Synthesis and Antifungal Activities of Trichodermin Derivatives as Fungicides on Rice

by **Xiaojun Xu**[a]), **Jingli Cheng**[a]), **Yong Zhou**[a]), **Chulong Zhang**[a]), **Xiaoming Ou**[c]), **Weike Su**[b]), **Jinhao Zhao***[a])[b]), and **Guonian Zhu**[a])

[a]) Institute of Pesticide and Environmental Toxicology, Zhejiang University, Hangzhou 310029, P. R. China (phone: +86-571-86971220; fax: +86-571-86430193; e-mail: jinhaozhao@zju.edu.cn)
[b]) College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou, P. R. China
[c]) Hunan Research Institute of Chemical Industry, Changsha, China

Twenty new trichodermin derivatives, **2a**–**5**, containing alkoxy, acyloxy, and Br groups in 4-, 8-, 9-, 10- and 16-positions were synthesized and characterized. The antifungal activities of the new compounds against rice false smut (*Ustilaginoidea virens*), rice sheath blight (*Rhizoctonia solani*), and rice blast (*Magnaporthe grisea*) were evaluated. The results of bioassays indicated that the antifungal activities were particularly susceptible to changes at 4-, 8-, and 16-positions, but low to changes at 9- and 10-positions. Most of these target compounds exhibited good antifungal activities at the concentration of 50 mg l^{-1}. Compound **4** (9-formyltrichodermin; EC_{50} 0.80 mg l^{-1}) with an CHO group at 9-position displayed nearly the same level of antifungal activity against *Ustilaginoidea virens* as the commercial fungicide prochloraz (EC_{50} 0.82 mg l^{-1}), while compound **3f** ((8*R*)-8-{[(*E*)-3-phenylprop-2-enoyl]oxy}-trichodermin; EC_{50} 3.58 and 0.74 mg l^{-1}) with a cinnamyloxy group at C(8) exhibited much higher antifungal activities against *Rhizoctonia solani* and *Magnaporthe grisea* than the commercial fungicides prochloraz (EC_{50} 0.96 mg l^{-1}) and propiconazole (EC_{50} 5.92 mg l^{-1}), respectively. These data reveal that compounds **3f** and **4** possess high antifungal activities and may serve as lead compounds for the development of fungicides in the future.

Introduction. – First isolated from *Trichoderma viride* found in New Guinea soil [1], trichodermin (**1**; *Fig. 1*) is a member of a family of fungal metabolites, which possess an olefinic bond and an epoxy group in a trichothecane ring system and are characterized as 12,13-epoxytrichothecenes [2]. Trichodermin and other sesquiterpene antibiotics (trichothecin, trichodermol, verrucarin A, fusarenon X, nivalenol, diacetoxicirpenol, and T-2 toxin) of the same group are effective inhibitors of the peptidyl transferase center of eukaryotic ribosomes and, therefore, block peptide-bond formation [3–10], and they show broad-spectrum antifungal activities with low toxicity to nontarget organisms such as mammals, vegetables, and fruit [11–14].

Trichodermin-BL, which was applied as pre-sowing seed treatment and on growing plants to decrease disease incidence, increasing the yield, and raising flax fiber quality,

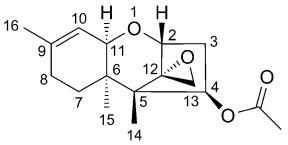


Fig. 1. *Chemical structure of trichodermin* (**1**)

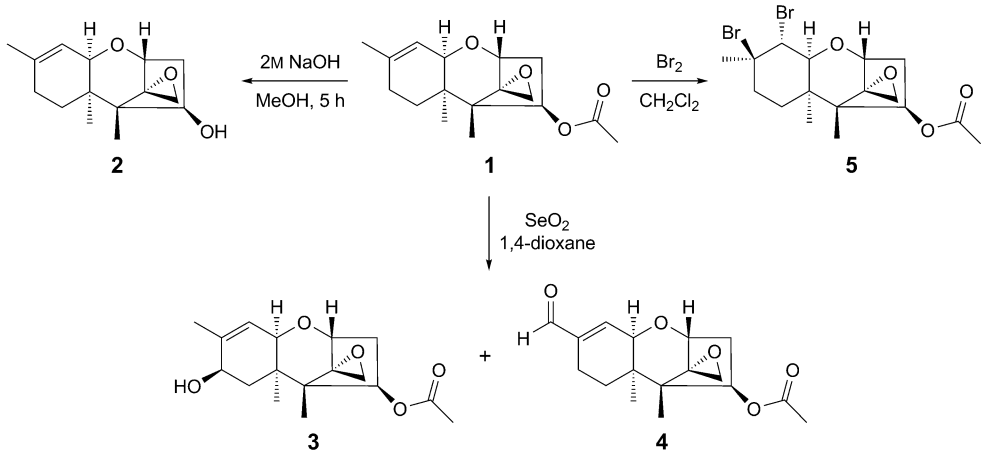
was widely used in Russia as the first commercial bioproduct [15][16]. Trichodermin was also used for removing toxic effects of crude oil hydrocarbons on soils and agricultural plants and increasing productivity of plants [17]. In recent years, it was reported that trichodermin had good efficacy on disease and pest control against the gall nematode (*Meloidogyne*), root rots of cucumber, *Rhizoctonia solani*, *Botrytis cinerea*, and *Artemia salina* [18][19]. Although some reports had appeared on trichodermin derivatives and analogs [20–26], their antifungal activities in controlling rice pathogen have been only rarely reported. With increasing human population, decreasing amounts of land available for agriculture [27], and growing drug resistance and new rice pathogens, development of novel and 'green' chemical fungicides with low hazard to environment and human being is becoming more and more necessary and important [28].

In our previous work, a new endophytic fungus *Trichoderma taxi* sp. *nov.* (ZJUF0986) was isolated, which could produce trichodermin in good yield, and preliminary derivatives had been synthesized [29–32]. To develop novel fungicides with low toxicity on crops, especially on rice, 29 novel trichodermin derivatives **2a**–**5** were designed and synthesized, and their antifungal activities against rice false smut (*Ustilaginoidea virens*), rice sheath blight (*Rhizoctonia solani*), and rice blast (*Magnaporthe grisea*) were evaluated. Compounds **3f** and **4** exhibited high antifungal activities against rice false smut (*Ustilaginoidea virens*) and rice blast (*Magnaporthe grisea*). In addition, the crystal structure of (8*R*)-8-{[(*E*)-3-phenylprop-2-enoyl]oxy}-trichodermin (**3f**) was established (*cf. Fig. 2* in the *Exper. Part*).

