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ENZYMIC SYNTHESIS OF OLIGOSACCHARIDES TERMINATING IN THE TUMOR-ASSOCIATED SIALYL-LEWIS-a DETERMINANT

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ABSTRACT

The isomeric sialyl-Le^a-terminating pentasaccharide derivatives, α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-O(CH$_2$)$_8$COOMe (**1**) and α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc-(1→6)-β-D-Gal*p*-O(CH$_2$)$_8$COOMe (**2**), have been prepared by the action in sequence of a porcine submaxillary (2→3)-α-sialyltransferase and a human-milk (1→3/4)-α-fucosyltransferase on the chemically synthesized trisaccharides β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)- (**4**) and -(1→6)-β-D-Gal*p*-O(CH$_2$)$_8$COOMe (**5**), respectively.

INTRODUCTION

The sialyl-Le^a antigen, α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc (**C**), is widely regarded as a tumor-associated antigen, and monoclonal antibodies, especially 1116NS 19-9, which detect this structure are important in the diagnosis of human gastrointestinal cancers[1–3]. The sialyl-Le^a tetrasaccharide terminus was demonstrated originally on the sialylated lacto-*N*-fucopentaose II glycolipid[4,5] and later in oligosaccharides from human milk[6,7], where it is attached to the 3-position of a β-D-Gal*p* residue. The antigen has been detected also on serum mucins of cancer patients[8] and in mucin-like glycoproteins in human seminal plasma and milk[9,10]. The detailed structural work on these latter oligosaccharides is in progress[11]. The antigen has also recently been detected in asparagine-linked oligosaccharides[12].

Since the sialyl-Le^a structure is a sialylated and fucosylated Type 1 [β-D-Gal*p*-

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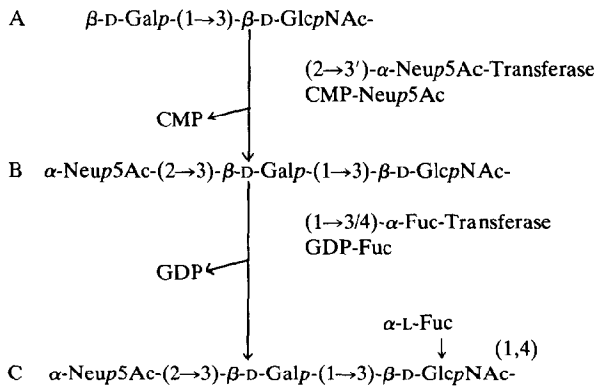
(1→3)-β-D-Glc*p*NAc] chain, the possibility for the expression of this antigen exists wherever Type 1 chains occur and, in fact, wherever β-D-Glc*p*NAc residues occur. We are involved in producing and better characterising the specificity of monoclonal antibodies against sialyl-Le^a-containing structures, using synthetic carbohydrate antigens in immunization and in screening[13,14], and we now report the preparation of α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-OR (**1**) and α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc-(1→6)-β-D-Gal*p*-OR (**2**), where R = $(CH_2)_8COOMe$. Total chemical syntheses of **1** and **2** were possible, but a combined chemical/enzymic approach was explored which would be applicable to other, more complex, sialyl-Le^a-containing structures. The use of glycosyltransferases in the synthesis of oligosaccharides now has sufficient precedent[15–21] to render this approach frequently the method of choice for the rapid production of such molecules in quantities sufficient for biological assays.

