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Chemoenzymatic synthesis of the sialyl Lewis X glycan and its derivatives

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Abstract

A combination of recombinant FKP and α-(1→3)-fucosyltransferase allows the facile synthesis of the sialyl Lewis X tetrasaccharide glycan and its derivatives in excellent yield. In this system, the universal fucosyl donor, guanidine 5′-diphosphate-β-L-fucose (GDP-fucose), or its analogues can be generated *in situ* by cofactor recycling using pyruvate kinase.

Keywords

Chemoenzymatic synthesis; transferases; fucose; sialic acid

1. Introduction

The kaleidoscopic functions of cell-surface glycans are governed by their diverse structures and their dynamic interactions with glycan-binding proteins (i.e., lectins).[1] Multivalent glycan–lectin interactions either convey changes in the extracellular environment or modulate cell–cell/cell–pathogen communications. For example, sialyl Lewis X (sLe^x), a tetrasaccharide glycan constitutively expressed on leukocytes, mediates the recruitment of these cells from the bloodstream into surrounding tissues during the early stage of inflammation.[2-4] SLe^x binds to E- and P-selectins, upregulated on the surface of endothelial cells, leading to leukocyte tethering and extravasation.[5] In addition, the sLe^x–E-selectin interaction is also crucial for directing the migration of hematopoietic stem cells through the blood, across the endothelial vasculature to different organs and to their bone marrow niches, the first and essential step in clinical stem cell transplantation.[6]

As revealed by NMR spectroscopyand X-ray crystallography, the Le^x domain of the sLe^x tetrasaccharide assumes a very rigid structure, displaying two surfaces along the NeuAc-Gal-

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Fuc axis of opposite hydrophility.[7] The stability of this highly compact, bipolar structure stems from the stacking of its fucose ring on top of the galactose residue with its exocyclic C-5 methyl group forming key van der Waals contacts with the hydrophobic surface of the galactose. Studies have shown the formation of this stack to be important in the binding of sLe^x to target lectins. Removal of the methyl group leads to a five-fold decrease in binding affinity of sLe^x to its target protein, E-selectin.[8]

In inflammatory diseases (i.e., rheumatoid arthritis, asthma and transplant rejection), the body's immune system inappropriately triggers an inflammatory signal and causes damage to its own tissues. One approach to treat these diseases is to disrupt the recruitment of leukocytes, a process mediated by sLe^x–selectin interactions. The possibility that inhibitors of selectin-mediated cell adhesion could serve as broad-spectrum anti-inflammatory agents has sparked significant efforts in both the pharmaceutical industry and academic laboratories to design sLe^x-based small-molecule inhibitors as selectin antagonists.[5] In order to evaluate the therapeutic value of sLe^x-based selectin antagonists, it is necessary to develop efficient methods for the synthesis of sLe^x and its derivatives bearing unnatural functionalities in each of the monosaccharide building blocks for structure–activity relationship studies.

Chemical syntheses of sLe^x have been pursued intensively,[5, 9] and have permitted the elucidation of the key functional groups and structural features that contribute to the sLe^x–selectin interaction. Despite recent advances in glycosylation methodologies, the chemical synthesis of complex fucosides is still complicated by tedious protecting group manipulations, and the use of harsh reagents and stringent anhydrous conditions.[10] This problem has been elegantly addressed by several groups using alternative approaches based on enzymatic glycosylation. Pioneering studies on the chemoenzymatic synthesis of the sLe^x tetrasaccharide were performed by Wong and co-workers who employed a recombinant human α-(1→3)-fucosyltransferase produced in eukaryotic cells.[7, 11] In this process, the universal fucosyl donor, guanidine 5′-diphosphate-β-L-fucose (GDP-fucose), was generated from mannose-1-phosphate (Man-1-P) via the combination of three enzymes: GDP-mannose pyrophosphorylase, GDP-mannose 4,6-dehydrase (GMD) and GDP-keto-6-deoxymannose 3,5-epimerase/4-reductase (GMER). The latter two enzymes are found in the *de novo* biosynthetic pathway of GDP-fucose.[12] The human α-(1→3)-fucosyltransferase, being a type II transmembrane glycoprotein, is difficult to produce in large quantities. Additionally, Man-1-P, although commercially available, is prohibitively expensive for large-scale synthesis.

We recently reported a facile and cost-effective method for the chemoenzymatic synthesis of GDP-fucose and Le^x derivatives.[13] This method exploits FKP (L-fucokinase/GDP-fucose pyrophosphorylase), a bifunctional enzyme isolated from *Bacteroides fragilis* 9343, which converts L-fucose to fucose-1-phosphate (Fuc-1-P) and thence to GDP-fucose.[14, 15] This transformation is found in the salvage pathway of *B. fragilis* 9343 GDP-fucose production and is conserved in all *Bacteroides* species. We found that a His_6-tagged recombinant FKP, expressed in *E. coli*, has relaxed specificity toward fucose analogues bearing unnatural substituents at the C-5 position and is capable of generating GDP-fucose derivatives *in vitro* with high efficiency. Furthermore, we demonstrated that the activities of FKP can be combined with a *Helicobacter pylori* α-(1→3)-fucosyltransferase for preparative-scale syntheses of Le^x trisaccharide glycans and its structurally related derivatives.[13] Herein, we report a novel chemoenzymatic method for the synthesis of the sLe^x tetrasaccharide glycan and its derivatives on a preparative scale using the recombinant FKP and the α-(1→3)-fucosyltransferase (Scheme 1). Importantly, this approach regiospecifically incorporates fucose or its synthetic analogues to the acceptor glycan sialyl *N*-acetyllactosamine (sLacNAc). Moreover, we demonstrate that the atom economy of this synthetic process can be improved by simply introducing a biologically inspired cofactor recycling system, in which both ATP and GTP are formed *in situ* using a commercially available pyruvate kinase.

