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Two efficient ways to 2-*O*- and 5-*O*-feruloylated 4-nitrophenyl α-L-arabinofuranosides as substrates for differentiation of feruloyl esterases

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Abstract—4-Nitrophenyl 2-*O*-(*E*)-feruloyl-α-L-arabinofuranoside **1** and 4-nitrophenyl 5-*O*-(*E*)-feruloyl-α-L-arabinofuranoside **2** have been synthesized by two different routes. Monoferuloylation was accomplished by a chemoenzymatic sequence employing a regioselective transesterification catalyzed by lipases. The feruloyl group was introduced to enzymatically prepared 2,3- and 3,5-diacetates of 4-nitrophenyl α-L-arabinofuranoside by reaction with 4-*O*-acetylferuloyl chloride. Removal of the protecting acetyl groups yielded **1** and **2**. An alternative chemical synthesis suitable for preparation of larger quantities of **1** and **2** also is presented. The new substrates represent convenient tools to differentiate feruloyl esterases on the basis of their substrate specificity.

The hydroxycinnamic acids (HCAs) associated with plant hemicelluloses play an important role in cell wall integrity and protection of plant tissues against digestion by plant-invading microorganisms.[1a,b] It is well documented that the most abundant HCA is ferulic acid, (*E*)-4-hydroxy-3-methoxycinnamic acid (FA). FA occurs as an ester-linked acid to the C-5 position of α-L-Ara*f* residues in arabinoxylans, to the C-2 position of α-L-Ara*f* residues in arabinan, to the C-6 position of β-D-Gal*p* residues in pectic substances and galactans, and to the C-4 position of D-Xyl*p* units of xyloglucans. Nature has designed enzymes that can attack ester linkages between HCAs and carbohydrates. Esterases liberating HCAs from plant cell walls have become common components of hemicellulolytic enzyme systems in a variety of microorganisms, and feruloyl esterase (FE) has also been reported to be endogenous to plants. A few reports indicate that microorganisms produce at least two types of FEs which differ in the affinity for 2-*O*- and 5-*O*-feruloylated α-L-Ara*f* residues.[2] Substrates for such differentiation of FEs have so far been isolated from plant cell walls. Their preparation involves enzymatic steps followed by tedious purification procedures.

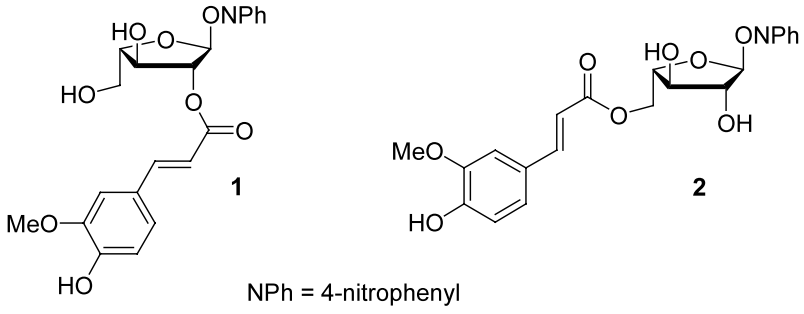

Figure 1.

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In this paper we report a synthesis of 4-nitrophenyl 2-*O*-(*E*)-feruloyl-α-L-arabinofuranoside **1** and 4-nitrophenyl 5-*O*-(*E*)-feruloyl-α-L-arabinofuranoside **2** (Fig. 1), as an alternative to the isolation of naturally occuring feruloylated oligosaccharides. We assume that the 4-nitrophenyl aglycon moiety in these compounds will mimic the second carbohydrate residue in a similar manner to 4-nitrophenyl glycosides, chromogenic substrates of glycosidases. The new FE substrates are intended to be used in an α-L-arabinofuranosidase-coupled photometric assays.[3]

The regioselective synthesis of monoferuloylated arabinofuranosides **1** and **2** requires the application of various mild protecting/deprotecting strategies with groups easily introduced or cleaved. For this purpose, we chose chemoenzymatic as well as classical chemical approaches. Commercially available 4-nitrophenyl α-L-arabinofuranoside[4] (NPh α-L-Ara*f*) **3** was used as the starting material in our synthesis.

