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Synthesis of the Host-Selective Phytotoxin Destruxin B. Avoiding Diketopiperazine Formation from an *N*-Methyl Amino Acid Dipeptide by Use of the Boc-Hydrazide Derivative.

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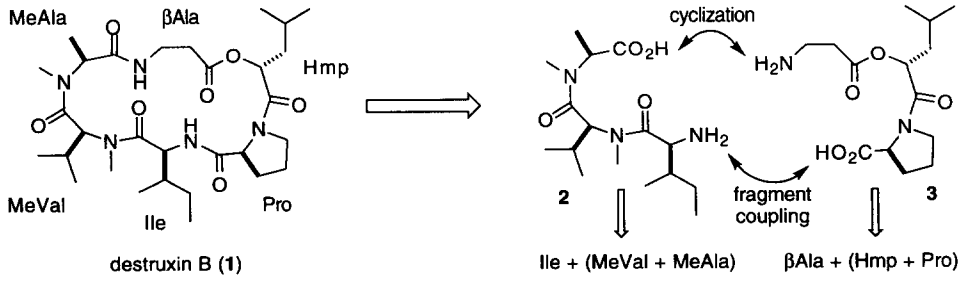
Abstract : An efficient synthesis of the cyclic hexdepsipeptide destruxin B from its component residues is described that involves a [3+3] fragment coupling followed by cyclization via the azide method. A novel feature of the synthesis is the use of the Boc-hydrazide protecting group for the C-terminal *N*-methylalanine residue. This group serves both to inhibit facile diketopiperazine formation from the *N*-methylvalyl-*N*-methylalanine dipeptide and as a latent activating group for hexadepsipeptide cyclization.

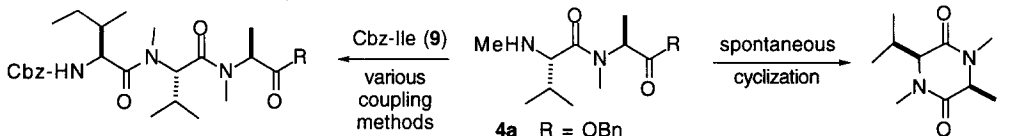
We are evaluating the role of host-selective phytotoxins[1] as part of an integrated research program aimed at understanding mechanisms of plant disease resistance.[2] Destruxin B (**1**) is a host-selective toxin produced both *in vitro*[3] and *in planta*[4] by the fungal pathogen *Alternaria brassicae* (Berk.) Sacc., the causative agent of alternaria blackspot of brassicas. From a global perspective this is the most destructive fungal disease of the economically important oilseeds rapeseed (*Brassica napus* and *B. rapa*) and canola (*B. napus* and *B. rapa*).[5] None of the commercially available rapeseed and canola crops show significant resistance to the pathogen. Destruxin B (**1**) causes chlorotic foliar lesions on brassicas at concentrations higher than 10^{-5} M.[6] Interestingly, the range of reactions of brassica pollen to destruxin B correlates with the plant resistance to the pathogen.[7] While some of the physiological effects of destruxin B have been studied,[6,8] the molecular basis of its selectivity is not understood. Towards this end we required ^{14}C-labeled **1** in order to trace its metabolic fate in the presence of resistant plant cells. Because the yields of **1** obtained from fungal cultures were not conducive to incorporations of radiolabelled precursors, its synthesis appeared as a more viable alternative. Herein we report a practical and efficient synthesis of destruxin B (**1**).[9]

Scheme 1



The synthesis of cyclic depsipeptides (and peptides) typically proceeds by coupling (linear or convergent) of intact hydroxy acid and amino acid fragments followed by cyclization.[10] Such an approach focuses the strategic decisions on the site of cyclization and the order of the residue coupling. Application of this strategy to destruxin B (**1**) leaves the introduction of the consecutive *N*-methylamino acid residues as a major concern because is has been established that these residues can be difficult to incorporate by standard peptide coupling methods.[11] The only reported synthesis of **1** involved formation of the βAla-Hmp bond by lactonization following linear coupling of the 6 residues.[12] Although this synthesis served to prove the structure of destruxin B, the low overall yield precludes any preparative application.[13] A successful synthesis of a destruxin analogue (cf. **1** with Hmp replaced by *D*-lactic acid) based on a [4+2] fragment coupling followed by cyclization of the hexadepsipeptide at the Pro-Ile site has been reported;[14] however, the efficiency of this approach was unsatisfactory for labelling purposes.[15]

