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Preparation and characterization of *N*-[2-(glycosyloxy)-ethyl]chitosan derivatives*

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ABSTRACT

Allyl glycosides of 8 simple sugars have been prepared and characterized, including ^{1}H- and ^{13}C-n.m.r. assignments. Formylmethyl glycosides, obtained by reductive ozonolysis of the allyl glycosides, have been reductively *N*-alkylated to chitosan with typical yields of 80%. The glycosides of α- and β-D-glucopyranose, α- and β-D-galactopyranose, 2-acetamido-2-deoxy-α- and β-D-glucopyranose, β-D-glucuronic acid, and β-lactose have been incorporated by this method. The degree of substitution (d.s.) of the products was controlled by varying the molar ratio of glycoside to free amine groups of chitosan by between 0.5 and 3.0. Derivatives of degree of substitution >0.3 were typically water soluble, and compounds of higher d.s. generally gave less-viscous aqueous solutions. Assignment of ^{13}C-n.m.r. chemical shifts verified the structure of these derivatives. The linewidths of the branch resonances (5–100 Hz) provided qualitative information about the relationship between d.s. and branch mobility. The resonances of high-d.s. products were narrower and more intense than analogous low-d.s. derivatives. The chitosan resonances of the backbone were generally broader (50–200 Hz) and less intense, and as a result were difficult to assign fully.

INTRODUCTION

Some natural branched polysaccharides, such as xanthan[1] and guar gum[2], are known to possess unique aqueous solution properties[1–3]. It has also been established that many branched exocellular polysaccharides have immunogenic activity[4,5], which renders them potentially useful in biomedical and pharmacological applications. These two factors have helped to direct attention toward the synthesis of polysaccharides bearing pendant carbohydrate substituents.

The synthesis of branched derivatives of polysaccharides has been accomplished by a variety of different approaches[6,7]. One notable method involves the reaction of acetylated glycosyl bromides under glycosylation conditions with amylose and cellulose to produce branched derivatives[8,9]. Carbohydrate 1,2-orthoesters have found application in coupling to amylose and cellulose derivatives via the cyclic orthoester glycosidation method[10–13]. Kochetkov *et al.*[13] have reported the reaction of 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(*tert*-butyl orthoacetate) with randomly substituted cellulose diace-

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tate, giving a product substituted mainly at primary positions. Pfannemuller *et al.*[10–12] used both 1,2-(*tert*-butyl orthoacetate) and 1,2-(ethyl orthoacetate) derivatives of 3,4,6-tri-*O*-acetyl-α-D-glucopyranose in reactions with 2,3-di-*O*-phenylcarbamoylamylose and cellulose derivatives. The products (d.s. 0.25–0.30) contained largely β-(1→6) branches with a small amount of α-(1→6).

Starch components have been treated with 3,4-dihydro-2*H*-pyran[14], to give derivatives bearing tetrahydropyran-2-yl acetal groups. These compounds are water-soluble at low levels of substitution and organic-soluble at high levels. Although the pendant group in this case is not a carbohydrate, it is coupled "glycosidically" and indicates the potential of using known carbohydrate glycals to prepare derivatives with 2-deoxysaccharide branches.

A variety of linear and branched synthetic polysaccharides have been prepared by polymerization of 1,6-anhydro sugar derivatives[6,15]. Also, polymerization of mono- and oligo-saccharides using 1,2-cyanoethylidene derivatives has been used to generate both homo and hetero polymers[13,16].

The methods just mentioned show significant potential and constitute a first generation of synthetic methods for making branched polysaccharides. However, various disadvantages are evident in most cases, including (*1*) requirement for specific protection, (*2*) multistep synthetic produces, (*3*) activation of the carbohydrate moiety, (*4*) low coupling efficiencies, (*5*) poor site-selectivity, and (*6*) harsh, degradative reaction conditions.

Reductive *N*-alkylation of chitosan with reducing mono- and di-saccharides has been used to produce chitosan derivatives having acyclic carbohydrate branches[17]. Some of these compounds showed interesting solution behavior, however, the branches differ substantially in structure from those on natural polysaccharides.

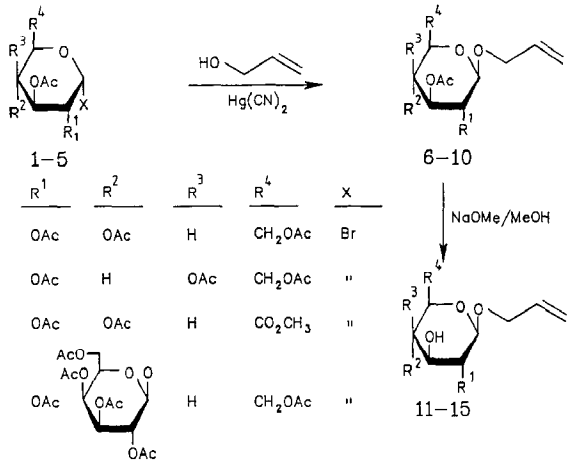
We describe here a new method for controlled solubilization of chitosan by introduction of carbohydrate-derived hydrophilic branches.

RESULTS AND DISCUSSION

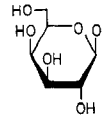
Many allyl glycosides have been prepared for use in biochemical studies[19,20] and as intermediates in carbohydrate syntheses[21]. Those used here were prepared as described by Lee and Lee[19]. The β-D-glycosides were prepared from the respective peracetylated α-D-glycopyranosyl halides.

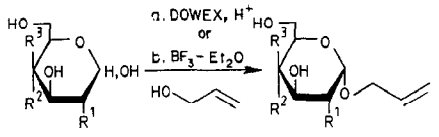
The acetylated glycosyl bromides or chlorides **1**–**5** were prepared by standard methods[22]. Koenigs–Knorr glycosidation with allyl alcohol, provided the peracetylated allyl β-D-glycosides **6**–**10**. Subsequent *O*-deacetylation gave the unprotected allyl β-D-glycopyranosides **11**–**15**. The allyl α-D-glycopyranosides **19** and **20** were prepared by acid-catalyzed glycosidation of D-glucose and D-galactose. The reported yields of allyl 2-acetamido-2-deoxy-α-D-glucopyranoside by this method were very low (10%)[19]; use of boron trifluoride–etherate as catalyst, however, gave **21** in 40% yield from **18**. Melting points and specific rotations of the products agreed with literature values[19]. Although published ^{1}H- and ^{13}C-n.m.r. spectroscopic data for allyl glycosides were not

available in many instances, the ^{1}H- and ^{13}C-n.m.r. spectra obtained (Tables I–III) agreed closely with published chemical-shift and coupling-constant values for the respective methyl glycosides[23,24].



