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DESIGN AND SYNTHESIS OF 6-(6-D-RIBITYLAMINO-2,4-DIHYDROXYPYRIMIDIN-5-YL)-1-HEXYLPHOSPHONIC ACID, A POTENT INHIBITOR OF LUMAZINE SYNTHASE

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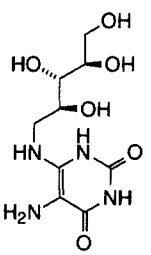
Abstract: A novel inhibitor of lumazine synthase, the penultimate enzyme in the biosynthesis of riboflavin, has been synthesized. The inhibitor was designed by computer graphics molecular modeling using a hypothetical structure of the enzyme-inhibitor complex. The new compound is relatively potent when compared with the known inhibitors, and displays a K_I of 109 μM.

The later stages of riboflavin biosynthesis involve the lumazine synthase-catalyzed reaction of 5-amino-6-D-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione (**1**) with 3,4-dihydroxy-2-butanone 4-phosphate (**2**) to form 6,7-dimethyl-8-D-ribityllumazine (**3**) and inorganic phosphate.[1] Two molecules of the lumazine **3** then undergo a novel riboflavin synthase-catalyzed dismutation reaction in which a four-carbon unit is transferred from one molecule of **3** to another one, resulting in riboflavin (**4**) and regeneration of the pyrimidinedione **1**, which can then be recycled.[2–4] This pathway offers a target for the development of new antibiotics, since enterobacteria such as *Escherichia* and *Salmonella* species lack a riboflavin uptake system and are therefore absolutely dependent on endogenous synthesis of the vitamin.[5]



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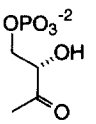

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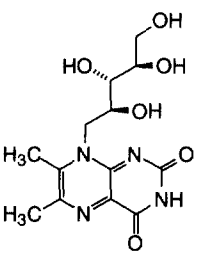

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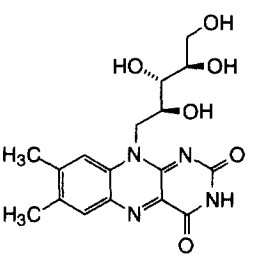

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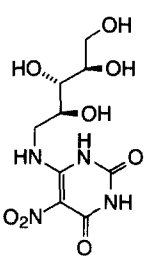

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The crystal structure of the active site of lumazine synthase containing bound **5** provides a starting point for the design of potential enzyme inhibitors.[6,7] The structure suggests a hypothetical model[7] for the binding of the Schiff base **6**, which would be formed from **1** and **2**, and is a thought to be involved in the conversion of **1** and **2** to **3** through intermediates **7**, **8**, and **9** as shown in Scheme 1.[8] The model of **6** bound to the enzyme indicates that potential inhibitors with appropriate functionality corresponding to the phosphate group of **6**, but

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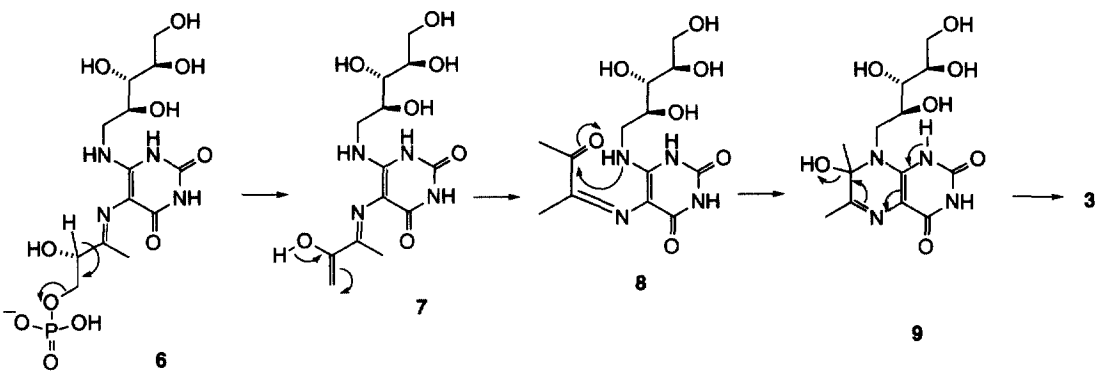
are more stable than **6** when bound to the enzyme, might in fact serve to inhibit the enzyme. Figure 1 shows the hypothetical structure of a potential phosphonate inhibitor **15** (Scheme 2) bound to lumazine synthase. The model was constructed by overlapping the ribitylaminopyrimidine fragment of **15** with the X-ray structure of bound **5**, with the phosphate group of **15** occupying the space where buffer-derived inorganic phosphate is found in the X-ray structure.[6,7] The structures of **5** and inorganic phosphate were then removed, the protein "frozen", and the energy minimized while allowing bound **15** to move. This procedure was carried out using Sculpt® 2.5 (Interactive Simulations, Inc.) software. According to this structure, the proposed inhibitor **15** fits nicely within the active site of lumazine synthase, with the phosphate group positioned near Arg127, Thr86, and H_2O, and the pyrimidine ring of the inhibitor stacked with Phe22.

