Carbohydrate Research, 167 (1987) 95–103
Elsevier Science Publishers B.V., Amsterdam - Printed in The Netherlands

A SIMPLE STRATEGY FOR CHANGING THE REGIOSELECTIVITY OF GLYCOSIDASE-CATALYSED FORMATION OF DISACCHARIDES*

KURT G. I. NILSSON
Swedish Sugar Co. Ltd., R & D, Box 6, S-232 00 Arlöv (Sweden)

(Received August 14th, 1986; accepted for publication, January 15th, 1987)

ABSTRACT

The regioselectivity of glycosidase-catalysed formation of disaccharides can be changed by using α- or β-glycosyl acceptors with various aglycons. The preponderant formation of other than (1→6) linkages can be effected with glycosidases which normally give (1→6) linkages. Thus, an α-D-galactosidase can be induced to catalyse the formation mainly of α-(1→2)-, α-(1→3)-, or α-(1→6)-linked digalactosides. Both the structure of the aglycon and the configuration of the glycosidic linkage can have a pronounced influence on the regioselectivity of disaccharide formation. Enzymic syntheses, in yields of 20–30%, are described of α-D-Gal*p*-(1→3)-α-D-Gal*p*-OMe, β-D-Gal*p*-(1→3)-β-D-Gal*p*-OMe, β-D-Gal*p*-(1→6)-α-D-Gal*p*-OMe, α-D-Man*p*-(1→2)-α-D-Man*p*-OMe, α-D-Man*p*-(1→6)-α-D-Man*p*-OMe, α-D-Gal*p*-(1→2)-α-D-Gal*p*-OPhNO$_2$-*o*, α-D-Gal*p*-(1→3)-α-D-Gal*p*-OPhNO$_2$-*p*, α-D-Man*p*-(1→2)-α-D-Man*p*-OPhNO$_2$-*p*, and α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-OMe. Soluble and immobilised enzymes have been used.

INTRODUCTION

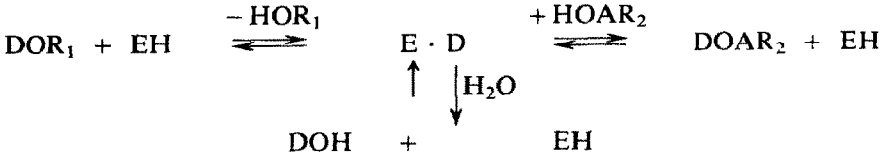
Knowledge of the carbohydrate structures present as glycoconjugates in living organisms is now extensive[1,2]. Chemical syntheses of such structures are limited, at least for larger scale work, by the many protection and deprotection steps that are necessary[3]. Biotechnological synthesis strategies are complementary to organochemical approaches and have been found to be valuable[4–6].

Glycosidases catalyse not only the hydrolysis but also the stereospecific formation of glycosidic bonds[7]. Synthesis has been achieved by either an equilibrium approach (reversed hydrolysis) or by a kinetic approach (transglycosidation). These principles have been used for protease-mediated synthesis of peptides[8,9]. However, the wider use of glycosidases for the synthesis of biologically active oligosaccharides has been limited, since preponderant formation of (1→6) linkages occurs and (1→2), (1→3), and (1→4) linkages are formed to a lesser extent[6,7]. Furthermore, purification of the complex mixtures of products may be difficult.

*Presented at the XIIIth International Carbohydrate Symposium, Ithaca, August 10–15, 1986.

0008-6215/87/$03.50

The regioselectivity of many glycosidases can be changed by using a simple strategy. Thus, several glycosidases, which synthesise mainly (1→6) linkages with glycosyl acceptors (HOA) that have a reducing moiety, will catalyse the preponderant formation of other linkages if the appropriate glycosides ($HOAR_2$) are used as acceptors. In the annexed scheme[7,10,11], HOA symbolises the carbohydrate portion of the acceptor, R_2 is a suitable α- or β-bound non-carbohydrate aglycon, $DOAR_2$ is the product oligosaccharide, DOR_1 is the donor, and EH is the enzyme. The strategy provides an approach to glycosides suitable for use as inhibitors, affinity labels, and enzyme substrates, and for attachment to lipids, peptides, proteins, chromatography supports, *etc.* Moreover, purification of the products is simplified, even if isomers are formed, since anomerisation of the acceptor cannot occur.



p-Nitrophenyl α- or β-D-glycosides have been used as glycoside donors, and methyl and nitrophenyl α- or β-D-glycosides as acceptors.

