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Studies on the Phosphorylation of LY303366

Uko E. Udodong,* William W. Turner,* Bret A. Astleford, Frank Brown Jr., Marcella T. Clayton, Steven E. Dunlap, Scott A. Frank, John L. Grutsch, Lisa M. LaGrandeur, Daniel E. Verral, and John A. Werner

Lilly Research Laboratories, Eli Lilly and Company

Lilly Corporate Center, Indianapolis, Indiana 46285

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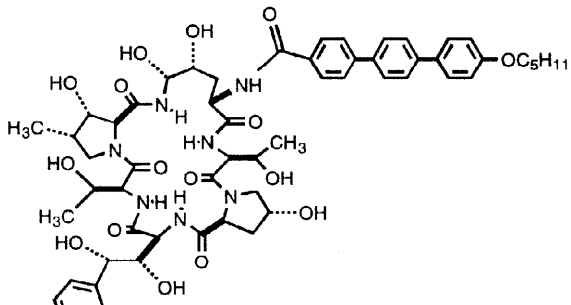
Abstract: Phosphorylation of LY303366 (**1**) was studied in THF and DMF. Benzyl phosphate **2** could be prepared in excellent yield using LiOH as the base. Both **2** and the derived phosphonic acid monosodium salt **3** were prone to undergo hydrolytic dephosphorylation.

The antifungal echinocandin natural product class has been the subject of chemical modification in several laboratories.[1] Our research group has developed LY303366 (**1**), a semisynthetic analog of echinocandin B, as a potential treatment for serious systemic *Candida* or *Aspergillus* infections.[2] The water solubility of **1** is low (<0.1mg/ml) which makes formulation difficult for IV administration. Research done on a related class of antifungals, the pneumocandins, by a Merck group has shown that water solubility can be significantly increased for this type of molecule by addition of ionizable groups to the cyclic peptide moiety.[3] Using a prodrug approach, the phenolic hydroxyl is a convenient attachment point for a variety of solubilizing groups of which phosphate has proven to have the best combination of solubility and activity properties. This paper presents the synthetic optimization studies for the production of LY307853 (**3**), the phosphate prodrug of LY303366.



1, R = H,
2, R = P(O)(OBn)$_2$
3, R = P(O)(OH)ONa

Given the structural complexity of **1** and the availability of multiple protonation and deprotonation sites, the polypeptide domain was expected to have defined ranges of acid and base stability. Seminal work

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revealed that **1** was stable in the pH range of 4-7. Of relevance to the phosphorylation chemistry was the observed propensity of **1** to undergo ring opening under basic conditions. The ring opening event was apparently initiated by the abstraction of the hemi-aminal hydroxyl proton. Once opened, a subsequent 5-membered ring closure occurs to form the unstable product **4**.[4] Two phosphorylating agents, dibenzyl chlorophosphate (DBCP)[5] and tetrabenzyl pyrophosphate (TBPP)[6], were selected. For reasons related to solubility and product isolation, THF and DMF were the only suitable solvents.

Tables 1 and 2 show the results of the phosphorylation of **1** (0.2M solution) with 1.4 molar equivalents of DBCP and TBPP, respectively. To minimize ring opening, it was essential to add the base at such a rate as to avoid accumulation. An excess of the phosphorylating agent was required. TBPP was 98% pure and was prepared by DCC-mediated self condensation of dibenzyl phosphate in THF.[6] Purity of TBPP as low as 90% could be used; however, there was a direct correlation between the percent of dibenzyl phosphate (major contaminant of TBPP) and the amount of ring opening by-product formed. TBPP was introduced as a solution in THF. Up to 20% THF (based on the total volume of the reaction) could be tolerated. Higher levels of THF retarded the reaction and encouraged ring opening.

Table 1. Phosphorylation of 1 with DBCP

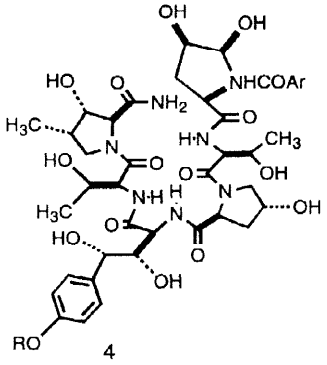
Entry	Solvent	Base	Temp (°C)	% **2**[7]
1	THF	tBuOLi	0	33
2	DMF	tBuOLi	0	0
3	4:1 THF/DMF	tBuOLi	0	30
4	THF	LiOTMS	0	30
5	DMF	Bu_4NOH	-10	51
6	THF	nBuLi	-60→ 0	40
7	DMF	nBuLi	-60→ 0	59

DBCP gave unsatisfactory results with a variety of bases (Table 1). Using *t*-BuOLi as the base, the yield of **2** from DBCP was higher at 0 °C than at room temperature; but the reaction tended to stall at 0 °C, leading eventually to the ring opening of **1** and **2**. Excellent yield of **2** was obtained with TBPP when 3M aqueous LiOH was added to 0.2M solution of **1** in DMF at -30 °C (Table 2).[8] Although 3M aqueous NaOH and KOH could also be employed, the rate of the reaction decreased in the order Li>Na>K. By-products resulting from the cleavage of **1** and **2** invariably accompany NaOH and KOH reactions.

