10.1071/CH19159_AC

Australian Journal of Chemistry 2019, 72(8), 637-643

Supplementary Material

n-Octyl (thio)glycosides as Potential Cryoprotectants: Glass Transition Behaviour, Membrane Permeability, and Ice Recrystallization Inhibition Studies

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1.0 Materials and Methods

General

Analytical thin layer chromatography (TLC) was performed on commercially prepared silica plates (ChemSupply Silica Gel 60 0.20 mm F254). Flash column chromatography was performed using 230–400 mesh Kieselgel 60 silica eluting with distilled solvents as described. Solvents and reagents were purchased from Sigma-Aldrich and ChemSupply and used without further purification. Solvents for anhydrous reactions were distilled and dried accordingly. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Ascend 500 NMR spectrometer at frequencies of 500 MHz and 125 MHz, respectively. Chemical shifts are reported as parts per million (ppm) downfield shift relative to the tetramethylsilane (TMS) internal standard ($CDCl_3$ only). The data are reported as chemical shift (δ), multiplicity, relative integral, coupling constant (J = Hz) and assignment where possible. IR spectra were recorded on a Perkin Elmer FT-IR (ATR) spectrometer. Optical rotation was measured on a Rudolph Research Analytical Autopol 1 automatic polarimeter (589 nm) using a 10 mL cell. ESI mass spectra were recorded on an Agilent 6120 single quadrupole mass spectrometer operating in positive mode unless otherwise stated.

Differential scanning calorimetry

DSC thermograms on warming were obtained with TA Instruments DSC 2920 Modulated differential scanning calorimeter using 5–10 mg of sample in hermetically sealed aluminum pans. Octyl (thio)glycosides were studied as neat samples and as 20% (w/v) aqueous solutions. For solutions, the samples were warmed from -120°C to 25°C at 5^{o}C/min with one-minute equilibration times at the end points. Neat surfactants samples (5–10 mg) were hermetically sealed in aluminum sample pans and cycled between 25 ^{o}C and -160 ^{o}C at a rate of 10 ^{o}C/min, with one-minute equilibration times at the endpoints. For all samples, measurements were made on several (at least 2) successive runs to confirm the consistency in thermal behaviour. T_g values were interpreted as the extrapolated mid-point from the inflection of the curve.

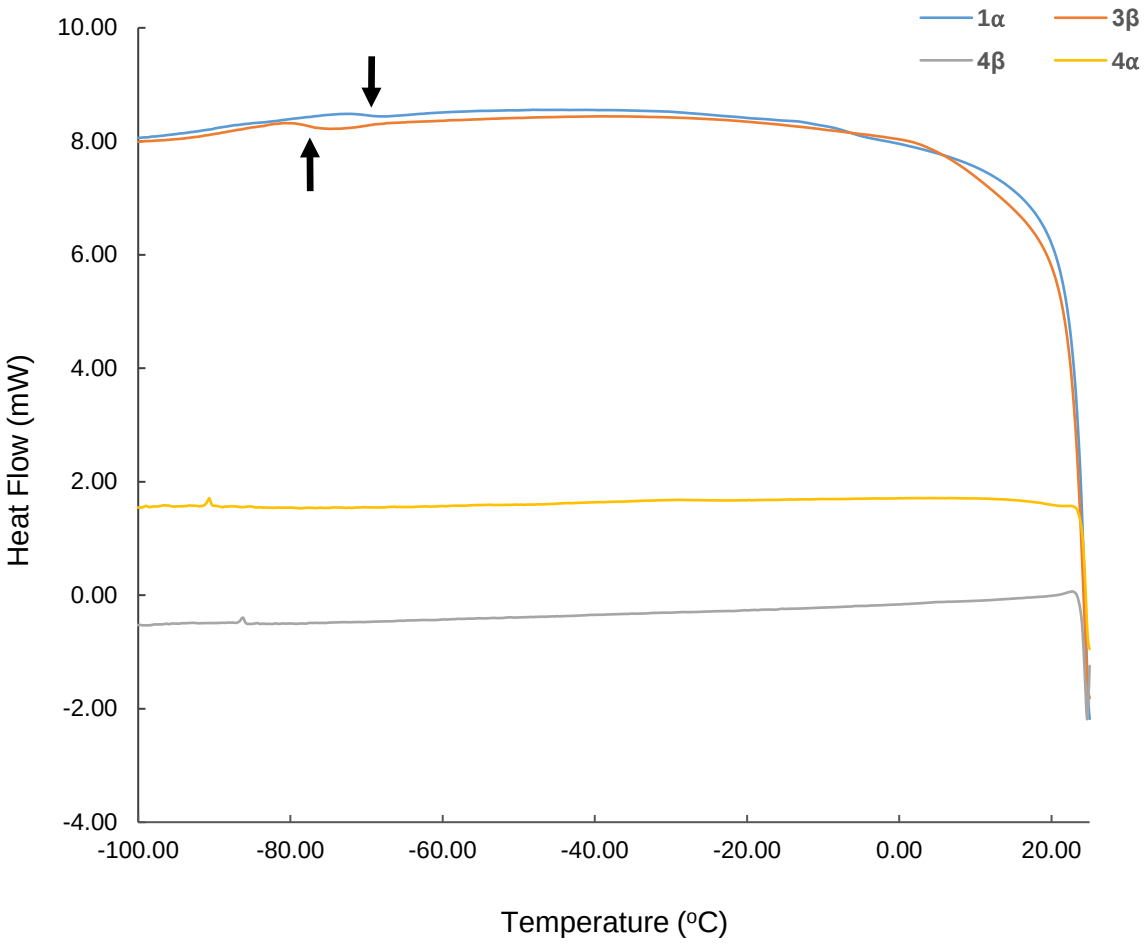


Figure S1. DSC warming thermograms for compounds neat surfactants **1α**, **3β**, **4α**, and **4β**. Only the regions of interest are shown. Scans have been vertically offset for clarity. Possible glass transitions are indicated by bold arrowheads.

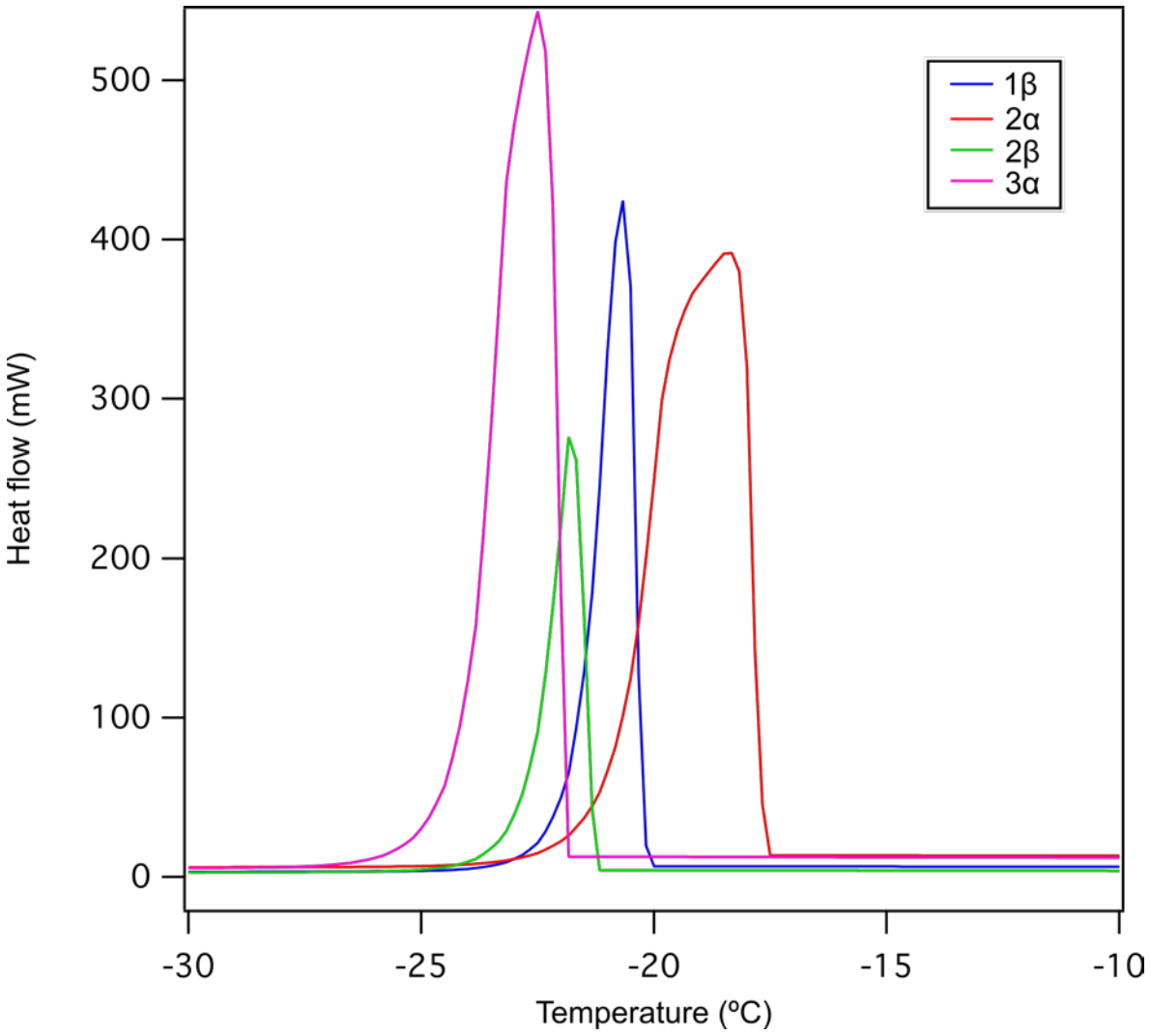


Figure S2. DSC cooling thermograms for soluble compounds (**1β**, **2α**, **2β**, and **3α)**, as 20% wt. aqueous solutions. Only the relevant temperature range is shown. The large endothermic peaks represent ice crystallisation.

