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N-Alkylation of chitin and some characteristics of the novel derivatives

Keisuke Kurita (✉), Satoko Mori, Yasuhiro Nishiyama, Manabu Harata*

Department of Applied Chemistry, Faculty of Engineering, Seikei University, Musashino-shi, Tokyo 180-8633, Japan
Tel.: +81-422-37-3745; Fax: +81-422-37-3871; e-mail: kurita@ch.seikei.ac.jp

* *Present address:* Department of Pathology and Laboratory Medicine, University of Texas-Houston Medical School, Houston, Texas 77030, USA

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Summary

Introduction of simple alkyl groups at the C-2 nitrogen of chitin and some properties of the resulting *N*-alkyl-chitins have been examined. Chitosan was fully deacetylated and treated with three kinds of aldehydes, formaldehyde, acetaldehyde, and pentanal. The Schiff bases of chitosan, whose extents of substitution were dependent on the amount of aldehydes, were reduced with sodium cyanoborohydride to N-alkylated chitosans. The *N*-alkyl-chitosans were then transformed into the corresponding *N*-alkyl-chitins by acetylation with acetic anhydride followed by transesterification to remove partly formed *O*-acetyl groups. The resulting *N*-methyl-, ethyl-, and pentyl-chitins were amorphous and showed improved affinity for organic solvents.

Introduction

Despite its extraordinary abundance in nature and distinctive biological activities, chitin remains an unutilized biomass resource primarily because of the lack of solubility in solvents except some special ones such as fluorinated solvents, *N*,*N*-dimethylacetamide/LiCl (1), and methanol/$CaCl_2$ (2). Solubilization would be accomplished by loosening the tight arrangement of chitin molecules and thereby preventing the strong intermolecular forces, and partial N-deacetylation or introduction of bulky groups was found to be effective. Randomly 50% deacetylated chitin (3) and chitin derivatives having tosyl, iodo (4), trimethylsilyl (5) and glycosyl groups (6) are, for example, soluble in water or common organic solvents. Chitosan derivatives, too, exhibit solubility when they are appropriately substituted as exemplified by N-phthaloylation (7) and 50% N-acetylation (8). Theses derivatives have proved important as soluble precursors for further modification reactions.

To use chitin derivatives as precursors for modifications and as model chitin analogues, those having simple substituents are often desirable in view of the close resemblance to chitin. In this respect, N-alkylation of chitin is expected to be practical for preparing simple chitin analogues with lowered crystallinity as well as for improving solvent affinity, since the resulting structure around C-2 is analogous to that of *N*,*N*-dimethylacetamide, an extremely good solvent exhibiting high affinity for a wide variety of substances. An attempt was made to synthesize polymers having *N*,*N*-dimethylacetamide moieties in the backbones by ring-opening polymerization of 2-oxazolines, and actually the resulting polymers showed high affinity for solvents (9). Here we report on some preliminary results of the introduction of methyl, ethyl, and

pentyl groups at the C-2 nitrogen of chitin and the influence on crystallinity and solubility.

Experimental

General

Pulverized chitin from shrimp shells was treated with 40% aqueous sodium hydroxide at 110 °C for 5 h in nitrogen, and the product was washed with deionized water. The deacetylation procedure was repeated two more times to give an almost colorless powdery chitosan, whose degree of deacetylation was 1.0 as determined by conductometric titration. Formaldehyde (36% aqueous solution), acetaldehyde (80% aqueous solution), pentanal (>97%), and sodium cyanoborohydride were used as received.

IR spectra were recorded on a Shimadzu FTIR-8900 spectrometer by the KBr method. Elemental analysis was carried out with a Perkin-Elmer 2400 instrument. X-ray diffraction diagrams were obtained by the powder method with the use of Ni-filtered Cu Kα radiation with a MAC Science M03X-HF 1013 instrument. The degree of substitution (ds) of derivatives was calculated principally from the C/N value of elemental analysis.

