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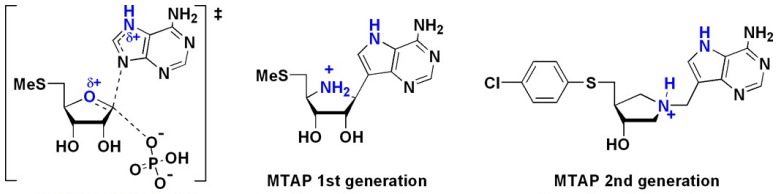

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Second Generation Transition State Analogue Inhibitors of Human 5′-Methylthioadenosine Phosphorylase

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The polyamine biosynthetic pathway is a therapeutic target for proliferative diseases because cellular proliferation requires elevated levels of polyamines. A byproduct of the synthesis of spermidine and spermine is 5′-methylthioadenosine (MTA). In humans MTA is processed by 5′-methylthioadenosine phosphorylase (MTAP) so that significant amounts of MTA do not accumulate. Products of the MTAP reaction (adenine and 5-methylthio-α-D-ribose-1-phosphate) are recycled to *S*-adenosylmethionine, the precursor for polyamine synthesis. Potent inhibitors of MTAP might allow the build-up of sufficient levels of MTA to generate feedback inhibition of polyamine biosynthesis and/or reduce *S*-adenosylmethionine levels. We recently reported the design and synthesis of a family of potent transition state analogue inhibitors of MTAP. We now report the synthesis of a second generation of stable transition state analogues with increased distance between the ribooxocarbenium ion and purine mimics. These compounds are potent inhibitors with equilibrium dissociation constants as low as 10 pM. The first and second generation inhibitors represent synthetic approaches to mimic early and late features of a dissociative transition state.

Introduction

Recently we reported the design,[1] synthesis,[2] and biological activity[1,2] of a first generation series of inhibitors of human methylthioadenosine phosphorylase (MTAP), the 5′-alkylthio and 5′-arylthio immucillins. MTAP is a key enzyme in the catabolism of methylthioadenosine (MTA), a byproduct of polyamine synthesis (Figure 1).

In humans it is proposed that inhibition of MTAP and accumulation of methylthioadenosine (MTA) could lead to feedback inhibition of spermidine and spermine synthases and thus down-regulate polyamine biosynthesis. The polyamines putrescine, spermidine, and spermine play important roles in all mammalian cells, protozoa, bacteria, and fungi. They are involved in replication, transcription and translation, but their roles in growth-related processes are best defined.[3−5] Cellular proliferation requires increased levels of polyamines, and compared to quiescent cells, rapidly dividing cells have elevated polyamine pools.

In humans MTA is rapidly degraded by MTAP so that no significant concentration of MTA is known to accumulate. MTAP is the only enzyme in humans that processes MTA, and human genomic studies indicate only a single gene locus for the enzyme. The phosphorolysis reaction that it catalyses produces 5-methylthio-α-D-ribose-1-phosphate and adenine (Figure 2).[6,7] Potent inhibitors of MTAP might be expected to raise the intracellular concentration of MTA sufficiently to achieve feed-back inhibition of polyamine biosynthesis and have antiproliferative activity.[8−12] This has made the polyamine biosynthetic pathway a therapeutic target for the treatment of proliferative diseases such as cancer and parasitic infections.[13−18] A less-recognized feature of the polyamine pathway is the recycling of 5-methylthio-α-D-ribose-1-phosphate to methionine and adenine to ATP, the precursors of *S*-adenosylmethionine. These steps complete the cycle of the polyamine pathway since *S*-adenosylmethionine is the precursor for polyamine synthesis.

The transition state for the reaction catalyzed by human MTAP was inferred through our knowledge of the transition state for human purine nucleoside phosphorylase (PNP)[19−21] and confirmed by transition state analysis of human MTAP (Figure 2).[22] The characteristics of an early transition state for an S_N1 mechanism i.e., the ribooxocarbenium ion character in the 5′-methylthioribosyl group and the elevated pK_a for the purine leaving group with little participation of the phosphate ion, have been partially captured by the first generation inhibitors. To better mimic the cationic charge which develops at the anomeric carbon of a fully dissociative transition state, we designed a second generation of inhibitors in which the nitrogen atom was moved to the position of the anomeric carbon, the ring oxygen was replaced by a methylene group, and the ribosyl mimic and the deazapurine were increased in separation through insertion of a methylene bridge.[23]

