

## Synthesis of a Photoaffinity-labeling Analog of Alternariolide (AM-toxin I), a Host-specific Phytotoxin

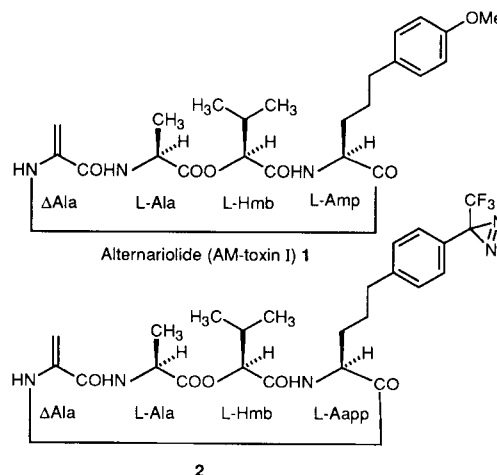
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A photoaffinity-labeling analog of alternariolide (AM-toxin I) which contains L-2-amino-5-[4-(1-aziridin-2,2,2-trifluoroethyl)phenyl]pentanoic acid (**10**) was synthesized.

Alternariolide (AM-toxin I, **1**) produced by *Alternaria mali* has been found to be responsible for the necrotic brown spots on certain apple leaves, which is the first example of a host-specific phytotoxin.<sup>1</sup> This toxin should work as a host recognition factor of the fungal pathogen at the infection site of the plants.<sup>2</sup> To study the host recognition process, we synthesized a photo-affinity labeling analog of alternariolide containing L-2-amino-5-[4-(1-aziridin-2,2,2-trifluoroethyl)phenyl]pentanoic acid (L-Aapp, **10**)<sup>3</sup> as a new labeling component. Since the diazirine group produces carbene by UV irradiation and it forms a covalent bond with a certain functional group around the receptor,<sup>4</sup> it has been used as a useful reagent to investigate the structure of its binding site. To synthesize the photoaffinity-labeled amino acid, we used Nassal's<sup>5</sup> and Kanaoka's<sup>4</sup> procedures.

Chain elongation of aldehyde **3** by the Horner-Emmons reaction followed by reduction with LiAlH<sub>4</sub> to give an alcohol<sup>6</sup> which was then protected with the TBS group to afford **4**. The halogen-metal exchange of **4** using n-BuLi and the resulting lithium compound was trapped by CF<sub>3</sub>CO<sub>2</sub>Et to give ketone **5** in 79% yield. The carbonyl group of **5** was converted to the oxime which was then



reacted with TsCl to give **6**. The tosylate **6** was exposed to ammonia in Et<sub>2</sub>O to give diaziridine which was then oxidized to diazirine by *N*-chlorination using *t*-BuOCl followed by dehydrochlorination with Et<sub>3</sub>N. To construct an amino acid functionality, the TBS group of **7** was removed using CSA in MeOH and the resulting hydroxyl group was converted to the

