Glycoconjugate Journal 16, 757–765, 1999

Synthesis of a highly hydrophobic dimeric Lewis X containing glycolipid: A model for the study of homotypic carbohydrate–carbohydrate interaction

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This paper describes the synthesis of an octasaccharidic glycolipid 1 based on a stereo- and regioselective glycosylation between a Lewis X trisaccharidic donor and a pentasaccharidic acceptor. A highly hydrophobic lipid moiety of a new type was selected, making the compound 1 a good candidate for the study of the interaction of Lewis X functionalised vesicles.

Keywords: **Lewis X, glycosylation, thioglycoside, octasaccharide, carbohydrate–carbohydrate interaction**

Introduction

The new biological concept of divalent calcium-mediated carbohydrate–carbohydrate interaction has mainly been advocated by Hakomori et al. [1]. A typical example is the homotypic Lewis X–Lewis X interaction [2] which may play a critical role in the compaction process [3], that is to say during early mammalian development [4,5]. This interaction was evidenced by the observed selective self-aggregation of liposomes containing cholesterol and purified Lewis X natural sphingoglycolipids. This has recently been confirmed [6] using rat basophilic leukemia cells. MS [7] and NMR [8,9] investigations have also shown that Le^X-Le^X interactions do exist in the presence of Ca^{2+}.

We recently studied the adhesion between giant vesicles that included lipids carrying Le^X groups. The initially tested natural glycosphingolipid shown in Figure 1 proved too soluble in water for accurate adhesion studies. A more hydrophobic synthetic glycolipid (see Figure 1) made out of three phytyl chains was thus selected [10] and the adhesion was demonstrated [11].

In order to quantitatively investigate the influence on the homotypic interaction of the frequently occurring interaction of the trisaccharidic Le^X determinant in natural glycolipids, we

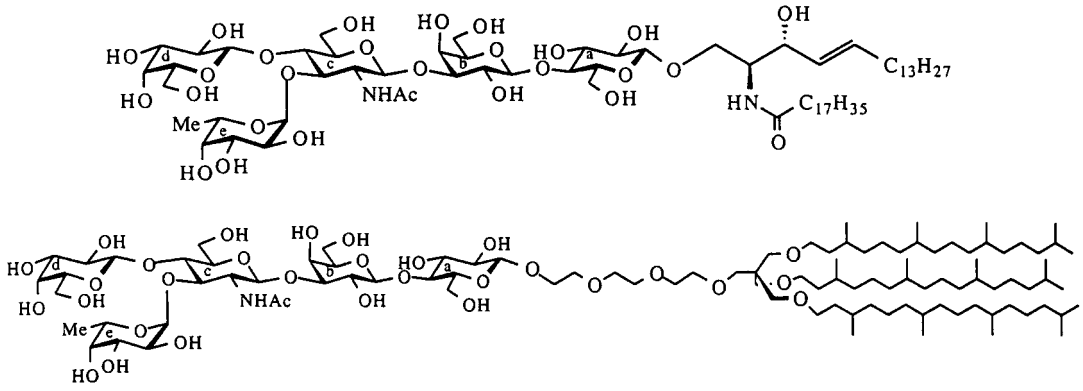

Figure 1. Natural Le^X containing sphingoglycolipid (upper part) and synthetic highly hydrophobic glycolipid previously included in giant vesicles (lower part).

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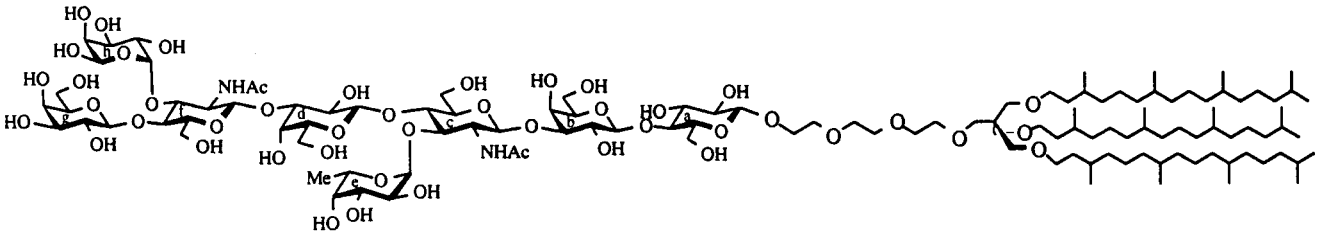


Figure 2. Synthetic dimeric Le^X neoglycolipid **1**.

decided to study the behaviour of the dimeric Le^X neoglycolipid **1** (Figure 2). This article is dealing with the chemical synthesis of this novel type of glycolipid.

Several syntheses of dimeric Le^X have been reported, making use of the imidate glycosylation method [12–14] or the Mukaiyama [15]–Suzuki [16] fluoride glycosylation technique [17]. We describe here the classical use of thioglycoside as a latent leaving group.

Results and discussion

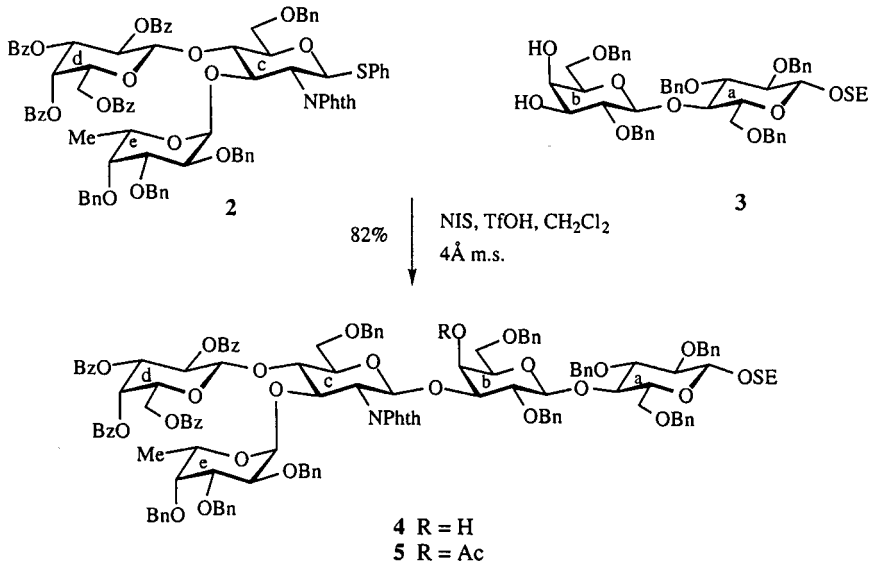
Coupling of the previously described donor **2** [18] with the known lactoside **3** [19] was performed in dichloromethane, promoted by *N*-iodosuccinimide (NIS)–trifluoromethanesulfonic acid (TfOH), at −20°C for 1 h, then 12 h at room temperature. The desired pentasaccharide **4** was generated in 82% yield (Scheme 1).

