Carbohydrate Research, 244 (1993) 149–159
Elsevier Science Publishers B.V., Amsterdam

Use of the "core-2"-*N*-acetylglucosaminyltransferase in the chemical–enzymatic synthesis of a sialyl-Le^X-containing hexasaccharide found on O-linked glycoproteins

Reinhold Oehrlein *, Ole Hindsgaul and Monica M. Palcic

Department of Chemistry, University of Alberta, Edmonton, Alberta, T6G 2G2 (Canada)

(Received November 18th, 1992; accepted December 25th, 1992)

ABSTRACT

A simple preparation of the "core-II" *N*-acetylglucosaminyltransferase (UDP-D-Glc*p*NAc:β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc (GlcNAc to GalNAc) β-(1 → 6)-GlcNAc-transferase, GlcNAcT, EC 2.4.1.102) from commercial mouse kidney acetone powder is reported. The enzyme obtained in a single step of affinity chromatography is suitable for use in preparative oligosaccharide synthesis. In conjunction with previously described preparations of β-(1 → 4)-galactosyltransferase (EC 2.4.1.22), α-(2 → 3)-sialyltransferase (EC 2.4.99.6) and α-(1 → 3/4)-fucosyltransferase (EC 2.4.1.65), the GlcNAcT was used in the first step of a sequence which converted the disaccharide β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc-OR into the sialyl-Le^X-containing structure α-D-Neu*p*Ac-(2 → 3)-β-D-Gal*p*-(1 → 4)-[α-L-Fuc*p*-(1 → 3)]-β-D-Glc*p*NAc-(1 → 6)-{β-D-Gal*p*-(1 → 3)}-α-D-Gal*p*NAc-OR (**5**), where R = $(CH_2)_8CO_2Me$. Hexasaccharide **5**, thus assembled in only one week once the enzymes were prepared, was characterized by ^1H and ^{13}C NMR spectroscopy and fast-atom bombardment mass spectrometry, as were all intermediate oligosaccharides. The core II GlcNAcT thus joins the expanding repertoire of readily available reagents for the rapid assembly of oligosaccharides.

INTRODUCTION

Accumulating evidence that complex oligosaccharides may serve as recognition markers to direct important biological processes make these molecules increasingly important as targets for organic synthesis. Particularly in the areas of cancer[1,2], inflammation[3], and infectious diseases[4], complex oligosaccharides have potential as drug candidates since they may directly inhibit the carbohydrate-mediated binding of cells. Numerous reports[5–8] exist describing very elegant chemical syntheses of complex oligosaccharides by routes involving alcohol protection–deprotection and stereospecific glycosylation. It is a tribute to the progress in this field that almost any oligosaccharide of up to 6–8 sugar residues in size can now be

* Corresponding author (present address): Ciba Geigy AG, Central Research Laboratories, Rosental R1060.1.10, CH-4002 Basel, Switzerland.

assembled in a reasonably predictable fashion. Such total chemical syntheses, however, remain long and difficult, very demanding in experience, tedious in chromatography, and correspondingly expensive[9]. Typically, many months of dedicated work by several individuals can be required to produce a few mg of a new oligosaccharide for biological evaluation.

