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SYNTHESIS AND MASS SPECTRA OF THE PARTIALLY METHYLATED AND PARTIALLY ETHYLATED ANHYDRO-D-MANNITOL ACETATES DERIVED BY REDUCTIVE CLEAVAGE OF PERMETHYLATED AND PERETHYLATED *Saccharomyces cerevisiae* D-MANNANS*

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ABSTRACT

Reductive cleavage of per-*O*-ethylated or per-*O*-methylated *Saccharomyces cerevisiae* D-mannans and subsequent acetylation had previously been shown to produce the expected derivatives of 1,5-anhydro-D-mannitol. Described herein is the independent synthesis of each of these derivatives, namely, 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-, -2,3,4,6-tetra-*O*-ethyl-, -2-*O*-acetyl-3,4,6-tri-*O*-methyl-, -2-*O*-acetyl-3,4,6-tri-*O*-ethyl-, -3-*O*-acetyl-2,4,6-tri-*O*-methyl-, -3-*O*-acetyl-2,4,6-tri-*O*-ethyl-, -6-*O*-acetyl-2,3,4-tri-*O*-methyl-, -6-*O*-acetyl-2,3,4-tri-*O*-ethyl-, -2,6-di-*O*-acetyl-3,4-di-*O*-methyl-, -2,6-di-*O*-acetyl-3,4-di-*O*-ethyl-, -3,6-di-*O*-acetyl-2,4-di-*O*-methyl-, and -3,6-di-*O*-acetyl-2,4-di-*O*-ethyl-D-mannitol. The ^{1}H-n.m.r. spectra, chemical-ionization (NH_3) mass spectra, and electron-impact mass spectra for all of these derivatives are tabulated.

INTRODUCTION

We recently described[1] a new method for the determination of polysaccharide structure wherein the linkage position(s) and ring form of each monosaccharide residue are established simultaneously. This method is based upon the reductive cleavage of all glycosidic carbon–oxygen bonds in a fully methylated polysaccharide with triethylsilane in the presence of either boron trifluoride etherate[1] or trimethylsilyl trifluoromethanesulfonate[2]. Pyranosides are thus cleaved to produce partially methylated 1,5-anhydroalditols, and furanosides are cleaved to partially methylated 1,4-anhydroalditols; these are analyzed as their acetyl derivatives by combined gas–liquid chromatography–mass spectrometry.

In order to demonstrate the efficacy of this approach, we have begun a series of model studies employing polysaccharides having well established structures. In

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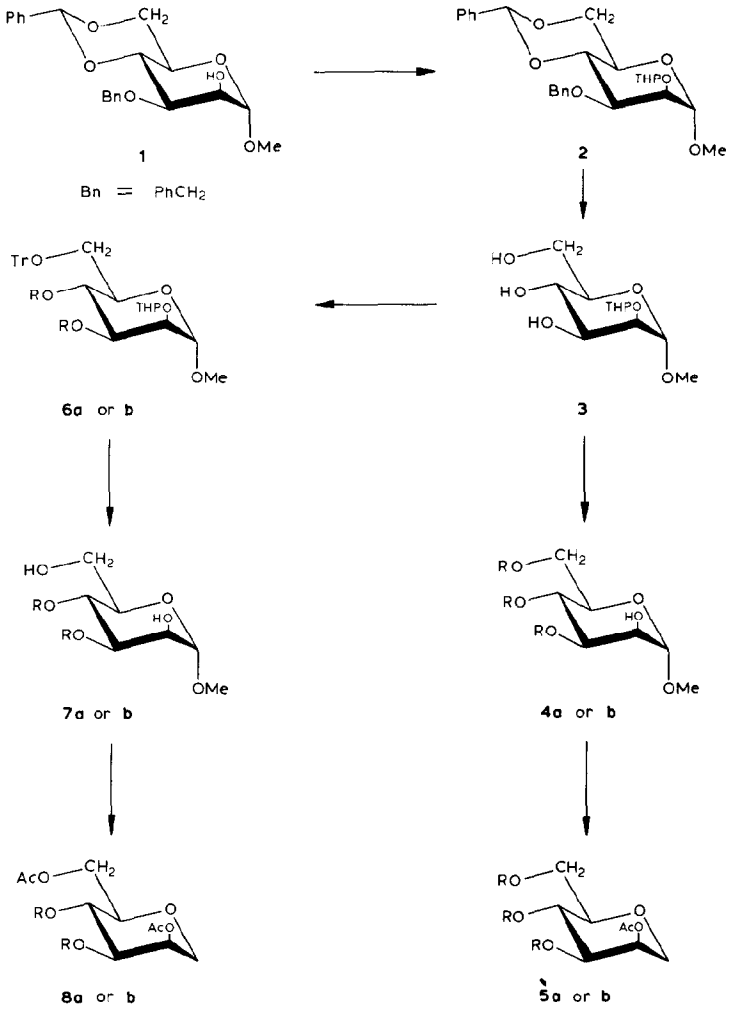
the application of this method to the analysis of linkage positions in the D-mannans derived from *Saccharomyces cerevisiae* X2180 and its mutants *mnn*1, *mnn*2, and *mnn*4, the expected derivatives of 1,5-anhydro-D-mannitol were obtained[3]. Per-*O*-ethylated D-mannans produced better quantitative results than per-*O*-methylated D-mannans in this case, because of loss of the volatile 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D-mannitol, formed from terminal D-mannopyranosyl groups. Qualitatively, the same results were, however, obtained from both per-*O*-ethylated and per-*O*-methylated D-mannans.

