(14) Siegfried, D. L.; Manson, J. A.; Sperling, L. H. *J. Polym. Sci., Polym. Phys. Ed.* **1978**, *17*, 583.
(15) Miyata, S.; Hata, T. *Rep. Prog. Polym. Phys. Jpn.* **1969**, *12*, 313.
(16) Kato, K. *J. Polym. Sci., Part B* **1966**, *B4*, 35.
(17) Matsuo, M.; Kwei, J. K.; Klempner, D.; Frisch, H. L. *Polym. Eng. Sci.* **1970**, *10*, 327.
(18) American Society for Testing and Materials, ASTM D-1505-68.

Some Chemical and Analytical Aspects of Polysaccharide Modifications.[1] 3. Formation of Branched-Chain, Soluble Chitosan Derivatives[2]

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ABSTRACT: Specific attachment of carbohydrates to the 2-amino functions of chitosan transforms this water-insoluble, linear polymer into branched-chain water-soluble derivatives. Facile conversions can be achieved by reductive alkylation using sodium cyanoborohydride and any aldehydo or keto sugar, by Schiff base formation, or by amidation reactions using carboxylic acid or lactone derivatives. Experimental results are presented for a series of mono-, di-, tri-, and polysaccharides, including D-glucose, *N*-acetylglucosamine, D-glucosamine, D-galactose, D-galactosamine, D-fructose, D-glucoheptonic acid γ-lactone, lactose, cellobiose, maltose, melibiose, maltotriose, streptomycin sulfate, C^6-*aldehydo*-cycloheptamylose, and dextran. These procedures facilitate the preparation of polymer derivatives with a variety of comblike, treelike, and other branching types. Many of these products are amenable to further, specific chemical modifications; this is demonstrated by the introduction, via galactose oxidase treatment, of C-6 aldehyde functions into the pendant galactose residues of derivatives **8** and **13**. The synthetic chitosan derivatives exhibit a number of useful and uncommon properties in terms of their solution characteristics. Thus, aqueous solutions of derivative **8** were compatible with a wide range of salts, and derivatives **11** and **13** were stable in 50% aqueous ethanol. Derivative **8** formed inclusion complexes with iodine, lactose, and 4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl. Solubility modifications were accomplished by coreaction of hydrophilic (lactose) and hydrophobic (various alkyl) residues, affording products, such as **24**, which were soluble in both aqueous and organic media. Reductive alkylation of chitin afforded the 1-deoxylactit-1-yl derivative **27**, which was water insoluble but formed sols in water and several organic solvents. Factors affecting the solution behavior of chitosan and its branched derivatives have been evaluated and mechanisms have been discussed for solute interactions and conformational transitions.

Introduction

In contrast to most other polysaccharides, both chitin, a β(1-4)-linked 2-deoxy-2-acetamido-D-glucopyranosyl polymer, and chitosan, its *N*-deacetylated derivative, exhibit basic properties (pK_a of chitosan is 6.3) which impart them with unique characteristics in terms of solution properties, membrane-forming ability, and metal chelation capacity.[4] Chitin is widely distributed in nature with an estimated[5] annual natural production of 10^{10}–10^{11} tons, yet this inexpensive biopolymer has attracted, by comparison with for example cellulose, relatively little attention mainly as a result of its intractability. Thus, both chitin and chitosan are insoluble in common organic solvents, water, dilute acids, or cold alkalis of any concentration. Only a few solvent systems reportedly do not give rise to hydrolysis of the amide or glycosidic linkages;[4] these include hexafluoro-2-propanol, hexafluoroacetone sesquihydrate, certain chloro alcohols, and lithium chloride in *N*,*N*-dimethylacetamide solutions for chitin[6] and a number of organic acids, such as acetic and formic acid, for chitosan. Clearly, these solvents offer only very limited utility for chemical derivatizations in homogeneous solution, particularly for large-scale applications.

In this study we report the use of reductive alkylation as a facile and versatile procedure for covalent attachment of a diverse range of carbohydrates and other substrates to the primary amine functions of chitin and chitosan. As part of a general program of selective chemical modifications of polysaccharides, this work pursued two major interests. Our first objective was to develop derivatization methods which could ultimately lead to a greater commercial utilization of chitin and chitosan. The second, conceptually more challenging goal involved the synthesis of model compounds which would facilitate systematic investigations of the structure/function relationship of polysaccharides. Although some of the properties of polysaccharides are known to follow certain trends, a methodical understanding of the relation of their primary structure to their aqueous solution characteristics and solute interactions, etc., remains to be established.[5,7]

Clearly, one of the keys to any successful large-scale utilization of chitin/chitosan lies in the resolution of the intractability of these polymers. It would be desirable to design versatile synthetic routes for the formation of water- or organic-soluble products or, ideally, of derivatives whose solubility and hydrophobicity could be tailored. Previous specific derivatizations of the primary amine group of chitosan[8] have invariably afforded insoluble products. Although certain water soluble ether and salt derivatives of chitosan are known,[4] there have been no efforts to affect solubilization by introducing suitable hydrophilic moieties onto the polymer backbone. Similarly, there are only a few examples of derivatized chitins and chitosans which are soluble in organic solvents;[6,9] none of the reported derivatives is soluble in both organic and aqueous solvents. As we shall demonstrate, the methods described here allow the preparation of chitosan derivatives which are of the latter type.

Prerequisite to any systematic investigations of the structure/function relationship of polysaccharides is the availability of versatile strategies which would allow the synthesis of branched polymers, whose branch type, branch

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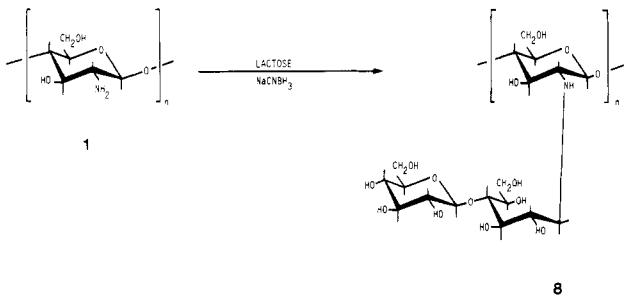
Scheme I



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stereochemistry, and branch length as well as degree of branching are readily controllable. Considerable efforts have been directed at the conversion of linear polysaccharides into branched-chain analogues which by themselves are of interest for a variety of other reasons,[10] including the investigation of lectin–carbohydrate reactions,[11] the preparation of model compounds in the fields of allergy,[12] enzymology,[13] and immunology,[14] the study of the physical properties of branched-chain derivatives,[15] and the synthesis of analogues of natural, branched polysaccharides such as xanthan gum, with improved properties for commercial applications. Previous studies have applied to cellulose, amylose, alginic acid, and other polysaccharides various synthetic routes,[15] including (a) copolymerization,[17] (b) reaction with orthoesters,[15] aceto bromo sugars,[18] or hydrazones,[19] and (c) enzymic glycosylations.[20] These procedures, however, suffer from various limitations since they require (i) specific protection of the linear polysaccharide, such as in the reaction of 1,2-orthoacetate sugars with 2,3-di-*O*-phenylcarbamoyl derivatives of amylose and cellulose, (ii) activation and/or partial protection of the sugar which is to form the side chain and removal of the protecting groups subsequent to the reaction, or (iii) reaction conditions which may lead to partial or extensive polysaccharide degradation, e.g., using hydrazine hydrate. Most of the above reactions are also laborious and low yielding, all reasons which mitigate against routine or large-scale synthesis. As we shall illustrate here, reductive alkylation provides a particularly facile and versatile procedure for preparing polysaccharide derivatives with a wide variety of branch types and branch lengths.

Results and Discussion

Synthesis. By use of chitosan as an exemplar, a method was devised which is suitable for the transformation of linear, amine-containing polysaccharides into stable, branched-chain derivatives. The reaction itself, summarized in Scheme I, represents a further example of the reductive amination procedure[21] which is compatible with essentially any aldehydo or keto sugar. Under typical conditions, chitosan **1**, dissolved in a mixture of dilute aqueous acetic acid and methanol, was reductively alkylated by a solution of the carbonyl-containing sugar (1.1–16.7 mol equiv per hexosamine residue (mol/GlcN)) at ambient temperature. The reactions of chitosan with various aldehydo, keto, and lactone sugars are summarized in Table I (structures in Charts I and II).

