Supporting Information for

Probing 3-hydroxyflavone for *in vitro* glycorandomization of flavonols by YjiC

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General methods for cloning, expression and purification

The *yjiC* gene was amplified by using primer pairs: *yjiC*-F/*yjiC*-R: *yjiC*F-5'-**GGATCC**ATGGGACATAAACATATCGCG-3' *Bam*HI and *yjiC*R-5'-**CTCGAG**TTATTTTACTCCTGCGGGTGCTAA-3' *Xho*I (Bold and underlined sequence represents the restriction enzyme site) using total genomic DNA of *Bacillus licheniformis* DSM-13 as a template. The 1,191 bp long fragment of *yjiC* was excised from pGEM® -T vector by *Bam*HI/*Xho*I and was cloned into the same sites of pET28a (+) to generate the recombinant expression vector pET28-YjiC. pET28-YjiC was transformed into *E. coli* BL21 (DE3) using the heat shock transformation method. 50 μL of *E. coli* BL21 (DE3) harboring pET28-YjiC was cultured overnight and was inoculated into 50 mL LB medium and incubated at 37℃ until absorbance at 600 nm (OD_{600}) reached to 0.5-0.7. The culture was induced with 0.5 mM of isopropyl-*β*-D-thiogalactopyranoside (IPTG) followed by continued growth at 20℃ with shaking for 20 h. The cells were harvested by centrifugation at 3,000 rpm for 10 min, washed twice with 100 mM Tris-Cl (pH: 8.0) and resuspended in 1 mL of the same buffer. The cells were lysed by sonication and the lysate was cleared by centrifugation (12,000 rpm*)* for 30 min at 4℃. Cleared lysate was mixed with 1 mL of Ni-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen, Valencia, CA, USA) and was purified according to the manufacturer's instructions. Fractions containing purified protein were assessed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12% SDS-PAGE), and were concentrated using Amicon Ultra-15 (Millipore, 10K NMWL centrifugal filters). The purified protein was stored in the buffer containing 100 mM Tris-HCl (pH 8.0) and 20% [v/v] glycerol until use. Protein concentration was determined by the Bradford assay kit from Bio-Rad (Hercules, CA, USA). 3-hydroxyflavone, fisetin, quercetin, kaempferol, and myricetin were purchased from Sigma-Aldrich (USA). UDP-*α*-D-glucose, UDP-*α*-D-galactose, UDP-*α*-D-glucuronic acid, and

UDP-*N*-acetyl glucosamine were purchased from Sigma-Aldrich (USA). TDP-L-rhamnose and GDP-L-fucose was purchased from GeneChem (Daejeon, Korea) TDP-*α*-D-2-deoxyglucose and TDP-*α*-D-viosamine was gifted by Dr. Dae Hee Kim (GeneChem, Daejeon, Korea). All other chemicals used in the study are of highest chemical grade.

Glycosyltransferase activity assay

The reactions for YjiC were carried out in a total volume of 100 µl of reaction mixture containing 100 mM Tris-Cl (pH 8.8), 4 mM NDP-sugar, 2 mM acceptor substrate, 2 mM $MgCl_2$ and 40 µg/mL of appropriately diluted enzyme. The reaction mixture was incubated at 30℃ for 4 h. The assay mixtures lacking enzyme served as controls. The reaction was quenched by addition of double volume of chilled methanol, centrifuged to remove proteins, and the reaction product was monitored by HPLC-PDA after proper dilution.

Analytical methods

Reverse-phase HPLC-PDA analysis was performed with a C18 column (Mightysil RP-18 GP (4.6x250, 5µm) connected to a photo diode array using binary condition of H_2O (0.1% trifluroacetic acid buffer) and 100% acetonitrile (ACN) at a flow rate of 1 mL/min for 25 minutes. The concentration of ACN is as follows: 20% (0-5) min, 50% (5-10) min, 70% (10-15) min, 90% (15-20) min, 10% (20-25) min. The molecular weight of the products were confirmed by high resolution mass spectra obtained in positive mode from LC-QTOF-ESI-MS/MS [ACQUITY (UPLC, Waters Corp., USA)-SYNAPT G2-S (Waters Corp., USA)].

Supplementary results

Supplementary table 1. HPLC-PDA analysis of flavonols sharing 3-hydroxyflavone backbone.