We now report the independent synthesis and mass spectra of the methylated and ethylated 1,5-anhydro-D-mannitol derivatives formed from these D-mannans, as an aid to those who may use this method in the structural characterization of other D-mannans.

RESULTS

S. cerevisiae D-mannans contain 2-, 3-, and 6-linked D-mannopyranosyl residues, 2,6- and 3,6-linked, branch-point D-mannopyranosyl residues, and nonreducing (terminal) D-mannopyranosyl groups. Reductive cleavage of the methylated or ethylated D-mannans, and acetylation of the products, therefore yields partially methylated or ethylated 1,5-anhydro-D-mannitol derivatives containing acetyl groups at O-2, O-3, O-6, O-2,6, or O-3,6. In addition, per-*O*-methylated or per-*O*-ethylated 1,5-anhydro-D-mannitol is formed from the nonreducing, terminal groups. Each of these derivatives was independently synthesized, and its electron-impact mass spectrum was recorded.

Synthesis. — 2-*O*-Acetyl-1,5-anhydro-3,4,6-tri-*O*-methyl- (**5a**) and -3,4,6-tri-*O*-ethyl-D-mannitol (**5b**) and 2,6-di-*O*-acetyl-1,5-anhydro-3,4-di-*O*-methyl- (**8a**) and -3,4-di-*O*-ethyl-D-mannitol (**8b**) were prepared as shown in Scheme 1. With one exception, the various manipulations employed in the syntheses were standard, and so the intermediates were not isolated and fully characterized. The product of each reaction was, however, checked by ^{1}H-n.m.r. spectroscopy, to ensure completion of protection or deprotection. The key intermediate in the synthesis of **5a,b**, as well as of **8a,b**, was methyl 2-*O*-tetrahydropyran-2-yl-α-D-mannopyranoside (**3**), which was prepared from methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[4] (**1**) by sequential treatment with dihydropyran in the presence of *p*-toluenesulfonic acid, and catalytic hydrogenolysis. Alkylation of **3** with methyl iodide or ethyl iodide by the procedure of Hakomori[5], and selective removal of the tetrahydropyranyl ether[6], gave **4a** and **4b**, respectively; these were respectively converted into **5a** and **5b** by a route employing a newly developed procedure for the synthesis of anhydroalditols[7]. Compounds **4a** and **4b** in dichloromethane were each treated with trifluorobis(trimethylsilyl)acetamide, and, after silylation was complete, reductive cleavage of the glycoside was effected by the addition of triethylsilane and trimethylsilyl trifluoromethanesulfonate. Removal of the trimethylsilyl protecting group and acetylation of the product gave **5a**



a R = Me
b R = Et

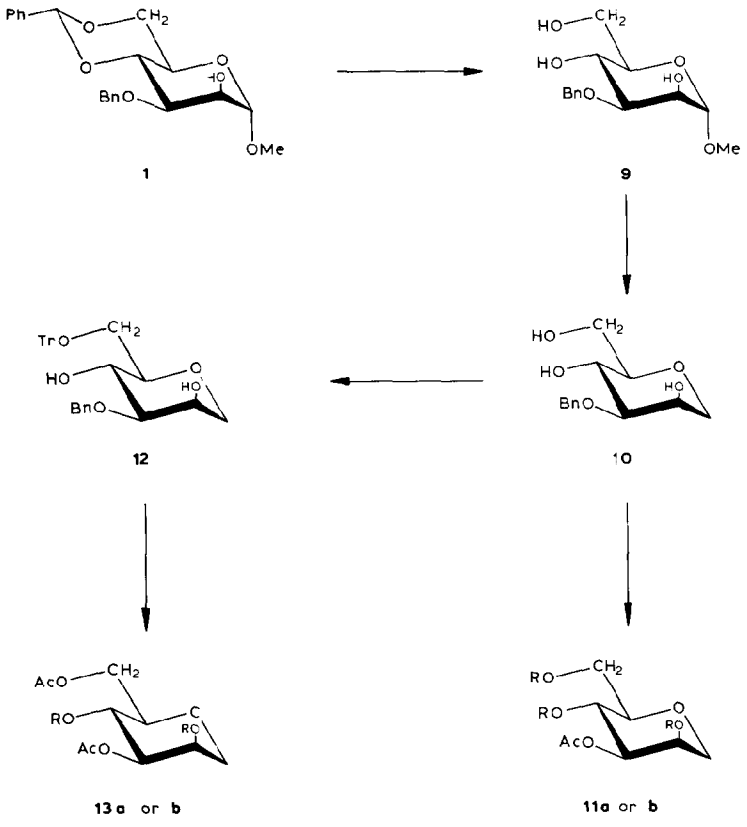
THP = tetrahydropyran-2-yl
Tr = Ph_3C

Scheme 1

and **5b**, which were isolated in chromatographically and analytically pure form by preparative g.l.c. Compounds **8a** and **8b** were prepared from **3** by a similar route, except that, prior to alkylation, tritylation of **3** was accomplished, and the ether alkylated, to give **6a** and **6b**. Removal of the tetrahydropyran-2-yl[6] and trityl (H_2, Pd) protecting-groups gave **7a** and **7b**, which were converted into **8a** and **8b**, respec-

tively, as described for the synthesis of **5a** and **5b**. Analytically and chromatographically pure **8a** and **8b** were obtained by preparative g.l.c.

