SUPPORTING INFORMATION

Synthesis of 12-*O*-Mono- and Di-glycosyl-oxystearates, a New Class of Agonists for the C-type Lectin Receptor Mincle

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Materials and methods

All of the used chemical reagents were of analytical grade and used as obtained from commercial companies including: Sigma-Aldrich (2,4,6-Tri-tert-butylpyrimidine-TTBP, dibutyltin(IV) oxide), Merk (benzyl bromide), Tokyo chemical industry (isomaltose, trifluoroacetic anhydride, tetrabutylammonium iodide (Bu_4NI), hydrogen bromide (30% in acetic acid, ca. 5.1mol/l), Funakoshi (gentiobiose octa-acetate), Wako (maltose monohydrate, cellobiose, Pd/C 10%, sodium methoxide, sodium hydride, sodium carbonate, melibiose, ethanne thiol, mannose), Kishida chemical (Mannitol), Nacalai techtesque (silver carbonate, *meta*-chloroperoxybenzoic acid (*m*-CPBA), Chemical Chameleon (galactose). Solvents used in water sensitive reactions were in commercial available anhydrous solvents, and further dried over 3Å molecular sieves (for benzaldehyde and dichloromethane) or 4 Å molecular sieves (for other solvents). All of the reactions were followed by analytical thin layer chromatography (TLC-Merck GF254). TLC plates were visualized under UV light, and/or treated with a solution of ethanol-sulphuric acid (95:5) which was followed by heating to observe. Column chromatography was performed using silica gel 60 (230–240 mesh, Machery & Nagel). NMR spectra were recorded on a Varian 400 MHz spectrometer or Varian 600 MHz spectrometer in the deuterated chloroform ($CDCl_3$), Methanol-*d*4 (CD_3OD), and Pyridine-d_5 with residual protonated solvent as internal standard. The abbreviations for multiplicity are: s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, dq = dq doublet of quartets, t = triplet, td = triplet of doublets, q = quartet, quint = quintet, m = multiplet, and br = broad. High resolution mass spectra (HRMS) were conducted with the positive ionizing mode by using electro-spray ionization and a time-of-flight mass analyzer (ESI-TOFMS) (Errors were less than 4 ppm).

General procedure 1 (for benzoylation)

The suspension of free sugar (1mmol) in pyridine (5ml) was dropwise added benzoyl chloride (1.5 *eq.* per hydroxyl group) at 0 ℃, and stirred overnight at rt.. The reaction mixture was followed by TLC, and quenched by ice-cold water. After filtered, the

remaining gum was dissolved in $CHCl_3$, added water, and evaporated at 80 ℃ until dry as white amorphous solid.

General procedure 2 (for Debenzoylation)

The starting material (10 mg) was treated with the suspension of NaOMe in methanol (0.4 M, 1ml). The resulting mixture was stirred vigorously in for 30 minutes, neutralized by Amberlite IR 120 ion-exchange, and filtered off the ion exchanger. The filtrate was concentrated in rotary evaporator, and followed by flash chromatography to obtain the desire product.

General procedure 3 (for Bromination)

The benzoylated or acetylated compound (0.5 mmol) in CH_2Cl_2 (1.8 ml) was dropwise treated with HBr/ HOAc (0.5 ml, 30% w/w) at rt., stirred, and followed by TLC until completion. Upon completion (~2 hr), the reaction was quenched by ice cold water (10 ml), extracted with Et_2O (60 ml), washed with saturated $NaHCO_3$ (3*15 ml). The organic phase was separated, dried with Na_2SO_4, concentrated in rotary evaporator, and purified by column chromatography to afford the brominated compound.

General procedure 4 (for benzylation)

The NaH (2 *eq.* per hydroxyl group) was prewashed with hexane, dried in *in vacuo*, suspended in anhydrous DMF. The prepared NaH was then added to the suspension of the indicated compound in anhydrous DMF (*N*, *N*-dimethylformamide) at rt. to provide the final concentration of 0.2 M of the indicated compound. The resulting suspension was stirred for 45 minutes, cooled in ice bath, and dropwise treated with benzyl bromide (1.5 *eq.* per hydroxyl group). After 20 minutes, the ice bath was removed, and the reaction mixture was stirred overnight at rt.. Methanol was slowly added to reaction mixture to destroy the excess NaH, and the solvent was co-evaporated with toluene in evaporator

until dryness. The residue was dissolved in ethyl acetate, and washed twice with cold water, saturated solution of $NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4, and removed by evaporator. The residue was purified by column chromatography to furnish the benzylated product.

General procedure 5 (for the mannosyl sulfoxide)

The *m*-CPBA (1.02 *eq.)* was added to the stirred solution of ethyl 1-thiomannopyranoside (1.1 mmol, 1 *eq.*) in dry CH_2Cl_2 (10 mL) at -78 ℃. After flushed with argon gas, the reaction mixture was stirred at -78 ℃ for 4 hr, warmed t-*O*-20 °C, and quenched with saturated aq. Na_2CO_3. The solution was washed twice with saturated aq. Na_2CO_3, brine, and dried over anhydrous Na_2SO_4. The organic phase was concentrated in evaporator and the residue was recrystallized with the ethyl acetate and hexane system to provide the mannosyl sulfoxide as a white solid.

Glycosylation 6 (for the sulfoxide glycosylation)

a) The prepared mixture of indicated sulfoxide donor (1.0 *eq.*, 0.78 mmol), TTBP (2 *eq.)*, and MS_4A (1.0 weight *eq.)* in anhydrous CH_2Cl_2 (20 ml) was stirred for 3 hr under argon gas at -78 ℃ before being dropwise treated with Tf_2O (1.3 *eq.)* at this temperature. After 15 minutes, the appropriate acceptor in anhydrous CH_2Cl_2 (2 *eq*., 0.5 M.) was cooled, and injected dropwise into the mixture. The resulting solution was further stirred for 5 hr at -78 °C, slowly warmed t-*O*-30 °C, quenched with cooled MeOH, and filtered off MS_4A. The filtrate was washed twice with saturated Na_2CO_3, once with brine, and dried over anhydrous Na_2SO_4. The organic phase was concentrated in rotary evaporator, and the residue was purified by column chromatography over silica gel to afford the corresponding glycosylated product.

b) Similar protocol 6a. However, the equivalent of reagents were changed to be: sulfoxide donor (2.0 *eq.*, 0.78 mmol), TTBP (4 *eq.*), MS_4A (1.0 weight *eq.)*, Tf_2O (2.6 *eq.)*, and the appropriate acceptor (1 *eq.*).

General procedure 7 (for deprotection of benzyl or benzylidene protected group)

The starting material (10 mg) was treated with the suspension of the 10% Pd/C (100 mg) in the heterogeneous mixture of the EtOAC/ THF/ n-propanol/ H_2O (4.5 ml, 2:1:1:1). After degasification, the hydrogenation was initiated under an atmosphere of H_2 (1 atm) at rt., and followed by TLC until completion (48 hr). The catalyst was separated by filtration through a filter paper, and washed twice by both THF and H_2O. The combined filtrate was concentrated under reduced pressure, dried in *vacuo*. 2 days, and purified by silica gel and/or RP8 and/or sephadex chromatography columns to obtain the deprotected product.

General procedure 8 (for glycosylation by Koenigs–Knorr approach)

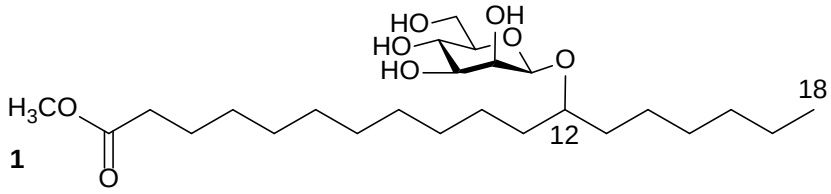
A mixture of acceptor (0.3 mmol, 1 *eq.*), glycosyl bromide (1.2 - 1.6 *eq.)*, and MS4A (2g) in anhydrous CH_2Cl_2 (10 ml) was stirred 3 hr at rt. The reaction mixture was quickly added $AgClO_4$ (1.5 *eq.*) and Ag_2CO_3 (1.5 *eq.*), flushed with argon, and stirred at rt. until completion (5 hr). The filtrate of the reaction mixture was evaporated in rotary evaporator, and purified by both silica gel and/or RP8 chromatography columns to provide the desired product.

General procedure 9 (for deprotection of *p*-methoxyl benzyl protected group)

The DDQ (59.2 mg, 0.261 mmol, 2.3 *eq.*) was charged into the solution of the starting material in a mixture of CH_2Cl_2/H_2O (5.3 ml, 17/1) at 0 ℃. The reaction mixture was kept

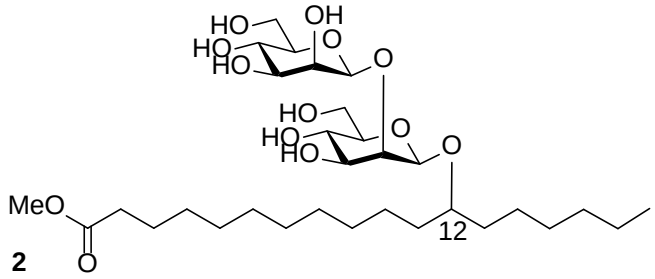
on stirring at this temperature for 30 minutes and then at rt. for 1 hour. The resulting mixture was quenched with saturated aq. Na_2CO_3, and extracted twice with $CHCl_3$. The combined organic phase was then washed twice with saturated aq. Na_2CO_3, brine. The organic phase was dried over anhydrous Na_2SO_4 and removed by evaporator. The solid residue was purified by column chromatography over silica gel to furnish the product.

12-*O*-β-D-mannopyranosyl-oxystearate (1)



Compound **17** (11.9 mg, 15.9 µmol) was hydrogenolysis using general procedure 7 to afford compound **1** in 91% yield (6.9 mg, 14.4 µmol) as white solid [Scheme 1, (e)]. ^{1}H NMR (600 MHz, py-d5) δ 4.92 (s, 1H, H1), 4.51 (t, *J* = 9.4 Hz, 1H, H4), 4.49 – 4.42 (m, 2H, H2, H6), 4.30 (dd, *J* = 11.5, 5.4 Hz, 1H, H6), 4.14 (dd, *J* = 9.3, 3.3 Hz, 1H, H3), 3.95 – 3.90 (m, 1H, H12), 3.87 – 3.81 (m, 1H, H5), 3.61 (s, 3H, C*H*$_3$), 2.27 (t, *J* = 7.4 Hz, 2H, C*H*$_{2\alpha}$), 1.77 – 1.01 (m, 28H), 0.78 (t, *J* = 6.9 Hz, 3H, C*H*$_3$). ^{13}C NMR (151 MHz, py-d5) δ 174.21 (CO), 100.57 (C1, $^1J_{C1H1}$ = 155.36), 79.00 (C12), 78.36 (C5), 75.41 (C3), 72.52 (C2), 68.58 (C4), 62.59 (C6), 51.34 (O*C*H$_3$), 35.27, 33.99, 31.87, 29.99, 29.69, 29.68, 29.64, 29.52, 29.32, 29.14, 25.49, 25.16, 25.05, 22.69, 14.11 (*C*H$_3$). (ESI-TOF) MS: $C_{25}H_{48}NaO_8^+$ calculated for $(M+Na)^+$ was 499.3241 and found 499.3258 (Δ = 3.40 ppm).

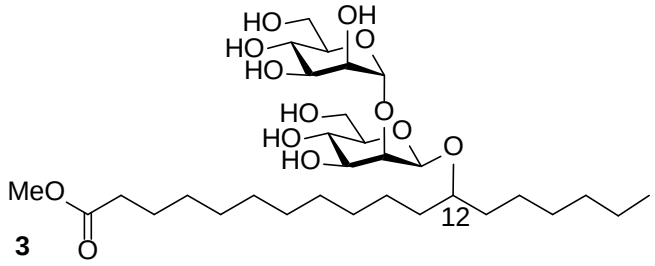
12-*O*-(2-*O*-β-D-mannopyranosyl)-β-D-mannopyranosyl-oxystearate (2)



Compound **21** (18.1 mg, 16.7 μmol) was followed general procedure 7, and purified by the reverse phase HPLC to afford compound **2** as white solid in 80% yield (8.5mg, 13.3 μmol). NMR data of compound **2** was measured at 30 ℃ and 25 ℃ in pyridine [Scheme 1, (e)]. At 30 ℃: ^{1}H NMR (600 MHz, py-d5) δ 5.46 (s, 1H, H1`), 4.99 (s, 1H, H1), 4.73 (d, *J* = 2.9 Hz, 1H, H2`), 4.67 (d, *J* = 3.4 Hz, 1H, H2), 4.53 (t, *J* = 9.4 Hz, 1H, H4`), 4.50 – 4.44 (m, 2H, H6, H6`), 4.36 – 4.29 (m, 2H, H4, H6`), 4.21 (dd, *J* = 11.6, 6.2 Hz, 1H, H6), 4.11 (td, *J* = 9.4, 3.3 Hz, 2H, H3, H3`), 3.96 (quint, *J* = 5.8 Hz, 1H, H12), 3.89 (ddd, *J* = 9.4, 5.6, 2.5 Hz, 1H, H5'), 3.83 (ddd, *J* = 9.1, 6.2, 2.7 Hz, 1H, H5), 3.62 (s, 3H, OCH_3), 2.30 (t, *J* = 7.5 Hz, 2H, C$H_{2\alpha}$), 1.77 – 1.10 (m, 34H), 0.84 (t, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, py-d5) δ 174.25 (CO), 102.07 (C1', $^{1}J_{C'1H'1}$=159.57), 99.82 (C1, $^{1}J_{C\ 1H1}$ =156.05), 78.93 (C2), 78.62 (C5'), 78.51 (C12), 78.43 (C5), 75.20 (C3'), 74.19 (C3), 71.52 (C2'), 68.89 (C4), 68.14 (C4'), 62.44 (C6), 62.20 (C6'), 51.30 (O*C*H$_3$), 34.79, 33.92, 33.36, 31.78, 29.87, 29.62, 29.60, 29.52, 29.42, 29.25, 29.06, 25.22, 25.08, 24.97, 22.63, 14.05 (*C*H$_3$). At 25 ℃: ^{1}H NMR (600 MHz, py-d5) δ 5.41 (s, 1H, H1'), 4.95 (s, 1H, H1), 4.69 (d, *J* = 3.1 Hz, H2'), 4.64 (d, *J* = 3.4 Hz, 1H, H2), 4.48 – 4.40 (m, 3H, H4', H6, H6'), 4.32 (t, *J* = 9.5 Hz, 1H, H4), 4.25 (dd, *J* = 11.8, 5.9 Hz, 1H, H6'), 4.17 (dd, *J* = 11.7, 6.2 Hz, 1H, H6), 4.10 (dd, *J* = 8.7, 2.5 Hz, 1H, H3), 4.08 (dd, *J* = 8.5, 2.3 Hz, 1H, H3'), 3.93 (quint, *J* = 5.8 Hz, 1H, H12), 3.87 – 3.78 (m, 2H, H5', H5), 3.61 (s, 1H, OCH_3), 2.26 (t, *J* = 7.5 Hz, 1H, C$H_{2\alpha}$), 1.70 – 1.03 (m, 28H), 0.80 (t, *J* = 6.9 Hz, 3H,

CH_3). ^{13}C NMR (151 MHz, py-d5) δ 174.25 (CO), 102.07 (C1', $^1J_{C'1H'1}$=158.84), 99.82 (C1, $^1J_{C\ 1H1}$ =156.30), 78.93 (C2), 78.62 (C12), 78.51 (C5'), 78.43 (C5), 75.20 (C3'), 74.19 (C3), 71.52 (C2'), 68.89 (C4), 68.14 (C4'), 62.44 (C6), 62.20 (C6'), 51.30 (O*C*H$_3$), 34.79, 33.92, 33.36, 31.78, 29.87, 29.62, 29.60, 29.52, 29.42, 29.25, 29.06, 25.22, 25.08, 24.97, 22.63, 14.05 (*C*H$_3$). (ESI-TOF) MS: $C_{31}H_{58}O_{13}Na^+$ calculated for $(M+Na)^+$ was 661.3770 and found 661.3798 (Δ = 4.23 ppm).

12-*O*-(2-*O*-α-D-mannopyranosyl)-β-D-mannopyranosyl-oxystearate (3)

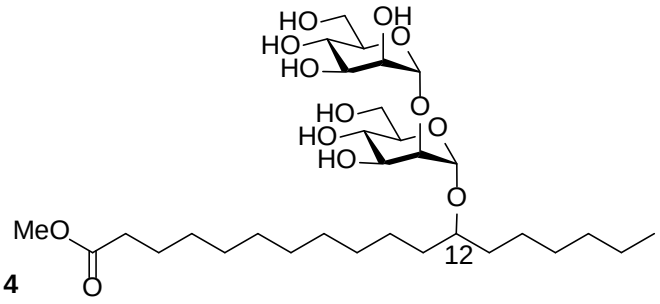


Compound **24** (22.4 g, 18.2 μmol) was removed benzoyl protecting group by using general procedure 2 to provided intermediate as off white solid in 90% yield (13.4 mg, 16 μmol). This intermediate was followed the general procedure 7 to afford compound **3** as white solid in 84% yield (8.7 mg, 13.6 μmol) [Scheme S1, (d)]. The NMR data was measured in pyridine and CD_3OD.

^{1}H NMR (600 MHz, CD_3OD) δ 5.21 (d, *J* = 1.4 Hz, 1H, H1'), 4.57 (s, 1H, H1), 4.12 (dt, *J* = 9.4, 2.8 Hz, 1H, H5'), 3.98 (d, *J* = 1.2 Hz, 1H, H2), 3.95 (dd, *J* = 3.0, 1.7 Hz, 1H, H2'), 3.86 (dd, *J* = 11.7, 2.5 Hz, 1H, H6), 3.83 – 3.75 (m, 2H, H3',H4', H6'), 3.74 – 3.67 (m, 3H, H6, H6', H12), 3.65 (s, 3H, OC*H*$_3$), 3.59 – 3.55 (m, 2H, H4, H3), 3.19 (ddd, *J* = 8.6, 5.8, 2.5 Hz, 1H, H5), 2.31 (t, *J* = 7.4 Hz, 2H, C*H*$_{2α}$), 1.58 (m, 3H), 1.49 (m, 3H), 1.42 – 1.17 (m, 22H), 0.92 (t, J = 6.9 Hz, 3H, C*H*$_3$). ^{13}C NMR (151 MHz, CD_3OD) δ 176.05 (CO), 102.56 (C1', $^1J_{C'1H'1}$=177.5), 100.64 (C1, $^1J_{C\ 1H1}$=154.0), 80.74 (C12), 78.51 (C5),

77.37 (C2), 76.20 (C3), 73.42 (C5'), 72.46 (C3'), 72.17 (C2'), 69.03 (C4), 67.95 (C4'), 63.12 (C6), 62.43 (C6'), 51.96 (O*C*H$_3$), 35.99, 34.82, 34.70, 33.05, 30.92, 30.76, 30.69, 30.59, 30.39, 30.20, 26.45, 26.06, 26.03, 23.73, 14.45 (*C*H$_3$). (ESI-TOF) MS: $C_{31}H_{58}O_{13}Na^+$ calculated for $(M+Na)^+$ was 661.3770 and found 661.3796 (Δ = 3.93 ppm).

12-*O*-(2-*O*-α-D-mannopyranosyl)-α-D-mannopyranosyl-oxystearate (4)



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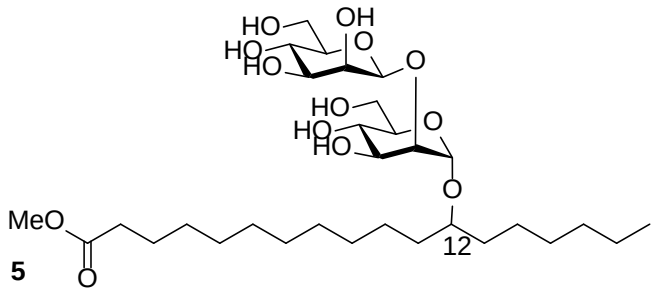
Compound **27** (18.7 mg, 15.2 µmol) was removed the benzoyl protecting group by using general procedure 2 to provide the intermediate as white syrup in 61% yield (7.6 mg, 9.3 µmol) [Scheme S1, (h), i)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (dd, *J* = 7.7, 2.0 Hz, 1H), 7.36 – 7.17 (m, 8H), 5.57 (s, 1H), 5.10 (s, 1H), 4.81 (s, 1H), 4.77 (d, *J* = 12.2 Hz, 1H), 4.61 (d, *J* = 12.2 Hz, 1H), 4.14 (dd, *J* = 9.4, 3.9 Hz, 1H), 4.10 – 3.99 (m, 2H) 3.99 – 3.86 (m, 5H), 3.85 – 3.67 (m, 3H), 3.61 (s, 3H, OC*H*$_3$), 3.59 (s, 1H), 3.52 – 3.43 (m, 1H), 2.25 (t, *J* = 7.5 Hz, 2H, C*H*$_{2\alpha}$), 1.79 – 1.33 (m, 6H), 1.22 (s, 22H), 0.84 (t, *J* = 6.8 Hz, 3H, C*H*$_3$). (ESI-TOF) MS: $C_{45}H_{68}O_{13}Na^+$ calculated for $(M+Na)^+$ was 839.4552 and found 839.4525 (Δ = 3.22 ppm). This intermediate (7.6 mg, 9.3 µmol) was followed general procedure 7 to afford compound **4** as off white solid in 72% yield (4.25 mg, 6.7 µmol) [Scheme S1, (h), ii)]. ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 9/1) δ 5.12 (d, *J* = 1.7 Hz, 1H, H1), 4.96 (d, *J* = 1.8 Hz, 1H, H1'), 3.97 (dd, J = 3.2, 1.8 Hz, 1H, H2'), 3.83 – 3.77 (m, 3H, H6^a, H6'^{a}, H5'), 3.76 (dd, *J* = 3.2, 1.8 Hz, 1H, H2), 3.74 – 3.69 (m, 4H, H5, H6^a, H6'^{a}, H3'), 3.65 (s, 3H, OC*H*$_3$), 3.66 – 3.57 (m, 4H, H12, H4, H4', H3), 2.31 (t, *J* = 7.5

Hz, 2H, C$H_{2\alpha}$), 1.63 – 1.44 (m, 6H), 1.43 – 1.22 (m, 22H), 0.90 (t, *J* = 7.0 Hz, 3H, CH_3). (*Value with the same letter may be exchangeable*). ^{13}C NMR (151 MHz, $CD_3OD/CDCl_3$ = 9/1) δ 176.02 (CO), 104.07 (C1', $^1J_{C'1H'1}$ = 168.2), 98.64 (C1, $^1J_{C\ 1H1}$ = 170.1), 81.01 (C2), 78.76 (C12), 74.74 (C3), 74.69 (C3'), 72.35 (C5), 72.15 (C5), 71.82 (C2'), 68.81 (C4), 68.56 (C4'), 62.84 (C6, C6'), 51.98 (O*C*H_3), 35.55, 34.79, 34.06, 32.94, 30.91, 30.67, 30.60, 30.53, 30.49, 30.31, 30.13, 26.50, 25.99, 25.97, 23.67, 14.45 (*C*H_3). (ESI-TOF) MS: $C_{31}H_{58}O_{13}Na^+$ calculated for $(M+Na)^+$ was 661.3770 and found 661.3770 (Δ = 0.0 ppm).

