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Diastereoselectivity in the transglycosidation of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-D-glucopyranoside, -galactopyranoside, and -mannopyranoside with racemic 2-butanol under reductive-cleavage conditions

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Abstract

The fully methylated derivatives of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside, methyl 2-acetamido-2-deoxy-β-D-galactopyranoside, and methyl 2-acetamido-2-deoxy-α-D-mannopyranoside were subjected to reductive-cleavage conditions in the presence of various promoters, and the rates of formation of the respective oxazolinium ions were established by ^{1}H NMR spectroscopy. When oxazolinium ion formation was complete, racemic 2-butanol was added to each reaction, and the time-course for transglycosidation to form the respective 2-butyl glycosides was established by gas–liquid chromatography. These studies established the minimum times required for conversion of these acetamido sugars to their respective oxazolinium ions under various reductive-cleavage conditions and the minimum times required for their transglycosidation with 2-butanol. In separate experiments, oxazolinium ions having the *gluco*, *galacto*, and *manno* configuration were formed in the presence of trimethylsilyl trifluoromethanesulfonate as the promoter, then reacted with racemic 2-butanol either in the presence or absence of a proton acceptor (2,6-di-*tert*-butylpyridine), and the time-course for formation of the individual diastereomeric glycosides was established by gas–liquid chromatography. For all three oxazolinium ions, diastereoselectivity was observed in their conversion to the respective diastereomeric (*R*)- and (*S*)-2-butyl glycosides. When the transglycosidations were conducted in the absence of a proton acceptor, the kinetically preferred diastereomers were, in all three cases, present in the lowest proportion at equilibrium, whereas when the transglycosidations were conducted in the presence of a proton acceptor, the ratios of the diastereomers were constant throughout the reaction and the kinetically preferred diastereomers predominated. These experiments have established the

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ratios of the diastereomeric 2-butyl glycosides to be expected when the reductive-cleavage method is employed for the analysis of acetamido sugar-containing polysaccharides.

Keywords: Acetamido sugars; Reductive cleavage; Transglycosidation

1. Introduction

Chiral alcohols have previously been used as glycosyl acceptors in order to establish the absolute configuration of sugar residues in polysaccharides [1,2]. The same strategy has been employed to establish the absolute configuration of acetamido sugars in fully methylated polysaccharides when the reductive-cleavage method is employed [3]. In the latter case, the oxazolinium ions formed under reductive-cleavage conditions were found to serve as glycosyl donors when the reactions were quenched with (*S*)-2-butanol. However, when this method was tested on an O-antigenic polysaccharide having a repeat unit thought to be composed of equimolar amounts of 2-linked β-D-glucopyranosyl (D-Glc), 3-linked 2-acetamido-2,6-dideoxy-α-L-galactopyranosyl (L-FucNAc), and 3-linked β-D-FucNAc residues, the diastereomeric (*S*)-2-butyl glycosides of the L-FucNAc and D-FucNAc residues were observed in a ratio of 2.69:1 rather than 1:1, as expected [4]. These results indicated that either the D- and L-FucNAc residues were not in equimolar proportions in the polysaccharide or that there was considerable diastereoselectivity in the reaction of the intermediate oxazolinium ions with (*S*)-2-butanol. In the present study, the transglycosidation of acetamido sugars with racemic 2-butanol under reductive-cleavage conditions was reexamined in order to establish the degree of diastereoselectivity that was obtained.

2. Results

Synthesis.—Methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-D-glucopyranoside (**1**) was prepared from 2-acetamido-2-deoxy-D-glucose as previously described [3]. Methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-D-galactopyranoside (**2**) was prepared from 2-acetamido-2-deoxy-D-galactose by sequential Fischer glycosidation in the presence of methanol [5] and permethylation [6]. The mixture of α and β glycosides was subjected to reductive-cleavage at 70 °C in 1,2-dichloroethane [7], and the reaction was quenched with methanol. The reaction mixture was then deionized with mixed-bed resin, and the product (**2**) was obtained after purification by flash column chromatography. Methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-mannopyranoside (**3**) was synthesized from 2-acetamido-2-deoxy-D-mannose by sequential Fischer glycosidation [5], permethylation [8], *N*-deacylation [9], and acetylation. The last two steps were necessitated by the fact that Hakomori methylation gave not only the *O*- and *N*-methylated product but *C*-methylated products such as the *N*-propionyl and *N*-isobutyryl derivatives. After deacylation of the permethylated product with hydrazine [9] and purification by flash column chromatography, the product was acetylated to give the α-glycoside **3** in pure form.

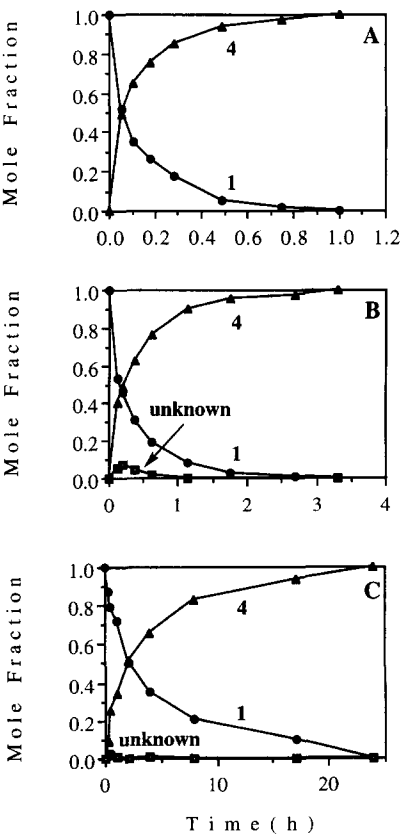


Fig. 1. Time-course of formation of oxazolinium ion **4** from methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-D-glucopyranoside (**1**) in the presence of (A) $Me_3SiOSO_2CF_3$, (B) $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$, and (C) $BF_3 \cdot OEt_2$.

Kinetics of oxazolinium ion formation from acetamido sugar derivatives **1–3**.—Compounds **1**, **2**, and **3** were reacted separately with one of three Lewis acid promotors, namely trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$) (5 equiv) [10], a mixture of trimethylsilyl methanesulfonate (Me_3SiOSO_2Me) (5 equiv) and boron trifluoride etherate ($BF_3 \cdot OEt_2$) (1 equiv) [11] or $BF_3 \cdot OEt_2$ (5 equiv) alone [12], and the rates of formation of the respective oxazolinium ions (**4–6**) were established by ^1H NMR spectroscopy. For the *gluco* isomer **1**, the rate of formation of oxazolinium ion **4** was measured by integration of characteristic signals at δ 6.628 (d, J 8.0 Hz, H-1), δ 4.616 (broad multiplet, H-2), and δ 3.893 (t, J 2.5 Hz, H-3) [13]. Time-course experiments (Fig. 1) demonstrated that formation of oxazolinium ion **4** was complete after 1.0 h for $Me_3SiOSO_2CF_3$, 3.5 h for $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$, and 24 h for $BF_3 \cdot OEt_2$ alone. For the *galacto* isomer **2**, characteristic signals for the oxazolinium ion **5** appeared at δ 6.532 (d, J 7.5 Hz, H-1), δ

