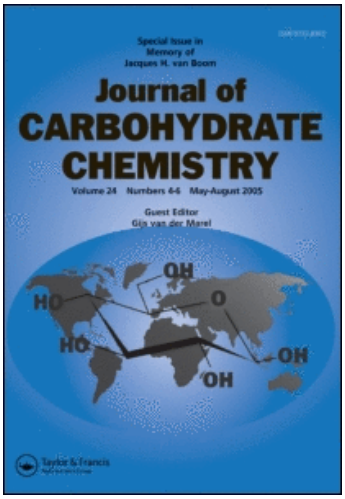

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An Aminated GDP-Fucose Analog Useful in the Fucosyltransferase Catalyzed Addition of Biological Probes onto Oligosaccharide Chains[1]

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AN AMINATED GDP-FUCOSE ANALOG USEFUL IN THE FUCOSYLTRANSFERASE CATALYZED ADDITION OF BIOLOGICAL PROBES ONTO OLIGOSACCHARIDE CHAINS[1]

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ABSTRACT

An analog (**1**) of GDP-fucose, where C-6 is derivatized with an eight-atom spacer terminating in a primary amino group, was chemically synthesized. This amino group in sugar nucleotide **1** can be acylated using an *N*-hydroxysuccinimide ester of biotin and it can be coupled to another molecule that also contains an amino group using squaric acid diethyl ester as the coupling reagent. In this way, biotin and a blood group A-active trisaccharide were linked to C-6 of fucose in GDP-fucose. Both complex sugar nucleotides thus prepared were active as donors for a human milk fucosyltransferase, which transferred the derivatized α-linked fucose residue to a glycoside of *N*-acetyllactosamine, thus labeling this sequence with either biotin or the blood-group A trisaccharide. Compound **1** is proposed as a general and versatile reagent which should permit the addition of biological probes to the sugar chains of cell surface glycoproteins or glycolipids.

INTRODUCTION

A tool allowing the specific addition of biological probes to the oligosaccharide chains of glycolipids and glycoproteins, both isolated and when present at the cell surface, would greatly assist research on the function of glycosylation.[2,3] For example, if addition of specific oligosaccharide sequences to proteins were possible, this could affect not only their circulation lifetimes, but also result in targeting of specific cells via carbohydrate-recognizing protein-receptors.[2] Also, if designed biological probes could be added in a mild manner to the cell membrane, the behavior of the resulting tagged cells could be similarly affected.[3]

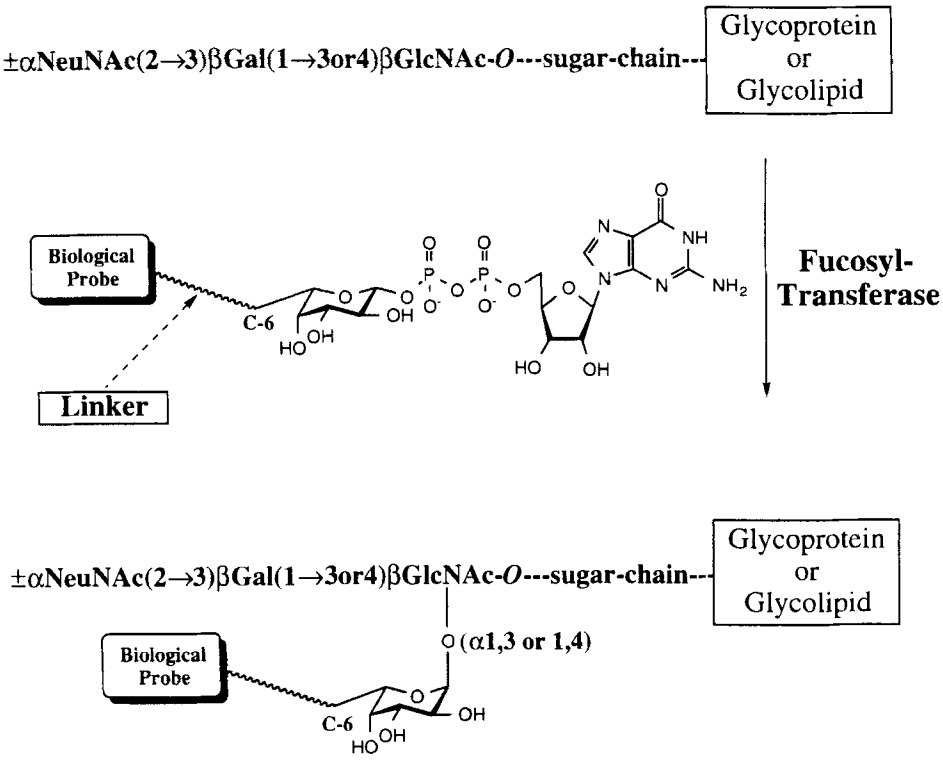


Figure 1. Linking of a biological probe of interest to C-6 of the fucose residue in GDP-fucose followed by transfer using a fucosyltransferase is proposed as a general method for covalently labeling the sugar chains of glycoproteins and glycolipids.

Since all animal cells contain cell surface glycoproteins and glycolipids with their sugar chains exposed to interact with their external environment, these sugar chains are ideal candidates to which biological probes could be attached. Such probes have been previously chemically attached to cell-surface glycoconjugates (by periodate oxidation followed by reductive amination) in order to add the molecule of interest covalently.[4] The feasibility of much milder and specific derivatizations has been demonstrated and involves the enzymatic addition of monosaccharide residues tagged with the biological probe of interest. The pioneering work in this area involved the use of sialyltransferases.[5] Later, fucosyltransferases were used for the same purpose, the strategy being summarized in Figure 1.[3] The advantage of using fucosyltransferases is that they can transfer to several oligosaccharide sequences that are already sialylated.

