Supplementary data for article:

Milivojević, A.; Ćorović, M.; Carevic, M.; Banjanac, K.; Vujisić, L. V.; Velickovic, D.; Bezbradica, D. Highly Efficient Enzymatic Acetylation of Flavonoids: Development of Solvent-Free Process and Kinetic Evaluation. *Biochemical Engineering Journal* **2017**, *128*, 106–115. https://doi.org/10.1016/j.bej.2017.09.018

Supporting Information

Highly efficient enzymatic acetylation of flavonoids: Development of solvent-free process and kinetic evaluation

Ana Milivojević[a], Marija Ćorović[a*], Milica Carević[a], Katarina Banjanac[a], Ljubodrag Vujisić[b], Dušan Veličković[c], Dejan Bezbradica[a]

[a]Department of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia

[b]Faculty of Chemistry, University of Belgrade, Studentskitrg 12, 11000 Belgrade, Serbia

[c] Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99354

[*]Corresponding author: M.Ćorović, Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia.E-mail address: mstojanovic@tmf.bg.ac.rs. Tel.: (+38111)3303727. Fax: (+38111)3370387.

1. Kinetic model

Set of ordinary differential equations (Eqs. S1-S3) that describes the rate of formation of each species

$$\frac{d([F])}{dt} = -\left(\frac{\frac{Vf_1 \cdot [F]}{Kms_1} - \frac{Vr_1 \cdot [FA]}{Kmp_1}}{1 + \frac{[F]}{Kms_1} + \frac{[FA]}{Kmp_1}}\right) \quad \text{(S1)}$$

$$\frac{d([FA])}{dt} = \left(\frac{\frac{Vf_1 \cdot [F]}{Kms_1} - \frac{Vr_1 \cdot [FA]}{Kmp_1}}{1 + \frac{[F]}{Kms_1} + \frac{[FA]}{Kmp_1}}\right) - \left(\frac{\frac{Vf_2 \cdot [FA]}{Kms_2} - \frac{Vr_2 \cdot [FDA]}{Kmp_2}}{1 + \frac{[FA]}{Kms_2} + \frac{[FDA]}{Kmp_2}}\right) \quad \text{(S2)}$$

$$\frac{d([FDA])}{dt} = \left(\frac{\frac{Vf_2 \cdot [FA]}{Kms_2} - \frac{Vr_2 \cdot [FDA]}{Kmp_2}}{1 + \frac{[FA]}{Kms_2} + \frac{[FDA]}{Kmp_2}}\right) \quad \text{(S3)}$$

where the letters inside the square brackets represent the concentrations of the respective species, while subscripts 1 and 2 of parameter names indicate the first (Eq. 2) and second (Eq. 3) reaction of kinetic model.

