Electronic Supplementary information

Pelargonidin-3-*O*-rutinoside as a Novel α-glucosidase Inhibitor for Improving Postprandial Hyperglycemia

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5. References

1. Experimental procedures

Materials and reagent Strawberry (*Fragaria × ananassa D.*), blueberry (*Vaccinium corymbosum L.*), mulberry (*Morus spp.*), cranberry (*Vaccinium macrocarpon*), bayberry (*Myrica rubra*) and raspberry (*Rubus hirsutus Thunb.*) were purchased from the local supermarket. α-glucosidase from saccharomyces cerevisiae was purchased from Sigma-Aldrich with a category number of G5003-100UN and the substrate pNPG (4-Nitrophenyl α-D-glucopyranoside) with a purity over 99% was purchased from the same company with a category number of N1377-1G. Acarbose with a purity over 98% was purchased from Yuan Ye Biotechnology. Other mentioned chemicals were in the grade of analytical purity.

Anthocyanin purification protocol 1000 g of fresh berry fruits or vegetables was extracted with 4L of 70% ethanol aqueous solution containing 0.1% HCL (v/v). The ethanol extract was centrifuged at 4000 rpm for 10 min and the supernatant was concentrated at 40°C. The collected concentrate was loaded on AB-8 macroporous resin column and then eluted with distilled water, 5%, 10% and 20% ethanol aqueous solution (v/v) in sequence. 10% and 20% ethanol eluates were collected, evaporated and then lyophilized. The lyophilized powder was dissolved in ethanol and recrystallized by ethyl acetate to yield anthocyanin extracts powder.

For separation of monomeric anthocyanins from anthocyanin extracts, a combination of semi-preparative HPLC and HSCCC technique was employed. Anthocyanin extracts was first purified by semi-preparative HPLC (Waters 600 system, Waters Corporation, Milford, MA) using a semi-preparative column (Unitary C18, 20.0 mm × 250 mm, 10 μm). The mobile phase consists of acetonitrile (solvent A) and 5% formic acid aqueous solution (solvent B). The elution process was performed as follows: 5% to 15% A for 15 min, 15% to 28% A for 6 min, 28% to 40% A for 1 min, 40% to 60% A for 2 min, 60% to 5% A for 3 min and 5% to 5% A for 3 min. The flow rate was 10 mL/min and the monitoring wavelength was 280 nm. The eluate was collected according to the peaks presented in HPLC chromatogram. After purification by semi-preparative HPLC, each collected eluate was lyophilized respectively and further purified by HSCCC (TBE-300C, Tauto Biotechnique Company, Shanghai, China) to obtain pure monomeric anthocyanins. The two-phase solvent system of n-butanol-methyl-tert-butyl ether-acetonitrile-water-trifluoroacetic (2:2:1:5:0.01, v/v) was used. The upper phase was treated as stationary phase, while the lower phase was considered as mobile phase. The apparatus was run at a revolution speed of 850 rpm and the flow rate of lower phase was set at 3 mL/min. After hydrodynamic equilibrium, 10 to 200 mg sample was dissolved in 15 mL lower phase and then injected into HSCCC to achieve separation. The UV detector was adjusted to 280 nm and the column temperature was maintained at 25 °C. The eluate was collected every 3 min based on the HSCCC chromatogram. The collected eluate was concentrated and analyzed by analytical HPLC. The targeted anthocyanins with a purities of at least 95% were collected and lyophilized for following structural characterization and biological evaluation.

Determination of anthocyanins Berry anthocyanin extracts or purified anthocyanins were determined by HPLC (Dionex ultimate 3000, ThermoFisher Scientific, USA) using Ultimate LP-C18 column (4.6 × 250 mm, 5 μm). The mobile phase consists of acetonitrile (solvent A) and 1.5% formic acid aqueous solution (solvent B), and the elution process was performed as follows: 5% to 15% A for 15 min, 15% to 28% A for 6 min, 28% to 40% A for 1 min, 40% to 60% A for 2 min, 60% to 5% A for 3 min and 5% to 5% A for 3 min.. The flow rate was 0.8 mL/min and the monitoring wavelength was 280 nm.

Structural characterization The structures of obtained monomeric anthocyanins were characterized by LC-MS/MS and NMR. ^{1}H and ^{13}C NMR analysis were performed on a Bruker AVANCE™ III spectrometer (14.1 Tesla), operating at Larmor frequencies of 600 MHz for ^{1}H and 150 MHz for ^{13}C. samples were dissolved in 0.5 mL of CD_3OD. The purities of all tested monomeric anthocyanins were at least 95% by HPLC analysis.

Assay for α-glucosidase inhibition α-glucosidase from was dissolved in 0.1M PBS solutions with a pH of 6.8, and diluted to be a 0.5 U/mL solution. The substrate pNPG was dissolved in PBS to be a 1 mM solution. Acarbose

was dissolved in DMSO and diluted to a series of concentrations from 0.1μM to 10 mM. Anthocyanins were dissolved in 0.1% hydrochloric acid/water and further diluted to a series of concentrations from 0.1 μM to 10 mM. *In vitro* α-glucosidase inhibitory assay was performed according to a previously described method with slight modification [1]. Briefly, 20 μL of 0.5 U/mL enzyme solution was first mixed with 10 μL of acarbose or anthocyanin solution, then 130 μL PBS solution was added, and the mixed solution was incubated in the water bath of 37 ℃ for 10 min. 40 μL of 1 mM substrate solution (pNPG) was subsequently added and further incubated in the water bath of 37 ℃ for 30 min, after which 200 μL of 1 M Na_2CO_3 solution was added to terminate the reaction. At last, the resulting reaction solution was centrifuged at 12000×*g* and the supernatant was analyzed by HPLC. The mobile phase consisted of acetonitrile (solvent A) and 1.5% formic acid aqueous solution (solvent B), and the elution process was performed as follows: 5% to 5% A for 2 min, 5% to 60% A for 6 min, 60% to 90% A for 2 min, 90% to 90% A for 2 min, 90% to 5% A for 2 min and 5% to 5% A for 1 min. The flow rate was 1 mL/min and the monitoring wavelength was 320 nm. The inhibitory rate was calculated using the following equation: Inhibitory rate% = (1-A_1/A_0) × 100%, in which A_1 is the content of generated 4-nitrobenzene in acarbose or anthocyanins group, and A_0 is that in negative control group.

Enzymatic kinetics of α-glucosidase The mode of α-glucosidase inhibition by inhibitors (Pg3R, Pg3G, M3A and acarbose) were evaluated using Lineweaver-Burk equation. pNPG with a concentration range of 40–1000 μM and α-glucosidase were incubated with different concentrations of inhibitor for 10 min, respectively. 20 μL of 0.5 U/mL enzyme solutions were first mixed with 10 μL of inhibitors or PBS solutions, then 130 μL PBS solutions were added, and the mixed solutions were incubated in the water bath of 37 ℃ for 10 min. At last, 40 μL of pNPG solutions (40, 100, 200, 400 and 1000 mM) were added, and the mixed solutions were further incubated in the water bath of 37 ℃ for 10 min, The absorbance of reaction solution was measured at 410 nm every minute.

Fluorescence quenching analysis The intrinsic fluorescence spectra (300-500 nm) were measured using cary eclipse spectrophotometer (Varian, USA). α-glucosidase (6 μM) was titrated with different concentrations of Pg3R (0-30 μM) at 291K, 303K, and 310 K, respectively. After equilibration for 5 min, the fluorescence spectra of mixed solutions were determined at an excitation wavelength of 278 nm. Both the excitation and emission bandwidths were set at 1 nm. The thermodynamic parameters (ΔH and ΔS), quenching rate constant (K_q), binding constant (K_a) and the number of the binding sites (n) were calculated according to the Stern-Volmer equation [2], which were listed as follows:

$$F_0/F=1+K_{sv}[Q]=1+K_q\ \tau_0[Q] \quad (1)$$

$$log\frac{F_0-F}{F}=logK_a+nlogQ \quad (2)$$

$$InK_a=-\frac{1}{T}\left(\frac{\Delta H}{R}\right)+\frac{\Delta S}{R} \quad (3)$$

Where F_0 and F represent the fluorescence intensities in the presence or absence of Pg3R, [Q] denote the concentration of Pg3R, τ_0 is the constant of the lifetime of fluorophore (10^{-8} s) and R is the gas constant of 8.31 J/(mol×K).

Circular Dichroism (CD) spectroscopy The CD measurements were performed using a circular dichroism spectrometer (J-1500-150ST, Jasco Corp, Tokyo, Japan) in a wavelength range of 190–250 nm at a speed of 60 nm/min. All measurements were carried out at 298 K using 1.0 mm path length quartz cuvette and sodium phosphate buffer (pH 6.8) was considered as a blank. The concentration of α-glucosidase was 1.4 μM, whereas the molar ratios of inhibitors (50 μM and 100 μM) to α-glucosidase were 36:1 and 72:1. All the results were expressed as ellipticity in mill degrees, and the percentage of each secondary structure (α-helix, β-sheet, β-turn and random coil) was calculated by Jasco Protein Secondary Structure Estimation Program (Jasco Corp, Tokyo, Japan) [3] based on the CD spectra of reference proteins [4].

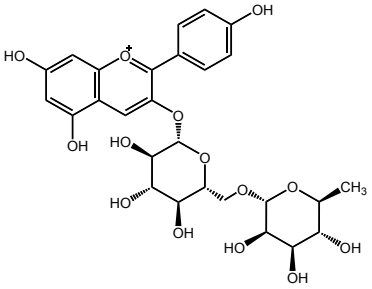
Molecular docking Based on previous report[5], the software Modeller (version 9.19) was selected to carry out the homology modelling, using the amino acid sequence of MAL12 and the structures of oligo-1,6-glucosidase (PDB ID: 1UOK) and GSJ (GH13 α-glucosidase, PDB ID: 2ZE0). EasyModeller (version 4.0) was used as the GUI (Graphical User Interface) of Modeller 9 models were generated, with the loop structures of each model automatically defined, and the one with the lowest DOPE (Discrete Optimized Protein Energy) score was selected for further optimization using Chiron server (http://redshift.med.unc.edu/chiron/index.php) to minimize clashes. The quality of optimized model was verified using Ramachandran Plot analysis and Verify_3D assessment. The verified model was then prepared using MGLTools (version 1.5.6), in which the center of a grid box with the size of 22.5 Å×22.5 Å×22.5 Å was determined using the conformational pose of α-D-glucose in its co-crystalized structure with isomaltase from saccharomyces cerevisiae (PDB ID: 3A4A). The 3D structures of anthocyanins were generated and then energetically minimized with MM2 force field to a minimum RMS (Root Mean Square) gradient of 0.005 using Chembio3D Ultra 2010 (Version 12.0) and prepared using MGLTools. The map files of prepared MAL12 and anthocyanins files were generated using Autogrid (version 4.2.6). Then the molecular docking was carried out with Autodock (version 4.2.6)[6, 7], using defined Lamarckian GA (Genetic Algorithm). The docked pose with the lowest predicted binding energy was selected as the proposed binding conformation for each anthocyanin and analyzed using PyMOL (The PyMOL Molecular Graphics System, Version 2.0.6 Schrödinger, LLC) and Discovery Studio 2017R2 (Dassault Systèmes BIOVIA, Discovery Studio Client, v17.2.0, San Diego: Dassault Systèmes, 2016).

Experimental animals SPF grade male ICR mice, six weeks old, weighting 20-25 g, were obtained from the SLAC Company Limited (Shanghai, China). The animals were housed in Experimental Animal Center of Zhejiang Chinese Medical University under 12 h light-dark cycle at controlled temperature (22 ± 1 ℃), and provided with a standard pellet diet and water ad libitum. The mice were adapt to diet and general conditions of vivarium for 1 week before the experiment. All animal experiments were performed in compliance with the relevant laws and institutional guidelines for the care and use of laboratory animals in China (GB/T 35892-2018 and GB/T 35823-2018). The experiments were also approved by the laboratory animal management and ethics committee of Zhejiang Chinese Medical University, and the animal ethical approval number is IACUC-20180425-01.

Oral disaccharide (sucrose and maltose) tolerance test Normal ICR mice were divided into five groups of nine animals each. Sucrose or maltose, as well as the inhibitors (Pg3R and acarbose), was dissolved in sterile water. Pg3R was tested at dose of 50 mg/kg and 150 mg/kg, whereas acarbose was evaluated at dose of 25 mg/kg and 50 mg/kg. All doses were in mg/kg of body weight. Mice were fasted 16 h and then administrated orally Pg3R or acarbose *via* a stomach tube, 15 min later, 2 g/kg of sucrose or 2 g/kg of maltose solution was given to the animals. Blood samples were obtained from the tail vein at 0, 15, 30, 60, and 120 min after sucrose or maltose loading and blood glucose was measured with Accu-Chek Performa glucometer. Area under the curve (AUC) over a period of 120 min was calculated based on the trapezoidal method.

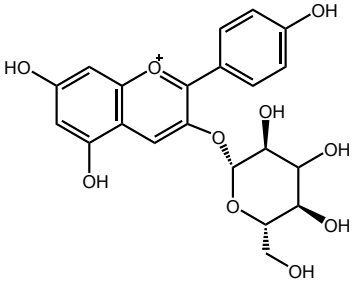
2. Structural characterization

Compound 1, Pelargonidin-3-*O*-rutinoside (Pg3R), $C_{27}H_{31}O_{14}^{+}$



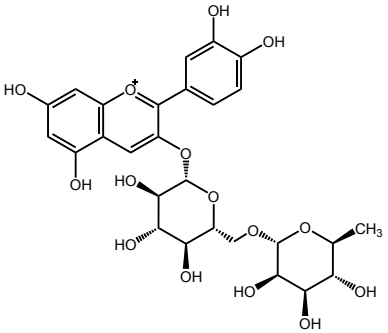
^{1}H NMR (CD_3OD, 600 MHz): δ 8.80 (s,1H), 8.41 (d, *J*=9.0 Hz, 2H), 6.87 (d, *J*=9.0 Hz, 2H), 6.75 (s, 1H), 6.53 (s, 1H), 5.13 (d, *J*=7.8 Hz, 1H), 4.54 (s, 1H), 3.94 (d, *J*=10.2 Hz, 1H), 3.69-3.70 (m, 1H), 3.58-3.60 (m, 1H), 3.41-3.51 (m, 5H), 3.27-3.30 (m, 1H), 3.19-3.22 (m, 1H), 1.04 (d, *J*=6.6 Hz, 3H). **^{13}C NMR** (150 MHz, CD_3OD) δ 170.7, 166.6, 164.1, 159.1, 157.7, 145.4, 136.8, 135.7, 120.7, 117.9, 113.4, 103.6, 103.5, 102.1, 95.3, 78.0, 77.4, 74.7, 73.8, 72.4, 71.8, 71.2, 69.7, 67.8, 17.9. **ESI-LC/MS/MS**, [M^+] 579.0 (MS), 270.8 (MS/MS), **Retention time**, 21.297 min **Purity**, 97.76% (HPLC).

Compound 2, Pelargonidin-3-*O*-glucoside (Pg3G), $C_{21}H_{21}O_{10}^{+}$



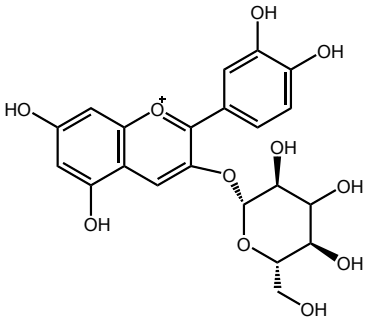
^{1}H NMR (CD_3OD, 600 MHz): δ 8.70 (s,1H), 8.24 (d, *J*=9.0 Hz, 2H), 6.71 (d, *J*=9.0 Hz, 2H), 6.60 (s, 1H), 6.36 (s, 1H), 5.05 (d, *J*=7.8 Hz, 1H), 3.70-3.72 (m, 1H), 3.50-3.53 (dd, *J*=12 Hz, 6.0Hz, 1H), 3.39-3.42 (m, 1H), 3.32-3.37 (m, 2H), 3.21-3.24 (m, 1H). **^{13}C NMR** (150 MHz, CD_3OD) 170.5, 166.5, 163.8, 159.2, 157.5, 145.3, 137.0, 135.6, 120.6, 117.8, 113.3, 103.6, 103.3, 95.2, 78.7, 78.1, 74.7, 71.0, 62.3. **ESI-LC/MS/MS**, [M^+] 433.0 (MS), 270.9 (MS/MS), **Retention time**, 21.170 min, **Purity**, 96.34% (HPLC).

Compound 3, Cyanidin-3-*O*-rutinoside (C3R), $C_{27}H_{31}O_{15}^{+}$



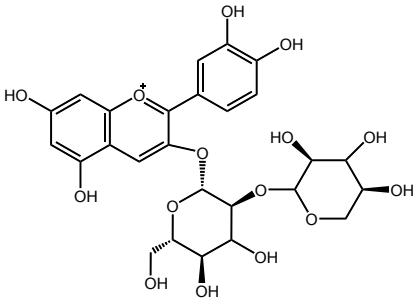
^{1}H NMR (CD_3OD, 600 MHz): δ 8.83 (s,1H), 8.17-8.19 (dd, *J*=9.0 Hz, 2.4 Hz, 1H), 7.91 (d, *J*=1.8 Hz, 1H), 6.92 (d, *J*=9.0 Hz, 1H), 6.81 (d, *J*=1.2 Hz, 1H), 6.60 (d, *J*=1.8 Hz ,1H), 5.22 (d, J=7.8Hz, 1H), 4.59 (d, *J*=1.2 Hz, 1H), 4.00-4.02 (m, 1H), 3.74-3.75 (m, 1H), 3.65-3.68 (m, 1H), 3.48-3.61 (m, 5H), 3.33-3.37 (m, 1H), 3.25-3.28 (m, 1H), 1.10 (d, *J*=6.0 Hz, 3H). **^{13}C NMR** (151 MHz, CD_3OD) δ 170.4, 163.9, 159.0, 157.5, 155.8, 147.4, 145.6, 135.9, 128.4, 121.1, 118.2, 117.4, 113.2, 103.4, 103.4, 102.1, 95.2, 78.0, 77.4, 74.6, 73.9, 72.4, 71.8, 71.2, 69.7, 67.7, 17.9. **ESI-LC/MS/MS**, [M^+] 595.2 (MS), 287.0 (MS/MS), **Retention time**, 19.373 min, **Purity**, 98.33% (HPLC).

Compound 4, Cyanidin-3-*O*-glucoside (C3G), $C_{21}H_{21}O_{11}^+$



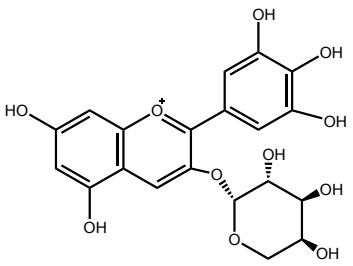
^{1}H NMR (CD_3OD, 600 MHz): δ 8.95 (s,1H), 8.19 (dd, *J*=9.0 Hz, 2.4 Hz, 1H), 7.96 (d, *J*=2.4 Hz, 1H), 6.95 (d, *J*=9.0 Hz, 1H), 6.83 (d, *J*=1.8 Hz, 1H), 6.61 (d, *J*=1.8 Hz, 1H), 5.27 (d, *J*=7.8 Hz, 1H), 3.90-3.92 (m, 1H), 3.70-3.73 (m, 1H), 3.64-3.67 (m, 1H), 3.52-3.57 (m, 2H), 3.42-3.45 (m, 1H). **^{13}C NMR** (150 MHz, CD_3OD) δ 170.4, 164.0, 159.2, 157.5, 155.8, 147.4, 145.6, 136.7, 128.3, 121.2, 118.3, 117.4, 113.3, 103.7, 103.3, 95.1, 78.8, 78.1, 74.8, 71.1, 62.4. **ESI-LC/MS/MS**, [M^+] 448.9 (MS), 286.9 (MS/MS), **Retention time**, 18.900 min, **Purity**, 98.27% (HPLC).

Compound 5, Cyanidin-3-*O*-sambubinoside (C3S), $C_{26}H_{29}O_{15}^+$



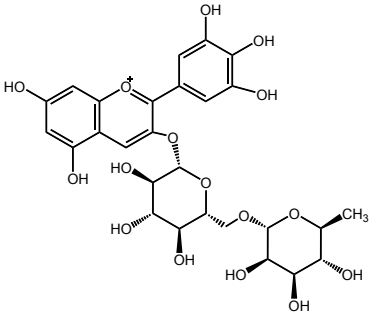
^{1}H NMR (CD_3OD, 600 MHz): δ 8.80 (s,1H), 8.15-8.17 (dd, *J*=8.4 Hz, 1.8 Hz, 1H), 7.88 (d, *J*=1.8 Hz, 1H), 6.87 (d, *J*=9.0 Hz, 1H), 6.77 (s, 1H), 6.53 (s, 1H), 5.36 (d, *J*=7.2 Hz, 1H), 4.67(d, *J*=7.8 Hz, 1H), 3.85-3.88 (m, 1H), 3.81-3.83 (m, 1H), 3.67-3.70 (m, 1H), 3.59-3.64 (m, 3H), 3.49-3.52 (m, 1H), 3.37-3.40 (m, 1H), 3.29-3.32 (m, 1H), 3.05-3.08 (m, 1H), 2.95-2.99 (m, 1H). **^{13}C NMR** (150 MHz, CD_3OD) δ 170.2, 163.9, 159.1, 157.4, 155.8, 147.4, 145.3, 135.9, 128.8, 121.1, 118.3, 117.3, 113.1, 105.6, 103.2, 101.3, 95.0, 81.5, 78.7, 78.2, 77.8, 75.7, 70.9, 70.7, 67.2, 62.2. **ESI-LC/MS/MS**, [M^+] 580.9 (MS), 286.7 (MS/MS), **Retention time**, 18.500 min, **Purity**, 99.45% (HPLC).

Compound 6, Delphinidin-3-*O*-arabinoside (D3A), $C_{20}H_{19}O_{11}^+$



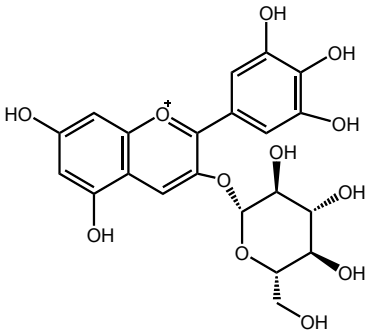
^{1}H NMR (CD_3OD, 600 MHz): δ 8.74 (s,1H), 7.65 (s, 2H), 6.75 (s, 1H), 6.54 (s, 1H), 5.21 (d, *J*=6.6 Hz, 1H), 3.93-4.00 (m, 3H), 3.72-3.76 (m, 2H). **^{13}C NMR** (150 MHz, CD_3OD) δ 170.2, 163.9, 158.9, 157.4, 147.4, 145.6, 144.7, 135.6, 119.8, 113.0, 112.5, 104.2, 103.2, 95.0, 73.8, 72.0, 69.1, 67.3. **ESI-LC/MS/MS**, [M^+] 434.9 (MS), 302.9 (MS/MS), **Retention time**, 17.243 min, **Purity**, 97.25% (HPLC).

Compound 7, Delphinidin-3-*O*-rutinoside (D3R), $C_{27}H_{31}O_{16}^{+}$



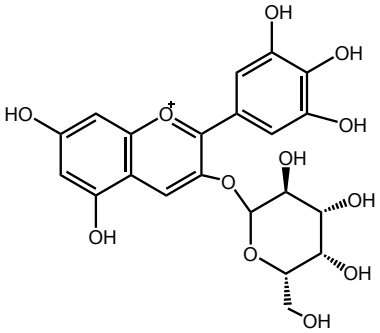
^{1}H NMR (CD_3OD, 600 MHz): δ 8.60 (s,1H), 7.46 (s, 2H), 6.63 (s, 1H), 6.47 (s, 1H), 5.17 (d, *J*=7.8 Hz, 1H), 4.54 (s, 1H), 3.95 (d, *J*=10.8 Hz, 1H), 3.69-3.70 (m, 1H), 3.59-3.62 (m, 1H), 3.42-3.57 (m, 5H), 3.29-3.32 (m, 1H), 3.18-3.21 (m, 1H), 1.02 (d, *J*=6 Hz, 3H). **^{13}C NMR** (150 MHz, CD_3OD) δ 170.2, 162.9, 158.8, 157.1, 147.2, 145.5, 144.6, 134.7, 119.7, 112.9, 112.4, 103.4, 102.9, 102.1, 95.2, 77.8, 77.3, 74.5, 73.8, 72.3, 71.7, 71.1, 69.7, 67.6, 17.8. **ESI-LC/MS/MS**, [M^+] 611.0 (MS), 302.8 (MS/MS), **Retention time**, 17.863 min, **Purity**, 96.84% (HPLC).

Compound 8, Delphinidin-3-*O*-glucoside (D3G), $C_{21}H_{21}O_{12}^{+}$



^{1}H NMR (CD_3OD, 600 MHz): δ 8.81 (s,1H), 7.61 (s, 2H), 6.73 (s, 1H), 6.53 (d, *J*=4.8 Hz, 1H), 5.25 (d, *J*=7.2 Hz, 1H), 3.85-3.87 (m, 1H), 3.67-3.69 (m, 1H), 3.63-3.66 (m, 1H), 3.49-3.52 (m, 2H), 3.38-3.42 (m, 1H). **^{13}C NMR** (150 MHz, CD_3OD) δ 170.2, 163.5, 159.1, 157.4, 147.4, 145.7, 144.7, 135.7, 119.8, 113.1, 112.4, 103.4, 103.2, 95.0, 78.7, 78.0, 74.7, 71.0, 62.3. **ESI-LC/MS/MS**, [M^+] 464.9 (MS), 302.8 (MS/MS), **Retention time**, 16.697 min, **Purity**, 95.64% (HPLC).

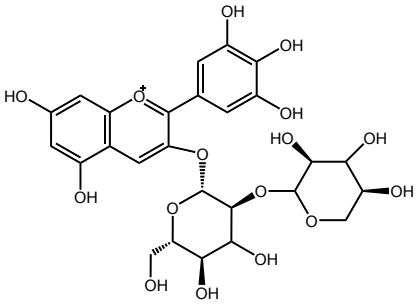
Compound 9, Delphinidin-3-*O*-galactoside (D3Ga), $C_{21}H_{21}O_{12}^{+}$



^{1}H NMR (CD_3OD, 400 MHz): δ 8.81 (s,1H), 7.62 (s, 2H), 6.73 (s, 1H), 6.53 (d, *J*=1.6 Hz, 1H), 5.21 (d, *J*=7.6 Hz, 1H), 3.94-3.98 (m, 1H), 3.91 (d, *J*=3.2 Hz, 1H), 3.70-3.78 (m, 3H), 3.64-3.67 (dd, *J*=9.6, 3.2 Hz, 1H). **^{13}C NMR** (100 MHz, CD_3OD) δ 170.2, 163.7, 159.1, 157.4, 147.4, 145.8, 144.6, 136.0, 119.8, 113.1, 112.4, 104.4, 103.2, 95.0, 77.8, 74.8, 72.1, 70.1, 62.4. **ESI-LC/MS/MS**, [M^+] 464.8 (MS), 302.9 (MS/MS), **Retention time**, 15.587 min,

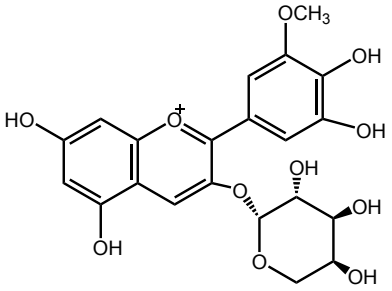
Purity, 96.04% (HPLC).

