Supplementary material

Enzymatic synthesis of apigenin glucosides by glucosyltransferase (YjiC) from *Bacillus licheniformis* DSM 13

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Running title: Enzymatic Synthesis of Apigenin glucosides

Supplementary Figure Legends

Figure S1. SDS-PAGE analysis of the crude soluble protein prepared from *E.coli* harboring pET302-YjiC (S) and affinity purified fractions (Y1-Y4) of YjiC, 44.7 kDa. M, marker.

Figure S2. HRLC-ESI-Q-TOF-MS/MS analysis of *in-vitro* glucosylation reaction mixture. (**a**) MS spectra of APG1, a monoglucoside of apigenin (*m/z* 433.1112 $[M+H]^+$) and substrate, apigenin (*m/z* 271.0538 $[M+H]^+$); (**b**) MS spectra of APG2, a monoglucoside of apigenin (*m/z* 433.1092 $[M+H]^+$) and substrate, apigenin (*m/z* 271.0518 $[M+H]^+$); (**c**) MS spectra of APG3, a diglucoside of apigenin (*m/z* 595.1677 $[M+H]^+$) and substrate, apigenin (*m/z* 271.0520 $[M+H]^+$).

Figure S3. a) ^{1}H NMR analysis of aglycone, APG (apigenin). **b)** ^{13}C NMR analysis of aglycone, APG (apigenin).

Figure S4. a) ^{1}H NMR analysis of APG1 (apigenin 4'-*O*-glucoside). **b)** ^{13}C NMR analysis of APG1 (apigenin 4'-*O*-glucoside).

Figure S5. a) ^{1}H NMR analysis of APG2 (apigenin 7-*O*-glucoside). **b)** ^{13}C NMR analysis APG2 (apigenin 7-*O*-glucoside).

Figure S6. a) ^{1}H NMR analysis of APG3 (apigenin 4',7-*O*-diglucoside). **b)** ^{13}C NMR analysis of APG3 (apigenin 4',7-*O*-diglucoside).

Figure S1.

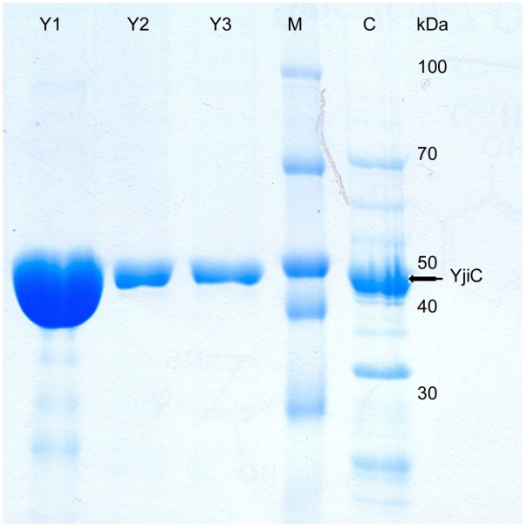
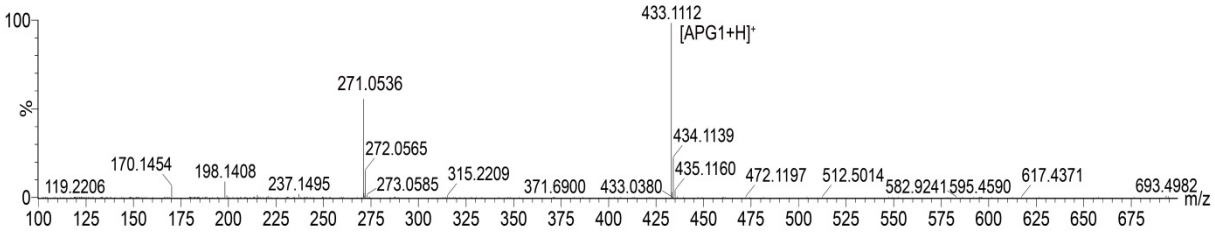
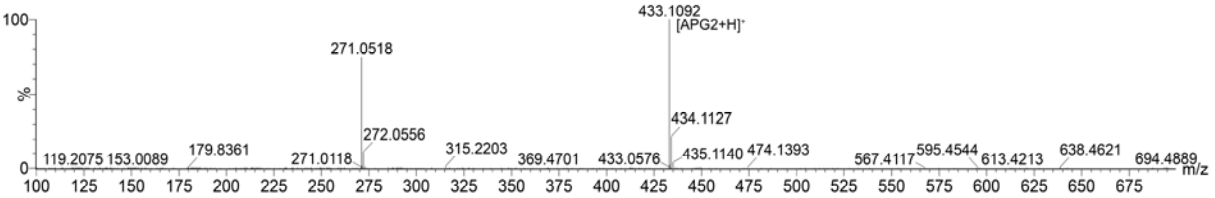