Cell permeability studies

An aliquot of cell suspension (50–100 µL) was added to each well of a micro well plate. After the cells settled, the thioglycoside samples were added as small droplets slowly through the side walls of the wells avoiding any violent perturbation to the cell solution to ensure elimination of imaging ambiguities. Samples were prepared in RPMI1640 cell medium and used for cell perfusion with final concentrations 0.65M and 0.33M. For each sample and perfusion experiment, fresh cell suspensions were loaded to the micro wells from the incubator. The cell volume excursion history was recorded with live cell imaging using video microscopy. Video was recorded at 20 frames / second until osmotic equilibrium was obtained. All perfusion

images were captured for at least 5–10 minutes at room temperature (~22°C). An inverted microscope (Olympus IX71 inverted optical microscope) equipped with a CCD camera at 1024×1024 resolution and a CMOS sensor (Mikrotron MC1362) was utilized for visualization. Automated time-lapse image acquisition was used for periods of up to 15 min under the control of StreamPix software (NorPix, Montreal) to capture the cell volume excursion history.

The captured video was converted into image frames by exporting the full sequence at 1 frame/sec. Cells were cropped from each frame of the image. Images were extracted and processed using ImageJ (https://imagej.nih.gov/ij/). Several images were analyzed at regular time intervals to understand the volume response (shrinking and swelling) of the cells. The areas of the selected cells were manually measured using oval/free hand selections. Radii and normalized volumes were calculated assuming the cells to be spheres.

Ice recrystallization inhibition (IRI) analysis

The ice recrystallization inhibition (IRI) activities of the thioglycosides were measured using the splat cooling assay.1 A 10 µL aliquot of the compound dissolved in phosphate-buffered saline (PBS) was dropped from a height of 2 metres onto a polished aluminium block cooled to approximately -80 °C using dry ice. The resulting circular ice wafer (approximately 1 cm in diameter and 20 µm thick) was transferred to a cryostage which was maintained at -6.4 °C using a programmable Peltier unit (S3 Series 800 temperature controller, Alpha Omega Instruments). The ice wafer was annealed for 30 minutes at -6.4 °C. Using a digital camera (Nikon CoolPix 5000) fitted to a microscope, images of the ice wafer were captured between crossed polarizing filters. A novel domain recognition software was then used to measure the cross-sectional areas of randomly-selected ice crystals in each of the three images analyzed per sample. The resulting mean grain size (MGS) of ice crystals was then calculated. IRI activities of the compounds are reported as percent mean grain size (% MGS) and compared to the % MGS of the positive control, PBS. The error bars in Figure 5 represent the percent standard error of the mean (% SEM). Testing for each compound was performed in triplicate.

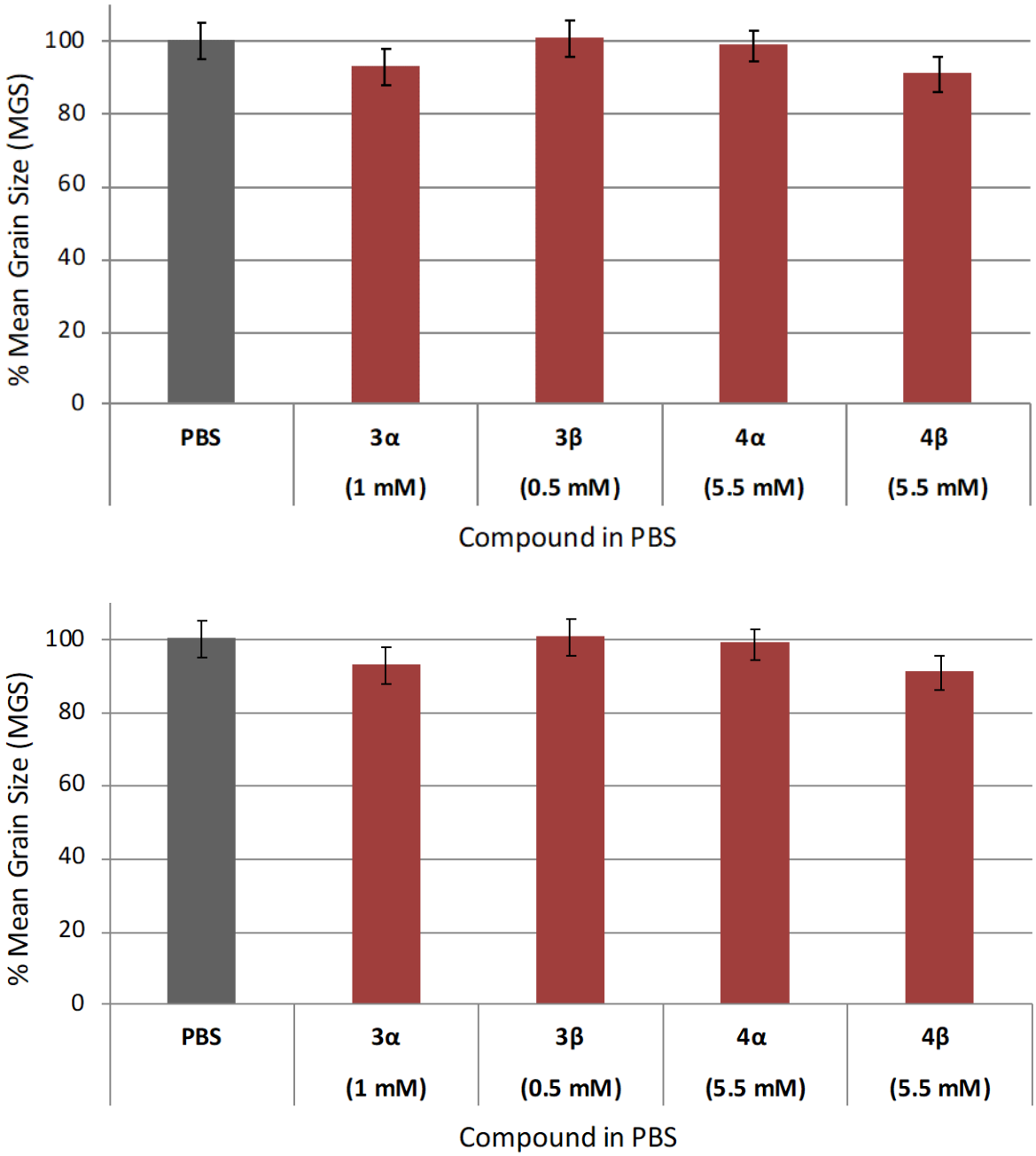
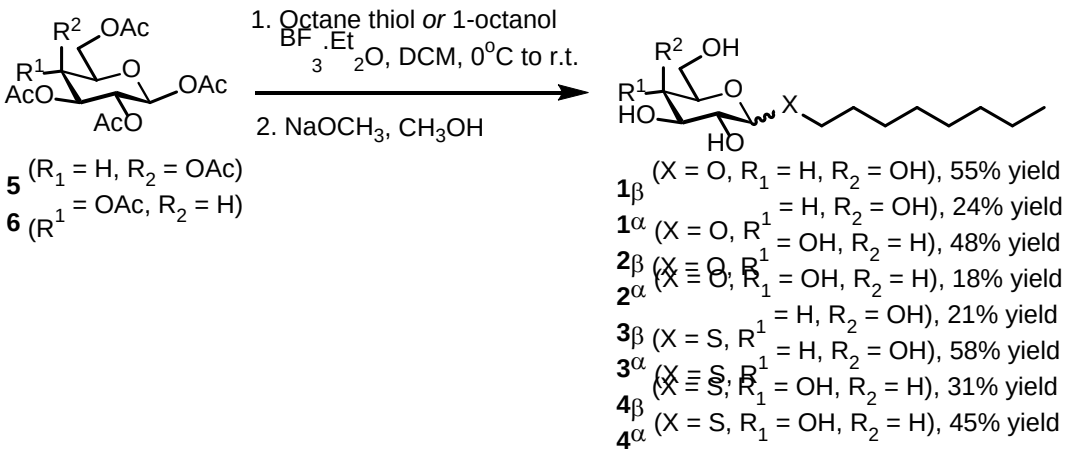


Figure S3. IRI activities of thioglycosides **3α/β** and **4α/β** at their upper solubility limits in PBS. Inhibitory activity is depicted as percent mean grain size (% MGS) with error bars representing percent standard error of the mean (% SEM).

2.0 Synthesis



Scheme S1. Synthesis of *n*-octyl (thio)glycosides **1–4.**

General method 1 – synthesis of *O*-glycosides.[2]

The synthesis was adapted from a procedure by Lindhorst and co-workers.[2] To a stirred solution of the sugar pentaacetate (2.0 g, 5.1 mmol) in dry CH_2Cl_2 (10 mL) under N_2 atmosphere was successively added 1-octanol (1.35 mL, 10.2 mmol, 2.1 equiv) and $BF.Et_2O$ (1.6 mL, 12.8 mmol, 2.5 equiv) drop-wise. The solution was then stirred at room temperature for 4 h, at which time TLC analysis (1:1 ethyl acetate/petroleum spirit) indicated consumption of starting material and formation of product. The reaction was carefully neutralized by the drop-wise addition of saturated aqueous $NaHCO_3$ solution (*ca*. 20 mL). CH_2Cl_2 (20 mL) was then added and the organic layer was separated and washed successively with saturated aqueous $NaHCO_3$ solution (2 x 20 mL) and brine (1 x 20 mL). The organic layer was dried with anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by flash silica chromatography (1:5 v/v ethyl acetate-petroluem spirit) to give the optically pure α- and β-D-glycosides (**1α/1β-OAc** and **2α/2β-OAc**).

