THE DETOXIN COMPLEX. – A NATURALLY OCCURRING SAFENER

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Abstract – *The detoxin complex is a group of depsipeptide metabolites produced by the organism Streptomyces caespitosus var. detoxicus 7072 GC$_1$ as well as other strains of streptomyces. The complex is the first isolated natural product which displays a unique detoxification effect in plant and animal cells as its biological activity. The structural investigations carried out to elucidate the structures of the congeners and the stereochemistry of the stereogenic centers are described. Structure-activity relationships are discussed. The different synthetic approaches to the parent amino acid, (-)detoxinine, are compared. The syntheses of detoxins (-)-B$_1$, (-)-B$_3$, (-)-D$_1$, and (+)-valyldetoxinine are described.*

1. INTRODUCTION

The synthesis of unusual highly functionalized amino acids, which are found as components of biologically active peptides, is a topic of current interest.[1] (-)-Detoxinine, the parent component of the detoxin complex, is one of these amino acids, and its synthesis has received considerable attention from several research groups. The production, isolation and the biological activities of some members of the detoxin complex were first reported by Yonehara *et al.*[2] The organism which produces the detoxins was isolated from soils and classified as a species of *Streptomyces caespitosus* var. *detoxicus* 7072 GC_1. The complex shows potent antagonistic activity to the cytotoxicity of the antibiotic blasticidin S, a fungicide used in the treatment of rice blast disease.[3,4] The detoxin complex is the first isolated natural product displaying detoxification as its biological activity. This group of depsipeptides is of great interest because of this unique detoxifying effect in both plant and animal cells.

1.1 Isolation of the Detoxin Complex

In 1968, Yonehara *et al.* obtained the detoxin complex from a cultured seed of *Streptomyces caespitosus* var. *detoxicus* 7072 GC_1.[2] The production of the detoxin complex was carried out in a jar fermentor at 27 °C.[2,5-7] The time course studies on the fermentation of the detoxin complex indicated that its production paralleled the cell growth (mycelium) and reached a maximum at 48 h after inoculation.[5]

Purification of the individual components of the detoxin complex was achieved by a series of chromatographic and extractive procedures as shown in **Scheme 1**. The fermentation broth was filtered, and the filtrate was

subjected to ion exchange chromatography. The active fractions were detected by spraying with 10% aqueous sulfuric acid or with a ninhydrin solution and also located simultaneously by bioautogram. After concentration of the active fractions, the resulting oil was placed on an activated carbon column and eluted with 60% aqueous methanol containing 3% acetic acid. Removal of the solvent gave a solid which was triturated twice with methanol at 50 °C. Spray drying of the methanol solution under reduced pressure afforded the detoxin complex (5 g) as a light yellow powder.[2]

The detoxin complex was found to contain components with different specific activities. Their isolation was carried out by column chromatography. The collected fractions were assayed for their antagonism to the inhibitory action of blasticidin S against *Bacillus cereus*. This test led to the characterization of eight principal components which were termed A-H, based on their elution order. Detoxins C and D were found to be the most active. Paper and thin-layer chromatography revealed that most of these fractions were made up of several constituents.

Each individual group from A to E was further purified by gel filtration and partition chromatography. Groups F to H were not further purified due to the scarcity of the samples.[7] The purification of the detoxin C group will serve as an example to illustrate the separation of groups A, B, and E into their individual components. The detoxin C group was first subjected to gel filtration chromatography. The active fractions were concentrated and lyophilized to give a yellow powder. This material was then further purified by silica gel partition column chromatography to give detoxins C_1, C_2, and C_3 in pure form. Using the purification sequence outlined in **Scheme 1**, two tons of culture filtrate afforded detoxin A_1 (**1**, 20 mg), B_1 (**4**, 30 mg), C_1 (**6**, 60 mg), C_2 (**7**, 3 mg), C_3 (**8**, 80 mg), and E_1 (**14**, 60 mg).[8]

The most active component of the detoxin complex, detoxin D_1 (**9**), was obtained using a procedure similar to that described above.[6] The pure detoxin D_1 was obtained by additional gel filtration chromatography. Subsequent GC/ms spectral studies of the derivatives of the members of this group revealed that there were five congeners, which were labeled D_1, D_2, D_3, D_4, and D_5 (**9-13**) respectively.[9]

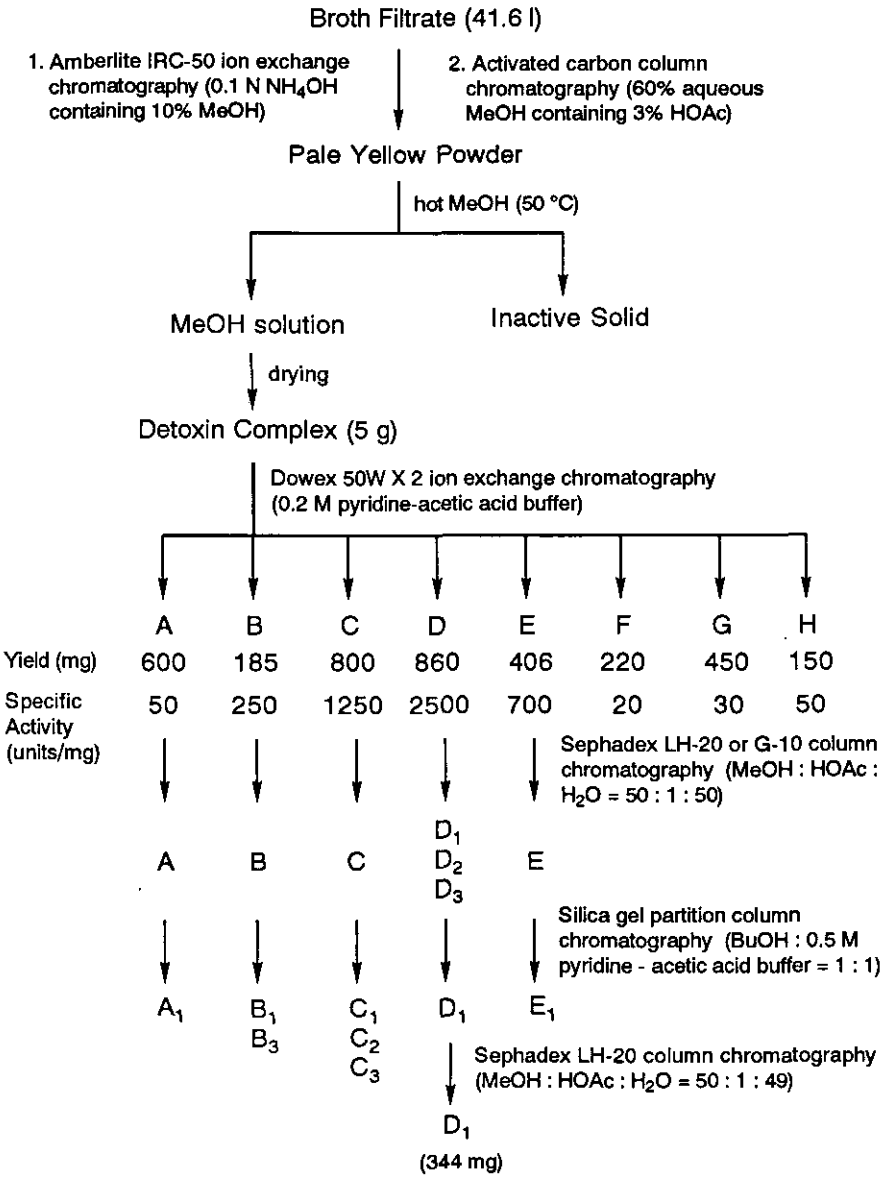
Full characterization of the detoxin complex constituents was carried out.[10-15] All detoxins contained the parent amino acid detoxinine (**2a**) (except detoxins B_1, **4** and B_3, **5**), L-valine (except detoxin E_1, **14**), and *N*-acylated phenylalanine (except detoxin A_1, **1**) as shown in **Figure 1**. The detailed structural determination of the detoxins will be discussed later.

1.2 Chemical and Physical Properties of the Detoxin Complex

The members of the detoxin complex were isolated as amphoteric powders which gave positive ninhydrin tests and had similar isoelectric points. These data indicated the presence of a free amino function and a carboxylic acid group in every member.

Detoxin C_1 (**6**) was recrystallized from water to afford micro needles of mp 142-144 °C, $[\alpha]_D^{25}$ -23° (*c* 1,

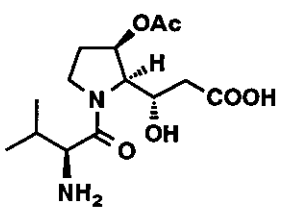
Scheme 1[5-7]



MeOH).[16] Potentiometric titration of detoxin C_1 revealed its amphoteric nature (pKa 8.0 and 3.9 with titration equivalent 590).[6] This data was consistent with the molecular formula $C_{29}H_{44}N_4O_9$ derived from the elemental analysis.

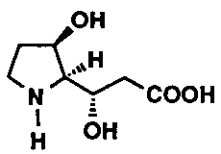
Detoxin D_1 (**9**), $C_{28}H_{41}N_3O_8$, mp 156-158 °C, $[\alpha]_D^{25}$ -16° (*c* 1, MeOH), was an amphoteric compound (pKa 4.0 and 8.0 with titration equivalent 570).[6] The infrared and ^1H nmr (100 MHz) spectral data of this

compound suggested the presence of one acetyl, one phenyl, four methyl, an amide, a carboxylic acid and an ester functionalities. Reported data on the physical properties of the other members of the complex are scarce.



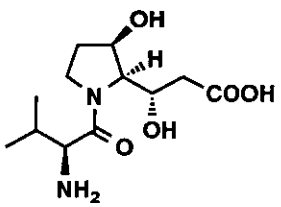
detoxin A_1

1



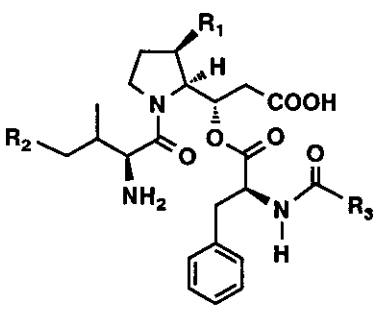
detoxinine

2a



valyldetoxinine

3

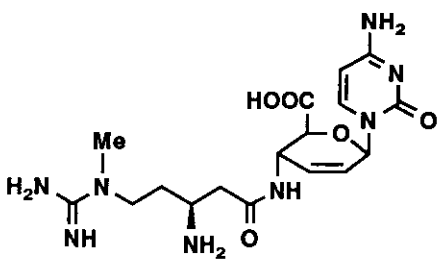


Compound Number	Detoxin	R_1	R_2	R_3
4	B_1	H	H	CH_3
5	B_3	H	H	$CH(CH_3)_2$
6	C_1	OAc	H	CH_3
7	C_2	OAc	H	CH_2CH_3
8	C_3	OAc	H	$CH(CH_3)_2$
9	D_1	OAc	H	$CH(CH_3)CH_2CH_3$
10	D_2	$OC(O)CH_2CH_3$	H	$CH(CH_3)CH_2CH_3$
11	D_3	$OC(O)CH(CH_3)_2$	H	$CH(CH_3)CH_2CH_3$
12	D_4	$OC(O)CH_2CH_2CH_3$	H	$CH(CH_3)CH_2CH_3$
13	D_5	$OC(O)CH_2CH(CH_3)_2$	H	$CH(CH_3)CH_2CH_3$
14	E_1	$OC(O)CH_3$	CH_3	$CH(CH_3)CH_2CH_3$

Figure 1. Structures of the members of the detoxin complex and their derivatives.

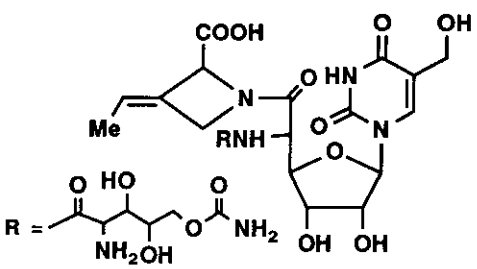
2. BIOLOGICAL ACTIVITIES OF THE DETOXIN COMPLEX

Blasticidin S (**15**) is a nucleoside antibiotic produced by *Streptomyces griseochromogenes*, which has been used extensively as a fungicide against the virulent fungus *Piriculuria oryzae*, the cause of rice blast disease in Japan.[3,4] However, the curative effect of blasticidin S (**15**) usually required dosages which caused



blasticidin S

15



polyoxin A

16

phytotoxicity in rice plants. During investigations of the biological transformation of blasticidin S, the detoxin complex was found to exhibit a remarkable detoxification effect for this antibiotic (**15**) in both animal and plant cells. The detoxin complex drastically reduced the phytotoxicity caused by blasticidin S without reducing its effectiveness. The detoxin complex is the first selective antagonist among natural products to exhibit such a unique detoxification activity.