We report here an important simplification of our earlier strategy for labeling cell-surface sugar chains using fucosyltransferases.[3] A GDP-fucose derivative labeled at C-6

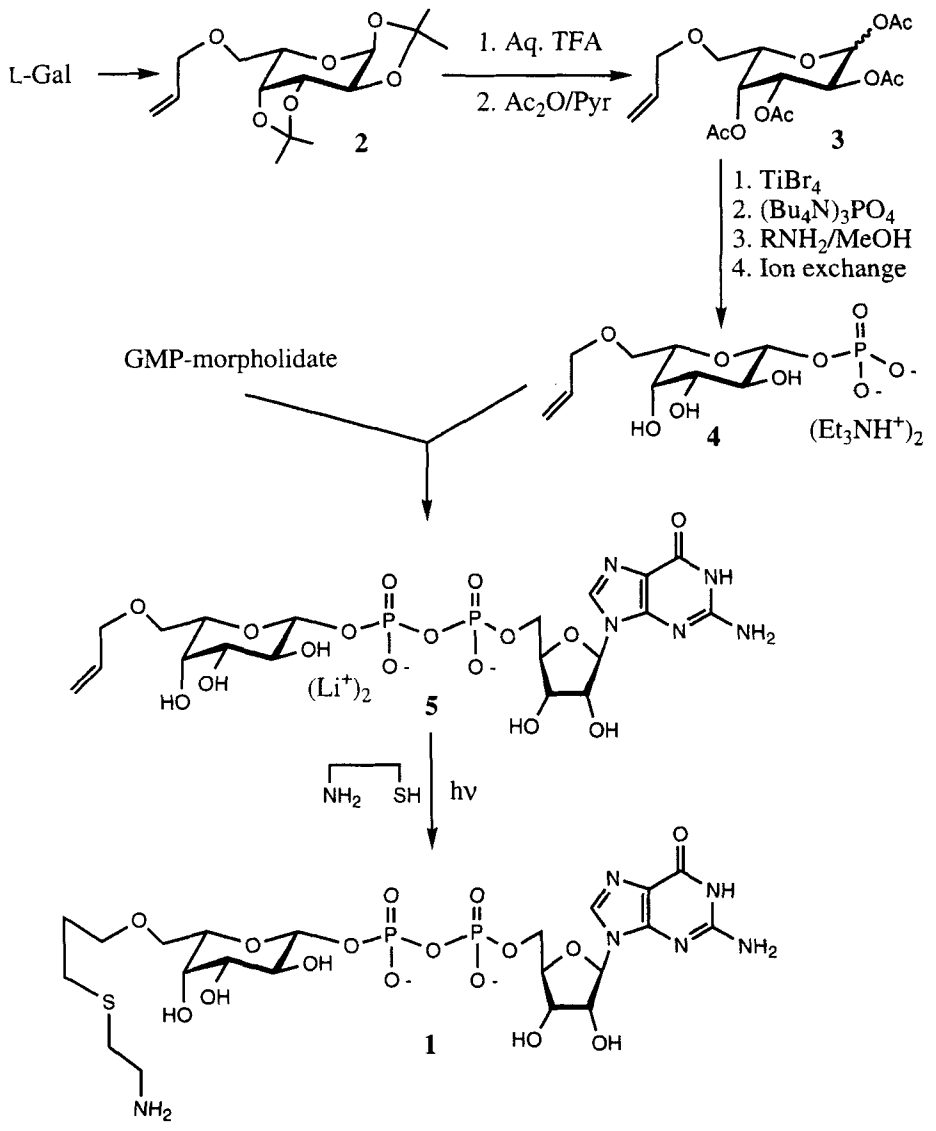
with the biological probe of interest is essential in this approach (Figure 1). However, the synthesis of such probes was very cumbersome and many chemical steps would need to be repeated for each new probe. In the present work, we prepare a single GDP-fucose analog, the aminated derivative (**1**), and we demonstrate that complex molecules possessing either an activated carboxyl-group or another amine can be added to **1** in simple high-yielding steps. Furthermore, the GDP-fucose derivatives prepared in this manner are shown to be enzymatically active.

RESULTS AND DISCUSSION

The synthesis of the GDP-fucose analog **1** was straightforward using chemistry well established in the D-galactose series. Briefly, acetonation of L-galactose gave the 1,2:3,4-diacetonide[6] which was then 6-*O*-allylated to give **2**. Hydrolysis of the isopropylidene group in **2**, followed by acetylation gave **3**. Attempted conversion of **3** to the glycosyl bromide with HBr failed, but was successful using $TiBr_4$. Reaction of the resulting glycosyl bromide (not isolated) with tetrabutylammonium phosphate, followed by de-*O*-acetylation, gave the required 6-*O*-allyl-β-L-galactopyranosyl phosphate **4**. Conventional coupling of **4** with GMP-morpholidate[7] yielded the sugar nucleotide **5**. Reaction of **5** with cysteamine (2-aminothioethanol) under UV-irradiation gave the target **1**.

