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Rapid synthesis of partially O-methylated alditol acetate standards for GC–MS: some relative activities of hydroxyl groups of methyl glycopyranosides on Purdie methylation

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Abstract—Mixtures containing the majority of partially O-methylated alditol acetates (PMAAs), necessary for the GC–MS based identification of glycosidic linkages in oligo- and polymeric structures were prepared. Rha, Fuc, Rib, Ara, Xyl, Man, Gal, and Glc were converted to their Me glycosides, and the products were progressively O-methylated using the Purdie reagent at 25 °C. Resulting PMGs were assayed by TLC and at times that were optimum for formation of mono-*O*-methyl derivatives and later for higher degrees of methylation; they were converted to PMAAs, in a process incorporating NaB^2H_4 reduction. The majority of these can be used as standards for simultaneous identification of pyranosides and some furanosyl units particularly in heteropolysaccharides. The relative reactivities of OH-groups were determined by GC–MS as: Me α- and β-Glc*p*, HO-2 > HO-4 > HO-3 > HO-6, Me α- and β-Gal*p*, HO-3 > HO-2 > HO-4 > HO-6, Me α-Man*p*, HO-3 > HO-2 > HO-4 > HO-6, Me β-Man*p*, HO-3 > HO-4 ⩾ HO-6 > HO-2, Me α-Rha*p*, OH-3 > OH-2 > OH-4; Me αβ-Fuc*p*, OH-2 > OH-3 > OH-4, and Me αβ-Xyl*p*, OH-2 > OH-4 > OH-3. The results differ from those obtained with Haworth, Hakomori, and Ciucanu methylation techniques, although some similarities occurred with the more rapid Kuhn method.

Keywords: Partially O-methylated alditol acetates; GC–MS standards; Purdie methylation; OH reactivity

1. Introduction

Methylation analysis, in conjunction with NMR determination, is the most utilized method for determination of carbohydrate structure, providing the structure of monosaccharide units in oligo-, and polysaccharides and in glycoconjugates. Several methods have been described to give fully methylated carbohydrates, such as those of Haworth,[1] Kuhn et al.,[2] Hakomori,[3] and the most used frequently nowadays being that of Ciucanu and Kerek.[4]

These methods are sometimes used sequentially to finally form fully O-methylated products that can be converted to partially O-methylated alditol acetates (PMAAs) via successive hydrolysis, reduction with sodium borohydride, and acetylation, and which can be identified by GC–MS using their characteristic GC retention times and EIMS fingerprints (the method is extremely sensitive, requiring ⩾50 μg samples).[5] This method has been refined by Carpita and Shea,[6] who used sodium borodeuteride in the reduction step and avoided the problem of formation of identical, symmetrical derivatives from different partially O-methylated aldoses (example: 2,3-Me_2xylitol ≡ 3,4-Me_2xylitol). However, for the analysis of polysaccharides with more than one monosaccharide component, it is necessary to compare the retention times of the PMAAs. Such syntheses can be time consuming, if done individually, so we have now carried out partial O-methylation of methyl glycosides to simultaneously provide a wide range of products (PMGs), whose degree of methylation was assayed by TLC, and whose structure was

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confirmed by GC–MS examination of derived PMAAs. The graded methylation method of choice was that of Purdie (Ag_2O–MeI).[7] That method was selected as it is relatively slow and easier to handle than that of Doares et al.[8] and it provides a wide spectrum of substitution, as shown by Gagnaire and Odier.[9] They found that Me β-Glc*p* had relative reactivities of HO-2 > HO-6 > HO-3 > HO-4, compared with the slow Haworth method, which provided a large excess of 6-*O*- and very little 3-*O*-substitution, in the order HO-6 > HO-2 > HO-4 > HO-3.[10] The Purdie methylation appears analogous to that of Kuhn et al.,[2] which is more rapid however, having DMF as an additional component. In the case of Me α-Man*p*, the relative activities were HO-2 > HO-3 > HO-4 ⩾ HO-6.[11] We have now prepared partially PMAAs, starting from methyl glycosides of Glc, Man, Gal, Ara, Xyl, Fuc, and Rha, and have identified them by EIMS profiles and retention times.[5,6]

