Chitosan Derivatives Bearing C_{10}-Alkyl Glycoside Branches: A Temperature-Induced Gelling Polysaccharide

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ABSTRACT: Undecenyl β-D-glycosides of glucose, galactose, and lactose have been ozonolyzed to give formylnonyl glycosides, which were reductively N-alkylated to chitosan. These chitosan derivatives, having mixed hydrophobic/hydrophilic branches, were prepared with degree of substitution values as high as 1.5. Solutions of derivatives bearing pendant monosaccharides with ds >1.0, in 1% aqueous acetic acid, gelled upon heating to 50 °C. Lower ds samples did not exhibit this property, nor did derivatives having a disaccharide pendant residue. Further control over the properties of these derivatives was possible by preparing mixed-branched derivatives having short- and long-chain alkyl glycoside pendant groups.

Introduction

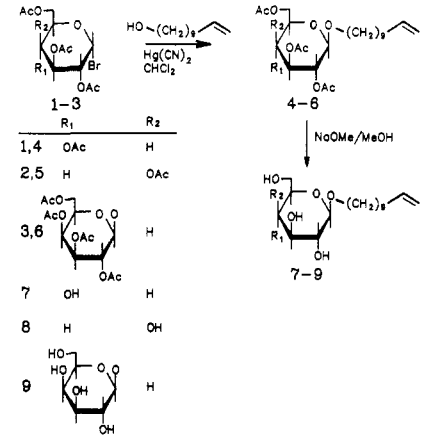
This laboratory has been interested in the chemical modification of chitosan, a β-(1–4)-2-amino-2-deoxy-D-glucose homopolymer, for the purpose of preparing controlled solubility derivatives of this intractable polymer. A previous report on chitosan derivatives bearing carbohydrate-derived acyclic branches showed that hydrophilic groups could be used to solubilize chitosan.[1] Subsequent work, in which allyl glycosides were employed as synthons for preparing alkyl glycoside branched chitosan derivatives, showed that aqueous solution properties of branched polysaccharides could be controlled by varying the degree of substitution (ds) and the pendant glycoside functionality.[2,3] Also, the rheological behavior of these synthetically branched polysaccharides was best rationalized by using explanations developed for the self-association of legume seed galactomannans,[4] which are important natural branched polysaccharides. The utility of modifying chitosan with hydrophobic branches for controlling solubility properties has also been demonstrated.[1]

In this study, we will report on an extension of the previously reported allyl glycoside methodology, whereby long-chain alkenyl glycosides serve as precursors for generating novel branched chitosan products having both hydrophilic and hydrophobic character in the pendant group.[5]

Results and Discussion

Synthesis and Characterization. The 10′-undecenyl β-D-glycosides of glucose (**7**), galactose (**8**), and lactose (**9**) were prepared by methods[6] similar to those for the synthesis of allyl β-D-glycosides, as outlined in Scheme I. The acetobromo sugars **1–3** were reacted under Koenigs–Knorr glycosidation conditions with 2 mol equiv of 10-undecen-1-ol in chloroform to give the intermediate peracetylated glycosides **4–6**. The crude residue was directly de-O-acetylated to yield the desired 10′-undecenyl β-D-glycopyranosides **7–9**. It was noted by ^{1}H NMR that some α-D-glycoside impurity was present in the β-lactoside product. Liquid chromatography of the crude material, using methods reported for long-chain alkyl glycosides,[7] afforded the compounds **7–9** as waxy solids.

Scheme I



Characterization of the 10′-undecenyl β-D-glycosides was best accomplished by ^{1}H and ^{13}C NMR. Since similar molecules are known to behave as nonionic surfactants and form micelles in aqueous solution[7-9] and because their water solubility was limited, the glycosides were dissolved in methanol-*d*.[4] This served to reduce aggregation of the molecules and allowed better resolved spectra to be obtained. It was found that spectra recorded at 50 °C were better resolved than those determined at 20 °C. Thus, assignment of ^{1}H and ^{13}C NMR spectra recorded at 400 and 100.6 MHz, respectively, at 50 °C was possible. The ^{13}C NMR chemical shift data for the three glycosides are given in Table I.

Despite attempts at purification by liquid chromatography, the β-lactoside product contained some α-isomer (~10%) impurity and was carried through as such. Obtaining analytically pure samples for optical rotation determinations and elemental microanalyses was precluded because of difficulty in the crystallization and drying of the glycosides. However, fast atom bombardment (FAB) mass spectrometry provided the expected parent peaks as proof of product molecular weights.

Ozonolysis of the alkenyl β-D-glycopyranosides **7** and **8** was performed at −78 °C in methanol. Somewhat surprisingly, it was necessary to use a chloroform–methanol mixture (1:5) to solubilize the disaccharide **9**. After workup, the aldehydes **10–12** were directly employed in reactions with chitosan. It was found that, upon sitting for over 1 day, the aldehydes became insoluble in methanol and water but dissolved slowly if small amounts of acetic or hydrochloric acid were added. Apparently oligo- and

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Table I
100.6-MHz ^{13}C NMR Chemical Shift Data (ppm), for Saccharide and Some Aglycon Resonances, of the 10′-Undecenyl β-D-Glycopyranosides in CD_3OD (Referenced to External TMS)

sample no.	sugar	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-9′	C-10′	C-11′
7	β-Gal	102.5	73.3	76.0	70.0	76.3	61.1	69.1	32.9	138.3	112.8
8	β-Glc	103.1	70.8	73.3	69.0	74.7	60.7	68.5	32.9	138.2	112.8
9	β-Lac										
	(β-Gal)	103.0	70.8	72.8[a]	69.7	75.1	60.7				
	(β-Glc)	102.2	72.8[a]	74.5[a]	79.0	74.5[a]	60.2	68.4	32.9	138.3	113.2

[a] Assignments may be reversed.

