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α and β L-Fucopyranosyl oxyamines: key intermediates for the preparation of fucose-containing glycoconjugates by oxime ligation

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Abstract—We report herein the synthesis of new α and β aminooxylated L-fucopyranosyl derivatives for the preparation of glycoclusters through oxime ligation. The glycosylation reaction between activated triacetylated L-fucopyranosyl fluoride and *N*-hydroxyphthalimide was carried out in the presence of boron trifluoride–diethyl etherate and the stereochemical outcome of glycosylation was compared in dichloromethane, acetonitrile or tetrahydrofuran. Interestingly, an unexpected α and β anomer ratio was obtained in spite of the presence of an acetate participating group at the carbon 2, particularly the 1,2-*cis* glycosylation was largely favoured in acetonitrile. The resulting α and β *N*-oxyphthalimido fucopyranosyl derivatives were finally deprotected with methylhydrazine to obtain the corresponding free aminooxylated fucopyranosyls. The structure of single-crystal α anomer **12** was analysed by X-ray diffraction.

Keywords: Aminoxylated fucopyranosyl; Glycosylation; Anomeric mixture; Oxime ligation; Crystal structure; Glycocluster

1. Introduction

As part of our programme to develop new chemical tools for glycomics,[1–3] we focussed recently our synthetic efforts on the L-fucose motif which is a relevant part of ligands involved in a wide range of biological processes.[4,5] In mammals, fucose-containing glycans play a crucial role in the recognition events related to fertilisation, development or adhesion of leukocytes to vascular endothelial cells, which is mediated by interactions between selectin and glycans at the initial state of inflammation processes.[6] Moreover, fucosylated glycoconjugates are involved in many diseases, going from pathogen infections[7] to cancers.[8] For example, it has recently been shown that the chronic colonisation of the lung with *Pseudomonas aeruginosa*, which is responsible for infections in patients affected with cystic fibrosis, starts with the adhesion of the bacterium to the host cells through fucose-binding PA-IIL lectin present at its surface.[9]

Inhibiting these host/pathogen recognition processes, which occur mostly through multivalent interactions, might efficiently prevent infections. For this purpose, the synthesis of glycoclusters appears as one of the most promising approaches to discover new active and selective therapeutics.[10] Among the very large panel of reported synthetic methods, chemoselective ligations have emerged as an attractive strategy for the assembly of biomolecules.[11,12] In our laboratory, we have chosen chemoselective oxime bond formation to prepare cyclic decapeptides exhibiting clusters of carbohydrates in solution as well as on solid support for diagnostic or therapeutic applications.[1–3] Our oxime-based strategy relies on the convergent assembly of aminooxylated carbohydrates onto templates presenting aldehyde functions. Herein, we report the synthesis of new fucopyranosyl derivatives **12** and **13** bearing at the anomeric centre both α and β aminooxy functionalities that are essential for the evaluation of the influence of anomeric configurations on recognition (Fig. 1). These derivatives represent key intermediates for further assembly onto scaffolds to form clusters and evaluation

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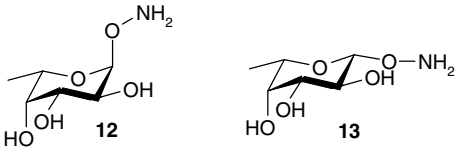


Figure 1. Structure of aminooxylated α and β L-fucopyranosyl **12** and **13**.

of the resulting fucose-containing conjugate as anti-infective agents.