Herein we report the synthesis of a family of these second generation transition state analogue inhibitors of human MTAP. Their inhibitory properties against human MTAP extend to dissociation constants of 10 pM and establish these compounds as powerful inhibitors for human MTAP while at the same time delineating some structure−activity relationships. We have de-

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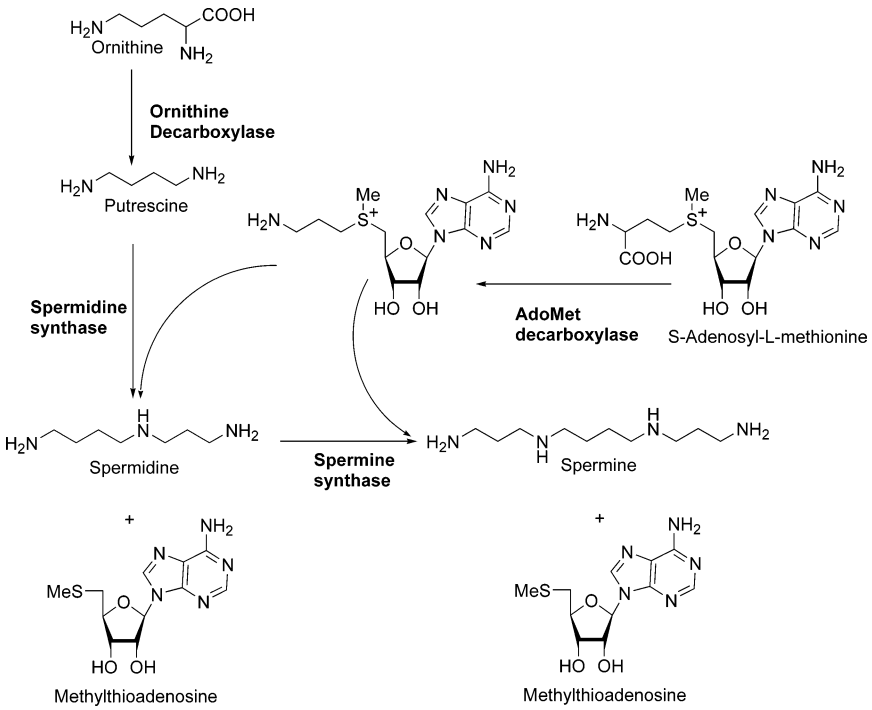


Figure 1. Polyamine biosynthesis from ornithine to spermine.

