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Asymmetric total synthesis of AK-toxins

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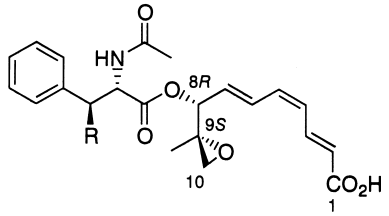
Abstract—A practical total synthesis of AK-toxins (AK-toxin I, **1**; II, **2**), host-specific toxins against the Japanese pear, has been achieved in 10% total yield, starting from the key intermediate **6**. The (2*E*,4*Z*,6*E*)-conjugated triene system was successfully constructed by the use of the Stille coupling reaction between the (*E*,*Z*)-bromodiene (**17**) and (*E*)-stannylacrylate (**21**). The (*E*,*Z*)-configuration of **17** was established with excellent geometrical purity by palladium-catalyzed stereoselective hydrogenolysis of *gem*-dibromide (**15**), which was derived from **6** via the Wittig–Horner reactions.

1. Introduction

AK-toxins (AK-toxin I, **1**; II, **2**) were first isolated as host-specific toxins (HSTs) produced by *Alternaira alternata* Japanese pear pathotype, the causal fungus of black spot disease in the Japanese pear.[1–3] The structures of the toxins were elucidated by Nakashima et al. in 1982 as shown in Fig. 1.[4,5] They cause severe necrosis on the fruit and the leaf of the susceptible cultivars of Japanese pear at an extraordinary low concentration,[6] resulting in large scale harvest damage. Microscopic analysis suggests that the primary site of action for AK-toxins is on the plasma membrane of the pear cells.[7,8] However the exact mechanism of their toxicity is not yet clear. The difficulty in elucidating the mechanism of action is partly due to the fact that the toxins can only be isolated from the natural source in small amounts. Thus, their practical preparation method has been the major requirement for elucidating the mode of action.



1, AK-toxin I ; R = CH_3
2, AK-toxin II ; R = H

Figure 1. Structure of AK-toxins.

AK-toxins are esters consisting of a phenylalanine derivative and a hydroxydecatrienoic acid. The latter has two characteristic substructures, a conjugated triene carboxylic acid with (2*E*,4*Z*,6*E*)-configuration (C1–C7) and a chiral methylglycidol (C8–C10) with two neighboring chiral centers. A number of researchers have been intrigued by the precise stereochemistry required for the synthesis of these compounds and the insufficient availability of a natural source. Accordingly, several efforts have been made to achieve the complete chemical synthesis of AK-toxins. Irie et al. pioneered in this field, and achieved the first total synthesis of the methyl ester of **2**.[9–12] They used the acetonide **3**, which was derived from ascorbic acid, as the chiral source for establishing the configuration at C8 of AK-toxins (Scheme 1). They also prepared all the possible stereoisomers of an AK-toxin analogs, to demonstrate that the configuration of C8 and C9 was critical for the phytotoxicity.[13] In another synthetic strategy adopted by Ando et al.[14] the stereochemistry of C8 and C9 was derived from the chiral intermediate **4**, which was prepared from D-fructose.[15] Taking advantage of its stereochemistry, compound **4** was also used in the synthesis of [^{3}H]-labeled AK-toxin I methyl ester by Okada et al.[16] However, compound **4** is a poor starting material, since the conversion of D-fructose to **4** requires extended reaction times (2–3 months) to provide even low yields (ca. 10%). In contrast to these strategies relying on natural chiral sources, Crombie et al. tried to chemically construct the stereostructure by utilizing Sharpless asymmetric epoxidation (SAE) for the kinetic resolution of the racemic tetraene alcohol **5**.[17–19] The targeted glycidol derivative, however, was obtained only with moderate stereoselectivity and in a low yield. Thus, all the preceding methods of AK-toxin preparation were unsatisfactory for our needs, both in terms of stereoselectivity and total yield, affording only small amounts of AK-toxins with great difficulty. In order to establish a more facile method of preparation, we recently developed an effective and stereoselective synthesis of a chiral glycidol

Keywords: AK-toxin; host-specific toxin; asymmetric total synthesis; palladium-catalyzed stereoselective hydrogenolysis; Stille reaction.
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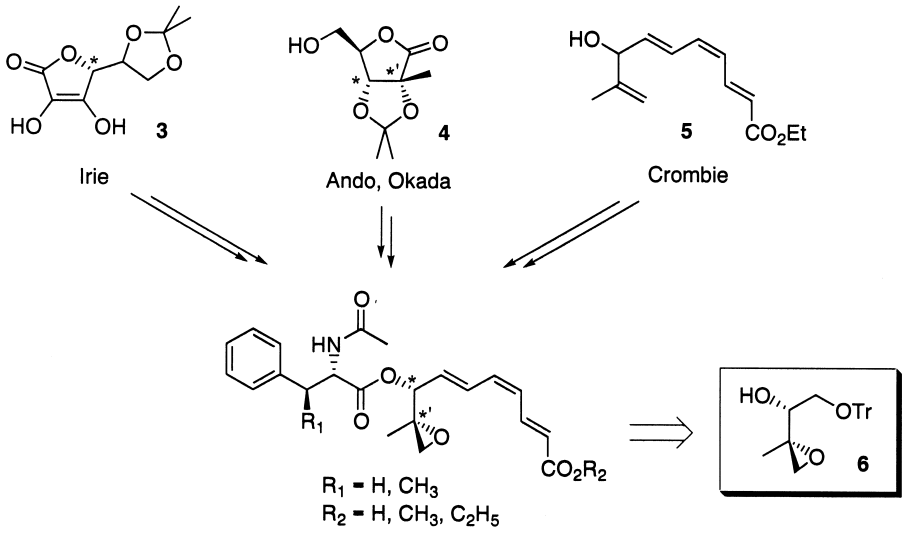


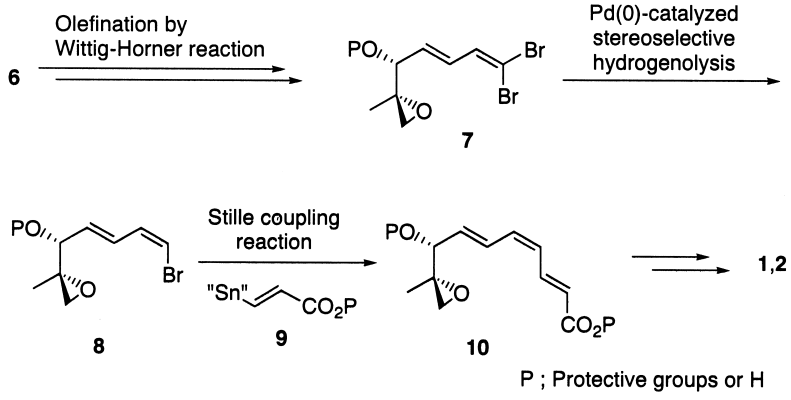
Scheme 1. Preceding strategies for the preparation of AK-toxins.

intermediate **6**, of which configuration agrees with those of AK-toxins.[20] In this paper, we report the transformation of **6** to achieve the total synthesis of AK-toxin I and II.