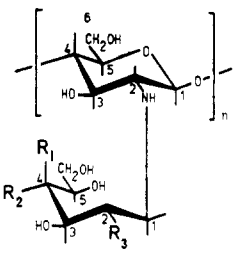
From Table I it is evident that the reactions of aldehydo sugars and chitosan generally proceed smoothly, yielding products with mostly high degrees of substitution (ds). Almost all of these reactions were accompanied by the formation of soft to very rigid, transparent or white gels with, in the latter case, attendant synereses. Not surprisingly, monosaccharides afforded products and gels at almost twice the rate of disaccharides and, for a given reaction time, the ds of the corresponding chitosan derivatives increased with the concentration of aldehydo sugar. Conversely, high ds values could be obtained at relatively low sugar concentrations by extending the reaction period or by elevating the temperature. We found that, for a given concentration of aldehydo sugar and under otherwise identical reaction conditions, Schiff's base products, such as derivative **8** which was prepared by condensation of lactose and chitosan, had lower ds values (**8**, ds 0.1) than the analogous amine derivatives. Presumably this is largely a reflection of the hydrolytic lability of the imine linkages of the Schiff's bases; although this is a disadvantage in this context, it may be valuable for other applications, such as drug release formulations.[22]

As can be seen from Table I, the derivatization of chitosan with reducing sugars can be extended to trisaccharides, e.g., maltotriose (**14**, ds 0.54) and higher oligo- and polysaccharides as exemplified here by dextran ($\bar{M}_w$ = 10 000). Facile alkylation reactions can be similarly performed with keto sugars, e.g., fructose (**15**, ds 0.7), lactones, e.g., α-glucoheptonic acid γ-lactone (**16**, ds 0.75), and other carbonyl-containing carbohydrates, such as streptomycin sulfate and specifically oxidized cyclodextrins.[23a] Thus, branched, comb-like chitosan derivatives can be prepared with practically any desired side-chain length and ds value.

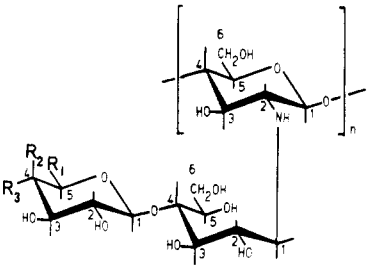
The versatility of this procedure is demonstrated by the fact that many of the synthetic chitosan derivatives are themselves amenable to further chemical modifications; this is exemplified here by the specific oxidation of the pendant galactose residues of **8** and **13** using galactose oxidase (EC 1.1.3.9.)[1] to afford the corresponding C-6 aldehyde derivatives **17** and **18** which constitute useful intermediates for specific chain elongation (via, e.g., reductive amination[23b]) or other types of reactions. Similarly, the primary amine function of the pending deoxyglycit-1-yl residues of **5** and **7** provides a convenient locus for further reductive alkylation reactions which would afford more complex (tree-like) branching patterns. Other branching types can be alternately obtained by introduction of suitable spacer groups, such as diaminopimellic acid (via carbodiimide-mediated coupling reactions); derivative **23** was prepared as a model glycopeptide.[5]

The reductive alkylation procedure can be employed with equal facility for attaching nonsaccharidic residues, such as aliphatic and aromatic aldehydes and ketones, to chitosan, as exemplified by cyclohexanone (**22**) and various salicylaldehyde-type derivatives.[24] While our discussion in this paper focuses mainly on carbohydrate derivatives of chitosan, we also draw attention to the possibility of tailoring the solubility, hydrophobicity, and other properties of products by coreacting nonsaccharidic carbonyl compounds in admixture with an aldehydo sugar. A few illustrative examples may suffice here to indicate the scope of this method. When a mixture (1:1.3) of lactose and propionaldehyde was employed in the reductive alkylation of chitosan, we obtained a water soluble product **24**, which (in contrast to derivative **8**) was also completely compatible with several organic solvents, including acetone, ether, ethanol, and chloroform. Similarly, chitosan derivatives were prepared from mixtures containing lactose as hydrophilic substituent and either *N*-cyclohexyl-2-pyrrolidone (**25**) or *N*-cocoalkyl-2-pyrrolidone (**26**). We will describe the diverse range of rheological characteristics of these "mixed" derivatives elsewhere. Although a few polysaccharide derivatives, such as certain hydroxypropylcelluloses, are known to be both water and organic soluble, our method seems to offer the advantages of simplicity and versatility in tailoring solubility; the overall

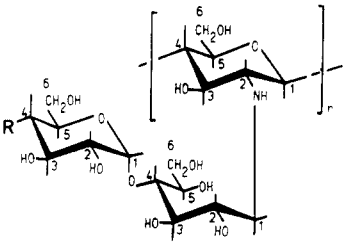
Chart I



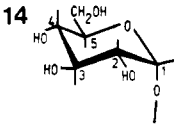
	R_1	R_2	R_3
3	H	OH	OH
4	H	OH	$NHCOCH_3$
5	H	OH	NH_2
6	OH	H	OH
7	OH	H	NH_2



	R_1	R_2	R_3
8	CH_2OH	OH	H
11	CH_2	H	OH
17	CHO	OH	H



	R
12	H



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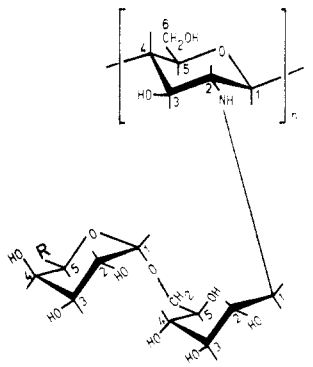
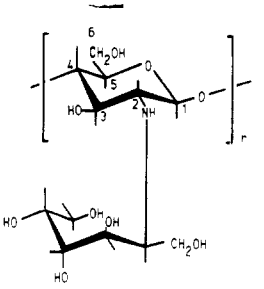


	R
13	CH_2OH
33	CHO



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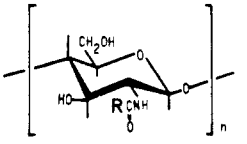

