Supporting Information for:

Regioisomeric SCFA Attachment to Hexosamines Separates Metabolic Flux from Cytotoxicity and MUC1 Suppression

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SYNTHESIS AND CHARACTERIZATION OF ANALOGS

Materials and Methods

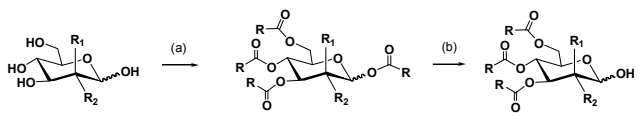
The starting materials *N*-acetyl-D-glucosamine (GlcNAc) was purchased from Sigma-Aldrich and *N*-acetyl-D-mannosamine (ManNAc) was purchased from Pfanstiehl, and the corresponding fully acylated derivatives, namely, 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-butanoyl-α,β-D-mannopyranose (**1**), 1,2,3,4,6-penta-*O*-butanoyl-α,β-D-mannopyranose (**2**), 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl-α,β-D-mannopyranose (**5**), 2-acetamido-2-deoxy-1,3,4,6-tetra-*O*-butanoyl-α,β-D-glucopyranose (**8**), and (1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(4-oxopentanoyl)amino-α,β-D-mannopyranose (**11**) were synthesized according to reported methods (*1-4*). The synthesis and characterization of **3**, **4**, **6**, **7**, **9**, **10**, **12**, and **13** is reported below**.**

Commercial reagents were used without further purification. Thin layer chromatography (TLC) was performed on silica gel coated glass plates (Cat. No. 21521). Column chromatography was performed using silica gel 60 Å. ^{1}H and ^{13}C NMR spectra were obtained using a 400 MHz Bruker instrument at 22 ^{o}C; the chemical shifts values are reported in 'δ' and coupling constants (*J*) in Hz. Mass spectrometry was performed using either ESI-MS, high resolution FAB-MS, or MALDI-TOF (Voyager DE-STR, Applied Biosystems). Molecular sieves 4Å (Sigma-Aldrich) was activated at 150 °C overnight, cooled in a desiccator and powdered freshly before use. Solvent evaporations were performed on a rotary evaporator under reduced pressure at 30–35 °C.

General Notes on Analog Solubility, Stability, and Anomers

Stock solutions of analogs were made at 10 mM in ethanol or at 50 mM in DMSO to maintain sterility and also because the SCFA-derivatized sugars typically were not soluble in aqueous solutions (e.g., in tissue culture media) above ~500–700 μM. When stored at either 4^{o}C or –20^{o}C the analogs were stable in for solution for several months (i.e., migration of SCFA groups to the free hydroxyl of triacetylated or tributanoylated analogs was not observed). Analogs were used as the α/β mixtures obtained from column chromatography except for **4** and **7** that were isolated as pure anomers.

General Procedure for the Synthesis of the 2-Acetamido-3,4,6-tri-*O*-acyl-2-deoxy-α,β-D-manno/glucopyranoses (3, 6 & 9)



Scheme 1. Scheme for the synthesis of the 2-acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy-α,β-D-mannopyranose (**3**), 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α,β-D-mannopyranose (**6**) and 2-acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy-α,β-D-glucopyranose (**9**); Conditions: (a) $(RCO)_2O$, pyridine, DMAP, 22 °C, 24 h; (b) Molecular sieves 4 Å, MeOH, 22 °C, 7–12h (*5*).

Synthesis of 3, 6 & 9

The 2-acetamido-1,3,4,6-tetra-*O*-acyl-2-deoxy-α,β-D-manno/glucopyranose **1**, **5** or **8** (2.0 mmol) was mixed with activated and powdered molecular sieves 4Å (4.0 g) in methanol (100 ml) and stirred at 22 °C. The reaction mixture was monitored by TLC {hexanes : ethyl acetate (AcOEt)} to maximize conversion to the hemi-acetal while minimizing de-acylation at positions other than C1. After ~ 7–12 h, the reaction mixture was filtered through a pad of celite, washed twice with methanol (10 ml) and the combined filtrate was concentrated. Column chromatography of the residue (hexanes : ethyl acetate (AcOEt)) was done to separate unreacted starting material, respectively from the hemiacetals (**3**, **6** & **9**).

Characterization of 2-Acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy-α,β-D-mannopyranose (3)

Crystalline solid, Yield: 80 % (mixture of anomers, major : minor = 88 : 12): ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.04 (d, 0.12H, *J* = 8.4 Hz, NH), 5.88 (d, 0.88H, *J* = 8.8 Hz, NH), 5.42 (dd, 0.88H, *J* = 4.4 and 10.0 Hz), 5.22-5.02 (m, 2.12 H), 5.00 (s, 0.12H) 4.65 (m, 0.12H, H-2), 4.58 (m, 0.88H, H-2), 4.43 (m, 0.88H) 4.32-4.05 (m, 2.88H), 3.70 (m, 0.12H, H-5), 2.45-2.13 (m, 6H), 2.10 (s, 0.36H, NHAc), 2.05 (s, 2.64H, NHAc), 1.90-1.50 (m, 6H), 1.10-0.80 (m, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 173.6, 173.2, 173.0, 173.0, 172.9, 172.8, 171.2 (NHCO), 171.2 (NHCO), 94.1 (C-1), 93.8 (C-1), 73.0, 72.0, 69.3, 68.4, 66.8, 66.0, 62.8 (C-6), 62.8 (C-6), 52.4 (C-2), 51.8 (C-2), 36.4, 36.4, 36.3, 23.6, 18.7, 18.7, 18.5, 14.0, 14.0, 14.0; FAB-MS : Calcd for C_{20} H_{34} NO_9 ($[M+H]^+$): 432.2234, found: 432.2234.

Characterization of 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α,β-D-mannopyranose (6)

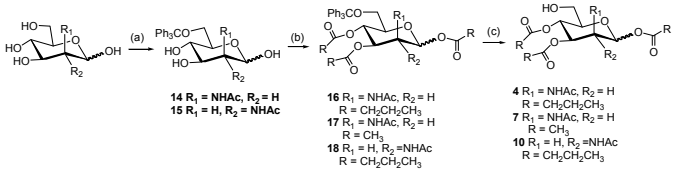
Crystalline solid, Yield: 90 % (mixture of anomers; major : minor = 88:12). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.15 (d, 0.12H, *J* = 8.4 Hz, NH), δ 5.98 (d, 0.88H, *J* = 8.8 Hz, NH), 5.42 (dd, 1H, *J* = 4.4 & 10.4 Hz, H-3), 5.35 (s, 0.12H), 5.20-5.03 (m, 2.12H, H-1), 4.70 (bs, 0.88H), 4.65 (m, 0.12H, H-2), 4.60 (m, 0.88H, H-2), 4.35-4.05 (m, 2.88H), 3.75 (m, 0.12H, H-5), 2.12, 2.11, 2.07, 2.07(4s, 6H), 2.06 (s, 2.64H, NHAc), 2.06 (s, 0.36H, NHAc), 2.03 (s, 0.36H, $COCH_3$), 2.00 (s, 2.64H, $COCH_3$); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 172.5, 170.9, 170.8, 170.7, 170.7, 170.2, 170.2, 170.2 (NHCO), 170.1 (NHCO), 93.9 (C-1), 93.5 (C-1), 72.5, 71.6, 69.0, 67.9, 66.2, 65.8, 62.7 (C-6), 62.6 (C-6), 52.0 (C-2), 51.1 (C-2), 23.4, 23.3, 21.1, 20.8, 20.8, 20.7, 20.7; FAB-MS : Calcd for $C_{14}H_{22}NO_9$ ($[M+H]^+$): 348.1295, found: 348.1294.

