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Synthesis of two lipopentasaccharides related to the nodulation factors of the nitrogen-fixing bacterium *Rhizobium* sp. NGR 234

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Experimental

^{1}H- and ^{13}C-spectra recorded on Bruker AC-200 200.13 MHz and 50.34 MHz correspondingly). Chemical shifts of NMR spectra were recorded using the following internal standards: ^{1}H-NMR - $CHCl_3$ (δ_H 7.27); ^{13}C-NMR - $CHCl_3$ (δ_C 77.0); in the case of D_2O as a solvent – 1,4-dioxane was used as external standard (δ_C 67.4).

High resolution mass-spectrometry was recorded on ion-cyclotron resonance "Bruker Daltonix ESI FT ICR Apex II" (Germany) with density of magnetic flux 7 T and ion source Apollo. Samples were prepared in concentration of 10ng/ml in a mixture of 2-propanol-water-thriethyl-amine (50:50:0.001 v/v) and were injected into the source using syringe with a speed of 2 μl/min. Electric potential of capillary inlet 3.8 KV, gas carrier temperature 150^{o} C. Mass-spectra were recorded and processed by standard software from Bruker. Spectra containing peaks with different charges were transformed into spectra of neutral molecules. The presented in the paper molecular masses respond to neutral monoisotope peaks in transformed spectra. Dissociation on capillary scimmer (CSD) was performed by increasing of voltage on the exit from capillary from minus 100 V to minus 350 V.

TLC was performed on DC-Alufolien Kieselgel 60 F_{254} (Merck) in a systems of solvents, presented in Experimental part for every experiment. TLC plates were developed by 98% H_2SO_4-water (1:1 v/v) followed by heating on a hot plate (150^{o} C). Column chromatography was performed on silica gel "Silpearl"(Chemapol) or on Silica gel 60 (Fluka) with gradient elution. Solutions were concentrated in vacuum at 40^{o} C.

Dichloromethane and chloroform were distilled over diethanolamine and then over CaH_2. Absolute acetonitrile was distilled over P_4O_{10}; absolute dimethylforamide "HPLC grade" was stored over calcined molecular sieves 4Å.

Optical rotation was recorded on digital polarimeter DIP-360 (Jasco) at 20-25^{o}C.

Compounds 3 – 7, 13 – 22 were obtained as solid foams, and results of elementary analysis were in agreement with calculated data.

2-Azido-3,4,6-tri-O-benzyl-2-deoxy-1-O-(2,2,2-trichloroacetimidoyl)-α-D-glucopyranose (8a). In the atmosphere of dry Ar with magnetically stirring 0.25 ml of DBU (1.6 mmol) was added to a solution of 1 g (2.1 mmol) of **8** in a mixture of 5 ml abs. DCM and 1.5 ml of CCl_3CN. The reaction mixture was stirred at +4°C during 16 h. Solvents were evaporated in vacuum and the solids were dissolved in a mixture of 8 ml of abs. DCM and 8 ml of hexane, solution was applied on a layer of SiO_2, wetted by DCM, in a funnel with porous bottom (d = 4 cm, h = 2 cm), followed by elution with 40 ml of hexane – EtOAc 6:1 (v/v) mixture, then 60 ml of hexane – EtOAc 4:1 (v/v) mixture. The combined fractions containing **8a** were concentrated in vacuum giving 1.059 g of the substance. R_f 0.71 (hexane – EtOAc 3:2 v/v + one drop of Et_3N). $[\alpha]_D^{20}$ = + 51.5° (c 1, $CHCl_3$). ^{1}H-NMR spectrum: (C_6D_6, δ, ppm., *J*/Hz): 6.48 (d, $^3J_{1,2}$ = 3.5, 1 H, H-1); 4.10 (dd, $^3J_{3,2}$ = 10.5, $^3J_{3,4}$ = 9.5, 1 H, H-3; m, 1 h, H-5); 3.83 (t, $^3J_{3,4}$ = $^3J_{3,5}$ = 9.5, 1 H, H-4); 3.70 (dd, $^3J_{6,5}$ = 3.0, $^3J_{6,6'}$ = 12.0, 1 H, H-6); 3.53 (dd, $^3J_{6',5}$ = 2.0, $^3J_{6,6'}$ = 12.0, 1 H, H-6'); 3.12 (dd, $^3J_{1,2}$ = 3.5, $^3J_{3,2}$ = 10.51 H, H-2).

