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Chemical modification of chitin and chitosan 2: preparation and water soluble property of *N*-acylated or *N*-alkylated partially deacetylated chitins

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Abstract

N-Acylated partially deacetylated chitin (DAC-88) derivatives were prepared via ring-opening reactions with various cyclic acid anhydrides in aqueous MeOH system. *N*-Alkylation of DAC-88 were also performed in aqueous MeOH with various aldehydes, monosaccharides, and disaccharides. The water solubility of *N*-acylated and *N*-alkylated chitosan derivatives at various pHs were studied.

Keywords: *N*-acylated partially deacylated chitin (DAC-88); Degree of deacetylation; Gel permeation chromatography

1. Introduction

Chitosan is a polysaccharide formed primarily of repeating units of β-(1–4)2-amino-2-deoxy-D-glucose (D-glucosamine). Generally, chitosan is soluble in aqueous medium in the presence of a small amount of acids such as AcOH, lactic acid, HCl and so on. Though the chitosan dissolves in aqueous acidic medium below pH 6.5, it precipitates above this pH by the addition of alkali solution like aq. NaOH. The application of chitosan was limited owing to the insolubility at neutral or high pH region.

To improve the soluble property of chitosan, generally, partial *N*-acetylation or chemical modification is required. For example, it was reported that partially *N*-acetylated chitosans with the degree of deacetylation (DDA) around 50% were soluble at neutral pH values (Sannan and Kurita, 1976; Varum et al., 1994). For the chemical modification, the reaction of chitosan with some cyclic acid anhydrides was reported (Hirano and Moriyasu, 1981; Yamaguchi et al., 1981). Reductive *N*-alkylation of chitosan was performed with various aldehydes (Muzzarelli et al., 1982a,b; Muzzarelli and Tanfani, 1985; Tong et al., 1991). Moreover, the modified chitosan with various saccharides was also prepared (Yalpani and Hall, 1984). In some cases, however, ^{1}H and ^{13}C NMR spectra of these chitosan derivatives were not clarified sufficiently and those were not described as the water soluble properties, especially for the correlation between the pH and solubility of these chitosan derivatives. To reveal the earlier problems, herein we report the preparation and water soluble property at various pHs of *N*-acylated and *N*-alkylated chitosan.

2. Results and discussion

2.1. N-Acylation of DAC-88

The *N*-acylation of 88% deacetylated chitin (DAC-88) with various cyclic acid anhydrides are summarized in Scheme 1 and Table 1. The DS values of products were 0.21–0.44 by the addition of 3 equivalent of anhydrides per amino group of DAC-88. From the data reported by Yamaguchi et al., 1981, the DS value of *N*-succinylation was 0.61–0.66 (which were determined by following three methods: method A, measured the amount of succinic acid released by saponification; B, measured the amount of unsubstituted amino groups with ninhydrin; C, elemental analysis) by the addition of 3.67 equivalent of anhydride per amino group of chitosan. The DS values with other cyclic acid anhydrides except for trimellitic anhydride were reported from 0.53–0.80 determined by elemental analysis) by the addition of 3–5 equivalent of anhydride (Hirano and Moriyasu, 1981). Though the DS values in

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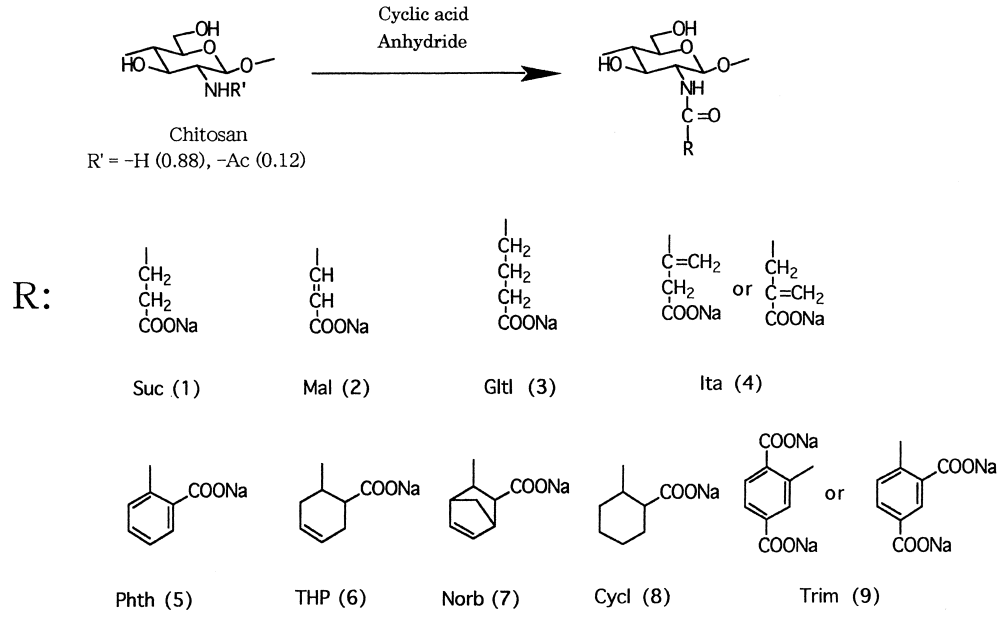


Scheme 1.

Table 1
N-acylation of DAC-88 with various cyclic acid anhydrides[a]

Entry	Anhydride[b]	Equiv	Reaction mix.[c]	Recovery (wt% (g/g))[d]	DS[e]	FW of MR[f]	Yield (%)[g]	$\overline{Mn}$[h]
1	Suc	3	gel	120	0.32	205	97	37 000
2		6	gel	135	0.47	223	100	16 000
3		9	gel	130	0.55	233	93	17 000
4		12	gel	140	0.65	245	95	26 000
5	Mal	3	homo	116	0.24	195	99	43 000
6		6	gel	120	0.30	202	98	
7		12	gel	120	0.48	224	89	
8	Gltl	3	gel	146	0.43	224	108	36 000
9		6	gel	130	(0.50)	234	92	18 000
10	Ita	3	gel	113	0.32	209	90	
11		12	gel	148	0.67	256	96	
12	Phth	3	homo	135	0.34	224	100	27 000
13		12	t. insoluble	128	0.39	232	91	
14	THP	3	homo	120	0.32	222	90	45 000
15		12	gel	180	0.76	294	100	36 000
16	Norb	3	gel	143	0.33	244	97	
17	Cycl	1.5	homo	106	(0.36)	229	77	
18		3	gel	130	(0.44)	243	88	30 000
19		9	gel	175	(0.82)	310	94	31 000
20		15	gel	146	(0.84)	314	77	36 000
21	Trim	3	emulsion	144	0.21	216	111	24 000

[a] DAC-88, 1.0 g (5.3 mmol/–NH_2; $\overline{Mn}$ 24 000); 24 h; 30°C; solvent, lactic acid/H_2O/MeOH = 1/20/80 (ml/ml/ml).

[b] Mol equivalent/–NH_2. Suc, succinic; Mal, maleic; Gltl, glutalic; Ita, itaconic; Phth, phthalic; THP, *cis*-1,2,3,6-tetrahydrophthalic; Norb, 5-norbornyl-endo-2,3-dicarboxylic; Cycl, *cis*-1,2-cyclohexyl dicarboxylic; Trim, trimellitic anhydride.

[c] Homo, homogeneous solution; t. insoluble: contain swelled and transparent products; color, pale yellow.

[d] Recovery (%) = [Wt of product (g)/wt of DAC-88] × 100; color, pale yellow.

[e] Determined by ^{1}H NMR; DS was determined from the area ratio of substituted group protons and methyl proton of *N*-acetyl group (0.36 H); data in parentheses were determined from the area ratio of substituted group protons to H-3, H-4, H-5, H-6 protons of the hexosamine residue and H-2 proton of *N*-acylated hexosamine residue.

[f] For example, FW of *N*-suiccinyl DAC-88 was calculated as follows; FW of *N*-succinyl DAC-88 = (FW of *N*-succinylGlcN-18) × DS + (FW of GlcNAc-18) × 0.12 + (FW of GlcN-18) (1-DS-0.12).

[g] Yield (%) = [Monosaccharide residue (MR) of product/MR of DAC-88] × 100; FW of MR of DAC-88 = 166.

[h] Determined by GPC with pullulan as standard; $\overline{Mn}$, number average molecular weight.