The pathway of biosynthesis for the production of the sialyl-Le^a determinant has been proposed[22] to involve sialylation of Type 1 structures (**A**) followed by fucosylation as summarized in Scheme 1. The biosynthetic sialylation is catalyzed by a β-D-Gal*p*-(1→3/4)-β-D-Glc*p*NAc:CMP-Neu*p*5Ac (2→3′)-α-sialyltransferase (EC 2.4.99.5), an enzyme which has been purified to homogeneity from rat liver[23]. The utility in synthesis of this enzyme has been demonstrated[19], but it is tedious to purify and is not yet available commercially. However, a β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc:CMP-Neu5Ac (2→3′)-α-sialyltransferase (EC 2.4.99.4) from porcine submaxillary glands[24] is available commercially and cross-reacts[25,26], albeit poorly, with structures terminating in the Type 1 sequence **A**. The latter sialyltransferase has been used because of its availability. A fucosyltransferase activity which converts **B** into **C** (Scheme 1) has been detected in colonic tumor-cell extracts[22] and, presumably, is the Lewis (or 3/4)-α-fucosyltransferase. However, the Lewis fucosyltransferase from human milk was reported[22,27] *not* to fucosylate a milk oligosaccharide terminating in the structure **B**. These results contrast with the report[28] that a sialylated-Type 1-terminating milk oligosaccharide is a substrate for a highly purified human milk 3/4-α-fucosyltransferase. A major concern, therefore, was whether a readily accessible fucosyltransferase activity could be found that would convert **B** into **C** with our synthetic structures. We now report that a readily accessible, partially purified fucosyltransferase activity from the milk of a human Le^{a+b-} donor efficiently catalyzes this conversion. This same activity was also found, as observed by others[29], to fucosylate Type 2 structures terminating in α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc, thereby producing the so-called sialyl-Le^x determinant, also found on tumor-associated carbohydrates[30,31].

RESULTS AND DISCUSSION

The objective of this study was to evaluate the potential of the commercially available porcine (2→3)-α-sialyltransferase and the human milk (1→3/4)-α-fucosyl-



Scheme 1. Biosynthetic pathway for the sialyl-Le^a determinant.

transferase preparation as tandem enzymes in the preparative syntheses of sialyl-Le^a-terminating oligosaccharides. The reactions were performed only on a mg scale, although the syntheses can be scaled-up if required. The syntheses reported yielded material sufficient for characterization by ^{1}H-n.m.r. spectroscopy and for probes for studying antibody/antigen reactions.

The oligosaccharides used in this study (Table I) were prepared as 8-methoxycarbonyloctyl glycosides in order to allow their conversion into artificial antigens[13,14,32] and affinity matrices[33]. The presence of this hydrophobic spacer arm allows the rapid isolation of oligosaccharides on C_{18} reverse-phase chromatographic supports, and thereby provides a simple method for the assay of glycosyltransferase activity[34]. The kinetic characteristics for many of the enzymic reactions are included in Table I. Compounds **3**–**6** and **9**–**15** (Table I) had been prepared by total chemical synthesis and were available as structurally characterized enzyme substrates and products.

Sialyltransferase reactions. — The porcine submaxillary (2→3)-α-sialyltransferase catalyzes the transfer of sialic acid from CMP-Neu*p*5Ac to HO-3′ of β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→O)-Ser/Thr[24,25]. The synthetic "T" disaccharide (**15**) was an excellent substrate for this enzyme (K_m 44 μM; Table I). The K_m of this enzyme for the Type 1 structure **3** was 100-fold higher, but, as reported[26], extended reaction resulted in the formation of sialylated product. Reactions of **4** and **5** with the enzyme and CMP-Neu*p*5Ac for 5 days at 37° produced the sialylated tetrasaccharides **7** (30%) and **8** (45%), respectively, which were isolated readily on a mg scale. The superior yield of **8** is reflected in the relative rates of sialylation of **4** and **5**, which are presented in Table I. However, each compound was a poor substrate. Nevertheless, the sialylation can be viewed as a reasonable synthesis step, since unreacted **4** and **5** are neutral compounds that can be recovered after ion-exchange chromatography and recycled. As noted[25], Type 2 structures are poor acceptors for this glycosyltransferase and **11** showed very low activity under the

