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An Improved Strategy for the Stereoselective Synthesis of Glycosides Using Glycosidases as Catalysts

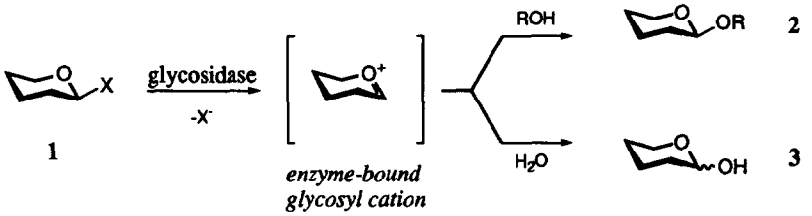
Anne Baker, Nicholas J. Turner*, and Matthew C. Webberley[1]

Department of Chemistry, University of Exeter, Stocker Road, Exeter EX4 4QD.

Abstract: A alternative strategy for the synthesis of glycosides, using glycosidase enzymes, has been developed. In contrast to previous procedures, this new method uses limiting amounts of the acceptor alcohol substrate in combination with an excess of the glycosyl donor. Using this procedure, a series of mono- and disaccharides have been prepared.

The development of stereoselective methods for the synthesis of oligosaccharides presents a considerable challenge to synthetic chemists. During the glycosidation step it is necessary to control both the stereoselectivity of the reaction (*i.e.* α versus β glycoside) together with the regioselectivity. Considerable advances have been made recently with the introduction of new reagents and catalysts for glycosidation and protecting group strategies.[2] Alternative procedures based on enzymic catalysis have been investigated and appear especially promising for the synthesis of certain types of oligosaccharides.[3]

Enzyme based methods employ either glycosyl transferases or glycosidases as catalysts. Glycosyl transferases are a large group of enzymes that are involved in the biosynthesis of oligosaccharides and have been used for the preparation of sialyl[4], fucosyl[5], and mannosyl[6] containing oligomers. In contrast, glycosidases are generally responsible *in vivo* for the degradation of oligosaccharides *via* hydrolysis of the glycosidic linkage. However by adjustment of the reaction conditions they can be used as catalysts for glycoside synthesis according to **Scheme 1**.[7]



Scheme 1

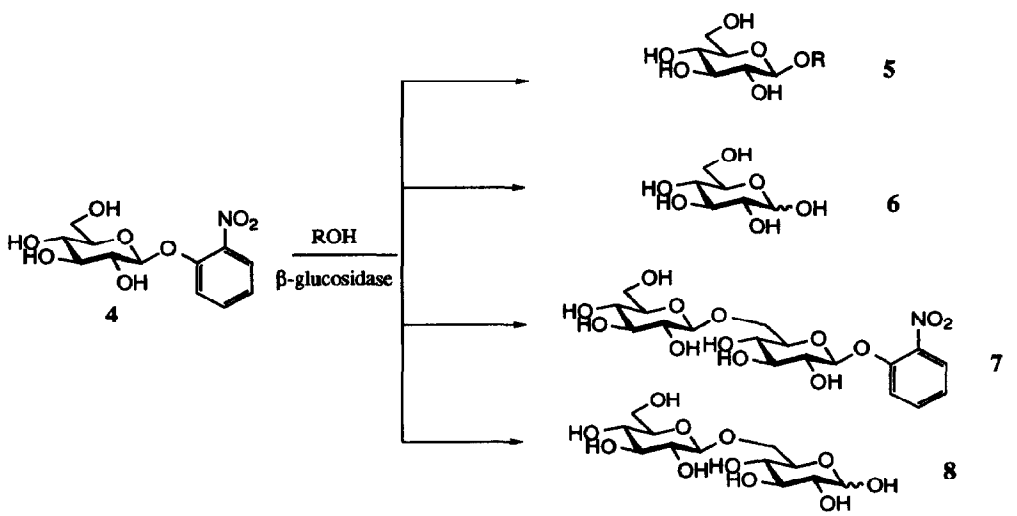
By the use of an appropriate glycosyl donor **1** (*e.g.* X = *o*-nitrophenyl) the enzyme bound glycosyl cation can be captured by an alcohol to yield a glycoside. The competing reaction involving attack by water leads to hydrolysis. Even when these reaction are carried out in water (55 M) using relatively low (~100 mM) concentrations of the alcohol, appreciable yields of **2** can be obtained, implying the preferential binding of alcohols compared to water at the active site.

