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Enzymatic synthesis of fucose-containing disaccharides employing the partially purified α-L-fucosidase from *Penicillium multicolor*

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Dedicated to Professor Günther Wulff on his 65th birthday

Abstract

The α-L-Fuc*p*-(1→3)-D-Glc*p*NAc disaccharide structure is a vital core unit of the oligosaccharide components of glycoconjugates isolated from human milk and blood group substances. α-L-Fucosidase from *Penicillium multicolor* catalyses the transfer of L-fucose from donor structures such as α-L-Fuc*p*O*p*NP and α-L-Fuc*p*F to various Glc*p*NAc derivatives and Glc*p*, forming α-(1→3) linkages. The synthesis of several biologically relevant disaccharides including α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOMe, α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOAll, α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAcOAll, α-L-Fuc*p*-(1→3)-D-Glc*p*NAc and α-L-Fuc*p*-(1→3)-D-Glc*p* has been achieved in up to 34% yields by application of this enzyme.

Keywords: Enzyme catalysis; Transglycosylation; α-L-Fucosidase; *Penicillium multicolor*; α-L-Fuc*p*-(1→3) linkage

1. Introduction

A number of L-fucose-containing glycoconjugates have important biological functions, such as growth regulation, receptor functions, cell–cell interactions, and antigenicity [1]. Further, they were shown to act as chain-stoppers in biosynthesis by controlling the extent of the chain elongation [2]. The occurrence of oligosaccharides containing fucose has been well-established in human milk [3] e.g. lacto-*N*-fucopentaoses I–III and in blood group substances e.g. H, Le^x, Le^a, Le^b determinants [4,5].

Classical organic chemical procedures have been developed for the synthesis of fucose-containing complex oligosaccharides [6,7], but they are frequently complicated because of many protection and deprotection steps or difficult separations of mixtures of anomers, which is reflected in decreased overall yields. Therefore, in recent years, attempts have been made to utilise fucose-transferring enzymes in syntheses of such oligosaccharides. Among enzymatic methods, especially the application of glycoside hydrolases (glycosidases) is advantageous, because of high stereo- and regioselectivity in the transglycosylation. Isolation and handling of hydrolases is comparatively facile and their application does not involve expensive donors and cofactors [8–11].

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α-L-Fucosidases (α-L-fucoside fucohydrolase, EC 3.2.1.51) are important enzymes in the metabolism of biological substances containing L-fucose. α-L-Fucosidases have been isolated from a number of prokaryotic and eukaryotic sources and are classified into four groups, namely, those from bacteria and moulds [12–16], molluscs [17,18], plants [19,20] and mammals [21–30]. The substrate specificities of these enzymes differ depending on their origin. Those from mammalian tissues and marine gastropods have a broad substrate specificity, and they cleave α-L-Fuc*p*-(1→3)-D-Glc*p*NAc, α-L-Fuc*p*-(1→4)-D-Glc*p*NAc, α-L-Fuc*p*-(1→6)-D-Glc*p*NAc and α-L-Fuc*p*-(1→2)-D-Gal*p* linkages, whereas the enzymes from microorganisms and almond emulsin cleave only the α-L-Fuc*p*-(1→2)-D-Gal*p* linkage.

The transglycosylation activity of fucosidases was observed in the following cases:

- porcine liver fucosidase by Svensson and Thiem in 1990 [27] and also by Murata et al. in 1999 [30];
- fucosidases from bovine kidney [28] and from bovine testes [29] by Nilsson et al.;
- fucosidases from *Aspergillus niger*, *Corynebacterium* sp, Ampullaria and *Penicillium multicolor* by Ajisaka et al. [12,31];
- the fucosidase from *A. niger* by Vetere et al. [32];
- a fucosidase from *Alcaligenes* sp. by Murata et al. [33].

We report here the application of *p*-nitrophenyl α-L-fucopyranoside (**1**) and α-L-fucopyranosyl fluoride (**2**) in the enzymatic synthesis of the following α-L-Fuc*p*-(1→3) structures by use of α-L-fucosidase from *P. multicolor*: α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOMe (**8**), α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOAll (**10**), α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAcOAll (**12**), α-L-Fuc*p*-(1→3)-D-Glc*p*NAc (**14**) and α-L-Fuc*p*-(1→3)-D-Glc*p* (**16**).

