Preparation and Thermal Dehydration of *N*-(Carboxy)acyl Chitosan Derivatives with High Stereoregularity

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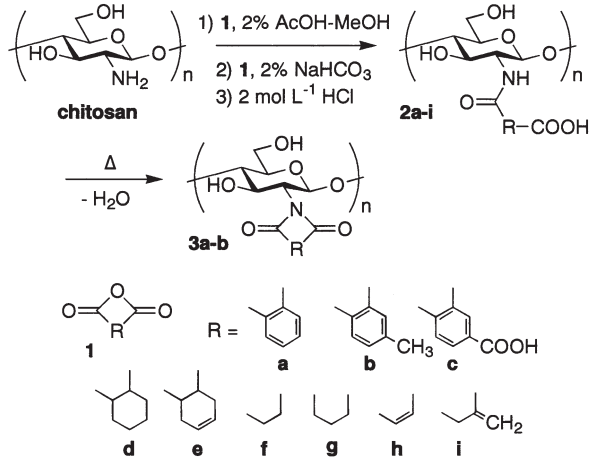
Complete N-acylation of chitosan has been achieved by treating with cyclic acid anhydrides in aqueous homogeneous media at pH 4 to 8. Some of the resulting *N*-(carboxy)acyl chitosans were successfully converted into the corresponding imido forms by thermal dehydration.

Chitosan is an amino polysaccharide obtained from a naturally abundant biopolymer, chitin. Chitosan, having such notable features as high structural regularity, multifunctionality, and chirality, has been regarded as a source of various functional materials, e.g., chiral selector[1] and liquid crystals.[2] To synthesize these chitosan-based materials, its precise and site specific reactions have been investigated.[3] However, its extremely low solubility in organic solvents and water resulted in limited yields, low selectivities, and irregular structures of the products. Although acylation of chitosan under homogeneous conditions were performed only in acidic aqueous media[4,5] or in dimethylsulfoxide (DMSO) containing (*1R*)-(−)-10-camphor-sulfonic acid,[6] the products in the former have incomplete N-substitution and those in the latter have *N*,*O*-acyl groups.

In the present work, we have reinvestigated N-acylation of chitosan using various cyclic acid anhydrides **1a**–**i** and found that high degree of N-acylation was attained by two step reactions in aqueous media. We also investigated thermal dehydration of the resulting *N*-(carboxy)acyl chitosan derivatives **2a**–**i**.



Scheme 1.

A solution of chitosan[7] in aqueous acetic acid–methanol was treated with phthalic anhydride (**1a**) according to the procedure reported. As the reaction proceeded, partially N-acylated chitosan aggregated and formed a gel with a degree of substitution (d.s.) of ca. 0.5. It has been reported that partially *N*-(carboxy)acyl chitosan derivatives are insoluble in the weak acidic to neutral media,[5] presumably due to ion-pair formation between amino and carboxyl groups on the polymer chain. Assuming, then, that the reactivity of the amino group would be restored by the ion-pair dissociation, we next examined further N-acylation under basic conditions.