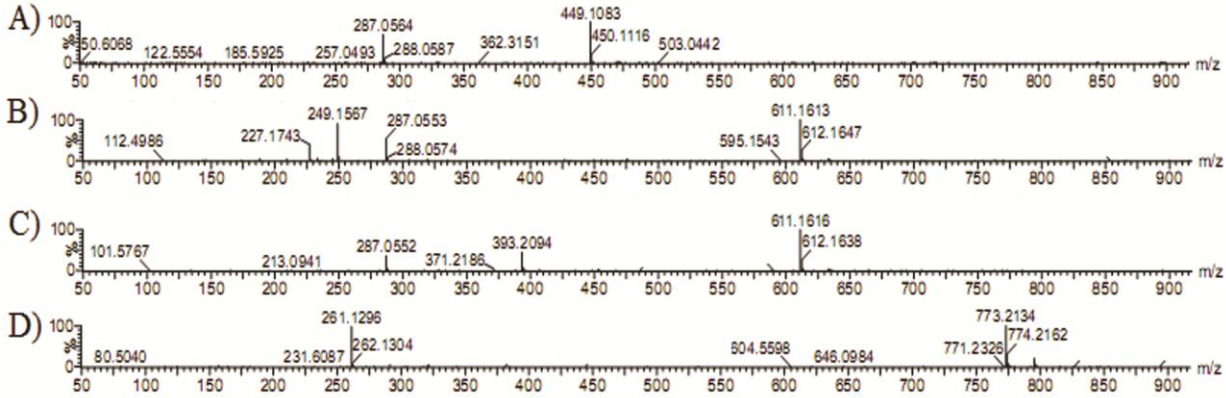
Compound	Types of glucoside	Retention time (t_R) min
Fisetin	Aglycone standard	14.6
F_1	Monoglucoside	11.8
F_2	Diglucoside	11.4
F_3	Diglucoside	10.7
F_4	Triglucoside	10.0
Quercetin	Aglycone standard	15.3
Q_1	Monoglucoside	14.2
Q_2	Diglucoside	13.1
Q_3	Diglucoside	12.7
Q_4	Triglucoside	12.2
Q_5	Triglucoside	11.3
Q_6	Tetraglucoside	10.7
Myricetin	Aglycone standard	14.5
M_1	Monoglucoside	12.1
M_2	Diglucoside	11.6
M_3	Triglucoside	11.3
M_4	Tetraglucoside	10.5
Kaempferol	Aglycone standard	16.5
K_1	Monoglucoside	13.9
K_2	Diglucoside	12.3
K_3	Diglucoside	12.0
K_4	Triglucoside	10.3

Supplementary table 2. HPLC-PDA and HPLC-PDA-QTOF-ESI/MS analysis of 3-HF glycorandomized reaction mixtures.