Scheme II

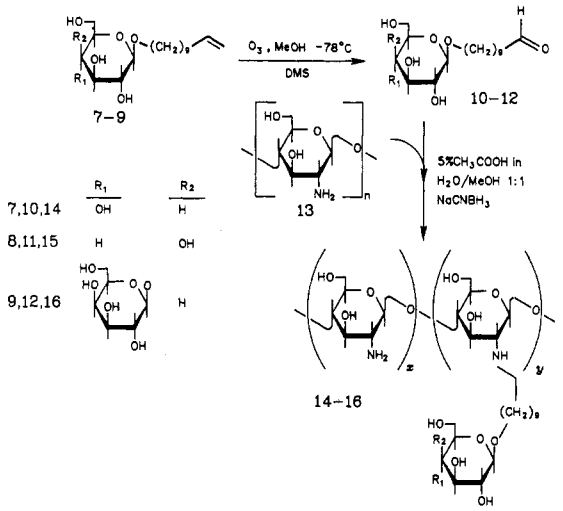


Table II
Characteristics for the
N-[10′-O-(β-D-Glycopyranosyl)decyl]chitosan Derivatives

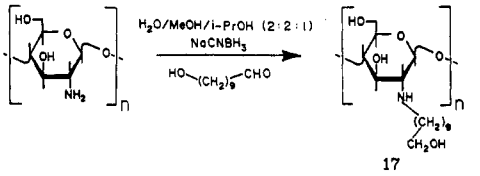
derivative	branch	A/C	ds (±0.05)	yield, %
14a	β-Glc	3.0	1.47	80
14b		2.1	0.81	85
15a	β-Gal	3.0	1.37	65
15b		1.5	0.22	85
16a	β-Lac	2.9	1.10	70
16b		1.6	0.50	70
17a		2.0	1.73	76
17b		1.0	1.00	82
19	α-Gal[a]		0.32	
	β-Gal	2.9	1.04	63

[a] The *N*-ethyl-α-galactosyl branch was present on 18 prior to its modification to give the mixed derivative **19**.

polyacetal compounds formed, as might be expected for these relatively unhindered long-chain aldehydes.

The reductive amination of the formylnonyl β-D-glycopyranosides **10** and **11** to chitosan (Scheme II) was performed in 5% aqueous acetic acid–methanol (1:1). The methanol was necessary to solubilize the aldehydes **10** and **11**; however, the aldehyde **12** was soluble in a totally aqueous system. Upon addition of sodium cyanoborohydride to the reaction solutions, a marked decrease in viscosity occurred. After the reactions were stirred for 24 h, they were dialyzed, filtered, and lyophilized. The reactions were performed by using two different aldehyde-to-chitosan ratios for each of the aldehydes **10**–**12**, to give the derivatives **14**–**16**, which are listed in Table II. The degree of substitution values, as determined from elemental microanalysis, immediately showed that the coupling efficiency of the long-chain aldehydes was much greater than for those of the allyl glycoside route. Thus, in this series, derivatives **14a** and **15a** had ds values of 1.47 and 1.37 when 3 equiv of aldehyde was employed, while most previous reports of chitosan alkylation had maximum ds values of 1.0. Derivatives with ds values lower than 1.0 were prepared by reducing the amount of aldehyde used in the coupling reaction. Obviously, this was a result of the less hindered nature of the formylnonyl aldehydes, which allowed a substantial amount of N,N-disubstitution. For comparison purposes the derivatives **17a** and **17b** were prepared by using standard conditions by reaction of chitosan with 10-hydroxydecan-1-al (Scheme III), which was obtained from ozonolysis of 10-undecen-1-ol. Derivative **17a** precipitated from the reaction solution and was collected by filtration, and **17b** was isolated by using procedures. Again high ds values were obtained at typical A/C ratios (Table II).

Scheme III



Disappointingly, none of the derivatives were water soluble. They were, however, all soluble in dilute organic or mineral acid solutions (e.g., 1–2% aqueous acetic acid). The high ds samples **14a**, **15a**, and **16a**, bearing pendant β-D-glucose, β-D-galactose, and β-D-lactose residues, respectively, gave thin, mobile solutions at 5.0% (w/w) polysaccharide concentration in 2% aqueous acetic acid, while the lower ds analogues **14b**, **15b**, and **16b** gave slightly more viscous solutions. Solution ^{13}C NMR spectra of **14a**, **15a**, and **16a** had easily discernible resonances for the pendant sugars and alkyl group but virtually no distinguishable signals from the chitosan backbone. This is indicative of freely rotating pendant sugars. Total assignments of the branch and alkyl ^{13}C resonances for derivatives **14a**, **15a**, and **16a** are presented in Table III, and they compare well with the methyl glycoside analogues[10,11] and the 10-undecenyl β-D-glycoside precursors (Table I).

To our chagrin, it was immediately apparent that these solutions had uninteresting rheology at ambient temperatures. Serendipitously, it was noted that upon heating of the 5.0% solutions of **14a** and **15a** to 50 °C a stiff opaque gel formed, which dissolved reversibly upon cooling. Solutions of 2.0% concentration did not exhibit this behavior. Interestingly, the gelation of **15a** is accompanied by increased solution opacity, which is probably related to the "cloud point" phenomena that occurs at the monomeric level.[7-9,12] Thus, it appears that the combination of hydrophobic character and polymeric structure necessary for temperature-induced gelation is present in these structures. The reversibility of the interaction indicates that reorientation of the polymer chains accompanies temperature reduction. Methylcellulose is known to exhibit similar hydrophobic-based behavior;[13-15] however, for these derivatives the exact mechanism of gelation remains unclear.

