic acid (12 mL). Addition of water (12 mL) to the heterogeneous mixture resulted in a clear solution. The mesylate salt of chitosan was then precipitated with EtOH (40 mL) resulting in a gel-like precipitate. The precipitate was filtered and washed two times with EtOH (30 mL) and acetone (30 mL), allowed to air-dry and further dried in a vacuum oven at 40 °C overnight to give 363 mg as a brownish solid (75% yield). FTIR (KBr): ν 3376 (br, OH), 2880 (m, C–H), 1677 (vs, C=O amide I), 1586 (vs, C=O amide II) cm^{-1}. ^{1}H NMR (400 MHz, D_2O) δ 2.0 (s, $CH_3C{=}O$), 2.7 (s, CH_3S), 3.1 (m, H-2 GlcN), 3.4–4.1 (m, H-2 GlcNAc, H-3, H-4, H-5, H-6, H-6′), 4.8 (H-1, partly overlapped by water peak). ^{13}C NMR (DMSO): δ 38.8–40.0 (CH_3S overlapped by solvent peak, 38.5 when recorded in D_2O), 55.7 (C-2), 60.2 (C-6), 70.1 (C-3), 74.8 (C-5), 77.6 (C-4), and 97.5 (C-1).

2.3.2. 3,6-di-*O*-TBDMS-chitooligosaccharide (2), (3), and (4) with TBDMSCl as reagent

Chitooligosaccharide HCl (G061023, 2.00 g, 9.70 mmol) was weighed into a flame-dried round-bottomed flask that had been flushed with N_2. The oligosaccharide was mixed with DMF (60 mL) forming a cloudy mixture. The mixture was then cooled down to 0 °C in an ice bath. Imidazole (6.78 g, 99.7 mmol) and TBDMSCl (4.5 g, 30 mmol) were dissolved in dry DMF (10 mL) and added dropwise into the reaction mixture. The reaction was carried out at 0 °C for 20 min, the ice bath was removed, and the reaction was carried out for an additional 24 h (**2**), 48 h (**3**), or 72 h (**4**) at 22 °C. Adding H_2O (60 mL) to the reaction mixture stopped the reaction. The material was extracted three times with

hexane (50 mL), and the combined hexane fractions were then washed three times with H_2O (50 mL) and twice with brine (50 mL), dried over $MgSO_4$, and filtered, and the solvent was evaporated. The white powdered oligomer material was then dissolved in acetone and precipitated with water and finally washed with cold acetonitrile. The white solid was dried in a vacuum oven at 40 °C overnight giving 570 mg (18% yield) after a 48 h reaction (**3**) and 2100 mg (51% yield) after a 72 h reaction (**4**) (*note*: the yield of **2** was not determined).

FTIR (KBr): *ν* 3376 (br, OH, NH), 2880 (s, C–H TBDMS), 1689 (vs, C=O amide I), 1527 (vs, C=O amide II) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) *δ* 0.00 (br s, $(CH_3)_2Si$), 0.83 (br s, $(CH_3)_3C$), 1.91 (br s, CH_3C=O), 2.65 (br s, H-2 GlcN), 2.8–4.0 (m, H-2 GlcNAc, H-3, H-4, H-5, H-6, H-6′), 4.0–5.5 (m, H-1, OH-3 and OH-6 residues), 8.05 (br s, NH/NH_2).

2.3.3. 3,6-Di-*O*-TBDMS-chitosan (5) with TBDMSCl as reagent

Chitosan polymer mesylate (1.00 g, 3.85 mmol) (**1**) was weighed into a flame-dried round-bottomed flask that had been flushed with N_2. The polymer was dissolved in DMSO (13 mL) and cooled down to 0 °C in an ice bath. Imidazole (6.78 g, 99.7 mmol) and TBDMSCl (4.5 g, 30 mmol) were dissolved in DMSO (10 mL) and added dropwise into the reaction mixture. The reaction was carried out at 0 °C for 20 min, the ice bath was removed, and the reaction was carried out for 24 h at 22 °C. After 24 h the same amount of TBDMSCl and imidazole was added to the reaction mixture, and the reaction was allowed to continue for another 24 h. The reaction was stopped by adding H_2O (60 mL) to the reaction mixture. The material was extracted three times with hexane (50 mL), and the combined hexane fractions were then washed three times with H_2O (50 mL), twice with brine (50 mL), dried over $MgSO_4$, and filtered, and the solvent was evaporated. The silylated crude product was washed with acetone and acetonitrile and allowed to air-dry. The white material was dried in a vacuum oven at 40 °C overnight giving 980 mg (58% yield) (**5**).

