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An improved procedure for the analysis of linkage positions in 2-acetamido-2-deoxy-D-glucopyranosyl residues by the reductive-cleavage method †

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ABSTRACT

The conditions of the reductive-cleavage method were modified to allow simultaneous analysis of 2-acetamido-2-deoxy-D-glucopyranosyl 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 transglycosidation under reductive-cleavage conditions when the reaction was quenched with an alcohol. Transglycosidation proceeded via an oxazolinium-ion intermediate, which then acted as a glycosyl donor to form an anomerically pure product. Time-course studies showed that in the presence of trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$), 4 h were required for complete conversion of the substrate into this intermediate, which was then trapped with methanol-d_4. When the reaction was conducted in the presence of a mixture of trimethylsilyl methanesulfonate (Me_3SiOSO_2Me) and boron trifluoride etherate ($BF_3 \cdot OEt_2$) or with $BF_3 \cdot OEt_2$ alone, 24 h and 48 h, respectively, were required for complete conversion. The α anomer was unreactive after 24 h under all conditions, confirming earlier results. Reaction with racemic 2-butanol yielded a pair of diastereomers, in a 1:1 ratio, which were distinguishable by their GLC retention times and their ^1H NMR spectra. Reaction with (*S*)-2-butanol gave only one of the diastereomeric products. These experiments demonstrated the feasibility of using the reductive-cleavage method to determine the absolute configuration of 2-acetamido sugars.

INTRODUCTION

In a previous study[1], conditions developed for the analysis of 2-acetamido sugar-containing carbohydrates by the reductive-cleavage method were found to give water-soluble derivatives which formed via hydrolysis of oxazolinium-ion intermediates. Accurate quantitation of acetamido sugar residues was therefore not possible by this technique because their derivatives were separated from the other cleavage products when aqueous sodium bicarbonate was added to neutralize the Lewis-acid promoter. Since oxazoline and oxazolinium-ion derivatives of 2-acetamido sugars have been used as glycosyl donors for the formation of

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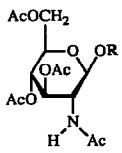
anomerically pure alkyl glycosides and oligosaccharides in the presence of Lewis acids[2–4], a reinvestigation of the previously established reductive-cleavage conditions was conducted. The objective of this study was to modify the procedure to generate acetamido sugar derivatives that could be analyzed simultaneously with derivatives of monosaccharide residues of other classes.

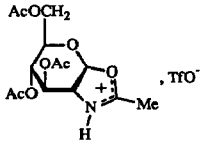
RESULTS

*Methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**2**).—A method was devised for preparing **2**, a precursor to the fully methylated β glycoside **4b**, from penta-*N*,*O*-acetyl compound **1**. The procedure of Nakabayashi et al.[2] for producing oxazoline **3** by heating **1** in ethylene dichloride in the presence of trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$) was modified to operate under conditions similar to those for reductive cleavage (room temperature, CH_2Cl_2), and glycosidation was effected in situ to produce anomerically pure **2** in one step. The transformation was effected by reacting **1** with a slight molar excess of $Me_3SiOSO_2CF_3$ under anhydrous conditions, followed by quenching with anhydrous methanol and deionization with mixed-bed resin. The product **2** was obtained in 65% yield after recrystallization.

Reaction times for both steps of this process were established by TLC (3:2 acetone–hexane). The conversion of **1** (R_f 0.40) to the oxazolinium triflate (**3**, R_f 0.50), or its neutral derivative[2], required at least 1 h. After the addition of methanol, the formation of **2** (R_f 0.44) was complete in 30 min.



