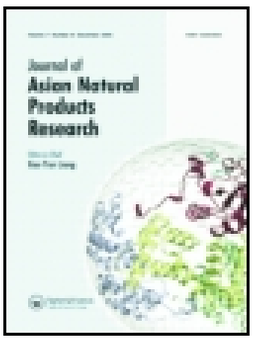

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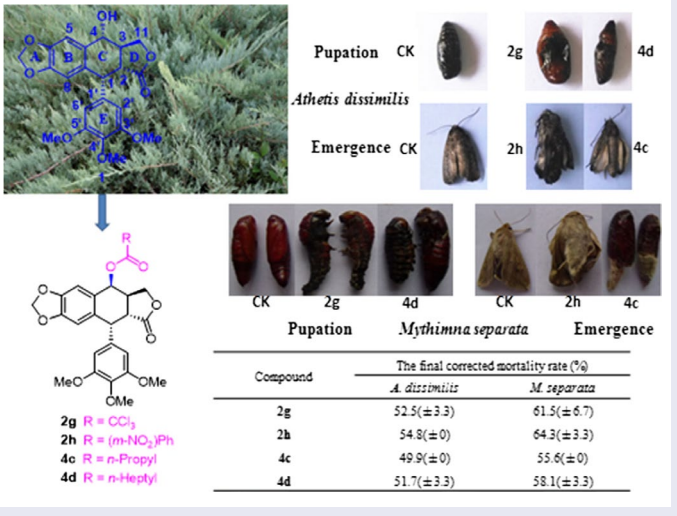
Stereoselective synthesis of 4β-acyloxypodophyllotoxin derivatives as insecticidal agents

Zhi-Ping Che[#], Yue-E Tian[#], Sheng-Ming Liu, Jia Jiang, Mei Hu and Gen-Qiang Chen

Laboratory of Pharmaceutical Design & Synthesis, Department of Plant Protection, College of Forestry, Henan University of Science and Technology, Luoyang 471003, China

ABSTRACT

As our ongoing work on research of natural-product-based insecticidal agents, some 4α/β-acyloxypodophyllotoxin derivatives were synthesized, and were evaluated against the pre-third-instar larvae of *B. mori*, *A. dissimilis* and *M. separate in vivo* at the concentration of 1 mg ml^{-1}, respectively. Among all derivatives, compounds **2 g**, **h** and **4c**, **d** showed more promising insecticidal activities than their precursors – podophyllotoxin and epipodophyllotoxin. Furthermore, derivatives **2 g**, **h** and **4c**, **d** exhibited more relative amicable activities than their precursors – podophyllotoxin and epipodophyllotoxin. This results indicated that 4β-acyloxy moiety in the podophyllotoxin derivatives was significant for obtaining the more potent compounds.



Compound	The final corrected mortality rate (%)	
	A. dissimilis	*M. separata*
2g	52.5(±3.3)	61.5(±6.7)
2h	54.8(±0)	64.3(±3.3)
4c	49.9(±0)	55.6(±0)
4d	51.7(±3.3)	58.1(±3.3)

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Podophyllotoxin; acyloxy; semisynthesis; botanical insecticide; insecticidal activity

CONTACT Zhi-Ping Che zhipingche@163.com
[#]These authors contributed equally to this work.

1. Introduction

Several thousand Lepidopterous insects are distributed in China. Some of them are known to be beneficial insects (such as *Bombyx mori* Linnaeus, an economically important insect) and some are noted to be agricultural pests (such as *Mythimna separata* Walker, a typical lepidopteran pest, is widely distributed around the world; *Athetis dissimilis* Hampson, a widespread and harmful lepidopteran pest threatening China recently). It is very important in modern insect control strategy to protect beneficial insects and prevent agricultural pests [1–4]. On the other hand, the contamination of the food, emergence of pest drug-resistance, and synthesis of insecticides with negative impacts on nontarget organisms, all of these factors stimulate the research and development of environmentally safe pesticides in pest control [5]. Nowadays, new theories and strategies for agricultural pest control are required to be used in rotation with or replacement of conventional insecticides [1,6].

Plant secondary metabolites, as botanical pesticides, provide a potential resource to develop more environment friendly and less toxic means of insecticides. The compounds originated from the interaction between the plants and the environment (life and non-life) during the long period of plants evolution may delay the development of insecticide-resistance of the pest and decrease the effects of environmental pressures [6]. Therefore, screening botanical insecticides originated from plant, and (or) developing botanical insecticides directly from plant secondary metabolites, or by using them as lead compounds for further structural optimizations, have been a promising route for the discovery of new insecticides recently [7–11].

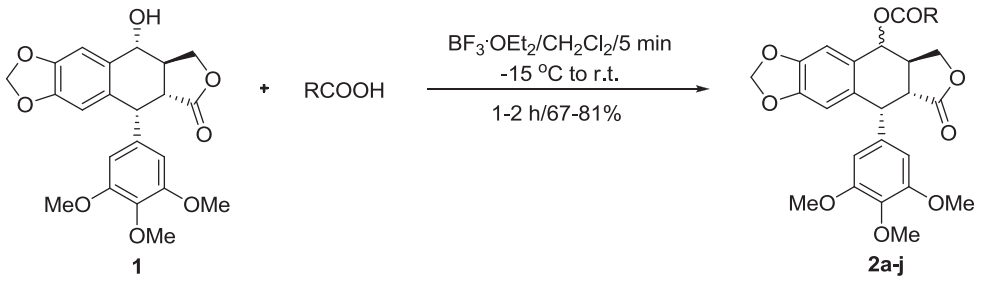
Podophyllotoxin (**1**), a naturally occurring cyclolignan, is isolated from the roots and rhizomes of *Podophyllum* species such as *Podophyllum hexandrum* and *Podophyllum peltatum*. To the best of our knowledge, compound **1** is an excellent candidate for the study of anticancer [12,13], antifungal [14,15], and insecticidal [16,17] activities. More recently, a large number of podophyllotoxin derivatives have been synthesized, and some derivatives displayed more promising insecticidal activity than toosendanin, a commercial botanical insecticide isolated from *Melia azedarach* [18–20]. But for all that, the insecticidal activity of podophyllotoxin and its analogs or derivatives has only been evaluated against very few species of pests, the structure-activity relationship (SAR) and mode of action have also not been well understand. On the other hand, compound **1** as well as its congeners and derivatives have shown antifeedant and toxic effects on several insect species, including various agricultural pests, but the action of these compounds on beneficial insects is rarely understood. Inspired by these previous observations, and the aim in our program is to discover and develop natural-product-based insecticidal agents [18,19], here we first report the results of the insecticidal activity of 4β-acyloxypodophyllotoxin derivatives against *B. mori*, *A. dissimilis* and *M. separate*, *in vivo*, respectively. Moreover, the SAR studies of these analogs were also described.

