Supporting Information

A dimethoxytriazine type glycosyl donor enables facile chemo-enzymatic route toward α-linked *N*-acetylglucosaminyl-galactose disaccharide unit from gastric mucin

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Supplementary Material

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Synthesis of **1**

4-(4,6-dimethoxy-1,3,5-triazine-2-yl)-4-methyl morphorinium chloride (DMT-MM, 533 mg, 2.0 mmol) was added to a solution of *N*-acetylglucosamine (221 mg, 1.0 mmol) and lutidine (0.23 ml, 2.0 mmol) in water (6.25 ml), and the resulting mixture was stirred for 24 h at room temperature. After concentration in vacuo, the residue was purified by silica gel column chromatography (eluent : ethyl acetate / methanol = 7/1) to give 4,6-dimethoxy-1,3,5-triazin-2-yl α-*N*-acetylgludosaminide (288 mg, 0.84 mmol, 84%).

^{1}H NMR(500 MHz, D_2O) : δ 6.38(1H, d, H-1, $J_{1,2}$ = 3.3 Hz), 4.03(1H, dd, H-2, $J_{1,2}$ = 3.3 Hz, $J_{2,3}$ = 10.8 Hz), 3.89(6H, s, -OC$\underline{H}_3$), 3.83(1H, dd, H-3, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 9.9 Hz), 3.74-3.66(3H, m, H-5, H-6a, H-6b), 3.51(1H, t, H-4, $J_{3,4}$ = $J_{4,5}$ = 9.4 Hz), 1.85(3H, s, -COC$\underline{H}_3$)

^{13}C NMR(126 MHz, D_2O) : δ 174.7, 173.2, and 171.7(triazine, -$\underline{C}$OCH$_3$), 94.3(C-1), 74.2(C-5), 70.4(C-3), 69.3(C-4), 60.1(C-6), 55.8(-O$\underline{C}$H$_3$), 52.8(C-2), 21.6(-CO$\underline{C}$H$_3$).

ESI-MS : *m/z* calcd for $C_{13}H_{20}N_4O_8$[M+Na]$^+$: 383.1173, Found : 383.1174.

Enzymatic hydrolysis of **1** by α-*N*-acetylglucosaminidase

A mixture of **1** (5 μmol, final conc.: 10 mM) and α-N-acetylglucosaminidase B1 (5.0 μg, 5 μl) in PBS (pH 7.4) was incubated at 37 °C. The reaction mixtures were analysed by HPLC (column; TOSOH TSK-Gel Amide-80 (ϕ4.6 × 250 mm), eluent; 75 vol.% CH_3CN, flow rate; 1.0 ml/min, column oven; 30 °C, detection; UV (214 nm)).

Synthesis of **6a**

A mixture of **1** (50 μmol, final conc.: 50 mM), *p*-methoxyphenyl β-galactoside (50 μmol, final conc.: 50 mM), and α-*N*-acetylglucosaminidase B1 (100 μg, 36.4 μl, PBS solution) in 100 mM sodium phosphate buffer (pH 6.5) was incubated at 37 °C. The reaction mixtures were analyzed by HPLC (column; Inertsil ODS-3 (ϕ4.6 × 250 mm, GL-Sciences), eluted by 5% CH_3CN to 15% CH_3CN gradient, flow rate; 1.0 ml/min, column oven; 30 °C, detection; UV (280 nm)). The products were purified by HPLC (column; COSMOSIL Cholester (20 × 250 mm, nacalai tesque), eluent; 8 vol.% CH_3CN, flow rate; 18.9 ml/min, column oven; 30 °C, detection; UV (280 nm)), concentrated *in vacuo*, and freeze-dried.

^{1}H NMR (500 MHz, CD_3OD): δ 7.08 (2H, d, Ph, J = 9.1 Hz), 6.86 (2H, d, Ph, J = 9.1 Hz), 4.95 (1H, d, H-1', J = 3.6 Hz), 4.84 (1H, d, H-1, J = 7.6 Hz), 4.29 (1H, ddd, H-5' , J = 2.5, 4.0, 9.9 Hz), 4.04 (1H, d, H-4, J = 2.8 Hz), 3.97 (1H, dd, H-2', J = 3.6, 10.9 Hz), 3.83 (1H, dd, H-6a', J = 2.2, 11.8 Hz), 3.79-3.71 (8H, m, H-2, H-5, H-6a, H-3', H-6'b, *O*CH$_3$), 3.69-3.65 (2H, m, H-3, H-6b), 3.46 (1H, t, H-4', J = 9.4, 9.5 Hz), 2.02 (3H, s, CH_3).

^{13}C NMR (126 MHz, CD_3OD): δ 173.8 (CO of Ac), 156.7 (Ph), 153.0 (Ph), 119.1 (Ph), 115.5 (Ph), 104.0 (C-1), 100.3 (C-1'), 77.7 (C-4), 76.8 (C-5), 74.3 (C-3), 73.7 (C-5'), 72.6 (C-3'), 72.4 (C-2),

72.0 (C-4'), 62.3 (C-6'), 60.7 (C-6), 56.1 (*O*CH_3), 55.5 (C-2'), 22.7 (CH_3 of Ac).

Synthesis of **6b**

A mixture of **1** (45 mg, 0.125 mmol, final conc.: 50 mM), methyl β-galactoside (73 mg, 0.375 mmol, final conc.: 150 mM), and α-*N*-acetylglucosaminidase B1 (385 μg, 134 μl, PBS solution) in 100 mM sodium phosphate buffer (pH 6.5 final volume 2.5 ml) was incubated at 37 °C for 1 hour. The product was purified by HPLC (column; Amide-80 (21.5 mm I.D. × 250 mm, Tosoh), eluent; 80 vol.% CH_3CN, flow rate; 8 ml/min, column oven; 30 °C, detection; UV (214 nm)), concentrated *in vacuo*, and freeze-dried to give methyl 4-*O*-(2-acetamido-2-deoxy-α-glucopyranosyl)-β-galactopyranoside (**6b**, 7.8 mg, 17%).

^{1}H NMR (500 MHz, CD_3OD) : δ 4.88(1H, d, H-1', *J* = 3.7 Hz), 4.24(1H, ddd, H-5', *J* = 2.5, 4.4, 10.2 Hz), 4.21(1H, d, H-1, *J* = 7.4 Hz), 3.97(H1, d, H-4, *J* = 3.0 Hz), 3.94(H1, dd, H-2', *J* = 3.7, 10.8 Hz), 3.79(1H, dd, H-6'a, *J* = 2.6, 11.8 Hz), 3.74-3.63(5H, m, H-6'b, H-6, H-3', H-6, H-5), 3.58(3H, s, -OC$\underline{H_3}$), 3.55(1H, dd, H-3, J = 3.0, 10.1 Hz), 3.50(1H, dd, H-2, *J* = 7.5, 10.1 Hz),3.43(1H, dd, H-4', *J* = 9.0, 10.1 Hz), 2.03(3H, s, -COC$\underline{H_3}$).

