Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5629–5632
Bioorganic & Medicinal Chemistry Letters

Synthesis and cytotoxicities of dioscin derivatives with decorated chacotriosyl residues

Shilei Zhu,[b] Yichun Zhang,[a] Ming Li,[a] Jia Yu,[a] Lihong Zhang,[a] Yingxia Li[b] and Biao Yu[a,*]

[a]*State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China*
[b]*Key Laboratory of Marine Drugs, The Ministry of Education of China, Marine Drug and Food Institute, Ocean University of China, Qingdao 266003, China*

Received 20 June 2006; revised 19 July 2006; accepted 2 August 2006
Available online 14 August 2006

Abstract—Two series of dioscin derivatives (**4a**–**o** and **5a**–**o**) with selected modifications at the 6′ and 4‴ positions of the chacotriosyl residue, respectively, were synthesized. All the 6′-*N*-acyl-dioscin derivatives did not show considerable inhibitory activities at 10 μM, while most of the 4‴-*O*-(2-*N*-acyl)ethyl-dioscin derivatives behaved as potent as dioscin, against the growth of tumor cells.

A quite common feature of spirostan saponins, which occur widely and abundantly in plants, is their inhibitory activities against the growth of tumor cells.[1] And the cytotoxic potency of spirostan saponins is highly dependent on their sugar residues.[2] Dioscin, diosgenin-3-yl α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside (chacotrioside), represents one of the most common plant spirostan saponins, which has been isolated from some twenty genera, including many vegetables and medicinal plants. Dioscin is among the most potent cytotoxic spirostan saponins[2,3] and is relatively easy to synthesize.[4] Therefore, we employed dioscin as a lead structure to decipher the structure–activity relationships and mechanism of action of spirostan saponins. To this end, we have synthesized all the eight possible mono-methylated derivatives of dioscin and found that the 6′- and 4‴-*O*-methyl derivatives (**1** and **2**) retained largely the cytotoxicities of dioscin but other mono-*O*-methyl compounds were nearly inactive.[5] The 4‴-*O*-acetyl-dioscin (**3**) also showed to be as toxic as dioscin,[6] and the 6′-*N*-(2-*N*-dansyl)ethyl-dioscin retained about 30% of the toxicity of dioscin.[7] These results prompted us to examine the influence on the cytotoxicity of a variety of modifications at the 6′ and 4‴ positions of dioscin, thus, we might be able to label dioscin properly to study its mechanism of action. Here, we report the convergent synthesis of such two series of dioscin derivatives (**4a**–**o** and **5a**–**o**) and their inhibitory activities against the growth of tumor cells (Fig. 1).

The synthetic route toward the 6′-*N*-acyl-dioscins **4a**–**o** is depicted in Scheme 1. Thus, diosgenin-3-yl β-D-glucopyranoside (trillin)[8] was subjected to selective sulfonylation at the primary 6′-OH with *p*-toluenesulfonyl chloride in pyridine, the desired 6′-*O*-tosyl derivative **6** was obtained in a satisfactory 73% yield. Treatment of **6** with NaN_3 in DMF at 90 °C gave 6′-azide **7** successfully (80%). Triol **7** was then selectively protected with pivaloyl chloride at the 3′-OH to afford diol **8** in 81% yield.[9] Glycosylation of the remaining 2′,4′-di-OHs in **8** with 2,3,4-tri-*O*-benzoyl-L-rhamnopyranosyl trichloroacetimidate (**9**)[10] under the promotion of TMSOTf led to the desired trisaccharide **10** in 76% yield. Removal of the benzoyl and pivaloyl groups in **10** with LiOH in MeOH provided the 6′-azido-dioscin **11** in 95% yield. Reduction of the 6′-azido into the 6′-NH_2 group with PPh_3 provided the desired key intermediate **12** quantitatively. Finally, selective coupling of the 6′-NH_2 in **12** with a variety of the acyl chlorides was achieved in the presence of Et_3N in CH_3OH, furnishing the 6′-*N*-acyl-dioscin derivatives **4a**–**o** in 70 ~ 95% yields.[11,12]