Investigations were also carried out to determine whether the antagonistic action of the detoxin complex extended to other antibiotics. Unfortunately, the only other nucleoside antibiotic to be susceptible to the detoxin complex was polyoxin A (**16**). However, the detoxification of this antibiotic was not selective in *B. cereus* IAM 1729 and *P. oryzae* NIAS. Therefore, the antagonistic action of the detoxin complex is limited to compounds structurally related to blasticidin S.

2.1 Effect of the detoxin complex on blasticidin S (15)

The detoxin complex has been found to negate completely the antimicrobial action of blasticidin S (**15**) in the case of *B. cereus* IAM 1729, *B. circulans* IAM-1112, *B. subtilis* PCI 219 *C. albicans* NIHJ 4905, and *M. ramonnianus* IAM 6128, but not in *E. coli* NIHJ, *P. fluorescence* IAM 1201, *M. phlei* IID Timothee, and *P. oryzae* NIAS.[2,5] As seen in **Table 1**, the selective antagonistic activity of the detoxin complex on the antibiotic is manifested on gram positive microorganisms but not on gram negative organisms. This selectivity suggested that its mechanism of action must involve cell membrane participation. Furthermore, assays of the detoxin complex against various kinds of microorganisms revealed that the complex alone showed neither antimicrobial activity nor toxicity. These results led to the testing of its biological activity in vivo when used with

Table 1.[5] Antagonistic Activity of the Detoxin Complex on Blasticidin S Against Various Microorganisms

Test Organism	Inhibition Zone (mm)		
	BS (1000 μg/ml)	DC (250 units/ml)	BS + DC (1000 μg/ml + 250 units/ml)
Bacillus cereus	28.0	0	0
Bacillus circulans	27.0	0	0
Bacillus subtilis	17.0	0	0
Candida albicans	20.0	0	0
Escherichia coli	16.0	0	16.0
Mucor ramonnianus	29.0	0	0
Mycobacterium phlei	24.5	0	24.5
Piricularia oryzae	27.0	0	30.0
Pseudomonas fluorescence	20.5	0	20.5

BS = blasticidin S; DC = detoxin complex.

blasticidin S in the treatment of rice blast disease in a greenhouse. Remarkably, the complex completely suppressed the phytotoxicity caused by blasticidin S without decreasing the curative effect of the antibiotic. In addition, animal studies showed that this safener decreased eye irritation caused by the antibiotic and exhibited a remarkable effect in depressing conjunctivitis in rats.[5]

The complex also exerted a detoxicating effect for the antibiotic in several commercially important crops and this detoxification was almost non-selective toward the type of plant (**Table 2**).[5] In practice, the phytotoxicity of the antibiotic can be completely inhibited by addition of a few μg/ml of the detoxin complex to the blasticidin S preparation.

Table 2.[5] Detoxification Effects of the Detoxin Complex Against Blasticidin S in Plants

		Test plants				
BS (μg/ml)	DC (unit/ml)	Rice	Tobacco	Tomato	Cucumber	Kidney Bean
60	40	0*	0	0	0	0
60	20	0	1	0	0	0
60	10	0	2	0	0	1
60	5	1	2	1	0	1
60	2.5	1	3	1	1	2
60	0	2	3	2	1	3

*0 = no toxicity, 1 = slightly toxic, 2 = toxicity, 3 = extremely toxic
BS = blasticidin S; DC = detoxin complex.

The mechanism of action of detoxin D in preventing the uptake of blasticidin S in *Bacillus cereus* was investigated.[17] Blasticidin S was shown to inhibit protein synthesis by binding to 50S ribosomal subunits and blocking peptidyltransferase activity in a cell-free system from *E. coli* as well as in intact cells.[18-20] The inhibition of protein synthesis by blasticidin S in *Bacillus cereus* was counteracted by the addition of detoxin D only in intact cells or protoplasts, but not in cell-free systems. Therefore, it was suggested that the detoxin D group affects the transportation of blasticidin S across the cell membrane. In the absence of detoxin D, blasticidin S accumulated in the cells, while in its presence transport into the cells was inhibited. Examination of this effect on ^{14}C-blasticidin S in *Bacillus cereus* revealed that detoxin D interfered only with active transport and not with carrier-mediated passive transport (namely facilitated diffusion).

2.2 Structure-Activity Relationships[7,16]

The congeners of the detoxin group showed different levels of inhibition. Original bioassays of the groups (A-H) isolated from the detoxin complex by ion exchange chromatography revealed that the detoxin D group was the most active, followed by the C group (**Scheme 1**). With *Bacillus cereus* as the test organism, the

minimum effective concentrations of the detoxins and their derivatives are shown in **Table 3.**[7] Detoxins D_1 (**9**) and E_1 (**14**) were identified as the most active compounds, while detoxin A_1 (**1**) and derivatives of detoxin D_1 (**17,18**) were found to be the least active. The following conclusions were reached, based on studies of structure vs. activity. Detoxins D_1 (**9**) and E_1 (**14**) differed only in the N-terminal amino acid (valine vs. isoleucine, respectively). Therefore, the type of N-terminal amino acid was not considered important for the biological activity. Replacement of the 2-methylbutyric acid in detoxin D_1 (**9**) with propionic acid (C_2), 1-butyric acid (C_3), or acetic acid (C_1) resulted in a two to fourfold decrease in activity. The detoxins B_1 (**4**) and B_3 (**5**) were 650 times less active than detoxins E_1 (**14**) and D_1 (**9**). Since the most notable structural difference was the absence of the acetoxy group in B_1 (**4**) and B_3 (**5**), the acetoxy substituent on the proline ring of detoxinine was assumed to play a significant role in the biological activities of detoxins. The phenylalanyl moiety was important for biological potency as demonstrated by the extremely low activity of detoxin A_1 (**1**) and valyldetoxinine (**3**). Finally, investigations showed that blocking the N-terminal amino acid [as in acetyl-detoxin D_1 (**18**)] or the carboxylic acid [as in detoxin D_1-methyl ester (**17**)] eliminated the activity.

Table 3.[7] Comparison of the Activities of the Detoxins and Their Derivatives Against *Bacillus Cereus*

Congener	Minimum Effective Concentration (μg/ml)
Detoxin D_1 (**9**)	0.025
Detoxin E_1 (**14**)	0.025
Detoxin C_2 (**7**)	0.05
Detoxin C_3 (**8**)	0.05
Detoxin C_1 (**6**)	0.1
Detoxin B_1 (**4**)	1.6
Detoxin B_3 (**5**)	1.6
Detoxin A_1 (**1**)	12.5
Valyldetoxinine (**3**)	12.5
Detoxin D_1-methyl ester (**17**)	250
Acetyl-detoxin D_1 (**18**)	>500

3. STRUCTURAL INVESTIGATIONS OF THE DETOXIN COMPLEX

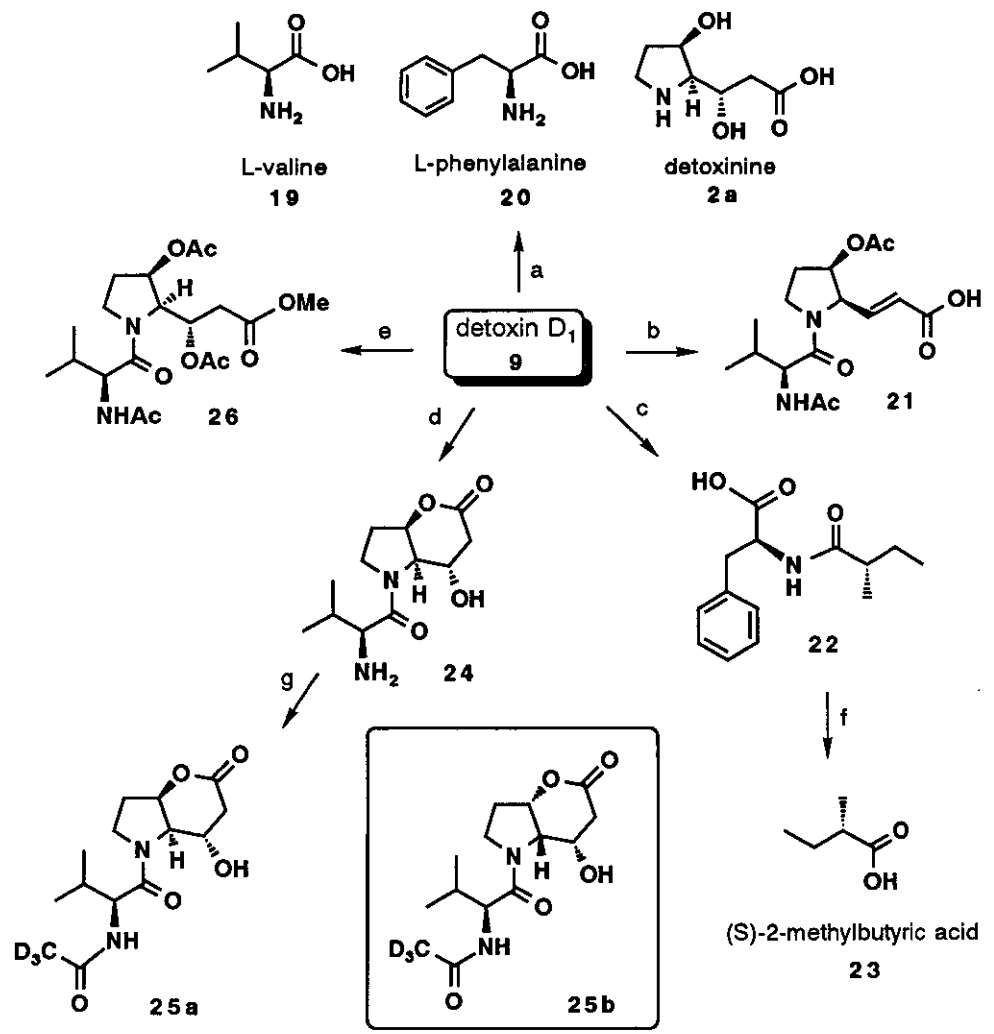
The structure and stereochemistry of the detoxin complex were originally determined from both chemical degradation and spectroscopic evaluation of the individual congeners. The absolute configuration of detoxinine was then revised by subsequent synthetic studies. We shall first summarize the various methods of chemical degradation and spectroscopy employed in the identification of the structural components of these molecules

and then review the use of synthesis in the revision of their stereochemistry.

3.1 Chemical Degradation[7,10,11,15]

Detoxin D_1 (**9**) was the major component of the detoxin complex and the first member of the complex to have its structure elucidated. The chemical degradation of detoxin D_1 is shown on **Scheme 2**. Complete acid hydrolysis afforded one equivalent of L-valine (**19**), L-phenylalanine (**20**) and a new amino acid designated as detoxinine (**2a**). Mild basic hydrolysis of detoxin D_1, followed by extraction with ether under acidic

Scheme 2



[a] 5.7 N HCl, 110 °C, 16 h; [b] Ac_2O, 100 °C, 2 h; [c] 0.1 N NaOH, 37 °C, 1 week, then pH 3.0; [d] 1. 1 N NaOH, 70 °C; 2. ion exchange; [e] 1. 0.1 N NaOH; 2. pH 7.0; 3. ion exchange; 4. Ac_2O, Pyr.; 5. CH_2N_2; [f] 6 N HCl; [g] CD_3COCl, Pyr.

conditions, gave (+)-S-2-methylbutyryl-L-phenylalanine (**22**). Further acid hydrolysis of acid (**22**) afforded (+)-S-2-methylbutyric acid (**23**) and L-phenylalanine (**20**). Alkaline hydrolysis of detoxin D_1 followed by ion exchange chromatography gave valyldetoxinine (**3**), which was converted to the corresponding triacetate methyl ester (**26**) and characterized by ^1H nmr, ir, and ms analyses. The binding position of the phenylalanine moiety was confirmed by treatment of detoxin D_1 with acetic anhydride. This reaction gave the α,β-unsaturated carboxylic acid acetate **21** of valyldetoxinine (**3**) by a selective β-elimination.

