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Anomalous Zemplén deacylation reactions of α- and β-D-mannopyranoside derivatives

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Dedicated to Professor András Lipták on the occasion of his 65th birthday

Abstract

Reaction of mono-, di-, and trisaccharide derivatives of methyl β-D- and octyl β-D-mannopyranosides bearing ester groups at isolated and non-isolated positions on the same molecule, under Zemplén conditions (catalytic amount of sodium methoxide in methanol) gave partially deacylated compounds, in which the *O*-acyl groups were retained at isolated sites. In the case of one disaccharide, all the benzoyl groups remained intact at the reducing end, while all the acetyl functions were removable from the nonreducing end. In another case, both isolated ester groups at positions 2 and 4 were retained at the reducing end. The isolated 2-*O*-acyl groups on methyl α-D-mannopyranoside compounds were more labile than on the corresponding β-mannosides under the same conditions. The mechanism of the reaction may be different for ester groups at isolated or non-isolated positions. In the latter case, acyl migration may take place and carry acyl groups into a less hindered position.

Keywords: Anomalous Zemplén deacylation; Temporary ester groups; Mannosides

1. Introduction

Zemplén deacylation[1] is one of the commonly used deblocking reactions in carbohydrate chemistry. Using this transesterification reaction, OH-functions can be regenerated under mild conditions, in methanol with a catalytic amount of sodium methoxide at room temperature. In most cases the reaction goes smoothly and, after the very simple workup procedure, the expected product can be obtained in good yield. However, during the synthesis of α-L-rhamnopyranosyl-(1→3)-D-galactose[2] it was found that under Zemplén conditions, benzyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside yielded a disaccharide in which the 2-*O*-benzoyl group was retained. There were only sporadic data[3–5] on this type of reaction called anomalous deacylation but later on it turned out to be more general and was observed among gluco derivatives, too.[6,7] Thus, in galacto and gluco compounds, acyl groups could not be removed from position 2 with a catalytic amount of sodium methoxide when an alkyl or glycosyl substituent was present at position 3. Bacterial oligosaccharides were prepared by this anomalous reaction and in special cases acyl groups were shown to serve as temporary

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protecting groups.[8,9] It is worth noting that a similar finding has recently been reported for deoxy compounds bearing acyl groups at isolated positions.[10] Among mannosides, the first observation had been made[11] with octyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-β-D-mannopyranoside (**1**) prepared according to the 'ulosyl bromide' approach.[12] Thus, on Zemplén deacylation of **1**, BzO-4,6 were removed but not BzO-2, and octyl 2-*O*-benzoyl-3-*O*-benzyl-β-D-mannopyranoside (**2**) was obtained in good yield.[11] Octyl 3,6-di-*O*-α-D-mannopyranosyl-β-D-mannopyranoside, a trisaccharide substrate of glycosyltransferases was successfully synthesised[11] using **2** as the key compound. We now report the anomalous Zemplén deacylation reactions of some other α- and β-D-mannopyranoside derivatives.

2. Results and discussion

The chemical synthesis of glycan chains of oligomannose type *N*-glycoproteins is just one of the ongoing research programmes in our laboratories.[11,13–17] After the preparation of a series of protected mannose derivatives, the investigation of the anomalous Zemplén deacylation reactions[2,6–9] was continued among methyl α-D-, methyl β-D-, and octyl β-D-mannopyranoside compounds.

Similar results have now been obtained with a methyl β-D- and another octyl β-D-mannopyranoside derivative as in the case of compound **1**. Mild acidic hydrolysis and subsequent acetylation of methyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D- (**3**)[18] and octyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-mannopyranoside (**4**)[19] gave the triacetates **5** and **6**, which, under Zemplén conditions, yielded exclusively the monoacetates **7** and **8**, respectively. In contrast, the α-mannoside derivative **10**, prepared from methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**9**)[20] gave a mixture of the monoacetate **11** and the fully deacylated product **12** (Fig. 1). The presence of the acetyl groups in monoacetates **7**, **8** and **11** were indicated by ^{1}H NMR, ^{13}C NMR, and MS data.

During the synthesis of mannobioses **14**[14] and **16**,[11] **13** gave easily **14** under Zemplén conditions but **15** having an isolated benzoyl function could only be converted into **16** with an equimolar amount of sodium methoxide in methanol at the boiling point.[11] In the present study, when deacylation was performed at room temperature with catalytic amount of the reagent, surprisingly, all the benzoates remained at the β-mannosidic unit yielding compound **17** (Fig. 2).