2. Results and discussion

Our synthetic route is based on previous reports that bacterial fucosyltransferases act efficiently on sialylated glycans with good activity.[16] To confirm the activity of the recombinant *H. pylori* α-(1→3)-fucosyltransferase toward sLacNAc, we prepared this acceptor trisaccharide glycan using the chemoenzymatic approach developed by Chen and co-workers for synthesizing α-(2→3)-linked sialosides.[17] In this method, sialic acid or its analogues are converted to the corresponding sialylated trisaccharides in a one-pot reaction using a combination of two enzymes: CMP-sialic acid synthetase and α-(2→3)-sialyltransferase. We cloned a *Neisseria meningitides* CMP-sialic acid synthetase[18] and a *Pasteurella multocida* α-(2→3)-sialyltransferase[19] and expressed them in *E. coli*. With these recombinant enzymes in hand, we successfully synthesized sLacNAc using the system shown in Scheme 1, in which CMP-sialic acid was generated *in situ* by the *N. meningitides* CMP-sialic acid synthetase.

To compare the activity of the α-(1→3)-fucosyltransferase toward LacNAc and sLacNAc, we measured the k_{cat} and K_M of the fucosylation reaction for both acceptor substrates using a coupled enzyme assay (Supplementary Data). Both of these substrates bear a short 2-azidoethyl spacer that allows fast copper-free click modification via azide–alkyne cycloaddition[20] to fabricate glycan microarrays for high-throughput screening of sLe^x–lectin interactions.[21] To our satisfaction, sialylation of the LacNac afforded a 50% increase in turnover number of the fucosyltransferase (Table 1). However, the incorporation of the bulky and charged sialic acid residue hampered the formation of the enzyme–substrate complex. The K_M value determined for 2-azidoethyl sLacNAc was 11-fold higher than that determined for 2-azidoethyl LacNAc.

After we confirmed the activity of the recombinant α-(1→3)-fucosyltransferase toward sLacNAc, the stage was set for the one-pot synthesis of the sLe^x tetrasaccharides. This task was accomplished by combining a fucose analogue and the acceptor trisaccharide, 2-azidoethyl sLacNAc with the recombinant FKP and α-(1→3)-fucosyltransferase. An inorganic pyrophosphatase (Sigma) was included to hydrolyze the pyrophosphate byproduct generated in the reaction and to drive the reaction to completion. Using this method, we synthesized a series of sLe^x tretrasaccharide derivatives (**7**–**12**, 17–22 mg each) bearing a variety of substituents at the fucose C-5 position (Table 2). SLe^x derivative **3** was functionalized with an alkynyl group that could enhance the van der Waals contacts between the fucose residue and the galactose. Fucose analogues **4**, **5** and **6** were modified with fluoride, hydroxyl and methoxyl groups, respectively. These groups might form additional hydrogen bonds with the neighboring galactose, thereby enforcing different conformational constraints on the final sLe^x tetrasaccharides. The resulting sLe^x derivatives bearing these functionalities will be valuable tools for probing the influence of the conformational constraints in sLe^x–selectin binding. The unnatural tetrasaccharide glycans in Table 2 were typically isolated in yields greater than 75%. In a few cases nearly quantitative formation of the desired tetrasaccharide was achieved.

Though this chemoenzymatic process is straightforward and highly efficient, the generation of one molecule of sLe^x product is accompanied by the formation of an equal amount of ADP and GDP byproducts. To overcome this drawback, we developed a regeneration system in which the ADP and GDP byproducts can be recycled for *in situ* generation of the universal fucosyl donor—GDP-fucose. During glycolysis, ADP is reconverted into ATP through the transfer of phosphate from phosphoenolpyruvate in a process catalyzed by pyruvate kinase. Wong and Thiem applied this principle to produce sugar nucleotides *in situ* using a coupled-enzymatic approach.[22, 23] Inspired by these precedents, we introduced readily available pyruvate kinase into the fucosylation system for cofactor recycling (Scheme 2). We supplied the reaction system with a catalytic amount of ADP and GDP. In the presence of pyruvate kinase and phosphoenolpyruvate (2 equiv to fucose), ADP and GDP were converted *in situ* into ATP and GTP, respectively, as the substrates for FKP to produce GDP-fucose. As reported

previously, pyruvate kinase has similar maximum velocities for ADP and GDP. However, its K_M for ADP is four-fold smaller than its K_M for GDP.[24] Since fucose phosphorylation is the rate-limiting step of the FKP-catalyzed GDP-fucose formation, the *in situ* regeneration of GDP-Fuc is sensitive to the ADP to GDP ratio.[13] In order to identify an optimal condition for the recycling system, we screened a combination of ADP and GDP at different molar ratios while maintaining the concentration of pyruvate kinase and fucosylation enzymes constant. We discovered that 1:2 ADP–GDP afforded the fastest reaction rate when the ADP loading was 10 mol % relative to fucose. Using this recycling system, the fucosylation reaction finished in four hours and afforded sLe^x in 83% yield, which is comparable to the yield reported above.