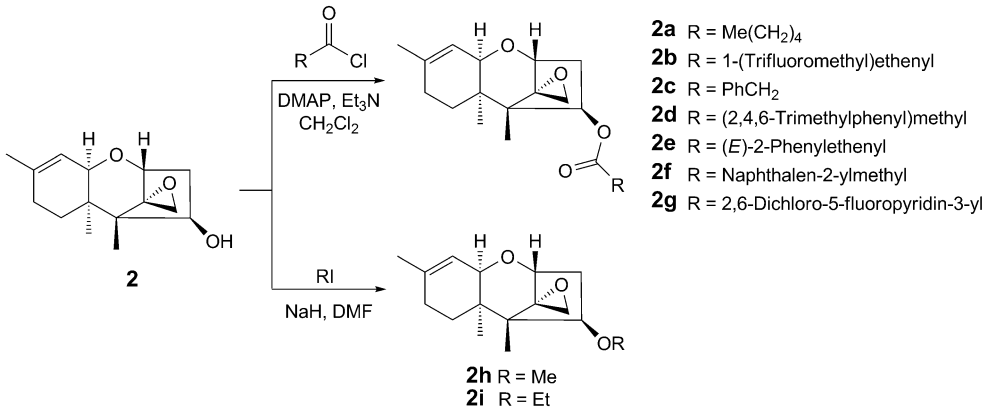
Results and Discussion. – *Design*. In our previous work, trichodermin (**1**) was modified at C(12) and C(13) by opening the epoxy group, but it was found that the derivatives obtained had no antifungal activities against the three rice fungi. So we retained the polycyclic skeleton of trichodermin, including the epoxy group, and changed the substituents at C(4), C(8), C(9), C(10), and C(16). To investigate the importance of the ester function at C(4) for activity, we prepared the derivatives **2a**–**2g** containing saturated, unsaturated, and aromatic acyloxy groups. For comparison, the ethers **2h** and **2j** with MeO and EtO groups at C(4) were prepared (*Schemes 1* and *2*). Similarly, the effect of the C(9)=C(10) bond was evaluated by comparing the activity with that of the dibromo derivative **5** (*Scheme 1*). The influence of the other functional groups at C(8) and C(16) of trichodermin on the activity was studied by treatment of compound **1** with SeO_2 in 1,4-dioxane (*Scheme 1*), and the derivatives with a OH group at C(8) (**3**) and an CHO group at C(9) (**4**) were obtained. Then, the OH group at C(8) was replaced with saturated, unsaturated, and aromatic acyloxy groups to provide compounds **3a**–**3i** (*Scheme 3*).

Chemistry. The synthetic routes to compounds **2**–**5** are outlined in *Scheme 1*, and the target compounds **2a**–**2i** and **3a**–**3i** were obtained in good yields (56.9–91.2%) *via* acylation or alkylation of **2** and **3**, respectively. To obtain intermediate **3** ((8β)-8-hydroxytrichodermin) with high purity, the method reported by *Kraus* and *Thomas* was modified by employing AcOEt as the extraction solvent, and petroleum ether and AcOEt as the eluent for column chromatography [33]. When trichodermin was oxidized by SeO_2 to give compound **3** [33], compound **4**, 9-formyltrichodermin, was obtained as a by-product surprisingly, and it was found to have an excellent antifungal

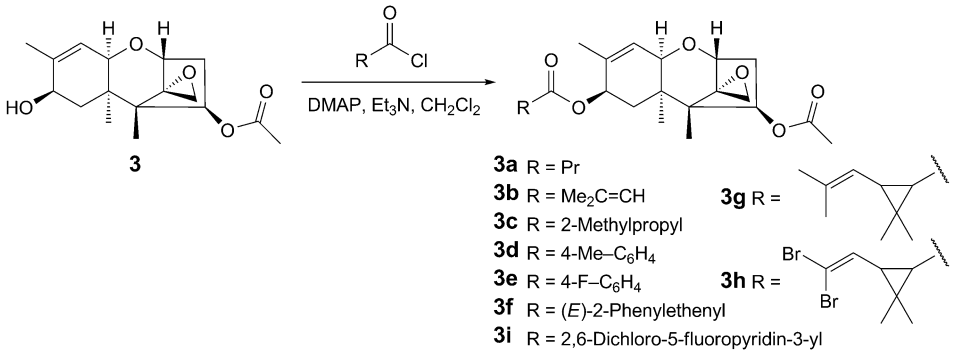
Scheme 1. *General Synthetic Routes to Compounds* **2**–**5**



Scheme 2. *General Synthetic Routes to Compounds* **2a**–**2i**



Scheme 3. *General Synthetic Route to Compounds* **3a**–**3i**



activity. To obtain an *anti* addition product of compound **5**, trichodermin was reacted with 2 equiv. of Br_2 by using pyridine as catalyst (*Scheme 1*). Compounds **2** and **3** were reacted with newly prepared acyl chlorides using Et_3N and DMAP (=4-(dimethylamino)pyridine) as base and catalyst, respectively, in dried CH_2Cl_2 afforded the target compounds **2a**–**2g** and **3a**–**3i** (*Schemes 2* and *3*).

The main characteristic of the ^{1}H-NMR spectra of **2a** was the presence of broad *multiplet*s at δ(H) 2.66–2.52 and 2.04–1.99 ppm, presumably arising due to the CH_2(3) group linking the two CH(2) and CH(4) groups at. A sharp peak at δ(H) 1.88–1.70 ppm was assigned to the Me group at C(9) directly attached to the C=C bond of the six-membered ring.

Biological Activity. Based on the data compiled in *Tables 1* and *2*, a clearcut, well-defined relation between chemical structure and biological activity was gradually developed. The effects of different kinds of electron-withdrawing, electron-donating, and spatially demanding groups on the fungicidal activity were studied. *Table 1* contains the antifungal activities of the target compounds **2a**–**5**, prochloraz, propiconazole, and compound **1** against rice false smut (*Ustilaginoidea virens*), rice sheath blight (*Rhizoctonia solani*), and rice blast (*Magnaporthe grisea*). The results indicated that most of the designed trichodermin derivatives with modifications at C(4), C(8), and C(9) had moderate antifungal activities. *Table 2* reveals that **3f** ((8*R*)-8-{[(*E*)-3-phenylprop-2-enoyl]oxy}trichodermin) and **4** (9-formyltrichodermin) exhibited the highest antifungal activities against *Ustilaginoidea virens*, *Rhizoctonia solani*, and *Magnaporthe grisea* than other derivatives, with EC_{50} values of 1.48, 3.58, and 0.74, and 0.80, 7.40, and 11.82 mg l^{-1}, respectively. Fortunately, **3f** and **4** had higher antifungal activities against *Ustilaginoidea virens* than the parent compound trichodermin (**1**; EC_{50} 2.30 mg l^{-1}), and **3f** displayed antifungal activities against *Magnaporthe grisea* even about six times better than that of **1** (EC_{50} 4.25 mg l^{-1}). While **4** displayed comparable antifungal activity against *Ustilaginoidea virens* as prochloraz (EC_{50} 0.82 mg l^{-1}), **3f** showed much higher antifungal activities against *Rhizoctonia solani* and *Magnaporthe grisea* than the commercial fungicides propiconazole (EC_{50} 5.92 mg l^{-1}) and prochloraz (EC_{50} 0.96 mg l^{-1}), respectively, it could be concluded that the C=O group at C(9) and [(*E*)-3-phenylprop-2-enoyl]oxy group at C(8) increase the antifungal activity. However, compound **5** brominated at C(9) and C(10) showed no or poor antifungal activities against the three rice funguses. Interestingly, trichodermin (**1**), as a starting material, exhibited the highest antifungal activity against *Rhizoctonia solani*, with an EC_{50} value of 0.72 mg l^{-1}.

