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Regioselective C-6 Hydrolysis of Methyl *O*-Benzoyl-pyranosides Catalysed by *Candida Rugosa* Lipase

Aslan Esmurziev,[a,b] Eirik Sundby,[b] and Bård Helge Hoff*[a]

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Hydrolysis of six methyl *O*-benzoyl-pyranosides has been investigated using *Candida rugosa* lipase in dioxane/buffer mixtures. The lipase catalysed the hydrolysis of all substrates in a regiospecific manner at C-6. The rate of reaction was dependent on pyranoside structure, reaction temperature and scale, dioxane concentration and agitation speed. Starting from their C-6 *O*-benzoyl precursors, the methyl 2,3,4-tri-*O*-benzoyl-pyranosides of α-D-galactose, β-D-galactose, α-D-glucose, and methyl 2,3-di-*O*-benzoyl-α-D-galactopyranoside could be isolated in 85–96% yield. In hydrolysis of methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside and methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside substrate inhibition were observed, which in part could be overcome by increasing the reaction volume. Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside and methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside were poor substrates for *Candida rugosa* lipase and low degree of conversion towards products were obtained under all conditions. No acyl migration was detected in any of the products.

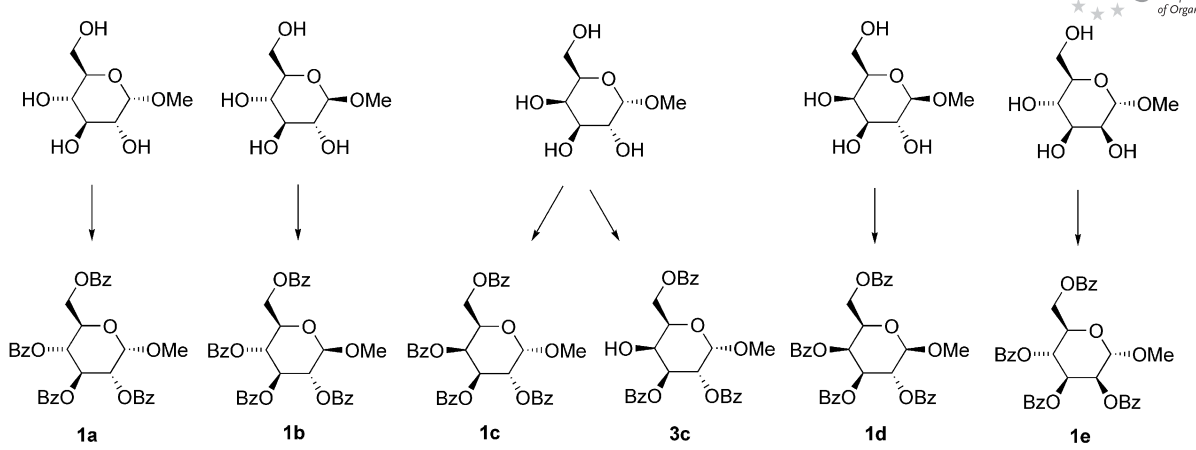
Introduction

Partially acylated pyranosides have widespread use in carbohydrate chemistry. Derivatives containing a free C-6 hydroxy, such as methyl 2,3,4-tri-*O*-acyl-pyranosides, are useful building blocks for uronic acid derivatives,[1] C-6 modified hexoses,[2,3] disaccharides,[4–8] and oligosaccharides.[9–12] However, regioselective deprotection of carbohydrates is a considerable challenge due to the presence of multiple hydroxy groups of similar reactivity. Therefore, non-enzymatic methods for synthesising 2,3,4-tri-*O*-benzoyl-pyranosides includes a 3-step sequence of C-6 protection, acylation, and cleavage of the protection group. Commonly used protection groups includes trityl,[13,14] bulky silane ethers,[15–17] bromoacetyl,[18] and formyl.[19]