12-*O*-(2-*O*-β-D-mannopyranosyl)-α-D-mannopyranosyl-oxystearate (5)



Compound **28** (15 mg, 13.8 µmol) was followed general procedure 7 to afford compound **5** as off white solid in 75% yield (6.6 mg, 10.3 µmol) [Scheme S1, (j)]. ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ = 9/1) 4.97 (s, 1H, H1), 4.62 (s, 1H, H1'), 3.98 (dd, *J* = 3.1, 1.8 Hz, 1H, H2), 3.92 (dd, *J* = 3.2, 0.8 Hz, 1H, H2'), 3.87 (dd, *J* = 12.0, 2.4 Hz, 1H, H6'), 3.76 (dd, *J* = 3.5, 1.7 Hz, 2H, H6), 3.74 – 3.66 (m, 4H, H6', H5, H4, H12), 3.65 (s, 3H, O*C*H_3), 3.61 (dt, *J* = 9.3, 3.5 Hz, 1H, H3), 3.57 (t, *J* = 9.5 Hz, 1H, H4'), 3.47 (dd, *J* = 9.4, 3.2 Hz, 1H, H3'), 3.23 (ddd, *J* = 9.6, 6.1, 2.4 Hz, 1H, H5'), 2.31 (t, *J* = 7.5 Hz, 1H, 2H, C$H_{2\alpha}$), 1.64 – 1.45 (m, 6H), 1.44 – 1.20 (m, 22H), 0.90 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (151 M

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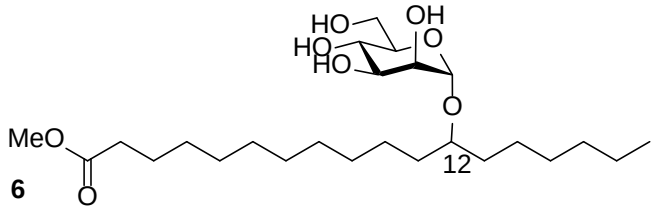
62.63 (C6'), 62.31 (C6), 51.97 (O*C*H_3), 35.51, 34.78, 33.97, 32.88, 30.84, 30.62, 30.55, 30.48, 30.45, 30.26, 30.09, 26.40, 26.01, 25.92, 23.63, 14.43, 1.47 (*C*H_3). (ESI-TOF) MS: $C_{31}H_{58}O_{13}Na^+$ calculated for $(M+Na)^+$ was 661.3770 and found 661.3777 (Δ = 1.06 ppm).

12-*O*-α-D-mannopyranosyl-oxystearate (6)

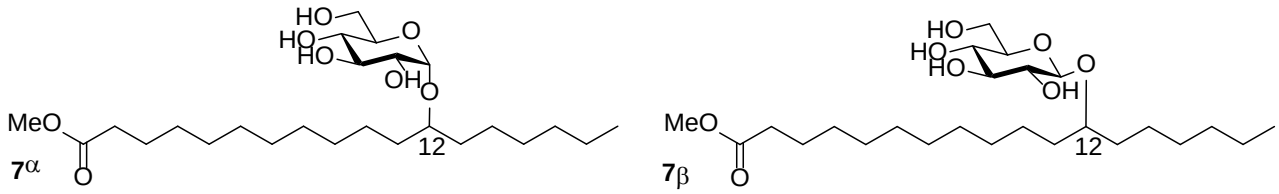


The preparation of the compound **6** was followed the procedure described in Scheme S2, including the benzoylation of-D-mannose by the general procedure 1, the bromination by general procedure 3, the glycosylation by general procedure 8 (glycosylation by Köenings-Knorr approach), and the debenzoylation by general procedure 2 to furnish the compound **6** as white syrup in 65% in total yield. NMR data was measured in pyridine and CD_3OD. ^{1}H NMR (600 MHz, py-d5) δ 5.47 (d, *J* = 1.3 Hz, 1H, H1), 4.67 (t, *J* = 9.4 Hz, 1H, H4), 4.54 (dd, J = 9.1, 3.4 Hz, 1H, H3), 4.52 (dd, J = 3.3, 1.6 Hz, 1H, H2), 4.47 (dd, *J* = 11.5, 2.5 Hz, 1H, H6), 4.39 (dd, *J* = 11.6, 5.3 Hz, 1H, H6), 4.36 – 4.32 (m, 1H, H5), 3.91 – 3.85 (m, 1H, H12), 3.62 (s, 3H, C*H*$_3$), 2.29 (t, *J* = 7.5 Hz, 2H, C$H_{2\alpha}$), 1.70 – 1.38 (m, 6H), 1.31 – 1.07 (m, 22H), 0.76 (t, *J* = 7.0 Hz, 3H, C*H*$_3$).^{13}C NMR (151 MHz, py-d5) δ 174.22 (CO), 99.93 (C1, $^1J_{C1H1}$=165.5), 76.91 (C12), 75.29 (C5), 72.79 (C3), 72.43 (C2), 68.57 (C4), 62.66 (C6), 51.37 (O*C*H_3), 34.93, 34.05, 33.27, 31.92, 30.02, 29.72, 29.66, 29.64, 29.56, 29.52, 29.39, 29.21, 25.84, 25.12, 22.81, 14.17 (*C*H_3). ^{1}H NMR (600 MHz, CD3OD) 4.85 (s, 1H, H1), δ 3.79 (dd, *J* = 11.7, 2.3 Hz, 1H, H6), 3.75 (dd, *J* = 3.1, 1.8 Hz, 1H, H2), 3.73 (dd, *J* = 11.7, 4.8 Hz, 1H, H6), 3.69 (dd, *J* = 9.1, 3.5

Hz, 1H, H3), 3.67 (t, *J* = 6.4 Hz, 1H, H12), 3.65 (s, 3H, OC*H*$_3$), 3.64 – 3.62 (m, 1H, H4), 3.62 – 3.60 (m, 1H, H5), 2.31 (t, *J* = 7.4 Hz, 2H, C*H*$_{2\alpha}$), 1.65 – 1.46 (m, 6H), 1.45 – 1.11 (m, 22H), 0.91 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, CD_3OD) δ 176.00 (CO), 100.36 (C1), 78.55 (C12), 74.81 (C5), 72.77 (C2), 72.66 (C3), 68.47 (C4), 62.80 (C6), 51.96 (O*C*H$_3$), 35.71, 34.80, 34.04, 33.00, 30.92, 30.68, 30.62, 30.59, 30.55, 30.36, 30.18, 26.60, 26.02, 25.93, 23.73, 14.46 (*C*H$_3$). (ESI-TOF) MS: $C_{31}H_{58}O_{13}Na^+$ calculated for $(M+Na)^+$ was 499.3241 and found 499.3265 (Δ = 4.81 ppm).

12-*O*-α/β-D-glucopyranosyl-oxystearate (7α/7β)

The preparation of glucosyl analogues **43** and **44** were followed the Koenigs–Knorr approach in 57% total yield (**7α/7β** = 1/ 2) (Scheme S2).



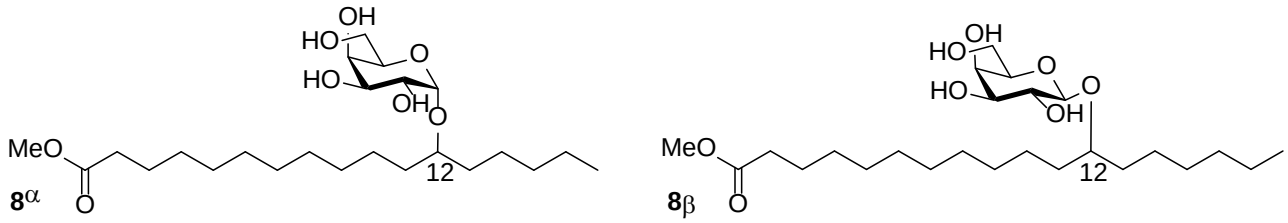
Characterization of compound **7α**: ^{1}H NMR (600 MHz, CD_3OD) δ 4.90 (d, J = 3.9 Hz, 1H, H1α), 3.76 (dd, J = 11.6, 2.4 Hz, 1H), 3.71 (t, *J* = 5.8 Hz, 1H), 3.69 – 3.60 (m, 7H), 3.36 (dt, *J* = 5.8, 3.9 Hz, 1H), 2.31 (t, *J* = 7.4 Hz, 2H, $CH_{2\alpha}$), 1.64 – 1.47 (m, 6H), 1.46 – 1.25 (m, 22H), 0.91 (t, *J* = 7.0 Hz, 3H, C*H*$_3$). ^{13}C NMR (151 MHZ, CD_3OD) δ 176.03 (CO), 98.75, 78.84, 75.05, 73.77, 73.68, 71.73, 62.53, 51.96 (O*C*H$_3$), 35.71, 34.81, 34.15, 33.02, 30.99, 30.67, 30.64, 30.60, 30.56, 30.36, 30.19, 26.69, 26.03, 25.94, 23.75, 14.46 (*C*H$_3$). (ESI-TOF) MS: $C_{25}H_{48}O_8Na^+$ calculated for $(M+Na)^+$ was 499.3241 and found 499.3264 (Δ = 4.61 ppm).

Characterization of compound **7β**: ^{1}H NMR (600 MHz, CD_3OD) δ 4.30 (d, *J* = 7.8 Hz, 1H, H1β), 3.84 (dd, *J* = 11.7, 2.4 Hz, 1H), 3.72- 3.65 (m, 2H), 3.65 (s, 3H, C*H*$_3$), 3.36-

3.28 (m, 2H), 3.23 (ddd, *J* = 9.4, 5.5, 2.4 Hz, 1H), 3.15 (dd, *J* = 9.0, 7.8 Hz, 1H), 2.31 (t, *J* = 7.5 Hz, 2H, C$H_{2\alpha}$), 1.65 – 1.47 (m, 6H), 1.44 – 1.26 (m, 22H) 0.90 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 176.02 (CO), 103.38 (C1), 80.44 (C12), 78.19, 77.72, 75.31, 71.80, 62.93, 51.96 (O*C*H_3), 35.91, 34.94, 34.81, 33.02, 30.86, 30.72, 30.69, 30.68, 30.58, 30.38, 30.19, 26.29, 26.03, 23.72, 14.45 (*C*H_3). (ESI-TOF) MS: $C_{25}H_{48}O_8Na^+$ calculated for $(M+Na)^+$ was 499.3241 and found 499.3255 (Δ = 2.80 ppm).

12-*O*-α/β-D-galactopyranosyl-oxystearate (8α/8β)

The preparation of galatosyl analogues **8α** and **8β** were followed the Koenigs–Knorr approach in 71% total yield (**8α/8β** = 1/ 5.3) (Scheme S2).



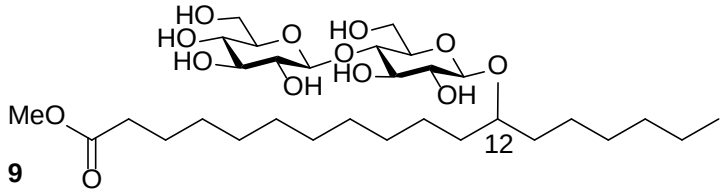
Characterization of compound **8α**: ^{1}H NMR (600 MHZ, CD_3OD) δ 4.92 (d, *J* = 3.8 Hz, 1H, H1α), 3.93 – 3.88 (m, 2H), 3.76 (dd, J = 10.2, 3.8 Hz, 1H), 3.72 (dd, *J* = 7.6, 2.6 Hz, 1H), 3.71 – 3.66 (m, 2H), 3.65 (s, 3H, O*C*H_3), 3.65 – 3.62 (m, 1H), 2.31 (t, *J* = 7.5 Hz, 2H, C$H_{2\alpha}$), 1.67 – 1.47 (m, 6H), 1.46 – 1.28 (m, 22H), 0.91 (t, *J* = 7.0 Hz, 3H, CH_3).^{13}C NMR (151 MHZ, CD_3OD) δ 176.02 (CO), 99.00 (C1), 78.78 (C12), 72.34 (C5), 71.58 (C3), 71.02 (C4), 70.34 (C2), 62.52 (C6), 51.95 (O*C*H_3), 35.69, 34.81, 34.11, 33.01, 30.98, 30.67, 30.64, 30.62, 30.56, 30.36, 30.19, 26.71, 26.03, 25.93, 23.74, 14.45 (*C*H_3). (ESI-TOF) MS: $C_{25}H_{48}O_8Na^+$ calculated for $(M+Na)^+$ was 499.3241 and found 499.3265 (Δ = 4.81 ppm).

Characterization of compound **8β**: ^{1}H NMR (600 MHZ, CD_3OD) δ 4.26 (d, *J* = 7.2 Hz, 1H, H1β), 3.85 (dd, *J* = 3.1, 1.0 Hz, 1H), 3.75 (dd, *J* = 11.0, 6.3 Hz, 1H, H), 3.72 – 3.67

(m, 2H), 3.65 (s, 3H, OCH_3), 3.49 – 3.43 (m, 3H, H), 2.31 (t, J = 7.5 Hz, 2H, $CH_{2\alpha}$), 1.65 – 1.46 (m, 6H), 1.46 – 1.24 (m, 22H), 0.91 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 176.00 (CO), 104.06 (C1), 80.44 (C12), 76.29, 75.10, 72.76, 70.10, 62.17, 51.96 (OCH_3), 35.94, 35.00, 34.81, 33.02, 30.85, 30.72, 30.70, 30.68, 30.59, 30.38, 30.19, 26.24, 26.08, 26.03, 23.72, 14.46 (CH_3). (ESI-TOF) MS: $C_{25}H_{48}O_8Na^+$ calculated for $(M+Na)^+$ was 499.3241 and found 499.3255 (Δ = 2.8 ppm).

12-*O*-β-D-cellobiosyl-oxystearate (9)

The preparation of compound **9** was followed the Koenigs–Knorr approach in 38% yield (Scheme S2).

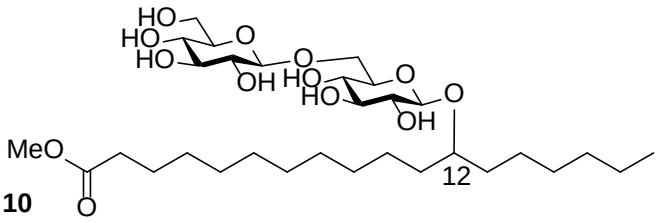


9

^{1}H NMR (600 MHz, CD_3OD) δ 4.43 (d, J = 7.9 Hz, 1H, H1'), 4.33 (d, J = 7.8 Hz, 1H, H1), 3.88 (dd, J = 12.3, 2.6 Hz, 1H, H6'), 3.86 (d, J = 3.4 Hz, 2H, H6), 3.70 – 3.64 (m, 5H, H6', H12, CH_3OCO), 3.57 (t, J = 8.9 Hz, 1H, H4), 3.51 (t, J = 9.0 Hz, 1H, H3), 3.39 – 3.35 (m, 2H, H3', H5), 3.35 – 3.33 (m, 1H, H5'), 3.33 – 3.30 (m, 1H, H4') 3.25 – 3.21 (m, 2H, H2, H2'), 2.31 (dd, J = 9.5, 5.4 Hz, 2H, C$H_2\alpha$), 1.64 – 1.46 (m, 6H), 1.46 – 1.24 (m, 22H), 0.90 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 176.00 (CO), 104.62 (C1'), 103.35 (C1), 81.13[a] (C4), 80.71[a] (C12), 78.10[b] (C5'), 77.85[b] (C5), 76.51 [c](C3), 76.26[c] (C3'), 74.96[d] (C2), 74.92[d] (C2'), 71.35 (C4'), 62.42[e] (C6), 62.21[e] (C6'), 51.97 (CH$_3$OCO), 35.89, 35.00, 34.81, 33.01, 30.82, 30.70, 30.68, 30.67, 30.57, 30.37, 30.18, 26.26, 26.05, 26.03, 23.71, 14.46 (CH_3).(*Value with the same letter may be*

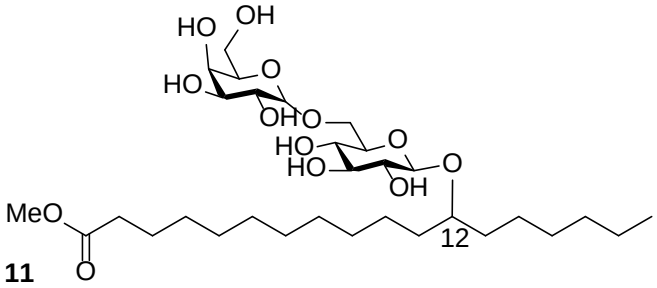
exchangeable)**.** (ESI-TOF) MS: $C_{31}H_{58}NaO_{13}^+$ calculated for $(M+Na)^+$ was 661.3770 and found 661.3798 (Δ = 4.23 ppm).

12-*O*-β-D-gentiobiosyl-oxystearate (10)



The preparation of compound **10** was followed the Koenigs–Knorr approach in 43% yield (Scheme S2). ^{1}H NMR (600 MHz, CD_3OD) δ 4.40 (d, *J* = 7.8 Hz, 1H, H1'), 4.31 (d, *J* = 7.8 Hz, 1H, H1), 4.10 (dd, *J* = 11.8, 1.9 Hz, 1H, H6), 3.87 (dd, *J* = 11.8, 2.1 Hz, 1H, H6'), 3.81 (dd, *J* = 11.8, 5.4 Hz, 1H, H6), 3.74 – 3.60 (m, 5H, H12, H6', C*H*$_3$OCO), 3.44 – 3.39 (m, 1H, H5), 3.38 – 3.33 (m, 3H, H3, H3', H4), 3.29 (d, *J* = 8.4 Hz, 1H, H4'), 3.28 – 3.24 (m, 1H, H5'), 3.21 (dd, *J* = 9.0, 8.0 Hz, 1H, H2'), 3.17 (t, *J* = 8.4 Hz, 1H, H2), 2.32 (t, *J* = 7.4 Hz, 2H, C*H*$_2$α), 1.64 – 1.46 (m, 6H), 1.46 – 1.23 (m, 22H), 0.91 (t, *J* = 6.8 Hz, 3H, C*H*$_3$). ^{13}C NMR (151 MHz, CD_3OD) δ 176.07 (CO), 104.99 (C1'), 103.28 (C1), 80.36 (C12), 78.07[a] (C3), 78.00[a] (C3'), 77.98[a] (C5'), 77.01 (C5), 75.26[b] (C2), 75.15[b] (C2'), 71.64[c] (C4'), 71.61[c] (C4), 69.97 (C6), 62.80 (C6'), 51.97 (*C*H$_3$OCO), 35.93, 34.93, 34.83, 33.03, 30.96, 30.77, 30.74, 30.71, 30.61, 30.40, 30.21, 26.41, 26.05, 25.98, 23.72, 14.46 (*C*H$_3$). (*Value with the same letter may be exchangeable*)**.** (ESI-TOF) MS: $C_{31}H_{58}NaO_{13}^+$ calculated for $(M+Na)^+$ was 661.3770 and found 661.3803 (Δ = 4.99 ppm).

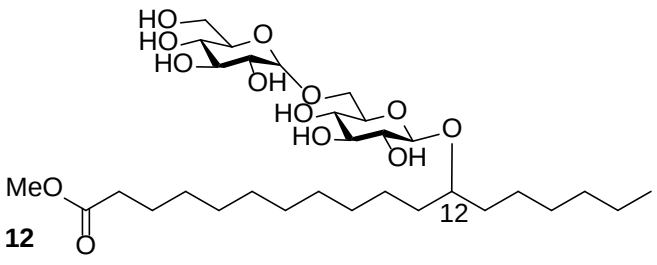
12-*O*-β-D-melibiosyl-oxystearate (11)



11

The preparation of compound **11** was followed the Koenigs–Knorr approach in 44% yield (Scheme S2). ^{1}H NMR (600 MHz, CD_3OD) δ 4.88 (d, *J* = 2.0 Hz, 1H, H1'), 4.33 (d, *J* = 7.8 Hz, 1H, H1), 3.96 (dd, *J* = 10.7, 4.8 Hz, 1H, H6), 3.91 – 3.90 (m, 1H, H4'), 3.88 (t, *J* = 6.1 Hz, 1H, H5'), 3.77 – 3.75 (m, 2H, H2', H3'), 3.71 (dd, *J* = 6.0, 2.0 Hz, 2H, H6'), 3.69 – 3.64 (m, 5H, H12, H6, C*H*$_3$OCO), 3.48 – 3.43 (m, 1H, H5), 3.41 – 3.34 (m, 2H, H3, H4), 3.20 – 3.16 (m, 1H, H2), 2.31 (t, *J* = 7.5 Hz, 2H, C*H*$_2$α), 1.64 – 1.48 (m, 6H), 1.31 (s, 22H), 0.91 (t, *J* = 7.0 Hz, 3H, C*H*$_3$). ^{13}C NMR (151 MHz, CD_3OD) δ 176.04 (CO), 103.79 (C1), 100.26 (C1'), 81.20 (C12), 78.14 (C3), 76.14 (C5), 75.25 (C2), 72.18 (C5'), 71.68 (2C, C4, C3'), 71.10 (C4'), 70.49 (C2'), 67.59 (C6), 62.73 (C6'), 51.98 (*C*H$_3$OCO), 49.00, 35.90, 35.01, 34.81, 33.00, 30.94, 30.72, 30.70, 30.58, 30.38, 30.19, 26.36, 26.03, 26.02, 23.70, 14.46 (CH_3). (ESI-TOF) MS: $C_{31}H_{58}NaO_{13}^{+}$ calculated for $(M+Na)^{+}$ was 661.3770 and found 661.3795 (Δ = 3.78 ppm).

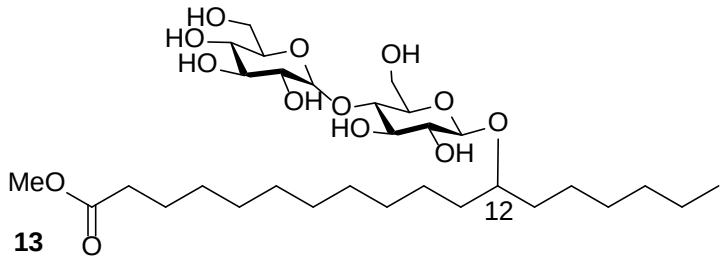
12-*O*-β-D-isomaltosyl-oxystearate (12)



12

The preparation of compound **12** was followed the Koenigs–Knorr approach in 70% yield (Scheme S2). ^{1}H NMR (600 MHz, CD_3OD) δ 4.85 (H1', suppressed by CD_3OD), 4.34 (d, *J* = 7.8 Hz, 1H, H1), 3.99 (dd, *J* = 10.8, 3.8 Hz, 1H, H6), 3.79 (dd, *J* = 11.6, 2.2 Hz, 1H, H6'), 3.72 – 3.62 (m, 8H, H5, H5', H6, H6', H12, C*H*$_3$OCO), 3.48 – 3.41 (m,, 2H, H4, H3'), 3.38 – 3.29 (m, 3H, H3, H2', H4'), 3.18 (dd, *J* = 9.2, 7.9 Hz, 1H, H2), 2.31 (t, *J* = 7.5 Hz, 2H), 1.65 – 1.49 (m, 6H), 1.46 – 1.24 (m, 22H), 0.91 (dd, *J* = 8.2, 5.8 Hz, 3H, C*H*$_3$). ^{13}C NMR (151 MHz, CD_3OD) δ 176.04 (CO), 103.64 (C1), 100.11 (C1'), 80.90 (C12), 78.20 (C3), 76.20 (C3'), 75.33[a] (C2), 75.27[a] (C5), 73.85 (C2'), 73.48 (C5'), 71.63[b] (C4'), 71.42[b] (C4), 67.40 (C6), 62.56 (C6'), 51.98 (*C*H_3OCO), 49.43, 49.28, 49.14, 49.00, 48.86, 48.72, 48.57, 35.85, 34.93, 34.82, 33.01, 30.93, 30.73, 30.72, 30.69, 30.60, 30.39, 30.20, 26.27, 26.03, 25.99, 23.71, 14.46 (CH_3) (*Value with the same letter may be exchangeable*). (ESI-TOF) MS: $C_{31}H_{58}NaO_{13}^{+}$ calculated for $(M+Na)^{+}$ was 661.3770 and found 661.3797 (Δ = 4.08 ppm).

