Combined Chemical and Enzymatic Synthesis of a Pentasaccharide Repeating Unit of the Capsular Polysaccharide of Type III Group B *Streptococcus* and One- and Two-Dimensional NMR Spectroscopic Studies†,‡

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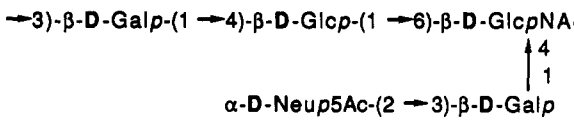
A combination of chemical and enzymatic methods is described for the synthesis of the branching pentasaccharide 3-OMe-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 6)-[α-D-Neu*p*5Ac-(2 → 3)-β-D-Gal*p*-(1 → 4)]-β-D-Glc*p*NAc-OMe (**4**). Pentasaccharide **4**, represents a complete repeating unit of the capsular polysaccharide of type III group B *Streptococci*, which will be used as a molecular probe in the study of the fine specificities of carbohydrate–protein interactions. Key intermediate was the alcohol methyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (**9**), which was β-D-galactosylated and chemoselectively deprotected to provide the β-D-Gal*p*-β-D-Glc*p*NAc acceptor block **13**. Two approaches are described for the synthesis of the β-D-Gal*p*-(1 → 4)-β-D-Glc*p* donor block **17**, which was coupled to acceptor **13** under the agency of trimethylsilyl trifluoromethanesulfonate. Removal of the blocking groups from the fully protected tetrasaccharide **24** by transesterification and hydrogenolysis provided tetrasaccharide **5**, which was sialylated enzymatically using a specific rat liver sialyltransferase to provide pentasaccharide **4**. Complete assignment of the ^1H and ^{13}C NMR spectra of the tetra-**5** and pentasaccharide **4** is presented, and their carbon spin–lattice relaxation times (T_1) are also reported.

Group B *Streptococcus* (GBS) is a major cause of neonatal sepsis and meningitis.[1,2] Although group B *Streptococci* share a highly complex common polysaccharide antigen,[3] they can be differentiated by serotype based on structurally distinct capsular polysaccharides.[2] The structures of these specific polysaccharides have been elucidated,[2,4] and while all serotypes have been implicated in invasive disease, type III capsular polysaccharide, which is composed of the pentasaccharide repeating unit **1**,[5] is responsible for more than 60% of all group B streptococcal infections.[1] The importance of capsular polysaccharide-



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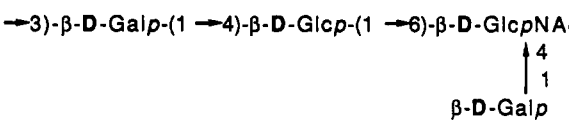
specific antibodies in immunity to type III group B streptococcal[6] infection has been established, and the type III polysaccharide has been used as an experimental vaccine in humans.[7]

It has been previously demonstrated[5] that neither the asialo polysaccharide **2** nor the repeating unit pentasaccharide **3** obtained by enzymatic depolymerization of



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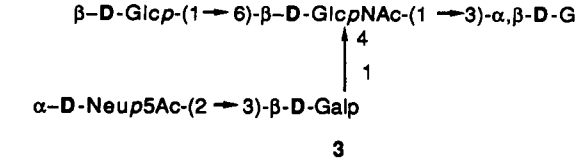

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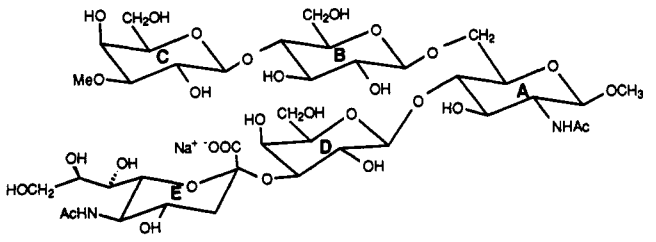
the native type III (GBS) polysaccharide with *endo*-β-D-galactosidase[5] inhibits the binding of the native type III polysaccharide to its homologous antibody, while binding was inhibited by a decasaccharide composed of two contiguous units of **3**.[5] This lack of binding by **3** can be attributed to either the failure of **3** to adopt the same conformation as it does in the decasaccharide or polysaccharide[8] or the fact that **3** lacks a critical structural feature present in the decasaccharide. To study the feasibility of this latter hypothesis, this laboratory has undertaken the synthesis of a comprehensive range of oligosaccharides related to the type III GBS polysaccharide, in order to provide further molecular probes for mapping the antibody binding site. This paper describes the synthesis of pentasaccharide glycoside **4**, which represents



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another of the three possible repeating units of the type III polysaccharide. Pentasaccharide **4** contains the β-D-Gal*p*(1 → 4)-β-D-Glc*p* bond that is still present in the

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‡ Synthetic Oligosaccharides Related to Group B Streptococcal Polysaccharides. 5. Part 4: see ref 9.

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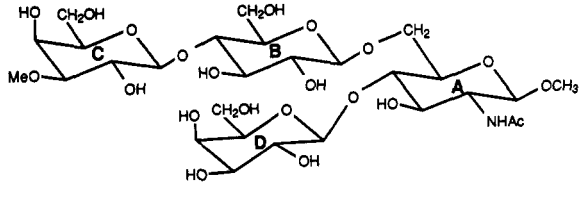
decasaccharide but that is broken during the formation of 3 by the enzymatic depolymerization of the type III GBS polysaccharide.[5] The synthesis of the asialotetrasaccharide fragment of 4 has been previously reported.[9]

Results and Discussion

Crucial to the synthetic strategy was the incorporation of the *N*-acetylneuraminic acid (Neu5Ac) portion in the target compound. Chemical syntheses of oligosaccharides containing Neu5Ac usually proceed with modest yields at best, and the construction of α-D-Neu5Ac-(2 → 3)-D-Gal interglycosidic linkages is especially low-yielding[10] due to several inherent structural factors. These include (i) the lack of an "assisting" group adjacent to the anomeric carbon atom, (ii) the tendency of Neu5Ac-derived glycosyl donors to form glycals, and (iii) the structurally unfavorable axial orientation of the carboxyl group in the final product. Although new approaches recently proposed by Hasegawa[11] and Ogawa[12] appear to alleviate this problem, e.g., by introducing[12] "directing" groups into Neu5Ac prior to glycosylation, the overall yields are still not within the range of glycosylations involving the usual sugars. As an alternative to chemical synthesis, enzymatic coupling of Neu5Ac to oligosaccharides has been proposed[13-15] using catalysis by highly specific mammalian sialyltransferases. Starting from corresponding asialo oligosaccharide precursors and employing cytidine 5′-(5-acetamido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid monophosphate) (CMP-Neu5Ac) as the source of Neu5Ac and GalB1,3(4)GlcNAcα2,3-sialyltransferase isolated from rat liver as the enzyme, Sabesan and Paulson[13] synthesized in excellent yields several oligosaccharides having terminal α-D-Neu5Ac-(2 → 3)-β-D-Gal*p* sequences. Although the fine substrate specificity of this enzyme remains to be established, it appears reasonable to assume that branching tetrasaccharide 5 would be recognized by the enzyme sialyltransferase, therefore the overall strategy for the preparation of pentasaccharide 4 is based on chemical synthesis of tetrasaccharide 5 followed by enzymatic sialylation at HO-3 of its galactose residue D.



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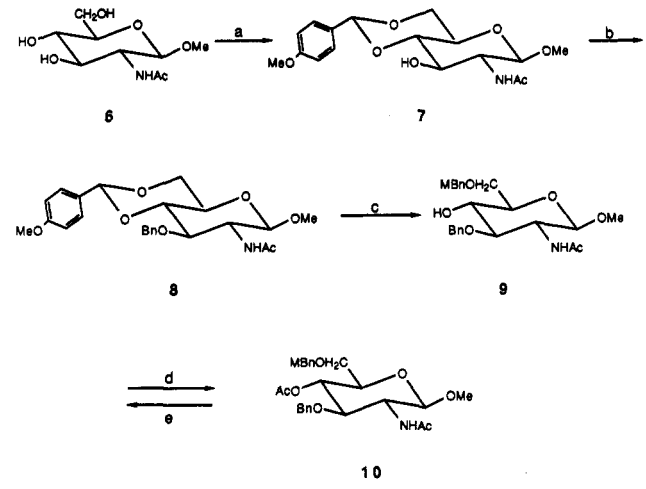
Our approach to the branched tetrasaccharide 5 was guided by the pattern recently established[9] for the synthesis of related structures in this laboratory. In this synthesis, a partially protected D-glucosamine glycoside having a selectively removable, temporary protecting group at HO-6 is first galactosylated at HO-4. Then, following the removal of the protecting group at HO-6, the newly formed nucleophilic center is glycosylated by use of a lactose synthon.

Scheme I[a]



[a] Key: (a) 4-$CH_3OC_6H_4CH(OCH_3)_2$, PTS, DMF, 12 h, 25 °C; (b) $C_6H_5CH_2Br$, BaO, $Ba(OH)_2 \cdot 8H_2O$, DMF, 24 h, 25 °C; (c) $NaCNBH_3$, TFA, DMF, 24 h, 25 °C; (d) Ac_2O, C_5H_5N, 12 h, 25 °C; (e) NaOMe, MeOH, 2 h, 25 °C.