^{13}C NMR (126 MHz, CD_3OD) : δ 173.7(-$\underline{C}OCH_3$), 106.3(C-1), 100.3(C-1'), 77.6(C-4), 76.7(C-5), 74.4(C-3), 73.6(C-5'), 72.7(C-3'), 72.6(C-2), 72.0(C-4'), 62.3(C-6'), 60.8(C-6), 57.8(-OCH_3), 55.5(C-2'), 22.7(-CO$\underline{C}H_3$)

Synthesis of **6c**

A mixture of **1** (40 mg, 0.11 mmol, final conc.: 50 mM), *i*-propyl 1-thio β-galactoside (79 mg, 0.33 mmol, final conc.: 150 mM), and α-*N*-acetylglucosaminidase B1 (340 μg, 118 μl, PBS solution) in 100 mM sodium phosphate buffer (pH 6.5, final volume 2.2 ml) was incubated at 37 °C for 1 hour. The product was purified by HPLC (column; Amide-80 (21.5 mm I.D. × 250 mm, Tosoh), eluent; 80 vol.% CH_3CN, flow rate; 8 ml/min, column oven; 30 °C, detection; UV (214 nm)), concentrated *in vacuo*, and freeze-dried to give *i*-propyl 1-thio 4-*O*-(2-acetamido-2-deoxy-α-glucopyranosyl)-β-galactopyranoside (**6c**, 14 mg, 29%).

^{1}H NMR (500 MHz, CD_3OD) : δ 4.89(1H, d, H-1', *J* = 3.7 Hz), 4.51(1H, d, H-1, *J* = 9.6 Hz), 4.22(1H, ddd, H-5', *J* = 2.5, 4.4, 10.1 Hz), 4.01(1H, d, H-4, *J* = 3.0 Hz), 3.95(1H, dd, H-2', *J* = 3.7, 10.8 Hz), 3.79(1H, dd, H-6'a, *J* = 2.5, 11.8 Hz), 3.73(1H, dd, H-6'b, *J* = 4.5, 11.8 Hz), 3.70-3.59(4H, m, H-3', H-5, H-6a, H-6b), 3.56(1H, dd, H-3, *J* = 3.0, 9.6 Hz), 3.48(1H, t, H-2, *J* = 9.6 Hz), 3.43(1H, dd, H-4', *J* = 9.0, 10.1 Hz), 3.25(1H, m, isopropyl, *J* = 6.8 Hz), , 2.03(3H, s, -COC$\underline{H_3}$). 1.34(3H, d, isopropyl, *J* = 4.2 Hz), 1.33(3H, d, isopropyl, *J* = 4.4 Hz).

^{13}C NMR (126 MHz, CD_3OD) : δ 173.7(-$\underline{C}OCH_3$), 100.53(C-1'), 87.3(C-1), 80.5(C-5), 78.3(C-4), 75.9(C-3), 73.6(C-5'), 72.7(C-3'), 72.0(C-4'), 71.7(C-2), 62.3(C-6'), 60.8(C-6), 55.5(C-2'), 24.4, 24.1(isopropyl), 22.7(-CO$\underline{C}H_3$).

Synthesis of **6d**

A mixture of **1** (32.4 mg, 90 μmol, final conc.: 75 mM), 2-pyridyl 1-thio-2-acetamido-2-deoxy-4-*O*-β-galactopyranosyl-β-glucopyranoside (14.3 mg, 30 μmol, final conc.: 25 mM), and α-*N*-acetylglucosaminidase B1 (120 μg, 43 μl, PBS solution) in 100 mM sodium phosphate buffer (pH 6.5, final volume 1.2 ml) was incubated at 37 °C for 3 hours. The product was purified by HPLC (column; Amide-80 (21.5 mm I.D. × 250 mm, Tosoh), eluent; 75 vol.% CH_3CN, flow rate; 8 ml/min, column oven; 30 °C, detection; UV (240 nm)), concentrated *in vacuo*, and freeze-dried to give 2-pyridyl 1-thio-2-acetamido-2-deoxy-4-*O*-(4-*O*-(2-acetamido-2-deoxy-α-glucopyranosyl)-β-galactopyranosyl)-β-glucopyranoside (**6d**, 8.2 mg, 39%).

^{1}H NMR (400 MHz, CD_3OD): δ 8.38, 7.68, 7,40, and 7.16(4H, Pyridyl), 5.45(1H, d, H-1, *J* = 10.7 Hz), 4.90(1H, d, H-1”, *J* = 3.7 Hz), 4.48(1H, d, H-1’, *J* = 7.4 Hz), 4.20(1H, ddd, H-5” , *J* = 2.4, 4.6, 7.8 Hz), 3.99(1H, dd, H-2, *J* = 9.6, 10.5 Hz), 3.95(1H, d, H-4’, *J* = 2.2 Hz), 3.93-3.89(3H, m, H-6a, H-6b, H-2”), 3.81(1H, dd, H-6”a, *J* = 2.5, 11.9 Hz), 3.78-3.54(10H, m, H-3, H-4, H-5, H-2’, H-3’, H-5’, H-6’a, H-6’b, H-3”, H-6”b), 3.42(1H, dd, H-4”, *J* = 8.9, 10.1 Hz), 2.01(3H, s, -COC*H*$_3$), 1.95(3H, s, -COC*H*$_3$).

^{13}C NMR (100 MHz, CD_3OD): δ 173.7 and 173.5(-*C*OCH_3), 158.9, 150.3, 138.5, 124.4, and 122.0(Pyridyl), 105.4(C-1’), 100.1(C-1”), 84.8(C-1), 80.9(C-5), 80.6(C-4), 78.1(C-4’), 77.2(C-5’), 75.7(C-3), 74.5(C-3’), 73.8(C-5”), 72.5(C-2’, C-3”), 72.1(C-4”), 62.4(C-6”), 61.8(C-6), 61.3(C-6’), 55.6(C-2”), 55.5(C-2), 22.9 and 22.7(-CO*C*H_3).

