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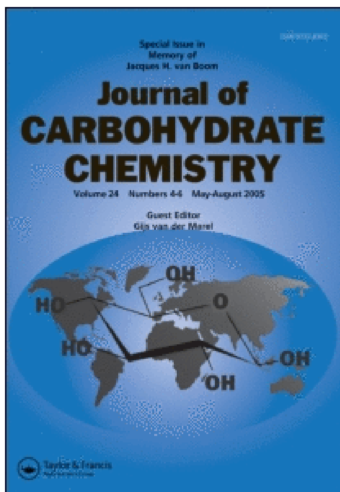

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Expeditious Synthesis of a Trisubstrate Analogue for α(1→3)Fucosyltransferase

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EXPEDITIOUS SYNTHESIS OF A TRISUBSTRATE ANALOGUE FOR α(1→3)FUCOSYLTRANSFERASE

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ABSTRACT

The synthesis of cyclohexyl 2-acetamido-2-deoxy-3-*O*-{2-*O*-[2-(guanosine 5'-*O*-phosphate)ethyl]-α-L-fucopyranosyl}-β-D-glucopyranoside (**1**), a potential inhibitor of α(1→3)fucosyltransferases, is described. Target compound **1** was assembled *via* fucosylation of cyclohexyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**6**) with ethyl 2-*O*-[2-(benzoylhydroxy)ethyl]-3,4-*O*-isopropylidene-1-thio-β-L-fucopyranoside (**5**) followed by debenzoylation, subsequent condensation of the resulting compound with 3',4'-di-*O*-benzoyl-5'-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)-2-*N*-diphenylacetylguanosine (**10**) and deprotection.

INTRODUCTION

Recently it has been revealed that the sialyl Lewis X (SLe^x: Neu5Ac (α2→3)Gal(β1→4)[Fuc(α1→3)]GlcNAc) determinant and other naturally occurring derivatives thereof are involved in the interaction with selectins (*i.e.* E-selectin, L-selectin and P-selectin).[1] The recognition of SLe^x located on leucocyte surfaces by E-selectin, which is expressed on activated endothelial cells, is a critical event in the adhesion of leucocytes to the vascular endothelium. The latter process eventually results in the migration of leucocytes from the bloodstream to sites of inflammation.

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Since excessive leucocyte recruitment is related to inflammatory diseases (*e.g.* rheumatoid arthritis), obstruction of the E-selectin-SLe^x interaction may be a promising approach towards the development of anti-inflammatory drugs. Biological studies[2] showed that the fucose residue is a crucial structural element of SLe^x for the binding with E-selectin. Incorporation of fucose is the final step in the biosynthesis of SLe^x and is mediated by a specific α(1→3)fucosyltransferase[3] [α(1→3)FucT]. Consequently, with the aim to develop anti-inflammatory therapeutics, effort has been devoted to the preparation of α(1→3)FucT inhibitors.[4] In order to achieve this goal, metabolically stable *substrate analogues* of guanosine 5'-(β-L-fucopyranosyl)-diphosphate (GDP-Fuc), the naturally occurring donor substrate of fucosyltransferases, have been prepared.[4b-d] Apart from this, Hindsgaul *et al.*[5] reported a second type of inhibitor for α(1→2)FucT, a so-called *bisubstrate analogue*. This analogue, which is characterized by the presence of a nucleotide (GDP) as well as an acceptor (phenyl β-D-galactopyranoside) residue, showed a slightly increased inhibitory effect on α(1→2)FucT compared to the endogenous inhibitor GDP.

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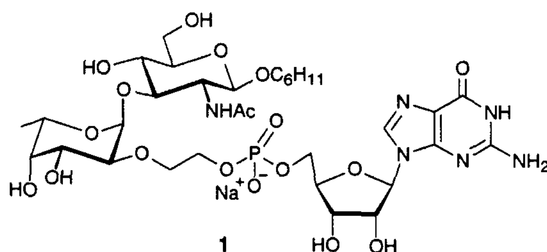
As part of a program directed towards the development of α(1→3)FucT inhibitors, we here describe the design and preparation of *trisubstrate analogue* **1**.



RESULTS AND DISCUSSION

It has been postulated[5] that the enzymatic transfer of a fucosyl moiety to an acceptor proceeds *via* an ion-pair mechanism, implying that the donor (GDP-Fuc) and acceptor are interacting simultaneously with the enzyme in the transition state. On the basis of this hypothesis we reasoned that incorporation of three important recognition sites (*i.e.* a guanosine, a fucose and an acceptor moiety) in a molecule may result in an active and selective inhibitor. One possible route to validate this

concept entails the design and synthesis of ***trisubstrate analogue* 1**, in which a L-fucopyranosyl residue is α-linked to the HO-3 function of cyclohexyl 2-acetamido-2-deoxy-β-D-glucopyranoside (*i.e.* the acceptor) while a guanosine moiety is anchored to the HO-2 group of the fucose *via* a phosphate-ethylene bridge. The flexibility of the spacer may enable the guanosine and fucose residues to adopt the correct relative orientation for optimal recognition by the enzyme.