Compound **1** contained the required 6'-amino group which allowed further very simple derivatization. The simplest derivatization methods are reaction with an activated ester and coupling with another amine using a heterobifunctional reagent. To demonstrate that **1** could be derivatized as expected, it was treated with a water soluble *N*-hydroxysuccinimide activated ester of biotin (**6**) which resulted in the production of **7**. To show that **1** could be coupled to other amines, we selected the method of Tietze and Arlt,[7] using commercial diethyl squarate, which shows extraordinary promise as a reagent for the very simple coupling of two amines. The blood-group-A active trisaccharide, as its synthetic 8-methoxycarbonyloctyl glycoside **8**,[9] was first treated with ethylenediamine to produce the required amine derivative **9**. Reaction of **9** with diethyl squarate gave the monoamide **10** which was further reacted with **1** to give the desired A-blood-group-tagged-GDP-Fuc derivative **11** (Scheme 2).

Finally, both of the GDP-Fuc derivatives **7** and **11** proved to be active as donors for a partially purified preparation of fucosyltransferase from human milk.[10] For demonstration purposes, incubations were performed using the *N*-acetyllactosamine derivative **12** which we use as a model for the corresponding terminal oligosaccharide of cell-surface glycoconjugates. Enzymatic glycosylation of **12** with either **7** or **11** as the donor produced the expected tagged oligosaccharides **13** and **14** which were characterized

