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Rational design and synthesis of novel nucleotide anti-*Giardia* agents

Dae-Hwan Suk,[a] Laurent Bonnac,[a] Christine C. Dykstra,[b] Krzysztof W. Pankiewicz[a] and Steven E. Patterson[a,*]

[a]*Center for Drug Design, Academic Health Center, University of Minnesota, Minneapolis, MN 55455, USA*
[b]*Department of Pathobiology, Auburn University, Auburn, AL 36832, USA*

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Abstract—Design and synthesis of a novel nucleotide anti-*Giardia* agent that is micromolar inhibitor of *Giardia* trophozoite growth in culture is described.

Protozoa are one of the more common causes of infections and illness in humans and animals worldwide.[1,2] *Giardia lamblia* is one of the most commonly diagnosed protozoal cause of diarrhea in developed nations and yearly there is an estimated 100 million cases of giardiasis worldwide. *Giardia* is also commonly found in livestock as well as companion animals and many mammals may serve as important reservoirs capable of transmitting disease to humans.[3–7] To date widely effective treatment, broad-spectrum antimicrobial agents for prophylaxis, or effective vaccines, for *Giardia* is not available.[8,9] In addition, clinical resistance has been reported for current chemotherapeutics, including cases where both metronidazole and albendazole failed in treatment of *giardiasis*.[7–10] *Giardia* infections are therefore a significant worldwide risk to the health of humans and animals.

Giardia has unique features that make it exceptionally well adapted for survival in, and dissemination by water and this further complicates treatment of infections. This pathogen is difficult to detect in water and cysts are highly resistant to common treatments such as chlorine and ozone. Resistance of these organisms to chemical treatment is such that 10% bleach or a mixture of 2% sulfuric acid and 2.5% potassium dichromate is necessary to fully inactivate cysts.[1,2]

While differing protozoal species have quite different life cycles this environmentally resistant and infective cyst is common to many protozoans.[11,12] Because encystation appears to occur in response to unfavorable growth conditions, it is highly likely that prevention of cyst formation would be fatal.[13] However, unlike bacterial cell wall synthesis protozoal cyst wall synthesis has not been well exploited as a drug target.[14] Yet because *Giardia* must encyst to complete its life cycle cyst wall synthesis is an extremely attractive target for chemotherapy.[15–17]

While protozoal cyst walls are not as fully characterized as cell walls in other organisms, it is known that some, that is *Giardia, Entamoeba*, and *Toxoplasma*, contain chitin or a chitin-like polysaccharide.[13,16–19] Recent description of enzyme activity termed cyst wall synthase in *Giardia*[16,17] inspired us to investigate this as a novel drug target. We report here design, synthesis, and activity of a novel potent anti-*giardial* agent that was designed as an inhibitor of protozoal cyst wall biosynthesis.

Biosynthesis of cyst walls in *Giardia* is mediated by an enzyme called cyst wall synthase (CWS). CWS catalyzes synthesis of the chitin-like poly β-1-3-linked *N*-acetylgalactosamine [poly(GalNAc)] that comprises about 63% of the *giardia* cyst wall. CWS has a high affinity (K_m = 0.048 mM) for its substrate and glycosyl donor, UDPGalNAc, V_{max} = 0.07 nmol/(min mg protein). This synthase requires (in order of preference) the divalent cations Ca^{2+}, Mg^{2+}, Co^{2+}, Mn^{2+}, and Zn^{2+}. Metal chelators such as EDTA inhibit CWS. Finally, CWS is specific for UDPGalNAc. UDP-glucose, UDPGlcNAc,

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* Corresponding author. Tel.: +1 612 625 7962; e-mail: patte219@umn.edu

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UDP-galactose, glucosamine, and galactosamine are not substrates.[16]

As with other glycosyl transferase processes, synthesis of poly(GalNAc) is proposed to proceed through a transition state with a positive charge on the hexose moiety of the UDPGalNAc substrate (Fig. 1).[20]

In *Giardia* UDPGalNAc is synthesized from UDPGlcNAc.[15] Because other protozoal species, such as *Entamoebae*, possess a cyst wall comprised of chitin which is synthesized from UDPGlcNAc, we therefore hypothesized that analogs containing a stable linker between uridine and an *N*-acetylglucosamine analog might inhibit cyst formation in a variety of protozoal species. We have designed and synthesized such a substrate analog that has an alkylphosphonate linkage between *N*-acetylglucosamine and the uridine moiety in place of the natural pyrophosphate linkage (Fig. 2). Our inhibitor retains the alpha-configuration at the anomeric center of the *N*-acetylglucosamine moiety to more accurately mimic the natural pyrophosphate which is also the alpha anomer. Similar C-glycosides containing phosphate linkages have been reported as inhibitors of glycosyl transferases and epimerases.[21,22]