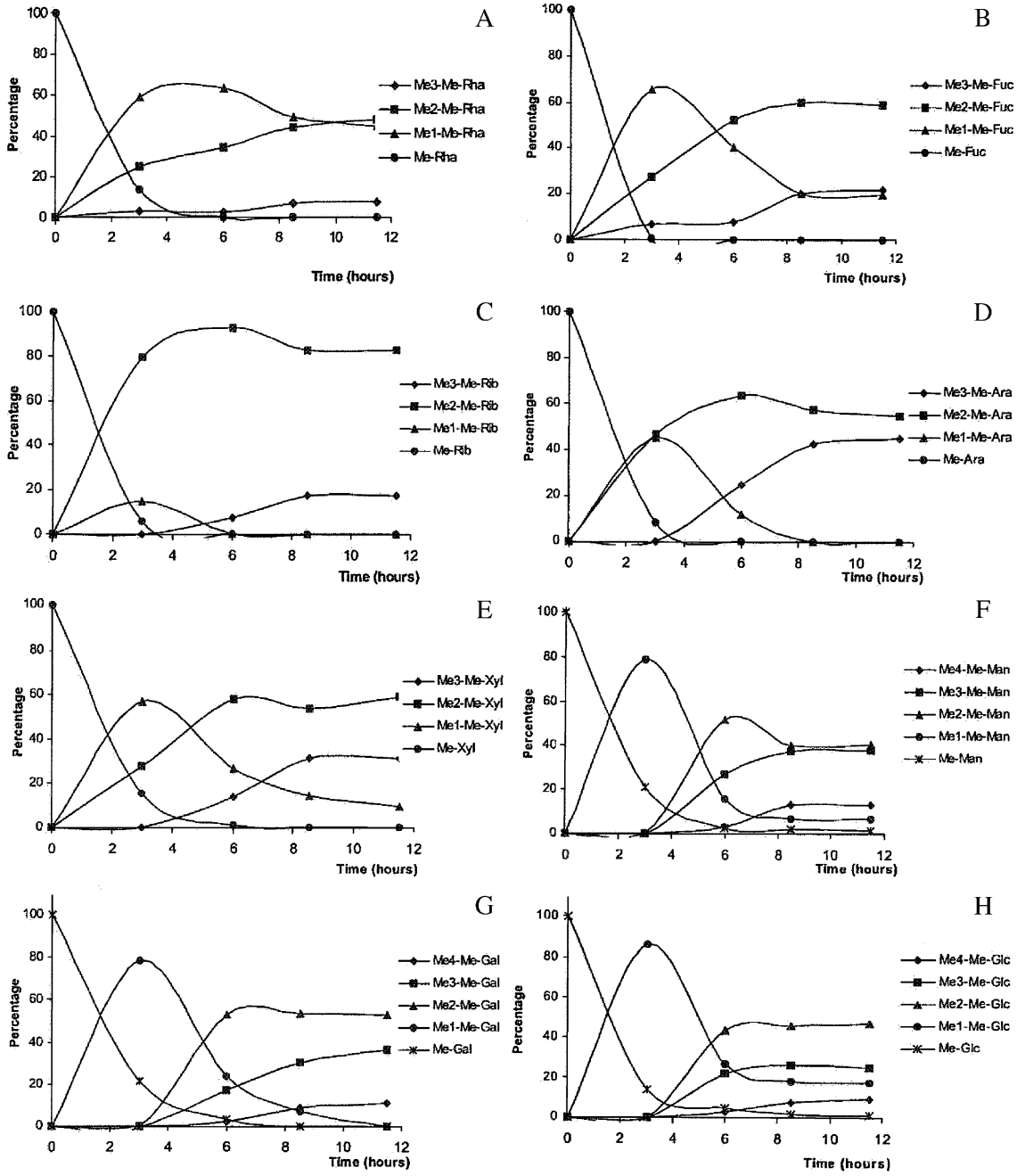

Figure 1. Progressive formation of PMGs on Purdie methylation of glycosides at 3 h intervals up to 12 h: Me Rha (A), Me Fuc (B), Me Ara (C), Me Xyl (D), Me Man (E), Me Gal (F), and Me Glc (G).

2. Experimental

2.1. Acquisition and preparation of methyl glycosides

Gal, Glc, Man, Fuc, Rha, Rib, Ara, Xyl, Me α-Glc*p*, Me β-Glc*p*, Me α-Gal*p*, Me β-Gal*p*, Me α-Man*p*, and Me β-Man*p* were obtained from Sigma–Aldrich (MO, USA).

Methyl glycosidation of Gal, Glc, Man, Rha, Fuc, Rib, Ara, and Xyl (10 mg each) was carried out by refluxing in 3% MeOH–HCl (1.0 mL) for 2 h, followed by neutralization ($AgCO_3$), filtration, and evaporation. In the case of Rha and Man, the α-pyranosides were formed almost exclusively.

2.2. Purdie methylation of methyl glycosides

The glycosides were submitted to shaking in CH_3I (1.5 mL), containing Ag_2O (250 mg) at 25 °C over a period of 12 h. They gradually dissolved in the suspension, and the degree of methylation was monitored at 3 h intervals using aliquots, which were removed on decantation, and resulting PMGs were detected by TLC and converted to PMAAs for GC–MS examination.

2.3. TLC determination of degree of O-methylation

TLC of a portion (0.2-mL aliquot) of each PMG mixture was performed on silica gel plates (10 × 7 cm; solvent: 4:1 *n*-PrOH–H_2O) for 15 min, which were developed using orcinol–H_2SO_4 spray at 100 °C for 5 min.[12] Quantification was carried out at 3 h intervals using TLC (solvent: 9:1 $CHCl_3$–EtOH; spray: as above). Products with increasing degrees of O-methylation were examined using the Scion Image Program (Scion Corporation, Maryland, USA), based on perception of the image of each spot, after obtaining a slope of displacement by intensity. A relation between the area under the peaks of intensity and their perceived concentration was obtained. The graphics were prepared using Microcal Origin v. 5.0 software.

2.4. Preparation of PMAAs and GC–MS examination

Another 0.2 mL aliquot of the PMG mixture was evaporated, and the residue was hydrolyzed with M H_2SO_4 for 8 h at 100 °C. The solution was neutralized ($BaCO_3$), and the mixture containing partially O-methylated aldoses was reduced with NaB^2H_4 (5 mg) for 4 h at room temperature. The solution was neutralized with 50 μL glacial HOAc, dried under reduced pressure, and co-distilled with 100 μL of MeOH at 50 °C. This step was repeated thrice. The product was acetylated with 1:1 Ac_2O–pyridine (500 μL), overnight at room temperature. The PMAAs were extracted with $CHCl_3$ and washed with 2% aq $CuSO_4$ solution, and the organic layer containing PMAAs was dried at room temperature, and the residue was dissolved in acetone before GC–MS analysis.

