Chemistry of Natural Compounds and Bioorganic Chemistry

Synthesis of lipooligosaccharides related to nodulation factors of *Rhizobium* sp. NGR234

1. Branched trisaccharides containing sulfated or acetylated residue of 2-*O*-methyl-L-fucose

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Trisaccharide analogs of natural nodulation factors from *Rhizobium* sp. NGR234, namely, 2-acetamido-2-deoxy-4-*O*-(2-deoxy-2-hexadecanamido-β-D-glucopyranosyl)-6-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-D-glucopyranose and its derivatives containing a 4-*O*-acetyl or a 3-*O*-sulfo group at the L-fucose residue, were synthesized. The oligosaccharides synthesized were shown to possess biological activity.

Key words: Nod-factors, trisaccharides, lipooligosaccharides, *Rhizobia*.

The concerted interaction of nitrogen-fixing bacteria of the *Rhizobium* species and legumes is established by exchange of signal molecules between the symbionts.[1,2] An important role in a series of complex processes, which eventually result in the formation of nodules containing nitrogen-fixing bacteria, belongs to the so-called nodulation factors (Nod-factors), whose structural features determine the specificity of interaction of *Rhizobia* with the host plant.

In the chemical aspect, Nod-factors are chitooligosaccharides, in which the glucosamine residue located on the non-reducing end of the chain is *N*-acylated with a higher fatty acid, while all of the other amino groups of the molecule are *N*-acetylated. A characteristic feature of these compounds is additional modification of the terminal residues of the reducing *N*-acetylglucosamine and non-reducing *N*-acylglucosamine.

Nod-factors produced by different bacteria differ in chain length, nature of the fatty acid, and modification of terminal monosaccharide residues (see reviews[3,4] and a paper[5] dealing with the structure of Nod-factors). The isolation of individual Nod-factors from natural sources is quite a laborious task, which encouraged the initiation

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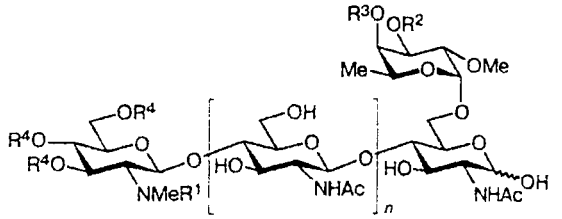
of studies into the synthesis of compounds belonging to this series.[6—12]

In the present communication, we start a series of publications on the chemical synthesis of lipooligosaccharides related to nodulation factors of *Rhizobium* sp. NGR234, a strain of unusually broad host-specificity, which interacts with over 110 genera of legumes and produces over 80 Nod-factors. The general structure of the latter can be represented by formula **1**.



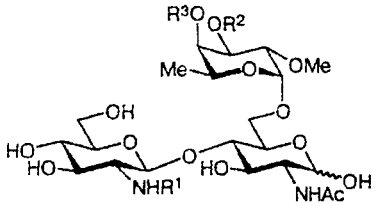
1

R^1 = (*Z*)-$CH_3(CH_2)_5CH{=}CH(CH_2)_9CO$; $CH_3(CH_2)_{14}CO$ (Palm)
R^2 = H, SO_3^-; R^3 = Ac, H; R^4 = NH_2CO, H
n = 2, 3

Unlike in the Nod-factors of other bacteria, the nonreducing terminal glucosamine residue in these compounds can be *N*-acylated with a saturated fatty (palmitic) acid. A characteristic modification of the *N*-acetylglucosamine residue on the reducing oligosaccharide end is substitution with 4-*O*-acetyl-2-*O*-methyl-α-L-fucopyranose or 2-*O*-methyl-3-*O*-sulfo-α-L-fucopyranose residues at its C(6) atom.

The purpose of this work is the synthesis of the smallest lipooligosaccharides, whose chitooligosaccharide chains contain substituents characteristic of natural Nod-factors of *Rhizobium* sp. NGR234.

The trisaccharide family of general formula **2*** was chosen as a primary synthetic goal.

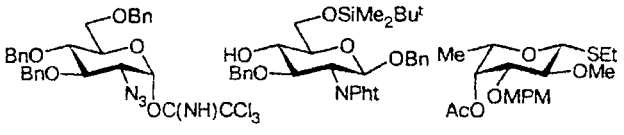


2a: R^1 = Palm; R^2 = R^3 = H
2b: R^1 = Palm; R^2 = SO_3^-; R^3 = H
2c: R^1 = Palm; R^2 = H; R^3 = Ac

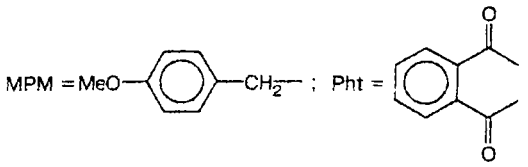
This choice is based on the following premises: first, trisaccharide **2** is the smallest fragment of lipooligosaccharide **1** possessing its characteristic pattern of substitution at amino groups and C(6) atom of the reducing oligosaccharide end; second, the chitobiose fragment bearing an *N*-acyl and an *N*-acetyl group undergoes cleavage with chitinases to the smallest degree; and third, the presence of an α(1→6)-linked 2-*O*-methyl-L-fucose residue should probably increase the stability of the glucosamine—glucosamine glycoside bond against chitinases of legumes[15] which inactivate natural Nod-factors.

