Regioselective Fucosylation Using L-Galactosyltransferase from *Helix pomatia*

Laurent Bornaghi[a], Lisa Keating[a], Hayley Binch[a], Hagen Bretting[b], and Joachim Thiem*[a]

Institut für Organische Chemie, Universität Hamburg[a],
Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

Zoologisches Institut und Zoologisches Museum, Universität Hamburg[b],
Martin-Luther-King-Platz 3, D-20146 Hamburg, Germany

Received May 4, 1998

Keywords: Chemoenzymatic synthesis / L-Fucose / L-Galactosyltransferase / H-Blood group determinant / *Helix pomatia*

The L-galactosyltransferase from *Helix pomatia* catalyses the transfer of L-galactose from GDP-L-galactose to various disaccharides having a D-galactose at the non-reducing position, forming an α(1→2) linkage. L-Fucose, an important part of the human blood determinant, is also transferred by this enzyme, allowing the formation of H-blood group determinant. The transfer of L-fucose has been studied with four disaccharides: the Galβ(1→3)GalβOMe, the Galβ(1→3)-GalNAcαOThr, the Galβ(1→3)GalαOMe, and the Galβ-(1→3)GlcNAcβOMe.

Introduction

Carbohydrates are among the most important biomolecules. In addition to their importance as energy sources and structural materials, carbohydrates and glycoconjugates are responsible for essential biological and physicochemical functions in all living systems. There have been numerous reports of their role in cell-cell recognition[1], in host-pathogen interaction[2], and as antigenic determinants[3].

The H-blood group determinant (types 1 and 2), defined by the sequence Fucα(1→2)Gal, has been reported in a wide variety of naturally occurring glycoproteins[4], glycolipids[5], and oligosaccharides[6]. Structures bearing this terminal chain are precursors of the A and B antigens (blood determinants) (Scheme 1). The enzyme α(1→2)-fucosyltransferase (EC 2.4.1.69) is involved in the formation of the H-blood group antigen and can catalyse the transfer of α-Fuc to a type-1, -2, -3 or -4 oligosaccharide[7].

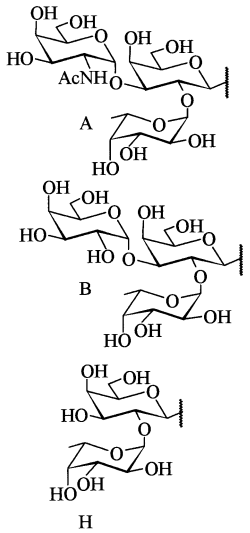
Type 1: Galβ(1→3)GlcNAcβ(OR)
Type 2: Galβ(1→4)GlcNAcβ(OR)
Type 3: Galβ(1→3)GalNAcα(OR)
Type 4: Galβ(1→3)GalNAcβ(OR)

Our source of transferases has been the albumen glands of *Helix pomatia*[8]. Snails of this species were collected from their natural habitat near Hamburg or Schwandorf (Germany) in May or June. Apparently, the enzyme shows the highest activity around this time, corresponding to the breeding season. At this period, the snails synthesize a highly branched polysaccharide in these glands, composed of D- and L-galactose. The D-Gal residues are β(1→3)- or β(1→6)-linked. The polysaccharide is irregularly branched and contains linear sections, in which β(1→3) linkages prevail over β(1→6) linkages. The galactan is the exclusive carbohydrate source for embryos and freshly hatched snails.

Using the L-galactosyltransferase, L-fucose is transferred from GDP-L-Fuc onto the 2-OH group of the non-reducing terminal Gal residue of the acceptor oligosaccharide structure. We can describe the fucosylation using the L-galactosyltransferase of *Helix pomatia* in terms of four disaccharides

Galβ(1→3)GalβOMe (**1**)
Galβ(1→3)GalNAcαOThr (**2**)
Galβ(1→3)GalαOMe (**6**)
Galβ(1→3)GlcNAcβOMe (**10**)

Scheme 1. The human A, B and H-blood group determinants



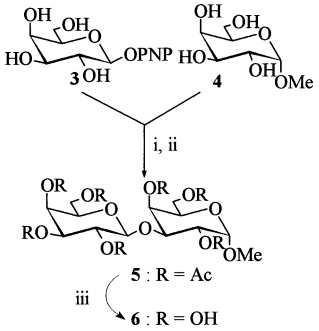
Results and Discussion

The disaccharide Galβ(1→3)GalβOMe (**1**) is readily recognized by *Helix pomatia* galactosyltransferase[8] and is commercially available. The T-antigen component, Galβ(1→3)GalNAcαOThr (**2**), has recently been synthesized in our laboratory[9].