The key features of the process shown in **Scheme 1** are i) the reaction is completely stereospecific ii) no protection of the glycosyl donor is required and iii) a range of glycosidase enzymes is commercially available (*e.g.* β-glucosidase, β-galactosidase) make the reaction of general use. In comparison with glycosyl transferases, the glycosidase enzymes are cheaper and more robust, and the required glycosyl donors are readily available. The major drawback of the reaction is that in order to obtain good yields of the product it is often necessary to use high concentrations of the alcohol acceptor in order to bias formation of **2** over **3**. In the case where the alcohol is cheap and readily available this presents no problem.

However the need for using high concentrations of the acceptor nucleophile clearly limits the scope and application of this reaction. We have now addressed this problem and report below a modified procedure for glycosidase catalysed glycosidations in which the acceptor alcohol substrate is used in limiting amounts.

Results and Discussion

Our initial idea was simply to reverse the relative concentrations of the two reactants such that now the glycosyl donor was in excess. However, as can be seen from **Scheme 2**, this approach generates an additional problem *i.e.* since the glycosyl donor *can also function as an acceptor*, high concentrations of **4** lead to rapid formation of the disaccharide **7**.



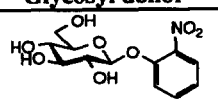
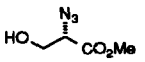
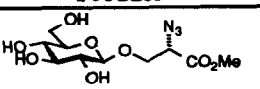
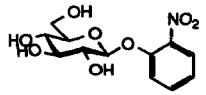
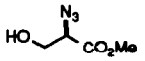
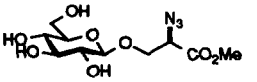
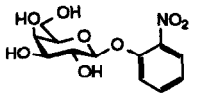
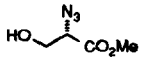
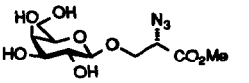
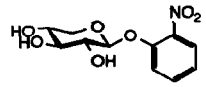
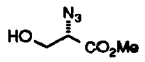
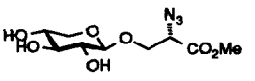
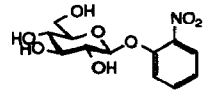
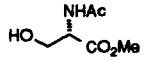
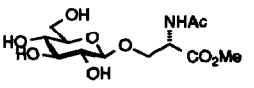
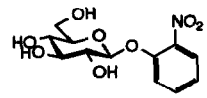
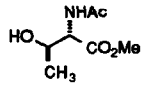
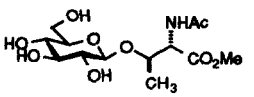
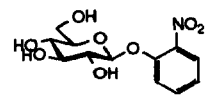
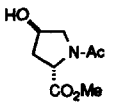
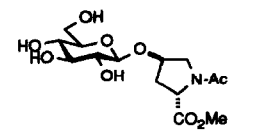
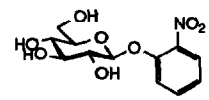
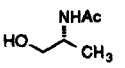
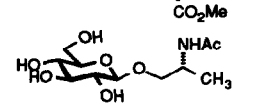
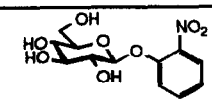
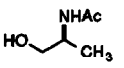
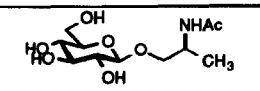
Scheme 2

In addition, any hydrolysed sugar **6** can similarly act as an acceptor leading to formation of the disaccharide **8**. To circumvent these problems and thereby maximise the yield of the desired glycoside **5** we devised a simple protocol in which the glycosyl donor was added over a period of approximately 24 hours to a solution containing the enzyme and acceptor alcohol. The donor sugar can be added either in several portions or continuously using a syringe pump. In this way it is possible to add excess glycosyl

donor (up to 10 equivalents) but avoiding high concentrations at any one time during the reaction. The reactions can be easily monitored by reverse phase h.p.l.c. and terminated when the concentration of the desired product is at a maximum.

