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Mechanism of anomerization of cyclohexyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α- and β-D-hexopyranosides under reductive-cleavage conditions

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Abstract

The fully methylated cyclohexyl glycosides of 2-acetamido-2-deoxy-α- and β-D-hexopyranoses having the *gluco*, *manno*, and *galacto* configurations were each subjected to reductive-cleavage conditions using one of three promoters, namely trimethylsilyl trifluoromethanesulfonate, a mixture of trimethylsilyl methanesulfonate and boron trifluoride etherate, or boron trifluoride etherate alone. As expected, the fully methylated 1,2-trans-linked acetamido sugar derivatives were rapidly converted to their respective oxazolinium ions with all three promoters. Surprisingly, however, the fully methylated 1,2-cis-linked acetamido sugar derivatives were also converted to their respective oxazolinium ions, albeit at a much slower rate. In the latter case, evidence was obtained for anomerization to the 1,2-trans-linked isomers under reductive-cleavage conditions. Since the anomerization was relatively slow at room temperature in dichloromethane, a modified procedure was developed in which the reaction was carried out at 70 °C in 1,2-dichloroethane. Using the modified procedure, all 1,2-cis- and 1,2-trans-linked acetamido sugar derivatives were rapidly converted into their respective oxazolinium ions and subsequent quenching of the reactions with anhydrous methanol gave the respective 1,2-trans-linked methyl glycoside derivatives in quantitative yield. The modified procedure is recommended for the total reductive cleavage of polysaccharides comprised of acetamido sugar residues. © 1996 Elsevier Science Ltd.

Keywords: Anomerization; Reductive cleavage

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1. Introduction

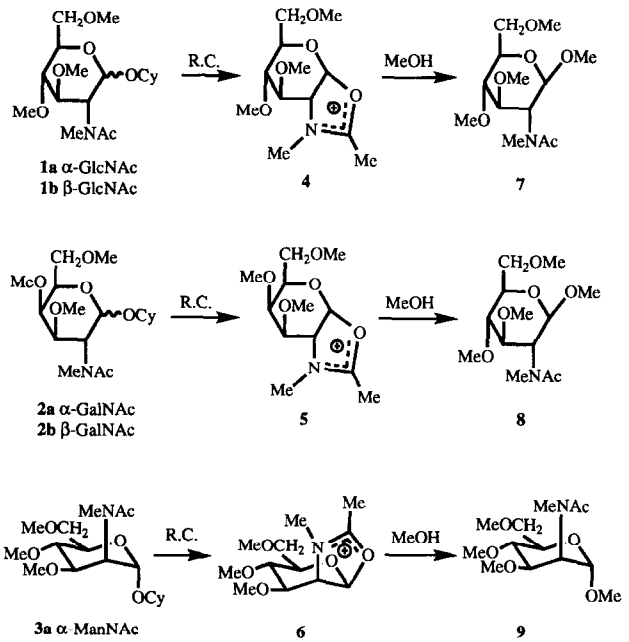
In a previous study [1], methyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α- and β-D-glucopyranosides were subjected to reductive-cleavage conditions, and the β anomer was found to undergo rapidly anomeric C–O bond cleavage to give an oxazolinium ion. The α anomer, in contrast, was found to be unreactive even after 24 h 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. From this result, it was assumed that all 1,2-cis-linked acetamido sugar residues in polysaccharides would be stable to reductive-cleavage conditions. However, when the reductive-cleavage procedure was tested on an O-antigenic polysaccharide containing 3-linked 2-acetamido-2,6-dideoxy-α-D-galactopyranosyl (α-D-FucNAc*p*; 1,2-cis-linked acetamido sugar) and 3-linked β-L-FucNAc*p* (1,2-trans-linked acetamido sugar) residues, complete cleavage at both acetamido sugar residues was observed [2]. Therefore, it became necessary to reinvestigate the previously established conditions for reductive cleavage of acetamido sugars in order to develop a better understanding of the mechanism of this reaction. In the present study, the fully methylated cyclohexyl glycosides of α- and β-D-GlcNAc*p*, α- and β-D-GalNAc*p* and α-D-ManNAc*p* were subjected to reductive cleavage in the presence of various promotors in order to establish the relative rates of cleavage of their glycosidic bonds. Based upon these studies, an efficient procedure for the cleavage of 1,2-cis-linked acetamido sugar residues was developed.

2. Results

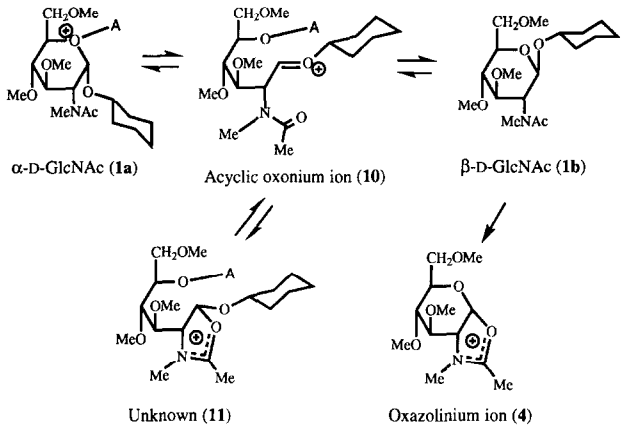
*Cyclohexyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-D-glucopyranoside* (**1b**).—Anomerically pure **1b** was synthesized by treatment of fully acetylated 2-acetamido-2-deoxy-D-glucose with a slight molar excess of trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$), followed by quenching of the intermediate oxazolinium triflate with anhydrous cyclohexanol and deionization with mixed-bed resin. The product, cyclohexyl 2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside, was obtained in 70% yield after recrystallization. Subsequent methylation [3] gave **1b** in pure form.

*Cyclohexyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α and β-D-hexopyranosides* (**1–3**).—Treatment of 2-acetamido-2-deoxy-D-glucose or 2-acetamido-2-deoxy-D-galactose with cyclohexanol under Fischer glycosidation conditions [4] gave a mixture of the respective α and β anomers, which were fully methylated [3]. Treatment of 2-acetamido-2-deoxy-D-mannose under the same conditions gave only the α anomer. Separation of the mixture of anomers could be achieved by flash column chromatography, but the procedure proved to be quite tedious. Alternatively, the mixture of anomers derived by Fischer glycosidation was subjected to benzoylation, and the benzoylated anomers were readily separated by HPLC. Debenzoylation then gave the individual anomers in pure form. Subsequent methylation [3] and purification as necessary gave the respective methylated derivatives (**1–3**).

Reactivity of cyclohexyl 2-deoxy-3,4,6-tri-O-methyl-2-(N-methylacetamido)-α- and β-D-glucopyranosides (**1a,b**) *under reductive-cleavage conditions.*—Compounds **1a** and **1b** were reacted separately with one of three Lewis acid promotors, namely trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$) (5 equiv) [5], a mixture of trimethylsilyl methanesulfonate (Me_3SiOSO_2Me) (5 equiv) and boron trifluoride etherate ($BF_3 \cdot OEt_2$) (1 equiv) [6] or $BF_3 \cdot OEt_2$ (5 equiv) alone [7]. Reaction mixtures were quenched at selected times with excess methanol and then deionized with mixed-bed resin. Experiments with **1a** as the substrate gave results which were quite different from those obtained using the corresponding methyl glycoside. These studies demonstrated that the cyclohexyl α-glycoside (**1a**) gave the transglycosidation product **7** for all three Lewis acid promotors when the reaction was quenched with methanol (Scheme 1). In time-course experiments at room temperature, 50% conversion to the oxazolinium-ion intermediate was found to require 4 days for $Me_3SiOSO_2CF_3$, 6 days for $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$ and 10 days for $BF_3 \cdot OEt_2$ alone. Mixtures containing compounds **1a**, **1b**, oxazolinium-ion intermediate **4**, and the Lewis acid promotor were analyzed by ^1H NMR spectroscopy. Characteristic signals for the oxazolinium-ion intermediate were observed at δ 6.628 (H-1), 4.616 (H-2) and 3.839 (H-3) [8]. During the reaction, the β anomer (**1b**) appeared at early time points as evidenced by the presence of its H-1 resonance at δ 4.67. An intermediate presumed to be **11** (see Scheme 2 and Discussion) also appeared at early time points as evidenced by the presence of its H-1 resonance at δ 6.32 (d, *J* 3.5 Hz). Under normal reductive-cleavage conditions, namely at room temperature in dichloromethane, the conversion of **1a** to



Scheme 1.



Scheme 2.

oxazolinium ion **4** was very slow. In order to accelerate the reaction, the cyclohexyl α anomer was subjected to reductive-cleavage conditions at 70 °C in 1,2-dichloroethane and at selected times, aliquots were withdrawn and treated with excess methanol. In time-course experiments at 70 °C, < 1% of the substrate remained after 3 h for $Me_3SiOSO_2CF_3$ while in the presence of $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$ and $BF_3 \cdot OEt_2$, respectively, the complete conversion of **1a** to the oxazolinium-ion intermediate, as measured by the appearance of **7**, required 16 h and 24 h, respectively. Product mixtures containing compounds **1a** and **7** were analyzed by integration of the GLC profiles using Method 1.

Using the same Lewis acid promotors, experiments with the β anomer **1b** demonstrated that the reaction was much faster than that of the corresponding α anomer (**1a**). In time-course experiments at room temperature, the substrate (**1b**) was completely converted to the oxazolinium-ion (**4**) within 3 h for $Me_3SiOSO_2CF_3$, 5 h for $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$ and 15 h for $BF_3 \cdot OEt_2$ alone. The kinetics of formation of oxazolinium-ion intermediate **4** were measured by ^1H NMR spectroscopy, which also revealed the presence of the same unknown (presumed to be **11**) observed in the reaction with **1a**. An additional experiment was performed in order to determine the minimum time necessary for complete reaction of oxazolinium-ion **4** with methanol. The result so obtained (70 min) was the same as previously reported [1].

*Reactivity of cyclohexyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α- and β-*D*-galactopyranosides* (**2a,b**) *under reductive-cleavage conditions.*—Compounds **2a** and **2b** were reacted separately with one of the three aforementioned Lewis acid promotors, and the reactions were quenched at selected times with excess methanol and then deionized with mixed-bed resin. The reactions with fully methylated cyclohexyl α-GalNAc*p* (**2a**) were first carried out at 70 °C, but formation of the oxazolinium-ion intermediate was too fast for its half-life to be determined. Therefore, the reactions were carried out at room temperature. In time-course experiments at room temperature, it was found that **2a** was completely converted to oxazolinium-ion **5**, as measured by the appearance of the transglycosidation product **8**, within 6 h for $Me_3SiOSO_2CF_3$, 12 h for

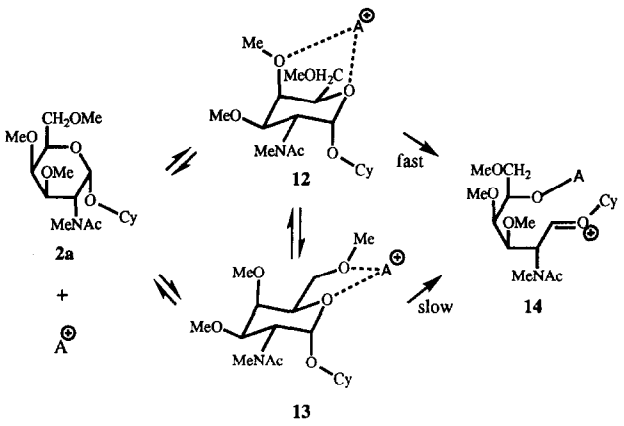
$Me_3SiOSO_2Me/BF_3 \cdot OEt_2$ and 39 h for $BF_3 \cdot OEt_2$. Product mixtures containing compounds **2a** and **8** were analyzed by integration of GLC profiles using Method 1.

