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

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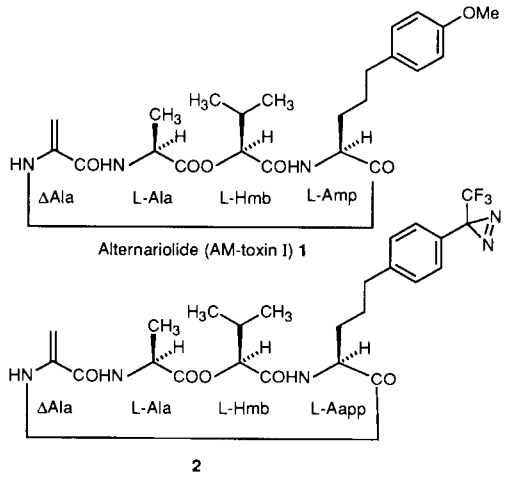
A photoaffinity-labeling analog of alternariolide (AM-toxin I) which contains L-2-amino-5-[4-(1-azi-2,2,2-trifluoro)ethyl-phenyl]pentanoic acid (**10**) was synthesized.

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.[1] This toxin should work as a host recognition factor of the fungal pathogen at the infection site of the plants.[2] To study the host recognition process, we synthesized a photo-affinity labeling analog of alternariolide containing L-2-amino-5-[4-(1-azi-2,2,2-trifluoroethyl)phenyl]pentanoic acid (L-Aapp, **10**)[3] 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,[4] 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[5] and Kanaoka's[4] procedures.



Chain elongation of aldehyde **3** by the Horner-Emmons reaction followed by reduction with $LiAlH_4$ to give an alcohol[6] 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_3CO_2Et 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_2O to give diaziridine which was then oxidized to diazirine by *N*-chlorination using *t*-BuOCl followed by dehydrochlorination with Et_3N. 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

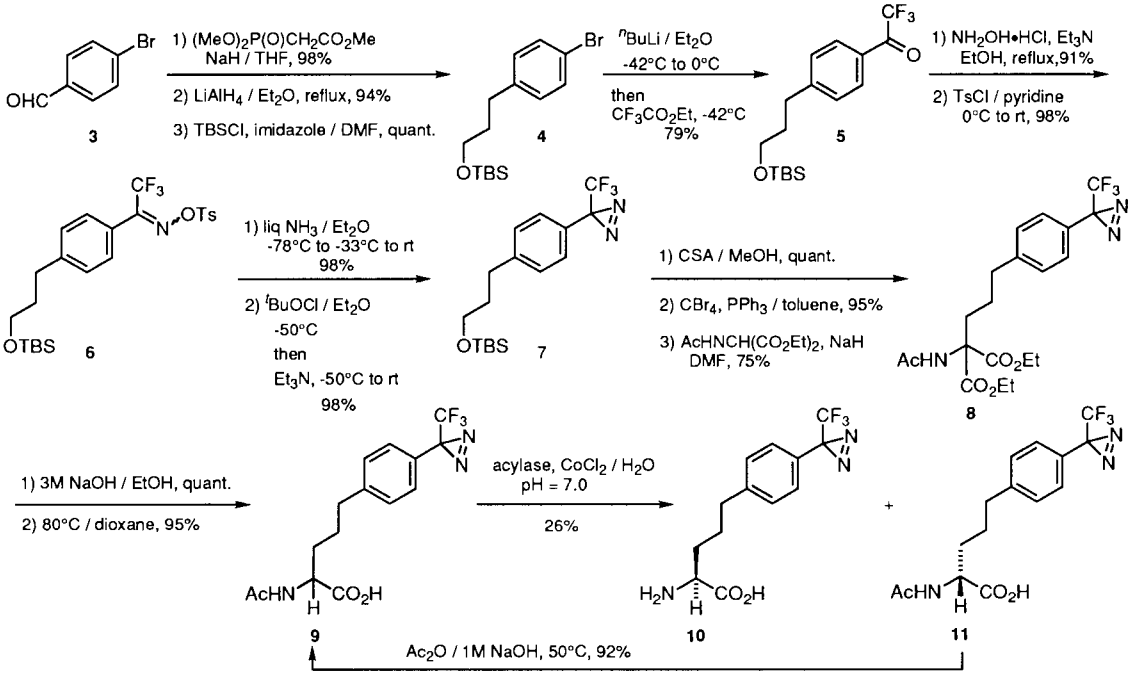


Figure 1.

