Biotransformations in Carbohydrate Synthesis. *N*-Acetylgalactosaminyl and *N*-Acetylglucosaminyl Transfer onto Methyl α- and β-Glucosides Catalysed by the β-*N*-Acetylhexosaminidase from *Aspergillus oryzae*

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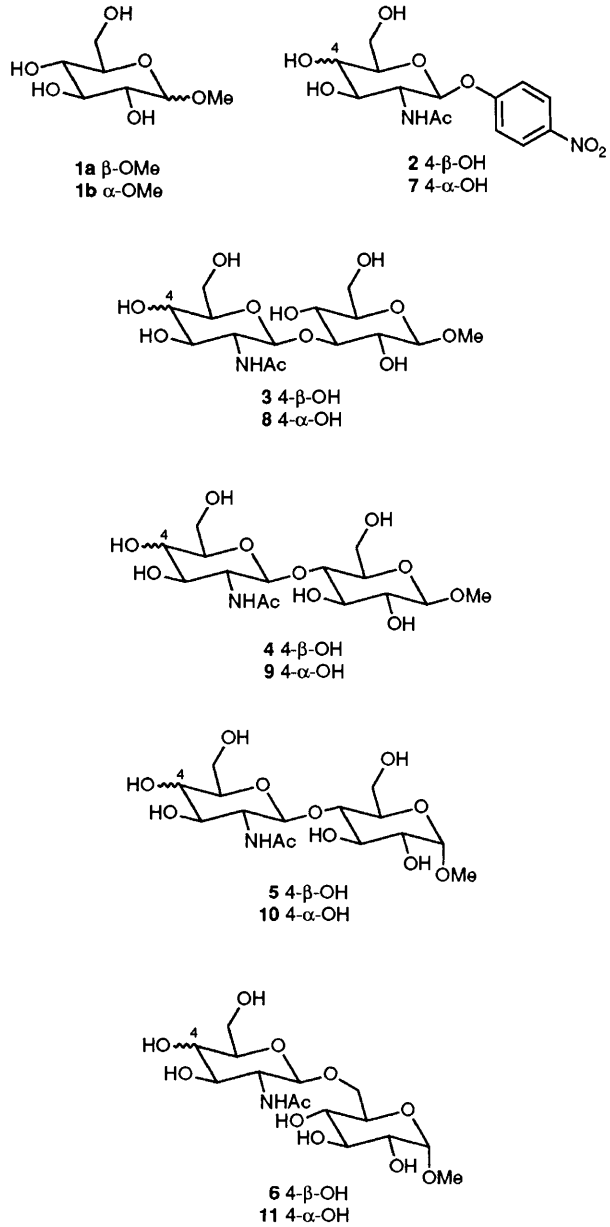
Regioselectivity of *N*-acetylhexosaminyl transfer onto methyl α- and β-D-glucosides catalysed by the *N*-acetylhexosaminidase from *Aspergillus oryzae* is strongly dependent on the configuration at the anomeric centre of the acceptor: with the β-glucoside, 1,3- and 1,4-transfer products are formed, whereas with the α-glucoside, 1,4- and 1,6-transfer products are obtained.

Oligosaccharides may be synthesised by the 'reverse hydrolytic' activity of glycosidases.[1] A glycosidic donor, often with an activating aglycone, is used to generate a glycosyl–enzyme intermediate that may be intercepted by a nucelophilic acceptor other than water, leading to glycoside formation. Such reactions are synthetically useful, since no protection is required and the configuration at the newly formed anomeric centre is determined by the specificity of the enzyme. Although glycosyl transfer is usually regioselective, this selectivity is rarely absolute. Transfer to more than one hydroxy group in the acceptor is usually observed. However, it has been shown by Nilsson[2] that regioselectivity of glycosyl transfer can be modulated by controlling the configuration of the anomeric centre in glycosidic acceptors. The objective of the present investigation was to develop a system for selective transfer of *N*-acetylgalactosaminyl residues onto C-4 of a glucosidic acceptor, based on this approach.

