Synthesis of a Potent Transition-State Inhibitor of 5′-Deoxy-5′-methylthioadenosine Phosphorylase

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Abstract: Human 5′-deoxy-5′-methylthioadenosine phosphorylase (MTAP) participates in the purine salvage pathway to generate adenine and methylthioribose-1-phosphate, which in turn is converted into adenine nucleotides and methionine. Hence, inhibition of MTA phosphorylase may be an effective target in the design of potential antiproliferative agents. Presented herein is the synthesis of 2-(4-amino-5*H*-pyrrolo-[3,2-*d*]pyrimidin-7-yl)-5-methylsulfanylmethylpyrrolidin-3,4-diol (**1**), a potent inhibitor of MTAP.

Introduction. 5′-Deoxy-5′-methylthioadenosine phosphorylase (MTAP) catalyzes the reversible phosphorolysis of 5′-deoxy-5′-methylthioadenosine (MTA) to adenine and 5-methylthio-D-ribose-1-phosphate. MTA is a byproduct of polyamine biosynthesis, which is essential for cell growth and proliferation. This salvage reaction is the principal source of free adenine in human cells.[1] Because of the importance in coupling the purine salvage pathway to polyamine biosynthesis, MTAP is a potential chemotherapeutic target. Biochemical evidence suggests that mammalian MTAP is a trimer made up of three identical units of 32 kDa. Protein sequence analysis has shown that human MTAP is about 25% identical with members of the mammalian purine nucleoside phosphorylase (PNP) family of trimeric enzymes.[2,3] While PNP plays a role in the purine salvage pathway to catalyze the phosphate-dependent cleavage of nucleosides, MTAP is highly specific for 6-aminopurine nucleoside substrates.[4]

The overall quaternary structure and subunit topology of MTAP are somewhat similar to mammalian PNP with some slight differences.[5] The crystal structure of human MTAP reveals that it is a trimer containing three subunits related by C_3 symmetry. The majority of the trimer surface is exposed to the solvent channels that make up a large percentage of the cell volume. The subunit contacts within the trimer are extensive and involve residues from strand β7 and the loop connecting β7 to helix α2 and helix α3 and the loop connecting β9 to α3. At the trimer interface, there is hydrophobic interaction of three Trp189 residues and hydrogen-bonding network with three Thr118 residues. The active site of MTAP contains three distinct regions or binding pockets that correspond to the purine base, methylthioribose, and sulfate/phosphate binding sites.

The loss of MTAP activity in several malignant human cell lines has been identified as being due to deletions on chromosome 9 of the MTAP and the p16/MTS1 tumor suppressor gene.[6,7] The absence of the tumor suppressor gene leads to the onset of the malignancy and development of cancer leading to loss of MTAP activity. Upon loss of MTAP activity, cells are unable to recycle adenine back to nucleotides. As a result MTAP$^-$ cells would be more susceptible to the growth inhibitory effects of chemotherapeutic drugs that target the purine biosynthesis pathway. It has been observed that cancer cell lines that lack MTAP are more sensitive toward known chemotherapeutic drugs such as methotrexate and azaserine in the presence of MTA, whereas cancer cell lines with MTAP activity (MTAP$^+$ cells) are not as severely affected. On the basis of these observations, it is possible to enhance the treatment of MTAP$^+$ tumors with a potent MTAP inhibitor along with traditional chemotherapeutic compounds that target the purine salvage pathway.[8]

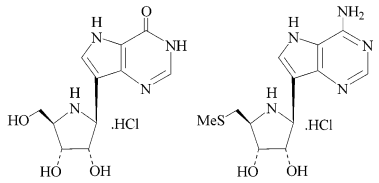

Figure 1. Inhibitor of MTAP, compound **1**.

Over the past 2 decades, various groups have identified several analogues of methylthioadenosine and deazaadenosine as potent inhibitors of MTAP.[9] Recently Schramm et al. have investigated kinetic isotope effects on PNP to predict the transition-state structure. This analysis has led to the development of a novel class of inhibitors that are very potent against PNP. These compounds are aza-C-nucleosides, and one is currently being developed as BCX-1777 by BioCryst Pharmaceuticals, Inc.[10,11] On the basis of the similarity of the crystal structures of PNP and MTAP, we proposed that a similarly designed BCX-1777 transition-state analogue could be constructed for MTAP. We proposed that the replacement of the 5′-hydroxy group with a 5′-thiomethyl moiety and the 6-oxo with 6-amine could give an inhibitor selective for MTAP because these groups take advantage of the unique binding properties of MTAP. Herein, we describe the synthesis of 2-(4-amino-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-5-methylsulfanylmethylpyrrolidine-3,4-diol (**1**) (Figure 1).

