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Design and Synthesis of a Trisubstrate Analogue for α(1→3)Fucosyltransferase: A Potential Inhibitor

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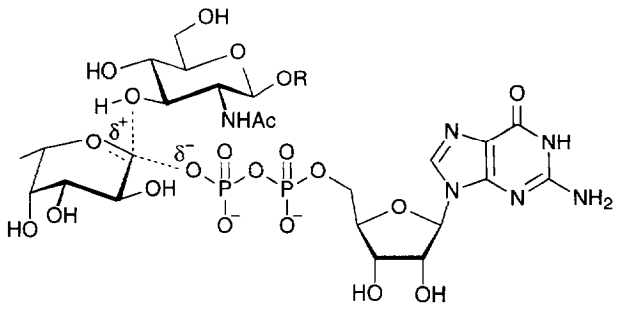
Abstract: *Phenyl 1-azido-3,4,5-tri-O-benzyl-1-deoxy-2-seleno-α-L-fuco-heptulopyranoside (**5**), readily accessible from perbenzylated L-fucono-1,5-lactone, is a key intermediate in the preparation of trisubstrate analogue **2**. NIS mediated condensation of fucosyl donor **5** with methyl 2,4,6-tri-O-benzyl-α-D-glucopyranoside (**7**) and ensuing reduction of the azido function furnished ketodisaccharide **10b**, which was elongated at the 1-amino group of the fucose residue with a malonic acid spacer. The thus obtained compound was deprotected and subsequently coupled with 5'-amino-5'-deoxy-guanosine to afford the target compound **2**.*

Introduction

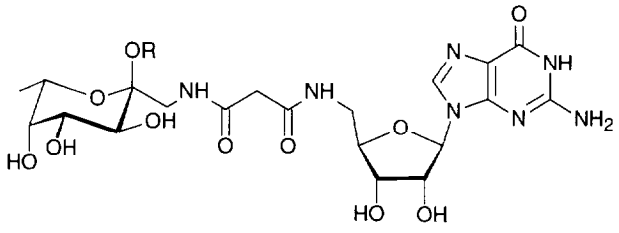
Fucosyltransferases (FucTs) catalyze the transfer of L-fucopyranoside residues from guanosine diphosphate fucose (GDP-Fuc) to glycoconjungate acceptors resulting in the formation of fucosylated glycoconjungates and GDP. Recently the naturally occurring fucose containing sialyl Lewis X determinant (SLe^x: Neu5Acα(2→3)Galβ (1→4)[Fucα(1→3)]GlcNAc), *inter alia* present on the extracellular surfaces of leukocytes, has gained considerable interest since it has been identified as a ligand for E-selectin. E-selectin, an endothelial glycoprotein, is a member of the selectin family of cell adhesion molecules. The E-selectin-SLe^x interaction is the first of several sequential adhesion events in the leukocyte extravasation to inflamed tissue[1], a process that is essential for host defense. On the other hand, various inflammatory diseases (*e.g.* adult respiratory distress syndrome) originate from misdirected or excessive leukocyte recruitment. Biological experiments revealed that the presence of a fucose residue in the SLe^x ligand is essential for the low affinity binding of leukocytes to E-selectin[2]. On the basis of this knowledge α(1→3)fucosyltransferase[3] [α(1→3)FucT] inhibitors have been proposed as potential drugs for the treatment of inflammatory diseases.

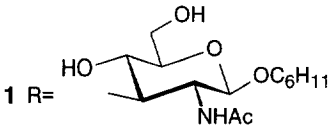
Thus far, the design and preparation of possible FucT inhibitors have mainly been restricted to metabolically stable GDP-Fuc analogues[4]. However, the inhibitory activity of these analogues is comparable to that of the endogenous inhibitor GDP. Several years ago, Hindsgaul *et al.*[5] postulated that enzymatic glycosyl transfer proceeds *via* an ion-pair mechanism, implying that GDP, fucose and the acceptor are simultaneously interacting with the enzyme in the transition state (see Figure 1). On the basis of the proposed transition state Hindsgaul prepared a *bisubstrate analogue* of α(1→2)FucT, lacking the

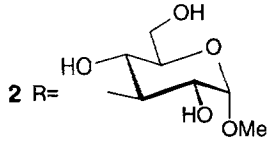


fucose moiety, which unfortunately showed only a slight increase in inhibitory activity with respect to GDP.

It occurred to us that a *trisubstrate analogue* might display an enhanced inhibitory effect, due to the presence of an additional fucose residue. In order to substantiate the viability of this concept we embarked on the synthesis of the *trisubstrate analogues* **1** and **2**, which may inhibit α(1→3)FucTs. The proposed inhibitors contain a malondiamido[6] instead of a pyrophosphate linkage between the guanosine and L-fucose moieties. It was anticipated that the non-charged amide bonds would facilitate membrane transport, which is a crucial element in the design of an effective inhibitor.