The stereochemistry of the newly introduced glycosidic linkage in pentasaccharide **4** was determined to be β on the basis of the H-1c, H-2c coupling constant ($J_{1c,2c} = 8.4$ Hz). The regiochemistry of **4** was readily assigned from the ^{1}H-NMR spectrum of **5**–obtained from **4** by acetylation–which revealed a deshielded signal for H-4b at 5.40 ppm (dd, $J_{4b,5b} < 1$ Hz, $J_{3b,4b} = 3.6$ Hz), indicating the position of the new glycosidic linkage in **4** as being OH-3b of the diol **3**.

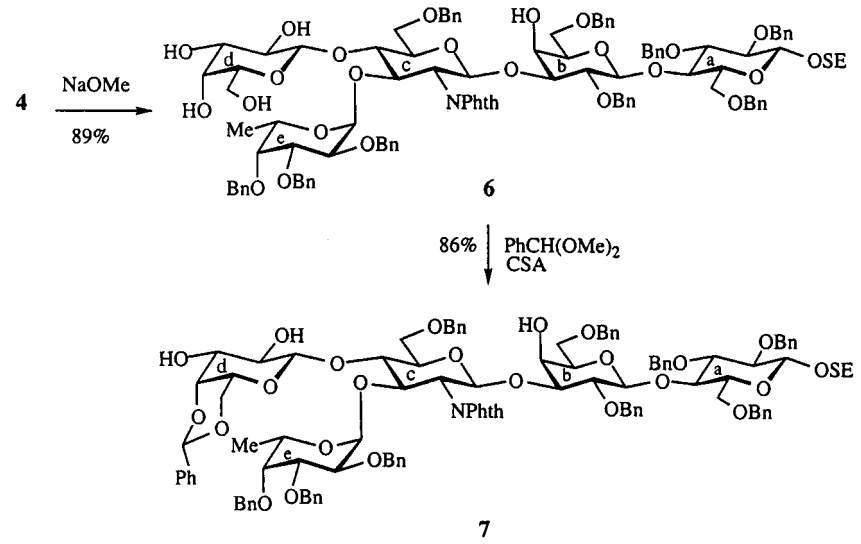
Treatment of **4** with sodium methoxide in methanol–dichloromethane gave compound **6** (89%) which, after reaction with benzaldehyde dimethylacetal in the presence of camphorsulphonic acid, gave the pentasaccharide **7** in 86% yield (Scheme 2).

Coupling of the previously used donor **2** with the pentasaccharidic triol **7**, in the presence of NIS-TfOH, regioselectively gave the protected octasaccharide **8** in 61% yield (Scheme 3).

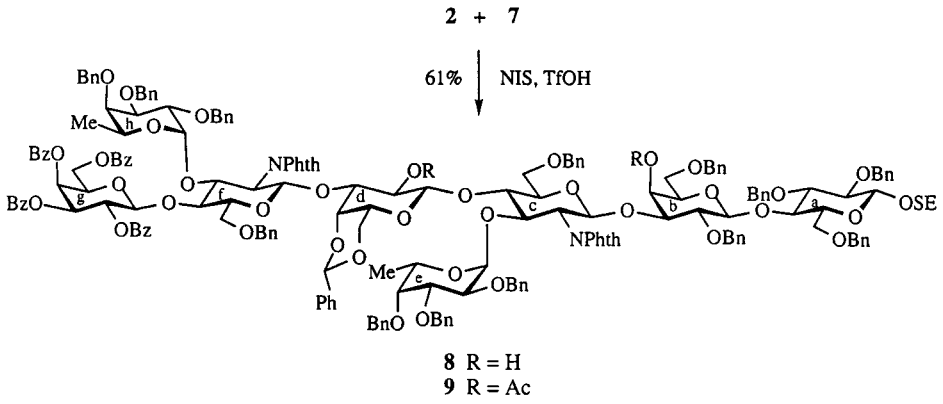
The stereochemistry of the newly introduced linkage in octasaccharide **8** was determined to be β on the basis of the H-1f, H-2f coupling constant ($J_{1f,2f} = 8.5$ Hz). The regiochemistry of **8** was assigned from the ^{1}H NMR spectrum of **9**, obtained from **8** by acetylation, which revealed in $CDCl_3$ solvent, a deshielded signal for H-4b at 5.42 ppm (dd, $J_{4b,5b} < 1$, $J_{3b,4b} = 3.3$ Hz); however the signal for H-2d was not clear, due to overlapping of several signals. To clarify this, a ^{1}H NMR was taken in $CDCl_3$–C_6D_6 (1 : 1) solution. The spectrum then showed a well resolved one proton signal at 4.99 ppm for H-2d (dd, $J_{1d,2d} = 8.3$ Hz, $J_{2d,3d} = 9.8$ Hz). We therefore concluded that the position of the newly formed



Scheme 1. A regioselective glycosylation of the lactoside **3**.



Scheme 2. Two step synthesis of the key triol **7**.



Scheme 3. Regioselective synthesis of the protected octasaccharide **8**.

glycosidic linkage in **8** was OH-3d of the acceptor **7**, which confirmed the stereo- and regioselectivity of the glycosylation.

Treatment of the octasaccharide **8** with hydrazine hydrate in refluxing ethanol, followed by acetylation with acetic anhydride in pyridine, gave the derivative **10** in 86% overall yield from **8** (Scheme 4).

Catalytic hydrogenolysis of **10** in methanol and ethyl acetate, followed by acetylation, gave the fully acetylated octasaccharide **11** in 73% overall yield (Scheme 5).