1–5 6–10 11–15

	R^1	R^2	R^3	R^4	X
1,6	OAc	OAc	H	CH_2OAc	Br
2,7	OAc	H	OAc	CH_2OAc	"
3,8	OAc	OAc	H	CO_2CH_3	"
4,9	OAc		H	CH_2OAc	"
5,10	NHAc	OAc	H	CH_2OAc	Cl
11	OH	OH	H	CH_2OH	
12	OH	H	OH	CH_2OH	
13	OH	OH	H	CO_2CH_3	
14	OH		H	CH_2OAc	
15	NHAc	OH	H	CH_2OAc	



16–18 19–21

	R^1	R^2	R^3
a. 16,19	OH	OH	H
a. 17,20	OH	H	OH
b. 18,21	NHAc	OH	H

The ^{13}C-n.m.r. spectrum of allyl β-D-glucopyranuronic acid (**13**) was comparable to that of the corresponding methyl glycoside[23], and contained additionally the characteristic allyl group carbon resonances. This compound was not obtained crystalline and analytically pure, but the crystalline precursor, methyl (allyl 2,3,4,tri-*O*-acetyl-β-D-glucopyranoside)uronate (**8**), was fully characterized. *O*-Deacetylation of **8** under

TABLE I

^{1}H-N.m.r. data for peracetylated allyl β-D-glycopyranosides **6**–**10**

Resonance	*Chemical shifts*					
	6	**7**	**8**	**(Gal) 9**	**(Glc)**	**10**
H-1	4.48 d	4.53 d	4.60 d	4.53 d	4.50 d	4.73 d
H-2	5.04 dd	5.16–5.34 m	5.04 t	5.12 dd	4.94 t	3.90 dt
H-3	5.11 t	5.04 dd	5.16–5.32 m	4.97 dd	5.16–5.32 m	5.08 t
H-4	5.21 t	5.40 d	5.16–5.32 m	5.35 d	3.82 t	5.31 t
H-5	3.71 m	3.91 t	4.03 d	3.90 t	3.61 m	3.72 m
H-6a	4.28 dd	4.05–4.23 m	n.a.	4.04–4.15 m	4.47 dd	4.28 dd
H-6b	4.14 dd	4.05–4.23 m	n.a.	4.04–4.15 m	4.04–4.15 m	4.15 dd
H-1'a	4.35 dd	4.36 dd	4.36 dd		4.31 dd	4.35 dd
H-1'b	4.11 dd	4.05–4.23 m	4.09 dd		4.04–4.15 m	4.10 dd
H-2'	5.84 m	5.85 m	5.74 m		5.94 m	5.84 m
H-3'a	5.29 bd	5.16–5.34 m	5.16–5.32 m		5.16–5.32 m	5.28 bd
H-3'b	5.22 bd	5.16–5.34 m	5.16–5.32 m			5.21 bd
CO_2CH_3			3.76			
N*H*Ac						5.76 d
$NHCOCH_3$						1.95 s
	Coupling constants[a]					
$J_{1,2}$	8.0	8.0	8.0	8.0	8.0	8.0
$J_{2,3}$	9.5	10.0	9.0	10.0	9.0	10.0
$J_{3,4}$	9.5	3.5	n.a.	3.0	9.0	10.0
$J_{4,5}$	9.5	n.a.	9.0	<0.5	9.0	10.0
$J_{5,6a}$	2.0	7.0	n.a.	7.0	2.0	5.0
$J_{5,6b}$	4.5	7.0	n.a.	7.0	n.a.	2.0
$J_{6a,6b}$	13.0	n.a.	n.a.	n.a.	12.0	13.0
$J_{1'a,1'b}$	14.0	13.0	13.5		13.0	13.0
$J_{1'a,2'}$	5.0	4.5	5.0		5.0	5.0
$J_{1'b,2'}$	6.5	n.a.	6.5		n.a.	6.0
$J_{2',3'a}$	17.0	n.a.	n.a.		n.a.	18.0
$J_{2',3'b}$	12.0	n.a.	n.a.		n.a.	11.0

[a] N.a. denotes not assigned.

standard Zemplén conditions showed (t.l.c.) a major component having an R_F value higher than that expected for **13**. Treatment with aqueous sodium hydroxide converted this component into a lower-R_F material corresponding to the product.

The allyl glycosides **11**–**15**, and **19**–**21** were reductively ozonolyzed[25] to provide the respective formylmethyl glycosides **22**–**29**. Aldehydes of this type exist in a number of equilibrium states, including the gem-diol, various intramolecular cyclic hemiacetals, and acetal oligomers; direct characterizations were not attempted. Previous work[26,27] has established that the ozonolysis of allyl glycosides proceeds virtually quantitatively, and thus, in most cases, the aldehyde products were used directly in the subsequent step. Aldehyde **24** was reduced and the product characterized by ^{13}C-n.m.r. spectroscopy in order to establish the stability of glycosiduronic acids to ozonolytic conditions. The

TABLE II

^{1}H-N.m.r. data for allyl glycopyranoside products **11**, **12**, **14**, **15**, and **19–21**