Our retrosynthetic analysis of destruxin B is shown is Scheme 1. Lactamization is more facile than lactonization and, of the five amide bonds in **1**, we selected the MeAla-βAla site as the most favorable for cyclization on the basis of minimal steric hindrance.[10a] The Pro-Ile bond in the requisite linear hexadepsipeptide presents a logical disconnection because C-terminal proline residues are resistant to isomerization during fragment coupling.[10a] This synthetic design not only provides for maximum convergence but, by localizing the 2 difficult *N*-methyl amide bonds in a single tripeptide fragment **2**, also should maximize the overall yield in the longest linear sequence. A potential disadvantage of this approach concerns the synthesis of the fragment **2** which could be thwarted because *N*-methylamino acid containing dipeptides are prone to cyclize to diketopiperazines.[11] Indeed, we found that the dipeptide **4a** spontaneously cyclized to **5**[16] with a half-life of ca. 30 min,



protected derivative of **2**

16 R = NHNH-Boc

4a R = OBn
4b R = $NHNH_2$
4c R = NHNH-Boc

5

thereby preventing further peptide coupling.[17] In an effort to attenuate the propensity of **4** to cyclize, we considered alternatives to the benzyl ester protecting group. The use of a *tert*-butyl ester for this purpose has been reported.[18] Reasoning that a hydrazide protecting group should not only slow the rate of diketopiperazine formation but also could serve as a latent activating group for the eventual cyclization of the hexadepsipeptide (i.e. the azide method),[10] we chose to examine the stability of **4b**. We were pleased to note that the half-life for the conversion of **4b** to **5** in $CDCl_3$ solution was ca. 16 h. The Boc-protected hydrazide **4c** showed similar stability and we were able to successfully convert **4c** into the tripeptide **16** under a variety of conditions (*vide infra*).

Our synthesis of destruxin B is summarized in Scheme 2.[19] The Cbz protecting group was selected for the new *N*-terminal amino acid residues during preparation of tripeptide **16** because this group gives higher yields than the Boc group in acylations of *N*-methylamino acids.[20] The remaining protecting groups we chosen to provide the necessary orthogonality with the fewest deprotection steps. Various coupling methods were examined for Steps C, D, and E and the results are summarized in Table 1. The dipeptide **14** was produced in excellent yield from **10** and **12** by using PyBrop[21] or BOP-Cl[22] (Step C); expectedly,[11] yields were much lower with the more common carbodiimide procedures.[10a] The coupling of **9** with **4c** to give **16** (Step D) is particularly difficult because both amino acid residues are hindered, the amine is secondary, and **4** has a propensity to cyclize to the

diketopiperazine **5**. The use of PyBrop or BOP-Cl gave acceptable yields of **16**. Monitoring the reactions by TLC suggested that the cyclization of **4c** was faster under the coupling conditions than in $CDCl_3$ solution.[17] Although diketopiperazine formation is often base catalyzed,[11] further experimentation revealed[17] that **4c** was *more stable* in the presence of $^{i}Pr_2EtN$. Thus, the yields for BOP-Cl mediated coupling (the less expensive reagent) were greatly improved in the presence of excess DIEA, albeit with a concomitant increase in isomerization. Fragment coupling of **15** with **16** (Step E) proceeded in excellent yield, even using a "routine" protocol.[10a] Finally, the cyclization of **17** was also very efficient giving destruxin B (**1**) which was identical in all respects with an authentic sample.

In summary, the synthesis of destruxin B proceeds in 6 operations from readily available starting materials (i.e. **6-10** & **12**).[19] The average yield per operation is >88% and the overall yields for the longest linear sequences (4 steps) are 57% (from **8**) and 51% (from **12**). The synthesis is practical and amenable to the preparation of analogues, including radiolabelled congeners. The novel use of a Boc-hydrazide protecting group to inhibit diketopiperazine formation of an *N*-methylamino acid dipeptide is noteworthy.

