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# Polyoxypregnane Glycosides from the Roots of *Marsdenia tenacissima* and Their Anti-HIV Activities

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## ABSTRACT

A continuous phytochemical study on the roots of *Marsdenia tenacissima* led to the isolation and identification of 13 new polyoxypregnane glycosides named marstenacissides B10–B17 (**1**, **2**, **4**, **7**, **8**, **11**, **12**, and **14**) and marstenacissides A8-A12 (**3**, **9**, **10**, **13**, and **15**) in addition to two known polyoxypregnane glycosides marsdenosides M and L (**5** and **6**). Their structures were established by spectroscopic techniques and by comparison with the reported data in the literature. Moreover, the anti-HIV activities of these isolates and the previous isolated marstenacissides A1–A7 and B1–B9 were assessed, some of which exhibited slight or negligible effects against HIV-1.

## Introduction

*Marsdenia tenacissima* (Roxb.) Moon (Asclepiadaceae), a perennial climber recorded in the Flora of China, is extensively distributed in the Yunnan province of China. The roots of this plant are widely used as a traditional herbal medicine of Dai nationality, called "Dai-Bai-Jie" in Chinese, due to its pharmacological functions of clearing heat, expelling miasma, decreasing swelling, alleviating pain, etc. In addition, these roots are also used as the main medicinal materials for preparing a series of preparations [1]. The isolation and structural identification of 16 polyoxypregnanes glycosides (marstenacissides A1–A7 and B1–B9) from the roots of *M. tenacissima* have been reported in our previous paper [2]. A continuous phytochemical study on the roots of *M. tenacissima* led to the further isolation of 13 new chemicals (**1**–**4** and **7**–**15**) and 2 known polyoxypregnane glycosides (**5** and **6**; ▶ **Fig. 1**).

The traditional Chinsese medicine with functions of heat clearing and detoxicating is supposed to have specific antiviral activity based on the correlation analysis between this kind of traditional medicine and antiviral drugs [3]. Therefore, the extracts of *M. auricularis* roots were subjected to a preliminary screening of activity against HIV-1, and it was found that the 95% EtOH extract of *M. auricularis* roots, especially its EtOAc-soluble fraction, exhibited a significant HIV-1 inhibitory effect. In view of the above significant HIV-1 inhibitory effect, all 31 isolated polyoxypregnane glycosides were screened, followed by chemical work.

## Results and Discussion

After a series of purification steps, 15 compounds were obtained from the roots of *M. auricularis*, and all of the compounds were identified to be $C_{21}$ steroidal glycosides with 2-deoxysugar units. Among them, two known compounds were marsdenosides M (**5**) and L (**6**), determined by comparing their $^{13}C$ NMR data with the

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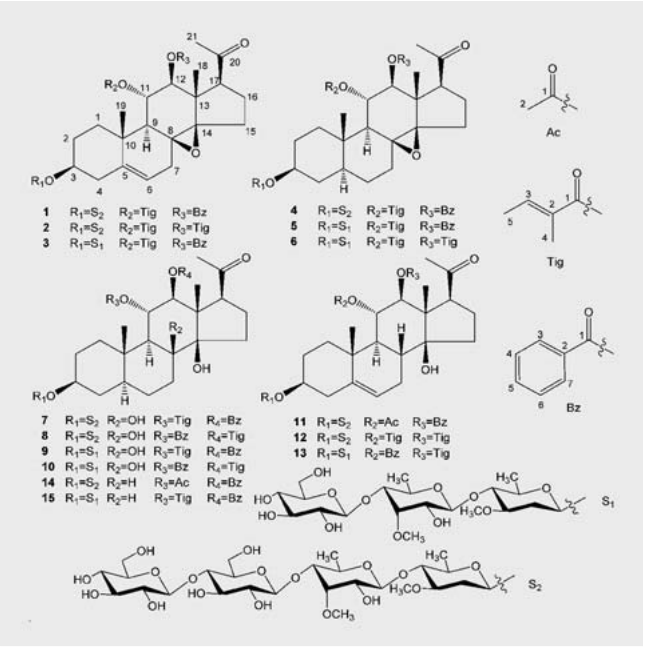
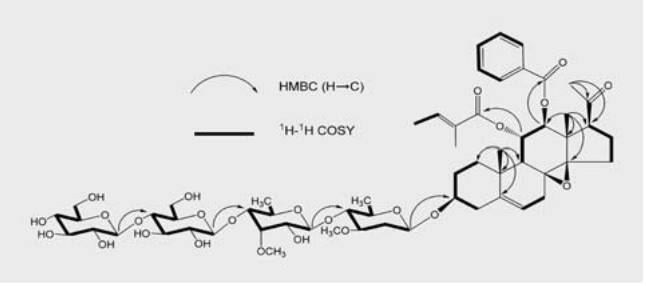
literature reported [4], and the other compounds (**1**–**4** and **7**–**15**) were identified to be new polyoxypregnane glycosides by analysis of NMR (including 1D and 2D NMR) and MS spectra.

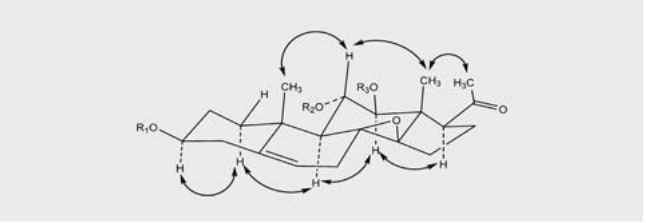
Compound **1** had a molecular formula of $C_{59}H_{84}O_{24}$ based on the HRESIMS ion $[M + Na]^+$ at *m/z* 1199.5303. In the $^1H$ NMR spectrum, three methyl singlet signals at *δ* 1.55 (3 H, s, H-18), 1.54 (3 H, s, H-19), and 1.99 (3 H, s, H-21) and three signals at *δ* 3.80 (1 H, m, H-3), 5.97 (1 H, t, *J* = 10.1 Hz, H-11), and 5.59 (1 H, d, *J* = 10.1 Hz, H-12) corresponding to carbons with secondary oxidation were observed. Combination of $^1H$ and $^{13}C$ NMR data indicated **1** had a $C_{21}$ steroidal skeleton. The proton signals at *δ* 6.77 (1 H, qq, *J* = 7.1, 1.4 Hz, Tig-H-3), 1.33 (3 H, d, *J* = 7.1, Tig-H-4), 1.50 (3 H, s, Tig-H-5), 8.15 (2 H, dd, *J* = 7.8, 1.3 Hz, Bz-H-3, 7), 7.36 (2 H, dd, *J* = 7.8, 7.4 Hz, Bz-H-4, 6), and 7.46 (1 H, t, *J* = 7.4 Hz, Bz-H-5) as well as two groups of characteristic carbon signals at *δ* 167.0 (Tig-C-1), 128.6 (Tig-C-2), 138.7 (Tig-C-3), 14.1 (Tig-C-4), and 11.7 (Tig-C-5), 166.5 (Bz-C-1), 130.6 (Bz-C-2), 130.0 (Bz-C-3, 7), 128.8 (Bz-C-4, 6), and 133.5 (Bz-C-5) indicated the existence of a tigloyl and a benzyl group in the molecular structure. By the combined use of HSQC, HMBC, and $^1H$-$^1H$ COSY experiments (▶ **Fig. 2**), the proton and carbon signals of the aglycone moiety were assigned. In the ROESY spectrum, the dipolar interaction of H-11/H-19, H-12/H-17, H-12/H-9, H-21/H-18, H-9/H-4a, H-9/H-1a, H-3/H-4a, H-3/H-1a, and H-3/H-2a deduced the orientations of H-3, H-11, H-12, and H-17 to be *α, β, α*, and *α*, respectively (▶ **Fig. 3**). Therefore, the aglycone structure of **1** was identified as 8*β*,14*β*-epoxy-3*β*,11*α*,12*β*-trihydroxyprengn-5-en-20-one, which is a new $C_{21}$ steroid skeleton. On the basis of HMBC correlations between *δ* 167.0 (Tig-C-1) and 5.97 (H-11), and between *δ* 166.5 (Bz-C-1) and 5.59 (H-12), the position of the tigloyl and benzyl groups were determined at C-11 and C-12, respectively (▶ **Fig. 2**). The anomeric regions in the $^1H$ and $^{13}C$ NMR spectra presented four protons at *δ* 5.24 (1 H, d, *J* = 8.8 Hz), 5.19 (1 H, d, *J* = 7.9 Hz), 4.90 (1 H, d, *J* = 7.8 Hz), and 4.76 (1 H, dd, *J* = 9.5, 1.4 Hz) and four carbon signals at *δ* 106.6, 105.0, 101.9, and 97.9, suggesting the existence of four sugar units in **1**. The *β* configurations of the four sugars were determined based on each large coupling constant ($^3J_{1,2} > 7$ Hz). Moreover, two methyl doublets at *δ* 1.56 (3 H, d, *J* = 5.3 Hz), 1.61 (3 H, d, *J* = 6.2 Hz) and two methyl singlets at *δ* 3.48 (3 H, s), 3.79 (3 H, s) in the $^1H$ NMR spectrum indicated that two of the four sugar units were 6-deoxy-3-*O*-methly pyranoses [5]. Eventually, the sugar units were identified as oleandrose, 6-deoxy-3-*O*-methyl-allose, and two glucoses by NMR spectroscopic data analysis as well as by comparison with previously reported values. Using $^1H$-$^1H$ COSY, HSQC, and HMBC spectra, the proton and carbon resonances of each sugar were fully assigned. The connectivity of the sugars was established by HMBC correlations between *δ* 5.24 (Allo-H-1) and 83.3 (Ole-C-4), between *δ* 4.90 ($Glc_1$-H-1) and 83.4 (Allo-C-4), and between *δ* 5.19 ($Glc_2$-H-1) and 81.5 ($Glc_1$-C-4) (▶ **Fig. 2**). Therefore, the sugar moiety could be deduced as 3-*O*-*β*-glucopyranosyl-(1 → 4)-*β*-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-*β*-allopyranosyl-(1 → 4)-*β*-oleandropyranoside, well in agreement with those in the compounds isolated from the same plant [6–8]. Also, previous phytochemical studies suggested that the absolute configuration of the glucose, 6-deoxy-3-*O*-methyl-allose, and oleandrose from *M. auricularis* should be D [4–11]. Furthermore, the glycosidation site was determined by HMBC correlations between *δ* 4.76 (Ole-H-1) and 77.0 (C-3) (▶ **Fig. 2**). Consequently, the structure of **1** was elucidated as 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1 → 4)-*β*-D-oleandropyranosyl-11-*O*-tigloyl-12-*O*-benzyl-8*β*,14*β*-epoxy-3*β*,11*α*,12*β*-trihydroxyprengn-5-e-20-one and named marstenacisside B10.




