

Full Paper

## Isolation, Synthesis and Structures of Cytotoxic Ginsenoside Derivatives

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**Abstract:** Four known ginsenosides: ginsenoside-Rb<sub>1</sub> (**1**), Rb<sub>3</sub> (**2**), Rd (**3**) and Re (**4**) were isolated from the methanolic extract of the traditional Chinese medicine *Panax ginseng* C. A. Meyer. Further enzyme reactions and chemical modifications led us to obtain ginsenoside-M<sub>1</sub> (**5**) and synthesize three novel mono-esters of ginsenoside-M<sub>1</sub>, ginsenoside-DM<sub>1</sub> (**6**), PM<sub>1</sub> (**7**) and SM<sub>1</sub> (**8**) 30 - 50% of yield *via* a facile and green synthetic strategy. The structures were elucidated on the basis of extensive 1D- and 2D-NMR, as well as high resolution ESI-TOF mass spectroscopic analyses. The isolated and synthetic compounds were tested in an anti-tumor bioassay, and compounds **5-8** showed considerable cytotoxicity (SRB) against several human cancer cell lines (breast cancer MCF-7, skin melanoma SK-MEL-2 and human ovarian carcinoma B16), but moderate effects on lung carcinoma COR-L23. The other ginsenosides showed no effects.

**Keywords:** Isolation, Synthesis, Ginsenoside-DM<sub>1</sub>, Ginsenoside-PM<sub>1</sub>, Ginsenoside-SM<sub>1</sub>.

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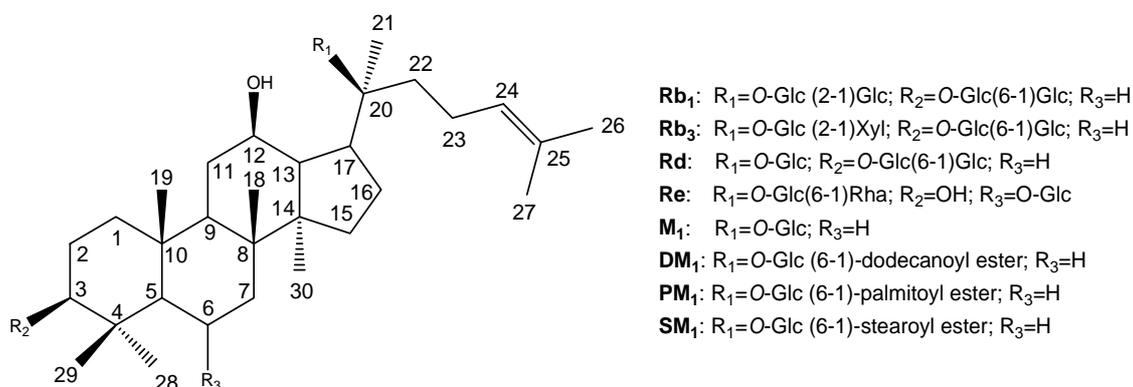
### Introduction

Ginseng (*Panax ginseng* C. A. Meyer) has been used in China for thousands of years as a traditional medicine and proven to possess a wide range of pharmacological properties, such as anti-

fatigue, memory stimulating and anti-diabetes, as well as in the prevention of cancer and the ageing process [1-7]. Recent publications also indicated some rare ginsenosides such as Rh<sub>2</sub>, Rg<sub>3</sub> and M<sub>1</sub> showed significant *in vivo* anti-tumor activities [8-12]. Pharmaceutical studies have shown that orally ingested ginsenoside passes through the stomach and small intestine without decomposition, but the colonic bacteria cleave the oligosaccharide connected to the aglycone stepwise from the terminal sugar to afford the major metabolite, ginsenoside-M<sub>1</sub> [13-14]. Previous studies also showed that ginsenoside-M<sub>1</sub> was further esterified with fatty acids which could be sustained longer in the body; this result indicated that fatty acid ester of the M<sub>1</sub> might be the real anti-tumor active species *in vivo* [15].

In our continued studies [16-18] on bioactive ginsenosides from *Panax ginseng*, four known ginsenosides were isolated, a ginsenoside was obtained from enzyme reaction and three novel fatty acid esterified ginsenosides derivatives were synthesized. Their structures were elucidated on the basis of high resolution ESI-TOF-MS, <sup>1</sup>H- and <sup>13</sup>C-NMR, together with 2D-NMR spectroscopic analyses. The isolated and synthetic compounds were tested in an anti-tumor bioassay, and compounds **5-8** showed considerable cytotoxicity (SRB) against several human cancer cell lines: breast cancer MCF-7, skin melanoma SK-MEL-2 and human ovarian carcinoma B16, but moderate effects on lung carcinoma COR-L23, while the other ginsenosides showed no effects. To our knowledge, this is first time these fatty acid esterified ginsenosides have been synthesized. In this paper, we describe the isolation and structure elucidation of the novel ginsenoside derivatives and their bioactivity results.