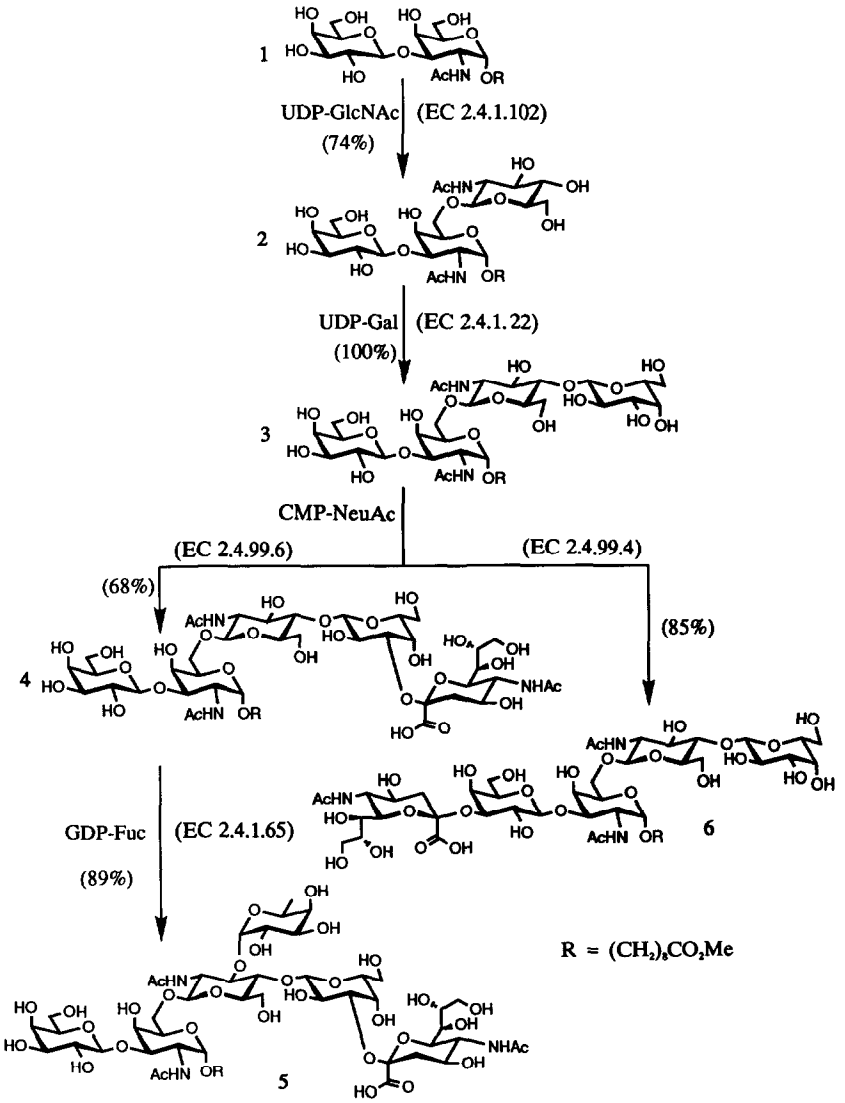
The use of glycosyltransferases to elaborate small synthetic oligosaccharide primers has gained increasing interest recently[9,10] with the promise that these enzymes may soon become more readily available through genetic engineering[11–13]. The advantage to using glycosyltransferases, of course, is that the enzymes add sugar residues to oligosaccharide acceptors both stereospecifically and regiospecifically, thus potentially obviating the need for both protection and separation of isomeric products. Thereby, target oligosaccharides can be assembled in days rather than weeks or months[9]. The major single impediment to this approach, however, is that very few enzymes are currently commercially available. Most literature reports on the purification of glycosyltransferases[14] from biological tissues involve procedures which are laborious, lengthy, and require expertise not normally available in an organic chemistry laboratory where the interest in producing a target oligosaccharide may arise. As a consequence, the use of glycosyltransferases in the combined chemical–enzymatic synthesis of oligosaccharides has not gained universal popularity but has remained largely in the domain of several specialist laboratories.

We introduce here the enzyme: UDP-D-Glc*p*NAc: β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc (GlcNAc to GalNAc) β-(1 → 6)-GlcNAc-transferase to the repertoire of readily available reagents for the rapid assembly of oligosaccharides. This enzyme, also referred to[13] as the "core-2"-GlcNAc-transferase (GlcNAcT, EC 2.4.1.102), catalyses the biosynthetic reaction shown in Scheme 1, where R is either a serine or a threonine residue on a glycoprotein[13]. The enzyme has been purified to homogeneity from bovine tracheal epithelium[15]. The GlcNAcT has recently been cloned[16]. It is known to act on oligosaccharides where R is a simple alkyl or aryl aglycon and small quantities of product, sufficient for characterization by NMR spectroscopy, have been produced using the enzyme from lymphoid tumor cells[17].

We sought a readily available source of the enzyme for synthetic purposes. For such purposes, a glycosyltransferase need not be purified to homogeneity but simply to be "functionally pure" in the sense that only one reaction is catalyzed by the enzyme preparation: the desired synthetic reaction. In the case of the GlcNAcT described here, the required level of functional purity could be achieved starting from a commercial acetone powder from mouse kidney in a single step of affinity chromatography which yielded sufficient enzyme to produce more than 20 mg of a trisaccharide product. The enzyme was then used in the key step, in a set of reactions involving four sequential glycosyltransferases, to convert the synthetic T-disaccharide glycoside **1** to the O-linked sialyl-Le^X derivative **5**. Hexasaccharide **5** has been reported[18–20] to exist on O-linked glycoproteins and is a candidate as a ligand for the selectins implicated in the initiation of the inflammatory process[3].