Scheme 1



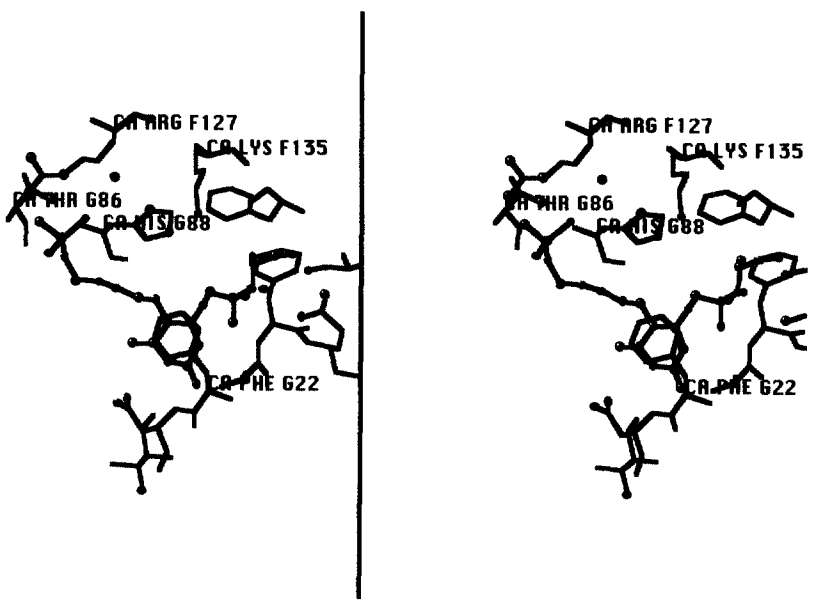
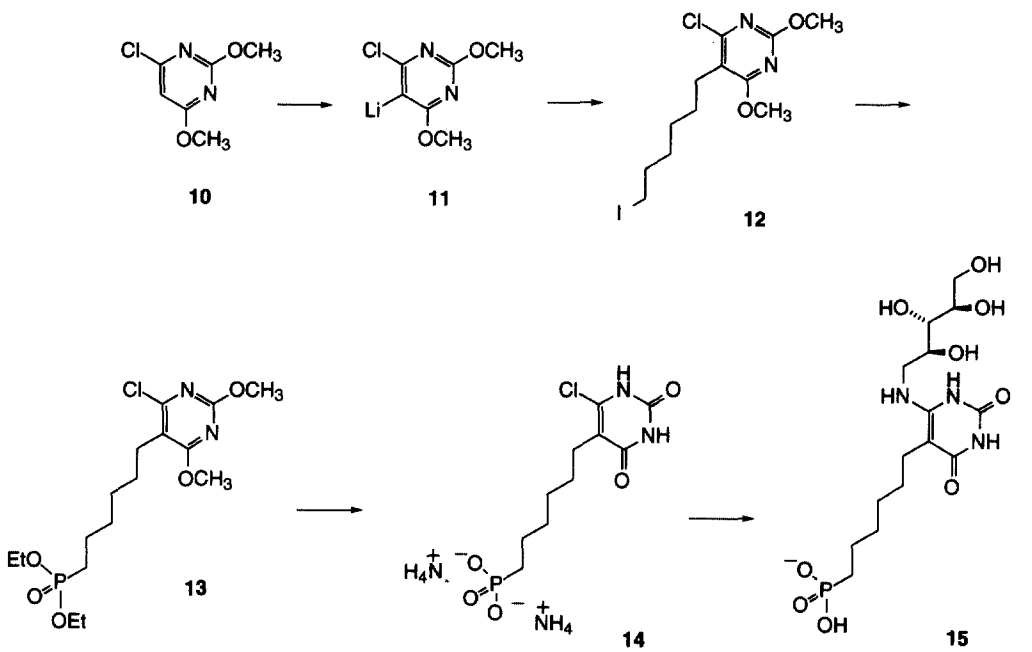