Such compounds have not been isolated from natural objects, and their study aimed at the detection of structural elements responsible for biological activity is of considerable interest.

The following considerations were taken into account in retrosynthetic analysis: first, the amino groups should be acylated with different acids; second, the synthesis of all three compounds should preferably start from a common trisaccharide precursor, which should be modified in a minimum number of final steps; and third, because of the low reactivity of the OH(4) group of the glucosamine residue in glycosylation reactions, a glycosyl acceptor with the minimum steric hindrance should be used for the construction of the chitobiose fragment. The combination of these requirements led us to three key intermediates: **3**, **4**, and **5**.



3 **4** **5**

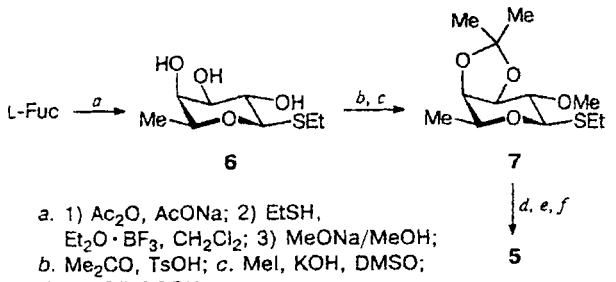


The previously described[16] trichloroacetimidate **3** incorporating an azido group at the C(2) atom was used as the glycosyl donor, and phthalimide derivative **4** was used as the glycosyl acceptor. The conditions for the transformation of phthalimido and azido groups into free amino groups differ, which makes it possible to introduce different acyl substituents at the amino groups in GlcN residues. Protective groups in glycosyl donor **5** enable selective introduction of substituents at the C(3) and C(4) atoms of the fucose residue.

Thiofucoside **6** was obtained in high yield by the reaction of L-fucose acetate with EtSH in the presence of $BF_3 \cdot Et_2O$. Acetonation[9] of thiofucoside **6** followed by methylation with MeI in the presence of KOH afforded methylated acetonide **7** (Scheme 1). Mild removal of the 3,4-*O*-isopropylidene protection by aqueous CF_3CO_2H occurred smoothly, and the diol obtained in quantitative yield was *p*-methoxybenzylated without isolation, using a method based on the selective alkyla-

* According to the currently recommended nomenclature for Nod-factors, compounds **2a**, **2b**, and **2c** are named NodNGR-II(C16:0)(MeFuc), NodNGR-II(C16:0)(MeFuc,S), and NodNGR-II(C16:0)(MeFuc,Ac), respectively.

Scheme 1

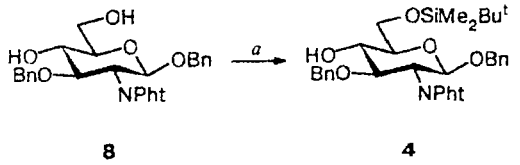


a. 1) Ac_2O, AcONa; 2) EtSH, $Et_2O \cdot BF_3$, CH_2Cl_2; 3) MeONa/MeOH;
b. Me_2CO, TsOH; *c*. MeI, KOH, DMSO;
d. aq. CF_3COOH;
e. 1) Bu_2SnO, PhH, Δ; 2) MPMCl, Bu_4NBr;
f. Ac_2O, Py

tion of a stannylidene derivative.[17] Subsequent acetylation gave crystalline thioglycoside **5**.

Selective silylation[18] of reported[19] diol **8** with Bu^tMe_2SiCl in DMF in the presence of Et_3N and 4-dimethylaminopyridine (DMAP) gave (Scheme 2) selectively protected phthalimide derivative **4** in high yield.

Scheme 2



a. Bu^tMe_2SiCl, Et_3N, DMAP, DMF

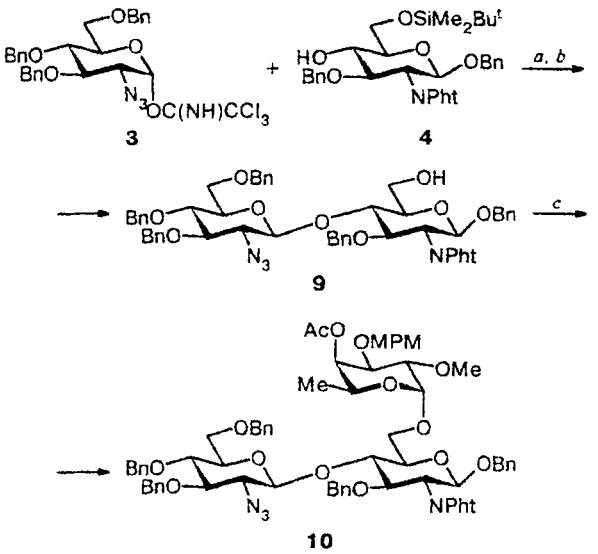
Glycosylation of compound **4** with trichloroacetimidate **3** [16] catalyzed by $BF_3 \cdot Et_2O$ in a MeCN—CH_2Cl_2 mixture at −40 °C (Scheme 3) followed by removal of silyl protection by acid hydrolysis without isolation of the intermediate gave disaccharide derivative **9**, which had the only free hydroxyl group at the C(6) atom of the reducing monosaccharide residue, in 78% yield. Performing the glycosylation and desilylation in one preparative step made it possible to simplify the chromatographic isolation of product **9** from the reaction mixture.