It was interesting to note that the lower ds monosaccharide derivatives, **14b** and **15b**, and the lactose deriv-

Table III
100.6-MHz ^{13}C NMR Chemical Shift (ppm) Data for Pendant Residues of the *N*-Decyl β-D-Glycopyranosides, in 1.0% CD_3COOD/D_2O (Referenced to External TMS)

derivative	branch	C-1	C-2	C-3	C-4	C-5	C-6	C-10′
14a	β-Glc	100.8	71.8	74.5	69.0	74.6	59.5	68.4
15a	β-Gal	101.3	67.0	71.4	69.2	73.4	59.2	68.8
16a	β-Lac							
	(β-Gal)	102.5	70.5	72.2	70.0	75.8	60.5	
	(β-Glc)	101.7	72.4	74.1	78.5	74.3	60.0	68.1
19	β-Gal	97.0	66.7	68.0	67.6	69.9	60.4	
	β-Gal	101.2	67.0	71.3	69.1	73.3	59.2	68.8

atives, **16a,b**, even at 7.5% concentration, did not gel at elevated temperatures. Thus, substantial hydrophobic character appears to be necessary, and a large pendant group precludes gel formation. The latter is likely a result of the increased hydrophilicity of the disaccharide, counteracting or interfering in the hydrophobic interactions. It has been reported[8] that alkyl lactosides are not as prone to micelle formation and behave poorly as surfactants. This is likely a further manifestation of that property. Interestingly, of the derivatives **17a** and **17b**, which lacked the pendant carbohydrate, **17a** was insoluble in aqueous acetic acid and **17b** gave a highly viscous solution showing no observable change upon heating. This gratifyingly indicated that gel formation was dependent on the hydrophilic character of the pendant moiety. Both **17a** and **17b** gave clear stiff gels in 1% acetic acid–methanol, while the branched derivatives gave gels in aqueous acetic acid–methanol systems.

^1H and ^{13}C NMR Investigations. In order to follow gel formation and to perhaps gain insight into the mechanism, ^1H and ^{13}C NMR experiments were undertaken. It was felt that the mobility of the components of the derivative could be probed by observing the temperature dependence of the T_1 relaxation of resonances in the ^1H NMR spectrum of **15a**. As such, T_1 relaxation measurements of three resonances, representing the pendant sugar, the alkyl chain, and the solvent, in the ^1H NMR spectrum were performed at 20, 40, 60, and 80 °C by using the inversion recovery method (Figure 1). The T_1 values obtained are given in Table IV. The increase in the T_1 relaxation time of the sugar and alkyl protons indicates that the correlation time (τ_c) of the polymer is sufficiently slow at 20 °C that it has past the minima in the T_1 vs τ_c curve.[16] This is expected since at 300 MHz, a $\tau_c \sim 3 \times 10^{-9}$ s^{-1} would result in a T_1 minimum, while chitosan derivatives in solution have been shown to have correlation times of 10^{-9}–10^{-8} s^{-1}.[17] Thus, increased T_1 values of 40, 60, and 80 °C result from reduced mobility of the respective groups in the gel state. The decreasing T_1 value of the solvent or HOD resonance is supportive of reduced solvent mobility upon gelation. In this case, the water molecules having $\tau_c \sim 10^{-12}$–10^{-11} s^{-1} in solution, are "trapped" in the gel matrix, and their reduced mobility causes a reduction in T_1, in the direction of the T_1 minima at $\tau_c \sim 3 \times 10^{-9}$ s^{-1}. Unfortunately, there were no obvious chemical shift changes upon heating that could help illuminate the gelling mechanism. As expected, a general broadening of resonances occurred upon heating, due to the dependence of T_2 and line width, on correlation time. As in the ^{13}C NMR spectrum, no ^1H NMR resonances from the chitosan main chain were discernable. Gelation was also monitored by ^{13}C NMR spectroscopy. In Figure 2, the ^{13}C NMR spectrum of **15a** at 30 and 50 °C is given. Substantial line broadening is immediately apparent at 50 °C, with line widths for C-1 being ~15 and 150 Hz, respectively, for the 30 and 50 °C spectra, reflecting the substantially reduced mobility of the pendant galactose unit in the gel.

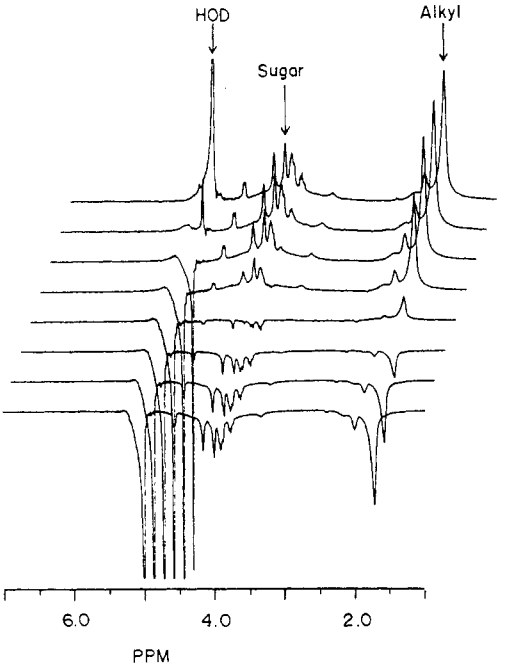


Figure 1. Stacked plot of the 300-MHz ^1H NMR spectra of **14a**, in 1.0% CD_3COOD/D_2O, at 20 °C, indicating the resonances for which T_1 values were determined by using the inversion-recovery method.