Table 2
Solubility of *N*-acylated DAC-88 in water of various pHs[a]

Sample	DS	Solubility[b] pH: 1 3 5 7 9 11 13
Suc	0.32	
Gltl	0.43	
Phth	0.34	
THP	0.32	
Norb	0.33	
Cycl	0.36	
Trim	0.20	
DAC-88		

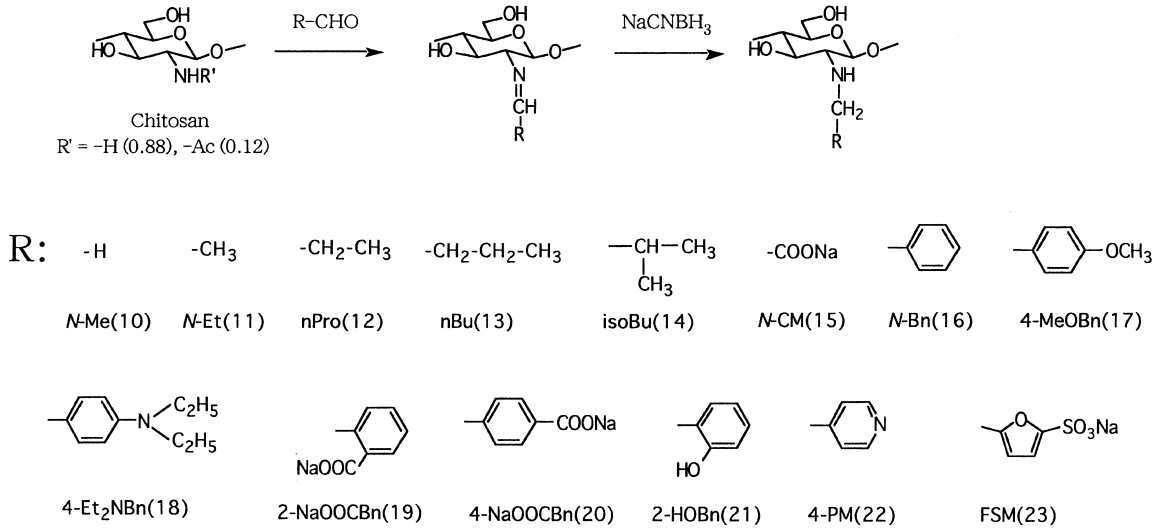
[a] Solid sample (100 mg; DDA = 88%) was dispersed in H_2O (20 ml). The pH of the solution was adjusted with 0.5% (w/v) aqueous HCl and NaOH.
[b] white bar, soluble; black bar, insoluble.

our cases were somewhat low, the desired DS value of products could be obtained by use of excess amount of anhydride (6–15 equiv) for the preparation of *N*-succinyl and *N*-(2-carboxycyclohexancarbonyl) DAC-88.

Table 2 shows the solubility of DAC-88 and *N*-acylated DAC-88 of DS 0.20–0.43 in water of various pH. DAC-88, which is the starting material in this study, dissolved only in the acidic region below pH 6.5. The *N*-acylated derivatives showed the solubility even in the basic region above pH 7–8. As these derivatives (DS = 0.20–0.43) have both amino (0.45–0.56 per monosaccharide residue) and carboxy group (0.32–0.43 per monosaccharide residue, trimellitic derivative (DS = 0.2) has two carboxy groups in substituted group), these derivatives have a property as polyampholyte. The solubility in acidic region would be caused by the protonation of amino group (changed from $-NH_2$ to $-NH_3^+$). The solubility in alkaline region would also be caused by the change of carboxy group to carboxylate ion (from $-COOH$ to $-COO^-$). The insolubility between pH 3.5–7.0 would be owing to the isoelectric point which exists equimolar of $-NH_3^+$ and $-COO^-$ groups in the molecule. The reason why several samples have different region of insoluble pH would be caused by the scatter of substituted functional groups in the product. Namely, the number of carboxy group will be different in each polysaccharide included in the same products. The DS value shown in Table 2 is the average value in each polysaccharide. Therefore, the insoluble pH region of samples were widely distributed (pH 3.5–7.0). The maleoyl (Mal) DAC-88 and itaconyl (Ita) DAC-88 of various DS, which have vinyl groups, did not dissolve in both acidic and basic water after standing for more than 1–2 months in air, though these showed the same solubility as *N*-acylated derivatives listed in Table 2 after the preparation for 1–2 weeks. This change of solubility would be caused by either the slow reformation of hydrogen bond, crosslinking, or grafting owing to the vinyl group in substituted moiety.

2.2. N-Alkylation of DAC-88 with various aldehydes

Scheme 2 and Table 3 show the *N*-alkylation of DAC-88 with various aldehydes. The degree of *N*-alkylation (DS) was relatively high independent on the molecular size of aldehyde used in this study. In the case of HCHO, the DS value was over 0.88 which would be caused by a part of *N*,*N*-disubstitution of amino group. The solubility of *N*-alkylated DAC-88 are shown in Table 4. Almost all of DAC-88 derivatives having no anionic group showed similar property of solubility to original DAC-88, though the pH of the insoluble point were different from each other. *N*-alkylated derivatives having aromatic moiety such as *N*-benzyl (*N*-Bn), *N*-(4-methoxybenzyl) (4-MeOBn),



Scheme 2.

N-(4-diethylaminoethylbenzyl) (4-Et_2NBn), *N*-(4-pyridinylmethyl) (4-PM), and *N*-(2-hydroxybenzyl) (2-HOBn) DAC-88 showed lower pH of the insoluble point compared with original DAC-88 and other *N*-alkylated DAC-88 derivatives. The insoluble point of *N*-ethyl DAC-88 (*N*-Et) was almost the same as DAC-88. The insoluble point of pH of *N*-methyl (*N*-Me), *N*-npropyl (nPro), *N*-nbutyl (nBu), *N*-isobutyl (isoBu) DAC-88 were slightly lower than that of original DAC-88. These differences would be due to the hydrophobicity or the interaction between substituted groups of these derivatives. The protonation of secondary amino group and DS would also affect the difference of insoluble point. *N*-(2-Carboxybenzyl) (2-NaOOCBn) and *N*-(4-carboxybenzyl) (4-NaOOCBn) DAC-88 having carboxy group showed similar solubility to *N*-acylated DAC-88 derivatives listed in Table 2. However, the insolubility of these derivatives

Table 3
N-Alkylation of DAS-88 with various aldehydes[a]

Entry	Aldehyde[b]	Equiv	Time (h)	$NaCNBH_3$(equiv)[c]	Recovery wt.%(g/g)[d]	DS[e]	FW of MR[f]	Yield (%)[g]	$\overline{Mn}$
1	HCHO	3	3	4	110	1.28	184	99	34 000
2	CH_3CHO	3	3	4	108	0.81	188	96	13 000
3	CH_3CH_2CHO	3	3	4	120	0.77	198	100	12 000
4	$CH_3(CH_2)_2CHO$	3	3	4	132	0.70	205	100	6 000
5	$(CH_3)_2CHCHO$	3	3	4	115	0.80	211	91	13 000
6	GOA	3	24	4	145	1.03	248	97	
7	C_6H_5CHO	3	3	4	142	0.79	237	90	
8	4-MeO–C_6H_4CHO	3	3	4	160	0.86	269	95	
9	4-Et_2N–C_6H_4CHO	3	3	4	153	0.88	308	83	24 000
10	2-FBA	3	3	4	127	0.69	274	77	
11	4-FBA	3	24	10	138	0.86	300	77	12 000
12	4-FBA	3	3	4	150	0.86	300	83	
13	4-FP	3	24	4	130	0.72	232	93	22 000
14	SA	12	24	10	104	0.45	214	81	
15	SA	3	3	4	116	0.79	250	77	
16	FFSA	3	3	4	190	0.78	308	100	43 000

[a] DAC-88, 1.0 g (5.3 mmol/–NH_2; $\overline{Mn}$ 24 000); 30°C; solvent, lactic acid/H_2O/MeOH = 1/20/80 (ml/ml/ml).

[b] Mol equivalent/–NH_2. GOA,glyoxillic acid; 2-FBA, 2-formylbenzoic acid; 4-FBA, 4-formylbenzoic acid; 4-FP, 4-formylpyridine; SA, salicylaldehyde; FFSA, 2-formyl-5-furansulfonic acid.

[c] Time, 24 h.

[d] Recovery(%) = [Wt of product (g)/wt of DAC-88] × 100; color, pale yellow.

[e] Determined by ^{1}H NMR; DS was determined from the area ratio of substituted group protons and methyl proton of *N*-acetyl group (0.36 H).

[f] For example, FW of *N*-methyl DAC-88 was calculated as follows: FW of *N*-methyl DAC-88 = (FW of methyl GlcN-18) × DS + (FW of GlcNAc-18) × 0.12 + (FW of GlcN-18) (1-DS-0.12).

[g] Yield(%) = [Monosaccharide residue (MR) of product/MR of DAC-88] × 100; FW of MR of DAC-88 = 166.