Characterization of 2-Acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy-α,β-D-glucopyranose (9)

Crystalline solid, Yield: 80 % (mixture of anomers, major : minor = 95:5): ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.35 (d, 0.05H, NH), 5.95 (d, 0.95H, *J* = 9.2 Hz, NH), 5.40-4.95 (m, 3H), 4.60 (m, 0.05H, H-2), 4.30 (m, 0.95H, H-2), 4.27-3.95 (m, 3.95H), 3.70 (m, 0.05H, H-5), 2.43-2.13 (m, 6H), 2.05 (s, 0.15H, NHAc), 1.96 (s, 2.85H, NHAc), 1.74-1.52 (m, 6H), 1.10-0.86 (m, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 175.0, 174.5, 174.2, 173.7, 173 .6, 172.0, 170.5 (NHCO), 170.4 (NHCO), 95.0 (C-1), 91.7 (C-1), 72.2, 72.0, 70.6, 68.0, 67.8, 67.8, 66.0, 61.9 (C-6), 58.0, 52.5 (C-2 major), 36.2, 36.2, 36.0,36.0, 36.0, 36.0, 23.2, 23.0, 18.5, 18.5 18.4, 18.4,18.4, 18.3, 13.7, 13.7, 13.6,13.6, 13.6, 13.6; MALDI-MS : Calcd for C_{20} H_{33} NO_9 Na ($[M+Na]^+$): 454.2053, found: 454.3054.

General Procedure for the Synthesis of the 2-Acetamido-1,3,4-tri-*O*-acyl-2-deoxy-α,β-D-manno/glucopyranoses 4, 7 and 10

Compounds **4**, **7** and **10** were synthesized by following the general procedure outlined in Scheme 2. The synthesis and characterization of these compounds, along with the synthesis of intermediates **14**–**18** (synthesized following a similar reported procedure for corresponding GalNAc compound) (*6*), are described below in this section.



Scheme 2. Synthesis of 2-acetamido-1,3,4-tri-*O*-butanoyl-2-deoxy-6-*O*-hydroxy-α,β-D-mannopyranose (**4**), 2-acetamido-1,3,4-tri-*O*-butanoyl-2-deoxy-6-*O*-hydroxy-α,β-D-mannopyranoses (**7**), and 2-acetamido-1,3,4-tri-*O*-butanoyl-2-deoxy-6-*O*-hydroxy-α,β-D-mannopyranoses (**10**); Conditions: (a) Ph_3C-Cl, pyridine, RT–60 °C, 48 h; (b) $(RCO)_2O$, pyridine, DMAP, 0 °C–22 °C, 24 h (c) Acetic acid : water (3 : 1), 60 °C, 3–4 h.

Synthesis of 2-Acetamido-2-deoxy-6-*O*-triphenylmethyl-α,β-D-manno & glucopyranose (14 & 15)

To a stirred mixture of ManNAc or GlcNAc (0.835 mmol) in pyridine (2.7 ml) was added triphenylmethyl chloride (3.0 g, 1.07 mmol) at 22 °C. After 48 h, the reaction mixture was heated at 60 °C for 1.0 h and monitored by TLC (AcOEt). The reaction mixture was concentrated with toluene (3 x 20 ml). The residue was dissolved in AcOEt and washed with water. The organic layers were collected, dried over anh. Na_2SO_4, filtered and concentrated to obtain **14** or **15** as a crude product which was taken to the next step without further purification.

Synthesis of 16 and 17 from 14 and 18 from 15

To a stirred solution of **14** or **15** (2.16 mmol) in pyridine (1.46 ml, 18 mmol) at 0 °C (ice-water bath), the respective acid anhydride (either acetic anhydride or butyric anhydride) (12 mmol) was added. The reaction mixture was allowed to warm to 22 °C and monitored by TLC (hexanes:AcOEt 3:1). After 24 h, the mixture was concentrated with toluene (3 x 10 ml), and extracted using a mixture of dichloromethane (100 ml) and water (50 ml). The organic layers were collected, dried over anh. Na_2SO_4, filtered and concentrated. Column chromatography of the residue (hexanes : AcOEt) gave either the tri-butyrate **16** and **18** or the tri-acetate **17** as a mixture of anomers.

Characterization of 2-Acetamido-1,3,4-tri-*O*-butanoyl-2-deoxy-6-*O*-triphenylmethyl-α,β-D-mannopyranose (16)

Syrup. Yield: 67 % (2 steps) (mixture of anomers; major : minor = 60:40) from ManNAc. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.50- 7.15 (m, 15H, 3 x Ph), 6.15 (d, 0.6H, *J* = 2.0 Hz, H-1), 5.92 (d, 0.4H, *J* = 1.6 Hz, H-1), 5.83 (d, 0.4H, *J* = 9.6 Hz, NH), 5.76 (d, 0.6H, *J* = 9.6 Hz, NH), 5.40-5.25 (m, 1.6H), 5.03 (dd, 0.4H, *J* = 4.0 & 10.0 Hz), 4.78 (m, 0.4H, H-2), 4.68 (m, 1H, H-2), 4.00 (m, 0.6H, H-5). 3.70 (m, 0.4H, H-5), 3.38 (dd, 0.4H, J = 2.4 & 10.4 Hz), 3.30 (dd, 0.6H, J = 2.4 & 10.8 Hz), 3.13-3.10 (m, 1H), 2.50-2.17 (m, 6H), 2.12 (s, 1.2H, NHAc), 2.10 (s, 1.8H, NHAc), 1.80-1.35 (m, 6H,), 1.08-0.79 (m, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 172.7, 172.7, 171.9, 171.8, 171.0, 170.9, 170.4 (NHCO), 170.0 (NHCO), 143.8, 143.4, 129.1, 128.4, 128.3, 128.0, 127.3, 127.1, 91.6 (C-1), 90.5 (C-1), 86.8, 86.7, 74.8, 71.9, 71.6, 69.1, 65.4, 65.3, 61.8 (C-6), 61.7 (C-6), 49.9 (C-2), 49.5 (C-2), 36.0, 35.9, 35.8, 35.7, 23.4, 23.3, 18.3, 18.3, 18.2, 18.1, 18.0, 18.0, 14.2, 13.7, 13.6, 13.6, 13.5, 13.5; MALDI-MS : Calcd for $C_{39}H_{47}NO_9Na$ ($[M+Na]^+$): 696.3149, found: 696.3350.

Characterization of 2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-6-*O*-triphenylmethyl-α,β-D-mannopyranose (17)

Syrup, Yield: 70 % (2 steps) (mixture of anomers, major : minor 55:45) from ManNAc: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.50- 7.15 (m, 15H, 3 x Ph), 6.13 (d, 0.55H, *J* = 2.0 Hz, H-1), 5.88 (d, 0.45H, *J* = 2.0 Hz, H-1), 5.86 (d, 0.45H, *J* = 9.6 Hz, NH), 5.78 (d, 0.55H, *J* = 9.2 Hz, NH), 5.36 (t, 0.55H, *J* = 10.0), 5.32 (t, 0.45H, *J* = 10.0 Hz), , 5.31 (dd, 0.55H, *J*= 4.4 and 10.0 Hz), 5.00 (dd, 0.45H, J= 3.6 & 10.0 Hz), 4.78 (m, 0.45H, H-2), 4.65 (m, 0.55H, H-2), 3.93 (m, 0.55H, H-5), 3.68 (m, 0.45H, H-5), 3.36 (dd, 0.55H, J = 2.4 & 10.8 Hz), 3.32 (dd, 0.45H, J = 2.4 & 10.0 Hz), 3.12 (dd, 0.55H, J = 4.0 & 10.0 Hz), 3.08 (dd, 1H, J = 4.8 & 10.0 Hz), 2.18, 2.17, 2.14, 2.12, 2.11, 2.06, 2.03 (s, 1.35H, NHAc), 2.02 (s, 1.65H, NHAc); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 171.1, 170.3, 170.2, 169.9, 169.9, 168.7, 168.2 (NHCO), 168.0 (NHCO), 143.4, 143.0, 128.9, 128.7, 128.2, 127.9, 127.3, 127.2, 126.6, 91.8 (C-1), 90.2 (C-1), 86.8, 86.3, 73.4, 71.7, 71.5, 69.3, 69.3, 65.8, 61.5 (C-6), 61.3 (C-6),49.4 (C-2), 49.3 (C-2), 23.4, 23.1, 20.9, 20.8, 20.5, 20.4, 20.4, 20.2. MALDI-MS : Calcd for $C_{33}H_{35}NO_9Na$ ($[M+Na]^+$): 612.2209, found: 612.3708.

