# A new triterpenoid saponin from *Pulsatilla cernua* predicted by NMR-based mosaic method

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**Abstract**

A saponin (**1**) with gypsogenin as aglycone was isolated from the roots of *Pulsatilla cernua*. The aglycone of compound **1** was considered as gypsogenin which was rarely found in this genus. Its structure was predicted by NMR-based “mosaic” method rapidly, and further confirmed on the basis of spectroscopic data, including 2D NMR spectra and chemical evidence. This work suggested that NMR-based mosaic method is suitable for most of saponins from common species of genus *Pulsatilla*.

## Experimental Section

### General Experimental Procedures.

The NMR spectra were measured in pyridine-$d_5$, on a Bruker AV600 instrument. HR-TOF-MS spectra were performed on Agilent LC/MS spectrometer (Agilent, USA). HPLC was performed on JAI LC9103 Recycling preparative HPLC (Japan Analytical Industries, Japan) equipped with JAIGEL-ODS-AP-P column and JAIGEL-GS310 column using a JAI refractive index detector and a JAI UV-3702 detector with MultiChro 2000 workstation. TLC was performed on pre-coated $GF_{254}$ plates (Merck, Germany) and detected by spraying with 10 % $H_2SO_4$ followed heating. GC analyses were performed using an Agilent GC 6890 instrument on an HP-5 column (320 μm × 30 m, 0.25 μm).

### Materials.

The roots of *P. cernua* were collected in May 2011 at Qingyuan (*N* 42°10.750′, *E* 125°03.323′, *H* 385m), Fushun, Liaoning, China, and authenticated by Professor Jin-Cai Lu (The School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University). A voucher specimen has been deposited in our laboratory (voucher No. pc-2011-001).

### Extraction and isolation.

The dry roots of *P. cernua* (40 kg) were extracted three times with 70 % aqueous ethanol under reflux. The extracts were combined and concentrated under a vacuum to yield a residue (4 kg), which was then suspended in water and partitioned sequentially with petroleum ether, EtOAc and n-BuOH. The n-BuOH extract part (550 g) was chromatographed over a D101 macroporous resin column (15 × 150 cm), eluted successively with aqueous ethanol (water, 30% EtOH, 50 % EtOH, 70 % EtOH and 95 % EtOH, respectively) as reported in our previous study (Fan et al., 2013).

The 70% EtOH crude extract (150 g) was chromatographed on P-HPLC using reversed phase chromatographic column (ODS) with MeOH-$CH_3CN$-$H_2O$ (3:2:1) at 210 nm wavelength to obtain two main fractions.The flow rate was 5 mL $min^{-1}$. The first fraction was further purified by P-HPLC using normal phase chromatographic column (GS310) with MeOH-n-Hexane (5:3) to yield **1**.

### NMR measurement.

NMR measurement were recorded at 600 MHz 1H frequency and carried out at 298.2 K. Chemical shifts (δ) are expressed in ppm with reference to the residual solvent signals, and coupling constants (J) are given in Hz. The 1D $^1H$ NMR spectra were recorded, an acquisition time of 2.7264 s, and a relaxation delay of 60 s.

### Pulsatillacernuoside G (1)

White, amorphous powder; $[\alpha]_D^{20}$ +50(c 0.10, MeOH); (+)-HR-ESI-MS *m/z* 1403.6454 $[M+Na]^+$ (calcd for $C_{65}H_{104}O_{31}Na$, 1403.6459); $^1H$ NMR (600 MHz,

pyridine-$d_5$) δ 9.67 (1H, s, H-23), 6.18 (1H, d, *J*=8.4 Hz, glc4-1), 6.06 (1H, br s, rha1-1), 5.80 (1H, br s, rha3-1), 5.30 (1H, s, H-12), 5.10 (1H, d, *J*=7.8 Hz, glc1-1), 4.95 (1H, d, *J*=8.4 Hz, glc5-1), 4.94 (1H, m, glc4-5), 4.70 (1H, m, H-3), 4.65 (1H, m, rha1-2), 4.65 (1H, m, rha3-2), 4.64 (1H, m, glc4-6b), 4.60 (1H, d, *J*=6.0 Hz, ara-1), 4.59 (1H, m, rha1-5), 4.55 (1H, m, ara-3), 4.55 (1H, m, rha1-3), 4.55 (1H, m, rha3-3), 4.49 (1H, m, glc1-6b), 4.44 (1H, m, ara-5b), 4.42 (1H, m, ara-2), 4.40 (1H, m, glc4-3), 4.35 (1H, m, glc1-6a), 4.35 (1H, m, rha1-4), 4.35 (1H, m, rha3-1) 4.33 (1H, m, glc4-6a), 4.33 (1H, m, glc4-4), 4.25 (1H, m, ara-4), 4.20 (1H, m, glc5-6b), 4.20 (1H, m, glc1-3), 4.20 (1H, m, glc1-4), 4.15 (1H, m, glc4-2), 4.13 (1H, m, glc5-3), 4.09 (1H, m, glc5-4), 4.09 (1H, m, glc5-6a), 4.02 (1H, m, glc1-2), 3.93 (1H, m, glc5-2), 3.92 (1H, m, glc4-5), 3.90 (1H, m, glc1-5), 3.69 (1H, d, *J*=5.0 Hz, ara-5a), 3.62 (1H, m, glc5-5), 3.08 (1H, dd, *J*=12.0, 6.0 Hz, H-18), 2.20 (1H, m, H-16b), 2.17 (1H, m, H-2b), 2.17 (1H, m, H-11), 2.02 (1H, m, H-16a), 2.01 (1H, m, H-15b), 1,95 (1H, m, H-2a), 1.93 (1H, m, H-9), 1.93 (1H, m, H-1b), 1.89 (1H, m, H-22b), 1.82 (1H, m, H-22a), 1.79 (1H, m, H-19b), 1.75 (1H, m, H-5b), 1.75 (1H, m, H-7b), 1.66 (3H, d, *J*=6.0 Hz, glc4-6), 1.65 (3H, d, *J*=6.0 Hz, rha1-6), 1.60 (1H, m, H-21b), 1.59 (1H, m, H-5), 1.49 (1H, m, H-7a), 1.41 (1H, m, H-21a), 1.39 (3H, s, H-24), 1.29 (1H, m, H-19a), 1.27 (1H, m, H-1a), 1.22 (1H, m, H-15a), 1.13 (3H, s, H-27), 1.12 (1H, m, H-6a), 0.95 (3H, s, H-26), 0.81 (3H, s, H-25), 0.81 (3H, s, H-30), 0.79 (3H, s, H-29). $^{13}$C NMR (151 MHz, pyridine-$d_5$) δ 205.5 (C-23), 176.3 (C-28), 144.8 (C-13), 122.6 (C-12), 106.2 (glc1-1), 104.8 (ara-1), 104.6 (glc5-1), 102.5 (rha3-1), 101.3 (rha1-1), 95.4 (glc4-1), 83.8 (C-3), 79.3 (ara-4), 78.5 (glc1-5), 78.4 (glc1-3), 78.2 (glc4-3), 78.0 (glc5-4), 77.8 (glc4-5), 76.9 (glc5-5), 76.3 (glc5-3), 76.2 (glc1-2), 75.5 (ara-2), 75.1 (glc5-2), 73. 9 (ara-3), 73.7 (rha3-4), 73.7 (rha1-4), 73.6 (glc4-2), 72.3 (rha3-3), 72.2 (rha3-2), 72.2 (rha1-3), 72.0 (rha1-2), 71.0 (glc1-4), 70.5 (glc4-4), 70.1 (rha3-5), 69.4 (rha1-5), 69.0 (glc4-6), 64.5 (ara-5), 62.3 (glc1-6), 61.0 (glc5-6), 55.8 (C-4), 48.5 (C-9), 48.1 (C-5), 47.2 (C-17), 46.4 (C-19), 42.3 (C-14), 41.8 (C-18), 40.3 (C-8), 38.5 (C-1), 36.3 (C-10), 34.1 (C-21), 33.3 (C-29), 32.7 (C-7), 32.6 (C-22), 30.9 (C-20), 28.4 (C-15), 26.3 (C-27), 25.7 (C-2), 23.9 (C-11), 23.9 (C-30), 23.5 (C-16), 20.8 (C-6), 18.3 (rha1-6), 18.3 (rha3-6), 17.6 (C-26), 15.8 (C-25), 11.0 (C-24).

