Biotransformations in Carbohydrate Synthesis. *N*-Acetylgalactosaminyl Transfer on to Methyl *N*-Acetyl-β-D-glucosaminide (Methyl 2-Acetamido-2-deoxy-β-D-glucopyranoside) and Methyl *N*-Acetyl-α-D-glucosaminide (Methyl 2-Acetamido-2-deoxy-α-D-glucopyranoside) Catalysed by a β-*N*-Acetylgalactosaminidase from *Aspergillus oryzae*

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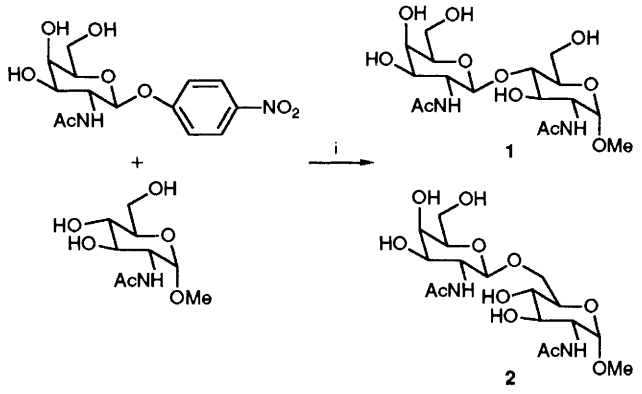
Using a crude *N*-acetylgalactosaminidase from *Aspergillus oryzae*, the β-*N*-acetylgalactosaminyl moiety of *p*-nitrophenyl *N*-acetylgalactosaminide was transferred to the C-4 and C-6 hydroxy groups of methyl *N*-acetyl-β-D-glucosaminide and methyl *N*-acetyl-α-D-glucosaminide and, for the latter, with high efficiency and selectivity for transfer to the C-4 position.

Currently, there is great interest in oligosaccharide synthesis owing to the important role that oligosaccharides play in a wide variety of molecular recognition processes in biological systems.[1] Powerful methods for oligosaccharide synthesis have been developed. However, the sheer complexity of oligosaccharide structures creates many opportunities for the development of further methods, including methods based on an enzymatic approach. Biotransformations applied to oligosaccharide synthesis have been based either on the natural (biosynthetic) glycosyl transferases or on the so-called 'reverse hydrolytic' activity of glycosidases.[2] The latter method has been most widely used as it does not require sugar nucleotides as substrates, as when the sugar nucleotide–dependent glycosyl transferases are used, and glycosidases as a class are more readily available than such nucleotide–dependent glycosyl transferases. The advantages of the method are that no protection–deprotection sequences are required and there is total control over the configuration at the newly generated anomeric centre. The major problem with this approach is that transfer is not completely regioselective and mixtures of products usually result. However, although the factors determining regioselectivity are imperfectly understood, it has been shown by Nilsson[3] that regioselectivity can be modulated by control of the configuration at the anomeric centre of the acceptor sugar, by what can loosely be described as anomeric control.

We report here a remarkably efficient glycosyl transfer reaction catalysed by a crude β-*N*-acetyl-D-galactosaminidase from *Aspergillus oryzae*,[4] in which anomeric control was used to optimise the formation of the desired 1–4 linked disaccharide product.

The transfer reaction was initiated by using *p*-nitrophenyl β-*N*-acetyl-D-galactosaminide as glycosyl donor. To determine the effect of anomeric control, the α- and β-anomers of methyl *N*-acetyl-D-glucosaminide were used in the transfer reactions.† With methyl *N*-acetyl-α-D-glucosaminide as acceptor, two disaccharide products were formed in 89% isolated yield. The major product (90% of disaccharide formed) was shown by ^{1}H, ^{13}C NMR spectroscopy and mass spectrometry to be the 1–4 linked product **1**, and the minor product (10% of disaccharide formed) was shown to be the 1–6 linked product **2** (Scheme 1). Transfer efficiency (based on donor glycoside) was 91% as determined by HPLC. With methyl *N*-acetyl-β-D-glucosaminide as acceptor, the efficiency of transfer was lower (43% by HPLC) and two products were formed in nearly equal amounts (47 : 53). The first of these (47% of product) was identified as the 1–4 linked product **3** (Scheme 2). The other component has not been identified.

