Carbohydrate Research 345 (2010) 1094–1098

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Preparation of regioselectively feruloylated *p*-nitrophenyl α-L-arabinofuranosides and β-D-xylopyranosides—convenient substrates for study of feruloyl esterase specificity

Mária Mastihubová *, Peter Biely

Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia

ARTICLE INFO

Article history:
Received 11 January 2010
Received in revised form 19 March 2010
Accepted 25 March 2010
Available online 8 April 2010

Keywords:
p-Nitrophenyl glycoside ferulates
Feruloyl esterase substrates
ZnO acylation
Bu_2SnO deacetylation

ABSTRACT

p-Nitrophenyl α-L-arabinofuranoside and β-D-xylopyranoside mono-*O*-ferulates were prepared by 4-O-acetylferuloylation of corresponding enzymatically prepared di-*O*-acetates followed by deacetylation. An alternative mild acylation catalysed by zinc oxide was tested on xylopyranoside derivatives. The chemoselective methanolysis of the acetyl groups using neutral catalyst dibutyltin oxide at reflux was used as deacetylation method. Under these conditions a significant feruloyl migration was observed mainly on *p*-nitrophenyl 3-*O*-feruloyl-β-D-xylopyranoside resulting in low yields of the positional isomers. Investigation of substrate and positional specificity of different types of feruloyl esterases on the presented compounds in enzyme-coupled assays was reported previously.

1. Introduction

Ferulic acid esterases (FAEs, E.C. 3.1.2.73) are well-defined components of microbial plant cell wall-degrading enzyme systems.[1–5] As a subclass of the carboxylic acid esterases, they are able to hydrolyse the ester bond between saccharides and hydroxycinnamic acids or their dehydrodimers. The classification of FAEs into four types (A–D) was proposed based on protein sequence identities and similarities in hydrolytic activity profiles against methyl esters of partly hydroxylated or methoxylated hydroxycinnamic acids or their dimers.[2] Despite this effort the structure–function relationship of FAEs is far from being understood. The reason is the great diversity of esterification of ferulic acid in plants.

Ferulic acid [(2*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid] has been found regioselectively attached to polysaccharide backbone of hemicelluloses. In dicotyledonous plants, such as spinach and sugar beet, the acid is attached to primary *C*-6 hydroxyls of 1,4-galactans and to *C*-2 hydroxyls of 1,5-L-arabinans.[6,7] In monocotyledonous plants and grasses, such as bamboo, sugar cane, wheat and maize, ferulic acid is esterified by *C*-4 hydroxyls of D-xylose in xyloglucans and by primary *C*-5 hydroxyls of L-arabinose in arabinoxylans.[8–12]

Although a relationship between the specificity of FAEs in hydrolysis of methyl phenylalkanoates[13,14] and release of ferulic acid or their dehydrodimers from plant cell walls saccharides[15–23] has been investigated, the specificity of FAEs regarding the saccharide moiety and the position of acylation is not well understood. A few reports indicate that microorganisms produce at least two types of FAEs, which differ in the affinity for 2-*O*- and 5-*O*-feruloylated α-L-arabinofuranoside residues.[1,24]

Understanding of the role of FAEs in biodegradation of plant cell walls requires detailed knowledge of their catalytic properties. Consequently, there is a growing demand for a variety of regioselectively feruloylated *p*-nitrophenyl glycosides that could serve as chromogenic substrates for investigation of catalytic properties of these enzymes. A number of substrates in which ferulic acid was esterified with chromogenic non-saccharidic alcohol[25,26] or with hydroxyl groups of L-arabinofuranoside[27–30] have previously been synthesised and used in FAE assays.[24,26,27,30,31] Our synthesis of *p*-nitrophenyl 2-*O*- and 5-*O*-feruloyl α-L-arabinofuranoside was published earlier as a preliminary communication.[29] These compounds were found to be convenient chromogenic substrates for determination of activity and differentiation of FAEs according to substrate specificity in an α-L-arabinofuranosidase-coupled UV-spectrophotometric assay.[24]

In this full paper we present improved and integral synthesis of all positional isomers of *p*-nitrophenyl α-L-arabinofuranoside and β-D-xylopyranoside mono-*O*-ferulates using enzymatic synthesis of suitably acetylated starting compounds in combination with convenient acylations and chemoselective deacetylations under mild conditions. These products, mimicking the naturally occurring feruloylated pentoses, served as substrates in enzyme-coupled assays to study the substrate and positional specificity of types A, B

* Corresponding author. Tel.: +421 2 59410246; fax: +421 2 59410222.
E-mail address: chemjama@savba.sk (M. Mastihubová).

0008-6215/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2010.03.034

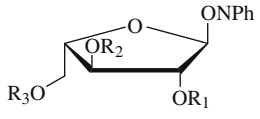
and C FAEs.[32] The coupling of the action of FAEs with that of *Aspergillus niger* α-L-arabinofuranosidase or *A. niger* β-D-xylosidase, respectively, renders feruloylated *p*-nitrophenyl pentosides to chromogenic substrates of FAEs.

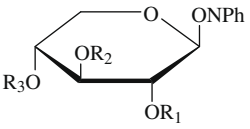
2. Results and discussion

Several different strategies have been published for regioselective preparation of feruloyl glycosides. The straightforward methods were described like was the acylation exploiting a slightly higher reactivity of sterically less hindered primary hydroxyl of methyl α-L-arabinofuranoside[27,29] or dibutyltin oxide-mediated one-step acylation of methyl β-D-xylopyranoside.[33] More complicated protection–deprotection strategies limited to suitable deprotection of primary position of per-O-silylated[28] or per-O-acetylated[29,30] α-L-arabinofuranosides were developed as well. Diacetates of *p*-nitrophenyl α-L-arabinofuranoside **1**–**3** and *p*-nitrophenyl β-D-xylopyranoside **4**–**6** serving as starting compounds in our syntheses were prepared either by regioselective acetylation of corresponding glycosides or by deacetylation of their per-O-acetylated derivatives by commercial immobilised lipases[34,35] (Chart 1).

The free hydroxyl groups in di-*O*-acetates **1–6** were acylated with 4-*O*-acetylferuloyl chloride[25,27] using DMAP and Et_3N to give 4-*O*-acetylferuloyl glycosides **7**–**12** in high yields within 3 h. An exception was the acetylferuloylation of 2,4-di-*O*-acetyl-β-D-xylopyranoside **5** which needed more than 6 h of reaction time.