EXPERIMENTAL

General. — α-D-Galactosidase (EC 3.2.1.22; coffee bean), β-D-galactosidase (EC 3.2.1.23; *E. coli*, grade VIII), and all the monosaccharide glycosides were obtained from Sigma, α-D-mannosidase (EC 3.2.1.24; jack bean) from Boehringer, and Tresyl-activated Sepharose from Pharmacia.

The enzymic reactions were performed in mixtures of buffer and *N,N*-dimethylformamide (0–30%). The reactions were monitored by t.l.c., h.p.l.c. (see below), and spectrophotometry (400 nm for *p*-nitrophenol and 420 nm for *o*-nitrophenol) of liberated nitrophenol. The methods for the isolation and characterisation of the products have been reported[4,12]. Solvents were removed with a rotary evaporator and then at <0.1 Torr. T.l.c. was performed on Kieselgel 60 F_{254} (Merck) with detection by u.v. light or charring with sulfuric acid. Column chromatography was performed on Kieselgel 60 (Merck, 230–400 mesh) or Sephadex G10 (Pharmacia). Chloroform–methanol–water, ethyl acetate–iso-octane (for acetylated compounds), or water (Sephadex G10) were used as eluents unless otherwise indicated. The purity and yields were determined by h.p.l.c. [Waters pump, refractometer 410, spectrophotometer 481; columns: Spherisorb, NH_2-silica (5 μm) and Nucleosil, C_{18}-silica (5 μm); elution with water–acetonitrile] by integrating the peak areas. Acetylation was effected conventionally with pyridine–acetic anhydride (2:1) and deacetylation with methanolic sodium methoxide. Duolite C-6 (H^+) resin (methanol-washed and dried over P_2O_5) was used for neutralisations.

The structure of the acetylated glycosides was determined by ^{1}H- and ^{13}C-n.m.r. spectroscopy (Varian XL 200 instrument); internal Me_4Si or sodium 3-(trimethylsilyl)propionate-d_4 (TSP). The assignments were based on double-resonance and DEPT-experiments[4,5,12]. That a linkage was (1→2) or (1→3) was indicated by the marked up-field shifts of the H-2 or H-3 resonances, respectively. Similarly, (1→6) linkages were indicated by the marked downfield shift of the C-6 resonance. Methylation analyses[13] were carried out when literature n.m.r. data were not available.

Immobilisation of α- and β-D-galactosidase on tresyl-agarose. — A standard procedure was used[9]. Tresyl-agarose (3 g) was washed with mM HCl (600 mL) at 4° and then with cold coupling buffer (100 mL of 0.1M sodium phosphate, mM $MgCl_2$, pH 8), and portions (5 and 6 g, respectively) were added to α-D-galactosidase (4 mL, 20 mg of protein, 200 U) and β-D-galactosidase (17.5 mg, 10200 U) each dissolved in the coupling buffer (6 mL). The gels were agitated gently for 15 h at 4°, then washed with coupling buffer and 30mM sodium phosphate (pH 7) (3 × 10 vol. each), and stored at 4°. The yields of bound α- and β-D-galactosidase were 55 and 84%, respectively, as determined from the u.v. absorption (280 nm) of the collected washings.

Enzymic syntheses with α-D-galactosidase. — (*a*) p-*Nitrophenyl 2-O-* (**1**) and *3-O-α-D-galactopyranosyl-α-D-galactopyranoside* (**2**). To a solution of *p*-nitrophenyl α-D-galactopyranoside (4.5 g) in 0.03M sodium phosphate (70 mL, pH 6.5) and *N,N*-dimethylformamide (30 mL) was added α-D-galactosidase (1.2 mL; 60 U). The mixture was stored at room temperature, and the reaction was monitored spectrophotometrically and by reversed-phase h.p.l.c. After 52 h, the reaction was stopped by heating at 75° for 10 min. The products were isolated by column chromatography on Kieselgel 60 (chloroform–methanol–water, 6:5:0.5) and on Sephadex G10 (the isomers separated on this support on elution with water). This gave, as white powders, **1** (60 mg, purity 90%) contaminated with the (1→6)-linked isomer, and **2** (440 mg, purity 97%). The products were crystallised from ethyl acetate–2-propanol–water.