Table 1. Partially O-methylated alditol acetates obtained in syntheses following Purdie methylation for 12 h of methyl glycosides of Rha, Fuc, Rib, Ara, Xyl, Gal, Glc, and Man[a]

OMe-Alditol acetates[b]	t_R[b]	%[c]	OMe-Alditol acetates	t_R	%	OMe-Alditol acetates	t_R	%	OMe-Alditol acetates	t_R	%
2,3,5-Me_3-Rha	7.9	8.5	2,3,5-Me_3-Ara	7.8	11.6	2,3,6,-Me_3-Man	13.5	35.1	2,4-Me_2-Gal	22.3	1.2
3,4-Me_2-Rha	9.7	16.1	2,3,4-Me_3-Ara	8.4	30.0	2,3,4,-Me_3-Man	14.2	5.2	2,3-Me_2-Gal	21.2	2.8
2,3-Me_2-Rha	9.9	42.0	3,5-Me_2-Ara	9.4	1.1	4,6-Me_2-Man	16.5	tr	3,4-Me_2-Gal	23.0	0.8
2-Me-Rha	11.7	3.8	2,5-Me_2-Ara	9.7	7.6	2,6-Me_2-Man	16.6	tr	2-Me-Gal	25.8	tr
4-Me-Rha	12.1	2.2	2,3-Me_2-Ara	10.5	25.1	3,6-Me_2-Man	18.3	4.1	3-Me-Gal	30.4	tr
3-Me-Rha	12.6	25.6	2,4-Me_2-Ara	10.6	13.1	2,3-Me_2-Man	19.1	7.3	2,3,5,6-Me_4-Glc	9.8	2.4
2,3,5-Me_3-Fuc	8.3	5.8	3,4-Me_2-Ara	11.0	0.3	3,4-Me_2-Man	20.5	1.6	2,3,4,6-Me_4-Glc	10.1	7.3
2,3,4-Me_3-Fuc	8.5	27.9	5-Me-Ara	11.4	1.3	2,4-Me_2-Man	20.9	0.7	3,4,6-/2,3,4-Me_3-Glc[f]	13.0	16.7
2,3-Me_2-Fuc	10.3	47.9	2-Me-Ara	13.1	6.1	2-Me-Man	24.4	0.2	2,3,6,-Me_3-Glc	14.4	36.5
3,4-Me_2-Fuc	10.6	3.6	3-Me-Ara	13.7	3.1	3-/4-Me-Man	28.1	0.8	2,6-Me_2-Glc	17.6	2.8
2-Me-Fuc	11.8	10.2	2,3,5-Me_3-Xyl	8.0	1.2	2,3,5,6-Me_4-Gal	10.4	10.4	3,6-Me_2-Glc	18.5	2.3
4-Me-Fuc	12.6	tr[d]	2,3,4-Me_3-Xyl	8.5	27.9	2,3,4,6-Me_4-Gal	10.7	22.6	2,4-Me_2-Glc	20.2	5.8
3-Me-Fuc	12.9	3.7	2,5-Me_2-Xyl	10.1	2.0	2,5,6,-Me_3-Gal	13.2	9.9	2,3-Me_2-Glc	20.6	14.1
2,3,5-Me_3-Rib	7.6	17.4	2,4-Me_2-Xyl	10.5	31.6	2,4,6,-Me_3-Gal	13.7	4.9	3,4-Me_2-Glc	20.8	10.3
2,3,4-Me_3-Rib	7.7	16.8	2,3-/3,4-Me_2-Xyl	10.9	27.6	2,3,6,- Me_3-Gal	14.0	22.2	2-Me-Glc	26.2	tr
2,5-Me_2-Rib	9.5	5.7	2-/3-/4-Me-Xyl[e]	14.1	9.4	2,3,5,-Me_3-Gal	15.8	13.4	3-Me-Glc	28.2	1.7
2,4-Me_2-Rib	9.8	7.3	2,3,4,6-Me_4-Man	10.0	28.9	2,3,4-Me_2-Gal	16.1	3.9	4-Me-Glc	30.9	tr
2,3-/3,4-Me_2-Rib	9.9	42.3	3,4,6,-Me_3-Man	12.9	15.6	2,6-Me_2-Gal	17.0	6.6			
2-/3-Me-Rib	12.7	10.1	2,4,6,-Me_3-Man	13.3	0.4	3,6-Me_2-Gal	18.6	1.3			

[a] DB-225 at 215 °C.
[b] Retention time in minutes.
[c] Relative percentage.
[d] Trace.
[e] The 2-/4-mixture was resolved from the 3-derivative using DB-210.
[f] Resolved at 200 °C with DB-225.

Each PMAA product was examined by GC–MS using a Varian model 3300 gas chromatograph linked to a Finnigan ion trap, model 800R mass spectrometer. PMAA mixtures were applied to a capillary column of DB-225 (30 m × 0.25 mm i.d.). Conditions were: electron impact at 70 eV; injector temp: 250 °C; initial temp: 50 °C (1 min); temp program: 40 °C min^{-1} to 215 °C, maintained for 40 min. In order to resolve 3,4,6- and 2,3,4-Me_3Glc derivatives, the temp was reduced to 200 °C. A DB-210 column at 180 °C was used to resolve the acetates of 2-/4- and 3-Me-xylitol. Post run analysis of the partially PMAAs was performed after optimized separation.

