Homogeneous *N,O*-Acylation of Chitosan in Dimethyl Sulfoxide with Cyclic Acid Anhydrides[1]

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By using soluble chitosan (1*R*)-(–)-10-camphorsulfonate salt (chitosan-CSA), *N,O*-acylated chitosans were prepared in dimethyl sulfoxide (DMSO) with cyclic acid anhydride under homogeneous conditions.

Chitosan **1** is a polysaccharide composed of β-(1-4)-2-amino-2-deoxy-D-glucopyranose repeating unit. Most chemical modifications of chitosan under homogeneous conditions have been carried out in aqueous acetic acid (AcOH) or in mixed aqueous AcOH and organic solvents such as MeOH.[2] However, there is no report on the homogeneous chemical modifications of chitosan in neat organic solvents, because chitosan itself is essentially insoluble in any common organic solvents. In our previous work, *O*-acylation of 20% partially deacetylated chitin (DAC-20) in a mixture of LiCl/*N,N*-dimethylacetamide (DMAc)[3] or selective *N*-acylation of chitosan in aqueous medium[4] was carried out effectively with cyclic acid anhydrides. More recently, we reported that chitosan can dissolve in DMSO by salt formation with *p*-toluenesulfonic acid or with (*1R*)-(–)-10-camphorsulfonic acid (CSA).[5] We report herein the highly substituted *N,O*-acylation of chitosan-CSA salt in DMSO with cyclic acid anhydrides under complete homogeneous conditions.

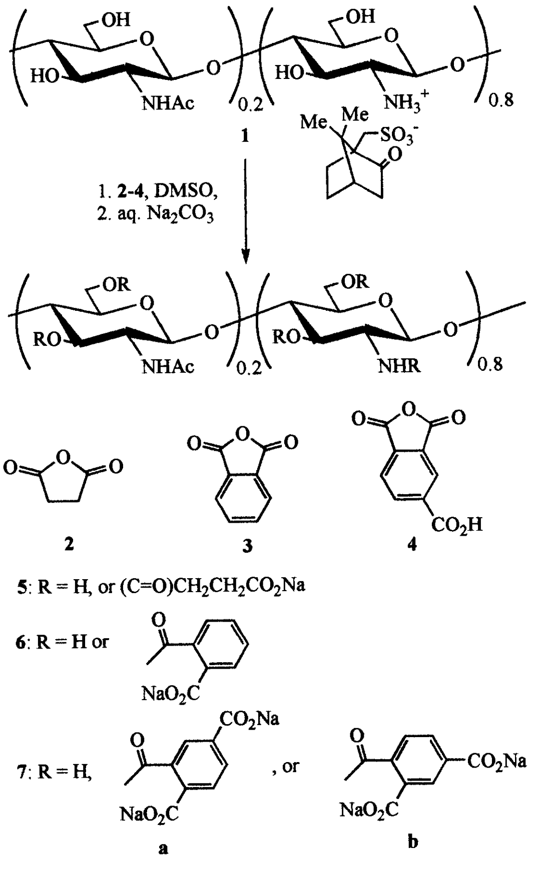
Chitosan (NHAc = 0.2, M_n = 24000) was purchased from Kyowa Tecnos Co., Japan. The degree of *N*-acetylation was determined by ^{1}H NMR spectroscopy and M_n was determined by GPC using pullulan as standard. Chitosan-CSA salt (NH_2 : CSA = 1:1) was prepared according to previous report (chitosan 1.0 g; CSA, 1.1 g; H_2O, 50 cm^3; dissolved at rt for 1 day; lyophilized).[5] A typical procedure for the acylation of chitosan-CSA is as follow. Chitosan-CSA (200 mg: 0.47 mmol NH_2) was dissolved in DMSO (7 cm^3) and then various amounts of cyclic acid anhydrides were added (Table 1). After stirring for selected reaction time, sat. aqueous Na_2CO_3 (10 cm^3) was added to the solutions, which was then dialyzed (MW cut off 12000) against H_2O for 2 days, and lyophilized to provide *N,O*-acylated chitosan (Scheme 1). All compounds showed satisfactory NMR (Bruker AMX 500 MHz).[6]