Figure S2.

a)



b)



c)

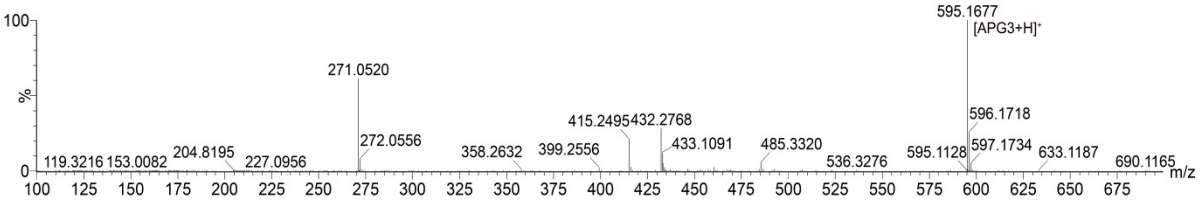
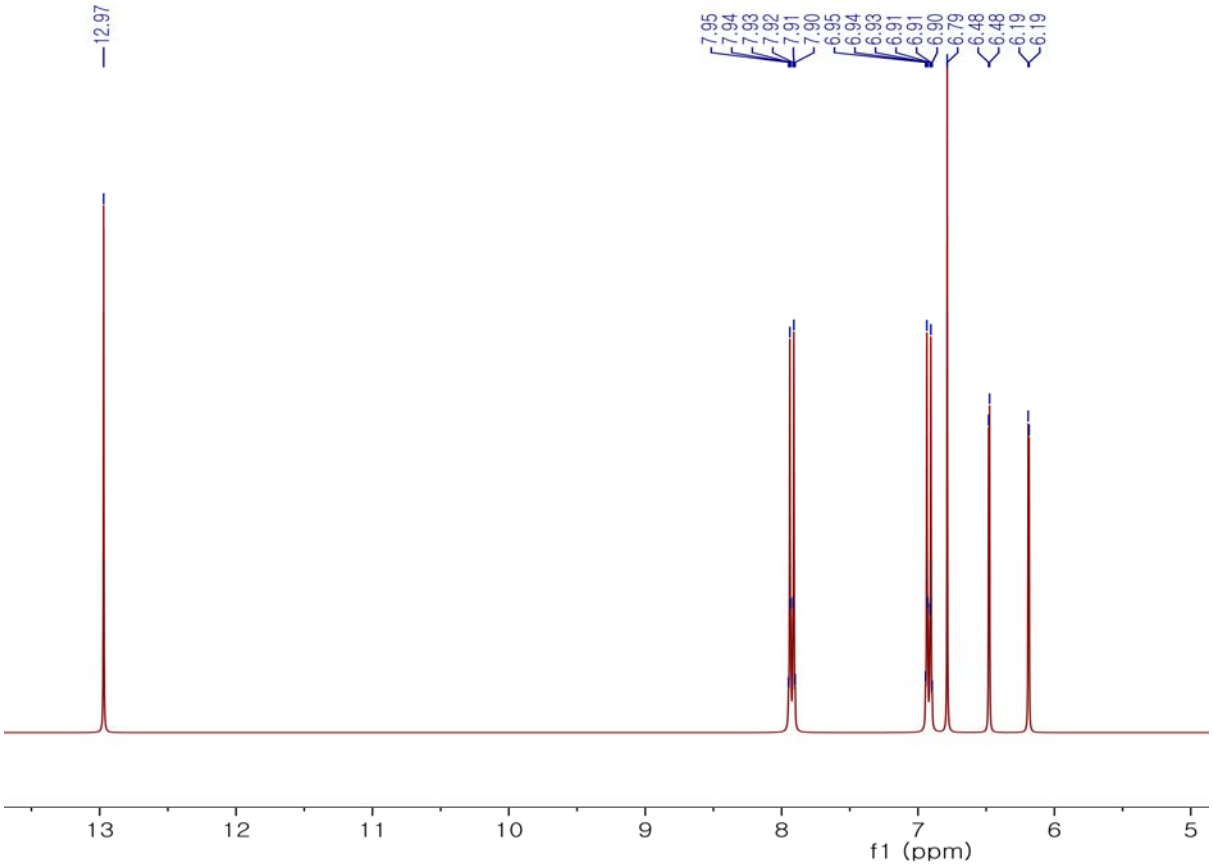