The synthesis of 3-*O*-acetyl-1,5-anhydro-2,4,6-tri-*O*-methyl- (**11a**) and -2,4,6-tri-*O*-ethyl- (**11b**), and 3,6-di-*O*-acetyl-1,5-anhydro-2,4-di-*O*-methyl- (**13a**) and -2,4-di-*O*-ethyl-D-mannitol (**13b**) was accomplished as shown in Scheme 2. The

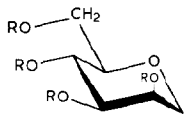


Scheme 2

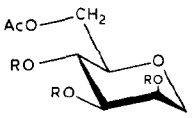
starting material in these syntheses was also compound **1**. Selective hydrogenolysis[8] of the benzylidene protecting-group of **1** gave methyl 3-*O*-benzyl-α-D-mannopyranoside (**9**), which was converted into 1,5-anhydro-3-*O*-benzyl-D-mannitol (**10**) as described for the reductive cleavage of **4a** and **b** and **7a** and **b**. Alkylation[5] of **10** with methyl iodide or ethyl iodide, followed by catalytic hydrogenolysis of the benzyl group, and acetylation of the product, gave **11a** and **11b**, respectively. Compounds **13a** and **13b** were prepared from **10** by a similar route, except that trityla-

tion of **10** was first accomplished, to give 1,5-anhydro-3-*O*-benzyl-6-*O*-trityl-D-mannitol (**12**). After alkylation of **12**, the trityl and benzyl ether groups were removed by catalytic hydrogenolysis, prior to acetylation. Compounds **11a**, **11b**, **13a**, and **13b** were obtained in chromatographically and analytically pure form by preparative g.l.c.

The remaining anhydro-D-mannitol derivatives examined in this study were prepared from 1,5-anhydro-D-mannitol[9]. 1,5-Anhydro-2,3,4,6-tetra-*O*-methyl- (**14a**) and -2,3,4,6-tetra-*O*-ethyl-D-mannitol (**14b**) were prepared from 1,5-anhydro-D-mannitol by direct alkylation[5], whereas 1,5-anhydro-6-*O*-acetyl-2,3,4-tri-*O*-methyl- (**15a**) and -2,3,4-tri-*O*-ethyl-D-mannitol (**15b**) were prepared by a route involving sequential tritylation, alkylation, detritylation[10], and acetylation. The crude products were subjected to preparative g.l.c., in order to obtain analytically and chromatographically pure samples.



14a R = Me
14b R = Et



15a R = Me
15b R = Et

^{1}H-N.m.r. spectra. — The positions of substitution of the acetyl groups in the partially methylated or ethylated 1,5-anhydro-D-mannitol derivatives were readily verified by 300-MHz, ^{1}H-n.m.r. spectroscopy. In the spectra of all twelve derivatives (**5a** and **b**, **8a** and **b**, **11a** and **b**, **13a** and **b**, **14a** and **b**, and **15a** and **b**), H-1*e* was observed as the expected doublet of doublets ($J_{1e,1a}$ 13, $J_{1e,2}$ 2.2 Hz) at δ ~4.1. In the spectra of derivatives containing a 2-*O*-acetyl group (**5a** and **b**, and **8a** and **b**), the H-2 resonance was observed at δ 5.3 as an unresolved multiplet, reflecting *gauche* couplings to H-1*e*, H-1*a*, and H-3. For derivatives lacking a 2-*O*-acetyl substituent, the H-2 multiplet was observed upfield at δ ~3.7. For derivatives containing a 3-*O*-acetyl group (**11a** and **b**, and **13a** and **b**), H-3 was observed at δ 4.8 as a doublet of doublets ($J_{2,3}$ 3.4 and $J_{3,4}$ 9.6 Hz), reflecting *gauche* coupling to H-2 and *trans*-diaxial coupling to H-4. For derivatives in which a 6-*O*-acetyl substituent was present (**8a** and **b**, **13a** and **b**, and **15a** and **b**), the methylene protons on C-6 were observed as two doublets of doublets at δ 4.23 ($J_{5,6}$ 5.8, $J_{6,6'}$ 11.8 Hz) and δ 4.36 ($J_{5,6'}$ 1.8, $J_{6,6'}$ 11.8 Hz). In all cases, the H-6 resonance at lowest field displayed the smaller coupling to H-5. In addition to the foregoing characteristic methine and methylene resonances of the 1,5-anhydro-D-mannitol backbone, *O*-acetyl, *O*-methyl, and *O*-ethyl resonances were observed at their expected chemical shifts.