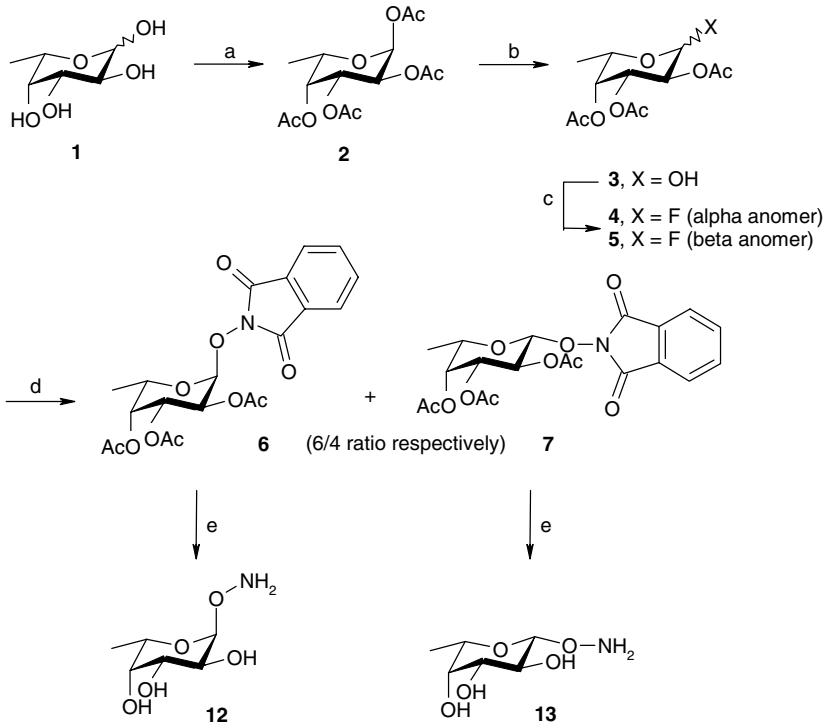
2. Results and discussion

2.1. Synthesis

We reported previously a general method for the preparation of aminooxylated carbohydrate-based ligands[13] and cancer-related antigens[14] using a glycosylation reaction between glycosyl fluoride donors and *N*-hydroxyphthalimide as the key step. This efficient synthetic strategy has been applied to the L-fucose series to prepare the corresponding aminooxylated derivatives (Scheme 1). The commercial L-fucopyranose **1** was first protected with acetates in the presence of pyridine and acetic anhydride. The peracetylated compound **2** was then deprotected regioselectively at the anomeric position by treatment with a solution of ethylenediamine/acetic acid (1/1)[15] in tetrahydrofuran to obtain **3** in 86% yield. A fluoride was introduced as an anomeric leaving group to the triacetylated fucopyranose **3** by using diethylaminosulfur trifluoride (DAST) in THF.[16] The fluorinated compound has been obtained as an α/β anomer mixture (1.2/1, 72% yield).

When the glycosylation reaction was carried out between crude anomer mixture **4/5** and *N*-hydroxyphthalimide in the presence of triethylamine and boron trifluoride–diethyl etherate ($BF_3{\cdot}Et_2O$)[17] as promoter in dichloromethane (Scheme 1), both the α and β fucopyranosyl-*N*-oxyphthalimide anomers **6** and **7** were formed and purified by silica gel chromatography and recrystallisation. They were characterised by using electrospray ionisation mass spectrometry and ^1H, ^{13}C and 2D (GCOSY and GHMQC) NMR experiments. The determination of the coupling constant (*J*) between protons H-1 and H-2 was in good agreement with the anomer configuration of **6** ($J_{1,2} = 4.0$ Hz) and **7** ($J_{1,2} = 8.1$ Hz). Interestingly, this glycosylation gave a poor anomeric ratio in favour of the α anomer (α/β = 1.5/1). While it is well established that the presence of an ester protecting group at C-2 enables the preferential formation of a 1,2-*trans* glycosidic linkage due to the assistance of a neighbouring participating group,[18–20] this result indicates a minor influence of the acetate on the stereoselectivity of this reaction.

Several publications report unexpected stereochemical outcomes for glycosylation. For example, it has been

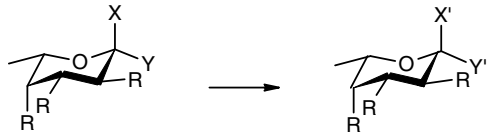


Scheme 1. Reagents and conditions: (a) Ac_2O, pyridine, 6 h, 97; (b) ethylenediamine, AcOH, THF, 12 h, 86%; (c) diethylaminosulfur trifluoride, THF, 1 h, 72% (α/β anomer, 1.2/1); (d) *N*-hydroxyphthalimide, $BF_3{\cdot}Et_2O$, Et_3N, dry CH_2Cl_2, 1 h, 77%; (e) $MeHNNH_2$, EtOH, 12 h, 51%.