Table IV
T_1 Values, at 20, 40, 60, and 80 °C, for the Resonances Indicated in the 300-MHz ^1H NMR Spectrum of **15a** (Figure 4), in 1% CD_3COOD/D_2O Solution

temp, °C	T_1 of resonances, s		
	sugar	alkyl	HOD
20	0.34	0.25	3.4
40	0.42	0.30	1.6
60	0.47	0.32	1.4
80	0.80	0.54	1.2

Again, no discernible chemical shift changes occur upon heating the sample.

Mixed-Branch Chitosan Derivatives. The concept of preparing cobranched chitosan derivatives in order to control or enhance solubility properties has been introduced.[1] In this study, we felt that *N*-10′-*O*-(β-D-glycopyranosyl)decyl branches, and the unique temperature dependence they impart, could be exploited in conjunction with the controlled solubility properties of the *N*-2-*O*-(D-glycopyranosyl)ethyl-branched chitosan derivatives reported by this laboratory[2,3] in order to give products having viscous properties that were stable or enhanced at elevated temperature. To this end, derivative **18** having an *N*-ethyl glycoside ds of 0.32 (prepared according to procedure in ref 2 and to be described in detail elsewhere[3] with a family of related compounds) was reductively N-alkylated with the formylnonyl glycoside **11** to give **19** (Scheme IV), as described in Table II. Disappointingly, it was found that while **19** would swell in water, it would not give a true

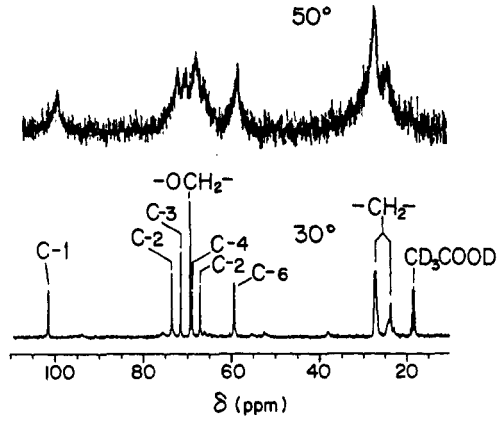
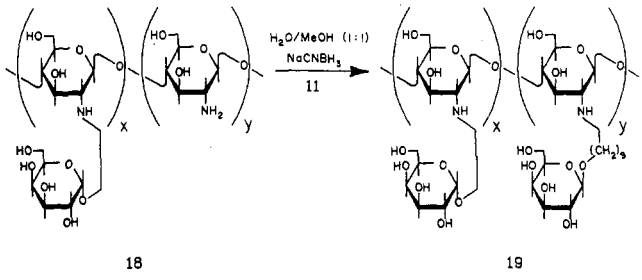


Figure 2. 100.6-MHz ^{13}C NMR spectra of **15a** in 1.0% CD_3COOD/D_2O, recorded at 30 and 50 °C.

Scheme IV



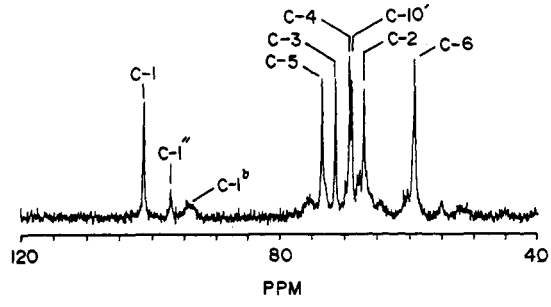


Figure 3. 100.6-MHz ^{13}C NMR spectra of **19** in 1.0% CD_3COOD/D_2O at 30 °C.

solution. Again, it was found that a viscous solution was obtained in 2.0% aqueous acetic acid. The ^{13}C NMR spectrum (Figure 3) of **19** contains resonances for both branch residues; however, mobility differences result in considerable suppression of the α-galactosyl branch compared with the more extended β-D-galactosyl branch.

Steady-shear viscometry on 2.0% (w/w) solutions of **19** and **18** in 2.0% aqueous acetic acid, at 20 and 50 °C, provided the data shown in the logarithmic rheogram in Figure 4. The rheology of the solution of **19** at 20 °C was interesting in that both pseudoplasticity and viscosity were appreciable, and the solution was considerably more viscous than that of **18**. However, the rheograms (and power law parameters, Table V) show that the temperature dependences of both **19** and **18** are similar and result in a reduction of both viscosity and, to a lesser extent, pseudoplasticity. As an exploratory experiment, these results were rewarding and are certainly indicative of further potential for mixed derivatives.

Conclusion

In this study it has been shown that chitosan derivatives having long-chain (C_{10}) alkyl glycoside pendant groups can be prepared from undecenyl glycoside precursors. Solutions of these novel branched chitosan derivatives gel

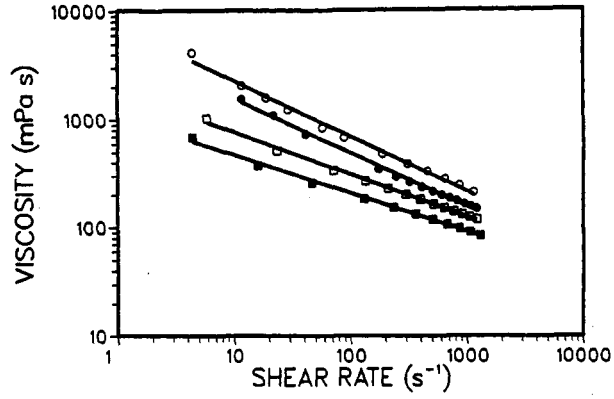


Figure 4. Rheograms of 1.0% solutions of derivatives **18** at 20 (□) and 50 °C (■) and **19** at 20 (○) and 50 °C (●), on logarithmic coordinates.