Structure–Activity Relationship. The results in *Tables 1* and *2* showed that the antifungal activities of derivatives with substituents containing conjugated C=C bonds were much better than those with single bonds. For example, the antifungal activities of compound **3b** ((8*R*)-8-[(3-methylbut-2-enoyl)oxy]trichodermin) against *Ustilaginoidea virens*, *Rhizoctonia solani*, and *Magnaporthe grisea* at 50 mg l^{-1} concentration were 80.5, 99.7, and 92.6%, respectively, as compared with 52.5, 98.9, and 41.7% for compound **3c** ((8*R*)-8-[(3-methylbutanoyl)oxy]trichodermin) at the same concentration. In particular, **3f** and **4** with conjugated C=C bonds had the highest antifungal activities against the three rice fungi in all the target compounds. At C(4), compounds **2h** ((4β)-4-methoxytrichodermin) and **2i** ((4β)-4-ethoxytrichodermin) that contained a MeO or a EtO substituent, respectively, had better antifungal activities than the other

Table 1. *Antifungal Activities*[a]) *of Compounds* **2a**–**5** *against Rice False Smut, Rice Sheath Blight, and Rice Blast*

Compound	Against rice false smut	Against rice sheath blight	Against rice blast
1	100	100	100
2	86.3	87.0	88.0
2a	70.5	63.0	77.0
2b	69.6	30.1	56.0
2c	30.8	57.7	43.0
2d	49.7	50.5	37.3
2e	32.0	49.8	57.5
2h	25.4	32.3	32.5
2g	28.1	20.0	68.4
2h	73.8	90.0	82.3
2i	71.5	87.8	81.9
3	31.3	34.0	56.6
3a	29.2	46.7	56.7
3b	80.5	99.7	92.6
3c	52.5	98.9	41.7
3d	36.7	32.6	43.1
3e	86.3	76.5	69.6
3f	100	97	100
3g	25.3	60.1	57.4
3h	20.9	36.2	31.6
3i	40.3	50.5	42.7
4	100.0	95	98
5	0.0	0.0	0.0
Prochloraz	100	–	100
Propiconazole	–	100	–

[a]) Antifungal activity [%] indicates the growth rate of rice fungi that the compound, at the concentration of 50 $mg\,l^{-1}$, inhibits as compared to the untreated fungus.

Table 2. *Antifungal Activities against Rice False Smut, Rice Sheath Blight, and Rice Blast of Compounds* **1**, **3f**, **4**, *Prochloraz, and Propiconazole Expressed as* EC_{50}[a])

Compound	EC_{50} [$mg\,l^{-1}$]		
	Toxicities against rice false smut	Toxicities against rice sheath blight	Toxicities against rice blast
1	2.30	0.72	4.25
3f	1.48	3.58	0.74
4	0.80	7.40	11.82
Prochloraz	0.82	–	0.96
Propiconazole	–	5.92	–

[a]) EC_{50} [$mg\,l^{-1}$] indicates the concentration that the compound inhibits the growth rate of rice fungi by 50% as compared to the untreated fungus.

derivatives against the three rice fungi, but a little lower than the intermediate trichodermol (**2**). On the whole, the antifungal activities of trichodermin derivatives modified at C(4) were not higher than that of those with acryl, 3-methoxylacryl, and crotonyl substituents [32]. In addition, the C(9)=C(10) bond exerts great influence on the activity and should not be modified.

Conclusions and Outlook. – As described above, 20 new trichodermin derivatives **2a**–**5**, synthesized by introducing various substituted groups at C(4), C(8), C(9), C(10), and C(16), were active fungicides against rice false smut (*Ustilaginoidea virens*), rice sheath blight (*Rhizoctonia solani*), and rice blast (*Magnaporthe grisea*). The antifungal activity was acceptable with changes at C(4), C(8), and C(16), but lowered with changes at C(9) and C(10). Some of them exhibited good antifungal activities at the concentration of 50 mg l^{-1}; particularly the compounds **3f** and **4** showed the highest antifungal activities. Therefore, the present work not only demonstrates that the antifungal activity of trichodermin derivatives can be significantly improved *via* structural modification at some positions, but also paves the way toward the synthesis, and antifungal-activity studies of new trichodermin derivatives modified at C(8) and C(16).

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Experimental Part

General. All starting materials and reagents were commercially available and used without further purification except as indicated. All fungal materials were obtained from the National Engineering Research Center. M.p.: *X-4* binocular microscope melting point apparatus (*Beijing Tech Instruments Co.*, Beijing, China); uncorrected. Optical rotation: *SEPA-300* spectropolarimeter (*Horiba, Ltd.*, Kyoto, Japan) equipped with a cell with a 10-cm optical path length at 589 nm and at 28°; concentrations of of samples in MeOH, 5 mg ml^{-1}. ^{1}H- and ^{13}C-NMR (500 and 100 MHz, resp.) spectra: *Bruker AVANCE III* in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-MS: *FTICR-MS* instrument (*Ionspec7.0T*); in *m*/*z*. Elemental analyses: *Yanaca CHN Corder MT-3* elemental analyzer.

Synthesis of Trichodermol (*=(4β)-12,13-Epoxytrichothec-9-en-4-ol*; **2**) [32]. A mixture of *trichodermin* (*=(4β)-12,13-epoxytrichothec-9-en-4-yl acetate*; **1**; 2.00 g, 6.85 mmol), NaOH (24 ml, 2 mol l^{-1}), and MeOH (30 ml) was stirred at r.t. for 5 h. After the reaction was completed, the solvent was evaporated under reduced pressure, and the product was purified by flash column chromatography (FC; silica gel petroleum ether (PE; 60–90°)/AcOEt 3 :1) to give 1.52 g of **2** (89%) as colorless crystals. M.p. 117–119°. ^{1}H-NMR (500 MHz, $CDCl_3$): 5.39 (*d*, *J*=5.0, H–C(10)); 4.34 (*s*, H–C(4)); 3.84 (*d*, *J*=7.0, H–C(2)); 3.52 (*d*, *J*=6.5, H–C(11)); 3.12 (*d*, *J*=5.0, 1 H, CH_2(13)); 2.82 (*d*, *J*=5.0, 1 H, CH_2(13)); 2.61 (*dd*, *J*=9.0, 19.5, 1 H, CH_2(3)); 2.00–1.96 (*m*, CH_2(8)); 1.94–1.91 (*m*, 1 H, CH_2(7)); 1.89–1.87 (*m*, 1 H, CH_2(3)); 1.71 (*s*, Me(16)); 1.46–1.43 (*m*, 1 H, CH_2(7)); 0.93 (*s*, Me(14)); 0.72 (*s*, Me(15)). ^{13}C-NMR (100 MHz, $CDCl_3$): 140.04 (C(9)); 118.69 (C(10)); 78.70, 73.91 (C(2,11)); 70.23 (C(4)); 65.71 (C(12)); 49.06, 48.34, 47.50 (C(5,13,6)); 40.04 (C(3)); 27.95 (C(8)); 24.36, 23.16 (C(7,16)); 15.75 (C(15)); 6.15 (C(14)). HR-ESI-MS: 273.1471 ($[M+Na]^+$, $C_{15}H_{22}NaO_3^+$; calc. 273.1467).

Synthesis of (4β,8β)-8-Hydroxy-12,13-epoxytrichothec-9-en-4-yl Acetate (**3**) *and (4β)-16-Oxo-12,13-epoxytrichothec-9-en-4-yl Acetate* (**4**). A soln. of **1** (4.00 g, 13.70 mmol) in 1,4-dioxane (30 ml) was added dropwise during 90 min to a refluxing soln. of SeO_2 (2.00 g, 18.02 mmol) in 1,4-dioxane (20 ml) [33].

After refluxing for 12 h, the mixture was cooled and concentrated. Then, 50 ml of AcOEt was added, and the mixture was washed with 5% aq. $NaHCO_3$, dried, and concentrated *in vacuo* to give 3.50 g of a yellow liquid. The crude product was purified by FC (SiO_2; PE/AcOEt 5 :1) to give **3** (2.10 g, 49.8%). Colorless crystals. M.p. 138–141°. $[\alpha]_D^{28} = +3.49$ ($c = 5$, acetone). ^{1}H-NMR (500 MHz, $CDCl_3$): 5.52 (*dd*, $J = 3.5, 7.5$, H–C(4)); 5.48–5.46 (*m*, H–C(10)); 4.06 (*d*, $J = 7.5$, H–C(8)); 3.84 (*d*, $J = 5.0$, H–C(2)); 3.63 (*d*, $J = 5.5$, H–C(11)); 3.13 (*d*, $J = 4.0$, 1 H, CH_2(13)); 2.85 (*d*, $J = 4.0$, 1 H, CH_2(13)); 2.53–2.49 (*m*, 1 H, CH_2(3)); 2.08 (*s*, Me); 2.02–1.98 (*m*, 1 H, CH_2(3)); 1.86–1.84 (*m*, 1 H, CH_2(7)); 1.70 (*s*, Me(16)); 1.53–1.52 (*m*, 1 H, CH_2(7)); 0.98 (*s*, Me(14)); 0.73 (*s*, Me(15)). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.94 (Me); 142.24 (C(9)); 121.02 (C(10)); 79.29, 74.11 (C(2,11)); 70.30, 68.92 (C(4,8)); 65.36 (C(12)); 49.03, 48.50, 47.74 (C(5,13,6)); 42.36, 36.61 (C(3,7)); 21.09 (Me); 18.75, 16.87 (C(15,16)); 5.78 (C(14)). HR-ESI-MS: 331.1518 ($[M+Na]^+$, $C_{17}H_{24}NaO_5^+$; calc. 331.1521).