As opposed to standard methods, regioselective hydrolysis catalysed by enzymes offers a two step route.[20–24] *Candida rugosa* lipase (CRL) has proven to be the catalyst of choice for deacetylation at C-6.[20,24] The lipase has a rather wide active site and the ability to tolerate high concentrations of organic co-solvents.[25–27] The hydrolysis of glucose pentaacetate has been investigated at different pH by several authors.[20,23,24] Reactions at pH 4–5 using CRL led to high regioselectivity favouring hydrolysis at C-6, whereas at pH 7 a mixture of the C-6 and C-4 deacetylated products were obtained. Also the hydrolysis of methyl and butyl 2,3,4,6-tetra-*O*-acetyl α-D- and β-D-glucopyranosides proceeded regioselectively at C-6 at pH 4 using 25% acetonitrile as a co-solvent.[20] Good regiocontrol was also observed in hydrolysis of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside.[28] Hydrolysis of acetylated hexapyranosides using a PEG-modified variant of CRL in 1,1,1-trichloroethane/water lead to mixtures of methyl 2,3-diacetyl hexapyranosides and methyl 2,3,4-triacetyl hexapyranosides.[29] As an alternative, ethanolysis reactions can be utilised. Using CRL, methyl 2,3,4-triacetyl-α-D-gluco-, -β-D-gluco- and -α-D-mannopyranosides have been prepared by this method.[28] Besides lipases, various esterases could be envisioned as suitable catalysts for regioselective hydrolysis.[21,30]

A disadvantage with pyranoside acetates is their tendency to undergo acyl migrations.[20,21,31] Benzoyl migration in carbohydrate chemistry is not unknown.[32,33] However, due to their higher stability towards base,[34] benzoate pyranosides are expected to be less problematic in this respect compared to their acetylated analogues. The benzoates also offer the possibility of analysing products by UV detectors,[35] or by circular dichroism spectroscopy,[36] which is often troublesome for acetate esters. In contrast to the methyl 2,3,4-tri-*O*-acetyl-pyranosides, enzymatic approaches have not been developed or investigated in the benzoyl series. This is probably due to expected challenges with solubility, and the increase in size of the substrates possibly limiting the number of useful catalysts for this transformation. On this background, the present work de-

[a] Department of Chemistry, Norwegian University of Science and Technology,
Høgskoleringen 5, 7491 Trondheim, Norway
Fax: +47-72593973
E-mail: bhoff@chem.ntnu.no
[b] Department of Chemistry and Material Science, Sør-Trøndelag University College,
E. C. Dahls gate 2, 7004 Trondheim, Norway



Scheme 1. Synthesis of starting materials: **a**: α-D-glucopyranoside, **b**: β-D-glucopyranoside, **c**: α-D-galactopyranoside, **d**: β-D-galactopyranoside, **e**: α-D-mannopyranoside.

scribes regioselective enzymatic hydrolysis of methyl *O*-benzoyl-pyranosides as a preparative route to derivatives having a free C-6 hydroxy group.

Results and Discussion

Preparation of Substrates

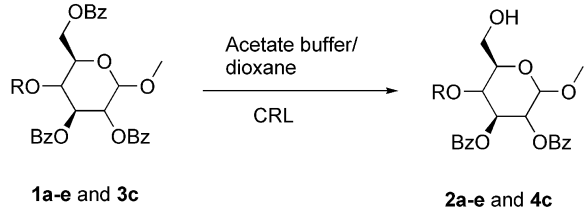
The methyl 2,3,4,6-tetra-*O*-benzoyl-pyranosides **1a**–**e** of α-D-glucose, β-D-glucose, α-D-galactose, β-D-galactose, and α-D-mannose, respectively, were synthesised from the corresponding methyl D-pyranosides using benzoyl chloride in pyridine (Scheme 1).

Benzoylation of methyl α-D-galactopyranoside to **1c** was sluggish, and methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**3c**) could easily be isolated.[37] Compound **3c** was included in the study to investigate the effect of steric crowd on the enzymatic hydrolysis, and possibly to provide a new method for the isolation of methyl 2,3-di-*O*-benzoyl-α-D-galactopyranoside (**4c**).