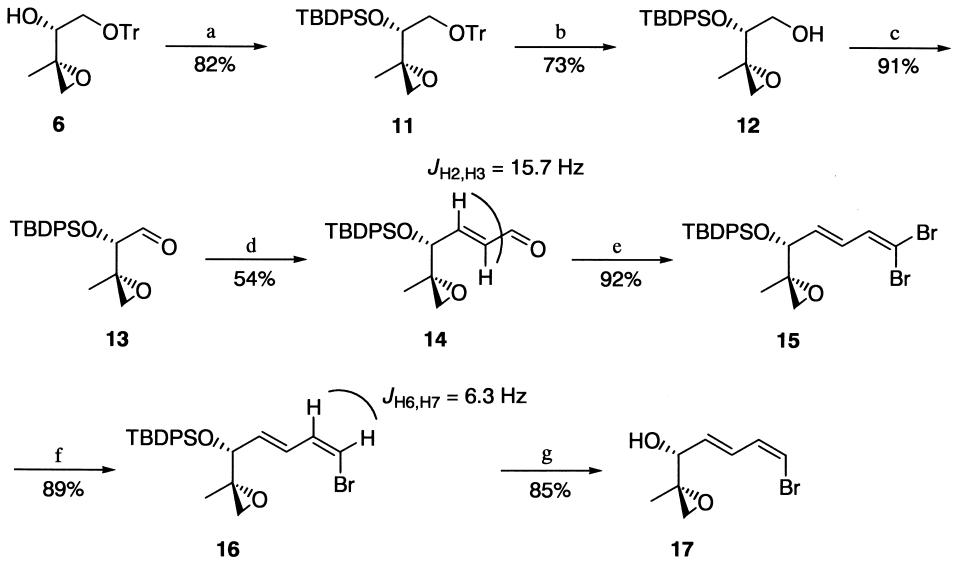
2. Results and discussion

Our synthetic plan to achieve the conjugated triene system of **6** is outlined in Scheme 2. We employed the Pd(0)-catalyzed hydrogenolysis to stereoselectively construct the (*Z*)-alkenyl unit from a *gem*-dibromide (**7**),[21,22] which was prepared from **6** via the transformation based on the Wittig–Horner reaction. The conjugated system of the resultant bromodiene (**8**) was then extended to a triene (**10**) by the Stille reaction with a stannylacrylate (**9**).[23]

First, the secondary hydroxyl group of **6** was protected as *t*-butyldiphenylsilyl (TBDPS) ether (Scheme 3). Treatment of **6** with TBDPS chloride, imidazole, and a catalytic amount of 4-(dimethylamino)pyridine in DMF afforded the corresponding glycidyl ether **11**. The trityl group of **11** was removed in 90% acetic acid at 50°C to give the primary alcohol **12** in 75% yield with small amount of **11** remained unreacted.[20] This reaction condition turned out to be the optimum, since a prolonged heating to complete the reaction caused a substantial reduction in yield, probably due to the hydrolysis of the oxirane ring of **12**. Other deprotection methods using Lewis acids, such as boron trichloride or zinc bromide, were all ineffective, giving complex mixture of products.[24,25] Oxidation of **12** was effected by Dess–Martin periodinane,[26,27] affording the corresponding aldehyde **13** in a stereochemically pure form and in an excellent yield (91%). Our preceding study showed that this transformation could not affect the stereochemistry at the carbon next to the carbonyl group.[20] The Horner–Wadsworth–Emmons reaction of **13**, with diethyl 2-oxoethylphosphonate under the conditions developed by Masamune provided the α,β-unsaturated aldehyde **14** in geometrically pure form in 54% yield.[28–30] The *E*-orientation of the introduced double bond was confirmed by the coupling constant of olefinic protons ($J_{H2,H3}$=15.7 Hz). Any diastereomeric byproduct was not found even in the crude product by ^{1}H NMR analysis. This implies that the absolute configuration of **13** was unchanged under this reaction condition, though an excess amount of base (6 equiv. *N*,*N*-diisopropylethylamine) was used. This retention of configuration is probably attributed to the sterical hindrance



Scheme 2. Synthetic strategy from the key intermediate **6**.

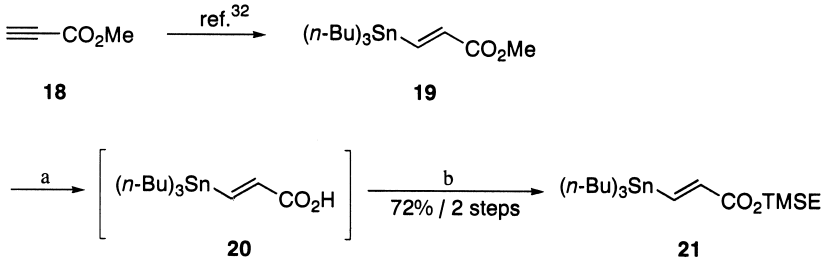


Scheme 3. *Reagents and conditions*: (a) TBDPSCl, imidazole, DMAP, DMF, rt, overnight; (b) 90% aq. AcOH, 50°C, 3 h; (c) Dess–Martin periodinane, CH_2Cl_2, rt, 30 min; (d) LiCl, *N*,*N*-diisopropylethylamine, $(EtO)_2P(O)CH_2CHO$, CH_3CN, rt, overnight; (e) CBr_4, PPh_3, CH_2Cl_2, −20°C, 1 h; (f) $Pd(OAc)_2$, PPh_3, $(n\text{-}Bu)_3SnH$, benzene, rt, 1.5 h; (g) $(n\text{-}Bu)_4NF$, THF, rt, overnight.