Compound **4** (0.80 g, 19.1%) was obtained as the second product. Pale yellow oil. $[\alpha]_D^{28} = -1.18$ ($c = 5$, acetone). ^{1}H-NMR (500 MHz, $CDCl_3$): 9.54 (*s*, CHO); 6.60–6.65 (*m*, H–C(10)); 5.61 (*dd*, $J = 3.5, 7.5$, H–C(4)); 3.89 (*d*, $J = 5.5$, H–C(2,11)); 3.63 (*d*, $J = 5.5$, H–C(11)); 3.12 (*d*, $J = 4.0$, 1 H, CH_2(13)); 2.85 (*d*, $J = 4.0$, 1 H, CH_2(13)); 2.58–2.53 (*m*, 1 H, CH_2(3)); 2.48–2.44 (*m*, 1 H, CH_2(3)); 2.08 (*s*, Me); 2.09–2.03 (*m*, CH_2(8)); 1.87–1.84 (*m*, 1 H, CH_2(7)); 0.97–0.95 (*m*, 1 H, CH_2(7)); 0.92 (*s*, Me(14)); 0.75 (*s*, Me(15)). ^{13}C-NMR (100 MHz, $CDCl_3$): 199.22 (CHO); 170.54 (Me); 137.80 (C(9)); 136.74 (C(10)); 79.20 (C(2)); 77.04, 74.59 (C(11,4)); 69.75 (C(12)); 49.23, 48.56, 47.74 (C(5,13,6)); 42.36, 40.10 (C(3,7)); 21.01 (Me); 18.75, 18.14 (C(15,8)); 5.28 (C(14)). HR-ESI-MS: 329.1360 ($[M+Na]^+$, $C_{17}H_{22}NaO_5^+$; calc. 329.1365).

Synthesis of (4β,9α,10α)-9,10-Dibromo-12,13-epoxytrichothecan-4-yl Acetate (**5**). In a flask, Br_2 (0.22 g, 1.39 mmol) in CH_2Cl_2 (5 ml) was added dropwise to a soln. of **1** (0.20 g, 0.68 mmol) and pyridine (0.11 g, 1.39 mmol) in 15 ml of CH_2Cl_2, and stirred for 3 h at r.t. [34]. The solvent was washed with dil. HCl and sat. Na_2CO_3 soln., dried, and concentrated *in vacuo* to 0.40 g of colorless liquid. The crude product was purified by FC (silica gel; PE/AcOEt 5 :1) to give **5** 0.27 g, 88.0%). White solid. The solid was filtered and recrystallized from 95.0% EtOH as colorless blocks. M.p. 170–172°. ^{1}H-NMR (500 MHz, $CDCl_3$): 5.57 (*dd*, $J = 3.5, 8.0$, H–C(4)); 4.62 (*s*, H–C(10)); 4.02 (*s*, H–C(11)); 3.87 (*d*, $J = 5.0$, H–C(2)); 3.21 (*d*, $J = 4.0$, 1 H, CH_2(13)); 2.94 (*d*, $J = 4.0$, 1 H, CH_2(13)); 2.49–2.41 (*m*, 1 H, CH_2(13)); 2.41–2.35 (*m*, 1 H, CH_2(8)); 2.07 (*s*, Me); 2.05 (*s*, Me(16)); 2.01–1.97 (*m*, H–C(3)); 1.96–1.95 (*m*, 1 H, CH_2(7)); 1.95–1.87 (*m*, 1 H of CH_2(8)); 1.46–1.42 (*m*, 1 H, CH_2(7)); 1.38 (*s*, Me(14)); 0.71 (*s*, Me(15)). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.20 (Me); 80.56 (C(2)); 77.35, 74.42 (C(11,4)); 68.78, 64.92 (C(12,9)); 52.11 (C(10)); 48.60, 48.23, 47.74 (C(5,13,6)); 42.36, 40.10 (C(3,8)); 35.10, 32.33 (C(16,7)); 21.20 (Me); 18.50, 18.23 (C(15,8)); 6.10 (C14). Anal. calc. for $C_{17}H_{24}Br_2O_4$: C 45.16, H 5.35; found: C 45.14, H 5.34.

Synthesis of (4β,8β)-4-(Acetyloxy)-12,13-epoxytrichothec-9-en-8-yl Methylbenzoate (**3d**). *Representative Procedure.* A soln. of 4-methylbenzoyl chloride (0.65 mmol) in dry CH_2Cl_2 (5 ml) was added dropwise to a soln. of **3** (0.20 g, 0.65 mmol), Et_3N (0.20 g, 1.95 mmol), and DMAP (0.007 g, 0.06 mmol) in CH_2Cl_2 (10 ml), and the mixture was stirred at r.t. for 8 h and then was washed successively with H_2O, sat. $NaHCO_3$ soln., and brine. The org. layer was dried (Na_2SO_4), and the solvent was removed to give a crude product, which was purified by FC (SiO_2; PE/AcOEt 5 :1) to afford **3d** (0.24 g, 85.9%). Colorless crystals. M.p. 132–134°. $[\alpha]_D^{28} = +11.41$ ($c = 5$, acetone). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.95–7.93 (*m*, Ar); 7.26–7.24 (*m*, Ar); 5.63–5.61 (*m*, H–C(10)); 5.60–5.58 (*m*, H–C(8)); 5.56 (*dd*, $J = 4.0, 8.0$, H–C(4)); 3.88 (*d*, $J = 5.0$, H–C(2)); 3.69 (*d*, $J = 5.5$, H–C(11)); 3.13 (*d*, $J = 4.0$, 1 H, CH_2(13)); 2.81 (*d*, $J = 4.0$, 1 H, CH_2(13)); 2.57–2.52 (*m*, 1 H, CH_2(3)); 2.08 (*s*, Me); 2.05–2.02 (*m*, 1 H, CH_2(3)); 2.01–1.95 (*m*, CH_2(7)); 1.77 (*s*, Me(16)); 1.11 (*s*, Me(14)); 0.72 (*s*, Me(15)). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.89 (C=O); 166.29 (C=O); 143.92 (Ar); 139.17 (C(9)); 129.73, 129.16 (2 Ar); 127.31 (Ar); 122.72 (C(10)); 79.41, 74.34, 71.46, 69.96 (C(2,11,8,4)); 65.30 (C(12)); 49.15, 48.42, 47.72 (C(5,13,6)); 42.24 (C(3)); 30.89 (C(7)); 21.64, 21.08, 18.87 (2 Me, C(16)); 16.99 (C(15)); 5.81 (C(14)). Anal. calc. for $C_{25}H_{30}O_6$: C 70.40, H 7.09; found: C 70.38, H 7.11.

Compounds **2a**–**2g**, **3a**–**3c**, and **3e**–**3i** were prepared as described for **3d**.