Keywords: Dioscin derivatives; Amide; Synthesis; Cytotoxicity.
* Corresponding author. Tel.: +0086 21 54925131; fax: +86 21 64166128; e-mail: byu@mail.sioc.ac.cn

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.08.019

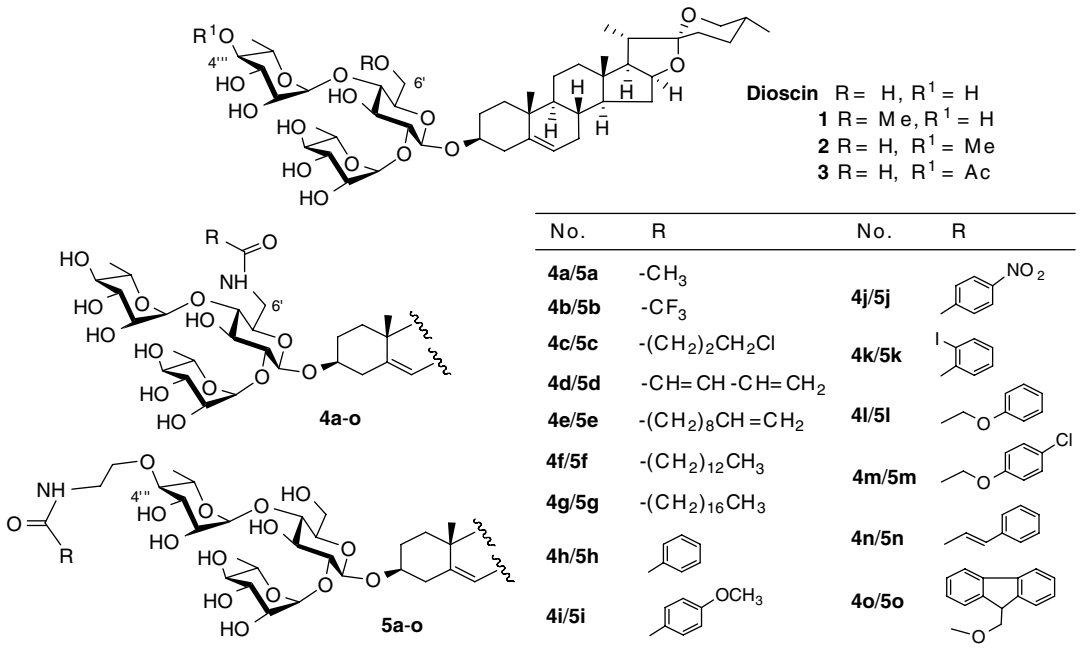
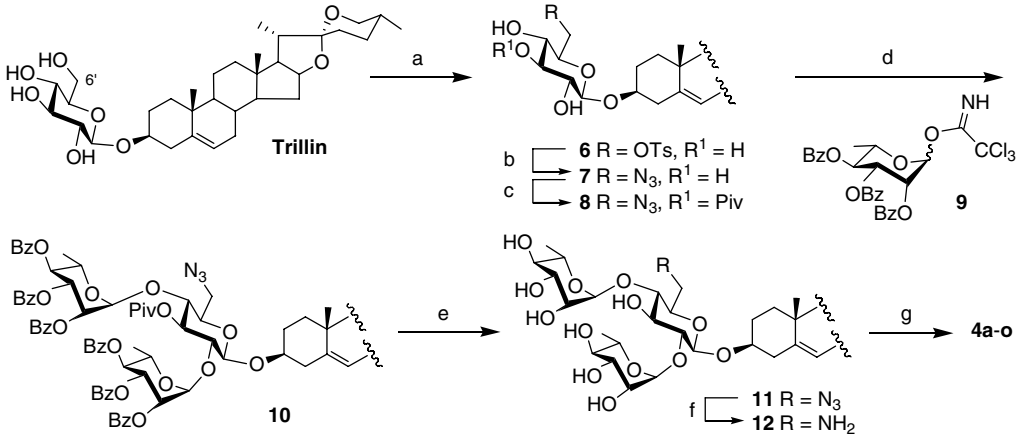