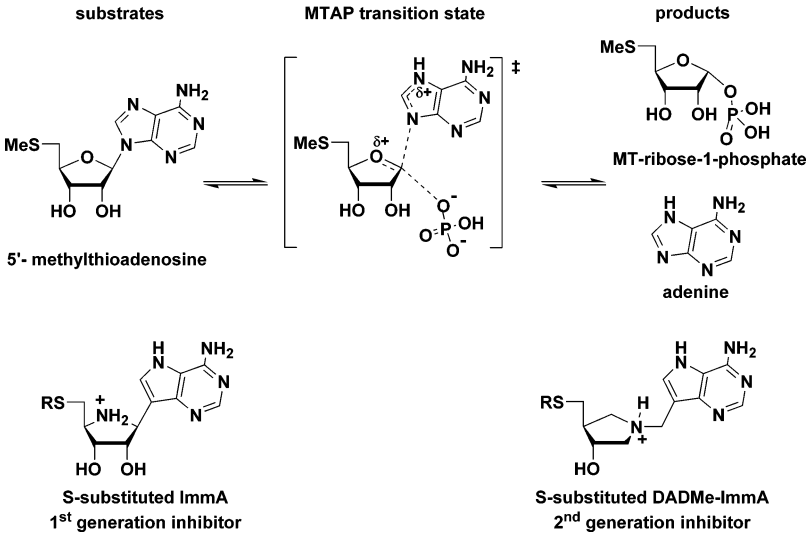


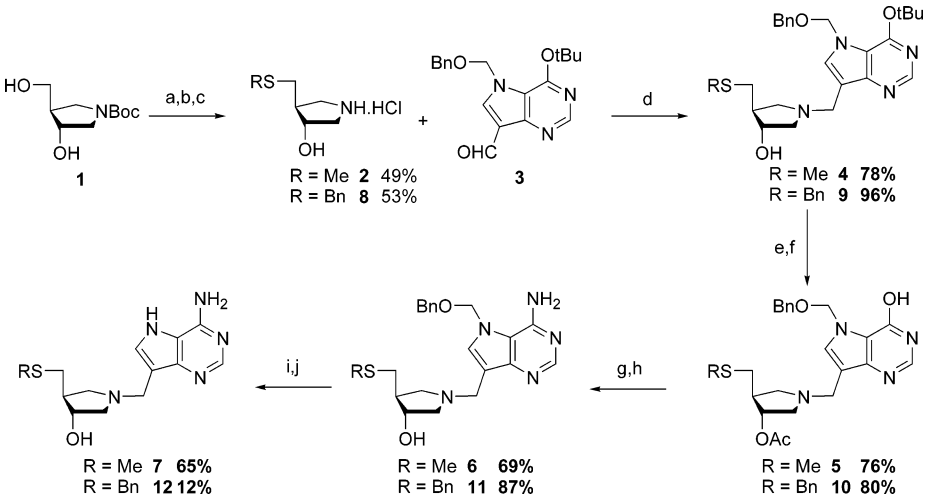
Figure 2. Phosphorolysis of methylthioadenosine catalyzed by MTAP, including the putative transition state, and the general structures of the first and second generation inhibitors of MTAP.

scribed the inhibitory properties of some of these compounds in an initial report that includes the X-ray crystal structure of human MTAP with one of these inhibitors.[1] Other members of this family have been shown to be powerful inhibitors of *E. coli* 5'-methylthioadenosine nucleosidase.[24]

Results and Discussion

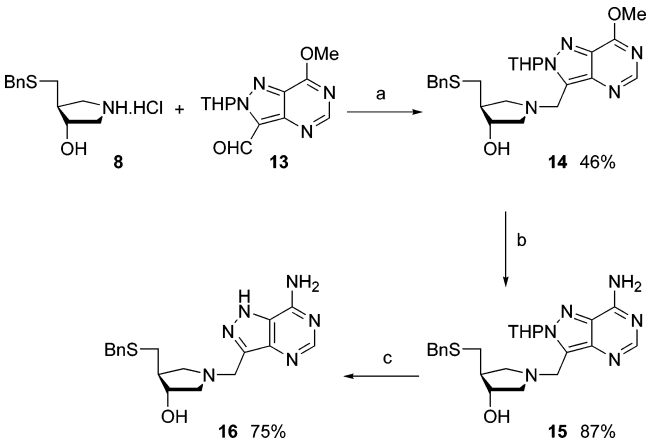
Synthesis. The *tert*-butoxycarbonyl-protected amine (**1**), prepared as previously described, was used for the preparation of the 9-deazaadenine derivatives **7** and **12** (Scheme 1).[23] Sulfonation of **1** with methanesulfonyl chloride and Hunigs base in CH_2Cl_2 at 0 °C afforded predominantly the primary mono-methanesulfonate, and this was readily displaced with sodium thiomethoxide, followed by removal of the *tert*-butoxycarbonyl protecting group, to provide the amine hydrochloride **2** in good overall yield. Reductive amination of **2** with 7-benzyloxymethyl-6-*O*-*tert*-butyl-9-deaza-9-formylhypoxanthine (**3**) using sodium cyanoborohydride could be carried out in an analogous manner to that previously described, to afford compound **4**.[23] Conversion of the protected deazahypoxanthine to a deazaadenine moiety was achieved via a series of synthetic steps which initially involved acetylation of the secondary hydroxyl, followed by removal of the *tert*-butyl group through acid deprotection to afford **5**. Compound **5** was then treated with refluxing phosphoryl chloride and the C-6 chlorine atom resultant in the product was displaced with concomitant removal of the acetate by heating with methanolic ammonia in a sealed tube to yield **6** containing the deazaadenine moiety. Global deprotection of **6** through treatment with cHCl, followed by conversion

Scheme 1[a]



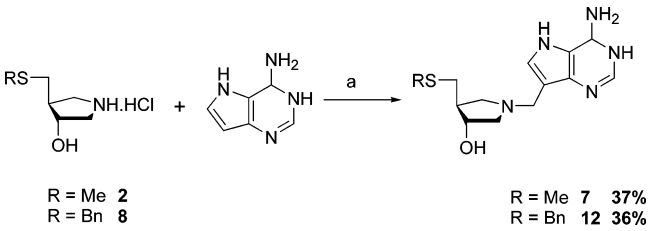
[a] Reagents: (a) MsCl, *i*PR_2NEt, CH_2Cl_2, 0 °C → room temp; (b) NaSMe or NaSBn, DMF, room temp; (c) cHCl, MeOH, room temp; (d) $NaCNBH_3$, MeOH, room temp; (e) Ac_2O, DMAP, Et_3N, CH_2Cl_2, room temp; (f) TFA, CH_2Cl_2, room temp; (g) $POCl_3$, reflux; (h) 7 N NH_3 in MeOH, 110 °C; (i) cHCl, reflux; (j) NH_4OH, room temp.

