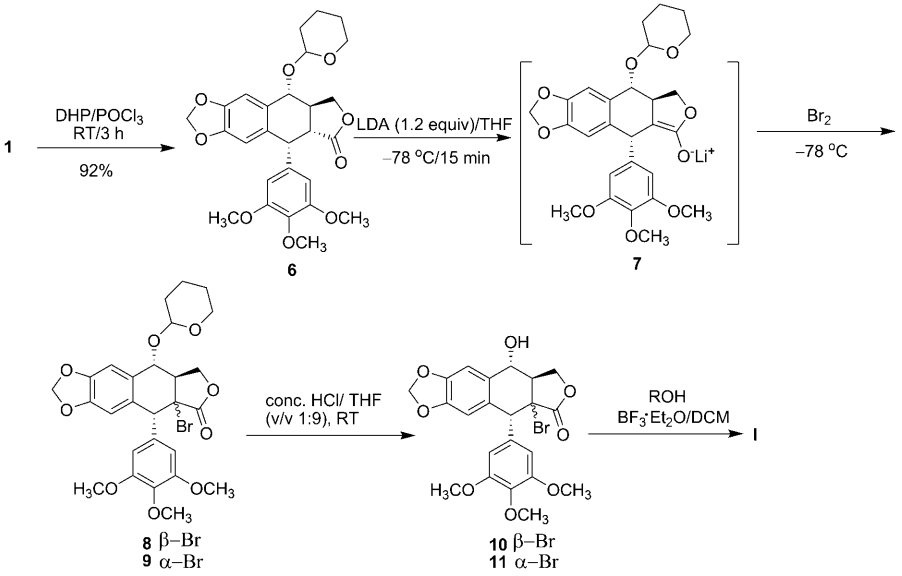
DOI: 10.1002/chem.201100855

Stereoselective Synthesis of 4α-Alkyloxy-2-α/β-Bromopodophyllotoxin Derivatives as Insecticidal Agents

Hui Xu,* Qingtian Wang, and Yong Guo[a]

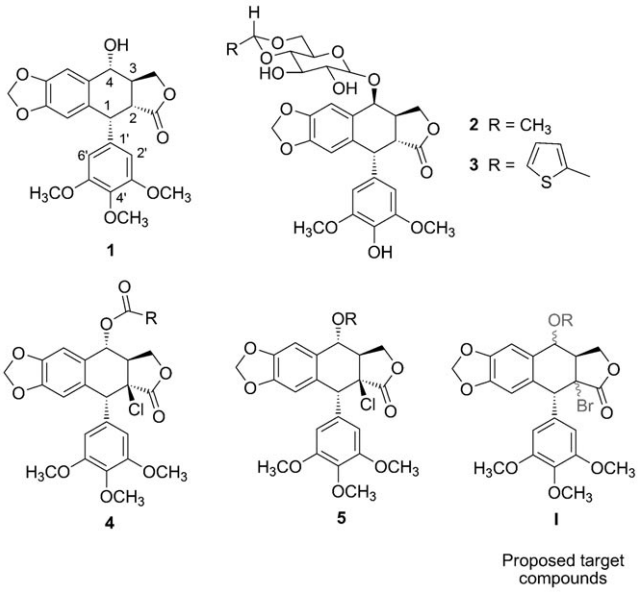
Podophyllotoxin (**1**), a naturally occurring aryltetralin lignan, is extracted as the main component from the roots and rhizomes of Podophyllum species such as *P. hexandrum* and *P. peltatum*. Since two semisynthetic derivatives of **1**, etoposide (VP-16, **2**) and teniposide (VM-26, **3**), have been used as DNA topoisomerase II inhibitors in chemotherapy for various types of cancer, more attention has recently been paid to extensive structural modifications on **1** to develop new antitumor drugs.[1] In addition, compound **1** has also exhibited interesting insecticidal activity.[2]



Scheme 1. The synthetic approach for target compounds **I**.

