Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Facile Anomer-oriented Syntheses of 4-Methylumbelliferyl Sialic Acid Glycosides

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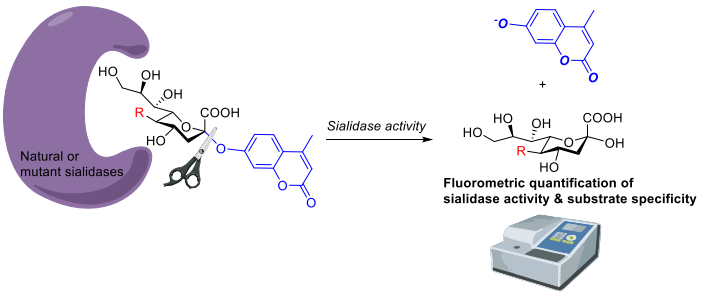
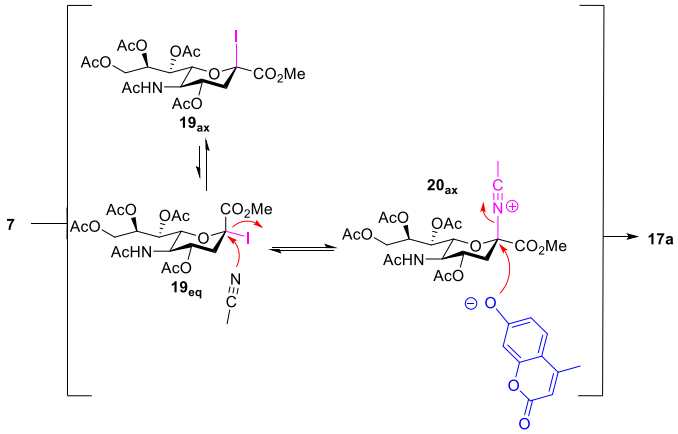
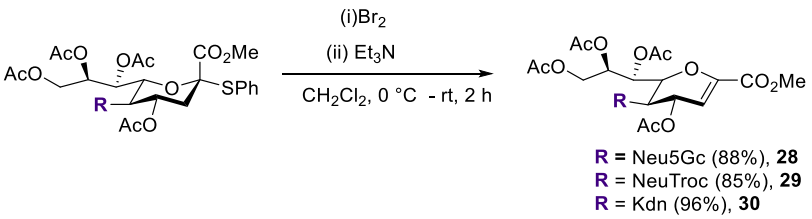


Scheme S1: Determining sialidase activity and specificity using fluorogenic sialic acid substrates



Scheme S2: Plausible mechanism involving TBAI and acetonitrile during the glycosylation reaction



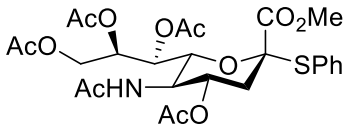
Scheme S3: Conversion of sialic acid thioglycosides to their corresponding glycals

Experimental Procedure

General Experimental

All the starting material chemicals were purchased from commercial suppliers (Carbosynth, Sigma Aldrich, Flourochem and Acros) and used without further purification. Unless otherwise stated, all reactions containing air- and moisture sensitive reagents were carried out under an inert atmosphere of nitrogen in oven-dried glassware with magnetic stirring. Anhydrous solvents were obtained from PureSolv-EN™ solvent purification system or purchased from Sigma-Aldrich in AcrosSeal® bottles. All reactions were monitored by thin-layer chromatography (TLC) on Merck DC-Alufolien plates precoated with silica gel 60 F254. TLC plates were visualised with UV-light (254 nm) and stained with H_2SO_4 (8%) and/or ninhydrin solution. Silica gel column chromatography was carried out using Davisil silica gel or with automated flash chromatography suite (Buchi Reveleris X2 AND Biotage SP4 HPFC). ^{1}H NMR (300, 400 or 500 MHz), ^{13}C NMR (101 MHZ or 125 MHz) spectra were recorded on Varian-inova spectrometers at 25 °C in chloroform-d1 ($CDCl_3$), methanol-d4 (CD_3OD), water-d2 (D_2O), acetone-d6 ($(CD_3)_2CO$), dimethylsulfoxide-d6 ($(CD_3)_2SO$). ^{1}H NMR spectra were standardised against the residual solvent peak ($CDCl_3$, δ = 7.26 ppm; CD_3OD, δ = 3.31 ppm; D_2O, δ = 4.79 ppm; $(CD_3)_2SO$ δ = 2.50 ppm; $(CD_3)_2CO$, δ = 2.84 ppm); or internal trimethylsilane, δ = 0.00 ppm). ^{13}C NMR spectra were standardised against the residual solvent peak ($CDCl_3$, δ = 77.16 ppm; CD_3OD, δ = 49.00 ppm; $(CD_3)_2SO$ δ = 39.52 ppm; $(CD_3)_2CO$, δ = 29.84 ppm). All ^{13}C NMR are ^{1}H decoupled. All NMR data is represented as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of doublets of doublets, dt = doublet of triplets, m = multiplet, br = broad signal, *a*d = apparent doublet, *a*t = apparent triplet), coupling constant in Hz, integration. Mass spectrometry was determined using Waters Quattro Micro LC-MS/MS in electronspray ionisation (ESI) mode.

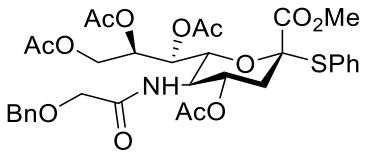
Methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-di-deoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranoside) onate (7)



Per-acetylated *N*-acetylneuraminic acid (19.7 g, 36.9 mmol) was dissolved in 1,2-dichloroethane (369 mL), and at O °C trimethyl(phenylthio)silane (24 mL, 129 mmol) and TMSOTf (6.7 mL, 36.9 mmol) were added sequentially. The solution was stirred at O °C for 30 min and then warmed up to room temperature and stirred at this temperature for 12 h. Upon completion of the reaction, the mixture was diluted with CH_2Cl_2 (100 mL), washed with sat. $NaHCO_3$ (100 mL), brine (100 mL), and dried over anhydrous Na_2SO_4. The organic solvent was evaporated under reduced pressure and the crude product was purified by column chromatography (Toluene: EtOAc, 20:80 v/v) to give **7** as an off-white foam (15.5 g, 26.6 mmol,

79%). R_f: 0.51, Toluene: EtOAc (5:95); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.52-7.33 (m, 5H, ArH), 5.70 (d, *J* = 10.3 Hz, 1H, NH), 5.31-5.23 (m, 2H, H-7, H-8), 4.87-4.80 (m, 1H, H-4), 4.39 (dd, J = 12.4, 2.8 Hz, 1H, H-9a), 4.19 (dd, J = 12.4, 5.5 Hz, 1H, H-9b), 3.99 (dd, J = 12.3, 8.6 Hz, 1H, H-5), 3.89 (dd, J = 10.9, 1.6 Hz, 1H, H-6), 3.56 (s, 3H, OMe), 2.80 (dd, J = 13.1, 4.8 Hz, 1H, H-3eq), 2.10 – 2.08 (m, 7H, H-3ax, OAc) , 2.07 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 1.88 (s, 3H, NHAc); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.4, 170.9, 170.3, 170.2, 170.0, 168.4, 167.8, 136.0, 129.6, 128.8, 128.4, 88.9, 74.7, 73.0, 69.0, 67.6, 61.9, 52.5, 49.0, 37.4, 21.0, 20.8, 20.74, 20.72, 20.6; **LRMS** (ESI) m/z calcd for $C_{26}H_{33}NNaO_{12}S$ [M +Na] 606.16, found 606.21.

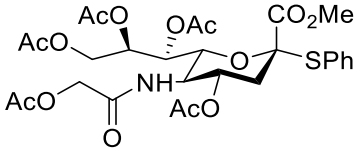
Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-5-benzyloxyacetamido-3,5-di-deoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranoside) onate (11)



Methanesulfonic acid (0.56 mL, 8.7 mmol) was added to a solution of **7** (5 g, 8.57 mmol) dissolved in anhydrous methanol (29 mL). The resulting mixture was heated to reflux (~68 °C) and stirred in the dark for 12 h. The solution was subsequently neutralized with Dowex $^-$OH ion exchange resin. Upon quenching, the resin was filtered, washed with MeOH and the resulting filtrate was concentrated *in vacuo*. The crude amine was used without further purification. Benzylglycolic acid *N*-hydroxysuccinimide ester **10** (2.7 g, 10.2 mmol) and Et_3N (1.51 mL, 10.8 mmol) were added to free amine **8** in CH_3CN: H_2O (20:1, 17 mL). The reaction mixture was stirred at room temperature for 24 h, and upon completion of the reaction (monitored by TLC), the solvent system was removed under reduced pressure. The crude product was solubilized in dry pyridine (72 mL) and treated with Ac_2O (5.48 mL, 58.0 mmol). The mixture was stirred at room temperature for 18 h. The resulting yellow solution was concentrated *in vacuo*, diluted in CH_2Cl_2 (100 mL) and washed sequentially with $NaHCO_3$ (100 mL), brine (2 x 50 mL) and H_2O (2 x 50 mL). The combined organic fractions were dried over Na_2SO_4 and the excess solvent was evaporated off under reduced pressure. The crude residue was purified by flash column chromatography (cHEX: EtOAc, 20:80 v/v) to give **11** as a white amorphous solid (3.60 g, 5.23 mmol, 61%). R_f: 0.51 (toluene: EtOAc 10:90, v/v); **^{1}H NMR** (500

MHz, $CDCl_3$) δ 7.53-7.51 (m, 2H, ArH), 7.40-7.32 (m, 8H, ArH), 6.32 (d, *J* = 10.3 Hz, 1H, NH), 5.33 - 5.29 (m, 2H, H-8, H-7), 4.86 (ddd, *J* = 11.7, 10.3, 4.6 Hz, 1H, H-4), 4.54 (p, 1H, CH_2Ph), 4.38 (dd, *J* = 12.6, 2.6 Hz, 1H, H-9a), 4.19 (dd, *J* = 12.4, 4.8 Hz, 1H, H-9b), 4.04 (q, *J* = 10.4 Hz, 1H, H-5), 3.94 (dd, *J* = 10.8, 1.9 Hz, 1H, H-6), 3.92-3.81 (m, 2H, CH_2CO), 3.58 (s, 3H, OMe), 2.86 (dd, *J* = 12.9, 4.7 Hz, 1H, H-3eq), 2.13 (s, 3H, OAc), 2.05-2.01 (m, 7H, 2 x OAc, H-3ax); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.8, 170.5, 170.2, 170.2, 170.0, 167.9, 136.6, 130.0, 129.0, 128.8, 87.6, 74.6, 73.7, 69.7, 69.6, 69.3, 67.6, 62.0, 52.9, 48.6, 38.4, 21.13, 21.10; **LRMS** (ESI) calculated for $C_{33}H_{39}NNaO_{13}S$ [M+Na] 712.20, found 712.41.

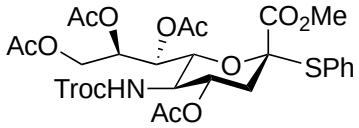
Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-5-acetoxyacetamido-3,5-di-deoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranoside) onate (12)



Methanesulfonic acid (0.73 mL, 11.31 mmol) was added to a solution of **7** (5.50 g, 9.43 mmol) dissolved in anhydrous methanol (37 mL). The resulting mixture was heated to reflux (68 °C) and stirred in the dark for 12 h. The solution was subsequently neutralized with Dowex $^-$OH ion exchange resin. Upon quenching, the resin was filtered, washed with MeOH and the resulting filtrate was concentrated *in vacuo*. The crude amine **8** was used without further purification. Acetylglycolic acid *N*-hydroxysuccinimide ester **9** (2.76 g, 12.9 mmol) and Et_3N (2.58 mL, 18.51 mmol) were added to free amine **8** in CH_3CN: H_2O (20:1, 25 mL). The reaction mixture was stirred at room temperature for 18 h, and upon completion of the reaction (monitored by TLC), the solvent system was removed under reduced pressure. The crude product was solubilized in dry pyridine (85 mL) and treated with Ac_2O (6.4 mL, 68.6 mmol). The mixture was stirred at room temperature for 18 h. The resulting yellow solution was concentrated *in vacuo*, diluted in CH_2Cl_2 (100 mL) and washed sequentially with $NaHCO_3$ (2 x 50 mL), brine (2 x 50 mL) and H_2O (2 x 50 mL). The combined organic fractions were dried over Na_2SO_4 and the excess solvent was evaporated off under reduced pressure. The crude residue was purified by flash column chromatography (cHEX: EtOAc, 20:80 v/v) to give **12** as a white amorphous solid (5.38 g, 8.39 mmol, 89%). R_f: 0.35, cHEX:EtOAc (10:90); **^{1}H NMR** (500 MHz,

$CDCl_3$) δ 7.55 – 7.12 (m, 5H, ArH), 6.16 (d, *J* = 10.3 Hz, 1H, NH), 4.83 – 4.78 (m, 2H, H-7, H-4), 4.39 – 4.32 (m, 1H, H-9a, H-8, H-6, H-5), 4.19 (dd, *J* = 12.4, 5.5 Hz, 1H, H-9b), 3.59 (s, 3 H, OMe), 2.26 (s, 3H, OAc) 2.21 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.04 (s, 3H, OAc); **LRMS** (ESI) calculated for $C_{28}H_{35}NNaO_{14}S$ [M + Na] 664.1, found 664.3. Spectral data matches those reported in literature.[1]

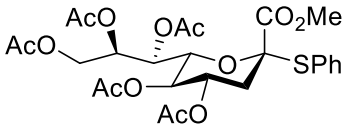
Methyl [phenyl 5-(2,2,2-trichloroethoxycarbonylamino)-4,7,8,9-tetra-*O*-acetyl-3,5-di-deoxy-2-thio-D-*glycero*-α--D-*galacto*-non-2-ulopyranosid] onate (13)



Methanesulfonic acid (0.41 mL, 6.37 mmol) was added to a solution of **7** (3.1 g, 5.31 mmol) dissolved in anhydrous methanol (21 mL). The resulting mixture was heated to reflux (68 °C) and stirred in the dark for 12 h. The solution was subsequently neutralized with Dowex $^-$OH ion exchange resin. Upon quenching, the resin was filtered, washed with MeOH and the resulting filtrate was concentrated *in vacuo*. Without further purification, crude amine **8** was solubilized in 1M $NaHCO_3$ (5.4 mL). A solution of succinimidyl 2,2,2-trichloroethyl carbonate (1.85 g, 6.37 mmol) in dioxane (5 mL) was added portion-wise and the resulting mixture was stirred at room temperature for 1 h until reaction when to completion. The dioxane layer was evaporated under reduced pressure and the remaining aqueous solution was extracted with EtOAc (2 x 50 mL) and concentrated *in vacuo*. The crude product **8** was solubilized in dry pyridine (26 mL) and treated with Ac_2O (4.01 mL, 42.48 mmol). The mixture was stirred at room temperature for 12 h. The resulting yellow solution was concentrated *in vacuo*, diluted in CH_2Cl_2 (100 mL) and washed sequentially with $NaHCO_3$ (2 x 50 mL), brine (2 x 50 mL) and H_2O (2 x 50 mL). The combined organic fractions were dried over Na_2SO_4 and the excess solvent was evaporated off under reduced pressure. The crude residue was purified by flash column chromatography (toluene: EtOAc, 20:80 v/v) to give **13** as a white amorphous solid (2.96 g, 4.14 mmol, 78%). R_f: 0.34 cHEX:EtOAc; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.65−7.63 (m, 2H, ArH), 7.37−7.30 (m, 3H, ArH), 4.96 (d, *J* = 12.3 Hz, 2H, H-9a, H-8), 4.70 (d, *J* = 12.3 Hz, 1H, H-9b), 4.59 (d, *J* = 10.3 Hz, 1H, H-7), 4.25 (ddd, *J* = 12.0, 11.8, 4.7 Hz, 1H, H-4), 3.88−3.55 (m, 2H,

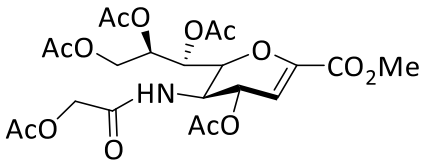
H-5, H-6), 3.45 (s, 3H, OMe), 2.69 (dd, *J* = 13.7, 4.7 Hz, 1H, H-3eq), 2.22 – 1.97 (m, 13H, OAc, H-3ax). Spectral data matches those reported in literature.[2]

Methyl (phenyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-2-thio-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid) onate (15)



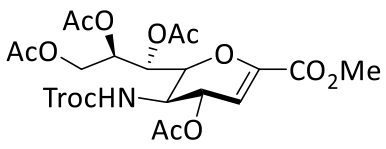
A solution of compound **7** (5.0 g, 8.5 mmol) in anhydrous CH_2Cl_2 (85 mL) was treated with anhydrous pyridine (6.8 mL, 85 mmol) and cooled to – 10 °C. After stirring at temperature for 30 min, $NOBF_4$ (3.97 g, 34 mmol) was added portion-wise. The reaction was stirred at – 10 °C until completion of the reaction (monitored by TLC). The solution was then diluted with cold CH_2Cl_2 (50 mL) and washed sequentially with 1N HCl (50 mL), sat. $NaHCO_3$ (50 mL) and brine (50 mL). The combined organic fractions were dried over Na_2SO_4 and concentrated under reduced pressure at 10 °C to afford the nitrosyl sialoside **14**, which was used in the next step without further purification. **14** was dissolved in dry CH_2Cl_2 (42.5 mL) and treated with trifluoroethanol (0.91 mL, 12.8 mmol). The solution was cooled to – 10 °C and then treated with 0.2 N sodium isopropoxide in isopropanol (51 mL, 10.2 mmol). The mixture was stirred for 5 min before charging the reaction vessel with pre-cooled solution of glacial acetic acid (10 mL) in CH_2Cl_2 (42.5 mL). After stirring at this temperature for 10 mins, the reaction was warmed to 0 °C and subsequently quenched with the addition of sat. $NaHCO_3$ (50 mL). The crude product was solubilized in dry pyridine (85 mL) and treated with Ac_2O (4.8 mL, 51 mmol). Upon completion of the reaction, the resulting organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4, concentrated *in vacuo* and the desired residue was purified by silica column chromatography to afford **15** as a white solid (1.88 g, 3.23 mmol, 38%). R_f: 0.21 toluene: EtOAc (10:90); **^{1}H NMR** (300 MHz, CD_3OD) δ 7.59 - 7.35 (m, 5H, ArH), 5.46 (dd, *J* = 9.9, 5.6 Hz, 1.3 Hz, 1H, H-6), 4.96 (ddd, *J* = 1H, H-4), 4.87 (dd, *J* = 9.2, 5.6 Hz 1H, H-7), 4.83 (dd, *J* = 10.9, 2.6 Hz, 1H, H-9a), 4.72 (ddd, *J* = 10.9, 9.2, 2.6 Hz, 1H, H-8), 4.66 (dd, *J* = 10.9, 5.3 Hz, 1H, H-9b), 3.71 (s, 3H, OMe), 3.58 (dd, *J* = 9.9, 9.2 Hz, 1H, H-5), 2.59 (dd, *J* = 13.9, 5.0 Hz, 1H, H-3eq), 2.25 – 1.97 (m, 15H, OAc), 1.88 (dd, *J* = 13.9 Hz, 11.9 Hz, 1H, H-3ax). Spectral data matches those reported in literature[3]

Methyl 4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-5-acetoxyacetamido-3,5-di-deoxy-D-*glycero*-D-*galacto*-non-2-enonate (28)



Molecular bromine (0.28 mL, 5.6 mmol) was added to a solution of **12** (3.1 g, 4.67 mmol) dissolved in anhydrous CH_2Cl_2 (46 mL) at 0 °C. The reaction mixture was warmed up to room temperature and stirred at this temperature for 1 h. Upon formation of the bromide intermediate (reaction progress was monitored by the disappearance of the starting material by TLC), anhydrous Et_3N (26.2 mL, 186.8 mmol) was added to the solution drop wise and the solution was stirred at room temperature for a further 1 h. Upon elimination of the bromide, the solution was diluted CH_2Cl_2 (50mL) and washed sequentially with $NaHCO_3$ (25 mL), water (25 mL) and brine (25 mL). The organic fractions were dried over Na_2SO_4 and concentrated *in vacuo*. The crude glycal was purified by flash column chromatography to give **28** (2.18 g, 4.11 mmol, 88%) as an off-white amorphous foam. R_f: 0.54 cHEX: EtOAc (20:80); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.34 (d, *J* = 9.5 Hz, 1H, NH), 5.98 (d, *J* = 2.9 Hz, 1H, H-3), 5.64 (dd, *J* = 7.5, 2.9 Hz, H-4), 5.34 – 5.31 (m, 2H, H-7, H-8), 4.63 (dd, *J* = 12.4, 7.1 Hz, H-9a), 4.56 (d, *J* = 13.1 Hz, 1H, CH_2OAc), 4.40 – 4.38 (m, 3H, H-9b, H-6, H-5), 4.37 (d, *J* = 13.1, 1H, CH_2OAc), 3.81 (s, 3H, OMe), 2.15 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.03 (s, 3H, OAc); **^{13}C NMR** (100 MHz, $CDCl_3$) δ 170.8–169.6 (C x 6), 161.4, 144.9, 108.1, 76.6, 70.8, 67.7, 67.5, 63.7, 61.8, 52.4, 46.3, 20.6–20.4 (C x 6); **LRMS** calculated for $C_{22}H_{30}NO_{14}$ [M + H] 532.16, found 531.6.

