Synthesis of Methyl Esters of AF-Toxin IIa and IIc, Toxins to Japanese White Pear Produced by *Alternaria alternata* Strawberry Pathotype

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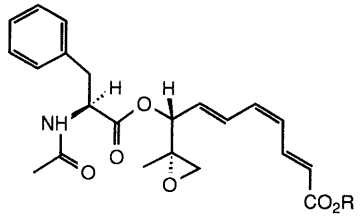
Methyl esters of AF-toxin IIa and IIc, toxic compounds to Japanese white pear produced by *Alternaria alternata* strawberry pathotype, were synthesized as the optically active forms starting from vitamin C as a chiral material.

Keywords AF-toxin; host-specific toxin; plant pathology; isoleucine; Mitsunobu reaction; α-hydroxy-2-methylpentanoic acid; Wadsworth–Emmons reaction; esterification

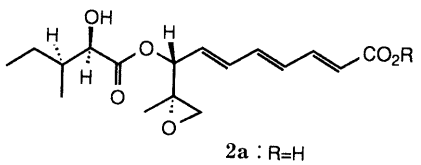
The fungi *Alternaria alternata* strawberry pathotype produce several kinds of compounds named AF-toxin, which are toxic not only to the host plant but also to Japanese white pear. Some of them were isolated in pure forms and the structures were elucidated by Nakatsuka *et al.*[1] AF-Toxins are closely related structurally to AK-toxins[2] in the C-11 trienoic acid moiety. Because of their biological activity and their novel structures, there have been several reports[3-5] concerning the synthesis of these toxins and their esters. We also reported the synthesis of the methyl ester (**1**) of AK-toxin II and pointed out that the stereochemistries of two chiral centers in the C-11 trienoic acid moiety played an important role in the toxicity–structure relationship.[6] As a continuation of our synthetic work on the toxins, we report here the synthesis of the methyl esters (**3** and **2**) of AF-toxin IIa and IIc, which differ from each other in the double bond geometry of the trienoic acid moiety. When we initially examined the synthesis of AF-toxins, the exact stereochemistry of the α-hydroxy-β-methylpentanoic acid moiety in the toxins had not been firmly elucidated.[7] Therefore, preparation of the diastereoisomeric acids (*threo* and *erythro*-forms) in optically active forms was required to complete the synthesis of these toxin methyl esters.

Treatment of isoleucine (**4**) with sodium nitrite in acetic acid gave the α-acetoxy-β-methylpentanoic acid (**5**) as a result of retention of the configuration of an α-amino group.[8] Esterification of the acid (**5**) with benzyl alcohol in benzene in the presence of *p*-toluenesulfonic acid with removal of water afforded a mixture consisting of the α-acetoxy-ester (**6**) and α-hydroxy-ester (**7**), the former of which was easily converted to the latter by hydrolysis with lithium carbonate in methanol. After protection of the hydroxyl group with a *tert*-butyldiphenylsilyl (TBDPS) group, hydrogenation of the resulting ester (**8**) on palladium carbon furnished the acid (**9**) in good yield. Esterification of the acid (**9**) with the oxide-ester (**10**)[6] with dicyclohexylcarbodiimide (DCC) in the presence of 4-pyrrolidinopyridine[9] gave the ester (**11**) in 86% yield without racemization at the α-carbon of the acid (**9**). De-silylation of the ester (**11**) with tetrabutylammonium fluoride (TBAF) gave the hydroxy-ester (**12**) which has the entire carbon framework corresponding to AF-toxin IIc methyl ester. Although the proton nuclear magnetic resonance (^{1}H-NMR, 400 MHz) spectrum of the synthetic compound (**12**) exhibited a close similarity to that of AF-toxin IIc methyl ester reported in the literature,[1] the chemical shifts of the primary (0.88, 3H, t) and secondary methyl (0.99, 3H, d) groups in the synthesized ester (**12**) showed remarkable differences from the methyl ester obtained from natural sources (0.97, 3H, t and 0.87, 3H, d). Thus, we aimed at the synthesis of the diastereoisomeric α-hydroxy-acid (**13**). Treatment of the α-hydroxy-ester (**7**) with ethyl diazodicarboxylate and triphenylphosphine in formic acid[10] gave the formyl ester (**14**). Mild hydrolysis of **14** with lithium carbonate in methanol gave the hydroxy-ester (**15**). Its ^{1}H-NMR spectrum and $[\alpha]_D$ value are different from those of the hydroxy-ester (**7**) mentioned above. The same reaction sequence on the hydroxy-ester (**15**) as for the ester (**7**) gave the acid (**16**) in 68% overall yield. The same reaction sequence (condensation of **10** and **16** followed by the de-silylation reaction) furnished AF-toxin IIc methyl ester (**2b**) in 75% yield. Accomplishment of the synthesis was confirmed by the identity of the ^{1}H-NMR spectral data of the synthetic compound with reported values.[1]

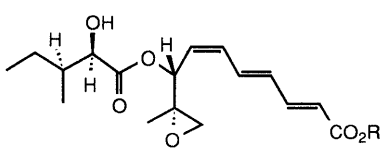
Next, we turned our attention to the synthesis of AF-toxin IIa methyl ester (**3b**), which has a 6,7-*cis* double bond in the trienoic acid moiety and shows strong toxicity to Japanese white pear. An attempt to form the *cis* olefin by Wittig reaction of the aldehyde (**17**)[6] with methyl 4-triphenylphosphonium crotonate and lithium methoxide[6] was unsuccessful, resulting in formation of the *trans–trans* ester (**18**), identical with an authentic sample,[6] in low yield. Then, we planned to form the *cis* double bond by a partial