1, R=Ac
2, R=Me



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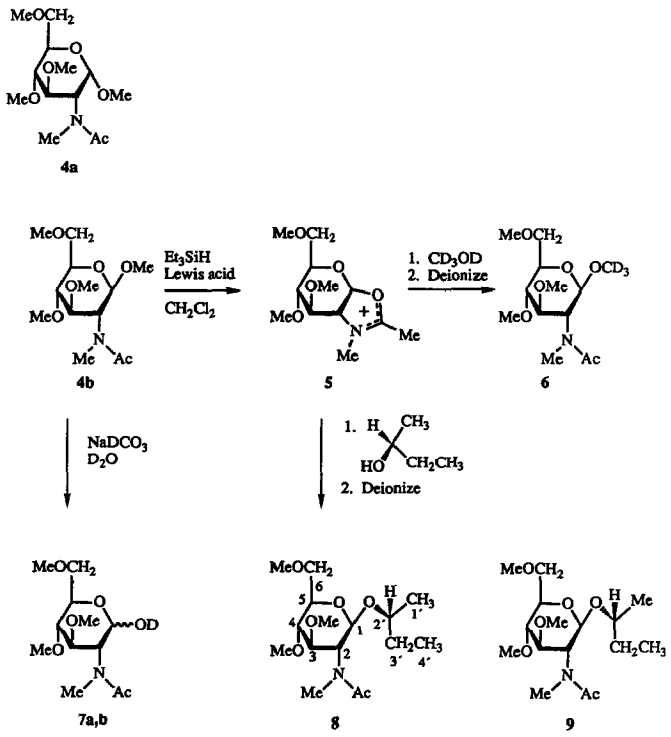
*Reactivity of methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α,β-*D*-glucopyranosides (**4a,b**) under reductive-cleavage conditions.*—The satisfactory results obtained in the synthesis of **2** suggested that Lewis acid-promoted, stereospecific transglycosidation of fully methylated 2-acetamido sugars, via alcoholysis of oxazolinium ions, might occur under reductive-cleavage conditions. To test this hypothesis, compounds **4a** and **4b** were subjected to reductive-cleavage conditions and subsequent quenching with methanol-d_4. Experiments were designed to determine (*a*) the feasibility of alcoholytic quenching for all reagent systems studied, (*b*) the minimum reaction time, and (*c*) the percent recovery of products. Compounds **4a** and **4b** were reacted separately with triethylsilane (Et_3SiH) (5 equiv) as the reducing agent and one of three Lewis-acid promoters—(*a*) $Me_3SiOSO_2CF_3$ (5 equiv)[5], (*b*) a mixture of trimethysilyl methanesulfonate (Me_3SiOSO_2Me) (5 equiv) and boron trifluoride etherate ($BF_3 \cdot OEt_2$) (1 equiv)[6], or (*c*) $BF_3 \cdot OEt_2$ (5 equiv)[7].



Reaction mixtures were quenched with excess methanol-d_4 and were deionized and processed as in the synthetic procedure for **2**. In time-course experiments, aliquots of the reaction mixtures were removed, quenched separately, and processed. Product mixtures were analyzed by GLC (Method 2) combined with chemical-ionization (NH_3) mass spectrometry (CIMS).

With all three reagent systems, experiments with **4a** as substrate demonstrated that no reaction occurred even after 24 h. Experiments with **4b** indicated that quenching with methanol-d_4 yielded the expected product, **6**, for all three reagent systems (Scheme 1). In time-course experiments (Fig. 1), $< 1\%$ of the substrate remained after 4 h for reagent (*a*) and after 24 h for reagent (*b*); for reagent (*c*), 2% of the substrate remained after 48 h. Product mixtures containing compounds **4b** and **6** were analyzed by integration of selected-ion GLC–CIMS peaks (**4b**, m/z 292; **6**, m/z 295). The ^{1}H NMR spectrum of **6** was essentially identical to that of **4b**, except for the absence of singlets at δ 3.45 (1 H) and 3.47 (2 H), corresponding to the anomeric methoxyl group of the two amide rotamers. Product recoveries in all experiments were quantitative, within experimental error, as determined by GLC peak areas relative to the peak for internal docosane.