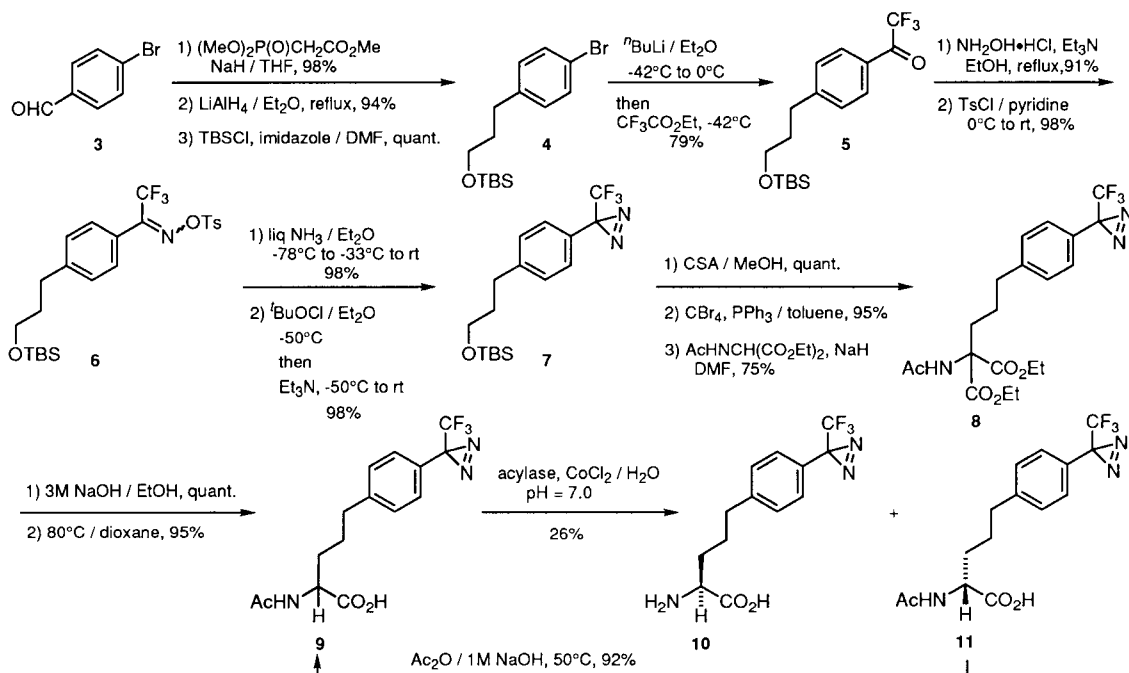


Figure 1.

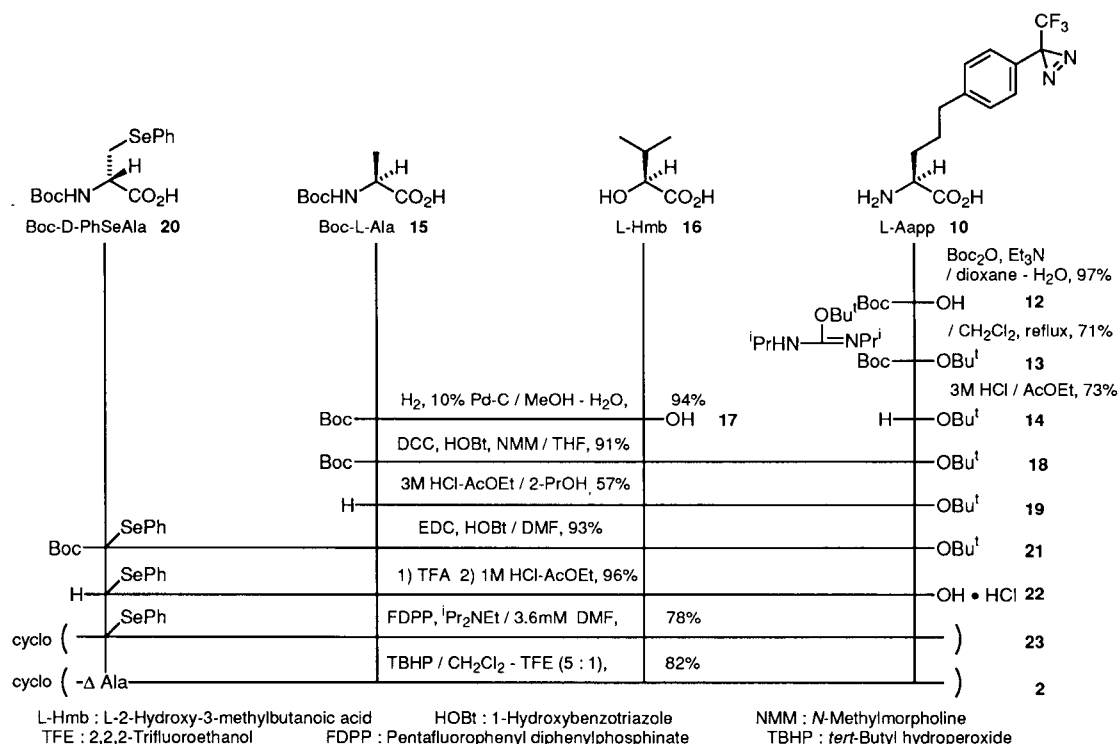


Figure 2.

bromide using  $\text{CBr}_4$  and  $\text{PPh}_3$ . The bromide was condensed with diethyl acetamidomalonate to give diester **8**. Hydrolysis of the esters under basic conditions followed by decarboxylation under neutral conditions gave **9**. The L-form of the acetamide **9** was hydrolyzed to the amine using acylase to give **10**. The residual D-form of the acetamide was racemized to **9** using 1M NaOH and  $\text{Ac}_2\text{O}$  at 50 °C which was later recycled.