Using the same Lewis acid promotors, experiments with the β anomer **2b** demonstrated that the reaction was somewhat faster than that of the corresponding α anomer **2a**. In time-course experiments at room temperature, **2b** was found to be completely converted to oxazolinium-ion **5**, as measured by the appearance of the transglycosidation product (**8**), within 4.6 h for $Me_3SiOSO_2CF_3$, 9 h for $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$ and 24 h for $BF_3 \cdot OEt_2$ alone. Product mixtures containing compounds **2b** and **8** were also analyzed by integration of the GLC profiles using Method 1. An additional experiment was performed in order to determine the minimum time necessary for complete reaction of oxazolinium-ion **5** with methanol, and the reaction was found to be complete in 60 min.

*Reactivity of cyclohexyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-*D*-mannopyranoside* (**3a**) *under reductive-cleavage conditions.*—Compound **3a** was reacted separately with the three aforementioned promoters and was found to undergo transglycosidation when the reactions were quenched with methanol. In time-course experiments at room temperature, complete conversion of **3a** to its oxazolinium-ion (**6**) was found to require 24 h for $Me_3SiOSO_2CF_3$, 48 h for $Me_3SiOSO_2Me/BF_3 \cdot OEt_2$ and 5 days for $BF_3 \cdot OEt_2$. The kinetics of formation of oxazolinium-ion intermediate **6** were measured by ^1H NMR spectroscopy. These experiments also revealed the presence of substantial proportions of an unknown at early time points, as evidenced by the presence of its H-1 signal at δ 6.40 (d, J 3.0 Hz). An additional experiment was performed in order to determine the minimum time necessary for complete reaction of oxazolinium-ion **6** with methanol to give **9**. ^1H NMR studies demonstrated that the reaction was complete in 2 h.

3. Discussion

Cyclohexyl glycosides, rather than methyl glycosides, of fully methylated acetamido sugars were chosen as model compounds in the present study in the belief that their reactivities might be more similar to those of the respective residues in polysaccharides. Indeed, the fully methylated cyclohexyl α-glycoside of GlcNAc*p* (**1a**) underwent transglycosidation under reductive cleavage conditions when the reactions were quenched with methanol, in contrast to the results obtained with the corresponding methyl glycoside [1]. Since a 1,2-cis orientation does not permit anchimeric assistance by the *N*-acetyl group in cleavage of the glycosidic C–O bond, it is proposed that transglycosidation occurs via an acyclic oxonium ion **10** as shown in Scheme 2. Collapse of the acyclic oxonium ion **10** could then occur to give the β anomer **1b**, which has already been shown [1] to give the oxazolinium ion **4**. In support of this proposal, small amounts of the β anomer **1b** were observed during the course of the reaction by ^1H NMR spectroscopy. Alternatively, the acyclic oxonium ion **10** could also give rise to an oxazolinium ion **11** having an acyclic carbohydrate residue, and it is proposed, based upon the chemical shift of its H-1 resonance, that oxazolinium ion **11** is the 'unknown' observed during the course of the reaction.



Scheme 3.

The fully methylated cyclohexyl α glycoside of GalNAc*p* (**2a**) was also found to undergo transglycosidation under reductive-cleavage conditions when the reactions were quenched with methanol. Surprisingly, however, the rate of conversion of the *galacto* isomer **2a** to its oxazolinium ion **5** was much faster than the rate of conversion of the *gluco* isomer **1a** to its oxazolinium ion **4**. Based upon these results, it is proposed (Scheme 3) that a complex **12** between **2a** and the Lewis acid promotor A^+ is formed in which the promotor coordinates to an axial electron lone pair of the ring oxygen and an unshared electron lone pair at O-4. The rate of formation of the acyclic oxonium ion **14** would thus be accelerated [5,9] due to the antiperiplanar relationship between the glycosidic C–O bond and the partial bond between the ring oxygen atom and Lewis acid promotor. This same argument has been advanced by Lee et al. [10] to explain anomerization of permethylated methyl glycopyranosides by trimethylsilyl trifluo-

Table 1
Kinetic data for oxazolinium-ion formation from cyclohexyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α,β-D-hexopyranosides (**1–3**) [a]

Lewis acid	Compound									
	1a		**1b**		**2a**		**2b**		**3a**	
	$t_{1/2}$ (1) [b]	$t_{1/2}$ (2) [c]	$t_{1/2}$ (1)	$t_{1/2}$ (2)	$t_{1/2}$ (1)	$t_{1/2}$ (2)	$t_{1/2}$ (1)	$t_{1/2}$ (2)	$t_{1/2}$ (1) [d]	$t_{1/2}$ (2)
$Me_3SiOSO_2CF_3$	0.55 [e]	0.61 [e]	0.14	0.49	0.51	0.60	0.36	0.59	–	2.52
Me_3SiOSO_2Me/ $BF_3 \cdot Et_2O$	1.85 [e]	2.02 [e]	0.34	0.72	1.21	1.49	0.71	1.20	–	10.8
$BF_3 \cdot Et_2O$	3.45 [e]	5.93 [e]	3.50	4.76	3.90	6.05	2.52	3.73	–	16.6

[a] Reactions were carried out at room temperature, except where noted.
[b] $t_{1/2}$(1): half-life in h for loss of starting material.
[c] $t_{1/2}$(2): half-life in h for appearance of oxazolinium-ion intermediate.
[d] The reaction was too fast for its half-life to be determined.
[e] The reaction was carried out at 70 °C in 1,2-dichloroethane.

romethanesulfonate. It is also conceivable that **2a** and the Lewis acid promotor form a complex **13** involving the unshared electron pairs of the ring oxygen atom (O-5) and O-6, but this same type of complex could also be formed in the case of the *gluco* isomer **1a** and thus would not explain the rate acceleration observed for the *galacto* isomer **2a**.