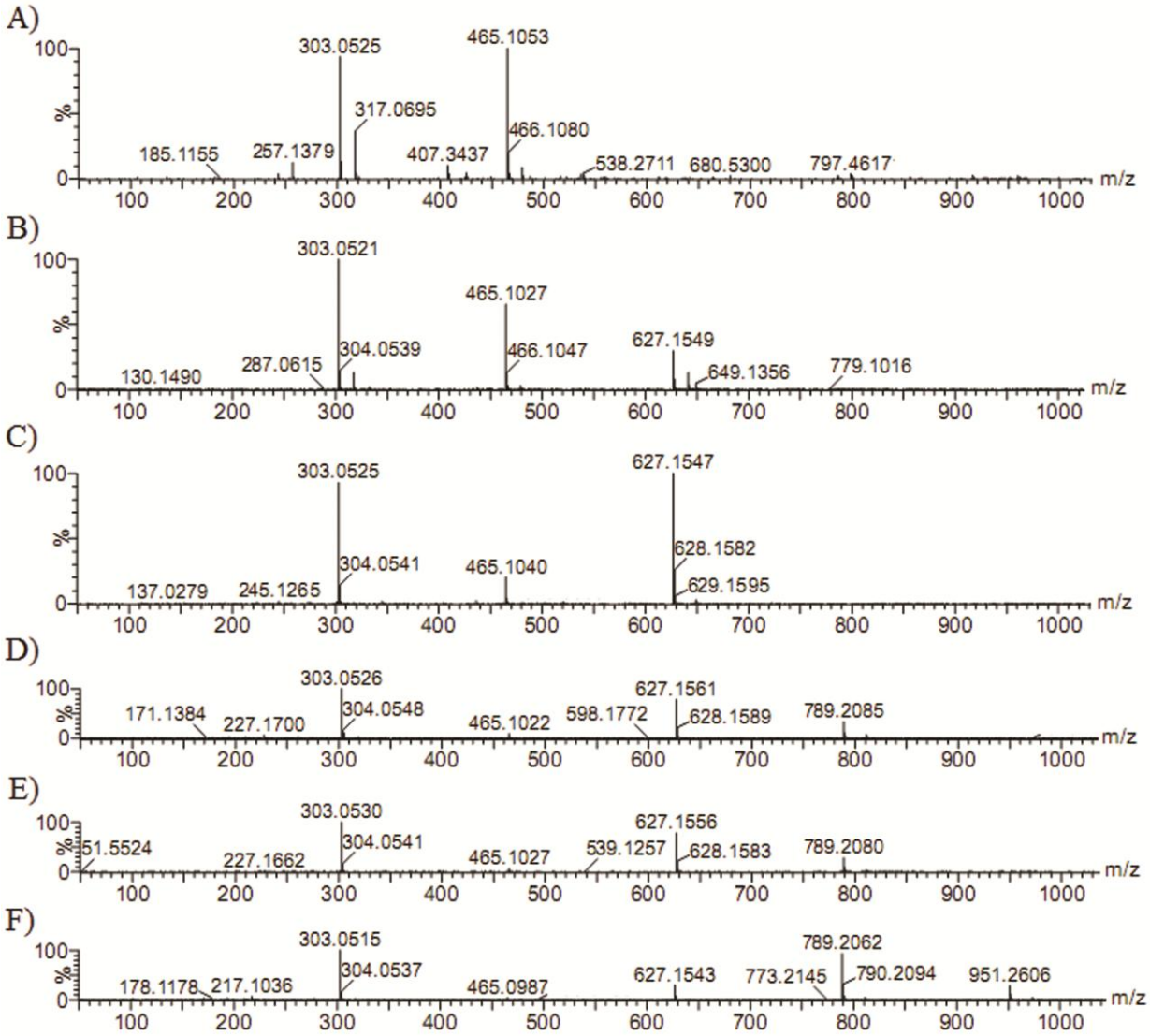
Compound	**Retention time (t_R) min**	**Ion formula**	**Calculated *m/z***	**Observed *m/z***
3-HF 3-*O*-*β*-D-glucoside	16.8	$C_{21}H_{21}O_8$ [M+glucose]+H^+ $C_{21}H_{20}NaO_8$ [M+glucose]+Na^+	401.123643 423.105588	401.1271 423.1054
3-HF 3-*O*-*β*-D-galactoside	16.8	$C_{21}H_{21}O_8$ [M+galactose]+H^+ $C_{21}H_{20}NaO_8$ [M+galactose]+Na^+	401.123643 423.105588	401.1298 423.1057
3-HF 3-*O*-*β*-D-viosaminoside	17.0	$C_{21}H_{22}NO_6$ [M+viosamine]+H^+	384.144713	384.1453
3-HF 3-*O*-*β*-D-2-deoxy-glucoside	17.0	$C_{21}H_{20}NaO_7$ [M+2-deoxyglucose]+Na^+	407.110673	407.1098
3-HF 3-*O*-*β*-L-fucoside	16.9	$C_{21}H_{20}NaO_7$ [M+fucose]+Na^+	407.1107	407.1108
3-HF 3-*O*-*β*-L-rhamnoside	16.8	$C_{21}H_{20}NaO_7$ [M+rhamnose]+Na^+	407.1107	407.1094
UDP-*N*-Acetylglucosamine	ND	ND	ND	ND
UDP-glucoronic acid	ND	ND	ND	ND

ND: not detected.