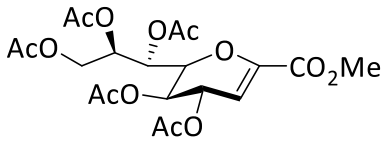
Methyl 4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-di-deoxy-5-trifluoropropanoyl-D-*glycero*-D-*galacto*-non-2-enonate (29)



Molecular bromine (0.22 mL, 4.19 mmol) was added to a solution of **13** (2.5 g, 3.49 mmol) dissolved in anhydrous CH_2Cl_2 (19 mL) at 0 °C. The reaction mixture was warmed up to room temperature and stirred at this temperature for 1 h. Upon formation of the bromide

intermediate (reaction progress was monitored by the disappearance of the starting material by TLC), anhydrous Et_3N (19.5 mL, 139 mmol) was added to the solution drop wise and the solution was stirred at room temperature for a further 1 h. Upon elimination of the bromide, the solution was diluted CH_2Cl_2 (40 mL) and washed sequentially with $NaHCO_3$ (20 mL), water (20 mL) and brine (20 mL). The organic fractions were dried over Na_2SO_4 and concentrated *in vacuo*. The crude glycal was purified by flash column chromatography to give **29** (1.79 g, 2.97 mmol, 85%) as an off-white amorphous foam. R_f: 0.49 cHEX: EtOAc (30:70); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.29 (d, J = 9.2 Hz, 1H, NH), 5.99 (d, *J* = 2.9 Hz, 1H, H-3), 5.65 (dd, *J* = 7.8, 2.9 Hz, 1H, H-4), 5.44 (dd, 1H, J = 4.6, 3.1 Hz, H-7), 5.35 (ddd, *J* = 7.0, 5.2, 3.5 Hz, 1H, H-8), 4.63 (dd, *J* = 12.2, 2.9 Hz, 1H, H-9), 4.57 (d, *J* = 15.3 Hz, 1H, CH_2Troc), 4.46 (dd, *J* = 8.2, 3.1 Hz, 1H, H-6), 4.39 – 4.37 (m, 1H), 4.36(td, *J* = 9.5, 7.8 Hz, 1H, H-5), 4.19 (dd, *J* = 12.2, 6.8 Hz, 2H, H-9), 3.81 (s, 3H,OMe), 2.17 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.06 (s, 3H, OAc). Spectral data matches those reported in literature.[4]

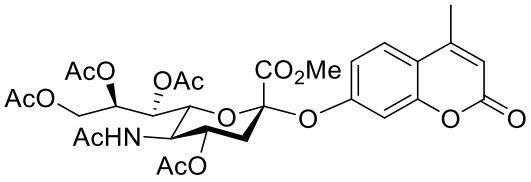
Methyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enonate (30)



Molecular bromine (0.16 mL, 3.08 mmol) was added to a solution of **15** (1.5 g, 2.57 mmol) dissolved in anhydrous CH_2Cl_2 (14.3 mL) at 0 °C. The reaction mixture was warmed up to room temperature and stirred at this temperature for 1 h. Upon formation of the bromide intermediate (reaction progress was monitored by the disappearance of the starting material by TLC), anhydrous Et_3N (14.3 mL, 102.8 mmol) was added to the solution drop wise and the solution was stirred at room temperature for a further 1 h. Upon elimination of the bromide, the solution was diluted CH_2Cl_2 (25mL) and washed sequentially with $NaHCO_3$ (50 mL), water (50 mL) and brine (50 mL). The organic fractions were dried over Na_2SO_4 and concentrated *in vacuo*. The crude glycal was purified by flash column chromatography to give **30** (1.16 g, 2.46 mmol, 96%) as an amorphous foam. R_f: 0.67 cHEX: EtOAc (30:70); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 5.92 (d, *J* = 3.8 Hz, 1H, H-3), 5.57 (dd, *J* = 7.3 Hz, 1H, H-7), 5.51 (q, *J* = 3.0 Hz, 1H, H-4), 5.35

(dt, *J* = 6.3 Hz, 1H, H-8), 5.23 (dd, *J* = 9.5, 7.5 Hz, 1H, H-6), 4.55 (dd, *J* = 12.5, 3.6 Hz, 1H, H-9a), 4.34 (dd, *J* = 9.5, 3.8 Hz, 1H, H-5), 4.11 (dd, *J* = 12.5, 6 Hz, 1H, H-9b), 3.87 (s, 3H), 2.12 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.069 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.05 (s, 3H, OAc); **^{13}C NMR** (125 MHz, CDCl3) δ: 170.8, 170.6, 170.1, 169.94, 169.91, 161.6, 145.5, 107.9, 75.8, 70.1, 69.0, 67.0, 65.8, 62.0, 52.8, 21.0, 20.9, 20.7; **HRMS** (ESI) calculated for $C_{20}H_{27}O_{13}$ [M + H] 475.1452, found 475.1450.

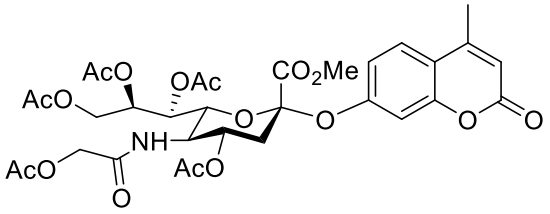
Methyl (4-methylcoumarin-7-yl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (17α)



A pre-oven dried round bottom flask containing compound **7** (3.5 g, 6.0 mmol), 4-methylumbelliferone (2.11 g, 12 mmol), pre-activated 4 Å molecular sieves and a stirring bar were dried on a Schlenk line for 45 min. The reaction vessel was then charged with anhydrous CH_2Cl_2 (15 mL) and CH_3CN (15 mL). The reaction mixture was cooled to -78 °C and sequentially NIS (2.7 g, 12 mmol), AgOTf (3.08 g, mmol) and TBAI (1.77 g, 4.8 mmol) were added to the solution. The suspension was stirred at -78 °C for 16 h, until the disappearance of the donor **7** was observed by TLC. The mixture was treated with Et_3N (5 mL), filtered through celite and the filtrate was concentrated *in vacuo*. The crude 4-Mu glycoside was purified by flash column chromatography (cHEX:EtOAc 1:9 → 100% EtOAc, v/v) to give **17α** (3.74 g, 5.76 mmol, 91%) as a white solid. R_f: 0.51 (cHEX:EtOAc, 1:9 v/v); **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.49 (d, *J* = 8.8 Hz, 1H, ArH), 7.04 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 6.98 (d, *J* = 2.4 Hz, 1H, ArH), 6.17 (d, *J* = 1.3 Hz, 1H, ArH), 5.71 (d, *J* = 10.0 Hz, 1H, NH), 5.35 – 5.32 (m, 2H, H-8, H-7), 4.95 (ddd, *J* = 12.1, 10.0, 4.7 Hz, 1H, H-4), 4.58 – 4.41 (m, 1H, H-6), 4.32 – 4.20 (m, 1H, H-9a), 4.13 – 4.05 (m, 2H, H-9b, H-5), 3.66 (s, 3H, OMe), 2.79 (dd, *J* = 13.0, 4.7 Hz, 1H, H-3eq), 2.39 (d, *J* = 1.3 Hz, 3H, 4-Mu-CH_3), 2.22 (dd, *J* = 13.0, 12.1 Hz, 1H, H-3ax), 2.11 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.89 (s, 3H, NHAc); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.7, 170.6, 170.3, 170.1, 169.9 (d, *J* = 0.8 Hz), 167.9, 160.8, 156.5, 154.3, 152.3, 125.7, 116.1, 115.6, 113.3, 107.6, 99.8,

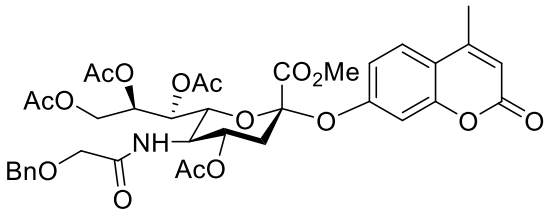
73.6, 69.0, 68.4, 67.3, 62.0, 53.1, 49.1, 38.1, 23.1, 20.9, 20.7, 20.7, 18.6; **HRMS** (ESI) calculated for $C_{30}H_{35}NNaO_{15}$ [M + Na] 672.1904, found 672.1909.

Methyl (4-methylcoumarin-7-yl-4,7,8,9-tetra-*O*-acetyl-5-acetoxyacetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (22α)



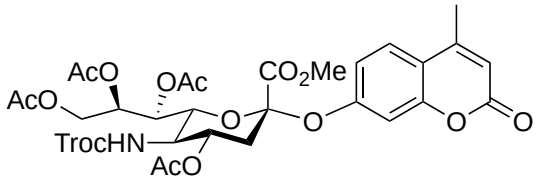
A pre-oven dried round bottom flask containing compound **12** (5.0 g, 7.79 mmol), 4-methylumbelliferone (2.74 g, 15.58 mmol), pre-activated 4 Å molecular sieves and a stirring bar were dried on a Schlenk line for 45 min. The reaction vessel was then charged with anhydrous CH_2Cl_2 (19mL) and CH_3CN (19 mL). The reaction mixture was cooled to -78 °C and sequentially NIS (3.5 g, 15.58 mmol), AgOTf (4.0 g, 15.58 mmol) and TBAI (2.30 g, 6.23 mmol) were added to the solution. The suspension was stirred at -78 °C for 24 h, until the disappearance of the donor **12** was observed by TLC. The mixture was treated with Et_3N (5 mL), filtered through celite and the filtrate was concentrated *in vacuo*. The crude 4-Mu glycoside was purified by flash column chromatography (cHEX:EtOAc 1:9 → 100% EtOAc, v/v) to give **22α** (3.97 g, 5.60 mmol, 72%) as a white solid. R_f: 0.56 (cHEX:EtOAc, 1:9 v/v); **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.54 (d, *J* = 9.1 Hz, 1H, ArH), 7.09 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.03 (d, *J* = 2.3 Hz, 1H, ArH), 6.22 (d, *J* = 1.3 Hz, 1H, ArH), 5.76 (d, *J* = 9.7 Hz, 1H, NH), 5.40 (dt, *J* = 2.9, 1.4 Hz, 2H, H-8, H-7), 5.01 (ddd, *J* = 12.0, 10.3, 4.7 Hz, 1H, H-4), 4.64 – 4.47 (m, 1H, H-6, CH_2OAc), 4.37–4.25 (m, 2H, H-9a, CH_2OAc), 4.19 – 4.10 (m, 2H, H-9b, H-5), 3.71 (s, 3H, OMe), 2.75 (dd, *J* = 13.0, 4.7 Hz, 1H, H-3eq), 2.44 (d, *J* = 1.3 Hz, 3H, 4-Mu-CH_3), 2.27 (dd, *J* = 13.0, 12.1 Hz, 4H, H-3ax, OAc), 2.17 (s, 3H, OAc), 2.16 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.06 (s, 6H, 2 x OAc); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.6, 170.4, 170.2, 170.0, 169.8, 167.8, 160.7, 156.4, 154.26, 152.23, 125.5, 116.0, 115.4, 113.2, 107.5, 99.7, 73.5, 68.9, 68.3, 67.2, 61.9, 53.0, 49.0, 38.0, 23.0, 20.8, 20.6, 20.5, 18.5, 14.0; **HRMS** (ESI) calculated for $C_{32}H_{38}NO_{17}$ [M + H] 708.2140, found 708.2137.

Methyl (4-methylcoumarin-7-yl-4,7,8,9-tetra-*O*-acetyl-5-benzyloxyacetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (21α)



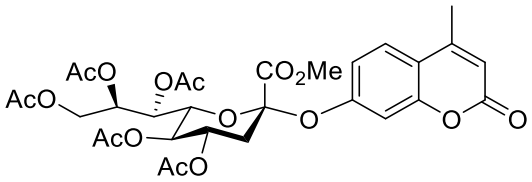
A pre-oven dried round bottom flask containing compound **11** (3.25 g, 4.72 mmol), 4-methylumbelliferone (1.66 g, 9.44 mmol), pre-activated 4 Å molecular sieves and a stirring bar were dried on a Schlenk line for 45 min. The reaction vessel was then charged with anhydrous CH_2Cl_2 (11 mL) and CH_3CN (11 mL). The reaction mixture was cooled to -78 °C and sequentially NIS (2.12 g, 9.44 mmol), AgOTf (2.43 g, 9.44 mmol) and TBAI (1.39 g, 3.77 mmol) were added to the solution. The suspension was stirred at -78 °C for 24 h, until the disappearance of the donor **11** was observed by TLC. The mixture was treated with Et_3N (8 mL), filtered through celite and the filtrate was concentrated *in vacuo*. The crude 4-Mu glycoside was purified by flash column chromatography (cHEX:EtOAc 1:9 → 100% EtOAc, v/v) to give **21α** (3.17 g, 4.20 mmol, 89%) as an amorphous foam. R_f: 0.54 (cHEX:EtOAc, 1:9 v/v); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.54 (d, *J* = 9.2 Hz, 1H, ArH), 7.09 (dd, *J* = 9.2, 2.1 Hz, 1H), 7.03 (d, *J* = 2.1 Hz, 1H, ArH), 6.22 (d, *J* = 1.3 Hz, 1H, ArH), 5.81 (d, *J* = 9.8 Hz, 1H, NH), 5.40 (dd, *J* = 4.8, 2.7 Hz, 1H, H-8), 5.38 - 5.36 (m, 1H, H-7), 4.90 (td, *J* = 11.2, 4.8 Hz, 1H, H-4), 4.63-4.51 (m, 2H, CH_2Ph), 4.31 (dd, *J* = 12.1, 2.6 Hz, 1H, H-9a), 4.21-4.07 (m, 2H, H-9b, H-5), 3.90-3.86 (m, 4H, OMe, H-6), 3.82 (s, 3H, OCH3), 2.76 (dd, *J* = 12.4, 4.8 Hz, 1H, H-3eq), 2.46 (d, *J* = 1.3 Hz, 3H, 4-Mu-CH_3), 2.18 (s, 3H, OAc), 2.13 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.97-1.93 (m, 1H, H-3ax); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.7, 170.6, 170.2, 170.2, 170.0, 167.9, 136.6, 130.0, 129.0, 128.8, 87.6, 74.6, 73.7, 69.7, 69.6, 69.3, 67.6, 62.0, 52.9, 48.6, 39.4, 21.4, 21.0; **HRMS** (ESI) calculated for $C_{37}H_{41}NNaO_{16}$ [M + Na] 778.2323, found 778.2327.

Methyl (4-methylcoumarin-7-yl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoropropanoyl-α-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (23α)



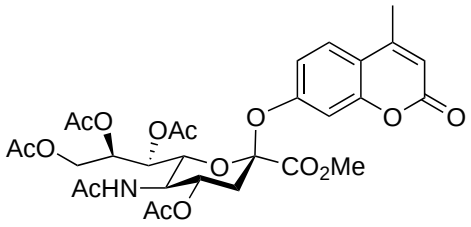
A pre-oven dried round bottom flask containing compound **13** (2.83 g, 3.96 mmol), 4-methylumbelliferone (1.39 g, 7.92 mmol), pre-activated 4 Å molecular sieves and a stirring bar were dried on a Schlenk line for 45 min. The reaction vessel was then charged with anhydrous CH_3CN and CH_2Cl_2 (19.8 mL). The reaction mixture was cooled to -78 °C and sequentially NIS (1.78 g, 7.92 mmol), AgOTf (2.03 g, 7.92 mmol) and TBAI (1.17 g, 3.17 mmol) were added to the solution. The suspension was stirred at -78 °C for 24 h, until the disappearance of the donor **13** was observed by TLC. The mixture was treated with Et_3N (5 mL), filtered through celite and the filtrate was concentrated *in vacuo*. The crude 4-Mu glycoside was purified by flash column chromatography (cHEX:EtOAc 1:9 → 100% EtOAc, v/v) to give **23α** (2.32 g, 2.97 mmol, 75%) as a white solid. R_f: 0.54 (cHEX:EtOAc 1:9 v/v); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.8 Hz, 1H, ArH), 7.08 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 7.02 (d, *J* = 2.4 Hz, 1H, ArH), 6.21 (d, *J* = 1.3 Hz, 1H, 4-Mu), 5.40 – 5.35 (m, 2H, H-8, H-7), 5.27 (d, *J* = 10.3 Hz, 1H, NH), 5.00 (ddd, *J* = 12.1, 10.3, 4.6 Hz, 1H, H-4), 4.52 (dd, *J* = 10.7, 1.5 Hz, 1H, H-6), 4.42 – 4.38 (m, 1H), 4.31 (dd, *J* = 12.6, 2.1 Hz, 1H, H-9a), 4.17 – 4.08 (m, 3H, H-9b, H-5), 3.71 (s, 3H, OMe), 2.74 (dd, *J* = 13.1, 4.6 Hz, 1H, H-3eq), 2.43 (d, *J* = 1.3 Hz, 3H, 4-Mu-CH_3), 2.27 (dd, *J* = 13.1, 12.1 Hz, 1H, H-3ax), 2.17 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.06 – 2.05 (m, 6H, 2 x OAc); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.0, 170.9, 170.6, 170.4, 170.3, 168.2, 161.2, 156.9, 154.6, 152.6, 126.0, 116.4, 115.9, 113.6, 107.9, 100.1, 74.0, 69.3, 68.7, 67.6, 62.3, 53.4, 49.5, 38.4, 23.4, 21.2, 21.1, 21.0, 18.9; **HRMS** (ESI) calculated for $C_{31}H_{34}Cl_3NNaO_{16}$ [M + Na] 804.0841, found 804.0836.

