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# Synthesis of a potent 5′-methylthioadenosine/*S*-adenosylhomocysteine (MTAN) inhibitor

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**Abstract**—MTAN has been known to occur in a variety of bacterial cell types. Due to the evolution of bacterial strains which are resistant to some of the most powerful antibiotics there has been a renewed interest in the development of novel anti-microbial agents. Presented herein is a synthesis of a potent MTAN inhibitor, namely 2-amino-4-[5-(4-amino-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3,4-dihydroxypyrrolidin-2-ylmethylsulfanyl]-butyric acid (**1**).


5′-Methylthioadenosine/*S*-adenosylhomocysteine nucleosidase (MTAN) catalyzes the hydrolysis of 5′ methylthioadenosine (MTA) to adenine and 5′-methylthioribose (MTR), and the hydrolysis of *S*-adenosylhomocysteine (SAH) to adenine and *S*-ribosylhomocysteine (SRH).[1] MTAN occurs in a variety of bacterial cell types (both Gram-positive and Gram-negative).

Inhibition of MTAN may have several important effects. First, MTA is produced as a byproduct during the formation of spermadine by the action of spermadine synthase.[2,3] MTA is a potent inhibitor of spermadine synthase. Therefore, the buildup of MTA, which may occur as a result of MTAN inhibition, may result in decreased polyamine biosynthesis.[4–8] Polyamines are postulated to play key roles in growth processes and the regulation of DNA synthesis. Therefore, inhibition of MTAN may impact the regulation of cell growth and/or DNA synthesis.

Second, MTAN inhibition would block the methionine salvage pathway in bacterial cells. In the bacterial methionine salvage pathway, MTA is converted to MTR by MTAN. MTR is then acted on by a pathway of bacterial enzymes to produce methionine and *S*-adenosylmethionine. *S*-Adenosylmethionine is an important methyl donor in a variety of intracellular reactions.[9,10] Inhibiting the conversion of MTA will block methionine salvage and will also impact reactions dependent on methyl transfer from *S*-adenosylmethionine.

Finally, MTAN inhibition may impact the production of various autoinducer (AI) molecules important for a variety of bacterial functions.[11,12] AI molecules are involved in a bacterial process termed 'quorum sensing', whereby the bacteria can monitor the presence of other bacteria in their surroundings by producing and responding to various AI molecules. In this manner the bacteria can determine a count of other bacteria and modulate their responses accordingly. A variety of behaviors are controlled by this quorum sensing pathway. These behaviors generally require a group of bacteria to carry out the behavior in synchrony to be effective and include bioluminescence, expression of virulence factors, biofilm formation, conjugation, and pigment production. MTAN is involved in the regulation of both the AI-1 and AI-2 pathways.

As a result, MTAN is an important target for the development of novel anti-microbial agents. Such new anti-microbial agents may provide alternate treatment to recently reported 'super-bugs' that are resistant to even the most powerful of the currently used antibiotics. Since MTAN is not present in humans, this new class of MTAN inhibitors would not be expected to harm the host.

MTAN shares certain structural features with 5′-methylthioadenosine phosphorylase (MTAP). MTAP is an enzyme found in a variety of organisms, including humans, and catalyzes the reversible phosphorolysis of

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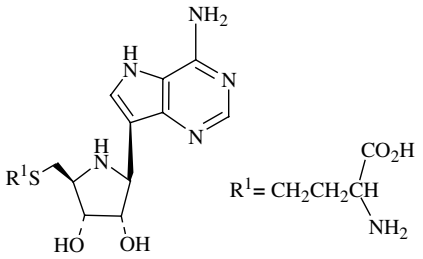