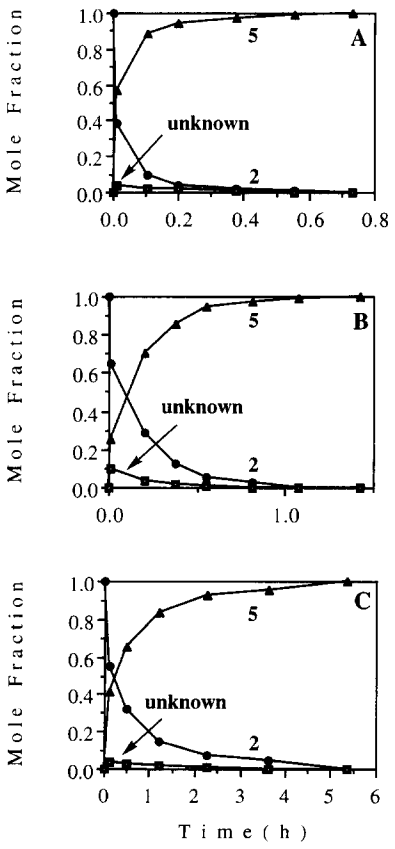


Fig. 2. Time-course of formation of oxazolinium ion **5** from methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-D-galactopyranoside (**2**) in the presence of (A) $Me_3SiOSO_2CF_3$, (B) $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$, and (C) $BF_3 \cdot OEt_2$.

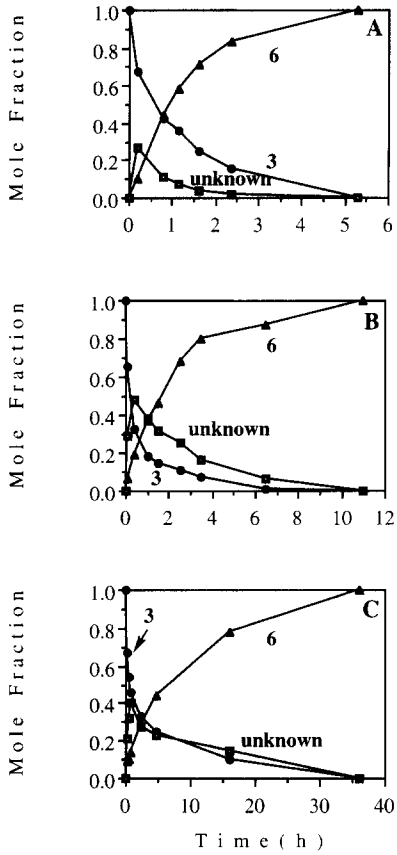
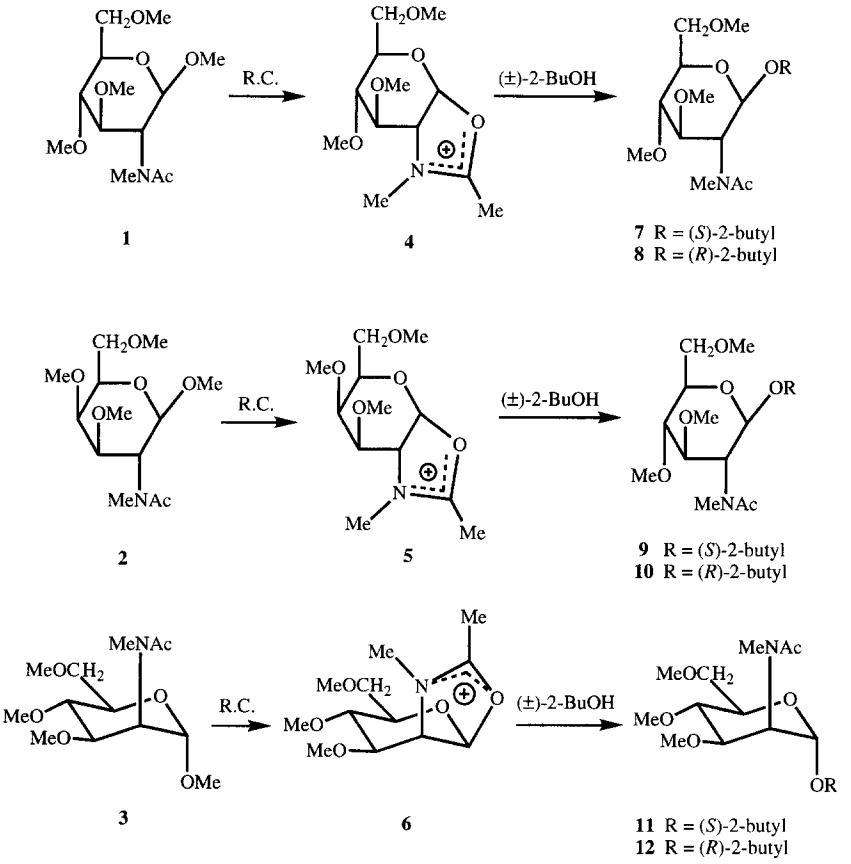


Fig. 3. Time-course of formation of oxazolinium ion **6** from methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-mannopyranoside (**3**) in the presence of (A) $Me_3SiOSO_2CF_3$, (B) $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$, and (C) $BF_3 \cdot OEt_2$.

4.137 (m, H-2), δ 3.982 (broad d, J 2.5 Hz, H-4), and δ 3.786 (dd, J 8.0 Hz, H-3). Time-course experiments (Fig. 2) demonstrated that formation of the oxazolinium ion **5** was complete after 45 min for $Me_3SiOSO_2CF_3$, 1.5 h for $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$, and 5.5 h for $BF_3 \cdot OEt_2$ alone. Oxazolinium ion (**6**) formation from the *manno* isomer **3** was followed by the appearance of signals for **6** at δ 6.465 (d, J 8.0 Hz, H-1), δ 4.859 (dd, J 4.0 and 8.0 Hz, H-2), δ 4.051 (m, H-5), and δ 3.768 (dd, J 4.0 and 9.0 Hz, H-3). Time-course studies (Fig. 3) demonstrated that the *manno* isomer (**3**) was completely converted to the oxazolinium ion (**6**) after 5.5 h for $Me_3SiOSO_2CF_3$, 11 h for $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$, and 36 h for $BF_3 \cdot OEt_2$ alone. It should be noted that for all three acetamido sugar derivatives (**1–3**), an intermediate of undetermined structure was present during the early stages of their conversion to the respective oxazolinium ion (see Figs. 1–3). Based upon the chemical shifts of their H-1 resonances, these unknowns are thought to be oxazolinium ions having an acyclic carbohydrate residue [7].



Scheme 1.

Kinetics of reaction of oxazolinium ions **4–6** *with (±)-2-butanol in the presence of different Lewis acid promotors.*—In a previous study [3], (±)-2-butanol was used as a quenching alcohol in the reductive-cleavage of **1** to give 2-butyl glycosides **7** and **8** (Scheme 1). In the present study, time-course experiments demonstrated that oxazolinium-ion **4** was completely converted to **7** and **8** after 7 h for $Me_3SiOSO_2CF_3$, 2 h for $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$ and 18 h for $BF_3 \cdot OEt_2$ alone (Fig. 4). Product mixtures containing **7** and **8** were analyzed by GLC (Method 1) by comparison of their integrated areas relative to that of docosane as an internal standard. Each of (*S*)-D- and (*R*)-D-diastereomers was distinguishable by GLC (Fig. 5) as well as by ^1H NMR spectroscopy; i.e. the H-1 resonances of the major rotamers of the (*S*)-D-diastereomer (**7**) and the (*R*)-D-diastereomer (**8**) were both observed as doublets at δ 4.47 (J 8.0 Hz) and 4.46 (J 8.0 Hz), respectively [3].