Ethyl-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-1-thio-2-phtalimido-β-D-glucopyranoside (5). A solution of 1 g (1.6 mmol) of **8a** and 573 mg (1.07 mmol) of **9** in 8 ml of abs. DCM was stirred during 1 h at room temperature under Ar with 800 mg of molecular sieves 4Å. Then suspension was chilled till –45°C (bath MeCN/ CO_2 solid), and 50 μl of solution of $BF_3{\cdot}Et_2O$ (0.08 mmol) in abs DCM was added drop vise. After 2.5 h of stirring at –45°C the second portion of 50 μl of solution of $BF_3{\cdot}Et_2O$ (0.08 mmol) in abs DCM was added. After additional 2.5 h stirring the chilling bath was removed, and when reaction mixture reached a room temperature, 0.50 ml of Et_3N were added. The reaction mixture was filtered and concentrated in vacuum. Column chromatography of the residue in gradient Ph → 25% of EtOAc in PhH gave rise to 566 mg (52%) of **5**. R_f 0.61 (hexane – EtOAc 3:2 v/v + 1 drop of Et_3N). $[\alpha]_D^{20}$ = + 9.5° (c 1.3, $CHCl_3$). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm., *J*/Hz): 1.25 (t, ^3J = 7.4, 3 H, $CH_2\underline{CH_3}$); 2.72 (q, ^3J = 7.4, 2H, $\underline{CH_2}CH_3$); 5.28 (d, $^3J_{1,2}$ = 9.8, 1 H, H-1'). ^{13}C-NMR spectrum ($CDCl_3$, δ, ppm): 14.9 ($CH_2\underline{C}H_3$); 23.8 ($\underline{C}H_2CH_3$); 54.8 (C-2); 67.0 (C-2'); 68.3 (C-6, C-6'); 79.2 (C-3'); 81.0 (C-3); 83.2 (C-1); 101.02 (C-1'); 167.5, 167.9 ($\underline{C}$=O).

Ethyl-3,6-di-O-benzyl-2-deoxy-1-thio-2-phtalimido-4-O-chloroacetyl-β-D-glucopyranoside (9a). A solution of 400 mg (2.34 mmol) of $(ClCH_2CO)_2O$ in 670 μl of DCM was added drop vise with magnetic stirring to a chilled at 0°C solution of 500 mg (0.937 mmol) of **9** in 5 ml of Py, and stirring was continued for additional 30 min at 0°C. 50 ml of chloroform were added to reaction mixture, and the organic solution was washed with 25 ml of 10% AcOH solution, 25 ml of saturated

$NaHCO_3$ solution and water. Organic layer was separated, dried by filtration through layer of celyte/ Na_2SO_4, concentrated in vacuum . Column chromatography of the residue in gradient Ph → 10% of EtOAc in PhH followed by crystallization from Et_2O – petroleum ether gave rise to 566 mg (52%) of **9a**. R_f 0.65 (PhH – EtOAc 19:1 v/v). $[\alpha]_D^{20}$ = + 49° (c 1, $CHCl_3$).

Benzyl-(3,6-di-O-benzyl-2-deoxy-2-phtalimido-4-O-chloroacetyl-β-D-glucopyranosyl)-(1→4)-3-O-benzyl-2-deoxy-6-O-(4-methoxyphenyl)-2-phtalimido-β-D-glucopyranoside (13). A solution of 400 mg (0.672 mmol) of **10** and 533 mg (0.874 mmol) of **9a** in 2 ml of abs. DCM was stirred during 1 h at room temperature under Ar with 200 mg of molecular sieves 4Å. Then suspension was chilled till –25°C (bath MeCN/ CO_2 solid), and 227 mg (1.0 mmol) of NIS dried in vacuum was added together with 0.2 ml of solution, prepared from 0.1 ml TESOTf in 0.9 ml of DCM (0.088 mmol of TESOTf). Mixture was stirred for 15 min at –25°C, then was attained till 0°C, and 1 ml of water solution containing both 10% of $NaHCO_3$ and 10% of $Na_2S_2O_3$. Reaction mixture was filtered through the celite layer, the latter was washed by 50 ml of $CHCl_3$. Combined organics were washed 3x50 ml of water, organic layer was separated, dried by passing through layer celite/ Na_2SO_4, concentrated in vacuum. . Column chromatography of the residue in gradient petroleum ether-10% of EtOAc → 35% of EtOAc in petroleum ether gave rise to 660 mg (86%) of **13**. R_f 0.68 ($CHCl_3$ – Me_2CO 19:1 v/v). $[\alpha]_D^{20}$ = + 31.5° (c 2, $CHCl_3$). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 5.29 (d, $^3J_{1',2'}$ = 8.1, 1 H, H-1'); 5.20 (dd, $^3J_{4',3}$ = $^3J_{4',5}$ = 9.1, 1 H, H-4'); 5.00 (1 H, H-1); 3.82 (s, 3 H, $C_6H_4OC\underline{H}_3$); 3.69 (s, 2 H, $ClC\underline{H}_2$).^{13}C-NMR spectrum ($CDCl_3$, δ, ppm.): 97.2 (C-1′); 96.8 (C-1); 69.4 (C-6'); 66.9 (C-6); 56.1 (C-2); 55.6 ($C_6H_4O\underline{C}H_3$); 55.5 (C-2'); 40.4 ($Cl\underline{C}H_2$).