RESULTS AND DISCUSSION

The GlcNAcT was readily obtainable from mouse kidney acetone powder (1 g) by detergent (Triton X-100) extraction followed by a single step of affinity chromatography on UDP-hexanolamine Sepharose[21] (see Experimental). Incubation of the synthetic T-disaccharide **1**[22] with a portion of this extract, in the presence of UDP-GlcNAc, resulted in the formation of a single product as evidenced by TLC (Table III). This product was readily isolated by chromatogra-



Scheme 1.

TABLE I

Selected ^{1}H NMR data [a] for compounds **1–6**

Residue	Proton	1	2	3	4	5	6
GalNAc	H-1	4.88 (3.5)	4.86 (4.0)	4.84 (3.5)	4.86 (3.5)	4.86 (2.5)	4.88 (3.5)
	H-2	4.31 (3.5, 11.0)	4.29 (4.0, 11.0)	4.28 (3.5, 11.0)	4.29 (3.5, 11.0)	4.29 (2.5, 10.5)	4.28 (3.5, 11.0)
	H-3	4.02 (3.5, 11.0)	4.01 (3.0, 11.0)	n.d.	n.d.	n.d.	n.d.
	H-4	4.24 (2.9)	4.21 (3.0)	4.20 (2.9)	4.21 (2.9)	4.21 (2.9)	4.21 (2.9)
	NAc	2.02	2.02	2.00	2.02	2.02	2.01
Galβ3	H-1	4.48 (8.0)	4.46 [b] (8.0)	4.45 * (8.0)	4.46 * (8.0)	4.46 (7.7)	4.54 * (7.9)
	H-4	3.91 (3.0)	3.90 (3.5)	3.91 [c] (3.0)	3.95 (3.0)	n.d.	n.d.
GlcNAc	H-1		4.52 [b] (8.0)	4.53 (8.0)	4.54 (8.0)	4.54 (8.2)	4.54 (8.5)
	NAc		2.01	1.99	2.00	1.99	2.00
Galβ4	H-1			4.45 * (8.0)	4.54 * (8.0)	4.51 (7.9)	4.46 * (8.5)
	H-3			n.d.	4.11 (2.5, 7.5)	n.d.	n.d.
	H-4			3.89 [c] (3.0)	n.d.	n.d.	n.d.
NeuAc	H-3e				2.75 (4.5, 12.0)	2.76 (4.4, 12.2)	2.75 (4.5, 12.4)
	H-3a				1.80 (12.0)	1.79 (12.3)	1.78 (12.4)
	NAc				2.03	2.03	2.02
Fuc	H-1					5.09 (3.0)	
	H-5					4.81 (6.3)	
	H-6					1.16 (6.3)	

[a] Numbers in parantheses give coupling constants in Hz. Cross-couplings of protons of each compound were confirmed by COSY-spectra; n.d., not determined. [b] May be interchangeable. [c] May be interchangeable.

phy on silica gel and adsorption[23] onto C_{18} reverse-phase Sep-Pak followed by elution with methanol. Trisaccharide **2** (22 mg) was thus obtained in 74% yield.

The structure of **2** was assigned on the basis of the NMR data presented in Tables I and II. In particular, the new glycosidic linkage is established as having the β configuration from the H-1 doublet (J 8.0 Hz) at δ 4.52, in accord with a previous assignment of the nitrophenyl glycoside of this trisaccharide[16]. The chemical shift of C-6 of the GalNAc residue in **2** is 8.45 ppm downfield from that in disaccharide **1** thereby establishing that O-6 is glycosylated. As with this, and all remaining oligosaccharides produced in this work, the fast-atom bombardment mass spectrum confirmed the monosaccharide composition and size (Table III).

