Tetrahedron 67 (2011) 3533–3539

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

The absolute configuration determination of naturally occurring diacetylenic spiroacetal enol ethers from *Artemisia lactiflora*

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ARTICLE INFO

Article history:
Received 5 January 2011
Received in revised form 1 March 2011
Accepted 10 March 2011
Available online 16 March 2011

Keywords:
Artemisia lactiflora
Diacetylenic spiroacetal enol ethers
Absolute configuration
Chemical transformation
X-ray crystallography
Circular dichroism

ABSTRACT

Six new naturally occurring diacetylenic spiroacetal enol ethers, Lactiflodiynes A–F (**1**–**6**), together with five known congeners (**7**–**11**), were isolated from the whole plant of *Artemisia lactiflora* (Compositae). The structures were elucidated by extensive spectroscopic methods, X-ray crystallography, chemical transformations, and CD. The absolute configuration of Lactiflodiyne A (**1**) was determined to be 2*R*, 5*S*, 6*S*, and 7*R* by an X-ray crystallographic diffraction experiment using Mo Kα radiation with the absolute parameter of 0.01(8). In combination with CD, the absolute configurations of compounds **2**–**11** were confirmed by chemical transformations using **1** as the starting material.

1. Introduction

Artemisia lactiflora Wall. ex DC. (Chinese name Bai-Bao-Hao), one species of genus *Artemisia*, belongs to the Compositae family. It is distributed mainly in southeast Asia, and has long been regarded as an edible and medicinal plant. So far, a few publications reported its chemical constituents,[1–4] and among them the most striking components are diacetylenic spiroacetal enol ethers.[4] Since Bohlmann first discovered compounds of this kind in 1961,[5] chemists have isolated an array of analogues[6–10] and achieved total synthesis on selected examples.[11–17] Not only did they possess amazing skeletons, but they also arose several challenging stereochemical questions.[18] Such as: (i) *E* or *Z* orientation of the 8,9-double bond, (ii) the absolute configurations at C-2, C-5, C-6, and C-7. Although Hofer et al. did experimental efforts on the absolute configurations determination by using the kinetic method of Horeau and CD analysis,[18,19] no X-ray proofs were available to support the absolute configuration of this scaffold. In order to further study the stereochemistry of the skeleton, we herein report the isolation, structural elucidation, and absolute configurations determination of six new diacetylenic spiroacetal enol ethers, Lactiflodiynes A–F (**1**–**6**), and five known anologues (**7**–**11**) from *A. lactiflora* by X-ray crystallography, chemical transformations, and CD (Fig. 1).

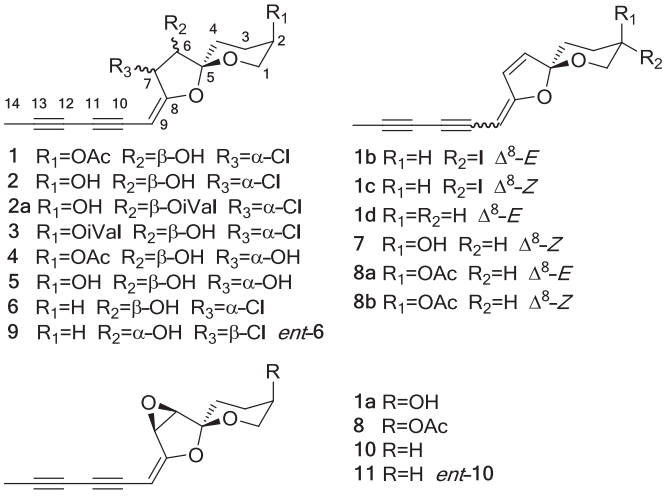

Fig. 1. Structures of isolated and synthetic compounds.

2. Results and discussion

Lactiflodiyne A (**1**), a yellowish crystalline adduct formed with $CHCl_3$, showed a molecular formula of $C_{16}H_{17}O_5Cl$ as determined by its HR-EI-MS, indicating 8 degrees of unsaturation. The molecular peak ion at *m*/*z* 324 $[M]^+$ and an isotopic ion at *m*/*z* 326 $[M+2]^+$ with ca. 30% intensity further proved the molecular formula. The

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0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.03.022

typical C≡C and C=C absorption bands at 2145 cm^{-1} and 1655 cm^{-1} in the IR spectrum combined with the pattern of the UV spectrum (Experimental part) suggested the presence of a conjugated ene-diyne unit. Further proofs were obtained from the ^{13}C NMR and ^{1}H NMR spectra (Tables 1 and 2). Four quaternary carbon resonances at δ_C 64.5, 68.4, 80.4, and 81.1 indicated a diacetylenic group. One of its two terminals was connected to a very high-field methyl at δ_C 4.7 [δ_H 1.99 (d, J=0.9, 3H)] due to the paramagnetic shielding effect, and the other to a trisubstituted oxygenated olefin at δ_C 165.5 and 86.0 [δ_H 5.21 (br s, 1H)] by reason of the strong inductive and conjugated effects of the oxygen atom and the diacetylenic group. The quaternary carbon resonance at δ_C 105.0 along with proton signals at δ_H 4.04 (dd, J=13.0, 1.6 Hz, 1H), δ_H 3.94 (d, J=13.0 Hz, 1H), and δ_H 1.85–2.15 (m, 4H) suggested the presence of a cyclic spiroacetal unit that is common in natural acetylenes.[6] 2D NMR (HMBC and HSQC) experiments further supported the existence of these two units as well as their connections. Hence, the 14-carbon diacetylenic spiroacetal enol ether skeleton with 7 degrees of unsaturation was confirmed. Since the skeleton contributed 6 degrees of unsaturation, the left one was ascribed to the acetyl group, which was supported by the IR absorption band at 1718 cm^{-1}, and NMR resonances at δ_C 170.5, 21.2, and δ_H 2.11 (s, 3H). The residuals of the formula were an OH group, which was indicated in the IR absorption band at 3448 cm^{-1} and a chlorine atom. The assignments of the three substituents were established by 2D NMR approaches (Fig. 2). The *E* orientation of the 8,9-double bond was based on the proton signal at δ_H 5.21 (br s, 1H).[18] Thus, the planar structure of compound **1** was proved.

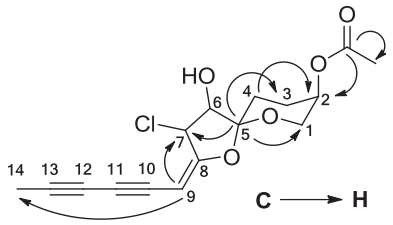


Fig. 2. Key HMBC correlations of compound **1**.

