Isolation, Modification, and NMR Assignments of a Series of Cellulose Oligomers

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Received February 22, 1999. Revised Manuscript Received June 14, 1999

Abstract: A homologous series of cellulose oligomers from two to eight repeating subunits have been isolated and size-fractionated from the hydrolysis products of microcrystalline cellulose. Chemical modification of cellotriose (**1**), cellotetraose (**2**), cellopentaose (**3**), and cellohexaose (**4**) to the corresponding β-methyl glycosides **13**−**16** proceeded in three steps in overall yields of 16−46%. Peracetylation produced oligomers **5**−**8** in 70−75% yield, and subsequent formation of the β-methyl glycosides gave **9**−**12** in 42−89% yield. Removal of the acetate-protecting groups employing guanidine provided **13**−**16** in 73−79% yield. This modification eliminated anomeric equilibration and permitted a detailed NMR solution study of these oligomers. The complete ^{1}H and ^{13}C chemical shift assignments of each peracetylated and deprotected oligomer were obtained through a combination of DQF−COSY, HMQC, HMBC, and HMQC-TOCSY experiments. All the resonances in methyl cellotriose (**13**) and methyl cellotetraose (**14**) were readily distinguishable from one another and directly assignable. Severe overlap of the resonances for the inner pyranose rings of methyl cellopentaose (**15**) and methyl cellohexaose (**16**) was observed and could only be resolved and assigned using a comprehensive battery of 3D pulse sequences. These results demonstrate the utility of multidimensional NMR experiments in assigning the signals from a repeating polysaccharide and represent the first necessary step in a comprehensive, systematic study of cellulose oligomers in solution.

Introduction

Carbohydrates represent an important class of biopolymers and are possibly the least understood contributors to biological structure and function.[1] Polysaccharides play essential roles in a myriad of biological processes such as cell−cell recognition,[2] signaling,[3] protein folding,[4] embryogenesis and development,[5] and cellular and extra-cellular structure.[6] Despite the obvious importance of polysaccharides, development of a comprehensive, detailed structural description of carbohydrates in solution is substantially less advanced compared to other important biopolymers such as proteins and nucleic acids.[1] Although a more complete understanding of the structure and dynamics of carbohydrates would provide insight into how these polymers function in vivo, their spectral overlap and flexibility hinder their comprehensive solution study by modern NMR methods.[1] The assignment of ^{1}H and ^{13}C NMR chemical shifts for an arbitrary carbohydrate remains a challenge, and unlike other biopolymers, enriching the NMR active isotopes in an oligosaccharide is a problem that has yet to be solved in a general sense. Presently, the assignment of linear carbohydrate polymers with simple repeating structures is typically limited to 3 or 4 residues.[7] Consequently, most studies of carbohydrate dynamics in solution have focused on dimers or trimers as models, which may not provide a complete picture of the more biologically relevant longer oligomers.[8] Recently, advances in solution NMR techniques and molecular modeling methods have progressed to where it is now feasible to undertake the systematic study of more complex carbohydrates including linear oligomers with known biological activity.[8,9]

Of all the possible glycoforms available for a systematic structural study, cellulose and its oligomers possess one of the simplest repeating motifs. Composed solely of repeating β-1,4-glucose monomers, this near-ubiquitous carbohydrate, by virtue of its simple composition, may serve as a model for other more complex carbohydrates in solution. Cellulose, found in most plants and some algae and bacteria, is the most abundant polymer found in nature[6,10] and has found use in a large variety of applications due to its solubility profiles, its rigidity, and its inherent lack of toxicity.[11] Cellulose and cellulose-derived compounds are found in a diverse number of disparate applications including pharmaceuticals, food stuffs, cosmetics, textiles, paper, and other material-based applications.[6,10] Despite exten-

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10.1021/ja990561u CCC: $18.00
Published on Web 07/21/1999

sive ongoing research on the value-added uses of cellulose, questions remain regarding the solution properties and dynamics of the polymer. A clearer picture of cellulose in solution is a necessary prerequisite for studies on cellulose chelation behavior and would have a broad impact in the areas of carbohydrate solution dynamics and protein−carbohydrate recognition events.

Remarkably, little is known about the structure of cellulose oligomers in solution, although considerable information is available on the solid-state properties of high-molecular-weight cellulose. Solid-state characterizations by fiber X-ray diffraction,[12] atomic force microscopy,[13] solid-state NMR,[14] IR spectroscopy,[15] Raman spectroscopy,[16] and small-angle X-ray scattering[17] have been reported. These studies have elucidated the gross morphology of the two main forms of cellulose fibers, cellulose-I and cellulose-II, which differ in the orientation of the individual polysaccharide chains with respect to one another. In cellulose-I, the ribbonlike chains are laid down in staggered parallel sheets and are held in place by interstrand hydrogen bonding. Cellulose-II is a modified form of cellulose, which is formed when cellulose-I is treated with base or when cellulose derivatives are saponified. These cellulose chains are oriented in antiparallel sheets that are no longer staggered.[10] Due to the insolubility of native cellulose in both forms and the lack of signal dispersion in the proton spectra, NMR studies on cellulose have generally been limited to solid-state ^{13}C experiments. Although these spectra have broad line widths that are useful in determining the allomorphs present in the solid state, they reveal little about specific interactions in solution.[18]

The short cellulose oligomers cellotetraose,[19] methyl β-cellobiose,[20] and methyl β-cellotriose,[21] have been crystallized and analyzed by X-ray diffraction, revealing additional details of molecular structure which have been used to infer details of the longer chains. Cellotetraose exists as a hemihydrate in the crystalline state with packing analogous to that of cellulose-II. The primary difference between the structure of high-molecular-weight cellulose-II and the short oligomers is the conformation about the C6−O6 bond. Diffraction studies on cellulose-II suggest that the C6−O6 conformation alternates between two different states.[18] By contrast, diffraction studies on the oligomers indicate that all C6−O6 bonds are predominately in one conformation, resulting in a single intramolecular hydrogen bond between OH3 and O5, and leaving all other hydroxyl groups free to form intermolecular hydrogen bonds.

Despite the obvious importance of cellulose, even short cellulose oligomers have defied a detailed description of their behavior in aqueous media. High-resolution NMR spectroscopy offers a powerful means to probe the properties of water-soluble species, including carbohydrates composed of simple repeating motifs.[22] In the case of high-molecular-weight cellulose fragments, insolubility has hindered NMR studies. Solubilizing agents that disrupt the hydrogen-bonding network of high-molecular-weight cellulose have been employed in some NMR studies but have revealed little about cellulose in its native state.[18,23]These studies sought to determine in which solvent systems cellulose could be dissolved, what, if any, covalent modification occurred in solution, and the degree of substitution in cellulose after partial derivatization. The resultant spectra were of poor quality, and broad line widths of at least 50 Hz were typically observed. Additionally, some of the agents that contained metal ions exhibited evidence of ion complexation to the cellulose, which does not give a true picture of naturally occurring cellulose.

Smaller water-soluble cellulose fragments have yet to be studied systematically because of the inherent resonance overlap problem that plagues most solution NMR investigations of carbohydrates. Small oligomers of cellulose ranging from cellobiose to celloheptaose are water-soluble, and details of their solution structure can serve as important models for the structure of linear oligosaccharides in aqueous media. A more complete understanding of the structure of cellulose oligomers in solution would also have a broad impact in a number of disciplines. For example, the interaction of cellulose with exogenous species such as the cellulose-binding proteins is of great interest.[24] Small, water-soluble cellulose fragments could serve as models for cellulose in NMR studies of cellulose-binding proteins such as CBH1. Current studies with CBH1 and cellulose fragments are plagued by a dual equilibria problem, where in addition to the equilibrium of cellulose binding to CBH1, the cellulose is also in anomeric equilibrium.[25−33]Additionally, the flexibility and dynamics of the glycosidic linkage continues to be an area of active research. The dynamic behavior of cellulose oligomers

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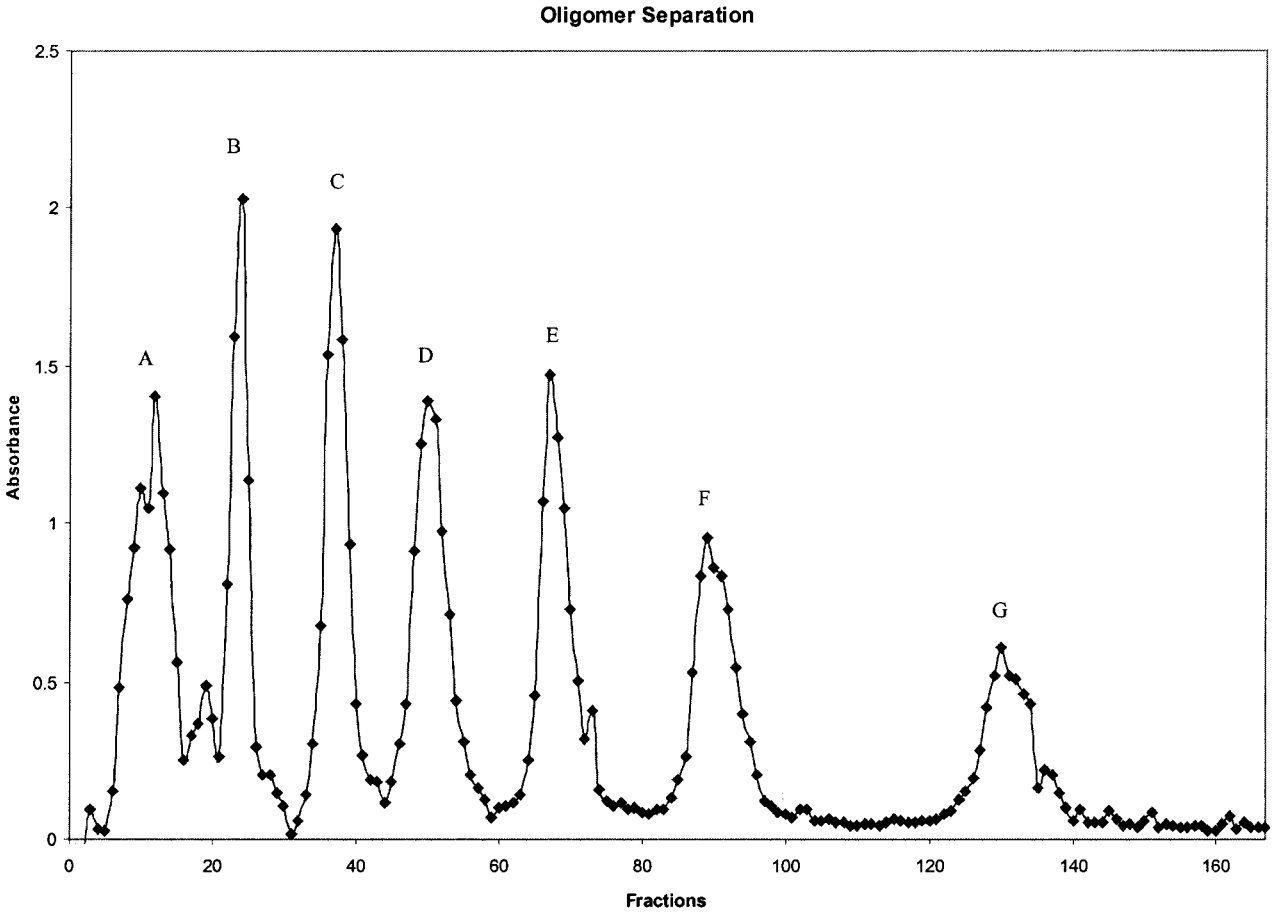

Figure 1. Absorption of cellulose oligomers versus fraction number during chromatographic run: peak A, Glucose; peak B, Cellobiose; peak C, Cellotriose; peak D, Cellotetraose; peak E, Cellopentaose; peak F, Cellohexaose; peak G, Celloheptaose.

in solution would serve to address this issue. Finally, cellulose can complex metal ions, but the conformational effects of this are largely unknown. *The prerequisite for all of these aforementioned studies is direct knowledge of the ^{1}H and ^{13}C chemical shifts of each center in each oligomer to be studied.*

The systematic study of cellulose oligomers presents several obstacles: the availability of the oligomers, the anomeric equilibrium of the reducing end that occurs in aqueous solution, and the inherent difficulty of NMR on carbohydrates.[34,35] Cellulose itself can be obtained from natural sources or can be synthesized chemically or biosynthetically.[36] However, there is presently no direct means to control polydispersity. Cellulose oligomers of uniform length must either be made synthetically, a complicated, time-consuming procedure,[37] or be isolated as the hydrolysis products of high-molecular-weight cellulose.