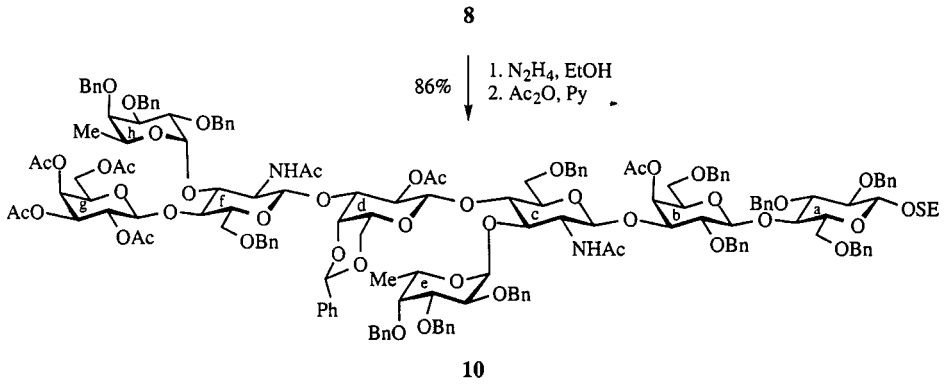
Acid catalysed cleavage of the 2-(trimethylsilyl)ethyl glycoside was performed in dichloromethane using trifluoroacetic acid [19] to give a hemiacetal which was directly treated with trichloroacetonitrile in the presence of DBU [20] to give the imidate **12** in 77% yield from **11** (Scheme 6). According to ^{1}H-NMR, **12** exists essentially in α form.

Coupling of imidate **12** with the alcohol **13** [10], promoted by TMSOTf (trimethylsilyl trifluoromethanesulfonate), gave the glycolipid **14** in 61% yield. The stereochemistry of the new glycosidic linkage was determined to be β, on the basis of H-1a, H-2a coupling constant from ^{1}H NMR ($J_{1a,2a} = 7.9$ Hz). De-*O*-acetylation of compound **14** in methanol-dichloromethane quantitatively afforded the target product **1** (Scheme 7).

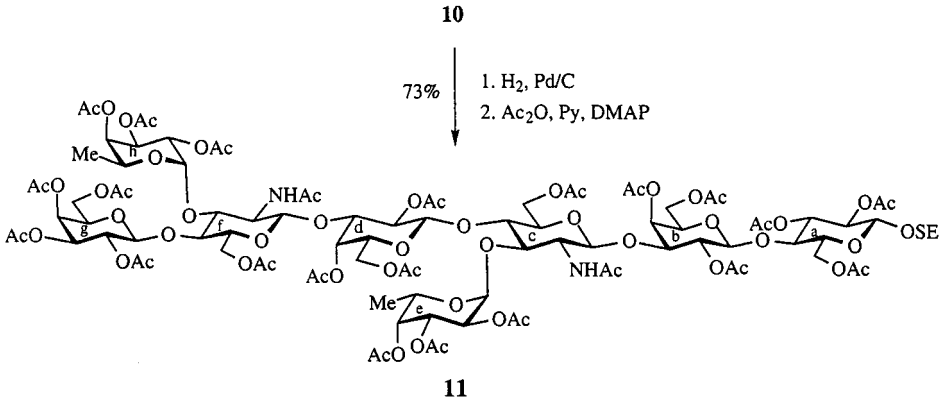
Materials and Methods

General methods

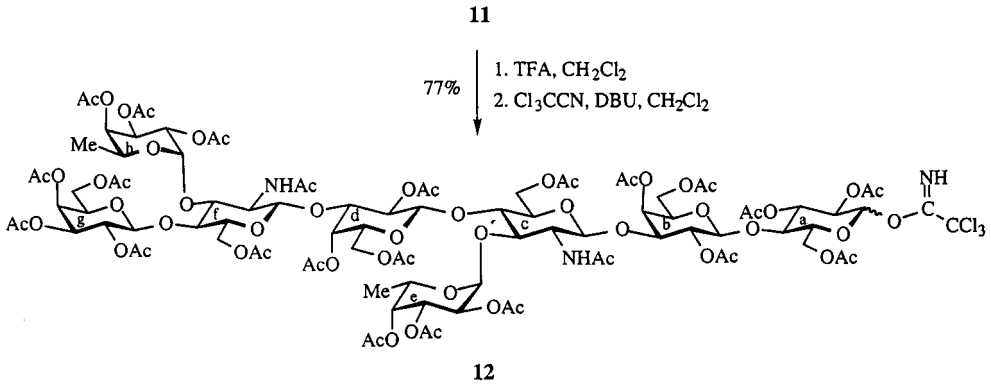
Melting points were determined with a Büchi model 510 m.p. apparatus and are uncorrected. Optical rotations were measured at 20 ± 2°C with a Perkin Elmer Model 241 digital polarimeter, using a 10 cm, 1 ml cell. Chemical Ionisation (CI ammonia) and Fast Atom Bombardment (FAB) mass spectra were obtained with a JMS-700 spectrometer. Elemental analyses were performed by Service de Microanalyse de 1'Université Pierre et Marie Curie, 4 Place Jussieu, 75005 Paris, France. ^{1}H-NMR spectra were recorded with a Bruker



Scheme 4. Hydrazinolysis and acetylation of the octasaccharide **8**.

