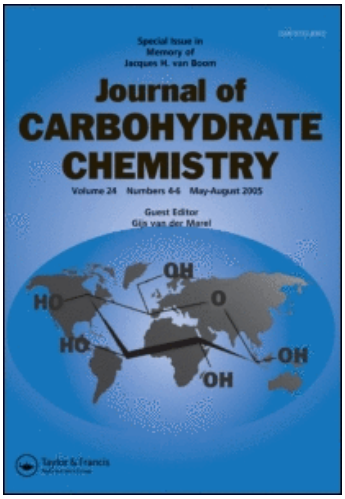

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An Efficient Synthesis of the Lewis A (Le[a]) Antigen Pentasaccharide Moiety

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AN EFFICIENT SYNTHESIS OF THE LEWIS A (Le^a) ANTIGEN PENTASACCHARIDE MOIETY

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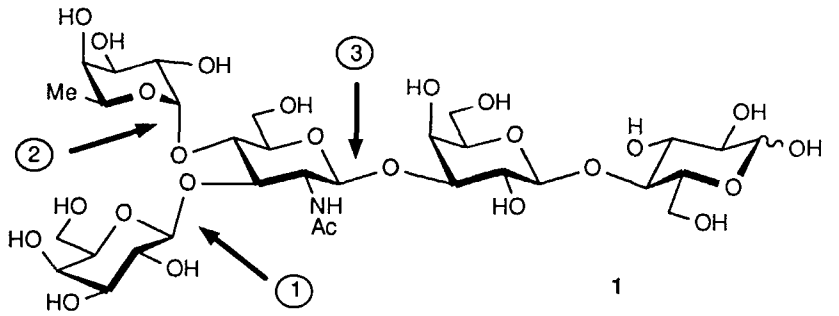
ABSTRACT

Starting material for the synthesis of Lewis A pentasaccharide (**1**) was azidoglucose derivative **2** which was readily transformed into the 3,4-*O*-unprotected derivative **3** or the 3-*O*-unprotected derivative **5**, respectively. Reaction of **3** and *O*-galactosyltrichloroacetimidate **6** led preferentially to the desired β(1-3)-connected disaccharide **8** which could be selectively obtained from donor **6** and acceptor **5** via disaccharide **9**. 4a-*O*-Fucosylation of **8** with fucosyl donor **10** furnished trisaccharide **11** which was transformed into triosyl donor **13**; glycosylation of lactose derivative **14** as acceptor furnished the desired pentasaccharide in high yield. Azide reduction and *N*-acetylation and *O*-deprotection afforded the title compound **1** in high overall yield.

INTRODUCTION

The Lewis A antigen is a blood group antigen which is found on the surface of erythrocytes and normal glandular and epithelial cells.[2] We present a synthesis of the pentasaccharide moiety **1** of this glycoconjugate which is based on the synthetic strategy outlined in Scheme 1 (arrows ① – ③ indicate the consecutive glycoside bond formations in our synthesis). It takes into account a reactivity difference of the hydroxy groups in 3- and 4-position of 6-*O*-protected azidoglucose[3] or, alternatively, selective generation of 4-*O*-unprotected 3-*O*-galactosylated azidoglucose from a 4,6-*O*-

Scheme 1



L-Fuc + D-Gal + D-GlcNAc + Lactose

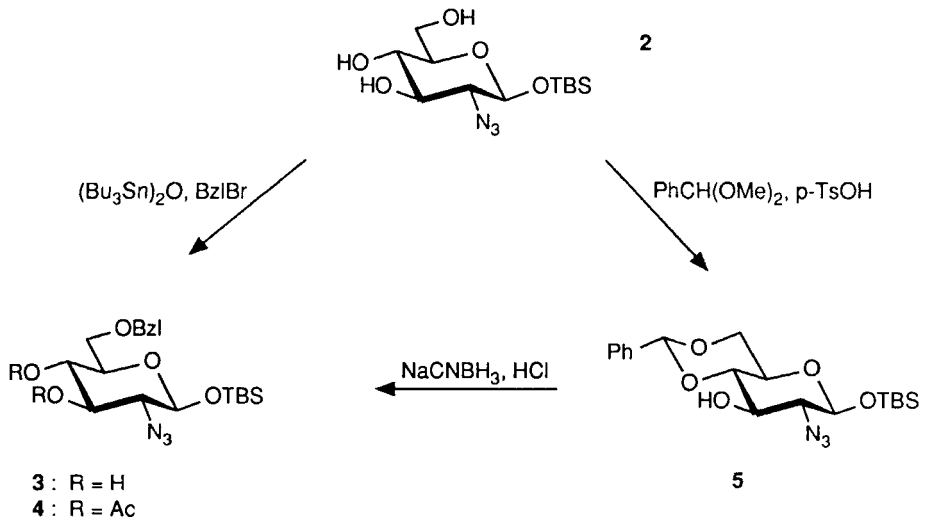
benzylidene protected precursor. Thus, via consecutive 3-*O*-galactosylation and 4-*O*-fucosylation the required trisaccharide building block should be obtained which is subsequently linked to a lactose moiety to yield the desired pentasaccharide. A different approach for the synthesis of Le^a pentasaccharide has been previously reported.[4]

RESULTS AND DISCUSSION

As basic starting material for the synthesis of pentasaccharide **1**, readily available 3,4,6-*O*-unprotected azidoglucose derivate **2**[5] was chosen (Scheme 2).

