Glycosidase-catalysed synthesis of oligosaccharides: trisaccharides with the α-D-Gal-(1 → 3)-D-Gal terminus responsible for the hyperacute rejection response in cross-species transplant rejection from pigs to man

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α-Galactosidases from *Coffea arabica*, *Aspergillus niger* and *A. oryzae* catalyse the transfer of α-galactosyl residues on to lactose thioglycosides to give 1 → 3 and 1 → 6 linked trisaccharides, including an unusual branched trisaccharide 11 and the isoglobotriose glycoside 4, which, as 'linear B type 6', has been recognised as of the type responsible for the hyperacute rejection response in pig-to-man xenotransplantation.

The α-D-Gal-(1 → 3)-linkage is found in a number of biologically significant glycoconjugates including oligosaccharide ceramides of transplanted tumour (pheochromocytoma) cells,[1] erythrocytes of New World primates and other mammals[2,3] and glycoproteins of Ehrlich carcinomas.[4] Although the α-D-Gal-(1 → 3)-D-Gal structural determinant formerly was not considered to occur in man, more recent antibody binding studies have indicated that human erythrocytes contain small amounts of complex glycosphingolipids bearing this structural feature. These may represent senescence antigens, as binding of anti-Gal antibody was demonstrated to human normal senescent and some pathological erythrocytes.[3] Galili and co-workers showed that 1% of circulating immunoglobulin G (IgG) binds to this carbohydrate fragment.[5] The significance of this finding has been thrown into high relief by evidence that α-D-Gal-(1 → 3)-D-Gal is the carbohydrate epitope in porcine tissue towards which the anti-Gal antibodies are directed in the hyperacute rejection response that has so far proved inimical to cross-species organ transplantation from pigs to man.[6] This disaccharide sequence is therefore significant in the light of its occurrence in glycoconjugates, and its importance in studies of cross-species organ transplantation and for investigations of ways in which the carbohydrate itself or analogues might be used to inhibit the hyperacute rejection response.[6] Accordingly, we have studied formation of the key α-D-Gal-(1 → 3)-D-Gal sequence by α-D-galactosyl transfer on to the α-D-galactosyl residue of lactose thioglycosides using *p*-nitrophenyl α-D-galactoside **1** as glycosyl donor. The application of glycosidase-catalysed glycosyl transfer avoids the need for protection–deprotection sequences in oligosaccharide synthesis and confers the additional benefit of complete control of configuration at newly created anomeric centres.

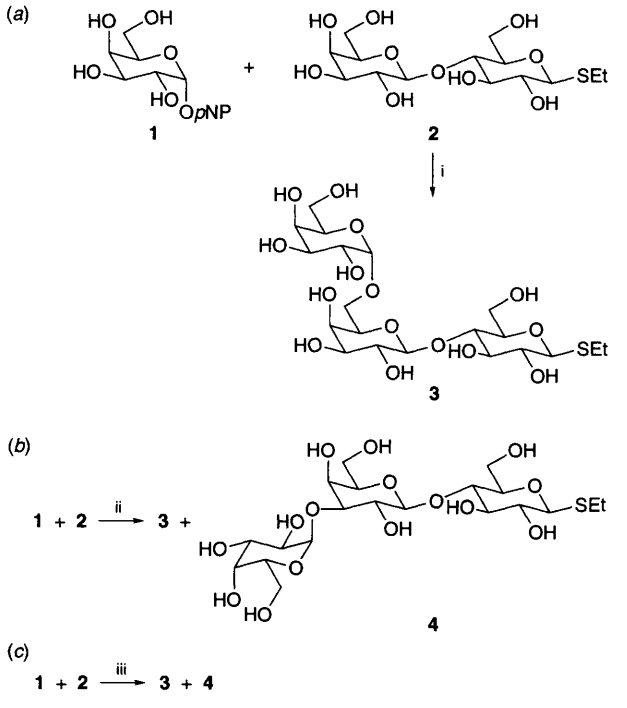
Thus with thioethyl β-D-lactoside **2** as acceptor and glycoside **1** as donor, the α-galactosidase of *Aspergillus niger*† catalysed glycosyl transfer to give a trisaccharide glycoside **3** as the sole product in 20% yield based on donor [Scheme 1(*a*)]. (The excess of acceptor is quantitatively recovered from the product mixture by Biogel P2 chromatography).‡

