Synthesis of a Fluorescent Analog of Alternariolide (AM-toxin I), A Host-specific Phytotoxin for Apple Leaves

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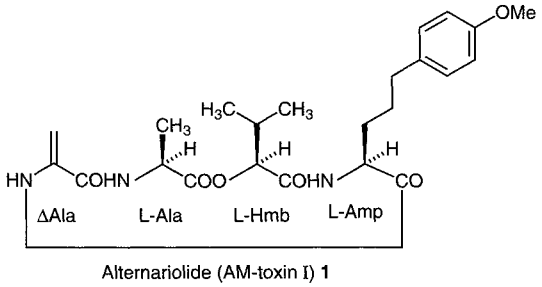
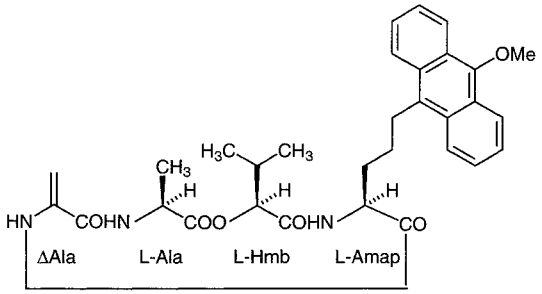
Received 1 July 1997

Abstract: A new fluorescent amino acid, L-2-amino-5-(10-methoxy-9-anthryl)pentanoic acid (L-Amap, **3**) was synthesized and incorporated into alternariolide instead of L-Amp (L-2-amino-5-(4-methoxyphenyl)-pentanoic acid).

Alternariolide (AM-toxin I, **1**)[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.[2] The host recognition process has been of great interest, that is, from which the specificity arises, resistance or susceptibility of the host plants. To reveal the exact process, we synthesized a fluorescent analog of alternariolide **2** which contains a new amino acid, L-2-amino-5-(10-methoxy-9-anthryl)pentanoic acid (L-Amap, **3**) as a fluorescent component, instead of L-Amp (L-2-amino-5-(4-methoxyphenyl)pentanoic acid).



Alternariolide (AM-toxin I) **1**



2

Anthraquinone (**4**) was monoalkylated using 3-butenylmagnesium bromide followed by reduction with $NaBH_4$ in MeOH to give the diol **5** (Fig. 1). The diol **5** was alkylated with MeI using NaH as a base in THF to give a mixture of dimethoxy **7** and monomethoxy compounds **6**