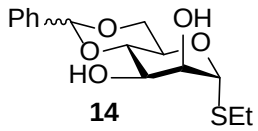
12-*O*-β-D-maltosyl-oxystearate (13)



The preparation of compound **13** was followed the Koenigs–Knorr approach in 70% total yield (Scheme S2). ^{1}H NMR (600 MHz, CD_3OD) δ 5.16 (d, *J* = 3.8 Hz, 1H, H1'), 4.32 (d, *J* = 7.8 Hz, 1H, H1), 3.89 – 3.80 (m, 3H, H6, H6'), 3.72 –3.64 (m, 6H, H5', H6', H12, C*H*$_3$OCO) 3.61 (t, *J* = 9.1 Hz, 2H, H3, H3'), 3.55 (t, *J* = 9.2 Hz, 1H, H4), 3.44 (dd, *J* = 9.7, 3.8 Hz, 1H, H2'), 3.34 (ddd, *J* = 9.6, 4.3, 2.1 Hz, 1H, H5), 3.26 (t, *J* = 9.1 Hz, 1H, H4'), 3.21 (dd, *J* = 9.3, 7.8 Hz, 1H, H2), 2.31 (t, *J* = 7.4 Hz, 2H, C*H*$_2$α), 1.67 – 1.48 (m, 6H), 1.45 – 1.23 (m, 22H), 0.90 (t, *J* = 7.0 Hz, 3H, C*H*$_3$). ^{13}C NMR (151 MHz, CD_3OD)

δ 176.01 (CO), 103.40 (C1), 102.87 (C1'), 81.36 (C4), 80.63 (C12), 77.90 (C3), 76.49 (C5), 75.08 (C3'), 74.86 (C2), 74.73 (C5'), 74.16 (C2'), 71.51 (C4'), 62.76 (C6'), 62.32 (C6), 51.97 (*C*H_3OCO), 35.89, 34.94, 34.81, 33.01, 30.84, 30.71, 30.68, 30.67, 30.57, 30.37, 30.18, 26.26, 26.03, 23.71, 14.46 (*C*H_3). (ESI-TOF) MS: $C_{31}H_{58}NaO_{13}^+$ calculated for $(M+Na)^+$ was 661.3770 and found 661.3801 (Δ = 4.69 ppm).

Ethyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (14)

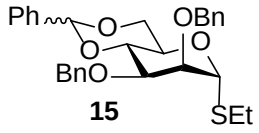


14

The preparation of the known Ethyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside **14** was developed from several previous reports [Scheme 1, (a)].[2-4] Hence, the dried crude of per benzoyl mannose prepared from the free-D-mannose (4.371 g, 24.3 mmol) by the general procedure 1 in round bottom flask was flushed with argon and dissolved in 1,2-dichloroethane (218 ml). This stirred solution was sequentially treated with Boron trifluoride diethyl etherate (5.5 ml, 43.7 mmol), Ethane thiol (3.6 ml, 48.5 mmol) at -5 °C, and kept for 5 minutes at this temperature. The reaction mixture was then stirred at 50 °C for 4 hr and neutralized with Et_3N (the reaction color was change from brown to transparent yellow). The organic solvent was removed by evaporator, and the solid residue was followed with general procedure 2 to generate ethyl 1-thio-α-D-mannopyranoside intermediate in 80.4% yield (4.374 g, 19.5 mmol) [Scheme 1, (a), i) ii) iii)]. Proton NMR data of the known ethyl 1-thio-α-D-mannopyranoside intermediate: ^{1}H NMR (400 MHz, CD_3OD) δ 5.16 (br, 1H, H1), 3.89 – 3.52 (m, 6H), 2.67 – 2.43 (m, 2H, SC*H*$_2$CH$_3$), 1.19 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$).

This powdered intermediate was then added to a solid mixture of benzaldehyde (dried by MS_3A, 7.5 ml, 73.4 mmol) and $ZnCl_2$ (2.516 g, 18.5 mmol) which was prepared by being stirred at room temperature for 2 minutes and applied in ultrasound for 2 minutes. The resulting mixture was flushed with argon, stirred for 30 seconds, subjected to ultrasound for 1 minute, and immediately quenched by 10 ml of ice-cold water. The reaction mixture was extracted with the solvent mixture of chloroform and methanol (900 ml, 30:1). The organic phase was concentrated in rotary evaporator, and purified by column chromatography to give **14** as a white solid in 53% yield (3.222 g, 10.3 mmol, equal to 61 % conversion yield based on recovered ethyl 1-thio-α-D-mannopyranoside intermediate) [Scheme 1, (a), iv)]. The Proton NMR data of the compound **14**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.43 (m, 2H), 7.43 – 7.32 (m, 3H), 5.55 (s, 1H, C*H*Ar), 5.35 (s, 1H, H1), 4.29 – 4.17 (m, 2H), 4.12 (s, 1H), 4.05 (d, *J* = 9.8 Hz, 1H), 3.95 (t, *J* = 9.1 Hz, 1H), 3.84 (t, *J* = 11.8 Hz, 1H), 2.79 (br, 1H, OH), 2.73 – 2.53 (m, 2H, SC*H*$_2$CH$_3$), 1.29 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$). (This data was comparable with reference).[5] (ESI-TOF) MS: $C_{15}H_{20}O_5SNa^+$ calculated for $(M+Na)^+$ was 335.0924 and found 335.0925 (Δ = 0.30 ppm).

Ethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (15)

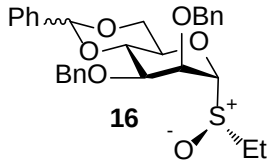


15

Compound **14** (2.248 g, 7.2 mmol) was benzylated by general procedure 4 to afford the ethyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside **15** as an off white syrup in 91% yield (3.217 g, 6.5 mmol) (Scheme S1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 7.2 Hz, 2H), 7.45 – 7.22 (m, 13H), 5.63 (s, 1H, C*H*Ar), 5.29 (s, 1H, H1), 4.82 – 4.69 (m, 3H, C*H*$_2$Ar), 4.62 (d, *J* = 12.2 Hz, 1H, C*H*$_2$Ar), 4.32 – 4.14 (m, 3H), 3.95 – 3.85

(m, 3H), 2.66 – 2.48 (m, 2H, SC*H*$_2$CH$_3$), 1.23 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$). (This data was comparable with reference).[6] (ESI-TOF) MS: $C_{29}H_{32}O_5SNa^+$ calculated for $(M+Na)^+$ was 515.1863 and found 515.1861 (Δ = 0.39 ppm).

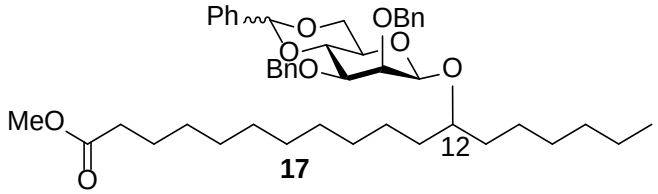
Ethyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-1-deoxy-1-thio-α-D-mannopyranoside S-oxide(16)



16

Compound **16** was prepared in 83% yield (2.746 g, 5.4 mmol) from compound **15** (3.205 g, 6.5 mmol) by general procedure 5 as a white solid (Scheme 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.22 (m, 15H), 5.63 (s, 1H, C*H*Ar), 4.83 (t, *J* = 12.8 Hz, 2H, C*H*$_2$Ar), 4.69 (t, *J* = 11.6 Hz, 2H, C*H*$_2$Ar), 4.61 (s, 1H), 4.51 (s, 1H), 4.34 (t, *J* = 9.5 Hz, 1H), 4.19 (dd, *J* = 9.2, 3.5 Hz, 1H), 4.12 (d, *J* = 9.9 Hz, 1H), 3.85 – 3.66 (m, 2H), 2.97 – 2.84 (m, 1H, SC*H*$_2$CH$_3$), 2.70 – 2.57 (m, 1H, SC*H*$_2$CH$_3$), 1.34 (t, *J* = 7.2 Hz, 1H, SCH$_2$C*H*$_3$)). (This data was comparable with reference).[7] (ESI-TOF) MS: $C_{29}H_{32}O_6SNa^+$ calculated for $(M+Na)^+$ was 531.1812 and found 531.1794 (Δ = 3.39 ppm).

12-*O*-4,6-*O*-benzylidene-2,3-*O*-benzyl-β-D-mannopyranosyl-oxystearate (17)

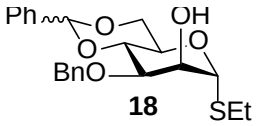


17

General procedure 6a using 400 mg of donor **16** was employed to generate compound **17** in 44% yield (256 mg, 344 μmol) as an off white syrup. (General procedure 6b did archive in the 57% yield) (Scheme 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 7.24 (m, 15H), 5.63

(s, 1H, C*H*Ar), 5.00 (d, *J* = 13.0 Hz, 1H), 4.89 (d, *J* = 13.2 Hz, 1H), 4.71 (d, *J* = 11.6 Hz, 1H), 4.61 (d, *J* = 11.3 Hz, 1H), 4.51 (s, 1H), 4.35 – 4.27 (m, 1H), 4.23 (t, *J* = 8.2 Hz, 1H), 3.96 (t, *J* = 10.4 Hz, 1H), 3.89 (s, 1H), 3.68 (s, 3H), 3.67 – 3.58 (m, 2H), 3.39 – 3.28 (m, 1H), 2.32 (t, *J* = 7.1 Hz, 2H, C$H_{2\alpha}$), 1.74 – 1.43 (m, 6H), 1.30 (s, 22H), 0.92 (t, *J* = 7.4 Hz, 3H, CH_3). (ESI-TOF) MS: $C_{46}H_{64}O_8Na^+$ calculated for $(M+Na)^+$ was 767.4493 and found 767.4457 (Δ = 4.69 ppm).

Ethyl 4, 6-*O*-benzylidene-3-*O*-benzyl-1-deoxy-1-thio-α-D-mannopyranoside (18)

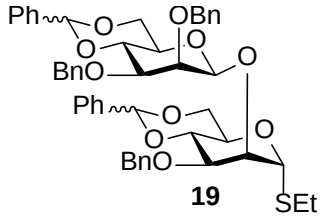


18

A solution of intermediate **14** (150 mg, 480 μmol) and $BuSn_2O$ (143.9 mg, 576 μmol) in 6 ml of Toluene was refluxed for 3 hr at 130 ℃, and then removed the solvent by evaporator. The white solid residue was dried completely in *vacuo.* for 30 minutes, added CsF (148.9 mg, 979 μmol), and dried again in *vacuo.* at 40 ℃ for 30 minutes. The dried solid was flushed with argon, dissolved in 3.5 ml of DMF, and then dropwise treated with benzyl bromide (125 μl, 1.1 mmol) at 0 ℃. After 30 minutes, the resulting mixture was turned to rt., and stirred overnight. The reaction mixture was poured in to sat. $NaHCO_3$, extracted by $CHCl_3$, washed with brine, and dried over Na_2SO_4. The organic phase was evaporated by rotary evaporator, and purified by column chromatography to give compound **18** as white syrup in 98% yield (189.1 mg, 470 μmol) (Scheme 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 6.8 Hz, 2H), 7.44 – 7.28 (m, 8H), 5.62 (s, 1H, C*H*Ar), 5.37 (s, 1H, H1), 4.86 (d, *J* = 11.7 Hz, 1H, CH_2Ar), 4.71 (d, *J* = 11.8 Hz, 1H, CH_2Ar), 4.29 – 4.20 (m, 2H), 4.18 – 4.10 (m, 2H), 3.94 – 3.84 (m, 2H), 2.81 (br, 1H, OH), 2.74 –

2.52 (m, 2H, SC*H*$_2$CH$_3$), 1.29 (t, *J* = 7.3 Hz, 3H, SCH$_2$C*H*$_3$). (ESI-TOF) MS: $C_{22}H_{26}O_5SNa^+$ calculated for $(M+Na)^+$ was 425.1393 and found 425.1377 (Δ = 3.76 ppm).

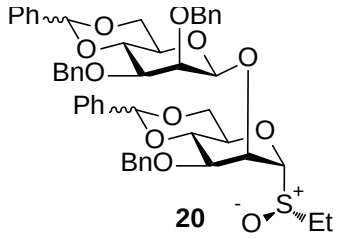
Ethyl 4,6-*O*-benzylidene-2-*O*-[4,6-*O*-benzylidene-2,3-di-*O*-benzyl-β-D-mannopyranosyl]-3-*O*- benzyl-1-thio-α-D-mannopyranoside (19)



19

The coupling of α-mannosyl sulfoxides **16** (109.8 mg, 216 µmol) and acceptor **18** (174.8 mg, 431 µmol) by general procedure 6a gave compound **19** as off white solid in 51% yield (91.1 mg, 109 µmol) (Scheme 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67– 7.10 (m, 25H), 5.53 (s, 1H, C*H*Ar), 5.42 (s, 1H, C*H*Ar), 5.25 (s, 1H, H1), 4.98 (d, *J* = 12.1 Hz, 1H, C*H*$_2$Ar), 4.90 (d, *J* = 12.4 Hz, 1H, C*H*$_2$Ar), 4.72 – 4.53 (m, 4H, C*H*$_2$Ar), 4.28 (s, 1H), 4.24 – 4.10 (m, 4H), 4.10 – 4.02 (m, 1H), 3.93 (s, 1H), 3.82 (dd, J = 24.7, 10.2 Hz, 2H), 3.71 (t, J = 10.9 Hz, 1H), 3.55 (d, J = 10.0 Hz, 1H), 3.27 (dd, J = 11.2, 7.5 Hz, 1H), 2.64 – 2.46 (m, 2H, SC*H*$_2$CH$_3$), 1.42 – 1.15 (m, 3H, SCH$_2$C*H*$_3$). (ESI-TOF) MS: $C_{49}H_{52}O_{10}SNa^+$ calculated for $(M+Na)^+$ was 855.3173 and found 855.3158 (Δ = 1.75 ppm).

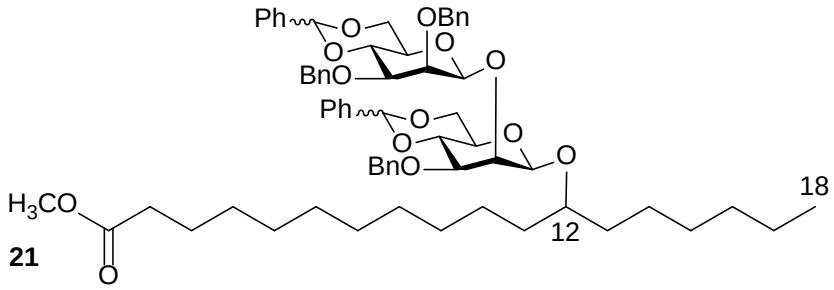
Ethyl 4,6-*O*-benzylidene-2-*O*-[4,6-*O*-benzylidene-2,3-di-*O*-benzyl-β-D-mannopyranosyl]-3-*O*- benzyl-1-thio-α-D-mannopyranoside S-Oxide (20)



20

Compound **19** (89 mg, 106 µmol) was oxidized by general procedure 5 to furnish the compound **20** as a white solid in 78% yield (70.6 mg, 83 µmol) (Scheme 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.20 (m, 25H), 5.58 (s, 1H, C*H*Ar), 5.48 (s, 1H, C*H*Ar), 5.05 – 4.91 (m, 2H), 4.89 – 4.57 (m, 6H), 4.52 (s, 1H), 4.31 – 4.05 (m, 5H), 3.98 (s, 1H), 3.81 (t, *J* = 10.3 Hz, 1H), 3.76 – 3.57 (m, 3H), 3.39 – 3.29 (m, 1H), 3.03 – 2.88 (m, 1H, SC*H*$_2$CH$_3$), 2.74 – 2.63 (m, 1H, SC*H*$_2$CH$_3$), 1.39 (t, *J* = 7.4 Hz, 3H, SCH$_2$C*H*$_3$). (ESI-TOF) MS: $C_{49}H_{52}O_{11}SNa^+$ calculated for $(M+Na)^+$ was 871.3123 and found 871.3116 (Δ = 0.8 ppm).

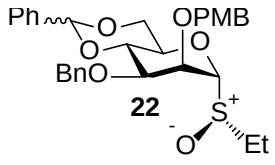
12-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,3-*O*-benzyl-4,6-*O*-benzylidene -β-D-mannopyranosyl)-β-D-mannopyranosyl-oxystearate (21).



The sulfoxide **20 (**50.1 mg, 59 µmol) was followed general procedure 6a to afford the glycosylated product **21** as off white syrup in 38% yield (24.2 mg, 22 µmol) [Scheme 1, (d)]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48 (d, *J* = 7.9 Hz, 2H), 7.42 (dd, *J* = 11.6, 7.7 Hz, 4H), 7.37 (d, *J* = 7.7 Hz, 2H), 7.33 – 7.25 (m, 6H), 7.25 – 7.21 (m, 4H), 7.21 – 7.15 (m, 7H), 5.54 (s, 1H, C*H*Ar), 5.36 (s, 1H, C*H*Ar), 5.02 (d, *J* = 12.3 Hz, 1H, C*H*$_2$Ar), 4.91 (d, *J* = 12.4 Hz, 1H, C*H*$_2$Ar), 4.78 (s, 1H, H1'), 4.72 (s, 2H, C*H*$_2$Ar), 4.50 (q, *J* = 12.4 Hz, 2H, C*H*$_2$Ar), 4.43 (s, 1H, H1) 4.22 (td, *J* = 10.5, 4.7 Hz, 2H, H6'), 4.16 (t, *J* = 9.5 Hz, 1H,

H4'), 4.12 (d, *J* = 3.2 Hz, 1H, H2'), 4.09 (d, *J* = 3.1 Hz, 1H, H2), 3.97 (t, *J* = 9.6 Hz, 1H, H4), 3.87 (t, *J* = 10.3 Hz, 1H, H6), 3.66 (t, *J* = 10.2 Hz, 1H, H6), 3.58 (s, 3H, OCH_3), 3.57 – 3.55 (m, 1H, H12), 3.55 – 3.53 (m, 1H, H_3), 3.48 (dd, *J* = 9.8, 2.9 Hz, 1H, H3'), 3.28 – 3.21 (m, 2H), H5, H5'), 2.21 (t, *J* = 7.6 Hz, 2H, C$H_{2\alpha}$), 1.58 – 1.00 (m, 28H, CH_2) 0.81 (t, *J* = 7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.40(CO), 139.24, 138.93, 138.55, 137.87, 137.69, 128.99, 128.91, 128.65, 128.35, 128.33, 128.27, 128.23, 127.69, 127.56, 127.48, 127.39, 103.85, 103.83 (C1'), 101.66 (C*H*Ar), 101.51 (C*H*Ar), 100.71 (C1), 79.93 (C12), 78.54 (C4), 78.46 (C4'), 77.87 (C3'), 77.75 (C2), 76.43 (C3), 75.89 (C2'), 74.81 (CH_2Ar), 71.93 (CH_2Ar), 71.30 (CH_2Ar) 68.98 (C6), 68.91 (C6'), 67.85 (C5'), 67.62 (C5), 51.55(O*C*H_3), 34.96, 34.24 (*C*$H_{2\alpha}$), 33.82, 31.93, 29.89, 29.83, 29.62, 29.55, 29.44, 29.30, 25.33, 25.19, 25.09, 22.76, 14.21 (*C*H_3). (ESI-TOF) MS: $C_{66}H_{84}O_{13}Na^+$ calculated for $(M+Na)^+$ was 1107.5804 and found 1107.5801 (Δ = 0.27 ppm).

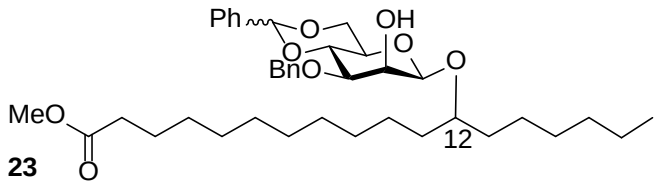
Ethyl 4,6-*O*-benzylidene-3-*O*-Benzyl-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside *S*-oxide (22)



The suspension of NaH in anhydrous DMF (49.4 mg, 1.2 mmol, prepared as described in general procedure 4) was added to a stirred solution of intermediate **18** (249 mg, 617 µmol) and Bu_4NI (40.7mg, 110 µmol) in anhydrous DMF in ice-bath (6.6 ml in final volume). The reaction mixture was stirred vigorously for 30 minutes and dropwise charged with PMBCl (109 µl, 773 µmol). The resulting mixture was stirred further one

hour in ice-bath and took out ice-bath to let the reaction run overnight at rt. The reaction mixture was quenched with MeOH (0.5 mL), extracted with ice-cold water (45 ml) and $CHCl_3$ (3*30ml), and washed with sat. $NaHCO_3$ and brine. The organic phase was concentrated on rotary evaporator, dried in *vacuo.* for 1 days, and purified by column chromatography over silica gel to furnish the compound Ethyl 4,6-*O*-benzylidene-3-*O*-benzyl-2-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside as a syrup in 81% yield (261.4 mg, 0.5 mmol) [Scheme S1, (a), i)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 6.4 Hz, 2H), 7.42 – 7.24 (m, 10H), 6.88 (d, *J* = 8.2 Hz, 2H), 5.64 (s, 1H, C*H*Ar), 5.28 (s, 1H), 4.79 (d, *J* = 12.0 Hz, 1H), 4.69 (s, 2H), 4.61 (d, J = 12.0 Hz, 1H), 4.33 – 4.14 (m, 3H), 3.91 (d, *J* = 11.1 Hz, 3H), 3.82 (s, 3H), 2.68 – 2.44 (m, 2H, SC*H*$_2$CH$_3$), 1.25 (t, *J* = 7.5 Hz, 3H, SCH$_2$C*H*$_3$). (ESI-TOF) MS: $C_{30}H_{34}O_6SNa^+$ calculated for $(M+Na)^+$ was 545.1968 and found 545.1972 (Δ = 0.73 ppm). This intermediate was followed the general procedure 5 to afford the compound **22** as a white solid in 91% yield (243.4 mg 452 μmol) [Scheme S1, (a), ii)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (dd, *J* = 6.6, 2.9 Hz, 2H), 7.43 – 7.29 (m, 10H), 6.88 (d, *J* = 8.6 Hz, 2H), 5.64 (s, 1H), 4.80 (t, *J* = 11.6 Hz, 2H), 4.67 (dd, *J* = 11.7, 1.8 Hz, 2H), 4.60 (s, 1H), 4.52 (d, *J* = 2.6 Hz, 1H), 4.34 (t, *J* = 9.6 Hz, 1H), 4.21 (dd, *J* = 10.0, 4.3 Hz, 1H), 4.12 (dd, *J* = 10.2, 3.5 Hz, 1H), 3.82 (s, 3H), 3.86 – 3.77 (m, 1H), 3.76 – 3.67 (m, 1H), 2.93 (m, 1H), 2.74 – 2.60 (m, 1H), 1.37 (t, *J* = 7.5 Hz, 3H). (ESI-TOF) MS: $C_{30}H_{34}O_7SNa^+$ calculated for $(M+Na)^+$ was 561.1917 and found 561.1901 (Δ = 2.85 ppm).