More recently, we have semisynthesized a series of 4α-acyloxy (**4**)[3]/4α-alkyloxy-2-chloropodophyllotoxins (**5**),[4] and found that some compounds displayed more pronounced insecticidal activity than toosendanin, a commercial insecticide derived from *Melia azedarach*. Consequently, these encouraging results promoted us to further design and prepare 4-alkyloxy-2-bromopodophyllotoxins (**I**) as insecticidal agents and to investigate if their insecticidal activity could be improved when the chlorine atom at the C2 position of **5** was substituted for a bromine atom.

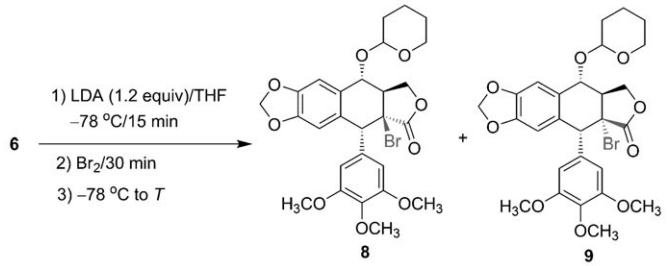
The synthetic route for the preparation of **I** is proposed in Scheme 1. First, as described in our previous paper, the 4-OH group of **1** was smoothly protected by a tetrahydropyranyl (THP) group in the presence of phosphorus oxychloride ($POCl_3$) and dihydropyran (DHP) at room temperature to afford 4-*O*-tetrahydropyranylpodophyllotoxin (**6**) in 92% yield.[4,5] Although Durst and co-workers reported the reaction of **6** with Br_2 in the presence of lithium diisopropylamide (LDA),[6] to our delight, herein we found that the relative ratio of two stereoisomers, 2β-bromo-4-*O*-tetrahydro-



[a] Prof. Dr. H. Xu, Q. Wang, Dr. Y. Guo
Laboratory of Pharmaceutical Design & Synthesis
College of Sciences, Northwest A & F University
Yangling 712100 (P.R. China)
Fax: (+86) 29-87091952
E-mail: orgxuhui@nwsuaf.edu.cn

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201100855.

Table 1. Investigation of **6** reacting with Br_2 in the presence of LDA.



Entry	T [°C]	t [h]	Yield [%][a]		Ratio of **8**/**9**
			8	**9**	
1	37	13	4.6	25.4	1:5.5
2	33	14	5.6	18.4	1:3.3
3	30	14.5	8.2	22.8	1:2.8
4	27	15	9.5	20.3	1:2.1
5	25	15.5	15.7	14.8	1:0.94
6	21	16	20.3	12.4	1:0.61

[a] Yield of isolated product.

pyranylpodophyllotoxin (**8**) and 2α-bromo-4-*O*-tetrahydropyranylpicropodophyllotoxin (**9**), could be well controlled by the reaction temperature. The results are described in Table 1. Following treatment of **6** with LDA at −78 °C in dry THF via the intermediate **7**, and subsequent substitution reaction with Br_2, the solution was allowed to warm slowly from −78 °C to a certain temperature over a number of hours. For example, when the solution was warmed slowly from −78 to 37 °C for 13 h, the molar ratio of **8** and **9** was 1/5.5; whereas when the solution was warmed slowly from −78 to 21 °C for 16 h, the molar ratio of **8** and **9** was 1/0.61.

To obtain the configurations of the lactones of **8** and **9**, the single-crystal structures of 2β-bromopodophyllotoxin (**10**), a hydrolysis product of **8**, and 4α-*n*-butanoyloxy-2α-bromopicropodophyllotoxin (**12**), a esterification product of 2α-bromopicropodophyllotoxin (**11**, a hydrolysis product of **9**) reacting with *n*-butyric acid in the presence of *N,N′*-diisopropylcarbodiimide (DIC), were determined by X-ray crystallography[7] (Figure 1). In these structures, the C2 bromine atoms of **10** and **12** adopted the β- and α configuration, respectively. That is, the configurations of the lactones of **8** and **9** were *trans* and *cis*, respectively.