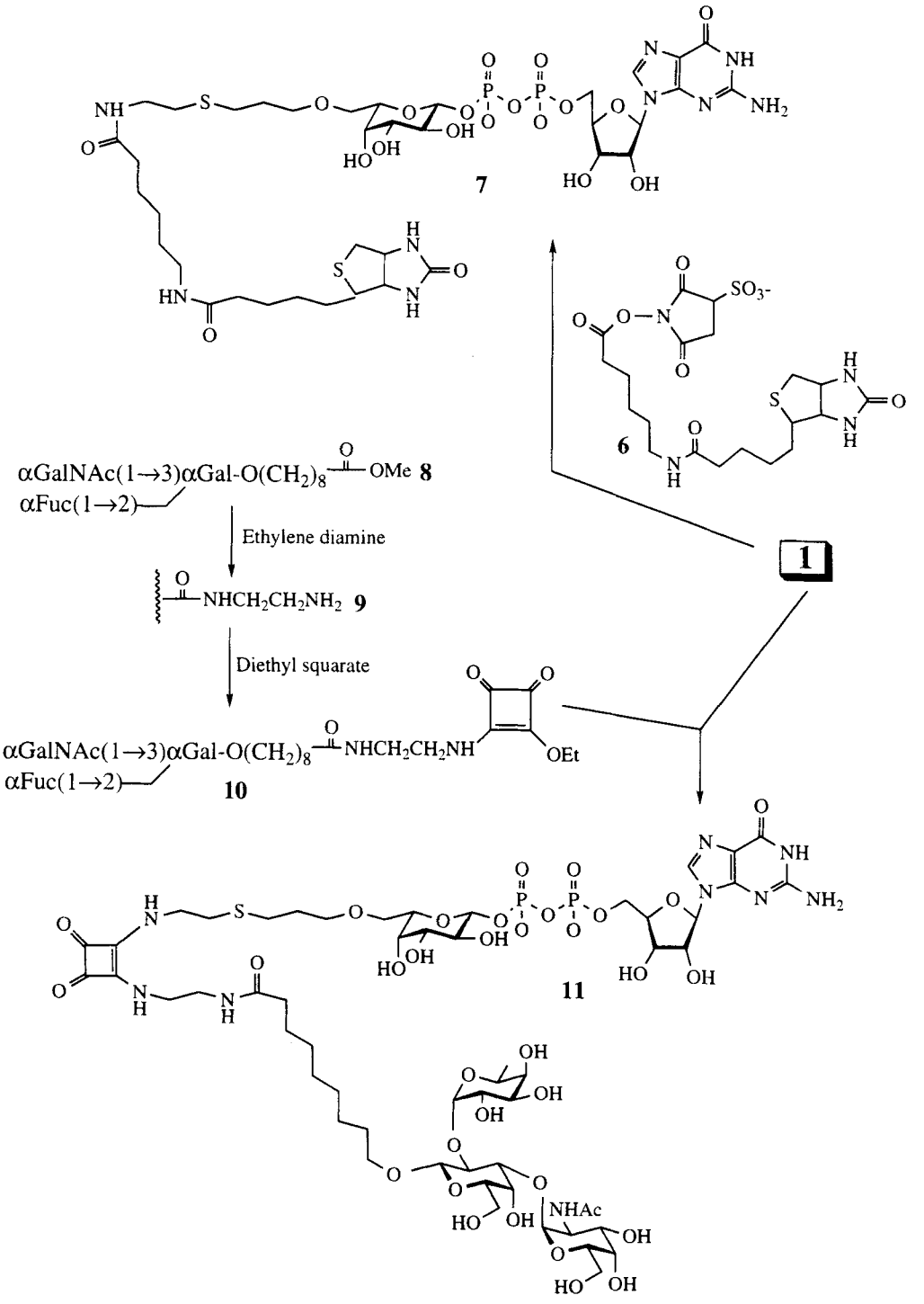


Scheme 1

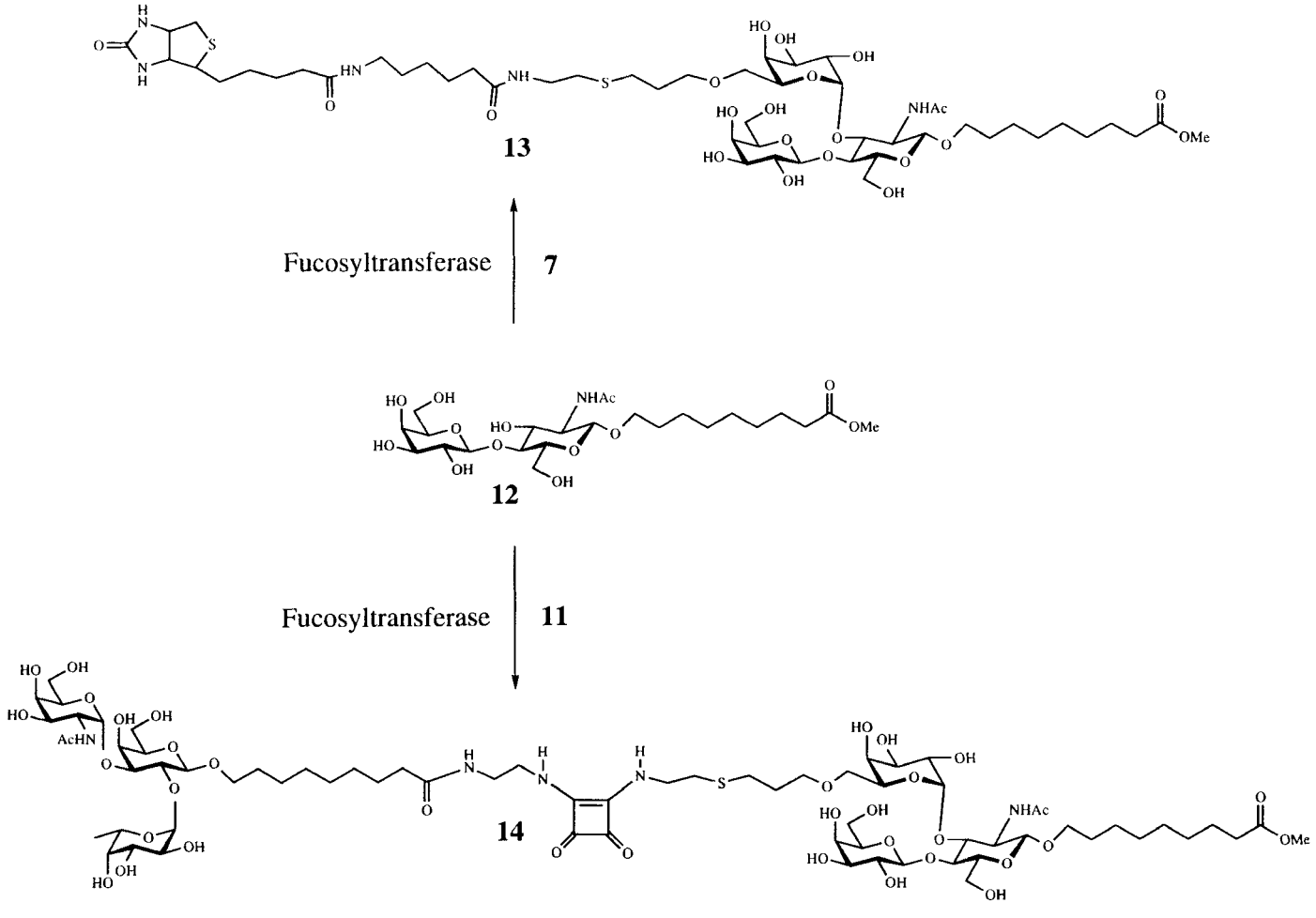
by ^{1}H NMR spectroscopy. The molecular weights of **13** and **14** were confirmed using MALDI-TOF mass spectrometry. The enzyme specificity requires the addition to occur at OH-3 of the GlcNAc residue as shown.

CONCLUSION

In summary, the GDP-fucose derivative **1**, reported in this work, is shown to react in a predictable way with active esters and amino-containing molecules to produce



Scheme 2

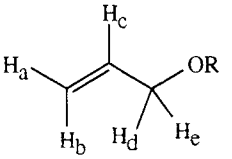


Scheme 3

derivatives that can be transferred enzymatically to oligosaccharide chains. This means that, in principle, any molecule containing an activatable carboxyl group, or a free amino group, can be covalently added to the carbohydrate chains of cell-surface glycoconjugates in a very simple and mild manner. Several such experiments are in progress.

EXPERIMENTAL

General methods. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 22 ± 2 °C. Analytical TLC was performed on Silica Gel 60-F_{254} (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with 8% sulfuric acid. The solvent systems used have been designated by letters: A, 3:1 toluene-ethyl acetate; B, 7:3:1 2-propanol-water-concd ammonia; C, 60:35:6 chloroform-methanol-water. All commercial reagents were used as supplied and chromatography solvents were distilled prior to use. Column chromatography was performed on Silica Gel 60 (E. Merck 40-60 μM, Darmstadt). C-18 Silica Gel was from Toronto Research Chemicals Inc. (35-70 μM), Millex-GV (0.22 μM) filter units were from Millipore (Missisuaga, ON), C-18 and QMA Sep-Pak sample preparation cartridges were from Waters Associates (Missisuaga, ON). The biotin active ester **6** was from Pierce (Rockford, IL). ^1H NMR spectra were recorded at 360 MHz (Bruker WM-360), 300 MHz (Bruker AM-300) or 500 MHz (Varian Unity 500) with either internal $(CH_3)_4Si$ (δ 0, $CDCl_3$, CD_3OD) or DOH (δ 4.80, D_2O). ^{13}C NMR spectra were recorded at 75.5 MHz (Bruker AM-300) with internal $(CH_3)_4Si$ (δ 0, $CDCl_3$, CD_3OD) or external acetone (δ 31.0, D_2O). ^{31}P NMR spectra were recorded at 81.0 MHz (Bruker WH-200) or 162.0 MHz (Bruker WH-400) with external H_3PO_4 (δ 0, $CDCl_3$, CD_3OD, D_2O). ^1H NMR data are reported as though they were first order. Protons in the 8-methoxycarbonyloctyl (MCO) spacer are designated H-1 to H-8 with increasing distance from the anomeric center and protons of the allyl group are designated as defined below.