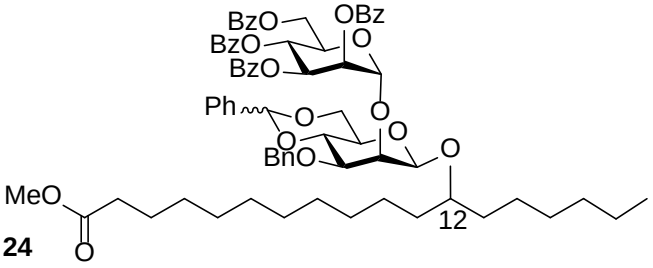
12-*O*-(4, 6-*O*-benzylidene-3-*O*-benzyl)-β-D-mannopyranosyl-oxystearate (23)



23

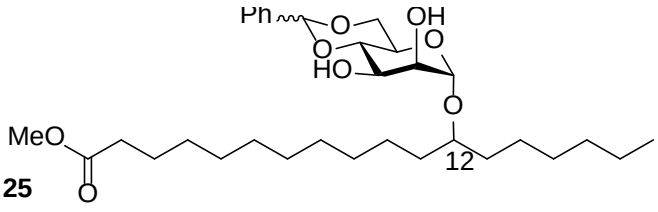
The preparation of the compound **23** was initiated with the glycosylation of the donor **22** (241 mg, 447 µmol) and the methyl 12-hydroxystearate as an acceptor (general procedure 6a) to afford intermediate 12-*O*-(4, 6-*O*-benzylidene-3-*O*-benzyl-2-*O*-*p*-methoxybenzyl)-β-D-mannopyranosyl-oxystearate as an off white syrup in 25% yield (87.6 mg, 113 µmol) [Scheme S1, (b), i)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 6.6 Hz, 2H), 7.45 – 7.23 (m, 10H), 6.84 (d, *J* = 7.2 Hz, 2H), 5.61 (s, 1H, C*H*Ar), 4.92 (d, *J* = 11.9 Hz, 1H), 4.81 (d, *J* = 12.1 Hz, 1H), 4.68 (d, *J* = 12.5 Hz, 1H), 4.57 (d, *J* = 12.4 Hz, 1H), 4.48 (s, 1H), 4.27 (dd, *J* = 9.8, 4.2 Hz, 1H), 4.20 (t, *J* = 9.4 Hz, 1H), 3.94 (t, *J* = 10.2 Hz, 1H), 3.86 (s, 1H), 3.81 (s, 3H), 3.67 (s, 3H), 3.65 – 3.53 (m, 2H), 3.35 – 3.25 (m, 1H), 2.30 (t, *J* = 7.3 Hz, 2H, C$H_{2\alpha}$), 1.68 – 1.50 (m, 6H), 1.51 – 1.20 (m, 22H), 0.91 (t, *J* = 6.6 Hz, 3H, CH_3). (ESI-TOF) MS: $C_{47}H_{66}O_9Na^+$ calculated for $(M+Na)^+$ was 797.4599 and found 797.4605 (Δ = 0.75 ppm). This intermediate was followed the general procedure 9 to furnish the compound **23** as an off white syrup in 79.5% yield (59 mg, 0.09 mmol) [Scheme S1, (b), ii)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 6.7 Hz, 2H), 7.43 – 7.23 (m, 8H), 5.60 (s, 1H, C*H*Ar), 4.87 (d, *J* = 12.8 Hz, 1H), 4.79 (d, *J* = 12.6 Hz, 1H), 4.54 (s, 1H), 4.29 (dd, *J* = 9.9, 4.2 Hz, 1H), 4.15 (t, *J* = 9.5 Hz, 1H), 4.07 (s, 1H), 3.87 (t, *J* = 10.2 Hz, 1H), 3.66 (s, 3H, OCH_3), 3.7 1– 3.61 (m, 1H), 3.38 – 3.26 (m, 1H), 2.53 (br, 1H, OH), 2.30 (t, *J* = 7.3 Hz, 2H, C$H_{2\alpha}$), 1.68 – 1.40 (m, 6H), 1.27 (s, 22H), 0.89 (t, *J* = 5.7 Hz, 3H, CH_3). (ESI-TOF) MS: $C_{39}H_{58}O_8$ Na^+ calculated for $(M+Na)^+$ was 677.4024 and found 677.4037 (Δ = 1.92 ppm)

12-*O*-(4,6-*O*-benzylidene-3-*O*-benzyl-2-*O*-(2,3, 4, 6-*O*-benzoyl- α-D-mannopyranosyl))-β-D-mannopyranosyl-oxystearate (24)



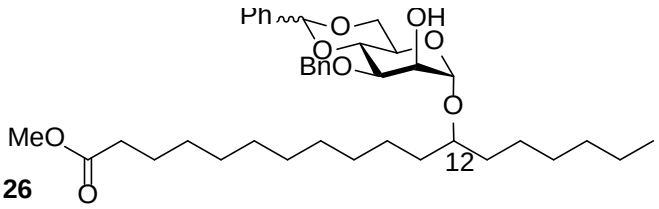
The general procedure 8 using the methyl 12-hydroxystearate as an acceptor and per-*O*-benzoyl-α-D-mannopyranosyl bromide as a donor was used to synthesize compound **24** as a syrup in 89% yield (44.4 mg, 36 µmol) [Scheme S1, (c)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 7.4 Hz, 2H), 8.03 (d, *J* = 7.1 Hz, 2H), 7.93 (d, *J* = 7.5 Hz, 2H), 7.84 (d, *J* = 7.5 Hz, 2H), 7.62 – 7.46 (m, 5H), 7.46 – 7.30 (m, 10H), 7.30 – 7.21 (m, 4H), 7.19 – 7.08 (m, 3H), 6.19 (t, *J* = 9.7 Hz, 1H), 6.00 (d, *J* = 10.7 Hz, 2H), 5.74 (s, 1H), 5.56 (s, 1H), 5.09 (d, *J* = 10.1 Hz, 1H), 4.90 (d, *J* = 13.0 Hz, 1H), 4.74 – 4.62 (m, 2H), 4.59 (s, 1H), 4.42 (d, *J* = 11.9 Hz, 1H), 4.38 – 4.31 (m, 1H), 4.31 – 4.21 (m, 2H), 4.06 (t, *J* = 10.4 Hz, 1H), 3.71 (d, *J* = 8.9 Hz, 2H), 3.65 (s, 3H), 3.41 – 3.30 (m, 1H), 2.27 (t, *J* = 7.3 Hz, 2H, C$H_{2\alpha}$) 1.63 – 1.17 (m, 28H), 0.86 (t, *J* = 5.5 Hz, 3H, CH_3). (ESI-TOF) MS: $C_{73}H_{84}O_{17}Na^+$ calculated for $(M+Na)^+$ was1255.5601 and found 1255.5572 (Δ = 2.31 ppm).

12-*O*-(4,6-*O*-benzylidene)-α-D-mannopyranosyl-oxystearate (25)



The procedure developed for compound **14** was employed to prepare the compound **25** in 31% yield (55 mg, 97.4 µmol, equal to 57 % conversion yield based on recovered **6**). Thus, the intermediate **6** (150 mg, 314.7 µmol) was initially mixed with 41 mg of $ZnCl_2$. The resulting mixture was then flushed with argon, added 120 µl of dried benzaldehyde, stirred for 30 seconds, subjected to ultrasound for 1 minute, immediately quenched by 10 ml of ice-cold water, and purified as the procedure developed for compound **14** [Scheme S1, (e)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.36 (m, 2H), 7.32 – 7.26 (m, 3H), 5.48 (s, 1H, C*H*Ar), 4.86 (s, 1H, H1), 4.15 (dd, *J* = 9.7, 3.1 Hz, 1H), 4.08 – 3.96 (m, 1H), 3.90 (s, 1H), 3.87 – 3.78 (m, 2H), 3.77 – 3.69 (m, 1H), 3.58 (s, 3H, OCH_3), 3.53 (quint, *J* = 5.8 Hz, 1H), 2.84 (d, *J* = 18.0 Hz, 2H, OH), 2.22 (t, *J* = 7.5 Hz, 2H, C$H_{2\alpha}$), 1.60 – 1.35 (m, 6H), 1.34 – 1.11 (m, 22H), 0.82 (t, *J* = 6.4 Hz, 3H, CH_3). (ESI-TOF) MS: $C_{32}H_{52}O_8Na^+$ calculated for $(M+Na)^+$ was 587.3554 and found 587.3559 (Δ = 0.85 ppm).

12-*O*-*(*4,6-*O*-benzylidene-3-*O*-benzyl)-α-D-mannopyranosyl-oxystearate (26)

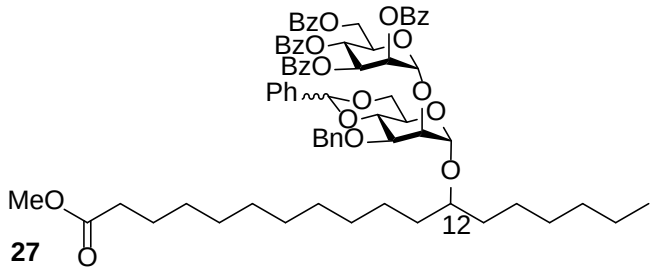


26

The procedure used to prepare compound **18** was employed to prepare compound **26** in 84% yield as white syrup (48.8 mg, 74.5 µmol) from the intermediate **25** (50 mg, 88 µmol) [Scheme S1, (f)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (dd, *J* = 7.7, 1.9 Hz, 2H), 7.38 – 7.21 (m, 3H), 5.58 (s, 1H, C*H*Ar), 4.92 (d, *J* = 1.0 Hz, 1H, H1), 4.85 (d, *J* = 11.8 Hz, 1H, CH_2Ar), 4.70 (d, *J* = 11.8 Hz, 1H, CH_2Ar), 4.20 (dd, *J* = 9.9, 4.5 Hz, 1H), 4.07 (t, *J* = 9.4 Hz, 1H), 4.00 (d, *J* = 3.2 Hz, 1H), 3.93 – 3.85 (m, 1H), 3.80 (t, *J* = 10.2 Hz, 1H), 3.63 (s, 1H, OCH_3), 3.57 (quint, *J* = 5.7 Hz, 1H), 2.68 (s, 1H, OH), 2.27 (t, *J* = 7.5

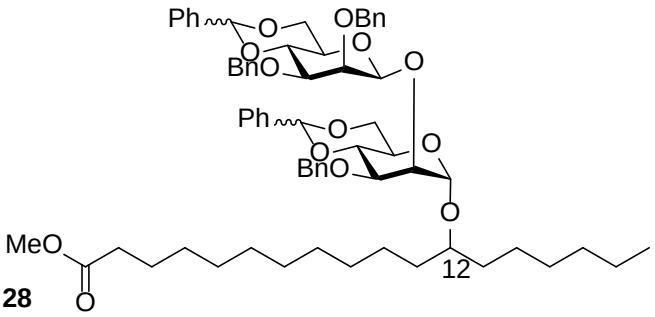
Hz, 2H, C$H_{2\alpha}$), 1.64 – 1.53 (m, 1H, 3H), 1.53 – 1.39 (m, 3H), 1.37 – 1.14 (m, 22H), 0.85 (t, *J* = 6.7 Hz, 3H, CH_3). (ESI-TOF) MS: $C_{39}H_{58}O_8Na^+$ calculated for $(M+Na)^+$ was 677.4024 and found 677.4010 (Δ = 2.07ppm).

12-*O*-(4,6-*O*-benzylidene-3-*O*-benzyl-2-*O*-(2,3,4,6-*O*-benzoyl-α-D-mannopyranosyl))-α-D-mannopyranosyl-oxystearate (27)



The acceptor **26** (10 mg, 15.3 µmol) and per-*O*-benzoyl-α-D-mannopyranosyl bromide as a donor (15.2 mg, 0.023 mmol) were used to synthesize compound **27** as off white syrup in 99% yield (18.7 mg, 15.2 µmol) by general procedure 8) [Scheme S1, (g)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 7.99 (m, 2H), 7.98 – 7.94 (m, 2H), 7.91 – 7.86 (m, 2H), 7.75 (dt, *J* = 8.4, 1.5 Hz, 2H), 7.55 – 7.40 (m, 5H), 7.39 – 7.15 (m, 15H), 7.12 – 6.99 (m, 2H), 6.04 (t, *J* = 9.9 Hz, 1H), 5.91 (dd, *J* = 10.1, 3.3 Hz, 1H), 5.88 – 5.84 (m, 1H), 5.69 (s, 1H), 5.35 (d, *J* = 1.8 Hz, 1H), 4.95 (d, *J* = 1.4 Hz, 1H), 4.84 (d, *J* = 12.2 Hz, 1H), 4.57 (dd, *J* = 18.1, 8.1 Hz, 2H), 4.46 – 4.38 (m, 2H), 4.21 (dt, *J* = 8.7, 6.3 Hz, 2H), 4.04 (dd, *J* = 3.2, 1.4 Hz, 1H), 3.95 (dd, *J* = 9.9, 3.1 Hz, 1H), 3.93 – 3.83 (m, 2H), 3.57 (s, 3H, OCH_3), 3.54 – 3.46 (m, 1H), 2.21 – 2.15 (m, 2H, C$H_{2\alpha}$), 1.54 – 1.31 (m, 6H), 1.31 – 1.09 (m, 22H), 0.83 (t, *J* = 6.9 Hz, 3H, CH_3). (ESI-TOF) MS: $C_{73}H_{84}O_{17}Na^+$ calculated for $(M+Na)^+$ was 1255.5601 and found 1255.5672 (Δ = 2.31ppm).

12-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(2,3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl))-α-D-mannopyranosyl-oxystearate (28)



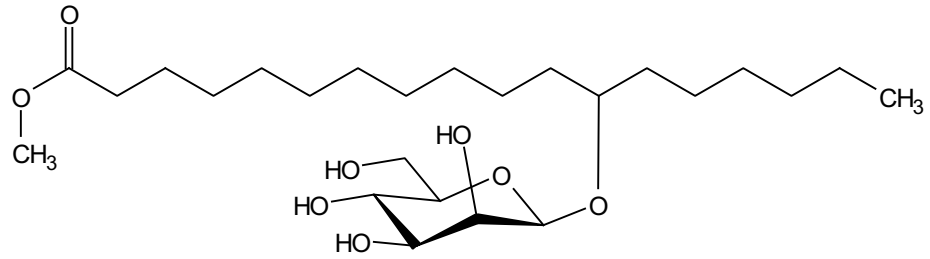
The donor **16** (36 mg, 71 µmol) was coupled with acceptor **26** by the general procedure 6b to afford the resulting adduct **28** as an off white syrup in 73% yield (29.9 mg, 27.5 µmol)) [Scheme S1, (i)]. ^{1}H NMR (600 MHz, $CDCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (t, *J* = 7.6 Hz, 6H), 7.40 (d, *J* = 7.6 Hz, 2H), 7.38 – 7.29 (m, 6H), 7.29 – 7.18 (m, 11H), 5.57 (s, 1H), 5.47 (s, 1H), 5.02 (d, *J* = 12.3 Hz, 1H), 4.93 (d, *J* = 12.2 Hz, 1H), 4.87 (s, 1H), 4.74 (s, 2H), 4.68 (d, *J* = 12.6 Hz, 1H), 4.59 (t, *J* = 6.2 Hz, 2H), 4.26 – 4.15 (m, 4H), 4.08 (t, *J* = 9.6 Hz, 1H), 3.98 – 3.92 (m, 2H), 3.86 (td, *J* = 10.0, 4.5 Hz, 2H), 3.71 (t, *J* = 10.2 Hz, 1H), 3.62 (s, 3H), 3.58 (dd, *J* = 9.9, 3.0 Hz, 1H), 3.56 – 3.49 (m, 1H), 3.29 (td, *J* = 9.6, 4.8 Hz, 1H), 2.24 (t, *J* = 7.5 Hz, 1H, C$H_{2\alpha}$), 1.63 – 1.52 (m, 3H), 1.51 – 1.35 (m, 3H), 1.24 (s, 22H), 0.85 (t, *J* = 6.5 Hz, 1H, CH_3). (ESI-TOF) MS: $C_{66}H_{84}O_{13}Na^+$ calculated for $(M+Na)^+$ was 1107.5804 and found 1107.5801 (Δ = 0.27 ppm).

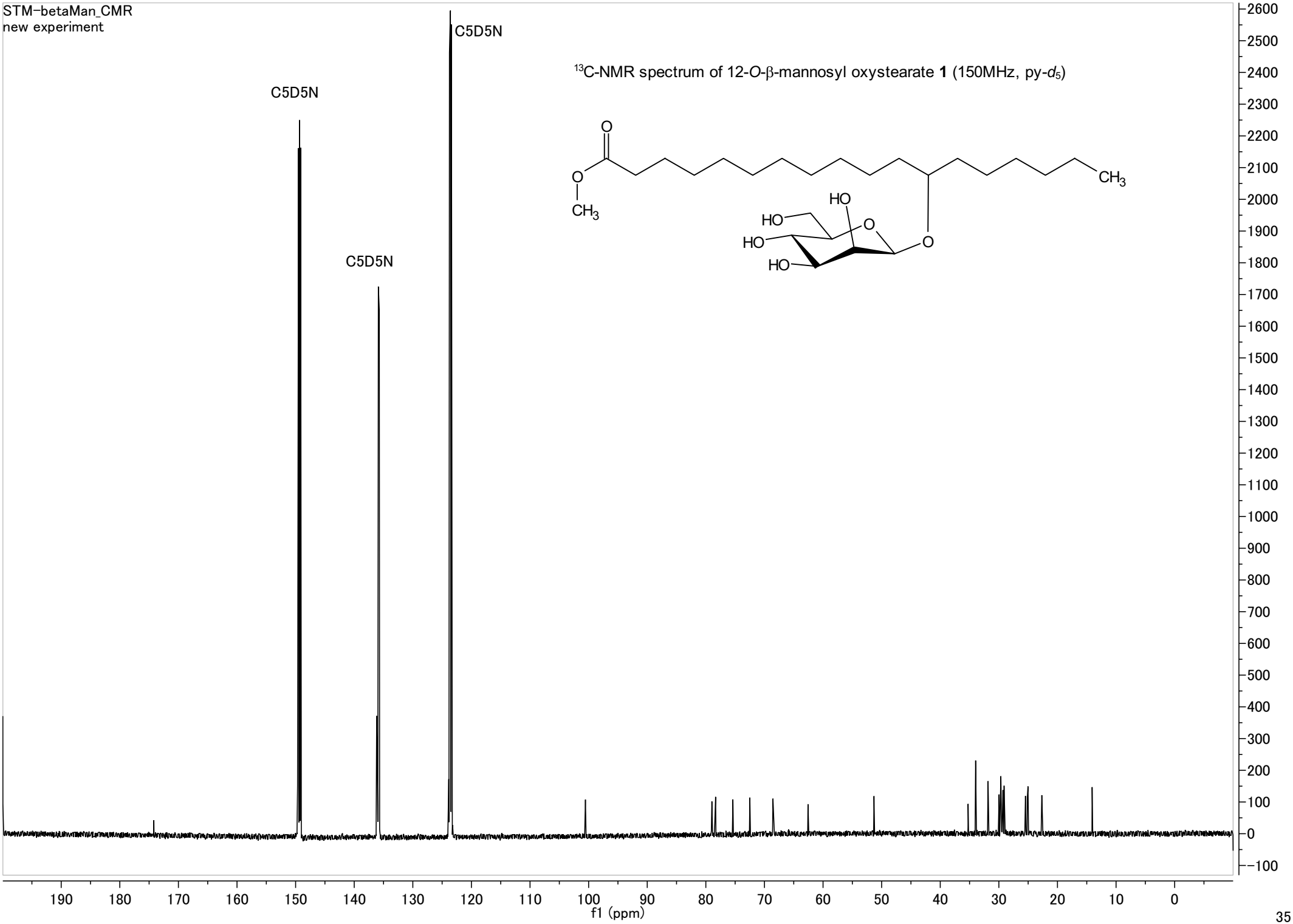
^{1}H and ^{13}C NMR spectra of compounds 1-13

All spectra were processed using MestReNova v11.0.4 (Mestrelab Research S.L.).

STM-betaMan_PMR2
STANDARD PROTON PARAMETERS

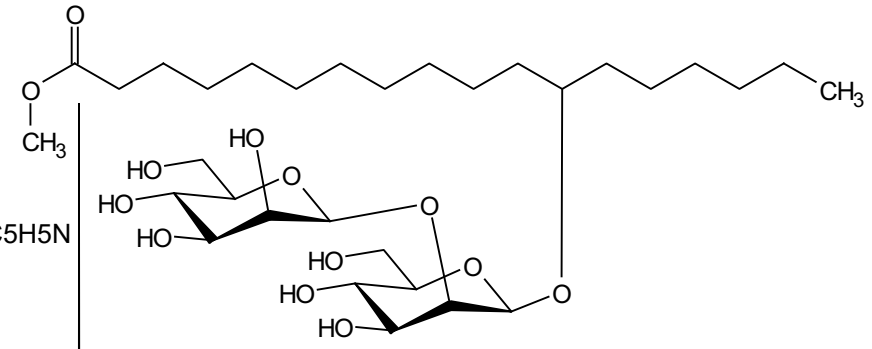
^{1}H-NMR spectrum of 12-*O*-β-mannosyl oxystearate **1** (600MHz, py-d_5)




STM-betaMan_CMR
new experiment
C5D5N
C5D5N
C5D5N
^{13}C-NMR spectrum of 12-*O*-β-mannosyl oxystearate **1** (150MHz, py-d_5)
O
O
CH_3
CH_3
HO
HO
HO
HO
O
O
2600
2500
2400
2300
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
−100
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

STM-bbManr_PMR
STANDARD PROTON PARAMETERS

^{1}H-NMR spectrum of 12-*O*-ββ-di-mannosyl oxystearate **2** (600MHz, py-d_5)

