Preparation of Tosylchitins as Precursors for Facile Chemical Modifications of Chitin

Keisuke Kurita,* Hitoshi Yoshino, Koji Yokota, Motonari Ando, Satoshi Inoue, Shigeru Ishii, and Shin-Ichiro Nishimura

Department of Industrial Chemistry, Faculty of Engineering, Seikei University, Musashino-shi, Tokyo 180, Japan

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ABSTRACT: Tosylation of chitin was accomplished by interfacial condensation to give tosylchitins expected to be useful as soluble and reactive precursors for chemical manipulations under mild conditions. The reaction was efficient even at low temperatures. Tosylation was quantitative with a 20-fold excess of tosyl chloride. Free amino groups in the tosylchitins were acylated to form well-defined structures. The tosylchitins with substitution degrees above 0.4 were soluble in common polar organic solvents. The resulting tosylchitins underwent efficiently reactions such as acetylation and iodination to fully acetylated tosylchitins and iodochitins. Iodochitins were more soluble than tosylchitins. Reduction of tosyl- and iodochitins with sodium borohydride led to deoxychitins.

Introduction

Much attention has been paid to chitin due to its attractive characteristics including abundance in nature, biodegradability, cellulose-like rigid structure, presence of amino groups, and inherent bioactivity.[1,2] Although many possibilities of chitin have been discussed as a special polymeric material, intractability has limited the studies on the chemical modification and utilization. We have been interested in the development of the chemical modification of chitin and found that solubilization can be achieved by random introduction of an appropriate amount of acetyl groups[3] or by introduction of bulky groups such as phthaloyl groups.[4,5] The tosyl group is anticipated to be sufficiently bulky to develop solubility, and, moreover, tosylated chitins should show a high reactivity that would allow controlled modification under mild conditions. On the basis of some preliminary results of tosylation,[6] detailed studies have been done to establish the preparative procedures for tosylchitins of well-defined structures. Tosylchitins have also been evaluated as versatile precursors to some novel derivatives.

Experimental Section

General Procedures. Dimethyl sulfoxide (DMSO) was distilled over calcium hydride at a reduced pressure under nitrogen and stored over 3-Å molecular sieves. All the chemicals were of reagent grade and used without further purification. Chitin isolated from shrimp shells was a gift from Katokichi Co., Ltd. It was a powdery material with a degree of deacetylation of 0.12 and assumed to be free from impurities judging from the colorlessness and IR spectroscopic data. The chitin was treated with 1 mol/L of aqueous sodium hydroxide at 100 °C for 8 h to ensure high purity and washed with deionized water. The degree of deacetylation was 0.18. IR spectra were recorded on a JEOL JIR-3510 or Jasco IR-700. ^{13}C NMR spectra were taken at 67.8 MHz with a JEOL JNM-GX270, tetramethylsilane being used as the internal reference. X-ray diffraction diagrams were obtained with a Rigaku RAD-IA diffractometer. The degree of deacetylation was determined by conductometric titration with a conductivity meter TOA CM-40S.

Tosylation of Chitin. (1) Tosylation. A mixture of 5.03 g of chitin and 100 mL of 42% aqueous sodium hydroxide was left standing at a reduced pressure by a water aspirator for 3 h. To this was added 250 g of crushed ice made of deionized water, and the mixture was stirred to give a clear alkali chitin solution. It was cooled in an ice bath, and 75 g (15 mol equiv to pyranose rings) of tosyl chloride dissolved in 200 mL of chloroform was added with vigorous stirring. After 2 h of reaction, the ice bath was replaced by a water bath of around 18 °C, and the mixture was stirred for an additional 2 h. The mixture was poured into a large amount of deionized water with stirring, and the precipitate was washed repeatedly by decantation with deionized water until neutral. The resulting white fibrous product was then washed with methanol and ether and dried to give 7.81 g of a white solid. The degree of tosylation was 0.95 as determined by the S/N ratio of elemental analysis.