Scheme 2[a]



[a] Reagents: (a) $NaCNBH_3$, MeOH, room temp; (b) 7 N NH_3 in MeOH, 110 °C; (c) cHCl, MeOH, room temp.

Scheme 3[a]



[a] Reagents: (a) 37% aq formaldehyde, NaOAc, dioxane, H_2O, 95 °C.

of the subsequent hydrochloride salt to the free base, afforded the target compound **7**.

Similarly displacement of the monomesylate of compound **1** with sodium thiobenzyloxide afforded, after deprotection, the *S*-benzyl derivative **8**. This was converted into compound **12** via compounds **9**, **10**, and **11**. The final deprotection of **11** to give **12** proceeded in poor yield (12%), but the reaction was not further investigated.

For the preparation of the 8-aza-9-deazaadenine derivative **16** the benzylthio amine hydrochloride **8** was also reductively aminated with 8-aza-9-deaza-9-formyl-6-methoxy-8-tetrahydropyranylpurine (**13**)[23] under standard conditions to yield **14** (Scheme 2). Displacement of the C-6 methoxy group was readily achieved in this case with 7 N ammonia in methanol in a sealed tube at 130 °C to afford **15**. Global deprotection yielded the target compound **16**, the 8-aza homologue of **12**.

Subsequently it was found that compounds **7** and **12** could be synthesized in a more direct manner via the Mannich reaction between the amine hydrochlorides **2** and **8**, 9-deazaadenine and formaldehyde (Scheme 3,) but we were unable to prepare compound **16** using this strategy.[25]

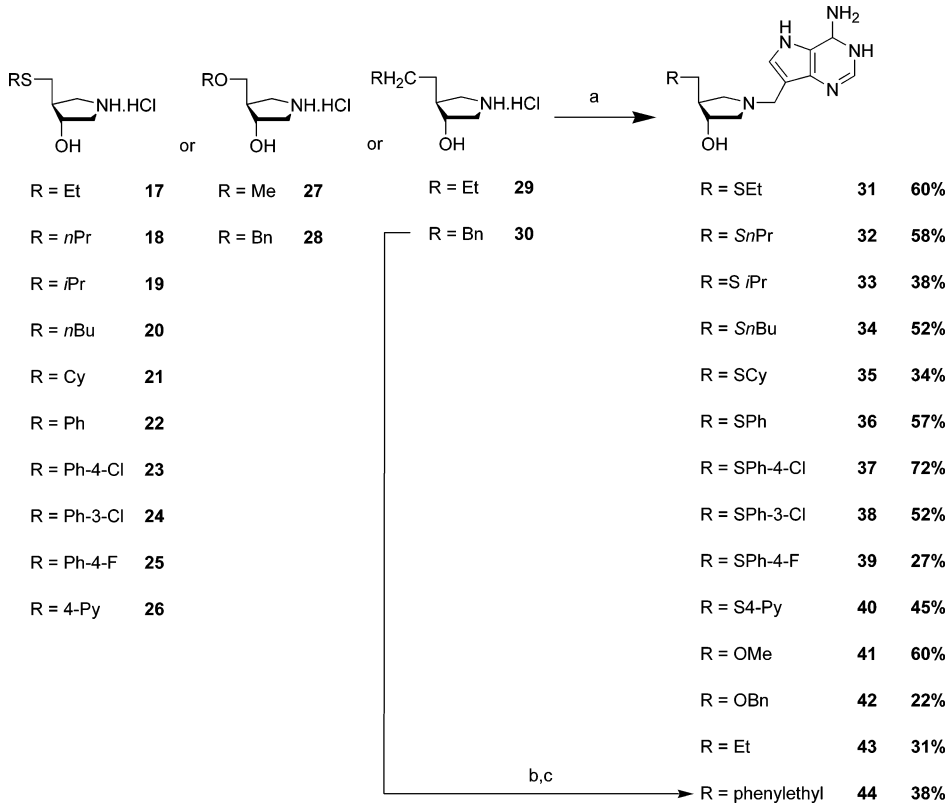
To study the SAR of this class of inhibitors, the MeS group was replaced with alternative substituents. The requisite thio-substituted amine hydrochlorides **17**−**26** were prepared from compound **1** in an analogous fashion to that used for compounds **2** and **8** (Scheme 4).