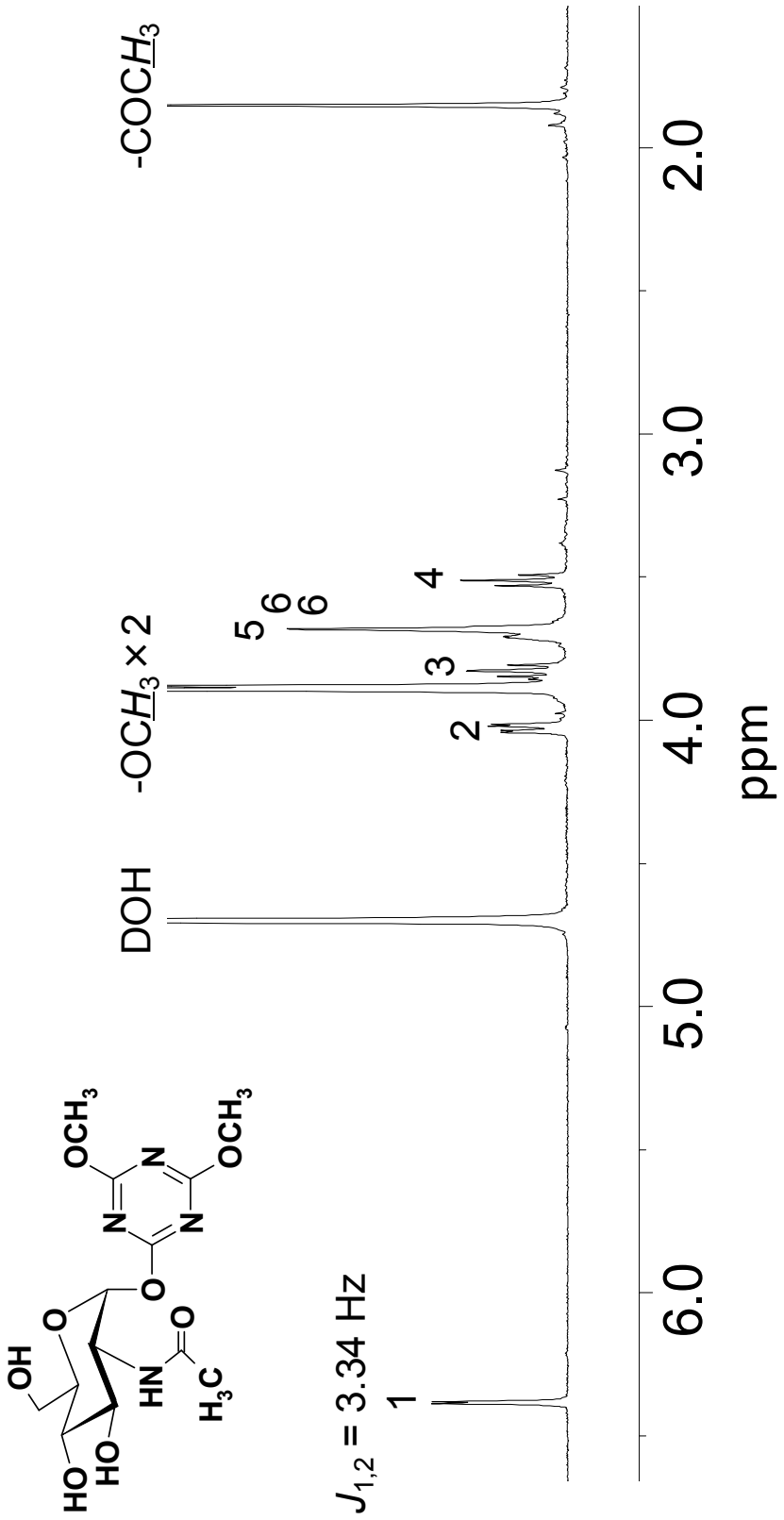
Synthesis of **6e**

A mixture of **1** (28.8 mg, 80 μmol, final conc.: 50 mM), 4,6-dimethoxy-1,3,5-triazine-2-yl 2-acetamido-2-deoxy-3-*O*-β-galactopyranosyl-α-D-galactopyranoside (20.9 mg, 40 μmol, final conc.: 25 mM), and α-*N*-acetylglucosaminidase B1 (240 μg, 88 μl, PBS solution) in 100 mM sodium phosphate buffer (pH 6.5, final volume 1.6 ml) was incubated at 37 °C for 4 hours. The product was purified by HPLC (column; Amide-80 (21.5 mm I.D. × 250 mm, Tosoh), eluent; 75 vol.% CH_3CN, flow rate; 8 ml/min, column oven; 30 °C, detection; UV (214 nm)), concentrated *in vacuo*, and freeze-dried to give 4,6-dimethoxy-1,3,5-triazine-2-yl 2-acetamido-2-deoxy-3-*O*-(4-*O*-(2-acetamido-2-deoxy-α-glucopyranosyl)-β-galactopyranosyl)-α-galactopyranoside (**6e**, 12.7 mg, 44%).

^{1}H NMR (400 MHz, CD_3OD): δ 6.63(1H, d, H-1, *J* = 3.72 Hz), 4.93(1H, d, H-1”, *J* = 3.7 Hz), 4.66(1H, dd, H-2, *J* = 3.7, 11.3 Hz), 4.56(1H, d, H-1’, *J* = 7.1 Hz), 4.27(1H, d, H-4, *J* = 2.1 Hz), 4.20-4.16(2H, m, H-3, H-5”), 4.02-4.00(7H, m, -OC*H*$_3$, H-5), 3.97(1H, d, H-4’, *J* = 2.8 Hz), 3.94(1H, dd, H-2”, *J* = 3.7, 10.9 Hz), 3.81(1H, d, H-6”a, *J* = 2.3 11.8 Hz), 3.76-3.54(9H, m, H-6a, H-6b, H-2’,

H-3', H-5', H-6'a, H-6'b, H-3", H-6"b), 3.42(1H, dd, H-4", J = 8.9, 10.0 Hz), 2.02(3H, s, -COC$\underline{H}_3$), 1.92(3H, s, -COC$\underline{H}_3$).

^{13}C NMR (101 MHz, CD_3OD): δ 175.1, 174.3, 173.8, and 173.7(triazine, -$\underline{C}OCH_3$), 106.1(C-1'), 100.1(C-1"), 96.4(C-1), 78.4(C-3), 78.1(C-4'), 76.8(C-5'), 74.8(C-5), 74.5(C-3'), 73.8(C-5"), 72.6(C-3"), 72.5(C-2'), 72.1(C-4"), 69.5(C-4), 62.5(C-6), 62.4(C-6"), 61.3(C-6'), 56.1(-O$\underline{C}H_3$), 55.6(C-2"), 49.6-48.4(C-2 in peak of CD_3OD), 22.7 and 22.6(-CO$\underline{C}H_3$).



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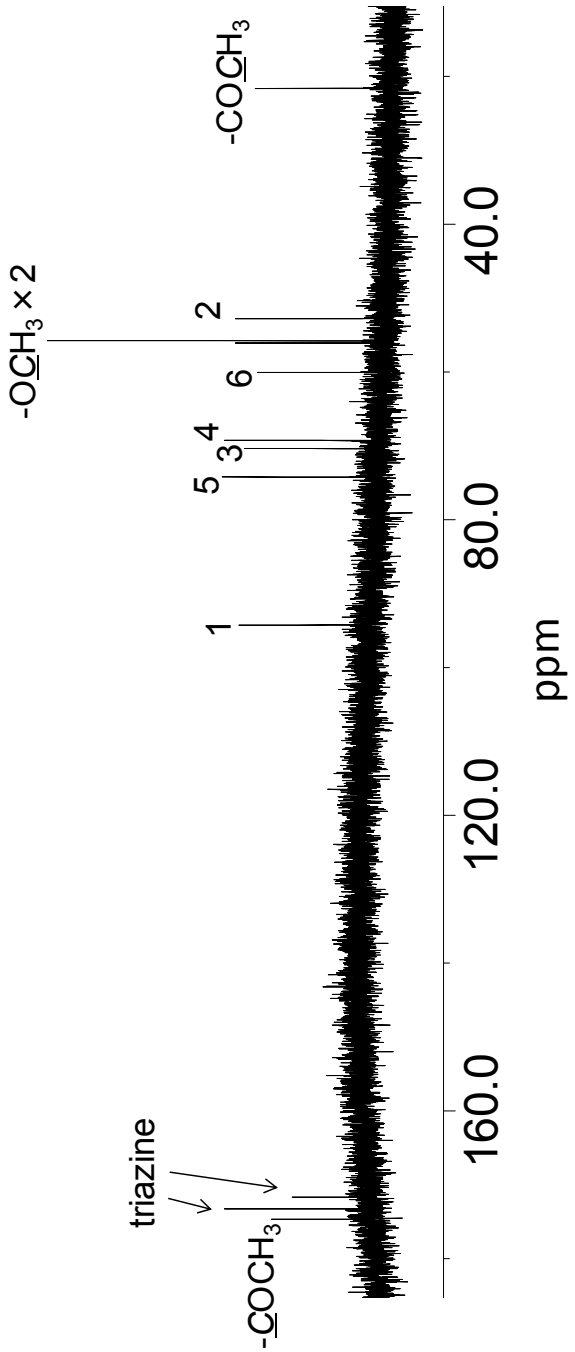