This report describes the isolation of cellulose oligomers from the acid-catalyzed hydrolysis of high-molecular-weight cellulose, the chemical modification of these fragments to eliminate their anomeric equilibration, and a complete assignment of all ^{1}H and ^{13}C resonances for each fragment. The NMR resonance assignments of the modified β-methyl cellulose oligomers and the peracetylated intermediates have also been determined by a battery of NMR experiments including 2D experiments such as COSY and HMQC, and 3D HMQC−COSY and HMQC-TOCSY experiments. This represents the initial step toward a comprehensive study of the structure and dynamics of cellulose in solution.

Results and Discussion

Isolation. Isolation of the oligomers from the acid-catalyzed hydrolysis of microcrystalline cellulose was achieved using the column chromatography method of Miller.[38] This proved effective for obtaining near-gram quantities of cellulose oligomers, representing a significantly higher yield than other reported methods.[39] Over a five-day period, oligomers ranging from glucose to celloheptaose were separated (Figure 1). This method yielded oligosaccharides ranging from 700 mg of cellotriose to 200 mg of celloheptaose from 10 g of high-molecular-weight cellulose. Previous work on the solubility of cellulose oligosaccharides has shown that celloheptaose represents the largest water-soluble oligocellulose that can be isolated by chromatographic separation.[40,41]

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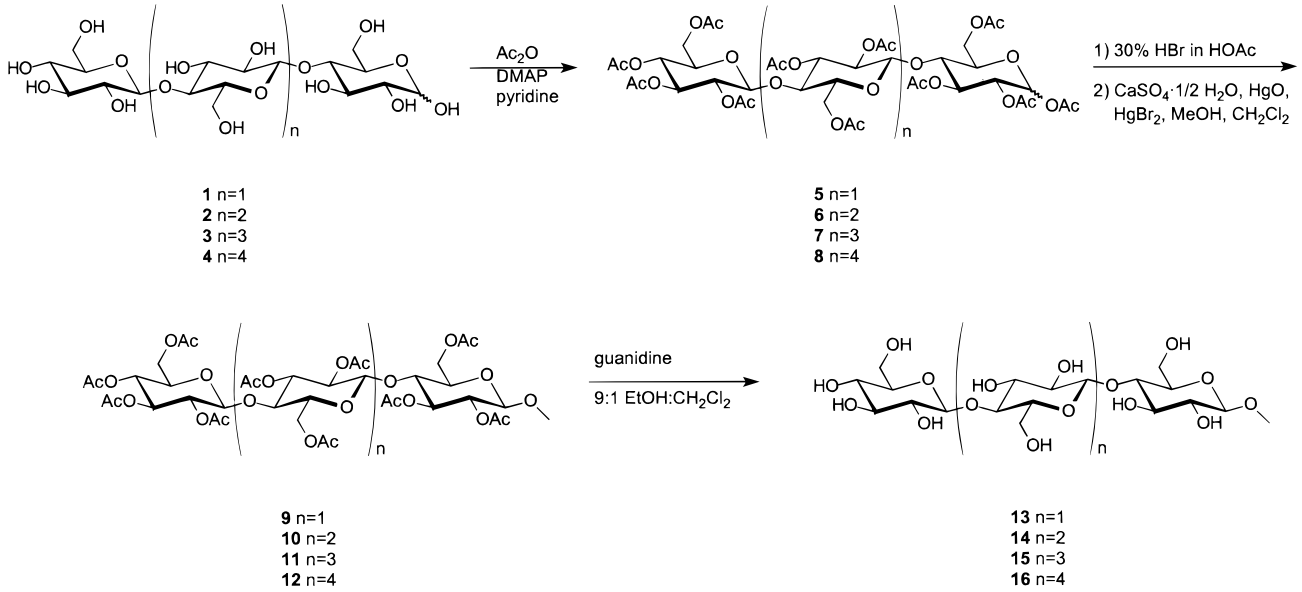
Scheme 1



Modification of the Isolated Oligomers. In aqueous solution reducing carbohydrates typically equilibrate between the α and β anomers.[10] The existence of both anomers further complicates the already severe spectral overlap of these oligomers. Modification to the corresponding β-methyl glycosides eliminates this process by producing a single anomerically pure compound. Furthermore, the β-methyl glycosides **13**−**16** would correspond more generally to the structure of high-molecular-weight cellulose than the corresponding α-anomers. Modification of the cellulose oligomers was achieved through a four-step reaction sequence to methylate the reducing end of cellotriose (**1**), cellotetraose (**2**), cellopentaose (**3**), and cellohexaose (**4**) (Scheme 1). These β-methyl oligomers **13**−**16** have previously been synthesized by enzymatic means to afford, starting from methyl β-cellobioside, a series of oligomers up to methyl β-cellopentaoside.[42] The yields for the longer oligomers were poor (14% **13**, 36% **14**, 6% **15**), and cellohexaose was not isolated. The chemical modification reported herein is moderate-yielding (from 16% to 46% overall yield in 4 steps) and should be applicable to other carbohydrate oligomers. Celloheptaose and cellooctaose, although available from our separation, were not modified as they are sparingly soluble in aqueous solution, which would hinder NMR studies in the absence of isotopic enrichment. Additionally, on the basis of our assignment data for **13**−**16**, we believe that the severe spectral overlap of these longer oligomers would make their complete assignments impossible.

The cellulose oligomers were peracetylated with acetic anhydride in the presence of pyridine and DMAP.[43] Reaction times ranged from 12 h for **1** to 3−4 days for the longer oligomers. We hypothesize that the longer reaction times are a function of the additional hydroxyl groups and the reduced initial solubility of the longer oligomers. Acetylation products were predominately β, with anomeric ratios ranging from 1:1 β/α (**5**) to 4:1 β/α (**8**) by NMR. The anomers were not separated, as the mixture could be elaborated to the corresponding glycosyl bromides.

Formation of the glycosyl bromides proceeded smoothly using 30% HBr in glacial acetic acid.[44] This reaction proceeded faster with the higher oligomers; **5** reacted in 1 h, while the reaction was complete in 30 min for **6**, 20 min for **7**, and 10 min for **8**. We believe this is due in part to the differing solubilities of the two anomers present in the reaction mixture, which becomes less important with increasing oligomer size. The β anomer, being more polar, is more likely to dissolve in the highly polar glacial acetic acid than the less polar *a* anomer. Thus, **5** with a 1:1 anomeric ratio reacts relatively slowly compared to the other oligomers with higher β anomer content and greater molecular weight. The reaction proceeds quantitatively, and the product was used without further purification. Methylation was performed under standard Koenigs-Knorr conditions using methanol and methylene chloride in the presence of $CaSO_4 \cdot {}^1/_2H_2O$, HgO, and $HgBr_2$.[44] On average the reactions were completed in 3 days. The resulting products were purified by flash column chromatography on silica gel when necessary. Column chromatography resulted in product decomposition and much lower yields. Crude yields ranged from 89% for **9** to 42% for **12**. Column chromatography decreased these yields to approximately 20%. Where chromatography was not performed, the products were carried on and purified following the subsequent step.

Glycoside **9** was deacetylated under Zemplén conditions using methanol and NaOMe.[44] These conditions were found to be ineffective for the higher oligomers due to their insolubility in methanol. Deprotection of the higher oligomers was instead accomplished using guanidine in ethanol and methylene chloride[45] and purified by either recrystallization or trituration in ethanol, yielding 73−79% of the deprotected products.

NMR Assignments. Complete NMR assignment of unprotected carbohydrates can be difficult due to severe overlap of ^{1}H and ^{13}C resonances. Cellulose oligomers, by nature of their simple repeating structure, have an especially severe spectral overlap problem. The bulk of ^{1}H resonances occur between 3.0 and 4.5 ppm, while most of the corresponding ^{13}C resonances occur between 55.0 and 80.0 ppm. The use of modern high field

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spectrometers coupled with multidimensional pulse sequences can disperse these resonances and allow for the complete assignment of proton and carbon signals.[46]

In this work, most proton spectra were recorded at a resonance frequency of 750 MHz and ^{13}C spectra were recorded at a resonance frequency of 188 MHz. A range of multidimensional spectra were employed to aid in the resonance assignments, including DQFCOSY,[47] HMQCPS,[48] COSYPS,[49] and HMBC[50] and the 3D experiments HMQC−COSY and HMQC-TOCSY.[51] Assignments were made for the β-methyl peracetylated oligomers **9**−**12** as well as the β-methyl oligocelluloses **13**−**16**.

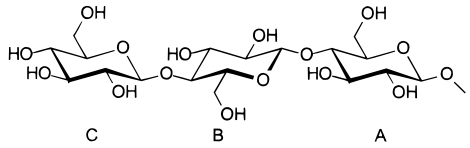
Assignment of Protected Oligomers. The proton and carbon resonances of the fully protected oligomers **9**−**12** were assigned using a combination of COSY, HMQC, and HMBC experiments. Methyl cellotriose decaacetate (**9**) was assigned using spectra taken at a proton resonance frequency of 500 MHz, while the longer oligomers were assigned with data taken at a proton resonance frequency of 750 MHz. Coupling networks were elucidated using COSY data. One-bond ^{13}C-^{1}H correlations were obtained from HMQC data, and the position of the glycosidic linkages was determined using HMBC data.