3. Conclusions

In conclusion, the chemoenzymatic method described here offers a practical and versatile approach for the synthesis of the sLe^x tetrasaccharide and its derivatives. As we routinely express the FKP and fucosyltransferase with high activity on a 100-miligram scale in a single day, this procedure can be easily extended for multigram synthesis (The specific activities of FKP and α-(1→3)-fucosyltransferase were determined to be 4.5 U mg^{-1} protein and 6–10 U mg^{-1} protein, respectively. One unit is defined as the amount of enzyme that is required to produce 1 μmol of product per minute at 37 °C). Not only does this method provide a facile means to produce sLe^x bearing neo-substituents at the fucose C-5 position, it is also directly applicable to the generation of sLe^x derivatives with unnatural functional groups incorporated at the sialic acid C-5 or C-9 position.[17, 25] Unnatural sLe^x binds to all three selectins (E-, L-, P-selectins) with similar affinity. By incorporating unnatural functionalities of various stereoelectronic properties, we may be able to generate sLe^x derivatives that are selective for a particular selectin. Currently, we are using this method to produce a sLe^x library for fabricating glycan microarrays to profile sLe^x–selectin interactions.

4. Experimental

4.1 Kinetic measurements

Initial velocity experiments were performed at various concentrations of one substrate in the presence of a fixed, saturating concentration of the second substrate. Initial velocities of the α-(1→3)-fucosyltransferase-catalyzed fucosylation reactions were assayed spectrophotometrically by coupling the formation of GDP to the reaction of pyruvate kinase and lactate dehydrogenase. The decrease in absorbance of NADH at 340 nm (ε = 6220 M^{-1} cm^{-1}) was measured at 37 °C using a BioTek Synergy 4 microplate reader. The standard reaction contained 100 mM Tris (pH 7.5), 1.0 mM $MnCl_2$, 1.0 mM phosphoenolpyruvate, 225 μM NADH, 3.73 units pyruvate kinase, and 4.53 units lactate dehydrogenase in addition to the substrates in a final volume of 200 μL. After incubation for 15 min at 37 °C, the reactions were initiated by the addition of α-(1→3)-fucosyltransferase (18.5 nM). The α-(1→3)-fucosyltransferase activities were corrected for background activity. The rate of product formation is proportional to the rate of NADH oxidation, where one molecule of NADH is oxidized for each molecule of Le^x or sLe^x formed. Individual substrate saturation kinetic data were fitted to Eq. 1 Graphpad Prism 5.00:

$$v = \frac{V_{\max}[A]}{K_M + [A]} \quad (1)$$

where V_{max} is the maximal velocity, [*A*] is the substrate concentration, and K_M is the Michaelis–Menten constant (K_M).

4.2 General procedure for preparative-scale (0.059 mmol) synthesis of the sLacNAc derivatives

Reactions were typically carried out in a 15-mL centrifuge tube with 5.0 mL Tris-HCl buffer (200 mM, pH 8.8) containing sialic acid sodium salt (27.7 mg, 0.088 mmol, 1.5 equiv), CTP disodium salt (46.4 mg, 0.088 mmol, 1.5 equiv), *N*-acetyllactosamine or 2-azidoethyl *N*-acetyllactosamine (22.6 or 26.5 mg, 0.059 mmol, 1 equiv), $MgCl_2$ (20 mM), *Neisseria meningitides* CMP-sialic acid synthetase (3.0 units), and *Pasteurella multocida* α2,3 sialyltransferase (1.5 units). The reaction mixture was incubated at 37 °C for 3 h with shaking (225 rpm). The reaction was monitored by TLC analysis using 4:2:1:0.1 EtOAc–MeOH–H_2O–HOAc as the developing solvent and the plates were stained with 5% H_2SO_4 in EtOH. After adding the same volume of ice-cold EtOH to quench the reaction, the alcoholic mixture was incubated on ice for 15 min. Insoluble material was removed by centrifugation (8,000 × *g*, 30 min), and the supernatant was concentrated *in vacuo*. The aqueous residues were lyophilized to dryness. The crude products were purified by Bio-Gel P2 gel-filtration chromatography (1.5 × 120 cm) eluted with aq NH_4HCO_3 (50 mM). Only the fractions containing the product were collected, lyophilized, and further purified using Bio-Gel P2 gel-filtration chromatography (1.5 × 120 cm) eluted with NH_4HCO_3 (50 mM). Lyophilized sialyl lactosamines were characterized by NMR spectroscopy and HRMS. Yield: sLacNAc, 35.9 mg (90%); 2-azidoethyl sLacNAc, 39.6 mg (90 %).