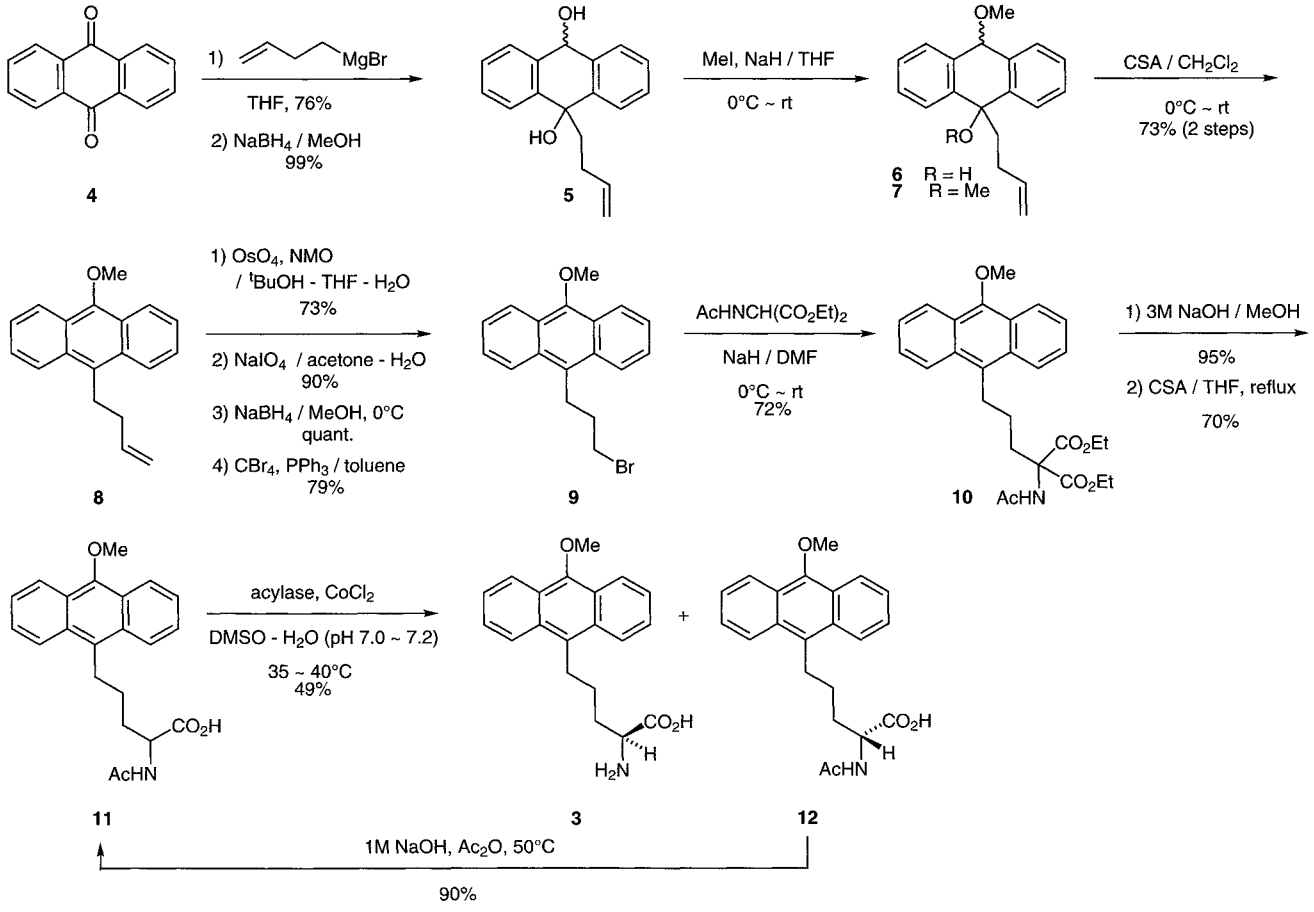


Figure 1

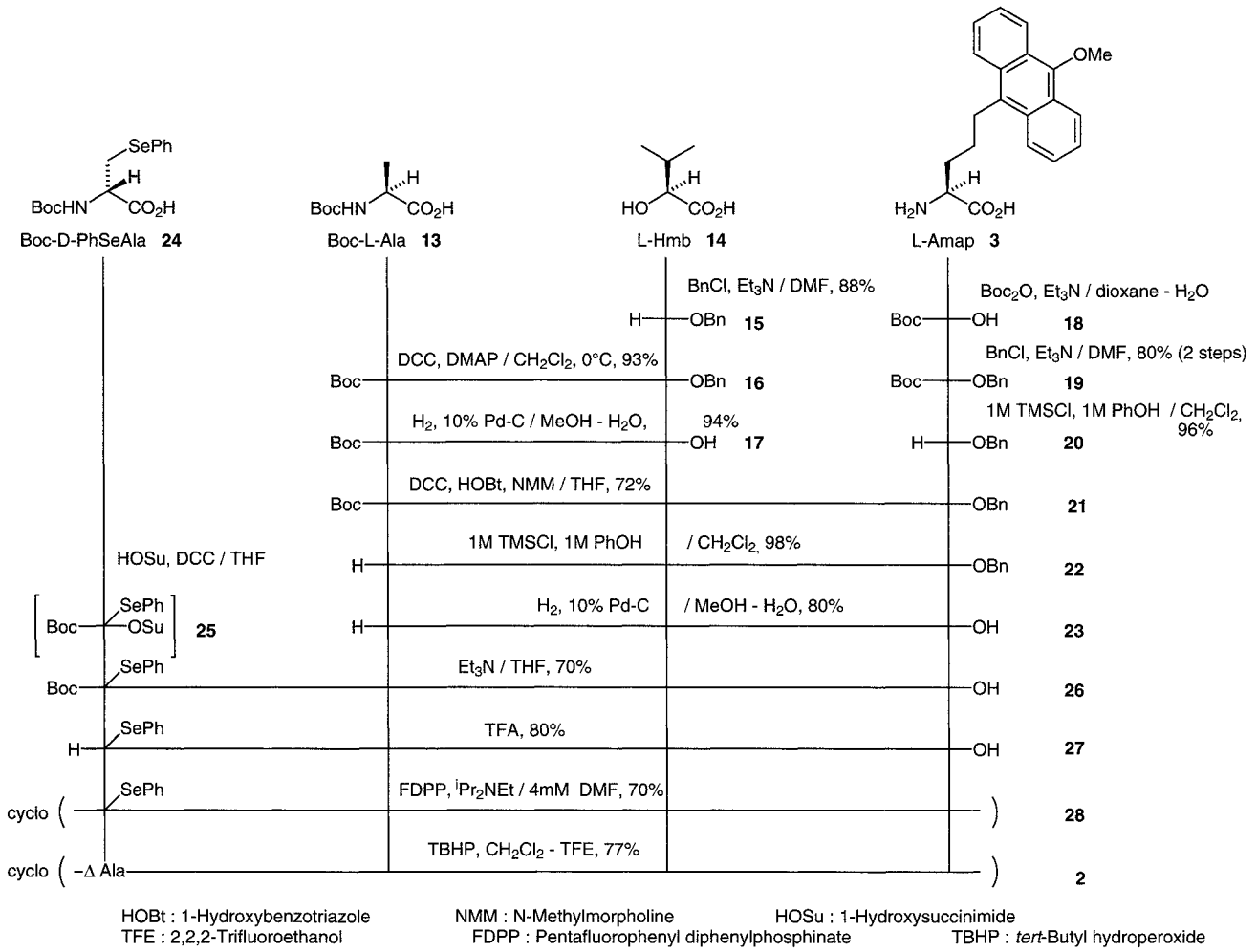


Figure 2

which was then exposed to acidic conditions to give aromatized compound **8**. Oxidative cleavage of the double bond of **8** was carried out through sequential two steps, diol formation using OsO_4 and then cleavage of the diol with $NaIO_4$. The anthracene ring with the methoxy group was highly reactive toward ozonolysis which gives a complex mixture. The resulting aldehyde was converted to the bromide **9** through reduction with $NaBH_4$ in MeOH followed by bromination using CBr_4 and PPh_3. To construct an α-amino acid functionality, diethyl acetamidomalonate was condensed with the bromide **9** to give **10**. The diester was hydrolyzed to the diacid under basic conditions and the resulting diacid was decarboxylated by exposure to CSA in refluxing THF to afford the acid **11**. Finally, the racemic acetamide acid **11** was subjected to hydrolysis using acylase (*Aspergillus genus*) under neutral conditions and the L-amino acid was selectively obtained. The residual D-acetamide acid **12** was racemized to the *dl*-form using Ac_2O and NaOH and the resulting racemate **11** was recycled.

The obtained amino acid **3** was protected as **20** for the peptide synthesis by the following sequence of reactions; (1) protection of the amine with Boc group, (2) esterification of the carboxylic acid with a benzyl group, and (3) removal of the BOC group using a mixture of 1M TMSCl and 1M PhOH in CH_2Cl_2[3] (Fig. 2).