Table 4
Solubility of *N*-alkylated DAC-88 in water of various pHs[a]

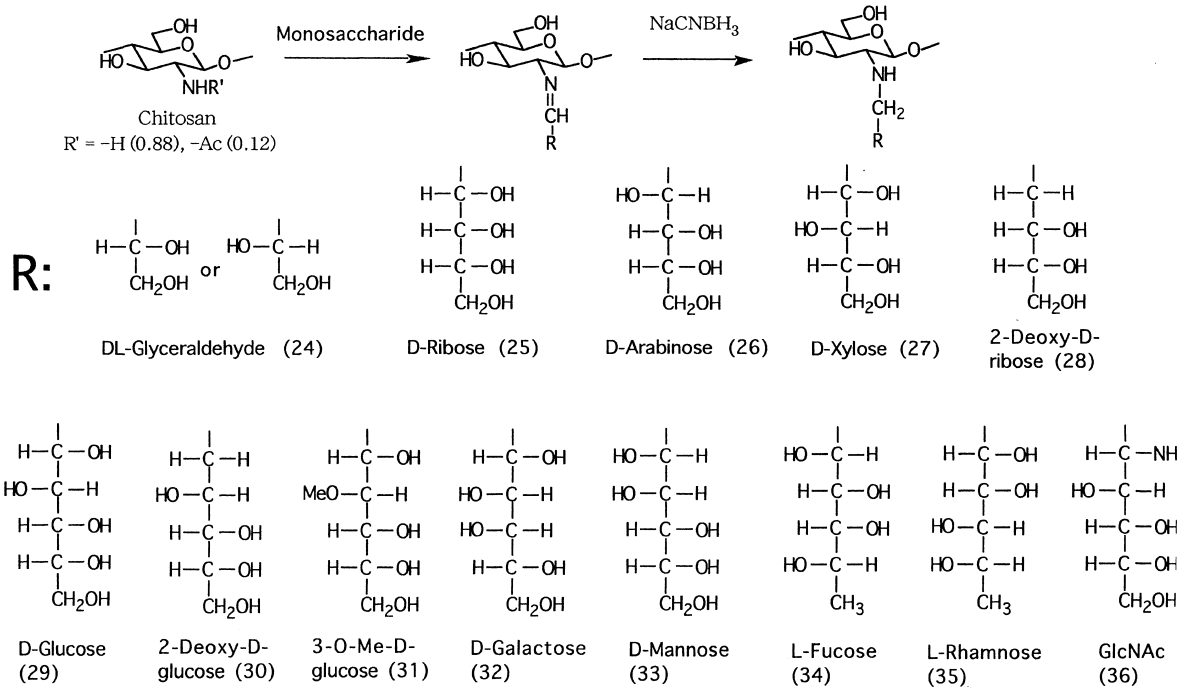
Sample	DS	Solubility[b] pH 0.5 1 3 5 7 9 11 13
N–Me	1.28	
N–Et	0.81	
nPro	0.77	
nBu	0.70	
isoBu	0.80	
N-Bn	0.79	
4-MeOBn	0.86	
4-Et_2NBn	0.88	
2-NaOOCBn	0.69	
4-NaOOCBn	0.86	
4-PM	0.72	
2-HOBn	0.45	
FSM	0.78	
DAC-88		

[a] Solid sample (100 mg; DDA = 88%) was dispersed in H_2O (20 ml). The pH of the solution was adjusted with 0.5% (w/v) aqueous HCl and NaOH.
[b] White bar, soluble; black bar, insoluble.

below pH 1–1.5 were unclear. *N*-(5-furansulfonyl-2-methyl) DAC-88 having sulfonic group also showed similar solubility to *N*-acylated DAC-88 derivatives.

2.3. N-Alkylation of DAC-88 with various mono and disaccharides

Yalpani and Hall, 1984 reported various *N*-alkylated chitosan derivatives with saccharides such as D-glucose, *N*-acetyl-D-glucosamine (GlcNAc), D-glucosamine, D-galactose, D-galactosamine, lactose, cellobiose, maltose, melibiose, maltotoriose, D-fructose, and dextran. They also reported the solubility of these derivatives in aqueous solution (pH 7), dilute acidic solution (pH 5–6), and dilute basic solution (pH 8). By partly including the earlier derivatives, we prepared additional kinds of *N*-alkylated DAC-88 derivatives using triose (glyceraldehyde), pentose (D-ribose, D-arabinose, D-xylose, 2-deoxy-D-ribose), and hexose (2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, D-mannose, L-fucose, L-rhamnose) according to their method (Yalpani and Hall, 1984). Scheme 3 and Table 5 shows the *N*-alkylation of DAC-88 with glycolaldehyde, glyceraldehyde (triose), pentoses and hexsoses. In the case of using glycolaldehyde (dimer), only water insoluble material was



Scheme 3.

obtained which would be caused by the crosslink formation between substituted glycolaldehyde dimer. Though the DS value of these derivatives varied from 0.37–1.02 under the same conditions, they were soluble in dilute acidic solution except for glycolaldehyde. The *N*-alkylation of DAC-88 with various disaccharides are also listed in Scheme 4 and Table 6. In the case of lactose and cellobiose, the addition of MeOH caused a decrease in DS value owing to the insolubility of disaccharide in H_2O and MeOH (20/80 and 50/50). Melibiose which was partially soluble in H_2O and MeOH (20/80) gave high DS value. Maltose was tested only in H_2O medium.

Table 7 shows the solubility of these derivatives in water at various pH. Some derivatives such as D-arabinose,

Table 5
N-alkylation of DAC-88 with various monosaccharides[a]

Entry	Monosaccharide	Recovery wt.%(g/g)[b]	DS[c]	FW of MR	Yield (%)[d]	$\overline{Mn}$
1	Glycolaldehyde	106	—[e]	—[e]	—[e]	
2	DL-Glyceraldehyde	131	0.69	217	100	20 000
3	D-Ribose	180	0.93	291	100	
4	D-Arabinose	170	0.84	279	100	25 000
5	D-Xylose	136	0.53	237	95	37 000
6	2-Deoxy-D-ribose	171	1.02	286	99	28 000
7	D-Gulcose	162	0.43	237	100	33 000
8	2-Deoxy-D-glucose	161	0.82	287	93	26 000
9	3-O-Me-D-Glucose	158	0.46	248	100	
10	D-Galactose	140	0.37	227	100	27 000
11	D-Mannose	163	0.60	264	100	45 000
12	L-Fucose	157	0.75	277	94	44 000
13	L-Rhamnose	163	0.72	273	99	29 000
14	GlcNAc	143	0.45	259	92	

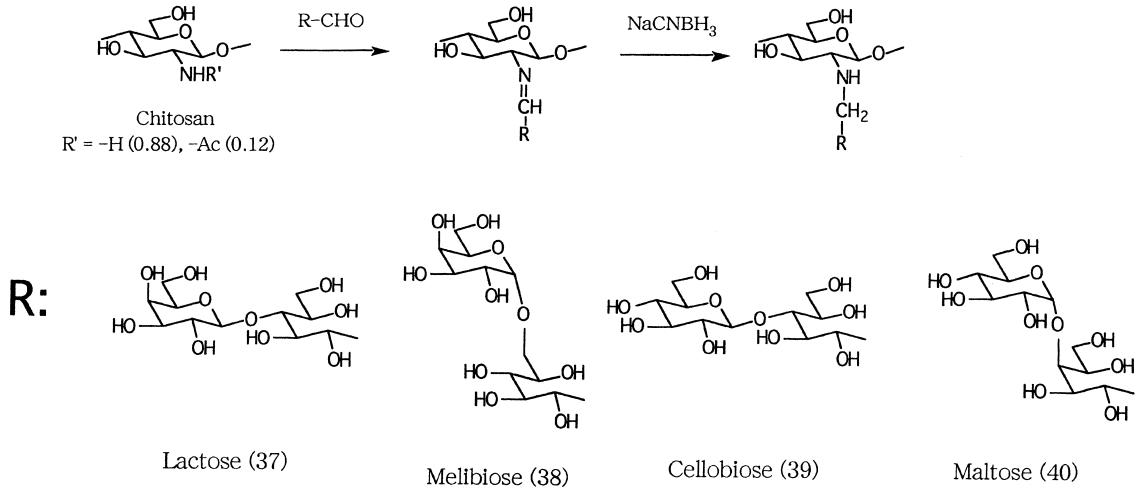
[a] DAC-88, 1.0 g (5.3 mmol/–NH_2; $\overline{Mn}$ 24 000); solvent, lactic acid/H_2O/MeOH = 1/20/80 (ml/ml/ml); monosaccharide, 3 equiv/–NH_2; 25°C; 3 h; $NaCNBH_3$, 4 equiv/–NH_2; 25°C; 24 h.

[b] Recovery(%) = [Wt of product (g)/wt of DAC-88] × 100; color, pale yellow.

[c] Determined by ^{1}H NMR.

[d] Yield(%) = [Monosaccharide residue (mol) of product/monosaccharide residue (mol) of DAC-88] × 100; FW of monosaccharide residue of DAC-88 = 166.

[e] The DS of this sample could not estimated by ^{1}H NMR owing to the insolubility iin D_2O, 0.5 M DCl/D_2O, and 5.5 M DCl/D_2O.



Scheme 4.