Methyl (4-methylcoumarin-7-yl-4,5,7,8,9-penta-*O*-acetyl-3-deoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (24α)



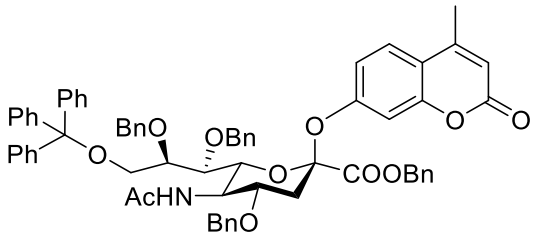
A pre-oven dried round bottom flask containing compound **15** (1.72 g, 2.94 mmol), 4-methylumbelliferone (1.04 g, 5.89 mmol), pre-activated 4 Å molecular sieves and a stirring bar were dried on a Schlenk line for 45 min. The reaction vessel was then charged with anhydrous CH_2Cl_2 (7.2 mL) and CH_3CN (7.2 mL). The reaction mixture was cooled to -78 °C and sequentially NIS (1.32 g, 5.89 mmol, 2eq), AgOTf (1.51 g, 5.89 mmol) and TBAI (0.87 g, 2.35 mmol) were added to the solution. The suspension was stirred at -78 °C for 24 h, until the disappearance of the donor **15** was observed by TLC. The mixture was treated with Et_3N (3 mL), filtered through celite and the filtrate was concentrated *in vacuo*. The crude 4-Mu glycoside was purified by flash column chromatography (cHEX:EtOAc 1:9 → 100% EtOAc, v/v) to give **24α** (1.28 g, 1.97 mmol, 67%) as a white solid. R_f: 0.49 (cHEX:EtOAc 1:9) ; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.50 – 7.45 (m, 2H, ArH), 6.99 (d, *J* = 2.6 Hz, 1H, ArH), 6.18 (d, *J* = 1.5 Hz, 1H, ArH), 5.36 – 5.32 (m, 2H, H-8, H-7), 4.97 (dd, *J* = 12.1, 4.7 Hz, 1H, H-4), 4.60 – 4.43 (m, 1H, H-8), 4.33 – 4.21 (m, 1H, H-9a), 4.15 – 4.06 (m, 2H, H-5, H-9b), 3.67 (s, 3H, OMe), 2.76 (dd, *J* = 12.8, 4.7 Hz, 1H,), 2.40 (d, *J* = 1.3 Hz, 3H, 4-Mu-CH_3), 2.17 – 2.13 (m, 4H, OAc, H-3ax), 2.12 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.02 (s, 6H, 2 x OAc); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.6, 172.4, 172.2, 172.0, 171.8, 169.8, 162.7, 158.4, 156.2, 154.2, 127.5, 117.9, 117.4, 115.2, 109.5, 101.7, 75.5, 70.9, 70.3, 69.1, 63.9, 55.0, 51.0, 40.2, 25.0, 22.8, 22.6, 22.54, 20.51, 20.4; **HRMS** (ESI) calculated for $C_{30}H_{34}NaO_{16}$ [M + Na] 673.1745, found 673.1750.

Methyl (4-methylcoumarin-7-yl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (17β)



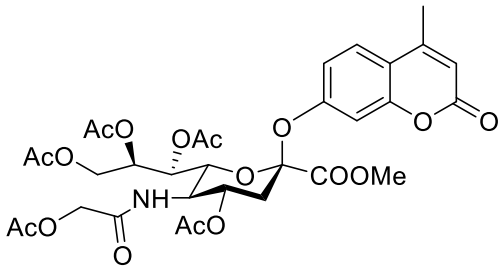
Compound **18**[5] (1.15 g, 2.43 mmol) was dissolved in anhydrous CH_3CN (7 mL) and CH_2Cl_2 (7 mL) and at O °C 4-Mu (1.07 g, 6.08 mmol), (Diacetoxyiodo)benzene (0.94 g, 2.92 mmol) and iodine (0.37 g, 1.46 mmol) were added sequentially. The reaction vessel was then removed from the ice bath and stirred at ambient temperature for 30 min. The reaction mixture was diluted in ethyl acetate (10 mL) and washed sequentially with Na_2SO_3 (2 x 10 mL), water (2 x 10 mL) and brine (2 x 10 mL). The combined organic fractions were dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash column chromatography (cHEX/EtOAc, 20:80 → 100% EtOAc, v/v) to give the desired epimer. The resulting residue was solubilised in toluene (10 mL), which was degassed by N_2 bubbling and freeze-pump-thaw method. Bu_3SnH (2.83 g, 9.72 mmol) and AIBN (59 mg, 0.36 mmol) were added. The reaction was heated to reflux overnight in the dark. The crude product was purified by flash column chromatography (cHEX/EtOAc, 70:30 v/v) to afford **17β** (947 mg, 1.46 mmol, 60%). R_f: 0.50, cHEX/EtOAc (10:90); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.50 (d, *J* = 8.8 Hz, 1H, ArH), 7.05 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 6.99 (d, *J* = 2.4 Hz, 1H, ArH), 6.19 (d, *J* = 1.3 Hz, 1H, ArH), 5.70 (d, *J* = 10.0 Hz, 1H, NH), 5.35 – 5.34 (m, 2H, H-8, H-7), 4.96 (ddd, *J* = 12.1, 10.0, 4.7 Hz, 1H, H-4), 4.51 – 4.41 (m, 1H, H-6), 4.32 – 4.22 (m, 1H, H-9a), 4.14 – 4.07 (m, 2H, H-9b, H-5), 3.68 (s, 3H, OMe), 2.51 (dd, *J* = 12.8, 4.7 Hz, 1H, H-3eq), 2.40 (d, *J* = 1.3 Hz, 3H, 4-Mu-CH_3), 2.22 (dd, *J* = 12.8, 12.1 Hz, 1H, H-3ax), 2.12 (s, 3H, OAc), 2.10 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.88 (s, 3H, NHAc); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.9, 170.7, 170.19, 170.12, 169.1, 167.6, 160.8, 157.1, 154.3, 152.3, 125.7, 119.1, 115.6, 113.3, 107.6, 99.8, 73.6, 69.0, 68.4, 67.3, 62.0, 54.2, 49.1, 38.1, 23.3, 20.8, 20.74, 20.70, 18.9; **HRMS** (ESI) calculated for $C_{30}H_{35}NNaO_{15}$ [M + Na] 672.1904, found 672.1901.

Benzyl (4-methylcoumarin-7-yl-5-acetamido-4,7,8-tri-*O*-benzyl-3,5-dideoxy-9-*O*-trityl-β-D-*glycero*-D-*galacto*-non-2- nonulopyranosid) onate (27)



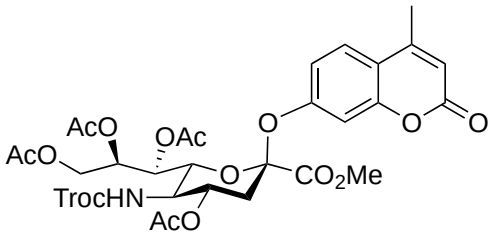
Compound **25** (1.23 g, 1.38 mmol) was dissolved in anhydrous CH_3CN (5.52 mL) and at O °C 4-Mu (0.61 g, 3.45 mmol), (Diacetoxyiodo)benzene (0.53 g, 1.66 mmol) and iodine (0.21 g, 0.83 mmol) were added sequentially. The reaction vessel was then removed from the ice bath and stirred at ambient temperature for 30 min. The reaction mixture was diluted in ethyl acetate (15 mL) and washed sequentially with Na_2SO_3 (2 x 10 mL), water (2 x 10 mL) and brine (2 x 10 mL). The combined organic fractions were dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash column chromatography (cHEX/EtOAc, 20:80 → 100% EtOAc, v/v) to give the desired epimer. The resulting residue was solubilised in toluene (5.5 mL), which was degassed by N_2 bubbling and freeze-pump-thaw method. Bu_3SnH (1.61 g, 5.52 mmol) and AIBN (1.38 g, 0.22 mmol) were added. The reaction was heated to reflux overnight in the dark. The crude product was purified by flash column chromatography (cHEX/EtOAc, 70:30 v/v) to afford **27** (1.31 g, 89%). R_f: 0.43, cHEX/EtOAc (20:80); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.62 – 7.48 (m, 10H, ArH), 7.41 – 7.34 (m, 8H, ArH), 7.30 – 7.17 (m, 20H, ArH), 7.17 – 7.12 (m, 2H, ArH), 6.17 (d, *J* = 1.5 Hz, 1H, ArH),), 5.23 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 5.16 (d, *J* = 12.3 Hz, 2H, CH_2Ph), 4.74 (ddd, *J* = 6.3, 4.3, 4.1 Hz, 1H, H-8), 4.68 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.51 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.44 (dd, *J* = 11.5, 6.3 Hz, 2H, H-7), 4.35 (d, *J* = 12.1 Hz, 1H, CH_2Ph), 4.27 – 4.23 (m, 2H, CH_2Ph, H-4), 4.21 – 4.17 (m, 1H, H-5), 3.92 (dt, *J* = 6.0, 4.1 Hz, 1H, H-6), 3.81 (dd, *J* = 10.1, 4.3 Hz, 1H, H-9a), 3.44 (dd, *J* = 10.1, 4.0 Hz, 1H, H-9b), 2.42 (dd, *J* = 13.0, 12.1 Hz, 1H, H-3eq), 2.37 (d, *J* = 1.5 Hz, 3H, 4-Mu-CH_3), 1.84 (s, 3H, NHAc);**^{13}C NMR** (151 MHz, CD_3OD) δ 166.9, 143.9, 137.8, 135.1, 129.42 – 125.69, 100.6, 77.2, 74.6, 74.4, 70.3, 67.2, 66.7, 21.6. **HRMS** (ESI) calculated for $C_{68}H_{64}NO_{11}$ [M + H] 1070.4479, found 1070.4481.

Methyl (4-methylcoumarin-7-yl-4,7,8,9-tetra-*O*-acetyl-5-acetoxyacetamido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (22β)



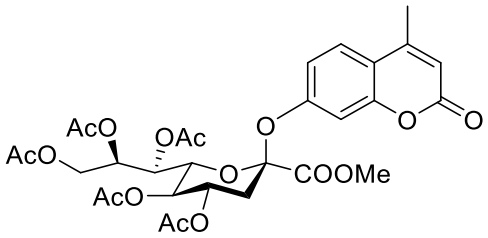
Compound **28** (1.98 g, 3.72 mmol) was dissolved in anhydrous CH_3CN (7.8 mL) and CH_2Cl_2 (7.8 mL), and at O °C 4-Mu (1.64 g, 9.3 mmol), (Diacetoxyiodo)benzene (1.44 g, 4.46 mmol) and iodine (0.57 g, 2.23 mmol) were added sequentially. The reaction vessel was then removed from the ice bath and stirred at ambient temperature for 30 min. The reaction mixture was diluted in ethyl acetate and washed sequentially with Na_2SO_3 (2 x 5 mL), water (2 x 5 mL) and brine (2 x 5 mL). The combined organic fractions were dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash column chromatography (cHEX/EtOAc, 20:80 → 100% EtOAc, v/v) to give the desired epimer. The resulting residue was solubilised in toluene (14 mL), which was degassed by N_2 bubbling and freeze-pump-thaw method. Bu_3SnH (4.33 g, 14.90 mmol) and AIBN (0.61 g, 3.72 mmol) were added. The reaction was heated to reflux overnight in the dark. The crude product was purified by flash column chromatography (cHEX/EtOAc, 10:90 → 100% EtOAc, v/v) to afford **22β** (1.79 g, 2.53 mmol, 68%). R_f: 0.48 (cHEX:EtOAc, 1:9 v/v); **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.54 (d, *J* = 9.1 Hz, 1H, ArH), 7.09 (dd, *J* = 9.1, 2.3 Hz, 1H, ArH), 7.03 (d, *J* = 2.3 Hz, 1H, ArH), 6.22 (d, *J* = 1.3 Hz, 1H, ArH), 5.76 (d, *J* = 9.7 Hz, 1H, NH), 5.40 – 5.39 (m, 2H, H-8, H-7), 5.01 (ddd, *J* = 12.0, 10.3, 4.7 Hz, 1H, H-4), 4.64 – 4.47 (m, 1H, H-6, CH_2OAc), 4.37 – 4.25 (m, 2H, H-9a, CH_2OAc), 4.19 – 4.10 (m, 2H, H-9b, H-5), 3.71 (s, 3H, OMe), 2.52 (dd, *J* = 12.5, 4.7 Hz, 1H, H-3eq), 2.44 (d, *J* = 1.3 Hz, 3H, 4-Mu-CH_3), 2.27 – 2.24 (m, 1H, H-3ax), 2.17 (s, 3H, OAc), 2.16 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.06 (s, 6H, 2 x OAc); **^{13}C NMR** (151 MHz, $CDCl_3$) δ 170.6, 170.4, 170.2, 170.0, 169.8, 167.8, 160.7, 156.4, 154.26, 152.23, 125.5, 116.0, 115.4, 113.2, 107.5, 99.7, 73.5, 68.9, 68.3, 67.2, 61.9, 53.0, 49.0, 38.0, 23.0, 20.8, 20.6, 20.5, 18.5, 14.0; **HRMS** (ESI) calculated for $C_{32}H_{37}NNaO_{17}$ [M + Na] 730.1959, found 730.1957.

Methyl (4-methylcoumarin-7-yl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoropropanoyl-β-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (23β)



Compound **29** (1.51 g, 2.49 mmol) was dissolved in anhydrous CH_3CN (4.9mL) and CH_2Cl_2 (4.9 mL), and at O °C 4-Mu (1.09 g, 6.23 mmol), (Diacetoxyiodo)benzene (0.53 g, 2.99 mmol) and iodine (0.38 g, 1.49 mmol) were added sequentially. The reaction vessel was then removed from the ice bath and stirred at ambient temperature for 30 min. The reaction mixture was diluted in ethyl acetate and washed sequentially with Na_2SO_3 (2 x 5 mL), water (2 x 5 mL) and brine (2 x 5 mL). The combined organic fractions were dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash column chromatography (cHEX/EtOAc, 20:80 → 100% EtOAc, v/v) to give the desired epimer. The residue was then solubilised in toluene (9 mL), which was degassed by N_2 bubbling and freeze-pump-thaw method. Bu_3SnH (2.90 g, 9.96 mmol) and AIBN (2.49 g 0.41 mmol) were added. The reaction was heated to reflux overnight in the dark. The crude product was purified by flash column chromatography (cHEX/EtOAc, 10:90 → 100% EtOAc, v/v) to afford **23β** (1.57 g, 2.02 mmol, 71%). R_f: 0.43 (cHEX:EtOAc, 1:9 v/v) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.55 (d, *J* = 8.7 Hz, 1H, ArH), 7.09 (dd, *J* = 8.7, 2.4 Hz, 1H, ArH), 7.02 (d, *J* = 2.4 Hz, 1H, ArH), 6.21 (d, *J* = 1.3 Hz, 1H, 4-Mu), 5.40 – 5.35 (m, 2H, H-8, H-7), 5.28 (d, *J* = 10.2 Hz, 1H, NH), 5.02 (ddd, *J* = 12.1, 10.3, 4.6 Hz, 1H, H-4), 4.53 (dd, *J* = 10.7, 1.5 Hz, 1H, H-6), 4.42 – 4.38 (m, 1H), 4.31 (dd, *J* = 12.6, 2.1 Hz, 1H, H-9a), 4.17 – 4.08 (m, 3H, H-9b, H-5), 3.71 (s, 3H, OMe), 2.48 (dd, *J* = 12.1, 4.6 Hz, 1H, H-3eq), 2.43 (d, *J* = 1.3 Hz, 3H, 4-Mu-CH_3), 2.27 -2.25 (m, 1H, H-3ax), 2.17 (s, 3H, OAc), 2.15 (s, 3H, OAc), 2.08 (d, *J* = 8.7 Hz, H, OAc), 2.06 – 2.05 (m, 6H, 2 x OAc); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.2, 170.9, 170.6, 170.4, 170.3, 168.2, 161.2, 156.9, 154.6, 152.6, 126.0, 116.4, 115.9, 113.6, 107.9, 100.1, 74.0, 69.3, 68.7, 67.6, 62.3, 53.4, 49.5, 38.4, 23.4, 21.2, 21.1, 21.0, 18.9; **HRMS** (ESI) calculated for $C_{31}H_{34}Cl_4NO_{16}$ [M + Cl] 816.0632, found 816.0641

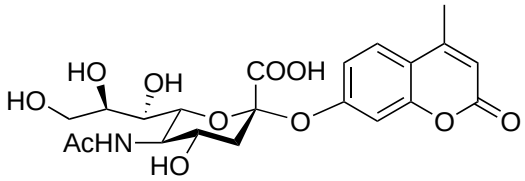
Methyl (4-methylcoumarin-7-yl-4,5,7,8,9-penta-*O*-acetyl-3-deoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (24β)



Compound **30** (960 mg, 2.02 mmol) was dissolved in anhydrous CH_3CN (4.1 mL) and CH_2Cl_2 (4.1 mL), and at O °C 4-Mu (0.89 g, 5.06 mmol), (Diacetoxyiodo)benzene (0.78 g, 2.42 mmol) and iodine (0.31 g, 1.2 mmol) were added sequentially. The reaction vessel was then removed from the ice bath and stirred at ambient temperature for 30 min. The reaction mixture was diluted in ethyl acetate and washed sequentially with Na_2SO_3 (2 x 5 mL), water (2 x 5 mL) and brine (2 x 5 mL). The combined organic fractions were dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash column chromatography (cHEX/EtOAc, 20:80 → 100% EtOAc, v/v) to give the desired epimer. The residue was solubilised in toluene (8.5 mL), which was degassed by N_2 bubbling and freeze-pump-thaw method. Bu_3SnH (2.35 g, 8.08 mmol) and AIBN (0.33 g, 2.02 mmol) were added. The reaction was heated to reflux overnight in the dark. The crude product was purified by flash column chromatography (cHEX/EtOAc, 10:90 → 100% EtOAc, v/v) to afford **24β** (906 mg, 1.39 mmol, 62%). R_f: 0.51(cHEX:EtOAc, 1:9 v/v) ; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.52 – 7.48 (m, 2H, ArH), 6.99 (d, *J* = 2.6 Hz, 1H, ArH), 6.18 (d, *J* = 1.5 Hz, 1H, ArH), 5.38 – 5.34 (m, 2H, H-8, H-7), 4.97 (dd, *J* = 11.9, 4.7 Hz, 1H, H-4), 4.60 – 4.43 (m, 1H, H-8), 4.33 – 4.21 (m, 1H, H-9a), 4.15 – 4.06 (m, 2H, H-5, H-9b), 3.67 (s, 3H, OMe), 2.56 (dd, *J* = 12.1, 4.7 Hz, 1H, H-3eq), 2.40 (d, *J* = 1.3 Hz, 3H, 4-Mu-CH_3), 2.18 – 2.15 (m, 4H, OAc, H-3ax), 2.12 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.05 (s, 6H, OAc); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.9, 172.4, 172.2, 172.0, 171.8, 169.8, 162.7, 158.4, 156.2, 154.2, 127.5, 117.9, 117.4, 115.2, 109.5, 101.7, 75.5, 70.9, 70.3, 69.1, 63.9, 55.0, 51.0, 40.2, 25.0, 22.8, 22.6, 22.5, 20.56, 20.53; **HRMS** (ESI) calculated for $C_{30}H_{35}O_{16}$ [M + H] 651.1925, found 651.1931.