Synthesis of 4 and 7, respectively, from 16 and 17 and 10 from 18

A stirred mixture of either **16**, **17** or **18** (0.743 mmol) in 80 % aqueous acetic acid (10 ml) was heated at 60 °C and monitored by TLC (hexanes:AcOEt). After ~4 h, the reaction mixture was concentrated with toluene (3 x 10 ml). Column chromatography of the residue (hexanes:AcOEt) gave the tri-butyrate **4** (as pure anomers which were separated) and tri-butyrate **10** (as a mixture of anomers) or the tri-acetate **7** (as pure anomers).

Characterization of 2-Acetamido-1,3,4-tri-*O*-butanoyl-2-deoxy-D-mannopyranose (4)

Semi-solid. Yield: 70 % yield. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.11 (d, 1H, *J* = 9.6 Hz, NH), 6.07 (d, 1H, *J* = 2.0 Hz, H-1), 5.43 (dd, 1H, *J* = 4.8 & 10.4 Hz, H-3), 5.19 (t, 1H, *J* = 10.4 Hz, H-4), 4.68 (m, 1H, H-2), 3.82 (m, 1H, H-5), 3.75 (m, 1H, H-6a), 3.60 (m, 1H, H-6b), 2.75 (m, 1H, C6-OH), 2.50-2.15 (m, 6H, 3 x CH_2), 2.08 (s, 3H, NHAc), 1.80-1.50 (m, 6H, 3 x CH_2), 1.08-0.80 (m, 9H, 3 x CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 173.8, 172.6, 171.0, 170.2 (NHCO), 91.8 (C-1), 72.5, 68.4, 65.5, 60.8 (C-6), 49.4 (C-2), 36.0, 36.0, 35.9, 23.2, 18.4, 18.2, 18.1, 13.6, 13.6, 13.6. MALDI-MS : Calcd for $C_{20}H_{33}NO_9Na$ ($[M+Na]^+$): 454.2053, found: 454.2036.

Characterization of 2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-D-mannopyranose (7)

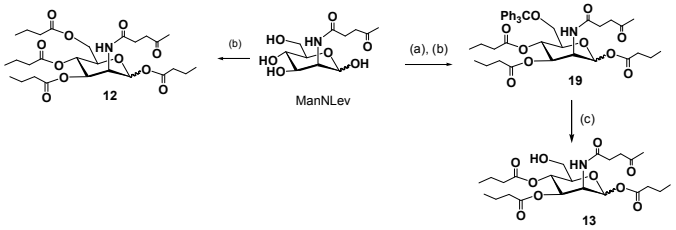
Yield: 50 %. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.14 (d, 1H, J = 9.2 Hz, NH), 6.05 (d, 1H, J = 1.6 Hz, H-1), 5.42 (dd, 1H, J = 4.0 & 10.4 Hz, H-3), 5.19 (t, 1H, J = 10.4 Hz, H-4), 4.64 (m, 1H, H-2), 3.82 (m, 1H, H-5), 3.73 (m, 1H, J = 2.0 & 13.3 Hz, H-6a), 3.60 (m, 1H, J = 3.6 & 13.3 Hz, H-6b), 2.72 (m, 1H, C6-OH), 2.18, 2.13, 2.06, 2.04 (s, 3H, NHAc); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.1, 170.9, 170.2, 170.2, 168.4 (NHCO), 91.9 (C-1), 72.4, 68.7, 65.7, 60.7 (C-6), 49.3 (C-2), 23.2, 20.9, 20.8, 20.7; MALDI-MS : Calcd for $C_{14}H_{21}NO_9Na$ ($[M+Na]^+$): 370.1113; found 370.0904

Characterization of 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α,β-D-glucoopyranose (10)

Yield 70 % Crystalline solid, Yield: 80 % (α : β = 78:22): ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.21 (d, 0.78H, J = 4.8 Hz, H-1), 5.70 (d, 0.22H, *J* = 11.6 Hz, H-1), 5.55 (d, 0.78H, J = 12.0 Hz, -NH), 5.54 (d, 0.22H, NH), 5.40-5.01 (m, 2H), 4.45 (m, 0.78H, H-2), 4.30 (m, 0.22H, H-2), 3.90-3.50 (m, 3H), 2.46-2.21 (m, 7H), 1.92 (s, 2.34H, NHAc), 1.90 (s, 0.66H, NHAc), 1.80-1.52 (m, 6H), 1.07-0.86 (m, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 174.5, 173.8, 172.7, 172.6, 172.4, 171.4, 169.8 (NHCO), 169.7 (NHCO), 92.6 (C-1), 90.5 (C-1), 75.0, 72.2, 72.1, 70.2, 67.8, 67.7, 61.0 (C-6), 60.4 (C-6), 53.1 (C-2), 51.4 (C-2), 36.0, 36.0, 36.0,35.9, 35.9, 35.9, 23.2, 23.0, 18.4, 18.4, 18.4, 18.0,18.0, , 13.6, 13.6, 13.5,13.5, 13.5, 13.4; MALDI-MS : Calcd for $C_{20}H_{33}NO_9Na$ ($[M+Na]^+$): 454.2053, found: 454.3140

Synthesis of 1,3,4,6-tetra-*O*-butanoyl-2-deoxy-2-(4-oxopentanoyl)-amino-α,β-D-mannopyranose (12) and 1,3,4-tri-*O*-butanoyl-2-deoxy-2-(4-oxopentanoyl)amino-α,β-D-mannopyranose (13)

The *N*-4-oxo-pentanoyl-α,β-D-mannosamine analogs **12** and **13** were synthesized as shown in Scheme 3 (the conditions for the synthesis and characterization of **12**, **19** and **13** are provided below).



Scheme 3: Conditions: (a) Ph_3C-Cl, pyridine, RT-60 °C, 48 h; (b) Butyric anhydride, pyridine, DMAP, 0–22 °C, 24 h (c) Acetic acid:water (3:1), 60 °C, 3–4 h.

Synthesis of 1,3,4,6-tetra-*O*-butanoyl-2-deoxy-2-(4-oxopentanoyl)-amino-α,β-D-mannopyranose (12)

To a stirred solution of ManNLev (*7*) (2.16 mmol) in pyridine (1.46 ml, 18 mmol) at 0 °C (ice-water bath), butyric anhydride (12 mmol) was added. The reaction mixture was allowed to warm up to 22 °C and monitored by TLC (hexanes : AcOEt 1:1). After 24 h, the mixture was concentrated with toluene (3 x 10 ml), and extracted using a mixture of dichloromethane (100 ml) and water (50 ml). The organic layers were collected, dried over anh. Na_2SO_4, filtered, and concentrated. Column chromatography of the residue (hexanes:AcOEt) gave the titled compound **12** as a mixture of anomers.

