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Analysis by the reductive-cleavage method of a polysaccharide containing 2-acetamido-2,6-dideoxy-D- and -L-galactopyranosyl residues †

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ABSTRACT

Modifications of the reductive-cleavage method for the analysis of 2-acetamido sugars were tested using a polysaccharide containing α-linked 2-acetamido-2,6-dideoxy-L-galactopyranosyl (Fuc*p*NAc) residues, β-linked D-Fuc*p*NAc residues and 2-linked D-glucopyranosyl residues. Reductive cleavage of the fully methylated O-antigenic polysaccharide of *Pseudomonas aeruginosa* ATCC 33358 in the presence of triethylsilane and trimethylsilyl trifluoromethanesulfonate, followed by quenching with methanol and subsequent acetylation, unexpectedly resulted in nearly complete cleavage of all glycosidic linkages to yield 2-*O*-acetyl-1,5-anhydro-3,4,6-tri-*O*-methyl-D-glucitol (**1a**) and methyl 3-*O*-acetyl-2,6-dideoxy-4-*O*-methyl-2-(*N*-methylacetamido)-β-D- and -L-galactopyranosides (**3a** and **4a**) as the major products. When the reductive-cleavage reaction was quenched with (*S*)-2-butanol, the major FucNAc derivatives were the diastereomeric (*S*)-2-butyl glycosides **15a** and **17a**, confirming the presence of enantiomeric FucNAc residues in the repeating unit of the polysaccharide. However, compounds **15a** and **17a** were not detected in equimolar proportions, presumably as a consequence of diastereoselectivity in the reaction of the chiral alcohol with the respective intermediate oxazolinium ions. Reductive cleavage of the fully methylated polysaccharide in the presence of a mixture of trimethylsilyl methanesulfonate and boron trifluoride etherate, followed by quenching with methanol, resulted in incomplete cleavage, giving rise to three disaccharide derivatives whose sequences overlap that of the trisaccharide repeating unit in the polysacharide. The lack of selectivity for cleavage at β-D-FucNAc residues suggests that the α-L-FucNAc residues underwent anomerization prior to transglycosidation.

INTRODUCTION

In a previous study[1], the conditions of the reductive-cleavage method[2] were modified to allow simultaneous analysis of 2-acetamido-2-deoxyhexopyranosyl residues and monosaccharides of other classes. Methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-D-glucopyranoside was found to undergo transglycosida-

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tion under reductive-cleavage conditions when reactions were quenched with an alcohol. Transglycosidation proceeded via an oxazolinium-ion intermediate, which then acted as a glycosyl donor to form an anomerically pure product. The use of an optically active alcohol as the glycosyl acceptor made possible the application of the reductive-cleavage method to the determination of the absolute configuration of acetamido sugar residues. In contrast, the corresponding α anomer was unreactive under reductive-cleavage conditions, presumably because the 1,2-*cis* orientation did not permit anchimeric assistance by the *N*-acetyl group in cleavage of the glycosidic bond. Thus, the resistance of a 1,2-*cis*-linked acetamido sugar residue to reaction under reductive-cleavage conditions could presumably be exploited to generate oligosaccharide derivatives which would yield sequence information.

In this study, the modified reductive-cleavage procedures were tested on the O-antigenic polysaccharide of *Pseudomonas aeruginosa* ATCC 33358 (international serotype 11) (ref 3), which has previously been reported[4,5] to have a repeating unit of the following structure:

$$[\rightarrow 2)\text{-}\beta\text{-D-Glc}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-L-Fuc}p\text{NAc-}(1\rightarrow 3)\text{-}\beta\text{-D-Fuc}p\text{NAc-}(1\rightarrow]_n$$

This polysaccharide was chosen because the two FucNAc residues provide suitable structural features for testing the selectivity of the method for cleavage at 1,2-*trans*-linked acetamido sugar residues as well as the method's usefulness in establishing the absolute configuration of acetamido sugars.

RESULTS

Isolation of the O-antigenic polysaccharide of P. aeruginosa *ATCC 33358.*—The lipopolysaccharide (LPS) produced by *P. aeruginosa* ATCC 33358 was isolated from dried cells in 22% crude mass yield (1–5% protein)[6] by a modification of the procedure of Darveau and Hancock[6,7]. Hydrolysis of the LPS with hot, dilute acetic acid followed by Sephadex G-50 gel-permeation chromatography of the water-soluble product gave the O-antigen (7% by mass from LPS), eluting at K_{av} 0.02 to 0.17, and two additional fractions assumed to contain core oligosaccharide, eluting at K_{av} 0.59 (lit.[8] K_{av} 0.53), and 3-deoxy-D-*manno*-octulosonic acid, eluting at K_{av} 1.00 (lit.[8] K_{av} 0.98).

Reductive cleavage of the fully methylated polysaccharide.—The fully methylated[9] O-antigen was subjected to reductive cleavage under conditions previously developed for *N*-acetylglucosamine-containing carbohydrates[1] that were shown to be selective for β-glycosidic linkages. Thus, the fully methylated O-antigen was treated with triethylsilane (Et_3SiH) as the reducing agent and one of two electrophilic promoters, namely trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$) (ref 2), or a mixture[10] of trimethylsilyl methanesulfonate (Me_3SiOSO_2Me) and boron trifluoride etherate ($BF_3 \cdot OEt_2$). At the end of the reaction period, methanol, (±)-2-butanol, or (*S*)-2-butanol was added to quench the

1a R = Ac
1b R = Bz
7 R = Me

AcO—3L-Fucp NAc(α)→3D-Fucp NAc(β)—OMe

2

AcO—2D-Glcp(β)→3L-Fucp NAc(β)—OMe

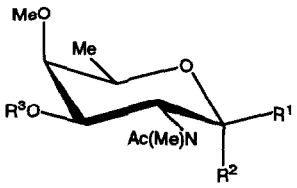
12

AcO—2D-Glcp(β)→3 1,5An-L-FucNAc-ol

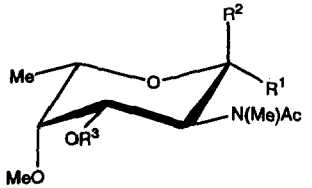
13

AcO—3D-Fucp NAc(β)→2 1,5An-D-Glc-ol

14



	R^1	R^2	R^3
3a	OMe	H	Ac
3b	OMe	H	Bz
5	H	OMe	Ac
8	OMe	H	Me
10a	H	H	Ac
10b	H	H	Bz
16	*(R)*-2-Bu	H	Ac
17a	*(S)*-2-Bu	H	Ac
17b	*(S)*-2-Bu	H	Bz



	R^1	R^2	R^3
4a	OMe	H	Ac
4b	OMe	H	Bz
6	H	OMe	Ac
9	OMe	H	Me
11a	H	H	Ac
11b	H	H	Bz
15a	*(S)*-2-Bu	H	Ac
15b	*(S)*-2-Bu	H	Bz
18	*(R)*-2-Bu	H	Ac

promoter and to provide a glycosyl acceptor for acetamido sugar-derived intermediates[1]. A portion of the product mixture was subsequently acetylated, and the derivatives so obtained were analyzed by gas–liquid chromatography (GLC) and GLC combined with mass spectrometry (GLC–MS) in the chemical-ionization (CI) and electron-ionization (EI) modes. The molar ratios for the products identified in these experiments are summarized in Table I.