Chemistry. Several synthetic routes were attempted to prepare **1**. The target aza-C-nucleoside (**1**) was successfully prepared using the methodology as outlined in Scheme 1. Our ultimate selection of the route was based on early work from our group in which a substituted aldehyde is used to construct a substituted aminopyrrole, which is further elaborated to a pyrrolo[3,2-*d*]pyrimidine derivative. This route was also used by Schramm et al. in the first reported synthesis of BCX-1777.[11c] Compound **2**, synthesized by a previously known method,[11d] was treated with a catalytic amount of DMAP and excess triethylamine followed by *p*-toluenesulfonyl chloride at ambient temperature. Upon workup of the reaction, the crude tosylate was taken directly to the next step without any further purifica-

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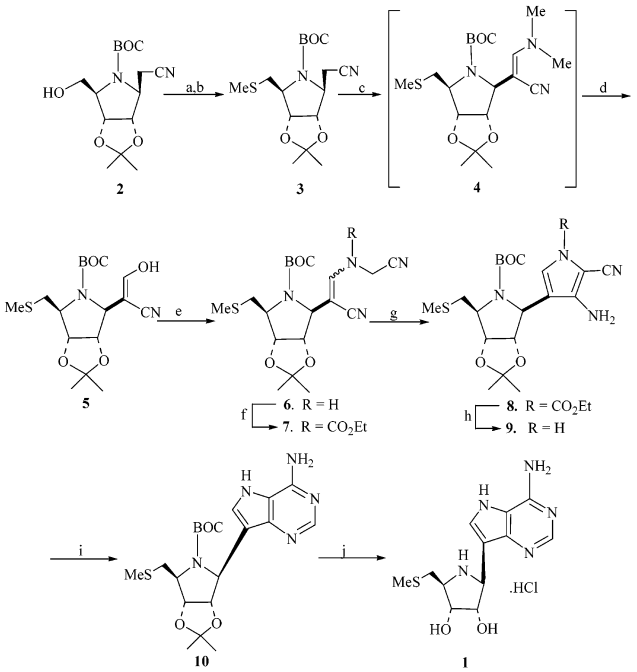
Scheme 1. Total Synthesis of MTAP Inhibitor **1**[a]



[a] Reagents: (a) TsCl, TEA; (b) NaSMe, MeOH (96%, two steps); (c) $BuOCH(NMe)_2$; (d) $THF/H^+/H_2O$ (58%, two steps); (e) NH_2CH_2CN, NaOAc (*E*/*Z* mixture, 78%; (f) ethyl chloroformate, DBU; (g) DBU; (h) 0.1 equiv, Na_2CO_3 (60%, three steps); (i) formamidine acetate, EtOH (quantitative); (j) H^+/MeOH (85%).

tion. The thiomethyl group was introduced using sodium thiomethoxide, which replaced the 5′-*O*-tosyl group. Purification of the sample gave the desired compound **3** as a syrup in 96% yield (two steps). Treatment of **3** with *tert*-butoxy-bis(*N*,*N*-dimethylamino)methane (Bredereck's reagent) in DMF at 70 °C for 1 h gave the enamine **4**, which was used without any purification. The crude enamine was subjected to mild acid hydrolysis to afford **5** as syrup (58%, two steps). The ^1H NMR spectra of **4** and **5** indicated that they were single isomers. Compound **5** was treated with aminoacetonitrile and sodium acetate at ambient temperature and chromatographed to give **6** as a mixture of *E*/Z diastereomers, as indicated by NMR spectra. On the basis of earlier experience, the enamine nitrogen had to be protected to effect a cyclization to the pyrrole under basic conditions. Conversion of **6** to **9** was conveniently carried out as a one-pot reaction. Compound **6** was temporarily protected as the carbamate by treatment with ethyl chloroformate in the presence of 1 equiv of DBU at 0 °C for 1 h. Addition of 1 equiv of DBU to the same pot and stirring at ambient temperature gave the pyrrole **8**. Evaporation of the solvent followed by treatment with sodium carbonate furnished **9** (60%, three steps). Treatment of **9** with formamidine acetate furnished **10** in quantitative yield. Finally, deprotection of compound **10** under acidic conditions furnished the target molecule **1** (85% yield). Compound **1**, an inhibitor of the MTAP enzyme, had an IC_{50} of 1.2 ± 0.3 nM and was 100- to 1000-fold more potent than any other known inhibitors.[12,13] Compound **1** was evaluated in MOLT-4 (T-cells from an acute lymphoblastic leukemia patient), which is an $MTAP^+$ cell in the presence and absence of methatrexate. Compound **1** did not inhibit the proliferation of MOLT-4 cells up to 10 μM. However, a combination of compound **1** in the presence of 3 μM MTA demonstrated inhibition with an IC_{50} of 1 μM. A combination of 100 nM methotrexate and 10 μM compound **1** demonstrated no significant inhibition.

Inhibition of MTAP by Compound 1. A radiochemical enzyme assay was used to determine the inhibitory potency of compound **1** against MTAP. Enzyme activity was measured following the conversion of 8-[^{14}C]-5′-deoxy-5′-methylthioadenosine (MTA) to 8-[^{14}C]-adenine. The reaction was started by the addition of human erythrocyte MTAP enzyme. The enzymatic product adenine was separated by chromatography, and the radioactivity was measured.

Cell Proliferation Assay. MOLT-4 cells were incubated with various concentrations of compound **1** in the presence and absence of 3 μM methylthioadenosine. In another experiment the MOLT-4 cells were incubated with various concentrations of compound **1** and 100 nM of methotrexate. After incubation for 72 h at 37 °C and 5% CO_2, the cells were pulsed for 4 h with [^{3}H]-thymidine, harvested, and incorporated. The radioactivity was quantitated by a β-counter. The IC_{50} is defined as the concentration of the drug at which 50% reduction in proliferation is observed.

Supporting Information Available: Description of the chemical synthesis and characterization of the intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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