Based on previous experience for related glucose systems,[4,6] selective 6-*O*-protection should provide an intermediate which exhibits strikingly higher reactivity in glycosylation for the 3-hydroxy group than for the 4-hydroxy group. Because acyl groups diminish the overall reactivity and sterically demanding groups in 6-*O*-position decrease access to the 4-*O*-position, the benzyl group was selected for the 6-*O*-protection. To this aim **2** was treated with bis(tributyltin)oxide[7] and then with benzyl bromide which resulted in a 70% yield of the desired 6-*O*-benzyl protected derivative **3**. The structural assignment was confirmed by *O*-acetylation of **3** in acetic anhydride/pyridine (→ **4**) which led to ^{1}H NMR low field shifts for H-3 and H-4 (from δ 3.33 and 3.58 to 4.88-5.03). Alternatively, compound **3** can be prepared from **2** by benzylidenation with benzaldehyde dimethyl acetal in the presence of *p*-toluenesulfonic

Scheme 2

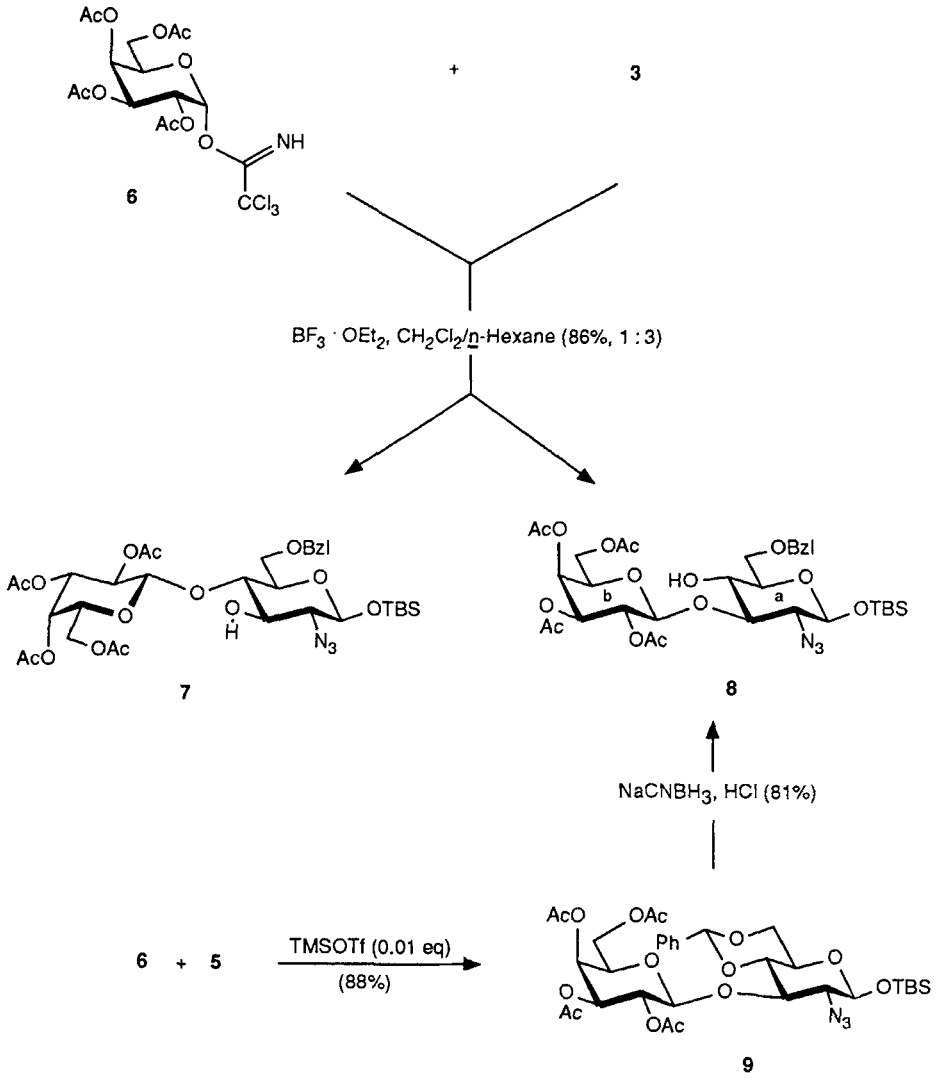


acid as catalyst to furnish **5** in practically quantitative yield. Reductive ring opening of **5** with sodium cyanoborohydride in the presence of HCl in ether[8] gave compound **3** in 76% yield.

For the ensuing galactosylation readily available galactosyl donor **6**[9] was chosen (Scheme 3). The reaction with acceptor **3** was carried out under mild conditions with diethyl ether-boron trifluoride catalysis at -25 °C. However, the regioselectivity was not as high as expected; the regioisomers **7** and **8** were obtained in a 1:3 ratio, although in very high overall yield. Separation of **7** and **8** by medium pressure chromatography precluded preparation of large amounts of disaccharide **8**. Therefore, reaction of donor **6** was carried out with 4,6-*O*-benzylidene protected acceptor **5** in the presence of catalytic amounts of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst which furnished, due to neighboring group participation, exclusively β(1-3)-connected disaccharide **9**. Reductive ring opening of the benzylidene moiety as described above led to the required disaccharide **8** in high overall yield.

Fucosylation of **8** was carried out with known 2,3,4-tri-*O*-benzyl protected fucosyl donor **10**[11] which gave, with TMSOTf as catalyst under "inverse procedure" conditions[11] (i.e., addition of the donor to an acceptor-catalyst solution), trisaccharide building block **11** in high yield (Scheme 4). Removal of the 1-*O*-silyl group was carried out by treatment with tetrabutylammonium fluoride (TBAF) in the presence of acetic acid[12] to give 1-*O*-unprotected trisaccharide **12**. Treatment of **12** with CCl_3CN and 1,8-