Methyl-(3,6-di-O-benzyl-2-deoxy-2-phtalimido-4-O-chloroacetyl-β-D-glucopyranosyl)-(1→4)-3-O-benzyl-2-deoxy-6-O-(4-methoxyphenyl)-2-phtalimido-β-D-glucopyranoside (14). Analogous to the synthetic procedure for **13**, from 100 mg (0.193 mmol) of acceptor **11** the protected oligosacharide **14** was obtained in 196 mg (95%). R_f 0.50 ($CHCl_3$ – Me_2CO 19:1 v/v). $[\alpha]_D^{20}$ = + 37.6° (c 1, $CHCl_3$). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 5.30 (d, $^3J_{1,2}$ = 8.0, 1 H, H-1); 5.21 (dd, $^3J_{4',3}$ = $^3J_{4',5}$ = 9.1, 1 H, H-4'); 4.95 (d, $^3J_{1',2'}$ = 7.7, 1 H, H-1'); 3.81 (s, 3 H, $C_6H_4OC\underline{H}_3$); 3.70 (s, 2 H, $ClC\underline{H}_2$); 3.28 (s, 3 H, $OC\underline{H}_3$). ^{13}C-NMR spectrum ($CDCl_3$, δ, ppm): 98.8 (C-1); 97.3 (C-1'); 69.5 (C-6'); 66.9 (C-6); 56.4 ($O\underline{C}H_3$); 56.2 (C-2'); 55.6 (C_6H_4 $O\underline{C}H_3$); 55.3 (C-2); 40.5 ($Cl\underline{C}H_2$).

Benzyl-(3,6-di-O-benzyl-2-deoxy-2-phtalimido-4-O-chloroacetyl-β-D-glucopyranosyl)-(1→4)-3-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (15). 1.07 g (1.94 mmol) of $(NH_4)_2Ce(NO_2)_6$ were added portion wise to a stirred solution of 533 mg (0.484 mmol) of **13** in a mixture of 7.2 ml of CH_3CN and 0.8 ml of water at 0°C. The stirring was continued at 0°C 15 min.

Reaction mixture was diluted with 50 ml of $CHCl_3$ and 25 ml of concentrated saline was added. Mixture was washed with 2x50 ml of water solution containing 10% of $NaHCO_3$ and 10% Na_2SO_3, finally with 2x50 ml of water. Organic layer was separated, dried by passing through layer celite/ Na_2SO_4, concentrated in vacuum. Column chromatography of the residue in gradient PhH - 10% of EtOAc → PhH - 35% of EtOAc in PhH gave rise to 439 mg (87%) of **15**. R_f 0.06 ($CHCl_3$ – Me_2CO 19:1 v/v) $[\alpha]_D^{20}$ = +10.8° (c 2, $CHCl_3$). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 5.41 (d, $^3J_{1',2'}$ = 8.3, 1 H, H-1); 5.25 (dd, $^3J_{4',3} = {}^3J_{4',5}$ = 9.3, 1 H, H-4'); 5.03 (d, $^3J_{1,2}$ = 7.8, 1 H, H-1'); 3.73 (s, 2 H, ClC$\underline{H}_2$);. ^{13}C-NMR spectrum ($CDCl_3$, δ, ppm): 97.2 (C-1′); 97.0 (C-1); 69.3 (C-6'); 60.5 (C-6); 55.9 (C-2); 55.4 (C-2'); 40.3 (Cl$\underline{C}H_2$).

Methyl-(3,6-di-O-benzyl-2-deoxy-2-phtalimido-4-O-chloroacetyl-β-D-glucopyranosyl)-(1→4)-3-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (16). Analogous to the synthetic procedure for **15**, from 107 mg (0.193 mmol) of disaccharide **14** the protected oligosacharide **16** was obtained in 90 mg (93%). R_f 0.09 ($CHCl_3$ – Me_2CO 19:1 v/v). $[\alpha]_D^{20}$ = + 15.4° (c 1, $CHCl_3$). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 5.41 (д, $^3J_{1,2}$ = 8.3, 1 H, H-1); 5.25 (дд, $^3J_{4',3} = {}^3J_{4',5}$ = 9.2, 1 H, H-4'); 4.93 (д, $^3J_{1',2'}$ = 8.3, 1 H, H-1'); 3.72 (c, 2 H, ClC$\underline{H}_2$); 3.28 (c, 3 H, OC$\underline{H}_3$). ^{13}C-NMR spectrum ($CDCl_3$, δ, ppm): 99.0 (C-1); 97.5 (C-1'); 69.5 (C-6'); 60.7 (C-6); 56.6 (O$\underline{C}H_3$); 56.1 (C-2'); 55.5 (C-2); 40.5 (Cl$\underline{C}H_2$).

Benzyl-(3,6-di-O-benzyl-2-deoxy-2-phtalimido-4-O-chloroacetyl-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl – (1→6)]-3-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (17). A solution of 419 mg (0.404 mmol) of **15** and 202 mg (0.525 mmol) of **12** in a mixture of 5 ml of abs. DCM and 1 ml of DMFA was stirred during 1 h at room temperature under Ar with 200 mg of molecular sieves 4Å. Then 180 mg (0.81 mmol) of $CuBr_2$ and 260 mg (0.81 mmol) of Bu_4NBr were added. After 72 h of stirring reaction mixture was filtered through the layer of celite, the filter was washed by 50 ml of $CHCl_3$, combined filtrates were washed with 50 ml of 10% water solution of $Na_2S_2O_3$ and with 3x50 ml of water. Organic layer was separated, dried by passing through layer celyte/ Na_2SO_4, concentrated in vacuum. Column chromatography of the residue in gradient petroleum ether - 30% of EtOAc → petroleum ether - 60% of EtOAc gave rise to 385 mg (71%) of **17**. R_f 0.53 (PhH – EtOAc 4:1 v/v). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 5.60 (d, $^3J_{1,2}$ = 8.3, 1 H, H-1); 5.35 (m, 2 H, H-4 Fuc; H-4'); 5.10 (d, $^3J_{1',2'}$ = 3.0, 1 H, H-1 Fuc); 5.00 (d, $^3J_{1',2'}$ = 8.3, 1 H, H-1');. ^{13}C-NMR spectrum ($CDCl_3$, δ, м.д.): 96.7, 96.5, 96.3 (C-1, C-1', C-1 Fuc).