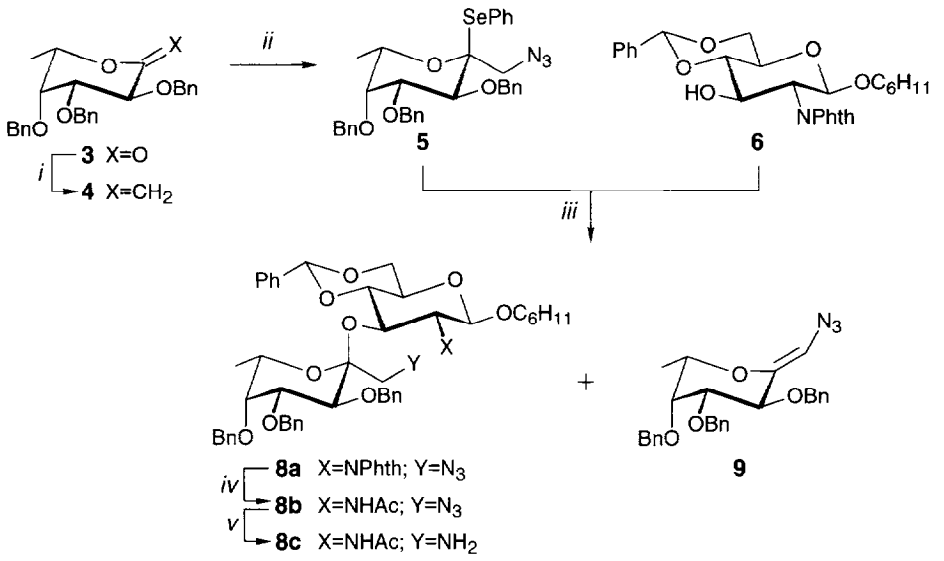




In this paper it will be shown that the synthesis of inhibitor **1** was hampered due to the intrinsic acid lability of the target molecule. Notwithstanding this failure, it will be demonstrated that the designed synthetic route can be followed successfully for the preparation of derivative **2**.

Results and discussion

Retrosynthetic analysis reveals that *trisubstrate analogues* **1** and **2** are in principle accessible by coupling of a protected anomerically branched L-fucopyranosyl donor with an appropriate acceptor and subsequent introduction of 5'-amino-5'-deoxy-guanosine *via* a malonic acid spacer at the equatorial branch of the fucose residue. It was expected that application of the recently reported[7] *anti*-Markovnikov azido-phenylselenation procedure to 1-methylene fucose **4** (Scheme 1) would lead to phenyl 1-azido-3,4,5-tri-*O*-benzyl-1-deoxy-2-seleno-L-*fuco*-heptulopyranoside **5**. The anomeric phenylselenyl function of **5** enables glycosylation[8-10], while reduction of the azido function permits attachment of the malonic acid spacer. Derivative **4** was prepared in 75% yield by treating tri-*O*-benzyl-L-fucono-1,5-lactone **3**[4d] with



Reagents and conditions:

(i) Cp_2TiMe_2, toluene (74%); *(ii)* NaN_3, $(PhSe)_2$, $PhI(OAc)_2$, CH_2Cl_2 (68%); *(iii)* IDCT, DCE, Et_2O (**8a**: 42%, **9**: 22%) or NIS, DCE, Et_2O (**8a**: 56%); *(iv)* (a) H_2NNH_2, EtOH; (b) Ac_2O, pyridine (90%, 2 steps); *(v)* 10% Pd/C, H_2, *i*-PrOH (74%).

Scheme 1

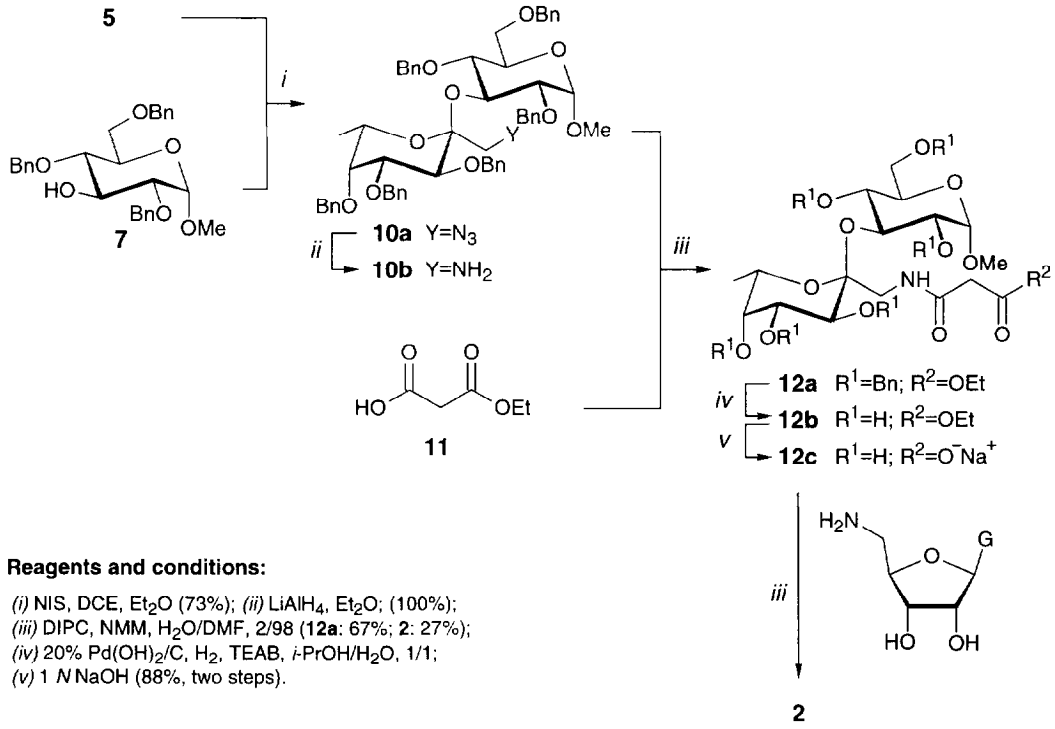
dimethyltitanocene[11]. Addition of *N*-phenylselenophtalimide and azidotrimethylsilane[7] to **4** afforded compound **5** in 30% yield. The *anti*-Markovnikov configuration of addition product **5** was *inter alia* assigned on the basis of the characteristic ^{13}C-NMR chemical shift of C-1 (56.6 ppm). It turned out that the earlier reported azido-phenylselenation method of Tingoli *et al.*[12] led to a better yield of the *anti*-Markovnikov addition product. Hence, treatment of **4** with sodium azide and diphenyldiselenide in the presence of (diacetoxyiodo)benzene gave phenylselenyl donor **5** in 68% yield[13].

