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Quantitative protection of chitin by one-step tritylation and benzylation to synthesize precursors for chemical modifications

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Abstract Protections of hydroxyl groups of chitin by triphenylmethylation and benzylation were studied in detail to synthesize precursors for efficient regioselective chemical modifications in organic solvents. The reactions of squid β-chitin with triphenylmethyl chloride and benzyl chloride proceeded much more facilely than those of shrimp α-chitin, and quantitative 6-*O*-triphenylmethylation and 3,6-*O*-dibenzylation of β-chitin were accomplished in the presence of dimethylaminopyridine and sodium hydride, respectively, in simple one-step reactions. The resulting protected derivatives exhibited high affinity for organic solvents and would allow modification reactions under homogeneous or almost homogeneous conditions in suitable solvents.

Keywords Chitin · Protection · Tritylation · Benzylation · Chemical modification · Precursor · Solubility

Introduction

Though chitin is a quite abundant, easily accessible, and environmentally benign amino polysaccharide, it still remains an almost unutilized biomass resource. However, increasingly more attention has been paid to chitin and the derived chitosan as their distinctive physicochemical properties and biological functions are being better understood [1–5]. They are expected to have great potential as specialty biopolymers in various fields including medicine, cosmetics, food, and agrochemicals.

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For promoting utilization of these invaluable biomaterials, chemical modification would be particularly promising among some possibilities, giving rise to the derivatives having desirable properties, and such attempts have been made extensively [6–10]. Preparation of the derivatives with well-defined structures is, however, usually difficult owing to the multifunctionality and insolubility in ordinary solvents, which has made difficult in-depth discussion on structure–property relationship of the derivatives. Controlled structural transformation will become feasible only when quantitatively protected organosoluble intermediates with proper groups are available to carry out regioselective reactions in solution under mild conditions. Development of such precursors is thus essential for constructing sophisticated molecular architectures based on these amino polysaccharides, and some groups have proved effectual for preparing soluble key intermediates to introduce substituents at a designated position of the backbone.

As protective and solubilizing groups, trimethylsilyl [11–13] and phthaloyl [14–22] are especially practical for chitin and chitosan to give derivatives with required molecular structures. For instance, sugar branches could be incorporated into chitin and chitosan through a series of site-specific reactions starting from 2-*N*-phthaloylchitosan; the resulting nonnatural branched chitins and chitosans were water-soluble and exhibited significant medicinal functions such as antimicrobial [16, 17, 19] and antitumor activities [22].

Of some other protective groups, triphenylmethyl (trityl) and benzyl are expected to be useful for providing C-6 monosubstituted and C-3,6 disubstituted chitins in view of many examples in carbohydrate chemistry. Despite that ordinary α-chitin from crab and shrimp shells is resistive to chemical reactions owing to the strong intermolecular forces, *β*-chitin from squid pens, when properly pulverized, exhibits considerable reactivity even under heterogeneous conditions [23], and is superior to α-chitin as a starting material for structural transformations. If quantitative protections with trityl and benzyl become possible in simple one-step reactions, it would be beneficial to expand the scope of regioselective modification reactions for preparing a new kind of functional polymers based on these highly prospective amino polysaccharides. The protecting reactions were thus studied in detail with *β*-chitin and also with α-chitin for comparison.

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. Conductometric titration was carried out with a DKK-TOA conductivity meter CM-20J to determine the extent of N-acetylation of chitin. Elemental analysis was performed with a Perkin Elmer 2400 II. *N*,*N*-Dimethyl-4-aminopyridine (DMAP) was purified by recrystallization from ethyl acetate. Other chemicals were of reagent grade and used without further purification. Solvents were purified in usual manners and stored over molecular sieves.

Chitin

β-Chitin isolated from squid pens [23, 24] had a degree of deacetylation around 0.1, and the free amino groups were acetylated by the method described previously [25]. In brief, 3.00 g of pulverized β-chitin was suspended in 200 mL of methanol, and 100 mL of acetic anhydride was added. After 48 h at 40 °C, the product was isolated in ice water and treated with 0.1 mol/L potassium hydroxide in methanol at room temperature for 5 h to give 2.70 g (89%) of fully N-acetylated chitin as confirmed by conductometric titration. α-Chitin isolated from shrimp shells was N-acetylated in a similar manner in a yield of 90%.

