

Supplementary Information

Efficient Enzymatic Synthesis of Mangiferin Glycosides in Hydrophilic Organic Solvents

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Analytical Methods

HPLC Analysis: HPLC analysis was conducted using a Dionex P680A HPLC system with a Discovery (ODS) column (250×4.6 mm, 5 μm) and a UV detector at 316 nm. Glycosides were separated from their respective aglycones using a linear gradient of methanol/0.1% formic acid (v/v) in H₂O: 20-60% at 1 mL/min over 20 min.

LC-HRMS Analysis: LC-HRMS was performed on a Waters ACQUITY UPLC system equipped with a binary solvent delivery manager and a sample manager, coupled with a Waters Micromass Q-TOF Premier Mass Spectrometer equipped with an electrospray interface (Waters Corporation, Milford, MA).

NMR Analysis: ¹H, ¹³C and 2D-NMR spectra of glycosides were obtained using a Bruker AV-500 spectrometer (Switzerland), operating at 500 MHz. Samples were dissolved in DMSO-*d*₆ at room temperature with tetramethylsilane (Me₄Si) as the chemical shift reference.

SDS-PAGE of β-Fructofuranosidase

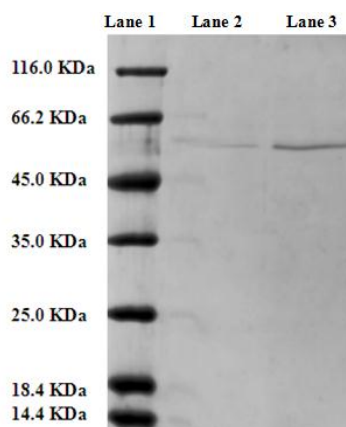


Figure S1. SDS-PAGE of culture from *Arthrobacter arilaitensis* NJEM01 and purified β-fructofuranosidase. Lane 1, molecular-mass standards; Lane 2, supernatant of culture (incubated 24h); Lane 3, purified enzyme.

Specificity of flavonoid substrate for glycosylation

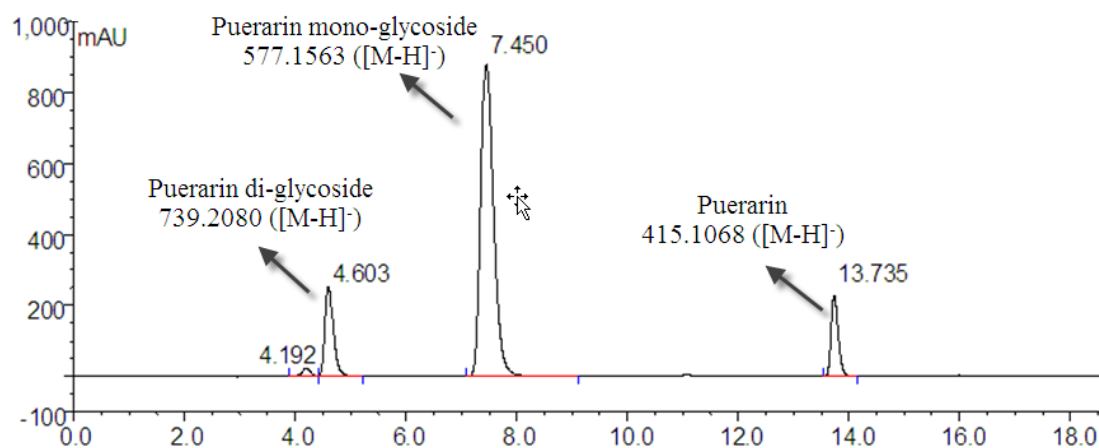


Figure S2. HPLC and MS analysis of puerarin glycosides.