The α-galactosidase from green coffee beans (*Coffea arabica*)§ is reported to be able to cleave α-D-Gal-(1 → 3)-D-Gal linkages.[7] It therefore possesses the intrinsic ability to generate this linkage. Incubation of donor **1** with acceptor **2** in the presence of this α-galactosidase gave a 1 : 0.57 mixture of the 1 → 6 (**3**) and (1 → 3) (**4**)-linked trisaccharide glycosides [Scheme 1(*b*)]. Although for characterisation purposes pure samples of glycosides **3** and **4** were isolated, separation of the products proved difficult, a problem that was later overcome (see below).

Recently we discovered that *A. oryzae* produces two α-galactosidases designated α-galactosidases I and II.¶ Incubation of donor **1** with acceptor **2** in the presence of α-galactosidase II gave trisaccharide glycosides **3** and **4** in 26% yield and in a ratio of 0.77 : 1 [Scheme 1(*c*)]. With this enzyme, the 1 → 3 linked glycoside is the major product. The carbohydrate component (isoglobotriose) of this glycoside is a highly specific ligand for the anti-Gal IgG that is responsible for the hyperacute rejection response in xenotransplantation of organs between the pig and man.[6] By changing the aglycone to thiobutyl **5** the yield of trisaccharide glycosides **6** and **7** increased to 31% and with thiophenyl glycoside **8** as acceptor the yield of trisaccharide glycosides **9** and **10** increased to 39%.

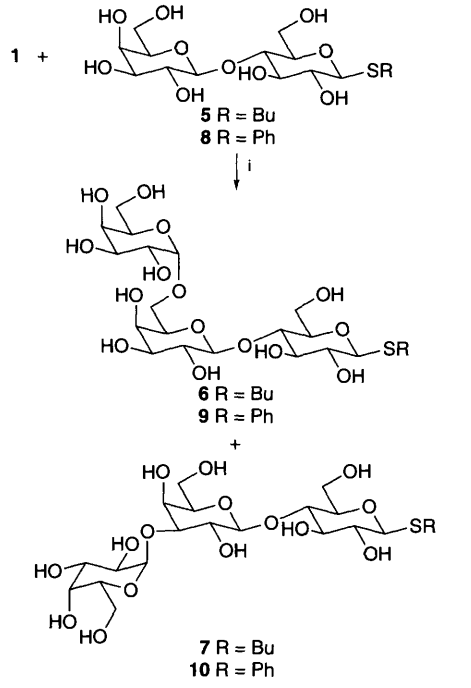
We have observed previously that glycosidase-catalysed glycosyl transfer to di- and higher oligo-saccharides is usually improved both in yield and selectivity compared with transfer to monosaccharides, an effect attributable to a recognition site on the enzyme extending over at least two monosaccharide units. The increased yields in the series of thioethyl, thiobutyl and thiophenyl lactosides indicates that the aglycones of these disaccharides are also recognised, to varying degrees.

When donor **1** was incubated with acceptor **2** in the presence of α-galactosidase I, an entirely unexpected result was obtained.

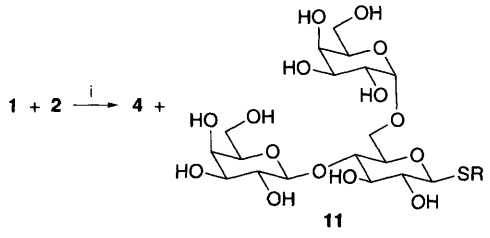


Scheme 1 *Reagents*: α-Galactosidases from i, *A. niger*; ii, *Coffea arabica*; iii, *A. oryzae* (α-galactosidase I). *p*NP = *para*-nitrophenyl.