In the reactions with 5 mol equiv or less amounts of tosyl chloride, the mixtures were dialyzed, concentrated, and poured into methanol/ether to isolate the products which were partially soluble in water.

(2) *N*-Acetylation of Tosylchitins. To a dispersion of 7.77 g of pulverized tosylchitin obtained above in 180 mL of methanol was added 10.2 mL (5 mol equiv to pyranose rings) of acetic anhydride. The mixture was stirred at room temperature overnight and poured into ice-water. The solid product was filtered, washed successively with methanol, acetone, and ether, and dried to give 7.58 g of a white powder. The acetylated tosylchitin, 7.31 g, was then treated with 164 mL of 0.1 mol/L of methanolic potassium hydroxide at room temperature for 3 h. The product was washed with methanol until free of alkali and then with ether and dried to give 5.26 g of *N*-acetylated tosylchitin as a white powdery material. The degree of tosylation was 0.56, as determined by the S/N ratio: IR (KBr) ν 3400 (OH), 1598 (phenylene), 1663 (amide I), 1540 (amide II), 1176 (SO_2), and 813 (phenylene) cm^{-1}.

Anal. Calcd for $(C_{15}H_{19}NO_7S)_{0.56}(C_8H_{13}NO_4)_{0.44}$: C, 49.45; H, 5.70; N, 4.84; S, 6.20. Found: C, 49.49; H, 5.45; N, 4.77; S, 6.15.

Full Acetylation of Tosylchitins. To a mixture of 0.50 g of tosylchitin with a substitution degree (ds) of 0.64 in 20 mL of pyridine were added 0.2 g of 4-(dimethylamino)pyridine and 2 mL of acetic anhydride. The mixture was stirred at room temperature for 24 h, and the resulting brown solution was poured into ice-water. The precipitate was filtered, washed with water and ethanol, and dried to give 0.30 g of fully acetylated tosylchitin as a white powder: IR (KBr) ν 3400 (NH), 1748 (*O*-acetyl), 1675 (amide I), 1598 (phenylene), 1522 (amide II), 1228 (*O*-acetyl), 1176 (SO_2), and 814 (phenylene) cm^{-1}; ^{13}C NMR (DMSO-d_6) δ 20.5 and 20.8 ($OCOCH_3$ and $NCOCH_3$), 22.3 (tosyl-CH_3), 53.5 (C-2), 68.4-7.31 (C-3-5, and -6), 88.9 (C-4), 100.3 (C-1), 125.6, 128.0, 130.0, and 137.7 (arom), and 170.6 and 170.8 (OCO and NHCO).

Anal. Calcd for $(C_{17}H_{21}NO_8S)_{0.64}(C_{12}H_{17}NO_7)_{0.36}\cdot 0.1H_2O$: C, 50.59; H, 5.52; N, 3.88; S, 5.69. Found: C, 50.36; H, 5.24; N, 3.69; S, 5.96.

Iodination of Tosylchitins. To 0.50 g of tosylchitin with a substitution degree of 0.48 was added 14 mL of DMSO, and the mixture was stirred in a nitrogen atmosphere to give a clear solution. A solution of 2.2 g (10 mol equiv to pyranose rings) of sodium iodide in 6 mL of DMSO was added, and the solution was

