Electronic Supplementary Information

Functional Characterization, Structural Basis, and Regio-Selectivity Control of a Promiscuous Flavonoid 7,4′-di-*O*-glycosyltransferase from *Ziziphus jujuba* var. *spinosa*

Zi-Long Wang,[‡a] Wanqing Wei,[‡b] Hai-Dong Wang,[‡a] Jia-Jing Zhou,[a] Hao-Tian Wang,[a] Kuan Chen,[a] Rong-Shen Wang,[a] Fu-Dong Li,[c] Xue Qiao,[a] Huan Zhou,*[d] Yong Liang,*[e] and Min Ye *[a]

[a] State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, China

[b] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China

[c] Hefei National Laboratory for Physical Science at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei 230036, China

[d] Shanghai Synchrotron Radiation Facility, Shanghai Advanced Research Institute, Chinese Academy of Sciences, 239 Zhangheng Road, Pudong District, Shanghai 201204, China

[e] State Key Laboratory of Coordination Chemistry, Jiangsu Key Laboratory of Advanced Organic Materials, Chemistry and Biomedicine Innovation Centre (ChemBIC), School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

[‡] These authors contributed equally.

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1. Experimental procedures

1.1 General

Compounds **1**-**8**, **10**-**12**, **14**, **18** and **20**-**34** were purchased from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Compounds **9**, **13**, **15**-**17** and **19** were previously purified and characterized in our lab. Methanol and acetonitrile (Fisher Scientific, USA) were of HPLC grade. All other chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Beijing Chemical Corporation (Beijing, China) unless otherwise specified. Substrate specificity and conversion rates were analyzed by Vanquish UHPLC (Thermo Fisher Scientific, Massachusetts, USA). The glycosylated products were isolated and purified by semi-preparative HPLC on an Agilent 1200 instrument (Agilent Technologies, Waldbronn, Germany). LC/MS analysis was performed on an UHPLC-Q-Exactive HRMS (Thermo Fisher Scientific, Massachusetts, USA) equipped with an electrospray ionization (ESI) source. NMR spectra were recorded on a Bruker AVANCE III-400 instrument at 400 (^{1}H) and 100 (^{13}C) MHz in DMSO-d_6. Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) are given in Hertz (Hz).

1.2 Plant materials

Seeds of *Ziziphus jujuba* var. *spinosa* were obtained from Shandong Province (China). The seed kernels were collected and washed, then immediately frozen in liquid nitrogen for RNA extraction.

1.3 Transcriptome data assembly and candidate gene screening

Transcriptome data of *Ziziphus jujuba* var. *spinosa* (SRR9721936, SRR9721937, SRR9721938, SRR9721939, SRR9721941 and SRR9721944) were download from NCBI (https://www.ncbi.nlm.nih.gov/), and were assembled through the SOAPdenovo-Trans-src-v1.04 program. For BLAST analysis, 24 functionally characterized plant glycosyltransferase gene sequences were used as homology-based templates to analyze the transcriptome data (Table S1). By blast with these query sequences, 24 open reading frames (ORFs) were obtained as candidate genes (Figure S90). PCR primers were designed containing the homologous sequences of pET28a (+) as shown in Table S2.

1.4 Molecular cloning, heterologous expression, and protein purification

1.4.1 Molecular cloning

The total RNA of the seed kernels of *Ziziphus jujuba* var. *spinosa* was extracted using the TransZol Reagent (Transgen Biotech, China) following the manufacturer's instructions. RNA degradation and contamination was monitored on 1% agarose gel. Purity and concentration of RNA were measured using NanoDrop (Thermo Scientific). The first-stranded complementary DNA (cDNA) was synthesized using TransScript one-step genomic DNA (gDNA) removal and cDNA synthesis SuperMix (Transgen Biotech, China). The PCR was performed using 2 μL of first strand cDNA as a template, ZjOGT3-F and ZjOGT3-R as primers, and *FastPfu* DNA Polymerase under the following conditions: 95°C for 3 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, followed by 72°C for 10 min. The amplified fragments were cloned into pET28a (+) vector (Invitrogen, USA) by Quick-change method. The genes were sequenced by Tsingke biological technology (Beijing, China). The recombinant vector pET28a(+)-ZjOGT3 was transformed into *E. coli* BL21(DE3) (TransGen Biotech, China) for heterologous expression. Transformed cells were selected on agar plates containing 50 μg/mL kanamycin.

1.4.2 Heterologous expression and protein purification

Single colonies harboring the desired expression construct were inoculated overnight at 37°C with shaking in LB culture medium containing 50 μg/mL kanamycin. Isopropyl thiogalactoside (0.1 mM) was added into the medium for the expression of recombinant protein at 16°C when optical density at 600 nm was about 0.6. After incubation with shaking for 20 h, *E. coli* was harvested by centrifugation. The recombinant proteins were purified using a Nickel-affinity column (Proteinlso Ni-NTA Resin, TransGen Biotech, Beijing, China), and concentrated using Amicon Ultra-15 Ultracel-30K (Merck Millipore). The purity of recombinant ZjOGT3 determined by SDS-PAGE is shown in Figure S1. The concentration of recombinant ZjOGT3 was measured using NanoDrop under parameter of E 1%=12 (Thermo Scientific).

1.5 Effects of reaction time, pH, temperature, and divalent metal ions

To investigate the enzymatic properties of ZjOGT3, the reaction time of glycosylation was firstly determined for 8 h (Figure S2). To optimize the reaction pH, the enzymatic reactions were carried out

in various reaction buffers ranged in pH value from 4.0-6.0 (Citric acid-sodium citrate buffer), 6.0-8.0 (Na_2HPO_4-NaH_2PO_4 buffer), 7.0-9.0 (Tirs-HCl buffer), and 9.0-10.0 (Na_2CO_3-$NaHCO_3$ buffer). To study the optimal reaction temperature, the enzymatic reactions were incubated at different temperatures (4-60°C). To test the dependence of divalent metal ions for ZjOGT3 activity, different divalent cations including Ca^{2+}, Mn^{2+}, Mg^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Ba^{2+}, Zn^{2+}, Cu^{2+} and EDTA in the final concentration of 5 mM were used individually. All enzymatic reactions (100 μL reaction mixtures including 0.5 mM UDP-Glc, 0.2 mM **1**, and 25 μg of purified ZjOGT3) were conducted in three parallel experiments (n=3). All the reactions were terminated with pre-cooling methanol (MeOH) and centrifuged at 15,000 rpm for 20 min for further UHPLC analysis as described in general methods. The samples were separated on an ACQUITY UPLC® HSS T3 column (100 mm × 2.1 mm, 1.8 μm) at a flow rate of 0.3 mL min^{-1}. The mobile phase was a gradient elution of solvents A (MeOH) and B (0.1% formic acid aqueous solution), and the gradient programs were listed in Table S3. The conversion rates in percent were calculated from peak areas of glycosylated products and substrates as analyzed by UHPLC (Figure S3).

1.6 Kinetic studies

All assays were performed in a final volume of 50 μL containing 50 mM NaH_2PO_4-Na_2HPO_4 buffer (pH 8.0) at 45°C for 10 min. For the first step (4′-*O*-glycosylation), 0.3365 μg protein, 2 mM of saturated UDP-Glc, and different concentrations of 1 (1, 5, 10, 20, 50 μM). For the second step (7-*O*-glycosylation), 3.0925 μg protein, 2 mM of saturated UDP-Glc, and different concentrations of **1a** (10, 20, 50, 80, 100, 200, 500 μM). The reactions were quenched with 200 μL ice cold MeOH. Samples were centrifuged at 15,000 rpm for 20 min and analyzed by UHPLC as described above. All experiments were performed in triplicate. The Michaelis-Menten constants (K_m) values were calculated by using the Lineweaver-Burk plot method (Figure S4).

1.7 Substrate promiscuity and sugar donor selectivity of ZjOGT3

To investigate the substrate promiscuity and sugar donor selectivity of ZjOGT3, different acceptors including compounds **1-34** and different sugar donors including UDP-glucose (UDP-Glc), UDP-galactose (UDP-Gal), UDP-xylose (UDP-Xyl), UDP-arabinose (UDP-Ara), UDP-glucuronic acid (UDP-GlcA), UDP-*N*-acetylglucosamine (UDP-GlcNAc), and UDP-rhamnose (UDP-Rha) were used. The reaction mixtures were individually performed in a final volume of 100 μL containing 50 mM

NaH_2PO_4-Na_2HPO_4 buffer (pH 8.0), 25 μg of purified ZjOGT3, 0.1 mM aglycone, and 0.5 mM sugar donor. All reactions were incubated at 45°C for 8 h and quenched with 200 μL ice cold MeOH. The mixtures were then centrifuged at 15,000 rpm for 20 min and analyzed by UHPLC/MS. The samples were analyzed by an ACQUITY UPLC® HSS T3 column (100 mm × 2.1 mm, 1.8 μm) at a flow rate of 0.3 mL min^{-1}. The mobile phase was a gradient elution of solvents A (MeOH) and B (0.1% formic acid aqueous solution), and the gradient programs were listed in Table S3. The conversion rates in percent were calculated from peak areas of glycosylated products and substrates as analyzed by UHPLC (Figure S5-S38). The flow rate was 0.3 mL/min. The column temperature was 55°C. The detection wavelength was 254 nm, 300 nm, and 340 nm. MS analysis was performed on a UHPLC-Q-Exactive HRMS instrument (Thermo Fisher Scientific, Massachusetts, USA). The MS parameters were as follows: sheath gas pressure 45 arb, aux gas pressure 10 arb, discharge voltage 4.5 kV, capillary temperature 350°C. MS^1 resolution was set as 70,000 FWHM, AGC target $1*E^6$, maximum injection time 50 ms, and scan range *m/z* 100-1000. MS^2 resolution was set as 17,500 FWHM, AGC target $1*E^5$, maximum injection time 100 ms, NCE 35.

1.8 Preparative-scale reactions and structural identification of glycosylated products

The substrates (50 mM) were dissolved in dimethyl sulfoxide (DMSO) as sugar acceptor, with 50 mM UDP-Glc as sugar donor. Preparative-scale reactions contain 2 ml buffer (50 mM NaH_2PO_4-Na_2HPO_4, pH 8.0), 40 μL sugar acceptor (50 mM), 90 μL sugar donor (50 mM), and ZjOGT3. The reactions were performed at the optimum condition for 12 h and terminated by adding MeOH. The reaction mixtures were then centrifuged at 15,000 rpm for 30 min, and the supernatants were concentrated and dissolved in 1.5 mL methanol. The glycosylated products were subsequently purified by reversed-phase semi-preparative HPLC and characterized by HR-ESI-MS and NMR analyses (Figures S39-S79).

1.9 Protein expression, purification, and crystallization

The full-length cDNA of ZjOGT3 was cloned into pET-28a (+) vector. The S-tag of pET-28a was removed. A TrxA-tag and 6 His-tag followed by thrombin site were added before N-terminus of the target protein to facilitate purification. The TrxA-His-thrombin-ZjOGT3 protein was expressed in *E. coli* (DE3) strain and purified by Ni-NTA affinity chromatography (GE healthcare). After purification, the recombinant protein was digested by thrombin to cut the TrxA-His tag. The sample was put on the Ni-NTA affinity beads for the second time to purify the protein. The flow-through was concentrated

and then applied to molecular sieve on a Superdex™ 200 Increase 10/300 GL column (GE healthcare, Sweden) for further purification. Then the protein buffer was changed to elution buffer (20 mM Tris-HCl, pH 7.5, 50 mM NaCl). Fractions containing ZjOGT3 were collected and concentrated to 20 mg/mL. The purified ZjOGT3 protein (20 mg/mL) was incubated with UDP-Glc (10 mM) for 2 hours. The crystals were prepared by hanging drop vapor diffusion. Crystals of ZjOGT3 were obtained after 2 days at 16°C in hanging drops containing 1.0 μL of protein solution and 1.5 μL of reservoir solution 0.2 M sodium acetate trihydrate, 26% *w*/*v* PEG 4000, 10 mM Tris-HCl, pH 8.0 (Figures S81).

1.10 Crystal structure determination and refinement

Diffraction data of the ZjOGT3 crystals were collected at beamlines BL18U1, Shanghai Synchrotron Radiation Facility (SSRF) and processed with HKL-2000. The structure was solved by molecular replacement with Phaser using the previously reported UGT74AC1 structure (PDB ID: 6L8X) as the search model. Crystallographic refinement was performed repeatedly using Phenix and COOT. The refined structure was validated by Phenix and the PDB validation server (https://validate-rcsb-1.wwpdb.org/). The final refined structure was deposited in Protein Data Bank with the access ID 8INH. The diffraction data and structure refinement statistics are summarized in Table S4.

1.11 Site-directed mutagenesis of ZjOGT3 and enzyme activity assay

The mutants of ZjOGT3 were constructed using a Fast Mutagenesis System kit (TransGen Biotech, China) according to the manufacturer's instructions. The corresponding degenerate primers designed to construct the site-directed mutants are listed in Table S2. After verification of the mutant sequences, the recombinant plasmids were transformed into *E. coli* BL21(DE3) for heterologous expression. The protein expression, purification, and enzyme activity assays of mutants were performed under the same conditions as described above (Figure S85).

1.12 DFT calculations

DFT calculations were performed with the Gaussian 16 package[1]. The geometry optimizations of minima and transition states involved were carried out at the B3LYP-D3/6-31+G(d,p) level. The vibrational frequency calculations were calculated at the same level to ensure that all of the stationary points were transition states (one imaginary frequency) or minima (no imaginary frequency) and to evaluate zero-point vibrational energies (ZPVE) and thermal corrections at 298K. Single-point energy calculations were performed at the B3LYP-D3 level with the 6-311++G(2d,p) basis set. Solvation by

water was taken into account by using the CPCM model[2-4] for all above calculations. Gibbs free energy is the sum of the electronic energy and ZPVE and thermal corrections.

1.13 Initial structural preparation

The initial structure of wild type ZjOGT3 in complex with UDP-Glc was based on the crystal structure (PDB ID: 7EFK). The geometry of UDP-Glc was fully optimized at the B3LYP-D3/6-31+G(d) level of Gaussian 16 using the CPCM model in water, and the partial charges were fitted with HF/6-31G(d) calculations and the restrained electrostatic potential (RESP)[5-6] protocol implemented by the Antechamber module. The protonation states of charged residues were determined at constant pH 8.0 based on pKa calculations via the H++ program[7] and the consideration of the local hydrogen bonding network. His19, 44, 155, 158, 191, 323, 351, and 360 were assigned as HIE, and the rest His were HID. All Asp and Glu residues were deprotonated, while Lys and Arg were protonated. The system was neutralized by adding Na^+ ions and solvated into a rectangular TIP3P[8] water box with a 10-Å buffer distance on each side. A representative MD pre-equilibrated binary complex structure was used as the target receptor for molecular docking.

1.14 Molecular docking

20000 snapshots uniformly distributed at equal intervals from the last 40 ns MD simulation (with time intervals of 2ps) were picked up and divided into ten groups using hierarchical agglomerative (bottom-up) approach[9]. The anionic intermediates of substrates **2**, **10** and **12** were fully optimized at the B3LYP-D3/6-31+G(d,p) level of Gaussian 16 using the CPCM model in water, and then docked into the active site of one representative group snapshot to gain the ternary complex. Molecular docking was performed using the Lamarckian genetic algorithm local search method in the AutoDock 4.2 and AutoDockTools-1.5.6[10]. The docking approach was employed on rigid-receptor conformation, while all the rotatable torsional bonds of all substrates were set free. A grid box was centered on the carbon atom being attacked of UDP-Glc and its size was set to 40 Å × 40 Å × 40 Å points with a 0.375 Å spacing. A total of 500 independent docking runs were undertaken with a maximum energy evaluation of 2.5×10^7. The obtained 500 docked conformations were clustered with 2.0 Å RMSD and ranked depending on an energy-based scoring function. The possible catalytically active binding modes were selected as initial configurations to perform MD simulations of the ternary complexes according to scoring function and reasonable conformation.

1.15 Molecular dynamics simulations

All molecular dynamics (MD) simulations were performed by Amber 16 package[11]. The pre-equilibrated ZjOGT3 in complex with UDP-Glc binary complex structures and possible catalytically reactive binding modes of substrates were used as the starting conformations for MD simulations on the ternary complexes. Acceptor substrates were fully optimized at the B3LYP-D3/6-31+G(d,p) level of Gaussian 16 using the CPCM model in water, and the partial charges were fitted with HF/6-31G(d) calculations and RESP protocol implemented by the Antechamber module. The force field parameters for substrates were adapted from the standard general amber force field 2.0 (gaff2)[12] parameters, while the standard Amber14SB force field was applied to describe the protein.

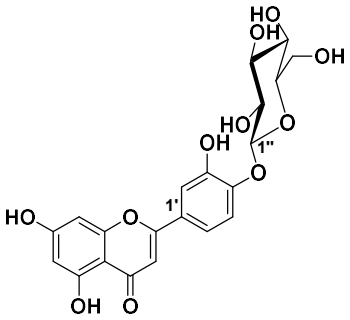
Each system was neutralized by adding Na^+ ions and solvated into a rectangular TIP3P water box with a 10-Å buffer distance on each side. After equilibrated with a series of minimizations interspersed by short MD simulations during which restraints on the protein backbone heavy atoms were gradually released [with force constant of 10, 2, 0.1 and 0 kcal/(mol·Å^2)], each system was heated from 0 to 318 K for 50 ps. Finally, 200 ns MD simulations with periodic boundary condition at constant temperature and pressure were carried out. The pressure was maintained at 1 atm and coupled with isotropic position scaling. The temperature was controlled at 318 K with Berendsen thermostat method. Long-range electrostatic interactions were treated with particle mesh Ewald (PME)[13] method and 12-Å cutoff was applied to both PME and van der Waals (vdW) interactions. Time step of 2 fs was employed along with SHAKE algorithm for hydrogen atoms, and periodic boundary condition was used. Each system was checked for stability (structure, energy, and temperature fluctuations) and convergence (root mean square deviations-RMSD of structures).

1.16 MM/GBSA calculations

The binding free energies of ZjOGT3 in complex with UDP-Glc and substrates were calculated using the Molecular Mechanics Generalized Born Surface Area (MM/GBSA) method in the MMPBSA.py program[14] in AMBER 16 package. Before calculation, the water and ions were stripped from the last 1 ns of MD trajectory of the complexes. ΔG_{bind} is based on the following equation: $\Delta G_{bind} = \Delta E_{vdw} + \Delta E_{ele} + \Delta G_{pol} + \Delta G_{nonpol} - T\Delta S$, where ΔE_{vdw} and ΔE_{ele} are van der Waals and electrostatic components in the gas phase, and ΔG_{pol} and ΔG_{nonpol} indicate the polar and nonpolar solvent interaction energies, respectively. ΔE_{vdw} and ΔE_{ele} were calculated using the Amber14SB force field (ff14sb). ΔG_{pol} was calculated by solving the Generalized Born (GB) equations. $\Delta G_{nonpol} = 0.0050 \times SASA + 0.000$, where

SASA referred to the solvent accessible surface buried upon binding. Since the normal mode calculation is extremely time-consuming for large systems, we estimated the ΔG_{bind} ignoring entropy. Here, we used GB model with igb=1 and the mbondi2 radii set.

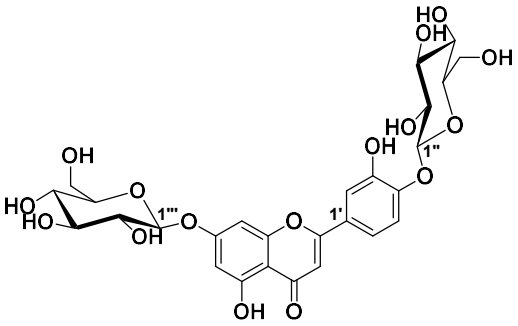
2. MS, ^{1}H and ^{13}C NMR spectral data for glycosylated products



3a

Luteolin 4'-*O*-*β*-D-glucoside (3a): ESI-MS *m*/*z* 447.0922 $[M-H]^{-}$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.91 (1H, s, 5-OH), 7.51 (1H, dd, *J*=8.5 2.1 Hz, H-6′), 7.49 (1H, d, *J*=2.1 Hz, H-2′), 7.24 (1H, d, *J*=8.5 Hz, H-5′), 6.83 (1H, s, H-3), 6.50 (1H, d, *J*=2.0 Hz, H-8), 6.21 (1H, d, *J*=2.0 Hz, H-6), 4.89 (1H, d, *J*=7.2 Hz, H-1″).[15]

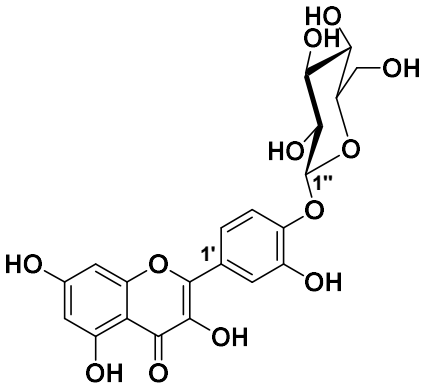
^{13}C NMR (DMSO-d_6, 100 MHz): δ 181.8 (C-4), 164.3 (C-7), 163.2 (C-2), 161.5 (C-5), 157.4 (C-9), 148.6 (C-4′), 147.0 (C-3′), 124.7 (C-1′), 118.6 (C-6′), 116.1 (C-5′), 113.6 (C-2′), 104.0 (C-10), 103.8 (C-3), 101.2 (C-1″), 99.0 (C-6), 94.1 (C-8), 77.3 (C-5″), 75.9 (C-3″), 73.3 (C-4″), 69.8 (C-2″), 60.7 (C-6″).[15]



3b

Luteolin 7,4'-di-*O*-glucoside (3b): ESI-MS *m*/*z* 609.1448 $[M-H]^{-}$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.91 (1H, s, 5-OH), 7.55 (1H, dd, *J*=8.4 2.1 Hz, H-6′), 7.54 (1H, d, *J*=2.1 Hz, H-2′), 7.26 (1H, d, *J*=8.4 Hz, H-5′), 6.92 (1H, s, H-3), 6.86 (1H, d, *J*=2.0 Hz, H-8), 6.45 (1H, d, *J*=2.0 Hz, H-6), 5.09 (1H, d, *J*=7.2 Hz, H-1‴), 4.89 (1H, d, *J*=7.2 Hz, H-1″).[15]

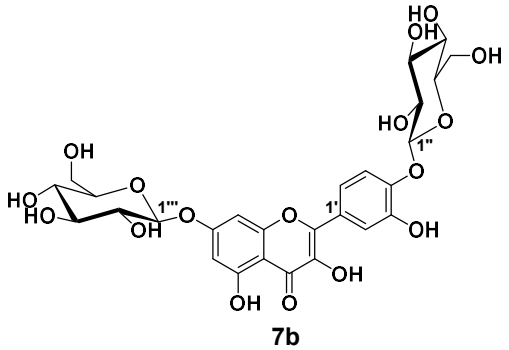
^{13}C NMR (DMSO-d_6, 100 MHz): δ 182.0 (C-4), 163.7(C-2), 163.0 (C-7), 161.1 (C-5), 157.0 (C-9), 148.7 (C-4′), 146.9 (C-3′), 124.5 (C-1′), 118.7 (C-6′), 116.0 (C-2′), 113.7 (C-5′), 105.4 (C-3), 104.2 (C-10), 101.3 (C-1″), 99.8 (C-1‴), 99.6 (C-6), 94.8 (C-8), 77.3 (C-3″), 77.1 (C-5″), 76.4 (C-5‴), 75.8 (C-3‴), 73.2 (C-2‴), 73.1 (C-2″), 69.8 (C-4″), 69.5 (C-4‴), 60.7 (C-6″), 60.6 (C-6‴).[15]



7a

Quercetin 4'-*O*-*β*-D-glucoside (**7a**)**:** ESI-MS *m*/*z* 463.0872 [M−H]$^-$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.41 (1H, s, 5-OH), 7.70 (1H, d, *J*=2.1 Hz, H-2′), 7.62 (1H, dd, *J*=8.8 2.1 Hz, H-6′), 7.24 (1H, d, *J*=8.8 Hz, H-5′), 6.45 (1H, d, *J*=2.0 Hz, H-8), 6.20 (1H, d, *J*=2.0 Hz, H-6), 4.84 (1H, d, *J*=7.2 Hz, H-1″).[16]

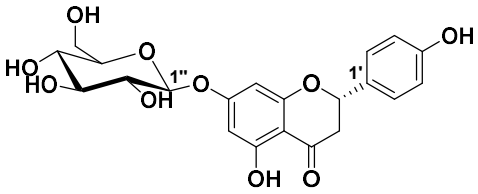
^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.0 (C-4), 164.1 (C-7), 160.7 (C-5), 156.2 (C-9), 146.8 (C-3′), 146.4 (C-2), 145.9 (C-4′), 136.4 (C-3), 125.1 (C-1′), 118.7 (C-6′), 115.8 (C-2′), 115.1 (C-5′), 103.1 (C-10), 101.4 (C-1″), 98.3 (C-6), 93.5 (C-8), 77.3 (C-3″), 76.0 (C-5″), 73.3 (C-2″), 69.8 (C-4″), 60.7 (C-6″).[16]



7b

Quercetin 7,4'-di-*O*-glucoside (**7b**): ESI-MS *m*/*z* 625.1355 [M−H]$^-$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.42 (1H, s, 5-OH), 7.75 (1H, d, *J*=2.1 Hz, H-2′), 7.65 (1H, dd, *J*=8.8, 2.1 Hz, H-6′), 7.26 (1H, d, *J*=8.8 Hz, H-5′), 6.82 (1H, d, *J*=2.0 Hz, H-8), 6.42 (1H, d, *J*=2.0 Hz, H-6), 5.09 (1H, d, *J*=7.2 Hz, H-1‴), 4.85 (1H, d, *J*=6.8 Hz, H-1″).[16]

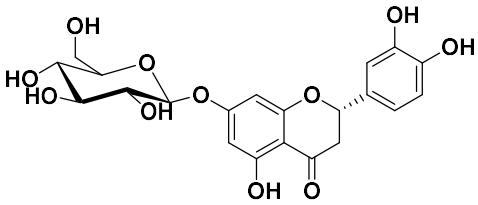
^{13}C NMR (DMSO-d_6, 100 MHz): δ 176.3 (C-4), 162.8 (C-7), 160.3 (C-5), 155.8 (C-9), 147.0 (C-3′), 146.6 (C-2), 146.4 (C-4′), 136.8 (C-3), 125.0 (C-1′), 119.6 (C-2′), 115.8 (C-5′), 105.7 (C-10), 101.4 (C-1″), 99.8 (C-1‴), 98.8 (C-6), 94.3 (C-8), 77.3 (C-3″), 77.1 (C-5‴), 76.4 (C-3‴), 75.9 (C-5″), 73.3 (C-2″), 73.1 (C-2‴), 69.8 (C-4″), 69.5 (C-4‴), 60.7 (C-6″), 60.6 (C-6‴).[16]



10a

Naringenin 7-*O*-*β*-D-glucoside (**10a**): ESI-MS *m*/*z* 433.1100 $[M-H]^-$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.06 (1H, s, 5-OH), 7.33 (2H, d, *J*=8.4 Hz, H-2′, H-6′), 6.80 (2H, d, *J*=8.4 Hz, H-3′, 5′), 6.15 (1H, d, *J*=2.2 Hz, H-8), 6.14 (1H, d, *J*=2.2 Hz, H-6), 5.50 (1H, dt, *J*=12.8 2.8 Hz, H-2), 4.98 (1H, d, *J*=7.6 Hz, H-1″).[17]

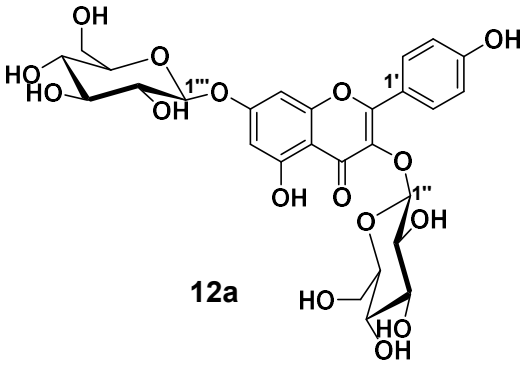
^{13}C NMR (DMSO-d_6, 100 MHz): δ 197.2 (C-4), 165.3 (C-7), 162.9 (C-5), 162.8 (C-9), 157.8 (C-4′), 128.6 (C-2′, C-6′), 128.4 (C-1′), 115.2 (C-3′, C-5′), 103.2 (C-10), 99.6 (C-1″), 96.5 (C-6), 95.4 (C-8), 78.7 (C-2), 77.1 (C-5″), 76.3 (C-3″), 73.0 (C-2″), 69.5 (C-4″), 60.6 (C-6″), 42.1 (C-3).[18]



11a

Eriodictyol 7-*O*-*β*-D-glucoside (**11a**): ESI-MS *m*/*z* 449.1050 $[M-H]^-$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.06 (1H, s, 5-OH), 6.88 (1H, s, H-2′), 6.75 (2H, s, H-5′, 6′), 6.14 (2H, m, H-6, 8), 5.44 (1H, dd, *J*=12.0 3.6 Hz, H-2), 4.97(1H, m, H-1″), 3.14 (1H, dd, *J*=18.4, 9.2 Hz, H_{ax}-3), 2.73 (1H, dd, *J*=18.4, 4.4 Hz, H_{eq}-3). [19]

^{13}C NMR (DMSO-d_6, 100 MHz): δ 197.2 (C-4), 165.3 (C-7), 162.9 (C-5), 162.7 (C-9), 145.8 (C-3′), 145.2 (C-4′), 129.2 (C-1′), 118.1 (C-6′), 115.3(C-2′), 114.4 (C-5′), 103.2 (C-10), 99.6 (C-1″), 96.4 (C-6), 95.4 (C-8), 78.7 (C-2), 77.0 (C-5″), 76.3 (C-3″), 73.0 (C-2″), 69.5 (C-4″), 60.6 (C-6″), 42.2 (C-3). [19]

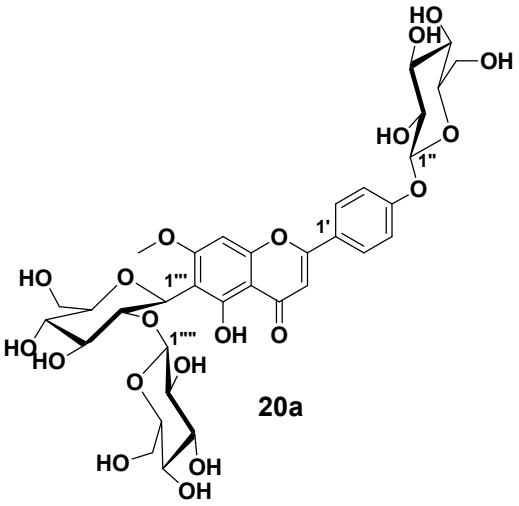


12a

Astragalin 7-*O*-*β*-D-glucopyranoside (**12a**): ESI-MS *m*/*z* 609.1443 $[M-H]^-$. ^{1}H NMR (DMSO-d_6,

400 MHz): δ 12.6 (1H, s, 5-OH), 8.06 (2H, d, *J*=8.8 Hz, H-2′, 6′), 6.90 (2H, d, *J*=8.8 Hz, H-3′, 5′), 6.79 (1H, d, *J*=2.0 Hz, H-8), 6.44 (1H, d, *J*=2.0 Hz, H-6), 5.48 (1H, d, *J*=7.2 Hz, H-1″), 5.08 (1H, d, *J*=7.2 Hz, H-1‴). [20]

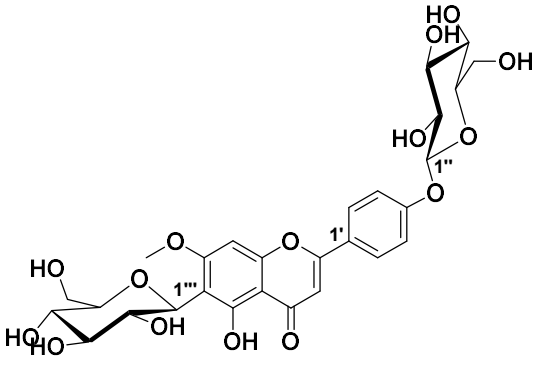
^{13}C NMR (DMSO-d_6, 100 MHz): δ 177.6 (C-4), 162.8 (C-7), 160.8 (C-5), 160.1 (C-4′), 156.8 (C-9), 131.0 (C-6′), 120.8 (C-1′), 115.1 (C-3′, C-5′), 105.6 (C-10), 100.7 (C-1″), 99.7 (C-1‴), 99.3 (C-6), 94.5 (C-8), 77.5 (C-5″), 77.2 (C-5‴), 76.4 (C-3″, C-3‴), 74.2 (C-2″), 73.1 (C-2‴), 69.9 (C-4″), 69.6 (C-4‴), 60.8 (C-6″), 60.6 (C-6‴). [20]



20a

Spinosin 4'-*O*-*β*-D-glucoside (**20a**): ESI-MS *m*/*z* 769.2177 [M−H]$^-$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.10 (2H, d, *J*=9.0 Hz, H-2′, 6′), 7.22 (2H, d, *J*=9.0 Hz, H-3′, 5′), 5.04 (1H, d, *J*=7.2 Hz, H-1′′′′), 4.69 (1H, d, *J*=6.9 Hz, H-1″), 4.17 (1H, d, *J*=8.0 Hz, H-1‴).

^{13}C NMR (DMSO-d_6, 100 MHz): δ 182.1 (C-4), 165.1 (C-7), 163.2 (C-2), 160.8 (C-4′), 160.5 (C-5), 157.3 (C-9), 128.2 (C-2′, 6′), 123.9 (C-1′), 116.6 (C-3′, 5′), 108.7 (C-6), 105.4 (C-1‴), 105.2 (C-10), 104.3 (C-3), 100.0 (C-1′′′′), 90.4 (C-8), 81.9 (C-5″), 81.9 (C-2″), 78.7 (C-3″), 77.2 (C-5‴), 77.2 (C-5′′′′), 76.7 (C-3′′′′), 76.6 (C-3‴), 74.6 (C-2‴), 73.2 (C-2′′′′), 71.1 (C-1″), 70.4 (C-4″), 69.7 (C-4‴), 69.5 (C-4′′′′), 61.5 (C-6″), 60.6 (C-6‴), 60.6 (C-6′′′′), 56.6 (7-OCH$_3$).

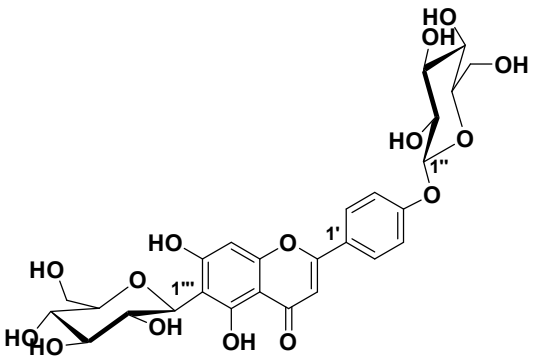


21a

Swertisin 4'-*O*-*β*-D-glucoside (**21a**): ESI-MS *m*/*z* 667.1614 [M−H]$^-$. ^{1}H NMR (DMSO-d_6, 400

MHz):δ 13.40 (1H, s, 5-OH), 8.09 (2H, d, *J*=9.0 Hz, H-2′, 6′), 6.97 (2H, d, *J*=9.0 Hz, H-3′, H-5′), 5.04 (1H, d, *J*=7.2 Hz, H-1‴), 4.59(1H, m, H-1″).[21]

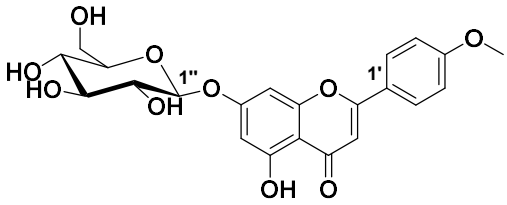
^{13}C NMR (DMSO-d_6, 100 MHz): δ 182.4 (C-4), 165.1 (C-7), 163.8 (C-2), 160.4 (C-5), 159.5 (C-4′), 156.9 (C-9), 128.2 (C-2′, 6′), 123.7 (C-1′), 116.6 (C-3′, 5′), 109.8 (C-6), 104.7 (C-3), 104.0 (C-10), 99.8 (C-1‴), 90.3 (C-8), 81.9 (C-5″), 79.1 (C-3″), 77.2 (C-5‴), 76.6 (C-3‴), 73.2 (C-2‴), 72.8 (C-1″), 70.8 (C-4″), 69.7 (C-2″), 69.6 (C-4‴), 61.7 (C-6″), 60.7 (C-6‴), 56.5 (7-OCH_3). [21]



22a

Isovitexin 4'-*O*-*β*-D-glucoside (**22a**): ESI-MS *m*/*z* 593.1497 [M−H]$^-$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.49 (1H, s, 5-OH), 8.04 (2H, d, *J*=8.9 Hz, H-2′, 6′), 7.19 (2H, d, *J*=8.9 Hz, H-3′, 5′), 6.89 (1H, s, H-3), 6.55 (1H, s, H-8), 5.03 (1H, d, *J*=7.3 Hz, H-1‴), 4.60 (1H, d, *J*=9.8 Hz, H-1″), 4.04 (1H, dd, *J*=9.8, 7.7 Hz, H-2″), 3.71 (1H, overlapped, H-6‴), 3.69 (1H, overlapped, H-6″), 3.48 (1H, m, H-6‴). [22]

^{13}C NMR (DMSO-d_6, 100 MHz): δ 182.0 (C-4), 163.4 (C-7), 162.9 (C-2), 160.6 (C-5), 160.3 (C-4′), 156.3 (C-9), 128.2 (C-2′, 6′), 123.9 (C-1′), 116.6 (C-3′, 5′), 109.0 (C-6), 103.8 (C-3), 103.5 (C-10), 99.9 (C-1‴), 93.7 (C-8), 81.6 (C-5″), 78.9 (C-3″), 77.2 (C-5‴), 76.5 (C-3‴), 73.2 (C-2‴), 73.0 (C-1″), 70.6 (C-4″), 70.2 (C-2″), 69.6 (C-4‴), 61.5 (C-6″), 60.6 (C-6‴). [22]



25a

Acacetin 7-*O*-*β*-D-glucoside (**25a**): ESI-MS *m*/*z* 491.1181 [M−H+HCOOH]$^-$. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.91 (1H, s, 5-OH), 8.07 (2H, d, *J* = 9.0 Hz, H-2′, 6′), 7.13 (2H, d, *J* = 9.0 Hz, H-3′, 5′), 6.95 (1H, s, H-3), 6.46 (1H, d, *J*=2.2 Hz, H-8), 6.86 (1H, d, *J*=2.2 Hz, H-6), 5.07 (1H, d, *J*=7.7 Hz, H-1″), 3.86 (3H, s, 4′-OCH_3).[23]

^{13}C NMR (DMSO-d_6 ,100 MHz): δ 182.1 (C-4), 163.1 (C-2), 162.9 (C-7), 162.1 (C-4′), 161.1 (C-5),

157.0 (C-9), 128.5 (C-2′, 6′), 122.7 (C-1′), 114.7 (C-3′, 5′), 105.5 (C-10), 103.4 (C-3), 100.0 (C-1″), 99.6 (C-6), 95.0 (C-8), 77.2 (C-3″), 76.5 (C-5″), 73.2 (C-2″), 69.5 (C-4″), 60.6 (C-6″), 55.6 (4′-OCH_3).[23]

3. Supplementary Tables

Table S1. Accession numbers of plant GTs used in this study.

Name	Organism	Genbank accession number
ZjOGT3	***Ziziphus jujuba* var. *spinosa***	**UGT84A68**
NmF4′GT (UGT88P1)	*Nemophila menziesii*	LC328827
UGT88A1	*Arabidopsis thaliana*	Q9LK73
UGT73A10	*Lycium barbarum*	AB360612
MdPh-4′-OGT	*Malus domestica*	AY786997
OsUGT-3	*Oryza sativa*	CT830931
UGT73A10	*Lycium barbarum*	AB360612
UGT74W1	*Bacopa monniera*	FJ586244
Sb3GT1 (UGT78B4)	*Scutellaria baicalensis*	MK577650
TcCGT1	*Trollius chinensis*	MK644229
UGT708D1	*Glycine max*	BAR73279
UGT708A6	*Zea mays*	NP_001132650.1
SbCGTa	*Scutellaria baicalensis*	MK894443
SbCGTb	*Scutellaria baicalensis*	MK894444
GgCGT	*Glycyrrhiza glabra*	MH998596
OsCGT	*Oryza sativa*	CAQ77160
MiCGT	*Mangifera indica*	ALD83754
GuCGTa	*Glycyrrhiza uralensis*	MK894447
GuCGTb	*Glycyrrhiza uralensis*	MK894448
GtUF6CGT1	*Gentiana triflora*	BAQ19550
PlUGT43	*Pueraria lobata*	A0A172J2G3.2

Table S2. PCR primers used in this study.

Name		Sequence (5′-3')
ZjOGT3	F	TGGACAGCAAATGGGTCGCCGGATGGGGCTACAACCTCCAATCC
	R	TGTCGACGGAGCTCGAATTCGGAGTGGCAGCTGGTTTTGAATTG
ZjOGT 2	F	TGGACAGCAAATGGGTCGCCGGATGGAATCTGAACCTCTTGCCC
	R	TGTCGACGGAGCTCGAATTCGGAATTACTGAATCGACAATCCCA
ZjOGT 6	F	TGGACAGCAAATGGGTCGCCGGATGAAGAAATCATTAGCAGAAC
	R	TGTCGACGGAGCTCGAATTCGGGGAAATATTCTCTATTACATCA
ZjOGT 10	F	TGGACAGCAAATGGGTCGCCGGATGAAGAAAATAAACCTTGTAG
	R	TGTCGACGGAGCTCGAATTCGGAGAGATGTTTGCTAGAATAGCC
ZjOGT 13	F	TGGACAGCAAATGGGTCGCCGGATGCAGAGCTCTAAACCACACG
	R	TGTCGACGGAGCTCGAATTCGGGGCATTCTGTTGAACCTCACAA
ZjOGT 15	F	TGGACAGCAAATGGGTCGCCGGATGAAGAAAGCAGAGTTAGTTT
	R	TGTCGACGGAGCTCGAATTCGGGGACACATTCTTCAAGATATCA
F14A	F	gcCCCAGCTCAAGGCCATATCAATCCCATG
	R	GCCTTGAGCTGGGgcGCAAACTAGAAAGAG
H19A	F	gcTATCAATCCCATGCTCAGATTAGCAAAAC
	R	CATGGGATTGATAgcGCCTTGAGCTGGGAAG
H44A	F	gcCGCCGGCAAAGACATACGACAAGCCAAC
	R	GTCTTTGCCGGCGgcCTCGGTTGTAGAGAAG
R85A	F	gcAGACCTCGACTTCTACGTTCCTCAGCTTG
	R	GAAGTCGAGGTCTgcTCGTTTCGGATCGTC
L87A	F	gcCGACTTCTACGTTCCTCAGCTTGAACTC
	R	GAACGTAGAAGTCGgcGTCTCGTCGTTTCGG
F124A	F	gcaATCCCTTGGGTCTGCGATGTCGCAGAAG
	R	GACCCAAGGGATtgcAGGGTTGTTCACGAAG
Q145A	F	gcATCCTGTGCTGTTTTCAGCTGTTACTATC
	R	AAACAGCACAGGATgcAATCCAAAGAGTTGC
L199A	F	gcAGGAACCGCCATTCTAGGCCAGTTCAAG
	R	GAATGGCGGTTCCTgcGATTTTATAAGGACTAG
I285A	F	gcAGCTTACATCAAGCAAGAGCAAGTGGAAG
	R	CTTGATGTAAGCTgcGGTTCCGAATGAAAT
W382A	F	gcGGGGGATCAGGTAACGAACGCCAAGTTC
	R	TACCTGATCCCCCgcTTGAGGATAAGCCAG

Note: The mutation sites are showed in lower case.

Table S3. UHPLC methods used in this study.

Method	Solvent A	Solvent B	Elution gradient	Substrates
A	Water containing 0.1% formic acid	MeOH	10-10% B, 3 min; 10-50% B, 7 min; 50-50% B, 3 min; 50-100% B, 1 min.	**1, 3, 5, 6, 12, 14, 16, 20-22, 30**
B	Water containing 0.1% formic acid	MeOH	20-45% B, 3 min; 45-45% B, 1 min; 45-100% B, 4.5 min; 100-100% B, 3.5 min.	**2, 4, 7-11, 13, 15, 17-19, 23-29, 31-34**

Table S4. Crystallographic data collection and refinement statistics[a].

	ZjOGT3/UDP
Data collection*	
Space group	$P2_1$
Cell dimensions	
a, *b*, *c* (Å)	61.73, 84.81, 103.63
α, *β*, *γ* (°)	90, 93.33, 90
Resolution (Å)	84.81-2.50 (2.60-2.50)[a]
R_{pim} [b] (%)	9.6 (61.2)
$CC_{1/2}$ (%)	98.4 (74.7)
$I/\sigma I$	5.4 (1.1)
Completeness (%)	97.5 (98.3)
Redundancy	4.1 (4.1)
Refinement	
No. reflections	35988
No. reflections (test sets)	1737
R_{work}/R_{free} (%)	23.98/27.61
No. atoms	7169
Proteins	7160
UDP/H_2O	50/114
B-factors (Å^2)	
Proteins	48.28
UDP/H_2O	41.05/41.53
R.m.s.deviations	
Bond lengths (Å)	0.002
Bond angles (º)	0.481
Ramachandran Plot (%)	
Favored region	96.12%
Allowed region	3.66%
Outliers	0.22%

[a] Numbers in brackets indicate values for the outermost resolution shell. One crystal was used to obtain this dataset.

[b] R_{pim} is a redundancy-independent R factor used to evaluate the diffraction data quality as proposed by Evans.

Table S5. The overall glycosylation free energy barriers (in kcal/mol) for substrates **2**, **10** and **12** from DFT calculations and the binding free energies (in kcal/mol) with standard errors for the anionic intermediates of substrate **2**, **10** and **12** calculated by MM/GBSA.

substrates	$\Delta G_{act,4'}$	$\Delta G_{act,7}$	$\Delta G_{act,4'}$-$\Delta G_{act,7}$	$\Delta G_{bind,4'}$	$\Delta G_{bind,7}$	$\Delta G_{bind,4'}$-$\Delta G_{bind,7}$
2	26.8	25.9	0.9	-21.4 ± 0.9	-15.0 ± 0.4	-6.4
10	30.6	27.3	3.3	-22.4 ± 0.7	-22.1 ± 0.9	-0.3
12	25.0	25.4	-0.4	-32.2 ± 0.8	-36.8 ± 1.1	4.6

4. Supplementary Figures

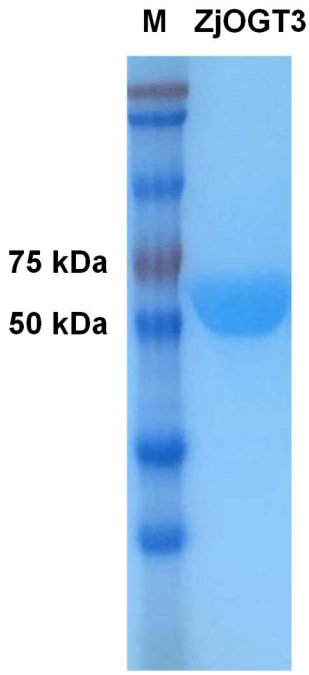


Figure S1. SDS-PAGE of the His-tagged ZjOGT3 purified by affinity chromatography. Lane M: Protein marker.