Zemplén reaction of disaccharide **18**, synthesised from **2** by selective mannosylation,[11] surprisingly gave **19** as the only product, in which not only the BzO-2 but the AcO-4 group was retained, as well. This experiment suggests that, for an anomalous reaction to occur, the isolated nature of the ester function is more important than its proximity to the steric relationship to the anomeric centre. This suggestion was verified by the fact that methyl 2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (**20**)[13] was stable for hours, under the usual reaction conditions. Moreover, a model disaccharide **22** was prepared from **21**[17] by conventional acetylation, and then converted into the 3-acetate **23** with a catalytic amount of sodium methoxide. In contrast, in the case of the methyl α-mannobioside **24**[17] the isolated acetyl group was not so

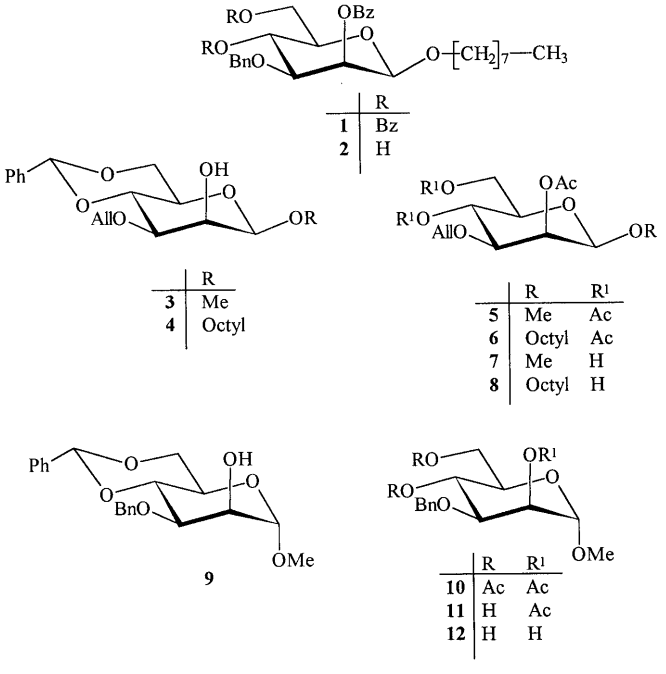

Fig. 1. Syntheses and deacylation reactions of mannose derivatives.

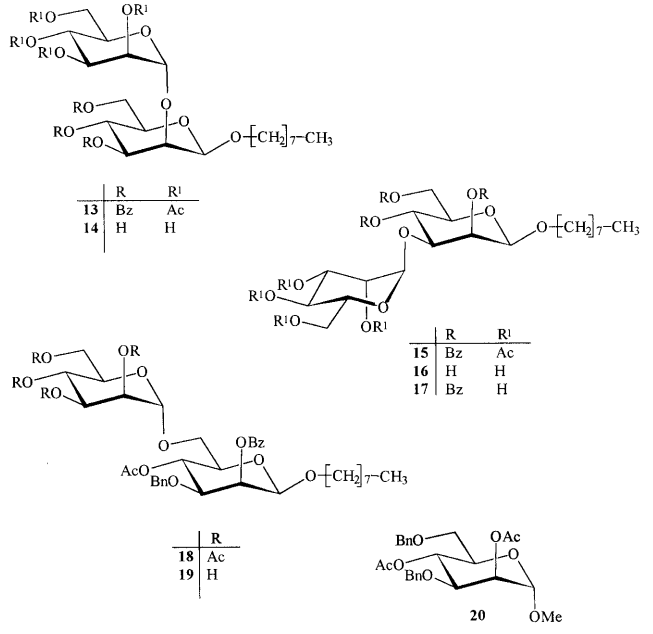

Fig. 2. Deacylation of mannose derivatives bearing ester groups at located and non-isolated positions.

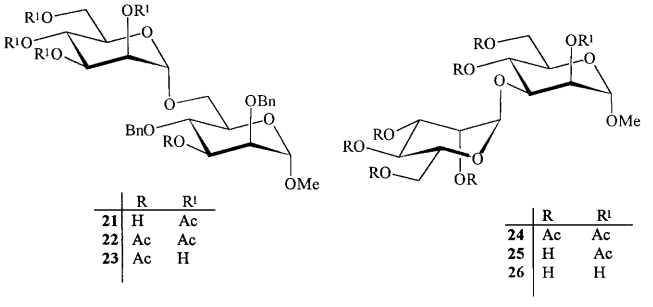

Fig. 3. Zemplén deacylations of mannobioses.

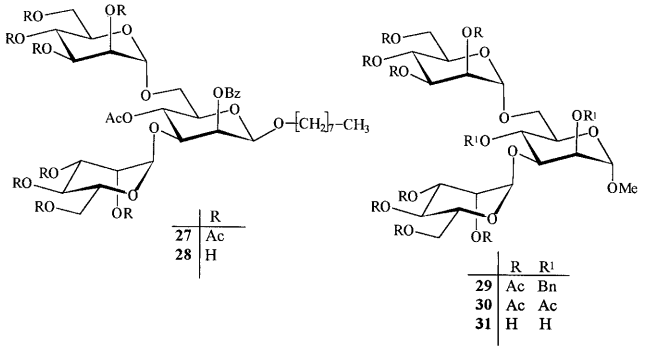

Fig. 4. Zemplén deacylations of mannotrioses.

stable in methanol in the presence of sodium methoxide and a mixture of methyl α-D-mannopyranosyl - (1 → 3) - 2 - *O* - acetyl - α - D-mannopyranoside (**25**) and the fully deprotected disaccharide (**26**) was obtained after 30 min at room temperature (Fig. 3).