The enzyme used in the present study was the crude β-galactosidase from *A. oryzae*. This material contains many glycosidase activities other than that of β-galactosidase. Although these activities individually may represent less than one per cent of the β-galactosidase activity, nevertheless, the enzyme is so cheap that it represents a useful source of these minor activities. For the present work, a useful enhancement of specific activity of the *N*-acetylhexosaminidase[3] was achieved by using an 80–100% ammonium sulphate fraction, in the preparation of which much of the β-galactosidase activity was removed.

When methyl-β-D-glucoside **1a** was used as acceptor and *p*-nitrophenyl-β-*N*-acetyl-D-galactosaminide **2** as *N*-acetyl-D-galactosaminyl donor (Scheme 1),† two disaccharide products only were observed by HPLC, in 49% overall yield (based on the donor glycoside) and in a ratio of 4:1. These were separated and purified by HPLC and were shown, on the basis of characteristic shifts in their ^{13}C NMR spectra,[4] to be the 1,3- (**3**, major isomer) and 1,4- (**4**, minor isomer) transfer products. NMR signal assignments were made using proton–proton and carbon–proton shift correlation spectroscopy (COSY). Structures were confirmed by negative ion FAB MS–MS (tandem) mass spectrometry.

When methyl α-D-glucoside **1b** was used as acceptor, a strikingly different result was obtained. In this case, only two products again were observed, in 36% overall yield. The major product (84%) was shown to be the 1,4-transfer product



Scheme 1

† In a typical procedure, *p*-nitrophenyl-*N*-acetyl-β-D-galactosaminide (35 mg, 0.102 mmol) and methyl β-D-glucopyranoside (199 mg, 1.02 mmol) in phosphate buffer (1.7 ml, 0.04 mol dm^{-3}, pH 6.5) were heated to 45–50 °C for 2 min to dissolve the substrates, cooled and incubated at 28 °C, and treated with the crude *N*-acetyl-β-D-hexosaminidase from *A. oryzae* (Sigma, grade XI) (80–100% ammonium sulphate fraction from the crude β-glactosidase, dialysed against the above phosphate buffer). Incubation was continued for 24 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 10 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 (81 : 19) as eluent and with UV detection at 210 nm. The total yield of transfer products as determined by HPLC was 49% based on *p*-nitrophenyl-*N*-acetyl-β-D-galactosaminide. The isolated yield was 34% (combined products).

5 and the minor isomer (16%) was shown to be the 1,6-transfer product **6**. Structure assignments were made as indicated above.

When *p*-nitrophenyl-β-*N*-acetyl-D-glucosaminide **7** was used as glycosyl donor, very similar results were obtained. With methyl β-D-glucoside **1** as acceptor, the 1,3- and 1,4-transfer products **8** and **9** were obtained in an overall yield of 23% and in a ratio of 55 : 45. With methyl α-D-glucoside as acceptor, the 1,4- and 1,6-transfer products **10** and **11** were obtained in an overall yield of 17% and in a ratio of 7 : 3.

Since a crude enzyme preparation was used in this study, it cannot be assumed with certainty that all of the transfer reactions observed with the respective substrates were catalysed by a single enzyme. From *A. niger*, for example, an *N*-acetylglucosaminidase has been isolated which has *N*-acetylgalactosaminidase activity.[5] Also, an *N*-acetylgalactosaminidase has been isolated which has no *N*-acetylglucosaminidase activity.[6] Nevertheless, assuming that with the two substrates **2** and **7** catalysis of transfer is respectively attributable mainly to a single enzyme, the present results indicate marked differences in the conformations of the bound acceptor depending on the configuration at the remote anomeric centre. Further study of these effects will clearly be of considerable interest in relation to molecular recognition of substrates by these enzymes, and for their applications in regiochemically controlled enzymatic synthesis of oligosaccharides.

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