Glycosylation of derivative **9** with thiofucoside **5** in the presence of $CuBr_2$ [20] gave the expected α-linked trisaccharide **10** in 73% yield. Its transformation into the target products is shown in Scheme 4.

Removal of the phthalimide protection from compound **10** on treatment with $N_2H_4 \cdot H_2O$ in Pr^nOH under reflux conditions followed by acetylation gave di-*N*,*O*-acetylated product **11** in 96% yield. The ^1H NMR spectrum of the latter confirmed the absence of a phthalimide group and the presence of two acetyl groups.

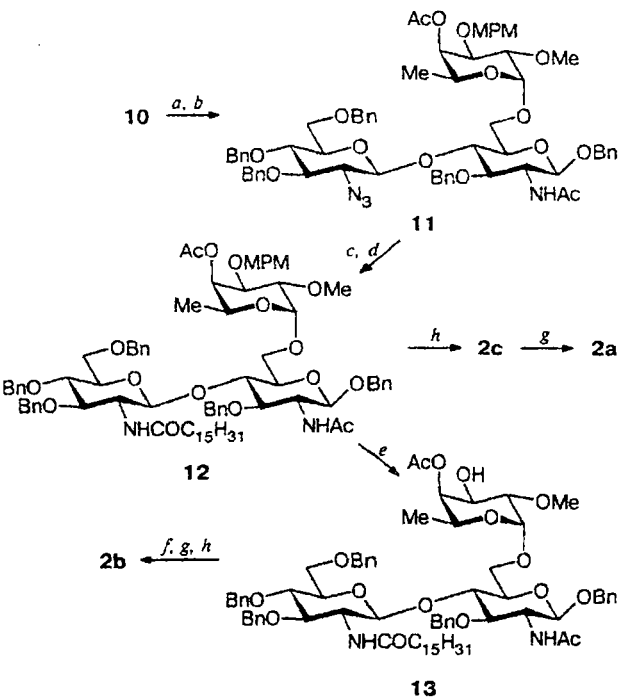
Reduction of the azido group in compound **11** with H_2S in aqueous Py [21] followed by *N*-palmitoylation on treatment with *N*-acylthiazolidine-2-thione (similarly to the procedure in Ref. 7) gave trisaccharide derivative **12** in 52% yield.

Scheme 3



a. $BF_3 \cdot Et_2O$, MeCN—CH_2Cl_2 1 : 1; *b*. TsOH · H_2O, CH_3CN;
c. **5**, $CuBr_2$, Bu_4NBr, DMF—CH_2Cl_2 1 : 5, MS 4 Å

Scheme 4



a. $N_2H_4 \cdot H_2O$, PrOH, D; *b*. Ac_2O, Py, DMAP;
c. H_2S, Py—H_2O; *d*. *N*-(palmitoyl)thiazolidine-2-thione, DMF; *e*. DDQ, CH_2Cl_2, H_2O; *f*. Py · SO_3, DMF, Py;
g. MeONa, MeOH; *h*. H_2, 10% Pd/C, THF—EtOH—H_2O 2 : 2 : 1

Hydrogenolysis of compound **12** resulted in trisaccharide **2c**, which was isolated in 56% yield after HPLC purification. The structure of this trisaccharide was confirmed by its $^1H—^1H$ COSY NMR spectrum. Four doublets at δ 5.00 ($J_{1,2}$ = 4.0 Hz), 4.94 ($J_{1,2}$ = 4.5 Hz), 4.56 ($J_{1,2}$ = 10.0 Hz), and 4.48 ($J_{1,2}$ = 10.0 Hz) were assigned to the four anomeric protons of the α-GlcNAc, α-L-Fuc, β-GlcNPalm, and β-GlcNAc residues, respectively. The downfield shift of the signal of H(4) of α-L-Fuc at δ 5.10 ($J_{3,4}$ = 3.6 Hz, $J_{4,5}$ = 0.5 Hz) unambiguously indicates the presence of an acetyl group at the O(4) atom of the α-L-fucose residue.

Saponification of trisaccharide **2c** followed by HPLC purification gave trisaccharide **2a**.

Treatment of trisaccharide derivative **12** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[22] resulted in removal of the 4-methoxybenzyl protective group, and trisaccharide derivative **13** with a free OH group at the C(3) atom of the fucose residue was isolated in 55% yield. Subsequent sulfation with $Py \cdot SO_3$,[7] saponification with MeONa in MeOH, hydrogenolysis, and HPLC gave the sodium salt of trisaccharide sulfate **2b** in high yield.

The structure of trisaccharide **2b** was confirmed by its $^1H—^1H$ COSY NMR spectrum. Four signals at δ 5.00 (br.d), 4.85 ($J_{1,2}$ = 4.5 Hz), 4.57 ($J_{1,2}$ = 9.5 Hz), and 4.48 ($J_{1,2}$ = 10.0 Hz) were assigned to the four anomeric protons of the α-GlcNAc, α-L-Fuc, β-GlcNPalm, and β-GlcNAc residues, respectively. The downfield shift of the signal of H(3) of the α-L-Fuc residue at δ 4.62 ($J_{3,4}$ = 3.6 Hz, $J_{3,2}$ = 8.5 Hz) unambiguously indicates that sulfate is located at position 3 of the fucose residue.