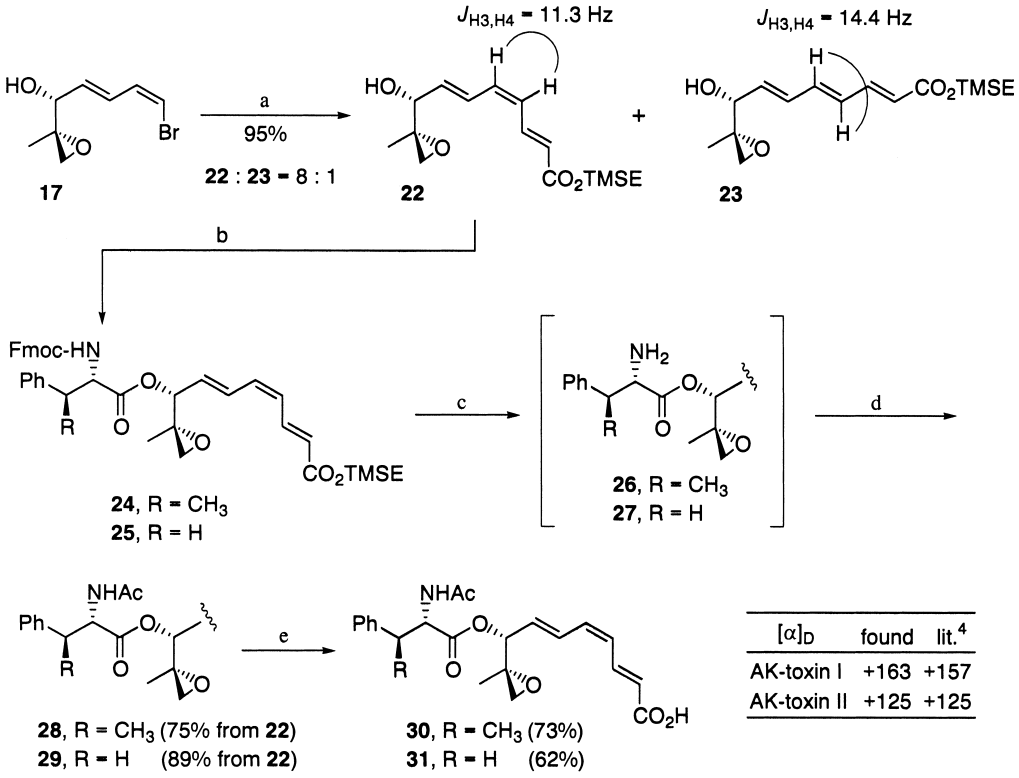
around the carbon bearing the TBDPSO group, as well as the relatively weak basicity of *N*,*N*-diisopropylethylamine. Compound **14** was then treated with dibromomethylenetriphenylphosphorane, which had been generated in situ from triphenylphosphine and tetrabromomethane,[31] to give the *gem*-dibromide (**15**) in 92% yield. The conversion of **15** to monobromide (**16**) was performed by the palladium-catalyzed stereoselective hydrogenolysis using the procedure recommended by Uenishi et al.[21,22] Palladium acetate (5 mol%) and triphenylphoshine (20 mol%) had been mixed to generate $Pd(PPh)_4$ as a catalyst, to which **15** and tributyltin hydride were added sequentially. The reaction proceeded smoothly to provide a single product, of which NMR spectra showed that the desired *Z*-alkenyl unit was successfully constructed ($J_{H6,H7}$=6.3 Hz). The key element of this reaction is the purity of the hydride source. When slightly cloudy tributyltin hydride was used, longer reaction time was required for completion and a significant reduction in yield was observed (89%, 1.5 h→67%, 3 h) due to a formation of a large number of byproducts. The TBDPS group of the resultant monobromide **16** was removed with tetrabutylammonium fluoride in THF to afford the substituted glycidol **17**, which was then subjected to the Stille reaction.

The coupling partner of **17**, 2-(trimethylsilyl)ethyl (*E*)-stannylacrylate (**21**), was synthesized as shown in Scheme 4. Methyl (*E*)-3-tributylstannylacrylate (**19**) was obtained from commercially available methyl propiolate (**18**).[32] Alkaline hydrolysis of **19** with aqueous sodium hydroxide in 1,4-dioxane gave the acid (**20**), which was then esterified without purification by using 2-(trimethylsilyl)ethanol and DCC to trimethylsilylethyl (TMSE) ester (**21**). The yield was 72% over 2 steps. The effectiveness of TMSE group as the protective group of terminal carboxyl moiety in AK-toxin synthesis has also been demonstrated by Ando et al.[14]

These two fragments were coupled by the Stille reaction (Scheme 5).[23] Compound **17** and **21** were treated with 10 mol% $Pd(PPh_3)_2Cl_2$ in DMF with mild heating (50°C), which was necessary to complete the reaction within reasonable time. Purification of the crude product afforded the targeted (2*E*,4*Z*,6*E*)-triene (**22**) and its all-*trans* isomer (**23**), which were separable by flash column chromatography. The yield was 95% and the isomeric ratio was 8:1 in favor of **22**. Their geometrical configurations were confirmed to be as such, based on the coupling constants in ^1H NMR spectrum ($J_{H3,H4}$=11.3 Hz for **22**, 14.3 Hz for **23**). Since the yield of **22** was excellent and **23** was successfully removed, further optimization of this reaction was not attempted.



Scheme 4. *Reagents and conditions*: (a) NaOH, H_2O, 1,4-dioxane, 60°C, 3 h; (b) DCC, 4-(dimethylamino)pyridine, 2-(trimethylsilyl)ethanol, CH_2Cl_2, rt, overnight.



$[\alpha]_D$	found	lit.[4]
AK-toxin I	+163	+157
AK-toxin II	+125	+125

Scheme 5. *Reagents and conditions*: (a) $Pd(PPh_3)_2Cl_2$, **21**, DMF, 50°C, 5.5 h; (b) DCC, 4-pyrrolidinopyridine, (2*S*,3*S*)-*N*-Fmoc-3-methylphenylalanine or (2*S*)-*N*-Fmoc-phenylalanine, EtOAc, rt, 1 h; (c) piperidine, CH_2Cl_2, rt, 5 h; (d) Ac_2O, pyridine, CH_2Cl_2, rt, 30 min; (e) $(n\text{-}Bu)_4NF$, THF, rt, 5 h.