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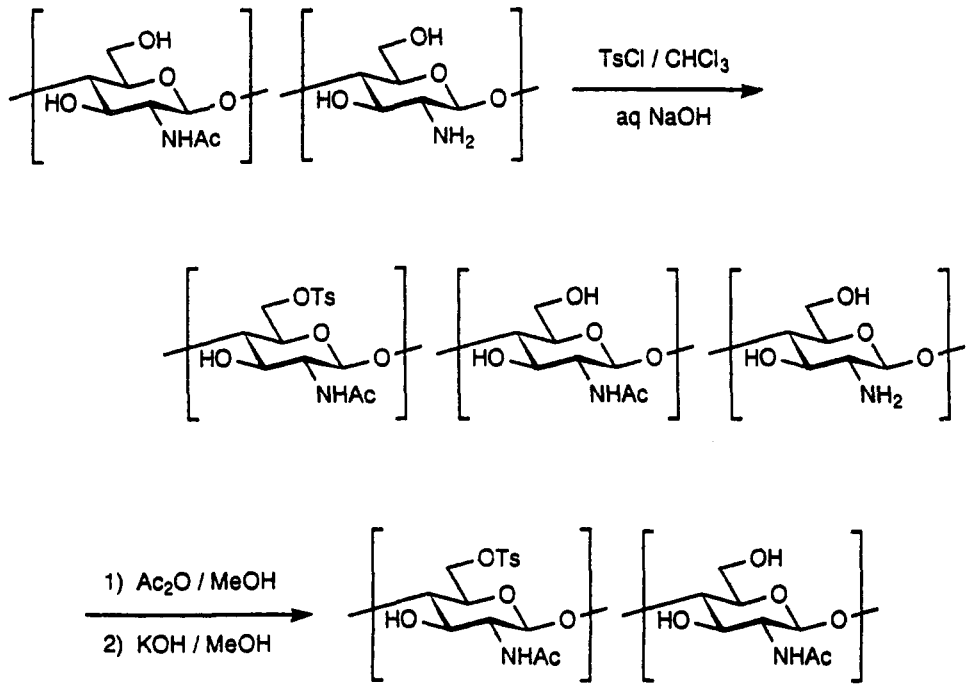
Scheme I



stirred under nitrogen at 85 °C. Heating was discontinued after 24 h, and the solution was poured into acetone. The precipitate was collected on a filter, washed with acetone and ether, and treated with 10 mL of 0.05 mol/L of methanolic potassium hydroxide at room temperature for 3 h. It was washed with methanol and then with ether. On drying, 0.30 g of iodochitin was obtained as a light-tan solid. The degree of substitution was 0.38 as determined by elemental analysis: IR (KBr) ν 3425 (OH), 1655 (amide I), and 1542 (amide II) cm^{-1}; ^{13}C NMR (DMSO-d_6) δ 23.1 (CH_3), 53.0 (C-2), 70.1–71.5 (C-3, -5, and -6), 78.9 (C-4), 98.8 (C-1), and 168.9 (C=C).

Anal. Calcd for $(C_8H_{12}NO_4I)_{0.38}(C_8H_{13}NO_5)_{0.62}$: C, 39.06; H, 5.17; N, 5.69. Found: C, 39.30, H, 5.67; N, 5.44.

Reduction of Tosylchitins to Deoxychitins. A mixture of 0.50 g of tosylchitin with a substitution degree of 0.90 and 0.27 g of sodium borohydride in 10 mL of DMSO was heated at 80 °C for 5 h under nitrogen with stirring. The resulting solution was poured into ethanol to give a cloudy mixture. It was dialyzed against deionized water, concentrated to a small volume under reduced pressure, and poured into acetone. The precipitate was collected by filtration, washed with ethanol and ether, and dried to give 0.16 g of deoxychitin as a white powdery material: IR (KBr) ν 3404 (OH), 1662 (amide I), and 1538 (amide II) cm^{-1}.

Anal. Calcd for $(C_8H_{13}NO_4)_{0.90}(C_8H_{13}NO_5)_{0.10}\cdot 1.3H_2O$: C, 45.27; H, 7.40; N, 6.60. Found: C, 45.20; H, 7.04; N, 6.33.