A study of the biological activity in the test for root-hair curling[23] showed the high activity of trisaccharide derivatives **2a—c** at concentrations $10^{-9}—10^{-6}$ mol L^{-1}.

Experimental

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or in CD_3OD at 300 K on a Bruker WM-250 spectrometer with acetone as the internal standard. Thin layer chromatography was carried out on plates with a fixed silica gel (Merck) layer. Column chromatography was performed on Silpearl (Chemapol) silica gel with gradient elution. HPLC was carried out on a modular chromatographic system (Altex pump, Rheodyne 7025 injector, Knauer ultraviolet detector (220 nm), Knauer chart recorder) with a C_8 column (250×16 mm) in a MeOH—water system (15 : 85), 5 mL min^{-1}. The solutions were concentrated *in vacuo* at 40 °C. Acetonitrile and methylene chloride were dried by distillation over CaH_2.

Ethyl 3,4-*O*-isopropylidene-2-*O*-methyl-1-thio-β-L-fucopyranoside (7). L-Fucose (1.64 g, 10.0 mmol) was added in portions with stirring and heating (100 °C) to a mixture of Ac_2O (10 mL, 10.82 g, 0.106 mol) and anhydrous AcONa (500 mg, 6.10 mmol). The mixture was poured into water (100 mL). After 16 h, the solution was extracted with $CHCl_3$ (3×50 mL), the organic layers were separated, and the combined extracts were concentrated *in vacuo*. The syrup obtained was dissolved in 5 mL of dry CH_2Cl_2, and then EtSH (1.11 mL, 0.932 g, 15.0 mmol) and $BF_3 \cdot Et_2O$ (0.127 mL, 142 mg, 1.0 mmol) were added with stirring. After 8 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (50 mL) and extracted with $CHCl_3$ (3×50 mL). The combined extracts were concentrated *in vacuo*, the syrup obtained was dissolved in anhydrous MeOH (20 mL), and 1 *M* NaOMe in MeOH (0.1 mL) was added at 20 °C. After 16 h, the solution was neutralized with a KU-2 cationite (H^+) to pH 7 (according to a universal indicator paper), the cationite was filtered off, and the filtrate was concentrated *in vacuo*. The thioglycoside syrup obtained was dissolved in anhydrous Me_2CO (10 mL), and $TsOH \cdot H_2O$ (0.1 g, 0.53 mmol) was added with stirring at 20 °C. After 24 h, $NaHCO_3$ (1 g) was added to the reaction mixture, and the mixture was stirred for 30 min. The solution was filtered, and the filtrate was concentrated *in vacuo*. The syrup obtained was dissolved in DMSO (5 mL), and the solution was added with stirring to a suspension of powdered KOH (4 g) in DMSO (10 mL) at such a rate that the temperature did not exceed 30 °C. After 5 min, MeI (2.00 mL, 4.56 g, 32.1 mmol) was added dropwise at such a rate that the temperature did not exceed 40 °C, and stirring was continued for 30 min. The reaction mixture was poured into ice water (100 mL) and extracted with Et_2O (4×30 mL). The combined ethereal extracts were concentrated *in vacuo*, and the syrupy residue was crystallized from hexane (20 mL). The yield of the product was 1.36 g (52%), m.p. 82 °C, $[\alpha]_D^{20}$ +11.8° (*c* 1.5, $CHCl_3$). ^1H NMR ($CDCl_3$), δ: 4.31 (d, 1 H, H-1, $J_{1,2}$ = 10.0 Hz); 4.10 (dd, 1 H, H-3, $J_{3,4}$ = 4.0 Hz); 4.30 (dd, 1 H, H-4, $J_{4,5}$ = 3.0 Hz); 3.80 (dq, 1 H, H-5, $J_{5,6}$ = 6.0 Hz); 3.58 (s, 3 H, OCH_3); 3.20 (dd, 1 H, H-2, $J_{2,3}$ = 6.0 Hz); 2.74 (m, 2 H, $C\underline{H}_2CH_3$); 1.55 (s, 3 H, CH_3); 1.40 (d, 3 H, H-6); 1.37 (s, 3 H, CH_3); 1.30 (t, 3 H, $CH_2C\underline{H}_3$).

Ethyl 4-*O*-acetyl-3-*O*-(4-methoxybenzyl)-2-*O*-methyl-1-thio-β-L-fucopyranoside (5). Compound 7 (750 mg, 2.86 mmol) was dissolved in $CHCl_3$ (10 mL), and 90% aqueous CF_3COOH (1 mL) was added at 20 °C. After 15 min, the solution was concentrated *in vacuo*, and an EtOH—toluene mixture (1 : 1) was added and evaporated repeatedly until CF_3COOH was completely removed from the residue. Bu_2SnO (1 g, 4.02 mmol) and benzene (20 mL) were added to the residue (700 mg), and the mixture was refluxed with a Dean—Stark trap until the precipitate dissolved completely. Et_4NBr (1.00 g, 4.76 mmol) and MPMCl (0.65 mL, 0.75 g, 4.79 mmol) were added to the solution, and the reaction mixture was refluxed until disappearance of the starting compound (TLC monitoring). Chromatography in a benzene→EtOAc gradient gave 832 mg of a 3-*O*-*p*-methoxybenzyl derivative (85%), $[\alpha]_D^{20}$ −10.5° (*c* 1, $CHCl_3$), which was acetylated in a mixture of Ac_2O (3 mL) and Py (3 mL). The solvent was evaporated, and toluene (4×5 mL) was added to the residue and evaporated. Chromatography in a benzene→EtOAc gradient gave 884 mg (95%) of compound **5**. $[\alpha]_D^{20}$ −12.0° (*c* 1.5, $CHCl_3$), m.p. 81—82 °C (from hexane).

