Articles

Regioselective Silylation of *N*-Phthaloylchitosan with TBDMS and TBDPS Groups

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N-Phthaloylchitosan represents a key intermediate for the regioselective modification of chitosan in organic media. Chemoselective protection of primary alcohols on *N*-phthaloylchitosan was achieved with *tert*-butyldimethylsilyl (TBDMS) and *tert*-butyldiphenylsilyl (TBDPS) groups in imidazole/DMF and DMAP/pyridine. Influence of experimental conditions such as solvent, choice of base, stoichiometry, temperature, and time of reaction was studied regarding the degree of substitution (ds) of silyl groups. TBDMS groups allow higher ds than TBDPS groups. Higher reaction temperatures in different conditions led to lower ds and incomplete substitution. However, regioselective silylation of *N*-phthaloylchitosan was realized with ds up to 0.92 at room temperature. Silylated derivatives were characterized by elemental analysis, IR, and CP/MAS ^{13}C NMR spectroscopies.

Introduction

Chitosan is a biopolymer derived from the N-deacetylation of chitin, which is the second most abundant polysaccharide on earth after cellulose. Sources of chitin are various where crustacean exoskeletons are the main industrial source; however, chitin also occurs in fungi and insects.[1] Chitosan is biodegradable, biocompatible, and nontoxic. Therefore, it is widely studied for numerous actual and potential application fields such as water treatment, materials, food, biotechnology, and pharmaceutical uses.[2] Efficient transformations and applications of chitosan are limited by its lack of organosolubility that can be explained by its high molecular weight and inter-unit hydrogen bonding. Complete trimethylsilylation of chitosan (ds of 2.9) and cellulose (ds of 3.00) has been reported to increase solubility in organic media.[3,4] Trimethylsilylated chitosan, however, is easily hydrolyzed in acidic medium and can be acetylated.[3] Moreover, regioselective silylation of starch at the 6-O positions has been achieved using thexyldimethylchlorosilane.[5]

tert-Butyldimethylsilyl (TBDMS) and *tert*-butyldiphenylsilyl (TBDPS) protective groups are known to be selectively introduced in mild conditions on primary alcohol functions due to their steric hindrance. They have been commonly used in the derivatization of monosaccharides for their stability toward several reaction conditions. Indeed, TBDMS and TBDPS groups possess excellent stability toward bases, although they can be slightly sensitive to acid.[6] Silyl groups can also be cleaved with numerous reagents in basic, acidic, oxidizing, and reducing conditions including fluoride ions.[6,7] On the other hand, the protection of amine groups by *N*-phthaloylation of chitosan induces an increase of solubility in organic solvents and facilitates the chitosan derivatization.[8,9] Regioselective protection of primary hydroxyl groups onto *N*-phthaloylchitosan has been reported with trityl groups, which are cleaved in acidic conditions.[10] The protection of O-6 positions with trityl chloride in hot pyridine has been reported with a ds on the order of 0.8.[10−12]

Herein, we report the regioselective protection of chitosan on primary hydroxyl groups in mild conditions with ds of 0.92 and 0.86, respectively, for TBDMS and TBDPS groups with the perspective to realize further chemoselective modifications.

Experimental Procedures

General Information. Completely deacetylated high molecular weight shrimp shell chitosan (547 kDa by GPC) was purchased from Marinard Biotech Inc. (Gaspé, Canada) and was mechanically ground to 33 mesh and used without prior chemical treatment. *N*,*N*-Dimethylformamide (DMF) and pyridine (py) were distillated over CaH_2 and under a nitrogen atmosphere. Reagents including TBDPSCl and TBDMSCl were purchased from Sigma-Aldrich and used without purification. *N*-Phthaloylchitosan with a ds of 1.0 and an absence of ester groups was synthesized according to the literature.[8] All reactions were realized under nitrogen atmosphere. FT-IR spectra and elemental analysis were recorded, respectively, with a PerkinElmer FT-IR 1600 in KBr pellets and with a Costech 4010 elemental analyzer. Solid-state ^{13}C NMR spectra were recorded on a Bruker DSX300 NMR spectrometer at 75 MHz with a 4 mm CP/MAS probe, a rotating speed of 12 kHz, a contact time of 1.5 ms, and a repetition time of 5 s, and 1400 scans were accumulated.