Lipase-Catalysed Regioselective Hydrolysis

Initially, a screening using several lipases was performed. Compound **3c** was selected as a test substance because it was expected to have the highest reactivity due to size effects and solubility. The screening revealed that only *Candida rugosa* lipase (CRL) had an acceptable reaction rate. No useful activity was observed using *porcine pancreas* lipase type 2, several formulations of *Candida antarctica* lipase A and B, *Humicola insolens* lipase (Lipozyme TL 100 L), *Rhizomucor miehei* lipase (Lipozyme RM IM) and *Rhizopus delemar* lipase. This parallels the activity observed for various lipases towards other acetylated carbohydrates.[23,24]

A challenge in hydrolysis of benzoylated pyranosides is their low solubility in water, causing mass-transfer limitations and rate retardation. Therefore the hydrolytic activity of CRL towards **3c** was examined in the presence of acetone, THF, dioxane and 2-propanol. A low activity was observed in acetone and 2-propanol, whereas reactions in dioxane and THF gave acceptable reaction rate. Dioxane (20 vol.-%) was selected as a co-solvent and hydrolysis of **1a**–**e** and **3c** were investigated at different temperatures on 25-mg scale (Scheme 2). Hydrolysis of all substrates proceeded in a regiospecific manner at C-6.



Scheme 2. CRL-catalysed hydrolysis of **1a**–**e** and **3c**. **1a**–**e**: R = Bz, **3c**: R = H.

The results of the screening experiments are summarised in Table 1. The degree of conversion depended on the pyranoside structure and the reaction temperature.

Table 1. Effect of pyranoside structure and reaction temperature on conversion in hydrolysis of **1a**–**e** and **3c** using CRL.

		Conversion (%)[b]				
Substrate	Enzyme loading[a]	20 °C[c]	30 °C[c]	40 °C[c]	30 °C[d]	Product
1a	0.5	35	71	47	85	**2a**
1b	1	0	6	4	6	**2b**
1c	0.5	66	67	88	75	**2c**
1d	0.5	42	70	91	86	**2d**
1e	1	0	4	4	6	**2e**
3c	0.5	98	99	97	99	**4c**

[a] Weigh amount relative to substrate. [b] By HPLC. [c] Reaction time: 20 h agitating at 140 rpm. [d] Reaction time 40 h agitating at 140 rpm.

The highest conversion rates were observed in hydrolysis of **3c**, possibly due to a more accessible site of reaction. Hydrolysis of the α-D-glucopyranoside **1a**, proceeded with the highest rate at 30 °C, whereas for the **1c**–**d** the best conversions were experienced at 40 °C. Methyl 2,3,4,6-tetra-*O*-

Table 2. Hydrolysis of selected substrates in acetate buffer containing 20% dioxane.[a]

Substrate	Agitation (rpm)	Enzyme loading (w/w)[b]	Reaction time [h]	Conversion (%)[c]	Isolated yield (%)	Product
1a	140	0.5	60	40	33	**2a**
1b	140	1	20	9	7	**2b**
1c	140	0.5	60	67	53	**2c**
1e	140	1	20	7	5	**2e**
3c	140	0.5	20	82	76	**4c**

[a] All reactions were performed in a 100-mg scale at 30 °C. [b] Weight amount relative to substrate. [c] By HPLC.

benzoyl-β-D-glucopyranoside (**1b**) and the α-D-mannopyranoside **1e**, were hydrolysed at a low rate at all temperatures, and were poor substrates for CRL.

When the reaction scale was increased to 100 mg, a lower degree of conversion, and thus a lower yield were the result in hydrolysis of all substrates, see Table 2. The highest conversion was obtained in hydrolysis of **3c**, reaching a moderate 82% conversion after 20 h reaction time.