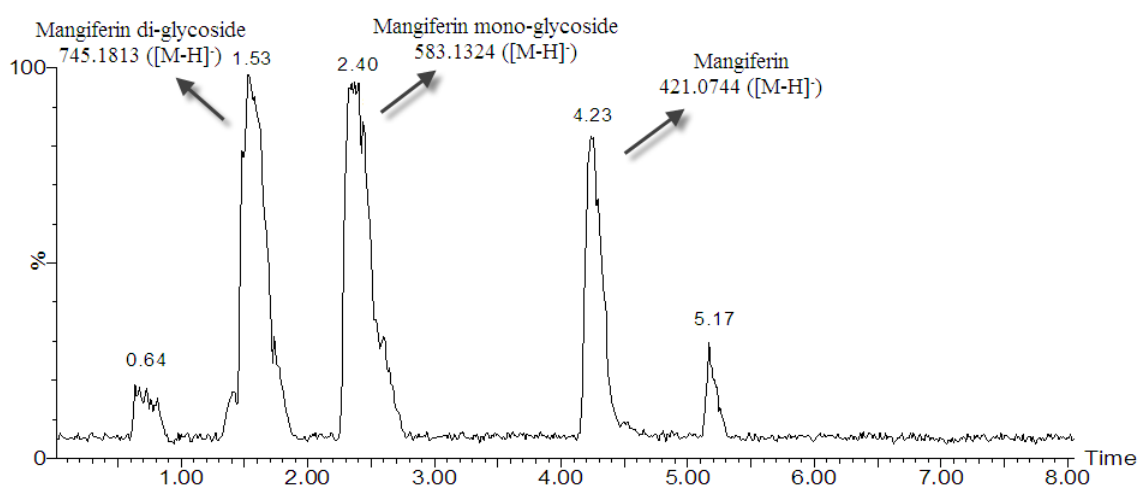


Figure S3. LC-MS analysis of mangiferin glycosides.

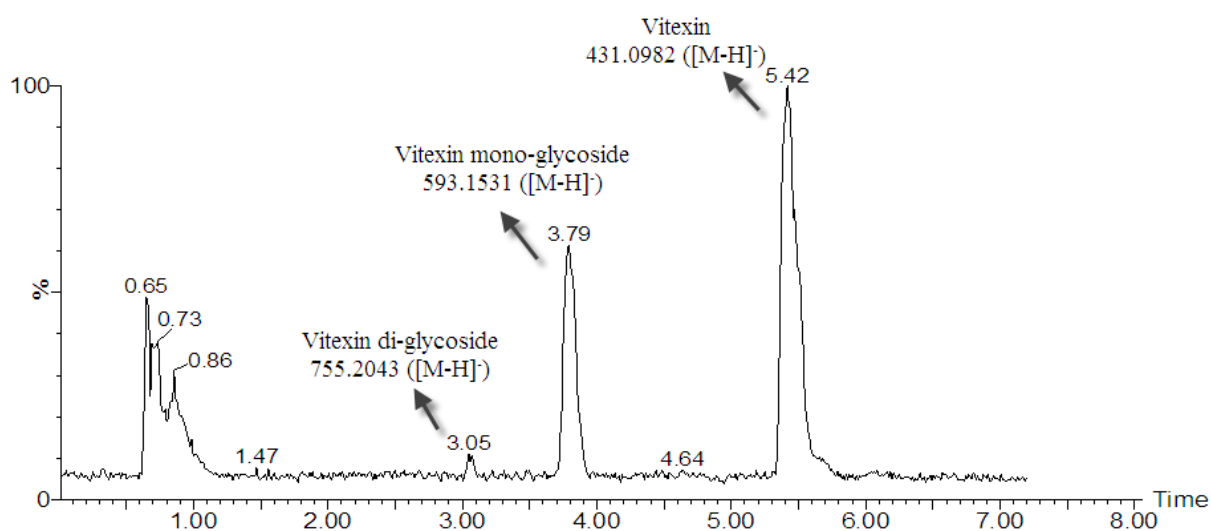


Figure S4. LC-MS analysis of vitexin glycoside

Identification of Mangiferin Glycosides by $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and HR-MS