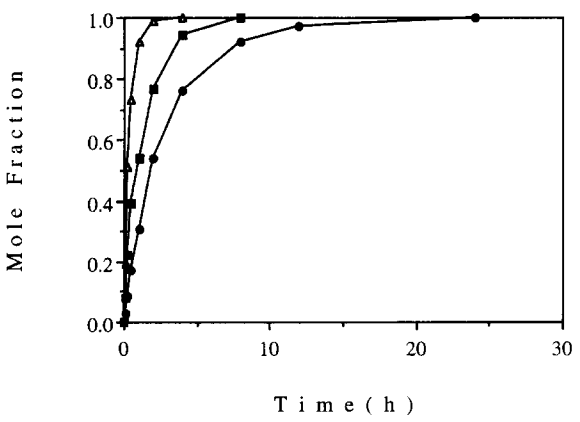


Fig. 4. Time-course of formation of 2-butyl glycosides (**7** and **8**) during the reaction of (±)-2-butanol with oxazolinium **4**. The oxazolinium ion (**4**) was first formed by treatment of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-D-glucopyranoside (**1**) with $Me_3SiOSO_2CF_3$ (■), $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$ (△), and $BF_3 \cdot OEt_2$ (●).

The oxazolinium ion (**5**) of the *galacto* configuration reacted with (±)-2-butanol much faster than the GlcNAc oxazolinium ion (**4**). In time-course experiments (Fig. 6), oxazolinium-ion **5** was completely converted to **9** and **10** after 4 h for $Me_3SiOSO_2CF_3$, 0.5 h for $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$, and 11 h for $BF_3 \cdot OEt_2$ alone. Product mixtures containing **9** and **10** were analyzed by GLC (Method 1) by comparison

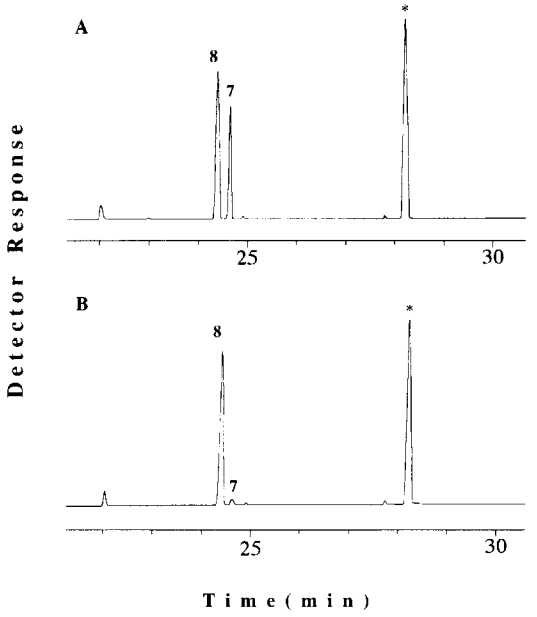


Fig. 5. Gas–liquid chromatograms of the products obtained after treatment of **1** with $Me_3SiOSO_2CF_3$, followed by quenching with racemic 2-butanol (A) or (*R*)-2-butanol (B). Docosane, added as an internal standard, is designated with an asterisk.

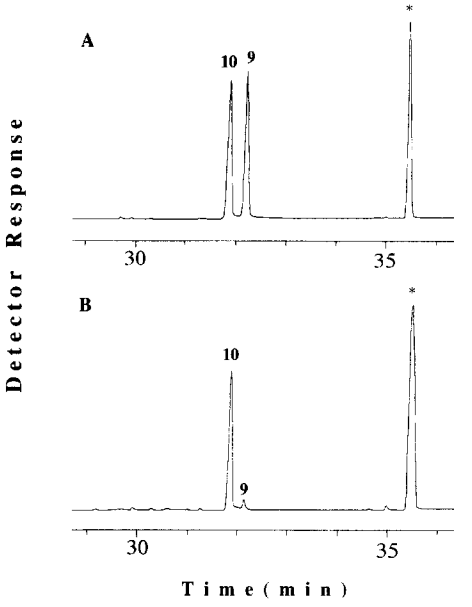


Fig. 7. Gas–liquid chromatograms of the products obtained after treatment of **2** with $Me_3SiOSO_2CF_3$, followed by quenching with (±)-2-butanol (A) or (*R*)-2-butanol (B). Docosane, added as an internal standard, is designated with an asterisk.

of their peak integrals to that of docosane as an internal standard. Each of (*S*)-D- and (*R*)-D-diastereomers was distinguishable by GLC (Fig. 7) and ^{1}H NMR spectroscopy; i.e. the H-1 resonances of the major rotamers of the (*S*)-D-diastereomer (**9**) and the (*R*)-D-diastereomer (**10**) were observed as doublets at δ 4.47 (*J* 8.0 Hz) and 4.46 (*J* 8.0 Hz), respectively.

The ManNAc-oxazolinium-ion (**6**) was quenched with (±)-2-butanol to give diastereomers **11** and **12**. In time-course experiments (Fig. 8), oxazolinium-ion intermediate **6** was completely converted to **11** and **12** after 10 h for $Me_3SiOSO_2CF_3$, 4 h for $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$, and 24 h for $BF_3 \cdot OEt_2$ alone. Product mixtures containing **11** and **12** were analyzed by GLC (Method 1) by comparison of their peak integrals to that of docosane as an internal standard. Each of (*S*)-D- and (*R*)-D-diastereomers was distinguishable by GLC (Fig. 9) and ^{1}H NMR

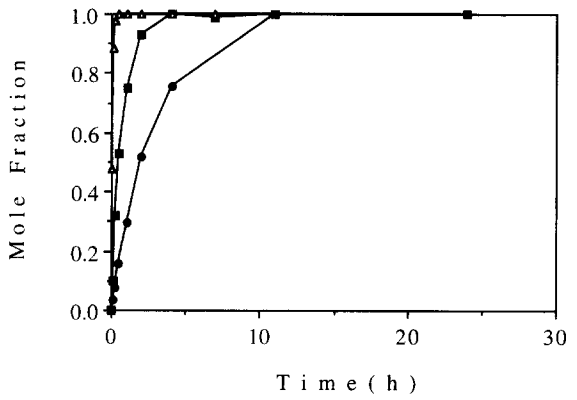


Fig. 6. Time-course of formation of 2-butyl glycosides (**9** and **10**) during the reaction of (±)-2-butanol with oxazolinium **5**. The oxazolinium ion (**5**) was first formed by treatment of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-D-galactopyranoside (**2**) with $Me_3SiOSO_2CF_3$ (■), $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$ (△), and $BF_3 \cdot OEt_2$ (●).