Figure S3.

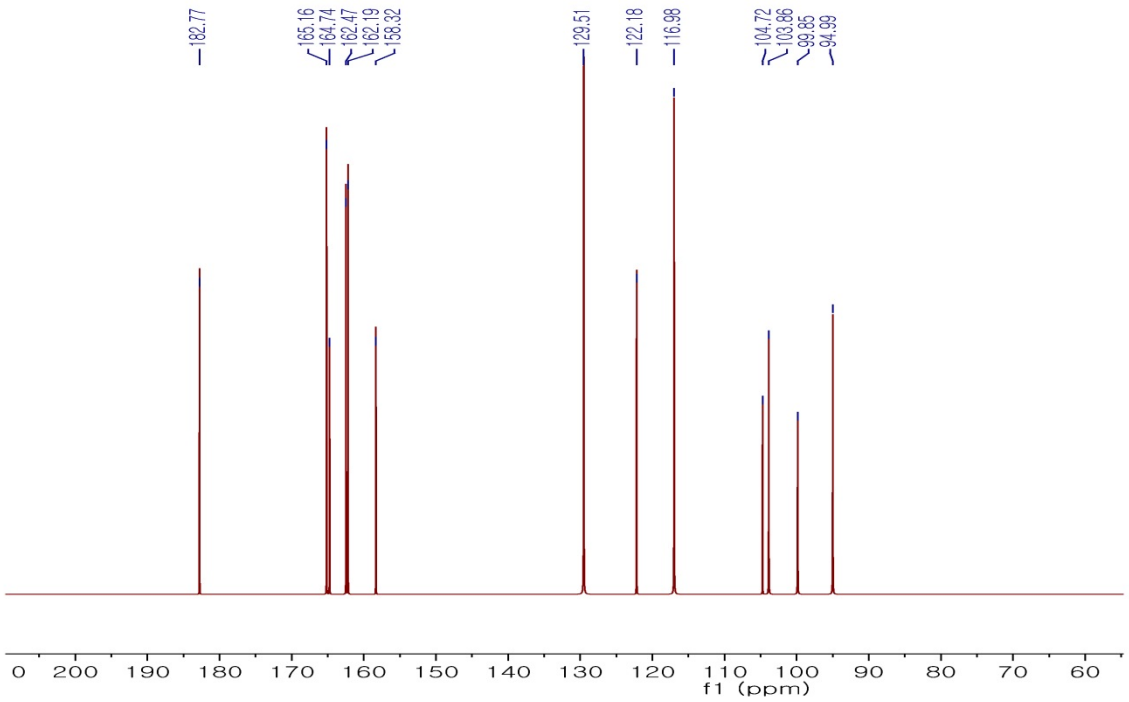
a)



^{1}H NMR data (in DMSO-d_6, 300 MHz) δ (ppm): 12.97 (1H, s, 5-OH), 7.92 (2H, d, J = 8.8 Hz, H-2', 6'), 6.92 (2H, d, J = 8.8 Hz, H-3', 5'), 6.79 (1H, s, H-3), 6.48 (1H, d, J = 2.1 Hz, H-8), 6.19 (1H, d, J = 2.1 Hz, H-6).