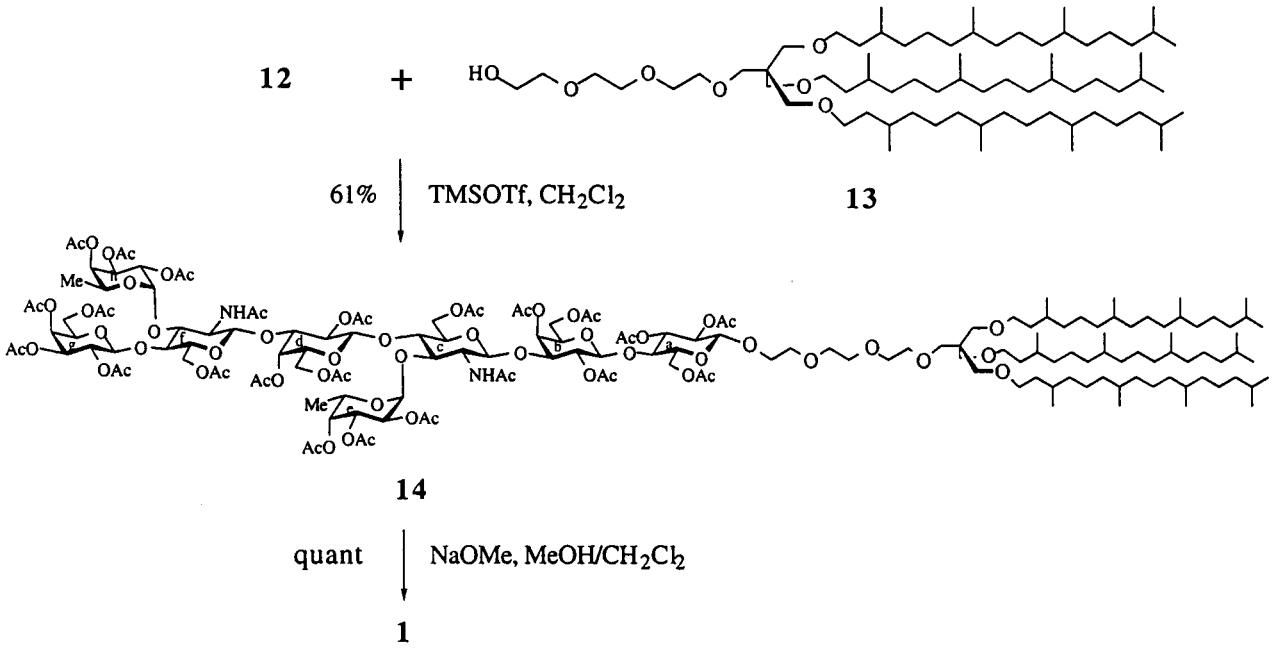


Scheme 5. Synthesis of the fully acetylated octasaccharide **11**.



Scheme 6. Synthesis of the glycosyl donor **12**.

AC 250 or a Bruker DRX 400 spectrometer for solutions in $CDCl_3$ at ambient temperature. Assignment were aided by COSY experiments. ^{13}C-NMR spectra were recorded at 62.9 MHz with a Bruker AC 250 or at 100.6 MHz with a Bruker DRX 400 for solutions in $CDCl_3$ adopting 77.00 ppm for the central line of $CDCl_3$. Assignments were aided by J-mod technique and proton-carbon correlation. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60 F_{254} (layer thickness 0.2 mm; E. Merck, Darmstadt, Germany) and detection by charring with sulfuric acid. Flash column chromatography was performed on silica gel 60 (230–400 mesh, E. Merck).



Scheme 7. Synthesis of the glycolipid **1**.

Trimethylsilylethyl O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1 → 3)]-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**4**)

A mixture of donor **2** (446 mg, 0.3 mmol), acceptor **3** (268 mg, 0.3 mmol), 4 Å powdered molecular sieves (1.5 g) and dry CH_2Cl_2 (20 ml) was stirred at room temperature for 30 min. N-iodosuccinimide (157 mg, 0.7 m mol) was added at room temperature. The reaction mixture was cooled to −20°C. Trifluoromethanesulfonic acid (2.65 µl, 30 µmol) was added. After stirring at −20°C for 1 h, then at room temperature for 12 h, the reaction mixture was filtered through a celite bed, washed with saturated aqueous $NaHCO_3$ solution, aqueous thiosulfate (10%), water, brine, dried over $MgSO_4$ and concentrated. The residue obtained was flash chromatographed (cyclohexane : ethyl acetate, 3 : 1) to afford **4** (560 mg, 82%) as an amorphous white solid: $[\alpha]_D + 8$ (*c* 0.88, $CHCl_3$).- TLC (cyclohexane : ethyl acetate, 3 : 1): R_F 0.35.- ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.87 (dd, 1 H, $J_{4d,5d} < 1$ Hz, $J_{4d,3d} = 3.56$ Hz, H-4d), 5.75 (dd, 1 H, $J_{2d,1d} = 8.2$ Hz, $J_{2d,3d} = 10.3$ Hz, H-2d), 5.43 (dd, 1H, $J_{3d,2d} = 10.3$ Hz, $J_{3d,4d} = 3.6$ Hz, H-3d), 5.31 (d, 1 H, $J_{1c,2c} = 8.4$ Hz, H-1c), 5.08 (d, 1 H, $J_{1e,2e} = 3.4$ Hz, H-1e), 5.05 (d, 1 H, $J_{1d,2d} = 8.2$ Hz, H-1d), 1.4 (d, 3 H, $J_{5e,6e} = 6.5$ Hz, H-6e), 1.05 (m, 2 H, OCH_2CH_2Si), 0.0 (s, 9 H, $Si(CH_3)_3$).- ^{13}C-RMN (100.6 MHz, $CDCl_3$) δ 165.85, 165.72, 165.31, 164.68 (4 benzoyl C=O), 138.97, 138.87, 138.73, 138.70, 138.48, 138.43, 138.25, 138.18, 137.53, 133.76, 133.50, 133.42, 133.37, 133.28, 131.17, 129.86, 129.70, 129.63, 129.40, 128.92, 128.78, 128.66, 128.31, 128.30, 128.26, 128.20, 128.19, 128.15, 127.98, 127.95, 127.89, 127.84, 127.82, 127.73, 127.59, 127.40, 127.36, 126.63, 126.160 (aromatic C), 102.96, 101.91, 99.81, 98.75, 96.75 (5 anomeric C), 83.26, 82.78, 81.11, 79.08, 77.92, 75.92, 75.58, 75.51, 74.56, 74.51, 72.53, 72.43, 71.71, 71.37, 69.80, 68.16, 67.37, 66.71 (19 ring C), 75.34, 75.06, 74.85, 73.99, 73.83, 73.24, 73.24, 72.83, 72.70, 71.87, 68.28, 67.77, 67.18 (9 $PhCH_2$, C-6a, C-6b, C-6c, $CH_2CH_2Si(CH_3)_3$), 61.34 (C-6d), 61.30 (C-2c), 18.36 ($CH_2CH_2Si(CH_3)_3$), 16.85 (C-6e), 0.0 ($Si(CH_3)_3$).- MS (FAB): $(M + Li)^+$ 2276.- Anal. Calcd for $C_{134}H_{137}NO_{30}Si$: C, 70.91; H, 6.08; N, 0.62. Found: C, 70.77; H, 6.27; N, 0.54.