2. Results and discussion

2.1. Chemistry

As illustrated in Table 1, podophyllotoxin (**1**) reacted with different carboxylic acids in the presence of $BF_3{\cdot}OEt_2$, only **2 g** (R as CCl_3) and **2 h** (R as (*m*-NO_2)Ph) were stereoselectively prepared in 78% and 81% yields, respectively. It may be due to the electronic effect of R

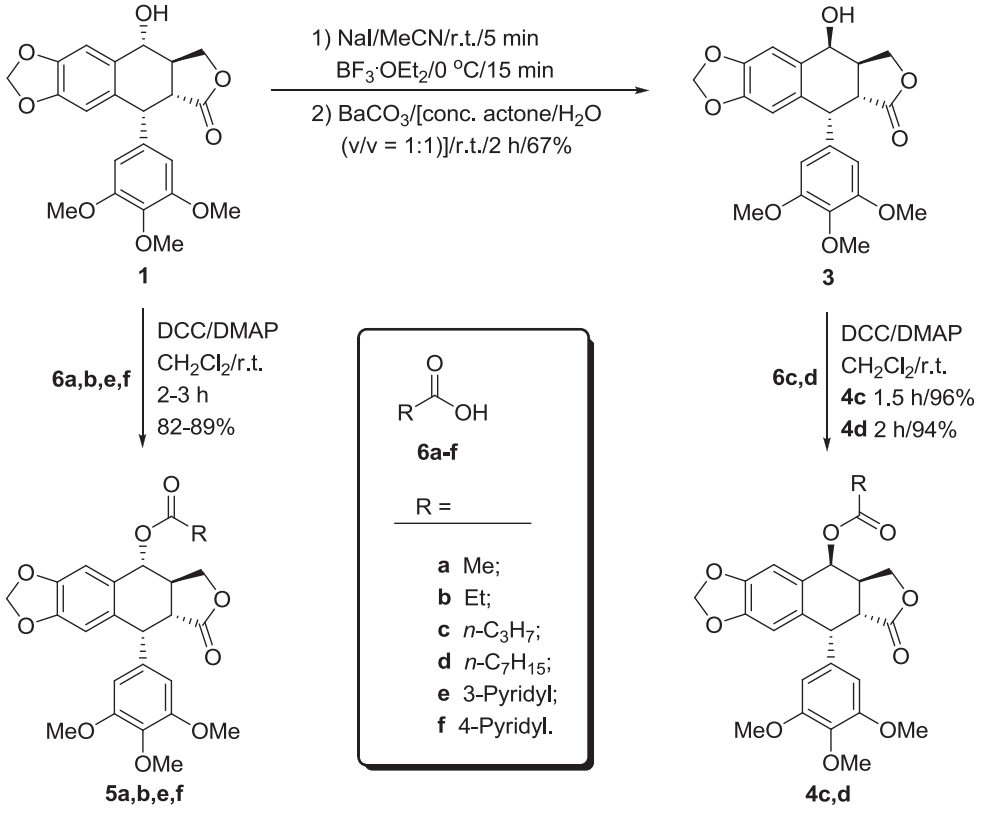
Table 1. Chart for the investigation of podophyllotoxin (**1**) reacting with carboxylic acids in the presence of $BF_3{\cdot}OEt_2$.



Compound	*R*	Isolated yield(%)	$\delta_{H\text{-}4}$ (ppm)	$J_{3,4}$ (Hz)	Configuration
2a	Me	73	5.89/6.15	9.0/3.0	α:β = 1.27:1
2b	Et	75	5.90/6.16	9.0/3.0	α:β = 1.27:1
2c	*n*-Propyl	79	5.90/6.16	9.0/3.0	α:β = 1.27:1
2d	*n*-Butyl	81	5.89/6.15	9.5/3.0	α:β = 1.27:1
2e	*n*-Heptyl	67	5.90/6.15	9.5/3.0	α:β = 1.27:1
2f	(*Z*)-9-*n*-$C_{17}H_{33}$	68	5.89/6.15	9.5/3.0	α:β = 1.27:1
2 g	CCl_3	78	6.23	3.0	β
2 h	(*m*-NO_2)Ph	81	6.45	3.5	β
2i	Ph	76	6.13/6.40	8.0/1.5	α:β = 1:1.17
2j	(*m*-Me)Ph	75	6.13/6.39	8.0/3.0	α:β = 1.17:1

that 4β-acyloxypodophyllotoxin derivatives (**2 g** and **2 h**, R as the electron withdrawing group) were stereoselectively obtained. Therefore, the relationship between the substituents introduced at the C-4 position of **2a-j** and their configuration of C-4 position is quite remarkable. The assignment of the configuration at the C-4 positions of **2a-j** was based on the Lee′s rule [21]. The configuration of the substituents introduced at the C-4 position of the above derivatives and their corresponding $J_{3,4}$ coupling constants were shown in Table 1. The C-4β-substituted derivatives have a $J_{3,4} \approx 4.0$ Hz due to a *cis* relationship between H-3 and H-4. If $J_{3,4} > 9.5$ Hz, it indicates that H-3 and H-4 is *trans* relationship, and the substituent at the C-4 position of **1** is in the α configuration. For example, the $J_{3,4}$ values of H-4 of **2 g** and **2 h** were 3.0 Hz and 3.5 Hz, respectively; therefore, the C-4 positions of **2 g** and **2 h** were all in the β configuration. In addition, there were two H-4 chemical shifts for **2a-f**, **2i**, and **2j**, and two scope corresponding $J_{3,4}$ values of H-4 were 8.0–9.5 Hz and 1.5–3.0 Hz, respectively; hence, 4-acyloxypodophyllotoxin derivatives **2a-f**, **2i**, and **2j** were a mixture of α and β isomers. Furthermore, the peak areas of two H-4 indicate that the ratio of α and β isomers of **2a-f** was all 1.27/1. Similarly, the ratios of α and β isomers of **2i** and **2j** were 1/1.17 and 1.17/1, respectively.

However, for stereoselective synthesis of a single configuration 4-acyloxypodophyllotoxin derivative, 4β-acyloxypodophyllotoxin derivatives (**4c, d**) and 4α-acyloxypodophyllotoxin derivatives (**5a, b, e, f**) modified in the C ring of C-4-hydroxyl group were synthesized as depicted in Scheme 1. Firstly, the essential intermediate of epipodophyllotoxin (**3**) was smoothly prepared by reaction of **1** with $BF_3{\cdot}OEt_2$/NaI followed by basic hydrolysis with barium carbonate. Then the epipodophyllotoxin (**3**) was treated with the corresponding carboxylic acids **6** in the presence of DCC and DMAP to afford 4β-acyloxypodophyllotoxin derivatives (**4c, d**) in 96% and 94% yields, respectively. Similarly, 4α-acyloxypodophyllotoxin derivatives (**5a, b, e, f**) were obtained by reaction of **1** with the corresponding carboxylic



Scheme 1. Route for the synthesis of 4-acyloxypodophyllotoxin derivatives **4c**, **d** and **5a**, **b**, **e**, **f**.

Table 2. Relationships of the configuration of C-4 position of compounds **3**, **4c, d** and **5a, b, e, f** with their corresponding $J_{3,4}$ coupling constants.

Compound	*R*	Isolated yield(%)	$\delta_{H\text{-}4}$ (ppm)	$J_{3,4}$ (Hz)	Configuration
3	/	72	4.86	3.0	β
4c	*n*-Propyl	95	6.15	3.0	β
4d	*n*-Heptyl	93	6.15	2.0	β
5a	Me	90	5.87	9.0	α
5b	Et	84	5.88	9.0	α
5e	3-Pyridyl	86	6.15	8.0	α
5f	4-Pyridyl	86	6.14	8.0	α

acids **6** in the presence of DCC and DMAP in 82–89% yields. The assignment of configuration of C-4 position of **3**, **4c, d** and **5a, b, e, f** was based on the above-mentioned Lee's rule [21]. Relationships of the configuration of C-4 position of compounds **3**, **4c, d** and **5a, b, e, f** with their corresponding $J_{3,4}$ coupling constants as shown in Table 2. For example, the $J_{3,4}$ values of H-4 of **3** and **4c, d** were 3.0, 2.4, and 1.6 Hz, respectively, so the C-4 position of **3** and **4c, d** were all in the β configuration. Additionally, the $J_{3,4}$ values of H-4 of **5a, b** and **5e, f** were 9.0, and 8.0 Hz, respectively; therefore, the 4-acyloxy groups at the C-4 position of 5**a, b** and **5e, f** were all in the α configuration.