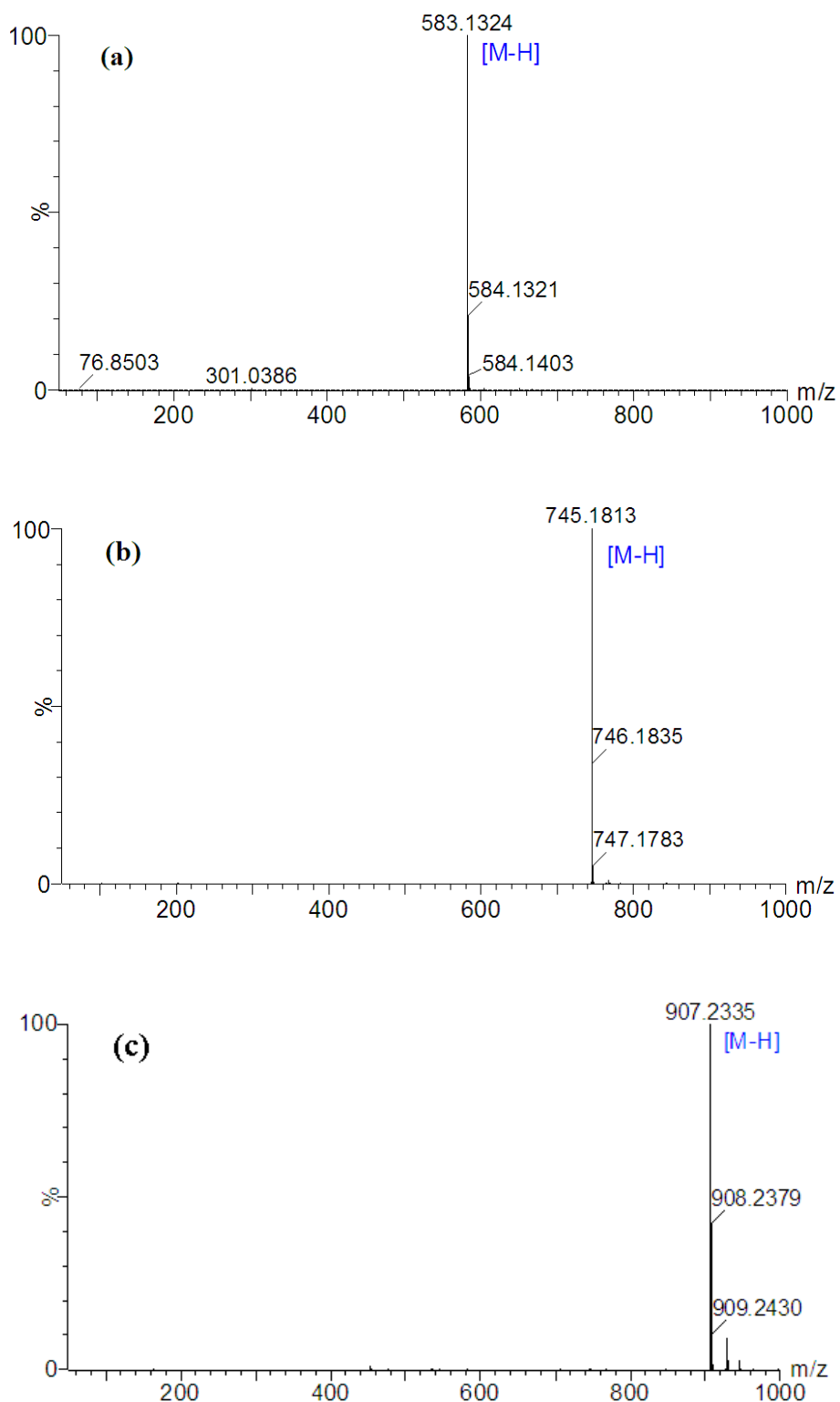


Figure S5. HRMS analysis of mangiferin glycosides.

(a) β -D-fructofuranosyl-(2 \rightarrow 6)-mangiferin (M1) (HRMS: 583.1324 ([M-H]⁺) (C₂₅H₂₇O₁₆, calc.583.1299, [M-H]⁺)),

(b) β -D-difructofuranosyl-(2 \rightarrow 6)-mangiferin (M2) (HRMS: 745.1813 ([M-H]⁺) (C₃₁H₃₇O₂₁, calc.745.1827, [M-H]⁺)),

(c) β -D-trifructofuranosyl-(2 \rightarrow 6)-mangiferin (M3) (HRMS: 907.2335 ([M-H]⁺) (C₃₇H₄₇O₂₆, calc.907.2356, [M-H]⁺)).

