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ENZYMIC SYNTHESIS OF DI- AND TRI-SACCHARIDE GLYCOSIDES, USING GLYCOSIDASES AND β-D-GALACTOSIDE 3-α-SIALYL-TRANSFERASE*

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ABSTRACT

The following disaccharide glycosides were obtained in yields of 10–35% from the appropriate donor and acceptor glycosides by employing glycosidases as catalysts: α-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-OEt (α-D-galactosidase), β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-OEt and β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-OEtBr (β-D-galactosidase), β-D-Glc*p*NAc-(1→6)-β-D-Gal*p*-OMe and β-D-Glc*p*NAc-(1→6)-α-D-Man*p*-OMe (β-*N*-acetylglucosaminidase). With β-D-Glc*p*NAc-OEtSiMe$_3$ as the acceptor, β-D-galactosidase gave β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-OEtSiMe$_3$ almost exclusively, whereas, with β-D-Glc*p*NAc-OMe as the acceptor, β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-OMe was formed in only slight excess over the analogous β-(1→4)-linked glycoside. The use of β-D-galactosidase and β-D-galactoside 3-α-sialyltransferase in sequence provided a convenient route to the trisaccharide glycosides α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-OEt, α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-OEtBr, and α-D-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-OMe.

INTRODUCTION

The oligosaccharides of glycolipids and glycoproteins are involved in a variety of biological processes[1,2], and sequences containing HexNAc and/or Neu5Ac are antigenic determinants, receptors for proteins, viruses, or bacteria, and important for cell–cell interaction, the circulation half-life of proteins, and changes during cellular differentiation[1–6]. In addition, β-D-GlcNAc residues are found at branch points in the core of glycoprotein glycans and are important for the conformation, rigidity, and biological activity of the glycans[4].

Methods for the syntheses of such structures in amounts sufficient for study are important. Chemical syntheses require multi-step procedures[7] and the stereospecific synthesis of α-sialosides is difficult[8,9]. Syntheses with enzymes are attractive

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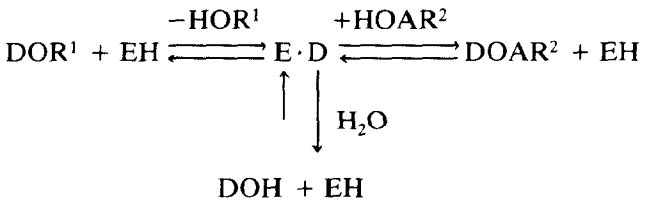
because of their stereospecificity and regioselectivity. Syntheses with glycosidases (EH) have been achieved[10,11] using the kinetic approach (transglycosylation). Thus, α- or β-D-glycosides (DOR^1) were used as donors and various α- or β-D-glycosides as acceptors ($HOAR^2$):



The use of glycosides as acceptors gives products ($DOAR^2$) that can be utilised variously[10,11], and simplifies purification since anomerisation is prevented. Moreover, the regioselectivity of glycosidases can be manipulated by changing the structure of the aglycon (R^2) or the anomeric configuration of the acceptor glycoside[10].

The synthesis of sialylated structures by the sequential use of β-D-galactosidase and sialyltransferase is now reported. The transferase reaction involved CMP-Neu5Ac, synthesised[12] from CTP and *N*-acetylneuraminic acid, as the donor, and disaccharide glycosides as the acceptors.

EXPERIMENTAL

General. — α-D-Galactosidase (EC 3.2.1.22; coffee bean) and β-*N*-acetylglucosaminidase (EC 3.2.1.30; jack bean) were obtained from Sigma. CMP-*N*-acetylneuraminate:β-D-galactoside 3-α-sialyltransferase (EC 2.4.99.4) was obtained from Genzyme or purified from porcine submaxillary glands. A described purification procedure was followed[13]. The eluate obtained after one affinity-column step was dialysed against 10mM 2-(4-morpholino)ethanesulphonic acid–HCl (MES–HCl, pH 6.5), and freeze-dried to give a preparation (460 mg, from 200 g of glands) which was used for sialylation. The crude extract of β-D-galactosidase (EC 3.2.1.23; bovine testes) and the ammonium sulphate precipitate were prepared as described[14,15]. The monosaccharide glycosides were commercial products.