The synthetic route to target compound **1** is outlined in Scheme 1 and comprises four stages: (i) preparation of the hydroxyethyl spacer containing fucosyl donor **5**, (ii) coupling of donor **5** to glucosamine acceptor **6**, (iii) introduction of the guanosine 5′-phosphate residue and finally (iv) cleavage of protective groups.

The requisite fucosyl donor **5** was prepared from readily accessible ethyl 3,4-*O*-isopropylidene-1-thio-β-L-fucopyranoside[6] **2** by the following three-step procedure (Scheme 1). The 2-*O*-(methoxycarbonylmethyl) substituent, as in **3**, was introduced by treatment of **2** with excess methyl bromoacetate and sodium hydride.[7] Reduction of the ester function in **3** with lithium aluminium hydride and subsequent benzoylation of the generated hydroxyl function (in **4**) with benzoyl chloride in pyridine afforded ethyl 2-*O*-[2-(benzoylhydroxy)ethyl]-3,4-*O*-isopropylidene-1-thio-β-L-fucopyranoside (**5**) in 66% overall yield.

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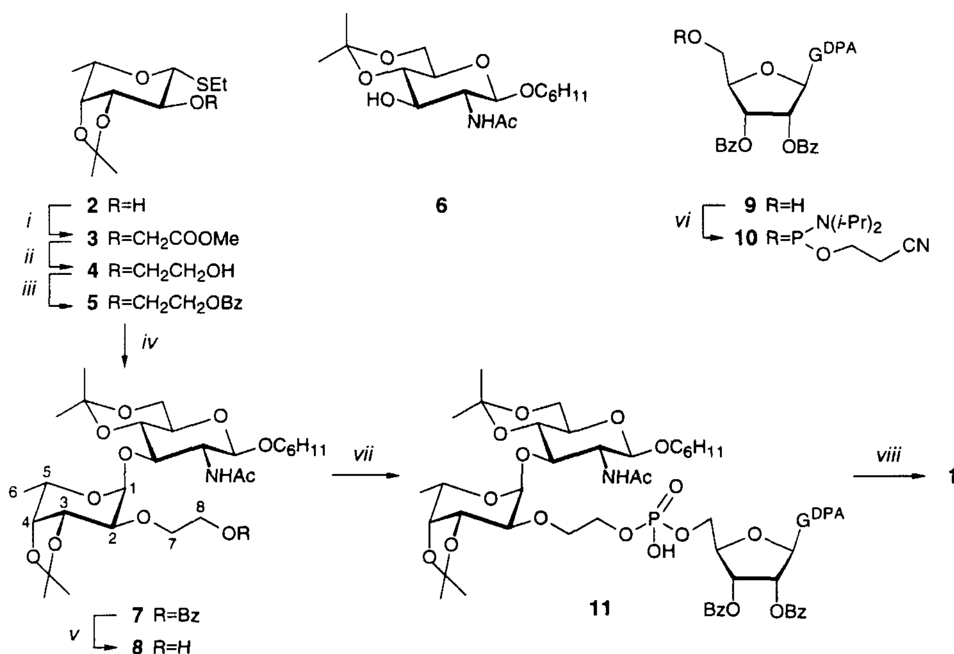
Having donor **5** in hand, we turned our attention to the glycosylation of acceptor cyclohexyl 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**6**), which was prepared as follows. First, glycosylation of cyclohexanol was achieved with readily accessible 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline[8] under the promotion of catalytic trifluoromethanesulfonic acid (TfOH). Subsequent Zemplén deacetylation and treatment with 2,2-dimethoxypropane and catalytic *p*-toluenesulfonic acid furnished acceptor **6**. Condensation of fucopyranosyl donor **5** with acceptor **6** under the agency[9] of *N*-iodosuccinimide (NIS) and catalytic TfOH gave disaccharide **7** as a mixture of anomers (α/β = 4/1) in 71% yield. On the other hand, a higher yield (94%) and a more favourable α/β ratio (7/1) was obtained by the use of the promoter system cupric bromide and tetrabutylammonium bromide as proposed by Ogawa.[10] Separation of the individual anomers by silica gel chromatography led to the α-linked



Reagents and conditions:

(i) NaH, $BrCH_2COOMe$, imidazole, Bu_4NI, THF, reflux (73%); *(ii)* $LiAlH_4$, THF, reflux; *(iii)* BzCl, pyridine (91%, 2 steps); *(iv)* **6**, $CuBr_2$, Bu_4NBr, DCE, DMF (94%, $\alpha/\beta=7/1$); *(v)* KO*t*-Bu, MeOH (91%); *(vi)* $ClP(OCH_2CH_2CN)[N(i\text{-}Pr)_2]$, DIPEA, CH_2Cl_2 (59%); *(vii)* (a) **10**, 1*H*-tetrazole, CH_2Cl_2, CH_3CN; (b) *t*-BuOOH; (c) TEA (79%, 3 steps); *(viii)* (a) PPTS, MeOH, 50 °C; (b) NH_4OH, 50 °C (90%, 2 steps).