**Figure 1.** Isolated, enzyme produced and synthetic ginsenosides.



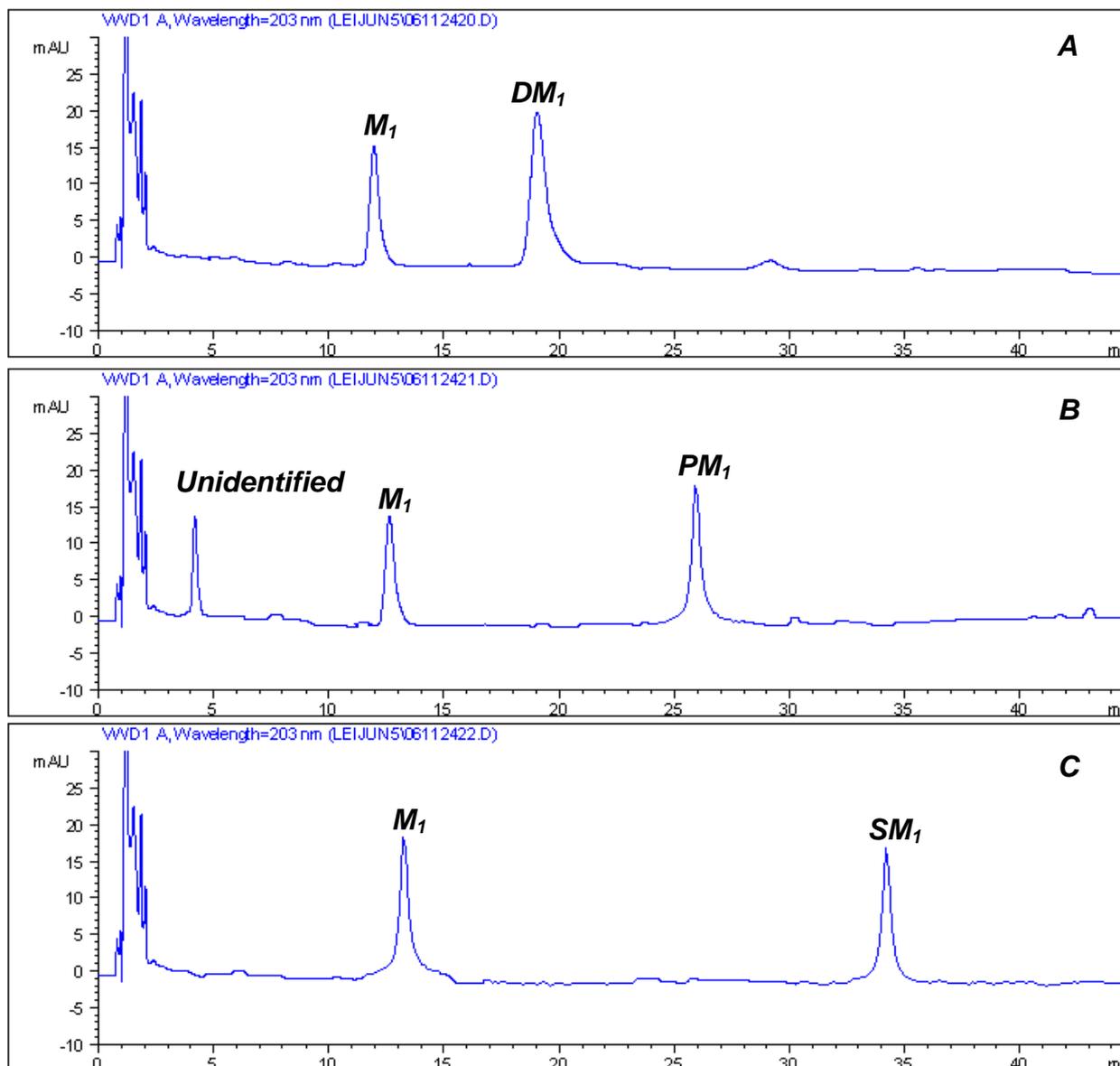
## Results and Discussion

### Characterizations of compounds **1** – **8**

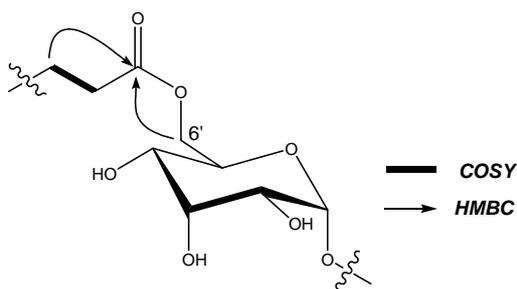
The known compounds were identified on the basis of comparison of their NMR data (see Table 1 and 2) with those of the literature [19]. Four compounds have been characterized as ginsenoside-Rb<sub>1</sub> (**1**), Rb<sub>3</sub> (**2**), Rd (**3**) and Re (**4**). Compound **1** had been used as a substrate in an enzymatic reaction to produce ginsenoside-M<sub>1</sub> (**5**), whose structure was elucidated by MS, 1D- and 2D-NMR data analysis (not shown). The process whereby ginsenoside M<sub>1</sub> was esterified by dodecanoyl chloride, palmitoyl chloride and stearoyl chloride, respectively, was monitored by HPLC. As shown in Figure 2, compounds **6-8** showed lower polarities compared with M<sub>1</sub> (**5**). Comparison of the <sup>13</sup>C-NMR data of **6-8** with those of **5**, indicated no significant chemical shifts changes in the main skeleton, but the C-6' of the 20-O-Glc group was shifted upfield to δ 63.3, 64.6, 64.4, respectively, suggesting that the fatty

acid ester substituent was connected to the C-6' position. This assumption had been verified by HMBC (see Figure 3) which showed a cross-peak between H-6' to the carboxyl carbon.

**Figure 2.** HPLC spectra of the reaction product of DM<sub>1</sub> (6), PM<sub>1</sub> (7) and SM<sub>1</sub> (8); **A:** M<sub>1</sub> reacted with dodecanoyl chloride; **B:** M<sub>1</sub> reacted with palmitoyl chloride; **C:** M<sub>1</sub> reacted with stearoyl chloride. HPLC conditions: gradient MeOH-H<sub>2</sub>O from 45% to 90% for 45 min.