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**Acid hydrolysis of compound 1.**

The acid hydrolysis method was carried as reported previously (Liu et al., 2012). Compound **1** (4 mg) was treated with 1M HCl (4 mL) at 90 °C for 2 h. Then the reaction mixture was extracted with $CHCl_3$ (3 × 5 mL). The aqueous layer was collected and the water was evaporated under vacuum with the repeated addition of MeOH to remove the solvent completely. The residue was redissolved in anhydrous pyridine (2 mL) and mixed with a pyridine solution of *L*-cysteine methyl ester hydrochloride (2 mL). After the mixed solution was heated at 60 °C for 1 h, trimethylchlorosilane (0.5 mL) was added and the resulting mixture was stirred at 60 °C for another 30 min. Then the solution was concentrated to dryness and taken up

inwater (3 × 1mL), followed by extraction with n-hexane (3 × 1 mL). The supernatant was analyzed by GC. Separations were carried out on HP-5 columns (320 μm× 30 m, 0.25 μm). Highly pure $N_2$ was employed as a carrier gas (1.0 mL/min), and the FID detector operated at 280 °C (column temperature 160-200 °C). The retention times of the monosaccharide derivatives were as follows: *L*-arabinose (12.69 min), *L*-rhamnose (12.80 min), and *D*-glucose (14.43 min), respectively.

**Cell culture**

Human lung cancer cells (A549), colon cancer cells (HCT-116), breast cancer cells (MDA-MB-231), liver cancer cells (SK-HEP-1), and stomach cancer cells (SNU-638) were provided by the Korean Cell Line Bank (Seoul, Korea). The cells were cultured in medium (DMEM for MDA-MB-231 and SK-HEP-1 cells; RPMI1640 for A549, HCT-116, and SNU-638 cells) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics antimycotics (PSF; 100 units/mL penicillin G sodium, 100 μg/mL streptomycin, and 250 ng/mL amphotericin B). The cells were incubated at 37 °C and 5% CO2 in a humidified atmosphere.

**In vitro cell proliferation assay**

Cell viability was determined by the sulforhodamine B (SRB) protein staining method. Cells were seeded in 96-well plates and incubated for 24 h, and were fixed (for zero day controls) or treated with test compounds for 72 h. After incubation, cells were fixed with 10% trichloroacetic acid (TCA), dried and stained in 0.4% sulforhodamine B (SRB) in 1% acetic acid solution. Unbound dye was washed and stained cells were dried and dissolved in 10 mM Tris (pH 10.0). Absorbance was measured at 515 nm and cell proliferation was determined as follows: cell proliferation (%) = (average absorbance $_{\text{compound}}$ − average absorbance $_{\text{zero day}}$) / (average absorbance $_{\text{control}}$ − average absorbance $_{\text{zero day}}$) × 100%. $IC_{50}$ values were calculated by non-linear regression analysis using the Table Curve 2D software (Version 5.01, Systant Software Inc., CA).


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Table S1 Structures of several saponins from common species of *Pulsatilla*

| No. | 3-O- | aglycone [a)] | 28-COO- | sources [b)] |
|---|---|---|---|---|
| 1 | H | VII | -glc(6-1)glc | 1Ye1996, 2Xu2011 |
| 2 | H | I | -glc(6-1)glc(4-1)rha | 2 Liu 2012,5Sun2009 |
| 3 | H | II | -glc(6-1)glc(4-1)rha | 1 Shi 2007,2 Liu 2012,3Li1990,5Sun2008 |
| 4 | H | III | -glc(6-1)glc(4-1)rha | 1 Shi 2007,2 Liu 2012 |
| 5 | H | VII | -glc(6-1)glc(4-1)rha | 1Ye1998, 2 Yang2010 |
| 6 | ara | II | -H | 2Xu2010,3Li1990,5 Zinova1992 |
| 7 | ara | VII | -H | 1Ye2002 |
| 8 | ara | I | -glc | 2 Liu 2015 |
| 9 | ara | II | -glc | 2 Fan 2013 |
| 10 | ara | VII | -glc | 2Yang2010 |
| 11 | ara | I | -glc(6-1)glc(4-1)rha | 5Sun2009 |
| 12 | ara | II | -glc(6-1)glc(4-1)rha | 1 Shi 2007, 2Xu2010,3Li1990,5 Zinova1992 |
| 13 | ara | VII | -glc(6-1)glc(4-1)rha | 1wu 1991, 2 Yang2010 |
| 14 | ara(2-1)rha | I | -H | 1 Mimaki 1999,2Fu 2008 |
| 15 | ara(2-1)rha | II | -H | 1 Mimaki 1999,2 Bang2005a,5 Sun2009 |
| 16 | ara(2-1)rha | V | -H | 2 Wang 2018 |
| 17 | ara(2-1)rha | VII | -H | 1 Ye1990,2 Bang2005a |
| 18 | ara(2-1)rha | I | -glc | 2 Fan 2013 |
| 19 | ara(2-1)rha | II | -glc | 2 Yang2010 |
| 20 | ara(2-1)rha | VII | -glc | 2 Yang2010 |
| 21 | ara(2-1)rha | II | -glc(6-1)glc | 2Zhang 2000b |
| 22 | ara(2-1)rha | VI | -glc(6-1)glc | 2 Yang2010 |
| 23 | ara(2-1)rha | I | -glc(6-1)glc(4-1)rha | 1 Shi 2007,2Fu 2008,5 Sun2009 |
| 24 | ara(2-1)rha | II | -glc(6-1)glc(4-1)rha | 1 Shi2007,2Kang1989 |
| 25 | ara(2-1)rha | VI | -glc(6-1)glc(4-1)rha | 1 Mimaki 2001,2 Yang2010 |
| 26 | ara(2-1)rha | VII | -glc(6-1)glc(4-1)rha | 1 Mimaki 2001,2 Yang2010 |
| 27 | ara(2-1)rha | VIII | -glc(6-1)glc(4-1)rha | 1 Mimaki 2001 |
| 28 | ara(2-1)rha(3-1)glc | I | -H | 1 Mimaki 1999,2 Bang2005a |
| 29 | ara(2-1)rha(3-1)glc | II | -H | 1 Mimaki 1999,2 Bang2005a |
| 30 | ara(2-1)rha(3-1)glc | VI | -H | 2 Bang2005b |
| 31 | ara(2-1)rha(3-1)glc | VII | -H | 2 Bang2005b |
| 32 | ara(2-1)rha(3-1)glc | II | -glc(6-1)glc | 2 Liu 2012 |
| 33 | ara(2-1)rha(3-1)glc | I | -glc(6-1)glc(4-1)rha | 2Fu 2008 |
| 34 | ara(2-1)rha(3-1)glc | II | -glc(6-1)glc(4-1)rha | 2 Bang 2005a |
| 35 | ara(2-1)rha(3-1)glc(4-1)glc | I | -H | 1 Mimaki 1999 |
| 36 | ara(2-1)rha(3-1)glc(4-1)glc | II | -H | 2 Bang2005a |
| 37 | glc(1-4)ara | II | -H | 1 Mimaki 1999, 2 Bang2005a,3Li1990 |
| 38 | glc(1-4)ara | V | -H | 2 Wang 2018 |
| 39 | glc(1-4)ara | VII | -H | 2 Bang2005b |
| 40 | glc(1-4)ara | II | -glc(6-1)glc(4-1)rha | 2Xu2010,3Li1990,5 Zinova1992 |
| 41 | glc(1-4)ara | III | -glc(6-1)glc(4-1)rha | 2Xu2010 |

| | | | | |
|---|---|---|---|---|
| 42 | glc(1-4)ara(2-1)rha | I | -H | 1 Mimaki 1999,2 Bang2005a |
| 43 | glc(1-4)ara(2-1)rha | II | -H | 1 Mimaki 1999,2 Bang2005a |
| 44 | glc(1-4)ara(2-1)rha | V | -H | 2 Wang 2018 |
| 45 | glc(1-4)ara(2-1)rha | VI | -H | 2 Bang2005b |
| 46 | glc(1-4)ara(2-1)rha | VII | -H | 1 Mimaki 1999,2 Bang2005b |
| 47 | glc(1-4)ara(2-1)rha | II | -glc | 2Zhang 2000b |
| 48 | glc(1-4)ara(2-1)rha | II | -glc(6-1)glc | 2 Liu 2015 |
| 49 | glc(1-4)ara(2-1)rha | I | -glc(6-1)glc(4-1)rha | 1 Shi 2007,2Fu 2008 |
| 50 | glc(1-4)ara(2-1)rha | II | -glc(6-1)glc(4-1)rha | 2 Fu 2008 |
| 51 | glc(1-4)ara(2-1)rha | VI | -glc(6-1)glc(4-1)rha | 2 Yang2010 |
| 52 | glc(1-4)ara(2-1)rha | VII | -glc(6-1)glc(4-1)rha | 2 Bang2005a |
| 53 | glc(1-4)ara(2-1)rha(3-1)glc | II | -H | 2 Fan 2013 |
| 54 | glc(1-4)ara(2-1)rha(3-1)glc | I | -glc(6-1)glc(4-1)rha | 2Zhang2000a |
| 55 | glc(1-4)ara(2-1)rha(3-1)glc | II | -glc(6-1)glc(4-1)rha | 2Zhang2000a |
| 56 | glc(1-4)ara(2-1)rha(3-1)glc | IV | -glc(6-1)glc(4-1)rha | 2 Liu 2012 |
| 57 | glc(1-4)ara(2-1)rha(3-1)glc(4-1)glc | I | -H | 1 Mimaki 1999 |
| 58 | glc(1-4)ara(2-1)rha(3-1)glc(4-1)glc | I | -glc(6-1)glc(4-1)rha | 2 Liu 2012 |
| 59 | rha(1-6)glc(1-4)ara(2-1)rha(3-1)glc | I | - H | 2 Fan 2013 |
| 60 | rha(1-6)glc(1-4)ara(2-1)rha(3-1)glc | I | -glc(6-1)glc(4-1)rha | 2 Liu 2012 |
| 61 | rha(1-6)glc(1-4)ara(2-1)rha(3-1)glc | II | -glc(6-1)glc(4-1)rha | 2 Liu 2012 |