Scheme 1

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dimer **7** in 82% yield. Removal of the benzoyl group in **7** by Zemplén deacylation afforded the requisite disaccharide **8**.

The next stage in the assembly of target molecule **1** entailed the introduction of the 5′-phosphate guanosine moiety in disaccharide **8**. To this end, the primary hydroxyl group of guanosine derivative **9**[11] was phosphitylated with chloro 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite[12] in the presence of *N*,*N*-diisopropylethylamine to furnish 3′,4′-di-*O*-benzoyl-5′-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite)-2-*N*-diphenylacetylguanosine (**10**). Phosphodiester **11** was obtained *via* a one-pot three-step procedure. Thus, condensation of disaccharide **8** with reagent **10** under the agency of 1*H*-tetrazole[13] was followed by *in situ* oxidation with *tert*-butyl hydroperoxide. Subsequent elimination of the cyanoethyl

group from the intermediate phosphotriester with triethylamine gave phosphodiester **11** (triethylammonium salt, δ_P -0.54 ppm) in 79% yield.

Finally, the protective groups in trisubstrate analogue **11** were unleashed by the following two-step protocol. Deacetonisation of **11** was effected by treatment with pyridinium *p*-toluene sulfonate (PPTS) in methanol. Saponification of the ester functions with aqueous ammonia and subsequent purification by gel filtration furnished target compound **1** in 90% yield. The structure of **1** was firmly established by ^1H 2D and CH COSY NMR experiments.

In conclusion, a straightforward synthesis of *trisubstrate analogue* **1** has been described. The synthetic route can readily be adopted for the assembly of *trisubstrate analogues* containing alternative spacers (*e.g.* a neutral phosphate bridge[14] or an isosteric spacer[15]) or different acceptor moieties in order to further assess the biological activity of this type of *trisubstrate analogues*. The inhibitory effect of compound **1** on α(1→3)FucT-VI is currently under investigation and will be reported in due course.

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EXPERIMENTAL

General procedures. 1,2-Dichloroethane, dichloromethane, diethyl ether and toluene were distilled from P_2O_5 (5 g/L). Acetonitrile and pyridine were dried by refluxing overnight with CaH_2 (5 g/L) and then distilled. *N,N*-Dimethylformamide (DMF) was stirred overnight with CaH_2 (5 g/L) and then distilled under reduced pressure. Methanol was dried by refluxing with magnesium methoxide and then distilled. Tetrahydrofuran (THF) was distilled from $LiAlH_4$. Methanol was stored on 0.3 nm and all other solvents 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. 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 Fractogel TSK HW-40. ^1H NMR (200 MHz), ^{13}C NMR (50.1 MHz) and ^{31}P NMR (80.7 MHz) spectra were recorded using a Jeol JNM-FX 200 spectrometer, unless stated otherwise. ^1H NMR (300 MHz) spectra

were recorded using a Bruker WM-300 spectrometer, ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using a Bruker MSL-400 spectrometer. ^1H and ^{13}C chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard, ^{31}P chemical shifts are given in ppm relative to 85% H_3PO_4 as external standard.

Ethyl 3,4-*O*-Isopropylidene-2-*O*-(methoxycarbonylmethyl)-1-thio-β-L-fucopyranoside (3). NaH (600 mg 60% dispersion, 15 mmol), imidazole (20 mg) and Bu_4NBr (20 mg) were added to a solution of compound **2** (1.38 g, 5.56 mmol), which was dried previously by evaporation with toluene (3 × 10 mL), in THF (25 mL). After stirring for 30 min, methyl bromoacetate (1.00 mL, 10.56 mmol) was added and the mixture was heated at reflux. After 16 h TLC analysis indicated complete conversion of the starting material to a less polar compound and the cooled mixture was quenched with methanol. The mixture was neutralized with dilute aqueous HCl and extracted with dichloromethane. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (diethyl ether/petroleum ether, 0:1 to 3:7 v/v) to give 1.30 g (73%) of pure **3**. ^1H NMR ($CDCl_3$) δ 1.30 (t, 3H, SCH_2CH_3), 1.34, 1.50 (2s, 6H, $C(CH_3)_2$), 1.39 (d, 3H, H-6, $J_{5,6}$ = 6.6 Hz), 2.74 (m, 2H, SCH_2CH_3), 3.45 (dd, 1H, H-2, $J_{1,2}$ = 9.6 Hz, $J_{2,3}$ = 6.9 Hz), 3.76 (s, 3H, OCH_3), 3.81 (dq, 1H, H-5, $J_{4,5}$ = 2.1 Hz), 4.04 (dd, 1H, H-4, $J_{3,4}$ = 5.6 Hz), 4.21 (dd, 1H, H-3), 4.39 (d, 1H, H-1), 4.42 (AB, 2H, H-7); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 13.7 (SCH_2CH_3), 14.3 (C-6), 23.8 (SCH_2CH_3), 25.8, 27.5 ($C(CH_3)_2$), 51.2 (OCH_3), 67.8 (C-7), 71.9, 76.0, 78.7, 79.7 (C-2, C-3, C-4, C-5), 82.3 (C-1), 109.1 ($C(CH_3)_2$), 169.8 (C=O).