Finally, reactions of trisaccharides **27** and **30** were investigated. In the light of the results mentioned above, not surprisingly, in the case of the β-octyl compound **27**[11] after 45 min the diacyl derivative **28** was successfully isolated. As it was indicated by FABMS data, the α-methyl derivative **30** prepared from **29**[17] by standard reactions, gave a complex mixture after 45 min. The sample contained traces of **31** and its triacetate and the corresponding mono- and diacetates as the main components. Within 2 h, the fully deprotected **31** was the main product. The minor component was not homogenous; ^{1}H NMR showed the presence of the 2-acetate and the 4-acetate of **31** in almost equal amounts (Fig. 4).

The accepted mechanism of Zemplén deacylation is that methylate anion makes a nucleophilic attack on the carbonyl carbon atom and forms a methyl ester. Then, the 'alcoholate' anion abstracts a proton from methanol through which process the methylate anion is regenerated. The nucleophilic attack may be unfavourable in a fully protected derivative because of steric hindrance. Additionally, the lone electron pairs of oxygen atoms, including the 'ring oxygen' may give rise to a 'stereoelectronic hindrance' discouraging attack by the negatively charged methylate anion. Thus, the reaction rate of removing ester groups from isolated positions can be very low. The available data strongly suggest that, in the case of non-isolated ester groups (e.g., 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl moiety), the mechanism of the deacylation reaction may be different. The nucleophilic attack takes place at the less hindered carbonyl carbon atom. Then, the 'alcoholate' anion, by an intramolecular nucleophilic attack against the next-door-neighbour's carbonyl carbon, may cause acyl migration. Thus, by the possible acyl migrations the ester groups may have less hindered position for the nucleophilic attack.[21] Consequently, the reaction rate of the deacylation reaction would be higher than the one for the isolated ester functions. The significant difference between the deacylation reactions of α- and β-mannosides can be explained by the configuration of C-1. In β-mannosides, the

molecule is more crowded around the carbonyl carbon of the C-2-*O*-acyl groups. Therefore, under Zemplén conditions ester functions at C-2 are more stable in the β anomers than those in the α anomers.

In summary, Zemplén deacylation reactions of mannose derivatives were investigated bearing ester groups at both isolated and non-isolated positions. The obtained results showed that isolated acyl groups in β-D-mannopyranoside derivatives were stable under Zemplén conditions. Some lability was observed for α-mannosides. This fact should be very important during the final step in the syntheses of oligosaccharides. Moreover, in certain compounds, ester groups can be used for temporary protection.

3. Experimental

General.—Melting points (uncorrected) were determined on a Kofler hot-stage apparatus. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. NMR spectra were recorded with a Bruker WP-200 SY spectrometer. The reactions were monitored by TLC on Kieselgel 60 F_{254} (E. Merck, Darmstadt) with detection by charring with H_2SO_4. Kieselgel 60 (E. Merck) was used for short-column chromatography. Fast-atom bombardment (FAB) mass spectrometric measurements have been performed using a VG-ZAB-2SEQ reverse geometry instrument operating at an accelerating voltage of 8 kV. A cesium ion gun producing 30 keV ions was utilised for ion bombardment. Glycerol was used as the FAB matrix. The electrospray (ESI) mass spectrometric measurements have been performed on PE SCIEX API 2000 triple quadrupole mass spectrometer (PE SCIEX, Toronto, Canada). The solvent applied was a 1:1 mixture of water–MeOH with 0.1% AcOH. The MALDI-TOF measurements were carried out with a Bruker Biflex III mass spectrometer, equipped with a 337 nm nitrogen laser. The instrument was used in reflectron mode at 19.0 kV voltage. 2,5-Dihydroxybenzoic acid was used as matrix and 100–200 laser shots were applied for each spectrum.

General description for Zemplén deacylation reactions.—To a soln of the model compound (1 mmol) in dry MeOH (10 mL) was added NaOMe (0.15 mmol). The mixture was kept at rt for the required time (the reaction was monitored by TLC), then neutralised with Amberlite IR 120 (H^+) resin, filtered, and concd. The residue was purified by short-column chromatography to give the partially deacylated product.

Methyl 2,4,6-tri-O-*acetyl-3*-O-*allyl-β*-D-*mannopyranoside* (**5**).—A mixture of methyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-mannopyranoside (**3**; 322 mg, 1 mmol)[18] and aq 60% AcOH (6 mL) was kept at 60 °C for 30 min, then concd and co-concd with toluene (3 × 5 mL). The residue was dissolved in pyridine (4 mL), Ac_2O (4 mL) was added and the mixture was kept at rt overnight, then concd and co-concd with toluene (3 × 5 mL). Column chromatography of the residue (19:1 CH_2Cl_2–EtOAc) gave **5** (329 mg, 91%); mp 104–105 °C (from EtOH); $[\alpha]_D$ −77.2° (*c* 0.29, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.89–5.70 (m, 1 H, –CH=), 5.53 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 5.29–5.16 (m, 2 H, CH_2=), 5.17 (t, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 4.48 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 4.30 (dd, 1 H, $J_{5,6a}$ 5.5, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.19–3.87 (m, 3 H, H-6b and –OCH_2–), 3.61 (m, 1 H, H-5), 3.56 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 3.53 (s, 3 H, OMe), 2.18, 2.09 and 2.08 (3 s, each 3 H, 3 OAc); ^{13}C NMR ($CDCl_3$): δ 170.67, 170.39 and 169.49 (3 C=O), 133.86 (–CH=), 117.41 (CH_2=), 99.91 (C-1), 76.35 (C-3), 72.25 (C-5), 70.17 (–OCH_2–), 67.33 (C-2,4), 62.65 (C-6), 57.18 (OMe), 20.84 and 20.69 (3 Me). ESIMS (+): *m/z* 383.2 $[M + Na]^+$, 378.1 $[M + NH_4]^+$, 361.0 $[M + H]^+$, 329.2 $[M + H - MeOH]^+$. Anal. Calcd for $C_{16}H_{24}O_9$ (360.14): C, 53.31; H, 6.72. Found: C, 53.49; H, 6.70.

