Novel *N*-Alkylation of Chitosan via Michael Type Reaction[1]

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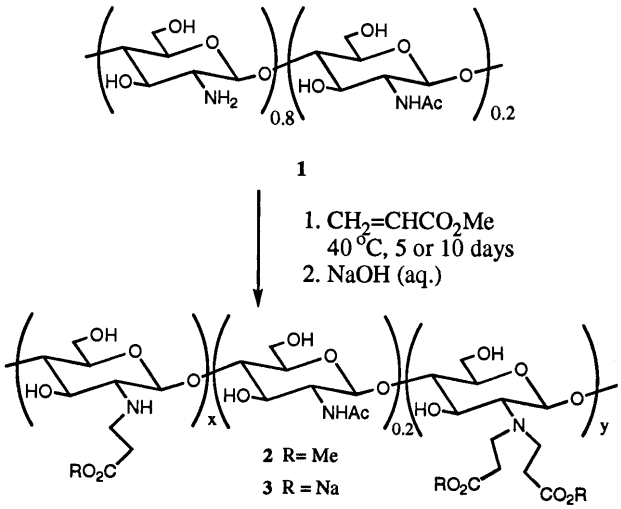
Using α,β-unsaturated carbonyl compounds, *N*-alkylated chitosans have been prepared efficiently via Michael type reaction under mild conditions.

Chitosan **1** is a polysaccharide mainly composed of β-(1-4)-2-amino-2-deoxy-D-glucopyranose repeating unit. Chitosan is a remarkable biomaterial because of its numerous biological and immunological activities.[2] Moreover, chitosan itself is a non-toxic and biodegradable biopolymer.[3] Most recently, it has been found that sulfated chitin or chitosan,[4] and other unrelated polyanions,[5] showed high anti-HIV-1 activity. Chitosan itself is insoluble in water at neutral pH, consequently possible application with chitosan is rather limited. Chemical modification of chitosan is an important issue to allow its solubility in water under neutral conditions. Although several reductive *N*-alkylations of chitosan with aldehyde exist,[6] there is no report for the *N*-alkylation of chitosan by conjugate addition. Michael type or 1,4-conjugate addition reaction is well known as an efficient procedure to attach α,β-unsaturated carbonyl compounds to amines. Poly(amidoamine) (PAMAM) dendrimer represents such an application. The *N*-alkylation of amine necessitates long reaction time (e.g., 5 days) at 40 °C.[7] Herein, we report a novel method for *N*-alkylation of chitosan via 1,4-conjugate addition.



Scheme 1.

A typical procedure is as follows. Chitosan (400 mg, 1.88 mmol of NH_2) was dissolved in H_2O (40 mL) containing AcOH (120 mg) and MeOH (40 mL). Prescribed amount of methyl acrylate and Et_3N (70 mg) was added to the solution which was stirred at 40 °C. After several days, an aliquot of the reaction mixture (40 mL) was quenched by precipitation with sat. $NaHCO_3$ (5 mL) and acetone (80 mL). The precipitate was collected by filtration, dispersed in H_2O (20 mL) containing sat. $NaHCO_3$ (5 mL), and the mixture was dialyzed against H_2O (4 L) for 2 days and lyophilized to give *N*-carboxyethyl chitosan methyl ester **2** (Scheme 1). To obtain *N*-carboxyethyl chitosan **3**, 0.1% NaOH (50 mL) was added to **2**, the mixture was stirred at rt for 2 h, dialyzed, and lyophilized as above. The molecular weight of the initial chitosan (*M*n = 24 kDa) was determined by GPC with pullulan as standard. All compounds showed satisfactory NMR (Bruker AMX 500 MHz). Selected data for **2** (DS = 0.5): δ_H (0.1 M DCl/D_2O) 2.06 (brs, 0.6 H, NH*Ac*), 2.91 (brs, 1.0 H, NH–C*H*$_2$–), 3.20 (br, 0.8 H, *H-2* of GlcN and *N*-alkylated GlcN), 3.4–4.1 (brm, 7.7 H, –COO*Me*, –C*H*$_2$–COOMe, *H-2* of GlcNAc, and *H-3,4,5,6* of GlcN and GlcNAc), 4.65 (0.2 H, *H-1* of GlcNAc), 4.85 (0.8 H, *H-1* of GlcN and *N*-alkylated GlcN); δ_C (0.1 M DCl/D_2O) 25.1 (NH*Ac*), 32.7 (NH–*C*H$_2$–), 45.8 (–*C*H$_2$–COOMe), 51.9 (COO*Me*), 58.8 (*C-2* of GlcN and GlcNAc), 63.0 and 63.6 (*C-6*), 64.8 (*C-2* of *N*-alkylated GlcN), 72.0–73.5 (*C-3*), 77.7 (*C-5*), 79.5 (*C-4*), 99.3 (*C-1* of *N*-alkylated GlcN), 100.4 (*C-1* of GlcN), 104.2 (*C-1* of GlcNAc), 176.0 (NH*C*O), 177.3 (*C*OOMe).