The β-methyl peracetylated oligomers (**9**−**12**) exhibited well-resolved signals, with overlap appearing in **11** and becoming problematic in **12**. As expected, the acetate-protecting groups caused chemical shift dispersion of the resonances relative to the free oligomer and consequently the peracetylated oligomers were much easier to completely assign. In each oligomer all of the resonances for each ring position clustered together, with the H5s resonating near 3.5 ppm, the H4s near 3.7 ppm, one set of H6s at 4.0 ppm, the H1s and remaining H6s together at 4.5 ppm, the H2s around 4.8 ppm, and the H3s near 5.0 ppm. The exception to this trend is the H4 of the terminal ring that is not part of a glycosidic linkage. This resonance occurs with the H3s near 5.0 ppm. In all of the oligomers, the ring next to the terminal ring on the nonreducing end ($n - 1$ ring) exhibits the most upfield resonances at the majority of the ring positions.

The carbon resonances also group together by ring position. The C6s occur the most upfield at around 62 ppm, with the C2s at 71.8 ppm, the C3s at 72.8 ppm, the C5s at 72.9 ppm, the C4s around 76 ppm, and the C1s around 100 ppm. Again, the exception is the C4 on the $n - 1$ ring, occurring at 67.8 ppm in all of the oligomers. The carbon resonances show no general trend as seen in the proton resonances where one ring is consistently the most upfield or downfield. In most of the positions the order in which the resonances occur is the same from oligomer to oligomer.

Previous work has been performed on the fully peracetylated oligomers with no terminal methyl group.[52] Most of the NMR experiments were done to determine the degree of acetate substitution, and not NMR assignments. Thomson and co-workers reported the ^{13}C chemical shifts for a series of α-peracetylated cellulose oligomers. These studies were performed at a proton resonance frequency of 100 MHz, which proved inadequate to assign the proton resonances now available from this study. Although the carbon resonances were assigned, the resonances of the interior residues all exhibited overlap.[53]

Table 1. Proton and Carbon Assignments for **13**



13

	^{1}H	multiplicity	^{13}C
ring A			
H1	4.403	d $J = 8.0$	102.94
H2	3.305	dd $J = 9.4, 7.6$	72.99
H3	3.633	m	74.13
H4	3.628	m	78.36
H5	3.597	ddd $J = 10.0, 5.1, 2.2$	74.62
H6	3.818	dd $J = 12.6, 5.4$	59.81
H6′	3.993	dd $J = 12.3, 2.2$	
ring B			
H1	4.533	d $J = 8.0$	102.21
H2	3.361	dd $J = 9.3, 8.0$	72.79
H3	3.648	m	73.90
H4	3.677	t $J = 8.8$	78.21
H5	3.618	ddd $J = 9.7, 5.2, 2.2$	74.67
H6	3.826	dd $J = 12.6, 5.4$	59.69
H6′	3.980	dd $J = 12.3, 2.3$	
ring C			
H1	4.509	d $J = 7.9$	102.43
H2	3.315	dd $J = 9.5, 7.9$	72.74
H3	3.507	t $J = 9.3$	75.32
H4	3.418	dd $J = 9.9, 9.1$	69.29
H5	3.487	ddd $J = 9.8, 5.9, 2.3$	75.82
H6	3.736	dd $J = 12.6, 6.0$	60.42
H6′	3.917	dd $J = 12.4, 2.3$	

Assignment of Deprotected Oligomers. The deprotected oligomers **13**−**16** were assigned using the same strategy as for **9**−**12**, with the addition of HMQC−COSY and HMQC−TOCSY data to assign the more crowded regions of the ^{1}H and ^{13}C spectra. As expected the chemical shift range of the unprotected oligomers was substantially smaller compared to their peracetylated counterparts. The optimal mixing times for the 3D experiments were determined by a series of TOCSY experiments utilizing mixing times of 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 ms. These data were used to determine the mixing times for TOCSY- and COSY-type data, which were slightly different for each oligomer. Methyl cellohexaose was determined to require only TOCSY-type data, as the signals could be assigned by analogy to the shorter oligomers. COSY, HMQC, and HMBC experiments were used to assign as much of the backbone as possible and, in the case of methyl cellotriose (**13**), were sufficient to completely assign the molecule. (Table 1) Higher oligomers could not be assigned directly and required the extra dispersion only possible with a 3D experiment.

The assignment strategy for methyl cellotetraose (**14**) started with assigning as many resonances as possible from the 2D data sets. This information was then used in the interpretation of the 3D data. The starting point for assigning the rings using HMQC−TOCSY and HMQC−COSY data was the anomeric

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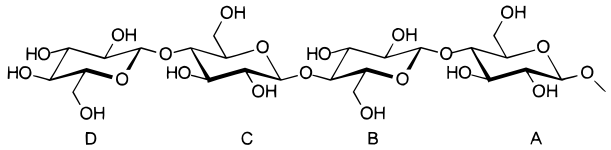
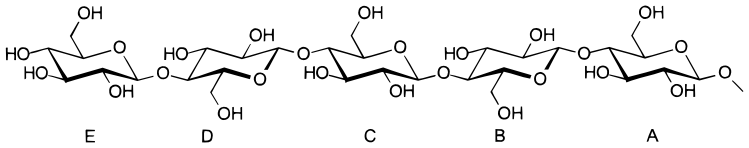
Table 2. Proton and Carbon Assignments for **14**



14

	^{1}H	multiplicity	^{13}C		^{1}H	multiplicity	^{13}C
ring A				ring C			
H1	4.404	d J = 7.97	102.92	H1	4.532	d J = 8.08	102.18 or 102.21
H2	3.304	t J = 8.03	72.99	H2	3.361	t J = 8.55	72.77 or 72.79
H3	3.636	m	74.12	H3	3.646	m	73.85
H4	3.634	m	78.36	H4	3.667	m	78.21
H5	3.597	m	74.62	H5	3.611	m	74.66
H6	3.818	dd J = 12.03, 6.40	59.82	H6	3.827	dd J = 12.22, 5.45	59.70 or 59.72
H6′	3.991	dd J = 12.15, 2.01		H6′	3.977 or 3.980	dd J = 12.28, 1.93 or dd J = 12.32, 1.79	
ring B				ring D			
H1	4.532	d J = 8.08	102.18 or 102.21	H1	4.506	d J = 7.83	102.40
H2	3.361	t J = 8.55	72.77 or 72.79	H2	3.316	t J = 8.73	72.73
H3	3.642	m	73.85	H3	3.509	t J = 9.25	75.32
H4	3.680	m	78.09	H4	3.417	t J = 9.47	69.30
H5	3.616	m	74.66	H5	3.488	ddd J = 9.99, 5.92, 2.15	75.83
H6	3.827	dd J = 12.22, 5.45	59.70 or 59.72	H6	3.736	dd J = 12.36, 5.88	60.41
H6′	3.977 or 3.980	dd J = 12.28, 1.93 or dd J = 12.32, 1.79		H6′	3.915	dd J = 12.41, 2.16	

Table 3. Proton and Carbon Assignments for **15**



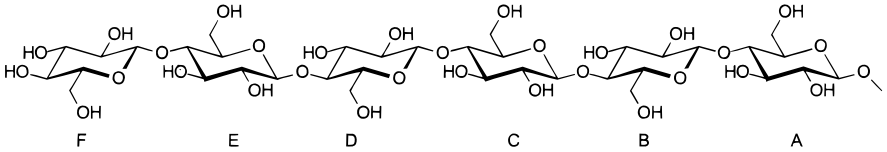
15

	^{1}H	multiplicity	^{13}C		^{1}H	multiplicity	^{13}C
ring A				ring D			
H1	4.403	d J = 8.01	102.87	H1	4.533	d J = 7.95	102.13
H2	3.304	dd J = 9.53, 8.29	72.97	H2	3.360	t J = 8.55	72.75
H3	3.64	m	74.06	3	3.65	m	73.80
H4	3.63	m	78.28	H4	3.69	m	78.16
H5	3.59	m	74.59	H5	3.62	m	74.64
H6	3.820	dd J = 12.20, 6.53	59.75	H6	3.829	dd = 12.06, 5.41	59.67
H6′	3.991	dd J = 12.26, 2.21		H6′	3.977	br d J = 11.92	
ring B				ring E			
H1	4.533	d J = 7.95	102.13	H1	4.510	d J = 7.95	102.35
H2	3.360	t J = 8.55	72.75	H2	3.315	dd J = 9.47, 8.03	72.70
H3	3.65	m	73.80	H3	3.507	t J = 9.25	75.27
H4	3.69	m	78.04	H4	3.416	t J = 9.49	69.23
H5	3.62	m	74.64	H5	3.486	ddd J = 9.70, 5.70, 2.46	75.83
H6	3.829	dd = 12.06, 5.41	59.67	H6	3.737	dd J = 12.42, 5.84	60.35
H6′	3.977	br d J = 11.92		H6′	3.916	dd J = 12.37, 2.25	
Ring C							
H1	4.533	d J = 7.95	102.13				
H2	3.360	t J = 8.55	72.75				
H3	3.65	m	73.80				
H4	3.69	m	78.04				
H5	3.62	m	74.64				
H6	3.829	dd = 12.06, 5.41	59.67				
H6′	3.977	br d J = 11.92					

protons, enabling assignment of each ring. (Table 2) Methyl cellopentaose (**15**) and methyl cellohexaose (**16**) were assigned in a similar fashion. (Tables 3 and 4)

The proton and carbon assignments were accomplished for the oligomers through methyl cellohexaose (**16**). Most of the protons and carbons for **13** and **14** were distinguishable as separate signals. By contrast, the inner ring protons and carbons of **15** and **16** exhibited overlap. The exception to this trend was the signal for C4 of the $n - 1$ ring. This signal was clearly separate in all oligomers. Presumably, proton and carbon signals for the inner rings of longer oligomers such as celloheptaose would simply overlap with the other interior ring signals and