The utility of the GlcNAcT as a reagent in the synthesis of biologically relevant oligosaccharides is demonstrated here by preparing a hexasaccharide (**5**) where the sialyl-Le^X tetrasaccharide[3] is attached to O-6 of β-D-Gal$p$-(1 → 3)-α-D-Gal$p$NAc-OR, the disaccharide core most commonly found on serine or threonine in O-linked glycoproteins. This hexasaccharide sequence has been reported as its

TABLE II

Selected ^{13}C NMR data [a] for compounds **1–6**

Residue	Carbon	1	2	3	4	5	6
GalNAc	C-1	97.81	97.60	97.56	97.54	97.54	97.51
	C-2	49.64	49.59	49.56	49.56	49.57	49.57
	C-3	78.17	77.97	77.94	77.96	77.96	78.11
	C-4	69.56	69.73	69.79	69.76	69.78	69.62
	C-5	71.84	70.12	70.13	70.13	70.05	70.14
	C-6	62.02	70.45	70.85	70.83	70.97	70.94
Galβ3	C-1	105.51	105.41	105.50 *	105.48	105.49	105.22 *
	C-2	71.33	71.54	71.46 *	71.46	71.47	69.94 *
	C-3	73.38	73.43	73.35 *	73.37	73.36	78.11 *
	C-4	69.45	69.46	69.43 *	69.44	69.44	68.23 *
	C-5	75.84	75.83	75.83	75.82	75.82	76.49
	C-6	61.84	61.83	61.83	61.81	61.82	61.82
GlcNAc	C-1		102.39	102.30	102.33	102.19 [b]	102.30
	C-2		56.37	55.84	55.84	56.50	55.86
	C-3		74.89	73.35	73.37 *	75.82 *	73.43
	C-4		70.89	79.41	79.25 *	76.50 *	79.38
	C-5		76.72	76.21	76.32 *	74.34 *	76.19
	C-6		61.74	60.93	60.94	60.55	60.92
Galβ4	C-1			103.75 *	103.44 *	102.50 [b]	103.73
	C-2			71.80 *	70.20 *	70.05	71.79
	C-3			73.43 *	76.02 *	76.17	73.34
	C-4			69.38 *	68.31 *	68.13	69.39
	C-5			75.62	75.61	75.73	75.61
	C-6			61.83	61.81	62.28	61.82
NeuAc	C-1				175.81	n.o.	175.84
	C-2				100.65	100.60	100.56
	C-3				40.47	40.64	40.57
	C-4				69.18	69.15	69.21
	C-5				52.52	52.54	52.51
	C-6				73.72	73.75	73.65
	C-7				68.94	68.96	68.90
	C-8				72.59	72.70 [c]	72.65
	C-9				63.41	63.44	63.35
Fuc	C-1					99.49	
	C-2					68.55	
	C-3					70.05	
	C-4					72.74 [c]	
	C-5					67.52	
	C-6					16.10	
	OCH_3	52.90	52.90	52.90	52.90	52.90	52.90
	CH_2CO_2	34.54	34.55	34.55	34.54	34.55	34.55
	OCH_2CH	68.94	68.53	68.43	68.43	68.40	68.43
	$OCCH_3$	22.86	22.89, 23.11	22.84, 23.10	22.85, 22.85, 23.10	22.85, 22.86, 23.17	22.87, 23.10
	OCNH	175.33	175.00, 175.27	n.o.	174.69, 174.96, 175.31,	n.o.	174.72, 174.94, 175.32

TABLE II (continued)

Residue	Carbon	1	2	3	4	5	6
	O=*C*OMe	178.75	178.64	n.o.	177.02	n.o.	178.73
	CCH_2C	29.24, 29.10, 29.03, 28.99, 26.02, 25.12	29.17, 29.11, 29.00, 26.09, 25.12	29.17, 29.15, 29.05, 29.02, 26.11, 25.13	29.14, 29.01, 26.10, 25.12	29.14, 29.01, 26.10, 25.12	29.15, 29.04, 29.02, 26.10, 25.14

[a] Except for compound **5** all chemical shifts have been obtained from APT-spectra; n.o., not observed. [b] Interchangeable. [c] Interchangeable.

alditol acetate after release from glycoproteins[18–20], but it has not been synthesized. The total chemical synthesis of this molecule, using the conventional synthetic methodology of carbohydrate chemistry, would have taken several months even if protected monosaccharide intermediates were available. Its enzymatic assembly, using synthetic **1** as the primer and 4 sequential enzyme reactions, required only one week once the enzymes were in hand. This includes the time required for isolation and spectral characterization of the intermediate compounds. Once partially purified, these enzymes are stable indefinitely and can also be used in other synthetic schemes.