FTIR (KBr): *ν* 3376 (br, OH, NH), 2880 (s, C–H TBDMS), 1689 (vs, C=O amide I), 1527 (vs, C=O amide II) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) *δ* 0.00 (br s, $(CH_3)_2Si$), 0.83 (br s, $(CH_3)_3C$), 1.91 (br s, CH_3C=O), 2.65 (br s, H-2 GlcN), 2.8–4.1 (m, H-2 GlcNAc, H-3, H-4, H-5, H-6, H-6′), 4.2 (**5**) (br s, H-1), 8.05 (br s, NH/NH_2).

2.3.4. 3,6-di-*O*-TBDMS-chitooligosaccharide (6) and (7) with TBDMSOTf as reagent

The silylated starting material **3** (200 mg) (Table 1) was dissolved in dry CH_2Cl_2 (2 mL) in flame-dried glassware under an N_2 atmosphere. The reaction mixture was cooled to 0 °C in an ice bath. 2,6-Lutidine (120 μL, 1.03 mmol) was added, followed by dropwise addition of TBDMSOTf (316 μL, 1.37 mmol). The reaction was carried out at 0 °C for 30 min and then at 22 °C for 30 min or 24 h to give compounds **6** and **7**, respectively. The material was worked up by washing the reaction mixture three times with water (10 mL) and three times with brine (10 mL). The organic phases were combined, dried with $NaSO_4$, and the filtered solutions were evaporated to dryness. The white powdered oligomeric material was dissolved in acetone (10 mL) and precipitated with water (10 mL). The oligomeric material was finally washed with acetonitrile (10 mL) and dried in a vacuum oven at 40 °C overnight. The 3,6-di-*O*-TBDMS-chitooligosaccharide (**6**), and 3,6-di-*O*-TBDMS-chitooligosaccharide (**7**) gave 120 mg (48% yield) and 192 mg (77% yield), respectively.

FTIR (KBr): *ν* 3376 (br, OH, NH), 2880 (s, C–H TBDMS), 1689 (vs, C=O amide I), 1527 (vs, C=O amide II), 1255, 837, 781 (vs, TBDMS) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) *δ* 0.00 (br s, $(CH_3)_2Si$), 0.83 (br s, $(CH_3)_3C$), 1.91 (br s, CH_3C=O), 2.65 (br s, H-2 GlcN), 2.8–4.0 (m, H-2 GlcNAc, H-3, H-4, H-5, H-6, H-6′), 4.0–5.5 (m, H-1, OH-3 and OH-6 residues), 8.05 (br s, NH/NH_2).

2.3.5. *N*-Acetyl-3,6-di-*O*-TBDMS-chitooligosaccharide/3,6-di-*O*-TBDMS-chitin oligomer (8), (9), (10), (11), and *N*-acetyl-3,6-di-*O*-TBDMS-chitosan/3,6-di-*O*-TBDMS-chitin polymer (12), (13)

The silylated material **4, 5**, or **7**, or partly N-acetylated silyl material **8**, **10**, or **12** (200 mg, Table 2) was dissolved in dry pyridine (2.5 mL) in a flame dried, N_2 filled round-bottomed flask. Triethylamine (TEA, 500 μL, 0.014 mmol) was added whilst stirring, and the solution was cooled down to 0 °C in an ice bath. Finally, Ac_2O (600 μL, 0.006 mmol) was added dropwise whilst stirring at 0 °C for 1 h. The reaction was then carried out for an additional 24 h at 22 °C. After 3 h the liquid changed color from white to yellowish. Precipitation was induced by adding H_2O (50 mL). The precipitate was filtered off, washed with copious amounts of H_2O and dried on the vacuum line overnight. The yield varied from 50% to 95% of a yellowish solid. FTIR (KBr): *ν* 3376 (br, OH, NH), 2880 (s, C–H TBDMS), 1689 (vs, C=O amide I), 1527 (vs, C=O amide II), 1255, 837, 781 (vs, TBDMS) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) *δ* 0.00 (br s, $(CH_3)_2Si$), 0.83 (br s, $(CH_3)_3C$), 1.91 (br s, CH_3C=O), 2.65 (br s, H-2 GlcN), 2.8–4.1 (m, H-2 GlcNAc, H-3, H-4, H-5, H-6, H-6′), 4.0-5.5 (m, H-1), 8.05 (br s, NH/NH_2). ^{13}C NMR (**12**) *δ* −6.2 ($(CH_3)_2Si$), 17.3 ($C(CH_3)_3$), 22.4 (CH_3), 24.9 ($(CH_3)_3C$), 54.7 (C-2), 57.4 (C-6), 65.6 (C-3), 71.2 (C-5), 73.1 (C-4), 99.1 (C-1), and 168.9 (C=O).

