CARBOHYDRATE
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Carbohydrate Research 298 (1997) 243–249

Reductive cleavage of the positional isomers of benzoylated and methylated methyl α-D-mannopyranoside

Christine R. Rozanas, Gary R. Gray *

Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA

Received 1 August 1996; accepted 11 November 1996

Abstract

The four *O*-benzoyl-tri-*O*-methyl positional isomers, the six di-*O*-benzoyl-di-*O*-methyl positional isomers, the four tri-*O*-benzoyl-*O*-methyl positional isomers, and the tetra-*O*-benzoyl derivative of methyl α-D-mannopyranoside were synthesized, characterized, and subjected to reductive cleavage in the presence of triethylsilane and trimethylsilyl trifluoromethanesulfonate. The reactions were monitored by ^{1}H NMR spectroscopy in order to establish the rates of the reactions and the identities of the products so formed. The tetra-*O*-benzoyl derivative, all tri-*O*-benzoyl-*O*-methyl positional isomers, and the 2,4-di-*O*-benzoyl-3,6-di-*O*-methyl positional isomer were fully stable to reductive-cleavage conditions, but the other di-*O*-benzoyl-di-*O*-methyl positional isomers and all *O*-benzoyl-tri-*O*-methyl positional isomers were converted to their respective 1,5-anhydro-D-mannitol derivatives via reductive cleavage of their anomeric carbon–oxygen bonds. For the mono-*O*-benzoyl positional isomers, the rate of reductive cleavage decreased in the order 2-*O*-benzoyl > 6-*O*-benzoyl > 3-*O*-benzoyl ≅ 4-*O*-benzoyl. Moreover, all mono-*O*-benzoyl positional isomers were cleaved at a much faster rate than the di-*O*-benzoyl positional isomers. These results suggest that both anchimeric assistance and inductive effects of the ester groups contribute to the observed reaction rate. Based upon these results it is suggested that reductive cleavage of benzoylated polysaccharides might serve as a means to cleave selectively at branched residues. © 1997 Elsevier Science Ltd. All rights reserved.

Keywords: Reductive cleavage; Methyl α-D-mannopyranoside; Benzoylated; Methylated derivatives

1. Introduction

The reductive-cleavage method [1] has previously been shown to be applicable to the structural analysis of polysaccharides comprised of a wide variety of sugar residues and covalently attached substituents. In particular, the stability of ester groups [2] to conditions which effect complete cleavage of glycosidic carbon–oxygen bonds makes possible the direct analysis of polysaccharides comprised of uronic acids [3–5], sialic acids [6], and covalently linked sub-

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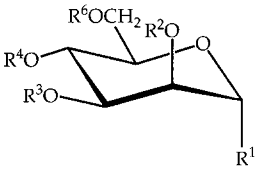
* Corresponding author.

PII S0008-6215(96)00322-9

stituents such as pyruvic acid acetals [7,8], carboxymethyl ethers [9], and carboxylic acid esters [10,11]. However, with regard to the analysis of polysaccharides bearing ester substituents, substantially different rates of reductive cleavage of the glycoside were observed in studies [10] of model compounds bearing a single ester substituent depending upon its position of substitution. Based upon these observations, it appeared that it might be possible to achieve selective reductive cleavage of glycosides depending both upon the number and positions of substitution of ester substituents. If so, the procedure might be useful in polysaccharide structural analysis as a means to generate oligomers of defined structure by selective reductive cleavage of the *fully* esterified polymer.

We have tested this proposal by examining the relative rates of reductive cleavage of the fifteen positional isomers of benzoylated and methylated methyl α-D-mannopyranoside (**1a**–**15a**). Described herein is the synthesis of these derivatives and the kinetics of their reductive cleavage.



	R^2	R^3	R^4	R^6
1	Bz	Me	Me	Me
2	Me	Bz	Me	Me
3	Me	Me	Bz	Me
4	Me	Me	Me	Bz
5	Bz	Bz	Me	Me
6	Bz	Me	Bz	Me
7	Bz	Me	Me	Bz
8	Me	Bz	Bz	Me
9	Me	Bz	Me	Bz
10	Me	Me	Bz	Bz
11	Bz	Bz	Bz	Me
12	Bz	Bz	Me	Bz
13	Bz	Me	Bz	Bz
14	Me	Bz	Bz	Bz
15	Bz	Bz	Bz	Bz

a series: R^1 = OMe

b series: R^1 = H

2. Results

Reductive cleavages.—Reductive cleavages were carried out in NMR tubes using triethylsilane (Et_3SiH, 5 equiv per equiv of acetal) as the reducing agent and trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$, 5 equiv) as the Lewis acid promoter [12] in dideuteriodichloromethane (CD_2Cl_2) as the solvent. The reactions were monitored by acquiring ^1H NMR spectra at various time intervals, and the ratio of starting material to product was determined by integration of the H-1 resonance (δ 4.8–5.0) of the starting material and the H-1e resonance (δ 4.1–4.3) of the anhydroalditol product, both of which were well separated from other resonances. When the reactions were complete or sufficiently so, they were quenched and processed in the usual way, and the ^1H NMR spectrum of the product was compared to that of an authentic sample [13] of the respective partially benzoylated and methylated 1,5-anhydro-D-mannitol.