suggested that the nature of a protecting group at C-4 may affect the selectivity of glycosylation through a long-range participation, particularly in the galactose and fucose series bearing a benzyl non-participating group at C-2.[21–23] More recently, it was reported that α glycosides can be formed from imidate or thioglycosyl donors despite the presence of acetate at C-2 being capable of neighbouring group participation.[24,25] These intriguing studies prompted us to investigate which factors could influence the stereoselectivity of the glycosylation of L-fucose with *N*-hydroxyphthalimide, in particular the effect of the configuration of the fluoride donor, of the nature of protecting groups and of the reaction solvent. Thus, we first performed the reaction between pure α and β fucopyranosyl fluoride donors **4** and **5** in dry dichloromethane, acetonitrile or tetrahydrofuran. Each fluoride anomer was thus separated by silica gel chromatography with hexane/diethyl ether as eluent to obtain 39% and 33% of pure anomers **4** and **5**, respectively. To evaluate the role of the protecting group at C-2, we then prepared and isolated fucopyranosyl fluorides **8** and **9** protected with benzyl groups as donors.[26] Glycosylations were achieved under the same experimental conditions except the solvent as shown in Table 1. The course of the reactions was followed using reverse phase analytical HPLC and the anomer composition in the crude mixture was determined by measuring the integration of the corresponding peaks. In polar aprotic solvent such as acetonitrile, the glycosylation from acetate protected fluoride donors **4** or **5** gave a large unexpected excess of α anomer **6** (α/β = 4.8/1 from **4**; α/β = 3.7/1 from **5**) in both cases. This stereoselectivity might be explained by a S_N1-type pathway in which the resulting oxonium cation is stabilised by solvation with acetonitrile. This may favour the formation of α glycosyl **6** due to the anomeric effect and decreases the influence of anchimeric assistance, which should have ensured the preferential formation of 1,2-trans β linkage by the nucleophilic attack of the *N*-hydroxyphthalimide at the α face. On the other hand, no trace of β glycosylation was observed when the carbon 2 of donors **8** and **9** is protected with a benzyl non-participating group, thus affording the more thermodynamically stable α anomer **10**.

The outcome of glycosylation was comparable in dichloromethane from benzylated fluoride donors **8** and **9** (α anomer only) for the same reason. However, when performed from acetate protected donors, the glycosylation from α fluoride **4** leads preferentially to α glycosyl **6** as the major product (α/β = 2.2/1), whereas a poor anomeric ratio was observed from **5**. Indeed, it can be postulated that in this apolar solvent the stabilisation of the oxonium cation is lower than in polar solvent. The formation of β glycosyl induced by the neighbouring group participation is thus higher than in acetonitrile, with a slight preference for β anomer **7**

Table 1. Glycosylation reaction with pure fucopyranosyl fluoride and *N*-hydroxyphthalimide



4: R = OAc; X = F; Y = H
5: R = OAc; X = H; Y = F
6: R = OAc; X' = O-NPht; Y' = H
7: R = OAc; X' = H; Y' = O-NPht
8: R = OBn; X = F; Y = H
9: R = OBn; X = H; Y = F
10: R = OBn; X' = O-NPht; Y' = H
11: R = OBn; X' = H; Y' = O-NPht

Solvent	Fluoride donor			Glycosylation product (yield %)
	Compound	Anomer	Protection at C-2	
Acetonitrile	**4**	Alpha	Acetate	α/β Anomers **6**/**7** = 4.8/1 (81)
	5	Beta	Acetate	α/β Anomers **6**/**7** = 3.7/1 (86)
	8	Alpha	Benzyl	α/β Anomer **10** only (68)
	9	Beta	Benzyl	α/β Anomer **10** only (73)
Dichloromethane	**4**	Alpha	Acetate	α/β Anomers **6**/**7** = 2.2/1 (88)
	5	Beta	Acetate	α/β Anomers **6**/**7** = 0.7/1 (81)
	8	Alpha	Benzyl	α/β Anomer **10** only (60)
	9	Beta	Benzyl	α/β Anomer **10** only (66)
Tetrahydrofuran	**4**	Alpha	Acetate	α/β Anomers **6**/**7** = 0.4/1 (80)
	5	Beta	Acetate	α/β Anomers **6**/**7** = 0.4/1 (75)
	8	Alpha	Benzyl	α/β Anomers **10**/**11** = 19/1 (50)
	9	Beta	Benzyl	α/β Anomers **10**/**11** = 16/1 (63)

All reactions have been performed at room temperature in anhydrous solvent in the presence of *N*-hydroxyphthalimide (1 equiv), $BF_3{\cdot}Et_2O$ (4 equiv) and triethylamine (1 equiv) for 2 h. Yields, which are unoptimised, and stereochemistry have been determined by peak integration of HPLC profile of crude mixtures at 214 nm after 1 h.

when the donor displays a 1,2-trans linkage ($\alpha/\beta = 0.7/1$ from **5**).

