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Reduction Studies of Antifungal Echinocandin Lipopeptides. One Step Conversion of Echinocandin B to Echinocandin C.

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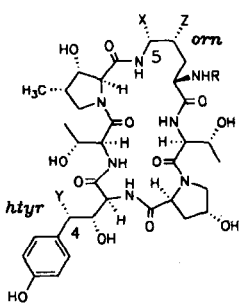
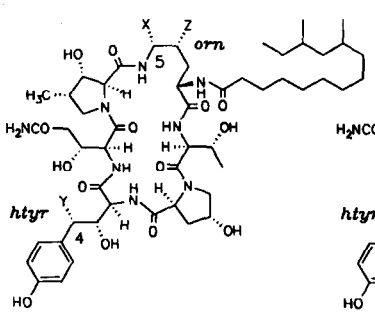
Abstract: Sodium cyanoborohydride in trifluoroacetic acid selectively reduced the C5-orn and C4-htyr carbinols to methylene groups in echinocandin lipopeptides. The selective reduction of either hydroxyl is also described. The first conversion of echinocandin B to echinocandin C was accomplished.

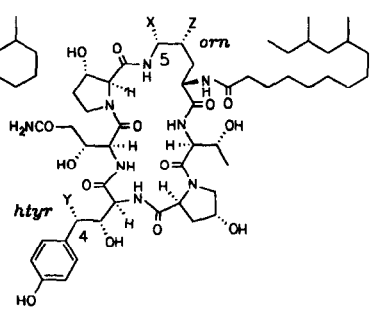
In 1974, Benz *et al.* reported the isolation and structure of a new cyclic peptide antifungal agent.[1] Echinocandin B **1a**, possessed potent fungicidal activity against *Candida albicans*, a clinically important pathogen, by inhibition of the synthesis of 1,3-β-glucan, an integral component of the fungal cell wall.[2] Subsequently, several related lipopeptides, echinocandin C **1b** and echinocandin D **1d**,[3] which differ from **1a** in the extent of hydroxylation, were isolated. A series of related natural products, the pneumocandins, have also been described.[4,5] These fungal metabolites vary in several of the amino acid residues, the lipophilic sidechain and the degree of oxidation, yet all retain potent antifungal properties. The structures of several of



Echinocandin



Pneumocandin A



Pneumocandin B

1a	X=Y=Z=OH	R=linoleoyl
1b	X=Z=OH, Y=H	R=linoleoyl
1c	X=Y=H, Z=OH	R=linoleoyl
1d	X=Y=Z=H	R=linoleoyl
2a	X=Y=Z=OH	R=stearoyl
2b	X=Z=OH, Y=H	R=stearoyl
2c	X=H, Y=Z=OH	R=stearoyl
2d	X=Y=H, Z=OH	R=stearoyl
3a	X=Y=Z=OH	R=p-octyloxybenzoyl
3b	X=Y=H, Z=OH	R=p-octyloxybenzoyl

4a	X=Y=Z=OH
4b	X=H, Y=Z=OH
4c	X=Z=OH, Y=H
4d	X=Y=H, Z=OH
5a	X=Z=H, Y=OH
5b	X=Y=Z=H

6a	X=Y=Z=OH
6b	X=Z=OH, Y=H
6c	X=H, Y=Z=OH
6d	X=Y=H, Z=OH

Table 1. Reduction of Cyclic Lipopeptides With Sodium Cyanoborohydride in TFA.[a]

Substrate	Product	Time (min)	Yield[b]
1a	**1c**	2	42%
2a[c]	**2d**	120	76%
3a[c]	**3b**	120	54%
4a	**4d**	30	44%
5a	**5b**	30	63%
6a	**6d**	30	60%
7b	**2c**	10	60%[d]
8b	**6c**	10	50%[d]

[a]See ref 10 for procedure. All products had satisfactory ^{1}H NMR and mass spectra. [b]All products were purified to >95% purity by reverse phase HPLC. [c]Dichloromethane used as solvent in reduction [d]Yield after hydrolysis of the methylcarbonate.