Synthesis of the A–D Disaccharide. As the acceptor in the first glycosylation step was selected key compound 9, which was obtained from methyl glycoside 6[16] as shown by Scheme I. Thus, triol 6[16] was treated with 4-methoxybenzaldehyde dimethyl acetal[9,17] in dry *N*,*N*-dimethylformamide (DMF) under catalysis by 4-toluenesulfonic acid (PTS) to give acetal 7 in 92% yield. Subsequent benzylation[18] under conditions[19] that avoid N-deacetylation and N-benzylation with benzyl bromide in the presence of barium oxide and barium hydroxide octahydrate in DMF gave compound 8 in 85% yield. A noteworthy feature of the ^1H NMR spectrum of 8 is the appearance of the H-1 signal as a multiplet pattern instead of the expected doublet. A related phenomenon has been interpreted[20] in the disaccharide methyl *N*,*N*′-diacetyl-β-chitobioside as arising from virtual couplign when H-2 and H-3 are strongly coupled. Treatment[21] of benzylidene acetal 8 with $NaCNBH_3$ and CF_3COOH gave the 6-*O*-(4-methoxybenzyl) derivative 9, which was purified as its acetate 10, in 73% yield. The location of the 4-methoxybenzyl group in 8 was verified by ^{13}C NMR spectroscopy. The primary C-6 carbon atom of the hexopyranose ring in 8 appears at δ 70.3, which is in agreement with the HO-6 hydroxyl group having a benzylic substituent.[9] Furthermore, in the ^1H NMR spectrum of 10 the H-4 proton appears at δ 5.00, indicating that acetylation occurred at HO-4. Treatment of alcohol 9 with acetobromogalactose (11) under Helferich conditions[22] ($Hg(CN)_2$, toluene) gave disaccharide 12 in 70% yield, together with the 4-*O*-acetate 10 (20%), which is formed by way of intermolecular *O*-acetyl group transfer. The 1,2-trans stereochemistry of the interglycosidic linkage in 12 was verified by NMR spectroscopy. The value of the three-bond, $^3J_{H\text{-}1,H\text{-}2}$ coupling constant of the galactosyl moiety is 7.7 Hz, whilst that of the one-bond $^1J_{C\text{-}1,H\text{-}1}$ coupling constant for that residue is 163 Hz, in agreement with the proposed structure.

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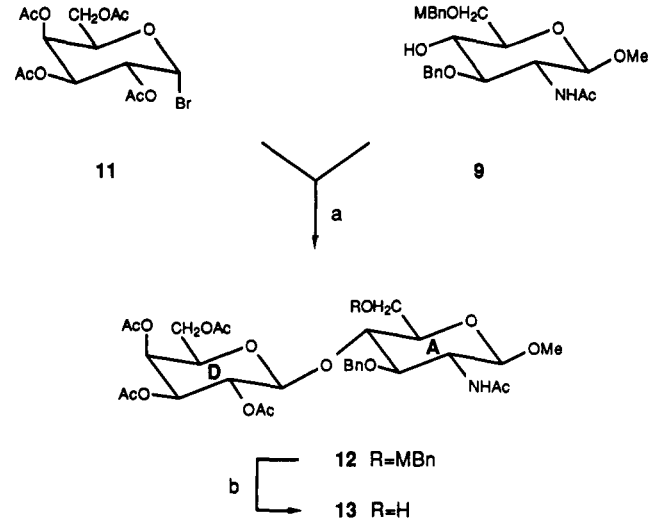
Scheme II[a]



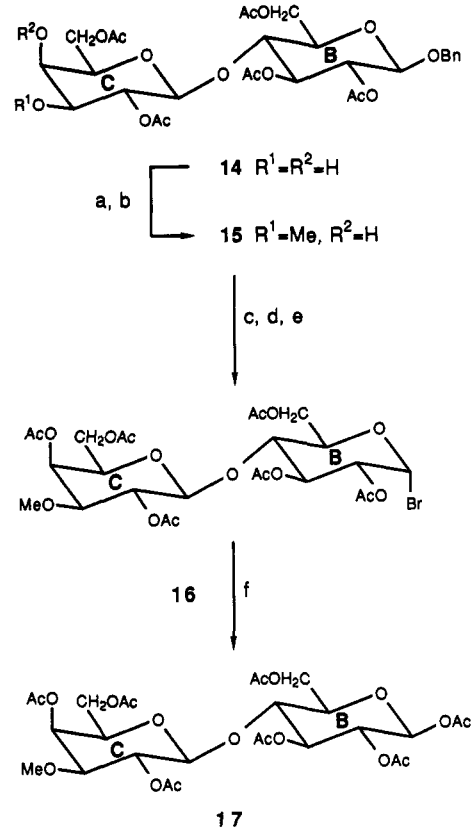
[a] Key: (a) $Hg(CN)_2$, $CH_6C_6H_5$, CH_3NO_2, 12 h, 25 °C; (b) DDQ, $CHCl_3$, H_2O, 3 h, 25 °C.

Oxidative removal[23] of the 4-methoxybenzyl protecting group using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone gave alcohol **13** in 67% yield (Scheme II).

Synthesis of the Lactosyl Donor. Earlier experimentation[9] in this laboratory established that attempted glycosylation of a disaccharide similar to **13** at HO-6 under Koenigs–Knorr conditions or modified versions[2,4] leads preferentially to an orthoester involving the glycosyl donor and the primary HO-6 group of the acceptor, whereas the use of 1,2-trans glycosyl acetates under Lewis acid catalysis affords the expected 1,2-trans interglycosidic linkage. Accumulated evidence[25] strongly indicates that glycosyl acetates must possess a 1,2-trans configuration to function as competent glycosyl donors under Lewis acid catalysis. Thus, heptaacetate **17** was selected as the lactose synthon, an integral part of which is a permanent methyl group at HO-3 of the galactose moiety, which was placed in **17** with the intent to prevent enzymatic sialylation at this site in compound **5**. Although cognizant of the potential influence of the methyl group on the shape of pentasaccharide **4** and therefore on the recognition process itself, this influence was thought to be minimal, especially since HO-3 of the main-chain galactose unit is not free in the native polysaccharide either.[5] Nevertheless, the need for selective protection clearly arises when attempting enzymatic glycosylation at only one of two or more identical glycoses.

Two approaches to compound **17** were devised. In the first approach, outlined in Scheme III, benzyl lactoside **14**[26] was used as the starting compound, which on treatment[27,28] with Bu_2SnO–MeI–Bu_4NI provided the regioselectively methylated disaccharide **15**. The site of methylation was verified by ^{13}C NMR spectroscopy; the signal of the C-3 carbon atom of the galactose moiety at δ 80.7 proved that methylation occurred at HO-3 of this unit, in agreement

Scheme III[a]



[a] Key: (a) Bu_2SnO, C_6H_6, 6 h, 80 °C; (b) MeI, Bu_4NI, C_6H_6, 24 h, 40 °C; (c) H_2, Pd/C, EtOH, AcOH, 24 h, 25 °C; (d) Ac_2O, NaOAc, 10 min, 100 °C; (e) HBr, AcOH, Ac_2O, 2 h, 0 °C; (f) $Hg(OAc)_2$, AcOH, 2 h, 25 °C.

with the empirical rule established for such processes.[27] Attempted one-step conversion of compound **15** to heptaacetate **17** by acetolysis (Ac_2O–H_2SO_4) was of limited value; NMR spectroscopy indicated the formation of a product mixture with ca. only 10% of compound **17**, the rest being mainly the corresponding 1,2-cis acetate. As an alternative route, benzyl lactoside **15** was converted to unstable bromide **16** by treatment with H_2–Pd/C, followed by Ac_2O–H_2SO_4, and then HBr–AcOH. Reaction of bromide **16** with AcOH in the presence of $Hg(OAc)_2$ provided pure 1,2-trans heptaacetate **17** in 60% yield. The structure of compound **17** was verified by ^1H NMR spectroscopy, which indicated that presence of one *O*-methyl and seven *O*-acetyl groups. The value of the three-bond coupling constant $^3J_{H\text{-}1,H\text{-}2}$, 8.2 Hz, clearly established its 1,2-trans anomeric configuration at the "reducing" end moiety.

The second approach (Scheme IV) to compound **17** utilized 1-thiolactoside **18**[29] as the starting compound, which was first treated with 2,2-dimethoxypropane in the presence of PTS to give acetal **19** in 90% yield. A small amount of the isomeric, thermodynamically unstable 4′,6′-*O*-isopropylidene acetal[29a] was also detected in the reaction mixture by thin-layer chromatography and could easily be removed by crystallization. The size, and therefore the position of the isopropylidene acetal in **19**, was verified by NMR spectroscopy. The signals in the ^{13}C NMR spectrum at δ 26.2, 27.9, and 111.8 clearly indicate the presence of a five-membered isopropylidene structure fused to a pyranose ring.[30] Acetylation (Ac_2O–C_5H_5N) of

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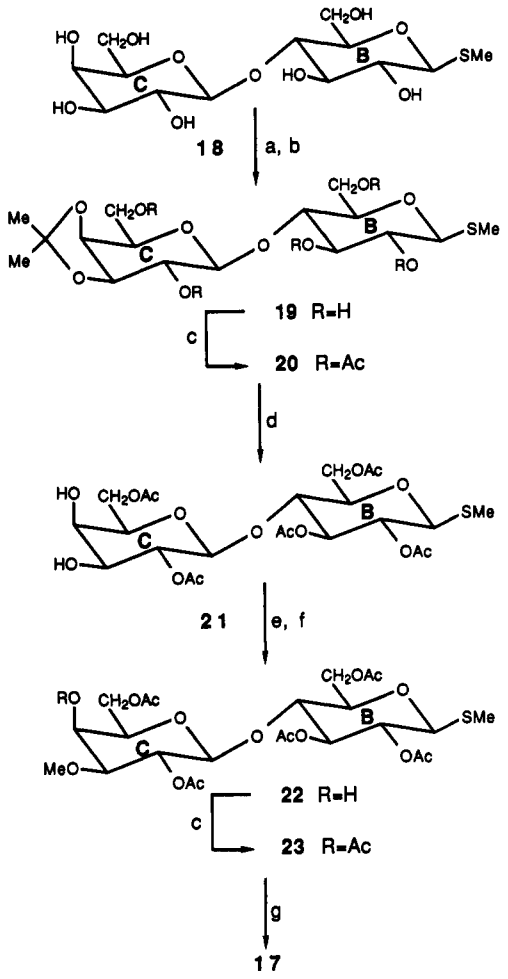
Scheme IV[a]



[a] Key: (a) $(CH_3O)_2C(CH_3)_2$, $(CH_3)_2CO$, DMF, PTS, 20 min, 60 °C; (b) MeOH, TFA, 10 min, 25 °C; (c) Ac_2O, C_5H_5N, 4 h, 25 °C; (d) HBF_4, AcOH, MeOH, 30 min, 40 °C; (e) Bu_2SnO, C_6H_6, 4 h, 80 °C; (f) MeI, Bu_4NI, C_6H_6, 12 h, 45 °C; (g) $NOBF_4$, Ac_2O, 30 min, 0 °C.