Octyl 2,4,6-tri-O-*acetyl-3*-O-*allyl-β*-D-*manno-pyranoside* (**6**).—Octyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-mannopyranoside (**4**; 210 mg, 0.5 mmol)[19] was converted into **6** as described for **5**. Column chromatography of the crude product (97:3 CH_2Cl_2–EtOAc) yielded **6** (205 mg, 89%) as a syrup; $[\alpha]_D$ −59.2° (*c* 0.32, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.86–5.72 (m, 1 H, –CH=), 5.61 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 5.30–5.16 (m, 2 H, CH_2=), 5.15 (t, 1 H, $J_{4,5}$ 9.8

Hz, H-4), 4.53 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.29 (dd, 1 H, $J_{5,6a}$ 5.7, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.17–3.81 (m, 3 H, –OCH_2–CH= and –OCH_2–CH_2–), 4.15 (dd, 1 H, $J_{5,6b}$ 2.6 Hz, H-6b), 3.59 (m, 1 H, H-5), 3.54 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 3.48 (dt, 1 H, –OCH_2–CH_2–), 2.18, 2.08 and 2.07 (3 s, each 3 H, 3 OAc), 1.61–1.26 (m, 12 H, 6 –CH_2–), 0.88 (t, 3 H, –CH_3); ^{13}C NMR ($CDCl_3$): δ 170.81, 170.51, 169.62 (3 C=O), 134.04 (CH=), 117.43 (CH_2=), 99.06 (C-1), 76.69 (C-3), 72.36 (C-5), 70.48 with double int. (2 –OCH_2–), 67.68 with double int. (C-2,4), 62.63 (C-6), 31.77, 29.67, 29.31 with double int., 25.82, 22.63 (6 –CH_2–), 20.80 with triple int. (3 Me), 14.08 (CH_2Me); ESIMS (+): m/z 481.3 $[M + Na]^+$, 476.3 $[M + NH_4]^+$, 459.2 $[M + H]^+$, 401.3 $[M + H - AllOH]^+$, 329.3 $[M + H - CH_3(CH_2)_7OH]^+$. Anal. Calcd for $C_{23}H_{38}O_9$ (458.25): C, 60.23; H, 8.36. Found: C, 60.26; H, 8.40.

*Methyl 2-*O*-acetyl-3-*O*-allyl-β-*D*-mannopyranoside* (**7**).—Compound **5** (180 mg, 0.5 mmol) was partially deacylated for 4 h. The product was purified by column chromatography (3:1 CH_2Cl_2–acetone) to give **7** (112 mg, 81%) as an amorphous solid; $[\alpha]_D$ −68.3° (*c* 2.66, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.97–5.78 (m, 1 H, –CH=), 5.50 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 5.34–5.18 (m, 2 H, CH_2=), 4.49 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 4.09 (m, 2 H, –OCH_2–), 3.53 (s, 3 H, OMe), 2.73 and 2.46 (2 bs, each 1 H, 2 OH, deuterable), 2.14 (s, 3 H, OAc); ^{13}C NMR ($CDCl_3$): δ 170.36 (C=O), 133.90 (–CH=), 118.07 (CH_2=), 100.02 (C-1), 79.07 (C-3), 75.67 (C-5), 70.20 (–OCH_2–), 67.22 (C-2), 66.72 (C-4), 62.34 (C-6), 57.23 (OMe), 20.73 (Me). ESIMS (+): m/z 299.2 $[M + Na]^+$, 294.1 $[M + NH_4]^+$, 277.0 $[M + H]^+$, 245.2 $[M + H - MeOH]^+$, 218.8 $[M + H - AllOH]^+$. Anal. Calcd for $C_{12}H_{20}O_7$ (276.12): C, 52.15; H, 7.30. Found: C, 51.98; H, 7.27.