Compound 10, Delphinidin-3-*O*-sambubinoside (D3S), $C_{26}H_{29}O_{16}^{+}$



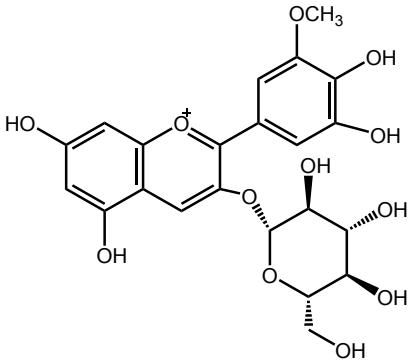
^{1}H NMR (CD_3OD, 600 MHz): δ 8.82 (s,1H), 7.70 (s, 2H), 6.80 (d, *J*=1.2 Hz, 1H), 6.58 (d, *J*=1.8 Hz, 1H), 5.42 (d, *J*=7.8 Hz, 1H), 4.62(d, *J*=7.2 Hz, 1H), 3.92-3.95 (m, 1H), 3.84-3.86(m, 1H), 3.65-3.73 (m, 3H), 3.50-3.53 (m, 2H), 3.43-3.46 (m, 1H), 3.20-3.23 (m, 1H), 3.08-3.11 (m, 1H), 2.88-2.92 (m, 1H). **^{13}C NMR** (150 MHz, CD_3OD) 170.1, 164.1, 159.1, 157.4, 147.5, 145.5, 144.7, 135.2, 120.0, 113.0, 112.7, 106.1, 103.2, 101.5, 94.9, 82.5, 78.6, 77.9, 77.7, 75.8, 70.8, 70.6, 67.0, 62.2. **ESI-LC/MS/MS**, [M^+] 596.9 (MS), 302.9 (MS/MS), **Retention time**, 15.847 min, **Purity**, 97.70% (HPLC).

Compound 11, Petunidin-3-*O*-arabinoside (Pet3A), $C_{21}H_{21}O_{11}^{+}$



^{1}H NMR (CD_3OD, 600 MHz): δ 8.86 (s,1H), 7.93 (d, *J*=1.8 Hz, 1H), 7.73 (d, *J*=1.2 Hz, 1H), 6.84 (s, 1H), 6.59 (s, 1H), 5.22 (d, *J*=6.6 Hz, 1H), 3.96-3.99 (m, 2H), 3.95 (s, 3H), 3.90 (s, 1H), 3.75-3.76 (m, 1H), 3.69-3.71 (m, 1H). **^{13}C NMR** (150 MHz, CD_3OD) 170.5, 164.2, 159.1, 157.7, 149.8, 147.5, 145.7, 145.3, 136.4, 119.9, 113.7, 113.3, 109.4, 104.4, 103.3, 95.2, 74.0, 72.1, 69.1, 67.3, 57.2. **ESI-LC/MS/MS**, [M^+] 449.0 (MS), 316.8 (MS/MS), **Retention time**, 21.253 min, **Purity**, 95.16% (HPLC).

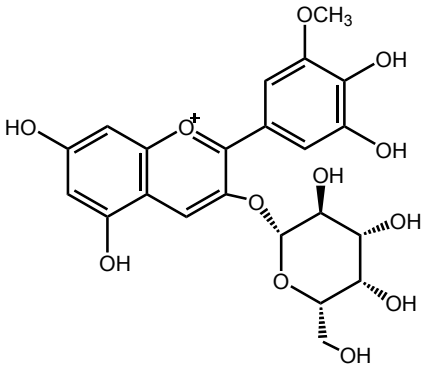
Compound 12, Petunidin-3-*O*-glucoside (Pet3G), $C_{22}H_{23}O_{12}^{+}$



^{1}H NMR (CD_3OD, 600 MHz): δ 8.92 (s,1H), 7.90 (s, 1H), 7.70 (s, 1H), 6.84 (s, 1H), 6.59 (s, 1H), 5.29 (d, *J*=7.8 Hz, 1H), 3.93 (s, 3H), 3.87 (d, *J*=12 Hz, 1H), 3.60-3.67 (m, 2H), 3.49-3.54 (m, 2H), 3.36-3.39 (m, 1H). **^{13}C NMR**

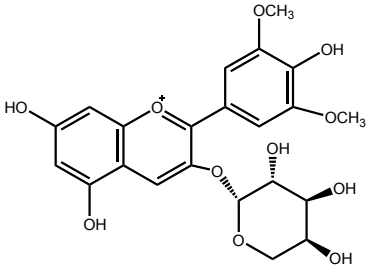
(150 MHz, CD_3OD) δ 170.4, 163.9, 159.2, 157.7, 149.7, 147.4, 145.8, 145.1, 136.4, 119.9, 113.6, 113.4, 109.2, 103.6, 103.2, 95.1, 78.8, 78.2, 74.9, 71.1, 62.3, 57.1. **ESI-LC/MS/MS**, [M^+] 479.0 (MS), 316.8 (MS/MS), **Retention time**, 19.970 min, **Purity**, 98.33% (HPLC).

Compound 13, Petunidin-3-*O*-galactoside (Pet3Ga), $C_{22}H_{23}O_{12}^+$



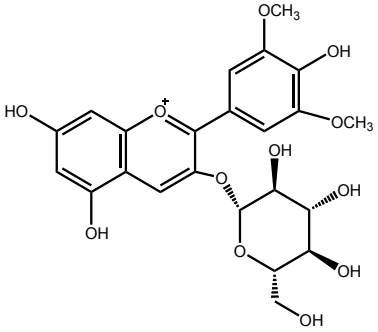
^{1}H NMR (CD_3OD, 600 MHz): δ 8.84 (s,1H), 7.86 (d, *J*=6.0 Hz, 1H), 7.60 (d, *J*=2.4 Hz, 1H), 6.78 (d, *J*=1.2 Hz, 1H), 6.54 (d, *J*=2.4 Hz, 1H), 5.26 (d, *J*=7.8 Hz, 1H), 3.93-3.96 (dd, *J*=9.6Hz, 7.8 Hz, 1H), 3.91 (s, 1H), 3.90 (s, 3H), 3.73-3.78 (m, 3H), 3.65-3.67 (m, 1H). **^{13}C NMR** (150 MHz, CD_3OD) δ 170.3, 163.4, 159.1, 157.4, 149.6, 147.3, 145.7, 145.1, 136.2, 119.7, 113.3, 113.2, 109.3, 104.2, 103.2, 95.1, 77.8, 75.0, 72.2, 70.1, 62.4, 57.1. **ESI-LC/MS/MS**, [M^+] 479.0 (MS), 316.9 (MS/MS), **Retention time**, 19.060 min, **Purity**, 98.73% (HPLC).

Compound 14, Malvidin-3-*O*-arabinoside (M3A), $C_{22}H_{23}O_{11}^+$



^{1}H NMR (CD_3OD, 600 MHz): δ 8.86 (s,1H), 7.91 (s, 2H), 6.89 (d, *J*=1.2 Hz, 1H), 6.58 (d, *J*=1.8 Hz, 1H), 5.21 (d, *J*=6.6 Hz, 1H), 3.95 (s, 6H), 3.91 (s, 2H), 3.75-3.76 (d, *J*=1.8 Hz, 1H), 3.73-3.74 (d, *J*=1.8 Hz, 1H), 3.68-3.70 (m, 1H). **^{13}C NMR** (150 MHz, CD_3OD) δ 170.7, 163.9, 159.1, 157.7, 149.7, 146.2, 145.6, 136.7, 119.7, 113.4, 110.5, 104.5, 103.3, 95.4, 74.1, 72.2, 69.2, 67.5, 57.3. **ESI-LC/MS/MS**, [M^+] 462.9 (MS), 330.9 (MS/MS), **Retention time**, 22.733 min, **Purity**, 95.06% (HPLC).

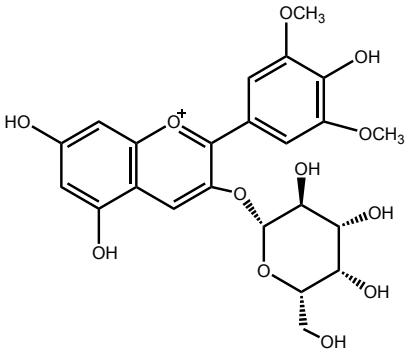
Compound 15, Malvidin-3-*O*-glucoside (M3G), $C_{23}H_{25}O_{12}^+$



^{1}H NMR (CD_3OD, 600 MHz): δ 8.84 (s,1H), 7.79 (s, 2H), 6.82 (d, *J*=1.2 Hz, 1H), 6.52 (d, *J*=1.8 Hz, 1H), 5.28 (d,

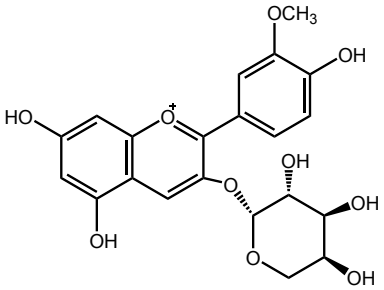
J=7.8 Hz, 1H), 3.89 (s, 6H), 3.85-3.87 (m, 1H), 3.63-3.66 (m, 1H), 3.55-3.68 (m, 1H), 3.49-3.52 (m, 2H), 3.34-3.37 (m, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 170.6, 163.1, 159.1, 157.5, 149.6, 146.1, 145.6, 136.4, 119.6, 113.4, 110.3, 103.6, 103.3, 95.4, 78.8, 78.2, 75.0, 71.1, 62.3, 57.2. **ESI-LC/MS/MS**, [M^+] 493.0 (MS), 331.0 (MS/MS), **Retention time**, 22.103 min, **Purity**, 95.01% (HPLC).

Compound 16, Malvidin-3-*O*-galactoside (M3Ga), $C_{23}H_{25}O_{12}^+$



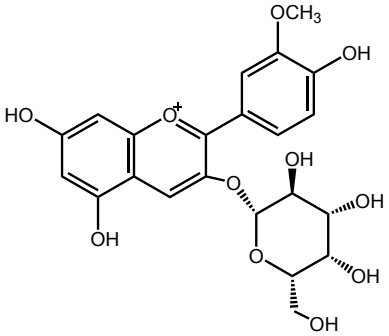
^{1}H NMR (CD_3OD, 600 MHz): δ 8.92 (s,1H), 7.88 (s, 2H), 6.87 (d, *J*=1.2 Hz, 1H), 6.56 (d, *J*=1.8 Hz, 1H), 5.26 (d, *J*=7.8 Hz, 1H), 3.94 (s, 6H), 3.91-3.93 (m, 1H), 3.88 (d, *J*=3 Hz, 1H), 3.69-3.78 (m, 3H), 3.63-3.66 (dd, *J*=9.6, 3.6 Hz, 1H). ^{13}C NMR (150 MHz, CD_3OD) δ 170.5, 163.6, 159.2, 157.7, 149.6, 146.1, 145.7, 136.7, 119.7, 113.5, 110.4, 104.4, 103.3, 95.3, 77.8, 75.0, 72.2, 70.1, 62.4, 57.3. **ESI-LC/MS/MS**, [M^+] 492.9 (MS), 330.9 (MS/MS), **Retention time**, 21.673 min, **Purity**, 97.77% (HPLC).

Compound 17, Peonidin-3-*O*-arabinoside (Peo3A), $C_{21}H_{21}O_{10}^+$



^{1}H NMR (CD_3OD, 600 MHz): δ 8.97 (s,1H), 8.33 (dd, *J*=8.4 Hz, 2.4 Hz, 1H), 8.24 (d, *J*=2.4 Hz, 1H), 7.05 (d, *J*=8.4 Hz, 1H), 6.95 (d, *J*=1.2 Hz, 1H), 6.66 (d, *J*=1.8 Hz, 1H), 5.29 (d, *J*=6.0 Hz, 1H), 4.03 (s, 3H), 3.96-4.02 (m, 3H), 3.76-3.80 (m, 2H). **^{13}C NMR** (150 MHz, CD_3OD) 170.6, 164.4, 159.1, 157.8, 156.6, 149.6, 145.5, 136.8, 129.2, 121.1, 117.6, 115.2, 113.4, 104.3, 103.4, 95.3, 73.8, 72.1, 68.8, 67.0, 57.0. **ESI-LC/MS/MS**, [M^+] 433.1 (MS), 301.1 (MS/MS), **Retention time**, 21.927 min, **Purity**, 99.11% (HPLC).

Compound 18, Peonidin-3-*O*-galactoside (Peo3Ga), $C_{22}H_{23}O_{11}^+$



^{1}H NMR (CD_3OD, 600 MHz): δ 8.99 (s,1H), 8.20 (dd, *J*=6.6 Hz, 2.4 Hz, 1H), 8.18 (d, *J*=1.8 Hz, 1H), 7.02 (d, *J*=8.4 Hz, 1H), 6.89 (d, *J*=1.2 Hz, 1H), 6.62 (d, *J*=1.8 Hz, 1H), 5.28 (d, *J*=7.8 Hz, 1H), 4.00 (s, 3H), 3.97-3.98 (m, 1H), 3.95 (d, *J*=3.6 Hz, 1H), 3.77-3.80 (m, 3H), 3.68-3.71 (dd, *J*=9.6, 3.0 Hz, 1H). **^{13}C NMR** (150 MHz, CD_3OD) 170.5, 163.9, 159.2, 157.7, 156.5, 149.4, 145.5, 137.0, 128.7, 121.0, 117.6, 115.3, 113.5, 104.4, 103.4, 95.3, 77.8, 75.0, 72.2, 70.1, 62.4, 56.9. **ESI-LC/MS/MS**, [M^+] 463.1 (MS), 301.0 (MS/MS), **Retention time**, 20.657 min, **Purity**, 97.86% (HPLC).

3. Supplementary tables

Table S1 Identification of major anthocyanins in the six kinds of berry fruits.

Peak	MS^+	MS/MS	Anthocyanin	Peak	MS^+	MS/MS	Anthocyanin
				Mulberry			
1	449	287	Cyanidin-3-*O*-glucoside	2	595	287	Cyanidin-3-*O*-rutinoside
				Blueberry			
1	465	303	Delphinidin-3-*O*-galactoside	7	419	287	Cyanidin-3-*O*-arabinoside
2	465	303	Delphinidin-3-*O*-glucoside	8	479	317	Petunidin-3-*O*-glucoside
3	449	287	Cyanidin-3-*O*-galactoside	9	449	317	Petunidin-3-*O*-arabinoside
4	435	303	Delphinidin-3-*O*-arabinoside	10	493	331	Malvidin-3-*O*-galactoside
5	449	287	Cyanidin-3-*O*-glucoside	11	493	331	Malvidin-3-*O*-glucoside
6	479	317	Petunidin-3-*O*-galactoside	12	463	331	Malvidin-3-*O*-arabinoside
				Strawberry			
1	449	287	Cyanidin-3-*O*-glucoside	3	579	271	Pelargonidin-3-*O*-rutinoside
2	433	271	Pelargonidin-3-*O*-glucoside				
				Raspberry			
1	449	287	Cyanidin-3-*O*-glucoside	3	579	271	Pelargonidin-3-*O*-rutinoside
2	433	271	Pelargonidin-3-*O*-glucoside				
				Cranberry			
1	449	287	Cyanidin-3-*O*-galactoside	3	463	301	Peonidin-3-*O*-galactoside
2	419	287	Cyanidin-3-*O*-arabinoside	4	433	301	Peonidin-3-*O*-arabinoside
				Bayberry			
1	449	287	Cyanidin-3-*O*-glucoside				

Table S2 The elution order of anthocyanins in HPLC and HSCCC system

Elution order in HSCCC																		
Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Anthocyanin	D3S	C3S	D3R	C3R	D3Ga	Pet3Ga	M3Ga	Pg3R	Peo3Ga	D3G	Pet3G	M3G	C3G	D3A	Pet3A	M3A	Peo3A	Pg3G
K	0.278	0.291	0.296	0.339	0.341	0.414	0.453	0.458	0.524	0.539	0.548	0.559	0.671	0.726	0.779	0.825	1.128	1.424
Elution order in HPLC																		
Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Anthocyanin	D3Ga	D3S	D3G	D3A	D3R	C3S	C3G	Pet3Ga	C3R	Pet3G	Peo3Ga	Pg3G	Pet3A	Pg3R	M3Ga	Peo3A	M3G	M3A
t_R (min)	21.92	23.45	25.13	28.65	28.89	31.45	31.56	33.77	35.68	37.40	38.30	38.81	40.61	41.41	42.24	42.81	43.26	44.29

t_R, retention time. K, partition coefficient. The value of t_R represents the elution order in HPLC, whereas the value of K represents the elution order in HSCCC.
Pg: pelargonidin, C: cyanidin, D: delphinidin, Pet: petunidin, Peo: peonidin, M: malvidin, G:glucoside, Ga: galactoside, A: arabinoside, R: rutinoside, S: sambubioside.

For HPLC purification, the elution process was performed as follows: 7% to 25% A for 35 min, 25% to 65% A for 10 min, 65% to 100% A for 1 min, 100% to 100% A for 4 min, 100% to 7% A for 3 min and 7% to 7% A for 2 min. The flow rate was 1 mL/min and the monitoring wavelength was 280 nm. The mobile phase consists of solvent A (acetonitrile : methanol : water : formic acid=22.5:22.5:41.5:8.5, V/V/V/V) and solvent B (water : formic acid = 91.5:8.5, V/V).

Table S3 The relevant parameters of fluorescence quenching on α-glucosidase by Pg3R

Parameters	Temperature (K)		
	291	303	310
K_q (10^{12} L/mol·s)	7.064	8.249	7.608
K_a (10^4 L/mol)	0.751	1.720	2.429
n	0.797	0.8640	0.899
ΔG (kJ/mol)	-21.439	-24.376	-26.089
ΔH (kJ/mol)		49.780	
ΔS (J/mol)		244.739	

Table S4 The percentage of secondary structure of α-glucosidase in the presence or absence of anthocyanins

Molar ratio of inhibitors to α-glucosidase		Secondary structures of α-glucosidase			
		α-helix (%)	β-sheet(%)	β-turn (%)	Random (%)
Without inhibitor	0:1	28.0	24.7	15.1	32.2
Pg3R	36:1	22.7	34.2	11.9	31.2
	72:1	17.1	47.1	7.3	28.5
Pg3G	36:1	20.1	37.9	12.4	29.6
	72:1	19.6	41.7	12.9	25.8
M3A	36:1	21.3	35.9	12.0	30.8
	72:1	19.1	42.6	9.5	29.8
D3S	36:1	25.5	28.3	24.9	21.3
	72:1	24.9	30.5	29.7	14.9

4. Supplementary Figures

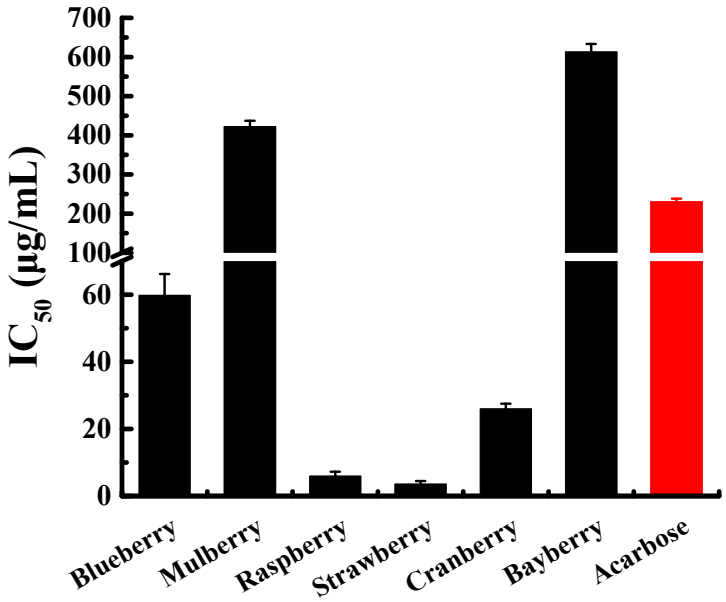


Fig. S1 α-glucosidase inhibitory activity of berry anthocyanin extracts (blueberry, mulberry, raspberry, strawberry, cranberry and bayberry). Acarbose was treated as a positive control.

We first determined the α-glucosidase inhibitory activities of berry anthocyanin extracts (strawberry, raspberry, blueberry, mulberry, bayberry and cranberry). Among these berry fruits, strawberry (IC_{50}=3.57 ± 0.20 μg/mL) and raspberry (IC_{50}=5.98 ± 0.27 μg/mL) anthocyanin extracts exhibited much more potent α-glucosidase inhibitory activity than acarbose (IC_{50}=230.16 ± 5.27 μg/mL) (**Fig. S1**). The major anthocyanins in these berry fruits were identified by LC-MS/MS and published data13. It can be seen from **Table S1 and Fig S2**, these berry fruits contain six kinds of anthocyanidins (cyanidin, pelargonidin, delphinidin, petunidin, peonidin and malvidin,) that are typical representative of the natural anthocyanins. Pelargonidin anthocyanins are major anthocyanins in strawberry and raspberry, as a result, it can be concluded that pelargonidin anthocyanins might play a key role in inhibition of α-glucosidase

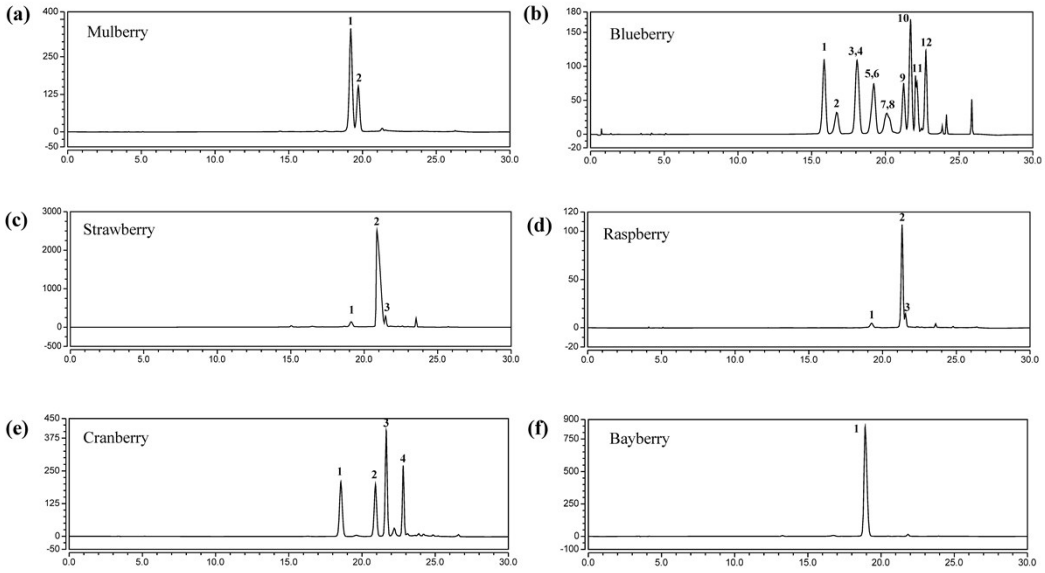


Fig. S2 HPLC chromatograms of berry anthocyanins extracts. **(a)** Mulberry anthocyanins extracts. **(b)** Blueberry anthocyanins extracts. **(c)** Strawberry anthocyanins extracts. **(d)** Raspberry anthocyanins extracts. **(e)** Cranberry anthocyanins extracts. **(f)** Bayberry anthocyanins extracts. The elution process was performed as follows: 5% to 15% A for 15 min, 15% to 28% A for 6 min, 28% to 40% A for 1 min, 40% to 60% A for 2 min, 60% to 5% A for 3 min and 5% to 5% A for 3 min. The mobile phase consists of acetonitrile (solvent A) and 1.5% formic acid aqueous solution (solvent B). The flow rate was 0.8 mL/min and the monitoring wavelength was 280 nm.

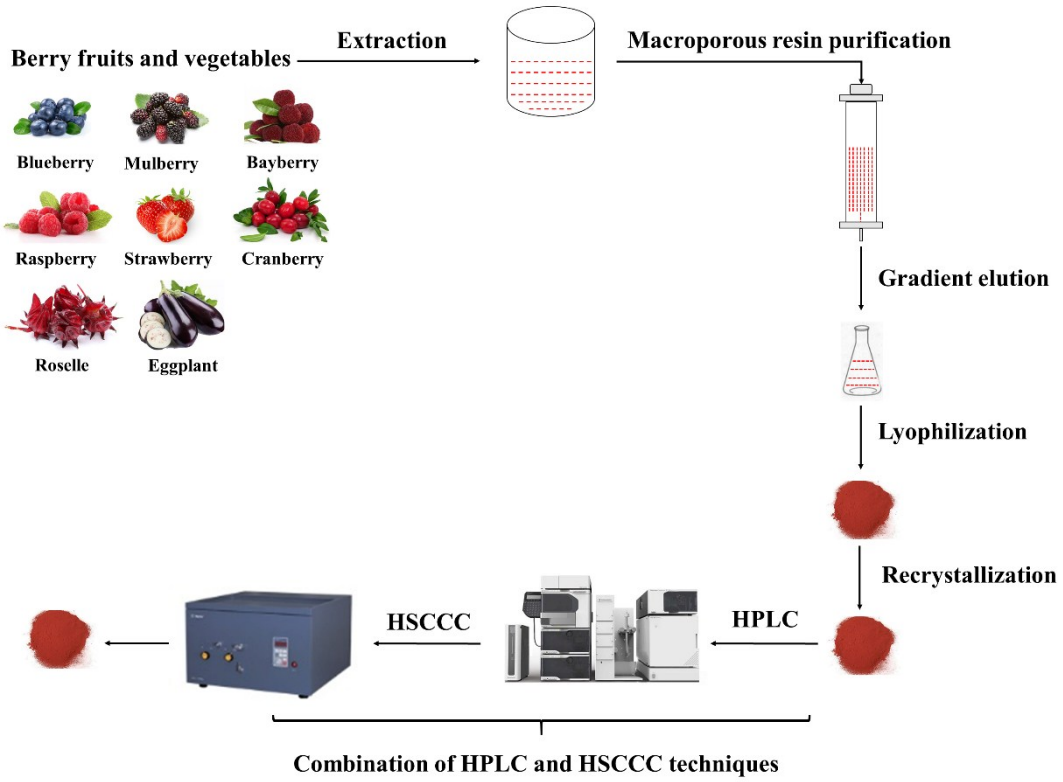


Fig. S3 The protocol for purification of anthocyanins.