1a : R=H
1b : R=Me

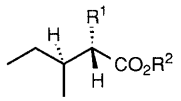


2a : R=H
2b : R=Me

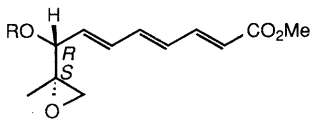


3a : R=H
3b : R=Me

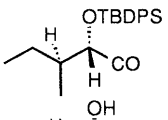
Chart 1



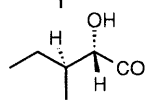
4 : R^1= NH_2; R^2= H
5 : R^1= OAc; R^2= H
6 : R^1= OAc; R^2= CH_2Ph
7 : R^1= OH; R^2= CH_2Ph
8 : R^1= OTBDPS; R^2= CH_2Ph
9 : R^1= OTBDPS; R^2= H



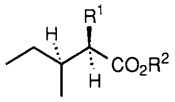
10 : R= H



11 : R=

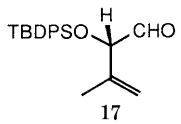


12 : R=



13 : R^1= OTBDPS; R^2=CH_2Ph
14 : R^1= OCHO; R^2=CH_2Ph
15 : R^1= OH; R^2= CH_2Ph
16 : R^1= OTBDPS; R^2= H

Chart 2



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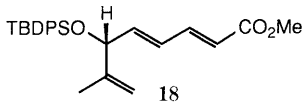

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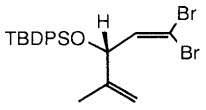

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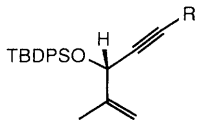
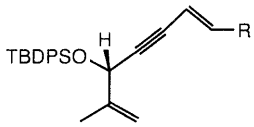
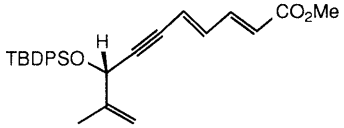


20 : R= H
21 : R= CHO



22 : R= CO_2Me
23 : R= CH_2OH
24 : R= CHO



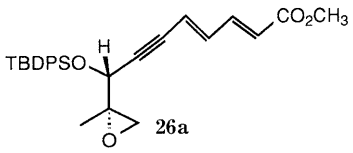
25

Chart 3

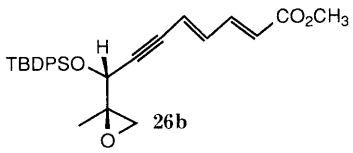
reduction of a triple bond.

Treatment of the aldehyde (**17**)[6] with carbon tetrabromide and triphenylphosphine[11] gave the dibromide (**19**) in 73% yield. The bromide was smoothly transformed to the acetylene (**20**) by treatment with *n*-butyllithium followed by water in 65% yield. Lithiation of the acetylene (**20**) with *n*-butyllithium and treatment of the resulting lithio compound with dimethylformamide (DMF) in tetrahydrofuran (THF) at −78 °C gave the aldehyde (**21**) in 86% yield. Direct treatment of the reaction mixture of the acetylene formation reaction with DMF did not give a good result. The aldehyde (**21**) was subjected to a Wadsworth–Emmons reaction with trimethyl phosphonoacetate, affording the ester (**22**) in 87% yield. The structure of this product was confirmed by its ^{1}H-NMR spectrum, which showed signals at δ 5.97 (1H, J=16 Hz) and 6.63 (1H, dd, J=16 and 1.8 Hz) assigned to two olefinic protons on a newly formed *trans*-double bond. Reduction of **22** with diisobutylaluminum hydride (DIBAL-H) followed by oxidation (MnO_2) gave the aldehyde (**24**) in good yield. Wadsworth–Emmons reaction on the aldehyde (**24**) gave the ester (**25**) having a *trans* diene. Epoxidation of **25** with *m*-chloroperbenzoic acid (*m*CPBA) gave a mixture (revealed by its ^{1}H-NMR spectrum (400 MHz)) of the oxides (**26a** and **26b**), but both oxides showed the same *Rf* values on thin layer chromatography with several solvent systems. An attempt to isolate each oxide in pure form was unsuccessful.

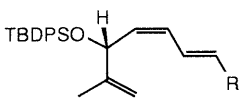
Hydrogenation of **22** with Lindlar catalyst gave the *cis–trans* diene-ester (**27**) in 87% yield. Its ^{1}H-NMR spectrum exhibited two doublet signals at δ 5.91 (t, J=11.7 Hz) and 5.71 (dd, J=11.7 and 8.4 Hz), confirming the *cis* geometry of the newly formed double bond. The same reaction sequence on **27** (DIBAL-H reduction, manganese dioxide oxidation, and Wadsworth–Emmons reaction) gave the *cis–trans–trans*-trienoic acid ester (**29**). The structure of **29** was also confirmed by its ^{1}H-NMR spectrum (7.04 and δ 5.80 (1H each, J=15.4 Hz)). Oxidation of **29** with *m*CPBA gave a mixture of two diastereoisomers (**30a**) and (**30b**) in a 1 : 1 ratio. In this case, both isomers were isolated in pure forms by preparative thin layer chromatography and flash chromatography. Stereostructures of both oxides were proposed on the basis of their ^{1}H-NMR spectra. Thus, one of the isomers showed signals at δ 2.53 and 2.64 (1H each, J=4.6 Hz) and the other showed signals at δ 2.55 and 2.63 (1H each, J=4.9 Hz), respectively, as a pair of AB-quartets assigned to the methylene protons of the oxide moiety. It is possible to



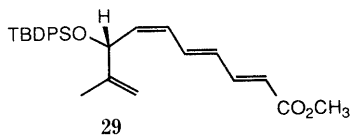
26a



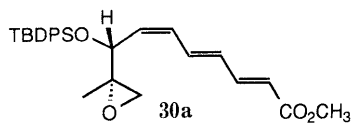
26b



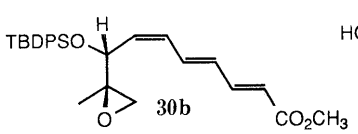
27 : R= CO_2Me
28 : R= CHO



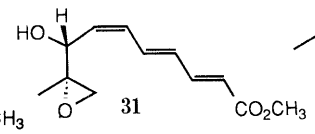
29



30a



30b



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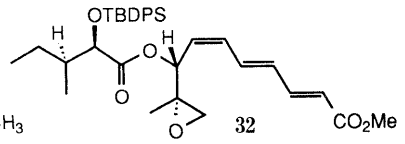