Resonance	*Chemical shifts*							
	11	**12**	**14**	**14**	**15**	**19**	**20**	**21**
H-1	4.51 d	4.43 d	4.49 d	4.40 d	4.56 d	4.98 d	4.89 d	4.93 d
H-2	3.28 t	3.53 dd	3.5–4.0	3.5–4.0	3.72 t	3.57 dd	3.8–3.9	3.65–3.95
H-3	3.51 t	3.64 dd	3.5–4.0	3.5–4.0	3.4–3.6	3.65–3.9	3.8–3.9	3.65–3.95
H-4	3.37 t	3.92 d	3.5–4.0	3.5–4.0	3.4–3.6	3.42 t	3.98 d	3.49 t
H-5	3.45 dd	3.67 dd	3.30 dd	3.5–4.0	3.4–3.6	3.65–3.9	3.96 d	3.65–3.95
H-6a	3.93 dd	3.77 dd	3.5–4.0	3.5–4.0	3.93 d	3.65–3.9	3.65–3.8	3.65–3.95
H-6b	3.71 dd	3.75 dd	3.5–4.0	3.5–4.0	3.74 dd	3.65–3.9	3.65–3.8	3.65–3.95
H-1′a	4.39 dd	4.40 dd		4.35 dd	4.34 dd	4.24 dd	4.25 dd	4.23 dd
H-1′b	4.21 dd	4.22 dd		4.19 dd	4.16 dd	4.07 dd	4.08 dd	4.03 dd
H-2′	5.85 m	5.97 m		5.94 m	5.90 m	5.99 m	5.99 m	5.94 m
H-3′a	5.39 d	5.39 d		5.33 d	5.31 d	5.38 d	5.39 d	5.36 d
H-3′b	5.30 d	5.28 d		5.24 d	5.26 d	5.27 d	5.28 d	5.27 d
	Coupling constants (Hz)[a]							
$J_{1,2}$	8.0	8.0	8.0	7.5	8.0	3.5	2.0	3.0
$J_{2,3}$	9.0	10.0	n.a.	n.a.	9.0	10.0	n.a.	n.a.
$J_{3,4}$	9.0	3.0	n.a.	n.a.	n.a.	10.0	1.0	10.0
$J_{4,5}$	9.0	<0.5	n.a.	n.a.	n.a.	10.0	n.a.	10.0
$J_{5,6a}$	2.0	7.0	7.0	n.a.	5.0	n.a.	8.0	n.a.
$J_{5,6b}$	6.0	4.0	6.0	n.a.	n.a.	n.a.	n.a.	n.a.
$J_{6a,6b}$	12.0	11.0	n.a.	n.a.	12.0	n.a.	n.a.	n.a.
$J_{1'a,1'b}$	13.0	13.0	n.a.	12.5	13.0	12.0	13.0	13.0
$J_{1'a,2}$	5.0	6.0		5.5	5.0	5.0	5.0	5.5
$J_{1'b,2'}$	6.5	7.0		6.0	6.0	6.0	6.0	6.5
$J_{2',3'a}$	17.0	16.0		17.0	17.0	17.0	17.0	16.5
$J_{2',3'b}$	10.0	10.0		10.0	10.0	10.0	11.0	11.0

[a] N.a. denotes not assigned.

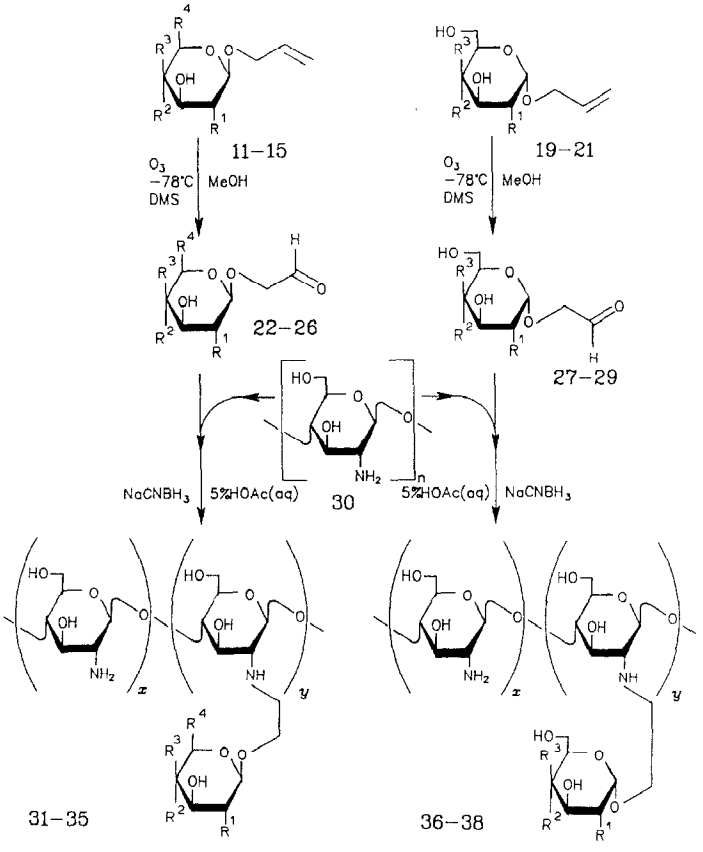
TABLE III

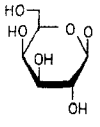
100.6-MHz ^{13}C-N.m.r. chemical-shift data (p.p.m.) for the allyl glycosides in D_2O solution (ref. external Me_4Si)

Derivative	*Branch*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1′*	*C-2′*	*C-3′*
11	β-Glc	99.6	71.5	74.2[a]	68.0[b]	74.2[a]	59.2	68.9[b]	131.8	117.0
12	β-Gal	100.2	69.1	71.1	67.0	73.4	59.3	68.9	131.9	116.9
13	β-GlcA	99.7	71.1	73.7	69.2	72.8	170.4	69.5	131.7	117.1
14	*B*-Lact									
	(β-Gal)	100.2	69.2	70.9[a]	66.8	73.5	59.3			
	(β-Glc)	99.4	71.1[a]	72.7[b]	76.8	73.0[b]	58.4	68.9	131.7	117.0
15	*B*-GlcNAc	98.8	54.2	72.5	68.7	74.5	59.5	68.9	132.3	116.5
19	αGlc	95.7	69.6	71.5	68.0	70.2	59.0	66.8	132.1	116.5
20	α-Gal	96.1	66.9[b]	68.0	67.7	69.3	59.6	66.7[a]	132.2	116.5
21	α-GlcNAc	94.5	52.0	69.4	68.4	70.3	59.0	66.8	132.1	116.2

[a] Assignments may be reversed. [b] Assignments may be reversed.

solution ^{13}C-n.m.r. spectrum of the product established that the carbohydrate portion of the molecule was unaltered, and that the expected 2-hydroxyethyl glycoside was formed.