The hydroxyl group of **22** was then acylated with (2*S*,3*S*)-*N*-Fmoc-3-methylphenylalanine or (2*S*)-*N*-Fmoc-phenylalanine using DCC as the condensing agent.[33] Although the structures of the resultant *N*-Fmoc derivatives (**24**, **25**) were confirmed by ^1H NMR, a small amount of impurities could not be removed even after chromatographic purification. Since they were found to be unstable, these semi-purified materials were immediately used for the next reaction without further purification. Treatment of **24** and **25** with piperidine in CH_2Cl_2 afforded the free amines (**26**, **27**) along with the dibenzofulvene and its piperidine adduct. This mixture was directly subjected to acetylation using acetic anhydride and pyridine in CH_2Cl_2, after most of the piperidine, which was expected to interfere with the acetylation, was removed by evaporation. The TMSE esters AK-toxin I and II (**28**, **29**) were thus obtained in good yields (75 and 89%, respectively). Neither recemization at C2′ nor geometrical isomerization occurred during this transformation. Finally, deprotection of the terminal carboxyl group was effected with tetrabutylammonium fluoride in THF to give the free acids (**30**, **31**), of which ^1H NMR spectra were practically identical with the literature data of naturally occuring compounds. Specific rotation of **30** and **31** were also in good agreement with the reported values, indicating that the stereochemically pure AK-toxins were successfully synthesized.

3. Conclusion

An efficient total synthesis of AK-toxins (**1**, **2**) has been achieved with an overall yield of 10%, requiring 12 steps starting from intermediate **6**. This procedure is highlighted by the excellent stereoselectivity, availability of **6**, and ease of product isolation without the use of HPLC. We have obtained **1** and **2** in stereochemically pure form on a several hundred milligram scale, and further scale-up is possible. With these synthetic AK-toxins in hand, we are now attempting to elucidate their mode of action against the Japanese pear.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were measured on a Bruker AC-300 spectrometer (300 MHz for ^1H; 75 MHz for ^{13}C), using tetramethylsilane as the internal standard. The solvents used are noted in each section below. Chemical shifts are reported in δ value by ppm unit and the coupling patterns are represented by the characters (s=singlet, d=doublet, t=triplet, m=multiplet, and combination of them). Optical rotations were measured on a JASCO DIP-1000 polarimeter. Elemental analyses were performed at the Microanalytical Center of Kyoto University. High-resolution mass spectra (HRMS) were taken on a JEOL JMS-600H instrument.

Reactions were carried out under inert atmosphere (Ar or N_2) in oven-dried glassware. Reaction solvents were dehydrated prior to use according to the standard protocols. Commercial reagents were used as purchased without further purification, except for *N*,*N*-diisopropyethylamine, which was distilled over CaH_2 prior to use. Diethyl 2-oxoethylphosphonate and (2*S*,3*S*)-*N*-Fmoc-3-methyl-

phenylalanine were prepared by the known procedure.[30,33] Preparation of compounds **6**, **11**–**13** was reported in our previous paper.[20] Flash column chromatography was performed on silica gel (Merck Kieselgel 60).

4.1.1. (2*E*,4*R*,5*S*)-4-(*tert*-Butyldiphenylsilyl)oxy-5,6-epoxy-5-methylhex-2-enal (14). To a stirred suspension of LiCl (2.54 g, 60 mmol) in CH_3CN (65 ml) was added *N*,*N*-diisopropylethylamine (21.0 ml, 120 mmol) and a solution of diethyl 2-oxoethylphosphonate (5.40 g, 30 mmol) in CH_3CN (10 ml). After 30 min, a solution of **13** in CH_3CN (10 ml) was introduced, and the resultant yellow solution was stirred overnight. The solution was poured onto saturated aqueous solution of NH_4Cl (200 ml) with vigorous stirring to afford a biphasic mixture, which was extracted wtih EtOAc (200 ml×3). The combined organic extract was washed with 1N aqueous HCl (200 ml), saturated aqueous $NaHCO_3$ (200 ml), and brine (200 ml). After dehydration with anhydrous Na_2SO_4, the solution was filtered and concentrated to a brown oil, which was purified by flash column chromatography (hexane/EtOAc=9:1, v/v) to afford **14** (4.11 g, 11 mmol, 54% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) 1.10 (s, 9H), 1.27 (s, 3H), 1.87 (d, *J*=4.6 Hz, 1H), 2.25 (d, *J*=4.6 Hz, 1H), 3.98 (dd, *J*=3.9, 1.7 Hz, 1H), 6.47 (ddd, *J*=15.7, 8.0, 1.7 Hz, 1H), 6.85 (dd, *J*=15.7, 3.9 Hz, 1H), 7.34–7.47 (m, 6H), 7.57–7.70 (m, 4H), 9.58 (d, *J*=8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) 15.7, 19.4, 27.0, 54.6, 57.6, 76.1, 127.8, 127.9, 130.2, 132.4, 132.6, 133.2, 134.8, 135.7, 135.8, 155.6, 193.2; $[\alpha]_D^{29}=-21.2$ (*c* 1.30, EtOH); Anal. Found: C, 72.19, H, 7.71. Calcd for $C_{23}H_{28}O_3Si$: C, 72.59, H, 7.42.

4.1.2. (4*E*,2*S*,3*R*)-7,7-Dibromo-3-(*tert*-butyldiphenylsilyl)-oxy-1,2-epoxy-2-methylhepta-4,6-diene (15). A solution of tetrabromomethane (4.97 g, 15 mmol) in CH_2Cl_2 (10 ml) was added dropwise to a stirred solution of triphenylphosphine (7.87 g, 30 mmol) in CH_2Cl_2 (60 ml), keeping the internal temperature below −15°C. After 30 min, a solution of **14** (4.00 g, 11 mmol) in CH_2Cl_2 (5 ml) was added slowly, and stirring was continued for 1 h. To the resultant orange solution was added triethylamine (3 ml). The reaction mixture was then warmed up to 0°C. Saturated aqueous Na_2CO_3 (4 ml) was added to the solution, which was then stirred vigorously for several minutes. This two-phase mixture was poured onto Et_2O (200 ml) and the resultant suspension was filtered through a pad of silica gel (∅ 60×20 mm^2). The filtrate was concentrated to crystallize triphenylphosphine oxide, which was then filtered off. The clear filtrate was concentrated and purified by flash column chromatography (hexane/EtOAc=14:1, v/v) to afford **15** as a colorless oil (5.16 g, 9.6 mmol, 92% yield). ^{1}H NMR ($CDCl_3$) 1.10 (s, 9H), 1.28 (s, 3H), 1.98 (d, *J*=4.8 Hz, 1H), 2.29 (d, *J*=4.8 Hz, 1H), 3.75 (dd, *J*=5.0, 1.4 Hz, 1H), 5.92 (dd, *J*=15.4, 5.0 Hz, 1H), 6.38 (ddd, *J*=15.4, 10.3, 1.4 Hz, 1H), 6.92 (d, *J*=10.3 Hz, 1H), 7.33–7.47 (m, 6H), 7.60–7.70 (m, 4H); ^{13}C NMR ($CDCl_3$) 15.9, 19.4, 27.0, 54.2, 58.0, 76.5, 91.6, 127.7 (×2), 128.1, 129.9, 130.0, 133.0, 133.6, 135.8, 135.9, 136.1, 136.2; $[\alpha]_D^{29}=-48.9$ (*c* 1.02, EtOH); Anal. Found: C, 53.83, H, 5.38. Calcd for $C_{24}H_{28}Br_2O_2Si$: C, 53.74, H, 5.26.