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Chart 4

discriminate the structures of these compounds based on the fact[6] that an oxide exhibiting larger chemical shift difference of the AB-signal has (*R*) and (*S*) configuration at the carbons bearing the silyloxy and the oxide oxygen, respectively. Thus, the former (**30a**) has (*R*) and (*S*) configurations and the other (**30b**) has (*R*) and (*R*) configurations at these carbons. The discrimination was ultimately confirmed by successful synthesis of AF-toxin IIa methyl ester. Treatment of **30a** with TBAF gave the (8*R*)-(9*S*)-hydroxy-*cis*–*trans*–*trans* ester (**31a**) in 45% yield. The acylation of **31** with the α-silyloxy-acid (**16**) in the same manner as mentioned above gave the ester (**32**) in 40% yield. Deprotection of the TBDPS group of **32** with TBAF in methylene chloride afforded AF-toxin IIa methyl ester (**3b**). Success in the synthesis of AF-toxin II methyl ester was confirmed by comparison of the ^{1}H-NMR spectral data with those[1] of AF-toxin II methyl ester obtained from natural sources. The synthetic AF-toxin IIa and IIc are toxic to Japanese white pear, as evaluated on leaves of the plant.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-408 spectrometer in chloroform. ^{1}H-NMR spectra were recorded on JEOL PMX-60, JEOL FX 90Q, and JNM-GX 400 NMR spectrometers with tetramethylsilane as an internal standard and chemical shifts are given in δ (ppm). Optical rotations were measured with a JASCO DIP-181 digital polarimeter and high-resolution mass (HR-MS) spectra were taken with a JEOL JMS-DX303 instrument. Column chromatography was performed with Kieselgel 60G (70—230 mesh) and flash column chromatography was performed with Kieselgel 60G (Art 7731). Homogeneities of the compounds cited in this report were confirmed by examination of the ^{1}H-NMR spectra and by thin layer chromatography.

2(*S*)-Acetoxy-3(*S*)-methylpentanoic Acid (5) $NaNO_2$ (4.8 g, 69.6 mmol) was added in portions to a stirred solution of L-isoleucine (**4**) (7.84 g, 59.8 mmol) in AcOH (72 ml) over 4.5 h at 30—35 °C, and the whole was allowed to stand overnight at room temperature. The solvent was evaporated off *in vacuo* to give a residue, which was shaken vigorously with a mixture of ether (120 ml), water (10 ml) and concentrated HCl (6 ml). After washing of the ethereal layer with water, the ethereal layer was extracted with 10% aqueous Na_2CO_3. The aqueous extracts were combined, and acidified with concentrated HCl, and extracted with ether. The ethereal layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated to dryness to give the acid (**5**) (8.9 g, 85%) as a yellow oil. IR ($CHCl_3$): 1720, 1740 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 0.85 (3H, t, J=7.2 Hz), 1.01 (3H, d, J=7.2 Hz), 1.30—1.62 (3H, m), 2.15 (3H, s), 4.95 (1H, d J=4.8 Hz), 10.3 (1H, s), $[\alpha]_D^{25}=+14.6°$ (c=1.00, EtOH). MS m/z: 174 (M^+).

Benzyl 2(*S*)-Acetoxy-3(*S*)-methylpentanoate (6) and Benzyl 2(*S*)-Hydroxy-3(*S*)-methylpentanoate (7) A mixture of the acid (**5**) (5.0 g, 28.7 mmol), *p*-toluenesulfonic acid (0.5 g) and benzyl alcohol (10.0 g, 92.6 mmol) in dry benzene (60 ml) was refluxed overnight with azeotropic removal of water under argon. The solvent was evaporated off *in vacuo* and the residue was dissolved in ether (100 ml). The ethereal solution was washed with 3% aqueous Na_2CO_3, and water, dried with $MgSO_4$ and concentrated to dryness to give a residue, which was chromatographed on silica gel in hexane–chloroform (1 : 1). Elution with the same solvent gave the benzyl acetoxypentanoate (**6**) (2.25 g, 32%) and the hydroxypentanoate (**7**) (2.40 g, 35.3%) in that order as colorless oils.

Benzyl 2(*S*)-Acetoxy-3(*S*)-methylpentanoate (**6**): IR ($CHCl_3$): 1740 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 0.88 (3H, t, J=7.2 Hz), 0.96 (3H, d, J=7.2 Hz), 1.15—1.90 (3H, m), 2.10 (3H, s), 4.93 (1H, d, J=4.8 Hz), 5.17 (2H, s), 7.33 (5H, br s). $[\alpha]_D^{27}=-27.6°$ (c=1.25, EtOH). HR-MS m/z: Calcd for $C_{15}H_{20}O_4$ (M^+): 264.1362. Found: 264.1341.

Benzyl 2(*S*)-Hydroxy-3(*S*)-methylpentanoate (**7**): IR ($CHCl_3$): 1725, 3540 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 0.86 (3H, t, J=7.2 Hz), 0.98 (3H, d, J=7.2 Hz), 1.15—1.90 (3H, m), 2.72 (1H, d, J=6.2 Hz), 4.12 (1H, dd, J=6.2, 4.8 Hz), 5.20 (2H, s), 7.35 (5H, s). $[\alpha]_D^{27}=-11.8°$ (c=1.00, EtOH). HR-MS m/z: Calcd for $C_{13}H_{18}O_3$ (M^+): 222.1256. Found: 222.1252.