2. NMR analyses

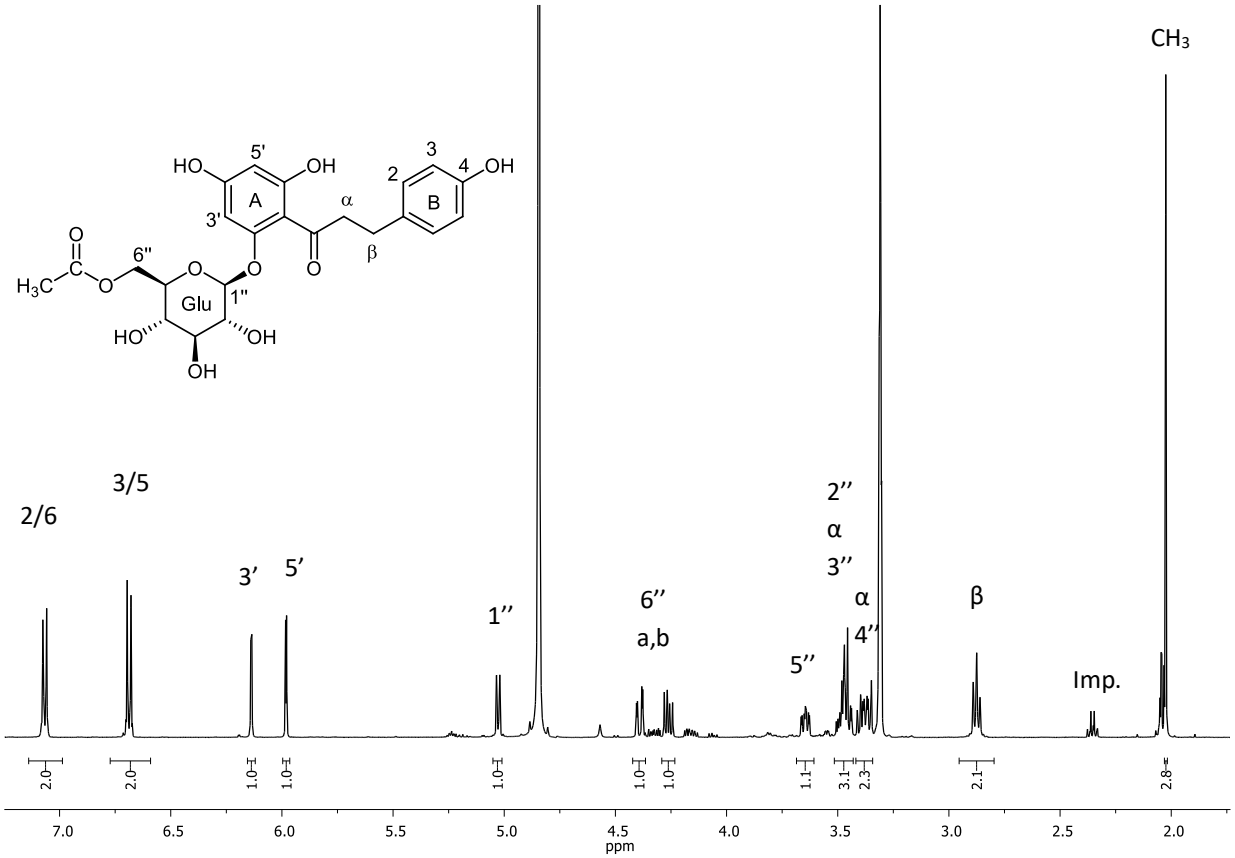


Figure S1. ^{1}H NMR (500 MHz, CD_3OD) spectrum of phloridzin-6''-*O*-acetate with complete assignments.

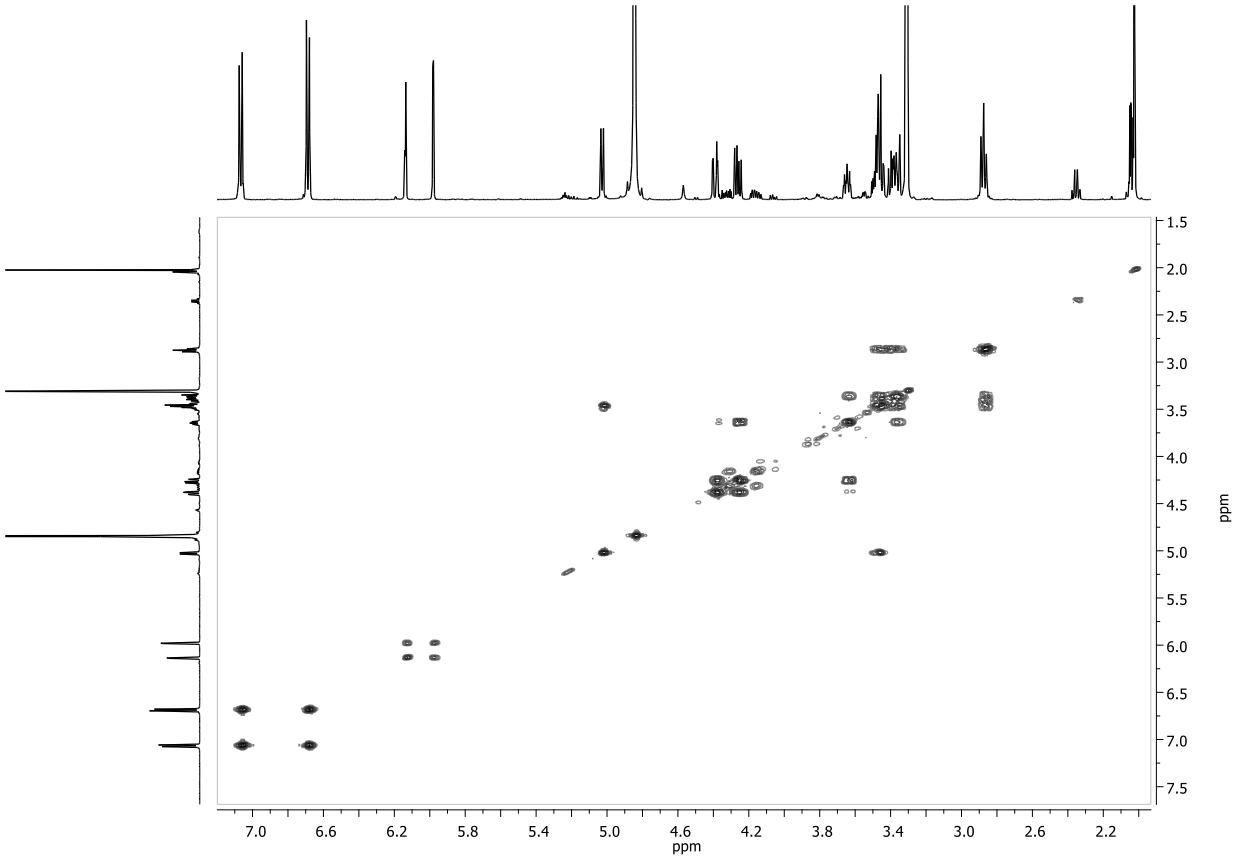


Figure S2. ^{1}H-^{1}H COSY (500 MHz, CD_3OD) NMR spectrum of phloridzin-6''-*O*-acetate.