19 gave pentaacetate **20**, hydrolysis of which in acetic acid containing tetrafluoroboric acid provided diol **21**. Diol **21** on treatment[27] with Bu_2SnO–MeI–Bu_4NI provided methyl ether **22**, from which hexaacetate **23** was obtained by acetylation (Ac_2O–C_5H_5N). The position of the *O*-methyl group in compound **23** was indicated by the appearance of the C-3 signal ^{13}C NMR spectrum of the galactose moiety at δ 79.8 and further corroborated by the downfield shift of the H-4′ proton in the ^1H NMR spectrum to δ 5.47 in **23** relative to the corresponding resonance in **22** at δ 4.05. Since thioglycosides can be converted to glycosyl bromides in near-quantitative yields by treatment[31] with bromine, compound **23** appears to be a suitable precursor for heptaacetate **17** via the intermediacy of bromide **16**, as shown in Scheme II. As an alternative to this two-step procedure, recent work in our laboratory established[32] that thioglycosides having a participating group at the carbon atom adjacent to the anomeric center can be converted directly to 1,2-trans glycosyl acetates in one step by treatment with Ac_2O under the agency of the thiophilic reagent $NOBF_4$. Indeed, these conditions transformed thioglycoside **23** into 1,2-trans acetate **17** in 91% yield. Considering operational simplicity, the second approach organized around the central idea of the one-step conversion of thioglycosides into glycosyl acetates,[32] is clearly superior to the first one (Scheme III), in which benzyl lactoside **14** was used as the starting compound.

Synthesis of Pentasaccharide 4. Disaccharide donor **17** and acceptor **13** were coupled (Scheme V) under the agency of the Lewis acid trimethylsilyl trifluoromethanesulfonate[24-26] in CH_2Cl_2 at 25 °C to provide protected tetrasaccharide **24** in 43% yield. Conventional deprotection ((i) NaOMe–MeOH; (ii) H_2–Pd/C) of **24** gave free tetrasaccharide **5** in 78% yield. Finally, treatment of compound **5** with the sodium salt of cytidine 5′-monophospho-*N*-acetylneuraminic acid in the presence of Galβ-1,3(4)GlcNAcα2,3-sialyltransferase as described[13] provided pentasaccharide **4** in 34% yield. Considering the efficiency of the sialylation step and the exclusive α-anomeric configuration of the Neu5Ac unit in the product, the combined chemical and enzymatic approach adopted in this work compares favorably with strategies employing entirely chemical synthetic techniques. Also, the example described in this paper demonstrates that the rat liver sialyltransferase, specific for transfering the Neu5Ac residue to HO-3 of the galactose unit, is capable of substrate recognition even with the adjacent GlcNAc unit being glycosylated, i.e., occupying a branch-point position.

NMR Spectroscopic Studies. One-dimensional ^1H and ^{13}C NMR spectroscopy including $^1H\{^1H\}$ homo- and $^{13}C\{^1H\}$ heteronuclear decoupling methods was routinely used to characterize all intermediates. The DEPT-135 pulse sequence was used to distinguish CH_2 carbons. One-bond ^{13}C–^1H heteronuclear coupling constants were measured in the gated decoupling mode. Structure of the free tetra- **5** and pentasaccharide **4** were further ascertained by unambiguous assignment of their ^1H and ^{13}C NMR spectra (Tables I and II) using a combination of one- and two-dimensional homo- and heteronuclear correlation methods including one-dimensional difference NOE spectroscopy, *J*-resolved spectroscopy, ^1H–^1H COSY,[33] RELAY–COSY,[34] ^{13}C–^1H heteronuclear correlation spectroscopy,[35] and two-dimensional NOE spectroscopy in rotating frame (ROESY[36]). In addition to structural verification, the eventual objective of these assignments is to gain information on the three-dimensional shape of oligosaccharides **4** and **5**, with the anticipation that these data might lead to a better understanding of the factors determining the shape of the native polysaccharide **1** itself. To this end, the ^1H and ^{13}C NMR spectra of polysaccharides **1** and **2** have also been assigned and are presented in Tables I and II. The assignment technique was assisted by established chemical shift patterns and by the well-documented effect of glycosylation on chemical shifts. For example, in the case of tetrasaccharide **5** the peak at δ 55.7 in the ^{13}C NMR spectrum was assigned to C-2 of the *N*-acetylglucosamine unit (residue A). The ^1H–^{13}C correlation map led immediately to the assignment of H-2_A at δ 3.748. Sequential connectivities in the ^1H–^1H COSY spectrum identified the H-1_A (δ 4.468) and H-3_A resonances (δ 3.687). The remaining signals belonging to the spin system of ring A were identified by the corresponding cross-peaks in the ^1H–^1H COSY and RELAY–COSY spectra. The combined use of the ^1H–^1H and ^1H–^{13}C COSY spectra was particularly advantageous since it

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Table I. ^{1}H NMR Data for Compounds 1, 2, 4, and 5[a]

	chemical shifts[b] (δ) for 1, 2, 4, and 5				coupling constants (Hz) for 4 and 5		
	compd					compd	
H-atom[c]	1	2	4	5	J_{H-H}	4	5
1_A	4.70	4.71	4.464	4.468	1_A-1_A	7.9	8.3
2_A	3.81	3.80	3.746	3.748	2_A-3_A	10.0	10.5
3_A	3.71	3.73	3.682	3.687	3_A-4_A	8.8	8.5
4_A	3.90	nd	3.86	3.855	4_A-5_A	10.0	9.8
5_A	3.70	3.71	3.72	3.723	5_A-6_A	3.9	4.0
6_A	3.97	3.97	3.967	3.963	5_A-$6'_A$	1.8	1.9
$6'_A$	4.27	4.27	4.313	4.302	6_A-$6'_A$	11.5	11.4
1_A	4.54	4.55	4.547	4.554	1_B-2_B	7.9	8.0
2_B	3.32	3.36	3.368	3.380	2_B-3_B	9.3	9.3
3_B	3.49	3.65	3.67	3.665	3_B-4_B	nd	nd
4_B	3.64	3.63	3.68	3.68	4_B-5_B	nd	9.6
5_B	3.67	3.60	3.64	3.600	5_B-6_B	4.9	5.0
6_B	3.81	3.78	3.823	3.815	5_B-$6'_B$	2.2	2.3
$6'_B$	3.99	3.97	3.980	3.982	6_B-$6'_B$	12.4	12.4
1_C	4.43	4.44	4.464	4.466	1_C-2_C	7.9	7.8
2_C	3.59	3.58	3.568	3.564	2_C-3_C	9.9	10.0
3_C	3.72	3.72	3.367	3.364	3_C-4_C	3.3	3.3
4_C	4.16	4.16	4.215	4.215	4_C-5_C	<1	0.6
5_C	3.70	nd	3.70	3.700	5_C-6_C	8.2	8.2
6_C	nd	nd	3.815	3.810	5_C-$6'_C$	4.1	4.0
$6'_C$	nd	nd	3.759	3.760	6_C-$6'_C$	11.9	11.8
1_D	4.62	4.54	4.608	4.533	1_D-2_D	7.9	7.8
2_D	3.57	3.53	3.560	3.535	2_D-3_D	9.8	10.0
3_D	4.08	3.66	4.089	3.670	3_D-4_D	3.1	3.3
4_D	3.97	3.92	3.961	3.919	4_D-5_D	<1	0.6
5_D	nd	nd	3.69	3.70	5_D-6_D	nd	8.2
6_D	nd	nd	3.76	3.777	5_D-$6'_D$	nd	3.9
$6'_D$	nd	nd	3.88	3.745	6_D-$6'_D$	nd	11.9
3_E	1.60; 2.55		1.810; 2.751		3_E^{ax}-3_E^{eq}	12.3	
					3_E^{ax}-4_E	12.3	
4_E	3.68		3.687		3_E^{eq}-4_E	4.6	
5_E	3.84		3.848		4_E-5_E	5.9	
6_E	nd		3.644		5_E-6_E	10.3	
7_E	nd		3.600		6_E-7_E	1.3	
8_E	nd		3.875		7_E-8_E	8.8	
9_E	3.65		3.949		8_E-9_E	4.9	
$9'_E$	3.86		nd		8_E-$9'_E$	nd	
					9_E-$9'_E$	12.4	
OCH_3 (aglycon)			3.502	3.504			
OCH_3 (ether)			3.442	3.445			
CH_3CON	2.04	2.04	2.03	2.03			

[a] At 500 MHz, in D_2O–acetone-d_6, 300 K. [b] First-order data. [c] Subscripts A–E refer to individual monosaccharide units as shown by the formulas.

verified the assignment. For example, the assignment of the peak at δ 4.855 to H-4_A could easily be confirmed by the observation in the ^{1}H–^{13}C COSY spectrum of a cross-peak between this resonance and the one at δ 78.58 in the ^{13}C dimension. The cross-peak originates from C-4_A based on the well-documented, strong deshielding effect of glycosylation. The strong deshielding effect of the methyl substituent in residue C of **5** required the assignment of the signal at δ 82.45 in the ^{13}C NMR spectrum to C-3_C, which, by use of the ^{1}H–^{13}C correlation map, identified the H-3_C resonance at δ 3.364. The cross-peaks observed in the ^{1}H–^{1}H COSY spectrum identified the remaining protons of spin system C. The doublet of doublets having coupling constants (3.3 and 0.6 Hz) characteristic for H-4 of galactopyranose residues was assigned to H-4 of ring D. Sequential connectivities made the subsequent assignment of resonances corresponding to ring-D atoms straightforward. The resonance at δ 79.32 in the ^{13}C NMR spectrum must originate from C-4 of the glucopyranosyl residue, on the basis of the deshielding effect of glycosylation. ^{1}H–^{13}C heteronuclear chemical shift correlations required then the assignment of the signal at δ 3.68 to H-4 of residue B. The remaining resonances (Tables I and II) were assigned on the basis of the ^{1}H–^{1}H and ^{1}H–^{13}C chemical shift correlation maps. The assignment of the signals in the ^{1}H and ^{13}C NMR spectra of compounds **1**, **2**, and **4** followed analytical approaches similar to those outlined previously.