General method 2 – synthesis of *S*-glycosides.[3]

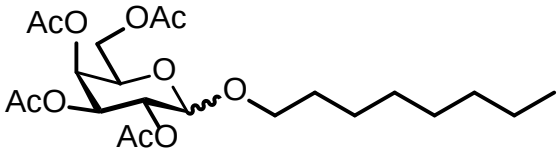
The synthesis was adapted from a procedure by Wynberg and co-workers.[3] To a stirred solution of the sugar pentaacetate (2.0 g, 5.1 mmol) in dry $CHCl_3$ (10 mL) under N_2 atmosphere was added 1-octanethiol (890 μL, 5.12 mmol, 1.0 equiv) followed by $BF.Et_2O$ (3.2 mL, 25.6 mmol, 5.0 equiv) drop-wise. The deep red solution was then stirred at room temperature for 2 h, at which time TLC analysis (1:1 ethyl acetate/petroleum spirit) indicated consumption of starting

material and formation of product. The reaction was carefully neutralized by the drop-wise addition of saturated aqueous $NaHCO_3$ solution (*ca*. 20 mL). $CHCl_3$ (20 mL) was then added and the organic layer was separated and washed successively with saturated aqueous $NaHCO_3$ solution (2 x 20 mL) and brine (1 x 20 mL). The organic layer was dried with anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by flash silica chromatography (petroluem spirit / ethyl acetate, 4:1) to give the optically pure α- and β-D-thioglycosides (**3α/3β-OAc** and **4α/4β-OAc**).

General method 3 – deprotection of glycosides under Zemplén conditions.[4]

Purified per-*O*-acetylated glycosides were deprotected using conditions adapted from Zemplén and Pascau.[4] The per-*O*-acetylated glycoside was dissolved in anhydrous methanol and a freshly prepared solution of sodium methoxide (1 M in methanol) was added. The mixture was stirred until TLC indicated consumption of starting material and formation of a single product (ethyl acetate / methanol, 19:1). The mixture was neutralized by the addition of Amberlite IR120 (H^+) resin, filtered, and evaporated to dryness to give pure *n*-octyl (thio)glycoside.

***n*-Octyl (2,3,4,6-tetra-*O*-acetyl) α/β-D-galactopyranoside (1α-Ac and 1β-Ac)**



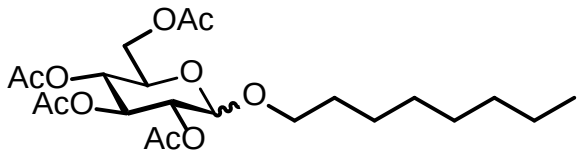
Compounds **1α-Ac** and **1β-Ac** were synthesised according to general method 1. The anomeric mixture (2:3 α/β) was purified by flash chromatography as described to yield the pure α-anomer (**1α-Ac**, 0.54 g, 1.17 mmol, 23%) and pure β-anomer (**1β-Ac**, 0.85 g, 1.83 mmol, 36%) as a colourless syrup.

β-anomer (1β-Ac): R_f = 0.24 (petroleum ether/ethyl acetate, 4:1); $[\alpha]_D^{20}$ = + 16.7 (*c* = 0.36, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.38 (dd, 1H, $J_{3,4}$ = 3.5 Hz , $J_{4,5}$ = 1.0 Hz, H-4), 5.20 (dd, 1H, $J_{2,3}$ = 10.57, $J_{1,2}$ = 8.08 Hz, H-2), 5.01 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.6 Hz, H-3), 4.45 (d, 1H, $J_{1,2}$ = 8.01 Hz, H-1), 4.18 (dd, 1H, $J_{6a\text{-}6b}$ = 11.25 Hz, $J_{5\text{-}6a}$ = 6.4 Hz, H-6a), 4.12 (dd, 1H, $J_{6a\text{-}6b}$ = 11.25 Hz, $J_{5\text{-}6b}$ = 7.05 Hz, H-6b), 3.91–3.86 (m, 2H, H-5, Gal-OC*H*H), 3.46 (dt, 1H, $J_{CH\text{-}CH}$ = 9.60 Hz, $J_{CH\text{-}CH2}$ = 6.61 Hz, Gal-OCH*H*), 2.14 (s, 3H, OAc CH_3), 2.05 (s, 3H, OAc CH_3), 2.04 (s, 3H, OAc CH_3), 1.98 (s, 3H, OAc CH_3), 1.62–1.51 (m, 2H, Gal-OCH_2-CH_2), 1.32–1.23 (m, 10H, 5 x CH_2), 0.88 (t, 3H, *J* = 6.85 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ =

170.56, 170.45, 170.36, 169.51 (4 x *C*OCH$_3$), 101.48 (C-1), 71.11 (C-3), 70.68 (C-5), 70.44 (Gal-O*C*H$_2$), 69.07 (C-2), 67.21 (C-4), 61.42 (C-6), 31.92 (CH$_2$), 29.53 (Gal-OCH$_2$*C*H$_2$), 29.40, 29.37, 25.92, 22.77 (4 x CH$_2$), 20.87, 20.81 (2), 20.73 (4 x CO*C*H$_3$), 14.21 (CH$_3$) ppm. MS (ESI+): calcd. for $C_{22}H_{36}O_{10}$: *m/z* = 483.22 $[M+Na]^+$; found: *m/z* = 483.2.

α-anomer (1α-Ac): R_f = 0.31 (petroleum ether/ethyl acetate, 4:1); $[\alpha]_D^{20}$ = +95.8 (*c* = 0.72, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ = 5.46 (dd, 1H, $J_{3,4}$ = 3.46 Hz, $J_{4,5}$ = 1.32 Hz, H-4), 5.37–5.34 (m, 1H, H-3), 5.12–5.09 (m, 2H, H-1, H-2), 4.24–4.21 (m, 1H, H-5), 4.13–4.06 (m, 2H, H-6a, H-6b), 3.68 (dt, 1H, $J_{CH\text{-}CH}$ = 9.81 Hz, $J_{CH\text{-}CH2}$ = 6.60 Hz, Gal-OC*H*H), 3.42 (dt, 1H, $J_{CH\text{-}CH2}$ = 9.89 Hz, $J_{CH\text{-}CH2}$ = 6.61 Hz, Gal-OCHC*H*), 2.14 (s, 3H, OAc CH$_3$), 2.07 (s, 3H, OAc CH$_3$), 2.05 (s, 3H, OAc CH$_3$), 1.99 (s, 3H, OAc CH$_3$), 1.61–1.55 (m, 2H, Gal-OCH$_2$C*H*$_2$), 1.36–1.26 (m, 10H, 5 x CH$_2$), 0.89 (t, 3H, *J* = 6.94 Hz, CH$_3$) ppm; ^{13}C NMR (125 MHz, CDCl$_3$): δ = 170.33, 170.32, 170.20, 169.97 (4 x *C*OCH$_3$), 96.11 (C-1), 68.71 (Gal-O*C*H$_2$), 68.33 (C-2), 68.19 (C-4), 67.74 (C-3), 66.19 (C-5), 61.85 (C-6), 31.85 (CH$_2$), 29.33 (Gal-OCH$_2$*C*H$_2$), 29.32, 29.30, 26.11, 22.68 (4 x CH$_2$), 20.79, 20.71 (2), 20.67 (4 x CO*C*H$_3$), 14.11 (CH$_3$) ppm. MS (ESI+): calcd. for $C_{22}H_{36}O_{10}$: *m/z* = 483.22 $[M+Na]^+$; found: *m/z* = 483.2.

***n*-Octyl (2,3,4,6-tetra-*O*-acetyl) α/β-D-glucopyranoside (2α-Ac and 2β-Ac)**



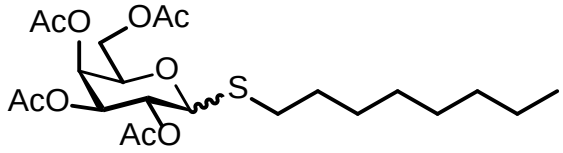
Compounds **2α-Ac** and **2β-Ac** were synthesised according to general method 1. The anomeric mixture (2:3 α/β) was purified by flash chromatography as described to yield the pure α-anomer (**2α-Ac**, 0.61 g, 1.32 mmol, 26%) and pure β-anomer (**2β-Ac**, 0.90 g, 1.93 mmol, 38%) as a colourless syrup.

β-anomer (2β-Ac): R_f = 0.22 (petroleum ether/ethyl acetate, 4:1); $[\alpha]_D^{20}$ = +14.6 (*c* = 0.48, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$): δ = 5.20 (dd~t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.57 Hz, H-3), 5.09 (dd~t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.61 Hz, H-4), 4.98 (dd, 1H, $J_{2,3}$ = 9.62, $J_{1,2}$ = 7.97, H-2), 4.49 (d, 1H, $J_{1,2}$ = 7.99 Hz, H-1), 4.26 (dd, 1H, $J_{6a,6b}$ = 12.23 Hz, $J_{5,6a}$ = 4.72 Hz, H-6a), 4.13 (dd, 1H, $J_{6a,6b}$ = 12.31 Hz, $J_{5\text{-}6b}$ = 2.45 Hz, H-6b), 3.87 (dt, 1H, $J_{CH\text{-}CH}$ = 9.70 Hz, $J_{CH\text{-}CH2}$ = 6.33 Hz, Glc-OC*H*H), 3.69 (ddd, 1H, $J_{4,5}$ = 9.90 Hz, $J_{5,6a}$ = 4.73 Hz, $J_{5,6b}$ = 2.43 Hz, H-5), 3.47 (dt, 1H, $J_{CH\text{-}CH}$ = 9.57 Hz, $J_{CH\text{-}CH2}$ = 6.63 Hz, Glc-OCH*H*), 2.08 (s, 3H, OAc CH$_3$), 2.04 (s, 3H, OAc, CH$_3$), 2.03 (s, 3H, OAc CH$_3$), 2.01 (s, 3H, OAc CH$_3$), 1.60–1.51 (m, 2H, Glc-OCH$_2$-C*H*$_2$), 1,34–1.23 (m, 10H, 5 x CH$_2$), 0.88

(t, 3H, *J* = 6.81 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.77, 170.39, 169.49, 169.35 (4 x *C*OCH_3), 100.90 (C-1), 72.95 (C-3), 71.80 (C-5), 71.43 (C-2), 70.31 (Glc-O*C*H_2), 68.56 (C-4), 62.08 (C-6), 31.87 (CH_2), 29.45 (Glc-OCH_2*C*H_2), 29.32 (2), 25.87, 22.71 (4 x CH_2), 20.81, 20.71, 20.70, 20.61 (4 x CO*C*H_3), 13.16 (CH_3) ppm. MS (ESI+): calcd. for $C_{22}H_{36}O_{10}$: *m/z* = 483.22 $[M+Na]^+$; found: *m/z* = 483.2.

