Supporting Information

Enzymatic Synthesis of Phloretin α-Glucosides Using a Sucrose Phosphorylase Mutant and its Effect on Solubility, Antioxidant Properties and Skin Absorption

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Supplementary Information

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Figure S1 - ESI-MS Glc-Phloretin-1
Figure S2 - ESI-MS Glc-Phloretin-2
Figure S3-A - 1H NMR spectrum of Glc-Phloretin-1. Two products were identified in the mixture: 4’-O-Glc-Phlo (blue dots) and the minor 4-O-Glc-Phlo (green dots)
Figure S3-B - a) 1H-NMR spectra of the mixture of compounds; b) 1D selective-NOESY from the anomeric proton of minor product; c) 1D selective-NOESY from the anomeric proton of major product
Figure S4 - a) 1H-NMR spectra of the fraction Glc-Phloretin-2; b) 1D-Selective TOCSY from the anomeric proton H1’’ of major product; c) 1D-Selective-ROESY from the anomeric proton H1’’ of major product; d) 1D-Selective TOCSY from the anomeric proton H1’’’ of major product; e) 1D-Selective-ROESY from the anomeric proton H1’’’ of major product.
Figure S5. 1H-13C HSQC-edited spectrum for the Glc-Phloretin-2 sample. The 1D-selective TOCSYs from H1’’ (red) and H1’’’ (green) are also superimposed. The 13C chemical shift associated to H3’’ (highlighted in the figure) indicates that the corresponding C3’’ is glycosylated.
Figure S6 - Energy profile of the solutions of p-derivative
Figure S7 - *In vitro* percutaneous absorption scheme
Figure S8 - Skin layers scheme