Trimethylsilylethyl O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1 → 3)]-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**5**)

Compound **4** (10 mg) was acetylated with acetic anhydride (0.3 ml) in pyridine (0.6 ml) for 15 h at room temperature. After concentration, the residue was coevaporated with toluene to give **5**: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.89 (dd, 1 H, $J_{4d,5d} < 1$ Hz, $J_{4d,3d} = 3.6$ Hz, H-4d), 5.75 (dd, 1 H, $J_{2d,1d} = 8.3$ Hz, $J_{2d,3d} = 10.3$ Hz, H-2d), 5.45 (dd, 1 H, $J_{3d,2d} = 10.3$ Hz, $J_{3d,4d} = 3.6$ Hz, H-3d), 5.41 (dd, 1 H, $J_{4b,5b} < 1$ Hz, $J_{3b,4b} = 3.5$ Hz, H-4b), 5.23 (d, 1 H,

$J_{1c,2c} = 8.2$ Hz, H-1c), 5.18 (d, 1 H, $J_{1d,2d} = 8.3$ Hz, H-1d), 5.05 (d, 1 H, $J_{1e,2e} = 3.5$ Hz, H-le), 2.09 (s, 3 H, OAc), 1.43 (d, 3 H, $J_{5e,6e} = 6.5$ Hz, H-6e), 1.01 (m, 2 H, OCH_2CH_2Si), 0.02 (s, 9 H, $Si(CH_3)_3$).

Trimethylsilylethyl O-(β-D-galactopyranosyl)-(1 → 4)-[(2, 3, 4-tri-O-benzyl-α-L-fucopyranosyl)-(1 → 3)]-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2, 3, 6-tri-O-benzyl-β-D-glucopyranoside (**6**)

To a solution of **4** (890 mg, 0.392 mmol) in methanol–dichloromethane (16 ml, 5 : 3) was added 0.1 M NaOMe solution in methanol (3 ml). After stirring for 3 h (monitoring by TLC) at room temperature, the mixture was neutralized by Amberlite resin (IR120, H^+ form), filtered and concentrated to dryness. The residue obtained was flash chromatographed (cyclohexane : ethyl acetate, 1 : 1) to afford **6** (650 mg, 89%) as an amorphous solid: TLC (cyclohexane : ethyl acetate, 1 : 2): R_F 0.32.- ^{1}H-NMR (250 MHz, $CDCl_3$) δ 7.5-6.7 (m, 49 H, aromatic H), 5.4 (d, 1 H, $J_{1c,2c} = 8.3$ Hz, H-1c), 1.05 (d, 3 H, H-6e), 0.95 (m, 2 H, OCH_2CH_2Si), 0.0 (s, 9 H, $Si(CH_3)_3$).- ^{13}C-RMN (62.9 MHz, $CDCl_3$) δ 166.24 (2 Pht. C=O), 103.15, 102.06, 99.97, 98.79, 98.52 (5 anomeric C), 62.88 (C-6d), 55.86 (C-2c), 16.58 (OCH_2CH_2Si), 16.44 (C-6e), 0.0 ($Si(CH_3)_3$).

Trimethylsilylethyl O-(4,6-O-benzylidene-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1 → 3)]-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(2, 6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**7**)

To a solution of **6** (650 mg, 0.351 mmol) in dry acetonitrile (12.5 ml) were added benzaldehyde dimethyl acetal (150 µl, 1.05 mmol) and camphorsulfonic acid (30 mg). The reaction mixture was stirred at room temperature for 1 h, solid potassium carbonate (2 g) was added. The mixture was stirred for 0.5 h, filtered through celite, and concentrated. The residue obtained was flash chromatographed (cyclohexane : ethyl acetate, 1 : 1) to afford **7** (588 mg, 86%) as a white amorphous solid: $[\alpha]_D - 25^\circ$ (*c* 1, $CHCl_3$).- TLC (cyclohexane : ethyl acetate, 1 : 2): R_F 0.37.- ^{1}H-NMR (250 MHz, $CDCl_3$) δ 6.8-7.55 (m, 54 H, H aromatic), 5.6 (s, 1 H, cetalic H of benzylidene), 5.3 (d, 1 H, $J_{1c,2c} = 8.4$ Hz, H-1c), 1.02 (d, 3 H, H-6e), 0.9 (m, 2 H, OCH_2CH_2Si), 0.0 (s, 9 H, $Si(CH_3)_3$).-^{13}C-NMR (62.9 MHz, $CDCl_3$) δ 167.79 (2 Pht C=O), 139.07-123.17 (aromatic C), 102.95, 101.91, 101.26, 100.07, 98.88, 97.98 (5 anomeric C, cetalic C of benzylidene), 83.23-66.55 (ring C, CH_2), 56.30 (C-2c), 16.35 ($CH_2CH_2Si(CH_3)_3$, 14.06 (C-6e), 0.0 ($Si(CH_3)_3$).- MS (CI): $(M + NH_4)^+$ 1958.7.- Anal. Calcd for $C_{113}H_{125}NO_{26}Si$: C, 69.91; H, 6.49; N, 0.72. Found: C, 69.72; H, 6.64; N, 0.64.

Trimethylsilylethyl O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1 → 3)] (6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(4, 6-O-benzylidene-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1 → 3)]-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(2, 6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**8**)