α-anomer (2α-Ac): R_f = 0.28 (petroleum ether/ethyl acetate, 4:1); $[\alpha]_D^{20}$ = +91.0 (*c* = 0.56, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.49 (dd~t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.80 Hz, H-3), 5.07–5.03 (m, 2H, H-2, H-4), 4.85 (dd, 1H, $J_{2,3}$ = 10.25 Hz, $J_{1,2}$ = 3.81 Hz, H-2), 4.26 (dd, 1H, $J_{6a,6b}$ = 12.21 Hz, $J_{5\text{-}6a}$ = 4.51 Hz, H-6a), 4.09 (dd, 1H, $J_{6a,6b}$ = 12.24 Hz, $J_{5,6b}$ = 2.41 Hz, H-6b), 4.02 (ddd, 1H, J4,5 = 10.25 Hz, $J_{5,6a}$ = 4.51 Hz, $J_{5,6b}$ = 2.41 Hz, H-5), 3.68 (dt, 1H, $J_{CH,CH}$ = 9.96 Hz, $J_{CH,CH2}$ = 6.58 Hz, Glc-OC*H*H), 3.42 (dt, 1H, $J_{CH,CH}$ = 9.82 Hz, $J_{CH,CH2}$ = 6.58 Hz, Glc-OCH*H*), 2.09 (s, 3H, OAc CH_3), 2.06 (s, 3H, OAc CH_3), 2.03 (s, 3H, OAc CH_3), 2.01 (s, 3H, OAc CH_3), 1.62-1.55 (m, 2H, Glc-OCH_2C*H*$_2$), 1.35-1.23 (m, 10H, 5 x CH_2), 0.89 (t, 3H, *J* = 6.85 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.76 (COCH3), 170.28 (*C*OCH_3), 170.25 (*C*OCH_3), 169.74 (*C*OCH_3), 95.27 (C-1), 71.05 (C-2), 70.37 (C-3), 68.83 (Glc-O*C*H_2), 68.74 (C-4), 67.22 (C-5), 62.05 (C-6), 31.91 (CH_2), 29.37 (Glc-OCH_2*C*H_2), 29.34 (2), 26.12, 22.74 (4 x CH_2), 20.81 (2), 20.76, 20.73 (4 x CO*C*H_3), 14.18 (CH_3) ppm. MS (ESI+): calcd. for $C_{22}H_{36}O_{10}$: *m/z* = 483.22 $[M+Na]^+$; found: *m/z* = 483.2.

***n*-Octyl (2,3,4,6-tetra-*O*-acetyl) α/β-D-thiogalactopyranoside (3α-Ac and 3β-Ac)**



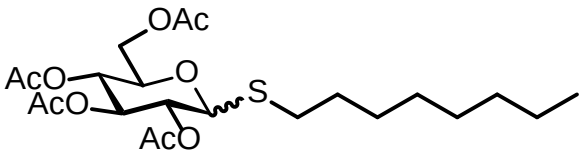
Compounds **3α-Ac** and **3β-Ac** were synthesised according to general method 2. The anomeric mixture (~2:1 α/β) was purified by flash chromatography as described to yield the pure α-anomer (**3α-Ac**, 1.31 g, 2.75 mmol, 54%) and pure β-anomer (**3β-Ac**, 0.78 g, 1.63 mmol, 32%) as a pale yellow syrup.

β-anomer (3β-Ac): R_f = 0.30 (petroleum ether/ethyl acetate, 4:1); $[\alpha]_D^{20}$ = -5.2 (*c* = 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.44 (dd, 1H, $J_{3,4}$ = 3.44 Hz, $J_{4,5}$ = 1.04 Hz, H-4), 5.23 (dd~t, 1H, $J_{2,3}$ = $J_{1,2}$ = 9.95 Hz, H-2), 5.05 (dd, 1H, $J_{2,3}$ = 9.95 Hz, $J_{3,4}$ = 3.42 Hz, H-3), 4.84 (d, 1H, $J_{1,2}$ = 10.04 Hz, H-1), 4.17 (dd, 1H, $J_{6a,6b}$ = 11.28 Hz, $J_{5,6a}$ = 6.73 Hz, H-6a), 4.11 (dd, 1H, $H_{6a,6b}$ =

11.28 Hz, $J_{5,6b}$ = 6.73 Hz, H-6b), 3.95–3.91 (m, 1H, H-5), 2.75–2.63 (m, 2H, Gal-SC*H*$_2$), 2.16 (s, 3H, OAc CH_3), 2.07 (s, 3H, OAc CH_3), 2.05 (s, 3H, OAc CH_3), 1.99 (s, 3H, OAc CH_3), 1.66–1.56 (m, 2H, Gal-SCH2C*H*$_2$), 2.41–2.34 (m, 2H, CH2), 1.30–1.25 (m, 8H, 4 x CH_2), 0.88 (t, 1H, J = 7.02 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.53, 170.38, 170.23, 169.71 (4 x *C*OCH_3), 84.40 (C-1), 74.53, 72.53, 67.45 (2), 61.59 (C-2, C-3, C-4, C-5, and C-6), 31.96, 30.42, 29.85, 29.33, 29.29, 28.97, 22.89 (7 x CH_2), 20.99, 20.85 (2) 20.77 (CO*C*H_3), 14.25 (CH_3) ppm. MS (ESI+): calcd. for $C_{22}H_{36}O_9S$: *m/z* = 499.2 $[M+Na]^+$; found: *m/z* = 499.2

α-anomer (3α-Ac): R_f = 0.37 (petroleum ether/ethyl acetate, 4:1); $[\alpha]_D^{20}$ = +154.7 (*c* = 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.72 (d, 1H, $J_{1,2}$ = 5.18 Hz, H-1), 5.45 (dd, 1H, $J_{3,4}$ = 3.31 Hz, $J_{4,5}$ = 1.15 Hz, H-4), 5.27 (dd, 1H, $J_{2,3}$ = 11.01 Hz, $J_{1,2}$ = 5.20 Hz, H-2), 5.22 (dd, 1H, $J_{2,3}$ = 11.02 Hz, $J_{3,4}$ = 3.27 Hz, H-3), 4.61–4.57 (m, 1H, H-5), 4.12 (dd, 1H, $J_{6a,6b}$ = 11.41 Hz, $J_{5,6a}$ = 6.15 Hz, H-6a), 4.09 (dd, 1H, $J_{6a,6b}$ = 11.41 Hz, $J_{5,6b}$ = 6.93 Hz, H-6b), 2.59–2.46 (m, 2H, Gal-SC*H*$_2$), 2.15 (s, 3H, OAc CH_3), 2.08 (s, 3H, OAc CH_3), 2.05 (s, 3H, OAc CH_3), 1.99 (s, 3H, OAc CH_3), 1.62–1.54 (m, 2H, CH_2), 1.39–1.22 (m, 10H, 5 x CH_2), 0.88 (t, J = 6.81 Hz, 3H, CH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.29, 170.13 (2), 169.82 (4 x *C*OCH3), 82.27 (C-1), 68.31, 68.14, 68.11, 61.95, 66.58 (C-2, C-3, C-4, C-5, and C-6), 31.89, 29.95, 29.45, 29.29, 29.21, 29.02, 22.75 (7 x CH_2), 20.94, 20.79, 20.74 (2) (CO*C*H_3), 14.20 (CH_3) ppm. MS (ESI+): calcd. for $C_{22}H_{36}O_9S$: *m/z* = 499.2 $[M+Na]^+$; found: *m/z* = 499.2

***n*-Octyl (2,3,4,6-tetra-*O*-acetyl) α/β-D-thioglucopyranoside (4α-Ac and 4β-Ac)**



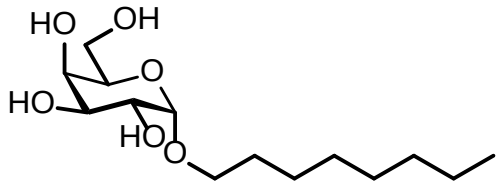
Compounds **4α-Ac** and **4β-Ac** were synthesised according to general method 2. The anomeric mixture (~2:1 α/β) was purified by flash chromatography as described to yield the pure α-anomer (**4α-Ac**, 1.24 g, 2.60 mmol, 51%) and pure β-anomer (**4β-Ac**, 0.85 g, 1.78 mmol, 35%) as a colourless syrup which slowly crystallized on standing in the refrigerator.

β-anomer (4β-Ac): R_f = 0.27 (petroleum ether/ethyl acetate, 4:1); $[\alpha]_D^{20}$ = -18.5 (*c* = 0.65, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.22 (dd~t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.36 Hz, H-3), 5.09 (dd~t, 1H, $J_{2,3}$ = $J_{1,2}$ = 9.70 Hz, H-2), 5.03 (dd, 1H, $J_{4,5}$ = 9.56 Hz, $J_{3,4}$ = 9.42 Hz, H-4), 4.48 (d, 1H, $J_{1,2}$ = 10.05 Hz, H-1), 4.25 (dd, 1H, $J_{6a,6b}$ = 12.41 Hz, $J_{5,6a}$ = 4.92 Hz, H-6a), 4.14 (dd, 1H, $J_{6a,6b}$ = 12.41 Hz, $J_{5,6b}$ = 2.42 Hz, H-6b), 3.71 (ddd, 1H, $J_{4,5}$ = 9.62 Hz, $J_{5,6a}$ = 4.91 Hz, $J_{5,6b}$ = 2.44 Hz,

H-5), 2.72–2.61 (m, 2H, Glc-SC*H*$_2$), 2.08 (s, 3H, OAc, CH_3), 2.06 (s, 3H, OAc, CH_3), 2.03 (s, 3H, OAc, CH_3), 2.01 (s, 3H, OAc, CH_3), 1.65–1.53 (m, 2H, Glc-SCH$_2$-C*H*$_2$), 1.40-1.22 (m, 10H, 5 x CH_2), 0.88 (t, 1H, *J* = 6.88 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.81, 170.37, 169.58, 169.55 (4 x *C*OCH3), 83.79 (C-1), 76.01 (C-2), 74.08 (C-3), 70.05 (C-4), 68.49 (C-5), 62.35 (C-6), 31.95, 30.17, 29.78, 29.33, 29.27, 28.95, 22.79 (7 x CH_2), 20.89 (2), 20.79, 20.76 (4 x CO*C*H$_3$), 14.25 (CH_3) ppm. MS (ESI+): calcd. for $C_{22}H_{36}O_9S$: *m/z* = 499.2 $[M+Na]^+$; found: *m/z* = 499.2.