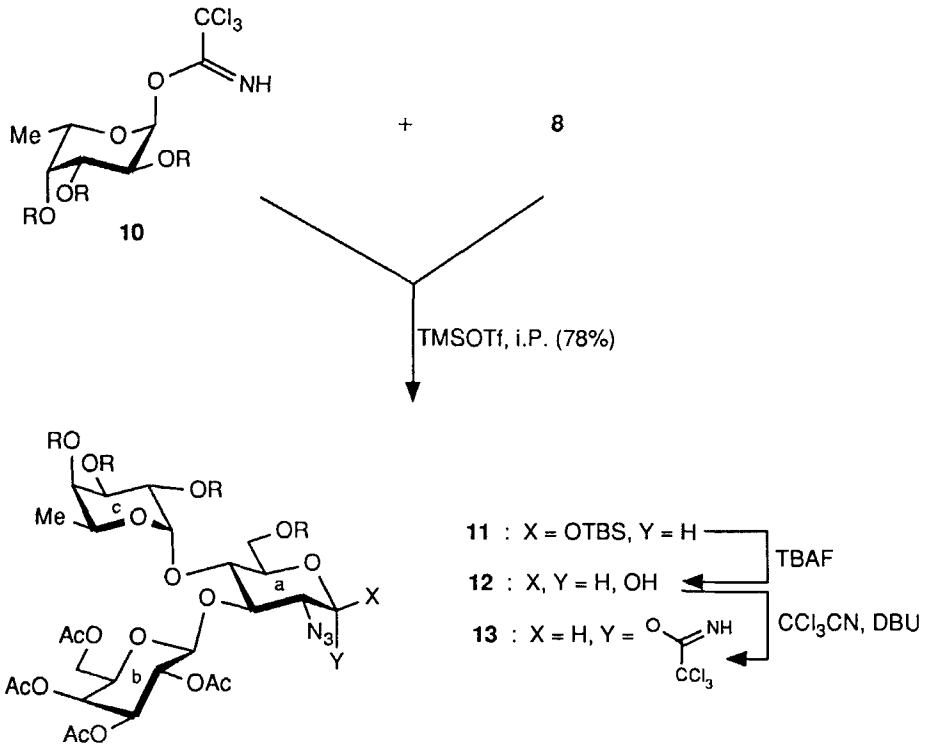
Scheme 3



diazabicyclo[5.4.0]undec-7-ene (DBU) as base afforded preferentially the α-trichloroacetimidate **13** and some β-anomer the latter being transformed into the thermodynamically more stable α-anomer **13** by addition of more base; thus, **13** was obtained in 80% overall yield.

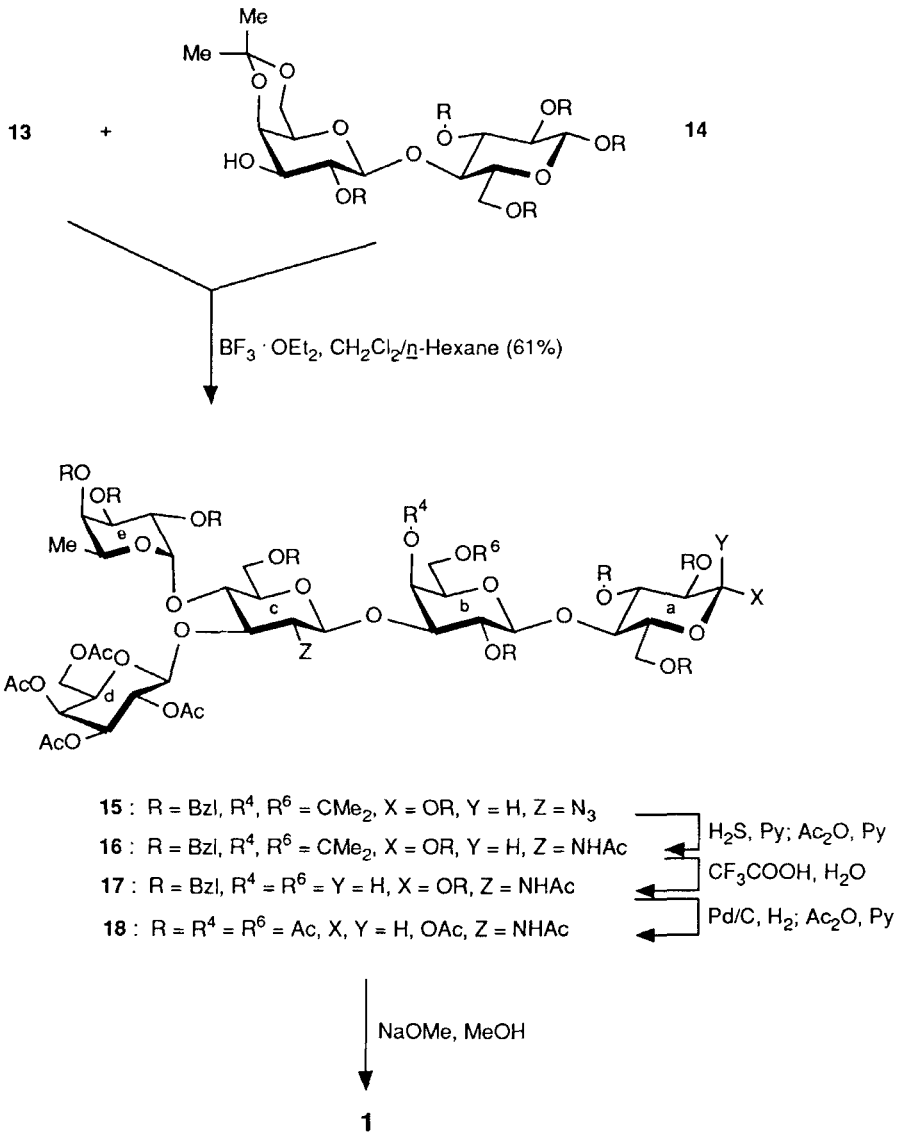
Triosyl donor **13** was reacted with known 3b-*O*-unprotected lactose derivative **14**[13] as acceptor with diethyl ether-boron trifluoride as catalyst at -20 °C to furnish the desired