Table 1
^{13}C NMR spectroscopic data of compounds **1**–**6** (100 MHz, δ in ppm)

Position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]	**6**[a]
1	65.1 t	67.3 t	65.2 t	64.4 t	68.3 t	63.9 t
2	65.9 d	63.5 d	65.6 d	66.0 d	64.9 t	24.4 t
3	22.7 t	24.7t	22.8 t	22.7 t	26.1 t	18.6 t
4	25.8 t	25.5 t	25.9 t	25.0 t	26.9 t	31.1 t
5	105.0 s	104.8 s	105.0 s	103.5 s	105.5 s	105.5 s
6	82.2 d	82.5 d	82.3 d	80.5 d	82.7 d	82.4 d
7	58.5 d	58.6 d	58.5 d	76.0 d	76.7 d	58.8 d
8	165.5 s	165.4 s	165.5 s	169.2 s	171.3 s	166.0 s
9	86.0 d	86.0 d	86.0 d	82.3 d	83.5 d	85.4 d
10	68.4 s	68.4 s	68.5 s	68.7 s	70.8 s	68.8 s
11	80.4 s	80.6 s	80.4 s	78.8 s	79.1 s	80.1 s
12	64.5 s	64.6 s	64.6 s	64.3 s	66.4 s	64.7 s
13	81.1 s	81.1 s	81.1 s	80.9 s	80.4 s	80.9 s
14	4.7 q	4.7 q	4.7 q	4.6 q	4.4 q	4.7 q
Acetate	170.5 s			170.7 s		
	21.2 q			21.2 q		
Isovalerate			172.5 s			
			43.5 t			
			25.8 d			
			22.3 q			
			22.3 q			

[a] In $CDCl_3$.
[b] In CD_3OD.

Lactiflodiyne B (**2**) gave a molecular formula of $C_{14}H_{15}O_4Cl$ containing 7 degrees of unsaturation. The IR spectrum exhibited absorption bands for OH (3415 and 3336 cm^{-1}), C≡C (2141 cm^{-1}), and C=C (1653 cm^{-1}). Compared with **1**, signals of an acetyl group disappeared in the ^{1}H and ^{13}C NMR spectra of **2**, and correspondingly, the chemical shift of H-2 moved to δ_H 3.89 due to the absence of the inductive effect of the acetyl group. Such an elucidation was further confirmed by its molecular formula and IR spectrum. Therefore, compound **2** was identified as a 2-deacetylated product of **1**.

Lactiflodiyne C (**3**) afforded a molecular formula of $C_{19}H_{23}O_5Cl$ with 8 degrees of unsaturation. The IR spectrum exhibited absorption bands for OH (3448 cm^{-1}), ester carbonyl (1723 cm^{-1}), C≡C (2239 and 2149 cm^{-1}), and C=C (1645 cm^{-1}). In the ^{1}H and ^{13}C NMR spectra, carbon resonances at δ_C 43.5, 25.8, and 22.3 and proton signals at δ_H 2.23 (d, J=6.9, 2H), 2.10 (m, 1H), 0.96 (d, J=6.6, 6H) revealed the presence of an isovaleryl group. In accordance with this, the chemical shift of H-2 in **3** was observed at δ_H 4.90 (br s, 1H) due to the inductive effect of the isovaleryl group. Thus, compound **3** was determined to be a 2-Oival derivative of **2**.

Lactiflodiyne D (**4**) showed a molecular formula of $C_{16}H_{18}O_6$ containing 8 degrees of unsaturation. The IR spectrum exhibited absorption bands for OH (3431 cm^{-1}), ester carbonyl (1736 cm^{-1}), C≡C (2233 and 2143 cm^{-1}), and C=C (1651 cm^{-1}). Compared with **1**, an OH group instead of a chlorine atom was present at C-7 in the molecule of **4**. 2D NMR experiments provided more evidence to confirm **4** to be a 7-OH substituted product of **1**.

Table 2
^{1}H NMR spectroscopic data of compounds **1**–**6** (300 MHz, δ in ppm, J in Hz)

Position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]	**6**[a]
1a	4.04 (dd, 13.0, 1.6)	3.98 (d, 12.1)	4.03 (dd, 12.9, 1.5)	3.95 (dd, 12.8, 1.7)	3.92 (d, 12.1)	3.90 (m)
1b	3.94 (d, 13.0)	3.81 (d, 12.1)	3.93 (d, 12.9)	3.84 (d, 12.8)	3.72 (d, 12.1)	3.87 (m)
2	4.89 (br s)	3.89 (br s)	4.90 (br s)	4.80 (br s)	3.77 (br s)	
3a	2.11 (m)	2.08 (d, 14.4)		2.09 (m)	2.02 (m)	
3b	1.94 (m)	1.86 (d, 12.4)	1.86–2.15 (m, 4H)	1.89 (m)	1.80 (d, 12.1)	1.60–1.98 (m, 6H)
4a	2.15 (m)	2.27 (td, 13.7, 4.8)		2.19 (m)	2.30 (td, 13.7, 4.8)	
4b	1.85 (m)	1.75 (d, 12.0)		1.57 (m)	1.54 (d, 13.7)	
6	4.10 (dd, 7.4, 4.4)	4.06 (d, 5.4)	4.09 (dd, 7.5, 4.5)	3.66 (d, 7.5)	3.66 (d, 7.4)	4.03 (dd, 7.6, 4.5)
7	4.79 (dd, 4.4, 1.5)	4.80 (d, 5.4)	4.78 (dd, 4.5, 1.5)	4.72 (dd, 7.5, 2.1)	4.73 (d, 7.5)	4.76 (dd, 4.5, 1.5)
9	5.21 (br s)	5.20 (br s)	5.20 (br s)	5.08 (br s)	5.08 (br s)	5.19 (br s)
14	1.99 (d, 0.9, 3H)	1.98 (s, 3H)	1.99 (d, 0.7, 3H)	1.93 (d, 1.4, 3H)	1.95 (br s, 3H)	1.99 (d, 1.0, 3H)
6-OH	2.90 (d, 7.4)		2.89 (d, 7.5)			2.89 (d, 7.6)
Acetate methyl	2.11 (s, 3H)			2.04 (s, 3H)		
Isovalerate						
2′			2.23 (d, 6.9, 2H)			
3′			2.10 (m)			
4′ and 5′			0.96 (d, 6.6, 6H)			

[a] In $CDCl_3$.
[b] In CD_3OD.

Lactiflodiyne E (**5**) possessed a molecular formula of $C_{14}H_{16}O_5$ with 7 degrees of unsaturation. The IR spectrum exhibited absorption bands for OH (3319 cm^{-1}), C≡C (2143 cm^{-1}), and C=C (1655 cm^{-1}). Compared with **4**, the absence of the acetyl group was clearly observed in the NMR spectra of **5** (Tables 1 and 2), which was further substantiated by the relatively high-fielded chemical shift of H-2 [δ_H 3.77 (br s, 1H)], and the molecular formula and the IR spectrum as well. Therefore, compound **5** was proved to be as a 2-deacetylated analogue of **4**.

