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THE SYNTHESIS OF WATER SOLUBLE PRODRUGS ANALOGS OF ECHINOCANDIN B

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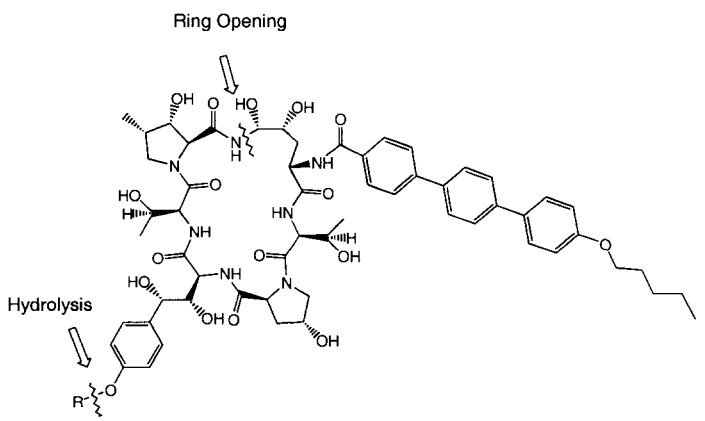
Abstract: A facile synthesis of phosphonate and phosphate ester prodrugs on the phenolic hydroxy of two echinocandin semisynthetic derivatives is reported. The water solubility and stability profiles of the ECB compounds varied with the choice of alkyl group used. In some cases, the ester prodrugs with small aliphatic side chains retained antifungal activity while enhancing water solubility.

The echinocandin class of compounds represent attractive drug candidates for treating fungal pathogens that affect the growing population of immunocompromised patients.[1] In order to improve the water solubility properties of this important class of antifungal compounds, phosphate analogs have been developed. As reported earlier, **2**, the phosphate prodrug of **1** (Figure 1) possessed greatly enhanced water solubility (>12 mg/mL) relative to **1** (<0.1 mg/mL).[2] Although **2** is more water soluble than **1** and is rapidly converted by tissue phosphatases to the parent compound when administered IP or orally, the stability of the molecule was not optimized for process development.

Figure 1. Decomposition of ECB Prodrug Analogs



1. R = H
2. R = P(O)(OH)$_2$

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The by-products seen in the synthesis of **2** resulted from the pH dependent hydrolysis of the phosphate moiety and cleavage of the cyclic peptide ring at the hydroxyl aminal position. At neutral or basic pH, the ring opened material was observed while at low pH, the hydrolysis of the prodrug to parent compound was dominant (Table 1).[3] In order to eliminate these by-products, an SAR study was initiated to find alternative prodrug analogs that provided both enhanced water solubility and stability.

Table 1. Decomposition Rates of **2**

Temperature	pH	% Ring Open	% Hydrolysis
45 °C	7.4	0.13/h	Not detected
55 °C	4.5	0.03/h	0.36/h

Results and Discussion

The synthesis of phosphate and phosphonate esters of the phenolic hydroxy group is outlined in Scheme 1. The use of base for the phosphorylation reaction without prior protection of the hydroxy aminal positions promotes cleavage and rearrangement of the cyclic peptide into inactive by-products.[4] To this end, the hydroxy aminal was protected as a 2-trimethylsilylethyl ether prior to the phosphorylation reaction. The use of 2-trimethylsilylethyl ether also improved the solubility properties of the natural product in organic solvents such as methylene chloride, tetrahydrofuran and dioxane. With the enhancement of organic solubility, a simplified procedure was developed for prodrug synthesis (**1→3→4→5**).

Typically, **1** was dissolved in dioxane followed by the addition of excess trimethylsilyl ethanol and a catalytic amount of *p*-toluene sulfonic acid.[5] After stirring for ~ 3 h at rt, the reaction was worked up by adding solid $NaHCO_3$ and concentrating the reaction volume to an oily residue. After purification by reversed-phase HPLC,[6] the material was lyophilized to give **3**. The silylated product **3** was then treated with LiHMDS before adding a THF solution of phosphorodichloridate $R'P(O)Cl_2$. After stirring for 30 mins at 0 °C, the reaction was quenched with water. After reversed-phase chromatography,[6] the final silyl deprotection step of **4** was accomplished at rt with excess $BF_3{\cdot}OEt_2$ in CH_2Cl_2. After the addition of water, ether was added to precipitate the product to yield pure material, **5**. A sample of prodrugs **5a-e** synthesized is reported in Table 2. Under hydrolytic conditions, the rate of hydrolysis of these prodrugs was negligible. As shown in Table 3, the methyl phosphonate prodrug **5c** is more stable to hydrolysis than **2**.