General Silylation Procedure. Preparation of Poly[β-(1 → 4)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-phthalimido-D-glucopyranose] (6-*O*-TBDMS−*N*-phthaloylchitosan). A suspension of *N*-phthaloylchitosan with a ds of 1.0 (0.304 g, 1.05 mmol) and an excess of imidazole (0.581 g, 8.53 mmol) in anhydrous DMF (40 mL) was treated with *tert*-butyldimethylsilyl chloride (1.14 g, 7.61 mmol). The suspension was stirred at room temperature for 48 h. The chitosan derivative was precipitated with 50 mL of a water/ethanol (1:1 v/v) solution. The solid was filtered and washed with 20 mL of water/ethanol solution followed by ethyl ether (3 × 5 mL). An off-white solid corresponding to a yield of 85% (0.361 g) based on the ds value from the elemental

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Scheme 1. Synthesis of Silylated *N*-Phthaloylchitosan

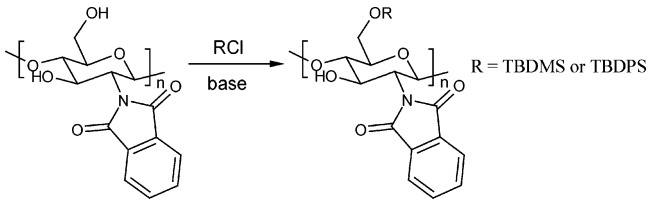

Table 1. Degree of Substitution of TBDMS Groups on *N*-Phthaloylchitosan under Various Conditions

entry	base/solvent	number of equiv[a]	temperature (°C)	time (h)	ds[b]
1	Im/DMF	4.8	25	48	0.92
2	Im/DMF	7.3	25	24	0.86
3	Im/DMF	7.3	25	48	0.88
4	DMAP/py	5.2	25	96	0.70
5	DMAP/py	2.2	115	71	0.25
6	DMAP/py	3.5	115	24	0.55
7	DMAP/py	3.5	115	48	0.66
8	DMAP/py	3.5	115	67	0.64
9	DMAP/py	5.1	115	96	0.70
10	Im/py, DMF (1:1)	6.0	25	24	0.76

[a] Number of silylating reagent equiv. [b] Degree of substitution calculated from the C/N ratio in elemental analysis.

Table 2. Degree of Substitution of TBDPS Groups on *N*-Phthaloylchitosan under Various Conditions

entry	base/solvent	number of equiv[a]	temperature (°C)	time (h)	ds[b]
11	Im/DMF	2.4	152	4	0.25
12	Im/DMF	2.4	152	27	0.32
13	Im/DMF	4.8	25	48	0.83
14	Im/DMF	7.3	25	24	0.85
15	Im/DMF	7.3	25	48	0.85
16	DMAP/py	5.1	25	24	0.09
17	DMAP/py	5.1	25	48	0.10
18	DMAP/py	2.5	115	24	0.09
19	DMAP/py	2.5	115	48	0.13
20	DMAP/py	5.2	115	48	0.46
21	Im/py, DMF (1:1)	6.0	25	24	0.45
22	Im/DMF	13.3	25	48	0.86

[a] Number of silylating reagent equiv. [b] Degree of substitution calculated from the C/N ratio in elemental analysis.