2-deoxy-D-glucose, lactose, cellobiose, and maltose directly dissolved in distilled water and dissolved at all pH regions. The *N*-alkylated derivatives with DL-Glyceraldehyde, D-ribose, D-glucose, D-galactose, D-mannose, and L-fucose showed the solubility at all pH regions after dissolving them with dilute HCl solution, though these derivatives were insoluble in distilled water directly. These phenomena would be explained by the fact that the hydration in acidic medium would be an important process to dissolve these derivatives in water. The same phenomenon was also observed in the case of partially *N*-acetylated chitosan with DDA = 50% reported by Aiba (1989). The *N*-alkylated derivatives with D-xylose showed slight emulsion at basic pH region (above pH 6.5). After standing for more than 3–6 months in air, some derivatives substituted with 2-deoxy-D-ribose, 3-O-methyl-D-glucose, GlcNAc, and L-rhamnose did not dissolve in dilute acidic pH region, though they showed the same solubility as DL-glycelaldehyde, D-ribose, etc., after the preparation for 1–2 months. The change of solubility would be caused by the slow reformation of the hydrogen bond of these derivatives.

In conclusion, the *N*-acyl and *N*-alkyl derivatives of DAC-88 having carboxy or sulfate group showed the solubility at basic pH region. Moreover, some of DAC-88 derivatives substituted with saccharides showed the solubility at all pH ranges and were stable for at least 2 weeks. These derivatives would be useful for the treatment at neutral or basic pH region to test biological activities in vitro and in vivo. Especially, the recognition or signaling properties against various animal cells of DAC-88 derivatives substituted with saccharide are of great interest.

3. Experimental section

3.1. Materials

Partially deacetylated chitin (DAC-88) from crab shell was purchased from Kyowa Technos Co., Ltd. Other reagents were purchased from Nacalai Tesque, Inc. and used without further purification.

Table 6
N-alkylation of DAC-88 with various disaccharides[a]

Entry	Disaccharide	Equiv	H_2O/MeOH (ml/ml)	Time (h)	Recovery (wt% (g/g))[b]	FW of MR	DS[c]	Yield (%)[d]	$\overline{Mn}$
1	Lactose	3	20/80	3	135	208	0.13	94	
2	Lactose	3	50/50	5	155	215	0.15	99	35 000
3	Lactose	6	100/0	1	244	400	0.71	100	43 000
4	Melibiose	3	20/80	3	135	410	0.74	55	53 000
5	Cellobiose	3	20/80	3	127	189	0.07	79	33 000
6	Cellobiose	3	100/0	0.5	200	322	0.48	100	25 000
7	Maltose	3	100/0	0.5	160	309	0.44	86	52 000

[a] DAC-88, 1.0 g (5.3 mmol/–NH_2; $\overline{Mn}$ 24 000); additive, lactic acid, 1 ml; 25°C; 3 h; $NaCNBH_3$, 4 equiv/–NH_2; 25°C; 24 h.
[b] Recovery (%) = [Wt of product (g)/wt of DAC-88] × 100; color, pale yellow.
[c] Determined by ^1H NMR.
[d] Yield(%) = [Monosaccharide residue (mol) of product/monosaccharide residue (mol) of DAC-88] × 100; FW of monosaccharide residue of DAC-88 = 166.

Table 7
Solubility of DAC-88 derivatives substituted with various saccharides in water at various pHs[a]

Sample	DS	Solubility[b] pH: 1 3 5 7 9 11 13
Glycolaldehyde	—	
DL-Glyceraldehyde	0.69	
D-Ribose	0.93	
D-Arabinose[c]	0.84	
D-Xylose	0.53	
D-Glucose	0.43	
2-Deoxy-D-glucose[c]	0.82	
D-Galactose	0.37	
D-Mannose	0.60	
L-Fucose	0.75	
Lactose[c]	0.71	
Cellobiose[c]	0.48	
Maltose[c]	0.44	

[a] Solid sample (100 mg; DDA mg; DDA = 88%) was dispersed in H_2O (20 mL) and the pH of the suspension was adjusted to 1.0 with 0.5% (w/v) aqueous HCl. The pH of the solution was adjusted with 0.5% (w/v) aqueous NaOH.
[b] White bar, soluble; shaded bar, partially soluble; black bar, insoluble.
[c] Soluble in H_2O.

3.2. General methods

^1H and ^{13}C NMR spectra were recorded for solutions in D_2O or 0.1 M DCl/D_2O on JEOL JMN-GX270 spectrometer, using sodium 3-(trimethylsilyl)propanesulfonate as an internal standard. Molecular weight of DAC-88 derivatives was determined by gel permeation chromatography (GPC) with pullulan as standard on a Shimadzu LC-6A apparatus (column, Asahipak GS-220H, GS-310H, and GS-510H; eluent, 0.1 M AcOH buffer containing 0.1 M NaCl (pH 4.7) or 0.1 M phosphate buffer (pH 7.4); flow rate, 1.0 mL/min; column temperature, 50°C.

3.3. N-acylation of DAC-88 (typical procedure)

DAC-88 (1.0 g) was dissolved in 4.8% (v/v) aq. lactic acid (21 mL), and then the pale yellow solution was diluted with 80 mL of MeOH. Prescribed amount of cyclic acid anhydride was added to the diluted solution and stirred at room temperature (rt). In many cases, the mixture was turned to gel or emulsion after 10–30 min. After standing

for 24 h, the pH of the mixture was adjusted to 5.0 with 5% w/v aq. NaOH (ca. 5 mL) to give a precipitate. The precipitate was collected by filtration and dispersed in 50 mL of H_2O. The pH of the dispersion was adjusted to 10–12 with 5% w/v aq. NaOH to give a pale yellow solution. The solution was dialyzed using dialysis membrane (molecular weight cut off, 12 000–14 000; Viskase Sales Corp.) at rt for 2–3 days and lyophilized. The lyophilized samples (1.2–1.8 g) were recovered from 1.0 g of DAC-88.

(1) *N*-succinyl DAC-88 (DS = 0.32): ^{1}H NMR(D_2O) δ = 2.03 (0.36 H, s, –NH(CO)CH_3), 2.44 (0.63 H, br s, –NH(CO)–CH_2–), 2.51 (0.62 H, br s, –CH_2–COONa), 2.78 (0.56 H, br m, H-2 of GlcN), 3.4–4.0 (br m, H-2 of *N*-acylated GlcN and H-3, H-4, H-5, H-6 of monosaccharide residue (MR)); DS = (0.63 + 0.62)/4 = 0.32; ^{13}C NMR δ = 24.8(–NH(CO)CH_3), 35.2, 35.5(–CH_2–CH_2–), 57.8–59.1(C-2), 62.8(C-6), 72.3–81.0 (C-3, C-4, C-5), 103.7(C-1), 177.2(–NH(*C*O)CH_3), 178.9 (–NH(*C*O)–CH_2–CH_2–), 183.7 (–*C*OONa).

(2) *N*-maleoyl DAC-88 (DS = 0.30): ^{1}H NMR(D_2O) δ = 2.04 (0.36 H, s, –NH(CO)CH_3,), 2.71 (0.58 H, br s, H-2 of GlcN), 3.4–4.0 (br m, H-2 of *N*-acylated GlcN and H-3, H-4, H-5, H-6 of MR); 5.94 (0.30 H, br s, –NH(CO)–C*H*=CH–); 6.41 (0.30 H, br s, –CH=C*H*–COONa); DS = (0.30 + 0.30)/2 = 0.30; ^{13}C NMR δ = 24.8 (–NH(CO)–*C*H_3), 57.1–59.4 (C-2), 62.9(C-6), 74.9–81.8 (C-3, C-4, C-5), 104.2 (C-1), 126.3 (–NH(CO)–*C*H=CH–), 140.5 (–CH=*C*H–COONa), 170.4 (–NH(*C*O)CH=CH–), 177.3 (–NH(*C*O)–CH_3 and –*C*OONa).

(3) *N*-glutalyl DAC-88 (DS = 0.43): ^{1}H NMR (0.5 M DCl/D_2O) δ = 1.84 (0.89 H, br m, –CH_2–CH_2–CH_2–); 2.05 (0.36 H, br s, –NH(CO)CH_3), 2.43 (1.66 H, br m, –NH(CO)–CH_2–CH_2–CH_2–COONa); 3.19 (0.45 H, br m, H-2 of GlcN), 3.4–4.0 (br m, H-2 of *N*-acylated GlcN and H-3, H-4, H-5, H-6 of MR); DS = (0.89 + 1.66)/6 = 0.43; ^{13}C NMR δ = 24.8(–NH(CO)*C*H_3 and CH_2–*C*H_2–CH_2–), 38.2, 39.3(–NH(CO)–*C*H_2–CH_2–*C*H_2–COONa), 57.0–58.2(C-2), 62.7(C-6), 72.4–81.0 (C-3, C-4, C-5), 101.2, 103.6 (C-1), 177.2 (NH(*C*O)CH_3), 179.5 (–NH(*C*O)–CH_2–), 185.0 (–*C*OONa).