Methanolysis/hydrolysis of esters in 17α and General procedure for the deprotection of C-5 functionalised 4-Mu-neuraminic acid derivatives:

4-Methylcoumarin-7-yl (5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulo-pyranosidonic acid (S1-α-Mu)



Compound **17α** (1.41 g, 2.17 mmol) was dissolved in methanol (10 mL) and NaOMe (11 mg, 0.43 mmol) was added to the solution at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred overnight. Upon complete de-acetylation (monitored by TLC analysis; EtOAc:MeOH, 90:10 v/v), water (10 mL) and further NaOMe (11 mg, 4.3 mmol) was added to the mixture. Upon the hydrolysis of the methyl ester (6 h), the reaction was quenched with Amberlyst® 15 hydrogen form resin and the stirred at room temperature for 20 min. The resin was subsequently filtered off and reaction mixture was concentrated *in vacuo* to give compound **S1-α-Mu** as a white amorphous solid (812 mg, 1.74 mmol, 80%). **^{1}H NMR** (600 MHz, D_2O) δ 7.70 – 7.66 (m, 1H, ArH), 7.13 – 7.10 (m, 2H, ArH), 6.22 (s, 1H, ArH), 3.99 (dd, *J* = 10.7, 1.4 Hz, 1H), 3.87 (t, *J* = 10.1 Hz, 1H), 3.81 – 3.77 (m, 2H), 3.73– 3.70 (m, 1H), 3.55 (dd, *J* = 12.0, 6.2 Hz, 1H), 3.51 (dd, *J* = 9.2, 1.4, 1H), 2.78 (dd, *J* = 12.6, 4.7 Hz, 1H), 2.39 (s, 3H), 1.96 (s, 3H); **^{13}C NMR** (125 MHz, D_2O) δ 178.6, 173.5, 164.9, 159.0, 153.6, 125.9, 117.4, 113.2, 111.2, 109.9, 108.2, 73.7, 76.9, 68.5, 67.6, 62.8, 52.1, 40.6, 22.3, 18.1; **LRMS** (ESI) calculated for $C_{21}H_{25}NNaO_{11}$ [M + Na] 490.13, found 490.13. Spectral data matches those reported in literature[6].

Apart from compounds **23α/β (**which requires prior Troc protecting group removal)**,** this conventional de-esterification (1-pot deacetylation and methyl ester saponification) strategy can be utilised to deprotect the remaining C-5 functionalised 4-methylumbelliferyl sialic acid derivatives prepared in this manuscript.

References:

(1) Marra, A.; Sinaÿ, P. *Carbohydr. Res.* 1989, **187** , 35–42.

(2) Crich, D.; Li, *J. Org. Chem.* 2007, **72**, 2387–2391.

(3) Akai, S.; Nakagawa, T.;. *J. Carbohydr. Chem.* 1999, **18**, 639–654.

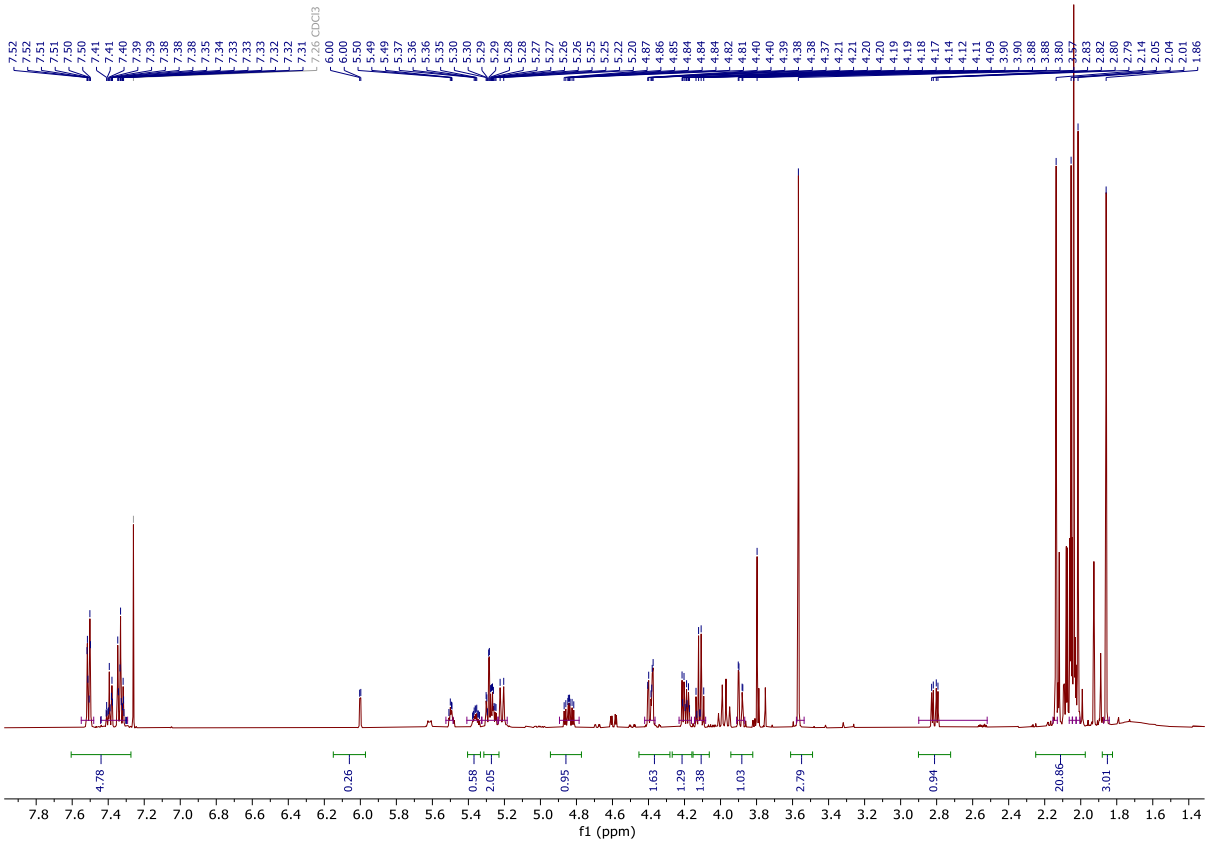
(4) Numata, M.; Sugimoto, M.; Shibayama, S.; Ogawa, T. A Total Synthesis of Hematoside, α-NeuGc-(2→3)-β-Gal-(1→4)-β-Glc-(1→1)-Cer. *Carbohydr. Res.* 1988, **174**, 73–85.

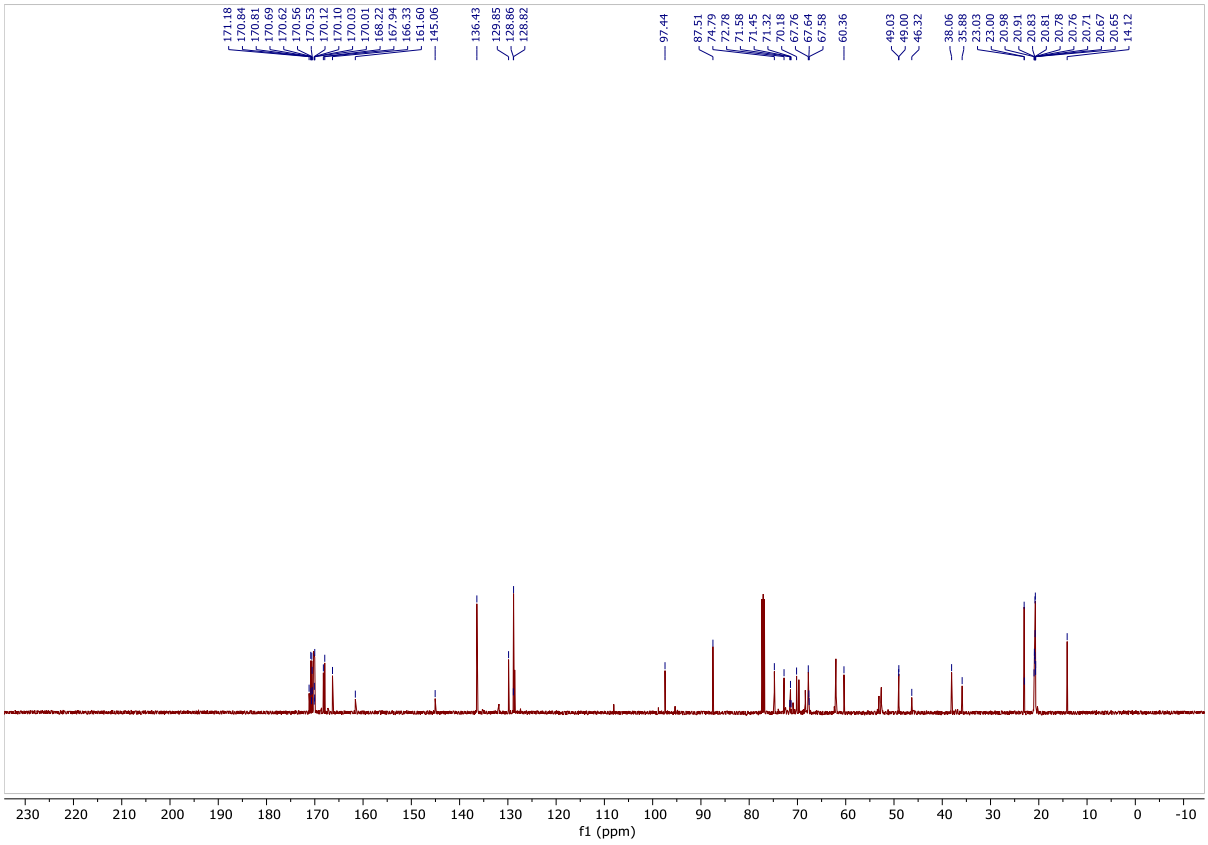
(5) A. A. Hassan and S. Oscarson, *Eur. J. Org. Chem.*, 2020, 6102-6108

(6) C. Y. Zamora, M. D'Alarcao and K. Kumar, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 3406–3410.

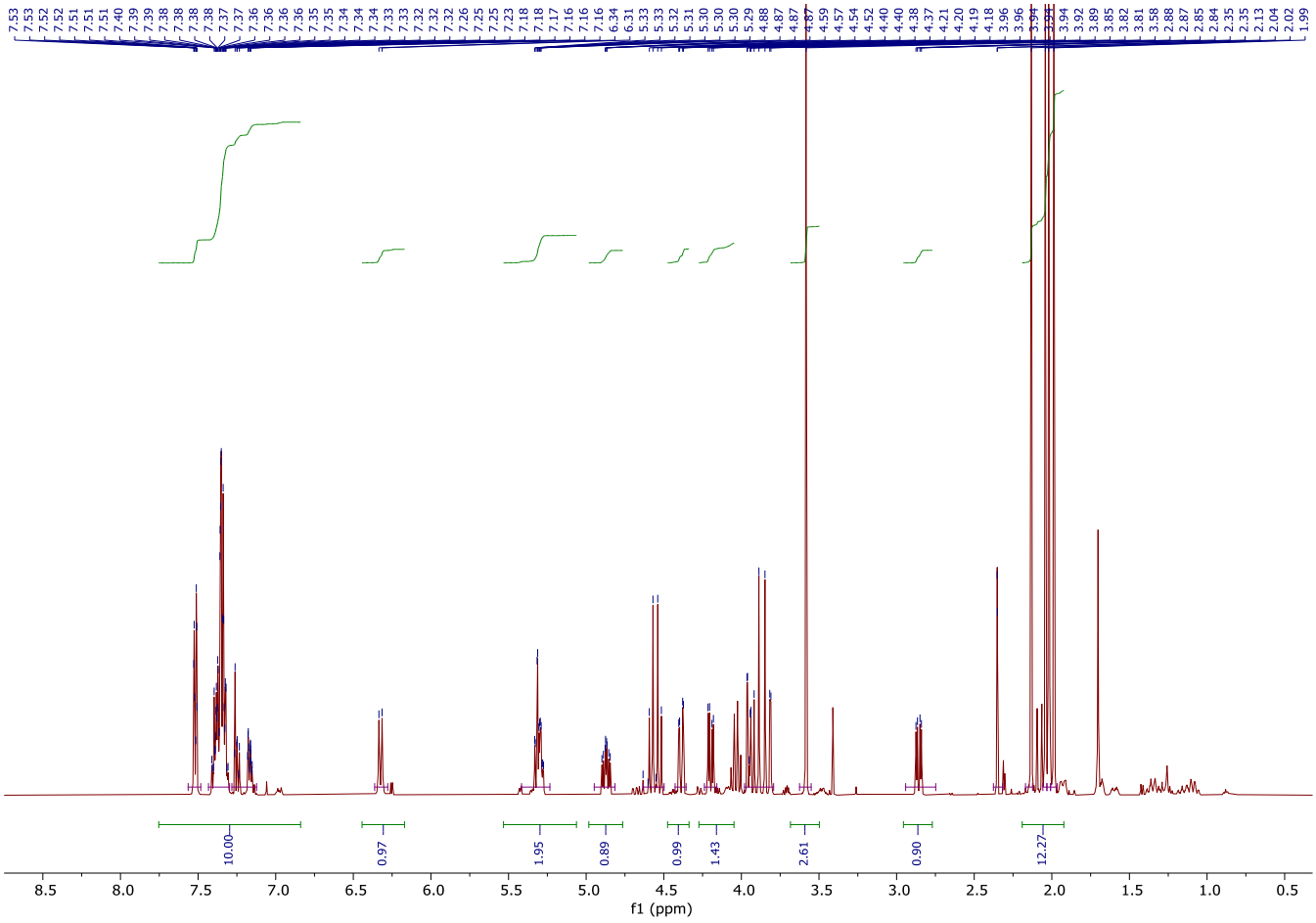
NMR Spectra

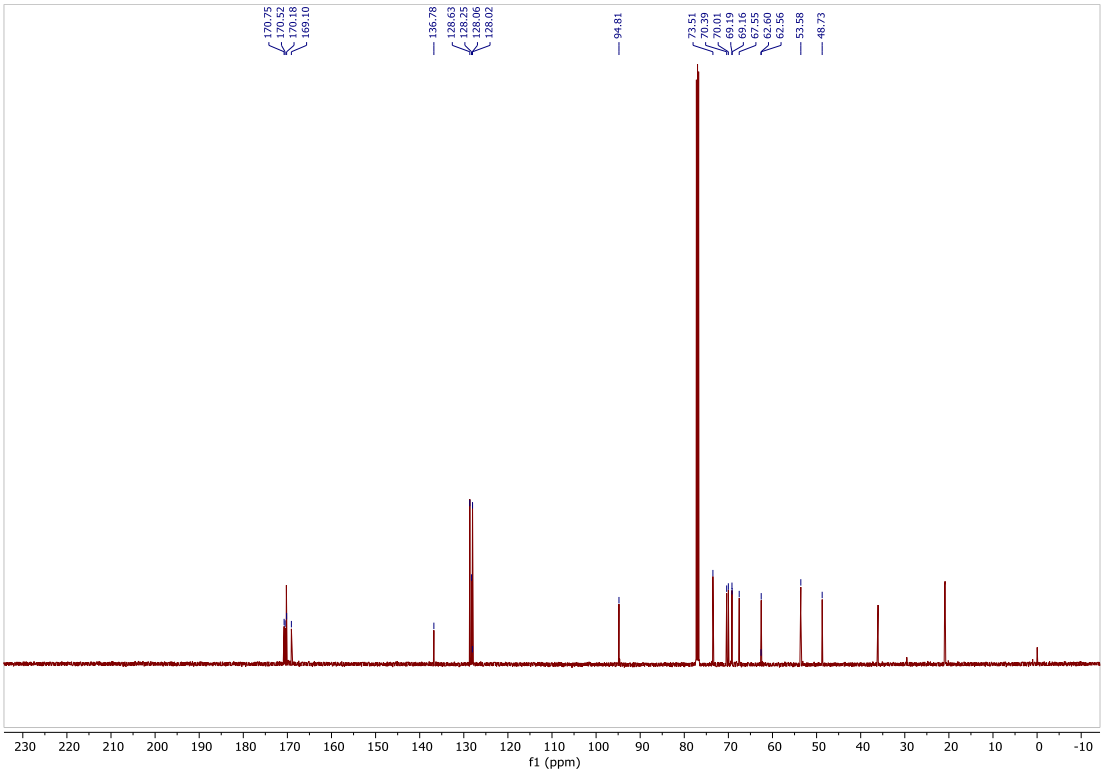
Methyl (phenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-di-deoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranoside) onate (7)