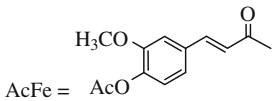
Our previous study of stability of acetylated xylopyranosides in aqueous media revealed that the acetyl moiety easily migrates along the flexible β-D-xylopyranoside ring.[35] Hence, acylations of xylopyranosides should be executed with carefully selected reagents and reaction conditions. Zinc oxide (ZnO) as a recently introduced efficient and eco-friendly acylation catalyst[36,37] was therefore applied in acylations of partially acetylated xylopyranosides **4**–**6**. The previously described heterogeneous reactions using liquid acylation reagents have been accomplished in solvent-free conditions.[36,37] Since 4-*O*-acetylferuloyl chloride and glycosides



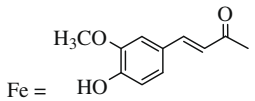


	R^1	R^2	R^3
1:	H	Ac	Ac
2:	Ac	H	Ac
3:	Ac	Ac	H
7:	AcFe	Ac	Ac
8:	Ac	AcFe	Ac
9:	Ac	Ac	AcFe
13:	Fe	H	H
14:	H	Fe	H
15:	H	H	Fe
19:	H	H	H

	R^1	R^2	R^3
4:	H	Ac	Ac
5:	Ac	H	Ac
6:	Ac	Ac	H
10:	AcFe	Ac	Ac
11:	Ac	AcFe	Ac
12:	Ac	Ac	AcFe
16:	Fe	H	H
17:	H	Fe	H
18:	H	H	Fe
20:	H	H	H



4'-*O*-acetylferuloyl



feruloyl

Chart 1.

Table 1
Dibutyltin oxide-catalysed deacetylation

Starting compound	Product[a]	Migration products[a]		NPhGlyc[a,b]
7	**13** (80%)	–	–	**19** (12%)
8	**14** (79%)	–	–	**19** (11%)
9	**15** (85%)	–	–	**19** (9%)
10	**16** (78%)	**17** (6%)	–	**20** (10%)
11	**17** (63%)	**16** (9%)	**18** (5%)	**20** (13%)
12	**18** (84%)	–	–	**20** (8%)

[a] Isolated yields, the residues were partially deacetylated starting compounds.
[b] NPhGlyc-4-nitrophenyl glycoside.

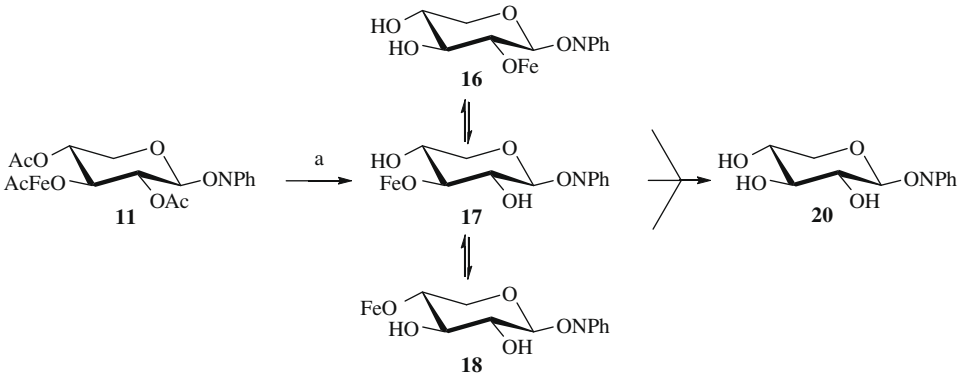
4–**6** are solid compounds, we have tested the reaction in dioxane, acetonitrile and dichloromethane. Using dry dichloromethane and 0.5 equiv of ZnO, the desired products **10** (79%), **11** (77%) and **12** (80%) were obtained within 1 h. Formation of positional isomers due to possible acyl migration was not observed. To our knowledge, ZnO has not been previously employed in acylations of carbohydrates.

The next step of our study was the investigation of conditions for chemoselective deacetylations on **7**–**12** which would cleave only acetyl esters and preserve the ferulate ester bond. In addition, the conditions should prevent acyl migration. The deacetylations with K_2CO_3 in CH_2Cl_2/MeOH (2:1) successfully applied to per-O-acetylated 2-*O*- and 5-*O*-feruloylated L-arabinofuranosides (**7** and **9**)[29] or pyrrolidine in ethanol previously used by Helm et al.[28] failed in the case of D-xylopyranoside derivatives **10**–**12** due to acyl migration along the xylopyranoside ring. The migration intermediates eventually decomposed to 4-nitrophenyl β-D-xylopyranoside and ferulic acid. Similarly, attempts to use several mild basic or acidic ways of deacetylation of 2-*O*-(4′-*O*-acetylferuloyl)-3,4-di-*O*-acetyl-β-D-xylopyranoside **10** failed due to unsatisfactory reactivity or chemoselectivity. In 2002, Liu et al. published a neutral, chemoselective and efficient methanolysis of *O*-acetyl groups with dibutyltin oxide (Bu_2SnO) in dry methanol.[38] Using this method, deacetylations of compounds **7**–**12** with Bu_2SnO (2 equiv to glycoside) in MeOH/CH_2Cl_2 (9:1) under mild reflux (60 °C in bath, 6 h) afforded the desired ferulates **13**–**18** in yields 63–85% (Table 1). Minor de-feruloylated products **19** and **20** were also isolated in 8–13%.

Feruloyl migration has been anyway observed in a limited extent due to the presence of protic solvent and higher reaction temperature. After deacetylation of 2,4-di-*O*-acetyl-3-*O*-(4′-*O*-acetylferuloyl)-β-D-xylopyranoside **11**, products **16** and **18** have been isolated in 9% and 5%, respectively (Table 1, Scheme 1). Similarly, 3,4-di-*O*-acetyl-2-*O*-(4′-*O*-acetylferuloyl) derivative **10** afforded a small amount (6%) of 3-*O*-feruloyl product **17**. The mono-*O*-feruloylated α-L-arabinofuranosides **13**–**15** as well as 4-*O*-feruloyl-β-D-xylopyranoside **12** showed remarkable stability to feruloyl migration. This phenomenon is in accordance with our previous results with partially acetylated 4-nitrophenyl glycosides.[34,35] There is no acetyl group migration along the L-arabinofuranosyl ring, neither between 5-O position and other free hydroxyl groups. In all cases, the deprotection of glycosides with Bu_2SnO left the 4-nitrophenyl aglycon fully intact.

