

Article

Total Syntheses and Stereochemical Assignment of Acremolides A and B

Yi Xiao ^{1,†}, Junyang Liu ^{1,2,†} , Yangyang Jiang ¹, Yian Guo ^{1,*}  and Tao Ye ^{1,3,*} 

¹ State Key Laboratory of Chemical Oncogenomics, Peking University Shenzhen Graduate School, Shenzhen 518055, China; 2001112078@pku.edu.cn (Y.X.); liujy@wyu.edu.cn (J.L.); yangyangjiang@stu.pku.edu.cn (Y.J.)

² School of Pharmacy and Food Engineering, Wuyi University, Jiangmen 529020, China

³ Qian Yan (Shenzhen) Pharmatech. Ltd., Shenzhen 518172, China

* Correspondence: yanguo@pku.edu.cn (Y.G.); yet@pkusz.edu.cn (T.Y.)

† These authors contributed equally to this work.

Abstract: The absolute stereochemical configurations of acremolides A and B were predicted by a biochemistry-based rule and unambiguously confirmed through their total syntheses. The features of the total syntheses include sequential Krische's Ir-catalyzed crotylation, Brown's borane-mediated crotylation, Mitsunobu esterification reaction, and cross-metathesis reaction. The efficient total synthesis enabled clear validation of the predicted stereochemistry for acremolides A and B.

Keywords: total syntheses; natural products; asymmetric crotylation; acremolide

1. Introduction

Originally identified by Capon et al. in 2007, acremolides A–D were isolated from *Acronium* sp. (MST-MF588a) discovered in an Australian marine-derived fungus [1]. Acremolides A–D represent a novel class of lipodepsipeptides characterized by a 12-membered macrocyclic lactam that is composed of a dipeptide unit and a substituted fatty acid fragment. Utilizing a novel C₃ Marfey's method [2], Capon and co-workers successfully determined the absolute configurations of the amino acid fragments within the molecule, namely, L-Pro and D-Phe. However, due to the unsuccessful coupling of the acremolides A and B with (S)-Mosher's reagent [3], the relative and absolute stereochemistry of the fatty acid within these natural products remains unassigned. Confirming the absolute stereochemistry of natural products through total synthesis is a well-established approach [4–8]. Prior efforts have not yet unveiled the stereochemistry of acremolides. In 2010, Cossy's group accomplished the total synthesis of the one and only stereoisomer of acremolide B. Putatively, the synthesis of the remaining 15 stereoisomers is necessary to determine its absolute stereochemistry [9]. In 2017, Pabbaraja's group endeavored to synthesize multiple isomers of acremolide B to elucidate its absolute configuration but encountered difficulties in macrocyclization via esterification [10]. Thus, the absolute stereochemistry of acremolides A–D remains elusive. For many years, our research has focused on elucidating the absolute stereochemistry of cyclic peptide natural products through total synthesis [11,12]. This work enables further investigations into the structure–activity relationships (SARs) of these biologically active natural products. We are interested in the absolute configurations of acremolides A and B, and to this end, we embarked on the synthesis of these two natural products aimed toward the assignment of their stereochemical structures.

Fungal HR-PKSs (highly reducing polyketide synthases) are single-module enzymes that catalyze the elongation of linear fatty acyl chains with exquisite stereochemical control. Recently, we and co-workers discovered a unified stereochemical course for polyhydroxy PKs, phialotides, phomenoic acid, and ACR-toxin (acyl carrier protein toxin), and we theorized the biochemistry-based rule for the configuration prediction of fungal-reduced



Citation: Xiao, Y.; Liu, J.; Jiang, Y.; Guo, Y.; Ye, T. Total Syntheses and Stereochemical Assignment of Acremolides A and B. *Molecules* **2024**, *29*, 3599. <https://doi.org/10.3390/molecules29153599>

Academic Editor: Xinghai Liu

Received: 25 June 2024

Revised: 24 July 2024

Accepted: 26 July 2024

Published: 30 July 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

polyketides [13]. Considerations on the biosynthesis of acremolides A and B and the rules related to the prediction of stereochemical configuration (please see the Supplementary Materials for details) led to the proposal that its previously unknown stereostructure should be represented as illustrated in Figure 1. Thus, the ketoreductase (KR) domain reduces the corresponding keto group to give the *R*-configured C11, *S*-configured C5, and *R*-configured C3 (marked in red); the enoyl reductase domain iteratively reduces enoyl moieties to give the *S*-configured C6 (marked in green); and the methyltransferase (MT) domain installs a methyl group at the α position to give the *R*-configured C2 (marked in blue). As such, we predict the absolute configuration of the acremolides A and B as depicted in Figure 1. The following task is to synthesize these compounds to substantiate our hypothesis.

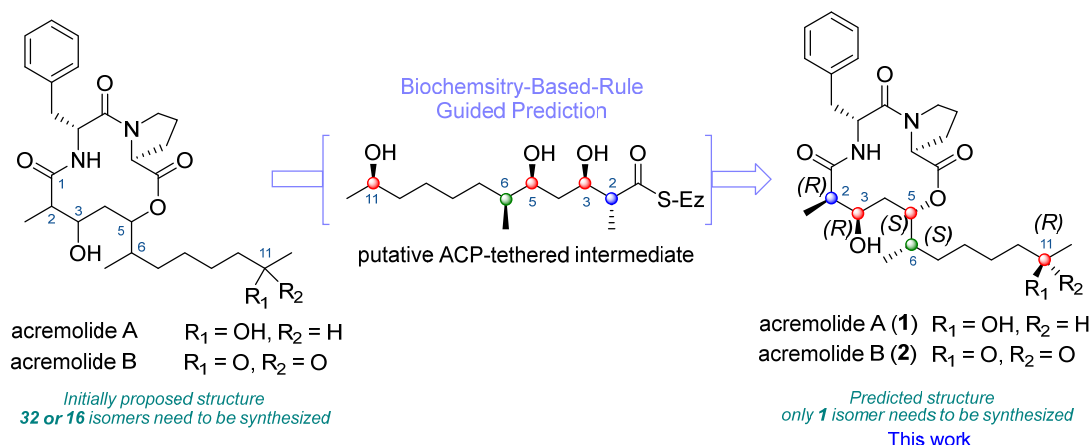
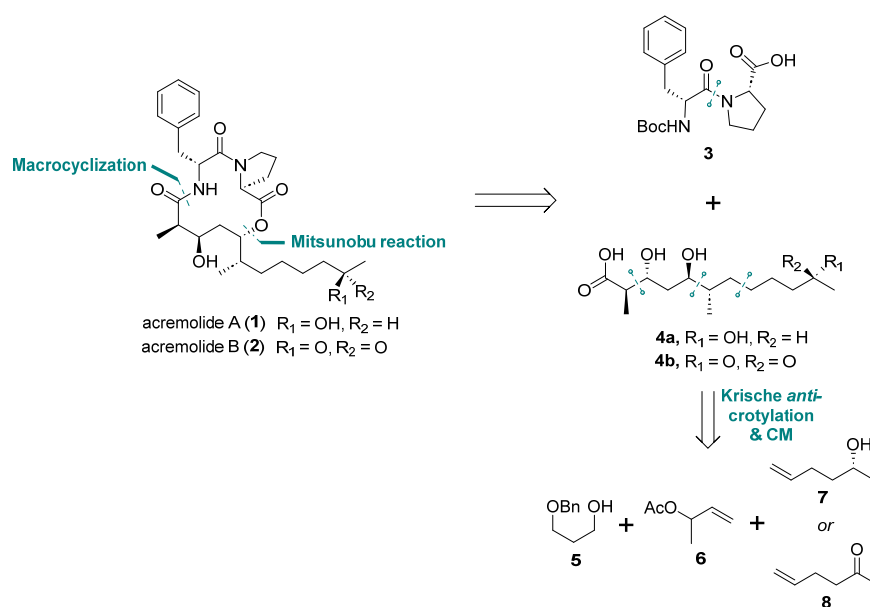


Figure 1. Biochemistry-based-rule guided prediction of the structure of acremolides A and B.

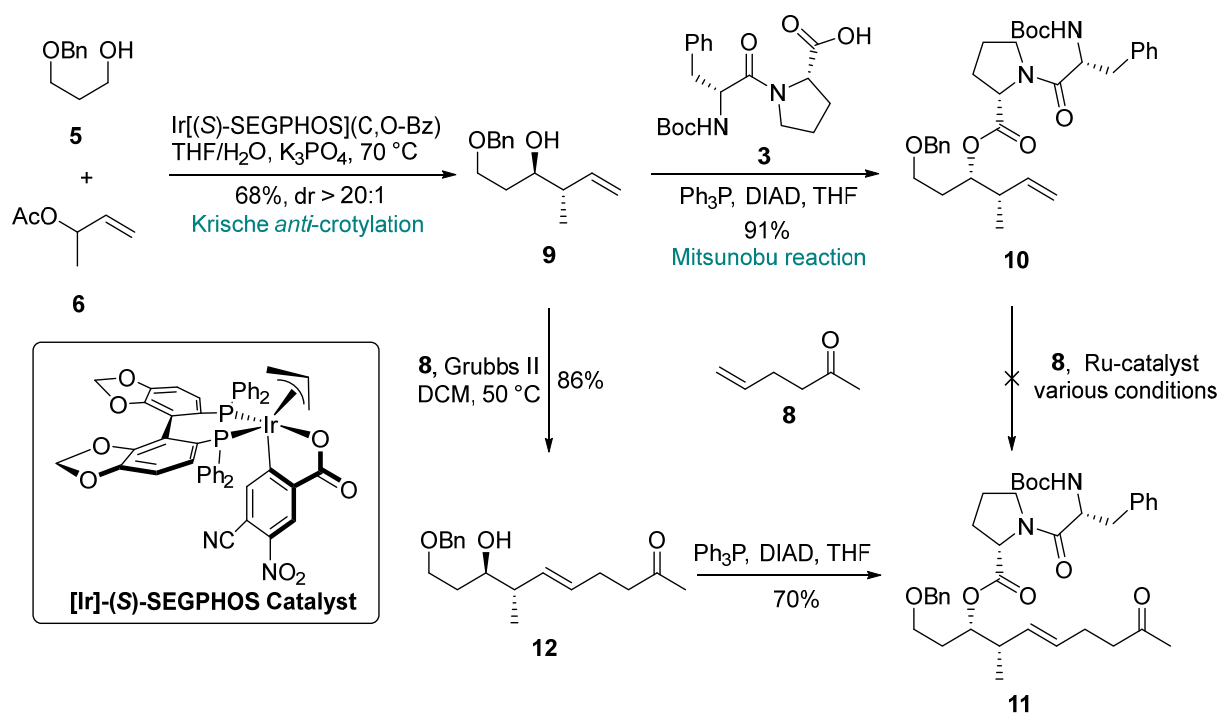
As shown in Scheme 1, our retrosynthetic analysis indicates that acremolides A and B could be prepared from dipeptide **3** and fatty acid **4** via a Mitsunobu reaction [14] and macrocyclization to form the 12-membered ring. It was further envisioned that intermediate **4** could be produced by a double Krische's asymmetric *anti*-crotylation [15] and an olefin cross-metathesis reaction [16,17], tracing back to four key compounds: **5**, **6**, **7**, and **8**. Compared to acremolide A, acremolide B has one less stereogenic center and requires fewer synthetic steps. Therefore, our initial endeavor was focused on the access to acremolide B.