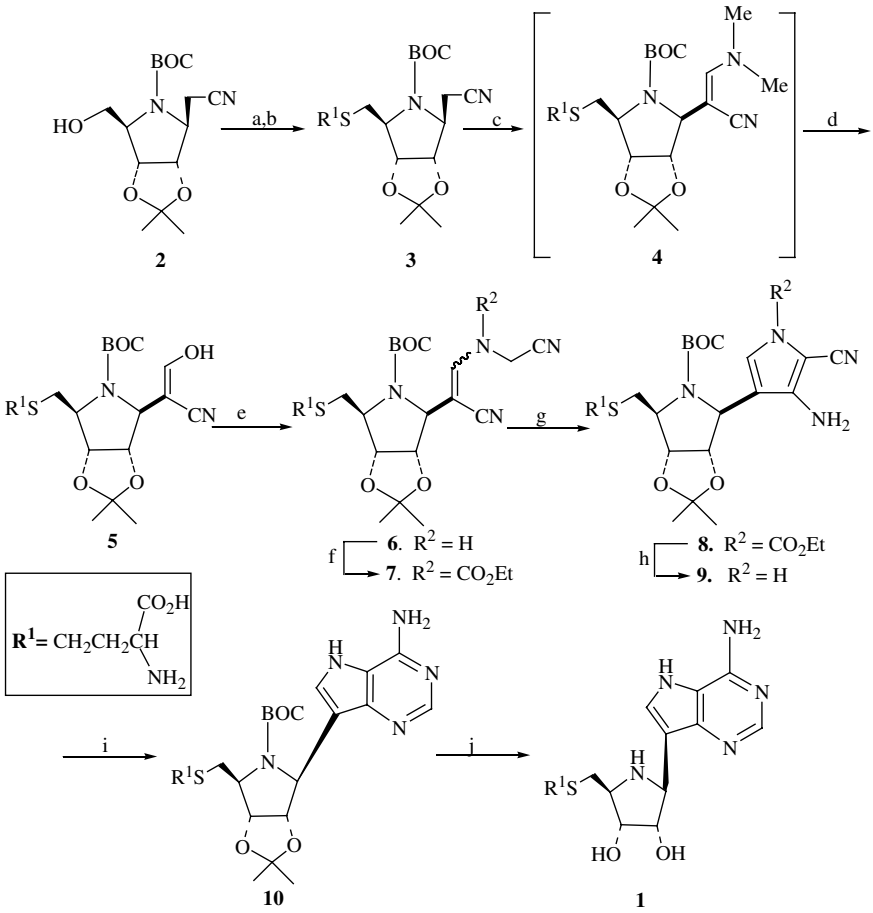
**Figure 1.** Inhibitor of MTAN, compound **1**.

MTA to adenine and 5′-methylthioribose-1-phosphate (MTR-1P).[13–16] Both MTAN and MTAP have active sites that can be divided into three discrete regions: (i) the adenine/purine binding region; (ii) the ribose binding region; and (iii) the 5′-alkylthio binding region. While MTAN and MTAP possess certain similarities, there are also dissimilarities. The ribose binding site of MTAN lacks the amino acid contacts to coordinate a phosphate anion. As a result, MTAN is a nucleosidase rather than a reversible phosphorylase. In addition, the 5′-alkylthio binding site is somewhat more extended in MTAN than in MTAP.

Over the past two decades various groups have investigated potent inhibitors of MTAP.[17] Recently, Schramm and co-workers have reported a novel class of aza-*C*-nucleosides which are very potent against MTAP.[18] Based on the similarity of the crystal structures of MTAP and MTAN, we proposed the replacement of the 5′-hydroxyl group with 5′-thio-L-homocysteine and 6-oxo with 6-amine which could give an inhibitor selective for MTAN. Herein we describe the synthesis of one of the potent inhibitor of MTAN (Fig. 1), namely 2-amino-4-[5-(4-amino-5*H*-pyrrolo[3,2-*d*]pyrimidin-7-yl)-3,4-dihydroxypyrrolidin-2-ylmethylsulfanyl]-butyric acid (**1**).

The target aza-*C*-nucleoside, **1**, was successfully prepared using the methodology as outlined in Scheme 1. Our ultimate selection of route was based on early work from our group in which a substituted aldehyde was used to construct a substituted amino pyrrole, which was further elaborated to a pyrrolo[3,2-*d*]pyrimidine derivative.[19]

Compound **2**, synthesized by a previously known method,[20] was treated with a catalytic amount of DMAP and triethylamine followed by methylsulfonyl chloride at ambient temperature. Upon workup of the reaction, the crude mesylate was taken directly to the next step without any further purification. The thio group was introduced at the 5′-position using BOC protected L-homocysteine thiolactone and sodi-



**Scheme 1.** Total synthesis of MTAN inhibitor **1**. Reagents and conditions: (a) MsCl, DMAP, TEA; (b) NaOMe, MeOH, [Boc]-L-homocysteine thiolactone (66%, two steps); (c) $BuOCH(NMe)_2$; (d) $THF/H^+/H_2O$ (65%, two steps); (e) $NH_2CH_2CN$, NaOAc (*E*/*Z* mixture, 92%); (f) ethyl chloroformate, DBN; (g) DBN; (h) 0.1 equiv $Na_2CO_3$ (54%, three steps); (i) formamidine acetate, EtOH (39%); (j) $H^+$/MeOH (88%).

um methoxide, which displaced the 5′-*O*-mesylate group. Purification of the sample gave the desired compound **3**, as syrup in 66% 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 (65%, two steps). The $^1$H NMR spectra of **4** and **5** indicated that they were single isomers.[21] 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.