Figure 1. Hypothetical model of the binding of phosphonate **15** to lumazine synthase. The figure is programmed for walleyed viewing.

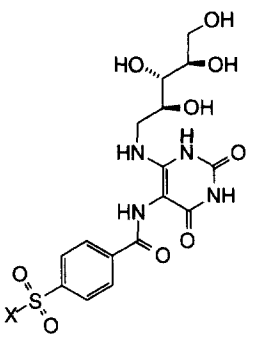
In Figure 1, the proposed inhibitor **15** is shown in purple in the enzyme active site, and the amino acid side chains are colored brown. The active site of the enzyme exists at the interface of two subunits, and some of the backbone fragments of these two subunits are represented in Figure 1 in orange and blue.

The starting point for the synthesis of the proposed inhibitor **15** was 6-chloro-2,4-dimethoxypyrimidine (**10**), which on treatment with *n*-butyllithium in THF at -78 °C for 15 min afforded the lithiated species **11**.[9,10] Intermediate **11** reacted with 1,6-diiodohexane at -78 °C to room temperature for 12 h to yield intermediate **12** in 84% yield. Treatment of diethylphosphite with sodium hydride in DMF at room temperature for 45 min afforded the corresponding anion, which reacted with the iodide **12** at room temperature for 45 min followed by 95 °C for 4 h to provide the diethylphosphonate **13** in 25% yield. The two methyl groups as well as the two ethyl groups were removed from **13** with trimethylsilyl iodide in methylene chloride at room temperature for 23 h to give **14** in 100% yield.[11] Reaction of the chloride **14** with D-ribitylamine[12] in 2-methoxyethanol at reflux for 23 h resulted in Michael addition of the amine to the α,β-unsaturated carbonyl moiety present in **14**, followed by chloride elimination to afford the desired product **15** in 53% yield.

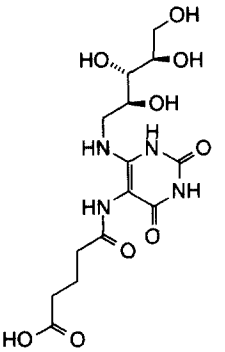
Scheme 2



The phosphonate **15** was tested as an inhibitor of lumazine synthase β_{60} capsids from *Bacillus subtilis*. It proved to be the most potent inhibitor of lumazine synthase reported to date, with a K_I of 109 μM. For comparison, the previously synthesized inhibitors had the following K_I values: **16** (200 μM),[13] **17** (360 μM),[13] **18** (430 μM),[13] **19** (470 μM),[14] and **20** (330 μM).[14] The corresponding pentamethylene and tetramethylene analogs of **15** were less potent, having K_I values of 123 and 410 μM, respectively. The relatively potent inhibitory activity of **15** may provide further insight that might be utilized in the design of more biologically active inhibitors of lumazine synthase.



16, X = F
17, X = ONa



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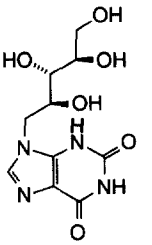

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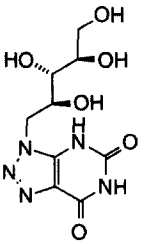

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The lumazine synthase inhibitor **15** or a related compound might also eventually be used to provide useful information about the binding of the hypothetical intermediate **6** to the enzyme. The existing hypothetical model of the binding of **6** is based on the X-ray structure of bound **5**.[6,7] The phosphate group of **6** in the published model was placed in a region of electron density corresponding to bound inorganic phosphate in the X-ray structure of the complex of the enzyme and **5**.[6,7] A crystal structure of **15** bound to the enzyme might provide more direct information about the binding of the hypothetical intermediate **6**.

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