Hydrolysis of the Acetate (6) A mixture of the acetoxycarboxylate **6** (270 mg, 1.0 mmol) and lithium carbonate (45 mg, 1.2 mmol) in methanol (10 ml) was stirred at room temperature for 15 h. The reaction mixture was concentrated *in vacuo* to afford a residue, which was extracted with ether. The ethereal solution was washed with 3% aqueous HCl, 3% aqueous Na_2CO_3 and water, and dried with $MgSO_4$. Removal of the solvent gave **7** (172 mg, 76%).

Benzyl 2(*S*)-(*tert*-Butyldiphenylsiloxy)-3(*S*)-methyl Pentanoate (8) A mixture of **7** (2.9 g, 13 mmol), *tert*-butylchlorodiphenylsilane (4.7 g, 17 mmol) and imidazole (1.2 g, 17 mmol) in dry DMF (20 ml) was stirred at room temperature for 12 h, diluted with 3% aqueous NH_4Cl, and extracted with ether. The ethereal extract was washed with 3% aqueous NH_4Cl, 3% aqueous Na_2CO_3 and water, dried with $MgSO_4$ and concentrated. The residue was chromatographed on silica gel in hexane–acetone (100 : 2) to give the TBDPS-ester (**8**) (4.5 g, 75%) as a colorless oil. IR ($CHCl_3$): 1740 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 0.82 (3H, t, J=6.8 Hz), 0.85 (3H, d, J=6.8 Hz), 1.08 (9H, s), 1.03—1.92 (3H, m), 4.16 (1H, d, J=4.6 Hz), 4.77 (2H, s), 7.20—7.81 (15H, m). $[\alpha]_D^{26}=-44.8°$ (c=1.35, EtOH). MS m/z: 460 (M^+).

2(*S*)-(*tert*-Butyldiphenylsiloxy)-3(*S*)-methylpentanoic Acid (9) A suspension of the TBDPS-ester (**8**) (1.0 g, 2.2 mmol) and 10% palladium carbon (2.0 g) in ethanol (50 ml) was stirred under H_2 for 2 h. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* to afford the acid (**9**) (470 mg, 75%) as a colorless oil. IR ($CHCl_3$): 3520—2510, 1770, 1720 cm^{-1}. ^{1}H-NMR (60 MHz in $CDCl_3$): 0.78 (3H, t, J=6.8 Hz), 0.85 (3H, d, J=6.8 Hz), 1.11 (9H, s), 1.01—1.85 (3H, m), 4.19 (1H, d, J=4.6 Hz), 7.21—7.85 (10H, m). HR-MS m/z: Calcd for $C_{22}H_{30}O_3Si$ (M^+): 370.1965. Found: 370.1977.

Methyl 9(*S*),10-Epoxy-8(*R*)-(2′(*S*)-*tert*-butyldiphenylsiloxy-3′(*S*)-methylpentanoyloxy)-9-methyl-deca-(*E*,*E*,*E*)-trienoate (11) A solution of DCC (332 mg, 1.5 mmol) and the acid (**9**) (596 mg, 1.5 mmol) in methylene chloride (4 ml) was stirred at room temperature under argon for 1.5 h, then the oxide-ester (**10**)[5b] (110 mg, 0.49 mmol) and 4-pyrrolidinopyridine (40 mg) were added and the resulting mixture was stirred at the same temperature for 12 h, diluted with ether (30 ml) and filtered. The filtrate was washed with 3% aqueous Na_2CO_3, 3% aqueous NH_4Cl and brine, dried with $MgSO_4$ and concentrated. The residue was chromatographed on silica gel in hexane–ethyl acetate (100 : 4) to give the ester (**11**) (269 mg, 95%) as a pale yellow oil. IR ($CHCl_3$): 1750, 1720 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 0.79 (3H, t, J=7.2 Hz), 0.93 (3H, d, J=7.2 Hz), 1.09 (9H, s), 1.14 (3H, s), 1.14—1.96 (3H, m), 2.47 (1H, d, J=4.8 Hz), 2.61 (1H, d, J=4.8 Hz), 3.74 (3H, s), 4.23 (1H, d, J=4.2 Hz), 4.99 (1H, d, J=7.3 Hz), 5.58 (1H, dd, J=14.3, 7.3 Hz), 5.81 (1H, d, J=15.4 Hz), 6.05—6.49 (3H, m), 7.14—7.72 (11H, m). $[\alpha]_D^{17}=-67.7°$ (c=0.98, EtOH). HR-MS m/z: Calcd for $C_{34}H_{44}O_6Si$ (M^+): 576.2908. Found: 576.2912.

The Diastereoisomer (12) of AF-Toxin IIc Methyl Ester (2a) TBAF (0.21 ml, 0.2 mmol, 1 M in THF) was added dropwise to a solution of the ester (**11**) (60 mg, 0.1 mmol) in dry THF (2 ml) at −10 °C under argon and the resulting mixture was stirred at the same temperature for 10 min and at room temperature for 1 h, and then diluted with ether (30 ml). The ethereal solution was washed with brine, dried with $MgSO_4$ and concentrated. The residue was chromatographed on silica gel in chloroform to afford the hydroxy-ester (**12**) (24 mg, 68%) as a pale yellow oil. IR ($CHCl_3$): 3540, 1750, 1720 cm^{-1}. ^{1}H-NMR (400 MHz in $CDCl_3$): 0.89 (3H, t, J=7.2 Hz), 0.99 (3H, d, J=7.2 Hz), 1.17—1.36 (2H, m), 1.37 (3H, s), 1.87 (1H, m), 2.64 (1H, d, J=4.8 Hz), 2.79 (1H, d, J=4.8 Hz), 3.76 (3H, s), 4.13 (1H, d, J=4.3 Hz), 5.32 (1H, d, J=7.5 Hz), 5.80 (1H, dd, J=14.5, 7.5 Hz), 5.93 (1H, d, J=15.8 Hz), 6.36 (1H, dd, J=15.1, 11.5 Hz), 6.43 (1H, dd, J=14.5, 10.8 Hz), 6.52 (1H, dd, J=15.1, 10.8 Hz), 7.29 (1H, dd, J=15.8, 11.5 Hz), $[\alpha]_D^{16}=+3.0°$ (c=0.67, EtOH). HR-MS m/z: Calcd for $C_{18}H_{26}O_6$ (M^+): 338.1730. Found: 338.1787.