In order to develop a reliable procedure for the cleavage of all acetamido sugar residues in polysaccharides under reductive-cleavage conditions, the use of other solvents and higher temperatures for the reductive cleavage reaction were explored. It was found that at 70 °C in 1,2-dichloroethane as solvent, the complete conversion of 1,2-cis-linked acetamido sugars such as **1a** and **2a** to their respective oxazolinium ions (**4** and **5**) could be readily achieved in a reasonable period of time (Table 1). This procedure is therefore recommended for the total reductive cleavage of polysaccharides comprised of acetamido sugar residues.

4. Experimental

General.—Melting points were determined with a Fisher–Johns apparatus and are uncorrected. 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 chromatography was performed on 230–400 mesh silica gel (E. Merck).

HPLC of benzoate derivatives was performed using Beckman 110B pumps equipped with a Beckman System Gold model 166 programmable detector, a Beckman System Gold model 406 analog interface module and an NEC controller. Reversed-phase chromatography was performed on a 5-μm particle-size Rainin Dynamax Microsorb semipreparative C_{18} reversed-phase column (1 × 25 cm) equipped with a guard column (1 × 5 cm) having the same packing. The column was eluted with 50% aqueous acetonitrile at a flow rate of 3 mL/min for 10 min, followed by a linear gradient to 95% acetonitrile over 25 min. The effluent was monitored at 245 nm. Solvents (HPLC grade) were used after deaeration and filtration through a 0.2-μm nylon membrane.

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 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 gas–liquid chromatographs were interfaced to a HP model 3365 Series II ChemStation. The injector temperature was set at 200 °C in order to avoid pyrolysis of the 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 RT_x-200 (0.25-μm film thickness; Restek Corp.), programmed from 80 to 300 °C at 2 °C/min with no initial hold time. 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) and/or a two way (Y) press-tight connector. Helium was used as the carrier gas at measured linear velocities (methane injection, oven temperature 80 °C) of 26.1 and 27.8 cm/s, respectively, for the DB-5 and RT_x-200 columns. Retention indices were calculated by the linear-temperature-programmed gas-liquid chromatographic retention index (LTPGLCRI) method as described by Elvebak et al. [11] 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 components had mass spectra identical to those of independently synthesized standards.

^{1}H 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 (Me_2SO), iodomethane, $Me_3SiOSO_2CF_3$, Me_3SiOSO_2Me, $BF_3 \cdot OEt_2$, benzoic anhydride, pyridine and methyl lithium were obtained from Aldrich Chemical Company. *N*-Acetyl-D-glucosamine, *N*-acetyl-D-galactosamine and *N*-acetyl-D-mannosamine were from ICN, Inc. 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. MeOH, cyclohexanol, dichloromethane, 1,2-dichloroethane and pyridine were distilled as described by Perrin and Armarego [12]. Alcohols were stored over 3 Å molecular sieves. Sodium hydroxide was pulverized with a mortar and pestle and stored under nitrogen. Sodium was freshly cleaned before use, and benzoic anhydride was crystallized from petroleum ether.

Time-course experiments.—The rate of formation of oxazolinium-ion intermediates was measured by dissolving 6.0 mg (0.017 mmole) of **1a**, **1b**, **2a**, **2b**, or **3a** in 500 μL of CD_2Cl_2 in a previously silylated NMR tube (No. 528). The solution was first analyzed by 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 and the emergence of the H-1 signal of the oxazolinium-ion intermediate (**4**, **5**, or **6**). The mole fractions of the starting material and product were determined from integrations of these resonances.

Improved method for the reductive-cleavage of fully methylated 1,2-cis-linked acetamido sugars.—Compound **1a** or **2a** (6.0 mg, 0.017 mmole) was dissolved in 2.0 mL

of 1,2-dichloroethane containing docosane (15 mole% relative to **1a** or **2a**). The solution was examined by gas–liquid chromatography (Method 1) in order to establish the integral values of starting material and docosane. One of the three Lewis-acid promotors was added to the solution and the solution was heated at 70 °C in an oil bath. Aliquots (100 μL) of the solution were removed at regular intervals and added to a solution of MeOH (300 μL) and 1,2-dichloroethane (100 μL). The solution was stirred at room temperature for 4 h, then deionized with mixed-bed resin. Each solution was analyzed by GLC (Method 1). The emergence of compound **7** or **8** and the disappearance of **1a** or **2a** were determined by comparison of the sum of their GLC peak areas, after correction for molar response [13], to that of internal docosane.

*Cyclohexyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-*D*-glucopyranoside* (**1a**).—*N*-Acetyl-D-glucosamine (1.0 g, 4.5 mmole) was dissolved in 20 mL of freshly distilled cyclohexanol in the presence of IRA-120 (H^+) (0.32 g), and the solution was heated at 65 °C in an oil-bath for 5 days. After removal of the solvent and resin, an anomeric mixture (820 mg, 60%) of cyclohexyl 2-acetamido-2-deoxy-α,β-D-glucopyranosides was obtained. An aliquot (200 mg, 0.66 mmole) of this mixture was methylated by the method of Ciucanu and Kerek [3], and the product was purified by flash column chromatography (7:3 hexane–acetone, R_f 0.19 for **1a**). The oily product, **1a**, (77 mg, 30%) was pure as determined by ^{1}H NMR spectroscopy and GLC (Method 2). GLC retention indices (LTPGLCRI method): DB-5, 2419.86: RT_x-200, 2949.42. GLC–EIMS spectrum (high resolution): *m/z* Calcd: 359.2308. Found: 359.2309. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.93 (d, 0.55 H, H-1 rotamer 1), 4.90 (d, 0.45 H, H-1, rotamer 2), 4.66 (dd, 0.55 H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 11.0 Hz, H-2, rotamer 1), 3.41, 3.49, 3.55 (3 s, 4.95 H, MeO, rotamer 1), 3.42, 3.53, 3.56 (3 s, 4.05 H, MeO, rotamer 2), 3.30–3.80 (complex, 6.45 H, H-2, rotamer 2, H-3, 4, 5, 6a, 6b, and H-1′), 3.02 (s, 1.65 H, MeN, rotamer 1), 2.97 (s, 1.35 H, MeN, rotamer 2), 2.12 (s, 1.65 H, AcN, rotamer 1), 2.11 (s, 1.35 H, AcN, rotamer 2), 1.02–2.00 (complex, 10 H, cyclohexyl ring protons); ^{1}H NMR (CD_3CN, 500 MHz): δ 4.93 (d, 0.27 H, H-1, rotamer 2), 4.76 (d, 0.73 H, H-1, rotamer 1), 4.36 (dd, 0.73 H, $J_{2,1}$ 3.5 Hz, $J_{2,3}$ 11.0 Hz, H-2, rotamer 1), 3.32, 3.47, 3.48 (3 s, 6.57 H, MeO, rotamer 1), 3.10–3.80 (complex, 8.70 H, H-2, rotamer 2, H-3, 4, 5, 6a, 6b, H-1′, and MeO, rotamer 2), 3.00 (s, 2.19 H, MeN, rotamer 1), 2.89 (s, 0.81 H, MeN, rotamer 2), 2.04 (s, 2.19 H, AcN, rotamer 1), 2.03 (s, 0.81 H, AcN, rotamer 2), 1.02–2.00 (complex, 10 H, cyclohexyl ring protons). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.25 (C=O), 97.32 (C-1), 82.11, 78.73, 76.28, 71.68 (C-3, 4, 5 and C-1′), 72.14 (C-6), 60.40, 59.14, 58.69 (3 MeO), 56.11 (C-2), 33.95, 33.19, 26.32, 24.50, 24.22 (C-2′, 3′, 4′, 5′ and 6′), 33.19 (MeN), 22.50 (AcN).

*Cyclohexyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-β-*D*-glucopyranoside* (**1b**).—2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose (250 mg, 0.70 mmole) was dissolved in 15 mL of freshly distilled CH_2Cl_2, and $Me_3SiOSO_2CF_3$ (150 μL, 0.78 mmole) was added. The reaction mixture was stirred at room temperature for 8 h, dry cyclohexanol (2.0 mL) was added, and the reaction was stirred for 6 h. Mixed-bed resin was added, and the solution was stirred until pH 7 (indicator paper) was reached. The resin was filtered and rinsed with MeOH. MeOH was removed by rotary evaporation and cyclohexanol was removed by vacuum distillation. Concentration of the filtrate yielded cyclohexyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside as a

white solid that was recrystallized from hexane (195 mg, 70.4%): mp 142 °C (dec.). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.42 (dd, 1 H, $J_{3,2}$ 6.5 Hz, H-3), 5.39 (d, 1 H, $J_{NH,2}$ 11.0 Hz, N–H), 5.04 (t, 1 H, $J_{4,3} = J_{4,5}$ 10.0 Hz, H-4), 4.86 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.26 (dd, 1 H, $J_{6b,5}$ 5.0 Hz, H-6b), 4.11 (dd, 1 H, $J_{6a,5}$ 2.5 Hz, $J_{6a,6b}$ 12.0 Hz, H-6a), 3.58–3.72 (complex, 3 H, H-2, 5, and H-1′), 2.02, 2.03, 2.07 (3 s, 9 H, AcO), 1.94 (s, 3 H, AcN), 1.02–1.90 (complex, 10 H, cyclohexyl ring protons). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.84 (C=O), 97.34 (C-1), 81.75, 80.40, 77.48, 74.50 (C-3, 4, 5 and C-1′), 71.12 (C-6), 63.33 (C-2), 60.43, 60.15, 59.31 (3 MeO), 33.21, 31.38, 25.49, 23.67, 23.41 (C-2′, 3′, 4′, 5′ and 6′), 28.05 (MeN), 22.00 (AcN).

Cyclohexyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside was *O*-deacetylated by treatment with 0.25 equiv of NaOMe in dry MeOH for 4 h, followed by neutralization by IRA-120 (H^+), and the product so obtained, cyclohexyl 2-acetamido-2-deoxy-β-D-glucopyranoside (cyclohexyl β-GlcNAc*p*), was methylated by the procedure of Ciucanu and Kerek [3]. Thus, cyclohexyl β-GlcNAc*p* (previously dried overnight under high vacuum; 50.0 mg, 0.16 mmole) was dissolved in 1.0 mL of Me_2SO, then pulverized NaOH (27.5 mg, 0.70 mmole) was added to the solution. The flask was sealed with a Mininert stopper valve, and the reaction mixture was stirred at room temperature for 1 h. Iodomethane (0.5 mL) was injected through the valve under an ice–water bath, and the mixture was stirred at room temperature for 2 h. The mixture was poured into 3.0 mL of ice water, the product was extracted with $CHCl_3$ (4 × 5 mL), and the organic layer was washed with water (3 × 10 mL). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation. The oily product **1b** was dried under high vacuum (41.0 mg, 71.3%). The purity of the product was determined by ^{1}H NMR spectroscopy and by GLC (Method 2). GLC retention indices (LTPGLCRI method): DB-5, 2284.16: RT_x-200, 2701.57. GLC–EIMS (high resolution): *m/z* Calcd: 359.2308. Found: 359.2289. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.49 (d, 0.73 H, H-1, rotamer 1), 3.21–3.70 (complex, 6.27 H, H-1, rotamer 2, H-3, 4, 5, 6a, 6b, and H-1′), 3.41, 3.49, 3.54 (3 s, 6.57 H, MeO, rotamer 1), 3.38, 3.45, 3.57 (3 s, 2.43 H, MeO, rotamer 2), 3.07 (s, 0.81 H, MeN, rotamer 2), 2.84 (s, 2.19 H, MeN, rotamer 1), 2.14 (s, 2.19 H, AcN, rotamer 1), 2.06 (s, 0.81 H, AcN, rotamer 2), 1.02–2.00 (complex, 10 H, cyclohexyl ring protons).

Compounds **1a** and **1b** were also prepared from *N*-acetyl-D-glucosamine (1.0 g, 4.5 mmole) by treatment with cyclohexanol (10 mL) in the presence of IRA-120 (H^+) (0.32 g) at 65 °C for 5 days. After removal of the solvent and resin, an anomeric mixture (820 mg, 60%) of cyclohexyl 2-acetamido-2-deoxy-α,β-D-glucopyranosides was obtained. An aliquot (60 mg, 0.198 mmole) of this mixture was reacted with benzoic anhydride (170 mg) in pyridine (0.5 mL), and the product was separated by reversed-phase HPLC. The fully benzoylated α anomer was found to elute at 34 min, whereas the fully benzoylated β anomer eluted at 31 min. Benzoylated positional isomers of the α anomer containing a free hydroxy group at the 3- and 4-positions were found to be present as a consequence of incomplete benzoylation, and these isomers did not separate (retention time 27 min) under reversed-phase HPLC conditions. The mixture was applied to a Regis Spherisorb S5W Hi-Chrom silica HPLC column (4.6 mm × 250 mm) equilibrated in 8:2 hexane–EtOAc at 3.0 mL/min, and the individual components were

separated under these conditions. All products were characterized by ^{1}H NMR spectroscopy and COSY.

For *cyclohexyl 2-acetamido-4,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside*: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.0–8.2 (complex, 4 H, H-2 and 6 of phenyl ring), 7.4–7.6 (complex, 6 H, H-3, 4 and 5 of phenyl ring), 5.746 (d, 1 H, $J_{NH,2}$ 10.0 Hz, N–H), 5.352 (dd, 1 H, $J_{4,3}$ 9.5 Hz, $J_{4,5}$ 11.0 Hz, H-4), 5.016 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.754 (dd, 1 H, $J_{6a,5}$ 5.0 Hz, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.556 (dd, 1 H, $J_{6b,5}$ 2.0 Hz, H-6b), 4.448 (m, 1 H, H-5), 4.128 (m, 1 H, H-2), 3.808 (t, 1 H, H-3), 3.605 (m, 1 H, H-1′), 3.101 (broad s, 1 H, 3-OH), 1.820 (s, 3 H, AcN), 1.0–2.0 (complex, 10 H, cyclohexyl ring protons).

For *cyclohexyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside*: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.0–8.2 (complex, 4 H, H-2 and 6 of phenyl ring), 7.2–7.6 (complex, 6 H, H-3, 4 and 5 of phenyl ring), 5.801 (d, 1 H, $J_{NH,2}$ 9.0 Hz, N–H), 5.272 (t, 1 H, $J_{3,2} = J_{3,4}$ 9.0 Hz, H-3), 5.020 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.570 (dd, 1 H, $J_{6a,5}$ 2.0 Hz, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.360 (dd, 1 H, $J_{6b,5}$ 6.5 Hz, H-6b), 4.270 (complex, 2 H, H-3 and 5), 3.974 (t, 1 H, H-2), 3.598 (m, 1 H, H-1′), 3.100 (broad s, 1 H, 4-OH), 2.056 (s, 3 H, AcN), 1.0–2.0 (complex, 10 H, cyclohexyl ring protons).

For *cyclohexyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-glucopyranoside*: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.9–8.1 (complex, 6 H, H-2 and 6 of phenyl ring), 7.3–7.6 (complex, 9 H, H-3, 4 and 5 of phenyl ring), 5.830 (d, 1 H, $J_{NH,2}$ 9.5 Hz, N–H), 5.674 (dd, 1 H, $J_{3,2}$ 10.5 Hz, $J_{3,4}$ 9.5 Hz, H-3), 5.624 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 5.106 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.568 (dd, 1 H, $J_{6a,5}$ 1.5 Hz, $J_{6a,6b}$ 11.0 Hz, H-6a), 4.552 (m, 1 H, H-2), 4.431 (m, 1 H, H-5), 4.407 (dd, 1 H, $J_{6b,5}$ 5.5 Hz, H-6b), 3.625 (m, 1 H, H-1′), 1.869 (s, 3 H, AcN), 1.2–2.0 (complex, 10 H, cyclohexyl ring protons).