Phase-transfer limitation was expected to be the main reason for the drop in conversion. Therefore, the effect of enzyme loading and agitation on conversion was investigated in 100-mg scale using **3c** as substrate. By increasing agitation from 140 to 200 rpm and doubling catalyst loading, full conversion could be obtained within 20 h when reacting at 30 °C. Also, hydrolysis of **1c** gave a high conversion (96%) when applying the same agitation and catalyst amount and reacting at 40 °C for 40 h.

Hydrolysis of the α-D-glucopyranoside **1a** proved more difficult to optimise. Experiments were undertaken varying the dioxane concentration and enzyme loading, see Table 3. The highest conversion obtained was 72%, applying 30% dioxane as co-solvent. Moderate activity was observed at lower (Entry 1) and higher (Entry 7) dioxane concentration. Surprisingly, increasing the enzyme loading did not affect degree of conversion to a significant extent (Entry 4 and 6). The reaction was also performed in the presence of 1 mol equivalent of benzoic acid (Entry 3). However, no significant effect on conversion was detected.

Table 3. Effect of dioxane concentration, enzyme loading and benzoic acid on conversion in hydrolysis of **1a**.[a]

Entry	Dioxane (%)	Enzyme loading (w/w)	Conversion (%)
1	10	1	42
2	20	1	65
3	20	1	63[b]
4	20	2	65
5	30	1	70
6	30	2	72
7	40	1	49

[a] Scale: 100 mg of **1a**, 200 rpm agitation at 30 °C for 60 h. [b] One mol equivalent of benzoic acid was added.

The moderate conversions being almost independent of enzyme loading was interpreted as either a deactivation of the enzyme by the co-solvent, or enzyme inhibition caused by some of the reaction components. The hypothesis of a time dependant inactivation of the lipase affected by dioxane was tested by adding a second portion of fresh enzyme after half of the reaction time (30 h). No change in conversion was the result. Therefore, deactivation of the enzyme by dioxane is a not a likely explanation.

Further, the effect of varying the volume and strength of the buffer medium was investigated in hydrolysis of **1a**, see Table 4. By increasing the volume of the buffer solution from 12 to 24 mL (Entry 2), the degree of conversion could be increased to 87%. The use of even higher volumes (Entry 3) did not improve the degree of conversion. Applying a stronger buffer medium (Entry 4) led to a slight rate retardation, possibly due to a salting out effect.

Table 4. Effect of buffer reaction volume and buffer strength in hydrolysis of **1a**.[a]

Entry	Buffer concentration [M]	Volume [mL]	Conversion (%)
1	0.2	12	65[b]
2	0.2	24	87
3	0.2	30	83
4	0.5	12	60

[a] Conditions: 100 mg of **1a**, temperature 30 °C, agitation: 200 rpm for 60 h, enzyme loading: 1. [b] Table 3 Entry 2.

Then the effect of the reaction components **1a** and **2a** on the performance of CRL was studied, using hydrolysis of **3c** as model system (Table 5). In the presence of **2a** (Entry 2), the degree of conversion of **3c** dropped from 99 to 80%. However, the effect of introducing **1a** (Entry 3) upon the equivalent reaction was much more profound and the degree of conversion dropped to 32%. By increasing the enzyme loading higher conversions could be obtained, but still the inhibiting action of **1a** was evident.

Table 5. Effect of **1a** and **2a** on hydrolysis of **3c** (140 rpm, 30 °C, 20 h).

Entry	Added substance (mol-equiv.)	Enzyme loading (w/w)	Conversion of **3c** (%)
1	none (0)	0.5	99
2	**2a** (1.2)	0.5	81
3	**1a** (1.0)	0.5	32
4	**1a** (1.0)	1	55
5	**1a** (1.0)	2	71

During investigation of the inhibition phenomenon, it was discovered that when a 1:1 mixture of **1a** and **2a** were subjected to fresh buffer and CRL, above 90% overall conversion could be obtained. Although inconvenient, the double batch protocol allowed for the isolation of **2a** and **2d** in 87 and 85% yield from **1a** and **1d**, respectively. More-

over, these experiments also confirm that the rate retardation compared to hydrolysis of **1c** is related to special effects caused by **1a** and **1d**.