Lactiflodiyne F (**6**) afforded a molecular formula of $C_{14}H_{15}O_3Cl$ containing 7 degrees of unsaturation. The IR spectrum exhibited absorption bands for OH (3464 cm^{-1}), C≡C (2143 cm^{-1}), and C=C (1653 cm^{-1}). Compared with the NMR data of **2**, a secondary carbon resonance at δ_C 24.4 replaced the tertiary resonance at δ_C 63.5 of **2**, which suggested a methylene rather than a hydroxyl methine was situated at C-2. The lack of the characteristic proton H-2 resonancing at δ_H 3.89 (br s, 1H) in **2** further verified that compound **6** was a 2-dehydroxylated analogue of **2**.

The six novel compounds along with five known congeners isolated from *A. lactiflora* all possess the same 14-carbon skeleton whose stereochemistry needed to be elucidated. Although the *Z*/*E* orientation of the 8,9-double bond could be precisely determined by its ^{1}H NMR spectrum,[18] the absolute configurations at C-2, C-5, C-6, and C-7 were yet to be illuminated. The correlations between 4-H and 6-H in the ROESY experiments confirmed the OH group and the oxygen of the oxane 6-ring were on the same face of the oxane 5-ring in compounds **1**–**6**. In order to clarify the absolute configurations of these eleven isolated compounds, X-ray crystallography, chemical transformations, and CD were carried out. The absolute configuration of **1** (Fig. 3) was determined to be 2*R*, 5*S*, 6*S*, and 7*R* by an X-ray crystallographic diffraction experiment using Mo Kα radiation with the absolute parameter of 0.01(8).[20] This is the first report on the determination of the absolute configuration of an acetylenic spiroacetal enol ether by a chloride-based X-ray crystallography. On this basis, the absolute configurations of compounds **2**–**11** were confirmed by chemical transformation experiments using **1** as the starting material. The determination methods were based on the identical TLC spots, optical rotation, ^{1}H NMR, and CD spectra of the synthetic compounds in comparison with those of either synthetic or isolated compounds.

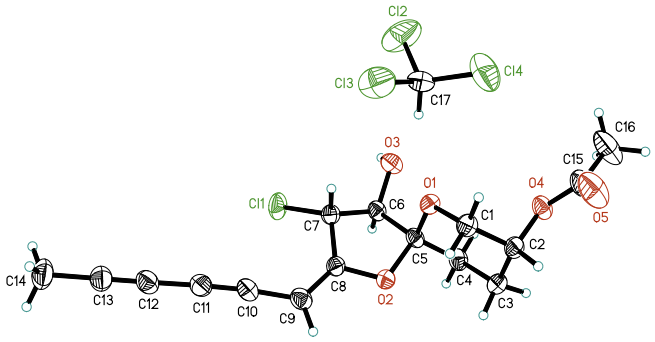
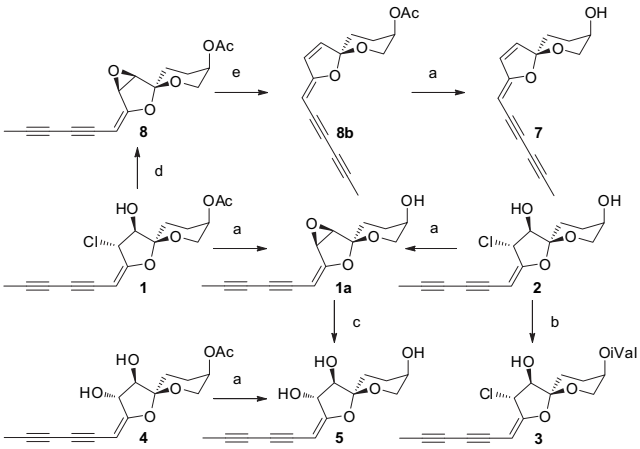


Fig. 3. Perspective ORTEP drawing for **1**.

Absolute configuration determination of C-2 substituted compounds **2**, **3**, **4**, **5**, and **8** (Scheme 1): compounds **1** and **2** were treated with concentrated aq K_2CO_3 solution in MeOH to give the identical compound **1a**. Compound **2** was treated with isovaleryl chloride and triethylamine in CH_2Cl_2 at 0 °C to give **2a** and **3** as a 1:1 mixture of isomers. Compound **4** was treated with satd K_2CO_3 (aq) in MeOH while **1a** was treated with satd KOH (aq) in 1,4-dioxane both to give **5** as the exclusive product. Compound **1** was treated with DBU in CH_2Cl_2 to give **8** in high yield (95%). Thus the absolute configurations of **2**, **3**, **4**, and **5** were deduced to be the same as **1**.

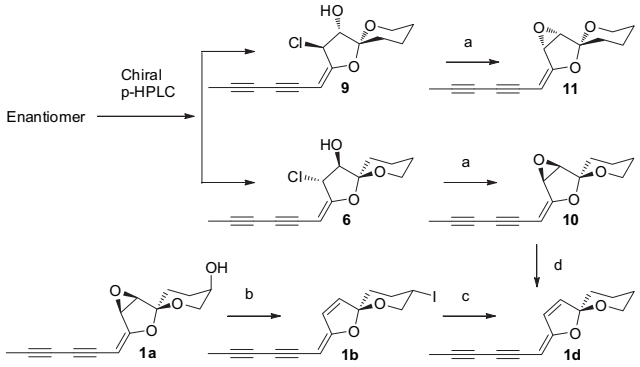


Reaction conditions: (a) K_2CO_3, MeOH, rt; (b) isovaleryl chloride, Et_3N, CH_2Cl_2, 0 °C to rt; (c) KOH, 1,4-dioxane, rt; (d) $NaHCO_3$, MeOH, rt; (e) PPh_3, I_2, imidazole, C_6H_6, reflux.

Scheme 1. Chemical transformations from **1** to **2**–**5**, **7**, **8**.

The stereochemistry of C-7 in **8** was inverted to the *S* configuration via the formation of an epoxide unit.

Absolute configuration determination of C-2 non-substituted compounds **6**, **9**, **10**, and **11** (Scheme 2): compound **1a** was treated with PPh_3, imidazole, and I_2 in benzene under reflux to give **1b** and **1c** as a 3:1 mixture of isomers. Next, **1b** was treated with $NaBH_4$ and AgOAc in MeOH to give **1d** in 50% yield. Compounds **6** and **9** were isolated as an enantiomeric mixture. Separation via chiral preparative HPLC yielded enantiomerically pure **6** and **9**, which were both treated with satd K_2CO_3 (aq) in MeOH to give compounds **10** and **11**, respectively. Compound **10** was treated with PPh_3, imidazole, and I_2 in CH_2Cl_2 at ambient temperature to give **1d** in 90% yield. Therefore the absolute configurations of **6** and **10** were deduced to be the same as **1** and **8**, respectively. Since compound **9** was the enantiomer of **6** and compound **11** was the enantiomer of **10**, their absolute configurations were established accordingly.



