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Note

Chemical modification of chitosan. Part 15: Synthesis of novel chitosan derivatives by substitution of hydrophilic amine using *N*-carboxyethylchitosan ethyl ester as an intermediate☆

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Abstract

The Michael type reaction of chitosan with ethyl acrylate has been investigated. Although this reaction was quite slow in the case of chitosan, the reiteration of the reaction was an effective means for increasing the degree of substitution (DS) of ethyl ester. The *N*-carboxyethylchitosan ethyl ester as an intermediate was successfully substituted with various hydrophilic amines, although the simultaneous hydrolysis of the ester to carboxylic acid also occurred. Water-soluble chitosan derivatives were obtained by substitution with hydroxyalkylamines and diamines.

Keywords: Chitosan; *N*-Carboxyethylchitosan ethyl ester; Michael reaction; Amidation

Chitosan **1** is the N-deacetylated product of chitin and an attractive material because of its biological properties such as immunological activity[2] or wound healing properties.[3] Although some work has been reported to obtain water-soluble derivatives of chitosan by N-acetylation in aqueous medium, only chitosans with around 50% of deacetylation (DAC-50) or N-acetylation (NAC-50) were soluble in water.[4] Attempts have also been made to modify the molecular structure of chitin by introduction of groups such as carboxymethyl, dihydroxyethyl, sulfuryl, or phosphoryl groups etc.[5] Recently, two papers have been published on the chemical modification of chitosan with methyl acrylate[6] or DAC-50 with ethyl acrylate[7] by Michael reaction. A second modification of chitosan using *N*-carboxyethylchitin methyl ester as a precursor to prepare chitosan-dendrimer hybrids was also reported.[8] However, the detailed reaction profile of chitosan with ethyl acrylate was not described. Herein we report a detailed study of the Michael type reaction of chitosan with ethyl acrylate and subsequent reaction of *N*-carboxyethylchitin ethyl ester **2** as an intermediate. Aliphatic diamines or aminoalkylalcohols were successfully attached to ethyl ester **2** without any protection.

Table 1 shows the preparation of *N*-carboxyethylchitin ethyl ester **2** by Michael reaction under the various conditions. Since chitosan is insoluble above pH 6.5, the reaction was performed under acidic conditions. At 25 °C, the reaction was quite slow, with the DS of CO_2Et in the product being only 0.41 after 10 days. The reaction rate was substantially increased at 50 °C for the first 2 days but decreased after 2 days. Reiteration of the reaction[9] was effective compared with elongation of reaction time (7 or 10 days), suggest-