Figure S3.

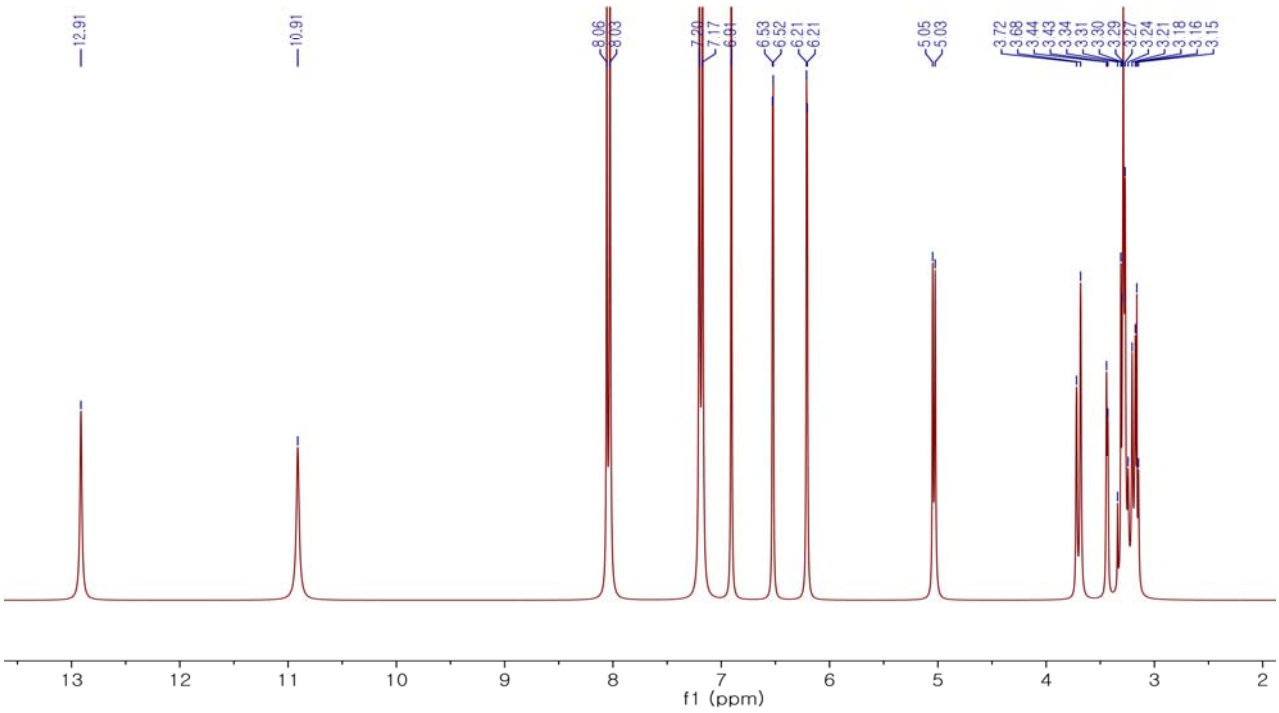
b)



^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 182.77 (C-4), 165.16 (C-2), 164.74 (C-7), 162.47 (C-9), 162.19 (C-4'), 158.32 (C-5), 129.51 (C-2', C-6'), 122.18 (C-1'), 116.98 (C-3', C-5'), 103.86 (C-10), 99.85 (C-6), 94.99 (C-8).

Figure S4.