**Figure 3.** Partial HMBC and COSY correlation of the synthetic compound.



**Table 1.** The  $^{13}\text{C}$  NMR data of compounds **1-8**<sup>a</sup>.

Position	Rb <sub>1</sub> (1)	Rb <sub>3</sub> (2)	Rd (3)	Re (4)	M <sub>1</sub> (5)	DM <sub>1</sub> (6)	PM <sub>1</sub> (7)	SM <sub>1</sub> (8)
1	39.2	39.3	39.2	39.7	39.3	38.9	39.5	39.1
2	26.3	26.3	26.7	26.6	28.1	27.3	28.2	28.0
3	88.9	89.0	89.0	78.1	77.9	78.8	78.0	77.8
4	39.6	39.6	39.1	39.9	39.4	38.9	39.4	39.3
5	56.1	56.7	56.3	60.7	56.2	55.8	56.4	56.1
6	18.6	18.6	18.5	74.2	18.6	18.2	18.7	18.5
7	35.1	35.2	35.1	45.8	35.0	34.7	35.2	34.9
8	39.8	39.9	40.4	41.3	39.9	39.7	39.5	39.8
9	50.0	50.2	50.2	49.5	50.2	49.8	50.3	50.0
10	36.7	37.0	37.1	39.6	37.2	37.0	37.3	37.1
11	30.8	31.0	31.1	30.7	30.7	30.3	30.7	30.7
12	70.1	70.1	70.2	70.1	70.1	70.6	70.1	69.8
13	49.3	49.5	49.6	49.4	49.3	47.8	49.5	49.3
14	51.3	51.4	51.5	51.3	51.3	51.7	51.4	51.3
15	30.8	30.8	30.7	30.9	30.7	30.6	31.0	30.5
16	26.6	26.8	26.6	26.6	26.5	26.6	26.7	26.5
17	51.5	51.6	51.6	51.6	51.5	51.3	51.6	51.2
18	16.0	16.0	17.7	17.7	16.2	16.1	16.3	16.1
19	16.1	16.2	16.4	17.8	15.9	15.7	16.0	15.8
20	83.1	83.5	83.1	83.3	83.2	84.3	83.4	83.1
21	22.3	22.3	22.4	22.4	22.3	21.2	22.9	22.7
22	36.2	36.1	36.2	35.8	36.0	35.4	36.1	35.9
23	23.0	23.0	23.2	23.3	23.1	22.0	23.0	22.8
24	125.8	126.0	125.8	126.0	125.8	124.5	126.0	125.8
25	131.1	131.0	131.0	130.9	130.8	131.5	130.9	130.7
26	25.8	25.8	25.7	25.8	25.7	25.7	25.8	25.5
27	17.9	17.9	18.0	17.6	17.7	17.6	17.8	17.6
28	28.0	28.3	28.1	32.3	28.6	28.0	28.6	28.4
29	16.6	16.5	16.3	17.3	16.2	15.3	16.3	16.1
30	17.1	17.4	17.7	17.3	17.3	16.9	17.4	17.2