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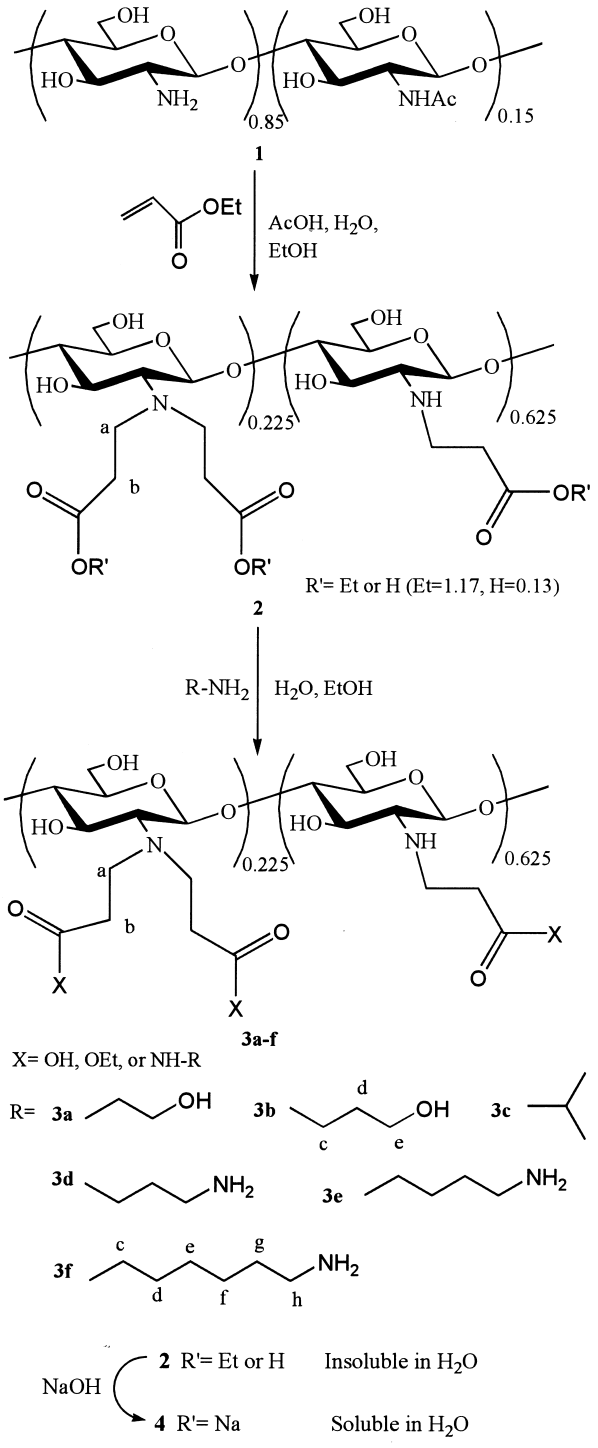
ing that the ethyl acrylate was gradually hydrolyzing to carboxyl groups. The maximum DS under these conditions was 1.30 ($CO_2Et + CO_2H$), which is similar to the previous report using methyl acrylate (DS = 1.40; 5 + 5 + 5 days at 40 °C).[6] The ethyl ester **2** was preferred to the methyl ester for the detailed structural analysis by ^1H NMR, because the differentiation signal at δ 1.28 (CH_3 of CO_2Et) allowed accurate determination of DS of ester groups (Scheme 1).

Several kinds of amine were reacted with ethyl ester **2** of the highest DS ($CO_2Et = 1.17$) under the aqueous acidic conditions to give a homogeneous reaction mixture. The results are summarized in Table 2. Using 20 equiv of hydroxyalkylamines such as aminoethanol or aminopropanol, the substitution of amines to esters was 16–17%. Using 50 equiv of aminopropanol, the substitution of amine (DS of –NHR) was effectively increased. The substitution for **3c** was also increased with increasing isopropylamine, although the reactivity was about half degree compared with hydroxyalkylamines. The low reactivity would be caused by the low reactivity of amino group linked to a secondary carbon ($Me_2CH–NH_2$). When using 2 equiv of amines to give **3a**–**c**, there was little substitution (DS of NHR under 0.02, data not shown). The substitution of these monoamines, required excess amounts of reagent. The products possessing hydroxyl groups **3a** and **3b** dissolved in H_2O, although the product **3c** was not. In accordance with the literature, an excess amount (20 equiv) of diamine such as ethylenediamine was used to prevent undesirable formation.[10] Therefore, of crosslinks during the preparation of poly(amidoamine) (PAMAM) dendrimer 20 equiv of diamine was used for



Scheme 1.

Table 1
Preparation of *N*-carboxyethylchitosan ethyl ester **2** [a]

Reaction conditions		DS		Reaction rate [b]
Temperature (°C)	Time (day)	CO_2Et	CO_2H	ΔDS/Δday
25	2	0.08	0.02	0.05
25	4	0.14	0.02	0.03
25	7	0.30	0.04	0.06
25	10	0.41	0.04	0.04
50	2	0.44	0.05	0.25
50	4	0.50	0.05	0.03
50	7	0.53	0.08	0.02
50	10	0.54	0.08	0.003
50	4+4	0.80	0.10	0.09
50	4+4+4	1.17	0.13	0.10

[a] Ethyl acrylate, 10 equiv/NH_2.