Reductive cleavage–methanolysis.—Reductive cleavage of the fully methylated O-antigen followed by quenching with methanol and acetylation was expected[1] to yield the product (**1a**) derived from 2-linked D-Glc*p* residues and the disaccharide derivative **2** in equimolar proportions owing to the presence of 1,2-*cis*-linked (α) L-Fuc*p*NAc residues. Indeed, treatment of the fully methylated O-antigen with

TABLE I

Molar ratios of products derived from reductive cleavage of the fully methylated O-antigen of *P. aeruginosa* ATCC 33358

Compound(s)	ECR [a]	Molar ratio [b]			
		A [c]	*B*	*C*	*D*
1a	1.09	1.00	1.00	1.00	1.00
2	2.63	0.11	0.33		
3a, 4a	1.46	1.76	1.35	0.18	0.14
5, 6	1.46	0.04			
7	1.00	0.01	0.07	0.05	0.04
8, 9	1.37	0.02	0.03		
10a, 11a	1.29	0.05	0.10		
12	2.43	0.12		0.17	0.16
13	2.26		0.18		
14	2.26		0.27		
15a [d]	2.06			0.93 [e]	0.45
17a [d]	2.06			1.06 [f]	1.21

[a] Effective carbon response; molar response factors were calculated as described in ref 21 and normalized to **7**. [b] Flame-ionization-detector response were corrected for molar response and normalized to **1a**. [c] Experimental conditions as designated in the text. [d] The values for **15a** and **17a** may be reversed; the assignments are based upon optical rotation data for the corresponding benzoates (**15b** and **17b**). [e] Includes the coeluting enantiomeric (*R*)-2-butyl glycoside (**16**). [f] Includes the coeluting enantiomeric (*R*)-2-butyl glycoside (**18**).

Et_3SiH and $Me_3SiOSO_2CF_3$ for 2 h, followed by quenching with methanol and then acetylation (Experiment *A*), yielded **1a** as a major product; however, only a trace of the expected disaccharide (**2**) was observed (Fig. 1A). Instead, the glycosidic linkages of both Fuc*p*NAc residues were essentially completely cleaved to yield **3a** and its L-enantiomer (**4a**) in a combined molar ratio of 1.76:1 relative to **1a** (Table I). Similar results were obtained when reductive cleavages were carried out for shorter (1 h) and longer (24 h) reaction periods. Compound **1a** was identified by comparison of its GLC retention indices and CI- and EI-mass spectra to those of an authentic standard (L.E. Elvebak II and G.R. Gray, unpublished work) whereas the **3a, 4a** mixture was identified on the basis of its CI-mass spectrum, which showed ions at *m/z* 290 $[(M + H)^+]$ and 258 $[(M - CH_3O)^+$, oxazolinium ion], and its EI-mass spectrum, which was essentially identical to that reported by Dmitriev et al.[4]. Disaccharide **2** was also identified on the basis of its CI-mass spectrum, which displayed a pseudomolecular ion at *m/z* 505 $[(M + H)^+]$, an abA_1^+ fragment (designated according to the notation of Kochetkov and Chizhov[11]) at *m/z* 473 $[(M - CH_3O)^+]$, a primary fragment ion at *m/z* 258 (aA_1^+), and a secondary fragment ion at *m/z* 198 $[(aA_1 - HOAc)^+$ or $(bA_1 + H - H_2O)^+]$.

Among the minor products observed in Experiment *A* were **5** and **6**, the α anomers of **3a** and **4a**, respectively, and **7**, which was identified by comparison of its GLC retention indices and CI- and EI-mass spectra to those of an authentic

standard (L.E. Elvebak II and G.R. Gray, unpublished work). The latter product, which was derived from nonreducing terminal glucopyranosyl residues, was expected based on the reported phase of the repeating unit in the polysaccharide[4,5]. However, compound **8** or its L-enantiomer (**9**) [CI-mass spectrum: *m/z* 279, $(M + NH_4)^+$; 262, $(M + H)^+$; 230, $(M - CH_3O)^+$], derived from nonreducing terminal FucNAc residues, was also detected. A trace of the 1,5-anhydroalditol derivative (**10a** or **11a**; M_r 259) of FucNAc also was produced by the unexpected reductive cleavage of the acetamidosugar residues. The **10a**, **11a** mixture was identified on the basis of its CI-mass spectrum, which displayed pseudomolecular ions at *m/z* 277 $[(M + NH_4)^+]$, and 260 $[(M + H)^+]$. Another disaccharide **12** was formed in a slightly higher proportion (Table I) than **2** and was identified by its CI-mass spectrum [*m/z* 494, $(M + H)^+$; 462, $(M - CH_3O)^+$, abA_1^+; 247, aA_1^+; and 198, $(bA_1 + H - H_2O)^+$].

The anomalous results obtained from Experiment *A* prompted another experiment in which the fully methylated O-antigen was subjected to reductive cleavage in the presence of Me_3SiOSO_2Me and $BF_3 \cdot OEt_2$ (Experiment *B*). Studies[1] with model compounds had demonstrated that 1,2-*trans*-linked acetamido sugars were cleaved slowly under these conditions and that 1,2-*cis*-linked acetamido sugars were not cleaved at all. Thus, the conditions of Experiment *B* were expected to yield a substantially greater proportion of disaccharide **2**, the formation of which would prove the reported[4,5] structure of the O-antigen. Indeed, disaccharide **2** was formed in a greater proportion in Experiment *B* than in Experiment *A* (Fig. 1B, Table I), but the expected selectivity of reductive cleavage was not observed; that is, **1a** and **2** were not formed exclusively. Instead, the glycosidic linkages of both FucNAc residues were substantially cleaved in Experiment *B*, as in Experiment *A*, to yield **1a** and the **3a**, **4a** mixture as major products. Furthermore, the incomplete cleavage observed in Experiment *B* was random, as evidenced by the presence of dimers (**2**, **13**, and **14**) representing all of the possibilities available from the trisaccharide repeating unit in the polysaccharide. The proportion of disaccharide derivatives produced under these conditions was reduced to trace levels when reductive cleavage was allowed to proceed for 24 h. Interestingly, compound **13**, which contains a reducing-terminal 2-acetamido-1,5-anhydro-2,6-dideoxyfucitol (1,5An-FucNAc) residue, was formed in preference to **12**, in which the reducing-terminal FucNAc residue occurs as the 1,2-*trans* methyl glycoside; the reason for this preference is unknown. Compounds **13** and **14**, which both have molecular masses of 463, were characterized on the basis of their CI-mass spectra, which distinguished the two products by their aA_1^+ fragment ions (**13**, *m/z* 247; **14**, *m/z* 258).