<sup>a</sup> Compound **6** was measured in  $\text{CDCl}_3$  and the other compounds were measured in pyridine- $d_5$  and chemical shifts are expressed in ppm.

**Table 2.**  $^{13}\text{C}$  and  $^1\text{H}$  NMR data of sugar and fatty acid ester moieties of compounds **5-8**<sup>a, b</sup>.

Position	$\text{M}_1$ (5)		$\text{DM}_1$ (6)		$\text{PM}_1$ (7)		$\text{SM}_1$ (8)	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$
<b>20-O-Glc</b>								
1'	98.1	5.14 (d, 7.5)	96.8	4.51 (d, 7.6)	98.0	5.11 (d, 7.5)	97.8	5.14 (d, 7.5)
2'	75.0	3.96 (t, 8.0)	73.3	3.36 (m)	75.0	3.95 (d, 8.0)	74.7	3.92 (d, 8.0)
3'	79.2	4.19 (m)	76.7	3.55 (m)	79.2	4.18 (m)	79.0	4.19 (m)
4'	71.5	4.12 (dd, 9.0, 8.5)	70.0	3.43 (m)	71.6	4.16 (m)	71.4	4.17 (m)
5'	78.2	3.88 (m)	73.4	3.44 (m)	78.0	3.98 (m)	77.8	3.98 (m)
6'	62.7	4.44, 4.27 (m)	63.3	4.30, 4.38 (m)	64.6	4.63, 5.02 (m)	64.4	4.65, 5.05 (m)
<b>Glc-6'-O-ester</b>								
1''			174.1		173.5		173.3	
2''			34.2	2.27, 2.35 (m)	34.4	2.43, 2.48 (m)	34.2	2.42, 2.49 (m)
3''			24.8	1.27 <sup>c</sup>	25.3	1.42	25.0	1.45
4''			29.6 <sup>c</sup>	1.27 <sup>c</sup>	29.4	1.27 <sup>c</sup>	30.7	1.27 <sup>c</sup>
5''			29.1 <sup>c</sup>	1.27 <sup>c</sup>	28.2	1.27 <sup>c</sup>	29.2	1.27 <sup>c</sup>
6''			29.2 <sup>c</sup>	1.27 <sup>c</sup>	29.6	1.27 <sup>c</sup>	29.3	1.27 <sup>c</sup>
7''			29.3 <sup>c</sup>	1.27 <sup>c</sup>	30.0 <sup>c</sup>	1.27 <sup>c</sup>	29.4	1.27 <sup>c</sup>
8''			29.4 <sup>c</sup>	1.27 <sup>c</sup>	30.0 <sup>c</sup>	1.27 <sup>c</sup>	29.7 <sup>c</sup>	1.27 <sup>c</sup>
9''			29.6 <sup>c</sup>	1.27 <sup>c</sup>	30.0 <sup>c</sup>	1.27 <sup>c</sup>	29.7 <sup>c</sup>	1.27 <sup>c</sup>
10''			31.9	1.36 (m)	30.0 <sup>c</sup>	1.27 <sup>c</sup>	29.7 <sup>c</sup>	1.27 <sup>c</sup>
11''			22.6	1.56 (m)	30.0 <sup>c</sup>	1.27 <sup>c</sup>	29.7 <sup>c</sup>	1.27 <sup>c</sup>
12''			14.1	0.85 (t, 6.0)	29.6	1.27 <sup>c</sup>	29.7 <sup>c</sup>	1.27 <sup>c</sup>
13''					29.8	1.27 <sup>c</sup>	29.5	1.27 <sup>c</sup>
14''					32.1	1.30 (m)	30.5	1.27 <sup>c</sup>
15''					22.1	1.48 (m)	29.7	1.27 <sup>c</sup>
16''					14.2	0.83 (t, 5.6)	31.9	1.36 (m)
17''							22.7	1.48 (m)
18''							14.0	0.85 (t, 0.56)

<sup>a</sup> Compound **6** was measured in  $\text{CDCl}_3$  and compounds **5**, **7-8** were measured in pyridine- $d_5$ ; chemical shifts are expressed in ppm; <sup>b</sup> Multiplicity and  $J$  values in Hz are given in parentheses; <sup>c</sup> Signals may be exchanged.