Scheme 4 R = Bzl



pentasaccharide **15** (Scheme 5). Azide group reduction in **15** was performed with H_2S in pyridine/H_2O,[14] followed by treatment of the resulting amino derivative with acetic anhydride in pyridine to give *N*-acetyl derivative **16**. Treatment of **16** with trifluoroacetic acid furnished deisopropylidenated pentasaccharide **17**; ensuing hydrogenolytic debenzylation with palladium on carbon as catalyst and then *O*-acetylation with acetic anhydride/pyridine afforded the peracetylated pentasaccharide **18** as a 1:1 mixture of anomers. Treatment with sodium methoxide in methanol led to the desired pentasaccharide **1** in high overall yield. The structural assignment is based on the ^1H NMR data: the signals (doublets) of the anomeric protons of the two galactose and the glucosamine moieties at δ 4.22, 4.29 and 4.48 with coupling constants of 7.8, 7.6, and 7.8 Hz, respectively, indicate β-glycosidic linkages; the α-linked fucose moiety shows a coupling constant of 3.7 Hz for the anomeric proton at δ 4.82. Accordance of the retention times (HPLC) of **1** and material obtained by isolation from natural sources was observed.[15]

Scheme 5



EXPERIMENTAL

General methods.- Optical rotations were determined with a Perkin-Elmer 241 MC polarimeter. ^1H NMR spectra were recorded of solutions in $CDCl_3$ (internal Me_4Si) and D_2O with a Bruker WM 250 (or AC 250) Cryospec instrument and a Jeol JNM-GX 400 instrument. R_F values refer to TLC performed on silica gel 60 F_{254} (Merck). Flash

chromatography was performed with silica gel (Baker, particle size 40 μm). Chromatography under elevated pressure (MPLC) was performed with LiChroprep Si 60 (Merck, 15-25 μm). The bp of the light petroleum was 35-65 °C.

***tert*-Butyldimethylsilyl 2-Azido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (3).** (a) Selective benzylation of **2**: A solution of *tert*-butyldimethylsilyl 2-azido-2-deoxy-β-D-glucopyranoside (**2**)[5] (26 g, 81.4 mmol) and bis(tributyltin)oxide (48.5 g, 81.4 mmol) in toluene (600 mL) was heated under reflux for 2.5 h (bath temperature 150 °C). The mixture was cooled to 95 °C, then tetrabutylammonium iodide (30 g, 81.4 mmol) and benzyl bromide (48.3 mL, 407 mmol) were added. After 8 h at the same temperature the cooled mixture was concentrated *in vacuo*. Flash chromatography (3:1→2:1→1:1 petroleum ether-methyl acetate) gave **3** (23.3 g, 70%) as a colourless oil. (b) Reductive ring opening of **5**: To a mixture of **5** (100 mg, 245 μmol), sodium cyanoborohydride (154 mg, 2.45 mmol) and molecular sieves (4Å, 100 mg) in dry THF was added dropwise a saturated solution of HCl in ether until the solution gave an acidic reaction. Solid $NaHCO_3$ was added, then the mixture was diluted with ether (25 mL) and saturated $NaHCO_3$ solution (10 mL) was added. After filtration through glasswool the layers were separated. The organic layer was washed with saturated $NaHCO_3$ solution until the formation of CO_2 had ceased, then with H_2O (10 mL) and thereafter concentrated *in vacuo*. Chromatography (3:2 petroleum ether-methyl acetate) yielded **3** (76 mg, 76%) as a colourless oil. **3**: R_F 0.31 (2:1 petroleum ether-methyl acetate. $[\alpha]_D$ - 5.0° (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$) δ 0.14 (s, 6H, 2SiMe), 0.91 (s, 9H, *t*-Bu), 2.70 (d, 1H, $J_{3,OH}$ = 2.5 Hz, OH-3), 3.02 (d, 1H, $J_{4,OH}$ = 2.2 Hz, OH-4), 3.22 (dd, 1H, $J_{1,2}$ = 7.4 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.33 (ddd, $J_{3,4}$ = 9.4 Hz, 1H, $J_{3,OH}$ = 2.5 Hz, H-3), 3.42 (dd, 1H, $J_{5,6}$ = 9.6 Hz, H-5), 3.58 (ddd, 1H, $J_{4,OH}$ = 2.2 Hz, H-4), 3.72 (m, 2H, H-6, H-6'), 4.54 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1), 4.57 (2 d, 2H, C*H*$_2$Ph), 7.31 (m, 5H, Ph).

Anal. Calcd for $C_{19}H_{31}N_3O_{15}Si$: C, 55.72; H, 7.63; N, 10.26. Found: C, 55.60; H, 7.73; N, 10.25.

***tert*-Butyldimethylsilyl 3,4-Di-*O*-acetyl-2-azido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (4).** For structure determination compound **3** (25 mg, 61 μmol) was treated with pyridine/acetic anhydride (1:1, 4 mL) for 24 h. Concentration *in vacuo*, coevaporation with toluene and chromatography (9:2 petroleum ether-methyl acetate) afforded **4** (25 mg, 82%). ^1H NMR (250 MHz, $CDCl_3$) δ 0.15, 0.16 (2s, 6H, 2SiMe), 0.92 (s, 9H, *t*-Bu), 1.89, 2.05 (2s, 6H, 2CH_3CO), 3.37 (dd, 1H, $J_{1,2}$ = 7.6 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.51 (m, 2H, H-6, H-6'), 3.60 (ddd, 1H, H-5), 4.50 (2d, 2H, J_{gem} = 11.9 Hz, C*H*$_2$Ph), 4.60 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.88-5.03 (2dd, 2H, H-3, H-4), 7.29 (m, 5H, Ph).

