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Chemoselective protection of chitosan by dichlorophthaloylation: preparation of a key intermediate for chemical modifications

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Abstract In order to expand the scope of protection of the amino group of chitosan for synthesizing a key intermediate that would allow regioselective modification reactions, dichlorophthaloylation behavior was studied in detail. The reaction proceeded in a similar manner to phthaloylation, and chemoselectively protected *N*-dichlorophthaloyl-chitosan could be prepared either by partial hydrolysis of *N*,*O*-dichlorophthaloylated chitosan or by one-step dichlorophthaloylation in *N*,*N*-dimethylformamide/water. Deprotection of *N*-dichlorophthaloyl-chitosan was achieved by hydrazinolysis followed by alkaline hydrolysis. GPC measurements revealed that the number–average molecular weight was reduced to about a half after the protection–deprotection process, indicating the cleavage of the main chain to be not pronounced. In the triphenylmethylation, *N*-dichlorophthaloyl-chitosan exhibited adequate reactivity for facile protection of the C6 hydroxy group. The dichlorophthaloyl group has thus proved to be a promising candidate to prepare a novel precursor for controlled chemical modifications of chitosan.

Keywords Chitosan · Chemical modification · Protection–deprotection · Dichlorophthaloylation · Molecular weight · Reactivity · Solubility

Introduction

Although chitosan exhibits distinctive biological activities and is considered to have high potential as a special biopolymer in various fields, especially medicine,

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biomaterials, antimicrobials, and hair and skin care products [1, 2], it remains an almost unutilized biomass resource because of the lack of solubility except in some aqueous acids. Furthermore, in addition to the limited solubility, it is multifunctional with three kinds of reactive groups per repeating unit, which is undoubtedly a primary obstacle to chemical modifications that would possibly lead to advanced utilization of this abundant amino polysaccharide [3–5].

In order to develop intelligent functions and to discuss the structure–properties relationship, it is crucial to exploit regioselective chemical modification reactions that make possible synthesis of the derivatives with well-defined structures [6–8].

Phthaloylation of chitosan has proved a convenient technique for this purpose; it is useful for protecting the amino group to distinguish the three kinds of functional groups as well as for solubilization in common organic solvents [9, 10]. *N*-Phthaloyl-chitosan is thus used widely for regioselective and controlled modification reactions of chitosan including the introduction of sugar branches [11–14] and many sorts of substituents [15–23]. After reactions, the phthaloyl group can be removed to regenerate the free amino group.

Introduction of electron-withdrawing groups such as nitro [24] and chloro [25] groups into the phthaloyl aromatic ring may facilitate deprotection after desired modification reactions. 4,5-Dichlorophthaloyl group is particularly interesting in that it could be removed under milder conditions than the phthaloyl group in disaccharide synthesis [26]. It is thus considered worthwhile to evaluate the dichlorophthaloyl group for the possible chemoselective and quantitative protection of the amino group of chitosan to expand the scope of protection methods for this bioactive amino polysaccharide.

Experimental

General

IR spectra were recorded on a Shimadzu FTIR-8900 instrument by the KBr method. ^{1}H NMR spectra were taken with a JEOL JNM-LA400D in dimethyl sulfoxide (DMSO)-d_6 at 90 °C. ^{13}C CP/MAS NMR spectra were taken with the same instrument at 20 °C and ^{13}C frequency of 100.40 MHz with TOSS (total suppression of spinning sidebands) and TOSDL (TOSS and dipolar dephasing) modes using hexamethylbenzene (17.36 ppm) as the external standard [27]. Conductometric titration was carried out with a DKK·TOA conductivity meter CM-20J. Molecular weight of chitosan was measured by gel permeation chromatography (GPC) with a Shimadzu LC-10AD system [column, Shodex OHpak SB-G + Shodex OHpak SB-804 HQ; solvent, CH_3CO_2H (0.07 mol/L)/CH_3CO_2Li (0.05 mol/L)/H_2O; flow rate, 0.5 mL/min; 40 °C] using pullulan standards. Elemental analysis was performed with a Perkin Elmer 2400 II. All the chemicals were of reagent grade and used without further purification. Solvents were purified in usual manners prior to use.