2.3.6. Chitin oligomer (14) and polymer (15), (16)

The acetylated material **9**, **12** or **13** (50 mg, 0.13 mmol) was dissolved in EtOH (2 mL), followed by addition of concd HCl (0.05 mL, 1.59 mmol). The liquid was stirred for 6 h at 22 °C. The reaction mixture was then co-evaporated with toluene (5 mL). The solid was finally washed with CH_2Cl_2 (3 × 20 mL) and dried on a vacuum line overnight, yielding between 50% and 86% white powder. FTIR (KBr): *ν* 3376 (br, OH), 2880 (m, C–H), 1750 (vs, C=O ester), 1677 (vs, C=O amide I), 1586 (vs, C=O amide II) cm^{-1}. ^1H NMR (400 MHz, D_2O) *δ* 2.0 (s, CH_3C=O), 3.1 (m, H-2 GlcN), 3.3–4.1 (m, H-2 GlcNAc, H-3, H-4, H-5, H-6, H-6′), 4.5 (H-1, partly overlapped

Table 1
Degree of substitution for the TBDMS-chitooligosaccharides

Starting material	Product	Base/solvent (time)	TBDMS [DS]	Ac [DS]
Chitosan oligomer	**2**	Im/DMF (24 h)	1.37	0.50
Chitosan oligomer	**3**[a]	Im/DMF (48 h)	1.29	0.50
Chitosan oligomer	**4**[b]	Im/DMF (72 h)	1.51	0.51
3	**6**	2,6-Lutidine/CH_2Cl_2 (1 h)	1.94	0.51
3	**7**	2,6-Lutidine/CH_2Cl_2 (24 h)	2.20	0.50

Note: The reported values are obtained from analysis of NMR spectra of materials used for further modification.

[a] The reaction for entry 3 was repeated, yielding TBDMS DS of 1.54 with and acetylation DS of 0.53.

[b] The reaction for entry 4 was repeated twice yielding TBDMS DS of 1.64 and 1.60 and with acetylation DS of 0.50 and 0.50, respectively.

Table 2
Degree of substitution for the TBDMS-chitomaterials after acetylation

Starting material	Product	TBDMS [DS]	Ac [DS]
Acetylation of the TBDMS-chitooligomer			
7	**8**	2.28	0.81
8	**9**	2.38	0.92
4	**10**	1.61	1.07
10	**11**	1.66	1.33
Acetylation of the TBDMS-chitopolymer			
5	**12**	1.99	0.89
12	**13**	2.07	1.04

by water peak). ^{13}C NMR: δ 22.1 (CH_3), 55.0 (C-2), 59.9 (C-6), 72.0 (C-3), 74.5 (C-5), 78.9 (C-4), 101.2 (C-1), and 174.5 (C=O).

2.4. Solubility properties

The chitomaterial (25 mg) was mixed with a solvent (250 μL) forming a 10% [w/v] solution that was stirred for 5 min, after which the solubility was evaluated by visual inspection. The solutions containing material that could not be dissolved were diluted to 2.5% [w/v] and stirred again for 5 min and inspected as before. The solubility was defined by the following categories: fully dissolved in 10% [w/v] concentration (+++), fully dissolved in 2.5% [w/v] concentration (++), dissolved but slightly opaque (+), swollen (+/−) and insoluble (−).

