Vinyl glycosides in oligosaccharide synthesis (part 4): glycosidase-catalysed preparation of substituted allyl glycosides

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But-3-en-2-yl glycosides have been obtained by glycosidase-catalysed transformations under thermodynamic conditions, and reaction parameters have been optimised. It has been shown that the enzymes can be immobilised on non-ionic Amberlite resin (XAD-4) and can conveniently be retrieved and re-used in a subsequent glycosylation. The enzyme-catalysed reactions display some diastereodifferentiation with a preference for the (*R*)- over the (*S*)-alcohol. An increase in size of the aliphatic substituent on the allyl alcohol gives a significant improvement of diastereoselection.

Introduction

Recently, we reported a novel latent-active glycosylation strategy based on the isomerisation of substituted allyl to vinyl glycosides which, in turn, can be glycosylated under mild Lewis acid conditions.[1] In this glycosylation strategy, the anomeric allyl group first acts as an anomeric protecting group (latent) but can be converted efficiently into a leaving group by isomerisation to a but-2-en-2-yl glycoside (active). The latter glycosyl donor can be coupled to a suitably protected but-3-en-2-yl glycosyl acceptor (latent) and the resulting disaccharide converted into a glycosyl donor by isomerisation of the but-3-en-2-yl moiety or into a glycosyl acceptor by selective removal of a protecting group. This strategy is particularly suitable for the preparation of oligosaccharide libraries and we have shown that four common allyl glycosyl building blocks can, in principle, be converted into 256 trisaccharides.[2]

The substituted vinyl glycosides were also shown to be suitable substrates for the preparation of anomeric phosphates[3] and the new methodology was applied to the synthesis of fluorinated uridine diphosphate (UDP)-galactopyranose derivatives. These compounds were used to investigate the enzymic interconversion of UDP-galactopyranose and UDP-galactofuranose at a molecular level.[4]

The isomerisations have been performed by a new isomerisation procedure[5] involving the treatment of tris[triphenylphosphine]rhodium(I) chloride with *n*-butyllithium. The rhodium-H catalyst formed is able to isomerise a wide range of substituted and unsubstituted allyl ethers and glycosides and has many advantageous properties over the use of conventional Wilkinson's catalyst.

The substituted allyl glycosides have been prepared by a modified Koenings–Knorr approach which involves the glycosylation of an acetoxy bromide with but-3-en-2-ol **2** in the presence of mercury salts.[1] This procedure is rather laborious and problematic from a health and environmental point of view.

We report here the enzymic synthesis of but-3-en-2-yl glycosides utilising glycosidases (glycosyl hydrolases) under reverse-hydrolysis conditions. Nature employs glycosidases for the degradation of oligosaccharides. However, the equilibrium of these reactions can be reversed to allow glycosidic-bond formation and these transformations have been accomplished under kinetically (trans-glycosylation)[6] and thermodynamically (reverse-hydrolysis)[7] controlled conditions. The thermodynamic procedures are based on a shift of the reaction equilibrium which normally lies in favour of the hydrolysis of a glycosidic linkage in aqueous medium, towards synthesis. Thermodynamic considerations indicate that this shift in equilibrium can be achieved by either increasing the substrate concentration or decreasing the water content of the reaction solvent. The best results have been obtained by using an alcoholic solution containing a small proportion of water.

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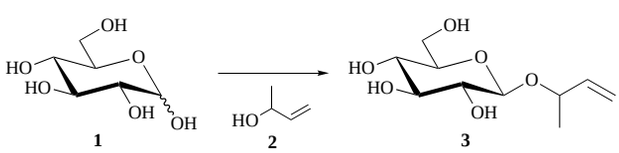
Results and discussion

The transformation of glucose **1** into but-3-en-2-yl glucoside catalysed by the enzyme almond β-glucosidase (Scheme 1) has