Mass spectra. — The molecular weights of the various 1,5-anhydro-D-mannitol derivatives were verified by positive-ion, chemical-ionization, mass spectrometry, with ammonia as the reagent gas. For all derivatives, the base peak in the

mass spectrum was the ammonium cluster-ion at (M + 18), but (M + 1) ions (M + H^+) were also observed in lesser amounts (see Experimental section).

The electron-impact, mass spectra of these derivatives were also recorded, in order to aid in their identification. Tabulated in the Experimental section are the *m/z* values observed, and their percent intensity (in parentheses) relative to the base peak. Listed are those ions, lying below *m/z*150, that comprise 10% or more of the intensity of the base peak, and those ions above *m/z* 150 that are prominent regardless of their absolute intensity. Inspection of these data revealed that each anhydromannitol derivative gives a characteristic spectrum, as expected. In the spectra of all of the acetyl derivatives, the base peak is *m/z* 43 (CH_3CO^+), as is observed for partially methylated alditol acetates as well[11]. Molecular ions were not observed, but (M + 1) ions of low intensity (<1%) were observed in the spectra of acetyl derivatives.

Some preliminary conclusions can be drawn that permit a distinction to be made among positional isomers. In the spectra of methylated 1,5-anhydro-D-mannitol derivatives (see Table I), characteristic fragments are observed due to the loss of the exocyclic, methoxymethyl group, (M − 45), the further elimination of methanol from the (M − 45) ion, to give (M − 77), and the elimination of one acetic acid (M − 60) or two acetic acid molecules (M − 120) from the molecular ion. As expected, the (M − 45) and (M − 77) ions are observed only in the spectra of 6-*O*-methyl derivatives (**5a**, **11a**, and **14a**). Interestingly, (M − 60) ions are observed for both the 3-*O*-acetyl derivative (**11a**) and the 6-*O*-acetyl derivative (**15a**), but not for the 2-*O*-acetyl derivative (**5a**). The failure of the 2-*O*-acetyl derivative (**5a**) to give an (M − 60) ion therefore distinguishes it from the 3-*O*-acetyl (**11a**) and 6-*O*-acetyl (**15a**) derivatives, whereas the failure of the 6-*O*-acetyl derivative (**15a**) to give (M − 45) and (M − 77) ions distinguishes it from the 3-*O*-acetyl derivative (**11a**). The two di-*O*-acetyl derivatives (**8a** and **13a**) can be distinguished, based on the intensities of ions arising from the elimination of acetic acid. The 2,6-di-*O*-acetyl derivative (**8a**) displays a relatively intense ion at (M − 60), due to the elimination of one mol of acetic acid, but an ion of weak intensity at (M − 120),

TABLE I

SELECTED FRAGMENTS OBSERVED IN THE ELECTRON-IMPACT, MASS SPECTRA OF THE ACETATES OF METHYLATED 1,5-ANHYDRO-D-MANNITOL DERIVATIVES

Derivative	*Mol. wt.*	*(M − 45)*	*(M − 60)*	*(M − 77)*	*(M − 120)*
OMe_4 (**14a**)	220	+	−	+	−
2-OAc-OMe_3 (**5a**)	248	+	−	+	−
3-OAc-OMe_3 (**11a**)	248	+	+	+	−
6-OAc-OMe_3 (**15a**)	248	−	+	−	−
2,6-OAc_2-OMe_2 (**8a**)	276	−	+	−	+(w)[a]
3,6-OAc_2-OMe_2 (**13a**)	276	−	+(w)	−	+

[a] w = weak

TABLE II

SELECTED FRAGMENTS OBSERVED IN THE ELECTRON-IMPACT, MASS SPECTRA OF THE ACETATES OF ETHYLATED 1,5-ANHYDRO-D-MANNITOL DERIVATIVES

Derivative	*Mol. wt.*	*(M − 59)*	*(M − 60)*	*(M − 105)*	*(M − 120)*
OEt_4 (**14b**)	276	+	−	+	−
2-OAc-OEt_3 (**5b**)	290	+	−	+	−
3-OAc-OEt_3 (**11b**)	290	+	+	+	−
6-OAc-OEt_3 (**15b**)	290	−	+	−	−
2,6-OAc_2-OEt_2 (**8b**)	304	−	+	−	+(w)[a]
3,6-OAc_2-OEt_2 (**13b**)	304	−	+(w)	−	+

[a]w = weak.

presumably arising from the elimination of two mol of acetic acid, is also observed. In contrast, the 3,6-di-*O*-acetyl derivative (**13a**) displays an intense ion at (M − 120), but an ion of low intensity at (M − 60). The relative intensities of the (M − 60) and (M − 120) ions in the spectra of the two di-*O*-acetyl derivatives are thus those expected, based on the relative ease of elimination of acetic acid from the mono-*O*-acetyl derivatives, as already noted.

Similar interpretations may be advanced for the fragmentations characteristic of the ethylated 1,5-anhydro-D-mannitol derivatives (see Table II). Derivatives containing a 6-*O*-ethyl substituent give rise to characteristic fragments at (M − 59), arising from the loss of the exocyclic ethoxymethyl moiety, and at (M − 105), arising from the further elimination of ethanol from the (M − 59) fragment. The (M − 59) and (M − 105) fragments are, therefore, homologous to the (M − 45) and (M −77) fragments, respectively, that were observed in the spectra of 6-*O*-methyl derivatives. In addition to these fragmentations, the mono- and di-*O*-acetyl ethylated anhydromannitol derivatives displayed patterns of elimination of one and two molecules of acetic acid, respectively, identical to those observed for the corresponding methylated derivatives.