### Bioactivity Results

The isolated and synthetic compounds were tested in an anti-tumor bioassay. Compounds **1-4** showed no cytotoxicity effects, while compounds **5-8** showed significant cytotoxicity (SRB) against several human cancer cell lines: breast cancer MCF-7, skin melanoma SK-MEL-2 and human ovarian carcinoma B16, but moderate effects on lung carcinoma COR-L23. The results are shown in Table 3. Compound **8** showed highest cytotoxic effect on skin melanoma SK-MEL-2 and human ovarian carcinoma B16; **6** showed highest cytotoxic effects on breast cancer cell lines MCF-7; Compounds **6-8** showed moderate effects on lung carcinoma COR-L23. All the tested compounds showed no cytotoxic activities towards normal cells at the concentration of 300  $\mu\text{g}/\text{mL}$ .

**Table 3.** Cytotoxic activities of compounds **5-8** (IC<sub>50</sub> values in µg/mL)

	M <sub>1</sub> (5)	DM <sub>1</sub> (6)	PM <sub>1</sub> (7)	SM <sub>1</sub> (8)
MCF-7	8.48	0.50	2.31	1.65
SK-MEL-2	14.71	1.46	1.88	0.17
B16	6.10	6.13	5.73	0.33
COR-L23	33.0	5.68	4.86	7.76

## Conclusions

The effects of some ginsenosides have been demonstrated on various models of tumor and endothelial cells. For example Rg<sub>3</sub> inhibited the proliferation and induced of apoptosis in bladder cancer cells [20], and Rh<sub>2</sub> shows effects on human lung adenocarcinoma A549 cells [21]. Most of the reported antitumor ginsenosides belongs to the protopanaxodiol-type saponins. In the present study, we isolated and transformed the protopanaxodiol-type ginsenosides Rb<sub>1</sub> into M<sub>1</sub>, and further chemical modification led to the synthesis of three ginsenoside mono- fatty acid esters. Comparison of the cytotoxic activity results suggested that the fatty acid esterified ginsenoside were more active against human tumor cells. The most probable reason was that when cells acquire the molecules and ions they need from their surrounding extracellular fluid (ECF), the membrane transportation of small molecules depends on their lipophilic abilities [22]. In this study, we synthesized ginsenoside-DM<sub>1</sub>, PM<sub>1</sub> and SM<sub>1</sub> used fatty acid acyl-chlorides, so these reaction products showed significant lower polarities compared with the ginsenosides as shown in Figure 2. These lipophilic compounds which could pass through the membrane easily led to their increased cytotoxic activities on tumor cells.

## Experimental

### General

The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were measured on a Bruker Avance DRX 500 NMR spectrometer in C<sub>5</sub>D<sub>5</sub>N or CDCl<sub>3</sub>, using TMS as an internal standard. Chemical shifts (δ) are expressed in parts per million (ppm), with the coupling constants (*J*) reported in Hertz (Hz). The HR-ESI-TOF mass spectra were obtained from a MDS SCIEX API QSTAR-MS instrument. Column chromatographies were carried out with silica gel 60M (200-300 mesh), MCI and ODS RP-18 (20 µm); TLC was performed with silica gel plates (Macherey -Nagel, SilG / UV<sub>254</sub>, 0.20mm); HPLC were carried out on an Agilent 1100 system.

### Chemicals and reagents

Dodecanoyl chloride, palmitoyl chloride and stearoyl chloride were purchased from ABCR GmbH & Co. KG. Sulforhodamine B (SRB) was purchased from Sigma (St. Louis, MO, USA). Enzyme NS 37040 was purchased from Novozymes Co. Ltd. (Holland). Other chemicals and reagents were purchased from the Chinese Chemical Group (Beijing, China).