4.3 General procedure for preparative-scale (0.025 mmol) synthesis of the sLe^x tetrasaccharide derivatives

One-pot reactions were performed in 15-mL centrifuge tubes with 5.0 mL Tris-HCl buffer (100 mM, pH 7.5) containing L-fucose or its C-5 substituted analogs (2.0 equiv, 0.05 mmol), 2-azidoethyl sLacNAc (18.6 mg, 0.025 mmol, 1.0 equiv,), ATP (25.4mg, 0.05 mmol, 2.0 equiv), GTP (26.2, 0.05 mmol, 2.0 equiv), $MnSO_4$ (20 mM). The enzymes inorganic pyrophosphatase (100 units, lyophilized form containing $MgCl_2$), FKP (9 units), and α-(1→3)-fucosyltransferase (2.4 units) were added to the solution. The reaction mixture was incubated at 37 °C for 3 h with vigorous shaking (225 rpm). The reaction was monitored by TLC analysis using 4:2:2:0.1 EtOAc–EtOH–H_2O–HOAc as the developing solvent, and the plates were stained with 5% H_2SO_4 in EtOH. After adding the same volume of ice-cold EtOH to quench the reaction, the alcoholic mixture was incubated on ice for 15 min. Insoluble material was removed by centrifugation (8,000 × *g*, 30 min), and the supernatant was concentrated *in vacuo*. The aqueous residues were lyophilized to dryness. Crude products were purified by Bio-Gel P2 gel-filtration chromatography (1.5 × 120 cm) eluted with aq NH_4HCO_3 (50 mM). Only the fractions containing the product were collected, lyophilized, and further purified using Bio-Gel P2 gel-filtration chromatography (1.5 × 120 cm) eluted with NH_4HCO_3 (50 mM). Lyophilized sLe^x derivatives were characterized by NMR spectroscopy and HRMS. Yield: 17.1–21.9 mg (76–95 %).

4.4 General procedure for preparative-scale (0.025 mmol) synthesis of the 2-azidoethyl sLe^x tetrasaccharide with *in situ* regeneration of GDP-fucose

The synthesis was performed in 15-mL centrifuge tubes with 5.0 mL of Tris-HCl buffer (100 mM, pH 7.5) containing L-fucose (8.20 mg, 0.05 mmol, 2.0 equiv), 2-azidoethyl sLacNAc (18.6 mg, 0.025 mmol, 1.0 equiv), ADP (2.13 mg, 0.005 mmol, 0.2 equiv), GDP (4.4 mg, 0.01 mmol, 0.4 equiv), $MnSO_4$ (20 mM), phosphoenolpyruvate potassium salt (20.6 mg, 0.10 mmol, 4.0 equiv), KCl (80 mM). The enzymes pyruvate kinase (400 units), inorganic pyrophosphatase (100 units, lyophilized form containing $MgCl_2$), FKP (9 units), and α-(1→3)-fucosyltransferase (2.4 units) were added to the solution. The reaction mixture was incubated at 37 °C for 4.5 h with vigorous shaking (225 rpm). The reaction was monitored by TLC analysis using 4:2:2:0.1 EtOAc–EtOH–H_2O–HOAc as the developing solvent, and the plates

were stained with 5% H_2SO_4 in EtOH. After adding the same volume of ice-cold EtOH to quench the reaction, the alcoholic mixture was incubated on ice for 15 min. Insoluble material was removed by centrifugation (8,000 × *g*, 30 min) and the supernatant was concentrated *in vacuo*. The aqueous residues were lyophilized to dryness. Crude product was purified by Bio-Gel P2 gel filtration chromatography (1.5 × 120 cm) eluted with aq NH_4HCO_3 (50 mM). Only the fractions containing the product were collected, lyophilized, and further purified using Bio-Gel P2 gel filtration chromatography (1.5 × 120 cm) eluted with NH_4HCO_3 (50 mM). Lyophilized 2-azidoethyl sLe^x was characterized by NMR spectroscopy and HRMS. Yield: 18.6 mg (83 %).

4.4.1 2-Azidoethyl (5-acetamido-3,5-dideoxy-D-g*lycero*-α-D-*galacto*-2-nonulo pyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)[(1→3)-α-L-fucopyranosyl]-2-acetamido-2-deoxy-β-D-glucopyranoside (7)—This compound was synthesized according to general procedure for synthesis of the sLex tetrasaccharide derivatives (17.5 mg, yield: 79%). ^{1}H NMR (600 MHz, D_2O): δ 1.18 (d, *J* = 5.8 Hz, 3H), 1.80 (t, *J* = 12.0 Hz, 1H), 2.04 (s, br, 6H), 2.77 (d, *J* = 11.6 Hz, 1H), 3.41-3.55 (m, 3H), 3.57-3.63 (m, 3H), 3.64-3.74 (m, 3H), 3.75-3.82 (m, 3H), 3.84-4.00 (m, 10H), 4.02-4.10 (m, 4H), 4.53 (d, *J* = 7.5 Hz, 1H), 4.61 (d, *J* = 8.0 Hz, 1H), 5.11 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 15.3, 22.0, 22.3, 39.8, 50.4, 51.7, 55.7, 59.7, 60.9, 61.5 62.6, 66.7, 67.3, 67.7, 68.1, 68.3, 68.7, 69.2, 69.3, 71.9, 72.9, 73.3, 74.85, 74.91, 75.3, 75.6, 98.6, 99.7, 100.8, 101.6, 173.9, 174.4, 175.0. HRMS: Calcd for $[C_{33}H_{55}N_5O_{23}+H]^+$: 890.3363. Found: 890.3367.