Scheme 1. Retrosynthetic analysis of acremolides A (1) and B (2).

2. Results and Discussion

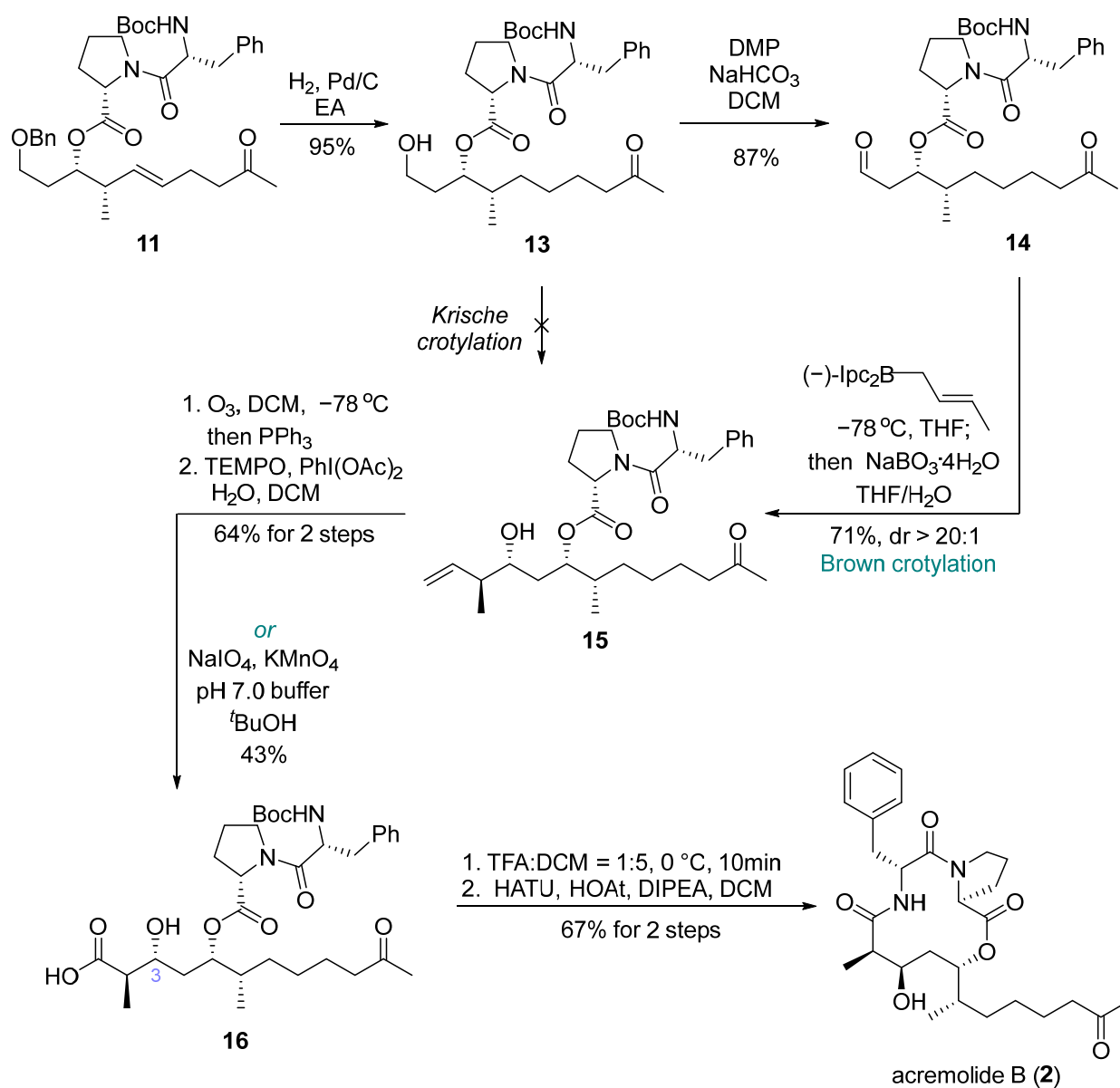
Our synthesis of acremolide B commenced with the conversion of the known compounds **5** and **6** into homoallylic alcohol **9** (Scheme 2). Utilizing Krische's methodology [15] with the chiral iridium catalyst Ir(S)-SEGPHOS, potassium phosphate, and heating at 70 °C in THF, we converted these compounds to product **9** with a remarkable yield of 68%, accompanied by an impressive stereoselectivity of dr > 20:1. Compound **9** was then esterified with dipeptide fragment **3** under Mitsunobu conditions (Ph₃P, DIAD), yielding ester **10** in a high yield of 91%. Our efforts then focused on introducing the fatty acid side chain to obtain the crucial intermediate **11** through an olefin cross-metathesis reaction involving terminal olefins **10** and **8**. Extensive conditions were surveyed, which included screening various catalysts such as the Hoveyda–Grubbs II [18,19] and Grubbs II catalysts [20,21], along with exploring different solvents and temperatures. However, these two fragments failed to undergo intermolecular olefin cross-metathesis reaction. This observed behavior can arguably be attributed to the spatial hindrance present at the olefinic α and β positions of olefin **10**, which was considered as a type IV olefin [22]. Alternatively, less hindered olefin **9** underwent cross-metathesis smoothly with olefin **8** in a heated (50 °C) sealed tube in the presence of Grubbs II catalyst, resulting in an 86% yield of product **12**. Subsequently, intermediate **12** was subjected to a Mitsunobu reaction to furnish the key fragment **11** with a moderate yield of 70%.



Scheme 2. Synthesis of ester **11**.

With compound **11** in hand, our phase was set for the total synthesis of acremolide B (Scheme 3). Hydrogenation of olefin **11** using 10% Pd/C under a hydrogen atmosphere afforded compound **13** with 95% high yield. We opted for Brown crotylation reaction [23,24] to achieve this transformation. Initially, alcohol **13** was converted to aldehyde **14** via Dess–Martin periodinane oxidation [25]. The resulting aldehyde was then subjected to standard Brown crotylation conditions, affording alcohol **15** in a moderate yield of 71%, with a high stereoselectivity of dr > 20:1. Under Krische's conditions, oxidative cleavage of the terminal double bond to carboxylic acid was achieved in a single step using NaIO₄ and KMnO₄ [26] as co-oxidants in a pH = 7.0 buffered solvent, albeit with a modest yield of 43%. To improve the yield of this transformation, a two-step process was implemented. It involved ozonolysis to cleave the terminal double bond to an aldehyde, followed by a Pin-

nick oxidation reaction to obtain carboxylic acid **16**. However, we found that the secondary hydroxyl group at C3 underwent an elimination reaction under the Pinnick oxidation conditions. To prevent the elimination reaction, we chose the milder TEMPO/PhI(OAc)₂ oxidation system [27], ultimately affording carboxylic acid **16** in two steps with an overall yield of 64%.