(4β)-12,13-Epoxytrichothec-9-en-4-yl Hexanoate (**2a**). Yield: 69.8%. Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 5.57 (*dd*, $J = 4.0, 8.0$, H–C(4)); 5.41–5.39 (*m*, H–C(10)); 3.82 (*d*, $J = 5.0$, H–C(2)); 3.61 (*d*, $J = 5.5$, H–C(11)); 3.12 (*d*, $J = 4.0$, 1 H, CH_2(13); 2.82 (*d*, $J = 4.0$, 1 H, CH_2(13)); 2.55–2.51 (*m*, 1 H, CH_2(13)); 2.34–2.31 (*m*, $COCH_2$); 1.99–1.98 (*m*, 1 H, CH_2(3)); 1.96–1.95 (*m*, CH_2(8)); 1.94–1.92 (*m*, 1 H, CH_2(7)); 1.71 (*s*, Me(16)); 1.64–1.61 (*m*, CH_2); 1.42–1.40 (*m*, 1 H, CH_2(7)); 1.39–1.32 (*m*, 2

CH_2); 0.93 (*s*, Me(14)); 0.92–0.89 (*m*, Me); 0.71 (*s*, Me(15)). ^{13}C-NMR (100 MHz, $CDCl_3$): 173.80 (C=O); 140.21 (C(9)); 118.67 (C(10)); 79.18, 74.86, 71.53, 70.56 (C(2,11,8,4)); 65.55 (C(12)); 48.90, 47.86 (C(5,6)); 40.46 (C(3)); 36.75 (C(13)); 34.42 (CH_2); 31.31, 30.91 (CH_2, C(7)); 28.03 (CH_2); 24.50 (CH_2); 22.31 (C(16)); 16.01 (C(15)); 5.88 (C(14)). HR-ESI-MS: 371.2199 ($[M+Na]^+$, $C_{21}H_{32}NaO_4^+$; calc. 371.2198).

(4β)-12,13-Epoxytrichothec-9-en-4-yl 2-(Trifluoromethyl)prop-2-enoate (**2b**). Yield: 56.9%. Pale-yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.75–6.74 (*m*, CH_2); 6.45 (*d*, *J* = 1.0, CH_2); 5.67 (*dd*, *J* = 3.0, 7.5, H–C(4)); 5.42–5.40 (*m*, H–C(10)); 3.86 (*d*, *J* = 5.5, H–C(2)); 3.61 (*d*, *J* = 5.5, H–C(11)); 3.13 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.84 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.64–2.61 (*m*, 1 H, CH_2(3)); 2.08–2.07 (*m*, 1 H, CH_2(3)); 2.06–2.04 (*m*, CH_2(8)); 1.97–1.93 (*m*, 1 H, CH_2(7)); 1.72 (*s*, Me(16)); 1.45–1.43 (*m*, 1 H, CH_2(7)); 0.95 (*s*, Me(14)); 0.75 (*s*, Me(15)). HR-ESI-MS: 395.1446 ($[M+Na]^+$, $C_{19}H_{23}F_3NaO_4^+$; calc. 395.1450).

(4β)-12,13-Epoxytrichothec-9-en-4-yl 2-Phenylacetate (**2c**). Yield: 86.1%. Colorless crystals. M.p. 150–153°. $[\alpha]_D^{28} = -8.77$ (*c* = 5, acetone). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.33–7.26 (*m*, Ph); 5.56 (*dd*, *J* = 3.5, 7.5, H–C(4)); 5.39–5.38 (*m*, H–C(10)); 3.82 (*d*, *J* = 5.0, H–C(2)); 3.65 (*d*, *J* = 1.5, 2 CH_2); 3.57 (*d*, *J* = 5.5, H–C(11)); 3.11 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.80 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.52–2.50 (*m*, 1 H, CH_2(3)); 2.00–1.97 (*m*, 1 H, CH_2(3)); 1.97–1.95 (*m*, CH_2(8)); 1.94–1.91 (*m*, 1 H, CH_2(7)); 1.70 (*s*, Me(16)); 1.40–1.39 (*m*, 1 H, CH_2(7)); 0.90 (*s*, Me(14)); 0.64 (*s*, Me(15)). Anal. calc. for $C_{23}H_{28}O_4$: C 74.97, H 7.66; found: C 75.01, H 7.62.

(4β)-12,13-Epoxytrichothec-9-en-4-yl (2,4,6-Trimethylphenyl)acetate (**2d**). Yield: 91.2%. Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.85 (*s*, Ar); 5.57 (*dd*, *J* = 4.0, 8.0, H–C(4)); 5.39–5.38 (*m*, H–C(10)); 3.82 (*d*, *J* = 5.0, H–C(2)); 3.67 (*s*, CH_2); 3.57 (*d*, *J* = 5.5, H–C(11)); 3.11 (*d*, *J* = 2.0, 1 H, CH_2(13)); 2.80 (*d*, *J* = 4 .0, 1 H, CH_2(13)); 2.50 (*m*, 1 H, CH_2(3)); 2.30 (*s*, Me); 2.01–1.98 (*m*, 1 H, CH_2(3)); 1.97–1.96 (*m*, CH_2(8)); 1.93–1.90 (*m*, 1 H, CH_2(7)); 1.69 (*s*, Me(16)); 1.46–1.43 (*m*, 1 H, CH_2(7)); 0.90 (*s*, Me(14)); 0.64 (*s*, Me(15)). HR-ESI-MS: 433.2355 ($[M+Na]^+$, $C_{26}H_{34}NaO_4^+$; calc. 433.2359).

*(4β)-12,13-Epoxytrichothec-9-en-4-yl (2*E*)-3-Phenylprop-2-enoate* (**2e**). Yield: 75.6%. Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.72 (*d*, *J* = 6.0, Ph); 7.54–7.52 (*m*, Ph); 7.38–7.37 (*m*, Ph); 7.38–7.37 (*m*, COCH); 6.50 (*d*, *J* = 16.0, CH); 5.72 (*dd*, *J* = 3.5, 7.5, H–C(4)); 5.44–5.42 (*m*, H–C(10)); 3.86 (*d*, *J* = 5.0, H–C(2)); 3.65 (*d*, *J* = 5.5, H–C(11)); 3.15 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.86 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.61–2.60 (*m*, 1 H, CH_2(3)); 2.11–2.10 (*m*, 1 H, CH_2(3)); 2.02–1.99 (*m*, CH_2(8)); 1.96 (*s*, 1 H of CH_2(7)); 1.73 (*s*, Me(16)); 1.46–1.43 (*m*, 1 H of CH_2(7)); 0.98 (*s*, Me(14)); 0.77 (*s*, Me(15)). ^{13}C-NMR (100 MHz, $CDCl_3$): 166.85 (C=O); 145.17 (CH); 140.24 (C(9)); 134.40 (Ph); 130.33 (Ph); 128.88 (Ph); 128.17 (Ph); 118.67 (C(10)); 118.11 (CO*C*H); 79.24 (C(2)); 75.24 (C(13)); 70.60 (C(4)); 65.63 (C(12)); 49.28 (C(5)); 47.92 (C(11)); 40.52 (C(6)); 36.76 (C(3)); 28.04 (C(8)); 24.53 (C(7)); 23.27 (C(16)); 16.05 (C(15)); 6.67 (C(14)). HR-ESI-MS: 403.1886 ($[M+Na]^+$, $C_{24}H_{28}NaO_4^+$; calc. 403.1882).