Methyl-(3,6-di-O-benzyl-2-deoxy-2-phtalimido-4-O-chloroacetyl-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl – (1→6)]-3-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (18). Analogous to the synthetic procedure for **17**, from oligosaccharide **14** the compound **18** was obtained in 155 mg (77%). R_f 0.32 (PhH – EtOAc 4:1 v/v). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 5.53 (d, $^3J_{1,2}$ = 8.3, 1 H, H-1); 5.39 (m, 2 H, H-4 Fuc; H-4'); 5.09 (d, $^3J_{1',2'}$ = 3.0, 1 H, H-1 Fuc); 4.88 (d, $^3J_{1',2'}$ = 8.3, 1 H, H-1');. ^{13}C-NMR spectrum ($CDCl_3$, δ, м.д.): 98.6, 96.4, 96.0 (C-1, C-1', C-1 Fuc).

Benzyl-(3,6-di-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl – (1→6)]-3-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (6). A solution of 278 mg (0.484 mmol) of trisaccharide **17** in a mixture of 4 ml of Py and 1 ml of water was kept at 80°C during 16 h. Reaction mixture was evaporated in vacuum, co-evaporated with EtOH and PhMe. Column chromatography of the residue in gradient PhH - 20% of EtOAc → PhH - 50% of EtOAc gave rise to 255 mg (98%) of **6**. R_f 0.16 (PhH – EtOAc 4:1 v/v). $[\alpha]_D^{20}$ = – 64.4° (c 2, $CHCl_3$). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 5.53 (d, $^3J_{1,2}$ = 8.1, 1 H, H-1); 5.39 (br.d, $^3J_{4,5}$ = 3.5, 1 H, H-4 Fuc); 5.02 (d, $^3J_{1',2'}$ = 7.7, 1 H, H-1′,); 5.09 (d, $^3J_{1,2}$ = 3.5, 1 H, H-1 Fuc); 4.35 (dd, $^3J_{3,2}$ = 9.5, $^3J_{3,4}$ = 10.5, 1 H, H-3); 4.25 (t, 1 H, H-4'); 4.22 (t, $^3J_{2,3}$ = $^3J_{2,1}$ = 9.5, 1 H, H-2); 4.18 (1 H, H-2'); 4.12 (1 H, H-3'); 3.95 (m, 1 H, H-5 Fuc); 3.90 (2 H, H-3 Fuc, H-4); 3.8 (s, 3 H, OC$\underline{H}_3$); 3.76 (1 H, H-6'); 3.7 (c, 3 H, $C_6H_4OC\underline{H}_3$); 3.7 (1 H, H-5); 3.54 (dd, $^3J_{1,2}$ = 3.5, $^3J_{2,3}$ = 8.0, 1 H, H-2 Fuc); 3.4 (1 H, H-6); 2.2 (s, 3 H, C$\underline{H}_3$CO); 1.1 (d, $^3J_{5,6}$ = 6, 1 H, H-6 Fuc). ^{13}C-NMR spectrum ($CDCl_3$, δ, м.д.): 96.8 (C-1'); 96.6 (C-1); 96.3 (C-1 Fuc); 78.5 (C-3); 77.7 (C-2 Fuc); 75.9 (C-3'); 75.8 (C-3 Fuc); 75.7 (C-4); 75.2 (C-4'); 73.5 (C-5'); 72.7 (C-5 Fuc); 71.3 (C-5); 71.1 (C-4 Fuc); 64.2 (C-6'); 59.5 ($C_6H_4OC\underline{H}_3$); 56.0 (C-2); 55.8 (C-2'); 55.0 (OC$\underline{H}_3$); 21.0 (C$\underline{H}_3$CO); 15.9 (C6 Fuc).