a) Aglycone: I oleanolic acid, II hederagenin, III bayogenin, IV gypsogenin, V kalopanax-genin L1, VI betulinic acid, VII 23-hydroxybetulinic acid, VIII 3β,20,23-trihydroxylupan-28-oic acid

b) Sources: 1. *P. chinensis*, 2. *P. cernua*, 3. *P. campanella*, 4. *P. patens* var. *multifida*, 5. *P. dahurica*, 6. *P. millefolium*


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Table S2 $^{1}$H NMR data of anomeric protons of saponins **1-62** from common species of *Pulsatilla*

| | C3-O- | | | | | | C28-O- | | | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| | ara | glc1 | glc2 | glc3 | rha1 | rha2 | glc4 | glc5 | rha3 | |
| 1 | - | - | - | - | - | - | 6.34 | 4.99 | - | Ye1996 |
| 2 | - | - | - | - | - | - | 5.28 | 4.30 | 4.88 | Liu 2012 |
| 3 | - | - | - | - | - | - | 6.19 | 4.96 | 5.79 | Li1990 |
| 4 | - | - | - | - | - | - | 6.21 | 4.98 | 5.82 | Liu 2012 |
| 5 | - | - | - | - | - | - | 6.29 | 4.96 | 5.78 | Ye1998 |
| 6 | 4.95 | - | - | - | - | - | - | - | - | Xu2010 |
| 7 | 4.97 | - | - | - | - | - | - | - | - | Ye1996 |
| 8 | 4.74 | - | - | - | - | - | 6.30 | - | - | Liu 2015 |
| 9 | 4.98 | - | - | - | - | - | 6.28 | - | - | Fan 2013 |
| 10 | 4.96 | - | - | - | - | - | 6.40 | - | - | Yang2010 |
| 11 | 4.73 | - | - | - | - | - | 6.21 | 4.95 | 5.83 | Sun2009 |
| 12 | 4.93 | - | - | - | - | - | 6.18 | 4.95 | 5.80 | Xu2010 |
| 13 | 4.97 | - | - | - | - | - | 6.36 | 4.97 | 5.87 | Wu1991 |
| 14 | 5.11 | - | - | - | 6.27 | - | - | - | - | Fu 2008 |
| 15 | 4.90 | - | - | - | 6.16 | - | - | - | - | Bang2005a |
| 16 | 5.07 | - | - | - | 6.23 | - | - | - | - | Wang 2018 |
| 17 | 4.38 | - | - | - | 5.20 | - | - | - | - | Ye1990 |
| 18 | 4.85 | - | - | - | 6.12 | - | 6.29 | - | - | Fan 2013 |
| 19 | 4.55 | - | - | - | 5.09 | - | 5.37 | | | Yang2010 |
| 20 | 5.05 | - | - | - | 6.11 | - | 6.33 | - | - | Yang2010 |
| 21 | 4.90 | - | - | - | 6.19 | - | 6.22 | 5.01 | - | Zhang 2000b |
| 22 | 4.92 | - | - | - | 6.19 | - | 6.20 | 5.05 | - | Yang2010 |
| 23 | 4.89 | - | - | - | 6.16 | - | 6.24 | 4.98 | 5.87 | Fu 2008 |
| 24 | 4.98 | - | - | - | 6.16 | | 6.23 | 5.10 | 5.85 | Kang1989 |
| 25 | 4.92 | - | - | - | 6.06 | - | 6.29 | 4.86 | 5.79 | Mimaki2001 |
| 26 | 4.94 | - | - | - | 5.11 | - | 6.36 | 6.26 | 5.87 | Wu1991 |
| 27 | 5.10 | - | - | - | 6.17 | - | 6.31 | 4.95 | 5.80 | Mimaki2001 |
| 28 | 4.82 | - | 5.49 | - | 6.21 | - | - | - | - | Bang2005a |
| 29 | 4.98 | - | 5.50 | - | 6.21 | - | - | - | - | Bang2005a |
| 30 | 4.81 | - | 5.46 | - | 6.17 | - | - | - | - | Bang2005b |
| 31 | 5.00 | - | 5.48 | - | 6.24 | - | - | - | - | Bang2005b |
| 32 | 4.95 | - | 4.97 | - | 6.20 | - | 6.19 | 5.43 | - | Liu 2012 |
| 33 | 4.96 | - | 4.79 | - | 6.18 | - | 6.23 | 5.45 | 5.83 | Fu 2008 |
| 34 | 5.00 | - | 4.98 | - | 6.29 | - | 6.25 | 5.50 | 5.85 | Bang2005 |

| | C3-O- | | | | | | C28-O- | | | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| | ara | glc1 | glc2 | glc3 | rha1 | rha2 | glc4 | glc5 | rha3 | |
| 35 | 4.83 | - | 5.43 | 5.19 | 6.18 | - | - | - | - | Mimaki 1999 |
| 36 | 4.97 | - | 5.37 | 5.10 | 6.17 | - | - | - | - | Bang2005 |
| 37 | 4.86 | 5.20 | - | - | - | - | - | - | - | Bang2005 |
| 38 | 4.87 | 5.22 | - | - | - | - | - | - | - | Wang 2018 |
| 39 | 4.88 | 5.16 | - | - | - | - | - | - | - | Bang2005b |
| 40 | 4.85 | 5.19 | - | - | - | - | 6.19 | 4.95 | 5.80 | Xu2010 |
| 41 | 4.91 | 5.22 | - | - | - | - | 6.21 | 4.91 | 5.82 | Xu2010 |
| 42 | 4.75 | 5.14 | - | - | 6.20 | - | - | - | - | Bang2005 |
| 43 | 4.99 | 5.13 | - | - | 6.28 | - | - | - | - | Mimaki 1999 |
| 44 | 4.95 | 5.08 | - | - | 6.25 | - | - | - | - | Wang 2018 |
| 45 | 4.76 | 5.13 | - | - | 6.17 | - | - | - | - | Bang2005b |
| 46 | 4.96 | 5.10 | - | - | 6.26 | - | - | - | - | Bang2005b |
| 47 | 5.01 | 5.09 | - | - | 6.23 | - | 6.31 | - | - | Zhang 2000b |
| 48 | 4.91 | 5.07 | - | - | 6.18 | - | 6.21 | 4.97 | - | Liu 2015 |
| 49 | 4.98 | 4.76 | - | - | 6.19 | - | 6.24 | 5.15 | 5.87 | Fu2008 |
| 50 | 4.96 | 4.70 | - | - | 6.21 | - | 6.22 | 5.11 | 5.83 | Fu2008 |
| 51 | 4.70 | 4.99 | - | - | 5.97 | - | 6.21 | 4.83 | 5.67 | Yang2010 |
| 52 | 4.97 | 5.10 | - | - | 6.25 | - | 6.34 | 4.93 | 5.85 | Ye2002 |
| 53 | 4.89 | 5.04 | 5.43 | - | 6.14 | - | - | - | - | Fan 2013 |
| 54 | 5.20 | 4.81 | 4.98 | - | 6.25 | - | 6.26 | 5.45 | 5.87 | Zhang2000b |
| 55 | 5.05 | 5.12 | 5.38 | - | 6.18 | - | 6.22 | 4.97 | 5.80 | Zhang 2000a |
| 56 | 4.91 | 4.99 | 5.11 | | 6.13 | | 6.14 | 4.90 | 5.76 | Liu 2012 |
| 57 | 4.70 | 5.11 | 5.42 | 5.15 | 6.14 | - | - | - | - | Mimaki 1999 |
| 58 | 4.61 | 5.10 | 5.42 | 5.15 | 6.19 | - | 6.22 | 4.96 | 5.84 | Liu 2012 |
| 59 | 4.78 | 5.10 | 5.41 | - | 6.18 | 5.39 | - | - | - | Fan 2013 |
| 60 | 4.76 | 5.11 | 5.40 | - | 6.23 | 5.39 | 6.21 | 4.96 | 5.85 | Liu 2012 |
| 61 | 4.95 | 5.06 | 5.39 | - | 6.23 | 5.37 | 6.20 | 4.92 | 5.81 | Liu 2012 |