Figure S1. ESI-MS Glc-Phloretin-1

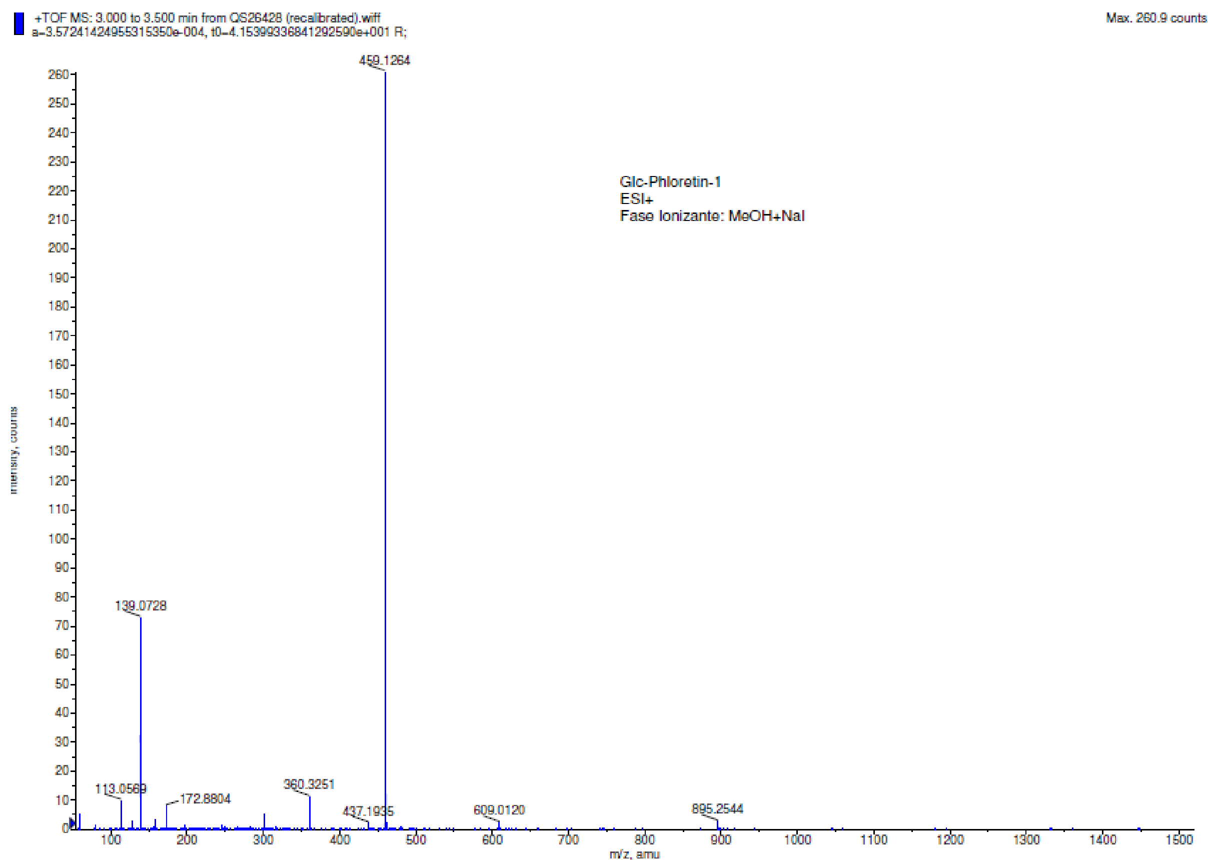


Figure S2. ESI-MS Glc-Phloretin-2

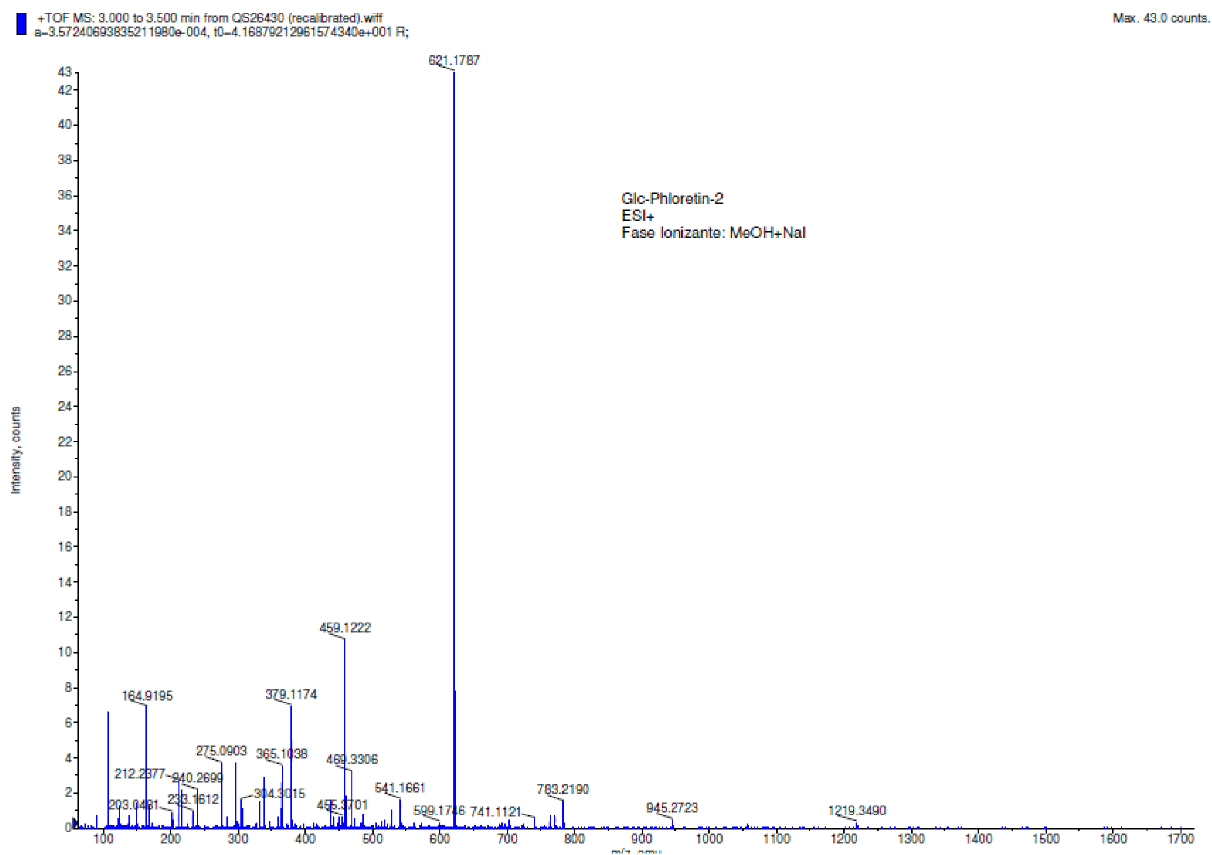


Figure S3-A. ^{1}H NMR spectrum of Glc-Phloretin-1. Two products were identified in the mixture: 4'-O-Glc-Phlo (blue dots) and the minor 4-O-Glc-Phlo (green dots). The key correlations to identify the structures from NOESY and HMBC spectra are marked on each structure.