^1H NMR ($CDCl_3$), δ: 5.35 (dd, 1 H, H-4, $J_{4,5}$ = 1.5 Hz); 4.32 (d, 1 H, H-1, $J_{1,2}$ = 8.5 Hz); 3.80 (s, 3 H, $C\underline{H}_3OC_6H_4$); 3.62 (dq, 1 H, H-5, $J_{5,6}$ = 6.0 Hz); 3.60 (s, 3 H, OMe); 3.50 (dd, 1 H, H-3, $J_{3,4}$ = 4.0 Hz); 3.25 (dd, 1 H, H-2, $J_{2,3}$ = 10.0 Hz); 2.74 (m, 2 H, $C\underline{H}_2CH_3$); 2.15 (s, 3 H, OAc); 1.33 (t, 3 H, $C\underline{H}_3CH_2$); 1.20 (d, 3 H, H-6).

Benzyl 3-*O*-benzyl-6-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (4). Bu^tMe_2SiCl (1.04 g, 6.90 mmol) was added with stirring at 20 °C to a solution of benzyl 3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **8** [18] (2.94 g, 6.01 mmol), Et_3N (1.68 mL, 12.05 mmol), and DMAP (73 mg, 0.60 mmol) in anhydrous DMF (12 mL). Stirring was continued for an additional 0.5 h, and then the reaction mixture was diluted with benzene (50 mL) and poured into water. The organic phase was separated, washed three

times with water, dried with $MgSO_4$, concentrated *in vacuo*, and chromatographed in the gradient 20 vol.% petroleum ether in benzene → 20 vol.% EtOAc in benzene to give 3.12 g (86%) of compound **4**, solid foam, R_f 0.38 (hexane—EtOAc 3 : 1), $[\alpha]_D^{20}$ −4.6° (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$), δ: 7.88—7.45 (m, 4 H, Pht); 7.13—6.82 (m, 10 H, C_6H_5); 5.16 (d, 1 H, H-1, $J_{1,2}$ = 8.5 Hz); 4.83—4.40 (m, 4 H, $PhC\underline{H}_2$); 4.25 (dd, 1 H, H-3, $J_{3,4}$ = 10.0 Hz); 4.17 (dd, 1 H, H-2, $J_{2,3}$ = 10.0 Hz); 3.85 (br.t, 1 H, H-4); 4.00—3.50 (m, 3 H, H-5 + H-6, H-6′); 3.40 (br.s, 1 H, OH); 0.93 (s, 9 H, Bu^t); 0.15 (s, 6 H, Me_2Si).

Benzyl 4-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9). A solution of compound **4** (226 mg, 0.374 mmol) and 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl trichloroacetimidate **3** [16] (295 mg, 0.476 mmol) in a mixture of anhydrous MeCN (5 mL) and anhydrous CH_2Cl_2 (1 : 1) was stirred for 1 h at 20 °C with 1 g of powdered MS (4 Å) in a dry argon atmosphere and then cooled to −40 °C, and a 0.5 *M* solution of $BF_3 \cdot Et_2O$ in anhydrous CH_2Cl_2 (95 μL, 0.0475 mmol) was added dropwise. The reaction mixture was stirred for 0.5 h at −40 °C, the $BF_3 \cdot Et_2O$ solution (95 μL) was added, the mixture was stirred for an additional 0.5 h at −40 °C, and Et_3N (0.15 mL, 0.11 g, 1.08 mmol) was then added. After warming to 20 °C, the mixture was filtered through Celite, and the residue was washed with $CHCl_3$ on the filter. The combined organic phases were washed three times with water, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was dissolved in MeCN (5 mL), and $TsOH \cdot H_2O$ (0.15 g, 0.79 mmol) was added. The mixture was heated for 1 h at 50 °C, concentrated *in vacuo*, and the residue was partitioned between 50 mL of $CHCl_3$ and 50 mL of saturated aqueous $NaHCO_3$. The organic layer was separated, dried with Na_2SO_4, concentrated *in vacuo*, and chromatographed in the gradient: benzene → 40 vol.% EtOAc in benzene to give 278 mg (78%) of compound **9**, m.p. 154—155 °C (benzene—hexane). R_f 0.08 (hexane—EtOAc 3 : 1), $[\alpha]_D^{20}$ −16.8° (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$), δ: 7.88—6.72 (m, 29 H, H_{arom}); 5.20 (d, 1 H, H-1, $J_{1,2}$ = 8.5 Hz); 4.70 (m, 10 H, $PhC\underline{H}_2$); 4.55 (d, 1 H, H-1′, $J_{1',2'}$ = 8.0 Hz); 4.33 (dd, 1 H, H-3, $J_{3,4}$ = 10.0 Hz); 4.20 (dd, 1 H, H-2, $J_{2,3}$ = 10.0 Hz); 3.50 (dd, 1 H, H-2′, $J_{2',3'}$ = 9.5 Hz); 2.18 (br.s, 1 H, OH).