Reductive cleavage–2-butanolysis.—Treatment of the fully methylated O-antigen with Et_3SiH and $Me_3SiOSO_2CF_3$ for 4 h, followed by quenching with (±)-2-butanol and subsequent acetylation (Experiment *C*), yielded nearly equal proportions (Table I) of **1a** and the diastereomeric (*R*)- and (*S*)-2-butyl-glycosides of the FucNAc residues [CI(NH_3)-mass spectra: $(M - C_4H_9O)^+$, *m/z* 258; $(M + H)^+$,

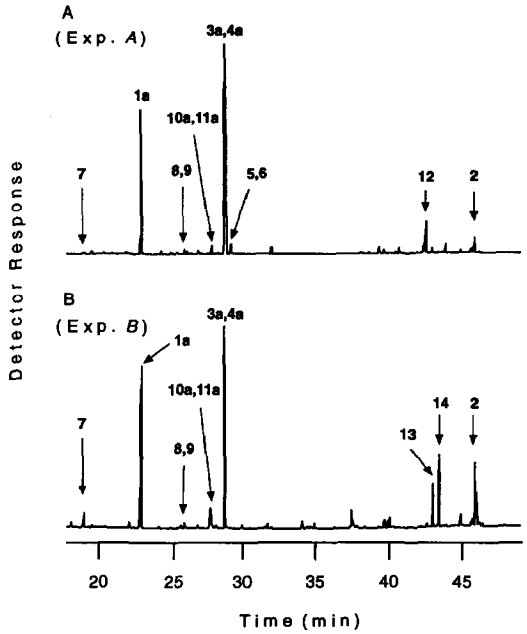


Fig. 1. Gas–liquid chromatograms of acetylated products obtained by reductive cleavage of the fully methylated O-antigen of *P. aeruginosa* ATCC 33358, under the conditions of (A) Experiment *A* and (B) Experiment *B*.

m/z 332; $(M + NH_4)^+$, *m/z* 349]. Gas–liquid chromatography of the product mixture obtained from Experiment *C* (Fig. 2A) demonstrated that the diastereomers were separable. Thus, **15a**, the (*S*)-2-butyl glycoside of L-Fuc*p*NAc, coeluted with its enantiomer (**16**), the (*R*)-2-butyl glycoside of D-FucNAc, and **17a**, the (*S*)-2-butyl glycoside of D-Fuc*p*NAc, coeluted with its enantiomer (**18**), the (*R*)-2-butyl glycoside of L-FucNAc. However, Experiment *C* did not prove that both the D- and L-forms of FucNAc were found in the polysaccharide, since the presence of either form alone would give the same result.

The use of (*S*)-2-butanol as the quenching agent and glycosyl acceptor (Experiment *D*) did, however, result in the formation of both **15a** and **17a** (Fig. 2B, Table I). Thus, the presence in the native polysaccharide of both the D- and L-forms of FucNAc was confirmed. However, the authentic standards of **15a** and **17a** were not available, so it was not possible to assign either of the peaks in the gas–liquid chromatogram to the appropriate diastereomer. Moreover, the diastereomeric glycosides (**15a** and **17a**) were formed in a ratio of 2.69:1, rather than 1:1 as would be expected if, indeed, the polysaccharide contained equimolar proportions of both D- and L-FucNAc. Examination of the product mixtures of Experiments *C* and *D* by ^1H NMR spectroscopy eliminated the possibility that the nonunity ratio of **15a** and **17a** was due to the presence of coeluting α anomers; that is, signals attributable to α anomers were not observed in the region of 4.9–5.0 ppm.

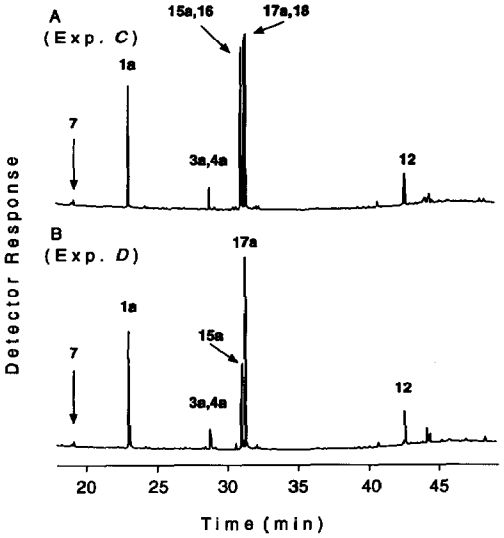


Fig. 2. Gas–liquid chromatograms of acetylated products obtained by reductive cleavage of the fully methylated O-antigen of *P. aeruginosa* ATCC 33358 under the conditions of (A) Experiment *C* and (B) Experiment *D*. The reactions were quenched with (±)-2-butanol (Experiment *C*) and (*S*)-2-butanol (Experiment *D*). Compounds **3a**, **4a**, and **12** arose due to the presence of residual methanol in the mixed-bed resin used for deionization.

Furthermore, the ratios of the integrals (1.1:1 in Experiment *C* and 2.7:1 in Experiment *D*) of the resonances for H-1′ of the 2-butyl group in **15a** and **17a**, confirmed the diastereomeric ratios obtained by GLC. From these results it was evident that either D- and L-FucNAc were not present in equimolar proportions in the polysaccharide or that the rates of reaction of (*S*)-2-butanol with the intermediate oxazolinium ions of the D- and L-FucNAc residues were significantly different.

Analysis of benzoylated reductive-cleavage products by ^1H NMR spectroscopy and mass spectrometry.—In order to confirm the structures of reductive-cleavage products, the fully methylated O-antigen was subjected to reductive cleavage in the presence of Et_3SiH and $Me_3SiOSO_2CF_3$ for 2 h, followed by quenching with methanol, Et_3SiH and Me_3SiOSO_2Me and $BF_3 \cdot OEt_2$ for 3 h, followed by quenching with methanol, and Et_3SiH and Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$ for 4 h, followed by quenching with (*S*)-2-butanol. The products so obtained were benzoylated and separated by semipreparative high-performance liquid chromatography with detection at 275 nm[12] (Fig. 3), and all the components were collected and analyzed by ^1H NMR spectroscopy and direct-probe CI(NH_3)-mass spectrometry. Compounds **1b**, **3b** plus **4b**, and **10b** plus **11b** were isolated in all three experiments, and for all three components, their CI-mass spectra gave the predicted molecular weights (see Experimental). The identity of **1b** was confirmed by comparison of its ^1H NMR spectrum to that of an independently synthesized

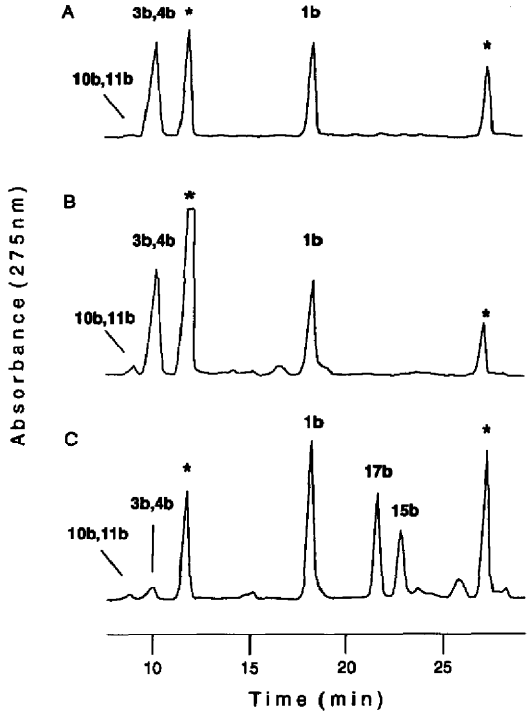


Fig. 3. High-performance liquid chromatograms of benzoylated products obtained by reductive cleavage of the fully methylated O-antigen of *P. aeruginosa* ATCC 33358 in the presence of Et_3SiH and $Me_3SiOSO_2CF_3$, followed by methanolysis (A); Et_3SiH and Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$, followed by methanolysis (B); and Et_3SiH and Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$, followed by (*S*)-2-butanolysis (C). Asterisks (*) denote peaks detected in a reagent blank.