3. Results and discussion

Ara, Xyl, Fuc, Rha, Gal, Glc, Man were each converted to mixtures of methyl glycosides with MeOH–HCl. Methylation of the products with the Purdie reagent over a period of time gave rise to methyl *O*-methyl glycosides (PMGs), whose increasing degree of methylation over a period was determined by TLC (spray: orcinol–sulfuric acid). The R_fs of the spots indicated the degree of methylation, and their areas were quantified by Scion Imaging (Fig. 1). The formation of the PMGs depended on the dissolution of the methyl glycosides in iodomethane, which was only gradual. But over a period of 12 h, those

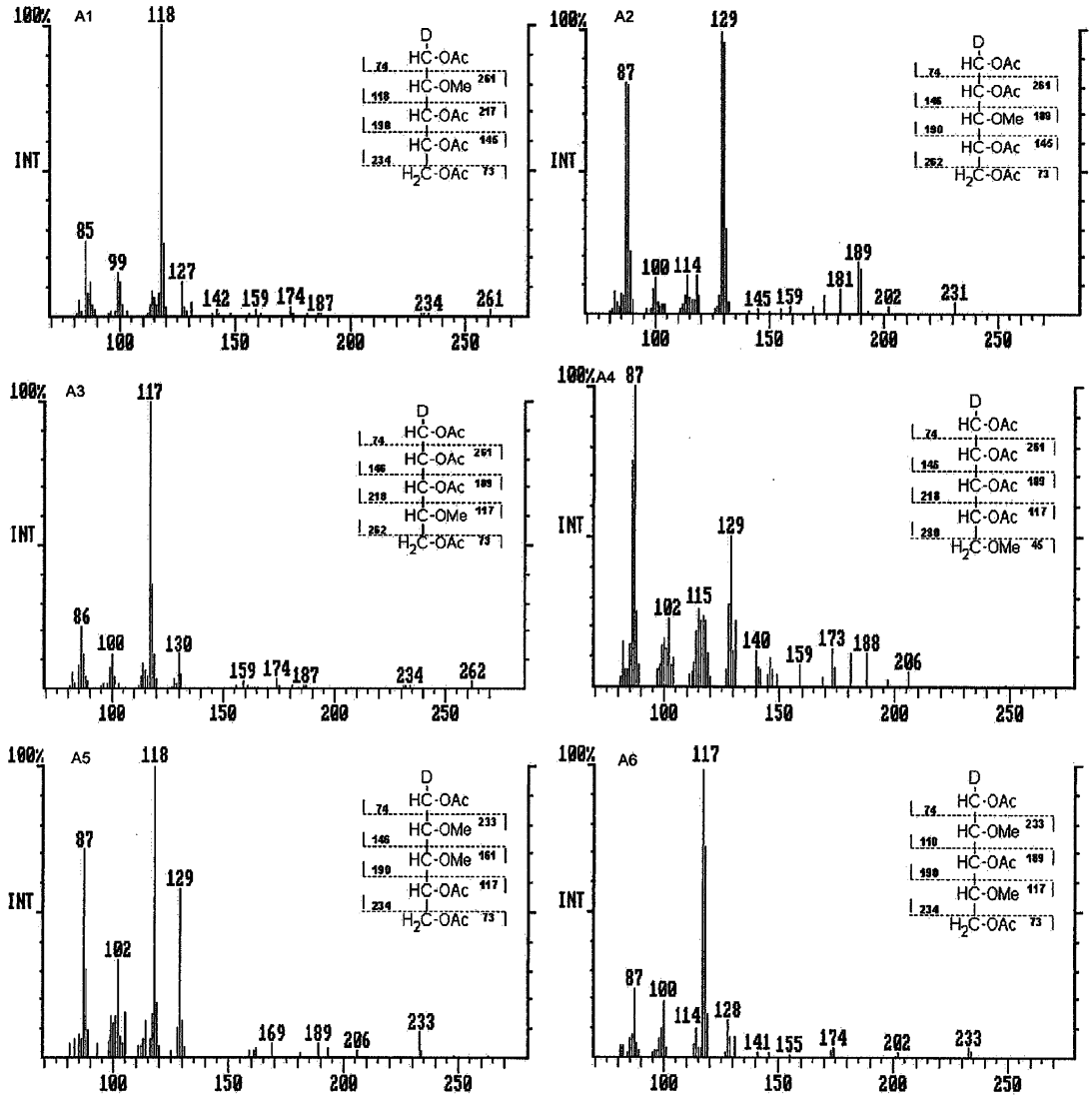

Figure 2. EIMS patterns at *m*/*z* 80 to 270 of PMAA standards, deuterated at C-1: 2-MeAra (A1), 3-MeAra (A2), 4-MeAra (A3), 5-MeAra (A4), 2,3,-Me_2Ara (A5), 2,4-Me_2Ara (A6), 2,5-Me_2Ara (A7), 3,4-/2,3-Me_2Xyl (A8), 3,5-Me_3Ara (A9), 2,3,4-Me_3-Ara (A10), 2,3,5-Me_3Ara (A11), 2-MeGal (B1), 3-MeGlc impure (B2), and 4-MeGlc (B3), 2,3-Me_2Gal (B4), 2,4-Me_2Gal (B5), 2,6-Me_2Gal (B6), 3,4-Me_2Gal (B7), 3,6-Me_2Gal (B8), 2,3,4-Me_3Gal (B9), 2,3,5-Me_3Gal (B10), 2,3,6-Me_3Gal (B11), 2,4,6-Me_3Gal (B12), 2,5,6-Me_3Gal (B13), 3,4,6-Me_2Man (B14), 2,3,4,6-Me_4Gal (B15), 2,3,5,6-Me_4Gal (B16), 2-MeFuc (C1), 3-MeFuc (C2), 4-MeFuc (C3), 2,3-Me_2Fuc (C4), 2,4-Me_2Fuc (C5), 3,4-Me_2Fuc (C6), 2,3,4-Me_3Fuc (C7), 2,3,5-Me_3Fuc (C8).