(4) *N*-itaconyl DAC-88 (DS = 0.32): ^{1}H NMR(D_2O) δ = 2.05 (0.36 H, s, –NH(CO)CH_3), 2.20 (0.20 H, s, –NH(CO)–CH_2–C(=CH_2)–); 2.83 (0.56 H, br m, H-2 of GlcN); 3.29 (0.44 H, s, –C(=CH_2)–CH_2–COONa), 3.4–4.0 (br m, H-2 of *N*-acylated GlcN and H-3, H-4, H-5, H-6 of MR), 5.52 (0.31 H, br s, –NH(CO)–C(=CH_2)–), 5.97 (0.32 H, br s, –C(=CH_2)–COONa); DS = (0.31 + 0.32)/2 = 0.32; ^{13}C NMR δ = 24.8(–NH(CO)*C*H_3), 43.1(–NH(CO)–*C*H_2– and –*C*H_2–COONa), 58.0–59.1(C-2), 62.9(C-6), 71.9–81.9(C-3, C-4, C-5), 103.5(C-1), 127.1((CO)–C(=*C*H_2)–), 142.3(–C(=*C*H_2)–COONa), 177.4 (–NH(*C*O)CH_3, –NH(*C*O)–CH_2–, and –*C*OONa).

(5) *N*-phthaloyl DAC-88 (DS = 0.34): ^{1}H NMR(D_2O) δ = 2.04 (0.36 H, s, –NH(CO)CH_3), 2.71 (0.54 H, br s, H-2 of GlcN), 3.4–4.0 (5.41 H, br m, H-2 of *N*-acylated GlcN and H-3, H-4, H-5, H-6 of MR); 7.52 (1.36 H, br m, *Ph*); DS = 1.36/4 = 0.34; ^{13}C NMR δ = 24.8 (–NH(*C*O)CH_3), 59.2(C-2), 62.9(C-6), 72.3–81.4(C-3, C-4, C-5), 103.5–105.3(C-1), 129.8, 131.0, 132.2, 133.5, 136.2, and 140.7(*Ph*), 177.5(–NH(*C*O)CH_3), 177.2(–NH(*C*O)–Ph), 179.0(–*C*OONa).

(6) *N*-tetrahydrophthaloyl DAC-88(DS = 0.32): ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.03(0.36H, s, –NH(CO)CH_3), 2.42(1.28 H, br s, –CH_2–), 3.08–3.20(0.99 H, br m, –C*H*– and H-2 of GlcN), 3.4–4.0 (br m, H-2 of *N*-acylated GlcN and H-3, H-4, H-5, H-6 of MR); 5.75(0.64 H, s, –C*H*=C*H*–); DS = 0.64/2 = 0.32; ^{13}C NMR δ = 24.8(–NH(CO)*C*H_3), 28.8–29.6(–*C*H_2–), 43.8–46.5(–*C*H–), 58.3(C-2), 63.3(C-6), 72.3–81.8(C-3, C-4, C-5), 103.2–104.0(C-1), 127.7, 129.3(–*C*H=*C*H–), 177.2(–NH(*C*O)CH_3), 180.0(–NH(*C*O)–CH–), 185.0(–*C*OONa).

(7) *N*-(2-carboxy-4-norbornenecarbonyl) DAC-88 (DS = 0.33): ^{1}H NMR(D_2O) δ = 1.36(0.66 H, s, –CH_2–), 2.03 (0.36 H, s, –NH(CO)CH_3), 2.69(0.55 H, br, H-2 of GlcN), 3.07(0.33 H, s, –C*H*–); 3.25(0.83 H, s, –C*H*–), 3.4–4.0(5.42 H, br m, H-2 of *N*-acylated GlcN and H-3, H-4, H-5, H-6 of MR); 6.20–6.35(0.84 H, br, –C*H*=C*H*–); DS = 0.66/2 = 0.33; ^{13}C NMR δ = 24.9(–NH(CO)*C*H_3), 49.5(–*C*H_2–), 51.5, 55.3(–*C*H–), 58.2, 59.2(C-2), 63.0(C-6), 77.5(C-3, C-5), 103.5–103.9(C-1), 137.0–139.6(–*C*H=*C*H–), 177.3(–NH(*C*O)CH_3), 179.2(–NH(*C*O)–CH), 183.8(–*C*OONa).

(8) *N*-(2-carboxylcyclohexanecarbonyl) DAC-88 (DS = 0.36): ^{1}H NMR(D_2O) δ = 1.43 and 1.67 (2.88 H, br m, –CH_2–), 2.04 (0.36 H, br m, –NH(CO)CH_3), 2.68 (1.36 H, br m, H-2 of GlcN and –C*H*–), 3.4–4.0 (6.00 H, br m, H-2 of *N*-acylated GlcN and H-3, H-4, H-5, H-6 of MR); DS = 3.16 × (5.12 + DS)/(6 × 8); ^{13}C NMR(D_2O) δ = 24.8(–NH(CO)*C*H_3), 25.6–26.7(–*C*H_2–), 45.6–48.4(–*C*H–), 59.0(C-2), 62.8(C-6), 72.3–81.8(C-3, C-4, C-5), 103.5–105.1(C-1), 177.2(–NH(*C*O)CH_3), 180.9–181.5(–NH(*C*O)–CH), 185.2 (–*C*OONa).

(9) *N*-(2,4-dicarboxybenzoyl) DAC-88 (DS = 0.21): ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.07(0.36 H, s, –NH(CO)CH_3), 3.24 (0.59 H, br s, H-2 of GlcN), 3.4–4.0 (br m, H-2 of *N*-acylated GlcN and H-3, H-4, H-5, H-6 of MR); 7.69–8.50 (0.62 H, br m, *Ph*); DS = 0.62/3 = 0.21; ^{13}C NMR δ = 24.8(–NH(CO)*C*H_3), 58.5(C-2), 62.8(C-6), 72.7(C-3), 77.4(C-5), 79.3(C-4), 100.1 (C-1), 131.0–136.0 (*Ph*), 177.2(–NH(*C*O)CH_3).

3.4. N-alkylation of DAC-88 with various aldehydes (typical procedure)

DAC-88 (1.0 g) was dissolved in 4.8% w/v aq. lactic acid (21 mL). The solution was diluted with MeOH (80 mL). Aldehyde (3 equiv/–NH_2 of DAC-88) was added to the diluted solution and stirred at rt. In many cases, the mixture was turned to gel or suspension after 1–6 min. After standing the mixture for the prescribed time, $NaCNBH_3$ (1.33 g: 4 equiv/–NH_2 of DAC-88) was added and stirred at rt for 24 h. The precipitate was collected by filtration, dispersed in 50 mL of H_2O, and the pH was adjusted to 10–12 with

5% w/v aq. NaOH. A clear solution was obtained to adjust pH 10–12 in the case of aldehydes such as 2-formylbenzoic acid (2-FBA), 4-formylbenzoic acid (4-FBA), 2-formyl-5-furansulfonic acid (FFSA). Glyoxillic acid (GOA) as aldehyde gave partially soluble material. Other aldehydes did not give a solution. The solution or suspension was dialyzed and lyophilized. The lyophilized samples (1.04–1.9 g) were recovered from 1.0 g of DAC-88.

(10) NMR data were report in detail by Domard et al.(1987).

(11) *N*-ethyl DAC-88 (DS = 0.80): ^{1}H NMR(0.5 M DCl/D_2O) δ = 1.44(2.44 H, br s, –CH_2–CH_3), 2.08(0.36 H, br s, –NH(CO)CH_3,), 3.4–4.4 (br m, H-2, H-3, H-4, H-5, H-6 of MR and –NH–CH_2–), 5.20 (0.80 H, br s, H-1 of *N*-alkylated GlcN); DS = 2.44/3 = 0.81; ^{13}C NMR (0.5 M DCl/D_2O) δ = 12.5–13.3(–CH_2–CH_3), 24.8(–NH(CO)CH_3), 51.5(–NH–CH_2–), 63.2(C-2), 66.1(C-6), 70.1(C-3), 77.2(C-5), 78.7(C-4), 81.7(C-4 of GlcNAc), 97.0–99.3(C-1), 103.8(C-1 of GlcNAc), 177.4(–NH(CO)CH_3).

(12) *N*-*n*-propyl DAC-88 (DS = 0.77): ^{1}H NMR(0.5 M DCl/D_2O) δ = 1.00(2.32 H, s, –CH_3), 1.83(1.50 H, br, –CH_2–CH_3), 2.05(0.36 H, br s, –NH(CO)CH_3), 3.3–4.4(br m, H-2, H-3, H-4, H-5, H-6 of MR and –NH–CH_2–); DS = 2.32/3 = 0.77; ^{13}C NMR δ = 13.1(–CH_3), 22.2(–CH_2–CH_2–), 25.1(–NH(CO)CH_3), 51.0(–NH–CH_2–), 63.5(C-2), 67.2(C-6), 70.4(C-3), 77.5(C-5), 78.7(C-4), 81.7(C-4 of GlcNAc), 98.2–99.4(C-1), 104.1(C-1 of GlcNAc), 177.7(–NH(CO)CH_3).