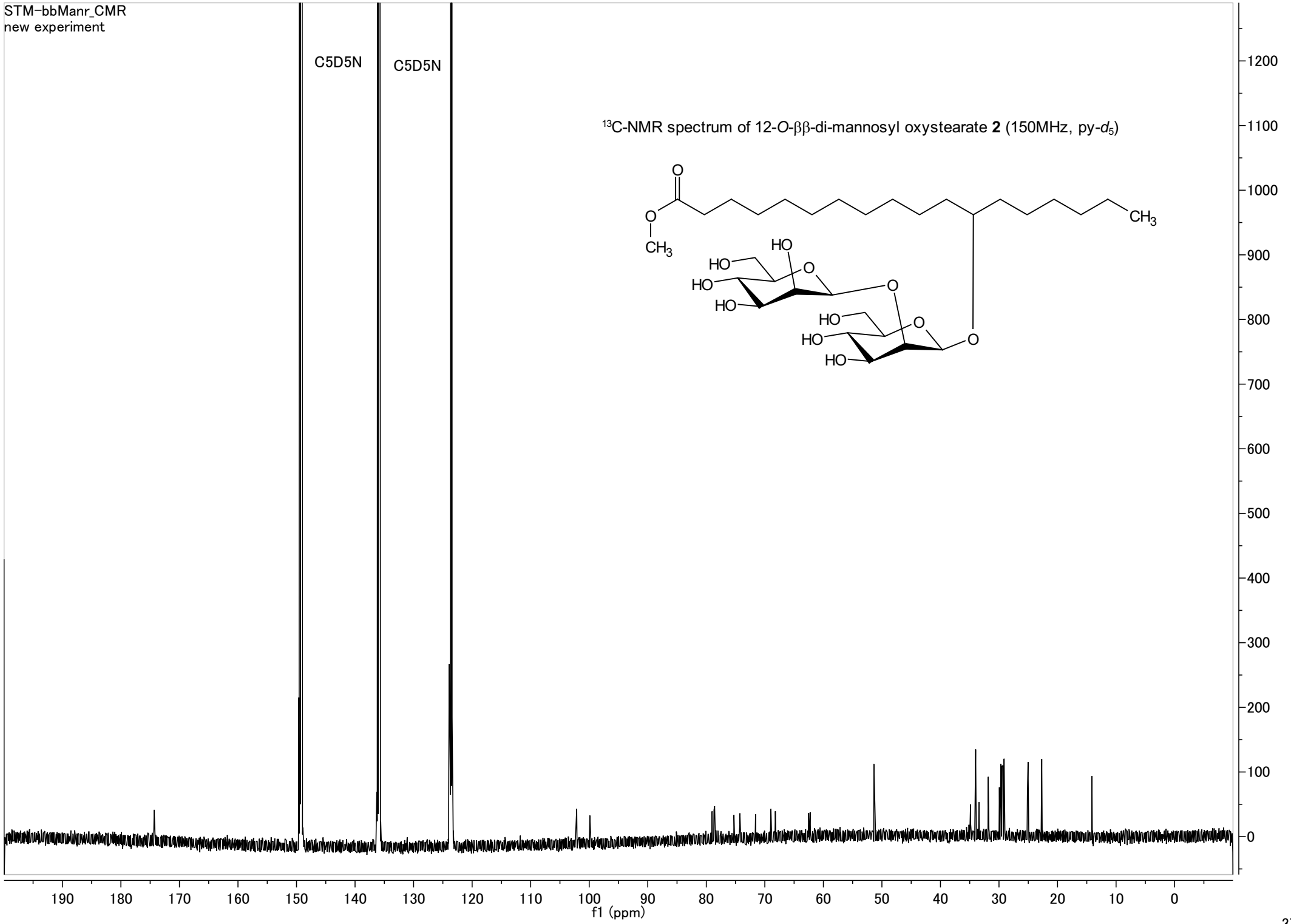


C5H5N

C5H5N

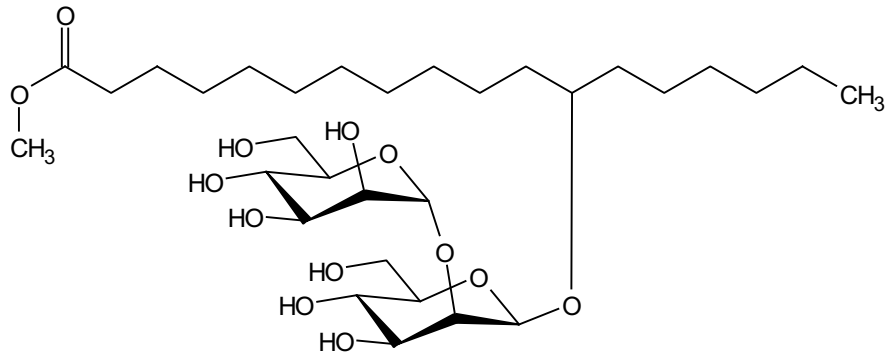
C5H5N

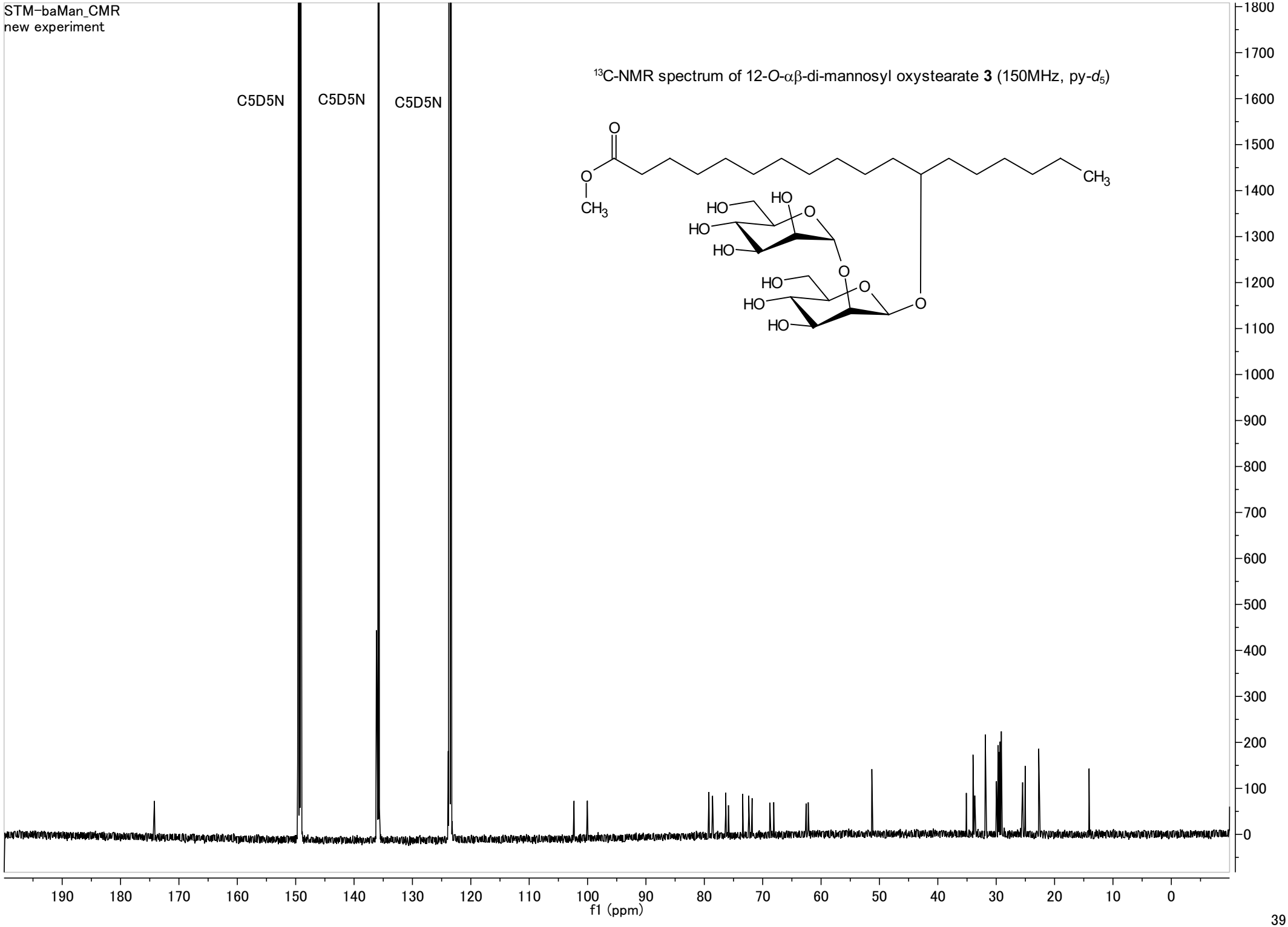
f1 (ppm)

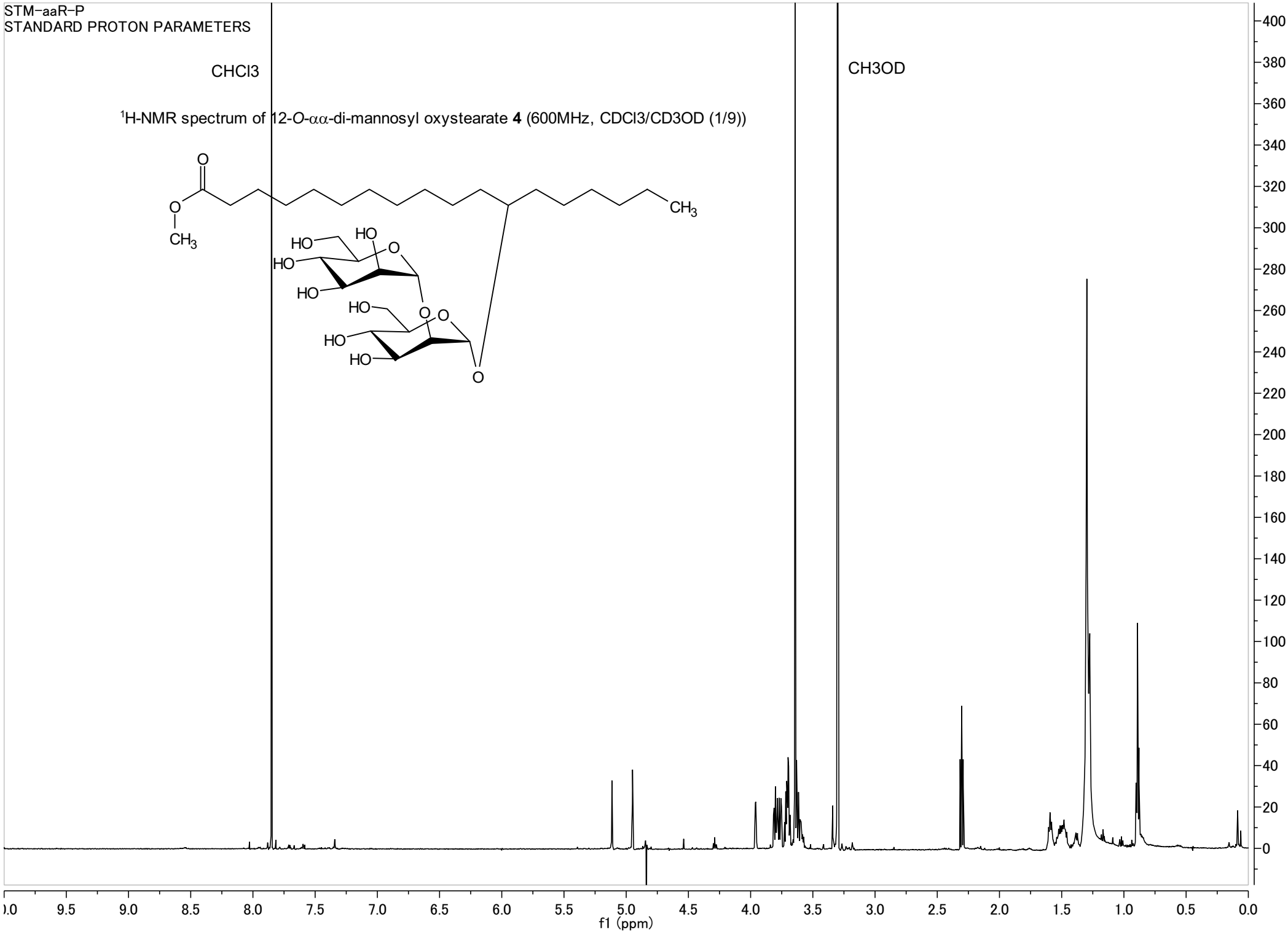

STM-bbManr_CMR
new experiment
C5D5N
C5D5N
^{13}C-NMR spectrum of 12-*O*-ββ-di-mannosyl oxystearate **2** (150MHz, py-d_5)
O
O
CH3
CH3
HO
HO
HO
HO
O
O
HO
HO
HO
O
O
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

STM-baMan_PMR
STANDARD PROTON PARAMETERS

^{1}H-NMR spectrum of 12-*O*-αβ-di-mannosyl oxystearate **3** (600MHz, py-d_5)

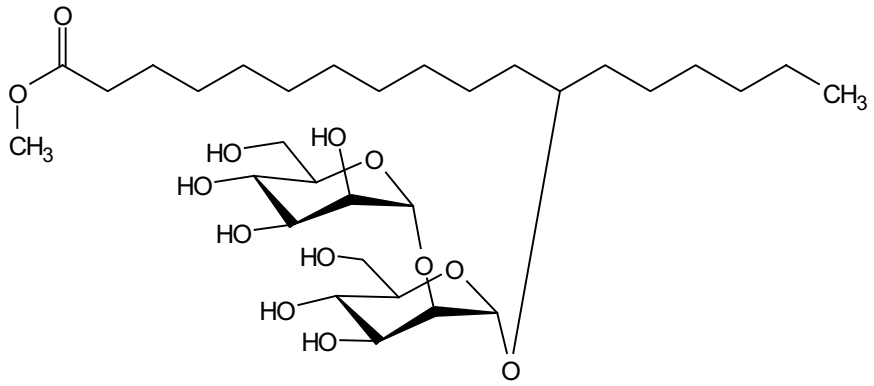



STM-baMan_CMR
new experiment
C5D5N
C5D5N
C5D5N
^{13}C-NMR spectrum of 12-*O*-αβ-di-mannosyl oxystearate **3** (150MHz, py-d_5)
O
O
CH3
CH3
HO
HO
HO
O
O
HO
HO
HO
O
O
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


STM-aaR-P
STANDARD PROTON PARAMETERS
CHCl3
CH3OD
^{1}H-NMR spectrum of 12-*O*-αα-di-mannosyl oxystearate **4** (600MHz, CDCl3/CD3OD (1/9))
O
O
CH3
CH3
HO
HO
HO
HO
O
HO
HO
HO
O
O
O
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
400
380
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0

STM-aaR-C
new experiment

^{13}C-NMR spectrum of 12-*O*-αα-di-mannosyl oxystearate **4** (150MHz, CDCl3/CD3OD (1/9))



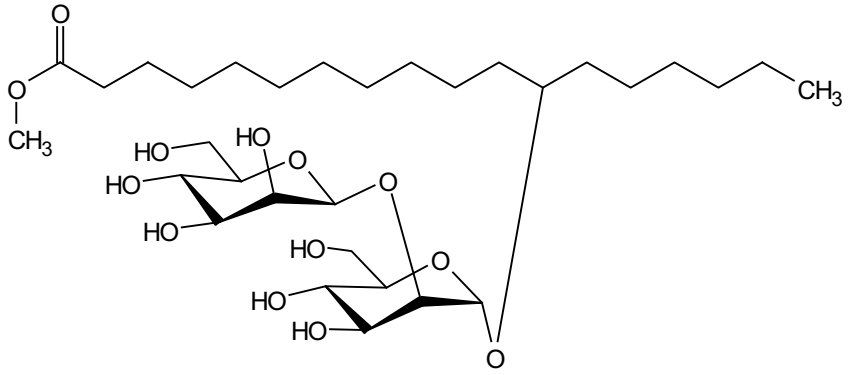
CD3OD

CDCl3

f1 (ppm)

STM-ab-P
STANDARD PROTON PARAMETERS

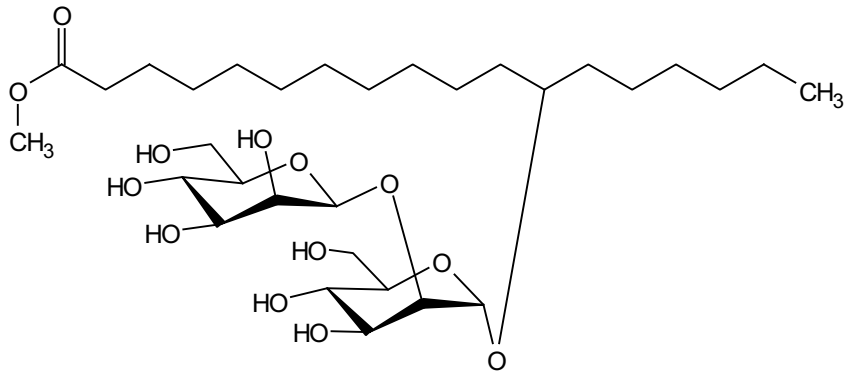
^{1}H-NMR spectrum of 12-*O*-βα-di-mannosyl oxystearate **5** (600MHz, CD3OD)



STM-ab-C
new experiment

CD3OD

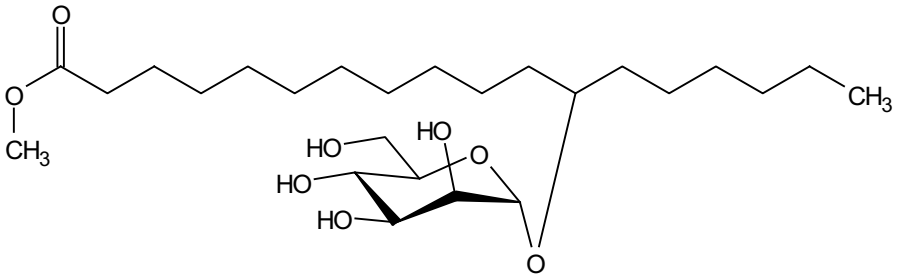
^{13}C-NMR spectrum of 12-*O*-βα-di-mannosyl oxystearate **5** (150MHz, CD3OD)



f1 (ppm)

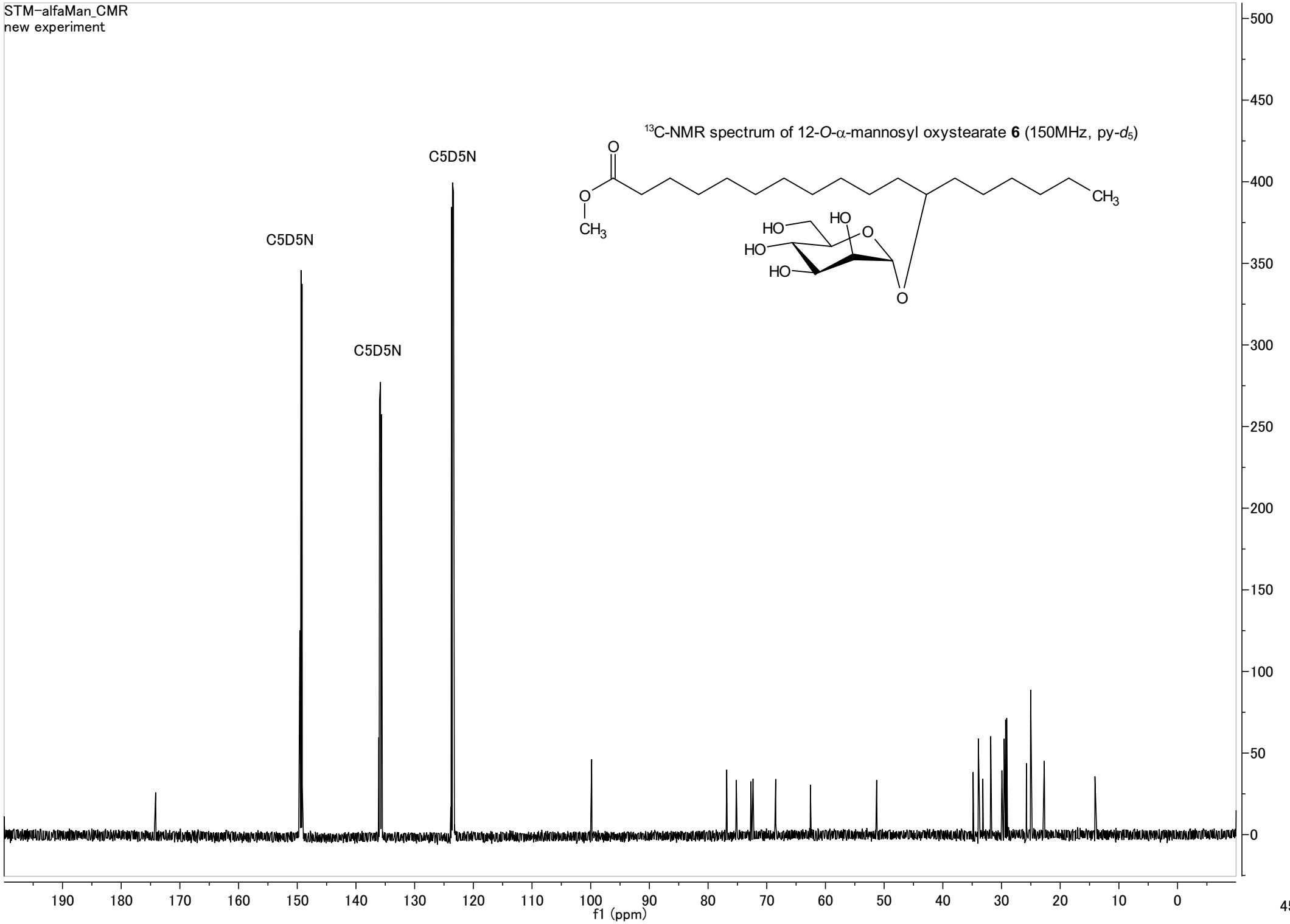
STM-alfaMan_PMR
STANDARD PROTON PARAMETERS

^{1}H-NMR spectrum of 12-*O*-α-mannosyl oxystearate **6** (600MHz, py-d_5)



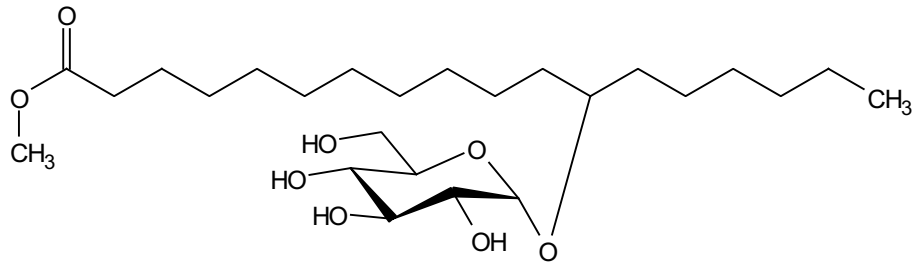
C5H5N C5H5N C5H5N

f1 (ppm)


STM-alfaMan_CMR
new experiment
^{13}C-NMR spectrum of 12-*O*-α-mannosyl oxystearate **6** (150MHz, py-d_5)
C5D5N
C5D5N
C5D5N
f1 (ppm)

ST-alfa-Glc-P
STANDARD PROTON PARAMETERS

^{1}H-NMR spectrum of 12-*O*-α-glucosyl oxystearate **7α** (600MHz,CD_3OD)



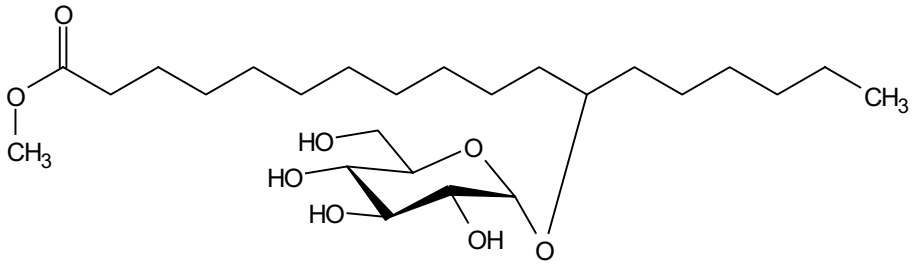
CH3OD

f1 (ppm)

ST-alfa-Glc-C
new experiment

CD3OD

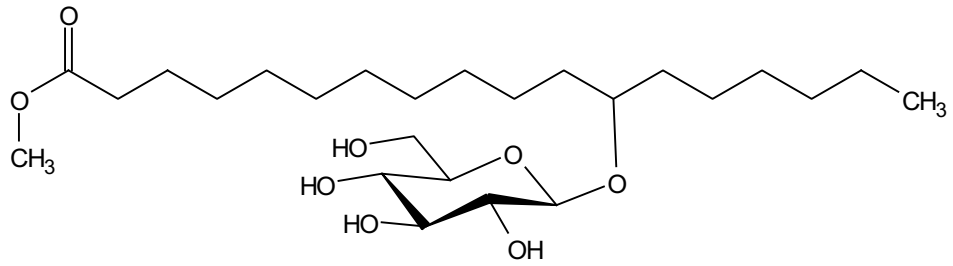
^{13}C-NMR spectrum of 12-*O*-α-glucoosyl oxystearate **7α** (150MHz,CD_3OD)



f1 (ppm)

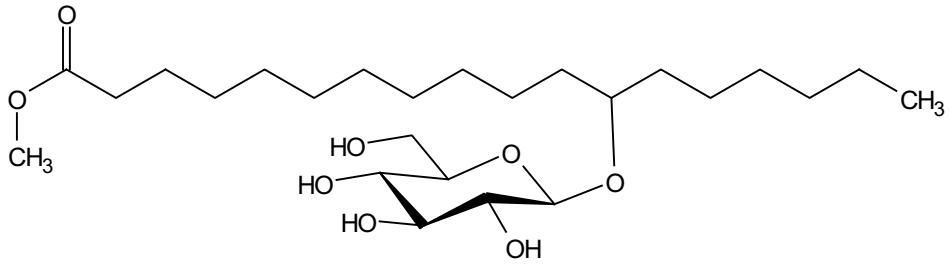
ST-beta-Glc-P
STANDARD PROTON PARAMETERS

^{1}H-NMR spectrum of 12-*O*-β-glucosyl oxystearate **7β** (600MHz,CD_3OD)



ST-beta-Glc-C
new experiment

^{13}C-NMR spectrum of 12-*O*-β-glucosyl oxystearate **7β** (150MHz,CD_3OD)

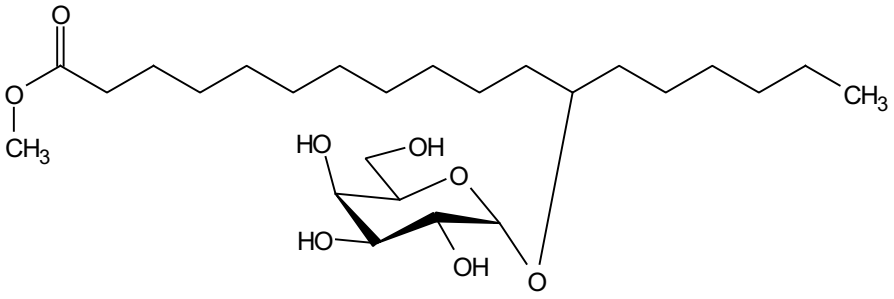


CD3OD

f1 (ppm)

ST-alfa-Gal-P
STANDARD PROTON PARAMETERS

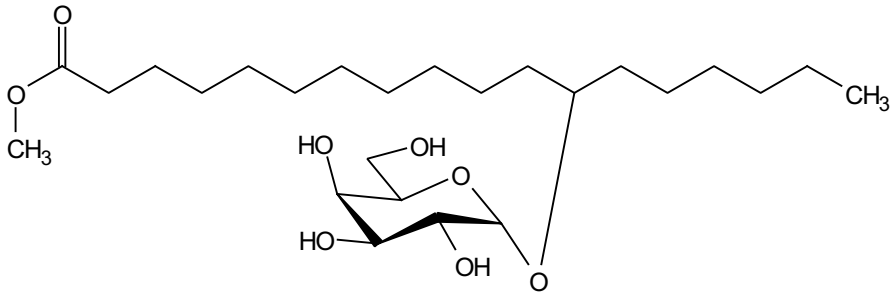
^{1}H-NMR spectrum of 12-*O*-α-galactosyl oxystearate **8α** (600MHz,CD_3OD)