Characterization of 1,3,4,6-tetra-*O*-butanoyl-2-deoxy-2-(4-oxo-pentanoyl)amino-α,β-D-mannopyranose (12)

Syrup, Yield: 80 % (mixture of anomers; major : minor = 78:22) from ManNLev: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.25 (d, 0.78H, J= 9.2 Hz, NH), 6.15 (d, 0.22H, J = 9.2 Hz, NH), 6.07 (s, 0.78H, J = 2.0 Hz, H-1), 5.90 (s, 0.22H, J = 1.6 Hz, H-1), 5.32 (dd, 0.78 H, J = 4.4 & 10.4 Hz, H-3 major), 5.25-5.09 (m, 1H), 5.05 (dd, 0.22H, J = 4.4 & 10.4 Hz, H-3 minor), 4.75 (m, 0.22H, H-2), 4.60 (m, 0.78H, H-2), 4.37-3.98 (m, 2.78H), 3.82 (m, 0.22H, H-5 minor), 2.85-2.10 (m, 12H, 4xCH_2, 2xCH_2,), 1.80-1.50 (m, 8H, 4xCH_2), 1.10-0.80 (m, 12H, 4xCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 207.6, 207.4 {2 x (-CO-)}, 173.3, 173.2, 172.5, 172.5, 172.5, 172.5, 172.2, 172.1, 171.1, 170.8, 91.6 (C-1), 90.5 (C-1), 73.4, 71.0, 70.4, 68.9, 65.1, 65.1, 61.8 (C-6), 61.7 (C-6), 49.3 (C-2), 49.3 (C-2), 38.7, 38.7, 35.9, 35.9, 35.8, 35.7, 30.0, 30.0, 30.0, 30.0, 29.9, 29.8, 29.8, 29.8, 18.4, 18.3, 18.0, 18.0, 18.0, 18.0, 18.0, 17.9, 13.6, 13.6, 13.6, 13.5, 13.5, 13.5, 13.5, 13.5; MALDI-MS : Calcd for $C_{27}H_{43}NO_{11}Na$ ($[M+Na]^+$): 580.6202, found: 580.5537.

Synthesis of 1,3,4-tri-*O*-butanoyl-6-*O*-triphenylmethyl-2-deoxy-2-(4-oxopentanoyl)amino-α,β-D-mannopyranose (19)

ManNLev(*7*) (2.0 g), pyridine (15 ml), and trityl chloride (2.0 g) were combined in a RB flask and stirred for 48 h at rt. The mixture was then stirred for one more hour at 65 °C. After completion of the reaction, as monitored by TLC in 100 % EtOAc, the reaction mixture was extracted with water/EtOAc. The organic layer was collected, coevaporated with toluene and taken directly to next step. The crude reaction mixture was combined with butyric anhydride (12 ml) and pyridine (10 ml) and then stirred for 24 h at rt. After completion of the reaction, as monitored by TLC (EtOAc:hexane = 1:4), the reaction mixture was coevaporated with toluene and extracted with water. The organic layer was collected, concentrated, and purified by column chromatography to obtain **19** with a 60 % overall yield.

Characterization of 1,3,4-tri-*O*-butanoyl-6-*O*-triphenylmethyl-2-deoxy-2-(4-oxopentanoyl)amino-α,β-D-mannopyranose (19)

Syrup, Yield: 60 % (2 steps) (mixture of anomers; major : minor = 53:47) from ManNLev. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.52- 7.15 (m, 15H, 3 x Ph), 6.20-6.07 (d, 1.53H, H-1major, NH-major and NH-minor), 5.89 (s, 0.47H, H-1-minor), 5.35-5.21 (m, 1.53H), 5.01 (m, 0.47H, H-3 minor), 4.74 (m, 0.53H, H-2 major), 4.61 (m, 0.47H, H-2 minor), 3.98 (m, 0.53H, H-5), 3.71 (m, 0.47H, H-5), 3.40-3.10 (m, 2H), 2.85-2.75 (m, 2H), 2.69-2.50 (m, 2H) 2.45-1.90 (m, 9H), 1.81-1.38 (m, 6H), 1.10-0.78 (m, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 207.2, 207.0 {2 x (-CO-)}, 172.7, 172.7, 172.4, 172.1, 171.8, 171.8, 171.2, 170.8, 143.5, 143.5, 128.8, 128.8, 128.6, 128.6, 127.2, 127.2, 127.1, 91.6 (C-1), 90.5 (C-1), 86.8, 86.7, 74.8, 72.0, 71.5, 69.8, 69.3, 65.5, 62.1 (C-6), 62.0 (C-6),49.5 (C-2), 49.5 (C-2), 38.6, 38.5, 36.0, 35.9, 35.8, 35.7, 30.0, 30.0, 30.0, 30.0, 29.8, 18.3, 18.2, 18.2, 18.1, 18.0, 18.0, 13.6, 13.6, 13.6, 13.6, 13.5, 13.5; MALDI-MS : Calcd for $C_{42}H_{51}NO_{10}Na$ ($[M+Na]^+$): 752.3411; found : 752.6177.

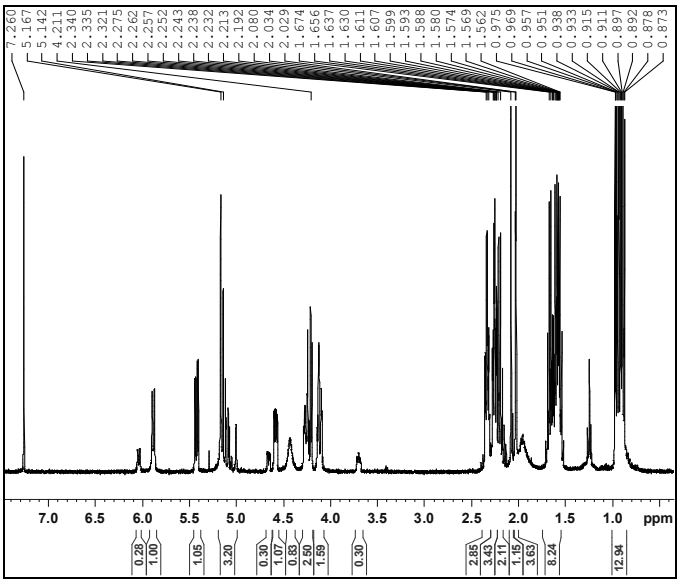
Synthesis of 1,3,4-tri-*O*-butanoyl-2-deoxy-2-(4-oxopentanoyl)-amino-α,β-D-mannopyranose (13) from 19

A stirred mixture of 1,3,4-tri-O-butanoyl-6-*O*-trityl-2-deoxy-*N*-levulinoyl-α,β-D-mannosamine (0.743 mmol) in 80 % aqueous acetic acid (10 ml) was heated at 60 °C and monitored by TLC (hexanes:AcOEt). After ~4 h, the reaction mixture was concentrated with toluene (3 x 10 ml). Column chromatography of the residue (hexanes:AcOEt) gave the tri-butyrate **13** (as a mixture of anomers) in a 75 % yield.

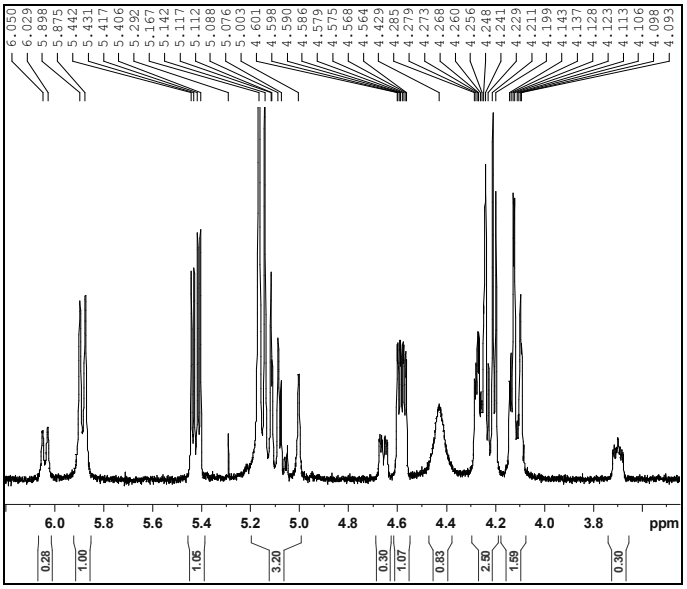
Characterization of 1,3,4-tri-*O*-butanoyl-2-deoxy-2-(4-oxopentan-oyl)-amino-α,β-D-mannopyranose (13)

Syrup, Yield: 75 % (2 steps) (mixture of anomers; major : minor = 56:44): ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.62 (d, 0.44H, J= 9.2 Hz, NH), 6.46 (d, 0.56H, J = 9.2 Hz, NH), 6.14 (s, 0.44H, H-1), 5.89 (s, 0.56H, H-1), 5.50-5.02 (m, 2H), 4.78 (m, 0.56H, H-2), 4.62 (m, 0.44H, H-2), 3.00-2.10 (m, 11.68H, 3xCH_2, 2xCH_2, NHAc), 1.88 (s, 1.32H, NHAc), 1.70-1.40 (m, 6H, 3xCH_2), 1.10-0.76 (m, 9H, 3xCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) : δ 207.8, 207.5, 173.6, 173.5, 172.6, 172.6, 172.6, 172.2, 171.3, 171.0, 143.5, 91.7 (C-1), 90.6 (C-1), 75.5, 71.1, 68.6, 65.4, 65.4, 60.8(C-6), 60.8 (C-6),49.7 (C-2), 49.3 (C-2), 38.6, 38.5, 36.0, 35.9, 35.8, 35.7, 29.9, 29.9, 29.8, 29.8, 18.4, 18.3, 18.2, 18.1, 18.0, 18.0, 13.6, 13.6, 13.6, 13.6, 13.5, 13.5; MALDI-MS : Calcd for $C_{23}H_{37}NO_{10}Na$ ($[M+Na]^+$): 510.5304; found 510.5321.