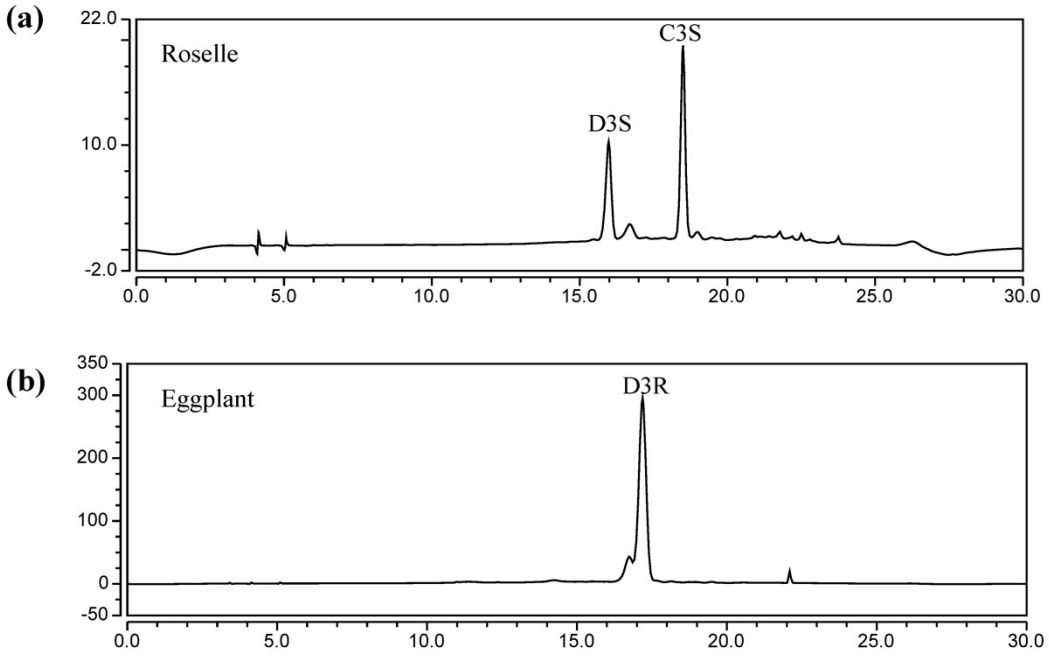


Fig. S4 The major anthocyanins in Roselle (a) and eggplant extracts (b). The mobile phase consists of acetonitrile (solvent A) and 1.5% formic acid aqueous solution (solvent B), and the elution process was performed as follows: 5% to 15% A for 15 min, 15% to 28% A for 6 min, 28% to 40% A for 1 min, 40% to 60% A for 2 min, 60% to 5% A for 3 min and 5% to 5% A for 3 min. The flow rate was 0.8 mL/min and the monitoring wavelength was 280 nm.

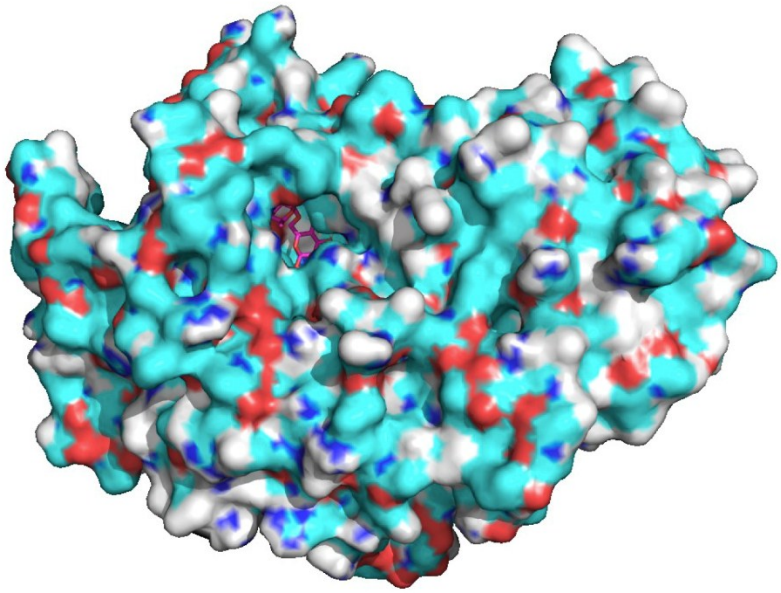
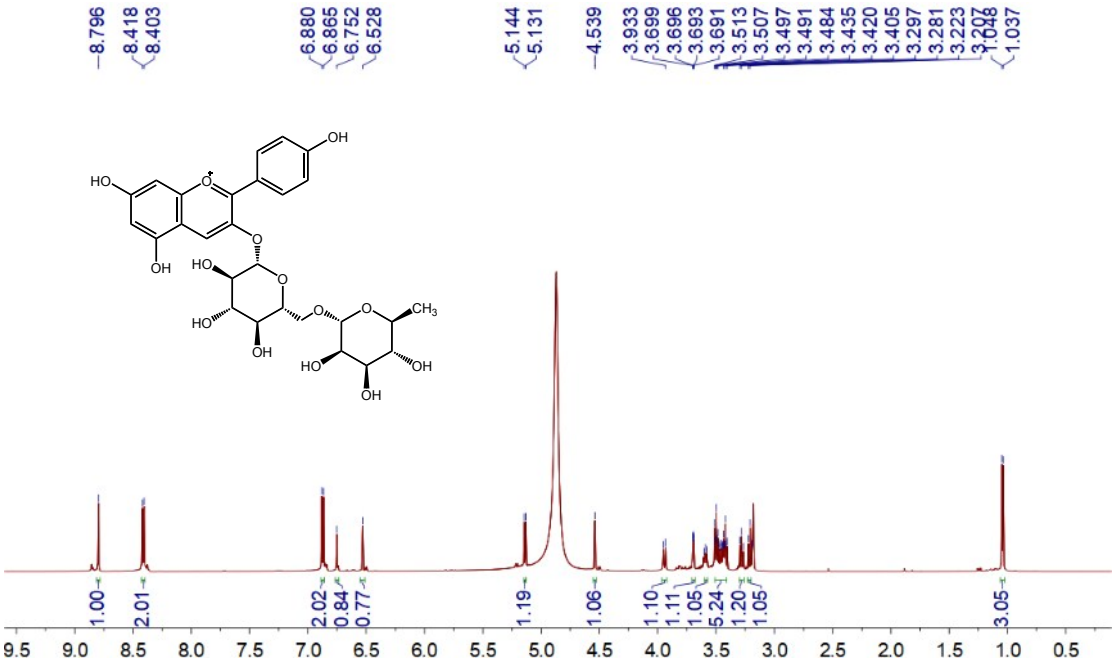
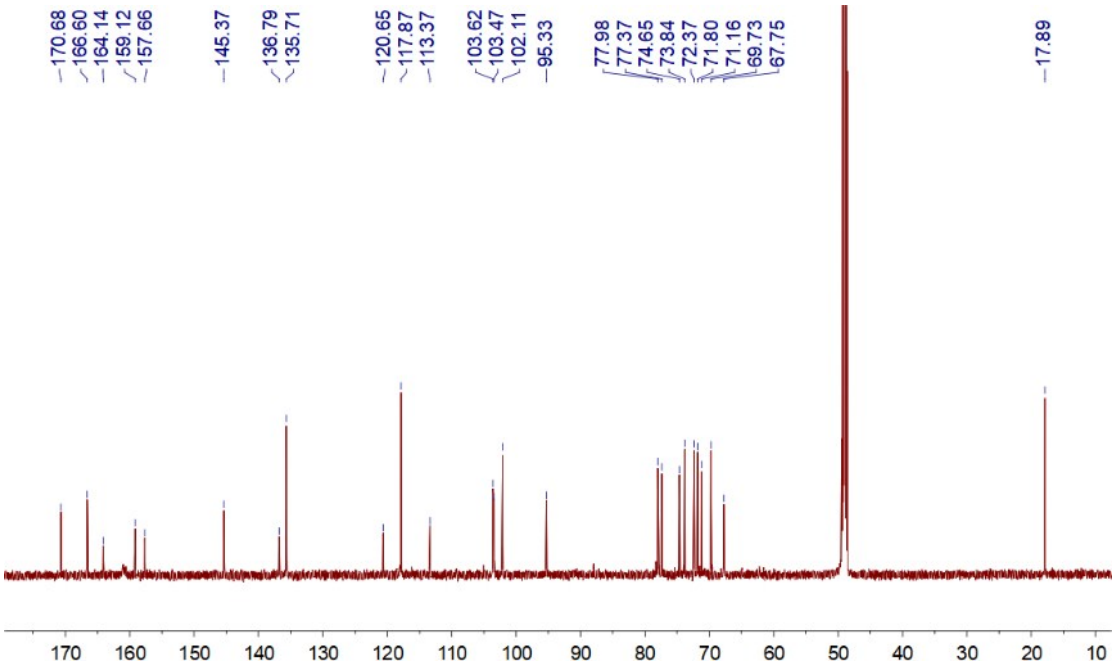