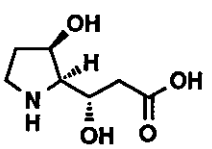
When detoxin D_1 was hydrolyzed under basic conditions, followed by ion exchange chromatography, a monobasic compound (L-valyldetoxininolactone, **24**) was obtained as an amorphous powder.[11] This compound was then treated with deuteroacetyl chloride to give the *N*-deuteroacetyl derivative **25a** selectively. Initial ^1H nmr decoupling experiments and optical rotatory dispersion (ord) studies based on Klyne's lactone sector rule led to the assignment of structure (**25b**) for the monodeuteroacetate, but subsequent synthetic studies led to the revised correct structure (**25a**). From the evidence cited above and subsequent synthetic investigations, the structure of detoxin D_1 as shown in **Figure 1** was proposed. Analysis of the ^{13}C nmr spectra of detoxin D_1 and its structural components verified this structure.[14]

Basic hydrolysis of the detoxin D group afforded acetic acid (D_1), propionic acid (D_2), isobutyric acid (D_3), 1-butyric acid (D_4), and 2-methylbutyric acid (D_5). These fatty acids in the hydrolysate of the detoxin were identified by gas liquid chromatographic analysis. The structure of the minor congeners of the detoxin D group (D_2 - D_5) were then elucidated by gas chromatographic and mass spectral (GC/ms) analyses of their *N*-trifluoroacetyl methyl ester derivatives.[9]

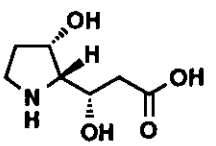
Since the determination of the structure of detoxin D_1, the other minor constituents of the detoxin complex were also identified by using similar procedures, as well as nmr, elemental analyses, and mass spectrometry data (**Figure 1**).[7,8]

3.2 Revised Stereochemistry of Detoxinine[12,13]

As mentioned in the last section (**Scheme 2**), the stereochemistry of detoxinine was initially assigned as in **2b** by ^1H nmr and ord studies on L-valyldetoxininolactone derivatives isolated from the hydrolysis of detoxin D_1 (**Figure 2**).



2a

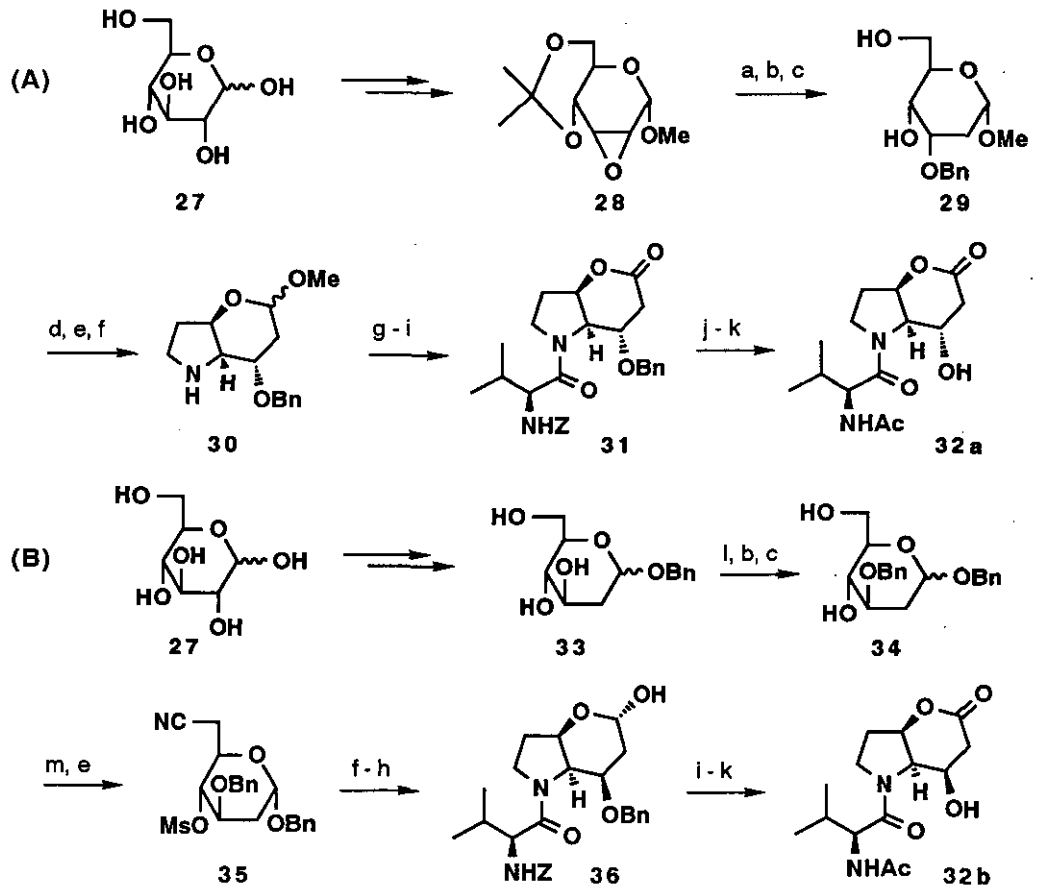


2b

Figure 2. Revised stereochemistry of detoxinine (2a) and its previously assigned configuration (2b).

Subsequently, the absolute configuration of detoxinine was questioned when the biosynthesis of a microbial amino acid, (3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid (AHMHA), was reported in 1974. ^{14}C Radiolabelling experiments showed that AHMHA was produced from L-leucine and malonic acid during the biosynthesis of pepstatin. These results suggested that pepstatin biosynthesis proceeded on a multienzyme system similar to fatty acid biosynthesis.[21] Therefore, the absolute configuration of detoxinine would be reversed (**2a**) if detoxinine were biosynthesized from L-proline as AHMHA from L-leucine (**Figure 2**). To verify the assigned stereochemistry, Kakinuma *et al.* synthesized both diastereomers of the N-acetyl derivative of L-valyldetoxininolactone (**32a** and **32b**), epimeric at the hydroxyl center, from D-glucose (**Scheme 3**).[12,13] Since the syntheses of these two L-valyldetoxininolactone derivatives involve similar transformations,

Scheme 3[12,13]



[a] *LAH;* [b] *BnCl, KOH, DMSO;* [c] *AcOH (aq.);* [d] *TsCl, Pyr.;* [e] *NaCN, DMSO;* [f] 1. $NaBH_4$, $CoCl_2$, *MeOH;* 2. KOH, MeOH; [g] Z-Val-OH, DCC; [h] HCl(aq.); [i] PCC, CH_2Cl_2; [j] H_2, Pd/C; [k] Ac_2O, MeOH; [l] 2,2-dimethoxypropane, TsOH, DMF; [m] Pyr., MsCl.

only the synthetic route to compound (**32a**) will be discussed. The key step of this synthesis was the formation of the pyrrolidine compound (**30**), which was accomplished under reductive conditions employing sodium borohydride in the presence of cobalt chloride, followed by alkaline treatment. The pyrrolidine (**30**) was then coupled to Z-valine using dicyclohexylcarbodiimide (DCC). Acid hydrolysis, followed by pyridinium chlorochromate oxidation, gave the lactone (**31**). After removal of the benzyl and benzyloxycarbonyl groups, the resulting amino group was acetylated to give compound (**32a**). The corresponding epimeric hydroxylactone (**32b**) was prepared in a similar manner.

These two epimeric compounds (**32a** and **32b**) were then compared to *N*-deuteroacetyl-L-valyldetoxininolactone (**25a**) obtained from the degradation of detoxin D_1. Comparison of the ^1H nmr of compound (**32a**) with compound (**25a**) showed that they were identical (except for the *N*-acetyl singlet at 2.03 ppm), while the ^1H nmr of compound (**32b**) was quite different. Compound (**32a**) also showed a negative ord maximum at 243 nm as did compound (**25a**) obtained from the degradation of detoxin D_1 (**9**). Based on these data, the absolute stereochemistry of detoxinine was revised to (2S,3R,1'S)-2-(2'-carboxy-1'-hydroxyethyl)-3-hydroxypyrrolidine (**2a**) as shown in **Figure 2**.

4. SYNTHETIC STUDIES OF DETOXINS AND DETOXININE

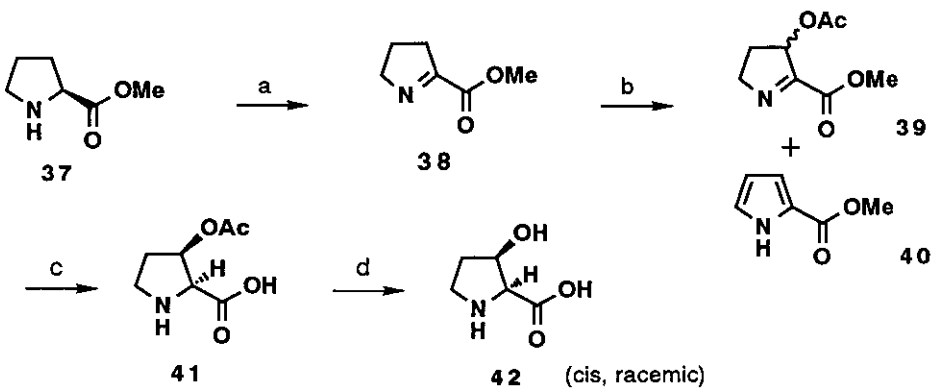
Since the initial synthetic investigations concerning the determinations of the absolute stereochemistry of detoxinine in 1980, there have been a number of syntheses of detoxins reported in the literature. In this section, we shall review the synthetic work of previous investigators in a chronological order, for a better understanding of the development of methodology designed for the components of the detoxin complex. We shall first focus on the investigations dealing with detoxinine, the parent amino acid of most detoxins.

4.1 Syntheses of Detoxinine

4.1.1 Synthetic Strategy of Häusler's Group[22,23]

The first synthesis of detoxinine in racemic form was reported by Häusler.[23] The synthesis (**Scheme 4**) began with L-proline methyl ester (**37**), which was converted to the corresponding *N*-chloride with *tert*-butyl hypochlorite. Dehydrohalogenation with triethylamine gave pyrroline (**38**) in 82-85% yield. Lead tetraacetate oxidation resulted in an inseparable mixture of the 3-acetoxypyrroline (**39**), which was used in the next step without further purification and a side product, 2-methoxycarbonylpyrrole (**40**), in a 9 : 1 ratio. Reduction of the imine double bond under basic conditions using sodium borohydride afforded *cis*-3-acetoxyproline (**41**) in 26% from pyrroline (**38**). After removal of the acetoxy group and ion exchange chromatography, *cis*-3-hydroxyproline (**42**) was obtained in 94% yield.

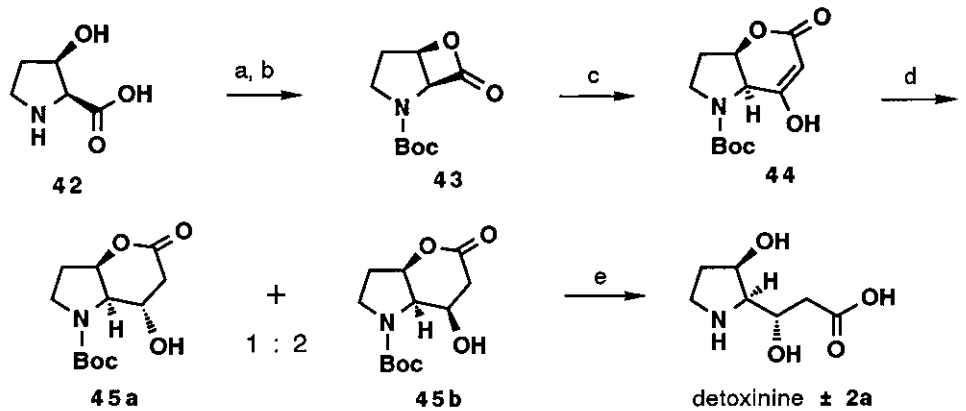
Scheme 4[22,24]



[a] 1. *tert*-butyl hypochlorite, Et_2O; 2. Et_3N, 82 -85%; [b] $Pb(OAc)_4$, benzene, 70%; [c] 0.5 N NaOH, $NaBH_4$, then pH 6.0, 37%; [d] 1. 2 N HCl; 2. ion exchange chromatography, 94%.