	R
2	CH_3
16	$(CHOH)_5CH_2OH$
23	$(NH_2)CH(CH_2)_3CH(NH_2)COOH$

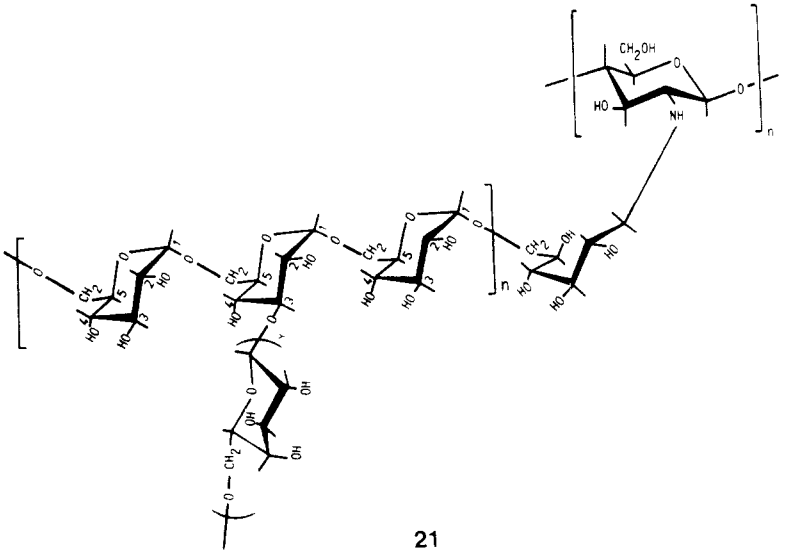

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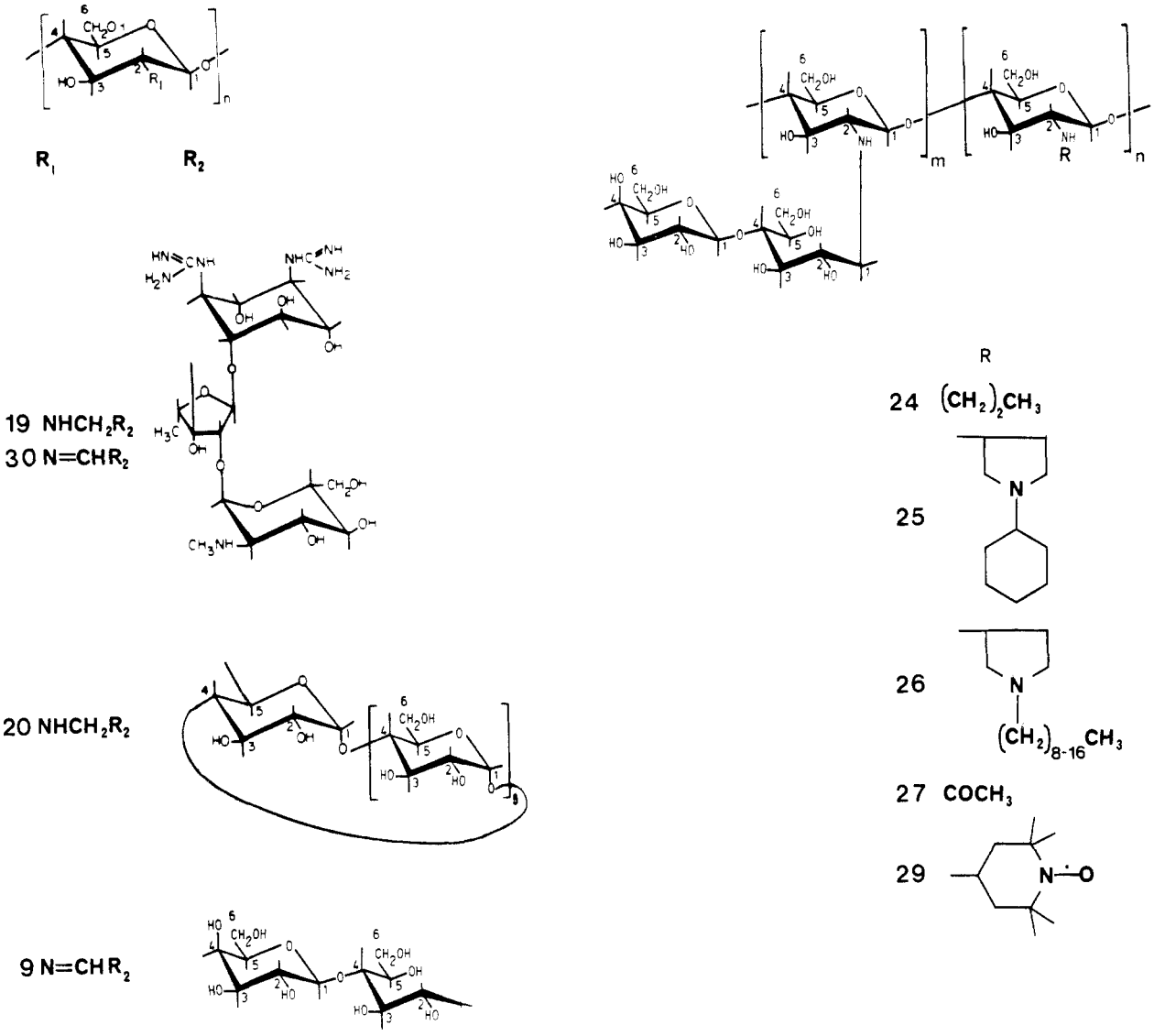
Table I
***N*-Alkylation of Chitosan with Carbohydrates**

reaction conditions			product					C		H		N	
carbohydrate used	mol/GlcN	time, h	gel[a]	code	ds[b]	solubility[c]	formula	calcd	found	calcd	found	calcd	found
D-glucose	1.33	8	–	**3**	*d*								
	3.00	8	+[5]		0.9	2, 5	$[(C_8H_{13}NO_5)_{0.02}(C_6H_{11}NO_4)_{0.08}(C_{12}H_{23}NO_9]\cdot 0.51H_2O$	43.05	42.80	7.22	7.10	4.39	4.60
N-acetylglucosamine	1.6	12	+[4]	**4**	*d*								
	1.6	24	+[5]		0.97	2, 5	$[(C_8H_{13}NO_5)_{0.03}(C_{14}H_{26}N_2O_9)_{0.97}]\cdot 2.9H_2O$	40.20	39.99	7.44	6.90	6.69	6.55
D-glucosamine	1.3–1.6	8	+[3]	**5**	0								
	2.7	72	+[3]		0								
	7.8	48	+[5]		0.67	2, 5	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.30}(C_{12}H_{26}N_2O_9)_{0.67}]\cdot 0.1H_2O$	42.37	42.55	7.52	7.47	8.19	7.83
D-galactose	2.03	4	+[4]	**6**	0.76	2, 5	$[(C_8H_{13}NO_5)_{0.02}(C_6H_{11}NO_4)_{0.22}(C_{12}H_{23}NO_9)_{0.76}]\cdot 0.6H_2O$	42.88	42.59	7.26	7.20	4.72	4.97
	2.22	4	+[4]		0.97	2, 5	$[(C_8H_{13}NO_5)_{0.02}(C_6H_{11}NO_4)_{0.01}(C_{12}H_{23}NO_9)_{0.97}]\cdot 1.8H_2O$	40.26	40.09	7.38	7.58	3.96	3.97
D-galactosamine	1.17–2.73	72	–	**7**	0								
	7.8	48	+		0.67	2, 5	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.30}(C_{12}H_{26}N_2O_9)_{0.67}]\cdot 0.89H_2O$	40.36	40.74	7.70	6.72	7.80	7.40
lactose	1.17	10	–	**8**	0.25	1, 2	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.72}(C_{18}H_{33}NO_{14})_{0.25}]\cdot 0.1H_2O$	44.26	44.29	6.88	7.27	5.70	5.96
	1.50	30	–		0.14	1, 2	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.83}(C_{18}H_{33}NO_{14})_{0.14}]\cdot 0.73H_2O$	42.00	41.69	7.11	7.03	6.33	6.60
	1.54	144	+[5]		0.80	1, 2	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.17}(C_{18}H_{33}NO_{14})_{0.80}]\cdot 2.8H_2O$	39.67	39.29	7.29	6.88	2.96	3.00
	4.0	96	+[5]		0.95	1, 2	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.02}(C_{18}H_{33}NO_{14})_{0.95}]\cdot 1.05H_2O$	42.67	42.88	6.99	7.69	2.85	2.86
	2.92–2.94	24	+[5]	**10**	"2.0"[e]	1, 2	$[(C_8H_{13}NO_5)_{0.03}(C_{18}H_{33}N_{14})_{0.97} + C_{12}H_{22}O_{11}]\cdot 2.0H_2O$	41.59	41.49	6.87	6.96	1.63	1.55
cellobiose	1.17	12	–[6]	**11**	*d*								
	2.20	36	–[6]		0.3	1, 2, 3, 4	$[(C_8H_{13}NO_5)_{0.05}(C_6H_{11}NO_4)_{0.65}(C_{18}H_{33}NO_{14})_{0.3}]\cdot 0.72H_2O$	42.47	42.38	7.04	7.06	5.11	5.15
	2.92	48	+[5]		0.4	1, 2, 3, 4	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.56}(C_{18}H_{33}NO_{14})_{0.4}]\cdot 0.95H_2O$	42.02	41.96	7.07	7.12	4.54	4.60
	2.92	456	+[5]		0.7	1, 2, 3, 4	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.27}(C_{18}H_{33}NO_{14})_{0.70}]\cdot 2.6H_2O$	39.66	39.42	7.29	6.15	3.20	3.31
maltose	1.7	12	–[6]	**12**	*d*								
	1.7	30	+[5]		0.6	1	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.37}(C_{18}H_{33}NO_{14})_{0.6}]\cdot 1.92H_2O$	40.53	40.14	7.21	6.72	3.57	3.67
	2.92	168	+[5]		0.87	1	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.1}(C_{18}H_{33}NO_{14})_{0.87}]\cdot 2.72H_2O$	39.99	39.77	7.26	6.76	2.83	2.78
melibiose	1.11	4	+[3]	**13**	*d*								
	1.11	18	+[5]		0.6	2, 4	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.37}(C_{18}H_{33}NO_{14})_{0.6}]\cdot 0.59H_2O$	43.17	43.05	6.96	6.87	3.80	3.80
maltotriose	1.3	12	–	**14**	0								
	1.78	456	–		0.14	1, 5	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.83}(C_{24}H_{44}NO_{19})_{0.14}]\cdot 1.61H_2O$	39.62	39.33	7.33	6.59	5.39	5.43
	4.16	144	–		0.54	1, 5	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.43}(C_{24}H_{44}NO_{19})_{0.54}]\cdot 2.37H_2O$	40.36	40.00	7.22	6.40	2.98	3.05
D-fructose	2.2–3.2	30	–[6]	**15**	0								
	5.6	456	–		*d*								
	5.6	168	–		0.1	2	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.87}(C_{12}H_{23}NO_9)_{0.1}]\cdot 1.23H_2O$	39.78	39.54	7.38	6.80	6.97	6.93
	16.7	72	–		0.7	2	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.27}(C_{12}H_{23}NO_9)_{0.7}]\cdot 1.35H_2O$	40.84	40.58	7.41	6.88	4.64	4.69
	20.4	624	+[5]		0.7	2	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.27}(C_{12}H_{23}NO_9)_{0.7}]\cdot 1.2H_2O$	41.21	40.94	7.37	6.99	4.69	4.73
α-glucoheptonic acid γ-lactone	3.2	72	–	**16**	0								
	3.65	144	+[3,5]		0.77	1, 2	$[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.2}(C_{13}H_{23}NO_{11})_{0.77}]\cdot 1.15H_2O$	41.52	41.22	6.88	7.20	4.23	4.22
dextran	0.17	40	–	**21**	ca. 0.15[g]	1	*f*		40.42		6.78		1.08

[a] Key: (+) gel formed, (–) gel not formed; (1) firm, (2) elastic, (3) very soft, (4) transparent, (5) white gel, (6) increase in viscosity of reaction mixture. [b] Obtained from microanalysis. [c] Solubility at ambient temperature and 1.0–3.0% (w/w) polymer concentration: (1) aqueous solution (pH 7), (2) dilute acidic solution (pH 5–6), (3) dilute basic solution (pH 8), (4) 50% aqueous ethanol, (5) with gel formation. [d] Not determined. [e] See text for discussion. [f] Unknown. [g] Based on $\bar{M}_w$ 10 000.