Reaction conditions: (a) K_2CO_3, MeOH, rt; (b) PPh_3, I_2, imidazole, C_6H_6, reflux; (c) (i) $NaBH_4$, MeOH, (ii) AgOAc; (d) PPh_3, I_2, imidazole, CH_2Cl_2, rt.

Scheme 2. Chemical transformations from **1a** to **6**, **9**–**11**.

Absolute configuration determination of 6,7-olefinic compound **7** (Scheme 1): compound **8** was treated with PPh_3, imidazole, and I_2 in benzene under reflux to give **8a** and **8b** as a 3:1 mixture of isomers. Then **8b** was treated with satd K_2CO_3 (aq) in MeOH to give **7** as the exclusive product. Therefore the absolute configuration of **7** was deduced to be 2*R*, 5*S*.

The CD spectra of compounds **1**–**11**, **1a**, and **1d** have been recorded. Compounds **1**, **2**, **3**, and **6** showed analogue CD spectra (Fig. 4). This phenomenon indicated that they should have the identical absolute configurations. The same observation for the CD

spectra referring to compounds **8**, **10**, and **1a** (Fig. 5) as well as **4** and **5** (Fig. 6) has been determined. Enantiomerically pure compounds **6** and **9** as well as **10** and **11** showed completely inversed CD spectra (Fig. 7). All these evidences obtained from the CD spectra supported the relationships of the absolute configurations among all these isolated congeners.

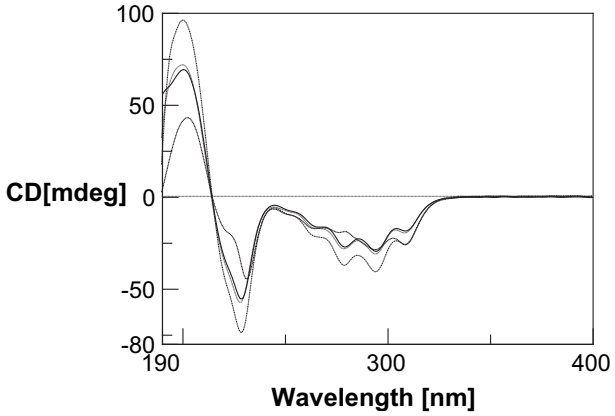


Fig. 4. CD curves of compounds **1** (solid line), **2** (long dash), **3** (short dash) and **6** (dot and dash).

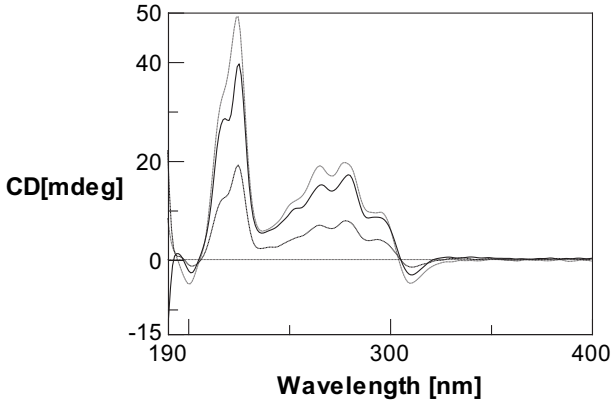


Fig. 5. CD curves of compounds **8** (solid line), **10** (long dash), **1a** (short dash).

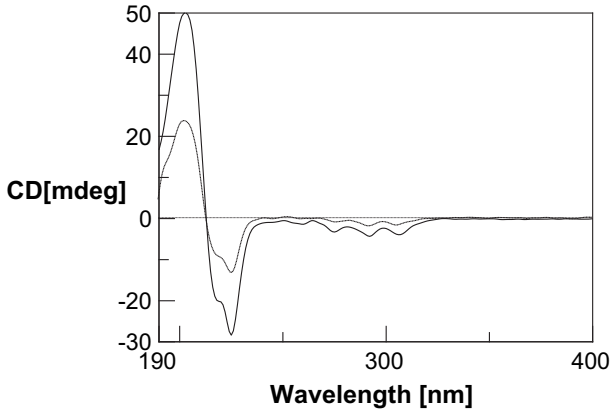


Fig. 6. CD curves of compounds **4** (solid line), **5** (long dash).

The characteristic functionalities of isolated natural products consist of unsaturated bonds, ketal as well as epoxide. In order to keep the vulnerable scaffold intact, we applied mild methods for their transformations. DBU, as a non-nucleophilic base, has been chosen to construct the epoxide moiety in **8** without undesired

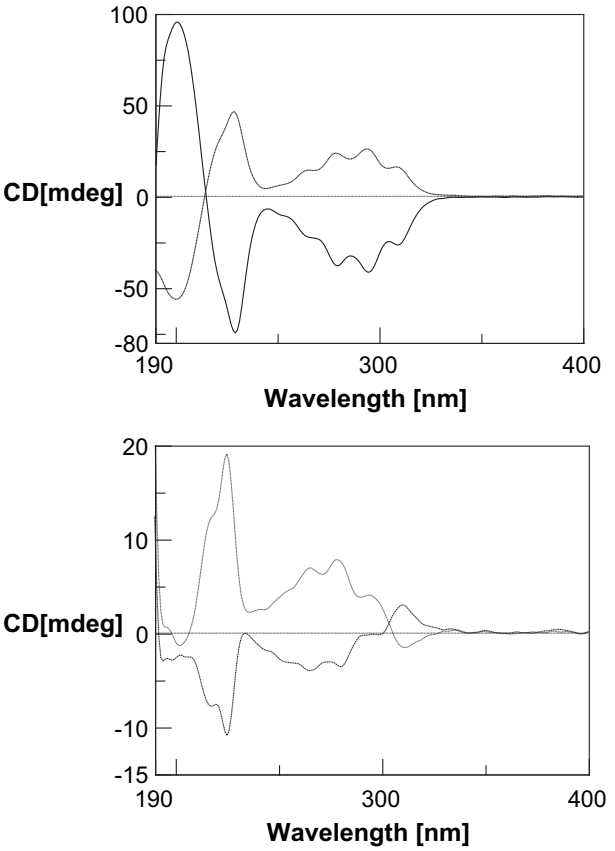


Fig. 7. CD curves of compounds **6** (solid line), **9** (long dash), **10** (short dash), and **11** (dot and dash).