	R^1	R^2	R^3	R^4
31,36	OH	OH	H	CH_2OH
32,37	OH	H	OH	CH_2OH
33	OH	OH	H	CO_2H
34,38	NHAc	OH	H	CH_2OH
35	OH		H	CH_2OH

Reductive amination of chitosan with the aldehydes **22–29**, was performed as outlined in the accompanying formulas, to yield derivatives **31-38**. The aldehydes were dissolved in 10–15 mL of 5% aqueous acetic acid and added to a viscous solution (~ 1 mmol/10 mL) of chitosan. Treatment with an excess of sodium cyanoborohydride resulted in appreciable foaming which dissipated over time. The mixture was stirred for

24 h and then filtered to remove insoluble material. Filtration was not possible in the preparation of **33a** as the product precipitated from the reaction. Varying the molar ratio of aldehyde to chitosan (A:C) gave products having a range of degree of substitution (d.s.) values, as shown in Table IV. Thus, a molar ratio of ~3 was used to give fully or highly substituted derivatives (such as **32a–37a**), whereas a ratio of 0.50 gave products having low d.s. values (**34d**, **36d**, and **38e**). The degrees of substitution were determined from C, H, and N analyses. For the β-lactosyl derivative **34a**, an A:C ratio of 3.0 resulted in a d.s. of 0.90, indicating that the size of the substituent influenced the coupling efficiency. This observation is further supported by the results with derivatives **38c** and **38d**, where A:C ratios of 1.5 and 0.75 provided d.s. values 0.35 and 0.19, respectively, being thus significantly lower than results for the **32**, **34**, and **36** series of derivatives. For the lactosyl derivatives, the size effect seemed to be manifested mainly at high d.s. values, hindering complete substitution, whereas for the acetamido derivatives, products of relatively lower d.s. were obtained at all A:C ratios less than 3.0. This latter trend in d.s. may not arise from steric effects alone, but may be related to molecular associations or repulsions involving free amino functionalities on the backbone, and acetamido groups on the branch.

TABLE IV

Characteristics of *N*-[2-(D-glycopyranosyloxy)ethyl]chitosan derivatives

Derivative	*Branch*	*A:C*	*d.s. (±0.05)*	*Yield (%)*
31a	β-Glc	3.1	1.00	95
32a	*B*-Gal	2.7	1.00	60
32b		1.3	0.70	85
32c		0.75	0.38	80
33a	β-GlcA	3.0	1.00	70
33b		1.0	0.67	80
34a	β-Lac[a]	3.1	0.90	95
34b		1.5	0.76	85
34c		0.75	0.35	95
34d		0.50	0.32	95
34e		0.35	0.24	87
35a	β-GlcNAc	3.0	1.00	85
36a	α-Glc	3.0	1.00	95
36b		1.5	0.59	60
36c		0.75	0.38	70
36d		0.5	0.26	80
37a	α-Gal	3.1	1-00	60
37b		2.0	0.86	55
37c		1.0	0.48	95
37d		0.75	0.32	95
38a	α-GlcNAc	3.1	1.00	90
38b		3.1	1.00	85
38c		1.5	0.35	95
38d		0.75	0.19	95
38e		0.5	0.17	95

[a] Lac = lactose.

^{13}C-N.m.r. spectra were recorded for all of the highly substituted derivatives for each sugar branch. These derivatives were very soluble and gave free-flowing 5% (w/w) solutions in D_2O. The lindewidths of the branch carbon resonances were relatively narrow (5–10 Hz) in comparison to the chitosan main-chain resonances (100–200 Hz), as shown in Fig. 1. The ^{13}C chemical-shift assignments (Table V) were readily accomplished by comparison to published values for methyl glycosides[23,24] or to values given in Table III for the respective allyl glycosides. The ^{13}C-n.m.r. spectra of derivatives **32b**, **36c**, and **37c**, having low d.s. values, show substantially broader signals for branch carbons (60, 100, and 10 Hz for respective C-1 resonances) than do the high-d.s. analogues **32a**, **36a**, and **37b** (5–10 Hz), as may be seen in Figs. 1, 2, and 3. This is indicative of interrelationships between the degree of substitution, solution viscosity and branch mobility, as manifested in the correlation-time (τ_c) dependence of T_2 and linewidth ($\nu_{1/2}$)[28]. Noteworthy is the increased linewidth and decreased intensity of the C-1′ carbon of the branch, when compared to C-6 or other ring-carbon resonances on the same derivative, illustrating the decreased mobility of branch positions closer to the main chain. Thus, not only does ^{13}C-n.m.r. spectroscopy provide proof of structural

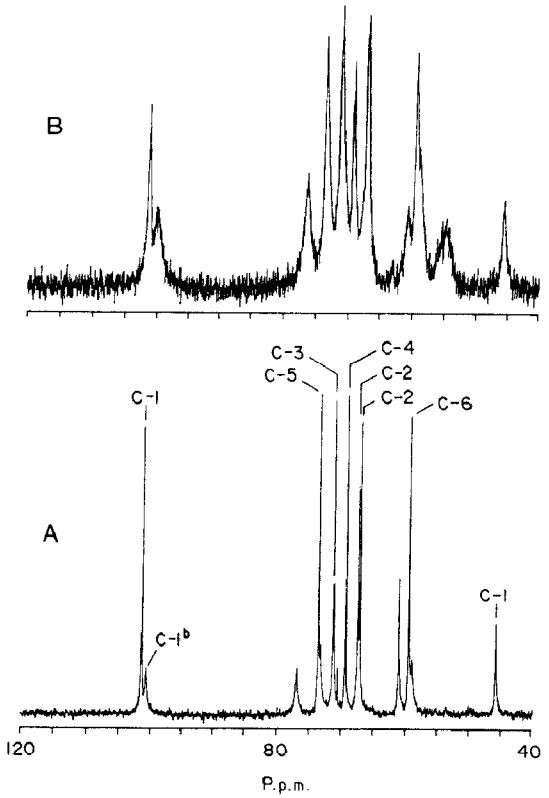


Fig. 1. Expanded region of the 100-MHz ^{13}C-n.m.r. spectra of (a) **32a** (d.s. 1.00) and (b) **32b** (d.s. 0.70), in D_2O solution referenced to external Me_4Si.

modification, but it also allows differentiation of resonances on the basis of mobility and provides a qualitative indication of relative viscosities.

The resonances arising from the chitosan backbone are evident to some extent in most of the derivative spectra, and also exhibit linewidth variations attributable to d.s. differences (50 *vs.* 150 Hz in Figs. 1A and 1B, respectively). In general, the resonances of the anomeric (C-1^b) and C-4^b carbon atoms (^{b} denotes backbone) are discernible. No attempt has been made here to assign all of the chitosan resonances, particularly in samples of intermediate and low d.s. where the "resonance splitting" caused by substituted and unsubstituted residues complicated assignment, and broad lines obscured the peaks. In all cases, it is possible to assign the C-1^b signal to the 98–102 p.p.m. region (depending on substitution) and C-4^b to the 75–80 p.p.m. range.