▶ **Fig. 1** Structures of **1**–**15**.




▶ **Fig. 2** Key HMBC and $^1H$-$^1H$ COSY correlations for **1**.



▶ **Fig. 3** Key ROESY correlations of the aglycone for **1**.

Compound **2**, with the molecular formula of $C_{57}H_{86}O_{24}$ determined by the HRESIMS ion $[M + Na]^+$ at *m/z* 1177.5454, had the same sugar moiety and $C_{21}$ steroid skeleton as **1** based on comparison of their NMR data. In the $^{13}C$ NMR spectrum of **2**, the characteristic carbon signals at *δ* 167.1, 167.0, 138.6, 138.4, 128.9, 128.8, 14.3, 14.2, 12.0, and 12.0 suggested the existence of double tigloyl groups in **2**. By the combined use of HSQC, HMBC, and $^1H$-$^1H$ COSY experiments, the structure of **2** was finally elucidated as 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1 → 4)-*β*-D-oleandropyranosyl-11,12-*O*-ditigloyl-8*β*,14*β*-epoxy-3*β*,11*α*,12*β*-tridroxypregnane-5-en-20-one and named marstenacisside B11.

Compound **3**, with the molecular formula of $C_{53}H_{74}O_{19}$ determined by the HRESIMS ion $[M - H]^-$at *m/z* 1013.4787, had the same $C_{21}$ steroid skeleton and ester groups as **1** by comparing their NMR data. The $^1H$ and $^{13}C$ NMR spectra of **3** showed three anomeric proton signals at *δ* 4.78 (1 H, d, *J* = 9.4 Hz), 4.99 (1 H, d, *J* = 7.7 Hz), and 5.27 (1 H, d, *J* = 8.0 Hz) and three anomeric carbon signals at *δ* 97.9, 101.9, and 106.6, suggesting that the sugar moiety of **3** was composed of three sugar units. The large coupling constants ($^3J_{1,2} > 7$ Hz) deduced the *β* configurations of the three sugars. Finally, the sugar units were identified as oleandrose, 6-deoxy-3-*O*-methyl-allose, and glucose by NMR spectroscopic data analysis as well as by comparison with previously reported values. Using $^1H$-$^1H$ COSY, HSQC, and HMBC spectra, the proton spin systems and the carbon resonances of three sugars were fully assigned. The HMBC correlations between *δ* 5.27 (Allo-H-1) and 83.2 (Ole-C-4), and between *δ* 4.99 (Glc-H-1) and 83.3 (Allo-C-4) established the sequence of the sugar chain. Therefore, the sugar moiety could be deduced as 3-*O*-*β*-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-*β*-allopyranosyl-(1 → 4)-*β*-oleandropyranoside and well coincided with neo-condurangotriose in other compounds that were also isolated from *M. tenacissima* [9]. The glycosidation site was confirmed by the HMBC correlation between *δ* 4.78 (Ole-H-1) and 76.2 (C-3). Finally, the structure of **3** was elucidated to be 3-*O*-*β*-D-glucopyranosyl-(1 → 4)- *β*-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1 → 4)-*β*-D-oleandropyranosyl-11-*O*-tigloyl-12-*O*-benozyl-8*β*,14*β*-epoxy-3*β*,11*α*,12*β*-tridroxypregnane-5-en-20-one and named marstenacisside A8.

Compound **4** had the molecular formula $C_{59}H_{86}O_{24}$, determined by the HRESIMS ion $[M - H]^-$ at *m/z* 1177.5488, with two mass units more than that of **1**. NMR data comparison suggested that **4** and **1** had an almost identical structure except for the difference in the A-ring portion. In the $^{13}C$ NMR spectrum of **4**, the carbon signals of C-3 (*δ* 76.0), C-4 (*δ* 35.0), C-5 (*δ* 44.2), C-6 (*δ* 27.4), C-7 (*δ* 32.5), and C-19 (*δ* 13.1) deduced that **4** had the $sp^3$ carbons of C-5 and C-6. Thus, the $C_{21}$ steroid skeleton of **4** could be deduced as 5*α*-8*β*,14*β*-epoxy-3*β*,11*α*,12*β*-tridroxypregnane-20-one, which is well in agreement with 17*β*-tenacigenin B [5, 11]. Confirmed by the combined use of $^1H$-$^1H$ COSY, HSQC, and HMBC experiments, the structure of **4** was finally elucidated as 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1 → 4)-*β*-D-oleandropyranosyl-11-*O*-tigloyl-12-*O*-benozyl-5*α*-8*β*,14*β*-epoxy-3*β*,11*α*,12*β*-tridroxypregnane-20-one and named marstenacisside B12.

Compound **7**, with a molecular formula of $C_{59}H_{88}O_{25}$ determined by the HRESIMS ion $[M - H]^-$ at *m/z* 1195.5596, had two hydrogen atoms and one oxygen atom more than **4**. The $^{13}C$ NMR data of **7** suggested that it had almost the same carbon signals as **4** except for significant differences at C-8 and C-14. The difference of molecular formula and chemical shifts at C-8 (*δ* 78.4, + 12.1 ppm) and C-14 (85.6, + 13.9 ppm) deduced, in the structure of **7**, two free hydroxyl groups linked to the C-8 and C-14 positions. Therefore, the $C_{21}$ steroid skeleton of **7** could be deduced as 5*α*-3*β*,11*α*,8*β*,12*β*,14*β*-pentadroxypregnane-20-one, which well coincided with tenacigenin C [10–12]. Confirmed by the combined use of $^1H$-$^1H$ COSY, HSQC, and HMBC experiments, the structure of **7** was elucidated to be 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1 → 4)-*β*-D-oleandropyranosyl-11-*O*-tigloyl-12-*O*-benozyl-5*α*-3*β*,11*α*,8*β*,12*β*,14*β*-pentadroxypregnane-20-one and named marstenacisside B13.

Compound **8** had the same molecular formula of $C_{59}H_{88}O_{25}$ as **7**, which was determined by the HRESIMS ion $[M - H]^-$ at *m/z* 1195.5588. The NMR data of **8** and **7** revealed that they had almost identical carbon signals. However, the carbon signals of **8** at *δ* 72.2 (C-11) and 78.8 (C-12) obviously differed from those of **7** at *δ* 71.3 (C-11) and 79.8 (C-12), suggesting they had the same ester groups but with different esterification positions. The HMBC correlations between *δ* 6.67 (H-11) and 166.0 (Bz-C-1), and between *δ* 5.55 (H-12) and 170.0 (Tig-C-1) determined the linkages of the benzoyl group to C-11 and the tigloyl group to C-12. By analyses of $^1H$-$^1H$ COSY, HSQC, and HMBC spectra, the NMR data of **8** were fully assigned. Thus, the structure of **8** was determined as 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1 → 4)-*β*-D-oleandropyranosyl-11-*O*-benozyl-12-*O*-tigloyl-5*α*-3*β*,11*α*,8*β*,12*β*,14*β*-pentadroxypregnane-20-one and named marstenacisside B14.

Compound **9**, with the molecular formula of $C_{53}H_{78}O_{20}$ determined by the HRESIMS ion $[M - H]^-$ at *m/z* 1033.5083, had the same aglycone moiety as **7** by comparison of their NMR data. In the $^1H$ and $^{13}C$ NMR spectra of **9**, three anomeric protons at *δ* 4.82 (1 H, d, *J* = 9.7 Hz), 4.99 (1 H, d, *J* = 7.7 Hz), and 5.29 (1 H, d, *J* = 8.0 Hz) and three anomeric carbons at *δ* 97.5, 101.9, and 106.6 were observed. Compound **9** had the same sugar moiety as **3** by comparing their NMR data of the sugar moiety. Therefore, the structure of **9** was elucidated to be 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1 → 4)-*β*-D-oleandropyranosyl-11-*O*-tigloyl-12-*O*-benozyl-5*α*-3*β*,11*α*,8*β*,12*β*, 14*β*-pentadroxypregnane-20-one and named marstenacisside A9.

Compound **10**, with the molecular formula of $C_{53}H_{78}O_{20}$ determined by the HRESIMS ion $[M - H]^-$ at *m/z* 1033.5059, was an isomer of **9**. The NMR data suggested that **10** had almost identical carbon signals as **9**. The carbon signals of **10** at *δ* 72.2 (C-11) and 78.8 (C-12), obviously different with those of **9** at *δ* 71.3 (C-11) and 79.8 (C-12), suggested that they had the same ester groups but with different esterification positions. Further comparison of the aglycone NMR data of **10** and **8** deduced that they had the same aglycone. Consequently, the structure of **10** was elucidated to be 3-*O*-*β*-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-*β*-D-allopyranosyl-(1 → 4)-*β*-D-oleandropyranosyl-11-*O*-benozyl-12-*O*-

tigloyl-5α-3β,11α,8β,12β,14β-pentadroxypregnane-20-one and named marstenacisside A10.