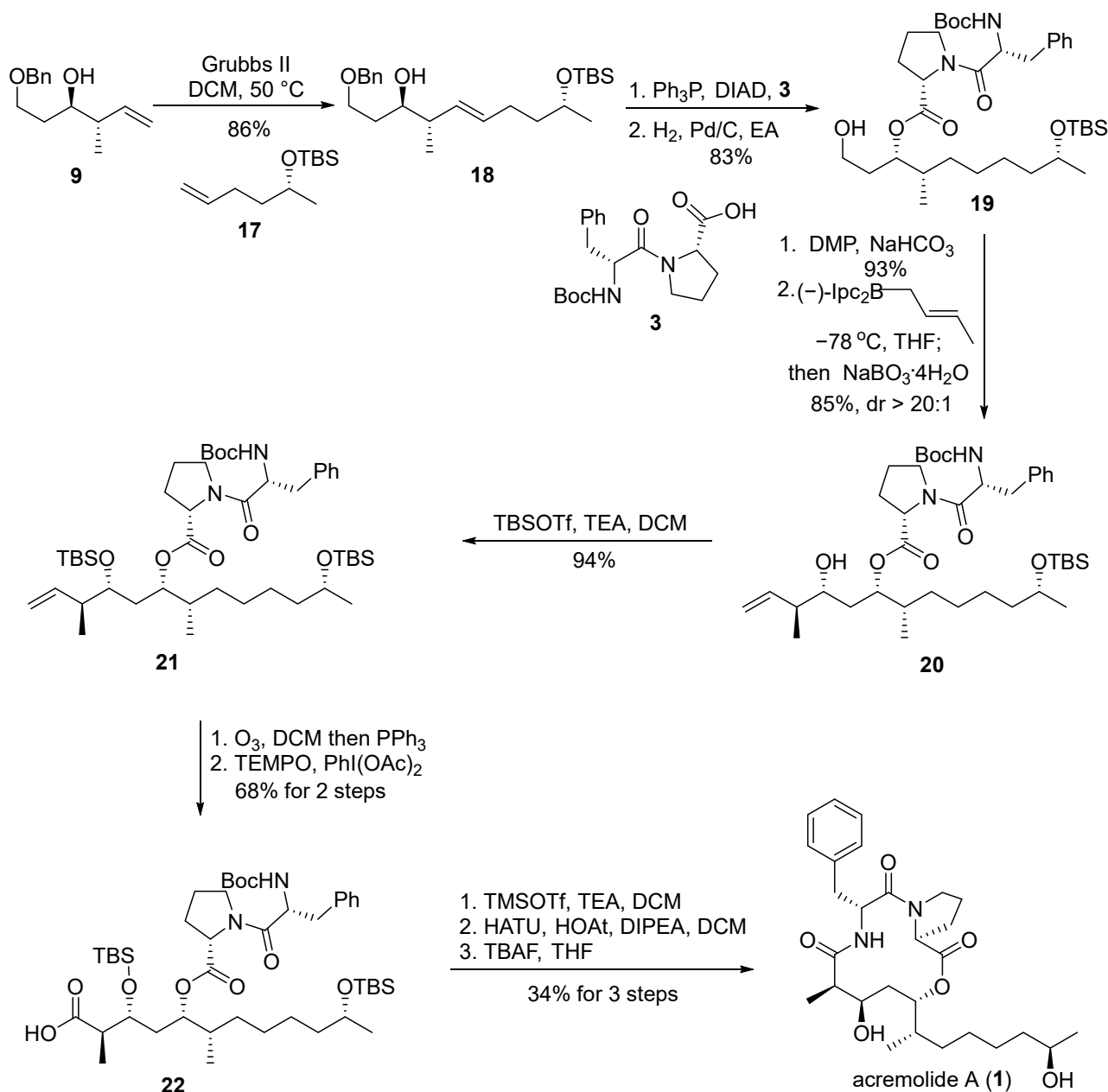


Scheme 3. Total synthesis of acremolide B (**2**).

Recognizing the potential risk of C3 secondary hydroxyl group elimination under acidic Boc-removal conditions, we optimized the reaction condition. Ultimately, we found that the Boc- group was successfully removed in 10 min by employing a TFA/DCM solvent with a volume ratio of 1:5 at 0°C , and the occurrence of the elimination reaction was also prevented effectively. Finally, the macrocyclization was performed under HATU/HOAt/DIPEA conditions, affording acremolide B (**2**) in two steps with an overall yield of 67%. The ^1H and ^{13}C NMR spectra data and the optical rotation for our synthetic acremolide B (**2**) were consistent with the literature values. As such, we assigned the structure of acremolide B (**2**) as depicted in Figure 1.

Upon completing the total synthesis of acremolide B and establishing its absolute stereochemistry, our next task is to explore the total synthesis of acremolide A and unveil its

stereochemistry. Given the structural similarity between acremolide A and B, the synthetic route to acremolide B was similarly applied to acremolide A, as shown in Scheme 4.



Scheme 4. Total synthesis of acremolide A (**1**).

The cross-metathesis of terminal olefins **9** and **17** with Grubbs II catalyst generated compound **18** in a good yield of 86%. Mitsunobu esterification of alcohol **18** and carboxylic acid **3** followed by hydrogenation of the double bond and simultaneous deprotection of the Bn- protecting group yielded **19** in an overall yield of 83% over two steps. Subsequently, Dess–Martin periodinane oxidation of **19** afforded an aldehyde (not shown), which was in turn subjected to Brown crotylation conditions, furnishing intermediate **20** in a good yield of 85%, with a remarkable stereoselectivity of dr > 20:1. Protection of the secondary hydroxyl group in **20** with TBS silyl group furnished **21** without incident. Following this, cleavage of the double bond by ozone and subsequent oxidation of the resulting aldehyde to a carboxylic acid under TEMPO/PhI(OAc)₂ conditions resulted in compound **22** in 68% overall yield. Eventually, deprotection of the Boc- protection group under TMSOTf/TEA conditions, followed by macrocyclization using HATU/HOAT/DIPEA conditions, and

subsequent removal of the TBS silyl group, resulted in the synthesis of natural product acremolide A in 34% overall yield in three steps (1). The ^1H and ^{13}C NMR spectra data and the optical rotation for our synthetic acremolide A (1) were consistent with the literature values. On this basis, we assigned the structure of acremolide A (1) as depicted in Figure 1.

3. Materials and Methods

3.1. General Experimental Details

All reactions were conducted in flame-dried or oven-dried glassware under an atmosphere of dry nitrogen or argon. Oxygen and/or moisture-sensitive solids and liquids were transferred appropriately. The concentration of solutions in vacuo was accomplished using a rotary evaporator fitted with a water aspirator. Residual solvents were removed under a high vacuum (0.1–0.2 mm Hg). All reaction solvents were purified before use: Tetrahydrofuran (THF) was distilled from Na/benzophenone. Toluene was distilled over molten sodium metal. Dichloromethane (DCM), 1,2-dichloroethane (DCE) and trimethylamine (Et_3N) were distilled from CaH_2 . Methanol (MeOH) was distilled from Mg/I_2 . The reagents were purchased at the highest commercial quality and used without further purification unless otherwise stated. Flash column chromatography was performed using the indicated solvents on silica gel 60 (230–400 mesh ASTM E, Qingdao, China). Reactions were monitored using thin-layer chromatography (TLC), which was carried out using pre-coated sheets (Qingdao silica gel 60-F250, 0.2 mm). Compounds were visualized with UV light, iodine, and ceric ammonium molybdate stainer phosphomolybdic acid in EtOH. The ^1H NMR spectra were recorded on Avance 400 MHz spectrometers (Bruker, Karlsruhe, Germany). Chemical shifts were reported in parts per million (ppm), relative to either a tetramethylsilane (TMS) internal standard or the signals due to the solvent. The following abbreviations are used to describe the spin multiplicity: s = singlet; d = doublet; t = triplet; q = quartet; qn = quintet; m = multiplet; br = broad; dd = doublet of doublets; dt = doublet of triplets; dq = doublet of quartets; ddd = doublet of doublet of doublets. Other combinations are derived from those listed above. Coupling constants (J) are reported in Hertz (Hz) for corresponding solutions, and chemical shifts are reported as parts per million (ppm) relative to residual CHCl_3 δH (7.26 ppm). ^{13}C -NMR nuclear magnetic resonance spectra were recorded at 100 MHz, 125 MHz, or 150 MHz for corresponding solutions, and chemical shifts are reported as parts per million (ppm) relative to residual CDCl_3 δC (77.16 ppm). High-resolution mass spectra were measured on an ABI Q-star Elite (Applied Biosystems, Beijing, China). Optical rotations were recorded on a Rudolph AutoPol-I polarimeter (Shanghai, China) at 589 nm with a 50 mm cell. Data are reported as follows: specific rotation (c (g/100 mL), solvent).

Synthesis of Compounds [28]

(3R,4S)-1-(benzyloxy)-4-methylhex-5-en-3-ol 9:

A pressure tube was charged with alcohol 5 (43 mg, 0.26 mmol, 1.0 equiv.), catalyst [Ir] (14 mg, 0.013 mmol, 0.05 equiv.), alkene 6 (89 mg, 0.78 mmol, 3.0 equiv.), and potassium phosphate (62 mg, 0.29 mmol, 1.1 equiv.). The pressure tube was carefully purged with argon and degassed tetrahydrofuran (0.26 mL, 1.0 M) was added, followed by the addition of distilled water (24 μL , 1.3 mmol, 5.0 equiv.). The tube was sealed, avoiding air contamination, and then heated to 70 $^\circ\text{C}$ for 48 h in an oil bath. The reaction mixture was allowed to cool to room temperature and then diluted with dichloromethane (10 mL). Solids were filtered off using a celite pad and rinsed with dichloromethane (3 \times 5 mL). Solvents were evaporated to give a brown oil. The crude material was absorbed on silica gel and purified by flash chromatography (ethyl acetate/hexanes = 1/9) to afford alcohol 9 (39 mg, 68%) as a colorless oil. TLC: R_f = 0.30 (silica gel, ethyl acetate/hexanes = 1/9). UV and PMA stain. $[\alpha]_D^{26} = -4.3$ (c 1.0, CH_3OH). ^1H NMR (400 MHz, CDCl_3) δ 7.54–7.17 (m, 5H), 6.10–5.70 (m, 1H), 5.13 (s, 1H), 5.10 (d, J = 8.8 Hz, 1H), 4.56 (s, 2H), 3.83–3.63 (m, 3H), 2.82 (s, 1H), 2.89–2.74 (m, 1H), 1.78 (q, J = 7.8, 6.8 Hz, 2H), 1.08 (d, J = 6.9 Hz, 3H). ^{13}C NMR (101 MHz,

CDCl₃) δ 140.6, 138.0, 128.5, 127.8, 115.6, 74.3, 73.4, 69.3, 44.1, 33.6, 15.9. HRMS (ESI, m/z) for C₁₄H₂₁O₂⁺ [M+H]⁺: Calcd. 221.1536; found: 221.1537.