Chitosan

Chitosan was prepared by deacetylating shrimp chitin with 40% aqueous sodium hydroxide at 110 °C for 4 h in a nitrogen atmosphere. After pulverization, it was treated with sodium hydroxide under the same conditions two more times to give thoroughly deacetylated chitosan. The degree of deacetylation (*dd*) of the product was 1.00 as determined by conductometric titration.

Dichlorophthaloylation of chitosan in *N*,*N*-dimethylformamide (DMF)

To a solution of 1.21 g (5.58 mmol) of dichlorophthalic anhydride in 6 mL of DMF was added 0.300 g (*dd* 1.00, 1.86 mmol) of chitosan. The mixture was heated at 120 °C to give a brown solution in 5 h. After 8 h, the solution was cooled to room temperature and poured into 500 mL of ice water. The precipitate was collected by filtration, washed with 150 mL of methanol overnight, and dried to give 0.704 g of the product as a pale tan powdery material. The degree of substitution (*ds*) was 1.40 as calculated from the C/N ratio of elemental analysis, and the yield was 85% based on the *ds* value. IR (KBr): ν 2,700–2,600 (COOH), 1,780 (imide C=O), 1,716 (broad, ester and imide C=O), 1,150-1,000 (pyranose), 773 (C–Cl), and 746 cm^{-1} (arom). ^{1}H NMR (DMSO-d_6): δ 3.3–5.2 (pyranose), 7.5–7.9 (*O*-dichlorophthaloyl), and 8.02 ppm (*N*-dichlorophthaloyl).

Anal. Calcd for $(C_{14}H_{11}NO_6Cl_2)_{0.60}(C_{22}H_{13}NO_9Cl_4)_{0.40}\cdot 0.4H_2O$: C, 45.48; H, 2.80; N, 3.08. Found: C, 45.59; H, 3.07; N, 3.08.

Removal of *O*-dichlorophthaloyl group from *N*,*O*-dichlorophthaloylated chitosan

The *N*,*O*-dichlorophthaloylated chitosan (*ds* 1.40, 100 mg) obtained above was added portionwise to 2 mL of DMF/water (95/5), and the resulting solution was stirred at 120 °C for 5 h in nitrogen to give a pale tan mixture. After cooling to room temperature, it was poured into 100 mL of ice water. The precipitate was collected on a filter, washed with methanol, and dried. The yield of *N*-dichlorophthaloyl-chitosan was 62 mg (77%). IR (KBr): ν 1,780 and 1,716 (imide C=O), 1,150–1,000 (pyranose), 773 (C–Cl), and 746 cm^{-1} (arom). ^{1}H NMR (DMSO-d_6): δ 3.3–5.2 (pyranose) and 8.02 ppm (*N*-dichlorophthaloyl).

Anal. Calcd for $C_{14}H_{11}NO_6Cl_2\cdot 0.5H_2O$: C, 45.55; H, 3.28; N, 3.79. Found: C, 45.45; H, 3.14; N, 3.80.

Selective hydrolysis was also possible by treating *N*,*O*-dichlorophthaloylated chitosan with methanol/conc HCl (5/3) under gentle reflux for 24 h to give *N*-dichlorophthaloyl-chitosan.

Anal. Calcd for $C_{14}H_{11}NO_6Cl_2\cdot 0.7H_2O$: C, 45.07; H, 3.36; N, 3.75. Found: C, 44.98; H, 3.17; N, 3.71.