Table V
Power Law Parameters Obtained from Rheological Evaluation of 18 and 19, at 20 and 50 °C, in 1.0% Aqueous Acetic Acid Solution

derivative	temp, °C	n	m, mPa·s	R^2	no. of points
19	20	0.488	7330	0.994	12
	50	0.499	4920	0.996	14
18	20	0.607	1880	0.997	13
	50	0.644	1060	0.997	11

upon heating as a result of the hydrophobic character imparted by the alkyl group. The carbohydrate moiety also appears to be important although more in terms of aiding aqueous dissolution. A mixed derivative having both long and short alkyl branches was prepared in hope that the temperature-dependent nature of the long-chain derivatives could be combined with the water solubility of the short-chain family.[3] It is shown that this mixed derivative does have different rheological properties, being more pseudoplastic, and more viscous than the **18** precursor, despite the high level of substitution. Unfortunately like the other long-chain derivatives, it was not water soluble. It did, however, swell to a greater extent and overall indicates that this approach offers potential for rationally tailoring the properties of polysaccharides.

Experimental Section

General Methods. All evaporations were performed under diminished pressure on a Buchi rotary evaporator. Melting points were determined with a Fisher-Johns apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 710B infrared spectrophotometer and were calibrated by using the 1601-cm^{-1} band of polystyrene film. Optical rotations were obtained by a Perkin-Elmer 141 polarimeter. Low-resolution mass spectra were recorded on a Varian/MAT CH4B or Kratos/AEI MS 50 mass spectrometer. Analytical gas–liquid chromatography (GLC) was performed on a Hewlett-Packard 5832A gas chromatograph with a 6 ft × 0.125 in. stainless steel column packed with OV-17 on 80–100-mesh Chromosorb W (HP). Carbon, hydrogen, and nitrogen elemental microanalyses were performed by Mr. P. Borda, Microanalytical Laboratory, University of British Columbia. Analytical thin-layer chromatography (TLC) was done with 0.20-mm precoated aluminum-back sheets of silica gel 60 F_{254} (E. Merck, Darmstadt, Germany). Solvent systems employed for TLC analyses were (a) ethyl acetate–hexane (3:2), (b) ethyl acetate–isopropyl alcohol–water (9:4:2), (c) chloroform–methanol (15:1), and (d) chloroform–methanol (4:1). For detection of components, TLC sheets were sprayed with (a) 30% sulfuric acid in 95% ethanol, followed by heating on a hot plate (for carbohydrate) or (b) 2.0% ammonium molybdenate in 10% sulfuric acid–ethanol, followed by heating on a hot plate. Flash liquid chromatography was performed by using 230–400-mesh silica gel (Kieselgel 60; E. Merck, Darmstadt, Germany).

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

Ozonolyses were performed at −78 °C by using a Welsbach Ozonator (90 V, 2 psi of input O_2 pressure) ozone source. The ozone was bubbled into the cooled solution via a sintered-glass bubbling tube until the reaction mixture turned pale blue. The ozone source was turned off and the solution purged with O_2 gas until colorless. Two equivalents of dimethyl sulfide (DMS) was added, and the reaction mixture was allowed to warm to room temperature with stirring for 2 h.

^{1}H NMR: Proton NMR spectra were typically measured at 270 MHz using a home-built unit based on a Nicolet Model 1180 computer. Where indicated, 400-MHz spectra were recorded on a Bruker WH-400 spectrometer and 300-MHz spectra on a Varian XL-300 spectrometer. Samples dissolved in deuterated chloroform were referenced relative to internal tetramethylsilane (TMS), and those dissolved in deuterium oxide, relative to internal sodium 3-(trimethylsilyl)propionate-*2,2,3,3,-d*$_4$ (TSP).

^{13}C NMR: Proton-decoupled ^{13}C NMR spectra were recorded at 100.6 MHz on 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 NMR 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. Chemicals and reagents were purchased from suppliers as follows. Dimethyl sulfide, hydrogen bromide in acetic acid (30% w/w), Dowex 50X8, H^+ (100–200 mesh) ion-exchange resin, sodium cyanoborohydride, and 10-undecen-1-ol were obtained from Aldrich Chemical Co. Mercuric cyanide was from ICN Pharmaceutical, Inc., D-lactose was from Eastman Kodak Co., D-Glucose was from Fischer Scientific, and D-galactose was from Merck and Co. Chitosan (from crab shell, *N*-acetyl < 5%) was purchased from Sigma Chemical Co.

General Synthetic Procedures. Synthesis of 10-Formylnonyl β-D-Glycosides 10–12. The starting 10-undecenyl β-D-glycoside was dissolved in methanol (at 5–10 mL/mmol) (methanol–chloroform 4:1 for undecenyl β-D-lactose) and subjected to ozonolytic conditions. The solvent was removed, and the residue was repeatedly taken up in a minimum of ethanol and precipitated with ether. The gummy residue was dried in vacuo to give a waxy solid, usually in 85–95% yields. It was found that the aldehyde was best used immediately (within 24 h) because a methanol-insoluble residue formed when the aldehyde was left standing at room temperature. This residue dissolved slowly on addition of acetic or dilute hydrochloric acid.