Compound **11** had a molecular formula of $C_{56}H_{82}O_{24}$ as determined by the HRESIMS ion $[M - H]^-$ at *m/z* 1137.5137. The NMR data of **11** suggested that it had the same sugar moiety as **1**. In the $^{13}C$ NMR spectra of **11**, two olefinic carbon signals at δ 139.7 and 122.6 deduced the double bond at C-5 and C-6, and four oxidative carbons at δ 77.3 (C-3), 71.8 (C-11), 78.6 (C-12), and 84.1 (C-14) suggested the existence of four hydroxyl groups in the aglycone. By comparing the NMR data of **11** with those compounds in the literature, the $C_{21}$ steroid skeleton of **11** was deduced to be 3β,11α,12β,14β-tetrahydroxyprengn-5-en-20-one, well in agreement with drevogenin P [13]. Characteristic carbon signals at δ 170.2 and 21.3 along with 166.8, 133.4, 130.2 (× 2), 130.1, and 129.2 (× 2) indicated the existence of an acetyl group and a benzyl group in the molecular structure. Based on the HMBC correlations between δ 170.4 (Ac-C-11) and 5.85 (H-11), and between δ 166.8 (Bz-C-1) and 5.43 (H-12), the linkages of an acetyl group to C-11 and a benzoyl group to C-12 were determined. Finally, confirmed by the combined use of $^1H$-$^1H$ COSY, HSQC, and HMBC experiments, the structure of **11** was elucidated as 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-11-*O*-acetyl-12-*O*-benozyl-3β,11α,12β,14β-tetrahydroxyprengn-5-en-20-one and named marstenacisside B15.

Compound **12**, with a molecular formula of $C_{57}H_{88}O_{24}$ as determined by the HRESIMS ion $[M - H]^-$ at *m/z* 1155.5636, had the same $C_{21}$ steroidal skeleton structure as **11** by comparing their NMR data. In the $^{13}C$ NMR spectrum of **12**, characteristic carbon signals at δ 167.9, 167.1, 138.6, 138.5, 129.0, 128.5, 14.3, 14.3, 12.1, and 12.0 suggested the existence of double tigloyl groups in the molecule. By the combined use of $^1H$-$^1H$ COSY, HSQC, and HMBC experiments, the structure of **12** was elucidated as 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-11,12-*O*-ditigloyl-3β,11α,12β,14β-tetrahydroxyprengn-5-en-20-one and named marstenacisside B16.

Compound **13** had a molecular formula of $C_{53}H_{76}O_{19}$ determined by the HRESIMS ion $[M - H]^-$ at *m/z* 1015.4949. The NMR data of **13** suggested that it had same $C_{21}$ steroid skeleton as **11** and **12**. In the $^{13}C$ NMR spectrum of **13**, two groups of characteristic carbon signals at δ 166.2, 133.4, 130.8, 130.1 (× 2), and 128.8 (× 2) and at δ 167.8, 128.3, 138.8, 14.2, and 11.9, coincided well with those of **8**, suggesting that **13** had a benzoyl group and a tigloyl group in the molecule. By comparing the carbon chemical shifts of the ester groups as well as the carbon chemical shifts of C-11 and C-12, the linkages of benzoyl to C-11 and tigloyl to C-12 were also the same as those in **8**. Moreover, the sugar moiety of **13** was deduced to be the same as that of **3** by comparing their NMR data. Thus, the structure of **13** was elucidated as 3-*O*-β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-11-*O*-benozyl-12-*O*-tigloyl-3β,11α,12β,14β-tetrahydroxyprengn-5-en-20-one and named marstenacisside A11.

Compound **14** had a molecular formula of $C_{56}H_{84}O_{24}$ as determined by the HRESIMS ion $[M - H]^-$ at *m/z* 1139.5333, two mass units more than that of **11**. The NMR data of **14** suggested that it had the almost identical $C_{21}$ steroid skeleton as **11**, except for the significant difference in the A-ring portion. In the $^{13}C$ NMR spectrum of **14**, carbon signals of C-3 (δ 76.2), C-4 (δ 35.5), C-5 (δ 44.7), C-6 (δ 29.4), C-7 (δ 28.4), and C-19 (δ 12.4) indicated that **14** had the $sp^3$ carbons of C-5 and C-6. Thus, the $C_{21}$ steroid skeleton was deduced to be 5α-3β,11α,12β,14β-tetrdroxypregnane-20-one, well in agreement with decaylconduragogenin A [12]. Therefore, by using $^1H$-$^1H$ COSY, HSQC, and HMBC experiments, the structure of **14** was elucidated as 3-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-11-*O*-acetyl-12-*O*-benozyl-5α-3β,11α,12β,14β-tetrdroxypregnane-20-one and named marstenacisside B17.

Compound **15** had a molecular formula of $C_{53}H_{78}O_{19}$ as determined by the HRESIMS ion $[M - H]^-$ at *m/z* 1017.5085. The NMR data suggested that its $C_{21}$ steroid skeleton is same as that of **14**. In the $^{13}C$ NMR spectrum of **15**, two groups of characteristic carbon signals at δ 167.4, 128.9, 138.6, 14.1, and 11.7 and at δ 166.8, 133.7, 130.3, 130.1 (× 2), and 128.9 (× 2) suggested that it had a tigloyl group and a benzoyl group. The linkages of the tigloyl group to C11 and the benzoyl group to C-12 were determined by the HMBC correlations between δ 167.4 (Tig-C-1) and 5.87 (H-11), and between 166.8 (Bz-C-1) and 5.43 (H-12). The sugar moiety of **15** was deduced to be the same as **3** by comparing the NMR data. Consequently, confirmed by the combined use of $^1H$-$^1H$ COSY, HSQC, and HMBC experiments, the structure of **15** was elucidated as 3-*O*-β-D-glucopyranosyl-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1 → 4)-β-D-oleandropyranosyl-11-*O*-tigloyl-12-*O*-benozyl-5α-3β,11α,12β,14β-tetrdroxypregnane-20-one and named marstenacisside A12.

Isolates **1–15** above and the previously isolated 16 polyoxypregnane glycosides, marstenacissides A1–A7 and B1–B9 [2], were further evaluated for anti-HIV and cytotoxic activities. As illustrated in ▶ **Table 1**, treatment of HIV-1-infected SupT1 cells with **6, 11, 12, 14**, marstenacissides A3, A4, and marstenacissides B1, B3, and B9 (30 μM) slightly inhibited HIV-1 replication, indicating that these compounds only had a marginal inhibitory effect on HIV-1, while the other compounds exhibited negligible effects since their inhibition rates were far below average. The toxicity test showed that all 31 compounds at the concentration of 30 μM displayed different cytoxicities against SupT1 cells (cell survival rate from 30% to 71%), while no linear relationship between toxicity and anti-HIV activity was observed.

The EtOAc-soluble fraction of the 95% EtOH extract from *M. auricularis* roots showed a significant HIV-1 inhibitory effect, while all of the isolated polyoxypregnane glycosides showed marginal and negligible inhibitory effects on HIV-1, which suggested that a further chemical investigation on this traditional medicine is needed to explore bioactive constituents against HIV-1.

## Materials and Methods

### General experimental procedures

NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 MHz for $^1H$ NMR and 125 MHz for $^{13}C$-NMR) and a Varian UNITY INOVA 600 spectrometer (600 MHz for $^1H$ NMR and

150 MHz for $^{13}$C-NMR) in pyridine-$d_5$ (Sigma-Aldrich), and the chemical shifts are given in $\delta$ (ppm). HRESIMS was performed on the Synapt MS (Waters Corporation). Optical rotations were measured with the Perkin-Elmer 343 polarimeter (PerkinElmer) and JASCO J-810 polarimeter (JASCO Corporation). UV data were recorded on a UV-2500 spectrophotometer (MAPADA Corporation). HPLC analyses were performed on an Agilent 1100 series (Agilent Technologies) equipped with an Alltech 2000 evaporative light scattering detector (temp: 110 °C, gas: 2.4 L/min, Alltech Corporation) and a Techmate C18 column (4.6 mm × 250 mm, ODS, 5 µm, Techmate Co. Ltd.). Semipreparative HPLC separations were carried out using a system consisting of an NP7000 module (Hanbon Co. Ltd.), a Shodex RID 102 detector (Showa Denko Group), and a Venusil XBP C18 column (8.0 mm × 250 mm, ODS, 5 µm, Bonna-Agela Technologies). Silica gel H (Qingdao marine Chemical), MCI resin (50 µm, Mitsubishi Chemicals), and ODS (Octadecylsilyl) silica gel (120 Å, 50 µm, YMC) were used for column chromatography performance.

## Plant material

The roots of *M. tenacissima* were collected from Zhenyuan, Simao, Yunnan province of China and were identified by Prof. Li-Xia Zhang. A voucher specimen (NO.111 010) was deposited in the herbarium of the Beijing Institute of Radiation Medicine, Beijing, China.

## Extraction and isolation

The dried roots of *M. tenacissima* (3 kg) were crushed and extracted with 95 % EtOH (24 L) at 120 °C three times (each time for 1 h). The filtrate was concentrated *in vacuo* to yield an extract and then partitioned between EtOAc and $H_2O$. After concentration, the residue (78 g) of the EtOAc extract was separated on a silica gel column (10 cm × 24 cm) eluted with a gradient consisting of $CH_3Cl_3$-MeOH (v/v, 50 : 1 → 7 : 1) to afford 374 fractions (Fr.$_1$ 1–374, each fraction 150 ml).