ST-alfa-Gal-C
new experiment

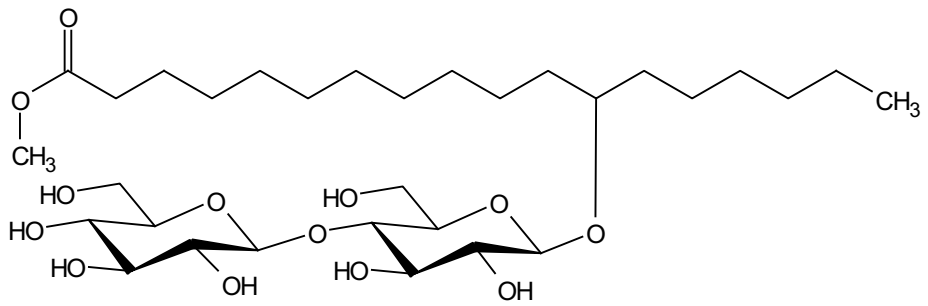
CD3OD

^{13}C-NMR spectrum of 12-*O*-α-galactosyl oxystearate **8β** (150MHz,CD_3OD)



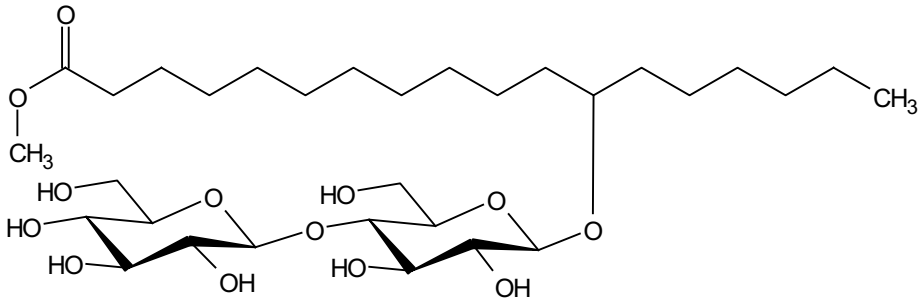
f1 (ppm)

^{1}H-NMR spectrum of 12-*O*-β-cellobiosyl oxystearate **9** (600MHz,CD_3OD)



09 -ST-Cello-C
new experiment

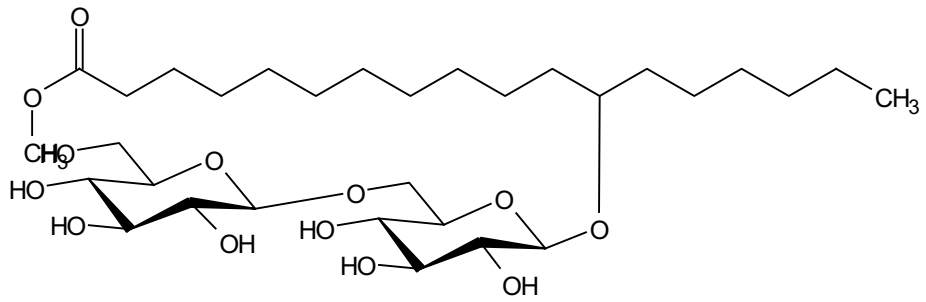
^{13}C-NMR spectrum of 12-*O*-β-cellobiosyl oxystearate **9** (150MHz,CD_3OD)



CD3OD

f1 (ppm)

^{1}H-NMR spectrum of 12-*O*-β-gentiobiosyl oxystearate **10** (600MHz,CD_3OD)



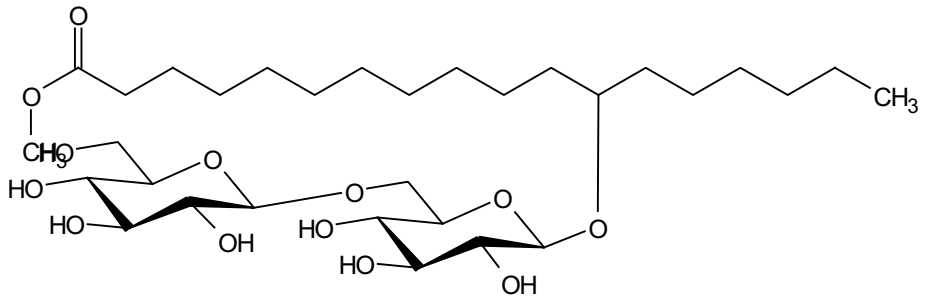
CH3OD

f1 (ppm)

10- STM-GEN_CMR
new experiment

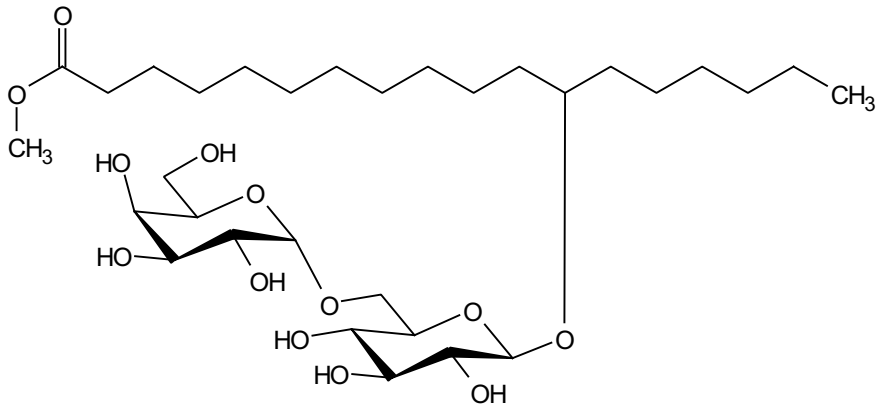
CD3OD

^{13}C-NMR spectrum of 12-*O*-β-gentiobiosyl oxystearate **10** (150MHz,CD_3OD)



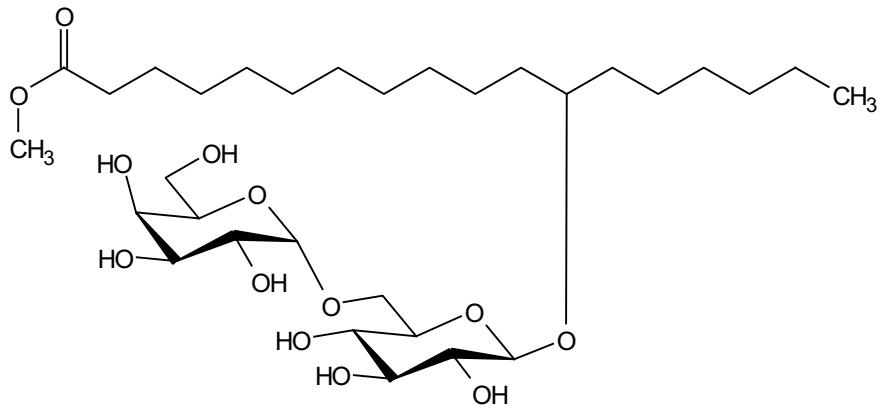
f1 (ppm)

^{1}H-NMR spectrum of 12-*O*-β-melibiosyl oxystearate **11** (600MHz,CD_3OD)



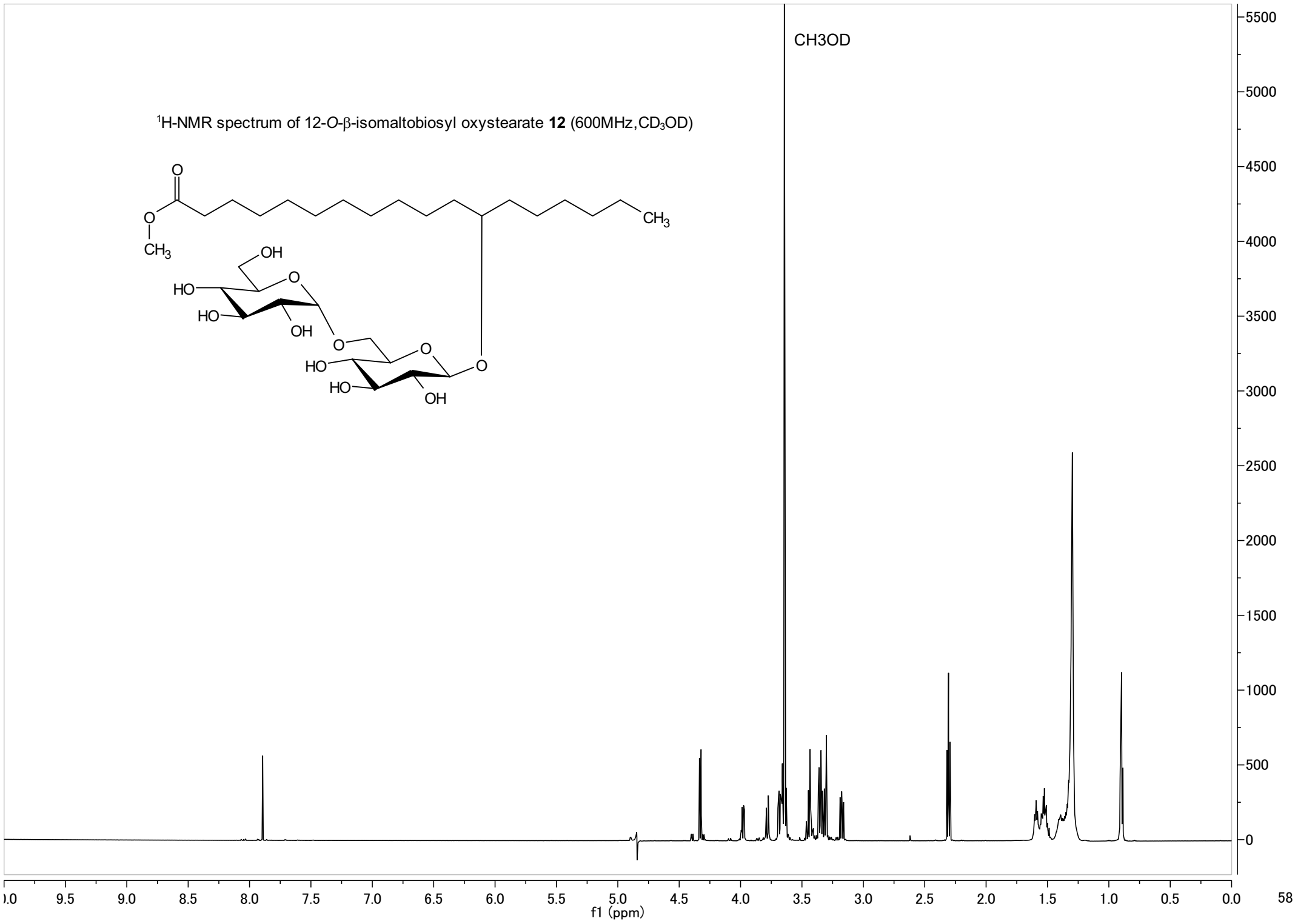
11- St-Melib-C
new experiment

^{13}C-NMR spectrum of 12-*O*-β-melibiosyl oxystearate **11** (150MHz,CD_3OD)



CD3OD

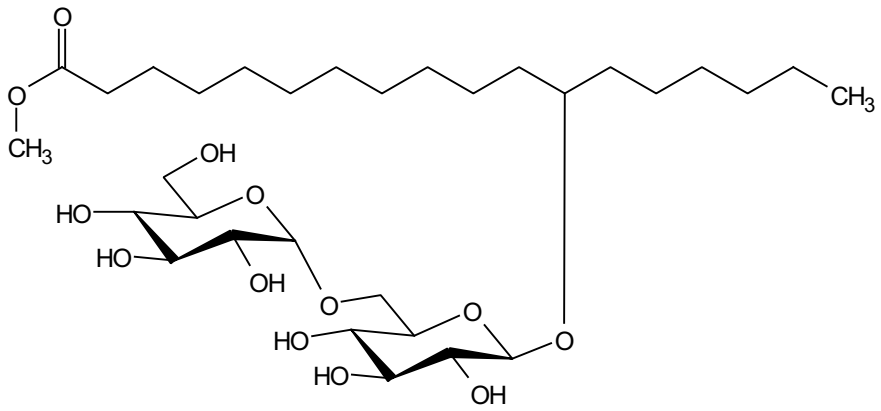
f1 (ppm)


CH3OD
^{1}H-NMR spectrum of 12-*O*-β-isomaltobiosyl oxystearate **12** (600MHz,CD_3OD)
O
O
CH_3
CH_3
OH
HO
O
HO
OH
O
O
HO
O
HO
OH
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

12-IsoMalt-C
new experiment

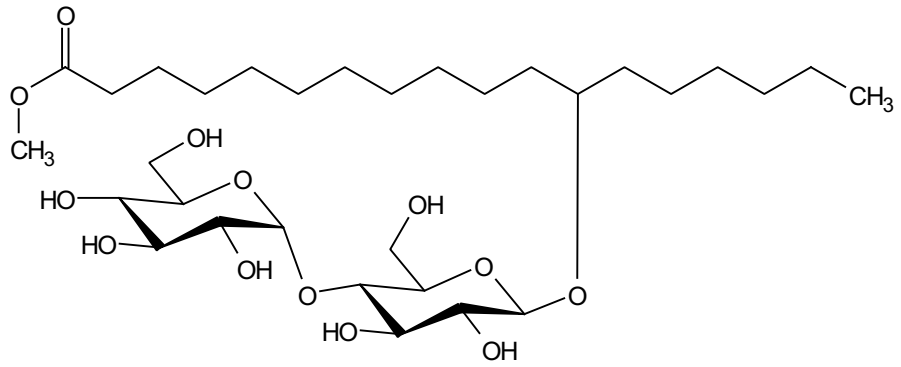
CD3OD

^{13}C-NMR spectrum of 12-*O*-β-isomaltobiosyl oxystearate **12** (150MHz,CD_3OD)



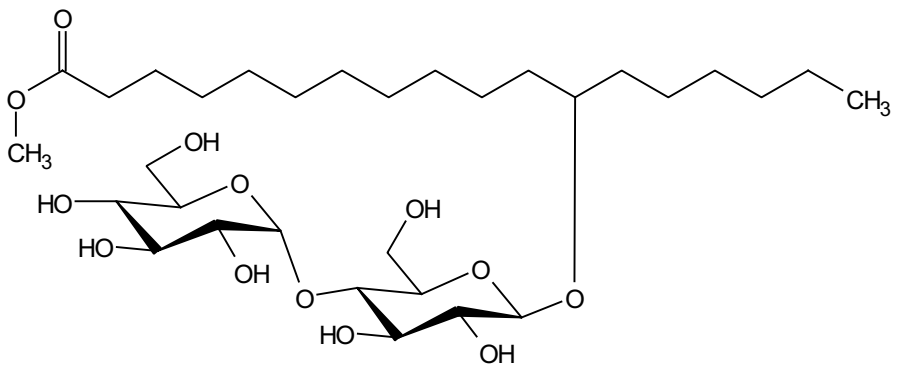
f1 (ppm)

^{1}H-NMR spectrum of 12-*O*-β-maltobiosyl oxystearate **13** (600MHz,CD_3OD)



13-ST-Malt-C
new experiment

^{13}C-NMR spectrum of 12-*O*-β-maltobiosyl oxystearate **13** (150MHz,CD_3OD)



CD3OD

f1 (ppm)

ESI-TOFMS spectra of compounds 1-13

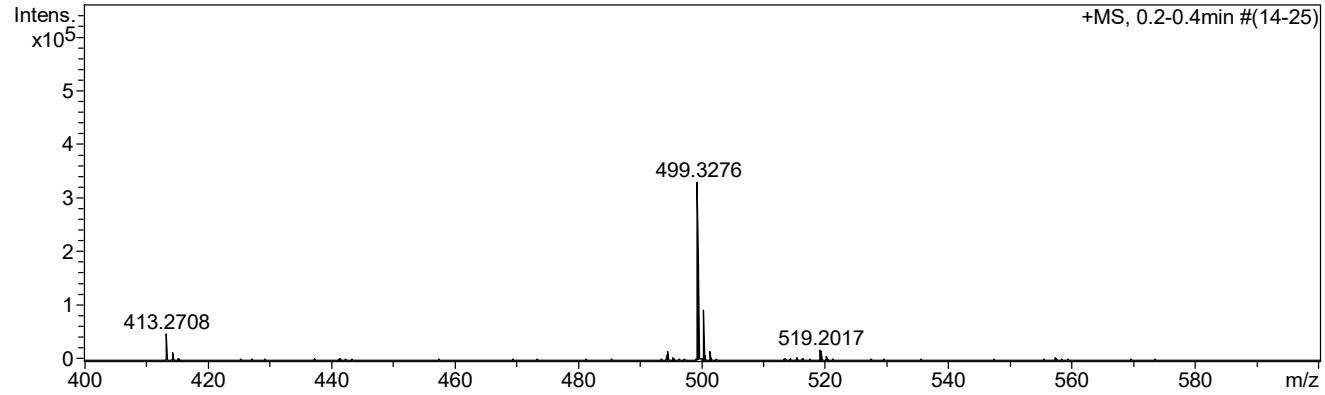
Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date	2017/03/15 15:57:19		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\STb ma-pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	STb ma	Instrument / Ser#	micrOTOF 10326
Comment			

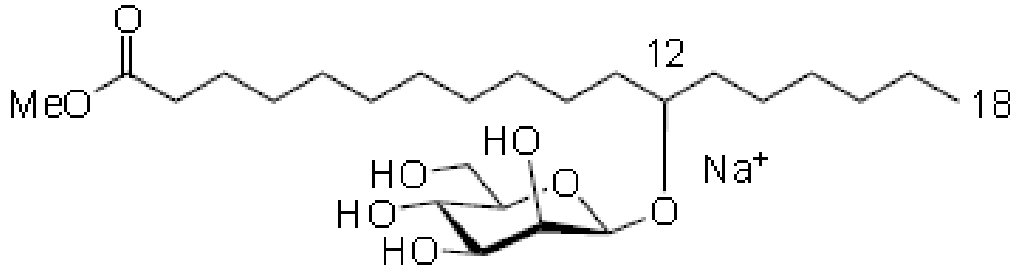
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	eP Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
499.3276	1	C 25 H 48 Na O 8	499.3241	-6.9	-7.1	1.5	ok	even	5.12	0.0078	0.0035	0.0039	0.0004	0.8427

12-O-β-mannosyl oxystearate **1**



Chemical Formula: $C_{25}H_{48}NaO_8^+$

Exact Mass: 499.3241

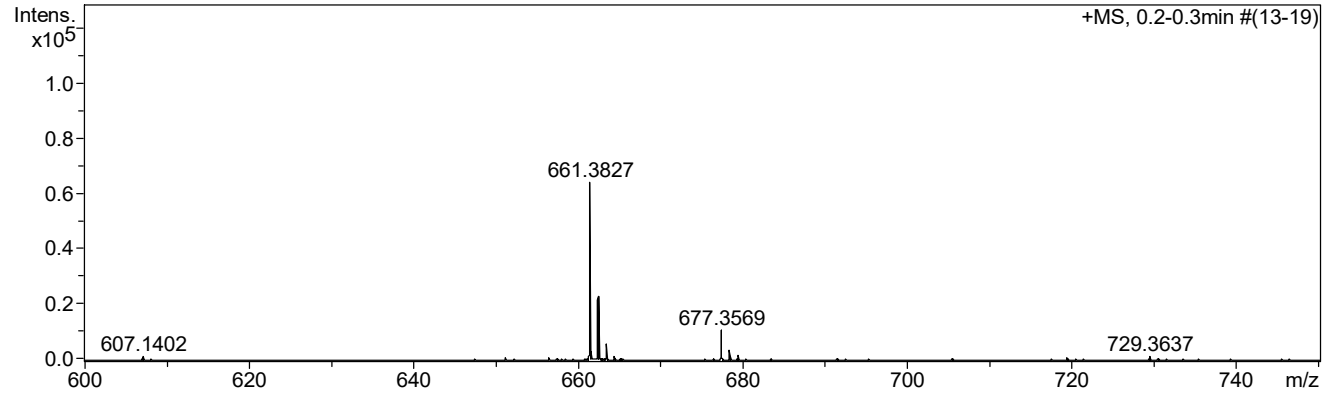
Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date	2017/03/15 16:27:16		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\ST bb 2ma -pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	ST bb 2ma pos	Instrument / Ser#	micrOTOF 10326
Comment			

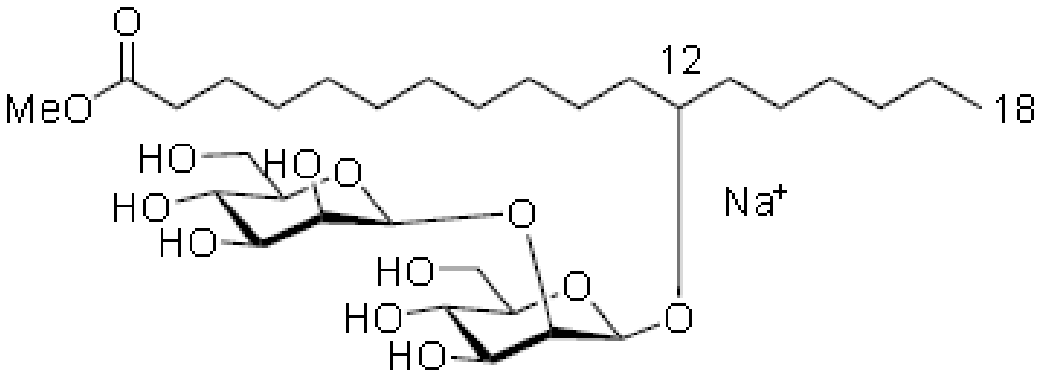
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	ePConf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
661.3827														
	1	C 31 H 54 N 6 Na O 8	661.3895	10.3	10.5	7.5	ok	even	5.25	0.0092	0.0069	0.0042	0.0003	0.8635
	2	C 31 H 58 Na O 13	661.3770	-8.7	-8.5	2.5	ok	even	7.84	0.0124	0.0057	0.0040	0.0004	0.8373
	3	C 31 H 45 N 14 O 3	661.3794	-5.1	-5.0	16.5	ok	even	16.34	0.0211	0.0033	0.0082	0.0002	0.8743

12-O-ββ-di-mannosyl oxystearate **2**



Chemical Formula: $C_{31}H_{58}NaO_{13}^{+}$

Exact Mass: 661.3770

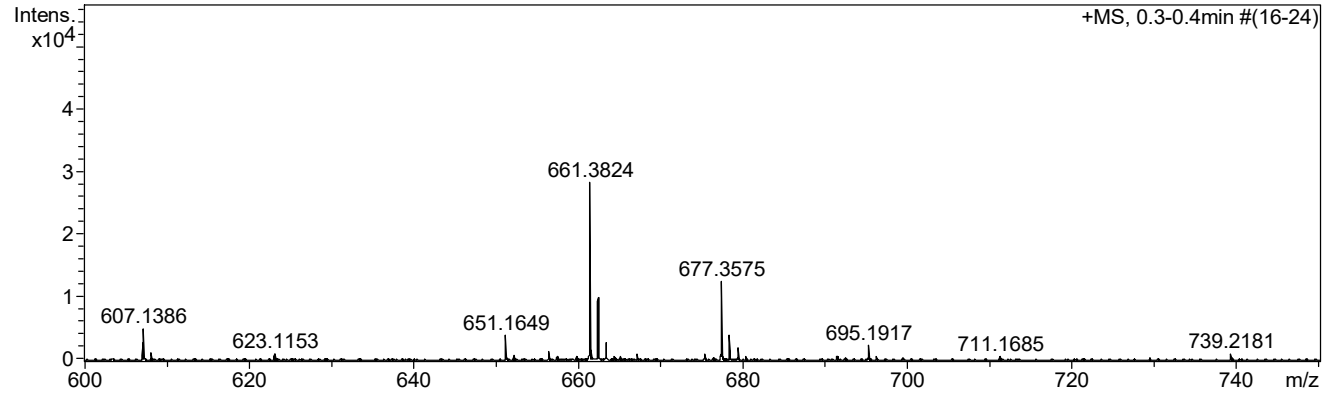
Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date	2017/03/15 16:23:49		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\ST ab 2ma -pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	ST ab 2ma pos	Instrument / Ser#	micrOTOF 10326
Comment			