2.2. Insecticidal activity

The insecticidal activity of 4α/β-acyloxypodophyllotoxin derivatives (**2a-j**, **4c**, **d** and **5a**, **b**, **e**, **f**) against the pre-third-instar larvae of *B. mori*, *A. dissimilis* and *M. separata in vivo* were screened by the leaf dipping method at the concentration of 1 mg/ml, respectively. Toosendanin, a commercial botanical insecticide extracted from *Melia azedarach*, was used as the positive control at a concentration of 1 mg/ml. Leaf discs dealt with acetone alone were used as a blank control group. Podophyllotoxin derivatives, in a time-dependent manner, unlike other conventional neurotoxic agents (e.g. organophosphates, carbamates, and pyrethroids), usually displayed delayed insecticidal activity, which coincided with our previous papers [18,19]. It is also shown that the insecticidal mechanisms of these samples are quite different from those of conventional neurotoxic agents, and the lethal symptoms of *B. mori*, *A. dissimilis* and *M. separata* during the different periods were also completely different. The symptoms of the tested *M. separata* were also characterized in the same way as in our previous reports [18,19]. The corresponding corrected mortality rates caused by these compounds were calculated and described in Table 3.

As illustrated in Table 3, some 4β-acyloxypodophyllotoxin derivatives exhibited better activity than toosendanin. Interestingly, among all 4α/β-acyloxypodophyllotoxin derivatives, compounds **2 g**, **h** and **4c**, **d** showed more promising insecticidal activities than their precursors podophyllotoxin and epipodophyllotoxin, and the final corrected mortality rates of **2 g**, **2 h**, **4c**, **4d**, **1** and **3** against *A. dissimilis* and *M. separata* were 52.5%/54.8%/49.9%/51.7%/28.6%/36.5%, and 61.5%/64.3%/55.6%/58.1%/29.3%/42.9%, respectively. On the other hand, **2 g**, **h** and **4c**, **d** exhibited more relative amicable activities than their precursor podophyllotoxin, epipodophyllotoxin, and toosendanin, and the final corrected mortality rates of **2 g**, **2 h**, **4c**, **4d**, **1**, **3** and toosendanin against *B. mori* were 43.3%/43.3%/43.3%/46.7%/60.0%/56.7% and 86.4%, respectively. In general,

Table 3. Insecticidal activity of 4α/β-acyloxypodophyllotoxin derivatives (**2a-j**, **4c**, **d** and **5a**, **b**, **e**, **f**) at 1 mg/ml against *B. mori*, *A. dissimilis* and *M. separate*, respectively.

	The final corrected mortality rate (%)		
Compound	*B. mori*	*A. dissimilis*	*M. separata*
1	60.0(±0)	28.6(±6.7)	29.3(±3.3)
2a	53.3(±3.3)	27.7(±3.3)	32.1(±3.3)
2b	53.3(±3.3)	27.5(±0)	32.1(±3.3)
2c	56.7(±3.3)	29.3(±3.3)	35.4(±5.8)
2d	50.0(±0)	30.7(±0)	33.6(±3.3)
2e	53.3(±3.3)	39.9(±6.7)	43.6(±3.3)
2f	50.0(±0)	34.9(±3.3)	38.4(±5.8)
2 g	43.3(±3.3)	52.5(±3.3)	61.5(±6.7)
2 h	43.3(±3.3)	54.8(±0)	64.3(±3.3)
2i	56.7(±3.3)	33.3(±3.3)	40.7(±3.3)
2j	53.3(±3.3)	38.4(±5.8)	45.6(±0)
3	56.7(±3.3)	36.5(±3.3)	42.9(±3.3)
4c	43.3(±3.3)	49.9(±0)	55.6(±0)
4d	46.7(±3.3)	51.7(±3.3)	58.1(±3.3)
5a	50.0(±0)	31.5(±6.7)	34.6(±3.3)
5b	58.3(±3.3)	30.8(±0)	34.6(±3.3)
5e	53.3(±3.3)	34.5(±3.3)	36.5(±3.3)
5f	50.0(±6.7)	33.6(±3.3)	35.8(±0)
Toosendanin	86.4(±5.8)	47.9(±3.3)	53.6(±3.3)

4β-acyloxypodophyllotoxin derivatives (**2 g**, **2 h**, **4c,** and **4d**) displayed better activity than the 4α-acyloxypodophyllotoxin derivatives (**5a**, **5b**, **5e,** and **5f**), and the final corrected mortality rates of **2 g**, **2 h**, **4c,** and **4d** against *B. mori*, *A. dissimilis* and *M. separate* were 43.3%/43.3%/43.3%/46.7%, 52.5%/54.8%/49.9%/51.7%, and 61.5%/64.3%/55.6%/58.1%, respectively; however, the final corrected mortality rates of **5a**, **5b**, **5e,** and **5f** against *B. mori*, *A. dissimilis,* and *M. separata* were 50.0%/58.3%/53.3%/50.0%, 31.5%/30.8%/34.5%/33.6%, and 34.6%/34.6%/36.5%/35.8%, respectively. This results indicated that 4β-acyloxy moiety in the podophyllotoxin derivatives were significant for obtaining the more potent compounds. However, for alkylacyloxypodophyllotoxin derivatives, the effect of the length of the side chain at the C-4 position on the activity was not very obvious.

3. Experimental

3.1. General experimental procedures

Melting points were taken on a X-6 microscopic melting point apparatus (Beijing Tech instrument Co., Ltd., Beijing, China) and are uncorrected. Optical rotations were determined on a SGW-2 automatic polarimeter (Ningbo Biocotek Scientific Equipment Co., Ltd., Ningbo, China) in chloroform solution. Proton nuclear magnetic resonance (^{1}H NMR) and carbon nuclear magnetic resonance (^{13}C NMR) spectra were carried out with a Bruker Avance 500 MHz instrument (Bruker Daltonik, Bremen, Germany) in $CDCl_3$ (^{1}H at 500 MHz, and ^{13}C at 125 MHz) using TMS (tetramethylsilane) as the internal standard. Electrospray ion-trap mass spectrometry (ESI-TRAP-MS) was recorded on a Bruker ESI-TRAP Esquire 3000 plus a mass spectrometry instrument. Reactions were monitored by analytical thin-layer chromatography (TLC) and purified by preparative thin-layer chromatography (PTLC), performed on silica gel glass plates containing 60 GF_{254} (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). Column chromatography was performed with 200–300 mesh silica gel (Qingdao Haiyang Chemical Co., Ltd., China). Podophyllotoxin was purchased from Gansu Gerui Medicinal Materials Co., Ltd. All chemicals and reagents were purchased and used without further purification. Solvents were of reagent grade used directly or purified according to standard methods before use.

3.2. General procedure for the synthesis of 4-acyloxypodophyllotoxin derivatives 2a-j

To a mixture of **1** (166 mg, 0.4 mmol) and the corresponding acids RCO_2H (0.48 mmol) in dry dichloromethane (CH_2Cl_2, 10 ml), a solution of $Et_2O{\cdot}BF_3$ (68 mg, 0.48 mmol) in dry CH_2Cl_2 (5 ml) was added dropwise to keep the temperature below −15 °C. After adding, the reaction temperature was raised from −15 °C to ambient temperature. When the reaction was completed for 1–2 h according to TLC analysis, water (H_2O, 15 ml) was added to the mixture and extracted with CH_2Cl_2 (3 × 30 ml). The combined organic phase was then washed by 5% aqueous sodium bicarbonate ($NaHCO_3$, 30 ml) and saturated sodium chloride (NaCl, 30 ml), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by PTLC to give the pure 4-acyloxypodophyllotoxin derivatives **2a-j** in 67–81% yields. The data for **2a-j** are shown as follows.