(4β)-12,13-Epoxytrichothec-9-en-4-yl 2-(Naphthalen-2-yl)acetate (**2f**). Yield: 84.9%. Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 8.03–8.00 (*m*, Naph); 7.86–7.84 (*m*, Naph); 7.53–7.26 (*m*, Naph); 5.58 (*dd*, *J* = 3.5, 8.0, H–C(4)); 5.37–5.36 (*m*, H–C(10)); 4.10 (*s*, CH_2); 3.81 (*d*, *J* = 5.0, H–C(2)); 3.55 (*d*, *J* = 6.0, H–C(11)); 3.11 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.80 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.52–2.51 (*m*, 1 H, CH_2(3)); 2.01–1.99 (*m*, 1 H, CH_2(3)); 2.00–1.96 (*m*, *J* = 4.0, CH_2(8)); 1.99–1.95 (*m*, 1 H, CH_2(7)); 1.68 (*s*, Me(16)); 1.37–1.25 (*m*, 1 H, CH_2(7)); 0.85 (*s*, Me(14)); 0.60 (*s*, Me(15)). HR-ESI-MS: 441.2042 ($[M+Na]^+$, $C_{20}H_{30}NaO_4^+$; calc. 441.2045).

(4β)-12,13-Epoxytrichothec-9-en-4-yl 2,6-Dichloro-5-fluoropyridine-3-carboxylate (**2g**). Yield: 71.1%. Colorless crystals. M.p. 141–144°. $[\alpha]_D^{28} = -7.17$ (*c* = 5, acetone). ^{1}H-NMR (500 MHz, $CDCl_3$): 8.05 (*d*, *J* = 7.0, Py); 5.79 (*dd*, *J* = 3.0, 7.5, H–C(4)); 5.43 (*d*, *J* = 4.5, H–C(10)); 3.90 (*d*, *J* = 5.0, H–C(2)); 3.64 (*d*, *J* = 5.5, H–C(11)); 3.17 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.87 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.86–2.65 (*m*, 1 H, CH_2(3)); 2.16–2.11 (*m*, 1 H, CH_2(3)); 2.01–1.96 (*m*, CH_2(8)); 2.00–1.95 (*m*, 1 H, CH_2(7)); 1.73 (*s*, Me(16)); 1.47–1.44 (*m*, 1 H, CH_2(7)); 0.99 (*s*, Me(14)); 0.79 (*s*, Me(15)). Anal. calc. for $C_{21}H_{22}Cl_2FNO_4$: C 57.02, H 5.01, N 3.17; found: C 57.26, H 4.97, N 3.16.

(4β,8β)-4-(Acetyloxy)-12,13-epoxytrichothec-9-en-8-yl Butanoate (**3a**). Yield: 80.0%. Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 5.60–5.58 (*m*, H–C(10)); 5.53 (*dd*, *J* = 3.5, 8.0, H–C(4)); 5.40–5.37 (*m*, H–C(8)); 4.26–4.21 (*m*, H–C(2)); 3.86 (*d*, *J* = 5.0, 1 H, CH_2(13)); 3.66 (*d*, *J* = 5.5, H–C(11)); 3.13 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.80–2.78 (*m*, 1 H, CH_2(7)); 2.54–2.50 (*m*, 1 H, CH_2(3)); 2.08 (*s*, Me); 2.02–2.00 (*m*,

1 H, CH_2(3)); 1.99–1.83 (*m*, 2 CH_2); 1.86–1.83 (*m*, 1 H, CH_2(7)); 1.72 (*s*, Me(16)); 1.06–1.03 (*m*, Me); 1.04 (*s*, Me(14)); 0.71 (*s*, Me(15)). HR-ESI-MS: 401.1940 ($[M+Na]^+$, $C_{21}H_{30}NaO_6^+$; calc. 401.1943).

(4β,8β)-4-(Acetyloxy)-12,13-epoxytrichothec-9-en-8-yl 3-Methylbut-2-enoate (**3b**). Yield: 67.2%. Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.09 (*s*, CH); 5.53–5.58 (*m*, CH_2(8)); 5.41–5.39 (*m*, H–C(10)); 3.83 (*d*, *J* = 5.5, H–C(2)); 3.62 (*d*, *J* = 5.5, H–C(11)); 3.09 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.78 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.53 (*dd*, *J* = 7.5, 15.5, 1 H, CH_2(3)); 2.18 (*s*, Me); 2.08 (*s*, Me); 2.01–1.97 (*m*, H–C(3,7)); 1.91 (*s*, Me); 1.88 (*s*, Me(16)); 1.05 (*s*, Me(14)); 0.70 (*s*, Me(15)). HR-ESI-MS: 413.1940 ($[M+Na]^+$, $C_{22}H_{30}NaO_6^+$; calc. 413.1944).

(4β,8β)-4-(Acetyloxy)-12,13-epoxytrichothec-9-en-8-yl 3-Methylbutanoate (**3c**). Yield: 74.3%. Pale yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 5.57–5.56 (*m*, H–C(4)), 5.53 (*dd*, *J* = 4.0, 8.0, H–C(8)); 5.36–5.33 (*m*, H–C(10)); 3.85 (*d*, *J* = 5.0, H–C(2)); 3.64 (*d*, *J* = 5.0, H–C(11)); 3.13 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.80 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.54 (*dd*, *J* = 8.0, 15.5, 1 H, CH_2(3)); 2.22 (*d*, *J* = 5.0, CH_2); 2.13–2.10 (*m*, CH); 2.08 (*s*, Me); 2.02–2.10 (*m*, 1 H, CH_2(3)); 2.00–1.98 (*m*, CH_2(7)); 1.70 (*s*, Me(16)); 1.15 (*s*, Me(14)); 0.97 (*d*, *J* = 7.0, 2 Me); 0.71 (*s*, Me(15)). HR-ESI-MS: 415.2097 ($[M+Na]^+$, $C_{22}H_{32}NaO_6^+$; calc. 415.2102).

(4β,8β)-4-(Acetyloxy)-12,13-epoxytrichothec-9-en-8-yl 4-Fluorobenzoate (**3e**). Yield: 70.0%. Colorless crystals. M.p. 142–144°. $[\alpha]_D^{28} = +3.61$ (*c* = 5, acetone). ^{1}H-NMR (500 MHz, $CDCl_3$): 8.08–8.05 (*m*, Ar); 7.15–7.11 (*m*, Ar); 5.64–5.63 (*m*, H–C(10)); 5.60–5.58 (*m*, H–C(8)); 5.56 (*dd*, *J* = 3.5, 8.0, H–C(4)); 3.88 (*d*, *J* = 5.0, H–C(2)); 3.69 (*d*, *J* = 5.5, H–C(11)); 3.13 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.81(*d*, *J* = 4.0, 1 H, CH_2(13)); 2.55 (*dd*, *J* = 8.0, 15.5, 1 H, CH_2(3)); 2.08 (*s*, Me); 2.04–1.99 (*m*, 1 H, CH_2(3)); 1.99–1.97 (*m*, CH_2(7)); 1.76 (*s*, Me(16)); 1.11 (*s*, Me(14)); 0.73 (*s*, Me(15)). Anal. calc. for $C_{24}H_{27}FO_6$: C 66.96, H 6.32; found: C 66.89, H 6.38.