Fr.$_1$ 195–214 (6.5 g) was subjected to an MCI resin column (5 cm × 20 cm) eluted with MeOH-$H_2O$ to afford 17 fractions (v/v, 70 : 30 for Fr.$_2$ 1–13; v/v, 80 : 20 for Fr.$_2$ 14–17, each fraction 250 ml). Fr.$_2$ 8 (225 mg) was separated by semipreparative HPLC with $CH_3OH$-$H_2O$ (v/v, 69 : 31, flow rate 5.0 mL/min) to give compound **6** (12.6 mg, $t_R$ 23.2 min), and Fr.$_2$ 9 (261 mg) was purified by semipreparative HPLC with $CH_3OH$-$H_2O$ (v/v, 70 : 30, flow rate 4.5 mL/min) to yield an additional compound **6** (23.8 mg, $t_R$ 25.0 min). Fr.$_2$ 14–15 (745 mg) was loaded on an ODS C18 column (2 cm × 30 cm) eluted with $CH_3OH$-$H_2O$ (v/v, 67 : 33) to give 16 fractions (Fr.$_3$ 1–16, each 50 mL). Then Fr.$_3$ 9–11 (293 mg) was separated by semipreparative HPLC with $CH_3OH$-$H_2O$ (v/v, 69 : 31, flow rate 4.5 mL/min) to give mixture A ($t_R$ 27.0 min), and Fr.$_3$ 13–16 (290 mg) was separated by semipreparative HPLC with $CH_3OH$-$H_2O$ (v/v, 70 : 30, flow rate 4.5 mL/min) to give mixtures B ($t_R$ 20.5 min) and C ($t_R$ 22.5 min). Furthermore, mixture A was purified by semipreparative HPLC with $CH_3CN$-$H_2O$ (v/v, 45 : 55, flow rate 4.5 mL/min) to yield compounds **5** (66.8 mg, $t_R$ 38.0 min) and **3** (24.6 mg, $t_R$ 42.5 min). Mixture B was purified by semipreparative HPLC with $CH_3CN$-$H_2O$ (v/v, 45 : 55, flow rate 4.5 mL/min) to yield compounds **9** (21.4 mg, $t_R$ 39.0 min) and **10** (45.5 mg, $t_R$ 42.1 min). Mixture C was purified by semipreparative HPLC with $CH_3CN$-$H_2O$ (v/v, 45 : 55, flow rate 5.0 mL/min) to yield compounds **15** (21.4 mg, $t_R$ 36.2 min) and **13** (45.5 mg, $t_R$ 39.6 min).

Fr.$_1$ 300–323 (6.0 g) was subjected to an MCI resin column (5 cm × 20 cm) eluted with MeOH-$H_2O$ to afford 30 fractions (v/v, 70 : 30 for Fr.$_4$ 1–21; v/v, 75 : 25 for Fr.$_4$ 22–30, each fraction 250 mL). Fr.$_4$ 16–19 (346.5 mg) was separated by semipreparative HPLC with $CH_3OH$-$H_2O$ (v/v, 66 : 34, flow rate 3.5 mL/min) to yield compound **12** (50.1 mg, $t_R$ 50.1 min), and mixtures D (21.5 mg, $t_R$ 32.3 min) and E (45.4 mg, $t_R$ 42.4 min). Fr.$_4$ 20–21 (214.4 mg) was separated by semipreparative HPLC with $CH_3OH$-$H_2O$ (v/v, 66 : 34, flow rate 3.5 mL/min) to obtain the additional mixtures D (43.1 mg, $t_R$ 30.1 min) and E (24.5 mg, $t_R$ 40.5 min). Fr.$_4$ 22–25 (750 mg) was loaded on an ODS $C_{18}$ column (2 cm × 30 cm) eluted

▶ **Table 1** HIV-1 inhibition rate and cell survival rate ($\bar{x} \pm s$, $n = 3$) of 31 polyoxypregnane glycosides from the roots of *M. tenacissima*.

| Compounds | Dose | Inhibition rate (%) | Cell survival rate (%) |
|---|---|---|---|
| Efavirenz | 0.0-1 µM | 99.91 ± 0.10 | 99.51 ± 0.03 |
| §1 | 30 µM | 13.70 ± 4.66 | 43.37 ± 14.98 |
| 2 | 30 µM | 20.90 ± 5.17 | 50.21 ± 5.94 |
| 3 | 30 µM | 13.478 ± 4.66 | 53.18 ± 13.03 |
| 4 | 30 µM | 21.19 ± 5.36 | 49.36 ± 2.12 |
| 5 | 30 µM | 8.26 ± 2.77 | 57.47 ± 5.78 |
| 6 | 30 µM | 39.26 ± 5.56 | 48.56 ± 13.77 |
| 7 | 30 µM | 29.09 ± 1.87 | 42.48 ± 1.03 |
| 8 | 30 µM | 31.36 ± 6.96 | 57.86 ± 3.49 |
| 9 | 30 µM | 29.77 ± 5.90 | 71.21 ± 8.46 |
| 10 | 30 µM | 25.57 ± 0.99 | 35.62 ± 9.40 |
| 11 | 30 µM | 47.75 ± 4.73 | 39.26 ± 0.54 |
| 12 | 30 µM | 37.81 ± 4.23 | 58.56 ± 3.15 |
| 13 | 30 µM | 20.06 ± 2.46 | 38.64 ± 5.81 |
| 14 | 30 µM | 36.87 ± 8.27 | 41.10 ± 10.73 |
| 15 | 30 µM | 25.52 ± 3.87 | 64.60 ± 8.02 |
| Marstenacisside A1 | 30 µM | 14.12 ± 1.75 | 42.05 ± 6.51 |
| Marstenacisside A2 | 30 µM | 16.48 ± 1.39 | 34.07 ± 1.68 |
| Marstenacisside A3 | 30 µM | 53.48 ± 9.48 | 30.29 ± 1.91 |
| Marstenacisside A4 | 30 µM | 39.74 ± 4.56 | 50.61 ± 0.88 |
| Marstenacisside A5 | 30 µM | 24.78 ± 4.41 | 38.12 ± 3.01 |
| Marstenacisside A6 | 30 µM | 25.14 ± 7.21 | 29.44 ± 6.52 |
| Marstenacisside A7 | 30 µM | 15.63 ± 3.82 | 69.90 ± 4.80 |
| Marstenacisside B1 | 30 µM | 41.32 ± 5.32 | 51.75 ± 3.61 |
| Marstenacisside B2 | 30 µM | 28.35 ± 3.69 | 56.53 ± 11.76 |
| Marstenacisside B3 | 30 µM | 48.83 ± 7.96 | 45.64 ± 1.69 |
| Marstenacisside B4 | 30 µM | 17.02 ± 6.04 | 62.22 ± 5.64 |
| Marstenacisside B5 | 30 µM | 33.81 ± 8.56 | 31.55 ± 2.68 |
| Marstenacisside B6 | 30 µM | 24.15 ± 5.03 | 39.63 ± 8.07 |
| Marstenacisside B7 | 30 µM | 22.54 ± 6.11 | 55.77 ± 2.13 |
| Marstenacisside B8 | 30 µM | 32.42 ± 6.73 | 60.34 ± 3.64 |
| Marstenacisside B9 | 30 µM | 39.20 ± 5.73 | 48.32 ± 0.61 |

with $CH_3OH$-$H_2O$ (v/v, 64:33–70:30) to give $Fr._5$ 12–16 (each 50 mL) and $Fr._5$ 17 (MeOH elution). $Fr._5$ 12–16 (239.5 mg) was separated by semipreparative HPLC with $CH_3OH$-$H_2O$ (v/v, 69:31, flow rate 4.0 mL/min) to afford an additional mixture E (42.6 mg, $t_R$ 35.3 min) as well as mixture F (83.8 mg, $t_R$ 39.5 min). $Fr._5$ 17 (360 mg) was separated by semipreparative HPLC with $CH_3OH$-$H_2O$ (v/v, 70:30, flow rate 4.0 mL/min) to give mixture G (76.7 mg, $t_R$ 37.3 min). Then, the combined mixture D was purified by semipreparative HPLC with $CH_3CN$-$H_2O$ (v/v, 35:65, flow rate 4.5 mL/min) to give compounds **14** (13.8 mg, $t_R$ 49.7 min) and **11** (26.7 mg, $t_R$ 52.0 min). The combined mixture E was purified by semipreparative HPLC with $CH_3CN$-$H_2O$ (v/v, 40:60, flow rate 4.5 mL/min) to give compound **2** (11.1 mg, $t_R$ 41.0 min). Mixture F was purified by semipreparative HPLC with $CH_3CN$-$H_2O$ (v/v, 40:60, flow rate 4.5 mL/min) to give compounds **4** (45.5 mg, $t_R$ 40.6 min) and **1** (15.5 mg, $t_R$ 41.4 min). Mixture G was purified by semipreparative HPLC with $CH_3CN$-$H_2O$ (v/v, 40:60, flow rate 4.0 mL/min) to give compounds **7** (20.5 mg, $t_R$ 35.2 min) and **8** (30.0 mg, $t_R$ 40.1 min).

## Characterization

*Marstenacisside B10* (**1**): white amorphous powder; $[\alpha]_D^{20}$ = + 14.2 ($c$ = 0.059, pyridine). UV (MeOH) $\lambda_{max}$ 225.8 ($\varepsilon$ 16354) nm. $C_{59}H_{84}O_{24}$, HRESIMS (positive): $m/z$ 1199.5303 $[M + Na]^+$ (calcd. for $C_{59}H_{84}O_{24}Na$: 1199.5250). $^1$H NMR data (600 MHz, pyridine-$d_5$): $\delta$ 3.80 (1 H, m, H-3), 5.97 (1 H, t, $J$ = 10.1 Hz, H-11), 5.59 (1 H, d, $J$ = 10.1 Hz, H-12), 3.11 (1 H, dd, $J$ = 11.2, 6.2 Hz, H-17), 1.55 (3 H, s, 18-$CH_3$), 1.54 (3 H, s, 19-$CH_3$), 1.99 (3 H, s, 21-$CH_3$), 6.77 (1 H, qq, $J$ = 7.1, 1.4 Hz, Tig-H-3), 1.33 (3 H, d, $J$ = 7.1 Hz, Tig-H-4), 1.50 (3 H, s, Tig-H-5), 8.15 (2 H, dd, $J$ = 7.8, 1.3 Hz, Bz-H-3, 7), 7.36 (2 H, dd, $J$ = 7.8, 7.4 Hz, Bz-H-4, 6), 7.46 (1 H, t, $J$ = 7.4 Hz, Bz-H-5), 4.76 (1 H, dd, $J$ = 9.4, 1.5 Hz, Ole-H-1), 1.71 (1 H, m, Ole-H-2a), 2.39 (1 H, m, Ole-H-2b), 3.56 (2 H, overlap, Ole-H-3,5), 3.55 (1 H, overlap, Ole-H-4), 3.53 (1 H, overlap, Ole-H-4), 1.56 (3 H, d, $J$ = 5.3 Hz, Ole-H-6), 3.48 (3 H, s, Ole-3-$OCH_3$), 5.24 (1 H, d, $J$ = 8.2 Hz, Allo-H-1), 3.80 (1 H, overlap, Allo-H-2), 4.39 (1 H, t, $J$ = 2.7 Hz, Allo-H-3), 3.66 (1 H, dd, $J$ = 9.6, 2.4 Hz, Allo-H-4), 4.23 (1 H, m, Allo-H-5), 1.61 (3 H, d, $J$ = 6.2 Hz, Allo-H-6), 3.79 (1 H, s, Allo-3-$OCH_3$), 4.90 (1 H, d, $J$ = 7.8 Hz, $Glc_1$-H-1), 3.99 (1 H, dd, $J$ = 8.2, 7.8 Hz, $Glc_1$-H-2), 4.25 (1 H, m, $Glc_1$-H-3), 4.28 (1 H, m, $Glc_1$-H-4), 3.93 (1 H, m, $Glc_1$-H-5), 4.50 (1 H, m, $Glc_1$-H-6a), 4.43 (1 H, dd, $J$ = 11.6 Hz, $Glc_1$-H-6b), 5.19 (1 H, d, $J$ = 7.9 Hz, $Glc_2$-H-1), 4.10 (1 H, dd, $J$ = 8.2, 7.9 Hz $Glc_2$-H-2), 4.20 (1 H, m, $Glc_2$-H-3), 4.19 (1 H, m, $Glc_2$-H-4), 4.01 (1 H, m, $Glc_2$-H-5), 4.52 (1 H, d, $J$ = 11.4 Hz, $Glc_2$-H-6a), 4.30 (1 H, m, $Glc_2$-H-6b). $^{13}$C NMR data (150 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