hydrolysis of the acetyl group. Compound **5** could be smoothly obtained by reaction of **1a** with KOH. From compound **8** to compounds **8a** and **8b**, the formations of two olefinic isomers were assumed to proceed via a carbon cation intermediate. The generated carbenium ion could rearrange from C-7 to C-9 via an allylic 1,3-shift. Thereafter, the single bond between C-8 and 9 could rotate to form both *Z* and *E* isomers. The same mechanism seems feasible for the transformation from **1a** to **1b** and **1c**. Besides, nucleophilic substitution of the OH group at C-2 in compound **1a** by applying the methodology of Garegg yielded the iodinated product **1b** and **1c**.[21] The addition of AgOAc facilitated the attempted reduction of **1b** by $NaBH_4$ in acceptable isolated yield. Because this approach did not affect the original stereochemistry, the absolute configuration of **1d** was determined to be 5*S*. Since the absolute configuration at C-5 was not affected during the chemical transformations, the remaining chiral centers could be determined via ROESY experiments. Compound **1d** was also available starting from compound **6** to evaluate the 5*S* configuration in **6**. Thus the stereochemistry of the other C-2 non-substituted compounds **9**, **10**, and **11** were enabled to be confirmed accordingly. Epoxide-derivative **10** has been chosen as starting material to synthesize **1d** according to previously described methods.[4,16] Modification of the reaction conditions published by Paryzek and Wydra[22] through the use of imidazole as an additive[21] gave the desired olefinic-derivative **1d** in 90% isolated yield. Studies to extend this synthetic methodology to further applications are currently in progress.

From the literature we notice that both 5*R* and 5*S* analogues exist in the plants. Based on our research, if C-2 was not substituted, the metabolites were a mixture of 5*R* and 5*S*. However, on condition that C-2 was substituted, only 5*S* derivatives were identified in this

plant. In conclusion, we have determined the absolute configurations of 11 isolated natural products from *A. lactiflora* via chemical transformations as well as CD. Additionally, we have provided suitable methods for further functionalizations of these examined diacetylenic spiroacetal enol ethers.

3. Experimental

3.1. General experimental procedures

Optical rotations were taken on a Perkin–Elmer 341 polarimeter. The melting points were determined by an XT-4 binocular microscope (Beijing Tech Instrument Co., China) and were not corrected. IR spectra were recorded on a Nicolet Magna FTIR 750 spectrophotometer using KBr disks. CD spectra were obtained on a JASCO 810 spectrometer. UV spectra were recorded on a Shimadzu UV-2550 spectrophotometer. NMR spectra were recorded on Bruker AM-400 and INVOR-600 NMR spectrometers. The chemical shift (δ) values are given in parts per million with TMS as internal standard, and coupling constants (J) are in hertz. EI-MS and HR-EI-MS spectra were recorded on a Finnigan MAT-95 mass spectrometer. ESIMS and HRESIMS spectra were recorded on a Micromass LC–MS mass spectrometer. Silica gel was used for flash chromatography and was produced by Qingdao Marine Chemical Industrials. MCI gel CHP20P (75–150 μm, Mitsubishi Chemical Industries, Japan), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) were used for column chromatography (CC). TLC was carried out on precoated silica gel GF_{254} plates (Yantai Chemical Industrials) and the TLC spots were viewed at 254 nm and visualized by 5% sulfuric acid in alcohol containing 10 mg/mL vanillin. Analytical HPLC was performed on a Waters 2690 instrument with a 996 PAD (photodiode array detector) and coupled with an Alltech ELSD 2000 detector. X-ray crystallographic analysis was carried out on a Bruker Smart Apex CCD diffractometer with graphite-monochromated Mo Kα radiation λ=0.71073 Å.

3.2. Plant material

The whole plants of *A. lactiflora* were collected in Gongcheng County in Guangxi province, China and identified by Professor Jingui Shen, from Shanghai Institute of Materia Medica. A voucher (20071024) was deposited at the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

3.3. Extraction and isolation

The dried and powdered plants of *A. lactiflora* (10 kg) were extracted with 95% EtOH (3×40 L, 3 days each) at room temperature. After evaporation of the solvent, the obtained residue was dissolved in water, and then extracted with petroleum ether (PE) and EtOAc. The PE extract (200 g) was subjected to CC over silica gel and eluted successively with PE, PE/acetone (from 10:1 to 1:1), acetone and MeOH to yield six fractions (Frs. 1–6). Fr. 2 (20 g) was subjected to CC (silica gel) eluting with PE/CH_2Cl_2 (from 2:1 to 1:1), CH_2Cl_2 to give five subfractions Frs. 2a–2e, which were then purified by Sephadex LH-20 ($CHCl_3$/MeOH=1:1) to yield **10** and **11** (260 mg) as an enantiomeric mixture, **6** and **9** (430 mg) as an enantiomeric mixture, and **3** (470 mg). Then **6** and **9** (30 mg) were separated by chiral preparative HPLC (Chiralcel OJ-H column (250×20 mm, 5 μm)); detected at 220 nm; *n*-hexane/*i*-propanol (90:10); flow (15 ml/min). Compounds **6** (9 mg) and **9** (10 mg) were obtained, respectively. Fr. 3 (30 g) was first subjected to CC (silica gel) eluting with CH_2Cl_2 to yield Frs. 3a–3c. Next they were subjected to CC (silica gel) eluting with PE/CH_2Cl_2 (1:2) to give **8** (200 mg) and **1** (990 mg). Fr. 4 (10 g) was separated by MCI eluting with MeOH/H_2O (from 6:4 to 10:0) to give six subfractions Frs. 4a–4f. Fr. 4c was subjected to CC (silica gel) eluting with PE/acetone (from 5:1 to 4:1), and then purified by Sephadex LH-20 (MeOH) to yield **4** (15 mg) and **7** (4 mg). Fr. 5 (20 g) was separated by MCI eluting with MeOH/H_2O (from 5:5 to 10:0) to give seven subfractions Frs. 5a–5g. Fr. 5e was subjected to CC eluting with PE/acetone (from 2:1 to 1:1) to yield Frs. 5e1–5e3. Compound **2** (200 mg) was obtained from Fr. 5e2, which was purified by Sephadex LH-20 ($CHCl_3$/MeOH=1:1). The EtOAc extract (100 g) was subjected to CC over silica gel and eluting with $CHCl_3$/MeOH (from 100:1 to 0:1) to yield six fractions (Frs. 1–6). Fr. 3 (25 g) was subjected to MCI eluting with EtOH/H_2O (from 2:8 to 10:0) to give five subfractions Frs. 2a–2i. Fr. 2c (1.5 g) was subjected to CC (silica gel) eluting with PE/acetone (from 1:1 to 1:2), and then purified by Sephadex LH-20 (MeOH) to yield **5** (515 mg).

