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Ivorenolide B, an Immunosuppressive 17-Membered Macrolide from *Khaya ivorensis*: Structural Determination and Total Synthesis

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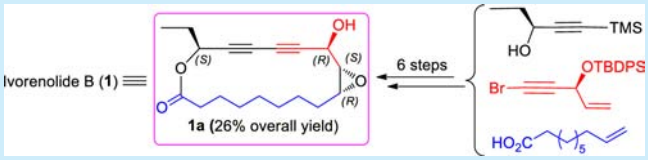
Supporting Information

ABSTRACT: Ivorenolide B (**1**), an unprecedented 17-membered macrolide featuring conjugated acetylenic bonds and four chiral centers, was isolated from *Khaya ivorensis*. The structure of **1** was fully determined by spectroscopic analysis and total syntheses of its four most possible stereoisomers. Compound **1** showed significant immunosuppressive activity.



Khaya ivorensis A. Chev. (Meliaceae), an African ethnobotanical also cultivated in China, contains the limonoids as the major metabolites[1] and has been demonstrated to exhibit anti-inflammatory and antimalarial activities.[2] Our recent study on *K. ivorensis* led to the isolation of an immunosuppressive 18-membered macrolide, ivorenolide A.[3] As a continuation of our studies of immunosuppressive agents from natural sources, an unprecedented 17-membered macrolide, ivorenolide B (**1**) (Figure 1), featuring conjugated acetylenic bonds and four oxygenated chiral centers, has been further isolated from the same material. The presence of the conjugated acetylenic bonds and the 9,10-epoxy group on the northern hemisphere of the molecule results in a planar loop conformation for the 17-membered macrolide of **1**, which made the stereochemical assignment, particularly the configuration of C-16, very challenging. In addition, the gumlike physical characteristic of **1** prevented the use of single-crystal X-ray diffraction. To determine the absolute configuration of **1**, a concise and efficient total synthetic strategy, utilizing ring-closing metathesis (RCM) as the key step, was carried out to produce four of its most possible stereoisomers **1a**–**d**, which allowed the unambiguous assignment of its absolute configuration. We report herein the isolation and structural elucidation via the total syntheses of its stereoisomers as well as the evaluation of their immunosuppressive activity.

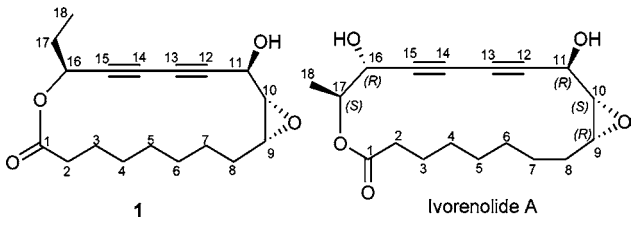


Figure 1. Structures of ivorenolides A and B (**1**).

Ivorenolide B (**1**) was obtained as a colorless, gumlike material with $[\alpha]^{25}_{D}$ −13.0 (*c* 0.126, MeOH). The molecular formula, $C_{18}H_{24}O_4$, was established by an HREIMS ion at *m*/*z* 304.1666 [M^+] (calcd for $C_{18}H_{24}O_4$, 304.1675) and indicated seven indices of hydrogen deficiency. The IR spectrum revealed the presence of hydroxy (3419 cm^{-1}) and carbonyl (1739 cm^{-1}) groups. In accordance with the molecular formula, 18 carbon signals were resolved in the ^{13}C NMR spectrum (Table S1, Supporting Information), which included a carbonyl (δ 173.2), a methyl (δ 9.8), eight sp^3 methylenes, four oxygenated sp^3 methines (δ 56.8, 62.0, 62.8, and 65.7), and four sp quaternary carbons (δ 70.0, 70.0, 79.0, and 80.5). In the ^1H NMR spectrum (Table S1, Supporting Information), a broad singlet at δ 8.48, which showed no correlation with any carbon in the HSQC spectrum, was assigned to the hydroxy group. The aforementioned functionalities accounted for 5 degrees of unsaturation, indicating that compound **1** is bicyclic.