3.2.1. Data for 2a (a:β = 1.27:1)

Yield = 73%, white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.87 (s, 0.45H, H-5), 6.77 (s, 0.56H, H-5), 6.55 (s, 0.44H, H-8), 6.54 (s, 0.56H, H-8), 6.39 (s, 1.11H, 2′, 6′-H), 6.27 (s, 0.85H, 2′, 6′-H), 6.15 (d, J = 3.0 Hz, 0.44H, H-4), 6.00 (d, J = 4.5 Hz, 1.12H, OCH_2O), 5.98 (d, J = 3.0 Hz, 0.86H, OCH_2O), 5.89 (d, J = 9.0 Hz, 0.56H, H-4), 4.67 (d, J = 5.0 Hz, 0.45H, H-1), 4.61 (d, J = 4.0 Hz, 0.58H, H-1), 4.34–4.40 (m, 1H, H-11), 4.22 (t, J = 10.0 Hz, 0.57H, H-11), 3.93 (t, J = 10.0 Hz, 0.53H, H-11), 3.81 (s, 1.73H, 4′-OCH_3), 3.80 (s, 1.35H, 4′-OCH_3), 3.76 (s, 3.41H, 3′, 5′-OCH_3), 3.74 (s, 2.64H, 3′, 5′-OCH_3), 3.25 (dd, J = 14.0, 5.0 Hz, 0.43H, H-2), 2.97–3.02 (m, 0.41H, H-3), 2.94 (dd, J = 14.5, 4.5 Hz, 0.60H, H-2), 2.81–2.87 (m, 0.58H, H-3), 2.19 (s, 1.68H, CH_3), 2.12 (s, 1.25H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.1, 173.6, 171.4, 170.5, 152.7, 152.6, 148.9, 148.1, 147.6, 147.4, 137.1, 134.8, 134.5, 132.9, 132.3, 128.3, 127.8, 110.2, 109.7, 109.5, 108.1, 107.0, 101.7, 101.6, 73.6, 71.3, 68.1, 67.4, 60.7, 56.2, 56.1, 45.6, 43.8, 43.7, 41.5, 38.7, 36.7, 21.1, 21.0. MS (ESI-TRAP) m/z (%): 479 ([M+Na]$^+$, 100).

3.2.2. Data for 2b (a:β = 1.27:1)

Yield = 75%, white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.86 (s, 0.46H, H-5), 6.76 (s, 0.55H, H-5), 6.55 (s, 0.44H, H-8), 6.54 (s, 0.56H, H-8), 6.39 (s, 1.11H, 2′, 6′-H), 6.28 (s, 0.90H, 2′, 6′-H), 6.16 (d, J = 3.0 Hz, 0.44H, H-4), 6.00 (d, J = 4.0 Hz, 1.15H, OCH_2O), 5.98 (s, 0.89H, OCH_2O), 5.90 (d, J = 9.0 Hz, 0.56H, H-4), 4.67 (d, J = 5.0 Hz, 0.46H, H-1), 4.61 (d, J = 4.0 Hz, 0.56H, H-1), 4.34–4.39 (m, 1H, H-11), 4.23 (t, J = 10.0 Hz, 0.57H, H-11), 3.91 (t, J = 10.0 Hz, 0.50H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.76 (s, 3.45H, 3′, 5′-OCH_3), 3.74 (s, 2.69H, 3′, 5′-OCH_3), 3.25 (dd, J = 14.0, 5.0 Hz, 0.46H, H-2), 2.98–3.03 (m, 0.40H, H-3), 2.95 (dd, J = 14.5, 4.5 Hz, 0.62H, H-2), 2.82–2.88 (m, 0.58H, H-3), 2.44–2.49 (m, 1.13H, $C\underline{H}_2CH_3$), 2.36–2.42 (m, 0.88H, $C\underline{H}_2CH_3$), 1.16–1.22 (m, 3H, $CH_2C\underline{H}_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.8, 174.2, 174.0, 173.6, 152.7, 152.6, 148.8, 148.1, 147.6, 147.4, 137.3, 137.1, 134.8, 134.5, 132.8, 132.3, 128.4, 127.9, 110.2, 109.7, 109.5, 108.1, 108.0, 107.0, 101.7, 101.6, 73.4, 71.4, 67.8, 67.4, 60.7, 56.2, 56.1, 45.6, 43.8, 43.7, 41.5, 38.7, 36.8, 27.7, 27.6, 9.1. MS (ESI-TRAP) m/z (%): 493 ([M+Na]$^+$, 100).

3.2.3. Data for 2c (a:β = 1.27:1)

Yield = 79%, white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.85 (s, 0.44H, H-5), 6.75 (s, 0.56H, H-5), 6.55 (s, 0.44H, H-8), 6.54 (s, 0.56H, H-8), 6.39 (s, 1.15H, 2′, 6′-H), 6.28 (s, 0.91H, 2′, 6′-H), 6.16 (d, J = 3.0 Hz, 0.46H, H-4), 5.99 (d, J = 2.0 Hz, 1.13H, OCH_2O), 5.98 (d, J = 1.0 Hz, 0.87H, OCH_2O), 5.90 (d, J = 9.0 Hz, 0.59H, H-4), 4.67 (d, J = 5.0 Hz, 0.45H, H-1), 4.61 (d, J = 4.0 Hz, 0.58H, H-1), 4.34–4.38 (m, 1H, H-11), 4.23 (t, J = 10.0 Hz, 0.57H, H-11), 3.91 (t, J = 10.0 Hz, 0.42H, H-11), 3.81 (s, 1.75H, 4′-OCH_3), 3.80 (s, 1.37H, 4′-OCH_3), 3.76 (s, 3.47H, 3′, 5′-OCH_3), 3.74 (s, 2.71H, 3′, 5′-OCH_3), 3.24 (dd, J = 14.0, 5.0 Hz, 0.45H, H-2), 2.97–3.00 (m, 0.46H, H-3), 2.95 (dd, J = 14.5, 4.5 Hz, 0.57H, H-2), 2.80–2.84 (m, 0.58H, H-3), 2.39–2.43 (m, 1.10H, $C\underline{H}_2CH_2CH_3$), 2.36 (t, J = 7.5 Hz, 0.96H, $C\underline{H}_2CH_2CH_3$), 1.67–1.72 (m, 2H, $CH_2C\underline{H}_2CH_3$), 1.01 (t, J = 7.5 Hz, 1.71H, $CH_2CH_2C\underline{H}_3$), 0.97 (t, J = 7.5 Hz, 1.33H, $CH_2CH_2C\underline{H}_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.2, 174.0, 173.6, 173.2, 152.7, 152.6, 148.8, 148.1, 147.6, 147.4, 137.3, 137.1, 134.8, 134.5, 132.8, 132.3, 128.4, 127.9, 110.2, 109.7, 109.5, 108.1, 108.0, 107.0, 101.7, 101.6, 73.3, 71.4, 67.8, 67.4, 60.7, 56.2, 56.1, 45.5, 43.8, 43.7, 41.5, 38.8, 36.7, 36.3, 36.2, 18.5, 13.7. MS (ESI-TRAP) m/z (%): 507 ([M+Na]$^+$, 100).