Methyl-(3,6-di-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl – (1→6)]-3-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (7). Analogous to the synthetic procedure for **6**, from trisaccharide **18** the compound **7** was obtained in 99 mg (95%). R_f 0.16 (PhH – EtOAc 4:1 v/v). $[\alpha]_D^{20}$ = – 47.3° (c 1.9, $CHCl_3$). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 5.53 (d, $^3J_{1,2}$ = 8.1, 1 H, H-1); 5.39 (bd, $^3J_{4,5}$ = 3.5, 1 H, H-4 Fuc); 5.09 (d, $^3J_{1,2}$ = 3.5, 1 H, H-1, Fuc); 4.9 (d, $^3J_{1',2'}$ = 7.7, 1 H, H-1′,);3.8 (s, 3 H, OC$\underline{H}_3$); 3.65 (s, 3 H, $C_6H_4OC\underline{H}_3$); 3.25 (s, 3 H, OCH_3); 2.2 (s, 3 H, C$\underline{H}_3$CO); 1.1 (d, $^3J_{5,6}$ = 6, 1 H, H-6 Fuc). ^{13}C-NMR spectrum ($CDCl_3$, δ, ppm): 98.6 (C-1); 96.4 (C-1'); 96.0 (C-1 Fuc); 64.2 (C-6'); 59.2 ($C_6H_4OC\underline{H}_3$); 55.9 (C-2, OC$\underline{H}_3$); 55.5 (C-2'); 55.1 (OC$\underline{H}_3$); 21.0 (C$\underline{H}_3$CO); 15.9 (C6 Fuc).

Benzyl-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl)- (1→4)- (3,6-di-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl – (1→6)]-3-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (3). A solution of 76 mg (0.059 mmol) of acceptor **6** and 77 mg (0.077 mmol) of donor **5** in 2 ml of abs. DCM was stirred during 1 h at room temperature under Ar with 200 mg of molecular sieves 4Å. Then suspension was chilled till –25°C (bath MeCN/ CO_2 solid), and 20 mg (0.089 mmol) of NIS dried in vacuum was added together with 50 μl of solution, prepared from 1.3 μl TESOTf (0.019 mmol) in 48.7 μl of DCM. Mixture was stirred for 15 min at –25°C, then was attained till 0°C, and 1 ml of water solution containing both 10% of $NaHCO_3$ and 10% of $Na_2S_2O_3$. Reaction mixture was filtered through the celite layer, the latter was washed by 50 ml of $CHCl_3$. Combined organics were washed 3x50 ml of water, organic layer was separated, dried by passing through layer celite/ Na_2SO_4, concentrated in vacuum. . Column chromatography of the residue in gradient 5% of EtOAc in PhH → 20% of EtOAc in PhH gave rise to 109 mg (83%) of **3**. R_f 0.45 (PhH – EtOAc 4:1 v/v). $[\alpha]_D^{20} = -34.7°$ (c 1, $CHCl_3$). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 5.33 (d, $^3J_{1,2}$ = 8.7, 1 H, H-1 C); 5.26 (d, $^3J_{4,3}$ = 3.7, 1 H, H-4 E); 5.23 (m 1 H, H-1 B); 4.93 (d, $^3J_{1,2}$ = 8.4, 1 H, H-1 A); 4.89 (d, $^3J_{1,2}$ = 3.5, 1 H, H-1 E); 4.42 (1 H, H-1 D); 4.38 (1 H, H-4 B); 4.35 (1 H, H-3 C); 4.21 (2 H, H-2 C, H-4 C); 4.19 (2 H, H-2 B, H-3 B); 4.12 (1 H, H-3 A); 4.08 (1 H, H-5 E); 4.04 (2 H, H-2 A, H-4 A); 3.85 (s, 3 H, OC$\underline{H}_3$); 3.81. (d, $^3J_{6',5}$ = 9.8, 1 H, H-6' C); 3.70 (2 H, H-6 C, H-3 E); 3.69 (1 H, H-6' B); 3.67 (m, 1 H, H-6 A); 3.47 (2 H, H-5 B, H-6 B); 3.38 (2 H, H-2 D, H-5 C); 3.36 (1 H, H-2 E); 3.26 (m, 1 H, H-6' A); 3.21 (d, $^3J_{5,6'}$ = 9.5, 1 H, H-5 A); 2.90 (s, 3 H, $C_6H_4OC\underline{H}_3$); 2.13 (s, 3 H, $C\underline{H}_3CO$); 1.01 (d, $^3J_{6,5}$ = 6.3, 3 H, H-6 E). ^{13}C-NMR spectrum ($CDCl_3$, δ, ppm): 100.5 (C-1 D); 96.3 (C-1 C, C-1 A); 95.9 (C-1 B, C-1 E); 83.0 (C-2 E); 75.6 (C-3 E); 70.9 (C-4 E); 68.0 (C-6 D); 67.2 (C-6 B); 67.0 (C-6 C); 64.0 (C-5 E); 63.7 (C-6 A); 15.3 (C-6 E).

Methyl-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl)- (1→4)- (3,6-di-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl – (1→6)]-3-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (4). Analogous to the synthetic procedure for **3**, from 185 mg (0.155 mmol)of acceptor **7** the compound **4** was obtained in 275 mg (83%). R_f 0.36 (PhH – EtOAc 4:1 v/v). $[\alpha]_D^{20} = -24.1°$ (c 1, $CHCl_3$). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 5.42 (d, $^3J_{1,2}$ = 8.7, 1 H, H-1 C); 5.35 (d, $^3J_{4,3}$ = 3.7, 1 H, H-4 E); 5.30 (m, 1 H, H-1 B); 4.93 (d, $^3J_{1,2}$ = 8.4, 1 H, H-1 A); 4.97 (d, $^3J_{1,2}$ = 3.5, 1 H, H-1 E); 4.52 (1 H, H-1 D); 4.45 (1 H, H-4 B); 4.44 (1 H, H-3 C); 4.30 (2 H, H-2 C, H-4 C); 4.28 (2 H, H-2 B, H-3 B); 4.19 (1 H, H-3 A); 4.10