analysis for the introduction of TBDMS groups was obtained. IR 3478 (OH), 2942 (CH), 1779 and 1720 (C═O phth), 1254 (Si−CH_3), 1122 (Si−O−C aliph), 1150−980 (C−O pyranosyl), 837 (Si−O−CH_3 def), 719 (aromatic) cm^{-1}. CP/MAS ^{13}C NMR δ −5.75 (CH_3Si), 18.39 ($C_{4°}$), 25.81 (CH_3 *tert*-butyl), 56.15 (C-2), 63.64 (C-6), 71.06 (C-3), 75.58 (C-5), 83.52 (C-4), 99.21 (C-1), 123.28, 132.52 (aromatic carbons), 167.76 (Phth C═O) ppm. Anal. calcd for $C_{20}H_{27}NO_6Si$ with a ds of 0.92: C, 59.15; H, 6.58; N, 3.53. Found: C, 58.50; H, 6.64; N, 3.61.

Poly[β-(1 → 4)-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-2-phthalimido-D-glucopyranose] (6-*O*-TBDPS−*N*-phthaloylchitosan) Yield of 88% (0.482 g) on the basis of a ds value for the introduction of TBDPS groups. IR 3475 (OH), 2930 (CH), 1779 and 1719 (C═O phth), 1113 (Si−O−C aliph), 1150−900 (C−O pyranosyl), 823 (Si−O−CH_3 def), 719 (aromatic) cm^{-1}. CP/MAS ^{13}C NMR δ 19.10 ($C_{4°}$), 26.91 (CH_3), 56.4 (C-2), 63 (C-6), 70 (C-3), 75.6 (C-5), 81 (C-4), 99 (C-1), 122.77, 128.19, 131.74, 134.90 (aromatic carbons), 167.37 (Phth C═O) ppm. Anal. calcd for $C_{30}H_{31}NO_6Si$ with a ds of 0.86: C, 67.07; H, 5.95; N, 2.82. Found: C, 67.65; H, 5.89; N, 2.88.

Results and Discussion

In view of selectively protecting primary hydroxyl groups on *N*-phthaloylchitosan, the introduction of steric hindrance TBDMS and TBDPS silyl groups on primary alcohols was achieved. To reach a high ds to maintain a homopolymer (ds of 1.0), the protective group must be used in excess and in mild conditions. In the cases of cellulose and starch, silylations have been realized in liquid ammonia; in contrast, trimethylsilylation of chitin[13] and chitosan with hexamethyldisilazane and trimethylchlorosilane occurs in pyridine. Silylations with TBDMS and TBDPS are recognized to be selective to primary alcohols even in the presence of secondary alcohols and have successfully been applied in carbohydrate synthesis.[6]

Silylations. *N*-Phthaloylchitosan reacts with silyl chloride derivatives in almost homogeneous conditions to give silylated chitosan derivatives (Scheme 1). Degrees of silylation were followed noting the reaction time in two different experimental conditions; imidazole/DMF (Im/DMF) and a second one with 4-(*N*,*N*-dimethylamino)pyridine/pyridine (DMAP/py) where pyridine acts as a solvent and a base. Precipitation and purification conditions were investigated to eliminate unreacted silyl agents and side reaction products from the reactional mixture and to obtain a high recovery of the chitosan derivatives. Purification attempts were made with acetone, ethyl ether, methanol, and ethanol. Workup with a water−ethanol (1:1 v/v) solution followed by an ethyl ether washing gave a clean product where the precipitation of the polymer was spontaneous and complete. Silylations gave yields above 85%.

Protection assays are presented in Tables 1 and 2, respectively, for TBDMS and TBDPS protective groups. Some ds data shown in the tables were obtained by following the same reaction mixture in time. When silylation reactions are realized with 1.2−1.5 equiv of silylating reagent, the obtained ds are lower than 0.05. This observation can be explained by the presence, in *N*-phthaloylchitosan, of an average of 0.7 water molecules per repetitive unit as determined by elemental analysis and in accordance with cited literature.[8] Chitosan is known to be a hygroscopic biopolymer. It was noted, for various experimental conditions, that increasing the number of equivalents over 2.2 of the silylating agent allows the beginning of the silylation of *N*-phthaloylchitosan (entries 5, 11, 12, 18, and 19). Additions of more than 5 equiv of silylating reagents (entries 2, 3 and 15, 22), however, did not permit ds higher than 0.92 and 0.86 to be obtained, respectively, for TBDMS