(3*S*,4*S*)-1-(benzyloxy)-4-methylhex-5-en-3-yl(*tert*-butoxycarbonyl)-*D*-phenylalanyl-*L*-proline **10**:

To a solution of alcohol **9** (34 mg, 0.16 mmol, 1.0 equiv.) and acid **3** (112 mg, 0.31 mmol, 2.0 equiv.) in anhydrous THF (2.0 mL, 0.08 M) at 0 °C under an argon atmosphere, PPh₃ (122 mg, 0.47 mmol, 3.0 equiv.) and DIAD (61 μ L, 0.31 mmol, 2.0 equiv.) were added. After being stirred at room temperature for 4 h, the reaction mixture was quenched with saturated aqueous solution of NaHCO₃ (10 mL) and extracted with EtOAc (3 \times 10 mL). The combined organic layers were washed with saturated aqueous solution of brine (20 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica gel (hexanes/EtOAc = 4/1) to afford **10** (80 mg, 91%) as a colorless oil. TLC: R_f = 0.40 (silica gel, ethyl acetate/hexanes = 1/4). UV and PMA stain. $[\alpha]_D^{26} = -48.60$ (c 1.0, CH₃OH). ¹H NMR (400 MHz, CDCl₃) δ 7.39–7.32 (m, 4H), 7.32–7.20 (m, 6H), 5.75 (ddd, $J = 17.5, 9.8, 7.2$ Hz, 1H), 5.41 (d, $J = 8.6$ Hz, 1H), 5.14–5.05 (m, 2H), 5.03 (d, $J = 4.9$ Hz, 1H), 4.66 (td, $J = 9.0, 5.4$ Hz, 1H), 4.53 (q, 2H), 4.33 (dd, $J = 8.2, 3.7$ Hz, 1H), 3.64–3.44 (m, 3H), 3.08 (dd, $J = 12.8, 5.5$ Hz, 1H), 2.95 (dd, $J = 12.7, 9.4$ Hz, 1H), 2.61 (dt, $J = 9.5, 6.8$ Hz, 1H), 2.43 (q, $J = 6.7$ Hz, 1H), 2.00–1.73 (m, 5H), 1.43 (s, 9H), 1.37–1.32 (m, 1H), 1.02 (d, $J = 6.8$ Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.5, 170.1, 155.0, 139.7, 138.5, 136.6, 129.6, 128.4, 128.4, 128.0, 127.6, 127.0, 115.4, 79.7, 74.7, 73.3, 66.8, 59.1, 53.6, 46.8, 41.5, 40.5, 31.9, 29.1, 28.4, 24.4, 21.8, 14.8. HRMS (ESI, m/z) for C₃₃H₄₅N₂O₆⁺ [M+H]⁺: Calcd. 565.3272; found: 565.3270.

(7*S*,8*R*,*E*)-10-(benzyloxy)-8-hydroxy-7-methyldec-5-en-2-one **12**:

To a solution of **9** (16 mg, 0.073 mmol, 1.0 equiv.) and alkene **8** (14 mg, 0.15 mmol, 2.0 equiv.) in DCM (1.0 mL, 0.07 M) at room temperature, Grubbs II catalyst (13 mg, 0.015 mmol, 0.2 equiv.) was added. The reaction mixture was heated at 50 °C for 9 h before it was cooled back to room temperature and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica (hexanes/EtOAc = 4/1) to afford **12** (18 mg, 86%) as a colorless oil. TLC: R_f = 0.20 (silica gel, ethyl acetate/hexanes = 1/4). UV and PMA stain. $[\alpha]_D^{28} = -13.20$ (c 1.0, CH₃OH). ¹H NMR (400 MHz, CDCl₃) δ 7.45–7.30 (m, 5H), 5.61–5.32 (m, 2H), 4.56 (s, 2H), 3.88–3.50 (m, 3H), 2.76 (s, 1H), 2.54 (t, $J = 7.3$ Hz, 2H), 2.42–2.27 (m, 2H), 2.19 (d, $J = 6.3$ Hz, 1H), 2.17 (s, 3H), 1.82–1.70 (m, 2H), 1.04 (d, $J = 6.9$ Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 208.6, 138.1, 133.0, 129.8, 128.5, 127.8, 74.4, 73.4, 69.3, 43.4, 43.1, 33.8, 31.5, 30.1, 27.0, 16.6. HRMS (ESI, m/z) for C₁₈H₂₇O₃⁺ [M+H]⁺: Calcd. 291.1955; found: 291.1955.

(3*S*,4*S*,*E*)-1-(benzyloxy)-4-methyl-9-oxodec-5-en-3-yl(*tert*-butoxycarbonyl)-*D*-phenylalanyl-*L*-proline **11**:

To a solution of alcohol **12** (90 mg, 0.31 mmol, 1.0 equiv.) and acid **3** (230 mg, 0.63 mmol, 2.0 equiv.) in anhydrous THF (4.0 mL, 0.08 M) at 0 °C under an argon atmosphere, PPh₃ (246 mg, 0.94 mmol, 3.0 equiv.) and DIAD (130 μ L, 0.62 mmol, 2.0 equiv.) were added. After being stirred at room temperature for 9 h, the reaction mixture was quenched with saturated aqueous solution of NaHCO₃ (10 mL) and extracted with EtOAc (3 \times 10 mL). The combined organic layers were washed with saturated aqueous solution of brine (20 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica gel (hexanes/EtOAc = 3/1) to afford **11** (139 mg, 70%) as a colorless oil. TLC: R_f = 0.40 (silica gel, ethyl acetate/hexanes = 1/3). UV and PMA stain. $[\alpha]_D^{26.9} = -38.80$ (c 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 7.39–7.36 (m, 4H), 7.28–7.22 (m, 6H), 5.50–5.30 (m, 2H), 5.04–4.96 (m, 1H), 4.70–4.61 (m, 1H), 4.61–4.44 (m, 2H), 4.33 (dd, $J = 8.2, 3.5$ Hz, 1H), 3.62–3.47 (m, 3H), 3.09 (dd, $J = 12.8, 5.4$ Hz, 1H), 2.95 (dd, $J = 12.8, 9.4$ Hz, 1H), 2.66–2.57 (m, 1H), 2.55–2.47 (m, 2H), 2.35 (q, $J = 6.8$ Hz, 1H), 2.31–2.23 (m, 2H), 2.16 (s, 3H), 1.98–1.87 (m, 2H), 1.87–1.74 (m, 3H), 1.43 (s, 9H), 0.97 (d, $J = 6.8$ Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 208.4, 171.6, 170.1, 155.0, 138.6, 136.6, 132.4, 129.6, 129.5, 128.4, 128.4, 128.0, 127.6, 127.0, 79.7, 75.1, 73.3, 66.8, 59.0, 53.6, 46.8, 43.3,

40.7, 40.5, 32.2, 30.0, 29.1, 28.4, 26.7, 24.4, 15.7. HRMS (ESI, m/z) for $C_{37}H_{51}N_2O_7^+$ $[M+H]^+$: Calcd. 635.3691; found: 635.3694.

(3*S*,4*S*)-1-hydroxy-4-methyl-9-oxodecan-3-yl(*tert*-butoxycarbonyl)-*D*-phenylalanyl-*L*-prolinate **13**:

To a solution of **11** (437 mg, 0.69 mmol, 1.0 equiv.) in EtOAc (15 mL, 0.05 M), palladium on charcoal (200 mg, 10% Pd, 0.19 mmol, 0.28 equiv.) was added under argon atmosphere. The reaction flask was evacuated and purged with hydrogen three times. The reaction mixture was stirred under a hydrogen atmosphere at room temperature for 9 h; the flask was then evacuated and purged with nitrogen three times, and the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated under reduced pressure to afford crude product, which was purified by flash chromatography on silica gel (hexanes/EtOAc = 1/1) to afford **13** (358 mg, 95%) as a colorless oil. TLC: R_f = 0.50 (silica gel, ethyl acetate/hexanes = 1/1). UV and PMA stain. $[\alpha]_D^{20.7} = -45.80$ (c 1.0, $CHCl_3$). 1H NMR (400 MHz, $CDCl_3$) δ 7.22–7.09 (m, 5H), 5.41 (d, J = 8.6 Hz, 1H), 5.04–4.92 (m, 1H), 4.60–4.50 (m, 1H), 4.17 (dd, J = 8.4, 4.1 Hz, 1H), 3.67–3.55 (m, 2H), 3.49–3.38 (m, 1H), 2.98 (dd, J = 12.8, 5.4 Hz, 1H), 2.84 (dd, J = 12.8, 9.5 Hz, 1H), 2.61–2.49 (m, 1H), 2.34 (t, J = 7.3 Hz, 2H), 2.05 (s, 3H), 1.92–1.83 (m, 1H), 1.81–1.73 (m, 2H), 1.72–1.65 (m, 2H), 1.49–1.40 (m, 3H), 1.36 (s, 9H), 1.28–1.16 (m, 4H), 1.10–0.99 (m, 1H), 0.81 (d, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 209.2, 172.6, 170.4, 155.0, 136.4, 129.5, 128.4, 127.0, 79.7, 75.1, 59.1, 58.6, 53.6, 46.9, 43.6, 40.3, 36.8, 34.7, 32.8, 29.9, 29.1, 28.4, 26.6, 24.6, 23.8, 14.5. HRMS (ESI, m/z) for $C_{30}H_{47}N_2O_7^+$ $[M+H]^+$: Calcd. 547.3378; found: 547.3377.

(3*S*,4*R*,6*S*,7*S*)-4-hydroxy-3,7-dimethyl-12-oxotridec-1-en-6-yl(*tert*-butoxycarbonyl)-*D*-phenylalanyl-*L*-prolinate **15**:

To a solution of alcohol **13** (237 mg, 0.43 mmol, 1.0 equiv.) in dry DCM (10 mL, 0.043 M) at 0 °C under argon atmosphere, Dess–Martin periodinane (221 mg, 0.52 mmol, 1.2 equiv.) was added. After being stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous solution of Na_2SO_3 (10 mL) and extracted with EtOAc (3 \times 10 mL). The combined organic extracts were washed with saturated aqueous solution of copper sulfate (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo. The residue was used directly in the next step without further purification.