*Marstenacisside B11* (**2**): white amorphous powder; $[\alpha]_D^{20}$ = + 23.7 ($c$ = 0.038, pyridine). UV (MeOH) $\lambda_{max}$ 215.6 ($\varepsilon$ 14112) nm. $C_{57}H_{86}O_{24}$, HRESIMS (positive): $m/z$ 1177.5454 $[M + Na]^+$ (calcd. for $C_{57}H_{86}O_{24}Na$: 1177.5407). $^1$H NMR data (500 MHz, pyridine-$d_5$): $\delta$ 3.79 (1 H, m, H-3), 5.88 (1 H, t, $J$ = 10.2 Hz, H-11), 5.44 (1 H, d, $J$ = 10.2 Hz, H-12), 3.06 (1 H, dd, $J$ = 11.2, 6.2 Hz, H-17), 1.49 (3 H, s, 18-$CH_3$), 1.54 (3 H, s, 19-$CH_3$), 2.10 (3 H, s, 21-$CH_3$), 6.95 (1 H, qq, $J$ = 7.1, 1.3 Hz, $Tig_1$-H-3), 1.57 (3 H, d, $J$ = 7.1 Hz, $Tig_1$-H-4), 1.78 (3 H, s, $Tig_1$-H-5), 6.93 (1 H, qq, $J$ = 7.1, 1.3 Hz, $Tig_2$-H-3), 1.57 (3 H, d, $J$ = 7.1 Hz, $Tig_2$-H-4), 1.84 (3 H, s, $Tig_2$-H-5), 4.77 (1 H, d, $J$ = 9.7 Hz, Ole-H-1), 3.50 (3 H, s, Ole-3-$OCH_3$), 5.27 (1 H, d, $J$ = 8.1 Hz, Allo-H-1), 3.81 (1 H, s, Allo-3-$OCH_3$), 4.92 (1 H, d, $J$ = 7.8 Hz, $Glc_1$-H-1), 5.22 (1 H, d, $J$ = 7.9 Hz, $Glc_2$-H-1). $^{13}$C NMR data (125 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

*Marstenacisside A8* (**3**): white amorphous powder; $[\alpha]_D^{20}$ = + 10.8 ($c$ = 0.051, pyridine). UV (MeOH) $\lambda_{max}$ 225.6 ($\varepsilon$ 22516) nm. $C_{53}H_{74}O_{19}$, HRESIMS (negative): $m/z$ 1013.4787 $[M - H]^-$ (calcd. for $C_{53}H_{73}O_{19}$: 1013.4746). $^1$H NMR data (500 MHz, pyridine-$d_5$): $\delta$ 3.81 (1 H, m, H-3), 6.00 (1 H, t, $J$ = 10.1 Hz, H-11), 5.62 (1 H, d, $J$ = 10.1 Hz, H-12), 3.11 (1 H, dd, $J$ = 11.5, 6.2 Hz, H-17), 1.57 (3 H, s, 18-$CH_3$), 1.56 (3 H, s, 19-$CH_3$), 2.01 (3 H, s, 21-$CH_3$), 6.79 (1 H, q, $J$ = 7.1 Hz, Tig-H-3), 1.35 (3 H, d, $J$ = 7.0 Hz, Tig-H-4), 1.52 (3 H, s, Tig-H-5), 8.18 (2 H, d, $J$ = 7.9 Hz, Bz-H-3, 7), 7.38 (2 H, dd, $J$ = 7.9, 7.4 Hz, Bz-H-4, 6), 7.48 (1 H, t, $J$ = 7.4 Hz, Bz-H-5), 4.78 (1 H, d, $J$ = 9.4 Hz, Ole-H-1), 1.74 (1 H, m, Ole-H-2a), 2.46 (1 H, m, Ole-H-2b), 3.58 (2 H, overlap, Ole-H-3, 5), 3.57 (1 H, overlap, Ole-H-4), 3.55 (1 H, overlap, Ole-H-4), 1.59 (3 H, d, $J$ = 4.9 Hz, Ole-H-6), 3.51 (3 H, s, Ole-3-$OCH_3$), 5.27 (1 H, d, $J$ = 8.0 Hz, Allo-H-1), 3.81 (1 H, overlap, Allo-H-2), 4.48 (1 H, br s, Allo-H-3), 3.75 (1 H, dd, $J$ = 9.6, 2.0 Hz, Allo-H-4), 4.27 (1 H, m, Allo-H-5), 1.61 (3 H, d, $J$ = 6.1, Allo-H-6), 3.83 (1 H, s, Allo-3-$OCH_3$), 4.99 (1 H, d, $J$ = 7.7 Hz, $Glc_1$-H-1), 4.04 (1 H, dd, $J$ = 8.2, 7.8 Hz, Glc-H-2), 4.25 (1 H, m, Glc-H-3), 4.23 (1 H, m, Glc-H-4), 4.00 (1 H, m, Glc-H-5), 4.55 (1 H, m, Glc-H-6a), 4.39 (1 H, br d, $J$ = 11.6 Hz, Glc-H-6b). $^{13}$C NMR (125 MHz, pyridine-$d_5$), data see ▶ **Tables 2** and **3**.

*Marstenacisside B12* (**4**): white amorphous powder; $[\alpha]_D^{20}$ = + 34.4 ($c$ = 0.0465, pyridine). UV (MeOH) $\lambda_{max}$ 225.0 ($\varepsilon$ 13750) nm. $C_{59}H_{86}O_{24}$, HRESIMS (negative): $m/z$ 1177.5488 $[M - H]^-$ (calcd. for $C_{59}H_{85}O_{24}$: 1177.5431). $^1$H NMR data (600 MHz, pyridine-$d_5$): $\delta$ 3.84 (1 H, m, H-3), 5.84 (1 H, t, $J$ = 10.2 Hz, H-11), 5.52 (1 H, d, $J$ = 9.9 Hz, H-12), 3.07 (1 H, dd, $J$ = 11.7, 6.3 Hz, H-17), 1.53 (3 H, s, 18-$CH_3$), 1.26 (3 H, s, 19-$CH_3$), 2.00 (3 H, s, 21-$CH_3$), 6.74 (1 H, qq, $J$ = 7.1, 1.2 Hz, Tig-H-3), 1.34 (3 H, d, $J$ = 7.1 Hz, Tig-H-4), 1.52 (3 H, s, Tig-H-5), 8.14 (2 H, dd, $J$ = 8.0, 1.2 Hz, Bz-H-3, 7), 7.35 (2 H, dd, $J$ = 7.8, 7.2 Hz, Bz-H-4, 6), 7.46 (1 H, t, $J$ = 7.2 Hz, Bz-H-5), 4.77 (1 H, br d, $J$ = 9.2 Hz, Ole-H-1), 3.49 (3 H, s, Ole-3-$OCH_3$), 5.26 (1 H, d, $J$ = 8.1 Hz, Allo-H-1), 3.79 (1 H, s, Allo-3-$OCH_3$), 4.92 (1 H, d, $J$ = 7.7 Hz, $Glc_1$-H-1), 5.19 (1 H, d, $J$ = 7.8 Hz, $Glc_2$-H-1). $^{13}$C NMR data (125 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

*Marstenacisside B13* (**7**): white amorphous powder; $[\alpha]_D^{25}$ = + 35.8 ($c$ = 0.0608, MeOH). UV (MeOH) $\lambda_{max}$ 225.4 ($\varepsilon$ 13167) nm. $C_{59}H_{88}O_{25}$, HRESIMS (negative): $m/z$ 1195.5596 $[M - H]^-$ (calcd. for $C_{59}H_{87}O_{25}$: 1195.5536). $^1$H NMR data (500 MHz, pyridine-$d_5$): $\delta$ 3.91 (1 H, m, H-3), 6.61 (1 H, t, $J$ = 10.6 Hz, H-11), 5.60 (1 H, d, $J$ = 10.0 Hz, H-12), 3.35 (1 H, dd, $J$ = 9.0, 5.2 Hz, H-17), 1.72 (3 H, s, 18-$CH_3$), 1.53 (3 H, s, 19-$CH_3$), 2.06 (3 H, s, 21-$CH_3$), 6.82 (1 H, q, $J$ = 7.1 Hz, Tig-H-3), 1.38 (3 H, d, $J$ = 7.1 Hz, Tig-H-4), 1.54 (3 H, s, Tig-H-5), 8.26 (2 H, d, $J$ = 7.6 Hz Bz-H-3, 7), 7.42 (2 H, t, $J$ = 7.7 Hz, Bz-H-4, 6), 7.51 (1 H, t, $J$ = 7.4 Hz, Bz-H-5), 4.81 (1 H, br d, $J$ = 9.6 Hz, Ole-H-1), 3.49 (3 H, s, Ole-3-$OCH_3$), 5.28 (1 H, d, $J$ = 8.0 Hz, Allo-H-1), 3.81 (1 H, s, Allo-3-$OCH_3$), 4.92 (1 H, d, $J$ = 7.7 Hz, $Glc_1$-H-1), 5.21 (1 H, d, $J$ = 7.9 Hz, $Glc_2$-H-1). $^{13}$C NMR data (125 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