In contrast, a significant difference in reactivity was observed in tetrahydrofuran. First, the expected β linkage was commonly formed ($\alpha/\beta = 0.4/1$) whatever the anomer configuration of the acetate protected fluoride donors **4** or **5** was. More surprisingly, while the formation of 1,2-*cis* glycoside due to the anomeric effect was predominantly favoured, traces of β anomer **11** were obtained from donors **8** and **9** protected with a benzyl non-participating group. This outcome could be explained by the participation of the solvent in glycosylation, forming an axial oxonium cation which helps the formation of the 1,2-trans linkage ($\alpha/\beta = 19/1$ or 16/1 from **8** and **9**).

The final deprotection was performed by the treatment of **6** and **7** with methylhydrazine in ethanol overnight (Scheme 1). Pure aminooxylated α fucose **12** was obtained as single crystals after evaporation and crystallisation in ethanol, whereas β anomer **13** was isolated after silica gel chromatography with dichloromethane/ethanol (1/1) as eluent. The measurement of coupling constant between H-1 and H-2 of **12** and **13** ($J_{1,2}$ 3.4 Hz, $J_{1,2}$ 8.2 Hz, respectively) was in good agreement with the expected configurations of the anomer centre.

2.2. X-ray crystallographic analysis

Standard results on the structure determination are reported in Table 2.[27] The crystal structure of *O*-α-L-fucopyranosyl oxyamine **12** (Fig. 2) was solved from a pseudo-orthorhombic twin crystal. The real system is a monoclinic crystal with $\beta \sim 90°$ twinned via a twofold rotation about **a**.[28] The asymmetric cell contains two independent molecules and four water molecules. The secondary structure comes out of a very strong net of hydrogen bonds (22 on the all) between the water molecules and **12** (16 bonds) or between two molecules **12** (5 bonds). One hydrogen bond is intramolecular along O(9)–H(9)–O(10). Compound **12** displays the expected distances and angles.[29] The Cremer and Pople parameters[30] calculated for the ring $Q = 0.575(2)$ Å, $\sigma_2 = 115.8(3)°$, $\Theta = 175.3(3)°$ show that the ring is very close to a perfect chair conformation. The absolute configuration which was deduced from the L-configuration of the starting material **1** confirms the stereochemistry of the anomeric centre of compound **12**.

Table 2. Crystal data and structure refinement for compound **12**

Empiric formula	$[C_6H_{13}NO_5]_2{\cdot}2H_2O$
Formula weight (g/mol)	430.41
Temperature (K)	150(2)
Wavelength (Å)	0.71073
Crystal system	Twined monoclinic
Space group	$P12_11$
Unit cell dimensions	
a (Å)	4.808(5)
b (Å)	9.828(5)
c (Å)	20.726(5)
β (°)	90.00(5)
V (Å^3)	979.4(12)
Z	2
D_{calcd} (Mg/m^3)	1.46
Absorption coefficient (mm^{-1})	0.133
$F(000)$	464
Crystal size (mm^3)	$0.34 \times 0.18 \times 0.10$
Crystal colour	Colourless
θ Range for data collection (°)	2.07–33.01
Index ranges	$-7 \leqslant h \leqslant 6$ $-14 \leqslant k \leqslant 14$ $-31 \leqslant l \leqslant 31$
Reflections collected/unique	15,094/6605
Refinement method	Against F^2
Data/restrain/parameters	6412/0/316
Final R indices	0.0303
R Indices (all data)	0.0901
Goodness-of-fit on F^2	0.76

3. Experimental

3.1. General

All chemical reagents and solvents were purchased from Aldrich (Saint Quentin Fallavier, France) or Acros (Noisy-Le-Grand, France) and were used without further purification. Thin layer chromatographies were performed on 0.2 mm silica 60 coated aluminium foils with F-254 indicator (Merck) and detected under UV light and developed with aqueous sulfuric acid (100 mL, H_2SO_4/H_2O 15%) containing molybdic acid (2 g) and cerium(IV) sulfate hydrate (1 g). Preparative column chromatographies were done using silica gel (Merck 60, 200–63 μm). Melting points were measured on an Electrothermal Serie IA9100 apparatus. ^{1}H and ^{13}C