***tert*-Butyldimethylsilyl 2-Azido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (5).** To a solution of *tert*-butyldimethylsilyl 2-azido-2-deoxy-β-D-glucopyranoside

2[5] (14.37 g, 45 mmol) in dry CH_3CN (350 mL) were added benzaldehyde dimethyl acetal (10.3 g, 67.5 mmol) and *p*-toluenesulfonic acid (80 mg). After 1 h dry K_2CO_3 (2 g) was added and the mixture was shaken for 30 min. After filtration the filtrate was concentrated *in vacuo*. Flash chromatography (5:1 petroleum ether-methyl acetate) gave **5** (17.06 g, 93%) as a colourless syrup. R_F 0.34 (5:1 petroleum ether-methyl acetate). The physical data were in agreement with published values.[5,16]

***tert*-Butyldimethylsilyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-azido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside** (**7**) and ***tert*-Butyldimethylsilyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-azido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside** (**8**). (a) From **3** and **6** under diethyl ether-boron trifluoride catalysis: To a solution of **3** (20.48 g, 50 mmol) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate **6**[9] (24.64 g, 50 mmol) in CH_2Cl_2-hexane (1:1, 200 mL) at -25 °C was added dropwise diethyl ether-boron trifluoride (2 mL of a 1 M solution in CH_2Cl_2). After 1 h the mixture was neutralized with $NaHCO_3$ (1 g), filtered and concentrated *in vacuo*. Flash chromatography (2:1 petroleum ether-methyl acetate) gave a mixture of **7**/**8** (1:3, 31.81 g, 86%). Compound **7** and **8** were separated by MPLC (4:13 petroleum ether-ether). (b) Selective preparation of **8** from **9**: Compound **8** was prepared from **9** (0.96 g, 1.3 mmol) as described for **3** (method b). Flash chromatography (2:1 petroleum ether-methyl acetate) yielded **8** (0.78 g, 81%) as a colourless foam. **7**: R_F 0.28 (1:3 petroleum ether-ether). $[\alpha]_D$ + 8.5° (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.12, 0.14 (2s, 6H, 2SiMe), 0.91 (s, 9H, *t*-Bu), 1.95, 1.96, 2.07, 2.13 (4s, 12H, $4CH_3CO$), 3.22 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.8 Hz, H-2a), 3.38 (ddd, 1H, $J_{4,5}$ = 9.8 Hz, H-5a), 3.48 (ddd, 1H, $J_{3,4}$ = 10.0 Hz, $J_{3,OH}$ = 1.5 Hz, H-3a), 3.60 (m, 3H, H-4a, H-6a, H-6'a), 3.93 (ddd, 1H, $J_{4,5}$ = 1.0 Hz, $J_{5,6}$ = 8.1 Hz, $J_{5,6'}$ = 5.1 Hz, H-5b), 4.03 (d, 1H, $J_{3,OH}$ = 1.5 Hz, OH-3a), 4.07 (dd, 1H, $J_{5,6}$ = 8.1 Hz, $J_{6,6'}$ = 11.5 Hz, H-6b), 4.14 (dd, 1H, $J_{6,6'}$ = 11.5 Hz, H-6'b), 4.46 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1a), 4.68 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1b), 4.50, 4.65 (2d, 2H, J_{gem} = 12.2 Hz, C*H*$_2$Ph), 4.93 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.4 Hz, H-3b), 5.15 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.5 Hz, H-2b), 5.34 (dd, 1H, $J_{3,4}$ = 2.7 Hz, H-4b), 7.29-7.36 (m, 5H, Ph). **8**: R_F 0.23 (1:3 petroleum ether-ether). $[\alpha]_D$ + 12.5° (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.15, 0.16 (2s, 6H, 2SiMe), 0.92 (s, 9H, *t*-Bu), 1.97, 2.00, 2.10, 2.14 (4s, 12H, $4CH_3CO$), 3.15 (dd, 1H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 8.3 Hz, H-3a), 3.26 (dd, 1H, $J_{1,2}$ = 7.6 Hz, $J_{2,3}$ = 10.0 Hz, H-2a), 3.37 (ddd, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 5.9 Hz, $J_{5,6'}$ = 2.0 Hz, 1H, H-5a), 3.51 (ddd, 1H, $J_{4,OH}$ = 1.2 Hz, H-4a), 3.62 (dd, 1H, $J_{6,6'}$ = 10.8 Hz, H-6a), 3.78 (dd, 1H, $J_{6,6'}$ = 10.3 Hz, H-6'a), 3.79 (d, 1H, $J_{4,OH}$ = 1.2 Hz, OH-4a), 3.97 (ddd, 1H, J = 6.8 Hz, H-5b), 4.10 (2dd, 2H, H-6b, H-6'b), 4.52 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1a), 4.54 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1b), 4.56 (s, 2H, C*H*$_2$Ph), 5.01 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.4

Hz, H-3b), 5.24 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.5 Hz, H-2b), 5.37 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1 Hz, H-4b), 7.25-7.31 (m, 5H, Ph).

Anal. Calcd for $C_{33}H_{49}N_3O_{14}Si$: C, 53.57; H, 6.68; N, 5.68. Found: **7**: C, 53.49; H, 6.63; N, 5.69; **8**: C, 53.62; H, 6.70; N, 5.58.