standard (L.E. Elvebak II and G.R. Gray, unpublished work). The identity of the **3b**, **4b** mixture was confirmed by its ^1H NMR spectrum that displayed an anomeric-proton doublet at δ 4.47 ($J_{1,2}$ 8.0 Hz) and a doublet of doublets ($J_{2,3}$ 11.0, $J_{3,4}$ 3.3 Hz) for H-3 at δ 5.34. The ^1H NMR spectrum of the **10b**, **11b** mixture was fully in accord with its structure. In addition to the signals for *C*-methyl, *N*-methyl, *O*-methyl, *N*-acetyl, and *O*-benzoyl groups, the spectrum of **10b**, **11b** also contained two H-1 resonances, namely H-1a (δ 3.60 t, $J_{1a,1e}$ 11.0, $J_{1a,2}$ 11.0 Hz) and H-1e (δ 3.99 dd, $J_{1e,2}$ 8.0 Hz). Also, the presence of the ester methine resonance (δ 5.35) as a doublet of doublets ($J_{2,3}$ 11.0, $J_{3,4}$ 3.3 Hz) confirmed that the benzoyl group was present at O-3. The ^1H NMR spectra of the **3b**, **4b** and **10b**, **11b** mixtures were complicated by the detection of rotamers of the 2-deoxy-2-(*N*-methylacetamido) group, as evidenced by the duplication of the signals for the *C*-methyl, *N*-methyl, *O*-methyl, *N*-acetyl, and *O*-benzoyl groups.

Reductive cleavage of the fully methylated O-antigen with Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$ followed by the addition of (*S*)-2-butanol gave **15b** and **17b** as major products (Fig. 3C). The CI-mass spectra of these two compounds were identical;

however, they were clearly distinguished on the basis of their ^{1}H NMR spectra, which were identical except for the signals arising from their (*S*)-butyl groups. Specifically, the chemical shifts of H-2′, H-3′, and H-4′ of the 2-butyl group of the later-eluting component were downfield from the same signals in the earlier-eluting component, and the H-1′ signal of the (*S*)-2-butyl group of the later-eluting component appeared upfield from that of earlier-eluting component. Although the amounts of these components isolated by HPLC were insufficient to obtain accurate specific rotations, polarimetric measurements at several wavelengths showed that the earlier-eluting diastereomer was dextrorotatory, whereas the later-eluting diastereomer was levorotatory. Since (*S*)-2-butanol is dextrorotatory {$[\alpha]_D^{20} + 14.2°$, $[\alpha]_{546}^{20} + 16.6°$ (*c* 10, H_2O); Certificate of Analysis, Fluka Chemical Co.} and 2-acetamido-2,6-dideoxy-L-galactopyranose is levorotatory {$[\alpha]_D^{23} - 115 \rightarrow -82°$ (equil., H_2O)[13]}, a preliminary assignment of absolute configurations of the diastereomers can be proposed; specifically, the later-eluting component is the L-Fuc*p*NAc derivative (**15b**), and the earlier-eluting component is the D-Fuc*p*NAc derivative (**17b**).

Analysis of the O-antigen by ^{1}H and ^{13}C NMR spectroscopy.—Based on previous results[1] with the fully methylated methyl glycosides of *N*-acetylglucosamine, reductive cleavage of the fully methylated O-antigen was expected to yield products **1a** and **2** in equimolar amounts when the reactions were quenched with methanol. However, the experiments just described did not result in the anticipated selectivity. To verify the reported structure of the O-antigen, particularly the assignments of anomeric configurations, ^{1}H and ^{13}C NMR experiments were performed on the native polysaccharide. The combination of these techniques, which included two-dimensional homonuclear shift correlation (COSY) and total correlation (TOCSY) spectroscopy, made possible the full assignment of the one-dimensional ^{1}H NMR spectrum of the O-antigen (Table II).

The ^{1}H NMR spectrum (500 MHz, 60°C) included three anomeric signals at δ 4.57 (d, $J_{1,2}$ 7.6 Hz), 4.65 (d, $J_{1,2}$ 8.3 Hz), and 5.02 (d, $J_{1,2}$ 3.6 Hz), two signals for acetamido groups at δ 2.03 and 2.05, and two CH_3C signals at δ 1.29 (d, $J_{5,6}$ 6.4 Hz) and 1.30 (d, $J_{5,6}$ 6.4 Hz). The ^{13}C NMR spectrum (125 MHz, 60°C), contained 22 signals, indicating a trisaccharide repeating unit. The spectrum showed signals for acetamido groups[14] at δ 174.9 and 175.2 ($NHCOCH_3$) and at δ 23.2 and 23.3 ($NHCOCH_3$), as well as C-2 signals for 2-acetamido sugars[16] at δ 48.7 and 52.5. Signals for anomeric carbons appeared at δ 99.9, 100.0, and 103.5, and those for C-6 appeared at δ 16.2 and 16.3 (CH_3C) and at δ 61.8 (CH_2OH). The remaining ring-methine carbon resonances appeared in the range of δ 69.0–82.0. These data are consistent with previous findings[4,5] of a polysaccharide with a trisaccharide repeat unit containing one Glc*p* and two Fuc*p*NAc residues with one alpha and two beta linkages. The downfield (δ 5.02) anomeric proton resonance was assigned to one of the FucNAc residues. This signal was coupled to a signal at δ 4.23 (dd, *J* 3.6, 11.1 Hz), which in turn was coupled to another signal at δ 4.16 (dd, *J* 2.6, 11.1 Hz), consistent with a residue of the *galacto* rather than the *gluco* configuration

TABLE II

500-MHz ^{1}H NMR assignments for the O-antigenic polysaccharide of *P. aeruginosa* ATCC 33358

Resonance	δ_H multiplicity (J, Hz)		
	→ 2)-β-D-Glc*p*-(1 →	→ 3)-α-L-Fuc*p*NAc-(1 →	→ 3)-β-D-Fuc*p*NAc-(1 →
H-1	4.57d (7.6)	5.02d (3.6)	4.65d (8.3)
H-2	3.41dd (7.6, 8.8)	4.23dd (3.6, 11.1)	4.02dd (8.3, 10.7)
H-3	3.50t (8.8)	4.16dd (2.6, 11.1)	3.79dd (3.1, 10.7)
H-4	3.36dd (8.8, 9.6)	4.09br d [b] (2.6)	3.74br d [b] (3.1)
H-5	3.40ddd [a] (2.2, 5.7, 9.6)	4.12br q [a,c] (6.4)	3.78br q [a,c] (6.4)
H-6	3.72dd (5.7, 12.1) 3.92dd (2.2, 12.1)	1.29d [a] (6.4)	1.30d [a] (6.4)

[a] Assignment was established by two-dimensional ^{1}H NMR experiments. [b] Broadened doublet. [c] Broadened quartet.

(Table II). These data therefore established that one of the Fuc*p*NAc residues was α-linked and that the other Fuc*p*NAc residue and the Glc*p* were β-linked, as previously reported[4,5,15].