To a suspension of *t*BuOK (246 mg, 2.2 mmol, 5.0 equiv.) in anhydrous THF (10 mL, 0.045 M) at -78 °C, *trans*-2-butene (172 mg, 3.1 mmol, 7.0 equiv.) was added, followed by the dropwise addition of *n*BuLi (1.4 mL, 2.2 mmol, 1.6 M in hexane, 5.0 equiv.). After the addition of *n*BuLi, the reaction mixture was allowed to warm to -45 °C and stirred for 30 min. After being re-cooled to -78 °C, a solution of (-)-Ipc₂BOMe (840 mg, 2.6 mmol, 6.0 equiv.) in anhydrous THF (2 mL) was added and stirred for 30 min at -78 °C. $BF_3 \cdot Et_2O$ (0.45 mL, 3.5 mmol, 8.0 equiv.) was then added, followed by a solution of aldehyde **14** (183 mg, 0.44 mmol, 1.0 equiv.) in THF (2 mL). After being stirred at -78 °C for 3 h, the reaction mixture was quenched with MeOH (5 mL) and allowed to warm to room temperature. Solvent was removed in vacuo and the residue was redissolved in THF (6 mL) and H_2O (4 mL), and $NaBO_3 \cdot 4H_2O$ (339 mg, 2.2 mmol, 5.0 equiv.) was then added. The mixture was stirred at room temperature for 3 h and poured into a mixture of EtOAc (30 mL) and H_2O (10 mL). The layers were separated, and the aqueous layer was further extracted with EtOAc (2 \times 10 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica (hexanes/EtOAc = 2/1) to afford two separable diastereoisomers (187 mg, 71% total yield, dr > 20:1) with **21** (colorless oil) as the major product. TLC: R_f = 0.40 (silica gel, ethyl acetate/hexanes = 1/2). UV and PMA stain. $[\alpha]_D^{23.3} = -45.80$ (c 1.0, $CHCl_3$). 1H NMR (400 MHz, $CDCl_3$) δ 7.31–7.14 (m, 5H), 5.85 (ddd, J = 17.0, 10.8, 7.6 Hz, 1H), 5.45 (d, J = 8.5 Hz, 1H), 5.18–5.03 (m, 3H), 4.71–4.57 (m, 1H), 4.27 (dd, J = 8.2, 4.1 Hz, 1H), 3.70–3.62 (m, 1H), 3.55–3.47 (m, 1H), 3.09 (dd, J = 12.8, 5.4 Hz, 1H), 2.94 (dd, J = 12.8, 9.6 Hz, 1H), 2.61 (q, J = 7.6, 6.9 Hz, 1H), 2.45 (t, J = 7.3 Hz, 1H), 2.39–2.29 (m, 1H), 2.16 (s, 3H), 2.01–1.82 (m, 4H), 1.70–1.60 (m, 2H), 1.55 (t, J = 7.2 Hz,

1H), 1.47 (s, 9H), 1.38–1.26 (m, 6H), 1.10 (d, $J = 6.9$ Hz, 3H), 0.89 (d, $J = 6.7$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 209.2, 172.0, 170.4, 155.1, 140.3, 136.6, 129.6, 128.5, 127.1, 115.6, 79.8, 72.9, 59.3, 53.8, 47.1, 44.3, 43.7, 40.6, 36.9, 36.4, 32.9, 30.0, 29.1, 28.5, 28.3, 26.8, 24.6, 23.9, 15.5, 14.3. HRMS (ESI, m/z) for $\text{C}_{34}\text{H}_{53}\text{N}_2\text{O}_7^+$ $[\text{M}+\text{H}]^+$: Calcd. 601.3847; found: 601.3849.

(2R,3R,5S,6S)-5-(((*tert*-butoxycarbonyl)-*D*-phenylalanyl-*L*-prolyl)oxy)-3-hydroxy-2,6-dimethyl-11-oxododecanoic acid **16**:

To a solution of alkene **15** (76 mg, 0.13 mmol, 1.0 equiv.) in DCM at -78 °C, ozone was bubbled through the solution until the solution became slightly blue. PPh_3 (170 mg, 0.65 mmol, 5.0 equiv.) was added and the resultant solution was stirred at room temperature for 1 h. After concentration, the residue was used directly in the next step without further purification.

To a solution of the above crude aldehyde and $\text{PhI}(\text{OAc})_2$ (123 mg, 0.38 mmol, 3.0 equiv.) in DCM (10 mL, 0.013 M) at 0 °C, TEMPO (8 mg, 0.051 mmol, 0.4 equiv.) and H_2O (1 mL) were added. The reaction mixture was warmed up to ambient temperature and stirred for 12 h, and then quenched with a saturated aqueous solution of Na_2SO_3 (10 mL). The aqueous layer was extracted with EtOAc (3×10 mL), and the combined organic layers were washed with H_2O (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica (hexanes/EtOAc = 1/1) to afford acid **16** (50 mg, 64%) as a colorless oil. TLC: $R_f = 0.10$ (silica gel, ethyl acetate/hexanes = 1/1). UV and PMA stain. $[\alpha]_{\text{D}}^{26.3} = -31.20$ (c 1.0, CHCl_3). ^1H NMR (400 MHz, CDCl_3) δ 7.43–7.05 (m, 5H), 5.22–5.06 (m, 1H), 4.73–4.59 (m, 1H), 4.30–4.22 (m, 1H), 3.92–3.84 (m, 1H), 3.61–3.48 (m, 1H), 3.06 (dd, $J = 12.8, 5.6$ Hz, 1H), 2.99–2.86 (m, 1H), 2.72–2.57 (m, 2H), 2.48–2.37 (m, 2H), 2.14 (s, 3H), 2.00–1.77 (m, 5H), 1.59–1.49 (m, 4H), 1.45 (s, 9H), 1.37–1.19 (m, 8H), 0.88 (d, $J = 6.7$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 209.4, 171.6, 155.2, 137.5, 136.4, 132.2, 132.1, 130.3, 129.5, 128.5, 127.5, 127.0, 79.9, 77.3, 59.3, 53.8, 47.2, 43.6, 40.2, 36.5, 32.8, 30.0, 29.7, 29.1, 28.4, 26.6, 24.5, 23.9, 14.2. HRMS (ESI, m/z) for $\text{C}_{33}\text{H}_{51}\text{N}_2\text{O}_9^+$ $[\text{M}+\text{H}]^+$: Calcd. 619.3589; found: 619.3585.

Acremolide B (**2**):

To a solution of **16** (36 mg, 0.06 mmol, 1.0 equiv.) in DCM (4 mL, 0.015 M) at 0 °C, TFA (0.8 mL) was added. The reaction mixture was allowed to be stirred for 10 min at 0 °C. The mixture was concentrated in vacuo directly. The residue was used directly in the next step without further purification.

To a solution of the above crude amine in DCM (60 mL, 0.001 M) at 0 °C, DIPEA (0.11 mL, 0.60 mmol, 10.0 equiv.), HATU (114 mg, 0.30 mmol, 5.0 equiv.), and HOAt (16 mg, 0.12 mmol, 2.0 equiv.) were added. The reaction mixture was allowed to warm for 24 h at room temperature and then concentrated in vacuo and the residue was redissolved in EtOAc (30 mL) and quenched with 4% aqueous citric acid solution. The aqueous layer was extracted with EtOAc (3×10 mL), and the combined organic layers were washed with saturated aqueous solution of NaHCO_3 (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica gel (hexanes/EtOAc = 1/2) to afford acremolide B (**2**) (20 mg, 67%) as a colorless oil. TLC: $R_f = 0.30$ (silica gel, ethyl acetate/hexanes = 2/1). UV and PMA stain. $[\alpha]_{\text{D}}^{25} = -102$ (c 0.02, MeOH). Major *cis* conformer: ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.21 (d, $J = 8.8$ Hz, 1H), 7.38–7.12 (m, 5H), 5.00 (dd, $J = 8.4, 3.2$ Hz, 1H), 4.56–4.53 (m, 1H), 4.53–4.48 (m, 1H), 4.42 (d, $J = 6.1$ Hz, 1H), 3.78–3.69 (m, 1H), 3.57 (dd, $J = 7.5, 5.0$ Hz, 1H), 3.50–3.45 (m, 1H), 3.21 (dd, $J = 13.9, 4.1$ Hz, 1H), 2.83 (dd, $J = 13.9, 10.8$ Hz, 1H), 2.41 (t, $J = 7.3$ Hz, 2H), 2.33 (dd, $J = 7.3, 2.1$ Hz, 1H), 2.28–2.24 (m, 1H), 2.07 (d, $J = 3.8$ Hz, 3H), 1.97–1.88 (m, 2H), 1.83–1.79 (m, 1H), 1.78–1.75 (m, 1H), 1.74–1.71 (m, 1H), 1.70–1.66 (m, 1H), 1.44–1.42 (m, 1H), 1.38–1.37 (m, 1H), 1.22–1.13 (m, 1H), 1.17–1.14 (m, 1H), 0.96–0.89 (m, 1H), 0.83 (d, $J = 6.8$ Hz, 3H), 0.74 (d, $J = 7.4$ Hz, 3H). ^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ 208.1, 175.6, 171.2, 168.9, 137.5, 128.8, 127.9, 127.7, 126.0, 75.1, 69.1, 57.7, 54.9, 48.4, 42.3, 41.7, 38.4, 35.1, 34.7, 31.2, 30.7, 29.3, 26.0, 25.4, 23.1,

20.3, 15.1, 15.0. (only for major isomer) HRMS (ESI, m/z) for $C_{28}H_{40}N_2O_6Na^+$ $[M+Na]^+$: Calcd. 523.2799; found 523.2797.