*Marstenacisside B14* (**8**): white amorphous powder; $[\alpha]_D^{25}$ = + 29.7 ($c$ = 0.0738, MeOH). UV (MeOH) $\lambda_{max}$ 225.4 ($\varepsilon$ 19513) nm. $C_{59}H_{88}O_{25}$, HRESIMS (negative): $m/z$ 1195.5588 $[M - H]^-$ (calcd. for $C_{59}H_{87}O_{25}$: 1195.5536). $^1$H NMR data (500 MHz, pyridine-$d_5$): $\delta$ 3.86 (1 H, m, H-3), 6.67 (1 H, t, $J$ = 10.6 Hz, H-11), 5.55 (1 H, d,

▶ **Table 2** $^{13}$C chemical shifts of the aglycones of **1–15** (150 MHz for **1, 4, 6, 11**, and **12**, and 125 MHz for **2, 3, 5, 7–10**, and **13–15**, pyridine-$d_5$).

| Position | 1 | 2 | 3 | 4 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 38.8 | 38.7 | 38.8 | 37.7 | 39.6 | 39.6 | 39.6 | 39.6 | 38.8 | 38.8 | 38.8 | 38.1 | 38.3 |
| 2 | 30.0 | 29.9 | 29.9 | 29.7 | 30.0 | 29.9 | 30.0 | 29.9 | 30.4 | 30.4 | 30.3 | 30.3 | 30.3 |
| 3 | 77.0 | 77.0 | 76.9 | 76.0 | 76.3 | 76.2 | 76.3 | 76.2 | 77.3 | 77.3 | 77.2 | 76.2 | 76.1 |
| 4 | 39.6 | 39.6 | 39.6 | 35.0 | 35.7 | 35.7 | 35.7 | 35.7 | 39.8 | 39.8 | 39.8 | 35.5 | 35.4 |
| 5 | 141.5 | 141.5 | 141.5 | 44.2 | 45.8 | 45.7 | 45.7 | 45.7 | 139.7 | 139.8 | 139.7 | 44.7 | 44.7 |
| 6 | 119.6 | 119.6 | 119.6 | 27.4 | 25.4 | 25.4 | 25.4 | 25.4 | 122.6 | 122.5 | 122.6 | 29.4 | 29.4 |
| 7 | 33.1 | 33.1 | 33.1 | 32.5 | 35.2 | 35.2 | 35.2 | 35.2 | 28.2 | 28.3 | 28.3 | 28.4 | 28.4 |
| 8 | 64.3 | 64.2 | 64.3 | 66.3 | 78.4 | 78.5 | 78.4 | 78.5 | 37.5 | 37.6 | 37.7 | 40.0 | 40.3 |
| 9 | 49.7 | 49.7 | 49.6 | 52.5 | 51.4 | 51.5 | 51.4 | 51.5 | 47.9 | 48.1 | 48.1 | 50.1 | 50.3 |
| 10 | 40.4 | 40.4 | 40.4 | 39.7 | 38.5 | 38.5 | 38.5 | 38.5 | 39.5 | 39.5 | 39.5 | 37.8 | 38.0 |
| 11 | 68.7 | 68.7 | 68.7 | 68.5 | 71.3 | 72.2 | 71.3 | 72.2 | 71.8 | 71.9 | 72.7 | 71.6 | 71.7 |
| 12 | 79.9 | 79.1 | 79.8 | 80.1 | 79.8 | 78.8 | 79.8 | 78.8 | 78.6 | 77.7 | 77.6 | 79.1 | 79.1 |
| 13 | 47.4 | 47.5 | 47.4 | 47.5 | 55.8 | 55.7 | 55.8 | 55.7 | 54.9 | 54.9 | 54.9 | 55.0 | 55.0 |
| 14 | 70.9 | 70.8 | 70.8 | 71.7 | 85.6 | 85.6 | 85.6 | 85.6 | 84.1 | 84.2 | 84.2 | 83.9 | 84.0 |
| 15 | 28.3 | 28.4 | 28.3 | 28.2 | 36.3 | 36.4 | 36.3 | 36.4 | 34.6 | 34.8 | 34.8 | 33.9 | 34.0 |
| 16 | 26.5 | 26.5 | 26.5 | 26.4 | 24.8 | 24.8 | 24.8 | 24.8 | 24.0 | 24.1 | 24.1 | 24.3 | 24.4 |
| 17 | 61.4 | 61.3 | 61.4 | 61.3 | 59.5 | 59.4 | 59.5 | 59.4 | 58.3 | 58.3 | 58.3 | 58.4 | 58.4 |
| 18 | 11.9 | 11.9 | 11.9 | 11.9 | 14.1 | 14.1 | 14.1 | 14.1 | 11.7 | 11.7 | 11.7 | 11.9 | 11.9 |
| 19 | 19.2 | 19.2 | 19.2 | 13.1 | 13.5 | 13.5 | 13.5 | 13.5 | 19.3 | 19.3 | 19.4 | 12.4 | 12.4 |
| 20 | 207.8 | 208.0 | 207.8 | 207.8 | 213.9 | 214.0 | 214.0 | 214.0 | 213.5 | 213.7 | 213.8 | 213.4 | 213.4 |
| 21 | 31.3 | 31.4 | 31.3 | 31.2 | 31.8 | 31.8 | 31.8 | 31.8 | 31.8 | 31.8 | 31.8 | 31.7 | 31.7 |
| 11-*O*- | Tig | $Tig_1$ | Tig | Tig | Tig | Bz | Tig | Bz | Ac | $Tig_1$ | Bz | Ac | Tig |
| 1 | 167.0 | 167.0 | 167.0 | 167.2 | 167.1 | 166.0 | 167.1 | 166.0 | 170.2 | 167.1 | 166.1 | 170.4 | 167.4 |
| 2 | 128.6 | 128.9 | 128.6 | 128.8 | 129.0 | 131.0 | 129.0 | 131.0 | 21.3 | 129.0 | 130.8 | 21.3 | 128.9 |
| 3 | 138.7 | 138.6 | 138.8 | 138.5 | 138.6 | 130.1 | 138.6 | 130.1 | | 138.5 | 130.1 | | 138.6 |
| 4 | 14.1 | 14.2 | 14.1 | 14.1 | 14.1 | 128.8 | 14.1 | 128.8 | | 14.3 | 128.8 | | 14.1 |
| 5 | 11.7 | 12.0 | 11.7 | 11.7 | 11.8 | 133.3 | 11.8 | 133.3 | | 12.0 | 133.5 | | 11.7 |
| 6 | | | | | | 128.8 | | 128.8 | | | 128.8 | | |
| 7 | | | | | | 130.1 | | 130.1 | | | 130.1 | | |
| 12-*O*- | Bz | $Tig_2$ | Bz | Bz | Bz | Tig | Bz | Tig | Bz | $Tig_2$ | Tig | Bz | Bz |
| 1 | 166.5 | 167.5 | 166.5 | 166.5 | 166.9 | 168.0 | 166.9 | 168.0 | 166.8 | 167.9 | 167.8 | 166.8 | 166.8 |
| 2 | 130.6 | 128.8 | 130.6 | 130.7 | 130.4 | 128.4 | 130.4 | 128.4 | 130.1 | 128.5 | 128.3 | 130.1 | 130.3 |
| 3 | 130.0 | 138.4 | 130.0 | 130.0 | 130.1 | 138.5 | 130.1 | 138.5 | 130.2 | 138.7 | 138.8 | 130.2 | 130.1 |
| 4 | 128.8 | 14.3 | 128.8 | 128.8 | 128.9 | 14.1 | 128.9 | 14.1 | 129.2 | 14.3 | 14.2 | 129.2 | 128.9 |
| 5 | 133.5 | 12.0 | 133.5 | 133.4 | 133.6 | 11.8 | 133.6 | 11.8 | 134.0 | 12.1 | 11.9 | 133.9 | 133.7 |
| 6 | 128.8 | | 128.8 | 128.8 | 128.9 | | 129.0 | | 129.2 | | | 129.2 | 128.9 |
| 7 | 130.0 | | 130.0 | 130.0 | 130.1 | | 130.1 | | 130.2 | | | 130.2 | 130.1 |

$J$ = 10.0 Hz, H-12), 3.30 (1 H, dd, $J$ = 9.0, 5.2 Hz, H-17), 1.68 (3 H, s, 18-$CH_3$), 1.54 (3 H, s, 19-$CH_3$), 2.16 (3 H, s, 21-$CH_3$), 8.19 (2 H, d, $J$ = 7.5 Hz Bz-H-3, 7), 7.40 (2 H, dd, $J$ = 7.7, 7.2 Hz, Bz-H-4, 6), 7.47 (1 H, t, $J$ = 7.2 Hz, Bz-H-5), 6.88 (1 H, q, $J$ = 7.0 Hz, Tig-H-3), 1.40 (3 H, d, $J$ = 7.0 Hz, Tig-H-4), 1.60 (3 H, s, Tig-H-5), 4.78 (1 H, d, $J$ = 9.5 Hz, Ole-H-1), 3.48 (3 H, s, Ole-3-$OCH_3$), 5.27 (1 H, d, $J$ = 7.9 Hz, Allo-H-1), 3.80 (1 H, s, Allo-3-$OCH_3$), 4.92 (1 H, d, $J$ = 7.6 Hz, $Glc_1$-H-1), 5.21 (1 H, d, $J$ = 7.8 Hz, $Glc_2$-H-1). $^{13}$C NMR data (125 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

