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Synthesis of Glucosidic Derivatives with a Spacer Arm by Reverse Hydrolysis using Almond β-D-Glucosidase

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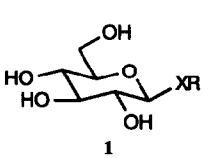
Abstract: Glucosidase-catalysed synthesis of glucosides with a spacer arm on the anomeric carbon is reported. By using the acceptor as solvent at an elevated temperature, much higher yields of product have been obtained than previously observed.

Recently the structural biology of oligosaccharides has undergone remarkable development. It is now known that carbohydrates play a key role in many biological phenomena[1,2]. To synthesise these compounds, the chemical approach is well developed but still requires multi-step procedures using sophisticated protecting group strategies[3]. Over the last decade, enzymatic synthesis has become an interesting alternative approach to time consuming conventional synthesis. In general, two types of enzymes are used for this purpose: the glycosyltransferases and the glycosylhydrolases. Although several carbohydrate structures have been obtained by using glycosyltransferases[4,5] the limited availability and high cost of these enzymes militate against their broad application. On the other hand, glycosylhydrolases are extensively used to synthesise carbohydrate derivatives using mainly transglycosylation[6-8] and more rarely reverse hydrolysis[9].

In previous work[10] we used a β-glucosylhydrolase: almond β-D-glucosidase (EC 3.2.1.21) to synthesise carbohydrate derivatives using reverse hydrolysis in an organic medium. This approach represents a thermodynamically controlled synthesis where the equilibrium normally in favour of hydrolysis in buffer, is shifted towards synthesis by using a high concentration of substrate or a medium of low water activity. We now report the synthesis of glucosidic derivatives (1) with a spacer group on the anomeric carbon by comparing three different variations of this method. These compounds can be used to make glycoconjugates[11] or they can be coupled to a solid phase for affinity supports[12] and immunoadsorbents[13].



1

1a

XR = O–(CH2)6–OH

1b

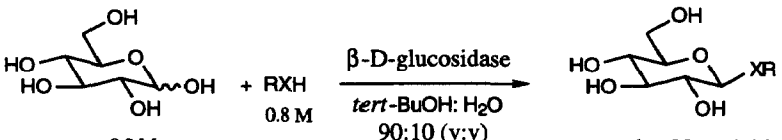
XR = O–(CH2)6–NHCOCF3

1c

XR = S–(CH2)3–SH

In the more general approach (Scheme 1) 80 or 90% (v:v) *tert*-butanol was used as organic co-solvent to decrease the water concentration and to solubilise the acceptor (alcohol or thiol). Using this method, mono-

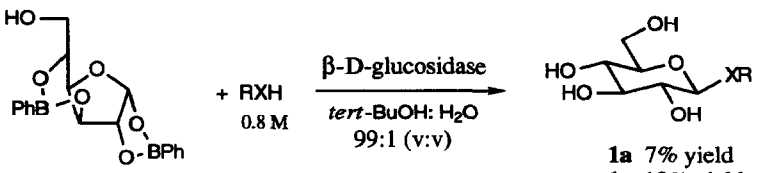
glycosylation of 1,6-hexanediol (**1a**) in 22% yield was observed. Glycoside **1b** was synthesised in 7% yield after protection of the amine because the unprotected amino alcohol inhibits the enzyme. The more unexpected result was the observation of S-glucosylation (**1c**) in 17% yield with 1,3-dithiopropane. This last result gives easy access to 1-thioglucosides which, moreover, can be chemically activated and used as glucosyl donors [14]. In this synthesis the yields depend on solvent concentration and the best results are obtained at around 80 to 90% (v:v) of solvent. Moreover the enzyme is very stable in this high concentration of *tert*-butanol; 90% of the enzyme activity remains after 72 h. The two obvious approaches to increasing the yields in this thermodynamic synthesis are first, to decrease the water concentration and second, to increase the substrate concentration. These two modifications have been studied.



1a 22% yield
1b 7% yield
1c 17% yield

Scheme 1

When *tert*-butanol is used as co-solvent, a minimum of 10% (v:v) of water is necessary in the medium to maintain enzymatic activity because this hydrophilic solvent strips essential water from the biocatalyst[15]. To avoid this effect a more hydrophobic solvent such as *tert*-butyl methyl ether was used which permitted the addition of a small amount of water, sufficient to maintain enzyme activity. Glucose was solubilised in this medium by presenting it as its phenyl boronate derivative[16]. As expected (Scheme 2), glycosides **1a** and **1c** were prepared using only 1% (v:v) of water in the medium but the yields are lower compared with *tert*-butanol. The use of a phenyl boronic acid derivative of glucose has already been shown to permit the synthesis of disaccharides in 14% yield[17].

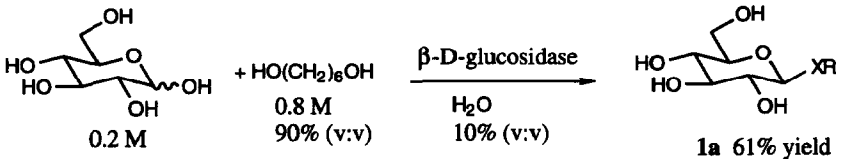


1a 7% yield
1c 10% yield

Scheme 2

The effect of substrate concentration was investigated by carrying out the reaction directly in 1,6-hexanediol. As this alcohol is liquid at 50 °C, a medium containing 90% (v:v) of diol and 10% (v:v) of water was used (Scheme 3). After 5 days, glucoside **1a** was obtained in 61% yield. If the percentage of water was increased (20% (v:v)) the reaction was faster but the yield was lower (43%). This method can be applied, in principle, to other alcohols if they have the following properties: they should be liquid at ≤50 °C, they should solubilise 10 to 20% (v:v) of water and they should not inhibit the biocatalyst. Applying these criteria we have

synthesised 1-allyl β-glucopyranoside in 62% yield (data not shown); several others derivatives are under investigation.