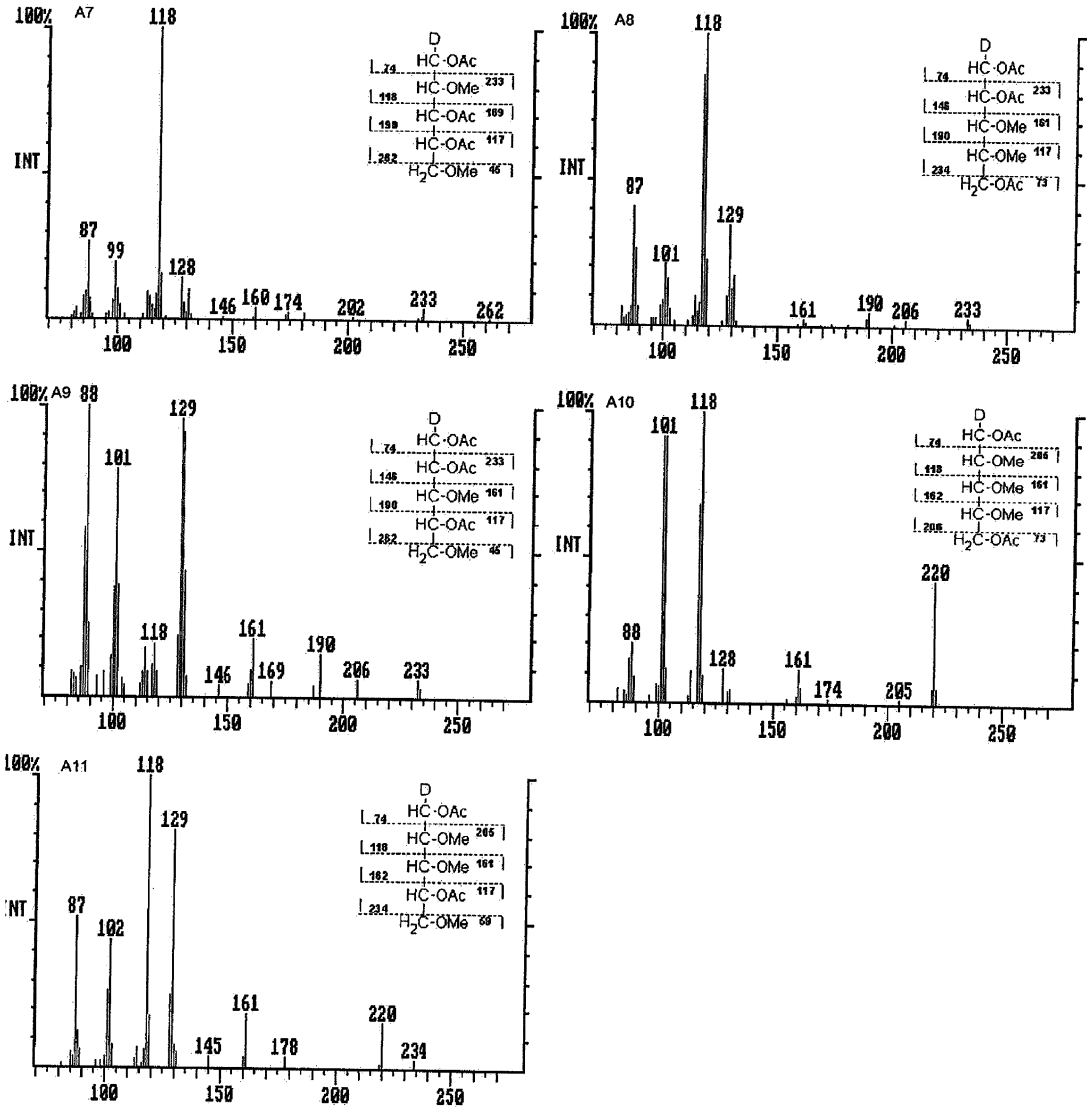

Figure 2. (*continued*)

of Fuc, Rha, and Ara dissolved completely after 4 h, whereas those of Gal, Glc, and Man took 6–8 h.

PMGs aliquots were removed from the methylation mixture after 2.5 h to obtain mainly mono-*O*-methyl derivatives and at 12 h for samples with higher degrees of methylation. Each batch was converted to PMAAs via successive hydrolysis, reduction with sodium borodeuteride, and acetylation. The resulting PMAA mixtures could then be employed as GC–MS standards in methylation analysis of polysaccharides, the majority of which contain pyranosyl, as well some containing Ara*f* and Gal*f* units (see Table 1).

The resolution of components of PMAAs of Gal, Glc, Man, Ara, Xyl, Rib, Rha and Fuc, on GC with a DB-225 capillary column at 215 °C was excellent, although it was sometimes necessary to reduce the temperature to 200 °C or to use a DB-210 column (Table 1). PMAA standards, necessary for identification of Gal*f* and Ara*f* units in oligomers and polymers, were also formed. Almost all of the PMAA structures were obtained in the rapid syntheses, exceptions being those of 4-MeAra, 4-MeGal, 2,4-Fuc, and 2,4-Rha, 2,5- and 3,5-Me_2Gal, and 3,5,6-Me_3Gal and 5-or 6-Me-Hex. The EIMS spectra for each of the neutral monosaccharide classes are represented in Figure 2.