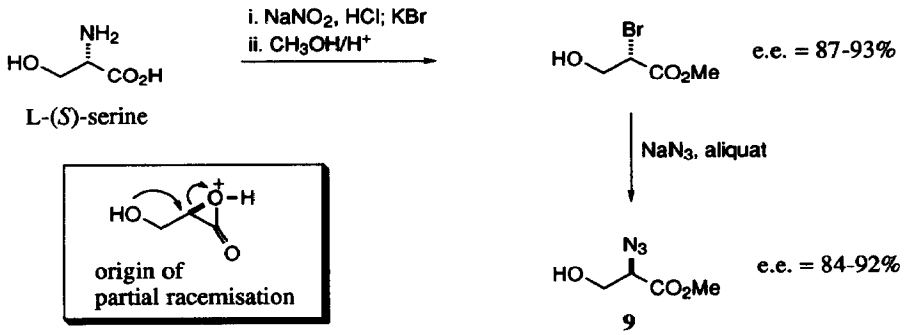
Using this procedure we have prepared a range of glycosides as shown in the **Table**.[8] The products can either be isolated by i) absorption onto silica followed by elution with chloroform/methanol or ii) preparative reverse-phase h.p.l.c. The yields have been calculated with respect to the glycosyl acceptor and thus represent far more efficient conversions than using previously reported methods.

Table: Synthesis of various glycosides using the 'syringe pump' method.

Glycosyl donor	Acceptor	Enzyme	Product	Yield/%
		β-glucosidase		25
		β-glucosidase		12
		β-galactosidase		5
		β-xylosidase		29
		β-glucosidase		25
		β-glucosidase		11
		β-glucosidase		9
		β-glucosidase		39
		β-glucosidase		11

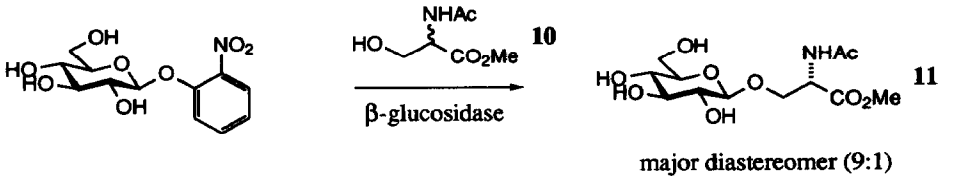
The products were characterised by conversion to the corresponding acetate derivatives which gave satisfactory spectroscopic and analytical data. Identification of the β-configuration of the anomeric centre was easily achieved *via* analysis of the H-1/H-2 coupling constant.

Initially we have focussed on simple alcohols as acceptors. Indeed, many of them are either simple derivatives or analogues of amino acids, thereby leading to the synthesis of glycosides which could be used as building blocks for the synthesis of more complex glycopeptides.[9] The α-azido esters proved to be good substrates, particularly with β-glucosidase and β-xylosidase. The acceptor alcohol methyl 2-azido-3-hydroxypropanoate was readily obtained from serine as shown in **Scheme 3**. However, we found that starting from either optically pure D or L-Serine, the bromo ester obtained had an e.e. of 87-93% and the azido ester an e.e. of 84-92%. This partial racemisation presumably occurs after displacement of the diazonium cation by the α-carboxyl group. The incipient α-lactone could undergo a competing inversion *via* S_N2 attack by the primary hydroxyl group.



Scheme 3

Using racemic alcohols as acceptors we have examined the possibility of diastereoselectivity in the glycosyl transfer step. Using N-acetyl DL-Serine methyl ester **10** as the acceptor substrate, the corresponding glucoside **11** was shown to have a diastereomeric excess of 80% (**Scheme 4**).