N-Pentylidene-chitosan

Fully deacetylated chitosan (0.50 g, 3.1 mmol pyranose) was dissolved in 15 mL of 2% aqueous acetic acid, and 15 mL of methanol was added. To the solution was added 2.67 g (31.0 mmol) of pentanal in 10 mL of methanol, and the mixture was stirred at room temperature for 24 h. The resulting white gel-like mixture was broken with a spatula, washed with 100 mL of acetone, and filtered. The product was pulverized, washed with 100 mL of acetone for 30 min with stirring, and filtered. It was then treated with 200 mL of 5% aqueous sodium hydrogen carbonate for 10 min at room temperature, filtered, and washed with deionized water repeatedly until neutral. After subsequent washing with 200 mL of methanol overnight, the product was filtered and dried under vacuum to give 0.573 g of a Schiff base (**1i**) as a white powdery material. IR (KBr): ν 3420 (OH), 2960, 2930, and 2870 (CH), 1635 (C=N), and 1150-1000 cm^{-1} (pyranose).

N-Pentyl-chitosan

N-Pentylidene-chitosan **1i** (0.10 g, 0.43 mmol pyranose based on ds 1.0) obtained above was dispersed in 10 mL of methanol, and the dispersion was stirred for 10 min. A solution of 0.027 g (0.43 mmol) of sodium cyanoborohydride in 10 mL of water was added, and the mixture was stirred at room temperature for 24 h. Filtration of the mixture gave a white powdery material, which was washed with methanol and dried to yield 77 mg of *N*-pentyl-chitosan (**2i**). IR (KBr): ν 3425 (OH and NH), 2960, 2930, and 2870 (CH), 1620 (NH), and 1150-1000 cm^{-1} (pyranose).

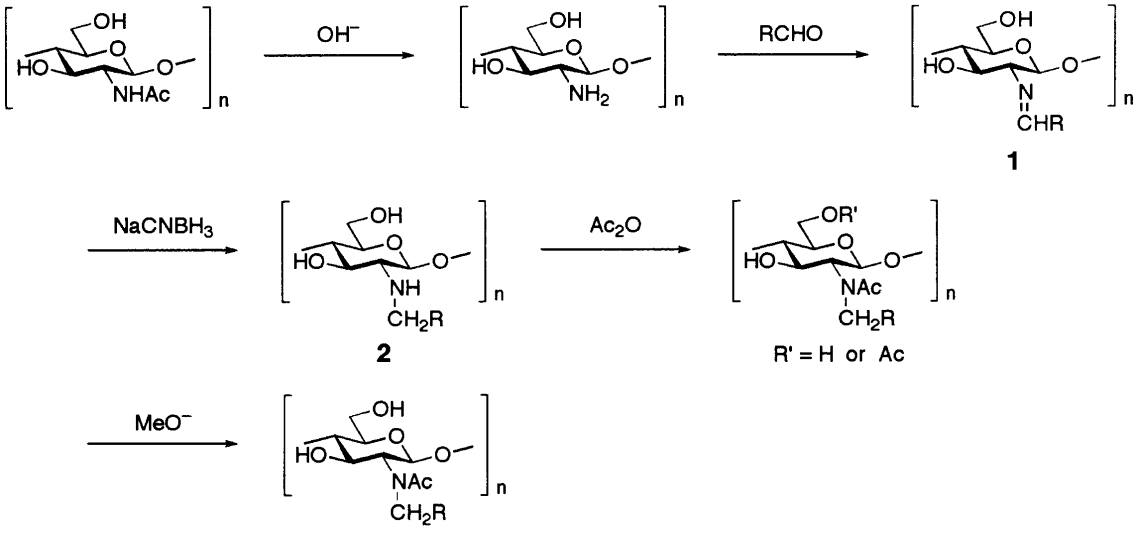
N-Pentyl-chitin

A dispersion of 60 mg (0.26 mmol pyranose based on ds 0.89) of *N*-pentyl-chitosan **2i** in 10 mL of pyridine was heated at 100 °C for 5 h in nitrogen for swelling. It was cooled to room temperature, and 0.83 g (8.0 mmol) of acetic anhydride was added. After stirring at room temperature for 24 h, the mixture was concentrated under reduced pressure, and 100 mL of methanol was added. The solid was collected by centrifugation and washed with methanol overnight. It was filtered, dried, and dispersed in 10 mL of dry methanol. Sodium metal (60 mg) was added to the dispersion, and the mixture was stirred at room temperature for 24 h in a nitrogen atmosphere. The mixture was filtered, and the product was washed thoroughly with methanol several times. On drying, 50 mg of *N*-pentyl-chitin (**3i**) was obtained as an off-white powdery material. IR (KBr): ν 3430 (OH), 2960,