*Marstenacisside A9* (**9**): white amorphous powder; $[\alpha]_D^{25}$ = + 47.0 ($c$ = 0.0885, MeOH). UV (MeOH) $\lambda_{max}$ 225.0 ($\varepsilon$ 15 189) nm. $C_{53}H_{78}O_{20}$, HRESIMS (negative): $m/z$ 1033.5083 $[M - H]^-$ (calcd. for $C_{53}H_{77}O_{20}$: 1195.5048). $^1$H NMR data (500 MHz, pyridine-$d_5$): $\delta$ 3.91 (1 H, m, H-3), 6.61 (1 H, t, $J$ = 10.6 Hz, H-11), 5.60 (1 H, d, $J$ = 10.1 Hz, H-12), 3.35 (1 H, dd, $J$ = 9.0, 5.2 Hz, H-17), 1.72 (3 H, s, 18-$CH_3$), 1.53 (3 H, s, 19-$CH_3$), 2.06 (3 H, s, 21-$CH_3$), 6.82 (1 H, q, $J$ = 7.1 Hz, Tig-H-3), 1.38 (3 H, d, $J$ = 7.1 Hz, Tig-H-4), 1.54 (3 H, s, Tig-H-5), 8.26 (2 H, d, $J$ = 7.6 Hz, Bz-H-3, 7), 7.42 (2 H, t, $J$ = 7.7 Hz, Bz-H-4, 6), 7.51 (1 H, t, $J$ = 7.4 Hz, Bz-H-5), 4.82 (1 H, d, $J$ = 9.7 Hz, Ole-H-1), 3.50 (3 H, s, Ole-3-$OCH_3$), 5.29 (1 H, d, $J$ = 8.1 Hz, Allo-H-1), 3.83 (1 H, s, Allo-3-$OCH_3$), 4.99 (1 H, d, $J$ = 7.7 Hz, Glc-H-1). $^{13}$C NMR data (125 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

▶ **Table 3** $^{13}C$ chemical shifts of the sugar moieties of **1–15** (150 MHz for **1, 4, 6, 11**, and **12**, and 125 MHz for **2, 3, 5, 7–10**, and **13–15**, pyridine-$d_5$).

| Position | 1 | 2 | 3 | 4 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ole-1 | 97.9 | 97.9 | 97.9 | 97.5 | 97.5 | 97.4 | 97.5 | 97.4 | 98.0 | 98.0 | 97.9 | 97.7 | 97.5 |
| – 2 | 37.7 | 37.7 | 37.8 | 37.8 | 37.8 | 37.8 | 37.8 | 37.8 | 37.8 | 37.8 | 37.8 | 37.8 | 37.8 |
| – 3 | 79.6 | 79.6 | 79.6 | 79.6 | 79.6 | 79.6 | 79.8 | 79.6 | 79.6 | 79.6 | 79.6 | 79.7 | 79.6 |
| – 4 | 83.2 | 83.2 | 83.2 | 83.2 | 83.3 | 83.2 | 83.3 | 83.2 | 83.3 | 83.2 | 83.2 | 83.3 | 83.3 |
| – 5 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 | 71.9 | 72.0 | 71.9 | 71.9 | 71.9 | 71.9 | 72.0 | 71.9 |
| – 6 | 19.0 | 19.0 | 19.0 | 19.0 | 19.0 | 19.0 | 19.1 | 19.1 | 19.0 | 19.0 | 19.0 | 19.1 | 19.0 |
| 3-$OCH_3$ | 57.2 | 57.2 | 57.2 | 57.2 | 57.2 | 57.1 | 57.2 | 57.2 | 57.2 | 57.2 | 57.2 | 57.2 | 57.3 |
| Allo-1 | 101.9 | 101.9 | 101.9 | 101.9 | 101.9 | 101.9 | 101.9 | 101.9 | 101.9 | 101.9 | 101.9 | 101.9 | 101.9 |
| – 2 | 72.7 | 72.7 | 72.7 | 72.7 | 72.7 | 72.7 | 72.7 | 72.7 | 72.7 | 72.7 | 72.8 | 72.7 | 72.7 |
| – 3 | 83.1 | 83.1 | 83.2 | 83.1 | 83.1 | 83.1 | 83.2 | 83.2 | 83.1 | 83.1 | 83.2 | 83.1 | 83.2 |
| – 4 | 83.4 | 83.4 | 83.3 | 83.4 | 83.4 | 83.4 | 83.4 | 83.3 | 83.4 | 83.4 | 83.3 | 83.4 | 83.3 |
| – 5 | 69.5 | 69.5 | 69.5 | 69.5 | 69.5 | 69.5 | 69.5 | 69.5 | 69.5 | 69.5 | 69.5 | 69.5 | 69.5 |
| – 6 | 18.3 | 18.3 | 18.3 | 18.3 | 18.3 | 18.3 | 18.3 | 18.3 | 18.3 | 18.3 | 18.3 | 18.3 | 18.3 |
| 3-$OCH_3$ | 61.7 | 61.7 | 61.7 | 61.7 | 61.7 | 61.7 | 61.7 | 61.7 | 61.7 | 61.7 | 61.7 | 61.7 | 61.7 |
| $Glc_1$-1 | 106.6 | 106.6 | 106.6 | 106.2 | 106.2 | 106.2 | 106.6 | 106.6 | 106.2 | 106.2 | 106.6 | 106.2 | 106.6 |
| – 2 | 75.0 | 75.0 | 75.5 | 75.0 | 75.0 | 75.0 | 75.5 | 75.5 | 75.0 | 75.0 | 75.5 | 75.0 | 75.5 |
| – 3 | 76.6 | 76.6 | 78.4 | 76.6 | 76.6 | 76.6 | 78.4 | 78.4 | 76.6 | 76.6 | 78.4 | 76.6 | 78.4 |
| – 4 | 81.5 | 81.5 | 71.9 | 81.5 | 81.5 | 81.5 | 72.0 | 72.0 | 81.5 | 81.5 | 71.9 | 81.5 | 71.9 |
| – 5 | 76.4 | 76.4 | 78.4 | 76.4 | 76.4 | 76.4 | 78.4 | 78.4 | 76.4 | 76.4 | 78.4 | 76.4 | 78.4 |
| – 6 | 63.4 | 62.4 | 63.0 | 62.5 | 62.5 | 62.4 | 63.0 | 63.0 | 62.4 | 62.4 | 63.0 | 62.4 | 63.0 |
| $Glc_2$-1 | 105.0 | 105.0 | | 105.0 | 105.0 | 105.0 | | | 105.0 | 105.0 | | 105.0 | |
| – 2 | 74.8 | 74.8 | | 74.8 | 74.8 | 74.8 | | | 74.8 | 74.8 | | 74.8 | |
| – 3 | 78.3 | 78.3 | | 78.3 | 78.3 | 78.3 | | | 78.3 | 78.3 | | 78.3 | |
| – 4 | 71.6 | 71.6 | | 71.6 | 71.6 | 71.5 | | | 71.5 | 71.6 | | 71.6 | |
| – 5 | 78.6 | 78.6 | | 78.6 | 78.6 | 78.6 | | | 78.6 | 78.6 | | 78.6 | |
| – 6 | 62.5 | 62.5 | | 62.5 | 62.4 | 62.5 | | | 62.5 | 62.5 | | 62.5 | |

*Marstenacisside A10* (**10**): white amorphous powder; $[\alpha]_D^{25}$ = + 35.3 ($c$ = 0.0962, MeOH). UV (MeOH) $\lambda_{max}$ 225.2 ($\varepsilon$ 14777) nm. $C_{53}H_{78}O_{20}$, HRESIMS (negative): $m/z$ 1033.5059 $[M - H]^-$ (calcd. for $C_{53}H_{77}O_{20}$: 1033.5048). $^1H$ NMR data (500 MHz, pyridine-$d_5$): $\delta$ 3.87 (1 H, m, H-3), 6.67 (1 H, t, $J$ = 10.7 Hz, H-11), 5.55 (1 H, d, $J$ = 10.1 Hz, H-12), 3.29 (1 H, dd, $J$ = 9.2, 5.4 Hz, H-17), 1.68 (3 H, s, 18-$CH_3$), 1.54 (3 H, s, 19-$CH_3$), 2.16 (3 H, s, 21-$CH_3$), 8.19 (2 H, d, $J$ = 7.5 Hz, Bz-H-3, 7), 7.41 (2 H, dd, $J$ = 7.5, 7.3 Hz, Bz-H-4, 6), 7.47 (1 H, t, $J$ = 7.3 Hz, Bz-H-5), 6.88 (1 H, q, $J$ = 7.0 Hz, Tig-H-3), 1.40 (3 H, d, $J$ = 7.0 Hz, Tig-H-4), 1.60 (3 H, s, Tig-H-5), 4.79 (1 H, d, $J$ = 9.5 Hz, Ole-H-1), 3.48 (3 H, s, Ole-3-$OCH_3$), 5.27 (1 H, d, $J$ = 8.2 Hz, Allo-H-1), 3.83 (1 H, s, Allo-3-$OCH_3$), 4.98 (1 H, d, $J$ = 7.8 Hz, Glc-H-1). $^{13}C$ NMR data (125 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

