ELSEVIER

Carbohydrate Research 279 (1995) 315–319

CARBOHYDRATE RESEARCH

Note

Synthesis of allyl and benzyl β-D-glucopyranosides, and allyl β-D-galactopyranoside from D-glucose or D-galactose and the corresponding alcohol using almond β-D-glucosidase

Gabin Vic, David H.G. Crout *

Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK

Received 20 February 1995; accepted 15 July 1995

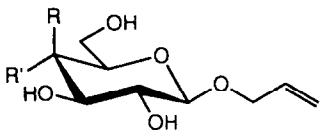
Keywords: Glucosidase; Glucopyranoside; Galactopyranoside; Low-water system; Reverse hydrolysis

Allyl β-D-glucopyranoside (**1**), benzyl β-D-glucopyranoside (**2**), and allyl β-D-galactopyranoside (**3**) are important starting intermediates in carbohydrate chemistry as temporary anomeric protected derivatives [1–4]. Moreover, allyl glycosides **1** and **3** are also useful monomers, used in the synthesis of glycopolymers [5,6]. Nevertheless, as common in carbohydrate chemistry, the chemical synthesis of these compounds is a multi-step procedure where at least three steps are necessary starting from D-glucose or D-galactose [7]. Shorter procedures can be devised using an enzymatic approach based on glycosidase action. There are many reports on the use of glycosidases for the synthesis of glycosidic structures and, in particular, of oligosaccharides [8–12]. Most of these reports describe a transglycosylation procedure where an activated glycosyl donor such as a *p*-nitrophenyl glycoside is used. This type of approach increases the cost of the synthesis. Also, it often makes the isolation of the products difficult because different side-products resulting from the transfer of the glycosyl donor onto itself can be generated in the medium. On the other hand, glycosidases can also be used in a reverse hydrolysis mode [13–15] where the thermodynamic equilibrium normally in favour of the hydrolysis in aqueous medium is shifted towards synthesis. There have been relatively few examples of this approach, although it is the most simple and the most cost-effective one. We have previously reported that almond β-D-glucosidase can

* Corresponding author. Tel: 01203 523 961; Fax: 01203 524 429.

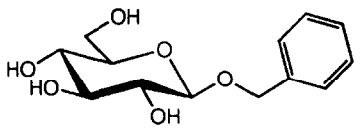
SSDI 0008-6215(95)00301-0

catalyse the synthesis of various alkyl β-D-glucopyranosides and thioglycosides in organic medium using only D-glucose and the corresponding alcohol or thiol as substrates [16,17]. In the present note we describe the enzymatic syntheses of three β-D-glycopyranosides (**1**, **2**, and **3**) using this reverse hydrolysis process starting directly from D-glucose or D-galactose and the corresponding alcohol. As an example, the synthesis of allyl β-D-glucopyranoside (**1**) has been optimised by studying the effect of D-glucose and water concentration in the medium.



1 R = H, R' = OH

3 R = OH, R' = H



2

To shift the thermodynamic equilibrium towards synthesis, the enzymatic reaction was carried out directly in the acceptor alcohol as solvent. It was found that a minimum amount of water was necessary to maintain enzymatic activity in such a medium. To find the best reaction conditions for the synthesis of **1**, the influences of water and D-glucose concentrations on the reaction were studied. As shown in Fig. 1, the reaction