*(4β,8β)-4-(Acetyloxy)-12,13-epoxytrichothec-9-en-8-yl (2*E*)-3-Phenylprop-2-enoate* (**3f**). Yield: 85.3%. Colorless crystals. M.p. 100–102°. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.72 (*d*, *J* = 16.0, COCH); 7.54–7.52 (*m*, Ph); 7.40–7.39 (*m*, Ph); 6.45 (*d*, *J* = 16, CH); 5.61 (*d*, *J* = 5.5, H–C(10)); 5.55 (*dd*, *J* = 3.5, 8.0, H–C(4)); 5.51–5.48 (*m*, H–C(8)); 3.87 (*d*, *J* = 5.0, H–C(2)); 3.68 (*d*, *J* = 5.5, H–C(11)); 3.14 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.83 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.56–2.51 (*m*, 1 H of CH_2(3)); 2.08 (*s*, Me); 2.04–1.98 (*m*, CH_2(7)); 1.93–1.91 (*m*, 1 H, CH_2(3)); 1.75 (*s*, Me(16)); 1.08 (*s*, Me(14)); 0.73 (*s*, Me(15)). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.90 (C=O); 166.60 (*C*OCH); 145.31 (CH); 139.04 (C(9)); 134.31 (Ph); 130.49 (Ph); 128.96, 128.16 (Ph); 122.81 (C(10)); 117.93 (*C*HCO); 79.42, 74.37, 71.07, 69.96 (C(2,11,8,4)); 65.33 (C(12)); 49.17, 47.75 (C(5,6)); 42.22 (C(3)); 36.69 (C(13)); 31.01 (C(7)); 21.10, 18.83 (Me, C(16)); 16.99 (C(15)); 5.84 (C(14)). Anal. calc. for $C_{26}H_{30}O_6$: C 71.21, H 6.90; found: C 71.18, H 6.83.

(4β,8β)-4-(Acetyloxy)-12,13-epoxytrichothec-9-en-8-yl 2,2-Dimethyl-3-(2-methylprop-1-en-1-yl)cyclopropanecarboxylate (**3g**). Yield: 76.8%. Colorless oil. $[\alpha]_D^{28} = +3.24$ (*c* = 5, acetone). ^{1}H-NMR (500 MHz, $CDCl_3$): 5.56–5.55 (*m*, H–C(10)); 5.53 (*dd*, *J* = 4.0, 8.0, H–C(4)); 5.41–5.38 (*m*, H–C(8)); 4.91 (*d*, *J* = 8.0, CH); 3.85 (*d*, *J* = 5.0, H–C(2)); 3.64 (*d*, *J* = 5.0, H–C(11)); 3.12 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.81 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.54–2.49 (*m*, 1 H, CH_2(3)); 2.07 (*s*, Me); 2.02–1.97 (*m*, 1 H of CH_2(3)); 1.96–1.91 (*m*, 1 H, CH_2(7)); 1.83–1.80 (*m*, 1 H, CH_2(7)); 1.71 (*s*, Me(16)); 1.70–1.76 (*m*, 2 Me); 1.80–1.79 (*m*, CH); 1.42–1.41 (*m*, COCH); 1.25 (*s*, Me); 1.15 (*s*, Me); 1.03 (*s*, Me(14)); 0.70 (*s*, Me(15)). HR-ESI-MS: 481.2562 ($[M+Na]^+$, $C_{27}H_{38}NaO_6^+$; calc. 481.2566).

(4β,8β)-4-(Acetyloxy)-12,13-epoxytrichothec-9-en-8-yl 3-(2,2-Dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate (**3h**). Yield: 60.8%. Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 6.79 (*d*, *J* = 8.5, Br_2CCH); 5.58–5.56 (*m*, H–C(10)); 5.53 (*dd*, *J* = 4.0, 8.0, H–C(4)); 5.37–5.35 (*m*, H–C(8)); 3.85 (*d*, *J* = 5.5, H–C(2)); 3.64 (*d*, *J* = 5.5, H–C(11)); 3.14 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.80 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.56–2.51 (*m*, 1 H, CH_2(3)); 2.18–2.16 (*m*, CH); 2.08 (*s*, Me); 2.03–1.92 (*m*, 1 H, CH_2(3), CH_2(7)); 1.87–1.80 (*m*, COCH); 1.72 (*s*, Me(16)); 1.30–1.21 (*m*, 2 Me); 1.03 (*s*, Me(14)); 0.71 (*s*, Me(15)). HR-ESI-MS: 609.0464 ($[M+Na]^+$, $C_{25}H_{32}Br_2NaO_6^+$; calc. 609.0461).

(4β,8β)-4-(Acetyloxy)-12,13-epoxytrichothec-9-en-8-yl 2,6-Dichloro-5-fluoropyridine-3-carboxylate (**3i**). Yield: 78.3%. Colorless crystals. M.p. 86–88°. $[\alpha]_D^{28} = +6.13$ (*c* = 5, acetone). ^{1}H-NMR (500 MHz, $CDCl_3$): 5.02 (*d*, *J* = 7.0, Py); 5.67–5.65 (*m*, H–C(10)); 5.64–5.62 (*m*, H–C(8)); 5.56 (*dd*, *J* = 3.5, 8.0, H–C(4)); 3.87 (*d*, *J* = 5.0, H–C(2)); 3.70 (*d*, *J* = 5.5, H–C(11)); 3.13 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.80 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.57–2.52 (*m*, 1 H, CH_2(3)); 2.08 (*s*, Me); 2.05–2.00 (*m*, 1 H, CH_2(3));

1.99–1.95 (*m*, CH_2(7)); 1.78 (*s*, Me(16)); 1.10 (*s*, Me(14)); 0.73 (*s*, Me(15)). Anal. calc. for $C_{23}H_{24}Cl_2FNO_6$: C 55.21, H 4.83, N 2.80; found: C 55.27, H 4.73, N 2.82.

Synthesis of (4β)-4-Methoxy-12,13-epoxytrichothec-9-ene (**2h**). A soln. of MeI (0.23 g, 1.63 mmol) in 5 ml of dry DMF was added dropwise to a soln. of **2** (0.10 g, 0.40 mmol) and NaH (0.029 g, 1.21 mmol) in DMF (10 ml) at 0°, and the mixture was stirred for 6 h. The solvent was removed under reduced pressure, and AcOEt (20 ml) was added. The AcOEt soln. was washed with H_2O and brine, and dried, filtered, and concentrated *in vacuo* to give a crude product, which was purified by FC (SiO_2; PE/AcOEt 8 : 1) to afford 0.09 g (85.2%) of **2h**. Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$): 5.40–5.38 (*m*, H–C(10)); 3.88 (*dd*, *J* = 3.5, 7.0, H–C(4)); 3.82 (*d*, *J* = 5.0, H–C(2)); 3.48 (*d*, *J* = 5.5, H–C(11)); 3.35 (*s*, Me); 3.09 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.80 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.39–2.35 (*m*, 1 H, CH_2(3)); 1.99–1.97 (*m*, 1 H, CH_2(3)); 1.96–1.94 (*m*, CH_2(8)); 1.94–1.93 (*m*, 1 H, CH_2(7)); 1.71 (*s*, Me(16)); 1.43–1.41 (*m*, 1 H of CH_2(7)); 0.86 (*s*, Me(14)); 0.76 (*s*, Me(15)). ^{13}C-NMR (100 MHz, $CDCl_3$): 140.10 (C(9)); 119.00 (C(10)); 80.13, 78.70, 74.53 (C(2,11,4)); 68.88 (C(12)); 59.46 (Me); 55.08, 48.96, 46.79 (C(5,13,6)); 39.56 (C(3)); 28.53 (C(8)); 24.74, 23.10 (C(7,16)); 16.10 (C(15)); 6.78 (C(14)). HR-ESI-MS: 287.1626 ($[M+Na]^+$, $C_{16}H_{24}NaO_3^+$; calc. 287.1623).