Unless otherwise stated, all reactions were carried out at room temperature. In the processing of reaction mixtures, solutions of organic solvents were washed with half their volumes of aqueous solutions. Organic solutions were dried (sodium sulfate) prior to concentration under vacuum at < 40 °C (bath). Microanalyses were carried out by the analytical services at this department and all samples submitted for elemental analyses were dried overnight under vacuum with phosphorous pentoxide at 56 °C (refluxing acetone).

Matrix assisted laser desorption ionization/time-of-flight (MALDI-TOF) mass spectra were recorded on a home-built instrument using a nitrogen laser and 2,5-dihydroxybenzoic acid as the matrix. The masses recorded have an accuracy of better than ±1 amu.

6-*O*-Allyl-1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (2). A solution of 1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (4.25 g, 16.3 mmol) and allyl bromide (1.7 mL, 19.6 mmol) in *N,N*-dimethylformamide (10 mL) was added dropwise to a vigorously stirred mixture of sodium hydride (735 mg, 24.5 mmol) in *N,N*-dimethylformamide (5 mL). After 30 min, methanol (5 mL) was added and the reaction mixture was stirred for an additional 5 min. The reaction mixture was partitioned between toluene and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated. Column chromatography (8:1 hexane-ethyl acetate) gave **2** (4.51 g, 92 %): $[\alpha]_{578}$ +72° (*c* 1.0, chloroform); R_f 0.61 (system A); ^{1}H NMR ($CDCl_3$) δ 1.33, 1.35, 1.45, 1.55 (s, 3 H each, 4 x CH_3), 3.59 (dd, 1 H, $J_{5,6a}$ = 6.8 Hz, $J_{6a,6b}$ = 10.1 Hz, H-6a), 3.66 (dd, 1 H, $J_{5,6b}$ = 5.8 Hz, H-6b), 3.98 (ddd, 1 H, $J_{4,5}$ = 1.9 Hz, H-5) 4.05 (dddd, 2H, $J_{Hd,He}$ 13.5 Hz, $J_{Hc,Hd} = J_{Hc,He}$ = 6.0 Hz, $J_{Ha,Hd} = J_{Hb,Hd} = J_{Ha,He} = J_{Hb,He}$ = 1.5, H_d, H_e), 4.27 (dd, 1 H, $J_{3,4}$ = 7.9 Hz, H-4), 4.31 (dd, 1 H, $J_{1,2}$ = 5.2 Hz, $J_{2,3}$ = 2.5 Hz, H-2), 4.61 (dd, 1 H, H-3), 5.18 (dddd, 1 H, $J_{Ha,Hc}$ = 10.5 Hz, $J_{Ha,Hb}$ = 1.4 Hz, H_a), 5.28 (dddd, 1 H, $J_{Hb,Hc}$ = 17.3 Hz, H_b), 5.55 (dd, 1 H, H-1), 5.93 (dddd, 1H, H_c).

Anal. Calcd for $C_{15}H_{24}O_6$: C, 60.0; H, 8.0. Found: C, 60.1; H, 8.1.

6-*O*-Allyl-1,2,3,4-tetra-*O*-acetyl-α,β-L-galactopyranose (3). Compound **2** (3.15 g, 10.5 mmol) was treated with 90% aqueous trifluoroacetic acid (100 mL) for 15 min and then concentrated. The aliquot was coevaporated three times with toluene (100 mL), dissolved in pyridine (75 mL) and treated with acetic anhydride (25 mL) at 60 °C for one h and then concentrated. Column chromatography (6:1 toluene-ethyl acetate) gave **3** (1.82 g, 45 %) as a mixture of anomers: R_f 0.38 (system A); ^{1}H NMR ($CDCl_3$) δ 2.00, 2.01, 2.02, 2.04, 2.11, 2.15, 2.16 (s, 3 H each, $COC\underline{H}_3$), 5.71 (d, 1 H, $J_{1,2}$ = 8.2 Hz, H-1β), 5.82 (dddd, 1 H, Hc), 6.39 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1α).

Anal. Calcd for $C_{17}H_{24}O_{10}$: C, 52.6; H, 6.2. Found: C, 52.8; H, 6.2.