Benzyl 6-*O*-[4-*O*-acetyl-3-*O*-(4-methoxybenzyl)-2-*O*-methyl-α-L-fucopyranosyl]-4-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (10). A mixture of disaccharide **9** (234 mg, 0.247 mmol), thioglycoside **5** (120 mg, 0.312 mmol), Bu_4NBr (201 mg, 0.624 mmol), and 0.5 g of powdered MS (4 Å) in anhydrous CH_2Cl_2 (2.5 mL) was stirred for 1 h at 20 °C in a dry argon atmosphere, then $CuBr_2$ (139 mg, 0.622 mmol) was added, and stirring was continued for three days. The mixture was filtered through Celite, and the residue was washed with $CHCl_3$ on the filter. The combined organic phases were washed with 5% aqueous $Na_2S_2O_3$, then washed three times with water, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed in the gradient: hexane → 50 vol.% EtOAc in hexane to give 230 mg (73%) of compound **10**, solid foam, R_f 0.38 (benzene—EtOAc 9 : 1), $[\alpha]_D^{20}$ −58.7° (*c* 1, $CHCl_3$)

^{1}H NMR ($CDCl_3$), δ: 7.92—7.52 (m, 4 H, Pht); 7.48—6.82 (m, 29 H, H_{arom}); 5.41 (dd, 1 H, H-4 Fuc, $J_{4,5}$ = 0.5 Hz); 5.15 (d, 1 H, H-1, $J_{1,2}$ = 8.5 Hz); 5.00 (d, 1 H, H-1, Fuc, $J_{1,2}$ = 3.5 Hz); 4.95 (d, 1 H, H-1′, $J_{1',2'}$ = 8.0 Hz); 4.25 (dq, H-5, Fuc, $J_{5,6}$ = 6.0 Hz); 4.23 (m, 2 H, H-2, H-3); 3.97 (dd, 1 H, H-3, Fuc, $J_{3,4}$ = 4.0 Hz); 3.77 (s, 3 H, $C\underline{H}_3OC_6H_4$); 3.60 (dd, 1 H, H-2, Fuc, $J_{2,3}$ = 8.5 Hz); 3.54 (t, 1 H, H-1′, $J_{1',2'}$ = 9.5 Hz); 3.51 (s, 3 H, OMe); 2.17 (s, 3 H, Ac); 1.13 (d, 3 H, H-6, Fuc). ^{13}C NMR ($CDCl_3$), δ: 100.9 (C-1); 97.2 (C-1′); 97.1 (C-1, Fuc); 59.7 (2 signals), 55.9, 55.2 (C-2 + C-2′ + MeO, Fuc + $\underline{C}H_3OC_6H_4$); 20.9 ($\underline{C}H_3CO$); 16.1 (C-6, Fuc).

Benzyl 2-acetamido-6-*O*-[4-*O*-acetyl-3-*O*-(4-methoxybenzyl)-2-*O*-methyl-α-L-fucopyranosyl]-4-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (11). A solution of compound **10** (310 mg, 0.244 mmol) in Pr^nOH (18 mL) and $N_2H_4 \cdot H_2O$ (2 mL, 41.2 mmol) was refluxed for 8 h in an argon atmosphere, and the solvent was concentrated *in vacuo* to dryness. The residue was dissolved in a mixture of Py (4 mL) and Ac_2O (4 mL), DMAP (10 mg, 82 μmol) was added, and the mixture was kept for one day at 20 °C. MeOH (5 mL) was added to the reaction mixture with stirring and cooling by ice water. After 5 min, the reaction mixture was concentrated *in vacuo* to dryness, and toluene (4×5 mL) was added to the residue and evaporated. The residue was chromatographed in the gradient: benzene → 25 vol.% Me_2CO in benzene to give 276 mg (96%) of derivative **11**, solid foam, R_f 0.46 (benzene—Me_2CO 5 : 1), $[\alpha]_D^{20}$ −67.2° (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$), δ: 7.44—7.30 (m, 27 H, H_{arom}); 6.87 (d, 2 H, $CH_3OC_6\underline{H}_4$); 5.42 (dd, 1 H, H-4, Fuc, $J_{4,5}$ = 0.5 Hz); 5.35 (d, 1 H, NH); 5.05 (d, 1 H, H-1, Fuc, $J_{1,2}$ = 3.5 Hz); 4.88 (d, 1 H, H-1′, $J_{1',2'}$ = 8.0 Hz); 4.82 (d, 1 H, H-1, $J_{1,2}$ = 8.5 Hz); 4.36 (dq, 1 H, H-5, Fuc, $J_{5,6}$ = 6.0 Hz); 4.14 (t, 1 H, H-4, $J_{4,5}$ = 9.5 Hz); 3.94 (t, 1 H, H-3, $J_{3,4}$ = 9.5 Hz); 3.94 (dd, 1 H, H-3 Fuc, $J_{3,4}$ = 4.0 Hz); 3.78 (s, 3 H, MeO, MPM); 3.55 (s, 3 H, MeO, Fuc); 3.48 (dd, 1 H, H-2′, $J_{2',3'}$ = 9.5 Hz); 2.13 (s, 3 H, OAc); 1.81 (s, 3 H, NAc); 1.15 (d, 3 H, H-6, Fuc).