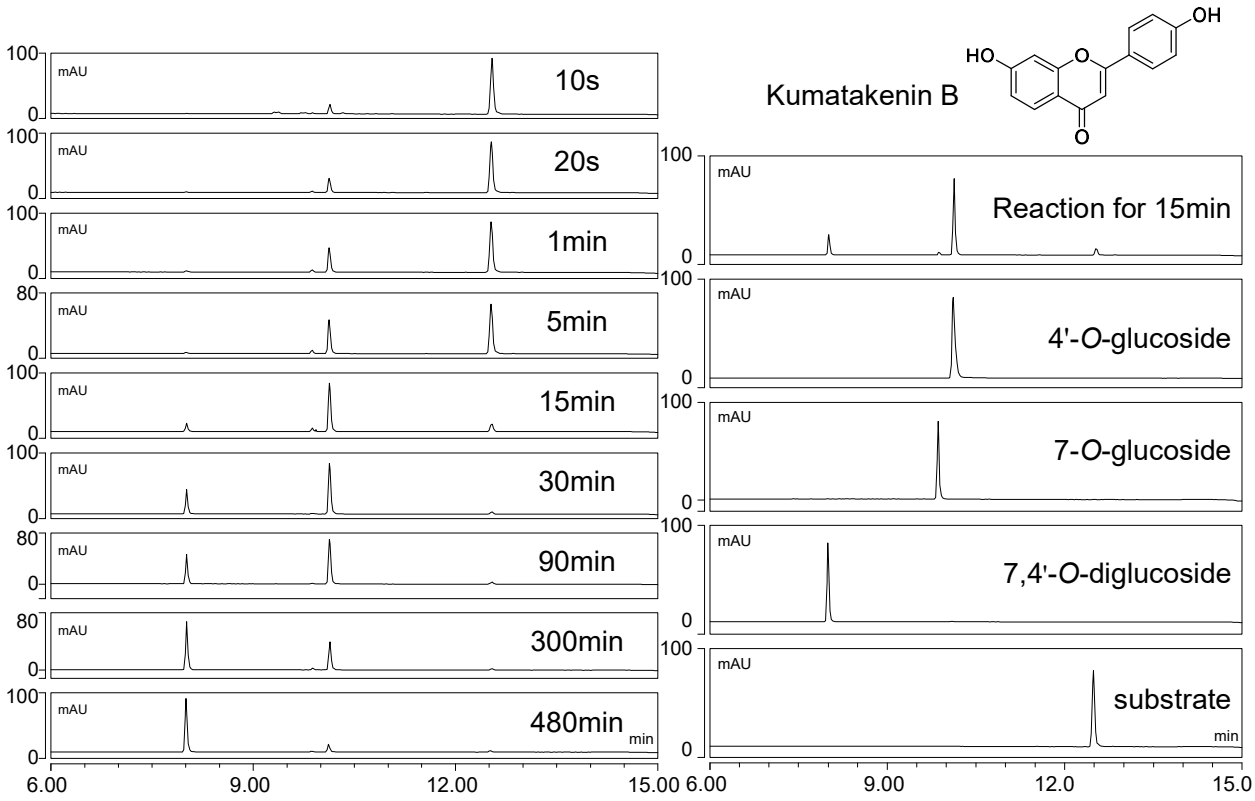


Figure S2. A time-course study of the enzymatic reaction of ZjOGT3. Kumatakenin B (**1**) was used as the acceptor and UDP-Glc was used as the sugar donor.

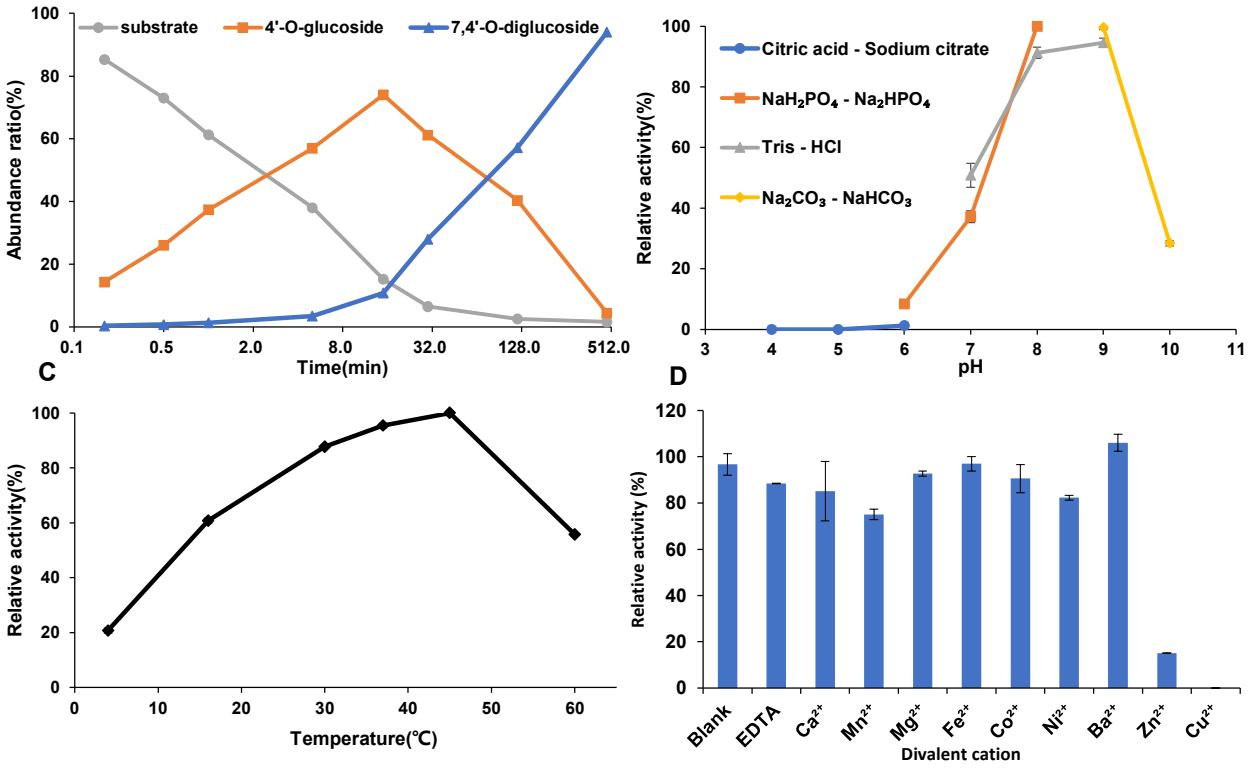


Figure S3. Effects of reaction time (A), reaction buffer (B), temperature (C), and divalent metal ions (D) on glycosylation activity of ZjOGT3. Kumatakenin B (**1**) was used as the acceptor and UDP-Glc was used as the sugar donor. An optimized reaction time of 480 min was used. ZjOGT3 exhibited its maximum activity at pH 8.0 (50 mM NaH_2PO_4-Na_2HPO_4 buffer) and 45ºC, and this enzyme was independent of divalent cations.

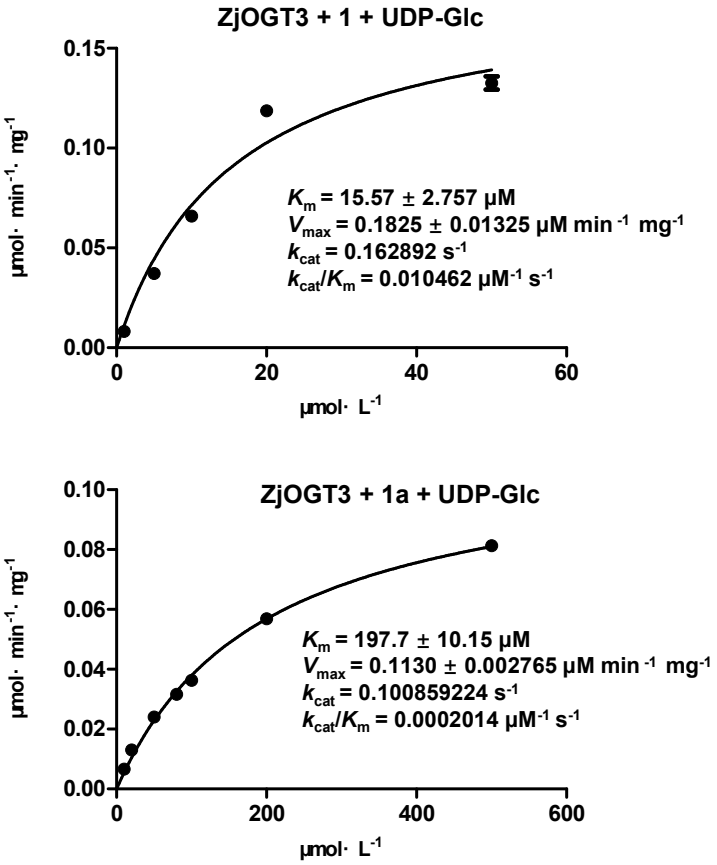


Figure S4. Determination of kinetic parameters for recombinant ZjOGT3. **1** and **1a** were used as sugar acceptor, respectively.

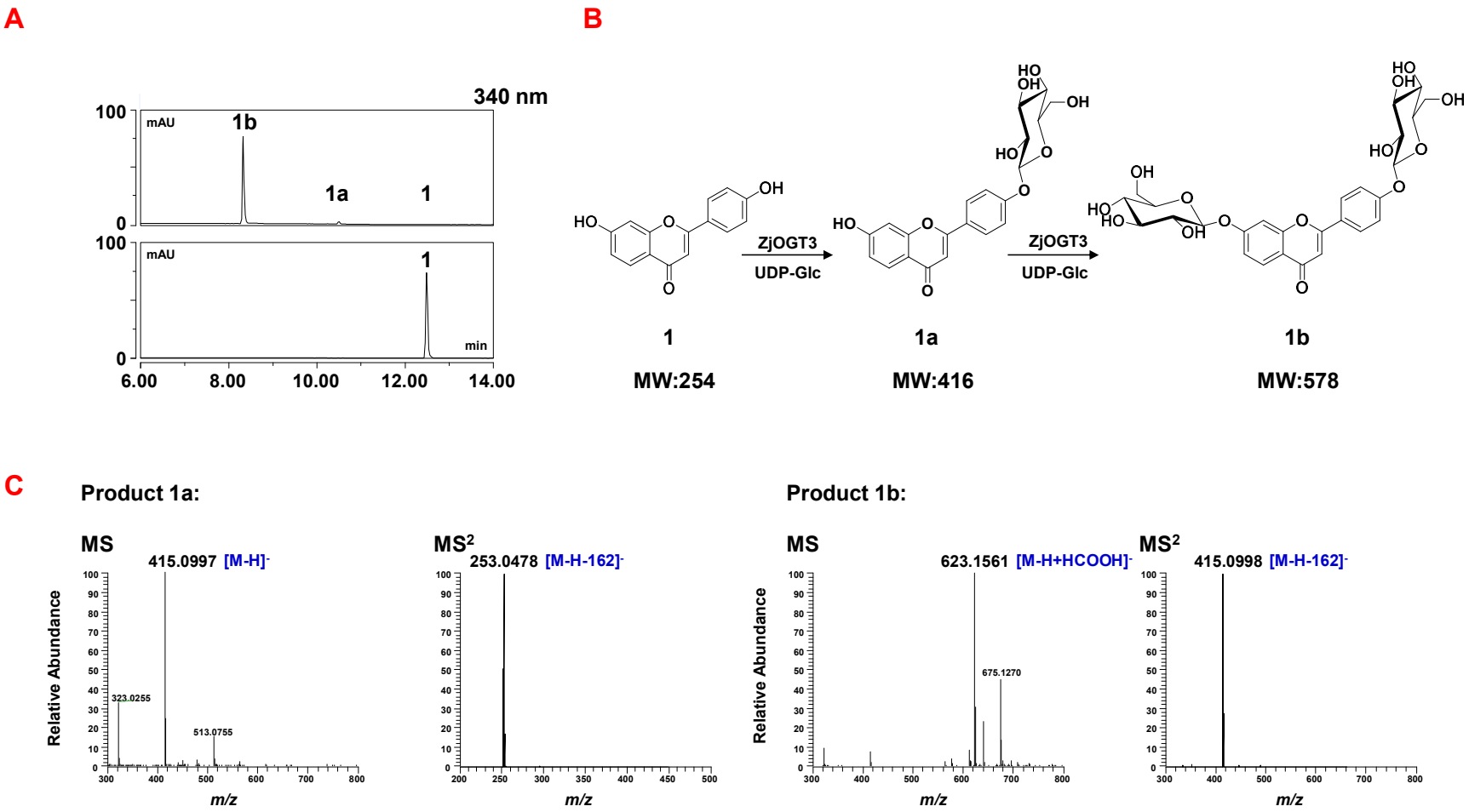


Figure S5. UHPLC/MS analysis of the enzyme catalytic reaction products of **1** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **1**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **1a** and **1b**. The analytical conditions are given in Table S3.

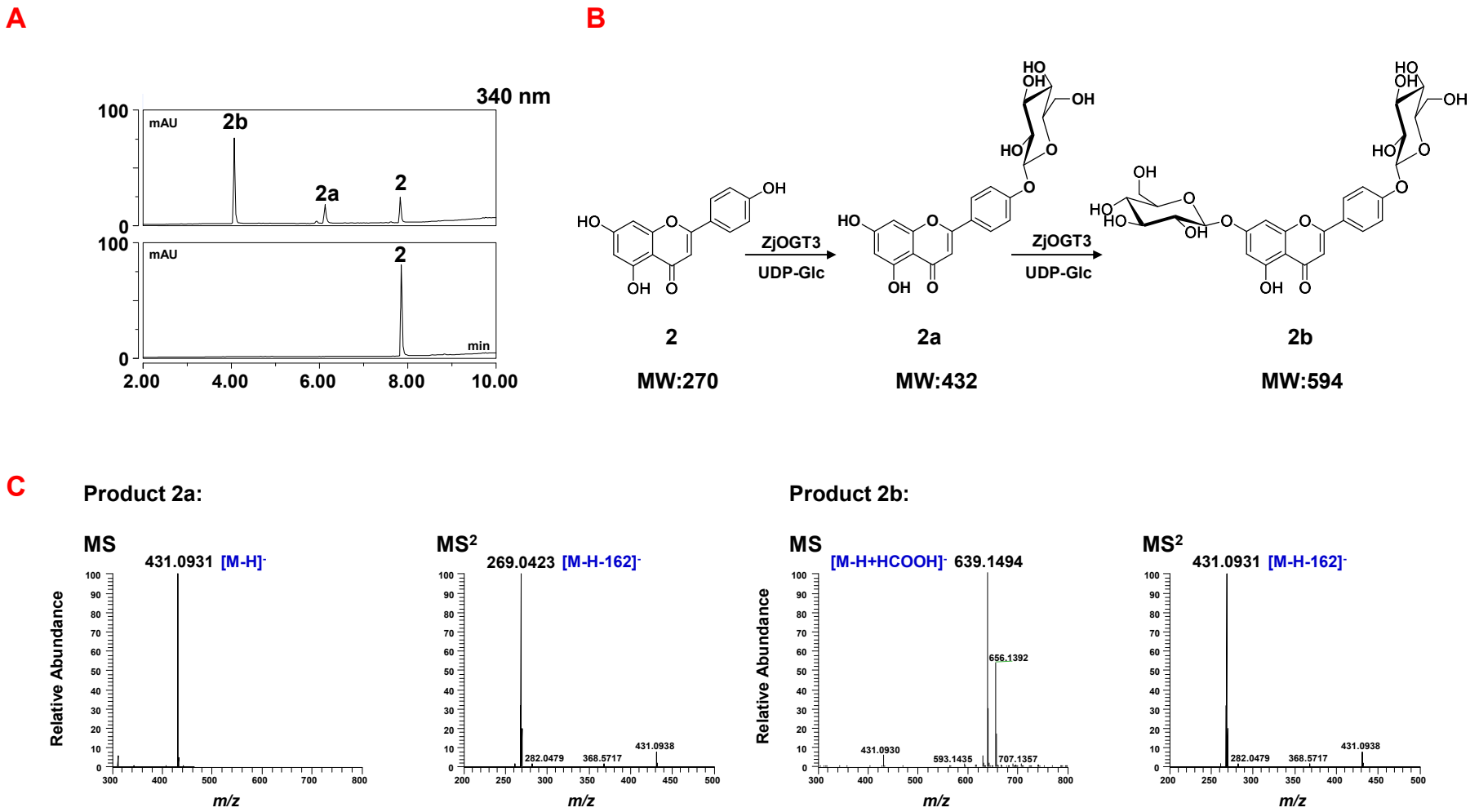


Figure S6. UHPLC/MS analysis of the enzyme catalytic reaction products of **2** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **2**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **2a** and **2b**. The analytical conditions are given in Table S3.

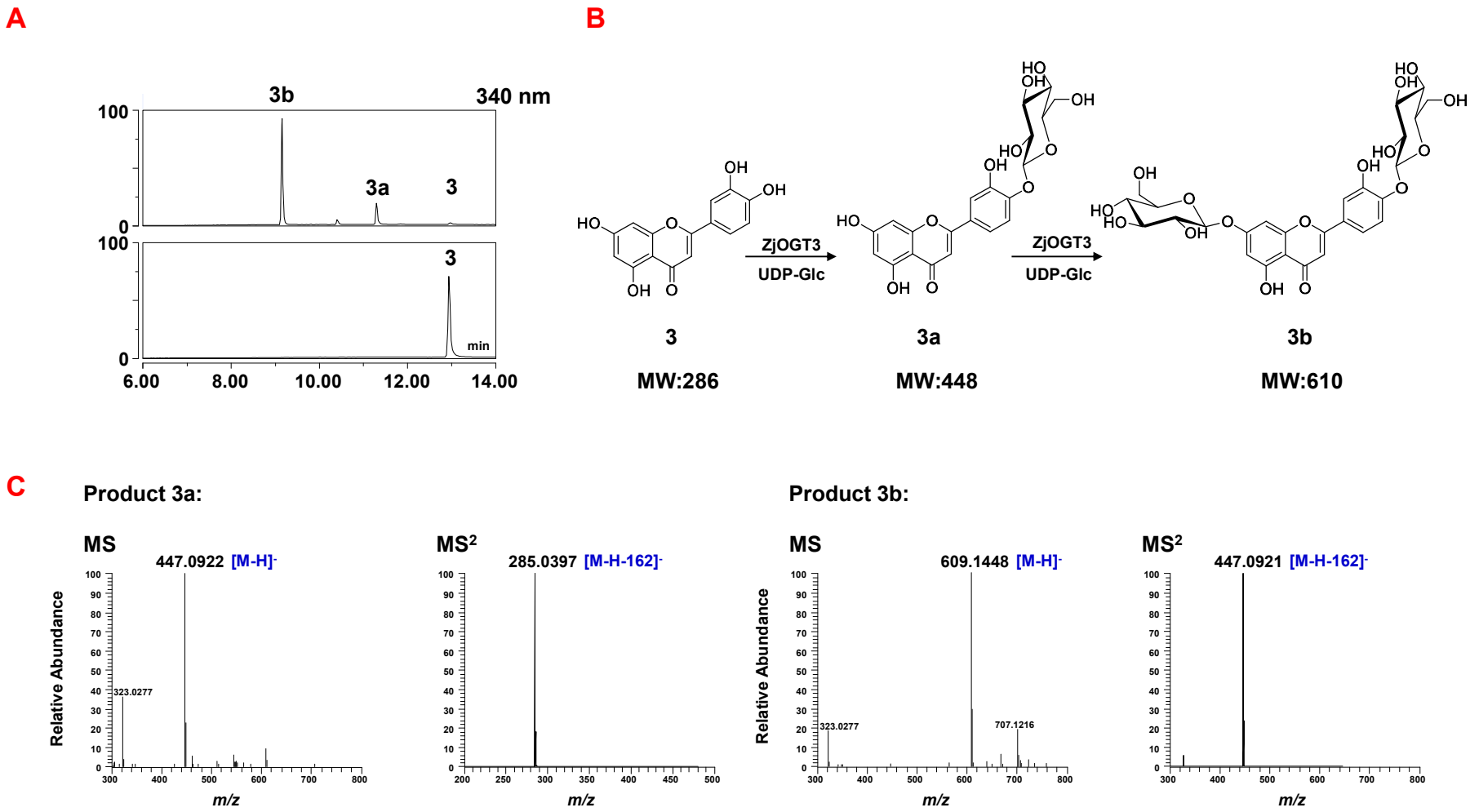


Figure S7. UHPLC/MS analysis of the enzyme catalytic reaction products of **3** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **3**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **3a** and **3b**. The analytical conditions are given in Table S3.

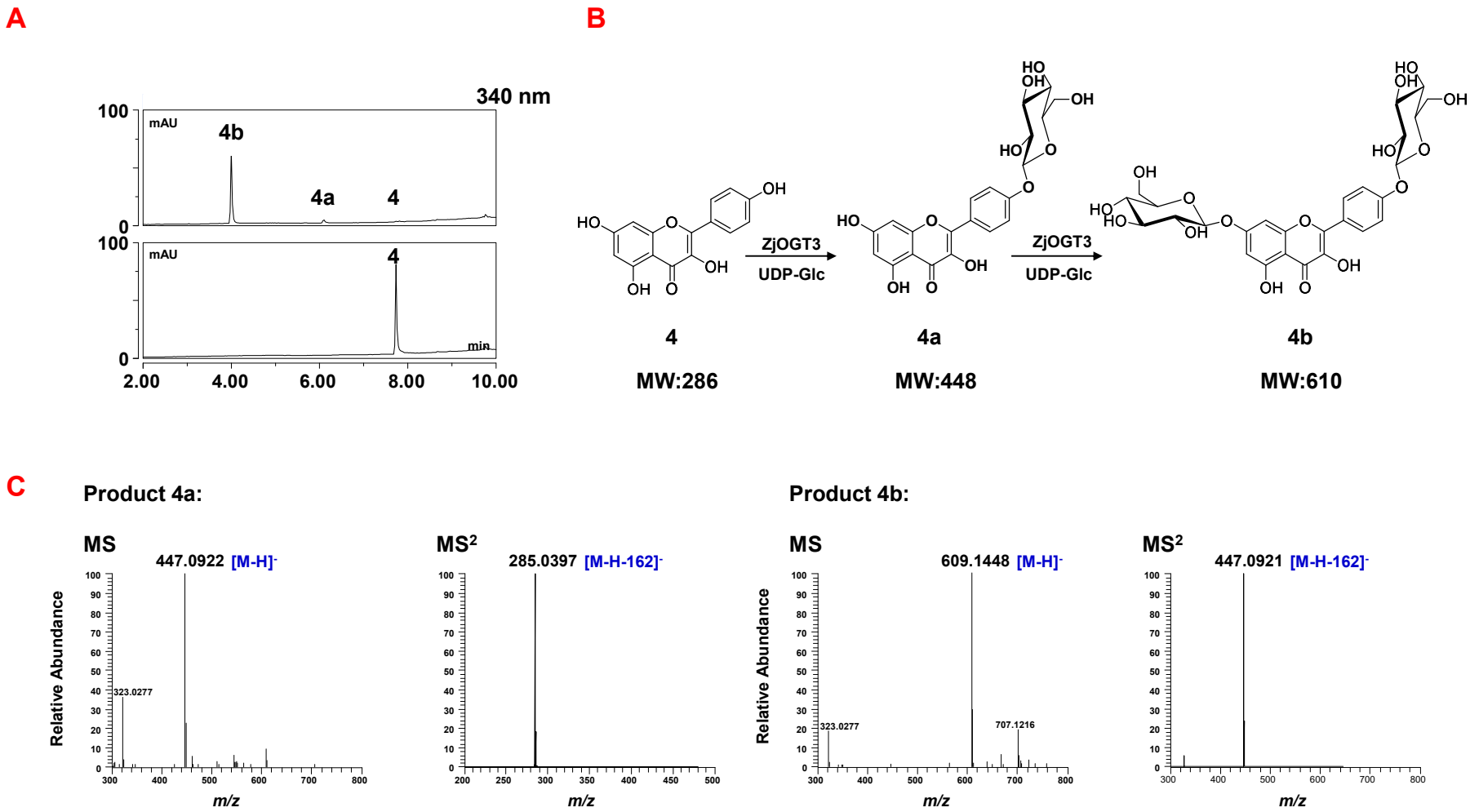


Figure S8. UHPLC/MS analysis of the enzyme catalytic reaction products of **4** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **4**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **4a** and **4b**. The analytical conditions are given in Table S3.

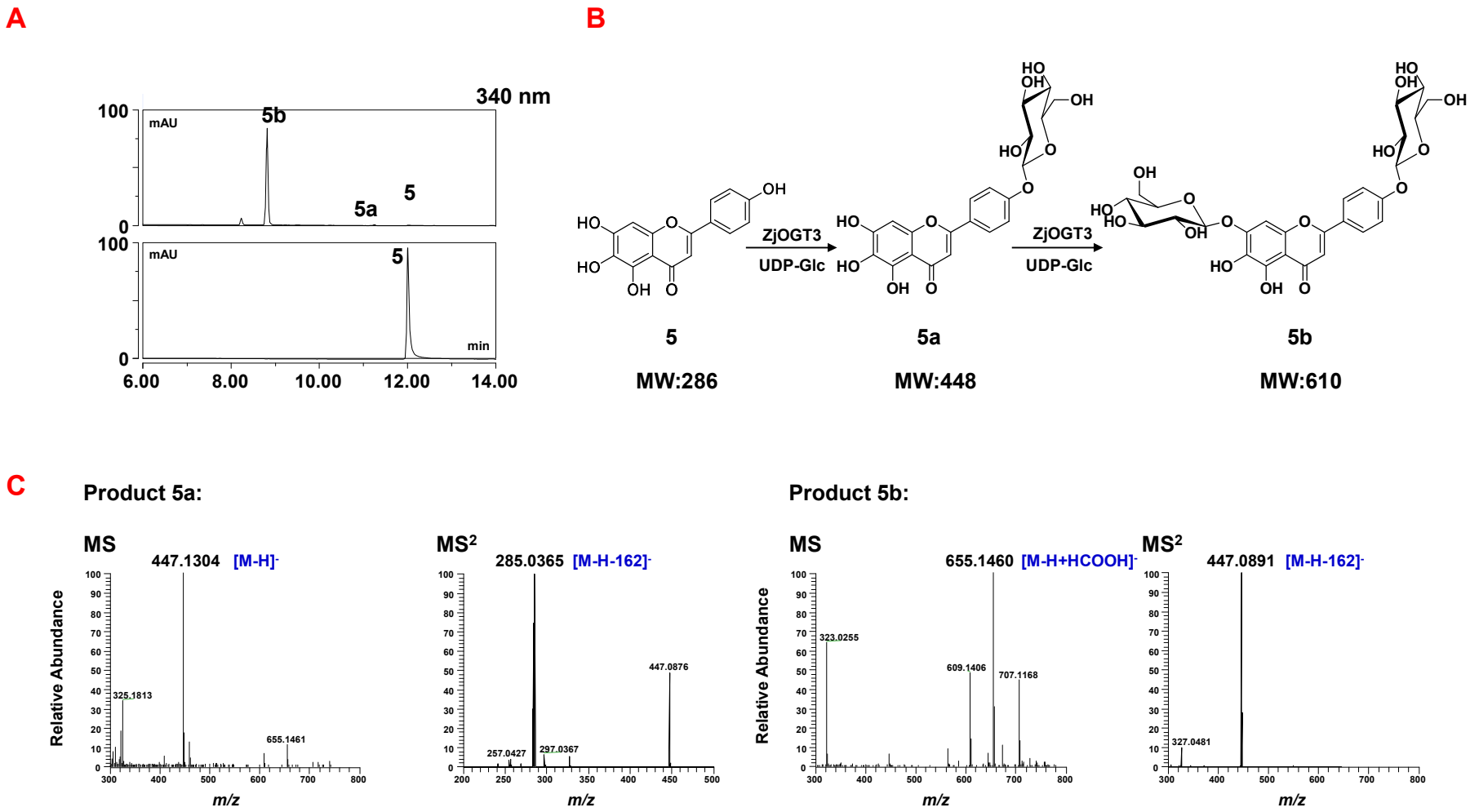


Figure S9. UHPLC/MS analysis of the enzyme catalytic reaction products of **5** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **5**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **5a** and **5b**. The analytical conditions are given in Table S3.

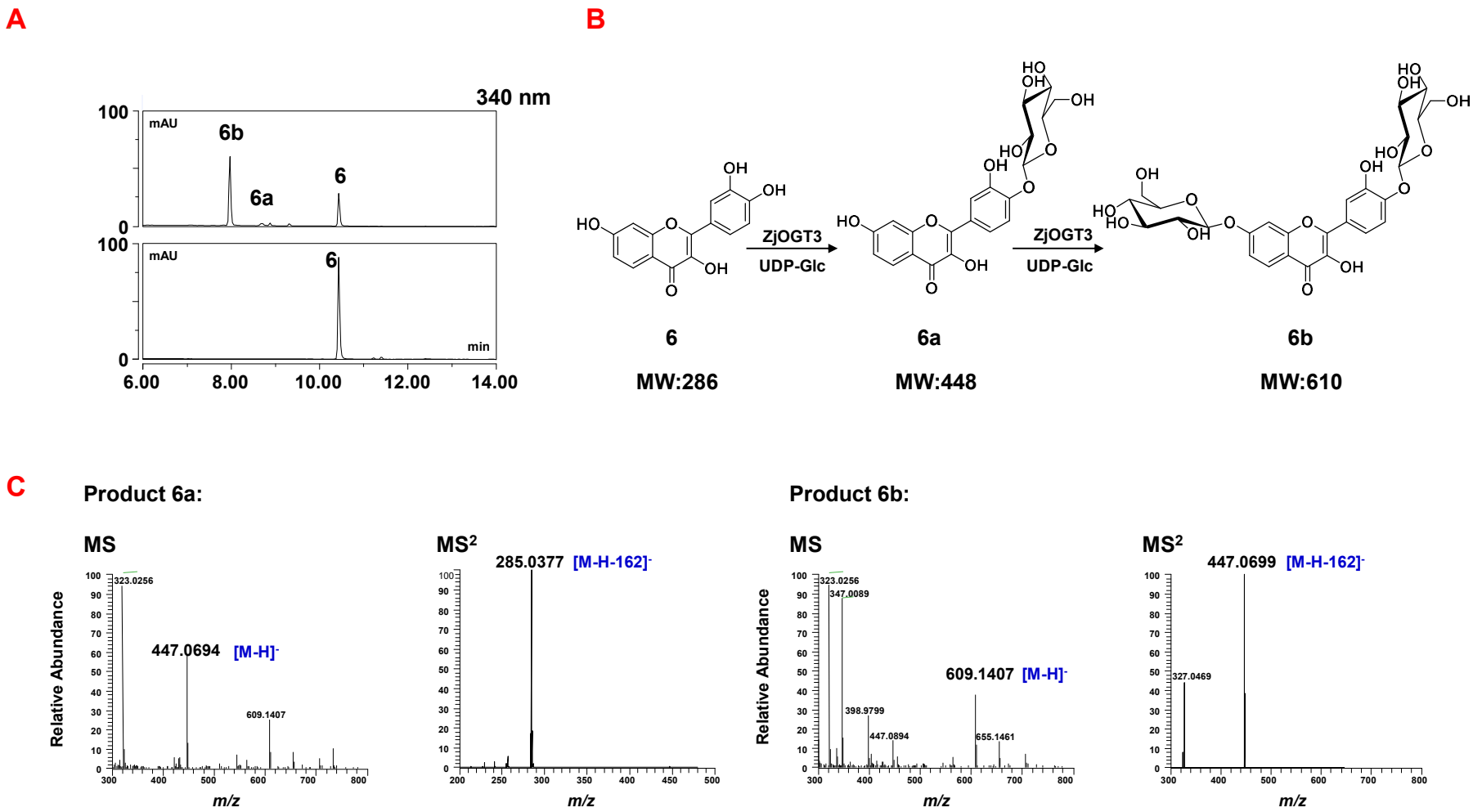


Figure S10. UHPLC/MS analysis of the enzyme catalytic reaction products of **6** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **6**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **6a** and **6b**. The analytical conditions are given in Table S3.

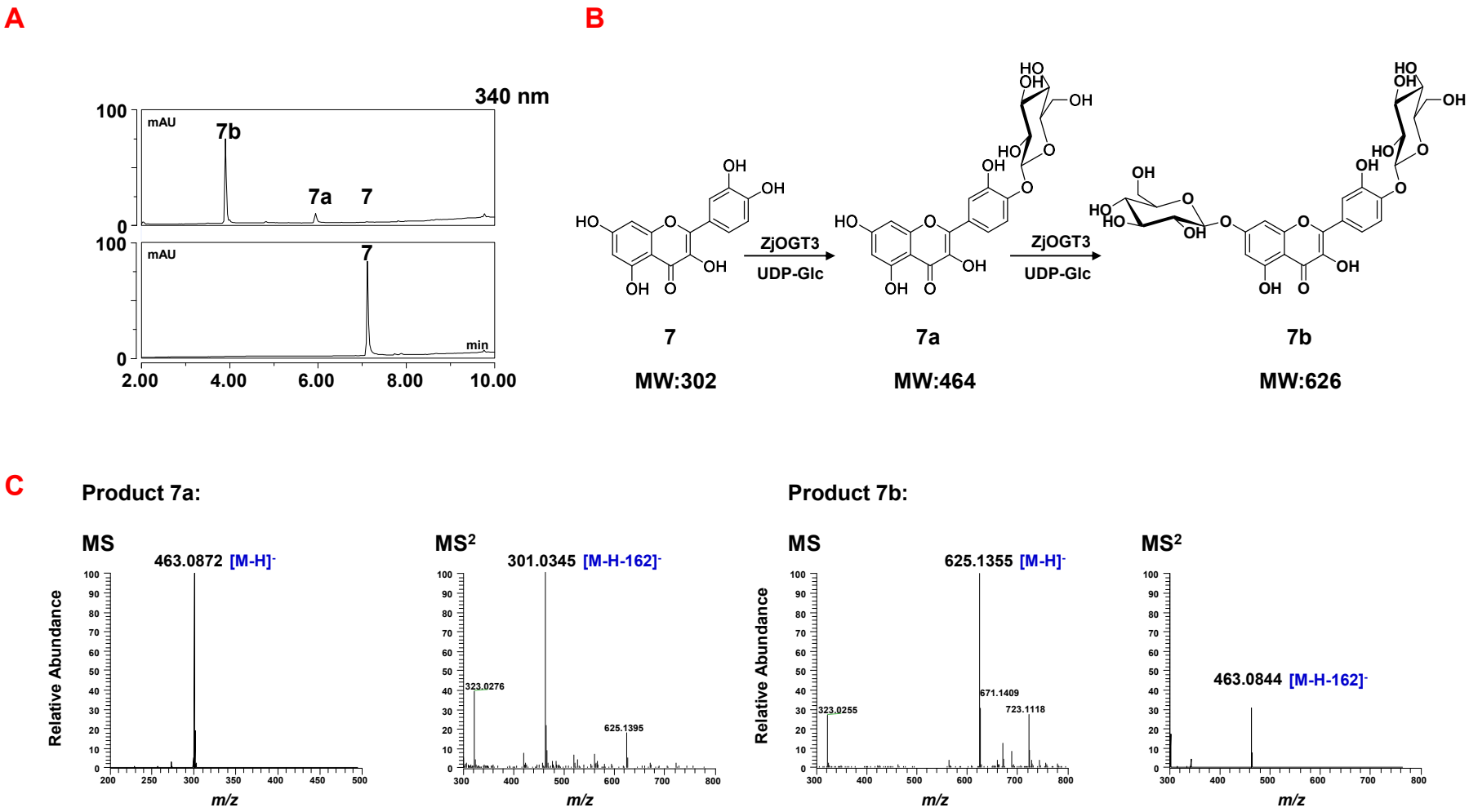


Figure S11. UHPLC/MS analysis of the enzyme catalytic reaction products of **7** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **7**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **7a** and **7b**. The analytical conditions are given in Table S3.

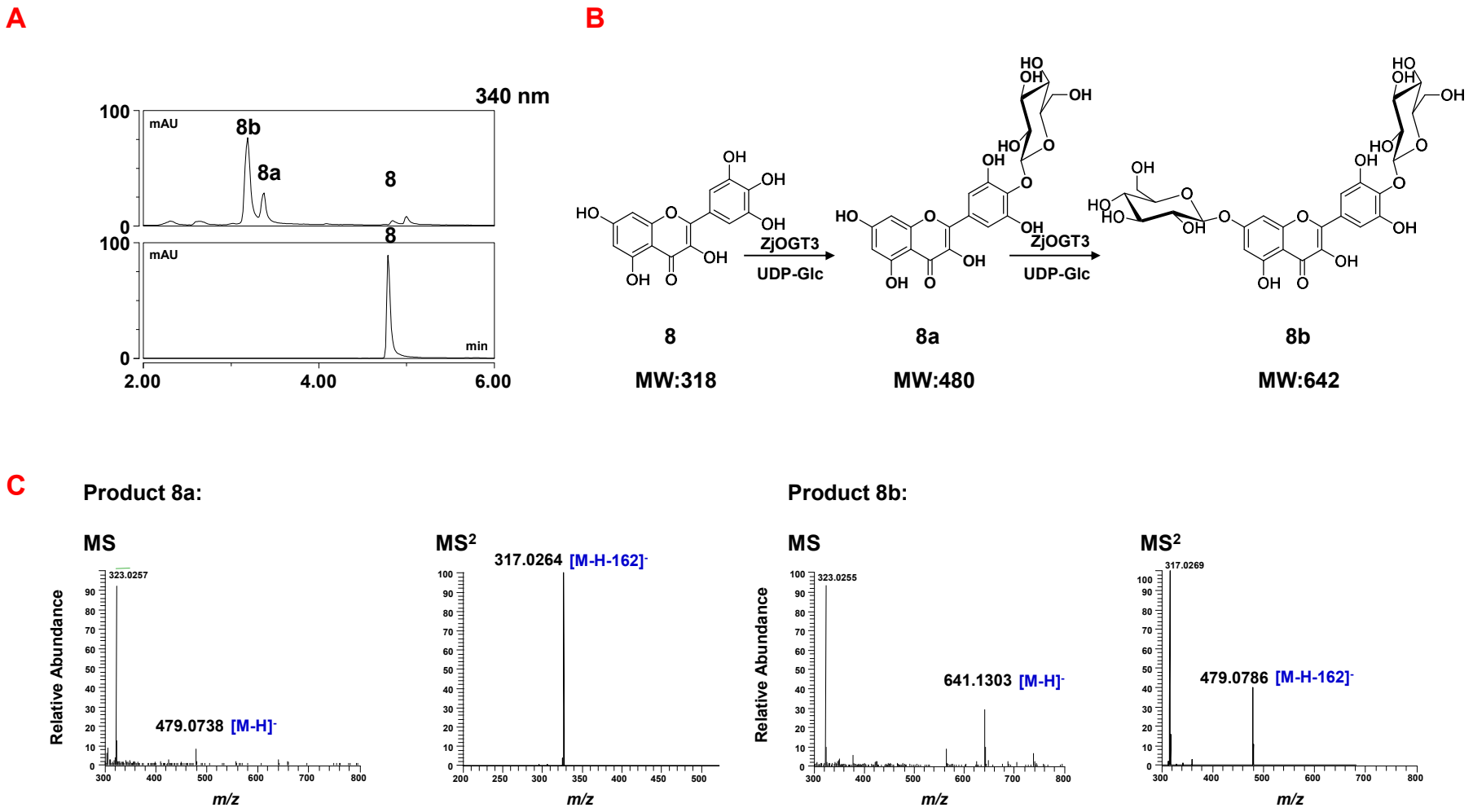


Figure S12. UHPLC/MS analysis of the enzyme catalytic reaction products of **8** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **8**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **8a** and **8b**. The analytical conditions are given in Table S3.

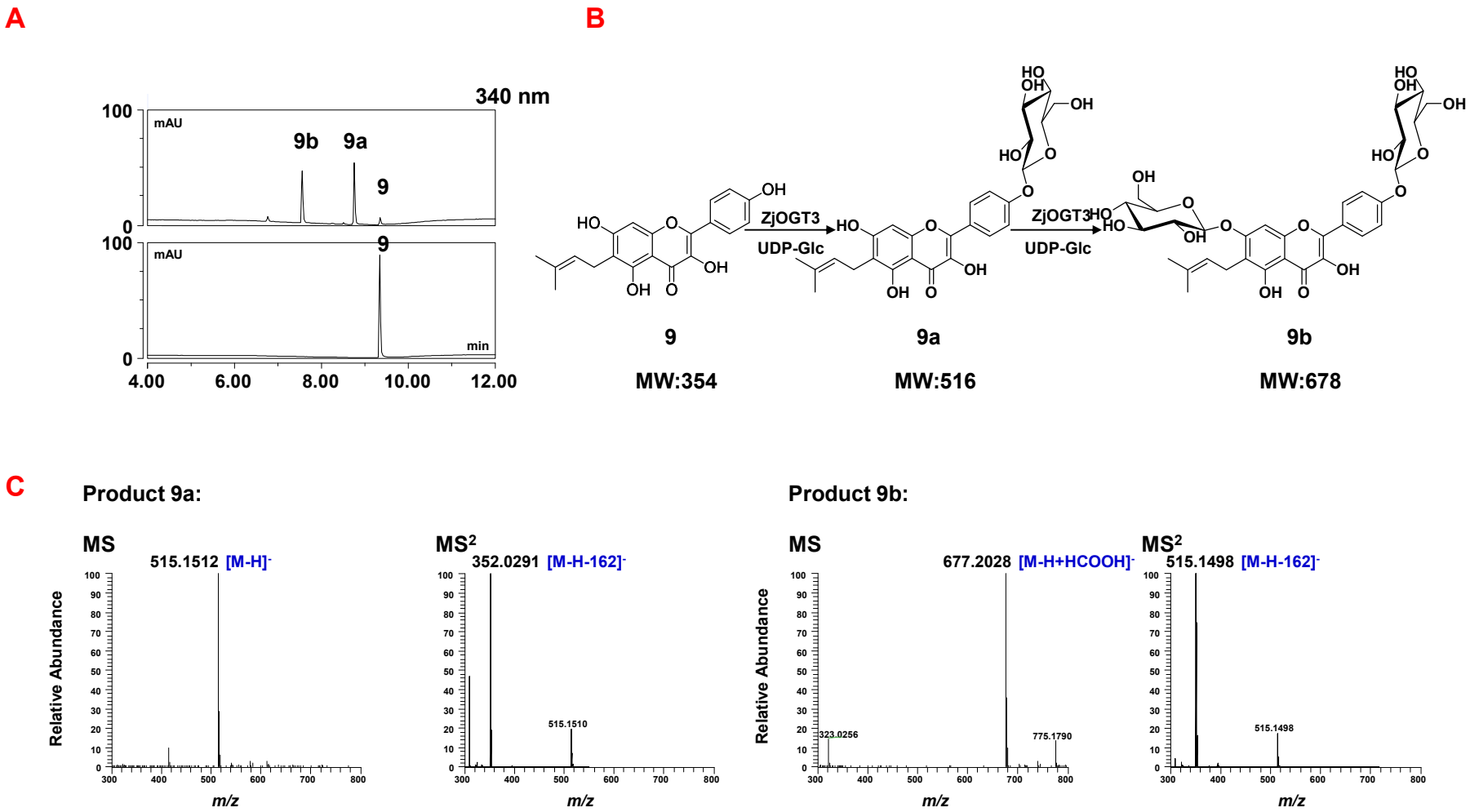


Figure S13. UHPLC/MS analysis of the enzyme catalytic reaction products of **9** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **9**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **9a** and **9b**. The analytical conditions are given in Table S3.

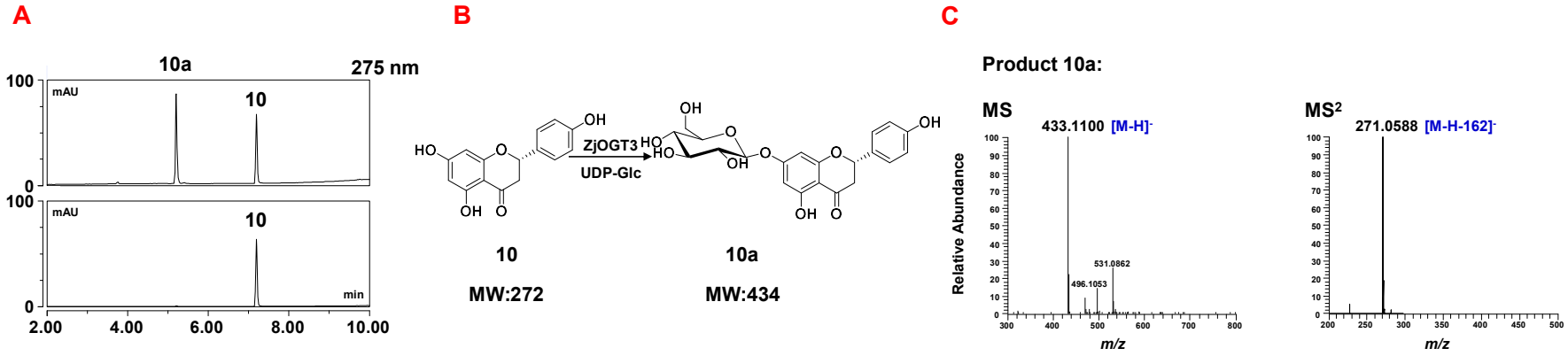


Figure S14. UHPLC/MS analysis of the enzyme catalytic reaction products of **10** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **10**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **10a**. The analytical conditions are given in Table S3.

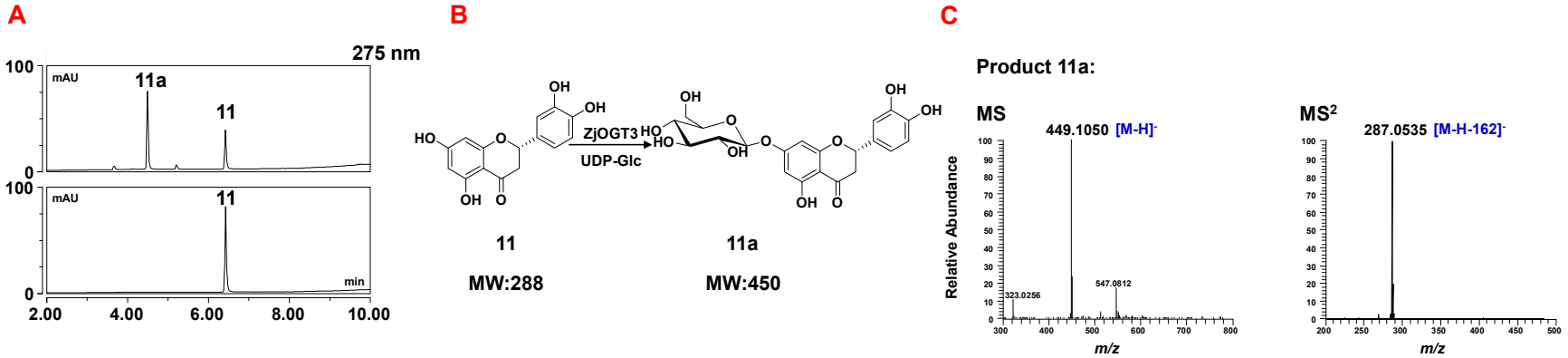


Figure S15. UHPLC/MS analysis of the enzyme catalytic reaction products of **11** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **11**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **11a**. The analytical conditions are given in Table S3.