Synthesis of (4β)-4-Ethoxy-12,13-epoxytrichothec-9-ene (**2i**). Compound **2i** was prepared as described for **2h** to give a colorless oil (0.08 g, 75.0%). ^{1}H-NMR (500 MHz, $CDCl_3$): 5.39–5.38 (*m*, H–C(10)); 3.97 (*dd*, *J* = 3.5, 7.5, H–C(4)); 3.79 (*d*, *J* = 5.0, H–C(2)); 3.61–3.56 (*m*, CH_2O); 3.43–3.39 (*m*, CH_2O); 3.49 (*d*, *J* = 5.5, H–C(11)); 3.08 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.80 (*d*, *J* = 4.0, 1 H, CH_2(13)); 2.40–2.35 (*m*, 1 H, CH_2(3)); 1.99–1.97 (*m*, 1 H, CH_2(3)); 1.96–1.94 (*m*, CH_2(8)); 1.94–1.93 (*m*, 1 H, CH_2(7)); 1.70 (*s*, Me(16)); 1.43–1.41 (*m*, 1 H, CH_2(7)); 1.21–1.18 (*m*, Me); 0.86 (*s*, Me(14)), 0.76 (*s*, Me(15)). ^{13}C-NMR (100 MHz, $CDCl_3$): 140.02 (C(9)); 118.51 (C(10)); 81.40, 78.70, 72.53 (C(2,11,4)); 68.50, 64.53 (C(12), Me*C*H_2O); 54.89, 48.40, 46.10 (C(5,13,6)); 39.17 (C(3)); 27.89 (C(8)); 24.39, 22.95 (C(7,16)); 16.13, 15.32 (C(15), *Me*CH_2O); 6.90 (C(14)). HR-ESI-MS: 301.1784 ($[M+Na]^+$, $C_{17}H_{26}NaO_3^+$; calc. 301.1780).

Antifungal Activity. Magnaporthe grisea, *Ustilaginoidea virens*, and *Rhizoctonia solani* were routinely maintained on potato dextrose agar (PDA) slants, and kept for stock at 4°. The antifungal activities were tested according to the method described in [35][36]. Cultures were obtained by transplanting mycelium disks, 10 mm in diameter, from a single culture in stationary phase. These were incubated at 25 ± 1° on PDA (pH 5.6 ± 0.2) on thin sterile sheets of cellophane, until the logarithmic phase of growth was reached, and then transferred to *Petri* dishes containing the medium supplemented with the compound to be tested. Each compound was dissolved in DMF, and a proper dilution was aseptically added to the medium at 45° to obtain a final concentration of 50 μg ml^{-1}. The DMF concentration of the final soln. was adjusted to 0.1%. Controls were set up with equivalent quantities (0.1%) of acetone. The growth rate was determined by measuring daily colony diameter for 4 d after the transport of the fungus onto dishes containing the substance to be tested. Four replicates were used for each concentration. Percentage inhibition was expressed as the mean values obtained in three independent experiments. For comparative purposes, the commercial fungicides prochloraz and propiconazole were tested under the same conditions as the title compounds. Compounds **1**, **3f**, and **4**, with high antifungal activities, were tested at 10, 5, 2.5, 1.25, and 0.625 μg ml^{-1} concentration.

X-Ray Crystal Structure Analysis of **3f** (*Fig. 2*). Crystal data were collected with a *Rigaku R-AXIS-RAPID* diffractometer equipped with a graphite-monochromatized MoK_α radiation (λ = 0.71073 Å). Compound **3f** was recrystallized from acetone to give colorless crystals, and its crystal structure was established using X-ray crystallographic diffraction analysis as shown in *Fig. 2*. The crystal was monoclinic with space group *C*2, and the cell parameters were *a* = 23.2430(13) Å, *b* = 7.8970(7) Å, *c* = 14.2170(9) Å, β = 99.233(2)°, *V* = 2575.3(3) Å^3, *Z* = 2. The final R_1 = 0.0619, wR_2 = 0.1674, *S* = 1.102, the difference densities were 0.26 e Å^{-3} (max.) and −0.42 e Å^{-3} (min.). The CCDC No. is 883766. The H-atoms of H_2O were located from a difference *Fourier* map with O – H distance of 0.88 Å, and the other H-atoms were placed in calculated positions with C – H distances between 0.93 and 0.98 Å according to their own hybrid way. All the H-atoms were included in the final cycles of refinement in riding model, with U_{iso}(H) of 1.2 or 1.5 U_{eq} of the carrier atoms. Surprisingly, there exists a positive hole that contains H_2O occupying a third of the total volume. It plays an important role in the formation of the single crystal.

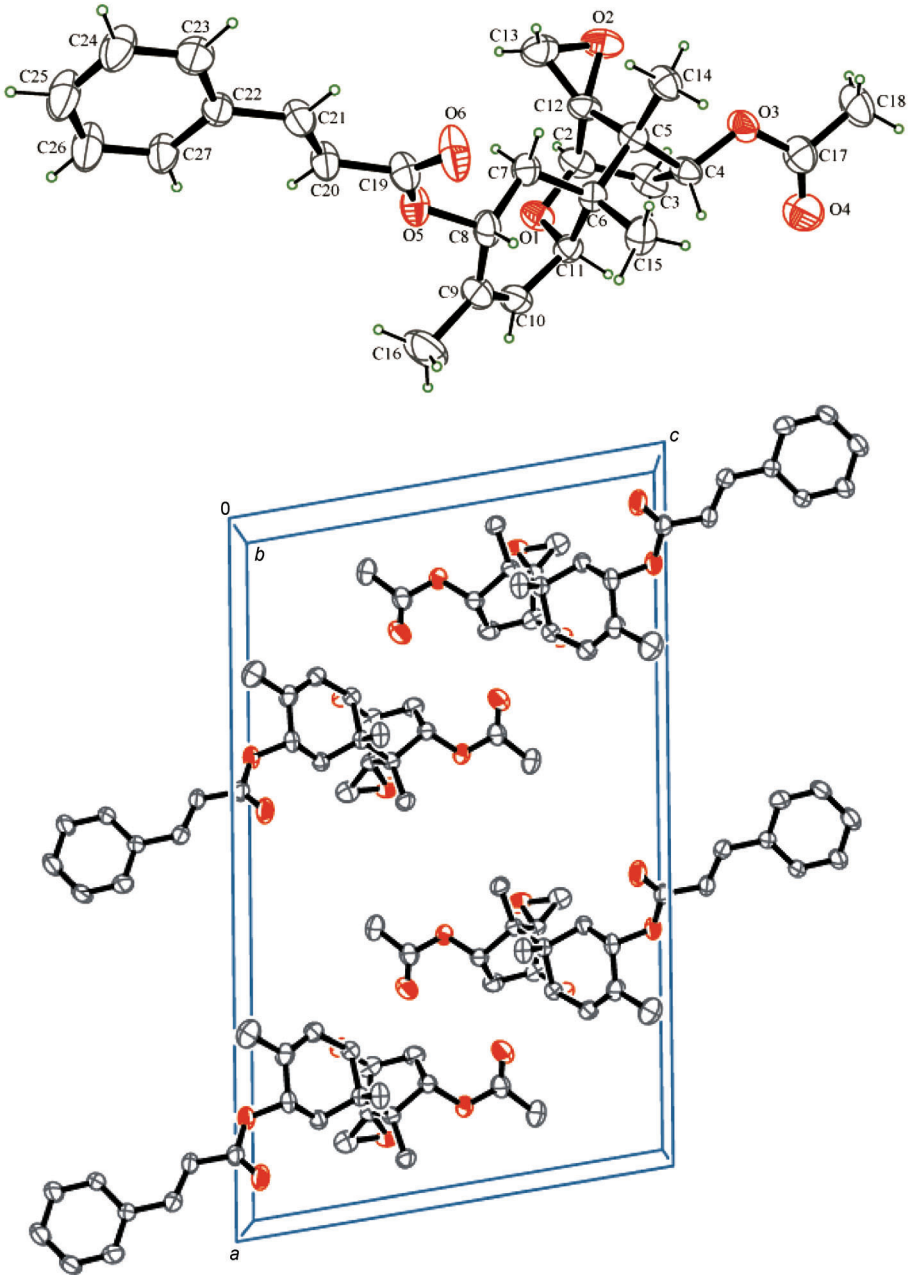

Fig. 2. *Molecular structure and packing diagram of* **3f**

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