[b] Example for $0.05 = (0.08+0.02-0)/(2-0)$; $0.03 = (0.14+0.02-0.08+0.02)/(4-2)$.

the reaction as with **3a**–**c**. A similar reactivity (21–25%) was shown independent of the length of methylene chain **3d**–**f** and gave water-soluble products. The hydrolysis of ester would be caused by the H_2O in the reaction mixture under the basic conditions in the presence of excess of amine and gave carboxyl groups as a major component (Scheme 2). The intermediate

Table 2
Reaction of various amines to ethyl ester **2** [a]

Compound	R–NH_2	DS					Solubility [c]	
		equiv	–NHR	(% [b])	CO_2Et	CO_2H	H_2O	Aqueous AcOH
2	–	–	–	–	1.17	0.13	X	O
3a	$HO(CH_2)_2NH_2$	20	0.20	17	0.15	0.95	O	O
3b	$HO(CH_2)_3NH_2$	20	0.19	16	0.10	1.01	O	O
3b	$HO(CH_2)_3NH_2$	50	0.43	37	0.05	0.82	O	O
3c	$(CH_3)_2CHNH_2$	20	0.11	9	0.30	0.89	X	O
3c	$(CH_3)_2CHNH_2$	50	0.20	17	0.10	1.00	X	O
3d	$H_2N(CH_2)_3NH_2$	20	0.24	21	0.07	0.99	O	O
3e	$H_2N(CH_2)_4NH_2$	20	0.25	21	0.07	0.98	O	O
3f	$H_2N(CH_2)_6NH_2$	20	0.29	25	0.02	0.99	O	O
–	H_2NPh	20	0	0	0.40	0.90	X	O
4		–	–	–	0	1.30	O	O

[a] Temperature, 70 °C; time, 1 day.
[b] % = [DS(–NHR)/1.17] × 100.
[c] O, soluble; X, insoluble; concentration of sample = 1 mg/mL; H_2O soluble sample was also dissolved at all pH region (pH 1–13).

ester **2** was soluble in aqueous medium with AcOH and it could be kept in homogeneous state. It did not dissolve in organic solvents such as alcohols or other aprotic solvents. Therefore, the partial hydrolysis of esters was unavoidable under these conditions. The aromatic amine ($PhNH_2$) did not react under the tested conditions and only partial hydrolysis of ester was observed. Moreover, the neutral water insoluble ester **2** was transformed to water-soluble sodium carboxylate **4** by treating with aqueous NaOH.

In this study, water-soluble chitosan derivatives possessing amine, hydroxyl, or carboxyl groups by using *N*-carboxyethylchitosan ethyl ester **2** as intermediate were obtained. This procedure can be utilized for the direct substitution of various bioactive amines without requiring protection groups.

1. Experimental

1.1. Materials

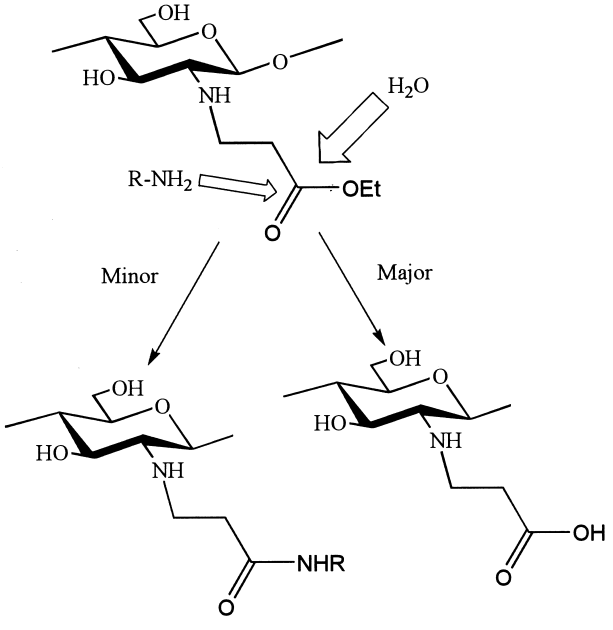
Chitosan (Flonac C: NHAc = 0.15; Mn = 26,000; Mw = 75,000) was purchased from Kyowa Technos Co., Japan.