Fig. S5 Predicted conformation of the complex formed by Pg3R and α-glucosidase

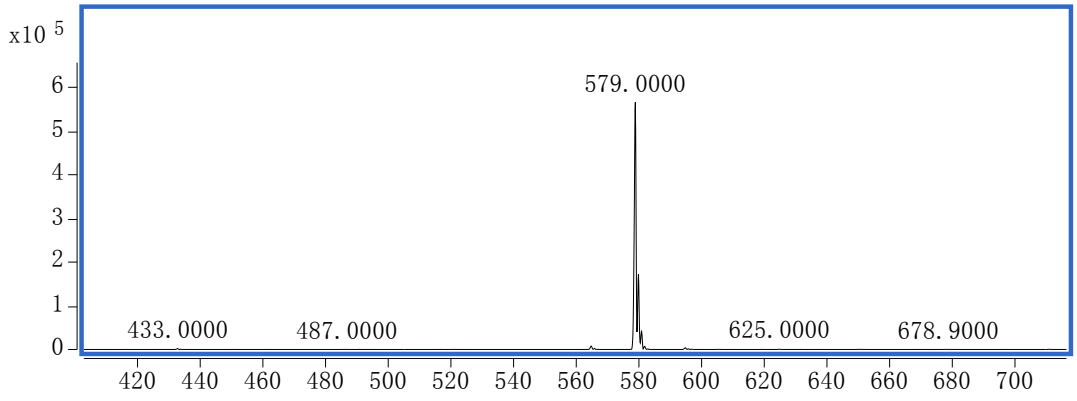
Fig. S6



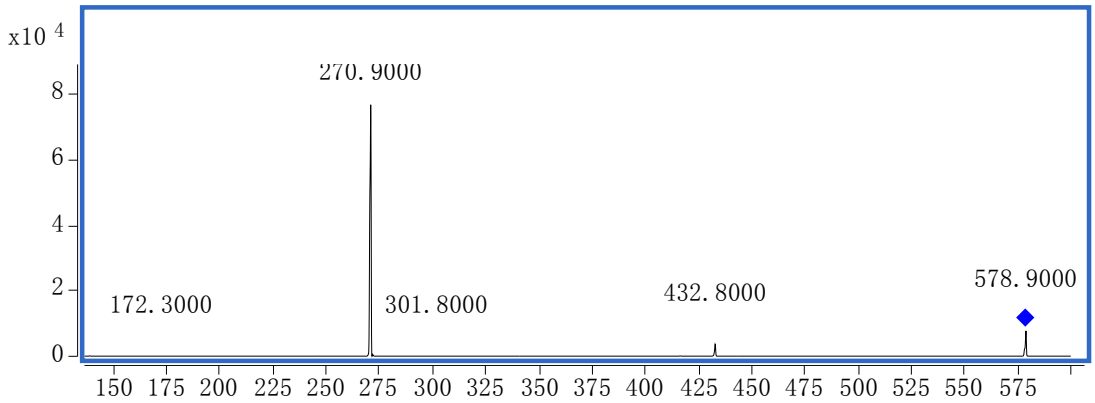
(a) ^{1}H NMR of Pg3R



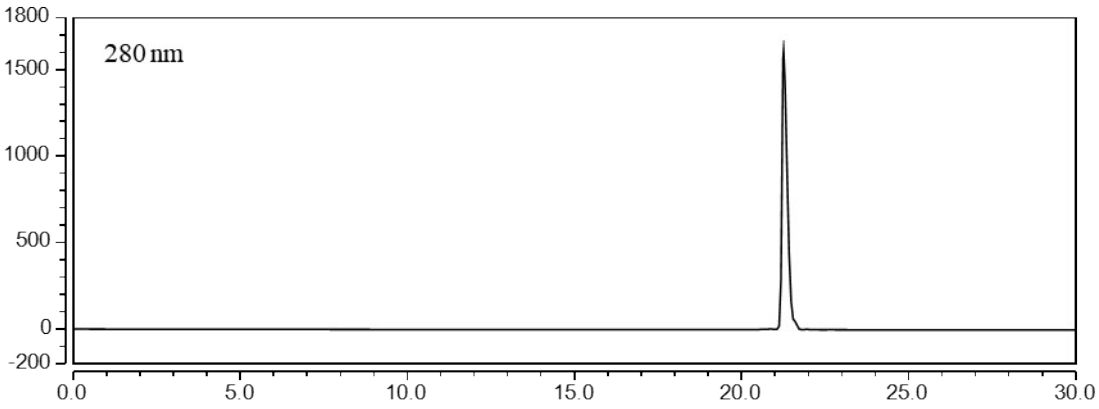
(b) ^{13}C NMR of Pg3R



(c) MS of Pg3R

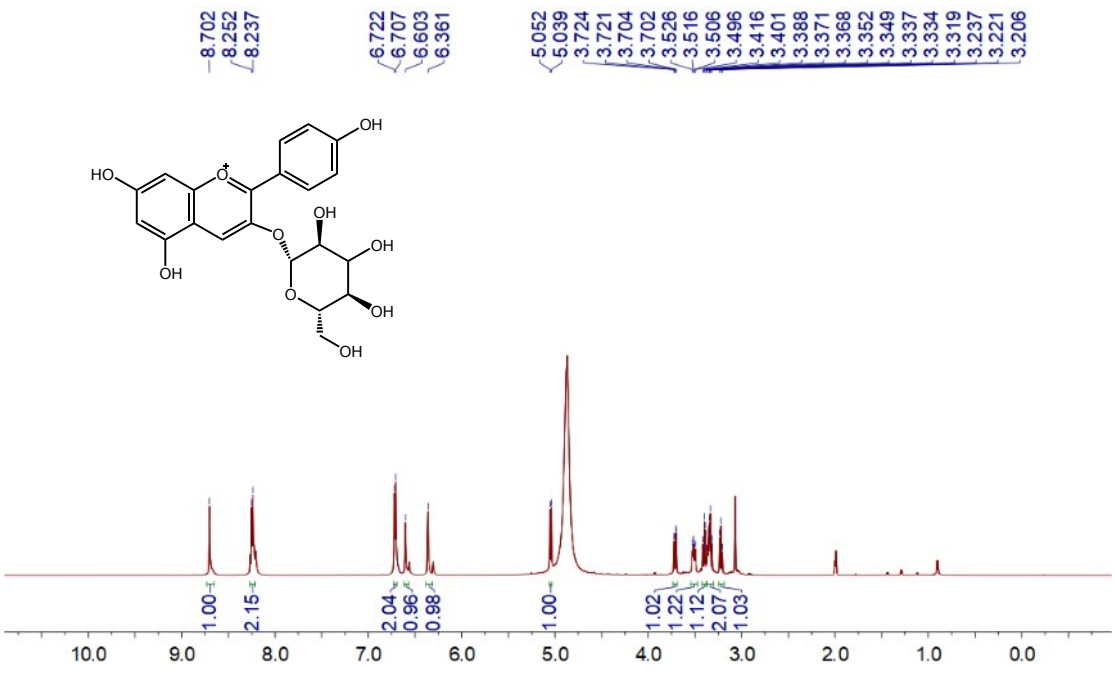


(d) MS/MS of Pg3R

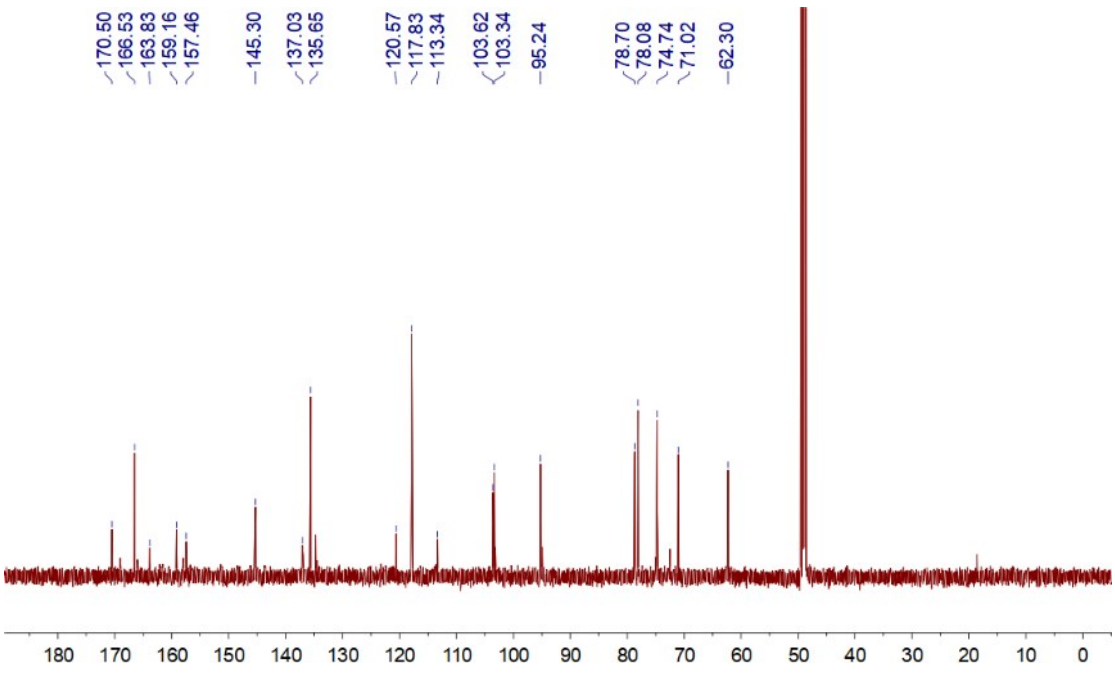


(e) HPLC for purified Pg3R

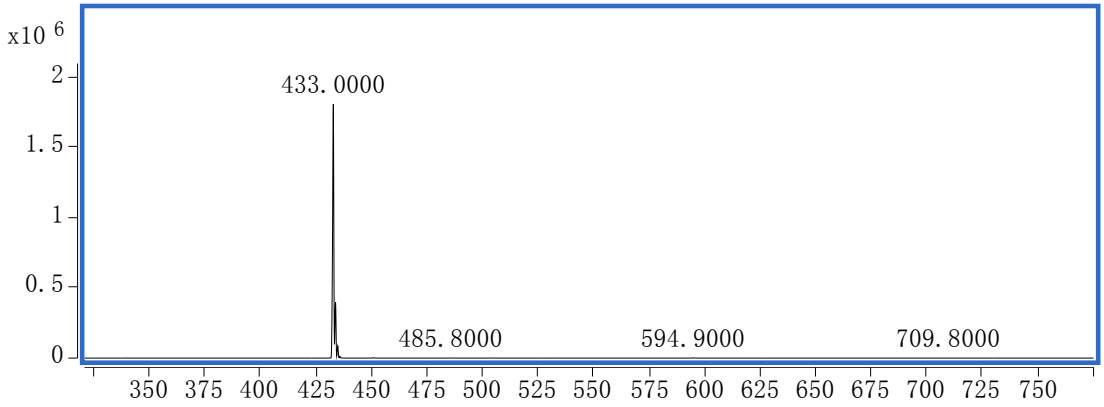
Fig. S7



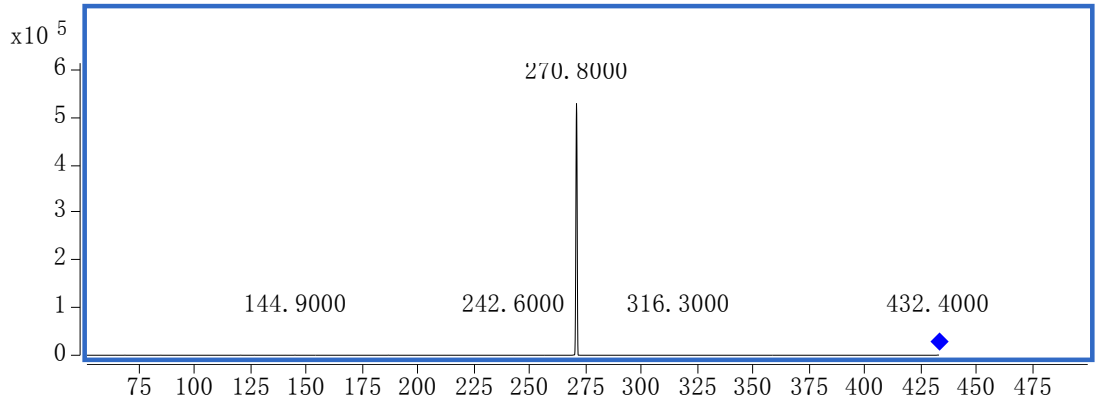
(a) ^{1}H NMR of Pg3G



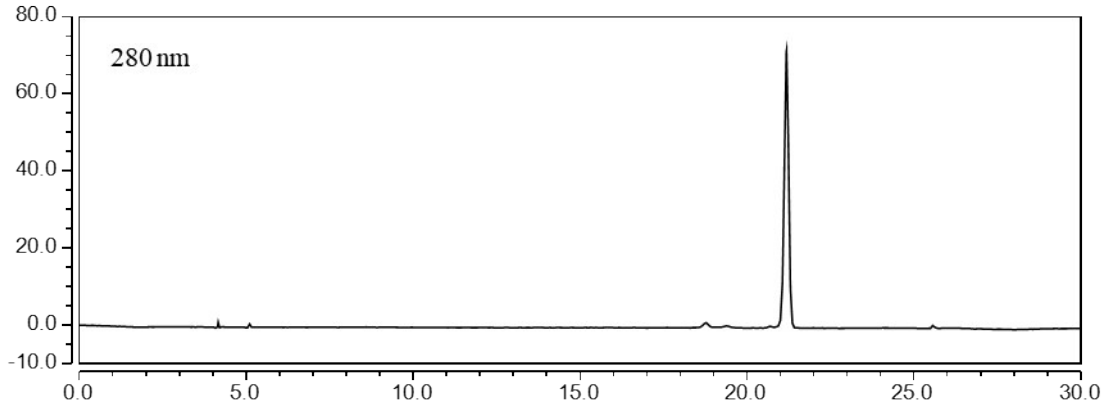
(b) ^{13}C NMR for Pg3G



(c) MS of Pg3G

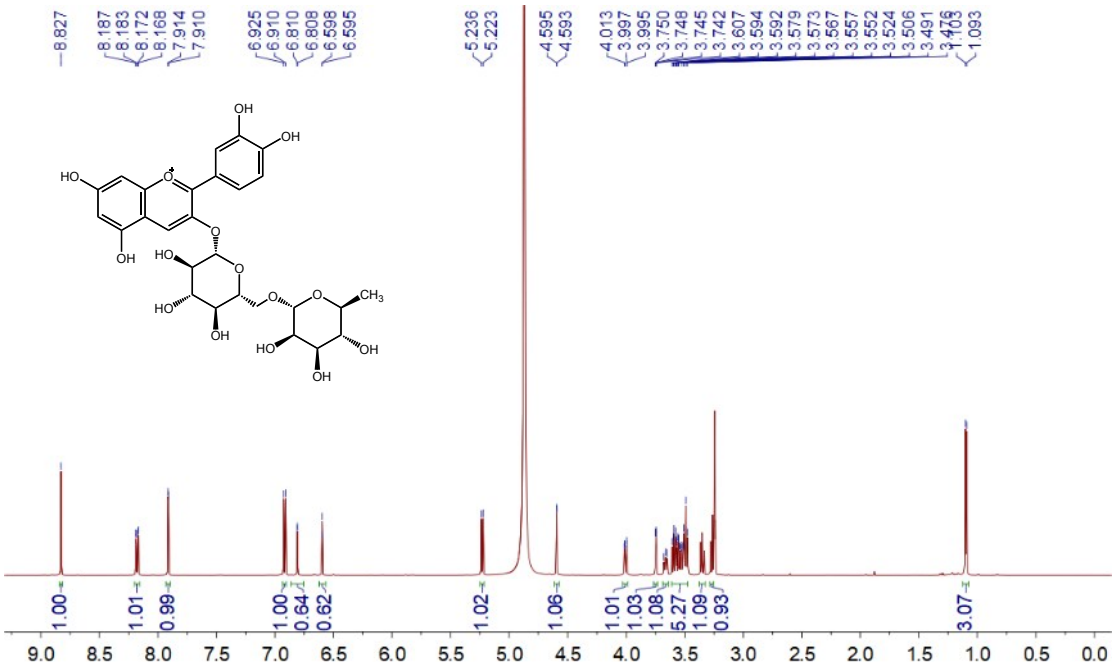


(d) MS/MS of Pg3G

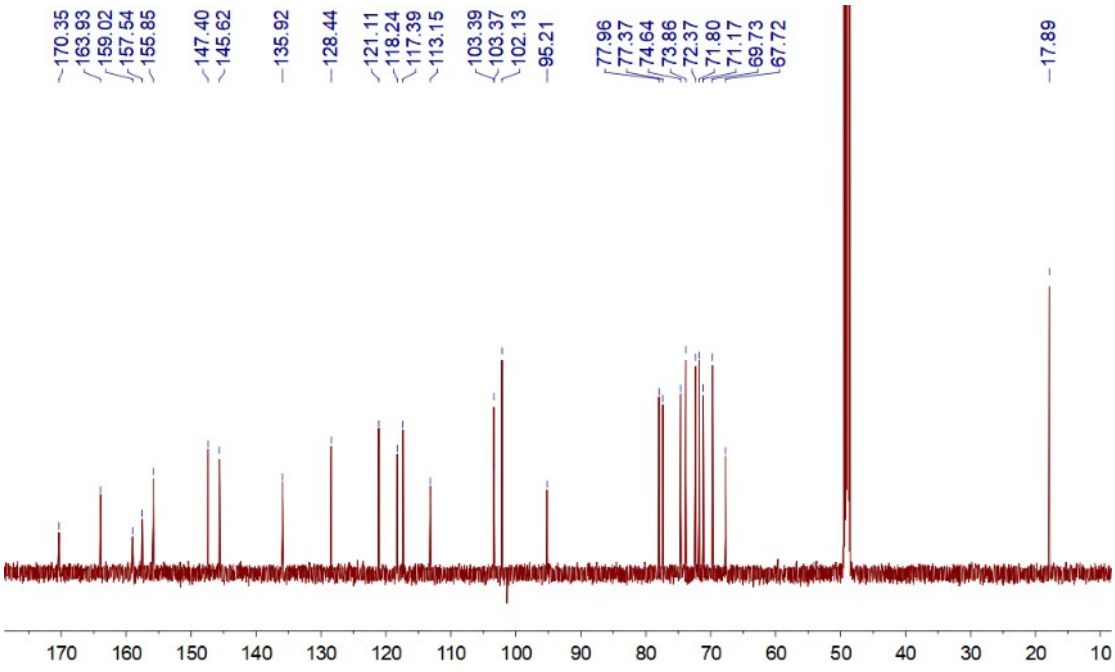


(e) HPLC for purified Pg3G

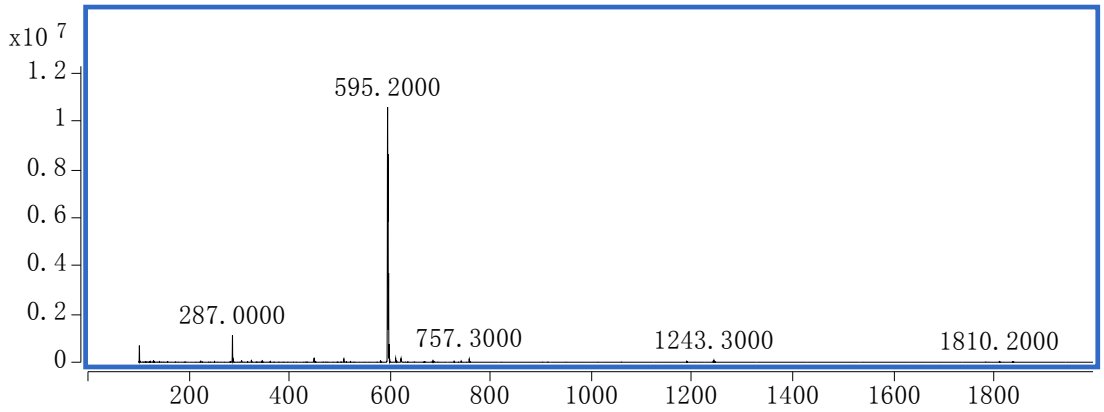
Fig. S8



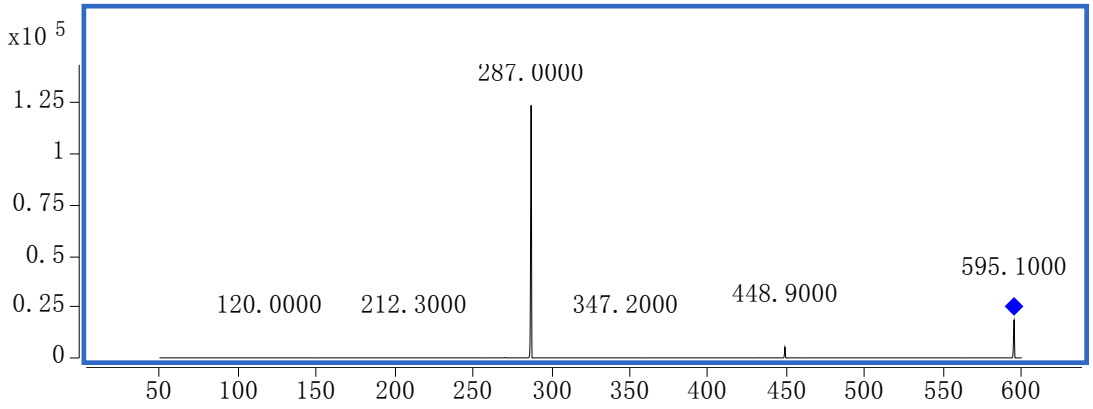
(a) ^{1}H NMR of C3R



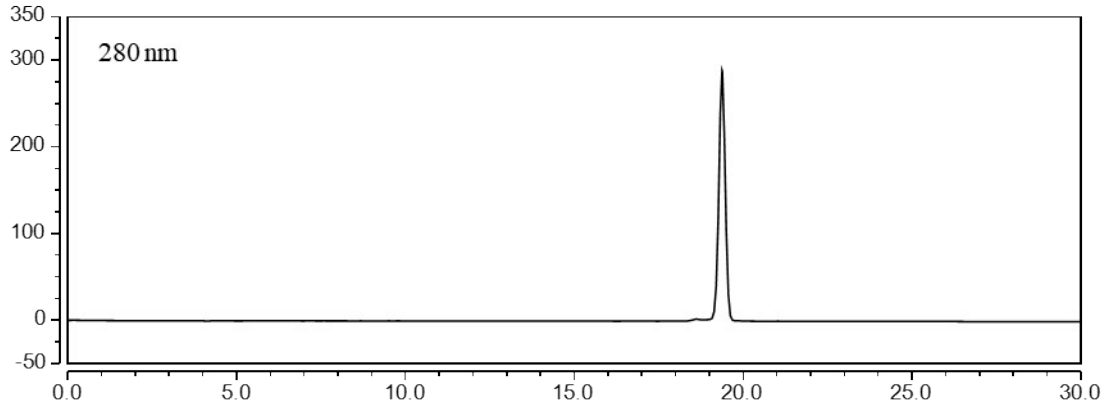
(b) ^{13}C NMR for C3R



(c) MS of C3R

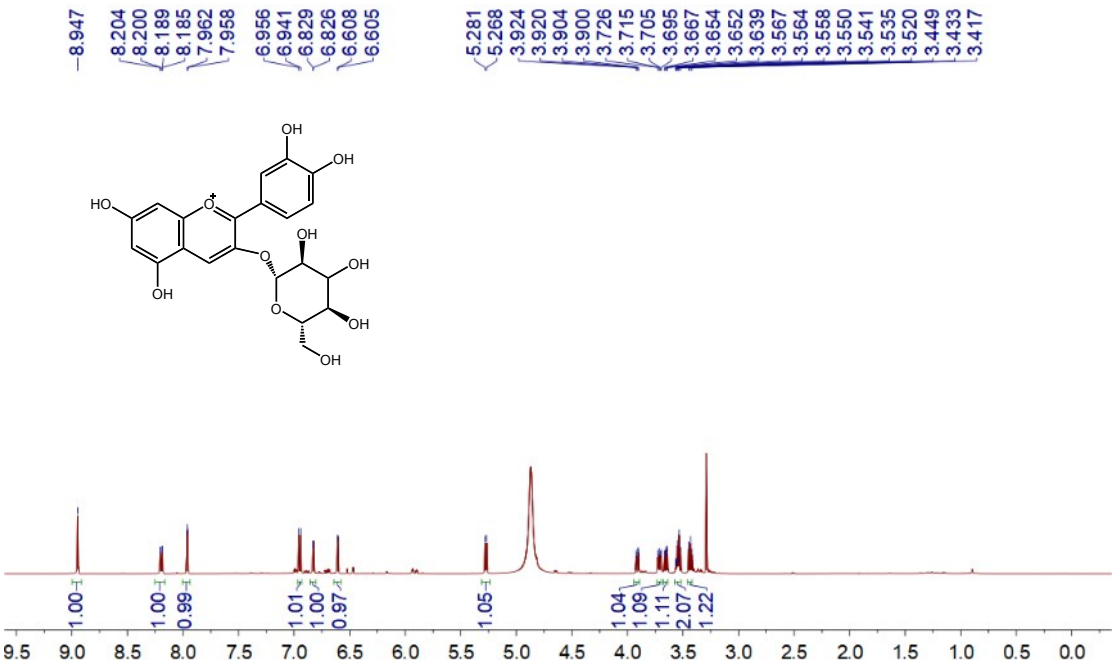


(d) MS/MS of C3R

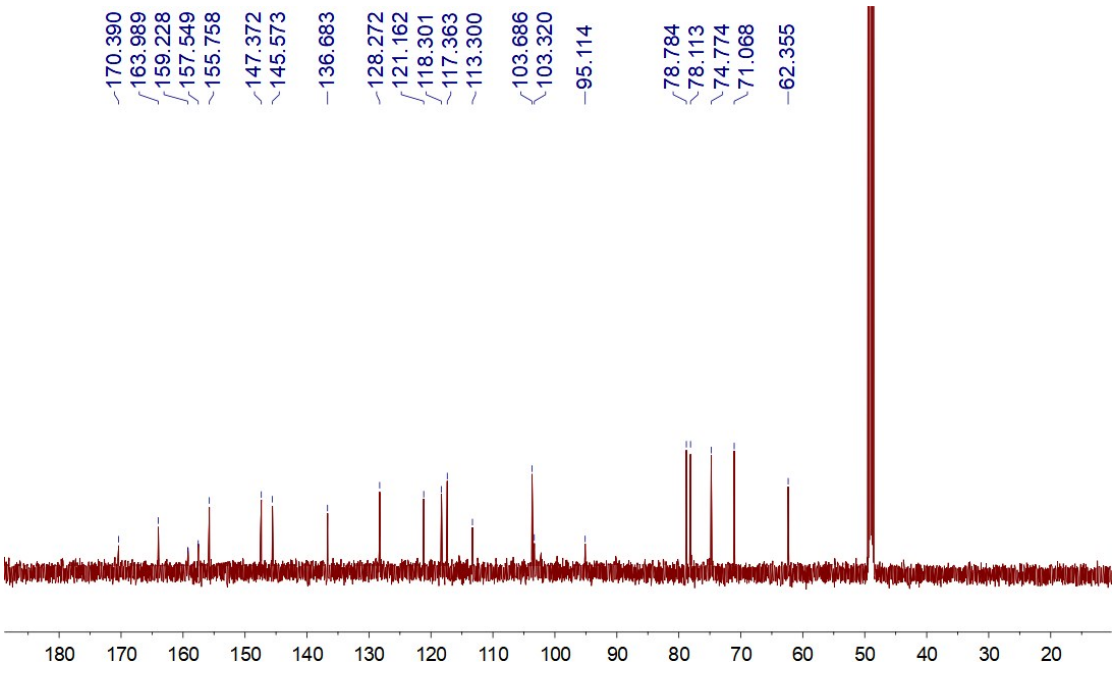


(e) HPLC for purified C3R

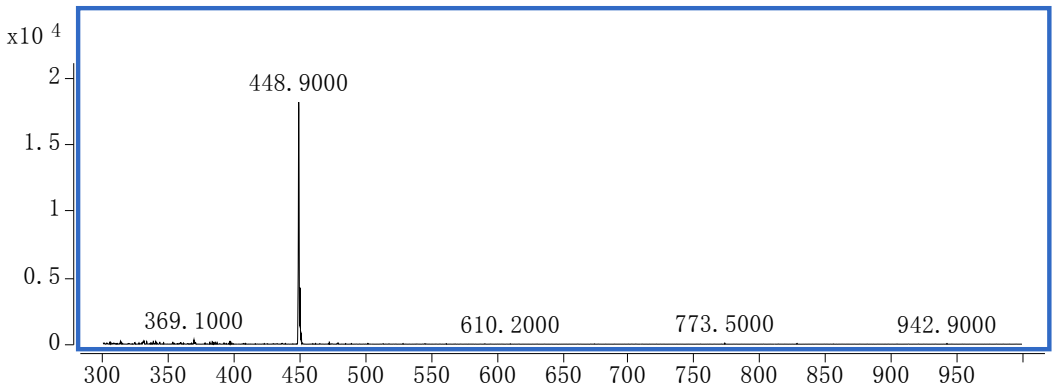
Fig. S9



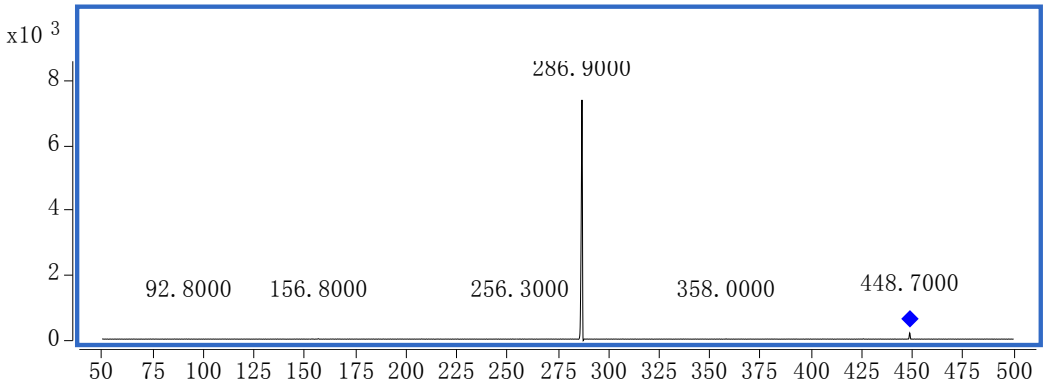
(a) ^{1}H NMR of C3G



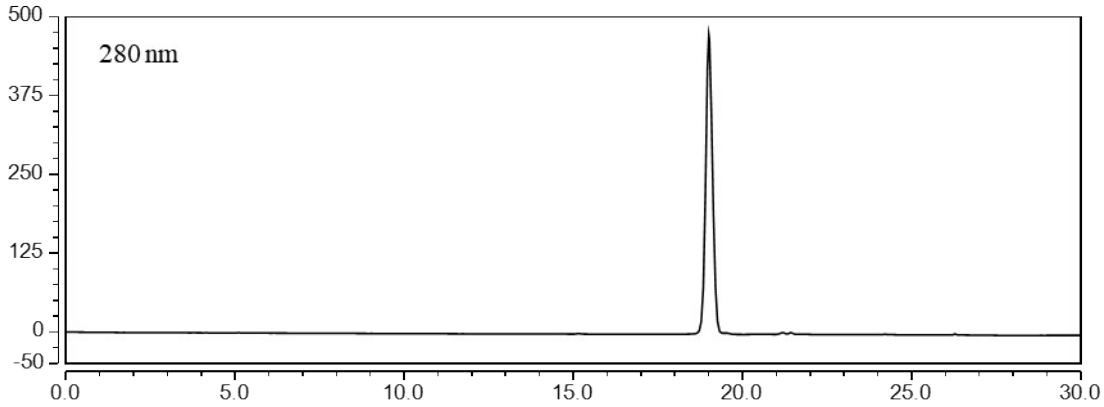