An additional experiment was performed to determine more accurately the minimum time for the reaction of the oxazolinium-ion intermediate **5** with

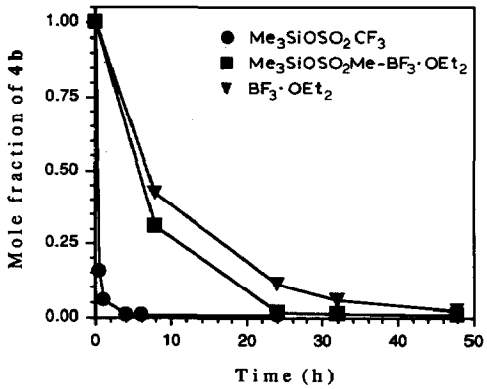


Fig. 1. Time-course of product formation during reductive cleavage of **4b** in the presence of Lewis-acid catalysts. Reactions were quenched with methanol-d_4, and the relative amounts of starting material and product (**6**) were monitored by GLC–CIMS as described in the text.

methanol. Compound **4b** in CD_2Cl_2 was treated with $Me_3SiOSO_2CF_3$ for the minimum period (4 h) as determined above, after which the ^1H NMR spectrum of **5** was recorded. Methanol-d_4 was then added, and the disappearance of the intermediate, as indicated by the decreasing area of the H-1 doublet at δ 6.61, was monitored at regular intervals. The data showed that the reaction was complete in ~ 70 min.

Another experiment was performed with compound **4b** and $Me_3SiOSO_2CF_3$ in order to determine the fate of the product arising from hydrolysis. After 4 h, the reaction in CD_2Cl_2 was quenched with a 5% solution of $NaDCO_3$ in D_2O (Scheme 1). The aqueous layer was then analyzed directly by ^1H NMR spectroscopy, which revealed the presence of an anomeric mixture of the free-sugar analogue (**7a,b**) of the substrate (δ 4.78, d, J 7.8 Hz, **7b**, H-1β, rotamer 2; δ 4.87, d, J 7.9 Hz, **7b**, H-1β, rotamer 1; δ 5.00, d, J 3.0 Hz, **7a**, H-1α, rotamer 1; δ 5.14, d, J 3.0 Hz, **7a**, H-1α, rotamer 2). Analysis of the organic layer by GLC demonstrated that **4b** was totally consumed and that no other products were formed. This experiment demonstrated that **7a,b**, a neutral compound, was indeed water-soluble; thus, the results of the previous study[1] were confirmed.

2-Butanolysis experiments.—Substitution of 2-butanol for methanol as the quenching alcohol in the reductive-cleavage of **4b** was expected to produce the corresponding *sec*-butyl glycosides. Furthermore, racemic 2-butanol was expected to produce chromatographically distinct diastereomeric glycosides, but the optically pure alcohol was expected to yield only one diastereomer[8]. Indeed, *sec*-butyl glycosides **8** and **9** were formed in quantitative yield when the mixture from the reductive cleavage of **4b** with reagent *a* was quenched with racemic 2-butanol. In this case, glycosidation was allowed to proceed for 24 h. Analysis of the product by GLC revealed the presence of two compounds in equimolar amounts (Fig. 2A), and analysis of the mixture by GLC–CIMS (NH_3) confirmed that **8** and **9**

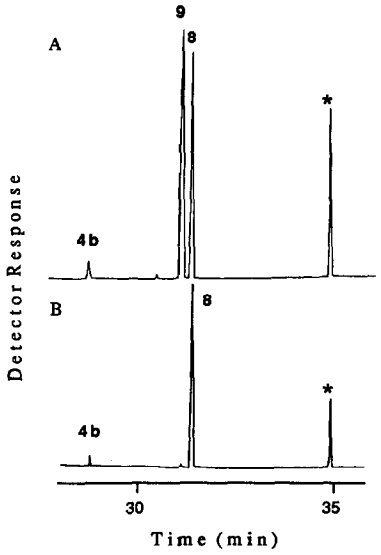


Fig. 2. Gas–liquid chromatograms of products obtained after treatment of **4b** with Et_3SiH and $Me_3SiOSO_2CF_3$, followed by quenching with (±)-2-butanol (A) or (*S*)-2-butanol (B). Peaks are designated by compound numbers, and docosane, added as an internal standard, is designated with an asterisk.