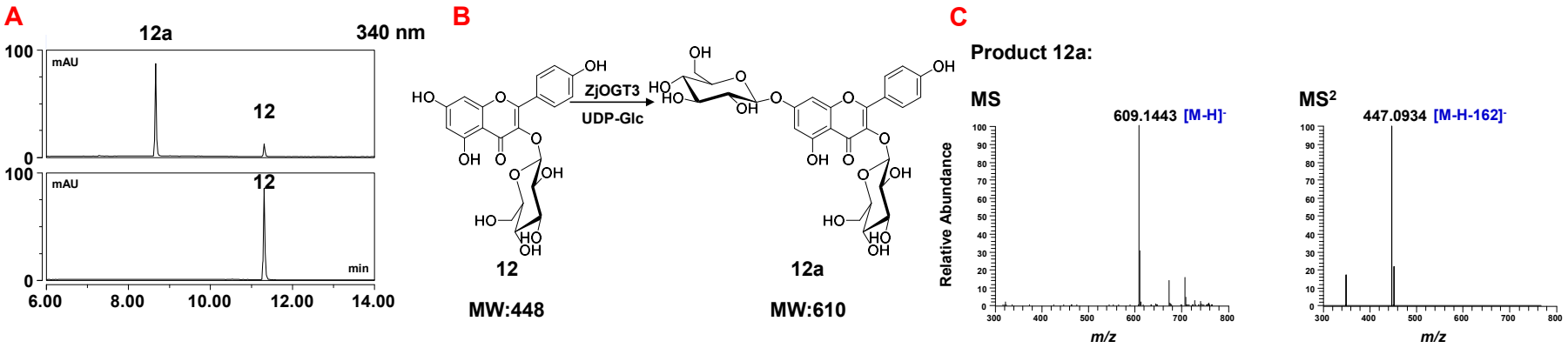


Figure S16. UHPLC/MS analysis of the enzyme catalytic reaction products of **12** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **12**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **12a**. The analytical conditions are given in Table S3.

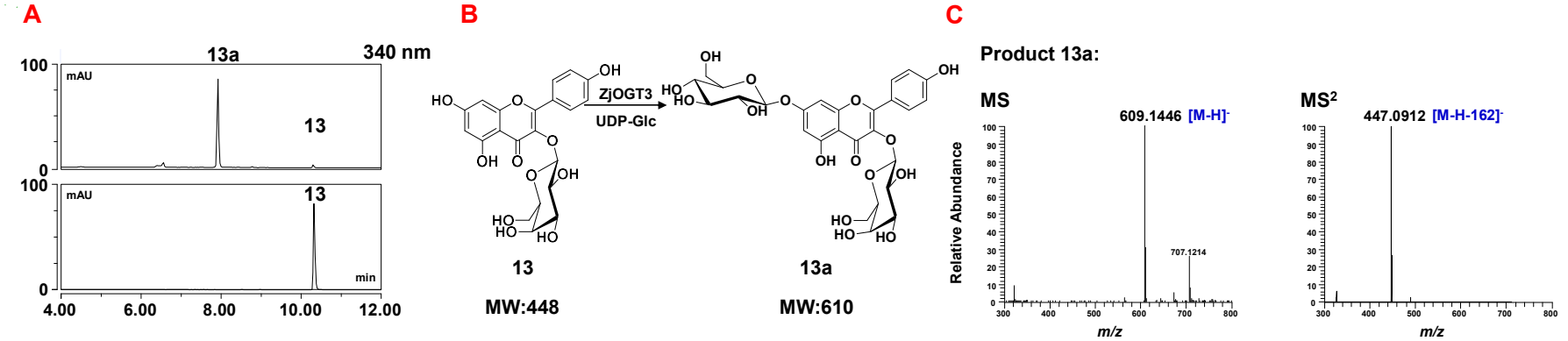


Figure S17. UHPLC/MS analysis of the enzyme catalytic reaction products of **13** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **13**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **13a**. The analytical conditions are given in Table S3.

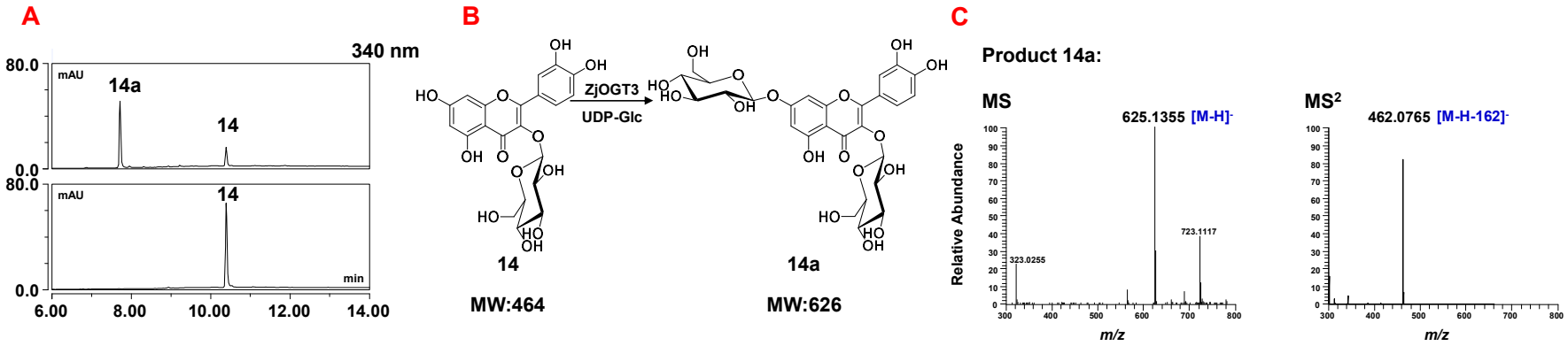


Figure S18. UHPLC/MS analysis of the enzyme catalytic reaction products of **14** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **14**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **14a**. The analytical conditions are given in Table S3.

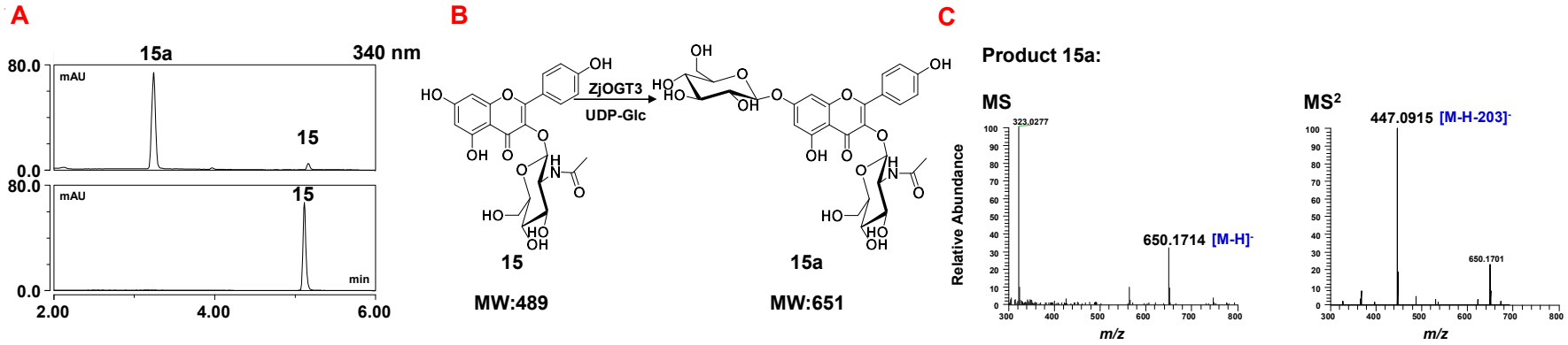


Figure S19. UHPLC/MS analysis of the enzyme catalytic reaction products of **15** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **15**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **15a**. The analytical conditions are given in Table S3.

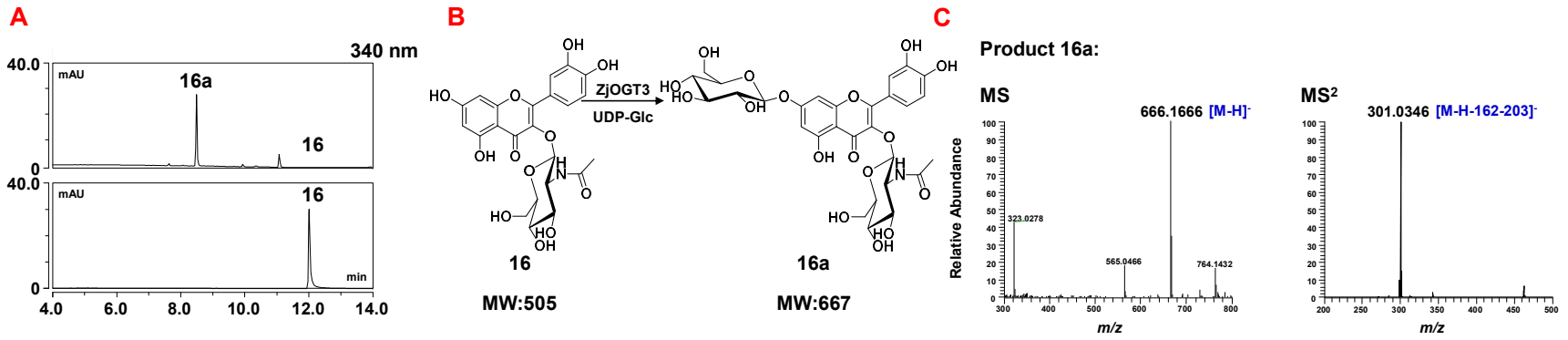


Figure S20. UHPLC/MS analysis of the enzyme catalytic reaction products of **16** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **16**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **16a**. The analytical conditions are given in Table S3.

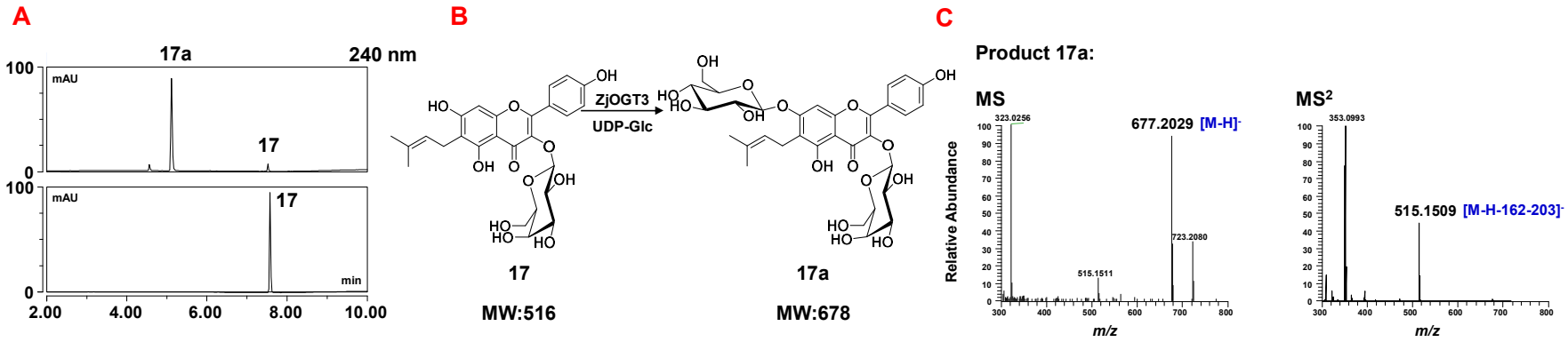


Figure S21. UHPLC/MS analysis of the enzyme catalytic reaction products of **17** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **17**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **17a**. The analytical conditions are given in Table S3.

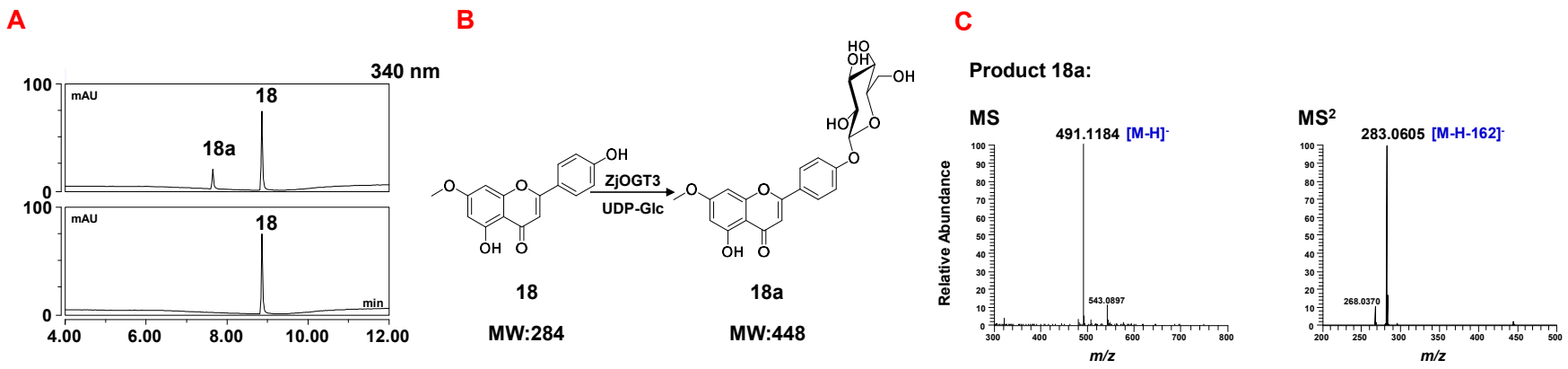


Figure S22. UHPLC/MS analysis of the enzyme catalytic reaction products of **18** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **18**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **18a**. The analytical conditions are given in Table S3.

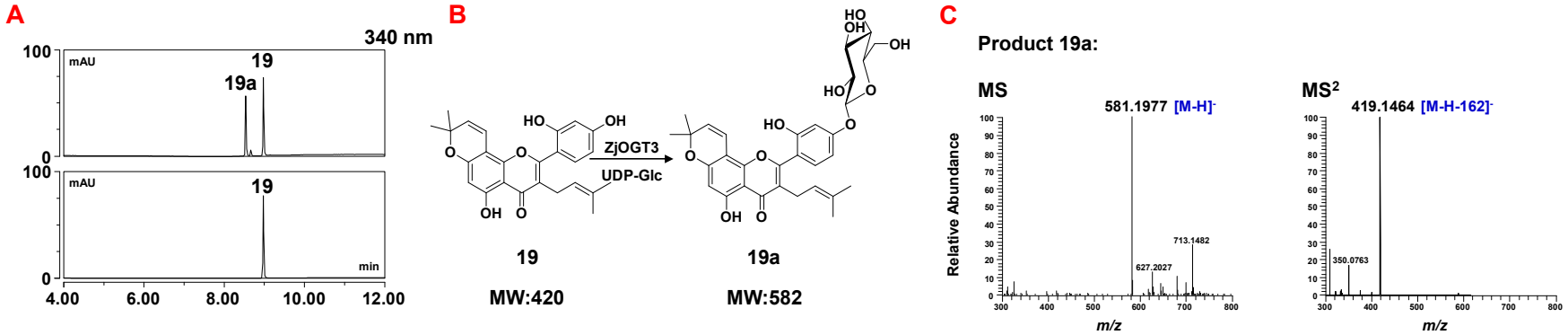


Figure S23. UHPLC/MS analysis of the enzyme catalytic reaction products of **19** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **19**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **19a**. The analytical conditions are given in Table S3.

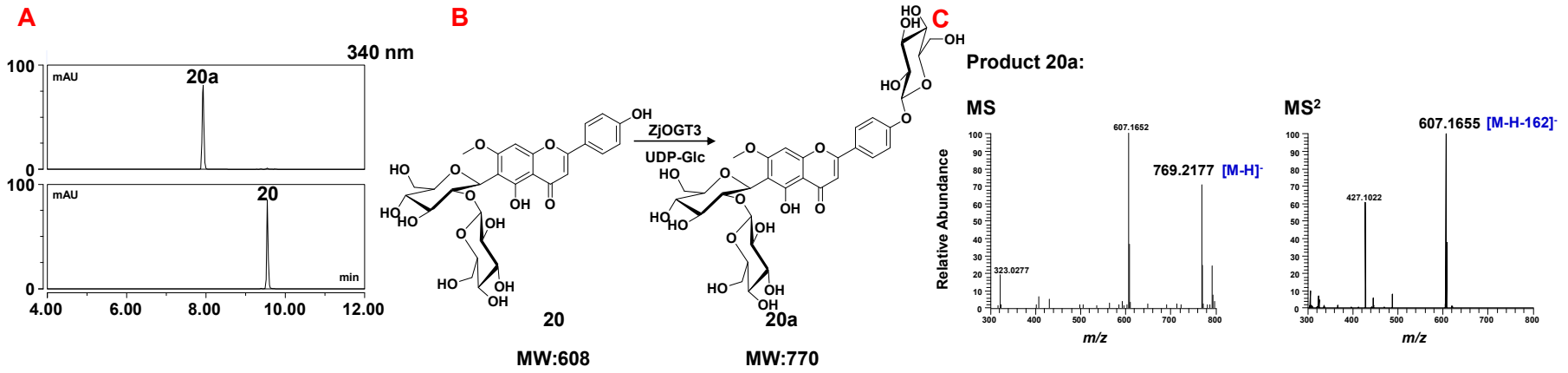


Figure S24. UHPLC/MS analysis of the enzyme catalytic reaction products of **20** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **20**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **20a**. The analytical conditions are given in Table S3.

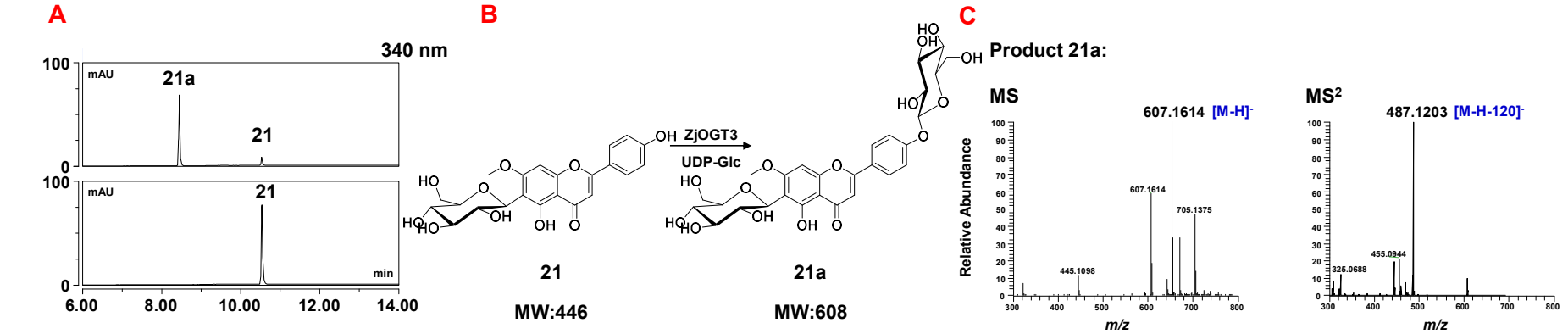


Figure S25. UHPLC/MS analysis of the enzyme catalytic reaction products of **21** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **21**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **21a**. The analytical conditions are given in Table S3.

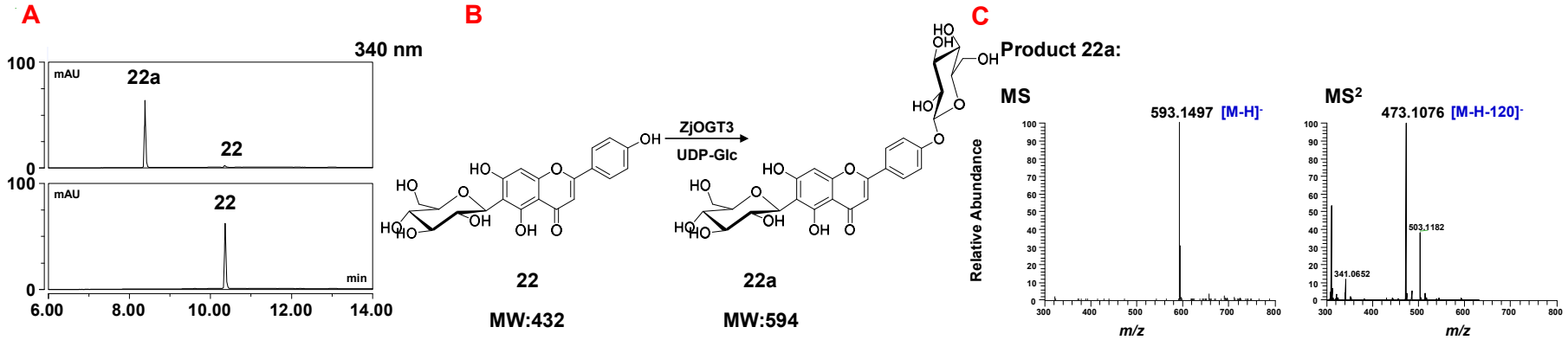


Figure S26. UHPLC/MS analysis of the enzyme catalytic reaction products of **22** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **22**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **22a**. The analytical conditions are given in Table S3.

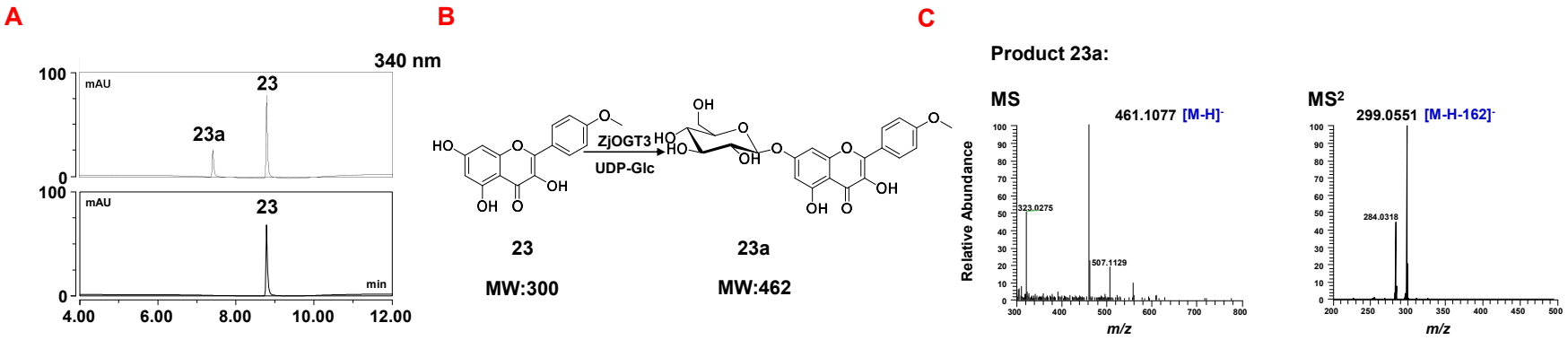


Figure S27. UHPLC/MS analysis of the enzyme catalytic reaction products of **23** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **23**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **23a**. The analytical conditions are given in Table S3.

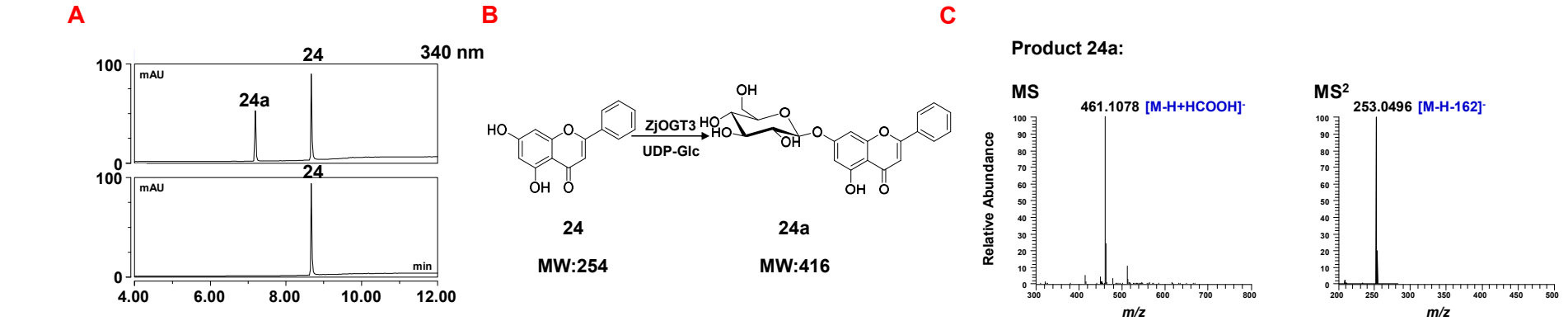


Figure S28. UHPLC/MS analysis of the enzyme catalytic reaction products of **24** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **24**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **24a**. The analytical conditions are given in Table S3.

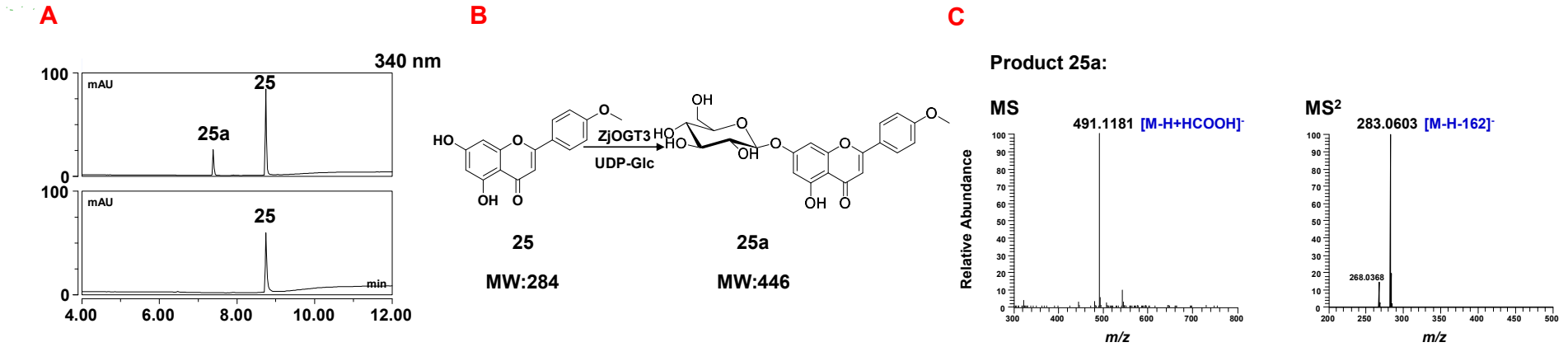


Figure S29. UHPLC/MS analysis of the enzyme catalytic reaction products of **25** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **25**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **25a**. The analytical conditions are given in Table S3.

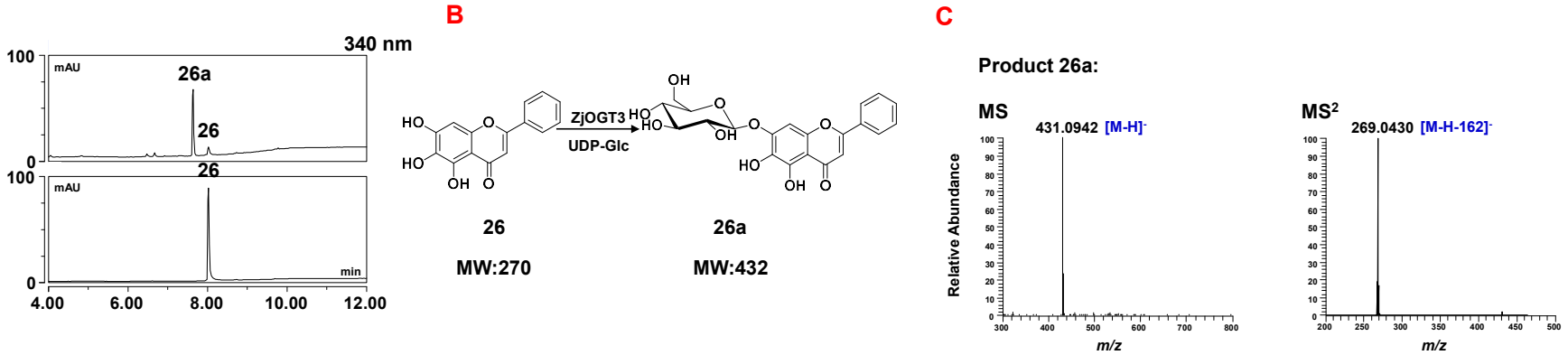


Figure S30. UHPLC/MS analysis of the enzyme catalytic reaction products of **26** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **26**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **26a**. The analytical conditions are given in Table S3.

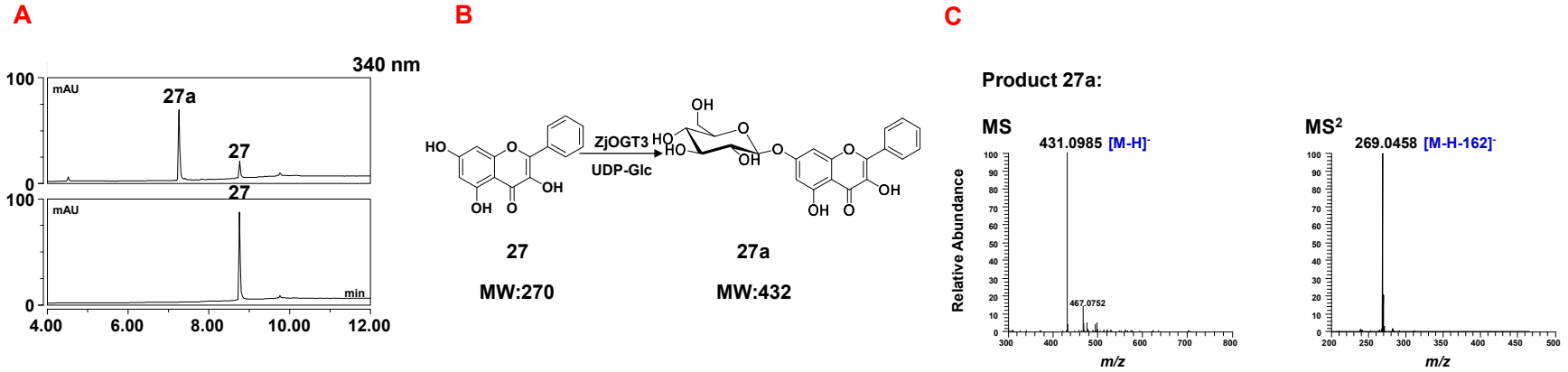


Figure S31. UHPLC/MS analysis of the enzyme catalytic reaction products of **27** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **27**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **27a**. The analytical conditions are given in Table S3.

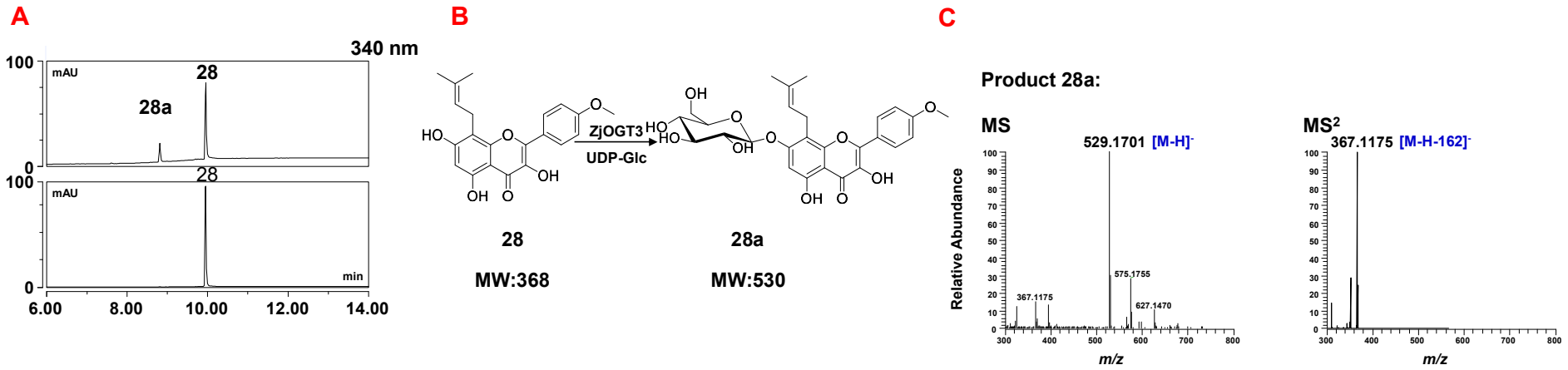


Figure S32. UHPLC/MS analysis of the enzyme catalytic reaction products of **28** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **28**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **28a**. The analytical conditions are given in Table S3.

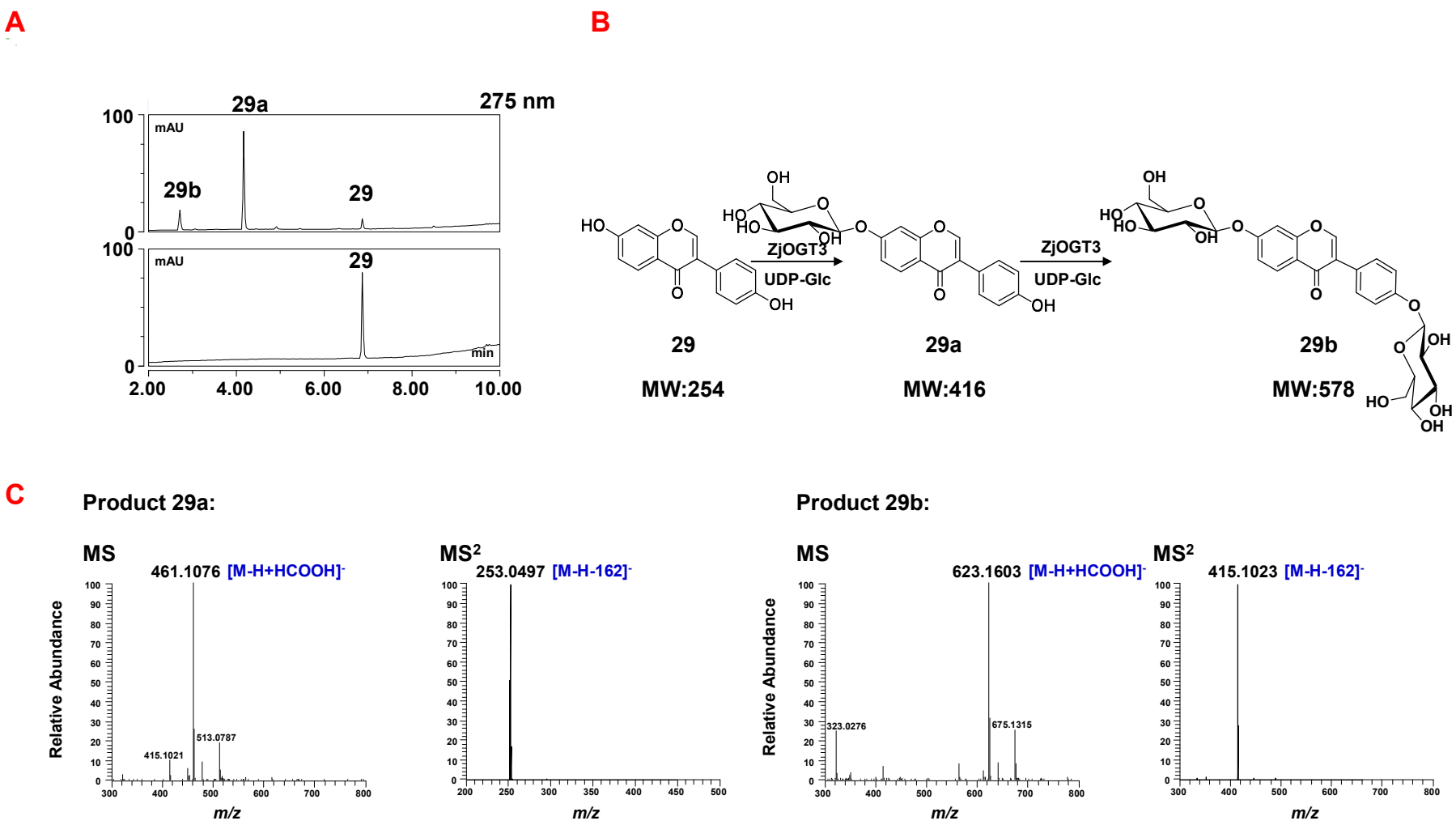


Figure S33. UHPLC/MS analysis of the enzyme catalytic reaction products of **29** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **29**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **29a** and **29b**. The analytical conditions are given in Table S3. **29a** and **29b** were confirmed by comparing with reference standards.

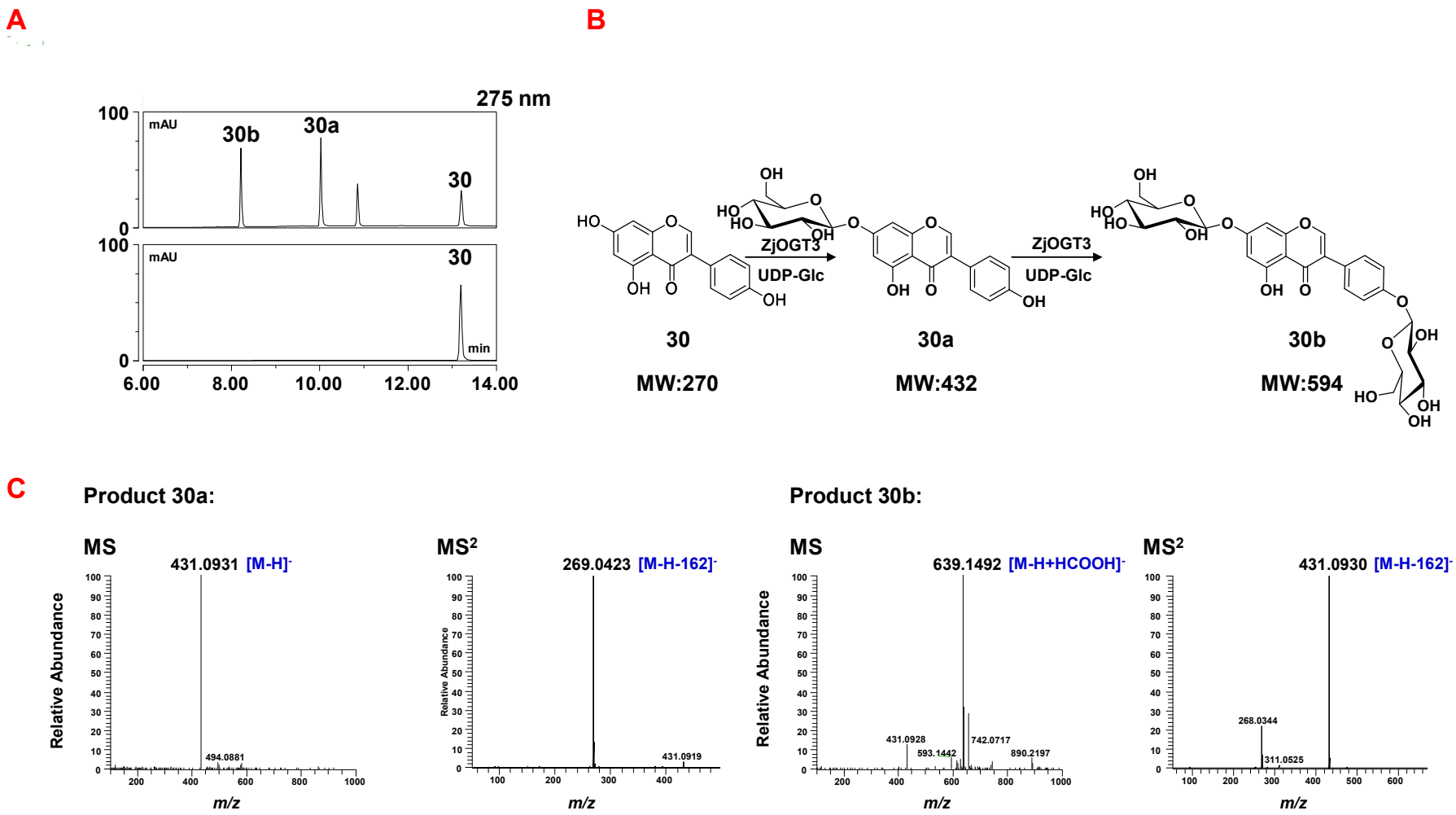


Figure S34. UHPLC/MS analysis of the enzyme catalytic reaction products of **30** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **30**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **30a** and **30b**. The analytical conditions are given in Table S3.

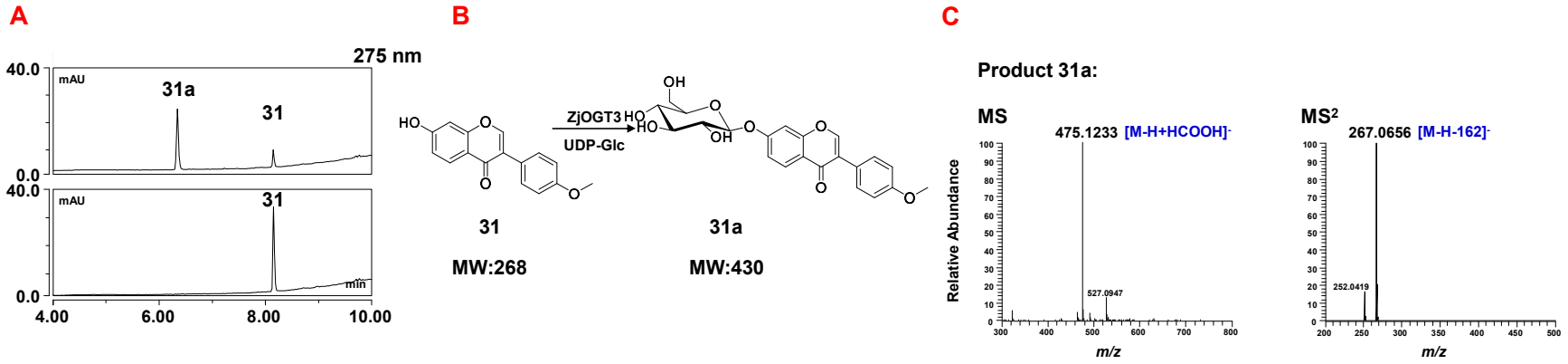


Figure S35. UHPLC/MS analysis of the enzyme catalytic reaction products of **31** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **31**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **31a**. The analytical conditions are given in Table S3.

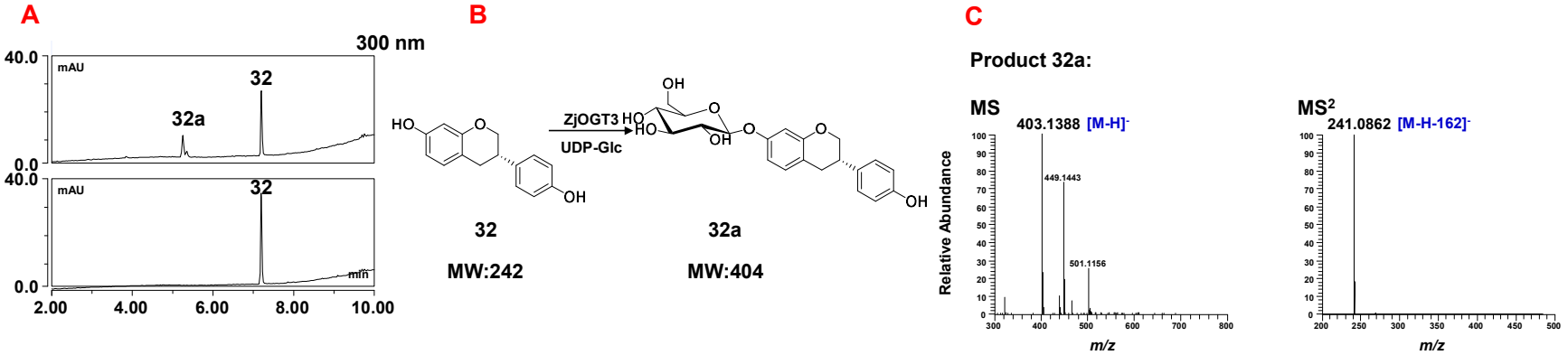


Figure S36. UHPLC/MS analysis of the enzyme catalytic reaction products of **32** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **32**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **32a**. The analytical conditions are given in Table S3.

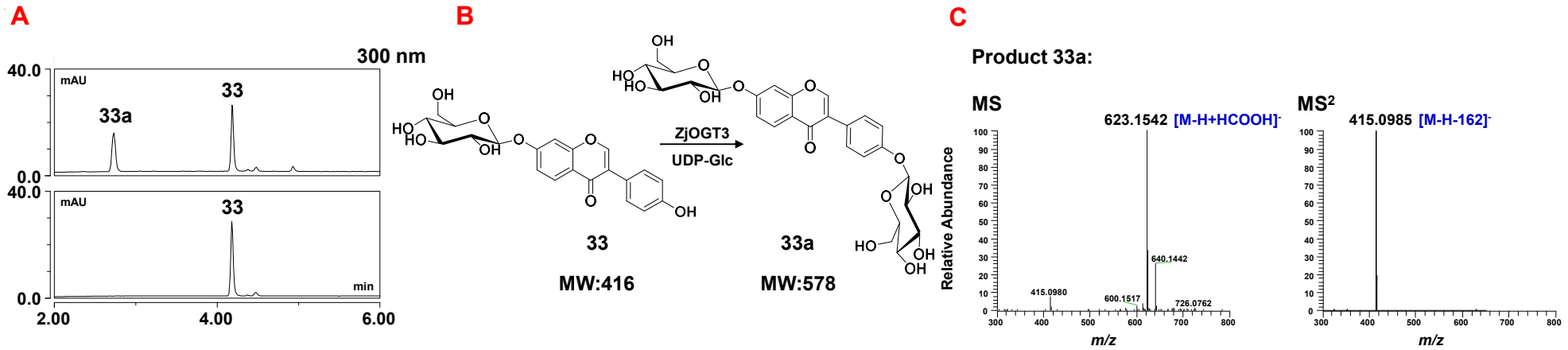


Figure S37. UHPLC/MS analysis of the enzyme catalytic reaction products of **33** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **33**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **33a**. The analytical conditions are given in Table S3. **33a** were confirmed by comparing with reference standards. **29b** and **33a** are the same compound.