Having the requisite donor in hand, we turned our attention to the coupling of **5** with the acceptors cyclohexyl 2-deoxy-2-phtalimido-4,6-*O*-benzylidene-β-D-glucopyranoside[14] (**6**) and methyl 2,4,6-tri-*O*-benzyl-α-D-glucopyranoside[15] (**7**). Studies from our laboratory[9] revealed that phenyl selenoglycosides can be employed successfully in iodonium-ion promoted glycosylations. Indeed, condensation (Scheme 1) of **5** with an equimolar amount of **6** under the influence of iodonium di-*sym*-collidine triflate[16] (IDCT) at -30°C furnished the expected α-linked disaccharide **8a** in 42% yield, the anomeric configuration of which was ascertained by NMR spectroscopy. In this particular case, the glycosylation was accompanied by the occurrence, as evidenced by NMR spectroscopy, of the elimination product **9** (22%). The elimination was diminished to less then 5% by executing the condensation in the presence of *N*-iodosuccinimide (NIS). On the other hand, NIS assisted coupling of the same donor with acceptor **7** (Scheme 2) afforded exclusively the corresponding α-linked dimer **10a** in 73% yield.

The final stage in the synthesis of *trisubstrate analogue* **1** comprises the introduction of a malonic acid bridge between the fully deprotected derivative of *fuco*-ketodisaccharide **8a** and 5'-amino-5'-deoxy-guanosine. To this end, fully protected **8a** was converted into **8b** by hydrazinolysis of the phtalimido

function and subsequent acetylation of the resulting amine. Furthermore, hydrogenolysis of the azido group in **8b** in the presence of catalytic 10% palladium on carbon furnished compound **8c**. Unfortunately, removal of the benzyl ethers and benzylidene acetal in **8c** by treatment with hydrogen in the presence of catalytic 10% palladium on carbon and acetic acid resulted in concomitant cleavage of the glycosidic linkage. In contrast, exclusive removal of the benzyl ethers was observed when hydrogenolysis of **8c** was performed under neutral hydrogen-transfer conditions (*e.g.* catalytic 10% palladium on carbon and 1,4-cyclohexadiene).

The results described so far indicate that deprotection of **8c** was prohibited due to the acid lability of the α-L-*fuco*-heptulopyranosidic linkage. The difficulties encountered above were an impetus to prepare analogue **2** from the fully benzylated disaccharide **10a** (Scheme 2). In the first step the azido function in



Scheme 2

10a was reduced with lithium aluminium hydride to yield primary amine **10b**. Subsequent coupling of **10b** with partially protected malonic acid **11** in the presence of *N,N*-diisopropylcarbodiimide (DIPC) and *N*-methylmorpholine (NMM) afforded compound **12a**. Debenzylation of **12a** by hydrogenolysis under buffered (triethylammonium bicarbonate, TEAB) conditions gave compound **12b**. In this respect it is of interest to note that hydrogenolysis of **12a** in the absence of TEAB buffer led to cleavage of the glycosidic bond. Saponification of the ethyl ester in **12b** with *N* sodium hydroxide furnished intermediate **12c**, which was purified by gel filtration and condensed with 5'-amino-5'-deoxy-guanosine[17] under the influence of DIPC and NMM. The resulting coupling product[18] was purified by gel filtration and subsequent silica gel chromatography to afford analogue **2** in 27% yield. The ^{13}C NMR, ^{1}H NMR (2D COSY) and mass

spectral data of **2** are in full accordance with the proposed structure.

The results presented in this paper indicate that the easily accessible intermediate **5** is a suitable synthon in the preparation of α-linked L-*fuco*-ketodisaccharides. However, a successful synthesis of this class of extremely acid labile disaccharides demands a judicious choice of protective groups in the acceptor moiety. The latter is nicely illustrated in the preparation of *trisubstrate analogue* **2**, a potential inhibitor of α(1→3)fucosyltransferases. The inhibitory effect of **2** on α(1→3)FucT-VII is currently under investigation.

Experimental

General procedures.

1,2-Dichloroethane, dichloromethane, diethyl ether and toluene were distilled from P_2O_5 and stored on 0.4 nm molecular sieves. Schleicher and Schüll DC Fertigfolien F 1500 LS 254 were used for TLC analysis. Compounds were visualized by UV light (254 nm) and by charring with 20% sulfuric acid in methanol, amines were charred with ninhydrin. Column chromatography was performed on silica gel 60, 230-400 mesh (Merck). The petroleum ether used for elution during chromatography was light boiling (40-60°C). Gel filtration was performed on Sephadex LH-20 (Pharmacia) or Fractogel TSK HW-40. ^{1}H NMR (200 MHz) and ^{13}C NMR spectra (50.1 MHz) were recorded using a Jeol JNM-FX 200 spectrometer, unless stated otherwise. ^{1}H NMR (300 MHz) spectra were recorded using a Bruker WM-300 spectrometer, ^{1}H NMR (400 MHz) spectra were recorded using a Bruker MSL-400 spectrometer and ^{1}H NMR (600 MHz) spectra were recorded using a Bruker DMX-600 spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard.

2,6-Anhydro-3,4,5-tri-*O*-benzyl-1-deoxy-L-*fuco*-hept-1-enitol (4).