(b) ^{13}C NMR for C3G



(c) MS of C3G

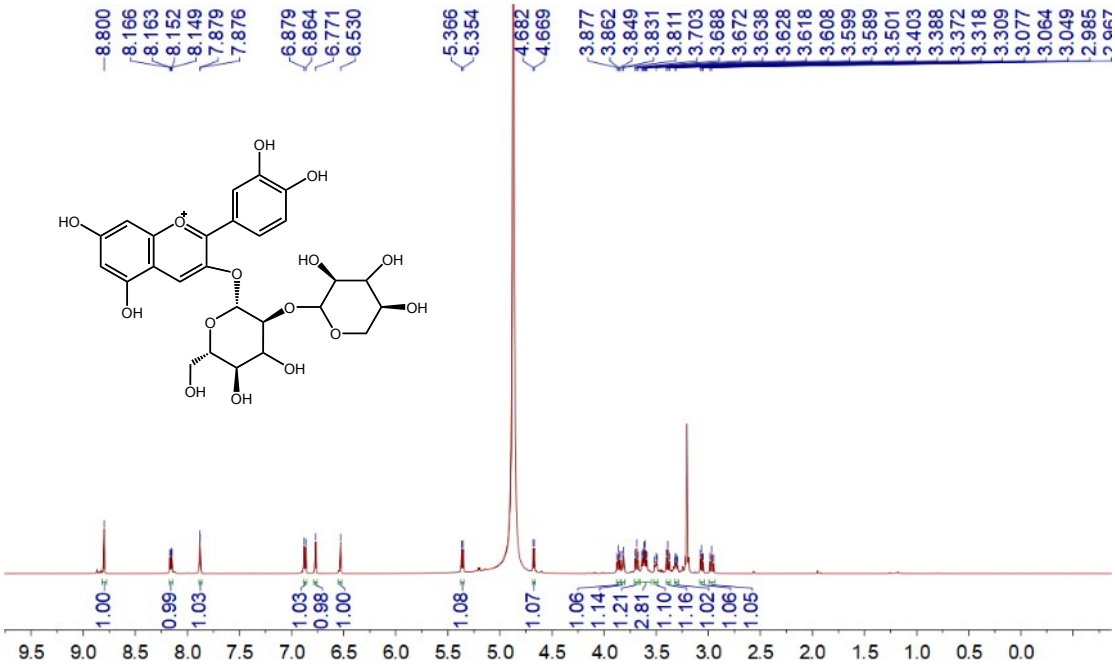


(d) MS/MS of C3G

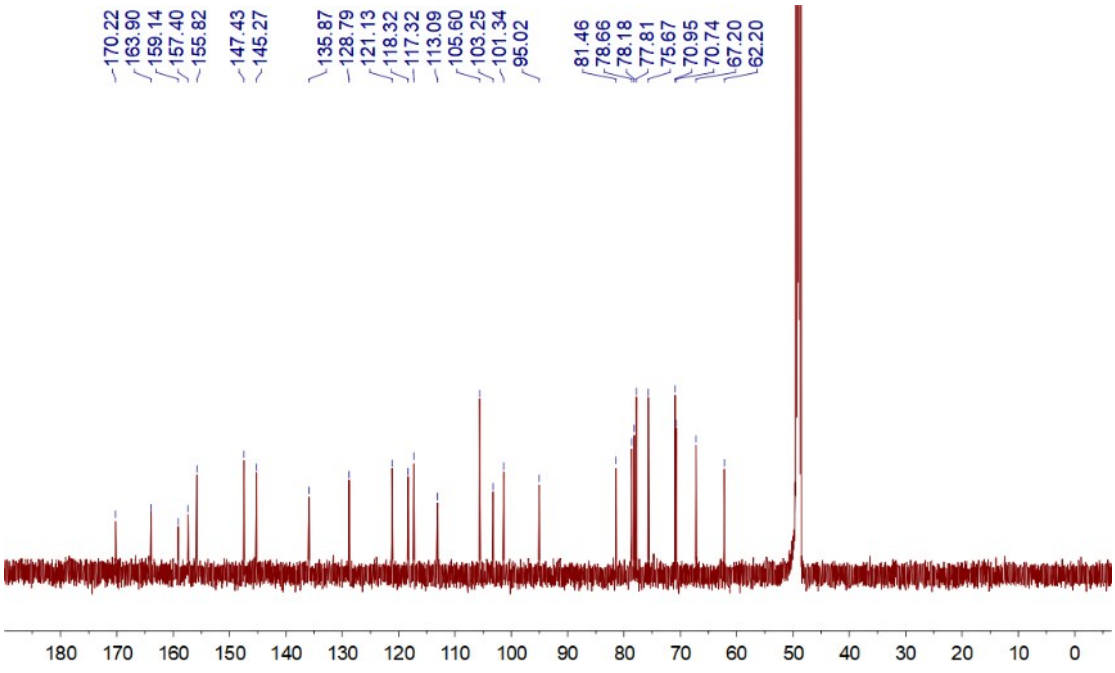


(e) HPLC for purified C3G

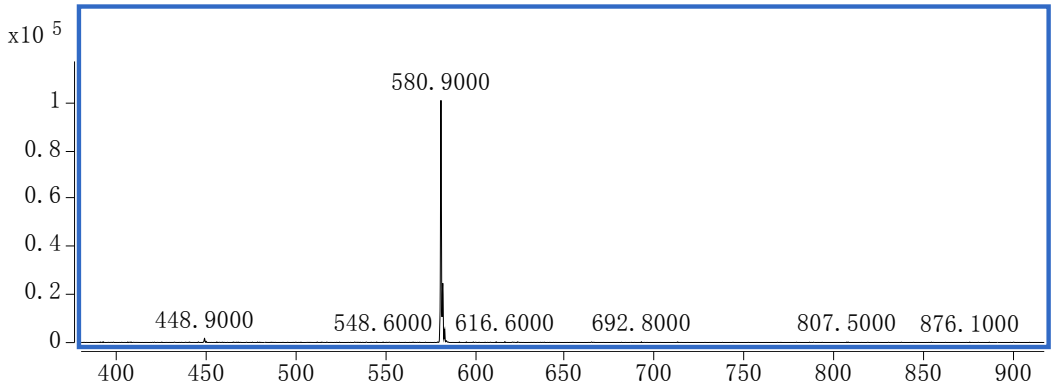
Fig. S10



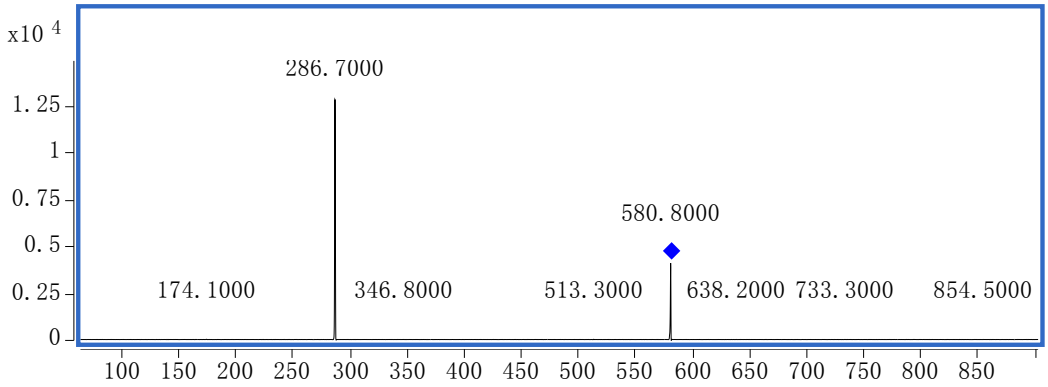
(a) ^{1}H NMR of C3S



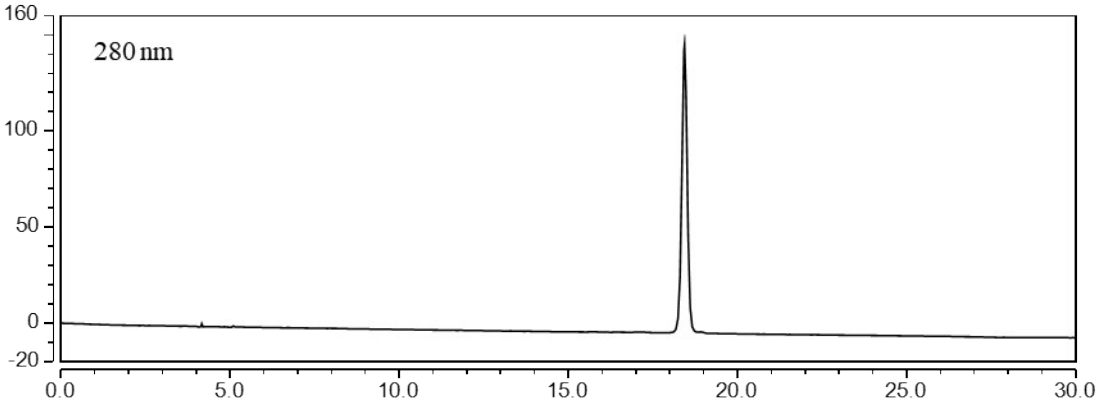
(b) ^{13}C NMR for C3S



(c) MS of C3S

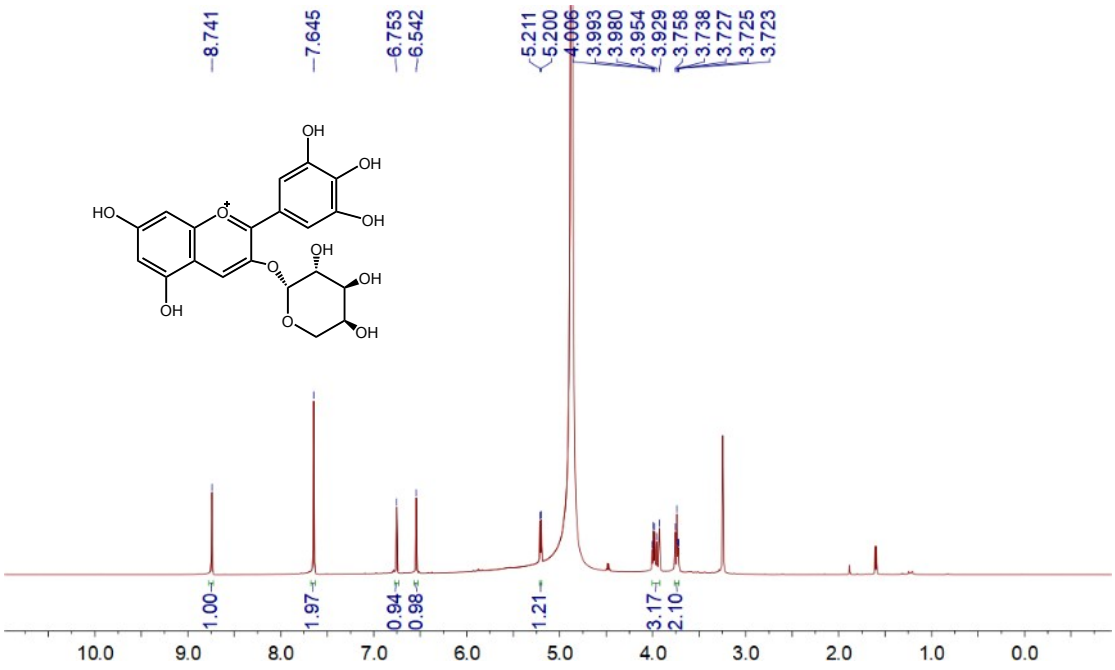


(d) MS/MS of C3S

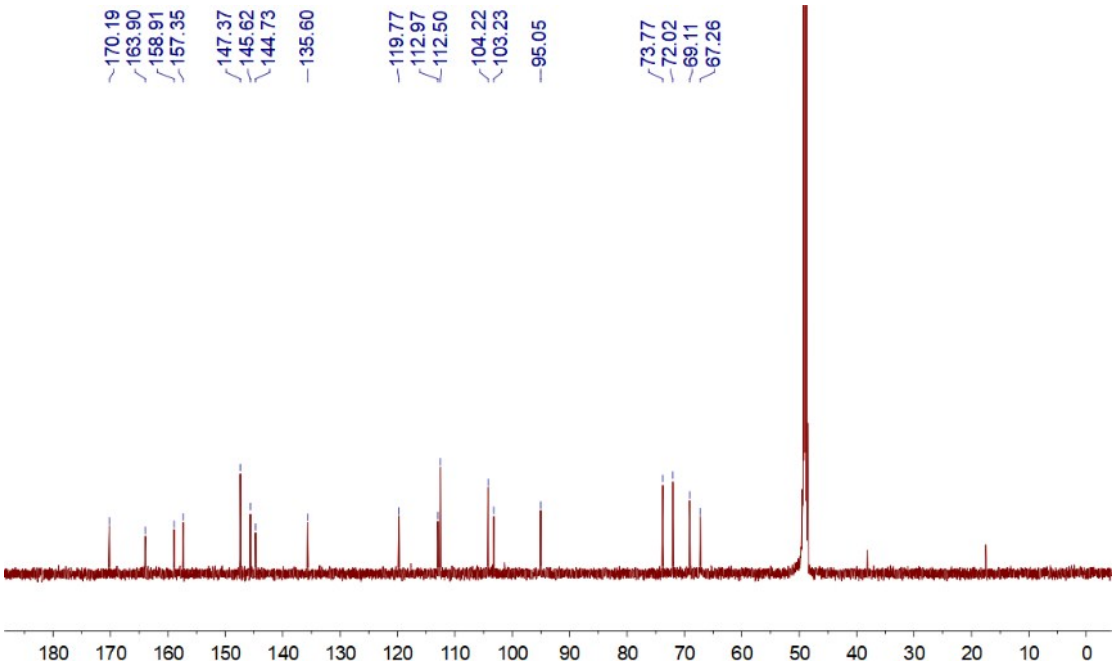


(e) HPLC for purified C3S

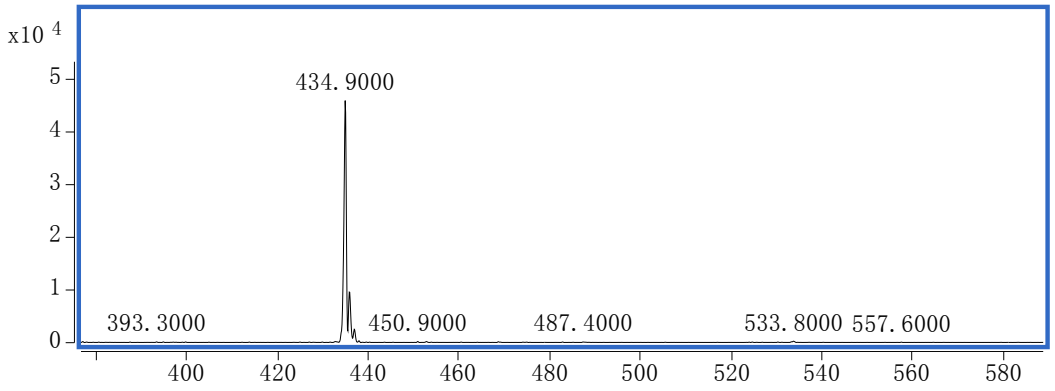
Fig. S11



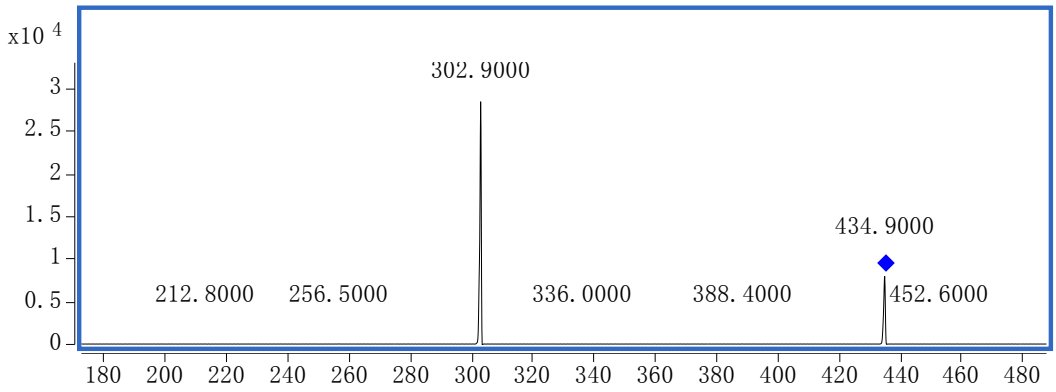
(a) ^{1}H NMR of D3A



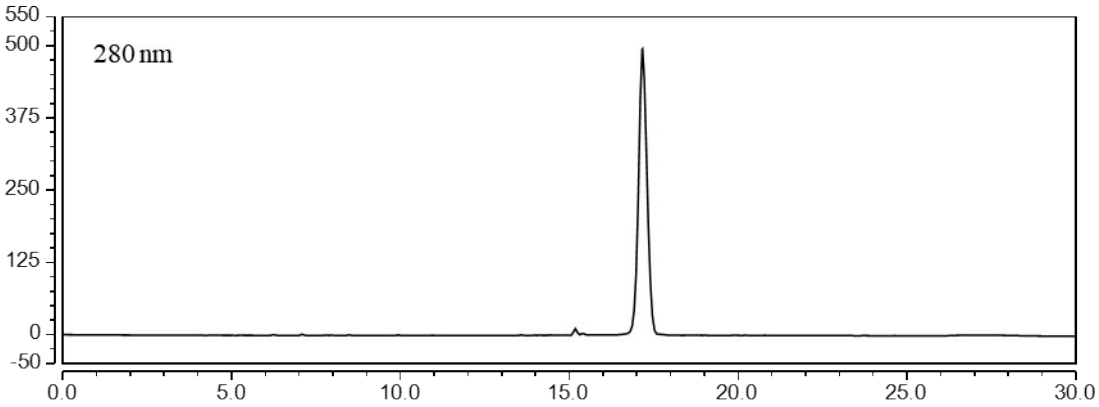
(b) ^{13}C NMR for D3A



(c) MS of D3A

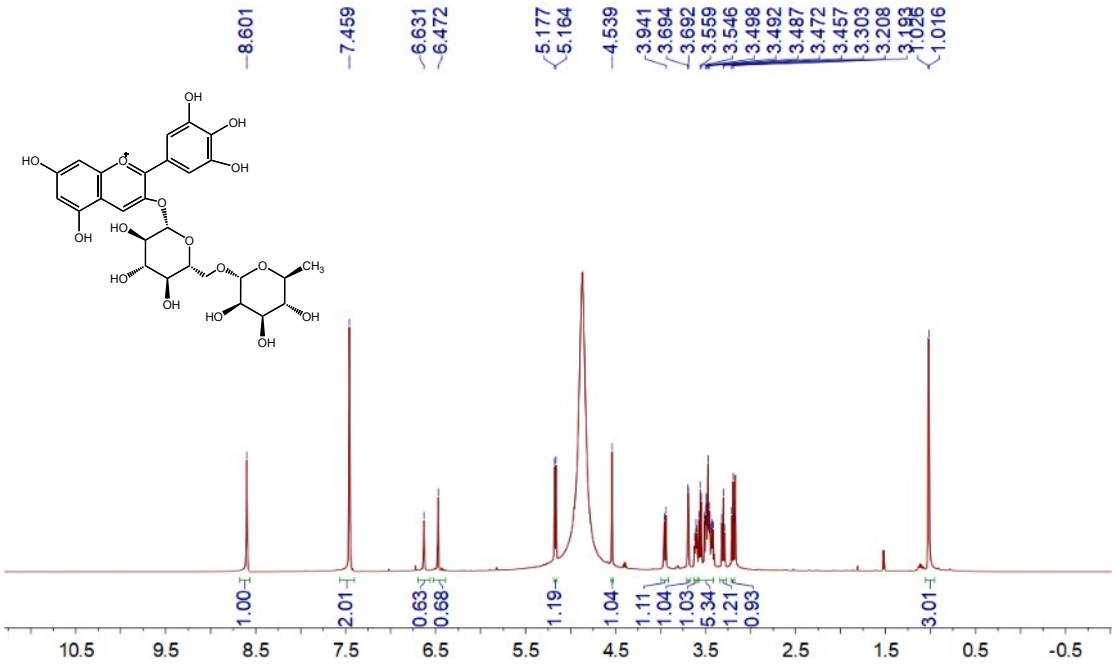


(d) MS/MS of D3A

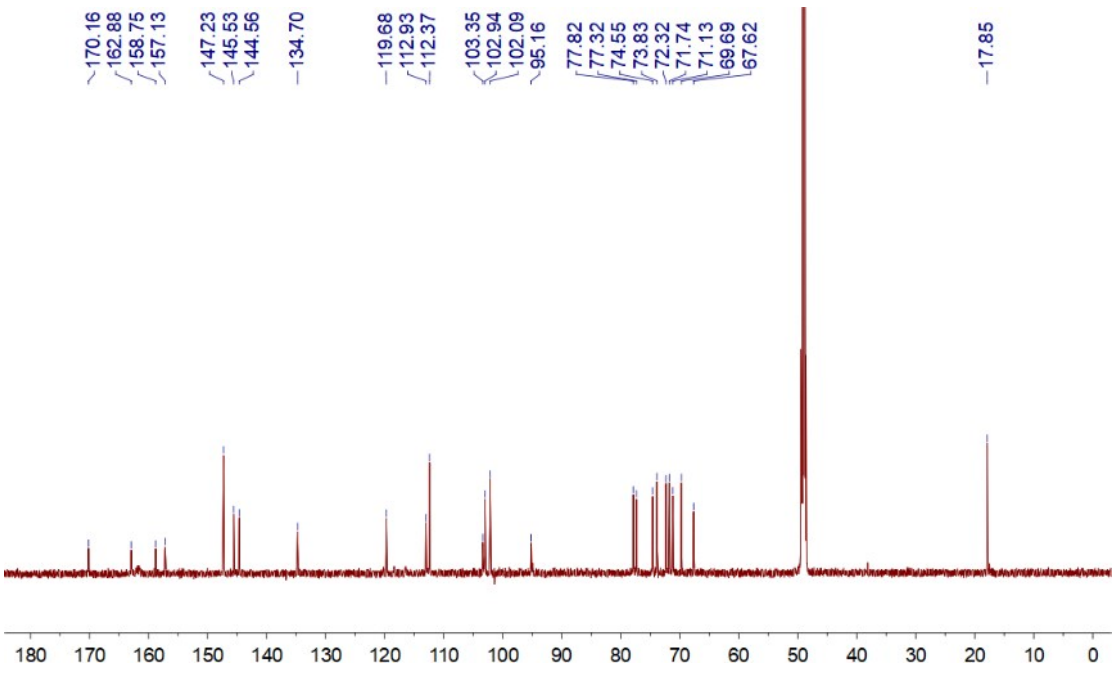


(e) HPLC for purified D3A

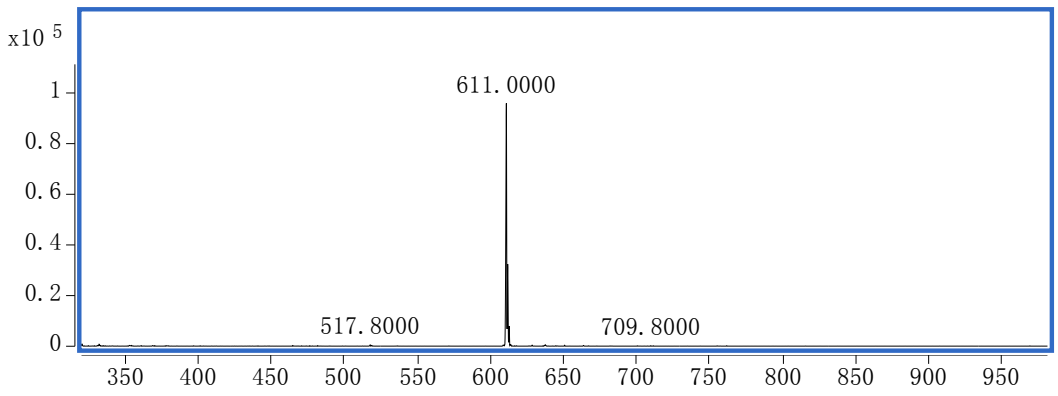
Fig. S12



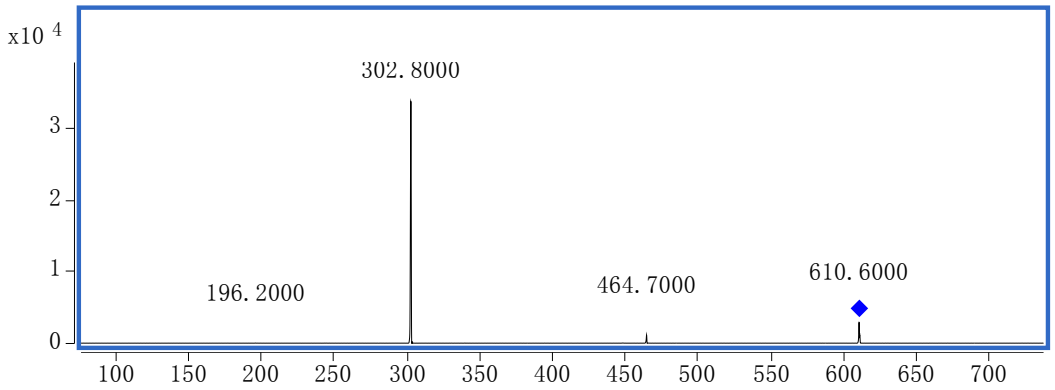
(a) ^{1}H NMR of D3R



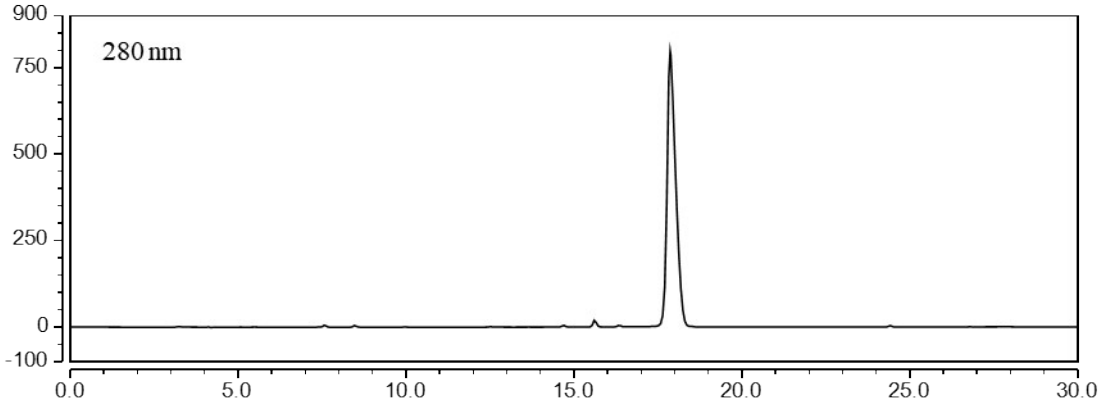
(b) ^{13}C NMR for D3R



(c) MS of D3R

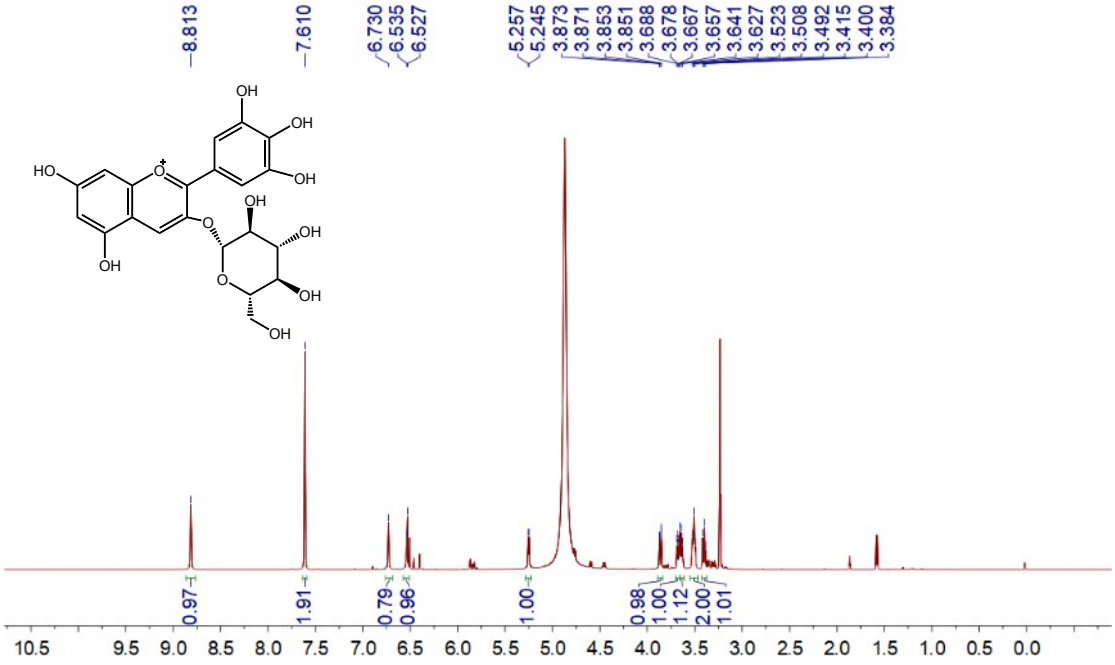


(d) MS/MS of D3R

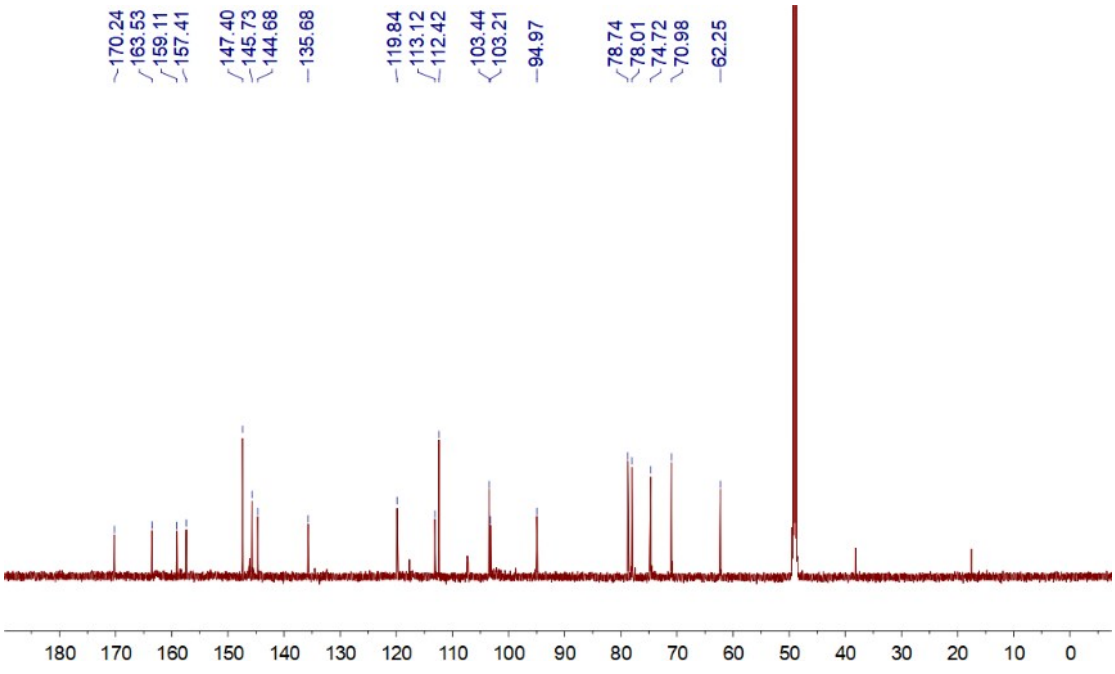


(e) HPLC for purified D3R

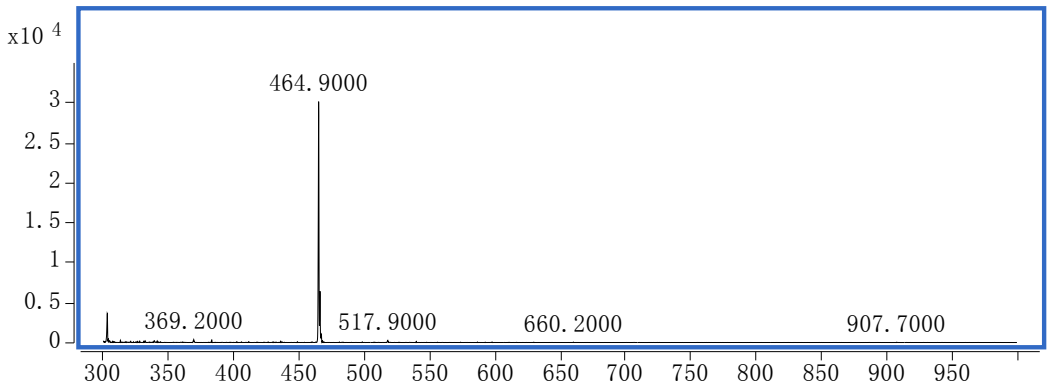