Bis(triethylammonium) 6-*O*-Allyl-β-L-galactopyranos-1-yl Phosphate (4). Titanium tetrabromide (3.2 g, 8.8 mmol) was added to a solution of **3** (1.14 g, 2.93 mmol) in dichloromethane-ethyl acetate (10:1, 50 mL) and the reaction mixture was stirred at room temperature for 48 h. Sodium acetate (1 g) was then added, and stirring was continued for 15 min. The mixture was filtered through Celite, diluted with dichloromethane and washed with ice cold water, dried and concentrated. The residue was dissolved in toluene (30 mL) and added to a stirred mixture of tetrabutylammonium phosphate (2.4 g, 6 mmol) in acetonitrile (60 mL) containing ground molecular sieves (4Å). The reaction was stirred an additional 4 h and then concentrated. The residue was

dissolved in water, washed with dichloromethane, concentrated, redissolved in water and passed through Dowex 50W-X8 (NH_4^+ form) resin. The carbohydrate containing fractions were pooled, concentrated, redissolved in a minimum amount of water, loaded on a C-18 Silica-gel column (1.5x36 cm). The column was eluted with water with a flow of 1 mL/min, 7 mL fractions were collected and those fractions containing carbohydrate but not inorganic phosphate (determined by a molybdate-color assay)[11] were pooled and lyophilized. The residue was dissolved in methanol-cyclohexylamine (1:1, 60 mL) and refluxed until TLC (system B) indicated complete conversion to a slower moving product. (2 h, R_f 0.75→0.68, system B). The solution was concentrated and partitioned between water and dichloromethane. The water phase was concentrated, redissolved in water and passed through Dowex 50W-X8 (Et_3NH^+ form) resin. The carbohydrate containing fractions were pooled and lyophilized to give **4** (418 mg, 28%) as a colorless powder: R_f 0.68 (system B); ^1H NMR (D_2O, 400 MHz) δ 1. 28 (t, 18 H, 7.3 Hz, Et_3N), 3.19 (q, 12 H, Et_3N), 3.55 (dd, 1 H, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.65-3.77 (m, 3 H, H-3, H-6a, H-6b), 3.86 (ddd, 1 H, H-5), 3.89 (dd, 1 H, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ <1 Hz, H-4), 4.09 (dddd, 2H, $J_{Hd,He}$ 13.5 Hz, $J_{Hc,Hd} = J_{Hc,He}$ = 6.0 Hz, $J_{Ha,Hd} = J_{Hb,Hd} = J_{Ha,He} = J_{Hb,He}$ = 1.5 Hz, H_d, H_e), 4.85 (dd, 1 H, $J_{1,P}$ = 7.8 Hz, H-1), 5.28 (dddd, 1 H, $J_{Ha,Hc}$ = 10.5 Hz, $J_{Ha,Hb}$ = 1.4 Hz, H_a), 5.35 (dddd, 1 H, $J_{Hb,Hc}$ = 17.2 Hz, H_b), 5.95 (dddd, 1H, H_c); ^{31}P NMR (D_2O; 162.0 MHz) δ 1.5

Dilithium 6-*O*-Allyl-β-L-galactopyranos-1-yl Guanosine 5'-Diphosphate (5). Compound **4** (418 mg, 832 μmol) was dissolved in dry pyridine (10 mL) and added to a suspension of guanosine 5'-monophosphomorpholidate 4-morpholine-*N*,*N*'-dicyclohexylcarboxamidine salt (604 mg, 832 μmol, Sigma). The activated nucleoside monophosphate and **4** were previously dried by coevaporating with dry pyridine three times. The mixture was coevaporated with pyridine one more time, then dissolved (suspended) in dry pyridine and vigorously stirred for 3.5 d. The mixture was concentrated, dissolved in a minimum amount of water and loaded onto a Dowex 1X2-200 (Cl^- form, 2.5x18 cm) column. The column was eluted with a linear LiCl gradient (0 → 0.5 M) made of water (800 mL) and of 0.5 M LiCl (800 mL) at 2 mL/min and 20 mL fractions were collected. The appropriate fractions (eluted at 0.4-0.5 M) were pooled, concentrated and desalted on a Biogel P-2 column (3x90 cm) to give **5** (226.4 mg, 41.4%) as a white powder: R_f 0.60 (system B); ^1H NMR (D_2O, 400 MHz) δ 3.62 (dd, 1 H, $J_{1'',2''}$ = 7.5 Hz, $J_{2'',3''}$ = 10.0 Hz, H-2"), 3.67-3.75 (m, 3 H, H-2", H-6"), 3.86 (ddd, 1 H, H-5"), 3.91 (dd, 1 H, $J_{3'',4''}$ = 3.3 Hz, $J_{4'',5''}$ = 0.8 Hz, H-4"), 4.06 (dddd, 2H, $J_{Hd,He}$ 13.5 Hz, $J_{Hc,Hd} = J_{Hc,He}$ = 5.9 Hz, $J_{Ha,Hd} = J_{Hb,Hd} = J_{Ha,He} = J_{Hb,He}$ = 1.5 Hz, H_d, H_e), 4.23 (dd, 2H, $J_{4',5a'}$ = 3.6 Hz, $J_{4',5b'}$ = 5.4 Hz, H-5'), 4.37 (ddd, 1 H, $J_{3',4'}$ = 3.1 Hz, H-4'), 4.55 (dd, 1H, $J_{2',3'}$ = 5.2 Hz, H-3'), 4.91 (dd, 1 H, $J_{1'',P}$ = 8.4 Hz, H-

1"), 5.26 (dddd, 1 H, $J_{Ha,Hc}$ = 10.5 Hz, $J_{Ha,Hb}$ = 1.6 Hz, H_a), 5.33 (dddd, 1 H, $J_{Hb,Hc}$ = 17.3 Hz, H_b), 5.93 (dddd, 1H, H_c), 5.94 (d, 1 H, $J_{1',2'}$ = 7.2 Hz, H-1'), 8.12 (s, 1H, H-8 guanosine); ^{31}P NMR (D_2O; 81.015 MHz) δ -12.6 (d, $^3J_{P,H}$ 20.6 Hz, Fuc-P), -10.7 (d, Rib-P).