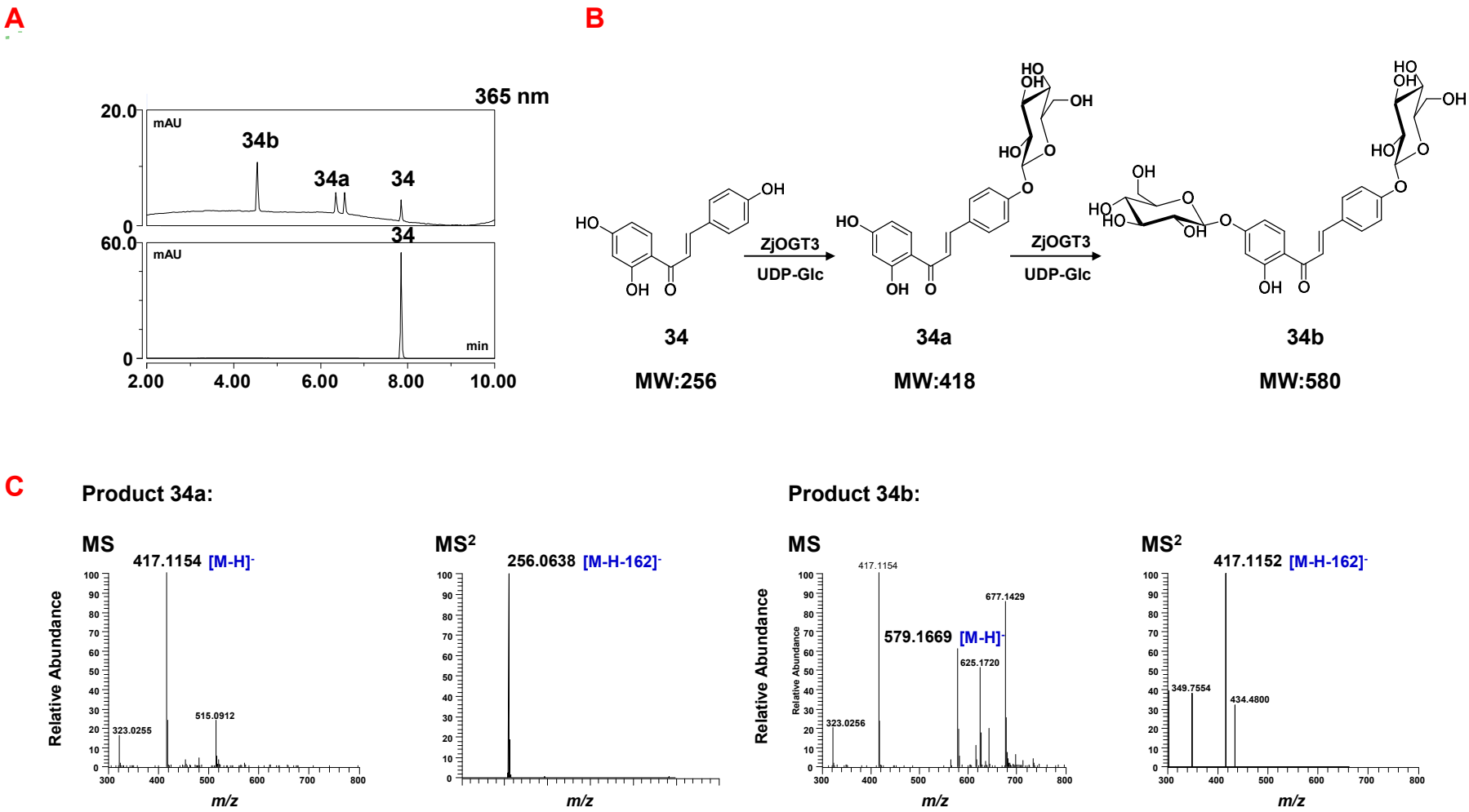


Figure S38. UHPLC/MS analysis of the enzyme catalytic reaction products of **34** by recombinant ZjOGT3. (A) HPLC chromatograms of the reaction products and reference standard **34**. (B) The reaction pathway. (C) (-)-ESI-MS and MS/MS spectra of **34a** and **34b**. The analytical conditions are given in Table S3.

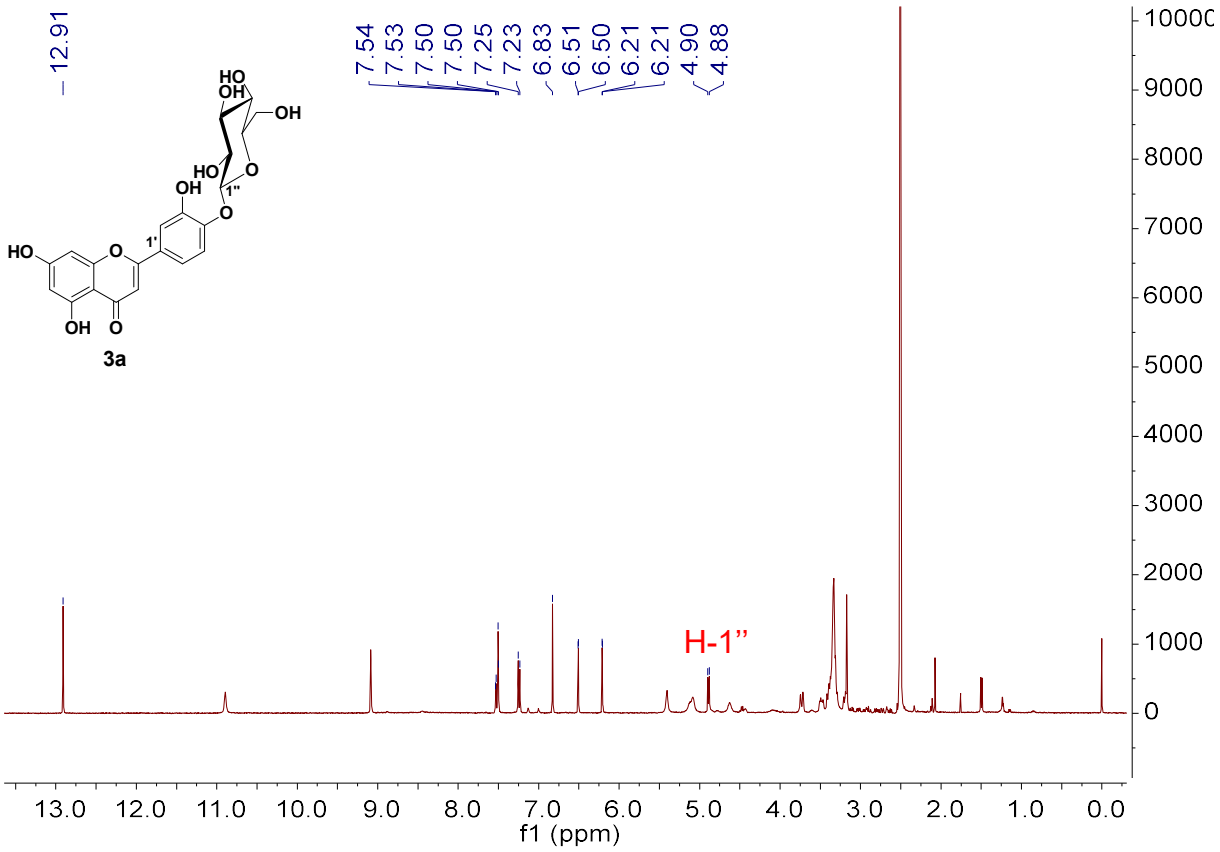


Figure S39. ^{1}H NMR spectrum of **3a** in DMSO-d_6 (400 MHz).

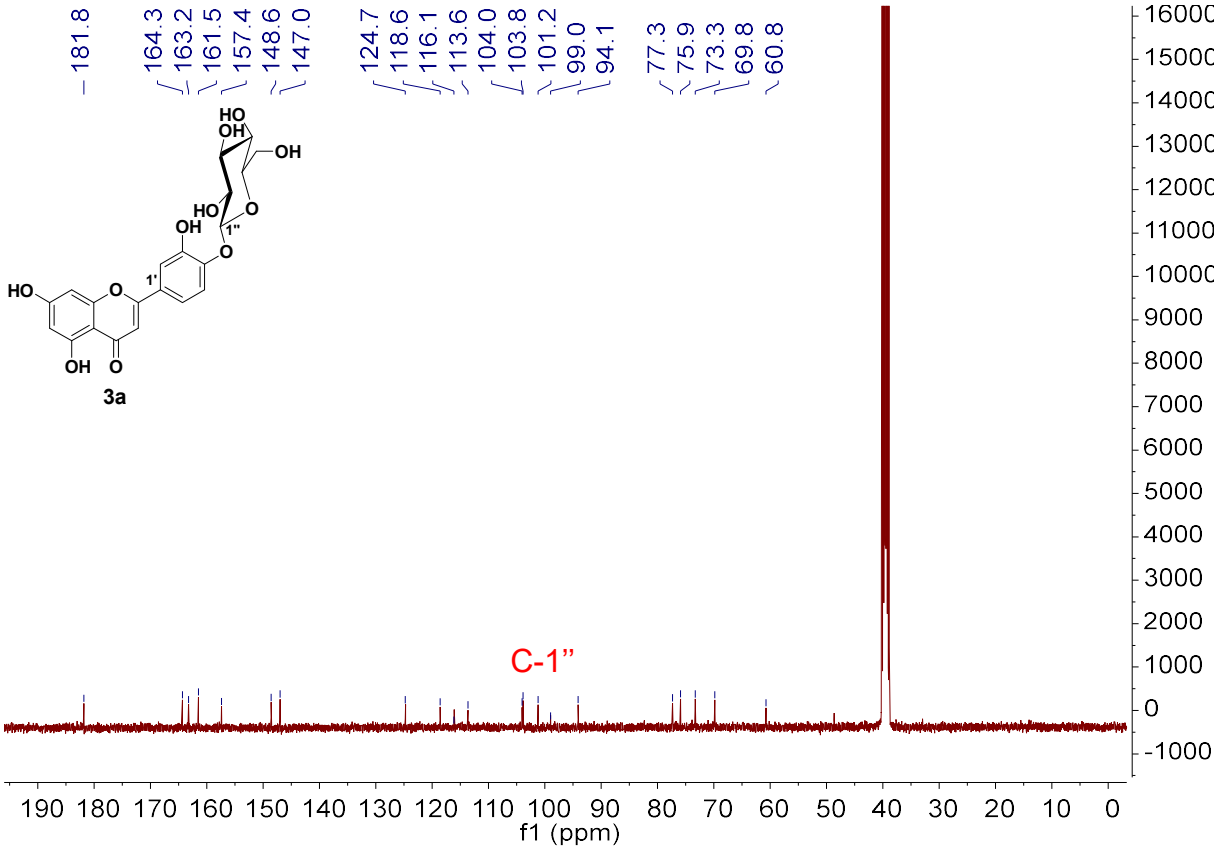


Figure S40. ^{13}C NMR spectrum of **3a** in DMSO-d_6 (100 MHz).

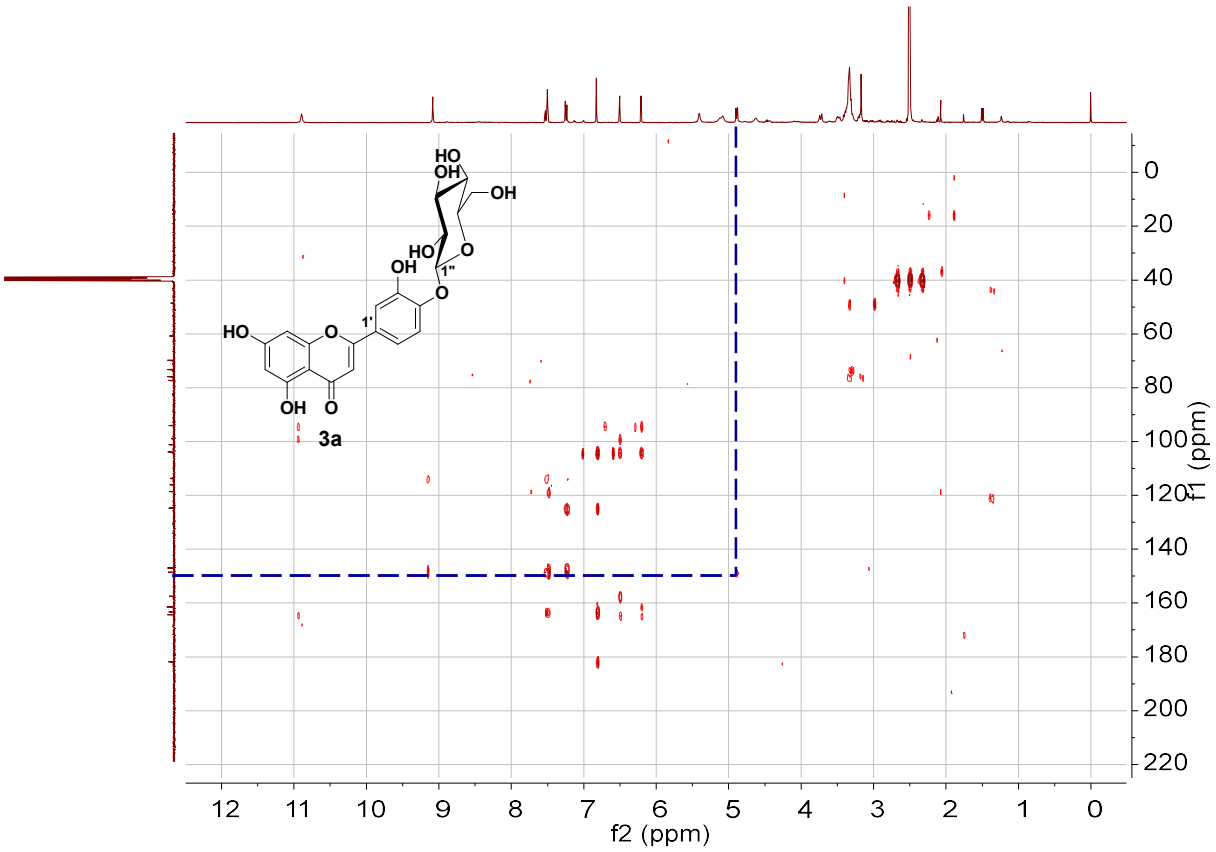


Figure S41. HMBC spectrum of **3a** in DMSO-d_6 (400 MHz).

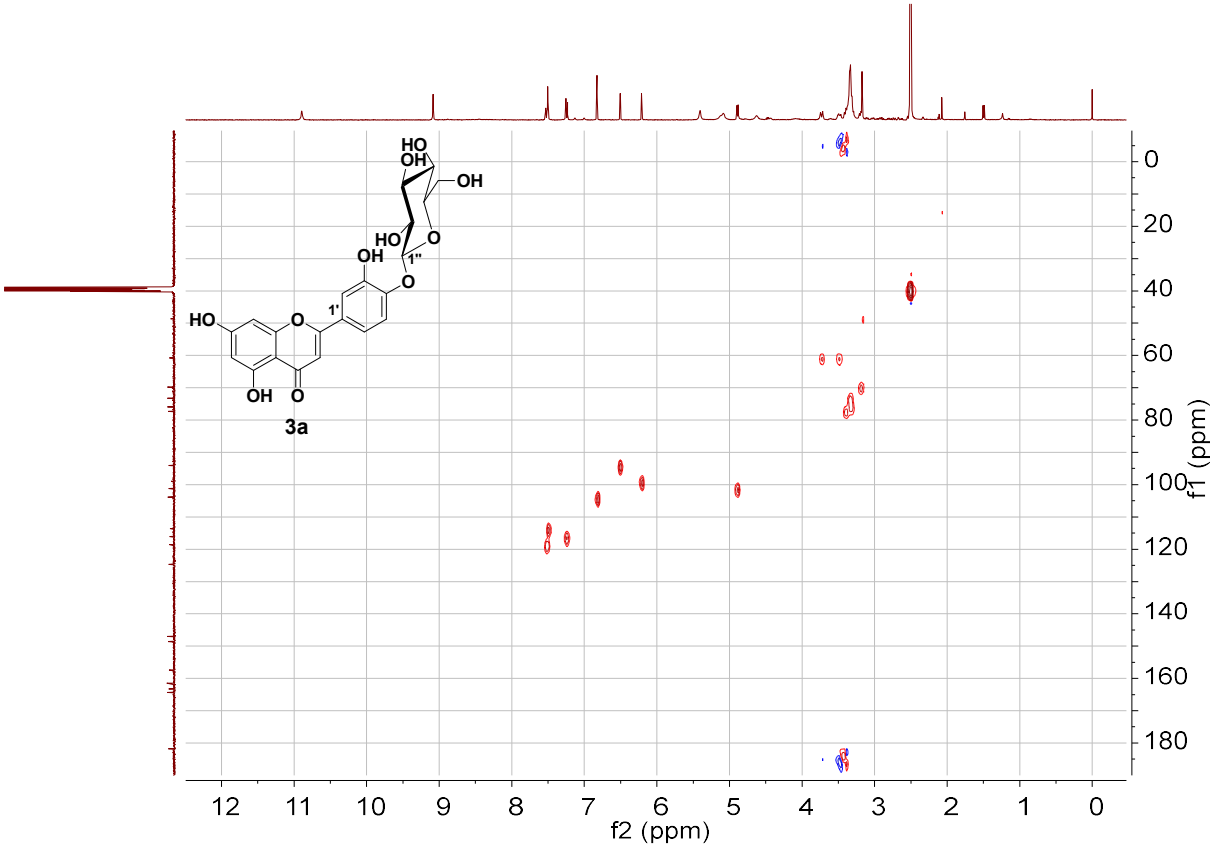


Figure S42. HSQC spectrum of **3a** in DMSO-d_6 (400 MHz).

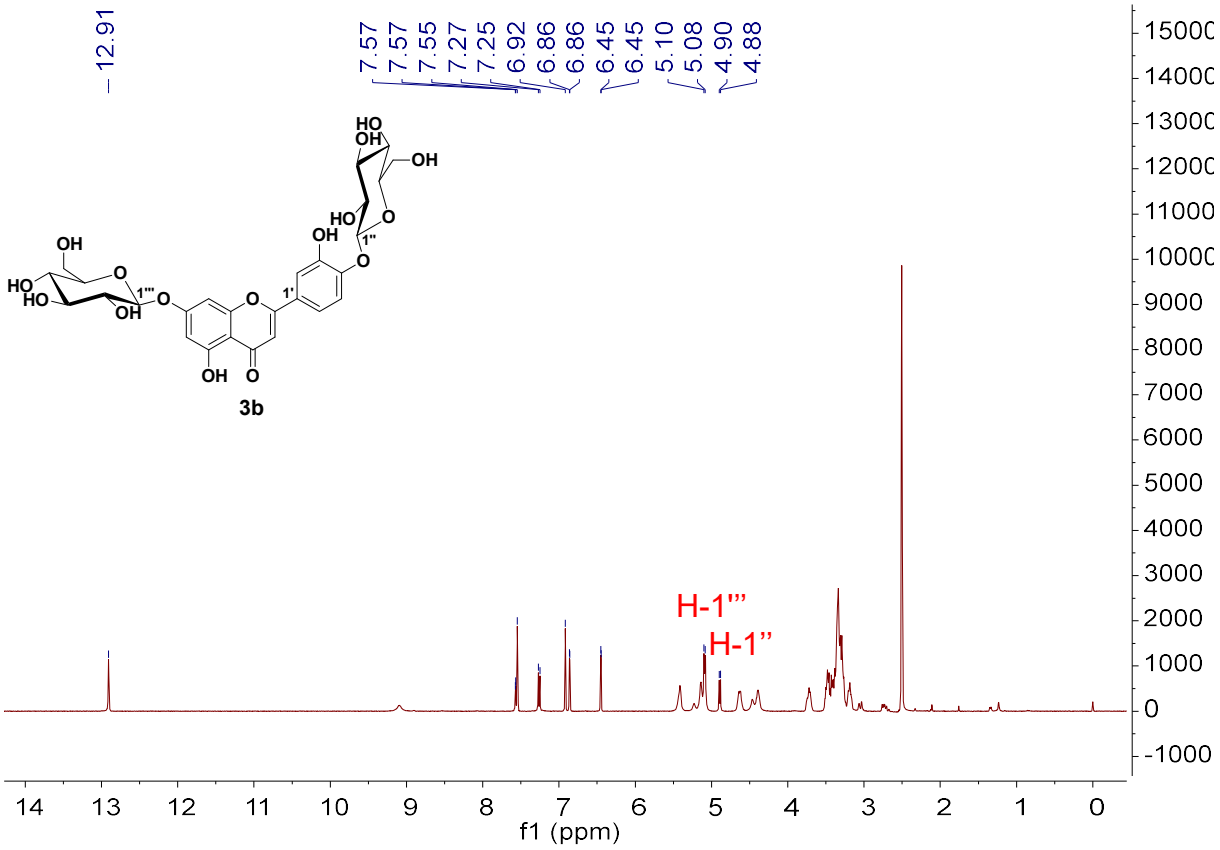


Figure S43. ^{1}H NMR spectrum of **3b** in DMSO-d_6 (400 MHz).

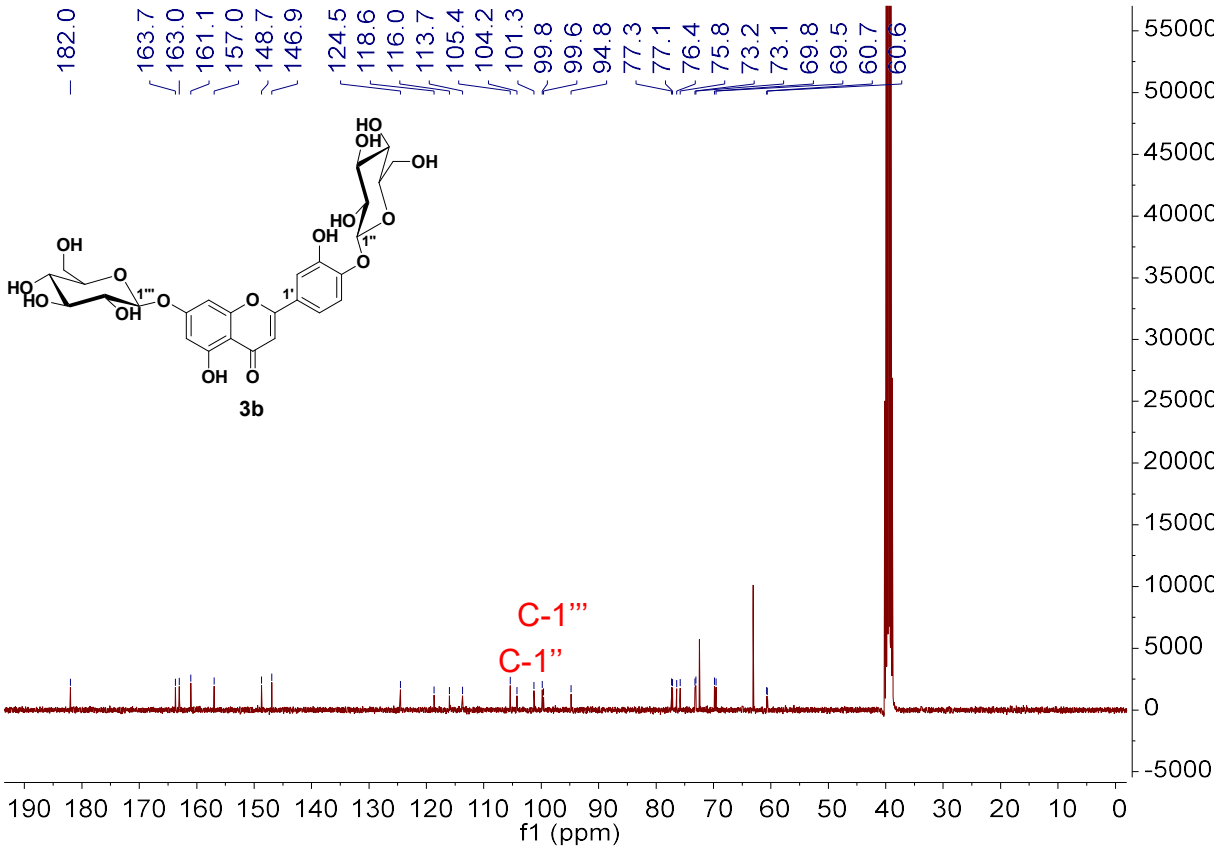


Figure S44. ^{13}C NMR spectrum of **3b** in DMSO-d_6 (100 MHz).

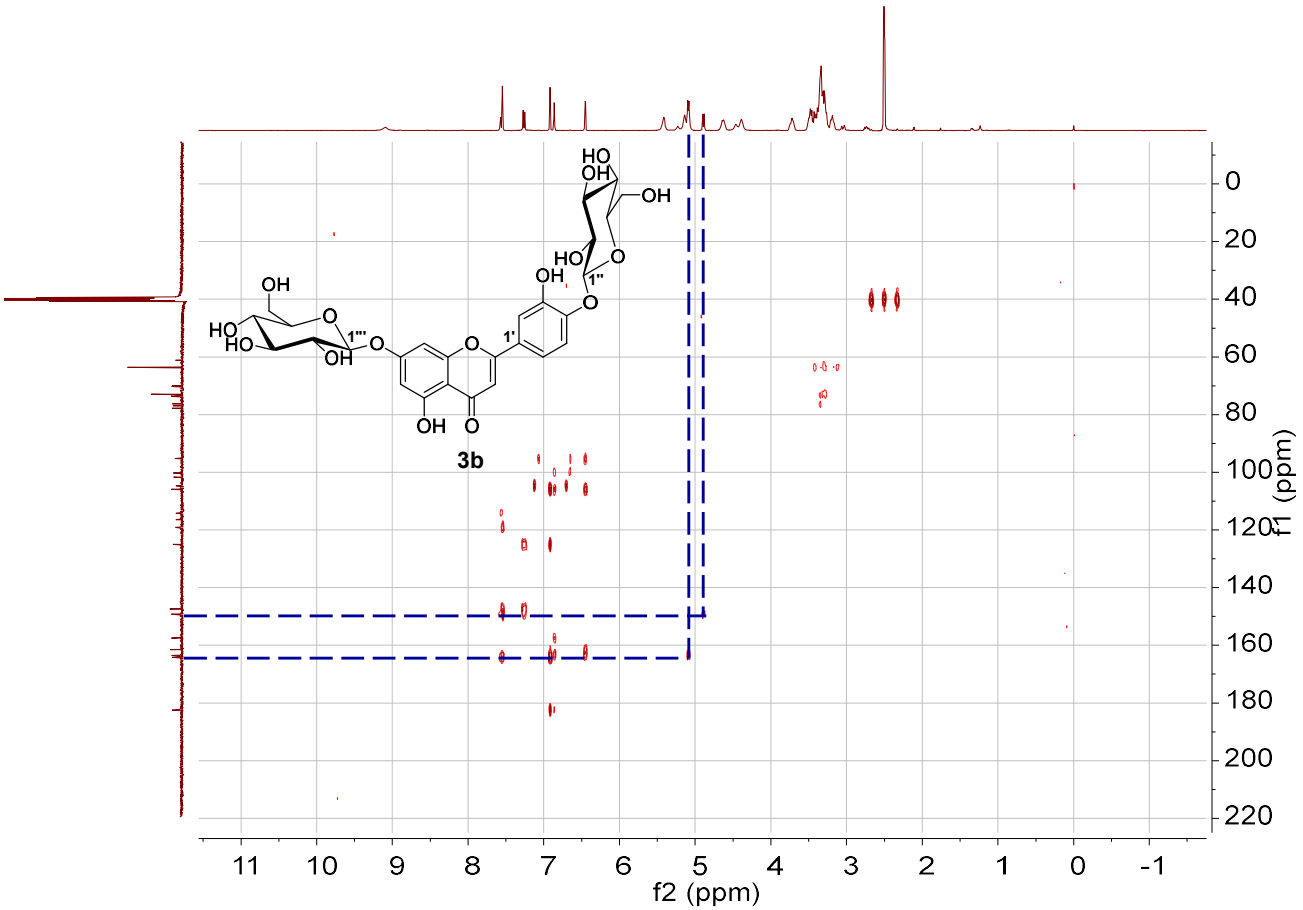


Figure S45. HMBC spectrum of **3b** in DMSO-d_6 (400 MHz).

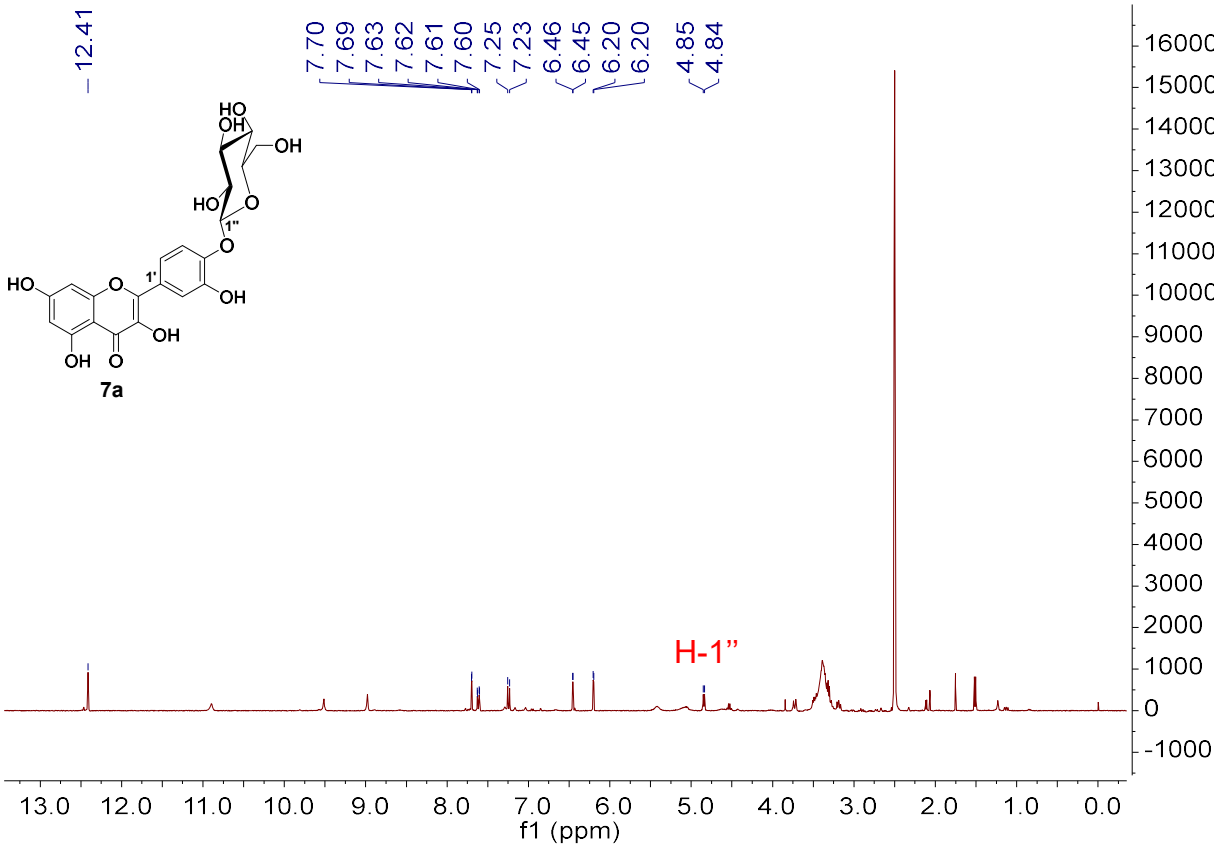


Figure S46. ^{1}H NMR spectrum of **7a** in DMSO-d_6 (400 MHz).

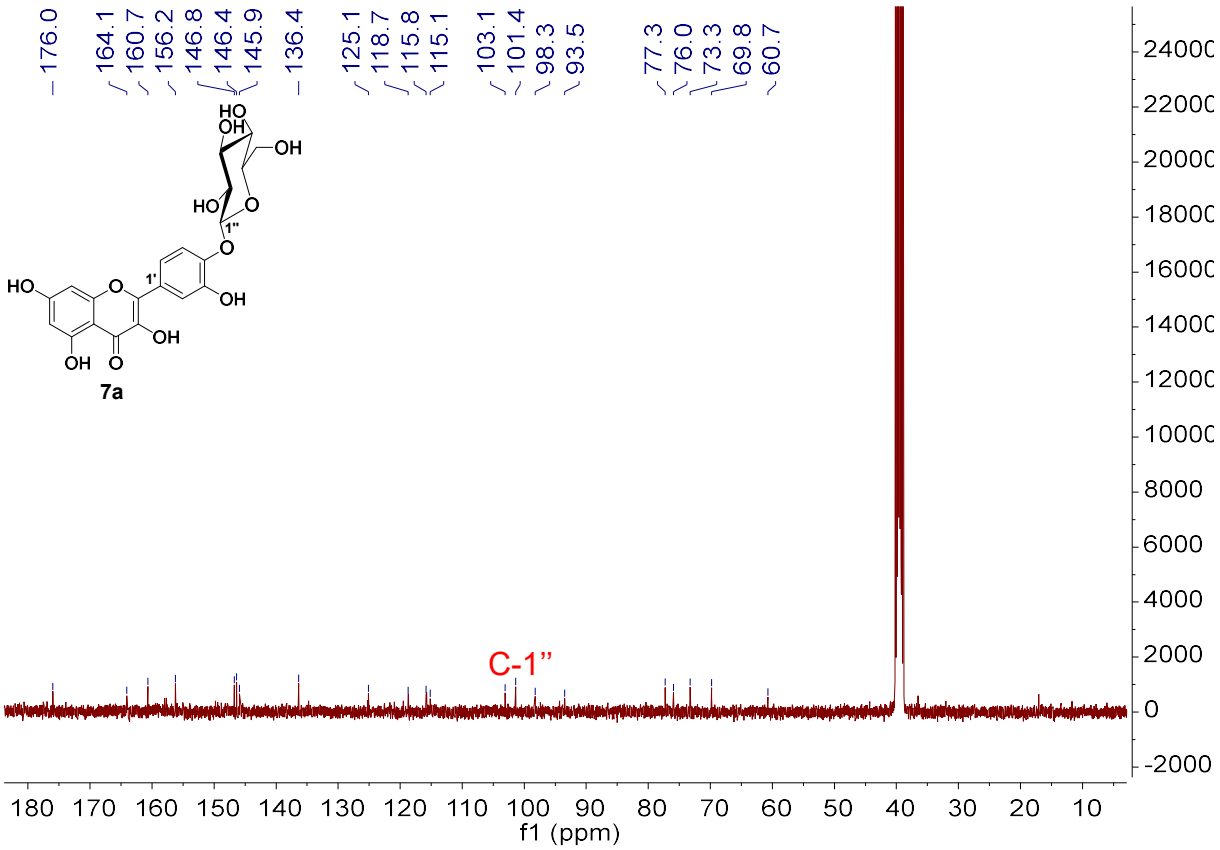


Figure S47. ^{13}C NMR spectrum of **7a** in DMSO-d_6 (100 MHz).

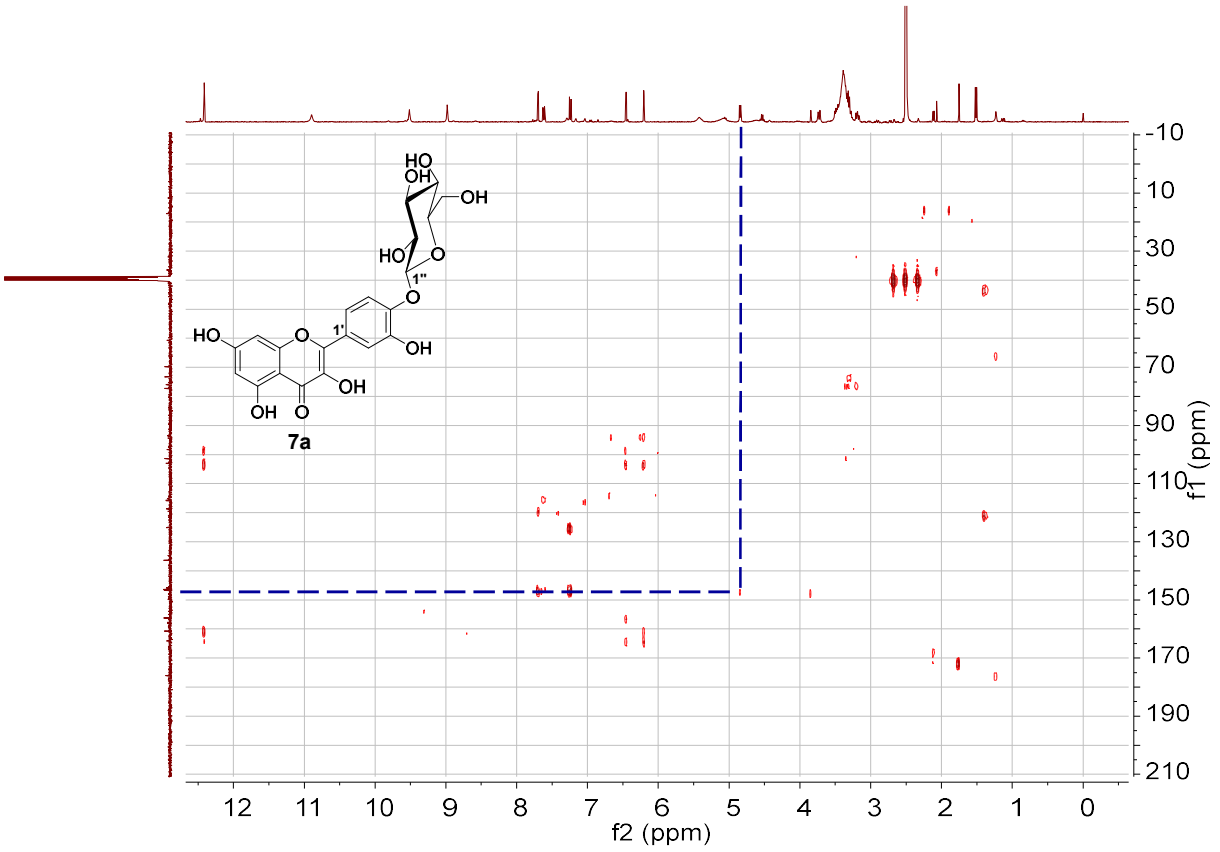


Figure S48. HMBC spectrum of **7a** in DMSO-d_6 (400 MHz).

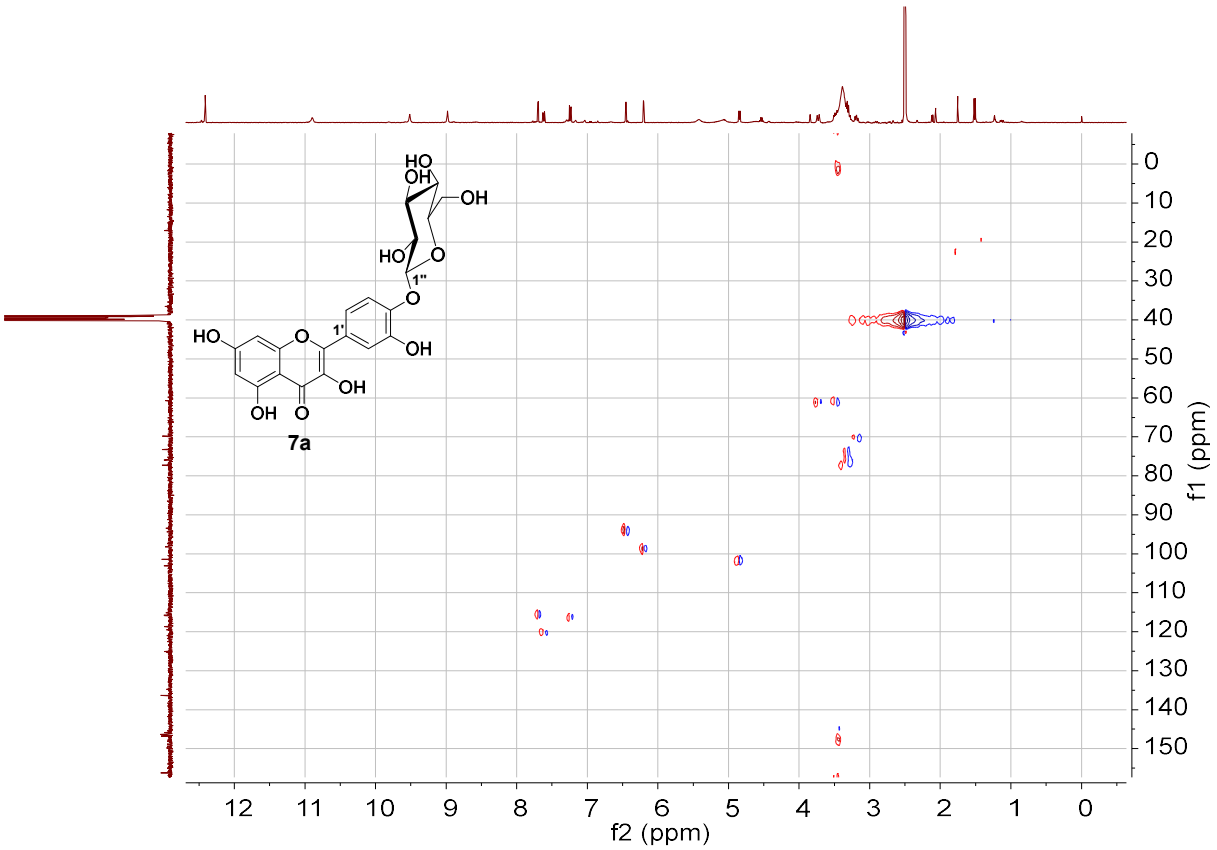


Figure S49. HSQC spectrum of **7a** in DMSO-d_6 (400 MHz).

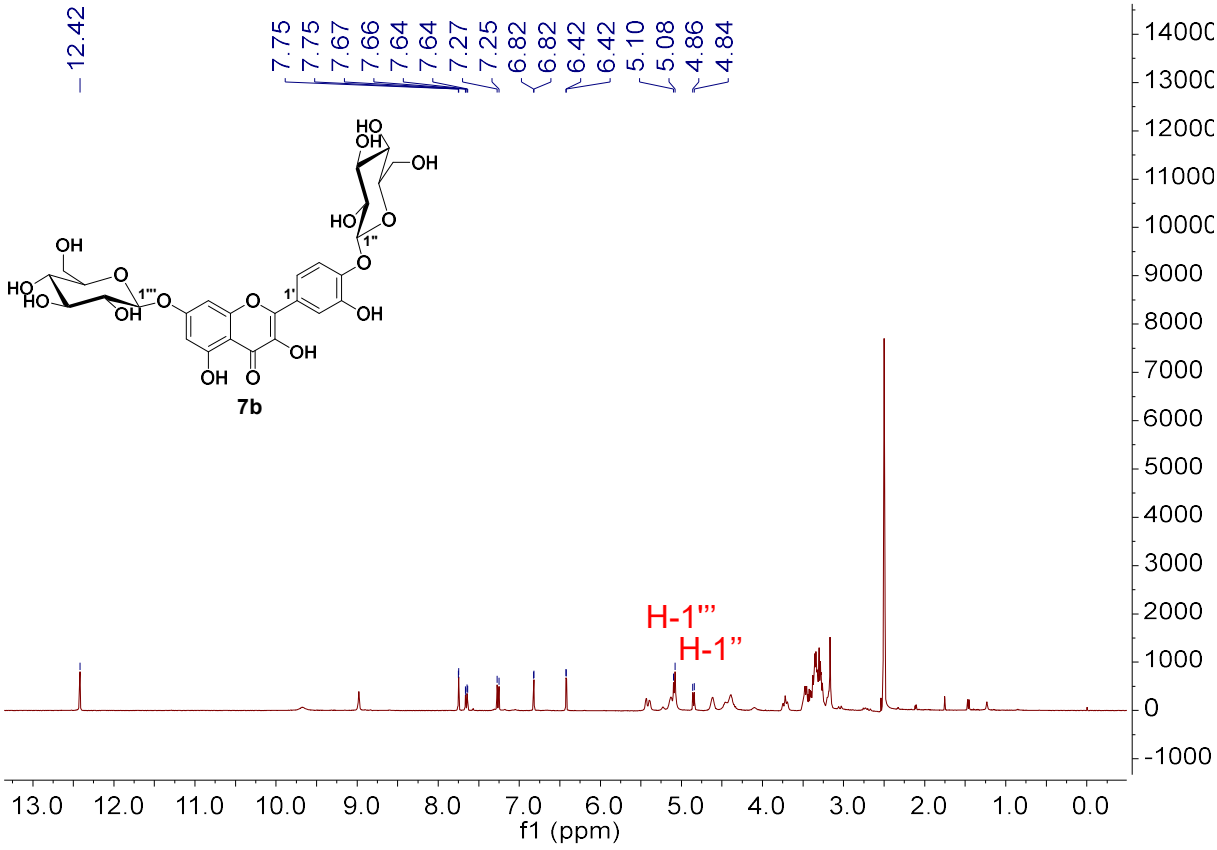


Figure S50. ^{1}H NMR spectrum of **7b** in DMSO-d_6 (400 MHz).

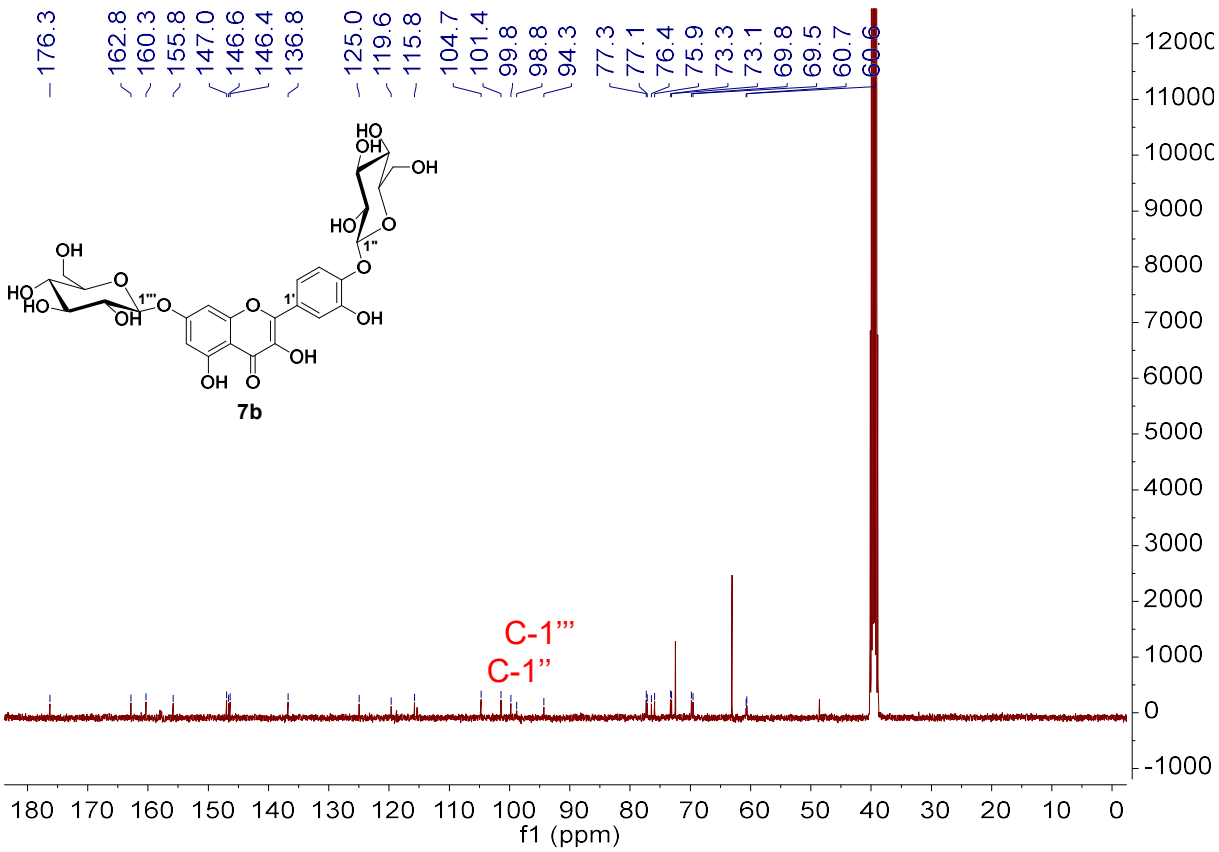


Figure S51. ^{13}C NMR spectrum of **7b** in DMSO-d_6 (100 MHz).