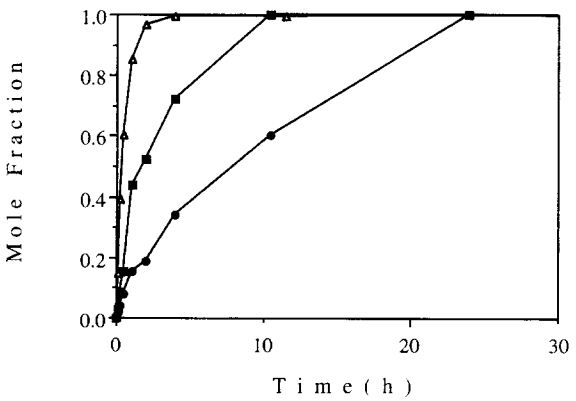


Fig. 8. Time-course of formation of 2-butyl glycosides (**11** and **12**) during the reaction of (±)-2-butanol with oxazolinium **6**. The oxazolinium ion (**6**) was first formed by treatment of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-mannopyranoside (**3**) with $Me_3SiOSO_2CF_3$ (■), $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$ (△), and $BF_3 \cdot OEt_2$ (●).

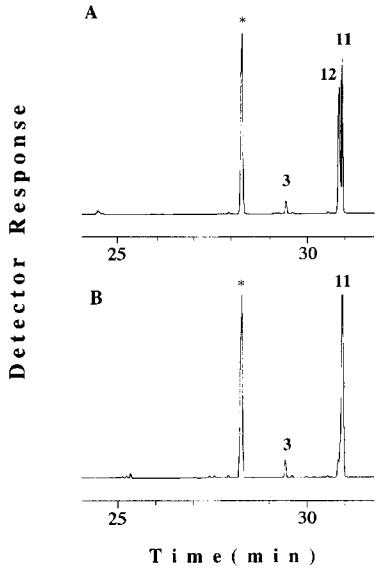


Fig. 9. Gas–liquid chromatograms of the products obtained after treatment of **3** with $Me_3SiOSO_2CF_3$, followed by quenching with (±)-2-butanol (A) or (*S*)-2-butanol (B). Docosane, added as an internal standard, is designated with an asterisk.

spectroscopy; i.e. for the (*S*)-D-diastereomer (**11**), H-1 of the major rotamer appeared as a doublet at δ 4.89 (*J* 1.2 Hz) whereas the H-1 resonance of the

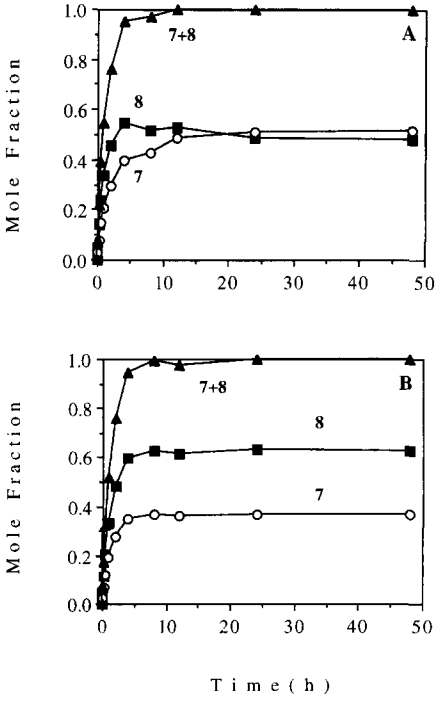


Fig. 10. Time-course of formation of 2-butyl glycosides **7** and **8** during transglycosidation of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-D-glucopyranoside (**1**) with $Me_3SiOSO_2CF_3$. Reactions were quenched with either (±)-2-butanol (A) or (±)-2-butanol and 2,6-di-*tert*-butylpyridine (B).

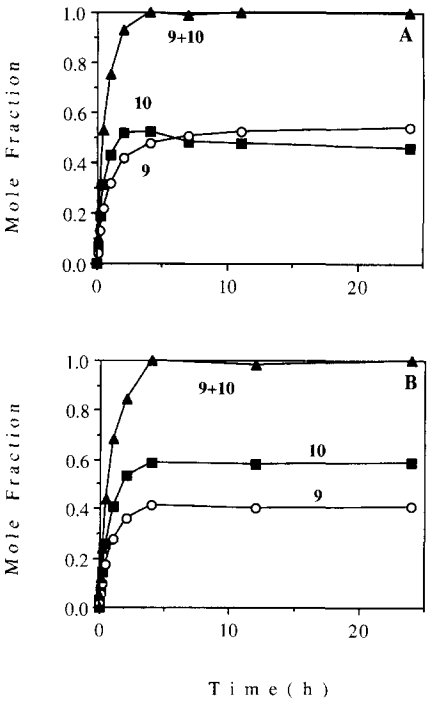


Fig. 11. Time-course of formation of 2-butyl glycosides **9** and **10** during transglycosidation of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-D-galactopyranoside (**2**) with $Me_3SiOSO_2CF_3$. Reactions were quenched with either (±)-2-butanol (A) or (±)-2-butanol and 2,6-di-*tert*-butylpyridine (B).

major rotamer of the (*R*)-D-diastereomer appeared as a doublet at δ 4.86 (*J* 1.2 Hz).

Diastereoselectivity in the reaction of oxazolinium ions **4**–**6** *with* (±)-*2-butanol.*—Oxazolinium ions **4**, **5**, and **6** were reacted separately with racemic 2-butanol in the presence or absence of 2,6-di-*tert*-butylpyridine, and the rates of formation of the diastereomeric (*R*)- and (*S*)-2-butyl glycosides were measured by GLC by integration of their peak areas with respect to the peak area of an internal standard (docosane). For all these acetamido sugar derivatives, diastereoselectivity was observed in the conversion of the oxazolinium ion to the respective 2-butyl glycoside. In the absence of a proton acceptor, the *gluco* and *galacto* oxazolinium ions (**4** and **5**, respectively) reacted with (*R*)-2-butanol faster than with (*S*)-2-butanol, but, in both cases, the (*S*)-2-butyl glycosides (**7** and **9**, respectively) predominated at equilibrium (Fig. 10A and Fig. 11A, respectively). In contrast, the *manno* oxazolinium ion (**6**) reacted with (*S*)-2-butanol faster than with (*R*)-2-butanol, but the (*R*)-2-butyl glycoside (**12**) predominated at equilibrium (Fig. 12A). In the presence of a proton acceptor, however, the ratios of the respective (*R*)- and (*S*)-2-butyl

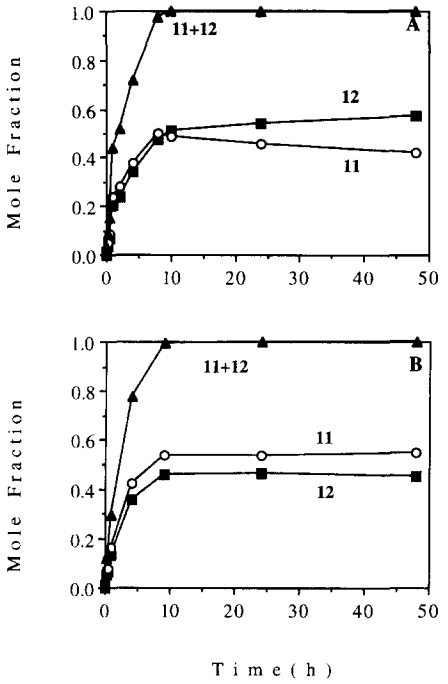


Fig. 12. Time-course of formation of 2-butyl glycosides **11** and **12** during transglycosidation of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-D-mannopyranoside (**3**) with $Me_3SiOSO_2CF_3$. Reactions were quenched with either (±)-2-butanol (A) or (±)-2-butanol and 2,6-di-*tert*-butylpyridine (B).