Table S3 NMR spectroscopic data of **1** (aglycone, pyridine-$d_5$)

| No | $\delta_C$ | $\delta_H$ | No | $\delta_C$ | $\delta_H$ |
|---|---|---|---|---|---|
| 1 | 38.5 | 1.27(1H,m), 1.93(1H,m) | 16 | 23.5 | 2.02(1H,m), 2.20(1H,m) |
| 2 | 25.7 | 1.95(1H,m), 2.17(1H,m) | 17 | 47.2 | —— |
| 3 | 83.8 | 4.70(1H,m) | 18 | 41.8 | 3.08(1H,dd,6,12) |
| 4 | 55.8 | —— | 19 | 46.4 | 1.29(1H,m), 1.79(1H,m) |
| 5 | 48.1 | 1.59(1H,m) | 20 | 30.9 | —— |
| 6 | 20.8 | 1.12(1H,m), 1.75(1H,m) | 21 | 34.1 | 1.41(1H,m), 1.60(1H,m) |
| 7 | 32.7 | 1.49(1H,m), 1.75(1H,m) | 22 | 32.6 | 1.82(1H,m), 1.89(1H,m) |
| 8 | 40.3 | —— | 23 | 205.5 | 9.67(1H,s) |
| 9 | 48.5 | 1.93(1H,m) | 24 | 11.0 | 1.39(3H,s) |
| 10 | 36.3 | —— | 25 | 15.8 | 0.81(3H,s) |
| 11 | 23.9 | 2.17(2H,m) | 26 | 17.6 | 0.95(3H,s) |
| 12 | 122.6 | 5.30(1H,s) | 27 | 26.3 | 1.13(3H,s) |
| 13 | 144.8 | —— | 28 | 176.6 | —— |
| 14 | 42.3 | —— | 29 | 33.3 | 0.79(3H,s) |
| 15 | 28.4 | 1.22(1H,m), 2.01(1H,m) | 30 | 23.9 | 0.81(3H,s) |

Table S4 NMR spectroscopic data of **1** (sugar moieties, pyridine-$d_5$)

| | $\delta_C$ | $\delta_H$ | | $\delta_C$ | $\delta_H$ |
|---|---|---|---|---|---|
| C3- | | | C28- | | |
| ara-1 | 104.8 | 4.60(1H,d,6.0) | glc4-1 | 95.4 | 6.18(1H,d,8.4) |
| 2 | 75.5 | 4.42(1H,m) | 2 | 73.6 | 4.15(1H,m) |
| 3 | 73.9 | 4.55(1H,m) | 3 | 78.2 | 4.40(1H,m) |
| 4 | 79.3 | 4.25(1H,m) | 4 | 70.5 | 4.33(1H,m) |
| 5 | 64.5 | 3.69(1H,d,6.0), 4.44(1H,m) | 5 | 77.8 | 3.92(1H,m) |
| glc1-1 | 106.2 | 5.10(1H,d,7.8) | 6 | 69.0 | 4.33(1H,m), 4.64(1H,m) |
| 2 | 76.3 | 4.02(1H,m) | glc5-1 | 104.6 | 4.95(1H,d,7.8) |
| 3 | 78.4 | 4.20(1H,m) | 2 | 75.1 | 3.93(1H,m) |
| F4 | 71.0 | 4.20(1H,m) | 3 | 76.3 | 4.13(1H,m) |
| 5 | 78.5 | 3.90(1H,m) | 4 | 78.0 | 4.09(1H,m) |
| 6 | 62.3 | 4.35(1H,m), 4.49(1H,m) | 5 | 76.9 | 3.62(1H,d,9.0) |
| rha1-1 | 101.3 | 6.06(1H,brs) | 6 | 61.0 | 4.09(1H,m), 4.20(1H,m) |
| 2 | 72.0 | 4.65(1H,m) | rha3-1 | 102.5 | 5.80(1H,brs) |
| 3 | 72.2 | 4.55(1H,m) | 2 | 72.2 | 4.65(1H,m) |
| 4 | 73.7 | 4.35(1H,m) | 3 | 72.3 | 4.55(1H,m) |
| 5 | 69.4 | 4.59(1H,m) | 4 | 73.7 | 4.35(1H,m) |
| 6 | 18.3 | 1.65(3H,d,6.0) | 5 | 70.1 | 4.94(1H,m) |
| | | | 6 | 18.3 | 1.66(3H,d,6.0) |

*O* overlapped

Table S5 Inhibitory effects of compound on the proliferation of human cancer cell lines

| Compound | $IC_{50}$ (μM)[a] | | | | |
|---|---|---|---|---|---|
| | A549 | MDA-MB-231 | SK-Hep-1 | SNU638 | HCT116 |
| **1** | >100 | >100 | >100 | >100 | >100 |
| Etoposide [b] | 4.1 | 50.1 | 0.6 | 4.1 | 7.8 |

A549: Human lung cancer cell lines

HCT-116: colon cancer cell lines

MDA-MB-231: breast cancer cell lines

SK-HEP-1: liver cancer cell lines

SNU-638: stomach cancer cell lines

[a] a half maximal (50%) inhibitory concentration;

[b] positive control

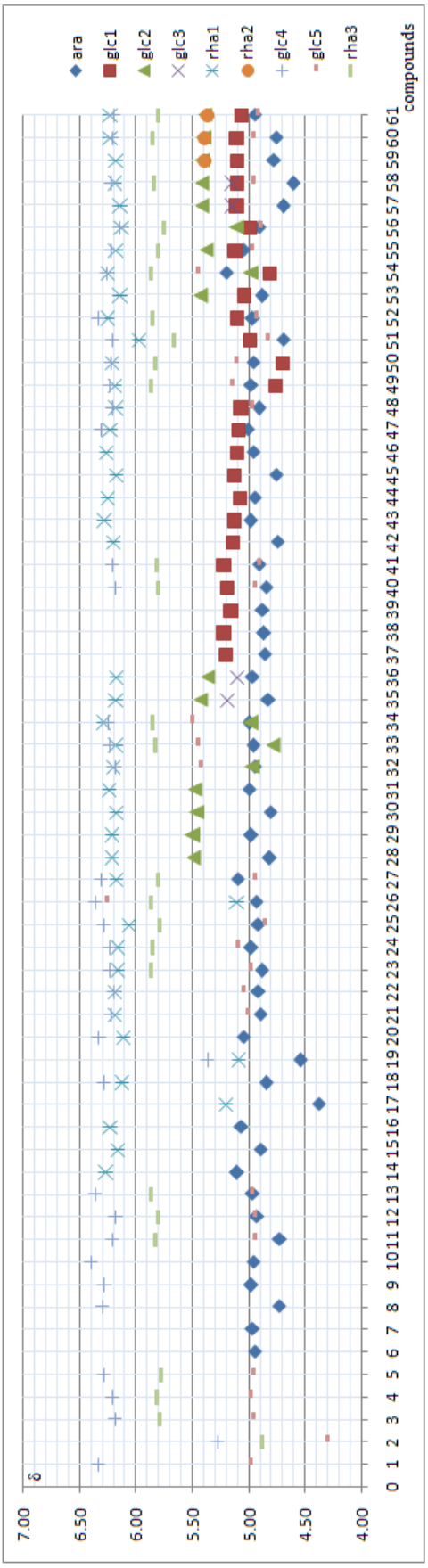



Figure S1 Chemical shifts of anomeric protons of **1**-**61** from common species of *Pulsatilla*