Results and Discussion

Tosylation of Chitin. Tosylation of chitin was unsuccessful under heterogeneous conditions in DMSO/pyridine. Although some reactions on chitin were much facilitated in homogeneous solutions as in acylation[7,8] and diethylaminoethylation,[9] chitin was insoluble in common inert solvents suitable for tosylation. The reaction was thus performed on alkali chitin by applying the technique of interfacial condensation between an aqueous alkali chitin solution and a chloroform solution of tosyl chloride (Scheme I). The resulting tosylchitins were isolated in water generally or in methanol/ether after dialysis for the samples with low substitutions because of the considerable solubility in water. The high hydrophilicity was developed probably as a result of effective interference with intermolecular hydrogen bonding by a randomly introduced small amount of substituents as in some other modifications of chitin[10,11] and chitosan.[3,12]

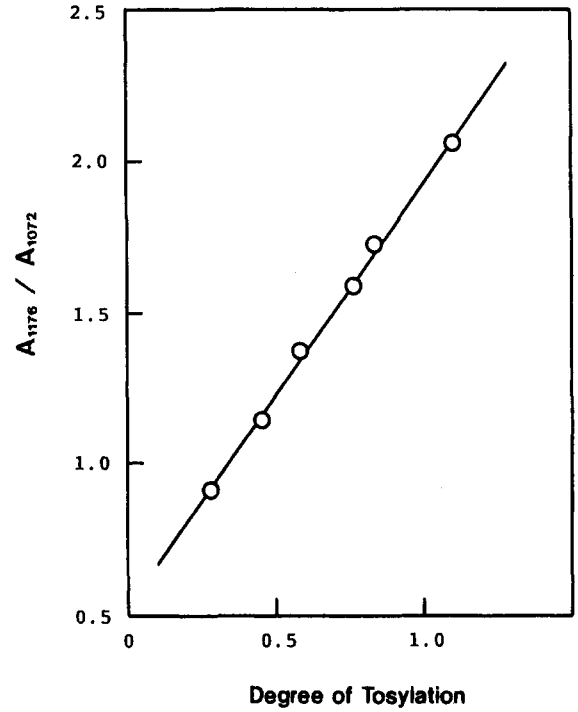


Figure 1. Calibration line to determine the degree of tosylation.

The degree of tosylation was determined by elemental analysis and also by a calibration line based on the relation between the absorbance ratio A_{1176}/A_{1072} (band at 1176 cm^{-1}, tosyl groups; band at 1072 cm^{-1}, pyranose rings) and the degree of substitution calculated by the S/N ratio as shown in Figure 1. The reaction was severely dependent on the stirring efficiency and temperature control because of the rapid reaction at the interface, and vigorous stirring at 0 °C in the initial stage was necessary. When the reaction mixture was allowed to warm to room temperature, the temperature sometimes rose to 40 °C especially with a large amount of tosyl chloride, leading to poor reproducibility and substitution. Cooling resulted in better reproducibility as well as high substitution degrees. The degree of tosylation steadily increased with the amount of tosyl chloride initially but leveled off at a

Table I
Preparation of Tosylchitins

		tosylchitin[b]		tosylchitin[c]									
						calcd				found			
chitin, g	TsCl/pyranose[a]	yield, g	ds[d]	yield, g	ds[e]	C	H	N	S	C	H	N	S
1.05	7	1.18	0.42	0.79	0.28	48.56	6.01	5.69	3.19[f]	49.17	6.12	5.06	3.23
1.05	10	1.55	0.73	0.62	0.56	48.25	5.83	4.72	6.05[g]	48.27	5.72	4.36	5.65
5.03	15	7.81	0.95	5.48	0.56	49.45	5.70	4.84	6.20[h]	49.49	5.45	4.77	6.15
1.01	20	1.68	1.01	1.03	0.70	49.80	5.57	4.50[i]		50.30	5.50	4.49	

[a] Molar ratio. [b] Tosylchitins before N-acetylation. [c] Tosylchitins after N-acetylation and alkaline treatment. [d] Degree of substitution determined by the calibration line. [e] Degree of substitution determined by elemental analysis. [f] $(C_{15}H_{19}NO_7S)_{0.28}(C_8H_{13}NO_5)_{0.72}$. [g] $(C_{15}H_{19}NO_7S)_{0.56}(C_8H_{13}NO_5)_{0.44}\cdot 0.4H_2O$. [h] $(C_{15}H_{19}NO_7S)_{0.56}(C_8H_{13}NO_5)_{0.44}$. [i] $(C_{15}H_{19}NO_7S)_{0.70}(C_8H_{13}NO_5)_{0.30}$.