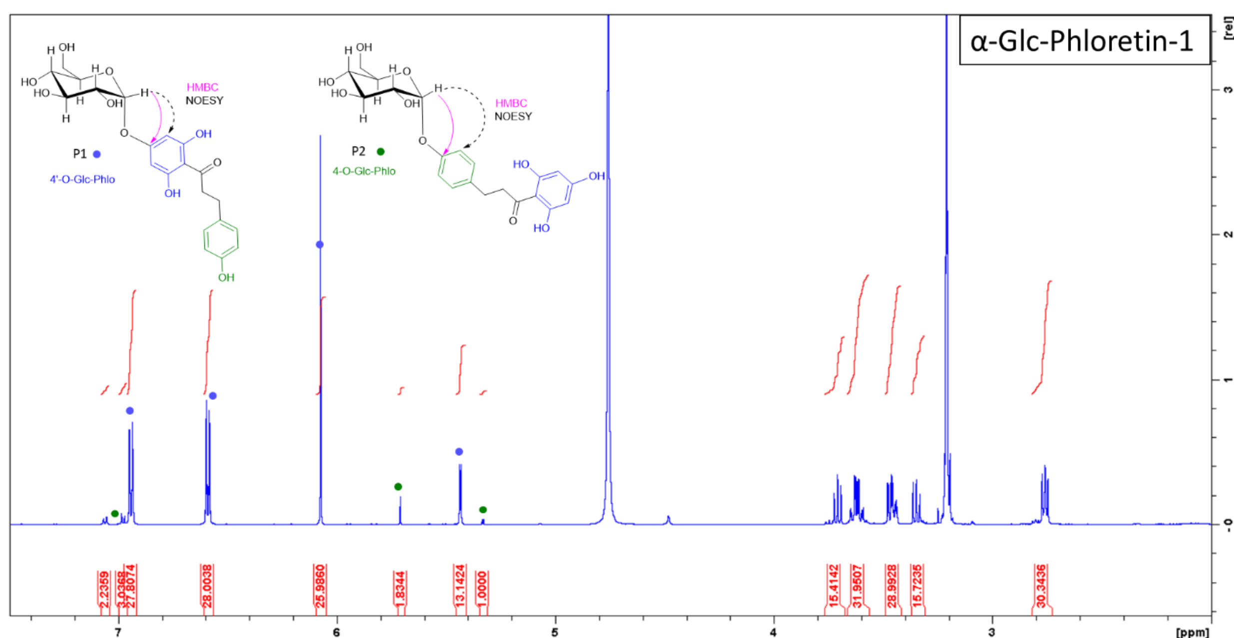


Figure S3-B: a) ^{1}H-NMR spectra of the mixture of compounds; b) 1D selective-NOESY from the anomeric proton of minor product; c) 1D selective-NOESY from the anomeric proton of major product.