2. Results and discussion

The enzymatic synthesis of Fucα-(1→3) glycosidically linked structures was achieved by transglycosylation using α-L-fucosidase from *P. multicolor* (EC 3.2.1.51.). In all cases the donors *p*-nitrophenyl α-L-fucopyranoside (**1**) and α-L-fucopyranosyl fluoride (**2**) exclusively transferred fucose to the 3-positions of the appropriate acceptor unit, resulting in the required α-(1→3) linkage. This enzymatic fucosylation could be successfully applied to the following acceptors: Glc*p*NAcαOMe **3**, Glc*p*NAcαOAll **4**, Glc*p*NAcβOAll **5**, Glc*p*NAc **6** and Glc*p* **7**.

α-L-Fucosidase from *P. multicolor* was purified according to the method of Ajisaka et al. [31]. The crude enzyme preparation (lactase-P) from *P. multicolor* contained β-D-galactosidase (66 U/g, measured on *p*-nitrophenyl β-D-galactopyranoside), and β-D-hexosaminidase (9 U/g, measured on *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside), as well as α-L-fucosidase (5 U/g, measured on *p*-nitrophenyl α-L-fucopyranoside) as minor components. This crude enzyme preparation was dialysed, then purified by HTP-gel column chromatography with gradient phosphate buffer (1–400 mM sodium phosphate buffer, pH 6.8), and after the second dialysis lyophilised. The fucosidase activity of this partially purified enzyme preparation employed in transfucosylation reactions was 21 U/g, but it still contained also β-D-galactosidase (7.4 U/g), and β-D-hexosaminidase (14 U/g).

In the reactions applying *p*-nitrophenyl α-L-fucopyranoside (**1**) as donor 5 equivalents of Glc*p*NAcαOMe **3** [33], Glc*p*NAcαOAll **4** [34] and Glc*p*NAcβOAll **5** [34] as acceptors were incubated in sodium acetate buffer (pH 5.0) with the enzyme preparation. After 30 h, the reaction was worked up and purified by Biogel P2 column chromatography with water as eluent. The disaccharide products were acetylated under standard conditions with acetic anhydride in pyridine and subsequently purified by column chromatography. The peracetylated α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOMe (**9**), α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOAll (**11**) and α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAcOAll (**13**) were isolated in 29, 34 and 25% yields, respectively (Table 1, Scheme 1).

In the reaction of the acceptor Glc*p*NAcβOAll **5**, as a side product β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAcOAll was also observed

in $<2\%$ yield, the formation of which was caused by the β-hexosaminidase activity of the applied enzyme preparation.

Because of the low solubility of *p*-nitrophenyl α-L-fucopyranoside in aqueous solutions, other glycosyl donors were also considered. Since glycosyl fluorides are useful substrates for hydrolases, α-L-fucopyranosyl fluoride (**2**) was synthesised [27].

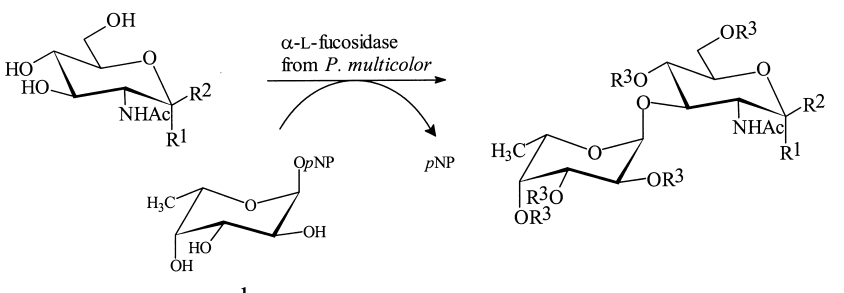
In the reactions applying α-L-fucopyranosyl fluoride (**2**) as donor, 5 equivalents of Glc*p*NAcαOMe **3**, Glc*p*NAcαOAll **4**, Glc*p*NAcβOAll **5**, Glc*p*NAc **6** and Glc*p* **7** as acceptors were incubated in sodium acetate buffer (pH 5.0) with the enzyme preparation. After 24 h the donor was completely hydrolysed, the reaction was worked up and purified by Biogel P2 column chromatography with water. The following fucose-containing disaccharide products were isolated and acetylated to give α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOMe (**9**), α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOAll (**11**), α-L-Fuc*p*-(1→3)-D-Glc*p*NAc (**15**) and α-L-Fuc*p*-(1→3)-D-Glc*p* (**17**) in 13, 7, 5 and 34% yields, respectively (Table 1). The generally lower yields applying α-L-fucopyranosyl fluoride as donor in comparison to the *p*-nitrophenyl α-L-fucopyranoside can be explained by the increased hydrolysis rate of the fluoride donor.