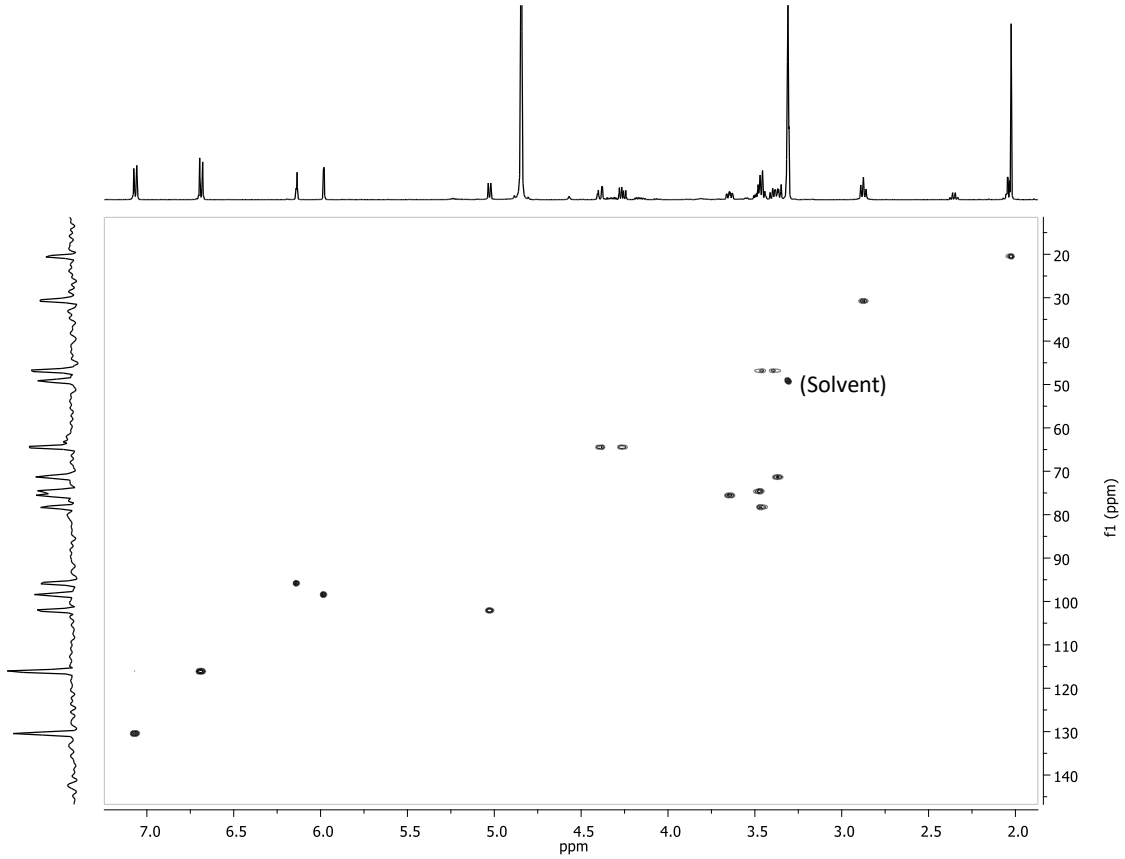


Figure S3. HSQC (500 MHz, CD_3OD) NMR spectrum of phloridzin-6''-*O*-acetate was used for assignments of 13 protonated carbons (nine CH, three CH_2 and one CH_3)