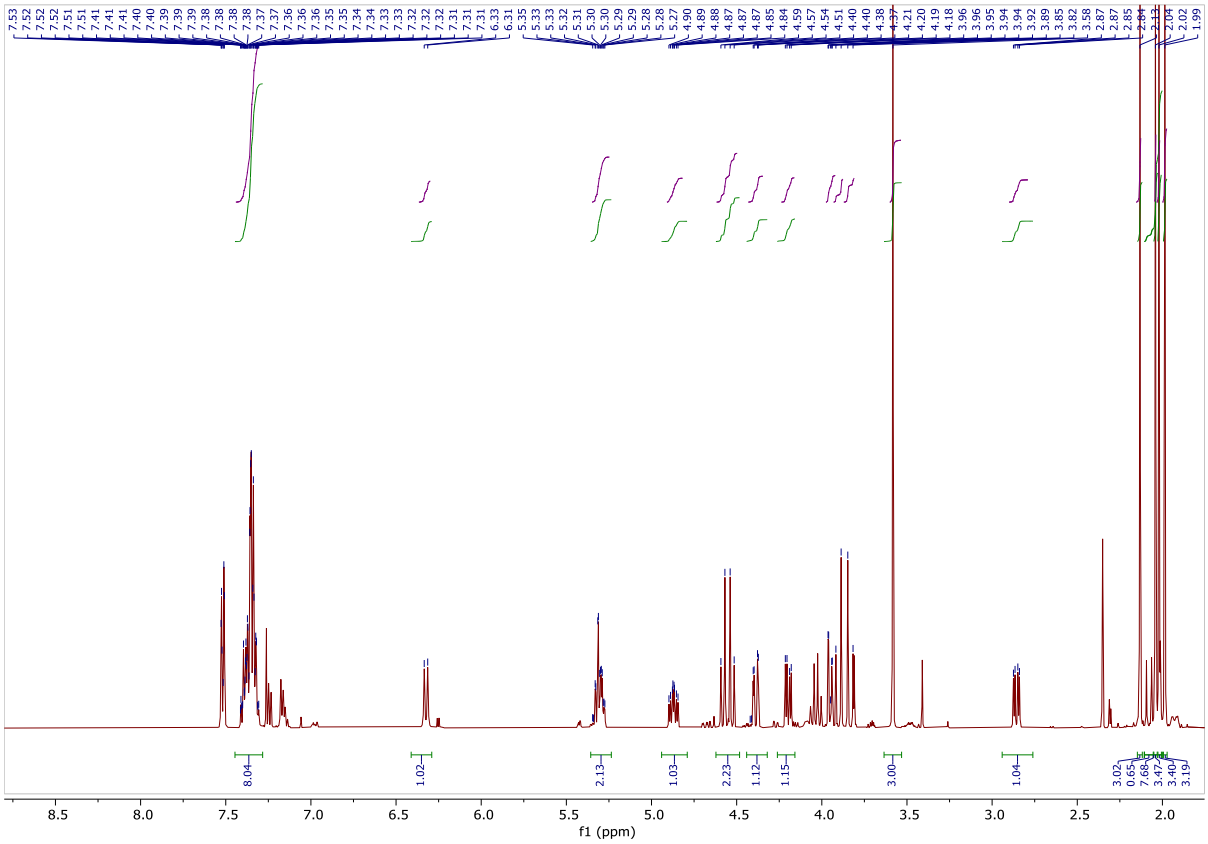


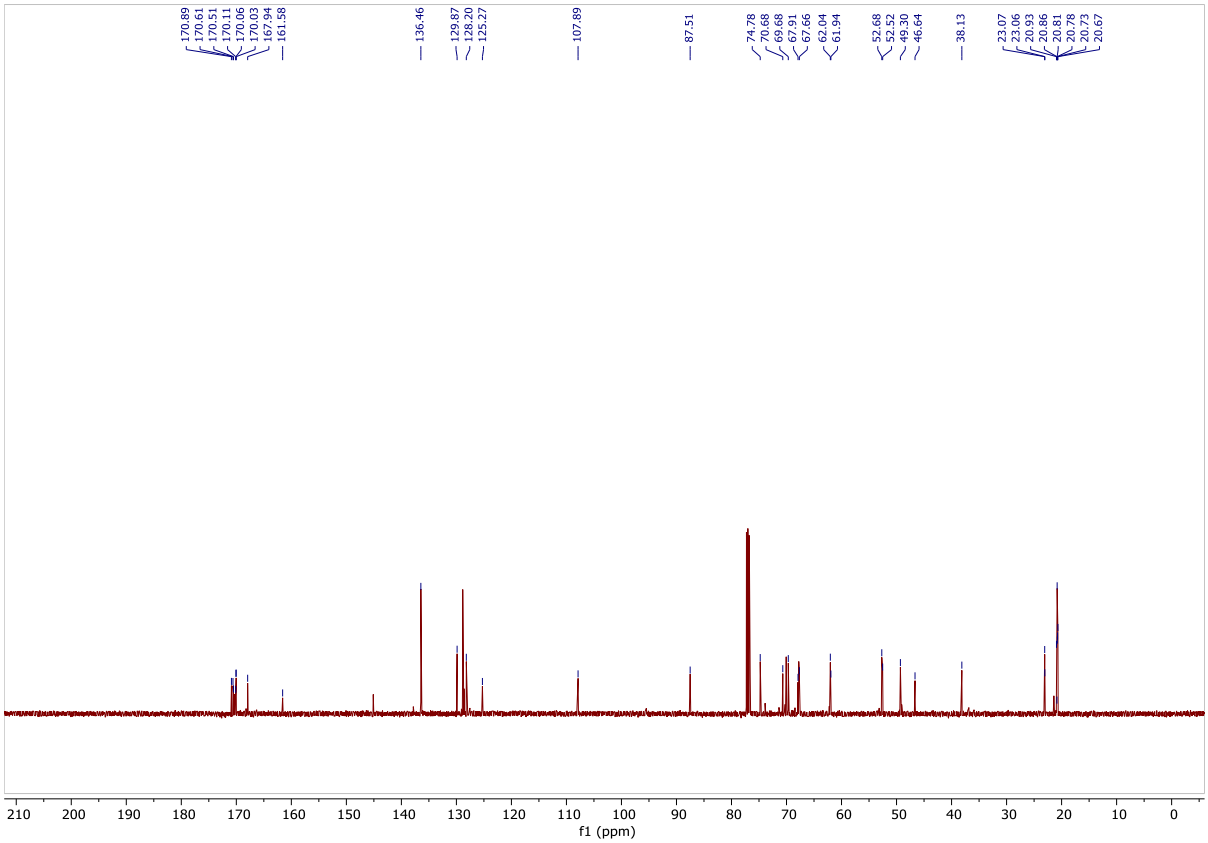
Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-5-benzyloxyacetamido-3,5-di-deoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranoside) onate (11)



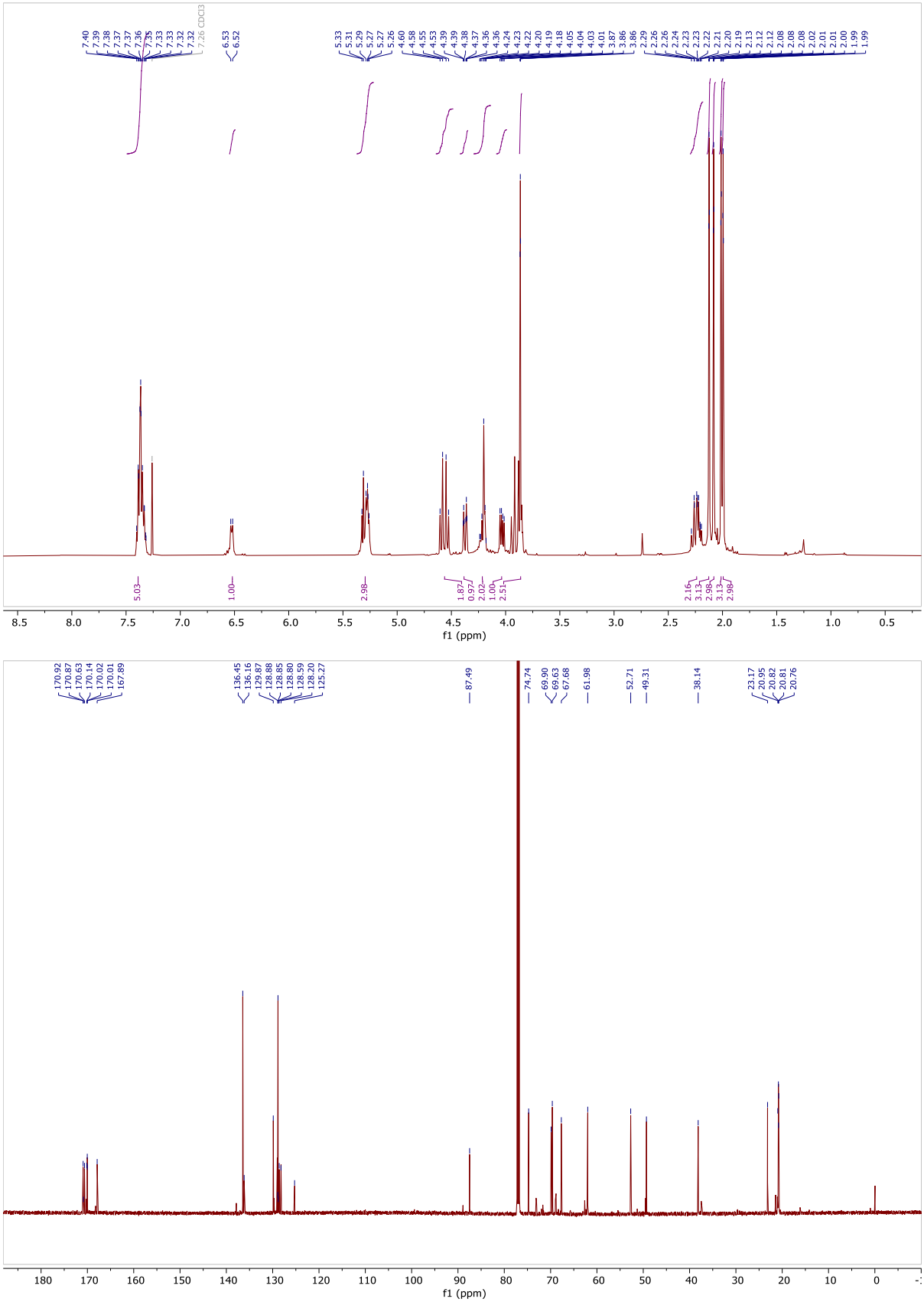


Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-5-(2,2,2-trichloroethoxycarbonylamino)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12)

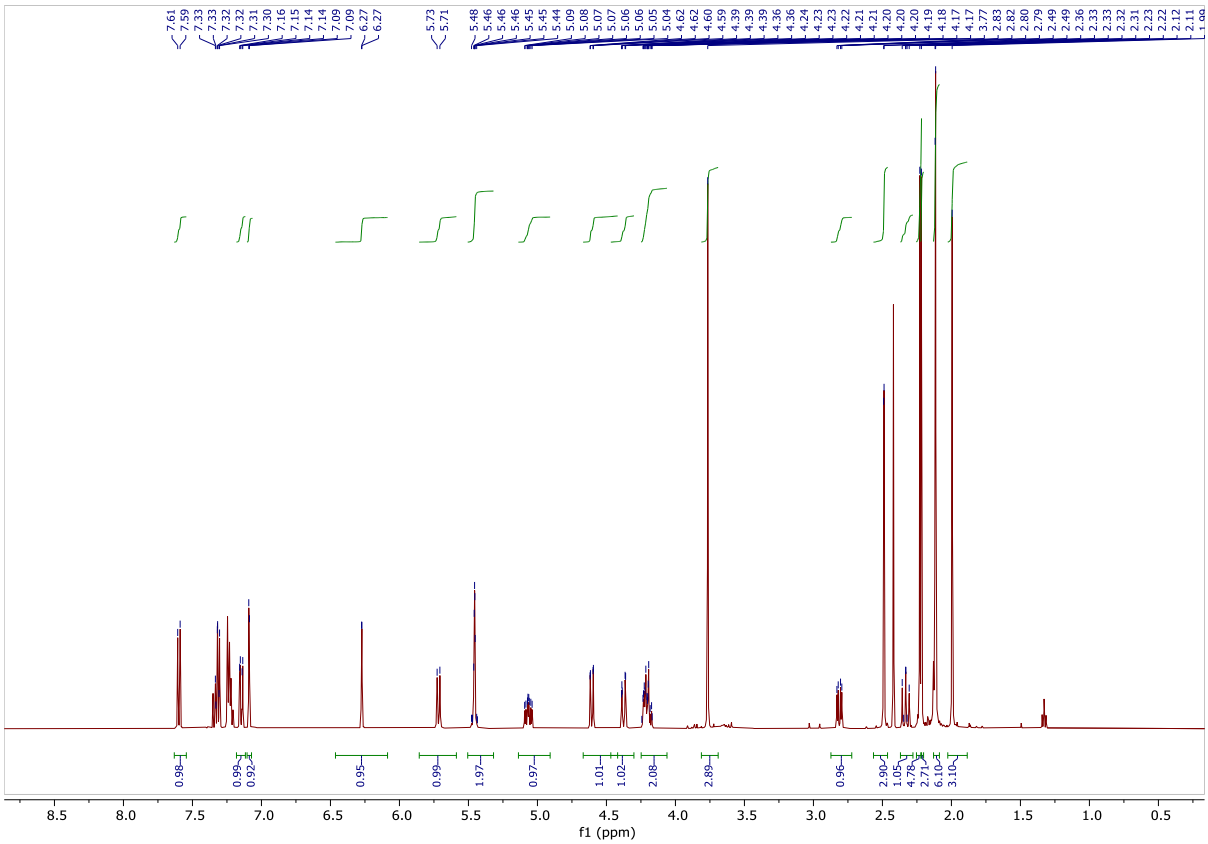


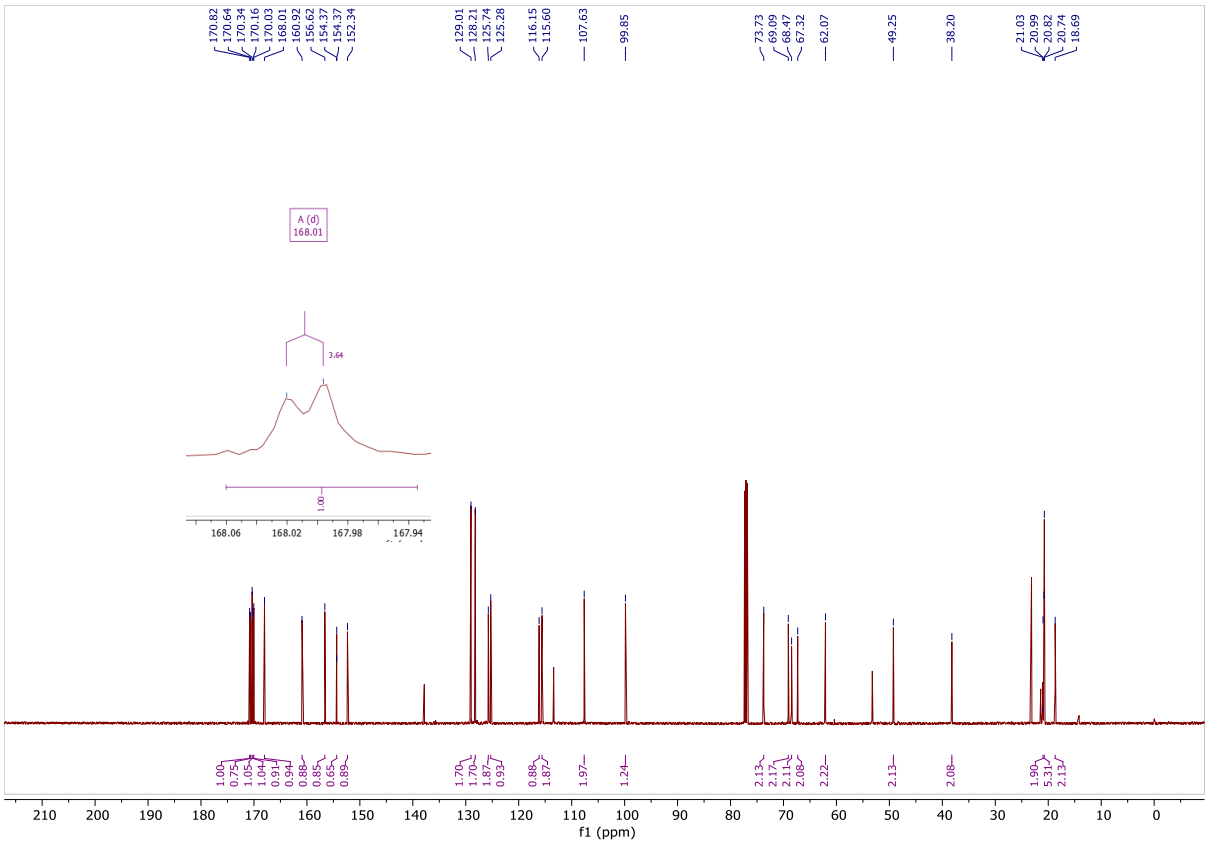


Methyl (phenyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-5-(2,2,2-trichloroethoxycarbonylamino)-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (13)

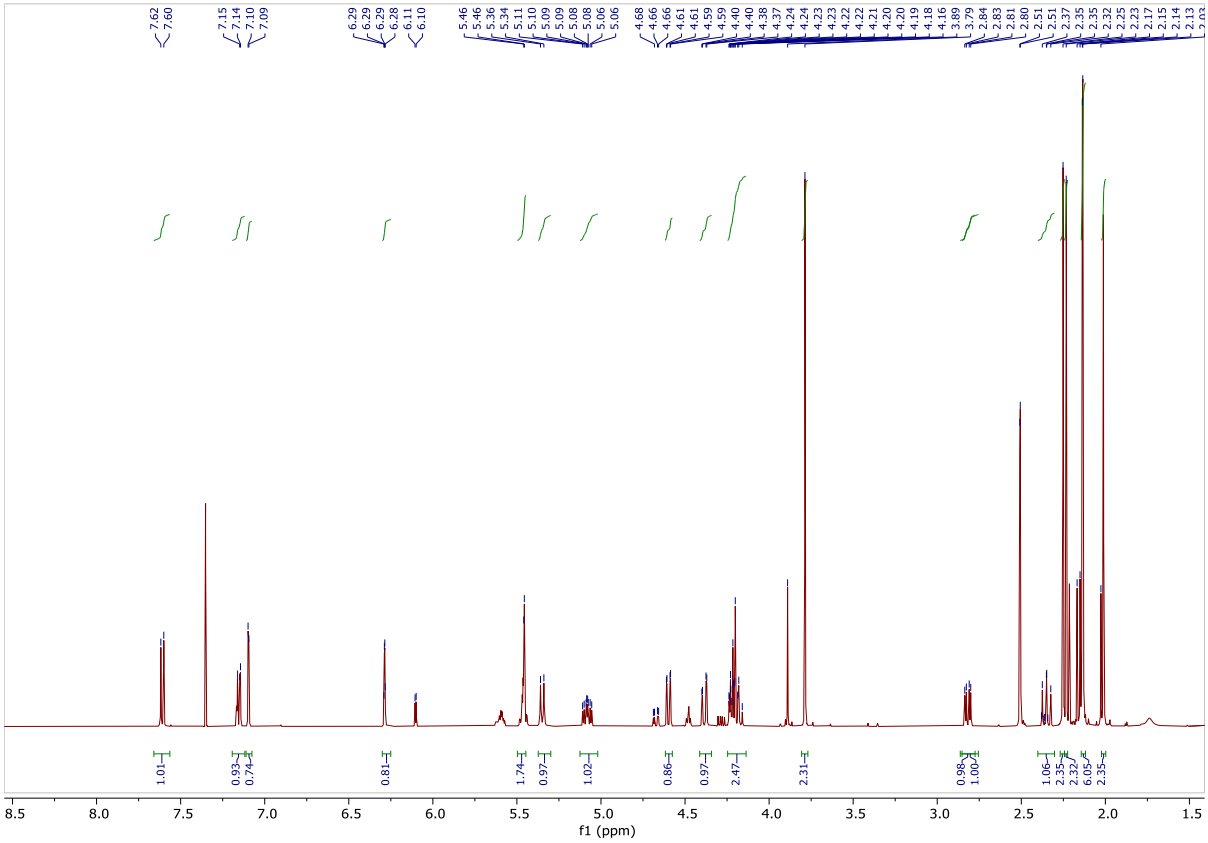


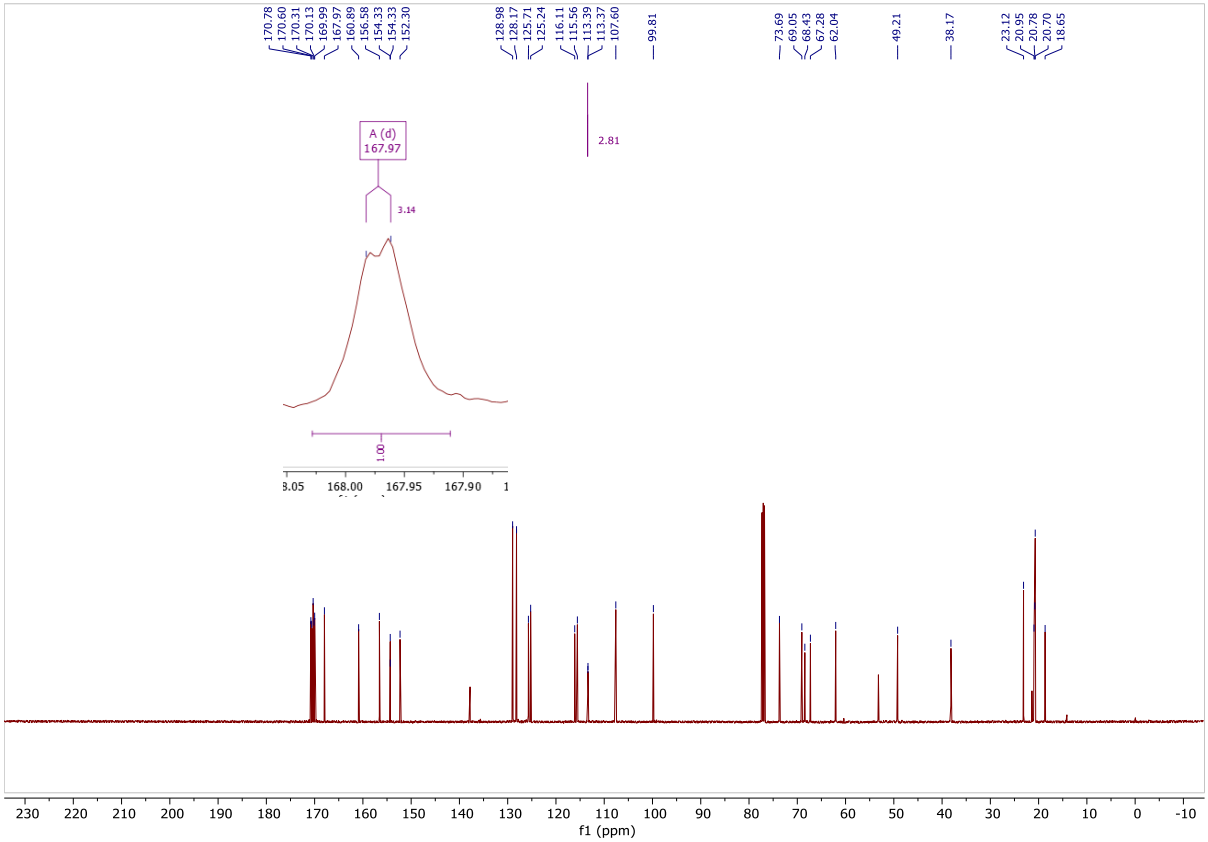
Methyl (4-methylcoumarin-7-yl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (17α)