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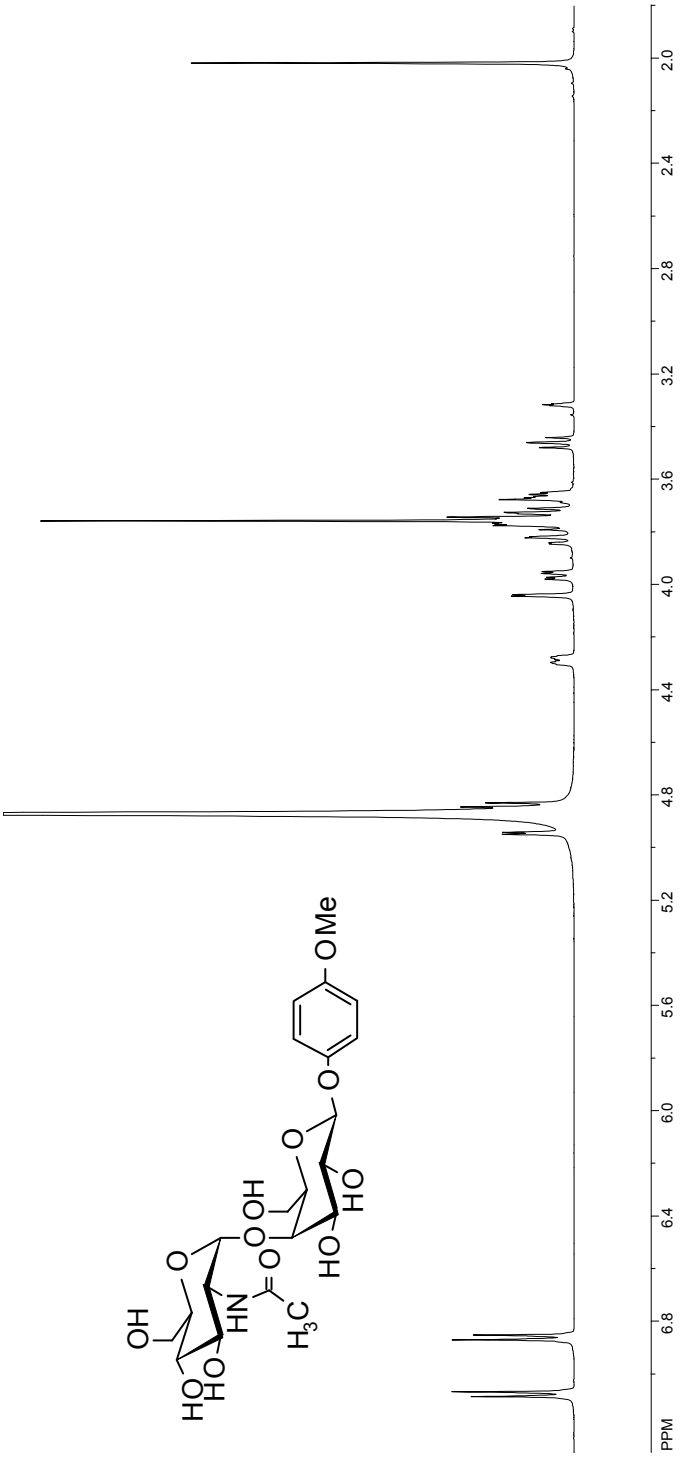

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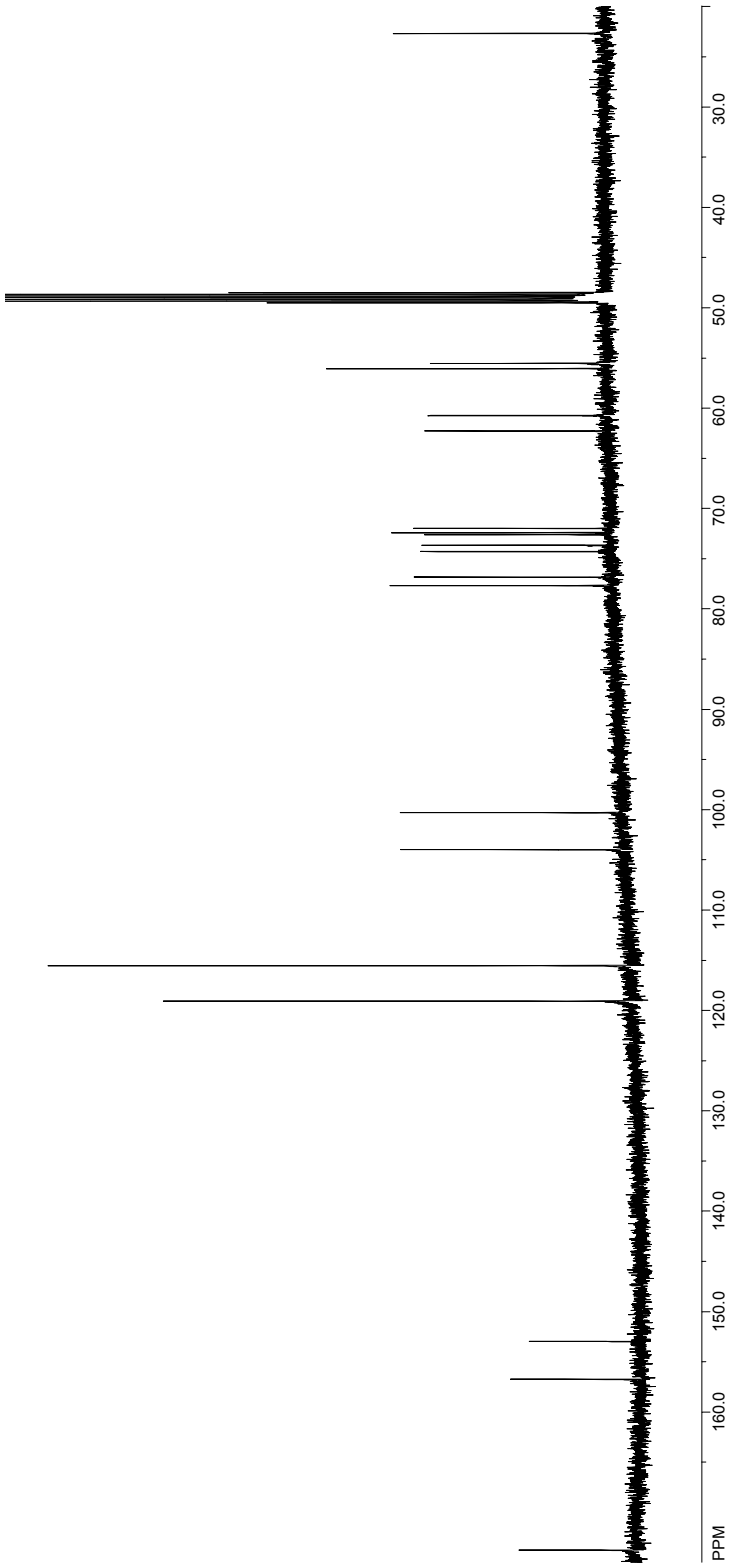