Tritylation

To a suspension of 0.200 g (0.985 mmol pyranose) of β-chitin in 10 mL of pyridine were added 2.74 g (9.85 mmol, 10 equiv. to pyranose) of trityl chloride and 0.36 g (2.95 mmol, 3 equiv. to pyranose) of DMAP. The mixture was stirred at 90 °C for 72 h under a nitrogen atmosphere. The resulting brown solution was allowed to cool to room temperature and poured into 150 mL of methanol. The fine precipitate was collected on a sintered glass filter, washed thoroughly with methanol, and dried to give 0.352 g of the product as a light brown powder. The degree of substitution (ds) was determined to be 0.75 from the *C*/*N* ratio of elemental analysis, and the yield was thereby calculated to be 93%.

Anal. Calcd for $(C_{27}H_{27}NO_5)_{0.75}(C_8H_{13}NO_5)_{0.25}\cdot 0.5H_2O$: C, 67.84; H, 6.27; N, 3.56. Found: C, 67.70; H, 6.16; N, 3.56.

In a similar manner, with 6 equiv. of DMAP, a fully substituted product (ds 1.00) was obtained in 84% yield. IR (KBr): ν 3408 (OH and NH), 3057 (arom CH), 1670 (amide I), 1597 (arom), 1522 (amide II), 1448 (arom), 1150–1000 (pyranose), and 764, 748, and 706 cm^{-1} (arom). ^1H NMR (DMSO-d_6): 1.53 (s, CO–CH_3), 3.0–4.5 (broad m, pyranose), and 7.19–7.38 ppm (m, phenyl).

Anal. Calcd for $C_{27}H_{27}NO_5\cdot 1.6H_2O$: C, 68.37; H, 6.42; N, 2.95. Found: C, 68.42; H, 6.30; N, 2.95.

IR calibration line for tritylation

Tritylated chitins (1 mg, ds 0.53–1.00) were ground with 50 mg of potassium bromide. Disks were made from a small portion of the mixtures, dried thoroughly with phosphorus pentoxide at 60 °C, and subjected to IR spectroscopy. Absorbance ratios of two bands were calculated using the baselines over 1800–800 cm^{-1} in the transmittance spectra and calibrated with ds values.

Benzylation of chitin

A suspension of 0.100 g (0.49 mmol pyranose) of β-chitin in 10 mL of DMSO was heated at 60 °C overnight, and 0.059 g (2.46 mmol, 5 equiv. to pyranose) of sodium hydride was added. After stirring the mixture at room temperature for 2 h, 0.620 g (4.9 mmol, 10 equiv. to pyranose) of benzyl chloride was added, and the mixture

was heated at 60 °C for 24 h in nitrogen with stirring. It was cooled to room temperature and poured into 100 mL of methanol to precipitate the product, which was collected by centrifugation, washed with water and methanol, and dried. The product was obtained as a white powdery material; yield 0.12 g. The ds was 1.33 as determined from the *C*/*N* ratio of elemental analysis. Based on the ds value, the yield was 75%.

Anal. Calcd for $(C_{22}H_{25}NO_5)_{0.33}(C_{15}H_{19}NO_5)_{0.67}\cdot 0.6H_2O$: C, 62.27; H, 6.70; N, 4.20. Found: C, 62.20; H, 6.67; N, 4.19.

Using 7 equiv. of sodium hydride, the product with ds 2.00 was obtained in 90% yield. IR (KBr): *v* 3285 (NH), 3063 and 3030 (arom CH), 1647 (amide I), 1554 (amide II), 1150–1000 (pyranose), and 737 and 698 cm^{-1} (arom).

Anal. Calcd for $C_{22}H_{25}NO_5\cdot 0.5H_2O$: C, 67.33; H, 6.68; N, 3.57. Found: C, 67.21; H, 6.39; N, 3.56.

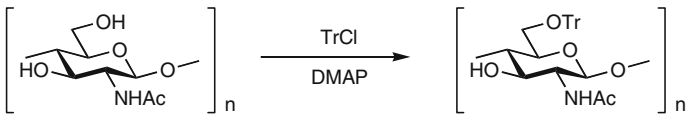
Results and discussion

The α- and *β*-chitins isolated from shrimp shells and squid pens were partially deacetylated, and they were therefore selectively N-acetylated to give structurally uniform chitins as starting materials for discussing the substitution behavior.

Tritylation

The trityl group is suitable for protecting primary hydroxyl groups, and tritylated chitin with ds 1.00 could be prepared by tritylation of trimethylsilylated chitin that shows high affinity for organic solvents [11]. However, *β*-chitin was found to exhibit higher reactivity than α-chitin in chemical modifications including acetylation and tritylation in our preliminary study [25], and direct tritylation of *β*-chitin was thus examined in detail in comparison with that of α-chitin to achieve quantitative protection in a simple manner by one-step reaction (Scheme 1).