Compound **1** had m.p. 219–220°, $[\alpha]_D^{21}$ +240° (*c* 0.2, 2:1 methanol–water). N.m.r. data (D_2O): ^{1}H, δ 8.29 and 7.36 (2 d, each 2 H, *J* 9 Hz, $C_6H_4NO_2$ group), 6.12 (d, 1 H, *J* 3.2 Hz, H-1), 5.15 (d, 1 H, *J* 4 Hz, H-1′), 4.25–3.71 (m, 12 H); ^{13}C, δ 98.74, 97.01 (C-1,1′), 75.00 (C-2), 74.65, 73.92, 72.17, 71.98, 71.93, 70.82, 70.73 (C-2′, C-3,3′, C-4,4′, C-5,5′), 63.98 and 63.78 (C-6,6′).

Anal. Calc. for $C_{18}H_{25}NO_{13}\cdot 0.5H_2O$: C, 45.76; H, 5.55; N, 2.96. Found: C, 45.40; H, 5.40; N, 2.87.

Compound **2** had m.p. 217°, $[\alpha]_D^{21}$ +298° (*c* 0.3, 2:1 methanol–water). N.m.r. data (D_2O): ^{1}H, δ 8.42 and 7.28 (2 d, each 2 H, *J* 9 Hz, $C_6H_4NO_2$ group), 5.90 (d, 1 H, *J* 3.2 Hz, H-1), 5.23 (d, 1 H, *J* 3.7 Hz, H-1′), 4.32–3.71 (m, 12 H); ^{13}C, δ 99.63, 98.05 (C-1,1′), 76.95 (C-3), 74.87, 73.91, 72.20, 72.12, 71.07, 69.15, 68.20 (C-2,2′, C-3′, C-4,4′, C-5,5′), 64.04 and 63.77 (C-6,6′).

Anal. Calc. for $C_{18}H_{25}NO_{13}\cdot 0.5H_2O$: C, 45.76; H, 5.55; N, 2.96. Found: C,

45.70; H, 5.41; N, 3.00.

The syntheses of *p*-nitrophenyl digalactosides with the α-D-galactosidase–agarose were performed as for the soluble enzyme, but the reactions were stopped by removal of the gel by centrifugation. The gel was washed with 0.03M sodium phosphate (pH 6.5) before re-use.

(*b*) *o-Nitrophenyl 2-O-α-D-galactopyranosyl-α-D-galactopyranoside* (**3**). The procedure in (*a*) was followed, but using *o*-nitrophenyl α-D-galactopyranoside (1.5 g) and α-D-galactosidase (0.4 mL) in a total reaction volume of 33 mL (10 mL of *N,N*-dimethylformamide). Column chromatography (Kieselgel 60, 6:2:1 EtOAc–2-propanol–H_2O) of the product and two crystallisations from the same solvent mixture gave **3** as a white solid (50 mg, purity >99%), m.p. 225–226°, $[\alpha]_D^{21}$ +221° (*c* 0.2, 2:1 methanol–water). N.m.r. data (1:1 D_2O–CD_3OD): ^1H, δ 7.95–7.25 (dd, m and t, 1, 2 and 1 H, respectively, $C_6H_4NO_2$-*o* group), 6.13 (d, 1 H, *J* 2.2 Hz, H-1), 5.15 (d, 1 H, *J* 3.4 Hz, H-1'), 4.25–3.73 (m, 12 H); ^{13}C, δ 101.69, 100.01 (C-1,1'), 78.13 (C-2), 76.92, 75.64, 74.02, 73.72, 73.43, 72.86, 72.60 (C-2', C-3,3', C-4,4', C-5,5'), 65.51 and 64.97 (C-6,6').

Anal. Calc. for $C_{18}H_{25}NO_{13}\cdot 0.5H_2O$: C, 45.76; H, 5.55; N, 2.96. Found: C, 45.80; H, 5.38; N, 2.93.