$[(M + H)^+, m/z\ 334]$ were produced. Transglycosidation was then performed with (*S*)-2-butanol (97.4% ee) as the glycosyl acceptor (Scheme 1). Analysis of the product by GLC (Fig. 2B) demonstrated that **8** was formed in 96% diastereomeric excess over **9**. Comparison of the ^1H NMR spectra of the products from these two experiments revealed that the diastereomeric glycosides are distinguishable by the resonances of their anomeric protons; that is, H-1 of **8**, the D*S*-diastereomer, was observed as a doublet at δ 4.47 (J 8.1 Hz). Thus, the remaining doublet at δ 4.46 (J 8.1 Hz) in the spectrum of the **8**,**9** mixture was assigned to H-1 of **9**, the D*R*-diastereomer.

A GLC experiment, conducted to determine the minimum time necessary for oxazolinium-ion intermediate **5** to react with (±)-2-butanol, showed this reaction to be complete in ~6 h. In this experiment, **5** was generated from **4b** in the presence of $Me_3SiOSO_2CF_3$ and subsequently treated with (±)-2-butanol. At regular intervals, aliquots were withdrawn, deionized and processed. The emergence of compounds **8** and **9** was monitored by GLC (Method 1) by comparison of their peak integrals to that of internal docosane.

DISCUSSION

The protected, anomerically pure β-glycoside **2** was synthesized in three steps from the free acetamido sugar. The key step, glycosidation of oxazoline **3** in situ, eliminated the need to isolate[9–12] the neutral oxazoline intermediate. Kiso and Anderson[3,13] similarly converted **1** to anomerically pure β glycosides by adapting

Bach and Fletcher's method (as described by Matta and Bahl[11]) for the $FeCl_3$-promoted production of oxazolines. The procedure described herein is more convenient than the latter in that the reaction proceeds in homogeneous solution and requires less time (90 min vs. overnight). In addition, the product can be isolated directly from the reaction mixture in reasonable yield and purity after deionization with mixed-bed resin. Satisfactory results with this procedure, as with that of Kiso and Anderson[13], could be predicted for a variety of glycosyl acceptors, including appropriately protected monosaccharides.

The success of the two-step procedure for the synthesis of compound **2** presented the possibility that methanol, instead of aqueous sodium bicarbonate, could be used to quench reductive-cleavage reactions employing reagent (*a*), thus yielding products which remain soluble in CH_2Cl_2. Methanol was already the prescribed quenching agent for reagents (*b*) and (*c*) in order to convert $BF_3 \cdot OEt_2$ to volatile $B(OMe)_3$. Preliminary results from experiments in which a permethylated, amino sugar-containing polysaccharide was sequentially treated with reagent (*b*) then methanol indicated that transglycosidation was indeed occurring. Thus, investigation of the reactivities of the simpler model substrates **4a** and **4b** seemed warranted. Indeed, exposure of the β anomer (**4b**) to reductive-cleavage conditions followed by quenching with anhydrous methanol led to rapid ($\sim$ 70 min) conversion of the intermediate oxazolinium ion (**5**) to the trideuteriomethyl glycoside (**6**). The latter was recovered in quantitative yield after deionization and extraction with dichloromethane–methanol. In contrast to these results, the α anomer (**4a**) was fully stable to transglycosidation under these conditions, as was expected [1].

From the outcome of these experiments it was evident that substitution of a chiral alcohol for methanol in the transglycosidation reaction would provide a means for establishing the optical purity and indeed the absolute configuration of the acetamido sugar derivative. Thus, quenching of the oxazolinium ion **5** with the (*S*)-enantiomer of 2-butanol gave a single product (**8**), whereas quenching with the racemic alcohol yielded the diastereomeric glycosides (**8** and **9**), which were separable by GLC. Determination of the absolute configurations of amino sugar residues in a polysaccharide requires only that authentic standards for at least one of the enantiomers of each amino sugar be available.

Finally, the procedures described herein should be applicable to the simultaneous analysis of β-linked GlcNAc and neutral sugar residues in polysaccharides by the reductive-cleavage method.