Table 1. Preparation of *N*-carboxyethyl chitosan methyl ester **2**[a]

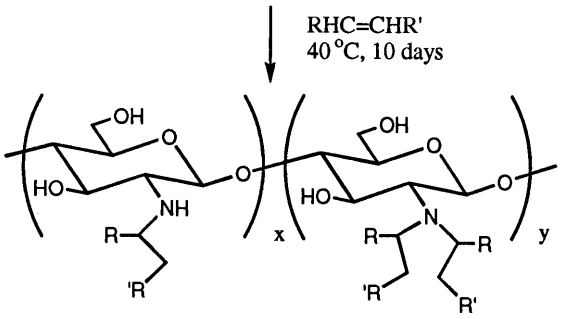
Entry	Reagent equiv	Solvent[b]	Time day	DS[c]	Ratio x : y
1	3	A	2	0.13	0.13 : 0
2	3	B	2	0.14	0.14 : 0
3	3	B	5	0.31	0.31 : 0
4	3	B	10	0.50	0.50 : 0
5	3	C	10	0.77	0.57 : 0.10
6	12	C	10	0.97	0.57 : 0.20
7	12	D	5d x 2[d]	1.20	0.40 : 0.40
8	12	D	5d x 3[d]	1.40	0.20 : 0.60

[a]Temp, 40 °C. [b]Solvent A: H_2O:MeOH (1:3), AcOH 1.1 equiv/-NH_2, Et_3N excess; B: H_2O:MeOH (1:3), AcOH 1.1 equiv; C: H_2O : MeOH (1:1), AcOH 1.1 equiv, Et_3N 0.4 equiv; D: H_2O : MeOH (1:10), Et_3N 0.4 equiv. [c]DS=x+2y. [d]5 days x 2 or 3 times.

Table 1 shows the *N*-alkylation of chitosan (NHAc=0.2) with methyl acrylate under various conditions. The yields of all compounds were over 80%. In our preliminary test, products with low substitution (DS 0.13) were obtained under heterogeneous conditions (entry 1). Under homogeneous conditions using solvent B, however, the degree of substitution[8] (DS) was

unchanged even after 2 days (entry 2). With longer reaction time (5 and 10 days), the DS value was gradually increased to 0.50 (entry 3,4). With the addition of a small amount of Et_3N, owing to the increased pH of the reaction mixture (solvent C: around pH 6), the DS value was also increased to 0.77 (entry 5). Repeating the reaction process was shown to be effective in increasing the DS of the products up to 1.40 (entry 7,8). Compounds **2** of high DS (DS > 0.77) were composed of a mixture of mono- and di-substituted units. The ratio (x:y) could be estimated by ^{1}H NMR analyses from the ratio of H-1 protons.[9] After saponification of **2** with aqueous NaOH, *N*-carboxyethyl chitosan **3** was obtained in 90–100% yields. The water solubility of **3** was observed above DS = 0.50.

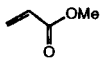
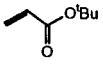
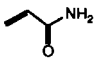
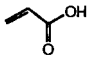
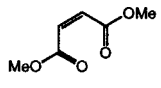
Chitosan **1**



4 : R = H, R' = COO^tBu
5 : R = H, R' = $CONH_2$
6 : R = H, R' = CN
7 : R, R' = COOMe

Scheme 2.