The utility of secondary modification of chitosan derivatives has been alluded to. The derivatives described here having d.s. < 1.0 are suitable candidates for homogeneous chemical reaction in aqueous media. To demonstrate this, two secondary modification sequences were undertaken. One comparison was to contrast the properties of the 2-acetamido-2-deoxy-α-D-glucose derivative (**38a**) with those of a derivative bearing pendant 2-amino-2-deoxy-α-D-glucose units. Derivative **38b** was treated with 40% aqueous NaOH at 100°. Elemental analyses and ^{13}C-n.m.r. spectra verified the absence

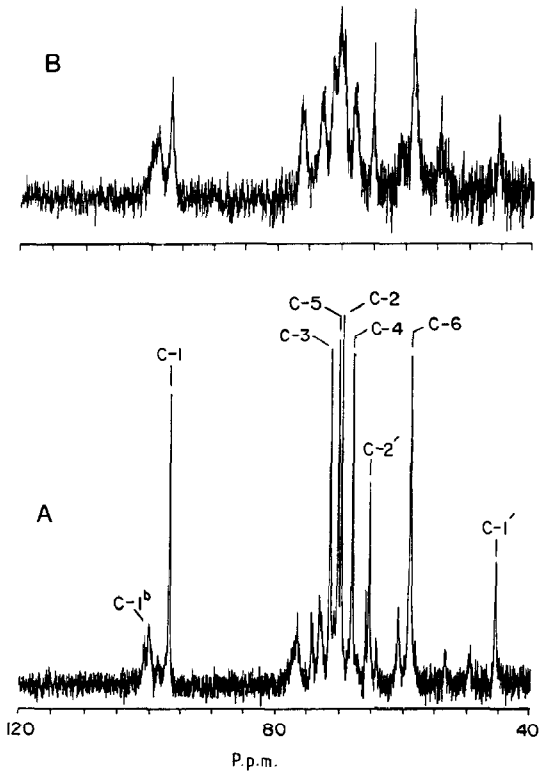


Fig. 2. Expanded region of the 100-MHz ^{13}C-n.m.r. spectra of (a) **36a** (d.s. 1.00) and (b) **36c** (d.s. 0.38), in D_2O solution referenced to external Me_4Si.

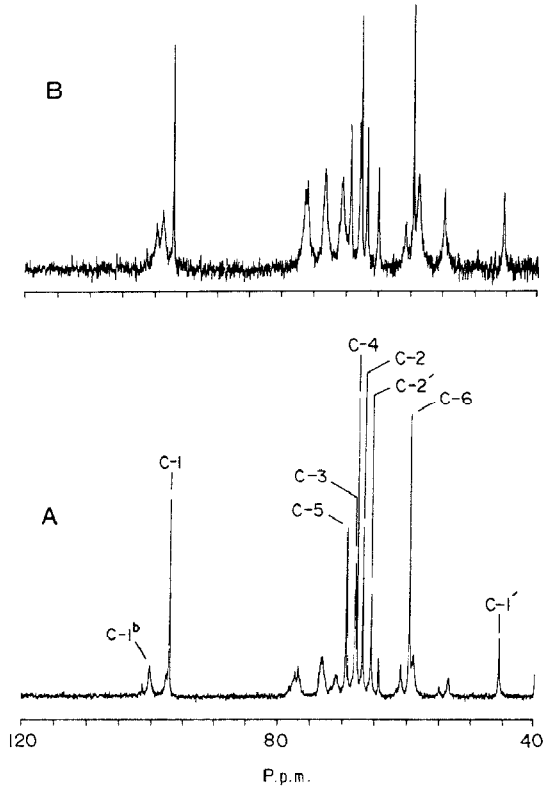


Fig. 3. Expanded region of the 100-MHz ^{13}C-n.m.r. spectra of (a) **37b** (d.s. 1.00) and (b) **37d** (d.s. 0.32), in D_2O solution referenced to external Me_4Si.

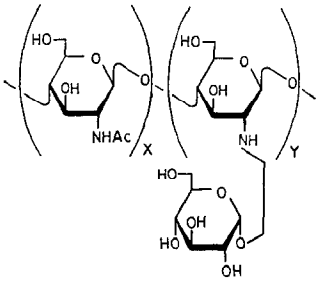
TABLE V

100.6-MHz ^{13}C-N.m.r. data for the *N*-[2-(glycosyloxy)ethyl]chitosan derivatives, showing chemical-shift values (p.p.m.) for pendant sugar resonances (ref. external Me_4Si)

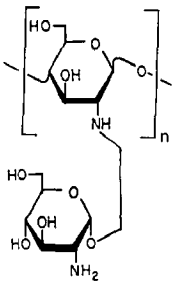
Derivative	*Branch*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-2'*	*C-1'*
31a	β-Glc	100.8	71.7	74.2	68.2	74.4	59.4	67.3	45.8
32a	β-Gal	101.4	67.2	71.2	69.3	73.6	59.5	67.5	45.8
33a	β-GlcA	100.7	71.6	74.1	70.4	74.4	174.1	67.4	45.8
34a	β-Lac								
	(β-Gal)	101.4	69.4	71.1	67.0	73.8	59.4		
	(β-Glc)	100.5	71.3	72.8	77.1	73.2	58.7	67.2	45.7
35c	β-GlcNAc	99.5	54.0	72.3	68.4	74.3	59.3	67.2	45.8
36a	α-Glc	97.0	69.9	71.6	68.2	70.4	59.2	65.5	45.6
37b	α-Gal	97.2	66.9	68.1	67.8	69.5	59.7	65.6	45.6
38a	α-GlcNAc	95.6	52.1	69.5	68.5	70.5	59.1	65.4	45.6
39	α-$GlcNH_2$	97.5	53.9	69.1	69.0	71.2	59.8	66.2	45.9
40a	(α-Glc)	96.9	69.8	70.3	68.0	71.5	59.0	65.0	45.5
	(β-GlcNAc)[a]	99.7	53.8	–	77.5	76.0	60.9		

[a] GlcNAc of backbone, NAc resonances; CH_3 20.5, C=O 173.2.

of *N*-acetyl groups from the product **39**; qualitative observations suggested little depolymerization. A second procedure, which offers a facile method for controlling or modifying the solubility properties of the *N*-(2-glycosyloxyethyl)chitosan derivatives is *N*-acetylation. Treatment of a solution of **36c** (d.s. 0.38) in aqueous methanol (2:1) with acetic anhydride provided derivative **40**, which showed characteristic *N*-acetate peaks in its ^{13}C-n.m.r. spectrum and whose analysis showed full *N*-acetylation at all unsubstituted amino groups (d.s. of NHAc 0.62). Compound **40**, having a high degree of *N*-acetylation, is thus an *N*-[2-(α-D-glucopyranosyloxy)ethyl]chitin.



40



39

We have thus prepared a family of structurally related water-soluble derivatives bearing pendant carbohydrates with varied functionality, glycosidic configuration, and size at a number of degrees of substitution. These are to be used (see following paper)[29] in studies relating solution properties to structural features.

