Stereospecific Attachment of Carbohydrates to Amino Acid Derivatives using β-Glucosidase and β-Xylosidase

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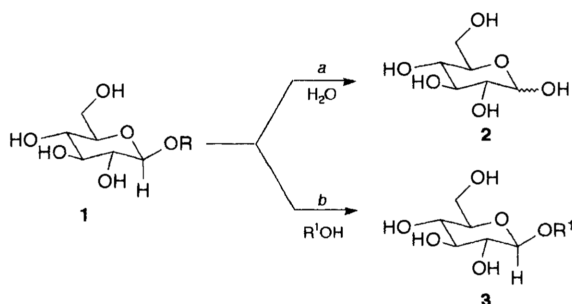
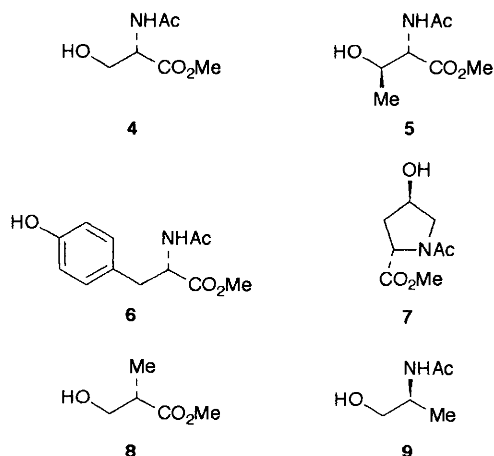
Both β-glucosidase from almonds and β-xylosidase from *Escherichia coli* have been used to catalyse the synthesis of β-glucosides and β-xylosides of amino acid derivatives, respectively.

There is currently considerable interest in new methods for the stereospecific synthesis of glycosidic linkages as a means of combining (*i*) two sugar residues,[1] (*ii*) a sugar and an amino acid[2] and (*iii*) a sugar and a lipid.[3] In addition to the development of more efficient methods for constructing glycosides, it has also been necessary to refine the strategies available for selectively protecting/deprotecting carbohydrates.[4] This combined problem of stereo- and regioselectivity in chemical synthesis has prompted the development of enzymatic approaches since a large number of the enzymes involved in carbohydrate and oligosaccharide biosynthesis are well documented and readily available.[5] For example β-glucosidase will catalyse the stereospecific hydrolytic cleavage of the β-glycosidic bond in **1** to give glucose **2** (Scheme 1, path *a*).

However, it is well known that if the reaction is carried out in the presence of a second nucleophile (*e.g.* an alcohol R^1OH) then appreciable transglycosylation occurs to give a new glucoside **3** exclusively with the β-configuration (Scheme 1, path *b*).[6] We were attracted by this reaction since if the second nucleophile was a suitably chosen amino acid residue of a peptide or protein it might offer an effective method of glycosylation under aqueous conditions without the need for any protection of the hydroxy groups. Such a process would have application in the rational preparation of novel glycopeptides and glycoproteins. To test this notion we decided to investigate simple protected amino acids as model substrates and herein we describe the synthesis of glycosylated amino acid derivatives using β-glucosidase and β-xylosidase.[7]



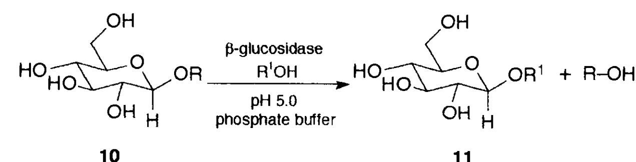
Scheme 1



We chose as candidate substrates the protected derivatives, **4–7**, of naturally occurring amino acids.

Initial experiments were conducted using β-glucosidase as the catalyst.† This enzyme is commercially available in large quantities and with high specific activity. Addition of the glycosyl donor **10** was carried out over a period of 2–60 h *via* a syringe pump to a solution of the acceptor substrate dissolved in phosphate buffer containing β-glucosidase. Reactions were monitored and products purified by reversed-phase HPLC.‡

Table 1

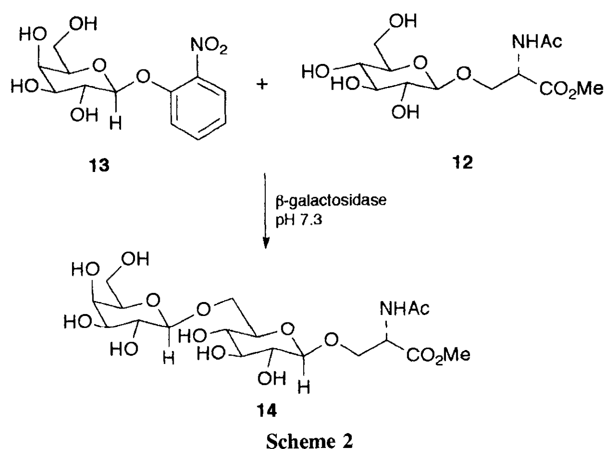


Entry	R	R^1OH	Ratio **10**: R^1OH	*t*/h	Yield of **11** (%)
1	$2-NO_2C_6H_4$	**4**	3:1	22	13
2	$2-NO_2C_6H_4$	**4**	6:1	24	25
3	$4-NO_2C_6H_4$	**4**	1:2	50	4
4	D-glucose	**4**	1:2	60	3
5	$2-NO_2C_6H_4$	**5**	6:1	24	11
6	$2-NO_2C_6H_4$	**6**	7:1	30	<1
7	$2-NO_2C_6H_4$	**7**	6:1	26	9
8	$2-NO_2C_6H_4$	**8**	1:4	2	27
9	$2-NO_2C_6H_4$	**9**	6:1	22	11

† β-Glucosidase from almonds (G-0395 Sigma).