α-anomer (4α-Ac): R_f = 0.37 (petroleum ether/ethyl acetate, 4:1); $[\alpha]_D^{20}$ = +135.4 (*c* = 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.65 (d, 1H, $J_{1,2}$ = 5.85 Hz, H-1), 5.37 (dd~t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.64 Hz, H-3), 5.04 (dd, 1H, $J_{4,5}$ = 10.21 Hz, $J_{3,4}$ = 9.50 Hz, H-4) 5.02 (dd, 1H, $J_{2,3}$ = 10.23 Hz, $J_{1,2}$ = 5.85 Hz, H-2), 4.43 (ddd, 1H, $J_{4,5}$ = 10.21 Hz, $J_{5,6a}$ = 4.71 Hz, $J_{5\text{-}6b}$ = 2.27 Hz, H-5), 4.30 (dd, 1H, $J_{6a,6b}$ = 12.23 Hz, $J_{5,6a}$ = 4.69 Hz, H-6a), 4.07 (dd, 1H, $J_{6a,6b}$ = 12.31 Hz, $J_{5,6b}$ = 2.31 Hz, H-6b), 2.58–2.46 (m, 2H, Gal-SC*H*$_2$), 2.09 (s, 3H, OAc CH_3), 2.06 (s, 3H, OAc CH_3), 2.03 (s, 3H, OAc CH_3), 2.01 (s, 3H, OAc CH_3), 1.39–1.21 (m, 10H, CH_2), 0.88 (t, 3H, *J* = 6.93 Hz, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.73, 170.08, 170.04, 169.78 (4 x *C*OCH_3), 82.13 (C-1), 70.90 (C-2), 70.67 (C-3), 68.72 (C-4), 67.63 (C-5), 62.09 (C-6), 31.91, 30.29, 29.51, 29.30, 29.22, 28.99, 22.76 (7 x CH_2), 20.89, 20.86, 20.81, 20.76 (4 x CO*C*H$_3$), 14.22 (CH_3) ppm. MS (ESI+): calcd. for $C_{22}H_{36}O_9S$: *m/z* = 499.2 $[M+Na]^+$; found: *m/z* = 499.2

***n*-Octyl α-D-galactopyranoside (1α)**

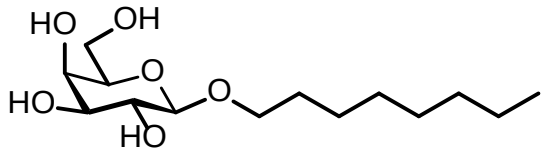


Compound **1α** were synthesised according to general method 3 and isolated as a waxy solid (352 mg, 1.12 mmol, 96%).

1α: R_f = 0.44 (ethyl acetate/methanol, 6:1); $[\alpha]_D^{20}$ = +95.3 (*c* = 0.53, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.79 (d, 1H, $J_{1,2}$ = 3.43 Hz, H-1), 3.89 (dd, 1H, $J_{3,4}$ = 3.03 Hz, $J_{4,5}$ = 1.13 Hz, H-4), 3.80 (td, $J_{5,6}$ = 9.21 Hz, $J_{3,4}$ = 1.13 Hz, H-5), 3.76 (dd, $J_{2,3}$ = 10.10 Hz, $J_{1,2}$ = 3.43 Hz, H-2), 3.74–3.73 (m, 1H, H-3), 3.73–3.69 (3H, Gal-OC*H*H, H-6a, H-6b), 3.44 (dt, 1H, $J_{CH,CH}$ = 9.69 Hz, $J_{CH,CH2}$ = 6.57 Hz, Gal-OCH*H*), 1.70–1.57 (m, 2H, Gal-OCH$_2$C*H*$_2$), 1.42–1.27 (m, 10H, 5 x CH_2), 0.90 (t, 3H, *J* = 7.12 Hz, CH3) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ ^{13}C NMR (125

MHz, CD_3OD): δ 100.47 (C-1), 72.41 (C-5), 71.69 (C-3), 71.26 (C-4), 70.42 (C-2), 69.35 (Gal-OCH_2), 62,86 (C-6), 33.16 (Gal-OCH_2CH_2), 30.74 (2), 30.56, 27.49, 23.86 (4 x CH2), 14.58 (CH3) ppm. MS (ESI+): calcd. for $C_{14}H_{28}O_6$: *m/z* = 315.2 $[M+Na]^+$; found: *m/z* = 315.2.

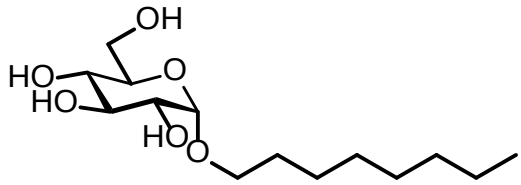
***n*-Octyl β-D-galactopyranoside (1β)**



Compound **1β** were synthesised according to general method 3 and isolated as a waxy solid (554 mg, 1.76 mmol, 96%).

1β: R_f = 0.36 (ethyl acetate/methanol, 6:1); $[\alpha]_D^{20}$ = -21.3 (*c* = 0.65, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.20 (d, 1H, $J_{1,2}$ = 7.48 Hz, H-1), 3.89 (dt, 1H, $J_{CH,CH}$ = 9.54 Hz, $J_{CH,CH2}$ = 6.89 Hz, Gal-OC*H*CH), 3.83 (dd, 1H, $J_{3,4}$ = 3.32 Hz, $J_{4,5}$ = 1.02 Hz, H-4), 3.75 (dd, 1H, $J_{6a,6b}$ = 11.30 Hz, $J_{5,6a}$ = 6.63 Hz, H-6a), 3.72 (dd, 1H, $J_{6a,6b}$ = 11.30 Hz, $J_{5,6b}$ = 5.60 Hz, H-6b), 3.56–3.47 (m, 3H, Gal-OCH*H*, H-2, H-5), 3.45 (dd, 1H, $J_{2,3}$ = 9.68 Hz, $J_{3,4}$ = 3.30 Hz, H-3), 1.68–1.59 (m, 2H, Gal-OCH2CH_2), 1.41–1.27 (m, 10H, 5 x CH_2), 0.90 (t, 3H, *J* = 7.19 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ 105.12 (C-1), 76.67 (C-5), 75.19 (C-3), 72.73 (C-2), 70.99 (Gal-OCH_2), 70.49 (C-4), 62.57 (C-6), 33.15 (CH_2), 30.98 (Gal-OCH_2CH_2), 30.73, 30.57, 27.27, 23.86 (4 x CH_2), 14.58 (CH_3) ppm. MS (ESI+): calcd. for $C_{14}H_{28}O_6$: *m/z* = 315.2 $[M+Na]^+$; found: *m/z* = 315.2.

***n*-Octyl α-D-glucopyranoside (2α)**

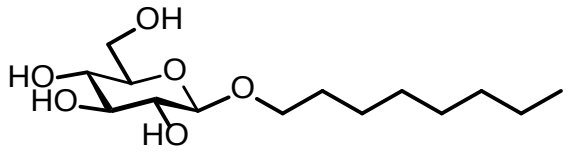


Compound **2α** were synthesised according to general method 3 and isolated as a waxy solid (416 mg, 1.32 mmol, 100%).

2α: R_f = 0.47 (ethyl acetate/methanol, 6:1); $[\alpha]_D^{20}$ = +95.6 (c = 0.54, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.78 (d, 1H, $J_{1,2}$ = 3.78 Hz, H-1), 3.79 (dd, 1H, $J_{6a,6b}$ = 11.82 Hz, $J_{5,6a}$ = 2.40 Hz, H-6a), 3.73 (dt, 1H, $J_{CH,CH}$ = 9.54 Hz, $J_{CH,CH2}$ = 6.61 Hz, Glc-OC*H*CH), 3.67 (dd, 1H, $J_{6a,6b}$ = 11.78 Hz, $J_{5,6b}$ = 5.46 Hz, H-6b), 3.63 (dd~t 1H, $J_{2,3}$ = $J_{3,4}$ = 9.22 Hz, H-3), 3.57 (ddd, 1H, $J_{4,5}$ =

9.92 Hz, $J_{5,6b}$ = 5.52 Hz, $J_{5,6a}$ = 2.40 Hz, H-5), 3.44 (dt, 1H, $J_{CH,CH}$ = 9.65 Hz, $J_{CH,CH2}$ = 6.38 Hz, Glc-OCH*H*), 3.38 (dd, $J_{2,3}$ = 9.67 Hz, $J_{1,2}$ = 3.73 Hz, 1H, H-2), 3.28 (dd, 1H, $J_{4,5}$ = 9.91 Hz, $J_{3,4}$ = 8.97 Hz, H-4), 1.70–1.57 (m, 2H, Glc-OCH_2CH_2), 1.43–1.26 (m, 10H, 5 x CH_2), 0.90 (t, 3H, J = 7.10 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ 100.22 (C-1), 75.28 (C-3), 73.77 (C-5), 73.72 (C-2), 72.02 (C-4), 69.26 (Glc-OCH_2), 62.80 (C-6), 33.16 (CH_2), 30.78, 30.74, 30.57, 27.28, 23.87 (5 x CH_2), 14.59 (CH_3) ppm. MS (ESI+): calcd. for $C_{14}H_{28}O_6$: *m/z* = 315.2 $[M+Na]^+$; found: *m/z* = 315.2.