substitution degree of 1 as summarized in Table I. This is reasonably ascribable to the preferential substitution at the C-6 position as revealed in carboxymethylation.[13] Hydroxyl groups are generally much more nucleophilic than amino groups under these strongly alkaline conditions as evident in the tosylation of aminophenols[14] and some other substitutions,[1] and thus *N*-toslylation, if it occurs, is considered to be of a low level. In the tosylation with 10 equiv or more of tosyl chloride, a weak shoulder at 1750 cm^{-1} was sometimes observed in the IR spectra, suggesting some wide reactions. This could be due to possible acetyl migration, since it disappeared easily on treatment with dilute alkali.

During the tosylation, alkaline hydrolysis of the acetamide groups and sometimes acetyl migration took place; while the degree of deacetylation of the original starting chitin was 0.18, those of tosylchitins were 0.25–0.29. In order to prepare tosylchitins with well-defined structures, free amino groups were acetylated with acetic anhydride in methanol. The N-acetylation was, however, not perfectly selective as indicated by a weak shoulder at 1750 cm^{-1} due to *O*-acetyl groups. The products were therefore treated with alkali to cleave the ester linkages. This alkali treatment, however, also removed tosyl groups to some extent, and even with alkali of low concentrations, the degree of tosylation decreased as listed in Table I.

The structures of tosylchitins obtained here were confirmed by elemental analysis and IR spectroscopy. IR spectra showed a characteristic absorption band at 1176 cm^{-1} attributable to tosyl groups. Absorption bands at 1598 and 831 cm^{-1} due to *p*-phenylene groups also supported the presence of tosyl groups as shown in Figure 2.

Tosylchitins were obtained as white fibrous or powdery materials in high yields. Qualitative solubility tests showed that the products with substitution degrees less than 0.3 were highly hydrophilic and partially soluble in water immediately after preparation. After drying, they were not soluble but still swelled in water. The tosylchitins with substitution degrees above 0.4 became hydrophobic and were soluble in common polar organic solvents as summarized in Table II. The solubility appeared to be much higher than that of tosylated cellulose prepared in pyridine under harsh heterogeneous conditions. The tosylcellulose with a substitution degree of 1.28 was, for example, insoluble in DMSO and only swelled.[15] The high solubility of tosylchitins prepared here is reasonably interpreted in terms of the randomness of substitution achieved by solution reaction as suggested by solubilization of chitosan by partial acetylation.[3] The X-ray diffraction diagrams showed that tosylation drastically lowered the crystallinity of the original chitin, and even the tosylchitins with low substitution degrees were amorphous. High solubility brought about by tosylation is ascribable in part to the destruction of the crystalline