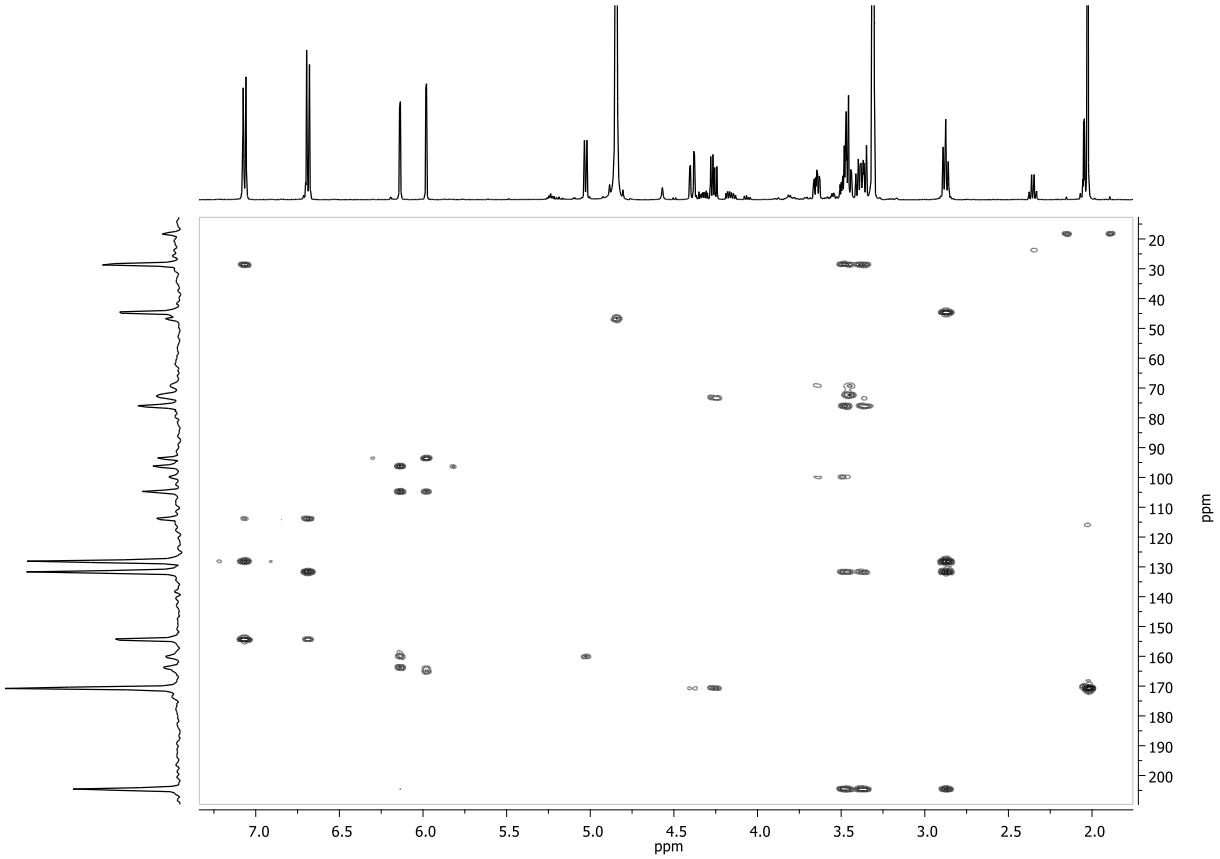


Figure S4. HMBC (500 MHz, CD_3OD) NMR spectrum of phloridzin-6''-*O*-acetate.

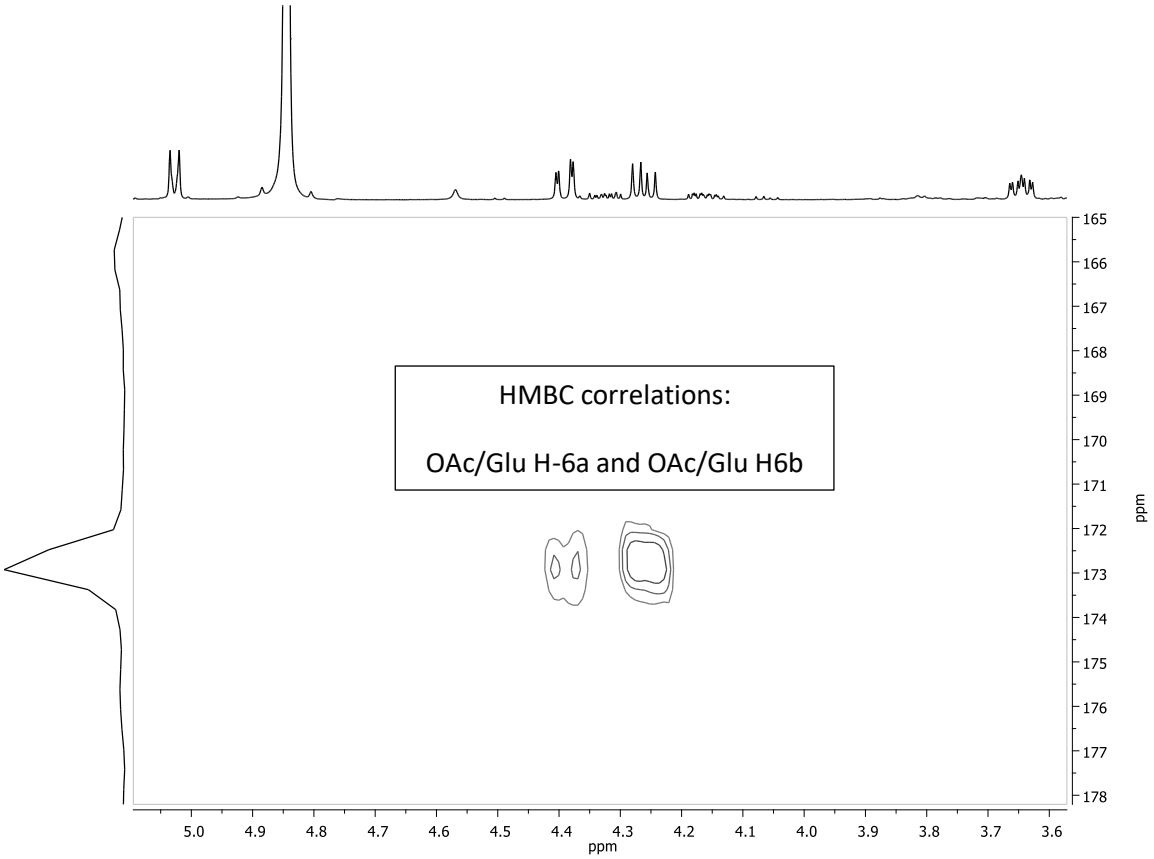
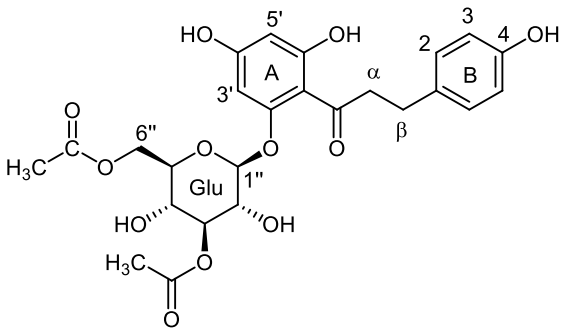


Figure S5. Partial HMBC (CD_3OD) NMR spectrum of phloridzin-6''-*O*-acetate showing the most important correlations between ester carbon and both Glu H-6 protons.