In an attempt to further characterize compounds **4** and **5**, carbon spin–lattice relaxation times (T_1 values) were measured for their protonated carbons by use of the inversion recovery technique. The observed T_1 values for such carbons are dominated by proton–carbon dipole–dipole relaxation and are proprotional to molecular mobility. In linear, neutral oligosaccharides the observed, average carbon T_1 values for the interchain residues are usually lower than those for the terminal ones,[37,38] indicating faster segmental motion for the end units. In the trisaccharide *N*-acetyleneuraminyl-(2 → 3)-lactose the Neu5Ac residue was shown[39] to act as an anchor with increasing segmental motion away from this residue. The carbon T_1 values for compounds **4** and **5** (Tables III and IV) indicate a highly restricted motion of the branching GlcNAc residue (unit A), which acts as the anchor in

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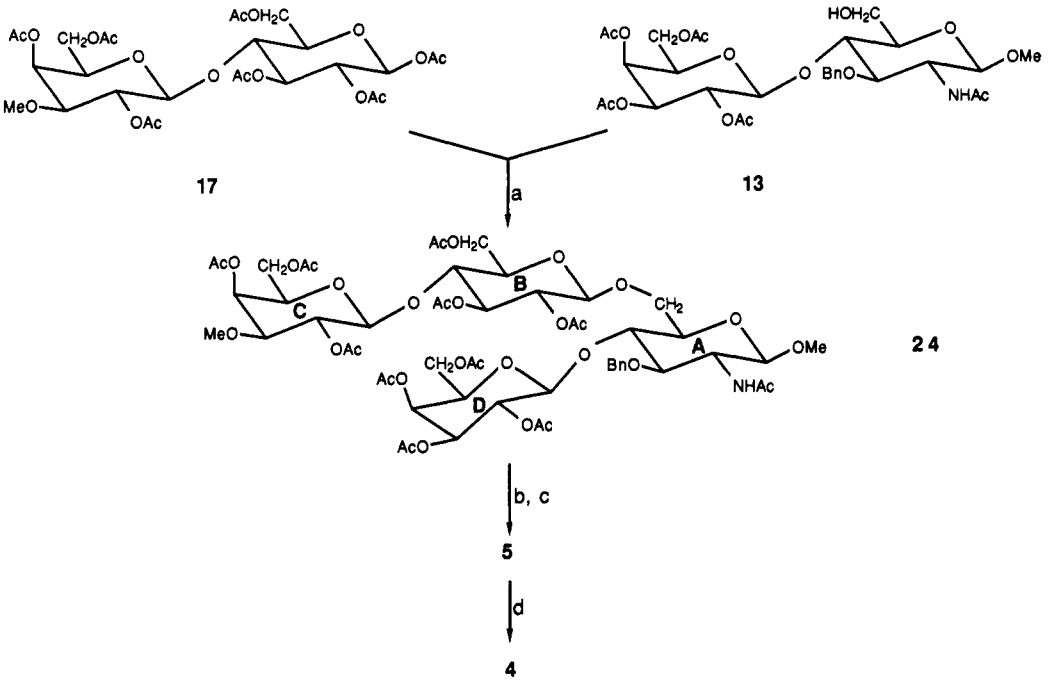
Scheme V[a]



[a] Key: (a) TMSOTf, CH_2Cl_2, 24 h, 25 °C; (b) NaOMe, MeOH, 24 h, 25 °C; (c) H_2, Pd/C, EtOH, AcOH, 48 h, 25 °C; (d) CMP-Neu5Ac, Galβ1,3(4)GlcNAcα2,3sialyltransferase, pH 6.5, 24 h, 37 °C.

Table II. ^{13}C NMR Chemical Shifts for Compounds 1, 2, 4, and 5[a]

	compd			
carbon atom[b]	1	2	4	5
1_A	103.87	103.75	102.92	102.78
2_A	55.97	56.02	55.81	55.70
3_A	72.81	nd	73.25	73.17
4_A	77.76	nd	78.29	78.58
5_A	74.0	74.1	74.40	74.22
6_A	68.38	68.33	68.30	68.00
1_B	103.41	103.41	103.30	103.15
2_B	73.45	73.48	73.56	73.6
3_B	nd	75.10	75.11	75.1
4_B	79.19	79.32	79.02	79.0
5_B	75.49	nd	75.48	75.41
6_B	60.89	60.92	60.88	60.78
1_C	104.00	103.81	103.67	103.59
2_C	70.81	70.81	70.76	70.65
3_C	83.09	83.05	82.47	82.45
4_C	69.15	69.08	64.94	64.83
4_C	75.9	nd	76.07	76.00
6_C	61.6	61.62	61.91	61.81
1_D	102.99	103.56	103.10	103.50
2_D	70.17	71.77	70.22	71.68
3_D	76.5	73.33	76.49	73.41
4_D	68.38	69.43	68.47	69.32
5_D	nd	nd	75.87	76.0
6_D	61.6	61.92	61.91	61.78
1_E	174.76		174.74	
2_E	100.86		101.02	
3_E	40.48		40.50	
4_E	69.15		69.17	
5_E	52.50		52.54	
6_E	nd		73.80	
7_E	nd		68.92	
8_E	nd		72.62	
9_E	63.41		63.47	
CH_3CON	23.22; 23.07	23.03	23.04; 22.88	22.99
CH_3CON	175.85; 176.06	175.74	175.95	175.36
			175.86	
CH_3O (aglycon)			56.08	57.92
CH_3O (ether)			56.08	56.97

[a] At 125 MHz, in D_2O–acetone-d_6, 300 K. [b] Subscripts A–E refer to individual monosaccharide units as shown by the formulas.

Table III. Observed Carbon *NT* Values[a] for Compounds 4 and 5

	compd	
carbon atom[b]	4[c]	5[d]
1_A	0.317	0.289
2_A	0.300	0.277
3_A	0.308*	0.291
4_A	0.272	0.266
5_A	0.272	0.270
6_A	0.308	0.286
1_B	0.343	0.313
2_B	0.332	0.307
3_B	0.318	0.315
4_B	0.351	0.298
5_B	0.340	0.305
6_B	0.336	0.326
1_C	0.414*	0.362
2_C	0.402	0.357
3_C	0.413*	0.364
4_C	0.345*	0.305
5_C	nd	nd
6_C	nd	nd
1_D	0.286*	0.324
2_D	0.300	0.321
3_D	0.293	0.334
4_D	0.289*	0.299
5_D	nd	nd
6_D	nd	nd
3_E	0.346	
4_E	0.322	
5_E	0.305	
6_E	0.284	
7_E	0.285	
8_E	0.309*	
9_E	0.472	

[a] In s. [b] For designations A–E, see formulas 4 and 5. [c] Standard deviation is less than 0.010 s, except for values with an * where it is less than 0.017 s. [d] Standard deviation is less than 0.005 s.

tetrasaccharide 5. The gradient in the average T_1 values (Table IV) shows increasing mobility away from the GlcNAc residue, the most mobile being the terminal unit C. In pentasaccharide 4, both the GlcNAc (unit A) and

Table IV. Average Carbon T Values[a] for Compounds 4 and 5

compd	sugar unit[b] A	B	C	D	E
4	0.30[c]	0.34[c]	0.39[d]	0.29[d]	0.3[e]
5	0.28[c]	0.31[c,d]	0.35[d]	0.32[d]	

[a] In s. [b] For designations A–E see formulas 4 and 5. [c] Average for C1–C5. [d] Average for C1–C4. [e] Average for C4–C8.

the Neu5Ac residue (unit E) act as anchors resulting in nearly isotropic mobilities for units A, D, and E. The T_{-1} gradient from unit A to B to C again indicates increasingly less restricted segmental motion in this part of the molecule. The motional independence of residue C in either 4 or 5 is considered as an indication of the lack of any significant nonbonded interaction between residue C and other residues in either 4 or 5.

To further probe possible interresidual, nonbonded interactions, two-dimensional NOE spectra in rotating frame (ROESY[38]) have been measured for both 4 and 5. This technique allows the detection of cross signals even in case of unfavorable correlation times. As expected, strong NOEs were found across the glycosidic linkages between the anomeric and the corresponding aglyconic protons in both 4 and 5. Strong NOEs were also detected between the H-3 of Neu5Ac residue and H-3 of the Gal residue D in 4, the occurrence of which has been documented.[40] No other interresidual C*H*–C*H* NOEs could be verified in either 4 or 5. This, together with the picture derived from the carbon T_1 data, supports the view that the C–B–A and the E–D–A segments lack significant, nonbonded interactions in pentasaccharide 4.

Immunochemical experiments performed with compounds 4 and 5 will be reported elsewhere.

Experimental Section

Melting points and optical rotations were measured, and column chromatography was performed as previously described.[9] Thin-layer chromatography was carried out on precoated aluminum sheets (E. Merck, 5562); solutions were dried over Na_2SO_4. ^1H and ^{13}C NMR spectra were run at 500 and 50 MHz, respectively.[9] Compounds 4 and 5 were freeze-dried from 99.95% D_2O twice before NMR measurements in 99.95% D_2O containing 5–10% acetone-d_6.