WXM-5 1H-NMR DMSO 303K AV-500

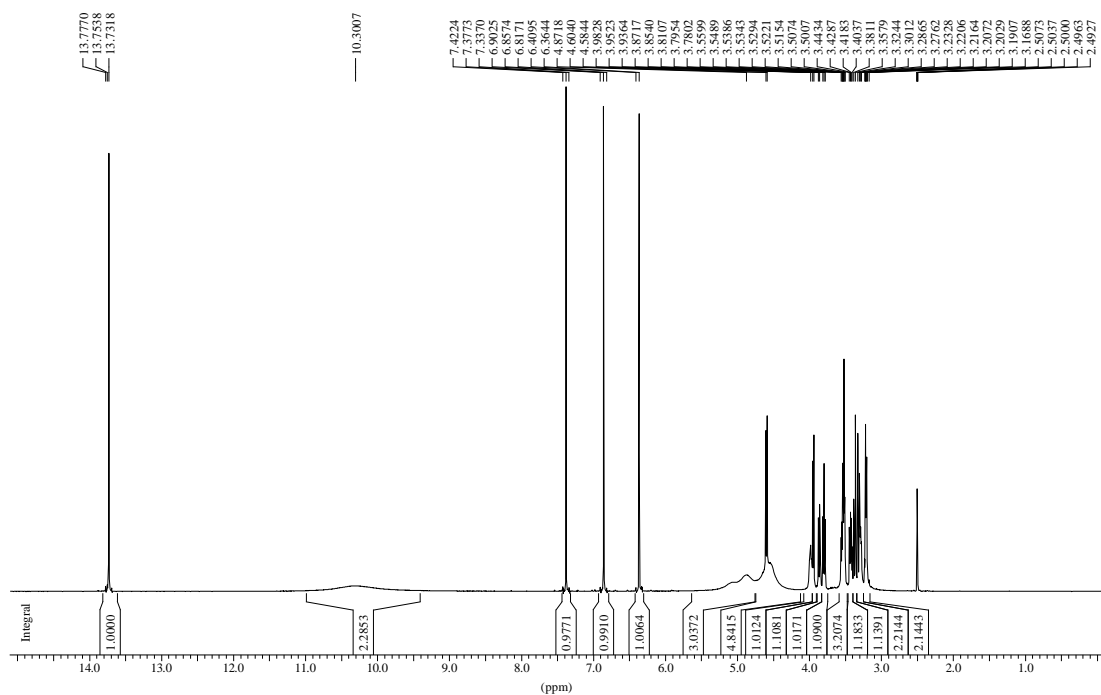


Figure S6. ¹H-NMR spectrum of β-D-fructofuranosyl-(2→6)-mangiferin.