Table 3. Solubility of Chitosan Derivatives in Common Organic Solvents[a]

solvents	*N*-phthaloylchitosan	6-*O*-TBDMS-*N*-phthaloylchitosan	6-*O*-TBDPS-*N*-phthaloylchitosan
acetone	−	−	−
chloroform	−	−	−
DMF	±	±	±
DMSO	±	±	±
methanol	−	−	−
pyridine	±	±	±

[a] +: soluble; ±: swelling; and −: insoluble.

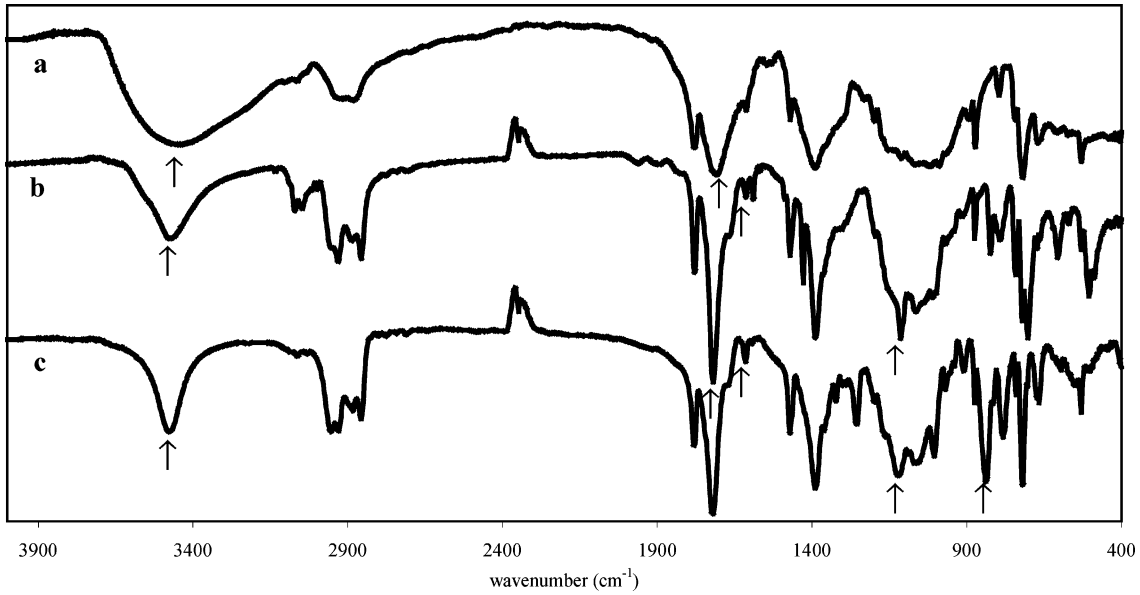

Figure 1. IR spectra of *N*-phthaloylchitosan and its silylated derivatives (a) *N*-phthaloylchitosan, (b) 6-*O*-TBDPS-*N*-phthaloylchitosan, and (c) 6-*O*-TBDMS-*N*-phthaloylchitosan.

