tions 10–49 were combined and again the solvent was evaporated *in vacuo*. The residue was crystallized from Et_2O–Skelly F to give 0.127 g of **11**, mp 177–178°; ORD (*c* 0.00207, dioxane) $[\phi]_{313}$ +2994, $[\phi]_{291}$ ±0, $[\phi]_{272}$ −1768; nmr δ 1.1 (s, 3 H, C-18 CH_3) and 1.25 (s, 3 H, C-17 CH_3).

Anal. Calcd for $C_{19}H_{30}O_2$: C, 78.57; H, 10.41. Found: C, 78.29; H, 10.35.

Registry No.—**1**, 5670-57-5; **2**, 20708-78-5; **3**, 4258-76-8; **4**, 20708-79-6; **5**, 20790-83-4; **6**, 20708-80-9; **7**, 20708-81-0; **8**, 6827-75-4; **9**, 20708-83-2; **10**, 20708-84-3; **11**, 20708-85-4; **12**, 20708-86-5.

Acknowledgment.—We wish to acknowledge the assistance of our colleagues in microanalysis and physical chemistry who obtained the data referred to in this paper. We particularly acknowledge the contributions of Messrs. M. M. Marsh and F. W. Beasley for the CD–RD data.

Selective Transglycosylation of Methylated 2-Acetamido-2-deoxy-β-D-glucopyranosides on a Microscale

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When heated at 125° in butyl acetate solution and in the presence of butyl alcohol and zinc chloride, methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranoside (β **1**) is converted into a mixture of the anomeric butyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-D-glucopyranosides (**2**), the β anomer preponderating. Under these conditions, α **1** is not attacked and the anomeric forms of methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside (**3**) are attacked to only a minor extent, and thus it appears that methylated 2-acetamido-2-deoxy-β-D-glucopyranosides may be selectively transglycosylated by this mixture of reagents. Since the anomeric forms of **2** are readily detected by gas–liquid partition chromatography (glpc) and thin layer chromatography (tlc), the method is applicable on a microscale. To explore the method further, it was applied to the chitobiose derivative **4**; the β linkage in this disaccharide was readily cleaved to give **2** and a second product which showed the chromatographic behavior expected of a butyl 2-acetamido-2-deoxy-3,6-di-*O*-methyl-β-D-glucopyranoside (**5**).

We have recently shown[2] that acetylated 2-acetamido-2-deoxy-β-D-glucopyranosides readily undergo a transglycosylation reaction when heated with benzyl alcohol at 125° in the presence of zinc chloride, giving benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside. An acetylated, β-linked disaccharide, "α-chitobiose octaacetate," was also, in part, cleaved under these conditions, and, since neither methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside nor methyl β-D-glucopyranoside tetraacetate was attacked under the transglycosylation conditions used, it is possible that a method of selective solvolysis is actually or potentially available here to cleave oligosaccharide chains at those points where a 2-acylamido-2-deoxyaldose is linked by a *trans* glycosidic bond. We have explored this reaction further, and will now describe the development of a modified transglycosylation procedure which is designed to be used on 1–5-mg quantities of material and which ought, in principle, to yield information regarding the point of attachment of the cleavage-susceptible sugar moiety.

In the earlier paper,[2] we suggested that the relative susceptibility of acetylated 2-acetamido-2-deoxy-β-D-glucopyranosides to transglycosylation arises through anchimeric assistance provided by the acetamido group in cleaving the *trans*-disposed glycosidic linkage. On the basis of evidence[3] obtained in this laboratory, it was assumed that an oxazoline was formed and that this, in turn, was attacked by the benzyl alcohol to form benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucoside. The *O*-acetyl groups were not deemed to be an essential feature of the reaction, except insofar as they masked hydroxyl groups. In adapting the process to small-scale work, gas–liquid partition chromatography of cleavage mixtures was contemplated and *O*-methyl, rather than *O*-acetyl, derivatives were used inasmuch as they would not only give products susceptible to glpc, but also the nature of these products should indicate the points of linkage in an oligosaccharide. Finally, substituted benzyl 2-acetamido-2-deoxy-D-glucopyranosides proved somewhat unsuitable for glpc examination, and we have turned to the more volatile butyl glycosides; this is a fortunate circumstance, since the medium of choice for the transglycosylation is butyl acetate, which, unless carefully purified, frequently contains a significant proportion of butyl alcohol.