Shown in Fig. 1 are the results obtained for the four *O*-benzoyl-tri-*O*-methyl derivatives of methyl α-D-mannopyranoside (**1a**–**4a**). As is evident in Fig. 1, all mono-*O*-benzoyl derivatives underwent reduc-

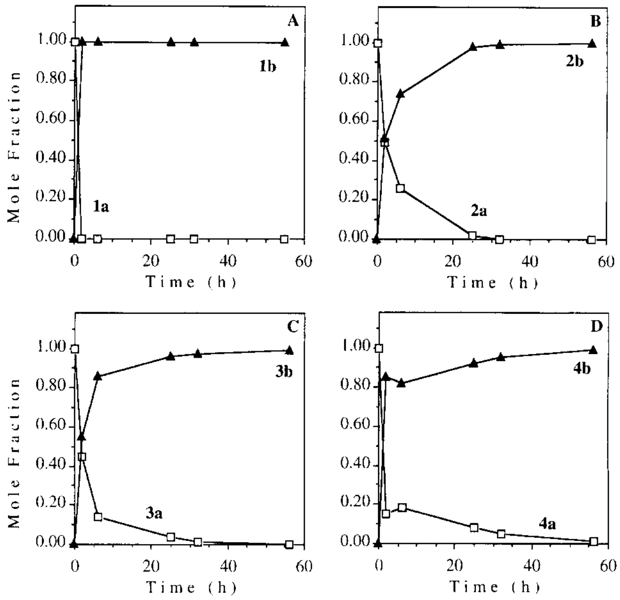


Fig. 1. Time course of product formation during reductive cleavage of (A) methyl 2-*O*-benzoyl-3,4,6-tri-*O*-methyl-α-D-mannopyranoside (**1a**), (B) methyl 3-*O*-benzoyl-2,4,6-tri-*O*-methyl-α-D-mannopyranoside (**2a**), (C) methyl 4-*O*-benzoyl-2,3,6-tri-*O*-methyl-α-D-mannopyranoside (**3a**), and (D) methyl 6-*O*-benzoyl-2,3,4-tri-*O*-methyl-α-D-mannopyranoside (**4a**) in the presence of Et_3SiH and $Me_3SiOSO_2CF_3$. Compounds **1b**–**4b** are the respective benzoylated/methylated derivatives of 1,5-anhydro-D-mannitol.

Table 1
Pseudo first-order reaction rate data for the reductive cleavage of the partially methylated and benzoylated positional isomers of methyl α-D-mannopyranoside in the presence of trimethylsilyl trifluoromethanesulfonate and triethylsilane

Starting material	Expected product	Position of benzoate	$t_{1/2}$ (h) [a]	K_{rel}
1a	**1b**	2	< 1.0 [b]	> 130
2a	**2b**	3	3.5	37
3a	**3b**	4	4.0	33
4a	**4b**	6	1.5 [b]	87
5a	**5b**	2,3	76 [c]	1.7
6a	**6b**	2,4	N.R. [d]	–
7a	**7b**	2,6	30	4.3
8a	**8b**	3,4	130 [c]	1.0
9a	**9b**	3,6	7.0	19
10a	**10b**	4,6	14	9.3
11a	**11b**	2,3,4	N.R.	–
12a	**12b**	2,3,6	N.R.	–
13a	**13b**	2,4,6	N.R.	–
14a	**14b**	3,4,6	N.R.	–
15a	**15b**	2,3,4,6	N.R.	–

[a] Determined from the equation of the best-fit line for the logarithmic graph of loss of starting material versus time.
[b] Half-lives are approximate since insufficient data were acquired at the early stages of the reaction.
[c] Half-lives obtained by extrapolation.
[d] No reaction.

tive cleavage completely in 60 h or less to yield the respective anhydroalditols (**1b**–**4b**) as the exclusive product. However, these derivatives were reductively cleaved at substantially different rates. The 2-*O*-benzoyl derivative (**1a**) was cleaved at the fastest rate, having been completely converted to product (**1b**) in 2 h (Fig. 1A). The 3-*O*-benzoyl (**2b**), 4-*O*-benzoyl (**3b**), and 6-*O*-benzoyl (**4b**) derivatives were cleaved somewhat more slowly (Fig. 1B–D), displaying $t_{1/2}$ values of approximately 3.5, 4.0, and 1.5 h, respectively (see Table 1).