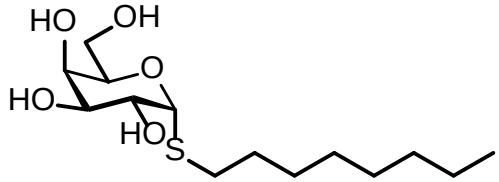
***n*-Octyl β-D-glucopyranoside (2β)**



Compound **2β** were synthesised according to general method 3 and isolated as a waxy solid (608 mg, 1.93 mmol, 100%).

2β: R_f = 0.65 (ethyl acetate/methanol, 6:1); $[\alpha]_D^{20}$ = -21.2 (*c* = 0.65, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.24 (d, 1H, $J_{1,2}$ = 7.82 Hz, H-1), 3.90 (dt, 1H, $J_{CH,CH}$ = 9.55 Hz, $J_{CH,CH2}$ = 6.68 Hz, Gal-OC*H*CH), 3.86 (dd, 1H, $J_{6a,6b}$ = 11.98 Hz, $J_{5,6a}$ = 2.01 Hz, H-6a), 3.66 (dd, 1H, $J_{6a,6b}$ = 11.98 Hz, $J_{5,6b}$ = 5.41 Hz, H-6b), 3.53 (dt, $J_{CH,CH}$ = 9.33 Hz, $J_{CH,CH2}$ = 6.79 Hz, Glc-OCH*H*), 3.34 (dd~t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.10 Hz, H-3), 3.28 (dd~t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.10 Hz, H-4), 3.26–3.23 (m, 1H, H-5), 3.16 (dd, 1H, $J_{2,3}$ = Hz, $J_{1,2}$ = Hz, H-2), 1.64–1.59 (m, 2H, Glc-OCH_2CH_2), 1.40–1.27 (m, 10H, 5 x CH_2), 0.90 (t, 3H, J = 7.12 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ 104.43 (C-1), 78.20 (C-3), 77.96 (C-5), 75.19 (C-2), 71.75 (C-4), 71.01 (Glc-OCH_2), 62.86 (C-6), 33.12 (CH_2), 30.90 (Glc-OCH2CH_2), 30.71, 30.53, 27.22, 23.83 (4 x CH_2), 14.58 (CH_3) ppm. MS (ESI+): calcd. for $C_{14}H_{28}O_6$: *m/z* = 315.2 $[M+Na]^+$; found: *m/z* = 315.2.

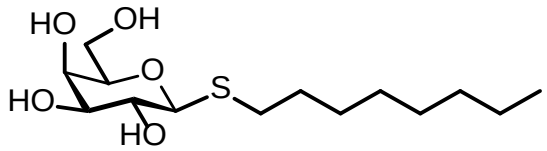
***n*-Octyl α-D-thiogalactopyranoside (3α)**



Compound **3α** were synthesised according to general method 3 and isolated as a colourless gym (910 mg, 2.75 mmol, 100%).

3α: R_f = 0.53 (ethyl acetate/methanol, 6:1); $[\alpha]_D^{20}$ = +200 (*c* = 0.8, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 5.37 (d, 1H, $J_{1,2}$ = 5.65 Hz, H-1), 4.21–4.18 (m, 1H, H-5), 4.07 (dd, 1H, $J_{2,3}$ = 10.16 Hz, $J_{1,2}$ = 5.64 Hz, H-2), 3.90 (dd, 1H, $J_{3,4}$ = 3.39 Hz, $J_{4,5}$ = 1.22 Hz, H-4), 3.74–3.72 (m, 2H, H-6a, H-6b), 3.62 (dd, 1H, $J_{2,3}$ = 10.13 Hz, $J_{3,4}$ = 3.36 Hz, H-3), 2.67–2.54 (m, 2H, Gal-SCH_2), 1.73–1.60 (m, 2H, Gal-SCH2-CH_2), 1.46–1.29 (m, 10H, 5 x CH_2), 0.92 (t, 3H, CH_3) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ 87.45 (C-1), 72.70 (C-5), 72.30 (C-3), 70.96 (C-4), 69.86 (C-2), 62.66 (C-6), 33.12, 30.88 (2), 30.49, 30.46, 30.20, 23.83 (7 x CH_2), 14.58 (CH_3) ppm. MS (ESI+): calcd. for $C_{14}H_{28}O_6$: *m/z* = 315.2 $[M+Na]^+$; found: *m/z* = 315.2.

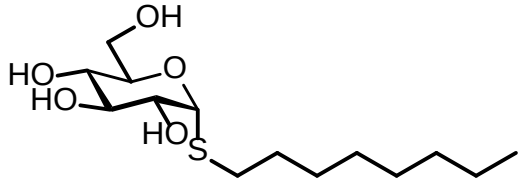
***n*-Octyl β-D-thiogalactopyranoside (3β)**



Compound **3β** were synthesised according to general method 3 and isolated as an amorphous solid (538 mg, 1.63 mmol, 100%).

3β: R_f = 0.56 (ethyl acetate/methanol, 6:1); $[\alpha]_D^{20}$ = -19.2 (*c* = 0.8, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.29 (d, 1H, $J_{1,2}$ = 9.54 Hz, H-1), 3.88 (dd, 1H, $J_{3,4}$ = 3.36 Hz, $J_{4,5}$ = 0.95 Hz, H-4), 3.53 (dd~t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.48 Hz, H-2), 3.73 (dd, 1H, $J_{6a,6b}$ = 11.37 Hz, $J_{5,6a}$ = 6.81 Hz, H-6a), 3.68 (dd, 1H, $J_{6a,6b}$ = 11.37 Hz, $J_{5,6b}$ = 5.43 Hz, H-6b), 3.53–3.49 (m, 1H, H-5), 3.45 (dd, 1H, $J_{2,3}$ = 9.27 Hz, $J_{3,4}$ = 3.35 Hz, H-3), 2.78–2.66 (m, 2H, Gal-SCH_2), 1.71–1.59 (m, Gal-SCH2-CH_2), 1.44–1.26 (m, 10H, 5 x CH_2), 0.90 (t, 3H, *J* = 6.92 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ 87.82 (C-1), 80.65 (C-5), 76.36 (C-3), 71.56 (C-2), 70.56 (C-4), 62.66 (C-6), 33.12, 31.21, 31.01, 30.48, 30.44, 30.13, 23.83 (7 x CH_2), 14.58 (CH_3) ppm. MS (ESI+): calcd. for $C_{14}H_{28}O_6S$: *m/z* = 331.2 $[M+Na]^+$; found: *m/z* = 331.2.

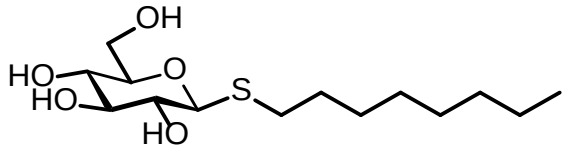
***n*-Octyl α-D-thioglucopyranoside (4α)**



Compound **4α** were synthesised according to general method 3 and obtained as an amorphous solid (860 mg, 2.60 mmol, 100%).

4α: R_f = 0.56 (ethyl acetate/methanol, 6:1); $[\alpha]_D^{20}$ = +218 (*c* = 0.38); ^{1}H NMR (500 MHz, CD_3OD): δ 5.30 (d, 1H, $J_{1,2}$ = 5.51 Hz, H-1), 3.95 (ddd, 1H, $J_{4,5}$ = 9.87 Hz, $J_{5,6b}$ = 7.73, Hz, $J_{5,6a}$ = 2.29 Hz, H-5), 3.79 (dd, 1H, $J_{5,6a}$ = 2.45 Hz, $J_{6a,6b}$ = 11.48 Hz, H-6a), 3.71 (dd, 1H, $J_{6a,6b}$ = 11.89 Hz, $J_{5,6b}$ = 5.47 Hz, H-6b), 3.68 (dd, 1H, $J_{2,3}$ = 9.72 Hz, $J_{1,2}$ = 5.48 Hz, H-2), 3.52 (dd~t, $J_{3,4}$ = $J_{4,5}$ = 9.21 Hz, 1H, H-4), 3.33–3.29 (m, 1H, H-3), 2.64–2.53 (m, 2H, GalS-OCH_2), 1.68–1.59 (m, 2H, GalSOCH_2-C*H*$_2$), 1.45–1.26 (m, 10H, 5 x CH_2), 0.90 (t, 1H, *J* = 7.14 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ 87.45 (C-1), 72.70 (C-5), 72.29 (C-2), 70.96 (C-3), 69.85 (C-4), 62.66 (C-6), 33.12, 30.88 (2), 30.49, 30.46, 30.20, 23.83 (7 x CH_2), 14.58 (CH_3) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ 87.30 (C-1), 73.30 (C-4), 74.04 (C-5), 75.80 (C-2), 71.85 (C-3), 62.66 (C-6), 33.14, 31.19, 30.91, 30.51, 30.46, 30.17, 23.84 (7 x CH_2), 14.58 (CH_3) ppm. MS (ESI+): calcd. for $C_{14}H_{28}O_6S$: *m/z* = 331.2 $[M+Na]^+$; found: *m/z* = 331.2.