3.4. Characteristics of compounds

*3.4.1. Lactiflodiyne A (**1**).* Yellow crystal; $[\alpha]_D^{25}$ −227.0 (*c* 0.1, $CHCl_3$); mp 83–85 °C; UV (MeOH) λ_{max} (log ε) 295 (4.08), 280 (4.11), 226 (4.16) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 200 (+1.36), 229 (−1.08), 245 (−0.09), 279 (−0.53), 286 (−0.44), 294 (−0.58), 304 (−0.33), 308 (−0.36) nm; IR (KBr) ν_{max} 3448, 3028, 2972, 2926, 2145, 1718, 1655, 1375, 1269, 1169, 891, 752 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; EI-MS *m*/*z* 326 $[M+2]^+$ (29), 324 $[M]^+$ (90), 288 (30), 246 (52), 154 (36), 111 (100), 104 (43), 83 (35), 76 (30); HR-EI-MS *m*/*z* 324.0761 $[M]^+$ (calcd for $C_{16}H_{17}O_5Cl$ 324.0764).

*3.4.2. Lactiflodiyne B (**2**).* White powder; $[\alpha]_D^{25}$ −333.0 (*c* 0.1, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 203 (+0.74), 232 (−0.78), 245 (−0.12), 294 (−0.50), 303 (−0.39), 309 (−0.46) nm; IR (KBr) ν_{max} 3415, 3336, 2947, 2908, 2141, 1653, 1444, 1379, 1169, 987, 744 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; EI-MS *m*/*z* 284 $[M+2]^+$ (21), 282 $[M]^+$ (64), 246 (33), 154 (43), 129 (100), 111 (69), 83 (20), 76 (26), 55 (22); HR-EI-MS *m*/*z* 282.0673 $[M]^+$ (calcd for $C_{14}H_{15}O_4Cl$ 282.0659).

*3.4.3. Lactiflodiyne C (**3**).* Colorless oil; $[\alpha]_D^{24}$ −276.0 (*c* 0.1, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 200 (+1.61), 228 (−1.29), 245 (−0.13), 279 (−0.64), 286 (−0.54), 294 (−0.70), 304 (−0.41), 309 (−0.45) nm; IR (KBr) ν_{max} 3448, 2963, 2932, 2873, 2239, 2149, 1723, 1645, 1372, 1185, 987, 808 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; EI-MS *m*/*z* 368 $[M+2]^+$ (14), 366 $[M]^+$ (45), 330 (15), 247 (26), 246 (100), 111 (47), 104 (20), 85(68), 83 (16), 57 (64), 55 (16); HR-EI-MS *m*/*z* 366.1248 $[M]^+$ (calcd for $C_{19}H_{23}O_5Cl$ 366.1234).

*3.4.4. Lactiflodiyne D (**4**).* White powder; $[\alpha]_D^{24}$ +78.0 (*c* 0.1, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 203 (+0.92), 225 (−0.52), 275 (−0.06), 281 (−0.04), 292 (−0.08), 299 (−0.04), 306 (−0.07) nm; IR (KBr) ν_{max} 3431, 2928, 2233, 2143, 1736, 1651, 1375, 1236, 1180, 1020 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; EI-MS *m*/*z* 306 $[M]^+$ (88), 288 (22), 246 (37), 201 (20), 172 (44), 149 (100), 136 (48), 111 (63), 99 (28), 83 (48), 77 (26), 57 (24), 55 (38); HR-EI-MS *m*/*z* 306.1099 $[M]^+$ (calcd for $C_{16}H_{18}O_6$ 306.1104).

*3.4.5. Lactiflodiyne E (**5**).* White powder; $[\alpha]_D^{24}$ +149.0 (*c* 0.1, MeOH); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (+0.38), 225 (−0.21), 292 (−0.03), 299 (−0.02), 306 (−0.03) nm; IR (KBr) ν_{max} 3319, 2931, 2906, 2143, 1655, 1444, 1385, 1267, 1182, 1095, 986, 922 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; EI-MS *m*/*z* 264 $[M]^+$ (100), 246 (23), 159 (25), 136 (59), 129 (64), 111 (59), 99 (23), 83 (29), 77 (22), 58 (24), 55 (23); HR-EI-MS *m*/*z* 264.0989 $[M]^+$ (calcd for $C_{14}H_{16}O_5$ 264.0998).

*3.4.6. Lactiflodiyne F (**6**).* Colorless oil; $[\alpha]_D^{24}$ −362.4 (*c* 0.1, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 200 (+1.53), 229 (−1.18), 245 (−0.10), 279 (−0.60), 286 (−0.51), 294 (−0.65), 304 (−0.39), 309 (−0.42) nm; IR (KBr) ν_{max} 3464, 2953, 2143, 2145, 1653, 1417, 1105, 968, 891, 889, 820 cm^{-1}; ^1H and ^{13}C NMR: see Tables 1 and 2; EI-MS *m*/*z* 268

$[M+2]^+$ (12), 266 $[M]^+$ (36), 154 (28), 113 (100), 76 (18); HR-EI-MS m/z 266.0693 $[M]^+$ (calcd for $C_{14}H_{15}O_3Cl$ 266.0709).

3.5. Single crystal X-ray crystallography of 1

Yellow, $C_{17}H_{18}O_5Cl_4$, FW 444.11, monoclinic, crystal size 0.421×0.369×0.234 mm, space group $P2(1)$, a=6.1287 (9) Å, b=19.323 (3) Å, c=8.8061 (13) Å, V=1030.4 (3) Å^3, Z=2, D_{calcd}=1.431 mg/m^3. F (000)=456, reflections collected 5643, reflection unique 3932 (R_{int}=0.0336), final R indices for $I>2\sigma$ (I), $R1$=0.0547, $wR2$=0.1409, R indices for all data $R1$=0.0664, $wR2$=0.1530, completeness to 2θ (25.99) 99.6%, maximum transmission 1.0000, minimum transmission 0.7523, absolute structure parameter is 0.01(8). The data collection was performed on a Bruker Smart Apex CCD diffractometer with graphite-monochromated Mo Kα radiation λ=0.71073 Å. The structure was solved by direct methods using the program SHELXS-97. Refinement method was full-matrix least-squares on F^2, and goodness-of-fit on F^2 is 0.977. The X-ray diffraction material has also been deposited in the Cambridge Crystallographic Data Center (CCDC 806091).

3.6. Chemical transformations

Compound **1** (10.0 mg, 30.9 μmol) was dissolved in MeOH (2 ml), followed by the addition of satd K_2CO_3 (aq) (1 ml). The reaction mixture was stirred at ambient temperature for 10 min, then diluted with water (5 ml) and extracted with EtOAc (3×10 ml). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4, and concentrated in vacuo to give **1a** (7.5 mg, 30.6 μmol, 99% yield). Compound **2** (10.0 mg, 35.5 μmol) was treated accordingly to give **1a** (8.6 mg, 35.1 μmol, 99% yield). Compound **1a**: white powder; $[\alpha]_D^{26}$ +289.0 (c 0.1, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (−0.07), 225 (+0.73), 237 (+0.08), 265 (+0.28), 271 (+0.25), 278 (+0.29), 310 (−0.07) nm; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.15 (br s, 1H, 9-H), 4.30 (d, J=2.8 Hz, 1H, 7-H), 4.03 (dd, J=12.3, 1.4 Hz, 1H, 1a-H), 3.91 (br s, 1H, 2-H), 3.86 (d, J=2.8 Hz, 1H, 6-H), 3.85 (d, J=12.3 Hz, 1H, 1b-H), 2.25–1.45 (m, 4H, 3,4-H), 1.98 (br s, 3H, 14-H); ESI-MS m/z 269 $[M+Na]^+$, 515 $[2M+Na]^+$.