Dichlorophthaloylation of chitosan in DMF/water

Chitosan (1.00 g, 6.20 mmol) was added to a solution of 4.04 g (18.60 mmol) of dichlorophthalic anhydride in 20 mL of DMF/water (95/5), and the mixture was

heated at 120 °C with stirring in nitrogen. It became a solution in about 1.5 h and then a gelatinous mixture in 2 h. After 8 h, the mixture was cooled to room temperature and poured into 500 mL of ice water to precipitate the product. It was washed with methanol and dried to give 1.62 g (90%) of *N*-dichlorophthaloyl-chitosan as an almost colorless powdery material. IR (KBr): ν 1,780 and 1,716 (imide C=O), 1,150–1,000 (pyranose), 775 (C–Cl), and 745 cm^{-1} (arom). ^{13}C CP/MAS NMR (TOSS mode): δ 58–101 (pyranose), 125.4 (arom C3,6), 131.7 (arom C1,2), 139.3 (arom C4,5), and 167.3 ppm (C=O); (TOSDL mode) δ 131.4 (arom C1,2), 138.9 (arom C4,5), and 167.1 ppm (C=O).

Anal. Calcd for $C_{14}H_{11}NO_6Cl_2 \cdot 0.5\ H_2O$: C, 45.55; H, 3.28; N, 3.79. Found: C, 45.63; H, 3.50; N, 3.81.

Deprotection of *N*-dichlorophthaloyl-chitosan

A mixture of 100 mg of *N*-dichlorophthaloyl-chitosan in 10 mL of hydrazine monohydrate was stirred at 80 °C for 7 h in a nitrogen atmosphere and cooled to room temperature. The product was isolated in 250 mL of ice water, washed with ethanol in a Soxhlet extractor for 3 h and then with methanol for 1 h at room temperature, and dried. It was added to 5 mL of 6 mol/L aqueous sodium hydroxide and heated at 50 °C for 3 h. After cooling to room temperature, the mixture was poured into 250 mL of water, and the solid was washed with deionized water until neutral and with methanol. On drying, 32 mg (72%) of chitosan was obtained as a white powdery material. IR (KBr): ν 1,591 (NH_2) and 1,150–1,000 cm^{-1} (pyranose).

Tritylation of *N*-dichlorophthaloyl-chitosan

To a solution of 1.55 g (5.56 mmol) of triphenylmethyl (trityl) chloride in 5 mL of pyridine was added 0.200 g (0.56 mmol) of *N*-dichlorophthaloyl-chitosan (*ds* 1.00), and the mixture was stirred at 90 °C for 24 h. The resulting brown solution was cooled to room temperature and poured into 150 mL of methanol to precipitate the tritylated product. It was collected by filtration, washed with 100 mL of methanol overnight, and dried to give 0.294 g (88%) of a light tan powdery material. The *ds* for trityl was calculated to be 1.00 from the C/N value of elemental analysis. IR (KBr): ν 3,057 (arom C–H), 1,782 and 1,724 (imide C=O), 1,150–1,000 (pyranose), 773 (C–Cl), and 766, 744, and 704 cm^{-1} (arom). ^1H NMR (DMSO-d_6): δ 3.8–4.8 (pyranose), 7.02 (trityl), and 7.83 ppm (*N*-dichlorophthaloyl).

Anal. Calcd for $C_{33}H_{25}NO_6Cl_2 \cdot 0.2\ H_2O$: C, 65.40; H, 4.22; N, 2.31. Found: C, 65.49; H, 4.21; N, 2.31.

Results and discussion

Dichlorophthaloylation in DMF

In order to discuss the reaction behavior quantitatively, fully deacetylated chitosan was used for the reaction with dichlorophthalic anhydride in DMF at 120 °C. The