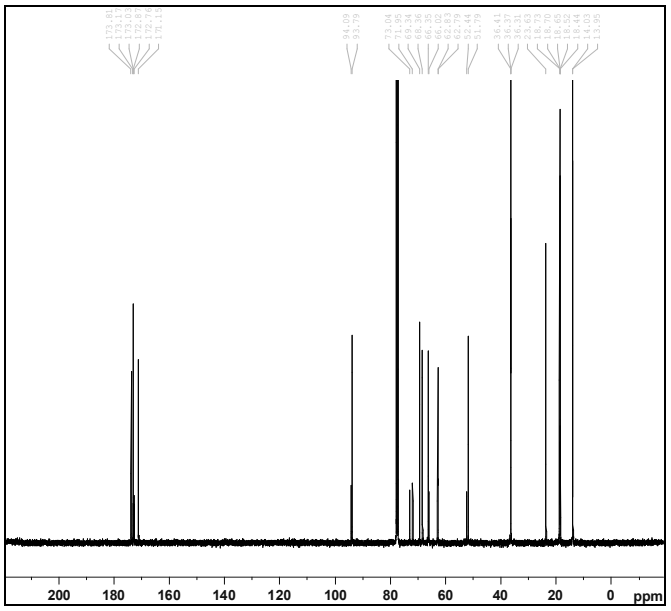
SPECTRA OF SELECTED COMPOUNDS



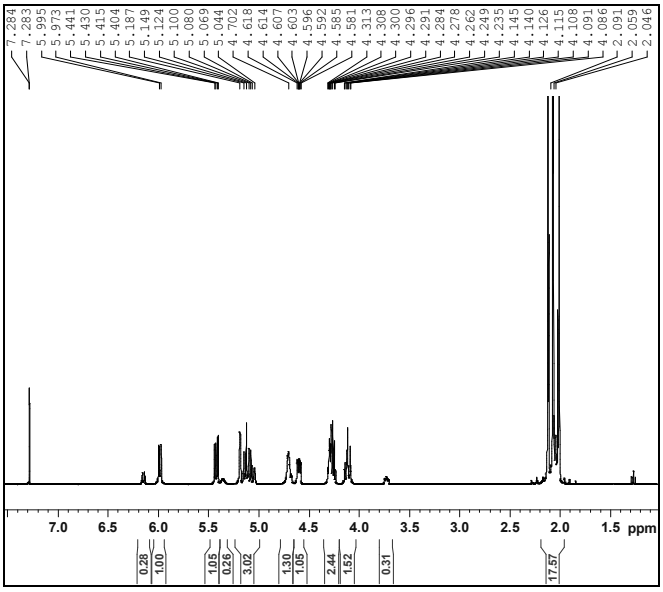
^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy-α,β-D-mannopyranose (**3**)



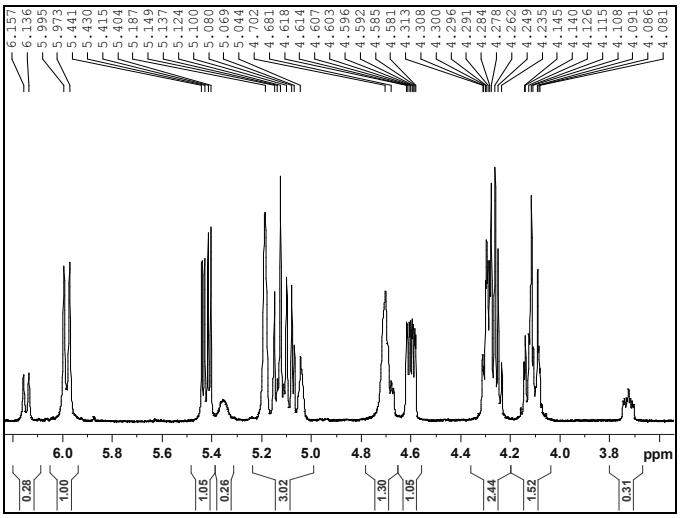
Expanded ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy-α,β-D-mannopyranose (**3**)



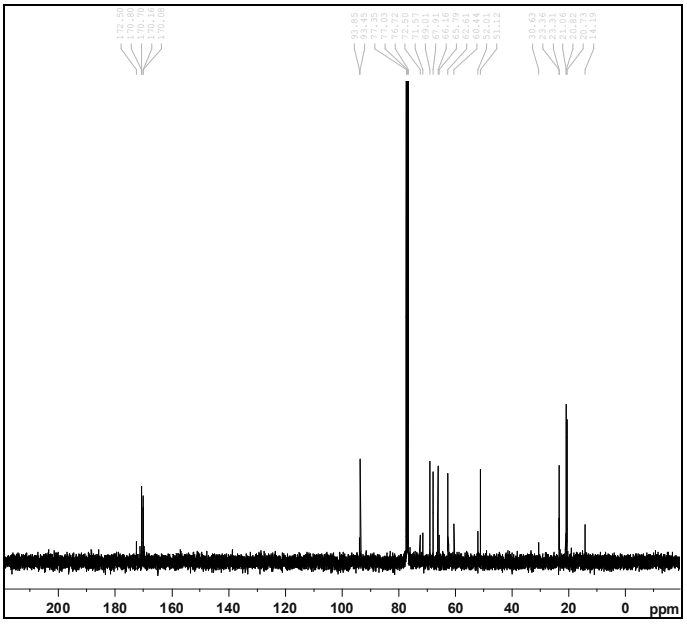
^{13}C-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy-α,β-D-mannopyranose (**3**)



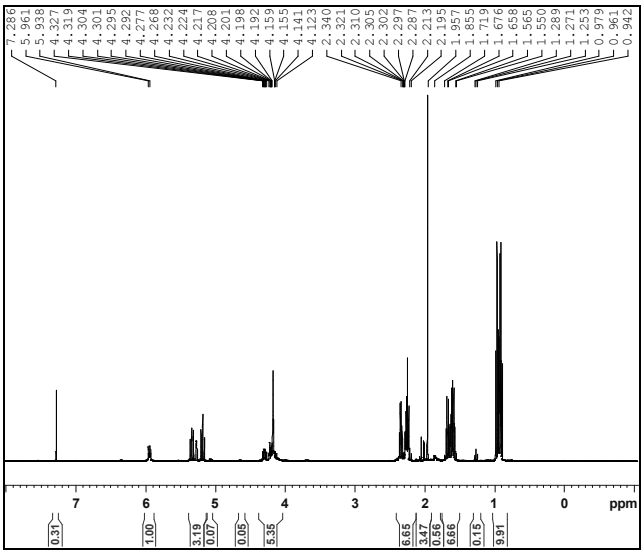
^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α,β-D-mannopyranose (**6**)