EXPERIMENTAL

General. — Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Preparative g.l.c. was performed in a column (6.35 mm × 244 cm) of OV-225 on 100–120 Supelcoport. All methylations and ethylations were conducted by the procedure described by Hakomori[5]. Tritylations and detritylations were performed as described by Helferich and Becker[10]. All catalytic hydrogenations were performed by using equal weights of starting material and 10% Pd–carbon, with methanol as solvent, at 276 kPa overnight. Acetylations were performed in 1:1 (v/v) acetic anhydride–pyridine for 1 h at 100°. ^{1}H-N.m.r. spectra of solutions in $CDCl_3$ were recorded with a Nicolet NT-300 spectrometer, and are refer-

enced to internal tetramethylsilane. Chemical-ionization and electron-impact mass spectra were recorded with a Finnigan 4000 mass spectrometer.

2-O-Acetyl-1,5-anhydro-3,4,6-tri-O-methyl-D-mannitol (**5a**). — Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[4] (**1**; 2.39 g) was converted into its tetrahydropyran-2-yl ether (**2**; 3.39 g, 86%) following the procedure described by Berry *et al.*[12]. Catalytic hydrogenolysis of **2** yielded methyl 2-*O*-tetrahydropyran-2-yl-α-D-mannopyranoside (**3**). Methylation[5] of **3**, and selective hydrolysis of the tetrahydropyran-2-yl ether[6] yielded methyl 3,4,6-tri-*O*-methyl-α-D-mannopyranoside (**4a**). The conversion of **4a** into 1,5-anhydro-3,4,6-tri-*O*-methyl-D-mannitol was accomplished by trimethylsilylation followed by reductive cleavage[7], as described for the synthesis of **10**. Acetylation of the product, and preparative g.l.c. at 170°, yielded pure **5a** as an oil; ^{1}H-n.m.r. ($CDCl_3$): δ 2.15 (s, 3 H, CH_3CO), 3.41, 3.43, 3.54 (3 s, 9 H, 3 MeO), 3.24–3.67 (complex, 6 H, H-1*a*,3,4,5,6,6′), 4.03 (dd, *J* 2.1, 13.1 Hz, 1 H, H-1*e*), and 5.30 (m, 1 H, H-2); e.i.-m.s.: *m/z* 43 (100), 45 (57), 59 (14), 71 (69), 74 (13), 87 (24), 88 (16), 101 (42), 102 (24), 111 (18), 129 (10), 147 (10), 156 (1), 171 (3), 203 (3), 216 (0.4), 217 (0.3), and 249 (0.5); c.i.-m.s. (NH_3, positive): *m/z* 249 (16) and 266 (100).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 52.99; H, 8.08.

2-O-Acetyl-1,5-anhydro-3,4,6-tri-O-ethyl-D-mannitol (**5b**). — Compound **5b** was prepared as described for **5a**, except that compound **3** was ethylated[5] to produce **4b**. For **5b**, ^{1}H-n.m.r. ($CDCl_3$): δ 1.12–1.30 (complex, 9 H, 3 ethyl CH_3), 2.14 (s, 3 H, CH_3CO), 3.27–3.91 (complex, 12 H, H-1*a*,3,4,5,6,6′, 3 ethyl CH_2), 4.03 (dd, *J* 2.0, 13.1 Hz, 1 H, H-1*e*), and 5.26 (m, 1 H, H-2); e.i.-m.s.: *m/z* 43 (100), 45 (19), 57 (29), 58 (13), 59 (23), 60 (12), 69 (10), 73 (16), 85 (43), 86 (36), 88 (11), 101 (19), 125 (10), 129 (34), 130 (13), 161 (5), 171 (3), 184 (1), 185 (3), 231 (1), 244 (0.5), 245 (0.6), 290 (0.2), and 291 (0.6); c.i.-m.s. (NH_3, positive): *m/z* 291 (29) and 308 (100).

Anal. Calc. for $C_{14}H_{26}O_6$: C, 57.91; H, 9.03. Found: C, 58.04; H, 8.91.

2,6-Di-O-acetyl-1,5-anhydro-3,4-di-O-methyl-D-mannitol (**8a**). — Compound **8a** was prepared as described for **5a**, except that tritylation[10] of **3** was accomplished prior to methylation, to produce **6a**. Selective hydrolysis[6] of the tetrahydropyran-2-yl ether of **6a**, followed by catalytic hydrogenolysis to remove the trityl ether, produced **7a**, which was converted into **8a** as described for the conversion of **4a** into **5a**. Preparative g.l.c. at 190° yielded pure **8a** as an oil; ^{1}H-n.m.r. ($CDCl_3$): δ 2.11, 2.15 (2 s, 6 H, 2 CH_3CO), 3.43, 3.53 (2 s, 6 H, 2 MeO), 3.30–3.49 (complex, 4 H, H-1*a*,3,4,5), 4.03 (dd, *J* 2.2, 13.1 Hz, 1 H, H-1*e*), 4.23 (dd, *J* 5.5, 11.9 Hz, 1 H, H-6), 4.37 (dd, *J* 1.6, 11.9 Hz, 1 H, H-6′), and 5.31 (m, 1 H, H-2); e.i.-m.s.: *m/z* 43 (100), 45 (13), 71 (30), 87 (40), 88 (13), 117 (10), 130 (12), 156 (1), 184 (0.7), 216 (3), and 277 (0.7); c.i.-m.s. (NH_3, positive): *m/z* 277 (15) and 294 (100).