Methyl 2-Acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (7). A solution of triol 6[16] (27 g, 115 mmol) in 100 mL of dry DMF was stirred with 4-methoxybenzaldehyde dimethyl acetal[9,17] (35 mL) and toluenesulfonic acid (400 mg) at 25 °C for 10 h, treated with triethylamine (2 mL), and poured into 400 mL of water. Crystalline 7 was isolated by filtration and thoroughly washed with diethyl ether: 37.4 g (92.3%); mp 265–270 °C; $[\alpha]_D$ −79° (*c* 1.2, DMF); ^1H NMR (DMSO-d_6) δ 1.84 (s, 3 H, CH_3CO), 3.35 (s, 3 H, CH_3O (aglycon)), 3.58 (m, H-2), 3.75 (s, 3 H, CH_3O (aromatic)), 4.20 (dd, 1 H, $J_{3,4}$ = 9.9 Hz, $J_{4,5}$ = 4.6 Hz, H-4), 4.40 (d, 1 H, $J_{1,2}$ = 7.5 Hz, H-1), 5.25 (d, 1 H, $J_{2,NH}$ = 5.3 Hz, N*H*), 5.51 (s, 1 H, *H*CO_2), 6.89 and 7.36 (2 d, 2 × 2 H, $J \approx 10$ Hz, aromatics), 7.81 (d, 1 H, $J_{3,HO}$ = 8.4 Hz, OH); ^{13}C NMR (DMSO-d_6) δ 21.1 (*C*H_3CO), 53.1 (C-2), 54.1 (2 *C*H_3O), 66.0 (C-6), 64.0 (C-5), 68.6 (C-3), 79.4 (C-4), 99.8 (C-1), 100.5 (*C*HO_2), 111.3 (2x) (C-3′,5′(*MBn*)), 125.7 (2x) [C-2′,6′(*MBn*)], 128.1 [C-1′ (*MBn*)], 157.6 [C-4′ (*MBn*)], 167.4 (*C*=O). Anal. Calcd for $C_{17}H_{23}NO_7$ (353.35): C, 57.78; H, 6.56; N, 3.96. Found: C, 57.82; H, 6.64; N, 3.89.

Methyl 2-Acetamido-3-*O*-benzyl-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-β-D-glucopyranoside (8). A stirred solution of compound 7 (34 g, 96.3 mM) in 600 mL of dry DMF was treated[18] with BaO (106 g), $Ba(OH)_2 \cdot 8H_2O$ (33 g) and benzyl bromide (30 mL, 43.2 g, 152 mmol). The mixture was stirred for 24 h at 25 °C and filtered and the filter cake was extracted with hot DMF (3 × 100 mL). The filtrate and extracts were combined and kept at 0 °C for 3 h. Filtration gave crystalline 8 (36.5 g, 85.5%): mp 300 °C; $[\alpha]_D$ −61° (*c* 0.5, DMF); ^1H NMR (DMSO-d_6) δ 1.89 (s, 3 H, CH_3CO), 3.42 ((s 3 H, CH_3O (aglycon)), 3.82 (s, 3 H, CH_3O (aromatic)), 4.30 (dd, 1 H, $J_{3,4}$ = 10.1 Hz, $J_{4,5}$ = 4.8 Hz, H-4), 4.52 (m, 1 H, H-1), 4.64 and 4.76 (2d, 2 H, $J \approx 12$ Hz, CH_2(Bn)), 5.71 (s, 1 H, *C*HO_2), 7.5–6.9 (m, 9 H, aromatic protons); ^{13}C NMR (DMSO-d_6) δ 20.8 (*C*H_3CO), 52.1 (C-2), 52.9 and 53.9 (2 *C*H_3O), 63.4 (C-5), 65.6 (C-6), 70.9 (*C*H_2 (Bn)), 76.5 (C-3), 78.7 (C-4), 97.9 (C-1), 100.1 (*C*HO_2), 111.2 (2x) (C-3′,5′ (*MBn*)), 125.1 (aromatic carbons), 125.9 (2x) (C-2′,6′ (*MBn*)), 169.4 (*C*H_3CO). Anal. Calcd for $C_{24}H_{29}NO_7$ (443.47): C, 65.00; H, 6.59; N, 3.16. Found: C, 65.04; H, 6.63; N, 3.28.

Methyl 2-Acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (9). A stirred mixture of acetal 8 (5 g, 11.3 mmol), 4A powdered molecular sieves, and dry DMF (50 mL) was treated[21] with sodium cyanoborohydride (5 g, 79.6 mmol) and trifluoroacetic acid (10 mL). After 12 h, more sodium cyanoborohydride (1 g) and trifluoroacetic acid (3 mL) were added. Stirring was continued for a further 12 h. Triethylamine (10 mL) was added, and the mixture was filtered and the filtrate partitioned between dichloromethane (150 mL) and 1 N NaOH (2 × 30 mL). The organic phase was dried and concentrated. The residual syrup was treated with dry pyridine (5 mL) and acetic anhydride (5 mL) for 12 h at 25 °C. Concentration gave a syrup that was crystallized from dichloromethane–ethyl acetate. Filtration gave a crystalline 10 (5.4 g, 78.3): mp 187–189 °C; $[\alpha]_D$ 20° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.86 (s, 3 H, CH_3COO), 1.89 (s, 3 H, CH_3CON), 3.34 (ddd, 1 H, H-2), 3.48 (s, 3 H, CH_3O, (aglycon)), 3.56 (m, 2 H, H-6,6′), 3.62 (m, 1 H, H-5), 3.77 (s, 3 H, CH_3O (aromatic)), 4.24 (t, 1 H, $J_{2,3} \approx J_{3,4}$ = 9.5 Hz, H-3), 4.45 and 4.58 (2s, 2 × 2 H, CH_2 (Bn), CH_2 (MBn)), 4.81 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1), 5.00 (t, 1 H, $J_{4,5}$ = 9.5 Hz, H-4), 6.21 (d, 1 H, $J_{2,NH}$ = 7.8 Hz, N*H*), 6.8–6.91 and 7.16–7.34 (m, 9 H, aromatic protons); ^{13}C NMR ($CDCl_3$) δ 20.2 (*C*H_3COO), 23.3 (*C*H_3CON), 55.1 (C-2), 56.7 and 57.1 (2 *C*H_3O), 69.1 (C-6), 71.5 and 73.0 (C-4,5), 73.0 and 73.6 (*C*H_2 (Bn), *C*H_2 (MBn)), 78.0 (C-3), 100.5 (C-1), 113.5 (2x) C-3′,5′ (MBn)), 127.6–129.8 (aromatic carbons), 138.0 (quaternary aromatic carbon), 159.1 (C-4 (MBn)), 169.7 and 170.7 (2 *C*=O). Anal. Calcd for $C_{26}H_{33}NO_8$ (487.51): C, 64.05; H, 6.82; N, 2.87. Found: C, 64.53; H, 7.14; N, 2.80.

A solution of compound 10 in anhydrous methanol (100 mL) was treated with a catalytic amount of sodium methoxide. After 4 h at 25 °C, the solution was neutralized (Dowex 50W, H^+), filtered, and concentrated to give crystalline 9 (4.6 g, 73.0%): mp 175–177 °C; $[\alpha]_D$ −4° (*c* 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.89 (s, 3 H, CH_3CON), 3.36 (m, 1 H, H-2), 3.45 (s, 3 H, CH_3O (aglycon)), 3.64 (t, 1 H, H-4), 3.79 (s, 3 H, CH_3O (aromatic)), 3.92 (dd, 1 H, J = 8.4 Hz, J = 9.9 Hz, H-3), 4.48 and 4.54 (2 d, 2 × 1 H, $J \approx$ 12 Hz, CH_2 (MBn)), 4.69 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1), 4.68 and 4.80 (2d, 2 × 1 H, $J \approx 12$ Hz, CH_2 (Bn)), 5.72 (d, 1 H, $J_{2,NH}$ = 7.8 Hz, N*H*), 6.82–6.92 and 7.21–7.38 (m, 9 H, aromatic protons); ^{13}C NMR ($CDCl_3$) δ 23.6 (*C*H_3CON), 55.2 (C-2), 56.5 and 56.6 (2 *C*H_3O), 70.2 (C-6), 73.3 and 74.0 (2 *C*H_2 (Bn and MBn), 73.3 and 73.6 (C-4,5), 80.4 (C-3), 100.9 (C-1), 113.8 (2x) (C-3′,5′ (MBn)), 127.8–129.4 (aromatic carbons), 170.6 (*C*=O). Anal. Calcd for $C_{24}H_{31}NO_7$ (445.49): C, 64.70; H, 7.01; N, 3.14. Found: C, 64.53; H, 7.14; N, 2.80.

Methyl 2-Acetamido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3-*O*-benzyl-2-deoxy-6-*O*-(4-methoxybenzyl)-β-D-glucopyranoside (12). A mixture of acceptor 9 (4.4 g, 9.87 mmol), bromide 11 (8.0 g, 19.5 mmol), $Hg(CN)_2$ (4.7 g), toluene (40 mL), and nitromethane (20 mL) was stirred at 25 °C for 12 h. Chloroform (100 mL) was added, and the mixture was extracted with 5% aqueous KI, then with water, followed by concentration. Chromatography of the residue with 1:1 ethyl acetate–hexane then ethyl acetate gave first 4-*O*-acetate 10 (0.8 g, 16.6%) then a ca. 1:1 mixture of 10 and 11 (0.4 g), followed by syrupy disaccharide 12 (5.5 g, 70.0%): $[\alpha]_D$ −14° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.87 (s, 3 H, CH_3CON), 1.95, 1.98, 2.10, 2.13 (4s, 4 × 3 H, 4 CH_3CO), 3.44 (s, 3 H, CH_3O (aglycon)), 3.71 (m, H-2_A) 3.81 (s, 3 H, CH_3O (aromatic)), 4.51 (d, 1 H, $J_{H\text{-}1_D}$, H-2_D = 7.7 Hz, H-1_D), 4.41, 4.60, 4.65, 4.77 (4d, 4 × 1 H, $J \approx 12$ Hz for each doublet, CH_2 (Bn) and CH_2 (MBn)), 4.92 (dd, $J_{H\text{-}2_D}$, H-3_D = 10.4 Hz, H-3_D), 5.13 (dd, 1 H, H-2_D), 5.32 (dd, $J_{H\text{-}4_D}$, H-5_D 1 Hz,

(40) Sabesan, S.; Bock, K.; Lemieux, R. U. *Can. J. Chem.* **1984**, *62*, 1034.