The reaction of **10** or **11** with alcohols in the presence of BF_3·Et_2O was then investigated as shown in Scheme 2. As anticipated, and as in our previous report for 2β-chloropodophyllotoxin,[4] when **10** was allowed to react with *n*-butanol in the presence of BF_3·Et_2O, only 4α-*n*-butoxy-2β-bromopodophyllotoxin (**13**) was obtained in a 54% yield due to steric effects of the C2β bromine atom, and the assignment of configuration of C4 position of **13** was based on the *J*-(H3,H4) coupling constant[8] and X-ray crystallography[7] (Figure 2). Interestingly, when compound **11** was allowed to react with different alcohols in the presence of BF_3·Et_2O for 100–150 min, only 4α-alkyloxy-2α- bromopicropodophyllotoxins (**14a–j**) were produced in 53–86% yields with excellent stereoselectivity. The configurations of C4 position of **14a–j** were assigned as follows: The 4-*n*-butanoyloxy and 4-

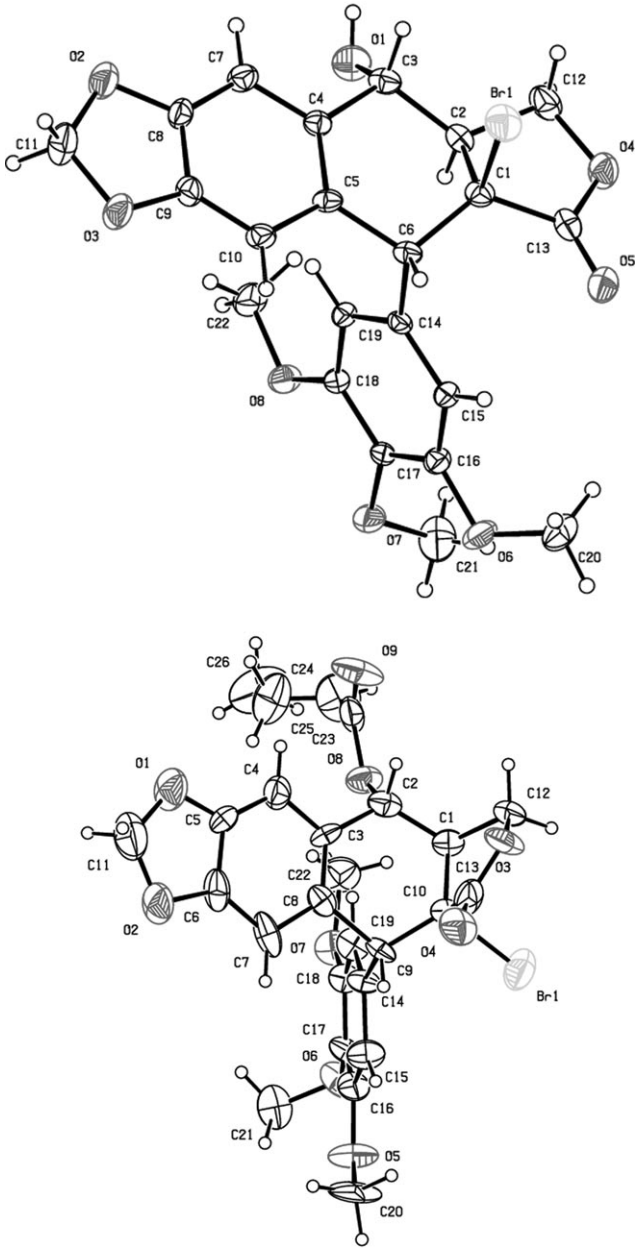
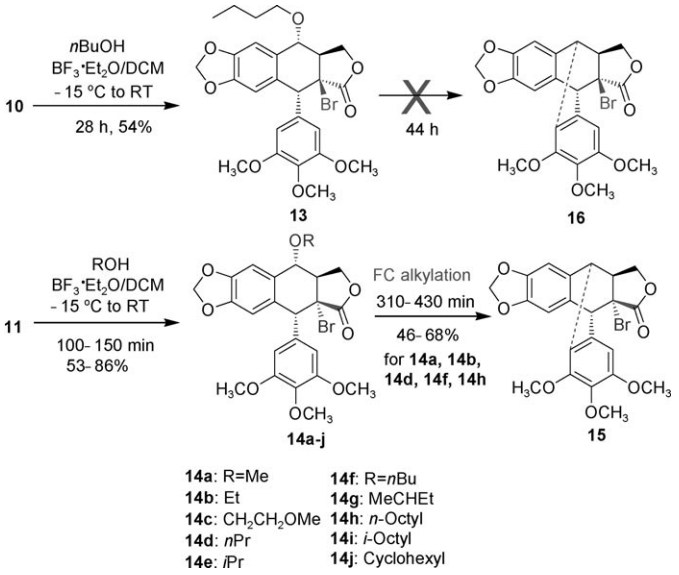