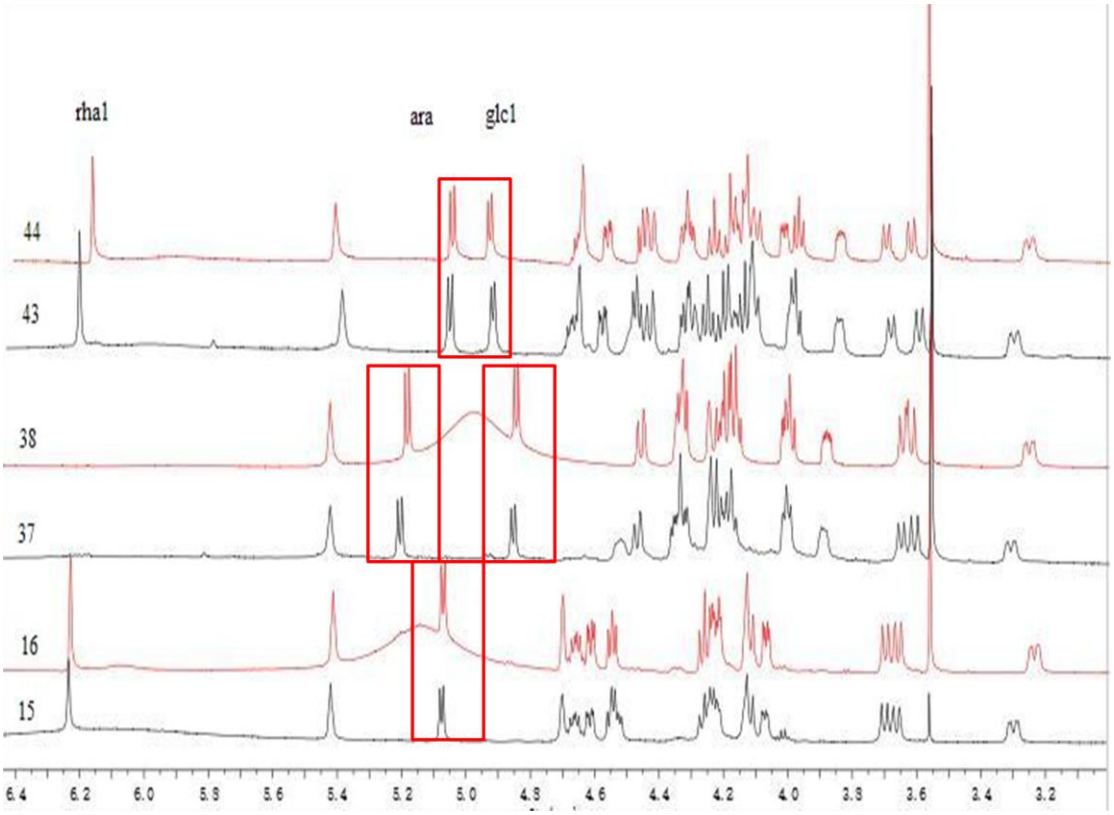



Figure S2 Anomeric proton signals of **16**, **38** and **44**, compared with those of **15**, **37** and **43**, respectively.

Not only the good agreement of NMRprofile of anomeric hydrogens signals but also the similar pattern of other signals of sugar residuesindicated that the structure of **16** contained same sugar chain as that of **15**. Similar conclusions can be obtained during analysis of compounds **38** and **37** which have same sugar chain with compounds **44** and **43**, respectively.

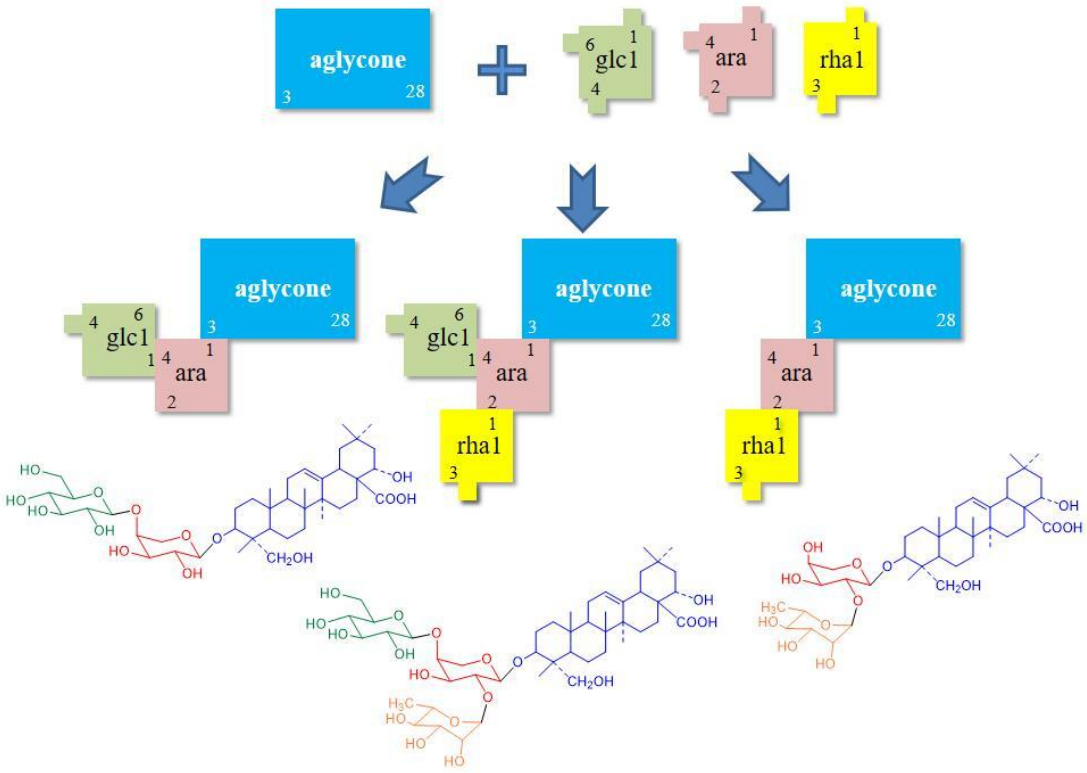



Figure S3 NMR mosaic method to predict the structures of **16**, **38** and **44**.

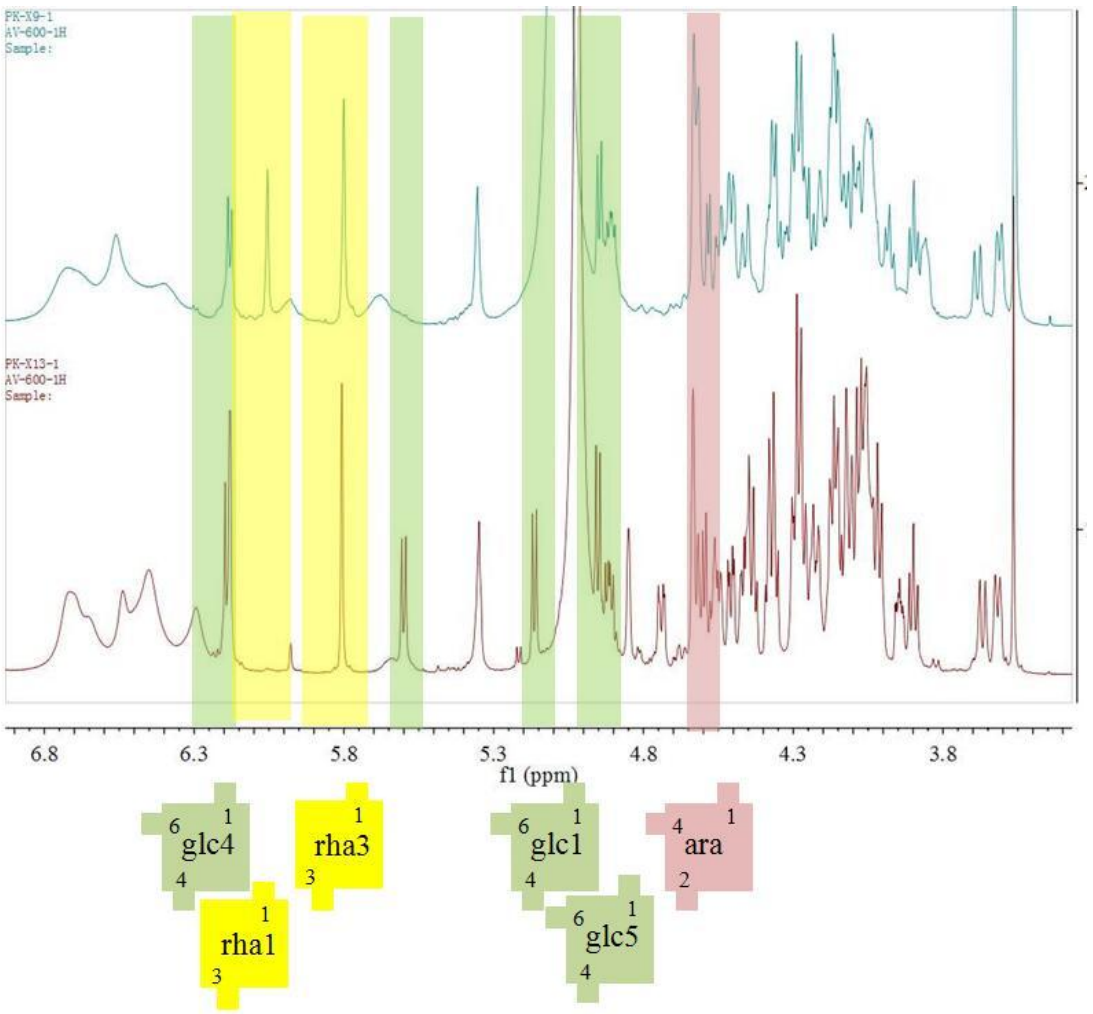



Figure S4 Part of $^{1}$H NMR spectra of compound **1** and **56** (Supplementary material) Compared with the signals of compound **56** which shared the same aglycone shown in Supplementary material, only glc2 was absent in compound **1**