Scheme 1 *Reagents:* Almond β-glucosidase (catalyst), aq. **2**

been studied and several parameters optimised. In the first instance, formation of glucoside **3** was examined as a function of the water content of the reaction medium. It has been reported that these glycosidase-catalysed glycosylations require a small amount of water to keep the protein properly hydrated and catalytically active.[7a,8] However, the amount of water needed depends on the polarity of the alcohol used and in general the more hydrophilic the alcohol the more water is needed to maintain adequate hydration of the enzyme. The experiments were performed with the enzyme supported on a non-ionic Amberlite resin (XAD-4). Such an experimental procedure allows the enzyme to be conveniently retrieved from the reaction mixture and re-used in a subsequent experiment. In this respect, it is important to note that some glycosidases are relatively expensive and therefore the ability to recover and re-use them is desirable. Thus, the supported glucosidase (100 mg enzyme/1 g resin) was added to a mixture of glucose and but-3-en-2-ol containing different amounts of water. The reaction mixtures were stirred for 48 h at 50 °C, after which they were filtered, the filtrates concentrated, and the residues subjected to silica gel flash column chromatography to give pure glycosides **3**. As can be seen from Table 1, an optimal conversion was obtained with a water content between 5 and 15%. The pH dependence of these transformations was also investigated. An acetate/phosphate buffer was included during the immobilisation of the enzyme; however, no significant differences in conversion were observed in the pH range 5.0–7.0. It has been

Table 1 Yield against water content of (*R*/*S*)-but-3-en-2-ol

Water content (% v/v)	Yield (%)
0	0
2.5	9.2
5	26.2
10	27.0
15	26.0
20	15.5

Reaction conditions: supported β-glucosidase, D-glucose (0.05 g ml^{-1}); 50 °C; 48 h. Each experiment was performed three times.

Table 2 Kinetic data of the transformation with the supported and unsupported enzyme

	Yield (%)	
Time (*t*/h)	Unsupported[a]	Supported[b]
3	9.7	4.7
6	13.9	7.7
12	18.8	12.5
24	23.1	18.8
48	24.2	25.5
96	24.1	26.1

[a] β-Glucosidase (100 mg), (*R*/*S*)-but-3-en-2-ol–water (20 ml; 9:1 v/v), D-glucose (0.05 g ml^{-1}), 50 °C, 48 h. [b] Supported β-glucosidase, (*R*/*S*)-but-3-en-2-ol–water (20 ml; 9:1 v/v), D-glucose (0.05 g ml^{-1}), 50 °C, 48 h. Each experiment was performed three times.

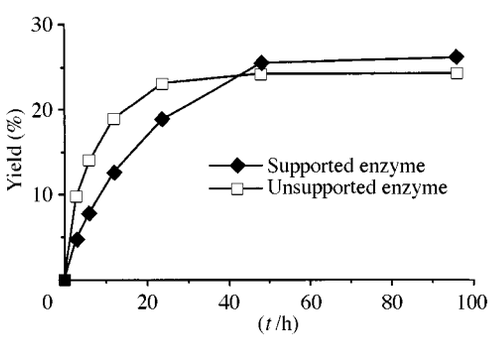


Fig. 1 Kinetic data for supported and unsupported enzymes

reported that pH = 6.0 is optimal for the preparation of glucosidic linkages by almond β-glucosidase in acetonitrile–water.[7b]

Next, we focused on the kinetic parameters of the transformation and the data from the use of supported enzyme and naked enzyme were compared. The reactions were performed at 50 °C with a water content of 10% and samples were taken at 3, 6, 12, 24, 48 and 96 h (Table 2). It was observed that the naked enzyme formed a hydrated mass in the alcoholic solvent, therefore it was expected that the transformation with the supported enzyme would be faster, as more of the enzyme would be exposed to the substrate. Surprisingly, the reaction catalysed by the naked enzyme had reached equilibrium after 24 h (Fig. 1). However, the reaction with the supported enzyme proceeded more slowly but after 48 h a somewhat higher conversion had been achieved. Possibly, the mass transport between the resin beads and solution is inefficient and slows down the transformation.