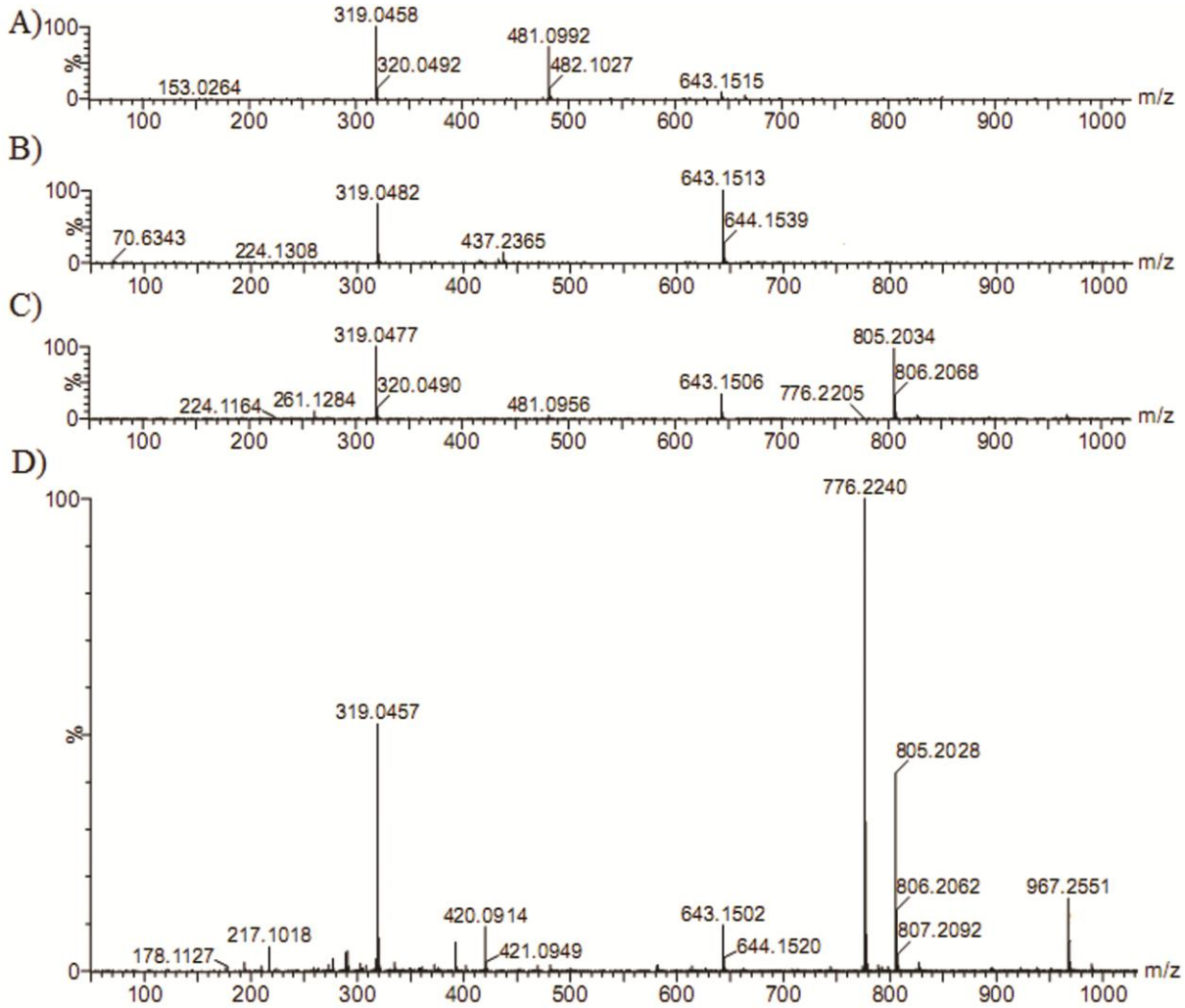
Supplementary Figures



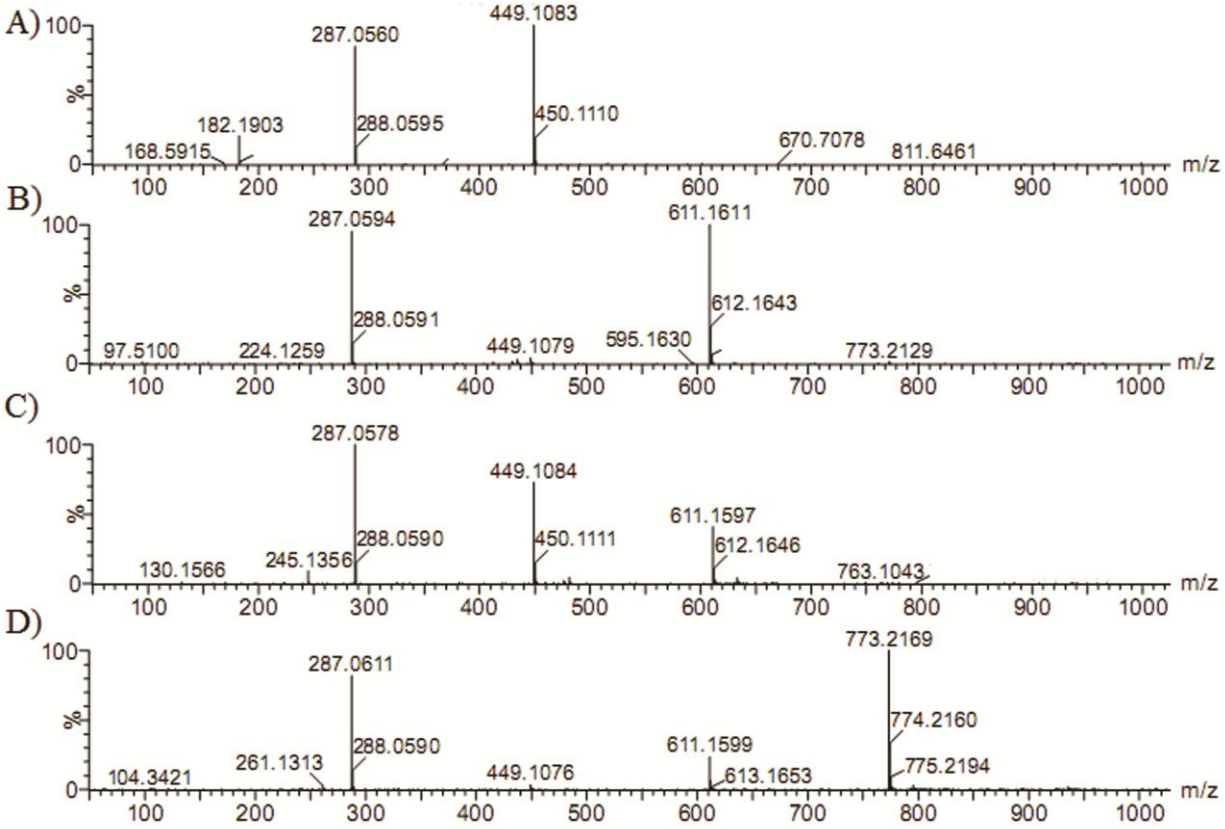
Supplementary figure S1. HPLC-PDA-QTOF-ESI/MS analysis of fisetin reaction mixture. A) Mass of fisetin monoglucoside F_1, t_R: 11.8 min, $[M+H]^+$ m/z^+~449.1083. B) Mass of fisetin diglucoside F_2, t_R: 11.4 min, $[M+H]^+m/z^+$~611.1613. C) Mass of fisetin diglucoside F_3, t_R: 10.7 min, $[M+H]^+m/z^+$~611.1616. D) Mass of fisetin triglucoside F_4, t_R: 10.0 min, $[M+H]^+$ m/z^+~773.2134. Mass of fisetin aglycone, $[M+H]^+$ m/z^+~287.0556.