Scheme 5 illustrates Häusler's strategy for the synthesis of (±)-detoxinine.[23] The amino group of acid (**42**) was protected as the *tert*-butoxycarbonyl (Boc) derivative, and then treated with 4-bromobenzenesulfonyl chloride to give the four-membered lactone (**43**) in 76% yield. The β-lactone (**43**) was alkylated with Meldrum's acid in the presence of 4-dimethylaminopyridine (DMAP), followed by refluxing in dioxane to afford the bicyclic pyrone (**44**) in 44% yield. Reduction of the pyrone was first attempted using catalytic hydrogenation (platinum or Raney nickel) but only polar side products were obtained. The pyrone system was found to be stable to sodium, zinc and tetrabutylammonium tetrahydroborate. Reduction was finally accomplished using a borane-ammonia complex to give a 1 : 2 mixture of epimeric alcohols (**45a**) and (**45b**) in

Scheme 5[23]



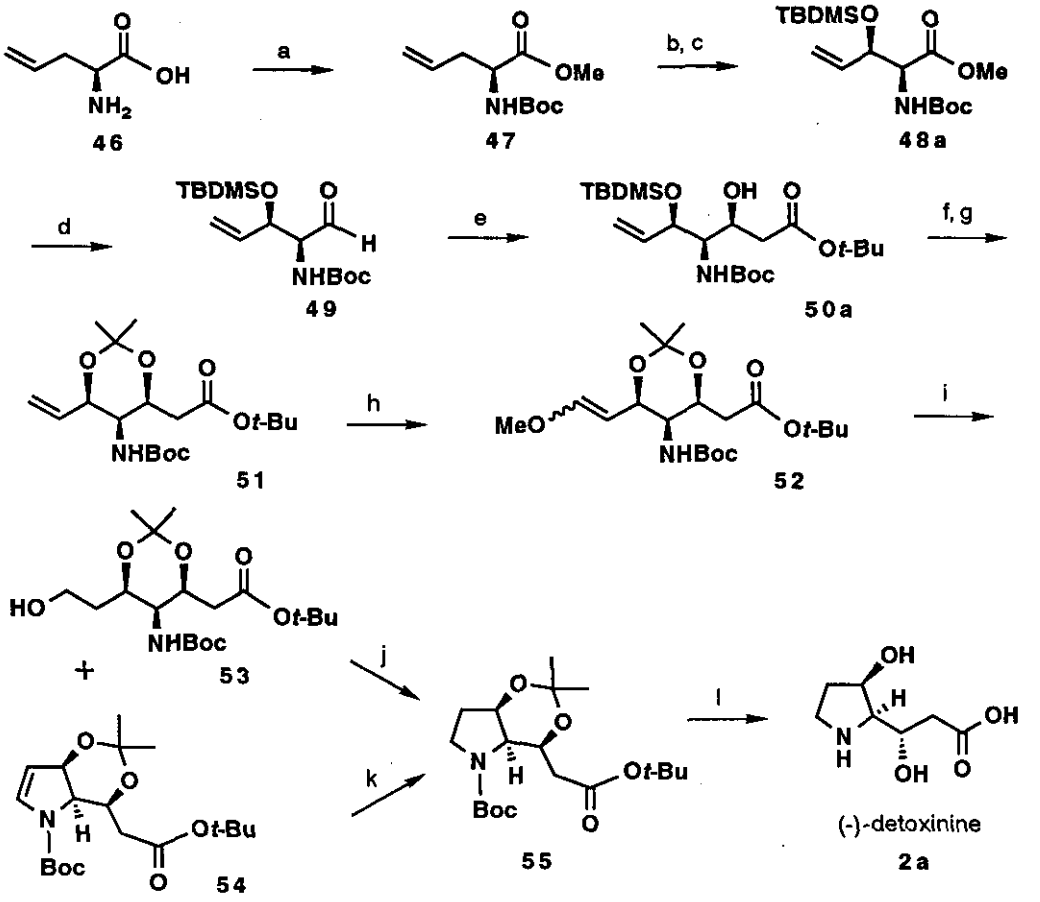
[a] Boc_2O, 97%; [b] bromobenzenesulfonyl chloride, Pyr., 76 - 84%; [c] 1. Meldrum's acid, DMAP; 2. H_3O^+; 3. dioxane, reflux, 44%; [d] $BH_3{\cdot}NH_3$, 90% MeOH (aq.), 80%; [e] 1. TFA; 2. NH_3, H_2O, 85%.

80% yield. The minor component (**45a**) possessed the correct relative configuration at the hydroxyl center. After removal of the Boc protecting group with trifluoroacetic acid (TFA), the resulting trifluoroacetate salt was placed onto an acidic ion exchange column and eluted with aqueous ammonium hydroxide. This treatment deprotected the amine and opened the lactone ring to produce (±) detoxinine (**2a**) in 85% yield.

4.1.2 Synthetic Strategy of Ohfune's Group[25]

The first stereocontrolled synthesis of optically active detoxinine (**2a**) was achieved by Ohfune and Nishio in 1984 as shown in **Scheme 6**.[25] The synthesis began with the protection of the primary amine and carboxylic

Scheme 6[25]



[a] 1.BocON, Et_3N; 2. CH_2N_2, 86%; [b] SeO_2, *t*-BuOOH, 55%; [c] TBDMSCl, imidazole, 70%; [d] 1. LAH, Et_2O; 2. PDC, CH_2Cl_2, 64%; [e] LDA, *t*-BuOAc, 96%; [f] *p*-TsOH, MeOH, 100%; [g] *p*-TsOH, 2,2-dimethoxypropane, 76%; [h] 1. O_3, Me_2S; 2. $Ph_3P(Cl)CH_2OMe$, sodium amylate, 81%; [i] 1. $Hg(OAc)_2$, then KI (aq.); 2. $NaBH_4$, EtOH, 73%; [j] 1. Ph_3P, NBS; 2. NaH, THF, 63%; [k] Pd/C, H_2, 90%; [l] 1. camphorsulfonic acid (CSA), MeOH; 2. CSA, CH_2Cl_2; 3.TFA; 4. ion exchange, 81%.

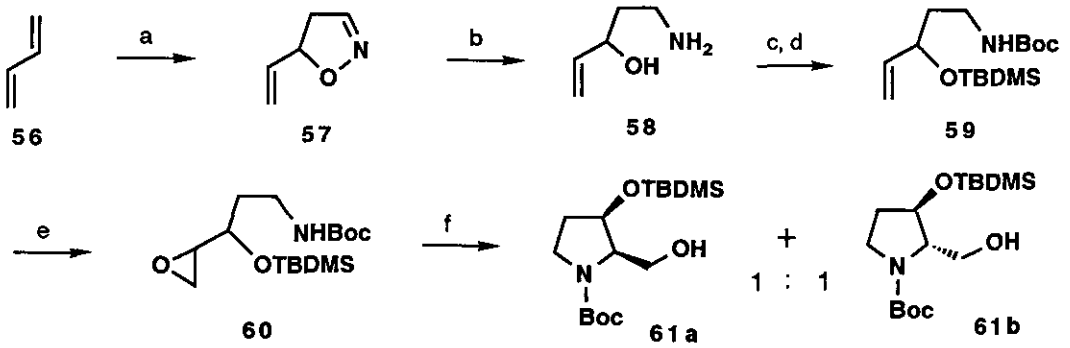
acid of allylglycine (**46**) to yield Boc-allylglycine methyl ester (**47**). Allylic oxidation of **47** with selenium dioxide gave a mixture of epimeric alcohols (3.8 : 1) which were separated after conversion to their corresponding *tert*-butyldimethylsilyl ethers. Subsequent reduction-oxidation of methyl ester (**48a**) led to the aldehyde (**49**) in 64% yield. The aldol condensation of **49** and lithium *tert*-butyl acetate gave a 6.5 : 1 ratio of β-hydroxy esters in favor of the desired *syn* diastereomer (**50a**). The absolute configuration of the major isomer (**50a**) was verified by decoupling and NOE experiments on the derived acetonide (**51**). Ozonolysis of the double bond of compound (**51**) to form the aldehyde was followed by a Wittig reaction [$Ph_3P(Cl)CH_2OMe$ and *t*-AmONa] to give the methylvinyl ether (**52**). Treatment of compound (**52**) with mercuric acetate followed by subsequent addition of aqueous potassium iodide and reduction with sodium borohydride afforded alcohol (**53**) (73% yield) and the unexpected product, $\Delta^{4(5)}$-pyrrolidine (**54**) (20% yield). Alcohol (**53**) was converted to pyrrolidine (**55**) by first forming the corresponding bromide (PPh_3, NBS) and then effecting its cyclization with sodium hydride. Compound (**54**) was hydrogenated to give the desired pyrrolidine (**55**) in 90% yield. Removal of the acetonide group and the *tert*-butyl group was accomplished with camphorsulfonic acid (CSA) in methanol and methylene chloride respectively. After TFA deprotection of the Boc group, the resulting trifluoroacetate salt of detoxinine was placed on an ion exchange column and eluted with 1 N NH_4OH to give (-) detoxinine in 81% yield from compound (**55**).

4.1.3 Synthetic Strategy of Joullié's Group[26,27]

In 1986, Joullié *et al.* reported a different route to detoxinine (**2a**), employing a stereoselective aldol condensation to construct the β-hydroxy acid side chain as the key step. The required aldehyde (**66**) was synthesized using two different approaches. The first approach represented the shortest synthesis of detoxinine (**2a**) but required resolution of the *cis* and *trans* pyrrolidines (**61a** and **61b**, **Scheme 7**).[26] The second approach involved an enantioselective synthesis of the pyrrolidine ring and gave a higher yield (**Scheme 8**).[27] The formation of the pyrrolidine ring from the first route is shown in **Scheme 7**.[26] The highly functionalized 1,3-amino alcohol (**58**) was synthesized according to the procedure of Das and Torssell,[28] from butadiene (**56**) and nitromethane, followed by lithium aluminum hydride (LAH) reduction of the resulting 5-vinyl-2-isoxazoline (**57**). The amino group of (**58**) was then converted to its *tert*-butoxycarbonyl (Boc) derivative using di-*tert*-butyldicarbonate in dichloromethane. At this point in the synthesis, the resolution of the resulting allylic alcohols was attempted using the Sharpless kinetic resolution, but the desired epoxide was obtained in less than 10% yield. After protection of the primary amine as its Boc derivative, the secondary hydroxyl group of compound (**58**) was protected as its silyl ether using *tert*-butyldimethylsilyl chloride. The double bond was then converted to the epoxide (**60**) in 83% yield using *m*-chloroperbenzoic acid (MCPBA). The resulting diastereomeric mixture could not be separated at this point but was used directly in the next step. Treatment of the epoxide (**60**) with magnesium triflate afforded *cis* (**61a**) and *trans* pyrrolidinols (**61b**) as a 1 : 1 separable

mixture and in 62% yield. The use of boron trifluoride etherate at -78 °C, in dichloromethane, to effect ring closure of substrate (**60**) gave rapidly and cleanly a 91% yield of isomeric pyrrolidinols (**61a**) and (**61b**). The low temperature was necessary to prevent removal of the protecting groups.[29]

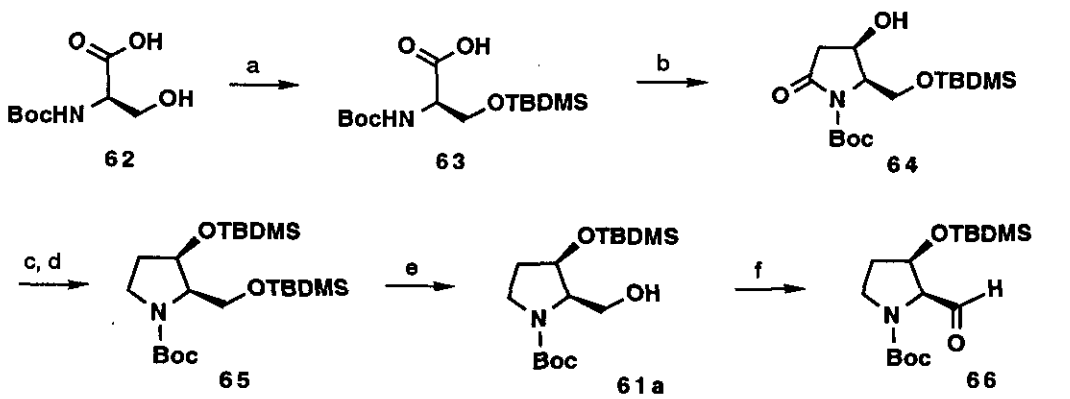
Scheme 7[26]



[a] 1. $MeNO_2$, Et_3N, Me_3SiCl, 2. TFA, 54%; [b] LAH, Et_2O, 91%; [c] $(Boc)_2O$, Et_3N, 85%; [d] TBDMSCl, DMF, imidazole, 99%; [e] MCPBA, $NaHCO_3$, 83%; [f] $Mg(OTf)_2$, $NaHCO_3$, 62%, or $BF_3{\cdot}Et_2O$, 91%.