2930, and 2870 (CH), 1661 and 1636 (amide I), 1556 (amide II), and 1150-1000 cm^{-1} (pyranose).

Results and discussion

Because of the difficulty in introducing alkyl groups directly at the C-2 nitrogen of chitin, monoalkylation of chitosan followed by N-acetylation seemed to be promising. N-Substitution of chitosan could be conveniently achieved by treatment with sugar aldehydes in the presence of a reducing agent, giving rise to branched products (10). In a similar manner, reductive methylation with formaldehyde and sodium borohydride resulted in a high extent of substitution up to N,N-dimethylation (11). To ensure introduction of only one alkyl group to the amino functionality of chitosan, therefore, Schiff bases should be isolated and subsequently reduced to prepare N-monoalkylated chitosans. *N*-Alkyl-chitins were thus prepared from chitin by a series of modification reactions involving full deacetylation to chitosan, Schiff base formation with aldehydes, reduction, and N-acetylation (Scheme 1).



Scheme 1.

Schiff base formation

The reaction of fully deacetylated chitosan with three kinds of aldehydes, formaldehyde, acetaldehyde, and pentanal, was thus conducted in a mixed solvent of aqueous acetic acid and methanol. With equimolar aldehydes, the reaction mixtures remained viscous solutions. When 5 or 10 equivalents of aldehydes were used, however, the mixtures became gels in the course of the reaction. The resulting Schiff base derivatives were washed with acetone and aqueous alkali to remove excess aldehydes and acetic acid, affording white powdery products (**1a-1i**).

As summarized in Table 1, the extents of substitution based on the C/N value of elemental analysis increased with an increase in the amount of aldehydes. When the reaction was performed with 10 equivalents of aldehydes, however, the calculated ds values of the products **1c**, **1f**, and **1i** were a little above 1.0 (1.04-1.18) most likely because of the difficulty in thorough removal of excess aldehydes by washing from the rather dense gels. Calculated analysis values of these products in Table 1 were thus based on the full substitution, 1.0. The IR spectra showed a band at around 1630 cm^{-1} due to C=N. Furthermore, it is noteworthy that the spectrum of a product from pentanal had especially strong C-H bands at 2870-2960 cm^{-1} owing to the introduction of pentylidene groups. A typical spectrum is shown in Figure 1.

Table 1. Schiff base formation from chitosan and aldehydes[a]

Aldehyde	-CHO/-NH_2[b]	Schiff base		
		ds[c]	Yield (%)[d]	Product no.
HCHO	1	0.45[e]	67	**1a**
HCHO	5	0.62[f]	73	**1b**
HCHO	10	1.00[g]	83	**1c**
CH_3CHO	1	0.07[h]	57	**1d**
CH_3CHO	5	0.39[i]	56	**1e**
CH_3CHO	10	1.00[j]	83	**1f**
$CH_3(CH_2)_3CHO$	1	0.13[k]	72	**1g**
$CH_3(CH_2)_3CHO$	5	0.70[l]	87	**1h**
$CH_3(CH_2)_3CHO$	10	1.00[m]	80	**1i**