4.4.2 2-Azidoethyl (5-acetamido-3,5-dideoxy-D-g*lycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)[(1→3)-α-D-arabinopyranosyl]-2-acetamido-2-deoxy-β-D-glucopyranoside (8)—This compound was synthesized according to general procedure for synthesis of the sLex tetrasaccharide derivatives (18.0 mg, yield: 82%). ^{1}H NMR (600 MHz, D_2O):δ 1.80 (d, *J* = 12.2 Hz, 1H), 2.04 (s, 3H), 2.05 (s, 3H), 2.77 (d, *J* = 12.5, 4.6 Hz, 1H), 3.41-3.45 (m, 1H), 3.47-3.50 (m, 1H), 3.52-3.58 (m, 2H), 3.59-3.63 (m, 3H), 3.65-3.72 (m, 4H), 3.74-3.79 (m, 2H), 3.84-3.91 (m, 6H), 3.93-3.95 (m, 2H), 3.96-4.07 (m, 4H), 4.10 (dd, *J* = 9.8, 3.1 Hz, 1 H), 4.55 (d, *J* = 7.8 Hz, 1H), 4.61 (d, *J* = 8.3 Hz, 1H), 4.65 (d, *J* = 12.5 Hz, 1H), 5.18 (d, *J* = 3.7 Hz, 1H). ^{13}C NMR (150 MHz, D_2O):δ 22.0, 22.3, 39.7, 50.4, 51.7, 55.6, 59.7, 60.0, 60.6, 61.4, 62.6, 63.6, 67.3, 68.0, 68.3, 68.4, 68.7, 69.1, 69.4, 71.9, 72.9, 73.2, 74.9, 75.1, 75.3, 75.6, 98.9, 99.7, 100.9, 101.5, 173.9, 174.4, 175.0. HRMS: Calcd for $[C_{32}H_{53}N_5O_{23}+H]^+$: 876.3210. Found: 876.3213.

4.4.3 2-Azidoethyl (5-acetamido-3,5-dideoxy-D-g*lycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)[(1→3)-6,7-deoxy-α-L-*galacto*-hept-6-ynopyranosyl]-2-acetamido-2-deoxy-β-D-glucopyranoside (9)—This compound was synthesized according to general procedure for synthesis of the sLex tetrasaccharide derivatives (17.1 mg, yield: 76%). ^{1}H NMR (600 MHz, D_2O): δ 1.82 (t, *J* = 12.2 Hz, 1H), 2.04 (s, 6H), 2.77 (dd, *J* = 12.3, 4.3 Hz, 1H), 2.01 (d, *J* = 2.0 Hz, 1H), 3.41-3.45 (m, 1H), 3.47-3.51 (m, 1H), 3.59-3.62 (m, 3H), 3.64-3.74 (m, 6H), 3.75-3.79 (m, 1H), 3.82-3.92 (m, 7H), 3.94-4.00 (m, 3H), 4.01-4.07 (m, 3H), 4.10 (dd, *J* = 9.8, 2.6 Hz, 1H), 4.54 (d, *J* = 7.9 Hz, 1 H), 4.62 (d, *J* = 8.4 Hz, 1H), 5.15 (d, *J* = 3.9 Hz, 1H), 5.58 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.0, 22.3, 39.8, 50.4, 51.7, 55.5, 59.6, 61.5, 62.6, 63.2, 67.2, 67.3, 68.1, 68.28, 68.34, 68.7, 69.1, 71.0, 71.8, 72.9, 73.3, 75.0, 75.3, 75.7, 76.1, 79.4, 99.0, 99.6, 100.9, 101.8, 173.8, 174.4, 175.0. HRMS: Calcd for $[C_{34}H_{53}N_5O_{23}+H]^+$: 900.3210. Found: 900.3189.