To construct the cyclic tetradepsipeptide **2** containing a dehydroalanine, employment of D-phenylselenoalanine (D-PhSeAla)<sup>7</sup> as a precursor of the dehydroalanine is essential. The newly obtained amino acid **10** was protected by the following sequence of reactions, i) protection of the amine with the Boc group, ii) esterification using the isourea method<sup>8</sup>, and iii) removal of the Boc group under acidic conditions to give an amine **14** which was condensed with an ester **17** using DCC, HOBT. The Boc group of the tridepsipeptide **18** was removed and the resulting amine **19** was coupled with Boc-D-PhSeAla (**20**) using EDC, HOBT to give a linear tetradepsipeptide **21**. The protective groups of both ends in **21** were removed with TFA and the product was isolated as a hydrochloride salt **22**. Cyclization of **21** smoothly proceeded using FDPP in 3.6mM DMF to give the cyclic depsipeptide **23**, which was then exposed to an oxidation reaction using TBHP in  $\text{CH}_2\text{Cl}_2$  to afford the dehydropeptide **2** in 82% yield without any change in the diazirine functionality. The photoaffinity-labeling peptide **2** showed biological activity (necrosis on apple leaves) as strong as the original toxin **1**.

## References

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- 2 J. D. Walton, *Biochem. Pept. Antibiotics.*, **1990**, 179; K. Kohmoto and H. Ohtani, *Experientia*, **47**, 755 (1991).
- 3 mp >170 °C (decomp),  $[\alpha]_D^{25} +30.6^\circ$  (c 0.28, DMF : 2M HCl = 1 : 1), Anal. Found: C, 51.84; H, 4.70; N, 13.63, Calcd for  $\text{C}_{13}\text{H}_{14}\text{F}_3\text{N}_3\text{O}_2$ : C, 51.83; H, 4.68; N, 13.95,  $^1\text{H}$  NMR (300 MHz,  $\text{D}_2\text{O}$ , HOD = 4.65 ppm)  $\delta$  1.60 (2H, m), 1.70 (2H, m), 2.61 (2H, t, J = 7.2 Hz), 3.58 (1H, t, J = 6.0 Hz), 7.16 (2H, d, J = 8.4 Hz), 7.26 (2H, d, J = 8.4 Hz), IR ( $\text{cm}^{-1}$ , nujol) 2924, 2855, 1586, 1155, 941, 723.
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- 8 V. E. Schmidt and F. Moosmüller, *Liebigs Ann. Chem.*, **567**, 235 (1955).
- 9 mp >170 °C (decomp),  $[\alpha]_D^{25} -87.9^\circ$  (c 0.17, DMF), EI-HRMS, Calcd for  $\text{C}_{24}\text{H}_{28}\text{O}_3\text{N}_3\text{F}_3$  ( $M^+$ ) 523.2042, Found 523.2048,  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ , DMSO = 2.49 ppm)  $\delta$  0.86 (3H, d, J = 7.0 Hz), 0.88 (3H, d, J = 7.0 Hz), 1.34 (3H, d, J = 7.4 Hz), 1.46 (1H, m), 1.59 (2H, m), 1.84 (1H, m), 1.94 (1H, m), 2.64 (2H, m), 4.31 (2H, m), 4.67 (1H, d, J = 5.8 Hz), 5.26, (ca1H, br), 5.39 (ca1H, br), 7.19 (2H, d, J = 8.2 Hz), 7.32 (2H, d, J = 8.2 Hz), 7.98 (1H, br), 8.07 (1H, d, J = 9.6 Hz), 9.05 (1H, br), IR ( $\text{cm}^{-1}$ , nujol) 3335, 3302, 3266, 2924, 2855, 1744, 1661, 1157, 1053.