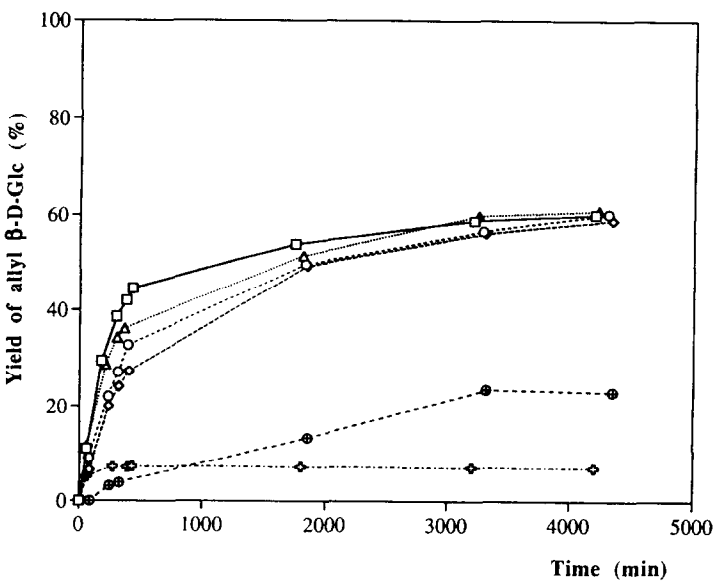


Fig. 1. Time course of the synthesis of allyl β-D-glucopyranoside catalysed by almond β-D-glucosidase in different allyl alcohol–water mixtures: (— · — · + · — · —) 80:20 (v/v); (— □ —) 90:10 (v/v); (· · · △ · · ·) 92:8 (v/v); (---○---) 94:6 (v/v); (---◇---) 96:4 (v/v); (---⊕---) 98:2 (v/v). D-Glucose concentration is fixed at 36 g/L. The yields, estimated by HPLC, were based on D-glucose added.

Table 1
β-D-Glucosidase-catalysed synthesis of allyl β-D-glucopyranoside for different D-glucose concentrations in 9:1 allyl alcohol–water

Glucose concentration in the medium (g/L)	36	54	72	90	108
Yield [a] of allyl β-D-Glc (%)	60	65	43	38	39

[a] Yields were based on D-glucose added and were determined by HPLC after reaction for 48 h.

time-course was followed for different concentrations of water in the medium (glucose concentration fixed at 36 g/L). The optimum yield (60% based on glucose added) was found to be the same for all water concentrations between 4 and 10% (v/v). The only difference was the rate of synthesis which increased with increasing water concentration. With 2% (v/v) of water, the yield and the rate of synthesis decreased dramatically and in neat allyl alcohol no synthesis was detected. If 20% (v/v) of water was added, the yield obtained was only 10% because the enzyme was rapidly inactivated under these conditions.

The effect of D-glucose addition in the medium is shown in Table 1. The best yield (65%) was obtained for 54 g/L of D-glucose in a 9:1 (v/v) allyl alcohol–water mixture. Higher concentrations of D-glucose gave lower yields.

The preparative scale reaction was carried out using the determined optimum conditions (9:1 allyl alcohol–water, 54 g/L of D-glucose) to give after purification by flash chromatography allyl β-D-glucopyranoside (**1**) in 62% yield. The same procedure applied to benzyl alcohol gave benzyl β-D-glucopyranoside (**2**) in 40% yield. In a previous report [18] it was noted that almond β-D-glucosidase can also catalyse the synthesis of β-D-galactosides. Since attempts to use the β-galactosidase from *Aspergillus oryzae* to synthesise glycoside **3** failed, almond β-D-glucosidase was used to catalyse the coupling of allyl alcohol to galactose. After 48 h of reaction at 30 °C allyl β-D-galactopyranoside (**3**) was obtained in 15% yield.

1. Experimental

General.—Almond β-D-glucosidase (EC 3.2.1.21) was purchased from the Sigma Chemical Co. (G-0395, 6.9 U/mg). Allyl and benzyl alcohols were obtained from the Aldrich Chemical Co. and dried over 4 Å molecular sieves. NMR spectra were recorded on a Bruker WH-400 spectrometer. Mass spectra were recorded on a Kratos MS80 spectrometer using the fast atom bombardment (FAB) mode. HPLC was performed on a Gilson system under the following conditions: column ODSII 5μ, 4.6 × 250 mm; solvent system, 20:80 MeCN–water; flow rate, 0.5 mL/min. Detection was by a light-scattering detector (Sedex 55). Melting points were determined with a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were determined with an Optical Activity Ltd. AA-1000 polarimeter. Flash chromatography was performed on Silica Gel 60 (Merck, 70–230 mesh) using 40:10:1 CH_2Cl_2–MeOH–H_2O as eluent.

Time course of reaction for the synthesis of allyl β-D-glucopyranoside (**1**).—The assay mixture (2 mL) contained D-glucose (0.4 mmol), water (0.2 mL), and allyl alcohol

(1.8 mL). The reaction was started by the addition of the enzyme (10 mg, 6.9 U) and stirred (100 rpm) at 50 °C. Aliquots (20 μL) were removed at timed intervals and quenched by addition of 180 μL of dimethylformamide. The samples were then analysed by HPLC.