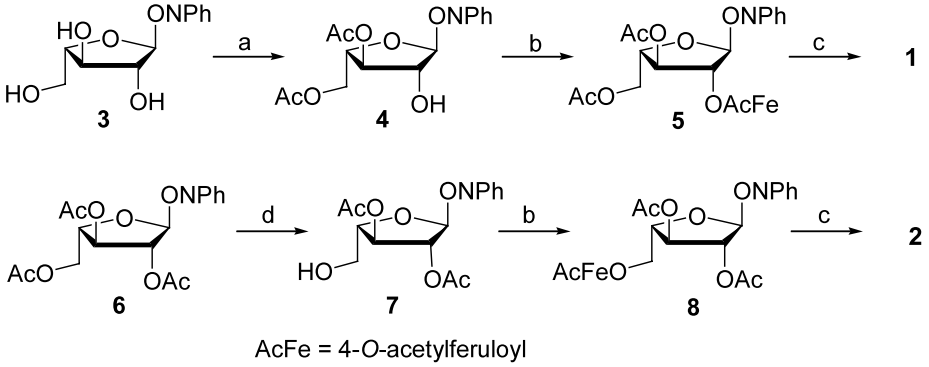
In order to shorten the lengthy protection/deprotection sequences usually associated with saccharide synthesis, the 2-*O*- and 5-*O*-monoferuloylated NPh α-L-Ara*f* **1** and **2** were first prepared by sequences employing highly selective enzyme reactions. The use of lipases or esterases for the regioselective acylation or deacylation of saccharide hydroxyl groups with comparable reactivity belongs to the classical tools of carbohydrate chemistry.[5a–c] In addition, enzymes work in neutral, weakly acidic or weakly basic media (pH range 4–8) and at room temperature, i.e. under conditions suitable for compounds sensitive to harsh chemical environments.

The 3,5-diacetate of NPh α-L-Ara*f* **4** which is used as an FA acceptor was obtained by selective acetylation of the corresponding glycoside **3** catalyzed by Lipase PS-30[6] (Amano) in vinyl acetate. The lipase catalyzed transesterification was carried out for 51 h whilst shaking at 40°C to afford **4**[7] in 75% yield. The 5-monoacetate and 2,5-diacetate of **3** were isolated by chromatography on silica gel in low yields as side products. The 2,3-diacetate **7**[7] was obtained in 60% yield by regioselective deacetylation of per-*O*-acetylated NPh α-L-Ara*f* **6** using Lipolyve CC[8] (Lyven) with shaking in a solution of 10% DMF in 0.1 M phosphate buffer (pH 6) at 37°C for 12 h (Scheme 1).

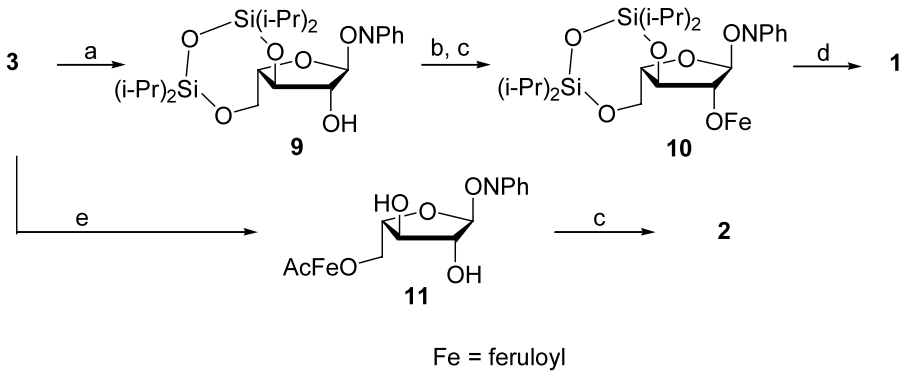
The free hydroxyl groups in diacetates **4** and **7** were esterified with 4-*O*-acetylferuloyl chloride[9] in DMAP, Et_3N, CH_2Cl_2 to give **5** and **8** in high yields. A similar feruloylation was applied in the preparation of 4-nitrophenyl ferulate,[10] a non-saccharide chromogenic FE substrate.[11] Next, we investigated chemoselective deacylation conditions, which cleaved only acetyl esters and preserved the ferulate ester bond. After unsuccessful attempts to deacetylate **5** upon mild treatment with pyrrolidine,[12] a literature survey revealed satisfying chemoselective and simple transesterification conditions.[13] Thus compounds **5** and **8** were deacetylated with a suspension of potassium carbonate in a 2:1 mixture of dichloromethane/methanol to give the products **1** and **2** in 67 and 76% yields as solids (Scheme 1).