For *cyclohexyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-glucopyranoside*: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.8–8.0 (complex, 6 H, H-2 and 6 of phenyl ring), 7.2–7.6 (complex, 9 H, H-3, 4 and 5 of phenyl ring), 5.872 (dd, 1 H, $J_{3,2}$ 10.5 Hz, $J_{3,4}$ 9.5 Hz, H-3), 5.601 (d, 1 H, $J_{NH,2}$ 8.5 Hz, N–H), 5.565 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 5.068 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.570 (dd, 1 H, $J_{6a,5}$ 3.5 Hz, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.471 (dd, 1 H, $J_{6b,5}$ 6.0 Hz, H-6b), 4.093 (m, 1 H, H-5), 3.920 (m, 1 H, H-2), 3.623 (m, 1 H, H-1′), 1.892 (s, 3 H, AcN), 1.1–2.0 (complex, 10 H, cyclohexyl ring protons).

The purified benzoylated compounds were separately treated with sodium in MeOH for 8 h at room temperature, and the solution was neutralized with mixed-bed resin. Each solution was evaporated under a stream of dry N_2 gas, then dried under vacuum. The product was methylated by the procedure of Ciucanu and Kerek [3]. The purity of products **1a** and **1b** was determined by ^{1}H NMR spectroscopy and GLC (Method 2).

*Cyclohexyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α,β-*D*-galactopyranosides* (**2a** and **2b**).—Compounds **2a** and **2b** were prepared from *N*-acetyl-D-galactosamine by sequential Fischer glycosidation [4] and permethylation [3]. The anomeric mixtures of cyclohexyl α-, and β-GalNAc*p* (**2a** and **2b**) were separated by flash column chromatography (silica gel, 1.5 × 20 cm, 230–400 mesh), eluted sequentially with hexane (50 mL), mixtures of hexane–EtOAc in ratios of 9:1 (100 mL), 4:1 (100 mL), 1:1 (100 mL), 1:4 (100 mL), and 1:9 (100 mL), and EtOAc (100 mL). The products (**2a** and **2b**) were obtained as oils, and their purities were checked by ^{1}H NMR spectroscopy and by GLC (Method 2).

Compounds **2a** and **2b** were also prepared from *N*-acetyl-D-galactosamine (0.5 g,

2.26 mmole) by treatment with cyclohexanol (5 mL) in the presence of IRA-120 (H^+) at 65 °C for 4 days. After removal of the solvent and resin, an anomeric mixture (390 mg, 56%) of cyclohexyl 2-acetamido-2-deoxy-α,β-D-galactopyranosides was obtained. An aliquot (50 mg, 0.165 mmole) of this mixture was treated with benzoic anhydride (162 mg) in pyridine (0.5 mL), and the benzoylated product was separated by reversed-phase HPLC. The fully benzoylated α anomer, which was present as a minor component, eluted at 33 min, whereas the fully benzoylated β anomer eluted at 26 min. Both products were characterized by ^{1}H NMR spectroscopy and COSY.

For *cyclohexyl 2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-α-D-galactopyranoside*: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.8–8.2 (complex, 6 H, H-2 and 6 of phenyl ring), 7.2–7.6 (complex, 9 H, H-3, 4 and 5 of phenyl ring), 5.889 (dd, 1 H, $J_{4,3}$ 3.5 Hz, $J_{4,5}$ 0.5 Hz, H-4), 5.709 (d, 1 H, $J_{NH,2}$ 10.0 Hz, N–H), 5.566 (dd, 1 H, $J_{3,2}$ 11.0 Hz, H-3), 5.200 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.878 (m, 1 H, H-2), 4.610 (m, 1 H, $J_{5,6a}$ 7.5 Hz, $J_{5,6b}$ 5.0 Hz, H-5), 4.513 (dd, 1 H, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.397 (dd, 1 H, H-6b), 3.615 (m, 1 H, H-1′), 1.876 (s, 3 H, AcN), 1.0–2.0 (complex, 10 H, cyclohexyl ring protons).

For *cyclohexyl 2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-β-D-galactopyranoside*: ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.0–8.2 (complex, 6 H, H-2 and 6 of phenyl ring), 7.2–7.6 (complex, 9 H, H-3, 4 and 5 of phenyl ring), 5.684 (dd, 1 H, $J_{NH,2}$ 10.0 Hz, N–H), 5.330 (dd, 1 H, $J_{3,2}$ 11.5 Hz, $J_{3,4}$ 2.5 Hz, H-3), 5.063 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1), 4.846 (m, 1 H, H-2), 4.608 (dd, $J_{6a,5}$ 5.0 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.525 (dd, 1 H, $J_{6b,5}$ 7.5 Hz, H-6b), 4.363 (m, 1 H, H-5), 3.601 (m, 1 H, H-1′), 1.869 (s, 3 H, AcN), 1.0–2.0 (complex, 10 H, cyclohexyl ring protons).