4.4.4 2-Azidoethyl (5-acetamido-3,5-dideoxy-D-g*lycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)[(1→3)- 6-

deoxy-6-fluoro-α-L-galactopyranosyl]-2-acetamido-2-deoxy-β-D-glucopyranoside (10)—This compound was synthesized according to general procedure for synthesis of the sLex tetrasaccharide derivatives (20.0 mg, yield: 88%). ^{1}H NMR (600 MHz, D_2O): δ 1.80 (t, *J* = 12.1 Hz, 1H), 2.03 (s, 3H), 2.04 (s, 3H), 2.77 (dd, *J* = 12.1, 3.7 Hz, 1H), 3.41-3.51 (m, 2H), 3.59-3.63 (m, 4H), 3.64-3.67 (m, 2H), 3.69-3.71 (m, 3H), 3.72-3.78 (m, 2H), 3.83-3.87 (m, 2H), 3.88-3.91 (m, 3H), 3.92-3.97 (m, 4H), 4.00-4.09 (m, 4H), 4.52 (d, *J* = 7.8 Hz, 1 H), 4.54-4.72 (m, 2H), 4.69 (d, *J* = 10.3 Hz, 1H), 5.07 (dd, *J* = 15.4, 5.4 Hz, 1H), 5.22 (d, *J* = 3.6 Hz, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.0, 22.3, 39.8, 50.4, 51.7, 55.7, 59.6, 61.4, 62.6, 67.3, 67.8, 68.1, 68.3, 68.7, 69.0, 69.1, 69.20, 69.25, 69.4, 71.8, 72.9, 73.6, 74.8, 75.3, 75.7, 83.5, 84.6, 98.6, 99.7, 100.8, 101.9, 173.8, 174.3, 174.9. HRMS: Calcd for $[C_{33}H_{54}FN_5O_{23}+H]^+$: 908.3272. Found: 908.3252.

4.4.5 2-Azidoethyl (5-acetamido-3,5-dideoxy-D-g*lycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)[(1→3)-α-L-galactopyranosyl]-2-acetamido-2-deoxy-β-D-glucopyranoside (11)—This compound was synthesized according to general procedure for synthesis of the sLex tetrasaccharide derivatives (18.1 mg, yield: 80%). ^{1}H NMR (600 MHz, D_2O): δ 1.80 (t, *J* = 12.2 Hz, 1H), 2.04 (s, 3H), 2.05 (s, 3H), 2.78 (dd, *J* = 12.5, 4.6 Hz, 1H), 3.42-3.51 (m, 2H), 3.56-3.58 (m, 1H), 3.59-3.67 (m, 5H), 3.68-3.73 (m, 6H), 3.74-3.79 (m, 2H), 3.84-3.88 (m, 2H), 3.89-3.92 (m, 2H), 3.93-3.98 (m, 5H), 4.00-4.01 (m, 1H), 4.03-4.07 (m, 2H), 4.11 (dd, *J* = 9.9, 3.1 Hz, 1 H), 4.54 (d, *J* = 7.8 Hz, 1 H), 4.61 (d, *J* = 7.8 Hz, 1H), 4.75-4.77 (m, 1H), 5.21 (d, *J* = 3.9 Hz, 1 H). ^{13}C NMR (150 MHz, D_2O): δ 22.0, 22.4, 39.8, 50.4, 51.7, 55.7, 59.7, 60.8, 61.3, 62.6, 67.4, 68.05, 68.12, 68.3, 68.7, 69.0, 69.2, 69.3, 70.0, 71.9, 72.9, 73.9, 74.6, 74.9, 75.4, 75.6, 89.4, 99.7, 100.9, 102.0, 173.8, 174.6, 175.1. HRMS: Calcd for $[C_{33}H_{55}N_5O_{24}+H]^+$: 906.3315. Found: 906.3320.