The phosphonates described here have an advantage over the previously described analogs because, in contrast to phosphates and pyrophosphates which do not readily cross cellular envelopes, phosphonates are able to penetrate cell membranes. In addition phosphonates are chemically and metabolically stable, whereas phosphates are subject to metabolic degradation.[23,24]

We began synthesis of target **1** by Wittig reaction of known uridine derivative **2**[25] (Scheme 1) with ylide **9** (Scheme 2) to give phosphonate **3** as exclusively the E isomer. Deprotection of the phosphonate moiety and concomitant reduction of the double bond were performed by treatment with hydrogen and palladium on barium sulfate. The resulting phosphonic acid, **4**, was coupled with C-glycoside **12** using DCC in pyridine with

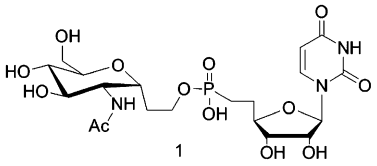

Figure 2. UDPGlcNAc analog.

Dowex-50 (H^+) and the protecting groups were removed using standard methods. Glycoside **12** was readily prepared from known C-glycoside **10** (Scheme 3).[21]

The target **1** was screened for activity against *G. lamblia* WB-6 strain. Key intermediates **10** and **12** were treated with ammonia in methanol (Scheme 4) and the resulting free alcohols, **13** and **14**, were also screened for activity against the same pathogen. Trophozoite inhibition was determined by culturing the parasites anaerobically for 48 h in the presence of 2-fold drug dilutions in triplicate. The XTT reagent was used to measure cell viability. Cyst development was determined in a similar manner except the assay was conducted for 4 d in encysting medium glass vials. The cysts were counted, washed in deionized water, and resuspended in trophozoite medium to evaluate cyst viability. Manine Darbey Bovine Kidney (MDBK) cells were used to evaluate host toxicity using a standard method.[26]

The results show that while all these compounds are active against trophozoite growth, with micromolar MIC values, the target molecule **1** is the least toxic and therefore has the greatest therapeutic index (Table 1).

In summary, we have designed and synthesized a novel, potent anti-*Giardia* agent. This new agent, **1**, is slightly more active against *Giardia* trophozoite culture than metronidazole (MIC 4.8 μM). Currently studies of the activity of this agent against CWS, chitin synthase as well as design and synthesis of second generation agents with improved activity and lower toxicity are in progress and will be reported in due course.

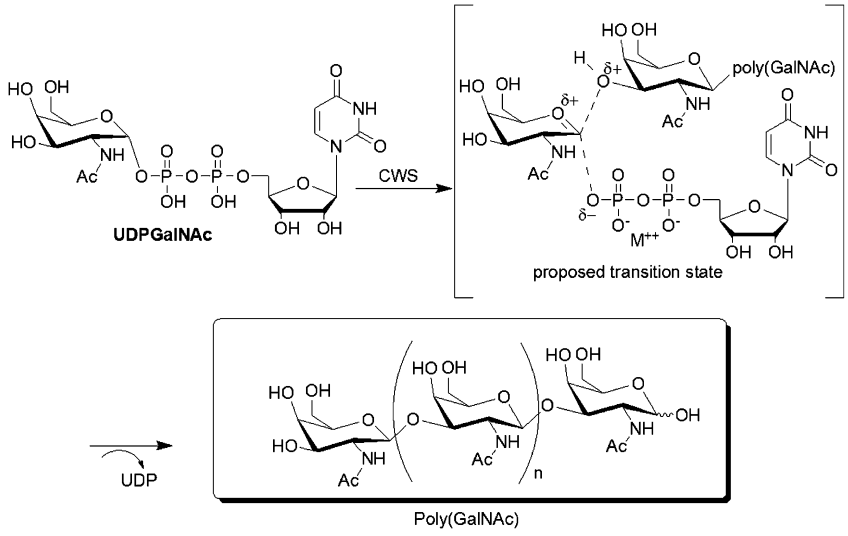

Figure 1. Synthesis of poly(GalNAc) by cyst wall synthase.