A mixture of donor **2** (316 mg, 0.212 mmol), acceptor **7** (374 mg, 0.193 mmol), 4 Å powdered molecular sieves (2 g) and dry CH_2Cl_2 (20 ml) was stirred at room temperature under argon for 30 min. NIS (130 mg, 0.58 mm mol) was added at room temperature. The reaction mixture was cooled to 0°C and triflic acid (3.42 µl, 38.6 µmol) was added. The reaction mixture was warmed gradually to room temperature and stirred for 6 h (monitoring by TLC). The mixture was filtered through celite, washed with saturated $NaHCO_3$ solution, aqueous sodium thiosulfate (10%), water, brine, dried over $MgSO_4$ and concentrated. The residue obtained was flash chromatographed (cyclohexane : ethyl acetate, 2 : 1) to afford **8** (390 mg, 61%) as an amorphous solid: $[\alpha]_D + 8$ (*c* 0.55, $CHCl_3$).- TLC (cyclohexane : ethyl acetate, 2 : 1): R_F 0.32.- ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.86 (dd, 1 H, $J_{4g,3g} = 3.6$ Hz, $J_{4g,5g} < 1$ Hz, H-4g), 5.78 (dd, 1 H, $J_{2g,1g} = 8.3$ Hz, $J_{2g,3g} = 10.3$ Hz, H-2g), 5.45 (s, 1 H, cetalic H of benzylidene), 5.41 (dd, 1 H, $J_{3g,2g} = 10.3$ Hz, $J_{3g,4g} = 3.6$ Hz, H-3g), 5.05 (d, 1 H, $J_{1g,2g} = 8.3$ Hz, H-1g), 1.49 (d, 3 H, $J_{6,5} = 6.5$ Hz, H-6e or H-6h), 1.02 (m, 2 H, OCH_2CH_2Si), 0.42 (d, 3 H, $J_{6,5} = 6.5$ Hz, H-6e or H-6h), 0.0 (s, 9 H, $Si(CH_3)_3$).- ^{13}C-NMR (100.6 Hz, $CDCl_3$) δ 165.88, 165.74, 165.26, 164.71 (4 C=O benzoyl), 139.30, 139.17, 138.97, 138.95, 138.85, 138.71, 138.47, 138.44, 138.24, 138.21, 138.10, 138.01, 137.86, 137.60, 134.08, 133.71, 133.57, 133.38, 133.30, 131.65, 131.03, 129.85, 129.70, 129.63, 129.60, 129.36, 129.17, 128.94, 128.75, 128.69, 128.61, 128.56, 128.50, 128.36, 128.31, 128.21, 128.17, 128.08, 127.97, 127.92, 127.87, 127.83, 127.72, 127.70, 127.67, 127.65, 127.44, 127.43, 127.38, 127.20, 126.56, 126.18, 125.80, 123.23, 123.18 (aromatic C), 102.94, 101.92, 100.78, 99.99, 99.55, 98.83, 98.51, 97.98, 96.68 (8 anomeric C, cetalic C of benzylidene), 83.03, 82.75, 81.78, 79.31, 79.16, 78.71, 78.40, 77.98, 75.87, 75.87, 75.16, 75.10, 75.04, 74.72, 74.56, 74.51, 74.27, 73.77, 72.51, 72.33, 71.67, 71.67, 71.38, 70.90, 68.26, 67.44, 67.42, 66.58, 66.53, 66.05 (30 ring C), 75.15, 75.01, 74.83, 74.51, 73.96, 73.78, 73.14, 73.01, 72.95, 72.95, 72.45, 72.00, 71.41, 68.95, 68.34, 67.78, 67.74, 67.69, 67.16 (13 $PhCH_2$, C-6a, C-6b, C-6c, C-6d, C-6f, $CH_2CH_2Si(CH_3)_3$), 61.33 (C-6g), 56.49, 56.23 (C-2c, C-2f), 18.35 ($CH_2CH_2Si(CH_3)_3$), 16.81, 15.61 (C-6e, C-6h), 0.0 ($Si(CH_3)_3$).- MS (FAB): $(M + Li)^+$ 3324.32.- Anal. Calcd for $C_{195}H_{198}N_2O_{45}Si$: C, 70.59; H, 6.20; N, 0.84. Found: C, 70.41; H, 6.15; N, 0.82.

Trimethylsilylethyl O-(2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)(1 → 3)]-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(4, 6-O-benzylidene-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1 → 3)]-(6-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 3)-(2, 6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**9**)

Compound **8** (9 mg) was acetylated with acetic anhydride (0.4 ml) in pyridine (0.8 ml) for 24 h at room temperature. After concentration, the residue was coevaporated with toluene to give **9**. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.85 (dd, 1 H, $J_{4g,3g} = 3.6$ Hz, $J_{4g,5g} < 1$ Hz, H-4g), 5.78 (dd, 1 H, $J_{2g,1g} = 8.3$ Hz, $J_{2g,3g} = 10.2$ Hz, H-2g), 5.50 (s, 1 H, cetalic H of benzylidene), 5.43 (dd, 1 H, $J_{3g,2g} = 10.2$ Hz, $J_{3g,4g} = 3.6$ Hz, H-3g), 5.42 (dd, 1 H, $J_{4b,3b} = 3.3$ Hz, $J_{4b,5b} < 1$ Hz, H-4b), 5.22, 5.13 (2 d, 2 H, $J_{1c,2c} = 8.3$ Hz, $J_{1f,2f} = 8.0$ Hz, H-1c, H-1f), 5.12 (d, 1 H, $J_{1,2} = 3.9$ Hz, H-1e or H-1h), 5.07 (d, 1 H, $J_{1g,2g} = 8.2$ Hz, H-1g), 2.07 (s, 3 H, Ac), 1.66 (s, 3 H, Ac), 1.45 (d, 3 H, $J_{6,5} = 6.5$ Hz, H-6e or H-6h), 1.01 (m, 2 H, OCH_2CH_2Si), 0.77 (d, 3 H, $J_{6.5} = 6.5$ Hz, H-6h or H-6e), 0.02 (s, 9 H, $Si(CH_3)_3$).

Trimethylsilylethyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1 → 3)]-(2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2-0-acetyl-4, 6-O-benzylidene-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-(1 → 3)]-(2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**10**)