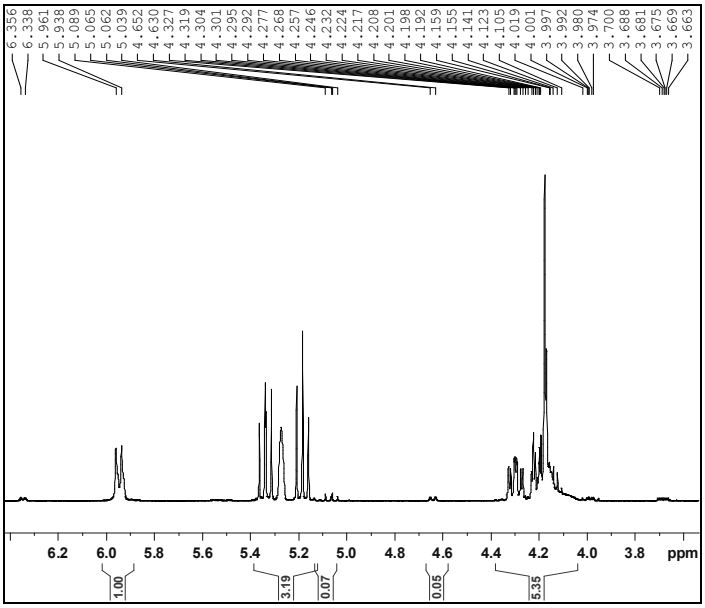
Expanded ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α,β-D-mannopyranose (**6**)



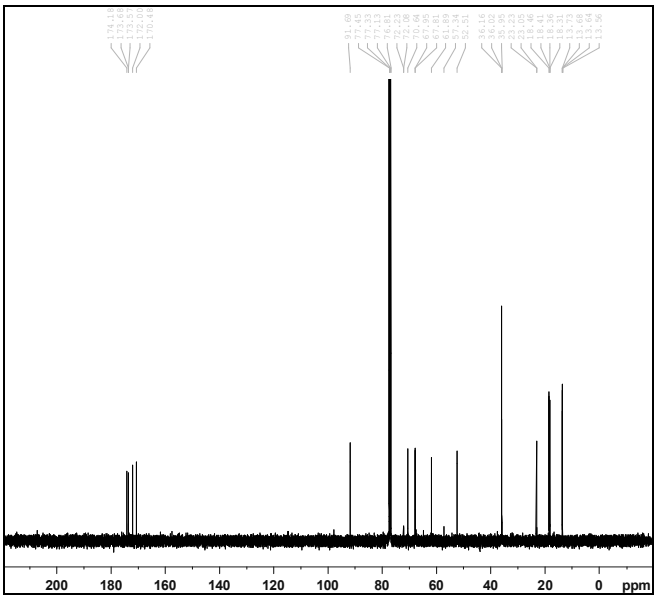
^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α,β-D-mannopyranose (**6**)



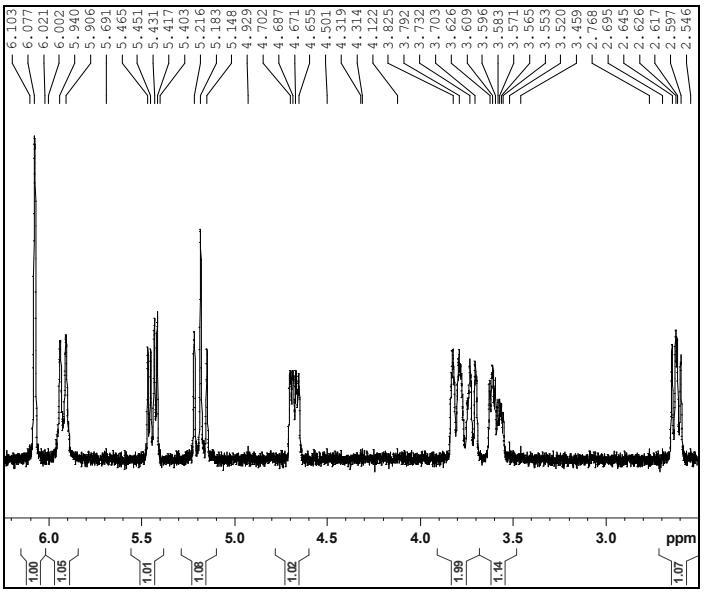
^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy-α,β-D-glucopyranose (**9**)



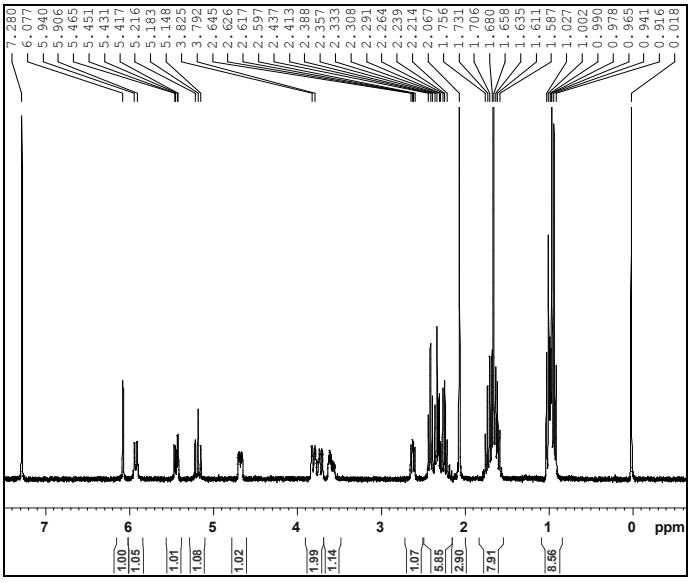
Expanded ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy-α,β-D-glucopyranose (**9**)



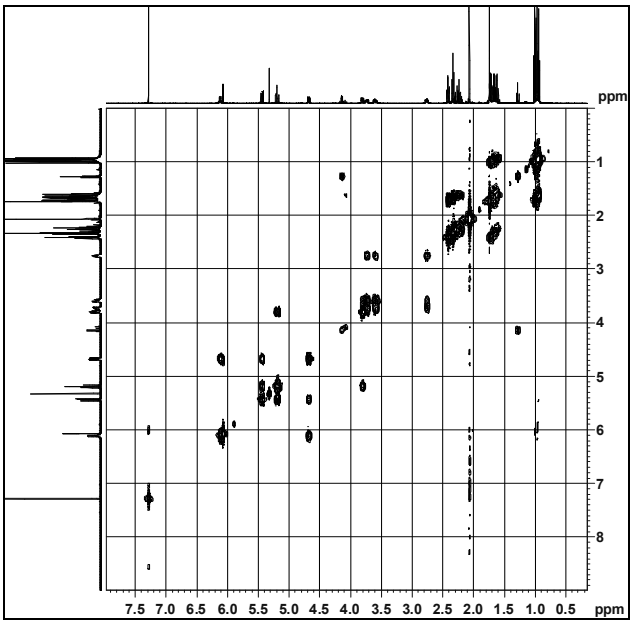
^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of 2-acetamido-3,4,6-tri-*O*-butanoyl-2-deoxy-α,β-D-glucopyranose (**9**)



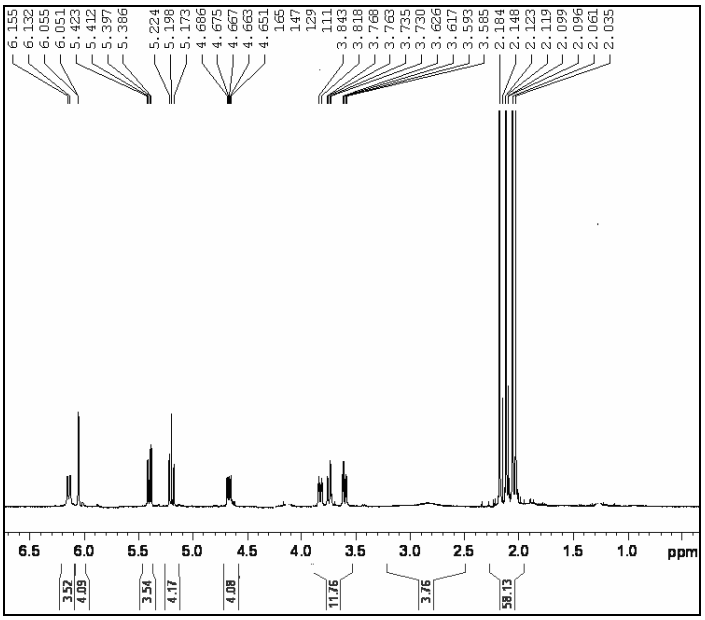
Expanded ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-1,3,4-tri-*O*-butanoyl-2-deoxy-D-mannopyranose (**4**)