The effect of glucose concentration was examined as shown in Table 3 and the best yield was obtained for 0.5 g glucose in 20 ml of solvent. However, 1.0 g of glucose in 20 ml of solvent proved optimal in terms of glucoside retrieved. Solubility problems were encountered with higher concentrations of glucose which gave less efficient conversions and, in each case, a similar amount of product was isolated. Thus, under the conditions applied, 0.5 g glucose per 100 mg enzyme apparently represents the maximum number of turn-overs to be achieved for the pseudo-first order reaction. The transformation was also

Table 3 Yield against D-glucose concentration[a]

D-Glucose (g ml^{-1})	Yield (%)	Product (g)
0.012	34	0.121
0.025	40	0.279
0.038	33	0.351
0.050	27	0.379
0.100	14	0.391
0.150	7.8	0.330
0.200	5.7	0.323

[a] (*R*/*S*)-But-3-en-2-ol–water (20 ml; 9:1 v/v), 50 °C, 48 h. Each experiment was performed three times.

Table 4 Data for the re-use of the supported β-glucosidase enzyme

No. of times catalyst used[a]	Yield (%)
1	26.1
2	23.1
3	21.4
4	18.4

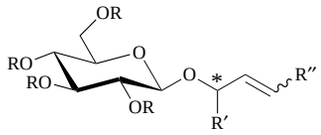
Conditions: (*R*/*S*)-but-3-en-2-ol–water (20 ml; 9:1 v/v), D-glucose (0.05 g ml^{-1}), 50 °C, 48 h. [a] Reaction mixture was filtered and retrieved catalyst was used immediately.

attempted in a mixture of acetonitrile–water–allyl alcohol; however, a disappointingly low yield of 5% was obtained.

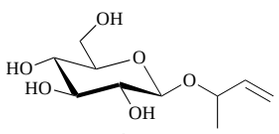
The potential for re-use of the supported enzyme was investigated. As can be seen from Table 4, each transformation results in some loss of activity; however, after 4 reaction cycles of 48 h at 50 °C sufficient catalytic activity had been retained to be synthetically useful. Attempted reactivation of the catalyst by treatment with 2-mercaptoethanol was unsuccessful. Thus, enzyme denaturation probably results from chain scissions and macro-unfolding rather than from disulfide cleavage.[9]

The ^{1}H NMR spectrum of the allyl glycoside **3** showed that some diastereodifferentiation had occurred and for all transformations the ratio between the (*R*)- and (*S*)-but-3-en-2-yl glucoside was 3:2. Thus, the enzyme has some preference for the (*R*)- over the (*S*)-alcohol. The assignments of the signals attributed to the (*R*)- and (*S*)-diastereoisomer was made by comparing the data of the product obtained by condensation of glucose with the pure (*R*)-alcohol. Enantiomerically pure but-3-en-2-ol **2** was easily prepared on a large scale by Sharpless epoxidation of (*E*)-but-2-en-1-ol followed by *in situ* tosylation of the epoxy alcohol and conversion of the iodide and subsequently reductive elimination by treatment with a Zn/Cu couple.[10] To the best of our knowledge, only one report deals with the resolution of a chiral secondary alcohol by β-glycosidase.[7b] However, this transformation was performed in a mixture of acetonitrile–water–alcohol and a very low yield of product was isolated. To study further the stereodifferentiation by the glucosidase, transformations were also performed with pent-1-en-3-ol, hex-1-en-3-ol and pent-3-en-2-ol (giving glycosides **4**, **5** and **6** respectively). As can be seen from Table 5, an increase in size of the aliphatic substituent on the allyl alcohol gave a significant improvement in diastereoselection. However, as expected, lower conversions were observed with the more bulky allyl alcohols. It is also important to note that the transformation with the (*R*)-but-3-en-2-ol (using 1 g glucose/20 ml solvent with 10% water) gave an improved conversion of 31% (product **7**). In this case, the aqueous (*R*)-but-3-en-2-ol solution could easily be reclaimed and re-used by filtration and careful distillation.

It has been reported that almond β-glucosidase can catalyse the coupling of galactose with several alcohols. However, treatment of a mixture of galactose–water–but-3-en-2-ol with the glucosidase gave no formation of product, and only galactose was reclaimed. Possibly, the previously reported procedure used a glucosidase that was contaminated with a galactosidase. Fortunately, the use of β-galactosidase from *Aspergillus*