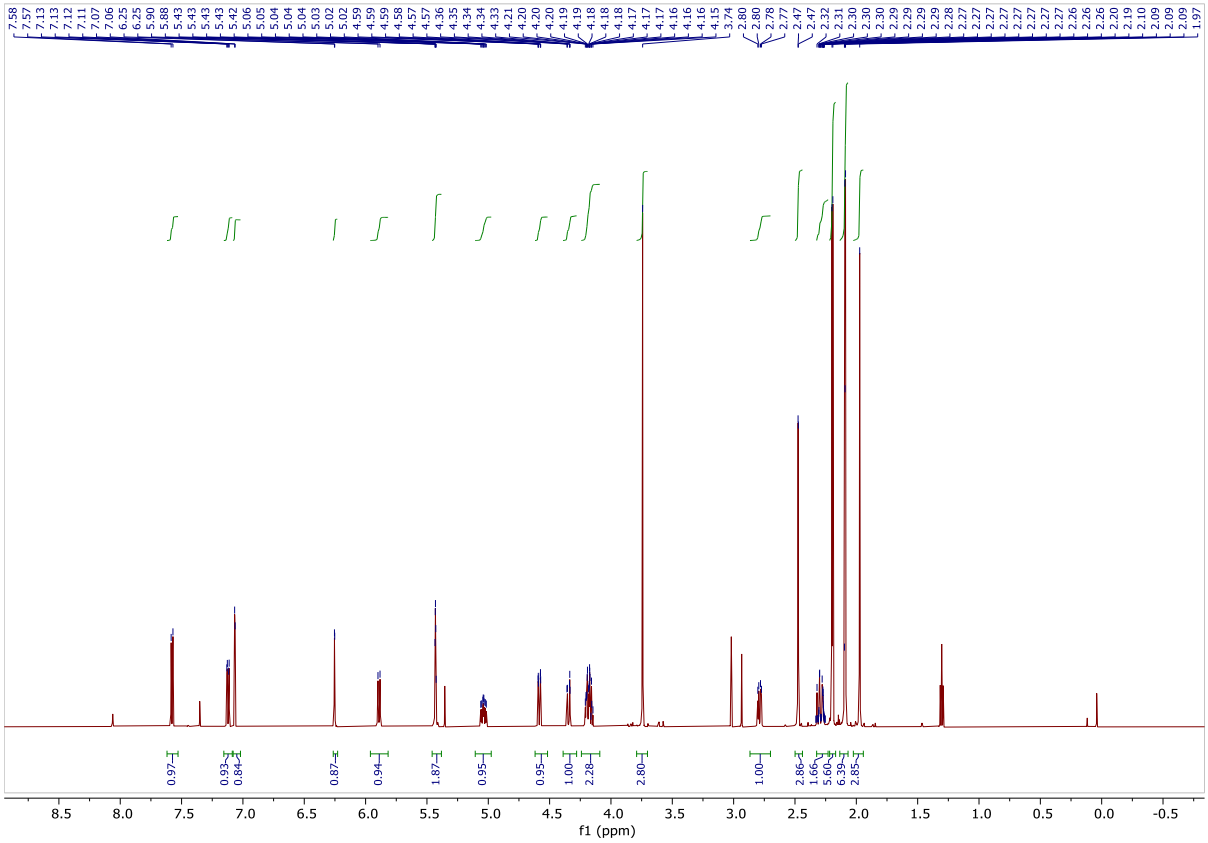


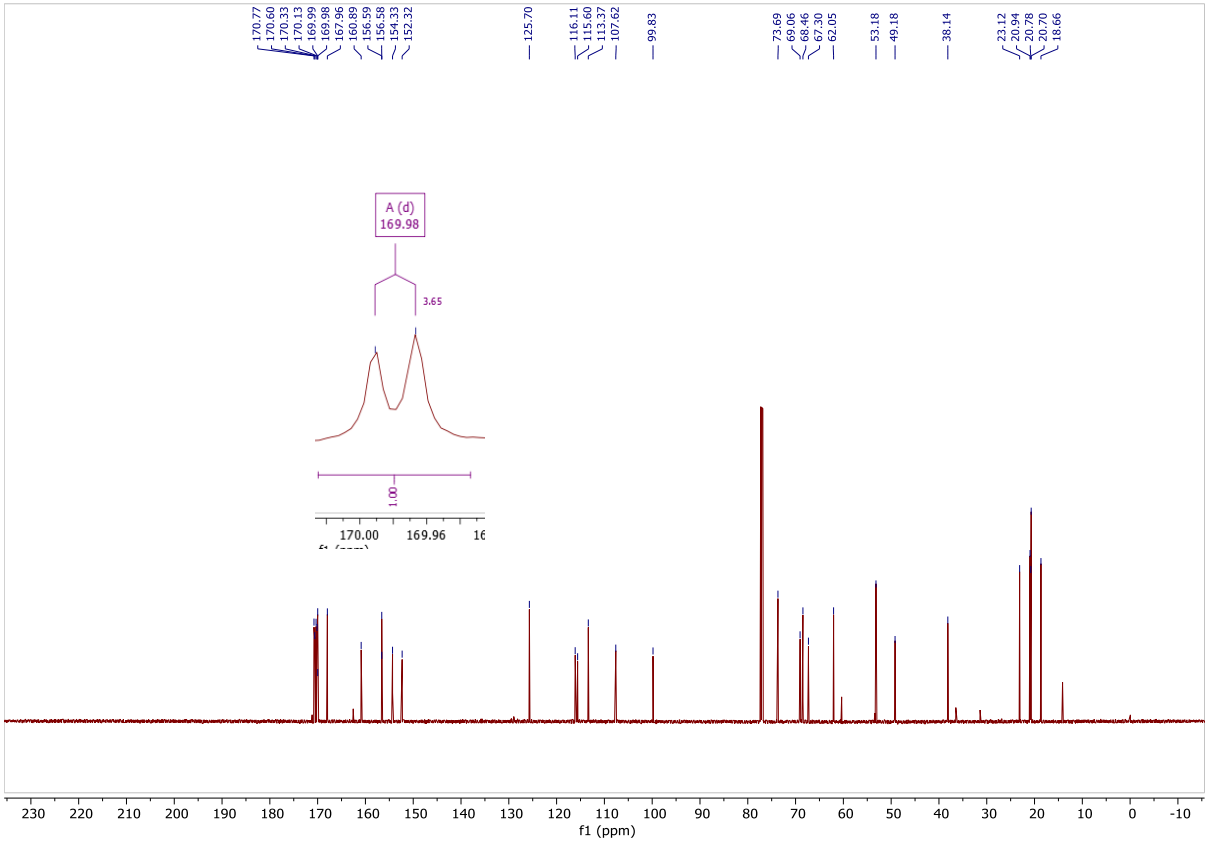
Methyl (4-methylcoumarin-7-yl 4,7,8,9-tetra-*O*-acetyl-5-acetoxyacetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (22α)



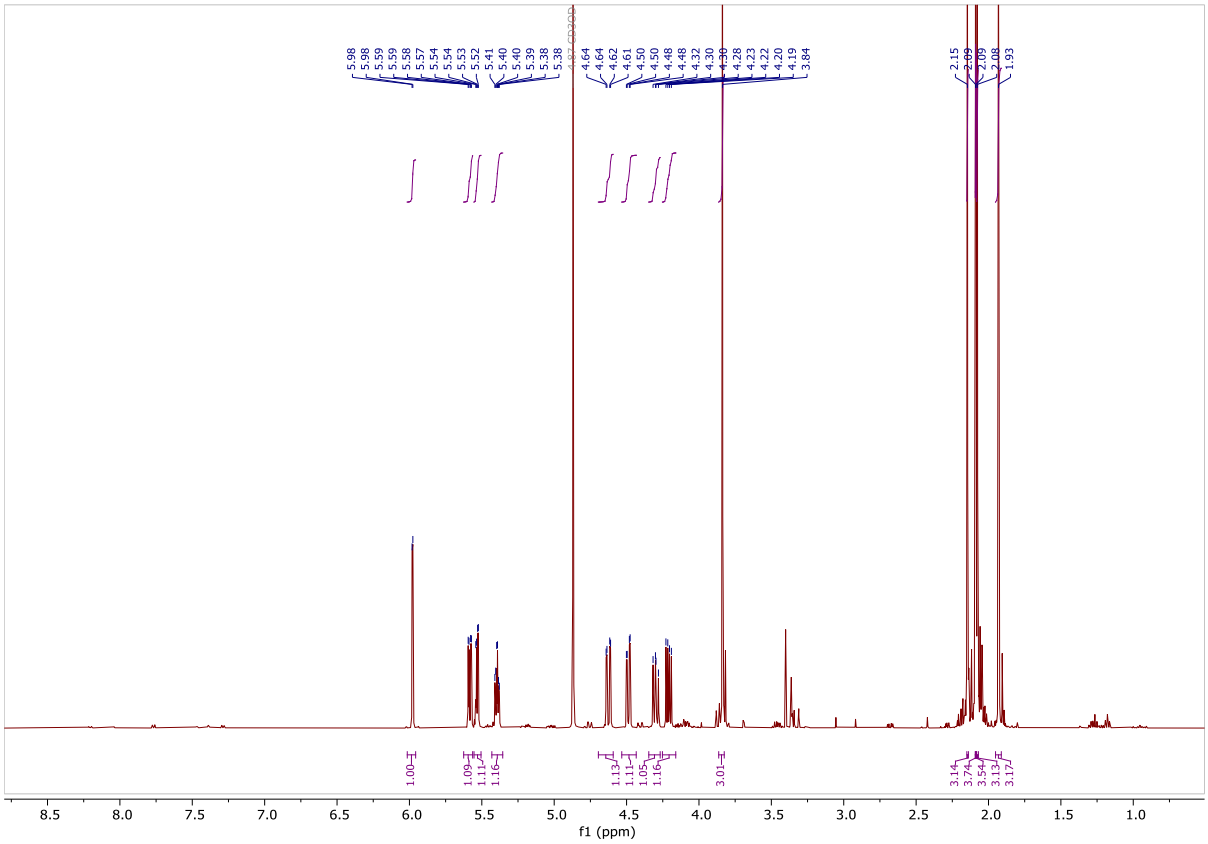


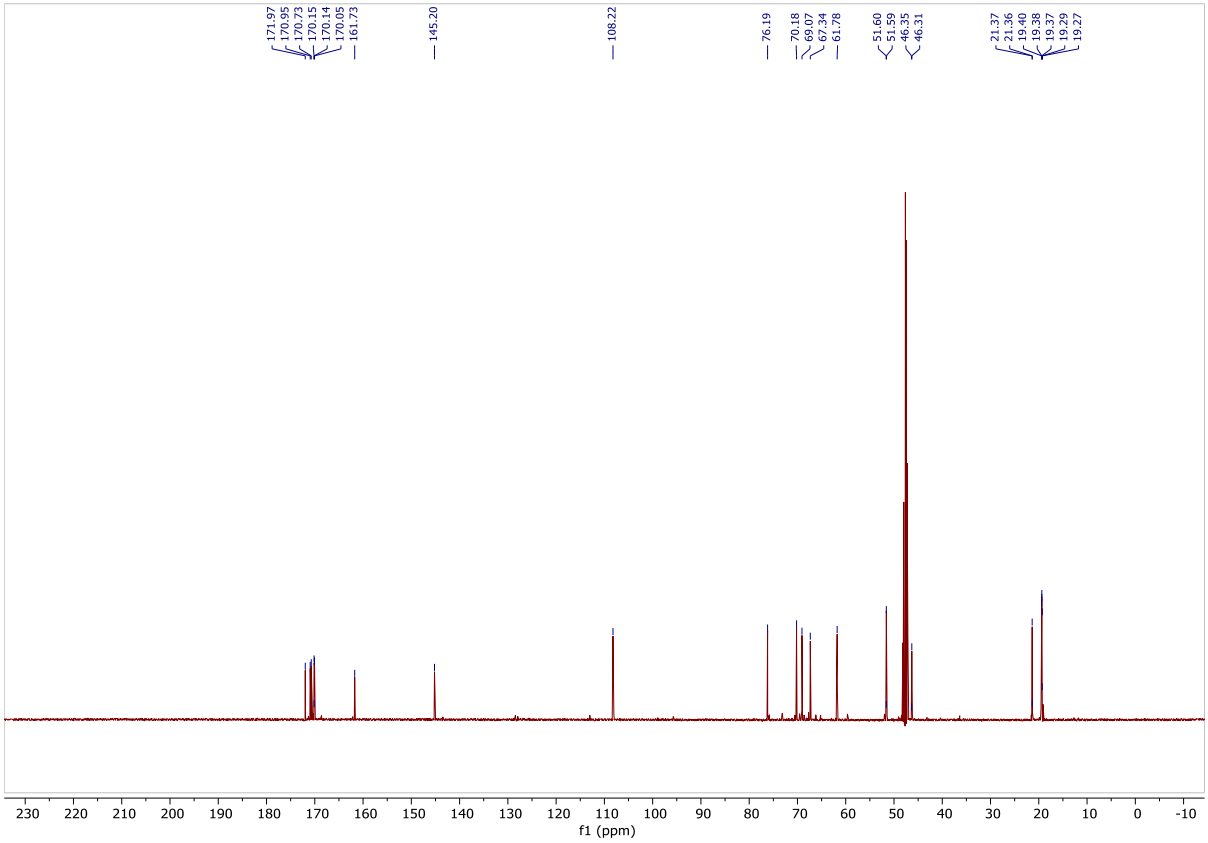
Methyl (4-methylcoumarin-7-yl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoropropanoyl-α-D-glycero-D-galacto-2-nonulopyranosid) onate (23α)



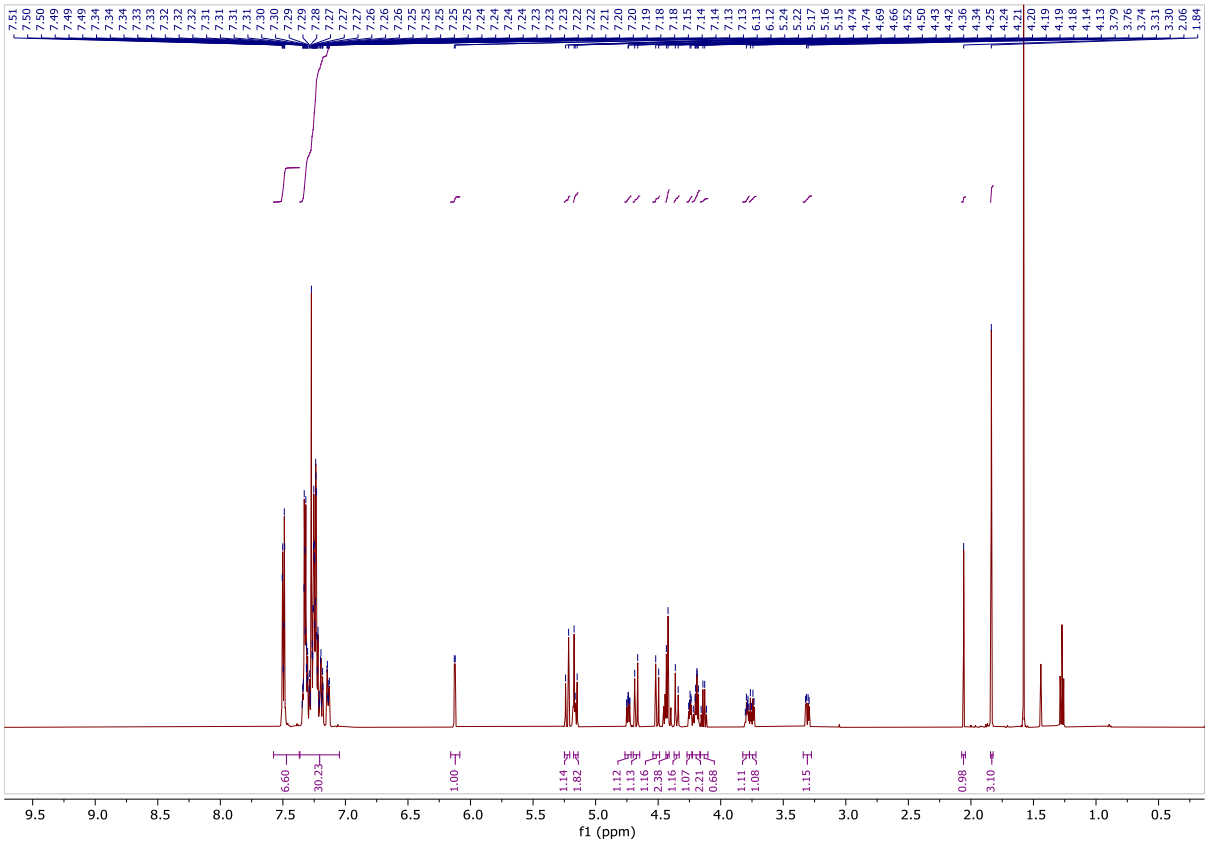


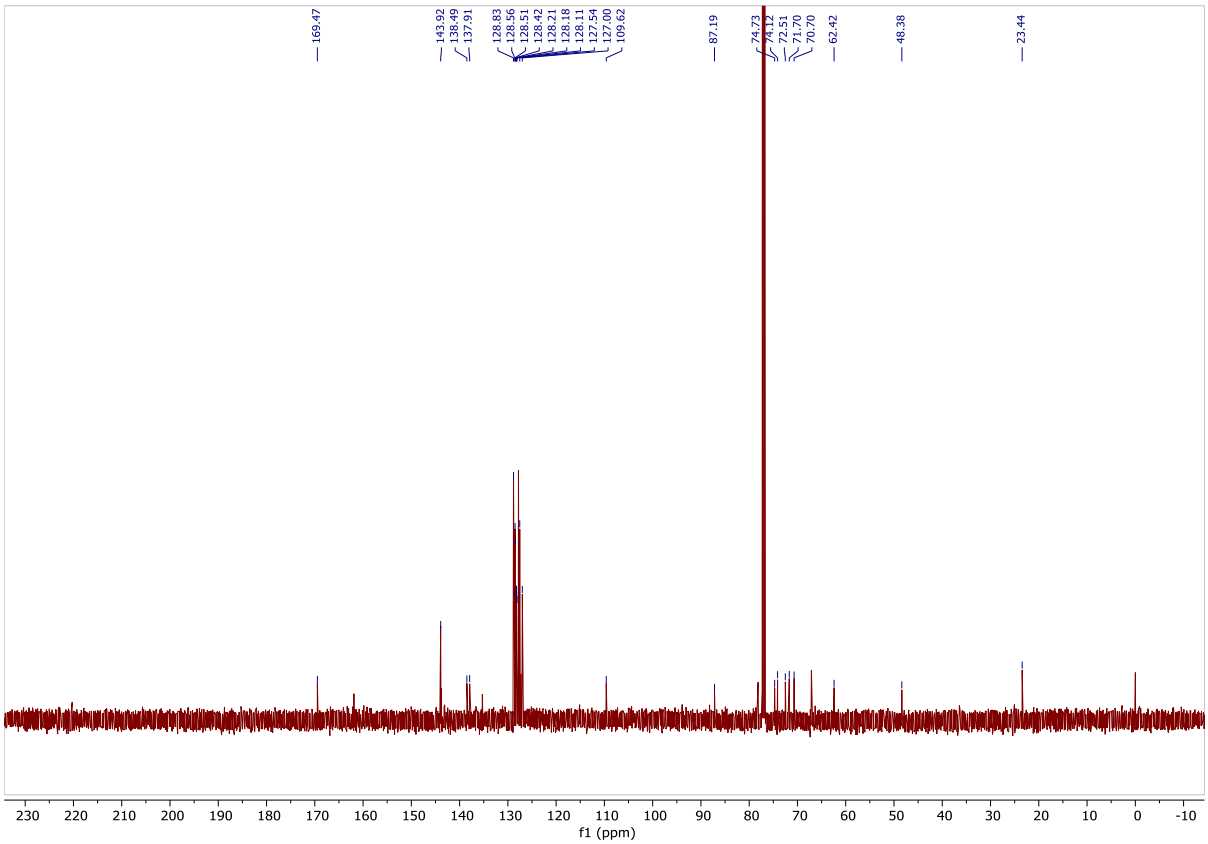
Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonate (18)