Hydrolysis of **1b** and **1d** were also attempted by this method, but as expected this did not improve the conversions towards **2b** and **2e** noteworthy.

The rate retardation in hydrolysis of **1a** and **1d** is not fully understood. However, all the results taken together indicate that the main cause is substrate inhibition. The effect of the inhibition could be reduced by increasing the reaction volume, indicating that solubility is important. D-Glucose has been shown to be a competitive inhibitor in CRL-catalysed esterifications forming a dead-end complex.[38] Probably also **1a** and **1d** could bind to the active site of the enzyme in a non-productive manner blocking further catalysis. Also, larger molecules as saponins, flavonoids, alkaloids and antimicrobial agents are known inhibitors of CRL without their detailed mechanism of action been clear.[39,40] A more thorough kinetic investigation of this inhibition is underway.

Benzoyl migration has been observed in structurally related compounds when exposed to bases.[32,33] No benzoyl migration was observed for **2a**–**e** and **4c** upon one month storage in $CDCl_3$ at 25 °C, as detected by NMR and HPLC. The presented methodology complements the established enzymatic procedures toward methyl 2,3,4-tri-*O*-acetyl-pyranosides, by providing building blocks which are less prone to acyl migration and that allow for tougher conditions in subsequent chemical steps.

Conclusions

The enzymatic hydrolysis of six methyl *O*-benzoyl-pyranosides has been investigated using *Candida rugosa* lipase. All substrates were hydrolysed in a regioselective manner at C-6. The highest conversions were observed in hydrolysis of methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**3c**). Also methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranoside (**1c**) could be hydrolysed at a reasonable time scale. This allowed for the isolation of methyl 2,3-di-*O*-benzoyl-α-D-galactopyranoside (**4c**) and methyl 2,3,4-tri-*O*-benzoyl-α-D-galactopyranoside (**2c**) in high yield. Hydrolysis of methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (**1a**) was complicated by what appeared as substrate inhibition. However, increasing the reaction volume, or by using a two-batch protocol, the product could be obtained in up to 87% isolated yield. The same method provided methyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (**2d**) in 85% yield. Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (**1b**) and methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (**1e**) were poor substrates for CRL. Acyl migration to the free hydroxy at C-6 was not observed for any of the product studied.

Experimental Section

General Information: Methyl α-D-mannopyranoside was prepared by a known method,[41] while methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, methyl α-D-galactopyranoside and β-D-galactopyranoside were purchased from Sigma–Aldrich. Benzoyl chloride and pyridine were from Fluka. Column chromatography was performed using silica gel 60A from Fluka (pore size 40–63 μm). *Candida rugosa* lipase was purchased from Sigma–Aldrich (type VII, ≥ 700 units/mg solid).

Analysis: The HPLC system consisted of an Agilent 1100 series quaternary pump, Agilent 1100 series variable wavelength UV-detector (200–315 nm) and a thermostatted column compartment. Conversion was analysed on a Supelcosil C18 column (5 μm particle size, 25 cm), UV detection (254 nm). All HPLC analyses were performed using a mixture of deionised water containing TFA (0.01%) and MeOH (30:70, vol.-%) with increase in flow rate after elution of the tribenzoate. **1a/2a**: Flow rate 1 mL/min. for 12 min, then 2 mL/min. for 33 min. Retention times **2a**: 11.9 min. and **1a**: 31.4 min. **1b/2b**: Flow rate 1 mL/min. for 9 min, then 2 mL/min. for 16 min. Retention time: **2b**: 8.5 min. and **1b**: 14.1 min. **1c/2c**: Flow rate 1 mL/min. for 12 min, then 2 mL/min. for 26 min. Retention time: **2c**: 11.4 min, **1c**: 24.7 min. **1d/2d**: Flow rate 1 mL/min. for 8 min, then 2 mL/min for 15 min. Retention time: **2d**: 7.6 min, **1d**: 12. 9 min. **1e/2e**: Flow rate 1 mL/min. 10 min, then 2 mL/min. for 22 min. Retention time: **2e**: 7.6 min, **1e**: 20.7 min. **3c/4c**: Flow rate 1 mL/min. 15 min. Retention time: **4c**: 5.0 min, **3c**: 14.4 min.