4.1.3. (4*E*,6*Z*,2*S*,3*R*)-7-Bromo-3-(*tert*-butyldiphenylsilyl)-oxy-1,2-epoxy-2-methylhepta-4,6-diene (16). Palladium acetate (105 mg, 0.47 mmol) and triphenylphosphine (492 mg, 1.90 mmol) were stirred in benzene (15 ml) to form a cloudy yellow solution in 30 min. By adding a solution of **15** (5.04 g, 9.4 mmol) in benzene (15 ml), a clear orange solution was obtained. To this solution was added dropwise tributyltin hydride (3.15 g, 10.8 mmol) over 30 min. In the course of addition, slight evolution of gas (H_2) was observed. After 1.5 h, the reaction mixture was diluted with Et_2O (200 ml) and washed with saturated aqueous KF (100 ml), water, and brine. The organic solution was dried over anhydrous $MgSO_4$, filtered, and concentrated to an oil, which was purified by flash column chromatography (hexane/EtOAc=14:1, v/v) to provide **16** (3.81 g, 8.3 mmol, 89% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$) 1.11 (s, 9H), 1.30 (s, 3H), 2.02 (d, *J*=4.8 Hz, 1H), 2.30 (d, *J*=4.8 Hz, 1H), 3.81 (d, *J*=5.0 Hz, 1H), 5.95 (dd, *J*=14.6, 5.0 Hz, 1H), 6.14 (d, *J*=6.3 Hz, 1H), 6.59 (dd, *J*=10.4, 6.3 Hz, 1H), 6.67 (dd, *J*=14.6, 10.4 Hz, 1H), 7.33–7.45 (m, 6H), 7.62–7.71 (m, 4H); ^{13}C NMR ($CDCl_3$) 16.0, 19.4, 27.0, 54.0, 58.1, 76.6, 108.4, 127.2, 127.6, 129.9 (×2), 131.8, 132.4, 133.1, 135.9, 136.1; $[\alpha]_D^{29}=-30.6$ (*c* 1.18, EtOH); Anal. Found: C, 63.17, H, 6.54. Calcd for $C_{24}H_{29}BrO_2Si$: C, 63.01, H, 6.39.

4.1.4. (4*E*,6*Z*,2*R*,3*S*)-7-Bromo-1,2-epoxy-2-methylhepta-4,6-dien-3-ol (17). To a solution of **16** (3.60 g, 7.9 mmol) in THF (40 ml) was added dropwise a 1 M solution of tetrabutylammonium fluoride in THF (16 ml, 16 mmol), and stirred overnight. The reaction mixture was diluted with EtOAc (200 ml), washed with saturated aqueous NH_4Cl (100 ml) and brine (100 ml). After being dried over anhydrous $MgSO_4$, the solution was filtered and concentrated. The residual oil was purified by flash column chromatography (hexane/EtOAc=3:1, v/v) to give **17** (1.47 g, 6.7 mmol, 85% yield) as a white waxy solid. This material was sufficiently pure for the next reaction, though further purification by recrystallizaion (hexane/ethyl acetate) gave colorless needles. Mp 59°C; ^{1}H NMR ($CDCl_3$) 1.37 (s, 3H), 1.45 (s, 1H), 2.63 (d, *J*=4.7 Hz, 1H), 2.93 (d, *J*=4.7 Hz, 1H), 4.22 (d, *J*=6.9 Hz, 1H), 5.86 (dd, *J*=14.6, 6.9 Hz, 1H), 6.23 (d, *J*=6.2 Hz, 1H), 6.65 (dd, *J*=10.2, 6.2 Hz, 1H), 6.71 (dd, *J*=14.6, 10.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) 17.9, 50.2, 58.6, 72.7, 109.3, 128.5, 131.6, 134.7; $[\alpha]_D^{26}=+73.1$ (*c* 1.02, EtOH); Anal. Found: C, 43.67, H, 4.87. Calcd for $C_8H_{11}BrO_2$: C, 43.86, H, 5.06.

4.1.5. 2-(Trimethylsilyl)ethyl (*E*)-3-(tributylstannyl)-acrylate (21). A 10% w/v aqueous NaOH (30 ml) was poured onto a solution of methyl (*E*)-3-(tributylstannyl)-acrylate (**19**, 4.92 g, 13 mmol) in 1,4-dioxane (30 ml), and the biphasic mixture was vigorously stirred at 60°C. After 2 h, the mixture was acidified with 1N aqueous HCl and extracted with EtOAc (100 ml×3). The combined extract was washed with brine (150 ml), dried over anhydrous $MgSO_4$, filtered, and concentrated. To the solution of the residual oil in CH_2Cl_2 (40 ml) was added 2-(trimethylsilyl)-ethanol (1.77 g, 15 mmol), 4-(dimethylamino)pyridine (0.12 g, 1 mmol), and a solution of 1,3-dicyclohexylcarbodiimide (3.09 g, 15 mmol) in CH_2Cl_2 (5 ml). After being stirred overnight, the resultant suspension was diluted with Et_2O (200 ml) and the precipitated dicyclohexylurea was filtered off. The filtrate was washed with 1N aqueous HCl (100 ml), saturated aqueous $NaHCO_3$ (100 ml), and brine

(100 ml). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated to an oil, which was purified by flash column chromatography (hexane/Et_2O=39:1, v/v) to provide **21** (4.38 g, 9.5 mmol, 72%) as a colorless oil. ^1H NMR ($CDCl_3$) 0.53 (s, 9H), 0.89 (t, J=7.2 Hz, 9H), 0.94–1.00 (m, 6H), 1.01–1.07 (m, 2H), 1.25–1.37 (m, 6H), 1.45–1.59 (m, 6H), 4.22–4.27 (m, 2H), 6.28 (d, J=19.4 Hz, 1H), 7.72 (d, J=19.4 Hz, 1H); ^{13}C NMR ($CDCl_3$) −1.5, 9.6, 13.7, 17.4, 27.2, 29.0, 62.6, 136.7, 152.1, 165.0; Anal. Found: C, 52.02, H, 9.32. Calcd for $C_{20}H_{42}O_2SiSn$: C, 52.07, H, 9.18.