Table 4. Proton and Carbon Assignments for **16**



16

	^{1}H	multiplicity	^{13}C		^{1}H	multiplicity	^{13}C
ring A				ring D			
H1	4.405	d $J = 8.1$	102.91	H1	4.533	d $J = 8.0$	102.18
H2	3.304	dd $J = 9.53, 8.29$	72.97	H2	3.358	br t $J = 8.5$	72.75
H3	3.64	m	74.10	H3	3.65	m	73.82
H4	3.63	m	78.32	H4	3.69	m	78.05
H5	3.59	m	74.60	H5	3.62	m	74.65
H6	3.819	dd $J = 12.3, 5.1$	59.70	H6	3.828	dd $J = 12.0, 5.0$	59.65
H6′	3.991	dd $J = 11.9, 2.0$		H6′	3.976	br d $J = 11.5$	
ring B				ring E			
H1	4.533	d $J = 8.0$	102.18	H1	4.533	d $J = 8.0$	102.18
H2	3.358	br t $J = 8.5$	72.75	H2	3.358	br t $J = 8.5$	72.75
H3	3.65	m	73.82	H3	3.65	m	73.82
H4	3.69	m	78.05	H4	3.69	m	78.18
H5	3.62	m	74.65	H5	3.62	m	74.65
H6	3.828	dd $J = 12.0, 5.0$	59.65	H6	3.828	dd $J = 12.0, 5.0$	59.65
H6′	3.976	br d $J = 11.5$		H6′	3.976	br d $J = 11.5$	
ring C				ring F			
H1	4.533	d $J = 8.0$	102.18	H1	4.509	d $J = 7.8$	102.39
H2	3.358	br t $J = 8.5$	72.75	H2	3.313	dd $J = 9.4, 8.0$	72.71
H3	3.65	m	73.82	H3	3.506	t $J = 9.3$	75.29
H4	3.69	m	78.05	H4	3.416	t $J = 9.5$	69.27
H5	3.62	m	74.65	H5	3.486	ddd $J = 9.6, 5.7, 2.4$	75.81
H6	3.828	dd $J = 12.0, 5.0$	59.65	H6	3.736	dd $J = 12.5, 5.9$	60.38
H6′	3.976	br d $J = 11.5$		H6′	3.915	dd $J = 12.6, 2.1$	

yield no additional information. All of the rings in the oligomers except the terminal, nonreducing ring exhibited roughly similar chemical shifts, with the H2s the most upfield near 3.4 ppm, the H5s at 3.7 ppm, the H4s and H3s near 3.8 ppm, the H6s at 3.9 and 4.0 ppm, and the H1s at 4.4 ppm. The signals from the interior rings are the most downfield signal for most ring positions. The carbon resonances exhibit this same clustering, with the same order as in the peracetylated oligomers, where C6 is the most upfield, followed by C2, C3, C5, C4, and C1. However, the nonreducing terminal ring signals do not cluster with the signals for the other rings. Presumably, this is because it is the only ring without a glycosidic linkage at C4. There is no general trend for the carbon signals as there is for the proton signals, where the signals for one ring are noticeably up- or downfield from the others, but the order of signals for each position in any given ring remains the same through the oligomer series. In addition, the proton and carbon chemical shifts did not markedly change with the size of the oligomer changed, indicating no dependence on oligomer length.

A limited amount of work has previously been done on the unmodified cellulose oligomers. The assignments for the oligomers **13**−**16** are similar to those for cellulose in NaOH/D_2O[23] and for cellohexaose (**4**),[34] although more peaks are discernible in the methyl cellohexaose (**16**). In addition, the methyl group shifts C1 downfield relative to its position in the unprotected cellulose oligomers. Gast and co-workers performed experiments on cellobiose, cellotriose (**1**), and cellotetraose (**2**).[35] As their experiments were performed at a proton resonance frequency of 100 MHz, little or no detail from the proton spectra could be obtained from their data. The carbon spectra only differentiated between terminal and interior residue signals. However, their assignments are comparable to ours, although they seem to be consistently 2 ppm downfield from the current findings. Heyraud and co-workers performed experiments on a series of cellulose oligomers up to cellohexaose (**4**) at a proton resonance frequency of 250 MHz.[34] They did not attempt proton assignments but were able to differentiate some of the signals in the carbon spectra; for instance, in cellotetraose all four C4 signals were different. They did identify the different signal for the C4 on the $n - 1$ ring but were unable to assign it to the $n - 1$ ring. However, most signals overlap, even in cellotetraose (**2**). Again, their results were comparable to the current results, although their assignments were consistently 3 ppm downfield from those in this study.

Conclusion

The isolation, modification, and NMR assignments for a series of cellulose oligomers have been completed. The modification procedure is a simple way of obtaining anomerically pure carbohydrates that are useful NMR substrates for many applications, including the study of protein−carbohydrate interactions and of metal chelates of carbohydrates. The NMR assignments represent the most complete NMR characterization to date of cellulose oligomers. This provides another method to characterize cellulose and its derivatives in addition to the methods currently available and enables the eventual elucidation of the solution dynamics of the cellulose oligomers. In addition, this represents an important step toward understanding carbohydrate solution behavior.

Experimental Section

General Information. Flash column chromatography following the method of Still employed EM Science silica gel 60 (230−400 mesh). Analytical thin-layer chromatography was performed with 0.25 mm coated commercial silica gel plates (E. Merck, DC-fertigplatten Kieselgel F254 or the corresponding Altech plate). Melting points were

obtained on a Buchi melting point apparatus in an open capillary tube and are uncorrected. High-resolution EI and FAB mass spectral data were obtained on either a 70-VSE or 70-SE-4F mass spectrometer. FAB spectra were obtained using either Na° or K° as the ionization source (as indicated).

NMR Experiments. All spectral data were obtained on either a Varian Unity-Plus spectrometer (^{1}H resonance frequency of 400 MHz), a Varian Unity-Plus spectrometer (^{1}H resonance frequency of 500 MHz), a Varian INOVA spectrometer (^{1}H resonance frequency of 500 MHz), or a Varian INOVA spectrometer (^{1}H resonance frequency of 750 MHz). Proton (^{1}H) chemical shifts are reported in delta (δ) units, parts per million (ppm) downfield from tetramethylsilane (TMS). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; sex, sextet; m, multiplet; and br, broad. Coupling constants are reported in Hertz (Hz). ^{13}C NMR chemical shifts are reported in parts per million relative to the center line at 77.23 ppm of the deuteriochloroform triplet. Spectra taken in D_2O are referenced to an internal benzene standard at 7.44 ppm. All spectra were acquired at 20 °C. All experiments were acquired with standard pulse sequences supplied with the software. The 3D data were processed with the Varian processing program VNMR 6.2. Two different mixing times were used in some of the HMQC−TOCSY experiments to give both TOCSY and COSY data. The number of data points taken for the 3D HMQC−TOCSY of methyl cellotetraose, with a mixing time of 100 ms, and with 4 scans per increment was 1024 × 48 × 24, which was linear predicted to 1024 × 128 × 128 and zero-filled to 2048 × 512 × 512. The data were processed with line broadening (lb = 0.82, lb1 = −13.6, lb2 = 15.6), Gaussian multiplication and shift (gf = 0.35, gfs = −0.0015, gf1 = 0.01, gfs1 = −0.0006, gf2 = 0.16), and sine bell multiplication and shift (sb = 0.56, sbs = −0.21, sb2 = 0.18, sbs2 = −0.02) as determined by the interactive weighting routine. The 3D HMQC−COSY of methyl cellotetraose was acquired with a mixing time of 10 ms and with 2 scans per increment and had 1024 × 48 × 16 data points, subsequently linear predicted to 1024 × 128 × 128 data points and zero-filled to 2048 × 512 × 512 data points. The data were processed with line broadening (lb = 0.84, lb1 = −49.4, lb2 = 2.3), Gaussian multiplication (gf = 0.38, gf1 = 0.009, gf2 = 0.18), and sine bell multiplication and shift (sb = 0.49, sbs = −0.21) as determined by the interactive weighting routine. The HMQC−TOCSY of methyl cellopentaose was acquired with mixing times of 60 ms and 10 ms and initial dimensions of 1024 × 64 × 24. The data were linear predicted to 1024 × 192 × 128 and zero-filled to 2028 × 512 × 512 data points. The data were processed with line broadening (lb = 1.3, lb1 = −20, lb2 = 17.5), Gaussian multiplication and shift (gf = 0.24, gfs = 0.042, gf1 = 0.006, gfs1 = 0.0003, gf2 = 0.05, gfs2 = 0.04), and sine bell multiplication and shift (sb = 0.35, sbs = −0.12, sb1 = 0.009, sbs1 = −0.009) as determined by the interactive weighting routine. The HMQC−TOCSY of methyl cellohexaose was taken with a mixing time of 80 ms and a data set size of 1024 × 64 × 24. The data were linear predicted to 1024 × 192 × 48 and zero-filled to 2048 × 512 × 512 data points. The data were processed with line broadening (lb = 3, lb1 = −20, lb2 = 5), Gaussian multiplication and shift (gf = 0.20, gf1 = 0.0059, gfs1 = 0.0002, gf2 = 0.013), and sine bell multiplication and shift (sb = 0.20, sbs = −0.047, sb1 = 0.0096, sbs1 = −0.009).

Isolation of Cellulose Oligomers. Microcrystalline cellulose (Avicel, 10 g) was hydrolyzed by stirring with fuming HCl solution (200 mL) for 2 h. The solution was diluted with cold Milli-Q water (600 mL) and neutralized with Na_2CO_3, then centrifuged. The supernatant was loaded onto a column of radius 5 cm, length 95 cm, and a reservoir volume of 1 L. The column resin consisted of 1:1 Darco G-60/Celite 545, successively equilibrated with solutions of 0.2% stearic acid in absolute ethanol, and 50% aqueous ethanol saturated with stearic acid. The resin was packed in the column and equilibrated with water prior to introduction of the oligosaccharides. The oligosaccharides were eluted with a water/ethanol gradient (0−45% ethanol over 5 days) and monitored by analyzing each fraction with an orcinol cocktail.[7,54] The fractions corresponding to each oligosaccharide were combined, dried, and verified by mass spectrometry. Cellotriose ($C_{18}H_{32}O_{16}Na$): mass calculated = 527.159, found = 527.159 (HRFABMS, Na° source). Cellotetraose ($C_{24}H_{43}O_{21}$): mass calculated = 667.230, found = 667.230 (HRFABMS, M+1). Cellopentaose ($C_{30}H_{53}O_{26}$): mass calculated = 829.283, found = 829.283. (HRFABMS, M + 1). Cellohexaose ($C_{36}H_{63}O_{31}$): mass calculated = 991.335, found = 991.335. (HRFABMS, M + 1). Celloheptaose ($C_{42}H_{72}O_{36}Na$): mass calculated = 1176.0, found = 1175.3. (LRFABMS, Na° source).