Table 2. Reduction of Cyclic Lipopeptides With Sodium Triacetoxyborohydride in TFA.[a]

Substrate	Products (ratio)[c]	Conversion	Yield[b]
1a	**1b/1c** (4:1)	62%	33%
2a	**2b/2d** (2:1)	75%	42%
6a	**6b/6d** (2:1)	86%	38%
6a[d]	**6b/6d** (5:95)	100%	e

[a]Reaction time was one minute except as noted. See ref 11 for procedure. All products had satisfactory ^{1}H NMR and mass spectra. [b]Isolated yield of monoreduced adduct. Compounds were purified to >95% purity by reverse phase HPLC except as noted. [c]Ratios were determined by HPLC [d]Reaction time 30 min. [e]Not isolated.

the pneumocandins, pneumocandin A_0 **4a**, pneumocandin A_2 **5a**, and pneumocandin B_0 **6a** are shown. Lipopeptides that possess a C5-*orn* hydroxyl group (X=OH) are unstable toward base[5] and acid.[3] We sought to develop a method to remove this functionality thereby increasing the stability of these compounds. Herein, we describe the selective reduction of the echinocandin and pneumocandin lipopeptides.

We first examined the reduction of the N-acyl hemiaminal group of tetrahydroechinocandin B **2a**.[3] Triethylsilane and trifluoroacetic acid in dichloromethane[6] required 22 h for reduction and gave substantial decomposition. In addition to the C5-*orn* hydroxyl, the C4-*htyr* hydroxyl (Y=OH) was also reduced.[7] Substitution of a more powerful, acid-stable reducing agent $NaCNBH_3$ (CAUTION! may ignite in air),[8] gave facile reduction to the bis-reduced product. Several lipopeptides including the semisynthetic agent cilofungin **3a**,[9] were reduced in this fashion. The yields are shown in Table 1.[10] It should be pointed out that these hydroxyl substitution patterns (X=Y=H, Z=OH) have not been observed in the natural products series.

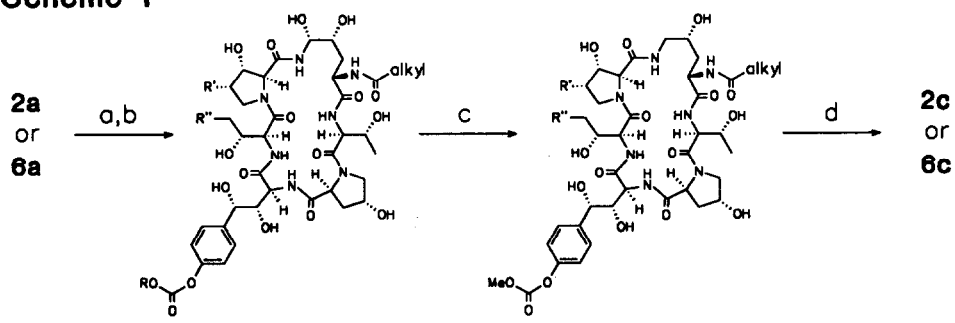
In order to slow down the reaction rate to allow the isolation of the monoreduced intermediates, tetrahydroechinocandin B **2a** was treated with $NaCNBH_3$ in HOAc. Reduction did not occur. When **2a** was treated with $NaCNBH_3$ in TFA and HOAc (1/11 v/v), reduction did occur at a slow rate. The monoreduced products **2b** and **2c** were obtained as a 1:8 mixture along with unreacted starting material (25%) and bis-reduced adduct **2d** (25%). The isolated yield of **2c** was 16%. In a similar fashion, the reduction of **4a** was carried out. In this case, however, the monoreduced products **4b** and **4c** were obtained in about a 1:1 ratio along with starting material (20%) and bis-reduced **4d** (40%). There appear to be subtle differences in the selective reduction of the echinocandins versus the pneumocandins. In order to facilitate the separation of **4b** and **4c**, the mixture was stirred with acidic methanol[3] to derivatize **4c** selectively, to give its methyl ether (X=OMe). The mixture was readily separated to give **4b** in 14% yield.

To explore the possibility that we were generating an acetoxyborohydride species in the above experiment, we substituted $NaBH(OAc)_3$ for $NaCNBH_3$ in TFA as solvent. The results are summarized in Table 2.[11] After 1 min, some over-reduction to the bis-reduced adduct occurred, however, the major product was the lipopeptide monoreduced at C4 of the homotyrosine. Surprisingly, very little product arising from

reduction at C5-*orn* was observed (<5%). A similar experiment using $NaCNBH_3$ in TFA and a 1 min reaction time, gave mostly bis-reduction. However, examination of the incomplete reaction mixture showed that the major monoreduced product was the one arising from reduction at C4-*htyr*. This shows that the distribution of monoreduced products is different in TFA and HOAc. The first entry in Table 2 illustrates the first conversion of echinocandin B to echinocandin C. The second entry is the analogous transformation in the tetrahydro series. The conversion of **2a** to **2b** has previously been described by a multistep procedure.[3]

We wished to control the chemoselectivity of the reduction in a predictable manner to give the lipopeptide exclusively monoreduced at C5-*orn*. Since these reductions presumably occur through a carbocation species, the C4-*htyr* position could be deactivated by acylation of the phenolic hydroxyl group. Mixed carbonates **7b** and **8b** were prepared by treating **7a** or **8a**[12] with methanol (Scheme 1). The methylcarbonates were isolated in 41% and 30% yields, respectively. Reduction of the carbonate was very selective to give **9** or **10**. Only small amounts of the bis-reduced adducts were produced. The carbonates were not isolated but hydrolyzed *in situ* with aqueous NaOH in methanol to give the desired phenolic products **2c** and **6c**, prior to purification.