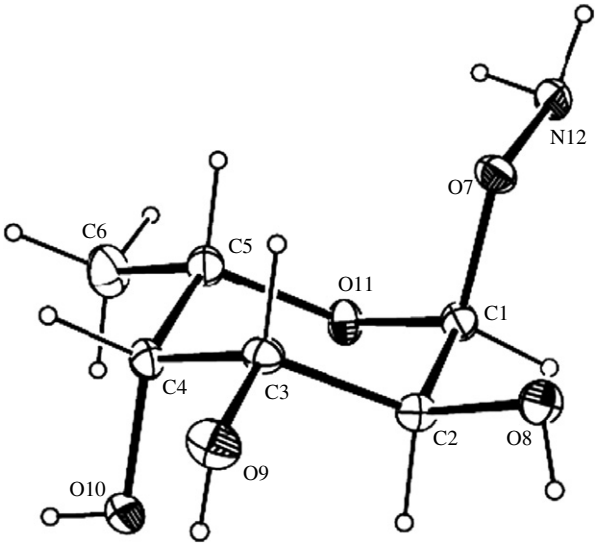


Figure 2. ORTEP drawing of compound **12**.

NMR spectra were recorded on Bruker AC300 spectrometers and chemical shifts (δ) were reported in parts per million (ppm). Spectra were referenced to the residual proton solvent peaks. Proton and carbon assignments were obtained from GCOSY and GHMQC experiments. The α-anomeric configuration of all carbohydrates was established by determination of the coupling constant (J) between H-1 and H-2. HRMS analyses were provided by the mass spectrometry service of the Department of Chemistry and Biochemistry, University of Berne, by electron spray ionisation (ES-MS) on an Applied Biosystems/Sciex QSTAR Pulsar in the positive mode. Reverse phase HPLC analyses were performed on Waters equipment using C_{18} columns. The analytical HPLC (Nucleosil 120 Å 3 μm C_{18} particles, 30×4.6 mm^2) was operated at 1.3 mL/min and the preparative HPLC (Delta-Pak 300 Å 15 μm C_{18} particles, 200×25 mm^2) at 22 mL/min flow with UV monitoring at 214 nm and 250 nm using a linear A–B gradient (buffer A: 0.09% CF_3CO_2H in water; buffer B: 0.09% CF_3CO_2H in 90% acetonitrile).

3.2. X-ray diffraction experiments

X-ray diffraction data for *O*-α-L-fucopyranosyl oxyamine **12** were recorded on a Bruker AXS-Enraf-Nonius Kappa-CCD diffractometer using a graphite monochromator (λ (Mo Kα): 0.71073 Å). The temperature of the crystal was maintained at the selected value 150(2) K using a 700 series Cryostream cooling device. The data were corrected for Lorentz and polarisation effects. Then the data were processed using WINGX.[31] The structure was solved by direct method with the use of the SIR92 software[32] and refined against F^2 by full-matrix least-squares techniques using SHELXL97.[33] The twin structure was solved within the $P12_11$ space group using the following twin law:

$$\begin{pmatrix} 1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \end{pmatrix}$$

with two domains and a BASF of 0.44896 displaying a ratio of almost 45–55% for the two domains. The final refinement involved an anisotropic model for all nonhydrogen atoms. The hydrogen atoms were set geometrically when ridding a carbon atom or for the hydroxyl groups. Hydrogen atoms from the water molecules or from the amino groups were set using the difference Fourier map. All of them were recalculated before the last refinement cycle.

3.3. (1,2,3,4-Tetra-*O*-acetyl)-α-L-fucopyranose 2

L-Fucopyranose **1** (5 g, 30.5 mmol) was dissolved in a solution of acetic anhydride and pyridine (1/2, 150 mL) and stirred at room temperature overnight. The solution was concentrated and taken up with ethyl acetate. The organic layer was washed successively four times with citric acid, then water, dried over sodium sulfate and evaporated to obtain **2** (9.7 g, 96%) as a white powder after precipitation from pentane; mp 90–92 °C; R_f 0.6 (ethyl acetate); ^{1}H NMR ($CDCl_3$): δ 6.36 (d, 1H, $J_{1,2}$ 2.7 Hz, H-1), 5.36–5.34 (m, 3H, H-2, H-3, H-4), 4.29 (br q, 1H, J_{5,CH_3} 6.5 Hz, H-5), 2.20 (s, 3H, $COCH_3$), 2.16 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 1.18 (d, 3H, J_{5,CH_3} 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.9 (C=O_{Ac}), 170.5 (C=O_{Ac}), 170.3 (C=O_{Ac}), 169.5 (C=O_{Ac}), 90.3 (C-1), 70.9 (C-2, C-3 or C-4), 68.2 (C-2, C-3 or C-4), 67.7 (C-5), 66.9 (C-2, C-3 or C-4), 21.3 ($COCH_3$), 21.1 ($COCH_3$), 21.0 ($COCH_3$), 20.9 ($COCH_3$), 16.3 (CH_3); ESIMS: m/z calcd for $C_{14}H_{20}O_9$ $[M+Na]^+$: 355.10; found: 354.92.