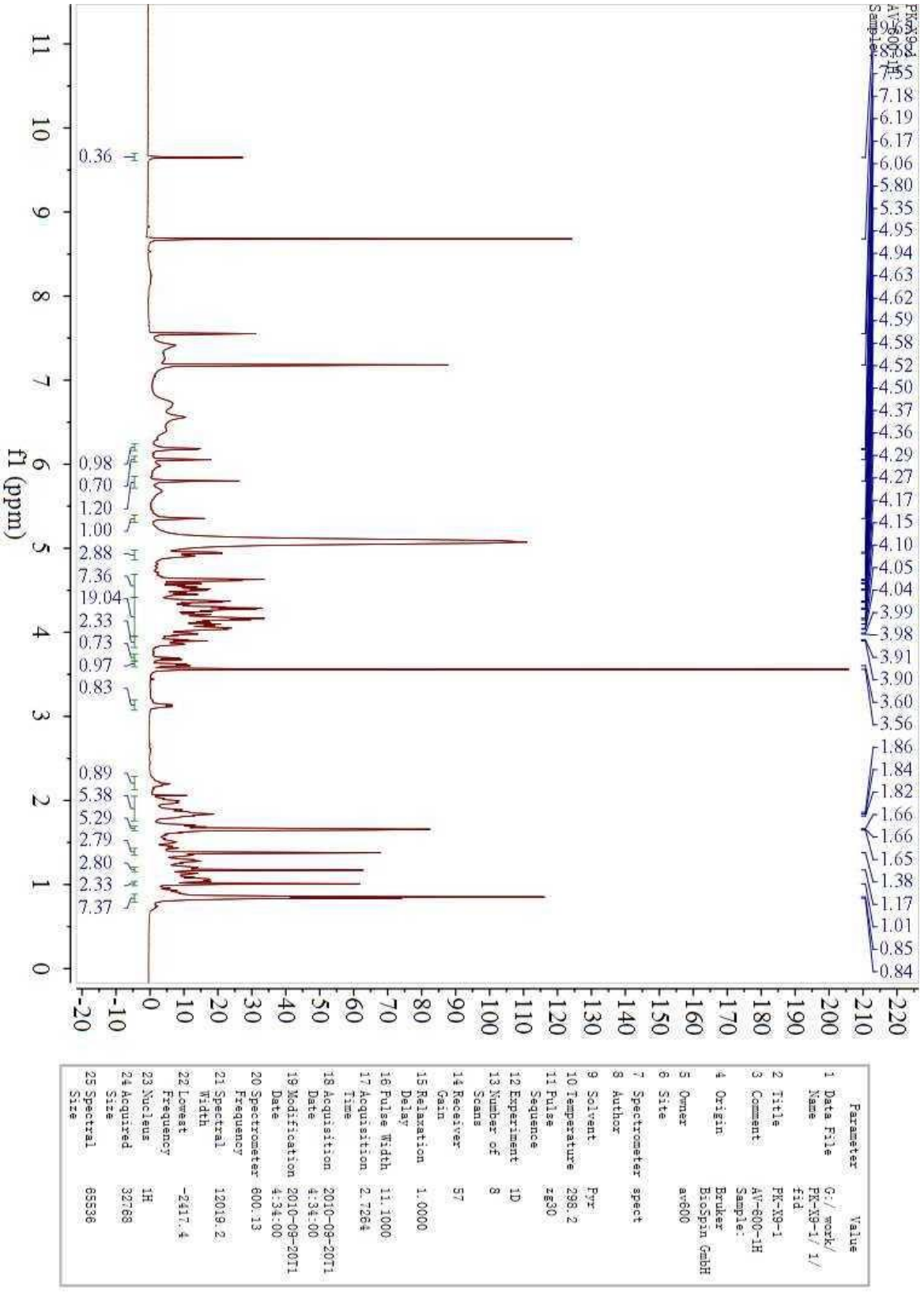



Figure S5 $^{1}$H NMR spectrum of compound **1** (600 MHz, pyridine-$d_5$)

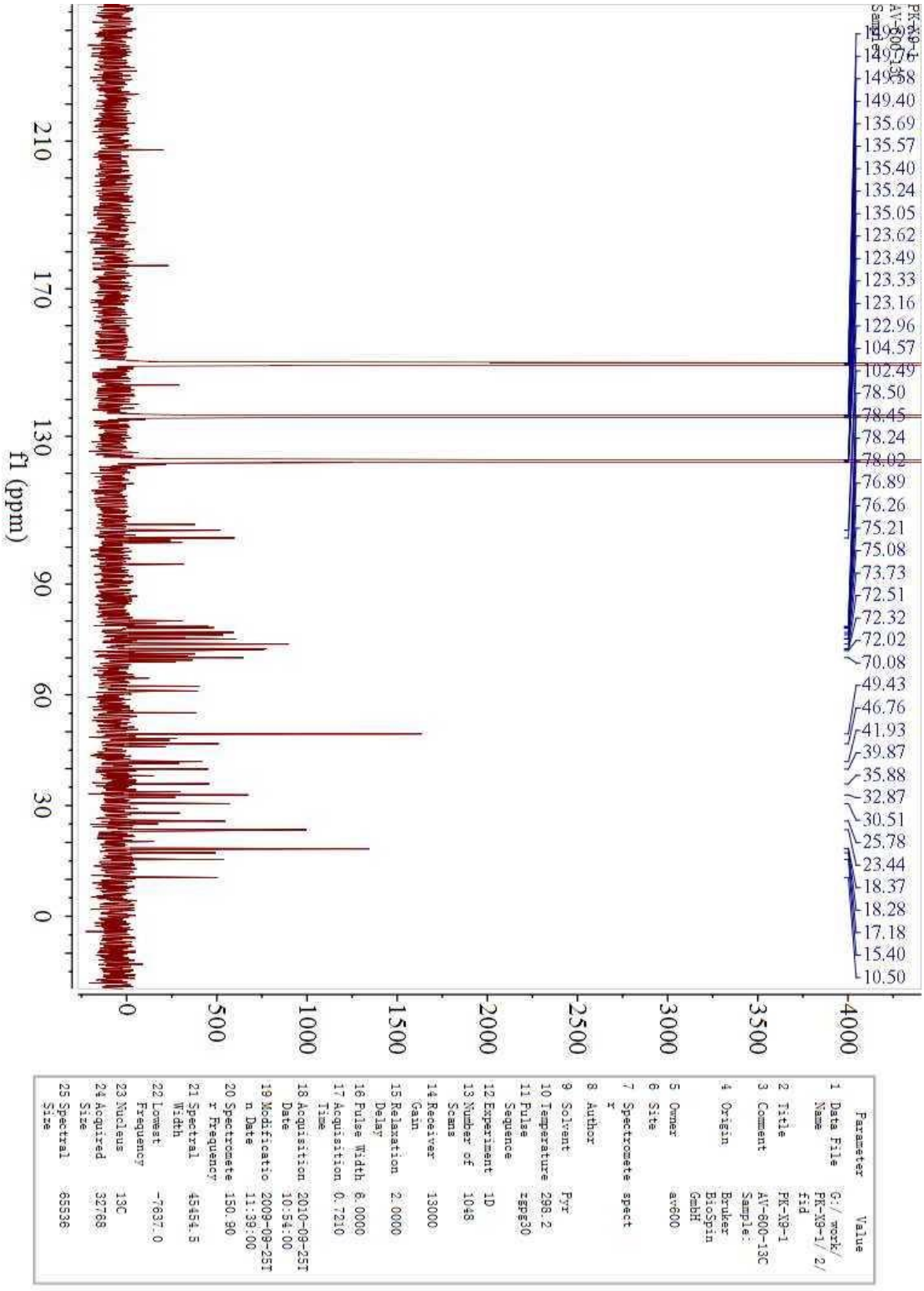



Figure S6 $^{13}$C NMR spectrum of compound **1** (150 MHz, pyridine-$d_5$)