Scheme 1



7a R = p-$NO_2C_6H_4$, R' = Me, R'' = H
7b R = Me, R' = Me, R'' = H
8a R = p-$NO_2C_6H_4$, R' = H, R'' = $CONH_2$
8b R = Me, R' = H, R'' = $CONH_2$

9 R' = Me, R'' = H
10 R' = H, R'' = $CONH_2$

a) $LiOH \cdot H_2O$, (bis)p-nitrophenylcarbonate, NMP; b) MeOH; c) $NaCNBH_3$, TFA;
d) 2N NaOH (aq), MeOH

Herein, we describe methodology for the selective removal of the C5-*orn* and/or C4-*htyr* hydroxyl groups of echinocandin and pneumocandin lipopeptides. Employment of $NaCNBH_3$ in TFA gives reduction at both the C5-*orn* and C4-*htyr* hydroxyl groups. If an electron withdrawing substituent is present on the homotyrosine phenol to destabilize a positive charge at C4-*htyr*, reduction occurs mainly at C5-*orn*. Use of $NaBH(OAc)_3$ in TFA with short reaction times gives mainly the C4-*htyr* monoreduced adduct. Lipopeptides that do not possess a hemiaminal group are base stable. A discussion of the biological activity of these analogues will be presented elsewhere.

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7. The ^{1}H NMR spectrum for **2d** (CD_3OD, 300 MHz) showed the appearance of a resonance at δ3.70 (1H, dd, J=14,3 Hz) and 2.97 (1H, dd, J=14,4 Hz) (C5-*orn*) and a shift of the aromatic protons from δ7.18 (1H, d, J=8 Hz) and 6.79 (1H, d, J=8 Hz) in **2a** to δ7.02 (1H, d, J=8 Hz) and 6.69 (1H, d, J=8 Hz), respectively, in **2d**. Also, the appearance of δ2.64 (1H, dd, J=13,6) and 2.56 (1H, dd, J=13,8 Hz) in **2d** is assigned to C4-*htyr*. Similar shifts were seen in analogous compounds. The δ3.70 resonance has been assigned to the β hydrogen of C5-*orn*, which lies in the deshielding region of the 3-hydroxy-4-methyl-proline carbonyl according to the crystal structure of the α-chloromercuriobenzyl ether derivative of **2a**. Indeed, the C5-*orn* β hydrogen of tetrahydroechinocandin B itself occurs at unusually high field, δ5.23.
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10. A typical procedure is as follows: Pneumocandin A_0 **4a** (1.02 g, 0.90 mmol) was dissolved in 5.0 mL of trifluoroacetic acid and sodium cyanoborohydride (0.307 g, 4.89 mmol, 5.4 eq) was added under a blanket of N_2 (CAUTION! On a larger scale, the sodium cyanoborohydride ignited on several occasions). The resultant solution was stirred for 30 min. The TFA was removed *in vacuo* and the white solid residue was purified immediately by reverse phase HPLC (2.12 X 25 cm C8 Zorbax, 45% H_2O/55% CH_3CN, 10 mL/min, λ=220, 277 nm). Lyophilization of the appropriate fractions gave the desired compound **4d** as a white solid.
11. A typical procedure for the monoreduction is as follows: Echinocandin B **1a** (100 mg, 74% pure, 0.0943 mmol) and $NaBH(OAc)_3$ (200 mg, 0.944 mmol, 10 eq) were mixed together. To the solids was added 2 mL of TFA at room temperature. After 1 min, the reaction was poured into 50 mL of water and the solid was collected by filtration. The crude product was purified by reverse phase HPLC (2.12 X 25 cm C18 Zorbax, 50% H_2O/50% CH_3CN, 20 mL/min, 20 mL fractions, λ=220, 277 nm). Fractions 12-14 were combined and lyophilized to yield 24 mg (33%) of echinocandin C **1b** and fractions 16-17 were lyophilized to give 7 mg (10%) of the bis-reduced adduct, **1c**.
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