H.p.l.c. of the reaction mixture revealed <1% of an unidentified isomeric glycoside.

(*c*) *Methyl 3-O-α-D-galactopyranosyl-α-D-galactopyranoside* (**4**). To a solution of *p*-nitrophenyl α-D-galactopyranoside (1.8 g) and methyl α-D-galactopyranoside (18 g) in 0.03M sodium phosphate (110 mL) and *N,N*-dimethylformamide (40 mL) was added α-D-galactosidase (0.2 mL, 10 U). The mixture was stored at room temperature for 170 h, the product was isolated as described in (*a*) and, after chromatography (Kieselgel 60), acetylated [small amounts of *p*-nitrophenyl digalactosides were also formed (t.l.c.), but were not isolated]. Column chromatography (Kieselgel 60) then gave the acetylated product [95% pure; contaminant probably the α-(1→6)-linked isomer], which was deacetylated to give **4** (0.60 g, purity 99.7%), m.p. 227–228°, $[\alpha]_D^{21}$ +252° (*c* 1, water). N.m.r. data (D_2O): ^1H, δ 5.13 (d, 1 H, *J* 3.7 Hz, H-1 or H-1'), 4.86 (d, 1 H, *J* 3 Hz, H-1 or H-1'), 4.21–3.70 (m, 12 H), 3,40 (s, 3 H, OMe); ^{13}C, δ 102.18, 97.77 (C-1,1'), 76.98 (C-3), 73.69, 73.39, 72.09, 71.99, 71.02, 69.42, 68.22 (C-2,2', C-3', C-4,4', C-5,5'), 64.04 and 63.81 (C-6,6'), and 57.84 (OMe).

Anal. Calc. for $C_{13}H_{24}O_{11}$: C, 43.81; H, 6.79. Found: C, 43.65; H, 6.83.

(*d*) *Methyl 3-O-* (**5**) and *6-O-α-D-galactopyranosyl-β-D-galactopyranoside* (**6**). The procedure in (*c*) was followed, but using *p*-nitrophenyl α-D-galactopyranoside (0.6 g) and methyl β-D-galactopyranoside (4 g) in a total reaction volume of 32 mL (9 mL of *N,N*-dimethylformamide). Compound **6** was 95% pure after deacetylation (125 mg), and, after recrystallisation from methanol–2-propanol, had m.p. 159–160°, $[\alpha]_D^{21}$ +104° (*c* 0.2, water); lit.[14] m.p. 161–162°, $[\alpha]_D^{25}$ +107° (*c* 1, water). The n.m.r. data corresponded well with those reported[14].

Compound **5** (65 mg) was obtained 85% pure [contaminated by the (1→6)-

linked isomer]. Column chromatography on Kieselgel 60 and crystallisation from methanol gave pure **5**, m.p. 199°, $[\alpha]_D^{21}$ +142° (*c* 0.5, water); lit.[15] m.p. 199°, $[\alpha]_D^{25}$ +127° (*c* 0.1, water). The ^{13}C-n.m.r. data corresponded well with those reported[16].

Enzymic syntheses with β-D-galactosidase. — (a) *Methyl 6-O-β-D-galactopyranosyl-α-D-galactopyranoside* (**7**). To a solution of *o*-nitrophenyl β-D-galactopyranoside (2.7 g) and methyl α-D-galactopyranoside (5 g) in 35 mL of buffer (0.05M Na phosphate, mM $MgCl_2$, 5mM mercaptoethanol, pH 6.8) and *N,N*-dimethylformamide (15 mL) was added a solution of β-D-galactosidase (1 mg, 620 U) in buffer (1 mL). The mixture was stored at room temperature for 5 h, and the reaction was stopped by heating for 10 min at 75°. The product (0.8 g), isolated as described above and acetylated, was 95% pure. Deacetylation gave **7** (0.32 g, purity 97%). Recrystallisation from methanol gave pure **7**, m.p. 123–124°, $[\alpha]_D^{21}$ +83° (*c* 0.7, water). N.m.r. data (D_2O): ^{1}H, δ 4.84 (s, t at 35°, 1 H, H-1), 4.45 (d, 1 H, *J* 7.5 Hz, H-1'), 4.12–3.48 (m, 12 H), 3.43 (OMe); ^{13}C, δ 106.15, 102.41 (C-1,1'), 77.97, 75.51, 73.62, 72.31, 72.07 (2 C), 71.44, 70.93 (C-2,2', C-3,3', C-4,4', C-5,5'), 71.97 (C-6), 63.82 (C-6'), 58.24 (OMe).