O-Alkyl-substituted amine hydrochlorides **27** and **28** were prepared via selective protection of the primary hydroxyl of **1** as a *tert*-butyldiphenylsilyl ether to afford **45** (Scheme 5). MOM protection of the secondary hydroxyl group yielded **46** and then removal of the silyl protecting group afforded **47**. Methylation or benzylation of the primary hydroxyl of **47** afforded compounds **48** and **49**, and removal of the *tert*-butoxycarbonyl protecting group afforded amine hydrochlorides **27** and **28**, respectively.

The chain-extended deoxygenated amines **29** and **30** were synthesized via the aldehyde **50**, obtained as a precursor during the synthesis of amine **1** (Scheme 6). The Wittig reaction between compound **50** and the appropriate ylid afforded the individual compounds **51** and **52** as *E*/*Z* mixtures of stereoisomers. Hydrogenation of compounds **51** and **52** followed by removal of the *tert*-butoxycarbonyl protecting group afforded the amines **29** and **30**, respectively.

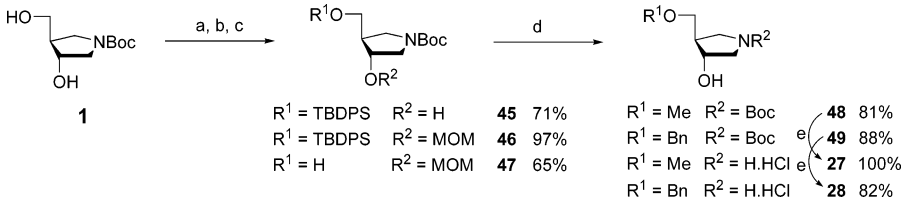
The Mannich reaction of the amine hydrochlorides **17**−**29** with 9-deazaadenine and formaldehyde (Scheme 4) allowed their rapid conversion into compounds **31**−**43** all of which were potent inhibitors of human MTAP (Table 1). The final analogue **44** was prepared via coupling with 6-chloro-9-deazapurine and displacement of the chlorine atom with ammonia followed by global

Scheme 4[a]



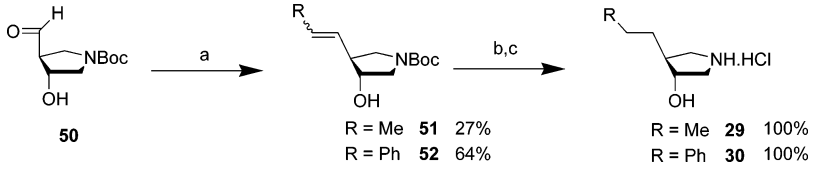
[a] Reagents: (a) 9-Deazaadenine, 37% formaldehyde, NaOAc, dioxane, H_2O, 95 °C; (b) 6-chloro-9-deazapurine, 37% formaldehyde, NaOAc, dioxane, H_2O, 95 °C; (c) 7 N NH_3 in MeOH, 130 °C.

Scheme 5[a]



[a] Reagents: (a) TBDPSCl, Im, DMF, room temp; (b) MOMBr, iPr_2NEt, CH_2Cl_2; (c) TBAF, THF; (d) NaH, RI, THF; (e) 4 M HCl, dioxane.

Scheme 6[a]



[a] Reagents: (a) $MeCH{=}PPh_3$ or $C_6H_5CH{=}PPh_3$, THF, 0 °C; (b) H_2, Pd/C, EtOH; (c) HCl, MeOH, room temp.

deprotection. Use of the Mannich reaction has enabled the synthesis of gram quantities of candidate MTAP inhibitors for in vivo trials.