Chart II



solubility characteristics can be adjusted by varying, for example, either the hydrophobicity of one of the substrates and/or the ratio of hydrophobic and hydrophilic substituents. Another application of this concept involves the solubilization of otherwise insoluble polymer products,[24] which may be of use for catalysis.

1-Deoxylactit-1-ylchitin. As a logical extension of the above work, we also prepared a branched-chain derivative of chitin. The reductive alkylation of the small percentage (ca. 10%) of free amine groups, however, required a somewhat different synthetic approach since chitin is insoluble in the acetic acid/methanol system. We found that 1-deoxylactit-1-ylchitin, **27** (ds 0.09), could be prepared either under homogeneous conditions or by brief alkali preactivation of **2** and subsequent heterogeneous reaction. Significantly, the relatively small extent of derivatization in these products manifests itself by substantial changes in the physical properties of chitin. Although 1-deoxylactit-1-ylchitin, unlike its chitosan analogue **8**, with an equivalent ds value, does not completely dissolve in aqueous solution (at pH values ranging from 1 to 7), it forms transparent sols in water (pH <6), dimethyl sulfoxide, *N,N*-dimethylformamide, pyridine, and several other organic solvents.

^{13}C NMR. ^{13}C NMR was employed for the characterization of chitosan and the 1-deoxyglycit-1-yl derivatives. The ^{13}C chemical shift assignments of chitosan and a selected number of the derivatives are summarized in Table II.

The spectral assignment of chitosan **1** itself in dilute acetic acid was readily accomplished by comparison with previous data reported for chitobiose and the monomeric aminoglycosides.[25,26] The anomeric signals of both the aminoglucose and acetamidoglucose residues of **1** were clearly resolved; the signal at lower field was attributed to the acetamido derivative while that of the amino derivative appeared 3 ppm upfield. The remaining ring carbon signals of the two types of hexosamine residues were indistinguishable at this pH (pD 4.0) and were assigned, on the basis of relative proportions of acetamidoglucose and glucosamine residues in **1**, to the latter (Table II). On lowering the pH (to pD 1.5) the resonances of **1** experienced an upfield shift of 2–3 ppm with a concomitant appearance of several additional signals, whose tentative assignments are listed in Table II. Prolonged storage (several weeks) of solutions of **1** at pD 1.5 and ambient temperature gave rise to a complex mixture of mono- and oligomeric hexosamine residues as evidenced by additional resonances in the anomeric region as well as in the spectral region between 60–77 ppm; these spectra were not further investigated.

Upon derivatization, the resonances of the polysaccharide backbone were found to be displaced by only small increments. For example, the hexosamine signals

Table II
Chemical Shift Assignment of Chitosan and Some Derivatives

compd	(ds)[h]		chemical shift,[a] ppm										
			C1	C2	C3	C4	C5	C6	C1′	C2′	C3′	C4′	C5′	C6′
1[b]	pD 4.0	β1,4-(2-deoxy-2-aminoglucopyranosyl)[k]	101.81	56.6	75.41	71.21	77.81	60.92						
		β1,4-(2-deoxy-2-acetamidoglucopyranosyl)[k]	98.61	nr	nr	nr	nr	nr						
	pD 1.5	β1,4-(2-deoxy-2-aminoglucopyranosyl)	98.31	53.13	72.00	68.51	74.36	57.51						
		β1,4-(2-deoxy-2-acetamidoglucopyranosyl)	94.91	52.91	71.61	67.85	nr	nr						
11	(0.3)	β1,4-(2-deoxy-2-aminoglucopyranosyl)	102.01	56.90	75.21	71.55	77.65	61.33						
		β-glucopyranosyl	103.06	74.14	76.34	nr	76.54	61.33						
		1-deoxyglucit-1-yl							51.37	73.91	75.54	80.62	71.55	62.61
8	(0.9)	β1,4-(2-deoxy-2-aminoglucopyranosyl)	102.87	56.84	75.70[c]	72.06[d]	77.71	61.88						
		β-galactopyranosyl	104.05	72.06	73.50	69.09	75.70[c]	nr						
		1-deoxyglucit-1-yl							51.35	72.06[d]	72.06[d]	79.09[e]	nr	62.96
10	("2.0")	lactose		nr	nr	73.50	69.60	76.17	nr	92.66[i]	nr	79.09[e]	75.70[c]	nr
										96.58				
8	(0.25)	β1,4-(2-deoxy-2-aminoglucopyranosyl)	102.77	57.00	74.91	nr	77.51	61.91						
		β-galactopyranosyl	104.02	72.02	72.88	69.19[f]	76.07	nr						
		1-deoxyglucit-1-yl							51.12	72.06	72.06	79.07	69.19[f]	63.01
22[b]	(0.5)	β1,4-(2-deoxy-2-aminoglucopyranosyl)	102.17	56.64	75.32	71.08	79.02	61.97						
			98.53					60.60						
	pD 4.0	cyclohexyl							59.87	30.52	25.05[g]	30.01	24.84[g]	30.52

[a] In D_2O relative to internal *p*-dioxane (67.40 ppm) at 315 K (± 0.07 ppm). [b] Solvent DOAc-d_4/D_2O. [c–f] Coinciding resonances. [g] Assignment may be reversed. [h] Degree of substitution. [i] α, β, respectively. [k] Resonances coincide except as indicated; nr = not resolved.

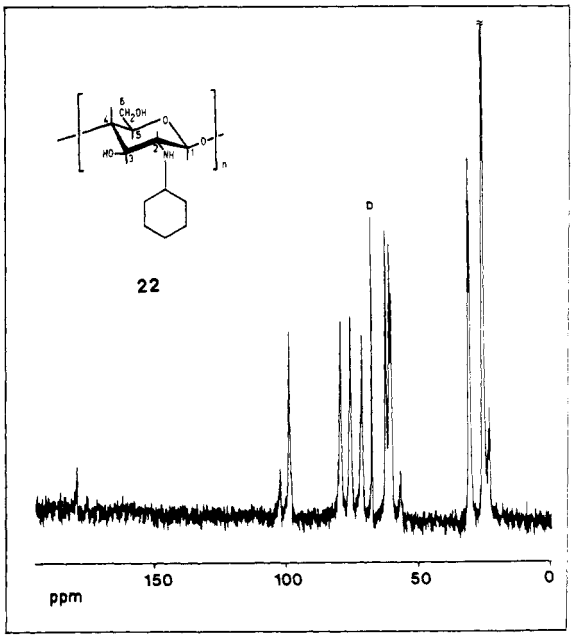


Figure 1. 100.6-MHz ^{13}C NMR spectrum of derivative **22** in D_2O/DOAc-d_4.

of C-1, C-2, C-4, and C-6 of derivative **11** were shifted downfield by approximately 0.3 ppm, while those of C-3 and C-5 were shifted upfield by 0.2 ppm. The resonances of the pending cellobiityl residues could be identified by comparison with the chemical shifts reported for cellobiose[27] and its 1-deoxy derivatives.[28]

The chemical shift of the deoxyglucityl C-1′ was displaced upfield by more than 40 ppm from that of the parent cellobiose. The assignments of the terminal glucopyranosyl residue of **11** were found to be in good agreement with previous results.[27]

The chemical shifts of the hexosamine residues of derivative **8** followed the same trend which was established for derivative **11**; the signals of the covalently linked lactityl residues were readily identified by comparison with previous assignments of lactose,[27] as well as of a series of related aryl- and alkyldeoxylactitylamine derivatives.[28,29] The C-1′ signal of the 1-deoxyglucit-1-yl residues of derivative **8** appeared at 51.35 ppm and was in accord with the corresponding signals of the alkyl derivatives of Hoagland et al.[29] Good agreement was also obtained between the remaining chemical shift assignments of **8** and ^{13}C NMR data reported elsewhere. The observed chemical shifts of this product agreed with those discussed above, revealing only minor displacements of some resonances.