3. Conclusion

Synthesis of targeted substrates *p*-nitrophenyl 2-*O*-, 3-*O*- and 5-*O*-feruloyl-α-L-arabinofuranosides **13–15** as well as *p*-nitrophenyl 2-*O*-, 3-*O*- and 4-*O*-feruloyl-β-D-xylopyranosides **16–18** was accomplished. The effective mild acylation method of starting compounds by zinc oxide was compared with conventional ways using partially acetylated xylopyranosides **4**–**6** as feruloyl acceptors. The neutral chemoselective de-O-acetylation procedure by dibutyltin oxide has been successfully applied for preparation of mono-*O*-feruloyl-D-glycosides **13**–**18**. The compounds **13–18** have



Scheme 1. Reagents and conditions: (a) Bu_2SnO (2 equiv), CH_2Cl_2/MeOH (1:9), 60 °C, 6 h.

already been used as the substrates for determination of activity and differentiation of FAEs according to substrate specificity in a UV-spectrophotometric assay.[24,32]

4. Experimental

4.1. General methods

Solvents were dried and distilled before use. The reagent Bu_2SnO (BDH Chemicals) was used as purchased without further purification. The ZnO powder (Aldrich) was activated for one hour at 400 °C before use. All reactions were monitored by TLC on Silica Gel 60 F_{254} plates (0.25 mm, E. Merck, Darmstadt, Germany). Spots were detected under UV lamp (λ_{max} = 254 nm) followed by dipping the plate in 5% ethanolic H_2SO_4 and heating at ca. 200 °C. Column chromatography was performed on silica gel (0.035–0.070 mm, pore diameter ca. 6 nm, Acrōs Organics). Melting points were determined on a Kofler hot-stage and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 20 °C. ^{1}H NMR spectra (300 MHz, Me_4Si as internal standard) and ^{13}C NMR spectra (75 MHz, shifts referenced to internal solvent) were recorded with Bruker AM 300. The assignment of resonances in the ^{1}H and ^{13}C NMR spectra of products was made by two-dimensional homonuclear and heteronuclear shift correlation experiments. Microanalyses were performed with a Fisons EA 1108 analyser.

4.2. General procedure for acetylferuloylation of 1–6

4.2.1. Procedure A using triethylamine/dimethylaminopyridine

Di-*O*-acetates of 4-nitrophenyl glycosides **1**–**6**[34,35] (0.355 g, 1 mmol) and 4-*O*-acetylferuloyl chloride[25,27] (0.306 g, 1.2 mmol) were dissolved in dry CH_2Cl_2 (10 ml). Triethylamine (0.139 ml, 1 mmol) and 4-dimethylaminopyridine (0.031 g, 0.25 mmol) were added at 0 °C. The reaction mixture was then stirred for 3 h at laboratory temperature. Then the mixture was diluted with CH_2Cl_2 (40 ml), washed with 1% HCl (10 ml), water (2 × 20 ml), dried over Na_2SO_4 and concentrated under reduced pressure. Products **7–12** were isolated by column chromatography of the residues on silica gel (toluene/EtOAc, 2:1).

4.2.1.1. *p*-Nitrophenyl 2-*O*-(4′-*O*-acetylferuloyl)-3,5-di-*O*-acetyl-α-L-arabinofuranoside (7). The starting compound **1** gave **7** (0.516 g, 90%) as white solid crystals; mp 126–128 °C (EtOH); $[\alpha]_D^{20}$ −90 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.22 (d, 2H, *J* 9.2 Hz, H-3′, H-5′), 7.73 (d, 1H, *J* 16.0 Hz, H-A), 7.17 (d, 2H, *J* 9.2 Hz, H-2′, H-6′), 7.16–7.06 (m, 3H, H-2″, H-5″, H-6″), 6.41 (d, 1H, H-B), 5.88 (s, 1H, H-1), 5.51 (s, 1H, H-2), 5.23 (bd, 1H, $J_{3,4}$ 2.9, H-3), 4.49–4.32 (m, 3H, H-4, H-5a, H-5b), 3.88 (s, 3H, OCH_3), 2.33 (s, 3H, $COCH_3$), 2.18 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 170.5 ($COCH_3$), 170.0 ($COCH_3$), 168.7 ($COCH_3$), 165.3 (O–C=O), 160.8 (C-1′), 151.5 (C-3″), 146.4 (C-A), 142.8 (C-4′), 142.0 (C-4″), 132.7 (C-1′), 2 × 125.8 (C-3′, C-5′), 123.4, 121.5 (C-6″, C-5″), 2 × 116.6 (C-2′, C-6′), 116.3 (C-B), 111.4 (C-2″), 103.9 (C-1), 82.2 (C-3), 81.2 (C-2), 76.8 (C-4), 63.0 (C-5), 55.9 (OCH_3), 20.7 (2 × $COCH_3$), 20.6 ($COCH_3$). Anal. Calcd for $C_{27}H_{27}NO_{13}$: C, 56.55; H, 4.75; N, 2.44. Found: C, 56.28; H, 5.02; N, 2.17.