Preparative scale reactions.—(a) Allyl β-D-glucopyranoside (**1**). To a solution of D-glucose (1.08 g, 6 mmol) in water (2 mL) and allyl alcohol (18 mL) was added almond β-D-glucosidase (100 mg, 690 U). The mixture was stirred (100 rpm) for 48 h at 50 °C, then filtered to remove the enzyme, and the excess of allyl alcohol was evaporated under reduced pressure. Flash chromatography of the residue gave **1** (0.82 g, 62%) as an amorphous solid; mp 101–102°; $[\alpha]_D^{27}$ −39.1° (*c* 0.95, MeOH) {lit. [19] mp 98–99°, $[\alpha]_D$ −49.1° (*c* 2.0, H_2O)}; ^{1}H NMR (D_2O): δ 3.21 (dd, 1 H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.2 Hz, H-2), 3.30 (dd, 1 H, $J_{3,4}$ 9.0, $J_{4,5}$ 9.6 Hz, H-4), 3.38 (m, 1 H, H-5), 3.41 (b, 1 H, $J_{2,3} = J_{3,4}$ 9.1 Hz, H-3), 3.64 (dd, 1 H, $J_{5,6a}$ 5.9, $J_{6a,6b}$ 12.3 Hz, H-6a), 3.84 (dd, 1 H, $J_{5,6b}$ 2.2 Hz, H-6b), 4.15 (m, 1 H, H-1′b), 4.32 (m, 1 H, H-1′a), 4.43 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 5.21 (m, 1 H, H-3′b), 5.31 (m, 1 H, H-3′a), 5.59 (m, 1 H, H-2′); ^{13}C (D_2O): δ 61.4 (C-6), 70.3 (C-4), 71.2 (C-1′), 73.7 (C-2), 76.4 (C-3 or C-5), 76.5 (C-3 or C-5), 101.8 (C-1), 119.4 (C-3′), 133.9 (C-2′); *m/z* (FAB$^+$) 243 (MNa^+).

(b) Benzyl β-D-glucopyranoside (**2**). To a solution of D-glucose (0.80 g, 4.4 mmol) in water (2 mL) and benzyl alcohol (18 mL) was added almond β-D-glucosidase (120 mg, 828 U). The mixture was stirred (100 rpm) for 48 h at 50 °C and worked up as described for glycoside **1**. Flash chromatography of the residue gave **2** (0.47 g, 40%) as an amorphous solid; mp 117–118 °C; $[\alpha]_D^{27}$ −55.1° (*c* 1, MeOH) {lit. [19] mp 119–120 °C, $[\alpha]_D$ −51.4° (*c* 2.0, H_2O)}; ^{1}H NMR (D_2O): δ 3.24 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.0 Hz, H-2), 3.35 (m, 3 H, H-3,4,5), 3.66 (dd, 1 H, $J_{5,6a}$ 5.7, $J_{6a,6b}$ 12.3 Hz, H-6a), 3.86 (dd, 1 H, $J_{5,6b}$ 2.0 Hz, H-6b), 4.46 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.69 (d, 1 H, *J* 11.6 Hz, H-1′b), 4.87 (d, 1 H, H-1′a), 7.37 (m, 5 H-aryl); ^{13}C (D_2O): δ 61.4 (C-6), 70.3 (C-4), 72.1 (C-1′), 73.7 (C-2), 76.4 (C-3 or C-5), 76.5 (C-3 or C-5), 101.8 (C-1), 129.1, 129.3, 129.4, 137.2 (C-aryl); *m/z* (FAB$^+$) 293 (MNa^+).