***tert*-Butyldimethylsilyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (9).** To a solution of **5** (1.5 g, 3.68 mmol) and *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl) trichloroacetimidate **6**[9] (2.7 g, 5.52 mmol) in as little ether as possible was added dropwise trimethylsilyl trifluoromethanesulfonate (0.5 mL of a 1 M solution in ether) while stirring. After 20 min the mixture was neutralized with $NaHCO_3$, filtered and concentrated *in vacuo*. Flash chromatography (14:1→12:1→11:1 toluene-acetone) yielded **9** (2.39 g, 88%). R_F 0.52 (7:1 toluene-acetone). $[\alpha]_D$ - 4.5° (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ 0.13, 0.14 (2s, 6H, 2SiMe), 0.91 (s, 9H, *t*-Bu), 1.92, 1.95, 2.06, 2.10 (4s, 12H, $4CH_3CO$), 3.31 (dd, 1H, $J_{1,2}$ = 7.6 Hz, $J_{2,3}$ = 9.3 Hz, H-2a), 3.33 (ddd, $J_{5,6'}$ = 4.9 Hz, 1H, H-5a), 3.58 (dd, 1H, J = 9.1 Hz, H-4a), 3.63-3.81 (m, 3H, H-3a, H-6a, H-5b), 3.86 (dd, 1H, $J_{5,6}$ = 5.8 Hz, $J_{6,6'}$ = 11.0 Hz, H-6b), 4.05 (dd, 1H, $J_{5,6'}$ = 7.8 Hz, $J_{6,6'}$ = 11.0 Hz, H-6'b), 4.25 (dd, 1H, $J_{6,6'}$ = 10.5 Hz, H-6'a), 4.59 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1a), 4.70 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1b), 4.97 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.4 Hz, H-3b), 5.25 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.5 Hz, H-2b), 5.31 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.0 Hz, H-4b), 5.52 (s, 1H, C*H*Ph), 7.31-7.46 (m, 5H, Ph).

Anal. Calcd for $C_{33}H_{47}N_3O_{14}Si$: C, 53.72; H, 6.42; N, 5.70. Found: C, 53.82; H, 6.38; N, 5.37.

***O*-(2,3,4-Tri-*O*-benzyl-α/β-L-fucopyranosyl) Trichloroacetimidate (10).** To a solution of 2,3,4-tri-*O*-benzyl-α/β-L-fucopyranose[17] (10.0 g, 23.0 mmol) in dry CH_2Cl_2 (50 mL) was added trichloroacetonitrile (10 g) and DBU (7 drops). After 30 min the mixture was concentrated *in vacuo*. The residue was purified over a short column of silica gel (3:1 petroleum ether-methyl acetate + 1% triethylamine) to yield **10** (11.85 g, 89%, α/β 1:4) as a colourless oil. R_F 0.76 (α-imidate, 3:1 petroleum ether-methyl acetate + 1% triethylamine). R_F 0.50 (β-imidate). The physical data were in agreement with published data.[9] β-Imidate: $[\alpha]_D$ -16.0° (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$) δ 1.22 (d, 3H, J = 6.4 Hz, CH_3), 3.62-3.70 (m, 3H, H-3, H-4, H-5), 4.09 (dd, 1H, J = 8.4 Hz, H-2), 4.69-5.06 (m, 6H, $3CH_2Ph$), 5.72 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 7.25-7.40 (m, 15H, 3Ph), 8.60 (s, 1H, NH).

Anal. Calcd for $C_{29}H_{30}Cl_3NO_5$: C, 60.17; H, 5.22; N, 2.42. Found: C, 60.10; H, 5.35; N, 2.66.

***tert*-Butyldimethylsilyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)-*O*-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)]-2-azido-6-*O*-benzyl-2-deoxy-β-**

D-glucopyranoside (11). To a solution of **8** (2.15 g, 2.91 mmol) in as little ether as possible was added trimethylsilyl trifluoromethanesulfonate (0.3 mL of a 0.1 M solution in ether). A solution of **10** (2.53 g, 4.37 mmol) in ether (10 mL) was added dropwise while stirring. The mixture was neutralized with $NaHCO_3$ (0.5 g), filtered and concentrated *in vacuo*. Flash chromatography (3:1 petroleum ether-methyl acetate) yielded **11** (2.62 g, 78%). R_F 0.40 (1:1 petroleum ether-ether). $[\alpha]_D$ - 12.0° (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 0.11, 0.13 (2s, 6H, 2SiMe), 0.91 (s, 9H, *t*-Bu), 1.26 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6c), 1.79, 1.94, 2.01, 2.09 (4s, 12H, $4CH_3CO$), 3.22 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 10.0 Hz, H-2a), 3.32 (ddd, 1H, J = 9.8 Hz, H-5a), 3.53 (2dd, 2H, H-3a, H-6a), 3.71 (dd, 1H, H-4c), 3.79-3.96 (m, 5H, H-3c, H-4a, H-5b, H-6'a, H-6b), 4.07 (dd, 1H, $J_{5,6'}$ = 8.3 Hz, $J_{6,6'}$ = 10.7 Hz, H-6'b), 4.14 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.3 Hz, H-2c), 4.35, 4.44 (2d, 2H, J_{gem} = 12.4 Hz, CH_2Ph), 4.45 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1a), 4.65, 4.83 (2d, 2H, J_{gem} = 11.7 Hz, CH_2Ph), 4.75 (m, 3H, CH_2Ph, H-5c), 4.78 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1b), 4.95-5.00 (m, 2H, H-3b, 1/2CH_2Ph), 5.06-5.13 (m, 3H, H-1c, H-2b, 1/2CH_2Ph), 5.33 (dd, 1H, $J_{3,4}$ = 2.7 Hz, H-4b), 7.23-7.34 (m, 20H, 4Ph).

Anal. Calcd for $C_{60}H_{77}N_3O_{18}Si$: C, 62.32; H, 6.71; N, 3.63. Found: C, 62.12; H, 6.70; N, 3.37.