  1434−193X/98/1111−2493 $ 17.50+.50/0

The chemoenzymatic synthesis of Galβ(1→3)GalαOMe (**6**) was achieved using β-D-galactosidase from bovine testes (EC 3.2.1.23)[9][10], employing transglycosylation. *p*-Nitrophenyl galactoside (**3**) was used as donor and the transfer took place at the 3-position of the galactosyl acceptor unit resulting in a β(1→3) linkage. The reaction was carried out in a phosphate/citrate buffer, and after acetylation, the disaccharide **5** was isolated in an overall yield of 16%. Deacetylation of compound **5** under Zemplén conditions gave compound **6** (Scheme 2).

Scheme 2. Chemoenzymatic synthesis of the disaccharide **6**: (i) phosphate/citrate buffer (50 mM, pH = 4.3), β-D-galactosidase, 37°C, 3 d; (ii) pyridine, Ac_2O, DMAP, room temp., 16 h, 16%; (iii) methanol, sodium methoxide, room temp., 18 h, 100%

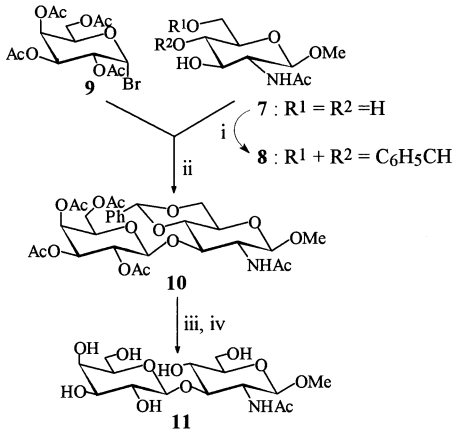


The preparation of the other disaccharide **11** was achieved by coupling of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**8**) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**9**). The acceptor **8** was synthesized from methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**7**) by protection of the 4- and 6-positions as benzylidene acetal. Coupling was carried out in the presence of mercuric cyanide in a mixture of toluene and nitromethane to give the protected disaccharide **10** in 30% yield. Disaccharide **10** was deprotected by removal of the acetyl groups followed by acidic cleavage of the benzylidene acetal to give the required disaccharide acceptor **11** in 96% yield (Scheme 3).

In a first set of experiments, the four disaccharides were incubated with the membrane fractions of the albumen glands containing L-galactosyltransferase, in the presence of GDP-L-fucose[11], in Tris/HCl buffer at 27°C for 3 days. The remaining GDP-L-Fuc was hydrolysed to fucose during the incubation. The reactions were terminated by boiling for 10 min, the protein was eliminated by centrifugation, and the supernatants were freeze-dried. In the case of compounds **1**, **6**, and **11**, the residues were acetylated. The trisaccharides **12**, **14**, and **15** were isolated in yields of 25, 21, and 22%, respectively. The trisaccharide **13**, an unblocked sugar, was isolated after chromatography on Biogel P2 in 19% yield.

With the aim of increasing the yields of the transferase products, optimization of the reaction conditions was undertaken. The sulfhydryl-reducing agent cysteine, which is known to enhance the activity of some enzymes such as

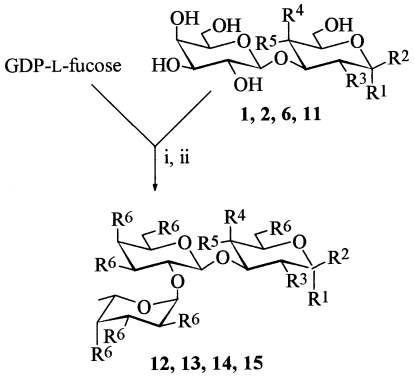
Scheme 3. Synthesis of the disaccharide **11**: (i) DMF, *p*-toluenesulfonic acid, dimethoxytoluene, 60°C, 3 h, 72%; (ii) toluene/nitromethane (1:1, v/v), $Hg(CN)_2$, $CaSO_4$, 40°C, 16 h, 30%; (iii) methanol, sodium methoxide, room temp., 15 h; (iv) water, acetic acid, 80°C, 3 h, 94%



α-amylase, was found to favour the transferase reaction. Addition of cysteine also allowed the reaction time and temperature to be reduced from 3 days to 1 day, and from 27°C to 20°C.