(13) *N*-*n*-butyl DAC-88 (DS = 0.70); ^{1}H NMR(0.5 M DCl/D_2O) δ = 0.93(2.11 H, s, –CH_3), 1.40(1.41 H, s, –CH_2–CH_3), 1.79(1.47 H, br s, –CH_2–CH_2–CH_3), 2.05(0.36 H, br s, –NH(CO)CH_3,), 3.3–4.4 (br m, H-2, H-3, H-4, H-5, H-6 of MR and –NH–CH_2–), 5.03, 5.19(0.66 H, br s, H-1 of *N*-alkylated GlcN); DS = 2.11/3 = 0.70; ^{13}C NMR δ = 15.7(–CH_3), 22.0(–CH_2–CH_3), 22.2(–CH_2–CH_2–), 25.1(–NH(CO)CH_3), 49.1(–NH–CH_2–), 63.4(C-2), 67.2(C-6), 70.5(C-3), 77.4(C-5), 78.8(C-4), 98.2–99.4(C-1), 104.1(C-1 of GlcNAc), 177.6(–NH(CO)CH_3).

(14) *N*-*iso*-butyl DAC-88 (DS = 0.80): ^{1}H NMR(0.5 M DCl/D_2O) δ = 1.01 (1.66 H, s, –CH_3), 2.04 (0.36 H, br s, –NH(CO)CH_3, (0.36 H) and –CH_2–CH–), 3.11 (br s, –NH–CH_2–), 3.29 (br s, H-2 of GlcN and *N*-alkylated GlcN), 3.4–4.2 (br m, H-2 of GlcNAc and H-3, H-4, H-5, H-6 of MR), 5.04 (0.22 H, br s, H-1 of *N*-alkylated GlcN); 6 × DS: 0.36 + DS = 1.66 H: 0.36 H, DS = 0.5/0.6 = 0.80; ^{13}C NMR δ = 21.6, 21.8(–CH_3), 24.8(–NH(CO)CH_3), 28.1(–CH_2–CH–), 55.9(–NH–CH_2–), 63.2(C-2), 64.1(C-6), 71.1(C-3), 77.2(C-5), 79.3(C-4), 81.5(C-4 of GlcNAc), 98.8(C-1), 103.9(C-1), 177.3(–NH(CO)CH_3).

(15) This derivative is also reported in detail by Muzzarelli et al., 1982a,b; Muzzarelli and Tanfani, 1985.

(16) *N*-benzyl DAC-88 (DS = 0.79): ^{1}H NMR (0.5 M DCl/D_2O) δ = 2.14 (0.36 H, br s, –NH(CO)CH_3,), 3.37 (0.88 H, br, H-2), 3.5–4.4 (br m, –NH–CH_2–, H-2 of GlcNAc, H-3, H-4, H-5, H-6 of MR), 7.60 (3.95 H, s, *Ph*); DS = 3.95/5 = 0.79; ^{13}C NMR δ = 25.1(–NH(CO)CH_3), 53.6(–NH–CH_2–), 63.6(C-2, C-6), 72.1(C-3), 77.4(C-5), 79.9(C-4), 99.5(C-1), 104.1(C-1 of GlcNAc), 132.3–134.0(*Ph*), 177.6(–NH(CO)CH_3).

(17) *N*-(4-methoxybenzyl) DAC-88 (DS = 0.86): ^{1}H NMR (0.5 M DCl/D_2O) δ = 2.21(0.36 H, br s, –NH(CO)CH_3,), 3.41(0.88 H, br s, H-2 of GlcN and *N*-alkylated GlcN), 3.6–4.4(br m, –NH–CH_2–, Ph–O–CH_3, H-2 of GlcNAc, H-3, H-4, H-5, H-6 of MR), 7.23 and 7.61 (3.45 H, s, *Ph*); DS = 3.45/4 = 0.86; ^{13}C NMR δ = 25.1(–NH(CO)CH_3), 53.1(–NH–CH_2–), 58.4(Ph–O–CH_3), 63.6(C-2, C-6), 72.1(C-3), 77.4(C-5), 79.9(C-4), 99.5(C-1), 104.2(C-1 of GlcNAc), 117.6, 125.6, 134.8, and 162.9 (*Ph*), 177.6(–NH(CO)CH_3).

(18) *N*-(4-diethylaminoethylbenzyl) DAC-88 (DS = 0.88): ^{1}H NMR (0.5 M DCl/D_2O) δ = 1.19 (5.29 H, s, –CH_3), 2.10 (0.36 H, br s, –NH(CO)CH_3), 3.37 (br s, H-2 of GlcN and *N*-alkylated GlcN), 3.5–4.4(br m, –NH–CH_2–, Ph–N–CH_2–, H-2 of GlcNAc, H-3, H-4, H-5, H-6 of MR), 7.71 and 7.81 (3.52 H, *Ph*); DS = 5.29/6 = 0.88; ^{13}C NMRδ = 12.3(–CH_3), 24.7(–NH(CO)CH_3), 52.4(–NH–CH_2–Ph), 56.4(Ph–N–CH_2–CH_3), 62.7(C-2), 63.4(C-6), 71.8(C-3), 77.1(C-5), 79.7(C-4), 99.2(C-1), 125.0, 1334.9, 135.0, 140.4(*Ph*), 177.3(–NH(CO)CH_3).

(19) *N*-(2-carboxybenzyl) DAC-88 (DS = 0.69): ^{1}H NMR(D_2O) δ = 2.05 (0.36 H, br s, –NH(CO)CH_3), 2.67(0.88 H, br s, H-2 of GlcN and *N*-alkylated GlcN), 3.5–4.0 (br m, –NH–CH_2–, H-2 of GlcNAc, H-3, H-4, H-5, H-6 of MR), 4.17 (1.39 H, br s, NH–CH_2–Ph), 7.41 –7.58 (2.75 H, br m, Ph); DS = 2.75/4 = 0.69; ^{13}C NMRδ = 24.8(–NH(CO)CH_3), 52.8(–NH–CH_2–Ph), 63.0(C-2), 63.6(C-6), 75.1(C-3), 77.6(C-5), 79.5(C-4) 104.0(C-1), 130.7, 132.1, 133.2, 137.5, and 141.8 (*Ph*), 177.3 (–NH(CO)CH_3), 179.9 (Ph–COONa).

(20) *N*-(4-carboxybenzyl) DAC-88 (DS = 0.86): ^{1}H NMR(D_2O) δ = 2.09(0.36 H, br s, –NH(CO)CH_3,), 2.69 (0.86 H, br s, H-2 of *N*-alkylated GlcN), 3.5–4.3 (br m, H-2 of GlcNAc, H-3, H-4, H-5, H-6 of MR and NH–CH_2–Ph), 7.45 and 7.89 (3.42 H, *Ph*); DS = 3.42/4 = 0.86; ^{13}C NMR δ = 24.8(–NH(CO)CH_3), 54.3 (–NH–CH_2–Ph), 58.0 (C-2 of GlcNAc), 63.0 (C-2), 64.7 (C-6), 75.3 (C-3), 77.5 (C-5), 80.3 (C-4) 104.8 (C-1), 131.0, 131.8, 138.0, and 144.8 (Ph), 177.3 (–NH(CO)–CH_3), 178.1 (Ph–COONa).

(21) *N*-(2-hydroxybenzyl) DAC-88 (DS = 0.45): ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.07 (0.36 H, br s, –NH(CO)CH_3), 3.27 (br s, H-2 of GlcN and *N*-alkylated GlcN), 3.5–4.3 (br m, H-2 of GlcNAc, H-3, H-4, H-5, H-6 of MR), 7.05–7.80 (1.82 H, brm, *Ph*); DS = 1.82/4 = 0.45; ^{13}C NMR was not measured.

(22) *N*-(4-pyridinylmethyl)DAC-88 (DS = 0.72): ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.08 (0.36 H, s, –NH(CO)CH_3), 3.45 (br s, H-2), 3.6–4.2 (br m, H-2 of GlcNAc, H-3, H-4, H-5, H-6 of MR), 4.89 (1.88 H, br s, –CH_2–Py), 8.26, 8.94 (2.87 H, br s, *Py*); DS = 2.87/4 = 0.72; ^{13}C NMRδ = 24.8(–NH(CO)CH_3), 52.4(–CH_2–Py), 58.6(C-2 of GlcNAc), 63.2(C-2), 64.5(C-2), 73.2(C-3), 77.2(C-5), 79.7(C-4)

100.2, 101.4, 103.8(C-1 of MR), 128.8, 129.6, 143.9, 144.4, 150.6, and 157.6 (*Py*), 177.2(–NH(*C*O)CH_3).