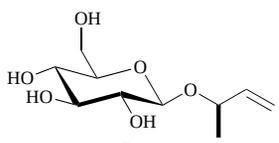
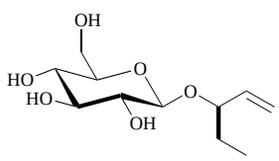
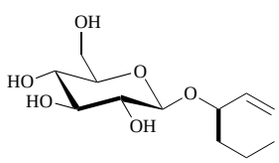
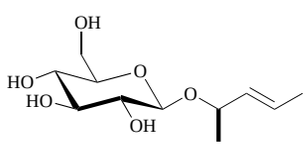


4 R = Ac, R′ = Et, R″ = H; * = *RS*
5 R = Ac, R′ = Pr, R″ = H; * = *RS*
6 R = H, R′ = R″ = Me; * = *RS*
7 R = R″ = H, R′ = Me; * = *R*



8

Table 5 Diastereodifferentiation by β-glucosidase (ex. almond)

Entry	Product	*R*:*S*	Yield (%)
1	 **7**	3:2	27.0
2		7:3	12.0[a]
3		9:1	5.0[a]
4	 (*R*)-**6**	13:6	17.0

[a] The products were isolated as the acetylated derivatives.

oryzae gave, under standard conditions, the expected galactoside **8** in 21% yield. In this case, some stereodifferentiation was also observed in favour of the (*R*)-isomer (*R*/*S* = 65/35). These conditions are not optimised.

Conclusions

We have demonstrated that glycosidase-catalysed transformations, under thermodynamic conditions, provide a powerful method for the synthesis of but-3-en-2-yl glycosides giving yields comparable to the 4-step synthesis, involving Koenigs–Knorr methodology, required previously. Immobilisation of the enzyme on a non-ionic Amberlite resin allows the enzyme to be retrieved and re-used in a subsequent transformation.This procedure allows gram-scale preparation and it is envisaged that it would be suitable for a continuous-batch-type process.

Experimental

General methods and materials

^{1}H, ^{1}H 2D-homonuclear chemical-shift correlation (COSY) (45) and ^{13}C NMR spectra were recorded on a Bruker AC300 spectrometer, equipped with a B-ACS 60 autochanger and an Aspect 3000 off-line editing computer. Chemical shifts were measured in ppm using tetramethylsilane as internal standard, and *J* values are given in Hz. Fast-atom bombardment (FAB) mass spectra were recorded using a VG Zabspec spectrometer with *m*-nitrobenzyl alcohol as matrix. Almond β-D-glucosidase (EC 3.2.1.21) (G-0395, 5.6 U/mg) and β-galactosidase (EC 3.2.1.23) from *A. oryzae*, (G-7138, 9 U/mg) were purchased from Sigma Chemical Co. (±)-But-3-en-2-ol was purchased from Fluka. Column chromatography was carried out on silica gel (Merck 7734). Flash silica ES70X was obtained from Crosfield Catalysts, Warrington, UK. TLC analysis was conducted on silica gel plates (Merck 1.05554 Kieselgel 60 F254). Compounds were visualised by UV light (254 nm) or by dipping with conc. H_2SO_4–methanol (1:10, v/v) and subsequent charring.

Preparation of supported β-glucosidase

Amberlite XAD-4 (1.0 g, pre-washed with water and dried) was added to a stirred solution of almond β-glucosidase (100 mg, 560 U) and 50 mM, pH 5.0, acetate buffer (2 ml). The mixture was then lyophilised (0.2 mmHg; −20 °C) to give a dry solid. This supported enzyme was stored at −20 °C until use.

Preparation of supported β-galactosidase

Immobilised β-galactosidase from *A. oryzae* (62 mg, 560 U) on Amberlite XAD-4 (1.0 g) was prepared and stored as described above.

General enzymic glycosylation procedure

Supported β-glycosidase (1.1 g) was added to a stirred solution of D-glucose or galactose (1.08 g, 6 mmol) in a mixture of but-3-en-2-ol **2** and water (20 ml) and the reaction mixture was stirred for 48 h at 50 °C. The reaction mixture was filtered and the immobilised enzyme was retrieved for re-use. The filtrate was concentrated under reduced pressure and the residue was chromatographed (SiO_2; CH_2Cl_2–MeOH 4:1 v/v). The appropriate fractions were concentrated under reduced pressure to give the desired glycoside.