Ethyl 2-*O*-(2-Hydroxyethyl)-3,4-*O*-isopropylidene-1-thio-β-L-fucopyranoside (4). Compound **3** (1.30 g, 4.06 mmol) was dried by evaporation with toluene (3 × 10 mL) and dissolved in THF (20 mL). $LiAlH_4$ (600 mg, 15.79 mmol) was added and the mixture was heated at reflux. After 16 h TLC analysis showed completion of the reaction and excess $LiAlH_4$ was decomposed with ethyl acetate (45 mL). A 20% KOH solution (60 mL) and water (100 mL) were added and the mixture was extracted with diethyl ether (2 × 30 mL). The combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* to give **4** in a quantitative yield

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(1.19 g). ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, SCH_2CH_3), 1.36, 1.54 (2s, 6H, $C(CH_3)_2$), 1.40 (d, 3H, H-6, $J_{5,6}$ = 6.6 Hz), 2.74 (m, 2H, SCH_2CH_3), 2.95 (bs, 1H, OH), 3.33 (dd, 1H, H-2, $J_{1,2}$ = 10.1 Hz, $J_{2,3}$ = 6.6 Hz), 3.71-3.88 (m, 5H, H-3, H-4, H-5, H-8), 4.04-4.21 (m, 2H, H-7), 4.28 (d, 1H, H-1); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 14.1 (SCH_2CH_3), 15.9 (C-6), 23.4 (SCH_2CH_3), 25.4, 27.2 ($C(CH_3)_2$), 60.7 (C-8), 72.9 (C-7), 71.5, 75.6, 78.6, 79.7 (C-2, C-3, C-4, C-5), 82.5 (C-1), 108.7 ($C(CH_3)_2$).

Ethyl 2-*O*-[2-(Benzoyloxy)ethyl]-3,4-*O*-isopropylidene-1-thio-β-L-fucopyranoside (5). Compound **4** (4.06 mmol) was dried by evaporation with pyridine (3 × 10 mL), dissolved in pyridine (20 mL) and benzoylchloride (0.50 mL, 4.31 mmol) was added. After stirring for 1 h the mixture was quenched with water and concentrated *in vacuo*. The residue was redissolved in dichloromethane, the solution was washed with saturated aqueous $NaHCO_3$, water, dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (diethyl ether/petroleum ether, 0:1 to 3:7 v/v) to give 1.61 g (92%) of pure **5**. R_f 0.87 (diethyl ether); ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, SCH_2CH_3), 1.34, 1.53 (2s, 6H, $C(CH_3)_2$), 1.39 (d, 3H, H-6, $J_{5,6}$ = 6.6 Hz), 2.70 (q, 2H, SCH_2CH_3), 3.36 (dd, 1H, H-2, $J_{1,2}$ = 9.9 Hz, $J_{2,3}$ = 6.6 Hz), 3.80 (dq, 1H, H-5, $J_{4,5}$ = 2.0 Hz), 4.02-4.19 (m, 4H, H-3, H-4, H-7), 4.34 (d, 1H, H-1), 4.48 (t, 2H, H-8), 7.38-8.13 (m, 5H, $H_{arom.}$); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 14.1 (SCH_2CH_3), 16.1 (C-6), 23.6 (SCH_2CH_3), 25.6, 27.4 ($C(CH_3)_2$), 63.3 (C-8), 69.2 (C-7), 71.6, 75.7, 78.8, 80.2 (C-2, C-3, C-4, C-5), 82.4 (C-1), 108.7 ($C(CH_3)_2$), 127.5, 129.0, 132.1 ($CH_{arom.}$), 129.6 ($C_{arom.}$), 165.5 (C=O).

Anal. Calcd for $C_{20}H_{28}O_6S$ (396.51): C, 60.59; H, 7.12. Found: C, 60.73; H, 7.19.

Cyclohexyl 2-Acetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (6). 2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (7.9 g, 24.0 mmol) was dried by evaporation with toluene and dissolved in dichloromethane (50 mL). Cyclohexanol (3.7 mL, 35.6 mmol) and TfOH (210 μL, 2.4 mmol) were added and the mixture was stirred for 2 h, when TLC analysis indicated completion of the reaction. The mixture was neutralized with TEA, washed with saturated aqueous $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated *in vacuo*. The crude crystalline product was recrystallized from ethanol to give pure cyclohexyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (6.9 g, 67%).