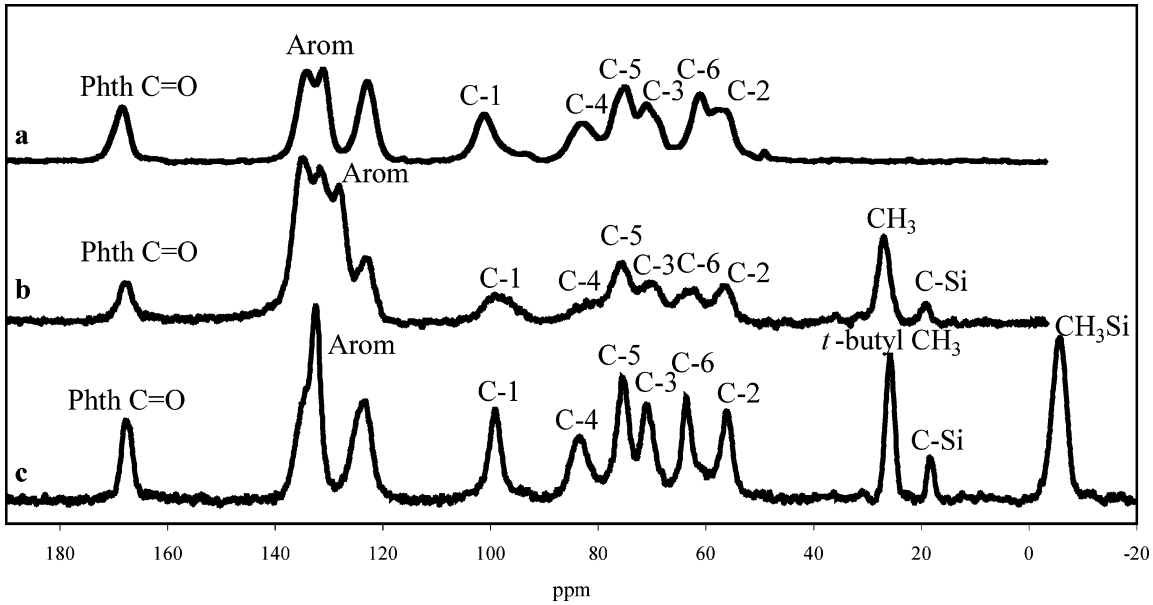

Figure 2. CP/MAS ^{13}C NMR spectra of chitosan derivatives (a) *N*-phthaloylchitosan, (b) 6-*O*-TBDPS-*N*-phthaloylchitosan, and (c) 6-*O*-TBDMS-*N*-phthaloylchitosan.

and TBDPS derivatives. Silylations with TBDMSCl seem to proceed faster than those with TBDPSCl in comparable experimental conditions (entries 1, 13; 2, 14; 3, 15; and 10, 21). Entries (6−8, 18, and 19) that were realized at 115 °C in pyridine with a catalytic amount of DMAP led to a small increase of ds between 24 and 48 h. When silylations are realized at reflux in DMF or in pyridine, the products are brownish, and the calculated ds are lower than 1.0; these silylations also gave equal or higher ds than for comparable conditions at room temperature (entries 4, 9 and 17, 20). Therefore, the use of higher temperatures is no improvement for this type of reaction due to partial decomposition. Silylations with TBDPSCl proceed faster in Im/DMF than in DMAP/pyridine (entries 13, 17 and 12, 18), which is consistent with the literature.[6] To observe the influence of solvents, silylations were made in a py/DMF (1:1 v/v) mixture (entries 10 and 21) that led to lower ds than those obtained with Im/DMF (entries 2 and 14). Our best silylating conditions to achieve regioselective protection of primary hydroxyl groups were realized with 4.8 equiv in Im/DMF at room temperature and are reported in entries 1 and 13. In the course of this work, ds higher than 1.00 was never reached, even with the use of an excess of reagents, extended reaction times, or with high-temperature reactions. On the other hand, protection of secondary alcohols with TBDMSCl has already been reported only in strong silylation conditions like solvent-free conditions or the utilization of a strong base as TBDMSCl/KH/18-Crown-6 in THF.[14]