The ^1H–^1H COSY data established the presence of two proton-bearing and spin-coupled structural units, A (C-2 to C-11) and B (C-16 to C-18), as drawn in bold (Figure 2).

Analysis of the HMBC data (Figure 2) showed the connectivity between units A and B, the four sp quaternary carbons, and the ester group, which form the 17-membered macrolide backbone of **1**, indicating that it belongs to the ring-constrained series of ivorenolide A.[3] As shown in Figure 2, the ester carbonyl C-1 (δ 173.2) was shown to be attached to C-2 by the HMBC correlations of H_2-2 and H_2-3 to C-1. The presence of a 9,10-epoxy moiety was evident by the diagnostic chemical shifts of CH-9 (δ_C 56.8, δ_H 3.11) and CH-10 (δ_C 62.0, δ_H 3.60) and corroborated by the multiple HMBC correlations

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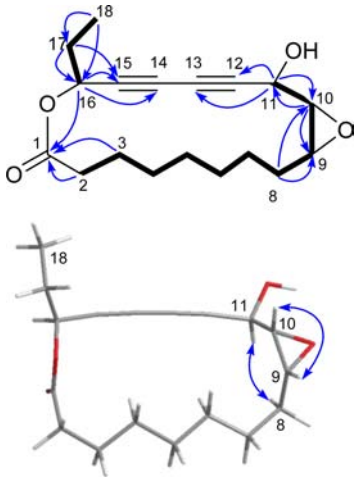
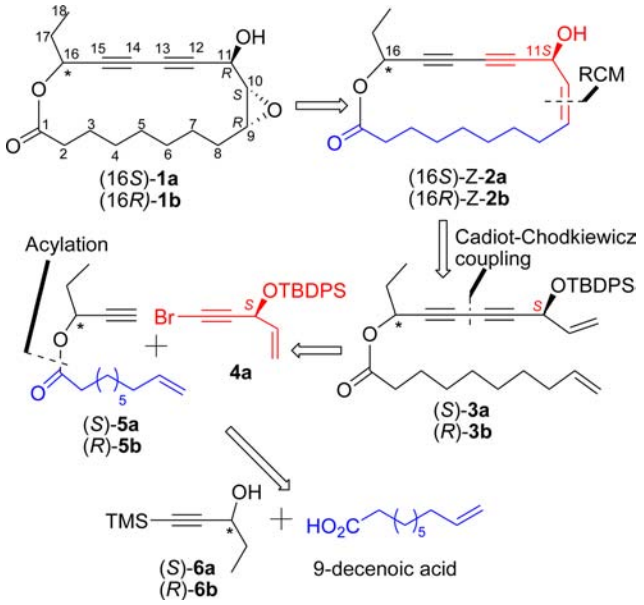


Figure 2. $^{1}H-^{1}H$ COSY (—), selected HMBC (H→C), and key ROESY (↔) correlations of **1**.

of H_2-8 to C-9 and C-10, H-10 to C-9 and C-11, and H-11 to C-10. The hydroxy group was assigned to CH-11 (δ_C 62.8, δ_H 4.87) by the downfield chemical shifts and the mutual HMBC correlations of H-11 to C-10, and H-10 to C-11. The HMBC correlations of H-11 to C-12 (δ_C 80.5) and C-13 (δ_C 70.0) and of H-16 to C-14 (δ_C 70.0) and C-15 (δ_C 79.0) indicated that a conjugated alkynyl moiety (C-12 to C-15, four sp quaternary carbons) was bridged between C-11 and C-16.[3,4] The presence of a 1,16-lactone was indicated by the strong HMBC correlation from H-16 to C-1 and was supported by the downfield chemical shift of H-16 (δ 5.49). The planar structure of **1** was thus determined. The ROESY (Figure 2) correlation between H-9 and H-10 and the small coupling constant ($J_{9,10}$ = 4.4 Hz) revealed that H-9 and H-10 are *cis*-configured, and they were arbitrarily assigned the β-orientation. Consequently, the coupling constant ($J_{10,11}$ = 7.6 Hz) between H-10 and H-11 and the ROESY correlation between H-11 and H-8b (δ 1.38) indicated an α-configuration for H-11. The relative stereochemistry of C-16 could not be assigned with the available data. Compared with ivorenolide A,[3] the highly similar NMR data from C-9 to C-11 in two compounds supported a *trans*-orientation for the 9,10-epoxy and 11-OH in **1**, which thus indicated either a 9*R*,10*S*,11*R* configuration or a 9*S*,10*R*,11*S* configuration. The structure of ivorenolide B (**1**) features an unprecedented 17-membered macrolide that incorporates conjugated acetylenic bonds and four chiral centers.