The influences of reaction temperature and the amount of DMAP are shown in Table 1. The reaction proceeded smoothly at 90 °C, and to avoid discoloration of the product, the reaction was also attempted at 80 °C only to result in low substitution. The progress of tritylation was dependent on the amount of DMAP, and 6 equiv. was found appropriate to attain full substitution in one step to give 6-*O*-trityl-chitin (**1**). However, the ds decreased with a further increase in the amount of DMAP probably because of the degradation of chitin as implied from discoloration of the reaction mixture. The structure of the product was supported by



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Scheme 1 Tritylation of chitin

Table 1 Tritylation of chitins (trityl chloride, 10 equiv.)

Chitin	DMAP/pyranose[a]	Temperature (°C)	Time (h)	Yield (%)	ds[b]
β-Chitin	0	90	72	–	0
β-Chitin	1	90	72	93	0.53[c]
β-Chitin	1.7	90	24	96	0.61[d]
β-Chitin	1.7	90	72	98	0.65[e]
β-Chitin	3	90	72	93	0.75[f]
β-Chitin	5	90	72	89	0.89[g]
β-Chitin	6	80	96	97	0.45[h]
β-Chitin	6	90	72	86	1.00[i]
β-Chitin	7	90	72	89	0.84[j]
β-Chitin	15	90	72	94	0.75[k]
α-Chitin	1.7	90	24	87	0.11[l]
α-Chitin	5	90	72	88	0.12[m]

[a] Mole ratio

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

[c] Anal. Calcd for $(C_{27}H_{27}NO_5)_{0.53}(C_8H_{13}NO_5)_{0.47}\cdot 0.4H_2O$: C, 64.06; H, 6.31; N, 4.13. Found: C, 64.09; H, 6.34; N, 4.14

[d] Ref. 25

[e] Ref. 25

[f] See "Experimental" section

[g] Anal. Calcd for $(C_{27}H_{27}NO_5)_{0.89}(C_8H_{13}NO_5)_{0.11}\cdot 0.4H_2O$: C, 69.63; H, 6.25; N, 3.26. Found: C, 69.74; H, 6.05; N, 3.26

[h] Anal. Calcd for $(C_{27}H_{27}NO_5)_{0.45}(C_8H_{13}NO_5)_{0.55}$: C, 63.66; H, 6.23; N, 4.49. Found: C, 63.98; H, 6.56; N, 4.52

[i] See "Experimental" section

[j] Anal. Calcd for $(C_{27}H_{27}NO_5)_{0.84}(C_8H_{13}NO_5)_{0.16}\cdot 0.2H_2O$: C, 70.13; H, 6.18; N, 3.41. Found: C, 70.12; H, 6.09; N, 3.41

[k] Anal. Calcd for $(C_{27}H_{27}NO_5)_{0.75}(C_8H_{13}NO_5)_{0.25}\cdot 0.6H_2O$: C, 67.53; H, 6.29; N, 3.54. Found: C, 67.53; H, 6.32; N, 3.56

[l] Anal. Calcd for $(C_{27}H_{27}NO_5)_{0.11}(C_8H_{13}NO_5)_{0.89}\cdot 0.4H_2O$: C, 51.12; H, 6.52; N, 5.91. Found: C, 51.01; H, 6.27; N, 5.86

[m] Anal. Calcd for $(C_{27}H_{27}NO_5)_{0.12}(C_8H_{13}NO_5)_{0.88}$: C, 53.16; H, 6.37; N, 6.03. Found: C, 53.09; H, 6.44; N, 6.07

elemental analysis and by comparing the IR and NMR spectra with those of the authentic sample prepared from trimethylsilylated chitin [11]. Compared to β-chitin, α-chitin showed quite poor reactivity as revealed in some modification reactions owing to its strong intermolecular hydrogen bonding and crystallinity [8, 25].

The ds value can be calculated from the *C*/*N* ratio of elemental analysis, but the IR calibration line would be much more convenient to estimate it. Among several combinations of absorbances of characteristic bands, A_{1448}/A_{1670} was confirmed proper judging from the correlation coefficient of the data, and Fig. 1 has proved a reliable and suited tool to determine the ds quickly.