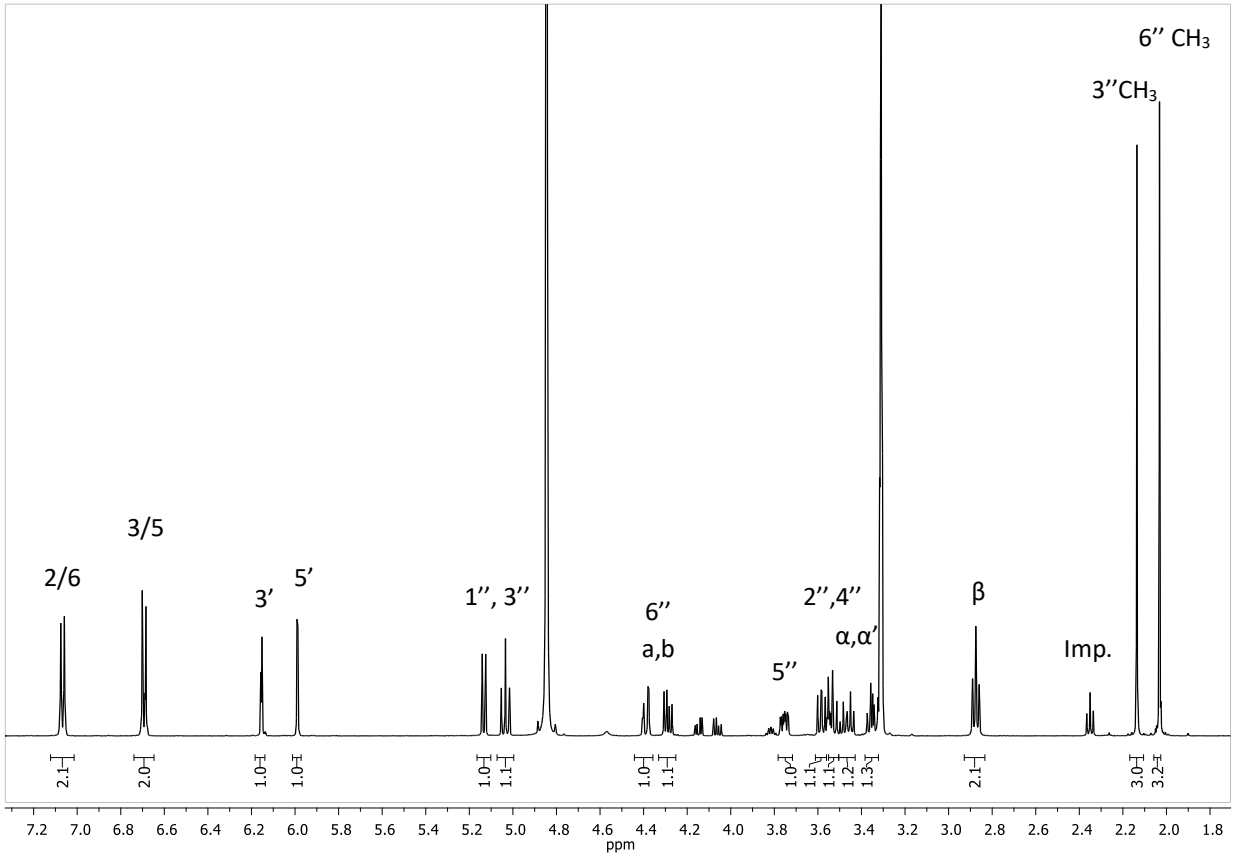


Figure S6. ^{1}H NMR (500 MHz, CD_3OD) spectrum of phloridzin-3'',6''-*O*-diacetate with complete assignments.