*Octyl2-*O*-acetyl-3-*O*-allyl-β-*D*-mannopyranoside* (**8**).—Compound **6** (138 mg, 0.3 mmol) was partially deacylated for 4 h. The product was purified by column chromatography (4:1 CH_2Cl_2–acetone) to give **8** (101 mg, 90%) as an amorphous solid; $[\alpha]_D$ −79.1° (*c* 0.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.97–5.78 (m, 1 H, –CH=), 5.49 (dd, 1 H, $J_{2,3}$ 2.7 Hz, H-2), 5.35–5.19 (m, 2 H, CH_2=), 4.57 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 4.08 (m, 2 H, OCH_2–CH=), 2.87 and 2.37 (2 bs, each 1 H, 2 OH, deuterable), 2.15 (s, 3 H, OAc), 1.61–1.26 (m, 12 H, 6 –CH_2–), 0.88 (t, 3 H, –CH_3); ^{13}C NMR ($CDCl_3$): δ 170.35 (C=O), 133.88 (CH=), 118.21 (CH_2=), 99.12 (C-1), 79.39 (C-3), 75.57 (C-5), 70.32 with double int. (2 –OCH_2–), 67.37 with double int. (C-2,4), 62.82 (C-6), 31.76, 29.31 with triple int., 25.62, 22.62 (6 –CH_2–), 20.87 (Me), 14.07 (CH_2Me); ESIMS (+): m/z 397.3 $[M + Na]^+$, 392.2 $[M + NH_4]^+$, 375.1 $[M + H]^+$, 357.1 $[M + H - H_2O]^+$, 317.2 $[M + H - AllOH]^+$, 245.2 $[M + H - CH_3(CH_2)_7OH]^+$. Anal. Calcd for $C_{19}H_{34}O_7$ (374.23): C, 60.93; H, 9.16. Found: C, 61.13; H, 9.11.

*Methyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α-*D*-mannopyranoside* (**10**).—Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-mannopyranoside (**9**; 745 mg, 2 mmol)[20] was converted into **10** as described for **5**. Column chromatography of the crude product (19:1 CH_2Cl_2–EtOAc) gave **10** (755 mg, 92%) as a syrup; $[\alpha]_D$ 0° (*c* 0.65, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36–7.23 (m, 5 H, aromatic), 5.34 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 5.22 (t, 1 H, $J_{4,5}$ 10 Hz, H-4), 4.73 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.52 (ABq, 2 H, PhCH_2), 4.24 (dd, 1 H, $J_{5,6a}$ 5.5, $J_{6a,6b}$ 12 Hz, H-6a), 4.11 (dd, 1 H, $J_{5,6b}$ 2.2 Hz, H-6b), 3.83 (m, 2 H, H-3,5), 3.37 (s, 3 H, OMe), 2.15, 2.09 and 2.00 (3 s, each 3 H, 3 OAc); ^{13}C NMR ($CDCl_3$): δ 170.52, 170.12, 169.53 (3 C=O), 137.56, 128.18, 127.56 (all for Ph), 98.70 (C-1), 74.30 (C-3), 71.17 (–OCH_2–), 68.36, 67.92, 67.24, 62.61 (C-6), 54.98 (OMe), 20.82 and 20.62 with double int. (3 Me); FABMS (+): m/z 411 $[M + H]^+$, 379 $[M + H - MeOH]^+$, 351 $[M + H - CH_3COOH]^+$, 303 $[M + H - BnOH]^+$. Anal. Calcd for $C_{20}H_{26}O_9$ (410.16): C, 58.51; H, 6.39. Found: C, 58.79; H, 6.33.

*Methyl 2-*O*-acetyl-3-*O*-benzyl-α-*D*-mannopyranoside* (**11**).—Compound **10** (616 mg, 1.5 mmol) was partially deacylated for 4 h. The product was purified by column chromatography (3:1 CH_2Cl_2–acetone) to give **11** (64 mg, 13%) as a glass $[\alpha]_D$ −4.8° (*c* 0.62, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.36–7.27 (m, 5 H, aromatic), 5.34 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 4.69 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.57 (ABq, 2 H, PhCH_2), 3.36 (s, 3 H, OMe), 2.83 and 2.38 (2 bs, each 1 H, 2 OH, deuterable), 2.11 (s, 3 H,

OAc); ^{13}C NMR ($CDCl_3$): δ 170.25 (C=O), 137.49, 128.51, 128.05 (all for Ph), 99.08 (C-1), 77.49 (C-3), 71.79 (C-5), 71.50 (–OCH_2–), 67.84, 66.85, 62.45 (C-6), 55.03 (OMe), 20.88 (Me); ESIMS (+): m/z 349.0 $[M+Na]^+$, 344.2 $[M+NH_4]^+$, 327.1 $[M+H]^+$, 309.1 $[M+H-H_2O]^+$, 295.3 $[M+H-MeOH]^+$, 218.8 $[M+H-BnOH]^+$. Anal. Calcd for $C_{16}H_{22}O_7$ (326.14): C, 58.87; H, 6.80. Found: C, 58.95; H, 6.81.