Anal. Calc. for $C_{12}H_{20}O_7$: C, 52.16; H, 7.30. Found: C, 51.91; H, 7.15.

2,6-Di-O-acetyl-1,5-anhydro-3,4-di-O-ethyl-D-mannitol (**8b**). — Compound **8b** was prepared as described for **8a** except that **6b** was formed by tritylation of **3**

and ethylation[5] of the product. For **8b**, ^{1}H-n.m.r. ($CDCl_3$): δ 1.17 (t, J 7.0 Hz, 3 H, ethyl CH_3), 1.18 (t, J 6.9 Hz, 3 H, ethyl CH_3), 2.10, 2.15 (2 s, 6 H, 2 CH_3CO), 3.34–3.92 (complex, 8 H, H-1*a*,3,4,5, 2 ethyl CH_2), 4.02 (dd, J 2.1, 13.1 Hz, 1 H, H-1*e*), 4.25 (dd, J 5.2, 11.9 Hz, 1 H, H-6), 4.36 (dd, J 1.7, 11.9 Hz, 1 H, H-6′), and 5.28 (m, 1 H, H-2); e.i.-m.s.: *m/z* 43 (100), 44 (11), 45 (12), 57 (11), 69 (13), 85 (13), 88 (21), 101 (22), 129 (11), 153 (1), 154 (3), 155 (2), 156 (1), 157 (2), 170 (1), 184 (2), 187 (1), 214 (0.8), 244 (0.6), 262 (0.3), and 305 (0.1); c.i.-m.s. (NH_3, positive): *m/z* 305 (12) and 322 (100).

Anal. Calc. for $C_{14}H_{24}O_7$: C, 55.25; H, 7.95. Found: C, 55.24; H, 8.02.

3-O-Acetyl-1,5-anhydro-2,4,6-tri-O-methyl-D-mannitol (**11a**). — Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside[4] (**1**; 1.8 g) was *O*-debenzylidenated by selective, acid hydrolysis, using the conditions described by Choy and Unrau[8] for *O*-debenzylidenation of the corresponding 3-*O*-tosyl derivative, to produce **9** in 87% yield. The conversion of **9** into **10** was accomplished by reductive cleavage, as described by Bennek and Gray[7]. Briefly, **9** (1.1 g) was trimethylsilylated by heating for 1 h at 40° in a mixture of dry CH_2Cl_2 (5 mL) and trifluoro-*N,O*-bis(trimethylsilyl)acetamide (2.43 mL, 1.5 eq.). After cooling to room temperature, triethylsilane (3.25 mL, 5 eq.) and Me_3Si trifluoromesylate (3.90 mL) were successively added, and the mixture was stirred for 40 h at room temperature, and cooled to 0°. The reaction was quenched by the addition of an excess of methanol, and the solution was treated batchwise with AG 501-X8(D) mixed-bed resin (Bio-Rad, 50 mL), the resin filtered off, and the filtrate evaporated to dryness *in vacuo*. Several additions and evaporations of H_2O yielded **10** (0.95 g, 96%) as a clear syrup. Methylation of **10**, catalytic hydrogenolysis of the product, and acetylation, yielded **11a**, which was obtained pure by preparative g.l.c. at 190°; ^{1}H-n.m.r. ($CDCl_3$): δ 2.16 (s, 3 H, CH_3CO), 3.40 (s, 6 H, 2 MeO), 3.47 (s, 3 H, MeO), 3.30–3.62 (complex, 5 H, H-1*a*,4,5,6,6′), 3.66 (m, 1 H, H-2), 4.12 (dd, J 2.1, 12.9 Hz, 1 H, H-1*e*), and 4.84 (dd, J 3.4, 9.8 Hz, 1 H, H-3); e.i.-m.s.: *m/z* 43 (100), 45 (61), 58 (18), 59 (15), 69 (10), 71 (19), 74 (38), 75 (53), 101 (44), 115 (10), 143 (18), 156 (5), 157 (3), 158 (5), 159 (1), 171 (2), 188 (3), 189 (1), 203 (1), 217 (0.3), and 249 (0.6); c.i.-m.s. (NH_3, positive): *m/z* 249 (8) and 266 (100).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 53.35; H, 8.21.