Anal. Calc. for $C_{13}H_{24}O_{11}\cdot 1.5H_2O$: C, 40.72; H, 7.10. Found: C, 40.55, H, 7.03.

(*b*) *Methyl 3-O-* (**8**) *and 6-O-β-D-galactopyranosyl-β-D-galactopyranoside* (**9**). These compounds were synthesised simultaneously when methyl β-D-galactopyranoside (5 g) was used as acceptor under the conditions in (*a*). Column chromatography of the acetylated products gave acetylated **8** (1.3 g, purity 95%), and acetylated **9** (160 mg, purity 90%). Deacetylation of the former product gave **8** (0.55 g, 98% pure), m.p. 203–204°, $[\alpha]_D^{21}$ +25° (*c* 0.9, water); lit.[17] m.p. 201–202°, $[\alpha]_D^{25}$ +24.5° (*c* 1, water). The latter product required column chromatography after deacetylation. Crystallisation from methanol then gave **9** (purity 99%), m.p. 210–211°, $[\alpha]_D^{21}$ −11° (*c* 0.2, water); lit.[14] m.p. 218–219°, $[\alpha]_D^{25}$ −9.5° (*c* 1.25, water). The n.m.r. data for **8** and **9** corresponded well with those reported[14,17,18].

Immobilised β-D-galactosidase was used in a one-pot preparation of **8** (3.0 g) and **9** (0.4 g) from *o*-nitrophenyl β-D-galactopyranoside (9 g) and methyl β-D-galactopyranoside (15 g).

Enzymic syntheses with α-D-mannosidase. — (*a*) *Methyl 2-O-* (**10**) *and 6-O-α-D-mannopyranosyl-α-D-mannopyranoside* (**11**). To a solution of *p*-nitrophenyl α-D-mannopyranoside (0.6 g) and methyl α-D-mannopyranoside (6 g) in sodium phosphate buffer (38 mL, pH 6.5) and *N,N*-dimethylformamide (12 mL) was added α-D-mannosidase (0.3 mL, 15 U), and the mixture was stored for 14 h at room temperature. The products were isolated and acetylated by the procedures described above. Column chromatography then gave almost pure acetylated **10** (0.3 g) and acetylated **11** (0.06 g). Deacetylation gave, as amorphous powders, **10** (0.15 g, purity 99%), $[\alpha]_D^{21}$ +69.5° (*c* 0.5, methanol) {lit.[19] $[\alpha]_D^{25}$ +72° (*c* 0.5, methanol)}; and **11** (28 mg, purity 99%), $[\alpha]_D^{21}$ +90.5° (*c* 0.7, water) {lit.[20] $[\alpha]_D^{25}$ +90.3° (*c* 0.7, water)}. The n.m.r. data for **10** and **11** corresponded well with those reported[21].

Compounds **10** (3.5 g) and **11** (0.7 g) were also synthesised on a larger scale

from *p*-nitrophenyl α-D-mannopyranoside (20 g) and methyl α-D-mannopyranoside (45 g). In addition, α-Man-(1→2)-α-Man-(1→2)-α-Man-OMe (300 mg) was obtained (purity 90%), and identified by methylation analysis and n.m.r. spectroscopy.

(*b*) p-*Nitrophenyl 2-O-α-D-mannopyranosyl-α-D-mannopyranoside* (**12**). This product was synthesised from *p*-nitrophenyl α-D-mannopyranoside (1.6 g; total reaction volume, 80 mL; 20 mL of *N,N*-dimethylformamide). After column chromatography on Kieselgel 60, the product (100 mg) was 95% pure. Precipitation from dichloromethane–ethyl acetate–methanol then gave **12** as an amorphous powder (purity 99%), $[\alpha]_D^{21}$ +85° (*c* 1.05, water); lit.[22] $[\alpha]_D^{20}$ +85.1° (*c* 1.2, water). The n.m.r. data corresponded well with those reported[23].