In the reactions using α-L-fucopyranosyl fluoride (**2**) as donor and Glc*p*NAcβOAll **5** as acceptor, β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*-NAcOAll was obtained as the main product

Table 1
Regioselective synthesis of α-L-fucose-containing disaccharides utilising *P. multicolor* α-L-fucosidase

Donor	Acceptor	Fucosylated product (peracetylated)	Yield (%) [a]
α-L-Fuc*p*O*p*NP **1**	Glc*p*NAcαOMe **3**	α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOMe **9**	29
α-L-Fuc*p*O*p*NP **1**	Glc*p*NAcαOAll **4**	α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOAll **11**	34
α-L-Fuc*p*O*p*NP **1**	Glc*p*NAcβOAll **5**	α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAcOAll **13**	25
α-L-Fuc*p*F **2**	Glc*p*NAcαOMe **3**	α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOMe **9**	13
α-L-Fuc*p*F **2**	Glc*p*NAcαOAll **4**	α-L-Fuc*p*-(1→3)-α-D-Glc*p*NAcOAll **11**	8
α-L-Fuc*p*F **2**	Glc*p*NAcβOAll **5**	α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAcOAll **13**	–
α-L-Fuc*p*F **2**	Glc*p*NAc **6**	α-L-Fuc*p*-(1→3)-D-Glc*p*NAc **15**	7
α-L-Fuc*p*F **2**	Glc*p* **7**	α-L-Fuc*p*-(1→3)-D-Glc*p* **17**	34

[a] Isolated yield of the peracetylated derivatives based on the donor substrate.

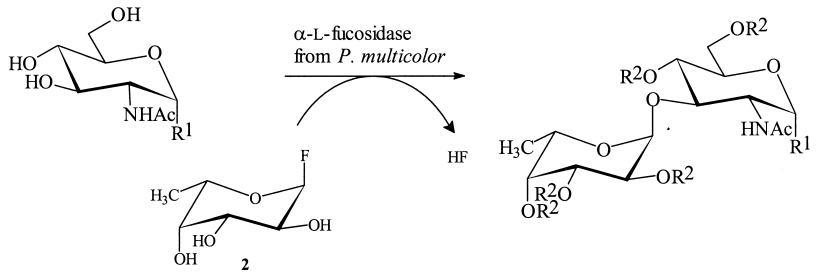


1

	R^1	R^2
3	OMe	H
4	OAll	H
5	H	OAll

	R^1	R^2	R^3
8	OMe	H	H
9	OMe	H	Ac
10	OAll	H	H
11	OAll	H	Ac
12	H	OAll	H
13	H	OAll	Ac

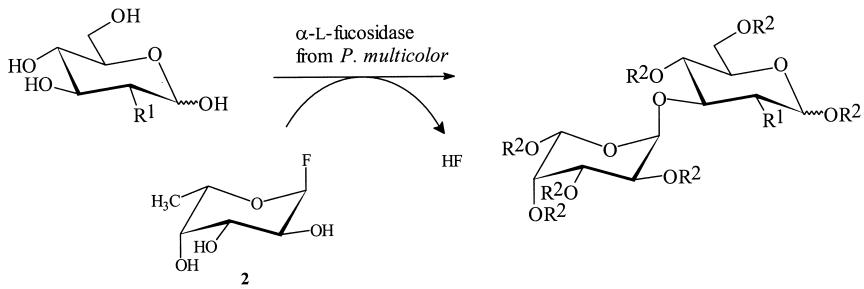
Scheme 1. Preparation of fucose-containing disaccharides using *p*-nitrophenyl α-L-fucopyranoside as glycosyl donor.