The general and instrumental methods were as described[10,11,16]. Thus, solvents were removed with a rotary evaporator and then at <0.1 Torr. T.l.c. was performed on Kieselgel 60 (Merck) with detection by u.v. light or charring with sulfuric acid. Column chromatography was performed on Kieselgel 60 (Merck, 230–440 mesh) and Sephadex G-10 or G-15 (Pharmacia), using *A*, water (Sephadex); *B*, 25:2.5:8.5:2.0 dichloromethane–ethanol–methanol–water; *C*, 6:1 ethyl acetate–toluene; *D*, 6:1 ethyl acetate–iso-octane; *E*, 5:65:25 acetonitrile–2-propanol–2.5M NH_4OH; or *F*, 10:10:1 trichlorotrifluoroethane–ethyl acetate–methanol. Non-sialylated structures were acetylated conventionally with pyridine–

acetic anhydride, and the products were deacetylated with methanolic sodium methoxide. Duolite C-6 (H^+) resin (methanol-washed and dried over P_2O_5) was used for neutralisation. The structure of the acetylated glycosides was determined by ^1H- and ^{13}C-n.m.r. spectroscopy (Varian XL 200 instrument). The assignments were based on double-resonance and DEPT-experiments[10,11,16]. (1→3)-Linkages were indicated by the marked upfield shift of the H-3 resonance, and (1→6)-linkages by the marked downfield shift of the C-6 resonance. The ^1H-chemical shift data for the sialylated structures were as expected for (2→3)-linked α-Neu*p*5Ac (*e.g.*, the H-3*a*,3*e* resonances)[17] and accorded with data (^1H and ^{13}C) reported for identical or analogous glycosides[18,19]. Methylation analysis was carried out when literature n.m.r. data were not available[20].

*Enzymic synthesis with β-*N*-acetylglucosaminidase. — (a) Methyl 6-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranoside* (**1**). To a solution of *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (0.68 g) and methyl α-D-mannopyranoside (3.6 g) in sodium phosphate buffer (26 mL; 0.04M, pH 6.5) and *N,N*-dimethylformamide (6 mL) was added β-*N*-acetylglucosaminidase (0.30 mL). After storage for 65 h at room temperature, the mixture was extracted with dichloromethane. Column chromatography (Sephadex G10), acetylation, and column chromatography (Kieselgel 60, solvent *D*) then gave acetylated **1** (130 mg). Deacetylation gave **1** (70 mg), m.p. 244° (from methanol); lit.[21] m.p. 259–260° (from 2-propanol). The ^1H- and ^{13}C-n.m.r. data corresponded with those reported[21].

*(b) Methyl 6-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-β-*D*-galactopyranoside* (**2**). To a mixture of *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (0.68 g) and methyl β-D-galactopyranoside (3.6 g) in sodium phosphate buffer and *N,N*-dimethylformamide as in (*a*) was added β-*N*-acetylglucosaminidase (0.30 mL). After storage for 65 h at room temperature, the mixture was processed as in (*a*) to give acetylated **2** (110 mg). Deacetylation gave **2**, m.p. 246–247° (from ethanol); lit.[22] m.p. 256–257°. The ^{13}C-n.m.r. data corresponded well with those reported[22].