A mixture (300 mg) of **11** and methyl 3-*O*-benzyl-α-D-mannopyranoside (**12**) was also eluted from which an analytical sample of pure **12** was obtained by rechromatography as an amorphous solid; $[\alpha]_D$ +38.8° (*c* 0.47, $CHCl_3$); ^{1}H NMR ($CDCl_3$–D_2O): δ 7.36–7.26 (m, 5 H, aromatic), 4.65 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.61 (ABq, 2 H, PhCH_2), 4.01 (t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 3.88–3.71 (m, 3 H, H-2,6a,6b), 3.62 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.7 Hz, H-3), 3.44 (m, 1 H, H-5), 3.26 (s, 3 H, OMe); ^{13}C NMR ($CDCl_3$): δ 137.61, 128.38, 128.15, 127.91 (all for Ph), 100.64 (C-1), 79.46 (C-3), 72.26 (C-5), 71.99 (–OCH_2–), 67.93, 64.89, 60.86 (C-6), 54.75 (OMe); Anal. Calcd for $C_{14}H_{20}O_6$ (284.13): C, 59.13; H, 7.09. Found: C, 59.02; H, 7.11.

Octyl α-D-*mannopyranosyl-(1→3)-2,4,6-tri*-O-*benzoyl-β-D-mannopyranoside* (**17**).—Octyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-mannopyranoside[11] (**15**; 23.4 mg, 25 μmol) was partially deacetylated for 45 min. Column chromatography of the crude product (17:3 CH_2Cl_2–MeOH) resulted in **17** (15.3 mg, 80%); glass; $[\alpha]_D$ −52.6° (*c* 1.53, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.09–7.26 (m, 15 H, aromatic), 5.75 (t, 1 H, $J_{3,4}=J_{4,5}$ 9.8 Hz, H-4), 5.66 (d, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 4.88 (bs, 1 H, H-1′), 4.75 (bs, 1 H, H-1), 4.68 (dd, 1 H, $J_{5,6a}$ 2.8, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.43 (dd, 1 H, $J_{5,6b}$ 5.0 Hz, H-6b), 1.50–1.14 (m, 12 H, 6 –CH_2–), 0.82 (t, 3 H, CH_3); ^{13}C NMR ($CDCl_3$): δ 166.17 with double int. and 165.37 (3 C=O), 133.64, 133.15, 133.01, 130.01, 129.90, 129.73, 129.65, 128.79, 128.67, 128.38 (all for 3 Ph), 102.46 (C-1), 99.00 (C-1′), 73.08, 72.23, 71.49, 70.88, 70.54, 69.82 (–OCH_2–), 69.18, 66.11, 63.30 (C-6), 60.99 (C-6′), 31.70, 29.39, 29.18 with double int., 25.75, and 22.60 (6 –CH_2–), 14.06 (C*Me*); ESIMS (+): m/z 789.4 $[M+Na]^+$, 784.3 $[M+NH_4]^+$, 605.2 $[M-C_6H_{10}O_5]^+$, 587.2 $[M-C_6H_{12}O_6]^+$, 475.3 $[M-C_6H_{10}O_5-CH_3(CH_2)_7OH]^+$; MALDI-TOF HRMS: Calcd for $C_{41}H_{50}NaO_{14}$ (789.3093). Found: 789.0450 $[M+Na]^+$. Resolution: 3060.

Octyl α-D-*mannopyranosyl-(1→6)-4*-O-*acetyl-2*-O-*benzoyl-3*-O-*benzyl-β-D-mannopyranoside* (**19**).—Compound **18** (86 mg, 0.1 mmol)[11] was partially deacylated for 30 min. Column chromatography (9:1 CH_2Cl_2–MeOH) of the product yielded **19** (58 mg, 84%) as an amorphous solid; $[\alpha]_D$ −52.6° (*c* 0.86, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.09–7.20 (m, 10 H, aromatic), 5.78 (d, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 5.27 (t, 1 H, $J_{4,5}$ 9.2 Hz, H-4), 4.83 (bs, 1 H, H-1′), 4.59 (ABq, 2 H, PhCH_2), 4.56 (bs, 1 H, H-1), 1.98 (s, 3 H, OAc), 1.54–1.11 (m, 12 H, 6 –CH_2–), 0.83 (t, 3 H, –CH_3); ^{13}C NMR ($CDCl_3$): δ 170.16, 166.01 (2 C=O), 137.39, 133.08, 129.98, 128.35, 127.77 (all for 2 Ph), 100.14 (C-1), 98.95 (C-1′), 76.60 (C-3), 72.91, 72.37, 71.56, 70.71 and 69.91 (2 –OCH_2–), 68.57, 68.04, 66.90 (C-6), 66.28, 60.91 (C-6′), 31.69, 29.29 with triple int., 25.82, and 22.59 (6 –CH_2–), 20.91 (Me), 14.05 (C*Me*); FABMS (+): m/z 691 $[M+H]^+$, 631 $[M+H-CH_3COOH]^+$, 583 $[M+H-BnOH]^+$, 569 $[M+H-PhCOOH]^+$, 561 $[M+H-CH_3(CH_2)_7OH]^+$. Anal. Calcd for $C_{36}H_{50}O_{13}$ (690.33): C, 62.58; H, 7.30. Found: C, 62.50; H, 7.35.