Lactone **3** (6.48 g, 15.00 mmol), dried by evaporation with toluene (3 × 5 mL) and dissolved in toluene (5 mL), was added to a solution of Cp_2TiMe_2 (30 mmol) in toluene (40 mL) and the mixture was kept at 70°C under a blanket of argon (under the exclusion of light). After 16 h TLC analysis (diethyl ether/petroleum ether, 2/1, v/v) indicated complete disappearance of the lactone. The mixture was diluted with petroleum ether (100 mL), filtered through Celite and the filtrate was concentrated *in vacuo*. The residue was applied to a column of silica gel, which was eluted with petroleum ether/diethyl ether/TEA (99/0/1 to 85/14/1, v/v). Pure **4** was obtained as a colourless oil in 74% yield (4.80 g). R_f 0.90 (diethyl ether/petroleum ether, 2/1, v/v); ^{1}H NMR ($CDCl_3$) δ 1.23 (d, 3 H, H-7, $J_{6,7}$ = 6.4 Hz), 3.64 (dd, 1 H, H-4, $J_{3,4}$ = 9.4 Hz, $J_{4,5}$ = 3.0 Hz), 3.66-3.73 (m, 2 H, H-5, H-6), 4.40 (dt, 1 H, H-3, $J_{1,3}$ = -1.7 Hz), 4.66-4.70 (m, 2 H, H-1), 4.64-5.02 (2 AB, 4 H, 2 × CH_2 benzyl), 4.77 (s, 2 H, CH_2 benzyl), 7.23-7.40 (m, 15 H, $H_{arom.}$); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 17.3 (C-7), 73.2, 74.1, 74.9 (3 × CH_2 benzyl), 76.1, 77.1, 77.6, 83.0 (C-3, C-4, C-5, C-6), 94.1 (C-1), 127.7-128.6 ($CH_{arom.}$), 138.6-138.9 ($C_{arom.}$), 158.7 (C-2).

Phenyl 1-Azido-3,4,5-tri-*O*-benzyl-1-deoxy-2-seleno-α-L-*fuco*-heptulopyranoside (5).

Compound **4** (3.42 g, 7.95 mmol) was dried by evaporation with toluene (3 × 5 mL) and dissolved in dichloromethane (40 mL). $(PhSe)_2$ (2.25 g, 7.21 mmol), $PhI(OAc)_2$ (3.59 g, 11.13 mmol) and NaN_3 (1.24 g, 19.09 mmol) were added and the mixture was stirred under nitrogen. After 6 h TLC analysis (diethyl ether/petroleum ether, 1/2, v/v) indicated complete conversion to a higher running product. The reaction mixture was poured out in saturated aqueous $NaHCO_3$ and the resulting mixture was extracted with diethyl ether. The organic layer was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by flash chromatography (petroleum ether/diethyl ether/TEA, 99/0/1 to 85/19/1, v/v) and pure **5** was isolated as an amorphous yellow solid (3.39 g, 68%). R_f 0.65 (diethyl ether/petroleum ether, 1/2, v/v); ^{1}H NMR ($CDCl_3$) δ 1.10 (d, 3 H, H-7, $J_{6,7}$ =

6.5 Hz), 3.59 (AB, 2 H, H-1), 3.75 (bd, 1 H, H-5, $J_{4,5}$ = 2.8 Hz), 4.11 (dd, 1 H, H-4, $J_{3,4}$ = 9.7 Hz), 4.14 (bq, 1 H, H-6), 4.44 (d, 1 H, H-3), 4.67-5.07 (AB, 4 H, 2 × CH_2 benzyl), 4.75 (s, 2 H, CH_2 benzyl), 7.25-7.56 (m, 20 H, $H_{arom.}$);$^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 16.5 (C-7), 56.6 (C-1), 71.2, 76.4, 76.7, 82.3 (C-3, C-4, C-5, C-6), 72.5, 74.3, 75.4 (3 × CH_2 benzyl), 94.7 (C-2), 127.3-128.7, 137.1 ($CH_{arom.}$), 138.3 ($C_{arom.}$).

Cyclohexyl 3-(1-Azido-3,4,5-tri-*O*-benzyl-1-deoxy-α-L-*fuco*-heptulopyranosyl)-4,6-*O*-benzylidene-2-deoxy-2-phtalimido-β-D-glucopyranoside (8a).

Method A: Compound **5** (190 mg, 0.30 mmol) and acceptor **6** (145 mg, 0.30 mmol) were dried by repeated evaporation with 1,2-dichloroethane (3 × 2 mL), dissolved in 1,2-dichloroethane/diethyl ether (5 mL, 1/3, v/v) and stirred for 30 min with crushed molecular sieves (0.4 nm) under a nitrogen atmosphere. IDCT (200 mg, 0.39 mmol) was added at -30°C and stirring was continued for 1 h, when TLC analysis (acetone/toluene, 5/195, v/v) showed complete disappearance of the donor. The reaction mixture was filtered, diluted with diethyl ether and washed with a 1 M $Na_2S_2O_3$ solution, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (acetone/toluene/TEA, 0/199/1 to 4/195/1, v/v), byproduct **9** was isolated first (31 mg, 22%), R_f 0.66 (acetone/toluene, 5/195, v/v); ^1H NMR ($CDCl_3$) δ 1.31 (d, 3 H, H-7, $J_{6,7}$ = 6.4 Hz), 3.67 (dd, 1 H, H-4, $J_{3,4}$ = 8.8 Hz, $J_{4,5}$ = 2.5 Hz), 3.71-3.84 (m, 2 H, H-5, H-6), 4.37 (dd, 1 H, H-3, $J_{1,3}$ = -1.4 Hz), 4.64-4.99 (3 AB, 6 H, 3 × CH_2 benzyl), 5.44 (d, 1 H, H-1), 7.26-7.34 (m, 15 H, $H_{arom.}$); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 16.4 (C-7), 72.9, 73.8, 74.4 (3 × CH_2 benzyl), 74.7, 76.4, 82.3 (C-3, C-4, C-5, C-6), 108.7 (C-1), 127.5-128.4 ($CH_{arom.}$), 137.9 ($C_{arom.}$), 144.3 (C-2). Further elution gave dimer **8a** as an oil (120 mg, 42%).