TABLE I

STRUCTURE AND ACCEPTOR PROPERTIES OF THE OLIGOSACCHARIDES **1–15**

Compound	*Designation*[a]	*Structure*	*Sialyltransferase*		*Fucosyltransferase*	
			Relative rate (%)[b]	K_m (μM)	K_m (μM)	*Relative* V_{max} (%)[c]
Type 1 Structures						
3	Type 1	β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-OR[d]	15	4100 ±480	68 ±3	90
4	Type 1→3-Gal	β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-OR	4		151 ±40	17
5	Type 1→6-Gal	β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→6)-β-D-Gal*p*-OR	7		326 ±64	76
6	SA→Type 1	α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-OR			116 ±25	100
7	SA→Type 1→3-Gal	α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-OR			145 ±22	18
8	SA→Type 1→6-Gal	α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→6)-β-D-Gal*p*-OR			176 ±16	65
9	Le[a]	α-L-Fuc*p*-(1→4) β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-OR				
10	SA +Le[a]	α-L-Fuc*p*-(1→4) α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-OR				
1	SA +Le[a] →3-Gal	α-L-Fuc*p*-(1→4) α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→3)-β-D-Gal*p*-OR				
2	SA +Le[a] →6-Gal	α-L-Fuc*p*-(1→4) α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→6)-β-D-Gal*p*-OR				
Type 2 Structures						
11	Type 2	β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-OR	0.3	2600 ±500	45 ±7	100
12	SA→Type 2	α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-OR			13 ±4	95
13	Le[x]	α-L-Fuc*p*-(1→3) β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-OR				
14	SA-Le[x]	α-L-Fuc*p*-(1→3) α-Neu*p*5Ac-(2→3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc-OR				
15	T	β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-OR	100	44 ±4		

[a]SA refers to α-Neu*p*5Ac-(2→3). [b]Determined at mM acceptor concentration and 200μM CMP-Neu*p*5Ac. [c]100% V_{max} was 14.3 pmol/min fucose transferred; other conditions are given in the Experimental. [d]R = $(CH_2)_8COOMe$.

assay conditions. Neither the Le^a nor Le^x trisaccharides (**9** and **13**, respectively) were acceptors for the enzyme.

Fucosyltransferase reactions. — A fucosyltransferase activity, in human milk, capable of fucosylating both Type 1 and 2 structures has long been known[27,35,36]. This enzyme has been recognized more recently as the Lewis-fucosyltransferase, which is a 3/4-α-fucosyltransferase[37]. This activity was isolated readily by passage of an ammonium sulfate precipitate of defatted milk from a human Le^{a+b-} donor onto CM-Sephadex and elution of the retained protein, using stepwise increments of salt concentration. This simple procedure resulted in a 175-fold purification of the fucosyltransferase. The preparation was free of (1→2)-α-fucosyltransferase activity, when assayed using phenyl β-D-galactopyranoside as an acceptor[34], was stable for at least four months at 4°, and could be used directly for the fucosylation of oligosaccharides.

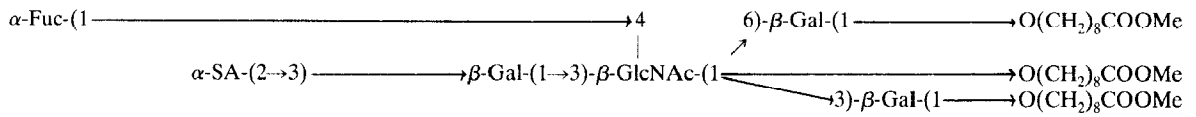
As seen from the kinetic data in Table I, the fucosyltransferase was active against both Type 1 and 2 substrates, non-substituted as well as sialylated. The Type 1 disaccharide **3** has a K_m of 68μM, which is similar to that of the Type 2 disaccharide **11** (K_m 45μM), and the values of V_{max} are comparable, in agreement with the results[27,29] obtained with the corresponding reducing disaccharides as acceptors. The synthetic sialylated Type 2 structure **12** was the best acceptor for the fucosyltransferase, with a K_M of 13μM, the decreased K_m being in line with results obtained with reducing oligosaccharides[29]. The sialylated Type 1 sequence **6** was also an excellent acceptor for the fucosyltransferase. The products of the incubation of **6** and **12** with the enzyme preparation and unlabelled GDP-fucose were isolated and shown by ^{1}H-n.m.r. spectroscopy to be identical to the chemically synthesized sialyl-Le^a (**10**) and sialyl-Le^x (**14**) tetrasaccharides, respectively, thereby confirming the specificity of these reactions. The ^{1}H-n.m.r. spectra of **10** and **14**, as well as those of all structures containing the Le^a or Le^x trisaccharides, are characterized by the uniquely deshielded[13,38] signal for H-5 (dq) of the fucose residue which resonates near 4.8 p.p.m. (see Table II). The enzyme was also active against both the Type 1-terminating trisaccharides **4** and **5**, although these were clearly distinguished kinetically.