4.2.1.2. *p*-Nitrophenyl 3-*O*-(4′-*O*-acetylferuloyl)-2,5-di-*O*-acetyl-α-L-arabinofuranoside (8). The title compound **8** (0.538 g, 94%) was obtained from diacetate **2** as a white foam; $[\alpha]_D^{20}$ −102 (*c* 1.0, $CHCl_3$); δ ^{1}H NMR ($CDCl_3$): δ 8.22 (d, 2H, *J* 9.3 Hz, H-3′, H-5′), 7.71 (d, 1H, *J* 16.0 Hz, H-A), 7.17 (d, 2H, *J* 9.3 Hz, H-2′, H-6′), 7.18–7.07 (m, 3H, H-2″, H-5″, H-6″), 6.43 (d, 1H, H-B), 5.82 (s, 1H, H-1), 5.49 (d, 1H, $J_{2,3}$ 1.4 Hz, H-2), 5.27 (dd, 1H, $J_{3,4}$ 4.4 Hz, H-3), 4.51–4.46 (m, 2H, H-4, H-5a), 4.34 (dd, 1H, $J_{5a,5b}$ 12.7, $J_{4,5b}$ 6.4 Hz, H-5b), 3.88 (s, 3H, OCH_3), 2.33 (s, 3H, $COCH_3$), 2.19 (s, 3H, $COCH_3$), 2.13 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 170.5 ($COCH_3$), 169.6 ($COCH_3$), 168.7 ($COCH_3$), 165.8 (O–C=O), 160.8 (C-1′), 151.5 (C-3″), 146.1 (C-A), 142.8 (C-4′), 141.9 (C-4″), 132.8 (C-1′), 2 × 125.8 (C-3′, C-5′), 123.4, 121.5 (C-6″, C-5″), 3 × 116.6 (C-2′, C-6′, C-B), 111.4 (C-2″), 103.9 (C-1), 82.2 (C-4), 81.1 (C-2), 77.2 (C-3), 63.0 (C-5), 56.0 (OCH_3), 20.7 ($COCH_3$), 20.6 (2 × $COCH_3$). Anal. Calcd for $C_{27}H_{27}NO_{13}$: C, 56.55; H, 4.75, N 2.44. Found: C, 56.68; H, 5.09; N, 2.28.

4.2.1.3. *p*-Nitrophenyl 5-*O*-(4′-*O*-acetylferuloyl)-2,3-di-*O*-acetyl-α-L-arabinofuranoside (9). The starting compound **9** (0.498 g, 87%) was obtained from **3** as a white solid; mp 78–80 °C (EtOH); $[\alpha]_D^{20}$ −56 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.21 (d, 2H, *J* 9.2 Hz, H-3′, H-5′), 7.69 (d, 1H, *J* 16.0 Hz, H-A), 7.16 (d, 2H, *J* 9.3 Hz, H-2′, H-6′), 7.14–7.05 (m, 3H, H-2″, H-5″, H-6″), 6.41 (d, 1H, H-B), 5.81 (s, 1H, H-1), 5.41 (d, 1H, $J_{2,3}$ 1.1 Hz, H-2), 5.21 (br d, 1H, $J_{3,4}$ 3.3 Hz, H-3), 4.59–4.41 (m, 3H, H-4, H-5a, H-5b), 3.86 (s, 3H, OCH_3), 2.32 (s, 3H, $COCH_3$), 2.17 (s, 3H, $COCH_3$), 2.15 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 170.0 ($COCH_3$), 169.5 ($COCH_3$), 168.7 ($COCH_3$), 166.2 (O–C=O), 160.8 (C-1′), 151.4 (C-3″), 145.1 (C-A), 142.7 (C-4′), 141.7 (C-4″), 133.0 (C-1′), 2 × 125.7 (C-3′, C-5′), 123.3, 121.3 (C-6″, C-5″), 117.3 (C-B), 2 × 116.5 (C-2′, C-6′), 111.3 (C-2″), 103.8 (C-1), 82.2 (C-4), 81.1 (C-2), 76.7 (C-3), 62.9 (C-5), 55.9 (OCH_3), 20.7 (2 × $COCH_3$), 20.6 ($COCH_3$). Anal. Calcd for $C_{27}H_{27}NO_{13}$: C, 56.55; H, 4.75; N, 2.44. Found: C, 56.54; H, 4.94; N, 2.22.

4.2.1.4. *p*-Nitrophenyl 2-*O*-(4′-*O*-acetylferuloyl)-3,4-di-*O*-acetyl-β-D-xylopyranoside (10). The compound **10** (0.517 g, 90%) was obtained from diacetate **4** as a white solid; mp 114–116 °C (EtOH);

$[\alpha]_D^{20}$ −23.0 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.21 (d, 2H, *J* 9.2 Hz, H-3′, H-5′), 7.71 (d, 1H, *J* 15.9 Hz, H-A), 7.11–7.07 (m, 5H, H-2′, H-6′, H-2″, H-5″, H-6″), 6.36 (d, 1H, *J* 15.9 Hz, H-B), 5.39–5.34 (m, 3H, H-2, H-1, H-3), 5.06 (m, 1H, H-4), 4.27 (dd, 1H, $J_{5a,5b}$ 12.3, $J_{4,5b}$ 4.3 Hz, H-5a), 3.86 (s, 3H, OCH_3), 3.66 (dd, 1H, $J_{4,5b}$ 6.7 Hz, H-5b), 2.32 (s, 3H, $COCH_3$), 2.11 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 2 × 169.8 (*C*OCH_3), 168.7 (*C*OCH_3), 165.0 (O–C=O), 161.1 (C-1′), 151.5 (C-3″), 146.1 (C-A), 143.1 (C-4′), 141.9 (C-4″), 132.7 (C-1′), 2 × 125.8 (C-3′, C-5′), 123.4, 121.6 (C-6″, C-5″), 2 × 116.6 (C-2′, C-6′), 116.5 (C-B), 111.3 (C-2″), 97.8 (C-1), 69.8, 69.4 (C-2, C-3), 68.2 (C-4), 62.0 (C-5), 55.9 (OCH_3), 20.8 (CO*C*H_3), 20.7 (CO*C*H_3), 20.6 (CO*C*H_3). Anal. Calcd for $C_{27}H_{27}NO_{13}$: C, 56.55; H, 4.75; N, 2.44. Found: C, 56.49; H, 5.05; N, 2.07.