4.4.6 2-Azidoethyl (5-acetamido-3,5-dideoxy-D-g*lycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)[(1→3)-6-O-methyl-α-L-galactopyranosyl]-2-acetamido-2-deoxy-β-D-glucopyranoside (12)—This compound was synthesized according to general procedure for synthesis of the sLex tetrasaccharide derivatives (21.9 mg, yield: 95%). ^{1}H NMR (600 MHz, D_2O): δ 1.82 (t, *J* = 12.2 Hz, 1H), 2.04 (s, 3H), 2.05 (s, 3H), 2.60 (s, 2H), 2.77 (dd, *J* = 12.5, 4.6 Hz, 1H), 3.40 (s, 3H), 3.42-3.45 (m, 1H), 3.46-3.52 (m, 1H), 3.57-3.65 (m, 8H), 3.66-3.68 (m, 1H), 3.69-3.74 (m, 4H), 3.75-3.79 (m, 1H), 3.84-3.92 (m, 4H), 3.93-3.98 (m, 5H), 4.01-4.06 (m, 2H), 4.09 (dd, *J* = 9.9, 3.2 Hz, 1H), 4.51 (d, *J* = 7.8 Hz, 1 H), 4.61 (d, *J* = 7.1 Hz, 1H), 4.86 (dd, *J* = 7.6, 4.2 Hz, 1H), 5.21 (d, *J* = 3.8 Hz, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.04, 22.34, 24.53, 39.77, 50.39, 51.70, 55.73, 58.57, 59.66, 61.38, 62.61, 67.45, 67.92, 68.12, 68.33, 68.65, 68.73, 69.00, 69.22, 71.88, 71.91, 72.91, 73.49, 74.27, 74.88, 75.30, 75.62, 98.22, 99.73, 100.88, 101.89, 173.88, 174.35, 175.02. HRMS: Calcd for $[C_{34}H_{57}N_5O_{24}+H]^+$: 920.3472. Found: 920.3470.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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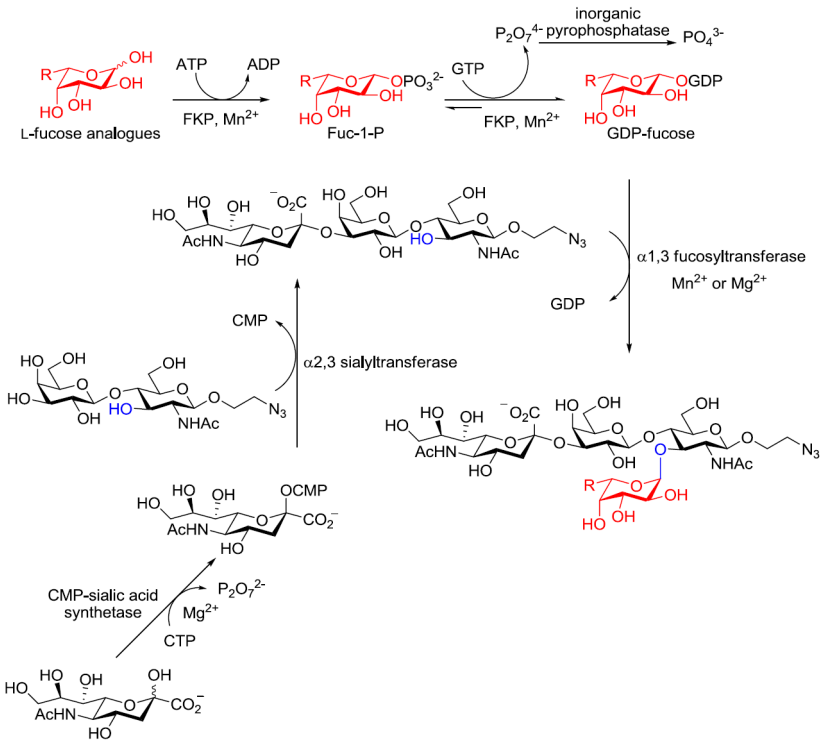
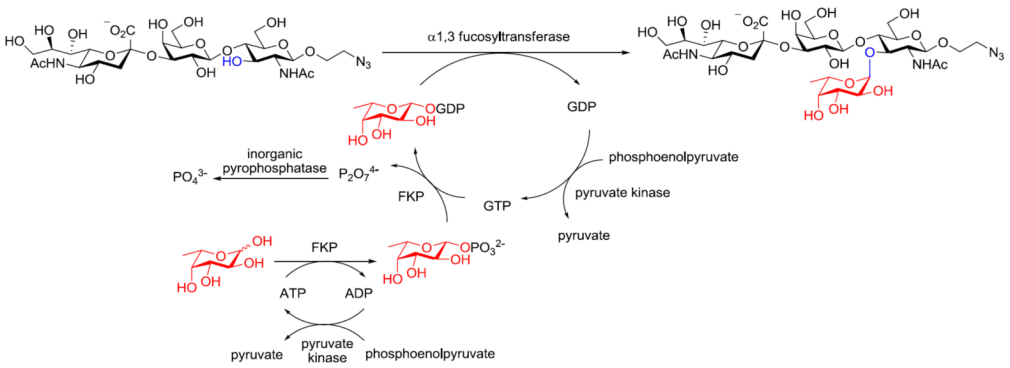


Scheme 1.
A chemoenzymatic approach for the synthesis of the sLe^x tetrasaccharide derivatives.



Scheme 2.
Synthesis of the sLe^x tetrasaccharide with *in situ* cofactor regeneration.