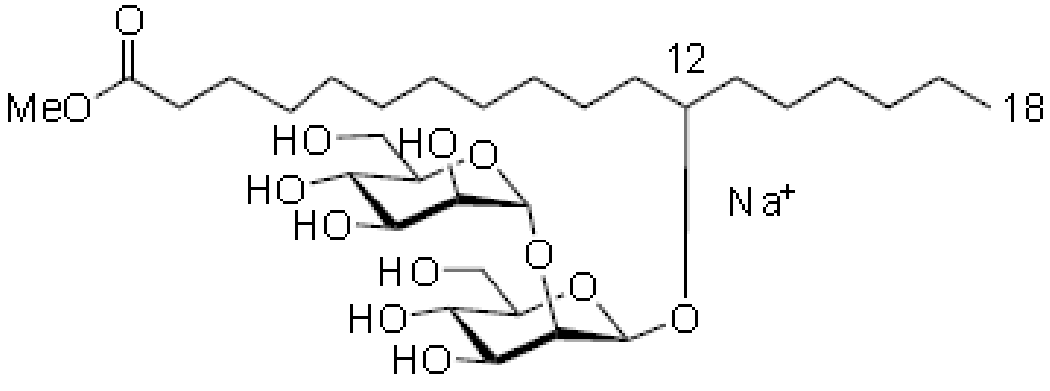
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	ePConf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
661.3824														
	1	C 31 H 58 Na O 13	661.3770	-8.3	-8.1	2.5	ok	even	6.73	0.0126	0.0054	0.0049	0.0004	0.8047
	2	C 31 H 54 N 6 Na O 8	661.3895	10.7	10.9	7.5	ok	even	9.71	0.0151	0.0072	0.0070	0.0003	0.8726
	3	C 31 H 45 N 14 O 3	661.3794	-4.6	-4.5	16.5	ok	even	21.25	0.0276	0.0030	0.0112	0.0003	0.8442

12-O-αβ-di-mannosyl oxystearate **3**



Chemical Formula: $C_{31}H_{58}NaO_{13}^{+}$

Exact Mass: 661.3770

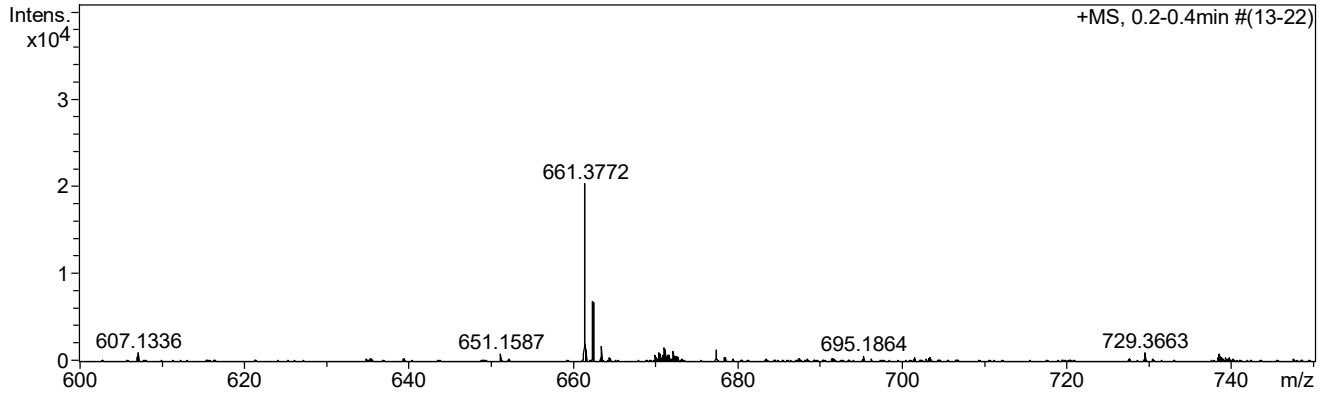
Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date	2017/08/21 16:13:36		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\Huy-20170821\24-pos.d		
Method	esi_pos_wide.m	Operator	BDAL
Sample Name	24	Instrument / Ser#	micrOTOF 10326
Comment			

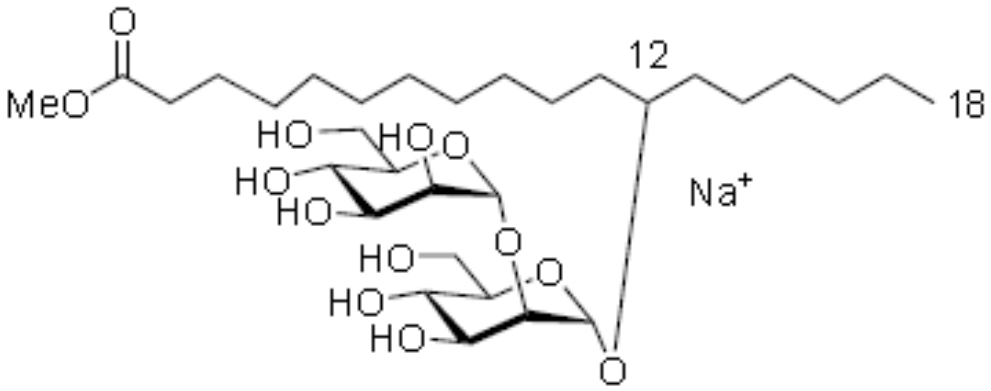
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	200 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	eP Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
661.3772														
	1	C 31 H 58 Na O 13	661.3770	-0.3	-0.2	2.5	ok	even	4.02	0.0063	0.0005	0.0034	0.0005	0.8427
	2	C 31 H 45 N 14 O 3	661.3794	3.3	3.4	16.5	ok	even	25.77	0.0336	0.0023	0.0124	0.0005	0.9962

12-O-αα-di-mannosyl oxystearate **4**



Chemical Formula: $C_{31}H_{58}NaO_{13}^{+}$

Exact Mass: 661.3770

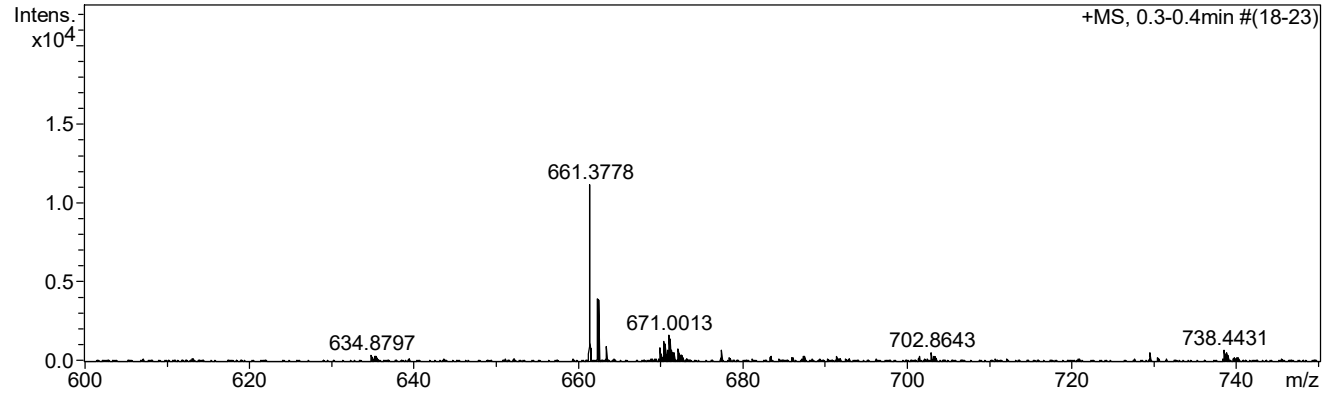
Mass Spectrum SmartFormula Report

Analysis Info

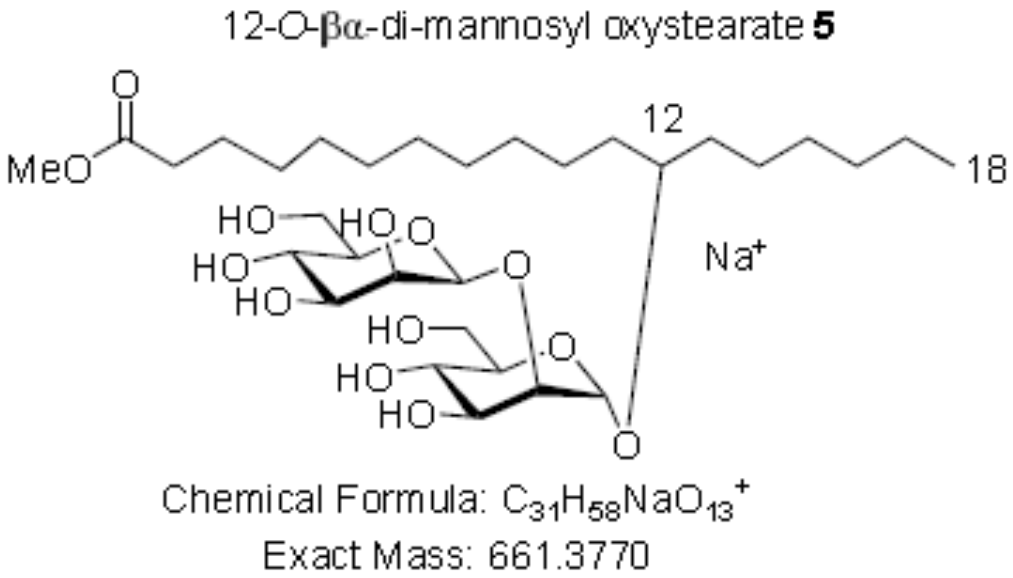
Acquisition Date	2017/08/21 16:51:19		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\Huy-20170821\29-pos.d		
Method	esi_pos_wide.m	Operator	BDAL
Sample Name	29	Instrument / Ser#	micrOTOF 10326
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Not active			Set Dry Heater	200 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	2000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	eP Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
661.3778														
	1	C 31 H 58 Na O 13	661.3770	-1.3	-1.5	2.5	ok	even	3.47	0.0061	0.0012	0.0023	0.0007	0.8427
	2	C 31 H 45 N 14 O 3	661.3794	2.3	2.1	16.5	ok	even	20.32	0.0264	0.0014	0.0097	0.0007	0.9745



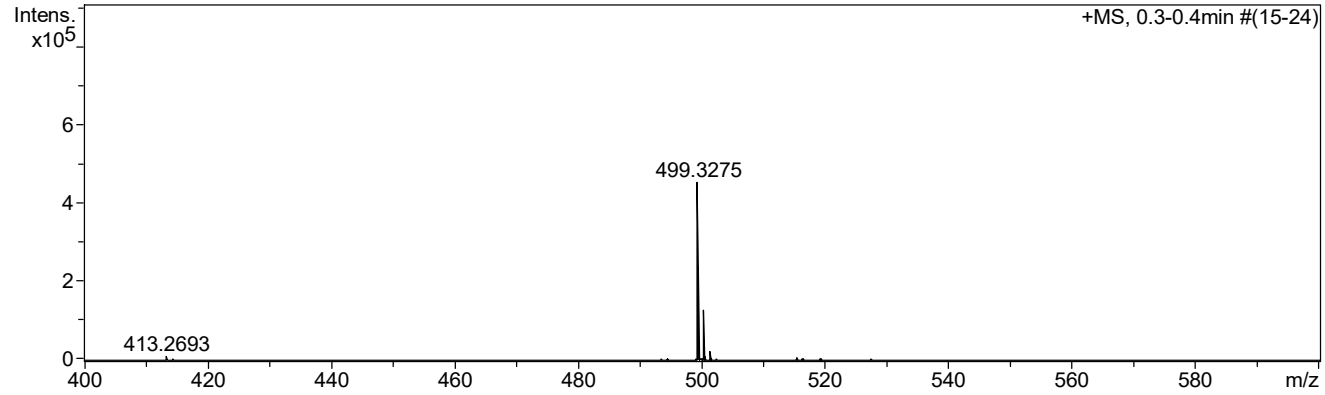
Mass Spectrum SmartFormula Report

Analysis Info

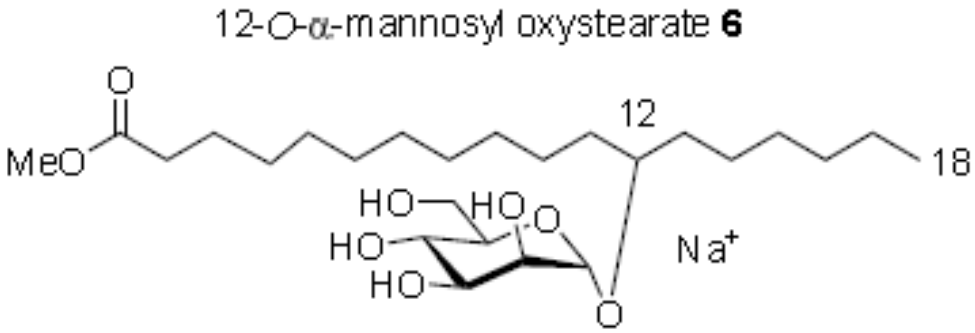
Acquisition Date	2017/03/15 15:49:42		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\STa ma-pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	STa ma	Instrument / Ser#	micrOTOF 10326
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	eP Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
499.3275	1	C 25 H 48 Na O 8	499.3241	-6.7	-6.6	1.5	ok	even	3.93	0.0059	0.0033	0.0033	0.0013	0.8427



Chemical Formula: $C_{25}H_{48}NaO_8^+$

Exact Mass: 499.3241

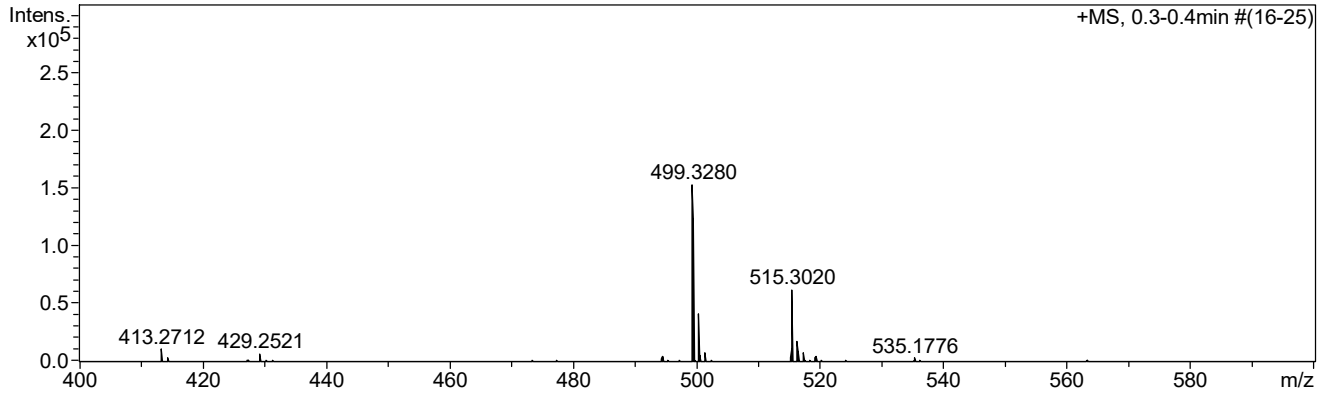
Mass Spectrum SmartFormula Report

Analysis Info

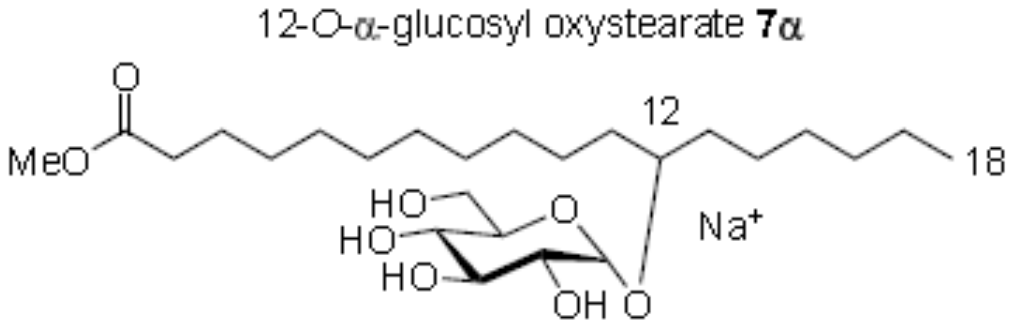
Acquisition Date	2017/03/15 16:18:31		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\ST a gu-pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	ST a gu pos	Instrument / Ser#	micrOTOF 10326
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	eP Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
499.3280														
	1	C 25 H 48 Na O 8	499.3241	-7.7	-8.1	1.5	ok	even	5.40	0.0088	0.0041	0.0037	0.0008	0.8723
	2	C 24 H 39 N 10 O 2	499.3252	-5.6	-6.0	10.5	ok	even	19.56	0.0275	0.0031	0.0122	0.0011	0.9536



Chemical Formula: $C_{25}H_{48}NaO_8^+$

Exact Mass: 499.3241

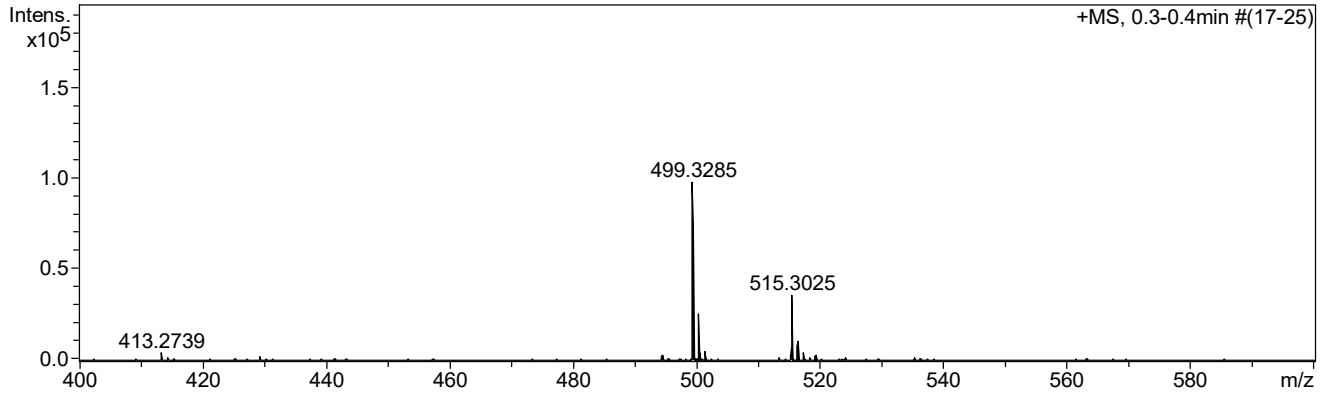
Mass Spectrum SmartFormula Report

Analysis Info

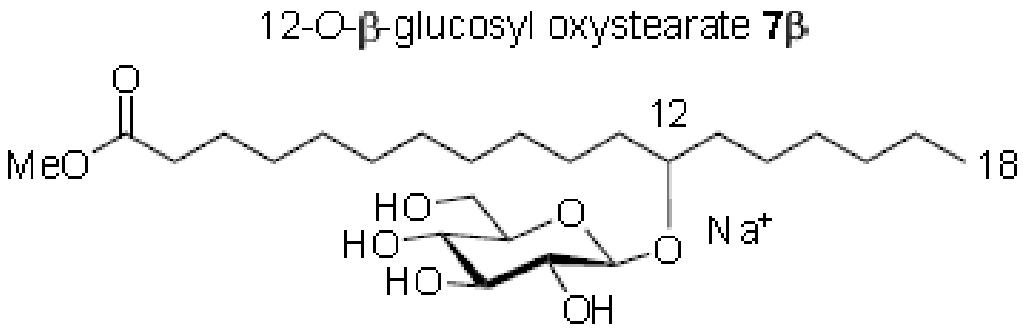
Acquisition Date	2017/03/15 16:14:50		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\STb gu-pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	STb gu pos	Instrument / Ser#	micrOTOF 10326
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	eP Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
499.3285														
	1	C 18 H 39 N 14 O 3	499.3324	7.7	7.0	6.5	ok	even	8.78	0.0158	0.0036	0.0080	0.0019	0.7842
	2	C 17 H 43 N 10 O 7	499.3311	5.1	4.3	1.5	ok	even	21.18	0.0344	0.0023	0.0148	0.0019	0.8013
	3	C 24 H 39 N 10 O 2	499.3252	-6.7	-7.4	10.5	ok	even	22.77	0.0327	0.0038	0.0143	0.0019	0.8487



Chemical Formula: $C_{25}H_{48}NaO_8^+$

Exact Mass: 499.3241

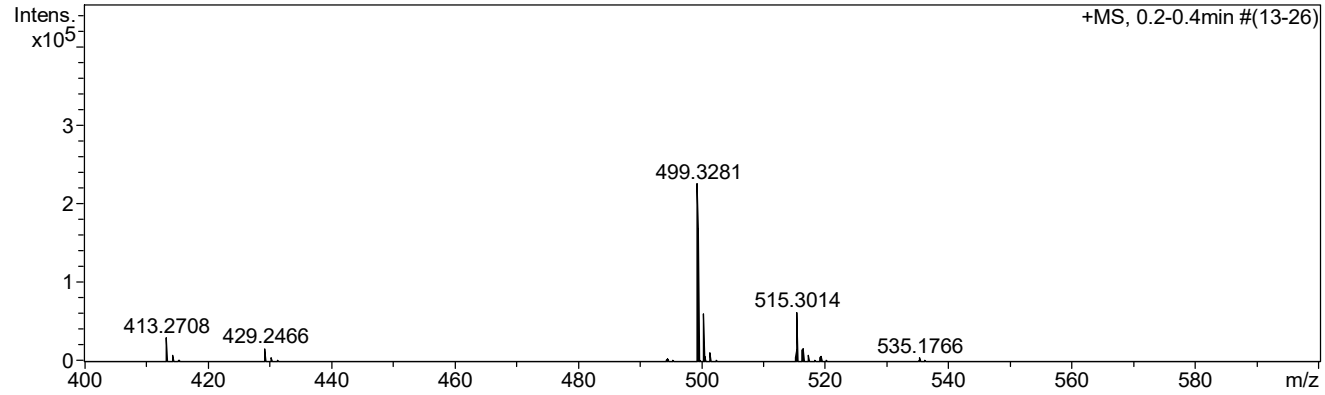
Mass Spectrum SmartFormula Report

Analysis Info

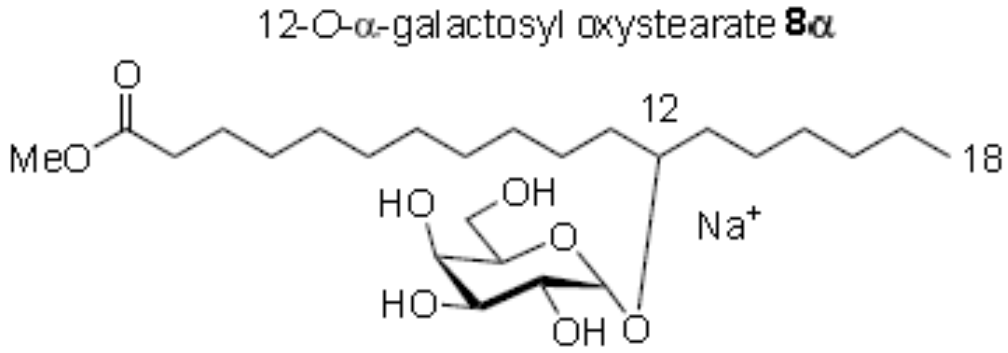
Acquisition Date	2017/03/15 16:10:32		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\STa ga-pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	STa ga pos	Instrument / Ser#	micrOTOF 10326
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	eP Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
499.3281	1	C 25 H 48 Na O 8	499.3241	-7.9	-8.4	1.5	ok	even	4.90	0.0075	0.0042	0.0039	0.0009	0.8427