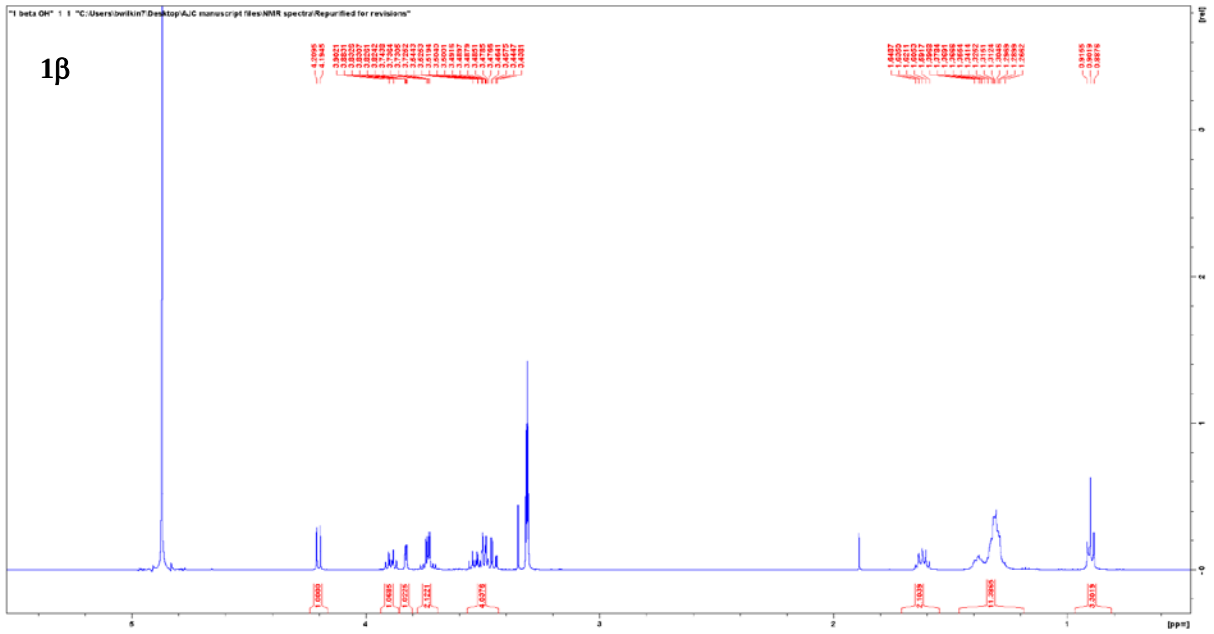
***n*-Octyl β-D-thioglucopyranoside (4β)**

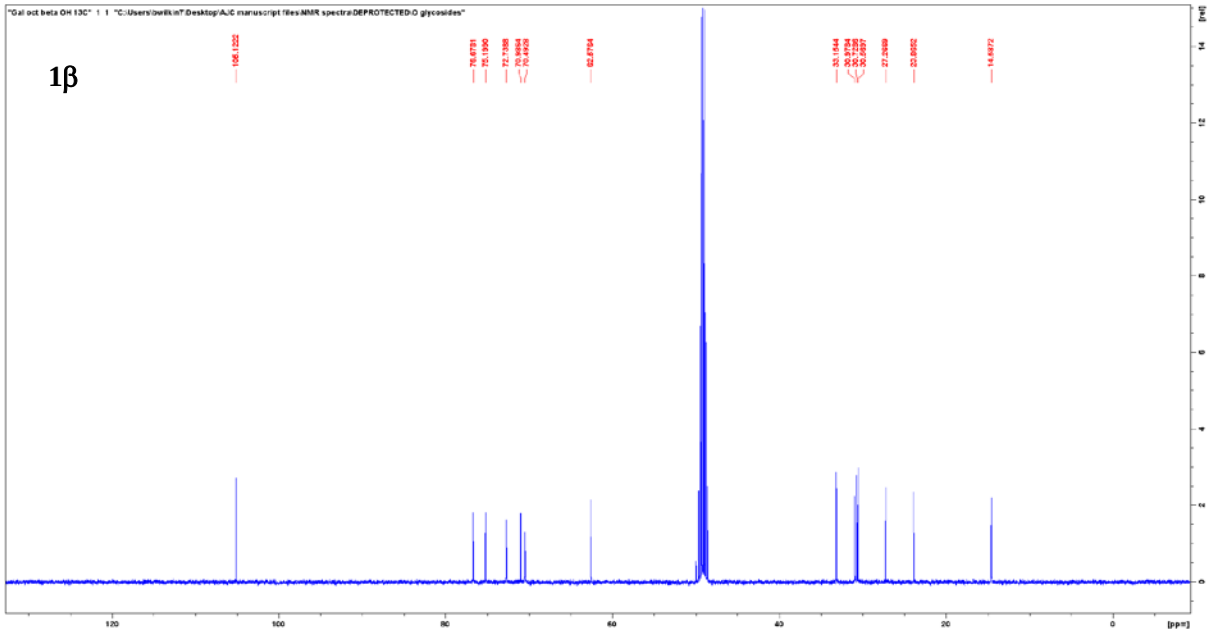


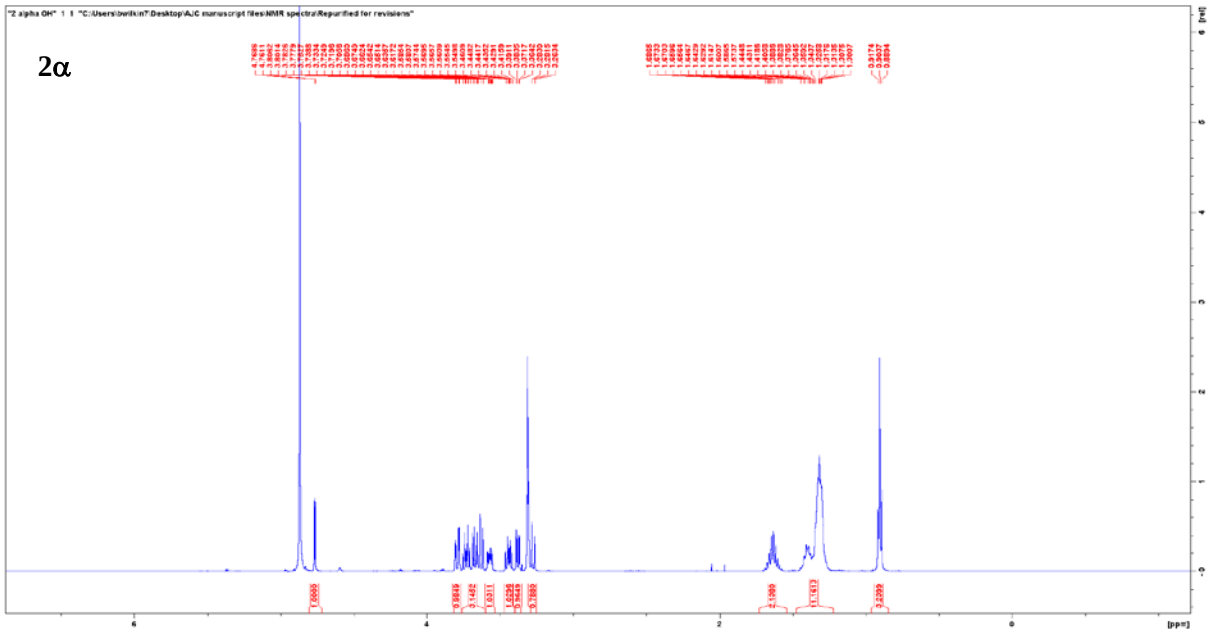
Compound **4β** were synthesised according to general method 3 and obtained as an amorphous solid (589 mg, 1.78 mmol, 100%).

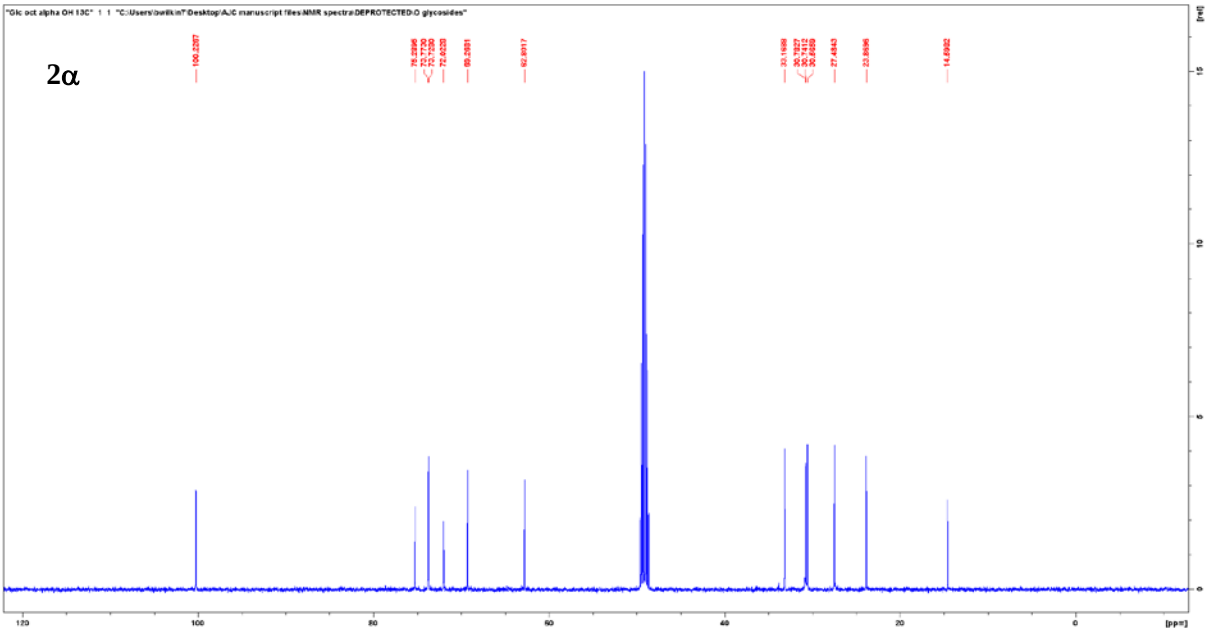
4β: R_f = 0.59 (ethyl acetate/methanol, 6:1); $[\alpha]_D^{20}$ = -47.6 (*c* = 0.46, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.35 (d, 1H, $J_{1,2}$ = 9.73 Hz, H-1), 3.86 (dd, 1H, $J_{6a,6b}$ = 12.09 Hz, $J_{6a,5}$ = 2.19 Hz, H-6a), 3.66 (dd, 1H, $J_{6a,6b}$ = 12.04 Hz, $J_{5,6b}$ = 5.49 Hz, H-6b), 3.37-3.25 (m, 3H, H-3, H-4 and H-5), 3.19 (dd, 1H, Hz, $J_{1,2}$ = 9.78 Hz, $J_{2,3}$ = 8.54 Hz, H-2), 2.79–2.67 (m, 2H, GlcS-OCH_2), 1.67-1.61 (m, 2H, GalS-OCH_2-C*H*$_2$), 1.45-1.28 (m, 10H, 5 x CH_2), 0.91 (t, *J* = 6.94 Hz, CH_3) ppm; ^{13}C NMR (125 MHz, CD_3OD): δ 87.23 (C-1), 82.07 (C-5), 79.70 (C-3 or C-4), 74.48 (C-2), 71.57 (C-3 or C-4), 63.01 (C-6), 33.12, 31.16, 30.97, 30.48, 30.44, 30.14, 23.83 (7 x CH_2), 14.58 (CH_3) ppm. MS (ESI+): calcd. for $C_{14}H_{28}O_6S$: *m/z* = 331.2 $[M+Na]^+$; found: *m/z* = 331.2.