The purified benzoylated compounds were treated separately with sodium in MeOH for 8 h at room temperature and neutralized with mixed-bed resin. Each solution was evaporated under a stream of dry N_2 gas, then dried under vacuum and methylated [3]. The purity of products **2a** and **2b** was determined by ^{1}H NMR spectroscopy and by GLC (Method 2). For **2a**: GLC retention indices (LTPGLCRI method): DB-5, 2284.16: RT_x-200, 2701.57. GLC–EIMS spectrum (high resolution): *m/z* Calcd: 359.2308. Found: 359.2308. ^{1}H NMR (CD_2Cl_2, 500 MHz): δ 4.896 (d, 0.30 H, $J_{1,2}$ 3.5 Hz, H-1, rotamer 2), 4.846 (d, 0.70 H, $J_{1,2}$ 3.5 Hz, H-1, rotamer 1), 4.762 (dd, 0.70 H, $J_{2,3}$ 12.0 Hz, H-2, rotamer 1), 4.080 (dd, 0.30 H, $J_{2,3}$ 12.0 Hz, H-2, rotamer 2), 3.963 (t, 0.30 H, $J_{5,6}$ 6.5 Hz, H-5, rotamer 2), 3.926 (t, 0.70 H, $J_{5,6}$ 6.5 Hz, H-5, rotamer 1), 3.821 (broad m, 1 H, H-4), 3.729 (dd, 0.30 H, $J_{3,4}$ 2.5 Hz, H-3, rotamer 2), 3.664 (dd, 0.70 H, $J_{3,4}$ 2.5 Hz, H-3, rotamer 1) 3.522, 3.420, 3.358 (3s, 6.30 H, MeO, rotamer 1), 3.40–3.60 (complex, 5.70 H, MeO, rotamer 2, H-6a, 6b and H-1′), 2.946 (s, 2.10 H, MeN, rotamer 1), 2.877 (s, 0.90 H, MeN, rotamer 2), 2.061 (s, 0.90 H, AcN, rotamer 2), 2.11 (s, 2.10 H, AcN, rotamer 2), 1.00–1.90 (complex, 10 H, cyclohexyl ring protons). ^{13}C NMR (CD_3CN, 125 MHz): δ 172.50 (C=O), 97.77 (C-1), 76.74, 76.23, 75.21, 70.03 (C-3, 4, 5 and C-1′), 72.26 (C-6), 61.36, 59.38, 55.53 (3 MeO), 53.25 (C-2), 34.09, 32.11, 26.45, 24.61, 24.34 (C-2′, 3′, 4′, 5′ and 6′), 32.90 (MeN), 22.78 (AcN). For **2b**: GLC retention indices (LTPGLCRI method): DB-5, 2284.16; RT_x-200, 2701.57. GLC-EIMS (high resolution): *m/z* Calcd: 359.2308. Found: 359.2298. ^{1}H NMR (CD_2Cl_2, 500 MHz): δ 5.238 (d, 0.25 H, $J_{1,2}$ 8.0 Hz, H-1, rotamer 2), 4.503 (d, 0.75 H, $J_{1,2}$ 8.0 Hz, H-1, rotamer 1), 4.367 (dd, 0.25 H, $J_{2,3}$ 10.5 Hz, H-3, rotamer 2), 3.804 (dd, 0.75 H, $J_{2,3}$ 10.5 Hz, H-2, rotamer 1), 3.761 (d, 1 H, $J_{4,3}$ 3.0 Hz, H-4, rotamer 1), 3.554, 3.410,

3.371 (3s, 6.75 H, MeO, rotamer 1), 3.30–3.60 (complex, 7.50 H, MeO, rotamer 2, H-2 and 4, rotamer 2, H-3, rotamer 1, H-5, 6a, 6b and H-1′), 3.044 (s, 0.75 H, MeN, rotamer 2), 2.756 (s, 2.25 H, MeN, rotamer 1), 2.075 (s, 2.25 H, AcN, rotamer 1), 2.005 (s, 0.75 H, AcN, rotamer 2), 1.10–1.90 (complex, 10 H, cyclohexyl ring protons). ^{13}C NMR (CD_2Cl_2, 125 MHz): δ 166.38 (C=O), 97.83 (C-1), 76.74, 76.23, 75.21, 70.03 (C-3, 4, 5 and C-1′), 71.14 (C-6), 79.41, 76.73, 73.08 (3 MeO), 59.95 (C-2), 33.64, 31.77, 25.88, 24.71, 22.24 (C-2′, 3′, 4′, 5′ and 6′), 25.88 (MeN), 22.24 (AcN).

*Cyclohexyl 2-deoxy-3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)-α-*D*-mannopyranoside* (**3a**).—Compound **3a** was synthesized from *N*-acetyl-D-mannosamine as described for compound **2**. The product (**3a**) was purified by flash column chromatography (silica gel, 1.5 × 20 cm, 230–400 mesh, eluted sequentially with hexane (50 mL), mixtures of hexane–EtOAc in ratios of 9:1 (100 mL), 4:1 (100 mL), 1:1 (100 mL), 1:4 (100 mL), and 1:9 (100 mL), and EtOAc (100 mL). The purity of product **3a** was determined by ^{1}H NMR spectroscopy and GLC (Method 2). GLC retention indices (LTPGLCRI method): DB-5, 2317.35; RT_I-200, 2730.56. GLC–EIMS (high resolution): *m/z* Calcd: 359.2308. Found: 359.2306. ^{1}H NMR (CD_2Cl_2, 500 MHz): d 5.069 (dd, 0.80 H, $J_{1,2}$ 2.0 Hz, $J_{2,3}$ 6.0 Hz, H-2, rotamer 1), 4.992 (d, 0.20 H, $J_{1,2}$ 3.5 Hz, H-1, rotamer 2), 4.881 (d, 0.80 H, H-1, rotamer 1), 4.012 (dd, 0.80 H, $J_{2,3}$ 5.0 Hz, H-2, rotamer 2), 3.471, 3.362, 3.333 (3 s, 7.20 H, MeO, rotamer 1), 3.35–3.65 (complex, 7.80 H, MeO, rotamer 2, H-3, 4, 5, 6a, 6b and H-1′), 3.070 (s, 2.40 H, MeN, rotamer 1), 2.968 (s, 0.60 H, MeN, rotamer 2), 2.086 (s, 3 H, AcN), 0.80–1.90 (complex, 10 H, cyclohexyl ring protons). ^{13}C NMR (CD_2Cl_2, 125 MHz): δ 171.92 (C=O), 97.78 (C-1), 80.62, 77.26, 74.78, 70.74 (C-3, 4, 5 and C-1′) 72.10 (C-6), 60.18 (C-2), 60.14, 59.43, 57.80 (3 MeO), 34.25 (MeN), 33.56, 32.26, 26.00, 24.47, 24.18 (C-2′, 3d′, 4′, 5′ and 6′), 22.47 (AcN).

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