Table 1 Dichlorophthaloylation of chitosan

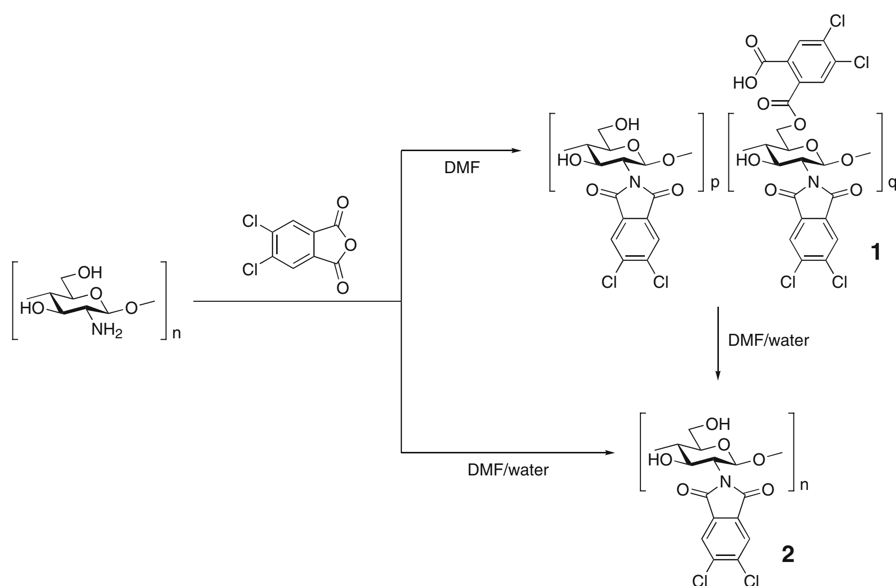
Solvent (v/v)	Time (h)	*ds*[a]	Yield (%)[b]
DMF	8	1.40	85
DMF	24	1.13	80
DMF/water (99/1)	8	1.12	83
DMF/water (95/5)	6	1.00	92
DMF/water (95/5)	8	1.00	90
DMF/water (92/8)	8	1.01	81
DMF/water (90/10)	8	1.01	80

Chitosan, 0.300 g; dichlorophthalic anhydride, 3 equiv; solvent, 6 mL; reaction temperature, 120 °C

DMF *N*,*N*-dimethylformamide

[a] Degree of substitution calculated from the C/N value of elemental analysis

[b] Calculated on the basis of the *ds* value



Scheme 1 Dichlorophthaloylation of chitosan

reaction mixture became a solution in 5 h, and after 8 h reaction, the *ds* of the product was found to be 1.40 (Table 1), the substitution taking place at some hydroxy groups in addition to the amino groups (Scheme 1).

Figure 1 shows the IR spectrum of the *N*,*O*-dichlorophthaloylated chitosan (**1**, *ds* 1.40). The bands at 1,782 and 1,716 cm^{-1} are attributable to the imide structure. The presence of *O*-dichlorophthaloyl group is apparent from the typical bands in the spectrum such as those at 2,700–2,600 cm^{-1} (COOH) and 1,300–1,240 cm^{-1} (C–O–C). The band centered at 1,716 cm^{-1} is broad because of the C=O of both imide and ester. The ^{1}H NMR spectrum in Fig. 2 also supported *N*,*O*-substitution; peaks due to *O*-dichlorophthaloyl are observed at 7.5–7.9 ppm in addition to the

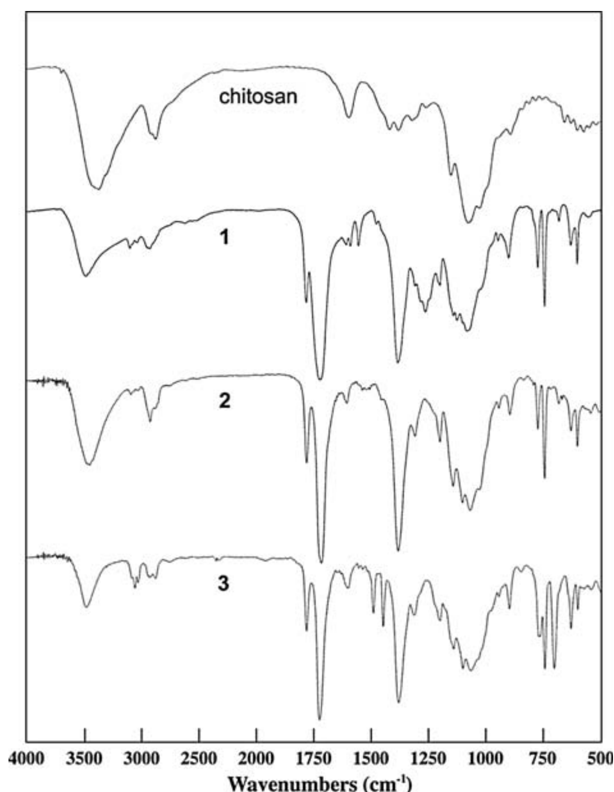


Fig. 1 IR spectra of chitosan and the derivatives: chitosan (*dd* 1.00), **1** (*ds* 1.40) prepared in DMF, **2** (*ds* 1.00) prepared in DMF/water (95/5), and **3** (*ds* 1.00 each for dichlorophthaloyl and trityl)