Disodium 6-*O*-3-(*S*-2-Aminothioethyl)-propyl-β-L-galactopyranos-1-yl Guanosine 5'-Diphosphate (1). Cysteamine (24 mg, 210 μmol) was added to a solution of **5** (14.1 mg, 21.4 μmol) in water (3 mL) and irradiated for 2 h in a quartz vial using a Hanovia UV-lamp (R_f 0.60→0.36 system B). The reaction mixture was passed through a Dowex 50W-X8 (Na^+ form) resin and the appropriate fractions were collected, concentrated, redissolved in a minimum amount of water and desalted on a Biogel P-2 column (3x90 cm) to give **1** (11.8 mg, 69%): R_f 0.36 (system B); ^{1}H NMR (D_2O, 300 MHz) δ 1.83 (quintet, 2 H, $NCH_2C\underline{H}_2CH_2S$), 2.63 (t, 2 H, CH_2), 2.84 (t, 2 H, CH_2), 3.22 (t, 2 H, CH_2), 3.58-3.77 (m, 4 H, H-2", H-3", H-5", H-6"), 3.86 (ddd, 2 H, H-5'"), 3.91 (dd, 1 H, $J_{3",4"}$ = 3.4 Hz, $J_{4",5"}$ <1 Hz, H-4"), 4.24 (dd, 2 H, $J_{4',5'}$ = 3.5 Hz, $J_{4',5'}$ = 5.2 Hz, H-5'), 4.37 (ddd, 1 H, H-4'), 4.54 (dd, 1 H, $J_{2',3'}$ = 5.0 Hz, $J_{3',4'}$ = 3.4 Hz, H-3'), 4.98 (dd, 1 H, $J_{1",2"} = J_{1",P}$ = 7.5 Hz, H-1"), 5.95 (d, 1H, $J_{1',2'}$ = 6.1 Hz, H-1'), 8.13 (s, 1 H, H-8 guanosine).

Disodium 6-*O*-3-[*S*-2-{6-(Biotinamido) Hexanoate} Amidothioethyl]-propyl-β-L-galactopyranos-1-yl Guanosine 5'-Diphosphate (7). Sulfosuccinimidyl 6-(biotinamido) hexanoate (**6**, 6.6 mg, 7.3 μmol) was added to a stirred solution of **1** (8.6 mg, 6.6 μmol) in saturated aqueous sodium hydrogencarbonate (0.5 mL, pH 9.45) and stirred overnight (R_f 0.36→0.31 system B). The reaction mixture was loaded directly on a Biogel P-2 column (3x90 cm) and gave **7** (9.1 mg, 80%): R_f 0.31 (system B); ^{1}H NMR (D_2O, 360 MHz) δ 1.84 (q, 2H, 7.3 Hz), 2.19 (t, 2 H, 7.5 Hz), 2.26 (t, 2 H, 7.4 Hz), 3.60-3.75 (m, 3 H, H-2", H-3", H-6"a, H-6"b), 3.86 (ddd, 1 H, H-5"), 3.90 (dd, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ < 1 Hz, H-4"), 4.22 (dd, 2 H, H-5'), 4.36 (ddd, 1 H, H-4'), 4.44 (dd, 1 H, J = 7.9 Hz, J = 4.4 Hz), 4.54 (dd, 1 H, $J_{2',3'}$ = 5.1 Hz, $J_{3',4'}$ = 3.2 Hz, H-3'), 4.62 (dd, 1 H, J = 4.4 Hz, J = 7.8 Hz), 4.96 (dd, 1 H, $J_{1",2"} = J_{1",P}$ = 7.7 Hz, H-1"), 5.94 (d, 1 H, $J_{1',2'}$ = 6.2 Hz, H-1'), 8.12 (s, 1 H, H-8 guanosine).

8-(*N*-2-Aminoethyl)-carboxamidooctyl 3-*O*-(2-Acetamido-2-deoxy-α-D-galactopyranosyl)-[2-*O*-(α-L-fucopyranosyl)-β-D-galactopyranoside (9). Trisaccharide ethyl ester **8**[9] (57.4 mg, 80.4 mmol) was treated with ethylenediamine (2 mL) at 70 °C for 2 d (R_f 0.64→0.45 system B). The solution was diluted with water (20 mL), cooled to room temperature and loaded onto two consecutive Sep-Pak C-18 cartridges. The cartridges were washed with water (20 mL) and **9** was eluted with methanol (20 mL), concentrated, redissolved in water (7 mL), and filtered through a Millipore filter. The washings were loaded onto another Sep-Pac cartridge and taken

through the same cycle of washing, elution and filtering. Lyophilization of both fractions gave **9** (57.6 mg, 98%) as a white powder: R_f 0.45 (system B); ^{1}H NMR (D_2O, 360 MHz) δ 1.24 (d, 1 H, $J_{5,6}$ = 6.6 Hz, H-6 fucose), 1.33 (m, 8 H, H-3 to H-6, MCO), 1.62 (m, 4 H, H-2, H-7 MCO), 2.06 (s, 3 H, acetamido CH_3), 2.28 (t, 2 H, H-8 MCO), 2.89 (t, 2 H, $C\underline{H}_2NHCO$), 3.35 (t, 2 H, $C\underline{H}_2NH_2$), 4.49 (q, 1 H, $J_{4,5}$ < 1 Hz, H-5 fucose), 4.55 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1 Gal), 5.19 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-1 GalNAc†), 5.34 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1 Fuc†); † may be interchanged.