The cyclohexyl derivative **22** produced a well-dispersed spectrum (at pD 4.0) in which the methylenic ring carbons were observed in the region between 24 and 31 ppm, in close agreement with the corresponding signals of *N*-methylcyclohexylamine[30] (Figure 1); the cyclohexyl C-1′ resonance appeared at 59.87 ppm, 1.2 ppm downfield from that of the corresponding *N*-methyl derivative.

The chemical shifts of the carbons of the polymer backbone were found to be again in good agreement with those of the previous derivatives with the exception of the C-5 signal which was displaced downfield by 1.2 ppm with respect to that of **1**. The hexosamine resonances in the spectral region between 70 and 80 ppm were further characterized by the presence of incompletely resolved signals at lower field which presumably arise from the unbranched hexosamine residues since the branched residues produced, in general, sharper signals. The C-6 resonances of both the glucosamine and acetamidoglucose

residues of derivative **22** were clearly resolved and could be assigned on the basis of the corresponding monomeric aminoglycosides.[25]

The carbonyl and methyl resonances of the *N*-acetate groups were observed at 175 (±0.6) and at 22 (±0.9) ppm, respectively, for all chitosan derivatives discussed here.

Solubility and Compatibility. In contrast to other known covalent derivatives, all of the branched-chain chitosan derivatives were soluble in either neutral or slightly acidic (pH 5–6) aqueous medium (see Table I). Water solubility could be achieved even at relatively low ds values, such as for **8** (ds 0.14). At concentrations above 1–5%, derivatives **3**, **8**, **10**, **11**, **12**, **14**, and **16** formed rigid or elastic gels in neutral aqueous solution, while derivatives **4** and **6** gelled in slightly acidic medium.

Derivatives **10** and **11** exhibited stability in alkaline solution, and **11** and **13** were also compatible with 50% aqueous ethanol. While chitosan can be precipitated from solution by a number of salts containing large anionic species, such as ammonium sulfate, potassium chromate, sodium sulfite, and sodium phosphate, aqueous solutions of the deoxylactit-1-yl derivative **8** were compatible with the above as well as with a series of other species, including sodium citrate, sodium hypophosphate, ferrous sulfate, boric acid, the chlorides of sodium, calcium, chromium, and tin, or several combinations of these.

Interestingly, the derivative **11**, which by itself did not gel, was found to form rigid white gels (which contracted after a few hours), when mixed with alginate, and viscous solutions, when mixed with either guaran or locust bean gum.[31]

Solute Interactions of Chitosan Solutions. Previous studies have demonstrated that acidified blends of chitosan and polyols, such as sorbitol and glycerol, produce high-viscosity solutions,[32a] while aqueous oxalic acid solutions of chitosan afford gels.[32b] In an attempt to gain an understanding of some of the pronounced changes in solution behavior (such as gelation and sharp viscosity increase) which we observed in many of our chitosan reactions, we examined the interactions of chitosan solutions with a series of solutes.

First we found that addition of melezitose solutions (1.05 mol/GlcN) to chitosan induced a drastic rise in viscosity and ultimately (over a period of 10 days) led to the formation of viscoelastic gels. Surprisingly, however, several other nonreducing saccharides, including sucrose and trehalose, did not evoke comparable behavior. Gel formation could also not be provoked either thermally (by cooling to 5 °C) or by extended (2 weeks) exposure to sodium cyanoborohydride (6.2 mol/GlcN), borate ions (5.4 mol/GlcN), or any of a series of other solutes, including alkali earth, lanthanide, and transition-metal salts.

In light of the above results and the observations made during the alkylation reactions (Table I), it appears that several distinct phenomena are operative in the gelation of chitosan. In the case of melezitose (and similarly, sorbitol and glycerol), the solute can be considered to act, on the one hand, as competing agents for solvent thereby leading to a decreased solvation of the polysaccharide chains and, on the other, as promoters of interchain hydrogen bonding; both modes of action resulting in gel formation.[33] This explanation appears to also apply to those reductive alkylation attempts where gelation occurred without the formation of a branched-chain product, e.g., for glucosamine (at 1.3–2.7 mol/GlcN). A very similar effect of nonelectrolytes has been observed for hypnean dispersions, where only small quantities of sugar lead to the formation of very strong gels.[34]

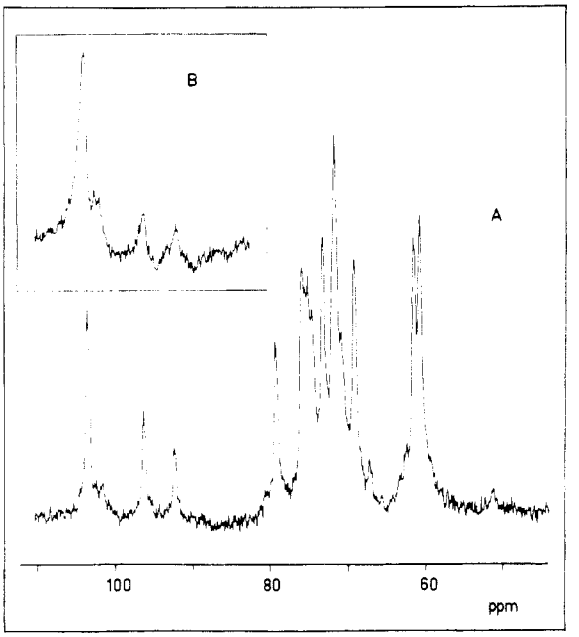


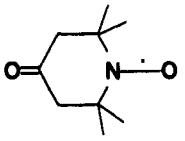
Figure 2. 100.6-MHz ^{13}C NMR spectrum of derivative **10** (A) before and (B) after dialysis (anomeric region between 80–110 ppm); see text for discussion.

In the case of the various branched-chain derivatives, on the other hand, gel formation is presumable largely due to conformational transitions of chitosan molecules in solution: removal of the positive charge (by *N*-alkylation), which, by electrostatic repulsions, maintains the chitosan chains in an extended, rigid-rod shape, allows for a more proximate intra- and interchain association of the resulting random coils. Although the precise nature of the gelling mechanism of the branched-chain chitosan derivatives remains to be established, we can identify certain features. The extent and regularity of the substitutions on chitosan appear to be of importance for gelation, since all derivatives with high ds formed rigid gels, whereas no gels (or only increases in viscosity) were observed for derivatives with ds <0.3, e.g., for cellobiose and lactose derivatives.[35]

Inclusion Complex Formation. A number of the branched-chain chitosan derivatives were found to form inclusion complexes with different solutes. For example, derivative **8**, like chitosan itself,[36] formed a complex with iodine. We also found in the preparation of derivative **8**, that under certain conditions, i.e., with lactose concentrations of about 2.9 mol/GlcN, a product (**10**) with a ds value of "2.0" was obtained (see Tables I and II).

However, the ^{13}C NMR spectrum of this product (Figure 2A) established the presence of 1 equiv of unbound lactose per hexosamine equivalent, as evidenced by the anomeric (α and β) glucopyranose signals in the region between 90 and 100 ppm. Extensive dialysis of this material failed to quantitatively remove the free lactose (Figure 2B), indicating a relatively strong complex between 1-deoxylactit-1-ylchitosan and lactose. These findings were also reflected in the ultrastructure of these materials as evidenced by scanning electron microscopy.[37] 1-Deoxylactit-1-ylchitosan also forms similar complexes with nonsaccharidic substrates as demonstrated by an experiment in which the residual (ca. 20%) free amine functions of derivative **8** (ds 0.8) were reductively alkylated with 4-oxy-2,2,6,6-tetramethylpiperidine-1-oxyl, **28**; after extensive purification, the ESR spectrum of the resulting product **29** revealed the presence of both bound and free nitroxide radical populations.

Although we have not, at this stage, thoroughly examined all of the branched-chain chitosan derivatives, it ap-



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pears that the formation of inclusion complexes is not restricted to derivative 8, as similar phenomena have been observed for several other derivatives.

Conclusions

We have demonstrated the utility of several chemical procedures for the efficient transformation of chitosan into a variety of soluble derivatives. Several of these derivatives can already be envisaged to have applications in specific areas. The cyclodextrin derivative **20**, for instance, could be employed for catalysis, metal complexation, and pharmaceutical preparations;[38] the streptomycin derivatives **19** and **30** exemplify other products of biomedical interest.[39] While recognition of the full application potential of the various branched chitosan derivatives obviously requires further detailed characterization, the results from our current investigations of their rheology,[40] metal chelation capacity,[23b,24] etc. are already indicative of a range of valuable and, in many cases, unusual properties.