*Enzymic synthesis with α-*D*-galactosidase: ethyl 2-acetamido-2-deoxy-3-*O*-α-*D*-galactopyranosyl-α-*D*-galactopyranoside* (**3**). — To a solution of *p*-nitrophenyl α-D-galactopyranoside (1 g) and ethyl 2-acetamido-2-deoxy-α-D-galactopyranoside (1.5 g) in sodium phosphate buffer (20 mL; 0.04M, pH 6.5) was added α-D-galactosidase (0.8 mL). After 36 h, more glycosyl donor (1.0 g) was added. The reaction was continued at room temperature for 64 h, then stopped by heating at 75° for 10 min. Column chromatography (Kieselgel 60, solvent *B*), acetylation, and column chromatography (solvent *C*) gave acetylated **3** (470 mg). Deacetylation gave **3**, m.p. 263° (from ethanol), $[\alpha]_D$ +212° (*c* 0.5, water). N.m.r. data (D_2O): ^1H, δ 5.11 (d, 1 H, *J* 3.6 Hz, H-1 or H-1′), 4.89 (d, 1 H, *J* 3.8 Hz, H-1 or H-1′), 4.39–3.39 (m), 2.04 (s, 3 H, Ac), 1.20 (t, 3 H, CH_3); ^{13}C, δ 176.88 (C=O), 99.51, 97.80 (C-1,1′), 75.47, 73.87, 73.16, 72.01, 71.74, 70.67, 67.35, 66.43 (C-3,4,5,2′,3′,4′,5′, OCH_2), 63.91, 63.49 (C-6,6′), 50.59 (C-2), 24.66 (Ac), 16.71 (CH_3).

Anal. Calc. for $C_{16}H_{29}NO_{11}$: C, 46.72; H, 7.06; N, 3.41. Found: C, 46.10; H, 7.13; N, 3.45.

Enzymic syntheses with β-D-galactosidase. — (a) Ethyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-α-D-galactopyranoside (**4**). To a solution of *p*-nitrophenyl β-D-galactopyranoside (1 g) and ethyl 2-acetamido-2-deoxy-α-D-galactopyranoside (0.35 g) in sodium phosphate buffer (10 mL; 0.05M, pH 5.3) and *N,N*-dimethylformamide (3 mL) was added ammonium sulphate precipitate (3.0 g) containing β-D-galactosidase (see above). The mixture was agitated gently at 37°. After 48 h, more glycosyl donor (0.5 g) was added. The reaction was stopped after 96 h (total time) by heating for 10 min at 75°. Column chromatography (dichloromethane–methanol–water, 60:40:5), acetylation, and column chromatography (solvent *F*) then gave acetylated **4** (300 mg). Deacetylation gave **4** (150 mg), m.p. 235° (from ethanol–iso-octane), $[\alpha]_D$ +107° (*c* 0.5, water). N.m.r. data (D_2O): ^1H, δ 4.90 (d, 1 H, *J* 3.7 Hz, H-1), 4.46 (d, 1 H, *J* 7.6, H-1′), 4.40–3.49 (m), 2.03 (s, 3 H, Ac), 1.22 (t, 3 H, *J* 6.5 Hz, CH_3); ^{13}C, δ 177.19 (C=O), 107.39 (C-1′), 99.58 (C-1), 79.90 (C-3), 77.61, 75.15, 73.23, 73.13, 71.47, 71.22 (C-4,5,2′,3′,4′,5′), 66.42 (OCH_2), 63.87, 63.62 (C-6,6′), 51.25 (C-2), 24.65 (Ac), 16.71 (CH_3).

Anal. Calc. for $C_{16}H_{29}NO_{11}$: C, 46.72; H, 7.06; N, 3.41. Found: C, 46.20; H, 7.52; N, 3.43.

(b) 2-Bromoethyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-galactopyranoside (**5**). This compound was prepared[23] from *o*-nitrophenyl β-D-galactopyranoside (1 g) and 2-bromoethyl 2-acetamido-2-deoxy-β-D-galactopyranoside (400 mg) employing commercial β-D-galactosidase (bovine testes, 2 U; Boehringer). Reaction for 4 days at 37° and work-up by column chromatography (solvent *B*), acetylation, column chromatography (ethyl acetate–toluene, 9:1), and deacetylation gave **5** (40 mg), m.p. 185° (from ethanol), $[\alpha]_D$ −1.5° (*c* 0.3, water). N.m.r. data (D_2O): ^1H, δ 4.57 (d, 1 H, *J* 8.4 Hz, H-1), 4.45 (d, 1 H, *J* 7.3 Hz, H-1′), 4.19–3.48 (m), 2.05 (s, 3 H, Ac); ^{13}C, δ 177.61 (C=O), 107.50 (C-1′), 104.04 (C-1), 82.32 (C-3), 77.62, 77.47, 75.06, 73.21, 72.52, 71.19, 70.61 (C-4,5,2′,3′,4′,5′, $-CH_2$), 63.63, 63.53 (C-6,6′), 53.80 (C-2), 34.00 (CH_2Br), 25.08 (Ac).