### Extraction and isolation

Crude powder of *Panax ginseng* (16 Kg) was extracted with 70% MeOH at room temperature, and the extract was concentrated to give a brown oily residue (3,700 g). The extract was directly subjected to chromatography on a silica column, eluted with a gradient mixture of CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (50: 10: 1, 7: 3: 0.5, 13: 7: 2), to yield eight fractions (F<sub>A</sub> - F<sub>H</sub>). The seventh fraction F<sub>G</sub> was subjected to silica gel, eluted with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O = 7: 3: 0.5 to afford compound **1** (2.1 g) and **2** (1.7 g). The fifth fraction F<sub>E</sub> was subjected to ODS RP-18 column eluted with 70% MeOH to afford compound **3** (1.3 g). The fourth fraction F<sub>D</sub> was subjected to further chromatography on MCI column, with 55% MeOH elution, to afford compound **4** (2.6 g).

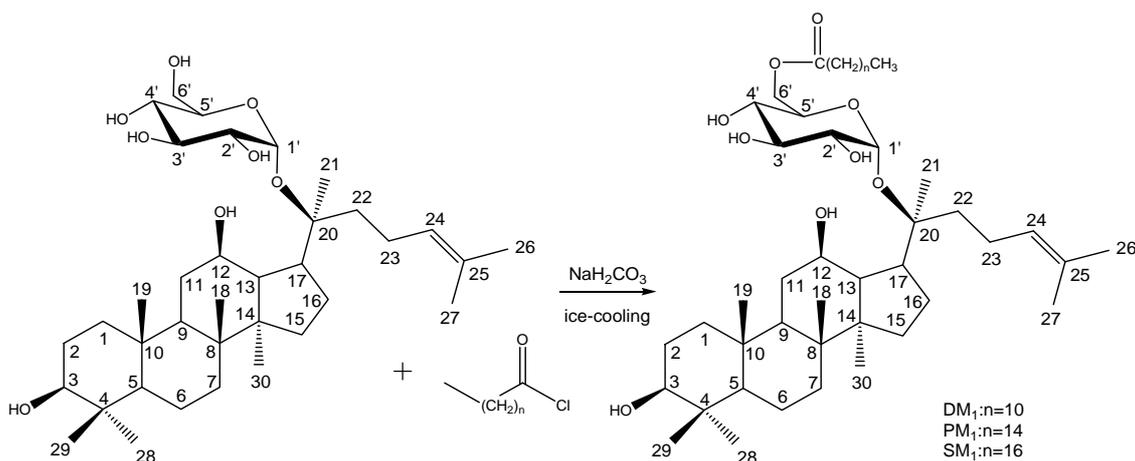
### Enzymatic reaction

Ginsenoside-Rb<sub>1</sub> (1,500 mg) was dissolved in H<sub>2</sub>O (100 mL), enzyme NS37040 (30 mL) was added and the mixture was shaken at 45 °C for 3 days with control of the pH at a value of 4.8. The reaction was terminated by heating to 80 °C, then the enzyme reaction mixture was centrifuged under 10,000 rpm for 5 min and the supernatant was subjected to silica gel eluted with CHCl<sub>3</sub>-MeOH (10:1) to give purified compound **5** (581 mg).

### Synthesis of DM<sub>1</sub>, PM<sub>1</sub> and SM<sub>1</sub>

Compound M<sub>1</sub> (1.0 mmol) was dissolved in CHCl<sub>3</sub> (200 mL) under ice-cooling, then aqueous saturated NaH<sub>2</sub>CO<sub>3</sub> (10 mL) was added slowly. Dodecanoyl chloride (2.0 mmol) was added to the aqueous CHCl<sub>3</sub> mixture with a measuring pipette under stirring, which was continued for 2 h. The aqueous layer was washed five times with CHCl<sub>3</sub> (100 mL). The CHCl<sub>3</sub> solution was concentrated under reduced pressure, then analyzed by TLC with CHCl<sub>3</sub>-MeOH = 15:1 as the developing solvent. Crude DM<sub>1</sub> (**6**, 62 mg) were purified by silica gel column chromatography, eluted by CHCl<sub>3</sub>-MeOH = 18:1, then the purity was analyzed by HPLC (80% MeOH, Hypersil C<sub>18</sub>, 40°C, 0.5 mL/min). The same experimental procedure (see Scheme 1) was followed using palmitoyl chloride and stearoyl chloride for the production of the corresponding compounds **7** (PM<sub>1</sub>, 77 mg) and **8** (SM<sub>1</sub>, 58 mg), respectively.