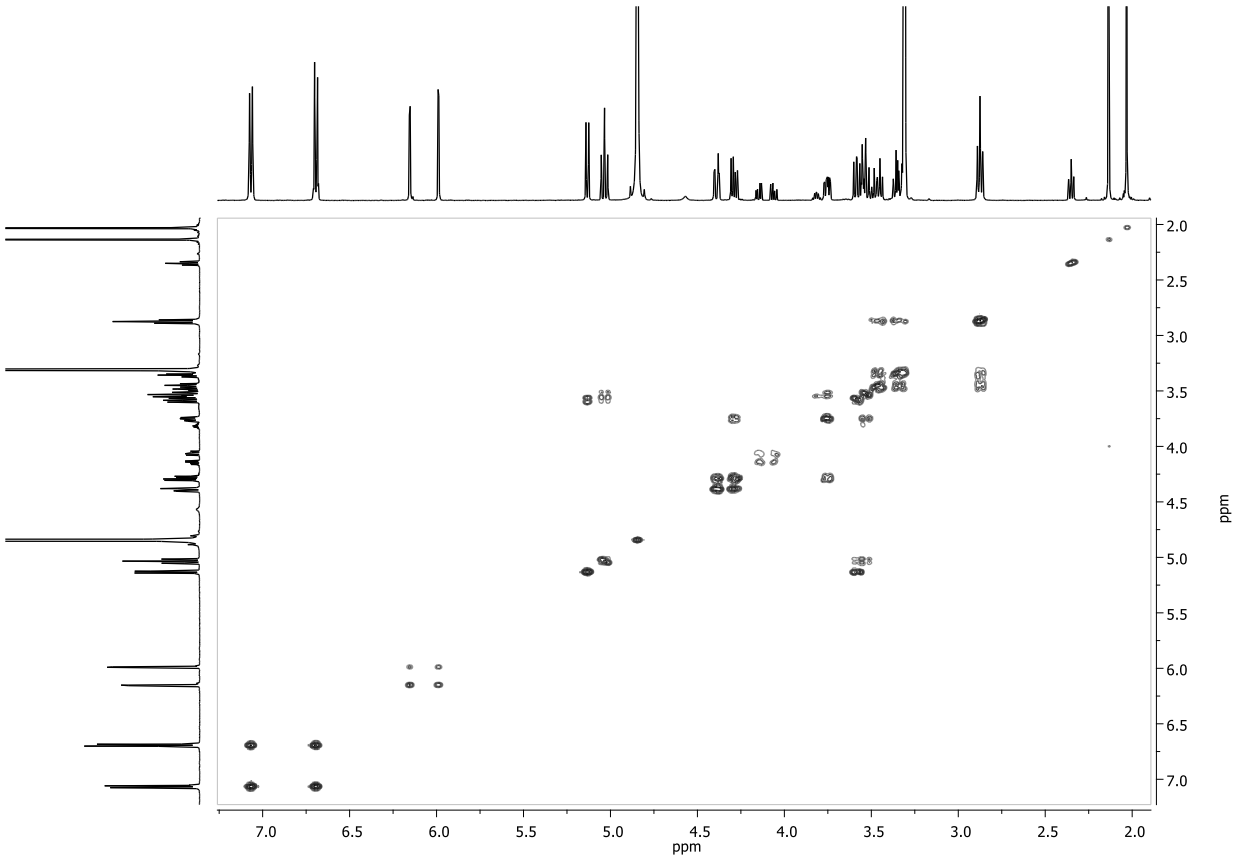


Figure S7. ^{1}H-^{1}H COSY (500 MHz, CD_3OD) NMR spectrum of phloridzin-3’’,6’’-*O*-diacetate.

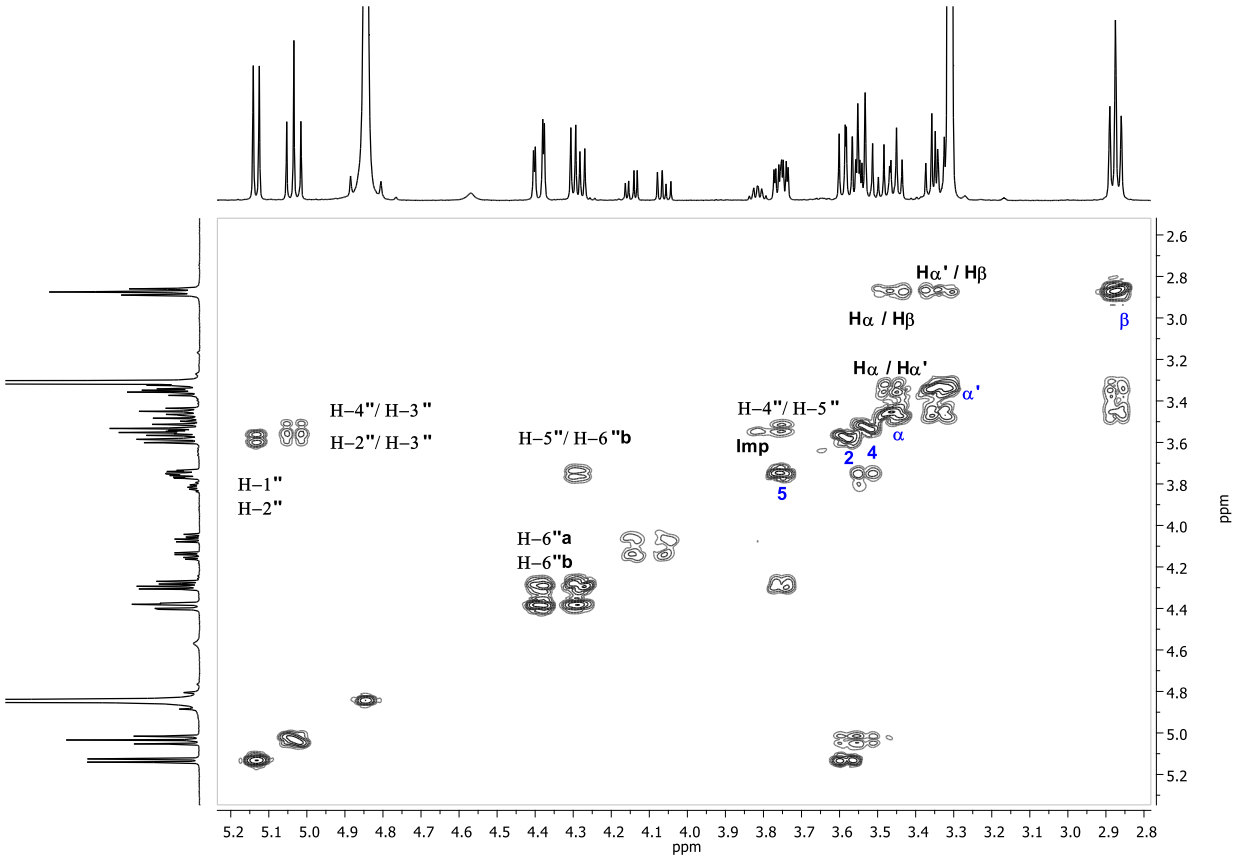


Figure S8. Partial ^{1}H-^{1}H COSY (500 MHz, CD_3OD) NMR spectrum of phloridzin-3'',6''-*O*-diacetate showing the most important correlations.