EXPERIMENTAL

General methods. — Evaporations were performed under diminished pressure on a Büchi rotary evaporator. Melting points were determined with a Fisher–Johns apparatus and are uncorrected. I.r. spectra were recorded with a Perkin–Elmer model 710B infrared spectrophotometer, and were calibrated using the 1601 cm^{-1} band of polystyrene. Optical rotations were obtained using a Perkin–Elmer 141 polarimeter. Low-resolution mass spectra (m.s.) were recorded with a Varian/MAT CH4B or Kratos/AEI MS-50 mass spectrometer. Elemental microanalyses (C, H, N) were performed by Mr. P. Borda, Microanalytical Laboratory, University of British Columbia. Copper microanalyses were done by Canadian Microanalytical Ltd., Vancouver, Canada. Analytical t.l.c. was performed with 0.20-mm precoated aluminum back sheets of Silica Gel 60 F_{254} (E. Merck, Darmstadt, Germany). Solvent systems employed for t.l.c. analyses were; (*A*) 3:2 EtOAc–hexane, (*B*) 9:4:2 EtOAc–2-propanol–water. For detection of components, t.l.c. sheets were sprayed with: (*a*) 30% H_2SO_4 in 95% EtOH followed by heating on a hot plate (for carbohydrates), (*b*) 2.0% ammonium molybdate in 10% H_2SO_4–EtOH solvent, and subsequent heating on a hot plate, and (*c*) 1% neutral, $KMnO_4$ (for unsaturated compounds). Flash-column chromatography was performed with 230–400 mesh silica gel (Kieselgel 60, E. Merck, Darmstadt, Germany) according to the procedure of Still *et al.*[30].

Workup and purification of reactions involving polysaccharides generally involved exhaustive dialysis (Spectrapor, membrane tubing, m.w. cutoff 6000–8000) against distilled water, followed by freeze-drying. Polysaccharide samples were dried for 48 h at 70° *in vacuo* (0.05 mmHg) and stored in Schlenk tubes under nitrogen prior to elemental microanalysis.

Ozonolyses were performed at −78°, by using a Welsbach Ozonator (90 V, 2 lb.in^{-2} input O_2 pressure) ozone source. Ozone was bubbled into the cooled solution via a sintered-glass bubbling tube until the mixture turned pale blue. The ozone source was turned off and the solution purged was gaseous O_2 until colourless. Two equiv. of Me_2S were added to the mixture, which was allowed to warm to room temperature with stirring for 2 h.

Nuclear magnetic resonance spectroscopy. — *^{1}H-N.m.r.* Proton n.m.r. spectra were typically measured at 270 MHz using a home-built unit comprised of an Oxford Instruments 63.4 KG superconducting solenoid, a Nicolet Model 1180 computer (32K), and a Bruker WP-60 console. Where indicated, 400-MHz spectra were recorded with a Bruker WH-400 spectrometer, and 300-MHz spectra with a Varian XL-300 spectrometer. Samples dissolved in $CDCl_3$ were referenced relative to internal Me_4Si, those in D_2O relative to internal sodium 4,4-dimethyl-4-silapentanoate-2,2,3,3-d_4 (TSP).

^{13}C-N.m.r. Proton-decoupled carbon-13 n.m.r. spectra were recorded at 100.6 MHz with a Bruker WH-400 spectrometer, or at 75.5 MHz with a Varian XL-300 spectrometer. Spectra were typically obtained at temperatures of 305–310 K, unless otherwise specified. Polysaccharide samples were prepared directly in the 10-mm n.m.r. tube to avoid handling of the viscous or gelatinous materials. Concentrations were typically 5% (w/w), unless further dilution was necessary for dissolution, in which case the concentrations are specified.

Materials. — Chitosan (from crab shell, *N*-acetyl <5%) and D-glucosamine hydrochloride were purchased from Sigma Chemical Co., lactose from Eastman Kodak Co., and D-glucurono-1,4-lactone from Eastman Organic Chemicals Ltd. *N*-Acetyl-D-glucosamine and 1,2:3,4-di-*O*-isopropylidene-D-galactose were obtained from Koch–Light Laboratories, D-glucose from Fisher Scientific Co., and D-galactose from Merck and Co. Purification and distillation of reagents were performed according to standard procedures[30]. The α-glycosyl halides used as starting materials for allyl glycoside synthesis were prepared by standard literature procedures[22,32].

Synthesis of allyl glycosides. — The allyl β-D-glycosides were prepared from the respective α-glycosyl halides via $Hg(CN)_2$-catalyzed Koenigs–Knorr glycosidation, followed by O-deacetylation under Zemplén conditions, essentially as reported by Lee and Lee[19]. The allyl α-D-glycosides were also prepared according to literature methods[19] by Lewis acid-catalyzed glycosidation with either Dowex 50X8 (H^+) ion-exchange resin (**19** and **20**) or with $BF_3{\cdot}OEt_2$ complex (**21**). Characterization data for the final glycosides agreed with published values. A more-complete listing of n.m.r. data for the allyl glycosides is given in Tables I–III.

*Synthesis of formylmethyl glycosides (**22–29**).* — A solution of the allyl D-glycoside in MeOH (with the exception of allyl β-lactose which required 2:1 MeOH–

water), (~ 5–10 mL/mmol) was cooled to −78° and saturated with ozone. The pale-blue solution was then purged with oxygen until colorless and then treated with an excess (2–4 equiv.) of Me_2S. The stirred mixture was warmed to room temperature during 2 h and concentrated. The syrupy residue was taken up in EtOH, and the product was precipitated with ether and decanted. This procedure was repeated, and the resultant gummy precipitate dried *in vacuo* (0.05 mmHg) to give a foamy solid that gave a streak by t.l.c. analysis at an R_F value (solvent *B*) lower than the starting material. Recovery of material was typically 85–95%, and the product was used directly in the subsequent reaction without further characterization.

Synthesis of branched chitosan derivatives. — General procedure for the preparation of N-*[2-(*D-*glycopyranosyloxy)ethyl]chitosans.* Chitosan flakes were dissolved in 5.0% aq. AcOH (~10 mL, 1.0 mmol) with stirring. A solution of the aldehyde (0.5–3.0 molar equivalents) in 5.0% aq. AcOH (10–15 mL) was added, followed by treatment with $NaCNBH_3$ (~4.0 molar equivalents) for 24 h. The mixture was then dialyzed against distilled water (6 × 2 L) for 6 days, filtered through a medium-pore glass frit filter, and freeze-dried. Yields varied between 55 and 95%.