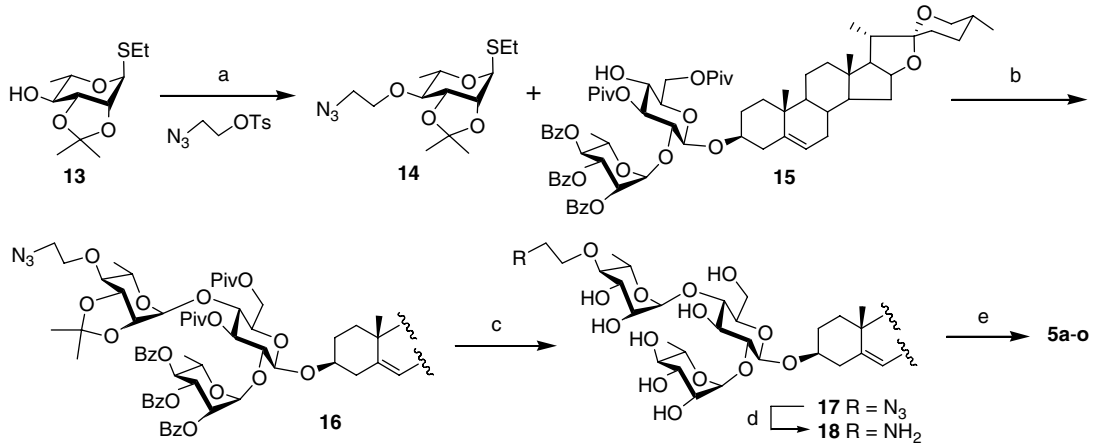
Figure 1. Dioscin and its derivatives with modifications at the 6′ and 4‴ positions.



Scheme 1. Reagents and conditions: (a) TsCl, pyridine, 0 °C → rt, 24 h, 73%; (b) NaN_3, DMF, 90 °C, 4 h, 80%; (c) PivCl, pyridine, 0 °C, 4 h, 81%; (d) TMSOTf (0.1 equiv), 4 Å MS, CH_2Cl_2, 0 °C → rt, 1 h, 76%; (e) LiOH, THF–MeOH–H_2O (1:1:1), 40 °C, overnight, 95%; (f) PPh_3, THF–H_2O (4:1), 60 °C, 2 h, 100%; (g) acyl chloride, CH_3OH, Et_3N, 0 °C → rt, 1 h, 70 ~ 95%.

The 4‴-*O*-(2-*N*-acyl)ethyl-dioscin derivatives **5a**–**o** were synthesized starting from ethyl 2,3-*O*-isopropylidene-1-thio-L-rhamnopyranoside (**13**)[13] (Scheme 2). Treatment of **13** with 1-tosyloxy-2-azidoethane[14] in the presence of NaH in THF provided **14** in a good 63% yield. Coupling of the readily available disaccharide **15**[9b] with thioglycoside **14** under the action of NIS/TfOH afforded the trisaccharide **16** in 81% yield. Removal of all the protective groups (benzoyl, pivaloyl, and isopropylidene groups) with LiOH followed with H^+ resin provided the 4‴-*O*-(2-azido)ethyl-dioscin **17** in 86% yield. Reduction of the terminal $-N_3$ into $-NH_2$ group with PPh_3 led to the key intermediate **18**. Finally, selective coupling of the appending NH_2 group in **18** with a variety of the acyl chlorides in the presence of Et_3N in CH_3OH furnished the desired 4‴-*O*-(2-*N*-acyl)ethyl-dioscins **5a**–**o** in 70 ~ 95% yields.[11,15]