The possibility of affecting or modifying polymer properties, such as solubility, compatibility, and viscosity, constitutes a particularly attractive and important aspect of the work reported here. With the exception of some of the solubility features, the changes in product properties induced here could admittedly not be predicted in either qualitative or quantitative fashion. We believe, however, that our chemical methodology, by virtue of its efficiency and simplicity, and its potential for systematic variations of a host of branch parameters could be instrumental in enhancing our understanding of the polysaccharide structure/function relationship, so that product properties could eventually be tailored in a specific manner. This can be more clearly discussed in the context of Figure 3.

Polymer derivatives with varying length of short, straight-chain branches (from C_3 to C_7 and higher residues), schematically represented in Figure 3A–D, are readily obtainable by using appropriate aldehydo, keto, or lactone sugars. Concurrently, for a given branch length, the stereochemistry at almost all carbon centers of the side chain can be altered, by selecting suitable stereoisomers. Similarly, medium-sized (C_{12}–C_{18}···residues) or long branches may be derived from appropriate di- or oligosaccharides (Figure 3E,F) or polysaccharides (Figure 3G) or by extension of preformed short branches, via, e.g., the combined galactose oxidase/reductive amination procedure exemplified by derivatives **17** and **18** (Figure 3H).

It is feasible, on the other hand, to introduce varying degrees of structural irregularities into the polymer side chains by selecting suitably functionalized carbohydrate residues, such as 2-keto sugars (Figure 3I) or disaccharides with glycosidic linkages other than 1–6 (e.g., derivative **8**, Figure 3J) or by chemical derivatization of branches bearing functional groups, such as primary amines (e.g., derivatives **5**, **7**; Figure 3K). More complex branching patterns can be produced by incorporation of oligosaccharides such as streptomycin (Figure 3L), cyclodextrin (Figure 3M), etc.

We note that for certain biomedical applications where it may be desirable to avoid acyclic structures in the carbohydrate side chains, facile alternative procedures, such as reductive ozonlysis/reductive amination using alkenyl glycosides[41] (Figure 3N) or carbodiimide-mediated coupling using uronic acids (Figure 3O), could be employed.

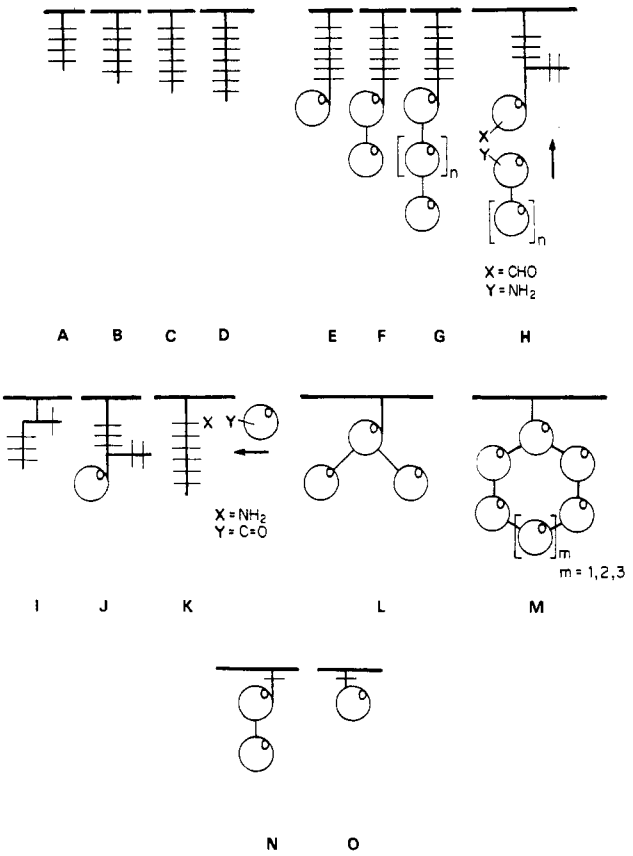

Figure 3. Schematic illustration of various branching patterns for the chitosan derivatives; see text for discussion. The heavy horizontal lines represent the chitosan backbone to which carbohydrate residues are attached, acyclic carbohydrate residues are represented by heavy vertical (and horizontal for H, I, and J) lines (in which each carbon center is indicated by a perpendicular line), and cyclic sugar residues are represented by circles.

Other polymer parameters amenable to chemical modification include the extent of branching, i.e., the ds, which is readily adjustable by substrate concentrations during the reaction, and the overall charge, which can be altered by incorporation or derivatization of suitable functional groups, e.g., aldehydes, amines, or carboxylic acids.[1]

While the list of structural parameter variations described above can undoubtedly be extended, it does indicate the broad range of branched polysaccharide structures which can be obtained by these methods.

Experimental Section

Materials. Chitin (from crab shells, ca. 10% *N*-deacetylated) and chitosan (from shrimp shells, ca. 15% *N*-acetylated) were purchased from Sigma Chemical Co. and used without purification. Anal. Calcd for chitosan, $[(C_8H_{13}NO_5)_{0.15}(C_6H_{11}NO_4)_{0.85}]\cdot 0.23H_2O$: C, 44.08; H, 6.91; N; 8.16. Found: C, 44.23; H, 6.98; N, 8.16. Anal. Calcd for chitin, $[(C_8H_{13}NO_5)_{0.90}(C_6H_{11}NO_4)_{0.10}]\cdot 0.28H_2O$: C, 45.92; H, 6.60; N 6.87. Found: C, 45.74; H, 6.78; N 6.87. Maltose, cellobiose, maltotriose, galactosamine hydrochloride, *N*-acetylglucosamine, D-glucose, hexafluoro-2-propanol, and sodium cyanoborohydride were from Aldrich Chemical Co.; melezitose, trehalose, and α-glucoheptonic acid γ-lactone were from Pfanstiehl Laboratories; β-lactose and melibiose were from Eastman Chemicals; fructose from BDH Chemicals; D-galactose and sucrose from Merck Co.; glucosamine hydrochloride, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC); LL,DD-diaminopimelic acid, galactose oxidase, and catalase were from Sigmal Chemical Co. Streptomycin sulfate was purchased from Allen and Hambury's, Toronto,

Canada. 4-Oxo-2,2,6,6-tetramethylpiperidine-1-oxyl was purchased from Aldrich Chemical Co. *N*-Cyclohexyl-2-pyrrolidone and *N*-cocoalkyl-2-pyrrolidone (containing a mixture of C_8–C_{16} alkyl groups) were gifts from GAF Chemicals. C^6-*aldehydo*-Cycloheptamylose was available from another study.[23a]

Analytical Methods. ^{13}C NMR. Proton-decoupled carbon NMR spectra were recorded at 100.6 MHz on a Bruker WH-400 spectrometer. Final sample concentrations ranged between 6 and 10% (w/v). Unless indicated otherwise, spectra were obtained at 303-310 K and were referenced with respect to internal 1,4-dioxane at 67.40 ppm from external tetramethylsilane.

ESR. ESR spectra were recorded at X-band on a Varian E-3 instrument with methods described previously.[42]

Degree of Substitution Determinations. C, H and N microanalysis was performed by P. Borda of the Microanalytical Laboratory, Department of Chemistry, University of British Columbia and by Canadian Microanalytical Service Ltd., Vancouver, following careful and extensive (24–48 h) drying of the samples in vacuo (56 °C, 0.1 torr) over sodium hydroxide. The reported analyses correspond to [(residual *N*-acetylglucosamine units) (underivatized glucosamine units) (derivatized glucosamine units)], unless indicated otherwise. Calculated analytical values were obtained by using a calculator program which matched formulas with observed C/N ratios. The chemical derivatization of chitosan resulted in a general lowering of the residual acetyl content of the products. This was supported by ^{13}C NMR, which was also used to confirm product composition whenever applicable.

General Synthetic Methods. All chitin and chitosan derivatizations were performed at ambient temperature, unless indicated otherwise. The polysaccharide reaction products were purified either by extensive dialysis against distilled water containing 0.2% sodium azide, repeated washings, gel chromatography on Sephadex LG-25 or by a combination of these methods. Product formation was in all cases confirmed by ^{13}C NMR spectroscopy.