4.1.6. 2-(Trimethylsilyl)ethyl (2*E*,4*Z*,6*E*,8*R*,9*S*)-9,10-epoxy-8-hydroxy-9-methyldeca-2,4,6-trienoate (22). To a solution of dichlorobis(triphenylphophine)palladium (248 mg, 0.35 mmol) and **17** (744 mg, 3.5 mmol) in DMF (20 ml) was added a solution of **21** (2.30 g, 5.0 mmol) in DMF (5 ml) with stirring at 50°C. After 5.5 h, the reaction mixture was poured onto water (150 ml) with vigorous stirring. The resultant suspension was extracted with Et_2O (100 ml×3). The combined extract was washed with saturated aqueous $NaHCO_3$ (150 ml) and brine (150 ml), dried over anhydrous $MgSO_4$, filtered, and concentrated. The resultant crude oil was purified by flash column chromatography (hexane/Et_2O=1:1, v/v) to provide **22** (540 mg, 1.7 mmol, 51%), along with a 3:1 mixture of **22** and **23** (460 mg, 1.5 mmol, 44%). The isomeric ratio of **22** to **23** was 8:1 in total. The latter fraction was further purified by flash column chromatography using the same mobile phase. Compound **22**: ^1H NMR ($CDCl_3$) 0.64 (s, 9H), 1.01–1.07 (m, 2H), 1.38 (s, 3H), 2.35 (s, 1H), 2.64 (d, J=4.7 Hz, 1H), 2.93 (d, J=4.7 Hz, 1H), 4.22–4.29 (m, 3H), 5.83 (dd, J=15.1, 6.7 Hz, 1H), 5.91 (d, J=15.2 Hz, 1H), 6.13 (t, J=11.3 Hz, 1H), 6.32 (t, J=11.3 Hz, 1H), 6.95 (dd, J=15.1 Hz, 11.4 Hz, 1H), 7.74 (ddd, J=15.2, 11.7, 0.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) −1.4, 17.4, 18.1, 50.1, 58.8, 62.7, 72.6, 122.7, 127.3, 127.4, 135.2, 135.7, 138.4, 167.1; $[\alpha]_D^{27}$=+84.0 (*c* 1.02, EtOH); HRMS (EI) *m/z* Found: 310.1610. Calcd for $C_{16}H_{26}O_4Si$: 310.1600; Compound **23**: ^1H NMR ($CDCl_3$) 0.05 (s, 9H), 1.01–1.07 (m, 2H), 1.37 (s, 3H), 2.27 (s, 1H), 2.63 (d, J=4.7 Hz, 1H), 2.92 (d, J=4.7 Hz, 1H), 4.22–4.29 (m, 3H), 5.84 (dd, J=14.4, 6.6 Hz, 1H), 5.90 (d, J=15.2 Hz, 1H), 6.35 (dd, J=14.3, 11.1 Hz, 1H), 6.45 (ddd, J=14.3, 10.7, 1.1 Hz, 1H), 6.55 (dd, J=14.3, 10.7 Hz), 7.28 (dd, J=15.2, 11.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) −1.4, 17.4, 18.1, 50.1, 58.8, 62.6, 72.5, 122.0, 131.0, 132.1, 134.8, 139.1, 143.8, 167.1; $[\alpha]_D^{26}$=+49 (*c* 0.82, EtOH); HRMS (EI) *m/z* Found: 310.1615. Calcd for $C_{16}H_{26}O_4Si$: 310.1600.

4.1.7. 2-(Trimethylsilyl)ethyl (2*E*,4*Z*,6*E*,8*R*,9*S*,2′*S*,3′*S*)-9,10-epoxy-8-[2′-(9-fluorenylmethoxycarbamino)-3′-phenylbutanoyloxy]-9-methyldeca-2,4,6-trienoate (24). 1,3-Dicyclohexylcarbodiimide (0.53 g, 2.6 mmol) in EtOAc (12 ml) was added to a solution of (2*S*,3*S*)-*N*-Fmoc-3-methylphenylalanine (0.89 g, 2.2 mmol), 4-pyrrolidinopyridine (20 mg, 0.13 mmol), and **22** (531 mg, 1.7 mmol) with stirring. After 1 h, the resultant white suspension was diluted with Et_2O (100 ml) and stirred vigorously for several minutes. The white precipitate was filtered off, and the filtrate was washed with aqueous 1N HCl (50 ml), aqueous saturated $NaHCO_3$ (50 ml), and brine (50 ml). After drying over anhydrous $MgSO_4$, the solution was filtered and concentrated to give a crude oil. Purification by flash column chromatography (hexane/Et_2O=3:2, v/v) afforded **24** (1.4 g) as a viscous oil with some impurities detected on TLC. This material was immediately used for the next reaction without further purification. ^1H NMR ($CDCl_3$) 0.06 (s, 9H), 1.01–1.06 (m, 2H), 1.27 (s, 3H), 1.36 (d, J=7.1 Hz, 3H), 2.57 (d, J=4.7 Hz, 1H), 2.71 (d, J=4.7 Hz, 1H), 3.35–3.44 (m, 1H), 4.18–4.28 (m, 3H), 4.33 (dd, J=10.5, 6.9 Hz, 1H), 4.44 (dd, J=10.5, 7.2 Hz, 1H), 4.65 (dd, J=8.7, 5.1 Hz, 1H), 5.08 (d, J=8.7 Hz, 1H), 5.28 (d, J=7.9 Hz, 1H), 5.76 (dd, J=15.2, 7.9 Hz, 1H), 5.92 (d, J=15.1 Hz, 1H), 6.17 (t, J=11.1 Hz, 1H), 6.27 (t, J=10.9 Hz, 1H), 6.89 (dd, J=15.0, 10.9 Hz, 1H), 7.13 (d, J=6.8 Hz, 2H), 7.20–7.33 (m, 5H), 7.40 (t, J=7.5 Hz, 2H), 7.54 (t, J=6.6 Hz, 2H), 7.68 (dd, J=15.1, 11.4 Hz, 1H), 7.76 (d, J=7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) −1.4, 17.3, 17.4, 17.6, 42.0, 47.2, 52.3, 56.5, 59.1, 62.8, 67.1, 77.1, 120.0, 123.5, 125.0, 125.2, 127.1, 127.7, 127.8, 128.6, 128.9, 130.1, 130.4, 134.8, 137.9, 140.3, 141.3, 143.7, 143.9.; $[\alpha]_D^{26}$=+80 (*c* 0.56, EtOH); Anal. Found: C, 70.92, H, 6.85, N, 2.01. Calcd for $C_{41}H_{47}NO_7Si$: C, 70.97, H, 6.83, N, 2.02.