Benzyl 2(*R*)-Formyloxy-3(*S*)-methylpentanoate (14) Diethylazodicarboxylate (226 mg, 1.3 mmol) in dry ether (2 ml) was added dropwise to a solution of the (*S*)-benzyl ester (**7**) (144 mg, 0.65 mmol), triphenylphosphine (340 mg, 1.3 mmol) and formic acid (60 mg, 1.3 mmol) in dry ether (4 ml) and the resulting mixture was stirred at room temperature for 24 h, diluted with ether (35 ml), and filtered. The filtrate was washed with 3% aqueous $NaHCO_3$, 3% aqueous NH_4Cl and water, dried with $MgSO_4$ and concentrated. The residue was chromatographed on silica gel in hexane–ethyl acetate (100 : 4) to give the formyl-ester (**14**) (166 mg, 72%) as a colorless oil. IR ($CHCl_3$): 1720 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 0.90 (3H, t, J=7.2 Hz), 0.92 (3H, d, J=7.2 Hz), 1.18—1.51 (2H, m), 1.69—2.13 (1H, m), 5.19 (2H, s), 5.24 (1H, d, J=4.3 Hz), 7.34 (5H, s),

8.16 (1H, s). $[\alpha]_D^{22} = +23.3°$ ($c = 1.20$, EtOH). MS m/z: 256 (M^+).

Benzyl 2(*R*)-Hydroxy-3(*S*)-methylpentanoate (15) A mixture of the formyl ester (**14**) (650 mg, 2.6 mmol) and lithium carbonate (260 mg, 3.1 mmol) in methanol–water (14 : 1) was stirred at room temperature for 2 h and concentrated *in vacuo* to give a residue, which was extracted with ether. The ethereal solution was washed with 3% aqueous Na_2CO_3, 3% aqueous NH_4Cl and water, and dried with $MgSO_4$. Removal of the solvent afforded the hydroxy-ester (**15**) (554 mg, 96%) as a colorless oil. IR ($CHCl_3$): 3500, 1715 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 0.78 (3H, d, $J = 7.2$ Hz), 0.92 (3H, t, $J = 7.2$ Hz), 1.00—1.98 (3H, m), 2.67 (1H, d, $J = 5.8$ Hz), 4.22 (1H, dd, $J = 3.2$, 5.8 Hz), 5.21 (2H, s), 7.32 (5H, s). $[\alpha]_D^{23} = +9.0°$ ($c = 1.05$, EtOH). HR-MS m/z: Calcd for $C_{13}H_{18}O_3$ (M^+): 222.1256. Found: 222.1244.

2(*R*)-(*tert*-Butyldiphenylsiloxy)-2(*S*)-methylpentanoic Acid (16) By use of the same procedure as described for the preparation of the acid (**9**) from **7**, the hydroxy-ester (**15**) (256 mg, 1.15 mmol) gave the acid (**16**) (374 mg, 87% overall yield) as a colorless oil. IR ($CHCl_3$): 3520—2510, 1760, 1720 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 0.76 (3H, t, $J = 7.2$ Hz), 0.91 (3H, d, $J = 7.2$ Hz), 1.11 (9H, s), 1.10—1.83 (3H, m), 4.19 (1H, d, $J = 4.1$ Hz), 7.24—7.73 (10H, m). $[\alpha]_D^{22} = +18.5°$ ($c = 1.01$, EtOH). HR-MS m/z: Calcd for $C_{22}H_{30}O_3Si$ (M^+): 370.1965. Found 370.1952.

AF-Toxin IIc Methyl Ester (2a) By use of the procedure as described for the preparation of **12**, coupling of the epoxy-ester (**10**) (123 mg, 0.33 mmol) and **16** (150 mg, 0.40 mmol) gave AF-toxin IIc methyl ester (**2a**) (72 mg, 65% overall yield) as a colorless oil. IR ($CHCl_3$): 1620, 1720, 1720 cm^{-1}. ^{1}H-NMR (400 MHz in $CDCl_3$): 0.86 (3H, d, $J = 6.9$ Hz), 0.98 (3H, t, $J = 7.4$ Hz), 1.36 (3H, s), 1.36—1.40 (2H, m), 1.83 (1H, m), 2.61 (1H, d, $J = 5.8$ Hz), 2.63 (1H, d, $J = 4.8$ Hz), 2.78 (1H, d, $J = 4.8$ Hz), 3.75 (3H, s), 4.23 (1H, dd, $J = 2.9$, 5.8 Hz), 5.35 (1H, d, $J = 7.3$ Hz), 5.80 (1H, dd, $J = 7.3$, 14.8 Hz), 5.94 (1H, d, $J = 15.3$ Hz), 6.38 (1H, dd, $J = 11.1$, 14.5 Hz), 6.43 (1H, dd, $J = 10.8$, 14.8 Hz), 6.53 (1H, d, $J = 14.5$, 10.8 Hz), 7.29 (1H, dd, $J = 11.1$, 15.3 Hz). HR-MS m/z: Calcd for $C_{18}H_{26}O_6$ (M^+): 338.1730. Found: 338.1787.