Method B: Donor **5** (200 mg, 0.32 mmol) and acceptor **6** (160 mg, 0.33 mmol) were dried by repeated evaporation with 1,2-dichloroethane (3 × 2 mL), dissolved in 1,2-dichloroethane/diethyl ether (5 mL, 1/3, v/v) and stirred for 30 min with crushed molecular sieves (0.4 nm) under a nitrogen atmosphere. NIS (80 mg, 0.36 mmol) was added at -40°C and stirring was continued for 1 h, when TLC analysis (acetone/toluene, 5/195, v/v) showed complete disappearance of the donor. Work-up and purification of the reaction mixture as described for *method A* yielded dimer **8a** in 56% (170 mg). TLC Analysis indicated that byproduct **9** was formed in less than 5%, which was not isolated. R_f 0.45; ^1H NMR (600 MHz 2D COSY) ($CDCl_3$) δ 0.99 (d, 3 H, Fuc: H-7, $J_{6,7}$ = 6.5 Hz), 1.00-1.83 (m, 10 H, 5 × CH_2 cHex), 2.85 (d, 1 H, Fuc: H-1, $J_{1,1'}$ = -11.9 Hz), 3.47 (dd, 1 H, Fuc: H-5, $J_{4,5}$ = 2.6 Hz, $J_{5,6}$ = 1.4 Hz), 3.55-3.64, (m, 1 H, OCH cHex), 3.56 (t, 1 H, GlcNPhth: H-4, J = 8.4 Hz), 3.60 (dt, 1 H, GlcNPhth: H-5, $J_{5,6'}$ = 4.7 Hz, J = 9.3 Hz), 3.69, 4.15 (AB, 2 H, CH_2 benzyl), 3.81 (d, 1 H, Fuc: H-1'), 3.84 (t, 1 H, GlcNPhth: H-6, J = 10.1 Hz), 3.90 (dd, 1 H, Fuc: H-4, $J_{3,4}$ = 10.2 Hz), 3.98 (d, 1 H, Fuc: H-3), 4.08 (dq, 1 H, Fuc: H-6), 4.27 (dd, 1 H, GlcNPhth: H-2, $J_{1,2}$ = 8.6 Hz, $J_{2,3}$ = 10.2 Hz), 4.42 (dd, 1 H, GlcNPhth: H-6', $J_{5,6'}$ = 4.6 Hz, $J_{6,6'}$ = -10.6 Hz), 4.56, 4.91 (AB, 2 H, CH_2 benzyl), 4.60 (s, 2 H, CH_2 benzyl), 4.64 (dd, 1 H, GlcNPhth: H-3), 5.36 (d, 1 H, GlcNPhth: H-1), 5.54 (s, 1H, CHPh), 6.81, 7.08-7.78 (m, 24 H, $H_{arom.}$); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 16.7 (Fuc: C-7), 23.4, 23.7, 25.3, 31.5, 33.2 (5 × CH_2 cHex), 50.7 (Fuc: C-1), 55.9 (GlcNPhth: C-2), 66.3, 68.2, 70.2, 75.2, 77.4, 78.3, 80.5, 82.2 (Fuc: C-3, C-4, C-5, C-6, GlcNPhth: C-3, C-4, C-5, OCH cHex), 68.7 (GlcNPhth: C-6), 73.1, 74.1 (3 × CH_2 benzyl), 97.1 (GlcNPhth: C-1), 100.9 (CHPh), 101.6 (Fuc: C-2), 123.0-133.7 ($CH_{arom.}$), 137.0-139.2 ($C_{arom.}$). ^1H NMR (600 MHz 2D COSY): NOE effects were observed between H-1 and H-3 of the Fuc moiety and between H-1 of the Fuc and H-3 of the GlcNPhth moiety.

Anal. calcd. for $C_{55}H_{58}O_{11}N_4$ (951.09): C 69.46, H 6.15, N 5.90; found C 69.53, H 6.22, N 5.82%.

Cyclohexyl 2-Acetamido-3-(1-azido-3,4,5-tri-*O*-benzyl-1-deoxy-α-L-*fuco*-heptulopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (8b).

Hydrazine hydrate (0.47 mL) was added to a solution of **8a** (185 mg, 0.19 mmol) in 96% ethanol (3 mL) and the mixture was heated under reflux for 16 h. Water (3 mL) was added and after stirring for 30 min the mixture was concentrated *in vacuo*. The remaining traces of hydrazine and water were removed by evaporation with toluene (3 ×

5 mL) and the residue was redissolved in pyridine (2 mL) and acetic anhydride (1 mL). After 1 h at room temperature water (5 mL) was added and the mixture was extracted with dichloromethane. The organic layer was washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (acetone/dichloromethane, 0/1 to 2/98, v/v) to give 151 mg (90%) of **8b**. R_f (acetone/dichloromethane, 3/97, v/v); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 16.2 (Fuc: C-7), 22.8 (C(O)$\underline{C}H_3$), 23.5, 23.8, 25.3, 31.6, 33.3 (5 × CH_2 cHex), 51.5 (Fuc: C-1), 58.9 (GlcNAc: C-2), 65.6, 68.2, 71.2, 76.6, 77.3, 77.5, 80.1, 81.6 (Fuc: C-3, C-4, C-5, C-6, GlcNAc: C-3, C-4, C-5, OCH cHex), 68.7 (GlcNAc: C-6), 71.9, 74.2, 75.5 (3 × CH_2 benzyl), 98.1 (GlcNAc: C-1), 101.6 (CHPh), 101.6 (Fuc: C-2), 126.2-128.9 ($CH_{arom.}$), 137.0-138.9 ($C_{arom.}$), 170.6 (C=O).