Five of the six di-*O*-benzoyl-di-*O*-methyl positional isomers of methyl α-D-mannopyranoside, i.e. **5a**, **7a**, **8a**, **9a**, and **10a**, were also found to undergo reductive cleavage to give the respective 1,5-anhydro-D-mannitol derivatives (**5b**, **7b**, **8b**, **9b**, and **10b**) as the exclusive product (Fig. 2). However, the rates of cleavage of these derivatives were substantially slower than those observed for the mono-*O*-benzoyl positional isomers (Table 1). Interestingly, and for reasons that are not understood, the 2,4-di-*O*-benzoyl positional isomer **6a** completely failed to undergo reductive cleavage (Fig. 2B) under these conditions.

The four tri-*O*-benzoyl-*O*-methyl positional isomers (**11a**–**14a**) and the tetra-*O*-benzoyl derivative (**15a**) of methyl α-D-mannopyranoside were also subjected to reductive cleavage under the same conditions as just described, and, in all cases, no product was observed by ^{1}H NMR spectroscopy even after 48 h of reaction. These reactions were processed in the usual way, and the products were again analyzed by ^{1}H NMR spectroscopy. In all cases, only the starting material was recovered.

Synthesis of starting materials.—Compounds **1a**–**15a** were synthesized from methyl α-D-mannopyranoside by a route [14] involving partial Hakomori methylation [15] and benzoylation in situ. The resul-

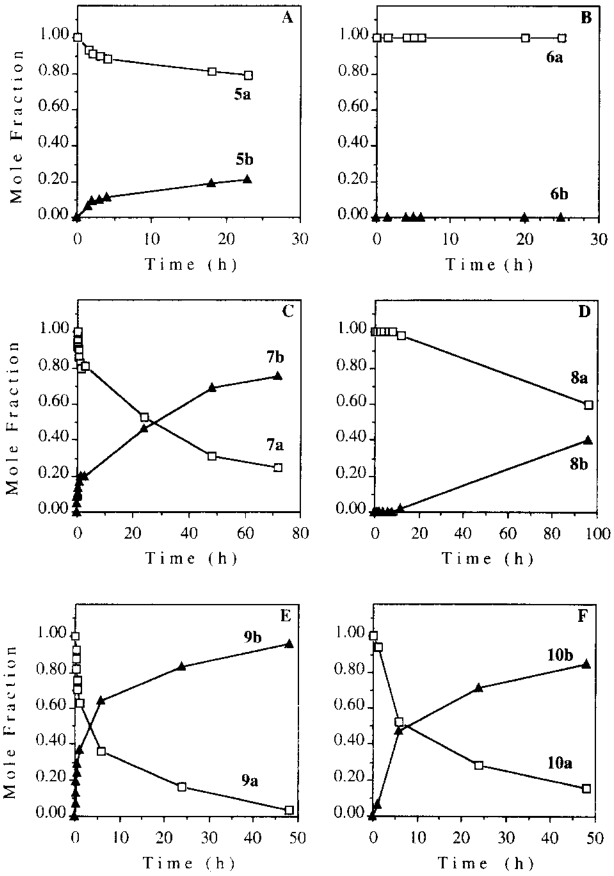


Fig. 2. Time course of product formation during reductive cleavage of (A) methyl 2,3-di-*O*-benzoyl-4,6-di-*O*-methyl-α-D-mannopyranoside (**5a**), (B) methyl 2,4-di-*O*-benzoyl-3,6-di-*O*-methyl-α-D-mannopyranoside (**6a**), (C) methyl 2,6-di-*O*-benzoyl-3,4-di-*O*-methyl-α-D-mannopyranoside (**7a**), (D) methyl 3,4-di-*O*-benzoyl-2,6-di-*O*-methyl-α-D-mannopyranoside (**8a**), (E) methyl 3,6-di-*O*-benzoyl-2,4-di-*O*-methyl-α-D-mannopyranoside (**9a**), and (F) methyl 4,6-di-*O*-benzoyl-2,3-di-*O*-methyl-α-D-mannopyranoside (**10a**) in the presence of Et_3SiH and $Me_3SiOSO_2CF_3$. Compounds **5b**–**10b** are the respective benzoylated/methylated derivatives of 1,5-anhydro-D-mannitol.

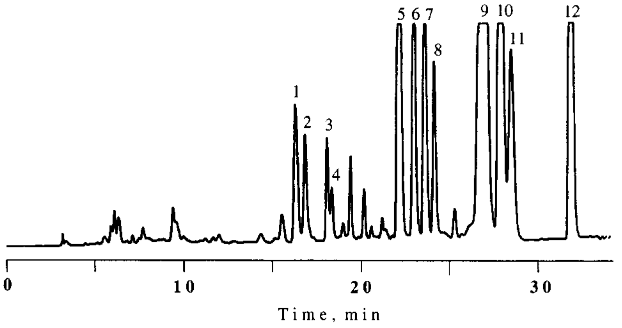


Fig. 3. High-performance liquid chromatogram of the methylated and benzoylated positional isomers of methyl α-D-mannopyranoside derived from the latter by sequential partial methylation and benzoylation in situ. The numbered peaks were identified as follows: (1) **3a**, (2) **4a**, (3) **2a**, (4) **1a**, (5) **8a** and **10a**, (6) **5a** and **6a**, (7) **9a**, (8) **7a**, (9) **11a** and **14a**, (10) **13a**, (11) **12a**, and (12) **15a**. Unlabeled peaks were not identified.