Anal. Calc. for $C_{16}H_{28}BrNO_{11} \cdot 1.5\ H_2O$: C, 37.13; H, 6.00; N, 2.71. Found: C, 37.00; H, 5.90; N, 2.76.

(c) Trimethylsilylethyl 2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-glucopyranoside (**6**). A crude extract (150 mL) containing β-D-galactosidase from bovine testes was added to *o*-nitrophenyl β-D-galactopyranoside (10 g) and trimethylsilylethyl 2-acetamido-2-deoxy-β-D-glucopyranoside (2 g). The reaction was carried out for 3 days at 37°. Toluene (0.5 mL) was added to minimise bacterial growth. The reaction was stopped by heating for 10 min at 75°. The mixture was extracted with dichloromethane. After acetylation, column chromatography (iso-octane–ethyl acetate, 1:3) gave a 2:1 solid mixture of acetylated **6** and acetylated β-D-GlcNAc-OEtSiMe$_3$ (2.35 g). Deacetylation and column chromatography (Sephadex G15) then gave **6** (0.98 g), m.p. 226–227° (from methanol), $[\alpha]_D$ −26° (*c* 0.7, water). N.m.r. data (D_2O): ^1H, δ 4.67 (d, 1 H, *J* 7.0 Hz, H-1), 4.50 (d, 1 H,

J 7.2 Hz, H-1'), 4.21–3.56 (m), 2.10 (s, 3 H, Ac), 1.14–0.94 (m, 2 H, CH_2Si), 0.09 (Me_3Si); ^{13}C, δ 176.81 (C=O), 105.94 (C-1'), 102.48 (C-1), 85.23 (C-3), 77.75, 77.63, 74.89, 73.05, 71.15, 70.89 (C-4,5,2',3',4',5'), 70.62 (OCH_2), 63.36, 63.15 (C-6,6'), 56.87 (C-2), 24.72 (Ac), 19.50 (CH_2Si), 0.12 (Me_3Si).

Anal. Calc. for $C_{19}H_{37}NO_{11}Si$: C, 47.20; H, 7.66; N, 2.89. Found: C, 46.60; H, 7.75; N, 2.89.

Acetylated β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-OEtSiMe$_3$, β-D-Gal*p*-(1→6)-β-D-Glc*p*NAc-OEtSiMe$_3$, β-D-Gal*p*-(1→3)-β-D-Gal*p*-OPhNO$_2$-*o*, and β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-OEtSiMe$_3$ were also obtained in almost pure form (~100 mg each; structures confirmed by methylation analysis).

Enzymic synthesis with β-D-galactoside 3-α-sialyltransferase. — *(a)* *α-D-Neup5Ac-(2→3)-β-D-Galp-(1→3)-α-D-GalpNAc-OEt* (**7**). CMP-Neu*p*5Ac (30 mg) and β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-OEt (40 mg) were dissolved in 0.1M MES–HCl (pH 6.7, 10 mL) containing bovine serum albumin (5 mg), Tween X-100 (35 μL), and freeze-dried 3-α-sialyltransferase preparation (150 mg, see above). After storage for 2 days at 37°, more CMP-Neu*p*5Ac (30 mg) was added. After 2 more days at 37°, the product was isolated by column chromatography [Sephadex G15 and Kieselgel 60 (solvent *E*)]. Freeze-drying gave **7** as a white solid (45 mg), $[\alpha]_D$ +52° (*c* 0.4, water). N.m.r. data (D_2O): ^1H, δ 4.93 (d, 1 H, *J* 3.6 Hz, H-1), 4.55 (d, 1 H, *J* 8.0, H-1'), 4.37–3.51 (m), 2.77 (dd, 1 H, $J_{3''e,3''a}$ 12.0, $J_{3''e,4''}$ 3.8 Hz, H-3''*e*), 2.05 (bs, 6 H, 2 Ac), 1.80 (t, 1 H, H-3''*a*), 1.23 (t, 3 H, *J* 7.0 Hz, CH_3); ^{13}C, δ 177.78, 177.35, 176.65 (2 C=O, COOH), 107.19 (C-1'), 102.50 (C-2''), 99.67 (C-1), 80.16 (C-3), 78.43 (C-3'), 77.54, 75.58, 74.60, 73.28, 71.83, 71.42, 71.13, 70.86, 70.17 (C-4,5,2',4',5',4'',6'',7'',8''), 66.59 (OCH_2), 65.30 (C-9''), 64.01, 63.75 (C-6,6'), 54.46 (C-5''), 51.40 (C-2), 42.51 (C-3''), 24.84 (2 C, 2 Ac), 16.81 (CH_3).