3.2.4. Data for 2d (α:β = 1.27:1)

Yield = 81%, white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.85 (s, 0.47H, H-5), 6.75 (s, 0.56H, H-5), 6.55 (s, 0.44H, H-8), 6.54 (s, 0.56H, H-8), 6.39 (s, 1.09H, 2′, 6′-H), 6.28 (s, 0.87H, 2′, 6′-H), 6.15 (d, J = 3.0 Hz, 0.44H, H-4), 5.99 (d, J = 1.0 Hz, 1.12H, OCH_2O), 5.97 (s, 0.86H, OCH_2O), 5.89 (d, J = 9.5 Hz, 0.57H, H-4), 4.66 (d, J = 5.0 Hz, 0.46H, H-1), 4.61 (d, J = 4.0 Hz, 0.57H, H-1), 4.33–4.38 (m, 1H, H-11), 4.23 (t, J = 10.0 Hz, 0.57H, H-11), 3.90 (t, J = 10.0 Hz, 0.51H, H-11), 3.81 (s, 1.70H, 4′-OCH_3), 3.80 (s, 1.32H, 4′-OCH_3), 3.76 (s, 3.42H, 3′, 5′-OCH_3), 3.74 (s, 2.66H, 3′, 5′-OCH_3), 3.24 (dd, J = 14.0, 5.0 Hz, 0.45H, H-2), 2.97–2.99 (m, 0.40H, H-3), 2.94 (dd, J = 14.5, 4.5 Hz, 0.62H, H-2), 2.81–2.84 (m, 0.58H, H-3), 2.41–2.44 (m, 1.06H, $C\underline{H}_2CH_2CH_2CH_3$), 2.37 (t, J = 7.5 Hz, 0.92H, $C\underline{H}_2CH_2CH_2CH_3$), 1.61–1.68 (m, 2H, $CH_2C\underline{H}_2CH_2CH_3$), 1.34–1.40 (m, 2H, $CH_2CH_2C\underline{H}_2CH_3$), 0.90–0.96 (m, 3H, $CH_2CH_2CH_2C\underline{H}_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.3, 174.2, 173.6, 173.4, 152.7, 152.6, 148.8, 148.1, 147.6, 147.4, 137.3, 137.1, 134.8, 134.5, 132.8, 132.3, 128.4, 127.9, 110.2, 109.7, 109.5, 108.1, 107.0, 101.7, 101.6, 73.3, 71.4, 67.8, 67.4, 60.7, 56.2, 56.1, 45.6, 43.8, 43.7, 41.5, 38.8, 36.7, 34.1, 27.1, 27.0, 22.2, 13.6. MS (ESI-TRAP) m/z (%): 521 ([M+Na]$^+$, 100).

3.2.5. Data for 2e (α:β = 1.27:1)

Yield = 67%, white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.85 (s, 0.47H, H-5), 6.75 (s, 0.55H, H-5), 6.55 (s, 0.44H, H-8), 6.54 (s, 0.56H, H-8), 6.39 (s, 1.14H, 2′, 6′-H), 6.28 (s, 0.95H, 2′, 6′-H), 6.15 (d, J = 3.0 Hz, 0.49H, H-4), 5.99 (d, J = 2.5 Hz, 1.14H, OCH_2O), 5.98 (d, J = 3.5 Hz, 0.88H, OCH_2O), 5.90 (d, J = 9.5 Hz, 0.55H, H-4), 4.66 (d, J = 5.0 Hz, 0.49H, H-1), 4.60 (d, J = 4.0 Hz, 0.55H, H-1), 4.33–4.38 (m, 1H, H-11), 4.22 (t, J = 10.0 Hz, 0.56H, H-11), 3.91 (t, J = 10.0 Hz, 0.53H, H-11), 3.81 (s, 1.75H, 4′-OCH_3), 3.80 (s, 1.35H, 4′-OCH_3), 3.76 (s, 3.43H, 3′, 5′-OCH_3), 3.74 (s, 2.67H, 3′, 5′-OCH_3), 3.24 (dd, J = 14.0, 5.0 Hz, 0.48H, H-2), 2.97–2.99 (m, 0.42H, H-3), 2.94 (dd, J = 14.5, 4.0 Hz, 0.62H, H-2), 2.79–2.86 (m, 0.59H, H-3), 2.40–2.44 (m, 1.07H, $C\underline{H}_2(CH_2)_5CH_3$), 2.36 (t, J = 7.5 Hz, 1.01H, $C\underline{H}_2(CH_2)_5CH_3$), 1.62–1.69 (m, 2H, $CH_2C\underline{H}_2(CH_2)_4CH_3$), 1.29–1.34 (m, 8H, $CH_2CH_2(C\underline{H}_2)_4CH_3$), 0.87–0.88 (m, 3H, $CH_2(CH_2)_5C\underline{H}_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.3, 174.2, 173.6, 173.4, 152.7, 152.6, 148.8, 148.1, 147.6, 147.4, 137.2, 134.8, 134.5, 132.8, 132.3, 128.4, 127.9, 110.2, 109.7, 109.5, 108.2, 108.1, 107.0, 101.6, 101.5, 73.3, 71.4, 67.8, 67.4, 60.7, 56.2, 56.1, 45.6, 43.8, 43.7, 41.5, 38.8, 36.7, 34.4, 34.3, 31.6, 29.1, 29.0, 28.9, 28.8, 25.1, 25.0, 22.6, 22.5, 14.0. MS (ESI-TRAP) m/z (%): 563 ([M+Na]$^+$, 100).

3.2.6. Data for 2f (α:β = 1.27:1)

Yield = 68%, colorless liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.85 (s, 0.46H, H-5), 6.75 (s, 0.55H, H-5), 6.55 (s, 0.44H, H-8), 6.54 (s, 0.56H, H-8), 6.39 (s, 1.13H, 2′, 6′-H), 6.28 (s, 0.92H, 2′, 6′-H), 6.15 (d, J = 3.0 Hz, 0.47H, H-4), 5.99 (s, 1.14H, OCH_2O), 5.97 (s, 0.88H, OCH_2O), 5.89 (d, J = 9.5 Hz, 0.58H, H-4), 5.34–5.37 (m, 2H, $(CH_2)_7C\underline{H}$ = $C\underline{H}(CH_2)_7CH_3$), 4.66 (d, J = 5.0 Hz, 0.46H, H-1), 4.60 (d, J = 4.0 Hz, 0.57H, H-1), 4.30–4.37 (m, 1H, H-11), 4.22 (t, J = 10.0 Hz, 0.57H, H-11), 3.90 (t, J = 10.0 Hz, 0.50H, H-11), 3.81 (s, 1.71H, 4′-OCH_3), 3.80 (s, 1.30H, 4′-OCH_3), 3.76 (s, 3.42H, 3′, 5′-OCH_3), 3.74 (s, 2.66H, 3′, 5′-OCH_3), 3.24 (dd, J = 14.5, 5.0 Hz, 0.46H, H-2), 2.97–2.99 (m, 0.42H, H-3), 2.94 (dd, J = 14.5, 4.5 Hz, 0.60H, H-2), 2.75–2.78 (m, 0.65H,H-3), 2.40–2.43 (m, 1.03H, $C\underline{H}_2(CH_2)_6CH$ = $CH(CH_2)_7CH_3$), 2.36 (t, J = 7.5 Hz, 0.96H, $C\underline{H}_2(CH_2)_6CH$ = $CH(CH_2)_7CH_3$), 2.00–2.05 (m, 3.16H, $CH_2C\underline{H}_2(CH_2)_4C\underline{H}_2CH$ = $CHC\underline{H}_2(CH_2)_6CH_3$), 1.60–1.67 (m, 3.09H, $CH_2C\underline{H}_2(CH_2)_4C\underline{H}_2CH$ = $CHC\underline{H}_2(CH_2)_6CH_3$), 1.25–1.32 (m, 20H, $CH_2CH_2(C\underline{H}_2)_4CH_2CH$ = $CHCH_2(C\underline{H}_2)_6CH_3$),

0.86–0.88 (m, 3H, $(CH_2)_7CH$ = $CH(CH_2)_7CH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.2, 174.1, 173.6, 173.4, 152.7, 152.6, 148.8, 148.1, 147.6, 147.4, 137.3, 137.2, 134.8, 134.5, 132.8, 132.3, 130.2, 129.9, 128.8, 128.4, 128.1, 127.9, 127.8, 110.2, 109.7, 109.5, 108.1, 106.9, 101.6, 101.5, 73.4, 71.4, 67.8, 67.4, 60.7, 56.2, 56.1, 45.6, 43.8, 43.7, 41.5, 38.7, 36.7, 34.3, 31.9, 31.5, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 27.2, 27.1, 25.6, 25.0, 22.6, 22.5, 14.1, 14.0. MS (ESI-TRAP) *m/z* (%): 701 ($[M+Na]^+$, 73).