tant mixture of positional isomers was subjected to reversed-phase high-performance liquid chromatography (HPLC) using a semipreparative Rainin C_{18} column (Fig. 3). The individual components were isolated and, after removal of solvent, were identified by ^1H NMR spectroscopy. The positions of substitution of the *O*-benzoyl and *O*-methyl groups were readily discerned based upon a straightforward analysis of the chemical shifts and coupling constants of the ring hydrogen resonances. Using this procedure, nine of the fifteen possible positional isomers were isolated in pure form. The remaining six positional isomers were present in the mixture as three co-eluting pairs, i.e. in peaks 5, 6, and 9 (Fig. 3). These pairs of positional isomers were readily separable, however, by normal-phase HPLC on a silica column (see Experimental).

3. Discussion

The sequencing of polysaccharides by chemical methods is a difficult task because of the wide variety of sugar residues that can be encountered. Toward this end, many procedures for the selective chemical cleavage of polysaccharides have been developed. One of our goals in the development of the reductive-cleavage method for carbohydrate structural analysis has been to develop conditions for the selective reductive cleavage of glycosyl linkages in polysaccharides in order that the method might be useful for sequence analysis, in addition to compositional and ring form/linkage position analysis. Indeed, combinations of reducing agent and promoter have been found that are quite selective in accomplishing reductive cleavage [16–18] and, based upon selective reductive cleavage, a general procedure for sequencing permethylated polysaccharides was proposed [19].

It could also be envisaged, however, that selective reductive cleavage of polysaccharides could be accomplished through alteration of the susceptibilities of their glycosyl residues toward reductive cleavage. Indeed, the well-known acetolysis procedure [20] for selective hydrolysis of glycosidic linkages in polysaccharides is based upon this concept. This proposal was tested using the positional isomers of benzoylated and methylated methyl α-D-mannopyranoside (**1a**–**15a**) as model compounds. Derivatives of methyl α-D-mannopyranoside were chosen both because of the importance of α-D-mannopyranosyl residues as constituents of polysaccharides and the fact that a *trans* 2-*O*-acyl group, when present, would be expected [10] to greatly assist reductive cleavage of the glycoside. Benzoyl esters were chosen for study because of their stability under acidic conditions and the fact that their ultraviolet absorbance facilitated purification of starting materials and products. Nonesterified positions were methylated in order to prevent ester migration and in order to approximate the effect of linked sugar residues. Indeed, these studies demonstrated that the susceptibility of the glycoside to reductive cleavage was dependent upon the number of ester groups present and their positions of substitution. The fact that the tetra-*O*-benzoyl derivative (**15a**) and all tri-*O*-benzoyl derivatives (**11a**–**14a**) were fully stable to reductive cleavage would suggest that terminal (nonreducing) and singly linked α-D-mannopyranosyl residues in benzoylated polysaccharides would not be cleaved. All mono-*O*-benzoyl derivatives (**1a**–**4a**) and five of the six di-*O*-benzoyl positional isomers (**5a** and **7a**–**10a**) readily underwent reductive cleavage, however, suggesting that triply linked and doubly linked α-D-mannopyranosyl residues in benzoylated polysaccharides might be cleaved under reductive cleavage conditions. If these findings can be extended to benzoylated polysaccharides, then the procedure should be of use for selective cleavage at branched residues.

The selectivity that is observed in this study appears to be due primarily to inductive effects, as evidenced by the decreasing rate of reductive cleavage with an increasing number of ester groups. Anchimeric assistance by the 2-*O*-benzoyl group was also in evidence, as expected [10], but only for the mono-*O*-benzoyl derivative (**1a**). It should also be noted that in cases where cleavage occurred, the respective 1,5-anhydro-D-mannitol derivatives were the only products formed. Similarly, in cases where cleavage did not occur, the starting materials were completely stable to the reaction conditions. The reaction is thus quite clean, which is of benefit should it be useful for the selective cleavage of polysaccharides.

4. Experimental

General.—^{1}H NMR spectra were acquired using a Varian 300-MHz or 500-MHz NMR spectrometer in $CDCl_3$ or CD_2Cl_2 as the solvent, and were referenced to internal tetramethylsilane. High-performance liquid chromatography (HPLC) was performed with a Beckman 110B solvent delivery module equipped with a Beckman System Gold model 166 programmable detector module with a System Gold model 406 analog interface module and an NEC controller. The columns used were a Rainin Dynamax Microsorb 5-μm particle-size C_{18} semipreparative column (10 mm × 25 cm) fitted with a guard column and a Regis reversible 5-μm particle-size silica column (4.6 mm × 25 cm).