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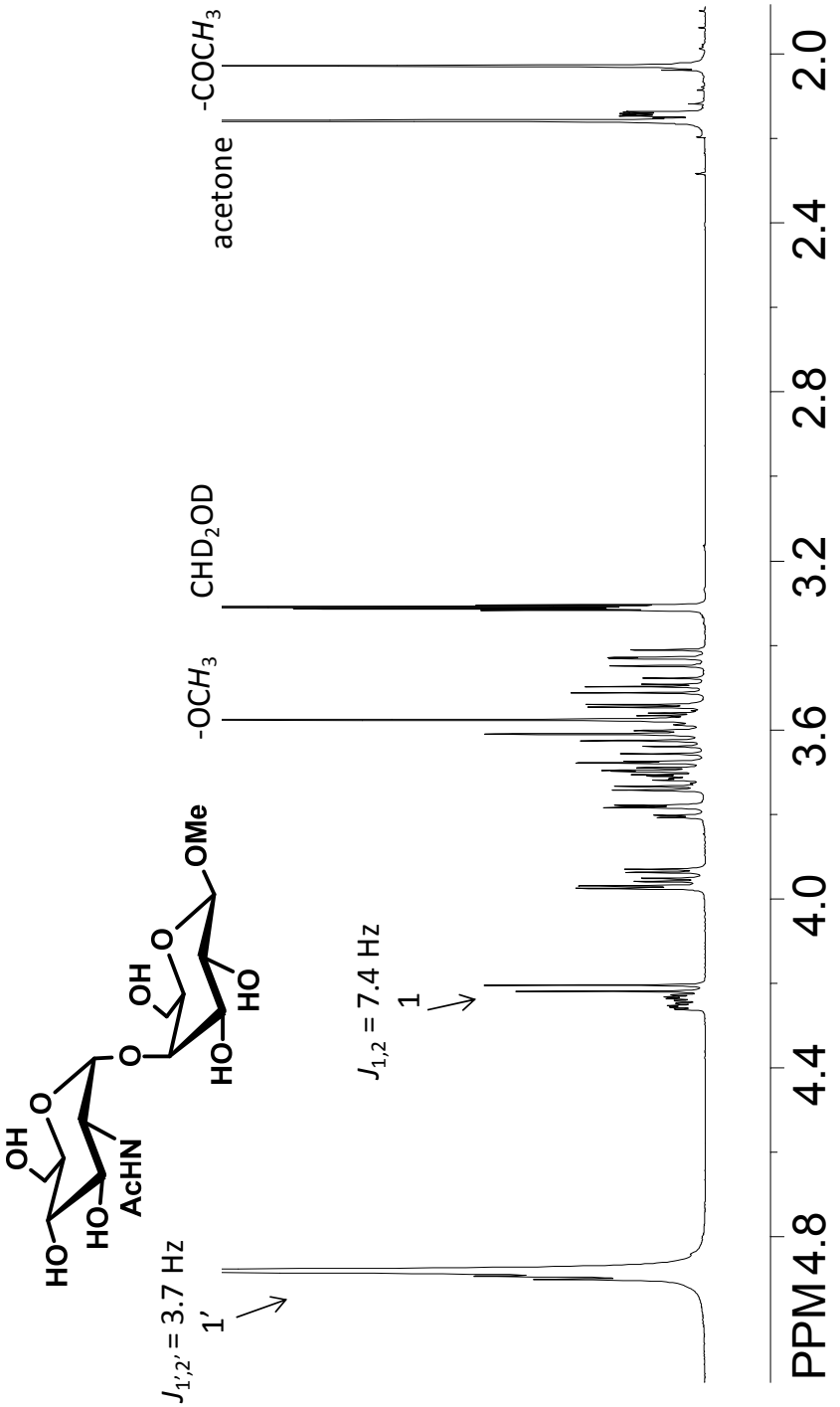

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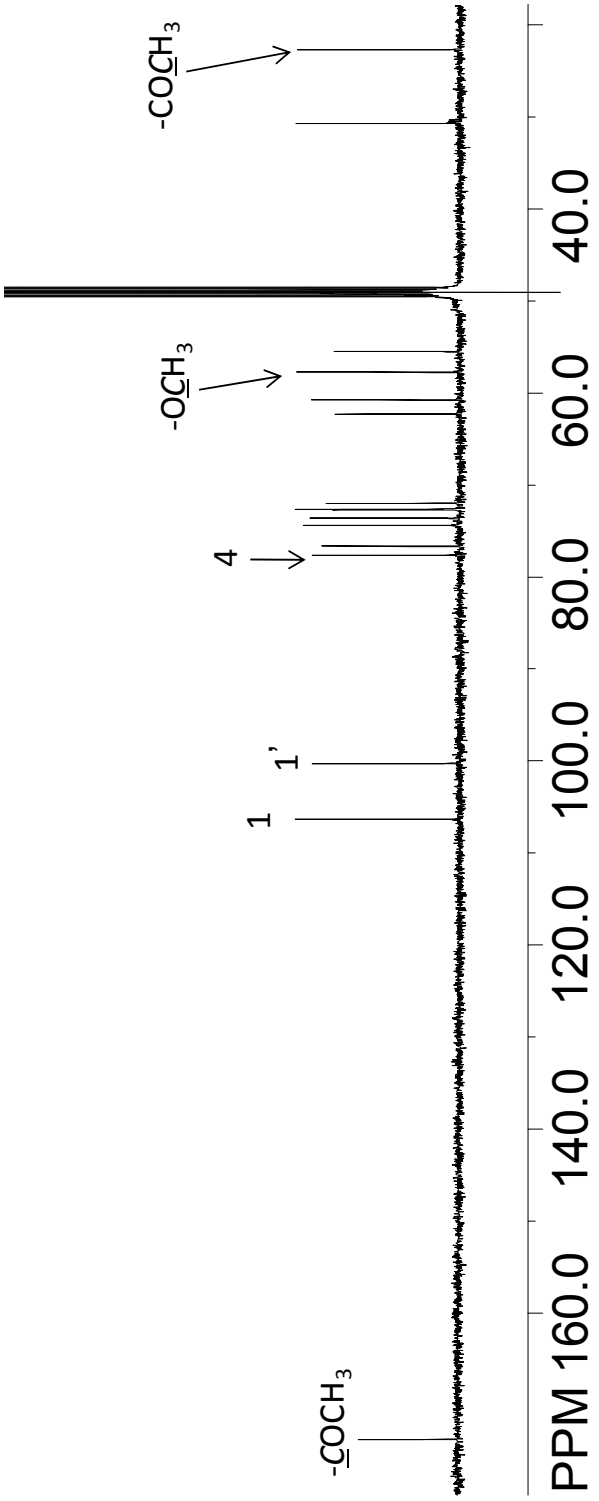