	R1
3	OMe
4	OAll

	R1	R2
8	OMe	H
9	OMe	Ac
10	OAll	H
11	OAll	Ac

Scheme 2. Preparation of fucose-containing disaccharides using α-L-fucopyranosyl fluoride as glycosyl donor.



	R1
6	NHAc
7	OH

	R1	R2
14	NHAc	H
15	NHAc	Ac
16	OH	H
17	OAc	Ac

Scheme 3. Preparation of further fucose-containing disaccharides using α-L-fucopyranosyl fluoride as glycosyl donor.

in 12%, however, the desired α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAcOAll (**12**) could not be isolated (Schemes 2 and 3).

3. Experimental

General methods.—The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60 F_{254}, E. Merck). Compounds were visualised by UV irradiation and/or spraying with 20% sulfuric acid in EtOH, followed by charring at 150 °C. Column chromatography was performed on Silica Gel 60 M (0.040–0.063 mm, E. Merck) or Biogel P2. Optical rotations were determined at rt with a Perkin–Elmer 241 and 341 polarimeter. NMR spectra were accumulated with a Bruker AMX 400 spectrometer. Chemical shifts are given in ppm (δ) (Table 2). Mass spectra were recorded with a Bruker MALDI-TOF mass spectrometer (with N_2 laser operating at 337 nm and 5 μL of 2,5-dihydroxybenzoic acid as matrix) and FAB with a VG 70-250S mass spectrometer (*m*-nitrobenzylalcohol as matrix). Lactase-P from *P. multicolor* was a kind gift from K-I Chemical Industrial Co., Ltd. (Shizuoka, Japan). *p*-Nitrophenyl α-L-fucopyranoside, *p*-nitrophenyl β-D-galactopyranoside and *p*-nitrophenyl *N*-acetyl-β-D-glucopy-

ranoside were purchased from Sigma, Germany.

General procedures

α-L-Fucosidase assay. 0.1 mL enzyme solution (2 mg enzyme powder/mL) was added to 0.1 mL of 2 mM *p*-nitrophenyl α-L-fucopyranoside in 0.1 M sodium acetate buffer (pH 5.0) and 0.1 mL of 0.1 M sodium acetate buffer (pH 5.0) and the mixture was incubated at 37 °C for 30 min. The reaction was stopped by adding 1 mL of 0.1 M glycine buffer (pH 10.0) and the liberated *p*-nitrophenol was determined spectrophotometrically. The absorbance was measured in cells with 1-cm light path at 410 nm. One unit of activity was defined as the amount of the enzyme releasing 1 μmol *p*-nitrophenol per min.

β-D-Galactosidase assay. 0.025 mL enzyme solution (2 mg enzyme powder/mL) was added to 0.05 mL of 2 mM *p*-nitrophenyl β-D-galactopyranoside in 0.1 M sodium acetate buffer (pH 5.0) and 0.1 mL of 0.1 M sodium acetate buffer (pH 5.0) and the mixture was incubated at 37 °C for 30 min. The reaction was stopped by adding 1 mL of 0.1 M glycine buffer (pH 10.0) and the liberated *p*-nitrophenol was determined spectrophotometrically. The absorbance was measured in cells with 1-cm light path at 410 nm.

β-D-Hexosaminidase assay. 0.1 mL enzyme solution (2 mg enzyme powder/mL) was added to 0.1 mL of 2 mM *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside in 0.1 M sodium acetate buffer (pH 5.0) and 0.1 mL of 0.1 M sodium acetate buffer (pH 5.0), and the mixture was incubated at 37 °C for 30 min. The reaction was stopped by adding 1 mL of 0.1 M glycine buffer (pH 10.0) and the liberated *p*-nitrophenol was determined spectrophotometrically. The absorbance was measured in cells with 1-cm light path at 410 nm.