3. Results and discussion

3.1. Silylation of the chitooligosaccharides

Initial investigations with the TBDMS protection group were done with oligomeric chitosaccharides due to better solubility in organic solvents, as compared to the polymeric chitosan. The oligomeric material was reacted with TBDMSCl using imidazole as a catalyst and a base in DMF (Scheme 1). The chitooligosaccharide was only partly soluble in this solvent, and the solution was cloudy although the concentration was only 3.3% [w/v]. However, after 24 h the reaction mixture had become clear. The reaction was stopped after 24 h (**2**), 48 h (**3**) or 72 h (**4**) and analyzed for the time-dependent evolution of the DS. The materials were analyzed by FTIR spectroscopy, which showed distinct C–H stretches at 2880 cm^{-1} for the alkyl groups of the TBDMS protecting group along with characteristic stretches for the Si–CH_3 vibration at 1255 cm^{-1} (Fig. 1). However, an O–H bend around 3200 cm^{-1} indicated that the O-silylated oligomers **2**, **3**, and **4** were not fully O-substituted. This was also confirmed by the DS calculated from integrals in the ^{1}H NMR spectra. Figure 2 shows that, after 24 h reaction with TBDMSCl, the DS was only 1.37 (**2**), and after 72 h the DS increased to only 1.64 (**3**). Thus, the number of OH-groups that have not been substituted is approximately equal to the number of acetyl groups in this material. These observations are consistent with the interpretation that the 3-OH groups adjacent to the amino groups are readily accessible for the reagent, but the 3-OH groups adjacent to the acetamido groups are sterically hindered and therefore react much slower. Binnette and Gagnon have reported that the 3-OH groups of *N*-phthaloyl chitosan could not be sylilated under comparable conditions, even after extensive heating.[19] The material that was isolated after 48 h (**3**) had good organosolubility, and it was therefore possible to modify this material further in a reaction with TBDMSOTf in CH_2Cl_2, using 2,6-lutidine as the base. The triflate is more reactive and has been shown to react with sterically hindered hydroxyl groups.[28] This was expected to reduce the reaction time and facilitate full silylation of the more sterically hindered 3-OH groups. Indeed, the chiooligosaccharide was rapidly silylated under these conditions (Fig. 2). After a 24 h reaction with TBDMSOTf, the oligomer derivative **7** yielded 2.20 DS as seen in Figure 2, which is consistent with silylation of all hydroxyl groups. The reason for a DS value over 2.00 is that the 1-O alcohol at the reducing end and 4-O alcohol at the non-reducing end can also be silylated.

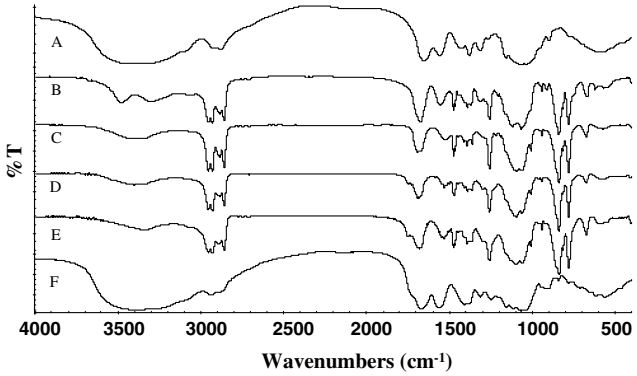
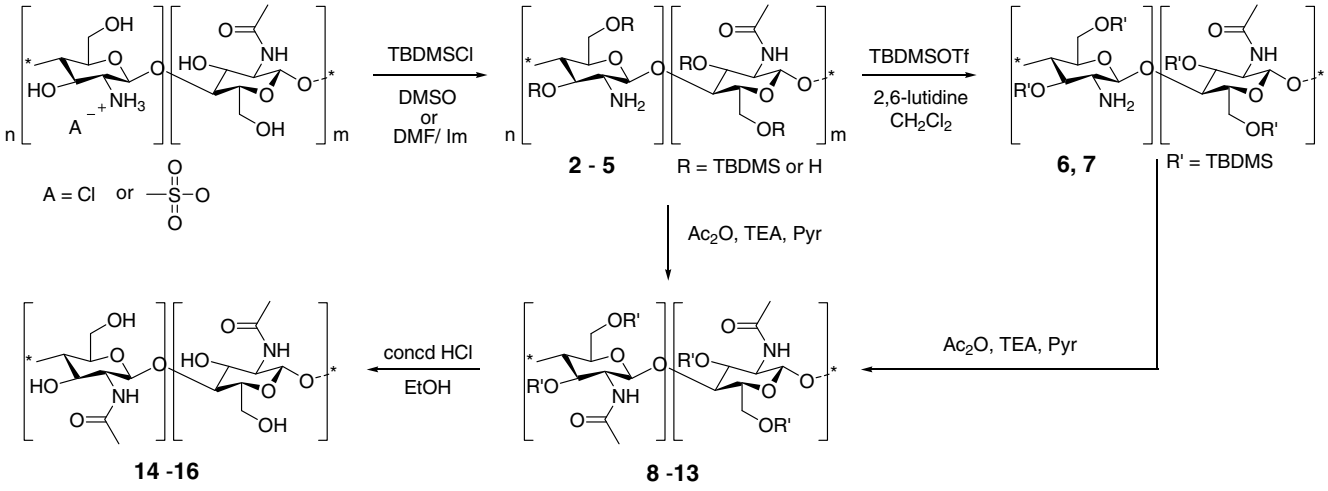