DISCUSSION

Three glycosidases have been examined for the syntheses of disaccharide glycosides by transglycosidation. Nitrophenyl glycosides were chosen as glycosyl donors since they are good substrates for most glycosidases, which results in rapid accumulation of products. Thus, although the reactions were not optimised, the yields of products were often >20%. The reactions could be followed easily by spectrophotometric measurement of the liberated nitrophenol.

Although the enzymic reactions were not completely regioselective, column chromatography of the isomeric products was usually straightforward, since the glycosidations were stereospecific and no anomerisation of the glycoside substrates occurred. Thus, the purity of the products was ≥99%. The structures of the products were unambigously determined by n.m.r. spectroscopy (double-resonance, DEPT) and methylation analysis.

The results obtained with α-D-galactosidase, β-D-galactosidase, and α-D-mannosidase are summarised in Table I, which shows that the anomeric configuration of the acceptor has a pronounced influence on the regioselectivity. Thus, with α-D-galactosidase, methyl β-D-galactopyranoside gave mainly the (1→6)-linked digalactoside, whereas the α-anomer gave almost exclusively the (1→3)-linked digalactoside. The reverse result was found with β-D-galactosidase.

The nature of the aglycon of the acceptor had a pronounced influence on the regioselectivity. Thus, with α-D-galactosidase, the α-(1→2)-linked digalactoside was the main product when *o*-nitrophenyl α-D-galactopyranoside was the acceptor, whereas, with the corresponding *p*-nitrophenyl glycoside, the α-(1→3)-linked product preponderated and there was only a small proportion of α-(1→2)-linked isomer. With the corresponding methyl glycoside, preponderant formation of the α-(1→3) linkage occurred and only small proportions of the α-(1→6)-linked isomer were formed. With α-D-mannosidase, the ratio of α-(1→2)- and α-(1→6)-linked products was 19:1 with *p*-nitrophenyl α-D-mannopyranoside as the acceptor, whereas, with the corresponding methyl glycoside, the ratio was 5:1. Other glycosides and glycosidases are being investigated.

The trisaccharide glycoside α-Man-(1→2)-α-Man-(1→2)-α-Man-OMe was

TABLE I

FORMATION OF DISACCHARIDE GLYCOSIDES USING α-D-GALACTOSIDASE, β-D-GALACTOSIDASE, AND α-D-MANNOSIDASE

Glycosyl donor	*Glycosyl acceptor*	*Initial conc. of acceptor (mM)*	*Main glycosides formed*	*Yield (%)*
α-D-*Galactosidase*				
α-Gal-$OPhNO_2$-*p*	α-Gal-OMe	600	α-Gal-(1→3)-α-Gal-OMe	27
			α-Gal-(1→6)-α-Gal-OMe	<2
α-Gal-$OPhNO_2$-*p*	β-Gal-OMe	600	α-Gal-(1→3)-β-Gal-OMe	9
			α-Gal-(1→6)-β-Gal-OMe	18
α-Gal-$OPhNO_2$-*p*	α-Gal-$OPhNO_2$-*p*	150	α-Gal-(1→2)-α-Gal-$OPhNO_2$-*p*	2
			α-Gal-(1→3)-α-Gal-$OPhNO_2$-*p*	16
α-Gal-$OPhNO_2$-*o*	α-Gal-$OPhNO_2$-*o*	150	α-Gal-(1→2)-α-Gal-$OPhNO_2$-*o*	6
			α-Gal-(1→3)-α-Gal-$OPhNO_2$-*o*	<1
β-D-*Galactosidase*				
β-Gal-$OPhNO_2$-*o*	α-Gal-OMe	500	β-Gal-(1→6)-α-Gal-OMe	14
			+ other isomers	<1
β-Gal-$OPhNO_2$-*o*	β-Gal-OMe	500	β-Gal-(1→6)-β-Gal-OMe	3
			β-Gal-(1→3)-β-Gal-OMe	22
α-D-*Mannosidase*				
α-Man-$OPhNO_2$-*p*	α-Man-OMe	600	α-Man-(1→2)-α-Man-OMe	18
			α-Man-(1→6)-α-Man-OMe	4
α-Man-$OPhNO_2$-*p*	α-Man-$OPhNO_2$-*p*	70	α-Man-(1→2)-α-Man-$OPhNO_2$-*p*	8
			α-Man-(1→6)-α-Man-$OPhNO_2$-*p*	<0.1
			+ other isomers	0.4