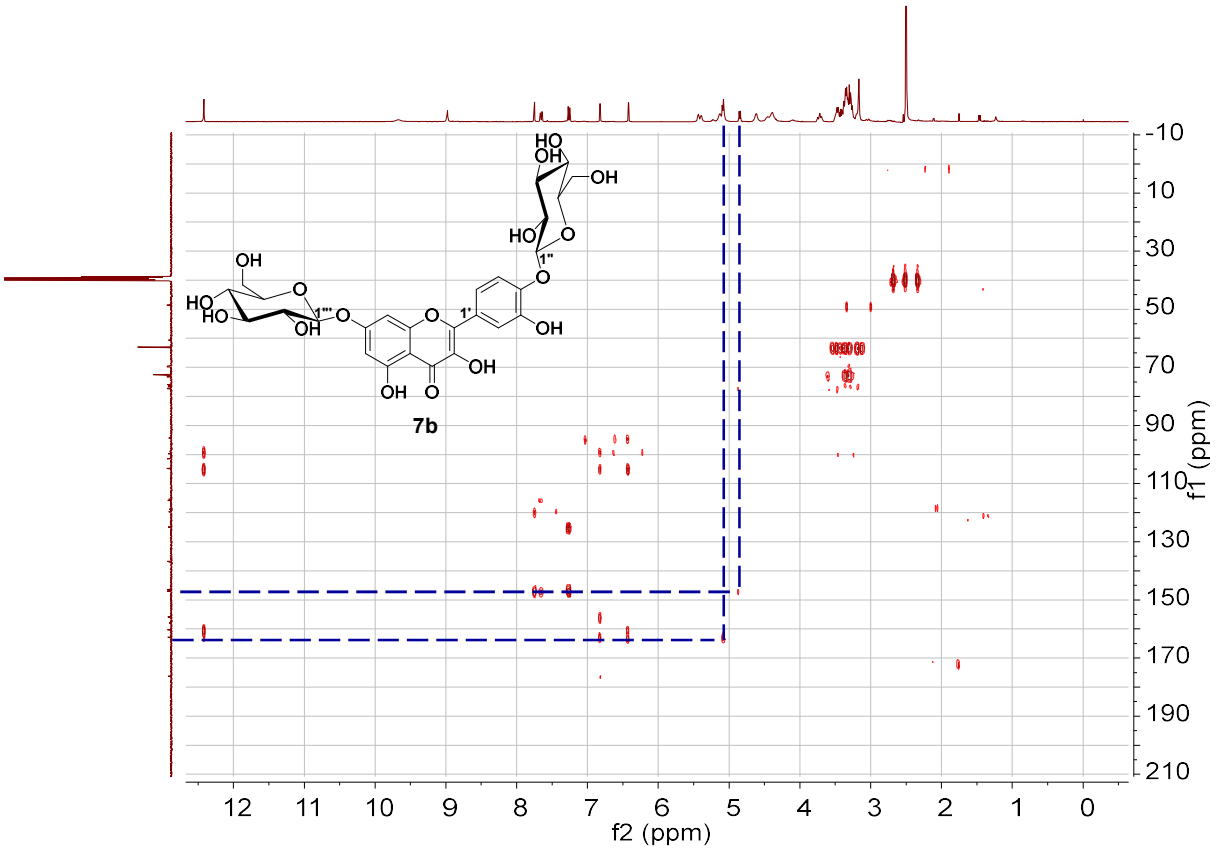


Figure S52. HMBC spectrum of **7b** in DMSO-d_6 (400 MHz).

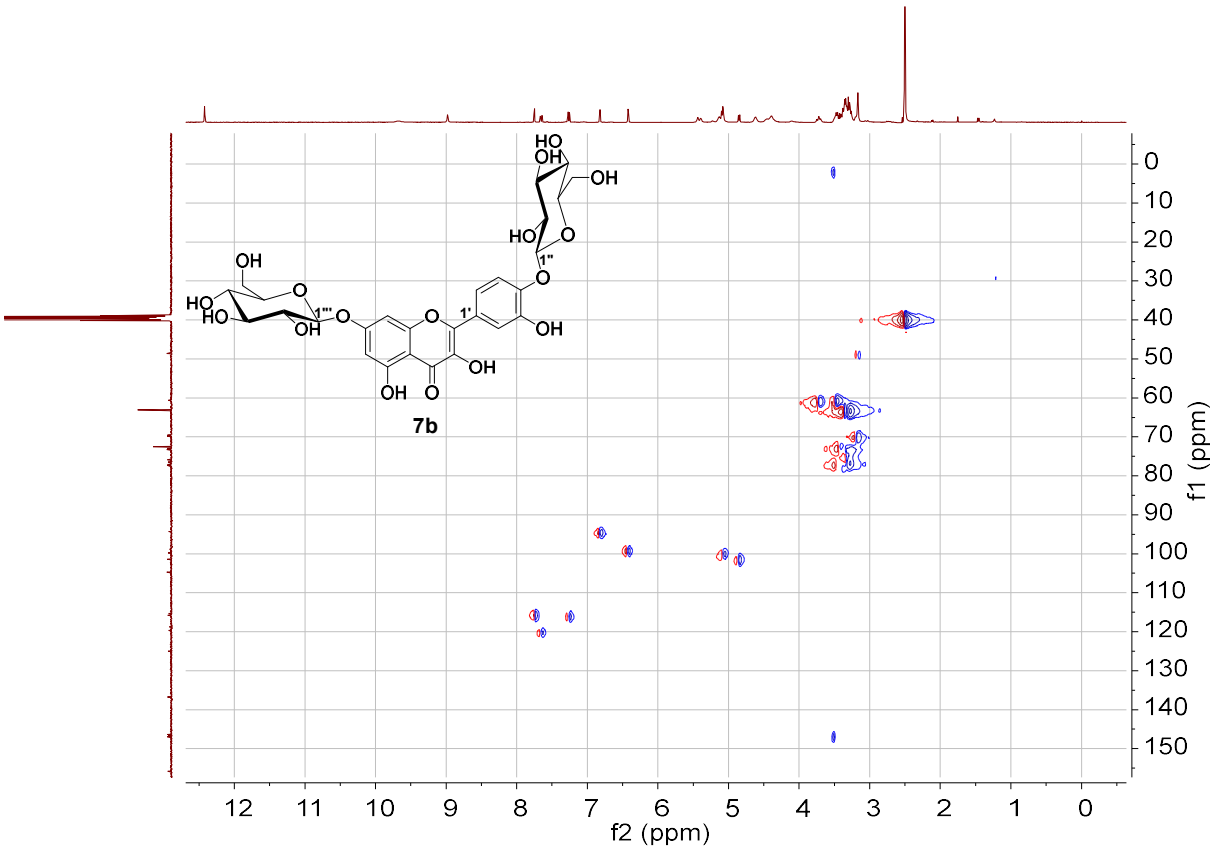


Figure S53. HSQC spectrum of **7b** in DMSO-d_6 (400 MHz).

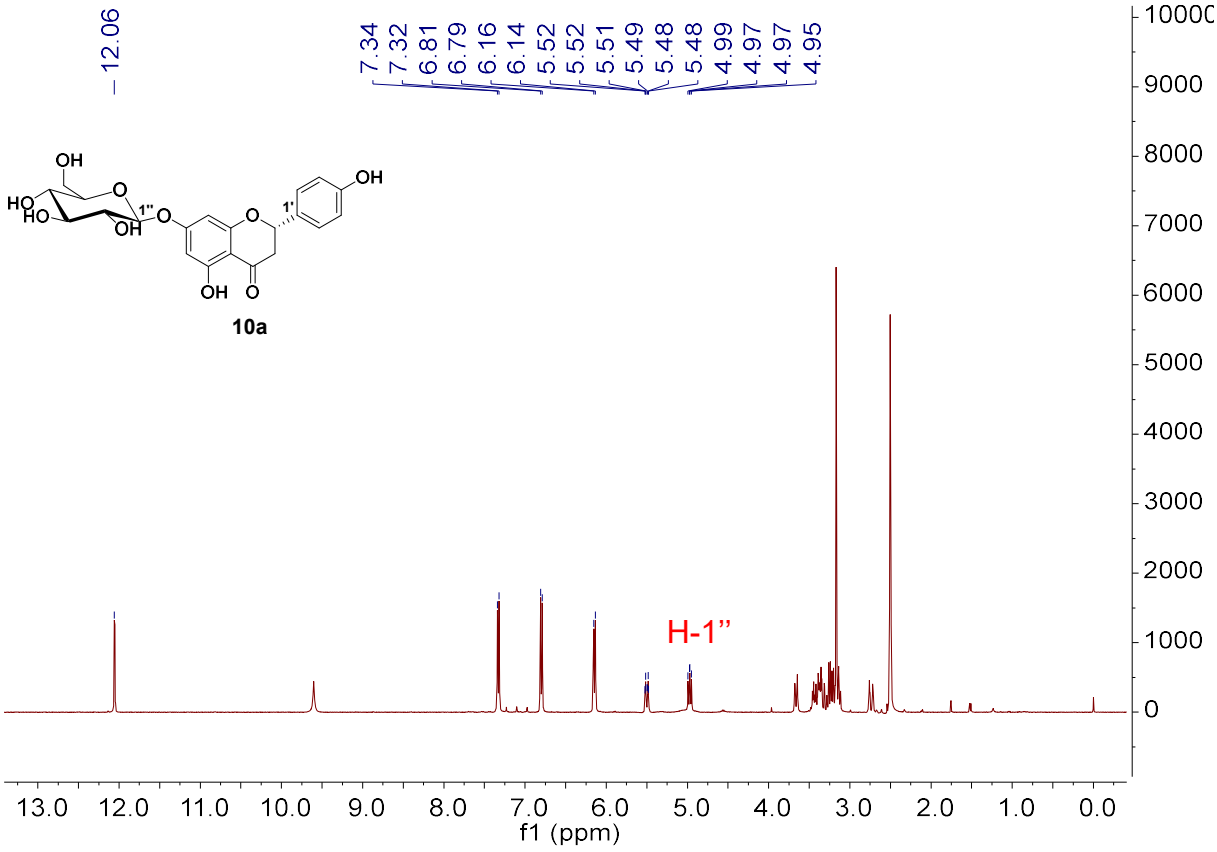


Figure S54. ^{1}H NMR spectrum of **10a** in DMSO-d_6 (400 MHz).

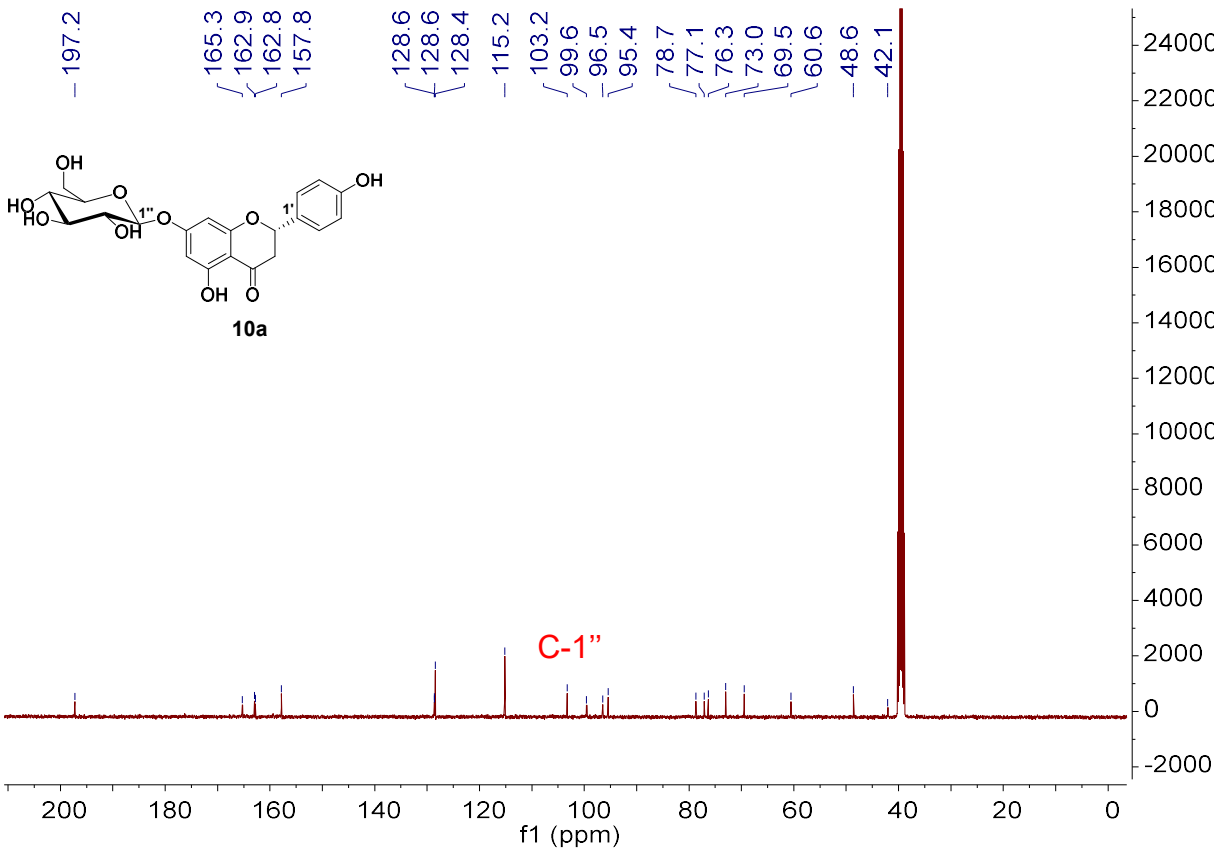


Figure S55. ^{13}C NMR spectrum of **10a** in DMSO-d_6 (100 MHz).

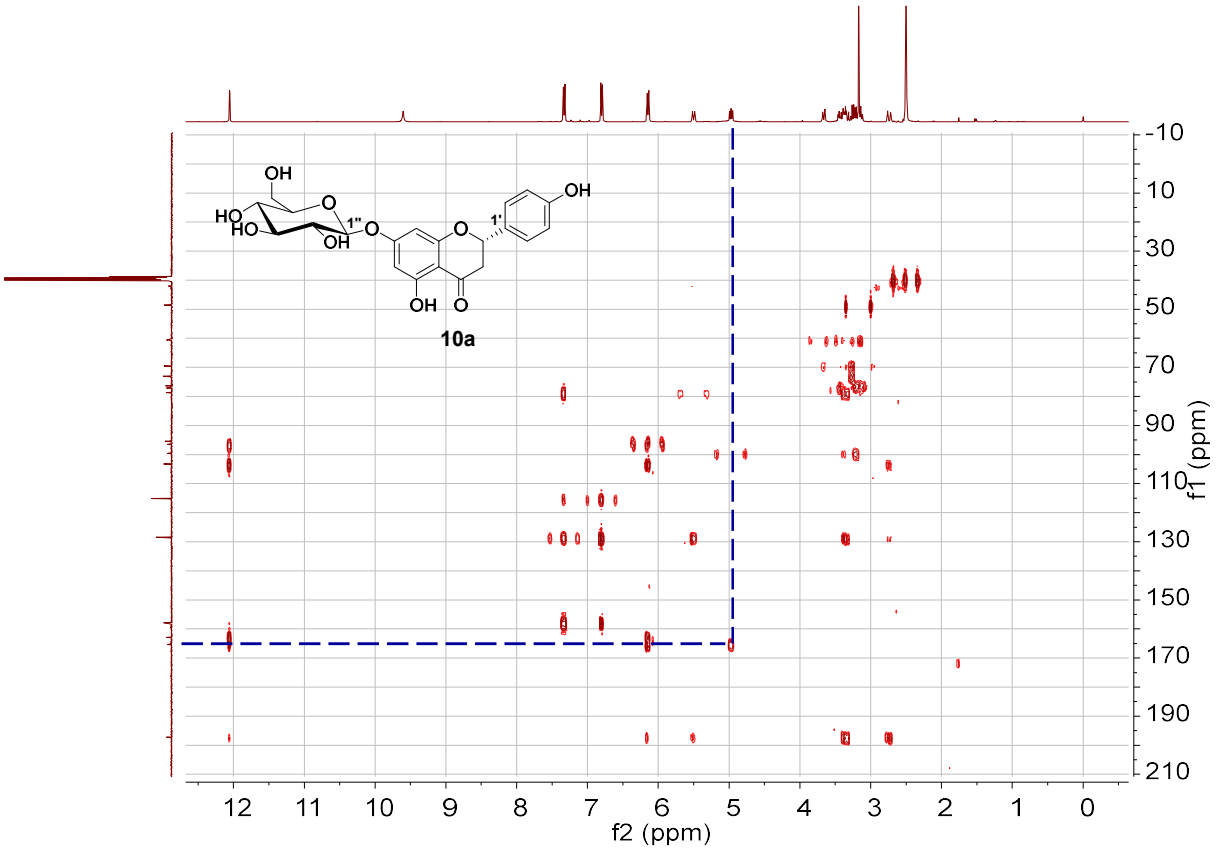


Figure S56. HMBC spectrum of **10a** in DMSO-d_6 (400 MHz).

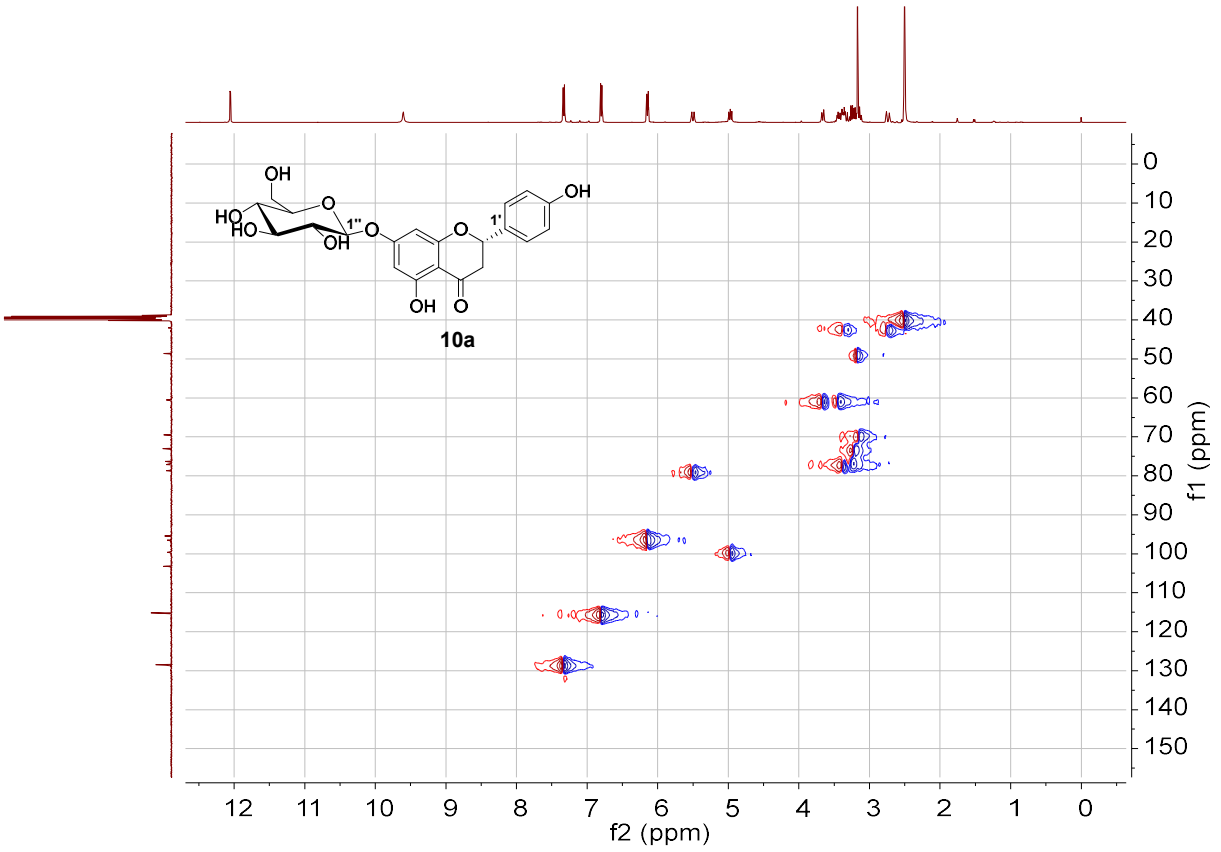


Figure S57. HSQC spectrum of **10a** in DMSO-d_6 (400 MHz).

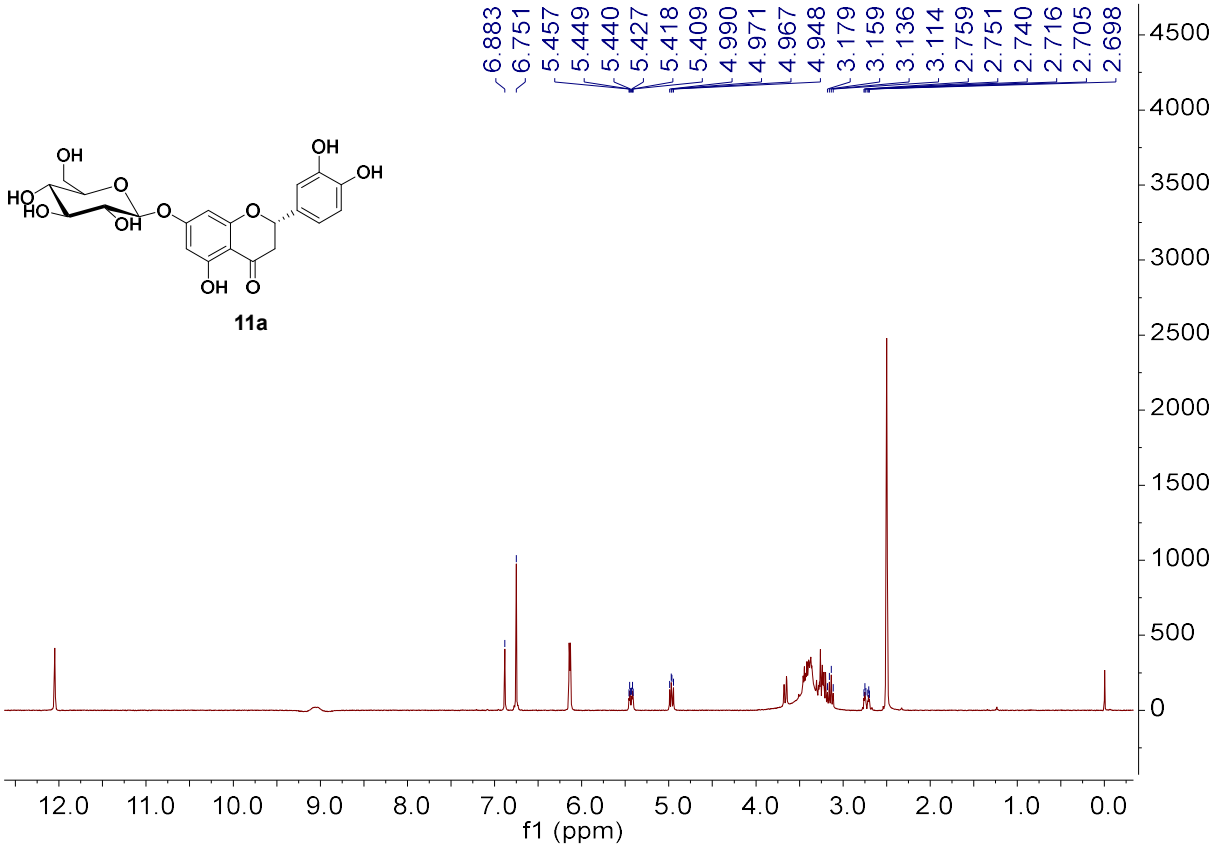


Figure S58. ^{1}H NMR spectrum of **11a** in DMSO-d_6 (400 MHz).

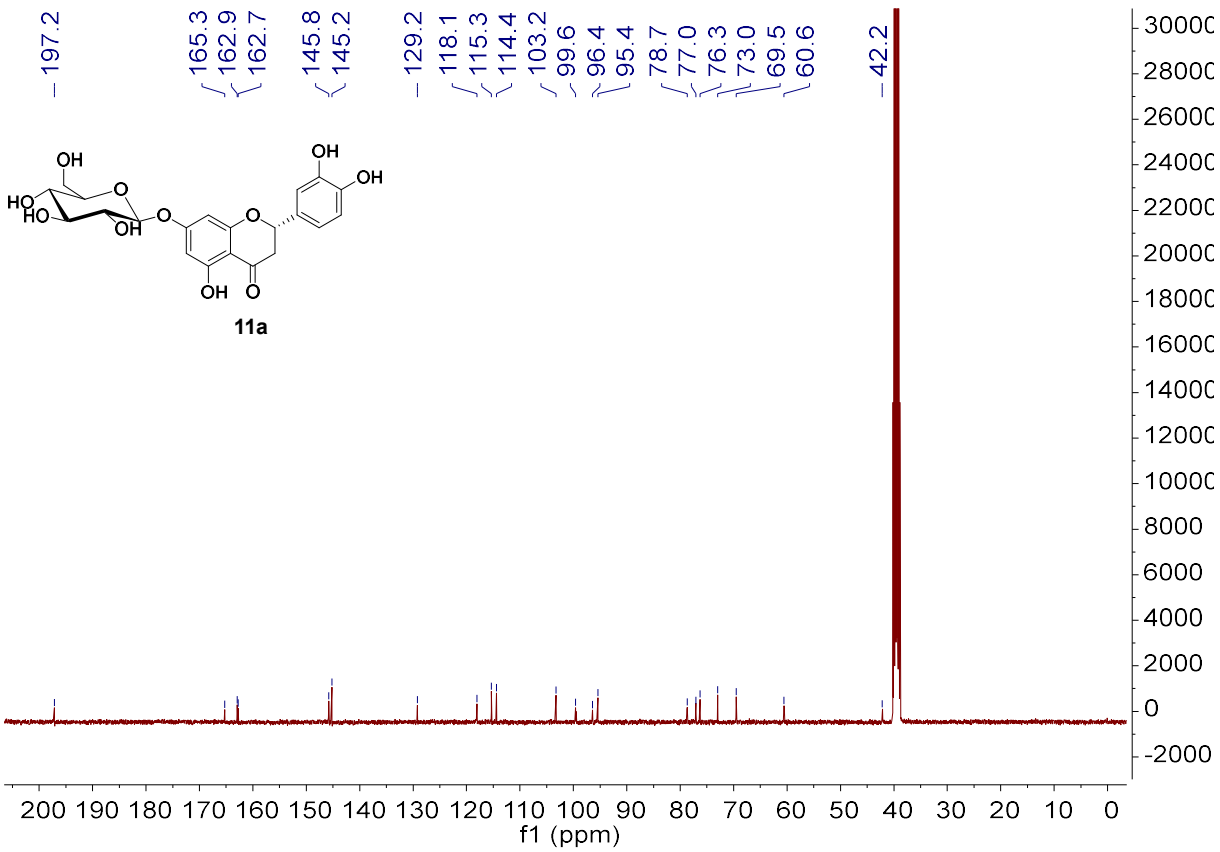


Figure S59. ^{13}C NMR spectrum of **11a** in DMSO-d_6 (100 MHz).

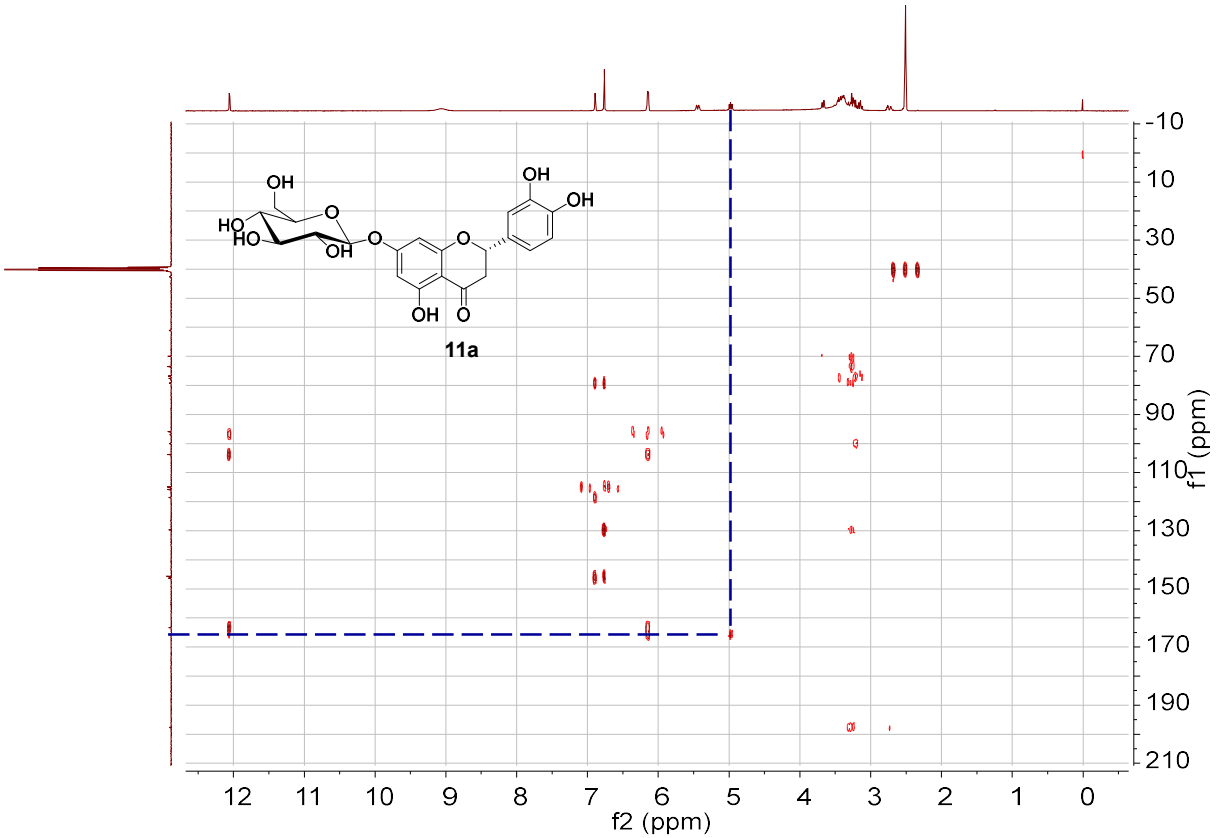


Figure S60. HMBC spectrum of **11a** in DMSO-d_6 (400 MHz).

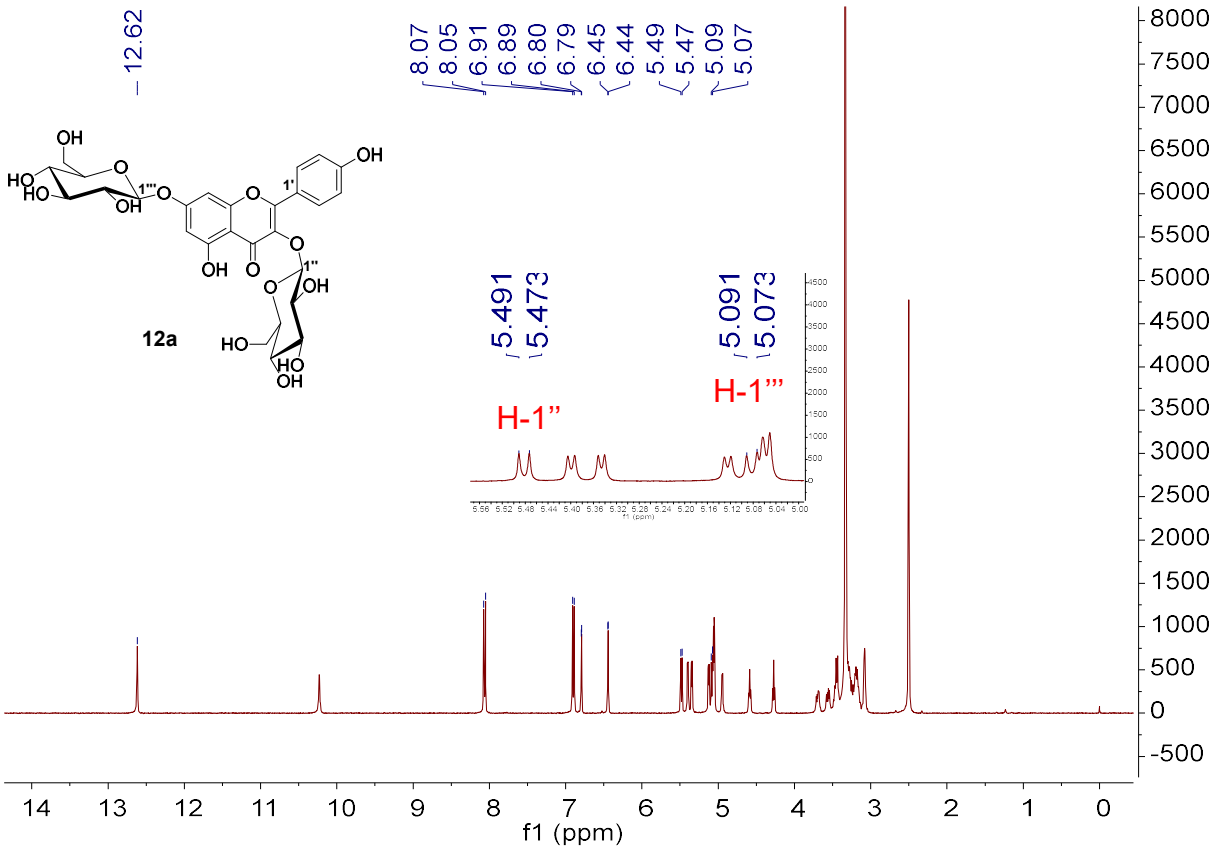


Figure S61. ^{1}H NMR spectrum of **12a** in DMSO-d_6 (400 MHz).

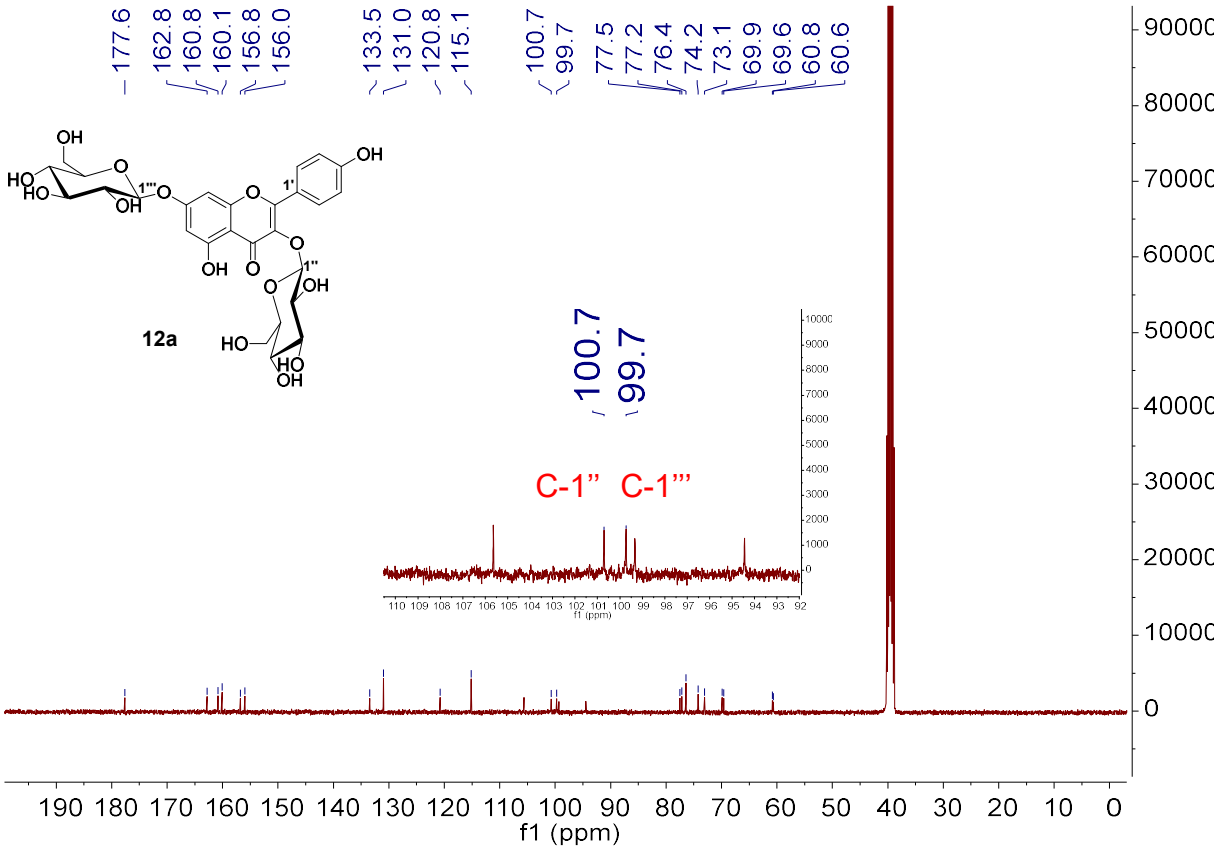


Figure S62. ^{13}C NMR spectrum of **12a** in DMSO-d_6 (100 MHz).

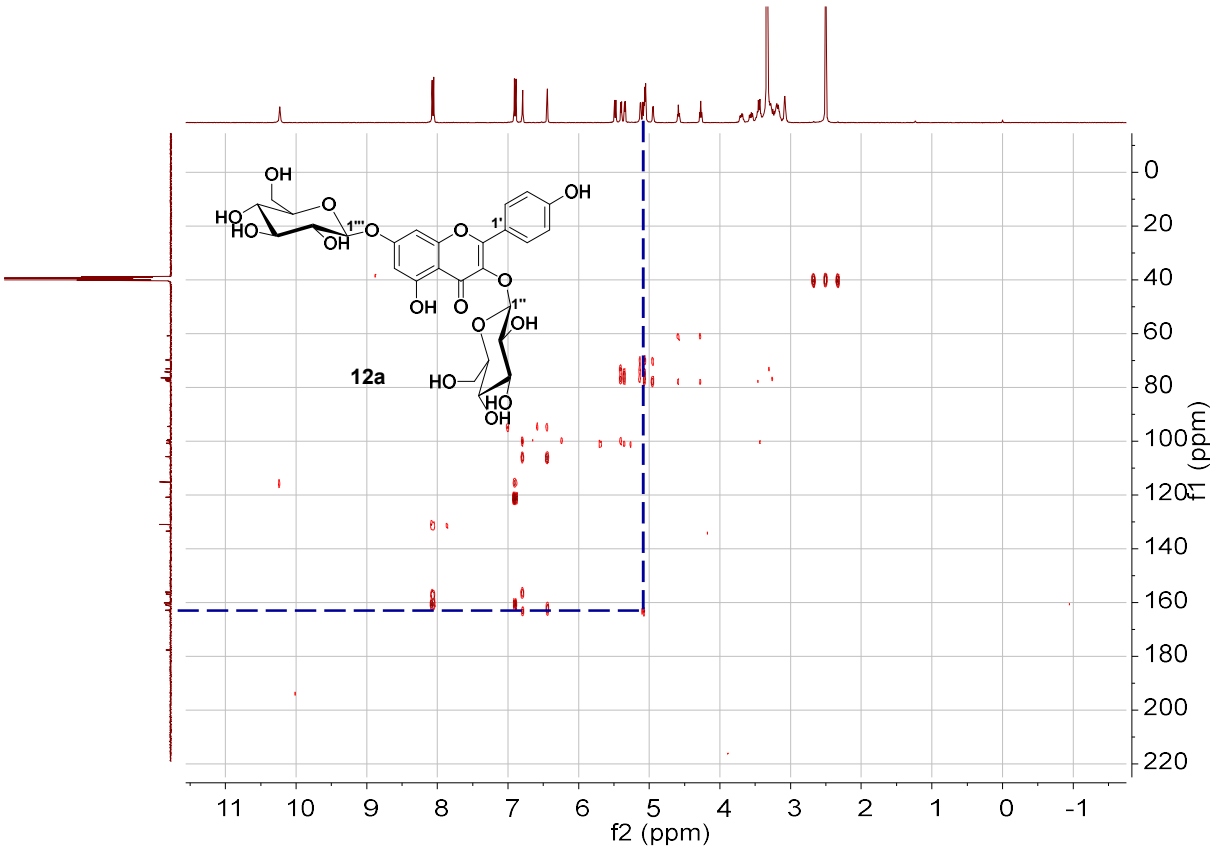


Figure S63. HMBC spectrum of **12a** in DMSO-d_6 (400 MHz).

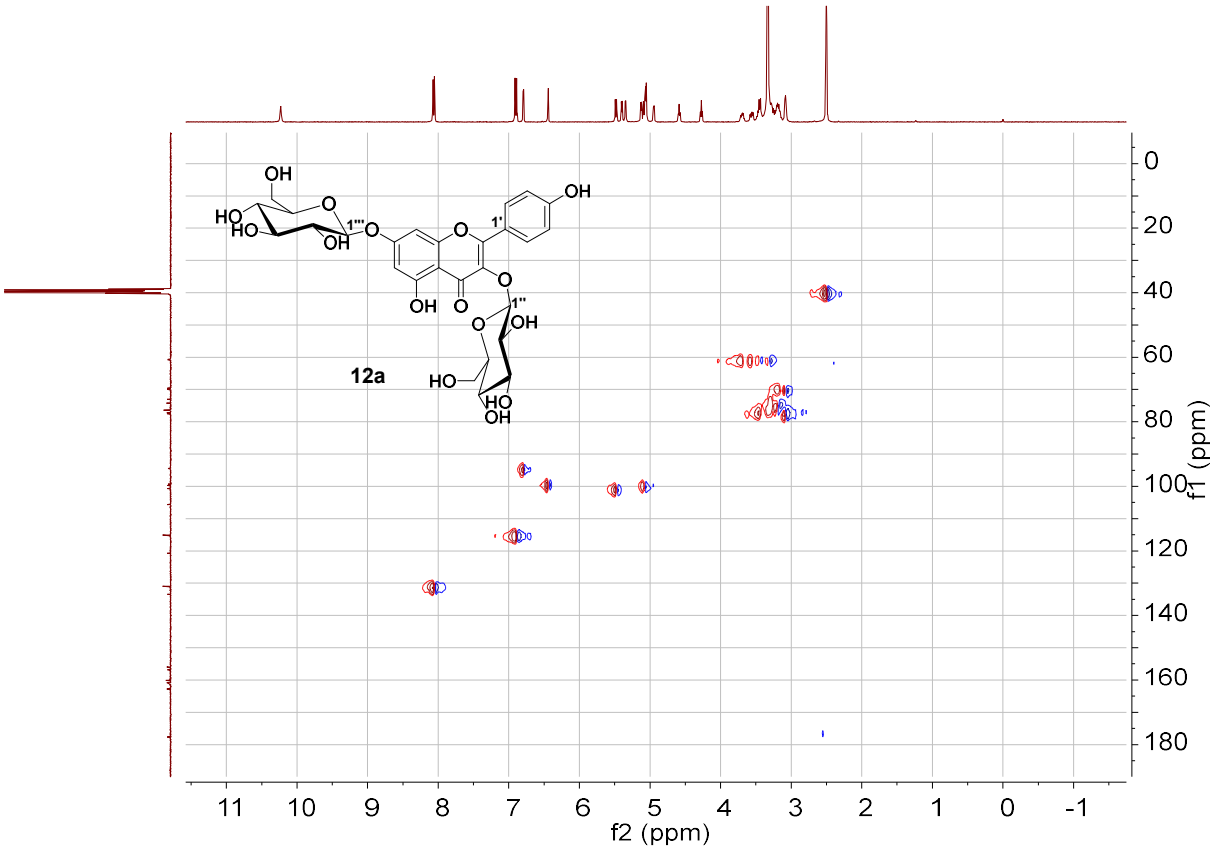


Figure S64. HSQC spectrum of **12a** in DMSO-d_6 (400 MHz).

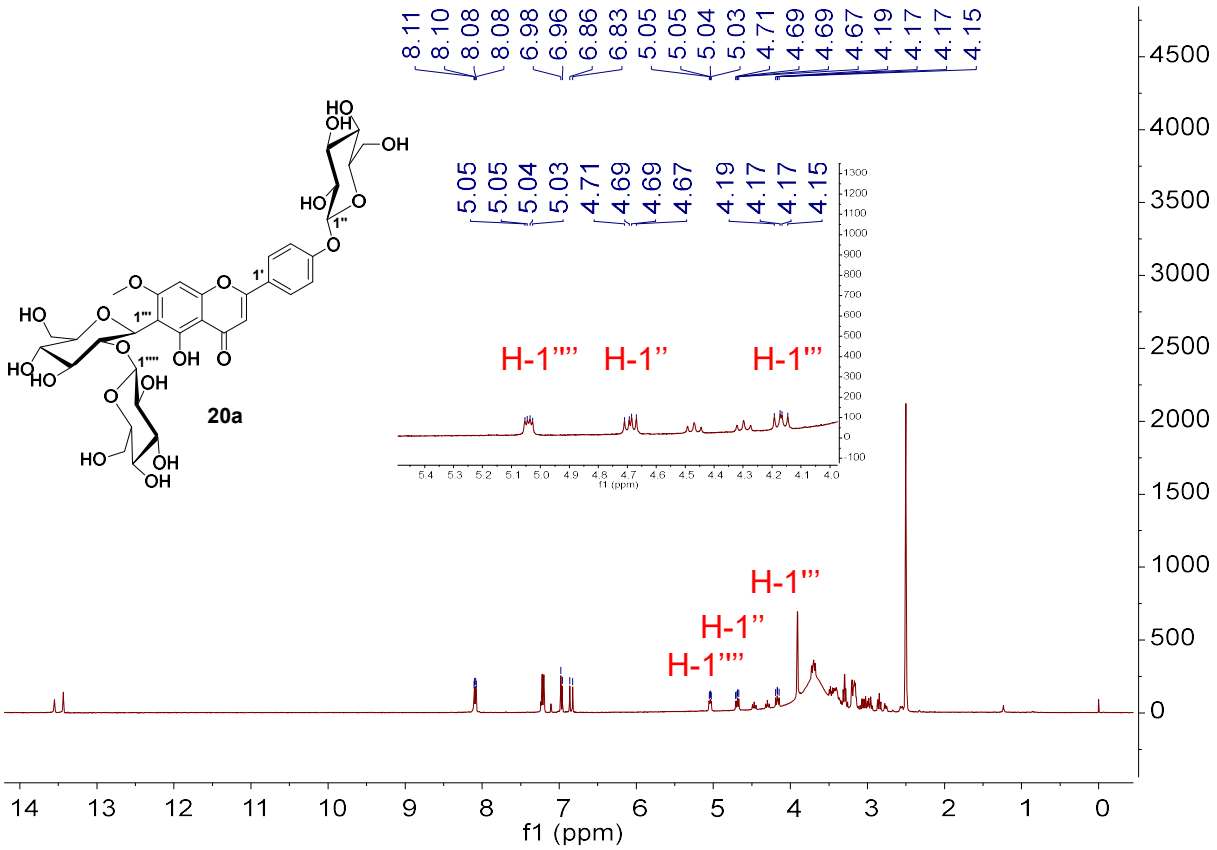


Figure S65. ^{1}H NMR spectrum of **20a** in DMSO-d_6 (400 MHz).