Supplementary figure S2. HPLC-PDA-QTOF-ESI/MS analysis of quercetin reaction mixture. A) Mass of quercetin monoglucoside Q_1, t_R: 14.2 min, $[M+H]^+m/z^+$~465.1053. B) Mass of quercetin diglucoside Q_2, t_R:13.1 min, $[M+H]^+m/z^+$~627.1549. C) Mass of quercetin diglucoside Q_3, t_R: 12.7 min, $[M+H]^+m/z^+$~627.1547. D) Mass of quercetin triglucoside Q_4, t_R: 12.2 min, $[M+H]^+m/z^+$~789.2085. E) Mass of quercetin triglucoside Q_5, t_R:11.3 min, $[M+H]^+m/z^+$~789.2080, and F) Mass of quercetin tetraglucoside Q_6, t_R: 10.7 min, $[M+H]^+m/z^+$~951.2606. Mass of quercetin aglycone, $[M+H]^+$ m/z^+~303.0505.



Supplementary figure S3. HPLC-PDA-QTOF-ESI/MS analysis of myricetin reaction mixture. A) Mass of myricetin monoglucoside M_1, t_R: 12.1 min, $[M+H]^+$ m/z^+~481.0992. B) Mass of myricetin diglucoside M_2, t_R: 11.6 min, $[M+H]^+m/z^+$~643.1513. C) Mass of myricetin triglucoside M_3, t_R: 11.3 min, $[M+H]^+$ m/z^+~805.2034, and D) Mass of myricetin tetraglucoside M_4, t_R: 10.5 min, $[M+H]^+m/z^+$~967.2551. Mass of myricetin aglycone, $[M+H]^+$ m/z^+~319.0454.



Supplementary figure S4. HPLC-PDA-QTOF HR-ESI/MS analysis of kaempferol reaction mixture. A) Mass of kaempferol monoglucoside K_1, t_R: 13.9 min, $[M+H]^+ m/z^+$~449.1083. B) Mass of kaempferol diglucoside K_2, t_R: 12.3 min, $[M+H]^+ m/z^+$~611.1611. C) Mass of kaempferol diglucoside K_3, t_R: 12 min, $[M+H]^+ m/z^+$~611.1597, and D) Mass of kaempferol triglucoside K_4, t_R: 10.3 min, $[M+H]^+ m/z^+$~773.2169. Mass of kaempferol aglycone, $[M+H]^+ m/z^+$~287.0556.