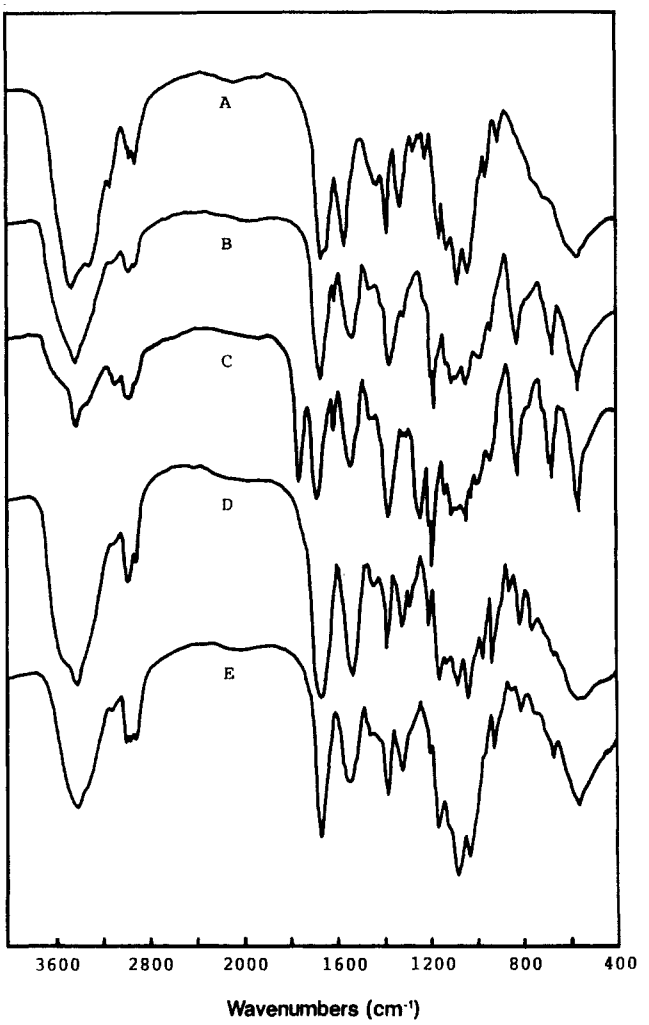


Figure 2. IR spectra of chitin and the derivatives (KBr method): (A) chitin, (B) tosylchitin (degree of substitution 0.56), (C) fully acetylated tosylchitin prepared from tosylchitin with a degree of substitution 0.77, (D) iodochitin (degree of substitution 0.38), (E) deoxychitin prepared from tosylchitin with a degree of substitution 0.90.

structure. Transparent films of tosylchitins could be cast from *N,N*-dimethylformamide (DMF) solution.

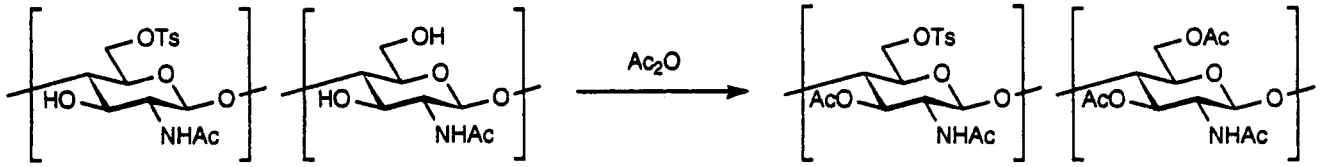
Full Acetylation of Tosylchitins. The remaining hydroxyl groups were then acetylated to confirm the reactivity and also to make possible further characterization of tosylchitins. The acetylation was attempted with acetic anhydride in pyridine under various conditions, but full acetylation was difficult, implying poor reactivity of the C-3 hydroxyl groups. Addition of 4-(dimethylamino)pyridine, a much superior catalyst to pyridine for *O*-acylation,[16] facilitated the reaction greatly, resulting in the formation of fully acetylated tosylchitins (Scheme II). The structures of the products were supported by elemental analysis and spectroscopic data. In the IR spectra, strong

Table II
Solubility Data of Tosylchitins and the Derivatives

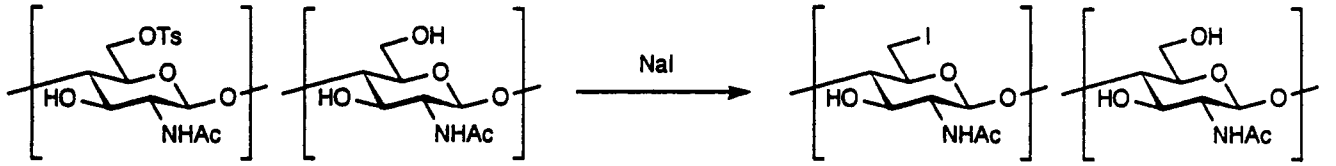
	solubility[a]								
	DMSO	DMAc	NMP	HMPA	HCOOH	$CHCl_3$	THF	MeOH	H_2O
tosylchitin (ds 0.29)	±	●	●	±	●	±	±	–	±
tosylchitin (ds 0.43)	+	+	+	●	+	±	±	±	–
tosylchitin (ds 0.74)	+	+	+	+	+	±	±	±	–
iodochitin (ds 0.38)	+	+	+	+	+	±	±	+	●
acetylated tosylchitin[b]	+	+	+	+	+	±	±	–	●
deoxychitin[c]	±	±	±	±	+	±	±	±	●