Oligomer Modification. (A) Cellotriose Undecaacetate (5). Cellotriose (**1**) (73.0 mg, 0.145 mmol, 1 equiv) and DMAP (1 mg, 0.008 mmol, 0.06 equiv) were placed in a flask which was purged with N_2, and pyridine (3.0 mL, 37 mmol, 260 equiv) and acetic anhydride (0.5 mL, 5.3 mmol, 37 equiv) were added via syringe. The solution was stirred 19 h at room temperature, then transferred to a flask containing 15 mL of ice water slurry and stirred for an additional 90 min. The mixture was extracted with $CHCl_3$ (5 × 10 mL). The organic layers were combined and washed with saturated $CuSO_4$ (4 × 10 mL), saturated $NaHCO_3$ (4 × 10 mL), and brine (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure. The residue was dried to yield 98.8 mg (70%) of an off-white solid that was a 1:1 mixture the α and β anomers of **5** by NMR: mp 105−109 °C. ^{1}H NMR (500 MHz, $CDCl_3$) 1.947 (s, 3H), 1.949 (s, 3H), 1.957 (s, 3H), 1.962 (s, 3H), 1.964 (s, 3H), 1.969 (s, 6H), 1.975 (s, 3H), 1.976 (s, 3H), 1.988 (s, 3H), 1.990 (s, 3H), 2.001 (s, 3H), 2.003 (s, 6H), 2.055 (s, 3H), 2.059 (s, 6H), 2.092 (s, 3H), 2.103 (s, 3H), 2.113 (s, 3H), 2.115 (s, 3H), 2.139 (s, 3H), 3.565 (ddd, J = 9.8, 5.2, 2.4 Hz, 1H), 3.570 (ddd, J = 9.8, 5.0, 2.6 Hz, 1H), 3.608 (ddd, J = 9.9, 4.4, 2.3 Hz, 1H), 3.610 (ddd, J = 9.9, 4.4, 2.3 Hz, 1H), 3.712 (ddd, J = 10.0, 4.6, 2.1 Hz, 1H), 3.737−3.793 (m, 4H), 3.950 (ddd, J = 10.1, 4.1, 2.1 Hz, 1H), 4.004 (dd, J = 12.5, 2.4 Hz, 1H), 4.009 (dd, J = 12.5, 2.5 Hz, 1H), 4.062 (dd, J = 12.4, 4.3 Hz, 1H), 4.066 (dd, J = 12.2, 4.6 Hz, 1H), 4.080 (dd, J = 12.2, 5.2 Hz, 1H), 4.096 (dd, J = 12.3, 5.1 Hz, 1H), 4.319 (dd, J = 12.5, 4.4 Hz, 1H), 4.324 (dd, J = 12.5, 4.4 Hz, 1H), 4.369 (dd, J = 12.1, 2.1 Hz, 1H), 4.375 (dd, J = 12.1, 2.0 Hz, 1H), 4.446 (d, J = 7.9 Hz, 1H), 4.452 (d, J = 7.9 Hz, 1H), 4.454 (dd, J = 12.1, 2.4 Hz, 2H), 4.458 (d, J = 7.9 Hz, 1H), 4.459 (d, J = 7.9 Hz, 1H), 4.815 (dd, J = 9.4, 7.9 Hz, 1H), 4.841 (dd, J = 9.4, 7.9 Hz, 1H), 4.868 (dd, J = 9.3, 7.9 Hz, 1H), 4.869 (dd, J = 9.2, 8.0 Hz, 1H), 4.962 (dd, J = 10.2, 3.4 Hz, 1H), 5.002 (t, J = 9.0 Hz, 1H), 5.018 (t, J = 9.8 Hz, 1H), 5.023 (t, J = 9.7 Hz, 1H), 5.085 (t, J = 9.2 Hz, 1H), 5.092 (t, J = 9.3 Hz, 2H), 5.099 (t, J = 9.2 Hz, 1H), 5.184 (t, J = 9.2 Hz, 1H), 5.385 (dd, J = 10.3, 9.3 Hz, 1H), 5.621 (d, J = 8.3 Hz, 1H), 6.211 (d, J = 3.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) 20.408, 20.478, 20.558, 20.611, 20.712, 20.732, 20.805, 20.867, 61.095, 61.342, 61.458, 62.097, 67.583, 69.253, 70.369, 70.641, 71.449, 71.585, 71.675, 71.931, 72.173, 72.526, 72.587, 72.648, 72.753, 72.788, 73.439, 75.840, 75.995, 76.083, 91.439, 100.308, 100.710, 100.751, 168.796, 168.824, 169.189, 169.228, 169.268, 169.420, 169.538, 169.695, 169.807, 170.088, 170.137, 170.469, 170.433. Exact mass calculated for $C_{40}H_{54}O_{27}K$ = 1005.249. Found = 1005.249 (HRFABMS, K° source).

(B) Cellotetraose Tetradecaacetate (6). Cellotetraose (**2**) (49.5 mg, 0.0743 mmol, 1 equiv) and DMAP (1 mg, 0.008 mmol, 0.1 equiv) were placed in a flask which was purged with N_2, and pyridine (3.0 mL, 37 mmol, 500 equiv) and acetic anhydride (0.5 mL, 5.3 mmol, 71 equiv) were added via syringe. The solution was stirred for 22 h at room temperature, then transferred to a flask containing 20 mL of ice water slurry and stirred for an additional 90 min. The mixture was extracted with $CHCl_3$ (5 × 10 mL). The organic layers were combined and washed with saturated $CuSO_4$ (4 × 10 mL), saturated $NaHCO_3$ (4 × 10 mL), and brine (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure. The residue was dried to yield 69.0 mg (74%) of an off-white solid which was a mixture of the α and β anomers of **6** by NMR. ^{1}H NMR (500 MHz, $CDCl_3$): 1.938 (s, 3H), 1.959 (s, 3H), 1.966 (s, 3H), 1.973 (s, 3H), 1.986 (s, 3H), 1.996 (s, 3H), 1.999 (s, 3H), 2.005 (s, 3H), 2.014 (s, 3H), 2.066 (s, 3H), 2.071 (s, 3H), 2.100 (s, 3H), 2.125 (s, 6H), 3.542 (ddd, J = 10.1, 5.1, 2.1 Hz; 1H), 3.562 (ddd, J = 10.0, 5.1, 2.1 Hz; 1H), 3.613 (ddd, J = 9.9, 4.4, 2.3 Hz; 1H), 3.716 (ddd, J = 9.9, 4.3, 2.1 Hz; 1H), 3.726 (t, J = 9.4 Hz, 1H), 3.733 (t, J = 9.4 Hz, 1H), 3.777 (dd, J = 9.9, 8.9 Hz; 1H), 4.010 (dd, J = 12.5, 2.3 Hz, 1H), 4.069 (dd, J = 12.2, 5.1 Hz, 1H), 4.073 (dd, J = 12.2, 5.2 Hz, 1H), 4.075 (dd, J =

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12.1, 4.6 Hz, 1H), 4.334 (dd, J = 12.5, 4.4 Hz, 1H), 4.376 (dd, J = 12.1, 2.1 Hz, 1H), 4.383 (dd, J = 12.2, 2.1 Hz, 1H), 4.422 (d, J = 7.79 Hz, 1H), 4.452 (d, J = 7.9 Hz, 2H), 4.457 (dd, J = 12.2, 2.0 Hz, 1H), 4.806 (dd, J = 9.4, 7.8 Hz, 1H), 4.812 (dd, J = 9.3, 7.8 Hz, 1H), 4.881 (dd, J = 9.2, 8.0 Hz, 1H), 5.013 (dd, J = 9.4, 8.5 Hz, 1H), 5.031 (t, J = 9.8 Hz, 1H), 5.078 (t, J = 9.2 Hz, 1H), 5.083 (t, J = 9.3 Hz, 1H), 5.101 (t, J = 9.3 Hz, 1H), 5.191 (dd, J = 9.5, 8.9 Hz, 1H), 5.630 (d, J = 8.3 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) 20.368, 20.412, 20.460, 20.449, 20.552, 20.668, 20.724, 61.342, 61.434, 61.925, 62.015, 67.561, 70.320, 71.409, 71.589, 71.638, 71.883, 72.113, 72.408, 72.498, 72.606, 72.646, 72.741, 73.390, 75.795, 75.943, 76.066, 76.070, 91.403, 100.252, 100.399, 100.684, 168.730, 168.969, 169.080, 169.172, 169.199, 169.346, 169.504, 169.597, 169.612, 170.057, 170.078, 170.126, 170.363. Exact mass calculated for $C_{52}H_{70}O_{35}Na$ = 1277.360. Found = 1277.360 (HRFABMS, Na° source).

(C) Cellopentaose Heptadecaacetate (7). Cellopentaose (**3**) (40.0 mg, 0.0483 mmol, 1 equiv) and DMAP (1 mg, 0.008 mmol, 0.2 equiv) were placed in a flask which was purged with N_2, and pyridine (2.0 mL, 25 mmol, 510 equiv) and acetic anhydride (0.2 mL, 2.1 mmol, 49 equiv) were added via syringe. The solution was stirred for 3 days at room temperature, then transferred to a flask containing 15 mL of ice water slurry and stirred for an additional 90 min. The mixture was extracted with $CHCl_3$ (5 × 10 mL). The organic layers were combined and washed with saturated $CuSO_4$ (4 × 10 mL), saturated $NaHCO_3$ (4 × 10 mL), and brine (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure. The residue was dried to yield 56.0 mg (75%) of an off-white solid which was a mixture of the α and β anomers of **7** by NMR. ^{1}H NMR (500 MHz, $CDCl_3$) 1.944 (s, 3H), 1.946 (s, 3H), 1.969 (s, 3H), 1.974 (s, 3H), 1.982 (s, 3H), 1.995 (s, 3H), 2.004 (s, 3H), 2.008 (s, 6H), 2.014 (s, 3H), 2.023 (s, 3H), 2.075 (s, 3H), 2.081 (s, 3H), 2.109 (s, 3H), 2.130 (s, 3H), 2.132 (s, 6H), 3.523−3.586 (m, 4H), 3.620 (ddd, J = 9.9, 4.5, 2.3 Hz, 1H), 3.701−3.803 (m, 4H), 4.020 (dd, J = 12.5, 2.1 Hz, 1H), 4.044−4.119 (m, 4H), 4.343 (dd, J = 12.6, 4.4 Hz, 1H), 4.367−4.481 (m, 4H), 4.419 (d, J = 7.9 Hz, 1H), 4.429 (d, J = 7.8 Hz, 1H), 4.460 (d, J = 7.9 Hz, 2H), 4.798 (dd, J = 9.3, 7.7 Hz, 1H), 4.814 (dd, J = 9.4, 7.7 Hz, 1H), 4.820 (dd, J = 9.4, 7.9 Hz, 1H), 4.889 (dd, J = 9.3 Hz, 8.0 Hz, 1H), 5.023 (t, J = 8.9 Hz, 1H), 5.040 (t, J = 9.8 Hz, 1H), 5.074 (t, J = 8.9 Hz, 1H), 5.086 (t, J = 9.2 Hz, 1H), 5.089 (t, J = 9.2 Hz, 1H), 5.110 (t, J = 9.3 Hz, 1H), 5.201 (dd, J = 9.5, 8.9 Hz, 1H), 5.639 (d, J = 8.3 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) 20.394, 20.453, 20.489, 20.581, 20.717, 20.767, 20.845, 61.407, 61.506, 61.921, 61.980, 62.095, 67.634, 69.239, 69.285, 70.403, 71.484, 71.653, 71.702, 71.766, 71.807, 71.958, 72.176, 72.412, 72.482, 72.558, 72.692, 72.723, 72.807, 73.474, 75.846, 75.998, 76.033, 76.088, 100.286, 100.404, 100.501, 100.752, 168.818, 169.063, 169.169, 169.255, 169.310, 169.437, 169.595, 169.675, 169.702, 169.724, 170.157, 170.223, 170.461. Exact mass calculated for $C_{64}H_{86}O_{43}K$ = 1581.418. Found = 1581.418 (HRFABMS, K° source).