Thus, trisaccharide **2** could be quantitatively converted to tetrasaccharide **3** by reaction with commercial β-(1 → 4)-galactosyltransferase (GalT, EC 2.4.1.22), an enzyme widely used[10,24] in the preparative synthesis of oligosaccharides, in the presence of UDP-galactose. The reaction was complete within one h. The β linkage in the product was assigned from its NMR spectra which showed a new anomeric H-1 (*J* 8.0 Hz) and C-1 (103.75 ppm), as well as a signal assigned to C-4 of the GlcNAc residue which was deshielded by 8.52 ppm.

Tetrasaccharide **3** was elongated by reaction with α-(2 → 3)-sialyltransferase (*N*-acetylneuraminyltransferase, NeuAcT, EC 2.4.99.6) partially purified from rat liver[25–27]. This enzyme selectively sialylates β-(1 → 4)-linked Gal residues. Less than 1 equiv of CMP-NeuAc was used to ensure that only mono-sialylation to produce pentasaccharide **4** occurred since this enzyme has also been reported[28] to act on β-Gal-(1 → 3)-GalNAc sequences though at an extremely low rate. The NMR spectra of the product contained the signals expected from the addition of the new sialic acid residue. To convincingly demonstrate that the sialylation had occurred as expected, the alternative sialylated pentasaccharide was also prepared by reaction of **4** with commercial porcine α-(2 → 3)-NeuNAcT (EC 2.4.99.4) which is known[29,30] to preferentially sialylate β-Gal-(1 → 3)-GalNAc sequences. The product **6**, thus obtained, was different from **4** as seen from the NMR data reported in Tables I and II.

Finally, pentasaccharide **4** was fucosylated using the human milk Lewis α-(1 → 3/4)-fucosyltransferase (EC 2.4.1.65), an enzyme known[30,31] to preparatively fuco-

TABLE III

Summary of reaction conditions and characteristic properties of the products of enzymatic glycosylation

Acceptor (μmol)	Donor (μmol)	Transferase (EC)	mU	Buffer solution (pH)	Total vol. (mL)	time (h)	Product (mg) yield	Isola-tion	Additives	R_f	Mass + Na^+ (found)
1, 22 mg (39.7)	UDP-GlcNAc (123.1)	GlcNAcT (2.4.1.102)	66.1	HEPES (0.05 M, 6.5)	29.0	43	**2**: 22.2 (74%)	*A*	24 U CIAP	0.28 [a]	779
2, 19 mg (24.7)	UDP-Gal (30.2)	Gal-T (2.4.1.22)	2000	Na-cacodylate (0.05 M, 7.4)	0.62	1	**3**: 22.7 (100%)	*B*	12 U CIAP, 1% BSA, 14 mM $MnCl_2$	0.19 [a]	941
3, 12 mg (12.5)	CMP-NeuNAc (11.9)	NeuNAc-T (rat) (2.4.99.6)	25	Na-cacodylate (0.025 M, 6)	1.0	61	**4**: 10.3 (68%)	*C*	12 U CIAP, 1% BSA, 30 mM $MnCl_2$	0.02 [a]	1232
4, 3.3 mg (2.8)	GDP-Fuc (3.9)	Fuc-T (2.4.1.65)	25	HEPES (0.25 M, 7)	1.0	6	**5**: 3.3 (89%)	*D*	5 mM ATP, 2% BSA, $MnCl_2$	0.30 [b]	1378
3, 3.4 mg (3.7)	CMP-NeuNAc (2.3)	NeuNAc-T (pig) (2.4.99.4)	20	Na-cacodylate (0.025 M, 6.2)	0.3	5	**6**: 2.4 (85%)	*E*	12 U CIAP, 1% BSA	0.03 [a]	1232

[a] In 13:7:1 $CHCl_3$–MeOH–H_2O. [b] In 5:4:1 $CHCl_3$–MeOH–H_2O. CIAP, calf intestine alkaline phosphatase, DNA-grade, Boehringer.

sylate sialylated *N*-acetyllactosamine containing sequences. The new anomeric doublet (δ 5.09, J 3.0 Hz) confirmed the new α linkage while the characteristically deshielded signal for H-5 of the new fucose residue (δ 4.81) confirmed the expected (1 → 3) linkage[32,33]. The mass spectrum also confirmed the addition of fucose. The present work thus complements the recent elegant enzymatic synthesis of simple sialyl-Le^X tetrasaccharide glycosides reported independently by Ball et al.[36] and Ichikawa et al.[37] Evaluation of **5** as a ligand for the E, P, and L selectins is in progress and the results will be reported elsewhere.