1,1-Dibromo-3(*R*)-*tert*-butyldiphenylsiloxy-4-methylpenta-1,4-diene (19) A solution of triphenylphosphine (1.48 g, 4 mmol) in anhydrous methylene chloride (3 ml) was added dropwise to a solution of carbon tetrabromide (633 mg, 2.0 mmol) in anhydrous methylene chloride (6 ml) under argon at 0 °C. The mixture was stirred at the same temperature for 5 min, then zinc powder (262 mg, 4 mmol) and the aldehyde (**17**)[6] (388 mg, 1 mmol) were added alternately and the resulting mixture was stirred at room temperature for 3 h and diluted with ether (50 ml). The ethereal solution was washed with water, dried with $MgSO_4$ and concentrated to give a residue, which was chromatographed on silica gel in chloroform. Elution with the same solvent afforded the dibromide (**19**) (360 mg, 73%). IR ($CHCl_3$): 1625 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 1.09 (9H, s), 1.72 (3H, s), 4.67 (1H, d, $J = 9.0$ Hz), 4.95 (1H, br s), 5.18 (1H, br s), 6.40 (1H, d, $J = 9.0$ Hz), 7.26—7.71 (10H, m). $[\alpha]_D^{21} = -80.6°$ ($c = 1.01$, EtOH). MS m/z: 494 (M^+).

3(*R*)-*tert*-Butyldiphenylsiloxy-4-methylpent-4-en-1-yne (20) *n*-Butyllithium (1 ml, 1.7 M in hexane) was added to a solution of the dibromide (**19**) (350 mg, 0.7 mmol) in dry THF (3 ml) at −78 °C under argon and the resulting mixture was stirred at the same temperature for 1 h, and at 25 °C for 1 h. After addition of water (2 ml), the reaction mixture was stirred at room temperature for 30 min and diluted with ether (50 ml). The ethereal solution was washed with water, dried with $MgSO_4$, and concentrated *in vacuo* to leave a residue, which was chromatographed on silica gel in chloroform. Elution with the same solvent gave the acetylene (**20**) (154 mg, 65%) as a colorless oil. IR ($CHCl_3$): 3290 cm^{-1}, ^{1}H-NMR (90 MHz in $CDCl_3$): 1.09 (9H, s), 1.85 (3H, br s), 2.35 (1H, d, $J = 2.2$ Hz), 4.37 (1H, m), 4.82 (1H, br s), 4.94 (1H, br s), 7.24—7.82 (10H, m). $[\alpha]_D^{24} = -47.3°$ ($c = 1.00$, EtOH). MS m/z: 334 (M^+).

4(*R*)-*tert*-Butyldiphenylsiloxy-5-methyl-hex-5-en-1-ynal (21) *n*-Butyllithium (1 ml, 2.3 mmol) was added dropwise to a solution of the acetylene (**20**) (480 mg, 1.48 mmol) in dry THF (5 ml) at −78 °C under argon, and the mixture was stirred at the same temperature for 2 h. Then DMF (0.1 ml) was added and the resulting mixture was stirred at −78 °C for 4 h and diluted with ether (30 ml). The ethereal solution was washed with water, dried with anhydrous $MgSO_4$ and concentrated *in vacuo* to give a residue, which was chromatographed on silica gel in hexane–ether (100 : 5). Elution with the same solvent afforded the aldehyde (**21**) (450 mg, 86%) as a colorless oil. IR ($CHCl_3$): 2200, 1665 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 1.09 (9H, s), 1.84 (3H, br s), 4.88 (1H, br s), 4.91 (1H, br s), 5.04 (1H, br s), 7.31—7.78 (10H, m), 9.30 (1H, d, $J = 0.7$ Hz). MS m/z: 326 (M^+).

Wadsworth–Emmons Reaction of the Aldehyde (21) A solution of trimethylphosphonoacetate (98 mg, 0.54 mmol) in dry benzene (2 ml) was added dropwise to a suspension of sodium hydride (13 mg, 0.45 mmol, 60% in oil) in benzene (5 ml) at room temperature and the resulting mixture was stirred at room temperature under argon for 30 min. Then a solution of the aldehyde (**21**) (130 mg, 0.36 mmol) in dry benzene (2 ml) was added and the whole was stirred at room temperature for 30 min, and diluted with ether (30 ml). The ethereal solution was washed with 3% aqueous $NaHCO_3$, 3% aqueous NH_4Cl and water, dried with $MgSO_4$ and concentrated to give a residue, which was chromatographed on silica gel in hexane–ether (100 : 2). Elution with the same solvent afforded the diene-acetylene (**22**) (130 mg, 87%) as a colorless oil. IR ($CHCl_3$): 1710 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 1.08 (9H, s), 1.84 (3H, br s), 3.73 (3H, s), 4.87 (2H, br s), 5.02 (1H, m), 5.97 (1H, d, $J = 15.8$ Hz), 6.63 (1H, dd, $J = 15.8$, 1.8 Hz), 7.24—7.79 (10H, m). $[\alpha]_D^{24} = -95.0°$ ($c = 1.30$, EtOH). HR-MS m/z: Calcd for $C_{26}H_{30}O_3Si$ (M^+): 418.1965. Found: 418.1942.

6(*R*)-*tert*-Butyldiphenylsiloxy-7-methyl-octa-2,7-dien-4-yn-1-ol (23) DIBAL-H (0.2 ml, 0.36 mmol, 25% (w/w) in *n*-hexane) was added to a solution of the ester (**22**) (100 mg, 0.24 mmol) in anhydrous methylene chloride (2 ml) at −78 °C under argon. The resulting mixture was stirred at the same temperature for 1 h. After addition of brine and chloroform (50 ml) to the mixture, the whole was filtered. The chloroform solution was washed with brine, dried with $MgSO_4$ and concentrated to give a residue, which was chromatographed on silica gel in benzene. The benzene eluate afforded the alcohol (**23**) (84 mg, 90%). IR ($CHCl_3$): 3600, 2255 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 1.08 (9H, s), 1.84 (3H, s), 4.14 (2H, dd, $J = 4.9$, 1.5 Hz), 4.83 (2H, br s), 4.98 (1H, br s), 5.60 (1H, dd, $J = 15.9$ Hz, 1.5 Hz), 6.04 (1H, dt, $J = 15.9$, 4.9 Hz), 7.27—7.28 (10H, m). $[\alpha]_D^{23} = -123°$ ($c = 1.01$, EtOH). MS m/z: 390 (M^+).