Synthesis of 10′-Undecenyl β-D-Glycosides. 10′-Undecenyl β-D-Glucopyranoside (7). A suspension of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1; 20.0 g, 48.7 mmol), 10-undecenol (16.58 g, 97.3 mmol), mercuric cyanide (13.53 g, 53.6 mmol), and Drierite (14 g) was stirred in chloroform (125 mL) at reflux for 10 h. The reaction mixture was filtered, washed with saturated brine solution (2 × 75 mL), dried over magnesium sulfate, filtered, and concentrated. The residue was taken up to anhydrous methanol and treated with 0.5 N methanolic sodium methoxide (10.0 mL). When the reaction was complete (TLC, solvent D), Dowex 50X8 (H^+, 100–200 mesh) ion-exchange resin was added to neutralize the solution, which was then filtered. The filtrate was concentrated, and the resultant residue was subjected to liquid chromatography (chloroform–methanol 5:1) to afford 12.9 g (80%) of a waxy solid, 7. ^{1}H NMR (270 MHz, CD_3OD δ 5.79 (m, 1 H, H-10′), 4.96 (br d, 1 H, J = 17.0 and 1.5 Hz, H-11′a), 4.90 (d, 1 H, J = 10.0 Hz, H-11′b), 4.26 (d, 1 H, J = 8.0 Hz, H-1), 3.86 (dd, 1 H, J = 11.5 and 2.4 Hz, H-6a), 3.69 (dd, 1 H, J = 11.5 and 5.2 Hz, H-6b), 3.55 (m, 2 H, H-1′a,b), 3.38 (t, 1 H, J = 9.0 and 8.5 Hz, H-3), 3.32 (t, 1 H, J = 9.0 and 8.5 Hz, H-4), 3.27 (m, 1 H, H-5), 3.19 (t, 1 H, J = 8.5 and 8.0 Hz, H-2), 2.04 (m, 2 H, H-9′a,b), 1.63 (m, 2 H, H-2′a,b), 1.43–1.29 (m, 12 H, alkyl protons). ^{13}C NMR (100.6 MHz, CD_3OD): δ 138.3 (C-10′), 112.8 (C-11′), 102.5 (C-1), 76.3 (C-5), 76.0 (C-3), 73.3 (C-2), 70.0 (C-4), 69.1 (C-1′), 61.1 (C-6), 32.9 (C-9′), 29.0–28.2 and 25.4 (C-2′ to 8′). MS (FAB): m/z 333 $(M + H)^+$.

10′-Undecenyl β-D-Galactopyranoside (8). A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (2; 17.9 g, 43.6 mmol), 10-undecenol (14.8 g, 87.6 mmol), mercuric cyanide (12.1 g, 48.0 mmol), and Drierite (18 g) in chloroform (125 mL) was stirred at reflux for 9 h under anhydrous conditions. The reaction mixture was cooled, diluted with ether (60 mL), filtered, and concentrated. The residue was dissolved in chloroform (200 mL), and the chloroform layer was washed with saturated brine (2 × 100 mL), dried over magnesium sulfate, and filtered. After the solvent was removed, the residue was dissolved in anhydrous methanol (80 mL) and deacetylated with 0.5 N sodium methoxide in methanol (10.0 mL). When the reaction was complete (TLC, solvent C), Dowex 50X8 (H^+, 100–200 mesh) was added and the mixture was filtered and concentrated. The crude product (16.0 g) was chromatographed (chloroform–methanol 5:1) to give 12.2 g (84%) of a waxy solid having a single spot by TLC analysis (solvent D). ^{1}H NMR (270 MHz, CD_3OH δ: 5.83 (m, 1 H, H-10′), 4.99 (br d, 1 H, J = 17.0 Hz, H-11′a), 4.93 (br d, 1 H, J = 17.0 Hz, H-11′b), 4.24 (d, 1 H, J = 7.2 Hz, H-1), 3.90 (m, 3 H, H-1′a,1′b,5), 3.77 (d, 1 H, J = 6.2 Hz, H-4), 3.63–3.45 (m, 4 H, H-6, 6b,2,3), 2.04 (m, 2 H, H-9′a,b), 1.63 (m, 2 H, H-2′a,b), 1.43–1.30 (br s, 12 H, alkyl protons). ^{13}C NMR (100.6 MHz, CD_3OD): δ 138.2 (C-10′), 112.8 (C-11′), 103.1 (C-1), 74.65 (C-5), 73.3 (C-3), 70.8 (C-2), 69.0 (C-4 or 1′), 68.5 (C-1′ or 4), 60.7 (C-6), 32.9 (C-9′), 29.0–28.2 and 25.2 (C-2′ to 8′). MS (FAB): m/z 333 $(M + H)^+$.