3-O-Acetyl-1,5-anhydro-2,4,6-tri-O-ethyl-D-mannitol (**11b**). — Compound **11b** was prepared as described for **11a**, except that **10** was ethylated prior to catalytic hydrogenolysis. For **11b**, ^{1}H-n.m.r. ($CDCl_3$): δ 1.15 (t, J 7.0 Hz, 3 H, ethyl CH_3), 1.19 (t, J 6.9 Hz, 3 H, ethyl CH_3), 1.22 (t, J 7.0 Hz, 3 H, ethyl CH_3), 2.13 (s, 3 H, CH_3CO), 3.32–3.74 (complex, 11 H, H-1*a*,4,5,6,6′, 3 ethyl CH_2), 4.06 (dd, J 2.2, 12.7 Hz, 1 H, H-1*e*), and 4.81 (dd, J 3.5, 9.7 Hz, 1 H, H-3); e.i.-m.s.: *m/z* 43 (100), 44 (15), 45 (16), 57 (15), 59 (20), 60 (10), 69 (44), 71 (10), 72 (10), 73 (15), 85 (12), 88 (27), 97 (14), 101 (11), 103 (16), 129 (37), 143 (11), 154 (1), 155 (3), 156 (2), 157 (2), 171 (6), 184 (2), 185 (4), 187 (2), 199 (2), 201 (1), 230 (2), 231 (0.8), and 291 (0.1); c.i.-m.s. (NH_3, positive): *m/z* 291 (10) and 308 (100).

Anal. Calc. for $C_{14}H_{26}O_6$: C, 57.91; H, 9.03. Found: C, 57.81; H, 8.85.

*3,6-Di-*O*-acetyl-1,5-anhydro-2,4-di-*O*-methyl-*D*-mannitol* (**13a**). — Compound **13a** was prepared as described for **11a**, except that tritylation of **10** was performed prior to methylation. Catalytic hydrogenolysis of the trityl and benzyl protecting groups, and acetylation of the product, gave **13a**, which was isolated pure by preparative g.l.c. at 190°; ^{1}H-n.m.r. ($CDCl_3$): δ 2.11, 2.17 (2 s, 6 H, 2 CH_3CO), 3.41, 3.47 (2 s, 6 H, 2 MeO), 3.38–3.49 (complex, 2 H, H-1*a*,5), 3.55 (t, *J* 9.6 Hz, 1 H, H-4), 3.69 (m, 1 H, H-2), 4.13 (dd, *J* 2.2, 13.0 Hz, 1 H, H-1*e*), 4.22 (dd, *J* 5.7, 11.9 Hz, 1 H, H-6), 4.36 (dd, *J* 2.0, 11.9 Hz, 1 H, H-6′), and 4.86 (dd, *J* 3.4, 9.6 Hz, 1 H, H-3); e.i.-m.s.: *m/z* 43 (100), 45 (13), 58 (12), 71 (10), 74 (25), 75 (19), 87 (10), 101 (23), 117 (11), 153 (2), 154 (1), 156 (10), 159 (1), 186 (1), 216 (0.4), 217 (0.6), 234 (0.3), 245 (0.4), and 277 (0.4); c.i.-m.s. (NH_3, positive): *m/z* 277 (9) and 294 (100).

Anal. Calc. for $C_{12}H_{20}O_7$: C, 52.16; H, 7.30. Found: C, 51.97; H, 7.22.

*3,6-Di-*O*-acetyl-1,5-anhydro-2,4-di-*O*-ethyl-*D*-mannitol* (**13b**). — Compound **13b** was prepared as described for **13a**, except that **12** was ethylated prior to catalytic hydrogenolysis and acetylation. For **13b**, ^{1}H-n.m.r. ($CDCl_3$): δ 1.15 (t, *J* 7.1 Hz, 3 H, ethyl CH_3), 1.20 (t, *J* 7.0 Hz, 3 H, ethyl CH_3), 2.11, 2.14 (2 s, 6 H, 2 CH_3CO), 3.39–3.74 (complex, 7 H, H-1*a*,4,5, 2 ethyl CH_2), 3.79 (m, 1 H, H-2), 4.05 (dd, *J* 2.1, 12.8 Hz, 1 H, H-1*e*), 4.28 (dd, *J* 5.8, 11.8 Hz, 1 H, H-6), 4.37 (dd, *J* 2.0, 11.8 Hz, 1 H, H-6′), and 4.82 (dd, *J* 3.4, 9.6 Hz, 1 H, H-3); e.i.-m.s.: *m/z* 43 (100), 57 (13), 60 (10), 69 (22), 71 (17), 72 (16), 73 (13), 85 (14), 88 (40), 101 (19), 103 (18), 129 (24), 131 (16), 153 (2), 154 (6), 155 (4), 156 (2), 157 (3), 169 (1), 170 (1), 171 (2), 184 (4), 185 (1), 187 (3), 214 (1), 244 (0.3), 245 (0.5), and 305 (0.9); c.i.-m.s. (NH_3, positive): *m/z* 305 (7) and 322 (100).

Anal. Calc. for $C_{14}H_{24}O_7$: C, 55.25; H, 7.95. Found: C, 55.14; H, 7.72.