At the outset, authentic samples of the anomeric butyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-D-glucopyranosides (α and β **2**) were synthesized *via* a conventional glycosylation of 2-acetamido-2-deoxy-D-glucose, followed by methylation and separation of the anomers by chromatography on silica gel; both proved to be readily crystallizable substances.

Turning now to the transglycosylation reaction, the behavior of methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranoside (β **1**) with butyl alcohol and zinc chloride in butyl acetate solution was first examined. After 4 hr at 125°, the reaction mixture was cooled and freed of zinc chloride. Glpc as well as tlc showed that only *ca.* 2.5% of β **1** remained, the bulk of the product being β **2**, along with some α **2**. While the immediate objective here was the development of a microanalytical method, the reaction described above was repeated on a preparative scale and β **2** was isolated in 82% yield.

That α **2** is formed from β **1** was somewhat unexpected in view of the presumed mechanisms involved. Glycosides such as α **1** (as well as α and β **3**) were not anomerized under the transglycosylation conditions,

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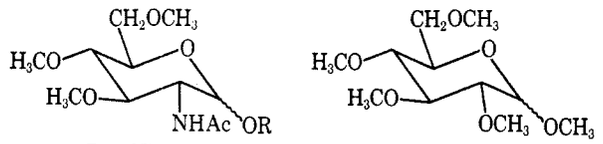
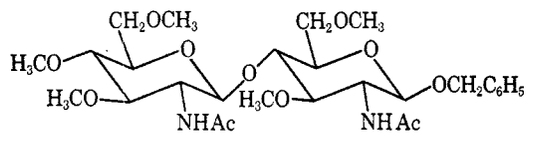


1, R = CH_3
2, R = $CH_3(CH_2)_3$

3



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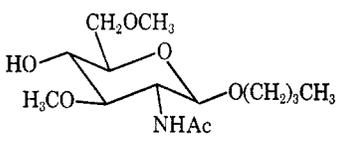

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and so it is unlikely that the α **2** found arose through the anomerization of the β **2** initially formed. We regard it as more likely that the intermediate oxazoline, formed from β **1**, was attacked by adventitious moisture to give 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-D-glucopyranose, and that this, in the presence of zinc chloride and butyl alcohol, afforded a mixture of α and β **2**. Yoshimura and his coworkers[4] have shown the effectiveness of a variety of Lewis acids in catalyzing the condensation of 2-acylamino-2-deoxy-D-glucoses with alcohols.

In contrast to methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranoside (β **1**), its anomer (α **1**) was not detectably attacked when subjected to the cleavage conditions. Similarly, the anomeric methyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranosides (α and β **3**) appeared to be resistant to cleavage, although minor products, chromatographically identified as the butyl 2,3,4,6-tetra-*O*-methyl- D- glucopyranosides, were detected. We next turned our attention to an intersaccharidic linkage of known structure and anomeric configuration and, for this purpose, chose a derivative of the disaccharide chitobiose, 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose. Benzyl 2-acetamido-4-*O*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranosyl)-2-deoxy-3,6-di-*O*-methyl-β-D-glucopyranoside (**4**) was prepared from the corresponding penta-*O*-acetyl derivative[2,5] and the methylated glycoside (**4**) was subjected to the transglycosylation conditions. While β **1** remained nearly colorless under these conditions, **4** darkened slightly. Glpc of the product revealed a major (91%) and a minor (9%) component; the former had the retention time of **2** (the anomeric forms of **2** migrate at the same rate in the system used). After trimethylsilylation, the retention time of the major product was unchanged, while that of the minor product was increased; on the basis of this evidence, it is assumed that the minor component is probably butyl 2-acetamido-2-deoxy-3,6-di-*O*-methyl-β-D-glucopyranoside (**5**). Tlc of the cleavage mixture showed that both anomers of **2** were present as well as a component with the rate of migration of **4**; whether this was indeed **4** or, as seems more likely, the corresponding butyl glycoside, was not ascertained. In any event, it is evident that **4** is cleaved under these transglycosylation conditions and that each of the two possible fragments may be detected by glpc. One would, of course, expect that **2** and **5** should be formed in equimolar amounts. That considerably less of **5** than of **2** is detected may be due to some special reactivity of **5**, which bears a free hydroxyl group; indeed, the colored materials produced in the cleavage of **4** may arise through the destruction of **5**.