(23) *N*-(5-furansulfonyl-2-methyl)DAC-88 (DS = 0.78): ^{1}H NMR(D_2O) δ = 2.06 (0.36 H, br s, –NH(CO)CH_3), 2.63(br, H-2), 3.4–4.4 (br m, H-2 of GlcNAc, H-3, H-4, H-5, H-6 of MR and NH–CH_2), 6.43 and 6.81(1.56 H, br s, –C=C*H*–); DS = 1.56/2 = 0.78; ^{13}C NMR δ = 24.9(–NH(CO)*C*H_3), 46.9(–NH–*C*H_2–), 63.0(C-2), 64.2(C-6), 75.6(C-3), 77.5(C-5), 80.4(C-4) 104.7(C-1), 111.8, 115.3, 153.2, 157.8(furan ring), 177.2(–NH(*C*O)–CH_3).

3.4. N-alkylation of DAC-88 with various monosaccharides

DAC-88 (1.0 g) was dissolved in 4.8% aq. lactic acid (21 mL). The solution was diluted with MeOH (80 mL). Monosaccharide (3 equiv/–NH_2 of DAC-88) was added to the solution and stirred at rt. All of monosaccharides were soluble in the mixed solvent of H_2O and MeOH (21/80). After standing the mixture for the prescribed time, $NaCNBH_3$ (1.33 g: 4 equiv/–NH_2) was added. A white solidified mass was formed in the mixed solvent of H_2O and MeOH after 3–6 h. After stirring the mixture at rt for 24 h, the precipitate was collected, dialyzed, and lyophilized in a similar manner as *N*-alkylation. The lyophilized samples (1.06–1.80 g) were recovered form 1.0 g of DAC-88.

(24) *N*-(2,3-dihydroxypropyl) DAC-88 (DL-glyceraldehyde, DS = 0.69[2]): ^{1}H NMR (D_2O) δ = 2.04 (0.36 H, s, –NH(CO)CH_3), 2.55 (br s, H-2 of *N*-alkylated GlcN), 2.80 and 2.92 (1.75 H, br, –CH_2–NH–), 3.4–4.4 (7.20 H, br m, H-2′, H-3′ substituted, H-2 of GlcNAc, and H-3, H-4, H-5, H-6 of MR); DS = (7.20–5.12)/3 = 0.69; ^{13}C NMR δ = 24.8 (–NH(CO)CH_3), 52.9 (–NH–*C*H_2–), 63.0 (C-2), 65.2–66.3 (C-6), 66.7 (C-3′ substituted), 72.9 (C-2′ substituted), 75.6 (C-3), 77.6 (C-2), 80.6 (C-4), 105.0 (C-1),177.2 (–NH(*C*O)CH_3).

(25) *N*-(1-deoxyribit-1-yl) DAC-88 (D-Ribose, DS = 0.93[2]): ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.06 (0.36 H, s, –NH(CO)CH_3,), 3.3–4.4 (12.52 H, br m, –NH–CH_2–, H-2′, H-3′ H-4′ H-5′ substituted, H-2, H-3, H-4, H-5, H-6 of MR); DS = (12.52–6)/7 = 0.93; ^{13}C NMR δ = 24.9(–NH(CO)*C*H_3), 50.9(–NH–*C*H_2), 63.2(C-2), 64.4(C-6), 65.3(C-5′ substituted), 69.5(C-2′ substituted), 71.6(C-3), 74.4(C-4′ substituted), 75.7(C-3′ substituted), 77.3(C-5), 79.3(C-4), 98.9(C-1).

(26) *N*-(1-deoxyarabit-1-yl) DAC-88 (D-Arabinose, DS = 0.84[2]): ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.07(0.36 H, s, –NH(CO)CH_3,), 3.3–4.4(11.88 H, br m, –NH–CH_2–H-2′, H-3′ H-4′ H-5′ substituted, H-2, H-3, H-4, H-5, H-6 of MR (6.0 H)); DS = (11.88–6)/7 = 0.84; ^{13}C NMR δ = 24.82(–NH(CO)*C*H_3), 53.4 (–NH–*C*H_2–, 58.3(C-2 of GlcNAc), 63.1 (C-2), 64.5 (C-6), 65.4(C-5′ substituted), 67.9(C-2′ substituted), 71.8(C-3), 73.3(C-4′ substituted), 74.1(C-3′ substituted), 77.3(C-5), 79.3(C-4), 99.4(C-1), 103.9(C-1 of GlcNAc), 177.3(–NH(*C*O)CH_3).

(27) *N*-(1-deoxyxylit-1-yl) DAC-88 (D-Xylose, DS = 0.53[2]): ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.06 (0.36 H, s, –NH(CO)CH_3,), 3.3–4.4 (9.68 H, br m, –NH–CH_2–, H–2′, H-3′ H-4′ H-5′ substituted, H-2, H-3, H-4, H-5, H-6 of MR(6.0 H)); DS = (9.68–6)/7 = 0.53; ^{13}C NMR δ = 24.9 (–NH(CO)*C*H_3), 51.9 (–NH–*C*H_2–), 58.4 (C-2 of GlcNAc), 63.2(C-2), 64.3(C-6), 65.1(C-5′ substituted), 69.5(C-2′ substituted), 71.6 (C-3), 74.1 (C-4′ substituted), 74.5(C-3′ substituted), 77.3 (C-5), 79.3 (C-4), 98.9 (C-1), 104.0 (C-1 of GlcNAc), 177.4 (–NH(*C*O)CH_3).

(28) *N*-(1,2-dideoxyribit-1-yl) DAC-88 (2-Deoxy-D-ribose, DS = 1.02): ^{1}H NMR (D_2O) δ = 1.63 and 1.79 (2.04 H, br m, –CH_2– substituted (H-2′)), 2.05 (0.36 H, s, –NH(CO)CH_3,), 2.61 (0.61 H, br s, H-2 of *N*-alkylated GlcN), 2.7–3.2 (1.89 H, br m, –NH–CH_2–), 3.3–4.4 (8.19 H, br m, H-2′, H-3′ H-4′ H-5′ substituted, H-2 of GlcNAc, H-3, H-4, H-5, H-6 of MR); DS = 2.04/2 = 1.02; ^{13}C NMR δ = 24.8 (–NH(CO)*C*H_3), 34.2 (–*C*H_2–substituted(C-2′)), 47.9 (–NH–*C*H_2–), 62.6 (C-2), 63.0 (C-6), 65.1 (C-5′ substituted), 73.1 (C-3′ substituted), 74.0–75.5 (C-3), 77.2 (C-4′ substituted and C-5), 80.7 (C-4), 104.3 (C-1), 177.2 (–NH(*C*O)CH_3).

(29) *N*-(1-deoxyglucit-1-yl) DAC-88 (D-Glucose, DS = 0.43[2]): ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.06 (0.36 H, s, –NH(CO)CH_3,), 3.0–4.4 (9.41 H, br m, –NH–CH_2–, H-2′, H-3′ H-4′ H-5′ H-6′ substituted, H-2, H-3, H-4, H-5, H-6 of MR (6.0 H)); DS = (9.41 − 6)/8 = 0.43; ^{13}C NMR δ = 24.9 (–NH(CO)*C*H_3), 51.5 (–NH–*C*H_2–), 58.4 (C-2 of GlcNAc), 63.3 (C-2), 64.3 (C-6), 65.3 (C-6′ substituted), 70.6 (C-2′ substituted), 71.6 (C-3), 73.4 (C-4′ and C-5′ substituted), 73.7 (C-3′ substituted), 77.3 (C-5), 79.5 (C-4), 98.9 (C-1).

(30) *N*-(1,2-dideoxyglucit-1-yl) DAC-88 (2-Deoxy-D-glucose, DS = 0.82): ^{1}H NMR(D_2O) δ = 1.69 (1.64 H, br m, –CH_2–substituted(H-2′)), 2.04 (0.36 H, s, –NH(CO)CH_3), 2.54 (0.75 H, br s, H-2), 2.89 (1.55 H, br m, –NH–CH_2–), 3.3–4.2 (8.32 H, br m, H-3′ H-4′ H-5′ H-6′ substituted, H-2 of GlcNAc, H-3, H-4, H-5, H-6 of MR (5.0 H)); DS = 1.64/2 = 0.82; ^{13}C NMR δ = 24.7 (–NH(CO)*C*H_3), 33.1 (–*C*H_2-substituted (C-2′)), 47.8 (–NH–*C*H_2–), 63.1 (C-2), 64.6 (C-6), 65.6 (C-6′ substituted), 71.1 (C-3′ substituted), 72.9 (C-3), 73.6 (C-5′ substituted), 75.5 (C-4′ substituted), 77.3 (C-5), 79.7 (C-4), 101.3 (C-1), 177.3 (–NH(*C*O)CH_3).