3.4. (2,3,4-Tri-*O*-acetyl)-α/β-L-fucopyranosyl fluoride 4 and 5

Acetic acid (2.35 mL, 41.0 mmol) was added in a solution of THF (400 mL) containing ethylenediamine (2.35 mL, 35.1 mmol). Compound **2** (9.7 g, 29.3 mmol) was added and the solution was stirred at room temperature overnight. After addition of water, the organic layer was taken up with ethyl acetate and washed successively with hydrochloric acid solution, aqueous solution of $NaHCO_3$, dried over sodium sulfate and evaporated to obtain **3** (7.3 g, 86%) as a colourless oil. Compound **3** (1.98 g, 6.8 mmol) was dissolved in dry THF (10 mL) and the solution was cooled at −30 °C. Diethylaminosulfurtrifluoride (1.08 mL, 8.2 mmol) was then added and the solution stirred at room temperature for 30 min. After quenching the reaction with methanol (2 mL) at −30 °C, the solution was concentrated and taken up with CH_2Cl_2. The organic layer was washed successively with aqueous solution of $NaHCO_3$, then water, dried over sodium sulfate and evaporated to obtain a colourless oil corresponding to the fucopyranosyl fluoride as anomer mixture. Each anomer was separated by silica gel chromatography (hexane/ether 4/6) to give pure α **4** and β **5** fluorides. α Anomer **4**: $[\alpha]_D^{20}$ −85.5 (c 2.5, $CHCl_3$); R_f 0.65 (hexane/ether, 4/6); ^{1}H NMR ($CDCl_3$): δ 5.87 (dd, 1H, $J_{1,2}$ 2.7 Hz, $J_{1,F}$ 53.7 Hz, H-1), 5.51–5.46 (m, 2H, H-3, H-4), 5.29 (dddd, 1H, $J_{2,4}$ 1.2 Hz, $J_{1,2}$ 2.7 Hz, $J_{2,3}$ 11.7 Hz, $J_{2,F}$ 23.6 Hz, H-2), 4.46 (br q, 1H, J_{5,CH_3} 6.5 Hz, H-5), 2.13 (s, 3H, $COCH_3$), 2.23 (s, 3H, $COCH_3$), 2.29 (s, 3H, $COCH_3$), 1.31 (d, 3H, J_{5,CH_3} 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.7 (C=O_{Ac}), 170.6 (C=O_{Ac}), 170.3 (C=O_{Ac}), 104.9 (d, $J_{C\text{-}1,F}$ 226 Hz, C-1), 70.7 (C-4), 67.8 (d, $J_{C\text{-}2,F}$ 30 Hz, C-2), 67.7 (d, $J_{C\text{-}2,F}$ 9 Hz, C-3), 65.6 (C-5), 20.9 ($COCH_3$), 21.0 ($COCH_3$), 21.1 ($COCH_3$), 18.2 (CH_3); HR-ESIMS: m/z calcd for $C_{12}H_{17}O_7FNa$: 315.0856; found: 315.0849. β Anomer **5**: $[\alpha]_D^{20}$ −48.1 (c 12.2,

$CHCl_3$); R_f 0.38 (hexane/ether, 4/6); ^1H NMR ($CDCl_3$): δ 5.21–5.13 (m, 2H, H-3, H-4), 5.03 (dd, 1H, $J_{1,2}$ 7.2 Hz, $J_{1,F}$ 51 Hz, H-1), 4.95–4.91 (m, 1H, H-2), 3.81 (br q, 1H, J_{5,CH_3} 6.4 Hz, H-5), 1.88 (s, 3H, $COCH_3$), 1.98 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 1.17 (d, 3H, J_{5,CH_3} 6.4 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 170.8 ($C{=}O_{Ac}$), 170.4 ($C{=}O_{Ac}$), 169.7 ($C{=}O_{Ac}$), 107.5 (d, $J_{C\text{-}1,F}$ 216 Hz, C-1), 70.8 (d, $J_{C\text{-}2,F}$ 45 Hz, C-2), 70.1 (C-4), 69.9 (C-5), 69.3 (C-3), 20.9 ($COCH_3$), 21.0 ($COCH_3$), 21.1 ($COCH_3$), 16.2 (CH_3); HR-ESIMS: *m*/*z* calcd for $C_{12}H_{17}O_7FNa$: 315.0856; found: 315.0846.