Scheme 8 outlines the second route to the required aldehyde (**66**).[27] Boc-D-Serine (**62**) was treated with excess *tert*-butyldimethylsilyl chloride followed by decomposition of the silyl ether using aqueous potassium carbonate in methanol and tetrahydrofuran. The acid (**63**) was converted to its corresponding mixed anhydride using isopropenyl chloroformate in the presence of Meldrum's acid and DMAP according to a reported procedure.[30] The resulting acyl-Meldrum's acid was then decomposed in refluxing ethyl acetate to give the

Scheme 8[27]

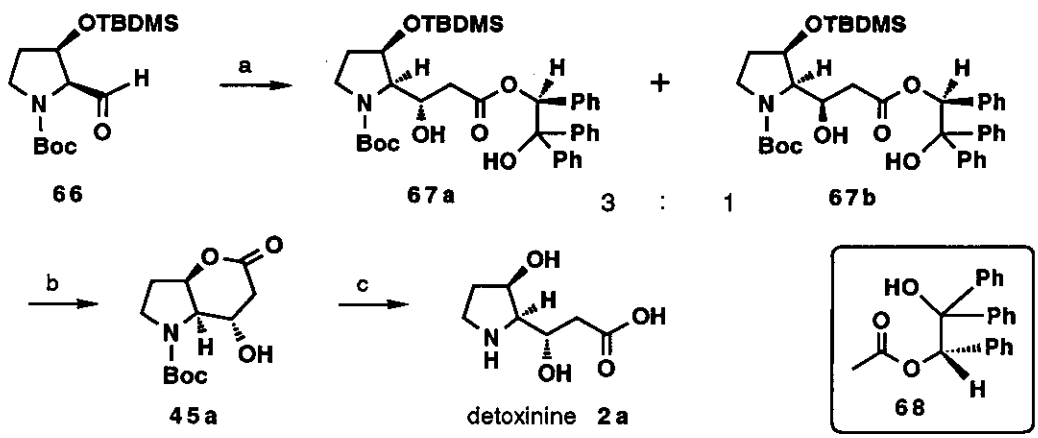


[a] 1.TBDMSCl, DMF, imidazole; 2. 1 M K_2CO_3, MeOH, THF, 87%; [b] 1. DMAP, isopropenyl chloroformate, Meldrum's acid; 2. EtOAc, reflux; 3. $NaBH_4$, AcOH, CH_2Cl_2, 41%; [c] TBDMSCl, imidazole, DMF, 99%; [d] $BH_3{\cdot}Me_2S$, THF, reflux, 74%; [e] AcOH, THF, H_2O, 93%; [f] TFAA, DMSO, Et_3N, 90%.

corresponding tetramic acid which was not isolated but reduced directly with sodium borohydride. This transformation gave only one diastereomeric alcohol (**64**) in a 41% overall yield from compound (**63**). The enantiomeric excess of compound (**64**) was 93% as determined by the ^{1}H nmr and HPLC analysis of its Mosher's ester. The secondary hydroxyl group was then protected as its silyl ether. Reduction of the Boc protected amide was accomplished in 74% yield using a borane-dimethyl sulfide complex in refluxing tetrahydrofuran. Ohfune and Tomita had found this complex to be the only reagent to effect the reduction of urethane protected amides.[31] Regioselective removal of the primary silyl group of compound (**65**) was accomplished using aqueous acetic acid in tetrahydrofuran at 0 °C. The *cis* pyrrolidinol (**61a**) was then oxidized to the corresponding aldehyde (**66**), under Swern oxidation conditions, with trifluoroacetic anhydride as the DMSO activator.

The completion of the synthesis is shown in **Scheme 9**.[26] The aldol condensation of aldehyde (**66**) with the magnesium enolate of **68** afforded a 3 : 1 chromatographically separable mixture of **67a** and **67b**. Treatment of **67a** with tetrabutylammonium fluoride produced **45a** in 85%. ^{1}H Nmr analysis of **45a** in the presence of the chiral shift reagent, tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato] europium(III) [Eu(hfc)$_3$], indicated the enantiomeric excess to be 60%. Removal of the Boc group with TFA, followed by ion exchange chromatography, gave detoxinine (**2a**) in 83% yield.

Scheme 9[26]



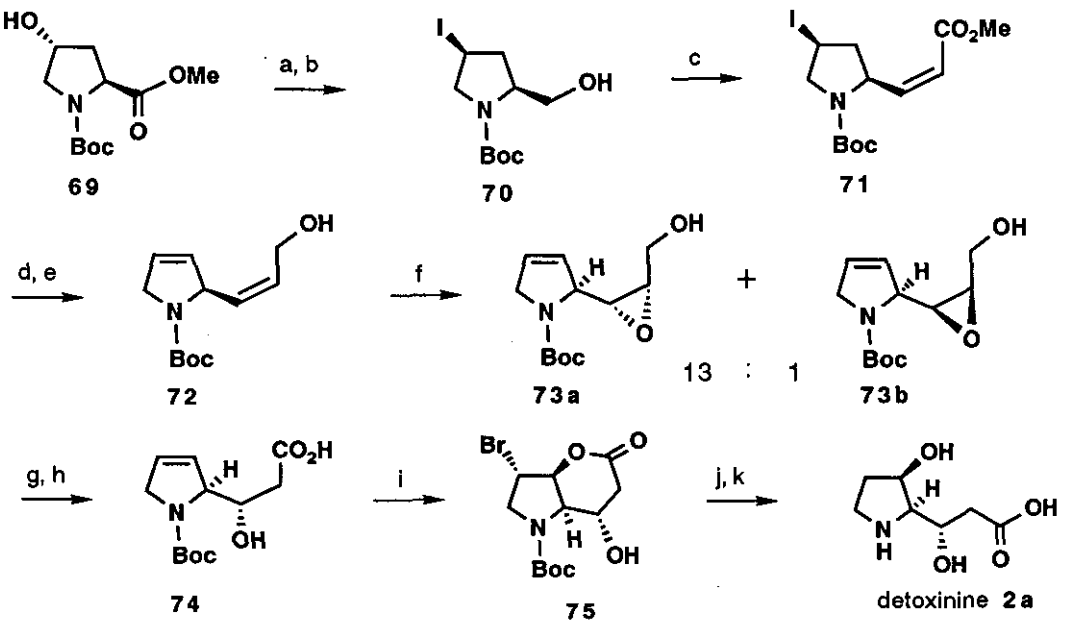
[a] LDA, (S)-2-acetoxy-1,1,2-triphenylethanol (**68**), $MgBr_2$, 40%; [b] $nBu_4N^+F^-$, THF, 85%; [c] 1. TFA; 2. ion exchange chromatography, 83%.

4.1.4 Synthetic Strategy of Kogen's Group[32]

Recently, Kogen *et al.* completed an efficient stereocontrolled synthesis of detoxinine *via* diastereoselective epoxidation of allylic alcohol (**72**) and bromolactonization of *syn*-β-hydroxy-γ-amino acid (**74**) (**Scheme**

10).[32] Treatment of Boc-(2S,4R)-4-hydroxyproline methyl ester (**69**) with triphenylphosphine, diethyl azodicarboxylate and methyl iodide,[33] followed by sodium borohydride reduction of the ester group provided alcohol (**70**) in 82% yield. Compound (**70**) was subjected to Swern oxidation and then converted to the α,β-unsaturated ester using Still's method.[34] This transformation gave the *cis*-isomer (**71**) in a greater than 30:1 ratio and in 86% yield. The *cis*-isomer (**71**) was converted to the alcohol (**72**) by first reducing the ester functionality and then effecting the double bond formation *via* elimination of the selenoxide. The *m*-chloroperbenzoic acid epoxidation of the allylic alcohol (**72**) proceeded with high diastereoselectivity to afford a >13:1 ratio of **73a** : **73b** in 94% yield. Reduction of the epoxide (**73**) and subsequent selective oxidation of the primary alcohol provided the *syn*-β-hydroxy-γ-amino acid (**74**) in 62% yield. The acid was converted to its sodium salt and then treated with bromine to give the bromolactone (**75**) in 95% yield. Tin hydride reduction of lactone (**75**) followed by TFA treatment afforded detoxinine (**2a**) in 87% yield after ion exchange resin purification.

Scheme 10[32]

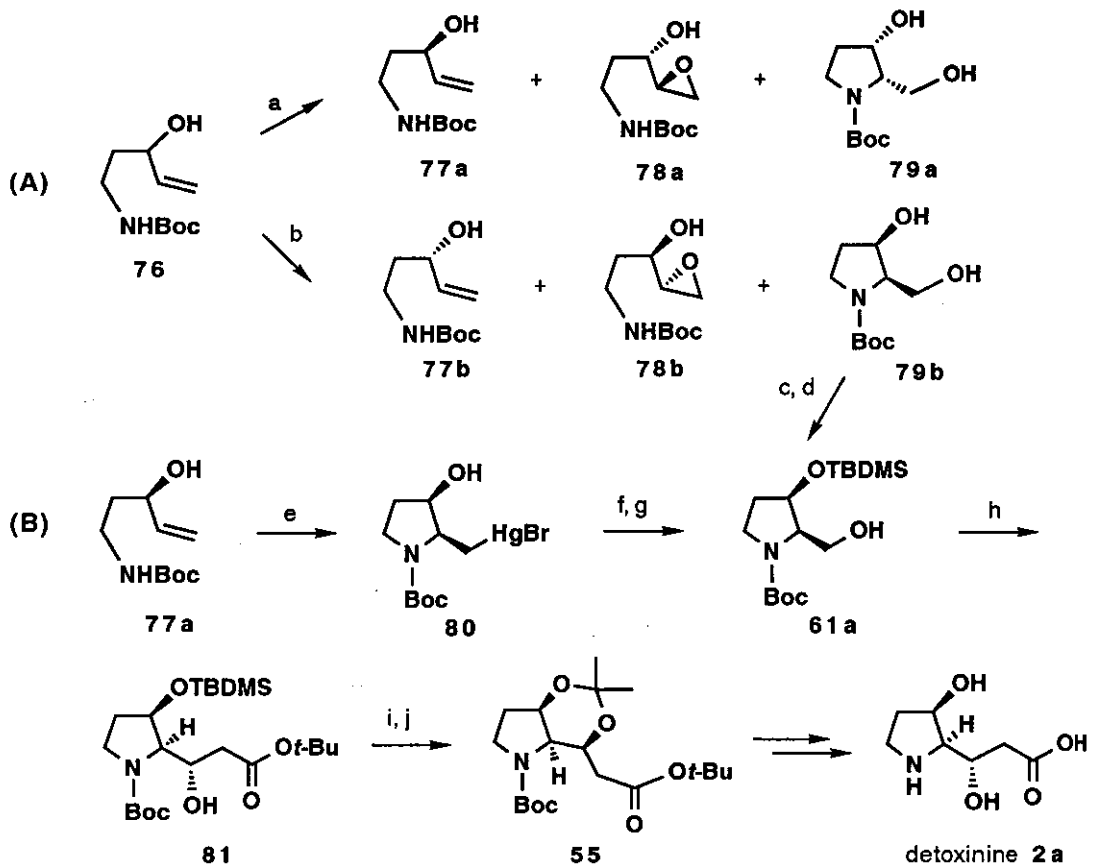


[a] Ph_3P, diethyl azodicarboxylate, MeI, 90%; [b] $NaBH_4$, LiCl, EtOH, THF, 92%; [c] 1. $(COCl)_2$, DMSO, Et_3N; 2. $(CF_3CH_2O)_2P(O)CH_2CO_2Me$, 18-Crown-6, THF, $(TMS)_2NK$, 86%; [d] DIBAL, CH_2Cl_2, 78%; [e] PhSeNa, EtOH, reflux, then H_2O_2, EtOH, THF, 92%; [f] MCPBA, CH_2Cl_2, 94%; [g] Red-Al®, THF, 74%; [h] Pt/O_2, H_2O, $NaHCO_3$, 84%, [i] 1. H_2O, $NaHCO_3$; 2. Br_2, EtOH, 95%; [j] $(Bu)_3SnH$, AIBN (cat.), THF, 97%; [k] 1. TFA; 2. ion exchange chromatography, 90%.