Preparation of fucose-containing disaccharides using p-*nitrophenyl α-L-fucopyranoside as glycosyl donor*. α-L-Fuc*p*O*p*NP 1 (20 mg, 0.07 mmol) was incubated with 5 equiv of Glc*p*NAcαOMe **3** [33], Glc*p*NAcαOAll **4** [34], or Glc*p*NAcβOAll **5** [34] and the enzyme preparation (0.4 U) in 0.5 mL of sodium acetate buffer (0.1 M, pH 5.0) at 37 °C. After 30 h the reactions were terminated by heating to 90 °C for 5 min. The solution was extracted with EtOAc to remove *p*-nitrophenol and lyophilised. The residue was applied to a Biogel P2 column and eluted with water to give compounds **8**, **10** and **12**. Compounds **8**, **10** and **12** were dissolved in pyridine and Ac_2O and DMAP were added. The reaction mixture was stirred at rt for 12 h and dried in vacuo. The residue was co-evaporated with toluene, concentrated and purified by column chromatography (17:3 CH_2Cl_2–acetone) to yield compounds **9**, **11** and **13** in 29, 34 and 25% yields, respectively.

Table 2
^{13}C chemical shifts of peracetylated α-L-fucose-containing disaccharides in $CDCl_3$ solution

Compound	Residue	Chemical shifts (δ)					
		C-1	C-2	C-3	C-4	C-5	C-6
9	GlcNAcαOMe	96.21	52.04	74.84	69.13	67.07	61.14
	Fuc	97.22	67.98	66.22	70.17	64.63	14.57
11	GlcNAcαOAll	95.51	52.10	74.86	68.05	67.35	61.10
	Fuc	96.25	69.14	66.21	70.16	64.68	14.59
13	GlcNAcβOAll	97.11	56.76	75.26	69.94	70.85	61.54
	Fuc	95.83	67.66	66.29	70.17	64.51	14.54
15	GlcNAc (α)	89.73	66.08	69.18	68.34	74.78	60.62
	Fuc	96.62	66.08	68.53	70.06	65.05	14.57
17	Glc (α)	88.19	69.25	72.88	67.69	69.93	60.69
	Glc (β)	90.73	67.99	71.97	67.69	70.00	60.69
	Fuc (α)	95.63	67.32	66.27	70.63	64.35	14.81
	Fuc (β)	95.38	67.30	66.16	70.55	64.13	14.68

Preparation of fucose-containing disaccharides using α-L-fucopyranosyl fluoride as glycosyl donor. α-L-Fuc*p*F **2** (13 mg, 0.08 mmol) was incubated with 5 equiv of Glc*p*NAcαOMe **3**, Glc*p*NAcαOAll **4**, Glc*p*NAcβOAll **5**, Glc*p*NAc **6** or Glc*p* **7** and the enzyme preparation (0.4 U) in 0.5 mL of sodium acetate buffer (0.1 M, pH 5.0) at 37 °C. After 24 h the reactions were terminated by heating to 90 °C for 5 min. The residue was applied to a Biogel P2 column and eluted with water to give compounds **8**, **10**, **14** and **16**. Compounds **8**, **10**, **14** and **16** were dissolved in pyridine and Ac_2O and DMAP were added. The reaction mixture was stirred at rt for 12 h and dried in vacuo. The residue was co-evaporated with toluene, concentrated and purified by column chromatography (17:3 or 4:1 CH_2Cl_2–acetone) to yield compounds **9**, **11**, **15** and **17** in 13, 7, 5 and 34% yields, respectively.

Methyl (2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranoside (**9**).—$[\alpha]_D^{20}$ −17° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.75 (d, 1 H, NH), 5.27 (dd, 1 H, $J_{3',4'}$ 3.05 Hz, H-3′), 5.23 (dd, 1 H, $J_{4',5'}$ 1.02 Hz, H-4′), 5.19 (d, 1 H, $J_{1',2'}$ 3.56 Hz, H-1′), 5.11 (dd, 1 H, $J_{2',3'}$ 10.7 Hz, H-2′), 5.07 (t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 4.82 (d, 1 H, $J_{1,2}$ 3.56 Hz, H-1), 4.25 (m, 1 H, $J_{2,3}$ 9.7 Hz H-2), 4.20–4.13 (m, 2 H, H-5′, H-6a), 4.06 (dd, 1 H, H-6b), 3.89 (dd, 1 H, $J_{3,4}$ 3.05 Hz, H-3), 3.79 (m, 1 H, H-5), 3.35 (s, 3 H, OCH_3), 2.08, 2.07, 2.03, 2.00, 1.94, 1.90 (6 s, 18 H, 5 OCOCH_3, 1 NHCOCH_3), 1.01 (d, 3 H, H-6′); ^{13}C NMR ($CDCl_3$): δ 169.78–168.39 (C=O), 97.22 (C-1), 96.21 (C-1′), 74.84 (C-3), 70.17 (C-4′), 69.13 (C-4), 67.98 (C-2′) 67.07 (C-5), 66.22 (C-3′), 64.63 (C-5′), 61.14 (C-6), 54.33 (OCH_3), 52.04 (C-2), 22.28 (NHCOCH_3), 19.96–19.61 (OCOCH_3), 14.57 (C-6′); FABMS *m/z* 592.2 $[M+H]^+$; $C_{25}H_{37}O_{15}N$ (591.22).