The reaction mixture, a suspension containing partial *N*-(2-carboxy)benzoyl chitosan, was made alkaline with 2% aqueous sodium hydrogen carbonate to give a clear solution, and the reaction was reinitiated by further addition of **1a**. Although concomitant hydrolysis of **1a** was observed in these basic aqueous media, further N-acylation of chitosan proceeded under homogeneous conditions. After acidification of the mixture, precipitated *N*-(2-carboxy)benzoyl chitosan (**2a**) was isolated by centrifugation and lyophilization in the yield of 82%.[8] The d.s. of **2a** calculated from data of elemental analysis was 1.00. In its IR spectrum, two C=O stretching vibration bands assignable to carboxylic acid and amide I were observed at 1710 and 1650 cm^{-1} together with an amide II band at 1550 cm^{-1}. Structural regularity of the product was examined by NMR spectroscopy. The ^{13}C NMR spectrum of **2a** (Figure 1, top) showed six peaks of pyranose residue and six peaks of asymmetric aromatic carbons at δ 59.2–103.2 and 130.0–141.0 ppm, respectively. The simple monosaccharide-like spectrum indicated a regular structure of the product. Furthermore, ^1H NMR spectrum of the product that **2a** treated with acetic anhydride in dilute ammonia–methanol showed no signals of *N*-acetyl group at δ 2.0–2.2 ppm, supporting the absence of unsubstituted amino groups in **2a**. Overall the NMR and the IR results are in full agreement with the expected structure of product **2a**, confirming that the reaction of N-acylation of chitosan proposed proceeds with highest selectivity and up to completion. In a similar way, this N-acylation method found to be extended to other cyclic acid anhydrides, i.e., 4-methylphthalic **1b**, trimellitic **1c**, cyclohexane-1,2-dicarboxylic **1d**, *cis*-1,2,3,6-tetrahydrophthalic **1e**, succinic **1f**, glutaric **1g**, maleic **1h**, and itaconic anhydrides **1i** giving the corresponding *N*-(carboxy)acyl chitosan **2b**–**i** (Table 1). The d.s. of the products **2b**–**i**, determined by elemental analyses and ^1H NMR spectroscopy after N-acetylation of the remaining amino group, ranged from 0.85 to 1.00.

Since dehydration of a (carboxy)amide is known to be an easy method for the preparation of the cyclic imide derivative, these *N*-(carboxy)acyl chitosans with high degrees of substitution would be good precursors for imido form chitosan derivatives[9] with high stereoregularity. We next carried out thermogravimetric (TG) analysis of **2a**–**i** together with original chitosan, in order to examine the dehydration process and to choose a suitable starting material. Although all of the compounds showed over 7% weight loss at above 230 °C, the aromatic *N*-acyl derivatives **2a**–**b** showed over 8% weight loss at below 200 °C. Stimulated by these

Table 1. The results of N-acylation of chitosan with various cyclic acid anhydrides

products	d.s.		yield/%
	^{1}H NMR[a]	Elemental Anal.[b]	
2a	1.00	1.00	82
2b	0.97	1.00	79
2c	0.92	0.91	74
2d	0.85	0.95	85
2e	0.95	0.95	80
2f	0.96	0.97	72
2g	0.90	0.93	92
2h	0.99	0.99	84
2i	0.95	0.97	78

[a]Calculation was based on the peak area ratio of (carboxy) acyl/acetyl group after N-acetylation. [b]Calculation was based on the C/N ratio.

results, **2a** was heated at 100, 130, 160, and 190 °C under reduced pressure. The reaction process was monitored by IR spectroscopy. Structural changes were observed at a temperature of 130 °C and above. Characteristic absorption of the imido group at 1775 and 1710 cm^{-1} increased, whereas those of amido group at 1650 and 1550 cm^{-1} decreased with increasing treatment temperature. After heating for 5 h at 190 °C, the latter peaks were almost disappeared suggesting that the (carboxy)amido groups in **2a** was completely converted into the imido groups to give *N*-phthaloyl chitosan (**3a**).[10,11] The structure of **3a** was also confirmed by ^{13}C NMR spectroscopy as shown in Figure 1 (bottom). Three peaks in the aromatic carbon region of the spectrum (123.5–135.0 ppm) and a single peak arising from the carbonyl carbon (168.2 ppm) revealed the symmetric structure of the *N*-phthaloyl group. Moreover, six peaks were attributable to the pyranose carbons (57.2–98.1 ppm), demonstrating the presence of a monosaccharide repeating unit. Although **3a** obtained by this method showed poor solubility in pyridine, it was slightly soluble in DMSO. Compound **2b** was next treated under the same conditions. The ^{1}H and ^{13}C NMR spectra of the product suggested that the imidization proceeded similarly to the phthaloyl derivative **2a**, although unchanged (carboxy)amido group remained slightly. It was noteworthy that *N*-(4-methyl)phthaloyl chitosan (**3b**)[11] had high solubility in DMSO, *N*,*N*-dimethylformamide, and pyridine. The IR spectrum of the heated sample of **2c** also revealed the drastic structural change similar to **2a**–**b**, however, the product was insoluble in any solvents. In contrast to these three derivatives, insufficient imidization was observed in the IR spectra of **2d**–**i**.