Cyclohexyl 2-Acetamido-3-(1-amino-3,4,5-tri-*O*-benzyl-1-deoxy-α-L-*fuco*-heptulopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (8c).

Compound **8b** (65 mg, 0.075 mmol) was dissolved in *i*-propanol (2 mL), 10% Pd/C (50 mg) was added and the suspension was stirred gently under a hydrogen atmosphere. After 16 h the mixture was filtered and the filtrate was concentrated *in vacuo* and subsequently purified by column chromatography (acetone/dichloromethane 0/1 to 2/98, v/v) to give 47 mg (74%) of **8c**. R_f (acetone/dichloromethane, 3/97, v/v); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 16.1 (Fuc: C-7), 23.2 (C(O)$\underline{C}H_3$), 23.6, 23.9, 25.5, 31.6, 33.3 (5 × CH_2 cHex), 44.5 (Fuc: C-1), 58.5 (GlcNAc: C-2), 65.8, 67.5, 68.9, 70.9, 75.9, 77.5, 80.6, 81.7 (Fuc: C-3, C-4, C-5, C-6, GlcNAc: C-3, C-4, C-5, OCH cHex), 68.9 (GlcNAc: C-6), 72.1, 74.4, 76.4 (3 × CH_2 benzyl), 98.9 (GlcNAc: C-1), 101.7 (Fuc: C-2), 101.8 (CHPh), 126.3-128.6 ($CH_{arom.}$), 137.1-138.6 ($C_{arom.}$), 170.8 (C=O).

Methyl 3-(1-Azido-3,4,5-tri-*O*-benzyl-1-deoxy-α-L-*fuco*-heptulopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (10a).

Donor **5** (660 mg, 1.05 mmol) and acceptor **7** (490 mg, 1.06 mmol) were treated with NIS (240 mg, 1.07 mmol) as described for the preparation of compound **8a**, *method B*. The crude product was purified by column chromatography (petroleum ether/diethyl ether, 1/0 to 7/3, v/v) to give 600 mg of **10a**. The impure fractions were further purified by Sephadex LH-20 gel filtration (dichloromethane/methanol, 2/1, v/v) to furnish another 105 mg of **10a** (total yield: 705 mg, 73%). R_f 0.45 (petroleum ether/diethyl ether, 1/1, v/v); ^1H NMR (300 MHz 2D COSY) ($CDCl_3$) δ 0.72 (d, 3 H, Fuc: H-7, $J_{6,7}$ = 6.3 Hz), 3.09 (d, 1 H, Fuc: H-1, $J_{1,1'}$ = -12.9 Hz), 3.22 (dd, 1 H, Fuc: H-5, $J_{4,5}$ = 2.4 Hz, $J_{5,6}$ = 1.4 Hz), 3.31 (s, 3 H, OCH_3), 3.33 (dd, 1 H, Glc: H-2, $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 9.5 Hz), 3.61 (t, 1 H, Glc: H-4, J = 9.3 Hz), 3.62-3.79 (m, 3 H, Glc: H-5, H-6), 3.78 (d, 1 H, Fuc: H-1'), 3.95 (dd, 1 H, Fuc: H-4, $J_{3,4}$ = 10.3 Hz), 4.27 (t, 1 H, Glc: H-3), 4.27 (dq, 1 H, Fuc: H-6), 4.32-4.98 (6 AB, 12 H, 6 × CH_2 benzyl), 4.41 (d, 1 H, Fuc: H-3), 4.42 (d, 1 H, Glc: H-1), 6.97-7.43 (m, 30 H, $H_{arom.}$); $13C\{^1H\}$ NMR ($CDCl_3$) δ 16.4 (Fuc: C-7), 53.4 (Fuc: C-1), 55.1 (OCH_3), 70.0, 70.5, 71.9, 76.0, 77.4, 77.8, 79.5, 80.3 (Fuc: C-3, C-4, C-5, C-6, Glc: C-2, C-3, C-4, C-5), 68.7 (Glc: C-6), 72.1, 73.2, 73.4, 73.8, 74.1, 75.9 (6 × CH_2 benzyl), 98.7 (Glc: C-1), 102.4 (Fuc: C-2), 126.4-128.4 ($CH_{arom.}$), 137.9-138.9 ($C_{arom.}$).

Anal. calcd. for $C_{56}H_{61}O_{10}N_3$ (936.12): C 71.86, H 6.57, N 4.49; found C 71.98, H 6.64, N 4.41%.

Methyl 3-(1-Amino-3,4,5-tri-*O*-benzyl-1-deoxy-α-L-*fuco*-heptulopyranosyl)-2,4,6-tri-*O*-benzyl-α-D-glucopyranoside (10b).

