- # Supplementary Material (ESI) for Chemical Communications
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Biosynthesis of conjugatable saccharidic moieties of GM₂ and GM₃ gangliosides by engineered E coli

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General methods

Reactions were monitored by TLC using silica gel 60 F254 precoated plates (E. Merck, Darmstadt) with CH₃CN/H₂O 8:2 (v/v) or Butanol/AcOH/H₂O 2:1:1 (v/v) as eluting solvent and detection by charring with either sulfuric acid solution (H₂SO₄/MeOH/H₂O 3:45:45) or orcinol/sulphuric acid solution (1g in H₂SO₄/EtOH/H₂O 3:70:20). For flash chromatography, Merck silica gel 60 was used. Glycoprotein **11** was purified by Econo-Pac[®] 10 DG column from Bio-Rad. Bradford test was performed with Bio-Rad protein assay kit. NMR spectra were recorded on Bruker AC 300 and Bruker Avance 400 at 298 K. Proton chemical shifts are reported in ppm relative to external SiMe₄ (0 ppm). Low-resolution FAB mass spectra were recorded in the positive mode of an R1010C quadripolar mass spectrometer (model 2000, Nermag, Reuil-Malmaison, France). MALDI-TOF measurements were performed on a Bruker Daltonics Autoflex apparatus. ES experiments were performed on a Waters Micromass ZQ spectrometer. IR spectra were recorded on a Beckman DU 640 spectrophotometer.

Biosynthesis and purification of GM₃ and GM₂ saccharidic portions were performed as previously described in reference 5:

T. Antoine, B. Priem, A. Heyraud, L. Greffe, M. Gilbert, W. W. Wakarchuk, J. S. Lam, E. Samain, *Chembiochem*, 2003, 4, 406.

High cell density cultivations were carried out in 2 litre reactors containing one litre of mineral culture medium as previously described. Antibiotics were added in both preculture and culture at the following concentration: ampicillin (50 mg L⁻¹), tetracycline (15 mg L⁻¹), chloramphenicol (20 mg L⁻¹). The temperature was maintained at 34°C and the pH regulated

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to 6.8. The cultivation strategy included 3 phases: a first exponential growth phase which started at the inoculation of the fermentor and lasted until exhaustion of the carbon substrate (glycerol) initially added to the medium at a concentration of 17.5 g L⁻¹; then a 5 h fed-batch phase with a high substrate feeding rate of 4.5 g L⁻¹ h⁻¹; and finally a 10-20 h fed-batch phase with a lower feeding rate of 2.7 g L⁻¹ h⁻¹. Allyl or propargyl lactoside (**1 or 2**) (4-8 mM) and sialic acid (1equiv.) were added at the beginning of the first fed-batch phase at the same time as the inducer (IPTG 50 mg L⁻¹) of the β -galactoside permease and of the recombinant genes that were under the control of the Lac repressor.

The fermentation process was monitored by TLC with a Butanol/AcOH/H₂O 2:1:1 (v/v) mixture as eluting solvent (2 runs). Culture samples (1ml) were immediately centrifuged in microfuge tubes (5 min, 12 000 x g). The supernatant constitutes the extracellular fraction. The pellets were resuspended in 1 ml of distilled water, boiled for 30 min and centrifuged as above. The second supernatant constitutes the intracellular fraction.

Purification of oligosaccharides

At the end of the fermentation time course, the bacterial cells were recovered by centrifugation (20 min at 7 000 x g). The pellets were suspended in a volume of distilled water equal to that of the original culture medium and the cells were permeabilized by autoclaving at 100°C for 50 min. After another centrifugation (20 min at 7 000 x g) the cell debris were discarded and the supernatant was mixed with activated charcoal. After filtration and thorough washing with distilled water, the oligosaccharides were eluted with aqueous ethanol 50% (v/v). Oligosaccharides were fixed on a Dowex 1X4-400 (HCO₃⁻ form) resin, then eluted with a linear NaHCO₃ gradient (10 to 50 mM). The sodium bicarbonate was then eliminated by a treatment with Dowex 50X4-400 (H⁺form) resin.

Allyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylunic acid)-

(2**⇒**3)-(β-D-galactopyranosyl)-(1**⇒**4)-β-D-glucopyranoside (3)



FAB-MS : *m*/*z* 696 [M+H]⁺, 718 [M+Na]⁺

¹H NMR (D₂O, 400 MHz)



¹³C NMR (D₂O, 100 MHz) δ 176.3, 175.2, 134.6, 120.0, 103.9, 102.3, 101.1, 79.5, 76.8, 76.4,
76.0, 75.7, 74.2, 74.1, 73.1, 72.0, 70.7, 69.6, 69.4, 68.8, 63.9, 62.3, 61.4, 53.0, 40.9, 23.3

Allyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylunic acid)-

(2⇒3)-[2-acetamido-2-deoxy-β-D-galactopyranosyl-(1⇒4)]-(β-D-galactopyranosyl)-(1⇒4)-

β-D-glucopyranoside (4)



ES-MS (negative mode) : m/z 875 [M-H]⁻



¹³C NMR (D₂O, 100 MHz) δ 176.3, 176.1, 175.4, 134.6, 120.1, 104.0, 103.9, 102.9, 102.3,
79.9, 78.4, 76.0, 75.9, 75.7, 75.6, 75.3, 74.3, 74.0, 73.6, 72.5, 71.9, 71.3, 70.0, 69.3, 69.1,
64.1, 62.4, 61.8, 61.4, 53.6, 52.9, 38.2, 23.9, 23.3

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Propargyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylunic

acid)-(2**⇒**3)-(β-D-galactopyranosyl)-(1**⇒**4)-β-D-glucopyranoside (5)



FAB-MS : *m/z* 694 [M+H]⁺, 716 [M+Na]⁺



¹³C NMR (D₂O, 100 MHz) *δ* 176.3, 175.2, 103.9, 101.7, 101.1, 80.0, 79.4, 77.6, 76.8, 76.5, 76.2, 75.6, 74.2, 73.9, 73.1, 70.7, 69.6, 69.4, 68.8, 63.9, 62.3, 61.3, 57.9, 53.0, 40.9, 23.3

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Propargyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylunic

acid)-(2**⇒**3)-[2-acetamido-2-deoxy-β-D-galactopyranosyl-(1**⇒**4)]-(β-D-galactopyranosyl)-

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(1■4)-β-D-glucopyranoside (6)
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ES-MS (negative mode) : m/z 873 [M-H]⁻



¹³C NMR (D₂O, 100 MHz) δ 176.3, 176.1, 175.4, 104.0, 103.9, 102.9, 101.6, 79.7, 78.4, 76.1,
76.0, 75.63, 75.59, 75.3, 74.4, 73.8, 73.5, 72.5, 71.3, 70.0, 69.3, 69.0, 64.1, 62.4, 61.8, 61.3,
57.9, 53.6, 52.9, 38.2, 23.9, 23.3

Propyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylunic acid)-

(2**■**3)-(β-D-galactopyranosyl)-(1**■**4)-β-D-glucopyranoside (7)





¹³C NMR (D₂O, 100 MHz) δ 176.3, 175.2, 103.9, 103.3, 101.1, 79.6, 76.8, 76.5, 76.1, 75.7,
74.2, 74.1, 73.6, 73.1, 70.7, 69.6, 69.4, 68.8, 63.9, 62.3, 61.4, 53.0, 40.9, 23.6, 23.3, 10.9

Propyl (5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylunic acid)-

(2⇒3)-[2-acetamido-2-deoxy-β-D-galactopyranosyl-(1⇒4)]-(β-D-galactopyranosyl)-(1⇒4)-

β-D-glucopyranoside (8)





¹³C NMR (D₂O, 100 MHz) δ 176.3, 176.1, 175.4, 104.0, 103.9, 103.2, 102.9, 83.0, 79.9, 78.4, 76.0, 75.7, 75.6, 75.3, 74.3, 74.1, 73.6, 73.5, 72.6, 71.3, 70.0, 69.3, 69.0, 64.1, 62.4, 61.8, 61.4, 53.6, 52.9, 38.2, 23.9, 23.5, 23.3, 10.9

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Compound 11

A solution of **3** (32 mg, 0.046 mmol) in MeOH (5 mL) is stirred at -50°C under a stream of ozone (0.25 g/h, 0.4 bar O₂, 160 V) generated by an OZ1-L ozonator from Kaufmann Umwelttechnik until complete disappearance of the starting material by TLC (1 hour). While still at -50°C, the flask is flushed with argon and dimethyl sulfide (17 μ l, 5 equiv.) is added. The solution is stirred for one hour at -50°C, at room temperature for one hour and concentrated. The crude aldehyde **9** is obtained quantitatively and used directly for the reductive amination with BSA.