The phosphate and phosphonate prodrugs of **1** did not prevent the formation of the ring open by-product. In order to design an analog that would be more stable at variable pH range, the hydroxy aminal was removed via a

Scheme 1. Preparation of Phosphate and Phosphonate ECB Prodrugs

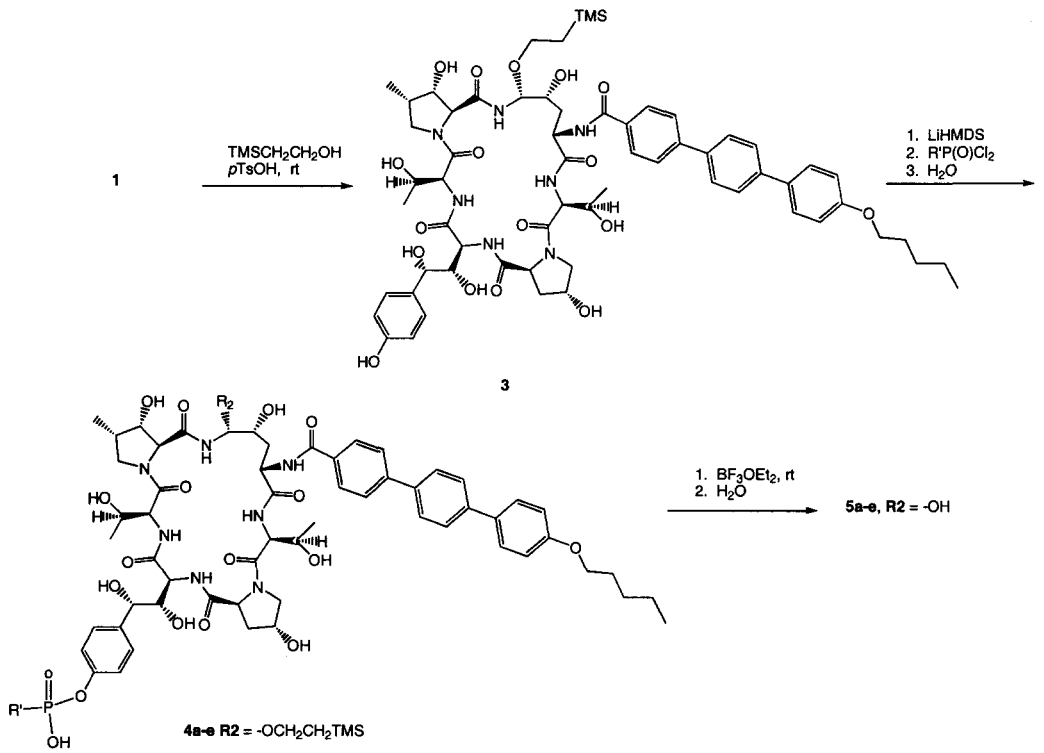


Table 2. Phosphate and Phosphonate Prodrugs of **1**

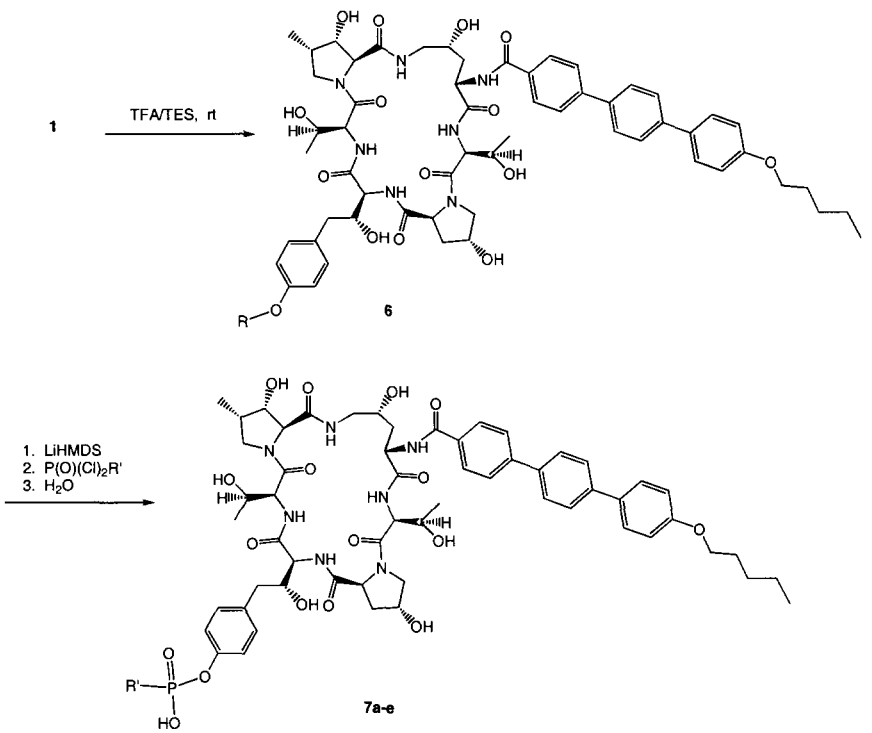
Prodrug , **5**	R'=
5a	$-OCH_3$
5b	$-OCH_2CH_3$
5c	$-CH_3$
5d	$-CH_2CH_3$
5e	-Ph