Figure 1. X-ray crystal structures of **10** (top) and **12** (bottom).

n-butoxy groups of **12** and **14f** clearly all adopted the α configuration according to X-ray crystallography (Figure 1 and Figure 2), and the *J*(H3,H4) values of H4 in compounds **12** and **14f** were 2.5 and 2.0 Hz, respectively. The *J*(H3,H4) values of H4 of **14a–j** were 2.0 Hz (see the Supporting Information), therefore, at this stage we proposed that the configurations of the 4-alkyloxy groups of **14a–j** were α. That is, in contrast to the podophyllotoxin derivatives,[8] the C4-substituted picropodophyllotoxin derivatives have a *J*(H3,H4) of approximately 2.0 Hz, which indicates that H3 and H4 are in a *trans* relationship, and the substituent on the C4 position of picropodophyllotoxin is in the α configuration. Based upon the X-ray crystallography of **14f**,[7] the lactone



Scheme 2. Investigation of **10** or **11** reacting with alcohols in the presence of BF_3·Et_2O.

adopted the *endo* configuration, and if the alkyloxy group on the C4 position adopted the β configuration, big steric effects might be observed between the lactone and the alkyloxy group. Consequently, the alkyloxy groups on the C4 position of **14a–j** adopting the α configuration was therefore reasonable.

On the other hand, it was noteworthy to investigate if the reaction of 4α-alkyloxy-2α-bromopicropodophyllotoxins (**14a–j**) completely depended on the reaction time (Scheme 2). The reaction time for the synthesis of **14a**, **14b**, **14d**, **14f**, and **14h** was investigated as examples. When the reaction time was prolonged to 310–430 min, they were all converted to the same byproduct **15** in 46–68% yields with the more rigid structure through an intramolecular Friedel–Crafts (FC) alkylation reaction, and its configuration was confirmed by X-ray crystallography (Figure 3).[7] However, for the reaction of 4α-*n*-butoxy-2β-bromopodophyllotoxin (**13**), even if the reaction time was prolonged to 44 h at room temperature or 17.5 h under reflux, the proposed byproduct **16** was not detected by thin-layer chromatography. Simultaneously, compound **13** in the presence of one equivalent of aq. HCl (0.12 M) in THF at room temperature for 19 h or under reflux for 12 h was also investigated, and no new compound was observed at all except **13**.

Finally, the insecticidal activity of **8**–**13**, **14a–j**, and **15** was evaluated against the pre-third-instar larvae of *Mythimna separata* Walker in vivo by