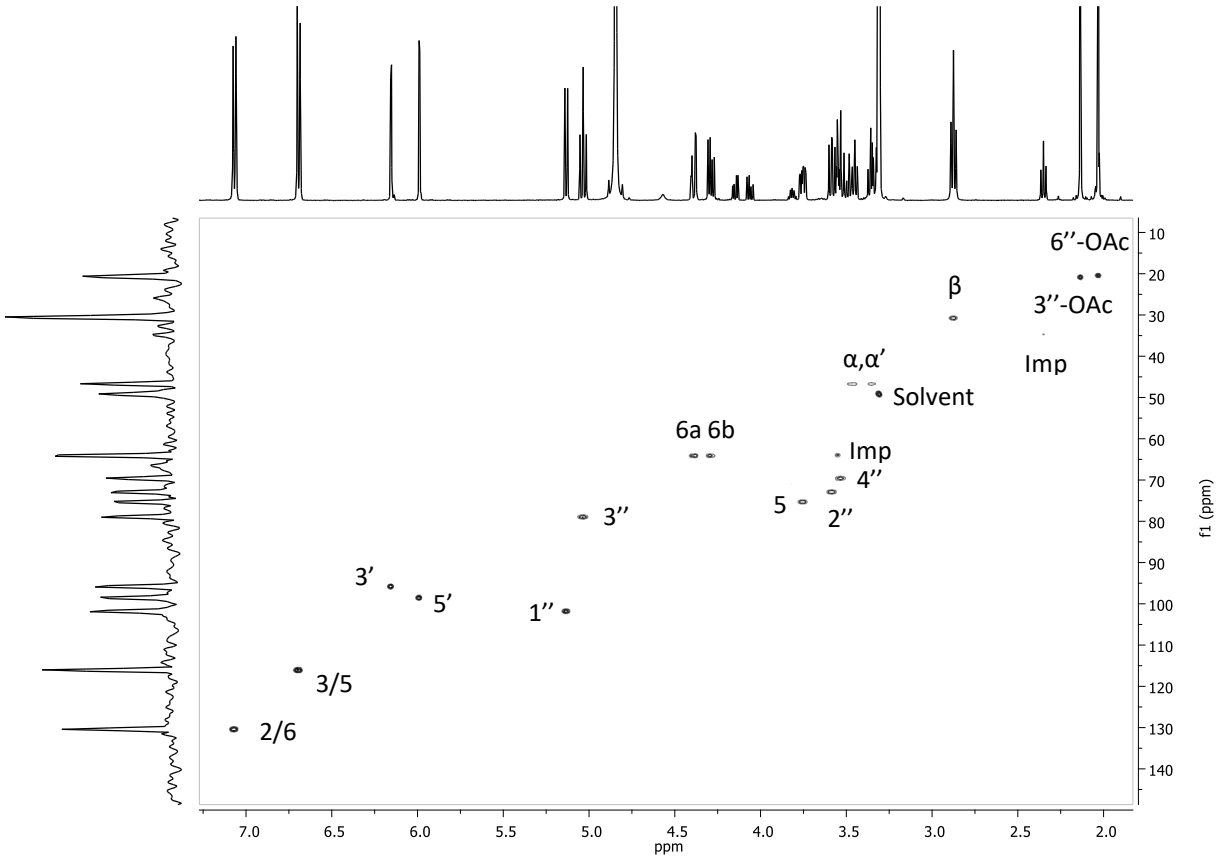


Figure S9. HSQC (500 MHz, CD_3OD) NMR spectrum of phloridzin-3'',6''-*O*-diacetate showing all assignments of protonated carbons.