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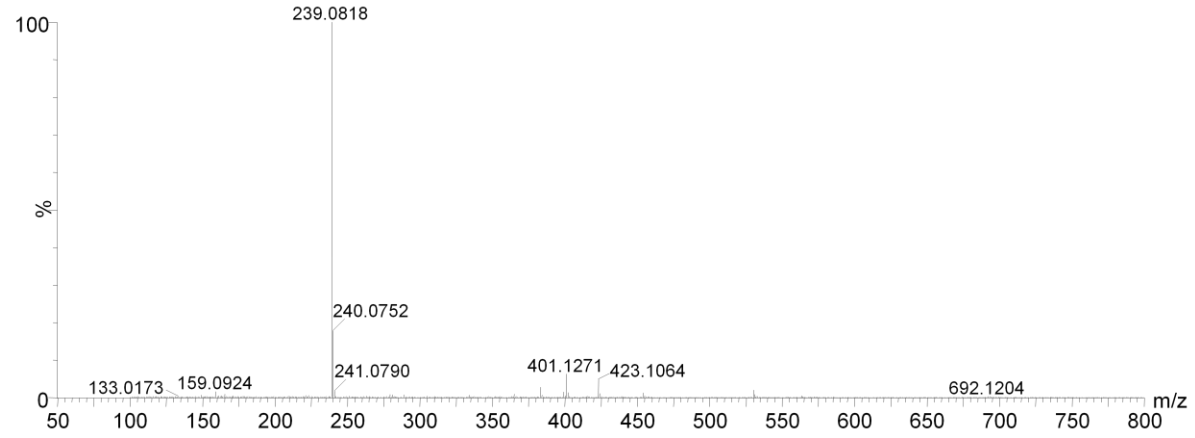
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m/z
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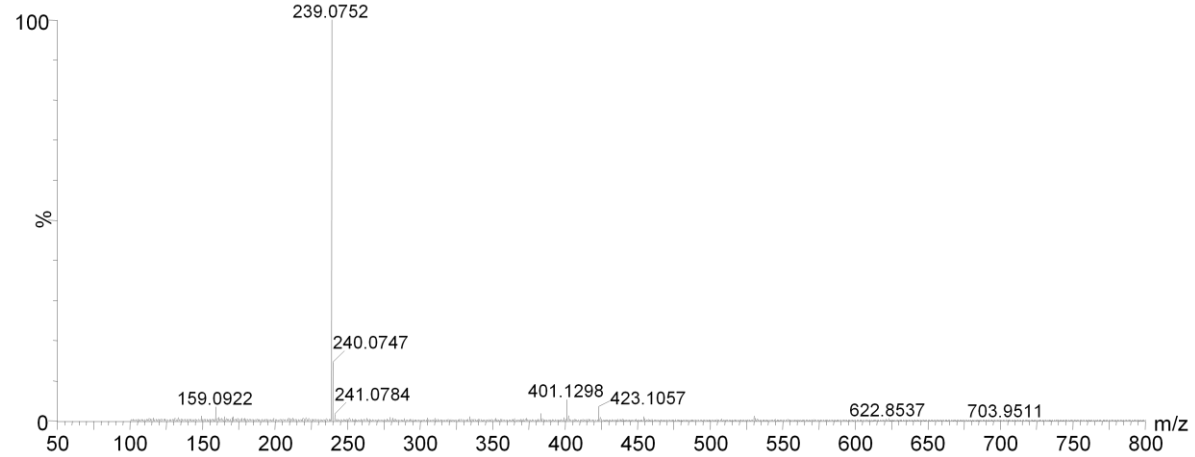
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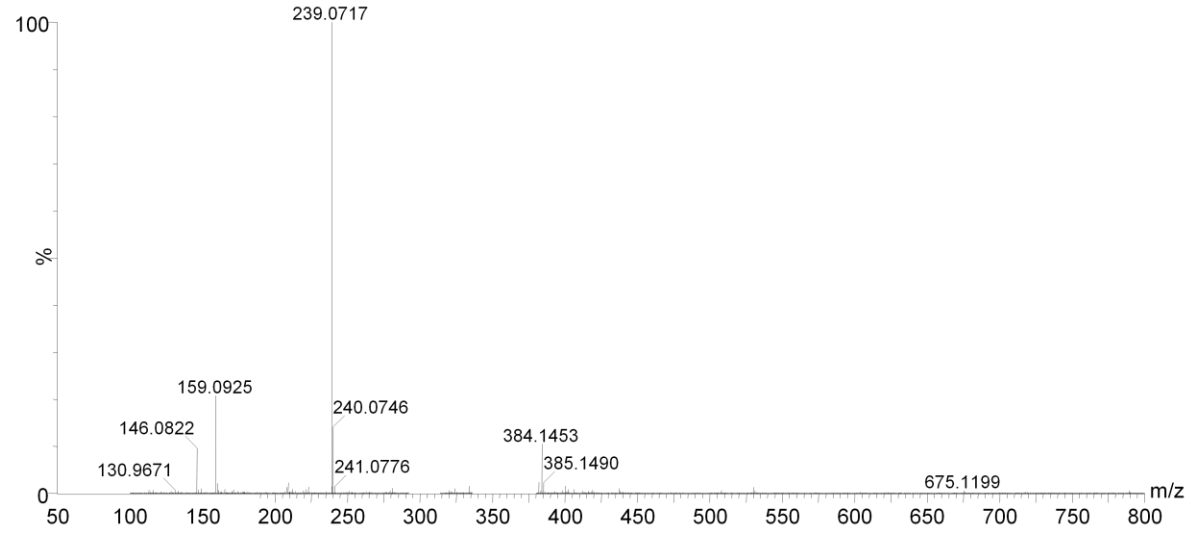
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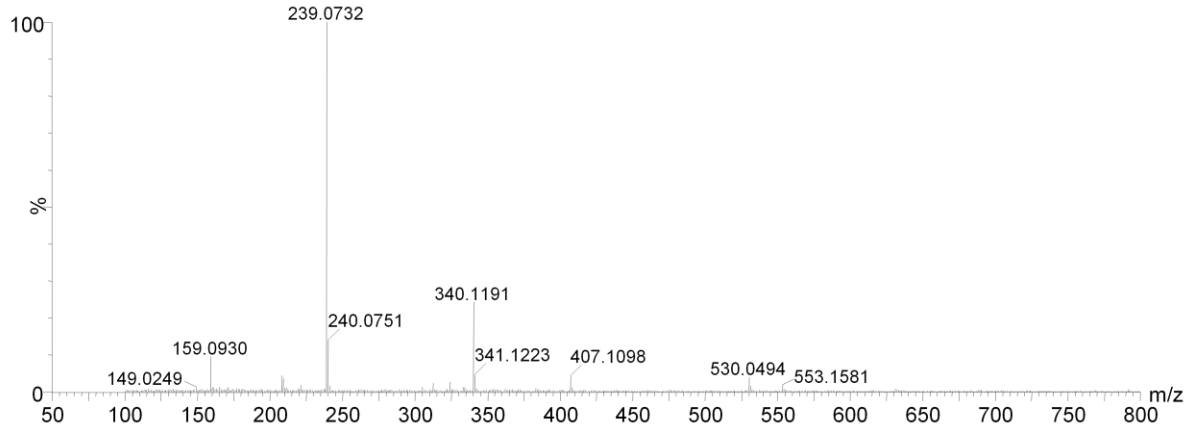