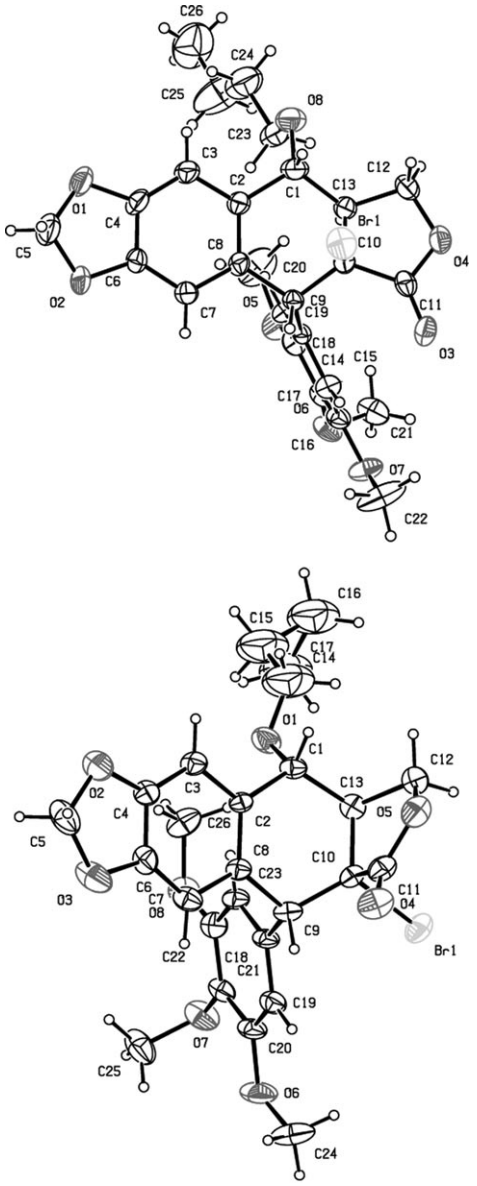


Figure 2. X-ray crystal structures of **13** (top) and **14f** (bottom).

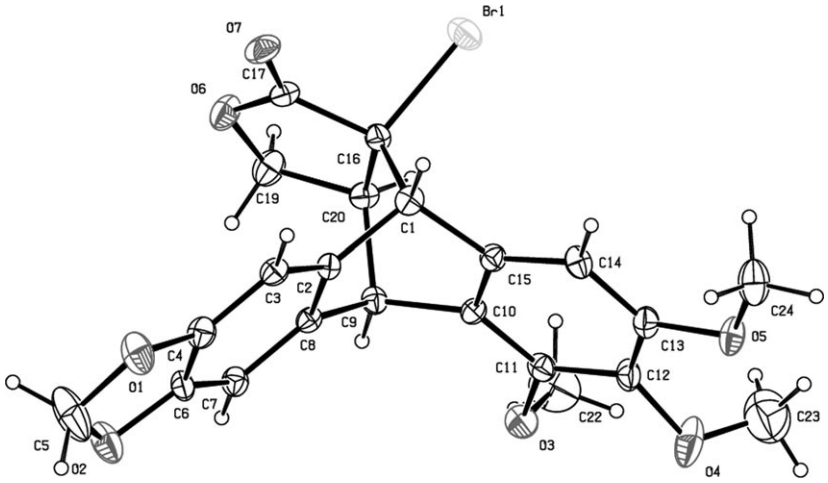


Figure 3. X-ray crystal structure of **15**.

using the leaf-dipping method at the concentration of 1 mg mL^{-1}.[9] Toosendanin was used as the positive control and the leaves that were treated with acetone alone were used as a blank control group. The corrected mortality rates of *M. separata* caused by **8**–**13**, **14a–j**, and **15** with the advance of time were shown in Table 2. The corresponding mortality rates after 35 days were far higher than those after

Table 2. Insecticidal activity of 2α/β-bromopodophyllotoxin derivatives **8**–**13**, **14a–j**, and **15** against *M. separata* at 1 mg mL^{-1}.

Compounds	Corrected mortality rate [%]		
	10 days	20 days	35 days
toosendanin	24.1 (±12.5)	32.1 (±12.5)	48.2 (±9.4)
1	10.4 (±12.5)	21.4 (±12.5)	44.4 (±14.1)
8	13.8 (±4.7)	28.6 (±12.5)	55.6 (±14.1)
9	10.4 (±9.4)	14.3 (±16.3)	44.4 (±0.0)
10	17.2 (±8.2)	25.0 (±14.1)	48.2 (±14.1)
11	10.4 (±12.5)	14.3 (±14.1)	44.4 (±16.3)
12	13.34 (±4.7)	20.7 (±4.7)	41.7 (±4.7)
13	10.4 (±4.7)	21.4 (±9.4)	51.9 (±9.4)
14a	10.4 (±9.4)	21.4 (±17.0)	55.6 (±8.2)
14b	3.5 (±4.7)	17.9 (±12.5)	48.2 (±4.7)
14c	17.2 (±14.1)	39.3 (±12.5)	63.0 (±4.7)
14d	13.8 (±17.0)	25.0 (±8.2)	59.3 (±12.5)
14e	13.8 (±12.5)	28.6 (±12.5)	59.3 (±12.5)
14f	3.5 (±9.4)	14.3 (±8.2)	40.7 (±9.4)
14g	6.95 (±8.2)	10.7 (±9.4)	37.0 (±12.5)
14h	10.4 (±9.4)	10.7 (±9.4)	48.2 (±4.7)
14i	10.4 (±12.5)	10.7 (±4.7)	40.7 (±4.7)
14j	10.4 (±12.5)	25.0 (±8.2)	37.0 (±12.5)
15	17.9 (±9.4)	25.0 (±8.2)	59.3 (±4.7)