(2 H, H-2 A, H-4 A); 3.95 (s, 3 H, OC$\underline{H}_3$); 3.89 (d, $^3J_{6',5}$ = 9.8, 1 H, H-6' C); 3.78 (1 H, H-6' B); 3.77 (2 H, H-6 C, H-3 E); 3.75 (1 H, H-6' D); 3.73 (m, 1 H, H-6 A); 3.68 (1 H, H-6 D); 3.55 (2 H, H-5 B, H-6 B); 3.46 (2 H, H-2 D, H-5 C); 3.45 (1 H, H-2 E); 3.30 (m, 3 H, H-5 A, H-5 D, H-6' A); 3.29 (s, 3H, OC$\underline{H}_3$); 3.00 (s, 3 H, C_6H_4OC$\underline{H}_3$); 2.22 (s, 3 H, C$\underline{H}_3$CO); 1.10 (d, $^3J_{6,5}$ = 6.3, 3 H, H-6 E).

Benzyl-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3,6-di-O-benzyl-2-deoxy -β-D-glucopyranosyl)- (1→4)- (2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl – (1→6)]- 2-acetamido-3-O-benzyl-2-deoxy-β-D-glucopyranoside (19). 2 ml of $N_2H_4.H_2O$ were added to a hot (100°C) solution of 153 mg (0.069 mmol) of pentasaccharide **3**. Reaction mixture was refluxed in Ar atmosphere during 8 h followed by concentration in vacuum. The residue was dissolved in a mixture of both 4 ml of Py and 4 ml of Ac_2O, 10 mg of DMAP were added and a solution was kept 40 h at room temperature. Then 5 ml of MeOH were added to a solution, mixture was concentrated in vacuum, co distilled in vacuum with PhMe, and was dried in vacuum. Column chromatography of the residue in gradient 10% of Me_2CO in PhH → 40% of Me_2CO in PhH gave rise to 120 mg (89%) of **19**. R_f 0.56 (PhH – Me_2CO 3:2 v/v). $[\alpha]_D^{20}$ = – 53.2° (c 1.9, $CHCl_3$). ^{1}H-NMR spectrum ($CDCl_3$, δ, м.д., *J*/Гц): 2.20 (s, 3 H, C$\underline{H}_3$CONH); 2.04 (s, 3 H, C$\underline{H}_3$CONH); 2.0 (s, 3 H, C$\underline{H}_3$CONH). ^{13}C-NMR spectrum ($CDCl_3$, δ, м.д.): 23.0, 21.1. (C$\underline{H}_3$CONH).

Methyl-(2-azido-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3,6-di-O-benzyl-2-deoxy -β-D-glucopyranosyl)- (1→4)- (2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl – (1→6)]- 2-acetamido-3-O-benzyl-2-deoxy-β-D-glucopyranoside (20). Analogous to the synthetic procedure for **19**, from 599 mg (0.280 mmol)of pentasaccharide **4** the compound **20** was obtained in 456 mg (87%). R_f 0.29 (PhH – Me_2CO 3:2 v/v). $[\alpha]_D^{20}$ = – 45.3° (c 1.9, $CHCl_3$). ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 2.08 (s, 3 H, C$\underline{H}_3$CONH); 2.05 (s, 3 H, C$\underline{H}_3$CONH); 1.82 (s, 3 H, C$\underline{H}_3$CONH). ^{13}C-NMR spectrum ($CDCl_3$, δ, ppm): 26.4, 23,3, 20.8 (C$\underline{H}_3$CONH).

Benzyl-(2-amino-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3,6-di-O-benzyl-2-deoxy -β-D-glucopyranosyl)- (1→4)- (2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl – (1→6)]- 2-acetamido-3-O-benzyl-2-deoxy-β-D-glucopyranoside (21). Solution of 90 mg (0.047 mmol) of pentasaccharide **19** in 10 ml of abs. THF was degassed from oxygen by triple vacuumation and refilling with Ar, then 1 ml of degassed water was added, solution was chilled to 0°C (water – ice bath) and 4.1 ml of 0.04M SmI_2 solution in abs. THF was added drop vise under

magnetic stirring till the steady green color was present during 30 sec. Reaction mixture was concentrated in vacuum, residue was dissolved in 30 ml of EtOAc and organic solution was washed by 20 ml of water solution containing both 10% of $NaHCO_3$ and 10% of $Na_2S_2O_3$. Separated water layer was extracted with 2x30 ml of EtOAc. Combined organics dried by passing through layer celyte/ Na_2SO_4, concentrated in vacuum. 88 mg of **21** were obtained. R_f 0.47 (PhH – Me_2CO 3:2 v/v).