Subsequently, a second set of experiments was carried out in the presence of cysteine, with the four disaccharides **1**, **2**, **6**, and **11**. By the same treatment as described previously, the trisaccharides **12**, **13**, **14**, and **15** were isolated in improved yields of 36, 24, 33, and 36%, respectively (Scheme 4).

Scheme 4. Synthesis of the different trisaccharides **12**, **13**, **14**, and **15**: (i) Tris/HCl buffer, GDP-L-Fuc, albumen glands, 20°C, 1 d, **13**: 24%; (ii) pyridine, Ac_2O, DMAP, room temp., 2 h, **12**: 36%, **14**: 33%, and **15**: 36%



1 : R1 = R5 = H, R2 = OMe, R3 = R4 = OH,
2 : R1 = OThr, R2 = R5 = H, R3 = NHAc, R4 = OH,
6 : R1 = OMe, R2 = R5 = H, R3 = R4 = OH,
11 : R1 = R4 = H, R2 = OMe, R3 = NHAc, R5 = OH.

12 : R1 = R5 = H, R2 = OMe, R3 = R4 = R6 = OAc,
13 : R1 = OThr, R2 = R5 = H, R3 = NHAc, R4 = R6 = OAc,
14 : R1 = OMe, R2 = R5 = H, R3 = R4 = R6 = OH,
15 : R1 = R4 = H, R2 = OMe, R3 = NHAc, R5 = R6 = OAc.

Conclusion

The chemoenzymatic approach using L-galactosyltransferase from *Helix pomatia* can be successfully used in fucosylation and can provide an easy access to various trisaccharides in good yield. These studies indicate that the yield of fucosylated products can be substantially increased by addition of the sulfhydryl-reducing agent cysteine.

The authors would like to thank Mr. *S. Kötter* for his assistance in obtaining mass spectra, Mr. *R. Gonzales Lio* for the preparation of the crude extract of the β-D-galactosidase, and Dr. *U. Gambert* for a gift of disaccharide **2**. We gratefully acknowledge support of this work by the *Deutsche Forschungsgemeinschaft*, the *Fonds der Chemischen Industrie*, and the *Commission of the European Communities, GDXII* (Programme CARENET).

Experimental Section

General Procedure: Solvents required absolute for reactions were distilled according to the procedures described by Perrin and Perrin (*Purification of Laboratory Chemicals*, Pergamon Press, **1983**). − Reactions were monitored by TLC analysis using aluminium-backed silica-gel plates (GF_{254} Merck). Compounds were visualized by spraying with 20% sulfuric acid in ethanol, followed by charring at 150°C and/or by UV irradiation. − Column chromatography was performed on silica gel 60 (0.040−0.063 nm mesh, Merck) or Biogel P2. − NMR spectra were accumulated with a Bruker AMX 400 spectrometer. Chemical shifts are given in ppm (δ). − Mass spectra were recorded with a Bruker MALDI-TOF mass spectrometer (with an N_2 laser operating at 337 nm and 5 μl of 2,5-dihydroxybenzoic acid as matrix).

Enzyme Preparation: Albumen glands (1 g) were homogenized with Tris/HCl buffer (50 mM, pH = 7.6, 5 ml) in a Potter-Elvehjem homogenizer, centrifuged at 4000 rpm for 30 min at 4°C, and the supernatant was separated from the pellet. This pellet was washed five times with buffer (5 ml) and used as the source of transferase.