*Marstenacisside B15* (**11**): white amorphous powder; $[\alpha]_D^{25}$ = + 7.0 ($c$ = 0.0938, MeOH). UV (MeOH) $\lambda_{max}$ 230.4 ($\varepsilon$ 9408) nm. $C_{56}H_{82}O_{24}$, HRESIMS (negative): $m/z$ 1137.5137 $[M - H]^-$ (calcd. for $C_{56}H_{81}O_{24}$: 1137.5118). $^1H$ NMR data (600 MHz, pyridine-$d_5$): $\delta$ 3.77 (1 H, m, H-3), 5.85 (1 H, t, $J$ = 10.2 Hz, H-11), 5.43 (1 H, d, $J$ = 10.2 Hz, H-12), 3.23 (1 H, dd, $J$ = 9.6, 4.8 Hz, H-17), 1.44 (3 H, s, 18-$CH_3$), 1.25 (3 H, s, 19-$CH_3$), 2.06 (3 H, s, 21-$CH_3$), 8.36 (2 H, d, $J$ = 7.9 Hz, Bz-H-3, 7), 7.49 (2 H, dd, $J$ = 7.9, 7.4 Hz, Bz-H-4, 6), 7.57 (1 H, overlap, Bz-H-5), 4.75 (1 H, d, $J$ = 9.2 Hz, Ole-H-1), 3.49 (3 H, s, Ole-3-$OCH_3$), 5.26 (1 H, d, $J$ = 8.0 Hz, Allo-H-1), 3.79 (1 H, s, Allo-3-$OCH_3$), 4.90 (1 H, d, $J$ = 7.7 Hz, $Glc_1$-H-1), 5.19 (1 H, d, $J$ = 7.8 Hz, $Glc_2$-H-1). $^{13}C$ NMR data (150 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

*Marstenacisside B16* (**12**): white amorphous powder; $[\alpha]_D^{25}$ = + 41.6 ($c$ = 0.0608, MeOH). UV (MeOH) $\lambda_{max}$ 215.2 ($\varepsilon$ 17679) nm. $C_{57}H_{88}O_{24}$, HRESIMS (negative): $m/z$ 1155.5636 $[M - H]^-$ (calcd. for $C_{57}H_{87}O_{24}$: 1155.5587). $^1H$ NMR data (600 MHz, pyridine-$d_5$): $\delta$ 3.76 (1 H, m, H-3), 5.88 (1 H, t, $J$ = 10.4 Hz, H-11), 5.29 (1 H, d, $J$ = 10.0 Hz, H-12), 3.18 (1 H, dd, $J$ = 9.3, 4.8 Hz, H-17), 1.38 (3 H, s, 18-$CH_3$), 1.31 (3 H, s, 19-$CH_3$), 2.17 (3 H, s, 21-$CH_3$), 6.92 (1 H, qq, $J$ = 7.1, 1.4 Hz, $Tig_1$-H-3), 1.57 (3 H, d, $J$ = 7.1 Hz, $Tig_1$-H-4), 1.78 (3 H, s, $Tig_1$-H-5), 7.06 (1 H, qq, $J$ = 7.1, 1.3 Hz, $Tig_2$-H-3), 1.64 (3 H, d, $J$ = 7.1 Hz, $Tig_2$-H-4), 1.89 (3 H, s, $Tig_2$-H-5), 4.74 (1 H, br d, $J$ = 9.2 Hz, Ole-H-1), 3.48 (3 H, s, Ole-3-$OCH_3$), 5.24 (1 H, d, $J$ = 8.1 Hz, Allo-H-1), 3.79 (1 H, s, Allo-3-$OCH_3$), 4.90 (1 H, d, $J$ = 7.7 Hz, $Glc_1$-H-1), 5.19 (1 H, d, $J$ = 7.8 Hz, $Glc_2$-H-1). $^{13}C$ NMR data (150 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

*Marstenacisside A11* (**13**): white amorphous powder; $[\alpha]_D^{25}$ = + 51.7 ($c$ = 0.0769, MeOH). UV (MeOH) $\lambda_{max}$ 225.8 (ε 14318) nm. $C_{53}H_{76}O_{19}$, HRESIMS (negative): $m/z$ 1015.4949 $[M - H]^-$ (calcd.

for $C_{53}H_{75}O_{19}$: 1015.4903). $^1$H NMR data (500 MHz, pyridine-$d_5$): $\delta$ 3.75 (1 H, m, H-3), 6.08 (1 H, t, $J$ = 10.2 Hz, H-11), 5.44 (1 H, d, $J$ = 10.0 Hz, H-12), 3.22 (1 H, dd, $J$ = 9.4, 4.7 Hz, H-17), 1.37 (3 H, s, 18-$CH_3$), 1.25 (3 H, s, 19-$CH_3$), 2.18 (3 H, s, 21-$CH_3$), 8.16 (2 H, d, $J$ = 7.8 Hz Bz-H-3, 7), 7.38 (2 H, dd, $J$ = 7.8, 7.4 Hz, Bz-H-4, 6), 7.48 (1 H, $J$ = 7.6, Bz-H-5), 6.92 (1 H, q, $J$ = 7.0 Hz, Tig-H-3), 1.46 (3 H, d, $J$ = 7.0 Hz, Tig-H-4), 1.66 (3 H, s, Tig-H-5), 4.72 (1 H, br d, $J$ = 9.5 Hz, Ole-H-1), 3.50 (3 H, s, Ole-3-$OCH_3$), 5.25 (1 H, d, $J$ = 8.0 Hz, Allo-H-1), 3.82 (1 H, s, Allo-3-$OCH_3$), 4.98 (1 H, d, $J$ = 7.7 Hz, Glc-H-1). $^{13}$C NMR data (125 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

*Marstenacisside B17* (**14**): white amorphous powder; $[\alpha]_D^{25}$ = + 8.2 ($c$ = 0.0500, MeOH). UV (MeOH) $\lambda_{max}$ 230.4 ($\varepsilon$ 16 817) nm. $C_{56}H_{84}O_{24}$, HRESIMS (negative): $m/z$ 1139.5333 $[M - H]^-$ (calcd. for $C_{56}H_{83}O_{24}$: 1139.5274). $^1$H NMR data (500 MHz, pyridine-$d_5$): $\delta$ 3.82 (1 H, m, H-3), 5.69 (1 H, t, $J$ = 10.2 Hz, H-11), 5.36 (1 H, d, $J$ = 9.8 Hz, H-12), 3.22 (1 H, dd, $J$ = 8.8, 5.0 Hz, H-17), 1.41 (3 H, s, 18-$CH_3$), 0.99 (3 H, s, 19-$CH_3$), 2.05 (3 H, s, 21-$CH_3$), 8.35 (2 H, d, $J$ = 7.9 Hz, Bz-H-3, 7), 7.49 (2 H, dd, $J$ = 7.9, 7.4 Hz, Bz-H-4, 6), 7.57 (1 H, overlap, Bz-H-5), 4.77 (1 H, br d, $J$ = 9.2 Hz, Ole-H-1), 3.50 (3 H, s, Ole-3-$OCH_3$), 5.27 (1 H, d, $J$ = 8.1 Hz, Allo-H-1), 3.79 (1 H, s, Allo-3-$OCH_3$), 4.90 (1 H, d, $J$ = 7.7 Hz, $Glc_1$-H-1), 5.19 (1 H, d, $J$ = 7.8 Hz, $Glc_2$-H-1). $^{13}$C NMR data (125 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

*Marstenacisside A12* (**15**): white amorphous powder; $[\alpha]_D^{25}$ = + 37.6 ($c$ = 0.0792, MeOH). UV (MeOH) $\lambda_{max}$ 225.2 ($\varepsilon$ 20 064) nm. $C_{53}H_{78}O_{19}$, HRESIMS (negative): $m/z$ 1017.5085 $[M - H]^-$ (calcd. for $C_{53}H_{77}O_{19}$: 1017.5059). $^1$H NMR data (500 MHz, pyridine-$d_5$): $\delta$ 3.82 (1 H, m, H-3), 5.87 (1 H, t, $J$ = 10.4 Hz, H-11), 5.43 (1 H, d, $J$ = 9.9 Hz, H-12), 3.28 (1 H, dd, $J$ = 9.1, 5.0 Hz, H-17), 1.46 (3 H, s, 18-$CH_3$), 1.07 (3 H, s, 19-$CH_3$), 2.08 (3 H, s, 21-$CH_3$), 6.78 (1 H, q, $J$ = 7.1 Hz, Tig-H-3), 1.40 (3 H, d, $J$ = 7.1 Hz, Tig-H-4), 1.54 (3 H, s, Tig-H-5), 8.27 (2 H, d, $J$ = 7.7 Hz, Bz-H-3, 7), 7.45 (2 H, dd, $J$ = 7.7, 7.5 Hz, Bz-H-4, 6), 7.53 (1 H, t, $J$ = 7.4 Hz, Bz-H-5), 4.77 (1 H, br d, $J$ = 9.7 Hz, Ole-H-1), 3.50 (3 H, s, Ole-3-$OCH_3$), 5.27 (1 H, d, $J$ = 8.0 Hz, Allo-H-1), 3.80 (1 H, s, Allo-3-$OCH_3$), 4.98 (1 H, d, $J$ = 7.8 Hz, Glc-H-1). $^{13}$C NMR data (125 MHz, pyridine-$d_5$), see ▶ **Tables 2** and **3**.

### HIV inhibition assay

SupT1 cells ($2 \times 10^5$) were co-transfected with 0.6 mg of pNL-Luc-E and 0.4 mg of pHIT/G. Then the VSV-G pseudo-typed viral supernatant (HIV-1) was harvested by filtration through a 0.45-µm filter after 48 h and the concentration of viral capsid protein was determined by p24 antigen capture ELISA (Biomerieux). SupT1 cells were exposed to VSV-G pseudo-typed HIV-1 (MOI = 1) at 37.8 °C for 48 h in the absence or presence of the test compounds (with the positive control, Efavirenz) [14]. A luciferase assay system (Promega) was used to determine the inhibition rate. The cytotoxicity was measured by the MTT method. SupT1 cells were seeded into a 96-well microtiter plate in the absence or presence of the test compounds (positive control, Efavirenz) in triplicate and incubated at 37.8 °C in a humid atmosphere of 5 % $CO_2$. After a 4-day incubation, cell viability was measured by the MTT method. Purities of all of the tested compounds were > 95 %, as detected by HPLC-ELSD. Efavirenz were obtained from NIH-AIDS Research and Reference Reagent Program with a purity of > 98 % (HPLC).

### Supporting information

NMR spectra of **1**–**15** are available as Supporting Information.

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### Conflict of Interest

There are no conflicts of interest for all authors.

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