4.1.8. 2-(Trimethylsilyl)ethyl (2*E*,4*Z*,6*E*,8*R*,9*S*,2′*S*)-9,10-epoxy-8-[2′-(9-fluorenylmethoxycarbamino)-3′-phenylpropanoyloxy]-9-methyldeca-2,4,6-trienoate (25). Compound **25** was synthesized from (2*S*)-*N*-Fmoc-phenylalanine (0.92 g, 2.4 mmol) and **22** (568 mg, 1.8 mmol) in a similar manner to that used for the preparation of **24**, using 1,3-dicyclohexylcarbodiimide (0.57 g, 2.7 mmol) and 4-pyrrolidinopyridine (20 mg, 0.13 mmol). The semipurified **25** (1.5 g) was subjected to the next reaction without further purification. ^1H NMR ($CDCl_3$) 0.59 (s, 9H), 1.01–1.07 (m, 2H), 1.29 (s, 3H), 2.59 (d, J=4.6 Hz, 1H), 2.73 (d, J=4.6 Hz, 1H), 3.08–3.16 (m, 2H), 4.18–4.29 (m, 3H), 4.35 (dd, J=10.6, 6.9 Hz, 1H), 4.44 (dd, J=10.5, 7.2 Hz, 1H), 4.67–4.74 (m, 1H), 5.26 (d, J=7.9 Hz, 1H), 5.28 (d, J=7.7 Hz, 1H), 5.68 (dd, J=14.9, 7.6 Hz, 1H), 5.93 (d, J=15.1 Hz, 1H), 6.17 (t, J=11.0 Hz, 1H), 6.25 (t, J=10.9 Hz, 1H), 6.85 (dd, J=15.0, 10.6 Hz, 1H), 7.09 (d, J=7.0 Hz, 2H), 7.20–7.33 (m, 5H), 7.30 (t, J=7.4 Hz, 2H), 7.40 (dd, J=7.4 Hz, 2H), 7.68 (dd, J=15.1 Hz, 1H), 7.76 (d, J=7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) −1.4, 17.4 (×2), 38.2, 47.2, 52.2, 54.8, 56.5, 62.8, 67.1, 77.1, 120.0, 123.5, 125.1 (×2), 127.1, 127.2, 127.7, 128.6, 128.7, 129.4, 130.0, 130.2, 134.8, 135.4, 138.0, 141.3, 143.7, 143.8; $[\alpha]_D^{26}$=+67.2 (*c* 1.10, EtOH); Anal. Found: C, 70.73, H, 6.63, N, 2.26. Calcd for $C_{40}H_{45}NO_7Si$: C, 70.66, H, 6.67, N, 2.06.

4.1.9. 2-(Trimethylsilyl)ethyl (2*E*,4*Z*,6*E*,8*R*,9*S*,2′*S*,3′*S*)-8-(2′-acetylamino-3′-phenylbutanoyloxy)-9,10-epoxy-9-methyldeca-2,4,6-trienoate: AK-toxin I TMSE ester (28). Piperidine (2.0 ml, 20 mmol) was added to a solution of **24** (1.4 g) in CH_2Cl_2 (30 ml) and the reaction mixture was stirred for 5 h. The resultant pale yellow solution was diluted with toluene (100 ml) and evaporated to dryness to remove the excess piperidine (repeated twice). The semi-solid material thus obtained was dissolved in CH_2Cl_2 (30 ml), followed by the addition of pyridine (3.0 ml, 37 mmol) and acetic anhydride (1.0 ml, 11 mmol). After 30 min, the solution was diluted with EtOAc (200 ml), and washed with 1N aqueous HCl (100 ml×2), saturated aqueous $NaHCO_3$ (100 ml), and brine (100 ml). The organic

phase was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residual oil was purified by flash column chromatography (hexane/ethyl acetate=1:1, v/v) to provide **28** (657 mg, 1.3 mmol, 75% from **22**) as a viscous oil. ^{1}H NMR ($CDCl_3$) 0.06 (s, 9H), 1.01–1.07 (m, 2H), 1.30 (s, 3H), 1.35 (d, J=7.1 Hz, 3H), 1.98 (s, 3H), 2.60 (d, J=4.7 Hz, 1H), 2.72 (d, J=4.7 Hz, 1H), 3.32–3.40 (m, 1H), 4.23–4.29 (m, 2H), 4.86 (dd, J=8.2, 5.8 Hz, 1H), 5.27 (d, J=7.9 Hz, 1H), 5.69 (d, J=8.1 Hz, 1H), 5.78 (dd, J=15.2, 7.9 Hz, 1H), 5.94 (d, J=15.1 Hz, 1H), 6.19 (t, J=11.1 Hz, 1H), 6.30 (t, J=10.9 Hz, 1H), 6.89 (dd, J=15.1, 11.1 Hz, 1H), 7.13–7.16 (m, 2H), 7.20–7.31 (m, 3H), 7.69 (dd, J=15.2, 11.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) −1.5, 17.3, 17.4, 17.7, 23.1, 41.9, 52.3, 56.5, 57.3, 62.7, 77.2, 123.5, 127.4, 127.7 (×2), 128.7, 128.8, 130.2, 134.8, 138.0, 140.6, 166.9, 170.0, 170.5; $[\alpha]_D^{25}$=+112 (*c* 0.80, EtOH); HRMS (FAB) *m/z* Found: 513.2563. Calcd for $C_{28}H_{39}NO_6Si$: 513.2547.