As a corollary to the Purdie methylation of methyl glycosides to give PMGs, some conclusions can be reached concerning the relative reactivities of their hydroxyl groups. The most relevant results were obtained by mono-methylation, since further methylation rates could be increased when a *vic*-methoxyl group is present. However, some conclusions on the relative reactivity order of each hydroxyl group can be reached (Table 2). We found that in the case of both Me α- and β-Glc*p*,

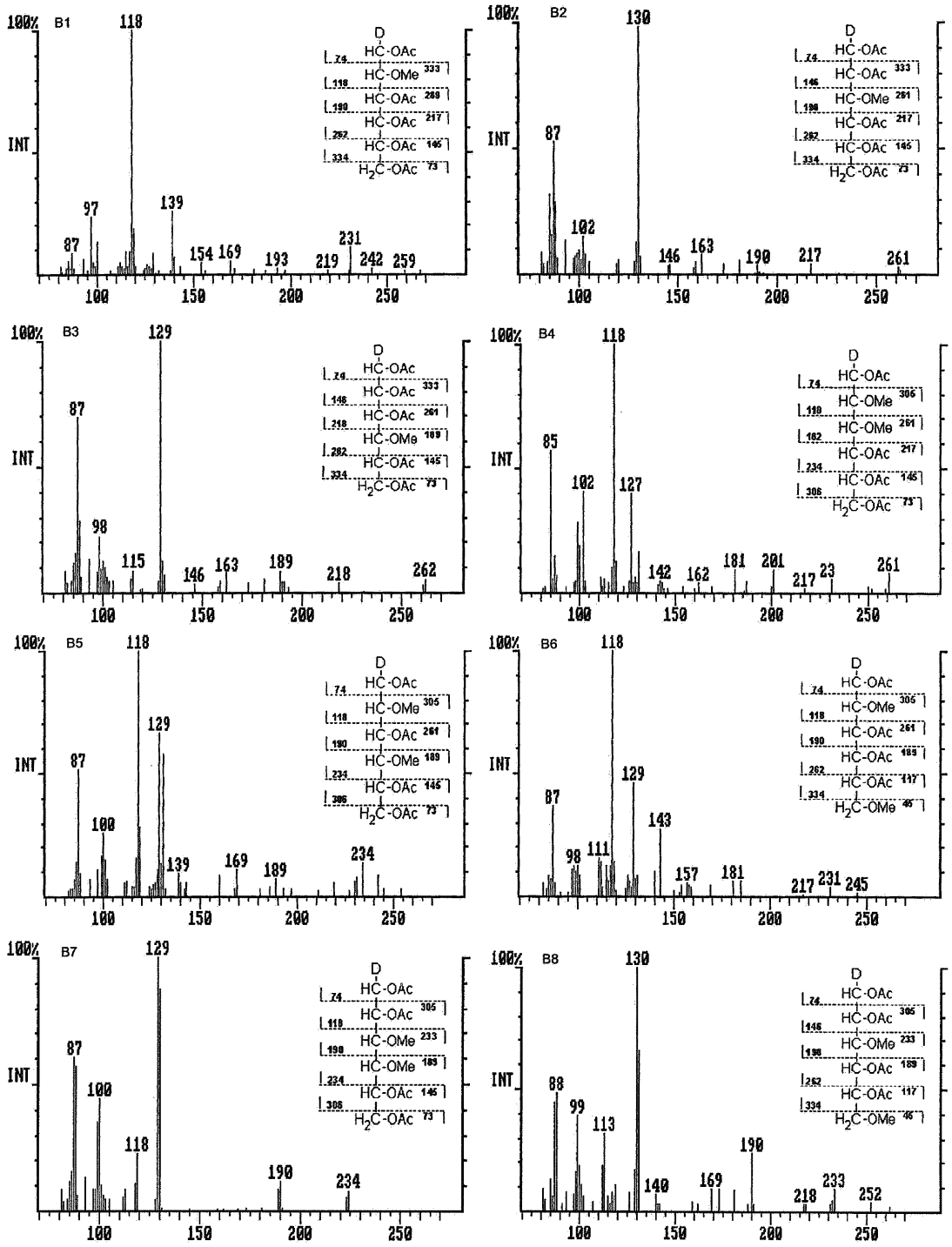

Figure 2. (*continued*)

the rate was similar with an order of HO-2 > HO-4 > HO-3 > HO-6, and that for Me α- and β-Gal*p* are identical in the order HO-3 > HO-2 > HO-4 > HO-6. The two anomeric forms of Me Man*p* were of interest since, after 3 h, Me β-Man*p* gave rise to 54% Me_3Man*p* and 4% Me Me_4-Man*p* derivatives, whereas the products from the α anomer were formed slowest of all, with significant amounts of Me_1-Man*p* (76%) and 24% of 2,3-Me_2-Man*p*. There were also significant differences in the order of reactivity, as it was observed for the β anomer that HO-3 > HO-4 ⩾ HO-6 > HO-2 and for the α anomer that HO-3 > HO-2 > HO-4 > HO-6. As previously observed for a Kuhn methylation of Me α-Man*p*, the order was not greatly different, with