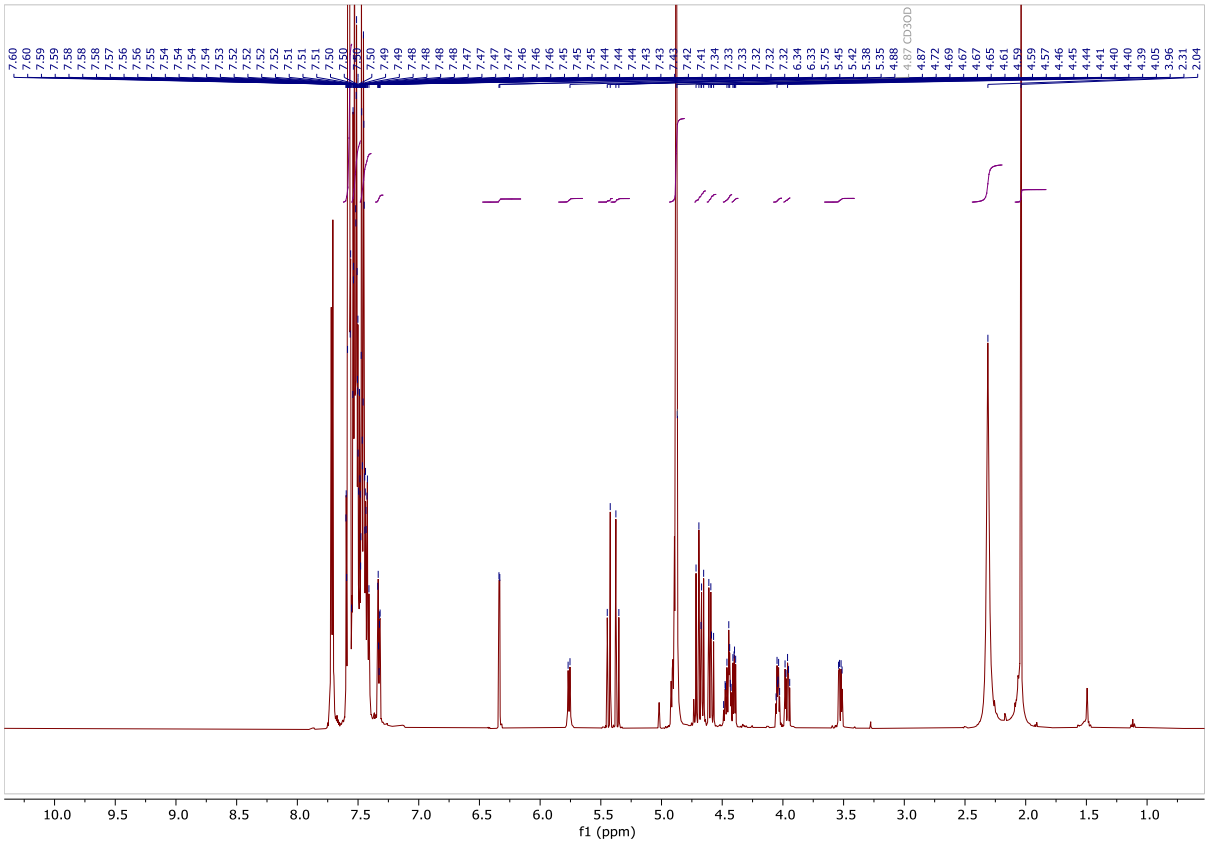


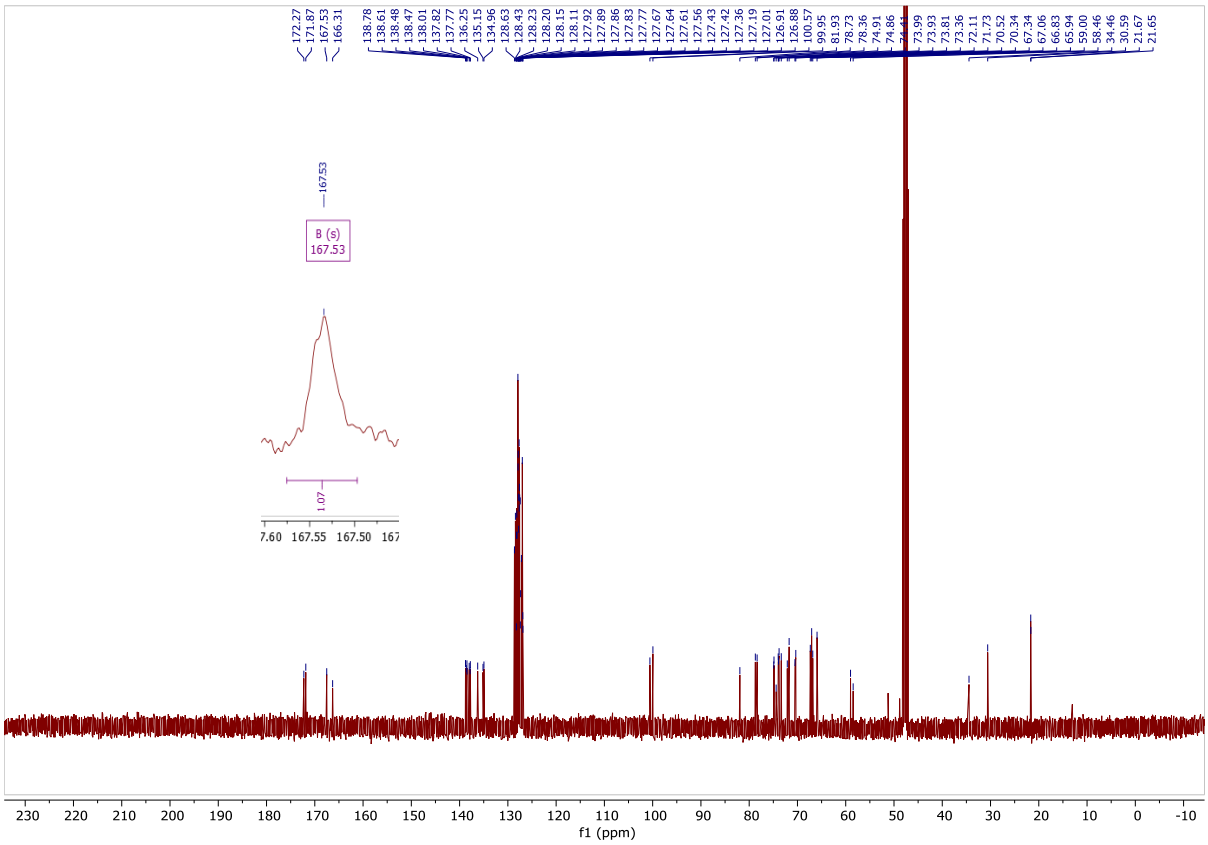
Benzyl 5-acetamido-2,6-anhydro-4,7,8-tri-*O*-benzyl-3,5-dideoxy-9-*O*-trityl-D-*glycero*-D-*galacto*-non-2-enonate (25)



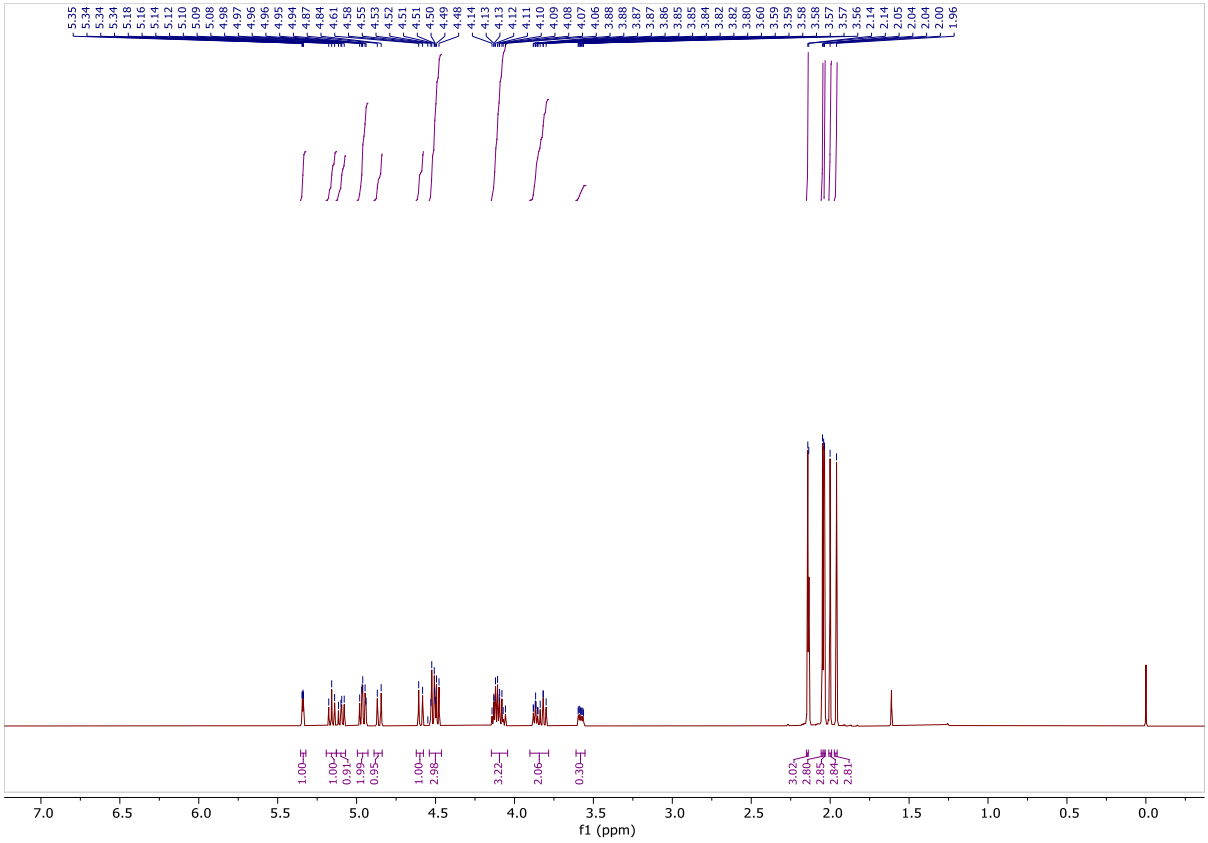


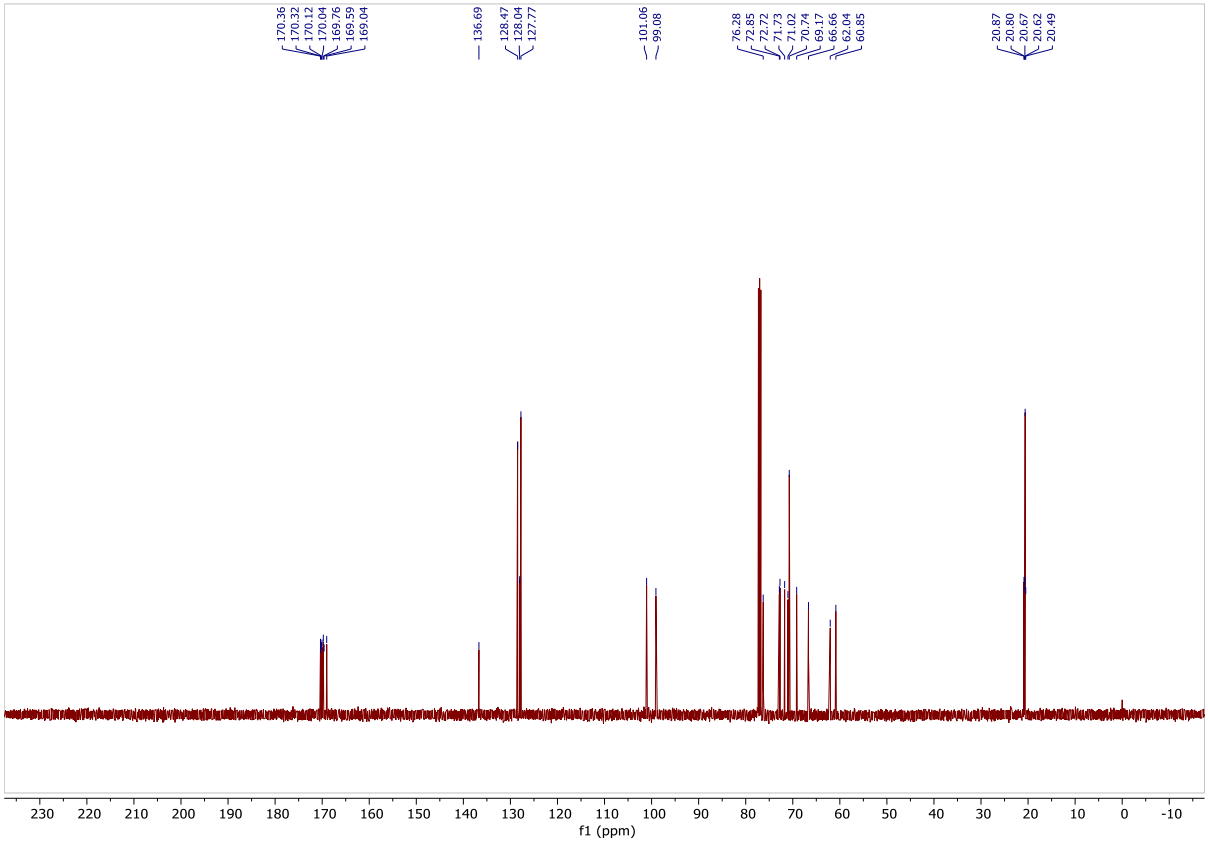
Benzyl 5-acetamido-2,6-anhydro-4,7,8-tri-*O*-benzyl-3,5-dideoxy-9-*O*-trityl-D-*glycero*-D-*galacto*-non-2-enonate (26)



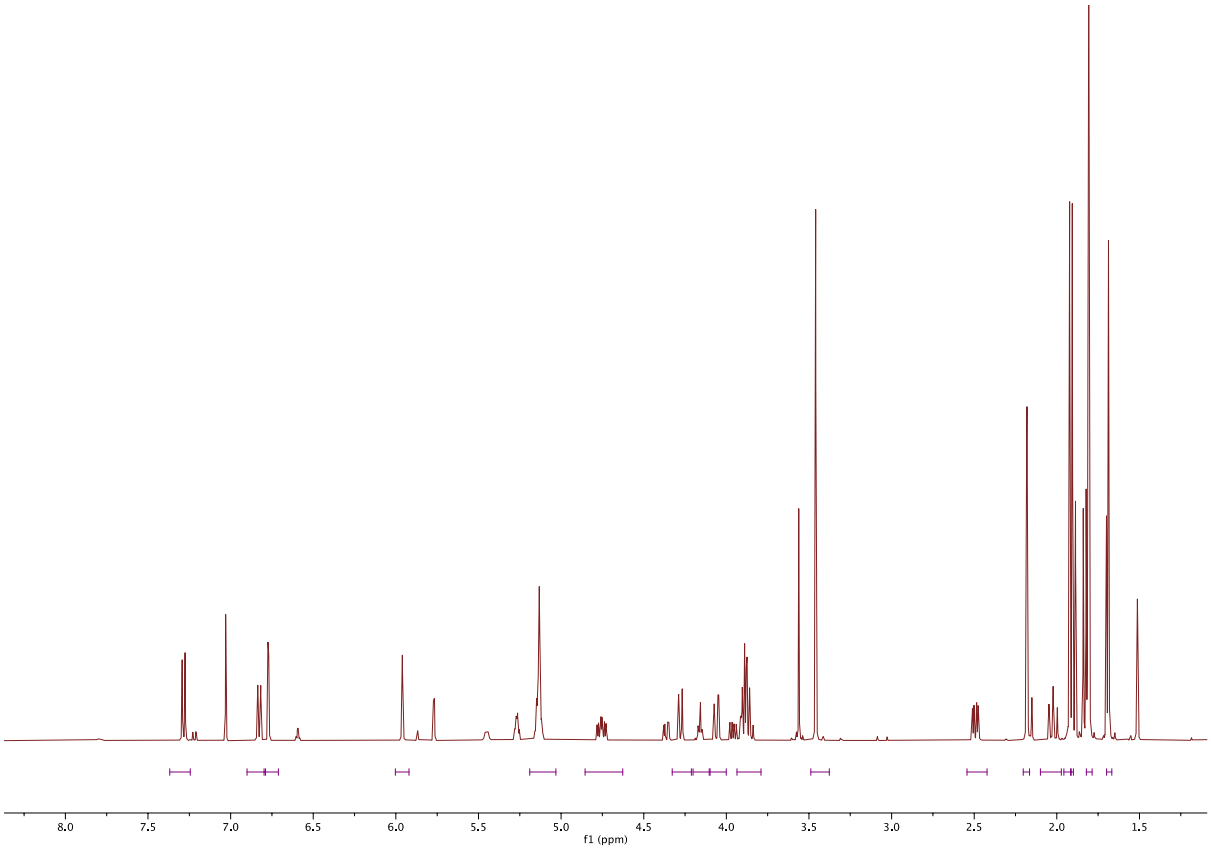


Methyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enonate (30)