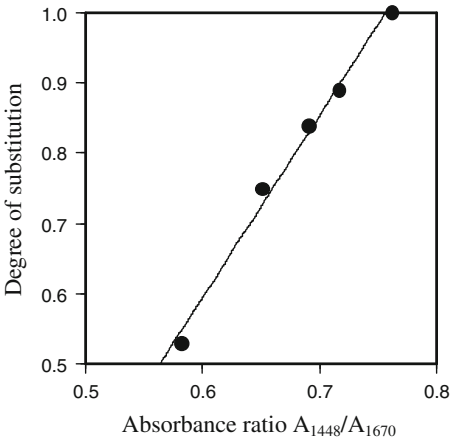
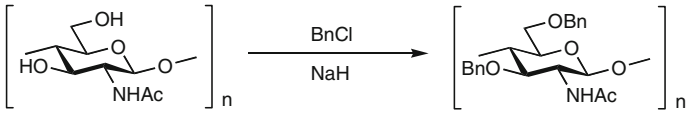


Fig. 1 Calibration line for determining the ds for trityl groups from absorbance ratio

Benzylation

The reaction of β-chitin with benzyl chloride (Scheme 2) was first attempted by the methylsulfinyl carbanion method where DMSO was treated with sodium hydride beforehand to form the corresponding methylsulfinyl carbanion [26–28] as suggested by the efficient benzylation of 2-*N*-phthaloyl-6-*O*-trityl-chitosan [29]. However, the reaction mixture assumed a dark color most likely due to the degradation of chitin backbone, and both the ds and yield were low as shown in Table 2 (Method A).

Benzylation of β-chitin was then carried out by adding sodium hydride and benzyl chloride to a suspension of chitin in DMSO (Method B), and the discoloration of the reaction mixture was less than that by the carbanion method. With twofold (sodium hydride) and fivefold (benzyl chloride) excess reagents at 60 or 80 °C, the products showed strong OH bands and weak phenyl bands in the IR spectra, indicating that the reaction proceeded only to low extents. With increases in the amounts of reagents to threefold (sodium hydride) and tenfold (benzyl chloride) excess, the ds was enhanced substantially as summarized in Table 2. As a reaction temperature, 70 °C seemed to give somewhat higher ds values, but 60 °C was judged more suitable from the light coloration of the reaction mixture. The ds increased steadily with the amount of sodium hydride, and full substitution (ds 2.00) was realized with sevenfold excess, resulting in the formation of 3,6-*O*-dibenzyl-chitin (**2**). With smaller amounts of sodium hydride and benzyl chloride, however, full substitution was also effected by repeating the reaction two times as included in



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Scheme 2 Benzylation of chitin

Table 2 Benzylation of chitins (reaction time, 24 h)

Method[a]	Chitin	NaH/ pyranose[b]	BnCl/ pyranose[b]	Temperature (°)	Repetition of reaction	Yield (%)	ds[c]
A	β-Chitin	3	10	60	1	65	0.66[d]
B	β-Chitin	2	5	60	1	–	nd
B	β-Chitin	2	5	80	1	–	nd
B	β-Chitin	3	10	60	1	93	0.90[e]
B	β-Chitin	3	10	70	1	99	1.16[f]
B	β-Chitin	4	10	60	1	88	1.09[g]
B	β-Chitin	5	10	60	1	75	1.33[h]
B	β-Chitin	6	10	60	1	61	1.43[i]
B	β-Chitin	7	10	60	1	90	2.00[j]
B	β-Chitin	3	8	60	2	43	2.00[k]
B	α-Chitin	2	6	60	2	–	nd
B	α-Chitin	3	8	60	2	61	1.00[l]

[a] NaH/DMSO (methylsulfinyl carbanion; method A) or NaH (method B) was added to chitin in DMSO

[b] Mole ratio

[c] Degree of substitution calculated from the C/N ratio of elemental analysis

[d] Anal. Calcd for $(C_{15}H_{19}NO_5)_{0.66}(C_8H_{13}NO_5)_{0.34}\cdot 0.3H_2O$: C, 56.54; H, 6.60; N, 5.22. Found: C, 56.35; H, 6.66; N, 5.22

[e] Anal. Calcd for $(C_{15}H_{19}NO_5)_{0.90}(C_8H_{13}NO_5)_{0.10}\cdot 0.2H_2O$: C, 59.69; H, 6.58; N, 4.86. Found: C, 59.81; H, 6.29; N, 4.87

[f] Anal. Calcd for $(C_{22}H_{25}NO_5)_{0.16}(C_{15}H_{19}NO_5)_{0.84}\cdot 0.5H_2O$: C, 61.13; H, 6.67; N, 4.42. Found: C, 61.21; H, 6.65; N, 4.42