Fucosylation of the sialylated Type 1 tetrasaccharides **7** and **8** proceeded to completion (>95%), within 72 h at 37°, as verified by the ^{1}H-n.m.r. spectra of the isolated products. The pure pentasaccharides **1** and **2** were obtained by isolation on C_{18} reverse-phase cartridges, followed by ion-exchange chromatography and desalting. The position of fucosylation was again evident from the chemical shift of the resonance of H-5 of the new fucosyl residue, as described above.

The key ^{1}H-n.m.r. spectral parameters for the compounds used in this study are presented in Table II. The spectra of several of these compounds have been reported previously, but they are given in Table II with the same concentration, temperature, field, and internal standard, in order to permit direct comparison. The most striking feature of the data in Table II is the invariance of the chemical shifts of the signals of the reporter-group protons of both the fucosyl and sialyl

TABLE II

SELECTED ^{1}H-N.M.R. DATA FOR **1–14**[a,b]



Compound	*H-1*	*H-5*	*H-6*	*H-3e*	*H-3a*	*NAc*	*H-1*	*H-1*	*NAc*	*H-1*	*H-1*	*H-4*	*OMe*	*CH_2CO*
Type 1 Structures														
3							4.545	4.269	2.023				3.687	2.388
4							4.439	4.746	2.026		4.373	4.140	3.687	2.387
5							4.435	4.587	2.022	4.366			3.689	2.391
6				2.756	1.784	2.028	4.549	4.993	2.022				3.688	2.390
7				2.758	1.786	2.027	4.507	4.750	2.027		4.374	4.137	3.687	2.388
8				2.760	1.782	2.028	4.502	4.586	2.029	4.367			3.687	2.392
9	5.021	4.874	1.178				4.514	4.485	2.030				3.687	2.388
10	5.005	4.873	1.171	2.768	1.767	2.028	4.526	4.526	2.034				3.689	2.392
1	5.013	4.874	1.174	2.769	1.770	2.028	4.547	4.701	2.037		4.368	4.139	3.688	2.388
2	5.004	4.871	1.170	2.770	1.763	2.028	4.538	4.555	2.033	4.364			3.689	2.393
Type 2 Structures														
11							4.472	4.521	2.030				3.686	2.388
12				2.757	1.800	2.030	4.513	4.552	2.026				3.685	2.388
13	5.108	4.845	1.174				4.453	4.552	2.026				3.685	2.388
14	5.101	4.826	1.168	2.764	1.794	2.031	4.520	4.520	2.019				3.685	2.387

[a]360 MHz, D_2O, ~22°, internal 0.01% acetone (δ 2.225). [b]Coupling constants: H-1 (α-Fuc), $J_{1,2}$ 4 Hz; H-5 (α-Fuc), $J_{4,5}$ 1, $J_{5,6}$ 6.5 Hz; H-1 (β-Gal, β-GlcNAc), $J_{1,2}$ 8 Hz; α-SA (H-3*a*), $J_{3a,3e}$ −12, $J_{3a,4}$ 11 Hz; α-SA (H-3*e*), $J_{3e,4}$ 4.5 Hz; H-4 (β-Gal), $J_{3,4}$ 4 Hz; CH_2CO (t), *J* 7.5 Hz. The variation in these coupling constants was $<\pm 0.3$ Hz.

residues within the same series, *i.e.*, Type 1 or 2. The signals for H-1,5,6 of the fucosyl residue and H-3*a*,3*e* of the sialic acid units are expected[39] to be sensitive to changes in the three-dimensional structures of these determinants. The differences in the chemical shift data between these structures are <0.03 p.p.m. and therefore negligible[39]. The tentative conclusion is that neither sialylation of the fucosylated structures nor fucosylation of the sialylated structures causes appreciable change in the conformation of these Type 1 and 2 antigens. Any changes observed in the antibody-reactivity of the new pentasaccharide structures **1** and **2**, relative to that of the sialyl-Le^a tetrasaccharide **10**, will probably reflect the recognition of the reducing-end galactose residues by the antibody-combining sites. Evaluation of the antibody-binding properties of these structures, as well as the enzymic preparation of additional sialyl-Le^a and sialyl-Le^x analogs, is in progress.