WXM-5 13C-NMR DMSO 303K AV-500

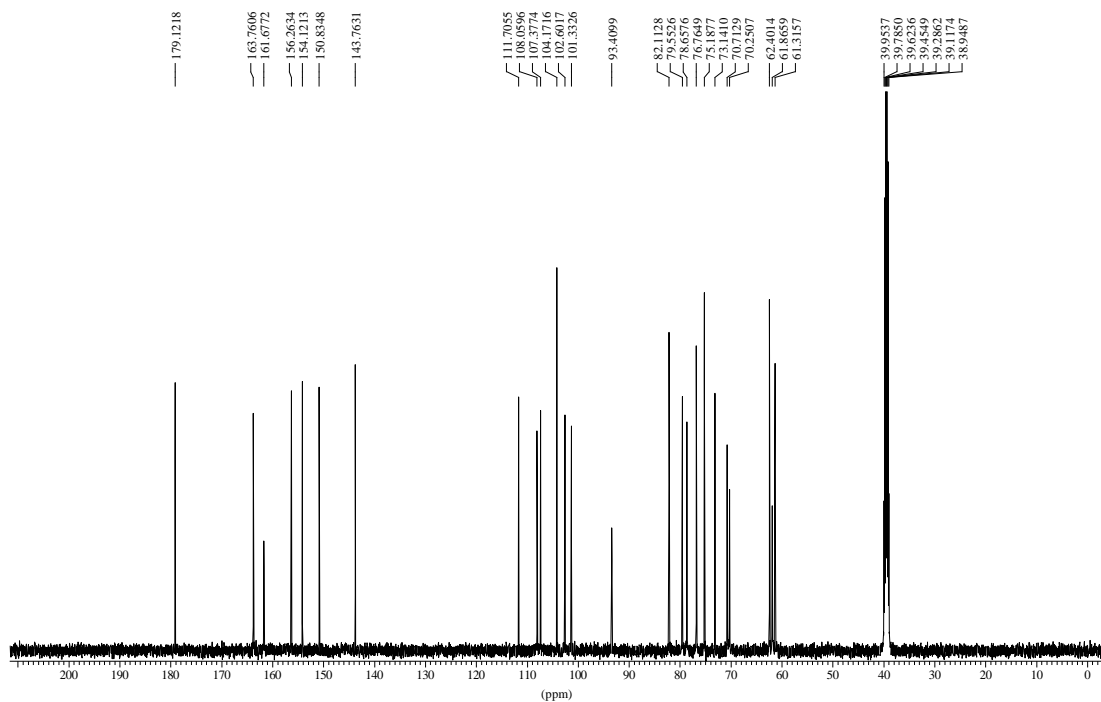


Figure S7. ¹³C-NMR spectrum of β-D-fructofuranosyl-(2→6)-mangiferin.

WXM-5 COSY DMSO 303K AV-300

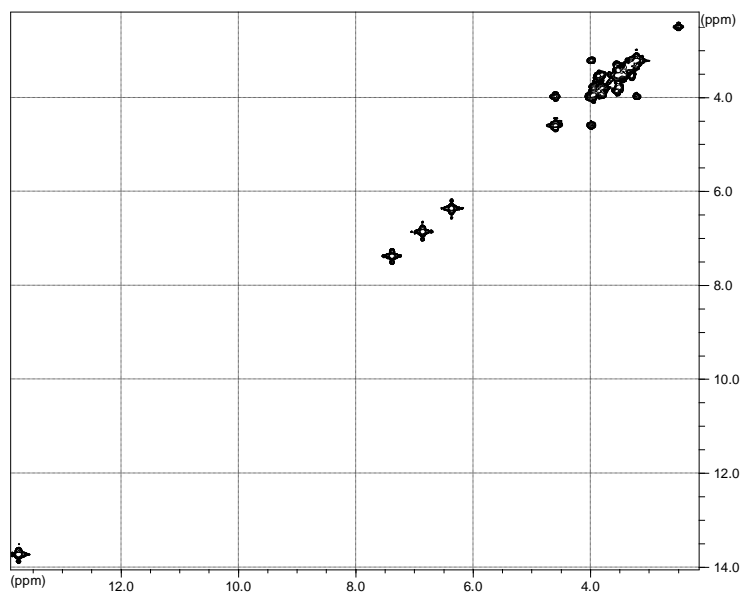


Figure S8. 2D ^1H - ^1H COSY spectrum of β -D-fructofuranosyl-(2 \rightarrow 6)-mangiferin.

WXM-5 HSQC DMSO 303K AV-300

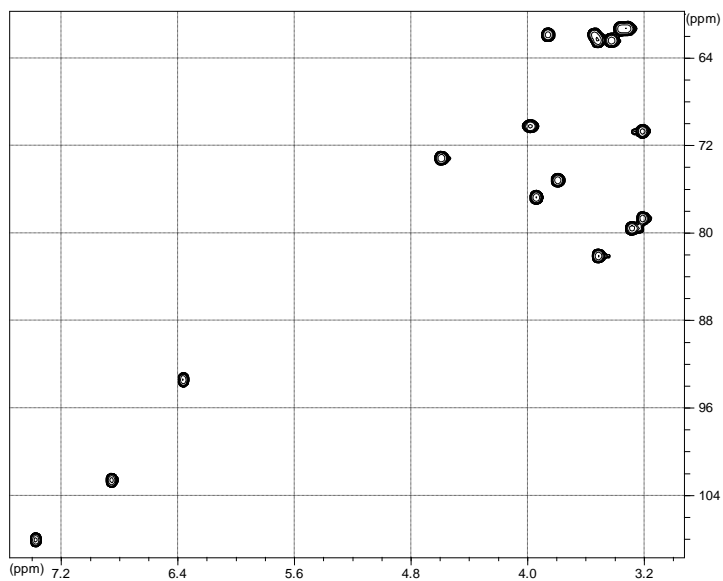


Figure S9. 2D ^1H - ^{13}C HSQC spectrum of β -D-fructofuranosyl-(2 \rightarrow 6)-mangiferin.