Squaric acid coupling of 9 and 1 to give 11. 3,4-Diethoxy-3-cyclobutene-1,2-dione (diethyl squarate, Aldrich, 1.88 μL, 13.5 μmol) was added to a stirred mixture of **8** (10.3 mg, 14.1 μmol) in ethanol (1 mL). After 3 h, TLC showed complete conversion to a faster moving spot (presumably **10**, R_f 0.05→0.73 system C). Compound **1** (9.0 mg, 11.2 μmol) dissolved in 1 mL of 0.1 M sodium bicarbonate buffer (pH 9.5) was added and stirring was continued for 16 h. The solution was diluted with water (5 mL) and loaded onto a Sep-Pak QMA cartridge. The cartridge was washed with water (7 mL), then the product was eluted with 3M sodium chloride solution (10 mL). The eluent was loaded onto a Sep-Pak C-18 cartridge and the cartridge was washed with water (10 mL) and **11** was eluted with methanol (10 mL), concentrated, redissolved in water (7 mL), and filtered through a Millipore filter. Lyophilization gave **11** (3.7 mg, 98%) as a white powder: R_f 0.40 (system B); ^{1}H NMR (D_2O, 360 MHz) δ 1.23 (d, 1 H, $J_{5,6}$ = 6.5 Hz, H-6 fucose), 1.26 (m, 8 H, H-3 to H-6 MCO), 1.49 (m, 2 H, H-2 MCO), 1.58 (quintet, 2 H, H-7 MCO), 1.82 (quintet, 2 H, $NCH_2C\underline{H}_2CH_2O$), 2.05 (s, 3 H, acetamido CH_3), 2.19 (t, 2 H, H-8 MCO), 2.63 (t, 2 H, CH_2), 2.80 (t, 2 H, CH_2), 3.42 (t, 2 H, CH_2), 4.35 (ddd, 1 H, H-4 ribose), 4.47 (q, 1 H, $J_{4,5}$ < 1 Hz, H-5 fucose), 4.51-4.54 (m, 2 H, H-1 D-Gal, H-3 Rib), 4.97 (dd, 1 H, $J_{1,2}$ = $J_{1,P}$ = 7.8 Hz, H-1 L-Gal), 5.18 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1 GalNAc†), 5.33 (d, 1 H, $J_{1,2}$ = 3.7 Hz, H-1 Fuc†), 5.92 (d, 1 H, $J_{1,2}$ = 6.2 Hz, H-1 Rib), 8.11 (s, 1 H, H-8 guanosine); † may be interchanged

Enzymatic Synthesis of 13 Using 7 as the Donor. α-3/4-Fucosyltransferase[10] (100 μL, 200 mU/mL) was added to a solution of LacNAc-MCO (**12**) (0.49 mg, 0.89 μmol) and **7** (0.59 mg, 0.60 μmol) in 440 μL buffer (20 mM HEPES, 20 mM $MnCl_2$, 0.2 % BSA, pH = 7) and incubated at 37 °C for 10 h (R_f 0.52→0.45 system C). Extra donor (1.00 mg) and extra enzyme (50 μL) were added and the solution was incubated for an additional 10 h. The solution was diluted with water (3 mL) and loaded onto a Sep-Pak C-18 cartridge, the cartridge was washed with water (30 mL) and **13** was eluted with methanol (10 mL), concentrated, redissolved in water (7 mL), and filtered through a Millipore filter. Lyophilization gave **13** (0.6 mg, 65%) as a white powder: R_f 0.45 (system C); ^{1}H NMR (D_2O, 360 MHz) δ 5.218 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1 Fuc), 2.030 (s, 3H, NHAc). MALDI-TOF MS: M + Na^+ obs. = 1195.4 (calcd $C_{51}H_{89}O_{21}N_5S_2 + Na^+$ = 1194.6).

Enzymatic Synthesis of 14 Using 11 as the Donor. α-3/4-Fucosyltransferase[10] (100 μL, 200 mU/mL) was added to a solution of LacNAc-MCO (**12**) (0.47 mg, 0.85 μmol) and **11** (0.90 mg, 0.60 μmol) in 440 μL buffer (20 mM HEPES, 20 mM $MnCl_2$, 0.2 % BSA, pH = 7) and incubated at 37 °C for 16 h (R_f 0.52→0.35 system C). Extra donor (1.00 mg, 0.94 μmol) and extra enzyme (50 μL) were added and the solution was incubated for an additional 16 h. The solution was diluted with water (3 mL) and loaded onto a Sep-Pak C-18 cartridge. The cartridge was washed with water (30 mL) and **14** was eluted with methanol (10 mL), concentrated, redissolved in water (7 mL), and filtered through a Millipore filter. Lyophilization gave **14** (0.9 mg, 65%) as a white powder: R_f 0.35 (system C); ^1H NMR (D_2O, 500 MHz) δ 5.323, 5.225 and 5.175 (each d, 1H, $J_{1,2}$ = 4.0 Hz, H-1α), 4.530 ($J_{1,2}$ = 7.9 Hz), 4.472 ($J_{1,2}$ = 7.0 Hz) and 4.457 ($J_{1,2}$ = 7.8 Hz) (each H-1 β), 2.044 and 2.031 (each s, 3H, NHAc). MALDI-TOF MS: M + Na^+ obs. = 1660.3 (calcd $C_{70}H_{119}O_{36}N_5S$ + Na^+ = 1660.7).

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