Preparation of (1-Deoxyglycit-1-yl)chitosan Derivatives. General Procedure. Chitosan (500 mg, 3 mmol) was dissolved with stirring in a mixture (1:1, pH 5.5) of methanol and 1% aqueous acetic acid (solvent A) or in the latter medium[13] (solvent B) (30 mL). To the resulting viscous solution was added with vigorous stirring a solution (10–20 mL) of the carbonyl-containing compound and sodium cyanoborohydride (20 mmol). The reaction mixture was left stirring at room temperature for various periods, usually until gel formation was observed. The solvent excluded by the gel was decanted and the gel was broken up, repeatedly (3–4 ×) washed with methanol (150 mL) and finally with diethyl ether (150 mL). The solid products thus obtained were first air-dried for several hours and then dried in vacuo. In the cases where no gel was formed or when inhomogeneities were observed, the products were dialyzed for 4–6 days. Product yields were in general greater than 80%.

Chitosan Schiff Base Derivative 9 and Lactose Complex 10. The reaction was carried out as for (1-deoxylactit-1-yl)chitosan with omission of the reducing agent, using a lactose to glucosamine (L/G) ratio of 3.90; no gel formed after 28 h and the resulting Schiff's base product, **9**, had ds 0.1. Anal. Calcd for $[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.87}(C_{18}H_{32}NO_{14})_{0.10}]\cdot 0.9H_2O$: C, 41.28; H, 7.14; N, 6.63. Found: C, 41.01; H, 7.01; N, 6.71.

When the reductive alkylation was performed with L/G 2.9 a white soft gel was formed within 24 h, which, after washing with methanol (9 × 150 mL) and ether (1 × 150 mL), produced a material **10**, whose elemental analysis indicated a fully substituted (ds 0.97) product containing 1 equiv of unreacted lactose per repeating unit.

Chitosan Streptomycin Derivatives 19 and 30. To a solution of chitosan (1 mmol) in solvent B (20 mL) was added a solution (15 mL) of streptomycin sulfate (2.50 g, 3.4 mmol) and $NaCNBH_3$ (1.2 g, 19 mmol). The reaction mixture was stirred for 15 h in the dark and then dialyzed (4 days) to yield **19** (ds 0.07) as sesquiacetate. Anal. Calcd for $[(C_8H_{13}NO_5)_{0.03}(C_6H_{11}NO_4)_{0.90}(C_{27}H_{49}N_8O_{15})_{0.07}(C_2H_4O_2)_{0.105}]\cdot 3.57H_2O$: C, 34.09; H, 7.87; N, 7.66 (C/N, 4.45). Found: C, 33.65; H, 6.92; N, 7.58 (C/N, 4.44).

The corresponding Schiff's base analogue was prepared as above with omission of the reducing agent to afford the yellowish sesquiacetate product **30** (ds 0.08). Anal. Calcd for $[(C_8H_{13}NO_4)_{0.03}(C_6H_{11}NO_4)_{0.89}(C_{27}H_{47}N_8O_{15})_{0.08}(C_2H_4O_2)_{0.12}]\cdot 3.87H_2O$: C, 33.69; H, 7.86; N, 7.69 (C/N, 4.38). Found: C, 33.23; H, 6.98; N, 7.66 (C/N, 4.34).

***N*-Cyclohexylchitosan, 22.** The milky white solution obtained on addition of cyclohexanone (4.8–13.5 mmol) produced no gel. The product **22** was isolated (0.60 g) after washing (hexane) and dialysis (10 day) (ds 0.5). Anal. Calcd for $[(C_8H_{13}NO_5)_{0.02}(C_6H_{11}No_4)_{0.48}(C_{13}H_{23}NO_4)_{0.5}]\cdot 0.41H_2O$: C, 52.68; H, 8.28; N, 6.44. Found: C, 52.56; H, 8.36; N, 6.44.

***N*-(2,6-Diamino-6-carboxyhexanoyl)chitosan, 23.** An aqueous solution of DD,LL-diaminopimelic acid (1.0 g, 4.58 mmol, 40 mL) was acidified with 3 drops of 4 N HCl before EDC (0.92 g, 5.3 mmol) was added. After stirring for 0.5 h, the mixture was combined with a solution of chitosan (0.5 g, 3 mmol) in aqueous HOAc, and the reaction mixture was stirred for 24 h at ambient temperature. The resulting clear solution was dialyzed (3 days) and lyophilized, to yield **23**; the microanalytical results (C, 37.47; H, 6.40; N, 9.89; C/N, 3.79) could not be exactly matched with the anticipated molecular formula (presumably because of some degree of lactamization of the side chains), but the ds corresponded approximately to 0.75 (calcd C/N, 3.82).

Chitosan Derivatives 24, 25, and 26. General Method. To chitosan was added a mixture containing lactose (3 mmol) and the carbonyl reagent (propionaldehyde, 4 mmol; *N*-cyclohexyl-2-pyrrolidone, 12 mmol; and *N*-cocoalkyl-2-pyrrolidone, 8 mmol, respectively) to afford after 2–3 days derivatives **24** (found: C, 26.85; H, 6.76; N, 9.40), **25** (found: C, 41.74; H, 7.28; N, 4.83), and **26** (found: C, 44.33; H, 7.60; N, 5.15), respectively.

(1-Deoxylactit-1-yl)chitin, 27. Method A. Chitin (0.50 g) was dispersed in hexafluoro-2-propanol (10 mL) and stirred for 12 h. After removal of undissolved material by filtration, a solution of lactose (0.50 g) and $NaCNBH_3$ (0.15 g) in the same solvent (15 mL) was added. After stirring the reaction mixture for 14 h, aqueous methanol (15 mL) was added. The resulting white precipitate was left standing for a further 12 h, filtered, washed with water (150 mL), and subsequently dialyzed for 3 days, yielding 153 mg of an ivory-colored, fluffy material, **27** (ds 0.09). Anal. Calcd for $[(C_8H_{13}NO_5)_{0.8}(C_6H_{11}NO_4)_{0.11}(C_{18}H_{33}NO_{14})_{0.09}]\cdot 0.65H_2O$: C, 44.09; H, 6.78; N, 5.93. Found: C, 44.30; H, 6.77; N, 5.97.

Method B. Chitin (1.00 g) was suspended in 40% aqueous sodium hydroxide (40 mL) for 30 min, filtered, pressed, and washed in a 1:1 mixture of 5% aqueous HOAc (50 mL) to which was added lactose (3.00 g) and $NaCNBH_3$ (0.4 g). The reaction mixture was stirred for 24 h and then filtered and exhaustively washed. Anal. Calcd for $[(C_8H_{13}NO_5)_{0.8}(C_6H_{11}NO_4)_{0.12}(C_{18}H_{33}NO_{14})_{0.08}]\cdot 0.67H_2O$: C, 44.10; H, 6.79; N, 6.01. Found C, 43.92; H, 6.85; N, 6.18.

[(1-Deoxylactit-1-yl)(1-oxy-2,2,6,6-tetramethylpiperidinyl)]chitosan, 29. A solution of 4-oxo-2,2,6,6-tetramethylpiperidine-1-oxyl (100 mg, 0.59 mmol) and $NaCNBH_3$ (340 mg, 6 mmol, 5 mL) was added to an aqueous solution of derivative **8** (ds 0.8) (50 mg, 5 mL) and stirred for 36 h. The resulting product was dialyzed (3 days) and found to contain (from ESR) unbound label. Further dialysis (5 days) of this material (found: C, 40.83; H, 7.30; N, 3.16) left the ESR spectrum unchanged.

Oxidations with Galactose Oxidase. (1-Deoxy-6′-*aldehydo*-lactit-1-yl)chitosan, 17. Compound **8** (103 mg, 0.13 mequiv galactose) was dispersed in phosphate buffer (25 mM, pH 7, 10 mL) affording a soft glassy gel, which was purged with O_2 for 1 min. Catalase (14 000 units) and galactose oxidase (90 units) solutions were added and a viscous, ropy material formed after a few hours. The polysaccharide was diluted with water (10 mL) after 2 days and precipitated from absolute ethanol (150 mL). The preecipitate was collected by centrifugation, washed, and dried, yielding 93 mg of the oxidized product **17**. Subsequent reductive amination of **17** with a nitroxide label afforded a product[23b] with ds 0.7 (from ESR), indicating a high degree of conversion of **8** to the C6′-aldehyde derivative **17**.

(1-Deoxy-6′-*aldehydo*-melibiit-1-yl)chitosan, 18. Compound **13** (100 mg, 0.13 mequiv galactose) was dissolved in dilute acetic acid and the pH was raised to 4.5 by addition of aqueous $NaHCO_3$ solution, yielding a gel which was treated as above. The oxidized product **18** (95 mg) was isolated. Subsequent reductive amination of **18** with nitroxide label afforded a product[23b] with ds 0.15.