4.2.1.5. *p*-Nitrophenyl 3-*O*-(4′-*O*-acetylferuloyl)-2,4-di-*O*-acetyl-β-D-xylopyranoside (11). The compound **11** was isolated from acetylferuloylation of diacetate **5** according to the general method described above. Due to slower process the reaction mixture was stirred for 6 h at laboratory temperature. The isolation of product was accomplished by the manner described above. The compound **11** (0. 510 g, 89%) was obtained from diacetate **5** as a white solid; mp 110–112 °C (from EtOH); $[\alpha]_D^{20}$ +14 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.23 (d, 2H, *J* 9.2 Hz, H-3′, H-5′), 7.70 (d, 1H, *J* 16.0 Hz, H-A), 7.16–7.07 (m, 5H, H-2′, H-6′, H-2″, H-5″, H-6″), 6.38 (d, 1H, *J* 16.0 Hz, H-B), 5.43–5.27 (m, 3H, H-2, H-1, H-3), 5.11 (m, 1H, H-4), 4.28 (dd, 1H, $J_{5a,5b}$ 12.2, $J_{4,5b}$ 4.5 Hz, H-5a), 3.89 (s, 3H, OCH_3), 3.66 (dd, 1H, $J_{4,5b}$ 7.0 Hz, H-5b), 2.33 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 169.8 (*C*OCH_3), 169.3 (*C*OCH_3), 168.7 (*C*OCH_3), 165.4 (O–C=O), 161.1 (C-1′), 151.5 (C-3″), 146.0 (C-A), 143.1 (C-4′), 141.9 (C-4″), 132.8 (C-1′), 2 × 125.8 (C-3′, C-5′), 123.4, 121.7 (C-6″, C-5″), 3 × 116.6 (C-2′, C-6′, C-B), 111.3 (C-2″), 97.8 (C-1), 70.1, 69.6 (C-2, C-3), 68.2 (C-4), 62.1 (C-5), 56.0 (OCH_3), 20.8 (CO*C*H_3), 20.6 (2 × CO*C*H_3). Anal. Calcd for $C_{27}H_{27}NO_{13}$: C, 56.55; H, 4.75; N, 2.44. Found: C, 56.81; H, 5.93 N, 2.60.

4.2.1.6. *p*-Nitrophenyl 4-*O*-(4′-*O*-acetylferuloyl)-2,3-di-*O*-acetyl-β-D-xylopyranoside (12). The title compound **12** (0.521 g, 91%), was obtained from **6** as a white solid; mp 181–183 °C (EtOH); $[\alpha]_D^{20}$ −52.0 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.22 (d, 2H, *J* 9.2 Hz, H-3′, H-5′), 7.69 (d, 1H, $J_{A,B}$ 15.9 Hz, H-A), 7.18–7.06 (m, 5H, H-2′, H-6′, H-2″, H-5″, H-6″), 6.38 (d, 1H, $J_{A,B}$ 15.9 Hz, H-B), 5.38–5.33 (m, 2H, H-1, H-3), 5.24 (dd, 1H, $J_{2,3}$ 7.3, $J_{1,2}$ 5.5 Hz, H-2), 5.13 (m, 1H, H-4), 4.32 (dd, 1H, $J_{5a,5b}$ 12.2, $J_{4,5a}$ 4.3 Hz, H-5a), 3.88 (s, 3H, OCH_3), 3.68 (dd, 1H, $J_{4,5b}$ 6.9 Hz, H-5b), 2.33 (s, 3H, $COCH_3$), 2.11 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$). ^{13}C NMR ($CDCl_3$): δ 169.8 (*C*OCH_3), 169.3 (*C*OCH_3), 168.7 (*C*OCH_3), 165.5 (O–C=O), 161.0 (C-1′), 151.5 (C-3″), 145.9 (C-A), 143.1 (C-4′), 141.9 (C-4″), 132.8 (C-1′), 2 × 125.8 (C-3′, C-5′), 123.4, 121.6 (C-6″, C-5″), 116.7 (C-B), 2 × 116.5 (C-2′, C-6′), 111.3 (C-2″), 97.6 (C-1), 69.8, 69.5 (C-2, C-3), 68.2 (C-4), 61.9 (C-5), 56.0 (OCH_3), 20.7 (3 × CO*C*H_3). Anal. Calcd for $C_{27}H_{27}NO_{13}$: C, 56.55; H, 4.75; N, 2.44. Found: C, 56.44; H, 5.09; N, 2.26.

4.2.2. Procedure B using zinc oxide

Di-*O*-acetates **4**–**6** (0.1725 g, 0.5 mmol) and 4-*O*-acetylferuloyl chloride (0.153 g, 0.6 mmol) were dissolved in dry CH_2Cl_2 (2 ml). Zinc oxide (0.02 g) was added in one portion. The heterogeneous mixture was then stirred for 1 h at laboratory temperature. Then the reaction mixture was diluted with CH_2Cl_2 (10 ml) and filtered. After concentration under reduced pressure, the residues were purified by column chromatography on silica gel (toluene/EtOAc, 3.5:1) to afford **10** (0.226 g, 79%), **11** (0. 221 g, 77%) and **12** (0.229 g, 80%) as white solids. The physicochemical data were identical to those of compounds **10**–**12** described above.

4.3. General procedure for deacetylation of 7–12

The respective di-*O*-acetyl-mono-*O*-(4′-*O*-acetylferuloyl)-glycosides **7**–**12** (0.287 g, 0.5 mmol) were suspended in a dry solution of MeOH/CH_2Cl_2 (9:1, 10 ml), followed by addition of Bu_2SnO (0.250 g, 1 mmol) in one portion. The reaction mixture was then kept under slight reflux (60 °C) for 6 h. The resulting homogeneous mixture was concentrated under reduced pressure and the product was isolated by column chromatography using gradient toluene/EtOAc (2:1→0:1). Completely deacylated 4-nitrophenyl glycosides were also isolated as minor products. Their physicochemical data were in accordance with literature.[39,40] Moreover, minor migration products were isolated after deacetylation of **10** and **11**.

4.3.1. *p*-Nitrophenyl 2-*O*-feruloyl-α-L-arabinofuranoside (13)

The compound **13** (0.172 g, 77%) was obtained as a pale yellow solid; mp 81–83 °C (CH_2Cl_2); $[\alpha]_D^{20}$ −152 (*c* 1.0, CH_3OH); ^1H NMR ($CDCl_3$): δ 8.23 (d, 2H, *J* 9.3 Hz, H-3′, H-5′), 7.69 (d, 1H, $J_{A,B}$ 15.9 Hz, H-A), 7.16 (d, 2H, *J* 9.3 Hz, H-2′, H-6′), 7.10 (dd, 1H, $J_{5'',6''}$ 8.2, $J_{2'',6''}$ 1.8 Hz, H-6″), 7.03 (d, 1H, H-2″), 6.94 (d, 1H, H-5″), 6.32 (d, 1H, H-B), 5.93 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1), 5.31 (d, 1H, $J_{2,3}$ 2.1 Hz, H-2), 4.30 (m, 2H, H-3, H-4), 3.99 (dd, 1H, $J_{5a,5b}$ 12.6, $J_{4,5a}$ 2.4 Hz, H-5a), 3.95 (s, 3H, OCH_3), 3.84 (dd, 1H, $J_{4,5b}$ 3.0 Hz, H-5b). ^{13}C NMR ($CDCl_3$): δ 167.7 (O–C=O), 161.1 (C-1′), 148.7, 146.9, 142.6 (C-4′, C-4″, C-3″), 147.3 (C-A), 126.3 (C-1″), 2 × 125.8 (C-3′, C-5′), 123.6 (C-6″), 2 × 116.5 (C-2′, C-6′), 114.9 (C-5″), 113.3 (C-B), 109.5 (C-2″), 103.9 (C-1), 87.2 (C-2), 84.3, 76.2 (C-3, C-4), 61.2 (C-5), 56.0 (OCH_3). Anal. Calcd for $C_{21}H_{21}NO_{10}$: C, 56.38; H, 4.73; N, 3.13. Found: C, 56.09; H, 5.04; N, 2.82. The minor deacylated product *p*-nitrophenyl α-L-arabinofuranoside was also isolated (0.016 g, 12%).