(D) Cellohexaose Icosaacetate (8). Cellohexaose (**4**) (38.7 mg, 0.039 mmol, 1 equiv) and DMAP (1 mg, 0.008 mmol, 0.2 equiv) were placed in a flask which was purged with N_2, and pyridine (3.0 mL, 37 mmol, 950 equiv) and acetic anhydride (0.2 mL, 2.1 mmol, 54 equiv) were added via syringe. The solution was stirred for 4 days at room temperature, then transferred to a flask containing 15 mL of ice water slurry and stirred for an additional 90 min. The mixture was extracted with $CHCl_3$ (5 × 10 mL). The organic layers were combined and washed with saturated $CuSO_4$ (4 × 10 mL), saturated $NaHCO_3$ (4 × 10 mL), and brine (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure. The residue was dried to yield 52.3 mg (73%) of an off-white solid which was a mixture of the α and β anomers of **8** by NMR: mp 129−136 °C. ^{1}H NMR (500 MHz, $CDCl_3$) 1.937 (s, 3H), 1.938 (s, 6H), 1.963 (s, 3H), 1.968 (s, 3H), 1.976 (s, 3H), 1.989 (s, 3H), 1.997 (s, 3H), 2.001 (s, 9H), 2.006 (s, 3H), 2.016 (s, 3H), 2.068 (s, 3H), 2.074 (s, 3H), 2.102 (s, 3H), 2.121 (s, 3H), 2.124 (s, 9H), 3.518−3.582 (m, 5H), 3.616 (ddd, J = 10.0, 4.3, 2.3 Hz, 1H), 3.688−3.756 (m, 4H), 3.777 (t, J = 9.4 Hz, 1H), 4.015 (dd, J = 12.5, 2.3 Hz, 1H), 4.039−4.095 (m, 5H), 4.335 (dd, J = 12.6, 4.4 Hz, 1H), 4.361−4.477 (m, 5H), 4.414 (d, J = 7.8 Hz, 2H), 4.423 (d, J = 7.8 Hz, 1H), 4.455 (d, J = 7.8 Hz, 2H), 4.789 (dd, J = 9.3, 7.8 Hz, 2H), 4.805 (dd, J = 9.3, 7.8 Hz, 1H), 4.812 (dd, J = 9.3, 7.9 Hz, 1H), 4.881 (dd, J = 9.3, 8.0 Hz, 1H), 5.015 (dd, J = 9.5, 8.4 Hz, 1H), 5.033 (t, J = 9.7 Hz, 1H), 5.066 (t, J = 8.9 Hz, 2H), 5.080 (t, J = 9.2 Hz, 1H), 5.086 (t, J = 9.7 Hz, 1H), 5.104 (t, J = 9.4 Hz, 1H), 5.194 (dd, J = 9.5, 8.9 Hz, 1H), 5.633 (d, J = 8.3 Hz, 1H). ^{13}C NMR (125 MHz. $CDCl_3$) 20.419, 20.469, 20.493, 20.515, 20.597, 20.748, 20.784, 61.457, 61.537, 61.962, 62.010, 62.131, 67.689, 70.448, 71.532, 71.688, 71.786, 71.999, 72.218, 72.464, 72.499, 72.582, 72.770, 72.834, 73.516, 73.570, 73.803, 75.864, 76.045, 76.115, 100.290, 100.420, 100.463, 100.516, 100.766, 168.863, 169.104, 169.227, 169.305, 169.355, 169.484, 169.653, 169.729, 169.769, 170.209, 170.234, 170.500. Exact mass calculated for $C_{76}H_{102}O_{51}Na$ = 1853.529. Found = 1853.528 (HRFABMS, Na° source).

(E) Methyl β-D-Cellotriose Decaacetate (9). Cellotriose undecaacetate (**5**) (97.0 mg, 0.100 mmol, 1 equiv) was dried by azeotropic distillation with anhydrous benzene distilled from sodium (3 × 10 mL). Residual benzene was removed in vacuo. A solution of 30% HBr in HOAc (0.2 mL, 1.0 mmol, 10 equiv) was added to the flask via syringe, and the mixture was stirred under N_2 for 1 h. CH_2Cl_2 (3 mL) was added to the flask, and the organic layer was washed with ice water (2 × 10 mL), ice/saturated $NaHCO_3$ (2 × 10 mL), and ice water (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure. The product was dissolved in $CHCl_3$ and transferred to a flask containing $CaSO_4 \cdot {}^1/_2\ H_2O$ (76.2 mg, 0.525 mmol, 5.25 equiv), HgO (96.9 mg, 0.447 mmol, 4.47 equiv), $HgBr_2$ (1 mg, 0.003 mmol, 0.03 equiv), CH_2Cl_2 (10 mL), and CH_3OH (1.5 mL, 37 mmol, 370 equiv), which had been stirring under N_2 for 90 min. The flask was wrapped in foil and allowed to stir under N_2 for 36 h. The reaction mixture was filtered through a pad of Celite 545. The solvent was removed under reduced pressure, and the resulting solid was redissolved in $CHCl_3$. This was filtered into a separatory funnel and washed with ice water (2 × 10 mL), ice/saturated $NaHCO_3$ (2 × 10 mL), and again with ice water (10 mL). The combined aqueous washes were back-extracted with $CHCl_3$ (10 mL), the organic layers were combined and dried ($MgSO_4$), and the solvent was removed under reduced pressure to yield 72.1 mg (76%) of **9** as an off-white solid: mp 94 (dec) ^{1}H NMR (500 MHz, $CDCl_3$) 1.960 (s, 3H), 1.964 (s, 3H), 1.980 (s, 3H), 1.990 (s, 3H), 2.007 (s, 3H), 2.019 (s, 3H), 2.020 (s, 3H), 2.073 (s, 3H), 2.115 (s, 3H), 2.123 (s, 3H), 3.452 (s, 3H), 3.565 (ddd, J = 9.9, 4.8, 2.1 Hz, 1H), 3.569 (ddd, J = 9.9, 4.9, 1.8 Hz, 1H), 3.619 (ddd, J = 9.9, 4.4, 2.3 Hz, 1H), 3.748 (t, J = 9.6 Hz, 1H), 3.752 (t, J = 9.5 Hz, 1H), 4.018 (dd, J = 12.5, 2.3 Hz, 1H), 4.069 (dd, J = 11.9, 4.7 Hz, 1H), 4.100 (dd, J = 12.1, 5.1 Hz, 1H), 4.338 (dd, J = 12.3, 4.5 Hz, 1H), 4.354 (d, J = 7.9 Hz, 1H), 4.384 (dd, J = 12.1, 2.1 Hz, 1H), 4.457 (d, J = 7.9 Hz, 1H), 4.468 (d, J = 7.9 Hz, 1H), 4.519 (dd, J = 12.0, 2.1 Hz, 1H), 4.835 (dd, J = 9.4, 7.9 Hz, 1H), 4. 862 (dd, J = 9.6, 7.9 Hz, 1H), 4.887 (dd, J = 9.3, 7.9 Hz, 1H), 5.037 (t, J = 9.7 Hz, 1H), 5.103 (t, J = 9.2 Hz, 1H), 5.106 (t, J = 9.3 Hz, 1H), 5.145 (t, J = 9.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) 20.434, 20.493, 20.536, 20.625, 20.672, 20.725, 20.835, 56.967, 61.467, 61.693, 62.107, 67.690, 71.531, 71.549, 71.746, 71.982, 72.426, 72.647, 72.677, 72.860, 76.098, 76.443, 100.538, 100.772, 101.392, 169.074, 169.297, 169.638, 169.774, 170.188, 170.317, 170.502. Exact mass calculated for $C_{39}H_{54}O_{26}K$ = 977.254. Found = 977.255 (HRFABMS, K° source).

(F) Methyl β-D-Cellotetraose Tridecaacetate (10). Cellotetraose tetradecaacetate (**6**) (68.0 mg, 0.0542 mmol, 1 equiv) was dried by azeotropic distillation with anhydrous benzene distilled from sodium (3 × 10 mL). Residual benzene was removed in vacuo. A solution of 30% HBr in HOAc (0.2 mL, 1.0 mmol, 19 equiv) was added to the flask via syringe, and the mixture was stirred under N_2 for 30 min. CH_2Cl_2 (3 mL) was added to the flask, and the organic layer was washed with ice water (2 × 10 mL), ice/saturated $NaHCO_3$ (2 × 10 mL), and ice water (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure. The product was dissolved in $CHCl_3$ and transferred to a flask containing $CaSO_4 \cdot {}^1/_2\ H_2O$ (66.8 mg, 0.460 mmol, 8.49 equiv), HgO (32.1 mg, 0.148 mmol, 2.73 equiv), $HgBr_2$ (1 mg, 0.003 mmol, 0.05 equiv), CH_2Cl_2 (10 mL), and CH_3OH (1.5 mL, 37 mmol, 680 equiv), which had been stirring under N_2 for 90 min. The flask was wrapped in foil and allowed to stir under N_2 for 36 h. The reaction mixture was filtered through a pad of Celite 545. The solvent was removed under reduced pressure, and the resulting solid was redissolved in $CHCl_3$. This was filtered into a separatory

funnel and washed with ice water (2 × 10 mL), ice/saturated $NaHCO_3$ (2 × 10 mL), and again with ice water (10 mL). The combined aqueous washes were back-extracted with $CHCl_3$ (10 mL), the organic layers were combined and dried ($MgSO_4$), and the solvent was removed under reduced pressure to yield 57.4 mg (86%) of **10** as an off-white solid: mp 106−108 °C. ^{1}H NMR (500 MHz, $CDCl_3$) 1.994 (s, 3H), 2.011 (s, 3H), 2.017 (s, 3H), 2.030 (s, 3H), 2.043 (s, 3H), 2.054 (s, 3H), 2.062 (s, 3H), 2.072 (s, 3H), 2.074 (s, 3H), 2.124 (s, 3H), 2.164 (s, 3H), 2.176 (s, 3H), 2.182 (s, 3H), 3.506 (s, 3H), 3.580−3.635 (m, 3H), 3.668 (ddd, J = 9.9. 4.3, 2.3 Hz, 1H), 3.782 (t, J = 9.5 Hz, 1H), 3.790 (t, J = 9.5 Hz, 2H), 4.065 (dd, J = 12.5, 2.3 Hz, 1H), 4.118 (dd, J = 12.0, 3.4 Hz, 1H), 4.126 (dd, J = 12.1, 2.1 Hz, 1H), 4.135 (dd, J = 12.1, 3.4 Hz, 1H), 4.394 (dd, J = 13.0, 4.3 Hz, 1H), 4.404 (d, J = 7.9 Hz, 1H), 4.423−4.450 (m, 2H), 4.475 (d, J = 7.8 Hz, 1H), 4.504 (d, J = 7.9 Hz, 1H), 4.506 (d, J = 7.9 Hz, 1H), 4.561 (dd, J = 12.1, 2.2 Hz, 1H), 4.865 (dd, J = 9.4, 7.8 Hz, 1H), 4.873 (dd, J = 9.3, 7.9 Hz, 1H), 4.914 (dd, J = 9.6, 7.9 Hz, 1H), 4.939 (dd, J = 9.3, 8.0 Hz, 1H), 5.070 (t, J = 9.6 Hz, 1H), 5.133 (t, J = 9.2 Hz, 1H), 5.139 (t, J = 9.1 Hz, 1H), 5.158 (t, J = 9.3 Hz, 1H), 5.195 (t, J = 9.3 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) 20.349, 20.372, 20.400, 20.424, 20.447, 20.461, 20.541, 20.664, 20.744, 56.878, 61.393, 61.687, 61.984, 62.002, 67.641, 71.469, 71.640, 71.781, 71.926, 72.333, 72.505, 72.543, 72.584, 72.624, 72.684, 72.780, 75.979, 76.076, 76.368, 100.432, 100.705, 101.320, 169.038, 169.197, 169.231, 169.277, 169.557, 169.694, 169.759, 170.121, 170.147, 170.285, 170.425. Exact mass calculated for $C_{51}H_{70}O_{34}K$ = 1265.339. Found = 1265.339 (HRFABMS, K° source).