Table 3. Decomposition Rates of **5c**

Temperature	pH	% Ring Open	% Hydrolysis
45 °C	7.4	0.19/h	Not detected
55 °C	4.5	0.06/h	Not detected

deoxygenation reaction before the phosphorylation reaction (**1**→**6**→**7**, Scheme 2). Typically, **1** was suspended in CH_2Cl_2 and treated with an excess of trifluoroacetic acid and triethylsilane. After 24 h, the solvent was evaporated and the residue subjected to reversed-phase HPLC purification.[6] The removal of both the aminal

Scheme 2. Synthesis of Dideoxygenated ECB Prodrugs



hydroxy and the benzylic hydroxy functional groups occurs under these conditions indiscriminately to produce **6**. Although the selective removal of the aminal hydroxy group over the benzyl hydroxy group has been reported by others,[7] the dideoxy compound was highly desired. The preparation of multiple grams of material using the TFA/TES conditions[8] was very reproducible and most importantly, **6** retained similar biological activity to that of the parent compound.

The use of the dideoxygenated core under strongly basic conditions posed no risk of decomposition. The removal of the two hydroxy groups posed another advantage similar to that found with **3**. Unlike **1**, **6** was

soluble in THF. The phosphorylation reaction was typically conducted by treating a THF solution of **6** with an excess of LiHMDS followed by the addition of the phosphorodichloridate. After quenching with water, the dideoxy prodrugs **7** were isolated and purified by reversed-phase chromatography.[6] A listing of phosphate and phosphonate prodrugs of **6** is given in Table 4.

Table 4. Phosphate and Phosphonate Prodrugs of **6**

Prodrug , 7	R'=
7a	$-OCH_3$
7b	$-OCH_2CH_2CH_2CH_3$
7c	$-OCH(CH_3)_2$
7d	$-CH_3$
7e	$-C_6H_{13}$

The combination of prodrug and natural product core modifications resulted in stable analogs. The water solubility of 20-30 mg/mL was maintained with the smaller alkyl groups (e.g., **7a** and **7d**). As the size of the prodrug ester was increased (e.g., **5e, 7b** and **7e**), the water solubility decreased. In addition to the solubility properties, these novel prodrugs have shown potent antifungal profiles comparable to the parent compound **1** in both in vitro and in vivo assays.[5] As shown in Table 5, the in vitro activity against *Candida albicans* for **7a** and **7d** was ≤0.312 μg/mL and the IP ED_{50} for **7a** and **7d** was 0.47 and 0.79 mg/kg, respectively.

Table 5. *Candida albicans* Antifungal Activity Profile for **1, 7a, 7d**

Prodrug	MIC (μg/mL)	IP ED_{50} (mg/kg)
1	0.01	0.32
7a	0.312	0.47
7d	0.039	0.79

Summary

We have described the preparation of novel echinocandin prodrug analogs. The alternative prodrug designs and the deoxygenation of the core nucleus provided analogs that maintained the desired water solubility profile while increasing the stability and antifungal properties. These compounds have shown promising features relative to that of **1**, but with the improved water solubility and stability these compounds have properties better suited for process development.

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References and Notes

1. Rodriguez, M. J.; Hitchcock, S. A. *Exp. Opin. Ther. Patents* **1997**, *7*, 829.

2. Turner, W.; Debono, L.; LaGrandeur, L; Burkhardt, F.; Rodriguez, M.; Zweifel, M.; Nissen, J.; Clingerman, K.; Gordee, R.; Zeckner, D.; Parr, T.; Tang, J. 33rd International Conference on Antimicrobial Agents and Chemotherapy, New Orleans, LA; American Society of Microbiology: Washington, DC, **1993**, Abstract 358.

3. A concentration of prodrug (1 mg/mL) in phosphate buffers was heated at the desired temperature for >48 h % ring open and hydrolysis by-products followed by reversed-phase HPLC.[6]

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6. Preparative reversed-phase HPLC analysis was performed with a Waters LC2000 prep system using 3X radial cartridges packed with Nova-pak® (C18- 40/100 mm, 50:50 acetonitrile/water solvent systems) with flow rate of 40 mL/min and using UV detection at 280 nm.

7. Balkovec, J. M.; Black, R. M. *Tetrahedron Lett.* **1992**, *33*, 4259.

8. To a suspension of **1** in methylene chloride (100 mg/mL) at 0 °C was added triethylsilane (25 molar equivalents) followed by slow addition of trifluoroacetic acid (25 molar equivalents). The reaction was allowed to stir at rt for 24-48 h. The reaction volume was reduced by rotary evaporation and the concentrated solution was purified by reversed-phase HPLC[6] to afford **6** in 70% yield.