4.3.2. *p*-Nitrophenyl 3-*O*-feruloyl-α-L-arabinofuranoside (14)

The compound **8** afforded product **14** (0.176 g, 79%) as a pale yellow foam; $[\alpha]_D^{20}$ −174 (*c* 1.0, CH_3OH); ^1H NMR ($CDCl_3$): δ 8.21 (d, 2H, *J* 9.2 Hz, H-3′, H-5′), 7.68 (d, 1H, $J_{A,B}$ 15.9 Hz, H-A), 7.14 (d, 2H, *J* 9.2 Hz, H-2′, H-6′), 7.11 (dd, 1H, $J_{5'',6''}$ 8.2, $J_{2'',6''}$ 1.8 Hz, H-6″), 7.04 (d, 1H, H-2″), 6.95 (d, 1H, H-5″), 6.33 (d, 1H, H-B), 5.82 (s, 1H, H-1), 5.12 (dd, 1H, $J_{2,3}$ 1.7, $J_{3,4}$ 4.4 Hz, H-3), 4.53 (d, 1H, H-2), 4.44–4.41 (m, 1H, H-4), 3.99–3.95 (m, 2H, H-5a, H-5b), 3.95 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$): δ 167.5 (O–C=O), 161.3 (C-1′), 148.6, 146.8, 142.5 (C-4′, C-4″, C-3″), 146.8 (C-A), 126.5 (C-1″), 2 × 125.8 (C-3′, C-5′), 123.5 (C-6″), 2 × 116.4 (C-2′, C-6′), 114.9 (C-5″), 113.8 (C-B), 109.5 (C-2″), 106.4 (C-1), 84.6 (C-4), 79.9 (C-3), 79.8 (C-2), 61.6 (C-5), 56.0 (OCH_3). Anal. Calcd for $C_{21}H_{21}NO_{10}$: C, 56.38; H, 4.73; N, 3.13. Found: C, 56.74; H, 4.93; N, 2.84. The minor deacylated product *p*-nitrophenyl α-L-arabinofuranoside was isolated (0.015 g, 11%).

4.3.3. *p*-Nitrophenyl 5-*O*-feruloyl-α-L-arabinofuranoside (15)

The compound **15** (0.191 g, 85%) was obtained as a pale yellow solid; mp 140–142 °C (EtOAc/toluene); $[\alpha]_D^{20}$ −87 (*c* 1.0, CH_3OH); ^1H NMR (CD_3OD): δ 8.20 (d, 2H, *J* 9.2 Hz, H-3′, H-5′), 7.63 (d, 1H, $J_{A,B}$ 15.9 Hz, H-A), 7.20 (d, 2H, *J* 9.3 Hz, H-2′, H-6′), 7.17 (d, 1H, $J_{2'',6''}$ 1.6 Hz, H-2″), 7.05 (dd, 1H, $J_{5'',6''}$ 8.2, $J_{2'',6''}$ 1.7 Hz, H-6″), 6.79 (d, 1H, H-5″), 6.38 (d, 1H, H-B), 5.70 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.44 (dd, 1H, $J_{5a,5b}$ 11.7, $J_{4,5a}$ 3.2 Hz, H-5a), 4.35–4.23 (m, 3H, H-2, H-4, H-5b), 4.06 (dd, 1H, $J_{2,3}$ 3.9, $J_{3,4}$ 6.2 Hz, H-3), 3.87 (s, 3H, OCH_3). ^{13}C NMR (CD_3OD): δ 168.9 (O–C=O), 163.3 (C-1′), 150.8, 149.4, 143.7 (C-4′, C-4″, C-3″), 147.4 (C-A), 127.7 (C-1″), 2 × 126.7 (C-3′, C-5′), 124.3 (C-6″), 2 × 117.7 (C-2′, C-6′), 116.5 (C-5″), 115.1 (C-B), 111.7 (C-2″), 107.8 (C-1), 84.2 (C-4), 83.7 (C-3), 78.9 (C-2), 64.8 (C-5), 56.5 (OCH_3). Anal. Calcd for $C_{21}H_{21}NO_{10}$: C, 56.38; H, 4.73; N, 3.13. Found: C, 56.24; H, 5.02; N, 2.93. Minor *p*-nitrophenyl α-L-arabinofuranoside (0.012 g, 9%) as a product of total deacylation was also isolated.

4.3.4. *p*-Nitrophenyl 2-*O*-feruloyl-β-D-xylopyranoside (16)