(3*R*,4*S*,9*R*,*E*)-1-(benzyloxy)-9-((*tert*-butyldimethylsilyl)oxy)-4-methyldec-5-en-3-ol **18**:

To a solution of **9** (100 mg, 0.46 mmol, 1.0 equiv.) and alkene **17** (195 mg, 0.91 mmol, 2.0 equiv.) in DCM (10 mL, 0.050 M) at room temperature, Grubbs II catalyst (77 mg, 0.091 mmol, 0.20 equiv.) was added. The reaction mixture was heated at 50 °C for 9 h before it was cooled back to room temperature and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica (hexanes/EtOAc = 9/1) to afford **12** (159 mg, 86%) as a colorless oil. TLC: R_f = 0.50 (silica gel, ethyl acetate/hexanes = 1/9). UV and PMA stain. $[\alpha]_D^{22} = -7.60$ (c 3.0, $CHCl_3$). 1H NMR (400 MHz, $CDCl_3$) δ 7.43–7.31 (m, 5H), 5.59–5.44 (m, 1H), 5.43–5.32 (m, 1H), 4.56 (s, 2H), 3.87–3.79 (m, 1H), 3.79–3.72 (m, 1H), 3.72–3.60 (m, 2H), 2.90 (d, J = 3.4 Hz, 1H), 2.23 (q, J = 6.8 Hz, 1H), 2.17–2.07 (m, 1H), 2.06–1.98 (m, 1H), 1.90–1.79 (m, 1H), 1.78–1.65 (m, 1H), 1.61–1.40 (m, 2H), 1.16 (d, J = 6.0 Hz, 3H), 1.07 (d, J = 6.9 Hz, 3H), 0.93 (s, 9H), 0.14–0.01 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.0, 132.4, 131.1, 128.5, 127.8, 127.8, 74.8, 73.4, 69.5, 68.2, 43.1, 39.6, 33.7, 29.1, 26.0, 23.9, 18.2, 16.0, –4.3, –4.6. HRMS (ESI, m/z) for $C_{24}H_{43}O_3Si^+$ $[M+H]^+$: Calcd. 407.2976; found: 407.2977.

(3*S*,4*S*,9*R*)-9-((*tert*-butyldimethylsilyl)oxy)-1-hydroxy-4-methyldec-3-yl (*tert*-butoxycarbonyl)-*D*-phenylalanyl-*L*-prolinate **19**:

To a solution of alcohol **18** (510 mg, 1.3 mmol, 1.0 equiv.) and acid **3** (480 mg, 1.3 mmol, 1.1 equiv.) in anhydrous THF (30 mL, 0.040 M) at 0 °C under an argon atmosphere, PPh_3 (990 mg, 3.8 mmol, 3.0 equiv.) and DIAD (0.5 mL, 2.5 mmol, 2.0 equiv.) were added. After being stirred at room temperature for 9 h, the reaction mixture was quenched with saturated aqueous solution of $NaHCO_3$ (20 mL) and extracted with EtOAc (3 \times 30 mL). The combined organic layers were washed with saturated aqueous solution of brine (30 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica gel (hexanes/EtOAc = 9/1) to afford Mitsunobu product (822 mg, 87%) as a colorless oil.

To a solution of Mitsunobu product (125 mg, 0.17 mmol, 1.0 equiv.) in EtOAc (10 mL, 0.020 M), palladium on charcoal (50 mg, 10% Pd, 0.047 mmol, 0.28 equiv.) was added under argon atmosphere. The reaction flask was evacuated and purged with hydrogen three times. The reaction mixture was stirred under a hydrogen atmosphere at room temperature for 9 h; the flask was then evacuated and purged with nitrogen three times, and the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated under reduced pressure to afford crude product, which was purified by flash chromatography on silica gel (hexanes/EtOAc = 4/1) to afford **19** (107 mg, 95%) as a colorless oil. TLC: R_f = 0.20 (silica gel, ethyl acetate/hexanes = 1/4). UV and PMA stain. $[\alpha]_D^{24.3} = -38.30$ (c 2.0, $CHCl_3$). 1H NMR (400 MHz, $CDCl_3$) δ 7.27–7.23 (m, 2H), 7.23–7.17 (m, 3H), 5.40 (d, J = 8.6 Hz, 1H), 5.09–4.98 (m, 1H), 4.70–4.54 (m, 1H), 4.24 (dd, J = 8.4, 4.1 Hz, 1H), 3.80–3.63 (m, 3H), 3.55–3.47 (m, 1H), 3.06 (dd, J = 12.8, 5.4 Hz, 1H), 2.91 (dd, J = 12.8, 9.5 Hz, 1H), 2.69–2.56 (m, 1H), 1.99–1.90 (m, 1H), 1.88–1.72 (m, 4H), 1.68–1.56 (m, 1H), 1.54–1.46 (m, 1H), 1.43 (s, 9H), 1.36–1.20 (m, 8H), 1.10 (d, J = 6.1 Hz, 3H), 0.92–0.84 (m, 12H), 0.04 (s, 3H), 0.03 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.8, 170.5, 155.1, 136.6, 129.6, 128.5, 127.0, 79.9, 75.6, 68.7, 59.2, 58.8, 53.8, 47.1, 40.5, 39.8, 37.1, 34.8, 33.2, 29.2, 28.5, 27.2, 26.0, 24.7, 23.9, 18.2, 14.7, –4.3, –4.6. HRMS (ESI, m/z) for $C_{36}H_{63}N_2O_7Si^+$ $[M+H]^+$: Calcd. 663.4399; found: 663.4401.

(3*S*,4*R*,6*S*,7*S*,12*R*)-12-((*tert*-butyldimethylsilyl)oxy)-4-hydroxy-3,7-dimethyltridec-1-en-6-yl (*tert*-butoxycarbonyl)-*D*-phenylalanyl-*L*-prolinate **20**:

To a solution of alcohol **19** (237 mg, 0.70 mmol, 1.0 equiv.) in dry DCM (10 mL, 0.070 M) at 0 °C under argon atmosphere, Dess–Martin periodinane (353 mg, 0.83 mmol, 1.2 equiv.) was added. After being stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous solution of Na_2SO_3 (10 mL) and extracted with EtOAc (3 \times 10 mL). The combined organic extracts were washed with saturated aqueous solution of copper sulfate (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 ,

filtered, and concentrated in vacuo. The residue was used directly in the next step without further purification.

To a suspension of *t*BuOK (365 mg, 3.3 mmol, 5.0 equiv.) in anhydrous THF (10 mL, 0.065 M) at $-78\text{ }^{\circ}\text{C}$ was added *trans*-2-butene (255 mg, 4.6 mmol, 7.0 equiv.), followed by the dropwise addition of *n*BuLi (2.0 mL, 3.3 mmol, 1.6 M in hexane, 5.0 equiv.). After the addition of *n*BuLi, the reaction mixture was allowed to warm to $-45\text{ }^{\circ}\text{C}$ and stirred for 30 min. After being re-cooled to $-78\text{ }^{\circ}\text{C}$, a solution of (-)-Ipc₂BOMe (1.2 g, 3.9 mmol, 6.0 equiv.) in anhydrous THF (2 mL) was added and stirred for 30 min at $-78\text{ }^{\circ}\text{C}$. BF₃·Et₂O (0.66 mL, 5.2 mmol, 8.0 equiv.) was then added followed by a solution of aldehyde in THF (2 mL). After being stirred at $-78\text{ }^{\circ}\text{C}$ for 6 h, the reaction mixture was quenched with MeOH (5 mL) and allowed to warm to room temperature. Solvent was removed in vacuo and the residue was redissolved in THF (6 mL) and H₂O (4 mL), and NaBO₃·4H₂O (500 mg, 3.3 mmol, 5.0 equiv.) was then added. The mixture was stirred at room temperature for 3 h and poured into a mixture of EtOAc (30 mL) and H₂O (10 mL). The layers were separated, and the aqueous layer was further extracted with EtOAc (2 × 10 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica (hexanes/EtOAc = 4/1) to afford two separable diastereoisomers (396 mg, 85% total yield, dr > 20:1) with **20** (colorless oil) as the major product. TLC: R_f = 0.40 (silica gel, ethyl acetate/hexanes = 1/4). UV and PMA stain. $[\alpha]_{\text{D}}^{24.9} = -30.60$ (c 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 7.31–7.14 (m, 5H), 5.86 (ddd, J = 16.4, 11.1, 7.6 Hz, 1H), 5.43 (d, J = 8.6 Hz, 1H), 5.17–5.04 (m, 3H), 4.69–4.55 (m, 1H), 4.31–4.22 (m, 1H), 3.83–3.73 (m, 1H), 3.72–3.63 (m, 1H), 3.57–3.44 (m, 1H), 3.09 (dd, J = 12.8, 5.3 Hz, 1H), 2.93 (dd, J = 12.8, 9.6 Hz, 1H), 2.69–2.53 (m, 1H), 2.40–2.28 (m, 1H), 2.04–1.81 (m, 4H), 1.80–1.72 (m, 1H), 1.70–1.59 (m, 2H), 1.47 (s, 9H), 1.39–1.30 (m, 3H), 1.32–1.22 (m, 5H), 1.17–1.05 (m, 6H), 0.95–0.81 (m, 12H), 0.10–0.02 (m, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 171.9, 170.3, 155.0, 140.3, 136.5, 129.6, 128.4, 127.0, 115.5, 79.7, 72.9, 68.7, 59.2, 53.8, 47.0, 44.3, 40.6, 39.7, 37.1, 36.3, 33.0, 29.7, 29.0, 28.4, 28.2, 27.2, 26.0, 24.5, 23.9, 18.2, 15.3, 14.4, -4.3, -4.7. HRMS (ESI, *m/z*) for C₄₀H₆₉N₂O₇Si⁺ [M+H]⁺: Calcd. 717.4869; found: 717.4866.