Chemical Formula: $C_{25}H_{48}NaO_8^+$

Exact Mass: 499.3241

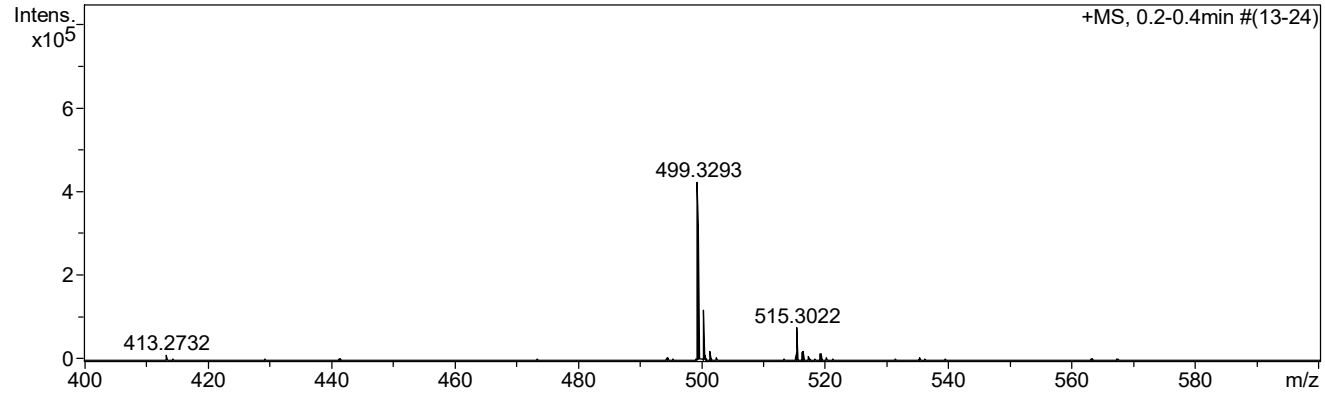
Mass Spectrum SmartFormula Report

Analysis Info

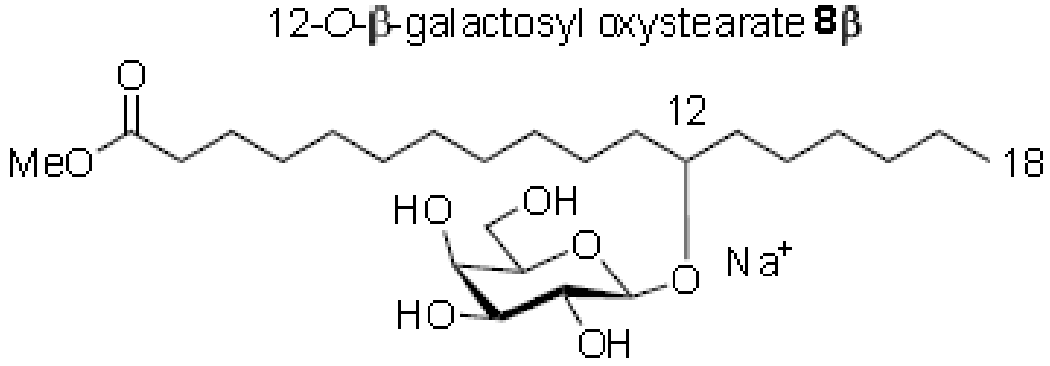
Acquisition Date	2017/03/15 16:06:27		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\STb ga-pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	STb ga pos	Instrument / Ser#	micrOTOF 10326
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	ePConf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
499.3293														
	1	C 25 H 48 Na O 8	499.3241	-10.3	-10.3	1.5	ok	even	4.39	0.0073	0.0052	0.0036	0.0009	0.8427
	2	C 25 H 44 N 6 Na O 3	499.3367	14.9	14.9	6.5	ok	even	9.03	0.0125	0.0074	0.0056	0.0003	0.9170



Chemical Formula: $C_{25}H_{48}NaO_8^+$

Exact Mass: 499.3241

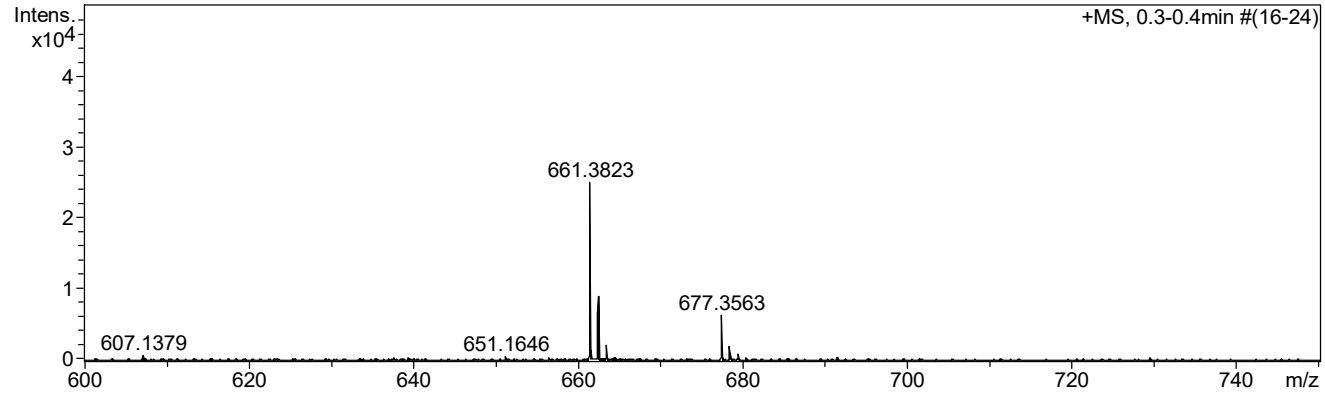
Mass Spectrum SmartFormula Report

Analysis Info

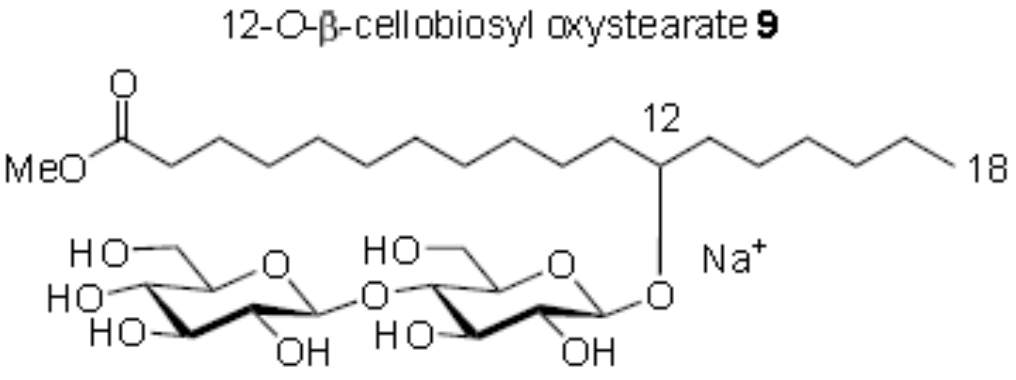
Acquisition Date	2017/03/15 16:42:54		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\ST b Cel pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	ST b Cel pos	Instrument / Ser#	micrOTOF 10326
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	ePConf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
661.3823														
	1	C 31 H 54 N 6 Na O 8	661.3895	10.9	11.0	7.5	ok	even	4.15	0.0060	0.0073	0.0028	0.0002	0.8752
	2	C 31 H 58 Na O 13	661.3770	-8.1	-8.0	2.5	ok	even	5.53	0.0081	0.0053	0.0026	0.0002	0.8340
	3	C 31 H 45 N 14 O 3	661.3794	-4.5	-4.4	16.5	ok	even	17.54	0.0229	0.0029	0.0081	0.0002	0.9169



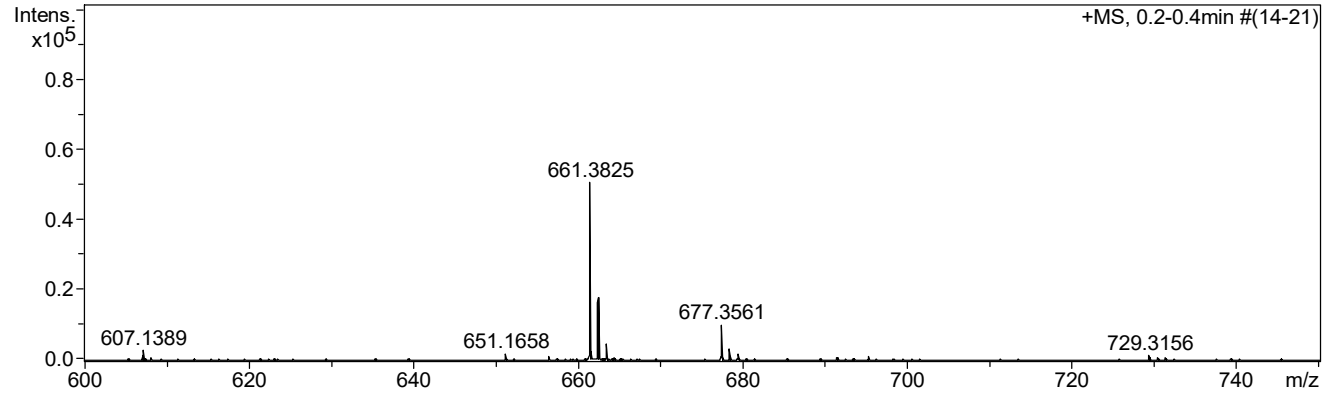
Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date	2017/03/15 16:30:23		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\ST gen -pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	ST gen pos	Instrument / Ser#	micrOTOF 10326
Comment			

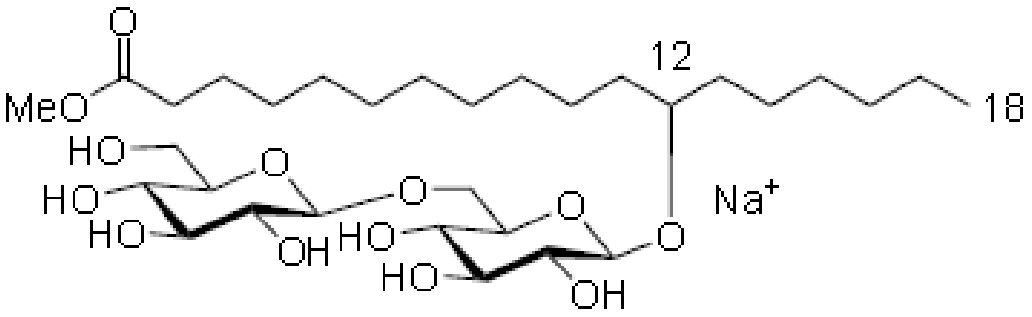
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	ePConf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
661.3825														
	1	C 31 H 58 Na O 13	661.3770	-8.4	-8.4	2.5	ok	even	3.86	0.0062	0.0056	0.0020	0.0004	0.7752
	2	C 31 H 54 N 6 Na O 8	661.3895	10.6	10.6	7.5	ok	even	6.42	0.0089	0.0070	0.0039	0.0005	0.8668
	3	C 31 H 45 N 14 O 3	661.3794	-4.8	-4.8	16.5	ok	even	19.75	0.0258	0.0032	0.0092	0.0005	0.8956

12-O-β-gentiobiosyl oxystearate **10**



Chemical Formula: $C_{31}H_{58}NaO_{13}^{+}$

Exact Mass: 661.3770

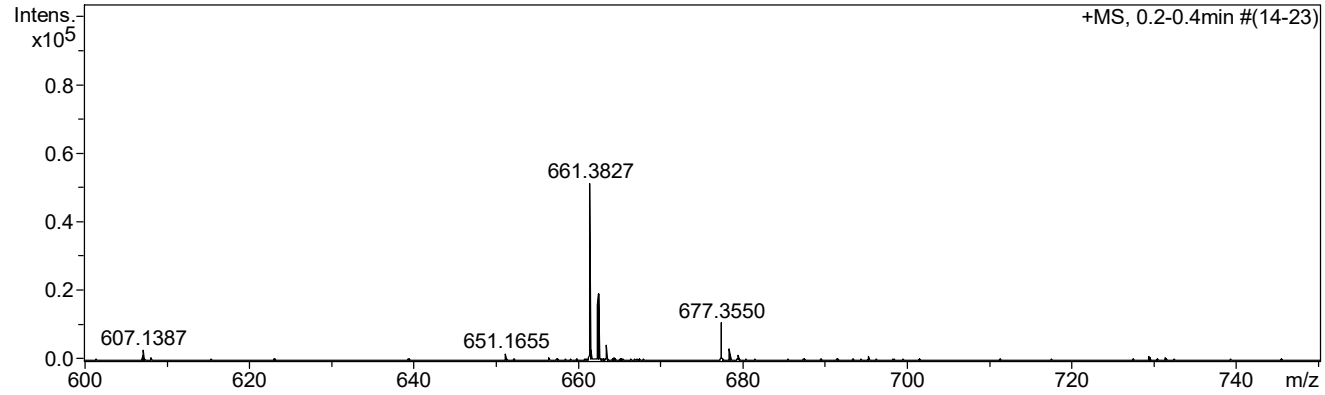
Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date	2017/03/15 16:36:26		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\ST b mel pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	ST b mel pos	Instrument / Ser#	micrOTOF 10326
Comment			

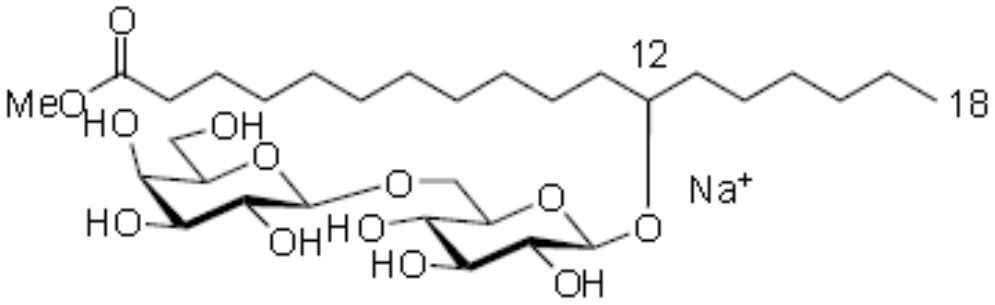
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	ePConf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
661.3827														
	1	C 31 H 54 N 6 Na O 8	661.3895	10.3	10.8	7.5	ok	even	4.72	0.0082	0.0072	0.0029	0.0013	0.8135
	2	C 31 H 45 N 14 O 3	661.3794	-5.1	-4.6	16.5	ok	even	11.04	0.0142	0.0031	0.0055	0.0013	0.7852
	3	C 31 H 58 Na O 13	661.3770	-8.7	-8.2	2.5	ok	even	12.19	0.0172	0.0055	0.0055	0.0013	0.8438

12-O-β-melibiosyl oxystearate **11**



Chemical Formula: $C_{31}H_{58}NaO_{13}^{+}$

Exact Mass: 661.3770

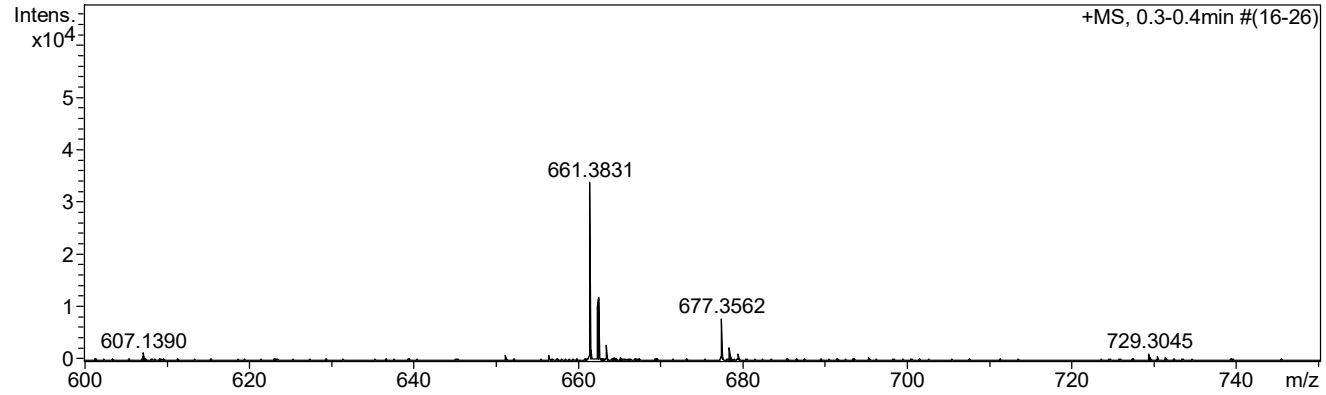
Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date	2017/03/15 16:33:43		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\ST b IsoMaltpos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	ST b IsoMalt pos	Instrument / Ser#	micrOTOF 10326
Comment			

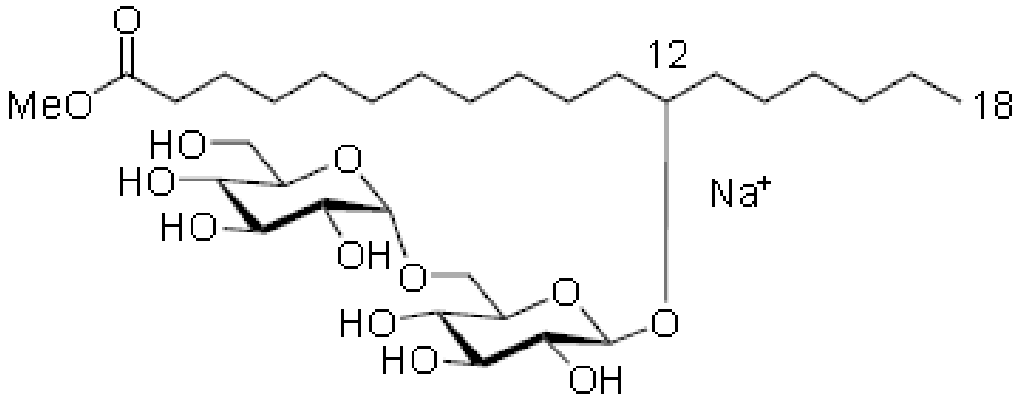
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	eP Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
661.3831														
	1	C 31 H 58 Na O 13	661.3770	-9.2	-9.0	2.5	ok	even	2.01	0.0028	0.0060	0.0013	0.0006	0.7488
	2	C 31 H 54 N 6 Na O 8	661.3895	9.8	10.0	7.5	ok	even	6.92	0.0093	0.0066	0.0032	0.0005	0.8530
	3	C 31 H 45 N 14 O 3	661.3794	-5.6	-5.5	16.5	ok	even	21.17	0.0284	0.0036	0.0094	0.0005	0.9208

12-O-β-isomaltobiosyl oxystearate **12**



Chemical Formula: $C_{31}H_{58}NaO_{13}^{+}$

Exact Mass: 661.3770

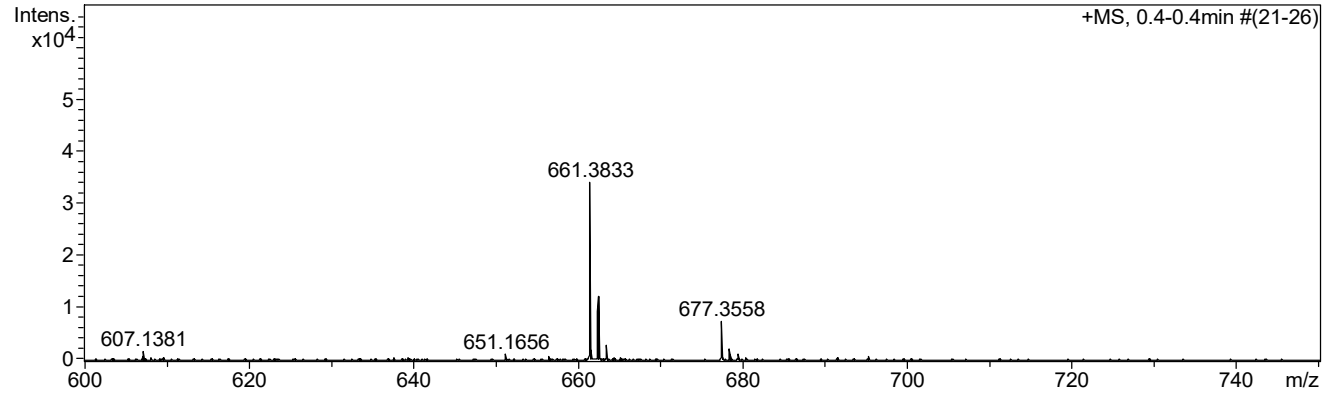
Mass Spectrum SmartFormula Report

Analysis Info

Acquisition Date	2017/03/15 16:39:46		
Analysis Name	C:\Users\Tom MIYAMOTO\Desktop\OL_2018\ESIMS\20170315\ST b Malt pos.d		
Method	esi_pos_low.m	Operator	BDAL
Sample Name	ST b Malt pos	Instrument / Ser#	micrOTOF 10326
Comment			

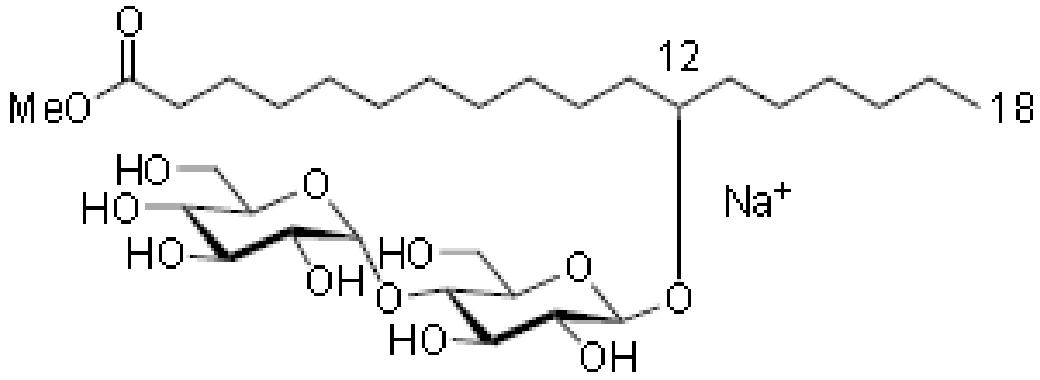
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	100 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	950 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	ePConf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
661.3833														
	1	C 31 H 54 N 6 Na O 8	661.3895	9.4	9.9	7.5	ok	even	2.66	0.0043	0.0066	0.0020	0.0009	0.8005
	2	C 31 H 58 Na O 13	661.3770	-9.6	-9.1	2.5	ok	even	7.34	0.0103	0.0060	0.0034	0.0009	0.8427
	3	C 31 H 45 N 14 O 3	661.3794	-6.0	-5.5	16.5	ok	even	15.63	0.0204	0.0037	0.0072	0.0008	0.8877

12-O-β-maltobiosyl oxystearate **13**



Chemical Formula: $C_{31}H_{58}NaO_{13}^{+}$

Exact Mass: 661.3770

Bioassay of synthesized compounds

The chemical study achieved thirteen compounds for further analyses on the structure-activity relationship with Mincle which was evaluated through the ability to induce the nuclear factor of activated T cells (NFAT)-green fluorescent protein (GFP) activation through Mincle. Briefly,

the 2B4-NFAT-GFP reporter cells expressing mouse or human Mincle were prepared as previously described.[8] For the *in vitro* stimulation, the synthesized glycolipids and TDB were dissolved in chloroform: methanol (2:1; v/v), diluted with isopropanol, added on 96-well plates at 20 μl/well, and followed by evaporation of the solvent as previously described.[9] 2B4-NFAT-GFP reporter cells were incubated for 16 hr at 37°C, and the GFP expression of reporter cells was evaluated by FACSCalibur flow cytometer (BD Biosciences).

Reference

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