Additionally, we synthesized **1** and **2** from **3**, by routes that are more suitable for large-scale preparations. As illustrated in Scheme 2, the preparation of **1** started with protection of the 3 and 5 positions of furanoside **3** by selective simultaneous silylation with 1 equiv. of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[14a,b] in pyridine to give **9** in 85% yield. Introduction of the feruloyl group to position 2 of **9** was achieved by reaction with 4-*O*-acetylferuloyl chloride in DMAP, Et_3N, CH_2Cl_2. Subsequent deacetylation with pyrrolidine in dichloromethane afforded **10** in high yield. Deprotective desilylation of **10** with nBu$_4$NF in THF afforded **1**[15] in 78% yield.

Due to the higher sensitivity of nitrophenyl aglycons to acidic, basic and aqueous conditions, we made use of the slightly higher reactivity of the primary hydroxy group towards acylation and coupled the NPh arabinofuranoside **3** with 4-*O*-acetylferuloyl chloride directly under mild reaction conditions.[9] After purification of **11** (54%) from the starting compound and by-products by chromatography on silica gel, the acetyl protecting group was removed with 10 equiv. of pyrrolidine in dichloromethane to give monoferuloylated **2**[16] in 90% yield.



Scheme 1. *Reagents and conditions*: (a) Lipase PS, vinyl acetate, 40°C, 51 h, 75%; (b) 4-*O*-acetylferuloyl chloride, DMAP, Et_3N, CH_2Cl_2, rt, 3 h, 90% for **5** and 92% for **8**; (c) K_2CO_3 (2 equiv.), 2:1 CH_2Cl_2/MeOH, /−5/→10°C, 5 h, 67% for **1** and 76% for **2**; (d) Lipolyve CC, 10% DMF in 0.1 M phosphate buffer pH 6, 37°C, 12 h, 60%.



Scheme 2. *Reagents and conditions*: (a) $(Cl(i\text{-}Pr)_2Si)_2O$, pyridine, rt, 6 h, 85%; (b) 4-*O*-acetylferuloyl chloride (1.2 equiv.), DMAP (0.25 equiv.), Et_3N (1 equiv.), CH_2Cl_2, rt, 3 h, 91%, (c) pyrrolidine (10 equiv.), CH_2Cl_2, rt, 2 h, 86% for **10** and 90% for **2**; (d) n-Bu_4F, THF, rt, 1 h, 78%; (e) 4-*O*-acetylferuloyl chloride, toluene, pyridine, /−5/°C for 3 h than 4°C overnight, 54%.

In conclusion, two methods for the synthesis of 4-nitrophenyl 2-*O*-(*E*)-feruloyl-α-L-arabinofuranoside **1** and 4-nitrophenyl 5-*O*-(*E*)-feruloyl-α-L-arabinofuranoside **2** are elaborated. The first, a chemoenzymatic route, seems to be simple and short, however, it requires large quantitites of enzymes. The second, a chemical route, which is more efficient and economically more feasible, is suitable for the preparation of larger quantities of **1** and **2**. Compounds **1** and **2** were found to be convenient substrates for determination of activity and differentiation of FeEs according to substrate specificity in a UV-spectrophotometric assay.[3] The coupling of the action of FeEs with α-L-arabinofuranosidase renders **1** and **2** chromogenic substrates of FeEs.[3]

