Glycosidase-catalysed synthesis of oligosaccharides: a two-step synthesis of the core trisaccharide of *N*-linked glycoproteins using the β-*N*-acetylhexosaminidase and the β-mannosidase from *Aspergillus oryzae*

Suddham Singh, Michaela Scigelova and David H. G. Crout*

Department of Chemistry, University of Warwick, Coventry, UK CV4 7AL

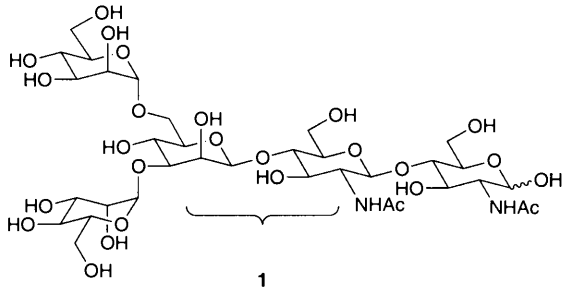
Using a partially purified β-mannosidase from *Aspergillus oryzae*, a β-mannosyl unit is transferred from *p*-nitrophenyl β-D-mannopyranoside 5 to di-*N*-acetylchitobiose 4 to give the core trisaccharide β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc 6 of the *N*-linked glycoproteins.

All *N*-linked (Asn-linked) glycoproteins contain the core pentasaccharide **1**. On this pentasaccharide are constructed the huge number of arrays of oligosaccharide structures found in *N*-linked glycoproteins and which profoundly influence their biological properties. With reference to the synthesis of the *N*-linked glycoproteins, it has been stated recently[1] that 'Among the several unresolved problems that remain, the most synthetically challenging by far is the construction of the β-glycosidic linkage between mannose and *N*-acetylglucosamine residues'.

The challenge of creating this linkage (bracketed in **1**) has stimulated a number of ingenious approaches: displacement of an α-anomeric group by the mannose acceptor, use of a tethered acceptor, or manipulation of a selectively exposed C-2 OH group in a corresponding glucoside, either by inversion or *via* an oxidation–reduction sequence.[1,2]

We have developed a one-step procedure for the preparation of di-*N*-acetylchitobiose **4** with *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside **2** as glycosyl donor and 2-acetamido-2-deoxy-D-glucopyranose (*N*-acetylglucosamine) **3** as acceptor using a partially purified β-*N*-acetylhexosaminidase from *A. oryzae* (Scheme 1).[3]

We have now discovered that from the same crude enzyme preparation, a β-mannosidase can be obtained that catalyses transfer of a β-mannosyl unit from *p*-nitrophenyl β-D-mannopyranoside **5** to the disaccharide **4** specifically to the 4-OH of the non-reducing unit, to give the core trisaccharide **6** of the *N*-linked glycoproteins (Scheme 1). A simple procedure was followed: the donor **5** (75 mg, 0.25 mmol) and acceptor **4** (690 mg, 1.63 mmol) in citrate-phosphate buffer (pH 4.5, 0.05 mmol dm^{-3}, 2 cm^3) were incubated with the partially purified β-mannosidase (46 mg protein cm^{-3}, 0.24 U mg^{-1}, 0.1 cm^3) at 30 °C for 5 h. The product **6** (38 mg, 26% yield based on donor) was isolated by HPLC [Hypersil 5APS column (25 cm × 20 mm), UV detection at 210 nm, eluent MeCN–H_2O (76:24), flow rate 10 cm^3 min^{-1}] and was identified by NMR[4] and mass spectroscopy. The product **6** was the only trisaccharide formed in the reaction.



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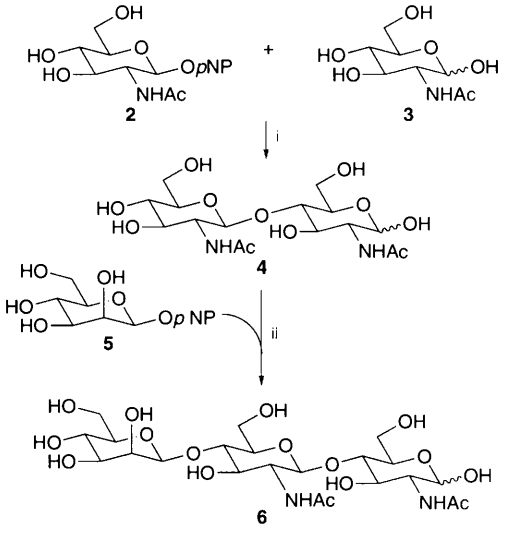
The reaction, which is not fully optimised, was carried out with limiting amounts of β-mannosyl donor. However, the excess of acceptor can be recovered quantitatively and re-used. When using glycosidases to catalyse formation of disaccharides, the presence of other glycosidase activities in the enzyme preparation is of little or no consequence. However, when tri- or higher oligosaccharides are to be prepared, it is essential that contaminating activities are removed that might catalyse hydrolysis of the acceptor. In the present case, the β-mannosidase, free of β-*N*-acetylhexosaminidase activity, was isolated from the crude β-galactosidase from *A. oryzae* (Sigma) by ammonium sulfate fractionation, hydrophobic interaction chromatography [Phenyl Sepharose (Pharmacia)] and anion exchange chromatography [(DEAE-Sepharose (Fluka)].

Since the acceptor **4** can be obtained in one step using the β-*N*-acetylhexosaminidase from *A. oryzae*, the trisaccharide **6** is now available in two glycosidase-catalysed steps (Scheme 1) from *N*-acetylglucosamine, the corresponding *p*-nitrophenyl β-D-glycopyranoside and *p*-nitrophenyl β-D-mannopyranoside. The disaccharide **4** is readily obtained on a multigram scale. (The alternative, commercial method is isolation from the mixture of products obtained by the partial hydrolysis of chitin.) Given that *p*-nitrophenyl β-D-mannopyranoside **5** is available in two steps from mannose following Garegg's procedure,[5] the method described here clearly can be adapted to provide the trisaccharide **6** in quantities that will permit its widespread use as a building block for the synthesis of oligosaccharides of *N*-linked glycoproteins. An alternative glycosidase-catalysed method has been published, but this requires a mannose trisaccharide as mannosyl donor and proceeds in very low yield.[4]



Scheme 1 *Reagents*: i, *N*-Acetylhexosaminidase from *A. oryzae*; ii, β-mannosidase from *A. oryza*. *p*NP = *p*-nitrophenyl.

We thank the BBSRC for financial support, the EPSRC Mass Spectrometry Unit, Swansea, for mass spectra and Dr Jeremy Hastings for NMR spectra.

References

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Received, 16th January 1996; Com 6/00329J