EXPERIMENTAL

General methods.—All reagents were commercially available unless otherwise stated. UDP-GlcNAc, UDP-Gal, and CMP-sialic acid were from Sigma (St. Louis, MO). GDP-Fuc was synthesized as previously described[34]. Disaccharide **1** was synthesized as previously described. TLC was performed on silica gel plates (60-F_{254}, E. Merck) with detection by charring after spraying with 5% H_2SO_4 in EtOH. Iatrobeads refers to a beaded silica gel (No. 6RS-8060) manufactured by Iatron Laboratories (Tokyo). ^{1}H NMR spectra were recorded at 500 MHz (Varian spectrometer) on solutions in D_2O with acetone (δ 2.225 in D_2O) as external standard at ambient temperature. ^{13}C NMR spectra were recorded at 125 MHz, on the same instrument, on solutions in D_2O with external 1,4-dioxane (δ 67.4) as reference. Only partial NMR data are reported. Other spectral features were in accord with the proposed structures. The ^{1}H NMR chemical shifts and coupling constants are reported as though they were first order. Assignments of ^{13}C resonances are tentative. FAB-mass spectra were obtained using an AEI MS-9 instrument with Xe as the bombarding gas, and with 5:1 1,4-dithiothreitol–1,4-dithioerythritol as matrix.

Preparation of glycosyltransferases.—*(a) Core-II GlcNAcT.* Mouse-kidney-acetone powder (1 g, Sigma) was suspended in 20 mL NaOAc buffer (*A*: 0.1 M, pH 6.0; 0.2 M NaCl, 0.01 M EDTA) and vigorously stirred for 30 min at 4°C. The slurry was centrifuged at 6000 g for 15 min at 4°C. The pellet was removed and again treated with 20 mL of buffer *A* and centrifuged as above. The pellet was subsequently stirred in 20 mL of water and centrifuged. The pellet was then extracted with 25 mL of HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (*B*: 0.05 M, pH 6.5; 0.4 M KCl, 6% Triton X-100) for 1 h and centrifuged as above. The supernatant was saved and the extraction of the pellet repeated once more with buffer *B*. The supernatants were combined, mixed with 5.87 mg of soybean trypsin-inhibitor (Sigma) and 1.4 mg of bovine aprotinin (Sigma), transferred into dialysis bags (12000–14000 mol. wt. cutoff) and dialyzed versus 1.5 L of HEPES buffer (*C*: 0.05 M, pH 6.5; 0.1% mercaptoethanol) for 20 h at 4°C. The dialyzate was again centrifuged. The volume of the supernatant was determined and assayed for core-II activity (about 527 mU). Approximately 90 g of a UDP-hexanolamine Sepharose 4B gel (11 μmol/g gel) were equilibrated with

buffer *C*. The crude extract was loaded onto the gel at a flow rate of 0.2 mL/min at 4°C. The gel was then washed with buffer *C* (150 mL) and subsequently eluted with 300 mL of buffer *D* (0.05 M, HEPES, pH 6.5; 0.1% Triton X-100, 0.1% mercaptoethanol, 0.5 M NaCl). Fractions (15 mL) were collected in plastic tubes, containing 150 μL aq bovine serum albumin (BSA, 10 mg/mL) and dialyzed against buffer *C* (4 L). The fractions showing core-II activity (total 383 mU, 73%) were pooled and concentrated using an Amicon ultrafiltration cell with a PM30 membrane. This concentrate (23 mL, 177 mU) was used for preparative glycosylation.

(b) Other glycosyltransferases. Bovine β-(1 → 4)-galactosyltransferase was obtained from Sigma. α-(1 → 3/4)-Fucosyltransferase (25 mU) with a specific activity of 107 mU/mg was partially purified from 300 mL of human milk by sequential chromatography on SP-Sephadex[35] and GDP-hexanolamine[30]. α-(2 → 3)-Sialyltransferase (30 mU) with a specific activity of 2.7 U/mg was isolated from 500 g of rat liver by extraction[25], followed by chromatography on Cibacron Blue[26,27] and β-D-Gal*p*-(1 → 3)-β-D-Glc*p*NAc-O-$(CH_2)_8CONH(CH_2)_2NH$-Sepharose[27].