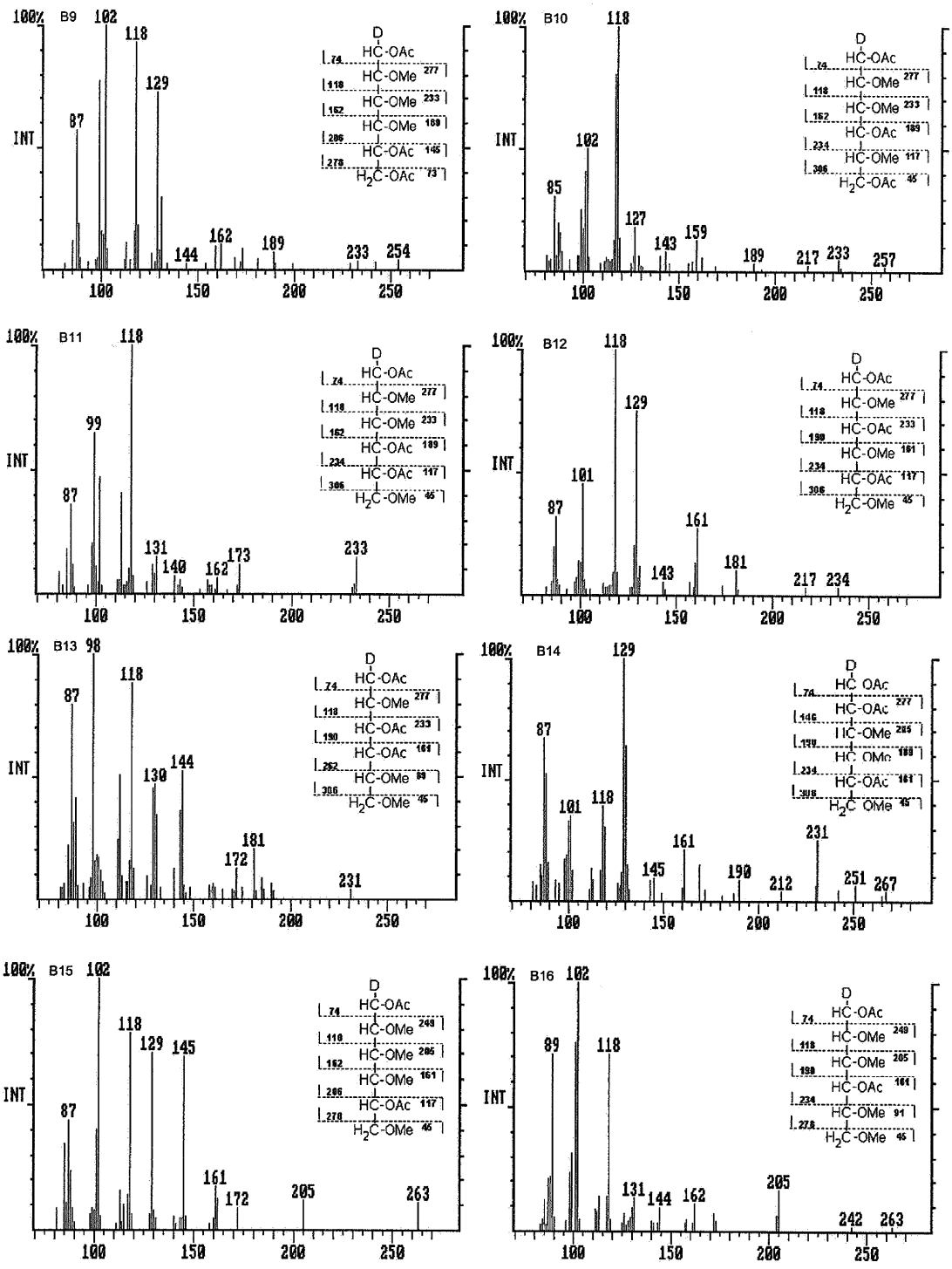

Figure 2. (*continued*)

HO-2 ⩾ HO-3 > HO-4 ⩾ HO-6.[11] For the structurally related Me α-Rha*p*, the most reactive group was HO-3, with an order of OH-3 > OH-2 > OH-4.

Other methyl glycosides that were methylated by the Purdie reagent contained both α- and β-pyranosides and sometimes furanosides. The mixture of Me αβ-Fuc*p* had the reactivity sequence HO-3 > HO-2 > HO-4, similar to that of Me α- and Me β-Gal*p*. The order of Me αβ-Xyl*p* was HO-2 > HO-4 > HO-3, which is identical to that observed by Ovodov and Evtushenko for Me β-Xyl*p*.[13] The methyl glycosides derived from Ara and