H-4$_D$), 5.98 (d, 1 H, N*H*), 6.8–6.9 and 7.2–7.4 (m, 9 H, aromatic protons); ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5 (2x), 20.7 (3s, 2 × 3 H and 6 H, 4 CH_3COO), 23.3 (s, 3 H, CH_3CON), 53.1 (C-2$_A$), 55.2 and 56.5 (2 CH_3O), 60.7 (C-6$_D$), 68.4 (C-6$_A$), 66.7, 69.2, 70.5 (2x) (C-2$_D$,3$_D$,4$_D$,5$_D$), 74.3, 75.0 (C-4$_A$,5$_A$), 76.7 (C-3$_A$), 99.6, 101.0 (C-1$_A$,1$_D$), 113.8 (2x) C-3′,5′ (MBn)), 127.6–129.9 (aromatic carbons), 138.4 (quaternary aromatic carbon), 156.1 (C-4′ (MBn)), 169.8, 169.9, 170.1, 170.2 (2x). (5 CH_3CO).

Methyl 2-Acetamido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (13). A stirred mixture of compound **12** (6.5 g, 8.52 mmol), dichloromethane (200 mL), and water (10 mL) was treated[23] with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.43 g, 10.7 mmol) at 25 °C for 3 h. The solution was extracted with 5% aqueous $NaHCO_3$, dried, and concentrated. Chromatography of the residue with ethyl acetate gave syrupy **13** (3.75 g, 67.2%): $[\alpha]_D$ −5° (*c* 1.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.89 (s, 3 H, CH_3CON), 1.97, 1.975, 2.08, 2.12 (4s, 4 × 3 H, 4 CH_3COO), 3.44 (s, 3 H, CH_3O (aglycon)), 3.53 (m, 1 H, H-2$_A$), 3.7–4.1 (m 8 H, H-3$_A$,4$_A$,5$_A$,6$_A$,6′$_A$,5$_D$,6$_D$,6′$_D$), 4.65 and 4.88 (2d, 2 H, $J \approx 12$ Hz for both, CH_2 (Bn)), 4.65 (d, 1 H, J H-1$_A$, H-2$_A$ = 7.5 Hz, H-1$_A$), 4.75 (d, 1 H, $J_{H\text{-}1_D,H\text{-}2_D}$ = 7.9 Hz, H-1$_D$), 5.03 (dd, 1 H, $J_{H\text{-}2_D,H\text{-}3_D}$ = 10.2 Hz, $J_{H\text{-}3_D,H\text{-}4_D}$ = 3.4 Hz, H-3$_D$), 5.20 (dd, 1 H, H-2$_D$), 5.34 (dd, 1 H, $J_{H\text{-}4_D,H\text{-}5_D} < 1$ Hz, H-4$_D$), 6.16 (d, 1 H, $J_{H\text{-}2_A,NH}$ = 8.2 Hz, N*H*), 7.15–7.58 (m, 5 H, aromatic protons); ^{13}C NMR ($CDCl_3$) δ 20.5 (3x), 20.7 (4 CH_3COO), 23.3 (CH_3CON), 55.2 (C-2$_A$), 56.8 (CH_3O (aglycon)), 60.7 (2x), (C-6$_{A,6D}$), 66.7, 69.5, 70.5, 70.8 (C-2$_D$,3$_D$,4$_D$,5$_D$), 73.7 (CH_2 (Bn)), 75.1, 76.1 (C-4$_A$,5$_A$), 77.8 (C-3$_A$), 100.3, 101.1 (C-1$_A$,1$_D$), 127.6–128.2 (aromatic carbons), 138.5 (quaternary carbon atom), 169.6, 170.0, 170.1, 170.2, 170.6 (5 CH_3CO).

Benzyl 2,3,6-Tri-*O*-acetyl-4-*O*-(2,6-di-*O*-acetyl-3-*O*-methyl-β-D-galactopyranosyl)-β-D-glucopyranoside (15). A mixture of diol **14**[26] (8.5 g, 13.2 mmol), dibutyltin oxide (3.6 g, 14.5 mmol) and 200 mL of dry benzene was stirred[27] under reflux by use of a Dean–Stark trap for 6 h. Benzene (100 mL) was distilled off, and the solution was cooled to ca. 40 °C and treated with tetrabutylammonium iodide (5.0 g, 13.5 mmol) and methyl iodide (25 mL). After the mixture was stirred for 24 h at 40 °C, the solution was concentrated. The residue was stirred with 100 mL of ethyl acetate at 0 °C for 2 h. The solids were removed by filtration, and the filtrate was concentrated. Purification of the residue by chromatography with 2:1 ethyl acetate–hexane as eluant gave crystalline **15** (6.7 g, 77.1%), which was recrystallized from ethanol: mp 153–155 °C, $[\alpha]_D$ −22° (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.99, 2.03, 2.07, 2.09, 2.13 (5s, 5 × 3 H, 5 CH_3CO), 3.23 (dd, 1 H, $J_{H\text{-}2_B,H\text{-}3_B}$ = 9.9 Hz, $J_{H\text{-}3_B,H\text{-}4_B}$ = 3.2 Hz, H-3$_B$), 3.38 (s, 3 H, CH_3O), 3.55–3.67 (m, 2 H, H-5$_b$,5$_C$), 3.78 (dd, 1 H, H-4$_B$), 4.04 (m, 1 H, H-4$_C$), 4.19 (dd, 1 H, $J_{H\text{-}5_B,H\text{-}6'_B}$ = 5.3 Hz, $J_{H\text{-}6_B,H\text{-}6'_B}$ = 12 Hz, H-6′$_B$), 4.30 (m, 2 H, H-6$_C$,6′$_C$), 4.32 (d, 1 H, $J_{H\text{-}1_B,H\text{-}2_B}$ = 7.7 Hz, H-1$_B$), 4.48 (dd, 1 H, $J_{H\text{-}5_B,H\text{-}6_B}$ = 1.6 Hz, H-6$_B$), 4.52 (d, 1 H, $J_{H\text{-}1_C}$, H-2$_C$ = 7.7 Hz, H-1$_C$), 4.58 and 4.85 (2d, 2 × 1 H, $J \approx 12$ Hz, CH_2 (Bn)) 4.96 (t, 1 H, H-2$_B$), 5.00 (t, 1 H, H-2$_C$), 5.13 (dd, 1 H, $J_{H\text{-}3_B,H\text{-}4_B}$ = 9.6 Hz, H-3$_B$), 7.22–7.40 (aromatic protons); ^{13}C NMR ($CDCl_3$) δ 20.6 (CH_3CO), 57.2 (CH_3O), 62.0, 62.5 (C-6$_B$,6$_C$), 70.5 (CH_2 (Bn)), 64.5, 70.5, 71.4, 72.0, 72.3, 72.6 (C-2$_B$,3$_B$,5$_B$,2$_C$,4$_C$,5$_C$), 75.8 (C-4$_B$), 80.1 (C-3$_C$), 98.9 (C-1$_B$), 100.7 (C-1$_C$), 127.4–128.2 (aromatic carbons), 136.6 (quaternary aromatic carbon), 169.2, 169.4, 169.9, 170.3, 170.5 (5 *C*=O). Anal. Calcd for $C_{30}H_{40}O_{16}$ (656.62): C, 54.87; H, 6.14. Found: C, 54.76; H, 6.18.

Methyl 4-*O*-(3,4-*O*-Isopropylidene-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (19). A stirred mixture of thioglycoside **18**[29] (10.0 g, 26.8 mmol) dry DMF (40 mL), acetone (200 mL), 2,2-dimethoxypropane (25 mL), and *p*-toluenesulfonic acid (300 mg) was refluxed for 90 min, cooled to 30–40 °C, and treated with methanol (10 mL) for 10 min. Addition of triethylamine (2 mL) followed by concentration gave a syrupy residue that was crystallized from ethyl acetate to give **19**: 10.0 g, 90.3%; mp 194–196 °C; $[\alpha]_D$ +14° (*c* 0.6, H_2O), ^{1}H NMR (D_2O) δ 1.39 and 1.54 (2s, 2 × 3 H, $(CH_3)_2C$), 2.22 (s, 3 H, CH_3S), 3.41 (dd, 1 H, $J_{H\text{-}2_B,H\text{-}3_B}$ = 8.8 Hz, H-2$_B$), 3.51 (dd, 1 H, $J_{H\text{-}2_C}$, H-3$_C$ = 8.0 Hz, H-2$_C$), 3.67 (H-3$_B$), 4.21 (dd, 1 H, $J_{H\text{-}3_C,H\text{-}4_C}$ = 5.1 Hz, H-3$_C$), 4.46 (d, 1 H, $J_{H\text{-}1_B,H\text{-}2_B}$ = 9.8 H, H-1$_B$), 4.50 (dd, 1 H, $J_{H\text{-}1_C,H\text{-}2_C}$ = 98.8 Hz, H-1$_C$); ^{13}C NMR (D_2O) δ 12.1 (CH_3S), 26.2 and 27.9 ($(CH_3)_2C$), 60.9, 61.6 (C-6$_B$,6$_C$), 72.2, 73.6, 74.2, 74.6, 76.5, 79.3, 79.4 (2x) (C-2$_B$,3$_B$,4$_B$,5$_B$,2$_C$,3$_C$,4$_C$,5$_C$), 86.2 (C-1$_B$), 102.9 (C-1$_C$), 111.8 [(C-$H_3)_2C$]. Anal. Calcd for $C_{16}H_{28}O_{10}S$ (412.44): C, 46.59; H, 6.84; S, 7.77. Found: C, 46.53; H, 7.02; S, 7.74.