1.2. General methods

^1H and ^{13}C NMR spectra were recorded on JEOL A-500 NMR spectrometer. Molecular weight was determined by means of GPC using pullulan as standards (column, Tosoh TSK Gel G4000pwxl and G3000pwxl; eluent, 0.5 M AcOH–0.5 M AcONa buffer; temp, 40 °C; flow rate, 1.0 mL/min; detection, RI).

1.3. *N*-carboxyethylchitin ethyl ester

Chitosan **1** (5 g) was dissolved in aq AcOH (AcOH = 1.5 mL and H_2O = 100 mL) and then diluted with EtOH (200 mL). To the solution was added ethyl acrylate (10 equiv/NH_2 in chitosan). After stirring at 25 or 50 °C for prescribed time, the reaction mixture was concentrated to approx 100 mL under reduced pressure to remove excess amount of ethyl acrylate and EtOH. The remaining mixture was dialyzed for 2 days and lyophilized to obtain *N*-carboxyethylchitin ethyl ester **2**



Scheme 2.

in quantitative yield (example for the degree of substitution per one sugar unit (DS) of $CO_2Et = 1.17$: Mn = 12,000; Mw = 49,000).

1.4. Reaction of amines with ethyl ester 2

Ethyl ester **2** (50 mg) was suspended in H_2O (10 mL) and EtOH (100 mL), and then amine (20 equiv/CO_2Et in **2**) was added to the suspension. After stirring at 70 °C for 1 day, the mixture was concentrated to approx 10 mL under the reduced pressure to remove EtOH. The remaining mixture was dialyzed for 2 days and lyophilized to obtain **3** in quantitative yield.

1.5. Structural analysis

The DS of CO_2Et and CO_2H in **2** was determined by ^1H NMR in 0.5 M DCl/D_2O from the peak area at δ 1.27 (–CO_2–CH_2CH_3) for DS (CO_2Et) and 2.91 (–CH_2–CO_2R) for DS ($CO_2Et + CO_2H$) against 4.90–5.15 (H-1 of N-alkylated and N,N-dialkylated GlcN: 0.85 H). The DS values of NHR in **3a**–**f** were described in the following spectral data.

Data for **2**: ^1H NMR: δ 1.27 (s, 3.51 H, CH_3 of CO_2Et), 2.06 and 2.09(s, 0.31 H, NHAc and AcOH), 2.91 (s, 2.6 H, –CH_{2b}–CO_2R), 3.31 (br, 0.85 H, H-2 of N-alkylated GlcN), 3.6-4.1 (m, *N*-CH_{2a}– of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.24 (d, 2.34 H, –CH_2– of CO_2Et), 4.60 (br, 0.15 H, H-1 of GlcNAc), 4.90–5.15 (m, H-1 of N-alkylated and N,N-dialkylated GlcN).

Data for **3a** (NHR = 0.20): ^1H NMR: δ 1.27 (s, 0.45 H, CH_3 of CO_2Et), 2.06 (s, 0.45 H, NHAc), 2.91 (s, 2.6 H, –CH_{2b}–CO_2R), 3.11 (s, 0.40 H, –CONH–CH_{2c}–), 3.30 (br, 0.85 H, H-2 of N-alkylated GlcN), 3.6–4.1 (m, –$CH_{2a,d}$– of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.24 (d, 0.30 H, –CO_2–CH_2CH_3 of CO_2Et), 4.90–5.15 (m, H-1 of N-alkylated and N,N-dialkylated GlcN). DS of –NHR was determined from the peak area at δ 3.11 (–NHCO–CH_{2c}–) against 4.90–5.15 (H-1 of N-alkylated and N,N-dialkylated GlcN: 0.85 H).

Data for **3b** (NHR = 0.19): ^1H NMR δ 1.27 (s, 0.30 H, CH_3 of CO_2Et), 1.90 (s, 0.38 H, –CH_{2d}–), 2.06 (s, 0.45 H, NHAc), 2.91 (s, 2.6 H, –CH_{2b}–CO_2R), 3.11 (s, 0.38 H, –CONH–CH_{2c}–), 3.30 (br, 0.85 H, H-2 of N-alkylated GlcN), 3.6–4.1 (m, –$CH_{2a,e}$– of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.24 (d, 0.20 H, –CO_2–CH_2CH_3 of CO_2Et), 4.90–5.15 (m, H-1 of N-alkylated and N,N-dialkylated GlcN). DS of –NHR was determined at δ 1.90 (–CH_{2d}–) against 4.90–5.15.