***O*- (2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)- (1→4) -*O*-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)]-2-azido-6-*O*-benzyl-2-deoxy-β-D-glucopyranose (12).** To a solution of **11** (2.5 g, 2.16 mmol) in dry THF (25 mL) at - 45 °C was added acetic acid (0.13 mL, 2.2 mmol). Thereafter tetrabutylammonium fluoride (2.5 mL of a 1 M solution in THF) was added dropwise. The mixture was warmed up to 0 °C, diluted with ether (250 mL) and the organic layer was washed with saturated NaCl solution (3 x 40 mL). Concentration of the organic layer and flash chromatography (5:4 petroleum ether-methyl acetate) yielded **12** (2.13 g, 94%) as a colourless foam. R_F 0.25 (3:2 petroleum ether-methyl acetate). $[\alpha]_D$ -8.0° (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$) δ 1.25 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6c), 1.78, 1.94, 2.00, 2.08 (4s, 12H, $4CH_3CO$), 3.22 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.8 Hz, H-2a), 3.42 (d, 1H, $J_{4,5}$ = 9.5 Hz, H-5a), 3.52-3.65 (m, 3H), 3.70 (dd, 1H, H-4c), 3.75-4.13 (m, 7H), 4.34 (2d, 2H, J_{gem} = 7.8 Hz, CH_2Ph), 4.50-5.11 (m, 12H, at δ 4.76: 1H, $J_{1,2}$ = 7.8 Hz, H-1b), 5.33 (dd, 1H, $J_{3,4}$ = 2.3 Hz, $J_{4,5}$ = 1 Hz, H-4b), 7.20-7.42 (m, 20H, 4Ph).

Anal. Calcd for $C_{54}H_{63}N_3O_{18}\cdot 1/2\ H_2O$: C, 61.70; H, 6.14; N, 4.00. Found: C, 61.75; H, 6.07; N, 4.07.

***O*- (2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)- (1→4) -*O*-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)]-2-azido-6-*O*-benzyl-2-deoxy-α-D-glucopyranosyl Trichloroacetimidate (13).** To a solution of **12** (520 mg, 0.5 mmol) in dry CH_2Cl_2 (25 mL) was added trichloroacetonitrile (4 mL) and DBU (9 drops). TLC control (4:1 toluene-

methyl acetate + 1% triethylamine) showed a product ratio of α/β 4:1. The α-imidate **13** (385 mg, R_F 0.43) was isolated by flash chromatography (3:2 petroleum ether-methyl acetate + 1% triethylamine); the β-imidate (R_F 0.37) together with unreacted starting material **12** (120 mg) dissolved in CH_2Cl_2 (5 mL) was treated again with trichloroacetonitrile (1 mL) and DBU (2 drops). Flash chromatography (see above) yielded **13**; (total yield 475 mg, 80%). $[\alpha]_D$ + 17.5° (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$) δ 1.31 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6c), 1.79, 1.95, 2.02, 2.09 (4s, 12H, $4CH_3CO$), 3.50-5.17 (m, 25H, 2H-1, 3H-2, 3H-3, 2H-4, 3H-5, 4H-6, $4CH_2Ph$), 5.36 (dd, 1H, $J_{3,4}$ = 2.6 Hz, H-4b), 6.54 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1a), 7.20-7.46 (m, 20H, 4Ph), 8.72 (s, 1H, NH).

Anal. Calcd for $C_{65}H_{63}N_4O_{18}Cl_3$: C, 56.69; H, 5.35; N, 4.72. Found: C, 56.72; H, 5.48; N, 4.90.

Benzyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)-*O*-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)]-(2-azido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-4,6-*O*-isopropylidene-β-D-galactopyranosyl)- (1→4) -2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (15). A solution of **13** (380 mg, 0.32 mmol) and benzyl *O*-(2-*O*-benzyl-4,6-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside **14**[13] (533 mg, 0.64 mmol) in CH_2Cl_2-hexane (1:1, 2 mL) was stirred with molecular sieves (4Å) for 3 h. At -20 °C to the solution was added dropwise diethyl ether-boron trifluoride (0.3 mL of a 0.1 M solution in CH_2Cl_2). The mixture was neutralized with $NaHCO_3$ (100 mg), filtered and concentrated *in vacuo*. Flash chromatography (15:2 toluene-acetone) yielded **15** (363 mg, 61%). R_F 0.73 (4:1 toluene-acetone). $[\alpha]_D$ - 19.5° (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 1.27 (d, 3H, $J_{5,6}$ = 6.4 Hz, H-6e), 1.35, 1.39 (2s, 6H, CMe_2), 1.79, 1.94, 2.04, 2.07 (4s, 12H, $4CH_3CO$), 2.90 (bs, 1H, H-5b), 3.16-5.01 (m, 47H, 5H-1, 4H-2, 5H-3, 4H-4, 4H-5, 8H-6, 17/2CH_2Ph, at δ 4.44: d, 1H, $J_{1,2}$ = 7.8 Hz, H-1, at δ 4.51: d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 5.10 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.5 Hz, H-2d), 5.15 (d, 1H, J_{gem} = 10.3 Hz, 1/2CH_2Ph), 5.35 (dd, 1H, $J_{3,4}$ = 2.7 Hz, H-4d), 7.20-7.53 (m, 45H, 9Ph).

Anal. Calcd for $C_{104}H_{117}N_3O_{28}$: C, 67,26; H, 6.35; N, 2.26. Found: C, 66.88; H, 6.41; N, 2.06.

Benzyl *O*- (2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)- (1→4) -*O*- [(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)] -*O*-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-4,6-*O*-isopropylidene-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (16). A solution of **15** (200 mg, 108 μmol) in pyridine (10 mL) and water (*ca.* 5 mL, **15** has to remain in solution) was saturated with H_2S (30 min) and left at room temperature for 10 d. Then the mixture was concentrated *in vacuo* and codistilled with toluene. The residue was treated with pyridine-acetic

anhydride (1:1, 4 mL). After 1 h the mixture was concentrated again and coevaporated three times with toluene. Flash chromatography (5:4 petroleum ether-methyl acetate) gave **16** (183 mg, 89%). R_F 0.33 (5:4 petroleum ether-methyl acetate). $[\alpha]_D$ -29.0° (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 1.20 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6e), 1.21, 1.37 (2s, 6H, CMe_2), 1.69, 1.81, 1.92, 2.00, 2.04 (5s, 15H, $5CH_3CO$), 2.62 (bs, 1H, H-5b), 3.25-5.17 (m, 49H, 5H-1, 5H-2, 5H-3, 4H-4, 4H-5, 8H-6, 9C*H*$_2$Ph), 5.33 (dd, 1H, $J_{3,4}$ = 2.9 Hz, H-4d), 5.41 (d, 1H, $J_{2,NH}$ = 7.3 Hz, NH-2c), 7.15-7.47 (m, 45H, 9Ph).