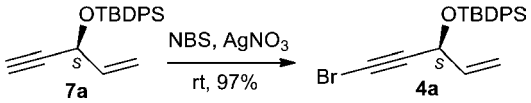
To fully determine the stereochemistry of **1**, a synthetic strategy (Scheme 1) involving a ring-closing metathesis (RCM) as the key step was implemented. This would allow the rapid synthesis of the four most likely stereoisomers, including **1a** (9*R*,10*S*,11*R*,16*S*), **1b** (9*R*,10*S*,11*R*,16*R*), **1c** (9*S*,10*R*,11*S*,16*R*), and **1d** (9*S*,10*R*,11*S*,16*S*). This strategy will also facilitate the modular construction of a small library of stereo analogues to study the immunosuppressive activity. With respect to the retrosynthetic analysis of **1a** and **1b**, it was envisioned that **1a** (**1b**) could be constructed via the asymmetric epoxidation of **2a** (**2b**), which could be accessible from **3a** (**3b**) through ring-closing metathesis (RCM). The cyclization precursor **3a** (**3b**) could be constructed through the assembly of bromoalkyne **4a** (**4b**) and alkyne **5a** (**5b**) via the Cadiot−Chodkiewicz coupling reaction. Finally, **5a** (**5b**) could be obtained by acylation of alcohol **6a** (**6b**) with commercially available 9-decenoic acid.

Scheme 1. Retrosynthetic Analysis of Ivorenolide B (1)



Bromoalkyne **4a** was produced from known starting material **7a**[5] using *N*-bromosuccinimide (NBS) and $AgNO_3$ (Scheme 2).

Scheme 2. Preparation of 4a



The synthesis of **8a** commenced with acylation of known alcohol **6a**[6] with commercially available 9-decenoic acid in the presence of DCC and DMAP. Compound **8a** was transformed to **5a** by removal of the trimethylsilyl (TMS) group. With **4a** and **5a** in hand, we next focused on the preparation of **3a**, which was obtained in good yield in the presence of Cu(I) via the Cadiot−Chodkiewicz coupling.[7] In an attempt to facilitate the ring-closing metathesis (RCM)[8] of **3a**, we first employed the Grubbs second-generation catalyst in CH_2Cl_2 at room temperature; however, no product was formed, even when the reaction was refluxed for 24 h. When we utilized toluene as the solvent and heated the reaction to 80 °C for 24 h, the desired RCM product **2a** (*Z*/*E* = 1/1.6) was obtained in 90% yield. Interestingly, when using the first-generation Grubbs catalyst and refluxing in CH_2Cl_2, the desired product **2a** was obtained in better yield (Table 1). Oxidation of **2a** (Z-form) with *m*-chloroperoxybenzoic acid (*m*-CPBA) afforded **9a** as the sole product; it is assumed that the *m*-CPBA approached the double bond from the sterically less hindered face of the molecule.[9] Finally, removal of the TBDPS ether afforded **1a** (Scheme 3). Similarly, by using **6b** (*R*-form) as the starting alcohol, compound **1b** was synthesized.