Methyl-(2-amino-3,4,6-tri-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido-3,6-di-O-benzyl-2-deoxy -β-D-glucopyranosyl)- (1→4)- (2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-3-O-(4-methoxybenzyl)-α-L-fucopyranosyl – (1→6)]- 2-acetamido-3-O-benzyl-2-deoxy-β-D-glucopyranoside (22). Analogous to the synthetic procedure for **21**, from 91 mg (0.049 mmol)of pentasaccharide **20** the compound **22** was obtained in 88 mg. R_f 0.20 (PhH – Me_2CO 3:2 v/v).

(2-amino-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido -β-D-glucopyranosyl)- (1→4)- (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-α-L-fucopyranosyl – (1→6)]- 2-acetamido-2-deoxy-D-glucopyranose (23). Solution of 88 mg (0.045 mmol) of pentasaccharide derivative **21** in a mixture of 10 ml EtOH, 1 ml AcOH and 55 μl HCOOH was placed into instrument for hydrogenolysis , 150 mg of 10% $Pd(OH)_2$ were added and instrument was filled with hydrogen. Hydrogenolysis was carried out at 36^{o}C. After 2.5 h additional potion (150 mg) of catalyst was added and hydrogenolysis was continued for additional 2.5 h. Catalyst was separated by centrifugation, washed 2x10 ml of EtOH. Combined supernatants were concentrated in vacuum, residue was dried in vacuum, dissolved in 2 ml of 0.1N AcOH and was purified by gel-filtration on Sephadex G-25 column (60 x 1.6 cm), eluent 0.1 AcOH, 1 ml/min. Fractions containing pentasacchride were concentrated in vacuum giving 40 mg of **23**. R_f 0.20 (MeOH – $CHCl_3$ – H_2O 9:9:2 v/v + 1 drop of Et_3N). Mass-spectrum (negative ions): *m/z* 989.39 [M – 1]; calculated for $C_{39}H_{66}N_4O_{25}$: 990.40

Methyl-(2-amino-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido -β-D-glucopyranosyl)-(1→4)- (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-α-L-fucopyranosyl – (1→6)]- 2-acetamido-2-deoxy-D-glucopyranose (24). Analogous to the synthetic procedure for **23**, from 88 mg (0.048 mmol) of pentasaccharide **22** the compound **24** was obtained in 88 mg (80%). R_f 0.20 (MeOH – $CHCl_3$ – H_2O 9:9:2 v/v + 1 drop of Et_3N). $[\alpha]_D^{20}$ = – 68.7° (c 1, MeOH). ^{1}H-NMR spectrum (D_2O, δ, ppm, *J*/Hz): 5.23 (d, $^3J_{4,3}$ = 3.2, 1 H, H-4 E); 5.17 (d, $^3J_{1,2}$ = 3.4, 1 H, H-1 E); 4.86 (d, $^3J_{1,2}$ = 8.3, 1 H, H-1 D); 4.62 (d, $^3J_{1,2}$ = 8.1, 1 H, H-1 C); 4.60 (d, $^3J_{1,2}$ = 7.8, 1 H, H-1 B); 4.43 (d, $^3J_{1,2}$ = 7.9, 1 H, H-1 A); 4.26 (q, $^3J_{5,6}$ = 6.3, 1 H, H-5 E); 4.13

(dd, $^3J_{3,2}$ = 10.5, $^3J_{3,4}$ = 3.2, 1 H, H-3 E); 3.93 (3 H, H-6 A, H-6 B, H-6 D); 3.89 (1 H, H-4 C); 3.87 (1 H, H-6 C); 3.79 (1 H, H-2 B); 3.78 (3 H, H-6' A, H-6' B, H-6' D); 3.77 (1 H, H-3 A); 3.75 (1 H, H-2 C); 3.71 (1 H, H-3 C); 3.70 (1 H, H-2 A); 3.69 (2 H, H-3 B, H-4 B); 3.68 (2 H, H-5 B, H-3 D); 3.67 (1 H, H-6' C); 3.65 (1 H, H-4 A); 3.64 (1 H, H-5 A); 3.58 (dd, $^3J_{2,3}$ = 10.5, $^3J_{2,1}$ = 3.4, 1 H, H-2 E); 3.54 (1 H, H-5 C); 3.53 (2 H, H-4 D, H-5 D); 3.52 (s, 3 H, OC<u>H</u>$_3$ E); 3.49 (s, 3 H, OC<u>H</u>$_3$ A); 3.13 (t, $^3J_{2,1}$ = $^3J_{2,3}$ = 8.1, 1 H, H-3 D); 2.22 (s, 3 H, C<u>H</u>$_3$CO E); 2.07 (s, 3 H, C<u>H</u>$_3$CO D); 2.00 (s, 3 H, C<u>H</u>$_3$CO C); 1.98 (s, 3 H, C<u>H</u>$_3$CO B), 1.13 (d, $^3J_{6,5}$ = 6.3, 3 H, H-6 E). ^{13}C-NMR spectrum ($CDCl_3$, δ, м.д.): 176.06, 175.95, 175.38 (CH_3<u>C</u>ONH A, B, C); 103.21 (C-1 A); 102.66 (C-1 B); 102.26 (C-1 C); 99.07 (C-1 D); 98.37 (C-1 E); 80.36 (C-4 A); 79.88 (C-4 B); 79.14 (C-2 E); 78.07 (C-4 C); 77.86 (C-5 D); 76.11 (C-5 C); 75.93 (C-4 E); 75.80 (C-5 B); 74.98 (C-5 A); 73.84 (C-3 A); 73.62 (C-3 B); 73.38 (C-3 C); 72.91 (C-3 D); 70.99 (C-4 D); 68.99 (C-3 E); 67.96 (C-6 A); 66.97 (C-5 E); 61.75 (C-6 B; C-6 D); 61.41 (C-6 C); 60.07 (O<u>C</u>H$_3$ E); 58.38 (O<u>C</u>H$_3$ A); 57.27 (C-2 D); 56.96 (C-2 B); 56.60 (C-2 C); 56.41 (C-2 A); 23.61 (<u>C</u>H$_3$CONH A, B, C); 21.65 (<u>C</u>H$_3$CO E); 16.56 (C-6 E). Mass-spectrum (negative ions): *m/z* 1003.41 [M – 1]; calculated for $C_{40}H_{68}N_4O_{25}$: 1004.42