NMR spectra were recorded with Bruker Avance DPX 400 operating at 400 MHz for ^1H, and 100 MHz for ^{13}C. Chemical shifts are in ppm relative to TMS. Coupling constants are in Hertz. All spectral assignments were confirmed by COSY, HMBC and HSQC experiments. Melting points were measured with a Mettler FP 5 melting point apparatus and are uncorrected. Optical rotations were measured using sodium D line at 589 nm with a Perkin–Elmer 243 B polarimeter.

Starting Materials: The compounds **1a**–**e** were prepared as described previously.[42] Compound **3c** was isolated after benzoylation of methyl α-D-galactopyranoside by column chromatography (silica, toluene/ethyl acetate, 9:1). Recrystallisation from ethanol gave methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside as a white solid, m.p. 135.0–135.5 °C, lit.[43] 135.5–137 °C, $[\alpha]_D^{23}$ = +118.0 (c = 1.00, $CHCl_3$), lit.[44] +119.8 (c = 1.01, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.08–7.94 (m, 6 H, Ar), 7.61–7.31 (m, 9 H, Ar), 5.75 (dd, ^3J = 2.9, 10.6 Hz, 1 H, 3-H), 5.70 (dd, ^3J = 10.6, 3.01 Hz, 1 H, 2-H), 5.22 (d, ^3J = 3.0 Hz, 1 H, 1-H), 4.66 (dd, ^3J = 6.0, 11.3 Hz, 1 H, 6_a-H), 4.58 (dd, ^3J = 6.8, ^2J = 11.3 Hz, 1 H, 6_b-H), 4.42 (m, 1 H, 4-H), 4.35 (t, ^3J = 6.0 Hz, 1 H, 5-H), 3.45 (s, 3 H, OCH_3) ppm. ^{13}C NMR spectrum was identical, but assignment differed from that reported previously.[44] ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 166.5 (C=O), 166.0 (C=O), 165.8 (C=O), 133.4–128.4 (18 C, Ar), 97.5 (C-1), δ = 70.8 (C-3), 68.9 (C-2), 68.2 (C-4), 67.7 (C-5), 63.4 (C-6), 55.5 (CH_3) ppm.

General Procedure Hydrolysis (25-mg Scale): The hydrolysis was performed by suspending compound **1a**–**e** or **3c** (25 mg, 0.041 mmol) in acetate buffer (2.4 mL, 0.2 M, pH: 4.8) and dioxane (0.6 mL) and adding *Candida rugosa* lipase (12.5 or 25 mg). The reaction mixture was shaken using an incubator at 140–200 rpm at the specified temperature and time. The reaction mixture was filtered through Celite, followed by washing of the Celite using ethyl acetate (15 mL). The combined organic fraction was washed with aqueous $NaHCO_3$ (20 mL). After drying with Na_2SO_4, and evaporation of the solvents, the residue was analysed by HPLC to determine conversion. Reported conversions are the results of averaging two runs.