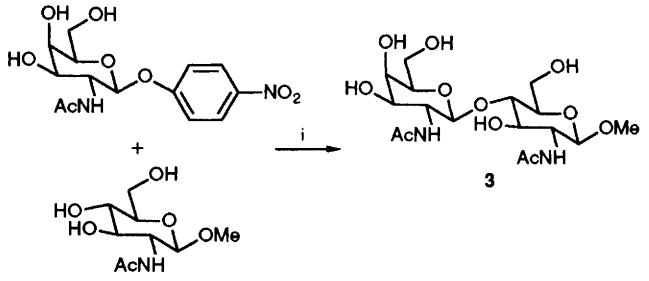
† In the following procedure, the enzyme used was a crude β-*N*-acetylgalactosaminidase isolated from the commercially available β-galactosidase from *A. oryzae*. In a typical procedure, *p*-nitrophenyl-*N*-acetyl-β-D-galactosaminide (25 mg, 0.07 mmol) and methyl *N*-acetyl-α-D-glucosaminide (100 mg, 0.43 mmol) in citrate–phosphate buffer (0.75 ml, 0.05 mol dm^{-3}, pH 4.5) were heated to 45–50 °C for 2 min to dissolve the substrates, cooled and incubated at 30 °C for 5 min, and treated with the crude *N*-acetyl-β-D-galactosaminidase (0.1 ml, 120 mg ml^{-1} protein) from *A. oryzae* (Sigma, grade XI) (80–100% ammonium sulfate fraction from the crude β-galactosidase, dialysed against the above phosphate buffer). Incubation was continued at 30 °C for 96 h, by which time all of the donor had been consumed. The enzyme activity was destroyed by heating the reaction mixture to 85–90 °C for 5 min. The *p*-nitrophenol was extracted with diethyl ether. The aqueous residue was lyophilised and the disaccharide products were separated by semi-preparative HPLC using a Magnasil 5H aminopropyl column with MeCN : H_2O (80 : 20) as eluent and with UV detection at 210 nm. The total yield of transfer products as determined by HPLC was 91% based on *p*-nitrophenyl-*N*-acetyl-β-D-galactosaminide. The isolated yield was 89% (combined products). The products were identified as GalNAcβ1–4GlcNAcOMe (90% of transfer products) and GalNAcβ1–6GlcNAcOMe (10% of transfer products) by ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry.



Scheme 1 *Reagent*: i, β-*N*-acetyl-D-galactosaminidase from *A. oryzae*

These experiments show that the presence of an α-anomeric linkage in the acceptor directs glycosyl transfer selectively towards the C-4 hydroxy group. A similar effect was observed during transfer on to methyl α- and β-glucosides.[4]

The foregoing results illustrate the remarkable efficiency with which disaccharides may be formed by this enzyme, even in the crude form† used here. The method is clearly open for further optimisation, particularly with respect to the concentration of acceptor, which is present in large excess. Investigation of the kinetic parameters of the reaction should make it possible to make more efficient use of the acceptors by reducing concentrations so as to achieve the optimum balance between transfer and hydrolysis.



Scheme 2 *Reagent*: i, β-*N*-acetyl-D-galactosaminidase from *A. oryzae*

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References

1 T. Feizi and R. A. Childs, *Biochem. J.*, 1987, **245**, 1; K. Drickamer, *J. Biol. Chem.*, 1988, **263**, 9557; M. Höök, L. Kjellen, S. Johansson and J. Robinson, *Ann. Rev. Biochem.*, 1984, **53**, 847.
2 K. G. I. Nilsson, *Trends Biotechnol.*, 1988, **6**, 256; E. J. Toone, E. S. Simon, M. D. Bednarski and G. M. Whitesides, *Tetrahedron*, 1989, **45**, 5365; G. L. Cote and B. Y. Tao, *Glycoconjugate J.*, 1990, **7**, 145.
3 K. G. I. Nilsson, *Carbohydr. Res.*, 1987, **167**, 95; 1988, **180**, 53.
4 D. H. G. Crout, O. W. Howarth, S. Singh, B. E. P. Swoboda, P. Critchely and W. T. Gibson, *J. Chem. Soc., Chem. Commun.*, 1991, 1550.