[a]Chitosan, 0.50 g; solvent, 2% AcOHaq (15 mL)/MeOH (25 mL); time, 24 h at room temperature. [b]Mole ratio. [c]Degree of substitution determined from the C/N value of elemental analysis. For g, j, and m, see Results and discussion section. [d]Calculated on the basis of the ds value. [e]Calcd for $(C_7H_{11}NO_4)_{0.45}(C_6H_{11}NO_4)_{0.55}\cdot 0.6H_2O$: C, 43.68; H, 6.93; N, 7.90. Found: C, 43.57; H, 7.88; N, 7.88. [f]Calcd for $(C_7H_{11}NO_4)_{0.62}(C_6H_{11}NO_4)_{0.38}\cdot H_2O$: C, 42.61; H, 7.02; N, 7.51. Found: C, 42.62; H, 7.42; N, 7.52. [g]Calcd for $C_7H_{11}NO_4\cdot H_2O$: C, 43.98; H, 6.85; N, 7.33. Found: C, 44.01; H, 7.56; N, 7.29. [h]Calcd for $(C_8H_{13}NO_4)_{0.07}(C_6H_{11}NO_4)_{0.93}\cdot 0.7H_2O$: C, 42.00; H, 7.20; N, 7.98. Found: C, 42.19; H, 7.66; N, 8.02. [i]Calcd for $(C_8H_{13}NO_4)_{0.39}(C_6H_{11}NO_4)_{0.61}\cdot 1.1H_2O$: C, 42.61; H, 7.37; N, 7.33. Found: C, 42.70; H, 7.51; N, 7.34. [j]Calcd for $C_8H_{13}NO_4\cdot 1.3H_2O$: C, 45.62; H, 7.47; N, 6.65. Found: C, 45.73; H, 7.83; N, 6.37. [k]Calcd for $(C_{11}H_{19}NO_4)_{0.13}(C_6H_{11}NO_4)_{0.87}\cdot 0.7H_2O$: C, 43.74; H, 7.42; N, 7.67. Found: C, 43.74; H, 7.16; N, 7.69. [l]Calcd for $(C_{11}H_{19}NO_4)_{0.70}(C_6H_{11}NO_4)_{0.30}\cdot 0.6H_2O$: C, 51.95; H, 8.17; N, 6.38. Found: C, 51.74; H, 8.57; N, 6.34. [m]Calcd for $C_{11}H_{19}NO_4\cdot 0.3H_2O$: C, 56.30; H, 8.42; N, 5.97. Found: C, 56.75; H, 8.87; N, 5.53.

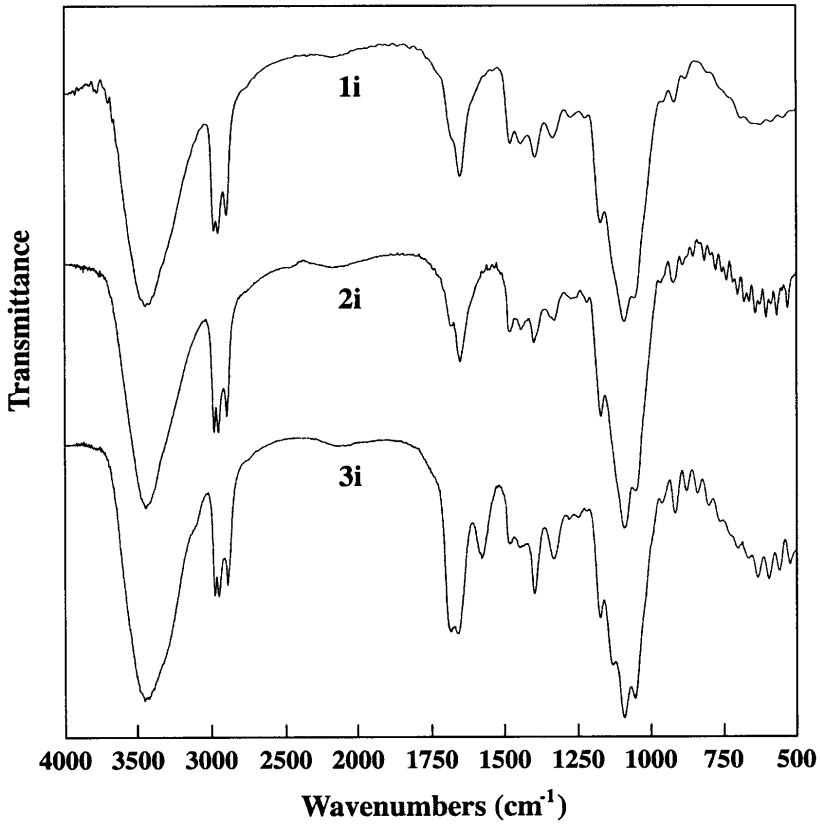


Figure 1. IR spectra of **1i**, **2i**, and **3i** (KBr method).