To a stirred solution of the crude aldehyde **9** (0.046 mmol) in distilled water (1 mL) are added a solution of BSA (10 mg in 400 μ L of water) and sodium cyanoborohydride (20 mg in 200 μ L of water). The mixture is stirred at 37°C on a rocking table for 2 days and the protein conjugate **11** is purified by filtration on a Econo-Pac[®] 10 DG column. The glycoprotein fractions were identified by Bradford protein assay and the average GM₃ content was determined by the phenol-sulfuric acid assay with 3'-sialyl-lactose as standard for the calibration curve.

4-(Pyrrol-1-yl)butanoic acid



A solution of 2,5-dimethoxytetrahydrofuran (4 mL, 31 mmol) and amino butyric acid (3.2 g, 1 equiv.) in acetic acid/water 1.5:1 v:v (30 mL) is stirred at 100°C for 45 min. The solution is cooled to room temperature, extracted with AcOEt twice, dried over anhydrous Na₂SO₄, concentrated and co-evaporated with toluene to remove acetic acid. After purification by flash chromatography with silica gel (AcOEt/ Petroleum ether 4:6), the pyrrolyl butanoic acid is isolated with 79 % yield (3.76 g).

¹H NMR (CDCl₃, 300 MHz) δ 6.63 (t, 2H, J = 2 Hz), 6.13 (t, 2H, J = 2 Hz), 3.94 (t, 2H, J = 7 Hz), 2.31 (t, 2H, J = 7 Hz), 2.09 (q, 2H, J = 7 Hz); ¹³C NMR (CDCl₃, 75 MHz) δ 150.8, 120.5, 108.3, 48.3, 30.6, 26.4

Pentafluorophenyl 4-(pyrrol-1-yl)butanoate



To a solution of pyrrolyl butanoic acid (1.56 g, 10.2 mmol) in dioxane (20 mL) is added pentafluorophenol (1.87 g, 1 equiv.) and dicyclohexylcarbodiimide (2.1 g, 1 equiv.). The mixture is stirred overnight under argon then filtered through a celite pad and concentrated. The resulting oil is taken up in hexane (70 mL) and placed in a freezer for 24h. After filtration and washing with cold hexane, pentafluorophenyl 4-(pyrrol-1-yl)butanoate is isolated with 79% yield (2.57 g) as golden needles.

¹H NMR (CDCl₃, 300 MHz) δ 6.65 (t, 2H, J = 2 Hz), 6.17 (t, 2H, J = 2 Hz), 4.01 (t, 2H, J = 7 Hz), 2.59 (t, 2H, J = 7 Hz), 2.20 (q, 2H, J = 7 Hz); ¹³C NMR (CDCl₃, 75 MHz) δ 168.8, 120.5, 108.6, 47.9, 29.9, 26.5

Reference 13: C. J. Pickett, K. S. Ryder, J. Chem. Soc., Dalton Trans., 1994, 14, 2181

Compound 12

A solution of **3** (30 mg, 0.043 mmol) and cysteamine hydrochloride (49 mg, 10 equiv.) in distilled water (1 mL) is degassed and flushed under argon. The mixture is irradiated at 254 nm under a UV lamp for 2 days and then freeze dried affording the crude amine **10**. To a stirred solution of the crude amine **10** in anhydrous dimethylformamide (2 mL) is added pentafluorophenyl 4-(pyrrol-1-yl)butanoate (225 mg, 16 equiv.) and triethylamine (90 μ L, 15

equiv.). The mixture is stirred overnight at room temperature and concentrated. After

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purification by flash chromatography with silica gel (CH₃CN/H₂O 9:1), **12** is isolated with 94% yield (37 mg).

The amino compound **10** can be purified by ion exchange chromatography on acidic Dowex 50WX4 resin and eluted with aqueous ammonia. The purified intermediate is then reacted with only 1.5 equiv. of activated pyrrole. The yield observed is slightly decreased.

MALDI-TOF : *m/z* 930 [M+Na]⁺





¹³C NMR (D₂O, 100 MHz) δ 177.1, 176.3, 175.2, 122.8, 108.9, 103.9, 103.4, 101.1, 79.5,
76.8, 76.4, 76.0, 75.6, 74.2, 74.1, 73.0, 70.6, 70.1, 69.6, 69.4, 68.7, 63.8, 62.3, 61.3, 53.0,
49.4, 40.9, 39.8, 34.0, 31.7, 30.1, 28.7, 28.2, 23.3