Based on our earlier experience the enamine nitrogen had to be protected to affect 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 one equivalent of DBN at 0 °C for 1 h. Addition of another equivalent of DBN to the same pot followed by stirring at ambient temperature gave the pyrrole **8**. Evaporation of the solvent followed by treatment with sodium carbonate furnished **9** (54%, three steps).[22] Treatment of **9** with formamidine acetate furnished **10** (39% yield). Finally, compound **10** was deprotected under acidic conditions to furnish the target molecule **1** (88% yield) as a white solid.[23]

Since MTAN catalyzes the hydrolysis of MTA to adenine and MTR, the activity of the enzyme can be determined by measuring the initial rate of reaction. The $K_i$ value was determined by carrying out the reaction in a final volume of 1 mL in the presence of fixed concentrations of the substrate MTA (250 μM), MTAN (10 nM), and potassium phosphate (100 mM) at pH 7.4. The inhibitor concentrations were varied from 0.01 to 1 μM. The enzyme was first pre-incubated with the inhibitor at rt for 10 min. The reaction was initiated by adding substrate into the tubes containing the pre-incubated enzyme plus the inhibitor. The reaction mixture was then immediately transferred into quartz cuvettes (10 mm), and the decrease in absorbance at 275 nm was recorded at 25 °C for 10 min using a Cary 3E UV-spectrophotometer (Varian).

The $K_i$ values for the inhibitor were extrapolated by fitting the initial rate and the corresponding inhibitor concentrations to the following equation:[3]

$$\frac{V_0'}{V_0} = \frac{K_m + [S]}{K_m + [S] + K_m[I]/K_i},$$

where $V_{0'}$ is the rate in the presence of inhibitor, $V_0$ is the rate in the absence of inhibitor, [*I*] is the inhibitor concentration, [*S*] is the substrate concentration, and $K_m$ is the Michaelis constant (0.5 μM). The $K_i$ value for compound **1** was determined to be 1.7 nM. Compound **1** was not tested on MTAP as we did not have easy access to the purified protein. Second, MTAN accepts both 5′-methylthioadenosine and *S*-adenosylhomocysteine as substrates, while MTAP recognizes only 5′-methylthioadenosine as the substrate. The compound described in this manuscript was an analog of *S*-adenosylhomocysteine and we believe that it maynot be a potent inhibitor of MTAP.