Reduction of Schiff bases

The Schiff bases **1a – 1i** prepared above were reduced with sodium cyanoborohydride, whose amount corresponded to the molar amount of pyranose units, in a mixed solvent of methanol and water (Scheme 1). The reactions proceeded under heterogeneous conditions, and the resulting *N*-alkyl-chitosans (**2a – 2i**) were obtained as white powdery materials.

The IR spectra of **2** were similar to that of chitosan, but as observed in the spectrum of *N*-pentyl-chitosan **2i** in Figure 1, C-H stretching bands at around 2900 cm^{-1} are quite evident. The ds values were close to those of the starting Schiff bases as listed in Table 2, supporting that the reduction was effected almost quantitatively under these conditions.

Table 2. Reduction of Schiff bases to *N*-alkyl-chitosans[a]

	N-Alkyl-chitosan		
Schiff base	ds[b]	Yield (%)[c]	Product no.
1a	0.37[d]	85	**2a**
1b	0.60[e]	76	**2b**
1c	0.99[f]	79	**2c**
1d	0.09[g]	60	**2d**
1e	0.37[h]	78	**2e**
1f	0.99[i]	77	**2f**
1g	0.11[j]	74	**2g**
1h	0.45[k]	96	**2h**
1i	0.89[l]	78	**2i**

[a]Schiff base, 0.10 g; reducing agent, $NaCNBH_3$; solvent, MeOH (10 mL)/water (10 mL); time, 24 h at room temperature. [b]Degree of substitution determined from the C/N value of elemental analysis. [c]Calculated on the basis of the ds value. [d]Calcd for $(C_7H_{13}NO_4)_{0.37}(C_6H_{11}NO_4)_{0.63}\cdot 0.5H_2O$: C, 43.63; H, 7.32; N, 7.99. Found: C, 43.69; H, 7.88; N, 8.00. [e]Calcd for $(C_7H_{13}NO_4)_{0.60}(C_6H_{11}NO_4)_{0.40}\cdot H_2O$: C, 42.26; H, 7.63; N, 7.47. Found: C, 42.39; H, 7.51; N, 7.49. [f]Calcd for $(C_7H_{13}NO_4)_{0.99}(C_6H_{11}NO_4)_{0.01}\cdot 1.3H_2O$: C, 42.33; H, 7.92; N, 7.05. Found: C, 42.19; H, 7.17; N, 7.04. [g]Calcd for $(C_8H_{15}NO_4)_{0.09}(C_6H_{11}NO_4)_{0.91}\cdot 0.7H_2O$: C, 42.11; H, 7.30; N, 7.95. Found: C, 42.20; H, 7.28; N, 7.96. [h]Calcd for $(C_8H_{15}NO_4)_{0.37}(C_6H_{11}NO_4)_{0.63}\cdot 1.1H_2O$: C, 42.31; H, 7.73; N, 7.32. Found: C, 42.13; H, 7.06; N, 7.30. [i]Calcd for $(C_8H_{15}NO_4)_{0.99}(C_6H_{11}NO_4)_{0.01}\cdot 1.1H_2O$: C, 45.92; H, 8.29; N, 6.70. Found: C, 45.80; H, 7.82; N, 6.69. [j]Calcd for $(C_{11}H_{21}NO_4)_{0.11}(C_6H_{11}NO_4)_{0.89}\cdot 0.8H_2O$: C, 42.92; H, 7.54; N, 7.64. Found: C, 42.95; H, 7.02; N, 7.67. [k]Calcd for $(C_{11}H_{21}NO_4)_{0.45}(C_6H_{11}NO_4)_{0.55}\cdot 0.5H_2O$: C, 49.12; H, 8.25; N, 6.94. Found: C, 49.33; H, 8.17; N, 6.97. [l]Calcd for $(C_{11}H_{21}NO_4)_{0.89}(C_6H_{11}NO_4)_{0.11}\cdot 0.5H_2O$: C, 53.97; H, 9.06; N, 6.02. Found: C, 53.83; H, 8.70; N, 6.05.