Figure 1. FTIR spectra of the oligomeric synthetic series: (A) chitooligosaccharide HCl; (B) 3,6-di-*O*-TBDMS-chitooligosaccharide (**3**) with *O*-DS of 1.29; (C) 3,6-di-*O*-TBDMS-chitooligosaccharide (**7**) with *O*-DS of 2.20; (D) 3,6-di-*O*-TBDMS-*N*-acetyl-chitooligosaccharide/3,6-di-*O*-TBDMS-chitin oligomer (**8**) with *O*- and *N*-DS of 2.28 and 0.81, respectively; (E) 3,6-di-*O*-TBDMS-*N*-acetyl-chitooligosaccharide/3,6-di-*O*-TBDMS-chitin oligomer (**9**) with *O*- and *N*-DS of 2.38 and 0.92, respectively; (F) chitin oligomer (**14**) with *N*-DS of 0.76.

3.2. Silylation of the chitosan polymers

The chitosan polymer had a very low solubility in dry DMF, and, therefore, it did not react under the conditions used for the silylation of the chitooligomer. Using different organic solvents and extensive heating did not yield any improvement (data not shown). Sashiwa et al. have reported that the mesylate salt of chitosan significantly increases the solubility in organic solvents,



Scheme 1. Synthetic route for the silylation, acetylation, and deprotection of the chitosaccharides. *Note:* TBDMS = *tert*-butyldimethylsilyl.

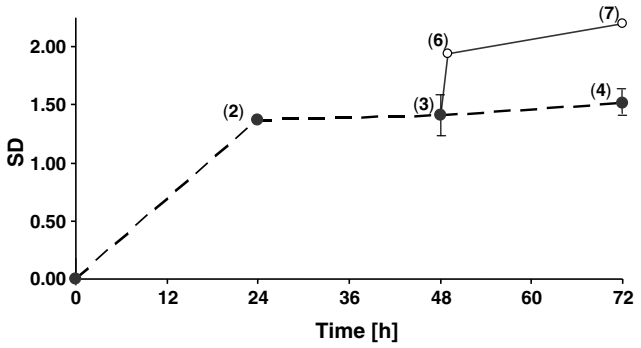


Figure 2. Graph showing the time course of the reaction of oligomeric material with TBDMSCl and DMF/imidazole (-●-), and TBDMSOTF and 2,6-lutidine (-○-). *Note*: The DS of compounds **3** and **4** are plotted as the mean value of two or three reactions, respectively. Error bars are shown to describe the variation (see footnote, Table 1).