4.1.5 Synthetic Strategy of Takahata's Group[35]

In 1991, a formal synthesis of detoxinine was reported by Takahata and collaborators in the course of their studies on asymmetric intramolecular amination of allylic alcohols (**Scheme 11**).[35] The key to this formal synthesis was the use of Sharpless asymmetric epoxidation. As discussed in **4.1.3**, this methodology was attempted on the same substrate but abandoned because the desired epoxide was obtained in less than 10% yield. Takahata *et al.* also obtained low yields of epoxide. However, these investigators found that in addition to promoting asymmetric epoxidation, the reaction also permitted optical resolution to provide two chiral building blocks used in the synthesis of several biologically active natural products.

Scheme 11[35]



[a] L-(+)-DIPT, TBHP, $Ti(O\text{-}i\text{-}Pr)_4$, molecular sieves 3Å; [b] D-(-)-DIPT, TBHP, $Ti(O\text{-}i\text{-}Pr)_4$, molecular sieves 3Å; [c] TBDMSCl, imidazole, DMF, 93%; [d] AcOH, THF, H_2O, 52%; [e] 1. $Hg(OAc)_2$; 2. KBr, $NaHCO_3$, 90%; [f] TBDMSCl, imidazole, DMF, 64%; [g] O_2, $NaBH_4$, DMF, 93%; [h] 1. TFAA, DMSO, Et_3N; 2. $MeCO_2t\text{-}Bu$, LDA, 80%; [i] nBu_4NF, 92%; [j] 2,2-dimethoxypropane, *p*-TsOH (cat.), 96%.

As shown in **Scheme 11**, the Sharpless asymmetric epoxidation and kinetic resolution of racemic **76** using L-(+)-diisopropyl tartrate (L-(+)-DIPT), *tert*-butyl hydroperoxide (TBHP), Ti(O-*i*-Pr)$_4$, and molecular sieves (3 Å) gave three compounds, optically pure starting material (**77a**) (36%), the epoxy alcohol (**78a**) (5%), and the pyrrolidine (**79a**) (33%). Similar reaction of racemic **76** using D-(-)-DIPT afforded **77b** (46%), **78b** (11%), and **79b** (33%). Stereoselective amidomercuration of **77a** with mercuric acetate followed by treatment with potassium bromide in the presence of sodium bicarbonate gave the mercuric bromide (**80**) as the only detectable regioisomer. *tert*-Butyldimethylsilylation of the mercuric bromide followed by reductive oxygenation afforded the alcohol (**61a**), which was also obtained from **79b** by selective protection of the secondary alcohol in two steps. Swern oxidation of the primary alcohol (**61a**) and subsequent aldol condensation of the resulting aldehyde with *tert*-butyl lithioacetate provided only one diastereomer (**81**). Desilylation of **81** with tetrabutylammonium fluoride, followed by ketalization, afforded intermediate (**55**), previously converted to detoxinine (**2a**).

4.1.6 Summary of Detoxinine Syntheses

A summary of the different syntheses of detoxinine are shown in **Table 4.** The syntheses are listed chronologically. The starting materials, number of steps, and yields are given.

Table 4. Synthetic Approaches to Detoxinine

Group	Year	Product	Precursor	Steps	% Yield
Häusler	1983	(±)-detoxinine	L-proline methyl ester	13	2.1
Ohfune	1984	(-)-detoxinine	L-allylglycine	19	3.2
Joullié	1986	(-)-detoxinine	butadiene	11	2.9
Joullié*	1988	(-)-detoxinine	Boc-D-serine	13	4.5
Kogen	1990	(-)-detoxinine	4-hydroxyproline	15	19.7
Takahata*	1991	(-)-detoxinine	N-Boc-3-hydroxy-4-pentenylamine	13	10.1

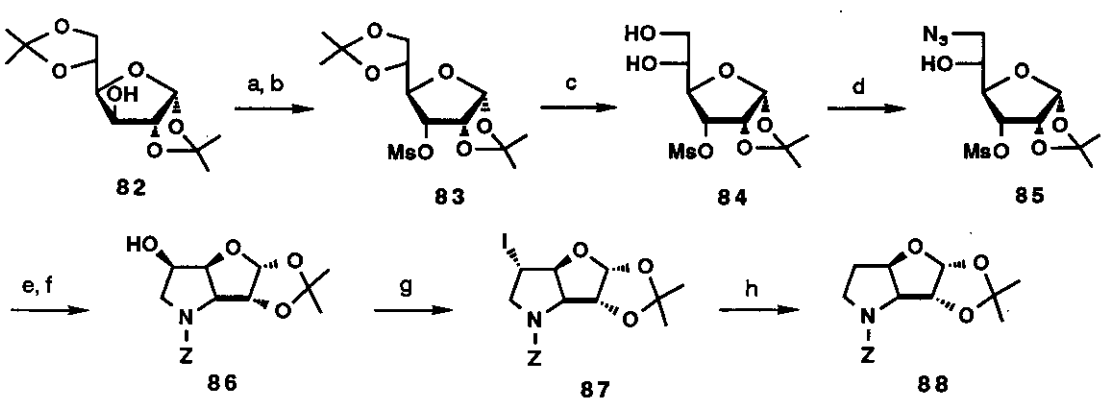
* Formal total synthesis. Yields are calculated based on the appropriate references.

4.2 Synthesis of Valyldetoxinine[36]

Valyldetoxinine was synthesized by Joullié and coworkers. As shown in **Scheme 12**, the pyrrolidinol ring was constructed by a modification of methodology previously employed for the synthesis of 1,4-dideoxy-1,4-imino-D-lyxitol hydrogen chloride.[37] The key step of this approach was the formation of the pyrrolidine ring in structure (**86**) *via* cyclization by attack of an amino group on a mesylate in the furanose ring. The synthesis began with diacetone D-glucose (**82**), which was oxidized with pyridinium chlorochromate (PCC) in the

presence of molecular sieves in methylene chloride. The crude ketone was then treated with sodium borohydride in ethanol to give the α-D-allofuranose (82% yield). Since one of the methyl groups of the 1,2-*O*-isopropylidene group sterically hinders the bottom face, the carbonyl group is only accessible from the least hindered top face. Treatment of the reduced product with methanesulfonyl chloride in pyridine gave mesylate (**83**) (95% yield).[38,39] Subsequent mild hydrolysis selectively removed the 5,6-*O*-isopropylidene group to afford 1,2-*O*-isopropylidene-3-*O*-methanesulfonyl-α-D-allofuranose (**84**) in 59-74% yield. Conversion of the primary alcohol (**84**) into the corresponding bromide was achieved with carbon tetrabromide and triphenylphosphine in THF (94% yield). Displacement of the bromide with sodium azide in DMF afforded intermediate (**85**) in 96% yield. Alternatively, the alcohol (**84**) was treated with carbon tetrabromide, triphenylphosphine, and lithium azide in DMF to provide the same product (**85**) in 96%.[40] The azide function in **85** was reduced to a primary amine in ethanol using a catalytic amount of palladium on carbon under a hydrogen atmosphere (30 psi). Subsequent cyclization with sodium acetate and protection of the resulting amine with benzyl chloroformate and triethylamine in THF afforded product **86** in 50% yield. When the secondary amine was treated with benzyl chloroformate and sodium carbonate in a mixture of acetone and water, the yield increased to 78% overall. Compound (**86**) was deoxygenated at C-5 *via* reductive radical cleavage of a halide. Treatment of **86** with 2,4,5-triiodoimidazole (ImI_3)[41] and triphenylphosphine in refluxing toluene gave the corresponding iodide (**87**) in 99% yield with inversion of configuration. Free radical reduction of the iodide with tributyltin hydride was initiated with azobis(isobutyronitrile) (AIBN) in

Scheme 12[36]

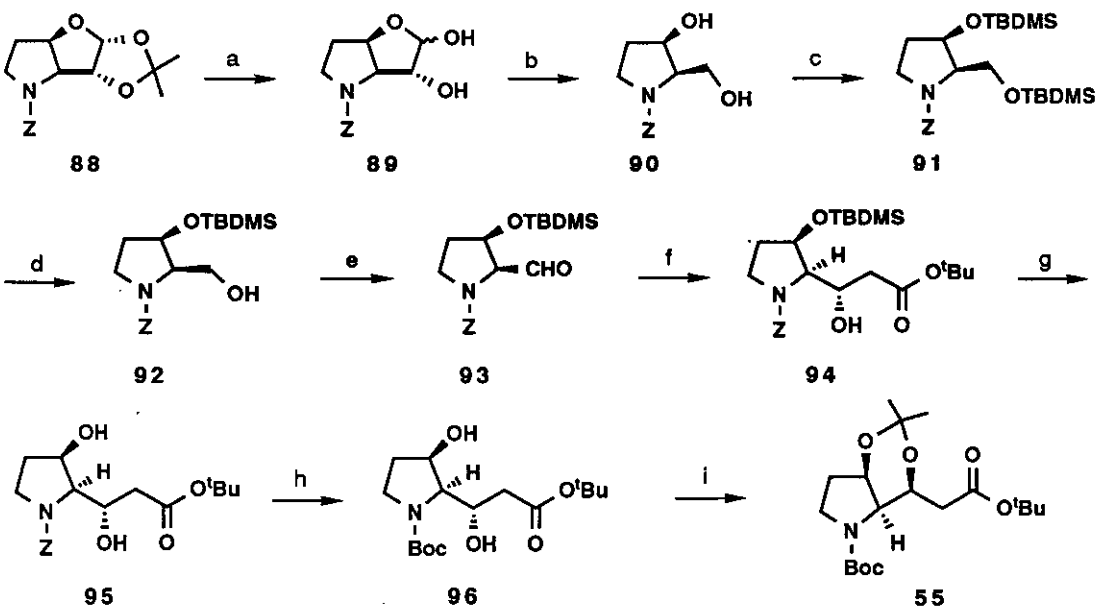


[a] 1. PCC, molecular sieves, CH_2Cl_2; 2. $NaBH_4$, EtOH, 82%; [b] MsCl, Pyr., 95%; [c] Dowex 50X4-400, dioxane, MeOH, H_2O, 0 °C, 59% or H_2SO_4 (aq.), 74%; [d] 1. Ph_3P, CBr_4, THF; 2. NaN_3, DMF, 90%; or Ph_3P, CBr_4, LiN_3, DMF, 96%; [e] 1. Pd/C, H_2 or Raney Ni, H_2; 2. NaOAc, EtOH, reflux; [f] benzyl chloroformate, Et_3N, THF, 50% from **85** or benzyl chloroformate, H_2O, acetone, Na_2CO_3, 78% from **85**; [g] ImI_3, imidazole, Ph_3P, toluene, 99%; [h] *n*-Bu_3SnH, benzene, AIBN, 97%.

benzene under reflux, and proceeded to completion to give compound (**88**) in 97% yield.