(G) Methyl β-D-Cellopentaose Hexadecaacetate (11). Cellopentaose heptadecaacetate (**7**) (72.5 mg, 0.0470 mmol, 1 equiv) was dried by azeotropic distillation with anhydrous benzene distilled from sodium (3 × 10 mL). Residual benzene was removed in vacuo. A solution of 30% HBr in HOAc (0.2 mL, 0.545 mmol, 11.6 equiv) was added to the flask via syringe, and the mixture was stirred under N_2 for 20 min. CH_2Cl_2 (3 mL) was added to the flask, and the organic layer was washed with ice water (2 × 10 mL), ice/saturated $NaHCO_3$ (2 × 10 mL), and ice water (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure. The product was dissolved in $CHCl_3$ and transferred to a flask containing $CaSO_4 \cdot {}^1/_2\ H_2O$ (50.4 mg, 0.347 mmol, 7.4 equiv), HgO (51.1 mg, 0.236 mmol, 5.0 equiv), $HgBr_2$ (1 mg, 0.003 mmol, 0.06 equiv), CH_2Cl_2 (10 mL), and CH_3OH (1.5 mL, 37 mmol, 790 equiv), which had been stirring under N_2 for 90 min. The flask was wrapped in foil and allowed to stir under N_2 for 36 h. The reaction mixture was filtered through a pad of Celite 545. The solvent was removed under reduced pressure, and the resulting solid was redissolved in $CHCl_3$. This was filtered into a separatory funnel and washed with ice water (2 × 10 mL), ice/saturated $NaHCO_3$ (2 × 10 mL), and again with ice water (10 mL). The combined aqueous washes were back-extracted with $CHCl_3$ (10 mL), the organic layers were combined and dried ($MgSO_4$), and the solvent was removed under reduced pressure to yield 39.1 mg (55%) of **11** as an off-white solid: mp 106−108 °C. ^{1}H NMR (750 MHz, $CDCl_3$) 1.937 (s, 3H), 1.938 (s, 3H), 1.955 (s, 3H), 1.963 (s, 3H), 1.976 (s, 3H), 1.990 (s, 3H), 2.000 (s, 3H), 2.001 (s, 3H), 2.008 (s, 3H), 2.017 (s, 3H), 2.019 (s, 3H), 2.069 (s, 3H), 2.109 (s, 3H), 2.117 (s, 3H), 2.126 (s, 3H), 2.127 (s, 3H), 3.452 (s, 3H), 3.546 (ddd, J = 9.9, 4.9, 2.0 Hz, 1H), 3.553 (ddd, J = 10.0, 5.1, 2.0 Hz, 1H), 3.567 (ddd, J = 10.1, 5.2, 2.0 Hz, 1H), 3.573 (ddd, J = 10.1, 5.0, 2.0 Hz, 1H), 3.614 (ddd, J = 9.9, 4.3, 2.3 Hz, 1H), 3.714 (t, J = 9.9 Hz, 1H), 3.720 (t, J = 9.4 Hz, 1H), 3.736 (t, J = 9.5 Hz, 2H), 4.011 (dd, J = 12.5, 2.3 Hz, 1H), 4.054 (dd, J = 11.9, 5.0 Hz, 1H), 4.064 (dd, J = 11.9, 4.9 Hz, 1H), 4.070 (dd, J = 11.9, 3.2 Hz, 1H), 4.080 (dd, J = 12.1, 3.2 Hz, 1H), 4.340 (dd, J = 12.5, 4.3 Hz, 1H), 4.350 (d, J = 7.9 Hz, 1H), 4.385 (dd. J = 11.9, 1.8 Hz, 1H), 4.392 (dd, J = 12.2, 1.9 Hz, 2H), 4.411 (d, J = 7.8 Hz, 1H), 4.421 (d, J = 7.8 Hz, 1H), 4.451 (d, J = 7.8 Hz, 1H), 4.453 (d, J = 7.9 Hz, 1H), 4.507 (dd, J = 12.0, 2.1 Hz, 1H), 4.793 (dd, J = 9.3, 7.5 Hz, 1H), 4.808 (dd, J = 9.3, 7.8 Hz, 1H), 4.817 (dd, J = 9.4, 7.9 Hz, 1H), 4.859 (dd, J = 9.6, 7.9 Hz, 1H), 4.884 (dd, J = 9.3, 7.9 Hz, 1H), 5.035 (t, J = 9.7 Hz, 1H), 5.064 (t, J = 9.3 Hz, 1H), 5.079 (t, J = 9.2 Hz, 1H), 5.082 (t, J = 9.2 Hz, 1H), 5.104 (t, J = 9.0 Hz, 1H), 5.139 (t, J = 9.1 Hz, 1H).^{13}C NMR (125 MHz, $CDCl_3$) 20.642, 20.733, 20.703, 20.752, 20.838, 20.887, 20.961, 20.982, 21.038, 57.189, 61.639, 61.925, 62.189, 62.229, 62.264, 67.861, 71.712, 71.885, 71.976, 72.010, 72.194, 72.586, 72.727, 72.798, 72.835, 72.882, 72.946, 73.047, 76.244, 76.294, 76.332, 76.657, 100.692, 100.701, 100.758, 100.997, 101.591, 169.314, 169.455, 169.507, 169.563, 169.850, 169.922, 169.952, 169.986, 170.009, 170.415, 170.554, 170.709. Exact mass calculated for $C_{63}H_{86}O_{42}Na$ = 1537.449. Found = 1537.449 (HRFABMS, Na° source).

(H) Methyl β-D-Cellohexaose Nonadecaacetate (12). Cellohexaose icosaacetate (**8**) (52.3 mg, 0.0286 mmol, 1 equiv) was dried by azeotropic distillation with anhydrous benzene distilled from sodium (3 × 10 mL). Residual benzene was removed in vacuo. A solution of 30% HBr in HOAc (0.2 mL, 0.55 mmol, 19 equiv) was added to the flask via syringe, and the mixture was stirred under N_2 for 10 min. CH_2Cl_2 (3 mL) was added to the flask, and the organic layer was washed with ice water (2 × 10 mL), ice/saturated $NaHCO_3$ (2 × 10 mL), and ice water (10 mL). The organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure. The product was dissolved in $CHCl_3$ and transferred to a flask containing $CaSO_4 \cdot {}^1/_2\ H_2O$ (74.6 mg, 0.514 mmol, 18.0 equiv), HgO (81.6 mg, 0.377 mmol, 13.2 equiv), $HgBr_2$ (1 mg, 0.003 mmol, 0.10 equiv), CH_2Cl_2 (10 mL), and CH_3OH (1.5 mL, 37.03 mmol, 1295 equiv), which had been stirring under N_2 for 90 min. The flask was wrapped in foil and allowed to stir under N_2 for 36 h. The reaction mixture was filtered through a pad of Celite 545. The solvent was removed under reduced pressure, and the resulting solid was redissolved in $CHCl_3$. This was filtered into a separatory funnel and washed with ice water (2 × 10 mL), ice/saturated $NaHCO_3$ (2 × 10 mL), and again with ice water (10 mL). The combined aqueous washes were back-extracted with $CHCl_3$ (10 mL), the organic layers were combined and dried ($MgSO_4$), and the solvent was removed under reduced pressure to yield 21.7 mg (42%) of **12** as a pale yellow solid: mp 114−116 °C. ^{1}H NMR (750 MHz, $CDCl_3$) 1.944 (s, 6H), 1.945 (s, 3H), 1.963 (s, 3H), 1.968 (s, 3H), 1.983 (s, 3H), 1.995 (s, 3H), 2.004 (s, 3H), 2.006 (s, 6H), 2.012 (s, 3H), 2.021 (s, 3H), 2.023 (s, 3H), 2.074 (s, 3H), 2.112 (s, 3H), 2.120 (s, 3H), 2.126 (s, 3H), 2.128 (s, 3H), 2.129 (s, 3H), 3.457 (s, 3H), 3.531 (ddd, J = 9.8, 4.9, 2.0 Hz, 1H), 3.551 (ddd, J = 9.4, 5.1, 2.3 Hz, 2H), 3.564 (ddd, J = 10.4, 5.2, 2.0 Hz, 1H), 3.571 (ddd, J = 10.1, 5.1, 2.0 Hz, 1H), 3.622 (ddd, J = 9.9, 4.4, 2.3 Hz, 1H), 3.712 (t, J = 9.4 Hz, 1H), 3.720 (t, J = 9.5 Hz, 1H), 3.723 (t, J = 9.3 Hz, 1H), 3.741 (t, J = 9.5 Hz, 2H), 4.024 (dd, J = 12.5, 2.3 Hz, 1H), 4.065 (dd, J = 12.4, 4.1 Hz, 2H), 4.076 (dd, J = 12.2, 4.8 Hz, 1H), 4.082 (dd, J = 12.2, 5.1 Hz, 1H), 4.085 (dd, J = 12.1, 4.6 Hz, 1H), 4.337 (dd, J = 12.5, 4.4 Hz, 1H), 4.357 (d, J = 7.9 Hz, 1H), 4.380 (dd, J = 12.6, 2.7 Hz, 2H), 4.384 (dd, J = 12.5, 3.0 Hz, 1H), 4.388 (dd, J = 12.1, 2.5 Hz, 1H), 4.422 (d, J = 7.8 Hz, 1H), 4.433 (d, J = 7.8 Hz, 1H), 4.461 (d, J = 7.8 Hz, 1H), 4.464 (d, J = 7.9 Hz, 2H), 4.510 (dd, J = 12.0, 2.2 Hz, 1H), 4.793 (dd, J = 9.3, 8.3 Hz, 1H), 4.794 (dd, J = 9.3, 7.8 Hz, 1H), 4.810 (dd, J = 9.3, 7.8 Hz, 1H), 4.819 (dd, J = 9.4, 7.9 Hz, 1H), 4.863 (dd, J = 9.6, 7.9 Hz, 1H), 4.887 (dd, J = 9.3, 8.0 Hz, 1H), 5.038 (t, J = 9.7 Hz, 1H), 5.071 (t, J = 9.2 Hz, 1H), 5.074 (t, J = 9.2 Hz, 1H), 5.087 (t, J = 9.1 Hz, 1H), 5.089 (t, J = 8.6 Hz, 1H), 5.111 (t, J = 9.3 Hz, 1H), 5.145 (t, J = 9.4 Hz, 1H).^{13}C NMR (188 MHz, $CDCl_3$) 20.657, 20.731, 20.760, 20.863, 20.919, 20.988, 21.101, 21.076, 22.879, 57.210, 61.635, 61.915, 62.170, 62.200, 62.221, 62.273, 67.836, 71.707, 71.869, 71.970, 72.003, 72.181, 72.570, 72.675, 72.715, 72.792, 72.831, 72.873, 72.932, 73.042, 76.236, 76.267, 76.323, 76.658, 100.675, 100.699, 100.744, 100.993, 101.582, 169.282, 169.431, 169.461, 169.484, 169.533, 169.824, 169.897, 169.929, 169.952, 169.985, 170.386, 170.533, 170.679. Exact mass calculated for $C_{76}H_{102}O_{49}Na$ = 1825.534. Found = 1825.535 (HRFABMS, Na° source).