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The peracetylated glycoside (6.9 g, 16.1 mmol) was dissolved in methanol (50 mL) and NaOMe (100 mg) was added. After 2 h the mixture was neutralized with Dowex 50 W X 4 [H^+], filtered and the filtrate was concentrated *in vacuo*. The residual methanol was removed by evaporation with toluene (3 × 10 mL). The deacetylated product was dissolved in acetone (50 mL) and dimethoxypropane (15 mL) and the acidity of the solution was adjusted to pH 4 with *p*-TsOH. After stirring for 4 h at 40 °C TLC analysis indicated disappearance of the starting material and the mixture was neutralized with TEA. The solvent was evaporated, the residue was redissolved in dichloromethane and washed with saturated aqueous $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (ethanol/petroleum ether, 0:1 to 5:95 v/v) to give pure **6**. Yield: 4.6 g (83%, 2 steps); R_f 0.54 (methanol/dichloromethane, 1:9 v/v); ^1H NMR ($CDCl_3$) δ 1.17-1.91 (m, 10H, 5 × CH_2 Hex), 1.44, 1.52 (2s, 6H, $C(CH_3)_2$), 2.04 (s, 3H, $COCH_3$), 3.03 (dt, 1H, H-5, J = 9.9 Hz, $J_{5,6b}$ = 5.6 Hz), 3.32 (dd, 1H, H-2, $J_{NH,2}$ = 5.35 Hz, $J_{2,3}$ = 9.6 Hz), 3.56-3.68 (m, 1H, OCH cHex), 3.58 (t, 1H, H-4, J = 9.3 Hz), 3.80 (t, 1H, H-6a, J = 10.3 Hz), 3.92 (dd, 1H, H-6b, $J_{6a,6b}$ = -10.3 Hz), 4.04 (t, 1H, H-3), 4.77 (d, 1H, H-1, $J_{1,2}$ = 8.1 Hz), 5.77 (bs, 1H, NH); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 18.7, 28.6 ($C(CH_3)_2$), 22.9 ($COCH_3$), 23.4, 23.6, 25.1, 31.4, 33.0 (5 × CH_2 cHex), 57.6 (C-2), 61.7 (C-6), 66.6, 70.8, 73.9, 77.3 (C-3, C-4, C-5, OCH cHex), 99.2 ($C(CH_3)_2$), 99.2 (C-1), 174.9 (C=O).

Anal. Calcd for $C_{17}H_{29}O_6N$ (343.42): C, 59.46; H, 8.51; N, 4.08. Found: C, 59.54; H, 8.47; N, 4.15.

Cyclohexyl 2-Acetamido-{3-*O*-[2-(benzoyloxy)ethyl]-3,4-*O*-isopropylidene-α-L-fucopyranosyl}-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (7). Donor **5** (500 mg, 1.26 mmol) and acceptor **6** (500 mg, 1.46 mmol) were dried by evaporation with 1,2-dichloroethane (3 × 5 mL), dissolved in 1,2-dichloroethane (5 mL) and DMF (1 mL) and stirred for 30 min with 0.4 nm molecular sieves under a nitrogen atmosphere. $CuBr_2$ (420 mg, 1.88 mmol) and Bu_4NBr (600 mg, 1.86 mmol) were added and stirring was continued. After 16 h TLC analysis showed complete disappearance of the donor and the reaction mixture was diluted with dichloromethane and filtered. The filtrate was washed with saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$) and concentrated *in vacuo*. Purification by column