(b) *α-D-Neup5Ac-(2→3)-β-D-Galp-(1→3)-β-D-GalpNAc-OEtBr* (**8**). This compound was synthesised[23] from CMP-Neu*p*5Ac (8 mg) and β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-OEtBr (4 mg), employing a commercial 3-α-sialyltransferase preparation (20 mU). Reaction for 3 days at 37° and isolation of the products by column chromatography [Kieselgel 60 (solvent *E*), Sephadex G15] gave **8** (6 mg). N.m.r. data (D_2O): ^1H, δ 4.56 (d, 1 H, *J* 8.4 Hz, H-1'), 4.52 (d, 1 H, *J* 9.8 Hz, H-1), 4.18–3.52 (m), 2.75 (dd, 1 H, $J_{3''e,3''a}$ 12.5, $J_{3''e,4''}$ 3.6 Hz, H-3''*e*), 2.03 (bs, 6 H, 2 Ac), 1.78 (t, 1 H, H-3''*a*); ^{13}C, δ 107.24 (C-1'), 104.06 (C-1), 102.32 (C-2''), 82.51 (C-3), 78.15 (C-3'), 77.47, 77.41, 75.41, 74.45, 72.51, 71.65, 71.04, 70.68, 70.49 (C-4,5,2',4',5',4'',6'',7'',8''), 69.99 (OCH_2), 65.10 (C-9''), 63.62, 63.57 (C-6,6'), 54.29, 53.66 (C-2,5''), 42.34 (C-3''), 33.98 (CH_2Br), 25.13, 24.65 (2 Ac).

(c) *α-D-Neup5Ac-(2→3)-β-D-Galp-(1→3)-β-D-GlcpNAc-OMe* (**9**). This compound was prepared[23] from CMP-Neup5Ac (20 mg) and β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-OMe (30 mg), employing a commercial 3-α-sialyltransferase preparation (40 mU). Reaction for 5 days at 37°, with purification by column chromatography as in (*b*), gave **9** (12 mg). The ^1H- and ^{13}C-n.m.r. data corresponded with those reported[18].

In an alternative procedure, the acceptor was not purified to homogeneity

before the sialylation reaction. The acceptor solution was obtained from *p*-nitrophenyl β-D-galactopyranoside (1 g) and methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (0.5 g) in sodium phosphate buffer (9 mL; 0.05M, pH 6.5) and *N,N*-dimethylformamide (1.5 mL), employing the ammonium sulphate precipitate (2 g) containing β-D-galactosidase. After 70 h at 37°, the mixture was applied to a column of Sephadex G15 (solvent *A*). Comparison of the n.m.r. data of the freeze-dried disaccharide fractions with literature data[18] for β-D-Gal-(1→3)-β-D-GlcNAc-OMe and the β-(1→4)-linked isomer revealed that these disaccharides were present in the ratio 0.55:0.50. Part (30 mg) of the preparation was sialylated as described for **7**. Purification as described above gave **9** (5 mg). The n.m.r. data corresponded with those reported[18].