Acknowledgements

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6. Lipase from *Burkholderia cepacia* (originally classified as *Pseudomonas cepacia*), LPSAZ0452412.
7. Compound **4**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.12 (s, 3H, $COCH_3$), 2.18 (s, 3H, $COCH_3$), 3.49 (bs, 1H, OH), 4.29 (dd, 1H, $J_{4,5a}=4.8$, $J_{5a,5b}=12.2$ Hz, H-5a), 4.41 (dd, 1H, $J_{4,5b}=3.0$ Hz, $J_{5a,5b}=12.2$ Hz, H-5b), 4.41–4.46 (m, 1H, H-4), 4.49 (bd, 1H, $J_{2,3}=2.5$ Hz, H-2), 4.84 (dd, 1H, $J_{3,4}=5.8$ Hz, H-3), 5.77 (s, 1H, H-1), 7.16 (dt, 2H, $J=2.2$, 3.3, and 9.3 Hz, NPh), 8.20 (dt, 2H, $J=2.2$, 3.3, and 9.3 Hz, NPh).
   Compound **7**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15 (s, 3H, $COCH_3$), 2.16 (s, 3H, $COCH_3$), 3.84 (dd, 1H, $J_{4,5a}=4.1$, $J_{5a,5b}=12.3$ Hz, H-5a), 3.92 (dd, 1H, $J_{4,5b}=3.5$ Hz, H-5b), 4.25 (bdd, 1H, $J_{4,5a}=4.1$, $J_{4,5b}=3.5$ Hz, H-4), 5.20 (dd, 1H, $J_{2,3}=1.8$, $J_{3,4}=5.1$ Hz, H-3), 5.43 (d, 1H, $J_{2,3}=1.8$ Hz, H-2), 5.79 (s, 1H, H-1), 7.15 (dt, 2H, $J=2.2$, 3.3, and 9.3 Hz, NPh), 8.21 (dt, 2H, $J=2.2$, 3.3, and 9.3 Hz, NPh).
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15. Compound **1**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (dd, 1H, $J_{4,5a}=2.9$, $J_{5a,5b}=12.5$ Hz, H-5a), 3.92 (s, 3H, OCH_3), 3.97 (dd, 1H, $J_{4,5b}=2.5$, $J_{5a,5b}=12.5$ Hz, H-5b), 4.25–4.33 (m, 2H, H-3, 4), 5.31 (bd, 1H, $J_{2,3}=2.0$ Hz, H-2), 5.90 (s, 1H, H-1), 6.30 (d, 1H, $J_{A,B}=15.9$ Hz, H-A), 6.92 (d, 1H, $J_{5',6'}=8.2$ Hz, H-5′), 7.02 (d, 1H, $J_{2',6'}=1.7$ Hz, H-2′), 7.07 (dd, 1H, $J_{2',6'}=1.7$, $J_{5',6'}=8.2$ Hz, H-6′), 7.15 (dt, 2H, $J=2.2$, 3.3 and 9.3 Hz, NPh), 7.67 (d, 1H, $J_{A,B}=15.9$ Hz, H-B), 8.20 (dt, 2H, $J=2.2$, 3.3, and 9.3 Hz, NPh).
16. Compound **2**: ^{1}H NMR (300 MHz, CD_3OD): δ 3.87 (s, 3H, OCH_3), 4.06 (dd, 1H, $J_{2,3}=3.9$, $J_{3,4}=6.2$ Hz, H-3), 4.23–4.35 (m, 3H, H-2, 4, 5a), 4.44 (dd, 1H, $J_{4,5b}=3.2$, $J_{5a,5b}=11.7$ Hz, H-5b), 5.70 (d, 1H, $J_{1,2}=1.5$ Hz, H-1), 6.38 (d, 1H, $J_{A,B}=15.9$ Hz, H-A), 6.79 (d, 1H, $J_{5',6'}=8.2$ Hz, H-5′), 7.05 (dd, 1H, $J_{2',6'}=1.7$, $J_{5',6'}=8.2$ Hz, H-6′), 7.17 (d, 1H, $J_{2',6'}=1.7$ Hz, H-2′), 7.20 (d, 2H, $J=9.2$ Hz, NPh), 7.63 (d, 1H, $J_{A,B}=15.9$ Hz, H-B), 8.20 (d, 2H, $J=9.2$ Hz, NPh).