The 1,2-*O*-isopropylidene group of **88** was hydrolyzed using an ion-exchange resin in a mixture of dioxane and water to give lactol **89** as shown in **Scheme 13**. Cleavage of the vicinal hydroxyl groups was achieved with sodium metaperiodate in dioxane and water. Immediate reduction of the aldehyde function using sodium borohydride in methanol afforded the 1,3-diol (**90**) in 95% from **89**. Both primary and secondary hydroxyl groups of **90** were protected as their silyl ethers in 98% yield. Selective removal of the primary silyl group of the diprotected intermediate (**91**) was accomplished in 83% yield using aqueous acetic acid and tetrahydrofuran. A modified Parikh-Doering reaction[42] (sulfur trioxide-pyridine complex), in the presence of dimethyl sulfoxide and triethylamine, gave the required aldehyde (**93**, 80% yield) to be used in the aldol condensation. The aldehyde (**93**) was treated with the lithium salt of *tert*-butyl acetate to afford only one diastereomer (**94**) in 87% yield. The formation of a single diastereomer can be explained by the predominance of the chelation-controlled Cram conformation in the transition state of **93** with the lithium *tert*-butyl acetate. The aldehyde-metal-urethane chelation forces the nucleophile to attack from the less hindered *si* face, resulting in preferential formation of the *syn* product.[43] A series of transformations was conducted to prove the stereochemistry at

Scheme 13[36]

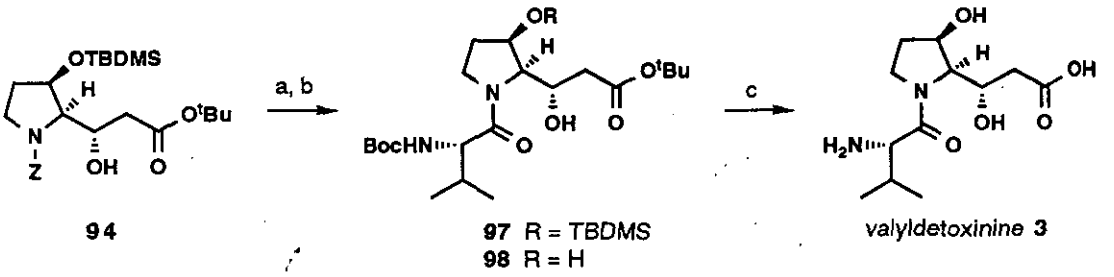


[a] Dowex 50X4-400, dioxane, H_2O, 40 °C; [b] 1. $NaIO_4$, dioxane, H_2O; 2. $NaBH_4$, MeOH, 0 °C to room temperature, 95%; [c] TBDMSCl, Im, DMF, 0 °C to room temperature, 98%; [d] AcOH, H_2O, THF, 0 °C to room temperature, 83%; [e] SO_3•Py, CH_2Cl_2, Et_3N, DMSO, 0 °C to room temperature, 80%; [f] $LiCH_2CO_2{}^tBu$, THF, -78 °C, 87%; [g] TBAF, THF, 0 °C, 98%; [h] 1. H_2, Pd/C, MeOH, room temperature; 2. $(Boc)_2O$, Et_3N, DMAP, THF, room temperature, 54%; [i] 2,2-dimethoxypropane, *p*-TsOH•H_2O, THF, Δ, 96%.

the β-hydroxy center of the side chain. The β-hydroxy ester (**94**) was desilylated using tetrabutylammonium fluoride in tetrahydrofuran to afford **95** in 98% yield. The Z group was removed by a standard procedure, followed by Boc group protection to give 1,3-diol (**96**) in 54% yield. This product (**96**) was ketalized using 2,2-dimethoxypropane to give intermediate (**55**), previously converted to (-)-detoxinine.[25,35] The physical data of compound (**55**) agreed with previously reported data.[25,35]

The elaboration of the β-hydroxy ester (**94**) to complete the total synthesis of (+)-valyldetoxinine (**3**) is shown in **Scheme 14**. Compound (**94**) was catalytically hydrogenated with palladium on carbon under an atmosphere of hydrogen. Subsequent coupling with Boc-L-valine, in the presence of dicyclohexylcarbodiimide and a racemization suppressing reagent, 1-hydroxybenzotriazole hydrate (HOBT), in methylene chloride, produced the fully protected valyldetoxinine precursor (**97**) in 75% yield. Treatment of **97** with 1.1 M TBAF in THF for 3 min at 0 °C provided **98** in 96% yield. Since removal of both the Boc and *tert*-butyl ester groups with trifluoroacetic acid did not give satisfactory results, compound (**98**) was treated with dry hydrogen chloride in ethyl acetate to afford the valyldetoxinine hydrochloride in 89% yield. The hydrochloride salt was purified by ion-exchange chromatography to afford (+)-valyldetoxinine (**3**) in 92% yield.

Scheme 14[36]



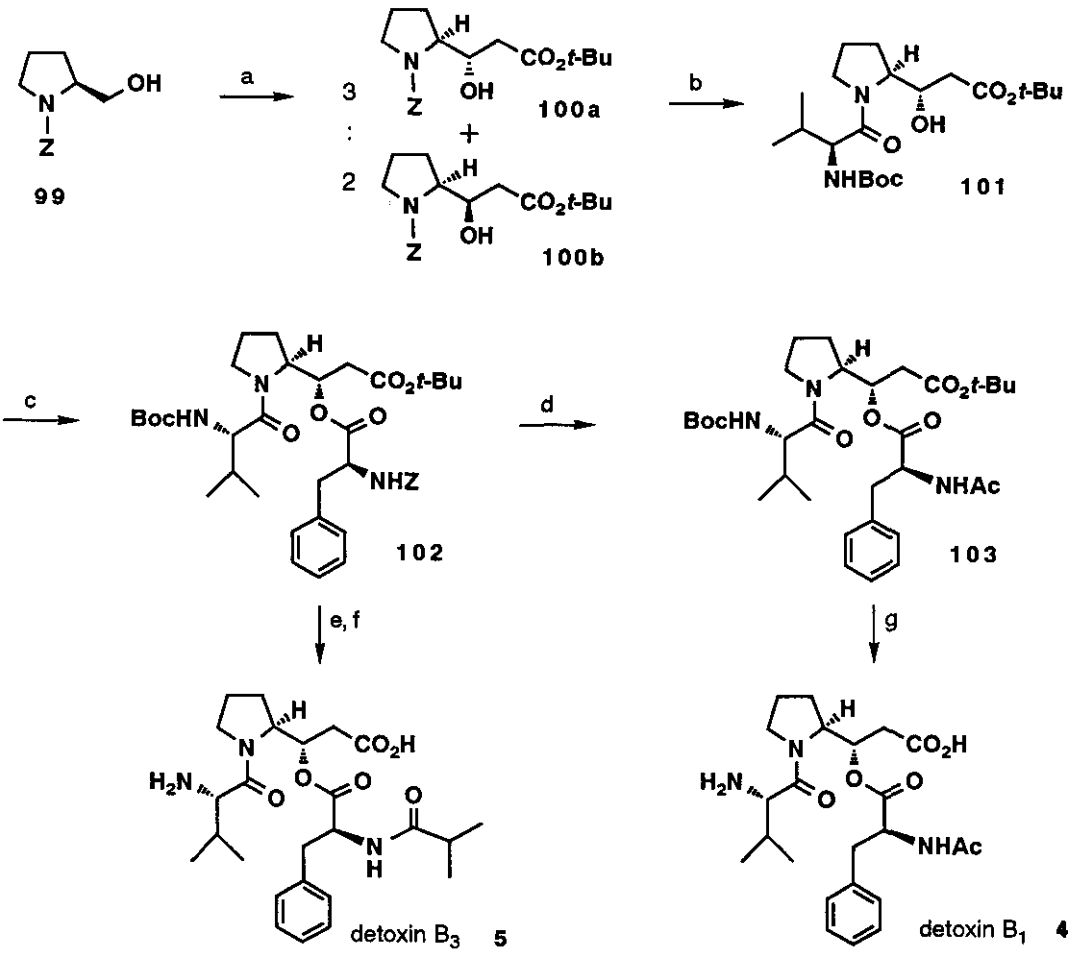
94 **97** R = TBDMS; **98** R = H; *valyldetoxinine* **3**

[a] 1. H_2, Pd/C, MeOH, room temperature; 2. Boc-L-Val-OH, DCC, HOBT, CH_2Cl_2, 0 °C to room temperature, 75%; [b] TBAF, THF, 0 °C, 96%; [c] 1. dry HCl, EtOAc, room temperature, 89%; 2. Ion-exchange, 92%.

4.3 Synthesis of Detoxins B_1 and B_3[44]

Detoxins B_1 (**4**) and B_3 (**5**) have been synthesized by Joullié *et al.* starting with L-proline (**Scheme 15**). After Z-L-prolinol (**99**) was generated, it was converted to Z-prolinal which was not isolated but treated directly with *tert*-butyl bromoacetate in the presence of a zinc-copper couple, under Reformatsky reaction conditions, to afford the β-hydroxy esters (**100a**) and (**100b**).[45-47] This method gave two chromatographically separable β-hydroxy esters in the ratio of 3 : 2, with the major product (**100a**) having the desired stereochemistry. After removal of the carbobenzyloxy protecting group, the secondary amine was coupled to Boc-L-valine under DCC/DMAP standard conditions to give dipeptide (**101**). Compound (**101**)

Scheme 15[44]



[a] 1. $(COCl)_2$, Et_3N, DMSO, CH_2Cl_2; 2. $BrCH_2CO_2t$-Bu, Zn-Cu, Et_2O, 80%; [b] 1. H_2, Pd/C, EtOH; 2. Boc-Val-OH, DCC, HOBT, 82%; [c] Z-Phe-OH, DCC, DMAP, 90%; [d] 1. H_2, Pd/C, EtOH; 2. AcCl, DMAP, CH_2Cl_2, 82%; [e] 1. H_2, Pd/C, EtOH; 2. isobutyric acid, DCC, HOBT, CH_2Cl_2, 90%; [f] TMSCl, NaI, MeCN; 60%; [g] TMSCl, NaI, MeCN, 57% or TFA, ion exchange, 78%.

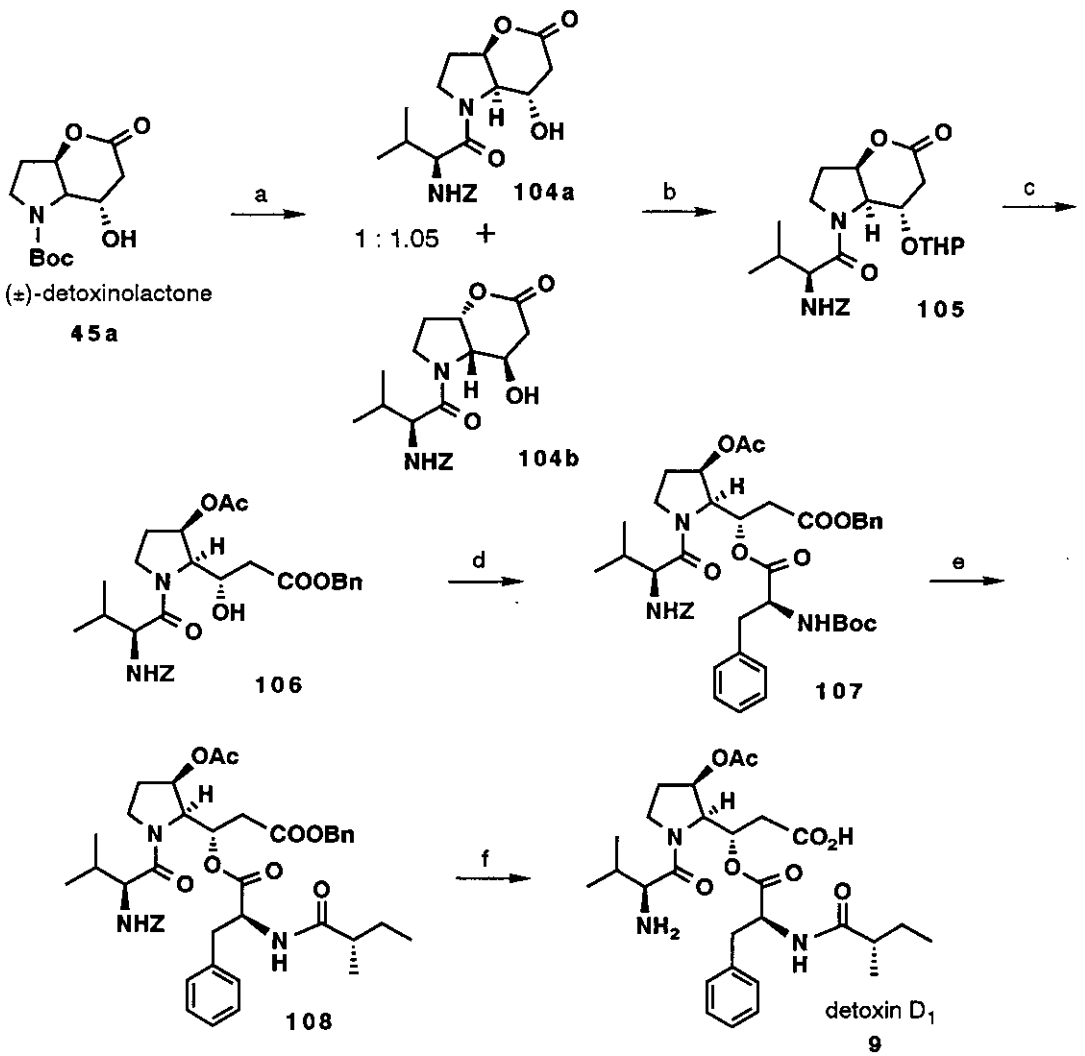
was then coupled with Z-L-phenylalanine to produce tripeptide (**102**) using DCC and DMAP. Hydrogenation, followed by treatment of the resulting amine with acetyl chloride and isobutyric acid, gave the fully protected detoxins B_1 (**4**) and B_3 (**5**) respectively. The Boc and *tert*-butoxy protecting groups were then removed simultaneously using chlorotrimethyl silane and sodium iodide to produce detoxins B_1 (**4**) or B_3 (**5**). Alternatively, detoxin B_1 (**4**) could be produced by treating fully protected tripeptide (**103**) with trifluoroacetic acid and purifying the resulting trifluoroacetate salt by ion exchange chromatography.