3.5. *O*-(2,3,6-Tri-*O*-acetyl)-α/β-L-fucopyranosyl-*N*-oxyphthalimide 6 and 7

The following procedure is typical for each glycosylation. To a stirring solution of α fluoride **4** (100 mg, 0.34 mmol), *N*-hydroxyphthalimide (55 mg, 0.34 mmol) and triethylamine (44 μL, 0.34 mmol) in THF was added $BF_3{\cdot}Et_2O$ (47 μL, 0.34 mmol). The reaction was stirred at room temperature for 1 h. After completeness of the reaction, CH_2Cl_2 was then added to the mixture and the organic layer was washed two times with 10% aqueous sodium hydrogenocarbonate, then water, dried under sodium sulfate and evaporated. A mixture of α and β anomers was obtained and each anomer was separated by flash chromatography (hexane/ether 4/6) to give pure compounds **6** and **7** (122 mg, 82%, α/β = 1/2.3). α Anomer **6**: mp 92–94 °C; $[\alpha]_D^{20}$ −157.1 (*c* 3.1, $CHCl_3$); R_f 0.34 (hexane–ether, 4/6); ^1H NMR ($CDCl_3$): δ 7.87–7.76 (m, 4H, H_{Pht}), 5.59 (dd, 1H, $J_{1,2}$ 3.9 Hz, H-1), 5.55 (dd, 1H, $J_{3,4}$ 3.3 Hz, $J_{2,3}$ 11.2 Hz, H-3), 5.45 (dd, 1H, $J_{4,5}$ 1.1 Hz, $J_{3,4}$ 3.3 Hz, H-4), 5.27 (dd, 1H, $J_{1,2}$ 3.9 Hz, $J_{2,3}$ 11.2 Hz, H-2), 5.09 (br q, 1H, J_{5,CH_3} 6.3 Hz, H-5), 2.23 (s, 3H, $COCH_3$), 2.20 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 1.21 (d, 3H, J_{5,CH_3} 6.3 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 171.2 ($C{=}O_{Ac}$), 170.8 ($C{=}O_{Ac}$), 170.2 ($C{=}O_{Ac}$), 163.5 ($C{=}O_{Pht}$), 135.1 (CH_{Pht}), 129.2 (C_{Pht}), 124.1 (CH_{Pht}), 102.5 (C-1), 71.3 (C-4), 67.6 (C-2 or C-3), 67.5 (C-2 or C-3), 67.4 (C-5), 21.2 ($COCH_3$), 21.1 ($COCH_3$), 20.9 ($COCH_3$), 16.2 (CH_3); HR-ESIMS: *m*/*z* calcd for $C_{20}H_{21}NO_{10}Na$: 458.1063; found: 458.1058. β Anomer **7**: mp 92–94 °C; $[\alpha]_D^{20}$ +7.6 (*c* 2.6, $CHCl_3$); R_f 0.17 (hexane/ether, 4/6); ^1H NMR ($CDCl_3$): δ 7.90–7.77 (m, 4H, H_{Pht}), 5.46 (dd, 1H, $J_{1,2}$ 8.3 Hz, $J_{2,3}$ 10.3 Hz, H-2) 5.29–5.27 (m, 1H, H-4), 5.12 (dd, 1H, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 10.3 Hz, H-3), 4.99 (q, 1H, $J_{1,2}$ 8.3 Hz, H-1), 3.84 (br q, 1H, J_{5,CH_3} 6.3 Hz, H-5), 2.24 (s, 3H, $COCH_3$), 2.23 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.27 (d, 3H, J_{5,CH_3} 6.3 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 171.2 ($C{=}O_{Ac}$), 170.5 ($C{=}O_{Ac}$), 170.3 ($C{=}O_{Ac}$), 163.1 ($C{=}O_{Pht}$), 135.1 (CH_{Pht}), 129.2 (C_{Pht}), 124.2 (CH_{Pht}), 106.8 (C-1), 71.4 (C-3), 70.6 (C-5), 70.1 (C-4), 67.5 (C-2), 21.2 ($COCH_3$), 21.1 ($COCH_3$), 20.9 ($COCH_3$), 16.4 (CH_3); HR-ESIMS: *m*/*z* calcd for $C_{20}H_{21}NO_{10}Na$: 458.1063; found: 458.1079.