Allyl (2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-di-O-acetyl-2-deoxy-α-D-glucopyranoside (**11**).—$[\alpha]_D^{20}$ −10° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.86 (m, 1 H, =CH–), 5.78 (d, 1 H, NH), 5.29–5.25 (m, 2 H, H-3′, -4′), 5.24–5.18 (m, 3 H, CH_2=, $J_{1',2'}$ 3.56 Hz, H-1′), 5.11 (dd, 1 H, $J_{2',3'}$ 11.7 Hz, H-2′), 5.07 (t, 1 H, H-4), 4.98 (d, 1 H, $J_{1,2}$ 3.56 Hz, H-1), 4.26 (m, 1 H, H-2), 4.20–4.10 (m, 3 H, H-5′, H-6a, –CH_2–), 4.04 (dd, 1 H, H-6b), 3.97 (m, 1 H, –CH_2–), 3.90 (dd, 1 H, $J_{2,3}$ 9.2, $J_{3,4}$ 10.7 Hz, H-3), 3.83 (m, 1 H, H-5), 2.13, 2.11, 2.08, 2.05, 1.98, 1.95 (6 s, 18 H, 5 OCOCH_3, 1 NHCOCH_3), 1.07 (d, 3 H, H-6′); ^{13}C NMR ($CDCl_3$): δ 169.75–168.37 (C=O), 132.30 (–CH=), 117.16 (CH_2=), 96.25 (C-1′), 95.51 (C-1), 74.86 (C-3), 70.16 (C-4′), 69.14 (C-2′), 68.05 (C-4), 67.77 (–CH_2–), 67.35 (C-5), 66.21 (C-3′), 64.68 (C-5′), 61.10 (C-6), 52.10 (C-2), 22.23 (NHCOCH_3), 19.91–19.60 (OCOCH_3), 14.59 (C-6′); FABMS *m/z* 618.5 $[M+H]^+$; $C_{27}H_{39}O_{15}N$ (617.23).

Allyl (2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1→3)-2-acetamido-4,6-di-O-acetyl-2-deoxy-β-D-glucopyranoside (**13**).—$[\alpha]_D^{20}$ −65° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.85 (m, 2 H, =CH, NH), 5.33–5.16 (m, 5 H, H-1′, -3′, -4′, CH_2=), 5.08 (dd, 1 H, $J_{1',2'}$ 3.57, $J_{2',3'}$ 10.7 Hz, H-2′), 5.00 (d, 1 H, $J_{1,2}$ 8.14 Hz, H-1), 4.94 (t, 1 H, H-4), 4.41 (t, 1 H, $J_{2,3}$ 9.66, $J_{3,4}$ 9.16 Hz, H-3), 4.30 (m, 1 H, –CH_2–), 4.20–4.03 (m, 4 H, H-6a,b, H-5′, –CH_2–), 3.61 (m, 1 H, H-5), 3.26 (m, 1 H, H-2), 2.13, 2.09, 2.07, 2.05, 1.98, 1.96 (6 s, 18 H, 5 OCOCH_3, 1 NHCOCH_3), 1.06 (d, 3 H, H-6′); ^{13}C NMR ($CDCl_3$): δ 169.74–168.3 (C=O), 132.58 (–CH=), 116.99 (CH_2=), 97.11 (C-1), 95.83 (C-1′), 75.26 (C-3), 70.82 (C-5), 70.17 (C-4′), 69.94 (C-4), 69.27 (–CH_2–), 67.66 (C-2′), 66.29 (C-3′), 64.51 (C-5′), 61.54 (C-6), 56.76 (C-2), 22.54 (NHCOCH_3), 20.03–19.61 (OCOCH_3), 14.54 (C-6′); FABMS *m/z* 618.5 $[M+H]^+$; $C_{27}H_{39}O_{15}N$ (617.23).