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chromatography (diethyl ether/petroleum ether, 1:1 to 1:0 v/v) gave 700 mg of pure α-linked dimer **7** and 105 mg of the β-anomer (total yield: 94%; α/β 7/1). R_f (α) 0.71 (methanol/dichloromethane, 1:9 v/v); ^{1}H NMR (300 MHz 2D COSY) ($CDCl_3$) δ 1.18-1.89 (m, 28H, Fuc: H-6, 2 × $C(CH_3)_2$, $COCH_3$, 5 × CH_2 cHex), 2.95 (ddd, 1H, GlcNAc: H-2, $J_{1,2}$ = 8.3 Hz, $J_{2,NH}$ = 7.2 Hz, $J_{2,3}$ = 9.9 Hz), 3.25 (dt, 1H, GlcNAc: H-5, $J_{4,5}$ = 9.9 Hz, $J_{5,6b}$ = 5.4 Hz), 3.44 (t, 1H, GlcNAc: H-4, J = 9.8 Hz), 3.46 (dd, 1H, Fuc: H-2, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 8.0 Hz), 3.55-3.62 (m, 1H, OCH cHex), 3.73 (t, 1H, GlcNAc: H-6a, J = 10.5 Hz), 3.83 (ddd, 1H, Fuc: H-7a, $J_{7a,8a}$ = 3.1 Hz, $J_{7a,8b}$ = 5.7 Hz, $J_{7a,7b}$ = -11.1 Hz), 3.87 (t, 1H, GlcNAc: H-6b), 3.99 (dd, 1H, Fuc: H-4, $J_{3,4}$ = 5.5 Hz, J = 2.6 Hz), 4.17 (t, 1H, GlcNAc: H-3), 4.19 (dd, 1H, Fuc: H-3), 4.22 (ddd, 1H, Fuc: H-7b, $J_{7b,8a}$ = 7.1 Hz, $J_{7b,8b}$ = 3.0 Hz), 4.41-4.49 (m, 2H, Fuc: H-5, H-8a), 4.73 (ddd, 1H, Fuc: H-8b, $J_{8a,8b}$ = -11.9 Hz), 4.83 (d, 1H, Fuc: H-1), 5.16 (d, 1H, GlcNAc: H-1), 6.52 (d, 1H, NH), 7.45-8.07 (m, 5H, $H_{arom.}$); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 16.1 (Fuc: C-6), 19.1, 26.3, 28.3, 29.0 (2 × $C(\mathit{C}H_3)_2$), 23.3 ($CO\mathit{C}H_3$), 23.6, 23.8, 25.5, 31.7, 33.3 (5 × CH_2 cHex), 59.2 (GlcNAc: C-2), 62.2 (GlcNAc: C-6), 62.7, 66.5, 73.3, 76.2, 77.4, 78.8 (Fuc: C-2, C-3, C-4, C-5, GlcNAc: C-3, C-4, C-5, OCH cHex), 64.0 (Fuc: C-8), 69.0 (Fuc: C-7), 98.2 (Fuc, GlcNAc: C-1), 98.9, 108.7 (2 × $\mathit{C}(CH_3)_2$), 128.5, 129.6, 133.2 ($CH_{arom.}$), 170.6 (C=O).

Anal. Calcd for $C_{35}H_{51}O_{12}N$ (677.80): C, 62.02; H, 7.58; N, 2.07. Found: C, 62.19; H, 7.66; N, 1.99.

7β: $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 16.5 (Fuc: C-6), 18.8, 26.0, 27.9, 28.9 (2 × $C(\mathit{C}H_3)_2$), 23.2 ($CO\mathit{C}H_3$), 23.6, 23.8, 25.4, 31.6, 33.3 (5 × CH_2 cHex), 57.7 (GlcNAc: C-2), 62.2 (GlcNAc: C-6), 64.2, 69.4 (Fuc: C-7, C-8), 66.5, 68.2, 74.3, 75.5, 76.1, 77.3, 81.3 (Fuc: C-2, C-3, C-4, C-5, GlcNAc: C-3, C-4, C-5, OCH cHex), 98.8 (GlcNAc: C-1), 99.1, 109.3 (2 × $\mathit{C}(CH_3)_2$), 102.3 (Fuc: C-1), 128.1, 129.5, 132.7 ($CH_{arom.}$), 170.6 (C=O).

Cyclohexyl 2-Acetamido-2-deoxy-3-*O*-[2-*O*-(2-hydroxyethyl)-3,4-*O*-isopropylidene-α-L-fucopyranosyl]-4,6-*O*-isopropylidene-β-D-glucopyranoside (8). Disaccharide **7** (155 mg, 0.23 mmol) was dissolved in methanol (2 mL) and KO*t*-Bu (10 mg, 0.09 mmol) was added. After 16 h the mixture was neutralized with Dowex 50 W X 4 [H^+], filtered and concentrated *in vacuo* to dryness. The crude product was purified by column chromatography (methanol/dichloromethane, 0:1 to 5:95 v/v) to

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give 120 mg (91%) of pure **8**. R_f 0.50 (methanol/dichloromethane, 1:9 v/v); ^{1}H NMR ($CDCl_3$) δ 1.21-1.90 (m, 10H, 5 × CH_2 cHex), 1.27 (d, 3H, Fuc: H-6, $J_{5,6}$ = 6.6 Hz), 1.33, 1.39, 1.43, 1.50 (4s, 12H, 2 × $C(CH_3)_2$), 1.96 (s, 3H, $COCH_3$), 3.16 (bq, 1H, GlcNAc: H-2, J = 7.7 Hz), 3.39 (dt, 1H, GlcNAc: H-5, J = 8.8 Hz, $J_{5,6b}$ = 4.4 Hz), 3.50-3.94 (m, 9H, Fuc: H-2, H-7, H-8, GlcNAc: H-4, H-6, OCH cHex), 4.00 (dd, 1H, Fuc: H-4, $J_{3,4}$ = 5.4 Hz, $J_{4,5}$ = 2.6 Hz), 4.19 (dd, 1H, Fuc: H-3, $J_{2,3}$ = 8.2 Hz), 4.28 (t, 1H, GlcNAc: H-3, J = 9.4 Hz), 4.53 (dq, 1H, Fuc: H-5), 4.98 (d, 1H, Fuc: H-1, $J_{1,2}$ = 3.6 Hz), 5.13 (d, 1H, GlcNAc: H-1, $J_{1,2}$ = 8.4 Hz), 6.58 (d, 1H, NH); 13C{1H} NMR ($CDCl_3$) δ 16.1 (Fuc: C-6), 19.1, 26.2, 28.2, 28.8 (2 × $C(CH_3)_2$), 23.3 ($COCH_3$), 23.8, 25.3, 31.8, 33.3 (5 × CH_2 cHex), 58.5 (GlcNAc: C-2), 61.7 (Fuc: C-8), 62.2 (GlcNAc: C-6), 62.5, 66.7, 73.0, 75.4, 76.1, 77.5, 78.1 (Fuc: C-2, C-3, C-4, C-5, GlcNAc: C-3, C-4, C-5, OCH cHex), 71.8 (Fuc: C-7), 96.9 (Fuc: C-1), 98.3 (GlcNAc: C-1), 99.0, 108.5 (2 × $C(CH_3)_2$), 171.1 (C=O).