DISCUSSION

These studies confirmed the utility of solvolysis with alcohols under reductive-cleavage conditions as a means to generate alkyl glycoside derivatives of 2-acetamidosugar residues that can be analyzed simultaneously with derivatives of other sugars. In this respect, the results of previous studies[1] with fully methylated methyl β-D-GlcNAc, which underwent transglycosidation under reductive-cleavage conditions, were borne out by the experiments with the fully methylated O-antigen of *P. aeruginosa* ATCC 33358.

However, the fact that all glycosidic linkages in the polysaccharide were cleaved led to the initial conclusion that the previously reported[4,5] structure of the repeating unit was in error. Specifically, since the 1,2-*cis*-linked L-Fuc*p*NAc residues unexpectedly yielded monosaccharide derivatives, the anomeric configurations of the L-Fuc*p*NAc and Glc*p* residues were thought to have been reversed. However, examination of the native polysaccharide by ^{1}H and ^{13}C NMR spectroscopy demonstrated that the anomeric assignments were indeed correct. Thus, the selectivity for 1,2-*trans*-linked acetamido sugar residues observed[1] in experiments with the α- and β-methyl glycosides of GlcNAc was not observed with the α- and β-linked FucNAc residues. Cleavage of the α-glycosidic linkage of the L-FucNAc residue most likely occurred as a consequence of anomerization of this residue, followed by cleavage of the now-1,2-*trans*-oriented glycosidic bond. This conclusion is consistent with the observation that glycosides of other types undergo anomerization under reductive-cleavage conditions[16].

The use of (*S*)-2-butanol as the quenching alcohol in these studies demonstrated the value of the reductive-cleavage method in distinguishing between the enantiomers of 2-acetamido-2-deoxy sugars. The high degree of cleavage in these experiments was helpful in determining the presence in the *P. aeruginosa* O-antigen of both enantiomeric forms of FucNAc. This task was made easier by the formation of both diastereomeric (*S*)-2-butyl glycosides (**15a** and **17a**); thus, isolation and further manipulation of the expected disaccharide product [the (*S*)-2-butyl analogue of **2**] containing both FucNAc residues was not necessary. Because authentic standards for these compounds (**15a** and **17a**) were not available, assignment of their absolute configuration was not possible. However, polarimetric measurements of their corresponding benzoates (**15b** and **17b**) did permit preliminary structural assignments based upon optical-rotation data available in the literature.

Unexpectedly, however, the diastereomeric (*S*)-2-butyl glycosides of D- and L-FucNAc were not obtained in equimolar proportions (Experiment *D*). Although this result could be explained by the presence of D- and L-FucNAc in unequal proportions in the polysaccharide, in this case the repeating unit of the polysaccharide could not be a trisaccharide. However, ^{1}H and ^{13}C NMR data conclusively prove that the polysaccharide is comprised of a trisaccharide repeating unit. Alternatively, the oxazolinium-ion intermediates resulting from cleavage of the FucNAc residues may react diastereoselectively with (*S*)-2-butanol. The remainder of the less reactive oxazolinium-ion intermediate then would have been removed from the product mixture by deionization with mixed-bed resin, causing product ratios to be skewed in favor of the derivative of the more reactive intermediate. The differences in the ^{1}H NMR chemical shifts of the aglyconic protons of the diastereomeric (*S*)-2-butyl glycosides (**15b** and **17b**) suggest that the electronic environments of the respective intermediate oxazolinium ions are sufficiently different that their rates of reaction with another chiral species could also be different. Further studies with derivatives of other 2-acetamido sugars and both enantiomers of (*S*)-2-butanol should be conducted to determine whether the transglycosidation reaction indeed proceeds diastereoselectively.

EXPERIMENTAL

General.—NMR spectra were recorded at room temperature (organic solutions) or 60°C (aqueous solutions) on a Varian VXR-500 spectrometer equipped with a VNMR data system. ^{1}H NMR spectra were recorded at 500 MHz. Chemical shifts in spectra recorded in $CDCl_3$ were referenced to internal tetramethylsilane; those recorded in D_2O were referenced to internal sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate (Aldrich Chemical Co.). The ^{13}C NMR spectrum of the native O-antigen was recorded in D_2O at 60°C and 125 MHz; chemical shifts were referenced to external 1,4-dioxane (δ 67.4) in D_2O. Two-dimensional homonuclear

^{1}H COSY[17] and TOCSY (homonuclear Hartmann–Hahn spin-lock)[18,19] experiments were performed on the native O-antigen using standard pulse sequences programmed into the Varian VNMR software.

Semipreparative reversed-phase high-performance liquid chromatography (HPLC) of benzoate derivatives was performed on a Beckman Instruments System Gold model 338 instrument equipped with a Hewlett–Packard model 3390A recording integrator. A stainless-steel column (4.6 mm × 25 cm), packed with 5-μm particle-size octadecylsilica (Kromasil KR100-5C18, Nobel Industries) and fitted with a glass water jacket, was maintained at 55°C by a circulating water bath. The column was eluted at a flow rate of 1.0 mL/min with 40% MeOH in H_2O, followed by a 25-min linear gradient to 80% MeOH. The effluent was monitored at 275 nm. Solvents (HPLC grade) were used after deaeration and filtration through a 0.22-μm nylon membrane.

Analytical GLC was performed on a Hewlett–Packard model 5890A gas–liquid chromatograph equipped with dual flame-ionization detectors, a cool on-column inlet, and a split-splitless inlet operated in the splitless mode. The detector temperature was set at 275°C. The following conditions were used: *Method 1.* On-column injection into a fused-silica capillary column (0.25 mm × 30 m), wall-coated with DB-5 (0.25-μm film thickness; J&W Scientific), programmed from 40 to 250°C at 6°C/min; *Method 2.* Splitless injection (injector temperature 240°C; septum purge resumed 0.5 min after injection) into the DB-5 column, programmed from 80 to 250°C at 6°C/min; *Method 3.* Split injection (injector temperature 250°C, split ratio 1:10) into the DB-5 column or a fused-silica capillary column (0.25 mm × 30 m) wall-coated with Rt_x-200 (0.25-μm film thickness; Restek Corp.), programmed from 80 to 250°C at 2°C/min. Each column was fitted with a J&W fused-silica guard column (0.32 mm × 1 m, Method 1; 0.25 mm × 1 m, Methods 2 and 3) via a press-tight connector (J&W or Supelco). Chromatography by GLC Method 3 was performed on the two columns simultaneously by inserting a two-way (Y) press-tight capillary-column splitter (Restek) between the guard column and the analytical columns. Helium was used as the carrier gas at measured linear velocities (methane injection, oven temperature 80°C) of 26.1 cm/s (Methods 2 and 3) and 28.3 cm/s (Method 1) for the DB-5 column and 27.8 cm/s for the Rt_x-200 column. Retention indices were derived from data acquired by GLC Method 3 by the linear-temperature-programmed gas-chromatographic retention index (LTPGCRI) method as described by Elvebak et al.[20]. Chromatograms and peak areas were recorded with a Hewlett–Packard model 3392A integrator or a PE-Nelson model 1020X computing integrator (Perkin–Elmer Corp.). Molar response factors were calculated based on the effective carbon response (ECR) method, as previously described[21].

GLC–MS analyses were performed using GLC Method 2 and a Hewlett–Packard 5890 Series II chromatograph connected by a deactivated fused-silica interface to a Finnigan–MAT model MAT-95 mass spectrometer. Column effluents were analyzed by electron-ionization (EI) mass spectrometry at 70 eV or by

chemical-ionization (CI) mass spectrometry using ammonia as the reagent gas. Direct-probe EI- or CI-mass spectra of isolated HPLC fractions were also acquired on the Finnigan–MAT instrument.