The conjugated acetylenic bonds and the 9,10-epoxy moiety cause the molecule to adopt a planar loop conformation. Thus, the NMR data (Table S1, Supporting Information) of the four stereoisomers were predicted to possess high similarities. To determine the absolute configuration of **1** and extend the scope of our medicinal chemistry studies, compounds **1c** and **1d** (Figure 3), the enantiomers of **1a** and **1b**, respectively, were

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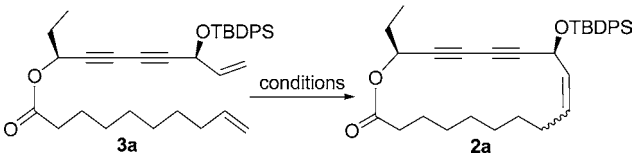
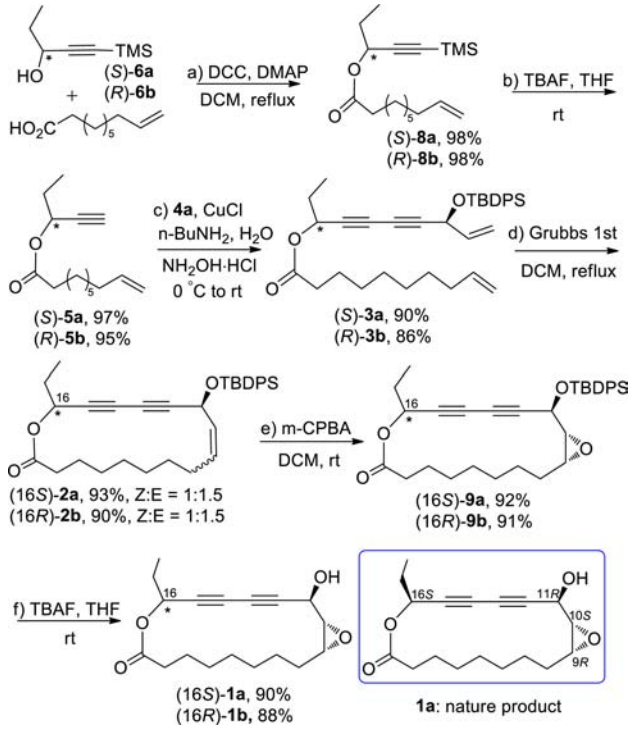
Table 1. Optimization of the RCM Conditions with 3a[a]



entry	catalyst	solvent	temp (°C)	time (h)	yield (%)
1	Grubbs II	CH_2Cl_2	rt	24	0
2	Grubbs II	CH_2Cl_2	43	24	0
3	Grubbs II	toluene	80	24	90 (*Z*:*E* = 1:1.6)
4	Grubbs I	CH_2Cl_2	43	24	93 (*Z*:*E* = 1:1.5)

[a]Reactions were performed in the indicated solvents (0.001 M) under argon, and the catalysts were added in one portion.

Scheme 3. Synthesis of 1a and 1b



synthesized from **4b** (*R*-form of **4a**) using the same strategy (Supporting Information).

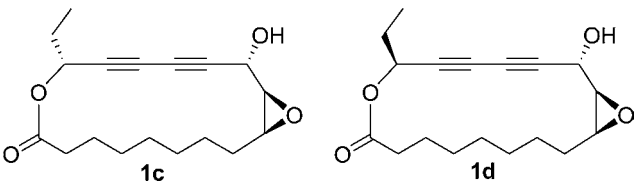


Figure 3. Structures of **1c** and **1d**.

The NMR spectra of compounds **1** and **1a**–**d** showed high similarities with slight variations of the proton and/or carbon chemical shifts of C-1, CH_2-3, CH_2-8, CH-9, CH-16, and CH_3-17 (Table S1, Supporting Information). Extensive comparison showed that the NMR spectra of compounds **1**, **1a**, and **1c** completely matched, indicating that their structures were the same and/or enantiomers, and the NMR spectra of **1b** also well matched those of **1d**, confirming that they were enantiomers.