Interaction of Chitosan with Nonreducing Sugars. A yellow, viscous solution was obtained when melezitose (1.70 g, 3.2

mmol) was mixed with chitosan (3 mmol) in solvent A. After standing for 10 days, an elastic gel was produced from the mixture.

Trehalose (1.5 g, 4 mmol), dissolved in solvent A (10 mL), was added to chitosan (3.0 mmol) and mixed for 12 h. No gel was formed. Similarly, no gels were produced when sucrose was employed.

Interaction of 11 with Other Polysaccharides. To each of three portions of the cellobiit-1-ylchitosan derivative 11 dissolved in distilled water (0.2 g, 10 mL) was added a solution (0.2 g, 25 mL) of (i) sodium alginate, (ii) guar gum, and (iii) locust bean gum. The mixtures were vigorously stirred and diluted to 40 mL. A white gel formed immediately for alginate; the gel volume contracted considerably (ca. 15 ×) over a 12 h period, leaving a viscous supernatent solution. No gels were produced in the other cases, but the guar gum mixture developed a considerable viscosity.

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References and Notes

(1) For part 2, see: Yalpani, M.; Hall, L. D. *J. Polym. Sci., Polym. Chem. Ed.* **1982**, *20*, 3399.
(2) For a preliminary communication, see: Hall, L. D.; Yalpani, M. *J. Chem. Soc., Chem. Commun.* **1980**, 1153. See also: Hall, L. D.; Yalpani, M. U.S. Patent 4 424 346, Jan 1984.
(3) Present address: B. C. Research, Chemical Technology Division, Vancouver, British Columbia V6S 2L2, Canada.
(4) Muzzarelli, R. A. A. "Chitin"; Pergamon Press: Oxford, 1977.
(5) Rees, D. A. "Polysaccharide Shapes"; Chapman and Hall: London, 1977.
(6) McCormick, C. L.; Lichatowich, D. K. *J. Polymn. Sci., Polym. Lett. Ed.*, **1979**, *17*, 479. McCormick, C. L.; Lichatowich, D. K.; Pelezo, J. A.; Anderson, K. W. In "Modification of Polymers"; American Chemical Society, Washington, DC, 1980; ACS Symp. Ser. No. 121, p 371.
(7) Morris, E. M. In "Techniques and Applications of Fast Reactions in Solution"; Gettins, W. J., Wyn-Jones, E., Eds.; D. Reidel: Boston, 1979; p 379.
(8) (a) Hirano, S.; Ohe, Y.; Ono, H., *Carbohydr. Res.* **1976**, *47*, 315. Hirano, S.; Matsuda, N.; Miura, O.; Tanaka, T. *Ibid.* **1979**, *71*, 339. (b) Moore, G. K.; Roberts, G. A. F. *Int. J. Biol. Macromol.* **1981**, *3*, 292.
(9) Kaifu, K.; Nishi, N.; Komai, T. *J. Polym. Sci., Polym. Chem. Ed.* **1981**, *19*, 2361. Fuji, S.; Kumakai, H.; Noda, M. *Carbohydr. Res.* **1980**, *83*, 389. Kurita, K.; Ichikawa, H.; Ishizeki, S.; Fujisaki, H.; Iwakura, Y. *Makromol. Chem.* **1982**, *183*, 1161. Moore, G. K.; Roberts, G. A. F. *Int. J. Biol. Macromol.* **1982**, *4*, 246.
(10) See for example; Graessley, W. W. *Acc. Chem. Res.* **1977**, *10*, 332. Graessley, W. W. *Adv. Polym. Sci.* **1974**, *16*, 1. Grisebach, H.; Schmidt, R. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 159. Small, P. A. *Adv. Polym. Sci.* **1975**, *18*, 1.
(11) Goldstein, I. J.; Poretz, R. D.; So, L. L.; Yang, Y. *Arch. Biochem. Biophys.* **1968**, *127*, 787.
(12) Grappel, S. F. *Experientia* **1971**, *27*, 329.
(13) Reese, E. T.; Parrish, F. W. *Biopolymers* **1966**, *4*, 1043.
(14) Cisar, J.; Kabat, E. A.; Dorner, M. M.; Lia, J. *J. Exp. Med.* **1975**, *142*, 435. See also: Vogelstein, B.; Dintzis, R. Z.; Dintzis, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 395.
(15) Pfannemüller, B.; Richter, G. C.; Husemann, E. *Carbohydr. Res.* **1977**, *56*, 139.
(16) For reviews see: Bochkov, A. F.; Zaikov, G. E. "Chemistry of the *O*-Glycosidic Bond"; Pergamon Press: New York, 1979. Szarek, W. A. *MTP Int. Rev. Sci.: Org. Chem. Ser. One* **1973**, *7*, 80.
(17) Ito, H.; Schuerch, C. *J. Am. Chem. Soc.* **1979**, *101*, 5797.
(18) Pfannemüller, B.; Richter, G. C.; Husemann, E. *Carbohydr. Res.* **1876**, *47*, 63.
(19) Andresz, H.; Richter, G. C.; Pfannemüller, B. *Makromol. Chem.* **1978**, *179*, 301.
(20) Neely, W. B. U.S. Patent 3 133 850, 1964; *Chem. Abstr.* **1964**, *61*, 5899*h*. Pfannemüller, B.; Emmerling, W. In "Mechanisms of Saccharide Polymerization/Depolymerization"; Marshall, J. J., Ed.; Academic Press: New York, 1980; p 413.
(21) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1966**, *88*, 1024.
(22) Miyazaki, S.; Ishii, K.; Nadai, T. *Chem. Pharm. Bull.* **1981**, *29*, 3067.
(23) (a) Yalpani, M.; Harris, J. M.; Brooks, D. E., manuscript in preparation. (b) Yalpani, M.; Hall, L. D., manuscript in preparation.
(24) Hall, L. D.; Yalpani, M. *Carbohydr. Res.* **1980**, *83*, C5.
(25) Yamaoka, N.; Usui, T.; Sugiyama, H.; Seto, S. *Chem. Pharm. Bull.* **1974**, *22*, 2196.
(26) Perkins, Sj.; Johnson, L. N.; Phillips, D. C.; Dwek, R. A. *Carbohydr. Res.* **1977**, *59*, 19.
(27) Hall, L. D.; Morris, G. A.; Sukumar, S. *J. Am. Chem. Soc.* **1980**, *102*, 1745.
(28) Yalpani, M.; Hall, L. D. *Can. J. Chem.* **1981**, *59*, 2934.
(29) Hoagland, P. D.; Pfeffer, P. E.; Valentine, K. M. *Carbohydr. Res.* **1979**, *74*, 135.
(30) Johnson, L. F.; Jankowski, W. C. "Carbon-13 NMR Spectra"; Wiley: New York, 1972.
(31) Detailed results will be reported elsewhere (Yalpani, M.; Brooks, D. E., manuscript in preparation).
(32) (a) Filar, L. J.; Wirick, M. G. "Proceedings of the First International Conference on Chitin/Chitosan"; Muzzarelli, R. A. A., Pariser, E. R., Eds.; MIT Sea Grant Program: Cambridge, MA, 1978; p 169; (b) Hayes, E. R.; Davies, D. H., In ref 32a, p 193.
(33) Robinson, G.; Morris, E. R.; Rees, D. A., *J. Chem. Soc., Chem. Commun.* **1980**, 152. Pippen, E.; Schultz, T. H.; Owens, H. *J. Colloid Sci.* **1953**, *8*, 97.
(34) Sand, R. E.; Glicksman, M. In "Industrial Gums"; Whistler, R. L., Ed.; Academic Press: New York, 1973; p 147; Humm, H. J.; Williams, L. G., *Am. J. Bot.* **1948**, *35*, 287.
(35) Schweiger, R. G. *Carbohydr. Res.* **1979**, *70*, 185.
(36) Shigeno, Y.; Kondo, K.; Takemoto, K. *Angew. Makromol. Chem.* **1980**, *91*, 55.
(37) Hall, L. D.; Yalpani, M.; Yalpani, N. *Biopolymers* **1981**, *20*, 1431.
(38) Bender, M. L.; Komiyama, M. "Cyclodextrin Chemistry"; Springer: New York, 1978.
(39) Hofman, V.; Ringsdorf, H.; Seganova, A.; Wagner, W.-H. *Makromol. Chem.* **1979**, *180*, 837.
(40) Yalpani, M.; Hall, L. D.; Tung, M. A.; Brooks, D. E. *Nature (London)* **1983**, *302*, 812.
(41) Bernstein, M. A.; Hall, L. D. *Carbohydr. Res.* **1980**, *78*, C1.
(42) Yalpani, M.; Hall, L. D. *Can. J. Chem.* **1981**, *59*, 3105.