While the evidence presented here tends to indicate that the method described is of potential utility in the investigation of oligosaccharide structure, it should be emphasized that many of the parameters involved await further investigation. In particular, a survey of the efficiency of Lewis acids other than zinc chloride would seem desirable.

Experimental Section

Melting points are equivalent to corrected values.

Thin layer chromatography was conducted on silica gel G_{254} (E. Merck AG, Darmstadt), components being detected by spraying with 10% sulfuric acid and heating at *ca.* 100°. Column chromatography was carried out with silica gel no. 7734 (0.05–0.2 mm) of E. Merck AG. Unless otherwise specified, dichloromethane–ether–methanol (20:10:1) was used for both tlc and column chromatography; for the latter, columns were packed in dichloromethane–ether (2:1) prior to use.

Gas–liquid partition chromatography was carried out with a Hewlett–Packard model 5750 chromatograph, using helium as a carrier gas and a flame ionization detector. The following columns were employed: (A) 0.25 in. o.d. × 6.5 ft of Apiezon N on Chromosorb P,[6] used isothermally at 250°; (B) 0.25 in. o.d. × 6.5 ft of 15% diethylene glycol succinate on Chromosorb WAW,[6] used isothermally at 180°. Trimethylsilyl derivatives were prepared using the "Tri-Sil" reagent of the Pierce Chemical Co., Rockford, Ill.

Reagent grade zinc chloride was fused and powdered and then stored over phosphorus pentaoxide. Reagent grade butyl alcohol and butyl acetate were stored over molecular sieve, type 4A (Fisher Scientific Co.), while *N*,*N*-dimethylformamide was stored over barium oxide. A stock solution of anhydrous zinc chloride in butyl acetate (100 mg/ml) was made up and stored over a small quantity of molecular sieve. All transglycosylation reactions were carried out under a reflux condenser equipped with a calcium chloride drying tube.

Nmr spectra were obtained using a Varian A-60 spectrometer and tetramethylsilane as an internal standard.

The Anomeric Methyl 2-Acetamido-2-deoxy-3,4,6-tri-*O*-methyl-D-glucopyranosides (α and β 1).—The α anomer was prepared from methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranoside[7] by methylation with a mixture of barium oxide, barium hydroxide, methyl iodide, and *N*,*N*-dimethylformamide:[8] mp 153–154.5°, $[\alpha]^{20}D$ 126.5° (*c* 1.35, chloroform) [lit.[9] mp 150°, $[\alpha]^{18}D$ 120.0° (*c* 0.4, chloroform)]. The β anomer was prepared by the direct methylation of 2-acetamido-2-deoxy-D-glucose as described by Kuhn and Trischmann:[8] mp 199.5–200.5° (lit.[8] mp 198–199°).

The Anomeric Butyl 2-Acetamido-2-deoxy-3,4,6-tri-*O*-methyl-D-glucopyranosides (α and β 2).—2-Acetamido-2-deoxy-D-glucose (2.0 g) and *p*-toluenesulfonic acid (200 mg) were added to butyl alcohol (50 ml) and the mixture was boiled under reflux for 5 hr. The clear, colorless solution was cooled and concentrated *in vacuo*, toluene being evaporated *in vacuo* from the residue. The syrup was dissolved in *N*,*N*-dimethylformamide (35 ml) and methylated by treatment with barium hydroxide octahydrate (5 g), barium oxide (7.4 g), and methyl iodide (18 ml) for 18 hr at room temperature. The reaction mixture was worked up in normal fashion and the crude product was chromatographed on a column of silica gel (2.9 × 34 cm). The first com-

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ponent eluted consisted of α **2** (2.56 g, 89%), which was crystallized from cold ether: mp 90–92°, $[\alpha]^{21}D$ 84° (*c* 2.7, chloroform); nmr ($CDCl_3$) τ 5.27 (H_1, doublet, $J_{1,2}$ = 3.7 Hz), 6.4–6.6 [18 H, 2 peaks, CH_3 and $CH_3(CH_2)_3$], and 8.0 (3 H, AcN).