*1,5-Anhydro-2,3,4,6-tetra-*O*-methyl-*D*-mannitol* (**14a**). — Compound **14a** was prepared by direct methylation[5] of 1,5-anhydro-D-mannitol[9], and was isolated pure after preparative g.l.c. at 160°; ^{1}H-n.m.r. ($CDCl_3$): δ 3.40, 3.45, 3.49, 3.53 (4 s, 12 H, 4 MeO), 3.23–3.66 (complex, 7 H, H-1*a*,2,3,4,5,6,6′), and 4.18 (dd, *J* 2.2, 12.9 Hz, 1 H, H-1*e*); e.i.-m.s.: *m/z* 43 (39), 45 (70), 58 (19), 59 (18), 71 (55), 72 (10), 73 (18), 75 (38), 87 (14), 88 (31), 99 (21), 101 (100), 102 (16), 143 (14), 156 (1), 157 (1), 158 (2), 175 (9), and 188 (2); c.i.-m.s. (NH_3, positive): *m/z* 221 (21) and 238 (100).

*1,5-Anhydro-2,3,4,6-tetra-*O*-ethyl-*D*-mannitol* (**14b**). — Compound **14b** was prepared by direct ethylation[5] of 1,5-anhydro-D-mannitol[9], and was isolated pure after preparative g.l.c. at 160°; ^{1}H-n.m.r. ($CDCl_3$): δ 1.17 (t, *J* 7.0 Hz, 3 H, ethyl CH_3), 1.21 (t, *J* 7.0 Hz, 3 H, ethyl CH_3), 1.22 (t, *J* 7.0 Hz, 3 H, ethyl CH_3), 1.25 (t, *J* 7.0 Hz, 3 H, ethyl CH_3), 3.26–3.95 (complex, 15 H, H-1*a*,2,3,4,5,6,6′, 4 ethyl CH_2), and 4.09 (dd, *J* 2.3, 12.6 Hz, 1 H, H-1*e*); e.i.-m.s.: *m/z* 43 (75), 44 (37), 45 (67), 47 (17), 55 (13), 57 (62), 58 (40), 59 (79), 60 (42), 61 (25), 69 (32), 71 (18), 72 (23), 73 (60), 75 (25), 82 (10), 83 (10), 85 (63), 86 (63), 87 (17), 88 (20), 98 (11), 99 (13), 101 (28), 103 (47), 113 (15), 116 (24), 129 (100), 130 (14), 155 (1.6), 156

(1.9), 157 (2.3), 171 (6), 173 (4), 200 (0.6), 203 (0.8), 217 (1.8), 230 (1.2), 276 (0.3), and 277 (0.5); c.i.-m.s. (NH_3, positive): *m/z* 277 (23) and 294 (100).

*6-O-Acetyl-1,5-anhydro-2,3,4-tri-O-methyl-*D*-mannitol* (**15a**). — Compound **15a** was prepared from 1,5-anhydro-D-mannitol[9] by successive tritylation[10], methylation[5], detritylation[10], and acetylation, and the final product was isolated pure by preparative g.l.c. at 170°; ^{1}H-n.m.r. ($CDCl_3$): δ 2.10 (s, 3 H, CH_3CO), 3.46, 3.50, 3.53 (3 s, 9 H, 3 MeO), 3.25–3.42 (complex, 4 H, H-1*a*,3,4,5), 3.63 (m, 1 H, H-2), 4.18 (dd, *J* 2.2, 12.9 Hz, 1 H, H-1*e*), 4.20 (dd, *J* 5.9, 11.8 Hz, 1 H, H-6), and 4.37 (dd, *J* 1.7, 11.8 Hz, 1 H, H-6′); e.i.-m.s.: *m/z* 43 (100), 45 (44), 58 (32), 59 (16), 69 (11), 71 (37), 73 (17), 75 (57), 87 (59), 88 (45), 101 (54), 130 (17), 156 (8), 175 (1), 188 (2), 216 (1), and 249 (1); c.i.-m.s. (NH_3, positive): *m/z* 249 (15) and 266 (100).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 53.37; H, 7.89.

*6-O-Acetyl-1,5-anhydro-2,3,4-tri-O-ethyl-*D*-mannitol* (**15b**). — Compound **15b** was prepared by the route described for **15a**, except that ethylation was performed. For **15b**, ^{1}H-n.m.r. ($CDCl_3$): δ 1.16 (t, *J* 7.0 Hz, 3 H, ethyl CH_3), 1.23 (t, *J* 6.9 Hz, 3 H, ethyl CH_3), 1.26 (t, *J* 6.9 Hz, 3 H, ethyl CH_3), 2.09 (s, 3 H, CH_3CO), 3.30–3.96 (complex, 11 H, H-1*a*,2,3,4,5, 3 ethyl CH_2), 4.09 (dd, *J* 2.3, 12.6 Hz, 1 H, H-1*e*), 4.21 (dd, *J* 6.0, 11.8 Hz, 1 H, H-6), and 4.35 (dd, *J* 2.0, 11.8 Hz, 1 H, H-6′); e.i.-m.s.: *m/z* 43 (100), 44 (16), 45 (18), 57 (19), 59 (15), 60 (16), 69 (14), 72 (14), 73 (20), 85 (19), 88 (13), 101 (50), 103 (24), 116 (12), 129 (17), 144 (12), 155 (1.5), 157 (1), 171 (1.7), 173 (1.5), 184 (2.1), 230 (1), 244 (0.7), and 291 (0.5); c.i.-m.s. (NH_3, positive): *m/z* 291 (15) and 308 (100).

Anal. Calc. for $C_{14}H_{26}O_6$: C, 57.91; H, 9.03. Found: C, 57.82; H, 8.85.

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