Methyl 2,3,4-Tri-*O*-acetyl-4-*O*-(2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (20). A solution of compound **19** (7.5 g, 18.2 mmol), dry ethyl acetate (200 mL), *N*,*N*-disopropylethylamine (25 mL), 4-(dimethylamino)pyridine (2.2 g), and acetic anhydride (15 mL) was stirred at 25 °C for 5 h and concentrated. A solution of the residual syrup in dichloromethane (100 mL) was successively extracted with 5% ice-cold hydrochloric acid, water, and 5% aqueous $NaHCO_3$, dried, and concentrated. Crystallization of the residual syrup from diethyl ether gave **20** (9.5 g, 83.9%): mp 125–127 °C; $[\alpha]_D$ +15° (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.32 and 1.53 [$(CH_3)_2C$], 2.05, 2.06, 2.08, 2.11, 2.13, 2.15 (5CH_3CO and SCH_3), 3.66 (H-5$_B$), 3.75 (t, 1 H, $J_{H\text{-}3_B,H\text{-}4_B}$ = 9 Hz, H-4$_B$), 3.93 (m, 1 H, H-4$_C$), 4.17 (H-3$_C$), 4.35 (d, H-1$_C$), 4.38 (H-1$_B$), 4.45 (H-6$_B$), 4.58 (H-6′$_B$), 4.87 (m, 1 H, H-2$_C$), 4.97 (dd, 1 H, $J_{H\text{-}1_B,H\text{-}2_B} \approx J_{H\text{-}2_B,H\text{-}3_B}$ = 9.8 Hz, H-2$_B$), 5.21 (dd, 1 H, $J_{H\text{-}3_B,H\text{-}4_B}$ = 9.7 Hz, H-3$_B$); ^{13}C NMR ($CDCl_3$) δ 11.5 (SCH_3), 20.6 (2x), 20.7 (2x) (4 CH_3CO), 26.0 and 27.2 ($(CH_3)_2C$), 62.3, 63.0 (C-6$_B$,6$_C$), 69.5, 70.8, 72.6, 72.9, 73.3, 75.9, 76.7, 76.9, (C-2$_B$,3$_B$,4$_B$,5$_B$,2$_C$,3$_C$,4$_C$,5$_C$), 82.9 (C-1$_B$), 100.4 (C-1$_C$), 110.7 ($(CH_3)_2C$). Anal. Calcd for $C_{26}H_{38}O_{15}S$ (622.62): C, 50.15; H, 6.15; S, 51.5. Found: C, 50.30; H, 6.25; S, 5.04.

Methyl 2,3,6-Tri-*O*-acetyl-4-*O*-(2,6-di-*O*-acetyl-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (21). A solution of thioglycoside **20** (3.3 g, 5.4 mmol) in 10 mL of methanol and 30 mL of acetic acid containing 50 μL of 50% aqueous tetrafluoroboric acid was kept at 40–45 °C for 30 min. Solid $NaHCO_3$ (2 g) was added, and the mixture was concentrated. A solution of the syrupy residue in chloroform (100 mL) was extracted with water (3 × 30 mL), dried, and concentrated to give crystalline **21** (2.96 g, 96.1%): mp 146–149 °C; $[\alpha]_D$ −5° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.05, 2.06, 2.11 (2x) (4 CH_3CO), 2.15 (CH_3S), 3.58 (dd, 1 H, $J_{H\text{-}2_C,H\text{-}3_C}$ = 9.8 Hz, $J_{H\text{-}3_C,H\text{-}_C}$ = 3.4 Hz, H-3$_C$), 3.68 (H-5$_B$), 3.71 (t, 1 H, H-4$_B$), 3.89 (dd, 1 H, $J_{H\text{-}4_C,H\text{-}5_C} < 1$ Hz, H-4$_C$), 4.17 (dd, 1 H, $J_{H\text{-}5_B,H\text{-}6_B}$ = 4.6 Hz, $J_{JH\text{-}6_B,H\text{-}6'_B}$ = 12.2 Hz, H-6′$_B$), 4.31 (d, 1 H, $J_{H\text{-}1_C,H\text{-}2_C}$ = 8.0 Hz, H-1$_C$), 4.38 (d, 1 H, $J_{H\text{-}1_B,H\text{-}2_B}$ = 9.8 Hz, H-1$_B$), 4.48 (dd, 1 H, $J_{H\text{-}5_B,H\text{-}6_B}$ = 1.1 Hz, H-6$_B$), 4.93 (dd, 1 H, $J_{H\text{-}2_C,H\text{-}3_C}$ = 9.8 Hz, H-2$_C$), 4.98 (t, 1 H, $J_{H\text{-}2_B,H\text{-}3_B}$ = 9.7 Hz, H-2$_B$), 5.19 (dd, 1 H, $J_{H\text{-}3,H\text{-}4}$ = 8.7 Hz, H-3$_B$); ^{13}C NMR ($CDCl_3$), δ 11.6 (CH_3S), 20.8, 20.9 (3x) (4 CH_3CO), 62.5, 62.7 (C-6$_B$,6$_C$), 68.7, 69.4, 72.5, 72.6, 73.1, 73.8, 76.6, 77.1 (C-2$_B$,3$_B$,4$_B$,5$_B$,2$_C$,3$_C$,4$_C$,5$_C$), 82.9 (C-1$_B$), 101.1 (C-1$_C$), 169.8, 170.7, 170.8, 171.0, 171.2 (5 CH_3CO). Anal. Calcd for $C_{23}H_{34}O_{15}S$ (582.56): S, 5.50. Found: S, 5.36.

Methyl 2,3,6-Tri-*O*-acetyl-4-*O*-(2,6-di-*O*-acetyl-3-*O*-methyl-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (22). A mixture of diol **21** (2.9 g, 5.1 mmol), dibutyltin oxide (1.27 g, 5.1 mmol), and dry benzene (150 mL) was stirred[27] under reflux by use of a Dean–Stark trap for 4 h. Benzene (80 mL) was distilled off, and the solution was cooled to ca. 50 °C. Tetrabutylammonium iodide (4 g) and methyl iodide (50 mL) were added, and the mixture was stirred at 40–50 °C for 12 h and then concentrated. A solution of the residue in chloroform (80 mL) was extracted with water (3 × 20 mL) and then concentrated. Chromatography of the residual syrup with 2:1 ethyl acetate–hexane then with ethyl acetate gave **22** as an amorphous solid (1.76 g, 59.2%): $[\alpha]_D$ +4° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.05, 2.06, 2.07, 2.12, 2.13 (5s, 5 × 3 H, 5 CH_3CO), 2.15 (CH_3S), 3.24 (dd, 1 H, $J_{H\text{-}2_C,H\text{-}3_C}$ = 10 Hz, $J_{H\text{-}3_C,H\text{-}4_C}$ = 3.4 Hz, H-3$_C$), 3.40 (s, 3 H, CH_3O), 3.66 (m, 1 H, H-5$_B$), 3.74 (dd, H-4$_B$), 4.05 (dd, 1 H, H-4$_C$), 4.16 (dd, 1 H, $J_{H\text{-}5_B,H\text{-}6'_B}$ = 5.0 Hz, $J_{H\text{-}6_B,H\text{-}6'_B}$ = 12.2 Hz, H-6′$_B$), 4.30 (m, 2 H, H-6$_C$,6′$_C$), 4.32 (d, $J_{H\text{-}1_C,H\text{-}2_C} \approx 8$ Hz, H-1$_C$), 4.38 (d, 1 H, $J_{H\text{-}1_B,H\text{-}2_B}$ = 10 Hz, H-1$_B$), 4.45 (dd, 1 H, $J_{H\text{-}5_B,H\text{-}6_B}$ = 1.8 Hz, H-6$_B$), 4.97 (dd, 1 H, $J_{H\text{-}2_B,H\text{-}3_B}$ = 9.7 Hz, H-2$_B$), 5.01 (dd, 1 H, $J_{2_C,3_C}$ = 7.8 Hz, H-2$_C$), 5.19 (dd, 1 H, $J_{H\text{-}3_B,H\text{-}4_B}$ = 9.0 Hz, H-3$_B$); ^{13}C NMR ($CDCl_3$) δ 11.4 (CH_3S), 20.6 (2x), 20.7 (3x) (5 CH_3CO), 57.4 (CH_3O), 62.3, 62.5 (C-6$_B$,6$_C$), 64.6, 69.4, 70.5, 72.0, 73.4, 75.8, 76.8 (C-2$_B$,3$_B$,4$_B$,5$_B$,2$_C$,4$_C$,5$_C$), 80.9 (C-3$_C$), 82.7 (C-1$_B$), 100.8 (C-1$_C$), 169.2, 169.6, 169.8, 170.4, 170.7 (5 CH_3CO).

Methyl 2,3,6-Tri-*O*-acetyl-4-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-galactopyranosyl)-1-thio-β-D-glucopyranoside (23). A solution of **22** (1.6 g, 2.68 mmol) in pyridine (3 mL) and acetic anhydride (3 mL) was kept at 25 °C for 12 h and concentrated. Crystallization of the residue from diethyl ether gave **23** (1.35 g, 78.9%): mp 152–153 °C; $[\alpha]_D$ −3° (*c* 1.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.04, 2.06, 2.08, 2.10 (2x) 2.12 (5s, 4 × 3 H and

6 H, 6 CH_3CO), 2.16 (s, 3 H, CH_3S), 3.30 (dd, 1 H, $J_{H\text{-}2_C,H\text{-}3_C}$ = 10.0 Hz, $J_{H\text{-}3_C,H\text{-}4_C}$ = 3.4 Hz, H-3$_C$), 3.36 (s, 3 H, CH_3O), 3.67 (ddd, 1 H, H-5$_B$), 3.79 (dd, 1 H, H-4$_B$), 3.81 (ddd, 1 H, H-5$_C$), 4.13 (d, 2 H, $J_{H\text{-}5_C,H\text{-}6_C}$ = $J_{H\text{-}5_C,H\text{-}6'_C}$ = 6.8 Hz, H-6$_C$ and H-6′$_C$), 4.18 (dd, 1 H, $J_{H\text{-}5_B,H\text{-}6'_B}$ = 5.2 Hz, $J_{H\text{-}6_B,H\text{-}6'_B}$ = 12.0 Hz, H-6′$_B$), 4.41 (d, 1 H, $J_{H\text{-}1_B,H\text{-}2_B}$ = 10.0 Hz, H-1$_B$), 4.42 (d, 1 H, $J_{H\text{-}1_C,H\text{-}2_C}$ = 8.0 Hz, H-1$_C$), 4.49 (dd, 1 H, $J_{H\text{-}5,H\text{-}6}$ = 1.6 Hz, H-6$_B$), 4.99 (dd, 1 H, $J_{H\text{-}1_B,H\text{-}2_B}$ = 9.6 Hz, H-2$_B$), 5.00 (dd, 1 H, $J_{H\text{-}1_C,H\text{-}2_C}$ = 7.6 Hz, H-2$_C$), 5.24 (t, 1 H, H-3$_B$), 5.47 (dd, 1 H, $J_{H\text{-}4_C,H\text{-}5_C}$ = 0.7 Hz H-4$_C$); ^{13}C NMR (CDCl$_3$) δ 11.5 (CH_3S), 20.6 (6 CH_3CO), 58.0 (CH_3O), 61.3 and 62.3 (C-6$_B$,6$_C$), 64.8 (C-4$_C$), 69.6 and 70.5 (C-2$_B$,2$_C$), 70.9 (C-5$_C$), 73.6 (C-3$_B$), 76.0 (C-4$_B$), 76.8 (C-5$_B$), 79.8 (C-3$_C$), 82.8 (C-1$_B$), 101.1 (C-1$_C$), 169.1, 169.5, 169.7, 170.1, 170.4 (2x) (6 C=O). Anal. Calcd for $C_{26}H_{38}O_{16}S$ (638.62): C, 48.89; H, 6.00; S, 5.02. Found: C, 49.00; H, 6.10; S, 4.91.