10″-Undecenyl 4′-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranoside (10″-Undecenyl β-D-lactoside; 9). Acetobromolactose (4; 25.0 g, 35.8 mmol), 10-undecenol (12.2 g, 72.0 mmol), mercuric cyanide (9.94 g, 39.4 mmol), and Drierite were stirred together in chloroform (100 mL) at reflux for 11 h. The reaction mixture was cooled and filtered, and the filtrate was washed with saturated brine solution (2 × 75 mL), dried over magnesium sulfate, and concentrated. The residue was dissolved in anhydrous methanol, and the resultant stirred solution was treated with 0.5 N sodium methoxide in methanol (10.0 mL). When the reaction was complete (TLC, solvent D), the reaction was neutralized with Dowex 50X8 (H^+, 100–200 mesh) ion-exchange resin and filtered. The solvent was removed, and the residue was precipitated from methanol to yield 14.9 g (84%) of **9** as a waxy solid. ^{1}H NMR (270 MHz, CD_3OD): δ 5.80 (m, 1 H, H-10″), 4.98 (br d, 1 H, J = 17.0 Hz, H-11″a), 4.93 (d, 1 H, J = 10.5 Hz, H-11″b), 4.42 (br d, 1 H, J = 7.5 Hz, H-1′), 4.28 (d, 1 H, J = 8.0 Hz, H-1), 3.94–3.83 (m, 3 H, H-6a,6b,4), 3.80 (br d, 1 H, J = 11.5 Hz, H-6′a), 3.74 (dd, 1 H, J = 11.5 and 4.5 Hz, H-6′b), 3.70–3.49 (m, 5 H, H-2,3,3′,4′,5), 3.46 (m, 1 H, H-5′), 3.30 (t, 1 H, J = 9.0 and 8.0 Hz, H-2′), 2.04 (m, 2 H, CH_2-9″), 1.63 (m, 2 H, CH_2-2″), 1.42–1.29 (br s, 12 H, alkyl protons). ^{13}C NMR (100.6 MHz, CD_3OD): δ 138.3 (C-10″), 113.2 (C-11″), 103.0 (C-1), 102.2 (C-1′), 79.0 (C-4′), 75.1 (C-5 or 5′ or 3′), 74.5 (C-5′ and 3′ or 5), 72.8 (C-2′ or 3), 72.78 (C-3 or 2′), 70.8 (C-2), 69.7 (C-4), 68.4 (C-1″), 60.7 (C-6), 60.2 (C-6′), 32.9 (C-9′), 28.9–28.2 and 25.1 (C-2″ to 8″). MS (FAB): m/z 495 $(M + H)^+$, 333.

General Procedure for the Preparation of *N*-[10-*O*-(β-D-Glycopyranosyl)decyl]chitosan Derivatives. A stirred solution of chitosan flakes in a mixture of 5% aqueous acetic acid–methanol (1:1, at 15 mL/mmol) was treated with a solution of the 10-formylnonyl β-D-glycoside (1.5–3.0 equiv) in the reaction media (10–15 mL) and subsequently with sodium cyanoborohydride (at 4.0 mol equiv). The resultant mixture was stirred for 24 h and then dialyzed for 3 days against methanol–water 1:1 (3 × 1 L) and 3 days against distilled water (3 × 1 L). The solution was filtered through a medium-pore sintered-glass filter and freeze-dried. Yields were 50–90%.

***N*-[10-*O*-(β-D-Glucopyranosidyl)decyl]chitosan (14).** (a) Chitosan (0.65 g, 4.0 mmol) was treated with the aldehyde **10** (2.8 g, 8.4 mmol) to give, after workup, 0.93 g (54%) of derivative **14a**.

Anal. Calcd for $[(C_6H_{11}NO_4)_{0.19}(C_{22}H_{41}N)_{10})_{0.81}]\cdot 0.83H_2O$: C, 52.49; H, 8.53: N, 3.23. Found: C, 52.49; H, 8.20; N, 3.23.

(b) When chitosan (0.45 g, 2.8 mmol) was reacted with the formylnonyl β-D-glycoside **10** (2.85 g, 8.5 mmol), 1.64 g (91%) of product **14b** was obtained.

Anal. Calcd for $[(C_{22}H_{41}NO_{10})_{0.53}\ (C_{38}H_{71}NO_{16})_{0.47})]\cdot 1.0H_2O$: C, 54.80; H, 8.83; N, 2.17. Found: C, 54.80; H, 8.60; N, 2.17.

N-[10-O-(β-D-Galactopyranosyl)decyl]chitosan (15). (a) Treatment of chitosane (0.65 g, 4.0 mmol) with the glycoside 11 (4.0 g, 12.0 mmol) provided 1.43 g (58%) of **15a**.

Anal. Calcd for $[(C_{22}H_{41}NO_{10})_{0.63}(C_{38}H_{71}NO_{16})_{0.37}]\cdot 0.82H_2O$: C, 54.79; H, 8.79; N, 2.29. Found: C, 54.79; H, 8.89; N, 2.29.

(b) Chitosan (0.65 g, 4.0 mmol) was reacted with the aldehyde 11 (2.0 g, 6.0 mmol) to yield 0.89 g (90%) of derivative **15b**.

Anal. Calcd for $[(C_6H_{11}NO_4)_{0.78}(C_{22}H_{41}NO)_{0.22}]\cdot 1.55H_2O$: C, 44.13; H, 8.00; N, 5.41. Found: C, 44.13; H, 7.83; N, 5.46.

N-[10-O-(β-D-Galactopyranosyl)-β-D-glucopyranosyl)decyl]chitosan or N-[10-O-(β-D-Lactosyl)decyl]chitosan (16). (a) Reaction of chitosan (0.65g, 4.0 mmol) with the aldehyde 12 (5.8 g, 11.7 mmol) provided 1.95 g (67%) of product **16a**.

Anal. Calcd for $[(C_{28}H_{51}NO_{15})_{0.90}(C_{50}H_{91}NO_{26})_{0.1}]\cdot 2.04H_2O$: C, 49.94; H, 8.14; N, 1.93. Found: C, 49.94; H, 8.11; N, 1.93.

(b) Treatment of chitosan (0.65 g, 4.0 mmol) with the aldehyde 12 (3.10 g, 6.3 mmol) yielded 1.18 g (69%) of lyophilized **16b**.

Anal. Calcd for $[(C_6H_{11}NO_4)_{0.50}(C_{28}H_{51}NO_{15})_{0.50}]\cdot 1.45H_2O$: C, 47.77; H, 7.94; N, 3.28. Found: C, 47.77; H, 8.05; N, 3.26.