Scheme 1 and Table 1 show the acylation of chitosan-CSA salt in DMSO with some cyclic acid anhydrides such as succinic anhydride **2**, phthalic anhydride **3**, and 1,2,4-benzenetricarboxylic anhydride **4**. All reactions were carried out under homogeneous conditions and gave water-soluble products in good yields. In the case of **2** at rt for 3 days (entry 1), the degree of substitution (DS) was only 0.55. Under higher temperature (70 °C, entry 2), the DS value increased moderately to 1.06. Using **3**, high DS (1.67) was obtained after just 1 day of reaction at rt (entry 4). The DS could be controlled by the amount of reagent added (entry 3, DS=0.51), or by the reaction time (entry 5, DS=2.28), although the effect of temperature was

Table 1. Acylation of chitosan-CSA salt in DMSO with cyclic acid anhydrides

Entry	Reagent[a]		Temp	Time	Yield[b]	DS[c]
		equiv.	°C	day	%	
1	**2**	2	rt	3	86	0.55
2	**2**	2	70	1	64	1.06
3	**3**	0.3	rt	1	86	0.51
4[d]	**3**	2	rt	1	72	1.67
5[d]	**3**	2	rt	3	72	2.28
6[d]	**3**	2	70	1	67	1.78
7[d]	**4**	2	rt	1	73	2.36
8[d]	**4**	2	rt	3	74	2.43

[a]Equiv., mol equivalent/OH and NH_2 in chitosan. [b]Yield determined by weight recovery and accounting for changes in FW according to the substitution level determined by ^{1}H NMR. [c]DS was determined by ^{1}H NMR with the ratio of δ 2.06-2.14 (0.6 H, NHAc) and 2.44-2.51 (CH_2 of Suc) for **5**, 7.5-7.7 (Ph) and at 7.5-8.2 (Ph) for **6** and **7**. [d] Insoluble in acidic water (pH<3).



Scheme 1.

not so great in this case (entry 6, DS=1.78). Anhydride **4** was more reactive than **3** and gave higher DS after 1 day of reaction (entry 7, DS=2.36). From these results, aromatic anhydrides such as **3** and **4** showed the highest reactivity compared with the aliphatic anhydride 2. All compounds were soluble in neutral water, however high DS for 3 and 4 (entry 4–8) resulted in insoluble products in acidic water (pH<3).

Table 2. DS distribution of *N,O*-acylated chitosan

Compd.	DS		
	Total	*N*-acyl[a]	*O*-acyl
5	0.55	0.32	0.23
5	1.06	0.34	0.72
6	0.51	0.19	0.32
6	1.67	0.80	0.87
6	2.28	0.80	1.48
7	2.36	0.80	1.56

[a] DS of *N*-acyl was determined by ^{1}H NMR from the ratio of δ 2.8 ppm (H-2 of GlcN residue) and 3.4-4.0 (H-2 of *N*-acylated GlcN and H-3-6 of hexosamine residue).

Table 2 shows the DS distribution of selected samples of *N,O*-acylated chitosans obtained in Table 1. At low DS (0.51–1.06), simultaneous *O*-acylation also occurred to a great extent (DS of *O*-acyl = 0.23–0.72). In contrast, in highly substituted products (DS = 1.67–2.36), the peak at δ 2.8 ppm (H-2 of GlcN residue) disappeared, thus indicating complete *N*-acylation. Furthermore, the ^{13}C NMR signal at δ 72.9 ppm was shifted downfield to 75.3–75.4, which suggests that *O*-acylation did proceed at both C-3 and C-6 hydroxyl groups at high DS values (DS of *O*-acyl = 1.48 and 1.56), though the ratio of C-3 and C-6 acylation could not be estimated. After the saponification of derivatives **5**–**7** (0.2 M NaOH, rt, 2 h, dialized and lyophilized), only *N*-acylated products were obtained and the DS values (*N*-acyl) showed good agreement with those observed before saponification. Highly *N*-substituted products were produced by this method in comparison to our previous report (DS = 0.21–0.39) [4] for **6** and **7**. In this study, imido formation of **5** was not observed from the ^{1}H NMR spectrum (δ 2.44 ppm of NHCO-CH_2 and 2.51 ppm of CH_2-CO$_2$H), although phthalimide formation of chitosan in DMF was reported.[7]

In conclusion, homogeneous chemical modifications of chitosan were first accomplished using chitosan-CSA salt in DMSO and highly water-soluble *N,O*-acylated chitosan derivatives were successfully obtained. The free carboxyl groups of these derivatives are useful for further modification such as in the scaffolding of chitosan-dendrimer hybrid. These applications will be published in due course.