Methyl 2,4,6-Tri-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (**5**): To compound **3** (100 mg, 0.33 mmol) and compound **4** (50 mg, 0.26 mmol) in phosphate/citrate buffer (50 mM, pH = 4.3, 3 ml), was added the β-D-galactosidase from bovine testes (50 mg, 1.5 U). The reaction mixture was stirred at 37°C for 3 d. The reaction was terminated by heating at 100°C for 10 min. Then, the solution was lyophylized, and the residue was acetylated in pyridine (5 ml) with acetic anhydride (5 ml) and DMAP (50 mg). The reaction mixture was stirred at room temperature for 16 h and then poured into ice/water. The resulting solution was extracted with CH_2Cl_2, washed with saturated solutions of $NaHCO_3$, $KHSO_4$ and NaCl, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography (petroleum ether/ethyl acetate, 3:7, v/v). Concentration of the appropriate fractions afforded **5** (27 mg, 16%). − R_f = 0.4 (petroleum ether/ethyl acetate, 1:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 2.40, 2.38, 2.37, 2.31, 2.30, 2.24, 2.21 (7 s, 21 H, $COCH_3$), 3.64 (s, 3 H, OCH_3), 4.10 (dd, 1 H, $J_{3,4}$ = 3.5 Hz, 3-H), 4.11 (dd, 1 H, $J_{5,6b}$ = 4.7 Hz, 6b-H), 4.41−4.25 (m, 4 H, 5-H, 5′-H, 6′a-H, 6′b-H), 4.45 (dd, 1 H, $J_{5,6a}$ = 3.5, $J_{6a,6b}$ = 11.0 Hz, 6a-H), 4.89 (dd, 1 H, $J_{1',2'}$ = 8.1 Hz, 1′-H), 5.15 (dd, 1 H, $J_{1,2}$ = 3.6 Hz, 1-H), 5.17 (dd, 1 H, $J_{3',4'}$ = 3.5 Hz, 3′-H), 5.33 (dd, 1 H, $J_{2',3'}$ = 9.8 Hz, 2′-H), 5.39 (dd, 1 H, $J_{2,3}$ = 11.0 Hz, 2-H), 5.46 (dd, 1 H, $J_{4',5'}$ = 1.0 Hz, 4′-H), 5.60 (d, 1 H, $J_{3,4}$ = 3.6 Hz, 4-H). − MALDI-TOF MS; $C_{27}H_{38}O_{18}$ (650.59): *m/z* = 673 $[M + Na]^+$.

Methyl 3-O-(β-D-Galactopyranosyl)-α-D-galactopyranoside (**6**): To compound **5** (20 mg, 0.031 mmol) in dry methanol (2 ml), was added sodium methoxide (1 M in methanol, 100 μl). The reaction mixture was stirred at room temperature for 18 h, and then neutralized with Amberlite IRN 120 $[H^+]$ resin until pH = 7 was reached, filtered, and concentrated. The residue was co-evaporated with water (5 ml), solubilized in water (2 ml), and lyophylized to give compound **6** (11.1 mg, 100%). − R_f = 0.1 (acetonitrile/water, 9:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 3.34 (s, 3 H, OCH_3), 3.91−3.5 (m, 12 H, 2-H, 3-H, 4-H, 5-H, 6a-H, 6b-H, 2′-H, 3′-H, 4′-H, 5′-H, 6′a-H, 6′b-H), 4.51 (d, 1 H, $J_{1',2'}$ = 7.1 Hz, 1′-H), 4.77 (d, 1 H, $J_{1,2}$ = 3.6 Hz, 1-H). − ^{13}C NMR ($CDCl_3$): δ = 55.54 (OCH_3), 61.61, 61.31 (C-6, C-6′), 79.97, 75.39, 72.80, 71.86, 71.45, 69.44, 68.95, 67.61 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 99.61 (C-1), 104.76 (C-1′). − MALDI-TOF MS; $C_{13}H_{24}O_{11}$ (356.33): *m/z* = 379 $[M + Na]^+$.

Methyl 2-Acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**8**): To triol **7** (200 mg, 0.85 mmol) in dry DMF (1.5 ml), was added *p*-toluenesulfonic acid (12 mg), followed by dimethoxytoluene (0.19 ml, 1.27 mmol). The reaction mixture was heated to 60°C under reduced pressure (15 mbar) for 3 h. Triethylamine (0.02 ml) was then added, and the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography (ethyl acetate/CH_2Cl_2/methanol, 5:5:1, v/v/v) to give compound **8** (198 mg, 72%) as a white solid: − R_f = 0.3 (CH_2Cl_2/acetone, 4:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 1.90 (3 s, 3 H, $COCH_3$), 3.45 (s, 3 H, OCH_3), 3.49 (t, 1 H, $J_{2,3}$ = 9.1 Hz, 2-H), 3.69−3.55 (m, 3 H, 3-H, 6a-H, 6b-H), 3.80 (t, 1 H, $J_{3,4}$ = 10.2, $J_{4,5}$ = 10 Hz, 4-H), 4.27 (dd, 1 H, $J_{4,5}$ = 10, $J_{5,6a}$ = 5 Hz, 5-H), 4.45 (d, 1 H, $J_{1,2}$ = 9.2 Hz, 1-H), 5.33 (d, 1 H, $J_{NH,2}$ = 6 Hz, NH), 5.67 (s, 1 H, CHAr), 7.75−7.25 (m, 5 H, ArH). − MALDI-TOF MS; $C_{16}H_{21}NO_6$ (323.35): *m/z* = 346 $[M + Na]^+$.