glycosides were constant throughout the reaction, and the kinetically favored diastereomers predominated even after extended reaction times. Thus, *gluco* oxazolinium ion **4** reacted with (*R*)-2-butanol at a rate 1.7 times faster than with (*S*)-2-butanol (Fig. 10B), whereas *galacto* oxazolinium ion **5** reacted with (*R*)-2-butanol 1.4 times faster than with (*S*)-2-butanol (Fig. 11B). Under the same conditions, *manno* oxazolinium ion **6** reacted with (*S*)-2-butanol 1.2 times faster than with (*R*)-2-butanol (Fig. 12B).

3. Discussion

In an effort to understand better the mechanism of transglycosidation of fully methylated acetamido sugars with chiral alcohols under reductive-cleavage conditions, the methyl glycosides of three commonly encountered acetamido sugars (GlcNAc, GalNAc, and ManNAc) were methylated fully, and the fully methylated products were exposed to reductive-cleavage conditions using various promotors. These studies were conducted in the absence of a reducing agent (Et_3SiH) since previous studies [13] had demonstrated that oxazolinium ion formation was not influenced by its presence. Once oxazolinium ion formation was complete, as indicated by ^1H NMR spectroscopy (Figs. 1–3), racemic 2-butanol was added to each of the reactions, and the time-course for formation of the diastereomeric 2-butyl glycosides was established by GLC (Figs. 4, 6 and 8). For all three acetamido sugar derivatives, the rate of formation of the 2-butyl glycosides was fastest when $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$ was used as the promoter, slowest when $BF_3 \cdot OEt_2$ was used as the promoter, and of intermediate rate when $Me_3SiOSO_2CF_3$ was used as the promotor. In separate experiments, oxazolinium ions having the *gluco* (**4**), *galacto* (**5**), and *manno* (**6**) configurations were formed in the presence of $Me_3SiOSO_2CF_3$ as the promoter, then reacted with racemic 2-butanol, and the time-course for formation of the individual diastereomeric 2-butyl glycosides (**7** and **8**, **9** and **10**, and **11** and **12**, respectively) was determined. Identification of the individual diastereomers was based upon a comparison of the GLC retention indices obtained using (±)-2-butanol and chirally pure 2-butanol as the glycosyl acceptor (Figs. 5, 7 and 9). Substantially different results were obtained in these experiments, however, depending upon whether the reaction with (±)-2-butanol was conducted in the presence or absence of a proton acceptor. When (±)-2-butanol was added directly to the reductive cleavage reaction mixtures (no proton acceptor), the individual diastereomers were formed at noticeably different rates but, in all three cases, the kinetically preferred diastereomer was present in the lower proportion at longer reaction times (Figs. 10A, 11A, and 12A). Since it would be expected that triflic acid, formed by the reaction of excess 2-butanol and $Me_3SiOSO_2CF_3$, would also catalyze transglycosidation and thus equilibration between the newly formed diastereomers, oxazolinium ions **4**, **5**, and **6** were also reacted with (±)-2-butanol in the presence of a proton acceptor (2,6-di-*tert*-butylpyridine) in order to measure the relative rates for formation of the individual diastereomeric glycosides (Figs. 10B, 11B, and 12B). Indeed, in these experiments the ratios of the diastereomeric glycosides (i.e. **7**:**8**, **9**:**10**, and **11**:**12**) were constant throughout the transglycosidation reaction. The diastereoselectivity was greatest for the oxazolinium ion having the *gluco* (**4**) configuration (**8**:**7** = 1.7), least for *manno* oxazolinium ion **6** (**11**:**12** = 1.2), and of intermediate value for *galacto* oxazolinium ion **5** (**10**:**9** = 1.4).

For the benefit of those who wish to use this method to establish the absolute configuration of the

acetamido sugar residues of polysaccharides, these experiments have established the minimum times necessary for their complete transglycosidation with 2-butanol under various reductive-cleavage conditions and the ratios of diastereomers to be expected in both the presence and absence of a proton acceptor.

4. Experimental

General.—TLC was performed on Silica Gel 60-F_{254} (E. Merck) with detection by UV light and/or charring with 5% H_2SO_4 in EtOH. Flash column chromatography was performed on 230–400 mesh silica gel (E. Merck).

Analytical GLC was performed on three Hewlett–Packard model 5890A gas–liquid chromatographs. One instrument was equipped with dual flame-ionization detectors, a cool on-column inlet, and a split–splitless inlet operated in the splitless mode; this instrument was used to perform quantitative gas–liquid chromatography using on-column injection. Another instrument was equipped with two split–splitless injection ports and two flame-ionization detectors; this instrument was used to perform retention index studies. The third instrument was equipped with dual flame-ionization detectors and two split–splitless inlets operated in the splitless mode; this instrument was used to check the purity of all starting materials and products. All of the above gas–liquid chromatographs were interfaced to a HP model 3365 Series II ChemStation. The injector temperature was set at 200 °C in order to minimize pyrolysis of acetamido sugar derivatives, and 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), programmed from 40 to 300 °C at 6 °C/min; *Method 2* — Splitless injection into the DB-5 column, programmed from 80 to 300 °C at 6 °C/min; *Method 3* — Split injection into the DB-5 column and a fused-silica capillary column (0.25 mm × 30 m) wall-coated with RTx-200 (0.25 μm film thickness; Restek Corp.) programmed from 80 to 300 °C at 2 °C/min. Each column was fitted with a J&W deactivated fused-silica capillary guard column (0.25 mm × 1 m) via a press-tight connector (J&W or Restek; Methods 1 and 2) and/or a two way (Y) press-tight connector (Method 3). Helium was used as the carrier gas at measured linear velocities (methane injection, oven temperature 89 °C) of 26.1 and 27.8 cm/s, respectively, for the DB-5 and RTx-200 columns. Retention index values were calculated by the linear-temperature-programmed gas-chromatographic retention index (LTPGCRI) method as described by Elvebak and Gray [14] using GLC Method 3.

GLC–MS analyses were performed using a Finnegan MAT 95 high-resolution double-focusing, reverse-geometry mass spectrometer equipped with a Hewlett–Packard 5890A Series II gas–liquid chromatograph and a DEC model 2100 workstation. Column effluents were analyzed by chemical-ionization (CI) mass spectrometry using ammonia as the reagent gas or by electron-ionization (EI) mass spectrometry at 70 eV in order to verify that eluted compounds had mass spectra identical to those of independently synthesized standards.