EXPERIMENTAL

General.—Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer model 241 polarimeter in a 1-dm semimicro cell. Flash chromatography[14] and medium-pressure liquid chromatography (MPLC) were performed on 230–400 mesh Silica Gel 60 (E. Merck). MPLC was carried out on an instrument consisting of a 0.8 × 25 cm

glass column, an Eldex model B-100-S-4 pump, a Scientific Systems model LP-21 pulse dampener, and a Rheodyne 7125 injector. Thin-layer chromatography was performed on glass plates precoated with Silica Gel GF (Analtech); spots were visualized by charring with 5% H_2SO_4 in EtOH.

^1H NMR spectra were recorded on Varian VXR-300 or VXR-500 spectrometers equipped with a VNMR data system at 300 and 500 MHz, respectively. Spectra recorded with $CDCl_3$ as the solvent were referenced to internal Me_4Si, whereas those recorded in CD_2Cl_2 were referenced to the instruments' internally set frequency for residual CH_2Cl_2 (δ 5.32). Spectra recorded in D_2O were referenced to internal sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate (Aldrich).

Analytical GLC was performed on a Hewlett–Packard model 5890A gas chromatograph equipped with a Hewlett–Packard model 3392A integrator, two flame-ionization detectors, a cool on-column inlet, and a split–splitless inlet operated in the split 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) coated with DB-5 (0.25-μm film thickness; J&W Scientific), programmed from 40 to 250°C at 6°C/min. *Method 2.* Split injection (injector temperature, 250°C, split ratio 40:1) 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 10:1) into the DB-5 column or a fused-silica capillary column (0.25 mm × 30 m) coated with Rt_x-200 (0.25-μm film thickness; Restek), programmed from 80 to 250°C at 2°C/min. Each column was fitted with a J&W fused-silica guard column (0.25 mm × 1 m) 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, over temperature 80°C) of 21.7 cm/s (*Method 1*) and 26.1 cm/s (*Methods 2* and *3*) for the DB-5 column and 27.8 cm/s for the Rt_x-200 column. Retention indices were obtained by the linear-temperature-programmed gas-chromatographic retention index (LTPGCRI) method as described by Elvebak et al.[15] using GLC *Method 3.*

GLC–MS analyses were performed using GLC *Method 2* and a Hewlett–Packard 5890A chromatograph connected by a deactivated fused-silica interface to a VG Analytical model VG 7070E-HF or a Finnigan-MAT model MAT-95 mass spectrometer. Column effluents were analyzed by chemical-ionization mass spectrometry using ammonia as the reagent gas.

Triethylsilane, trimethylsilyl trifluoromethanesulfonate, boron trifluoride etherate, methanesulfonic acid, chlorotrimethylsilane, methyl iodide, dimethyl sulfoxide (Me_2SO), (±)-2-butanol, 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose, and 2-acetamido-2-deoxy-α-D-glucopyranose (α-D-GlcNAc) were obtained from Aldrich Chemical Co. (*S*)-2-Butanol (97.4% ee) was from Fluka Chemical Co. Mixed-bed ion-exchange resin AG501 X-8(D) was obtained from

Bio-Rad Laboratories. All deuterated solvents were from Cambridge Isotope Laboratories. Trimethylsilyl methanesulfonate was prepared from methanesulfonic acid and chlorotrimethylsilane as previously described[6]. Trimethylsilyl trifluoromethanesulfonate, trimethylsilyl methanesulfonate, and boron trifluoride etherate were stored over 4A molecular sieves, and periodically redistilled. Methanol and CH_2Cl_2 were distilled as described by Perrin et al.[16] Alcohols were stored over 3A molecular sieves or anhyd $CaSO_4$ (Aldrich). Chlorinated, deuterated solvents were stored over anhyd K_2CO_3 (Aldrich).

Reductive cleavages.—Solutions of **4** and **4b** (0.15–0.20 M) were prepared in dry CH_2Cl_2. An aliquot of each solution containing ~25 μmol of glycoside was placed in a tared 1.0-mL conical vial whose inner surface had previously been silanized[5]. The solvent was evaporated under a stream of dry N_2, and the residue was dried overnight in a desiccator over anhyd $CaSO_4$ at atmospheric pressure. The mass of the residue was recorded, and it was redissolved in freshly distilled CH_2Cl_2 in an amount sufficient to give a final concentration of 0.1 M after the addition of all reagents.