Compound **10a** (705 mg, 0.76 mmol) was dried by evaporation with toluene (3 × 5 mL), dissolved in diethyl ether (2 mL) and added to a suspension of $LiAlH_4$ (50 mg, 1.32 mmol) in diethyl ether (5 mL) at 0°C under a nitrogen atmosphere. After 5 min TLC analysis (petroleum ether/diethyl ether, 2/1, v/v) showed complete conversion of the starting material to a more polar product and the reaction mixture was quenched carefully with water. Subsequently NH_4OH (1 mL) and Celite were added and the mixture was stirred for 30 min. The mixture was filtered and the filtrate was extracted with dichloromethane (3 × 20 mL). The organic layer was washed with brine, dried ($MgSO_4$)

and concentrated *in vacuo* to give pure **10b** in a quantitative yield (690 mg). R_f 0.46 (methanol/dichloromethane, 1/9, v/v); 1H NMR ($CDCl_3$) δ 2.75 (d, 1 H, Fuc: H-1, $J_{1,1'}$ = -12.9 Hz), 3.11 (d, 1 H, Fuc: H-1'); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 16.4 (Fuc: C-7), 45.6 (Fuc: C-1), 55.1 (OCH_3), 67.1, 70.3, 71.6, 74.0, 77.4, 78.0, 79.6, 81.0 (Fuc: C-3, C-4, C-5, C-6, Glc: C-2, C-3, C-4, C-5), 68.9 (Glc: C-6), 72.1, 72.5, 73.4, 74.1, 74.2, 75.0 (6 × CH_2 benzyl), 98.9 (Glc: C-1), 102.9 (Fuc: C-2), 126.3-128.3 ($CH_{arom.}$), 137.7-139.0 ($C_{arom.}$).

Methyl 2,4,6-Tri-*O*-benzyl-3-[1-(ethyl malonamido)-3,4,5-tri-*O*-benzyl-1-deoxy-α-L-*fuco*-heptulopyranosyl]-α-D-glucopyranoside (12a).

To a solution of compound **10b** (250 mg, 0.28 mmol) in H_2O/DMF (2 mL, 2/98, v/v) were added subsequently NMM (154 µL, 1.40 mmol), $HOOCCH_2COOEt$ (**11**, 62 µL, 0.56 mmol) and DIPC (60 µL, 0.42 mmol). After 15 min additional DIPC (60 µL) was added and stirring was continued for another 15 min, when TLC analysis (methanol/dichloromethane, 1/9, v/v) indicated completion of the reaction. The reaction mixture was diluted with diethyl ether and washed with brine. The organic layer was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by column chromatography (petroleum ether/diethyl ether, 1/1 to 0/1, v/v) to give 225 mg of the title compound. NMR-analysis indicated that the product was contaminated with 15% of diisopropyl ureum, consequently the actual yield is 191 mg (67%). R_f 0.79 (methanol/dichloromethane, 1/9, v/v); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 14.0 ($OCH_2\underline{C}H_3$), 16.2 (Fuc: C-7), 42.1, 42.8 (Fuc: C-1, $C(O)\underline{C}H_2C(O)$), 55.1 (OCH_3), 61.1 ($O\underline{C}H_2CH_3$), 67.5, 70.4, 72.0, 75.9, 77.7, 79.1, 80.6 (Fuc: C-3, C-4, C-5, C-6, Glc: C-2, C-3, C-4, C-5), 68.7 (Glc: C-6), 72.2, 73.2, 73.4, 74.3, 76.1 (6 × CH_2 benzyl), 99.2 (Glc: C-1), 101.3 (Fuc: C-2), 126.4-128.9 ($CH_{arom.}$), 137.5-139.1 ($C_{arom.}$), 168.2 ($\underline{C}(O)CH_2\underline{C}(O)$).

Methyl 3-[1-(ethyl malonamido)-1-deoxy-α-L-*fuco*-heptulopyranosyl]-α-D-glucopyranoside (12b).

Compound **12a** (94 mg, 0.091 mmol) was dissolved in *i*-PrOH/H_2O (3 mL, 1/1, v/v), 2 M TEAB solution (0.05 mL) and a suspension of 20% $Pd(OH)_2/C$ (50 mg) in the same solvent (1 mL) were added. After shaking under a hydrogen atmosphere (0.5 MPa) in a Parr-apparatus for 16 h, the mixture was filtered and the filtrate was concentrated *in vacuo*. The crude product was used without purification in the next step. $^{13}C\{^1H\}$ NMR (MeOD) δ 14.4 $_2\underline{C}H_3$), 16.6 (Fuc: C-7), 43.6 (Fuc: C-1, $C(O)\underline{C}H_2C(O)$), 55.5 (OCH_3), 62.4, 62.5 (Glc: C-6, $O\underline{C}H_2CH_3$), 69.2, 70.9, 71.1, 71.8, 72.4, 73.6, 78.0 (Fuc: C-3, C-4, C-5, C-6, Glc: C-2, C-3, C-4, C-5), 100.9 (Glc: C-1), 101.9 (Fuc: C-2), 172.6 ($\underline{C}(O)CH_2\underline{C}(O)$).

Methyl 3-[1-(sodio malonamido)-1-deoxy-α-L-*fuco*-heptulopyranosyl]-α-D-glucopyranoside (12c).