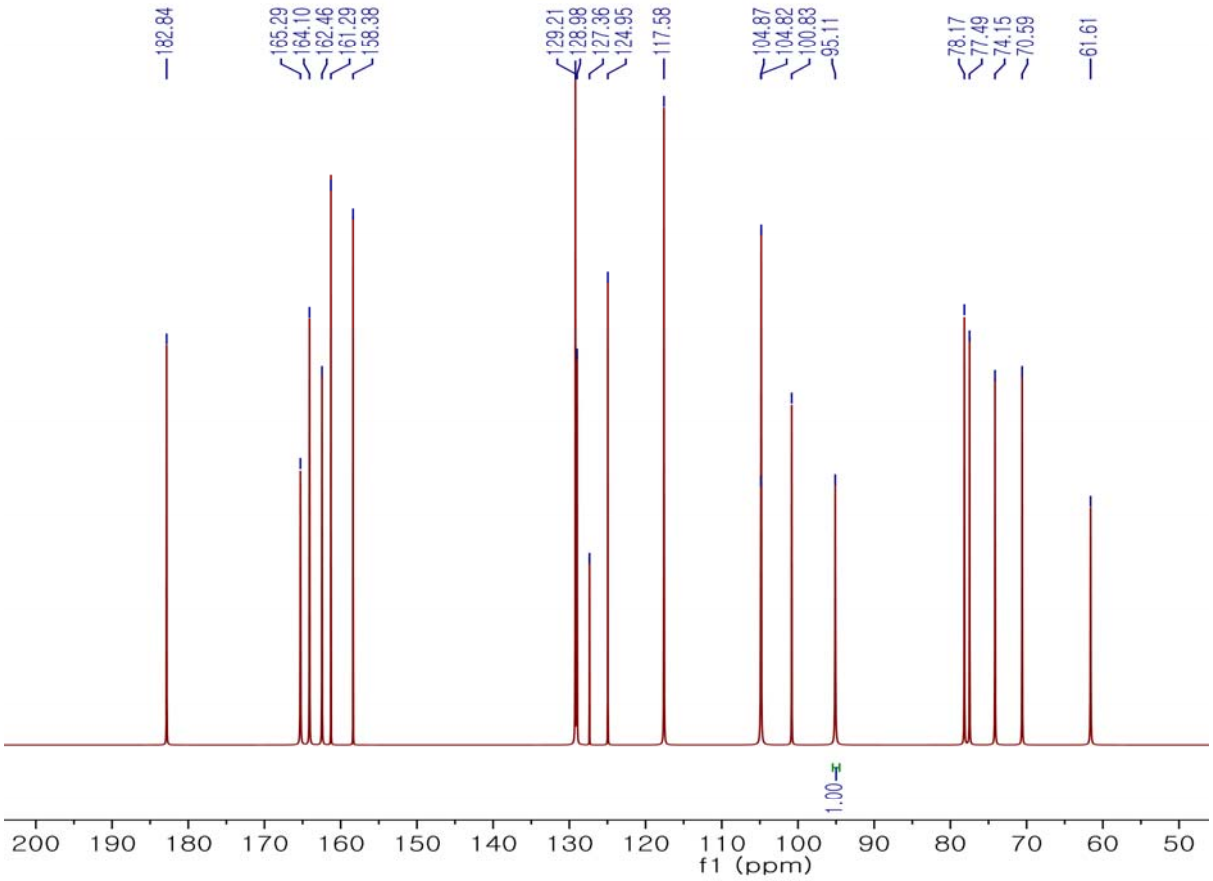
a)



^{1}H NMR data (in DMSO-d_6, 300 MHz) δ (ppm): 12.91 (1H, s, 5-OH), 10.91 (1H, s, 7-OH), 8.04 (2H, d, J = 8.8 Hz, H-2', 6'), 7.19 (2H, d, J = 8.8 Hz, H-3', 5'), 6.91 (1H, s, H-3), 6.52 (1H, d, J = 2.1 Hz, H-8), 6.21 (1H, d, J = 2.1, H-6), 5.04 (1H, d, J = 6.8 Hz, H-1"), 3.15 – 3.72 (5H, sugar protons).

Figure S4.