(c) Allyl β-D-galactopyranoside (**3**). To a solution of D-galactose (0.80 g, 4.4 mmol) in water (2 mL) and allyl alcohol (18 mL) was added almond β-D-glucosidase (100 mg, 690 U). The mixture was stirred (100 rpm) for 48 h at 30 °C, and worked up as described for glycoside **1**. Flash chromatography of the residue gave **3** (0.14 g, 15%) as an amorphous solid; mp 101–102 °C; $[\alpha]_D^{27}$ −10.1° (*c* 1.1, MeOH) {lit. [20] mp 102–103 °C, $[\alpha]_D^{20}$ −11° (*c* 2, H_2O)}; ^{1}H NMR (D_2O): δ 3.46 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.9 Hz, H-2), 3.59 (m, 2 H, H-3,5), 3.76 (m, 2 H, H-6a,b), 3.85 (d, 1 H, *J* 2.9 Hz, H-4), 4.16 (m, 1 H, H-1′b), 4.33 (m, 1 H, H-1′a), 4.37 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 5.22 (m, 1 H, H-3′b), 5.32 (m, 1 H, H-3′a), 5.91 (m, 1 H, H-2′); ^{13}C (D_2O): δ 61.6 (C-6), 69.2 (C-4), 71.2 (C-1′), 71.4 (C-2), 73.4 (C-3), 75.7 (C-5), 102.4 (C-1), 119.3 (C-3′), 133.0 (C-2′); *m/z* (FAB$^+$) 243 (MNa^+).

Acknowledgements

We thank Dr Jeremy J. Hastings for NMR measurements, Mr I. Katyal for MS measurements, and the Biotechnology Directorate (SERC) for the financial support.

References

[1] J. Gigg and R. Gigg, *J. Chem. Soc., C*, (1966) 82–86.
[2] P.A. Manthorpe and R. Gigg, *Methods Carbohydr. Chem.*, 8 (1980) 305–315.
[3] Y. Nishida and J. Thiem, *J. Carbohydr. Chem.*, 13 (1994) 1059–1063.
[4] K. Takeo, M. Kitajima, and T. Fukatsu, *Carbohydr. Res.*, 112 (1983) 158–164.
[5] K. Kobayashi, N. Nakishita, M. Okada, T. Akaike, and T. Usui, *J. Carbohydr. Chem.*, 13 (1994) 753–766.
[6] R. Roy, F.D. Tropper, and A. Romanowska, *J. Chem. Soc., Chem. Commun.*, (1992) 1611–1613.
[7] E.A. Talley, M.D. Vale, and E. Yanovsky, *J. Am. Chem. Soc.*, 67 (1945) 2037–2039.
[8] K.G.I. Nilsson, *Carbohydr. Res.*, 167 (1987) 95–103.
[9] K.G.I. Nilsson, *Carbohydr. Res.*, 167 (1988) 53–59.
[10] D.H.G. Crout, O.W. Howarth, S. Singh, B.E.P. Swoboda, P. Critchley, and W.T. Gibson, *J. Chem. Soc., Chem. Commun.*, (1991) 1550–1551.
[11] D.H.G. Crout, S. Singh, B.E.P. Swoboda, P. Critchley, and W.T. Gibson, *J. Chem. Soc., Chem. Commun.*, (1992) 704–705.
[12] S. Singh, J. Packwood, and D.H.G. Crout, *J. Chem. Soc., Chem. Commun.*, (1994) 2227–2228.
[13] E. Bourquelot and M. Bridel, *J. Pharm. Chim.*, 7 (1912) 569–573.
[14] K. Ajisaka, H. Nishida, and H. Fujimoto, *Biotechnol. Lett.*, 4 (1987) 243–248.
[15] R.A. Rastall, N.H. Rees, R Wait, M.W. Adlard, and C. Bucke, *Enzyme Microbiol. Technol.*, 14 (1992) 53–57.
[16] G. Vic and D. Thomas, *Tetrahedron Lett.*, 33 (1992) 4567–4570.
[17] G. Vic and D.H.G. Crout, *Tetrahedron: Asymmetry*, 5 (1994) 2513–2516.
[18] G. Vic, J. Biton. D. Le Beller, J.-M. Michel, and D. Thomas, *Biotechnol. Bioeng.*, 46 (1995) 109–116.
[19] T.E. Timell, *Can. J. Chem.*, 42 (1964) 1456–1472.
[20] A.Y. Chernyak, A.B. Levinsky, B.A. Dmitriev, and N.K. Kochetkov, *Carbohydr. Res.*, 128 (1984) 269–282.