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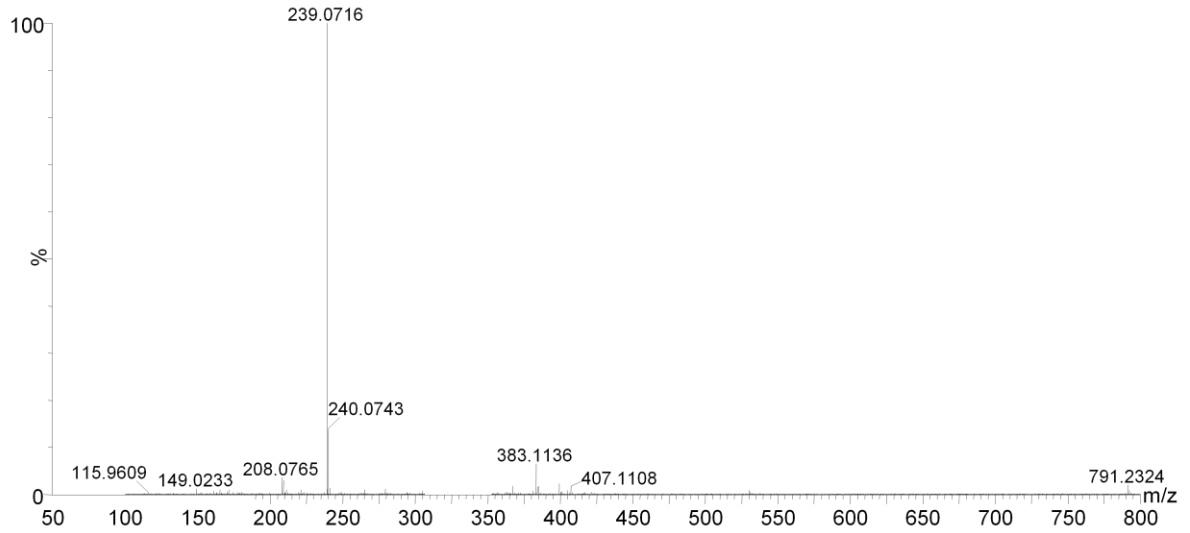