**Scheme 1.** Preparation of ginsenosides DM<sub>1</sub> (**6**), PM<sub>1</sub> (**7**) and SM<sub>1</sub> (**8**).



*Ginsenoside Rb<sub>1</sub>* (**1**). White amorphous powder, mp 199 - 202 °C; ESI-MS [+]: m/z = 1131.3 [M+Na]<sup>+</sup>; 407 [M-4xGlc]<sup>+</sup>; <sup>1</sup>H-NMR (pyridine-d<sub>5</sub>): δ 0.78 (3H, s, H-19), 0.93 (3H, s, H-30), 0.94 (3H, s, H-18), 1.08 (3H, s, H-29), 1.25 (3H, s, H-28), 1.57 (3H, s, H-21), 1.63 (3H, s, H-26), 1.64 (3H, s, H-27), 5.45 (1H, m, H-24), <sup>13</sup>C-NMR data, see Table 1.

*Ginsenoside Rb<sub>3</sub>* (**2**). White amorphous powder, mp 196 - 198 °C; IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 3322, 2930, 2893, 1651; ESI-MS [+]: m/z = 1079.0 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (pyridine-d<sub>5</sub>): δ 0.79 (3H, s, H-19), 0.93 (3H, s, H-30), 0.95 (3H, s, H-18), 1.09 (3H, s, H-29), 1.37 (3H, s, H-28), 1.53 (3H, s, H-21), 1.59 (3H, s, H-26), 1.62 (3H, s, H-27), 3.83 (1H, m, H-12), 5.40 (1H, brs, H-24), <sup>13</sup>C-NMR data, see Table 1.

*Ginsenoside Rd* (**3**). White amorphous powder, mp 205 - 206 °C; ESI-MS [+]: m/z = 947.6 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (pyridine-d<sub>5</sub>): δ 0.73 (3H, s, H-19), 0.89 (3H, s, H-30), 0.91 (3H, s, H-18), 1.05 (3H, s, H-29), 1.23 (3H, s, H-28), 1.56 (6H, s, H-26, 27), <sup>13</sup>C-NMR data, see Table 1.

*Ginsenoside Re* (**4**). White amorphous powder, mp 201 - 203 °C; ESI-MS [+]: m/z = 969.3 [M+Na]<sup>+</sup>; <sup>13</sup>C-NMR data, see Table 1.

*Ginsenoside M<sub>1</sub>* (**5**). White amorphous powder, mp 192 - 193 °C; IR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 3423, 2943, 2875, 1715, 1639; ESI-MS [+]: m/z = 645.2 [M+Na]<sup>+</sup>; <sup>1</sup>H-NMR (pyridine-d<sub>5</sub>): δ 0.85 (3H, s, H-19), 0.90 (3H, s, H-30), 0.94 (3H, s, H-18), 1.00 (3H, s, H-29), 1.19 (3H, s, H-28), 1.56 (6H, s, H-26, 27), 1.58 (3H, s, H-21), 5.21 (1H, brs, H-24); other NMR data, see Tables 1 and 2.

*Ginsenoside DM<sub>1</sub>* (**6**). Colorless oil; [α]<sub>D</sub><sup>25</sup> - 107.1 (c 0.70, MeOH); ESI-MS [+]: m/z = 805.3 [M+H]<sup>+</sup>; HR-ESI-TOF-MS [+]: m/z = 805.6186 [M+H]<sup>+</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 5.13 (1H, t, J = 6.0, H-24), 3.22 (1H, dd, J = 5.0, 11.0, H-3), 2.20 (1H, m, H-17), 2.18 (1H, m, H-23b), 1.98 (1H, m, H-23a), 1.82 (1H, m, H-11a), 1.80 (1H, m, H-13), 1.70 (3H, s, H-26), 1.73 (1H, s, H-1a), 1.66 (1H, m, H-16b), 1.63 (1H, m, H-2b), 1.62 (3H, s, H-27), 1.63 (1H, m, H-22b), 1.58 (1H, m, H-2a), 1.56 (1H, m, H-6b), 1.50 (1H, m, H-7b), 1.47 (1H, m, H-6a), 1.43 (1H, m, H-22a), 1.40 (1H, m, H-9a), 1.37 (3H, s, H-21), 1.28 (2H, m, H-7a, H-15b), 1.27 (2H, m, H-11a, H-16a), 1.00 (6H, s, H-18, 28), 0.99 (1H, m, H-2b), 0.91 (3H, s, H-30), 0.89 (3H, s, H-19), 0.88 (1H, m, H-15a), 0.80 (3H, s, H-29), 0.74 (1H, d, J = 11.0, H-5); other NMR data, see Tables 1 and 2.