3.6. *O*-(2,3,6-Tri-*O*-benzyl)-α-L-fucopyranosyl-*N*-oxyphthalimide 10

Compound **10** was obtained from **8** following the procedure described for **6** after purification by flash chromatography (hexane/ethyl acetate, 7/3); mp 53–55 °C; $[\alpha]_D^{20}$ −64.1 (*c* 4.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.86–7.73 (m, 4H, H_{Pht}), 7.56–7.29 (m, 15, H_{Bn}), 5.62 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.11–4.66 (m, 7H, H-5, $3\times CH_2$), 4.26 (d, 1H, $J_{1,2}$ 3.9 Hz, $J_{2,3}$ 10.3 Hz, H-2), 4.13 (d, 1H, $J_{3,4}$ 2.7 Hz, $J_{2,3}$ 10.3 Hz, H-3), 3.78 (br d, 1H, $J_{2,3}$ 2.7 Hz, H-4), 1.14 (d, 3H, J_{5,CH_3} 6.4 Hz, CH_3); ^{13}C NMR ($CDCl_3$): δ 163.5 ($C{=}O_{Pht}$), 138.8 (C_{Bn}), 138.4 (C_{Bn}), 138 (C_{Bn}), 134.4 (CH_{Pht}), 129.0 (C_{Pht}), 128.4 (CH_{Bn}), 128.4 (CH_{Bn}), 128.3 (CH_{Bn}), 128.2 (CH_{Bn}), 127.7 (CH_{Bn}), 127.6 (CH_{Bn}), 127.5 (CH_{Bn}), 127.5 (CH_{Bn}), 123.5 (CH_{Pht}), 102.7 (C-1), 78.5 (C-3), 77.8 (C-4), 75.3 (C-2), 75.0 (CH_2), 73.7 (CH_2), 72.8 (CH_2), 69.1 (C-5), 16.5 (CH_3); HR-ESIMS: *m*/*z* calcd for $C_{35}H_{33}NO_7Na$: 602.2154; found: 602.2162.

3.7. *O*-α-L-Fucopyranosyl oxyamine 12

Compound **6** (286 mg, 0.66 mmol) was dissolved in a solution of ethanol/methylhydrazine (10 mL, 1/1) and stirred at room temperature overnight. After evaporation, the fully deprotected aminoxylated fucose was recrystallised in ethanol to give **12** as single crystals (50 mg, 51%); mp 147–150 °C; $[\alpha]_D^{20}$ −131.2 (*c* 11.0, H_2O); ^1H NMR (D_2O): δ 4.96 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1), 4.10 (br q, 1H, J_{5,CH_3} 6.5 Hz, H-5), 3.83–3.80 (m, 3H, H-2, H-3, H-4), 1.24 (d, 3H, J_{5,CH_3} 6.5 Hz, CH_3); ^{13}C NMR (D_20): δ 102.2 (C-1), 72.2 (C-2, C-3 or C-4), 69.9 (C-2, C-3 or C-4), 68.0 (C-2, C-3 or C-4), 67.1 (C-5), 15.7 (CH_3); HR-ESIMS: *m*/*z* calcd for $C_6H_{14}NO_5$: 180.0871; found: 180.0866.

3.8. *O*-β-L-Fucopyranosyl oxyamine 13

Compound **13** was obtained from **7** following the procedure described for **12** after purification by flash chromatography (eluent: dichloromethane/ethanol, 7/3); mp 172–174 °C; $[\alpha]_D^{20}$ +2.8 (*c* 10.3, H_2O); ^1H NMR (D_2O): δ 4.50 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 3.81 (br q, 1H, J_{5,CH_3} 6.4 Hz, H-5), 3.77–3.75 (m, 1H, H-4), 3.67 (dd, 1H, $J_{3,4}$ 3.4 Hz, $J_{2,3}$ 9.6 Hz, H-3), 3.51 (dd, 1H, $J_{1,2}$ 8.2 Hz, $J_{2,3}$ 9.6 Hz, H-2), 1.28 (d, 3H, J_{5,CH_3} 6.4 Hz, CH_3); ^{13}C NMR (D_20): δ 105.9 (C-1), 73.4 (C-3), 71.7 (C-4), 71.2 (C-5), 69.5 (C-2), 15.7 (CH_3); HR-ESIMS: *m*/*z* calcd for $C_6H_{14}NO_5$: 180.0871; found: 180.0875.

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