Fig. S13



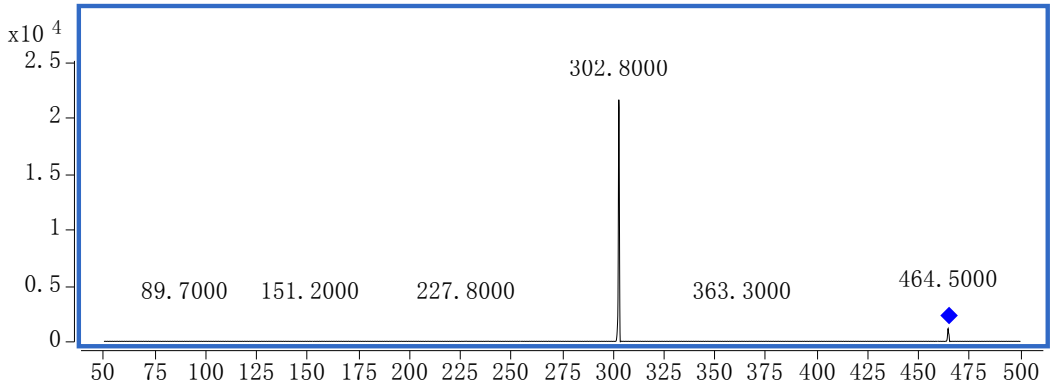
(a) ^{1}H NMR of D3G



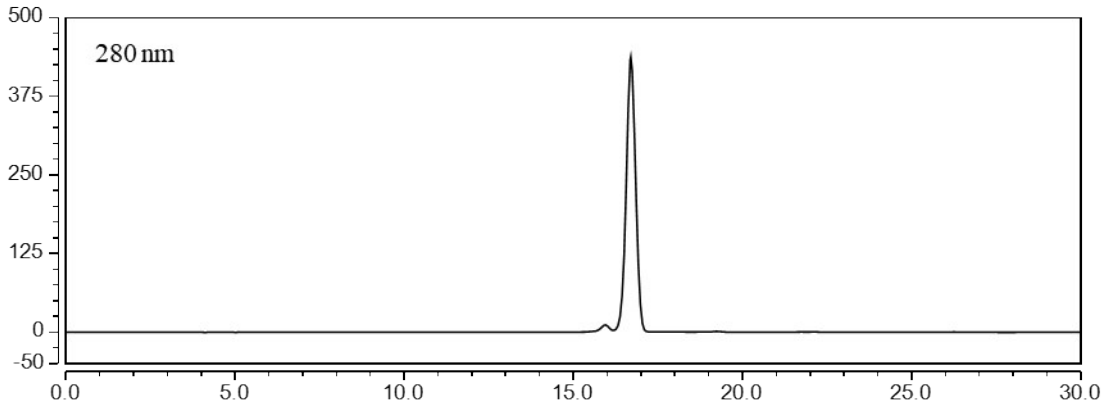
(b) ^{13}C NMR for D3G



(c) MS of D3G

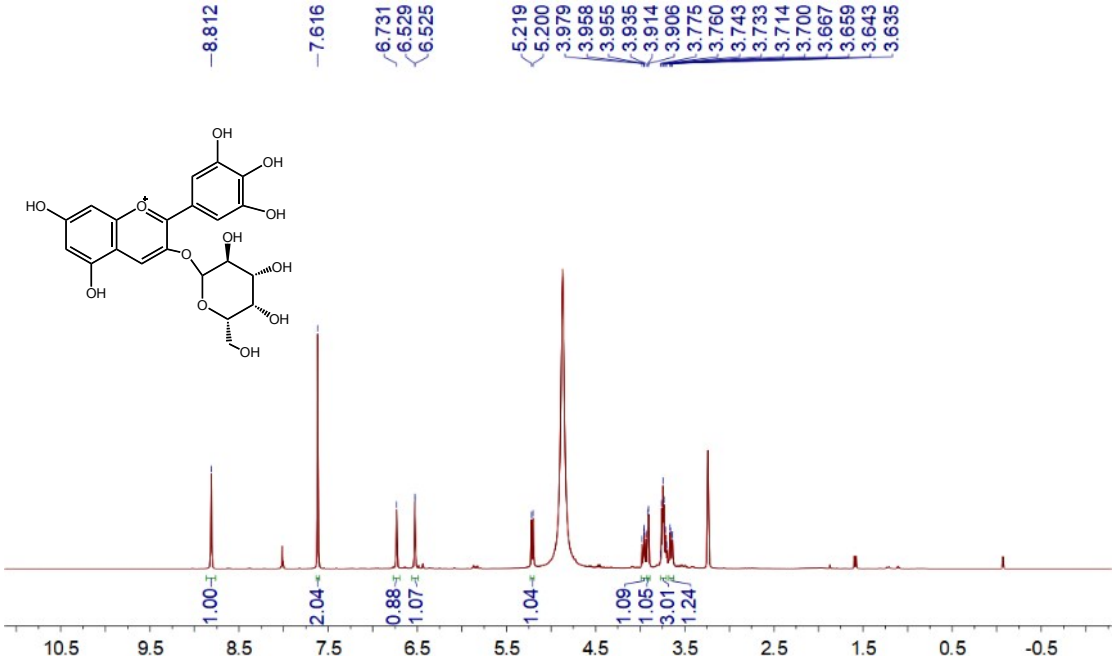


(d) MS/MS of D3G

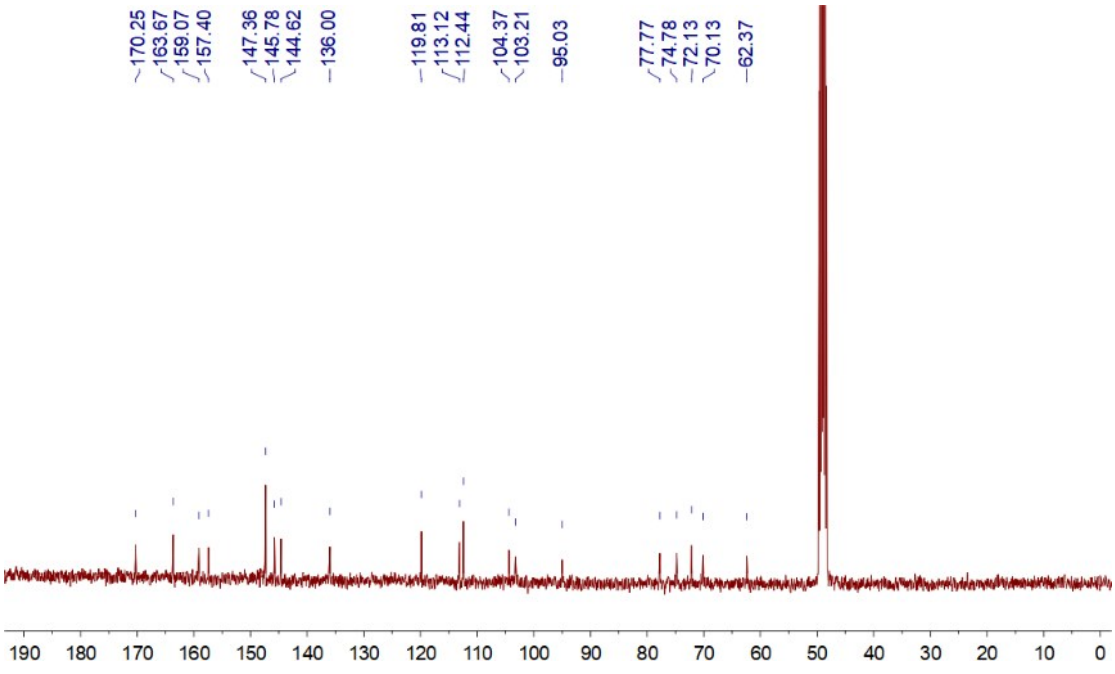


(e) HPLC for purified D3G

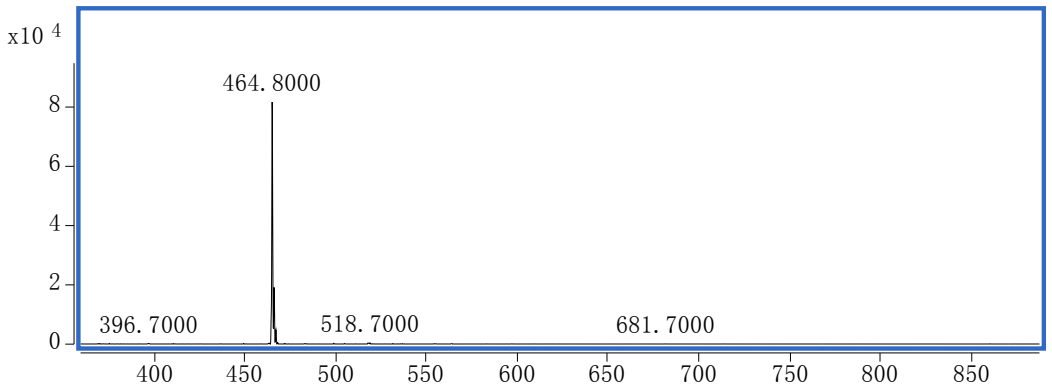
Fig. S14



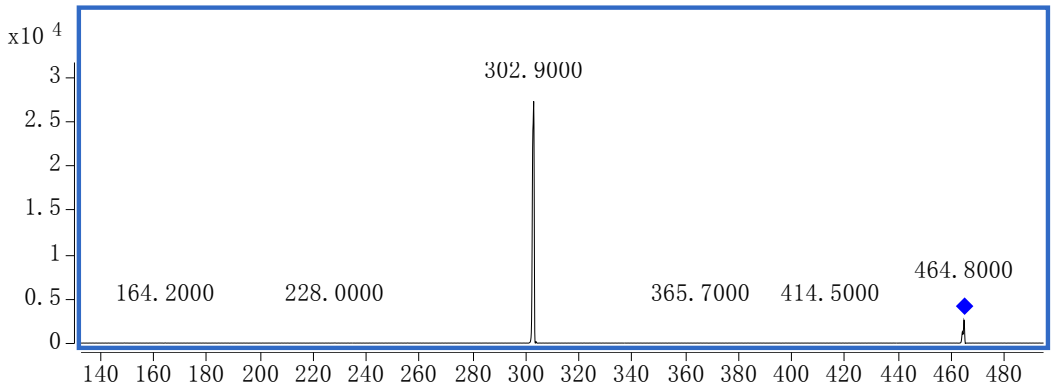
(a) ^{1}H NMR of D3Ga



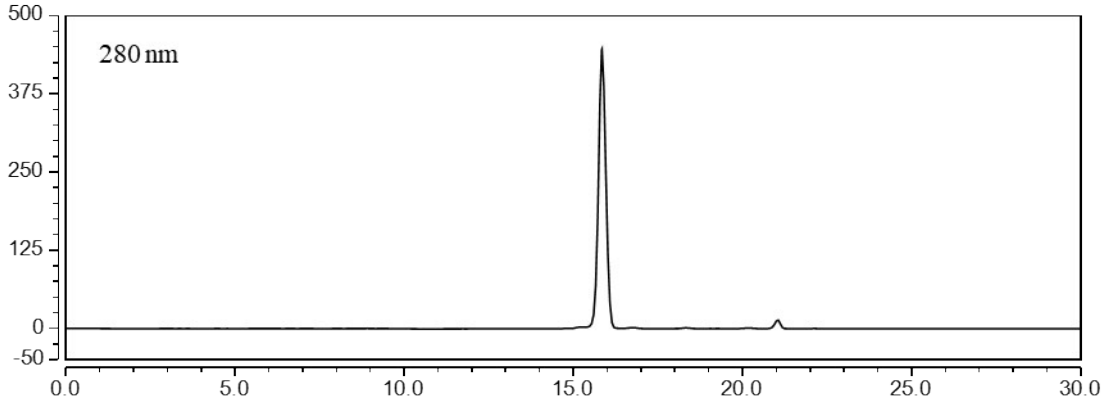
(b) ^{13}C NMR for D3Ga



(c) MS of D3Ga

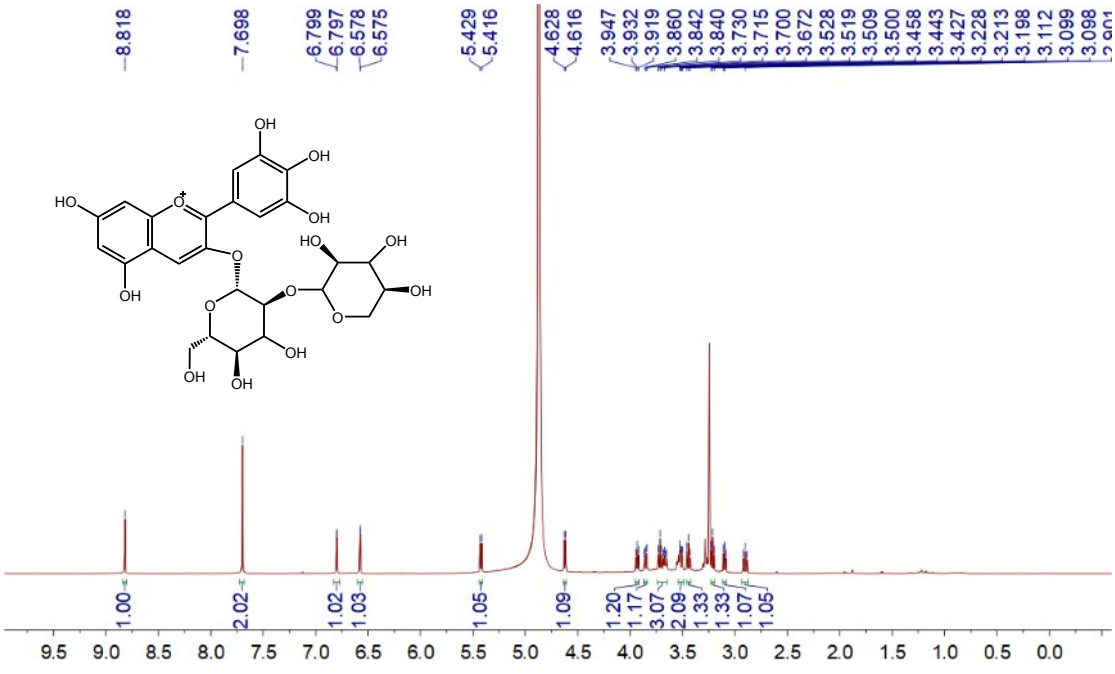


(d) MS/MS of D3Ga

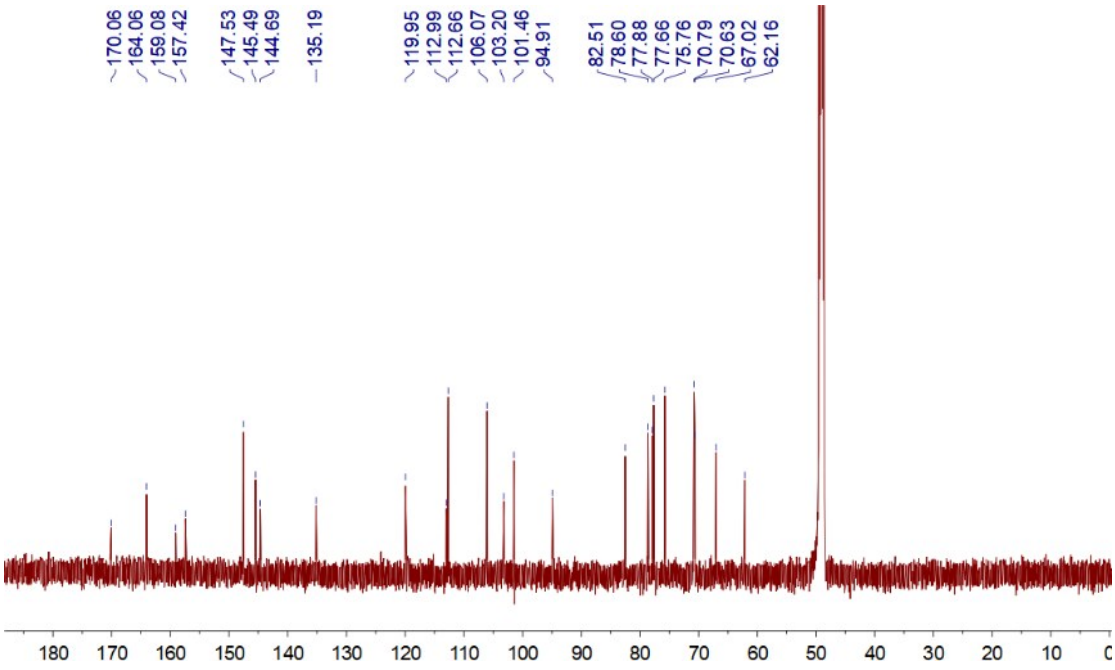


(e) HPLC for purified D3Ga

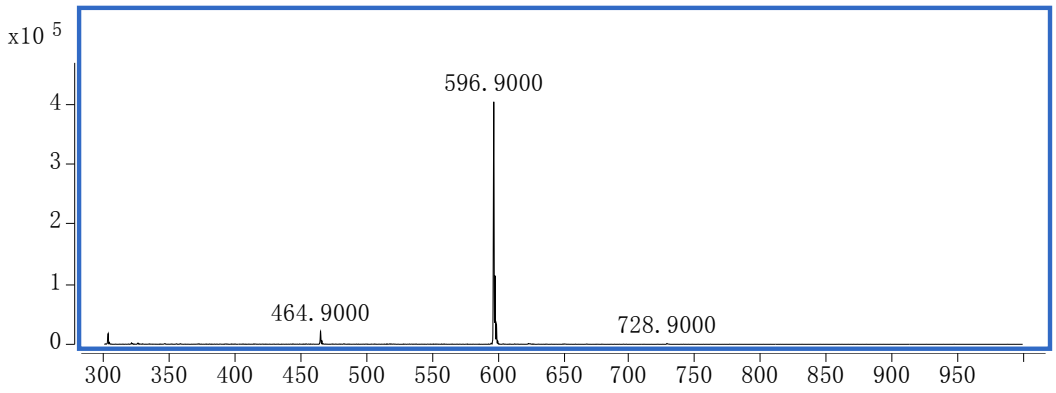
Fig. S15



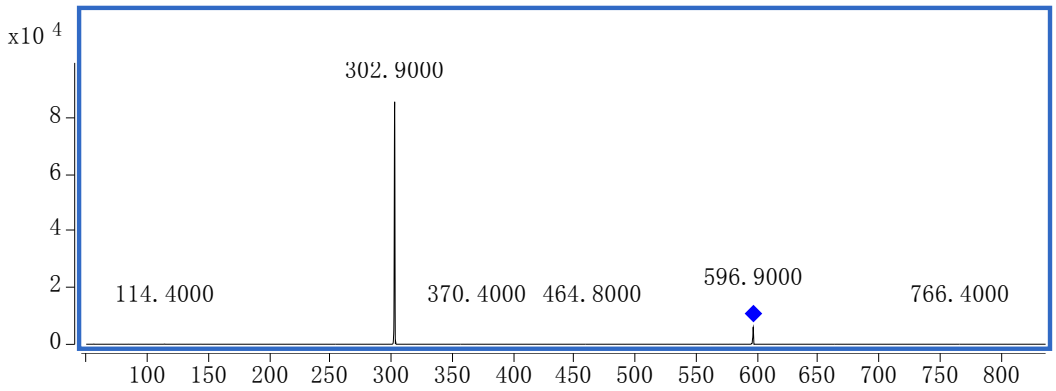
(a) ^{1}H NMR of D3S



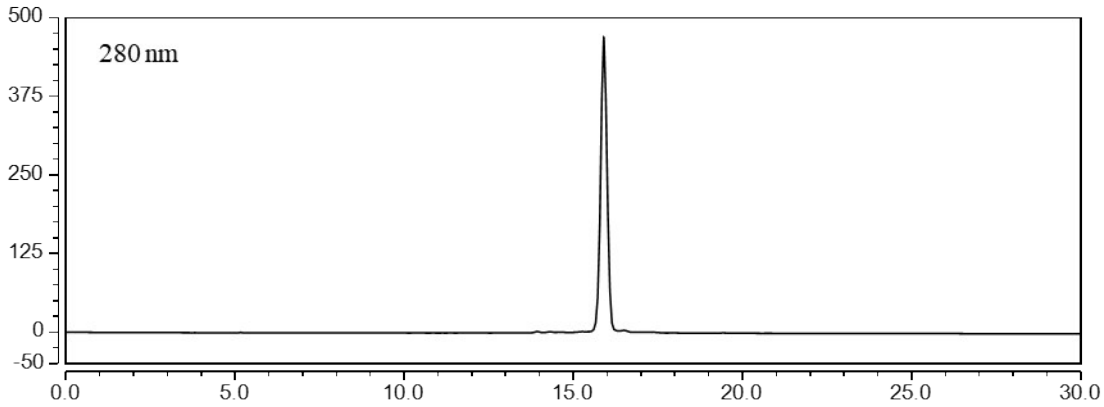
(b) ^{13}C NMR for D3S



(c) MS of D3S

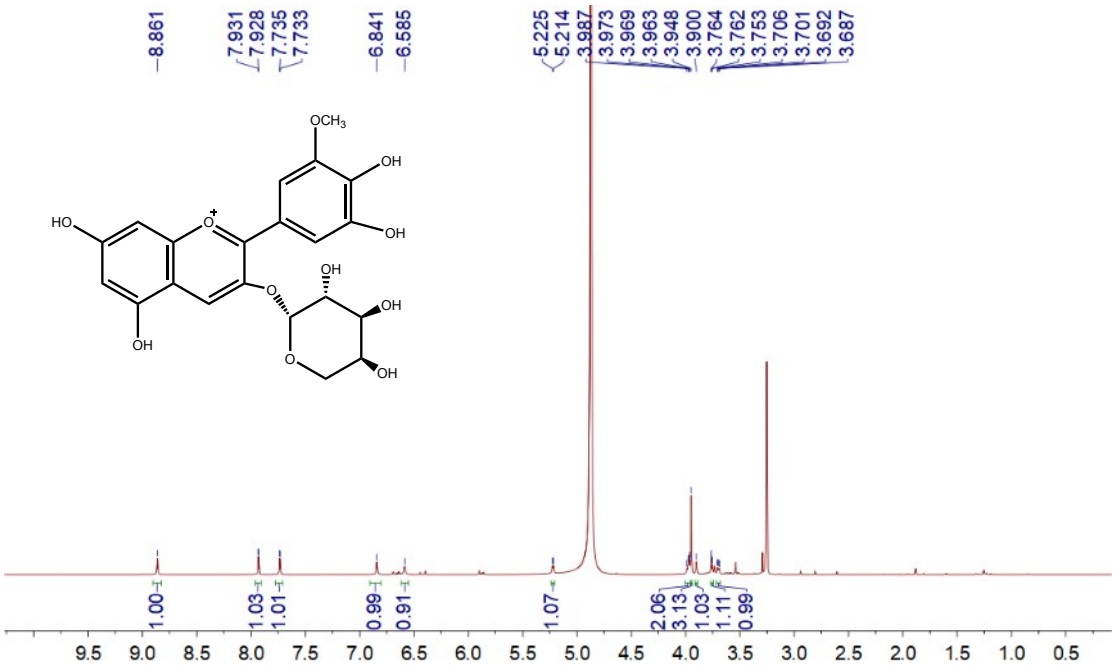


(d) MS/MS of D3S

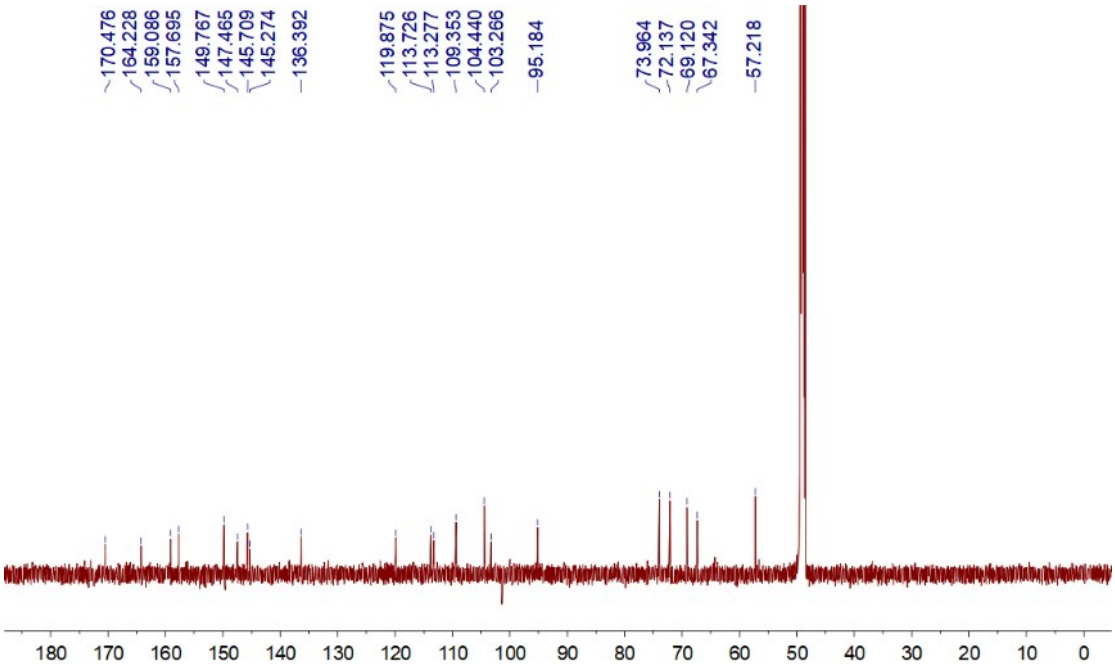


(e) HPLC for purified D3S

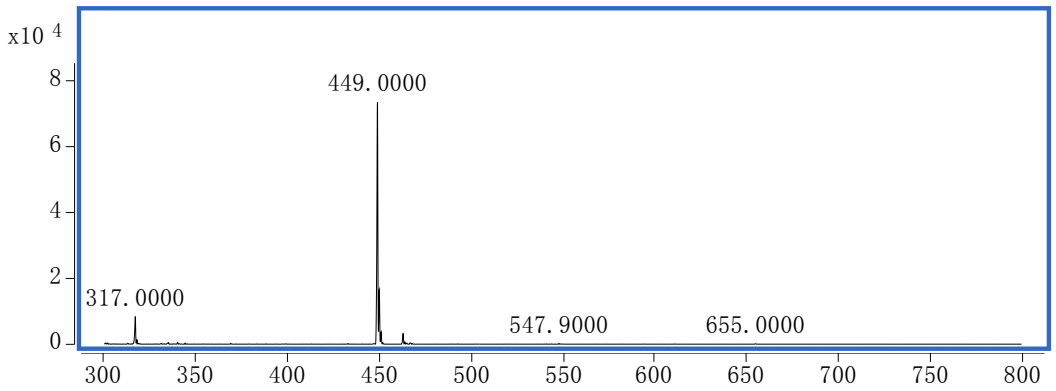
Fig. S16



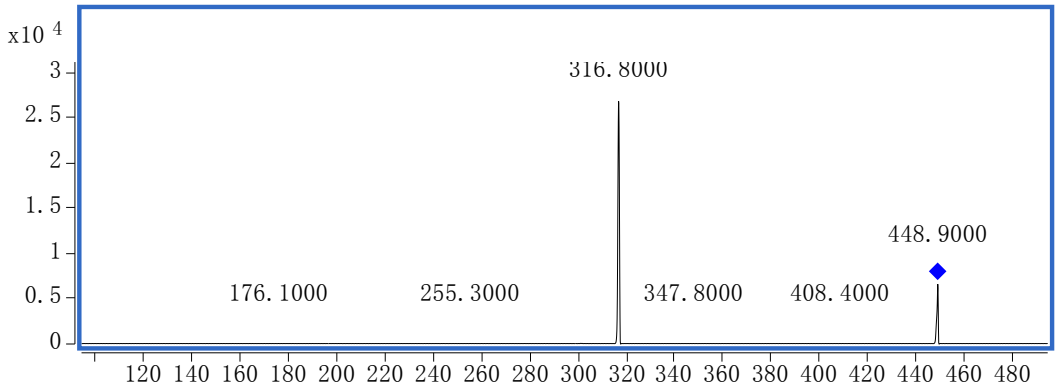
(a) ^{1}H NMR of Pet3A



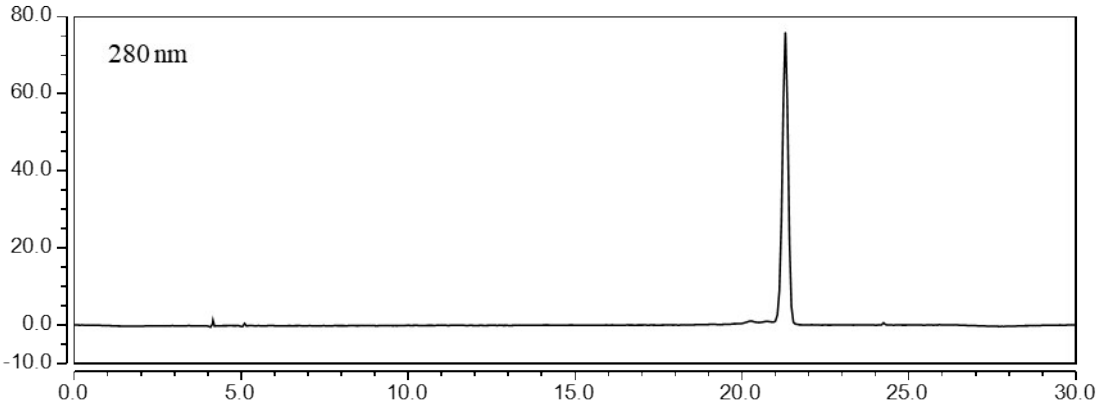
(b) ^{13}C NMR for Pet3A



(c) MS of Pet3A

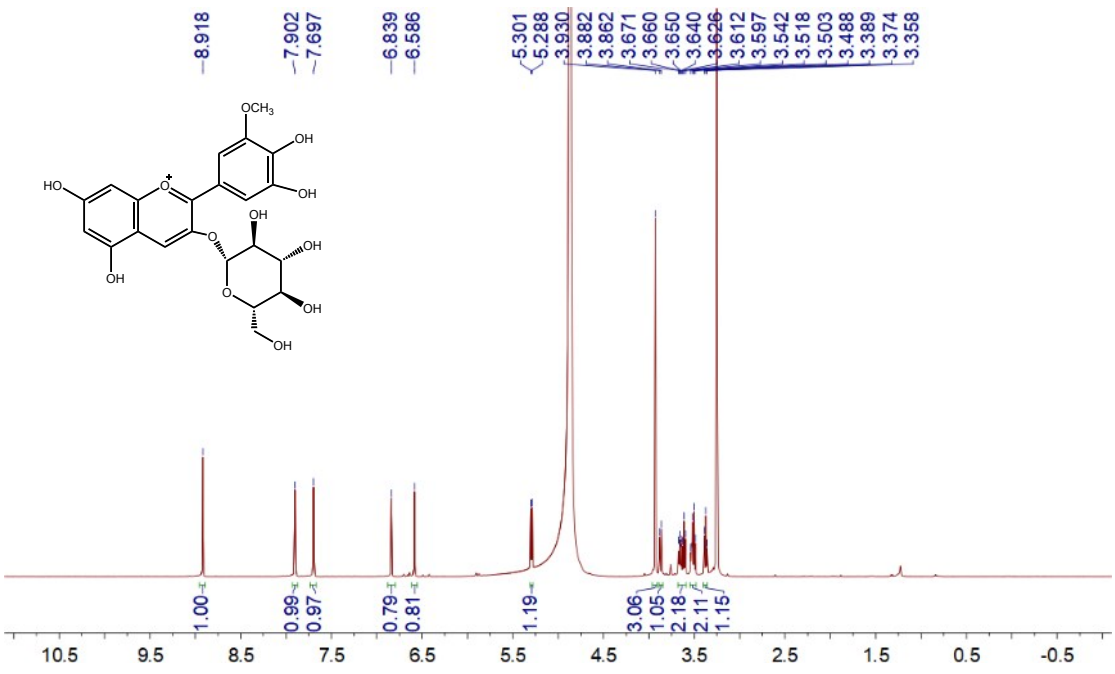


(d) MS/MS of Pet3A