(*R*/*S*)-But-3-en-2-yl β-D-glucopyranoside 3

Treatment of glucose **1** with the supported β-glucosidase in (*R*/*S*)-but-3-en-2-ol (**2**)–water (9:1, v/v) according to the general procedure gave *glucoside* **3** as an oil (0.379 g, 27%), δ_H(300 MHz; Me_2SO) 5.86 (0.4 H, ddd, J_{trans} 17.3, J_{cis} 10.7, J_{vic} 5.5, C*H*=CH_2, *S*), 5.68 (0.6 H, ddd, J_{trans} 17.3, J_{cis} 10.7, J_{vic} 5.5, C*H*=CH_2, *R*), 5.24 (0.6 H, d, *J* 17.3, CH=CH_2, *R*, *trans*), 5.15 (0.4 H, d, *trans*, *J* 17.3, CH=CH_2, *S*), 5.10 (0.6 H, d, *J* 10.7, CH=CH_2, *R*, *cis*), 4.99 (0.4 H, d, *cis*, *J* 10.7, CH=CH_2, *S*), 4.95–4.86 (3 H, m, 3 × OH), 4.53–4.42 (1 H, m, OH), 4.38–4.24 (1 H, m, C*H*CH=CH_2), 4.19 (0.4 H, d, *J* 8.1, 1-H, *S*), 4.10 (0.6 H, d, *J* 8.1, 1-H, *R*), 3.71–3.62 (1 H, m, 4-H), 3.47–3.38 (1 H, m, 5-H), 3.14–2.88 (4 H, m, 2-, 3-H, and 6-H_2) and 1.20 (3 H, d, *J* 6.5, CH_3, *R*/*S*); δ_C(75 MHz; Me_2SO) 141.0 (*C*H=CH_2, *S*), 139.7 (*C*H=CH_2, *R*), 116.2 (CH=*C*H_2, *R*), 114.2 (CH=*C*H_2, *S*), 100.7 (C-1, *S*), 100.6 (C-1, *R*), 76.1, 74.2, 74.1, 73.6, 73.3 and 70.0 (C-2, -3, -4, -5 and *C*HCH=CH_2, *R*/*S*), 61.0 (C-6), 21.7 (CH_3, *R*) and 19.7 (CH_3, *S*) (Found: $[M + Na]^+$, 257.1006. $C_{10}H_{18}NaO_6$ requires [*M* + Na], 257.1001).

(*R*,*S*)-Pent-1-en-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside 4

Treatment of glucose **1** with the supported β-glucosidase in (*R*/*S*)-pent-1-en-3-ol–water (9:1, v/v) according to the general procedure gave an oil, which was dissolved in a mixture of pyridine (2 ml) and acetic anhydride (2 ml). After stirring of the mixture for 1 h, TLC (CH_2Cl_2–acetone 97:3 v/v) showed complete consumption of the starting material (R_f 0.00) and one product (R_f 0.68). The reaction mixture was concentrated under reduced pressure and the residue was chromatographed (SiO_2; CH_2Cl_2). Concentration of the appropriate fractions gave *compound* **4** as an oil (0.3 g, 12%), δ_H(300 MHz; $CDCl_3$) 5.80 (0.3 H, ddd, J_{trans} 17.3, J_{cis} 10.7, J_{vic} 5.5, C*H*=CH_2, *S*), 5.12 (0.7

H, ddd, J_{trans} 17.3, J_{cis} 10.7, J_{vic} 5.5, C*H*=CH_2, *R*), 5.24–4.96 (5 H, m, CH=CH_2, *R*/*S*, 2-, 3- and 4-H), 4.54 (0.7 H, d, *J* 8.3, 1-H, *R*), 4.50 (0.3 H, d, *J* 8.3, 1-H, *S*), 4.23–3.86 (3 H, m, 6-H_2 and C*H*CH=CH_2, *R*/*S*), 3.66–3.58 (1 H, m, 5-H), 2.08–1.98 (12 H, m, 4 × Ac), 1.64–1.48 (2 H, m, C$H_2$$CH_3$) and 0.88–0.79 (3 H, m, CH_2CH_3); δ_C(75 MHz; $CDCl_3$) 170.7–168.3 (4 × C=O, Ac), 138.7 (*C*H=CH_2, *S*), 137.2 (*C*H=CH_2, *R*), 118.2 (CH=*C*H_2, *R*), 116.1 (CH=*C*H_2, *S*), 100.1 (C-1, *S*), 97.7 (C-1, *R*), 84.5, (*C*HCH=CH_2, *S*), 80.8 (*C*HCH=CH_2, *R*), 75.6, 73.0, 71.6 and 71.4 (C-2, -3, -4 and -5), 62.1 (C-6), 28.1 (*C*$H_2$$CH_3$), 20.7 (*C*$H_3$, Ac) and 20.6 ($CH_2$*C*$H_3$) (Found: $[M + Na]^+$, 439.1572. $C_{19}H_{28}NaO_{10}$ requires [*M* + Na], 439.1580).