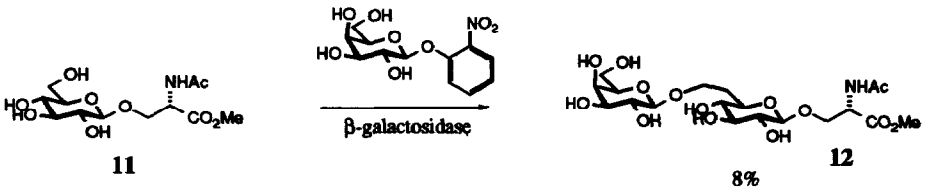


Scheme 4

The absolute configuration of the major diastereoisomer of the glucoside **11** was shown to be 2(*S*) by subsequent hydrolysis of the glucoside using β-glucosidase and determination of the optical rotation of the liberated N-acetyl serine methyl ester. However, when we ought to obtain further examples of diastereoselective glycosyl transfer, the results were disappointing. Thus using methyl (±)-2-azido-3-hydroxypropanoate **9** as substrate with *o*-nitrophenyl-β-D-glucoside and β-glucosidase, the corresponding glucoside was obtained as a 1:1 mixture of diastereomers. The relatively high diastereoselectivity

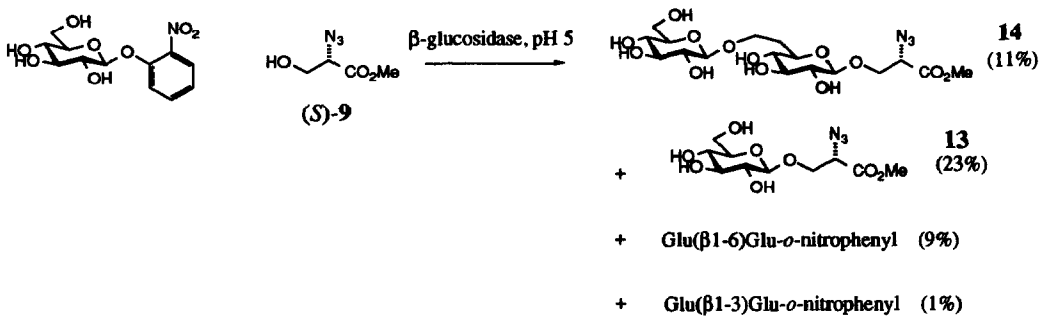
obtained with **10** as the acceptor substrate may be due to hydrogen bond interactions at the active site with the N-Acetyl group. It is noteworthy that with one exception[10], generally poor diastereoselectivity in these reactions has been observed.[8]

We next sought to apply our method to the synthesis of disaccharides. Again the approach involved using a fixed concentration of the acceptor alcohol (in this case a monosaccharide) and adding an excess of the sugar donor either continuously *via* the syringe pump or batchwise. Thus, using *o*-nitrophenyl β-D-galactose as the donor and the glucoside **11** as the acceptor we were able to prepare the disaccharide **12** in 8% yield with β-galactosidase as the catalyst (**Scheme 5**). The product disaccharide was characterised as its heptaacetate and could be clearly seen by n.m.r. analysis to be a single regio-isomer. The identity of the regioisomer was established both by comparison with an authentic sample prepared independently by synthesis[11] and by the characteristic downfield shift in the ^{13}C n.m.r. spectrum of C'-6 of the glucose moeity.



Scheme 5

When we attempted to repeat this reaction using the β-glucoside obtained from methyl 2(*S*)-2-azido-hydroxypropanoate none of the desired disaccharide was obtained. The reason for this is unclear, especially in view of the similarity of the acceptor substrates. It was however possible to obtain a related disaccharide using this substrate as shown in **Scheme 6**.



In this reaction, the azide ester (*S*)-**9** was used as the acceptor resulting initially in formation of the β-glucoside **13** as discussed above. However the glucoside was also able to function as an acceptor leading to the disaccharide **14** which could be isolated in 11% yield (based on the 23% yield of glucoside **13**). This unusual disaccharide results therefore from two sequential glucosidations of the alcohol (*S*)-**9**. Two

other disaccharides were also isolated from this reaction, namely the 1-6 (9%) and 1-3 (1%) Glu-Glu-*o*-nitrophenyl disaccharides.

In summary therefore we have demonstrated the practicality of synthesising a series of β-glycosides directly from the sugar donor and an alcohol acceptor using the appropriate glycosidase enzyme. In comparison with previously reported protocols, the method described herein utilises small, limiting amounts of the acceptor in combination with an excess of the donor. This approach results in good yields based on the amount of the acceptor and is therefore advantageous when the latter is available only in small quantities.

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10. H.-J. Gais, A. Zeissler, and P. Maidonis, *Tetrahedron Lett.*, **29**, 5743.
11. Full details of these experiments will be described in a subsequent publication.

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