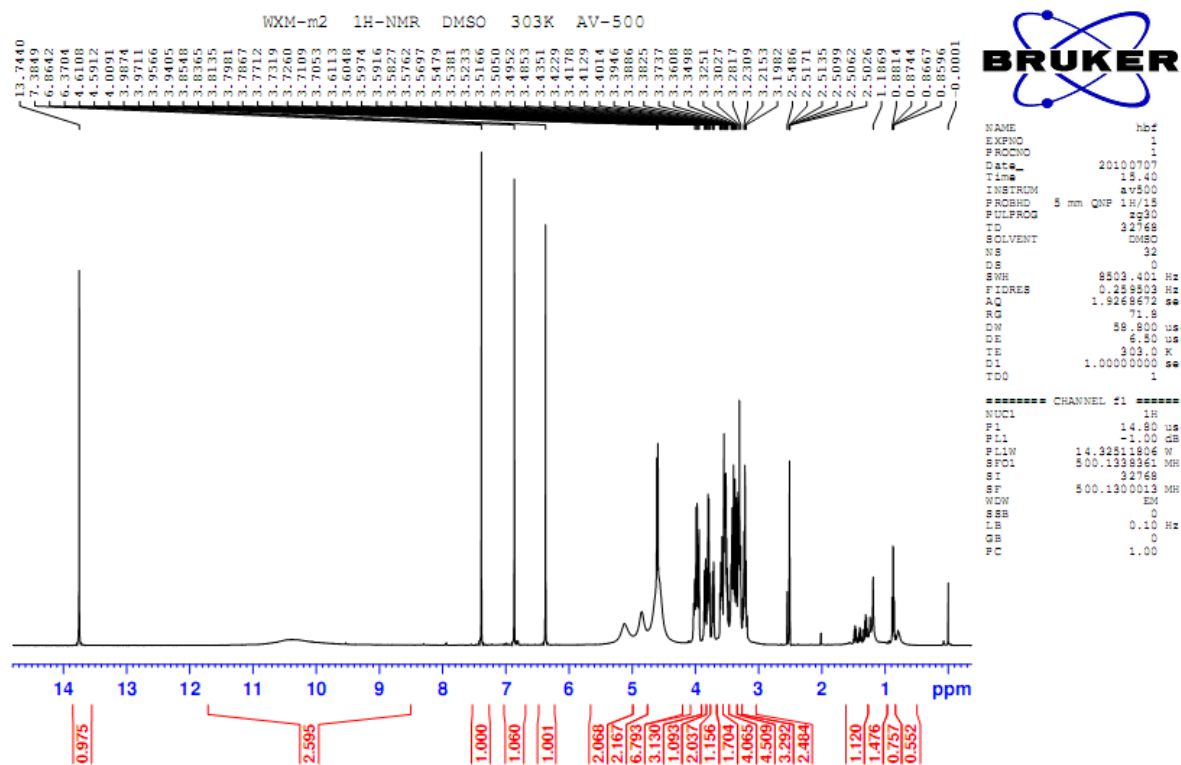


Figure S10. ¹H-NMR spectrum of β-D-difructofuranosyl-(2→6)-mangiferin.