Anal. Calcd for $C_{106}H_{121}NO_{29}$·1.5 H_2O: C, 67.00; H, 6.58; N, 0.74. Found: C, 67.03; H, 6.51; N, 1.00.

Benzyl *O*- (2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl) -(1→4)-*O*- [(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)] -*O*-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2-*O*-benzyl-β-D-galactopyranosyl) -(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (17). A solution of **16** (100 mg, 52.6 μmol) in CH_2Cl_2 (5 mL) was treated with trifluoroacetic acid (50%, 8 drops) for 24 h. The mixture was diluted with toluene (10 mL) and concentrated *in vacuo* to yield **17** (96 mg, 100%) as a colourless solid, which was used in the next step without further purification. R_F 0.38 (3:2 petroleum ether-methyl acetate). ^1H NMR (250 MHz, $CDCl_3$) δ 1.21 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6e), 1.59, 1.90, 1.93, 2.01, 2.02 (5s, 15H, $5CH_3CO$), 3.11-5.11 (m, 52H, 5H-1, 5H-2, 5H-3, 4H-4, 5H-5, 8H-6, 9C*H*$_2$Ph, 2OH), 5.30 (dd, 1H, $J_{3,4}$ = 2.9 Hz, H-4d), 5.75 (d, 1H, $J_{2,NH}$ = 7.4 Hz, NH-2c), 7.21-7.42 (m, 45H, 9Ph).

***O*-(2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl)-(1→4)-*O*-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)- (1→3)] -*O*-(2-acetamido-6-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)- (1→4) -1,2,3,6-tetra-*O*-acetyl-α/β-D-glucopyranose (18).** To a solution of **17** (96 mg, 52.6 μmol) in acetic acid, methanol and dioxane (1:1:1, 18 mL) was added palladium on carbon (10% Pd, 1.5 g) and the mixture was shaken in a H_2 atmosphere (4 bar) for 48 h (Parr hydrogenation) until TLC control (7:7:2 CH_2Cl_2-methanol-H_2O) showed a uniform product (R_F 0.63). The mixture was decanted and filtered through Celite. The palladium catalyst was suspended several times in methanol, the solution decanted and filtered. The combined filtrates were concentrated *in vacuo* and coevaporated with toluene. The solid residue was treated with pyridine-acetic anhydride (1:1, 4 mL) for 24 h. After concentration *in vacuo* and coevaporation with toluene, flash chromatography (1:4 petroleum ether-methyl acetate) gave **18** (43 mg, 95%). R_F 0.50 (1:4 petroleum ether-methyl acetate). $[\alpha]_D$ -32.0° (*c* 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 1.22 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6e), 1.92-2.16 (several s, 48H, $16CH_3CO$), 2.99-5.43 (m, 32H, 3H-1, 5H-2, 5H-3, 5H-4, 5H-5, 8H-6, at δ 4.57: d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 5.52 (d, 1H, $J_{2,NH}$ = 7.1 Hz, NH-2c), 5.64 (d, 0.5H, $J_{1,2}$ = 8.3 Hz, H-1a), 6.23 (d, 0.5H, $J_{1,2}$ = 3.7 Hz, H-1a).

Anal. Calcd for $C_{62}H_{85}NO_{40}\cdot 0.5\ H_2O$: C, 49.87; H, 5.80; N, 0.94. Found: C, 49.94; H, 6.14; N, 1.00.

***O*- (α-L-Fucopyranosyl)- (1→4) -*O*- [(β-D-galactopyranosyl)- (1→3)] -*O*- (2-acetamido-2-deoxy-β-D-glucopyranosyl) -(1→3) -*O*- (β-D-galactopyranosyl)- (1→4) -α/β-D-glucopyranose (1).** A solution of **18** (38.5 mg, 25.9 μmol) in dry methanol (10 mL) was treated with sodium methoxide (0.5 mL of a 0.2 M solution in methanol). After 48 h the solution was neutralized with Amberlite IR 120, filtered through cotton and concentrated *in vacuo*. The product **1** (24 mg, 100%) was dissolved in H_2O and lyophilized. R_F 0.61 (1:1:1 methyl acetate-2-propanol-H_2O). $[\alpha]_D$ -25.0° (t = O), -26.0° (t = 12 h) (*c* 1, H_2O). ^1H NMR (400 MHz, D_2O) δ 0.97 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6e), 1.82 (s, 3H, CH_3CO), 3.06-3.84 (m, 26H, 5H-2, 5H-3, 4H-4, 4H-5, 8H-6), 3.94 (dd, 1H, H-4b or H-4d), 4.22, 4.29 (2d, 2H, $J_{1,2}$ = 7.8 Hz, $J_{1,2}$ = 7.6 Hz, H-1b and H-1d), 4.45 (d, 0.5H, $J_{1,2}$ = 8.1 Hz, H-1a), 4.48 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1c), 4.67 (q, 1H, H-5e), 4.82 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1e), 5.01 (d, 0.5H, $J_{1,2}$ = 3.9 Hz, H-1a).

Anal. Calcd for $C_{32}H_{55}NO_{25}\cdot 4\ H_2O$: C, 41.51; H, 6.86; N, 1.51. Found: C, 41.58; H, 6.70; N, 1.50.

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