EXPERIMENTAL

General. — ^{1}H-N.m.r. spectra (0.01% internal acetone; δ 2.225) were recorded with a Bruker WM-360 instrument for mM solutions in D_2O at ~22°. Phenol–sulfuric acid assays were conducted according to Dubois *et al.*[40]. Glycosyltransferase assays were performed using a modification of the method described by Palcic *et al.*[34].

Sep-Pak C_{18} cartridges were obtained from Waters Associates, DEAE-Sephadex A25, CM-Sephadex C50, Sepharose 4B, and PD-10 columns from Pharmacia, guanosine diphospho-L-[U-^{14}C]fucose (248 mCi/mmol) and cytidine 5′-monophosphate-[9-^{3}H]Neu*p*5Ac (11.5 Ci/mmol) from New England Nuclear, CMP-Neu*p*5Ac from Sigma, and ACS liquid scintillation cocktail from Amersham International. GDP-fucose was synthesized as described[41]. (2→3)-α-Sialyltransferase from porcine submaxillary glands (EC 2.4.99.4) 120 munit/mL was purchased from Genzyme Corporation. Compounds **3** and **9**[13], **4** and **5**[42], **6, 10, 12,** and **14**[43], **11**[44], **13**[38], and **15**[45] were available from previous work. A protein assay kit, based on the method described by Bradford[46], was obtained from Bio-Rad Laboratories.

Preparative sialylation. — Acceptor **4** or **5** (1–2.5 mg), CMP-Neu*p*5Ac (0.7 equiv.), 6–7 munit (50–60 μL) of sialyltransferase, and 25–40 μL of 25mM sodium cacodylate buffer (pH 6.5) containing 0.5% of Triton X-100 and 1 mg/mL of bovine serum albumin (BSA) were incubated at 37° for 2 days. More CMP-sialic acid (0.7 equiv.) was then added. After 3 days, the mixture was diluted to 5 mL with H_2O and passed onto a C_{18} cartridge, conditioned as suggested by the manufacturer. The cartridge was washed with H_2O (3 × 5 mL) then with MeOH (3 × 5 mL). The MeOH eluate was concentrated to dryness and a solution of the residue in H_2O (2.0 mL) was passed on to a 1.0-mL column of DEAE-Sephadex A25 (Cl^- form). The column was washed with H_2O (5 × 2 mL). Phenol–sulfuric acid-positive material (**3** or **4**) was eluted only in the first 3 fractions. The sialylated products were eluted with M NaCl (3 × 2 mL) and desalted by adsorption onto a C_{18} cartridge

which was washed with H_2O (15 mL). Elution with MeOH (15 mL) followed by concentration and lyophilization from H_2O provided **7** or **8** as white powders. The yields were determined using the phenol–sulfuric acid assay with galactose as the reference, and by integration of relevant signals *vs.* internal acetone in the fully relaxed ^{1}H-n.m.r. spectra. The yields determined by these two methods agreed within 10%. Compounds **7** and **8** were obtained reproducibly in yields of 30% and 45%, respectively.

Sialyltransferase kinetics. — Reported methodologies[34] were adapted for assaying sialyltransferase activity. For studies of the relative rates, incubation mixtures contained, in a total volume of 64 μL, 200μM CMP-Neu*p*5Ac, 0.1 μCi of CMP-^{3}H-Neu*p*5Ac, mM acceptor saccharide, and enzyme (76 μunit for **15**, and 240 μunit for **3–5** and **11**) in 25mM sodium cacodylate buffer (pH 6.5) containing 0.5% of Triton X-100 and 1 mg/mL of BSA. After reaction at 37° for 1–3 h, the mixture was diluted with H_2O and applied to a Sep-Pak cartridge. Unreacted CMP-Neu*p*5Ac was eluted with 25 mL of H_2O, or until background counts were reached. Radiolabelled reaction products were eluted with MeOH (2 × 5 mL), and quantitated as d.p.m. in 10 mL of ACS liquid scintillation cocktail with a Beckman LS1801 scintillation counter. Kinetic studies were carried out in an analogous manner, but with various concentrations of acceptor, 8.3–100μM for **15**, or 0.4–4.0mM for Types 1 or 2 acceptors. Reaction was such that no greater than 10% turnover occurred to ensure initial rate conditions. The kinetic parameters V_{max} and K_m were determined using a computer program based on the method described by Wilkinson[47].