Table 2. *N*-Alkylation of chitosan by 1,4-conjugate addition.[a]

Entry	Reagent	Equiv	Time (Day)	DS	M.W.[b]
1		6	5	0.88	35
2		18	10	0.41	32
3		18	10	0.15	26
4		18	10	0.48	29
5		18	10	0.68	29
6		12	10	1.1	48

[a] Solvent, AcOH (1 equiv/NH_2), H_2O, MeOH; temp, 40 °C; Yield, over 80% in all examples.

[b] Initial chitosan M.W. (Mn = 24 kDa) by GPC using pullulan standard. M.W. calculated following : M.W. = F.W. x DP

Scheme 2 and Table 2 show the *N*-alkylation of chitosan with various α,β-unsaturated carbonyl compounds by Michael reactions. Compared to methyl acrylate (entry 1), the substitution by other reagents was somewhat less efficient, except for acrylonitrile (entry 5) and dimethyl maleate (entry 6). The 1,4-conjugate addition, however, did not proceed with methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (HEMA) because of steric hindrance or less reactivity of the β carbon. In the case of *tert*-butyl acrylate, steric hindrance by the *tert*-butyl group would affect the low substitution. The reaction with acrylonitrile (0.68) or dimethyl maleate (1.18) gave high DS values.

In conclusion, it has been shown that Michael type conjugate addition could be efficiently used for the *N*-alkylation of chitosan and high reactivity was shown with methyl acrylate, acrylonitrile, and dimethyl maleate. The biological properties of these chitosan derivatives will be published in due course.

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References and Notes

1 Chemical Modification of Chitosan. Part 2 of this series.
2 a) S. Minami, Y. Okamoto, A. Matsuhashi, H. Sashiwa, H. Saimoto, Y. Shigemasa, T. Tanigawa, Y. Tanaka, and S. Tokura, in "Advances in Chitin and Chitosan," ed. by C. J. Brine, P. A. Sandford, and J. P. Zikakis, Elsevier, London (1992), p. 61. b) K. Nishimura, S. Nishimura, N. Nishi, I. Saiki, S. Tokura, and I. Azuma, *Vaccine*, **2**, 93 (1984).
3 a) H. Sashiwa, H. Saimoto, Y. Shigemasa, R. Ogawa, and S. Tokura, *Int. J. Biol. Macromol.*, **12**, 295 (1990). b) Y. Shigemasa, K. Saito, H. Sashiwa, and H. Saimoto, *Int. J. Biol. Macromol.*, **16**, 43 (1994).
4 S. Nishimura, H. Kai, K. Shinada, T. Yoshida, S. Tokura, K. Kurita, H. Nakashima, N. Yamamoto, and T. Uryu, *Carbohydr. Res.*, **306**, 427 (1998).
5 a) A. Leydet, C. J. Segonds, P. Barthelemy, B. Boyer, and J. P. Roque, *Recl. Trav. Chim. Pays-Bas*, **115**, 421 (1996). b) A. Leydet, C. J. Segonds, C. Bouchitte, C. Moullet, B. Boyer, J. P. Roque, M. Witvrouw, J. Este, R. Snoeck, G. Andrei, and E. D. Clercq, *J. Med. Chem.*, **40**, 350 (1997).
6 a) M. Yalpani and L. D. Hall, *Macromolecules*, **17**, 272 (1984). b) H. Sashiwa and Y. Shigemasa, *Carbohydr. Polym.*, **39**, 127 (1999).
7 a) D. A. Tomalia, A.M. Naylor, and W. A. Goddard III, *Angew. Chem., Int. Ed. Engl.*, **29**, 138 (1990). b) K. Aoi, K. Itoh, and M. Okada, *Macromolecules*, **30**, 8072 (1997).
8 The DS of all compounds were estimated by ^{1}H NMR in 0.1 M DCl/D_2O with the ratio of δ 2.06 (0.6 H, NH*Ac*) and around 2.8–3.1 (NH–C*H*$_2$–R).
9 High DS of **2** (over 0.8): δ 4.85 (*H-1* of *N*-monoalkylated GlcN), 5.05 (*H-1* of *N,N*-dialkylated GlcN).