References and Notes

1 Chemical Modification of Chitosan. Part 5 of this series. Part 1: H. Sashiwa, Y. Makimura, Y. Shigemasa, and R. Roy, *Chem. Commun.*, **2000**, 909; Part 2: H. Sashiwa, Y. Shigemasa, and R. Roy, *Chem. Lett.*, **2000**, 862; Part 3: H. Sashiwa, Y. Shigemasa, and R. Roy, *Macromolecules*, (2000) in press. Part 4: H. Sashiwa, J. M. Thompson, S. K. Das, Y. Shigemasa, S. Tripathy, and R. Roy, *Biomacromolecules*, **1**, 303 (2000).

2 a) S. Hirano, Y. Ohe, and H. Ono, *Carbohydr. Res.*, **47**, 315 (1976). b) M. Yalpani and L. D. Hall, *Macromolecules*, **17**, 272 (1984).

3 Y. Shigemasa, H. Usui, M. Morimoto, Y. Okamoto, S. Minami, and H. Sashiwa, *Carbohydr. Polym.*, **39**, 237 (1999).

4 H. Sashiwa and Y. Shigemasa, *Carbohydr. Polym.*, **39**, 127 (1999).

5 H. Sashiwa, Y. Shigemasa, and R. Roy, *Chem. Lett.*, **2000**, 596.

6 Data for **5** (DS = 0.55): δ_H (D$_2$O) 2.06 (s, 0.6 H, NHAc), 2.44 (NHCO–CH_2), 2.51 (CH_2–CO$_2$H), 2.80 (br, 0.48 H, H-2 of GlcN unit), 3.4–4.0 (brm, 5.52 H, H-2 of *N*-acylated GlcN, H-2 of GlcNAc, and H-3,4,5,6 of hexosamine unit), 4.62 (br, 0.52 H, H-1 of *N*-acylated GlcN and GlcNAc unit), 4.90 (br, H-1 of GlcN unit); δ_C (0.1 M DCl/D$_2$O) 25.0 (NHAc), 31.7 (NHCO–CH$_2$), 31.9 (OCO–CH$_2$), 33.2 (CH$_2$–CO$_2$H), 58.5–58.7 (C-2), 62.7–63.3 (C-6), 72.9–74.1 (C-3), 77.2–77.9 (C-5), 79.4 (C-4), 100.3 (C-1 of GlcN), 103.8–104.1 (C-1 of *N*-acylated GlcN and GlcNAc), 177.5 (NHCO), 178.0 (OCO), 179.6 (CO$_2$H). Data for **6** (DS=2.28): δ_H (D$_2$O) 2.14 (s, 0.6 H, NHAc), 3.6–4.2 (brm, 6.0 H, H-2,3,4,5,6 of *N*-acylated GlcN and GlcNAc), 7.5–7.7 (m, 9.12 H, Ph); É¬$_C$ (D$_2$O) 25.0 (NHAc), 59.1 (C-2), 63.1 (C-6), 75.4 (C-3), 77.8 (C-5), 80.6 (C-4), 103.4 (C-1), 130.0–140.4 (Ph), 175.6 (NHCO), 177.5 (OCO), 178.7 (CO$_2$H). Data for **7** (DS=2.36): δ_H (D$_2$O) 2.14 (s, 0.6 H, NHAc), 3.6–4.2 (brm, 6.0 H, H-2,3,4,5,6 of *N*-acylated GlcN and GlcNAc), 7.5–8.2 (m, 7.08 H, Ph); δ_C (D$_2$O) 25.0 (NHAc), 59.1 (C-2), 63.0 (C-6), 75.3 (C-3), 77.7 (C-5), 80.6 (C-4), 103.4 (C-1), 130.1–143.4 (Ph), 175.1 (NHAc), 176.9 (NHCO), 177.1 (OCO), 178.5 (CO$_2$H).

7 a) S. Nishimura, O. Kohgo, K. Kurita, C. Vittavatvong, and H. Kuzuhara, *Chem. Lett.*, **1990**, 243. b) S. Nishimura, O. Kohgo, and K. Kurita, *Macromolecules*, **24**, 4745 (1991).