Anal. Calcd for $C_{15}H_{29}NO_6$ (319.41): C, 56.41; H, 9.15; N, 4.38. Found: C, 56.13; H, 9.14; N, 4.55.

The second component eluted (0.24 g, 8.3%), the β anomer (β **2**), was crystallized from cold ethyl acetate: mp 149.5–151°; $[\alpha]^{21}D$ 1.1° (*c* 0.81, chloroform); nmr ($CDCl_3$) τ 5.22 (doublet, H_1, $J_{1,2}$ = 7.5 Hz), 6.4–6.6 [18 H, 2 peaks, CH_3 and $CH_3(CH_2)_3$], and 8.0 (3 H, AcN).

Anal. Calcd for $C_{15}H_{29}NO_6$ (319.41): C, 56.41; H, 9.15; N, 4.38. Found: C, 56.21; H, 9.00; N, 4.56.

The Anomeric Methyl 2,3,4,6-Tetra-*O*-methyl-D-glucopyranosides (α **and** β **3**).—Methyl α-D-glucopyranoside and its anomer were methylated with methyl iodide and silver oxide in *N*,*N*-dimethylformamide solution.[10] The amorphous products were chromatographically homogeneous when examined by glpc on column B.

Benzyl 2-Acetamido-4-*O*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranosyl)-2-deoxy-3,6-di-*O*-methyl-β-D-glucopyranoside (**4**).—Benzyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside[2] (mp 273–274°) was methylated by the procedure of Kuhn and Trischmann[3] to give **4**, which was crystallized from cold chloroform solution: mp 258–260°, $[\alpha]^{21}D$ −40.2° (*c* 1.11, *N*,*N*-dimethylformamide).

Anal. Calcd for $C_{28}H_{44}N_2O_{11}$ (584.68): C, 57.52; H, 7.59; N, 4.79. Found: C, 57.67; H, 7.35; N, 4.79.

Behavior of Methyl 2-Acetamido-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranoside (β **1**) **with Zinc Chloride and Butyl Alcohol in Butyl Acetate Solution.**—Three milligrams (0.0108 mmol) of β **1** was added to butyl acetate (0.64 ml) containing zinc chloride (6.4 mg, 0.047 mmol) and butyl alcohol (10.6 μl, 0.116 mmol). The mixture was heated and stirred at 125° (bath) in a stoppered flask for 4 hr, the faintly yellow reaction mixture was cooled and diluted with dichloromethane, and the resulting solution was washed with water. Moisture was removed with sodium sulfate and the solution was concentrated to a volume of *ca.* 0.5 ml. Glpc of the residue on column A showed but a trace of starting material (2.5 %, estimated by peak areas), the bulk of the product being the anomeric butyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-D-glucopyranosides (α and β **2**) which are not resolved from each other on this column; a few minor peaks of low retention time were also observed but were not identified. Tlc of the mixture showed that β **2** was the preponderant product, although it was accompanied by some α **2**.

In order to ascertain the feasibility of the transglycosylation reaction as a preparative method, β **1** (294 mg) was dissolved in butyl acetate (29.4 ml), and to this solution was added anhydrous zinc chloride (588 mg) and butyl alcohol (1.2 ml). The reaction mixture was heated with stirring at 125° for 3 hr; it was then cooled, diluted with dichloromethane, and washed with water. Moisture was removed with sodium sulfate and the solution was concentrated *in vacuo* to yield a syrup which was dissolved in benzene–ether (1:1) and then chromatographed on a column of silica gel using benzene–ether–methanol (14:14:1) for elution. Fractions containing β **2** were pooled and concentrated, the residue being crystallized from ethyl acetate: yield 277 mg (82%), mp and mmp 150–151°.