(31) *N*-(3-O-methyl-1-deoxyglucit-1-yl) DAC-88 (3-O-Me-D-glucose, DS = 0.46[2]): ^{1}H NMR (0.5 M DCl/D_2O) δ = 2.08 (0.36 H, s, –NH(CO)CH_3,), 3.2–4.4(11.08 H, br m, H-1′, H-2′,H-3′ H-4′ H-5′ H-6′ and 3′–OCH_3(δ = 3.57) substituted, H-2, H-3, H-4, H-5, H-6 of MR(6.0 H)); DS = (11.08 − 6)/11 = 0.46; ^{13}C NMR δ = 24.8(–NH(CO)*C*H_3), 50.9(–NH–*C*H_2–), 58.5(C-2 of GlcNAc), 62.7(C-2), 63.1(–O–CH_3 substituted), 64.0(C-6), 65.5(C-6′ substituted), 69.2(C-2′ substituted), 71.6(C-3), 72.1(C-5′ substituted), 73.5 (C-4′ substituted), 77.3(C-5), 79.0(C-4), 100.1(C-1).

(32) *N*-(1-deoxygalctit-1-yl) DAC-88 (D-Galactose,

[2] The DS is a temporary value at the present stage because of the main peak of substituted group is overlapped with the peak of DAC-88 in ^{1}H NMR spectra.

DS = 0.37[2]); ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.07 (0.36 H, s, –NH(CO)CH_3,), 3.0–4.4 (8.96 H, br m, –NH–CH_2–, H-2′, H-3′ H-4′ H-5′ H-6′ substituted, H-2, H-3, H-4, H-5, H-6 of MR (6.0 H)); DS = (8.96 − 6)/8 = 0.37; ^{13}C NMR δ = 24.8 (–NH(CO)CH_3), 53.0 (–NH–CH_2–), 63.1 (C-2), 64.9 (C-6), 65.8 (C-6′ substituted), 69.7 (C-2′ substituted), 72.0, 72.6, 73.4 (C-3 of GlcN, C-3′, C-4′ and C-5′ substituted), 77.4 (C-5), 79.6 (C-4), 100.7 (C-1), 177.3 (–NH(CO)CH_3).

(33) *N*-(1-deoxymannit-1-yl) DAC-88 (D-Mannose, DS = 0.60[2]): ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.06 (0.36 H, s, –NH(CO)CH_3,), 3.2–4.4 (10.83 H, br m, –NH–CH_2–, H-2′, H-3′ H-4′ H-5′ H-6′ substituted, H-2, H-3, H-4, H-5, H-6 of MR (6.0 H)); DS = (10.83 − 6)/8 = 0.60; ^{13}C NMR δ = 24.8(–NH(CO)CH_3), 53.0(–NH–CH_2–), 63.0(C-2), 64.5(C-6), 65.7(C-6′), 69.7(C-2′ substituted), 71.8(C-4′ substituted), 73.4(C-5′ substituted), 73.7(C-3′ substituted), 77.3(C-5), 79.5(C-4), 100.8(C-1), 177.3(–NH(CO)CH_3).

(34) *N*-(1-deoxyfucit-1-yl) DAC-88 (L-Fucose, DS = 0.75): ^{1}H NMR(0.5 M DCl/D_2O) δ = 1.25 (2.26 H, s, –CH_3), 2.05(0.36 H, s, –NH(CO)CH_3), 3.2–4.3 (9.48 H, br m, –NH–CH_2–, H-2′, H-3′ H-4′ H-5′ of substituted, H-2, H-3, H-4, H-5, H-6 of MR (6.0 H)); DS = 2.26/3 = 0.75; ^{13}C NMR(0.5 M DCl/D_2O) δ = 21.3(–CH_3), 24.8(–NH(CO)CH_3), 53.3(–NH–CH_2–), 63.1(C-2), 64.8(C-6), 68.5(C-5′ substituted), 69.7(C-2′ substituted), 73.9(C-4′ substituted and C-3 of *N*-alkylated GlcN), 75.5(C-3′ substituted), 77.4(C-5), 79.8(C-4), 102.0(C-1), 104.0(C-1 of GlcNAc), 177.3 (–NH(CO)CH_3).

(35) *N*-(1-deoxyrhamnit-1-yl) DAC-88 (L-Rhamnose, DS = 0.72): ^{1}H NMR(0.5 M DCl/D_2O) δ = 1.27 (2.16 H, s, –CH_3), 2.05 (0.36 H, s, –NH(CO)CH_3,), 3.0–4.4 (12.13 H, br m, –NH–CH_2–, H-2′, H-3′ H-4′ H-5′ of substituted, H-2, H-3, H-4, H-5, H-6 of MR (6.0 H)); DS = 2.16/3 = 0.72; ^{13}C NMR(0.5 M DCl/D_2O) δ = 21.5(–CH_3), 24.9(–NH(CO)CH_3), 52.4(–NH–CH_2–), 63.3(C-2), 64.4(C-6), 69.4(C-5′ substituted), 69.7(C-2′ substituted), 71.7(C-3), 74.1(C-4′ substituted), 75.9(C-3′ substituted), 77.3(C-5), 79.3(C-4), 98.9(C-1).

(36) *N*-(2-acetamide-1,2-dideoxyglucit-1-yl) DAC-88 (GlcNAc, DS = 0.45[2]): ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.08 (1.01 H, s, –NH(CO)CH_3), 3.2–4.3 (5.67 H, br m, –NH–CH_2–, H-2′, H-3′ H-4′ H-5′ H-6′ of substituted, H-2, H-3, H-4, H-5, H-6 of MR (6.0 H)); 8 × DS + 6: 3 × S + 0.36 = 5.67: 1.01, DS = 4.03/8.9 = 0.45; ^{13}C NMR (0.5 M DCl/D_2O) δ = 24.9(–NH(CO)CH_3), 52.5(–NH–CH_2–), 62.7(C-2), 63.3(C-6), 65.1(C-2′ substituted), 65.3(C-6′ substituted), 71.9(C-3′ substituted), 72.3(C-3), 73.5(C-5′ substituted), 73.7(C-4′ substituted), 77.2(C-5), 79.0(C-4), 100.1(C-1), 177.4 (–NH(CO)CH_3 of DAC-88,) 177.9 (–NH(CO)CH_3 substituted).

3.5. N-alkylation of DAC-88 with various disaccharides

DAC-88 (1.0 g) was dissolved in H_2O (21 mL) containing 1 mL of lactic acid. The solution was diluted with H_2O or MeOH to adjust the ratio at 20/80, 50/50, and 100/0 (H_2O/MeOH (mL/mL); total volume, 101 mL). Disaccharide (3 equiv/–NH_2 of DAC-88) was added to the solution and stirred at rt. Most of the disaccharide was insoluble in the mixed solvent of H_2O and MeOH (20/80 and 50/50). In H_2O (100/0), however, all mixtures were a clear solution. After standing the mixture for the prescribed time, $NaCNBH_3$ (1.33 g: 4 equiv/–NH_2) was added. A white solidified mass was formed in the mixed solvent of H_2O and MeOH (20/80 and 50/50) after 3–6 h, though it was not formed in H_2O (100/0). After stirring the mixture at rt for 24 h, the precipitate was collected, dialyzed, and lyophilized in a similar manner as *N*-alkylation. The lyophilized samples (1.27–2.44 g) were recovered from 1.0 g of DAC-88.

(38) *N*-(1-deoxymelibit-1-yl) DAC-88 (Melibiose, DS = 0.74[2]): ^{1}H NMR(0.5 M DCl/D_2O) δ = 2.04 (0.36 H, br s, –NH(CO)CH_3), 3.2–4.4 (16.36 H, br m, H-2, H-3, H-4, H-5, H-6 of MR, –NH–CH_2–, H-2′, H-3′, H-4′, H-5′, H-6′, H-2″, H-3″, H-4″, H-5″, H-6″ substituted); DS = (16.36 − 6.0)/14 = 0.74; ^{13}C NMR δ = 24.8(–NH(CO)CH_3), 51.3(–NH–CH_2–), 58.6(C-2), 63.2, 63.8(C-6, C-6′, C-6″ substituted), 70.6 − 73.6(C-3, C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″ substituted), 77.3(C-5), 79.5 (C-4), 99.0(C-1), 100.1 (C″-1 substituted); C′, glucit-1-yl part; C″, galactose part substituted.

(40) *N*-(1-deoxymaltit-1-yl) DAC-88 (Maltose, DS = 0.44[2]): ^{1}H NMR(2.8 M DCl/D_2O) δ = 2.17 (0.36 H, s, –NH(CO)–CH_3), 3.4–4.3 (12.12 H, br m, –NH–CH_2– and H-2′, H-3′, H-4′, H-5′, H-6′, H-2″, H-3″, H-4″, H-5″, H-6″ substituted, H-2, H-3, H-4, H-5, H-6 of MR(6 H)); DS = (12.12 − 6)/14 = 0.44; ^{13}C NMR δ = 24.8(–NH(CO)CH_3), 52.0(–NH–CH_2–), 59.0 (C-2), 63.0–64.8 (C-6, C-6′, C-6″ substituted), 69.1–84.3(C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″ substituted), 98.3–103.2(C-1, C″-1 substituted), 177.3(–NH(CO)).

(37 and 39): NMR data ware reported byYalpani and Hall, 1984.

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