[a] DMSO, dimethyl sulfoxide; DMAc, *N,N*-dimethylacetamide; NMP, *N*-methyl-2-pyrrolidone; HMPA, hexamethylphosphoramide; +, soluble; ±, partially soluble or swelled; –, insoluble. [b] Fully acetylated tosylchitin prepared from tosylchitin with ds 0.77. [c] Prepared from tosylchitin with ds 0.90.

Scheme II



Scheme III



bands due to ester were observed at 1748 and 1228 cm^{-1} in addition to the peaks characteristic of tosylchitins as illustrated in Figure 2. Although the products are soluble in DMSO, the solution is highly viscous, which makes it difficult to take clear ^{13}C NMR spectra even at elevated temperatures as in the case of other chitin derivatives. A typical example in Figure 3 shows peaks due to acetyl methyls at around 21 ppm, tosyl methyls at 22 ppm, pyranose rings at 54–100 ppm, aromatic rings at 126–138 ppm, and carbonyls at 171 ppm. The acetylated products also showed a high solubility similar to that of tosylchitins as included in Table II. They gave transparent films by solution casting from DMF solutions.

Iodination of Tosylchitins. Tosylchitins obtained here are expected to show a high reactivity in addition to a high solubility and should be useful as precursors for controlled chemical manipulations. Among many possibilities, halogenation is considered of importance in view of the versatility of iodosugars in sugar synthetic chemistry, and, moreover, no halogenated chitins have been reported.

Tosylchitins were thus transformed to iodochitins that would also be useful for a variety of derivatizations. They were subjected to reaction with sodium iodide in a DMSO solution, and replacement of tosyloxy groups by iodo groups took place efficiently due to the solubility of both the starting material and the product. Although the reaction was complete in a few hours at 100–110 °C, the products assumed a dark-brown color. Replacement was not complete after 24 h at 60 °C even with a large excess of sodium iodide. At 85 °C, the reaction proceeded smoothly to give the expected iodochitins (Scheme III). The sulfur content of the product was 0.6–0.7% after 6 h, and it decreased to less than 0.1% after 24 h. Complete substitution was also confirmed by the negative result of qualitative analysis for sulfur.[17] The products, however, sometimes showed a weaker band at 1750 cm^{-1} in the IR spectra, implying some acetyl migration, and were thus treated with alkali.

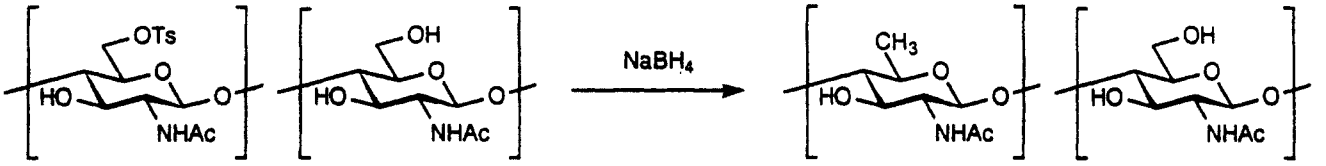
The IR spectra supported the absence of tosyl groups since the characteristic bands at 1598, 1176, and 813 cm^{-1} had disappeared completely as evident in Figure 2. The ^{13}C NMR spectra also indicated removal of tosyl groups as shown in Figure 3. The peaks due to C-1 to C-5 of pyranose rings are found at ordinary positions. No evident peak is, however, found at around 62 ppm where the C-6 peak usually appears,[18] probably because of a shift to the lower field on account of iodination leading to overlapping of the peak with C-3 and C-5 peaks. This again supports that C-6 is the favorable position for tosylation.