Preparative fucosylation. — The purification of human milk fucosyltransferase was carried out at 4° and was a modification of reported[27,48] procedures. Thawed milk (300 mL) from a Le^{a+b-} donor was defatted by centrifugation, and protein that precipitated at 65% $(NH_4)_2SO_4$ saturation was dialyzed against 25mM sodium cacodylate buffer (pH 6.5) (3 × 4 L) containing 5mM $MnCl_2$ (buffer *A*). After dialysis, any precipitated protein was removed by centrifugation and the supernatant solution was loaded onto a column (2.5 × 35 cm) of CM-Sephadex C50 equilibrated with buffer *A*. The column was washed with this same buffer until no further protein was eluted, then was developed stepwise with buffer *A* containing 0.1, 0.2, and 0.3M NaCl. Fractions containing fucosyltransferase activity, which were eluted generally with the 0.2M NaCl-containing buffer, were combined, and concentrated by ultrafiltration to 3 mL. The preparation thus obtained was purified 175-fold, and contained 34 munit of enzyme activity (80% yield), assayed as described below, with a protein content of 20 mg as determined by the Bradford assay[46] using BSA as a standard. This material was used without further purification.

Incubation mixtures contained, in 1.2 mL, acceptors **6, 7, 8,** or **12** (0.5–1.0 mg), GDP-fucose (1.7 equiv.), fucosyltransferase (1.6 munit), 8mM $MnCl_2$, 1.6mM ATP, 1.6mM ATP, 1.6mM NaN_3, and 25mM sodium cacodylate buffer (pH 6.5). After 24 h at 37°, 1.7 equiv. of GDP-fucose and 0.6 munit of fucosyltransferase

were added to the mixtures, which were kept at 37° for an additional 48 h. The products were isolated as described above for the sialylation reactions, except that elution from the column of DEAE was performed using 6 mL of 0.2M NaCl. Recoveries of the lyophilized powders varied in the range 50–75% and were not optimized. No unfucosylated starting materials could be detected by ^{1}H-n.m.r. spectroscopy, demonstrating that quantitative (>95%) fucosylation had occurred. No impurities were indicated by the ^{1}H-n.m.r. spectra.

For kinetic studies, the enzyme was purified further by affinity chromatography on a 2-mL GDP-hexanolamine Sepharose column[49] (4 μmol of ligand/mL of gel), equilibrated with 50mM sodium cacodylate buffer (pH 7.2) containing 25% of glycerol and 0.05% of NaN_3 (buffer *B*). The column was washed with this buffer, then buffer *B* containing 0.1M NaCl. Enzyme was eluted in 22 mL of buffer *B* containing 0.8M NaCl and 5mM GMP. This eluent (24 munit, 57% yield, 225 μg of protein) was brought to 0.14 mg/mL BSA to stabilize the fucosyltransferase, and immediately desalted on columns of PD-10 equilibrated with buffer *A*. Desalted samples were dialyzed against buffer *A* containing 50% of glycerol and concentrated to 3 mL by ultrafiltration. This preparation was stable for at least four months at 4°.

Fucosyltransferase kinetics. — Incubation mixtures contained in 50 μL, 48 μM GDP-fucose, 25,000 d.p.m. GDP-^{14}C-fucose, 25–500μM acceptor saccharide, fucosyltransferase (10 μunit), and 8mM $MnCl_2$, in 25mM sodium cacodylate buffer (pH 6.5). After reaction at 37° for 1 h, radiolabelled products were separated on Sep-Pak cartridges and counted in 10 mL of ACS cocktail as described above for sialyltransferase assays; less than 20% of the label was transferred to products. In order to conserve GDP-fucose, routine assays throughout the enzyme isolation were conducted with 3μM GDP-fucose, 500μM **3** or **11**, 8mM $MnCl_2$, 1.6mM NaN_3, 1.6mM ATP, and enzyme in 100 μL of 25mM sodium cacodylate (pH 6.5) for 1 h at 37°. In order to determine the yields at each step of the isolation, 48μM GDP-fucose and 500μM **3** were used with the remaining conditions being identical to those for routine assays. A unit is defined as the amount of enzyme transferring 1 μmol of fucose/min.

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