10 and 20 days, therefore these compounds, in a time-dependent manner (different from those conventional neurotoxic insecticides, such as organophosphates, carbamates, and pyrethroids) exhibited delayed insecticidal activity.[3,4] For example, the corrected mortality rate of **14a** against *M. separata* after 10 days was only 10.4%, but after 20 days the corresponding mortality rate was increased to 21.4%; however, after 35 days the corresponding mortality rate was rapidly increased to 55.6%, which was more than five times of the mortality rate after 10 days. Compared with toosendanin and the parent compound **1**, compounds **8**, **13**, **14a**, **14c–e**, and **15** exhibited more promising and pronounced insecticidal activity. Meanwhile, some interesting results with respect to structure–activity relationships were concluded. In general, the proper length of straight-chain or branched-chain alkyloxy at the C4 position of 2α-bromopicropodophyllotoxins was very important for the insecticidal activity. For example, the final mortality rates of **14a** (methoxy) and **14d** (*n*-propyloxy) were 55.6 and 59.3%, respectively, whereas the final mortality rates of **14b** (ethoxy), **14f** (*n*-butoxy), and **14h** (*n*-octyloxy) were only 48.2, 40.7, and 48.2%, respectively. Notably, this indicates that a β configuration of the C2 bromine atom of 2-bromopodophyllotoxins is usually necessary for the insecticidal activity. The final mortality rates of **8**, **10**, and **13** (for the C2β bromine series) were 55.6, 48.2, and 51.9%, respectively, whereas the final mortality rates of the corresponding compounds **9**, **11**, and **14f** (for the C2α bromine series) were only 44.4, 44.4, and 40.7%, respectively.

In conclusion, we found some interesting results that can be summarized as follows: 1) When 4-*O*-tetrahydropyranylpodophyllotoxin was allowed to react with Br_2 in the presence of LDA, the ratio of two stereoisomers **8** and **9** could be well controlled by the reaction temperature. 2) Due to the intramolecular steric effects, 4α-alkyloxy-2α/β-bromopodophyllotoxin derivatives were readily prepared with excellent stereoselectivity by the treatment of 2α-bromopicropodophyllotoxin or 2β-bromopodophyllotoxin with alcohols in the presence of $BF_3{\cdot}Et_2O$. 3) With respect to C4-substituted picropodophyllotoxin derivatives, a J(H3,H4) of approximately 2.0 Hz indicates that H3 and H4 have a *trans* relationship, and the substituent on the C4 position of picropodophyllotoxin is in the α configuration, which might be used as the supplement to Lee's rule.[8] 4) 4α-Alkyloxy-2α-bromopicropodophyllotoxins were all unexpectedly converted into **15** by an intramolecular FC alkylation reaction when the reaction time was prolonged (to a certain degree). 5) Of particular note, some derivatives displayed more potent and promising insecticidal activity than toosendanin.

Acknowledgements

The present research was partly supported by National Natural Science Foundation of China (No.31071737), the Program for New Century Excellent University Talents, State Education Ministry of China (NCET-06-0868), the Fok Ying Tong Education Foundation for Young Talents (No.121032), and the Special Funds of Central Colleges Basic Scientific Research Operating Expenses (QN2009045).