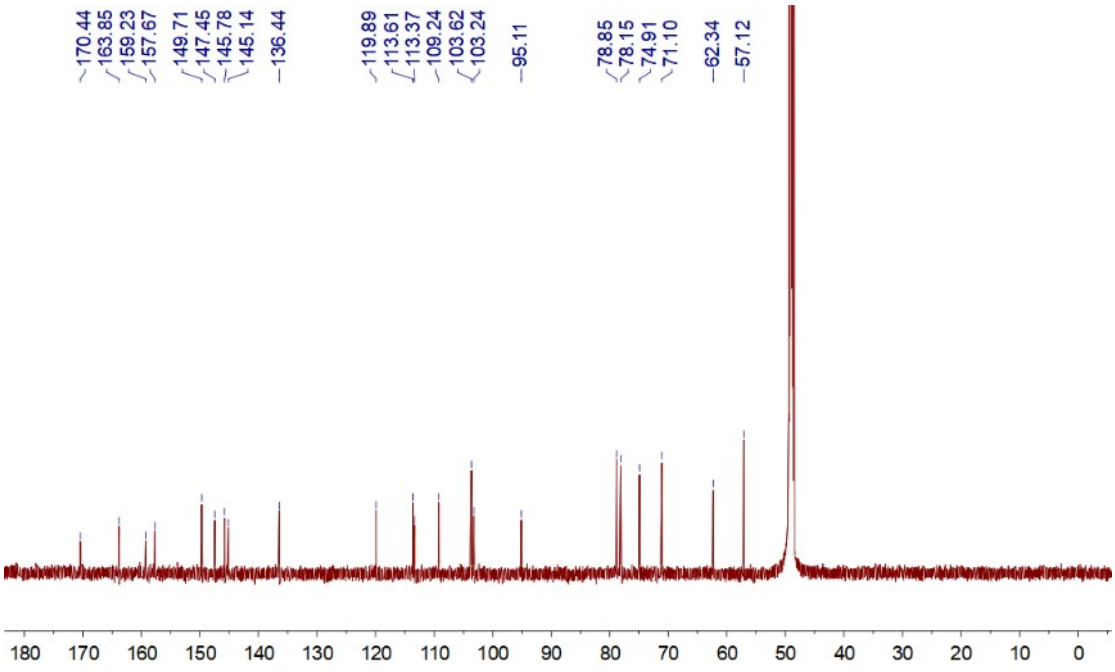


(e) HPLC for purified Pet3A

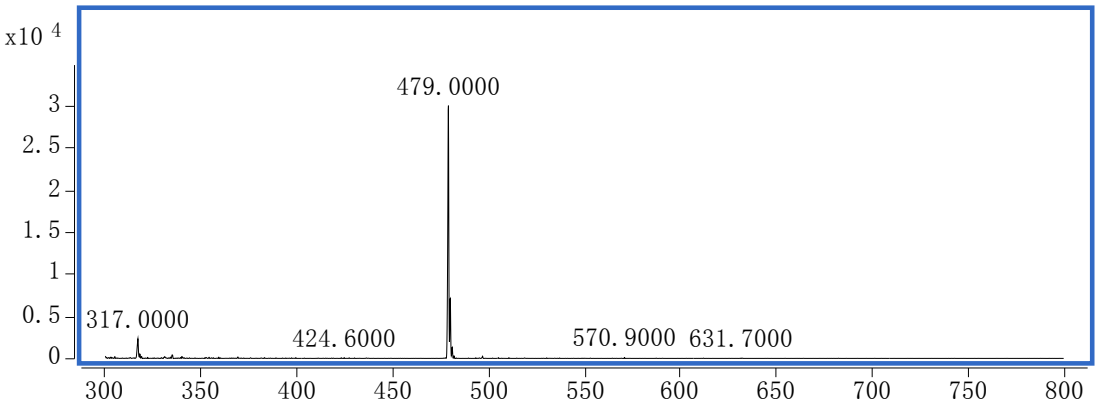
Fig. S17



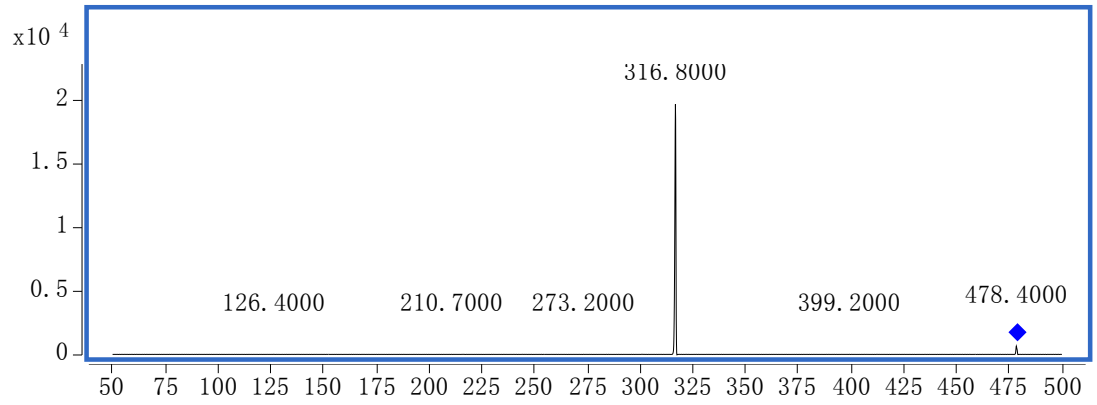
(a) ^{1}H NMR of Pet3G



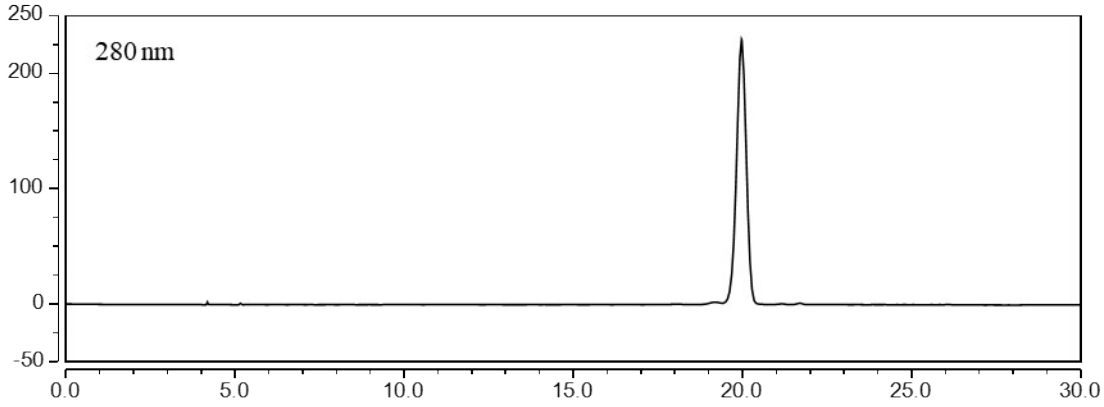
(b) ^{13}C NMR for Pet3G



(c) MS of Pet3G

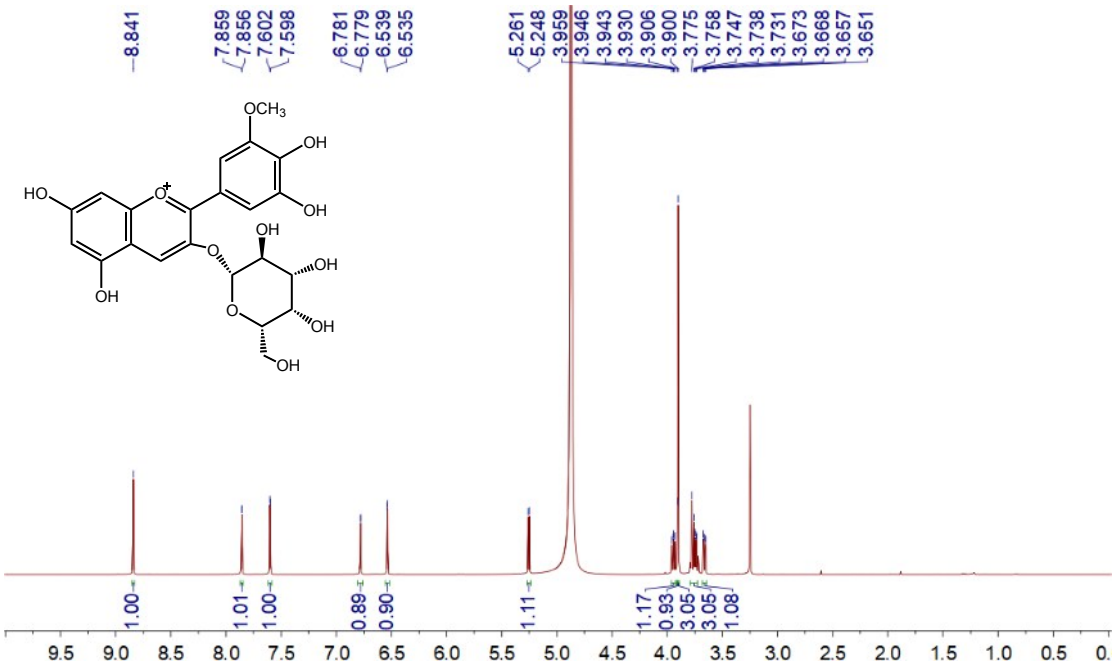


(d) MS/MS of Pet3G

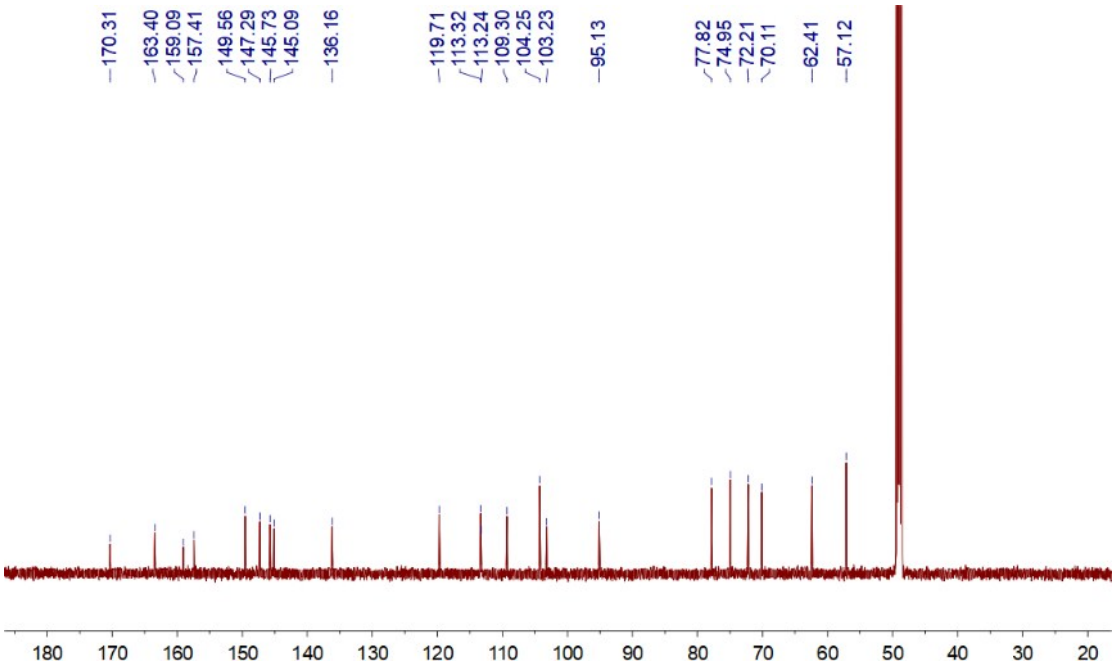


(e) HPLC for purified Pet3G

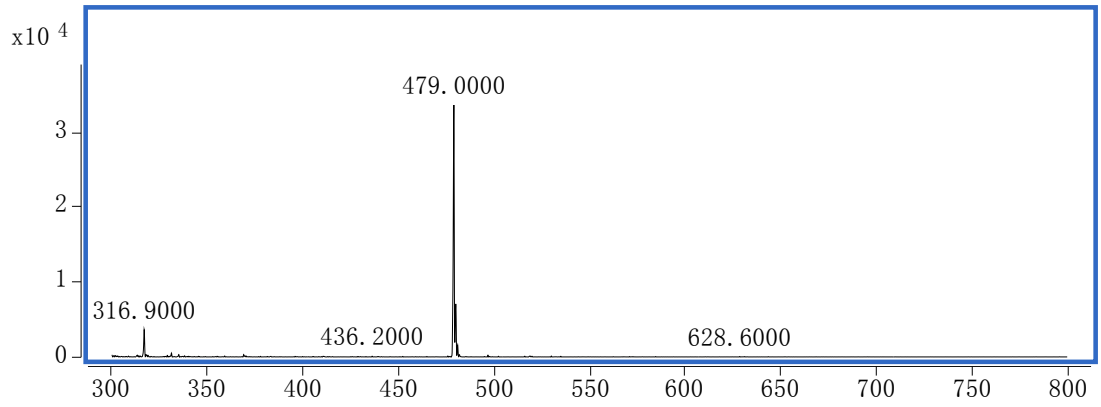
Fig. S18



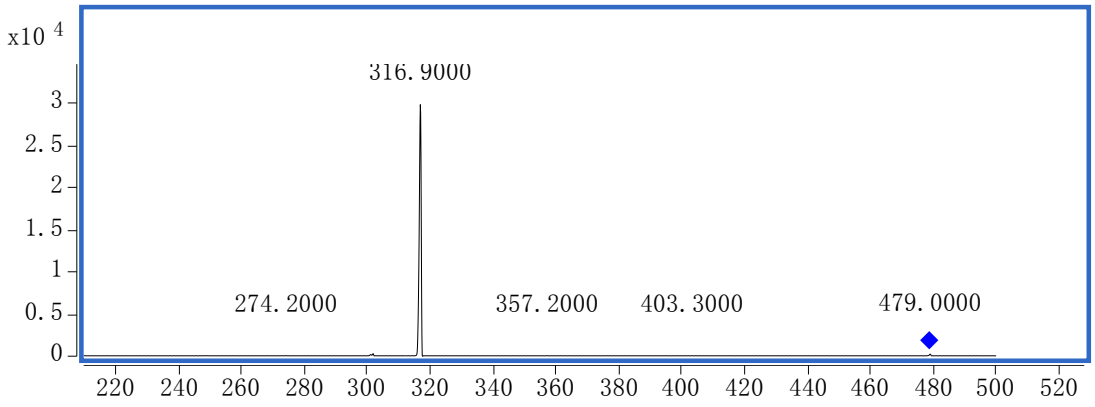
(a) ^{1}H NMR of Pet3Ga



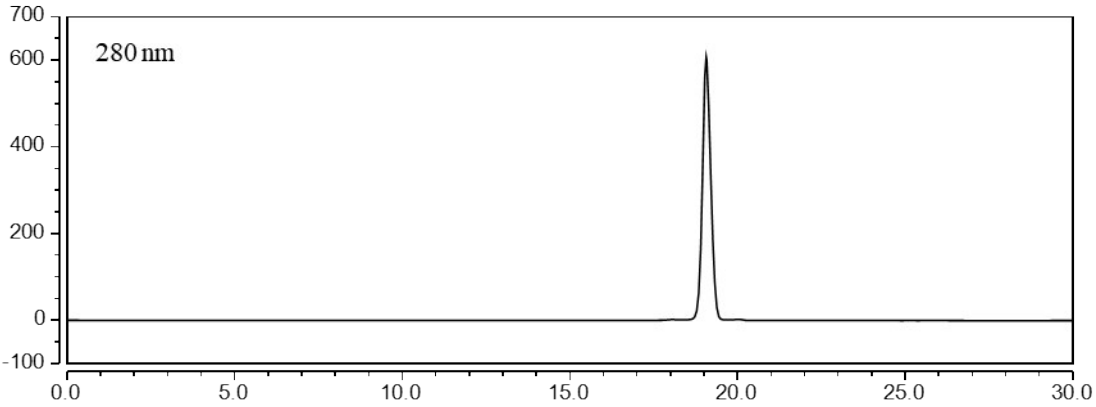
(b) ^{13}C NMR for Pet3Ga



(c) MS of Pet3Ga

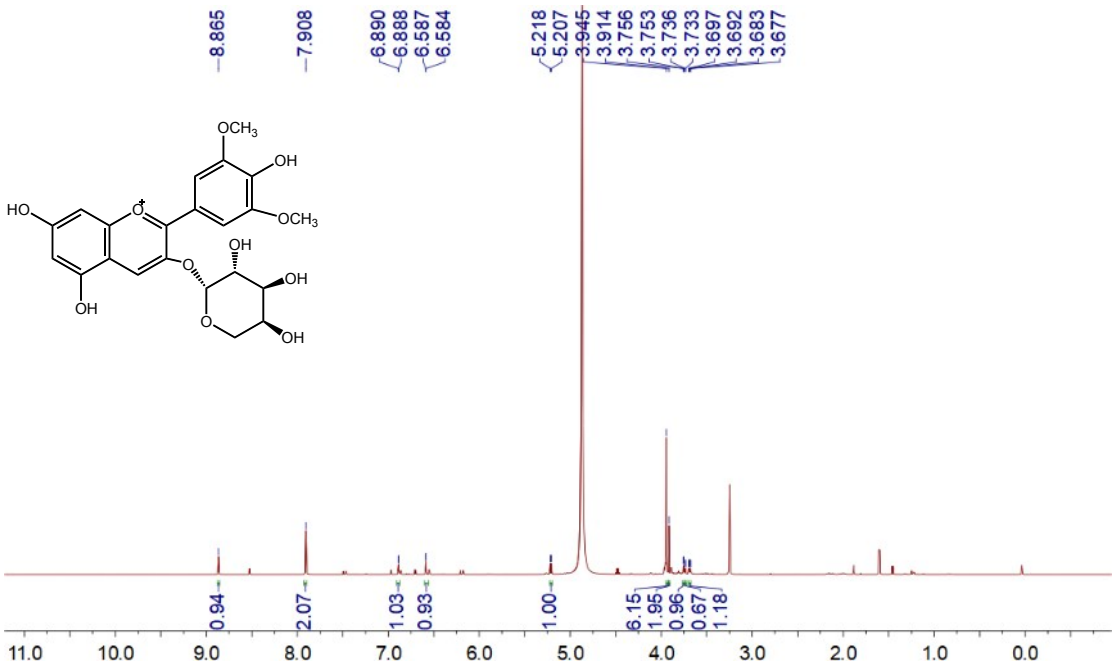


(d) MS/MS of Pet3Ga

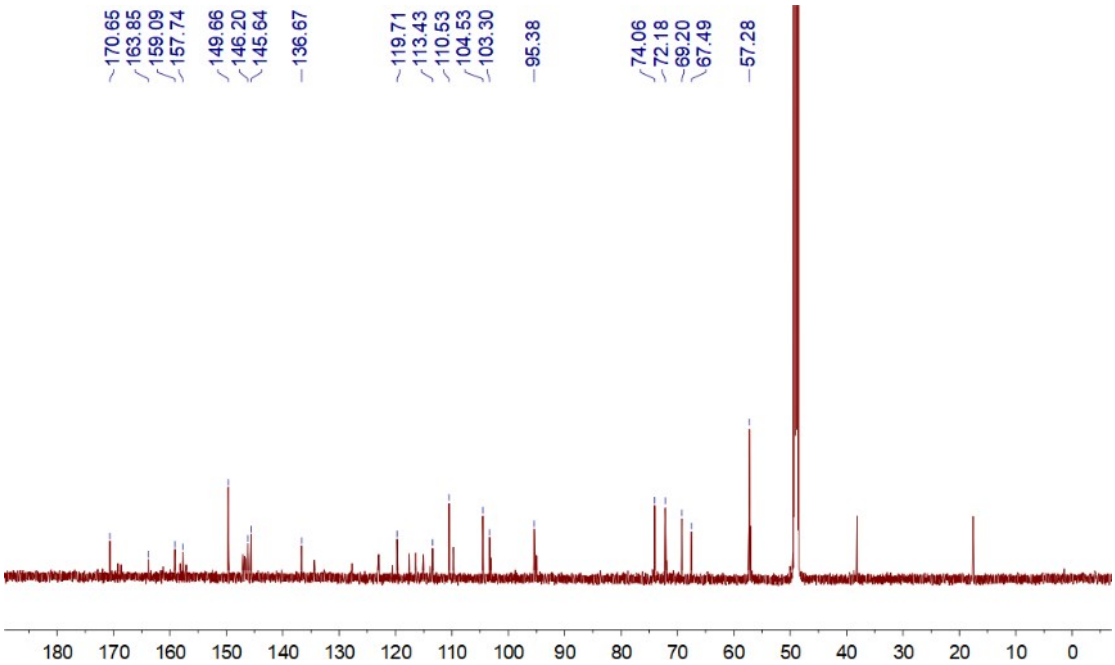


(e) HPLC for purified Pet3Ga

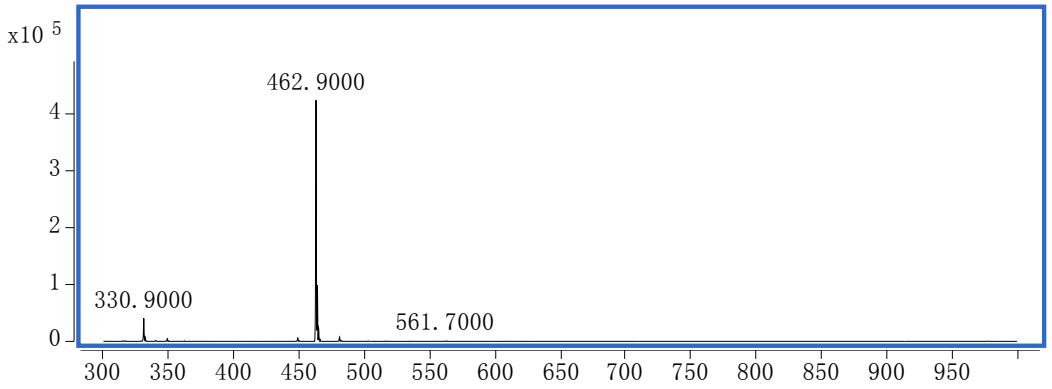
Fig. S19



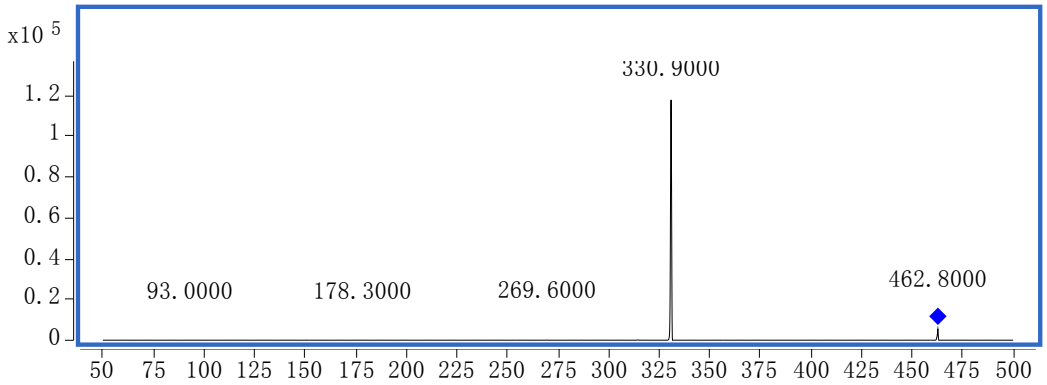
(a) ^{1}H NMR of M3A



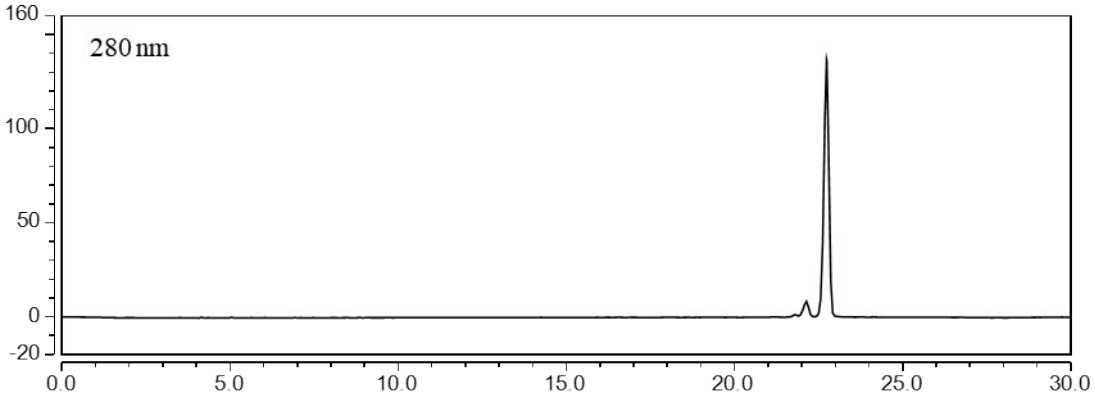
(b) ^{13}C NMR for M3A



(c) MS of M3A

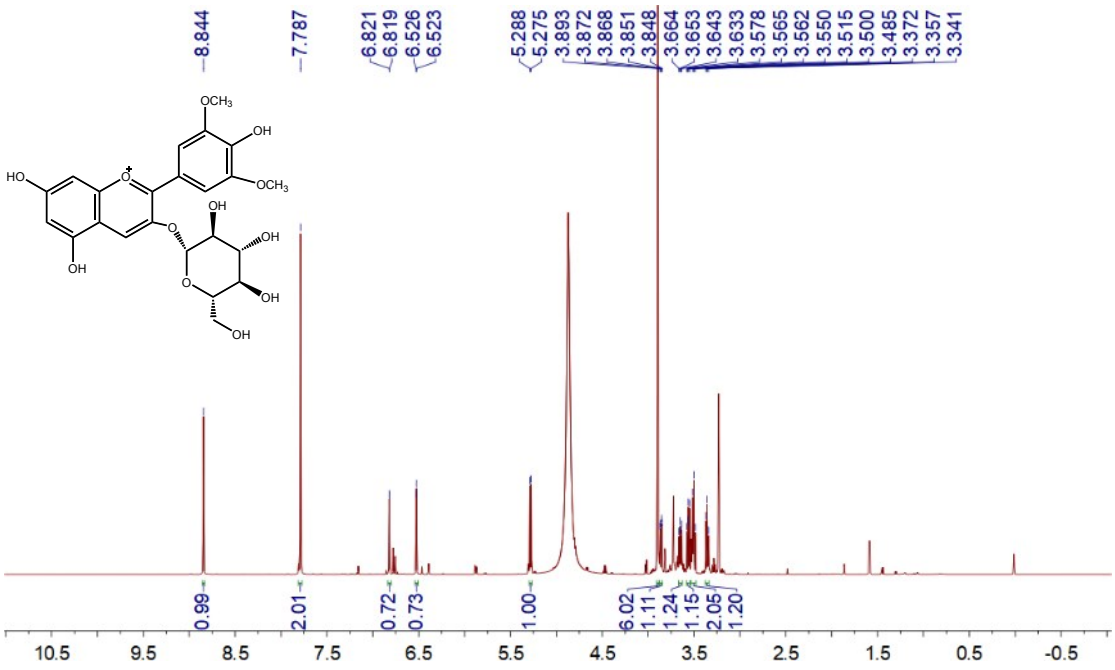


(d) MS/MS of M3A

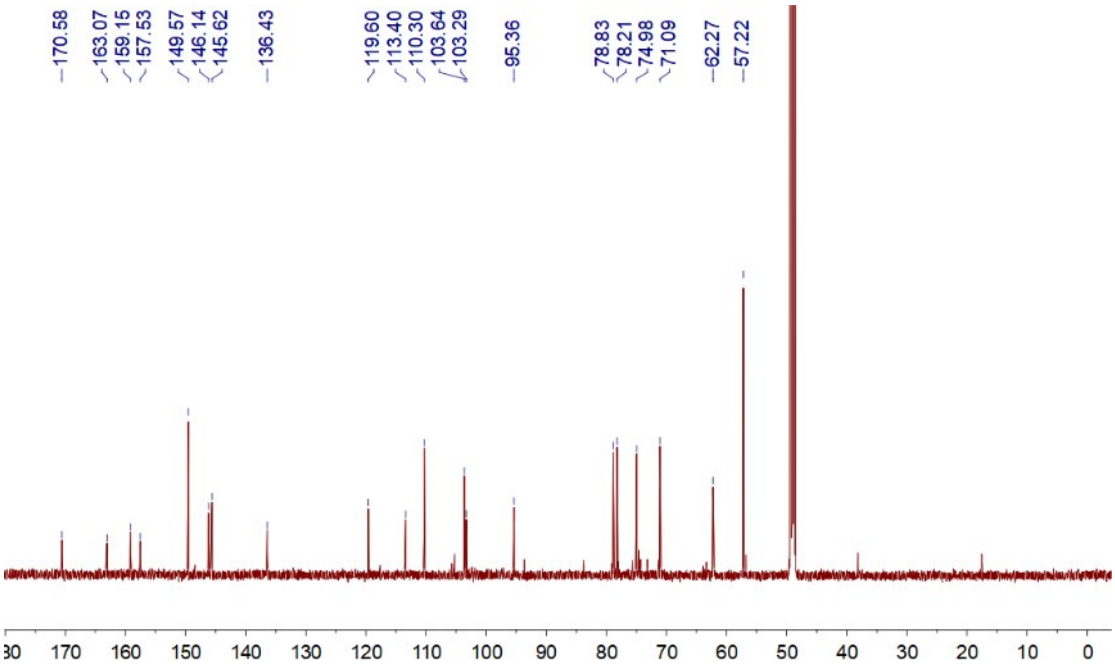


(e) HPLC for purified M3A

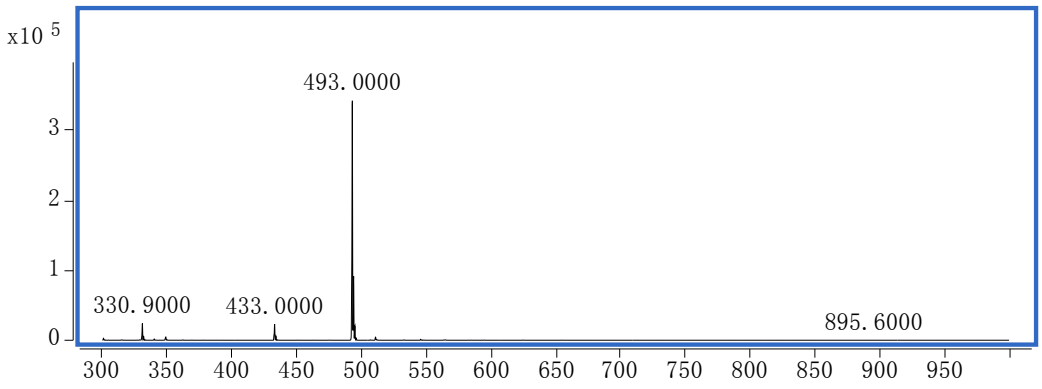
Fig. S20



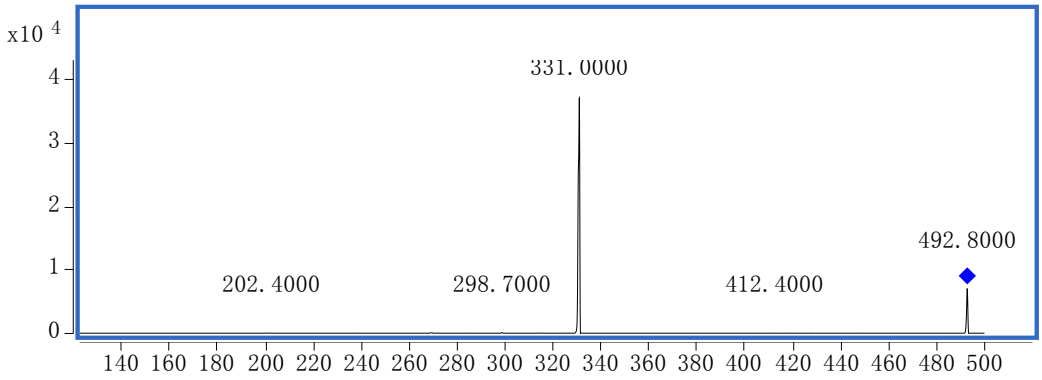
(a) ^{1}H NMR of M3G