The inhibitory activities of the dioscin derivatives **4a**–**o**, **5a**–**o**, **11**, **12**, **17**, and **18** against the growth of three tumor cell lines, that is, A549 (human lung carcinoma cell), BGC-823 (human gastric cancer cell), and HGC-27 (human gastric carcinoma cell), were evaluated following a standard MTT assay with dioscin as a positive control.[16] The results are listed in Table 1. All the derivatives with the 6′-*N*-modifications (**4a**–**o**, **11**, and **12**) did not show considerable inhibition at a concentration of 10 μM toward all the three cell lines. While most of the derivatives with the 4‴-*O*-substituents were remarkably active with the only exceptions of compounds **5f** and **5g** which bear the longest fatty chains. Compound **5e** bearing a $CH_2{=}CH(CH_2)_8$- residue was the most active compound, showing IC_{50}s of 2.6, 1.8, and 0.8 μM, respectively, toward the three cell lines. Interestingly, the aryl groups in the 4‴-*O*-(2-*N*-acyl)ethyl residue



Scheme 2. Reagents and conditions: (a) NaH, THF, rt → 80 °C, then $N_3CH_2CH_2OTs$, 50 °C, 48 h, 63%; (b) NIS, TfOH, 4 Å MS, CH_2Cl_2, 0 °C → rt, 1 h, 81%; (c) LiOH, THF–MeOH–H_2O (1:1:1), 40 °C, overnight, then H^+ resin, rt, 24 h, 86%; (d) PPh_3, THF–H_2O (4:1), 60 °C, 2 h, 87%; (e) acyl chloride, CH_3OH, Et_3N, 0 °C → rt, 1 h, 70 ~ 95%.

Table 1. Inhibitory activities of the dioscin derivatives against the growth of tumor cells

Compound	IC_{50} (μM)		
	A549	BGC-823	HGC-27
4a–o, 11,12	ND	ND	ND
5a	10.3	3.1	4.7
5b	3.7	4.0	8.3
5c	10.1	11.0	9.2
5d	4.9	1.9	3.3
5e	2.6	1.8	0.8
5f, 5g	ND	ND	ND
5h	11.2	5.1	1.3
5i	10.3	1.9	11.9
5j	15.3	1.6	15.0
5k	4.2	12.8	3.8
5l	7.7	16.4	4.2
5m	7.4	1.2	8.8
5n	11.2	2.6	13.1
5o	3.6	6.1	2.0
17	9.5	2.2	3.3
18	9.2	11.2	7.0
Dioscin	4.2	2.0	5.9

ND: IC_{50} not determined. These compounds did not show considerable inhibitory activities at a concentration of 10 μM.

affected the sensitivity of the dioscin derivatives toward different cell lines (i.e., **5h–n**). The present results indicate clearly that modifications on the 4‴-OH would not affect considerably the cytotoxicities of dioscin. Thus, the synthesis of the dioscin derivatives with a variety of the fluorescence and affinity labels at the 4‴-OH and the studies of their mechanisms of action on tumor cells become our current interest, and the results will be reported in due course.

Acknowledgments

This work was supported by the Chinese Academy of Sciences (KGCX2-SW-213 and KGCX2-SW-209) and the National Natural Science Foundation of China (20321202).