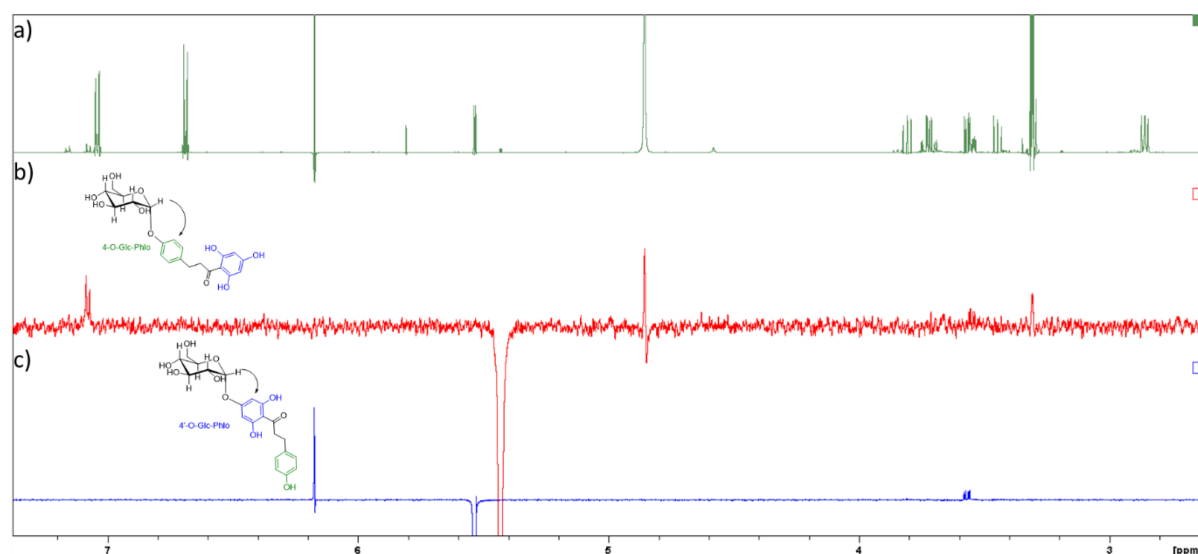


Figure S4. a) ^{1}H-NMR spectra of the fraction Glc-Phloretin-2; b) 1D-Selective TOCSY from the anomeric proton H1'' of major product; c) 1D-Selective-ROESY from the anomeric proton H1'' of major product; d) 1D-Selective TOCSY from the anomeric proton H1''' of major product; e) 1D-Selective-ROESY from the anomeric proton H1''' of major product.

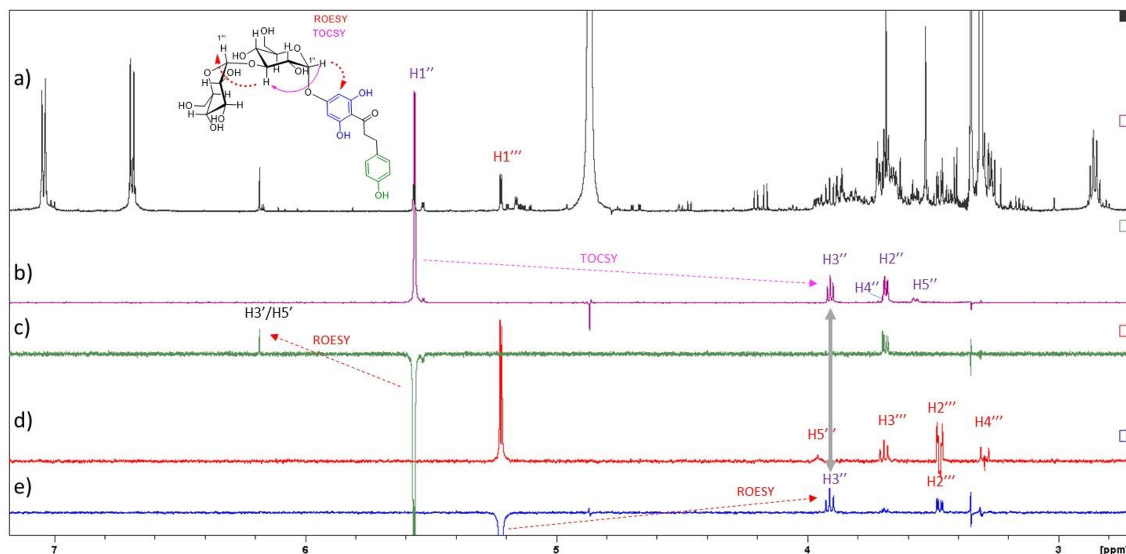


Figure S5. ^{1}H-^{13}C HSQC-edited spectrum for the Glc-Phloretin-2 sample. The 1D-selective TOCSYs from H1'' (red) and H1''' (green) are also superimposed. The ^{13}C chemical shift associated to H3'' (highlighted in the figure) indicates that the corresponding C3'' is glycosylated.