Methyl 2,3,4,6-tetra-O-*acetyl*-α-D-*mannopyranosyl-(1→6)-3*-O-*acetyl-2,4-di*-O-*benzyl*-α-D-*mannopyranoside* (**22**).—Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)-2,4-di-*O*-benzyl-α-D-mannopyranoside[17] (**21**; 70.5 mg, 0.1 mmol) was acetylated in 1:1 pyridine–Ac_2O (8 mL) at rt overnight, then the mixture was concd and co-concd with toluene (3 × 5 mL). Column chromatography of the residue (17:3 CH_2Cl_2–EtOAc) resulted in **22** (syrup, 69 mg, 92%); $[\alpha]_D$ +45.5° (*c* 1.73, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.38–7.25 (m, 10 H, aromatic), 5.37 (dd, 1 H, $J_{3',4'}$ 9.5 Hz, H-3′), 5.33 (dd, 1 H, $J_{2',3'}$ 3.3 Hz, H-2′), 5.27 (t, 1 H, $J_{4',5'}$ 9.5 Hz, H-4′), 5.21 (dd, 1 H, $J_{3,4}$ 8.8 Hz, H-3), 4.97 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.69 and 4.59 (2 ABq, each 2 H, 2 PhCH_2), 4.68 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.35

(s, 3 H, OMe), 2.14, 2.07, 2.04, 1.98, and 1.96 (5 s, each 3 H, 5 OAc); ^{13}C NMR ($CDCl_3$): δ 170.59, 169.75, 169.66 (all for 5 C=O), 137.97, 137.82, 128.33, 127.72, 127.44 (all for 2 Ph), 98.52 and 97.53 (C-1,1′), 75.66, 74.50 (–OCH_2–), 73.81, 73.25, 72.87 (–OCH_2–), 70.82, 69.41, 68.96, 68.28, 66.56 (C-6), 66.05, 62.41 (C-6′), 54.78 (OMe), 20.63 (C*Me*); ESIMS (+): *m*/*z* 769.3 $[M + Na]^+$, 764.2 $[M + NH_4]^+$, 747.4 $[M + H]^+$, 715.3 $[M + H - MeOH]^+$, 331.0 $[C_{14}H_{19}O_9]^+$. Anal. Calcd for $C_{37}H_{46}O_{16}$ (746.28): C, 59.50; H, 6.21. Found: C, 60.00; H, 6.18.

Methyl α-D-*mannopyranosyl-(1→6)-3*-O-*acetyl-2,4-di*-O-*benzyl*-α-D-*mannopyranoside* (**23**).—Compound **22** (44.8 mg, 60 μmol) was partially deacylated for 30 min and the crude product was subjected to column chromatography (19:1 CH_2Cl_2–MeOH) to yield syrupy **23** (27.1 mg, 78%); $[\alpha]_D$ +43.7° (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.35–7.22 (m, 10 H, aromatic), 5.19 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.2 Hz, H-3), 3.29 (s, 3 H, OMe), 1.92 (s, 3 H, OAc); ^{13}C NMR ($CDCl_3$): δ 170.10 (C=O), 138.02, 137.62, 128.41, 127.88, 127.69, 127.29 (all for 2 Ph), 100.27 and 98.56 (C-1,1′), 75.58, 74.51 (–OCH_2–), 73.81, 73.37, 72.89 (–OCH_2–), 72.28, 71.66, 70.73, 66.33 (C-6), 65.96, 60.75 (C-6′), 54.85 (OMe), 20.98 (C*Me*); ESIMS (+): *m*/*z* 601.3 $[M + Na]^+$, 596.5 $[M + NH_4]^+$, 579.4 $[M + H]^+$, 547.3 $[M + H - MeOH]^+$, 417.4 $[M - C_6H_9O_5]^+$; MALDI-TOF HRMS: Calcd for $C_{29}H_{38}NaO_{12}$ (601.2255). Found: 601.1524 $[M + Na]^+$. Resolution: 3862.

Methyl α-D-*mannopyranosyl-(1→3)-2*-O-*acetyl*-α-D-*mannopyranoside* (**25**).—Compound **24** (65 mg, 0.1 mmol)[17] was partially deacylated for 30 min into an inseparable mixture of **25** and **26**. FABMS (+):Calcd for $C_{15}H_{26}O_{12}$ (**25**, 398.14) and $C_{13}H_{24}O_{11}$ (**26**, 356.13). Found: *m*/*z* 399 $[M + H]^+$, 367 $[M + H - MeOH]^+$ for **25** and 357 $[M + H]^+$, 379 $[M + Na]^+$, 325 $[M + H - MeOH]^+$ for **26**.