2,5-dioxopyrrolidine-1-ester of vaccenic acid (26). Solution of 500 mg (1.77 mmol) vaccenic acid (**25**), 407 mg (3.45 mmol) NIS, 1.1 g (5.3 mmol) DCC and 22 mg (0.177 mmol) DMAP in 5 ml of DMFA was stored at room temperature for 24 h. The excess of DCC was decomposed by addition of 500 mg oxalic acid monohydrate in 20 ml of MeOH under stirring. Reaction mixture was diluted with 50 ml of $CHCl_3$, organic layer was washed 4x15 ml of water. Organic layer was separated, dried by passing through layer celite/ Na_2SO_4, concentrated in vacuum. Column chromatography of the residue in gradient 10% of EtOAc in petroleum ether → 30% of EtOAc in petroleum ether gave rise to 672 mg (100%) of **26**. R_f 0.33 (hexane – EtOAc 7:3 v/v). ЯМР ^{1}H-NMR spectrum ($CDCl_3$, δ, ppm, *J*/Hz): 5.34 (m, 2 H, C<u>H</u>=C<u>H</u>); 2.82 (s, 4H, C(O)C<u>H</u>$_2$C<u>H</u>$_2$CO); 2.59 (t, ^3J = 7.4, 2H, C<u>H</u>$_2$COO); 2.01 (m, 4H, C<u>H</u>$_2$CH=CHC<u>H</u>$_2$); 1.74 (m, 2H, C<u>H</u>$_2$CH$_2$COO); 1.28 (m, 20H, C<u>H</u>$_2$); 0.88 (t, ^3J = 6.7, 3H, C<u>H</u>$_3$). ^{13}C-NMR spectrum ($CDCl_3$, δ, м.д.): 169.1, 168.6 (<u>C</u>(O)CH$_2$CH$_2$<u>C</u>O); 129.9, 129.8 (<u>C</u>H=<u>C</u>H); 25.5 (C(O)<u>C</u>H$_2$<u>C</u>H$_2$CO); 14.1 (<u>C</u>H$_3$).

(2-[(11Z)-11-octadeceneamido]-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido -β-D-glucopyranosyl)- (1→4)- (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-α-L-fucopyranosyl – (1→6)]- 2-acetamido-2-deoxy-D-glucopyranose (1). One drop of Et_3N was added to a solution of 40 mg (0.04 mmol) of **23** and 46 mg (0.12 mmol) of **26** in 2 ml of DMSO. After mixing, the reaction mixture was stored at room temperature for 40 h protected from the day light. Solution was concentrated in vacuum at 45^{o}C, the liquid residue was dissolved in 20 ml of water, washed 2x20 ml of petroleum ether to remove non-polar admixtures. Water layer was

separated and concentrated in vacuum. Column chromatography of the residue in gradient 5% of water in a mixture CHCl3-MeOH 1:1 → 15% of water in a mixture CHCl3-MeOH 1:1 gave rise to 35 mg (70%) of **1**. R_f 0.63 (MeOH – $CHCl_3$ – H_2O 9:9:2 v/v). Mass-spectrum (negative ions): *m/z* 1253.66 [M – 1]; calculated for $C_{57}H_{98}N_4O_{26}$: 1254.65.

Methyl-(2-[(11Z)-11-octadeceneamido]-2-deoxy-β-D-glucopyranosyl)-(1→4)-(2-acetamido -β-D-glucopyranosyl)- (1→4)- (2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-[4-O-acetyl-2-O-methyl-α-L-fucopyranosyl – (1→6)]- 2-acetamido-2-deoxy-D-glucopyranose (2). Analogous to the synthetic procedure for **1**, from 29 mg (0.029 mmol) of pentasaccharide **24** the compound **2** was obtained in 26 mg (70%). R_f 0.65 (MeOH – $CHCl_3$ – H_2O 9:9:2 v/v). Mass-spectrum (negative ions): *m/z* 1267.67 [M – 1]; calculated for $C_{58}H_{100}N_4O_{26}$: 1268.66.