(5*R*,7*S*,8*S*,13*R*)-5-((*S*)-but-3-en-2-yl)-2,2,3,3,8,13,15,15,16,16-decamethyl-4,14-dioxo-3,15-disilaheptadecan-7-yl (*tert*-butoxycarbonyl)-*D*-phenylalanyl-*L*-prolinate **21**:

To a solution of alcohol **20** (4450 mg, 0.63 mmol, 1.0 equiv.) in dry DCM (5 mL, 0.15 M) at 0 °C under argon atmosphere, Et₃N (200 μL, 1.3 mmol, 2.0 equiv.) was added, followed by TBSOTf (220 μL, 0.95 mmol, 1.5 equiv.). The reaction mixture was warmed up to ambient temperature and stirred for 1 h, and then quenched with a saturated aqueous solution of NaHCO₃ (5 mL). The aqueous layer was extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with H₂O (10 mL) and brine (10 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica (hexanes/EtOAc = 9/1) to afford silyl ether **21** (490 mg, 94%) as a colorless oil. TLC: R_f = 0.40 (silica gel, ethyl acetate/hexanes = 1/9). UV and PMA stain. $[\alpha]_{\text{D}}^{25.8} = -35.40$ (c 1.0, CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 7.29–7.18 (m, 5H), 5.81 (ddd, J = 17.0, 10.5, 8.4 Hz, 1H), 5.39 (d, J = 8.4 Hz, 1H), 5.10–4.98 (m, 2H), 4.97–4.90 (m, 1H), 4.71–4.57 (m, 1H), 4.31 (dd, J = 8.2, 3.6 Hz, 1H), 3.88–3.68 (m, 2H), 3.56–3.44 (m, 1H), 3.08 (dd, J = 12.7, 5.4 Hz, 1H), 2.95 (dd, J = 12.7, 9.3 Hz, 1H), 2.71–2.58 (m, 1H), 2.48–2.35 (m, 1H), 2.03–1.90 (m, 1H), 1.90–1.79 (m, 4H), 1.73–1.65 (m, 2H), 1.46 (s, 9H), 1.39–1.23 (m, 8H), 1.13 (d, J = 6.1 Hz, 3H), 1.05 (d, J = 6.8 Hz, 3H), 0.93 (s, 9H), 0.91 (s, 9H), 0.85 (d, J = 6.9 Hz, 3H), 0.12 (s, 6H), 0.07 (s, 3H), 0.06 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.7, 169.8, 155.0, 140.3, 136.7, 129.6, 128.4, 126.9, 115.1, 79.6, 75.0, 72.2, 68.7, 59.1, 53.6, 46.6, 42.1, 40.6, 39.7, 37.0, 36.6, 33.0, 29.2, 28.4, 27.3, 26.0, 24.5, 23.8, 18.2, 18.1, 17.3, 14.0, -4.3, -4.3, -4.6, -4.6. HRMS (ESI, *m/z*) for C₄₆H₈₃N₂O₇Si₂⁺ [M+H]⁺: Calcd. 831.5733; found: 831.5735.

(3*S*,4*R*,6*S*,7*S*,12*R*)-12-((*tert*-butyldimethylsilyloxy)-4-hydroxy-3,7-dimethyltridec-1-en-6-yl (*tert*-butoxycarbonyl)-*D*-phenylalanyl-*L*-prolinate **22**:

In a solution of alkene **20** (441 mg, 0.53 mmol, 1.0 equiv.) in DCM at $-78\text{ }^{\circ}\text{C}$, ozone was bubbled through until the solution became slightly blue. PPh_3 (700 mg, 2.65 mmol, 5.0 equiv.) was added and the resultant solution was stirred at room temperature for 1 h. After concentration, the residue was used directly in the next step without further purification.

To a solution of the above crude aldehyde and $\text{PhI}(\text{OAc})_2$ (512 mg, 1.6 mmol, 3.0 equiv.) in DCM (20 mL, 0.026 M) at $0\text{ }^{\circ}\text{C}$, TEMPO (33 mg, 0.21 mmol, 0.40 equiv.), and H_2O (2 mL) were added. The reaction mixture was warmed up to ambient temperature and stirred for 12 h, and then quenched with a saturated aqueous solution of Na_2SO_3 (10 mL). The aqueous layer was extracted with EtOAc ($3 \times 10\text{ mL}$), and the combined organic layers were washed with H_2O (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica (hexanes/EtOAc = 4/1) to afford acid **22** (306 mg, 68%) as a colorless oil. TLC: $R_f = 0.40$ (silica gel, ethyl acetate/hexanes = 1/4). UV and PMA stain. $[\alpha]_{\text{D}}^{26.9} = -45.40$ ($c\ 1.0$, CHCl_3). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.30–7.10 (m, 5H), 5.32 (dd, $J = 8.7, 2.2\text{ Hz}$, 1H), 5.15–4.96 (m, 1H), 4.75–4.55 (m, 1H), 4.22 (dd, $J = 8.3, 4.0\text{ Hz}$, 1H), 4.10 (dt, $J = 8.4, 4.3\text{ Hz}$, 1H), 3.76 (q, $J = 5.9\text{ Hz}$, 1H), 3.65–3.43 (m, 1H), 3.05 (dd, $J = 12.9, 5.4\text{ Hz}$, 1H), 2.96–2.81 (m, 2H), 2.79–2.62 (m, 1H), 2.02–1.74 (m, 4H), 1.70–1.60 (m, 1H), 1.60–1.51 (m, 1H), 1.44 (s, 9H), 1.37–1.24 (m, 8H), 1.21 (d, $J = 7.0\text{ Hz}$, 3H), 1.11 (d, $J = 6.1\text{ Hz}$, 3H), 0.93 (s, 9H), 0.91–0.81 (m, 12H), 0.15 (d, $J = 5.0\text{ Hz}$, 6H), 0.05 (s, 3H), 0.04 (s, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 177.0, 171.3, 170.3, 155.0, 136.4, 129.5, 128.4, 127.0, 79.8, 77.4, 74.5, 71.3, 68.6, 59.1, 53.6, 46.9, 44.2, 40.3, 39.7, 36.8, 35.9, 32.9, 29.1, 28.4, 27.3, 26.0, 25.8, 24.5, 23.9, 23.8, 18.2, 17.9, 14.4, 13.8, -4.4 , -4.4 , -4.7 , -5.0 . HRMS (ESI, m/z) for $\text{C}_{45}\text{H}_{81}\text{N}_2\text{O}_9\text{Si}_2^+ [\text{M}+\text{H}]^+$: Calcd. 849.5475; found: 849.5479.

Acremolide A (**1**):

To a solution of **22** (77 mg, 0.091 mmol, 1.0 equiv.) in DCM (5 mL, 0.020 M) at $0\text{ }^{\circ}\text{C}$, Et_3N (0.25 mL, 1.8 mmol, 20.0 equiv.) was added, followed by dropwise addition of trimethylsilyl trifluoromethanesulfonate (0.41 mL, 1.8 mmol, 20.0 equiv.). The reaction mixture was allowed to warm for 1 h at room temperature and then quenched with saturated aqueous solution of NaHCO_3 (10 mL) and extracted with EtOAc ($3 \times 10\text{ mL}$). The combined organic layers were washed with saturated aqueous solution of NH_4Cl (10 mL), brine (10 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated in vacuo. The residue was used directly in the next step without further purification.

To a solution of the above crude amine in DCM (90 mL, 0.001 M) at $0\text{ }^{\circ}\text{C}$ was added DIPEA (0.16 mL, 0.91 mmol, 10.0 equiv.), HATU (173 mg, 0.46 mmol, 5.0 equiv.) and HOAt (37 mg, 0.27 mmol, 3.0 equiv.). The reaction mixture was allowed to warm for 9 h at room temperature and then concentrated in vacuo and the residue was redissolved in EtOAc (30 mL) and quenched with 4% aqueous citric acid solution. The aqueous layer was extracted with EtOAc ($3 \times 20\text{ mL}$), and the combined organic layers were washed with saturated aqueous solution of NaHCO_3 (15 mL) and brine (15 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica gel (hexanes/EtOAc = 2/1) to afford macrocyclization product (399 mg, 60%) as a colorless oil.

To a solution of macrocyclization product (16 mg, 0.028 mmol, 1.0 equiv.) in THF (4 mL, 0.007 M) at $0\text{ }^{\circ}\text{C}$ was added TBAF (2.0 mL, 2.0 mmol, 100 equiv., 1.0 M in THF). The reaction mixture was warmed up to ambient temperature and stirred for 6 h, and then quenched with a saturated aqueous solution of NH_4Cl (10 mL). The aqueous layer was extracted with EtOAc ($3 \times 10\text{ mL}$). The combined organic layers were washed with H_2O (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo. Purification of the crude product was performed by flash chromatography on silica (hexanes/EtOAc = 4/1) to afford acremolide A (**1**) (8.0 mg, 57%) as a colorless oil. TLC: $R_f = 0.40$ (silica gel, ethyl acetate/hexanes = 4/1). UV and PMA stain. $[\alpha]_{\text{D}}^{24.8} = -109$ ($c\ 0.05$, MeOH). Major *cis* conformer: $^1\text{H NMR}$ (400 MHz, $\text{DMSO}-d_6$) δ 8.23 (d, $J = 8.9\text{ Hz}$, 1H), 7.29–7.16 (m, 5H), 5.01 (dd, $J = 8.5, 3.0\text{ Hz}$, 1H), 4.59–4.47 (m, 2H), 4.28 (dd, $J = 6.9, 4.6\text{ Hz}$, 1H), 3.76–3.67 (m, 1H), 3.64–3.51 (m, 2H), 3.53–3.41 (m, 1H), 3.21 (dd, $J = 13.8, 3.9\text{ Hz}$, 1H),

2.83 (dd, $J = 13.8, 11.0$ Hz, 1H), 2.33 (d, $J = 7.3$ Hz, 1H), 2.32–2.20 (m, 1H), 1.96–1.85 (m, 2H), 1.84–1.75 (m, 2H), 1.75–1.71 (m, 1H), 1.71–1.65 (m, 1H), 1.61–1.57 (m, 1H), 1.53–1.48 (m, 1H), 1.27–1.17 (m, 4H), 1.02 (d, $J = 6.2$ Hz, 3H), 0.83 (d, $J = 6.3$ Hz, 3H), 0.73 (d, $J = 6.7$ Hz, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 175.9, 171.4, 169.0, 137.6, 129.0, 127.9, 126.2, 75.3, 69.4, 65.5, 57.8, 55.0, 48.6, 41.8, 38.6, 35.3, 34.9, 31.7, 31.3, 25.4, 23.5, 20.5, 15.4, 15.2. HRMS (ESI, m/z) for $\text{C}_{28}\text{H}_{42}\text{N}_2\text{O}_6\text{Na}^+$ [$M+\text{Na}$] $^+$: Calcd. 525.2936; found 525.2935.