N-*[2-(β-*D-*Glucopyranosyloxy)ethyl]chitosan* (**31**). (*a*) Chitosan (0.50 g, 3.11 mmol) reacted with formylmethyl β-D-glucopyranoside (**22**, 2.10 g, 9.50 mmol) to give 1.08 g, (95%) of compound **31a**.

Anal. Calc. for $[(C_{14}H_{25}NO_{10})_{1.00}]\cdot 0.51H_2O$: C, 44.66; H, 6.91; N, 3.72. Found: C, 44.67; H, 6.92; N, 3.62.

N-*[2-(β-*D-*Galactopyranosyloxy)ethyl]chitosan* (**32**). (*a*) Chitosan (0.65 g, 4.04 mmol) was treated with the formylmethyl glycoside **23** (2.7 equiv.) to give 0.86 g (58%) of derivative **32a**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.04}(C_{14}H_{25}NO_{10})_{0.96}]\cdot 2.3H_2O$: C, 41.12; H, 7.25; N, 3.43. Found: C, 41.12; H, 6.94; N, 3.51.

(*b*) Chitosan (0.55 g, 3.42 mmol) was treated with the aldehyde **23** (1.00 g, 4.50 mmol) to give 0.86 g (83%) of lyophilized product **32b**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.33}(C_{14}H_{25}NO_{10})0_{2.67}]\cdot 0.50H_2O$: C, 43.34; H, 7.12; N, 4.45. Found: C, 43.35; H, 7.11; N, 4.46.

(*c*) Chitosan (0.65 g, 4.04 mmol) reacted with aldehydo sugar **23** (0.67 g, 3.00 mmol) to yield 0.82 g (81%) of compound **32c**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.62}(C_{14}H_{25}NO_{10})_{0.38}]\cdot 0.63H_2O$: C, 43.31; H, 7.03; N, 5.59. Found: C, 43.31; H, 6.86; N, 5.59.

N-*[2-(β-*D-*Glucopyranosyloxyuronic acid)ethyl]chitosan* (**33**). (*a*) Coupling of the aldehyde **24** (1.75 g, 7.5 mmol) to chitosan (0.40 g, 2.5 mmol) gave a precipitated product after 15 min. The solution was made basic (pH ~8), dialyzed, and freeze-dried. The lyophilized product was dissolved in 0.5M NaOH (10 mL), precipitated with EtOH, filtered off, and washed with MeOH to give, after drying, 0.67 g (67%) of **33a**.

Anal. Calc. for $[(C_{14}H_{22}NO_{11}Na)]\cdot 0.32H_2O$: C, 41.40; H, 5.40; N, 3.45. Found: C, 41.40; H, 5.40; N, 3.18.

(*b*) Chitosan (0.40 g, 2.5 mmol), when treated with the aldehydo sugar **24** (0.60 g, 2.6 mmol), produced a viscous solution after 24 h. The solution was dialyzed and freeze-dried to yield 0.67 g (81%) of derivative **33b**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.33}(C_{14}H_{23}NO_{11})_{0.67}]\cdot 1.13H_2O$: C, 41.61; H, 6.47; N, 4.26. Found: C, 41.61; H, 6.43; N, 4.30.

N-*[2-(β-D-Lactosyloxy)ethyl]chitosan* (**34**). (*a*) Chitosan (0.40 g, 2.5 mmol) was treated with formylmethyl β-lactoside **25** (3.0 g, 7.8 mmol) to give 1.30 g (95%) of derivative **34a**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.11}(C_{20}H_{36}NO_{15})_{0.89}]\cdot 0.22H_2O$: C, 44.90; H, 6.83; N, 2.84. Found: C, 44.89; H, 6.84; N, 2.83.

(*b*) When chitosan (0.65 g, 4.0 mmol) was treated with compound **25** (2.30 g, 6.00 mmol), 1.52 g (86%) of derivative **34b** was obtained.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.24}(C_{20}H_{36}NO_{15})_{0.76}]\cdot 0.66H_2O$: C, 44.12; H, 6.92; N, 3.09. Found: C, 44.12; H, 7.17; N, 3.09.

(*c*) When chitosan (0.65 g, 4.04 mmol) was reacted with the aldehyde **25** (1.15 g, 3.00 mmol), 1.21 g (97%) of compound **34c** was isolated.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.65}(C_{20}H_{36}NO_{15})_{0.35}]\cdot 0.74H_2O$: C, 43.15; H, 7.00; N, 4.62. Found: C, 43.15; H, 7.19; N, 4.63.

(*d*) Chitosan (0.80 g, 5.00 mmol) and **25** (1.00 g, 2.60 mmol) were reacted to give 1.36 g (93%) of derivative **34d**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.67}(C_{20}H_{36}NO_{15})_{0.33}]\cdot 0.44H_2O$: C, 43.84; H, 6.92; N, 4.82. Found: C, 43.84; H, 7.27; N, 4.84.

(*e*) Chitosan (0.60 g, 3.70 mmol) and **25** (0.50 g, 1.30 mmol) were reacted to give 0.80 g (87%) of **34e**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.76}(C_{20}H_{36}NO_{15})_{0.24}]\cdot 0.49H_2O$: C, 43.47; H, 6.97; N, 5.41. Found: C, 43.46; H, 7.29; N, 5.37.

N-*[2-(2-Acetamido-2-deoxy-β-D-glucopyranosyloxy)ethyl]chitosan* (**35**). (*a*) Reaction of chitosan (0.30 g, 1.9 mmol) with the formylmethyl β-D-glycoside **26** (1.50 g, 5.6 mmol) gave 0.67 g (85%) of the derivative **35a**.

Anal. Calc. for $[(C_{16}H_{28}N_2O_{10})]\cdot 0.85H_2O$: C, 45.36; H, 7.02; N, 6.61. Found: C, 45.36; H, 7.33; N, 6.43.

N-*(2-(α-D-Glucopyranosyloxy)ethyl]chitosan* (**36**). (*a*) Chitosan (0.65 g, 4.04 mmol) when treated with formylmethyl α-D-glucopyranoside **27** (2.65 g, 11.9 mmol) gave 1.42 g (96%) of derivative **36a**.

Anal. Calc. for $[(C_{14}H_{25}NO_{10})_{1.00}]\cdot 0.61H_2O$: C, 44.44; H, 6.94; N, 3.70. Found: C, 44.45; H, 6.85; N, 3.40.