Hydrazine hydrate (1.2 ml) was added to a stirred solution of compound **8** (260 mg, 78.3 μmol) in 95% aqueous ethanol (22 ml) and refluxed for 14 h (monitoring by TLC). The solution was concentrated and coevaporated with ethyl acetate to dryness. The residue was then stirred with acetic anhydride (10 ml) in pyridine (20 ml) at room temperature for 12 h. The solution was concentrated and coevaporated with toluene. The residue was flash chromatographed on a column of silica gel (ethyl acetate : cyclohexane, 1.4 : 1), followed by a column of Sephadex (LH20), using dichloromethane–methanol (1 : 1) as eluate to yield **10** (210 mg, 86%) as an amorphous solid: $[\alpha]_D$-32 (*c* 1.8, $CHCl_3$).- TLC (ethyl acetate : cyclohexane, 1.4 : 1): R_F 0.35.- ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.69 (d, 1 H, N*H*Ac), 5.55 (s, 1 H, cetalic H of benzylidene), 5.50, 5.30 (2dd, 2 H, $J_{4,3} = 3.4$ Hz, $J_{4,3} = 3.3$ Hz, H-4d, H-4g), 5.47, 5.28 (2d, 2 H, $J_{1,2} = 8.0$ Hz, $J_{1,2} = 7.4$ Hz, 2 H-1), 5.15 (dd, 1 H, $J_{2d,3d} = 9.8$ Hz, $J_{2d,1d} = 8.4$ Hz, H-2d), 2.08-1.66 (8s, 24 H, 8 $COCH_3$), 1.23, 1.11 (2d, 6 H, H-6e, H-6h), 1.08 (m, 2 H, OCH_2CH_2Si), 0.0 (s, 9 H, $Si(CH_3)_3$).- ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ 170.44, 170.22, 170.00, 169.96, 169.81, 169.57, 169.02, 168.69 (8 $COCH_3$), 139.53-125.92 (aromatic C), 103.06, 102.09, 99.83, 99.74, 99.67, 99.55, 99.26, 97.57, 96.29 (8 anomeric C, cetalic C of benzylidene), 82.79-57.93 (32 ring C, 12 $PhCH_2$, 6 C-6, $CH_2CH_2Si(CH_3)_3$), 23.27, 22.91, 21.00, 20.94, 20.62, 20.55, 20.48, 20.48 (8 CH_3CO), 18.41 ($CH_2CH_2Si(CH_3)_3$), 16.71, 15.95 (C-6e, C-6h), 0.0 ($Si(CH_3)_3$).- MS (FAB): $(M + Li)^+$ 2984.29.-Anal. Calcd for $C_{167}H_{194}N_2O_{45}Si$: C, 67.36; H, 6.57; N, 0.94. Found: C, 67.26; H, 6.75; N, 0.88.

Trimethysilyethyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1 → 3)]-(2-acetamido-6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1 → 3)]-(2-acetamido-6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**11**)

A mixture of **10** (166 mg, 55.8 μmol), 10% Pd/C (21 mg) in methanol (21 ml) and ethyl acetate (6.4 ml) was stirred for 3.5 h under hydrogen (1.45 atm), filtered through celite. The celite pad was rinsed with methanol. The solution was concentrated and the residue was acetylated by acetic anhydride (5 ml) in pyridine (10 ml) at room temperature for 12 h, then 10 mg of DMAP was introduced and the reaction mixture was stirred at 40°C for 7 h. The reaction mixture was concentrated and coevaporated with toluene. The residue was flash chromatographed on a column of silica gel (dichloromethane : methanol, 25:1) to yield **11** (95 mg, 73%) as a white amorphous solid: $[\alpha]_D$-53 (*c* 1.3, $CHCl_3$).- TLC (dichloromethane : methanol, 25 : 1): R_F 0.3.- ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.46 (s, 1 H, N*H*Ac), 5.41 (dd, 1 H, $J_{3,4} = 3.19$ Hz, H-4 gal), 1.9-2.3 (23s, 69 H, 23 CH_3CO), 1.23, 1.18 (2d, 6 H, H-6e, H-6h), 0.92 (m, 2 H, OCH_2CH_2Si), 0.0 (s, 9 H, $Si(CH_3)_3$).- ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ 171.20, 171.05, 170.99, 170.98, 170.80, 170.66, 170.66, 170.54, 170.48, 170.46, 170.41, 170.31, 170.23, 169.99, 169.83, 169.76, 169.64, 169.55, 169.53, 169.49, 169.28, 168.94, 168.68 (23 $COCH_3$), 100.60, 100.40, 100.28, 99.87, 99.08, 99.03, 95.15, 95.08 (8 anomeric C), 75.86, 75.70, 75.65, 74.26, 74.04, 72.90, 72.88, 72.66, 72.48, 71.86, 71.84, 71.75, 71.54, 71.32, 71.26, 71.03, 70.03, 70.93, 70.93, 70.80, 70.78, 69.00, 68.94, 68.88, 68.70, 68.62, 67.86, 67.79, 66.57, 63.95, 63.91, 58.55, 58.48 (32 ring C), 67.39 ($CH_2CH_2Si(CH_3)_3$), 62.14, 61.46, 60.80, 60.45, 59.98, 59.50 (6 C-6), 20.97-20.45 (23 CH_3CO), 17.75 ($CH_2CH_2Si(CH_3)_3$), 15.72, 15.68 (C-6e, C-6h), 0.0 ($Si(CH_3)_3$).- MS (FAB): $(M+Li)^+$ 2354.9.- Anal. Calcd for $C_{99}H_{142}N_2O_{60}Si.4H_2O$: C, 49.12; H, 6.24; N, 1.16. Found: C, 49.16; H, 6.09; N, 1.09.

O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1 → 3)]-(2-acetamido-6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1 → 3)]-(2-acetamido-6-O-acetyl-2-deoxy-β–glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl trichloroacetimidate (**12**)

To a mixture of 75.5 mg (33 μmol) of **11** in dry dichloromethane (0.5 ml) under argon, was added dropwise at 0°C, 1 ml of trifluoroacetic acid (CF_3CO_2H), the mixture was stirred for 1 h at 0°C, then 4 h at room temperature. The mixture was diluted and washed with saturated $NaHCO_3$ solution, water, brine, dried over $MgSO_4$ and concentrated. To this amorphous solid (60 mg), was added dry dichloromethane (0.8 ml) and trichloroacetonitrile (0.1 ml). The mixture was cooled to −5°C, then 10 μl of DBU was introduced. After stirring at 0 °C for 3 h, TLC showed completion of reaction. The mixture was concentrated, the residue was flash chromatographed on a column of silica gel (dichloromethane : methanol, 14 : 1) to yield **12** (61 mg, 77%) as an amorphous solid: $[\alpha]_D$ − 25 (*c* 0.9, $CHCl_3$).- TLC (dichloromethane : methanol, 14 : 1): R_F 0.42.- ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1 H, *H*N=C), 6.52 (d, 1 H, $J_{1a,2a} = 3.8$ Hz, H-1a), 1.91-2.3 (23s, 69 H, 23 CH_3CO), 1.19, 1.23 (2d, 6 H, H-6e, H-6h).- ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ 171.47-168.73 (23 *C*OCH$_3$), 160.89 (O*C*(NH)CCl$_3$), 90.30 (*C*Cl$_3$), 61.50, 61.46, 60.84, 60.48, 60.42, 60.33 (6 C-6), 58.40, 58.26 (C-2c, C-2f), 23.35, 23.29 (2 NHCO*C*H$_3$), 20.97-20.40 (21*C*H$_3$CO), 15.74, 15.70 (C-6e, C-6h).- MS (FAB): $(M + Li)^+$ 2398.41.- Anal. Calcd for $C_{96}H_{130}N_3O_{60}H_2O$: C, 47.83; H, 5.52; N, 1.74. Found: C, 47.70; H, 5.51; N, 2.09.