Characterization. Solubilities of silylated chitosan derivatives are reported in Table 3. However, their limited solubilities do not allow the characterization by NMR in solution but can be suitable for synthetic applications. IR spectra of silylated chitosans show two characteristic bands at 1113 and 836 cm^{-1} assigned, respectively, to Si−O and Si−C stretching, where the Si−O band is superimposed with C−O stretching bands of pyranosyl units at 1160−1000 cm^{-1} (Figure 1). The degree of substitution could be estimated by the decreasing width and intensity of the hydroxyl band at 3300 cm^{-1}. Stretching of carbonyls of *N*-phthaloyl groups of silylated chitosan is observed

at higher wavenumbers (1719 vs 1702 cm^{-1}) and is narrower than that of *N*-phthaloylchitosan. This is explained by the lower hydrogen bonding donor ability of silylated chitosan derivatives. The solid-state ^{13}C NMR spectrum of 6-*O*-TBDMS−*N*-phthaloylchitosan shows characteristic peaks related to *tert*-butyl and methyl silyl groups, respectively, at around 19, 26, and −5.75 ppm (Figure 2). A downfield shift of 2 ppm is observed for C-6 carbon atoms of silylated chitosan derivatives as compared to *N*-phthaloylchitosan that is explained by the π acceptor ability of the silicon atoms. Pyranosyl carbons of 6-*O*-TBDMS−*N*-phthaloylchitosan are not superimposed and are easy to assign. Comparison of pyranosyl carbons between TBDMS− and TBDPS−*N*-phthaloylchitosan shows an upfield shift of 2 ppm for C-4 of the TBDPS derivative that also becomes broader and overlaps with C-5. This chemical shift variation could be explained from diamagnetic anisotropy created by phenyl groups of TBDPS moieties. C-4 carbons of 6-*O*-TBDMS and 6-*O*-TBDPS derivatives of methyl 2,3,4-triacetyl-α-D-glucopyranose also exhibit a shielding of 1.19 ppm for the TBDPS protected compound.[15] The phthaloyl group of the TBDMS chitosan derivative shows only two peaks as compared to *N*-phthaloylchitosan, where in the former two peaks are superimposed at 133 ppm. In solid-state ^{13}C NMR, peaks are broad due to the magnetic anisotropy environment and are known to be very sensitive to changes in the local structure. Chemical shifts of C-1 and C-4 carbon atoms in 1,4-linked carbohydrates are believed to be highly sensitive to changes in the glycosidic conformation.[16] Chemical shifts of these carbon atoms do not significantly change between silylated chitosans and *N*-phthaloylchitosan, indicating that they have similar glycosidic conformations. The peak widths are known to be related to the degree of cristallinity.[17] *N*-Phthaloylchitosan presents some crystallinity, and its line widths are similar to those observed for silylated chitosans that suggests that these derivatives present also comparable crystallinity.[8]

Even after many purification steps followed by a drying period of more than a week under vacuum, the silylated chitosan derivatives synthesized in DMF still show a small shoulder around 1612 cm^{-1} linked to traces of DMF, which were also observed in CP/MAS ^{13}C NMR spectra showing methyl carbons around 30 and 36 ppm. Traces of solvent are very difficult to eliminate due to the polymeric nature of these compounds. The presence of residual traces of DMF in chitosan derivatives would affect the quantification of silylated ds by a lower C/N ratio; the reported values of ds were, therefore, slightly underestimated. Series of elemental analysis was made on a single batch of a silyl chitosan derivative to substantiate the reliability of the method for the ds quantification; the calculated standard deviation of ds is 0.011 ($n = 10$), corresponding to a relative standard deviation of 1.2%.

Conclusion

Regioselective silylations of *N*-phthaloylchitosan with TBDMSCl and TBDPSCl were achieved at 6-O positions under mild conditions with ds near 1.0 at room temperature, and these new chitosan derivatives were successfully characterized by elemental analysis, IR, and solid-state ^{13}C NMR spectroscopies. Influences of experimental conditions such as time, temperature, and choice of base and solvent on ds of silylated *N*-phthaloylchitosan were studied. The highest ds were obtained at room temperature with an excess of silylating reagent after a reaction time of 48 h. The utilization of silylated *N*-phthaloylchitosan allows the further modification of 3-O positions and the design of novel and numerous chitosan architectures.

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