Polarimetric measurements were performed on a Perkin–Elmer model 241 polarimeter with a 1-dm semimicro cell (0.5 mL) containing samples dissolved in CH_2Cl_2. Optical rotations were recorded at the D-line of sodium (589 nm) and at 365, 406, 436, 546, and 578 nm using the polarimeter's mercury vapor lamp.

Benzoic anhydride, boron trifluoride etherate, chlorotrimethylsilane, 1,4-dioxane, iodomethane, magnesium chloride hexahydrate, methanesulfonic acid, *N*-methylimidazole, methyl sulfoxide (Me_2SO), triethylsilane, and trimethylsilyl trifluoromethanesulfonate were obtained from Aldrich Chemical Co. Trypticase tryptic soy broth (TSB) was obtained from BBL Microbiology Systems. Mixed-bed ion-exchange resin AG501 X-8(D) was obtained from Bio-Rad Laboratories. Deuterated solvents were obtained from either Aldrich or Cambridge Isotope Laboratories. Acetic anhydride and glacial acetic acid were obtained from Fisher Scientific Co. (*S*)-2-Butanol (97.4% ee; lot no. 288520/1), bovine pancreatic deoxyribonuclease I (DNase; EC 3.1.21.1), bovine pancreatic ribonuclease A (RNase; EC 3.1.27.5), Pronase E (EC 3.4.24.4), and tetrasodium ethylenediaminetetraacetate (Na_4EDTA) were obtained from Fluka Chemical Co. Sephadex G-50 (bead size, 20–80 μm), sodium dodecyl sulfate (SDS), and tris(hydroxymethyl)aminomethane (Tris), free base and hydrochloride (Tris · HCl), were obtained from Sigma Chemical Co. Trimethylsilyl methanesulfonate was prepared from methanesulfonic acid and chlorotrimethylsilane as previously described[11]. Boron trifluoride etherate, trimethylsilyl trifluoromethanesulfonate, and trimethylsilyl methanesulfonate were stored over 4A molecular sieves and periodically redistilled. Acetic anhydride, MeOH, NMI, pyridine, and CH_2Cl_2 were distilled and stored as described by Perrin et al.[22]. Alcohols were stored over 3A molecular sieves or anhyd $CaSO_4$ (Aldrich). Chlorinated, deuterated solvents were stored over anhyd K_2CO_3 (Aldrich). Deionized H_2O used in procedures other than HPLC was purified by a NANOpure II system (Barnstead/Thermolyne Corp.).

Preparation of the Pseudomonas aeruginosa *O-antigenic polysaccharide.—Pseudomonas aeruginosa* ATCC 33358 (IATS serotype 11, Fisher immunotype 2) (ref 3) was obtained as a freeze-dried pellet from the American Type Culture Collection. Bacteria were cultured at 37°C to late log phase from 2.5% (v/v) inocula in five concurrent 1-L batches of TSB; the cultures were aerated by rotation at 200 rpm in an orbital incubator. Cells were killed upon addition of aq formaldehyde to a final concentration of 0.37% (v/v). The cell paste, harvested by batchwise centrifugation, was washed by centrifugation from water and then acetone. The final pellet was lyophilized, yielding 12 g of dry cells.

Lipopolysaccharide was extracted by a modification of the procedure of Darveau and Hancock[7]. Bacterial cells (10 g) were suspended in 300 mL of 2 mM $MgCl_2$ in 20 mM Tris · HCl (pH 8.0) in an OmniMixer (Omni International) at high speed for 1 min. Nucleic acids were digested by the addition of DNase and RNase to

final concentrations of 100 μg/mL and 25μg/mL, respectively. The suspension was passed once through a cell fractionator at 20 000 psi. To ensure cell disruption, the suspension was sonicated (Sonifier Cell-Disruptor, Heat Systems/Branson) with two 30-s bursts at 75 W. Nucleases in the amounts given above were again added to the cell lysate, which was incubated at 37°C for 2 h.

To the nuclease digest, the following were added: (*a*) 100 mL of 0.5 M Na_4EDTA in 20 mM Tris · HCl (pH 8.0), (*b*) 50 mL of 20% SDS in 20 mM Tris · HCl (pH 8.0), and (*c*) 50 mL of 20 mM Tris · HCl (pH 8.0). The mixture was shaken well and then centrifuged at 48 000*g* (Beckman JA-20 rotor) at 20°C for 30 min. The pellet contained crude peptidoglycan. After addition of Pronase E, the supernatant was vigorously agitated overnight at 37°C. The digest was poured into 3 vol of cold (0°C) 0.375 M $MgCl_2$ in 95% EtOH. The resulting mixture, containing precipitated LPS, was allowed to stand for at least 3 h at −20°C. The LPS was then isolated by centrifugation at 8500*g* (Beckman JA-10 rotor) at 0°C for 15 min. The pellet was lyophilized to yield 2.2 g of LPS containing 1–5% protein. This preparation was sufficiently pure for isolation of the O-antigen[7].

A 1-g sample of crude LPS was suspended in 100 mL of 1% aq acetic acid, and the solution was heated at reflux for 5 h. The hydrolyzate was cooled in an ice bath, and the precipitated lipid A and contaminating protein were removed by low-speed centrifugation. Particulates in the supernatant were removed by vacuum filtration through a Nalgene disposable filtration unit (cellulose acetate membrane, 0.2-μm pore size). The filtrate was extracted three times with 1 vol each of $CHCl_3$. The volume of the final aq phase was reduced to 50 mL by evaporation under vacuum at 50°C and then lyophilized to yield 0.82 g of crude polysaccharide.

The crude polysaccharide was fractionated by gel-permeation chromatography through a column of Sephadex G-50 (2.5 × 90.5 cm, $V_0 = 159$ mL, $V_t = 415$ mL), eluted with 1% (v/v) pyridine and 0.4% (v/v) acetic acid (pH 5) (ref 23). In a typical run, a 260-mg sample of crude polysaccharide was dissolved in 15 mL of eluant and loaded onto the column with a syringe pump. The column, fitted with a flow adapter, was eluted by a peristaltic pump at 25 mL/h. Carbohydrate-containing fractions were detected by the phenol–H_2SO_4 acid assay[24] as modified by McKelvy and Lee[25]. Fractions eluting in the void volume of the column were combined and lyophilized to yield 20 mg of purified polysaccharide.

The purified O-antigen was methylated as described by Gunnarsson[9]. Specifically, a solution of the polysaccharide (29 mg, previously dried overnight in high vacuum) in 4.0 mL of Me_2SO was transferred to a conical flask containing finely powdered NaOH (155 mg) and equipped with a rubber septum and a dry N_2 line. Iodomethane (380 μL) was then added, the N_2 line was removed, and the mixture was stirred magnetically at room temperature for 60 min. The mixture was then neutralized with 4 mL of M acetic acid. To remove excess iodomethane, dry N_2 was bubbled through the mixture until a clear solution was obtained.