3.2.7. Data for 2 g

Yield = 78%, white solid, m.p. 105–106 °C. ^1H NMR (500 MHz, $CDCl_3$) δ: 6.92 (s, 1H, H-5), 6.59 (s, 1H, H-8), 6.27 (s, 2H, 2′, 6′-H), 6.23 (d, J = 3.0 Hz, 1H, H-4), 6.03 (d, J = 9.5 Hz, 2H, OCH_2O), 4.71 (d, J = 5.0 Hz, 1H, H-1), 4.44 (t, J = 8.0 Hz, 1H, H-11), 4.10 (t, J = 10.0 Hz, 1H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′, 5′-OCH_3), 3.31 (dd, J = 14.0, 5.0 Hz, 1H, H-2), 3.10–3.12 (m, 1H, H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.5, 161.6, 152.8, 149.7, 147.7, 137.5, 134.1, 133.5, 125.5, 110.5, 109.4, 108.2, 108.1, 101.9, 74.1, 66.7, 60.7, 56.3, 43.7, 41.3, 36.8. MS (ESI-TRAP) *m/z* (%): 581 ($[M+Na]^+$, 100), 583 ($[M+Na]^+$, 73), 585 ($[M+Na]^+$, 22).

3.2.8. Data for 2 h

Yield = 81%, pale yellow solid. ^1H NMR (500 MHz, $CDCl_3$) δ: 8.82 (s, 1H), 8.47 (d, J = 9.0 Hz, 1H), 8.36 (d, J = 9.0 Hz, 1H), 7.70 (t, J = 9.0 Hz, 1H), 6.91 (s, 1H, H-5), 6.61 (s, 1H, H-8), 6.45 (d, J = 3.5 Hz, 1H, H-4), 6.32 (s, 2H, 2′, 6′-H), 6.01 (d, J = 17.5 Hz, 2H, OCH_2O), 4.76 (d, J = 5.0 Hz, 1H, H-1), 4.44 (t, J = 8.0 Hz, 1H, H-11), 3.94 (t, J = 10.0 Hz, 1H, H-11), 3.82 (s, 3H, 4′-OCH_3), 3.77 (s, 6H, 3′, 5′-OCH_3), 3.37 (dd, J = 14.5, 5.0 Hz, 1H, H-2), 3.12–3.15 (m, 1H, H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.8, 164.2, 152.7, 149.2, 148.4, 147.6, 137.4, 135.4, 134.3, 133.3, 130.9, 129.9, 128.1, 126.9, 124.7, 110.4, 109.5, 108.1, 101.8, 70.0, 67.4, 60.7, 56.3, 43.8, 41.7, 36.9. MS (ESI-TRAP) *m/z* (%): 586 ($[M+Na]^+$, 100).

3.2.9. Data for 2i (α:β = 1:1.17)

Yield = 76%, white solid. ^1H NMR (500 MHz, $CDCl_3$) δ: 8.06 (d, J = 7.5 Hz, 1H), 8.02 (d, J = 7.5 Hz, 1H), 7.58–7.64 (m, 1H), 7.44–7.50 (m, 2H), 6.95 (s, 0.54H, H-5), 6.88 (s, 0.46H, H-5), 6.59 (s, 1H, H-8), 6.45 (s, 0.91H, 2′, 6′-H), 6.40 (d, J = 1.5 Hz, 0.58H, H-4), 6.32 (s, 1.09H, 2′, 6′-H), 6.13 (d, J = 8.0 Hz, 0.48H, H-4), 6.00 (s, 0.93H, OCH_2O), 5.98 (d, J = 11.5 Hz, 1.05H, OCH_2O), 4.73 (d, J = 4.5 Hz, 0.54H, H-1), 4.66 (d, J = 1.5 Hz, 0.49H, H-1), 4.44–4.47 (m, 0.47H, H-11), 4.42 (t, J = 8.5 Hz, 0.55H, H-11), 4.35 (t, J = 9.5 Hz, 0.51H, H-11), 3.99 (t, J = 10.0 Hz, 0.59H, H-11), 3.82 (s, 1.69H, 4′-OCH_3), 3.80 (s, 1.28H, 4′-OCH_3), 3.78 (s, 3.36H, 3′, 5′-OCH_3), 3.76 (s, 2.60H, 3′, 5′-OCH_3), 3.38 (dd, J = 14.0, 4.5 Hz, 0.57H, H-2), 3.08–3.10 (m, 0.57H, H-3), 2.98–3.00 (m, 0.94H, H-2, 3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.1, 173.6, 166.8, 166.1, 152.7, 152.6, 148.9, 148.2, 147.7, 147.5, 137.4, 137.2, 134.8, 134.5, 133.7, 133.0, 132.5, 129.8, 129.7, 129.2, 129.1, 128.7, 128.6, 128.4, 127.8, 110.2, 109.8, 108.2, 108.1, 107.1, 101.7, 101.6, 74.2, 71.5, 68.6, 67.6, 60.7, 56.3, 56.1, 45.7, 43.9, 43.8, 41.8, 38.9, 37.0. MS (ESI-TRAP) *m/z* (%): 541 ($[M+Na]^+$, 100).

3.2.10. Data for 2j (α:β = 1.17:1)

Yield = 75%, white solid. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.85 (s, 1H), 7.81 (s, 1H), 7.39–7.44 (m, 1H), 7.33–7.37 (m, 1H), 6.94 (s, 0.45H, H-5), 6.87 (s, 0.53H, H-5), 6.58 (s, 1H, H-8), 6.45 (s, 1.10H, 2′, 6′-H), 6.39 (d, J = 3.0 Hz, 0.50H, H-4), 6.32 (s, 0.94H, 2′, 6′-H), 6.13 (d, J = 8.0 Hz, 0.55H, H-4), 5.96–6.00 (m, 2H, OCH_2O), 4.73 (d, J = 4.5 Hz, 0.48H, H-1), 4.65

(d, J = 1.5 Hz, 0.56H, H-1), 4.43–4.46 (m, 0.48H, H-11), 4.41 (t, J = 8.5 Hz, 0.56H, H-11), 4.33 (t, J = 9.5 Hz, 0.58H, H-11), 3.98 (t, J = 10.0 Hz, 0.50H, H-11), 3.81 (s, 1.81H, 4′-OCH_3), 3.80 (s, 1.40H, 4′-OCH_3), 3.78 (s, 3.18H, 3′, 5′-OCH_3), 3.76 (s, 2.83H, 3′, 5′-OCH_3), 3.38 (dd, J = 14.0, 5.0 Hz, 0.47H, H-2), 3.08–3.10 (m, 0.48H, H-3), 2.97–3.00 (m, 1.12H, H-2, 3), 2.41 (s, 1.64H), 2.40 (s, 1.39H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.2, 173.6, 167.0, 166.3, 152.7, 152.6, 148.9, 148.2, 147.7, 147.5, 138.5, 137.2, 134.8, 134.6, 134.5, 133.0, 132.5, 130.3, 130.2, 129.2, 128.6, 128.5, 128.4, 127.8, 127.0, 126.8, 110.2, 109.7, 108.2, 108.1, 107.2, 101.7, 101.6, 74.0, 71.5, 68.5, 67.6, 60.7, 56.2, 56.1, 45.7, 43.9, 43.8, 41.8, 38.9, 37.0, 21.3, 21.2. MS (ESI-TRAP) m/z (%): 555 ([M+Na]$^+$, 100).