Table 2. Phosphorylation of 1 with TBPP

Entry	Solvent	Base	Temp (°C)	% **2**[7]
1	THF	tBuOLi	rt	64
2	1:1 THF/DMF	tBuOLi	rt	87
3	THF	tBuOLi	0	79
4	DMF	tBuOLi	0	83
5	DMF	nBuLi	-20	44
6	DMF	LiOH	-25	90
7	DMF	LiOH	-30	96
8	DMF	NaOH	-30	93
9	DMF	KOH	-30	67

A method for protecting the aminal hydroxyl was sought to prevent ring opening. When **1** was treated with benzaldehyde or *p*-methoxybenzaldehyde in the presence of catalytic amount of camphorsulphonic acid, the homotyrosine diol was selectively protected to give **6**, instead of the desired dihydroxyornithine protected product **5**. Attempts to prepare an isopropylidene ketal led to a mixture of **7** and **8**. While the homotyrosine diol of **7** and **8** could be liberated by aqueous acid hydrolysis, liberation of the dihydroxyornithine diol from **7b**, **8**, or **9a** (obtained by treating **1** in methanol with catalytic amount of HCl) was not possible under mild acid (aq HOAc) conditions. From these results, aminal protection in **1** by ketalization was not a viable strategy.



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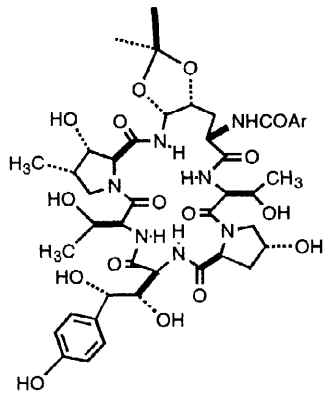

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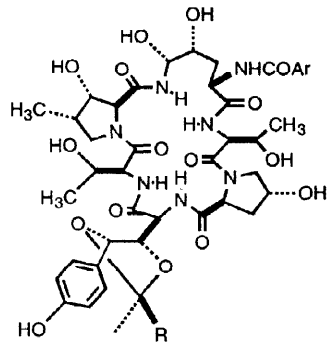
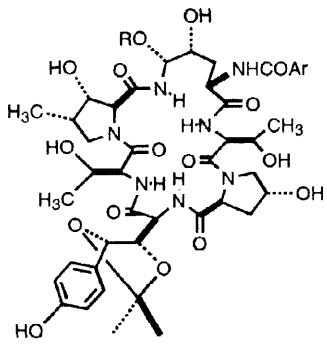
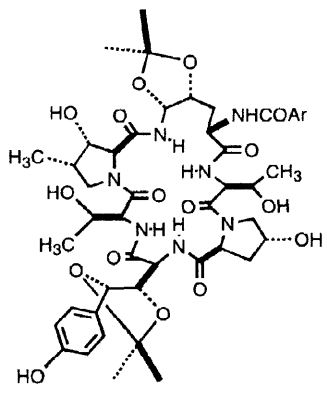


6a, R = C_6H_5
b, R = 4-MeO-C_6H_4



7a, R =H
b, R = Me



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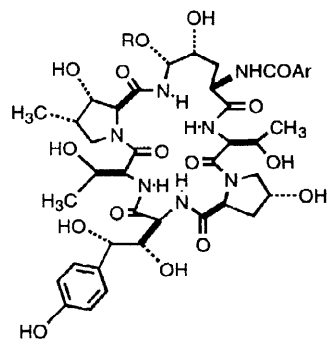

9a, R = Me
b, R = $C_6H_4CH_2$
c, R = 4-MeO-C_6H_4

Protection of the aminal by benzylic ether formation could be accomplished by reaction with benzyl alcohol in dioxane using catalytic p-toluenesulfonic acid. This protecting group did not prove useful because removal of the benzyl by hydrogenation was sluggish with variably low yields of free alcohol.

Removal of benzyl protection from phosphate **2** was achieved by hydrogenation over 5% Pd/C in acetic acid containing NaOAc, giving the phosphate monosodium salt **3**. A critical finding concerning these phosphates was that both **2** and **3** could be stored at -20 °C without significant loss of potency. Above -20 °C,

however, a slow degradation occurred (approximately 10% loss of potency in 6 weeks at 30 °C) with the principal degradation route being a hydrolytic dephosphorylation to return to **1**.

In summary, the phosphorylation of LY303366 (**1**) was examined in THF and DMF with a number of bases. Using tetrabenzyl pyrophosphate as the phosphorylating agent, 3M LiOH in DMF at -30 °C gave the best results. Base promoted ring opening of **1** and **2** is problematic in this reaction. Attempts to suppress this event through protecting of the aminal hydroxyl were unsuccessful. Benzyl phosphate **2** was converted to the monosodium phosphate by hydrogenation over Pd/C in the presence of sodium acetate. Compounds **2** and **3** are stable at -20 °C but hydrolytically dephosphorylate at warmer temperatures.

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References and Notes

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4 This 5-membered ring closure has also been proposed in the pneumocandin class (see ref. 3).

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7. Yields determined by HPLC (UV area percent).

8. **Typical phosphorylation procedure**: A solution of **1** (4.6 g, 4.0 mmol) in DMF (15 mL) at -30 °C under nitrogen was treated with a solution of freshly prepared TBPP (3.3 g, 6.1 mmol) in 1.2 g of THF. 2M aqueous LiOH was added dropwise over 45 min while keeping the temperature at -30 to -28 °C. After the addition, the reaction mixture was stirred for another 0.5 hr and monitored by HPLC. The reaction was quenched with acetic acid (3 mL) and allowed to warm to 0 °C. The mixture was poured into acetonitrile (100 mL). After being kept overnight in the freezer, the precipitated solid was collected by filtration and rinsed with acetonitrile and methyl t-butyl ether. The dried solid was washed with water and dried to obtain 4.3 g of **2** (Table 2, entry 7).