Radiochemical assay for core-II GlcNAcT.—A 90 mM GlcNAc (50 μL) solution, 30 μL of 1.31 mM aq UDP-GlcNAc, 30 μL of a 713 μM aq solution of acceptor **1**, and 0.5 μL of aq UDP-6-[^{3}H]GlcNAc (about 81 000 dpm, New England Nuclear) were combined in microfuge tubes and lyophilized. The residue was dissolved in 60 μL HEPES buffer (0.05 M, pH 6.5, 1% Triton X-100) and mixed with 20 μL of enzyme solution. The mixture was incubated for 30 min at 37°C, diluted with water to 10 mL and loaded onto a C_{18} Sep-Pak cartridge which was pre-equilibrated with water. The cartridge was washed with 20 mL of water and the product was eluted with 2 × 5 mL of MeOH. The radioactivity of the MeOH eluates were quantitated by liquid scintillation counting using 10 mL as the cocktail.

General incubation conditions for preparative reactions.—Donor sugar-nucleotides and acceptors were dissolved in the indicated buffer solutions and incubated at 37°C under the individual conditions described in Table III.

Product isolation procedures.—A: The reaction mixture was diluted with water to 60 mL and passed through three sequentially connected C_{18} Sep-Pak cartridges. The cartridges were washed with water (120 mL); the eluate contained the product. The Triton was removed from the Sep-Pak with 60 mL of MeOH and the water fraction again loaded onto the C_{18} Sep-Pak. The column was washed with water and eluted with 30 mL of MeOH. The MeOH was evaporated to dryness and the residue chromatographed on an Iatrobead-column (1.8 × 15 cm) with 20:10:1 CH_2Cl_2–MeOH–H_2O. The product fractions were combined, evaporated, and lyophilized from water.

B: The mixture was diluted to 30 mL with water, loaded onto three C_{18} Sep-Pak cartridges, washed with 60 mL of water and eluted with 60 mL of MeOH. The MeOH was evaporated and the residue lyophilized from water.

C: The mixture was diluted to 9 mL with water, loaded onto two C_{18} Sep-Pak cartridges, washed with 40 mL of water, and eluted with 50 mL of MeOH. The

MeOH was evaporated and the residue lyophilized from water. The resulting white powder was chromatographed on a 2 mL silica-gel column using 12:7:1 $CHCl_3$–MeOH–H_2O as eluent. Fractions containing product were pooled and concentrated. The residue was lyophilized from water after passing through a 0.22-μm filter.

D: The same procedure as in *B* was applied with only one C_{18} Sep-Pak cartridge.

E: The same procedure as in *C* was applied with only one C_{18} Sep-Pak cartridge.

ACKNOWLEDGMENTS

The authors acknowledge the invaluable assistance of Ms. C. Compston and Dr. S. Gosselin for providing the sialyl and fucosyltransferases, Mr. G. Alton for preparing the GDP-hexanolamine column and Mr. C. Bigam for recording the NMR spectra. This work was supported by grants from the Medical Research Council of Canada to both OH and MMP.