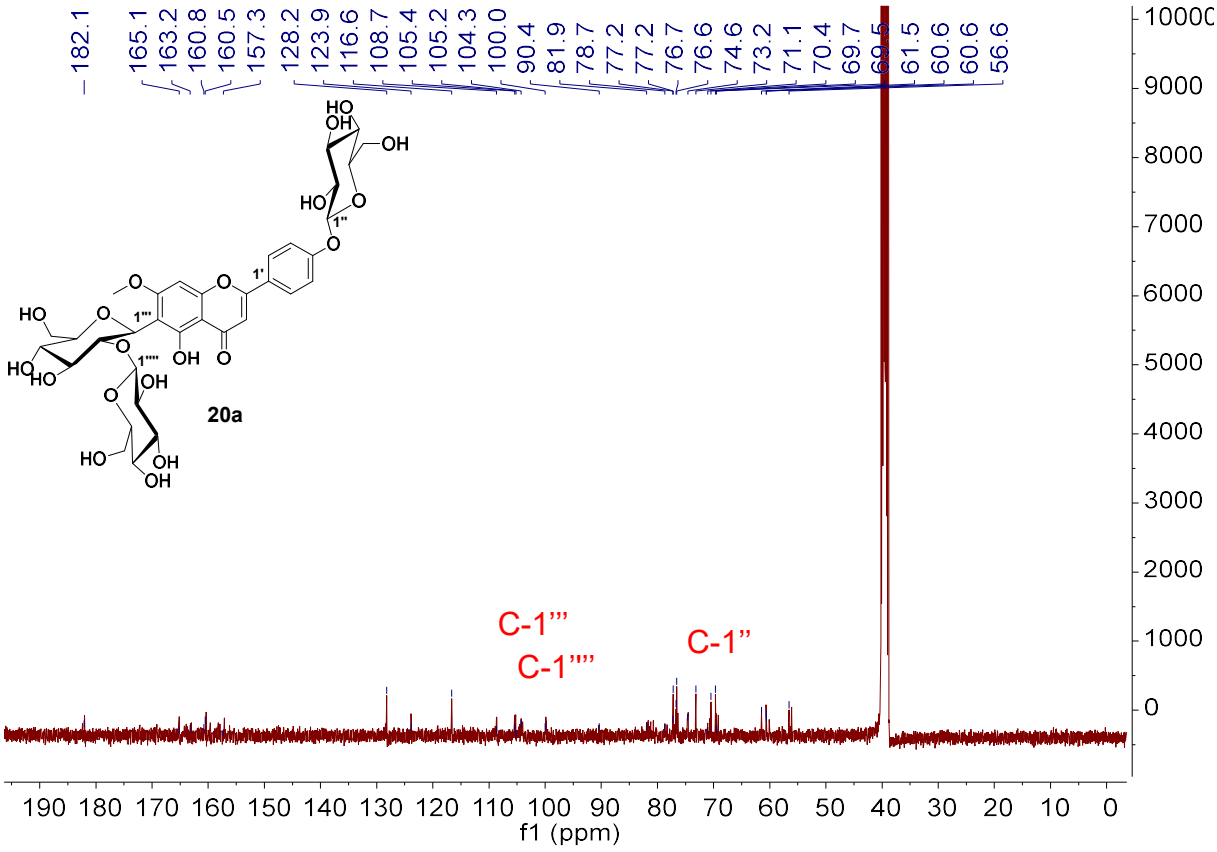


Figure S66. ^{13}C NMR spectrum of **20a** in DMSO-d_6 (100 MHz).

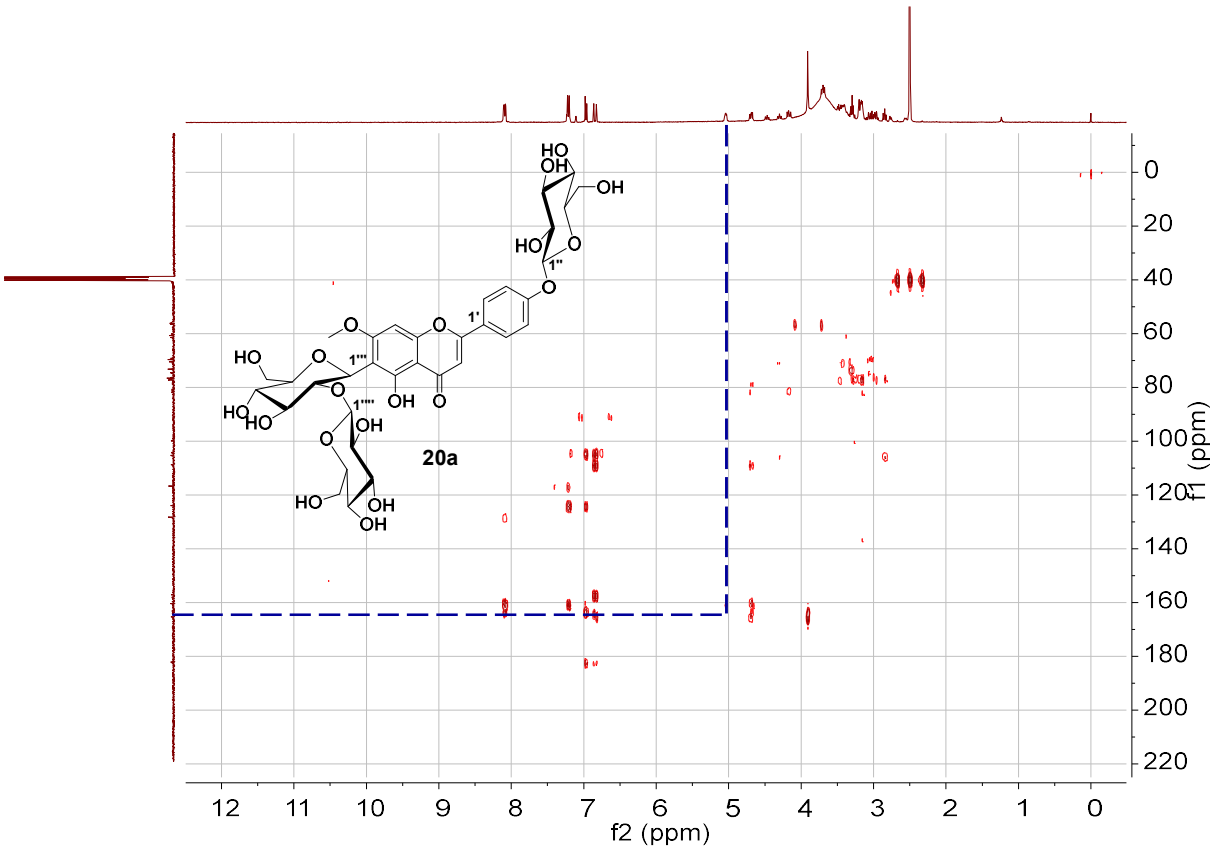


Figure S67. HMBC spectrum of **20a** in DMSO-d_6 (400 MHz).

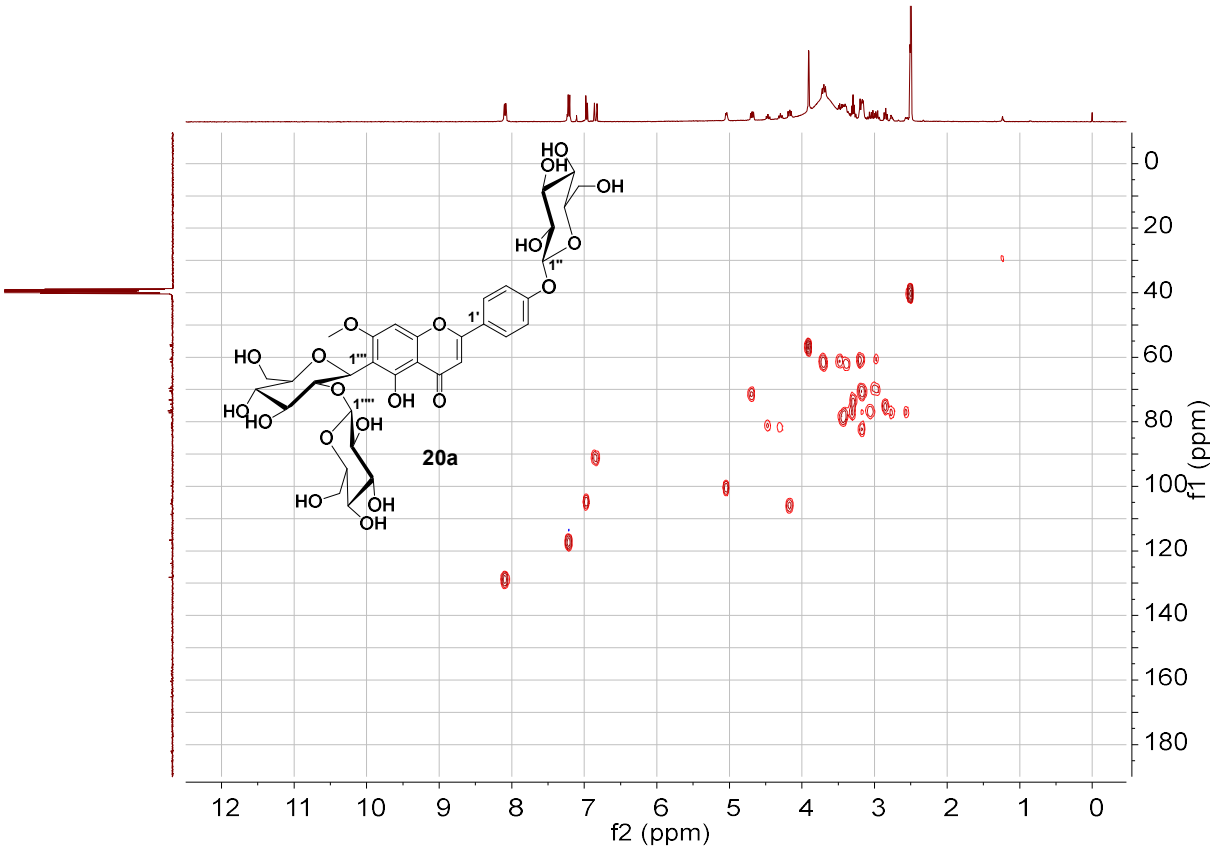


Figure S68. HSQC spectrum of **20a** in DMSO-d_6 (400 MHz).

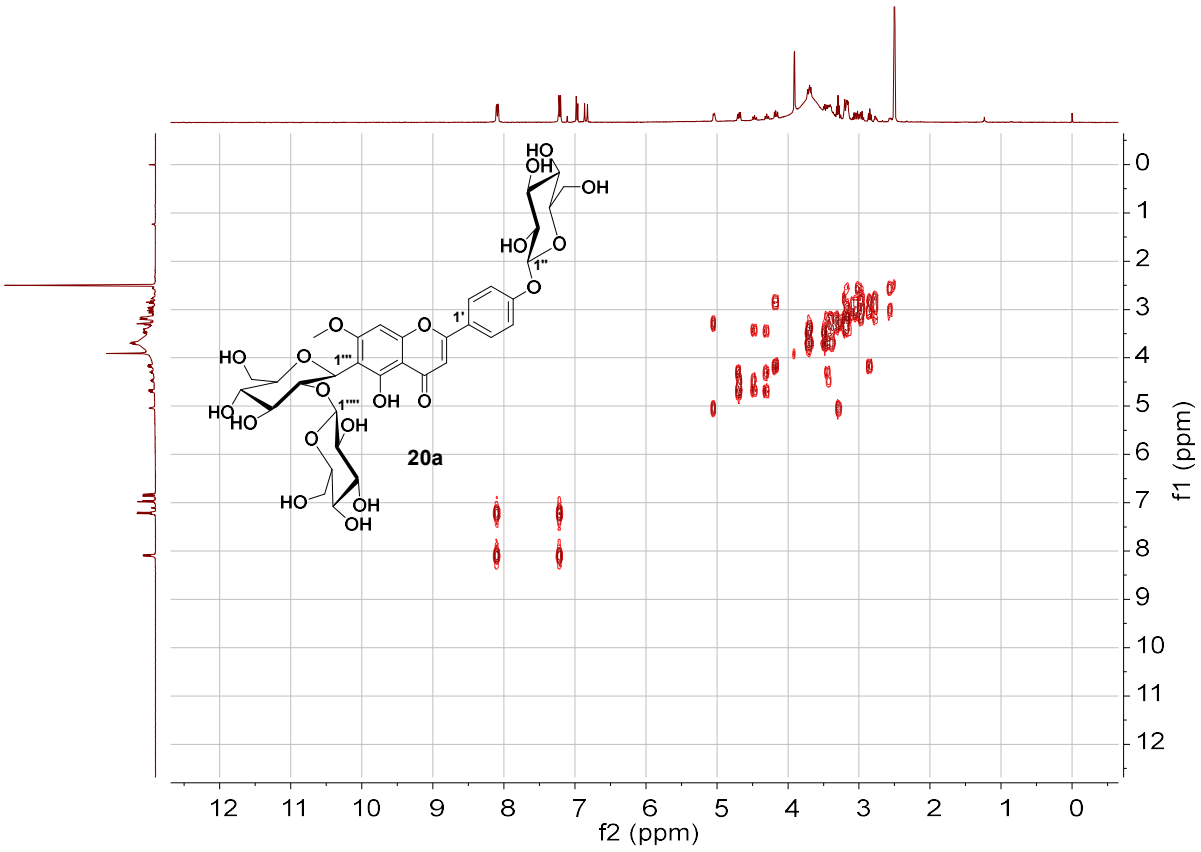


Figure S69. ^{1}H-^{1}H COSY spectrum of **20a** in DMSO-d_6 (400 MHz).

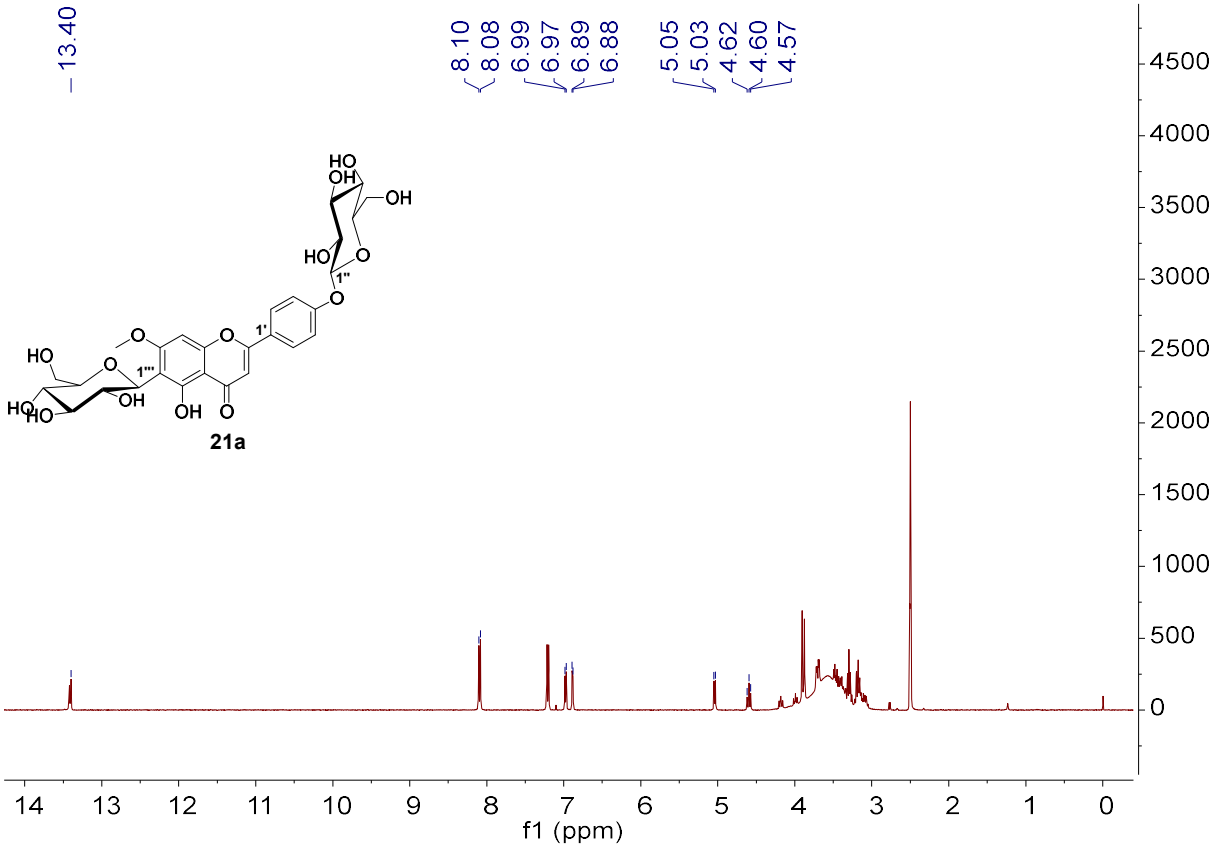


Figure S70. ^{1}H NMR spectrum of **21a** in DMSO-d_6 (400 MHz).

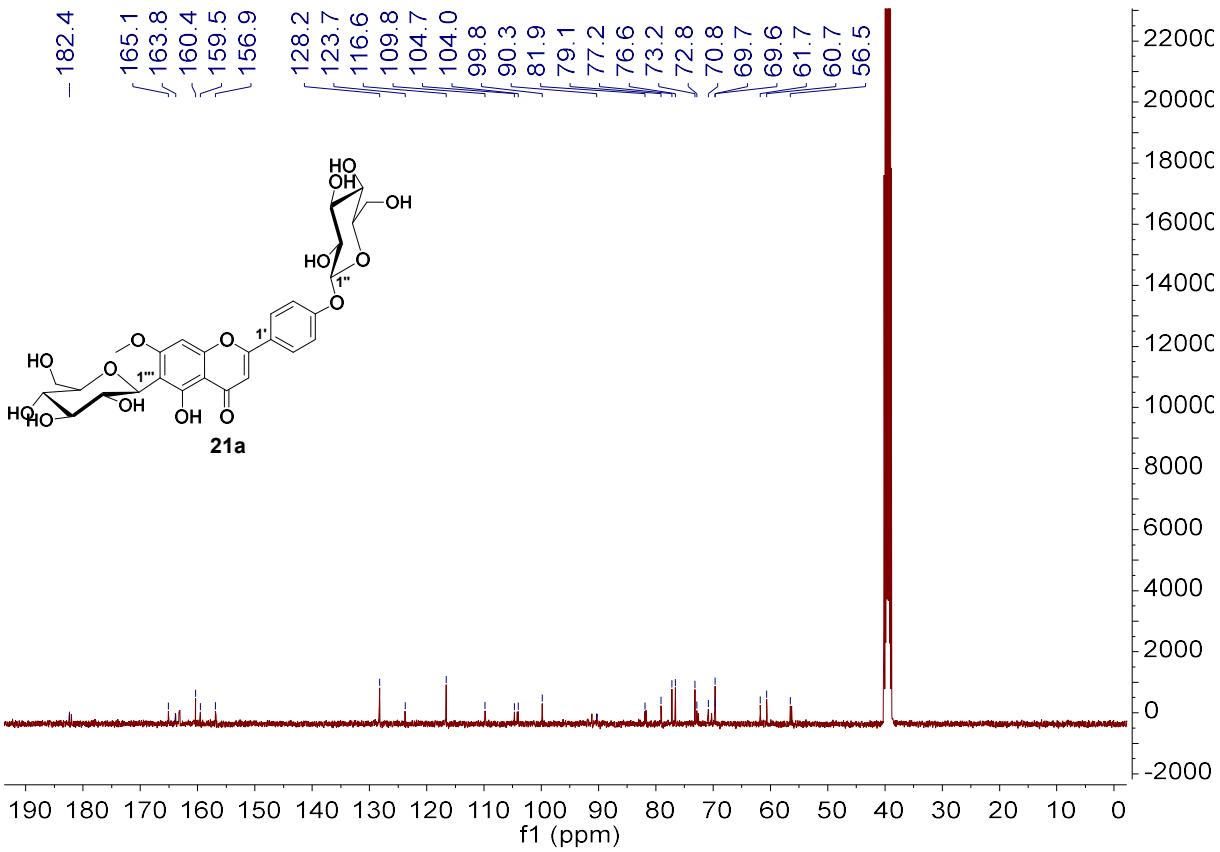


Figure S71. ^{13}C NMR spectrum of **21a** in DMSO-d_6 (100 MHz).

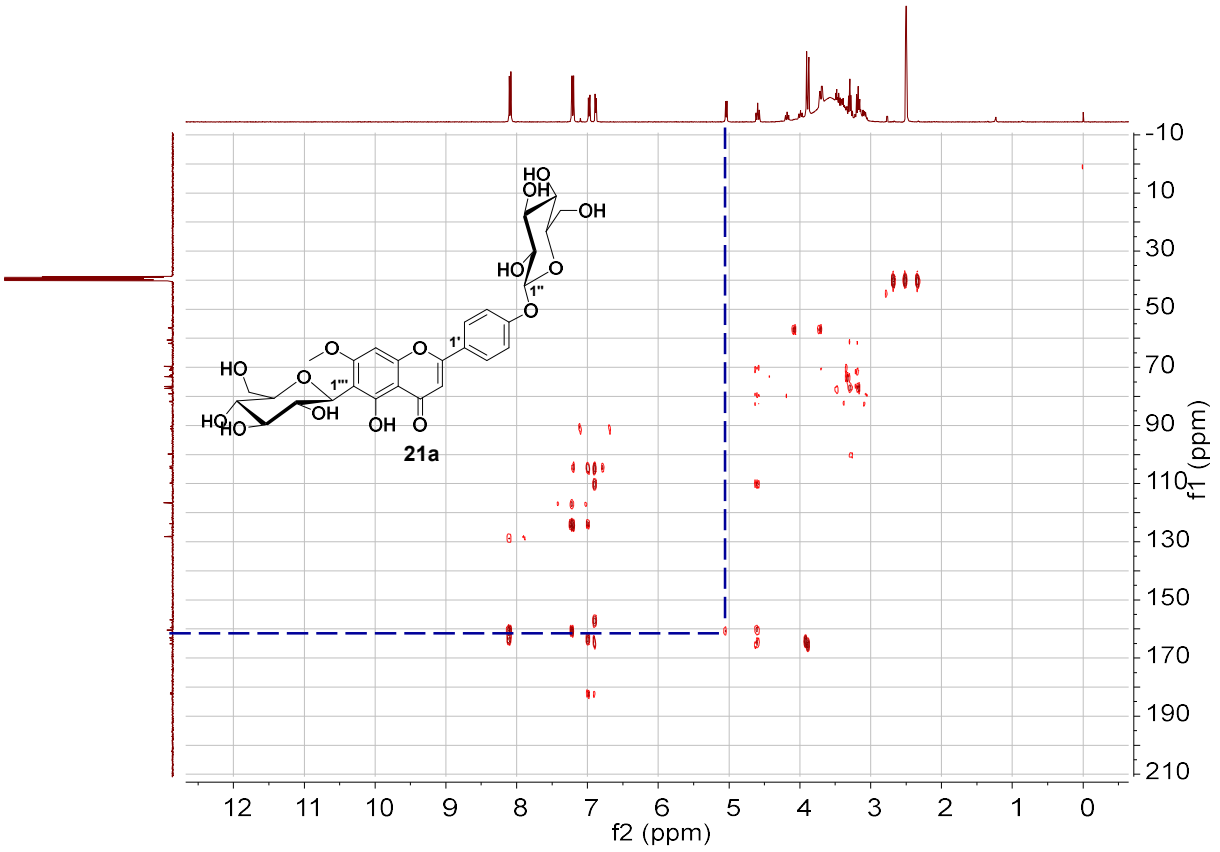


Figure S72. HMBC spectrum of **21a** in DMSO-d_6 (400 MHz).

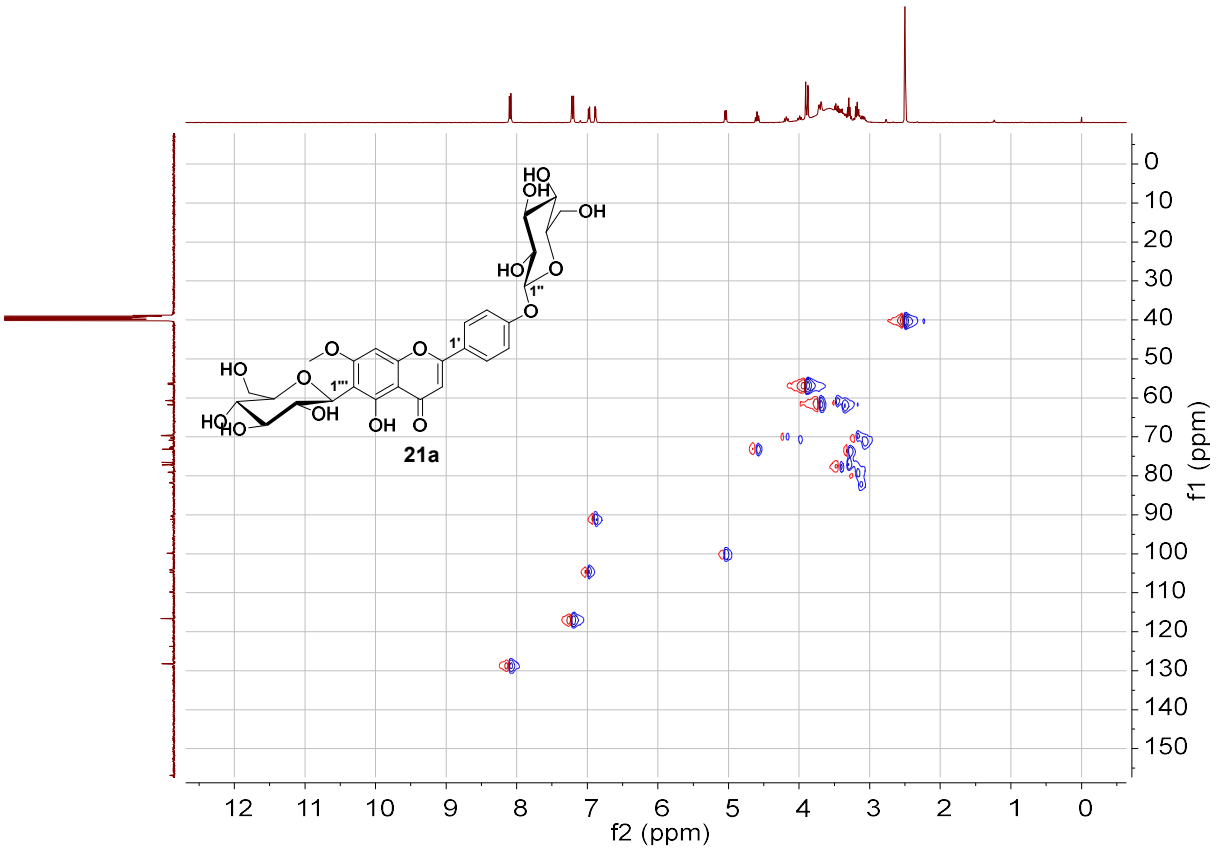


Figure S73. HSQC spectrum of **21a** in DMSO-d_6 (400 MHz).

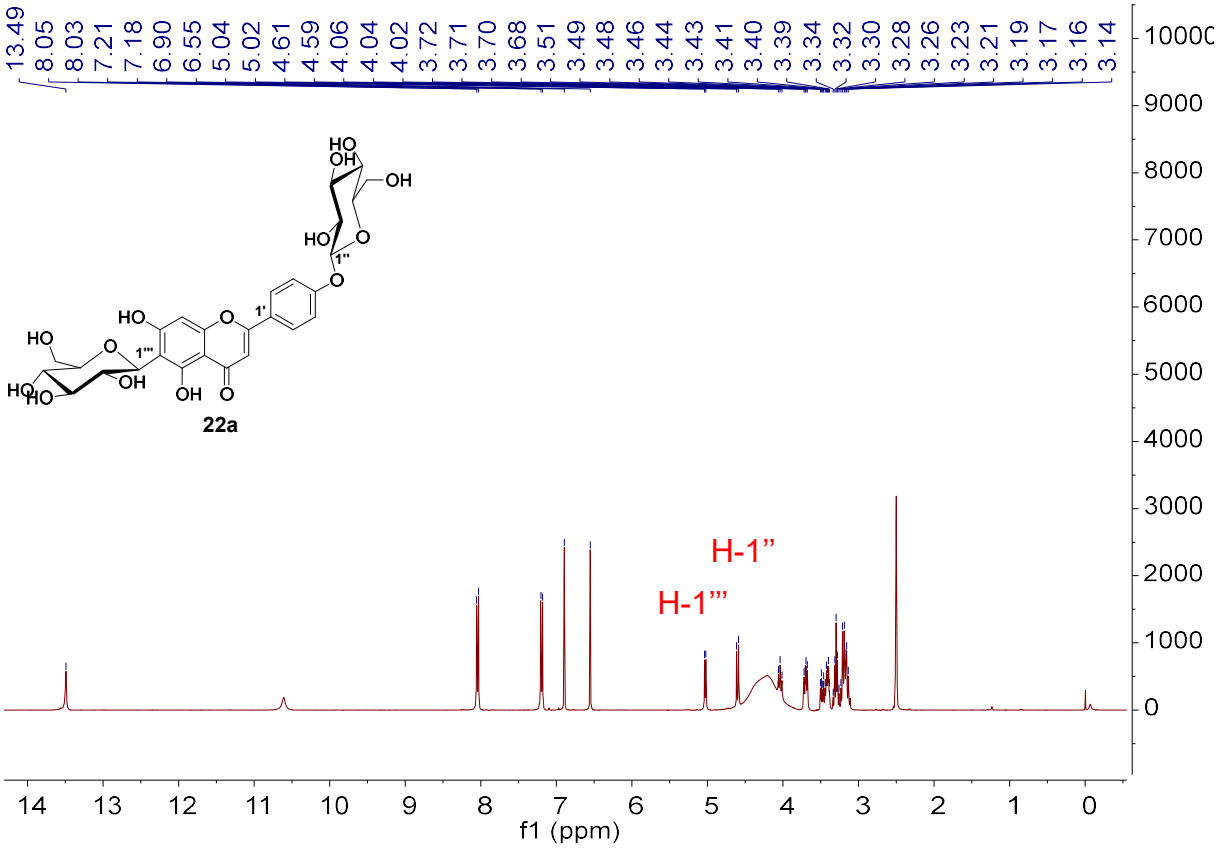


Figure S74. ^{1}H NMR spectrum of **22a** in DMSO-d_6 (400 MHz).

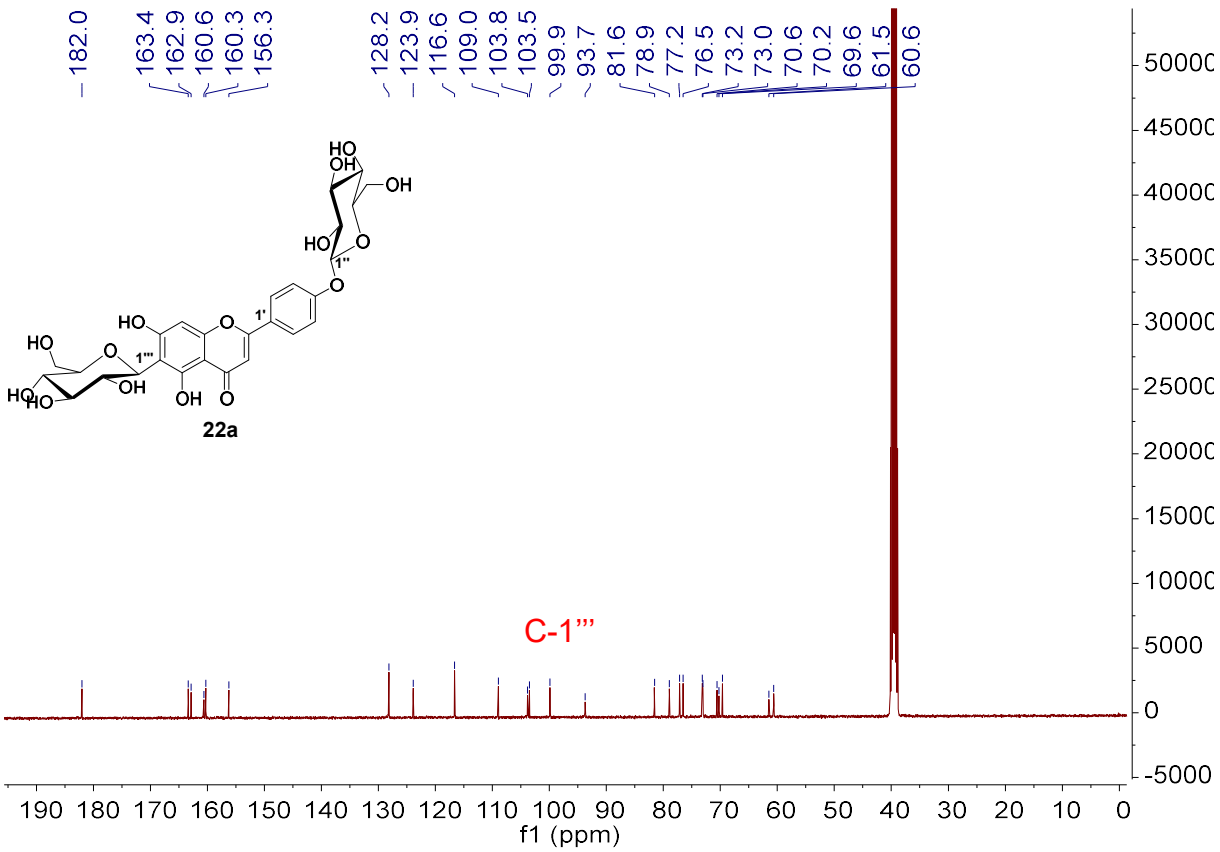


Figure S75. ^{13}C NMR spectrum of **22a** in DMSO-d_6 (100 MHz).

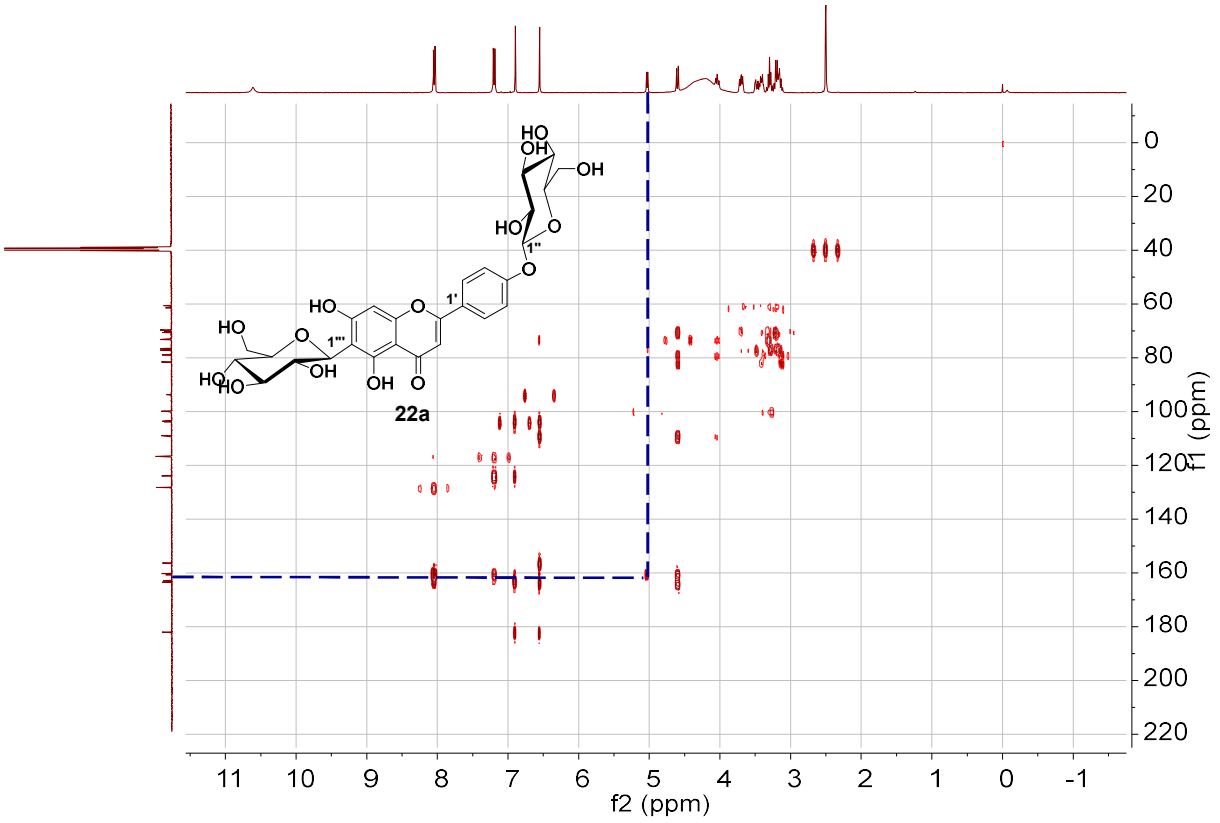


Figure S76. HMBC spectrum of **22a** in DMSO-d_6 (400 MHz).

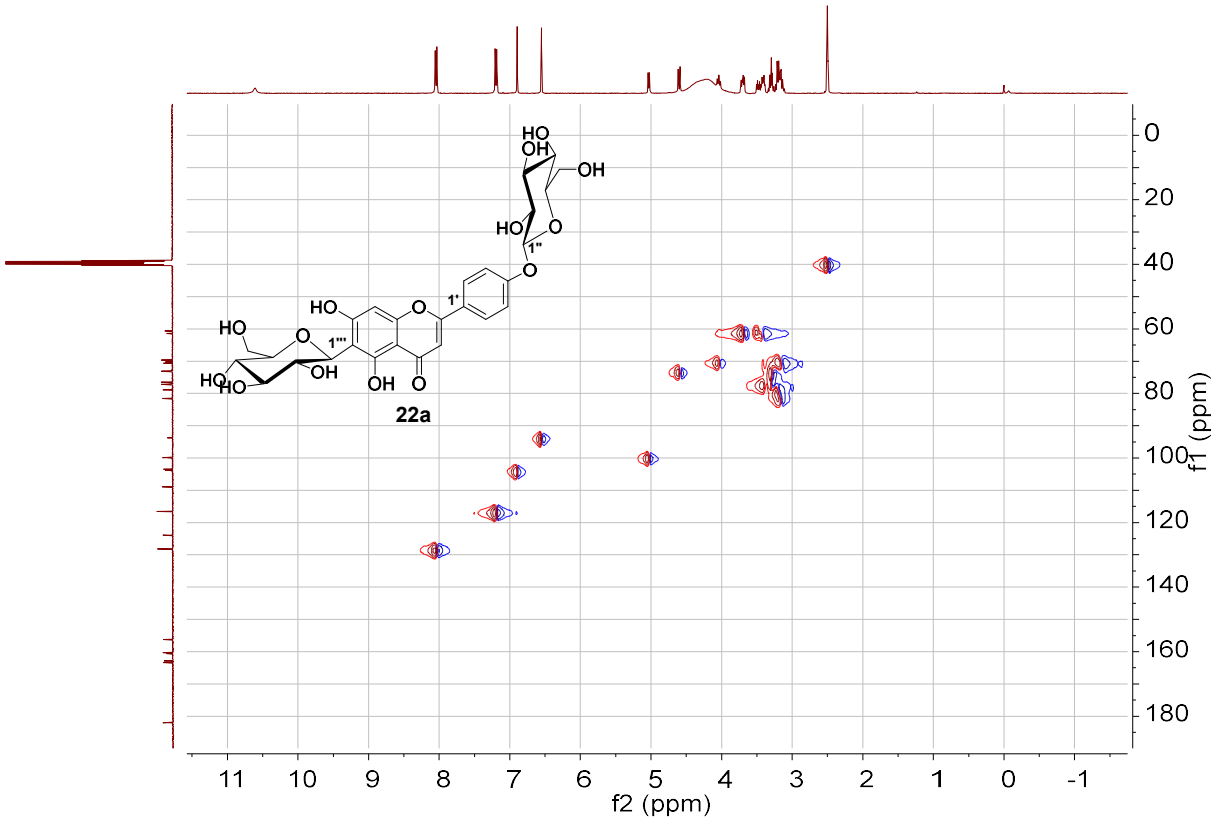


Figure S77. HSQC spectrum of **22a** in DMSO-d_6 (400 MHz).

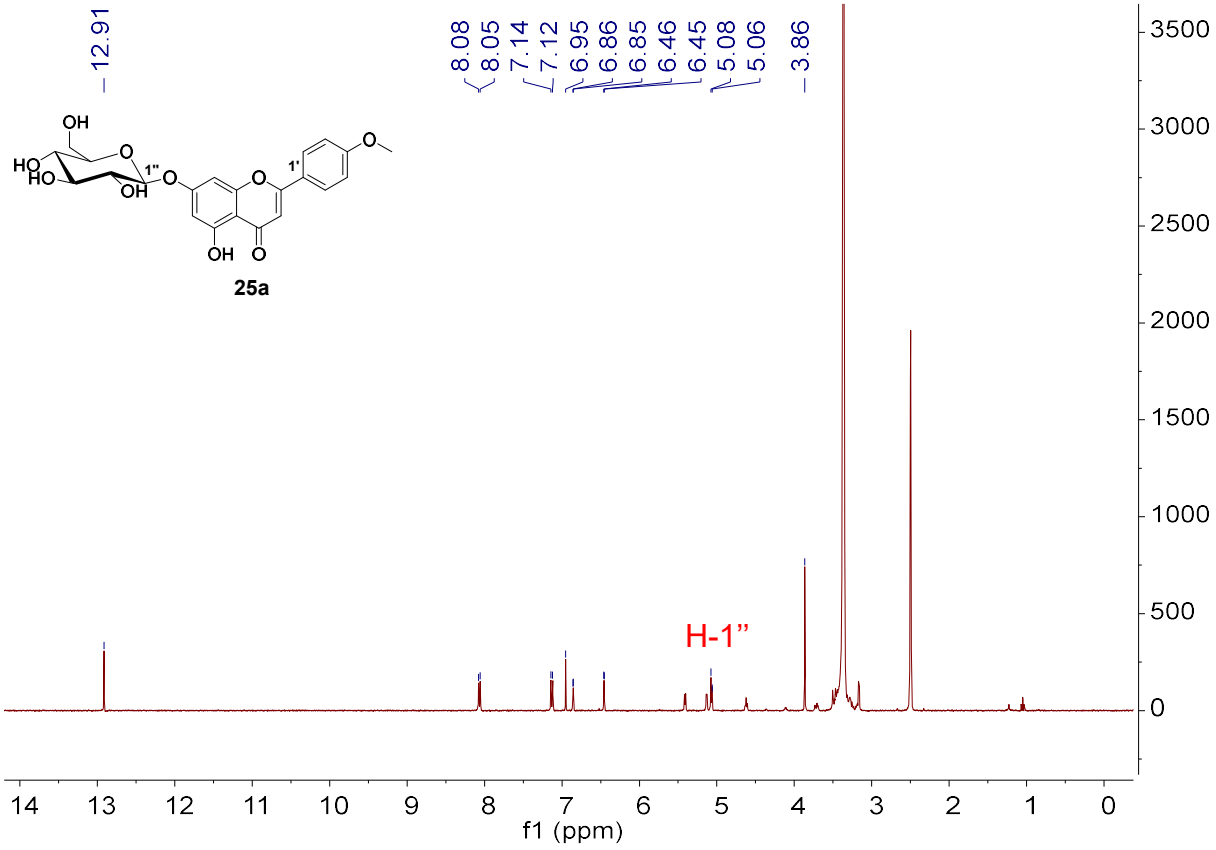


Figure S78. ^{1}H NMR spectrum of **25a** in DMSO-d_6 (400 MHz).

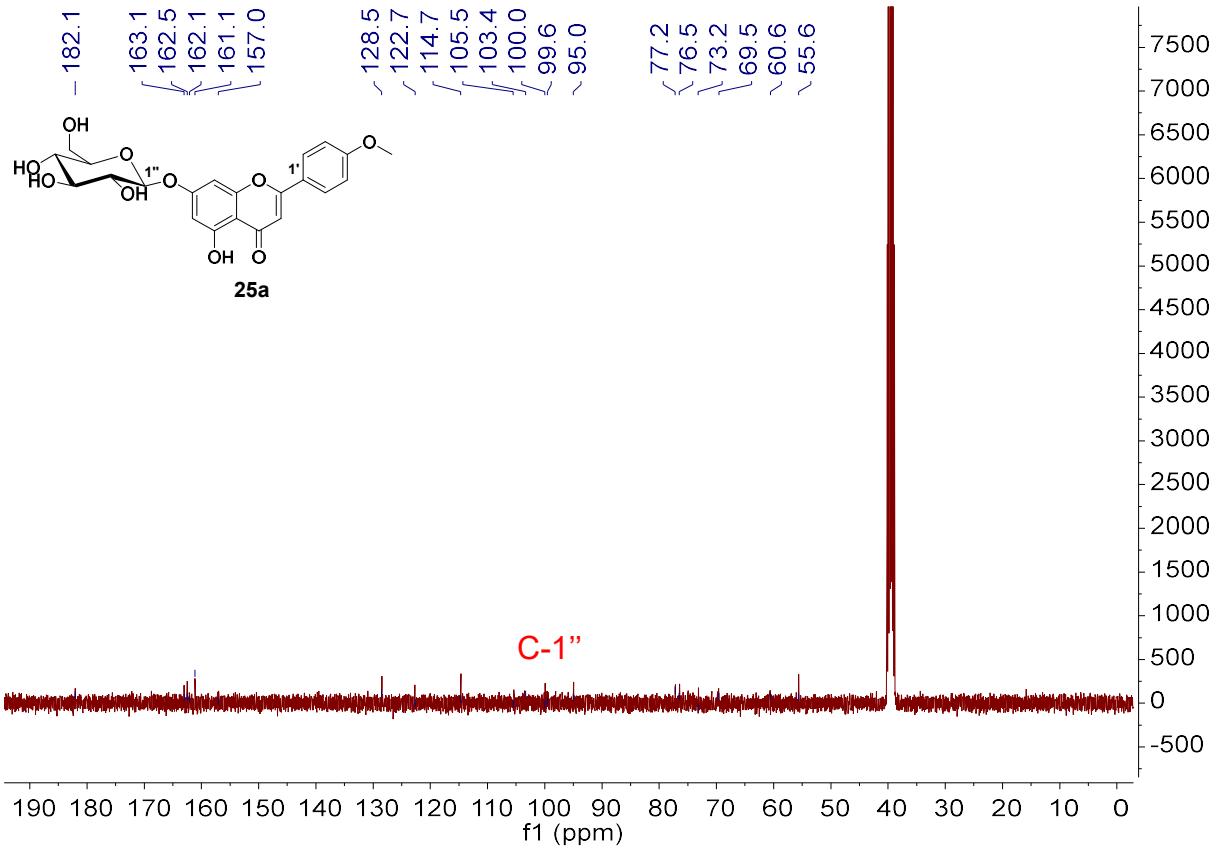


Figure S79. ^{13}C NMR spectrum of **25a** in DMSO-d_6 (100 MHz).

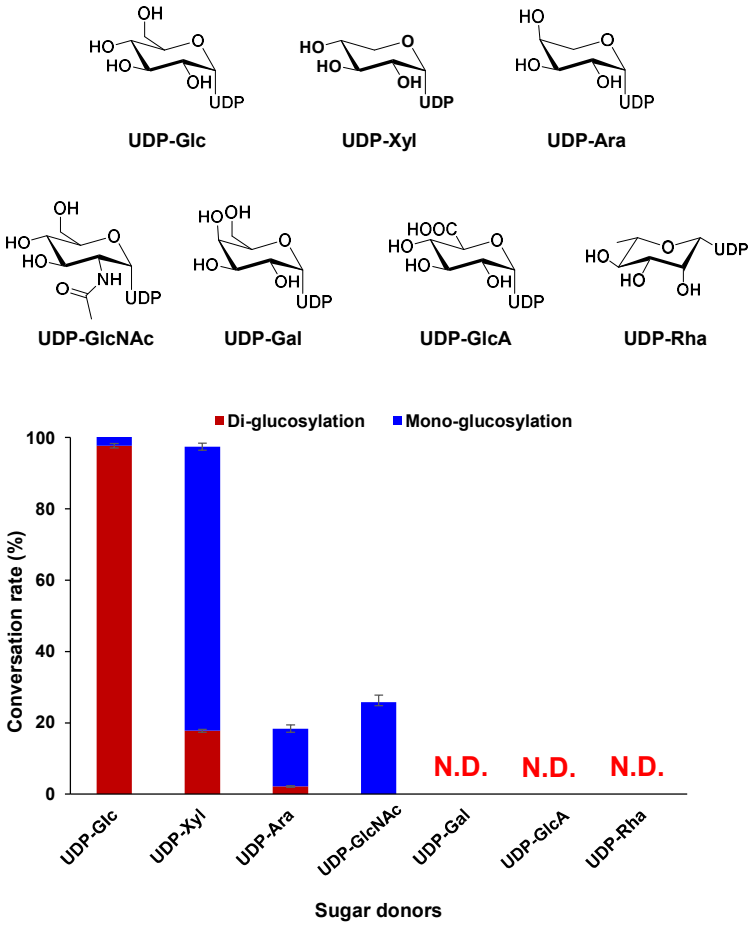


Figure S80. Sugar donor promiscuity of ZjOGT3**.** (A) Chemical structures of sugar donors used in this study. (B) Conversion rates of compound **1** using different sugar donors. N.D., products were not detected.