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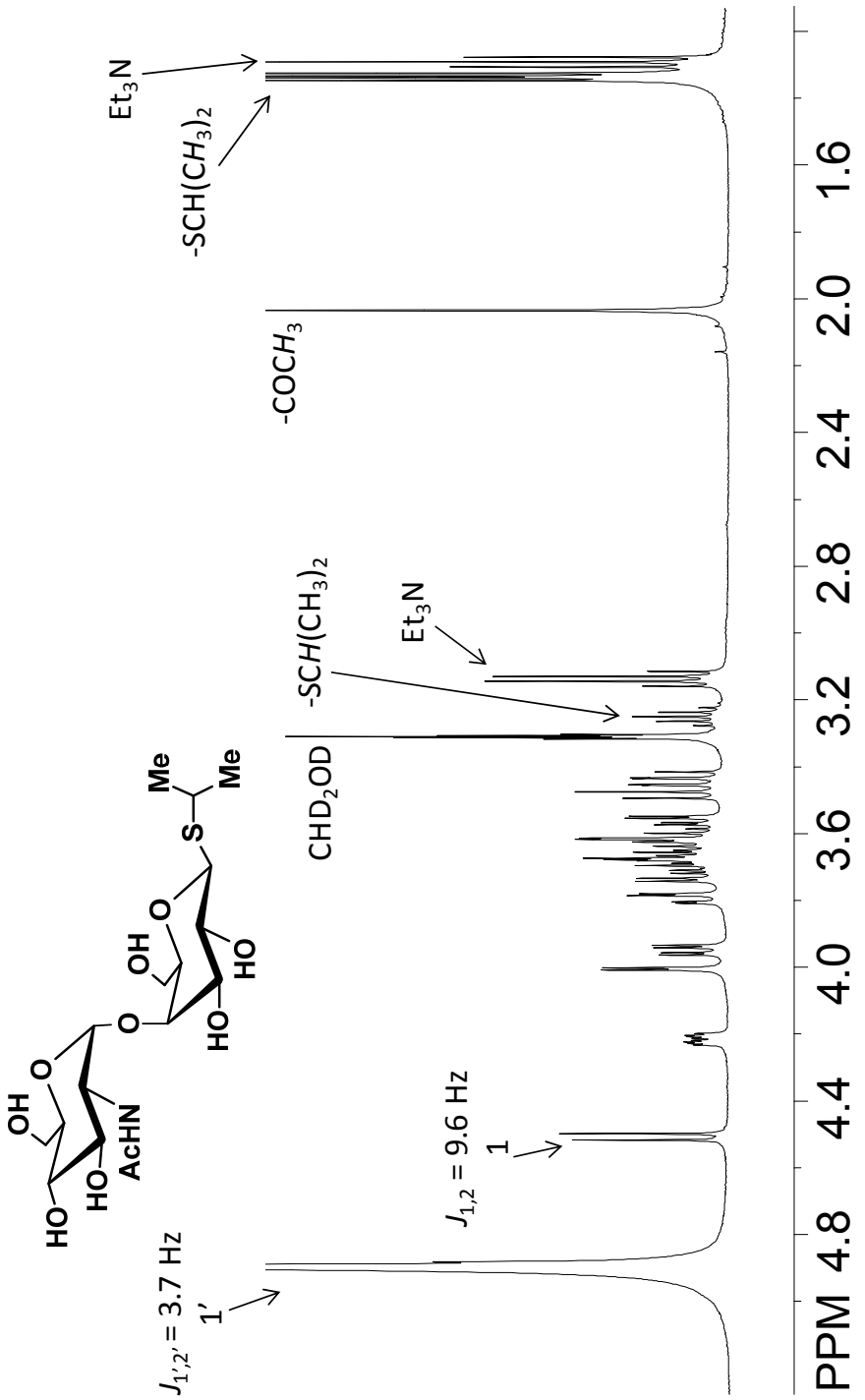

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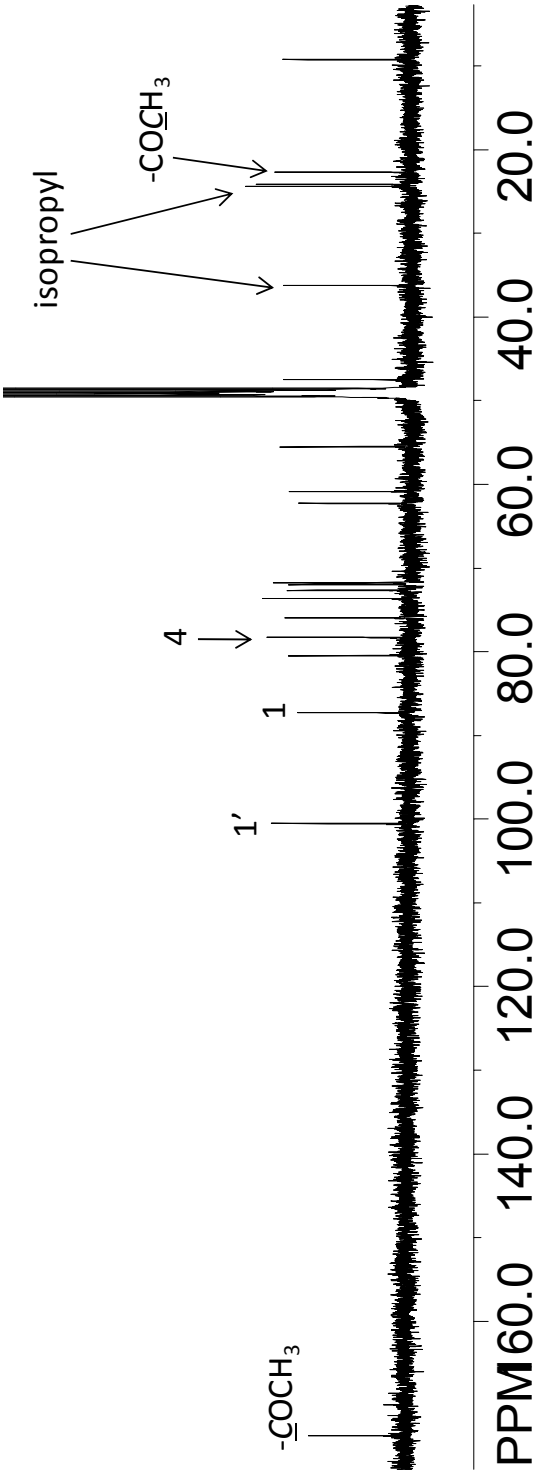