References and notes

1. Hostettmann, K.; Marston, A. *Saponins*; Cambridge University Press: Cambridge, UK, 1995.
2. (a) Mimaki, Y.; Yokosuka, A.; Kuroda, M.; Sashida, Y. *Biol. Pharm. Bull.* **2001**, *24*, 1286; (b) Nakamura, T.; Komori, C.; Lee, Y.-y.; Hashimoto, F.; Yahara, S.; Nohara, T.; Ejima, A. *Biol. Pharm. Bull.* **1996**, *19*, 564.
3. (a) Hu, K.; Dong, A. J.; Yao, X. S.; Kobayashi, H.; Iwasaki, S. *Planta Med.* **1996**, *62*, 573; (b) Chiang, H. C.; Tseng, T. H.; Wang, C. J.; Chen, C. F.; Kan, W. S. *Anticancer Res.* **1991**, *11*, 1911.
4. (a) Deng, S.; Yu, B.; Hui, Y.; Yu, H.; Han, X. *Carbohydr. Res.* **1999**, *317*, 53; (b) Yu, B.; Tao, H. *J. Org. Chem.* **2002**, *67*, 9099; (c) Zou, C.-C.; Hou, S.-J.; Shi, Y.; Lei, P.-S.; Liang, X.-T. *Carbohydr. Res.* **2003**, *338*, 721; (d) Hou, S.; Zou, C.; Zhou, L.; Lei, P.; Yu, D. *Chem. Lett.* **2005**, *34*, 1220.
5. Li, M.; Han, X.; Yu, B. *Carbohydr. Res.* **2003**, *338*, 117.
6. Yu, B.; Xing, G.; Hui, Y.; Han, X. *Tetrahedron Lett.* **2001**, *42*, 5513.
7. Yang, Z.; Wong, L. M. E.; Shum, Y. T. T.; Che, C. M.; Hui, Y. *Org. Lett.* **2005**, *7*, 669.
8. Deng, S.; Yu, B.; Xie, J.; Hui, Y. *J. Org. Chem.* **1999**, *64*, 7265.
9. (a) Jiang, L.; Chan, T. *J. Org. Chem.* **1998**, *63*, 6035; (b) Li, B.; Yu, B.; Hui, Y.; Li, M.; Han, X.; Fung, K. P. *Carbohydr. Res.* **2001**, *331*, 1.
10. Zhang, M.; Du, Y.; Kong, F. *Carbohydr. Res.* **2001**, *330*, 319.
11. A typical procedure for the selective *N*-acylation: to a stirred solution of compound **12** (70 mg, 0.08 mmol) and Et_3N (66 μL, 0.48 mmol) in MeOH (5 mL) was added benzoyl chloride (46 μL, 0.40 mmol) at 0 °C. After stirring at rt for 2 h, the mixture was concentrated to give a residue, which was purified by silica gel column chromatography (6:1 CH_2Cl_2–MeOH) to provide **4h** (69 mg, 89%) as a white solid.