Behavior of Methyl 2-Acetamido-2-deoxy-3,4,6-tri-*O*-methyl-α-D-glucopyranoside (α **1**) **with Zinc Chloride and Butyl Alcohol in Butyl Acetate Solution.**—The methylated glycoside α **1** (5.1 mg) was added to a solution of butyl acetate (1.07 ml) containing zinc chloride (10.7 mg) and butyl alcohol (18 μl). The reaction mixture was stirred and heated at 125° (bath) for 4 hr, remaining clear and colorless during this period; it was then cooled, diluted with dichloromethane (1 ml), and shaken once with water. The organic solution was dried over sodium sulfate, concentrated to a small volume, and examined by glpc on column A. Only one peak was observed, and this had the retention time of α **1**. Although this column does not resolve α and β **1**, it readily distinguishes these from **2**; that no **2** was detected clearly shows that no β **1** was formed.

Behavior of the Anomeric Methyl 2,3,4,6-Tetra-*O*-methyl-D-glucopyranosides (α **and** β **3**) **with Zinc Chloride and Butyl Alcohol in Butyl Acetate Solution.**—A mixture of methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside (α **3**, 25.2 mg), zinc chloride (58.5 mg), butyl alcohol (0.098 ml), and butyl acetate (5.85 ml) was heated at 125° (bath) for 4 hr. The cooled mixture was examined directly by glpc on column B and was found to consist largely of unchanged α **3**; a small (7%) peak of higher retention time was observed. In a similar fashion, methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside (β **3**, 27.5 mg) was heated with zinc chloride (56.8 mg), butyl alcohol (0.107 ml), and butyl acetate (5.14 ml) at 125° for 4 hr. Examination by glpc on column B showed β **3** to preponderate (86.2%); two smaller peaks (8.3% and 5.5%) with longer retention times were observed, but neither had the retention time of α **3**. 2,3,4,6-Tetra-*O*-methyl-D-glucopyranose was treated with zinc chloride, butyl alcohol, and butyl acetate at 125° for 4 hr and then examined by glpc on column B. Two peaks, presumably representing the anomeric butyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranosides, were obtained. The retention times of these products were identical with those of the two minor components detected after treatment of β **3**. The butyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside with the longer retention time was chromatographically identical with the minor component from the treatment of α **3**; the butyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranoside with the smaller retention time could not be resolved from α **3** on column B.

Behavior of Benzyl 2-Acetamido-4-*O*-(2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranosyl)-2-deoxy-3,6-di-*O*-methyl-β-D-glucopyranoside (**4**) **with Zinc Chloride and Butyl Alcohol in Butyl Acetate Solution.**—Compound **4** (12 mg) was heated at 125° (bath) with a mixture of zinc chloride (24 mg), butyl alcohol (0.04 ml), and butyl acetate (1.2 ml) for 4 hr. The amber-brown mixture was cooled, diluted with dichloromethane (1 ml), and washed once with water. Moisture was removed with sodium sulfate and the solution was concentrated to a small volume (0.5 ml); glpc on column A revealed two major components, the larger of which (91%) had the retention time of β **2**. The smaller peak (9%) was presumably **5**; trimethylsilylation caused this smaller peak to shift to a longer retention time while the larger peak was unaffected. Examination of the product by tlc showed the presence of both anomeric forms of **2** as well as of a component which migrated at the same rate as **4** but which may have been the butyl glycoside corresponding to **4**. The reaction mixture was chromatographed on a column (2.9 × 13.5 cm) of silica gel, and the first component to emerge was identical (tlc and glpc) with α **2**. The second component was similarly shown to be β **2**. Finally, a third component emerged; glpc on column A showed this to be the smaller of the two major peaks observed earlier. As before, its retention time on column A increased with trimethylsilylation. Since the liquid phase of column A (Apiezon N) is a hydrocarbon, trimethylsilylation of a compound such as **5** would be expected to increase the affinity of the compound for the liquid phase and thus increase the retention time.

Registry No.—α **1**, 7380-60-1; β **1**, 6195-86-4; α **2**, 20708-89-8; β **2**, 20708-90-1; α **3**, 605-81-2; β **3**, 3149-65-3; **4**, 20708-93-4; zinc chloride, 7646-85-7; butyl alcohol, 71-36-3.

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