Besides minor amounts of trisaccharide glycoside **4**, a 1 → 6-linked trisaccharide glycoside was obtained that was different from trisaccharide glycoside **3** (Scheme 2). The ratio of 1 → 6 to 1 → 3 linked products was 1 : 0.22. A detailed NMR investigation showed that the major product was the branched trisaccharide glycoside **11**. The structures of glycosides **3**, **4** and **11** were determined by a complete NMR shift assignment based on ^{1}H–^{1}H and ^{1}H–^{13}C shift-correlated 2D spectra. All carbon atoms bearing OH groups had shifts no more than 1.2 ppm above those of the corresponding monosaccharides, whereas all derivatised carbon atoms were deshielded by at least 5 ppm.



Scheme 2 *Reagent*: i, α-Galactosidase I from *A. oryzae*



Scheme 3 *Reagent*: i, α-Galactosidase II from *A. oryzae*

The switch from thioethyl lactoside **2** as acceptor to thiobutyl lactoside **5** as acceptor, besides giving an increased yield of product, offered an additional benefit in that it proved possible readily to separate the products **6** and **7** (Scheme 3) by charcoal–Celite chromatography. The increased differential retention of the two glycosides on the column was accompanied by stronger absorption. This effect may prove to be applicable to the separation of other trisaccharide mixtures.

The results described here significantly extend the range of synthetically useful glycosidases, particularly with respect to synthesis of the biologically significant isoglobotriose system as in **4**, **7** and **10**. Overall yields compare favourably with corresponding multi-step conventional procedures and lead directly to activatable products that can be used as building blocks for the synthesis of more complex oligosaccharides.

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Footnotes

† A gift from Novo Nordisk.

‡ Illustrative procedure: to a solution of acceptor **2** (0.61 g, 0.4 mol dm^{-3}) and donor **1** (0.10 g, 0.08 mol dm^{-3}) in citrate–phosphate buffer (50 mmol dm^{-3}, pH 5.0, 4 cm^3) was added the α-galactosidase (2.5–5 U). After all of the donor had been consumed, as determined by HPLC, the mixture was heated at 100 °C for 5 min. The mixture was filtered, the filtrate was diluted with water and the product was extracted with diethyl ether, applied to a Biogel P2 column and eluted with H_2O. The thiobutyl trisaccharides were further purified to homogeneity on a charcoal–Celite column eluted with an ethanol–water mixture (45 : 55).

§ Sigma Chemical Company Ltd.

¶ Isolated from the partially purified β-galactosidase of *A. oryzae*, a gift from the Sigma Chemical Company Ltd.

References

1 T. Ariga, R. K. Yu, J. N. Scarsdale, M. Suzuki, Y. Kuroda, H. Kitogawa and T. Miyatake, *Biochemistry*, 1988, **27**, 5335.
2 U. Galili, C. B. Basbaum, S. B. Shohet, J. Buehler and B. A. Macher, *J. Biol. Chem.*, 1987, **262**, 4683.
3 U. Galili, M. R. Clark, S. B. Shohet, J. Buehler and B. A. Macher, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 1369.
4 M. J. Elices and I. J. Goldstein, *J. Biol. Chem.*, 1989, **264**, 1375.
5 U. Galili, E. A. Rachmilewitz, A. Peleg and I. Flechner, *J. Exp. Med.*, 1984, **160**, 1519.
6 D. K. C. Cooper, E. Koren and R. Oriol, *Immunological Rev.*, 1994, **141**, 31.
7 A. Kobata and K. Yamashita, in *Glycobiology: a Practical Approach*, ed. M. Fukuda and A. Kobata, IRL Press, Oxford, 1993, p.168.

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