General Procedure Hydrolysis (100-mg Scale): The hydrolysis was performed by suspending compound **1a**–**e** or **3c** (100 mg,

0.163 mmol) in acetate buffer (9.6 mL, 0.2 M, pH: 4.8) and dioxane (2.4 mL) and adding *Candida rugosa* lipase (50–200 mg). The reaction mixture was shaken using an incubator agitating at 140 or 200 rpm at the specified temperature and time. The reaction mixture was filtered through Celite, followed by washing of the Celite using ethyl acetate (60 mL). The combined organic fraction was washed with aqueous $NaHCO_3$ (80 mL). After drying with Na_2SO_4, and evaporation of the solvents, the residue was analysed by HPLC to determine conversion. Selected reactions were purified by column chromatography (silica, toluene/ethyl acetate, 1:1). For preparative reactions, yields are reported for single runs.

Products

Methyl 2,3,4-Tri-*O*-benzoyl-α-D-glucopyranoside (2a):[12,45] Methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranoside (**1a**) (100 mg, 0.163 mmol) was suspended in 20% dioxane/acetate buffer at pH 4.8 (12 mL) using lipase from *Candida rugosa* (100 mg) at 30 °C agitating at 200 rpm for 40 h. Extraction, drying and concentration yielded a 58:42 mixture of **2a** and **1a**, which was treated once more under identical conditions. This gave a 92:8 mixture of **2a** and **1a**. Flash chromatography yielded 70 mg (87%) of **2a** as a white amorphous solid, m.p. 133.5–134.5 °C, $[\alpha]_D^{21}$ = +51.3 (*c* = 1.50, $CHCl_3$), lit.[19] +53.5 (*c* = 1.5, $CHCl_3$). ^1H NMR corresponded with data published by Verduyn et al.[12] ^{13}C NMR ($CDCl_3$) of the sugar backbone corresponded with data reported by Kovac et al.,[45] additional shifts for benzoyl C atoms, δ = 166.4 (C=O), 165.8 (C=O, 2 C), 133.5–128.2 (18 C, Ar) ppm.

Methyl 2,3,4-Tri-*O*-benzoyl-β-D-glucopyranoside (2b):[18] Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (**1b**) (100 mg, 0.163 mmol) was hydrolysed and purified using the exactly the same procedure as for substance **1a** to give methyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (**2b**) (6 mg, 8%) as a white solid, m.p. 82–83 °C. Optical rotation differed in sign of that reported previously; $[\alpha]_D^{21}$ = +6.8 (*c* = 1.1, $CHCl_3$), lit.[18] –6.6 (*c* = 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of the sugar backbond corresponded with data reported by Ziegler et al.,[18] additional shifts for benzoyl C atoms, δ = 166.2 (C=O), 165.9 (C=O), 166.3 (C=O), 133.0–128.4 (18 C, Ar) ppm.

Methyl 2,3,4-Tri-*O*-benzoyl-α-D-galactopyranoside (2c): [14,18] Methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranoside (**1c**) (100 mg, 0.163 mmol) was hydrolysed in 20% dioxane/acetate buffer (12 mL) using lipase from *Candida rugosa* (100 mg) at 40 °C agitating at 200 rpm for 40 h, yielding a 96:4 mixture of **2c** and **1c**. Purification by flash chromatography yielded 70 mg (86%) of **2c** as a white solid, m.p. 57.0–57.5 °C, lit.[14,46] amorphous solid, $[\alpha]_D^{21}$ = +246.6 (*c* = 1.2, $CHCl_3$), lit.[18] +248.4 (*c* = 1.2, $CHCl_3$). ^1H and ^{13}C NMR data ($CDCl_3$) corresponded with those reported.[14,18]

Methyl 2,3,4-Tri-*O*-benzoyl-β-D-galactopyranoside (2d):[47] Methyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside (**1d**) (100 mg, 0.163 mmol) was hydrolysed in 20% dioxane/acetate buffer (12 mL) using lipase from *Candida rugosa* (100 mg) at 40 °C for 40 h agitating at 200 rpm. Extraction, drying and concentration yielded a 55:45 mixture of **2d** and **1d**, which was subjected to enzymatic hydrolysis once more under identical conditions. This gave a 88:12 mixture of **2d** and **1d**, which upon flash chromatography yielded 69 mg (85%) of **2d** as a white amorphous solid, m.p. 89.0–90.0 °C, lit.[18] 90–91 °C, $[\alpha]_D^{21}$ = +124.0 (*c* = 1.0, $CHCl_3$). ^1H and ^{13}C NMR data corresponded with those reported for solutions in $[D_6]$DMSO[47] and $CDCl_3$.[16]