formed in the later stage of the reaction between *p*-nitrophenyl α-D-mannopyranoside and methyl α-D-mannopyranoside (see Experimental). The possibility of changing the regioselectivity of glycosidase-catalysed formation of trisaccharide derivatives by using disaccharide glycosides as acceptors remains to be investigated.

The observed regioselectivity of the above glycosidase-catalysed reactions reflects the properties of the glycosyl acceptor binding-sites thought to exist in this type of enzyme[7,10,24]. Since these binding sites are not well characterised, detailed interpretation of the results is not possible. The existence of hydrophobic regions in the acceptor site is indicated by the fact that most glycosidases have a considerably higher affinity for glycosides with hydrophobic aglycons than for the corresponding sugars[24]. Thus, the interaction of the acceptor and the enzyme will be influenced by the nature of the aglycon and the anomeric configuration. The results in Table I show that, in the glycosyl acceptors, the bulky, more hydrophobic aryl groups have larger effects on regioselectivity than the corresponding methyl groups. Furthermore, the yield of nitrophenyl digalactosides increased with a decreasing concentration of organic co-solvent (45-0% *N,N*-dimethylformamide)[25], which indicates that there is considerable hydrophobic interaction at the acceptor site.

The higher yields of products from the methyl glycosides than from the corresponding *p*- or *o*-nitrophenyl glycosides reflects differences in solubility. However, with *o*-nitrophenyl α-D-galactopyranosides, a significantly lower yield than from the corresponding *p*-nitrophenyl glycoside was obtained, which could be due to differences in binding to the enzyme or steric hindrance of HO-2 in the *ortho* compound.

The optimal time of reaction for enzymic transglycosidations is a function of the initial concentration of reactants and of the various dissociation and catalytic rate constants for the different steps shown in the scheme[10]. Thus, the yield of α-linked *p*-nitrophenyl digalactosides reached a maximum after ~70% conversion of the substrate, after which the enzymic hydrolysis of the products was more rapid than their formation.

Because of the simplicity of enzymic syntheses, the yields (10–30%) are sufficiently high to make the method attractive. Nitrophenyl and methyl glycosides are readily available. Preliminary investigations have shown that cheap oligosaccharides (*e.g.*, lactose, raffinose) may also be used as donors without affecting the regioselectivities. Recovery of excess of reagents by column chromatography is straightforward and the re-utilisation of enzymes is facilitated by their immobilisation. Thus, α- and β-D-galactosidase were immobilised on tresyl chloride-activated agarose in yields of 55 and 84%, respectively. The immobilised enzymes gave the same overall yield and relative yields of the isomers of α-linked *p*-nitrophenyl digalactosides (α-D-galactosidase) and β-linked methyl digalactosides (β-D-galactosidase) as obtained with the corresponding soluble enzymes.

Many of the disaccharide structures synthesised above occur widely in Nature. Thus, α-Gal-(1→3)-Gal is a structural unit of blood-group determinant B[1] and of several types of glycolipids[2], as is β-Gal-(1→3)-Gal. α-Man-(1→2)-Man and α-Man-(1→6)-Man are found in *N*-linked oligosaccharides[1]. The chemical syntheses of these structures involve multi-step procedures[14–22]. Nitrophenyl glycosides can be used as enzyme substrates[26] or can be reduced to aminophenyl glycosides capable of reacting with properly activated peptides, proteins, lipids, or chromatography media[27]. Alternatively, the amino function may be derivatised (*e.g.*, to *N*-bromoacetate, diazo, isothiocynate) for reactions with nucleophiles[27]. Methyl glycosides are useful for inhibition studies[28].