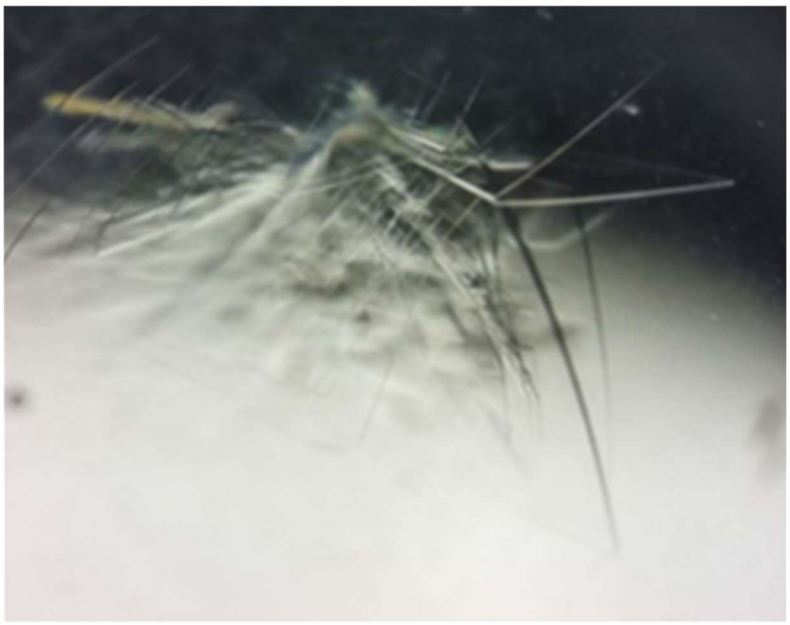


Figure S81. Photo of ZjOGT3 crystals. Purified ZjOGT3 (20 mg/mL) was incubated with 5 mM UDP-Glc at 4°C for 1 h before setting up the crystallization trays. The crystals were prepared by hanging drop vapor diffusion. The crystals were obtained in 0.2 M sodium acetate trihydrate, 0.1 M Tris-HCl 8.00, 26% *w*/*v* PEG 4000 after 2 days.

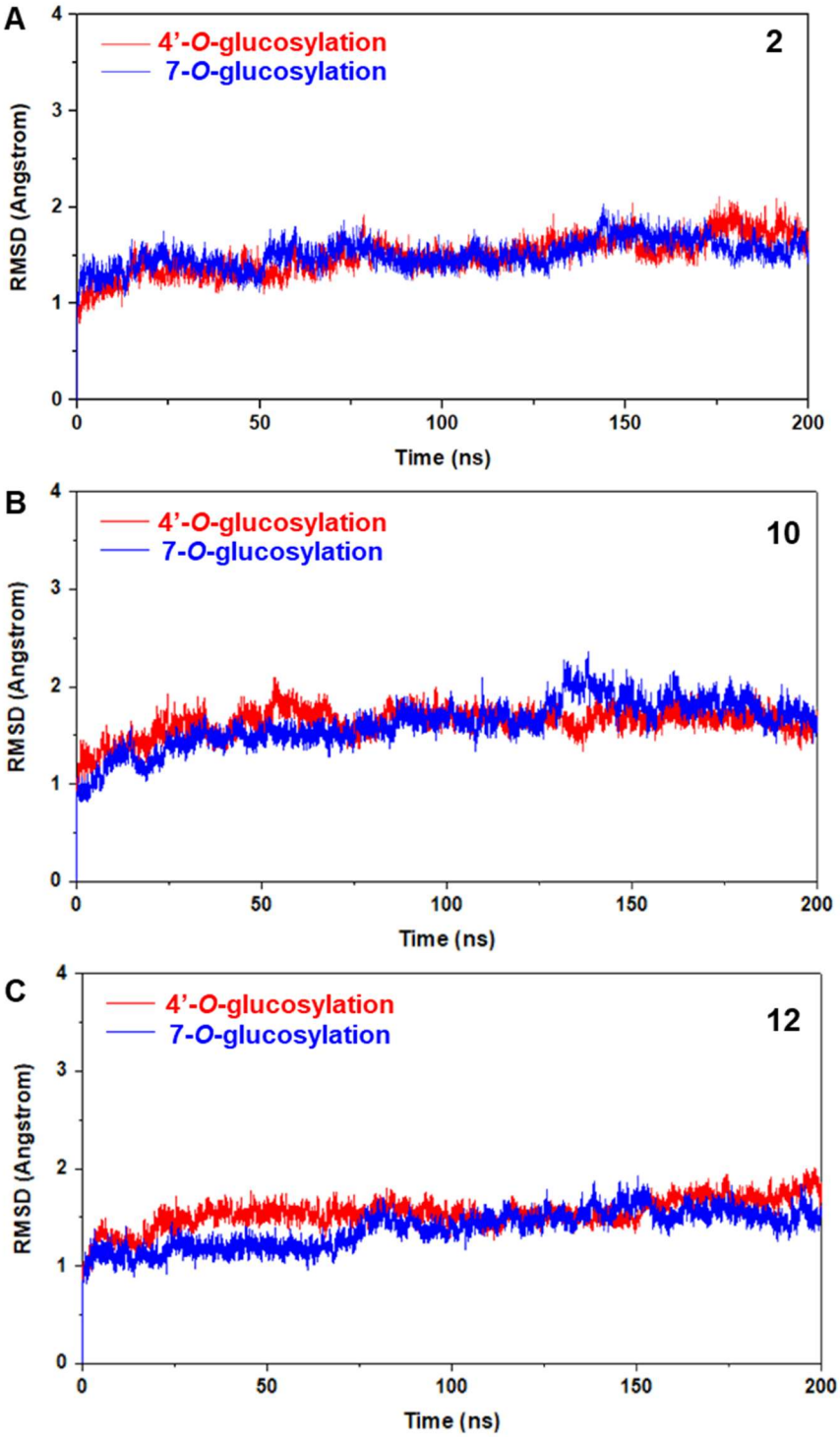


Figure S82. Root-mean-square deviation (RMSD) of backbone heavy atoms relative to the first snapshot during 200 ns MD simulation of these two anionic intermediates for the ternary complex (A) ZjOGT3:UDP-Glc:**2**, (B) ZjOGT3:UDP-Glc:**10**, (C) ZjOGT3:UDP-Glc:**12**.

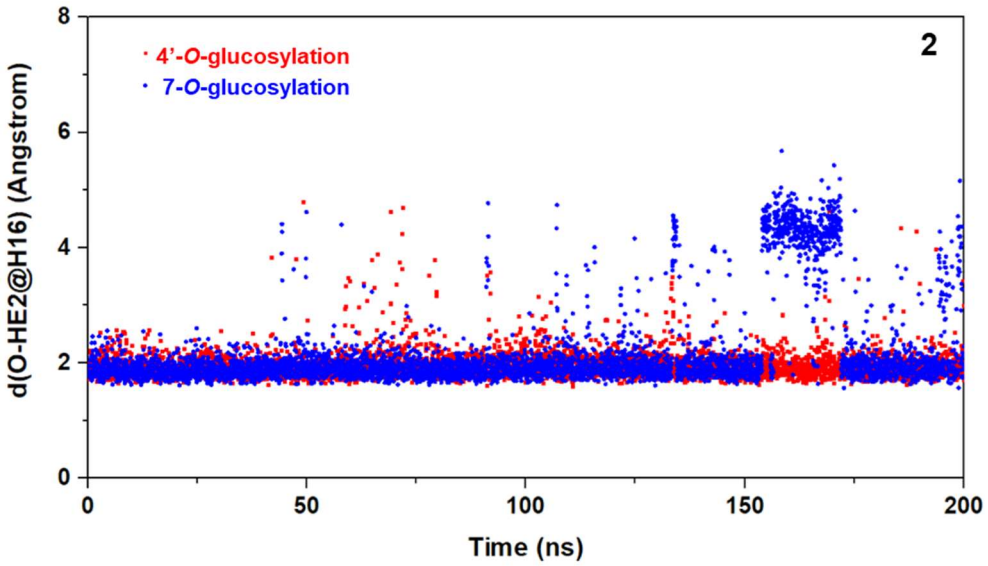


Figure S83. Distances between the imidazole ring hydrogen atom (HE2) of H16 and the phenol anion oxygen atom (O) of the two anionic intermediates during 200 ns MD simulation for the ternary complex ZjOGT3:UDP-Glc:**2**.

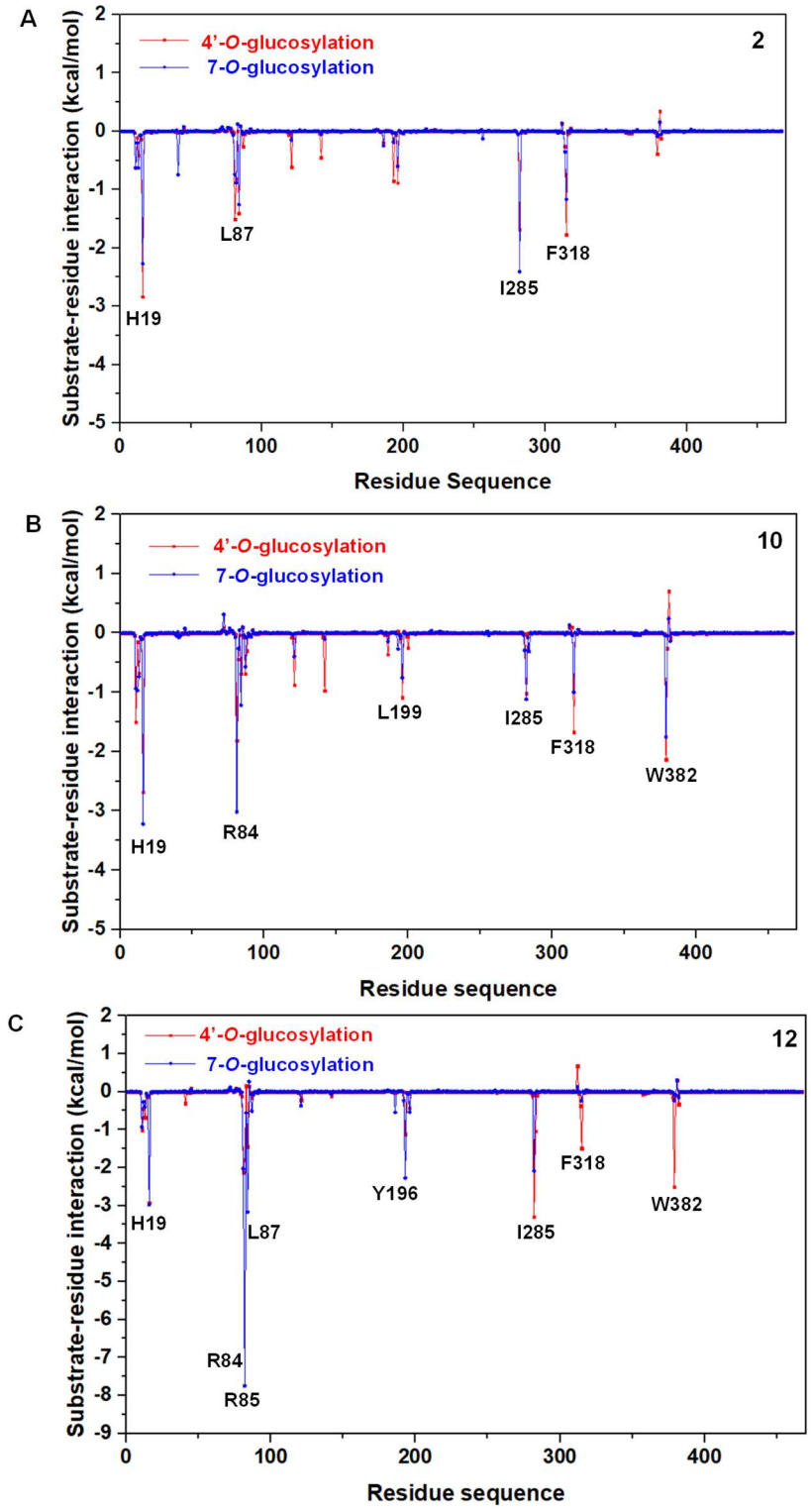


Figure S84. Per-residue contribution of binding energies and key residues of ZjOGT3 (| contribution | ≥1.0 kcal/mol) with 4′ and 7 anion orientation of acceptor substrates (A) **2**, (B) **10** and (C) **12**.

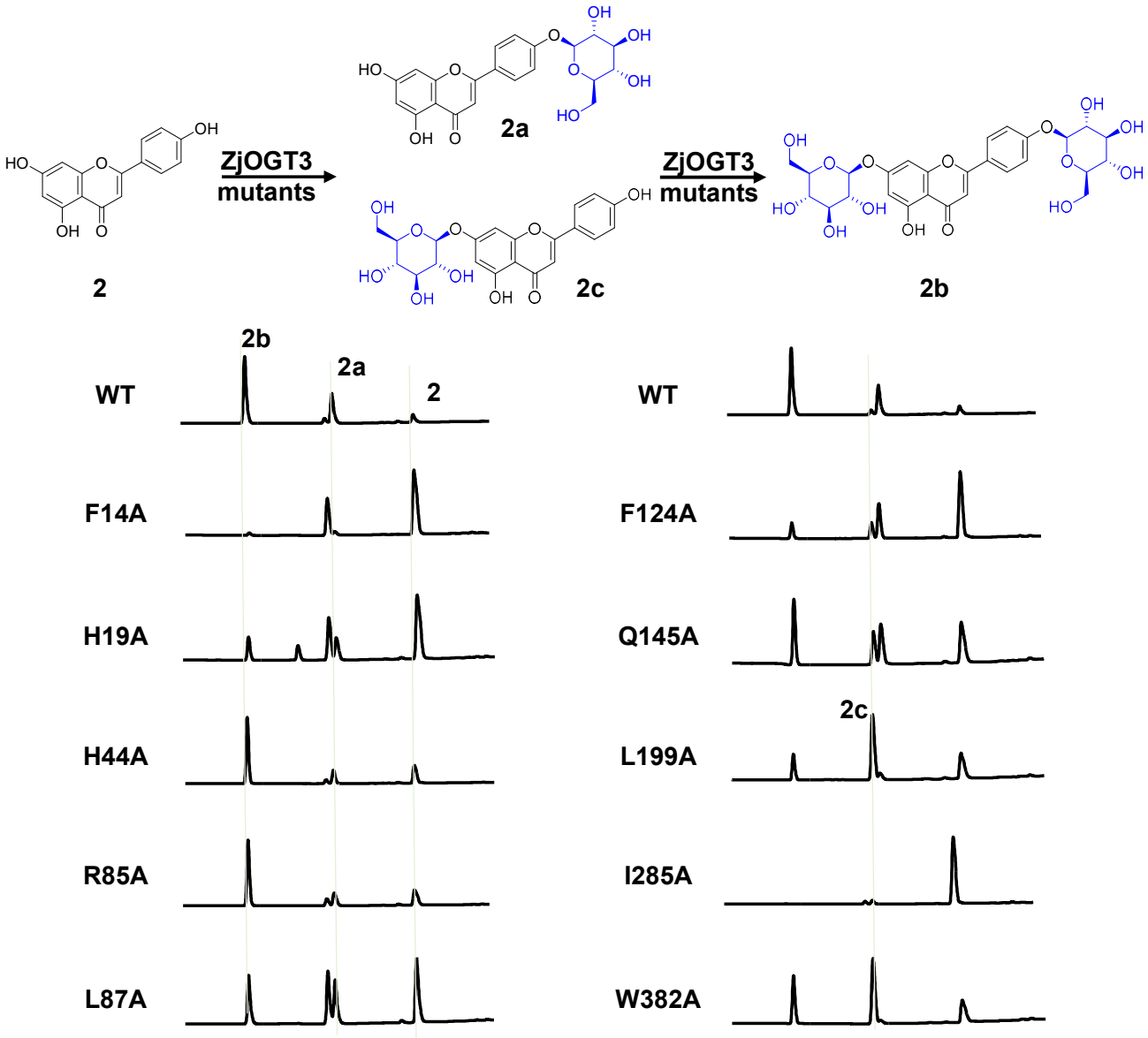


Figure S85. UHPLC analysis of reaction mixtures catalyzed by site-directed mutagenesis based on the results of molecule docking of ZjOGT3**.** The substrate was apigenin (**2**). The reaction mixtures were individually performed in a final volume of 100 μL containing 50 mM NaH_2PO_4-Na_2HPO_4 buffer (pH 8.0), 25 μg of purified ZjOGT3, 0.1 mM aglycone, 0.5 mM sugar-donor, and 45°C for 2 h.

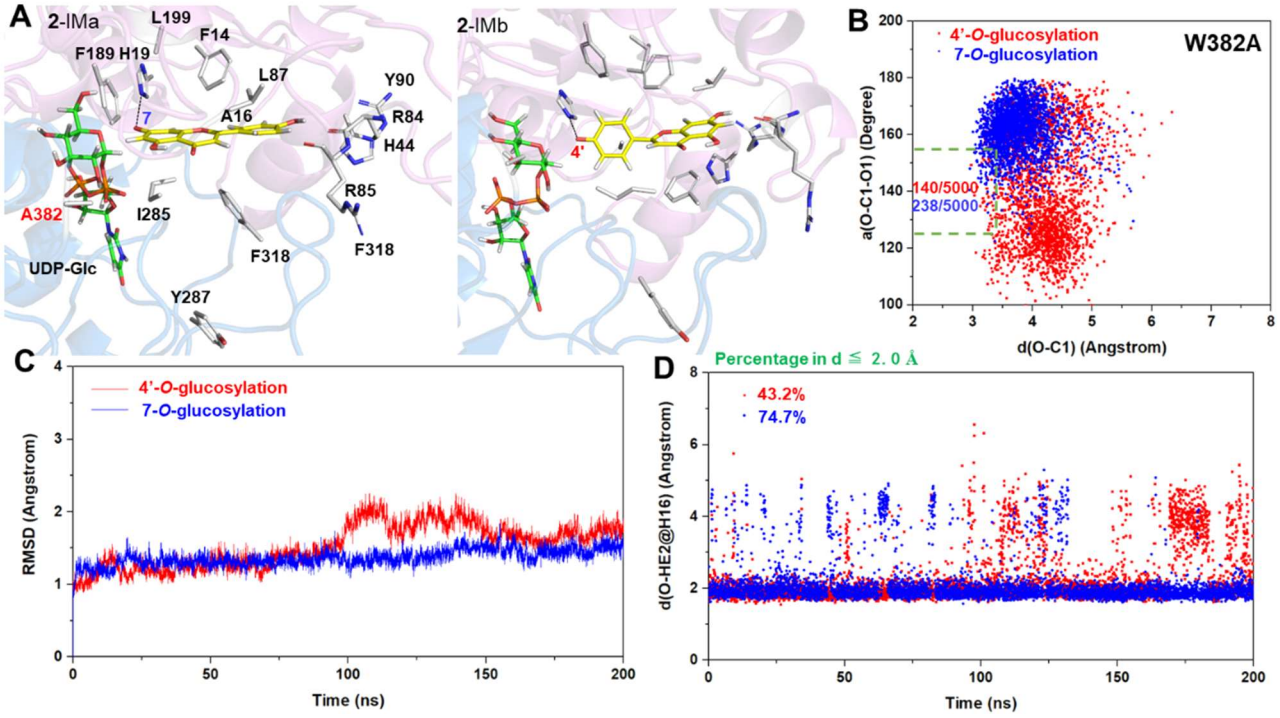


Figure S86. The molecular details for 7-*O*-glycosylation of **2** catalyzed by ZjOGT3-W382A mutant. (A) Close-up view of representative ternary complex MD snapshots of ZjOGT3-W382A active site in complex with UDP-Glc and the anionic intermediates of acceptor **2**. (B) MD plots for the distances to form the O-C bond d(O-C1) and the angles between the forming O-C bond and the breaking C-O bond a(O-C1-O1) for the two anionic intermediates in the ternary complex. (C) Root-mean-square deviation (RMSD) of backbone heavy atoms relative to the first snapshot during 200-ns MD simulation of these two anionic intermediates for the ternary complex ZjOGT3-W382A/UDP-Glc/**2**. (D) Distances between the imidazole ring hydrogen atom (HE2) of H16 and the phenolic anion oxygen atom (O) of these two anionic intermediates during 200-ns MD simulation for the ternary complex ZjOGT3-W382A/UDP-Glc/**2**.

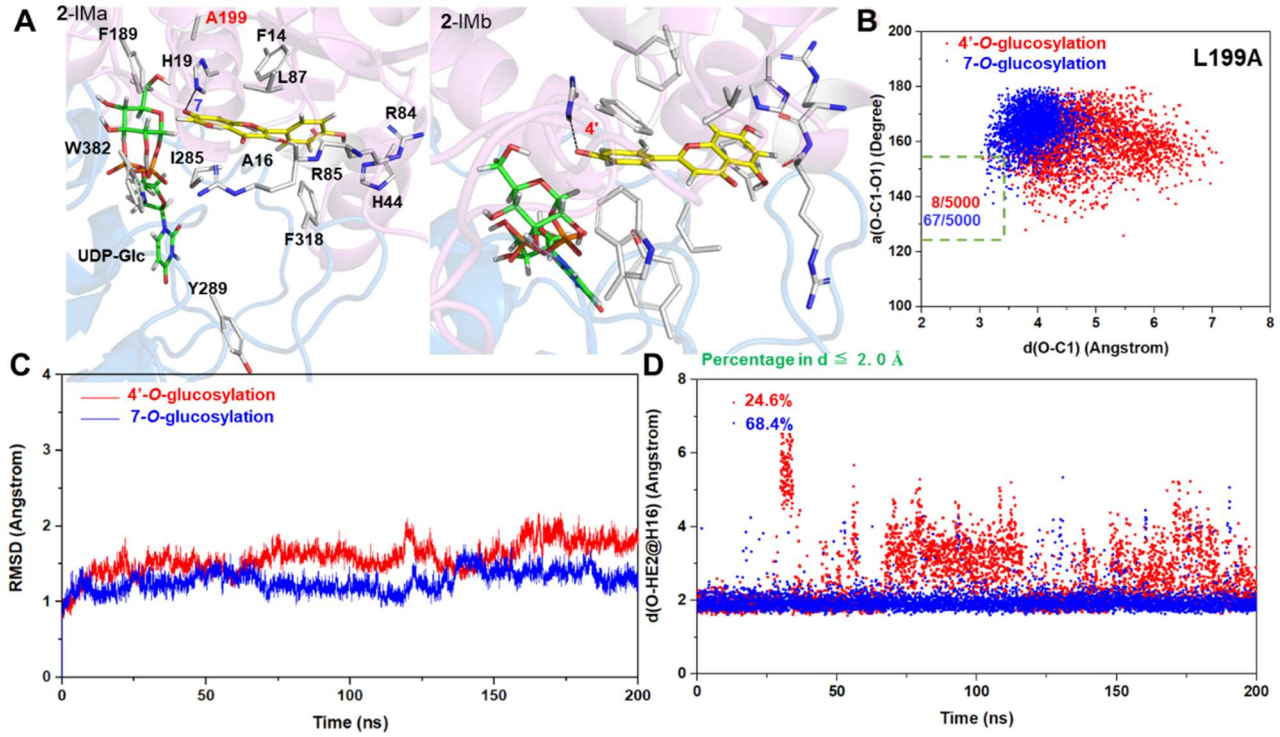


Figure S87. The molecular details for 7-*O*-glycosylation of ZjOGT3-L199A mutant. (A) Close-up view of representative ternary complex MD snapshots of ZjOGT3-L199A active site in complex with UDP-Glc and the anionic intermediates of acceptor **2**. (B) MD plots for the distances to form the O-C bond d(O-C1) and the angles between the forming O-C bond and the breaking C-O bond a(O-C1-O1) for the two anionic intermediates in the ternary complex. (C) Root-mean-square deviation (RMSD) of backbone heavy atoms relative to the first snapshot during 200-ns MD simulation of these two anionic intermediates for the ternary complex ZjOGT3-L199A/UDP-Glc/**2**. (D) Distances between the imidazole ring hydrogen atom (HE2) of H16 and the phenolic anion oxygen atom (O) of these two anionic intermediates during 200-ns MD simulation for the ternary complex ZjOGT3-L199A/UDP-Glc/**2**.

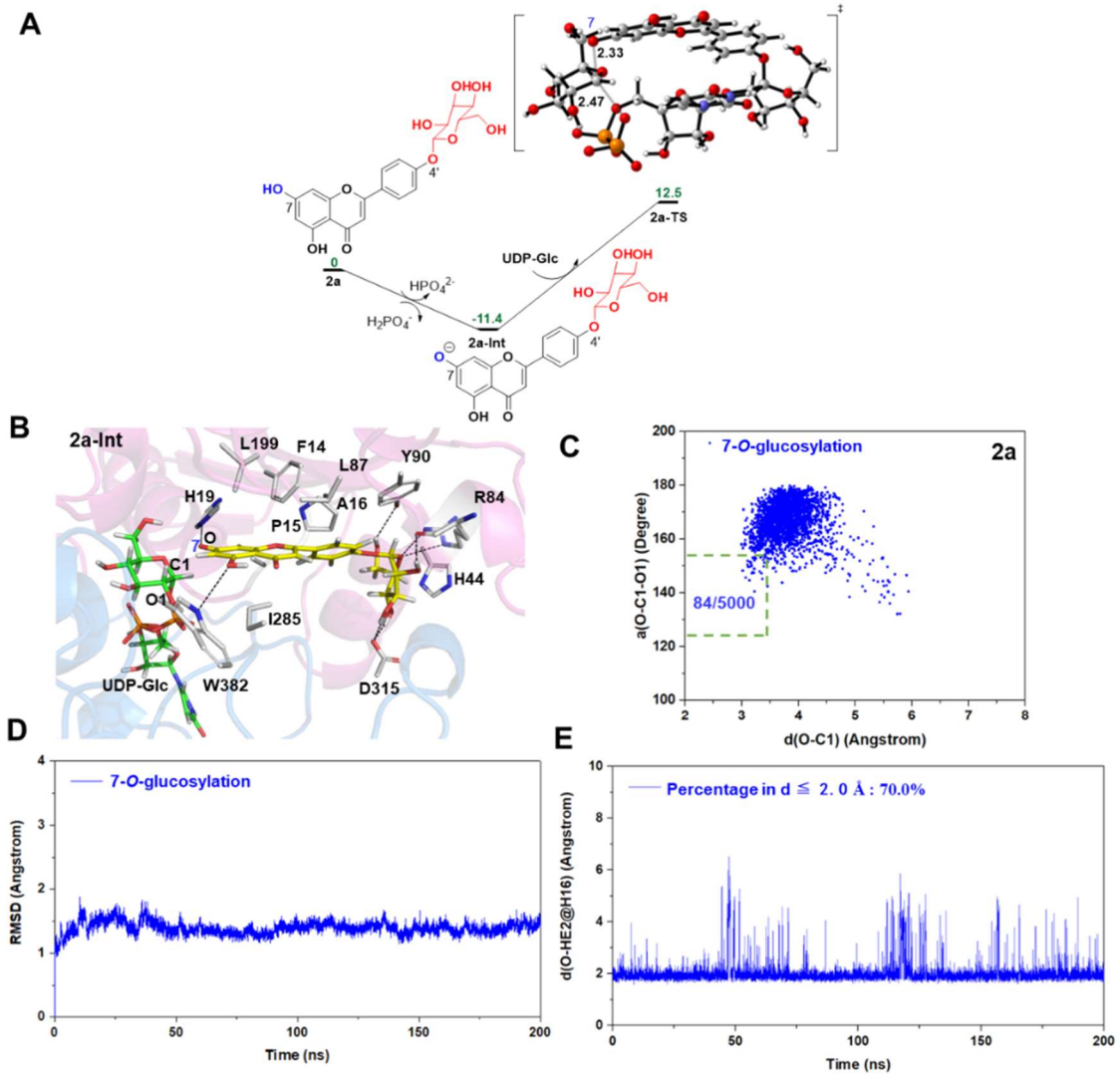


Figure S88. (A) Gibbs free energy profiles and DFT-optimized transition state structures for 7-*O*-glycosylation of **2a**. Computed at the CPCM(water)-B3LYP-D3/6-311++G(2d,p)//CPCM(water)-B3LYP-D3/6-31+G(d,p) level of theory (Carbon, gray; Hydrogen, white; Oxygen, red; Nitrogen, blue; Phosphorus, orange; distances are shown in Å). (B) Close-up view of representative ternary complex MD snapshots of ZjOGT3 active site in complex with UDP-Glc donor and the anionic intermediate of acceptor **2a** (2a-Int). (C) MD plots for the distances to form the O-C bond d(O-C1) and the angles between the forming O-C bond and the breaking C-O bond a(O-C1-O1) for the anionic intermediate in the ternary complex. (D) Root-mean-square deviation (RMSD) of backbone heavy atoms relative to the first snapshot during 200-ns MD simulation of the anionic intermediate for the ternary complex ZjOGT3/UDP-Glc/**2a**. (E) Distance between the imidazole ring hydrogen atom (HE2) of H16 and the phenolic anion oxygen atom (O) of the anionic intermediate during 200-ns MD simulation for the ternary complex ZjOGT3/UDP-Glc/**2a**.

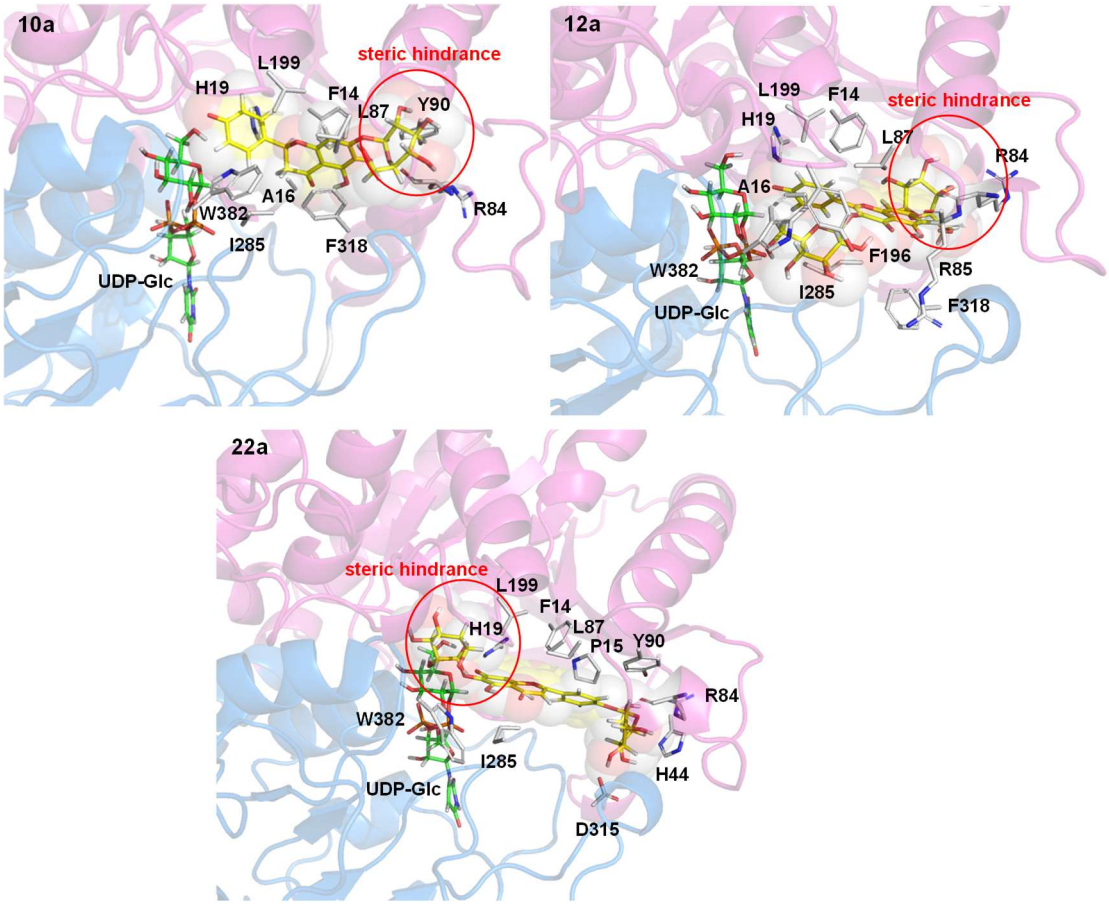


Figure S89. Close-up view of docking models of ZjOGT3 active site in complex with donor UDP-Glc and the anionic intermediate of acceptors (A) **10a**, (B) **12a** and (C) **22a**.

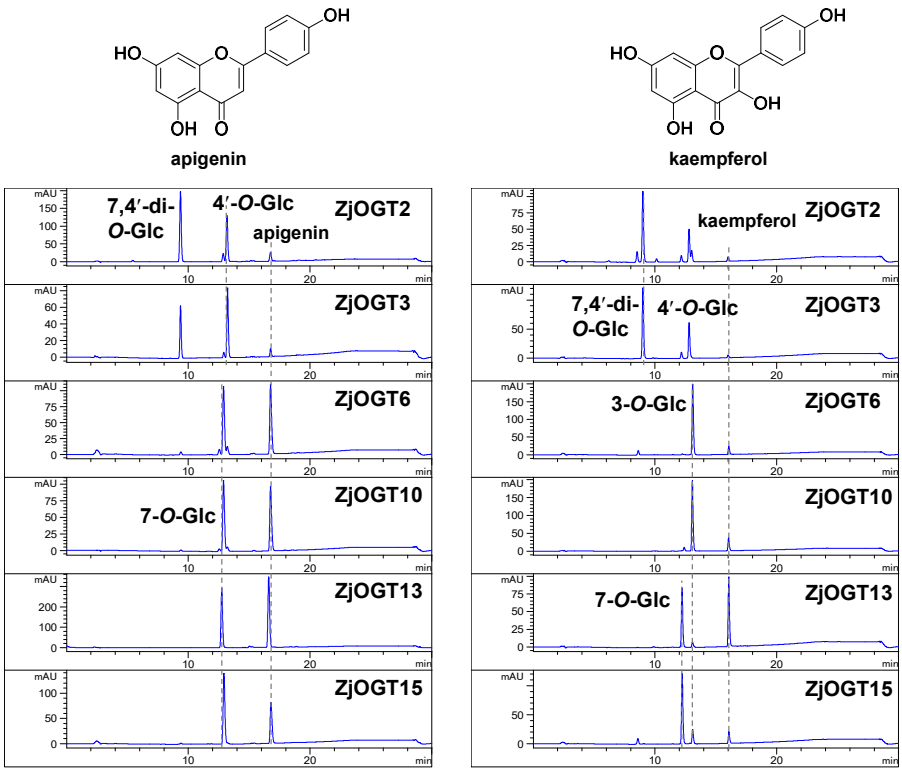


Figure S90. Functional characterization of ZjOGT 2/3/6/10/13/15. Apigenin and kaempferol were used as sugar acceptor, respectively. UDP-Glc was used as sugar donor.

Through blast with reported UGTs, 24 open reading frames (ORFs) were obtained as candidate genes from the transcriptome of *Z. jujuba* var. *spinosa*. After cloning and functional characterization, eight UGTs (ZjOGT 1/2/3/4/6/10/13/15) exhibited glycosylation activities. Among them, ZjOGT1 and ZjOGT4 were identified as 2-hydroxy flavanone CGTs, which were respectively named as ZjCGT1 and ZjCGT2 in our previous report (Zhang YQ, *et al*. *Chem. Commun.* 2022, *58*, 2472-2475.). ZjOGT 2/3/6/10/13/15 exhibited *O*-glycosylation activities. Apigenin and kaempferol were used as sugar acceptors to investigate their catalytic features.

ZjOGT2 and ZjOGT3 showed similar activities to produce 4′-*O*- and 7,4′-di-*O*-glycosides. ZjOGT6 and ZjOGT10 exhibited moderate 7-*O*-glycosylation activities towards apigenin, but potent 3-*O*-glycosylation activities towards kaempferol. ZjOGT13 and ZjOGT15 mainly showed moderate 7-*O*-glycosylation activities.

5. Calculated coordinates

UDP-Glc

G(Water) = -2656.886855 Hartree

N	-4.143224	0.432193	0.537576
C	-5.388458	0.978026	0.849138
N	-5.547703	2.296203	0.467188
C	-4.628995	3.118981	-0.200051
C	-3.374095	2.464210	-0.492144
C	-3.184873	1.177688	-0.117024
O	-6.283144	0.352294	1.414914
O	-4.935027	4.282315	-0.478838
C	-3.879037	-0.968454	0.928263
C	-3.791101	-1.938227	-0.261177
O	-4.375184	-3.171081	0.145791
C	-2.275639	-2.106528	-0.480323
C	-1.711894	-1.925331	0.947968
O	-2.639333	-1.037411	1.615775
O	-2.035191	-3.376513	-1.045520
C	-0.350993	-1.244593	1.085443
O	0.736769	-2.025191	0.573051
P	1.211249	-2.101199	-0.999632
O	0.511860	-3.271217	-1.672930
O	2.719752	-2.050594	-1.016776
O	0.515174	-0.762507	-1.656498
P	0.847793	0.857448	-1.693217
O	-0.482407	1.563422	-1.681857
O	1.857524	1.134912	-2.791730
O	1.563348	0.958489	-0.221160
C	2.426562	1.934363	0.371696
C	3.778284	2.072071	-0.357840
C	4.411175	0.686436	-0.444606
C	4.465865	-0.001460	0.930696
C	3.183454	0.155475	1.770607
C	3.413368	-0.099376	3.252975
O	3.658982	2.725851	-1.609009
O	5.737150	0.810267	-0.977605
O	4.763381	-1.390185	0.761688
O	2.644847	1.499127	1.689788
O	4.334283	0.826108	3.838585
H	-6.449629	2.703647	0.690541
H	-2.589286	2.998384	-1.008518
H	-2.242752	0.690003	-0.326118
H	-4.696182	-1.254241	1.588845
H	-3.867232	-3.872634	-0.296926
H	-1.075555	-3.417304	-1.322523
H	-4.303269	-1.541792	-1.145642
H	-1.903278	-1.311611	-1.138047
H	-1.706309	-2.901188	1.449372
H	-0.377004	-0.262254	0.612496
H	-0.135620	-1.099852	2.147172
H	1.918143	2.901567	0.432028
H	4.399107	2.719287	0.272371
H	3.802486	0.070815	-1.114215
H	5.305195	0.433100	1.483850
H	2.434198	-0.553635	1.408111
H	2.447079	-0.063471	3.774049
H	3.842768	-1.095914	3.381248
H	6.116439	-0.080770	-0.994146
H	4.051145	-1.758954	0.189201
H	3.073876	2.172053	-2.183728
H	4.002525	1.717879	3.656892

HPO_4^{2-}

G(Water) = -643.4070798 Hartree

P	0.140025	-0.028536	0.000036
O	0.290924	-0.860514	1.296781
O	0.908632	1.309850	-0.001621
O	0.291473	-0.864224	-1.294256
O	-1.494990	0.502912	-0.000990
H	-2.068688	-0.276157	0.000154

$H_2PO_4^-$

G(Water) = -643.8805478 Hartree

P	0.000843	0.095080	0.127473
O	-1.298632	-0.346939	-0.807045
O	0.017942	1.602809	0.135435
O	-0.011526	-0.736627	1.393960
O	1.294370	-0.375447	-0.801316
H	1.508652	-1.306107	-0.639683
H	-1.538532	-1.270462	-0.640677

2

G(Water) = -953.9117166 Hartree

C	-4.188341	-0.556969	0.024937
C	-3.478586	0.639240	-0.025248
C	-2.056390	0.634461	-0.018159

C	-1.403234	-0.610385	0.039485
C	-2.087527	-1.817560	0.089310
C	-3.486491	-1.772094	0.080584
H	-5.273098	-0.535398	0.019135
C	-1.279432	1.858199	-0.076839
H	-1.557679	-2.760756	0.133613
C	0.723430	0.453904	-0.007701
C	0.149883	1.692173	-0.080500
H	0.766189	2.577440	-0.161883
C	2.160785	0.161623	0.002392
C	3.104052	1.163423	0.309214
C	2.631592	-1.131095	-0.296146
C	4.464938	0.888693	0.307674
H	2.777595	2.163438	0.572459
C	3.994036	-1.414452	-0.301552
H	1.927412	-1.919329	-0.535428
C	4.916372	-0.403187	0.000020
H	5.188327	1.658994	0.552722
H	4.341355	-2.415243	-0.541972
O	-4.146085	1.809759	-0.082145
O	-4.130929	-2.969548	0.129060
H	-5.091358	-2.845899	0.115526
O	6.263082	-0.611779	0.016379
H	6.471251	-1.533190	-0.197215
O	-0.035506	-0.675338	0.054467
O	-1.836992	2.992915	-0.133794
H	-3.449084	2.526022	-0.113528

2-IMa

G(Water) = -953.45661 Hartree

C	-4.222927	-0.613105	0.027418
C	-3.527277	0.575858	-0.028021
C	-2.094856	0.598689	-0.019290
C	-1.438772	-0.657616	0.048452
C	-2.106149	-1.860675	0.104105
C	-3.548518	-1.890499	0.095095
H	-5.308856	-0.600761	0.018782
C	-1.338264	1.811942	-0.085068
H	-1.552696	-2.792270	0.156432
C	0.686201	0.435026	-0.004409
C	0.101421	1.663260	-0.086919
H	0.707673	2.555301	-0.176084
C	2.131161	0.160508	0.005941
C	3.061026	1.163218	0.345283
C	2.620597	-1.115953	-0.326459
C	4.426730	0.906639	0.339939
H	2.718779	2.150389	0.636499
C	3.987862	-1.382465	-0.334574
H	1.926049	-1.905420	-0.589415
C	4.896155	-0.369177	-0.002561
H	5.139000	1.679272	0.609806
H	4.348366	-2.372195	-0.601010
O	-4.195808	1.761274	-0.094252
O	-4.199169	-2.989814	0.144699
O	6.248251	-0.561651	0.012055
H	6.466450	-1.473684	-0.229031
O	-0.057351	-0.703217	0.066965
O	-1.890904	2.960389	-0.150820
H	-3.491259	2.469624	-0.128747

2-IMb

G(Water) = -953.4589379 Hartree

C	-4.149620	-0.540624	0.003683
C	-3.428673	0.652301	0.000069
C	-2.009209	0.636949	-0.001198
C	-1.362690	-0.609662	0.001407
C	-2.057379	-1.814583	0.004946
C	-3.455620	-1.760497	0.006116
H	-5.234411	-0.510707	0.004548
C	-1.218509	1.859672	-0.006460
H	-1.533746	-2.762509	0.007021
C	0.785902	0.440708	-0.002896
C	0.194086	1.691768	-0.008224
H	0.809562	2.581064	-0.015520
C	2.196071	0.143576	-0.001300
C	3.174655	1.175955	0.021581
C	2.674026	-1.196169	-0.021821
C	4.524495	0.897723	0.022615
H	2.864404	2.216536	0.040968
C	4.022726	-1.487295	-0.021292
H	1.958196	-2.011643	-0.039564
C	5.033463	-0.455630	0.001101
H	5.248560	1.708547	0.041803
H	4.358750	-2.521405	-0.038351
O	-4.083771	1.833088	-0.002767
O	-4.109270	-2.957989	0.009776
H	-5.068277	-2.824973	0.010368
O	6.284355	-0.716817	0.002899
O	0.002377	-0.684941	0.001141
O	-1.791248	2.999486	-0.010477

H	-3.364924	2.537328	-0.005805

2-TSa

G(Water) = -3610.304581 Hartree

N	2.252945	-1.755290	0.987418
C	3.564224	-1.768758	1.457971
N	3.920697	-0.661790	2.202908
C	3.111322	0.419953	2.577519
C	1.764420	0.333987	2.064015
C	1.393218	-0.728336	1.309148
O	4.349538	-2.689066	1.237490
O	3.573927	1.317708	3.287867
C	1.838607	-2.880019	0.106833
C	1.061561	-3.983122	0.836379
O	1.381999	-5.223597	0.214520
C	-0.408889	-3.611107	0.570596
C	-0.340864	-2.943255	-0.823812
O	1.000585	-2.394390	-0.919583
O	-1.184568	-4.792667	0.616300
C	-1.257228	-1.748036	-1.083380
O	-2.633343	-2.079281	-1.313804
P	-3.696585	-2.603312	-0.162534
O	-3.639935	-4.131428	-0.149234
O	-5.021248	-1.946691	-0.517386
O	-3.098552	-2.140506	1.247734
P	-2.672435	-0.825833	2.287286
O	-2.016798	-1.568669	3.448481
O	-4.000757	-0.104136	2.611826
O	-1.726368	0.045310	1.439893
C	-2.407680	1.869975	-0.075853
C	-3.778927	2.291865	0.398697
C	-4.815556	1.317945	-0.162458
C	-4.692315	1.187503	-1.681869
C	-3.248281	0.947979	-2.168301
C	-2.836789	1.817138	-3.353373
O	-3.804605	2.400920	1.798521
O	-6.114651	1.803877	0.177779
O	-5.545196	0.150268	-2.153056
O	-2.205267	1.197086	-1.141418
O	-2.969280	3.201942	-3.088068
H	4.875762	-0.660410	2.544601
H	1.055540	1.116533	2.295049
H	0.379916	-0.797972	0.937743
H	2.760430	-3.276265	-0.315838
H	0.550392	-5.729510	0.190683
H	-2.127229	-4.586523	0.347808
H	1.303981	-4.012858	1.904947
H	-0.749229	-2.889568	1.321315
H	-0.471082	-3.711065	-1.596662
H	-1.193499	-1.029923	-0.265018
H	-0.918249	-1.263327	-2.003061
H	-1.545551	2.018285	0.551895
H	-3.953265	3.289508	-0.026139
H	-4.655471	0.339593	0.299506
H	-5.057583	2.118907	-2.125812
H	-3.114086	-0.109860	-2.393697
H	-1.809389	1.554490	-3.642443
H	-3.497490	1.569676	-4.191038
H	-6.753569	1.206425	-0.239239
H	-5.341413	-0.669970	-1.629032
H	-3.857374	1.464399	2.171285
H	-2.376420	3.461746	-2.331146
C	0.213775	4.886062	0.253272
C	1.544660	4.807578	0.626469
C	2.371752	3.736897	0.169406
C	1.777661	2.787152	-0.688878
C	0.459903	2.858383	-1.099245
C	-0.371308	3.905880	-0.605184
H	-0.408198	5.688856	0.635306
C	3.747398	3.589352	0.570978
H	0.051019	2.094212	-1.747505
C	3.803453	1.534286	-0.759023
C	4.427526	2.418545	0.071579
H	5.440882	2.238305	0.404245
C	4.377387	0.299675	-1.309987
C	5.764742	0.054280	-1.269030
C	3.544228	-0.679805	-1.880421
C	6.298157	-1.126149	-1.769436
H	6.441519	0.793961	-0.855735
C	4.070171	-1.866303	-2.382561
H	2.472040	-0.533481	-1.906282
C	5.450162	-2.094951	-2.325510
H	7.366755	-1.310683	-1.738693
H	3.406150	-2.616431	-2.802830
O	2.076732	5.743952	1.453487
O	-1.650090	3.940056	-0.876975
O	6.030427	-3.238952	-2.790862
H	5.360789	-3.840524	-3.148109
O	2.515067	1.723556	-1.153647
O	4.319471	4.429391	1.333463
H	3.028089	5.475176	1.596459