Methyl 2-Acetamido-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**10**): To the benzylidene derivative **8** (198 mg, 0.61 mmol), mercuric cyanide (350 mg, 1.83 mmol) and calcium sulfate (780 mg) in a mixture of dry toluene (10 ml) and dry nitromethane (10 ml) were added at 40°C under argon. Then, bromide **9** (378 mg, 0.92 mmol) in a 1:1 mixture of toluene and nitromethane (5 ml) was added. The reaction mixture was stirred at 40°C under argon for 16 h. The solvent was then removed in vacuo, the residue was redissolved in CH_2Cl_2, and the resulting solution was washed with brine, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (toluene/ethyl acetate, 1:1, v/v). Concentration of the appropriate fractions gave compound **10** (120 mg, 30%). − R_f = 0.6 (CH_2Cl_2/acetone, 4:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 2.05, 1.94, 1.92, 1.89, 1.88 (5 s, 15 H, $COCH_3$), 3.00 (m, 1 H, 2-H), 3.42 (s, 3 H, OCH_3), 3.52−3.46 (m, 2 H, 5-H, 5′-H), 3.60 (t, 1 H, $J_{4,5}$ = 10.2 Hz, 4-H), 3.71 (t, 1 H, $J_{6'a,6'b}$ = 10.4 Hz, 6′b-H), 3.84 (dd, 1 H, $J_{5,6b}$ = 6.0, $J_{6a,6b}$ = 11.3 Hz, 6b-H), 3.99 (dd, 1 H, $J_{6a,6b}$ = 11.3, $J_{5,6a}$ = 4.0 Hz, 6a-H), 4.28 (dd, 1 H, $J_{6'a,6'b}$ = 10.4, $J_{5',6'a}$ = 4.1, 6′a-H), 4.61 (t, 1 H, $J_{3,4}$ = 10.2 Hz, 3-H), 4.70 (d, 1 H, $J_{1',2'}$ = 8.0 Hz, 1′-H), 4.85 (dd, 1 H, $J_{3',4'}$ = 3.4 Hz, 3′-H), 5.01 (d, 1 H, $J_{1,2}$ = 8.1 Hz, 1-H), 5.08 (dd, 1 H, $J_{2',3'}$ = 10.2 Hz, 2′-H), 5.23 (d, 1 H, $J_{4',3'}$ = 3.4 Hz, 4′-H), 5.45 (s, 1 H, CHAr), 5.75 (d, 1 H, $J_{NH,2}$ = 6 Hz, NH), 7.45−7.05 (m, 5 H, ArH). − MALDI-TOF MS; $C_{30}H_{39}NO_{15}$ (653): *m/z* = 676 $[M + Na]^+$.

Methyl 2-Acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (**11**): To disaccharide **10** (70 mg, 0.11 mmol) in dry methanol, was added sodium methoxide (1 M in methanol, 250 μl). The reaction mixture was stirred at room temperature for 1.5 h, and then neutralized with Dowex 50WX $[H^+]$ resin until pH = 7