Compound **2** (14.0 mg, 49.6 μmol) was dissolved in dry CH_2Cl_2 (2 ml), then dry triethylamine (11 μl, 74.4 μmol) was added, followed by dropwise addition of isovaleryl chloride (9 μl, 74.4 μmol). The reaction mixture was stirred at 0 °C for 5 h, then diluted with water (5 ml) and extracted with CH_2Cl_2 (3×10 ml). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4, and concentrated in vacuo. The remaining residue was purified by preparative TLC (PE/acetone=3:1) to give **2a** (6.0 mg, 16.5 μmol, 33% yield) and **3** (6.0 mg, 16.5 μmol, 33% yield). Compound **2a**: white powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.29 (d, J=4.9 Hz, 1H, 6-H), 5.21 (br s, 1H, 9-H), 4.99 (dd, J=4.9, 1.5 Hz, 1H, 7-H), 3.97 (d, J=12.2 Hz, 1H, 1a-H), 3.84 (br s, 1H, 2-H), 3.74 (d, J=12.2 Hz, 1H, 1b-H), 2.30 (d, J=7.4 Hz, 2H, 2′-H), 2.11 (m, 1H, 3′-H), 2.20–1.84 (m, 4H, 3,4-H), 1.99 (br s, 3H, 14-H), 0.98 (d, J=6.6 Hz, 6H, 4′,5′-H); ESI-MS m/z 389 $[M+Na]^+$.

Compound **4** (4.0 mg, 13.1 μmol) was dissolved in MeOH (1 ml), followed by the addition of satd K_2CO_3 (aq) (0.5 ml). The reaction mixture was stirred at ambient temperature for 10 min, then diluted with water (5 ml) and extracted with EtOAc (3×6 ml). The combined organic layers were washed with brine (5 ml), dried over Na_2SO_4, and concentrated in vacuo to give **5** (3.5 mg, 13.0 μmol, 99% yield). Compound **1a** (4.0 mg, 16.3 μmol) was dissolved in 1,4-dioxane (1 ml), then satd KOH (aq) (1 ml) was added. The reaction mixture was stirred at ambient temperature for 6 h, then diluted with water (5 ml) and extracted with EtOAc (3×6 ml). The combined organic layers were washed with brine (5 ml), dried over Na_2SO_4, and concentrated in vacuo. The remaining residue was purified by preparative TLC (PE/acetone=1:1) to give **5** (3.9 mg, 14.7 μmol, 90% yield).

Compound **1** (10.0 mg, 30.9 μmol) was dissolved in CH_2Cl_2 (2 ml), and DBU (9 μl, 60.8 μmol) was added. The reaction mixture was stirred at ambient temperature for 4 h, then diluted with water (5 ml) and extracted with CH_2Cl_2 (3×10 ml). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4, and concentrated in vacuo. The remaining residue was purified by preparative TLC (CH_2Cl_2) to give **8** (8.5 mg, 29.4 μmol, 95% yield). Compound **8**: colorless oil; $[\alpha]_D^{24}$ +223.0 (c 0.1, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 201 (−0.04), 225 (+0.69), 236 (+0.10), 266 (+0.27), 271 (+0.24), 279 (+0.30), 310 (−0.05) nm; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.16 (br s, 1H, 9-H), 4.90 (br s, 1H, 2-H), 4.31 (d, J=2.8 Hz, 1H, 7-H), 4.02 (dd, J=13.0, 1.7 Hz, 1H, 1a-H), 3.97 (d, J=13.0 Hz, 1H, 1b-H), 3.87 (d, J=2.8 Hz, 1H, 6-H), 2.20–1.50 (m, 4H, 3,4-H), 2.12 (s, 3H, Ac), 1.99 (br s, 3H, 14-H); ESI-MS m/z 311 $[M+Na]^+$, 599 $[2M+Na]^+$.

Compound **1a** (24.6 mg, 100 μmol) was dissolved in C_6H_6 (2 ml), followed by the addition of PPh_3 (78.7 mg, 300 μmol), imidazole (20.4 mg, 300 μmol), and I_2 (50.8 mg, 200 μmol). The reaction mixture was stirred at reflux for 2 h, then cooled to ambient temperature and diluted with 10% aq $Na_2S_2O_3$ solution (2 ml) and water (5 ml) and extracted with EtOAc (3×10 ml). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4, and concentrated in vacuo. The remaining residue was purified by preparative TLC (PE/acetone=5:1) to give **1b** (20.4 mg, 60 μmol, 60% yield) and **1c** (6.8 mg, 20 μmol, 20% yield). Compound **1b**: white powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.69 (d, J=5.8 Hz, 1H, 7-H), 6.15 (dd, J=5.8, 1.7 Hz, 1H, 6-H), 5.03 (br s, 1H, 9-H), 4.25–4.11 (m, 2H, 1-H), 4.00 (dd, J=11.5, 7.1 Hz, 1H, 2-H), 2.50–1.70 (m, 4H, 3,4-H), 1.99 (d, J=5.7 Hz, 3H, 14-H); ESI-MS m/z 341 $[M+H]^+$, 515 $[2M+H]^+$. Compound **1c**: white powder; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.25 (d, J=5.5 Hz, 1H, 7-H), 6.10 (d, J=5.5 Hz, 1H, 6-H), 4.67 (br s, 1H, 9-H), 4.35–4.10 (m, 2H, 1-H), 3.99 (d, J=9.1 Hz, 1H, 2-H), 2.70–1.75 (m, 4H, 3,4-H), 2.03 (br s, 3H, 14-H); ESI-MS m/z 341 $[M+H]^+$, 515 $[2M+H]^+$.