12. Analytical data for selected compounds. **11**: ^1H NMR (300 MHz, pyridine-d_5): δ 6.36 (s, 1H, H-1(Rha)), 5.52 (s, 1H, H-1(Rha)), 5.33 (d, 1H, J = 4.2 Hz), 5.03–4.78 (m, 4H), 4.65–4.33 (m, 6H), 4.28–4.17 (m, 2H), 3.97 (m, 2H), 3.78 (m, 1H), 3.78–3.50 (m, 4H), 2.77–2.65 (m, 2H), 1.78 (d, 3H, J = 6.3 Hz), 1.62 (d, 3H, J = 6.6 Hz), 1.15 (d, 3H, J = 6.3 Hz), 1.03 (s, 3H), 0.82 (s, 3H), 0.68 (d, 3H, J = 5.4 Hz); MALDI-MS: m/z $[M+Na]^+$ calcd 916.5.

Found: 916.9. **12**: ^{1}H NMR (400 MHz, pyridine-d_5): δ 6.43 (s, 1H, H-1(Rha)), 5.72 (s, 1H, H-1(Rha)), 5.43 (d, 1H, J = 4.7 Hz), 4.99 (m, 2H), 4.88 (m, 2H), 4.71–4.56 (m, 4H), 4.46–4.39 (m, 2H), 4.27–4.15 (m, 3H), 3.96 (m, 1H), 3.78–3.58 (m, 4H), 3.38 (m, 1H), 2.89–2.79 (m, 2H), 1.82 (d, 3H, J = 6.1 Hz), 1.69 (d, 3H, J = 4.2 Hz), 1.22 (d, 3H, J = 6.0 Hz), 1.12 (s, 3H), 0.91 (s, 3H), 0.78 (d, 3H, J = 5.3 Hz); MALDI-MS: m/z [M+Na]$^+$ calcd 890.5. Found: 890.9. **4a**: ^{1}H NMR (300 MHz, pyridine-d_5): δ 8.69 (m, 1H), 6.36 (s, 1H, H-1(Rha)), 5.65 (s, 1H, H-1(Rha)), 5.42 (d, 1H, J = 3.6 Hz), 5.04–4.92 (m, 3H), 4.85 (d, 1H, J = 1.5 Hz), 4.79 (m, 1H), 4.68–4.51 (m, 3H), 4.42 (m, 2H), 4.22–4.04 (m, 3H), 3.95–3.78 (m, 4H), 3.69–3.54 (m, 2H), 2.89–2.69 (m, 2H), 2.13 (s, 3H), 1.83 (d, 3H, J = 6.0 Hz), 1.67 (d, 3H, J = 6.2 Hz), 1.20 (d, 3H, J = 6.8 Hz), 1.14 (s, 3H), 0.92 (s, 3H), 0.78 (d, 3H, J = 5.1 Hz); MALDI-MS: m/z [M+Na]$^+$ calcd 932.5. Found: 932.8. **4j**: ^{1}H NMR (300 MHz, pyridine-d_5): δ 9.71 (br s, 1H), 8.34–8.27 (m, 4H), 6.31 (s, 1H, H-1(Rha)), 5.65 (s, 1H, H-1(Rha)), 5.23 (s, 1H), 4.90–4.79 (m, 5H), 4.63–4.56 (m, 3H), 4.41–4.34 (m, 2H), 4.22–4.18 (m, 3H), 3.96 (br s, 3H), 3.88–3.78 (m, 1H), 3.61–3.49 (m, 2H), 2.85–2.54 (m, 2H), 1.76 (d, 3H, J = 6.0 Hz), 1.63 (d, 3H, J = 6.0 Hz), 1.13 (d, 3H, J = 6.6 Hz), 0.94 (s, 3H), 0.82 (s, 3H), 0.70 (br s, 3H); MALDI-MS: m/z [M+Na]$^+$ calcd 1039.5. Found: 1039.9.