Keywords: alkylation · insecticidal activity · medicinal chemistry · stereoselectivity · structure–activity relationships

[1] a) S. Gupta, L. Das, A. B. Datta, A. Poddar, M. E. Janik, B. Bhattacharyya, *Biochemistry* **2006**, *45*, 6467–6475; b) T. Saitoh, K. Kuramochi, T. Imai, K. Takata, M. Takehara, S. Kobayashi, K. Sakaguchia, F. Sugawaraa, *Bioorg. Med. Chem.* **2008**, *16*, 5815–5825; c) S. W. Chen, R. Xiang, J. Liu, X. Tian, *Bioorg. Med. Chem.* **2009**, *17*, 3111–3117; d) J. Q. Zhang, Z. W. Zhang, L. Hui, S. W. Chen, X. Tian, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 983–986; e) D. Passarella, B. Peretto, R. B. Yepes, G. Cappelletti, D. Cartelli, C. Ronchi, J. Snaith, G. Fontana, B. Danieli, J. Borlak, *Eur. J. Med. Chem.* **2010**, *45*, 219–226; f) D. Guianvarc'h, M. Duca, C. Boukarim, L. Kraus-Berthier, S. Leonce, A. Pierre, B. Pfeiffer, P. Renard, P. B. Arimondo, C. Monneret, D. Dauzonne, *J. Med. Chem.* **2004**, *47*, 2365–2374; g) Y. J. You, *Curr. Pharm. Des.* **2005**, *11*, 1695–1717.

[2] a) M. Miyazawa, M. Fukuyama, K. Yoshio, T. Kato, Y. Ishikawa, *J. Agric. Food Chem.* **1999**, *47*, 5108–5110; b) H. Xu, M. Lv, X. Tian, *Curr. Med. Chem.* **2009**, *16*, 327–349; c) Y. Inamori, M. Kubo, Y. Kato, H. Tsujibo, M. Sakai, M. Kozawa, *Chem. Pharm. Bull.* **1986**, *34*, 2542–2549; d) M. Kozawa, K. Baba, Y. Matsuyama, T. Kido, M. Sakai, T. Takemoto, *Chem. Pharm. Bull.* **1982**, *30*, 2885–2888; e) X. D. Di, Y. Q. Liu, Y. Q. Liu, X. Y. Yu, H. Xiao, X. Tian, R. Gao, *Pestic. Biochem. Physiol.* **2007**, *89*, 81–87; f) R. Gao, C. F. Gao, X. Tian, X. Y. Yu, X. D. Di, H. Xiao, X. Zhang, *Pest. Manag. Sci.* **2004**, *60*, 1131–1136.

[3] H. Xu, X. Xiao, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5415–5418.

[4] H. Xu, X. Xiao, Q. T. Wang, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5009–5012.
[5] W. J. Gensler, C. D. Gatsonis, *J. Org. Chem.* **1966**, *31*, 4004–4008.
[6] M. B. Glinski, J. C. Freed, T. Durst, *J. Org. Chem.* **1987**, *52*, 2749–2753.
[7] Crystallographic data (excluding structure factors) for the structures of **15**, **12**, **13**, **10**, and **14f** in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-809704, 809705, 809706, 809707, 809708, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[8] K. H. Lee, Y. Imakura, M. Haruna, S. A. Beers, L. S. Thurston, H. J. Dai, C. H. Chen, S. Y. Liu, Y. C. Cheng, *J. Nat. Prod.* **1989**, *52*, 606–613. The C4β-substituted compounds have a J(H3,H4) of approximately 4.0 Hz due to a *cis* relationship between H3 and H4. If J(H3,H4) $\geqq$ 9.5 Hz, it indicates that H3 and H4 have a *trans* relationship, and the substituent on the C4 position of podophyllotoxin is in the α configuration. For example, the J(H3,H4) value of H4 of **13** was 9.0 Hz, therefore, the *n*-butoxy group on the C4 position of **13** was in the α configuration.
[9] H. Xu, J. J. Wang, H. J. Sun, M. Lv, X. Tian, X. J. Yao, X. Zhang, *J. Agric. Food Chem.* **2009**, *57*, 7919–7923.

Received: March 20, 2011
Published online: June 10, 2011