especially DMSO.[33] Thus, the chitopolymer was converted to the mesylate salt; however, this material was not soluble in DMF. On the other hand, it had good solubility in DMSO, which is also a polar aprotic solvent. By using DMSO as a solvent, it was possible to carry out the silylation reaction in a more concentrated solution than for the oligomer (Scheme 1). After 24 h, silylation with TBDMS in DMSO yielded only partial silylation of the polymer (data not shown). However, after another addition of TBDMSCl and imidazole to the reaction mixture and further reaction for 24 h, the fully silylated 3,6-di-*O*-TBDMS-chitosan (**5**) was obtained. For compound **5** the FTIR spectra showed a highly silylated material with only a very weak band corresponding to the O–H band. This was confirmed by ^{1}H NMR spectroscopy (Fig. 3), showing a 1.94 DS for the silylation for **5**. The distinctive peak for unsubstituted GlcN H-2 at 2.65 ppm, corresponding to one proton, also confirmed that the silylation was O-selective. To ensure that all sterically hindered hydroxyl groups were silylated, we conducted a silylation reaction using TBDMSOTf, but the DS did not increase (data not shown). This indicated that the chitosan starting material (**1**) had been fully O-silylated with TBDMSCl/imidazole in DMSO. DMSO was also tested as solvent for the chitooligosaccharides, yielding a material that was substituted to the same degree as that with DMF (data not shown), showing that complete silylation of the sterically hindered hydroxyl groups in the *N*-acetyl chitosaccharide required TBDMSOTf.

3.3. Acetylations

Acetylation was conducted on the silyl-protected material to confirm its usefulness for preparing N-substituted derivatives of chitosan, and to verify that the silylation reaction was O-selective, as has been demonstrated in the synthesis of small molecules.[26,27] The acetylation was performed under conventional reaction conditions using acetic anhydride in pyridine in the presence of TEA (Scheme 1). N-Acetylation can be readily confirmed by FTIR, since N- and O-acetylation gives a characteristic carbonyl absorption at 1680 and 1750 cm^{-1}, respectively. The reaction conditions have not been optimized, and, therefore, the reaction under conventional conditions had to be repeated to obtain highly N-acetylated product. Acetylation of a partly silylated chitooligosaccharide **4** (DS = 1.64) yielded partially O-acetylated material **11** as expected. On the other hand, when the acetylation was performed with the fully O-protected oligomer **7** (DS = 2.20), only a slight indication of O-acetylation was observed (Fig. 4). Thus, fully O-silylated material is required for N-selective substitution. When the fully silylated polymer **5** was acetylated, the N-acetylation was confirmed by an increase in the acetyl peak at δ 1.91 and a corresponding reduction in the intensity of the peak for unsubstituted H-2 at δ 2.65 (Fig. 3C). Furthermore, no indication of O-acetylation was observed. These data clearly show that *O*-TBDMS-protected chitosan and chitooligomers can be good starting materials for N-selective modifications.

3.4. Deprotection

One of the advantages of the TBDMS groups as a protective group for chitosaccharides is its relatively straightforward