peak at 8.02 ppm due to *N*-dichlorophthaloyl. The *ds* decreased to 1.13 after 24 as included in Table 1, and the IR bands at 2,700–2,600 and 1,300–1,240 cm^{-1} became weak. These results suggest that dichlorophthaloylation proceeded similarly to phthaloylation [27, 28].

An attempt to remove the resulting *O*-dichlorophthaloyl group by transesterification with sodium methoxide was not successful as evidenced by weakened imide bands, though no *O*-substituent remained. Although the treatment with a mixture of methanol and hydrochloric acid resulted in the formation of *N*-dichlorophthaloyl-chitosan (**2**), the use of such a strong acid would not be appropriate because of the most probable degradation of the chitosan main chain.

The *O*-dichlorophthaloyl group could be removed effectively by treating **1** with DMF/water (95/5) at 120 °C. The initial solution became a heterogeneous mixture, and the product isolated after 5 h was confirmed to be **2** by elemental analysis, IR spectroscopy, and NMR spectroscopy. In the IR spectrum, the bands due to ester and carboxy groups disappeared completely, and the imide band at 1,716 cm^{-1} became sharp. The peaks in the ^{1}H NMR spectrum at around 7.5–7.9 ppm due to *O*-dichlorophthaloyl also disappeared.

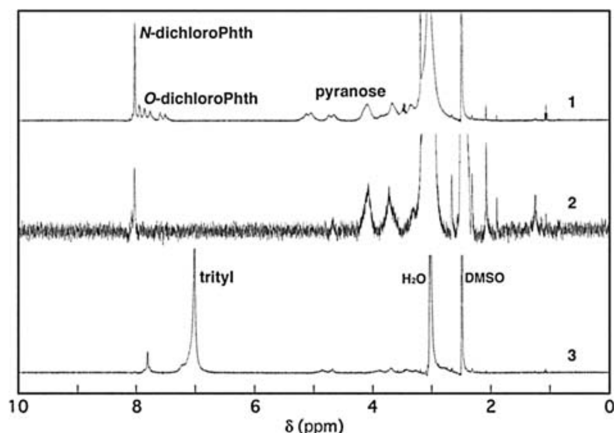


Fig. 2 ^{1}H NMR in DMSO-d_6: **1** (*ds* 1.40) prepared in DMF, **2** (*ds* 1.00) prepared in DMF/water (95/5), and **3** (*ds* 1.00 each for dichlorophthaloyl and trityl)

Dichlorophthaloylation in DMF/water

As implied from the phthaloylation [28], dichlorophthaloylation was then conducted in DMF/water to enhance the selectivity toward *N*-substitution. The initial heterogeneous mixture became a solution and then a gel-like mixture. As listed in Table 1, although *O*-substitution was still observed when the water content was as low as 1%, complete discrimination between the amino and hydroxy groups was achieved at a water content of 5–10% to give **2** in a simple one-step reaction. Conductometric titration of the product obtained in DMF/water (95/5) gave a sharp V-shaped profile, which indicated the absence of both free amino and carboxy groups. The elemental analysis and IR (Fig. 1) and NMR spectra (Fig. 2) also supported the formation of **2**.

The solid-state ^{13}C NMR spectra of **2** in the TOSS and TOSDL modes are shown in Fig. 3. In the TOSDL mode, peaks due to CH and CH_2 should disappear because of the short relaxation times, and as expected, three peaks were observed ascribable to the arom C1,2 (131.4 ppm), arom C4,5 (138.9 ppm), and carbonyl (167.9 ppm), whereas only two peaks (arom C1,2 at 131.7 and carbonyl at 169.1 ppm) for *N*-phthaloyl-chitosan [27].