was reached, filtered, and concentrated. The residue was partitioned between CH_2Cl_2 and water and the aqueous layer was lyophylized. The residue was treated with acetic acid (2.5 ml) and water (2.5 ml), the reaction mixture was heated at 80°C for 3 h, and then neutralized (2 M NaOH) and lyophylized. The residue was purified by Sephadex G10 chromatography, using water as eluent, to give the title compound **11** (41 mg, 94%). − R_f = 0.4 (ethyl acetate/methanol/water, 5:2:1, v/v/v). − ^{1}H NMR (D_2O): δ = 1.90 (s, 3 H, $COCH_3$), 3.39 (s, 3 H, OCH_3), 3.83−3.41 (m, 12 H, 2-H, 3-H, 4-H, 5-H, 6a-H, 6b-H, 2′-H, 3′-H, 4′-H, 5′-H, 6′a-H, 6′b-H), 4.36, 4.30 (both d, 2 H, $J_{1,2}$ = 8.1, $J_{1',2'}$ = 8.1 Hz, 1-H, 1′-H). − ^{13}C NMR (D_2O): δ = 22.6 ($COCH_3$), 57.5 (OCH_3), 61.4, 61.1 (C-6, C-6′), 83.0, 75.8, 75.7, 72.9, 71.1, 69.2, 68.9 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 103.9, 102.9 (C-1, C-1′), 177.5 ($COCH_3$). − MALDI-TOF MS; $C_{15}H_{27}NO_{11}$ (397.38): *m/z* = 420 [M + Na]$^+$.

Methyl 2,4,6-Tri-O-acetyl-3-O-[3,4,6-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-galactopyranoside (**12**): To albumen glands (0.5 g) in Tris/HCl buffer (50 mM, pH = 7.6, 1 ml), were added compound **1** (1 mg, 2.8 μmol), GDP-L-fucose (2.2 mg, 3.5 μmol, 1.2 equiv.), and a solution of cysteine (final concentration 20 mM). The reaction mixture was incubated at 20°C for 1 d, and then heated at 100°C for 10 min. After cooling, it was centrifuged for 10 min at 4000 rpm and the supernatant was extracted. The extraction procedure was performed twice with 1 ml buffer, and the aqueous fractions were pooled and lyophylized. The residue was redissolved in pyridine (0.5 ml), and acetic anhydride (0.5 ml) and DMAP (5 mg) were added. The reaction mixture was stirred at room temperature for 2 h and then poured into ice/water. The resulting solution was extracted with CH_2Cl_2, and the organic phase was washed with saturated solutions of $NaHCO_3$, $KHSO_4$, and NaCl, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography (chloroform/acetone, 15:1, v/v) to give compound **12** (0.9 mg, 36%). − R_f = 0.2 ($CHCl_3$/acetone, 15:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 1.20 (d, 3 H, 6′′-H), 2.37−2.18 (overlapping s, 27 H, $COCH_3$), 3.52 (s, 3 H, OCH_3), 3.91−3.84 (m, 3 H, 5-H, 2′-H, 5′-H), 3.99 (dd, 1 H, $J_{3,4}$ = 3.5 Hz, 3-H), 4.28−4.10 (m, 4 H, 6a-H, 6b-H, 6′a-H, 6′b-H), 4.36 (m, 1 H, 5′′-H), 4.39 (d, 1 H, $J_{1,2}$ = 8.0 Hz, 1-H), 4.60 (d, 1 H, $J_{1',2'}$ = 7.8 Hz, 1′-H), 5.03 (dd, 1 H, $J_{3',4'}$ = 3.5 Hz, 3′-H), 5.05 (dd, 1 H, $J_{3'',4''}$ = 3.5 Hz, 3′′-H), 5.18 (dd, 1 H, $J_{2,3}$ = 10.2 Hz, 2-H), 5.30 (dd, 1 H, $J_{2'',3''}$ = 11.0 Hz, 2′′-H), 5.39−5.31 (m, 3 H, 4′-H, 1′′-H, 4′′-H), 5.52 (d, 1 H, $J_{3,4}$ = 3.5 Hz, 4-H). − MALDI-TOF MS; $C_{37}H_{52}O_{24}$ (880.81): *m/z* = 903 [M + Na]$^+$.