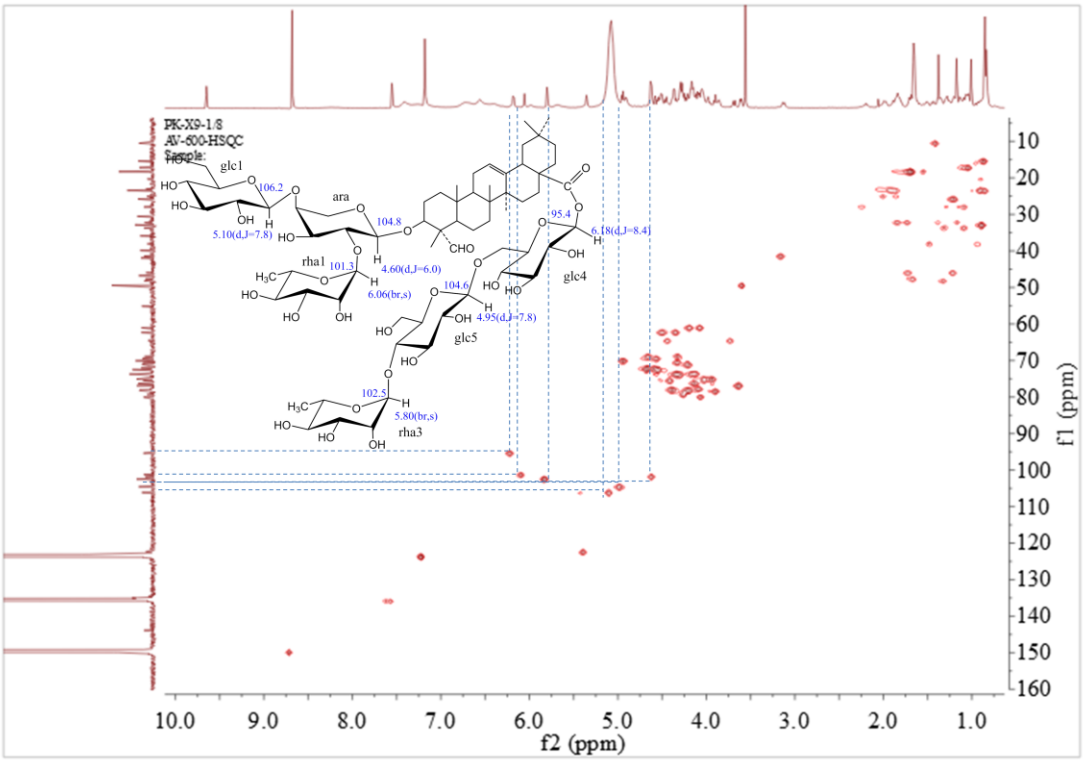
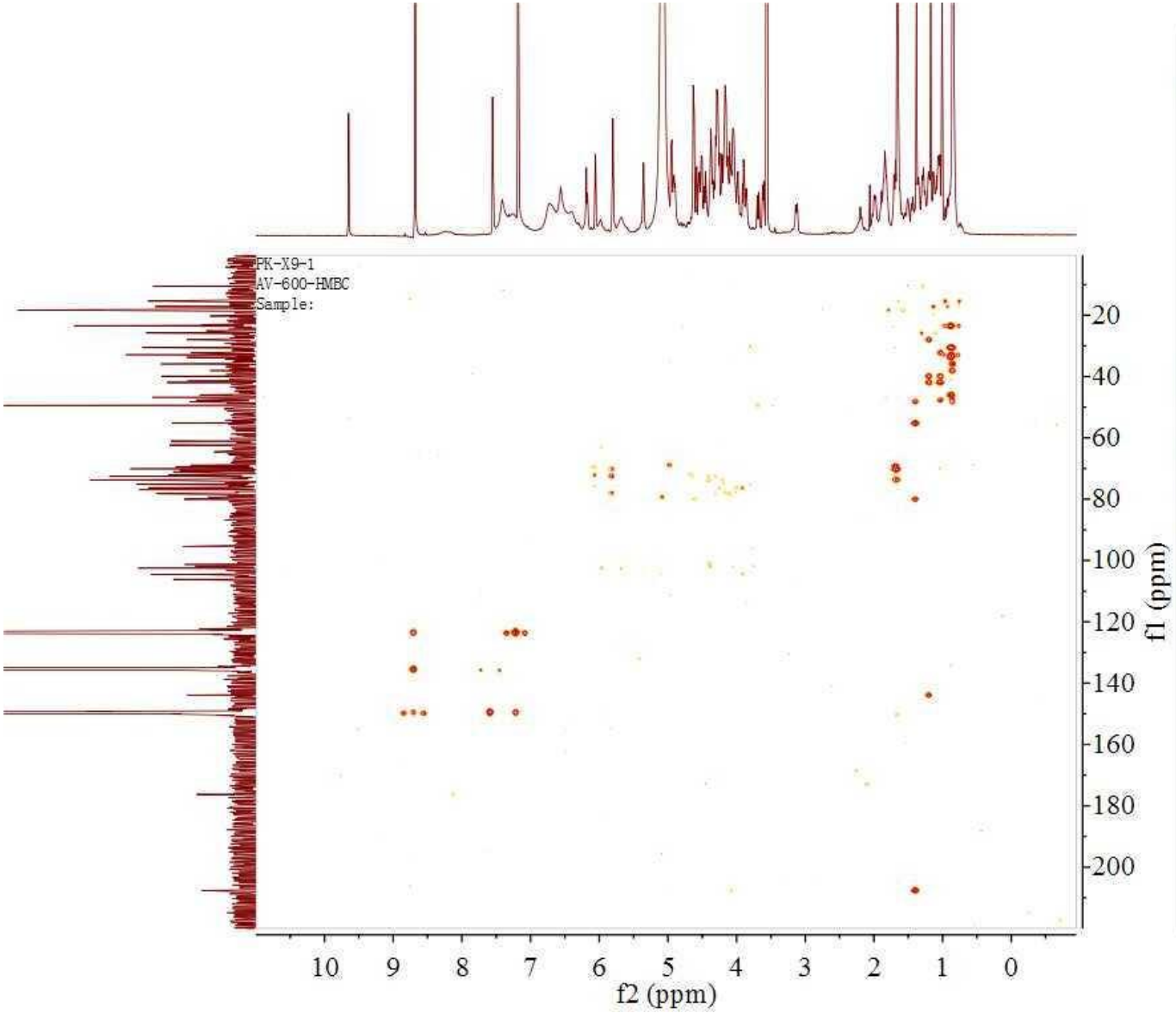



Figure S7 HSQC spectrum of compound **1**




| | Parameter | Value (f2, f1) |
|---|---|---|
| 1 | Data File Name | G:/ work/ PK-X9-1/ 9/ ser |
| 2 | Title | PK-X9-1 |
| 3 | Comment | AV-600-HMBC Sample: |
| 4 | Origin | Bruker BioSpin GmbH |
| 5 | Owner | av600 |
| 6 | Site | |
| 7 | Spectrometer | spect |
| 8 | Author | |
| 9 | Solvent | Pyr |
| 10 | Temperature | 296.5 |
| 11 | Pulse Sequence | hmbcgplpndqf |
| 12 | Experiment | 2D-HMBC |
| 13 | Number of Scans | 80 |
| 14 | Receiver Gain | 29100 |
| 15 | Relaxation Delay | 1.5000 |
| 16 | Pulse Width | 11.1000 |
| 17 | Acquisition Time | 0.0713 |
| 18 | Acquisition Date | 2011-06-13T19:34:56 |
| 19 | Modification Date | 2011-06-14T08:17:00 |
| 20 | Spectrometer Frequency | (600.13, 150.90) |
| 21 | Spectral Width | (7183.9, 33202.3) |
| 22 | Lowest Frequency | (-582.7, -4.6) |
| 23 | Nucleus | (1H, 13C) |
| 24 | Acquired Size | (512, 339) |
| 25 | Spectral Size | (512, 512) |