Triethylsilane (Et_3SiH), trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$), boron trifluoride etherate ($BF_3 \cdot Et_2O$), methanesulfonic acid, chlorotrimethylsilane, iodomethane, benzoyl chloride, and methyllithium (1.4 M soln in ether), were obtained from Aldrich. Methyl α-D-mannopyranoside was obtained from Sigma.

Iodomethane was distilled and stored cold over copper until use. Dimethyl sulfoxide was distilled from calcium hydride at reduced pressure and stored over 4 Å molecular sieves. Dichloromethane, *N*-methylimidazole, and pyridine were distilled prior to use. Lithium methylsulfinylmethanide was prepared from methyl lithium and Me_2SO, standardized, and stored frozen in 3-mL aliquots; the amount needed was thawed prior to use. Benzoic anhydride was used without further purification.

Reductive cleavages were carried out in silanized NMR tubes. The dried sample (3–6 mg) was transferred to the tube as a solution in dideuteriodichloromethane (0.5 mL), then triethylsilane (5 equiv per acetal) was added. An initial ^{1}H NMR spectrum was taken, then trimethylsilyl trifluoromethanesulfonate (5 equiv per acetal) was quickly added, and spectra were acquired by an automated acquisition program. In some experiments, spectra were acquired individually. In all experiments, the H-1 resonance of the starting material and the H-1e resonance of the product were integrated in order to determine the extent of reduction.

When the reactions were complete or nearly complete, they were quenched using satd aq $NaHCO_3$, and extracted with dichloromethane. The organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated, and the ^{1}H NMR spectrum of the product (in deuteriochloroform) was compared to the spectrum of an authentic standard [13].

Partially methylated and benzoylated positional isomers of methyl α-D-mannopyranoside.—Compounds **1a–15a** were synthesized in one reaction as follows. Three flame-dried flasks were prepared, each containing approximately equal amounts (20–40 mg) of methyl α-D-mannopyranoside. Dimethyl sulfoxide (1–2 mL) was added, along with a stirbar, and the flasks were sealed and purged with nitrogen. Aliquots of lithium methylsulfinylmethanide corresponding to 0.75, 1.5, and 2.5 equiv per acetal were added to each flask, and the reactions were stirred for one hour. Iodomethane (200 μL) was added, the reactions were stirred 15 min, then each flask was flushed with nitrogen for 15–30 min to remove the excess iodomethane. Pyridine (0.5 mL), benzoic anhydride (500 mg), and 1-methylimidazole (0.2 mL) were added to each flask, and stirring was continued for 30 min. Water was added carefully and the quenched reactions were vigorously stirred overnight. The three solutions were combined, extracted with dichloromethane, and the dichloromethane layer was washed sequentially (2 × each) with satd aq $NaHCO_3$, 2 M H_2SO_4, and water. The organic layer was then dried over anhyd Na_2SO_4 and evaporated to a syrup.

The residue was dissolved in acetonitrile, and the compounds were separated by reversed-phase HPLC on a Rainin Dynamax Microsorb C_{18} column equilibrated in 50% aq acetonitrile at 2 mL/min. The column was eluted with 50% MeCN for 10 min, then to 80% MeCN with a linear gradient over 5 min. After continued elution with 80% MeCN for 10 min, the column was eluted with a linear gradient to 95% MeCN over 3 min and was held at 95% MeCN until all compounds had eluted (see Fig. 3). The compounds were identified by ^{1}H NMR spectroscopy and, where mixtures were present, were further purified as indicated below.

Methyl 2-O-benzoyl-3,4,6-tri-O-methyl-α-D-mannopyranoside (**1a**).—^{1}H NMR ($CDCl_3$): δ 3.40, 3.44, 3.47, 3.55 (4 s, 12 H, 4 OMe), 3.5–3.7 (complex, 5 H, H-3, H-4, H-5, H-6, H-6′), 4.82 (d, *J* 1.6 Hz, 1 H, H-1), 5.51 (app. t, *J* 2.3 Hz, 1 H, H-2), 7.4–7.5 (m, 2 H, *m*-ArH), 7.5–7.6 (m, 1 H, *p*-ArH), 8.06 (d, *J* 7.2 Hz, 1 H, *o*-ArH).

Methyl 3-O-benzoyl-2,4,6-tri-O-methyl-α-D-mannopyranoside (**2a**).—^{1}H NMR (CDCl$_3$): δ 3.41, 3.43, 3.44, 3.47 (4 s, 12 H, 4 OMe), 3.5–3.7 (complex, 4 H, H-2, H-5, H-6, H-6′), 3.79 (app. t, *J* 9.2 Hz, 1 H, H-4), 4.79 (d, *J* 1.6 Hz, 1 H, H-1), 5.39 (dd, *J* 3.3, 8.9 Hz, 1 H, H-3), 7.4–7.5 (m, 2 H, *m*-ArH), 7.5–7.6 (m, 1 H, *p*-ArH), 8.12 (d, *J* 7.1 Hz, 2 H, *o*-ArH).