Benzyl 2-acetamido-6-*O*-[4-*O*-acetyl-3-*O*-(4-methoxybenzyl)-2-*O*-methyl-α-L-fucopyranosyl]-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-hexadecanamido-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranoside (12). Water (1.5 mL) was added to a solution of trisaccharide **11** (174 mg, 0.147 mmol) in Py (3.5 mL), and the mixture was saturated with H_2S at 20 °C for 20 min and kept for three days in a place protected from light. The reaction mixture, which according to TLC data did not contain the starting azide, was concentrated *in vacuo* to dryness. The residue was suspended in EtOAc, washed with saturated aqueous $NaHCO_3$, and then washed two times with water. The organic phase was separated, dried by filtering through a mixture of Celite and Na_2SO_4, and concentrated *in vacuo*. The residue was dissolved in a mixture of DMF (2 mL) and CH_2Cl_2 (1 mL), *N*-palmitoylthiazolidine-2-thione[24] (553 mg, 1.472 mmol) was added, and the mixture was kept for two days at 20 °C and then concentrated *in vacuo*. Toluene (4×5 mL) was added to the residue and evaporated. The residue was chromatographed in the gradient: benzene → 25 vol.% Me_2CO in benzene to give 107 mg (52%) of compound **12**, syrup, R_f 0.46 (benzene—Me_2CO 5 : 1), $[\alpha]_D^{20}$ −61.3° (*c* 1, $CHCl_3$).

^{1}H NMR ($CDCl_3$), δ: 7.50—7.12 (m, 27 H, H_{arom}), 6.84 (d, 2 H from $CH_3OC_6\underline{H}_4$); 6.23 (br.d, 1 H, NH); 5.92 (br.d, 1 H, NH); 5.37 (dd, 1 H, H-4, Fuc, $J_{3,4}$ = 4.0 Hz, $J_{4,5}$ = 0.5 Hz); 3.76 (s, 3 H, $C\underline{H}_3OC_6H_4$); 3.51 (s, 3 H, OMe); 2.15 (s, 3 H, OAc); 2.02 (br.t, 2 H, $\underline{C}H_2CO$); 2.00 (t, 2 H, CH_2CO); 1.93 (s, 3 H, NAc); 1.57 (m, 2 H, $C\underline{H}_2CH_2CO$); 1.38—1.13 (m, 24 H, $(CH_2)_{12}$); 1.01 (d, 3 H, H-6, Fuc); 0.92 (t, 3 H, $C\underline{H}_3CH_2$). ^{13}C NMR ($CDCl_3$), δ: 100.8 (C-1, GlcNAc); 100.1 (C-1, GlcNPalm); 97.5 (C-1, α-L-Fuc); 51.6, 51.3, 49.3 (C-2, GlcNAc + C-2, GlcNPalm + OMe); 29.7—29.4 (CH_2, Palm); 16.0, 14.1 (C-6, Fuc + CH_3, Palm).

Sodium salt of 2-acetamido-2-deoxy-4-*O*-(2-deoxy-2-hexadecanamido-β-D-glucopyranosyl)-6-*O*-(2-*O*-methyl-3-*O*-sulfo-α-L-fucopyranosyl)-D-glucopyranose (2b). Water (0.2 mL) and DDQ (12.0 mg, 52.9 μmol) were added to a solution of

trisaccharide **12** (50.0 mg, 35.9 μmol) in CH_2Cl_2 (1.5 mL), and the mixture was vigorously stirred for 1.5 h at 20 °C. A new portion of DDQ (4.0 mg, 17.6 μmol) was then added, and stirring was continued for 45 min. The reaction was then quenched by adding 5% aqueous Na_2SO_3 (3 mL). The reaction mixture was diluted with $CHCl_3$, and the organic layer was separated, washed three times with saturated aqueous $NaHCO_3$, washed with water, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed in the gradient: benzene → 50 vol.% Me_2CO in benzene to give 9.0 mg of the starting compound **12** (18% with respect to the starting amount) and 20.5 mg of benzyl 2-acetamido-6-*O*-(4-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl)-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-2-deoxy-2-hexadecanamido-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranoside (**13**) (55% with respect to the reacted compound **12**).

A solution of $Py \cdot SO_3$ (26 mg, 163 μmol) in anhydrous DMF (0.5 mL) was added dropwise with stirring in a dry argon atmosphere to a solution of product **13** (20.5 mg, 16.1 μmol) in anhydrous DMF (0.5 mL) and anhydrous Py (0.2 mL). The reaction mixture was kept for 2 h at 50 °C and cooled to 20 °C. MeOH (1 mL) was added, and the mixture was concentrated *in vacuo* to dryness. The residue was suspended in anhydrous CH_2Cl_2 (0.3 mL). The suspension was diluted with anhydrous MeOH (3 mL), and a 1 *M* solution of MeONa in MeOH (0.4 mL) was added. The mixture was heated for 3.5 h at 40 °C, and an excess of solid CO_2 was then added. Volatile compounds were distilled off *in vacuo*. The residue was partitioned between the upper and lower phase of the Bu^nOH—water system (2 mL of each). The upper phase was separated, and the lower phase was extracted with Bu^nOH saturated with water (2×2 mL). The combined extracts were concentrated *in vacuo*, and the residue (25 mg) was dissolved in 10 mL of a THF—EtOH—water mixture (2 : 2 : 1) and hydrogenated under atmospheric pressure at 36 °C over 10% Pd/C (25 mg). The catalyst was precipitated by centrifugation and washed with a THF—EtOH—water mixture (2 : 2 : 1, 3×10 mL) and a $CHCl_3$—MeOH—water mixture (10 : 10 : 1, 3×10 mL). The combined supernatants were concentrated *in vacuo*, and the residue was dissolved in water (5 mL), passed through a Sep-Pak C_{18} cartridge in water, and washed with water (5 mL). The adsorbed product was eluted with MeOH (10 mL), and the eluate was concentrated *in vacuo*. Trisaccharide **2c** was purified by HPLC (R_t = 5 min) to give 7.5 mg of the sodium salt of sulfate **2b** (52% with respect to compound **13**), $[\alpha]_D^{20}$ −12.4° (*c* 1, MeOH).