11-tri-(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1 → 3)]-(2-acetamido-6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-[(2,3,4-tri-O-acetyl-α-L-fucopyranosyl)-(1 → 3)]-(2-acetamido-6-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**14**)

A mixture of donor **12** (52 mg, 21.7 μmol), acceptor **13** (49 mg, 44 μmol), 4 Å powdered molecular sieves (0.5 g) and dry CH_2Cl_2 (1.2 ml) was stirred under argon at room temperature for 40 min. The reaction mixture was cooled to 0°C. Trimethylsilyl triflate (15 μl, 75 μmol) was added dropwise. After stirring at 0°C for 4 h, the reaction mixture was neutralized by triethylamine (0.1 ml), filtered through a celite bed, and concentrated. The residue obtained was flash chromatographed (cyclohexane : ethyl acetate, 1 : 6) to afford **14** (45 mg, 63%) as an amorphous solid: $[\alpha]_D$ − 32 (*c* 1.3, $CHCl_3$).- TLC (dichloromethane : methanol, 14 : 1): R_F 0.40.- ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.44, 5.40, 5.39 (3dd, 3 H, $J_{4,3} = 3.7$ Hz, $J_{4,3} = 3.3$ Hz, $J_{4,3} = 3.6$ Hz, H-4b, H-4d, H-4g), 4.64 (d, 1 H, $J_{1a,2a} = 7.9$ Hz, H-1a), 2.24-1.90 (23s, 69 H, 23 CH_3CO), 1.71-1.48, 1.46-1.25 (2 m, 72 H, 30 CH_2 and 12 C*H* of lipid chain), 1.23, 1.18 (2d, 6 H, H-6e, H-6h), 0.91, 0.90, 0.89, 0.87, 0.86 (5s, 45H, 15 CH_3 of lipid chain).- ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ 171.32, 171.16, 171.06, 171.06, 170.88, 170.76, 170.76, 170.61, 170.56, 170.50, 170.48, 170.40, 170.32, 170.08, 169.84, 169.84, 169.72, 169.61, 169.61, 169.61, 169.35, 169.02, 168.76 (23 *C*OCH$_3$), 100.69, 100.69, 100.50, 100.37, 99.12, 99.06, 95.24, 95.17 (8 anomeric C), 75.69, 75.75, 75.61, 74.35, 74.13, 72.98, 72.98, 72.67, 72.53, 71.89, 71.77, 71.45, 71.41, 71.35, 71.35, 71.13, 71.03, 71.03, 70.87, 70.87, 69.11, 69.02, 68.97, 68.82, 68.74, 67.96, 67.89, 66.65, 64.04, 63.99 (30 ring C), 58.73, 58.73 (C-2f, C-2c), 70.97, 70.75, 70.55, 70.43, 70.32, 70.15, 69.80, 69.80, 69.80, 69.71, 69.71, 69.71, 69.12 (13 O-*C*H$_2$ of lipid chain), 62.11, 61.54, 60.88, 60.52, 60.09, 59.56 (6 C-6), 44.33 (quaternary C of lipid chain), 32.52-36.65, 24.78-24.41 (*C*H$_2$ of lipid chain), 32.81, 32.80, 32.77, 32.75, 29.98, 29.67, 27.95 (*C*H of lipid chain), 23.44, 23.39 (2 NHCO*C*H$_3$), 22.71, 22.61, 21.06-20.53 (21 CO*C*H$_3$), 15.82, 15.77 (C-6e, C-6h).- MS (CI): $(M + NH_4)^+$ 3356.61.- Anal. Calcd for $C_{165}H_{272}N_2O_{66}$: C, 59.34; H, 8.21; N, 0.84. Found: C, 59.18; H, 8.45; N, 0.91.

11-tri-(3,7,11,15-tetramethylhexadecyloxymethyl)-3,6,9-trioxa-1-undecyl β-D-galactopyranosyl-(1 → 4)-[(α-L-fucopyranosyl)-(1 → 3)]-(β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1 → 4)-[(α-L-fucopyranosyl)-(1 → 3)]-(β-D-glucopyranosyl)-(1 → 3)-(β-D-galactopyranosyl)-(1 → 4)-β-D-glucopyranoside (**1**).

To a solution of **14** (40 mg, 12 μmol) in methanol–dichloromethane (2 ml, 5 : 3) was added 0.1 M NaOMe solution in methanol (0.2 ml) under argon. After stirring for 2 h (monitoring by TLC) at room temperature, the mixture was neutralized by Amberlite resin (IR120, H^+ form), filtered and concentrated to dryness. The residue obtained was purified by flash chromatography eluting with dichloromethane : methanol : water (6 : 3 : 0.3) to afford **1** (29 mg, 98%) as an amorphous solid: $[\alpha]_D$-24 (*c* 0.6, pyridine).- TLC (dichloromethane : methanol : water, 6 : 3 : 0.5): R_F 0.3.- ^{1}H-NMR (400 MHz, pyridine-d5) δ 5.94, 5.90 (2 d, 2 H, $J_{1,2} = 3.5$ Hz, $J_{1,2} = 3.6$ Hz, H-1e, H-1h), 5.58, 5.45 (2 q, 2 H, $J_{5,6} = 6.4$ Hz, $J_{5,6} = 6.5$ Hz, H-5e, H-5h), 2.04, 1.98 (2s, 6 H, 2 NHCOC*H*$_3$), 1.61, 1.55 (2 d, 6 H, $J_{6,5} = 6.4$ Hz, $J_{6,5} = 6.5$ Hz, H-6e, H-6h).- MS (FAB): $(M + Na)^+$ 2478.

Acknowledgements

The authors are grateful to Mrs Véronique Michon for NMR measurements (400 MHz), Mrs Nicole Morin for MS measurements, and CNRS for financial support (Programme Physique et Chimie du Vivant 1997).

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Received 31 March 2000, revised 31 March 2000, accepted 2 May 2000