‡ Preparation of 1-*O*-β-D-(*N*-acetyl-L-serine methyl ester) glucopyranoside using β-glucosidase from almonds: To a stirred solution of $2-NO_2$-phenyl-β-D-glucopyranoside (2-NP-Glc) (30 mg, 0.1 mmol) in phosphate buffer (pH 5, 70 mmol dm^{-3}, 0.36 ml) was added *N*-acetyl-L-serine methyl ester (16 mg, 0.1 mmol) and β-glucosidase (0.5 U). Over a period of 24 h, additional 2-NP-Glc (150 mg, 0.5 mmol) in phosphate buffer (2 ml) was added continuously by means of a syringe pump (Medical Magnetics MD50 with 2nd syringe and cloak; infusion rate set to 0.1 ml h^{-1}). Aliquots were removed and injected onto an HPLC column (ODSII, 5μ column; flow rate 1 ml min^{-1} of 99:1:0.05 H_2O–MeCN–HCO_2H at 220 nm), to determine the progress of the reaction.

After 24 hours, when HPLC showed the near complete disappearance of 2-NP-Glc, and a new peak, at 11.5 min, seemed to be at its maximum height, the reaction mixture was concentrated to dryness. The residue was dissolved in water (2 ml) and co-concentrated with methanol until all the 2-nitrophenol had been removed. Redissolving in methanol (10 ml), filtration and evaporation of the solvent removed inorganic salts. Purification was effected by HPLC (same conditions as above) to yield the title compound as an oil (8.2 mg, 25% with respect to *N*-acetyl-L-serine methyl ester); (Found M^+, 324.1295. $C_{12}H_{21}NO_4$ requires 324.1295); ^1H NMR [250 MHz, $(CD_3)_2SO$], δ 1.89 (3 H, s, Ac), 3.00 (2 H, m, β-H of serine), 3.63 (4 H, m), 4.11 (3 H, m), 4.18 (1 H, d, anomeric H, *J* 7 Hz), 4.55 (2 H, m), 8.21 (1 H, d, NH); ^{13}C NMR (125 MHz, CD_3OD), 22.36 (q, $COCH_3$), 52.92 (q, OCH_3), 54.23 (t), 62.76 (d), 70.24 (t), 71.63 (d), 75.09 (d), 78.11 (2 × d), 104.63 (d, anomeric CH), 172.05 (s), 173.37 (s).



Scheme 2

The results are summarised in Table 1. The structure of all of the product glycosides **11** was established by independent synthesis and comparison of spectroscopic data. In all cases only the β-glucoside was obtained. Prolonged reaction times (>24 h) generally resulted in decreased yields of the glucosides presumably due to competing hydrolysis of the product by β-glucosidase. 2-Nitrophenylglucopyranoside (entries 1 and 2) was found to be most effective glucosyl donor although the 4-nitrophenyl derivative (entry 3) and lactose (entry 4) also reacted.

An insight into the factors affecting the yields of these reactions was gained from the use of the glycosyl acceptors **8** and **9** wherein the -NHAc group and $-CO_2Me$ group respectively were replaced by -Me. The lower yields associated with -NHAc containing substrates **4** and **9** may be due to hydrogen bonding effects.

Attempts to generalise this reaction with substrates **4–7** using other glycosidases (with the corresponding glycosyl donors) were somewhat disappointing. Amongst a wide range tested only β-xylosidase was found to react with **4** giving the corresponding β-xyloside ($2-NO_2C_6H_4$-β-D-xylopyranoside : **4** = 3 : 4, 25 h, 7% yield). The reasons for this are not clear since β-galactosidase, for example, is known to catalyse *trans*-glycosylation reactions with simple alcohols as the nucleophilic component[6] and indeed could be used to catalyse the reaction between $2-NO_2C_6H_4$-β-D-galactopyranoside and **8** to give the corresponding β-galactoside (ratio = 1 : 4, 1 h, 62% yield).

Finally in order to extend the application of the methodology described herein we subjected the β-glucoside **12** derived from entry 2 to a second glycosylation reaction using 2-nitrophenyl-β-galactopyranoside **13** in the presence of β-galactosidase. The major product isolated was the 1,6-linked disaccharide **14** (Scheme 2) in which the new glycosidic linkage has been formed exclusively with the β-configuration. In this reaction there is no competing hydrolysis of the β-glucoside **12** since the β-galactosidase is highly specific for β-galactosides.

By using additional combinations of glycosidases and/or glycosyl transferases it should be possible to further extend the oligosaccharide chain to synthesise more complex structures in a defined manner. This goal, and the use of peptides/proteins as glycosyl acceptors, is currently under investigation in our laboratories.

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