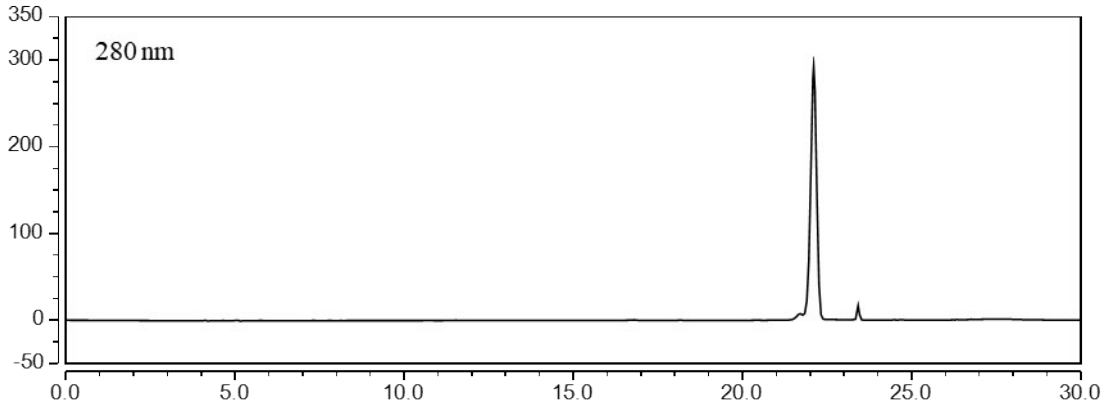
(b) ^{13}C NMR for M3G



(c) MS of M3G

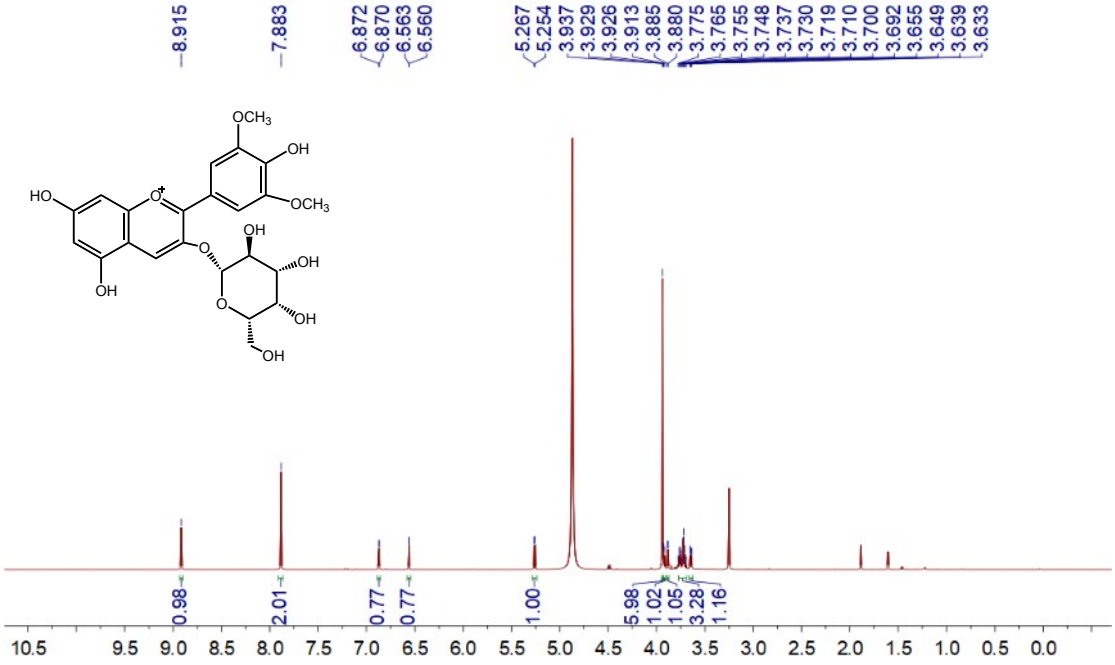


(d) MS/MS of M3G

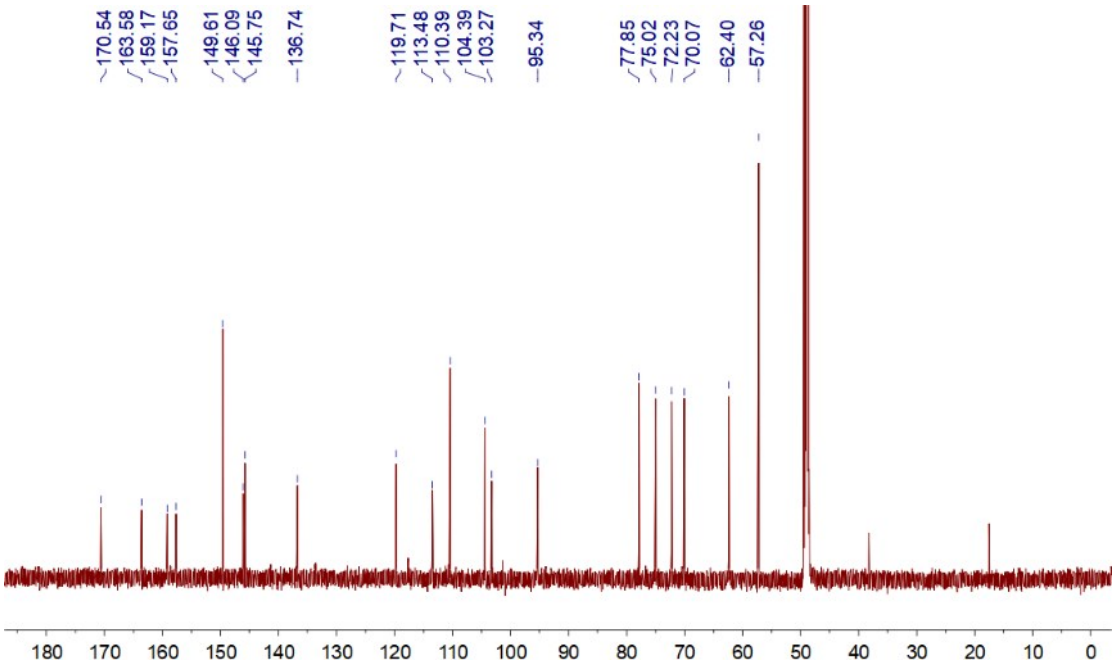


(e) HPLC for purified M3G

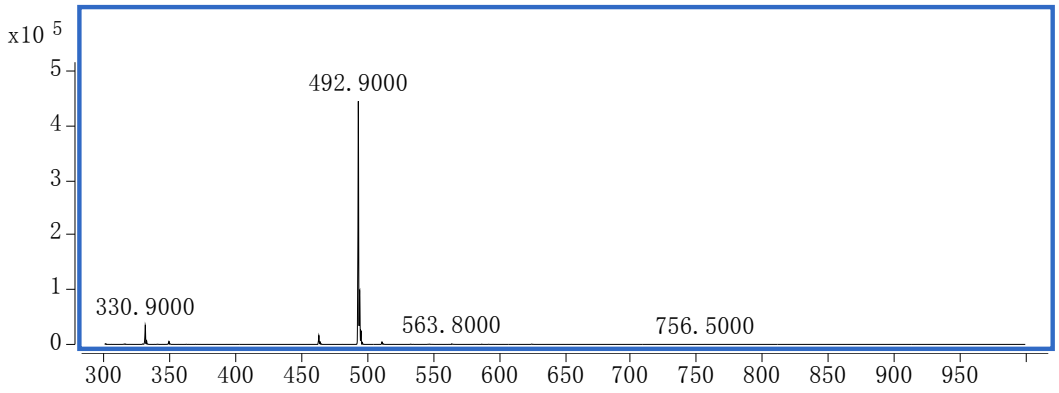
Fig. S21



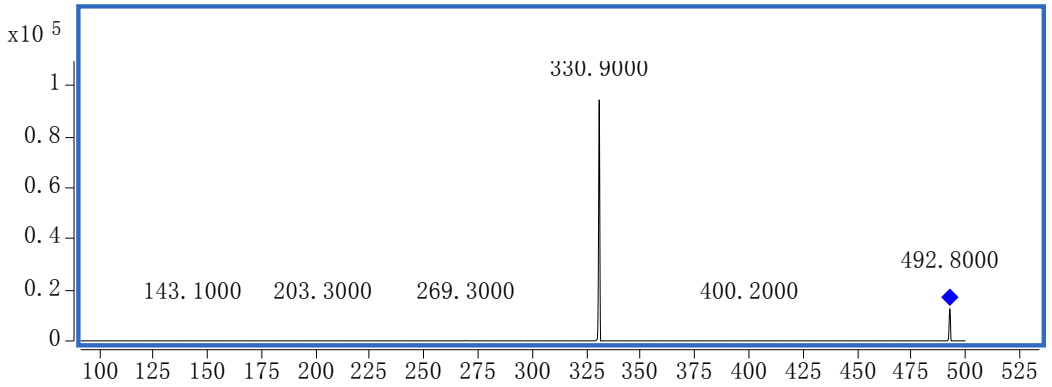
(a) ^{1}H NMR of M3Ga



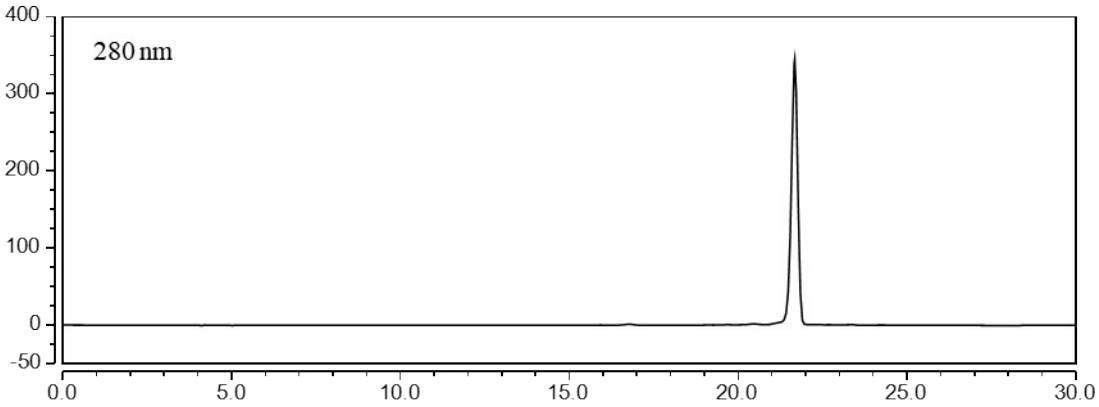
(b) ^{13}C NMR for M3Ga



(c) MS of M3Ga

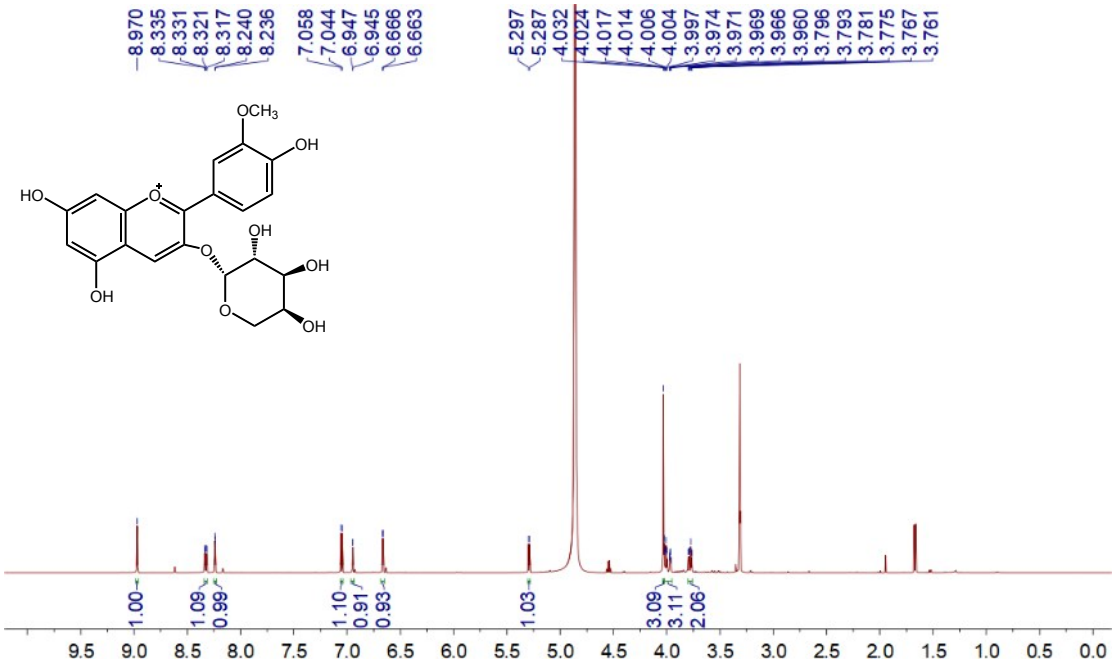


(d) MS/MS of M3Ga

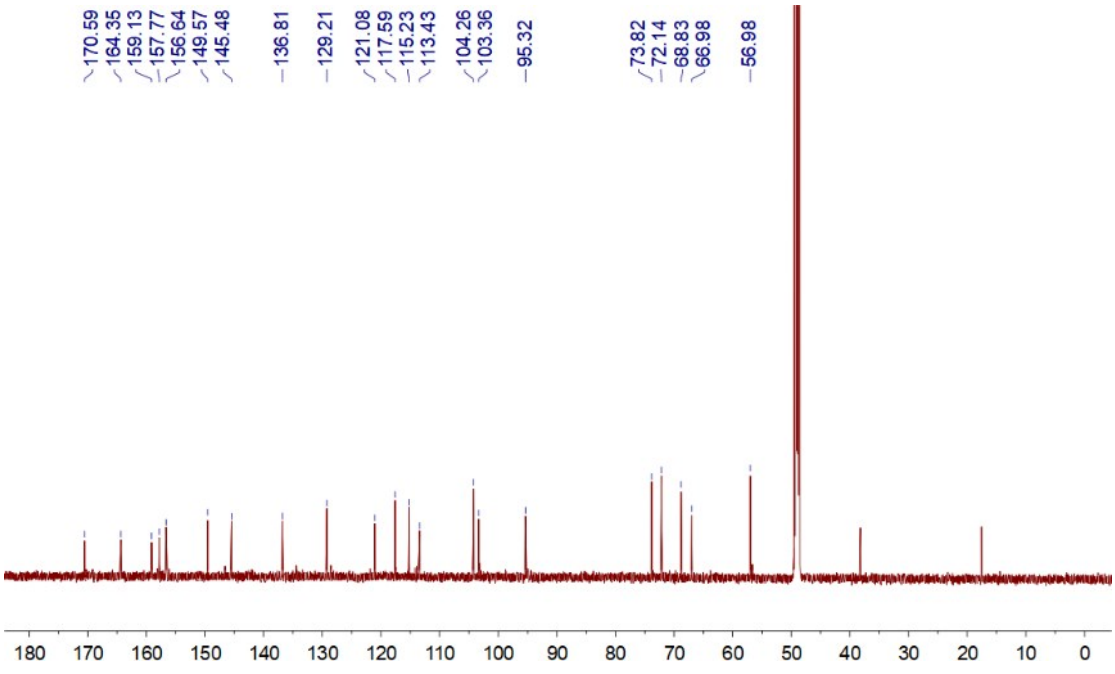


(e) HPLC for purified M3Ga

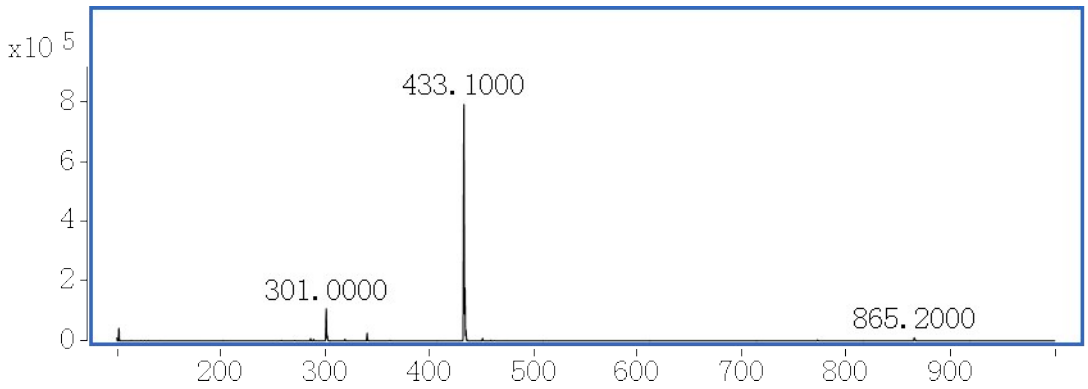
Fig. S22



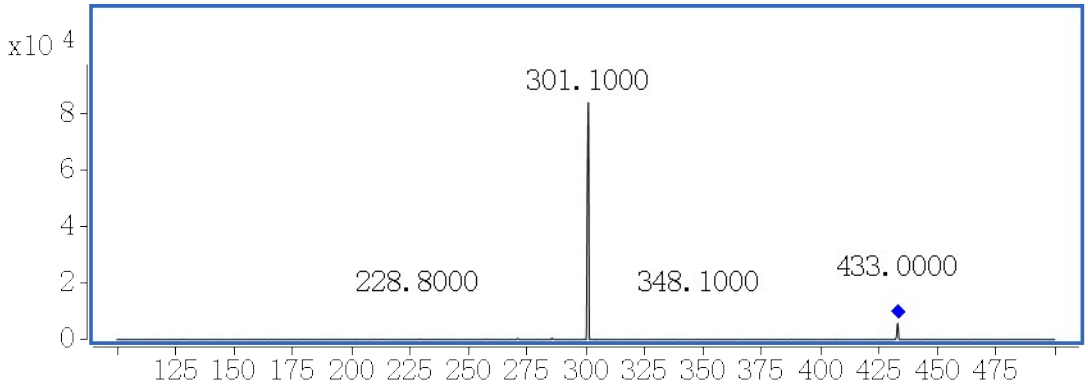
(a) ^{1}H NMR of Peo3A



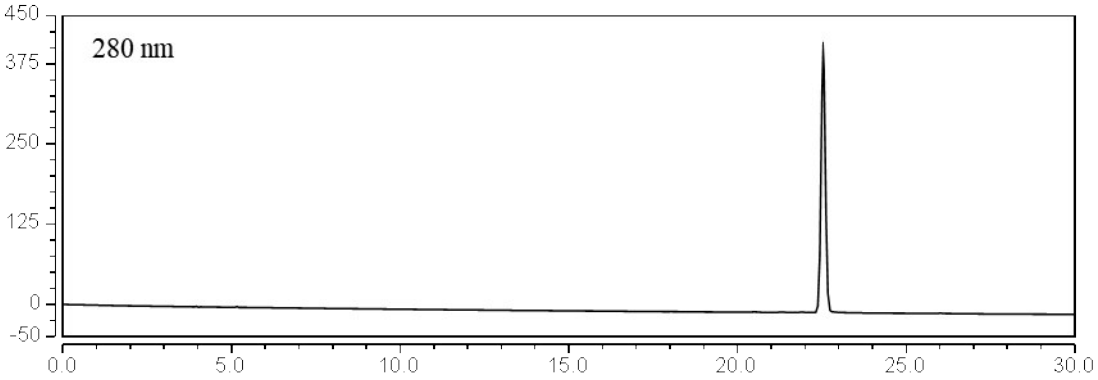
(b) ^{13}C NMR for Peo3A



(c) MS of Peo3A

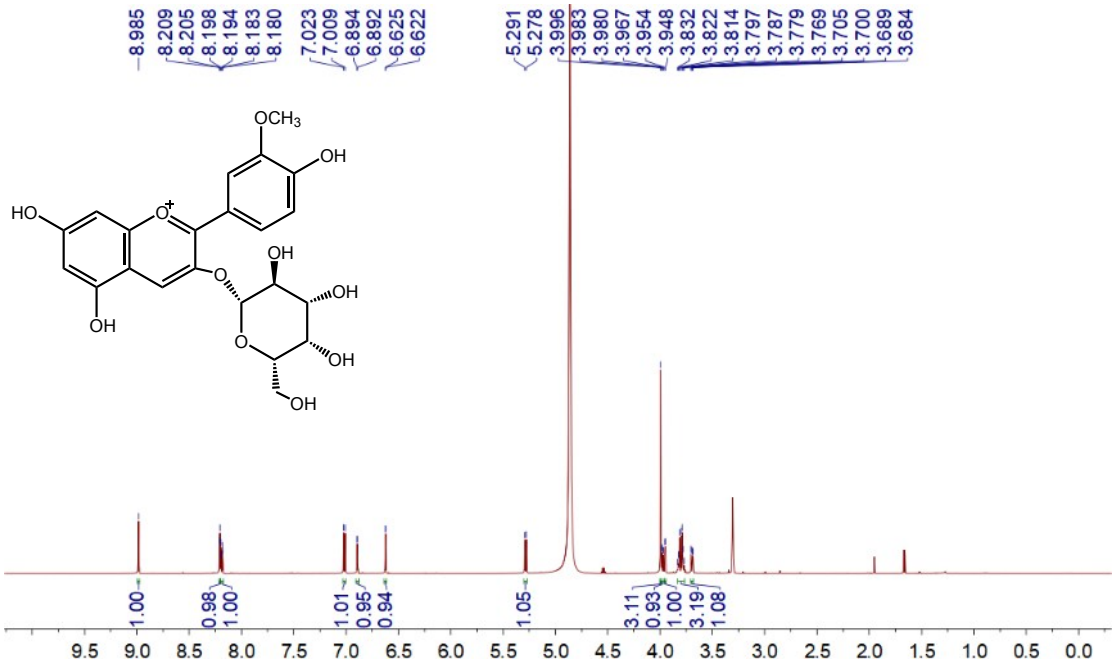


(d) MS/MS of Peo3A

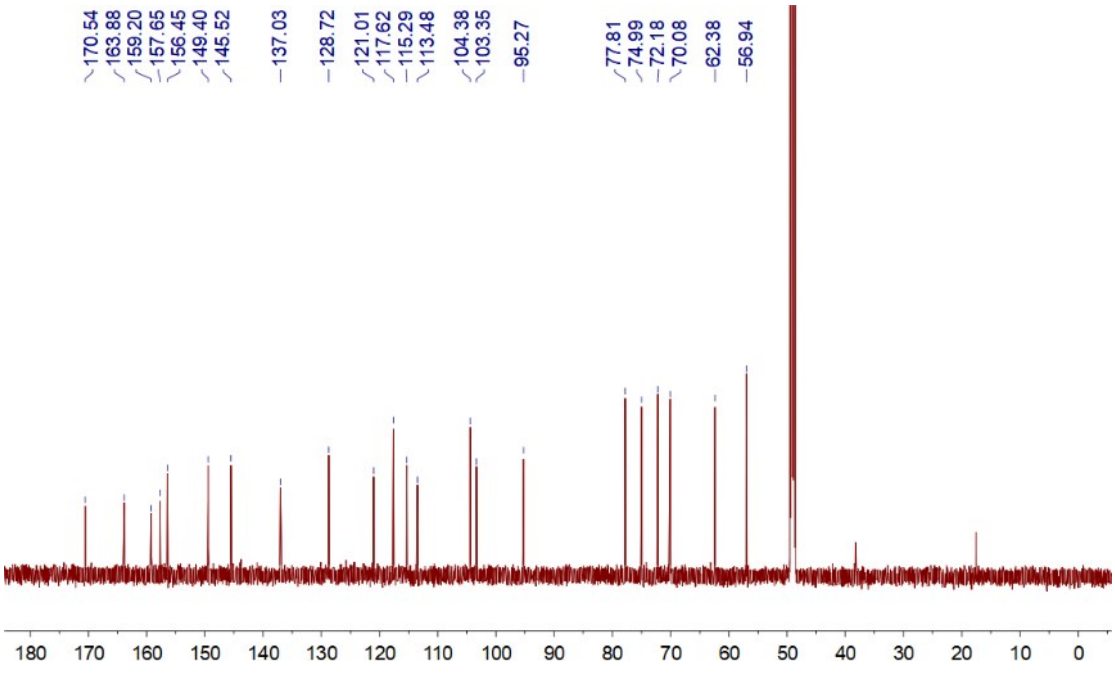


(e) HPLC for purified Peo3A

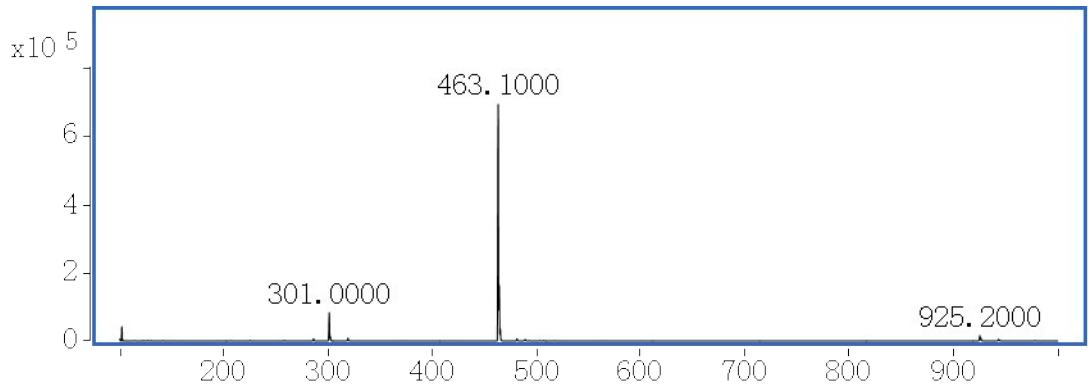
Fig. S23



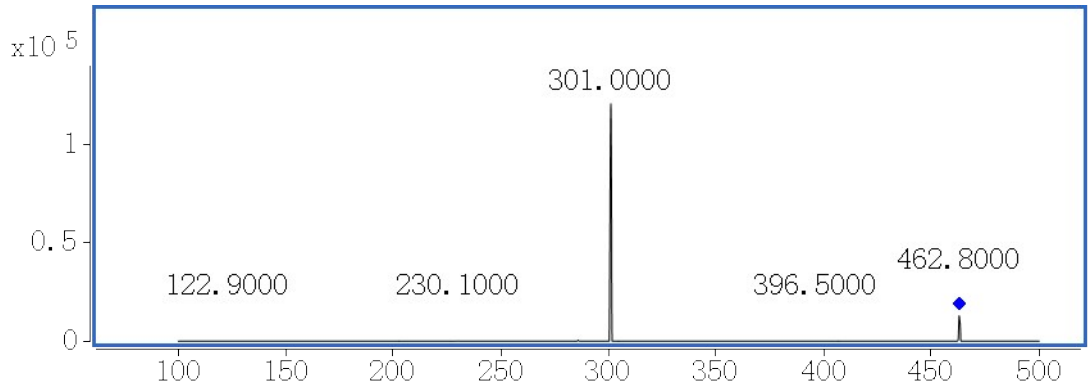
(a) ^{1}H NMR of Peo3Ga



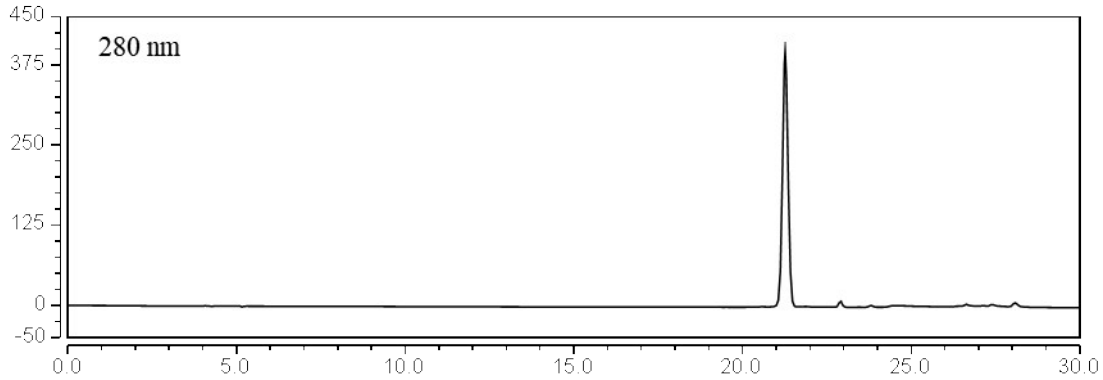
(b) ^{13}C NMR for Peo3Ga



(c) MS of Peo3Ga



(d) MS/MS of Peo3Ga



(e) HPLC for purified Peo3Ga

5. References

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