Anal. Calcd for $C_{28}H_{47}O_{11}N$ (573.69): C, 58.62; H, 8.26; N, 2.44. Found: C, 58.69; H, 8.37; N, 2.42.

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3′,4′-Di-*O*-benzoyl-5′-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-2-*N*-diphenylacetylguanosine (10). Compound **9** (1.38 g, 2.00 mmol) was dried by evaporation with 1,2-dichloroethane (3 × 5 mL), dissolved in 1,2-dichloroethane (10 mL) and kept under an atmosphere of nitrogen. DIPEA (0.70 mL, 4.02 mmol) and chloro 2-cyanoethyl-*N,N*-diisopropylphosphoramidite (0.63 mL, 2.80 mmol) were added and the mixture was stirred for 30 min, when TLC analysis (methanol/dichloromethane, 1:9 v/v) indicated complete conversion of the starting material. Water (10 mL) was added and the mixture was extracted with dichloromethane. The organic layer was washed with brine (2 ×) and saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo* to dryness. The crude product was purified by flash column chromatography (TEA/ethanol/diethyl ether, 1:0:99 to 1:4:95 v/v) to give 1.05 g (59%) of pure **10**. R_f 0.60 (methanol/dichloromethane, 1:9 v/v); ^{31}P{^{1}H} NMR ($CDCl_3$) δ 149.15, 149.82.

Cyclohexyl 2-Acetamido-3-*O*-{2-*O*-[2-(3′,4′-di-*O*-benzoyl-2-*N*-diphenylacetylguanosine 5′-*O*-phosphate)ethyl]-3,4-*O*-isopropylidene-α-L-fucopyranosyl}-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (11). Compound **8** (120 mg, 0.21 mmol) and **10** (230 mg, 0.26 mmol) were dried by

evaporation with toluene (3 × 5 mL) and dissolved in dichloromethane (3 mL). 1*H*-tetrazole (60 mg, 0.86 mmol), dried by evaporation with toluene (3 × 5 mL) and dissolved in acetonitrile (1 mL), was added. TLC analysis (R_f 0.55, methanol/dichloromethane, 1:9 v/v) indicated completion of the reaction after 10 min and *t*-BuOOH (115 μL, 0.50 mmol) was added. TLC analysis indicated completion of the oxidation after 30 min and TEA (1 mL) was added. After stirring for 4 h the mixture was concentrated *in vacuo* and the residue was purified by column chromatography (methanol/dichloromethane, 0:9 to 1:9 v/v) to give 230 mg (77%) of pure **11** in the triethylammonium form. R_f 0.37 (methanol/dichloromethane, 1:9 v/v); $^{13}C\{^1H\}$ NMR ($MeOD/CDCl_3$) δ 8.2 ($N(CH_2CH_3)_3$), 15.8 (Fuc: C-6), 18.7, 25.8, 27.8, 28.5 (2 × $C(CH_3)_2$), 22.6 ($COCH_3$), 23.4, 23.5, 25.0, 31.3, 33.0 (5 × CH_2 cHex), 45.8 ($N(CH_2CH_3)_3$), 56.7 ($CH(Ph)_2$), 57.2 (GlcNAc: C-2), 61.8 (GlcNAc: C-6), 62.5, 66.5, 71.8, 72.8, 73.0, 75.2, 75.9, 77.6, 78.1 (Fuc: C-2, C-3, C-4, C-5, GlcNAc: C-3, C-4, C-5, Rib: C-2, C-3, OCH cHex), 64.2, 64.7 (Fuc: C-8, Rib: C-5), 70.3 (Fuc: C-7), 82.4 (d, Rib: C-4, $J_{4,P}$ = 8.8 Hz), 86.8 (Rib: C-1), 97.2 (Fuc: C-1), 99.1 (GlcNAc: C-1), 98.9, 108.2 (2 × $C(CH_3)_2$), 120.9 (G: C-5), 124.1-129.2 ($CH_{arom.}$), 137.4, 137.7 ($C_{arom.}$), 133.4 (G: C-8), 147.8, 148.5 (G: C-2, C-4), 155.6 (G: C-6), 164.7, 165.0, 172.1, 175.0 (4 × C=O); $^{31}P\{^1H\}$ NMR (CH_2Cl_2) δ -0.54.