The permethylated polysaccharide was purified from the quenched mixture by solid-phase extraction on Sep-Pak Vac RC trifunctional-C_{18} cartridges (500 mg,

Waters Chromatography Div.). The cartridges were prewashed consecutively with MeCN (5 × 5 mL) and water (5 × 5 mL). The sample, combined with a water wash (0.5–1 mL) of the reaction flask, was split into equal portions, which were loaded into two cartridges by vacuum at 1–2 drops per second, until the liquid level was just above the bed. Each cartridge was eluted with four 5-mL vol of water to remove the more polar contaminants, and the polysaccharide was then eluted from each cartridge with 5 mL of MeCN. The MeCN fractions were combined and concentrated under reduced pressure, yielding 18 mg of a yellow film which was then dissolved in freshly distilled CH_2Cl_2 and stored as a stock solution in a 5-mL conical amber-glass vial sealed with a Teflon-lined screw cap.

Reductive cleavages.—In a typical experiment, the permethylated O-antigen (1–12 mg), previously dried in high vacuum for at least 2 h, was dissolved in an amount of dry CH_2Cl_2 sufficient to obtain a final concentration of 2 mg/100 μL after addition of the reagents specified below. Reaction volumes of at least 100 μL were used for less than 2-mg amounts of permethylated polysaccharide. The reactions were conducted in 1.0- or 3.0-mL conical vials whose inner surfaces had previously been silanized[26]; the vials were equipped with Teflon valve caps and spin vanes for stirring. To the polysaccharide solutions the following reagents were added: Et_3SiH and $Me_3SiOSO_2CF_3$, each to a final concentration of 0.5 M; or Et_3SiH (0.5 M final conc), Me_3SiOSO_2Me (0.5 M final conc) and $BF_3 \cdot OEt_2$ (0.1 M final conc). The mixtures were stirred at room temperature for the periods specified (see Results); 1 reaction vol of dry alcohol was then added, and stirring was continued for the minimum periods (MeOH, 1 h; 2-butanol, 6 h) as previously determined[1]. Mixed-bed resin (previously rinsed with MeOH and dried under high vacuum) was then added, followed by 3 to 4 vol of the alcohol used as the glycosyl acceptor. The mixture was then stirred vigorously until neutral, as determined by spotting of the mixture on pH paper, and the resin was removed by filtration under reduced pressure through an unpacked Poly-Prep column (Bio-Rad) with an integral 35-μm polyethylene frit. The resin was rinsed with MeOH–CH_2Cl_2, and the filtrate was then concentrated under vacuum. The residue was acetylated or benzoylated as described below.

Acetylations.—The residue was dissolved in 100 μL of Ac_2O, and the solution was stirred for 1 min; *N*-methylimidazole (10 μL) was then added, and the mixture was stirred for 10 min. The reaction was quenched by the addition of 1 mL of cold, satd aq $NaHCO_3$, and the mixture was stirred vigorously for 30 min. More $NaHCO_3$ solution (1 mL) was then added, and the aq solution was extracted with 1 mL of CH_2Cl_2. The organic phase was then washed with 1 mL each of M H_2SO_4 and water, dried over Na_2SO_4, and evaporated to dryness under dry N_2. The residue was redissolved in CH_2Cl_2 for analysis by GLC and GLC–MS.

Benzoylations.—The residue was dissolved in pyridine (100 μL), and *N*-methylimidazole (40 μL) was added. Benzoic anhydride (50–100 mg) was then added, and the mixture was stirred for 10–15 min. The reaction was quenched by the addition of 2 mL of cold, satd aq $NaHCO_3$, and the mixture was vigorously stirred

until a clear solution was obtained. More $NaHCO_3$ solution (3 mL) was added, and the aq solution was then extracted with 2 mL of CH_2Cl_2. The organic phase was then washed with 5 mL each of M H_2SO_4 and water, dried over Na_2SO_4, and evaporated to dryness under dry N_2. The residue was redissolved in MeOH (200 μL), and the resulting solution was filtered through a 0.2-μm pore-size PTFE syringe filter for preparative HPLC.

*Methyl 3-O-acetyl-2,6-dideoxy-4-O-methyl-2-(N-methylacetamido)-β-*D- *and* -L-*galactopyranoside* (**3a**, **4a**) *and 3-O-benzoyl-2,6-dideoxy-4-O-methyl-2-(N-methylacetamido)-β-*D- *and* L-*galactopyranoside* (**3b**, **4b**).—The **3a**, **4a** mixture was separated by GLC from the acetylated product mixture obtained from Experiments *A–D*. Retention indices (LTPGCRI method): DB-5, 1826.74; Rt_x-200, 2385.15. CI(NH_3)-mass spectrum; *m/z* 258 [$(M - CH_3O)^+$, 39%], 290 [$(M + H)^+$, 100%], 307 [$(M + NH_4)^+$, 1.3%]. GLC–EI-mass spectrum: *m/z* 43 (62%), 72 (65%), 73 (55%), 96 (22%), 98 (30%), 115 (100%), 128 (21%), 142 (21%), 157 (13%), 173 (8%), 184 (9%), 198 (2%), 216 (18%), 257 (1%), 258 (0.3%).

The **3b**, **4b** mixture was isolated by HPLC (Fig. 3) from the benzoylated reductive-cleavage product mixtures described above (see Results). ^1H NMR data (500 MHz, $CHCl_3$): δ 1.34 (d, 1 H, $J_{6,5}$ 6.2 Hz, H-6, rotamer 2), 1.38 (d, 2 H, $J_{6,5}$ 6.2 Hz, H-6, rotamer 1), 1.98 (s, 1 H, AcN, rotamer 2), 2.21 (s, 2 H, AcN, rotamer 1), 2.80 (s, 2 H, MeN, rotamer 1), 3.09 (s, 1 H, MeN, rotamer 2), 3.48, 3.49, 3.50, 3.55 (4 s, 6 H total, 2 MeO, rotamers 1 and 2), 3.61 (d, 1 H, $J_{4,3}$ 3.1 Hz, H-4), 3.70 (q, 1 H, H-5), 4.42 (dd, 1 H, $J_{2,1}$ 8.0, $J_{2,3}$ 11.2 Hz, H-2), 4.47 (d, 1 H, H-1), 5.34 (dd, 1 H, H-3), 7.47 (t, 2 H, $J_{m\text{-Ar},o\text{-Ar}}$ 7.6, $J_{m\text{-Ar},o\text{-Ar}}$ 7.6 Hz, *m*-Ar), 7.60 (t, 1 H, *p*-Ar), 7.99 (d, 1.5 H, *o*-Ar, rotamer 1), 8.05 (d, 0.5 H, *o*-Ar, rotamer 2). CI(NH_3)-mass spectrum: *m/z* 320 [$(M - CH_3O)^+$, 53%], 352 [$(M + H)^+$, 100%], 369 [$(M + NH_4)^+$, 2%].

*3-O-Acetyl-1,5-anhydro-2,6-dideoxy-4-O-methyl-2-(N-methylacetamido)-*D- *and* -L-*galactitol* (**10a**, **11a**) *and 1,5-anhydro-3-O-benzoyl-2,6-dideoxy-4-O-methyl-2-(N-methylacetamido)-*D- *and* L-*galactitol* (**10b**, **11b**).—Data for the **10a**, **11a** mixture were obtained as described for **3a**, **4a**. Retention indices (LTPGCRI method): DB-5, 1776.01; Rt_x-200, 2349.22. CI(NH_3)-mass spectrum: *m/z* 260 [$(M + H)^+$, 100%], 277 [$(M + NH_4)^+$, 5%]. EI-mass spectrum: *m/z* 43 (96%), 72 (85%), 74 (100%), 85 (69%), 98 (20%) 100 (33%), 112 (43%), 115 (57%), 139 (20%), 154 (99%), 168 (10%), 170 (35%), 200 (8%), 228 (5%), 259 (2%).