3.3. Synthesis of epipodophyllotoxin 3

To a solution of **1** (829 mg, 2 mmol) in dry acetonitrile (CH_3CN, 20 ml) at ambient temperature, NaI·$2H_2O$ (744 mg, 4 mmol) was added, and the mixture was then stirred for 5 min. To the above mixture at 0 °C, $Et_2O{\cdot}BF_3$ (852 mg, 6 mmol) was then added dropwise, and the mixture was stirred at ambient temperature for 15 min. Subsequently, the solvent was removed and the residue was diluted by H_2O/acetone (30 ml, 1:1, v/v). Anhydrous barium carbonate ($BaCO_3$, 789 mg, 4 mmol) was added to the mixture, which was stirred for 2 h at ambient temperature. When the reaction was completed according to TLC analysis, the solvent was removed. H_2O (10 ml) was then added to the residue and extracted with CH_2Cl_2 (3 × 30 ml). The combined organic phase was then washed by saturated NaCl (30 ml), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by silica gel column chromatography to afford **3** in a 72% yield as a white solid. The data for **3** are shown as follows.

3.3.1. Data for 3

m.p. 162–164 °C (Not reported) [22]; $[\alpha]_D^{20}$ −32 (c 3.6 mg/ml, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.88 (s, 1H, H-5), 6.55 (s, 1H, H-8), 6.28 (s, 2H, H-2′, 6′), 5.97 (d, J = 12.5 Hz, 2H, OCH_2O), 4.86 (d, J = 3.0 Hz, 1H, H-4), 4.60 (d, J = 5.0 Hz, 1H, H-1), 4.35–4.39 (m, 2H, H-11), 3.80 (s, 3H, 4′-OCH_3), 3.74 (s, 6H, 3′, 5′-OCH_3), 3.25 (dd, J = 14.0, 5.0 Hz, 1H, H-2), 2.82–2.85 (m, 1H, H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 175.0, 152.6, 148.6, 147.5, 137.3, 135.1, 132.0, 131.9, 110.5, 109.0, 108.3, 101.6, 67.6, 66.8, 60.8, 56.3, 43.9, 40.5, 38.3. MS (ESI-TRAP) m/z (%): 432 ([M+NH_4]$^+$, 100).

3.4. General procedure for synthesis of 4β-acyloxypodophyllotoxin derivatives 4c and 4d

A mixture of **3** (166 mg, 0.4 mmol), the corresponding acids RCO_2H (0.48 mmol), 4-dimethylaminopyridine (DMAP, 10 mg, 0.08 mmol), and *N*,*N*′-dicyclohexylcarbodiimide (DCC, 99 mg, 0.48 mmol) in dry CH_2Cl_2 (10 ml) was stirred at ambient temperature. When the reaction was completed for 2–3 h according to TLC analysis, the mixture was filtered, and the filtrate was diluted by CH_2Cl_2 (60 ml). The mixture was washed by 0.1 N HCl (30 ml), saturated sodium carbonate (Na_2CO_3, 30 ml) and saturated NaCl (30 ml), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by PTLC to obtain the pure 4β-acyloxypodophyllotoxin derivative **4c** in a 95% yield as a white solid and **4d** in a 93% yield as a white solid. The data for **4c** and **4d** are shown as follows.

3.4.1. Data for 4c

m.p. 153–154 °C (Reported 154–156 °C) [23]; $[\alpha]_D^{20}$ −98 (*c* 5.0 mg/ml, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) *δ*: 6.86 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.28 (s, 2H, H-2′, 6′), 6.15 (d, *J* = 3.0 Hz, 1H, H-4), 5.98 (d, *J* = 12.5 Hz, 2H, OCH_2O), 4.66 (d, *J* = 5.0 Hz, 1H, H-1), 4.34 (t, *J* = 10.0 Hz, 1H, H-11), 3.87–3.92 (m, 1H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′, 5′-OCH_3), 3.21 (dd, *J* = 14.0, 5.0 Hz, 1H, H-2), 2.97–3.03 (m, 1H, H-3), 2.33 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_2CH_3$), 1.65–1.71 (m, 2H, $CH_2C\underline{H}_2CH_3$), 0.94 (t, *J* = 7.5 Hz, 3H, $CH_2CH_2C\underline{H}_3$). MS (ESI-TRAP) *m/z* (%): 502 ($[M+NH_4]^+$, 100).

3.4.2. Data for 4d

m.p. 95–96 °C; $[\alpha]_D^{20}$ −57 (*c* 5.0 mg/ml, $CHCl_3$), ^1H NMR (500 MHz, $CDCl_3$) *δ*: 6.86 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.28 (s, 2H, H-2′, 6′), 6.15 (d, *J* = 2.0 Hz, 1H, H-4), 5.98 (d, *J* = 12.5 Hz, 2H, OCH_2O), 4.66 (d, *J* = 5.0 Hz, 1H, H-1), 4.34 (t, *J* = 10.0 Hz, 1H, H-11), 3.87 (t, *J* = 10.0 Hz, 1H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′, 5′-OCH_3), 3.21 (dd, *J* = 14.5, 5.0 Hz, 1H, H-2), 2.97–3.03 (m, 1H, H-3), 2.34 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2(CH_2)_5CH_3$), 1.62–1.66 (m, 2H, $CH_2C\underline{H}_2(CH_2)_4CH_3$), 1.27–1.29 (m, 8H, $CH_2CH_2(C\underline{H}_2)_4CH_3$), 0.86–0.89 (m, 3H, $CH_2(CH_2)_5C\underline{H}_3$). MS (ESI-TRAP) *m/z* (%): 558 ($[M+NH_4]^+$, 100).

3.5. General procedure for synthesis of 4α-acyloxypodophyllotoxin derivatives 5a, b, e, f

A mixture of **1** (166 mg, 0.4 mmol), the corresponding acids RCO_2H (0.48 mmol), DMAP (10 mg, 0.08 mmol), and DCC (99 mg, 0.48 mmol) in dry CH_2Cl_2 (10 ml) was stirred at ambient temperature. When the reaction was completed for 2–3 h according to TLC analysis, the mixture was filtered, and the filtrate was diluted by CH_2Cl_2 (60 ml). The mixture was washed by 0.1 N HCl (30 ml), saturated Na_2CO_3 (30 ml) and saturated NaCl (30 ml), dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and purified by PTLC to give the pure 4α-acyloxypodophyllotoxin derivatives **5a, b, e, f** in 84–90% yields. The data for **5a, b, e, f** are shown as follows.

3.5.1. Data for 5a

Yield = 90%, white solid; m.p. 210–212 °C (Reported 210–211 °C) [24]; $[\alpha]_D^{20}$ −149 (*c* 4.4 mg/ml, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) *δ*: 6.77 (s, 1H, H-5), 6.54 (s, 1H, H-8), 6.39 (s, 2H, H-2′, 6′), 5.98 (d, *J* = 5.5 Hz, 2H, OCH_2O), 5.87 (d, *J* = 9.0 Hz, 1H, H-4), 4.60 (d, *J* = 4.0 Hz, 1H, H-1), 4.37–4.40 (m, 1H, H-11), 4.18–4.22 (m, 1H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.76 (s, 6H, 3′, 5′-OCH_3), 2.90 (dd, *J* = 14.5, 4.5 Hz, 1H, H-2), 2.81–2.87 (m, 1H, H-3), 2.19 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) *δ*: 173.6, 171.4, 152.7, 148.2, 147.6, 137.3, 134.8, 132.4, 128.3, 109.7, 108.2, 107.0, 101.6, 73.7, 71.4, 60.8, 56.2, 45.6, 43.8, 38.7, 21.1. MS (ESI-TRAP) *m/z* (%): 479 ($[M+Na]^+$, 100).