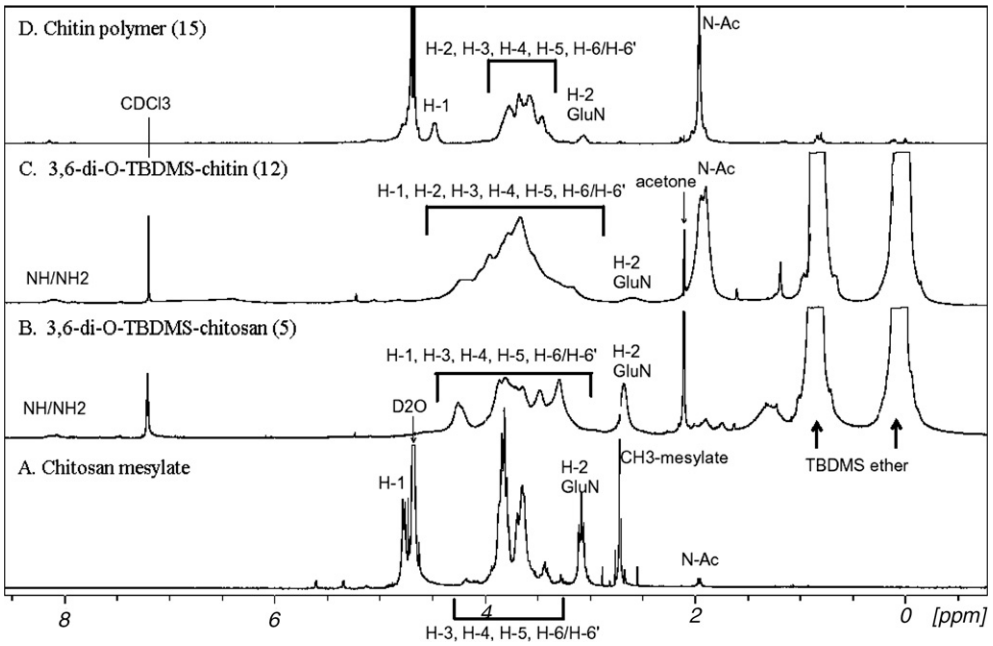


Figure 3. ^{1}H NMR spectra of the polymer synthetic series: (A) chitosan mesylate (**1**); (B) 3,6-di-*O*-TBDMS-chitosan (**5**) with *O*-DS of 1.94; (C) 3,6-di-*O*-TBDMS-*N*-acetyl-chitosan/3,6-di-*O*-TBDMS-chitin polymer (**12**); (D) chitin polymer (**15**).

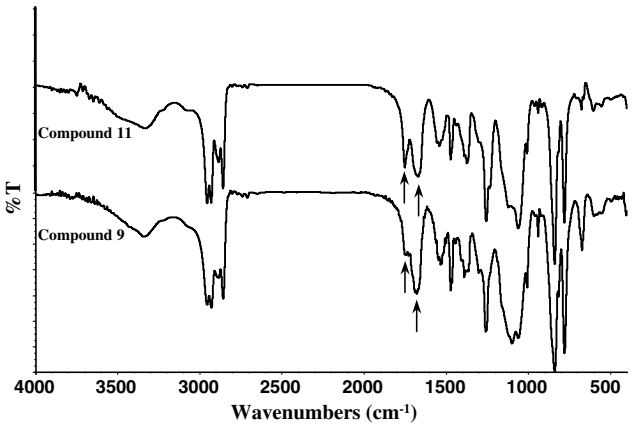


Figure 4. FTIR spectral difference between 3,6-di-*O*-TBDMS-*N*-acetyl-chitooligosaccharide **9** and **11**. The arrows show the O-acetylation (1750 cm^{-1}) and N-acetylation (1680 cm^{-1}) carbonyl peaks.

removal. There are several different procedures described in the literature that utilize fluoride ion, such as tetra-*n*-butylammonium fluoride (TBAF)[34] or HF/pyridine.[35] Another approach is to simply use a mixture of HCl/EtOH.[36] Indeed, treatment with HCl/EtOH yielded 50–86% of white material that precipitated during the course of the reaction (Scheme 1). The deprotected chitoologomer (**14**) had a degree of N-acetylation of 0.76 according to the ^{1}H NMR analysis. Reliable analysis of the degree of N-acetylation for the chitin polymer could not be performed due to the low solubility of the chitin in water.[1] Analysis of the deprotected and partly dissolved chitin material by ^{1}H NMR spectroscopy gave a DS of 0.74 and 0.78 for compounds **15** and **16**, respectively, but this probably underestimates the true DS, as the less acetylated material is more soluble. However, these results are sufficient to show that fully silylated chitosaccharides can be N-modified and deprotected to produce N-modified derivatives. Therefore, the 3,6-di-*O*-TBDMS derivatives could clearly become important precursors for regioselective N-derivatization of chitosan.

3.5. Solubility properties of *O*-TBDMS-protected chitomaterials

The solubility of the *O*-silyl protected materials in organic solvents is very important, as limited solubility can significantly restrict the scope of synthetic procedures that can be used. Solubility in common organic solvents was compared to the solubility of starting materials used in this study (Table 3). The mesylate salt of chitosan showed similar solubility to the hydrochloride salt of the chitosan polymer, with the exception that solubility in DMSO was improved considerably, confirming earlier studies.[33] For example, it was possible to record an NMR spectrum of the mesylate salt in DMSO, which was not possible for the HCl salt of the polymer.

The first indication that the silylation was successful a was dramatic increase organosolubility, to such an extent that it was possible to extract the compound from the reaction mixture with hexane or ethyl acetate. Table 2 shows that the 3,6-di-*O*-TBDMS-oligomer with 2.20 DS (**7**) had better organosolubility than the silylated oligomer with 1.64 DS (**3**), dissolving readily at the concentration of 10% [w/v]. Even the partially substituted 3,6-di-*O*-TBDMS-oligomer **2** could be useful in reactions using all of the listed solvents except water and acetonitrile.