Octyl α-D-*mannopyranosyl-(1→3)-[*α-D-*mannopyranosyl-(1→6)]-4*-O-*acetyl-2*-O-*benzoyl*-β-D-*mannopyranoside* (**28**).—Compound **27**[11] (22 mg, 0.02 mmol) was partially deacylated for 45 min. The product was purified by column chromatography (7:3 CH_2Cl_2–MeOH) to yield **28** (3.8 mg, 25%) as an amorphous solid; ^{1}H NMR (1:1 $CDCl_3$–CD_3OD): δ 8.10–7.47 (m, 5 H, aromatic), 5.69 (d, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 5.44 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 4.92 (d, 1 H, $J_{1'',2''}$ 1.5 Hz, H-1″), 4.87 (d, 1 H, $J_{1',2'}$ 1.3 Hz, H-1′), 4.67 (bs, 1 H, H-1), 2.14 (s, 3 H, OAc), 1.51–1.19 (m, 12 H, 6 –CH_2–), 0.85 (t, 3 H, –CH_3). ESIMS (+): *m*/*z* 785.5 $[M + Na]^+$, 780.4 $[M + NH_4]^+$, 763.3 $[M + H]^+$, 601.3 $[M - C_6H_{10}O_5]^+$, 439.3 $[M - C_{12}H_{20}O_{10}]^+$; MALDI-TOF HRMS: Calcd for $C_{35}H_{54}NaO_{18}$ (785.3202). Found: 785.1443 $[M + Na]^+$. Resolution: 2991.

Methyl 2,3,4,6-tetra-O-*acetyl*-α-D-*mannopyranosyl-(1→3)-[2,3,4,6-tetra*-O-*acetyl*-α-D-*mannopyranosyl-(1→6)]-2,4-di*-O-*acetyl*-α-D-*mannopyranoside* (**30**).—A mixture of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-benzyl-α-D-mannopyranoside (**29**; 517 mg, 0.5 mmol),[17] AcOH (20 mL), EtOH (20 mL) and Pd–C (100 mg) was stirred under a H_2 atmosphere at rt for 2 days. The catalyst was filtered off, the filtrate was concd and co-concd with toluene (4 × 30 mL). The product was purified by column chromatography (3:2 CH_2Cl_2–EtOAc) to give trisaccharide **30** (423 mg, 90%) as a foam; $[\alpha]_D$ +50.8° (c 1.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.38–5.02 (m, 8 H, H-2,4,2′,3′,4′,2″,3″,4″), 5.00 (bs, 1 H, H-1″), 4.83 (bs, 1 H, H-1′), 4.69 (bs, 1 H, H-1), 4.34–3.46 (m, 10 H, skeleton protons), 3.41 (s, 3 H, OMe), 2.22, 2.16, 2.13, 2.12, 2.07, 2.05, 199, and 1.98 (8 s, 30 H, 10 OAc); ^{13}C NMR ($CDCl_3$): δ 170.46, 170.31, 169.84, 169.61, 169.42 (all for 10 C=O), 98.64, 98.15, and 97.06 (C-1,1′,1″), 74.02 (C-3), 70.67, 69.82, 69.23, 68.83, 68.48, 68.25, 68.11, 66.61 (C-6), 65.83, 62.26 with double int. (C-6,6′), 54.92 (OMe), 20.67 and 20.48 (10 Me); FABMS (+): *m*/*z* 939 $[M + H]^+$. Anal. Calcd for $C_{39}H_{54}O_{26}$ (938.29): C, 49.88; H, 5.80. Found: C, 50.00; H, 5.82.

Zemplén reactions of compound **30**

Procedure A. Compound **30** (94 mg, 0.1 mmol) was deacylated for 1 day to give methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**31**, 48 mg, 93%) as a glass; $[\alpha]_D$ +79.3° (*c* 0.16, water), +103.2° (*c* 0.22, MeOH); lit. $[\alpha]_D$ +83.9° (water),[22] +96.7°

(MeOH);[23] ^{1}H NMR (D_2O): δ 5.09 (d, 1 H, $J_{1',2'}$ 1.4 Hz, H-1′), 4.90 (d, 1 H, $J_{1'',2''}$ 1.4 Hz, H-1″), 4.72 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), ^{13}C NMR (D_2O): δ 104.90 (C-1′), 103.50 (C-1), 101.89 (C-1″), 81.08 (C-3), 75.80, 75.19, 73.33, 73.09, 72.86, 72.46, 72.02, 69.22, 68.12, 67.69 (C-6), 63.44 (C-6′ and C-6″), 57.34 (OMe). FABMS (+): m/z 519 $[M+H]^+$.

Procedure B. Compound **30** (94 mg, 0.1 mmol) was partially deacylated. The reaction was monitored by TLC (2:1:1 *n*-butanol–MeOH–water). After 45 min the FABMS spectrum of a sample showed that it was a complex mixture in which the mono- and diacetate of **31** were the main components: m/z 645 $[M_{triacetate}+H]^+$, 625 $[M_{diacetate}+Na]^+$, 603 $[M_{diacetate}+H]^+$, 583 $[M_{monoacetate}+Na]^+$, 561 $[M_{monoacetate}+H]^+$, 519 $[M_{(31)}+H]^+$. After 2 h the working-up procedure gave a mixture of the monoacetates and the fully deacylated **31**. FABMS (+): m/z 519 $[M_{(31)}+H]^+$, 541 $[M_{(31)}+Na]^+$ and 561 $[M_{monoacetate}+H]^+$, 583 $[M_{monoacetate}+Na]^+$; ^{1}H NMR (Me_2SO-d_6): δ 5.12 (d, $J_{2,3}$ 4.9 Hz, H-2), 5.06 (t, $J_{3,4}=J_{4,5}$ 9.9 Hz, H-4).

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