Compound **1b** (10.0 mg, 29.4 μmol) was dissolved in MeOH (2 ml), then $NaBH_4$ (11.1 mg, 294 μmol) was added, followed by the addition of AgOAc (24.5 mg, 147 μmol). The reaction mixture was stirred at ambient temperature for 2 h, then filtered and the filtrate was diluted with water (5 ml) and extracted with EtOAc (3×10 ml). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4, and concentrated in vacuo. The remaining residue was purified by preparative TLC (PE/acetone=5:1) to give **1d** (4.0 mg, 18.7 μmol, 50% yield). Compound **6** (5.0 mg, 18.8 μmol) was dissolved in MeOH (1 ml), followed by the addition of satd K_2CO_3 (aq) (0.5 ml). The reaction mixture was stirred at ambient temperature for 10 min, then diluted with water (5 ml) and extracted with EtOAc (3×10 ml). The combined organic layers were washed with brine (10 ml), dried over Na_2SO_4, and concentrated in vacuo to give **10** (4.3 mg, 18.6 μmol, 99% yield). Compound **9** (5.0 mg, 18.8 μmol) was treated accordingly to give **11** (4.3 mg, 18.6 μmol, 99% yield). PPh_3 (5.1 mg, 19.5 μmol) and imidazole (1.3 mg, 19.5 μmol) were dissolved in CH_2Cl_2 (1 ml), followed by the addition of I_2 (4.0 mg, 15.6 μmol). The mixture was stirred at ambient temperature for 5 min and then a solution of compound **10** (3.0 mg, 13.0 μmol) in CH_2Cl_2 (0.5 ml) was added dropwise and the reaction mixture was stirred for 10 h at ambient temperature. Then the mixture was diluted with 10% $Na_2S_2O_3$ (aq) (1 ml) and water (3 ml) and extracted with CH_2Cl_2 (3×6 ml). The combined organic layers were washed with brine (5 ml), dried over Na_2SO_4, and concentrated in vacuo. The remaining residue was purified by preparative TLC (PE/acetone=5:1) to give **1d** (2.5 mg, 11.7 μmol, 90% yield). Compound **10**: colorless oil; $[\alpha]_D^{26}$ +106.7 (c 0.1, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 202 (−0.02), 225 (+0.27), 236 (+0.03), 278 (+0.11), 311 (−0.02) nm; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.14 (br s, 1H, 9-H), 4.27 (d, J=2.5 Hz, 1H, 7-H), 3.89 (m, 2H, 1-H), 3.79 (d, J=2.5 Hz, 1H, 6-H), 1.98 (br s, 3H,

14-H), 1.85–1.50 (m, 6H, 2,3,4-H); ESI-MS *m*/*z* 253 $[M+Na]^+$, 483 $[2M+Na]^+$. Compound **1d**: white powder; $[\alpha]_D^{22}$ +42.0 (*c* 0.1, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 197 (+0.13), 226 (−0.01), 250 (+0.01), 329 (−0.03) nm; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.66 (dd, *J*=5.8, 0.6 Hz, 1H, 7-H), 6.22 (dd, *J*=5.8, 1.7 Hz, 1H, 6-H), 4.96 (br s, 1H, 9-H), 3.98 (td, *J*=11.3, 3.6 Hz, 1H, 1a-H), 3.84 (dd, *J*=11.3, 2.5 Hz, 1H, 1b-H), 1.95–1.50 (m, 6H, 2,3,4-H), 1.99 (d, *J*=1.1 Hz, 3H, 14-H); ESI-MS *m*/*z* 215 $[M+H]^+$, 429 $[2M+H]^+$.

Compound **8** (28.8 mg, 100 μmol) was dissolved in C_6H_6 (2 ml), followed by the addition of PPh_3 (78.7 mg, 300 μmol), imidazole (20.4 mg, 300 μmol), and I_2 (50.8 mg, 200 μmol). The reaction mixture was stirred under reflux for 2 h, then cooled to ambient temperature and diluted with 10% $Na_2S_2O_3$ (aq) (2 ml) and water (5 ml) and extracted with EtOAc (3×10 ml). The combined organic layers were washed with brine (5 ml), dried over Na_2SO_4, and concentrated in vacuo. The remaining residue was purified by preparative TLC (CH_2Cl_2) to give **8a** (12.2 mg, 45 μmol, 45% yield) and **8b** (4.1 mg, 15 μmol, 15% yield). Compound **8a**: brownish oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.69 (dd, *J*=5.8, 0.7 Hz, 1H, 7-H), 6.27 (dd, *J*=5.8, 1.7 Hz, 1H, 6-H), 4.98 (br s, 1H, 9-H), 4.90 (br s, 1H, 2-H), 4.12 (dd, *J*=12.9, 1.7 Hz, 1H, 1a-H), 3.90 (dt, *J*=12.9, 1.9 Hz, 1H, 1b-H), 2.20–1.60 (m, 4H, 3,4-H), 2.11 (s, 3H, Ac), 1.97 (br s, 3H, 14-H); ESI-MS *m*/*z* 295 $[M+Na]^+$, 545 $[2M+H]^+$. Compound **8b**: brownish oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.25 (m, 2H, 6,7-H), 4.93 (br s, 1H, 2-H), 4.65 (br s, 1H, 9-H), 4.25 (d, *J*=12.9 Hz, 1H, 1a-H), 3.90 (d, *J*=12.9 Hz, 1H, 1b-H), 2.30–1.60 (m, 4H, 3,4-H), 2.14 (s, 3H, Ac), 2.01 (br s, 3H, 14-H); ESI-MS *m*/*z* 295 $[M+Na]^+$, 545 $[2M+H]^+$.

Compound **8b** (3.0 mg, 11.0 μmol) was dissolved in MeOH (1 ml), followed by the addition of satd K_2CO_3 (aq) (0.5 ml). The reaction mixture was stirred at ambient temperature for 10 min, then diluted with water (5 ml) and extracted with EtOAc (3×6 ml). The organic layer was washed with brine (5 ml), dried over Na_2SO_4, and concentrated in vacuo to give **7** (2.5 mg, 10.9 μmol, 99% yield). Compound **7**: brownish powder; $[\alpha]_D^{22}$ −6 (*c* 0.1, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 207 (−0.06), 225 (+0.02), 297 (−0.03) nm; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.23 (m, 2H, 6,7-H), 4.64 (br s, 1H, 9-H), 4.28 (dd, *J*=12.3, 0.7 Hz, 1H, 1a-H), 3.94 (s, 1H, 2-H), 3.80 (d, *J*=12.3, 1H, 1b-H), 2.30–1.55 (m, 4H, 3,4-H), 2.00 (br s, 3H, 14-H); ESI-MS *m*/*z* 253 $[M+Na]^+$.

Acknowledgements

The authors thank Professor Jie Sun from Shanghai Institute of Organic Chemistry for the X-ray diffraction experiment. Financial supports from the National Science and Technology Major Project 'Key New Drug Creation and Manufacturing Program', China (No. 2009ZX09301-001), the Ministry of Science and Technology (2007DFC31030), and the National Natural Science Funds for Distinguished Young Scholar (No. 30925043) are gratefully acknowledged. A.A. wishes to thank the Chinese Academy of Sciences for a fellowship for young international scientists.

Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.03.022. These data include MOL files and InChIKeys of the most important compounds described in this article.

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