(*R*/*S*)-Hex-1-en-3-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside 5

Treatment of glucose **1** with the supported β-glucosidase in (*R*/*S*)-hex-1-en-3-ol–water (9 : 1, v/v) according to the general procedure gave a clear oil, which was subsequently dissolved in a mixture of pyridine (2 ml) and acetic anhydride (2 ml). After stirring of the solution for 1 h, TLC (CH_2Cl_2–acetone 97 : 3 v/v) showed complete consumption of the starting material (R_f 0.00) and one major product (R_f 0.64). The reaction mixture was concentrated under reduced pressure and the residue was chromatographed (SiO_2; CH_2Cl_2). Concentration of the appropriate fractions gave *compound* **5** as an oil (0.13 g, 5%), δ_H(300 MHz; $CDCl_3$) 5.96–5.80 (0.1 H, m, C*H*=CH_2, *S*), 5.55 (0.9 H, ddd, J_{trans} 17.3, J_{cis} 10.7, J_{vic} 5.5, C*H*=CH_2, *R*), 5.21–4.94 (5 H, m, CH=CH_2, *R*/*S*, 2-, 3- and 4-H), 4.52 (1 H, d, *J* 8.2, 1-H, *R*), 4.26–4.07 (3 H, m, 6-H_2 and C*H*CH=CH_2), 3.65–3.59 (1 H, m, 5-H), 2.08–1.98 (12 H, m, 4 × Ac), 1.62–1.23 (4 H, m, RCH_2-C$H_2$$CH_3$) and 0.88 (3 H, t, *J* 7.4, CH_3); δ_C(75 MHz; $CDCl_3$) 170.4–169.3 (4 × C=O), 137.6 (*C*H=CH_2), 117.8 (CH=*C*H_2), 97.6 (C-1), 79.2 (*C*HCH=CH_2), 73.0, 71.6, 71.4 and 68.7 (C-2, -3, -4 and -5), 62.2 (C-6), 37.2 [CH(CH=CH_2)*C*H_2], 20.7 (CH_3, Ac), 18.1 (*C*$H_2$$CH_3$) and 13.3 ($CH_2$*C*$H_3$) (Found: $[M + Na]^+$, 453.1724. $C_{20}H_{30}NaO_{10}$ requires [*M* + Na], 453.1737).

(*R*/*S*)-Pent-3-en-2-yl β-D-glucopyranoside 6

Treatment of glucose **1** with the supported β-glucosidase in (*R*/*S*)-pent-3-en-2-ol–water (9 : 1, v/v) according to the general procedure gave compound **6** as an oil (0.252 g, 17%), δ_H(300 MHz; Me_2SO) 5.68–5.52 (2 H, m, CH=CH, *R*/*S*), 4.95–4.84 (3 H, m, 3 × OH), 4.51–4.38 (1 H, m, OH), 4.35–4.18 (1 H, m, C*H*CH=CH_2), 4.16 (0.35 H, d, *J* 8.1, 1-H, *S*), 4.11 (0.65 H, d, *J* 8.1, 1-H, *R*), 3.68–3.57 (1 H, m, 4-H), 3.45–3.35 (1 H, m, 5-H), 3.15–2.87 (4 H, m, 2-, 3-H and 6-H_2), 1.70–1.59 (3 H, m, CH=CHCH_3, *R*/*S*) and 1.18–1.12 (3 H, m, CH_3, *R*/*S*); δ_C(75 MHz; Me_2SO) 134.1 (CH*C*H=CHCH_3, *S*), 132.7 (CH*C*H=CHCH_3, *R*), 127.2 (CHCH=*C*HCH_3, *R*), 124.9 (CHCH=*C*HCH_3, *S*), 100.6 (C-1, *S*), 99.5 (C-1, *R*), 77.0, 76.8, 73.8, 73.5, 72.7 and 70.2 (C-2, -3, -4, -5 and *C*HCH=CH_2, *R*/*S*), 61.1 (C-6), 23.9 (*C*H_3CHCH=CH, *S*), 22.1 (*C*H_3CHCH=CH, *R*), 20.1 (CH=CH*C*H_3, *S*) and 17.6 (CH=CH*C*H_3, *R*).