(I) Methyl β-D-Cellotriose (13). Methyl-β-D-cellotriose decaacetate (**9**) (26.8 mg, 0.0285 mmol) was placed in a 10 mL flask. A solution of NaOMe in MeOH (1.5 mL) was made and added to the flask. The reaction was allowed to stir for 16 h. Small portions of cation-exchange resin (Dowex 50W-X8) were added until the solution was neutralized. The reaction was then filtered to remove the resin, and the methanol was removed under reduced pressure, resulting in 12.5 mg (84%) of **13** as a white solid. ^{1}H NMR (750 MHz, D_2O, benzene reference) 3.305 (dd, J = 9.5, 7.6 Hz, 1H), 3.315 (dd, J = 9.5, 7.9 Hz, 1H), 3.361 (dd, J = 9.3, 8.0 Hz, 1H), 3.418 (dd, J = 9.9, 9.1 Hz, 1H), 3.487 (ddd, J = 9.8, 5.9, 2.3 Hz, 1H), 3.507 (t, J = 9.3 Hz, 1H), 3.575 (s, 3H),

3.597 (ddd, J = 10.0, 5.1, 2.2 Hz, 1H), 3.618 (ddd, J = 9.7, 5.2, 2.2 Hz, 1H), 3.634−3.663 (m, 3H), 3.677 (t, J = 8.8 Hz, 1H), 3.736 (dd, J = 12.6, 6.0 Hz, 1H), 3.818 (dd, J = 12.6, 5.4 Hz, 1H), 3.826 (dd, J = 12.6, 5.4 Hz, 1H), 3.917 (dd, J = 12.4, 2.3 Hz, 1H), 3.980 (dd, J = 12.3, 2.3 Hz, 1H), 3.993 (dd, J = 12.3, 2.2 Hz, 1H), 4.403 (d, J = 8.0 Hz, 1H), 4.509 (d, J = 7.9 Hz, 1H), 4.533 (d, J = 8.0 Hz, 1H). ^{13}C NMR (125 MHz, D_2O, benzene reference) 57.109, 59.721, 59.806, 60.420, 69.303, 72.754, 72.809, 73.007, 73.908, 74.434, 74.623, 74.670, 75.328, 75.847, 78.202, 78.342, 102.221, 102.429, 102.947. Exact mass calculated for $C_{19}H_{34}O_{16}Na$ = 541.175. Found = 541.174 (HRFABMS, Na° source).

(J) Methyl β-D-Cellotetraose (14). A small piece of Na° was dissolved in EtOH (9 mL). CH_2Cl_2 (1 mL) was added to the solution, then guanidine·HCl (66.5 mg, 0.969 mmol). The resulting suspension was subjected to a short anion-exchange column (Dowex 2-X8) and dripped into a flask containing methyl cellotetraose tridecaacetate (**10**) (53.0 mg, 0.0432 mmol, 1 equiv) dissolved in a minimum amount of CH_2Cl_2. After the guanidine solution was added, a precipitate formed. The resulting suspension was stirred overnight, filtered, and dried. The residue was triturated in 9:1 CH_2Cl_2/MeOH, filtered, dried, and weighed to yield 21.5 mg (73%) of **14** as an off-white solid. ^{1}H NMR (750 MHz, D_2O, benzene reference) 3.304 (t, J = 8.0 Hz, 1H), 3.316 (t, J = 8.7 Hz, 1H), 3.361 (t, J = 8.6 Hz, 2H), 3.417 (t, J = 9.5 Hz, 1H), 3.488 (ddd, J = 9.3, 5.9, 2.2 Hz, 1H), 3.509 (t, J = 10.0 Hz, 1H), 3.574 (s, 3H), 3.588−3.680 (m, 9H), 3.736 (dd, J = 12.4, 5.9 Hz, 1H), 3.818 (dd, J = 12.0, 6.4 Hz, 1H), 3.827 (dd, J = 12.2, 5.5 Hz, 2H), 3.915 (dd, J = 12.4, 2.2 Hz, 1H), 3.977 (dd, J = 12.3, 1.9 Hz, 1H), 3.980 (dd, J = 12.3, 1.8 Hz, 1H), 3.991 (dd, J = 12.2, 2.0 Hz, 1H), 4.404 (d, J = 8.0, 1H), 4.509 (d, J = 7.8 Hz, 1H), 4.532 (d, J = 8.1 Hz, 2H). ^{13}C NMR (125 MHz, D_2O, benzene reference) 57.066, 59.690, 59.723, 59.807, 60.402, 69.290, 72.722, 72.769, 72.792, 72.989, 73.847, 73.866, 73.885, 74.280, 74.113, 74.609, 74.658, 75.308, 75.823, 78.090, 78.208, 78.350, 102.180, 102.207, 102.407, 102.924. Exact mass calculated for $C_{25}H_{44}O_{21}Na$ = 703.227. Found = 703.227 (HRFABMS, Na° source).

(K) Methyl β-D-Cellopentaose (15). A small piece of Na° was dissolved in EtOH (9 mL). CH_2Cl_2 (1 mL) was added to the solution, then guanidine·HCl (53.6 mg, 0.561 mmol). The resulting suspension was subjected to a short anion-exchange column (Dowex 2-X8) and dripped into a flask containing methyl cellopentaose hexadecaacetate (**11**) (37.4 mg, 0.0247 mmol, 1 equiv) dissolved in a minimum amount of CH_2Cl_2. After the guanidine solution was added, a precipitate formed. The resulting suspension was stirred overnight, filtered, and dried. The residue was triturated in 9:1 CH_2Cl_2/MeOH, filtered, dried, and weighed to yield 16.2 mg (77%) of **15** as an off-white solid. ^{1}H NMR (750 MHz, D_2O, benzene reference) 3.304 (dd, J = 9.5, 8.3 Hz, 1H), 3.315 (dd, J = 9.5, 8.0 Hz, 1H), 3.360 (t, J = 8.6 Hz, 3H), 3.416 (t, J = 9.5 Hz, 1H), 3.486 (ddd, J = 9.7, 5.7, 2.5 Hz, 1H), 3.507 (t, J = 9.3 Hz, 1H), 3.574 (s, 3H), 3.59−3.69 (m, 12H), 3.737 (dd, J = 12.4, 5.8 Hz, 1H), 3.820 (dd, J = 12.2, 6.5 Hz, 1H), 3.829 (dd, J = 12.1, 5.4 Hz, 3H), 3.916 (dd, J = 12.4, 2.3 Hz, 1H), 3.977 (br d, J = 12.0 Hz, 3H), 3.991 (dd, J = 12.3, 2.2 Hz, 1H), 4.403 (d, J = 8.0 Hz, 1H), 4.510 (d, J = 8.0 Hz, 1H), 4.533 (d, J = 8.0 Hz, 3H). ^{13}C NMR (188 MHz, D_2O, benzene reference) 57.066, 59.669, 59.749, 60.354, 69.227, 72.696, 72.749, 72.972, 73.795, 74.057, 74.592, 74.644, 75.266, 75.829, 78.041, 78.162, 78.283, 102.128, 102.348, 102.874. Exact mass calculated for $C_{31}H_{54}O_{26}Na$ = 865.280. Found = 865.280 (HRFABMS, Na° source).

(L) Methyl β-D-Cellohexaose (16). A small piece of Na° was dissolved in EtOH (9 mL). CH_2Cl_2 (1 mL) was added to the solution, then guanidine·HCl (53.6 mg, 0.561 mmol). The resulting suspension was subjected to a short anion-exchange column (Dowex 2-X8) and dripped into a flask containing methyl cellohexaose nonadecaacetate (**12**) (22.7 mg, 0.0126 mmol, 1 equiv) dissolved in a minimum amount of CH_2Cl_2. After the guanidine solution was added, a precipitate formed. The resulting suspension was stirred overnight, filtered, and dried. The residue was triturated in 9:1 CH_2Cl_2/MeOH, filtered, dried, and weighed to yield 9.3 mg (73%) of **16** as an off-white solid. ^{1}H NMR (750 MHz, D_2O, benzene reference) 3.304 (dd, J = 9.53, 8.29 Hz, 1H), 3.313 (dd, J = 9.4, 8.0 Hz, 1H), 3.358 (br t, J = 8.5 Hz, 4H), 3.486 (ddd, J = 9.6, 5.7, 2.4 Hz, 1H), 3.506 (t, J = 9.3 Hz, 1H), 3.574 (s, 3H), 3.59−3.69 (m, 15H), 3.736 (dd, J = 12.5, 5.9 Hz, 1H), 3.819 (dd, J = 12.3, 5.1 Hz, 1H), 3.828 (dd, J = 12.0, 5.0 Hz, 4H), 3.915 (dd, J = 12.6, 2.1 Hz, 1H), 3.976 (br d, J = 11.5 Hz, 4H), 3.991 (dd J = 11.9, 2.0, 1H), 4.405 (d, J = 8.1 Hz, 1H), 4.509 (d, J = 7.8 Hz, 1H), 4.533 (d, J = 8.0 Hz, 4H). ^{13}C NMR (188 MHz, D_2O, benzene reference) 57.066, 59.652, 59.701, 60.376, 69.269, 72.710, 72.750, 72.972, 73.815, 74.101, 74.601, 74.650, 75.290, 75.810, 78.048, 78.181, 78.323, 102.179, 102.389, 102.907. Exact mass calculated for $C_{37}H_{64}O_{31}Na$ = 1027.333. Found = 1027.333 (HRFABMS, Na° source).

Acknowledgment. This work has been supported by the National Institute of Health, the Petroleum Research Fund, and the American Heart Association. J.T.B. thanks the Colgate-Palmolive Corporation for an undergraduate fellowship. Mark Morris and Michael Williams are thanked for assistance in isolation of the cellulose oligomers. NMR spectra were obtained in the Varian Oxford Instrument Center for Excellence in NMR Laboratory. Funding for this instrumentation was provided in part from the W. M. Keck Foundation, the National Institutes of Health (PHS 1 S10 RR10444−01), and the National Science Foundation (NSF CHE 96−10502). The assistance of Vera Mainz and Feng Lin of the VOICE NMR Spectroscopy Lab at the University of Illinois is recognized. The members of the Petillo group are acknowledged for numerous helpful discussions.

Supporting Information Available: Tables of the proton and carbon assignments for **9**−**12**, HMQC−COSY and HMQC−TOCSY data for β-methyl cellotetraose, β-methyl cellopentaose, and β-methyl cellohexaose, and plots of the ^{13}C chemical shifts vs ring size for the reducing end ring, middle rings, and nonreducing end ring. This material is available free of charge via the Internet at http://pubs.acs.org.

JA990561U