N-Acetylation of N-alkyl-chitosans

To convert the *N*-alkyl-chitosans into *N*-alkyl-chitins, acetylation was carried out with **2c**, **2f**, and **2i** prepared with 10-fold excess aldehydes. The reaction with three equivalents of acetic anhydride in pyridine proceeded facilely at room temperature. The IR spectra of the products showed, however, weak to medium ester bands at 1740 and 1250 cm^{-1} in addition to the amide I and II bands characteristic of chitin, suggesting partial acetylation of hydroxy functionalities. The *O*-acetyl groups were thus removed by transesterification in methanol with sodium methoxide (Scheme 1), and the O-deacetylation was confirmed by disappearance of the ester bands in the IR spectra.

The resulting *N*-alkyl-chitins (**3c**, **3f**, and **3i**) were obtained as white to off-white powdery materials, and the ds values were up to 0.69 as shown in Table 3. The yields

were generally high in spite of small-scale reactions. The IR spectra were similar to that of chitin, and the amide I band was observed at around 1650 cm^{-1}. Chitin usually has a broad amide I band composed of a band at 1660 cm^{-1} and a rather strong shoulder at around 1640 cm^{-1} due to the presence of free and hydrogen-bonded amide carbonyls, respectively. It is noteworthy that **3i** exhibits the corresponding bands as two distinct ones as observed in Figure 1, whereas **3c** and **3f** showed a broad band similar to chitin. The C-H bands are again quite strong in the spectrum of **3i**.

Crystallinity of the products was examined by X-ray diffractiometry. As expected, the introduction of alkyl groups markedly lowered the crystallinity of the original chitin, and the diagrams in Figure 2 indicate that the alkylated chitins were amorphous in sharp contrast to crystalline chitin. Figure 2, however, implies that **3i** might have a somewhat ordered structure, though to only a low extent, compared to **3c** and **3f**.

Table 3. Acetylation of *N*-alkyl-chitosans to *N*-alkyl-chitins[a]

	N-Alkyl-chitin		
N-Alkyl-chitosan	ds[b]	Yield (%)[c]	Product no.
2c	0.69[d]	87	**3c**
2f	0.30[e]	95	**3f**
2i	0.63[f]	74	**3i**

[a]Acetylation with acetic anhydride (*N*-alkyl-chitosan, 60 mg; time, 24 h at room temperature) followed by transesterification with sodium methoxide (methanol, 10 mL; time, 24 h at room temperature). [b]Degree of substitution determined from the C/N value of elemental analysis. [c]Calculated on the basis of the ds value. [d]Calcd for $(C_9H_{15}NO_5)_{0.69}(C_8H_{13}NO_5)_{0.31}\cdot 1.9H_2O$: C, 42.24; H, 7.42; N, 5.67. Found: C, 42.17; H, 6.83; N, 5.66. [e]Calcd for $(C_{10}H_{17}NO_5)_{0.30}(C_8H_{13}NO_5)_{0.70}\cdot 0.9H_2O$: C, 45.34; H, 7.08; N, 6.15. Found: C, 45.52; H, 7.64; N, 6.17. [f]Calcd for $(C_{13}H_{23}NO_5)_{0.63}(C_8H_{13}NO_5)_{0.37}\cdot 0.4H_2O$: C, 52.60; H, 7.96; N, 5.50. Found: C, 52.79; H, 8.38; N, 5.51.