The **10b**, **11b** mixture was isolated by HPLC (Fig. 3) from the benzoylated product mixtures as described for **3b**, **4b**. ^1H NMR data (500 MHz, $CHCl_3$): δ 1.31 (d, 1.2 H, $J_{6,5}$ 6.4 Hz, H-6, rotamer 2), 1.34 (d, 1.8 H, $J_{6,5}$ 6.4 Hz, H-6, rotamer 1), 1.97 (s, 1.2 H, AcN, rotamer 2), 2.24 (s, 1.8 H, AcN, rotamer 1), 2.78 (s, 1.8 H, MeN, rotamer 1), 2.89 (s, 1.2 H, MeN, rotamer 2), 3.51 (s, 1.8 H, 2 MeO, rotamer 1), 3.57 (s, 1.2 H, 2 MeO, rotamer 2), 3.60 (t, 1 H, $J_{1a,1e}$ 11.0, $J_{1a,2}$ 11.0 Hz, H-1a), 3.61 (q, 1 H, H-5), 3.66 (d, 1 H, $J_{4,3}$ 3.3 Hz, H-4), 3.99 (dd, 1 H, $J_{1e,2}$ 8.0 Hz, H-1a), 4.48 (dt, 1 H, $J_{2,3}$ 11.0 Hz, H-2), 5.35 (dd, 1 H, H-3), 7.47 (t, 2 H, $J_{m\text{-Ar},o\text{-Ar}}$ 7.7, $J_{m\text{-Ar},o\text{-Ar}}$ 7.7 Hz, *m*-Ar), 7.60 (t, 1 H, *p*-Ar), 7.99 (d, 1.3 H, *o*-Ar, rotamer 1), 8.05

(d, 0.7 H, *o*-Ar, rotamer 2). CI(NH_3)-mass spectrum: *m/z* 321 [$(M + H)^+$, 100%], 339 [$(M + NH_4)^+$, 1%].

(S)-2-Butyl 3-O-acetyl-2,6-dideoxy-4-O-methyl-2-(N-methylacetamido)-β-L-galactopyranoside (15a) and (S)-2-butyl 3-O-benzoyl-2,6-dideoxy-4-O-methyl-2-(N-methylacetamido)-β-L-galactopyranoside (15b) (minor diastereomer, Figs. 2 and 3). —Data for **15a** were obtained by GLC (Method 1) of the acetylated product mixture obtained from Experiment *C*. Retention indices (LTPGCRI method): DB-5, 1955.99; Rt_x-200, 2489.05. CI(NH_3)-mass spectrum: *m/z* 258 [$(M - C_4H_9O)^+$, 30%], 332 [$(M + H)^+$, 100%], 349 [$(M + NH_4)^+$, 0.13%]. EI-mass spectrum: *m/z* 43 (62%), 72 (65%), 73 (55%), 96 (22%) 98 (30%), 115 (100%), 128 (21%), 142 (21%), 157 (13%), 173 (8%), 184 (9%), 198 (2%), 216 (18%), 257 (1%), 258 (0.3%).

Compound **15b** was isolated by HPLC (Fig. 3C) from the benzoylated product mixture obtained after reductive cleavage of the fully methylated O-antigen in the presence of Et_3SiH and Me_3SiOSO_2Me–$BF_3 \cdot OEt_2$ (see Results).^{1}H NMR data (500 MHz, $CHCl_3$) (Note: primed locants denote positions on the aglycons of **15b** and **17b**.): δ 0.88 (t, 1 H, $J_{4',3'}$ 7.3 Hz, H-4'), 1.05 (d, 0.75 H, $J_{1',2'}$ 6.2 Hz, H-1', rotamer 2), 1.07 (d, 2.25 H, $J_{1',2'}$ 6.2 Hz, H-1', rotamer 1), 1.31 (d, 0.75 H, $J_{6,5}$ 6.2 Hz, H-6, rotamer 2), 1.37 (d, 2.25 H, $J_{6,5}$ 6.2 Hz, H-6, rotamer 1), 1.49–1.63 (complex, 2 H, H-3a',3b'), 1.95 (s, 0.75 H, AcN, rotamer 2), 2.24 (s, 2.25 H, AcN, rotamer 1), 2.80 (s, 2.25 H, MeN, rotamer 1), 3.10 (s, 0.75 H, MeN, rotamer 2), 3.47 (s, 0.75 H, MeO, rotamer 2), 3.54 (s, 2.25 H, MeO, rotamer 1), 3.60 (d, 1 H, $J_{4,3}$ 3.1 Hz, H-4), 3.67 (q, 1 H, H-5), 3.80 (m, 1 H, H-2'), 4.21 (dd, 1 H, $J_{2,1}$ 8.0, $J_{2,3}$ 11.3 Hz, H-2), 4.61 (d, 1 H, H-1), 5.34 (dd, 1 H, H-3), 7.46 (t, 2 H, $J_{m\text{-Ar},o\text{-Ar}}$ 7.5, $J_{m\text{-Ar},o\text{-Ar}}$ 7.6 Hz, *m*-Ar), 7.60 (dt, 1 H, $J_{p\text{-Ar},o\text{-Ar}}$ 1.2 Hz, *p*-Ar), 7.99 (dd, 1.3 H, *o*-Ar, rotamer 1), 8.07 (dd, 0.7 H, *o*-Ar, rotamer 2). CI(NH_3)-mass spectrum: *m/z* 320 [$(M - C_4H_9O)^+$, 56%], 394 [$(M + H)^+$, 100%], 411 [$(M + NH_4)^+$, 0.3%].

(S)-2-Butyl 3-O-acetyl-2,6-dideoxy-4-O-methyl-2-(N-methylacetamido)-β-D-galactopyranoside (**17a**) *and (S)-2-butyl 3-O-benzoyl-2,6-dideoxy-4-O-methyl-2-(N-methylacetamido)-β-D-galactopyranoside* (**17b**) *(major diastereomer, Figs. 2 and 3).* —Data for **17a** was obtained as described for **15a**. Retention indices (LTPGCRI method): DB-5, 1970.41; Rt_x-200, 2512.94. CI(NH_3)-mass spectrum: identical to that of **15a**. EI-mass spectrum: identical to that of **15a**.

Compound **17b** was produced and isolated as described for **15b**. ^{1}H NMR data (500 MHz, $CHCl_3$): identical to that of **15b**, with the following exceptions: δ 0.83 (t, 1 H, $J_{4',3'}$ 7.3 Hz, H-4'), 1.21 (d, 0.75 H, $J_{1',2'}$ 6.2 Hz, H-1', rotamer 2), 1.24 (d, 2.25 H, $J_{1',2'}$ 6.2 Hz, H-1', rotamer 1), 1.38–1.51 (complex, 2 H, H-3a',3b'), 3.68 (m, 1 H, H-2'), 4.22 (dd, 1 H, $J_{2,1}$ 8.0, $J_{2,3}$ 11.3 Hz, H-2). CI(NH_3)-mass spectrum: identical to that of **15b**.

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