RESULTS AND DISCUSSION

Glycosidases are readily available and catalyse the stereospecific and regioselective formation of glycosidic linkages[24]. Glycosyltransferases, which are less readily available and require cofactors for synthesis[25], are stereo- and regio-specific and show a high selectivity for the acceptor saccharide. In order to synthesise trisaccharides and higher oligosaccharides, the more readily available glycosidases can be used to produce shorter saccharides and glycosyltransferases then used to catalyse the final steps when demands on regioselectivity are higher.

The glycosidase-catalysed reactions studied are summarised in Table I. Nitrophenyl glycosides were chosen as donors since they are good substrates for the glycosidases used.

The β-*N*-acetylglucosaminidase- and α-D-galactosidase-catalysed reactions as well as the syntheses of the β-D-Gal-(1→3)-D-GalNAc-glycosides with β-D-galacto-

TABLE I

FORMATION OF DISACCHARIDE GLYCOSIDES, USING β-*N*-ACETYLGLUCOSAMINIDASE, α-D-GALACTOSIDASE, AND β-D-GALACTOSIDASE

Donor	*Acceptor*	*Main product(s)*
β-N-Acetylglucosaminidase		
β-GlcNAc-OPhNO$_2$-*p*	β-Gal-OMe	β-GlcNAc-(1→6)-β-Gal-OMe
β-GlcNAc-OPhNO$_2$-*p*	α-Man-OMe	β-GlcNAc-(1→6)-α-Man-OMe
α-D-Galactosidase		
α-Gal-OPhNO$_2$-*p*	α-GalNAc-OEt	α-Gal-(1→3)-α-GalNAc-OEt
β-D-Galactosidase		
β-Gal-OPhNO$_2$-*p*	α-GalNAc-OEt	β-Gal-(1→3)-α-GalNAc-OEt
β-Gal-OPhNO$_2$-*o*	β-GalNAc-OEtBr	β-Gal-(1→3)-β-GalNAc-OEtBr
β-Gal-OPhNO$_2$-*o*	β-GlcNAc-OEtSiMe$_3$	β-Gal-(1→3)-β-GlcNAc-OEtSiMe$_3$
β-Gal-OPhNO$_2$-*p*	β-GlcNAc-OMe	β-Gal-(1→3)-β-GlcNAc-OMe β-Gal-(1→4)-β-GlcNAc-OMe

sidase were highly regioselective. This, together with the stereospecific transglycosylations and the use of glycosides as acceptors, facilitated column chromatography of products.

The nature of the aglycon of an acceptor can influence the regioselectivity of glycosidase-catalysed transglycosylations[10]. This effect was also observed in the reactions of β-D-galactosidase with β-D-GlcNAc-OMe and β-D-GlcNAc-OEtSiMe$_3$ as acceptors (Table I). Thus, with the trimethylsilylethyl glycoside, >80% of the β-(1→3)-linked isomer was formed whereas, with the methyl glycoside, the β-(1→3)-linked isomer was formed in only slight excess over the β-(1→4)-linked product [ratio 0.55:0.50; a small amount of the β-(1→6)-linked isomer was also observed]. The regioselectivity of the latter reactions depends on the source of the enzyme. Thus, *E. coli* β-D-galactosidase gave[26] mainly β-D-Gal*p*-(1→6)-β-D-Glc*p*NAc-OMe.

Although not optimised, the yields of the glycosidase-catalysed syntheses were in the range 10–35%. The simplicity of the method and the ready availability of glycosidases make the method attractive. Moreover, cheap, crude enzyme preparations can be used.

Some of the disaccharide glycosides produced with β-D-galactosidase as catalyst were sialylated with β-D-galactoside 3-α-sialyltransferase (EC 2.4.99.4) from porcine submaxillary glands. These reactions require CMP-Neu5Ac as glycosyl donor, which is commercially available but expensive. However, CMP-Neu5Ac can be prepared conveniently from CTP and Neu5Ac employing CMP-sialate synthase[27]. This enzyme can be coupled[12] in crude form with high retention of activity to tresyl chloride-activated supports[28] in a few h. Such immobilised preparations are suitable for the synthesis of CMP-Neu5Ac on a large scale.