Methyl 4-O-benzoyl-2,3,6-tri-O-methyl-α-D-mannopyranoside (**3a**).—^{1}H NMR (CDCl$_3$): δ 3.33, 3.40, 3.46, 3.53 (4 s, 12 H, 4 OMe), 3.4–3.6 (m, 2 H, H-6, H-6′), 3.67 (dd, *J* 2.1, 2.9 Hz, 1 H, H-2), 3.74 (dd, *J* 3.1, 9.5 Hz, 1 H, H-3), 3.93 (ddd, *J* 2.9, 6.7, 9.9 Hz, 1 H, H-5), 4.87 (d, *J* 1.6 Hz, 1 H, H-1), 5.42 (app. t, *J* 9.8 Hz, 1 H, H-4), 7.4–7.5 (m, 2 H, *m*-ArH), 7.5–7.6 (m, 1 H, *p*-ArH), 8.06 (d, *J* 7.1 Hz, 2 H, *o*-ArH).

Methyl 6-O-benzoyl-2,3,4-tri-O-methyl-α-D-mannopyranoside (**4a**).—^{1}H NMR (CDCl$_3$): δ 3.40, 3.50, 3.52, 3.55 (4 s, 12 H, 4 OMe), 3.5–3.6 (complex, 2 H, H-3, H-4), 3.61 (br s, 1 H, H-2), 3.78 (m, 1 H, H-5), 4.48 (dd, *J* 5.4, 11.8 Hz, 1 H, H-6), 4.60 (dd, *J* 2.0, 11.7 Hz, 1 H, H-6′), 4.83 (d, *J* 1.1 Hz, 1 H, H-1), 7.43 (app. t, *J* 7.8 Hz, 2 H, *m*-ArH), 7.55 (app. t, *J* 7.4 Hz, 1 H, *p*-ArH), 8.07 (d, *J* 7.6 Hz, 2 H, *o*-ArH).

Methyl 2,3-di-O-benzoyl-4,6-di-O-methyl-α-D-mannopyranoside (**5a**).—Compounds **5a** and **6a** eluted together on the reversed-phase HPLC column (Fig. 3). Further separation was achieved by HPLC on a Regis silica column eluted with 85:15 hexane–EtOAc. For **5a**: ^{1}H NMR (CDCl$_3$): δ 3.46, 3.49, 3.50 (3 s, 9 H, 3 OMe), 3.70 (dd, *J* 1.8, 10.6 Hz, 1 H, H-6), 3.77 (dd, *J* 4.0, 10.6 Hz, 1 H, H-6′), 3.87 (ddd, *J* 1.5, 3.8, 9.9 Hz, 1 H, H-5), 3.94 (app. t, *J* 9.9 Hz, 1 H, H-4), 4.89 (d, *J* 1.2 Hz, 1 H, H-1), 5.58 (app. t, *J* 2.5 Hz, 1 H, H-2), 5.63 (dd, *J* 3.4, 9.5 Hz, 1 H, H-3), 7.36, 7.47 (2 app. t, *J* 7.8 Hz, 4 H, *m*-ArH), 7.51, 7.60 (2 app. t, *J* 7.4 Hz, 2 H, *p*-ArH), 7.95, 8.06 (2 d, *J* 7.6 Hz, 4 H, *o*-ArH).

Methyl 2,4-di-O-benzoyl-3,6-di-O-methyl-α-D-mannopyranoside (**6a**).—^{1}H NMR (CDCl$_3$): δ 3.36, 3.38, 3.49 (3 s, 9 H, 3 OMe), 3.56 (dd, *J* 2.8, 10.6 Hz, 1 H, H-6), 3.61 (dd, *J* 6.1, 10.7 Hz, 1 H, H-6′), 3.93 (dd, *J* 3.4, 9.8 Hz, 1 H, H-3), 4.07 (ddd, *J* 3.1, 6.3, 9.8 Hz, 1 H, H-5), 4.93 (br s, 1 H, H-1), 5.56 (app. t, *J* 9.9 Hz, 1 H, H-4), 5.60 (app. t, *J* 2.4 Hz, 1 H, H-2), 7.46, 7.47 (2 app. t, *J* 7.8 Hz, 4 H, *m*-ArH), 7.58, 7.60 (2 app. t, *J* 7.0 Hz, 2 H, *p*-ArH), 8.08, 8.11 (2 d, *J* 7.8 Hz, 4 H, *o*-ArH).