Anal. Calcd for $C_{72}H_{92}O_{21}N_7P$ (1422.54): C, 60.79; H, 6.52; N, 5.89. Found: C, 60.98; H, 6.61; N, 6.80.

Cyclohexyl 2-Acetamido-2-deoxy-3-*O*-{2-*O*-[2-(guanosine 5′-*O*-phosphate)ethyl]-α-L-fucopyranosyl}-β-D-glucopyranoside (1). Compound **11** (140 mg, 0.098 mmol) was dissolved in methanol (1 mL) and PPTS (10 mg, 0.040 mmol) was added. After 16 h at 50 °C TLC analysis showed complete conversion to a more polar compound, the mixture was concentrated *in vacuo* and redissolved in 25% aqueous NH_4OH. After 48 h at 50 °C the mixture was concentrated *in vacuo*, taken up in water, washed with dichloromethane (3 ×) and lyophilized. The crude product was purified by gel filtration (water/methanol/2 M TEAB, 85:10:5 v/v, R_t 134 min), concentrated *in vacuo* and applied to a column of Dowex 50 W X 4 [Na^+], which was eluted with water. The resulting solution was concentrated *in vacuo*, the residue was redissolved in D_2O and lyophilized to give 76 mg (90%) of the title compound. R_f 0.41 (*i*-PrOH/NH_4OH/water, 6:3:1 v/v); ^1H NMR (400 MHz 2D COSY, CH

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COSY) (D_2O) δ 1.18 (d, 3H, Fuc: H-6, $J_{5,6}$ = 6.6 Hz), 1.21-1.94 (m, 10H, 5 × CH_2 cHex), 2.03 (s, 3H, $COCH_3$), 3.44-3.54 (m, 2H, GlcNAc: H-4, H-5), 3.63 (dd, 1H, Fuc: H-2, $J_{1,2}$ = 3.9 Hz, $J_{2,3}$ = 10.3 Hz), 3.70-3.76 (m, 6H, Fuc: H-7, GlcNAc: H-2, H-3, H-6a, OCH cHex), 3.80 (bd, 1H, Fuc: H-4, $J_{3,4}$ = 3.4 Hz), 3.89 (dd, 1H, Fuc: H-3), 3.92-4.00 (m, 3H, Fuc: H-8, GlcNAc: H-6b), 4.15-4.17 (m, 2H, Rib: H-5), 4.32-4.37 (m, 2H, Fuc: H-5, Rib: H-4), 4.53 (t, 1H, Rib: H-3, J = 4.5 Hz), 4.64 (d, 1H, GlcNAc: H-1, $J_{1,2}$ = 8.0 Hz), 4.80 (t, 1H, Rib: H-2), 5.18 (d, 1H, Fuc: H-1), 5.95 (d, 1H, Rib: H-1, $J_{1,2}$ = 5.7 Hz), 8.11 (s, 1H, G: H-8); $^{13}C\{^1H\}$ NMR (100 MHz) (D_2O) δ 15.9 (Fuc: C-6), 23.3 ($COCH_3$), 23.7, 23.9, 25.7, 31.7, 33.3 (5 × CH_2 cHex), 56.3 (GlcNAc: C-2), 61.6 (GlcNAc: C-6), 65.7 (d, Rib: C-5, $J_{5,P}$ = 4.4 Hz), 65.9 (d, Fuc: C-8, $J_{8,P}$ = 5.9 Hz), 67.6 (Fuc: C-5), 69.3 (Fuc: C-3), 69.6, 76.4 (GlcNAc: C-4, C-5), 70.5 (d, Fuc: C-7, $J_{7,P}$ = 7.3 Hz), 71.1 (Rib: C-3), 72.5 (Fuc: C-4), 74.4 (Rib: C-2), 77.3 (Fuc: C-3), 79.0, 79.8 (GlcNAc: C-3, OCH cHex), 84.4 (d, Rib: C-4, $J_{4,P}$ = 9.0 Hz), 87.6 (Rib: C-1), 97.5 (Fuc: C-1), 100.0 (GlcNAc: C-1), 117.0 (G: C-5), 138.1 (G: C-8), 152.6 (G: C-4), 154.8 (G: C-2), 159.8 (G: C-6), 174.9 (C=O); $^{31}P\{^1H\}$ NMR (D_2O) δ 0.91.

Anal. Calcd for $C_{32}H_{50}O_{18}N_6NaP$ (860.75): C, 44.65; H, 5.86; N, 9.76. Found: C, 44.57; H, 5.90; N, 9.87.

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