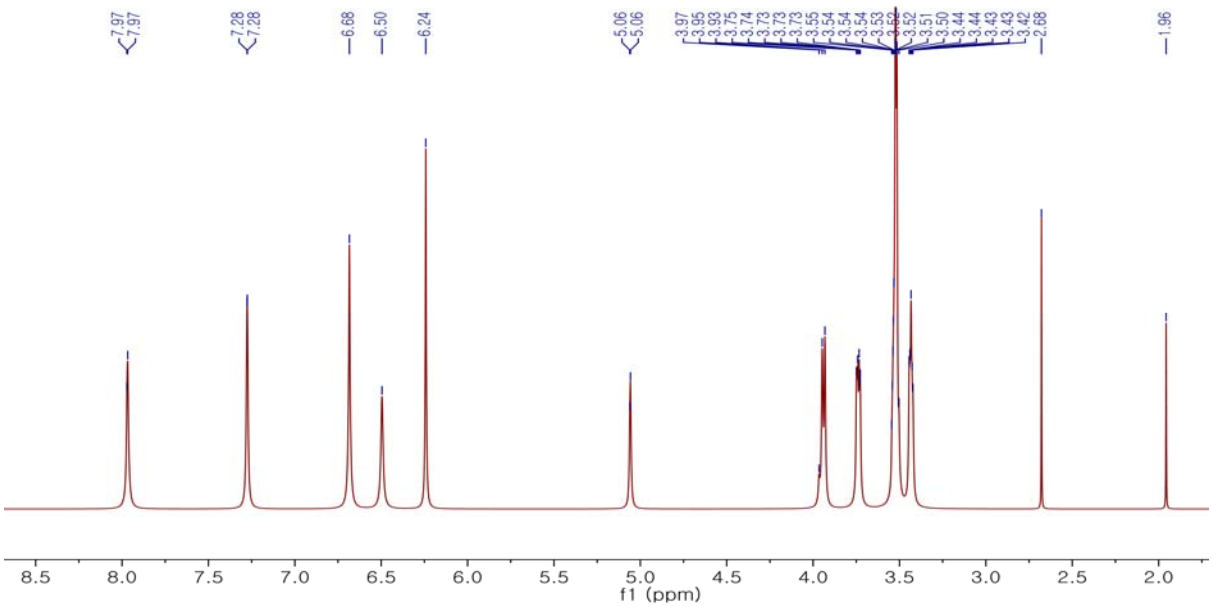
b)



^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 182.84 (C-4), 165.29 (C-2), 164.10 (C-5), 162.46 (C-4′), 161.29 (C-9), 129.21 (C-2’, C-6’), 124.95 (C-1’), 117.58 (C-3’, C-5’), 104.87 (C-10), 104.82 (C-3), 100.83 (C-1”), 95.11 (C-8), 78.17 (C-3”), 77.49 (C-5”), 74.15 (C-2”), 70.59 (C-4”), 61.61 (C-6”).

Figure S5.

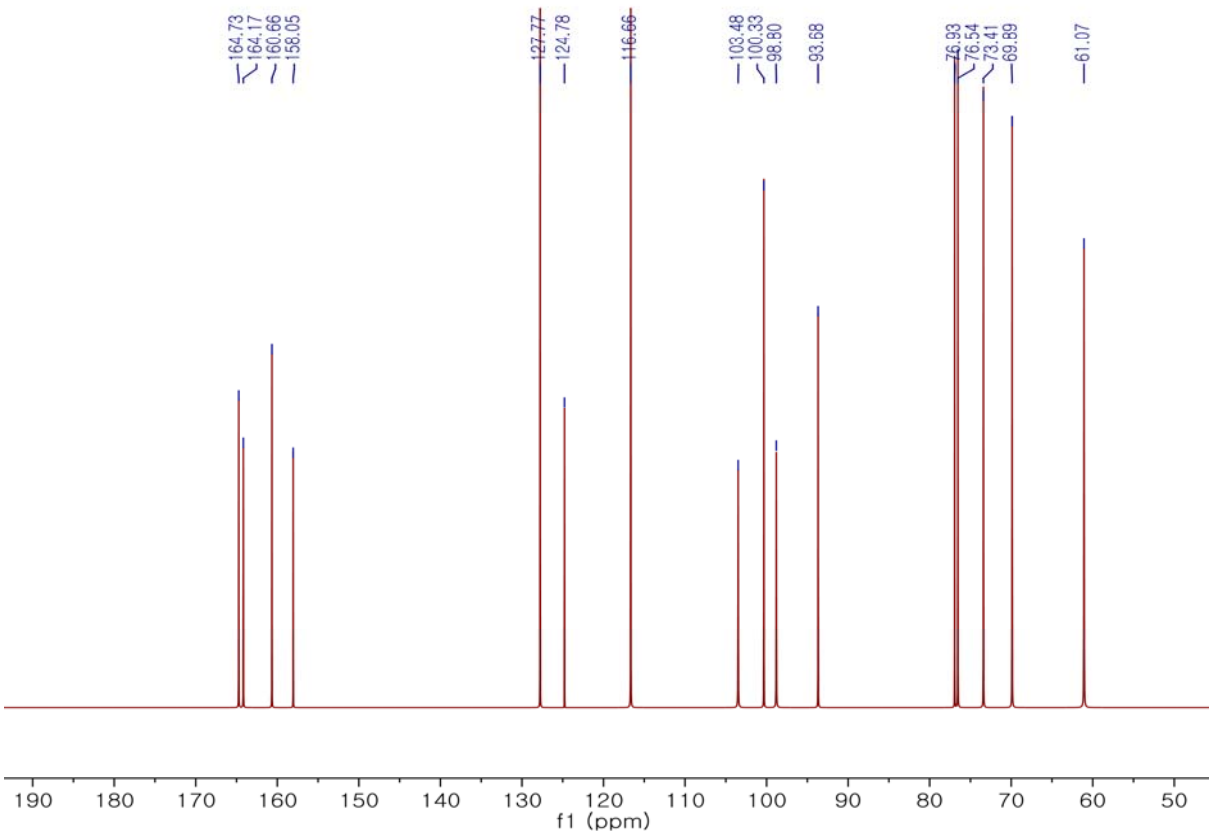
a)