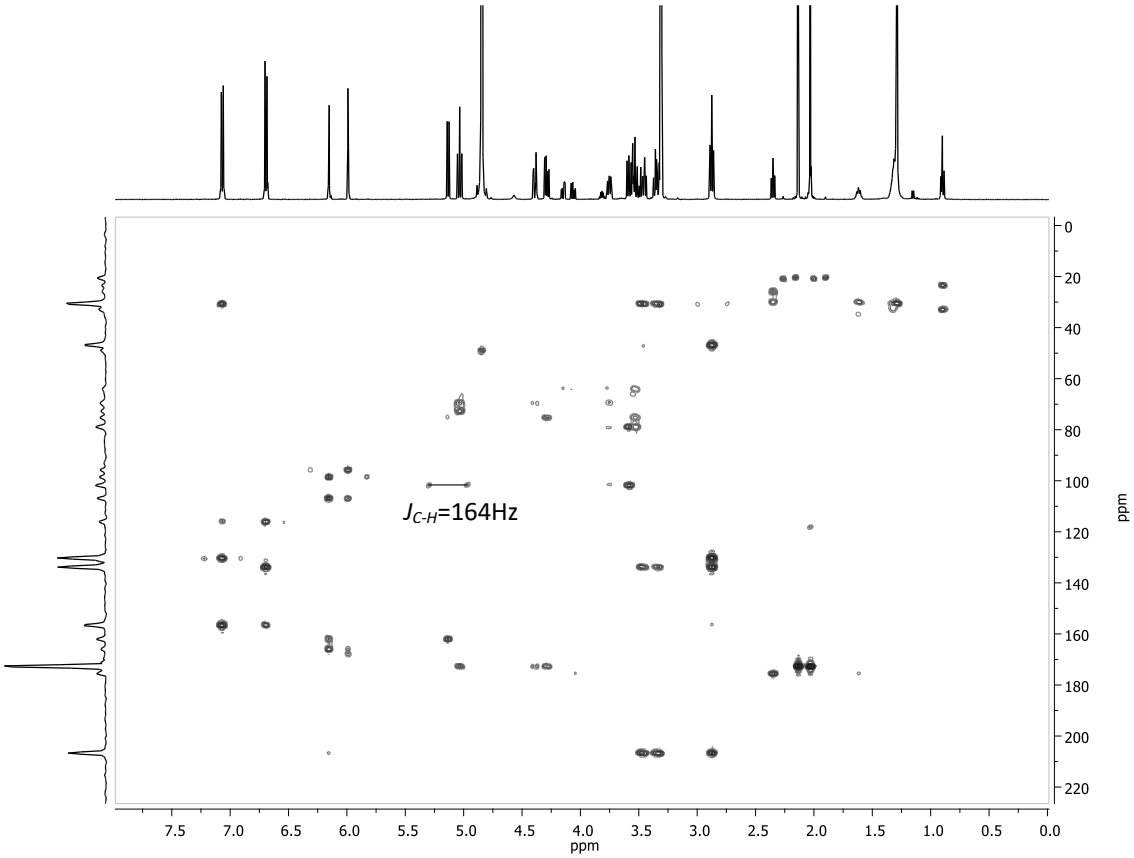


Figure S10. HMBC (500 MHz, CD_3OD) NMR spectrum of phloridzin-3’’,6’’-*O*-diacetate. Label is showing HSQC artefact of anomeric signal with coupling constant of 164 Hz between proton and carbon, characteristic for β-anomers.

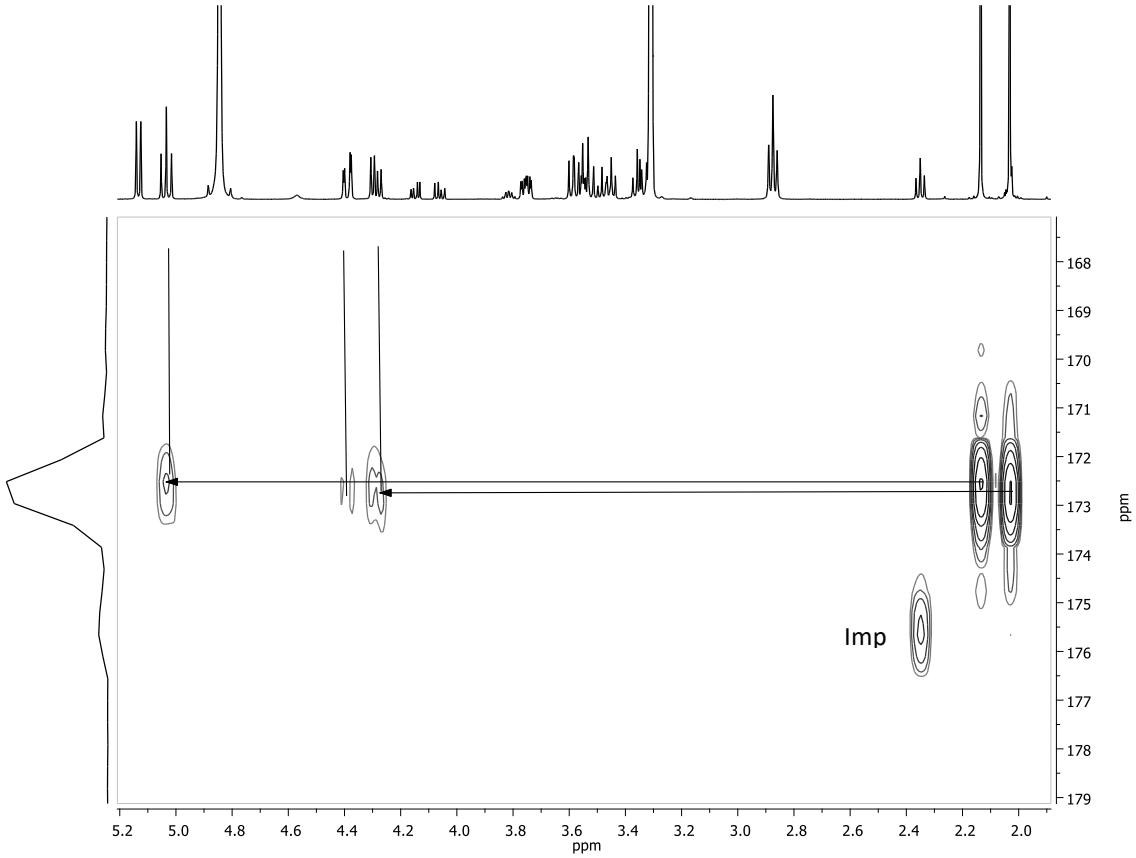


Figure S11. Partial HMBC (CD_3OD) NMR spectrum of phloridzin-3’’,6’’-*O*-diacetate showing the most important correlations between ester carbons and Glu H-3 and H-6 protons.

Table S1 Calculated values of coefficient of determination (R^2) for all variables in all experiment with model flavonoid used for kinetic modeling purposes.

	Initial concentration of phloridzin, mM			
	30	**50**	**75**	**100**
R^2 (Ph)	0.995	0.997	0.997	0.998
R^2 (PhAc)	0.978	0.983	0.987	0.994
R^2 (PhDAc)	0.997	0.998	0.998	0.998

*Ph, PhAc, PhDAc refer to phloridzin, phloridzin acetate andphloridzin diacetate, respectively.