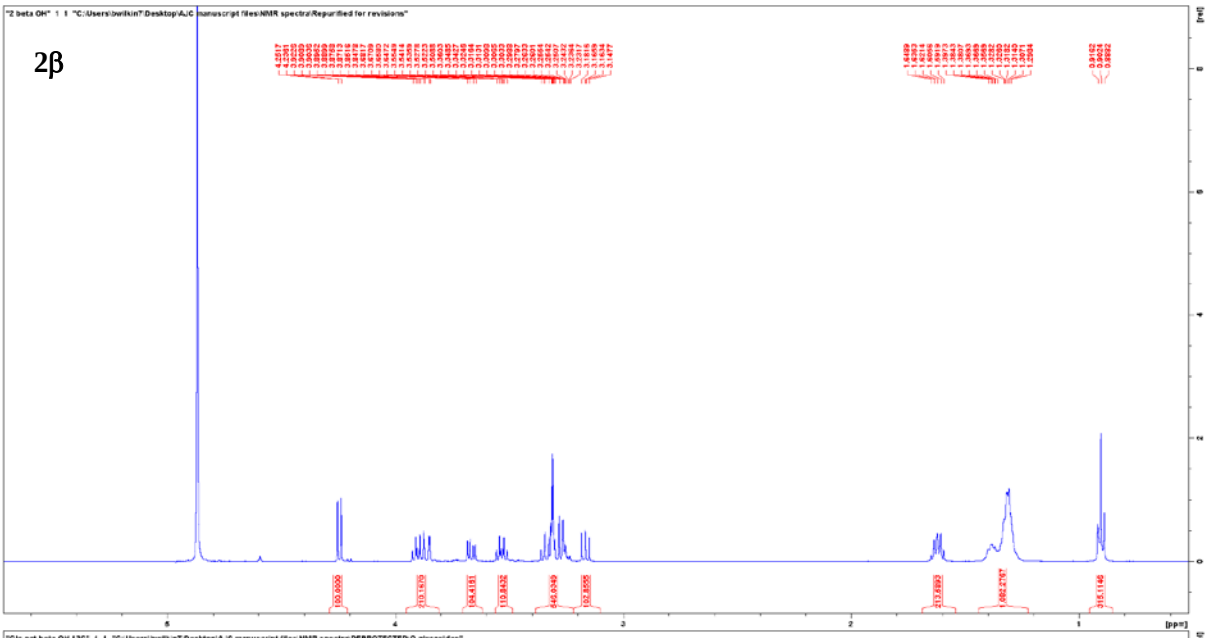
NMR spectra (compounds 1β, 2α, 2β and 3α).

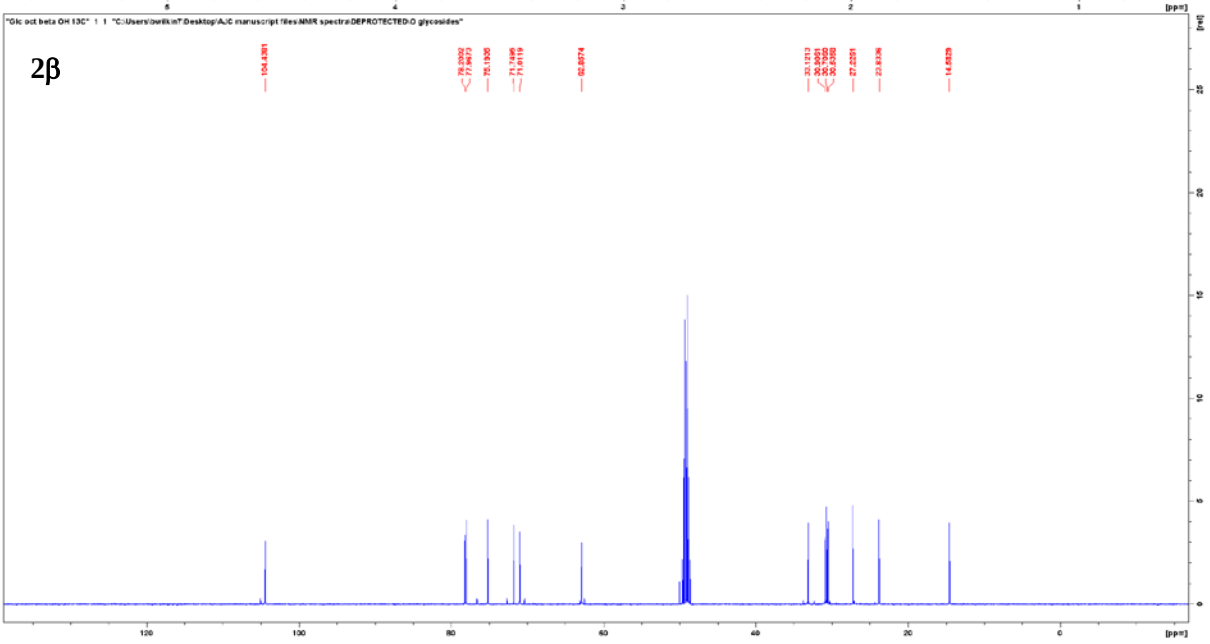


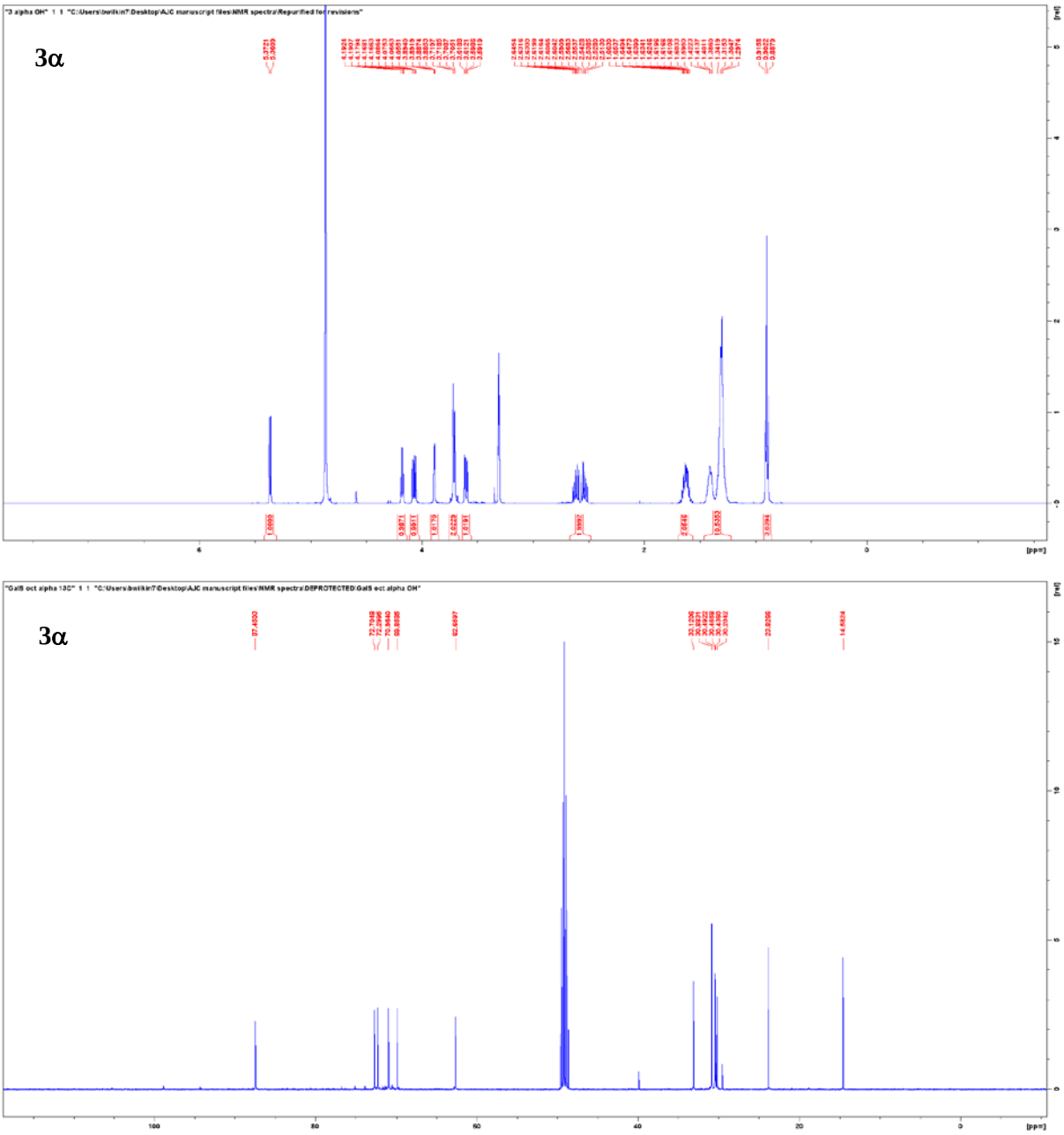



2α


2α


2β


2β



3.0 References

1. J. Jackman, M. Noestheden, D. Moffat, J. Pezacki, S. Findlay, R. N. *Biochem. Biophys. Res. Commun.* **2007**, *354*, 340–344.
2. J. Schmidt-Lassen, T. K. Lindhorst, *MedChemComm*, **2014**, *5*, 1218–1226.
3. H. A van Doren, R. van der Geest, R. M. Kellogg, H. Wynberg, *Carbohydr. Res.* **1989**, *194*, 71–77.
4. G. Zemplén and E. Pacsu, *Ber. Dtsch. Chem. Ges.* B, **1929**, *62*, 1613–1614.