^1H and ^{13}C NMR spectra were recorded on a Varian VXR-500 spectrometer equipped with a VNMR data system. Spectra recorded with $CDCl_3$ as the solvent were referenced to internal tetramethylsilane, whereas those recorded in CD_2Cl_2 were referenced to the instrument's internally set frequency (δ 5.32) for residual $CHDCl_2$.

Dimethyl sulfoxide, iodomethane, acetic anhydride, pyridine, (±)-2-butanol, 2,6-di-*tert*-butylpyridine, trimethylsilyl trifluoromethanesulfonate, trimethylsilyl methanesulfonate, boron trifluoride etherate, and methyl lithium were obtained from Aldrich. *N*-Acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, and *N*-acetyl-D-mannosamine were from ICN, Inc. (±)-2-Butanol, (*S*)-2-butanol (97.4% ee) and (*R*)-2-butanol (97.4% ee) were from Fluka. Mixed-bed ion-exchange resin AG 501 X-8(D) was obtained from Bio-Rad Laboratories. All deuterated solvents were from Cambridge Isotope Laboratories. Trimethylsilyl trifluoromethanesulfonate was stored over CaH_2, and Me_3SiOSO_2Me and $BF_3 \cdot OEt_2$ were stored over 4 Å molecular sieves and periodically redistilled. Methanol, dichloromethane, 1,2-dichloroethane, acetic acid, pyridine, and Me_2SO were distilled as described by Perrin et al. [15]. Alcohols were stored over 3 Å molecular sieves. Sodium hydroxide was pulverized with a mortar and pestle and stored under nitrogen.

Time-course experiments.—The rate of formation of oxazolinium-ion intermediates was measured by dissolving 5.0 mg (17.2 μmole) of **1**, **2**, or **3** in 500 μL of CD_2Cl_2 in a previously silylated NMR tube (No. 528). The solution was first analyzed by ^1H NMR to establish initial parameters, then one of three Lewis acid promoters, namely $Me_3SiOSO_2CF_3$ (5 equiv), Me_3SiOSO_2Me (5 equiv), and $BF_3 \cdot OEt_2$ (1

equiv) or $BF_3 \cdot OEt_2$ (5 equiv), was added. After mixing thoroughly on a vortex mixer, the spectrometer was re-shimmed, and a series of spectra were recorded at regular intervals in order to monitor the disappearance of the H-1 signal of the starting glycoside (**1–3**) and the emergence of the H-1 signal of the oxazolinium-ion intermediate **4–6**, and the H-1 signal of the intermediate of undetermined structure [7]. The mole fractions of the starting material and product were determined from integrations of these resonances.

Transglycosidation with (±)-2-butanol.—The starting glycoside (**1–3**) (5.0 mg, 17.2 μmol) was dissolved in 1.0 mL of CH_2Cl_2 containing docosane (15 mol% relative to **1–3**), and the solution was examined by gas–liquid chromatography (Method 1) in order to establish the integral values of starting material and docosane. One of three Lewis acid promotors was added to each reaction, and the reactions were stirred at room temperature until oxazolinium-ion formation was complete (see Figs. 1–3). Each reaction was then quenched by the addition of 200 μL of (±)-2-butanol and aliquots (50 μL) of each reaction were removed at regular intervals and added to a mixture of dichloromethane (500 μL) and aq $NaHCO_3$ (500 μL). After thorough mixing, the dichloromethane layer was analyzed by GLC (Method 1). The rates of appearance of the (*S*)- and (*R*)-diastereomers were determined by comparison of their peak areas, after correction for molar response [16], to that of internal docosane. The individual (*R*)- and (*S*)-diastereomers were identified in each case by conducting the transglycosidation in the presence of pure (*R*)- or (*S*)-2-butanol.

*Transglycosidation with (±)-2-butanol in the presence of 2,6-di-*tert*-butylpyridine.*—Compounds **1–3** (5.0 mg, 17.2 μmol) were dissolved separately in 1.0 mL of dichloromethane containing docosane (15 mol% relative to **1–3**), and the solution was examined by gas–liquid chromatography in order to establish the integral values of starting material and docosane. Trimethylsilyl trifluoromethanesulfonate (16.6 μL, 86 μmol) was added to each reaction and the reactions were stirred at room temperature until oxazolinium-ion formation was complete (see Figs. 1–3). The reactions were then quenched by the simultaneous addition of 200 μL of (±)-2-butanol and 40 μL of 2,6-di-*tert*-butylpyridine, the latter serving as a proton sponge [17]. Aliquots (50 μL) of the reactions were removed at regular intervals and added to a mixture of dichloromethane (500 μL) and aq $NaHCO_3$ (500 μL), and after thorough mixing the organic layer was removed and analyzed by GLC (Method 1). The rate of appearance of the (*S*)- and (*R*)- diastereomers was determined by comparison of their GLC peak areas, after correction for molar response [16], to that of internal docosane.

Methyl 2-deoxy-3,4,6-tri-O-methyl-2-(N-methylacetamido)-β-D-glucopyranoside (**1**).—Compound **1** was prepared from *N*-acetyl-D-glucosamine in four steps [3]. The GLC retention indices and ^1H NMR spectrum of the product were identical to those previously reported [3].

Methyl 2-deoxy-3,4,6-tri-O-methyl-2-(N-methylacetamido)-β-D-galactopyranoside (**2**).—*N*-Acetyl-D-galactosamine (0.25 g, 1.13 mmol) was dissolved in 10 mL of freshly distilled MeOH in the presence of IRA-120 (H^+) (0.1 g) [5]. The reaction mixture was heated at 65 °C for 5 days, and the resin was then filtered and washed with MeOH (10 mL). Concentration of the filtrate yielded a mixture of the α and β anomers as a white solid (60%). Without purification, a portion of the product was permethylated as described by Ciucanu and Kerek [6]. Thus, the product (0.1 g, 0.42 mmol, previously dried overnight under high vacuum) was dissolved in 1.0 mL of Me_2SO, pulverized NaOH (74 mg) was added, and the solution was stirred vigorously at room temperature for 8 h. Iodomethane (1.0 mL) was added to the solution under an ice-water bath and the mixture was stirred at room temperature for 4 h. The mixture was poured into ice-water (1.0 mL) and extracted with $CHCl_3$ (4 × 5 mL). The organic layer was washed with water (2 × 20 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum at < 40 °C. A portion of the crude product (20 mg, 0.069 mmol, previously dried overnight under high vacuum) was dissolved in 2.0 mL of freshly distilled 1,2-dichloroethane, $Me_3SiOSO_2CF_3$ (66 μL) was added, and the solution was heated at 70 °C for 8 h [7]. The reaction was then quenched with distilled MeOH (1.0 mL) and stirred at room temperature for 4 h. The solution was deionized with mixed-bed resin and then methanol was removed under a stream of dry N_2 gas. The product was purified by flash column chromatography (silica gel, 1.0 × 20 cm, 230–400 mesh) using hexane (50 mL), mixtures of hexane–EtOAc in ratios of 9:1 (50 mL), 4:1 (50 mL), 1:1 (50 mL), 1:4 (50 mL), and 1:8 (300 mL), and EtOAc (50 mL) as the eluent. The purity of the product was determined by ^1H NMR spectroscopy and GLC (Method 2). GLC retention indices (LTPGCRI method): DB-5, 1876.72; RTx-200, 2307.31. ^1H NMR ($CDCl_3$, 500 MHz) with COSY: δ