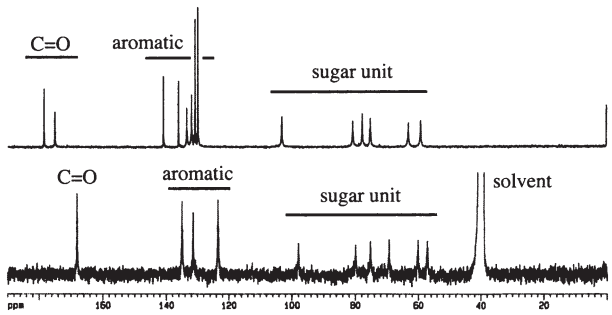


Figure 1. ^{13}C NMR spectra (75.48 MHz) of ammonium salt of **2a** in D_2O at 50 °C (top) and **3a** in DMSO-d_6 at 50 °C (bottom).

In conclusion, the highly substituted *N*-(carboxy)acyl chitosan derivatives **2a**–**i** were obtained by N-acylation in aqueous acidic media followed by in basic media. Furthermore, the chitosan derivatives having aromatic acyl group **2a**–**b** were successively converted into the imido form derivatives **3a**–**b** by heat treatment at 190 °C for 5 h under reduced pressure. Further studies now in progress to utilize these structural regular compounds as key intermediates for the chitosan-based functional materials.

References and Notes

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7 Chitosan with viscosity-average molecular weight of 110000 and degree of deacetylation of 0.99 was obtained by repeated N-deacetylation of Flonac C (Kyowa Technos Co. Ltd., Chiba, Japan).
8 To a solution of chitosan (3.00 g) in 2% AcOH–MeOH (300 mL, 1:1 (v/v)) **1a** (8.27 g) was added and the mixture was stirred at 35 °C for 12 h. The reaction mixture was dropped slowly into 2% aq. $NaHCO_3$ (1.20 L) with vigorous stirring to homogeneous solution of pH 8. To this solution **1a** (8.27 g) was added and stirred at 35 °C for 12 h again. Precipitate was formed by addition of aq. HCl (2 mol L^{-1}) to pH 3–4 and collected by centrifugation or filtration. After washing with H_2O and EtOH, **2a** was obtained as white powder by lyophilization (82% yield). ^{13}C NMR of ammonium salt (D_2O): δ 59.2, 63.1, 75.3, 77.8, 80.7, and 103.2, (pyranose), 130.0, 130.9, 132.0, 133.5, 136.2, and 141.0 (aromatic), 175.3 and 178.7 (C=O). ν_{max} (KBr) 1710 (C=O carboxyl), 1650 and 1550 (C=O and NH amide). Elemental Anal. Calcd for $C_{14}H_{15}NO_7 \cdot 0.2\ H_2O$: C, 53.74; H, 4.96; N, 4.48. Found: C, 53.59; H, 5.09; N, 4.63.
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11 Physical data of **3a**. ^{13}C NMR (DMSO-d_6): δ 57.2, 60.2, 69.3, 75.3, 80.0, and 98.1 (pyranose), 123.5, 131.5, and 135.0 (aromatic), and 168.2 (C=O). ν_{max} (KBr) 1775 and 1710 (C=O imide). Elemental Anal. Calcd for $C_{14}H_{13}NO_6 \cdot 0.5\ H_2O$: C, 56.00; H, 4.70; N, 4.66. Found: C, 55.84; H, 4.69; N, 4.77. Physical data of **3b**. ^{13}C NMR (DMSO-d_6): δ 21.4 (methyl) 56.7, 59.9, 69.1, 74.8, 79.7, and 97.9 (pyranose), 123.1, 123.6, 128.6, 129.3, 131.6, 135.0, and 145.6 (aromatic), and 167.8 (C=O). ν_{max} (KBr) 1770, 1710, and 740 (imide).