Docosane (10 mol% relative to **4a** or **4b**; 49 mM in CH_2Cl_2), used as an internal standard to determine product recovery by GLC (*Method 2*), was added to the glycoside solution along with a Teflon spin vane. The vial was sealed with a Teflon valve cap (e.g., Mininert, Dynatech Corp.) from which the nonresistant rubber septum had been removed. The solutions were analyzed by GLC (*Method 2*) in order to establish the initial proportions of substrate and internal standard. To each of the reactions was then added triethylsilane (5 equiv) and one of three Lewis-acid promoters, namely, (*a*) $Me_3SiOSO_2CF_3$ (5 equiv), (*b*) Me_3SiOSO_2Me (5 equiv) and $BF_3 \cdot OEt_2$ (1 equiv), or (*c*) $BF_3 \cdot OEt_2$ (5 equiv). The vials were then sealed and stirred at room temperature.

At the end of the reaction period, established as described below, the reactions were quenched with one volume of either MeOH-d_4, (±)-2-butanol, or (*S*)-2-butanol for periods determined as described below. Mixed-bed resin AG501 X-8(D) (previously rinsed with MeOH and dried under high vacuum) was then added, followed by 3 to 4 volumes of 2:1 MeOH–CH_2Cl_2 or 2:1 (±)-2-butanol–CH_2Cl_2, depending on the alcohol used in the glycosidation step. The mixture was then stirred vigorously until neutral, as determined by spotting of the mixture on pH paper. 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 thus obtained was analyzed by GLC, GLC–CIMS, and ^1H NMR spectroscopy.

Time-course experiments.—(*A*) *For consumption of substrate.* Aliquots (50 μL) of the above mixtures were removed at the times indicated in Fig. 1, transferred to dry, 1-dram screw-cap vials equipped with valve caps and stir bars, quenched with MeOH-d_4, and processed as described above. At the final time point, the remainder of the initial mixture was quenched and processed as described above.

(*B*) *For transglycosidation with 2-butanol.* Compound **4b** (9.2 mg, 32 μmol) was dissolved in 289 μL of CH_2Cl_2 containing docosane (10 mol% relative to **4b**). A 20-μL portion of this solution was withdrawn and diluted to 1.0 mL for analysis by GLC (*Method 1*) to establish initial parameters. The remainder of the solution was treated with $Me_3SiOSO_2CF_3$ (29 μL, 150 μmol, final reaction volume 300 μL). After stirring for 4 h, half of the mixture was quenched with 150 μL of (*S*)-2-butanol held for 4 h, deionized, and processed as described above. The remaining mixture was quenched with 150 μL of (±)-2-butanol. Aliquots (50 μL) of the quenched solution were removed at regular intervals, deionized in 0.5-dram vials, and processed as described above. Product mixtures were analyzed by GLC (*Method 1*). The emergence of compounds **8** and **9** was determined by comparison of the sum of their GLC peak areas to that of internal docosane.

(*C*) *For transglycosidation with methanol-*d_4. Compound **4b** (9.2 mg, 32 μmol) was treated with $Me_3SiOSO_2CF_3$ (29 μL, 150 μmol) in CD_2Cl_2 (final volume 300 μL) for 4 h. The mixture was then transferred to an NMR tube, diluted to 500 μL with CD_2Cl_2, and a ^1H NMR spectrum (500 MHz) was recorded. Methanol-d_4 (200 μL) was then added with thorough mixing, and, after reshimming the spectrometer (12 min), a series of six spectra were recorded at intervals of 10 min in order to monitor the disappearance of the H-1 signal (δ 6.61) of the oxazolinium-ion intermediate **5** and the emergence of the H-1 signal (δ 4.43) of the glycoside (**6**). Mole fractions of the two species were determined from the integrations of these resonances.

*Methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranoside* (**2**).—2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose (**1**) (1.99 g, 5.1 mmol) was dissolved in 40 mL of freshly distilled CH_2Cl_2 and $Me_3SiOSO_2CF_3$ (1.1 mL, 5.7 mmol) was added. After stirring for 60 min, dry MeOH (5 mL, 123 mmol) was added, and stirring was continued for 30 min. An excess (8 mL) of mixed-bed resin was then added, and after pH 6 (indicator paper) was reached, the resin was filtered and rinsed with MeOH. Concentration of the filtrate yielded **2** as a white solid which was recrystallized from EtOH (1.23 g, 65%); R_f 0.44 (3:2 acetone–hexane), mp 159–160°C; $[\alpha]_D^{25}$ −12.1° (*c* 1.0, $CHCl_3$); lit.[17,18] mp. 162°C (EtOH); $[\alpha]_D^{20}$ −12.0° (*c* 1.0, $CHCl_3$). ^1H NMR data (300 MHz, $CDCl_3$): δ 1.97 (s, 3 H, AcN), 2.03, 2.04, 2.09 (3 s, 9 H, AcO), 3.51 (s, 3 H, MeO), 3.70 (ddd, 1 H, $J_{5,6a}$ 2.5, $J_{5,6b}$ 4.7, $J_{5,4}$ 9.7 Hz, H-5), 3.87 (dt, 1 H, $J_{2,1} = J_{2,NH} = 8.6$, $J_{2,3}$ 10.5 Hz, H-2), 4.15 (dd, 1 H, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.28 (dd, 1 H, H-6b), 4.59 (d, 1 H, H-1), 5.09 (t, 1 H, $J_{4,3}$ 9.7 Hz, H-4), 5.28 (dd, 1 H, H-3), and 5.47 (d, 1 H, NH).

*Methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-*D*-glucopyranoside* (**4a**).—Compound **4a** was prepared as previously described[1], except that the product was purified by flash chromatography (45:55 acetone–hexane, R_f 0.33). The content of the β anomer (**4b**) was 7.6% as determined by ^1H NMR spectroscopy and 2.8% as determined by GLC (*Method 2*). GLC retention indices (LTPGCRI method): DB-5, 1922.78; Rt_x-200, 2394.36. The ^1H NMR spectrum was identical to that previously reported[1].

*Methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-*D*-glucopyranoside* (**4b**).—*Method* 1. All operations prior to workup were performed within a glove bag in a nitrogen atmosphere. Compound **2** (100 mg, 0.55 mmol), previously dried in high vacuum, was dissolved in 11 mL of dry Me_2SO, and NaOH (440 mg, 11 mmol) was pulverized and added to the solution with vigorous stirring. Addition of MeI (1.10 mL, 17.6 mmol) immediately caused the reaction color to fade. The reaction vessel was capped, and stirring was continued for 60 min. The mixture was then poured into one volume of 1 M acetic acid, and the product was extracted with two 20-mL portions of $CHCl_3$. The combined extracts were washed with four 200-mL portions of water, dried over anhyd Na_2SO_4, and concentrated under vacuum at $< 40°C$. The crude product (148 mg, 92%), was found to be 92% pure by GLC (*Method 2*); it contained residual Me_2SO, as determined by ^1H NMR spectroscopy.

Method 2. Compound **2** was *O*-deacetylated by treatment with 0.25 equiv of NaOMe in dry MeOH for 1 h followed by neutralization by Dowex 50-X8 (H^+), and the product thus obtained, methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (Me β-GlcNAc), was methylated by the procedure of Gunnarsson[19]. Thus, a 200-mg (0.43 mmol) quantity of Me β-GlcNAc, previously dried overnight under high vacuum, was dissolved in 9 mL of Me_2SO in a conical flask, and pulverized NaOH (690 mg, 17 mmol), previously dried under high vacuum, was added. The flask was sealed with a Mininert stopper valve, MeI (2.1 mL, 34 mmol) was injected through the valve, and the mixture was stirred for 20 min. The mixture was then processed as described for *Method 1*, yielding 164 mg of a yellow oil. The crude product was purified by MPLC (EtOAc, R_f 0.23), affording 59.3 mg (48%) of a clear, colorless oil. GLC retention indices (LTPGCRI method): DB-5, 1824.57; Rt_x-200, 2226.85. The ^1H NMR spectra of the products obtained by the two methods were identical to that previously reported[1].

*Trideuteriomethyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-*D*-glucopyranoside* (**6**).—Compound **6** was obtained by reacting **4b** with $Me_3SiOSO_2CF_3$ in CD_2Cl_2, followed by quenching with MeOH-d_4, as described above [see *Time-course experiments*, section (*C*)]. The mixture was deionized with mixed-bed resin and processed as described above. The chromatographic and spectral data for **6** were identical to those of **4b**, with the following exceptions. GLC–CI(NH_3)-mass spectrum: *m/z* 260 [$(M\text{-}CD_3O)^+$, 70%], 295 [$(M + H)^+$, 53%]. ^1H NMR data (300 MHz, $CDCl_3$): identical to **4b** except that singlets at δ 3.45 and 3.47 were absent.

*(R)- And (S)-*sec*-butyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-*D*-glucopyranosides* (**9** *and* **8**).—Compound **4b** (17.3 mg, 60 μmol) was dissolved in CH_2Cl_2 (final reaction volume 600 μL) containing 10 mol% of docosane (relative to **4b**) and treated with $Me_3SiOSO_2CF_3$ (58 μL, 300 μmol) for 6 h. The mixture was divided in half and one portion was quenched with (±)-2-butanol (300 μL), giving an equal mixture of **8** and **9**, and the other with (*S*)-2-butanol (300 μL), giving **8** in 96% diastereomeric excess by GLC (*Method* 1). For **8**: GLC retention indices (LTPGCRI method): DB-5, 1970.12; Rt_x-200, 2352.75 GLC–CI(NH_3)-mass

spectrum: m/z 260 [$(M - C_4H_9O)^+$, 19%], 334 [$(M + H)^+$, 100%], 351 [$(M + NH_4)^+$, 1.2%]. ^1H NMR data (300 MHz, $CDCl_3$): for rotamer 1, δ 0.82 (t, 2 H, $J_{4',3'}$ 7.4 Hz, H-4'), 1.21 (d, 2 H, $J_{1'2'}$ 6.3 Hz, H-1'), 2.15 (s, 2 H, AcN), and 2.86 (s, 2 H, MeN); for rotamer 2, δ 0.84 (t, 1 H, $J_{4',3'}$ 7.4 Hz, H-4'), 1.20 (d, 1 H, $J_{1',2'}$ 6.3 Hz, H-1'), 2.07 (s, 1 H, AcN), and 3.10 (br s, 1 H, MeN); for rotamers 1 and 2, δ 1.34–1.52 (complex, 2 H, H-3a',3b'), 3.25–3.66 (complex, 16 H, H-2,2',3,4,5,6a,6b, 3 MeO), and 4.47 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1).

For **9**: GLC retention indices (LTPGCRI method): DB-5, 1954.98; Rt_x-200, 2334.28 GLC–CI(NH_3)-mass spectrum: m/z 260 [$(M-C_4H_9O)^+$, 12%], 334 [$(M + H)^+$, 100%], 351 [$(M + NH_4)^+$, 1.1%]. ^1H NMR data (300 MHz, $CDCl_3$): for rotamer 1, δ 0.87 (t, 2 H, $J_{4',3'}$ 7.4 Hz, H-4'), 1.06 (d, 2 H, $J_{1',2'}$ 6.3 Hz, H-1'), 2.15 (s, 2 H, AcN), and 2.86 (s, 2 H, MeN); for rotamer 2, δ 0.88 (t, $J_{4',3'}$ 7.4 Hz, H-4'), 1.04 (d, 1 H, $J_{1',2'}$ 6.3 Hz, H-1'), 2.07 (s, 1 H, AcN), and 3.10 (br s, 1 H, MeN); for rotamers 1 and 2, δ 1.43–1.60 (complex, 2 H, H-3a',3b'), 3.25–3.66 (complex, 15 H, H-2,3,4,5,6a,6b, 3 MeO), 3.74 (sextet, 1 H, $J_{2',3'a,b}$ 6.3 Hz, H-2'), and 4.46 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1).

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