Deprotection

The introduction of chloro groups into the phthaloyl was expected to facilitate the deprotection for regenerating the amino group, and **2** was treated with hydrazine at 80 °C for 7 h. Though the IR bands at 1,780 and 1,716 cm^{-1} characteristic of imide disappeared, new weak bands were observed at 1,649 and 1,556 cm^{-1}, and the Beilstein test for chloro was positive, implying the possible formation of amide linkages by partial cleavage of the imide. The hydrazinolysis was repeated one more time for 7 h or was prolonged up to 24 h, but amide bands still remained though

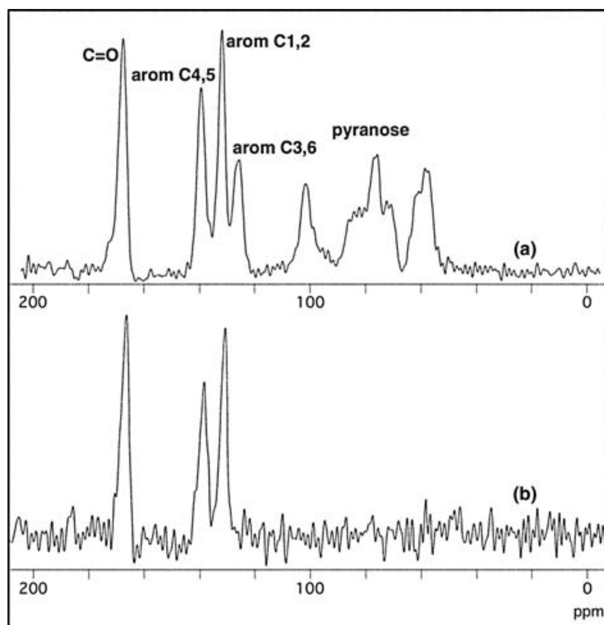


Fig. 3 ^{13}C CP/MAS NMR spectra of **2** (*ds* 1.00) prepared in DMF/water (95/5): **a** TOSS mode and **b** TOSDL mode

very weak. The product obtained after 7 h reaction with hydrazine was thus treated with 6 mol/L sodium hydroxide at 50 °C for 3 h, resulting in the full deprotection to chitosan. These results show that deprotection of the dichlorophthaloyl group needs somewhat severe conditions than that of the phthaloyl group where hydrazinolysis at 80 °C for 16 h or repeated treatment for 7 h two times was sufficient [27, 28].

Molecular weight determination

The molecular weight characteristics of the regenerated chitosans were determined by GPC using an aqueous acetic acid buffer solution. As listed in Table 2, the number–average molecular weight *M*n values of regenerated chitosans were about

Table 2 Molecular weight measurements by GPC

Chitosan	*M*n	*M*w	*M*w/*M*n
Original	51,000	168,000	3.29
Regenerated from **2**[a]	29,000	100,000	3.45
Regenerated from **2**[b]	22,000	74,000	3.36

Standard, pullulans

[a] With hydrazine at 80 °C for 7 h and then with 6 mol/L NaOH at 50 °C for 3 h

[b] By repeating the treatment with hydrazine two times at 80 °C for 7 h and then with 6 mol/L NaOH at 50 °C for 3 h

Table 3 Solubility

	Chitin	Chitosan	**1**[a]	**2**[b]
DMF	–	–	+	±
DMSO	–	–	+	±
Pyridine	–	–	±	±
m-Cresol	–	–	+	±
DCA	–	–	+	±
DMAc/LiCl	+	–	+	+
MeOH/$CaCl_2$	+	–	+	±
5% AcOHaq	–	+	–	–

DMF *N*,*N*-dimethylformamide, *DMSO* dimethyl sulfoxide, *DCA* dichloroacetic acid, *DMAc/LiCl* *N*,*N*-dimethylacetamide containing 8% LiCl, *MeOH/CaCl₂* methanol saturated with $CaCl_2$ dihydrate, + soluble, ± partially soluble or swelled, – insoluble