^{1}H NMR (CD_3OD), δ: 5.00 (br.d, 1 H, H-1, α-GlcNAc); 4.85 (d, 1 H, H-1, α-L-Fuc, $J_{1,2}$ = 4.5 Hz); 4.62 (dd, 1 H, H-3, α-L-Fuc, $J_{2,3}$ = 8.5 Hz, $J_{3,4}$ = 3.6 Hz); 4.57 (d, 1 H, H-1, β-GlcNPalm, $J_{1,2}$ = 9.5 Hz); 4.48 (br.d, 1 H, H-1′, β-GlcNAs, $J_{1',2'}$ = 10.0 Hz); 4.13 (dq, 1 H, H-5, α-L-Fuc, $J_{5,6}$ = 6.5 Hz); 4.03 (dd, 1 H, H-4, α-L-Fuc, $J_{4,5}$ = 0.5 Hz); 3.76 (dd, 1 H, H-2, β-GlcNPalm, $J_{2,3}$ = 9.5 Hz); 3.65 (dd, 1 H, H-2, α-L-Fuc, $J_{2,3}$ = 10.0 Hz); 3.60 (s, 3 H, OCH_3); 3.41 (dd, 1 H, H-4, β-GlcNPalm, $J_{4,5}$ = 9.5 Hz); 2.28 (t, 2 H, $COCH_2$); 2.00 (s, 3 H, NAc); 1.68 (m, 2 H, CH_2CH_2CO); 1.35 (m, 24 H, $(CH_2)_{12}$); 1.25 (t, 3 H, CH_3, Fuc); 0.93 (t, 3 H, CH_3CH_2).

2-Acetamido-6-*O*-(4-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl)-2-deoxy-4-*O*-(2-deoxy-2-hexadecanamido-β-D-glucopyranosyl)-D-glucopyranose (2c). Hydrogenolysis of derivative **12** (20 mg, 14.3 μmol) by a procedure similar to that for compound **2b** followed by HPLC (R_t = 35 min) gave 6.5 mg of acetate **2c** (56%), $[\alpha]_D^{20}$ −14.3° (*c* 1, MeOH).

^{1}H NMR (CD_3OD), δ: 5.10 (dd, 1 H, H-4, α-L-Fuc, $J_{4,5}$ = 0.5 Hz); 5.00 (d, 1 H, H-1, α-L-Fuc, $J_{1,2}$ = 4.0 Hz); 4.90 (d, 1 H, H-1, α-GlcNAs, $J_{1,2}$ = 4.5 Hz); 4.56 (d, 1 H, H-1, β-GlcNPalm, $J_{1,2}$ = 9.5 Hz); 4.48 (br.d, 1 H, H-1 β-GlcNAs, $J_{1,2}$ = 10.0 Hz); 4.18 (dq, 1 H, H-5, α-L-Fuc, $J_{5,6}$ = 6.5 Hz); 4.02 (dd, 1 H, H-3, α-L-Fuc, $J_{3,4}$ = 3.6 Hz); 3.82 (dd, 1 H, H-2, α-GlcNAs, $J_{2,3}$ = 9.5 Hz); 3.40 (dd, 1 H, H-2, α-L-Fuc, $J_{2,3}$ = 10.0 Hz); 3.64 (dd, 1 H, H-2, β-GlcNPalm, $J_{2,3}$ = 10.0 Hz); 3.53 (s, 3 H, OCH_3, Fuc); 2.20 (t, 2 H, $COCH_2$); 2.10 (s, 3 H, OAc); 1.92 (s, 3 H, NAc); 1.58 (m, 2 H, CH_2CH_2CO); 1.25 (m, 24 H, $(CH_2)_{12}$); 0.99 (t, 3 H, C(6), Fuc); 0.85 (t, 3 H, CH_3CH_2).

2-Acetamido-2-deoxy-4-*O*-(2-deoxy-2-hexadecanamido-β-D-glucopyranosyl)-6-*O*-(2-*O*-methyl-α-L-fucopyranosyl)-D-glucopyranose (2a). Compound **2c** (2 mg, 2.5 μmol) was saponified with 0.1 *M* MeONa in MeOH as described above. The trisaccharide **2a** obtained was isolated on Sep-Pak C_{18} similarly to compound **2b** to give 1.5 mg of compound **2a** (79%), R_t = 20 min (HPLC).

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