Figure S8 HMBC spectrum of compound **1**

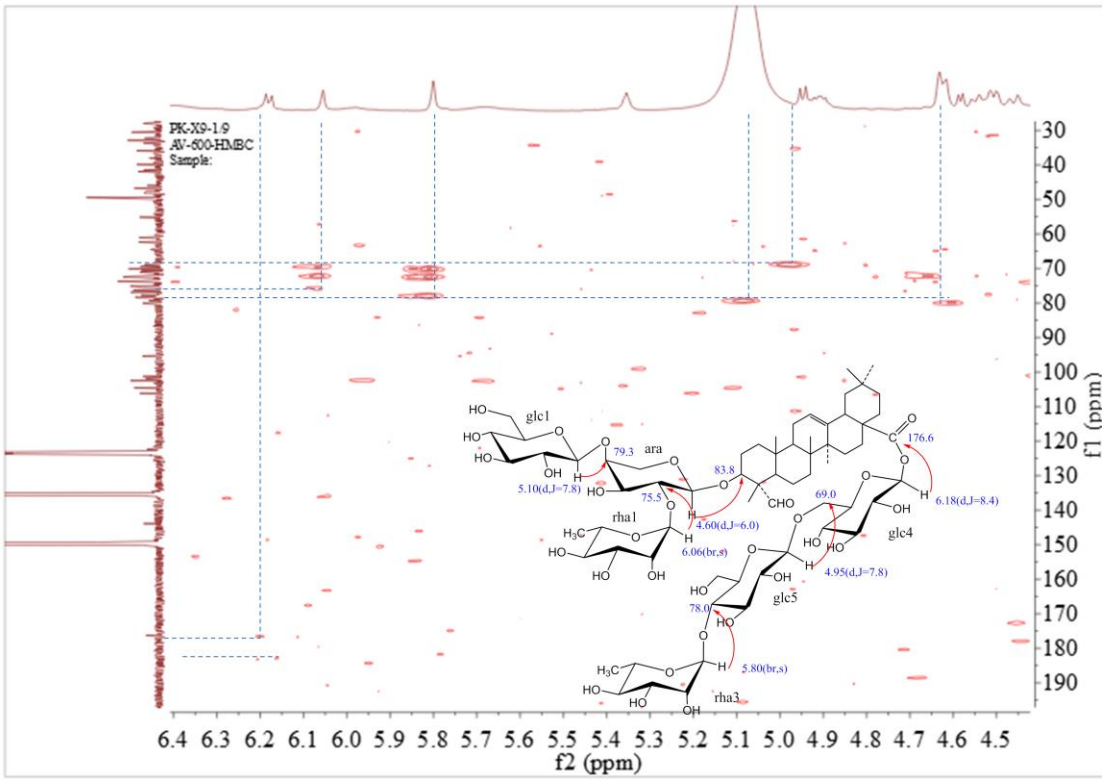



Figure S9 Part of HMBC spectrum of compound **1**

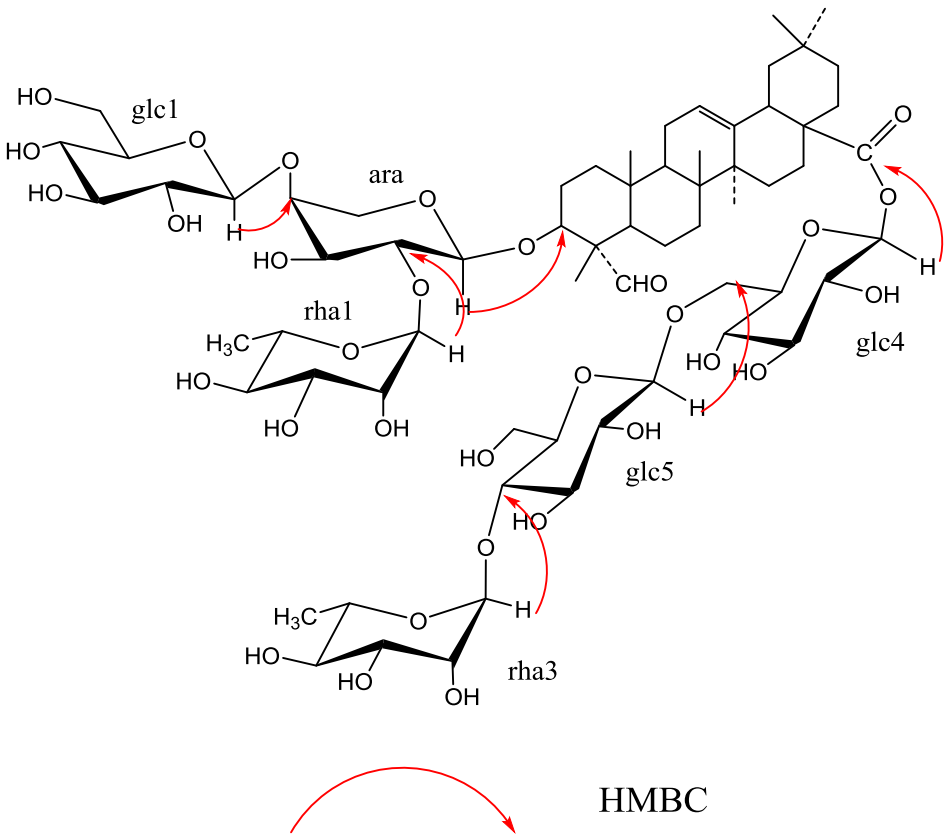



Figure S10 Structures and key HMBC (from H to C) of compounds **1** from the roots of *P. cernua*

# Mass Spectrum Molecular Formula Report

**Analysis Info**

| | | | |
|---|---|---|---|
| Analysis Name | D:\Data\20180612CEYANG\pk-x-9-1_1-D,6_01_12452.d | Acquisition Date | 6/12/2018 1:44:39 PM |
| Method | 20170322ceyang-neg.m | Instrument / Ser# | Bruker Customer |
| Sample Name | pk-x-9-1 | Operator | micrOTOF-Q 125 |
| Comment | | | |

**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 1.2 Bar |
| Focus | Active | Set Capillary | 4500 V | Set Dry Heater | 180 °C |
| Scan Begin | 50 m/z | Set End Plate Offset | -500 V | Set Dry Gas | 8.0 l/min |
| Scan End | 1500 m/z | Set Collision Cell RF | 400.0 Vpp | Set Divert Valve | Source |

**Generate Molecular Formula Parameter**

| | | |
|---|---|---|
| Formula, min. | | |
| Formula, max. | | |
| Measured m/z | Tolerance | Charge |
| Check Valence | Minimum | Maximum |
| Nirogen Rule | Electron Configuration | |
| Filter H/C Ratio | Minimum | Maximum |
| Estimate Carbon | | |

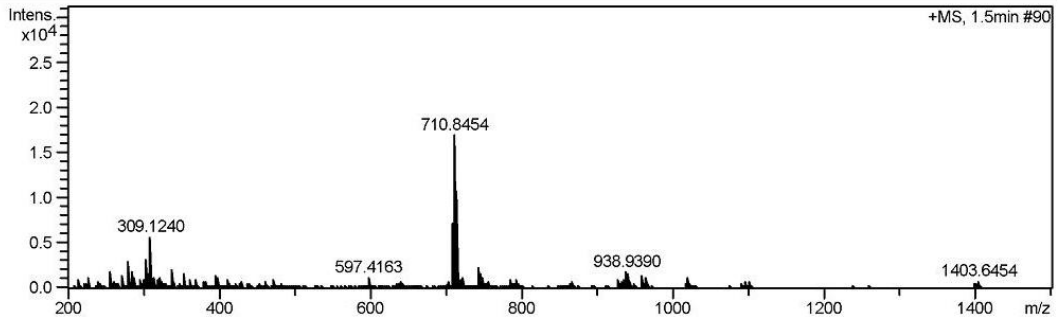





Figure S11 HR-ESI-MS spectrum of compound **1**

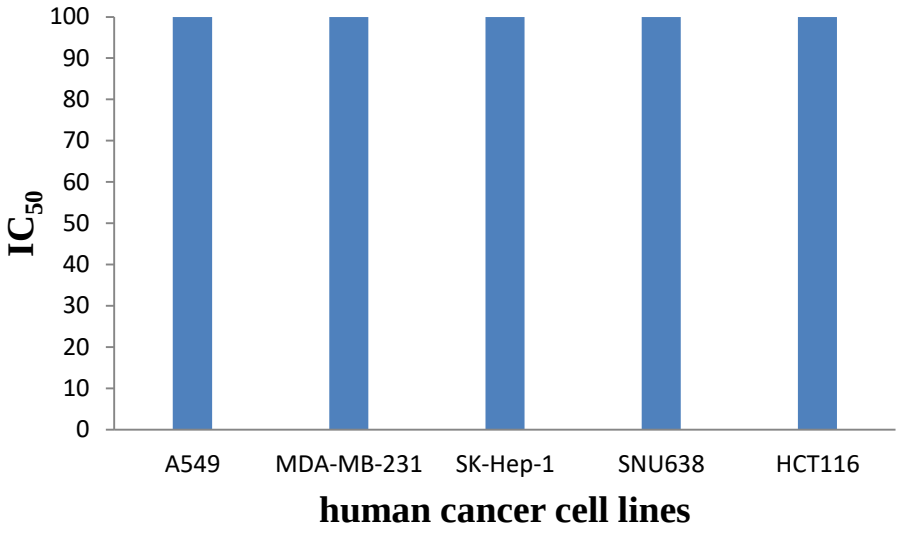



Figure S12 Cytotoxic activities of compound **1** against five human cancer cell lines