5.124 (d, 0.35 H, $J_{1,2}$ 7.5 Hz, H-1, rotamer 2), 4.378 (br d, 0.35 H, J 8.5 Hz, H-3, rotamer 2), 4.328 (d, 0.65 H, H-1, rotamer 1), 3.936 (dd, 0.65 H, $J_{2,1}$ 8.5 Hz, H-2, rotamer 1), 3.38–3.70 (complex, 15 H, H-5, 6a, 6b, and 4 MeO), 3.778 (d, 1 H, H-4), 3.312 (dd, 0.65 H, $J_{2,3}$ 11.0, $J_{3,4}$ 2.5 Hz, H-3, rotamer 1), 3.02-3.12 (br s, 1.4 H, MeN, and H-2, rotamer 2), 2.810 (s, 1.95 H, MeN, rotamer 1), 2.136 (s, 1.95 H, AcN, rotamer 1), and 2.074 (s, 1.05 H, AcN, rotamer 2). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.14 (C=O), 100.62 (C-1), 79.15, 73.37, 73.06 (C-3, 4, and 5), 70.68 (C-6), 61.19, 59.33, 57.52, 56.94 (4 MeO), 59.22 (C-2), 27.24 (MeN), and 22.14 (AcN).

Methyl 2 - deoxy - 3, 4, 6 - tri - O - methyl - 2 - (N - methylacetamido) - α - D - mannopyranoside (**3**).—*N*-Acetyl-D-mannosamine (3.0 g, 13.6 mmol) was dissolved in 15 mL of freshly distilled MeOH in the presence of IRA-120 (H^+) (1.0 g), the reaction was heated at 65 °C for 4 days, then the resin was filtered and washed with MeOH (10 mL). Concentration of the filtrate yielded a white solid (2.22 g, 70%). Without purification, the product was permethylated as described by Hakomori [8]. Thus, a portion of the product (0.1 g, 0.425 mmol, previously dried overnight under high vacuum) was dissolved in 8.0 mL of Me_2SO, and the solution was stirred at room temperature for 8 h. A solution of lithium methylsulfinylmethide, prepared by mixing 1.4 M CH_3Li (35 mL in ether) and Me_2SO (35 mL), was introduced to the sugar solution slowly, and the mixture was stirred at room temperature for 2.5 h. Iodomethane (6.8 mL) was then added under an ice-water bath, and the mixture was stirred at room temperature for 4.5 h. The mixture was poured into ice-water (300 mL) and extracted with $CHCl_3$ (4 × 70 mL). The organic layer was washed with water (2 × 100 mL), dried over anhyd Na_2SO_4, and concentrated under vacuum at < 40 °C. Without purification, the product (previously dried overnight under high vacuum) was treated with hydrazine hydrate (2.0 mL) in a side-arm tube at 120 °C for 20 h in an oil bath [9]. After removal of excess reagent by evaporation under vacuum at 50 °C, methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-methylamino-α-D-mannopyranoside (43 mg) was isolated by flash column chromatography (silica gel, 1.0 × 15 cm, 230–400 mesh) using 8:1 EtOAc–MeOH as the eluent. The purity of the deacetylated product was determined by ^{1}H NMR spectroscopy and GLC (Method 2). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.704 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 3.462, 3.391, 3.381, 3.346 (4 s, 12 H, 4 MeO), 3.10–3.60 (m, 6 H, H-3, 4, 5, 6a, and 6b), 2.895 (dd, 1 H, $J_{2,3}$ 4.5 Hz, H-2), 2.430 (s, 3 H, MeN), 1.952 (br s, 1 H, NH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 98.62(C-1), 80.76, 76.30, 70.68 (C-3, 4, and 5), 71.87 (C-6), 60.18 (C-2), 60.45, 59.22, 57.09, 54.94 (MeO), and 35.68 (MeN).

The deacylated compound (43 mg) was dissolved in CH_2Cl_2 (1.0 mL), the solution was cooled in an ice bath, and acetic anhydride (100 μL) and pyridine (200 μL) were added. The reaction was stirred at room temperature for 15 h, poured into ice water (5.0 mL), and extracted with dichloromethane (4 × 5 mL). The organic layer was washed with M H_2SO_4 (10 mL), $NaHCO_3$ (2 × 10 mL), and water (2 × 20 mL). The organic layer was dried over anhyd Na_2SO_4 and then concentrated and dried under high vacuum. The purity of the product (**3**) was determined by ^{1}H NMR spectroscopy and GLC (Method 2). GLC retention indices (LTPGCRI method): DB-5, 1869.61; RTx-200, 2271.69. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.23 (dd, 0.82 H, $J_{2,3}$ 6.35 Hz, H-2, rotamer 1), 4.75 (d, 0.18 H, H-1, rotamer 2), 4.63 (d, 0.82 H, $J_{1,2}$ 1.2 Hz, H-1, rotamer 1), 4.04 (dd, 0.18 H, $J_{2,1}$ 3.0, $J_{2,3}$ 5.0 Hz, H-2, rotamer 2), 3.20–3.70 (complex, 17 H, H-3, 4, 5, 6a, 6b, and 4 MeO), 3.08 (s, 2.46 H, MeN, rotamer 1), 3.00 (s, 0.54 H, MeN, rotamer 2), 2.11 (s, 2.46 H, AcN, rotamer 1), and 2.10 (s, 0.54 H, AcN, rotamer 2). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.18 (C=O), 101.89 (C-1), 80.43, 76.19, 70.44 (C-3, 4, and 5), 71.86 (C-6), 60.56, 59.60, 58.04, 54.90 (4 MeO), 51.49 (C-2), 34.48 (MeN), and 22.57 (AcN).

(S)- and (R)-2-Butyl 2-deoxy-3,4,6-tri-O-methyl-2-(N-methylacetamido)-β-D-glucopyranosides (**7** and **8**).—Compounds **7** and **8** were prepared as previously described [3]. Their GLC and ^{1}H NMR data were identical to those previously reported [3].

(S)- and (R)-2-Butyl 2-deoxy-3,4,6-tri-O-methyl-2-(N-methylacetamido)-β-D-galactopyranosides (**9** and **10**).—Compound **2** (5.0 mg, 0.0172 mmol) was dissolved in CH_2Cl_2 containing 15 mol% of docosane (relative to **2**) and treated with $Me_3SiOSO_2CF_3$ (16.6 μL) for 8 h. The mixture was divided into two portions, and one portion was quenched with (*S*)-2-butanol (100 μL), giving **9**, and the other with (*R*)-2-butanol (100 μL), giving **10**, both in 97% diastereomeric excess as determined by GLC (Method 1). For **9**: GLC retention indices (LTPGCRI method): DB-5, 1987.66; RTx-200, 2385.50. GLC–CI(NH_3)-mass spectrum: *m/z* 260 [$(M - C_4H_9)^+$, 26%], 334 [$(M + H)^+$, 100%], 351 [$(M + NH_4)^+$, 2.0%]. ^{1}H NMR (500 MHz, $CDCl_3$): for rotamer 1: δ 4.47 (d, 0.65 H, $J_{1,2}$ 8.0 Hz, H-1), 3.937 (dd, 0.65 H, H-2), 3.319 (dd, 0.65 H, $J_{3,2}$ 11.0, $J_{3,4}$ 2.5 Hz, H-3), 2.820