^{1}H NMR data (in MeOD, 800 MHz) δ (ppm): 7.97 (2H, d, J = 8.7 Hz, H-2', 6'), 7.28 (2H, d, J = 8.7 Hz, H-3', 5'), 6.68 (1H, s, H-3), 6.50 (1H, s, H-8), 6.24 (1H, s, H-6), 5.04 (1H, d, J = 6.9 Hz, H-1"), 3.42 – 3.97 (5H, sugar protons).

Figure S5.

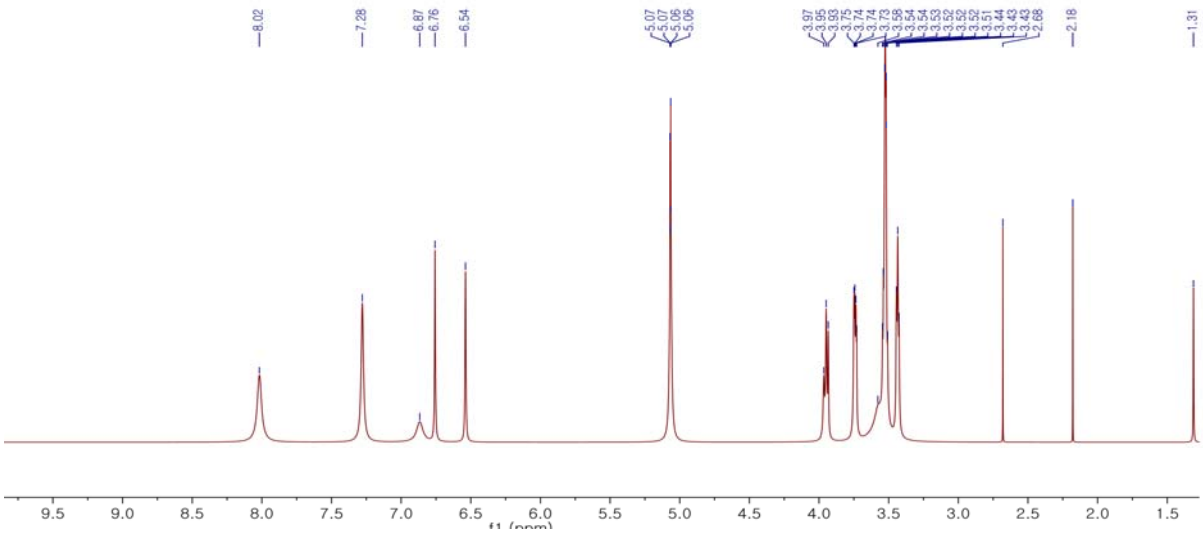
b)