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21. Compound **3**: compound **2** (15.5 g, 49.8 mmol) was dissolved in dry $CH_2Cl_2$ (300 mL) followed by the addition of TEA (52.0 mL, 37.3 mmol) and a catalytic amount of DMAP (0.12 g, 0.99 mmol). MsCl (5.80 mL, 74.7 mmol) was added slowly and the reaction mixture was stirred for 1 h. The mixture was washed with $H_2O$, dried, filtered, and evaporated to syrup. The crude sample was purified by column chromatography (hexanes/EtOAc, 7:3) to furnish the desired product (18.3 g, 94%) as a syrup. The sample was re-dissolved in MeOH (300 mL) followed by addition of 25% solution of sodium methoxide (21.3 mL, 93.5 mmol) and *tert*-butoxy carbonyl protected L-homocysteine (16.6 g, 76.8 mmol). The reaction mixture was stirred for 20 h at 60 °C and then neutralized with glacial acetic acid. The solvent was evaporated to a syrupy residue. The crude sample was purified by column chromatography (hexanes/EtOAc, 7:3) to furnish **3** (8.22 g, 32%) as a syrup. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 4.80–4.60 (m, 2H), 4.00–4.50 (m, 4H), 3.77 (s, 3H), 2.50–2.82 (m, 4H), 2.12 (s, 2H), 1.45–1.60 (m, 24H). HRMS $(M+Na)^+$ calcd for $C_{25}H_{41}N_3O_8S$: 566.2506. Found: 566.2516.
Compound **5**: compound **3** (7.87 g, 14.5 mmol) was dissolved in dry DMF (70 mL) followed by addition of *tert*-butoxy-bis(*N*,*N*-dimethylamino)methane (10.6 ml, 50.7 mmol). The reaction mixture was heated to 70 °C for 1 h. Toluene was added and the reaction mixture was washed with $H_2O$, dried, filtered, and evaporated to give compound **4**. The crude sample was re-dissolved in THF/acetic acid/$H_2O$ (1:1:1, v/v/v, 120 mL) and stirred at rt for 4 h. The reaction mixture was extracted with $CHCl_3$, washed with $H_2O$, satd $NaHCO_3$ and then dried, and evaporated. The crude sample was purified by column chromatography (hexanes/EtOAc, 7:3) to furnish **5** as a syrup (5.36 g, 65%, two steps). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.18 (s, 1H), 4.80–5.00 (m, 2H), 4.40 (m, 1H), 4.05 (dd, 1H), 3.75 (s, 3H), 3.71 (m, 1H), 2.75 (dd, 1H), 2.58–2.70 (m, 3H), 1.90–2.20 (m, 2H), 1.30–1.60 (m, 24H). HRMS $(M+Na)^+$ calcd for $C_{26}H_{41}N_3O_9S$: 594.2455. Found: 594.2466.
22. Compound **6**: compound **5** (5.36 g, 93.9 mmol) was dissolved in MeOH (100 mL) followed by addition of aminoacetonitrile (5.21 g, 56.3 mol) and NaOAc (7.70 g, 93.9 mmol). The reaction mixture was stirred at rt for 20 h. The solvent was evaporated to dryness and the crude sample was chromatographed using hexane/EtOAc (1:1) as eluent. The desired fractions were pooled together to furnish **6** (5.25 g, 92%). The mixture was taken directly to the next step. HRMS $(M+Na)^+$ calcd for $C_{28}H_{43}N_5O_8S$: 632.2724. Found: 632.2751.
Compound **9**: compound **6** (0.79 g, 1.30 mmol) was dissolved in dry $CH_2Cl_2$ (25 mL) followed by addition of DBN (0.32 mL, 2.60 mmol) and ethyl chloroformate (0.18 mL, 1.95 mmol). The reaction mixture was stirred at 0 °C for 1 h. Upon completion of the reaction as indicated by TLC the reaction mixture was removed from the cold bath and warmed to rt. DBN (0.32 mL, 2.60 mmol) was added and then stirred for 20 h. The solvent was evaporated to dryness and the crude product **8** was re-dissolved in MeOH (20 mL) followed by 0.1 equiv solid $Na_2CO_3$ and the mixture was stirred for 1 h. Upon completion of the reaction the solvent was evaporated to dryness and the crude sample was purified by column chromatography (hexanes/EtOAc, 1:1) to give **9** (0.42 g, 54%, three steps). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.00 (br s, 1H), 6.8 (d, 1H), 4.70–5.05 (m, 5H), 4.00–4.50 (m, 4H), 3.75 (s, 3H), 2.52–2.70 (m, 2H), 1.20–1.55 (m, 24H). HRMS $(M+H)^+$ calcd for $C_{28}H_{43}N_5O_8S$: 632.2724. Found: 632.2723.
23. Compound **10**: to a solution of **9** (0.42 g, 0.70 mmol) in EtOH (20 mL), formamidine acetate (0.44 g, 4.21 mmol) was added and the reaction mixture was refluxed for 20 h. The solvent was evaporated to dryness and chromatographed using ($CH_2Cl_2$/MeOH, 95:5) to give **10** (0.17 g, 39%). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.15 (br s, 3H), 7.60 (s, 1H), 7.45 (s, 1H), 7.45 (s, 1H), 5.23 (s, 2H), 4.81 (t, 1H), 4.00–4.30 (m, 3H), 3.65 (s, 1H), 2.55 (m, 2H), 2.00 (s, 3H), 1.2–1.5 (m, 24H). HRMS $(M+H)^+$ calcd for $C_{29}H_{44}N_6O_8S$: 637.3014. Found: 637.2997.
Compound **1**: compound **10** (0.17 g, 0.27 mmol) was dissolved in MeOH (5 mL) and 0.5 N NaOH. The reaction mixture was refluxed at 75 °C for 2 h. Upon completion of the reaction the solvent was evaporated to dryness. The crude mixture was re-dissolved in MeOH (20 mL) and concd HCl (3 mL) and then heated to 50 °C for 1 h. The solvent was evaporated to dryness and the residue was co-evaporated with EtOH to give a white powder. The product was purified by HPLC and the appropriate fractions were pooled together to give a solid residue. The residue was dissolved in $H_2O$ (8 mL), filtered through a Millipore filter (0.25 μ), and lyophilized to give **1** (88 mg, 88%) as a white solid. $^1$H NMR (300 MHz, $D_2O$) $\delta$ 8.24 (s, 1H), 7.85 (s, 1H), 4.87 (t, 2H), 4.43 (t, 1H), 3.87 (m, 1H), 3.82 (m, 1H), 3.00-3.30 (m, 2H), 2.80 (t, 2H), 2.20 (m, 2H). $^{13}$C NMR $\delta$ 174.4, 150.5, 147.4, 131.1, 113.8, 106.8, 73.4, 72.9, 63.6, 57.1, 54.1, 31.7, 30.6, 27.3, 8.8. HRMS $(M+H)^+$ calcd for $C_{15}H_{22}N_6O_4S$: 383.1496. Found: 383.1504.