Scheme 3

Experimental

Almond β-D-glucosidase (EC 3.2.1.21) was obtained from Sigma. The specific activity was 6.9 U/mg. *tert*-Butanol was purchased from BDH (< 0.10 H_2O) and *tert*-butyl methyl ether from Aldrich (< 0.005 H_2O). N.m.r. spectra were recorded on a Bruker WH-400 spectrometer. In assigning signals in ^{1}H-n.m.r. spectra, protons of the sugar residue are numbered conventionally 1 through 6 and the aglycone 1', 2', 3' etc... starting at the glucosylated end. Optical rotations were determined with an Optical Activity Ltd. AA-1000 polarimeter. Column chromatography was performed on silica gel 60, particle size 0.040-0.063 mm (Merck) using CH_2Cl_2 : CH_3OH : H_2O (40 : 10 : 1) as eluent.

Enzymatic glucosylation in tert-butanol (Method 1).

Glucose (2 mmol) was dissolved in water (1 cm^3) and *tert*-butanol (9 cm^3) was added (in the case of **1a** a 2 : 8 (v:v) water : *tert*-butanol mixture was used). The acceptor (8 mmol) was dissolved in the medium and almond β-D-glucosidase (50 mg) was added. The mixture was stirred (100 rpm) at 50 °C for 5 days, filtered and the solvent was evaporated under reduced pressure. The residue was dissolved in water (20 cm^3) and extracted with ethyl acetate (2 x 25 cm^3). The aqueous phase was concentrated and the crude residue was purified by column chromatography.

Enzymatic glucosylation using the boronate (Method 2).

The acceptor (8 mmol) was dissolved in *tert*-butyl methyl ether (9.9 cm^3) and water (0.1 cm^3). Almond β-D-glucosidase (50 mg) and boronate (2 mmol) were added. The mixture was stirred (100 rpm) at 50 °C for 5 days, filtered and evaporated to dryness under reduced pressure. The residue was dissolved in acetone : water (1:1, v/v, 20 cm^3) and incubated for 2 h at 30 °C. After removal of the solvent, the crude residue was worked-up as described in Method 1.

Enzymatic glucosylation in 1,6-hexanediol (Method 3).

Glucose (2 mmol) was dissolved in water (1 cm^3) and 1,6-hexanediol (9 cm^3), and almond β-D-glucosidase (50 mg) was added. The mixture was stirred (100 rpm) at 50 °C for 5 days and worked-up as described in Method 1.

6'-Hydroxyhexyl-*O*-β-D-glucopyranoside (1a): $[\alpha]_D^{27}$= -31.6 (c 0.28, H_2O). ^{1}H-n.m.r. (400 MHz, H_2O): δ 1.30 (m, 4 H, H-3', 4'), 1.56 (m, 4 H, H-2', 5'), 3.21 (dd, 1 H, J 8.0, 9.3 Hz, H-2), 3.40 (m, 3 H, H-3, 4, 5), 3.57 (t, 2 H, J 6.6 Hz, H-6'), 3.63 (m, 1 H, H-1a'), 3.68 (dd, 1 H, J 5.8, 12.3 Hz, H-6a), 3.89

(m, 2 H, H-6b,1'b), 4.41 (d, 1 H, J 7.9 Hz, H-1β). ^{13}C-n.m.r. (100 MHz, H_2O): δ 25.4, 25.4, 29.3, 31.8, 61.4, 62.4, 70.3, 71.2, 73.8, 76.5, 76.5 and 102.8.

6'-Trifluoroacetamidohexyl-*O*-β-D-glucopyranoside (1b): $[\alpha]_D^{28}$ = -17.1 (c 0.29, MeOH). ^{1}H-n.m.r. (400 MHz, H_2O): δ (1.31 m, 4 H, H-3', 4'), 1.54 (m, 4 H, H-2', 5'), 3.19 (t, 1 H, J 8 Hz, H-2), 3.27 (t, 2 H, J 7.5 Hz, H-6'), 3.33 (d, 1 H, J 8.9 Hz, H-4), 3.41 (m, 2 H, H-3, 5), 3.63 (m, 2 H, H-1'a, 6a), 3.86 (m, 2 H, H-1'b, 6b), 4.39 (d, 1 H, J 8.0 Hz, H-1β). ^{13}C-n.m.r. (100 MHz, H_2O): δ 25.2, 26.2, 28.2, 29.2, 40.3, 61.4, 70.3, 71.1, 73.8, 76.4, 76.5, 102.8, 112.3 (CF_3),115.1 (CF_3), 118.0 (CF_3), 120.8 (CF_3), 159.2 (C=O), 159.6 (C=O).

3'-Thiopropyl-*S*-β-D-glucopyranoside (1c): $[\alpha]_D^{26}$ = -49.6 (c 0.38, MeOH). ^{1}H-n.m.r. (400 MHz, H_2O): δ 1.88 (m, 2 H, H-2'), 2.59 (t, 2 H, J 6.9 Hz, H-3'), 2.79 (m, 2 H, H-1'), 3.25 (dd, 1 H, J 8.7, 9.8 Hz, H-2), 3.38 (m, 3 H, H-3,4,5), 3.64 (dd, 1 H, J 5.6, 12.3 Hz, H-6a), 3.84 (dd, 1 H, J 2.1 Hz, H-6b), 4.47 (d, 1 H, J 9.8 Hz, H-1β). ^{13}C-n.m.r. (100 MHz, H_2O): δ 23.1, 29.0, 33.8, 61.5, 70.1, 72.9, 77.8, 80.4 and 86.0.

Acknowledgements

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