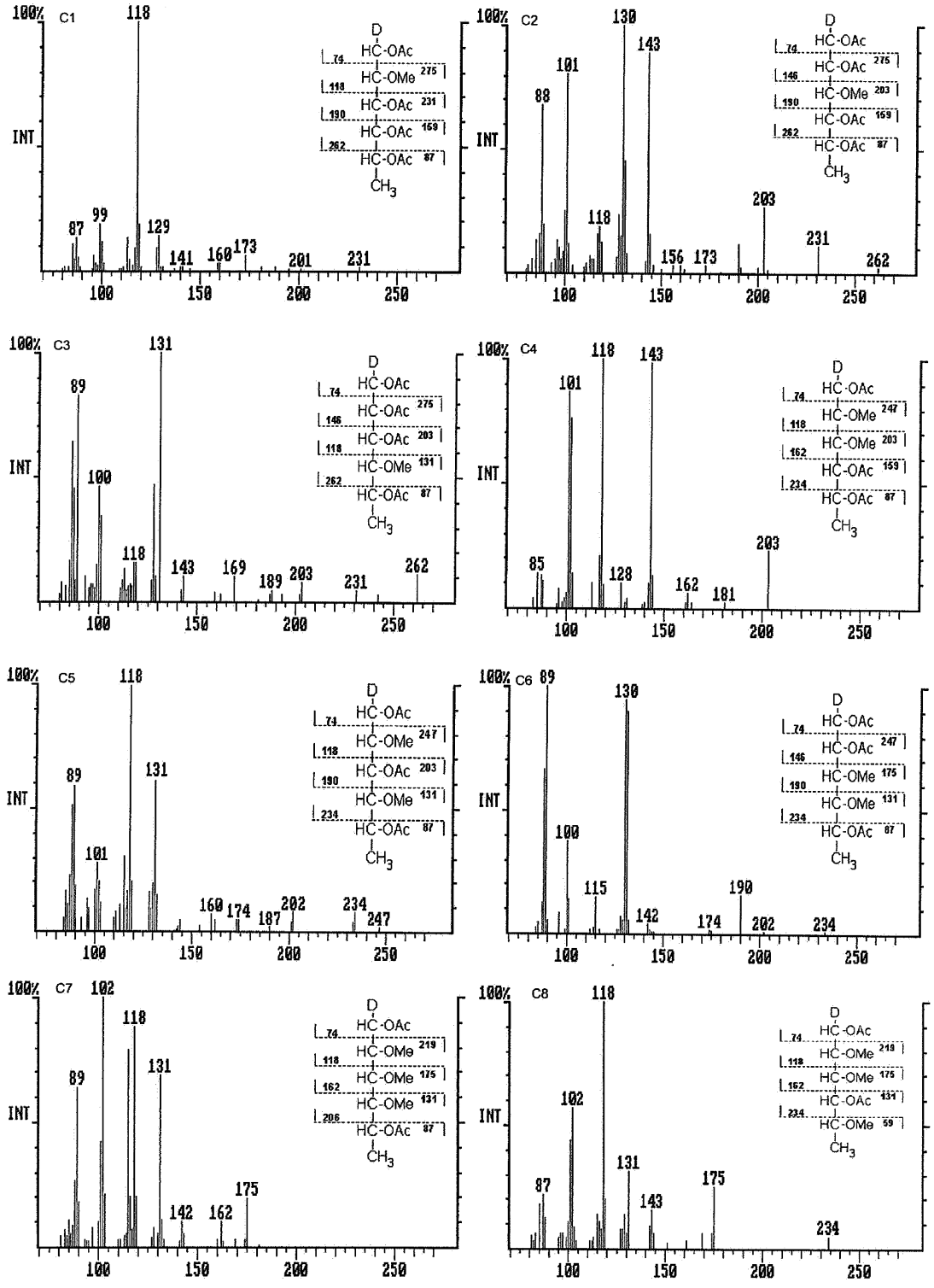

Figure 2. (*continued*)

Rib contained both furanosides and pyranosides, so that the order of reactivity was difficult to assess. However, in both systems, the HO-2 was the most reactive.

Thus in practical terms, for methylation analysis of carbohydrate-containing oligomers and polymers, partially O-methylated alditol acetate standards (PMAAs) have been simultaneously prepared for GC–MS in rapid syntheses, after determination of the degree of Purdie methylation of methyl glycoside precursors by TLC.

Table 2. Determination of the relative reactivities of the hydroxyl groups after 2.5 h of methylation and the influence of the anomeric configuration on the PMAA formation using Purdie methylation

OMe-Alditol acetate	α	β	αβ[a]	OMe-Alditol acetate	α	β
2,3,4-Me_3Rha*p*	6.3	—	—	3,6-Me_2Man*p*	—	11.7
3,4-Me_2Rha*p*	7.0	—	—	2,3-Me_2Man*p*	24.0	7.1
2,3-Me_2Rha*p*	65.0	—	—	3,4-Me_2Man*p*	—	7.9
2-Me-Rha*p*	3.2	—	—	2,4-Me_2Man*p*	—	2.6
4-Me-Rha*p*	1.1	—	—	2-MeMan*p*	27.3	0.5
3-Me-Rha*p*	16.6	—	—	3-/4-MeMan*p*	48.7	1.0
2,3,4-Me_3Fuc*p*	—	—	22.2	2,3,4,6-Me_4Gal*p*	0.9	1.1
2,3-Me_2Fuc*p*	—	—	44.1	2,4,6-Me_3Gal*p*	1.4	—
3,4-Me_2Fuc*p*	—	—	8.0	2,3,4-Me_3Gal*p*	—	3.3
2-MeFuc*p*	—	—	12.3	2,3,6-Me_3Gal*p*	6.2	6.2
3-MeFuc*p*	—	—	15.2	2,6-Me_2Gal*p*	8.0	8.6
2,3,4-Me_3Rib*fp*	—	—	8.2	4,6-Me_2Gal*p*	1.5	1.4
2,4-Me_2Rib*fp*	—	—	1.8	3,6-Me_2Gal*p*	3.9	3.9
2,3-Me_2Rib*fp*	—	—	66.0	2,4-Me_2Gal*p*	4.0	3.7
2-/4-MeRib*fp*	—	—	24.0	2,3-Me_2Gal*p*	8.5	8.0
2,3,5-Me_3Ara*fp*	—	—	0.3	3,4-Me_2Gal*p*	11.4	11.0
2,3,4-Me_3Ara*fp*	—	—	50.1	2-MeGal*p*	11.0	11.2
2,3-Me_2Ara*fp*	—	—	36.9	3-MeGal*p*	19.4	18.7
2,4-Me_2Ara*fp*	—	—	4.5	3,4,6-Me_3Glc*p*	5.4	6.7
2-MeAra*fp*	—	—	6.3	2,3,6-Me_3Glc*p*	4.1	6.1
4-MeAra*fp*	—	—	0.2	4,6-Me_2Glc*p*	1.8	1.3
3-MeAra*fp*	—	—	1.5	2,6-Me_2Glc*p*	3.2	4.9
2,3,4-Me_3Xyl*p*	—	—	18.8	2,4-Me_2Glc*p*	3.2	1.2
2,4-Me_2Xyl*p*	—	—	27.4	2,3-Me_2Glc*p*	4.7	10.2
2,3-MeXyl*p*	—	—	53.6	3,4-Me_2Glc*p*	22.4	39.5
2-/3-/4-MeXyl*p*[b]	—	—	0.1	6-MeGlc*p*	0.1	1.6
2,3,4,6-Me_4Man*p*	—	3.9	—	2-MeGlc*p*	23.1	12.1
3,4,6-Me_3Man*p*	—	23.7	—	3-MeGlc*p*	11.7	7.9
2,4,6-Me_3Man*p*	—	9.5	—	4-MeGlc*p*	20.1	8.3
2,3,6-Me_3Man*p*	—	21.7	—	—	—	—
4,6-Me_2Man*p*	—	10.4	—			

[a] Mixture of αβ anomers obtained on methanolysis.
[b] The 2-/3- were resolved from the 3-derivative using DB-210.

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