Oxidation of the Allyl Alcohol (23) A mixture of the allyl alcohol (**23**) (260 mg, 0.67 mmol) and active MnO_2 (1.5 g, 17 mmol) in methylene chloride (10 ml) was stirred at room temperature for 30 min. The reaction mixture was filtered and the filtrate was concentrated to afford the aldehyde (**24**) (217 mg, 87%) as a pale yellow oil. IR ($CHCl_3$): 2220, 1680 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 1.09 (9H, s), 1.85 (3H, s), 4.91 (2H, br s), 5.05 (1H, br s), 6.15 (1H, dd, $J = 15.8$, 7.1 Hz), 6.46 (1H, d, $J = 15.8$ Hz), 7.24—7.80 (10H, m), 9.48 (1H, d, $J = 7.1$ Hz). MS m/z: 388 (M^+).

Methyl 8(*R*)-*tert*-Butyldiphenylsiloxy-9-methyl-deca-2(*E*),4(*E*),9-trien-6-ynoate (25) By use of the procedure as described for the preparation of the ester (**22**), the aldehyde (**24**) (200 mg, 0.52 mmol) gave the ester (**25**) (192 mg, 84%) as a colorless oil. IR ($CHCl_3$): 2400, 1710 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 1.08 (9H, s), 1.85 (3H, br s), 3.75 (3H, s), 4.86 (2H, br s), 5.03 (1H, br s), 5.78 (1H, d, $J = 15.4$ Hz), 5.92 (1H, d, $J = 15.2$ Hz), 6.33 (1H, dd, $J = 15.4$, 11.1 Hz), 7.20 (1H, dd, $J = 15.2$, 11.1 Hz), 7.26—7.81 (10H, m). $[\alpha]_D^{26} = -198.0°$ ($c = 1.02$, EtOH). HR-MS m/z: Calcd for $C_{28}H_{32}O_3Si$ (M^+): 444.2122. Found: 444.2128.

Methyl 6(*R*)-*tert*-Butyldiphenylsiloxy-7-methyl-oct-2(*E*),4(*E*),7-trienoate (27) A mixture of quinoline (30 mg, 0.24 mmol), Lindlar catalyst (30 mg) and the methyl ester (**22**) (100 mg, 0.24 mmol) in ethyl acetate (5 ml) was stirred at 0 °C under H_2 for 2 d and filtered. The filtrate was concentrated to give a residue, which was submitted to flash chromatography in hexane–ether (9 : 1). Elution with the same solvent afforded the triene ester (**27**) (87 mg, 87%) as a colorless oil. IR ($CHCl_3$): 1705, 1640 cm^{-1}. ^{1}H-NMR (400 MHz in $CDCl_3$): 1.07 (9H, s), 1.65 (3H, s), 3.70 (3H, s), 4.83 (1H, br s), 4.95 (1H, d, $J = 8.4$ Hz), 5.09 (1H, br s), 5.69 (1H, d, $J = 15.4$ Hz), 5.71 (1H, dd, $J = 11.7$, 8.4 Hz), 5.91 (1H, dd, $J = 11.7$, 11.7 Hz), 7.04 (1H, dd, $J = 15.4$, 11.7 Hz), 7.29—7.66 (10H, m). $[\alpha]_D^{17} = +30.1°$ ($c = 1.01$, EtOH). HR-MS m/z: Calcd for $C_{26}H_{32}O_3Si$ (M^+): 420.2122. Found 420.2132.

6(*R*)-*tert*-Butyldiphenylsiloxy-7-methyl-oct-2(*E*),4(*E*),7-trienal (28) By use of the procedure described for the preparation of **24** from **22**, the *cis–trans*-diene ester (**27**) (100 mg, 0.24 mmol) gave the aldehyde (**28**) (79 mg, 85% overall yield) as a colorless oil. IR ($CHCl_3$): 1675, 1630 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 1.07 (9H, s), 1.68 (3H, s), 4.89 (1H, br s), 4.91 (1H, d, $J = 10.8$ Hz), 5.12 (1H, br s), 5.71—6.16 (2H, m), 6.74 (1H, dd, $J = 10.8$, 15.1 Hz), 7.24—7.71 (11H, m), 9.22 (1H, d, $J = 7.9$ Hz). MS m/z: 390 (M^+).

Wadsworth–Emmons Reaction of the Aldehyde (28) By use of the method described for the preparation of **22**, the aldehyde (**28**) (300 mg, 0.77 mmol) gave the *cis–trans–trans* ester (**29**) (295 mg, 86%) as a colorless oil. IR ($CHCl_3$): 1700, 1620 cm^{-1}. ^{1}H-NMR (400 MHz in $CDCl_3$): 1.06 (9H, s), 1.65 (3H, s), 3.73 (3H, s), 4.82 (1H, br s), 4.88 (1H, d, $J = 8.8$ Hz), 5.07 (1H, br s), 5.59 (1H, dd, $J = 8.8$, 11.0 Hz), 5.80 (1H, d, $J = 15.4$ Hz), 5.90 (1H, dd, $J = 11.0$, 11.0 Hz), 6.10 (1H, dd, $J = 15.7$, 10.6 Hz), 6.17 (1H, dd, $J = 15.7$, 11.0 Hz), 7.04 (1H, dd, $J = 15.4$, 10.6 Hz). $[\alpha]_D^{17} = +5.5°$

($c=0.80$, EtOH). HR-MS m/z: Calcd for $C_{28}H_{34}O_3Si$ (M^+): 446.2278. Found: 446.2262.

Epoxidation of the Ester (29) A mixture of the ester (**29**) (320 mg, 0.72 mmol) and *m*CPBA (186 mg, 1.1 mmol) in methylene chloride (8 ml) was allowed to stand overnight at room temperature in the dark, and then 10% aqueous $NaHSO_3$ and chloroform was added. The chloroform solution was washed with 3% aqueous Na_2CO_3, 3% aqueous NH_4Cl and water, and dried with $MgSO_4$. Removal of the solvent gave a residue, which was submitted to flash chromatography in ether–hexane (1 : 9). Elution with the same solvent afforded the epoxide (**30a**) (200 mg, 60%) as the faster-running portion and the epoxide (**30b**) (130 mg, 39%) as the slower-running one.