Iodochitins were obtained as pale-yellow to tan powdery materials in moderate to high yields. They showed a somewhat higher solubility than tosylchitins and were readily soluble in common solvents such as methanol as shown in Table II. The degrees of iodination determined by elemental analysis were generally lower that those of tosylation by 0.1–0.2 probably as a result of partial removal of the iodo groups in the alkaline treatment process. DMF solutions of iodochitins were cast to give transparent films.

Reduction of Tosyl- or Iodochitins. Since both tosyloxy and iodo groups are excellent leaving groups and, moreover, tosyl- and iodochitins showed solubility in organic solvents unlike chitin, these derivatives should be versatile reactive intermediates for chemical modification. Reduction of these derivatives was studied for preparing a novel type of chitin derivatives, deoxychitins.

Tosylchitins were reduced with sodium borohydride as in the reduction of tosyl- and iodosugars.[19,20] The reaction proceeded smoothly in homogeneous solution at 80 °C (Scheme IV). Complete reduction was achieved in 5 h as indicated by the absence of sulfur.[17] Elemental analysis supported the quantitative reduction to deoxychitins. In the IR spectra, bands due to tosyl groups disappeared completely. The spectra were quite close to that of chitin itself except that the bands due to hydroxyl groups, especially the one at 3400 cm^{-1}, became weak. Figure 2 includes a typical example of the IR spectra of the reduced products. Iodochitins also yielded similar products. The resulting deoxychitins were soluble in formic acid and showed high swelling in organic solvents due to a substantial decrease in the hydrogen bonding by the C-6 hy-

Scheme IV



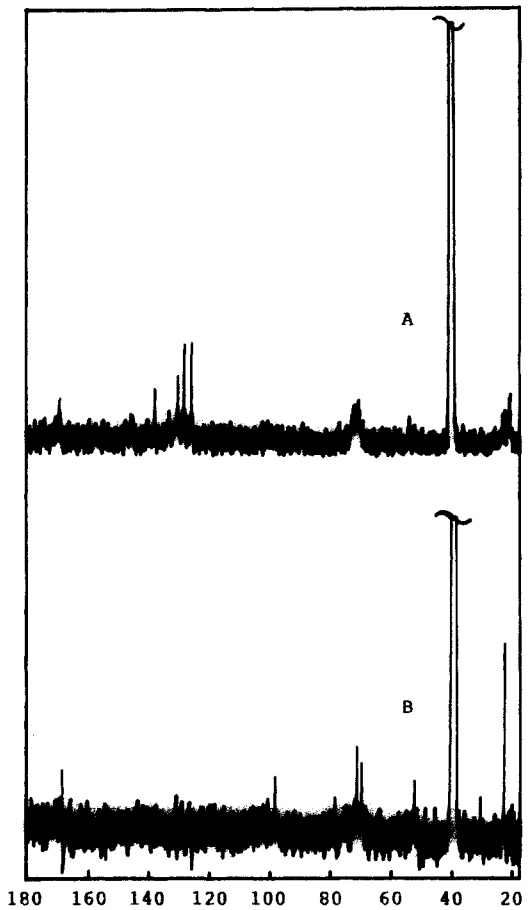


Figure 3. ^{13}C NMR spectra in DMSO-d_6: (A) fully acetylated tosylchitin prepared from tosylchitin with a degree of substitution 0.77; (B) iodochitin (degree of substitution 0.58).

droxyl groups. The solubility data are included in Table II.

Conclusions

A simple preparative procedure for tosylchitins has been established. The products are suitable precursors for controlled chemical manipulations due to the high solubility and reactivity and have been transformed efficiently to acetylated tosylchitins, iodochitins, and deoxychitins. These derivatives are also considered of interest from the viewpoint of the possibilities of a wide variety of modifications as well as physical properties characteristic of rigid polysaccharides soluble in organic solvents.

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