[a] *N*,*O*-Dichlorophthaloylated chitosan (*ds* 1.40)

[b] *N*-Dichlorophthaloyl-chitosan (*ds* 1.00)

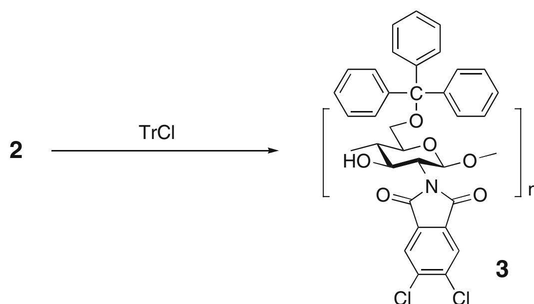
half that of the original chitosan, which revealed that the main chain was cleaved to a low extent under these protection–deprotection conditions. Furthermore, the molecular weight characteristics remained similar, judging from the polydispersity index *M*w/*M*n values.

Solubility

As summarized in Table 3, **1** exhibited high solubility in ordinary organic solvents because of the additional bulky groups at C6 of chitosan. The solubility of **2** was limited, but it was soluble in DMAc/LiCl and partially soluble in some solvents such as DMF and DMSO.

Tritylation of dichlorophthaloylated chitosan

To evaluate **2** as a potential reaction precursor for regioselective modifications of chitosan, tritylation was conducted in pyridine (Scheme 2). As listed in Table 4, the



Scheme 2 Tritylation of *N*-dichlorophthaloyl-chitosan **2**

Table 4 Tritylation of selectively *N*-protected chitosans

Protected chitosan	DMAP/pyranose[a]	Temp (°C)	Time (h)	*ds*[b]	Yield (%)[c]
2	–	70	24	0.78	75
2	–	80	24	0.85	88
2	5	80	24	0.87	82
2	–	80	48	0.94	79
2	–	90	24	1.00	88
2	–	100	24	1.00	79
N-Phthaloyl-chitosan	–	90	24	0.80	86
N-Phthaloyl-chitosan	–	100	24	1.00	88

Protected chitosan, 0.200 g; trityl chloride, 10 equiv; pyridine, 5 mL

DMAP dimethylaminopyridine

[a] Molar ratio

[b] Degree of substitution calculated from the C/N value of elemental analysis

[c] Calculated on the basis of the *ds* value

reaction was dependent on the temperature, and dimethylaminopyridine (DMAP) was not effective as a catalyst. Full substitution was attained in the reaction at 90 or 100 °C for 24 h. The structure of the product (**3**) was confirmed by elemental analysis and ^{1}H NMR spectroscopy. The IR spectrum in Fig. 1 supported the introduction of trityl as evident by intensified aromatic bands at 3,059, 744, and 704 cm^{-1}. Table 4 includes the results of tritylation of *N*-phthaloyl-chitosan for comparison. As shown there, tritylation proved to proceed a little more facilely with **2** than *N*-phthaloyl-chitosan. The resulting **3** was soluble in common solvents including pyridine, DMF, and DMSO.

Conclusions

Dichlorophthaloylation of chitosan proceeded smoothly, and the substitution profile was dependent on the solvent system. DMF as a solvent gave *N*,*O*-dichlorophthaloylated chitosan, which was transformed into *N*-dichlorophthaloyl-chitosan by *O*-deprotection. Selective and quantitative *N*-dichlorophthaloylation of chitosan could be attained in DMF/water, and a new simple one-step method for full *N*-protection was thus established. The influence of the protection–deprotection on the molecular weight of chitosan was allowable, which is significant for practical structural modifications involving a series of reactions. Though *N*-dichlorophthaloyl-chitosan exhibited limited solubility, its C6 hydroxy group showed higher reactivity than that of *N*-phthaloyl-chitosan to enable facile protection. These results indicate the useful nature of the dichlorophthaloyl group for the protection of the amino group of chitosan, and the resulting product would be a convenient precursor for controlled modification reactions to give chitosan derivatives with well-defined structures.