(s, 1.95 H, MeN), 2.169 (s, 1.95 H, AcN), 1.190 (d, 1.95 H, $J_{1',2'}$ 6.5 Hz, H-1'), 0.831 (t, 1.95 H, $J_{4',3'}$ 7.5 Hz, H-4'); for rotamer 2: δ 5.258 (d, 0.35 H, H-1), 4.397 (dd, 0.35 H, $J_{3,2}$ 11.0, $J_{3,4}$ 2.5 Hz, H-3), 3.083 (s, 1.05 H, MeN), 2.910 (dd, 0.35 H, $J_{2,1}$ 8.0 Hz, H-2), 2.051 (s, 1.05 H, AcN), 1.172 (d, 1.05 H, $J_{1',2'}$ 6.5 Hz, H-1'), 0.812 (t, 1.05 H, $J_{4',3'}$ 7.5 Hz, H-4'); for rotamers 1 and 2: δ 3.777 (d, 1 H, H-4), 3.30–3.70 (complex, 13 H, H-2', 5, 6a, 6b, and 3 MeO), and 1.32–1.51 (complex, 2 H, H-3a', and 3b').

For **10**: GLC retention indices (LTPGCRI method): DB-5, 1968.96; RTx-200, 2358.48, GLC–CI(NH_3)-mass spectrum: *m/z* 260 [$(M - C_4H_9)^+$, 19%], 334 [$(M + H)^+$, 100%], 351 [$(M + NH_4)^+$, 1.4%]. ^{1}H NMR (500 MHz, $CDCl_3$): for rotamer 1: δ 4.460 (d, 0.7 H, $J_{1,2}$ 8.0 Hz, H-1), 3.920 (dd, 0.7 H, H-2), 3.322 (dd, 0.7 H, $J_{3,2}$ 11.0, $J_{3,4}$ 2.5 Hz, H-3), 2.815 (s, 2.1 H, MeN), 2.161 (s, 2.1 H, AcN), 1.040 (d, 2.1 H, $J_{1',2'}$ 6.0 Hz, H-1'), 0.860 (t, 2.1 H, $J_{4',3'}$ 7.5 Hz, H-4'); for rotamer: 2, δ 5.230 (d, 0.3 H, H-1), 4.430 (dd, 0.3 H, $J_{3,4}$ 2.5 Hz, H-3), 3.071 (s, 0.9 H, MeN), 2.910 (dd, 0.3 H, $J_{2,3}$ 11.0, $J_{2,1}$ 8.0 Hz, H-2), 2.058 (s, 0.9 H, AcN), 1.028 (d, 0.9 H, $J_{1',2'}$ 6.0 Hz, H-1'), 0.870 (t, 0.9 H, $J_{4',3'}$ 7.5 Hz, H-4'); for rotamers 1 and 2: δ 3.780 (d, 1 H, H-4), 3.710 (sextet, 1 H, $J_{2',3ab'}$ 6.0 Hz, H-2'), 3.30–3.68 (complex, 12.7 H, H-3, 5, 6a, 6b, and 3 MeO), and 1.42–1.60 (complex, 2 H, H-3a', and 3b').

(S)- and (R)-2-Butyl 2-deoxy-3,4,6-tri-O-methyl-2-(N-methylacetamido)-α-D-mannopyranosides (**11** and **12**).—Compound **3** (5.0 mg, 0.0172 mmol) was dissolved in CH_2Cl_2 containing 15 mol% of docosane (relative to **3**) and treated with $Me_3SiOSO_2CF_3$ (16.6 μL) for 8 h. The mixture was divided into two portions, one portion was quenched with (*S*)-2-butanol (100 mL), giving **11**, and the other with (*R*)-2-butanol (100 μL), giving **12**, each in 97% diastereomeric excess as determined by GLC (Method 1). For **11**: GLC retention index (LTPGCRI method): RTx-200, 2373.16; GLC–CI(NH_3)-mass spectrum: *m/z* 228 [$(M - MeOH + C_4H_9)^+$, 30%], 260 [$(M - C_4H_9)^+$, 50%], 334 [$(M + H)^+$, 100%]. ^{1}H NMR (500 MHz, $CDCl_3$): for rotamer 1: δ 5.196 (dd, 0.77 H, $J_{2,3}$ 6.5 Hz, H-2), 4.893 (d, 0.77 H, $J_{1,2}$ 1.2 Hz, H-1), 3.118 (s, 2.31 H, MeN), 1.090 (d, 2.31 H, $J_{1',2'}$ 6.3 Hz, H-1'), 0.890 (t, 2.31 H, $J_{4',3'}$ 7.5 Hz, H-4'); for rotamer 2: δ 5.004 (d, 0.23 H, H-1), 4.018 (t, 0.23 H, $J_{2,1} = J_{2,3}$ 3.5 Hz, H-2), 3.968 (m, 0.23 H, H-2'), 3.041 (s, 0.69 H, MeN), 1.072 (d, 0.69 H, $J_{1',2'}$ 6.3 Hz, H-1'), 0.902 (t, 0.69 H, $J_{4',3'}$ 7.5 Hz, H-4'); for rotamers 1 and 2: δ 3.30–3.80 (complex, 14.77 H, H-2', 3, 4, 5, 6a, 6b, and 3 MeO), 2.146 (s, 3 H, AcN), and 1.38–1.62 (complex, 2 H, H-3a', and 3b').

For **12**: GLC retention index (LTPGCRI method): RTx-200, 2359.50. GLC–CI(NH_3)-mass spectrum: *m/z* 228 [$(M - MeOH + C_4H_9)^+$, 38%], 260 [$(M - C_4H_9)^+$, 57%], 334 [$(M + H)^+$, 100%]. ^{1}H NMR (500 MHz, $CDCl_3$): for rotamer 1: δ 5.220 (dd, 0.77 H, $J_{2,3}$ 6.3 Hz, H-2), 4.860 (d, 0.77 H, $J_{1,2}$ 1.2 Hz, H-1), 3.111 (s, 2.31 H, MeN), 2.141 (s, 2.31 H, AcN), 1.148 (d, 2.31 H, $J_{1',2'}$ 6.3 Hz, H-1'), 0.864 (t, 2.31 H, $J_{4',3'}$ 7.2 Hz, H-4'); rotamer 2: δ 4.978 (d, 0.23 H, H-1), 4.043 (t, 0.23 H, $J_{1,2} = J_{2,3}$ 3.5 Hz, H-2), 3.033 (s, 0.69 H, MeN), 3.970 (m, 0.23 H, H-2'), 2.149 (s, 0.69 H, AcN), 1.180 (d, 0.69 H, $J_{1',2'}$ 6.3 Hz, H-1'), 0.928 (t, 0.69 H, $J_{4',3'}$ 7.2 Hz, H-4'); for rotamers 1 and 2: δ 3.30–3.80 (complex, 14.77 H, H-1', 3, 4, 5, 6a, 6b, and 3 MeO), and 1.34–1.60 (complex, 2 H, H-3a', H-3b').

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