Data for **3c** (NHR = 0.20): ^1H NMR: δ 1.17 (s, 1.20 H, CH_3 of –NHR), 1.27 (s, 0.30 H, CH_3 of CO_2Et), 2.06 (s, 0.45 H, NHAc), 2.91 (s, 2.6 H, –CH_{2b}–CO_2R), 3.30 (br, 0.85 H, H-2 of N-alkylated GlcN), 3.6–4.1 (m, –CH_{2a}– and –CH– of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.24 (d, 0.20 H, –CO_2–CH_2CH_3 of CO_2Et), 4.90–5.15 (m, H-1 of N-alkylated and N,N-dialkylated GlcN). DS of –NHR was determined at δ 1.17 (–CH_3) against 4.90–5.15.

Data for **3d** (NHR = 0.24): ^1H NMR: δ 1.27 (s, 0.21 H, CH_3 of CO_2Et), 1.90 (s, 0.48 H, –CH_{2d}–), 2.06 (s, 0.45 H, NHAc), 2.92 (s, 2.6 H, –CH_{2b}–CO_2R), 3.12 (s, 0.48 H, –CONH–CH_{2c}–), 3.31 (br, 0.85 H, H-2 of N-alkylated GlcN), 3.6–4.1 (m, –$CH_{2a,e}$– of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.23 (d, 0.14 H, –CO_2–CH_2CH_3 of CO_2Et), 4.90–5.15 (m, H-1 of N-alkylated and N,N-dialkylated GlcN). DS of –NHR was determined at δ 1.90 (–CH_{2d}–) against 4.90–5.15.

Data for **3e** (NHR = 0.25): ^1H NMR: δ 1.28 (s, 0.21 H, CH_3 of CO_2Et), 1.76 (s, 1.00 H, –CH_{2d}–CH_{2e}–), 2.06 (s, 0.45 H, NHAc), 2.92 (s, 2.6 H, –CH_{2b}–CO_2R), 3.05 (s, 0.50 H, –CONH–CH_{2c}–), 3.31 (br, 0.85 H, H-2 of N-alkylated GlcN), 3.6–4.1 (m, –$CH_{2a,f}$– of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.23 (d, 0.14 H, –CO_2–CH_2CH_3 of CO_2Et), 4.90–5.15 (m, H-1 of N-alkylated and N,N-dialkylated GlcN). DS of –NHR was determined at δ 1.76 (–CH_{2d}–CH_{2e}–) against 4.90–5.15.

Data for **3f** (NHR = 0.29): ^1H NMR: δ 1.28 (s, 0.06 H, CH_3 of CO_2Et), 1.42 (s, 1.16 H, –CH_{2e}–CH_{2f}–), 1.69 (s, 1.16 H, –CH_{2d} and –CH_{2g}–), 2.07 (s, 0.45 H, NHAc), 2.93 (s, 2.6 H, –CH_{2b}–CO_2R), 3.01 (s, 0.58 H, –CONH–CH_{2c}–), 3.33 (br, 0.85 H, H-2 of N-alkylated GlcN), 3.6–4.1 (m, –$CH_{2a,h}$– of *N*-alkyl group, H-2 of GlcNAc, H-3,4,5,6 of GlcN and GlcNAc), 4.90–5.15 (m, H-1 of N-alkylated and N,N-dialkylated GlcN). DS of –NHR was determined at δ 1.42 (–CH_{2e}–CH_{2f}–) against 4.90–5.15.

–CH_{2a-h}–: Chitosan–NH–CH_{2a}–CH_{2b}–CONH–CH_{2c}–CH_{2d}–CH_{2e}–CH_{2f}–CH_{2g}–CH_{2h}–NH_2

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