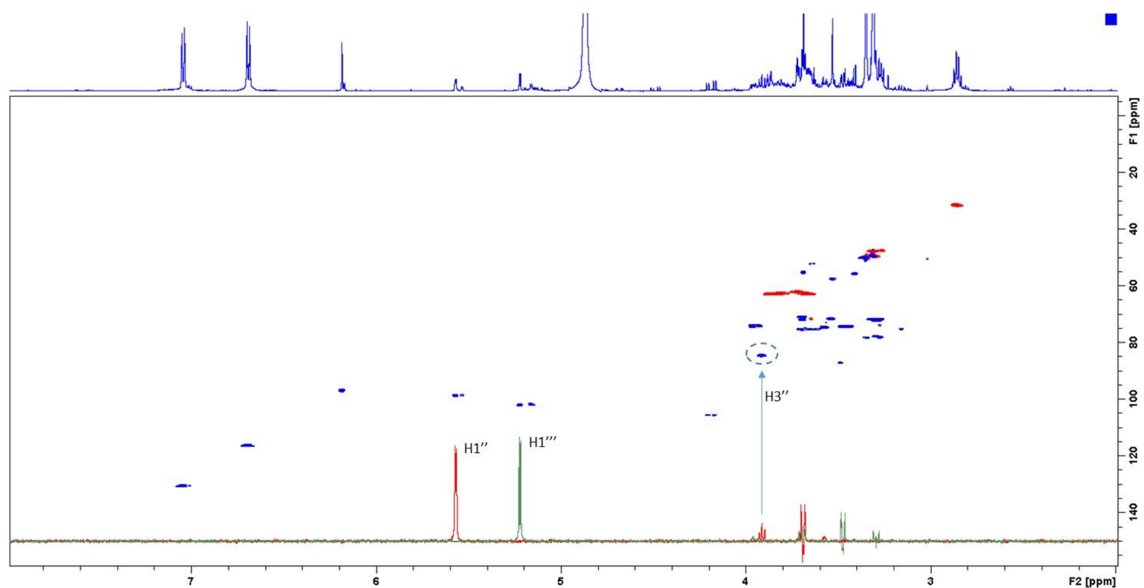
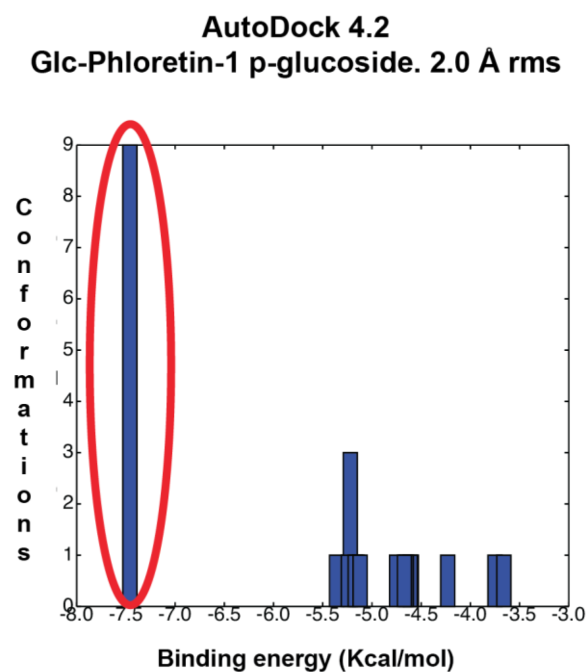
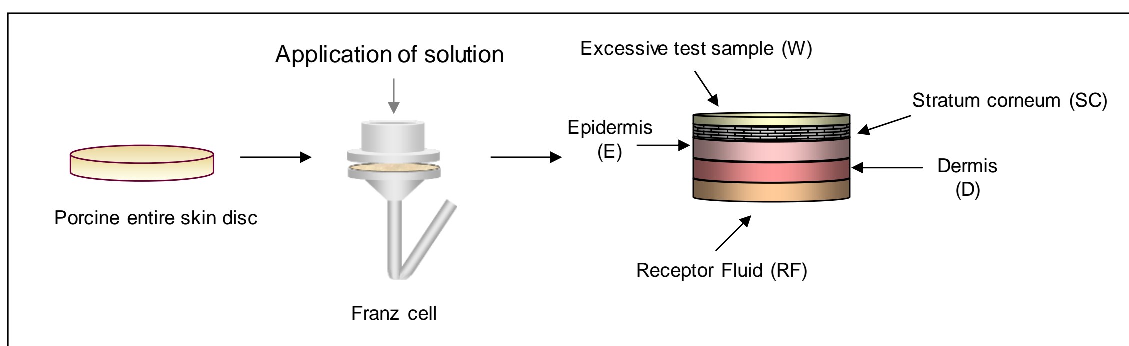


Figure S6. Energy profile for the solutions of *p*-derivative.



Cluster analysis and energy profile of the solutions obtained by a first run of modeling the binding of the p-derivative with AutoDock4.2

Figure S7. *In vitro* percutaneous absorption scheme.



The in vitro studies were carried out with pig skin on Franz static diffusion cells. The penetration cells were placed in a thermostated water bath. A sample of each tested solution was applied on the skin. At the end of the period of exposure (24 h), the skin surface was washed by a specific procedure to remove the excessive test sample. Then, the receptor fluid was recovered. Moreover, 8 strippings were carried out on the surface horny layers of stratum corneum with adhesive tapes (D-Squame, Cuderm Corporation, Dallas, USA) applied under controlled pressure. The epidermis was separated from the dermis. The different samples analyzed (receptor fluids, washing solutions, SC on tapes, epidermis and dermis) were extracted and/or diluted with an appropriate solvent.

Figure S8. Skin layers scheme.