REFERENCES

1 S. Hakomori, *Adv. Cancer Res.*, 52 (1989) 257–331.
2 J.W. Dennis, in M. Fukuda (Ed.), *Cell Surface Carbohydrates and Cell Development*, CRC Press, Boca Raton, FL, 1991, pp 161–194.
3 B.K. Brandley, *Seminars Cell Biol.*, 2 (1991) 281–287.
4 C.A. Lingwood, *Curr. Opin. Struct. Biol.*, 2 (1991) 693–700.
5 R.R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 212–235.
6 H. Paulsen, *Angew. Chem. Int. Ed. Engl.*, 29 (1990) 823–839.
7 P. Sinaÿ, *Pure Appl. Chem.*, 63 (1991) 519–528.
8 O. Kanie and O. Hindsgaul, *Curr. Opin. Struct. Biol.*, 2 (1992) 674–681.
9 O. Hindsgaul, *Seminars Cell Biol.*, 2 (1991) 319–326.
10 Y. Ichikawa, G.C. Look, and C.-H. Wong, *Anal. Biochem.*, 202 (1992) 215–238.
11 J.C. Paulson and K.C. Colley, *J. Biol. Chem.*, 264 (1989) 17615–17618.
12 J.B. Lowe, *Seminars Cell Biol.*, 2 (1991) 289–307.
13 H. Schachter, *Curr. Opin. Struct. Biol.*, 1 (1991) 755–765.
14 T.A. Beyer, J.E. Sadler, J.I. Rearick, J.C. Paulson, and R.L. Hill, *Adv. Enzymol.*, 52 (1981) 23–175.
15 P.A. Ropp, M.R. Little, and P.-W. Cheng, *J. Biol. Chem.*, 266 (1991) 23863–23871.
16 M.F.A. Bierhuuizen and M. Fukuda, *Proc. Natl. Acad. Sci. USA* 89 (1992) 9326–9330.
17 D. Zhuang, A. Grey, M. Harris-Brandts, E. Higgins, M.A. Kashem, and J.W. Dennis, *Glycobiology*, 1 (1991) 425–433.
18 G. Lamblin, A. Boersma, A. Klein, P. Roussel, H. van Halbeek, and J.F.G. Vliegenthart, *J. Biol. Chem.*, 259 (1984) 9051–9058.
19 E.J. Bregg, H. van Halbeek, J.F.G. Vliegenthart, G. Lamblin, M.C. Houvenhagel, and P. Roussel, *Eur. J. Biochem.*, 168 (1987) 57–68.
20 E.C. Yurewicz, F. Matsuura, and K.S. Moghissi, *J. Biol. Chem.* 262 (1987) 4733–4739.
21 R. Barker, K.W. Olsen, J.H. Shaper, and R.L. Hill, *J. Biol. Chem.*, 247 (1972) 7135–7147.
22 R.M. Ratcliffe, D.A. Baker, and R.U. Lemieux, *Carbohydr. Res.*, 93 (1981) 35–41.
23 M.M. Palcic, L.D. Heerze, M. Pierce, and O. Hindsgaul, *Glycoconj. J.*, 5 (1988) 49–63.
24 R. Schneider, M. Hammel, E.G. Berger, O. Ghisalba, J. Nueesch, and D. Gygax, *Glycoconj. J.*, 7 (1990) 589–600.

25 J. Weinstein, U. de Souza-e-Silva, and J.C. Paulson, *J. Biol. Chem.*, 257 (1982) 13835–13844.
26 U. Sticher, H.J. Gross, and R. Brossmer, *Biochem. J.*, 253 (1988) 577–580.
27 M.A. Mazid and M.A. Kashem, *PCT Int. Appl.* WO 90-12090 (18 October 1990).
28 J. Weinstein, U. de Souza-e-Silva, and J.C. Paulson, *J. Biol. Chem.* 257 (1982) 13845–13853.
29 J.I. Rearick, J.E. Sadler, J.C. Paulson, and R.L. Hill, *J. Biol. Chem.*, 254 (1979) 4444–4451.
30 M.M. Palcic, A.P. Venot, R.M. Ratcliffe, and O. Hindsgaul, *Carbohydr. Res.*, 190 (1989) 1–11.
31 J.P. Prieels, D. Monnom, M. Dolmans, T.A. Beyer, and R.L. Hill, *J. Biol. Chem.*, 256 (1981) 10456–10463.
32 R.U. Lemieux, K. Bock, L.T.J. Delbaere, S. Koto, and V.S. Rao, *Can. J. Chem.*, 58 (1980) 631–653.
33 Y.-C. Lin, C.W. Hummel, D.-H. Huang, Y. Ichikawa, K.C. Nicolaou, and C.-H. Wong, *J. Am. Chem. Soc.*, 114 (1992) 5452–5454.
34 U.B. Gokhale, O. Hindsgaul, and M.M. Palcic, *Can. J. Chem.*, 68 (1990) 1063–1071.
35 S. Eppenberger-Castori, H. Lotscher, and J. Finne, *Glycoconjugate J.*, 6 (1989) 101–114.
36 G.E. Ball, R.A. O'Neill, J.E. Schultz, J.B. Lowe, B.Y. Weston, J.O. Nagy, E.G. Brown, C.J. Hobbs, and M.D. Bednarski, *J. Am. Chem. Soc.*, 114 (1992) 5449–5451.
37 Y. Ichikawa, Y.-C. Lin, D.P. Dumas, G.-J. Shen, E. Garcia-Junceda, M.A. Williams, R. Bayer, C. Ketcham, L.E. Walker, J.C. Paulson, and C.-H. Wong, *J. Am. Chem. Soc.*, 114 (1992) 9283–9298.