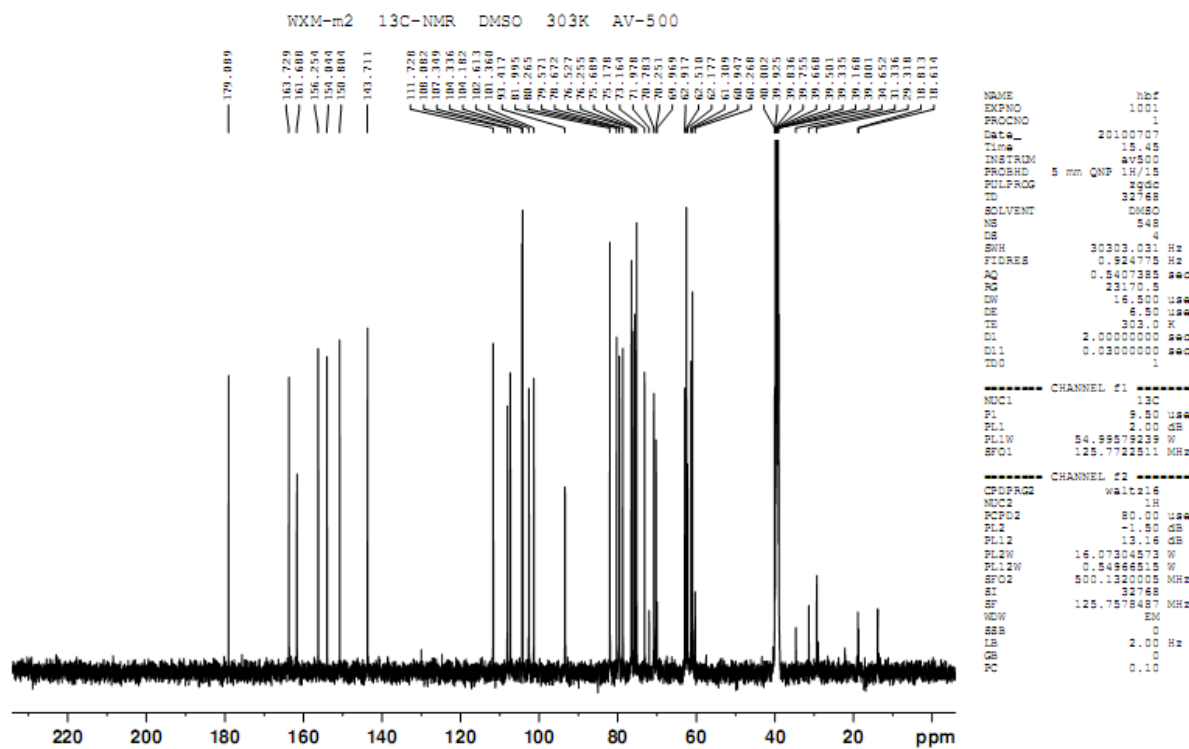


Figure S11. ¹³C-NMR spectrum of β-D-difructofuranosyl-(2→6)-mangiferin.