Methyl 8(*R*)-*tert*-Butyldiphenylsiloxy-9(*S*),10-epoxy-9-methyl-deca-2(*E*),4(*E*),6(*Z*)-trienoate (**30a**): IR ($CHCl_3$): 1705, 1620 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 1.07 (9H, s), 1.39 (3H, s), 2.53 (1H, d, $J=4.6$ Hz), 2.64 (1H, d, $J=4.6$ Hz), 3.75 (3H, s), 4.26 (1H, d, $J=8.8$ Hz), 5.60—6.09 (5H, m), 6.97 (1H, dd, $J=10.2$, 15.2 Hz), 7.25—7.73 (10H, m). $[\alpha]_D^{15}=+16.1°$ ($c=1.60$, EtOH). HR-MS m/z: Calcd for $C_{28}H_{34}O_4Si$ (M^+): 462.2227. Found: 462.2202.

Methyl 8(*R*)-*tert*-Butyldiphenylsiloxy-9(*R*),10-epoxy-9-methyl-deca-2(*E*),4(*E*),6(*Z*)-trienoate (**30b**): IR ($CHCl_3$): 1705, 1620 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 1.10 (9H, s), 1.36 (3H, s), 2.55 (1H, d, $J=4.9$ Hz), 2.63 (1H, d, $J=4.9$ Hz), 3.73 (3H, s), 3.90 (1H, d, $J=5.5$ Hz), 5.60—6.35 (5H, m), 7.10—7.74 (11H, m). $[\alpha]_D^{15}=-28.9°$ ($c=1.26$, EtOH). HR-MS m/z: Calcd for $C_{28}H_{34}O_4Si$ (M^+): 462.2227. Found: 462.2206.

Methyl 9(*S*),10-Epoxy-8(*R*)-Hydroxy-9-methyl-deca-2(*E*),4(*E*),6(*Z*)-trienoate (31) TBAF (3.12 ml, 3 mmol, 1 M in THF) was added dropwise to a solution of **30a** (200 mg, 0.45 mmol) in dry THF (5 ml) at -10 °C, and the resulting mixture was stirred at room temperature for 1 h then diluted with ether (50 ml). The ethereal solution was washed with brine, dried with $MgSO_4$ and concentrated to give a residue, which was chromatographed on silica gel in hexane–chloroform (1 : 4). Elution with the same solvent gave the alcohol (**31**) (117 mg, 68%) as a colorless oil. IR ($CHCl_3$): 3630, 1700, 1620 cm^{-1}. ^{1}H-NMR (90 MHz in $CDCl_3$): 1.33 (3H, s), 2.30 (1H, br s), 2.62 (1H, d, $J=4.6$ Hz), 2.96 (1H, d, $J=4.6$ Hz), 3.75 (3H, s), 4.64 (1H, d, $J=8.9$ Hz), 5.40—6.93 (6H, m). MS m/z: 224 (M^+).

Acylation of 31 with the Acid (16) By use of the method described for the preparation of **11**, the *cis–trans–trans* hydroxy-ester (**31**) (25 mg, 0.11 mmol) gave the ester (**32**) (36 mg, 40%) as an oil. IR ($CHCl_3$): 1745, 1705, 1620 cm^{-1}. ^{1}H-NMR (400 MHz in $CDCl_3$): 0.81 (3H, t, $J=7.3$ Hz), 0.94 (3H, d, $J=7.0$Hz), 1.08 (9H, s), 1.22 (3H, s), 1.45—1.75 (3H, m), 2.50 (1H, d, $J=4.8$ Hz), 2.63 (1H, d, $J=4.8$ Hz), 3.77 (3H, s), 4.22 (1H, d, $J=3.3$ Hz), 5.26 (1H, dd, $J=9.3$, 10.6 Hz), 5.47 (1H, d, $J=9.3$ Hz), 5.93 (1H, d, $J=15.4$ Hz), 6.17 (1H, dd, $J=10.6$, 11.3 Hz), 6.35 (1H, dd, $J=14.6$, 11.4 Hz), 6.79 (1H, dd, $J=14.6$, 11.3 Hz), 7.28—7.65 (11H, m). HR-MS m/z: Calcd for $C_{34}H_{44}O_6Si$ (M^+): 576.2908. Found: 576.2898.

AF-Toxin IIa Methyl Ester (3b) By use of the procedure described for the preparation of **12**, the ester (**32**) (24 mg, 0.041 mmol) gave AF-toxin IIa methyl ester (**3b**) (9 mg, 64%) as an oil. IR ($CHCl_3$): 3550, 1735 cm^{-1}. ^{1}H-NMR (400 MHz in $CDCl_3$): 0.87 (3H, d, $J=7.0$ Hz), 0.97 (3H, t, $J=7.3$ Hz), 1.36—1.40 (2H, m), 1.38 (3H, s), 1.83 (1H, m), 2.59 (1H, d, $J=5.7$ Hz), 2.62 (1H, d, $J=4.8$ Hz), 2.78 (1H, d, $J=4.8$ Hz), 3.76 (3H, s), 4.19 (1H, dd, $J=2.7$, 5.7 Hz), 5.52 (1H, dd, $J=9.2$, 10.6 Hz), 5.81 (1H, d, $J=9.2$ Hz), 5.95 (1H, d, $J=15.4$ Hz), 6.33 (1H, dd, $J=10.6$, 11.4 Hz), 6.41 (1H, dd, $J=11.4$, 15.0 Hz), 6.93 (1H, dd, $J=11.4$, 15.0 Hz), 7.37 (1H, dd, $J=11.4$, 15.4 Hz), HR-MS m/z: Calcd for $C_{18}H_{26}O_6$ (M^+): 338.1730. Found: 338.1761.

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