The compound **10** afforded product **16** (0.174 g, 78%) as a pale yellow solid; mp 170–171 °C (EtOAc/cyclohexane); $[\alpha]_D^{20}$ −31.0 (*c* 1.0, CH_3OH); ^1H NMR (CD_3OD): δ 8.18 (d, 2H, *J* 9.3 Hz, H-3′, H-5′), 7.66 (d, 1H, $J_{A,B}$ 15.9 Hz, H-A), 7.17 (d, 1H, $J_{2'',6''}$ 1.8 Hz, H-2″), 7.14 (d, 2H, *J* 9.3 Hz, H-2′, H-6′), 7.06 (dd, 1H, $J_{5'',6''}$ 8.2, $J_{2'',6''}$ 1.9 Hz, H-6″), 6.79 (d, 1H, H-5″), 6.39 (d, 1H, H-B), 5.31 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 5.11 (dd, 1H, $J_{2,3}$ 9.0, $J_{1,2}$ 7.6 Hz, H-2), 4.03 (dd, 1H, $J_{5a,5b}$ 11.3, $J_{4,5a}$ 4.3 Hz, H-5a), 3.72–3.68 (m, 2H, H-3, H-4), 3.86 (s, 3H, OCH_3), 3.52 (dd, 1H, $J_{4,5b}$ 9.8 Hz, H-5b). ^{13}C NMR (CD_3OD): δ 168.2 (O–C=O), 163.3 (C-1′), 150.8, 149.4, 144.2 (C-4′, C-4″, C-3″), 147.6 (C-A), 127.7 (C-1″), 2 × 126.7 (C-3′, C-5′), 124.3 (C-6″), 2 × 117.7 (C-2′, C-6′), 116.5 (C-5″), 115.1 (C-B), 111.8 (C-2″), 100.3 (C-1), 74.5 (C-2), 75.7, 70.9 (C-3, C-4), 67.1 (C-5), 56.5 (OCH_3). Anal. Calcd for $C_{21}H_{21}NO_{10}$: C, 56.38; H, 4.73; N, 3.13. Found: C, 56.33; H, 4.74; N, 3.13. The migration product **17** (0.014 g, 6%) and deacylated *p*-nitrophenyl β-D-xylopyranoside (0.013 g, 10%) were isolated as minor products.

4.3.5. *p*-Nitrophenyl 3-*O*-feruloyl-β-D-xylopyranoside (17)

The compound **17** was obtained from **11** as a pale yellow solid (0.141 g, 63%); mp 176–178 °C (CH_2Cl_2); $[\alpha]_D^{20}$ +67.0 (*c* 1.0, CH_3OH); ^1H NMR (CD_3OD): δ 8.22 (d, 2H, *J* 9.2 Hz, H-3′, H-5′), 7.69 (d, 1H, $J_{A,B}$ 15.9 Hz, H-A), 7.22 (d, 2H, *J* 9.2 Hz, H-2′, H-6′), 7.21 (d, 1H, $J_{2'',6''}$ 1.8 Hz, H-2″), 7.10 (dd, 1H, $J_{5'',6''}$ 8.2, $J_{2'',6''}$ 1.8 Hz, H-6″), 6.82 (d, 1H, H-5″), 6.45 (d, 1H, H-B), 5.19 (d, 1H, $J_{1,2}$ 7.3 Hz, H-1), 5.12 (dd, 1H, $J_{2,3}$ 9.0, $J_{3,4}$ 9.0 Hz, H-3), 4.03 (dd, 1H, $J_{5a,5b}$ 11.4, $J_{4,5a}$ 5.2 Hz, H-5a), 3.90 (s, 3H, OCH_3), 3.83 (m, 1H, H-4), 3.72 (dd, 1H, H-2), 3.58 (dd, 1H, $J_{4,5b}$ 10.0 Hz, H-5b). ^{13}C NMR (CD_3OD): δ 168.9 (O–C=O), 163.6 (C-1′), 150.7, 149.5, 144.1 (C-4′, C-4″, C-3″), 147.1 (C-A), 127.9 (C-1″), 2 × 126.7 (C-3′, C-5′), 124.1 (C-6″), 2 × 117.7 (C-2′, C-6′), 116.6 (C-5″), 115.7 (C-B), 111.9 (C-2″), 102.0 (C-1), 78.1 (C-3), 72.7 (C-2), 69.3 (C-4), 66.9 (C-5), 56.5 (OCH_3). Anal. Calcd for $C_{21}H_{21}NO_{10}$: C, 56.38; H, 4.73; N, 3.13. Found: C, 56.09; H, 5.00; N, 2.87. The migration products **16** (0.020 g, 9%) and **18** (0.011 g, 5%) were isolated along with *p*-nitrophenyl β-D-xylopyranoside (0.018 g, 13%).

4.3.6. *p*-Nitrophenyl 4-*O*-feruloyl-β-D-xylopyranoside (18)

The starting compound **12** gave product **18** (0.188 g, 84%) as a pale yellow solid; mp 184–186 °C (EtOAc/cyclohexane); $[\alpha]_D^{20}$ −7.0 (*c* 1.0, CH_3OH); ^1H NMR (CD_3OD): δ 8.22 (d, 2H, *J* 9.2 Hz, H-3′, H-5′), 7.68 (d, 1H, $J_{A,B}$ 15.9 Hz, H-A), 7.23 (d, 2H, *J* 9.3 Hz, H-2′, H-6′), 7.20 (d, 1H, $J_{2'',6''}$ 1.6 Hz, H-2″), 7.09 (dd, 1H, $J_{5'',6''}$ 8.2, $J_{2'',6''}$ 1.8 Hz, H-6″), 6.82 (d, 1H, H-5″), 6.40 (d, 1H, H-B), 5.13 (d, 1H, $J_{1,2}$ 7.3 Hz, H-1), 4.93–4.84 (m, 1H, H-4), 4.13 (dd, 1H, $J_{5a,5b}$ 11.5, $J_{4,5a}$ 5.3 Hz, H-5a), 3.90 (s, 3H, OCH_3), 3.79 (dd, 1H, $J_{2,3}$ 9.0, $J_{3,4}$ 9.0 Hz, H-3), 3.62 (dd, 1H, H-2), 3.57 (dd, 1H, $J_{4,5b}$ 9.7 Hz, H-5b). ^{13}C NMR (CD_3OD): δ 168.5 (O–C=O), 163.7 (C-1′), 150.9, 149.5, 144.1 (C-4′, C-4″, C-3″), 147.6 (C-A), 127.7 (C-1″), 2 × 126.7 (C-3′, C-5′), 124.3 (C-6″), 2 × 117.7 (C-2′, C-6′), 116.6 (C-5″), 115.0 (C-B), 111.9 (C-2″), 102.1 (C-1), 74.7, 74.6 (C-2, C-3), 72.8 (C-4), 64.0 (C-5), 56.5 (OCH_3). Anal. Calcd for $C_{21}H_{21}NO_{10}$: C, 56.38; H, 4.73; N, 3.13. Found: C, 55.99; H, 5.11; N, 2.93. The deacylated product *p*-nitrophenyl β-D-xylopyranoside (0.011 g, 8%) was also isolated.

Acknowledgements

This work was supported by the Slovak Grant Agency for Science VEGA No. 2/0145/08 and the Slovak State Programme Project No. 2003 SP 200280203.