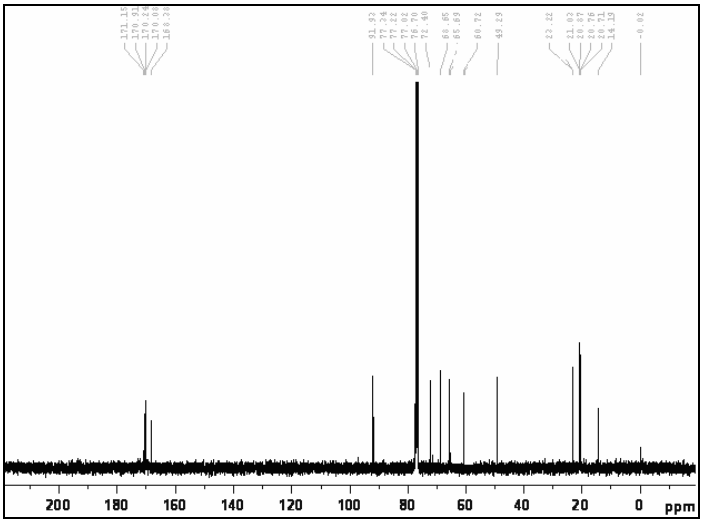
^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-1,3,4-tri-*O*-butanoyl-2-deox-D-mannopyranose (**4**)



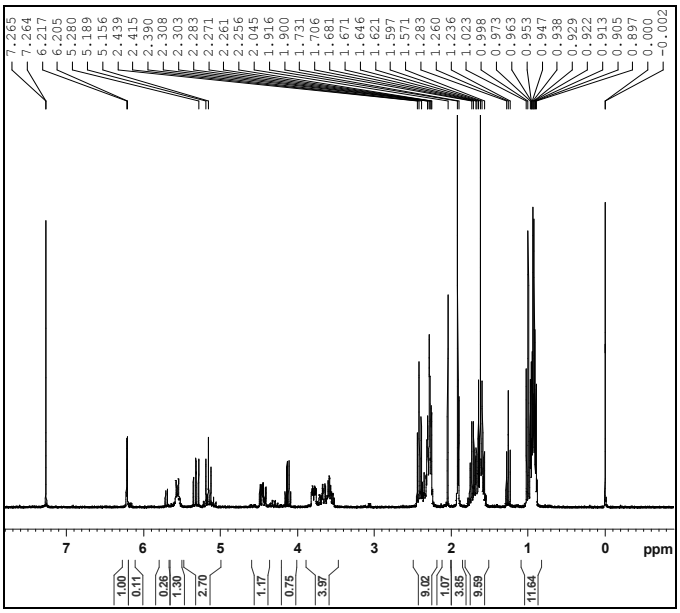
^{1}H-^{1}H COSY-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-1,3,4-tri-*O*-butanoyl-2-deoxy-D-mannopyraose (**4**)



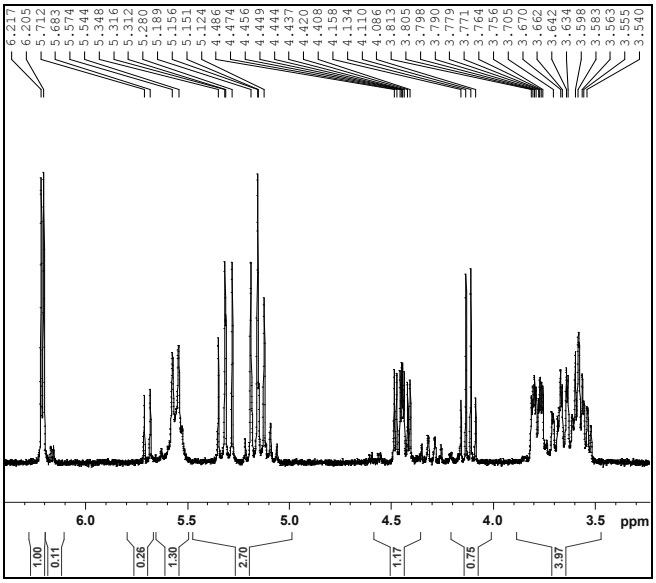
^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-D-mannopyranose (**7**)



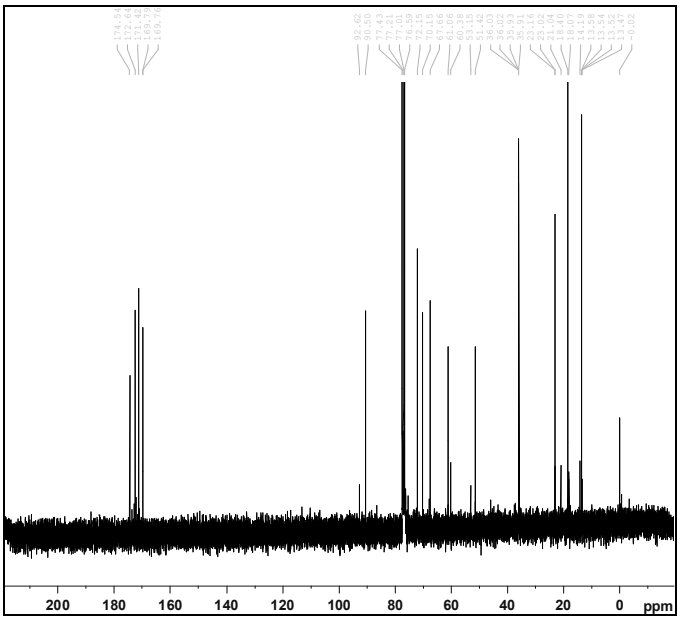
^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-D-mannopyranose (**7**)



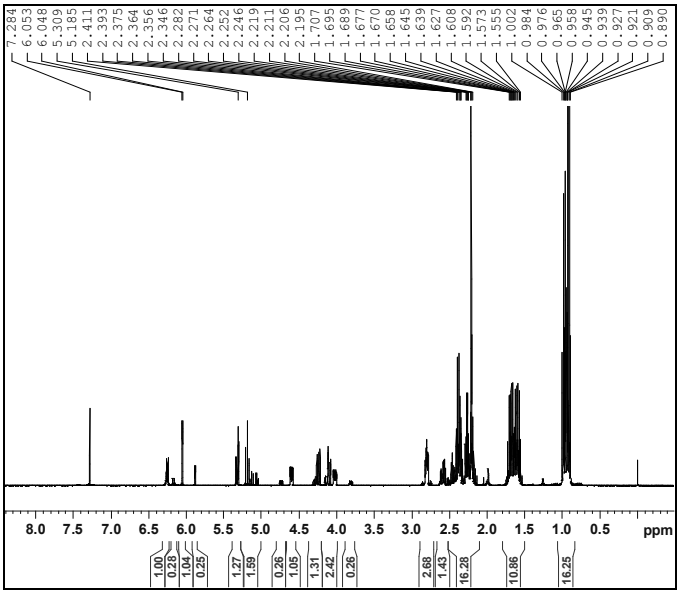
^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-1,3,4-tri-*O*-butanoyl-2-deoxy-α,β-D-glucopyranose (**10**)