4.4 Synthesis of Detoxin D_1

4.4.1 Synthetic Strategy of Häusler's Group[48]

In 1986, Häusler extended his synthesis of racemic detoxinine (**2a**) to detoxin D_1 (**9**).[48] **Scheme 16** outlines the conversion of (±)-Boc-detoxinolactone (**45a**) to detoxin D_1 (**9**). After removal of the Boc protecting group of **45a**, the trifluoroacetate salt was coupled to Z-valine anhydride to give two 1 : 1 diastereomeric

Scheme 16[48]



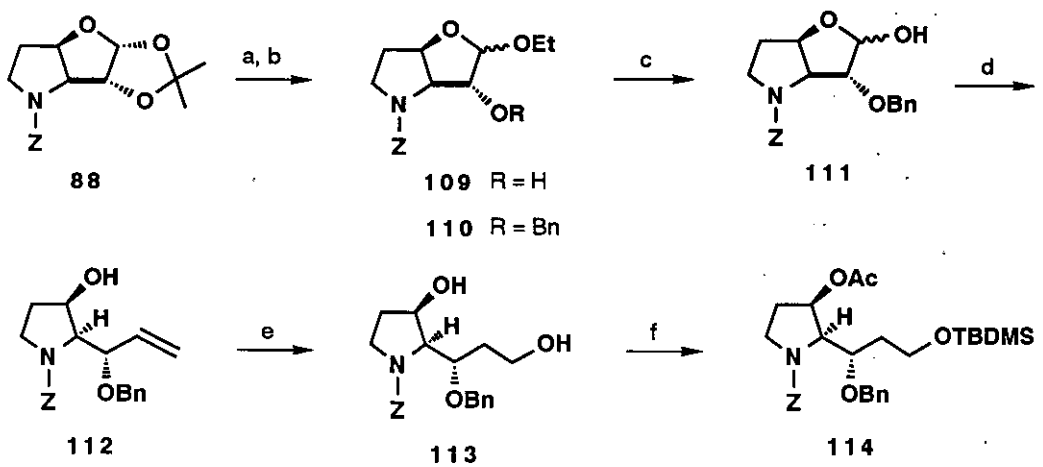
[a] 1. TFA; 2. $(Z\text{-Val})_2O$, NMM, MeCN, 76%; [b] dihydropyran, *p*-TsOH (cat.), 90%; [c] 1. 1 N NaOH, $MeOCH_2CH_2OMe$ (aq.); 2. BnBr, KI (cat.), DMF; 3. Ac_2O, DMF; 4. 4 N HCl (anhy.), MeOH, Et_2O, 29%; [d] $(Boc\text{-Phe})_2O$, DMAP, 87%; [e] 1.TFA; 2. 2-methylbutyric anhydride, NMM, 90%; [f] H_2, Pd/C, 88%.

dipeptides (**104a** : **104b**), which were separated by preparative thin layer chromatography. The desired dipeptide (**104a**) was obtained in 37% yield from lactone (**45a**). The free hydroxyl group of compound (**104a**) was protected as its tetrahydropyran (THP) ether (**105**) in 87-90% yield. Basic hydrolysis of the lactone ring with aqueous sodium hydroxide gave the free acid contaminated with a product resulting from the elimination of the ether group. The carboxylic acid was protected as the corresponding benzyl ester, followed by acetylation of the free hydroxyl group. After removal of the THP protecting group using a catalytic amount of 4 N HCl/Et_2O in MeOH and Et_2O, ester (**106**) was obtained in 29% yield. The free hydroxyl group was then coupled to Boc-L-phenylalanine anhydride to produce compound (**107**). Removal of the Boc protecting group followed by coupling to (S)-2-methylbutyric anhydride gave protected detoxin D_1 (**108**) in 90% yield. Removal of the benzyloxycarbonyl and benzyl groups by catalytic hydrogenation afforded detoxin D_1 (**9**) in optically pure form.

4.4.2 Synthetic Strategy of Joullié's Group[36,49]

In 1992, Joullié *et al.* reported the stereocontrolled synthesis of detoxin D_1 from D-glucose by extending their previously developed methodology.[37] **Scheme 17** shows the synthesis of fully protected pyrrolidinol (**114**) from compound (**88**) (**Scheme 12**). Treatment of the protected amine (**88**) with ethanol in the presence of 15% HCl/Et_2O at room temperature gave the ethyl glycoside (**109**), which was treated with benzyl bromide and potassium hydride to afford the benzyl ether (**110**) in 84% yield from **88**. Hydrolysis of the ethyl glycoside (**110**) with aqueous TFA gave the corresponding lactol (**111**) in 90% yield. Lactol (**111**) was treated with methylenetriphenylphosphorane to afford compound (**112**) in 71% yield, according to a literature

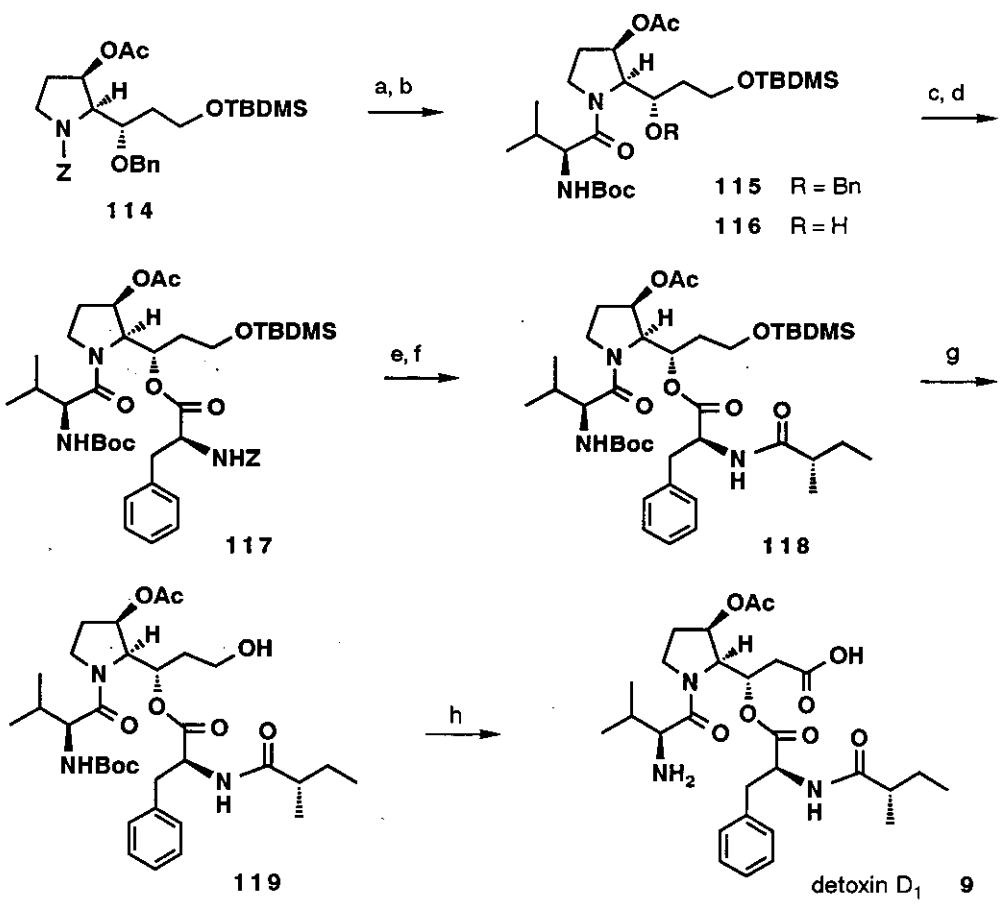
Scheme 17[36,49]



[a] EtOH, 15% HCl/Et_2O, 94%; [b] BnBr, KH, DMF, 89%; [c] TFA, H_2O, 90%; [d] $Ph_3P{=}CH_2$, THF, 71%; [e] disiamylborane; H_2O_2, NaOH, 83%; [f] TBDMSCl, Et_3N, DMAP, CH_2Cl_2; Ac_2O, Et_3N, 91%.

procedure.[50] Treatment of compound (**112**) with disiamylborane, followed by oxidation using 30% hydrogen peroxide and 2 N aqueous NaOH led to the diol (**113**) in 83% yield. The diol (**113**) was then transformed into the fully protected compound (**114**) by *tert*-butyldimethylsilylation and acetylation in one pot (91% yield). The completion of the synthesis is detailed in **Scheme 18.** The elaboration of the key intermediate (**114**) to a protected precursor of detoxin D_1 (**119**) was carried out using appropriate peptide and ester coupling reagents. The benzyloxycarbonyl protecting group of compound (**114**) was selectively removed by catalytic hydrogenolysis using Raney Ni as a catalyst under an atmosphere of hydrogen (40 psi). Coupling of the resulting

Scheme 18[36,49]



[a] Raney Ni, H_2, EtOAc, MeOH; [b] Boc-valine, BOP-Cl, Et_3N, CH_2Cl_2, 90%; [c] H_2, palladium black, EtOH, [d] Z-phenylalanine, DCC, DMAP, 10-camphorsulfonic acid (CSA), CH_2Cl_2, 88%; [e] Pd/C, H_2, EtOAc, MeOH; [f] (S)-2-methylbutyric acid (**23**), BOP, DIEA, CH_2Cl_2, 70%; [g] HOAc, THF, H_2O, 99%; [h] 1. TFAA, DMSO, Et_3N, CH_2Cl_2, then 1 M $KMnO_4$, 5%$NaHPO_4$;2. TFA, CH_2Cl_2, then ion exchange chromatography, 65-70%.

secondary amine with Boc-L-valine was then accomplished by using *N,N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) to afford the coupled product (**115**) in 90% yield.[51] Catalytic hydrogenation of the benzyl ether (**115**) in ethanol in the presence of palladium black, under 45 psi of hydrogen, gave the corresponding alcohol (**116**). Since the convergent approach for the synthesis of the protected precursor of detoxin D_1 (**118**) from alcohol (**116**) and (S)-2-methylbutyryl-L-phenylalanine (**22**) did not give satisfactory results, sequential coupling procedures were utilized. Treatment of dipeptide (**116**) with Z-L-phenylalanine, in the presence of DCC, a catalytic amount of DMAP, and 10-camphorsulfonic acid (CSA), in methylene chloride, afforded product (**117**) in 88% yield. Removal of the benzyloxycarbonyl protecting group by catalytic hydrogenolysis, and subsequent treatment with (S)-2-methylbutyric acid (**23**) in the presence of BOP reagent and *N,N*-diisopropylethylamine (DIEA), afforded the depsipeptide (**118**) in 70% yield.[52] The next step was the removal of the *tert*-butyldimethylsilyl protecting group in 99% yield, using HOAc : THF : H_2O (3 : 1 : 1). To synthesize detoxin D_1 (**9**), the primary alcohol in depsipeptide (**119**) had to be oxidized to a carboxylic acid. This operation was carried out in two steps. The primary alcohol (**119**) was first converted to the aldehyde by a Swern oxidation using trifluoroacetic anhydride (TFAA) as the DMSO activator.[53] The unstable aldehyde was immediately oxidized to the carboxylic acid, using a procedure developed by Masamune for oxygen-rich molecules containing acid sensitive groups.[54] Treatment of the aldehyde with 1 M potassium permanganate in *tert*-butyl alcohol, using 5% sodium hydrogen phosphate, produced the corresponding acid, which was used directly in the subsequent TFA deprotection to give detoxin D_1 (**9**) in 70% yield.

The route described represents the first total synthesis of detoxin D_1 in optically pure form, using the chiron approach from D-glucose. The advantages of this strategy are: (1) the oxidation of the primary hydroxyl group to a carboxylic acid is carried out in the last stage of the synthesis to avoid the elimination side reaction. Subsequent esterification is also facilitated. (2) The sequence does not involve diastereomeric separations and affords a better overall yield than the previous synthesis from racemic Boc-detoxino-lactone (Section *4.4.1*).

5. CONCLUSIONS

The unique biological activity of the detoxin complex is of great interest. This naturally occurring safener is essential to the understanding of the transport of blasticidin S across cell membranes in intact plant or animal cells. The structures of several members of the complex and that of the parent amino acid (-)-detoxinine are challenging synthetic targets for the development of stereocontrolled routes. Although the complex was originally obtained from culture filtrates, synthetic methodologies have been developed to prepare some of the congeners including its most active member, detoxin D_1.

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