Table 1

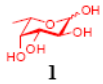
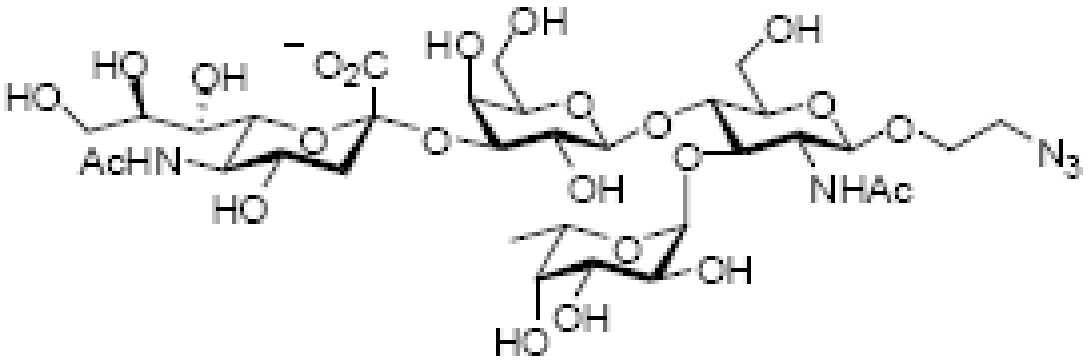
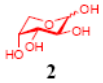
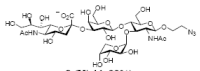
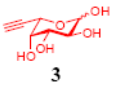
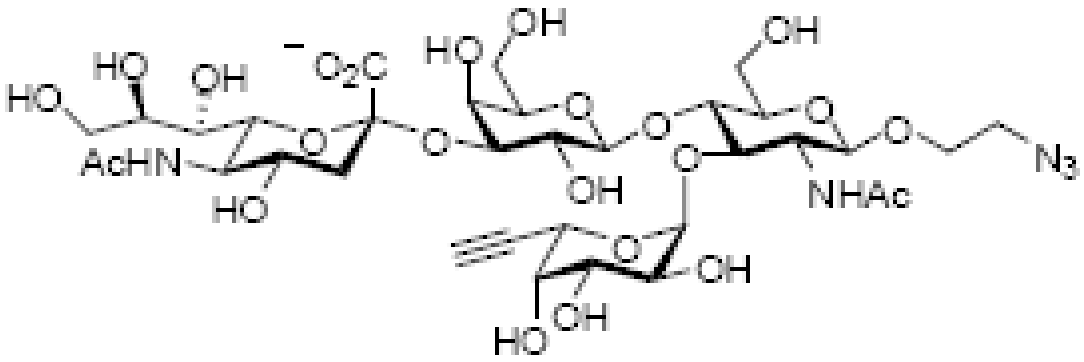
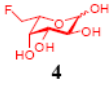
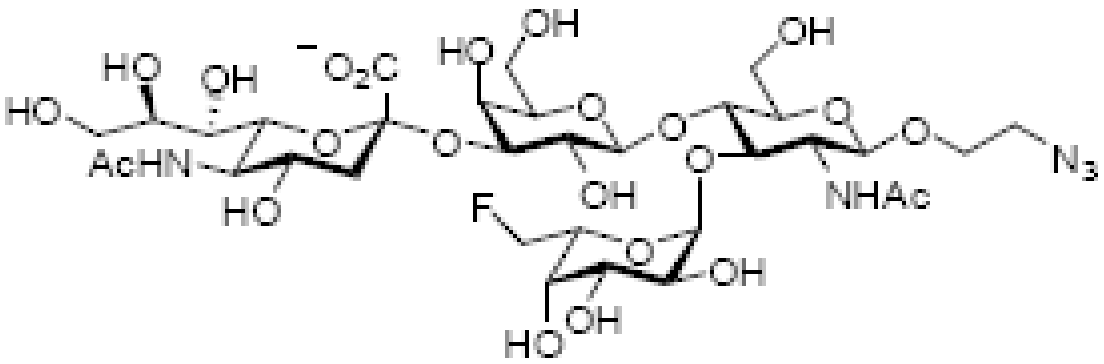
Activity and specificity of the α-(1→3)-fucosyltransferase

Substrate	K_M (mM)	k_c
	0.34 ± 0.05	
	1.3 ± 0.1	
	3.9 ± 0.2	
	0.18 ± 0.02	

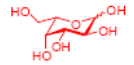
[a]The fucosylation activity of α-(1→3)-fucosyltransferase was measured using a coupled enzyme assay. All kinetic measurements were performed in 100 mM Tris-HCl, pH 7.5 at 37 °C. Each data point was collected in triplicate. Error margins were obtained from non-linear regression analyses of pooled triplicate measurements. The kinetic paramenters for GDP-L-fucose were measured using $LacNAcCH_2CH_2N_3$ as the acceptor substrate.

Table 2

Synthesis of sLe^x tetrasaccharide derivatives

L-Fucose analogues	sLe^x derivatives
 1	 7 (Yield: 79%)
 2	 8 (Yield: 82%)
 3	 9 (Yield: 76%)
 4	 10 (Yield: 88%)

L-Fucose analogues



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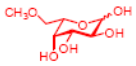
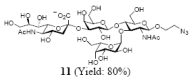
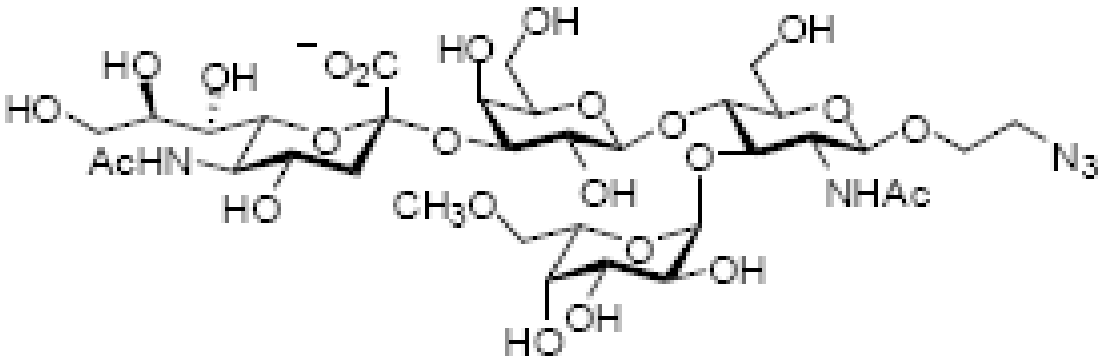


6

sLe^x derivatives



11 (Yield: 80%)



12 (Yield: 95%)