3.5.2. Data for 5b

Yield = 84%, white solid; m.p. 137–138 °C (Reported 136–138 °C) [24]; $[\alpha]_D^{20}$ −138 (*c* 3.6 mg/ml, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) *δ*: 6.76 (s, 1H, H-5), 6.54 (s, 1H, H-8), 6.40 (s, 2H, H-2′, 6′), 5.98 (d, *J* = 5.5 Hz, 2H, OCH_2O), 5.88 (d, *J* = 9.0 Hz, 1H, H-4), 4.60 (d, *J* = 4.0 Hz, 1H, H-1), 4.36–4.39 (m, 1H, H-11), 4.19 (t, *J* = 10.0 Hz, 1H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.76 (s, 6H, 3′, 5′-OCH_3), 2.91 (dd, *J* = 14.5, 4.0 Hz, 1H, H-2), 2.82–2.85 (m, 1H, H-3),

2.44–2.49 (m, 2H, $C\underline{H}_2CH_3$), 1.19 (t, J = 7.5 Hz, 3H, $CH_2C\underline{H}_3$). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.8, 173.7, 152.7, 148.1, 147.6, 137.2, 134.8, 132.4, 128.5, 109.7, 108.2, 107.0, 101.6, 73.5, 71.4, 60.7, 56.2, 45.6, 43.8, 38.8, 27.7, 9.2. MS (ESI-TRAP) *m/z* (%): 488 ([M+NH_4] $^+$, 100).

3.5.3. Data for 5e

Yield = 86%, white solid; m.p. 176–178 °C (Reported 177–178 °C) [25]; $[\alpha]_D^{20}$ −24 (*c* 3.5 mg/ml, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.25 (s, 1H), 8.85 (s, 1H), 8.32 (d, J = 8.0 Hz, 1H), 7.45–7.48 (m, 1H), 6.85 (s, 1H, H-5), 6.60 (s, 1H, H-8), 6.44 (s, 2H, H-2′, 6′), 6.15 (d, J = 8.0 Hz, 1H, H-4), 6.00 (d, J = 9.5 Hz, 2H, OCH_2O), 4.67 (s, 1H, H-1), 4.45–4.47 (m, 1H, H-11), 4.31–4.35 (m, 1H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.78 (s, 6H, 3′, 5′-OCH_3), 2.99–3.05 (m, 2H, H-2, 3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.4, 164.7, 152.7, 151.8, 148.8, 148.5, 147.9, 139.2, 137.3, 134.6, 132.7, 127.5, 126.3, 124.5, 110.0, 108.1, 106.9, 101.8, 75.5, 71.3, 60.7, 56.2, 45.6, 43.8, 38.8, 23.2. MS (ESI-TRAP) *m/z* (%): 542 ([M+Na]$^+$, 92).

3.5.4. Data for 5f

Yield = 86%, white solid; m.p. 172–173 °C (Reported 172–174 °C) [25]; $[\alpha]_D^{20}$ −46 (*c* 3.9 mg/ml, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.83 (d, J = 4.0 Hz, 2H), 7.85 (d, J = 4.5 Hz, 2H), 6.83 (s, 1H, H-5), 6.60 (s, 1H, H-8), 6.44 (s, 2H, H-2′, 6′), 6.14 (d, J = 8.0 Hz, 1H, H-4), 6.00 (d, J = 9.0 Hz, 2H, OCH_2O), 4.67 (s, 1H, H-1), 4.43–4.46 (m, 1H, H-11), 4.31–4.34 (m, 1H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.78 (s, 6H, 3′, 5′-OCH_3), 2.99–3.01 (m, 2H, H-2, 3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.3, 164.8, 152.7, 149.0, 148.6, 147.9, 138.3, 137.4, 134.6, 132.8, 127.4, 123.7, 110.0, 108.3, 106.8, 101.8, 75.8, 71.2, 60.8, 56.2, 45.6, 43.7, 38.7, 23.3. MS (ESI-TRAP) *m/z* (%): 542 ([M+Na]$^+$, 100).

3.6. Biological assay

The insecticidal activity of 4α/β-acyloxypodophyllotoxin derivatives **2a-j**, **4c**, **d** and **5a**, **b**, **e**, **f** were assessed as the mortality rates by the leaf-dipping method against the pre-third-instar larvae of *B. mori*, *A. dissimilis,* and *M. separate in vivo* at the concentration of 1 mg ml^{-1}, respectively [18,19]. The tested compounds **1**, **2a-j**, **3**, **4c**, **d**, **5a**, **b**, **e**, **f** and toosendanin (a botanical pesticide, used as a positive control) were dissolved in acetone and were prepared at 1 mg ml^{-1}. For each tested compound, 30 pre-third-instar larvae (10 larvae per dish) were assayed in each of these insects. Fresh mulberry (for *B. mori*) or maize (for *A. dissimilis* and *M. separata*) leaf discs (1 × 1 cm) were dipped into the corresponding compounds solution for 3–5 s and then taken out to dry in an ambient. Leaf discs dealt with acetone alone were used as control. Treated leaf discs were added in each dish (10 larvae in each dish), and the dishes were placed at 28 ± 1 °C with relative humidity (RH) of 65–80%, and on 12 h/12 h (light/dark) photoperiod. Additional treated leaf discs were added to each dish if they were consumed. 48 h later, all dishes were added with untreated fresh leaves to feed the larvae until the adult emergence. The corrected mortality rate values were calculated by the following formula:

Where T is the mortality rate in the treated group expressed as a percentage and C is the mortality rate in the untreated group expressed as a percentage. The results are described in Table 3.

Based on above discussion, firstly, the reaction of podophyllotoxin (**1**) with different carboxylic acids in the presence of $BF_3{\cdot}OEt_2$ was investigated, only **2 g** (R as CCl_3) and **2 h** (R as (*m*-NO_2)Ph) were stereoselectively prepared. Interestingly, it may be due to the electronic effect of R that 4β-acyloxypodophyllotoxin derivatives (**2 g** and **2 h**, R as the electron withdrawing group) were stereoselectively obtained. However, two approaches were applied for stereoselective synthesis of a single configuration 4-acyloxypodophyllotoxin derivative (e.g. **4c, d** and **5a, b, e, f**). On the one hand, the essential intermediate of epipodophyllotoxin (**3**) was smoothly prepared, then **3** was treated with the corresponding carboxylic acids **6** in the presence of DCC and DMAP to afford 4β-acyloxypodophyllotoxin derivatives (**4c, d**) in desired yields. On the other hand, 4α-acyloxypodophyllotoxin derivatives (**5a, b, e, f**) were obtained by reaction of **1** with the corresponding carboxylic acids **6** in the presence of DCC and DMAP in good yields. Moreover, the insecticidal activity of 4α/β-acyloxypodophyllotoxin derivatives (**2a-j**, **4c**, **d** and **5a**, **b**, **e**, **f**) against the pre-third-instar larvae of *B. mori*, *A. dissimilis* and *M. separata in vivo* were screened. Especially 4β-acyloxypodophyllotoxin derivatives (**2 g**, **h** and **4c**, **d**) exhibited the more promising activity as compared with toosendanin. This results indicated that 4β-acyloxy moiety in the podophyllotoxin derivatives was significant for obtaining the more potent compounds. This will pave the way for further design, structural modification of podophyllotoxins in the development of potential promising agents in crop protection.

Disclosure statement

No potential conflict of interest was reported by the authors.

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