2,3,4-Tri-O-acetyl-α-L-fucopyranosyl-(1→3)-2-acetamido-1,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranose (**15**).—$[\alpha]_D^{20}$ −19° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.31 (d, 1 H, $J_{1,2}$ 3.05 Hz, H-1α), 5.73 (d, 1 H, NH), 5.31 (dd, 1 H, $J_{2',3'}$ 10.7 Hz, H-2′), 5.24–5.20 (m, 2 H, H-4′, H-1′), 5.18–5.12 (m, 2 H, H-3′, H-4), 4.33 (m, 1 H, H-2), 4.20–4.14 (m, 2 H, H-5′, H-6a), 4.02 (dd, 1 H, H-6b), 3.93–3.85 (m, 2 H, H-3, H-5), 2.15, 2.13, 2.12, 2.07, 2.06, 1.98, 1.96 (7 s, 21 H, 6 OCOCH_3, 1 NHCOCH_3), 1.10 (d, 3 H, H-6′); ^{13}C NMR ($CDCl_3$): δ 169.76–167.43 (C=O), 96.62 (C-1′), 89.73 (C-1), 74.78 (C-5), 70.06 (C-4′), 69.18 (C-3), 68.53 (C-3′), 68.34 (C-4), 66.08 (C-2′), 65.05 (C-5′), 60.62 (C-6), 51. 38 (C-2), 22.09 (NHCOCH_3), 19.87–19.60 (OCOCH_3), 14.57 (C-6′);

MALDI TOF 624.3 $[M + Na]^+$, 658.2 $[M + K]^+$; FABMS m/z 620.4 $[M + H]^+$; $C_{26}H_{37}O_{16}N$ (619.21).

2,3,4-Tri-O-acetyl-α-L-fucopyranosyl-(1→3)-1,2,4,6-tetra-O-acetyl-α,β-D-glucopyranose (**17**).—$[\alpha]_D^{20}$ −61° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.23 (d, 1 H, $J_{1\alpha,2\alpha}$ 3.56 Hz, H-1α), 5.60 (d, 1 H, $J_{1\beta,2\beta}$ 8.14 Hz, H-1β), 5.37 (d, 1 H, $J_{1',2'}$ 4.07 Hz, H-1′α), 5.25–5.12 (m, 5 H, H-3′α, β, H-2α, β, H-1′β), 5.10–5.00 (m, 4 H, H-4α, β, H-2′α, β), 4.20–4.00 (m, 9 H, H-6a,b, α, β, H-4′α, β, H-5′α, β, H-3α), 3.99 (m, 1 H, H-5α), 3.92 (t, 1 H, H-3β), 3.69 (m, 1 H, H-5β), 2.15–1.94 (14 s, 42 H, OCOCH_3), 1.12 (2 d, 6 H, H-6′α, β)

^{13}C NMR ($CDCl_3$): δ 169.68–168.05 (C=O), 95.63 (C-1′β), 95.38 (C-1′α), 90.73 (C-1β), 88.19 (C-1α), 72.88 (C-3β), 71.97 (C-3α), 70.62 (C-4′β), 70.55 (C-4′α), 69.99 (C-5β), 69.93 (C-5α), 69.25 (C-2β), 67.99 (C-2α), 67.69 (C-4 α, β), 67.32 (C-2′β), 67.29 (C-2′α), 66.27 (C-3′β), 66.16 (C-3′α), 64.36 (C-5′β), 64.13 (C-5′α), 60.69 (C-6 α, β), 19.73–19.57 (OCOCH_3), 14.81 (C-6′β), 14.68 (C-6′α); FABMS m/z 561.5 $[M - HOAc + H]^+$; $C_{26}H_{36}O_{17}$ (620.20).

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