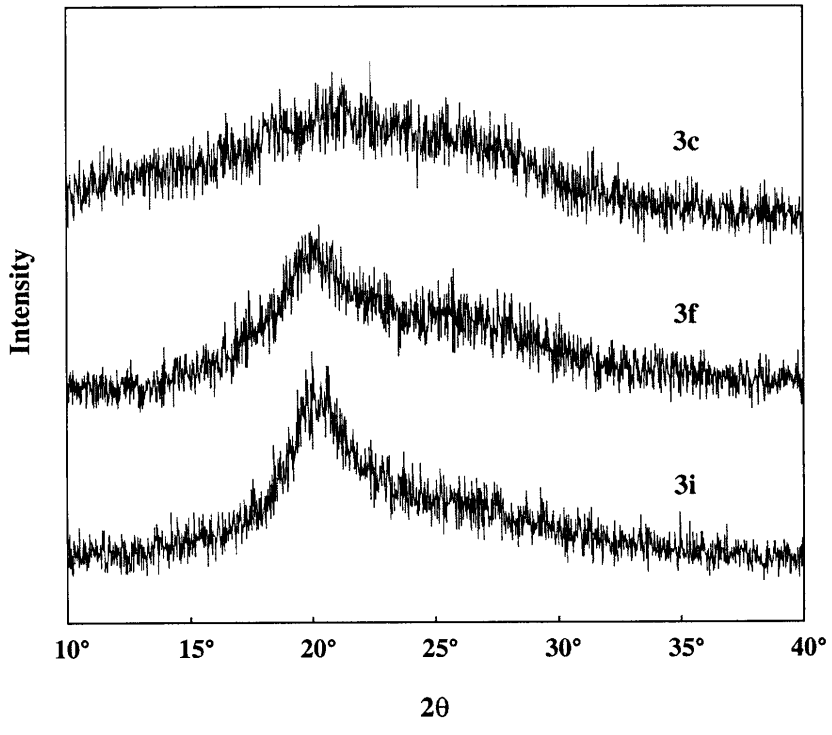


Figure 2. X-ray diffraction diagrams of *N*-alkyl-chitins.

A qualitative solubility test in some solvents revealed that N-alkylation improved affinity for solvents as evidenced in Table 4. Pentyl groups proved particularly effective

for enhancing the solubility compared to methyl and ethyl groups. Although the solubility is still limited, *N*-pentyl-chitin was soluble in dichloroacetic acid and swelled considerably in polar aprotic solvents such as dimethyl sulfoxide, *N,N*-dimethylacetamide, and pyridine.

Table 4. Solubility of **1**, **2**, and **3**[a]

	Schiff base			*N*-Alkyl-chitosan			*N*-Alkyl-chitin		
Solvent[b]	**1c**	**1f**	**1i**	**2c**	**2f**	**2i**	**3c**	**3f**	**3i**
$CHCl_3$	–	–	±	–	–	±	–	–	±
Pyridine	–	–	±	–	–	±	±	±	±
DMSO	–	–	–	–	–	–	–	–	±
DMAc	–	–	–	–	–	–	±	±	±
DCA	±	±	±	±	±	±	±	±	+

[a]+, soluble; ±, swollen; –, insoluble. [b]DMSO, dimethyl sulfoxide; DMAc, *N,N*-dimethylacetamide; DCA, dichloroacetic acid.

Conclusions

Alkyl groups such as methyl, ethyl, and pentyl groups were successfully introduced into chitin at the nitrogen of C-2 acetamido moiety through controlled 5-step modification reactions, key intermediates being *N*-monoalkyl-chitosans that were converted into *N*-alkyl-chitins by N-acetylation. This synthetic route is straightforward and efficient to provide well-defined novel chitin derivatives. The introduction of such alkyl groups was quite effective for destroying the crystalline structure of chitin. The influence is also apparent in the improved affinity for solvents. These results suggest the high possibility of *N*-alkyl-chitins as simple and tractable chitin analogues. A further study on the fine control of substitution including the choice of alkyl groups will enable to expand the scope of sophisticated modification reactions as well as to develop advanced functional materials based on the specialty biopolymer chitin.

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