O-{2-Acetamido-2-deoxy-3-O-[2-O-(α-L-fucopyranosyl)-β-D-galactopyranosyl]-α-D-galactopyranosyl}-L-threonine (**13**): To albumen glands (2.5 g) in Tris/HCl buffer (50 mM, pH 7.6, 2.5ml), were added compound **2** (2.5 mg, 5.2 μmol), GDP-L-fucose (4.1 mg, 6.5 μmol, 1.2 equiv.), and a solution of cysteine (final concentration 20 mM). The reaction mixture was incubated at 20°C for 1 d and then heated at 100°C for 10 min. After cooling, it was centrifuged for 10 min at 4000 rpm and the supernatant was extracted. The extraction procedure was performed twice with 1 ml buffer and the aqueous fractions were pooled and lyophylized. The residue was applied to a Biogel P2 column and eluted with water to give compound **13** (0.8 mg, 24%). − R_f = 0.3 (isopropanol/ethyl acetate/aq. ammonia/water, 6:4:1:5, v/v/v/v). − ^{1}H NMR (D_2O): δ = 1.12 (d, 3 H, 6′′-H), 1.36 (d, 3 H, $J_{\beta CH,CH3}$ = 6.9 Hz, ThrCH_3), 2.01 (s, 3 H, $COCH_3$), 3.78−3.56 (m, 13 H, 6a-H, 6b-H, 2′-H, 3′-H, 4′-H, 5′-H, 6′a-H, 6′b-H, 2′′-H, 3′′-H, 4′′-H, 5′′-H, ThrαCH), 3.85 (dd, 1 H, $J_{3,4}$ = 2.5 Hz, 3-H), 4.08−4.04 (m, 2 H, 4-H, 5-H), 4.14 (dd, 1 H, $J_{2,3}$ = 11.0 Hz, 2-H), 5.40−4.38 (m, 1 H, ThrαCH), 4.58 (d, 1 H, $J_{1',2'}$ = 7.9 Hz, 1′-H), 4.90 (d, 1 H, $J_{1,2}$ = 3.8 Hz, 1-H), 5.17 (d, 1 H, $J_{1'',2''}$ = 4.1 Hz, 1′′-H). − MALDI-TOF MS; $C_{24}H_{42}N_2O_{17}$ (630.62): *m/z* = 629 [M − H].

Methyl 2,4,6-Tri-O-acetyl-3-O-[3,4,6-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-α-D-galactopyranoside (**14**): To albumen glands (0.5 g) in Tris/HCl buffer (50 mM, pH = 7.6), were added compound **6** (1 mg, 2.8 μmol), GDP-L-fucose (2.2 mg, 3.5 μmol, 1.2 equiv.), and a solution of cysteine (final concentration 20 mM). The reaction mixture was incubated at 20°C for 1 d and then heated at 100°C for 10 min. After cooling, it was centrifuged for 10 min at 4000 rpm and the supernatant was extracted. The extraction procedure was performed twice with 1 ml buffer and the aqueous fractions were pooled and lyophylized. The residue was dissolved in pyridine (0.5 ml), and acetic anhydride (0.5 ml) and DMAP (5 mg) were added. The reaction mixture was stirred at room temperature for 2 h and then poured into ice/water. The resulting solution was extracted with CH_2Cl_2, and the organic phase was washed with saturated solutions of $NaHCO_3$, $KHSO_4$, and NaCl, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography (chloroform/acetone, 15:1, v/v) to give compound **14** (0.8 mg, 33%). − R_f = 0.2 ($CDCl_3$/acetone, 15:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 1.26 (d, 3 H, 6′′-H), 2.21−2.01 (overlapping s, 27 H, $COCH_3$), 3.33 (s, 3 H, OCH_3), 3.74 (t, 2 H, $J_{2',3'}$ = 9.8 Hz, 2′-H), 3.90 (t, 1 H, $J_{3,4}$ = 3.8 Hz, 3-H), 4.19−4.02 (m, 7 H, 5-H, 6a-H, 6b-H, 5′-H, 6′a-H, 6′b-H, 5′′-H), 4.59 (d, 1 H, $J_{1',2'}$ = 7.6 Hz, 1′-H), 4.88 (d, 1 H, $J_{1,2}$ = 3.6 Hz, 1-H), 4.90 (dd, 1 H, $J_{3'',4''}$ = 3.7 Hz, 3′′-H), 4.93 (dd, 1 H, $J_{3',4'}$ = 3.5 Hz, 3′-H), 5.03 (dd, 1 H, $J_{2'',3''}$ = 10.7 Hz, 2′′-H), 5.11−5.08 (m, 2 H, 1′′-H, 4′′-H), 5.21−5.20 (m, 2 H, 2-H, 4′-H), 5.42 (d, 1 H, $J_{3,4}$ = 3.8 Hz, 4-H). − MALDI-TOF MS; $C_{37}H_{52}O_{24}$ (880.81): *m/z* = 903 [M + Na]$^+$.