The approach described above should be applicable to a wide range of glycosidases, glycosyl donors and acceptor glycosides, and to the synthesis of trisaccharide glycosides. The one-pot preparation of mono- and oligo-saccharide glycosides from cheap oligosaccharides, such as lactose and raffinose, are also possible.

ACKNOWLEDGMENTS

My colleagues at the department are thanked for valuable discussions.

REFERENCES

1 V. GINSBURG AND P. W. ROBBINS (Eds.), *Biology of Carbohydrates,* Vol. 2, Wiley, New York, 1984.
2 S. HAKOMORI, *Annu. Rev. Biochem.*, 50 (1981) 733–764.
3 H. PAULSEN, *Chem. Soc. Rev.*, 13 (1984) 15–45.
4 J. DAHMÉN, T. FREJD, T. LAVE, F. LINDH, G. MAGNUSSON, G. NOORI, AND K. PÅLSSON, *Carbohydr. Res.*, 113 (1983) 219–224.
5 J. DAHMÉN, G. GNOSSPELIUS, A.-C. LARSSON, T. LAVE, G. NOORI, K. PÅLSSON, T. FREJD, AND G. MAGNUSSON, *Carbohydr. Res.*, 138 (1985) 17–28.
6 L. HEDBYS, P.-O. LARSSON, K. MOSBACH, AND S. SVENSON, *Biochem. Biophys. Res. Commun.* 123 (1984) 8–15.
7 K. WALLENFELS AND R. WEIL, in P. D. Boyer (Ed.), *The Enzymes,* 3rd edn., Vol. 7, 1972, pp. 617–663.
8 J. S. FRUTON, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 53 (1982) 239–306.
9 K. NILSSON AND K. MOSBACH, *Biotechnol. Bioeng.*, 26 (1984) 1146–1154.
10 R. E. HUBER, M. T. GAUNT, R. L. SEPT, AND M. J. BABIAK, *Can. J. Biochem. Cell. Biol.*, 61 (1983) 198–206.
11 M. L. SINNOTT, *FEBS Lett.*, 94 (1978) 1–9.
12 J. DAHMÉN, T. FREJD, G. MAGNUSSON, G. NOORI, AND A.-S. CARLSTRÖM, *Carbohydr. Res.*, 125 (1984) 237–245.
13 P.-E. JANSSON, L. KENNE, H. LIEDGREN, B. LINDBERG, AND J. LÖNNGREN, *Chem. Commun. Univ. Stockholm*, 8 (1976).
14 P. KOVÁC, E. A. SOKOLOSKI, AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 128 (1984) 101–109.
15 J. KOSCIELAK AND R. W. JEANLOZ, *Carbohydr. Res.*, 5 (1967) 220–223.
16 P. A. J. GORIN, *Carbohydr. Res.*, 101 (1982) 13–20.
17 P. KOVÁC, C. P. J. GLAUDEMANS, AND R. B. TAYLOR, *Carbohydr. Res.*, 142 (1985) 158–164.
18 U. K. SRIVASTAVA, S. J. SONDHEIMER, AND C. SCHUERCH, *Carbohydr. Res.* 86 (1980) 203–214.
19 T. OGAWA, K. KATANO, K. SASAJIMA, AND M. MATSUI, *Tetrahedron,* 37 (1981) 2779–2786.
20 T. OGAWA AND K. SASAJIMA, *Tetrahedron*, 37 (1981) 2787–2792.
21 T. OGAWA AND K. SASAJIMA, *Carbohydr. Res.*, 97 (1981) 205–227.
22 C. M. REICHERT, *Carbohydr. Res.*, 77 (1979) 141–147.
23 G. EKBORG AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 134 (1984) 83–87.
24 R. E. HUBER AND M. T. GAUNT, *Arch. Biochem. Biophys.*, 220 (1983) 263–271.
25 K. G. I. NILSSON, presented at a symposium on "Biocatalysis in Organic Media", Wageningen, The Netherlands, December 7–10, 1986.
26 S. A. ABBAS, J. J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, 98 (1981) 37–49.
27 G. EKBORG, B. VRANESIC, A. K. BHATTACHARJEE, P. KOVÁC, AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 142 (1985) 203–211.
28 N. SHARON AND H. LIS, *Science,* 177 (1972) 949–959.