Methyl 2,3,4-Tri-*O*-benzoyl-α-D-mannopyranoside (2e):[15,47] Methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (**1e**) (100 mg, 0.163 mmol) was hydrolysed and purified using exactly the same procedure as for substance **1a** to give methyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (**2e**) (5 mg, 6%) as a colourless syrup, $[\alpha]_D^{21}$ = –126.0 (*c* = 0.5, $CHCl_3$), lit.[18] –154 (*c* = 1.1, $CHCl_3$). ^1H and ^{13}C NMR spectra for a $[D_6]$DMSO solution were slightly shifted compared to those reported previously.[47] Assignments were verified by HSQC and HMBC experiments. ^1H NMR (400 MHz, $[D_6]$DMSO, 25 °C): δ = 8.02–7.33 (m, 15 H, Ar), 5.80 (t, ^3J = 10.0 Hz, 1 H, 4-H), 5.64 (dd, ^3J = 10.0, 3.4 Hz, 1 H, 3-H), 5.58 (dd, ^3J = 3.4, 1.7 Hz, 1 H, 2-H), 5.04 (d, ^3J = 1.7 Hz, 1 H, 1-H), 4.06 (m, 1 H, 5-H), 3.63 (m, 2 H, 6_a-H, 6_b-H), 3.48 (s, 3 H, OCH_3) ppm. ^{13}C NMR (100 MHz, $[D_6]$DMSO, 25 °C): δ = 164.7 (C=O), 164.6 (C=O), 164.6 (C=O), 133.9–128.6 (18 C, Ar), 97.6 (C-1), 70.8 (C-5), 70.6 (C-3), 69.8 (C-2), 66.4 (C-4), 60.0 (C-6), 54.7 (OCH_3) ppm.

Methyl 2,3-Di-*O*-benzoyl-α-D-galactopyranoside (4c):[43,48] Methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (**3c**) (100 mg, 0.197 mmol) was hydrolysed by CRL at 30 °C agitating at 200 rpm for 20 h, to yield a 1:99 mixture of **3c** and **4c**. Purification by flash chromatography gave 77 mg (96%) of **4c** as a white solid, m.p. 95.0–96.0 °C, lit.[48] amorphous solid, $[\alpha]_D^{21}$ = +198 (*c* = 1.3, $CHCl_3$), lit.[48] +200 (*c* = 1.2, $CHCl_3$). ^1H NMR (400 MHz, $[D_5]$pyridine, 25 °C): δ = 8.40–7.16 (m, 10 H, Ar) 6.44 (dd, ^3J = 10.7, 3.6 Hz, 1 H, 2-H), 6.21 (dd, ^3J = 10.7, 3.2 Hz, 1 H, 3-H), 5.50 (d, ^3J = 3.6 Hz, 1 H, 1-H), 5.00 (d, ^3J = 3.2 Hz, 1 H, 4-H), 4.45 (m, 1 H, 5-H), 4.40 (m, 2 H, 6_a-H, 6_b-H), 3.39 (s, 3 H, OCH_3) ppm. ^{13}C NMR data for a $[D_5]$pyridine solution were slightly shifted compared to those reported previously (25 MHz).[49] ^{13}C NMR (100 MHz, $[D_5]$pyridine, 25 °C): δ = 166.6 (C=O), 166.5 (C=O), 133.6–128.7 (12 C, Ar), 98.3 (C-1), 73.0 (C-3), 72.6 (C-5), 70.5 (C-2), 68.2 (C-4), 61.9 (C-6), 55.1 (CH_3) ppm.

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