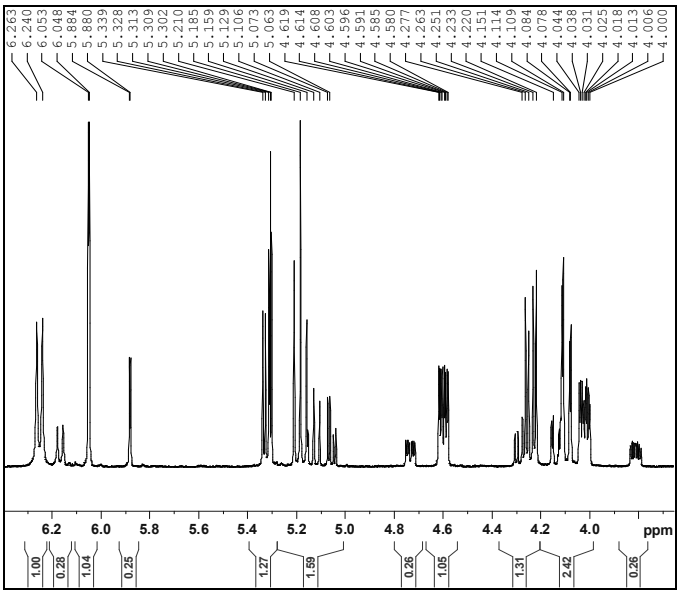
Expanded ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 2-acetamido-1,3,4-tri-*O*-butanoyl-2-deoxy-α,β-D-glucopyranose (**10**)



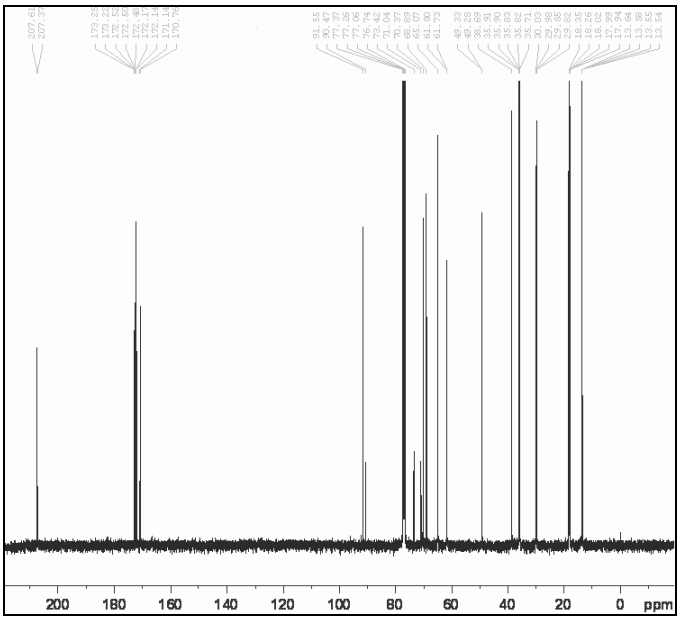
^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of 2-acetamido-1,3,4-tri-*O*-butanoyl-2-deoxy-α,β-D-glucopyranose (**10**)



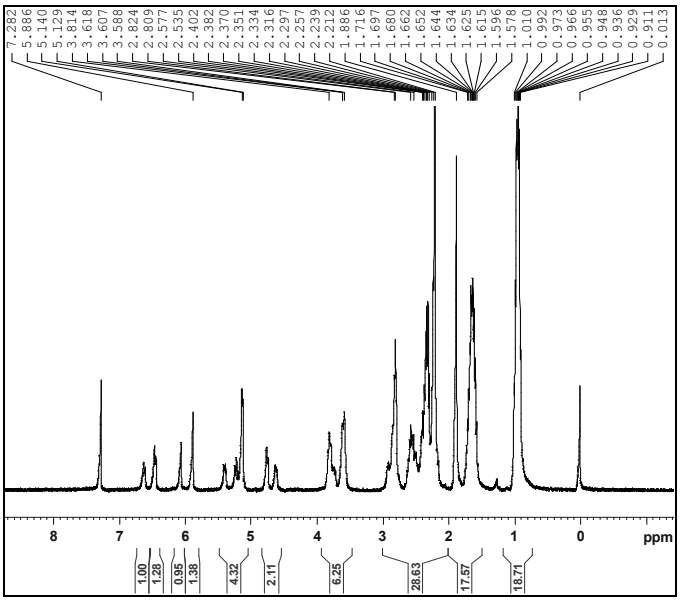
^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 1,3,4,6-tetra-*O*-butanoyl-2-deoxy-2-(4-oxopentanoyl)amino-α,β-D-mannopyranose **(12)**



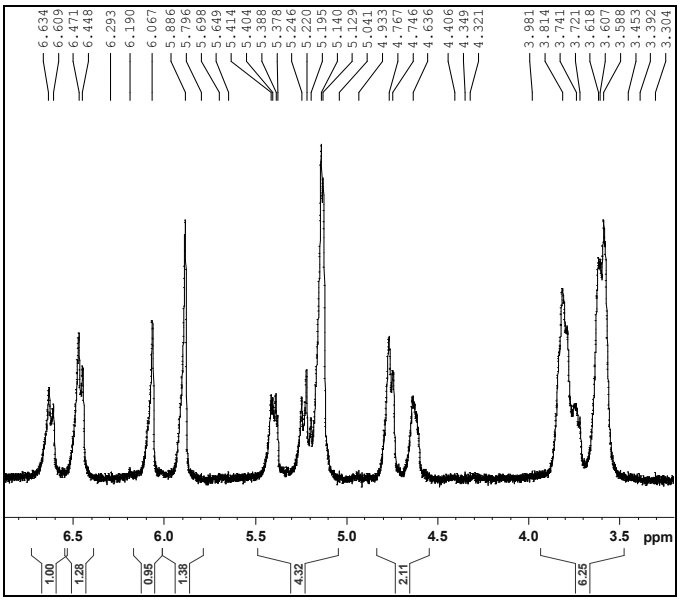
Expanded ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 1,3,4,6-tetra-*O*-butanoyl-2-deoxy-2-(4-oxopentanoyl)amino-α,β-D-mannopyranose **(12)**



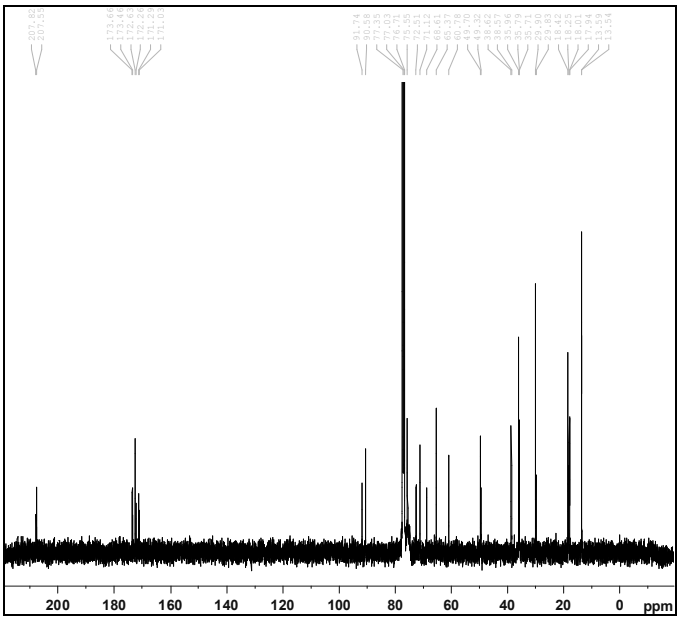
^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of 1,3,4,6-tetra-*O*-butanoyl-2-deoxy-2-(4-oxopentanoyl)amino-α,β-D-mannopyranose **(12)**



^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 1,3,4-tri-*O*-butanoyl-2-deoxy-2-(4-oxopentanoyl)amino-α,β-D-mannopyranose **(13)**



Expanded ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of 1,3,4-tri-*O*-butanoyl-2-deoxy-2-(4-oxopentanoyl)amino-α,β-D-mannopyranose **(13)**



^{13}C-NMR (100 MHz, $CDCl_3$) spectrum of 1,3,4-tri-*O*-butanoyl-2-deoxy-2-(4-oxopentanoyl)amino-α,β-D-mannopyranose **(13)**

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