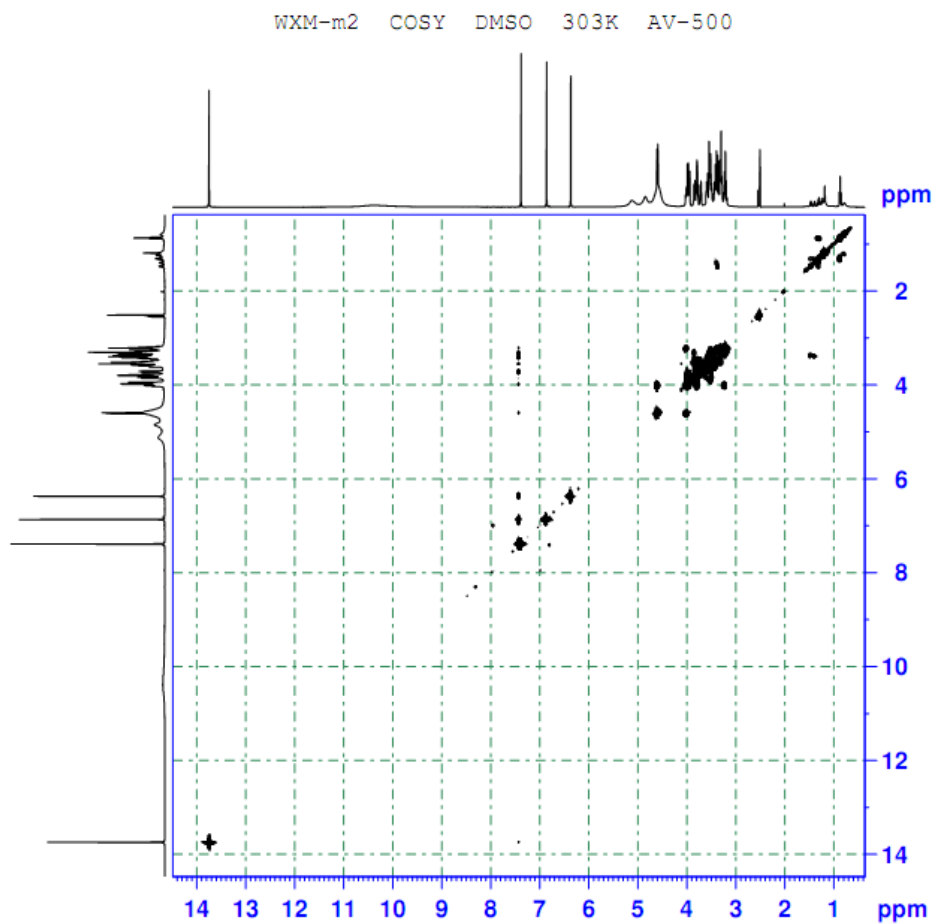


Figure S12. 2D ^1H - ^1H COSY spectrum of β -D-difructofuranosyl-(2 \rightarrow 6)-mangiferin.

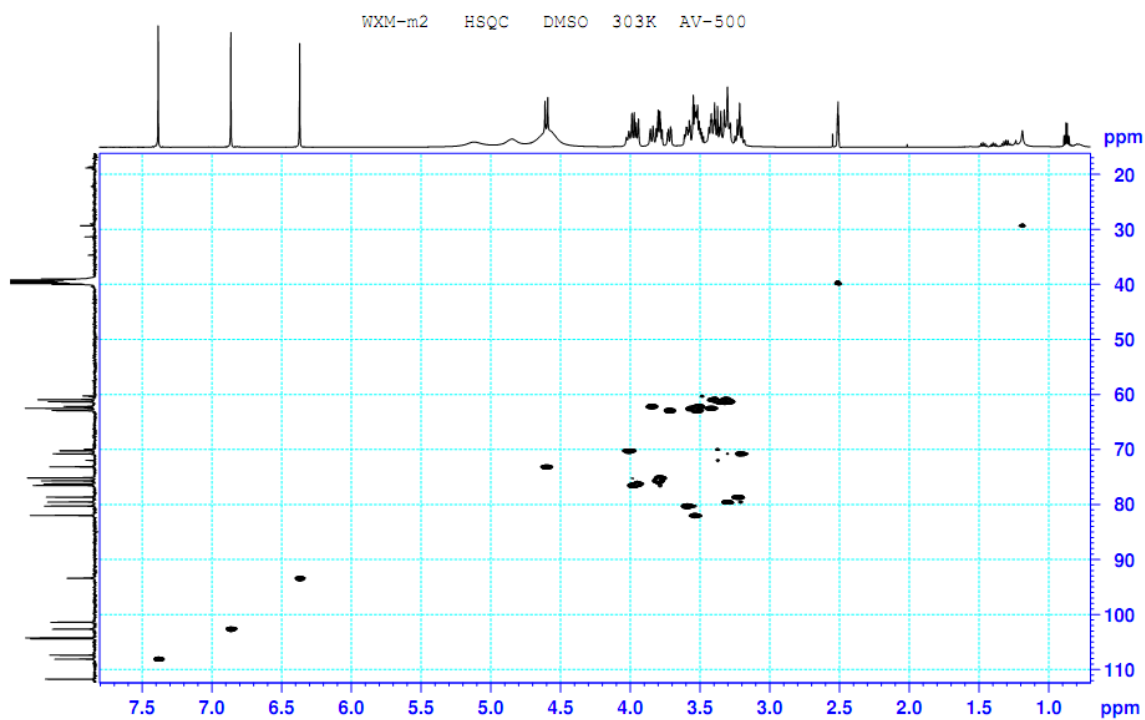


Figure S13. 2D ^1H - ^{13}C HSQC spectrum of β -D-difructofuranosyl-(2 \rightarrow 6)-mangiferin.