13. Yang, Z.; Yu, B. . *Carbohydr. Res.* **2001**, *333*, 105.
14. Nelissen, H. F. M.; Venema, F.; Uittenbogaard, R. M.; Feiters, M. C.; Nolte, R. J. M. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2045.
15. Analytical data for selected compounds. **17**: ^{1}H NMR (300 MHz, pyridine-d_5): δ 6.40 (s, 1H, H-1(Rha)), 5.83 (s, 1H, H-1(Rha)), 5.31 (d, 1H, J = 4.2 Hz), 4.99–4.83 (m, 4H), 4.64 (m, 2H), 4.58–4.51 (m, 2H), 4.44–4.35 (m, 3H), 4.23–4.16 (m, 3H), 4.04–3.83 (m, 4H), 3.61–3.46 (m, 4H), 3.36 (m, 1H), 2.81–2.66 (m, 2H), 1.77 (d, 3H, J = 6.3 Hz), 1.54 (d, 3H, J = 5.7 Hz), 1.15 (d, 3H, J = 6.6 Hz), 1.05 (s, 3H), 0.83 (s, 3H), 0.70 (d, 3H, J = 4.2 Hz); ^{13}C NMR (75 MHz, pyridine-d_5): δ 141.7, 122.7, 110.2, 103.2, 102.8, 101.2, 84.2, 82.0, 79.1, 79.0, 78.6, 77.8, 74.9, 73.7, 73.5, 73.3, 72.6, 70.4, 69.5, 67.8, 63.8, 62.1, 57.6, 52.7, 51.2, 42.9, 41.4, 40.8, 39.9, 38.4, 38.1, 38.0, 34.3, 33.2, 33.1, 32.7, 32.6, 31.5, 31.1, 30.2, 30.1, 26.9, 22.0, 20.3, 19.5, 19.2, 18.3, 18.2, 17.3, 16.0; ESI-HRMS: m/z [M+Na]$^+$ calcd 960.5027. Found: 960.5040. **18**: ^{1}H NMR (300 MHz, pyridine-d_5): δ 6.14 (s, 1H, H-1(Rha)), 5.83 (s, 1H, H-1(Rha)), 5.32 (d, 1H, J = 3.6 Hz), 4.96 (m, 2H), 4.82 (m, 2H), 4.67–4.51 (m, 4H), 4.42–4.34 (m, 2H), 4.22–4.18 (m, 3H), 4.06 (m, 1H), 3.93–3.84 (m, 3H), 3.62–3.46 (m, 3H), 3.13–2.99 (m, 2H), 2.82–2.68 (m, 2H), 1.78 (d, 3H, J = 6.0 Hz), 1.47 (d, 3H, J = 6.3 Hz), 1.15 (d, 3H, J = 6.9 Hz), 1.05 (s, 3H), 0.83 (s, 3H), 0.70 (d, 3H, J = 5.4 Hz); ^{13}C NMR (75 MHz, pyridine-d_5): δ 142.1, 123.1, 110.5, 103.6, 103.3, 101.6, 84.8, 82.4, 79.4, 79.2, 78.2, 75.4, 74.3, 74.1, 73.8, 73.3, 70.8, 70.1, 68.1, 64.2, 62.5, 57.9, 51.6, 43.4, 43.3, 41.8, 41.2, 40.3, 38.8, 38.4, 33.6, 33.5, 33.1, 33.0, 31.9, 31.5, 30.6, 23.0, 22.4, 20.7, 19.9, 19.6, 18.6, 17.6, 16.3; ESI-HRMS: m/z [M+Na]$^+$ calcd 934.5108. Found: 934.5135. **5a**: ^{1}H NMR (300 MHz, pyridine-d_5): δ 8.64 (s, 1H), 6.42 (s, 1H, H-1(Rha)), 5.83 (s, 1H, H-1(Rha)), 5.34 (s, 1H), 4.96 (m, 2H), 4.86–4.81 (m, 2H), 4.67–4.48 (m, 4H), 4.38 (m, 2H), 4.23–4.13 (m, 4H), 4.09–3.87 (m, 2H), 3.93–3.87 (m, 2H), 3.75–3.52 (m, 5H), 2.84–2.74 (m, 2H), 2.08 (s, 3H), 1.80 (d, 3H, J = 6.0 Hz), 1.49 (d, 3H, J = 6.0 Hz), 1.17 (d, 3H, J = 7.2 Hz), 1.07 (s, 3H), 0.85 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (75 MHz, pyridine-d_5): δ 170.3, 141.0, 121.9, 109.4, 102.5, 102.2, 100.5, 83.9, 81.3, 78.3, 78.1, 78.0, 77.1, 74.3, 73.0, 72.8, 72.7, 72.5, 72.3, 69.7, 69.0, 67.0, 63.1, 61.4, 56.8, 50.5, 46.1, 42.1, 41.0, 40.6, 40.0, 39.1, 37.7, 37.3, 32.5, 32.4, 32.0, 31.8, 30.7, 30.3, 29.4, 23.2, 21.3, 19.6, 18.8, 18.4, 17.5, 16.5, 15.2, 9.1; ESI-MS: (m/z) [M+Na]$^+$ calcd 976.5, found 976.6. **5k**: ^{1}H NMR (400 MHz, pyridine-d_5): δ 9.38 (t, 1H, J = 4.4 Hz), 7.94 (d, 1H, J = 7.9 Hz), 7.71 (dd, 1H, J = 7.6, 1.6 Hz), 7.34 (t, 1H, J = 7.5 Hz), 7.05 (dt, 1H, J = 7.7, 1.6 Hz), 6.45 (s,1H, H-1(Rha)), 5.87 (s, 1H, H-1(Rha)), 5.40 (d, 1H, J = 4.8 Hz), 5.03 (m, 2H), 4.90 (m, 2H), 4.71 (dd, 1H, J = 9.3, 3.3 Hz), 4.65–4.56 (m, 3H), 4.45–4.41 (m, 3H), 4.30–4.22 (m, 4H), 4.13–3.92 (m, 5H), 3.69–3.56 (m, 3H), 2.90–2.76 (m, 2H), 1.84 (d, 3H, J = 6.2 Hz), 1.58 (d, 3H, J = 6.2 Hz), 1.23 (d, 3H, J = 6.9 Hz), 1.13 (s, 3H), 0.91 (s, 3H), 0.79 (d, 3H, J = 5.5 Hz); MALDI-MS: (m/z) [M+Na]$^+$ calcd 1164.4. Found: 1165.1.
16. Liu, X.; Cui, Y.; Yu, Q.; Yu, B. *Phytochemistry* **2005**, *66*, 1671.