(*R*)-But-3-en-2-yl β-D-glucopyranoside 7

Treatment of glucose **1** with the supported β-glucosidase in (*R*)-but-3-en-2-ol–water (9 : 1, v/v) according to the general procedure gave compound **7** as an oil (0.435 g, 31%), δ_H(300 MHz; Me_2SO) 5.68 (1 H, ddd, J_{trans} 17.3, J_{cis} 10.7, J_{vic} 5.5, C*H*=CH_2), 5.28 (1 H, d, *J* 17.3, CH=CH_2, *trans*), 5.10 (1 H, d, *J* 10.7, CH=CH_2, *cis*), 5.08–4.85 (3 H, m, 3 × OH), 4.50–4.42 (1 H, m, OH), 4.34–4.27 (1 H, m, C*H*CH=CH_2), 4.08 (1 H, d, *J* 8.3, 1-H), 3.68–3.62 (1 H, m, 4-H), 3.45–3.34 (1 H, m, 5-H), 3.12–2.90 (3 H, m, 2-, 3- and 4-H) and 1.19 (3 H, d, *J* 6.5, CH_3); δ_C(75 MHz; $CDCl_3$) 139.8 (*C*H=CH_2), 116.1 (CH=*C*H_2), 100.0 (C-1), 76.9, 73.6, 73.4, 73.2 and 70.2 (C-2, -3, -4, -5, *C*HCH=CH_2), 62.8 (C-6) and 21.7 (*C*H_3); $[\alpha]_D^{25}$ (*c* 1.0, $CHCl_3$) 71.2 × 10^{-1} deg cm^2 g^{-1}.

(*R*/*S*)-But-3-en-2-yl β-D-galactopyranoside 8

Treatment of galactose with the supported β-galactosidase in (*R*/*S*)-but-3-en-2-ol–water (9 : 1, v/v) according to the general procedure gave compound **8** as an oil (0.295 g, 21%); δ_H(300 MHz; Me_2SO) 5.86 (0.35 H, ddd, J_{trans} 17.3, J_{cis} 10.7, J_{vic} 5.5, C*H*=CH_2, *S*), 5.68 (0.65 H, ddd, J_{trans} 17.3, J_{cis} 10.7, J_{vic} 5.5, C*H*=CH_2, *R*), 5.28–4.96 (2 H, m, CH=CH_2, *R*/*S*, *cis*/*trans*), 4.84–4.78 (1 H, m, OH), 4.70–4.62 (1 H, m, OH), 4.58–4.49 (1 H, m, OH), 4.35–4.18 (2 H, m, OH and C*H*CH=CH_2, *R*/*S*), 4.11–4.02 (1 H, m, 1-H, *R*/*S*), 3.63–3.58 (1 H, m, 4-H), 3.46–3.35 (1 H, m, 5-H), 3.28–3.19 (2 H, m, 6-H_2) and 1.19–1.15 (3 H, m, CH_3, *R*/*S*); δ_C(75 MHz; $CDCl_3$) 141.0 (*C*H=CH_2, *S*), 139.8 (*C*H=CH_2, *R*), 115.9 (CH=*C*H_2, *R*), 113.9 (CH=*C*H_2, *S*), 101.3 (C-1, *S*), 100.6 (C-1, *R*), 76.7, 75.0, 74.1, 73.5, 70.6 and 68.1 (C-2, -3, -4, -5 and *C*HCH=CH_2, *R*/*S*), 60.4 (C-6), 21.7 (CH_3, *R*) and 19.7 (CH_3, *S*).

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