4.1.10. 2-(Trimethylsilyl)ethyl (2*E*,4*Z*,6*E*,8*R*,9*S*,2′*S*)-8-(2′-acetylamino-3′-phenylpropanoyloxy)-9,10-epoxy-9-methyldeca-2,4,6-trienoate: AK-toxin II TMSE ester (29). Compound **29** was synthesized from **25** (1.5 g) in a similar manner to that used for the preparation of **28**, using piperidine (2.0 ml, 20 mmol), pyridine (3.0 ml, 37 mmol), and acetic anhydride (1.0 ml, 11 mmol). Purification by flash column chromatography (hexane/EtOAc=2:3, v/v) afforded **29** (818 mg, 1.6 mmol, 89% from **22**) as a viscous oil. ^{1}H NMR ($CDCl_3$) 0.06 (s, 9H), 1.02–1.07 (m, 2H), 1.31 (s, 3H), 2.01 (s, 3H), 2.61 (d, J=4.7 Hz, 1H), 2.74 (d, J=4.7 Hz, 1H), 3.12 (d, J=6.1 Hz, 2H), 4.24–4.29 (m, 2H), 4.91 (dt, J=7.6, 6.1 Hz, 1H), 5.27 (d, J=7.8 Hz, 1H), 5.68 (dd, J=15.2, 7.9 Hz, 1H), 5.91 (d, J=7.4 Hz, 1H), 5.94 (d, J=15.2 Hz, 1H), 6.19 (t, J=10.9 Hz, 1H), 6.28 (t, J=10.8 Hz, 1H), 6.84 (dd, J=15.2, 10.8 Hz, 1H), 7.07–7.10 (m, 2H), 7.20–7.26 (m, 3H), 7.68 (dd, J=15.2, 11.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) −1.4, 17.4 (×2), 23.1, 37.9, 52.2, 53.2, 56.5, 62.8, 77.0, 123.5, 127.2, 128.6, 128.8, 129.4, 130.0, 130.2, 134.8, 135.5, 138.0, 166.9, 169.6, 170.7; $[\alpha]_D^{20}$=+94 (*c* 1.10, EtOH); HRMS (FAB) *m/z* Found: 499.2364. Calcd for $C_{27}H_{37}NO_6Si$: 499.2390.

4.1.11. (2*E*,4*Z*,6*E*,8*R*,9*S*,2′*S*,3′*S*)-8-(2′-Acetylamino-3′-phenylbutanoyloxy)-9,10-epoxy-9-methyldeca-2,4,6-trienoic acid (30): AK-toxin I (1). To a solution of **28** (590 mg, 1.1 mmol) in THF (12 ml) was added 1 M tetrabutylammonium fluoride in THF (6 ml, 6 mmol) with stirring. After 5 h, the reaction mixture was diluted with EtOAc (150 ml), and washed with 1N aqueous HCl (100 ml) and brine (100 ml). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated. Purification of the residual oil by flash column chromatography ($CHCl_3$/MeOH=14:1, v/v) afforded **30** (349 mg, 0.84 mmol, 73%) as a white powder. ^{1}H NMR (CD_3OD) 1.30 (d, J=7.1 Hz, 3H), 1.31 (s, 3H), 2.61 (d, J=4.8 Hz, 1H), 2.76 (d, J=4.8 Hz, 1H), 3.21 (m, 1H), 4.73 (d, J=8.7 Hz, 1H), 5.35 (d, J=7.2 Hz, 1H), 5.88 (dd, J=15.1, 7.2 Hz, 1H), 5.95 (d, J=15.1 Hz, 1H), 6.25 (t, J=11.3 Hz, 1H), 6.35 (t, J=11.1 Hz, 1H), 7.15–7.31 (m, 5H), 7.77 (dd, J=15.2, 11.5 Hz, 1H); ^{13}C NMR (CD_3OD) 17.8, 19.1, 22.1, 43.0, 52.8, 57.8, 59.4, 77.5, 124.1, 128.1, 128.8, 129.4, 129.6, 130.6, 132.3, 136.6, 140.1, 143.4, 170.2, 171.7, 173.1; $[\alpha]_D^{23}$=+157 (*c* 0.16, MeOH); Anal. Found: C, 66.51, H, 6.70, N, 3.36, Calcd for $C_{23}H_{27}NO_6$: C, 66.81, H, 6.58, N, 3.39.

4.1.12. (2*E*,4*Z*,6*E*,8*R*,9*S*,2′*S*)-8-(2′-Acetylamino-3′-phenylpropanoyloxy)-9,10-epoxy-9-methyldeca-2,4,6-trienoic acid (31): AK-toxin II (2). Compound **31** was synthesized from **29** (712 mg, 1.4 mmol) as described in the preparation of **28**, using 1 M tetrabutylammonium fluride in THF (6 ml, 6 mmol). A 352 mg (0.88 mmol, 62%) of **31** was obtained as a white powder. ^{1}H NMR (CD_3OD) 1.29 (s, 3H), 1.94 (s, 3H), 2.61 (d, J=4.8 Hz, 1H), 2.75 (d, J=4.8 Hz, 1H), 3.00 (dd, J=13.7, 8.2 Hz, 1H), 3.10 (dd, J=13.7, 6.9 Hz, 1H), 4.67 (dd, J=8.1, 7.0 Hz, 1H), 5.22 (d, J=7.0 Hz, 1H), 5.71 (dd, J=15.2, 7.0 Hz, 1H), 5.95 (d, J=15.1 Hz, 1H), 6.22 (t, J=11.1 Hz, 1H), 6.33 (t, J=10.9 Hz, 1H), 6.81 (dd, J=15.1, 11.4 Hz, 1H), 7.15–7.29 (m, 5H), 7.74 (dd, J=15.1, 11.3 Hz, 1H); ^{13}C NMR (CD_3OD) 17.6, 22.2, 38.5, 53.1, 55.7, 57.8, 77.7, 124.0, 128.0, 129.2, 129.6, 130.2, 130.3, 132.3, 136.7, 137.9, 140.3, 170.3, 172.1, 173.2; $[\alpha]_D^{21}$=+125 (*c* 0.12, MeOH); Anal. Found: C, 66.10, H, 6.28, N, 3.53, Calcd for $C_{22}H_{25}NO_6$: C, 66.15, H, 6.31, N, 3.51.

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