*Ginsenoside PM<sub>1</sub>* (**7**). Colorless oil; ESI-MS [+]: m/z = 861.1 [M+H]<sup>+</sup>; HR-ESI-TOF-MS [+]: m/z = 861.6455 [M+H]<sup>+</sup>; <sup>1</sup>H-NMR (C<sub>5</sub>D<sub>5</sub>N): δ 5.32 (1H, t, J = 6.5, H-24), 3.39 (1H, dd, J = 5.0, 11.0, H-3), 2.59 (1H, m, H-17), 2.55 (1H, m, H-23b), 2.43 (1H, m, H-22a), 2.31 (1H, m, H-23a), 2.02 (1H, m, H-11a), 2.00 (1H, m, H-13), 1.67 (3H, s, H-26), 1.73 (1H, s, H-1a), 1.69 (1H, m, H-1b), 1.85 (1H, m, H-16b), 1.88 (1H, m, H-2b), 1.64 (3H, s, H-27), 1.82 (1H, m, H-22b), 1.80 (1H, m, H-2a), 1.59 (1H, m, H-6b), 1.50 (1H, m, H-7b), 1.47 (1H, m, H-6a), 1.43 (1H, m, H-22a), 1.40 (1H, m, H-9a), 1.64 (3H, s, H-21), 1.53 (1H, m, H-15b), 1.31 (1H, m, H-7a), 1.55 (1H, m, H-11a), 1.40 (1H, m, H-16a), 1.20 (3H, s, H-28), 0.87 (3H, s, H-18), 0.89 (1H, m, H-2b), 0.94 (3H, s, H-30), 0.98 (3H, s, H-19), 0.88 (1H, m, H-15a), 1.02 (3H, s, H-29), 0.79 (1H, d, J = 11.0, H-5); other NMR data, see Tables 1 and 2.

*Ginsenoside SM<sub>1</sub>* (**8**). Colorless oil; ESI-MS [+]:  $m/z = 889.3 [M+H]^+$ ; HR-ESI-TOF-MS [+]:  $m/z = 889.5203 [M+H]^+$ ; <sup>1</sup>H-NMR (C<sub>5</sub>D<sub>5</sub>N):  $\delta$  5.33 (1H, t,  $J = 6.5$ , H-24), 3.40 (1H, dd,  $J = 5.0, 11.0$ , H-3), 2.58 (1H, m, H-17), 2.56 (1H, m, H-23b), 2.41 (1H, m, H-22a), 2.32 (1H, m, H-23a), 2.01 (1H, m, H-11a), 2.00 (1H, m, H-13), 1.67 (3H, s, H-26), 1.73 (1H, s, H-1a), 1.69 (1H, m, H-1b), 1.85 (1H, m, H-16b), 1.64 (3H, s, H-27), 1.82 (1H, m, H-22b), 1.80 (1H, m, H-2a), 1.89 (1H, m, H-2b), 1.58 (1H, m, H-6b), 1.50 (1H, m, H-7b), 1.47 (1H, m, H-6a), 1.43 (1H, m, H-22a), 1.42 (1H, m, H-9a), 1.66 (3H, s, H-21), 1.53 (1H, m, H-15b), 1.33 (1H, m, H-7a), 1.53 (1H, m, H-11a), 1.39 (1H, m, H-16a), 1.20 (3H, s, H-28), 0.87 (3H, s, H-18), 0.89 (1H, m, H-2b), 0.94 (3H, s, H-30), 0.98 (3H, s, H-19), 0.88 (1H, m, H-15a), 1.02 (3H, s, H-29), 0.77 (1H, d,  $J = 11.0$ , H-5); other NMR data, see Tables 1 and 2.

### *In vitro* cytotoxicity assays

Five-day *in vitro* SRB cytotoxicity tests against human tumors cell lines were carried out at the Cell Culture Laboratory, Pharmaceutical College, Jilin University, using modified protocols for MCF-7 (breast cancer), SK-MEL-2 (skin melanoma), B16 (human ovarian carcinoma) and COR-L23 (lung carcinoma), the normal cells were used as control [23]. Generally,  $5 \times 10^3$ /mL cells were placed in a 24-well plate and treated with obtained compounds. The plate was incubated at 37 °C for 5 days. Then the medium was removed from the 24-well plate, and 10% ice-cold TCA (trichloroacetic acid, 1 mL) was added. The plate was kept at 4 °C for two hours after which was washed four times with cold water, then stained with SRB (Sulforhodamine B, Sigma St. Louis, MO, USA). After washing with 1% acetic acid, the bound dye was solubilized with Tris base A (Sigma) and 100  $\mu$ L of each sample were transferred into a 96-well plate, and then read at 492 nm.

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