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-galactopyranosyl)-β-D-glucopyranose (17). (a) A mixture of compound **15** (3.4 g, 5.18 mmol) and 10% palladium on carbon (200 mg) in ethanol (50 mL) and glacial acetic acid (50 mL) was stirred under hydrogen (1 atm) for 24 h at 25 °C. Removal of the catalyst by filtration followed by concentration gave a syrup that was dissolved in Ac_2O (60 mL) to which anhydrous NaOAc (5 g) was added. The solution was heated under stirring at reflux temperature for 5 min. The residue obtained after evaporation of the volatiles was partitioned between chloroform (100 mL) and water (2 × 50 mL), and the organic layer was dried and concentrated. The syrupy residue, which was shown by ^{1}H NMR spectroscopy in CDCl$_3$ to contain compound **17** and the corresponding α-anomer in a ratio of 1:1 (δ 6.58, d, $J_{H\text{-}1_B,H\text{-}2_B}$ = 8.1 Hz, H-1$_B$ (β); 6.14, d, $J_{H\text{-}1_B,H\text{-}2_B}$ = 3.6 Hz, H-1$_B$(α)) was dissolved in AcOH (30 mL) and then treated with 30% HBr in acetic acid (5 mL) at 0 °C for 2 h. The solution was diluted with chloroform (100 mL) followed by extraction with ice–water (5 × 100 mL) and drying. Concentration afforded unstable syrupy **16** (δ 6.61, $J_{H\text{-}1_B,H\text{-}2_B}$ = 4.1 Hz), which was dissolved in glacial acetic acid (50 mL). The solution was treated with Hg(OAc)$_2$ (4 g, 12.5 mmol) for 2 h at 25 °C. Water (100 mL) was added, and the solution was extracted with CHCl$_3$ (4 × 20 mL). The extracts were combined and washed with 5% aqueous NaHCO$_3$ (2 × 50 mL) and water (50 mL) followed by drying. Concentration left a semisolid that was purified by column chromatography in 2:1 ethyl acetate–hexane to provide crystalline **17** (2.0 g, 59.4%): mp 120–122; [α]$_D$ +9° (*c* 1.1, CHCl$_3$); ^{1}H NMR (CDCl$_3$) δ 2.03, 2.05, 2.08, 2.09 (2x), 2.12, 2.14 (6s, 5 × 3H, 6 H, 7 CH_3CO), 3.27 (dd, 1 H, $J_{H\text{-}2_C,H\text{-}3_C}$ = 10.0 Hz, $J_{H\text{-}3_C,H\text{-}4_C}$ = 3.4 Hz, H-3$_C$), 3.33 (s, 3 H, CH_3O), 3.74–3.88 (H-4$_B$,5$_B$,5$_C$), 4.10 (d, 2 H, $J_{H\text{-}5_C,H\text{-}6_C}$ = $J_{H\text{-}5_C,H\text{-}6'_C}$ = 6.7 Hz, H-6$_C$,6′$_C$), 4.17 (dd, 1 H, $J_{H\text{-}5_B,H\text{-}6'_B}$ = 4.6 Hz, $J_{H\text{-}6_B,H\text{-}6'_B}$ = 12.1 Hz, H-6′$_B$), 4.39 (d, 1 H, $J_{H\text{-}1_C,H\text{-}2_C}$ = 8.0 Hz, H-1$_C$), 4.43 (dd, 1 H, $J_{H\text{-}5_B,H\text{-}6_B}$ = 1.8 Hz, H-6$_B$), 4.96 (dd, 1 H, H-2$_C$), 5.04 (dd, 1 H, $J_{H\text{-}1_B,H\text{-}2_B}$ = 8.2 Hz, $J_{H\text{-}2_B,H\text{-}3_B}$ = 9.6 Hz,H-2), 5.23 (t, 1 H, H-3$_B$), 5.44 (dd, 1 H, $J_{H\text{-}4_C,H\text{-}5_C}$ = 1.1 Hz, H-4$_C$), 5.67 (d, 1 H, H-1$_B$); ^{13}C NMR (CDCl$_3$) δ 20.7 (CH_3CO), 57.9 (CH_3O), 61.4 (C-6$_C$), 61.8 (C-6$_B$), 64.9 (C-4$_C$), 70.4 (C-2$_B$,2$_C$), 70.9 and 73.5 (C-5$_B$,5$_C$), 72.5 (C-3$_B$), 75.4 (C-4$_B$), 79.6 (C-3$_C$), 91.4 (C-1$_B$), 100.8 (C-1$_C$), 168.8 (2x), 169.1 (2x), 169.5, 170.1, 170.4, (7 C=O). Anal. Calcd for $C_{27}H_{38}O_{18}$ (650.51): C, 49.85; H, 5.89. Found: C, 50.25; H, 6.05.

(b) A solution of thioglycoside **23** (2.1 g, 3.29 mmol) in acetic anhydride (50 mL) was treated[32] with nitrosyl tetrafluoroborate (400 mg, 3.45 mmol) at 0 °C for 30 min. Solid NaHCO$_3$ was added, and the mixture was concentrated. A solution of the residue in chloroform (80 mL) was extracted with water (3 × 20 mL), dried, and concentrated. Crystallization of the residue from diethyl ether gave **17** (1.95 g, 91.1%). This preparation had physical properties identical with those of the product obtained by method a.

Methyl 2-Acetamido-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-methyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (24). A stirred mixture of compound **13** (800 mg, 1.067 mmol), compound **17** (1000 mg, 1.53 mmol), powdered, 3A molecular sieves (2 g), and anhydrous dichloromethane (12 mL) was treated with trimethylsilyl trifluoromethanesulfonate (600 mL) at 25 °C for 24 h. Ice-cold, 5% aqueous NaHCO$_3$ (10 mL) was added, and the mixture was filtered. The filter cake was extracted with dichloromethane (5 × 5 mL). The dichloromethane fractions were combined and concentrated. Chromatography of the residual syrup with ethyl acetate gave **24** as an amorphous solid (650 mg, 42.7%): [α]$_D$ −22° (*c* 0.7, CHCl$_3$); ^{13}C NMR (CDCl$_3$) δ (partial data) 20.6 (CH_3COO), 23.1 (CH_3CON), 50.5 (C-2$_A$), 58.4 and 57.9 (2 CH_3O), 60.6, 61.3 and 62.0 (C-6,6$_C$,6$_D$), 64.6 (C-4$_C$), 66.6, 69.1, 70.5, 70.8 (C-2$_D$,3$_D$,4$_D$,5$_D$), 69.4 (C-6$_A$), 74.2 (C-5$_A$), 75.8 (C-4$_A$,4$_B$), 79.8 (C-3$_C$), 99.7 (C-1$_A$), 100.7 and 101.1 (2x) (C-1$_B$,1$_C$,1$_D$), 127.6, 128.2 (aromatic carbons), 138.0 (quaternary aromatic carbon), 169.1–170.3 (C=O).

Methyl 2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-6-*O*-[4-*O*-(3-*O*-methyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-D-glucopyranoside (5). A solution of tetrasaccharide **24** (200 mg, 0.16 mmol) in 20 mL of methanol was treated with sodium methoxide until the pH of the solution reached 11 (indicator paper), and then the solution was left standing at 25 °C for 24 h. The solution was neutralized (Dowex 50, H$^+$) and concentrated. A mixture of the residue and 10% palladium on carbon (300 mg) in 95% ethanol (10 mL) and glacial acetic acid (2 mL) was stirred under hydrogen (1 atm) at 25 °C for 24 h. The catalyst was removed by filtration, and the solution was concentrated. Purification of the residue through a column of Sephadex G-15 eluted with water gave **5** as an amorphous white powder (92 mg, 77.9%); [α]$_D$ +3° (*c* 1.0, H_2O). For ^{1}H and ^{13}C NMR data, see Tables I–IV.

Methyl 2-Acetamido-2-deoxy-4-*O*-[3-*O*-(α-D-*N*-acetylneuraminyl)-β-D-galactopyranosyl]-6-*O*-[4-*O*-(3-*O*-methyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-β-D-glucopyranoside (4). Tetrasaccharide **5** (50 mg, 68 μmol) was sialylated as described in ref 13, using CMP-Neu5Ac as *N*-acetylneuraminic acid donor under catalysis by β-galactoside-α2,3-sialyltransferase (rat liver) to give, after purification through Sephadex G-15 (water), pentasaccharide **4** (24 mg, 33.8%); [α]$_D$ +6° (*c* 0.5, H_2O). For ^{1}H and ^{13}C NMR data, see Tables I–IV.

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Supplementary Material Available: NMR spectra of **4**, **5**, **12**, **13**, **22**, and **24** (9 pages). Ordering information is given on any current masthead page.