The specific rotations $[\alpha]^{25}_D = -13.0$ (for **1**, *c* 0.126, MeOH), −8.3 (for **1a**, *c* 0.15, MeOH), and +9.7 (for **1c**, *c* 0.3, MeOH) further indicated that the absolute configuration of ivorenolide B (**1**) was identical to that of the synthetic **1a** (9*R*,10*S*,11*R*,16*S*). This conclusion was confirmed by CD analysis (Figure 4), in which the CD curve of **1** matched that of

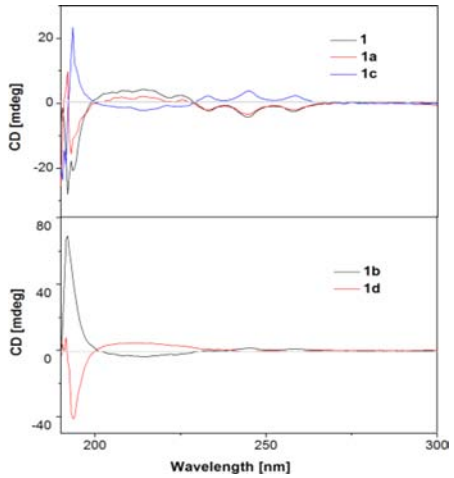


Figure 4. CD spectra of compounds **1** and **1a**–**d**.

1a and was opposite to **1c** that is the enantiomer of **1a**, and the CD curves of two enantiomers **1b** and **1d** were opposite, which was consistent with their specific rotations (Supporting Information). Thus, the absolute configuration of **1** was determined to be 9*R*,10*S*,11*R*,16*S*.

Ivorenolide B (**1**) and the synthetic compounds **1a**–**d** were then evaluated for immunosuppressive activity. Compound **1** and its synthetic equivalent **1a** exhibited significant inhibition of LPS-induced B-cell proliferation, while the synthetic enantiomer **1c** and stereoisomers **1b** and **1d** were inactive (Table 2).

Table 2. Immunosuppressive Effects of 1 and 1a–d on Murine Lymphocyte Proliferation Induced by ConA (5 μg/mL) or LPS (10 μg/mL)[a,b]

		ConA-induced T-cell proliferation		LPS-induced B-cell proliferation	
compd	CC_{50} (μM)	IC_{50} (μM)	SI	IC_{50} (μM)	SI
1	20.7	>10	–	7.2	2.9
1a	21.7	>10	–	8.5	2.6
1b	N	N	–	N	–
1c	N	N	–	N	–
1d	N	N	–	N	–

[a]The selectivity index (SI) is defined as the ratio of the concentration of the compound that reduced cell viability to 50% (CC_{50}) to the concentration of the compound needed to inhibit the proliferation by 50% relative to the control value (IC_{50}). [b]N indicates inactive, which was defined as inhibition rates lower than 30% at 10 μM.

In conclusion, this study identified an immunosuppressive agent, ivorenolide B (**1**), isolated from *K. ivorensis*. Compound **1** featured an unprecedented 17-membered macrolide with conjugated acetylenic bonds and four chiral centers. The absolute configuration of **1** was determined through asymmetric total synthesis, which was successfully achieved in six steps with a 26% overall yield from the known compounds. Compared to our recent 12-step synthesis of ivorenolide A,[3]

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this approach is more concise and effective involving a Cadiot–Chodkiewicz coupling and a ring-closing metathesis reaction as the key steps to construct the macrolide scaffold. With the same synthetic strategy, the enantiomer **1c** and two diastereoisomers **1b** and **1d** were also synthesized, thereby allowing the assignment of the absolute stereochemistry of **1**. Both the natural product **1** and its synthetic equivalent **1a** showed remarkable immunosuppressive activities. This finding and the formally reported immunosuppressive ivorenolide A have provided novel macrolide scaffolds for the development of immunosuppressive agents.

■ ASSOCIATED CONTENT

Supporting Information

Detailed synthetic information as well as spectroscopic characterization of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Author Contributions

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Notes

The authors declare no competing financial interest.

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