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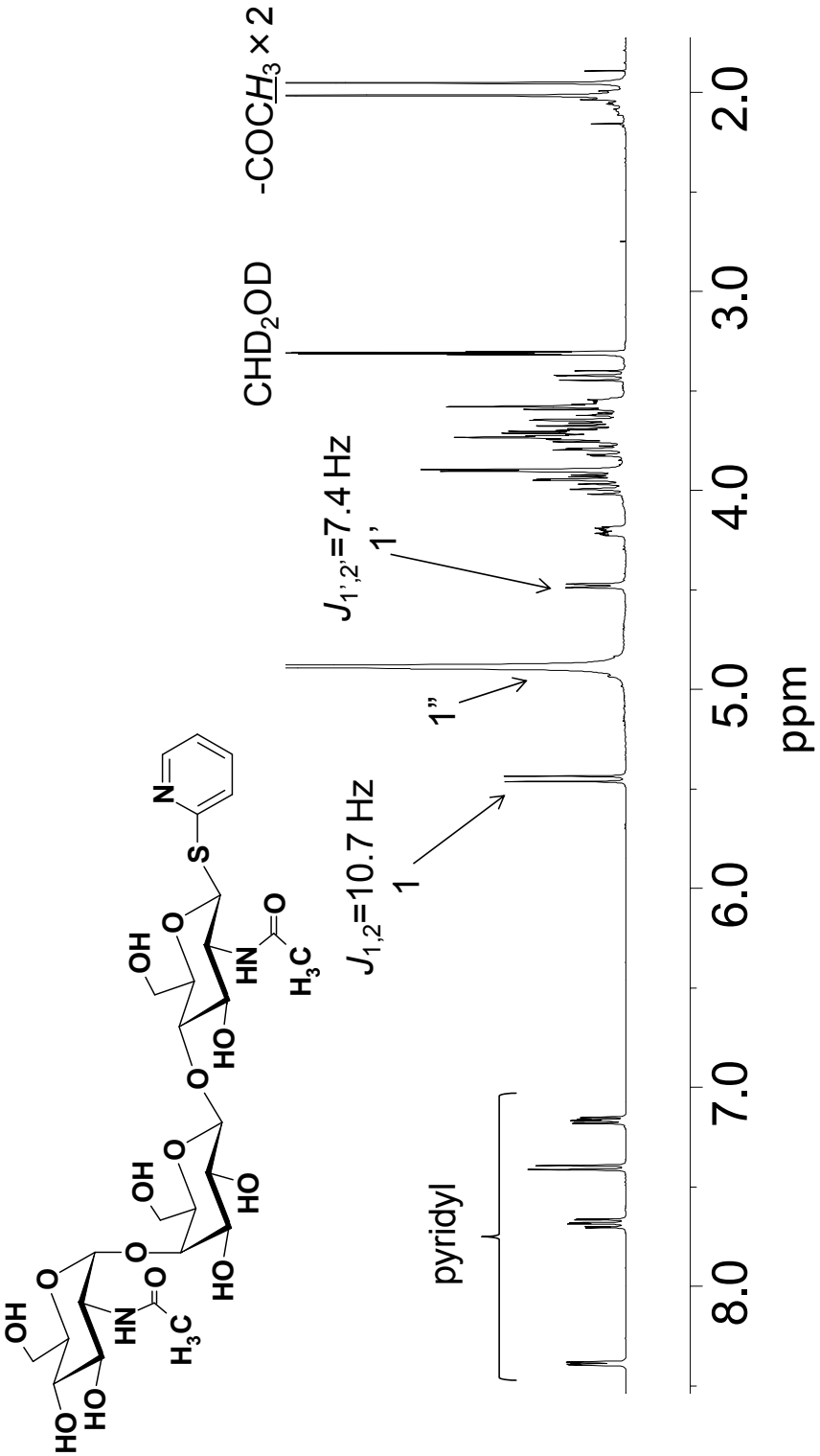

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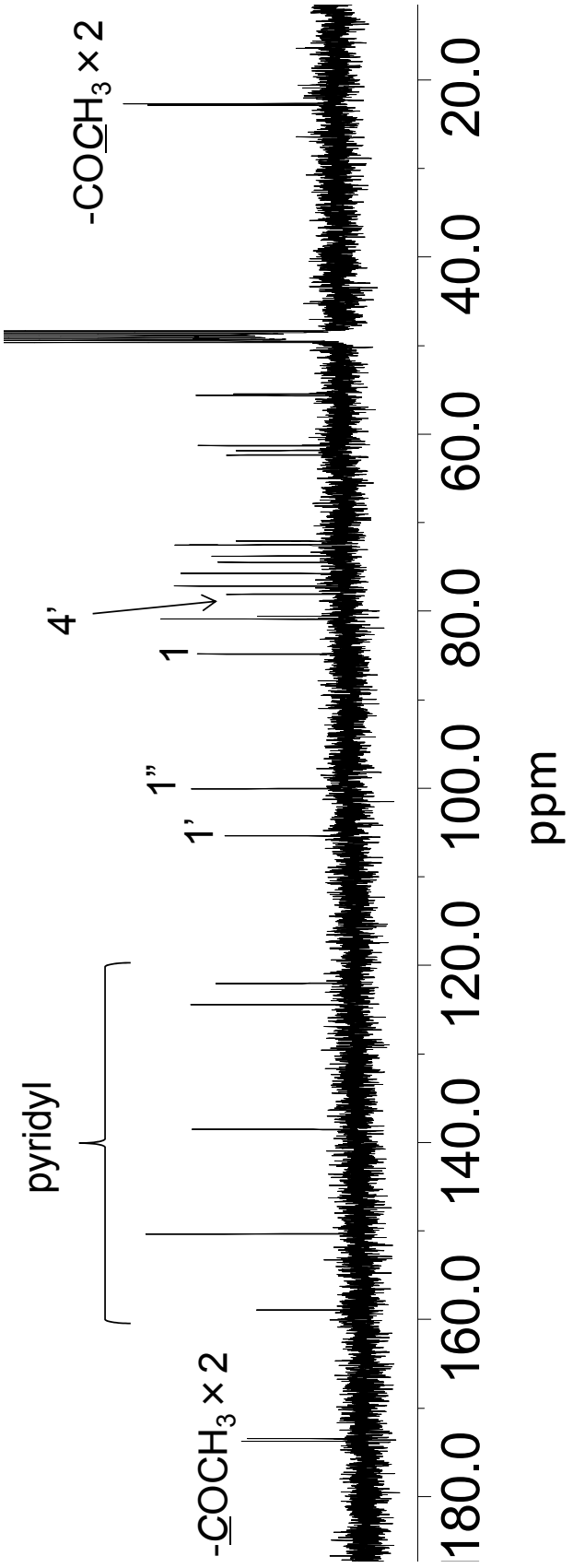