N-(10-Hydroxydecyl)chitosan (17). (a) A solution of chitosan (0.65 g, 4.0 mmol) in 5% aqueous acetic acid–methanol-isopropyl alcohol (5:4:1, 60 mL) was treated with 10 hydroxydecanal (2.1 g, 12.0 mmol) and subsequently with sodium cyanoborohydride (1.0 g, 16.0 mmol) for 24 h. A flocculent precipitate formed rapidly, which, upon completion of reaction, was collected by filtration and washed with water to give 1.30 g (76%) of derivative **17a**.

Anal. Calcd for $[(C_{10}H_{31}NO_5)_{0.27}(C_{26}H_{51}NO_6)_{0.73}]\cdot 0.23H_2O$: C, 64.26; H, 10.59; N, 3.22. Found: C, 64.26; H, 10.62; N, 3.22.

(b) Treatment of chitosan (0.65 g, 4.0 mmol) as in part a, with 10-hydroxydecanal (0.70 g, 4.0 mmol) and sodium cyanoborohydride (1.0 g, 16.0 mmol) for 24 h, provided a solution that after dialysis against methanol–water (2:1) for 2 days (2 × 1 L), methanol–water (1:1) for 2 days (2 × 1 L), and finally water for 2 days (2 × 2 L) gave 1.04 g (82%) of freeze-dried product **17b**.

Anal. Calcd for $[(C_{16}H_{31}NO_5)]\cdot 0.42H_2O$: C, 59.17; H, 9.81, N, 4.31. Found: C, 59.17; H, 9.65, N, 4.52.

Synthesis of Mixed N-(Ethyl- and N-(Decyl-D-glycopyranosyl)chitosan Derivatives (19). Derivative 18 (150 mg, 0.68 mmol) in 5% aqueous acetic acid–methanol (1:1) was stirred with the aldehyde 11 (0.65 g, 1.95 mmol) and sodium cyanoborohydride (0.30 g, 4.80 mmol) for 24 h. The reaction mixture was transferred to a dialysis sac, dialyzed against water–methanol (1:1) for 3 days (3 × 1 L) and against distilled water for 3 days (3 × 1 L), and freeze-dried to give 220 mg (63%) of **19**.

Anal. Calcd for $[(C_{14}H_{25}NO_{10})_{0.48}(C_{38}H_{71}NO_{16})_{0.52}]\cdot 1.28H_2O$: C, 51.79; H, 8.39; N, 2.28. Found: C, 51.78; H, 8.56; N, 2.29.

Viscometry. For viscometry, sample solutions of 1.0% (w/w) concentration were prepared in distilled water (containing 5 ppm sodium benzoate as stabilizer) or in 0.1 mM copper(II) sulfate (aqueous) solution. Dissolution was aided by periodic mixing (Vortex-Genie) over a 24-h period. Entrapped air bubbles were removed by centrifugation for 30 min on a benchtop centrifuge. Viscometric measurements were performed with a rotational, controlled stress rheometer (Visco-Elastic Analyzer, Sangamo Transucers, W. Sussex, England) with truncated cone and plate fixtures (diameter 50.0 mm, α 2.5°, gap 90 μm). A controlled-temperature glycol bath under the plate provided temperature control. All measurements were recorded at 20.0 (±0.2 °C. The gap setting was zeroed with no sample present by lowering the cone fixture until contact was just made with the plate. The cone fixture was then raised to allow sample loading. A sample solution (~1.5 mL) was loaded into a syringe with an 18-gauge needle. The sample was then discharged onto the center of the plate, taking care to avoid the formation of air bubbles. The cone fixture was lowered onto the sample to the desired 90-μm-gap setting. The sample was allowed to equilibrate to 200 °C, as verified by measurement with a thermocouple. The sample was then stepped through a series of increasing torque settings, applied to the rotating cone fixture, typically from 0.1 to 60 G·cm. For each torque setting, the resultant angular velocity (rad s^{-1}) was recorded on a strip chart, allowing the equilibrium value to be reached (usually within 30 s for the majority of samples). When either the maximum torque (60 G·cm) or the maximum angular velocity (100 rad s^{-1}) was approached, the torque was stepped through a similar decreasing series, and angular velocity was recorded. Duplicate measurements were performed on all samples. Three Newtonian standard oil samples were subjected to identical measurement, at all measured torque values, in order to calibrate the torque settings over the full range of observed viscosities. Shear stress (σ) is related to torque (T) according to eq 1 where T is torque in dynes per centimeter cm and r is the

$$\sigma = 3T/2\pi r^3 \tag{1}$$

radius; and shear rate ($\dot{\gamma}$) is to angular velocity (ω) according to eq 2. Substitution of the values from cone geometry can provide

$$\dot{\gamma} = \omega/(\tan \alpha) \tag{2}$$

shear stress and shear rate factors as seen in eqs 3 and 4. Apparent

$$\sigma = 2996.7T \text{ (mPa)} \tag{3}$$

$$\dot{\gamma} = 22.92\omega \text{ } (s^{-1}) \tag{4}$$

viscosity will then be given by eq 5. Corrected torque values

$$\eta = 130.76(T/\omega) \text{ (mPa·s)} \tag{5}$$

(T_{corr}) were calculated from standard oil measurements by rearranging eq 5, to give

$$T_{corr} = \frac{\eta_{oil}\omega}{130.76} \tag{6}$$

where η_{oil} is the known standard oil viscosity, and ω was the measured angular velocity. Corrected torque values and determined angular velocities provided shear stress, shear rates, and apparent viscosities from eqs 3–5. Rheograms of steady-shear viscometric data were plotted on linear coordinates as apparent viscosity vs shear rate.

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