Crude **12b** was dissolved in methanol/H_2O (2 mL, 1/1, v/v) and 0.5 M NaOH solution (0.5 mL) was added. After stirring for 1.5 h the mixture was neutralized with NH_4Cl (13 mg) and concentrated *in vacuo*. The residue was purified by HW-40 gel filtration (H_2O/methanol/2 M TEAB, 85/10/5, v/v, R_t 111 min) to give 46 mg (88%) of **12c**, 23 mg of which was applied to a column of Dowex 50 W X 4 [Na^+] and eluted with water. The resulting solution was concentrated *in vacuo*, the residue was redissolved in D_2O, lyophilized and used for NMR spectroscopy. ^1H NMR (300 MHz 2D COSY) (D_2O) δ 1.18 (d, 3 H, Fuc: H-7, $J_{6,7}$ = 6.6 Hz), 3.23 (AB, 1 H, $C(O)CH_2C(O)$), 3.42 (s, 3 H, OCH_3), 3.48 (dd, 1 H, Glc: H-3, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 8.5 Hz), 3.50 (d, 1 H, Fuc: H-1, $J_{1,1'}$ = -14.2 Hz), 3.63-3.68 (m, 1 H, Glc: H-5), 3.67 (dd, 1 H, Glc: H-2, $J_{1,2}$ = 3.7 Hz), 3.74 (d, 1 H, Fuc: H-3, $J_{3,4}$ = 10.2 Hz), 3.76 (dd, 1 H, Glc: H-6, $J_{5,6}$ = 5.4 Hz, $J_{6,6'}$ = -12.2 Hz), 3.80 (dd, 1 H, Fuc: H-5, $J_{4,5}$ = 3.3 Hz, $J_{5,6}$ = 0.9 Hz), 3.88 (dd, 1 H, Glc: H-6', $J_{5,6'}$ = 2.3 Hz), 3.91 (d, 1 H, Fuc: H-1'), 3.93 (dd, 1 H, Fuc: H-4), 4.01 (t, 1 H, Glc: H-4), 4.43 (dq, 1 H, Fuc: H-6), 4.82 (d, 1 H, Glc: H-1); 13C{1H} NMR ($CDCl_3$) δ 16.0 (Fuc: C-7), 42.9 ($C(O)\underline{C}H_2C(O)$), 45.8 (Fuc: C-1), 55.5 (OCH_3), 61.3 (Glc: C-6), 68.6, 69.6, 70.6, 71.0, 72.4, 72.5, 76.1 (Fuc: C-3, C-4, C-5, C-6, Glc: C-2, C-3, C-4, C-5), 99.9 (Glc: C-1), 101.2 (Fuc: C-2), 172.0 (NHC(O)), 175.7 (OC(O)).

N-[2-*O*-(methyl α-D-glucopyranos-3-yl)-1-deoxy-α-L-*fuco*-heptulopyranos-1-yl]-N'-(5'-deoxy-guanosin-5'-yl)-malonamide (2).

Compound **12c** (23 mg, 0.040 mmol) and 5'-amino-5'-deoxy-guanosine (17 mg, 0.060 mmol) were coupled employing the same procedure as described for the synthesis of compound **12a**, using NMM (10 mg, 0.099 mmol) and DIPC (20 mg, 0.16 mmol). After 24 h, the reaction mixture was concentrated *in vacuo*, the crude product was purified by LH-20 gel filtration (H_2O) and subsequent column chromatography (*i*-PrOH/NH_4OH/H_2O, 180/15/5 to 120/60/20, v/v). First a byproduct (14 mg) was isolated; R_f 0.79 (*i*-PrOH/NH_4OH/H_2O, 80/15/5, v/v). Further elution yielded 8 mg (27%) of the title compound. R_f 0.44; ^1H NMR (400 MHz, 2D COSY) (D_2O) δ 1.21 (d, 3 H, Fuc: H-7, $J_{6,7}$ = 6.6 Hz), 3.36 (AB, 1 H, C(O)CH_2C(O)), 3.46 (s, 3 H, OCH_3), 3.52 (dd, 1 H, Glc: H-4, $J_{3,4}$ = 8.6 Hz, $J_{4,5}$ = 9.9 Hz), 3.52 (d, 1 H, Fuc: H-1, $J_{1,1'}$ = -14.7 Hz), 3.66-3.72 (m, 2 H, Glc: H-2, H-5), 3.68 (d, 2 H, Ribo: H-5, $J_{4,5}$ = 6.0 Hz), 3.74 (d, 1 H, Fuc: H-3, $J_{3,4}$ = 10.4 Hz), 3.80 (dd, 1 H, Glc: H-6, $J_{5,6}$ = 5.4 Hz, $J_{6,6'}$ = -12.2 Hz), 3.85 (bd, 1 H, Fuc: H-5, $J_{4,5}$ = 3.3 Hz), 3.92 (dd, 1 H, Glc: H-6', $J_{5,6'}$ = 2.4 Hz), 3.94 (d, 1 H, Fuc: H-1'), 3.98 (dd, 1 H, Fuc: H-4), 4.02 (t, 1 H, Glc: H-3, J = 8.9 Hz), 4.27 (q, 1 H, Ribo: H-4, J = 5.0 Hz), 4.44 (t, 1 H, Ribo: H-3), 4.46 (bq, 1 H, Fuc: H-6), 4.82 (t, 1 H, Ribo: H-2), 4.86 (d, 1 H, Glc: H-1, $J_{1,2}$ = 3.6 Hz), 5.92 (d, 1 H, Ribo: H-1, $J_{1,2}$ = 5.0 Hz); $^{13}C\{^1H\}$ NMR (D_2O) δ 15.9 (Fuc: C-7), 41.2, 42.8, 43.6 (Fuc: C-1, Ribo: C-5, C(O)$\underline{C}H_2$C(O)), 55.5 (OCH_3), 61.2 (Glc: C-6), 68.6, 69.6, 70.6, 70.9, 71.4, 72.3, 72.5, 73.7, 76.1 (Fuc: C-3, C-4, C-5, C-6, Glc: C-2, C-3, C-4, Ribo: C-2, C-3), 83.0 (Ribo: C-4), 88.3 (Ribo: C-1), 99.9 (Glc: C-1), 101.1 (Fuc: C-2),154.4 (G: C-2), 159.5 (G: C-6), 169.9, 170.3 (C=O). *m/z* 719 $[M]^-$.

Anal. calcd. for $C_{27}H_{41}O_{16}N_7$ (719.66): C 45.06, H 5.74, N 13.62; found C 45.13, H 5.80, N 13.56%.

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