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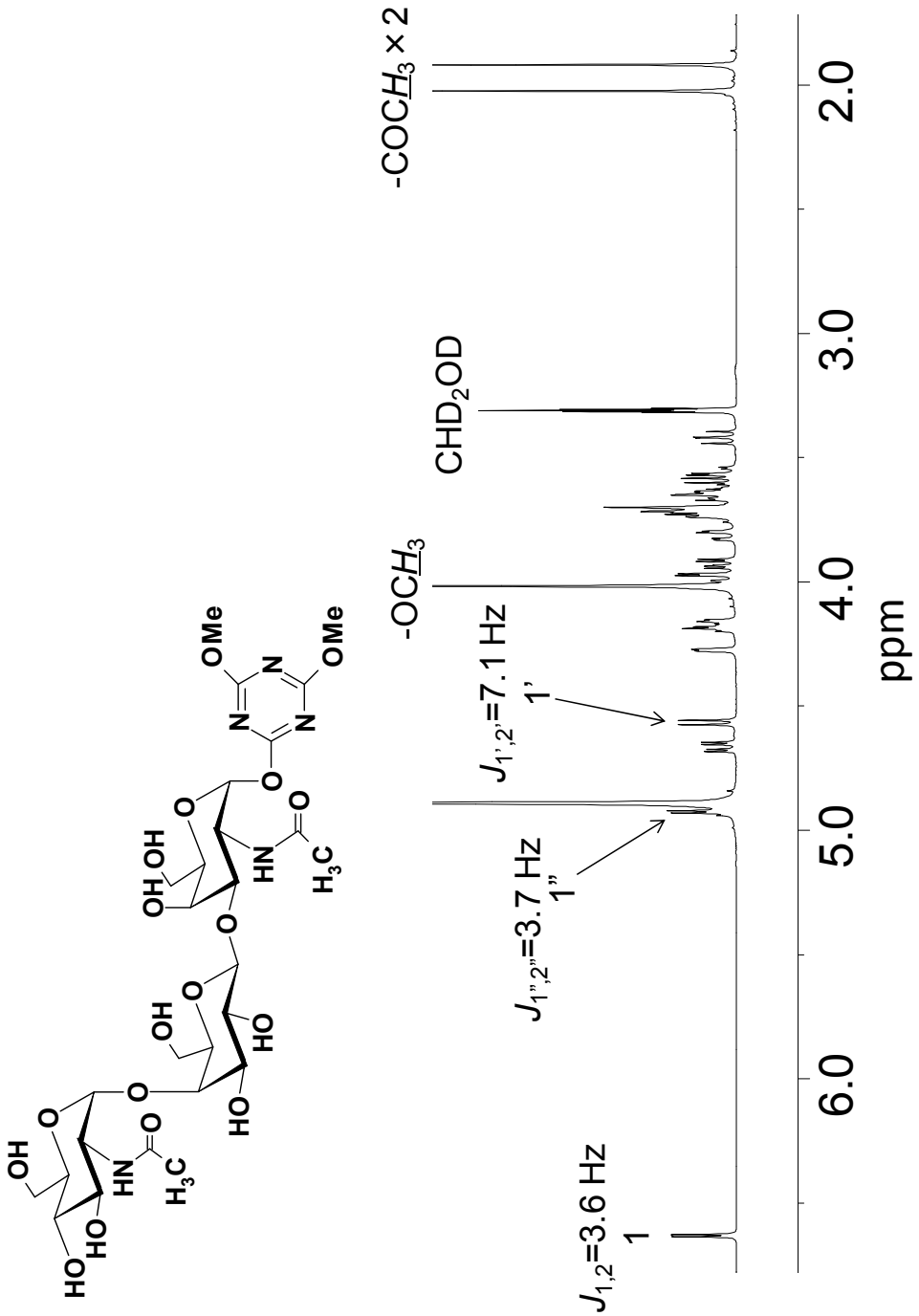

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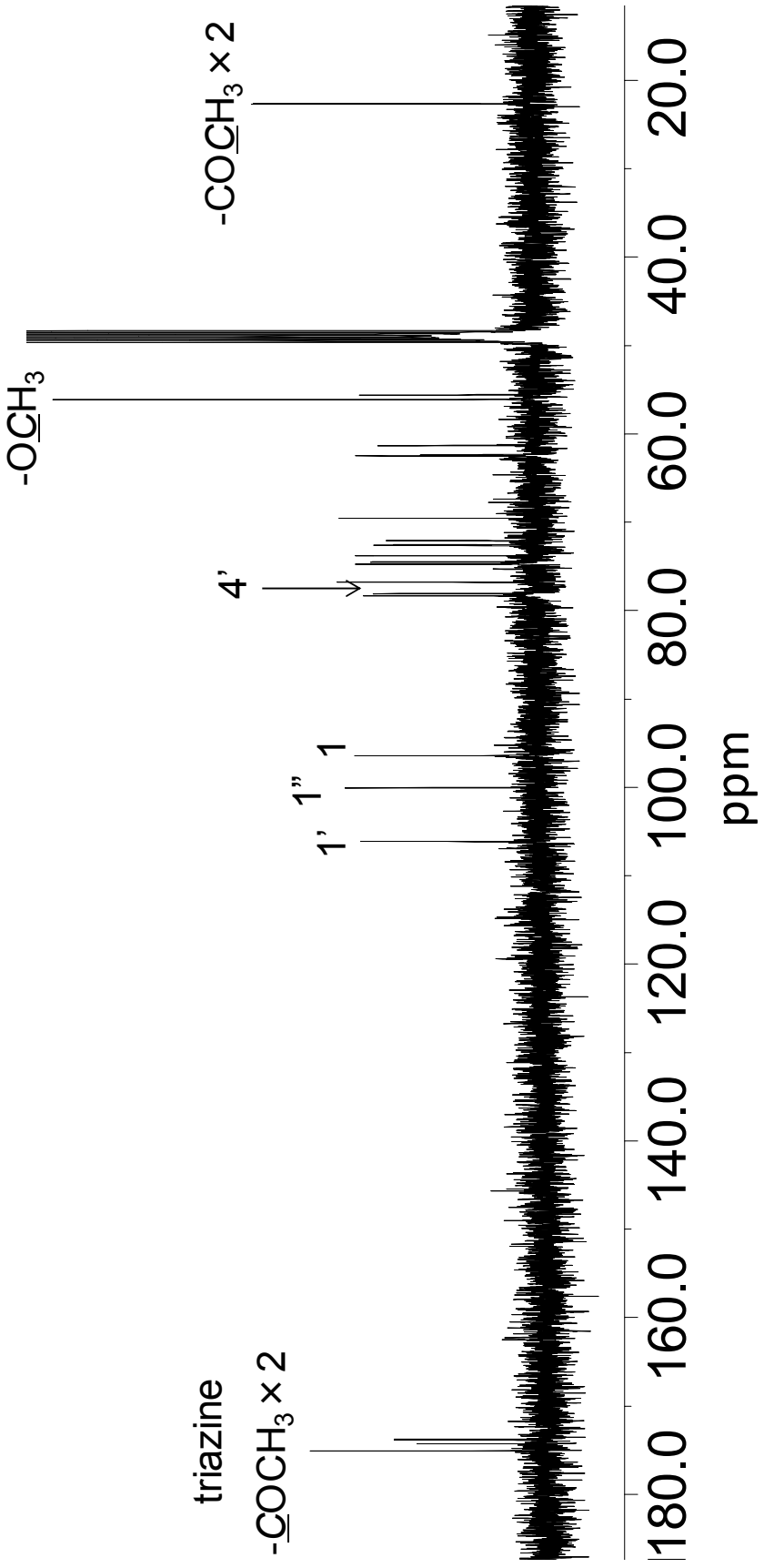

^{13}C NMR spectrum of **6e**