Boc-L-Ala **13** and the benzyl ester **15** prepared from L-Hmb **14** with BnCl and Et_3N was condensed using DCC and DMAP to give **16**, whose benzyl ester was removed under hydrogenation conditions. The resulting carboxylic acid **17** was coupled with amine **20** using DCC, HOBt and NMM to afford the tridepsipeptide **21**, whose protective groups on both ends were sequentially removed by acidic treatment and then hydrogenolysis. The obtained **23** was condensed with activated ester **25** prepared in situ from **24**[4] and HOSu with DCC to give the tetradepsipeptide **26** in the presence of Et_3N. After removal of the Boc group of **26** with TFA, the resulting **27** was treated with FDPP[5] in the presence of $^iPr_2NEt$ in 4mM DMF to give the cyclized product **28** in 70% yield. The cyclic peptide **28** was treated with excess TBHP in a mixture of CH_2Cl_2 and TFE[6] to give the target peptide **2** in 77% yield. The synthesized alternariolide analog fluoresces a blue color (λmax: 407, 430, 503 nm) upon exposure to UV light. The fluorescent light should be detectable without interference from major native fluorophores, such as protein (λmax: 348 (Trp), 304 (Tyr), 282 (Phe))[7], vitamin B2 (λmax: 531)[8], and NADH (λmax: 340, 450).[9]

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