^{13}C NMR (201 MHz, MeOD) δ (ppm): 182.84 (C-4), 164.73 (C-2), 164.17 (C-5), 160.66 (C-4′), 158.05 (C-9), 127.77 (C-2', C-6'), 124.78 (C-1'), 116.66 (C-3', C-5'), 103.48 (C-10), 100.33 (C-3), 98.80 (C-1"), 93.68 (C-8), 76.93 (C-3"), 76.54 (C-5"), 73.41 (C-2"), 69.89 (C-4"), 61.07 (C-6").

Figure S6.

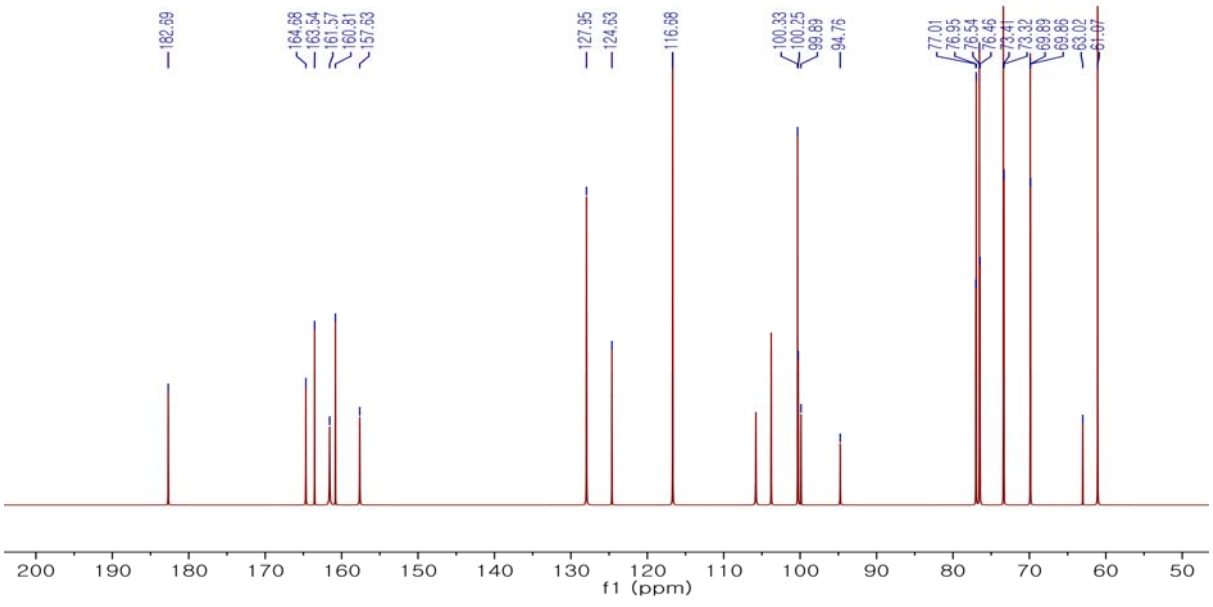
a)



^{1}H NMR data (in MeOD, 900 MHz) δ (ppm): 8.02 (2H, s, H-2′, 6′), 7.28 (2H, s, H-3′, 5′), 6.87 (1H, s, H-3), 6.76 (1H, s, H-8), 6.54 (1H, s, H-6), 5.07 (1H, d, H-1”), 5.06 (1H, d, H-1”’), 3.37- 3.97 (10H, sugar protons)

Figure S6.

b)



^{13}C NMR (226 MHz, MeOD) δ (ppm): 182.69 (C-4), 164.68 (C-5), 163.54 (C-2), 161.57 (C-4'), 160.81 (C-9), 157.63 (C-7), 127.95 (C-2', C-6'), 124.63 (C-1'), 116.68 (C-3', C-5'), 105.80 (C-3), 103.79 (C-10), 100.33 (C-1"'), 100.25 (C-1"), 99.89 (C-6), 94.76 (C-8), 77.01-61.07 (C-2"-C-6", C-2"'-C-6"').