[g] Anal. Calcd for $(C_{22}H_{25}NO_5)_{0.09}(C_{15}H_{19}NO_5)_{0.91}\cdot 0.8H_2O$: C, 59.43; H, 6.75; N, 4.44. Found: C, 59.34; H, 6.41; N, 4.43

[h] See "Experimental" section

[i] Anal. Calcd for $(C_{22}H_{25}NO_5)_{0.43}(C_{15}H_{19}NO_5)_{0.57}\cdot 0.7H_2O$: C, 62.73; H, 6.72; N, 4.07. Found: C, 62.79; H, 6.54; N, 4.07

[j] See "Experimental" section

[k] Anal. Calcd for $C_{22}H_{25}NO_5\cdot 0.7H_2O$: C, 66.72; H, 6.72; N, 3.54. Found: C, 66.78; H, 6.69; N, 3.58

[l] Anal. Calcd for $C_{15}H_{19}NO_5\cdot 0.4H_2O$: C, 59.95; H, 6.64; N, 4.66. Found: C, 59.80; H, 6.77; N, 4.65

Table 2. The IR spectrum of the fully protected product (Fig. 2) showed strong bands at 3063, 3030, 737, and 698 cm^{-1} characteristic of phenyl groups besides a weak NH band at 3285 cm^{-1}.

In contrast, the reaction of α-chitin was rather sluggish, and as shown in Table 2, the ds was 1.00 under the same reaction conditions where ds 2.00 was achieved with β-chitin.

Solubility

Qualitative solubility test was done with 0.5 mg of a sample in 0.75 mL of a solvent, and the substituted products, **1** and **2**, showed much improved solubility in

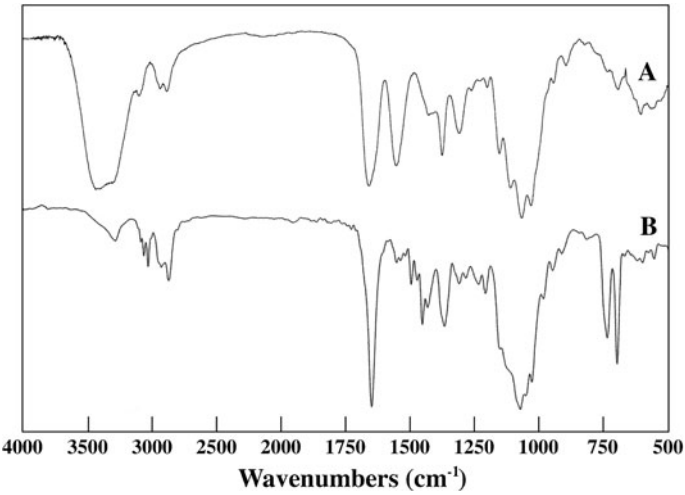

Fig. 2 IR spectra of β-chitin (*A*) and dibenzyl-chitin (*B*)

Table 3 Solubility of chitin and the derivatives

	DMSO	DMAc	DMF	Pyridine	$CHCl_3$	CH_2Cl_2	Acetone
Chitin	−	−	−	−	−	−	−
Trityl-chitin (**1**)[a]	+	+	+	+	+	+	−
Dibenzyl-chitin (**2**)[b]	±	±	±	+[c]	±	±	−

+ soluble, ± swelled, − insoluble, *DMSO* dimethyl sulfoxide, *DMAc N*,*N*-dimethylacetamide, *DMF N*,*N*-dimethylformamide

[a] Degree of substitution, 1.00

[b] Degree of substitution, 2.00

[c] Almost soluble but a small amount of highly swollen gel was observed in many cases

organic solvents as listed in Table 3. Tritylation was particularly effective for solubilization in organic solvents, and the noteworthy solubility of **1** in many common solvents is ascribable to the bulky nature of the substituent. Although benzylation was less effective in terms of solubilization, **2** swelled significantly in most organic solvents.

Conclusions

Quantitative protections of chitin by tritylation and dibenzylation were established in one step under appropriate reaction conditions using β-chitin to give 6-*O*-trityl-chitin and 3,6-*O*-dibenzyl-chitin, both of which exhibited high affinity for organic solvents due to the introduced bulky hydrophobic groups. The resulting protected products would allow various modification reactions: 6-*O*-trityl-chitin for C-2 and C-3 modifications and 3,6-*O*-dibenzyl-chitin for C-2 modifications. They are thus

considered promising as versatile precursors to conduct chemical modifications in regioselective manners to give well-defined chitin and chitosan derivatives.

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