Methyl 2-Acetamido-2-deoxy-4,6-di-O-acetyl-3-O-[3,4,6-tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-β-D-galactopyranosyl]-β-D-galactopyranoside (**15**): To albumen glands (2 g) in Tris/HCl buffer (50 mM, pH = 7.6, 2 ml), were added compound **11** (2 mg, 5.0 μmol), GDP-L-fucose (4.0 mg, 6.3 μmol, 1.3 equiv.), and a solution of cysteine (final concentration 20 mM). The reaction mixture was incubated at 20°C for 1 d and then heated at 100°C for 10 min. After cooling, it was centrifuged for 10 min at 4000 rpm and the supernatant was extracted. The extraction procedure was performed twice with 1 ml buffer and the aqueous fractions were pooled and lyophylized. The residue was dissolved in pyridine (2 ml), and acetic anhydride (2 ml) and DMAP (10 mg) were added. The reaction mixture was stirred at room temp. for 2 h and then poured into ice/water. The resulting solution was extracted with CH_2Cl_2, and the organic phase was washed with saturated solutions of $NaHCO_3$, $KHSO_4$, and NaCl, dried with Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (chloroform/ethyl acetate/methanol, 1:1:0→1:1:0.2) to give compound **15** (1.6 mg, 36%). − R_f = 0.3 ($CHCl_3$/acetone, 15:1, v/v). − ^{1}H NMR ($CDCl_3$): δ = 1.20 (d, 3 H, 6′′-H), 2.12−1.81 (overlapping s, 27 H, $COCH_3$), 2.70 (m, 1 H, 2-H), 3.46 (s, 3 H, OCH_3), 3.67 (m, 1 H, 5-H), 3.82−3.78 (m, 2 H, 2′-H, 5′-H), 4.08−4.01 (m, 3 H, 6-H, 6′a-H, 6′b-H), 4.25 (m, 1 H, 6-H), 4.37 (d, 1 H, $J_{1',2'}$ = 7.6 Hz, 1′-H), 4.57 (q, 1 H, $J_{5'',6''}$ = 6.6 Hz, 5′′-H), 4.82−4.75 (m, 3 H, 1-H, 3-H, 4-H), 4.90−4.87 (m, 2 H, 3′-H, 2′′-H), 5.14 (d, 2 H, $J_{1'',2''}$ = 3.6 Hz, 1′′-H), 5.17 (dd, 1 H, $J_{2'',3''}$ = 8.2, $J_{3'',4''}$ = 3.8 Hz, 3′′-H), 5.33 (d, 1 H, $J_{3'',4''}$ = 3.4 Hz, 4′′-H), 5.38 (d, 1 H, $J_{3',4'}$ = 3.5 Hz, 4′-H), 5.69 (s, 1 H, NH). − MALDI-TOF MS; $C_{37}H_{53}NO_{23}$ (879.82): *m/z* = 902 [M + Na]$^+$.

[1] G. M. Edelmann, *Science* **1983**, *219*, 450−457.
[2] D. C. Wiley, J. A Wilson, J. J. Skeiel, *Nature* **1981**, *289*, 373−378.
[3] T. Feizi, R. Childs, *Trends Biochem. Sci.* **1985**, *10*, 24−29.
[4] R. Kornfeld, S. Kornfeld, *Ann. Rev. Biochem.* **1976**, *45*, 217−237.
[5] J. M. McKiblin, *J. Lipid. Res.* **1978**, *19*, 131−147.
[6] A. Kobata in *The Glycoproteins*, vol. 1 (Eds.: M. I. Horowitz, W. Pigman), Academic Press, New York, **1977**, p. 423−440.
[7] A. Sarnesto, T. Kohlin, O. Hindsgaul, J. Thurin, M. Blaszczyk-Thurin, *J. Biol. Chem.* **1992**, *267*, 2737−2744.
[8] H. Lüttge, T. Heidelberg, K. Stangier, J. Thiem, H. Bretting, *Carbohydr. Res.* **1997**, *297*, 281−288.
[9] U. Gambert, J. Thiem, *Carbohydr. Res.* **1997**, *299*, 85−89.
[10] U. Gambert, R. Gonzales Lio, E. Farkas, J. Thiem, V. V. Bencomo, A. Liptak, *Bioorg. Med. Chem.* **1997**, *5*, 1285−1291.
[11] B. W. Murray, V. Wittmann, M. D. Burkart, S.-C. Hung, C.-H. Wong, *Biochemistry* **1997**, *36*, 823−831.

[98209]