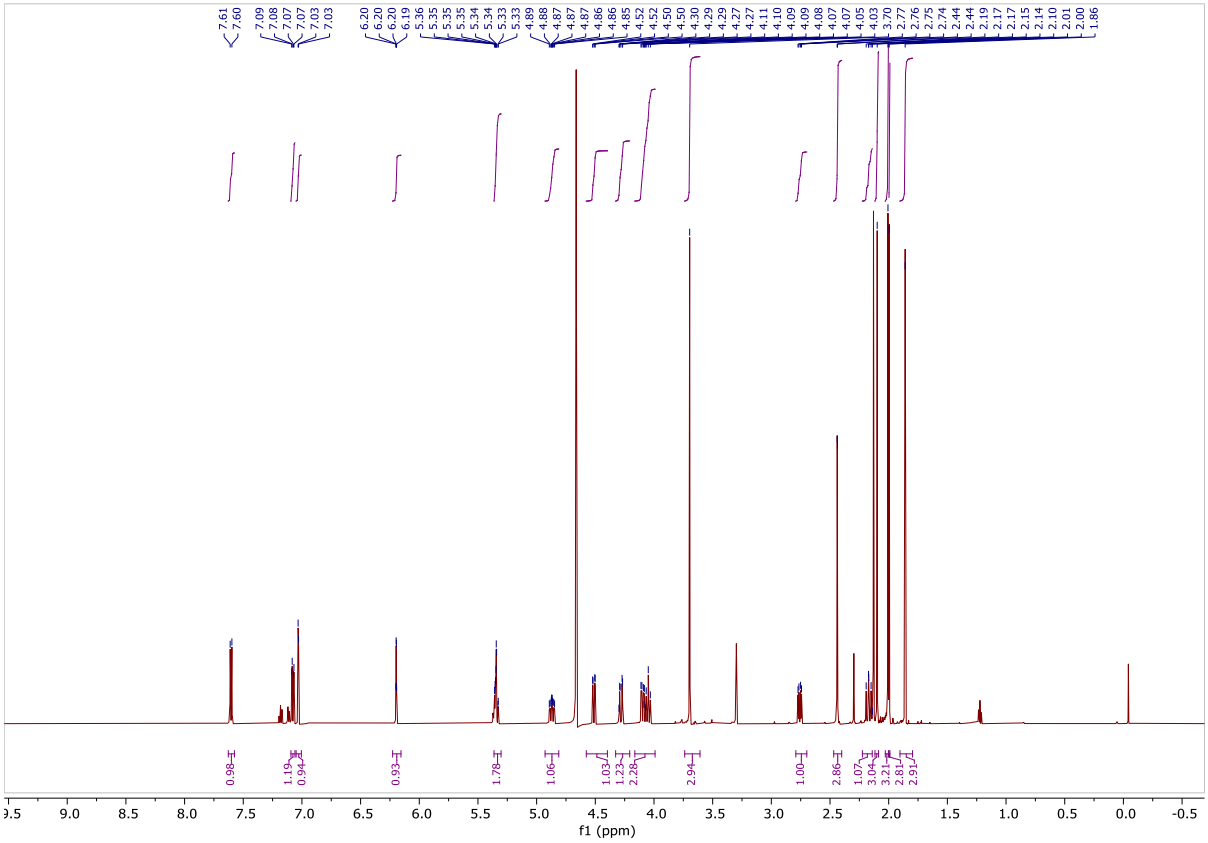


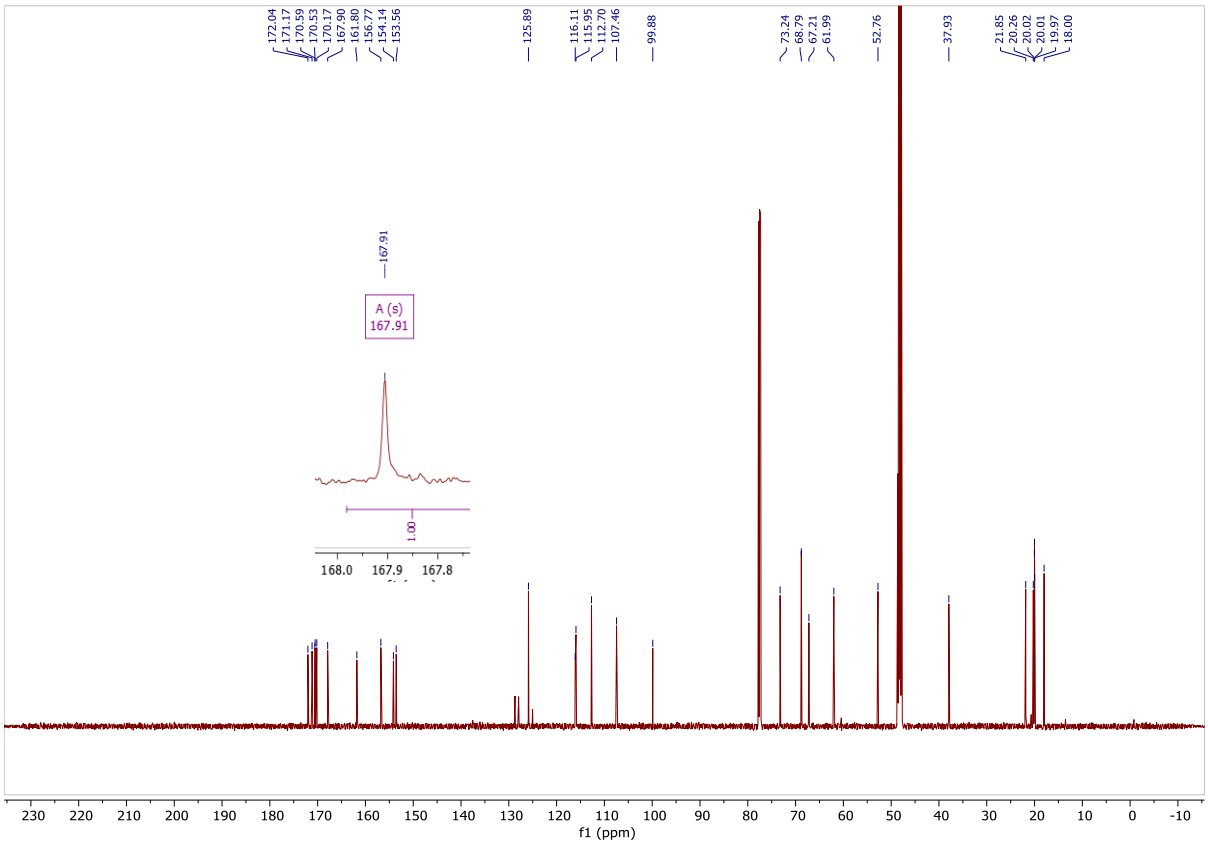


Methyl (4-methylcoumarin-7-yl-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (17β)

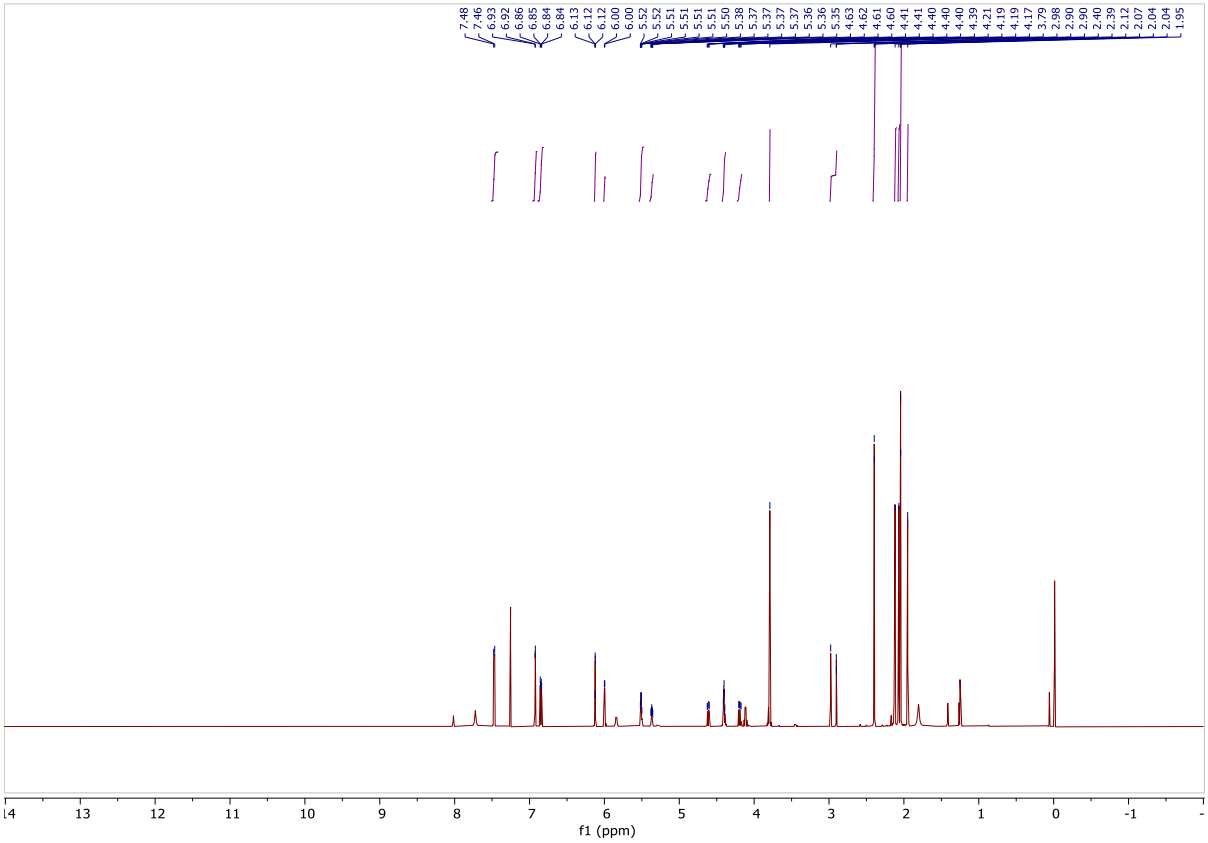


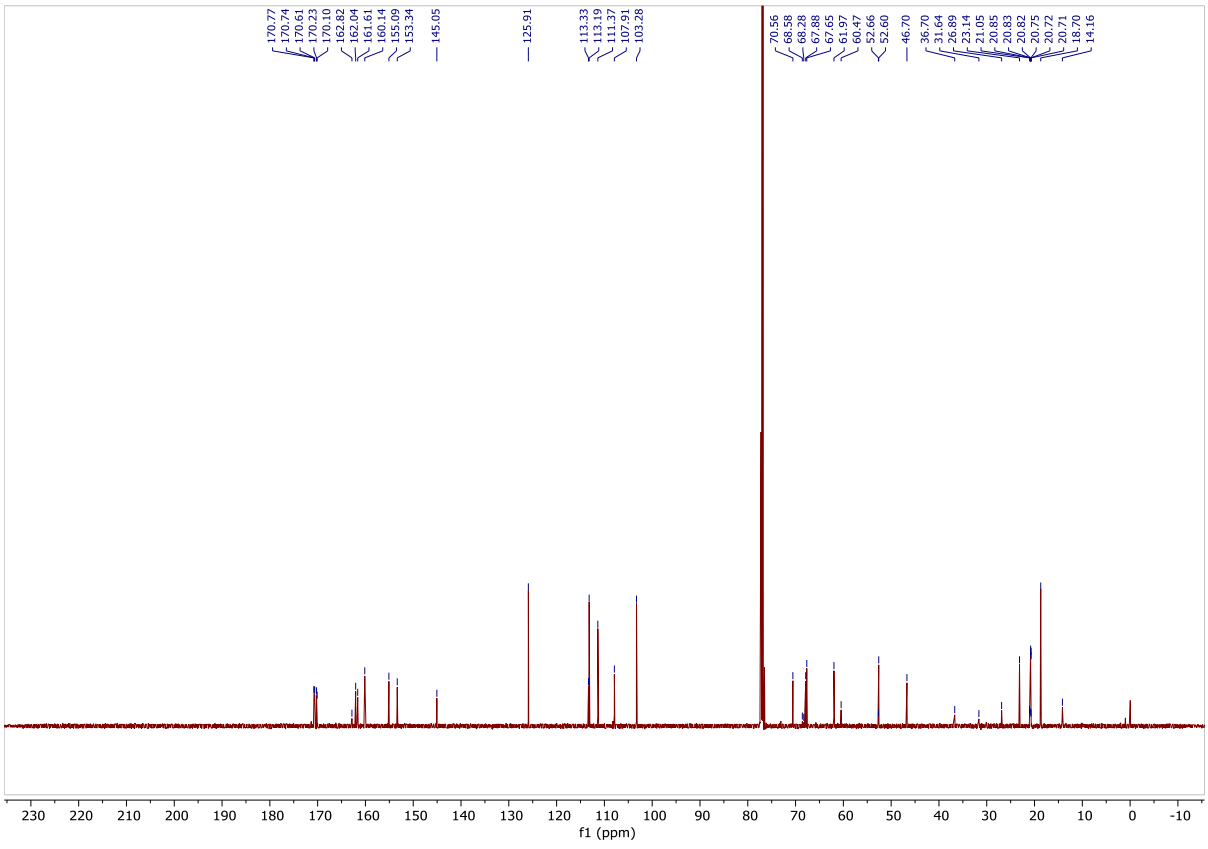
Methyl (4-methylcoumarin-7-yl 4,7,8,9-tetra-*O*-acetyl-5-acetoxyacetamido-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosid) onate (22β)



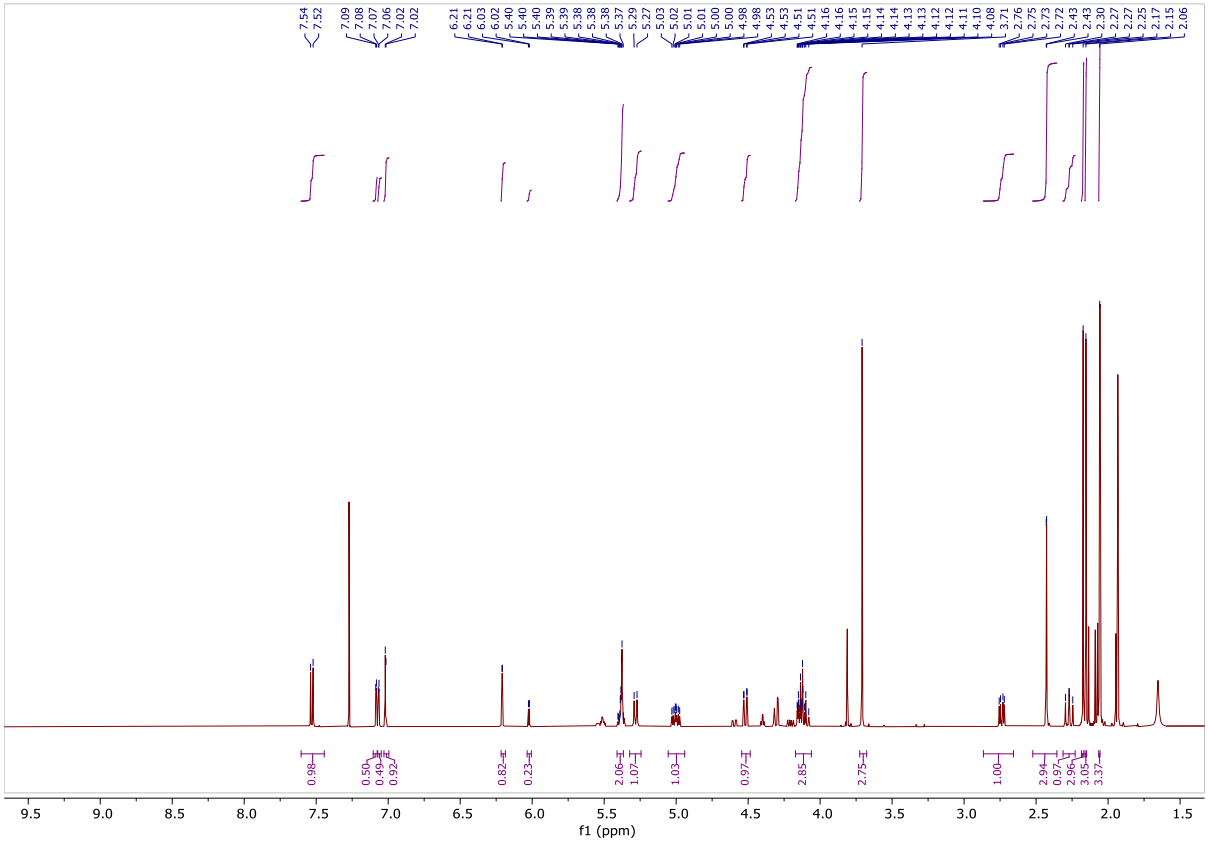


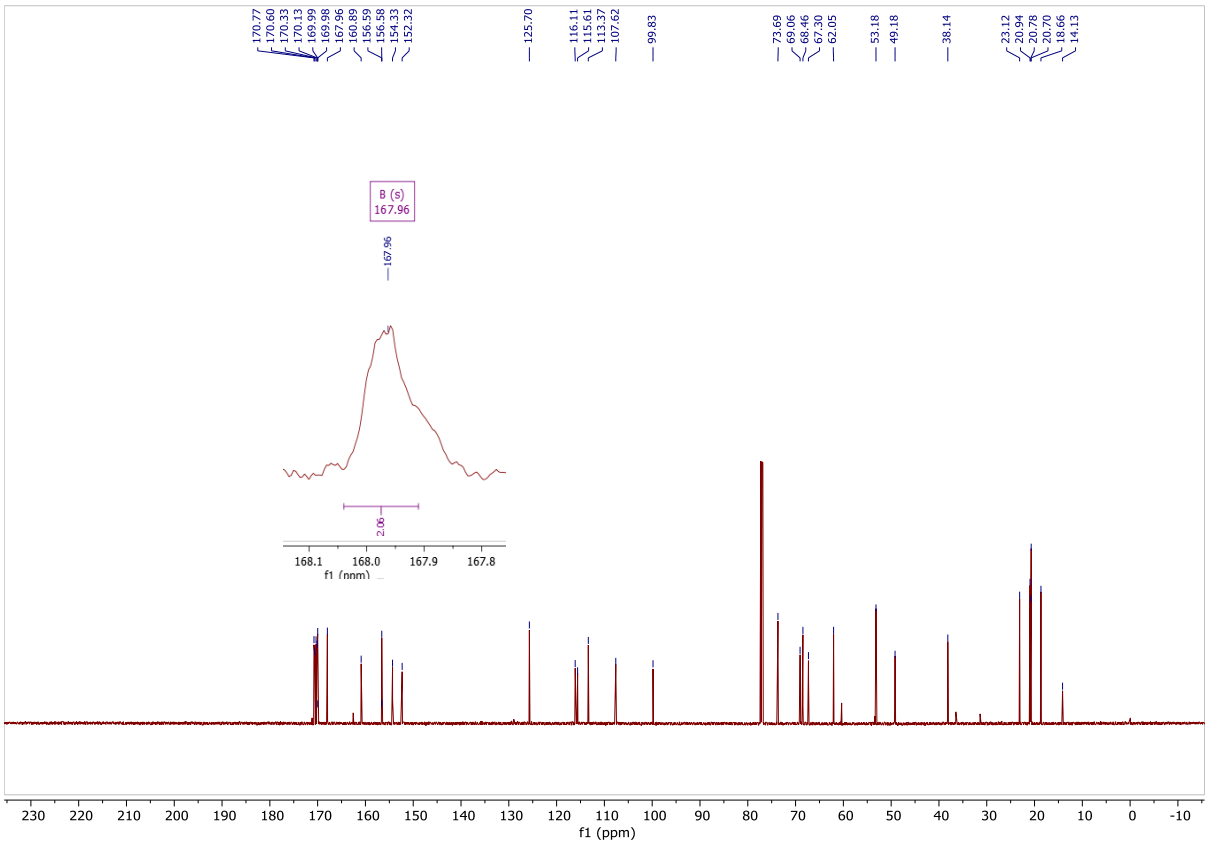
Methyl (4-methylcoumarin-7-yl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-trifluoropropanoyl-α-D-glycero-D-galacto-2-nonulopyranosid) onate (23β)





Methyl (4-methylcoumarin-7-yl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosid) onate (24β)





4-Methylcoumarin-7-yl (2-acetamido-3-deoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranoside (S1-α-Mu)