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References

1. Uragami T, Kurita K, Fukamizo T (eds) (2001) Chitin and Chitosan in Life Science. Kodansha Scientific, Tokyo
2. Domard A, Guibal E, Vårum KM (eds) (2007) Advances in Chitin Science, vol 9. 10th ICCC-EUCHIS'06, Montpellier
3. Roberts GAF (1992) Chitin Chemistry. Macmillan, London
4. Kurita K (1997) Chitin and chitosan derivatives. In: Arshady R (ed) Desk Reference of Functional Polymers: Syntheses and Applications. American Chemical Society, Washington, D. C, pp 239–259
5. Uragami T, Tokura S (eds) (2006) Material Science of Chitin and Chitosan. Kodansha Scientific, Tokyo
6. Kurita K (2001) Prog Polym Sci 26:1921
7. Kurita K (2006) Mar Biotechnol 8:203
8. Kurita K (2006) Introduction of biologically active branches through controlled modification reactions of chitin and chitosan. In: Uragami T, Tokura S (eds) Material Science of Chitin and Chitosan. Kodansha Scientific, Tokyo
9. Kurita K, Ichikawa H, Ishizeki S, Fujisaki H, Iwakura Y (1982) Makromol Chem 183:1161
10. Nishimura S, Kohgo O, Kurita K, Kuzuhara H (1991) Macromolecules 24:4745
11. Kurita K, Akao H, Kobayashi M, Mori T, Nishiyama Y (1997) Polym Bull 39:543
12. Kurita K, Shimada K, Nishiyama Y, Shimojoh M, Nishimura S (1998) Macromolecules 31:4764
13. Kurita K, Kojima T, Nishiyama Y, Shimojoh M (2000) Macromolecules 33:4711
14. Kurita K, Akao H, Yang J, Shimojoh M (2003) Biomacromolecules 4:1264
15. Nishimura S, Miura Y, Ren L, Sato M, Yamagishi A, Nishi N, Tokura S, Kurita K, Ishii S (1993) Chem Lett 1623
16. Nishimura S, Kai H, Shinada K, Yoshida T, Tokura S, Kurita K, Nakashima H, Yamamoto N, Uryu T (1998) Carbohydr Res 306:427
17. Nishiyama Y, Yoshikawa T, Ohara N, Kurita K, Hojo K, Kamada H, Tsutsumi Y, Mayumi T, Kawasaki K (2000) J Chem Soc Perkin Trans 1:1161
18. Kurita K, Hayakawa M, Nishiyama Y, Harata M (2002) Carbohydr Polym 47:7
19. Ouchi T, Nishizawa H, Ohya Y (1998) Polymer 39:5157
20. Holappa J, Nevalainen T, Svolainen J, Soininen P, Elomaa M, Safin R, Suvanto S, Pakkanen T, Másson M, Loftsson T, Järvinen T (2004) Macromolecules 37:2784
21. Holappa J, Nevalainen T, Soininen P, Elomaa M, Safin R, Másson M, Järvinen T (2005) Biomacromolecules 6:858
22. Yoksan R, Akashi M, Hiwatari KI, Chirachanchai S (2003) Biopolymers 69:386
23. Yoksan R, Matsusaki M, Akashi M, Chirachanchai S (2004) Colloid Polym Sci 282:337
24. Tsubouchi H, Tsuji K, Ishikawa H (1994) Synlett 63
25. Debenham JS, Madsen R, Roberts C, Fraser-Reid B (1995) J Am Chem Soc 117:3302
26. Shimizu H, Ito Y, Matsuzaki Y, Iijima H, Ogawa T (1996) Biosci Biotech Biochem 60:73
27. Kurita K, Ikeda H, Yoshida Y, Shimojoh M, Harata M (2002) Biomacromolecules 3:1
28. Kurita K, Ikeda H, Shimojoh M, Yang J (2007) Polym J 39:945