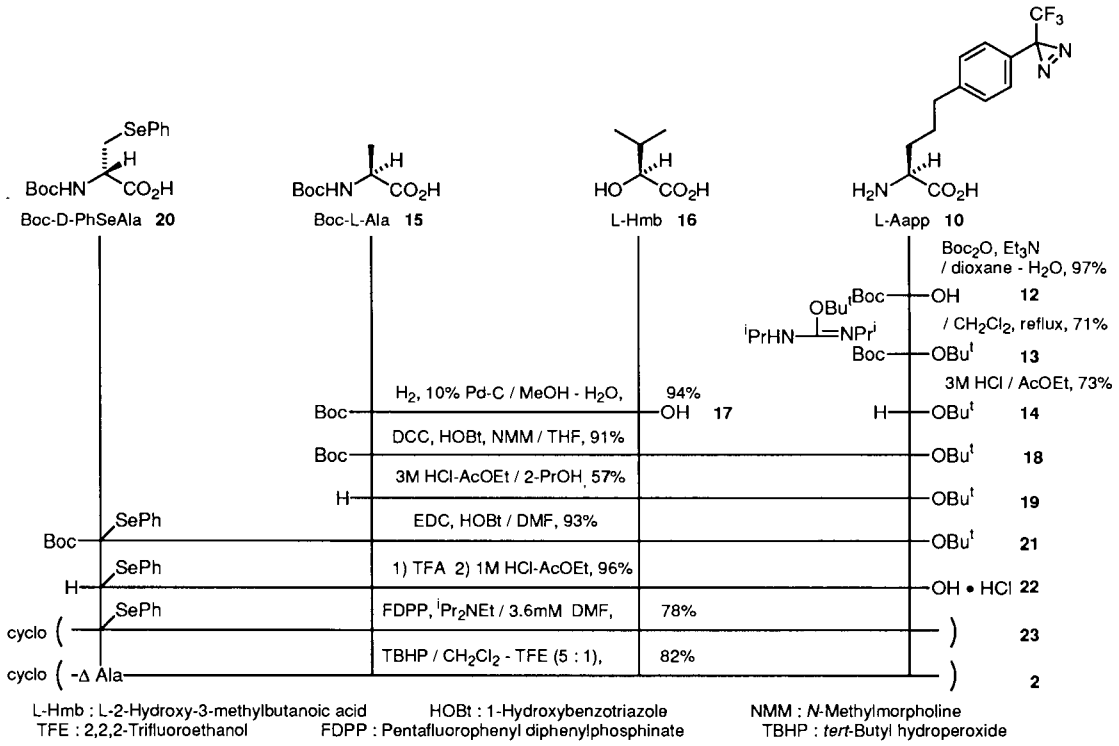


Figure 2.

bromide using CBr_4 and 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 Ac_2O at 50 °C which was later recycled.

To construct the cyclic tetradepsipeptide **2** containing a dehydroalanine, employment of D-phenylselenoalanine (D-PhSeAla)[7] 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[8], 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 CH_2Cl_2 to afford the dehydropeptide **2**[9] 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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3 mp >170 °C (decomp), $[\alpha]_D$ +30.6° (*c* 0.28, DMF : 2M HCl = 1 : 1), Anal. Found: C, 51.84; H, 4.70; N; 13.63, Calcd for $C_{13}H_{14}F_3N_3O_2$: C, 51.83; H, 4.68; N, 13.95, ^{1}H NMR (300 MHz, D_2O, HOD = 4.65 ppm) δ1.60 (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 (cm^{-1}, nujol) 2924, 2855, 1586, 1155, 941, 723.

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9 mp >170 °C (decomp), $[\alpha]_D$ -87.9° (*c* 0.17, DMF), EI-HRMS, Calcd for $C_{24}H_{28}O_5N_5F_3$ (M^+) 523.2042, Found 523.2048, ^{1}H NMR (300 MHz, DMSO-d6, DMSO = 2.49 ppm) δ0.86 (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 (cm^{-1}, nujol) 3335, 3302, 3266, 2924, 2855, 1744, 1661, 1157, 1053.