(*b*) Chitosan (0.60 g, 3.73 mmol) was coupled with the aldehyde **27** (1.24 g, 5.59 mmol) to give 0.66 g (61%) of **36b**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.41}(C_{14}H_{25}NO_{10})_{0.59}]\cdot 0.87H_2O$: C, 43.14; H, 7.04; N, 4.69. Found: C, 43.14; H, 7.10; N, 4.69.

(*c*) Chitosan (0.60 g, 3.73 mmol) was reacted with the aldehyde **27** (0.62 g, 2.80 mmol) to give 0.67 g (72%) of compound **36**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.62}(C_{14}H_{25}NO_{10})_{0.38}]\cdot 0.62H_2O$: C, 43.30; H, 7.00; N, 5.59. Found: C, 43.30; H, 7.01; N, 5.60.

(*d*) Chitosan (0.80 g, 5.0 mmol) was treated with aldehyde **27** (1.11 g, 5.00 mmol) to yield 0.91 g (80%) of **36d**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.74}(C_{14}H_{25}NO_0)_{0.26}]\cdot 0.66H_2O$: C, 42.82; H, 7.05; N, 6.18. Found: C, 42.82; H, 7.15; N, 6.17.

N-*[2-(α-D-Galactopyranosyloxy)ethyl]chitosan* (**37**). (*a*) Reaction of chitosan (0.45 g, 2.8 mmol) and the aldehyde (**28**, 1.90 g, 8.64 mmol) gave 0.60 g (57%) of lyophylized product.

Anal. Calc. for $[(C_{14}H_{25}NO_{10})]\cdot 0.63H_2O$: C, 44.40; H, 6.94; N, 3.70. Found: C, 44.41; H, 7.01; N, 3.74.

(*b*) Reaction of the aldehyde (**28**, 1.67 g, 7.52 mmol) and chitosan (0.60 g, 3.73 mmol) afforded 0.68 g (54%) of a white fluffy product **37b**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.14}(C_{14}H_{25}NO_1)_{0.86}]\cdot 1.06H_2O$: C, 43.27; H, 7.03; N, 3.92. Found: C, 43.27; H, 7.12; N, 3.91.

(*c*) When chitosan (0.80 g, 5.0 mmol) was reacted with the aldehyde **28** (1.11 g, 5.00 mmol), 1.34 g (96%) of product **37c** was obtained.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.52}(C_{14}H_{25}NO_{10})_{0.48}]\cdot 0.49H_2O$: C, 43.95; H, 6.97; N, 5.21. Found: C, 43.95; H, 7.19; N, 5.20.

(*d*) When chitosan (0.80 g, 5.0 mmol) was treated with the aldehyde **28** (0.83 g, 3.74 mmol), 1.10 g (95%) of product **37d** was obtained.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.68}(C_{14}H_{25}NO_{10})_{0.32}]\cdot 0.41H_2O$: C, 43.85; H, 6.96; N, 5.98. Found: C, 43.85; H, 7.30; N, 5.98.

N-*[2-(2-Acetamido-2-deoxy-α-D-glucopyranosyloxy)ethyl]chitosan* (**38**). (*a*) Chitosan (0.60 g, 3.7 mmol) was treated with the aldehydo sugar **29** (3.05 g, 11.5 mmol) to give 1.35 g (92%) of lyophilized product **38a**.

Anal. Calc. for $[(C_{16}H_{28}N_2O_{10})\cdot 0.36H_2O]$: C, 46.32; H, 6.92; N, 6.76. Found: C, 46.32; H, 6.61; N, 6.78.

(*b*) Reaction of chitosan (0.60 g, 3.7 mmol) with the formylmethyl glycoside **29** (3.00 g, 11.4 mmol) gave 1.25 g (85%) of product after freeze-drying.

Anal. Calc. for $[(C_{16}H_{28}NO_{10})\cdot 1.53H_2O]$: C, 44.08; H, 7.12; N, 6.43. Found: C, 44.08; H, 7.05; N, 6.23.

(*c*) Coupling of the aldehyde **29** (1.60 g, 6.0 mmol) to chitosan (0.65 g, 4.0 mmol) yielded 1.0 g (95%) of derivative **38c**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.63}(C_{16}H_{28}N_2O_{10})_{0.37}]\cdot 0.52H_2O$: C, 44.47; H, 7.01; N, 7.33. Found: C, 44.47; H, 6.86; N, 7.31.

(*d*) Treatment of chitosan (0.65 g, 4.0 mmol) with the aldehyde **29** (0.79 g, 3.0 mmol) gave 0.80 g (93%) of compound **38d**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.83}(C_{16}H_{28}N_2O_{10})_{0.17}]\cdot 0.58H_2O$: C, 43.30; H, 7.05; N, 7.68. Found: C, 43.30; H, 7.10; N, 7.71.

(*e*) Chitosan (80.80 g, 5.0 mmol) was treated with **29** (0.70 g, 2.5 mmol) to produce 1.03 g (95%) of product **38e**.

Anal. Calc. for $[(C_6H_{11}NO_4)_{0.81}(C_{16}H_{28}N_2O_{10})_{0.19}]\cdot 0.46H_2O$: C, 43.84; H, 7.00; N, 7.70. Found: C, 43.83; H, 7.18; N, 7.70.

N-*[2-(2-Amino-2-deoxy-α-D-glucopyranosyloxy)ethyl]chitosan* (**39**). Derivative **38b** (1.25 g, 3.10 mmol) was suspended in 40% aq. NaOH (60 mL) and heated for 6 h at 100° under N_2. The mixture was then cooled, made neutral, dialyzed, and freeze-dried to give 1.1 g (97%) of **39**.

Anal. Calc. for $[(C_{14}H_{26}N_2O_9)_{1.0}]\cdot 3.0H_2O$: C, 40.00; H, 7.63; N, 6.67. Found: C, 40.00; H, 7.97; N, 6.76.

N-*[2-(α-D-Glucopyranosyloxy)ethyl]chitosan* (**40**). Derivative **36c** (160 mg, 0.67 mmol) was dissolved in 1:1 water–MeOH (13 mL) and stirred with Ac_2O (70 mg, 65 μL, 0.67 mmol) for 24 h. Exhaustive dialysis against distilled water gave derivative **40** (95%).

Anal. Calc. for $[(C_8H_{13}NO_5)_{0.62}(C_{14}H_{25}NO_{10})_{0.38}]\cdot 0.83H_2O$: C, 44.03; H, 6.86; N, 5.00. Found: C, 44.03; H, 6.84; N, 4.80.

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