2-TSb

G(Water) = -3610.303106 Hartree

N	-2.365017	1.112288	0.808576
C	-3.625303	1.205852	1.391859
N	-3.807586	0.406644	2.502678
C	-2.859029	-0.410580	3.133061
C	-1.557633	-0.368401	2.511354
C	-1.362934	0.370552	1.392715
O	-4.511075	1.937447	0.953465
O	-3.180701	-1.071449	4.126753
C	-2.141735	1.931087	-0.418310
C	-1.672965	3.352981	-0.090008
O	-2.165319	4.222588	-1.104133
C	-0.141473	3.232905	-0.179226
C	0.037033	2.185901	-1.301615
O	-1.141838	1.338400	-1.214176
O	0.394371	4.506088	-0.470052
C	1.219474	1.226764	-1.195809
O	2.491831	1.845060	-1.437897
P	3.344498	2.732076	-0.331456
O	3.002371	4.204086	-0.569578
O	4.797466	2.323726	-0.508834
O	2.727906	2.359966	1.099101
P	2.603741	1.127222	2.314657
O	1.847635	1.873536	3.410997
O	4.059091	0.732740	2.665006
O	1.836680	-0.012692	1.622640
C	3.195411	-1.740433	0.243133
C	4.578041	-1.862228	0.829185
C	5.467487	-0.775251	0.220875
C	5.425599	-0.799893	-1.308646
C	4.002963	-0.907022	-1.894524
C	3.858659	-1.981348	-2.969799
O	4.506918	-1.810011	2.230612
O	6.804648	-0.988927	0.673729
O	6.079690	0.351115	-1.830176
O	2.949890	-1.227443	-0.892268
O	4.201800	-3.269933	-2.499272
H	-4.737928	0.428421	2.906488
H	-0.745723	-0.943087	2.933183
H	-0.392939	0.394612	0.916246
H	-3.092009	1.939812	-0.952120
H	-1.468331	4.885348	-1.250769
H	1.395700	4.442185	-0.511201
H	-2.015967	3.677775	0.898807
H	0.257549	2.839379	0.763948
H	0.038256	2.700776	-2.270417
H	1.232452	0.730210	-0.224428
H	1.110054	0.463712	-1.971066
H	2.333236	-1.971098	0.849125
H	4.955009	-2.852068	0.533369
H	5.102716	0.194547	0.573533
H	6.002951	-1.667642	-1.642079
H	3.679138	0.065808	-2.263722
H	2.836601	-1.946653	-3.369949
H	4.545737	-1.719805	-3.781965
H	7.352518	-0.305724	0.259072
H	5.626208	1.142425	-1.435557
H	4.312127	-0.846015	2.476145
H	3.536024	-3.576220	-1.816077
C	-6.672862	0.463054	-1.575180
C	-6.458906	-0.404552	-0.507534
C	-5.237976	-1.122488	-0.395046
C	-4.262976	-0.921226	-1.386691
C	-4.445630	-0.052819	-2.455911
C	-5.661880	0.634899	-2.533146
H	-7.609520	1.006492	-1.644872
C	-4.969196	-2.027468	0.709728
H	-3.669007	0.093769	-3.196034
C	-2.764877	-2.412811	-0.286326
C	-3.686733	-2.661804	0.701684
H	-3.449891	-3.349617	1.502360
C	-1.414904	-2.939819	-0.373126
C	-0.764670	-3.479479	0.761291
C	-0.687942	-2.885374	-1.585421
C	0.553275	-3.897410	0.701241
H	-1.282864	-3.521948	1.714009
C	0.625335	-3.316940	-1.656699
H	-1.166219	-2.490539	-2.475574
C	1.317471	-3.789864	-0.501846
H	1.055100	-4.261869	1.593341
H	1.165379	-3.259114	-2.596082
O	-7.415589	-0.562000	0.430197
O	-5.810728	1.485276	-3.586950
O	2.602091	-4.020295	-0.507030
O	-3.061932	-1.570284	-1.319983
O	-5.834569	-2.223048	1.618518
H	-7.043478	-1.217985	1.091570
H	-6.670092	1.929823	-3.548986

2a

G(Water) = -1564.736719 Hartree

C	3.811363	0.435997	-1.108283
C	3.901945	1.618839	-0.103459
C	5.246343	1.628612	0.628935
C	5.739655	0.192761	0.834017
C	6.024867	-0.453018	-0.529557
C	6.149998	-1.974600	-0.486668
O	3.607797	2.855059	-0.745821
O	5.069090	2.301328	1.869946
O	6.892285	0.154560	1.674767
O	5.036637	-0.067372	-1.527665
O	5.043625	-2.629733	0.126192
H	3.299499	0.762039	-2.017568
H	3.119122	1.492857	0.648391
H	5.986022	2.165287	0.011702
H	4.979424	-0.377574	1.375060
H	6.970410	-0.050193	-0.914063
H	6.300912	-2.330736	-1.515064
H	7.035639	-2.233668	0.100864
H	5.859387	2.133994	2.406631
H	7.645529	0.562544	1.219904
H	4.316040	3.074447	-1.369563
H	4.227564	-2.244361	-0.235158
C	-7.234103	0.985548	0.138698
C	-6.717118	-0.303528	0.228385
C	-5.313922	-0.524347	0.152393
C	-4.480164	0.596544	-0.016908
C	-4.970351	1.892083	-0.109351
C	-6.356569	2.069059	-0.029993
H	-8.306878	1.137097	0.198865
C	-4.735352	-1.851759	0.234174
H	-4.304088	2.735714	-0.238532
C	-2.547291	-0.788159	-0.026393
C	-3.300907	-1.917095	0.123325
H	-2.831909	-2.891678	0.142716
C	-1.085247	-0.729912	-0.149207
C	-0.279166	-1.822364	0.232405
C	-0.454500	0.419265	-0.653705
C	1.099605	-1.769284	0.098497
H	-0.725026	-2.716216	0.653850
C	0.931689	0.485203	-0.792293
H	-1.049654	1.275402	-0.948631
C	1.711816	-0.618322	-0.421673
H	1.716960	-2.609100	0.398443
H	1.377903	1.393900	-1.177479
O	-7.554178	-1.348769	0.387569
O	-6.809028	3.348525	-0.124565
O	3.076792	-0.668194	-0.521197
O	-3.121295	0.444519	-0.091491
O	-5.456646	-2.880290	0.382162
H	-6.977931	-2.164580	0.424545
H	-7.775091	3.378630	-0.064106

2a-IM

G(Water) = -1564.281401 Hartree

C	3.789170	0.394271	-1.119623
C	3.882498	1.605767	-0.148939
C	5.228743	1.635430	0.579069
C	5.713313	0.204574	0.833686
C	5.995532	-0.489165	-0.506831
C	6.105168	-2.009797	-0.412319
O	3.587253	2.824405	-0.824221
O	5.058900	2.352139	1.796270
O	6.864228	0.187005	1.677763
O	5.015372	-0.124554	-1.520213
O	4.993062	-2.633221	0.222681
H	3.282784	0.697321	-2.040072
H	3.101342	1.501165	0.607897
H	5.969547	2.146069	-0.058572
H	4.948393	-0.341511	1.392817
H	6.946512	-0.109102	-0.901234
H	6.251927	-2.401968	-1.428167
H	6.988881	-2.257393	0.183136
H	5.848630	2.196164	2.337218
H	7.623320	0.566369	1.208230
H	4.294227	3.026877	-1.455025
H	4.180522	-2.247112	-0.146654
C	-7.269028	1.038166	0.148164
C	-6.762340	-0.242142	0.214623
C	-5.352425	-0.486973	0.142613
C	-4.515974	0.650369	-0.001937
C	-4.992631	1.939941	-0.070825
C	-6.411229	2.193249	0.002019
H	-8.342181	1.194323	0.205260
C	-4.789994	-1.801435	0.200931
H	-4.305564	2.772332	-0.179669
C	-2.585994	-0.757723	-0.028008
C	-3.347555	-1.880175	0.095524
H	-2.887388	-2.859734	0.096206
C	-1.119484	-0.713132	-0.145599
C	-0.321289	-1.799898	0.265402

C	-0.478296	0.417939	-0.675023
C	1.059334	-1.760344	0.133845
H	-0.776456	-2.678741	0.708605
C	0.909943	0.471550	-0.811041
H	-1.068027	1.270403	-0.991299
C	1.681012	-0.627281	-0.411850
H	1.669802	-2.596948	0.456687
H	1.363327	1.367965	-1.216400
O	-7.601070	-1.307247	0.350091
O	-6.887315	3.377981	-0.058969
O	3.049225	-0.688383	-0.505160
O	-3.145632	0.482014	-0.071509
O	-5.507322	-2.848880	0.326421
H	-7.014044	-2.115700	0.372516

2a-TS

G(Water) = -4221.130185 Hartree

N	1.230388	0.329858	1.534176
C	2.182906	1.211555	2.041381
N	1.665100	2.361154	2.601603
C	0.314826	2.711757	2.749925
C	-0.597484	1.718695	2.232284
C	-0.117602	0.590425	1.656181
O	3.392743	0.991528	1.997390
O	0.012768	3.780315	3.289052
C	1.734849	-0.829348	0.758514
C	1.747063	-2.159473	1.517884
O	2.873002	-2.911495	1.048846
C	0.435296	-2.829387	1.076900
C	0.251060	-2.295126	-0.364129
O	0.939471	-1.017904	-0.389526
O	0.591207	-4.231589	1.162376
C	-1.167391	-1.960494	-0.822731
O	-1.995787	-3.092731	-1.114564
P	-2.644415	-4.116495	0.009064
O	-1.657059	-5.269204	0.198022
O	-4.032917	-4.447781	-0.514346
O	-2.648315	-3.322923	1.399355
P	-3.275171	-1.988689	2.304875
O	-2.468534	-2.105778	3.595244
O	-4.794456	-2.242781	2.431555
O	-2.961547	-0.754692	1.437906
C	-4.389423	0.217962	-0.327141
C	-5.776576	-0.293696	-0.011473
C	-5.902813	-1.725675	-0.534097
C	-5.530907	-1.815656	-2.014906
C	-4.205037	-1.108392	-2.363401
C	-4.282463	-0.214222	-3.597819
O	-6.050356	-0.168556	1.360301
O	-7.253163	-2.151182	-0.347345
O	-5.484071	-3.177743	-2.424398
O	-3.678875	-0.225679	-1.291257
O	-5.285073	0.780437	-3.489961
H	2.352098	3.015774	2.960121
H	-1.663164	1.882217	2.310227
H	-0.805424	-0.153378	1.275972
H	2.743981	-0.555984	0.467512
H	2.572698	-3.836485	0.983975
H	-0.239458	-4.676437	0.820696
H	1.815993	-2.019063	2.601003
H	-0.382402	-2.484123	1.718813
H	0.740951	-2.981639	-1.065869
H	-1.667069	-1.329225	-0.087559
H	-1.088271	-1.407558	-1.762774
H	-3.890053	0.893146	0.347116
H	-6.473101	0.353902	-0.560440
H	-5.232123	-2.365122	0.046646
H	-6.335113	-1.344503	-2.588648
H	-3.416712	-1.853471	-2.468881
H	-3.291146	0.224710	-3.778171
H	-4.531117	-0.851262	-4.453238
H	-7.322479	-3.036429	-0.735365
H	-4.897330	-3.668993	-1.789291
H	-5.574561	-0.922781	1.830345
H	-5.085122	1.382898	-2.723163
C	-4.249926	4.299622	-0.285811
C	-3.210688	5.127440	0.104323
C	-1.853033	4.778183	-0.169725
C	-1.630860	3.572887	-0.868825
C	-2.650359	2.743858	-1.295468
C	-3.999922	3.077267	-0.982223
H	-5.273297	4.566286	-0.043070
C	-0.736466	5.577553	0.265438
H	-2.418023	1.824630	-1.816836
C	0.727539	3.862840	-0.689720
C	0.575618	5.039813	-0.019639
H	1.436567	5.574506	0.358776
C	1.991299	3.154599	-0.955729
C	3.242875	3.752495	-0.710349
C	1.958249	1.819214	-1.397674
C	4.420596	3.020319	-0.843225

H	3.306551	4.787122	-0.392143
C	3.132077	1.087737	-1.543692
H	1.011561	1.330348	-1.584963
C	4.355992	1.678633	-1.228598
H	5.383604	3.467330	-0.621058
H	3.103418	0.046063	-1.843317
O	-3.472086	6.279362	0.774123
O	-4.982818	2.265571	-1.276423
O	-0.347971	3.166210	-1.144990
O	-0.893952	6.673087	0.887210
H	-2.585279	6.693789	0.967028
C	5.637654	-0.147991	-0.312643
C	5.649412	-1.507923	-1.050867
C	6.590887	-2.458808	-0.314118
C	8.006464	-1.874018	-0.395237
C	8.051848	-0.402308	0.088740
C	8.713732	0.546034	-0.913862
O	4.330552	-1.998587	-1.222110
O	6.542284	-3.738575	-0.935343
O	8.945436	-2.709025	0.290132
O	6.750171	0.077269	0.504421
O	8.110304	0.530317	-2.205765
H	4.778654	-0.086013	0.356679
H	6.038725	-1.353379	-2.062283
H	6.284128	-2.541315	0.741393
H	8.314131	-1.922736	-1.443966
H	8.639163	-0.360966	1.011586
H	8.715754	1.559116	-0.491044
H	9.752527	0.227400	-1.048807
H	7.274392	-4.260032	-0.572299
H	8.743199	-2.709769	1.238214
H	3.960765	-2.336197	-0.380626
H	7.221272	0.920236	-2.127213
O	5.517883	0.928361	-1.285587

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G(Water) = -955.0978179 Hartree

C	4.215440	-0.535354	0.022154
C	3.472988	0.641982	-0.005239
C	2.046517	0.610661	-0.015325
C	1.404136	-0.658322	0.014146
C	2.135715	-1.837158	0.034188
C	3.536938	-1.761491	0.033311
H	5.299112	-0.487224	0.025350
C	1.272085	1.820870	-0.151365
H	1.635450	-2.797237	0.065277
C	-0.701316	0.396714	0.463752
C	-0.223474	1.647327	-0.277669
H	-0.463843	1.565516	-1.345961
C	-2.165104	0.101442	0.268339
C	-3.061577	0.208866	1.339028
C	-2.663482	-0.257871	-0.991878
C	-4.425670	-0.025251	1.161866
H	-2.694673	0.480452	2.325146
C	-4.022355	-0.505904	-1.182236
H	-1.984692	-0.357168	-1.833640
C	-4.907013	-0.384537	-0.101439
H	-5.120125	0.060416	1.991275
H	-4.394861	-0.789635	-2.162875
O	4.125706	1.819978	-0.038812
O	4.200397	-2.946484	0.056767
H	5.158947	-2.806966	0.065876
O	-6.253441	-0.608312	-0.220306
H	-6.478944	-0.849096	-1.130304
O	0.049961	-0.767400	0.004389
O	1.795374	2.956022	-0.204448
H	3.432730	2.531569	-0.094770
H	-0.487001	0.511780	1.534244
H	-0.729042	2.535319	0.109859

10-IMa

G(Water) = -954.6443777 Hartree

C	4.249363	-0.583543	0.037732
C	3.519826	0.583730	0.000022
C	2.079551	0.576248	-0.028197
C	1.439342	-0.706630	-0.008969
C	2.153994	-1.879060	0.026248
C	3.602148	-1.874130	0.044341
H	5.334253	-0.542777	0.055269
C	1.326931	1.773536	-0.156346
H	1.632251	-2.830429	0.050526
C	-0.658306	0.354673	0.452591
C	-0.177645	1.626548	-0.250471
H	-0.458933	1.596072	-1.311516
C	-2.130048	0.085703	0.265895
C	-3.020782	0.209174	1.339619
C	-2.642433	-0.262683	-0.991810
C	-4.390504	0.001253	1.168897
H	-2.644390	0.472532	2.324566
C	-4.007130	-0.484352	-1.176638

H	-1.968147	-0.372979	-1.835750
C	-4.884484	-0.347436	-0.092173
H	-5.079226	0.098958	2.001904
H	-4.389810	-0.759775	-2.155891
O	4.163525	1.781939	-0.023407
O	4.269243	-2.960250	0.076499
O	-6.236704	-0.546035	-0.206040
H	-6.468710	-0.780650	-1.115940
O	0.069608	-0.794796	-0.055907
O	1.849686	2.926663	-0.218504
H	3.451719	2.477870	-0.095711
H	-0.434378	0.437850	1.525187
H	-0.656438	2.504980	0.191788

10-IMb

G(Water) = -954.6330653 Hartree

C	4.170384	-0.570059	0.021838
C	3.441981	0.615239	0.063443
C	2.016179	0.603807	0.012537
C	1.355151	-0.654552	-0.068805
C	2.076814	-1.841736	-0.116801
C	3.476973	-1.784754	-0.075062
H	5.254046	-0.537514	0.057315
C	1.259992	1.831494	-0.051696
H	1.565688	-2.795239	-0.169950
C	-0.756573	0.409735	0.421926
C	-0.232868	1.691759	-0.226814
H	-0.441740	1.686032	-1.304437
C	-2.214745	0.141203	0.230929
C	-3.029711	-0.191703	1.328710
C	-2.825702	0.182026	-1.038518
C	-4.387165	-0.459560	1.181858
H	-2.584765	-0.236641	2.322373
C	-4.177724	-0.090348	-1.205848
H	-2.228369	0.422591	-1.916110
C	-5.036951	-0.425271	-0.099897
H	-4.992155	-0.707216	2.051868
H	-4.622315	-0.053932	-2.198424
O	4.108045	1.784987	0.136950
O	4.129084	-2.976930	-0.120952
H	5.087790	-2.847873	-0.071133
O	-6.295243	-0.674325	-0.251507
O	0.004574	-0.741425	-0.119386
O	1.801405	2.960084	-0.001727
H	3.421416	2.505950	0.116939
H	-0.526889	0.436671	1.495468
H	-0.731838	2.564352	0.202264

10-TSa

G(Water) = -3611.487792 Hartree

N	2.151527	-2.565361	0.664900
C	3.257602	-3.126850	1.280203
N	3.574608	-2.602077	2.510398
C	2.913856	-1.568079	3.194610
C	1.764589	-1.055926	2.487065
C	1.429464	-1.565007	1.276653
O	3.922168	-4.035319	0.764218
O	3.333751	-1.194232	4.293268
C	1.782363	-3.115691	-0.678322
C	0.935851	-4.386590	-0.551818
O	1.204702	-5.205314	-1.684117
C	-0.499676	-3.836581	-0.609881
C	-0.349831	-2.644267	-1.580825
O	1.016031	-2.174642	-1.388045
O	-1.355640	-4.862182	-1.063647
C	-1.231657	-1.422159	-1.342628
O	-2.613777	-1.645060	-1.656122
P	-3.710716	-2.380630	-0.657438
O	-3.776942	-3.853702	-1.063507
O	-4.989913	-1.571278	-0.785282
O	-3.057215	-2.351045	0.805335
P	-2.653736	-1.317221	2.139787
O	-2.031289	-2.321347	3.105619
O	-3.984377	-0.681394	2.604880
O	-1.690360	-0.273570	1.539908
C	-2.353578	1.867400	0.530285
C	-3.696771	2.202925	1.132626
C	-4.793065	1.457928	0.371417
C	-4.686987	1.686063	-1.137263
C	-3.267946	1.473609	-1.698159
C	-2.838056	2.533787	-2.707593
O	-3.697895	1.946745	2.514123
O	-6.057972	1.928548	0.841766
O	-5.605285	0.842724	-1.824081
O	-2.194321	1.470154	-0.669966
O	-2.958243	3.855656	-2.210957
H	4.385502	-3.010144	2.964021
H	1.168752	-0.274518	2.937439
H	0.564559	-1.199299	0.742159
H	2.717969	-3.309008	-1.201961

H	0.360683	-5.630098	-1.915453
H	-2.301437	-4.523868	-1.072610
H	1.145233	-4.928379	0.377101
H	-0.800739	-3.473437	0.381197
H	-0.459183	-3.007318	-2.609882
H	-1.143156	-1.063776	-0.315671
H	-0.900046	-0.625235	-2.014223
H	-1.466898	1.895741	1.141568
H	-3.822063	3.284831	0.984964
H	-4.692502	0.389895	0.583421
H	-4.993276	2.716324	-1.342374
H	-3.187133	0.474698	-2.126539
H	-1.811942	2.313684	-3.033226
H	-3.497958	2.444628	-3.576940
H	-6.735226	1.479627	0.313768
H	-5.406846	-0.089176	-1.542269
H	-3.782009	0.947729	2.626282
H	-2.396257	3.963011	-1.400488
C	0.349788	5.438794	0.366918
C	1.729182	5.535026	0.331524
C	2.552260	4.402728	0.012580
C	1.881186	3.181360	-0.286596
C	0.507245	3.079827	-0.283729
C	-0.306553	4.204518	0.068658
C	3.978685	4.459414	0.073450
H	0.057070	2.139941	-0.572263
C	3.943483	2.211500	-1.062925
C	4.726122	3.159957	-0.155838
C	4.483849	0.809615	-1.195356
C	4.404517	0.148725	-2.429275
C	4.938125	0.089602	-0.082281
C	4.726339	-1.203947	-2.548896
H	4.052763	0.688659	-3.304448
C	5.282666	-1.256128	-0.191169
H	5.009417	0.564488	0.890788
C	5.139176	-1.917599	-1.418860
H	4.626949	-1.721590	-3.497545
O	2.323387	6.719053	0.625246
O	-1.604681	4.111487	0.110983
O	5.387332	-3.259362	-1.550018
O	2.583295	2.043874	-0.561264
O	4.615561	5.511592	0.348376
H	3.304827	6.559566	0.583677
H	5.642568	-1.800287	0.675455
H	5.171527	-3.701568	-0.706239
H	4.894741	2.709424	0.829938
H	-0.246994	6.306674	0.627269
H	5.705288	3.376815	-0.591334
H	3.859744	2.667027	-2.059061

10-TSb

G(Water) = -3611.482523 Hartree

N	-2.153979	1.644656	0.443710
C	-3.480774	1.762750	0.851583
N	-3.834693	0.944585	1.905924
C	-3.008354	0.076080	2.631582
C	-1.632556	0.086452	2.190938
C	-1.264545	0.848411	1.134177
O	-4.281489	2.525554	0.314421
O	-3.480556	-0.599123	3.551442
C	-1.746194	2.421252	-0.760290
C	-1.102991	3.770032	-0.419057
O	-1.436486	4.688062	-1.454682
C	0.403778	3.452037	-0.445901
C	0.491750	2.357540	-1.536125
O	-0.795193	1.682279	-1.495575
O	1.103109	4.641245	-0.748670
C	1.513611	1.236816	-1.348813
O	2.876741	1.623466	-1.574087
P	3.794935	2.525804	-0.538204
O	3.643928	3.991777	-0.947202
O	5.189387	1.924981	-0.604839
O	3.099495	2.401478	0.899767
P	2.714887	1.335359	2.207616
O	1.885330	2.254036	3.099738
O	4.069773	0.872200	2.788746
O	1.941856	0.175897	1.545719
C	2.865934	-1.865960	0.622424
C	4.212370	-2.019747	1.286049
C	5.226407	-1.128956	0.569188
C	5.239426	-1.400434	-0.935544
C	3.835953	-1.469434	-1.573807
C	3.645627	-2.682609	-2.482783
O	4.120154	-1.775575	2.666341
O	6.519698	-1.389637	1.118342
O	6.036954	-0.429843	-1.603555
O	2.714124	-1.546819	-0.602298
O	3.911070	-3.905524	-1.824360
H	-4.809348	0.991909	2.183401
H	-0.906633	-0.530915	2.700539
H	-0.238608	0.842854	0.791253

H	-2.653228	2.556986	-1.348822
H	-0.642338	5.234594	-1.590291
H	2.084354	4.442563	-0.822710
H	-1.439117	4.145610	0.554292
H	0.716803	3.045417	0.523119
H	0.614873	2.838767	-2.514411
H	1.424319	0.791603	-0.357119
H	1.303521	0.466057	-2.095485
H	1.959955	-1.994502	1.189069
H	4.491047	-3.073529	1.142724
H	4.954069	-0.085767	0.749869
H	5.729803	-2.366577	-1.088677
H	3.629301	-0.539927	-2.104622
H	2.628361	-2.653387	-2.897006
H	4.354395	-2.581353	-3.312506
H	7.149064	-0.851030	0.615743
H	5.713810	0.468714	-1.325079
H	4.073680	-0.776164	2.785809
H	3.241069	-4.058214	-1.085462
C	-7.247796	-0.017471	-0.102061
C	-6.257133	0.174352	-1.061031
C	-5.061812	-0.605689	-1.048484
C	-4.914996	-1.592348	-0.034657
C	-5.894972	-1.785840	0.928922
C	-7.049937	-0.991568	0.886212
C	-3.981530	-0.331779	-1.962233
H	-5.769289	-2.536167	1.699383
C	-2.971387	-2.497334	-1.120867
C	-2.717838	-1.129707	-1.767295
C	-1.674395	-3.153861	-0.711861
C	-0.825479	-3.677643	-1.699891
C	-1.211647	-3.129735	0.610807
C	0.469950	-4.091272	-1.396265
H	-1.169493	-3.729521	-2.731603
C	0.072929	-3.567455	0.930120
H	-1.853656	-2.743148	1.394789
C	0.984917	-3.995700	-0.073121
H	1.122061	-4.461496	-2.182542
O	-6.435896	1.122062	-1.998961
O	-7.973143	-1.224636	1.855302
O	2.255826	-4.213677	0.206294
O	-3.802494	-2.358106	0.061025
O	-4.048480	0.561307	-2.838017
H	-5.600471	1.149127	-2.540272
H	-8.740415	-0.643226	1.747961
H	0.417721	-3.533442	1.961144
H	-2.042156	-0.540129	-1.137756
H	-8.144635	0.592254	-0.125682
H	-2.218009	-1.258360	-2.730437
H	-3.527002	-3.130038	-1.827838

12

G(Water) = -1639.994289 Hartree

C	-5.522887	-1.105334	0.413103
C	-4.310819	-1.610919	-0.042365
C	-3.180836	-0.753566	-0.177684
C	-3.337983	0.602050	0.167594
C	-4.536630	1.129068	0.626472
C	-5.624536	0.256832	0.744056
H	-6.374799	-1.769893	0.511099
C	-1.897736	-1.232660	-0.625361
H	-4.626626	2.177081	0.882536
C	-1.058126	1.067028	-0.373188
C	-0.836689	-0.251542	-0.692238
C	-0.102768	2.178408	-0.406357
C	0.984704	2.202894	-1.301849
C	-0.284082	3.273380	0.461749
C	1.863308	3.279530	-1.322618
H	1.146520	1.376074	-1.979020
C	0.597923	4.348481	0.454713
H	-1.114645	3.277046	1.158281
C	1.675858	4.356180	-0.442213
H	2.696105	3.302025	-2.017491
H	0.452335	5.177757	1.140569
O	-4.213273	-2.919881	-0.350540
O	-6.786345	0.796383	1.196001
H	-7.482310	0.124813	1.248210
O	2.575378	5.375549	-0.507263
H	2.362121	6.066317	0.137510
O	-2.288370	1.468703	0.047771
O	-1.705081	-2.449577	-0.927753
H	-3.276318	-3.080293	-0.638187
C	1.302811	-1.166036	-0.152544
C	1.808245	-2.546699	-0.580687
C	2.948589	-2.977575	0.341719
C	4.035462	-1.907902	0.420681
C	3.405927	-0.565149	0.809198
C	4.391261	0.594583	0.762092
O	0.794754	-3.534661	-0.498513
O	3.571740	-4.179885	-0.113160
O	5.012847	-2.236639	1.405419

O	2.369469	-0.238835	-0.129870
O	3.782655	1.822225	1.158440
H	2.187986	-2.451482	-1.609275
H	2.539108	-3.131419	1.351566
H	4.506682	-1.808736	-0.568657
H	2.979535	-0.638683	1.821340
H	5.208987	0.401258	1.459069
H	4.805758	0.671897	-0.253127
H	2.910211	-4.886499	-0.097320
H	5.311784	-3.141390	1.230257
H	-0.059286	-3.157311	-0.808947
H	3.067969	2.013051	0.530849
O	0.389760	-0.695109	-1.134597
H	0.804617	-1.214122	0.827610

12-IMa

G(Water) = -1639.542089 Hartree

C	-5.554786	-1.069032	0.442550
C	-4.358217	-1.573209	-0.013000
C	-3.209138	-0.725629	-0.165497
C	-3.374754	0.642471	0.180403
C	-4.557870	1.172144	0.638532
C	-5.717489	0.326761	0.791770
H	-6.408512	-1.731066	0.550722
C	-1.948127	-1.204779	-0.611617
H	-4.627463	2.225843	0.885904
C	-1.081314	1.089224	-0.373656
C	-0.874526	-0.226157	-0.691620
C	-0.109058	2.191599	-0.403653
C	0.970465	2.214908	-1.307371
C	-0.264636	3.276180	0.480607
C	1.867641	3.277887	-1.319140
H	1.114346	1.394736	-1.996930
C	0.633959	4.339467	0.481531
H	-1.090439	3.281949	1.182956
C	1.704769	4.343992	-0.422336
H	2.695460	3.296700	-2.020325
H	0.505452	5.161345	1.179976
O	-4.247852	-2.895073	-0.322571
O	-6.830943	0.782685	1.214913
O	2.621421	5.352593	-0.479260
H	2.422238	6.034908	0.178547
O	-2.307149	1.505781	0.048081
O	-1.742283	-2.431277	-0.918139
H	-3.309043	-3.040186	-0.611486
C	1.254820	-1.165445	-0.163232
C	1.736558	-2.556866	-0.584551
C	2.870270	-3.002376	0.339061
C	3.976970	-1.953182	0.410643
C	3.371120	-0.597890	0.793504
C	4.375685	0.545112	0.742028
O	0.706558	-3.526118	-0.501276
O	3.469576	-4.220564	-0.107593
O	4.951093	-2.295046	1.394957
O	2.341163	-0.257445	-0.145804
O	3.785471	1.784940	1.128820
H	2.119099	-2.471077	-1.613365
H	2.459229	-3.141942	1.350445
H	4.448146	-1.866981	-0.579983
H	2.944241	-0.660608	1.806441
H	5.188931	0.343759	1.442019
H	4.793497	0.609510	-0.272847
H	2.789294	-4.909389	-0.098341
H	5.227813	-3.208218	1.227205
H	-0.153407	-3.122321	-0.777719
H	3.070024	1.979044	0.502648
O	0.349920	-0.681365	-1.140342
H	0.761231	-1.199980	0.820089

12-IMb

G(Water) = -1639.539692 Hartree

C	-5.481182	-1.146994	0.408189
C	-4.267059	-1.623386	-0.078552
C	-3.152039	-0.750605	-0.205369
C	-3.315202	0.588955	0.183963
C	-4.514829	1.088002	0.677452
C	-5.592518	0.202316	0.781468
H	-6.323895	-1.824664	0.497659
C	-1.860115	-1.201927	-0.675277
H	-4.611821	2.126742	0.967313
C	-1.037513	1.107852	-0.380147
C	-0.821693	-0.223332	-0.729857
C	-0.117876	2.214639	-0.404992
C	1.045202	2.230773	-1.222610
C	-0.368019	3.371635	0.390348
C	1.906017	3.309536	-1.232648
H	1.261364	1.379187	-1.852666
C	0.491728	4.447615	0.397780
H	-1.249026	3.394972	1.024043
C	1.684905	4.484904	-0.418821

H	2.777419	3.304137	-1.883257
H	0.287010	5.308167	1.029856
O	-4.153783	-2.923710	-0.425274
O	-6.757297	0.716873	1.265208
H	-7.444426	0.035608	1.300982
O	2.483324	5.479385	-0.420378
O	-2.278675	1.469905	0.075074
O	-1.670184	-2.425907	-0.995743
H	-3.209021	-3.052436	-0.718588
C	1.320024	-1.119818	-0.178318
C	1.810105	-2.519321	-0.562861
C	2.939337	-2.937241	0.379460
C	4.041285	-1.881209	0.438502
C	3.424538	-0.520079	0.781230
C	4.419601	0.630906	0.713763
O	0.784831	-3.492949	-0.461539
O	3.548708	-4.162268	-0.034455
O	5.005647	-2.197148	1.441084
O	2.405439	-0.213155	-0.178391
O	3.820155	1.872253	1.076866
H	2.198758	-2.457350	-1.590971
H	2.520663	-3.056276	1.390396
H	4.523181	-1.816365	-0.548603
H	2.986280	-0.560712	1.790631
H	5.232241	0.446344	1.419553
H	4.841235	0.678995	-0.300588
H	2.871664	-4.854077	-0.016627
H	5.290715	-3.111372	1.294450
H	-0.065439	-3.113064	-0.789871
H	3.131245	2.081651	0.421970
O	0.415824	-0.660288	-1.169353
H	0.821752	-1.132743	0.803302

12-TSa

G(Water) = -4296.388411 Hartree

N	-0.395407	1.963507	1.407329
C	-1.484753	2.275695	2.213009
N	-1.959311	1.226475	2.975288
C	-1.421757	-0.065249	3.082135
C	-0.245029	-0.267097	2.272141
C	0.211923	0.731872	1.477617
O	-1.995001	3.394498	2.238272
O	-1.957270	-0.893556	3.826171
C	0.052493	3.031171	0.459351
C	1.004248	4.035617	1.117709
O	0.819085	5.295332	0.480582
C	2.390058	3.457418	0.779155
C	2.151748	2.824699	-0.610438
O	0.745086	2.454059	-0.618720
O	3.332310	4.510611	0.793310
C	2.891278	1.529705	-0.935569
O	4.281881	1.697512	-1.242322
P	5.470214	1.983839	-0.128469
O	5.642518	3.500281	-0.027270
O	6.665304	1.169818	-0.598398
O	4.877971	1.520718	1.284340
P	4.367272	0.186681	2.264126
O	3.977422	0.902622	3.555067
O	5.592292	-0.753273	2.365251
O	3.209386	-0.446309	1.473129
C	3.598736	-2.195948	-0.310323
C	4.893821	-2.893343	0.026831
C	6.054812	-2.060516	-0.522406
C	5.879849	-1.758874	-2.012716
C	4.467659	-1.262890	-2.385483
C	3.845909	-1.981676	-3.579873
O	4.974383	-3.126514	1.409147
O	7.264121	-2.790884	-0.314898
O	6.858450	-0.815530	-2.433958
O	3.464521	-1.402605	-1.297925
O	3.759997	-3.381793	-3.387216
H	-2.766551	1.437855	3.552337
H	0.263259	-1.220222	2.302956
H	1.090370	0.592534	0.866693
H	-0.856823	3.517250	0.104239
H	1.703945	5.695878	0.420699
H	4.226453	4.165902	0.500568
H	0.830223	4.117862	2.196067
H	2.654574	2.677639	1.503452
H	2.326329	3.580619	-1.386036
H	2.786664	0.812123	-0.120540
H	2.441597	1.105073	-1.837141
H	2.768663	-2.230909	0.374942
H	4.865407	-3.863431	-0.488362
H	6.091121	-1.118514	0.033359
H	6.077914	-2.683550	-2.563607
H	4.490869	-0.185218	-2.545390
H	2.862546	-1.537883	-3.788513
H	4.489992	-1.797975	-4.446399
H	7.978998	-2.262891	-0.701014
H	6.790783	-0.026416	-1.832890

H	5.189374	-2.240212	1.845999
H	3.135287	-3.580564	-2.638684
C	0.369800	-4.559859	0.002384
C	-0.880875	-4.201846	0.468867
C	-1.453018	-2.935631	0.126253
C	-0.697037	-2.097566	-0.723108
C	0.538551	-2.446850	-1.228491
C	1.125604	-3.689212	-0.844862
H	0.803225	-5.509634	0.297532
C	-2.727842	-2.501600	0.606451
H	1.079384	-1.754935	-1.860063
C	-2.363849	-0.383431	-0.598765
C	-3.150123	-1.172985	0.202253
C	-2.622770	0.983541	-1.069081
C	-3.439684	1.878759	-0.356036
C	-1.999613	1.441486	-2.248579
C	-3.633178	3.182016	-0.804475
H	-3.923272	1.562431	0.556384
C	-2.197036	2.736138	-2.709294
H	-1.352643	0.775762	-2.807152
C	-3.013154	3.615445	-1.985398
H	-4.248162	3.866087	-0.227121
O	-1.569383	-5.049117	1.275822
O	2.339643	-4.003966	-1.200971
O	-3.162052	4.878251	-2.475170
O	-1.176477	-0.856767	-1.061282
O	-3.462373	-3.233471	1.347628
H	-2.426133	-4.600613	1.496492
O	-4.348461	-0.722203	0.714697
H	-1.716597	3.082971	-3.617824
H	-3.731987	5.405278	-1.895952
C	-5.520831	-0.974070	-0.044567
C	-6.551549	-1.647876	0.861964
C	-7.904153	-1.745487	0.149312
C	-7.205890	0.227716	-1.233476
C	-8.344416	-0.384391	-0.394302
O	-6.169700	-2.964391	1.222269
O	-8.906584	-2.235777	1.036838
O	-9.553599	-0.459050	-1.146682
C	-7.489842	1.658826	-1.668719
H	-8.409783	1.683483	-2.255557
H	-7.626462	2.283430	-0.774320
O	-6.449624	2.176325	-2.496857
O	-5.999991	0.289817	-0.458465
H	-5.276752	-1.604759	-0.912874
H	-7.033246	-0.392560	-2.128212
H	-6.662207	-1.009995	1.752558
H	-7.786766	-2.441104	-0.698799
H	-8.564810	0.279564	0.448640
H	-5.627974	2.170848	-1.980582
H	-9.457381	-1.123026	-1.845628
H	-8.573158	-3.061163	1.419456
H	-5.196893	-2.994013	1.391194

12-TSb

G(Water) = -4296.389056 Hartree

N	1.203927	0.106966	-2.365677
C	2.429643	-0.169372	-2.960454
N	2.919038	-1.436735	-2.708538
C	2.288610	-2.472788	-2.004416
C	0.987964	-2.114838	-1.495846
C	0.504509	-0.862472	-1.687709
O	3.034787	0.641507	-3.659964
O	2.858244	-3.562984	-1.877289
C	0.688170	1.495445	-2.517132
C	-0.175473	1.671612	-3.772589
O	0.020787	2.998048	-4.257735
C	-1.604389	1.499570	-3.231408
C	-1.484445	2.105268	-1.818188
O	-0.125250	1.816592	-1.406618
O	-2.505021	2.178186	-4.082780
C	-2.375434	1.523095	-0.726206
O	-3.740419	1.953631	-0.843463
P	-4.911271	1.127306	-1.676148
O	-4.869583	1.555952	-3.138136
O	-6.195981	1.402640	-0.905266
O	-4.535675	-0.410893	-1.462667
P	-3.605163	-1.757990	-2.043882
O	-3.253815	-1.478462	-3.498895
O	-4.601973	-2.917054	-1.797781
O	-2.429813	-1.773815	-1.050265
C	-3.257243	-1.793218	1.371275
C	-4.537744	-2.584105	1.463129
C	-5.734159	-1.678084	1.153151
C	-5.681536	-0.400008	1.997147
C	-4.313593	0.310621	1.988086
C	-3.876243	0.808582	3.364493
O	-4.455647	-3.737267	0.668824
O	-6.926393	-2.404001	1.457001
O	-6.698786	0.508383	1.599538
O	-3.181066	-0.535072	1.521210

O	-3.833449	-0.218578	4.334520
H	3.820014	-1.644795	-3.125363
H	0.407189	-2.848087	-0.955297
H	-0.473433	-0.589803	-1.315366
H	1.566168	2.140632	-2.539629
H	-0.837099	3.283685	-4.615422
H	-3.434099	1.966773	-3.776256
H	0.077011	0.937753	-4.546426
H	-1.856050	0.432423	-3.170170
H	-1.623572	3.192362	-1.880635
H	-2.315255	0.432315	-0.717425
H	-2.022975	1.896054	0.240251
H	-2.337918	-2.290099	1.102197
H	-4.600483	-2.898928	2.515705
H	-5.709608	-1.415108	0.093293
H	-5.909866	-0.687574	3.028292
H	-4.313660	1.129669	1.268593
H	-2.908444	1.318622	3.259983
H	-4.613838	1.550694	3.688528
H	-7.666815	-1.795982	1.311409
H	-6.514190	0.819205	0.669643
H	-4.499816	-3.452558	-0.307122
H	-3.164134	-0.919886	4.077458
C	7.196845	-2.837258	-0.669489
C	6.490478	-1.641362	-0.736986
C	5.266323	-1.490019	-0.026944
C	4.789100	-2.589739	0.706031
C	5.476718	-3.793352	0.790690
C	6.685215	-3.899624	0.095768
H	8.131269	-2.935742	-1.212140
C	4.489041	-0.276375	-0.074346
H	5.083067	-4.623306	1.363752
C	2.834335	-1.376704	1.378755
C	3.279437	-0.254062	0.700596
C	1.569371	-1.579291	2.058925
C	0.830302	-0.519067	2.635068
C	1.014481	-2.882880	2.124128
C	-0.407130	-0.742882	3.212734
H	1.236615	0.482109	2.635655
C	-0.234478	-3.104836	2.676202
H	1.559505	-3.716101	1.692979
C	-1.014626	-2.034391	3.213311
H	-0.954282	0.086231	3.650599
O	6.975116	-0.624557	-1.481100
O	7.338875	-5.089398	0.193696
O	-2.243637	-2.223792	3.605802
O	3.603326	-2.506602	1.372970
O	4.853504	0.713044	-0.791172
H	6.311029	0.114250	-1.416294
H	8.164249	-5.072700	-0.312443
O	2.457041	0.844560	0.564094
H	-0.667581	-4.101386	2.674089
C	2.931114	2.084544	1.057319
C	2.630405	3.162702	0.015202
C	2.964658	4.532253	0.604766
C	2.556718	3.591414	2.882909
C	2.245695	4.756764	1.931257
O	2.204366	2.356560	2.245030
O	3.369024	2.992650	-1.188465
O	2.575098	5.589314	-0.275087
O	2.676148	5.966930	2.553008
C	1.760995	3.675342	4.179622
H	1.861555	4.686355	4.590015
H	0.705338	3.491471	3.958523
O	2.147233	2.686384	5.135648
H	3.021739	2.912489	5.481852
H	3.057494	5.473819	-1.106942
H	3.862461	2.142115	-1.144875
H	2.618705	6.670735	1.889643
H	3.634325	3.598884	3.113410
H	4.005789	2.025381	1.278958
H	1.556209	3.108761	-0.203728
H	1.161148	4.786202	1.746684
H	4.049853	4.583535	0.783599

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6. References

1. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. *Gaussian 16 Rev. A.03*, Wallingford, CT, 2016.

2. Barone, V. & Cossi, M. Quantum calculation of molecular energies and energy gradients in solution by a conductor solvent model. *J. Phys. Chem. A* 1998, **102**, 1995-2001.

3. Cossi, M.; Rega, N.; Scalmani, G. & Barone, V. Energies, structures, and electronic properties of molecules in solution with the C-PCM solvation model. *J. Comput. Chem.* 2003, **24**, 669-681.

4. Takano, Y. & Houk, K. N. Benchmarking the conductor-like polarizable continuum model (CPCM) for aqueous solvation free energies of neutral and ionic organic molecules. *J. Chem. Theory Comput.* 2005, **1**, 70-77.

5. Jakalian, A.; Bush, B. L.; Jack, D. B. & Bayly, C. I. Fast, efficient generation of high-quality atomic charges. AM1-BCC model: I. Method *J. Comput. Chem.* 2000, **21**, 132-146.

6. Jakalian, A.; Jack, D. B. & Bayly, C. I. Fast, efficient generation of high-quality atomic charges. AM1-BCC model: II. Parameterization and validation. *J. Comput. Chem.* 2002, **23**, 1623-1641.

7. Gordon, J. C. et al. H++: a server for estimating pKa s and adding missing hydrogens to macromolecules. *Nucleic Acids Res.* 2005, **33**, W368-W371.

8. Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* 1983, *79*, 926-935.

9. Ward, J. H. Hierarchical grouping to optimize an objective function. *J. Am. Stat. Assoc.* 1963, **58**, 236-244.

10. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J., Automated docking using a lamarckian genetic algorithm and an empirical binding free Energy function. *J. Comput. Chem.* 1998, **19**, 1639-1662.

11. Case, D. A. et al. AMBER 16 (University of California, San Francisco, 2016).

12. Wang, J. M. et al. Development and testing of a general amber force field. *J. Comput. Chem.* 2004, **25**, 1157-1174.

13. Darden, T.; York, D. & Pedersen, L. Particle mesh Ewald: An N·log(N) method for Ewald sums in large systems. *J. Chem. Phys.* 1993, **98**, 10089-10092.

14. Miller, B. R., III; McGee, T. D., Jr.; Swails, J. M.; Homeyer, N.; Gohlke, H.; Roitberg, A. E. MMPBSA.Py: An efficient program for end-state free energy calculations. *J. Chem. Theory Comput.* 2012, **8**, 3314-3321.

15. Ming, H. L.; Son, Y. K.; Yong, N. H. Tissue factor inhibitory flavonoids from the fruits of *Chaenomeles sinensis*. *Arch. .Pharm. Res.* 2002, **25**, 842-850.

16. Gao, D.; Xu, M.; Yang, C.; Xu, M.; Zhang, Y. Phenolic antioxidants from the leaves of *Camellia pachyandra* Hu. *J. Agr. Food Chem.* 2010, **58**, 8820.

17. Yamamoto, H.; Kuribayashi, H.; Seshima, Y. Metabolism of administered (2*RS*)-naringenin in

flavonoid-producing cultured cells of *Sophora flavescens*. *Plant Tissue Culture Letters* 2004, **21**, 355-359.

18. Shimoda, K.; Kubota, N.; Taniuchi, K.; Sato, D.; Nakajima, N.; Hamada, H.; Hamada, H. Biotransformation of naringin and naringenin by cultured *Eucalyptus perriniana* cells. *Phytochemistry* 2010, **71**, 201-205.

19. Moretti, C.; Sauvain, M.; Lavaud, C.; Massiot, G.; Bravo, J.; Munoz, V. A novel antiprotozoal aminosteroid from *Saracha punctata*. *J. Nat. Prod.* 1998, **61**, 1390-1393.

20. Markham, K. R.; Ternai, B.; Stanley, R. Carbon-13 NMR studies of flavonoids-III. Naturally occurring flavonoid glycosides and their acylated derivatives. *Tetrahedron* 1978, **34**, 1389-1397.

21. Oyama, K. I.; Kondo, T. Total synthesis of flavocommelin, a component of the blue supramolecular pigment from *Commelina communis*, on the basis of direct 6-*C*-glycosylation of flavan. *J. Org. Chem.* 2004, **69**, 5240.

22. Hosoya, T.; Yun, Y. S.; Kunugi, A. Five novel flavonoids from *Wasabia japonica*. *Tetrahedron* 2005, **61**, 7037-7044.

23. Li, Y.; Li, J.; Wang, N.; Yao, X. Flavonoids and a new polyacetylene from *Bidens parviflora* Willd. *Molecules* 2008, **13**, 1931-1941.