The combined use of CMP-sialate synthase, β-D-galactosidase, and sialyltransferase constitutes a convenient route to the sialylated trisaccharide glycosides shown in Table II, which were obtained in quantitative yields.

The β-D-galactoside 3-α-sialyltransferase from porcine submaxillary glands exhibits a lower V_{max} and a higher K_m with β-D-Gal-(1→3)-D-GlcNAc than with β-D-Gal-(1→3)-D-GalNAc as acceptor[13]. However, by using a higher concentration of β-D-Gal-(1→3)-β-D-GlcNAc-OMe, a satisfactory yield of the sialylated product was obtained. A higher yield of this compound may be obtained with the β-Gal-(1→3/4)-GlcNAc 3-α-sialyltransferase (EC 2.4.99.5)[18]. The use of this enzyme may be beneficial when the acceptor is pure. However, the sialyltransferase used here

TABLE II

SIALYLATION OF DISACCHARIDES WITH β-D-GALACTOSIDE 3-α-SIALYLTRANSFERASE

Acceptor disaccharide	*Sialylated product*
β-Gal-(1→3)-α-GalNAc-OEt	α-Neu5Ac-(2→3)-β-Gal-(1→3)-α-GalNAc-OEt
β-Gal-(1→3)-β-GalNAc-OEtBr	α-Neu5Ac-(2→3)-β-Gal-(1→3)-β-GalNAc-OEtBr
β-Gal-(1→3)-β-GlcNAc-OMe	α-Neu5Ac-(2→3)-β-Gal-(1→3)-β-GlcNAc-OMe

showed a higher selectivity for β-D-Gal-(1→3)-D-GlcNAc than for the β-(1→4)-linked isomer[13]. Selective synthesis of α-D-Neu5Ac-(2→3)-β-D-Gal-(1→3)-β-D-GlcNAc-OMe was possible with a mixture of β-(1→3)- and β-(1→4)-linked Gal–GlcNAc-OMe and only one purification step of the β-D-galactosidase reaction mixture was required. The even higher selectivity for β-D-Gal-(1→3)-D-GalNAc-OR may allow a one-pot preparation of α-D-Neu5Ac-(2→3)-β-D-Gal-(1→3)-α-D-GalNAc-OR from suitable monosaccharide derivatives and this is being investigated with co-immobilised β-D-galactosidase and sialyltransferase.

Both commercial 3-α-sialyltransferase and a freeze-dried preparation obtained after affinity chromatography were used. The former enzyme is expensive but can be immobilised efficiently to tresyl chloride-activated agarose, which allows repeated use[12]. Alternatively, the enzyme can be purified conveniently in 1–2 weeks by affinity chromatography[13]. Recently, a facile purification from porcine liver has been described[29].

The di- and tri-saccharide structures synthesised above occur widely in Nature. Thus, β-D-GlcNAc-(1→6)-β-D-Gal and β-D-GlcNAc-(1→6)-α-D-Man are present in O- and N-glycoproteins, respectively[1,4]. Increased β-D-GlcNAc-(1→6)-α-D-Man-branching may be associated with metastasis[30]. α-D-Gal-(1→3)-D-GalNAc is α-linked to glycoproteins of the nervous system[31] and in teratocarcinoma cells[32]. β-D-Gal-(1→3)-α-D-GalNAc (often 3-α-sialylated) is a common structure in several O-glycoproteins[1,4] and gangliosides[2]. The Le^a and Le^b antigenic determinants and the type 1 human blood-group determinants are derived from β-D-Gal-(1→3)-β-D-GlcNAc (N-acetyl-lacto-*N*-biose I). The 3-α-sialylated structure is part of sialyl-lactotetraose and sialylated Le^a, which are well characterised cancer-associated antigens[2,5,33]. The trisaccharide may be the binding epitope of the C-50 monoclonal antibody[33]. The chemical syntheses of these structures require multi-step procedures[7–9,18,19,21,22]. The preparation of the above sialylated structures by the combined use of chemical synthesis and sialyltransferases has been described[18,19]. The sequential use of glycosidases and glycosyltransferases facilitates synthesis of oligosaccharides with a minimum of reaction steps.

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