4. Conclusions

The absolute stereochemical configurations of acremolides A and B were predicted based on the biochemistry-based rule, which was validated through total syntheses. The features of the syntheses include a sequential Krische's Ir-catalyzed crotylation and Brown's borane-mediated crotylation to establish the four stereogenic centers in the fatty acid substructure, a cross-metathesis reaction to link two fragments efficiently, and a Mitsunobu esterification reaction followed by a macrolactamization reaction to form the 12-membered ring. The total syntheses of acremolides A and B were achieved in 12 and 10 linear steps, with overall yields of 8.3% and 19%, respectively.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules29153599/s1>, Copies of NMR spectra (^1H and ^{13}C) of **1**, **2**, **9**, **13**, **15**, **16**, **18–22**.

Author Contributions: Y.X., J.L., Y.J., Y.G. and T.Y. conceived and designed this research; Y.X., J.L. and Y.J. prepared the compounds and collected their spectral data and analyzed the experimental data; J.L., Y.G. and T.Y. prepared the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by 2022 Shenzhen Sustainable Supporting Funds for Colleges and Universities (No.20220811103834002); Guangdong Basic and Applied Basic Research Foundation (2024A1515010958); Technology & Innovation Bureau of Longgang District (RCTDPT-2019-008); the Guangdong Natural Science Foundation, the Guangdong Department of Education (2021ZDJ097); National Natural Science Foundation of China (22171014, 21901013).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Acknowledgments: We are grateful to Robert J. Capon (The University of Queensland) for providing the original spectral data of Acremolides A and B.

Conflicts of Interest: Author Tao Ye was employed by the company Qian Yan (Shenzhen) Pharmatech. Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Ratnayake, R.; Fremlin, L.J.; Lacey, E.; Gill, J.H.; Capon, R.J. Acremolides A–D, Lipodepsipeptides from an Australian Marine-Derived Fungus, *Acromonium* sp. *J. Nat. Prod.* **2008**, *71*, 403–408. [[CrossRef](#)] [[PubMed](#)]
2. Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K.-i. A Nonempirical Method Using LC/MS for Determination of the Absolute Configuration of Constituent Amino Acids in a Peptide: Combination of Marfey's Method with Mass Spectrometry and Its Practical Application. *Anal. Chem.* **1997**, *69*, 5146–5151. [[CrossRef](#)]
3. Dale, J.A.; Dull, D.L.; Mosher, H.S. α -Methoxy- α -Trifluoromethylphenylacetic Acid, a Versatile Reagent for the Determination of Enantiomeric Composition of Alcohols and Amines. *J. Org. Chem.* **1969**, *34*, 2543–2549. [[CrossRef](#)]
4. Maier, M.E.; Maier, M.E. Structural revisions of natural products by total synthesis. *Nat. Prod. Rep.* **2009**, *26*, 1105–1124. [[CrossRef](#)]
5. Nicolaou, K.C.; Snyder, S.A. Chasing Molecules That Were Never There: Misassigned Natural Products and the Role of Chemical Synthesis in Modern Structure Elucidation. *Angew. Chem. Int. Ed.* **2005**, *44*, 1012–1044. [[CrossRef](#)]
6. Paul, D.; Kundu, A.; Saha, S.; Goswami, R.K.; Paul, D. Total synthesis: The structural confirmation of natural products. *Chem Commun.* **2021**, *57*, 3307–3322. [[CrossRef](#)]
7. Chhetri, B.K.; Lavoie, S.; Sweeney-Jones, A.M.; Kubanek, J.; Chhetri, B.K. Recent trends in the structural revision of natural products. *Nat. Prod. Rep.* **2018**, *35*, 514–531. [[CrossRef](#)] [[PubMed](#)]

8. Fuwa, H.; Fuwa, H. Structure determination, correction, and disproof of marine macrolide natural products by chemical synthesis. *Org. Chem. Front.* **2021**, *8*, 3990–4023. [[CrossRef](#)]
9. ElMarrouni, A.; Fukuda, A.; Heras, M.; Arseniyadis, S.; Cossy, J. Expedient Synthesis of a Stereoisomer of Acremolide B. *J. Org. Chem.* **2010**, *75*, 8478–8486. [[CrossRef](#)]
10. Yadav, J.S.; Amrutapu, S.K.; Pabbaraja, S. Studies Towards the Synthesis of Stereoisomer of Acremolide B. *ChemistrySelect* **2017**, *2*, 1850–1853. [[CrossRef](#)]
11. Zhang, T.; Feng, J.; Oikawa, H.; Guo, Y.; Ye, T. Synergy of Prediction Rule and Total Synthesis in Solving the Stereochemical Puzzle of Valactamides. *Precis. Chem.* **2024**, *2*, 120–126. [[CrossRef](#)]
12. Lei, H.; Yan, J.; Yu, J.; Liu, Y.; Wang, Z.; Xu, Z.; Ye, T. Total Synthesis and Stereochemical Reassignment of Mandelalide A. *Angew. Chem. Int. Ed.* **2014**, *53*, 6533–6537. [[CrossRef](#)] [[PubMed](#)]
13. Takino, J.; Kotani, A.; Ozaki, T.; Peng, W.; Yu, J.; Guo, Y.; Mochizuki, S.; Akimitsu, K.; Hashimoto, M.; Ye, T.; et al. Biochemistry-Guided Prediction of the Absolute Configuration of Fungal Reduced Polyketides. *Angew. Chem. Int. Ed.* **2021**, *60*, 23403–23411. [[CrossRef](#)] [[PubMed](#)]
14. Mitsunobu, O.; Yamada, M. Preparation of Esters of Carboxylic and Phosphoric Acid via Quaternary Phosphonium Salts. *Bull. Chem. Soc. Jpn.* **2006**, *40*, 2380–2382. [[CrossRef](#)]
15. Kim, I.S.; Han, S.B.; Krische, M.J. *anti*-Diastereo- and Enantioselective Carbonyl Crotylation from the Alcohol or Aldehyde Oxidation Level Employing a Cyclometallated Iridium Catalyst: α -Methyl Allyl Acetate as a Surrogate to Preformed Crotylmetal Reagents. *J. Am. Chem. Soc.* **2009**, *131*, 2514–2520. [[CrossRef](#)] [[PubMed](#)]
16. Delaude, L.; Noels, A.F. Metathesis. In *Kirk-Othmer Encyclopedia of Chemical Technology*; Wiley: Hoboken, NJ, USA, 2005.
17. Grubbs, R.H. Olefin-Metathesis Catalysts for the Preparation of Molecules and Materials (Nobel Lecture). *Angew. Chem. Int. Ed.* **2006**, *45*, 3760–3765. [[CrossRef](#)] [[PubMed](#)]
18. Gessler, S.; Randl, S.; Blechert, S. Synthesis and Metathesis Reactions of a Phosphine-Free Dihydroimidazole Carbene Ruthenium Complex. *Tetrahedron Lett.* **2000**, *41*, 9973–9976. [[CrossRef](#)]
19. Garber, S.B.; Kingsbury, J.S.; Gray, B.L.; Hoveyda, A.H. Efficient and Recyclable Monomeric and Dendritic Ru-Based Metathesis Catalysts. *J. Am. Chem. Soc.* **2000**, *122*, 8168–8179. [[CrossRef](#)]
20. Scholl, M.; Trnka, T.M.; Morgan, J.P.; Grubbs, R.H. Increased Ring Closing Metathesis Activity of Ruthenium-Based Olefin Metathesis Catalysts Coordinated with Imidazolin-2-Ylidene Ligands. *Tetrahedron Lett.* **1999**, *40*, 2247–2250. [[CrossRef](#)]
21. Scholl, M.; Ding, S.; Lee, C.W.; Grubbs, R.H. Synthesis and Activity of a New Generation of Ruthenium-Based Olefin Metathesis Catalysts Coordinated with 1,3-Dimesityl-4,5-dihydroimidazol-2-ylidene Ligands. *Org. Lett.* **1999**, *1*, 953–956. [[CrossRef](#)]
22. Chatterjee, A.K.; Choi, T.-L.; Sanders, D.P.; Grubbs, R.H. A General Model for Selectivity in Olefin Cross Metathesis. *J. Am. Chem. Soc.* **2003**, *125*, 11360–11370. [[CrossRef](#)] [[PubMed](#)]
23. Brown, H.C.; Jadhav, P.K. Asymmetric Carbon-Carbon Bond Formation via β -Allyldiisopinocampheylborane. Simple Synthesis of Secondary Homoallylic Alcohols with Excellent Enantiomeric Purities. *J. Am. Chem. Soc.* **1983**, *105*, 2092–2093. [[CrossRef](#)]
24. Brown, H.C.; Bhat, K.S. Chiral Synthesis Via Organoboranes. 7. Diastereoselective and Enantioselective Synthesis of Erythro- and Threo- β -Methylhomoallyl Alcohols Via Enantiomeric (*Z*)- and (*E*)-Crotylboranes. *J. Am. Chem. Soc.* **1986**, *108*, 5919–5923. [[CrossRef](#)] [[PubMed](#)]
25. Dess, D.B.; Martin, J.C. Readily Accessible 12-I-5 Oxidant for the Conversion of Primary and Secondary Alcohols to Aldehydes and Ketones. *J. Org. Chem.* **1983**, *48*, 4155–4156. [[CrossRef](#)]
26. Lemieux, R.U.; Rudloff, E.V. Periodate-Permanganate Oxidations: I. Oxidation of Olefins. *Can. J. Chem.* **1955**, *33*, 1701–1709. [[CrossRef](#)]
27. Epp, J.B.; Widlanski, T.S. Facile Preparation of Nucleoside-5'-carboxylic Acids. *J. Org. Chem.* **1999**, *64*, 293–295. [[CrossRef](#)]
28. Chen, J.; Jiang, Y.; Yan, J.; Xu, C.; Ye, T. Total Syntheses of Colletopeptide A and Colletotrichamide A. *Molecules* **2023**, *28*, 7194. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.