The silylated polymer showed even more interesting solubility properties, considering that the commonly used *N*-phthaloyl chitosan is not soluble in protic or nonpolar solvents but shows swelling or is almost soluble in polar aprotic solvents such as DMF, DMSO, and pyridine.[19] In spite of this limitation, the solubility of *N*-phthaloyl chitosan in those solvents is sufficient to make the derivative useful for further modifications. Kurita et al. showed that the solubility of 6-*O*-trityl chitosan is even less than that of *N*-phthaloyl chitosan, but still sufficient for modifications in polar aprotic solvents.[16] The TBDMS polymer with 1.94 DS (**5**) is soluble in a much wider range of solvents than 6-*O*-trityl chitosan, showing good solubility in non-polar, protic, and polar aprotic solvents. On the other hand, solubility is lower than for the TBDMS oligomers. Thus, the 3,6-di-*O*-TBDMS-chitosaccharides have, to our knowledge, much higher solubility than other derivatives that have been reported with free amino groups.

4. Conclusions

We have shown that all the hydroxyl groups of chitosan mesylate salt can be protected with the TBDMS group in a single reaction using TBDMSCl in DMSO, without prior protection of the amino groups. Furthermore, chitooligomers that are partially N-acetylated can be fully silylated by sequential treatment with TBDMSCl and TBDMSOTf, the latter silylation reagent required to silylate the sterically hindered secondary alcohols that are adjacent

Table 3
Solubility properties of the chitosan starting materials used and 3,6-di-*O*-TBDMS-chitoderivatives

Solvent	Boiling point [°C]	Polarity index [*P′*]	Chitosan oligomer HCl	Chitosan polymer HCl	Chitosan polymer mesylate (**1**)	3,6-Di-*O*-TBDMS-chitosan (**5**)	3,6-Di-*O*-TBDMS-chitooligo-saccharide (**3**)	3,6-Di-*O*-TBDMS-chitooligo-saccharide (**7**)
Water	100	9.0	+++	++	+	–	–	–
NMP	202	6.7	+/–	+/–	+/–	+	+++	+++
DMF	155	6.4	+/–	+/–	+/–	+	+++	+++
DMSO	189	7.2	++	+/–	++	+	+++	+++
Diethyl ether	35	2.8	–	–	–	+	+	+++
Triethylamine	89	1.8	–	–	+/–	+	+++	+++
Pyridine	115	5.3	+/–	+/–	+/–	++	+++	+++
THF	65	4.0	–	–	–	+	+++	+++
Acetone	56	5.1	–	–	–	–	+	+++
Acetonitrile	82	5.8	–	–	–	–	–	–
1-Butanol	118	3.9	–	–	–	++	+++	+++
2-Propanol	82	4.3	–	–	–	++	+++	+++
Ethyl acetate	77	4.4	–	–	–	++	+++	+++
Ethanol	78	5.2	–	–	–	+	++	+++
Methanol	65	5.1	–	–	–	–	+	+
Hexane	69	0.0	–	–	–	+	+	+
Dichloromethane	41	3.1	–	–	–	+	++	+++
Chloroform	61	4.1	–	–	–	+	+++	+++

Note: +++ = Completely soluble at 10% [w/v], ++ = completely soluble at 2.5% [w/v], + = slightly opaque at 2.5% [w/v], +/– = swollen material and – = hardly soluble/insoluble.

to the *N*-acyl groups. The silylated chitomaterials show unprecedented solubility in organic solvents. The combination of high organosolubility and stability of the TBDMS group toward a wide range of conditions makes TBDMS chitomaterials valuable intermediates for the preparation of various N-derivatives as important materials for pharmaceutical and other applications.

Acknowledgments

Financial support from the Iceland Centre for Research, the University of Iceland Research Fund, and the Bergþóru and Þorsteins Scheving Thorsteinssonar Fund is gratefully acknowledged. For the generous donation of chitosan and chitooligomer starting material and helpful advice we would like to thank Genis EHF and Dr. Jón M. Einarsson.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.08.014.

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