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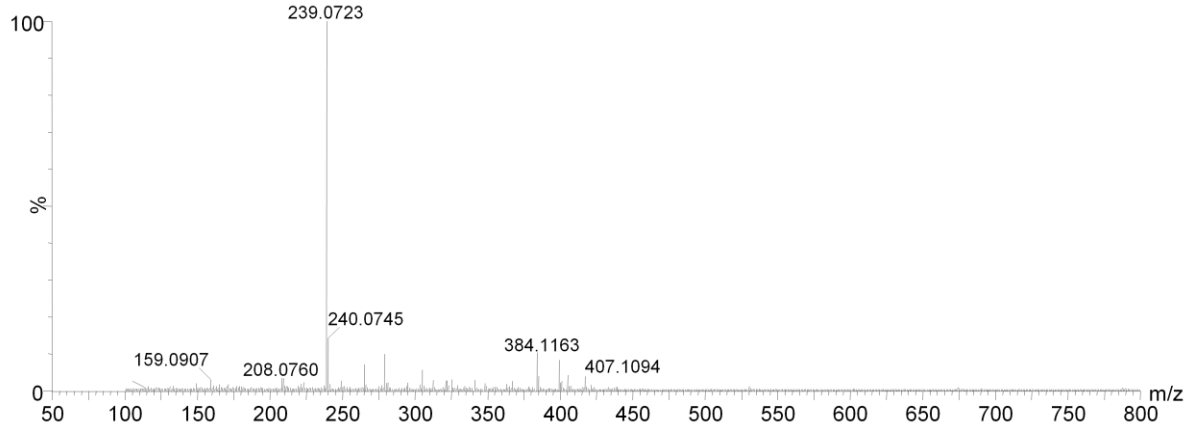
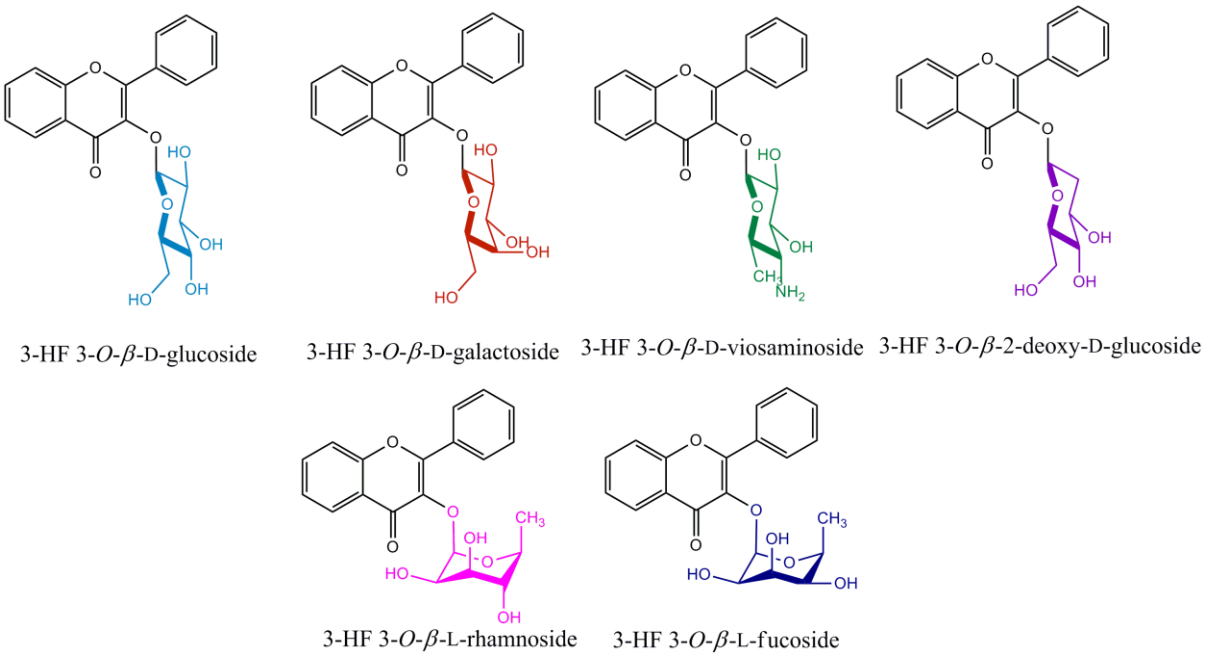


Supplementary figure S5. HPLC-PDA-QTOF-HR ESI/MS analysis of 3-HF with different

NDP-sugar donors and YjiC. A) 3-HF 3-*O*-*β*-D-glucoside, B) 3-HF 3-*O*-*β*-D-galactoside, C) 3-HF 3-*O*-*β*-D-viosaminoside, D) 3-HF 3-*O*-*β*-D-2-deoxy glucoside, E) 3-HF 3-*O*-*β*-L-fucoside, F) 3-HF 3-*O*-*β*-L-rhamnoside (See supplementary table 2 for observed mass, ion formula and calculated mass and for structures of each compounds see supplementary figure S6 or figure 4).



Supplementary figure S6. Structures of glycorandomized reaction products of YjiC catalyzed reactions of 3-HF and diverse kinds of NDP-D/L-sugars.