References

1. Williamson, G.; Kroon, P. A.; Faulds, C. B. *Microbiology* **1998**, *144*, 2011–2023.
2. Crepin, V. F.; Faulds, C. B.; Connerton, I. F. *Appl. Microbiol. Biotechnol.* **2004**, *63*, 647–652.
3. Topakas, E.; Vafiadi, C.; Christakopoulos, P. *Process Biochem.* **2007**, *42*, 497–509.
4. Fazary, A. E.; Ju, Y.-H. *Acta Biochim. Biophys. Sin.* **2007**, *39*, 811–828.
5. Koseki, T.; Fushinobu, S.; Ardiansyah; Shirakawa, H.; Komai, M. *Appl. Microbiol. Biotechnol.* **2009**, *84*, 803–810.
6. Fry, S. C. *Biochem. J.* **1982**, *203*, 493–504.
7. Colquhoun, I. J.; Ralet, M.-C.; Thibault, J.-F.; Faulds, C. B.; Williamson, G. *Carbohydr. Res.* **1994**, *263*, 243–256.
8. Ishii, T.; Hiroi, T. *Carbohydr. Res.* **1990**, *196*, 175–183.
9. Ishii, T.; Hiroi, T.; Thomas, J. R. *Phytochemistry* **1990**, *29*, 1999–2003.
10. Kato, Y.; Nevins, D. J. *Carbohydr. Res.* **1985**, *137*, 139–150.
11. Kato, A.; Azuma, J. I.; Koshijima, T. *Chem. Lett.* **1983**, 137–140.
12. Smith, M. M.; Hartley, R. D. *Carbohydr. Res.* **1983**, *118*, 65–80.
13. Kroon, P. A.; Faulds, C. B.; Brézillon, Ch.; Wiliamson, G. *Eur. J. Biochem.* **1997**, *248*, 241–245.
14. Topakas, E.; Christakopoulos, P.; Faulds, C. B. *J. Biotechnol.* **2005**, *115*, 355–366.
15. Ralet, M.-C.; Faulds, C. B.; Williamson, G.; Thibault, J.-F. *Carbohydr. Res.* **1994**, *263*, 257–269.
16. Faulds, C. B.; Kroon, P. A.; Saulnier, L.; Thibault, J. F.; Williamson, G. *Carbohydr. Polym.* **1995**, *27*, 187–190.
17. Kroon, P. A.; Garcia-Conesa, M. T.; Fillingham, I. J.; Hazlewood, G. P.; Williamson, G. *J. Sci. Food Agric.* **1999**, *79*, 428–434.
18. Kroon, P. A.; Williamson, G.; Fish, N. M.; Archer, D. B.; Belshaw, N. J. *Eur. J. Biochem.* **2000**, *267*, 6740–6752.
19. Saulnier, L.; Marot, C.; Elgorriaga, M.; Bonnin, E.; Thibault, J.-F. *Carbohydr. Polym.* **2001**, *45*, 269–275.
20. Faulds, C. B.; Sancho, A. I.; Bartolome, B. *Appl. Microbiol. Biotechnol.* **2002**, *60*, 489–494.
21. Faulds, C. B.; Mandalari, G.; LoCurto, R.; Bisignano, G.; Waldron, K. W. *Appl. Microbiol. Biotechnol.* **2004**, *64*, 644–650.
22. Faulds, C. B.; Mandalari, G.; LoCurto, R.; Bisignano, G.; Christakopoulos, P.; Waldron, K. W. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 622–629.
23. Shin, H.-D.; McClendon, S.; Le, T.; Taylor, F.; Chen, R. R. *Biotechnol. Bioeng.* **2006**, *95*, 1108–1115.
24. Biely, P.; Mastihubová, M.; van Zyl, W. H.; Prior, B. A. *Anal. Biochem.* **2002**, *311*, 68–75.
25. Mastihubová, M.; Mastihuba, V.; Kremnický, L.; Willet, J. L.; Côté, G. L. *Synlett* **2001**, 1559–1560.
26. Marmuse, L.; Asther, M.; Navarro, D.; Lesage-Meessen, L.; Asther, M.; Fort, S.; Driguez, H. *Carbohydr. Res.* **2007**, *342*, 2316–2321.
27. Hatfield, R. D.; Helm, R. F.; Ralph, J. *Anal. Biochem.* **1991**, *194*, 25–33.
28. Helm, R. F.; Ralph, J.; Hatfield, R. D. *Carbohydr. Res.* **1992**, *229*, 183–194.
29. Mastihubová, M.; Szemesová, J.; Biely, P. *Tetrahedron Lett.* **2003**, *44*, 1671–1673.
30. Marmuse, L.; Asther, M.; Fabre, E.; Navarro, D.; Lesage-Meessen, L.; Asther, M.; O'Donohue, M.; Fort, S.; Driguez, H. *Org. Biomol. Chem.* **2008**, *6*, 1208–1214.
31. Mastihuba, V.; Kremnický, L.; Mastihubová, M.; Willett, J. L.; Côté, G. L. *Anal. Biochem.* **2002**, *309*, 96–101.
32. Puchart, V.; Vršanská, M.; Mastihubová, M.; Topakas, E.; Vafiadi, Ch.; Faulds, C. B.; Tenkanen, M.; Christakopoulos, P.; Biely, P. *J. Biotechnol.* **2007**, *127*, 235–243.
33. Helm, R. F.; Ralph, J. *J. Org. Chem.* **1991**, *56*, 7015–7021.
34. Mastihubová, M.; Szemesová, J.; Biely, P. *Bioorg. Med. Chem.* **2006**, *14*, 1805–1810.
35. Mastihubová, M.; Biely, P. *Carbohydr. Res.* **2004**, *339*, 1353–1360.
36. Hosseini-Sarvari, M.; Sharghi, H. *Tetrahedron* **2005**, *61*, 10903–10907.
37. Tamaddon, F.; Amrollahi, M. H.; Sharafat, L. *Tetrahedron Lett.* **2005**, *46*, 7841–7844.
38. Liu, H.-M.; Yan, X.; Li, W.; Huang, C. *Carbohydr. Res.* **2002**, *337*, 1763–1767.
39. Fielding, A. H.; Hough, L. *Carbohydr. Res.* **1965**, *1*, 327–329.
40. Takeo, K.; Ohguchi, Y.; Hasegawa, R.; Kitamura, S. *Carbohydr. Res.* **1995**, *277*, 231–244.