Methyl 2,6-di-O-benzoyl-3,4-di-O-methyl-α-D-mannopyranoside (**7a**).—^{1}H NMR (CDCl$_3$): δ 3.43, 3.47, 3.58 (3 s, 9 H, 3 OMe), 3.68 (app. t, *J* 9.5 Hz, 1 H, H-4), 3.76 (dd, *J* 3.2, 9.3 Hz, 1 H, H-3), 3.91 (ddd, *J* 1.9, 4.1, 9.9 Hz, 1 H, H-5), 4.62 (dd, *J* 4.3, 11.9 Hz, 1 H, H-6), 4.68 (dd, *J* 2.0, 11.9 Hz, 1 H, H-6′), 4.83 (br s, 1 H, H-1), 5.55 (app. t, *J* 2.3 Hz, 1 H, H-2), 7.35, 7.44 (2 app. t, *J* 7.8 Hz, 4 H, *m*-ArH), 7.56, 7.60 (2 app. t, *J* 7.5 Hz, 2 H, *p*-ArH), 8.02, 8.11 (2 d, *J* 7.8 Hz, 4 H, *o*-ArH).

Methyl 3,4-di-O-benzoyl-2,6-di-O-methyl-α-D-mannopyranoside (**8a**).—Compounds **8a** and **10a** eluted together on the reversed-phase HPLC column (Fig. 3). Separation of **8a** and **10a** was achieved by HPLC on a Regis silica column using 85:15 hexane–EtOAc. ^{1}H NMR (CD$_2$Cl$_2$): δ 3.33, 3.48, 3.49 (3 s, 9 H, 3 OMe), 3.54 (m, 2 H, H-6, 6′), 3.83 (br d, *J* 1.7 Hz, 1 H, H-2), 4.06 (m, 1 H, H-5), 4.87 (br s, 1 H, H-1), 5.54 (dd, *J* 3.1, 10.1 Hz, 1 H, H-3), 5.68 (app. t, *J* 10.0 Hz, 1 H, H-4), 7.40 (app. t, *J* 7.6 Hz, 4 H, *m*-ArH), 7.53 (app. t, *J* 7.2 Hz, 2 H, *p*-ArH), 7.95 (d, *J* 7.2 Hz, 4 H, *o*-ArH).

Methyl 3,6-di-O-benzoyl-2,4-di-O-methyl-α-D-mannopyranoside (**9a**).—^{1}H NMR (CDCl$_3$): δ 3.44, 3.45, 3.50 (3 s, 9 H, 3 OMe), 3.79 (app. t, *J* 2.7 Hz, 1 H, H-2), 3.82 (app. t, *J* 9.6 Hz, 1 H, H-4), 3.98 (ddd, *J* 2.3, 5.7, 9.8 Hz, 1 H, H-5), 4.56 (dd, *J* 5.6, 11.8 Hz, 1 H, H-6), 4.64 (dd, *J* 2.1, 11.8 Hz, 1 H, H-6′), 4.82 (d, *J* 1.8 Hz, 1 H, H-1), 5.45 (dd, *J* 3.2, 9.3 Hz, 1 H, H-3), 7.45, 7.48 (2 app. t, *J* 7.8 Hz, 4 H, *m*-ArH), 7.5–7.6 (m, 2 H, *p*-ArH), 8.09, 8.13 (2 d, *J* 7.6 Hz, 4 H, *o*-ArH).

Methyl 4,6-di-O-benzoyl-2,3-di-O-methyl-α-D-mannopyranoside (**10a**).—^{1}H NMR (CDCl$_3$): δ 3.42, 3.45, 3.55 (3 s, 9 H, 3 OMe), 3.75 (app. t, *J* 2.5 Hz, 1 H, H-2), 3.78 (dd, *J* 3.1, 9.5 Hz, 1 H, H-3), 4.12 (ddd, *J* 3.2, 6.1, 9.7 Hz, 1 H, H-5), 4.39 (dd, *J* 6.1, 11.9 Hz, 1 H, H-6), 4.54 (dd, *J* 3.2, 11.9 Hz, 1 H, H-6′), 4.89 (d, *J* 1.8 Hz, 1 H, H-1), 5.63 (app. t, *J* 9.7 Hz, 1 H, H-4), 7.33–7.47 (m, 4 H, *m*-ArH), 7.48–7.60 (m, 2 H, *p*-ArH), 7.98–9.08 (m, 4 H, *o*-ArH).

Methyl 2,3,4-tri-O-benzoyl-6-O-methyl-α-D-mannopyranoside (**11a**).—Compounds **11a** and **14a** eluted together on the reversed-phase HPLC column (Fig. 3). Separation of **11a** and **14a** was achieved by HPLC on a Regis silica column using 95:5 hexane–EtOAc. ^{1}H NMR (CDCl$_3$): δ 3.41, 3.54 (2 s, 6 H, 2 OMe), 3.62 (dd, *J* 3.3, 10.6 Hz, 1 H, H-6), 3.65 (dd, *J*, 5.4, 10.6 Hz, 1 H, H-6′), 4.22 (ddd, *J* 3.3, 5.4, 9.3

Hz, 1 H, H-5), 5.00 (d, *J* 1.1 Hz, 1 H, H-1), 5.66 (br s, 1 H, H-2), 5.87–5.91 (m, 2 H, H-3, H-4), 7.26, 7.39, 7.43 (3 app. t, *J* 7.8 Hz, 6 H, *m*-ArH), 7.49, 7.53, 7.61 (3 app. t, *J* 7.7 Hz, 3 H, *p*-ArH), 7.83, 7.98, 8.10 (3 d, *J* 7.8 Hz, 6 H, *o*-ArH).

*Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-methyl-α-*D*-mannopyranoside* (**12a**).—^{1}H NMR ($CDCl_3$): δ 3.49, 3.51 (2 s, 6 H, 2 OMe), 3.99 (app. t, *J* 9.7 Hz, 1 H, H-4), 4.10 (app. dt, *J* 9.8, 3.1 Hz, 1 H, H-5), 4.70 (complex, 2 H, H-6, H-6′), 4.90 (d, *J* 1.2 Hz, 1 H, H-1), 5.62 (app. t, *J* 2.5 Hz, 1 H, H-2), 5.70 (dd, *J* 3.4, 9.6 Hz, 1 H, H-3), 7.3–7.6 (m, 9 H, *m*-,*p*-ArH), 7.96, 8.02, 8.14 (3 d, *J* 7.8 Hz, 6 H, *o*-ArH).

*Methyl 2,4,6-tri-*O*-benzoyl-3-*O*-methyl-α-*D*-mannopyranoside* (**13a**).—^{1}H NMR ($CDCl_3$): δ 3.38, 3.49 (2 s, 6 H, 2 OMe), 3.98 (dd, *J* 3.2, 9.8 Hz, 1 H, H-3), 4.26 (ddd, *J* 2.6, 4.9, 10.2 Hz, 1 H, H-5), 4.42 (dd, *J* 4.7, 12.1 Hz, 1 H, H-6), 4.68 (dd, *J* 2.7, 12.1 Hz, 1 H, H-6′), 4.93 (d, *J* 1.5 Hz, 1 H, H-1), 5.64 (app. t, *J* 2.5 Hz, 1 H, H-2), 5.81 (app. t, *J* 9.9 Hz, 1 H, H-4), 7.35–7.46 (complex, 6 H, *m*-ArH), 7.5–7.6 (m, 3 H, *p*-ArH), 8.07 (d, *J* 7.8 Hz, 4 H, *o*-ArH), 8.09 (d, *J* 7.8 Hz, 2 H, *o*-ArH).

*Methyl 3,4,6-tri-*O*-benzoyl-2-*O*-methyl-α-*D*-mannopyranoside* (**14a**).—^{1}H NMR ($CDCl_3$): δ 3.50 (s, 6 H, 2 OMe), 3.89 (br d, *J* 2.1 Hz, 1 H, H-2), 4.30 (ddd, *J* 3.4, 6.0, 9.7 Hz, 1 H, H-5), 4.48 (dd, *J* 5.7, 12.0 Hz, 1 H, H-6), 4.58 (dd, *J* 3.0, 12.0 Hz, 1 H, H-6′), 4.91 (br s, 1 H, H-1), 5.66 (dd, *J* 3.1, 10.0 Hz, 1 H, H-3), 5.92 (app. t, *J* 10.1 Hz, 1 H, H-4), 7.34, 7.37, 7.39 (3 app. t, *J* 8.0 Hz, 6 H, *m*-ArH), 7.46–7.54 (m, 3 H, *p*-ArH), 7.94, 7.99, 8.02 (3 d, *J* 7.8 Hz, 6 H, *o*-ArH).

*Methyl 2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranoside* (**15a**).—Compound **15a** was synthesized independently from methyl α-D-mannopyranoside (300 mg) using benzoyl chloride (1 mL, 1.2 equiv per OH) and pyridine (3 mL, 3 equiv per equiv of benzoyl chloride). After 1.5 h, the reaction was quenched with water, then $CHCl_3$ was added. The organic layer was extracted twice each with 2 M H_2SO_4, satd aq $NaHCO_3$, and water, dried over anhyd Na_2SO_4, and evaporated to a crystalline solid (837 mg, 89%). ^{1}H NMR ($CDCl_3$): δ 3.56 (s, 3 H, OMe), 4.42 (ddd, *J* 2.4, 4.4, 9.9 Hz, 1 H, H-5), 4.50 (dd, *J* 4.4, 12.1 Hz, 1 H, H-6), 4.72 (dd, *J* 2.4, 12.1 Hz, 1 H, H-6′), 5.01 (d, *J* 1.7 Hz, 1 H, H-1), 5.70 (dd, *J* 1.8, 3.3 Hz, 1 H, H-2), 5.92 (dd, *J* 3.3, 10.1 Hz, 1 H, H-3), 6.12 (app. t, *J* 9.9 Hz, 1 H, H-4), 7.2–8.2 (complex, 20 H, Ar–H).

Acknowledgements

This investigation was supported by Grant GM34710, awarded by the Department of Health and Human Services.

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