Scheme 2

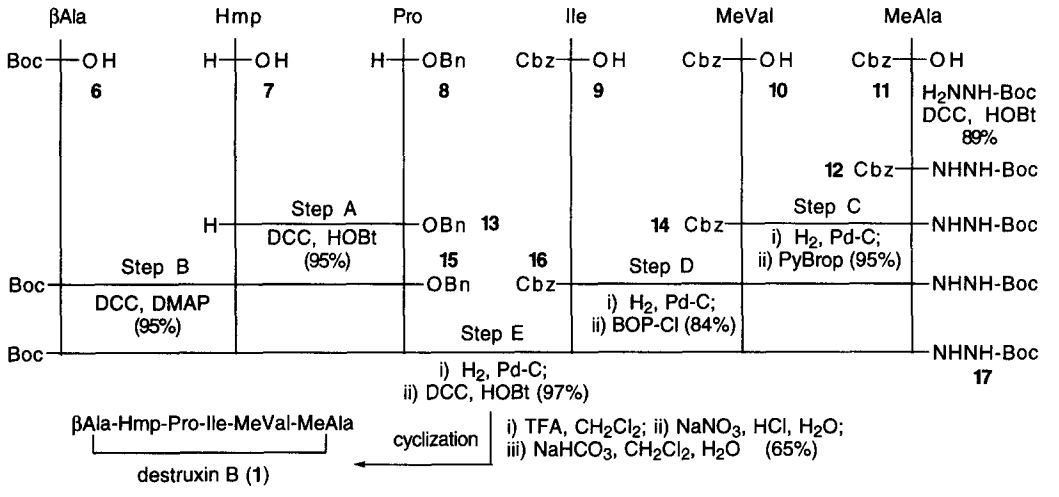


Table 1. Results of applying various coupling methods for steps C, D, and E (Scheme 2).

step	reagent	stoichiometry[a]	time (h)	% yield[b] (% isomerization)	step	reagent	stoichiometry[a]	time (h)	% yield[b] (% isomerization)
C	BOP-Cl	1.2:1.1:1:2	18	87 (<0.5)	D	PyBroP	1.2:1.1:1:2	14	60 (0.7)
	PyBroP	1.2:1.1:1:2	18	95 (<0.5)		BOP-Cl	1.2:1.1:1:2	14	60 (0.7)
	DCC/HOBt	1.1:1.1:1	48	55 (<0.5)		BOP-Cl	1.2:1.1:1:3	14	75 (1.5)
	EDC/HOBt	1.1:1.1:1	72	37 (<0.5)		BOP-Cl	1.2:1.1:1:4	14	84 (2.3)
	DCC/DMAP	1.1:1.1:0.1	24	72 (37)		HBTU	1.2:1.1:1	8	20 (16)
						DCC/HOBt	1.1:1.1:1	96	20 (<0.5)
E	DCC/HOBt	1.1:1.1:1	24	95		DCC/DMAP	1.1:1.1:1:0.1	36	21 (23)
	PyBroP	1.2:1.1:1:2	17	94					
	DPPA	1.2:1.1:1:2	24	97					

[a] reagent:carboxylic acid:amine:$^{i}Pr_2EtN$ (or DMAP), CH_2Cl_2, 0°C-rt; [b] isolated yield (% isomerization by HPLC)

Abbreviations: 3-aminopropanoic acid (βAla); *tert*-butoxycarbonyl (Boc); *N,N*-bis(2-oxo-3-oxazolidinyl)-phosphonic chloride (BOP-Cl); benzyloxycarbonyl (Cbz); dicyclohexylcarbodiimide (DCC); 4-(dimethylamino)pyridine (DMAP); diphenylphosphoryl azide (DPPA); 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC); 2-(benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU); (*R*)-2-hydroxy-4-methylpentanoic acid (Hmp); 1-hydroxybenzotriazole (HOBt); isoleucine (Ile); *N*-methylvaline (MeVal); *N*-methylalanine (MeAla); proline (Pro); bromotri(pyrrolidino)phosphonium hexafluorophosphate (PyBroP).

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