Biological Results. Inhibition Studies. Inhibition of MTAP by the transition state analogue inhibitors described here normally involves the two-step process of slow-onset tight-binding inhibition. The first step involves reversible competitive binding at the catalytic site: $E + I \leftrightarrow EI$ characterized by K_i as the dissociation constant. In the second step the EI complex undergoes a conformational change to tighten the binding: $EI \leftrightarrow E^*I$. This second step is usually slow relative to EI formation and equilibration. The overall equilibrium dissociation constant K_i^* is defined by the overall equilibrium dissociation constant: $E + I \leftrightarrow E^*I$. Experimentally, inhibition constants were estimated from two time periods of the reaction rate curve. In the first, the initial reaction rates ($t \rightarrow 0$) were fitted to the equation for competitive inhibition to give K_i, and the final reaction rates (after slow-onset was complete) were fitted to the equation for competitive inhibition to give K_i^*. These methods are valid for conditions where initial and final rates are clearly defined and delineated and the inhibitor concentration is >10× enzyme concentration.[26] Under high inhibitor (I) conditions, initial rates are difficult to measure because the tight-binding phase occurs rapidly. Inhibition curves were also fitted to the general integrated rate equation: $P = v_s t + (v_o - v_s)(1$

Table 1. Inhibition of Human MTAP by Second Generation Immucillins[a]

compd	R	K_i (nM)	K_i* (nM)
MTA		K_m = 5000 nM	
MT-ImmA		26.0 ± 0.8	1.0 ± 0.5
7	SMe	1.7 ± 0.2	0.09 ± 0.01
12	SBn	1.4 ± 0.2	0.7 ± 0.2
16		55 ± 4	ND
31	SEt	0.65 ± 0.08	0.034 ± 0.003
32	S*n*Pr	1.30 ± 0.04	0.12 ± 0.01
33	S$_i$Pr	0.9 ± 0.1	0.26 ± 0.03
34	S*n*Bu	0.28 ± 0.05	0.11 ± 0.02
35	SCy	0.76 ± 0.07	0.37 ± 0.03
36	SPh	1.5 ± 0.1	0.17 ± 0.02
37	SPh-4-Cl	0.36 ± 0.07	0.010 ± 0.005
38	SPh-3-Cl	2.0 ± 0.5	0.27 ± 0.15
39	SPh-4-F	0.81 ± 0.11	0.16 ± 0.01
40	S-4-Py	2.0 ± 0.1	0.160 ± 0.015
41	OMe	14 ± 2	8.0 ± 0.7
42	OBn	85 ± 7	42 ± 4
43	Et	22 ± 2	3.0 ± 0.4
44	Bn	>2000	ND

[a] K_i is the initial dissociation constant, and K_i* is the equilibrium dissociation constant obtained after slow-onset inhibition. ND indicates that no slow-onset phase was observed.

$- e^{-kt})/k$ where P, v_o, v_s, and k represent, respectively, product formed, initial rate, final steady-state rate, and first-order rate constant for attainment of the final rate. The value of k increases hyperbolically as a function of inhibitor concentration such that $k = k_6[1 + (I/(K_i^*(1 + A/K_a))]/[1 + (I/(K_i(1 + A/K_a))]$ where k_6 is the rate of conversion of E*I to EI and I and A are inhibitor and substrate concentrations.[27]

The 5′-MT-DADMe-ImmA (**7**) is a powerful inhibitor of MTAP and, after slow onset, has K_i^* = 90 pM. The K_m/K_i value of 55 555 for compound **7**, together with the chemical similarity to the proposed transition-state structure, supports the hypothesis that features of the transition state are being captured with this molecule. Extension of the methyl group shows a similar trend to that previously reported[2] with the K_i* for the ethyl analogue **31** falling to 34 pM with a K_m/K_i* value of 147 058. Weaker inhibitors are found as the chain length is either extended or branched as in compounds **31**−**35**. The inhibitory potency of the 8-aza analogue of **12**, compound **16**, is around 80 times less. In the 5′-arylthio series the 4-chlorophenyl analogue **37** is the most powerful of the inhibitors found with a K_i^* = 10 pM and a K_m/K_i* value of 500 000. Substitution of the sulfur atom of compound **7** with either an oxygen, as in compound **41**, or a carbon atom, as in compound **43**, affords inhibitors significantly less active with K_i* values of 8 and 3 nM, respectively.

Conclusions

A series of second generation transition state analogues of human 5′-methylthioadenosine phosphorylase have been designed and synthesized based on its dissociative transition state structure. Many of these compounds are potent inhibitors and display slow-onset tight binding characteristics that are consistent with action as transition state analogues.

Experimental Section

Aluminum-backed silica gel sheets (Merck or Riedel de Haen) were used for TLC. Column chromatography was performed on silica gel (230−400 mesh, Merck). Chromatography solvents were distilled prior to use. Anhydrous solvents were obtained from Aldrich or Acros. NMR spectra were recorded at 300 MHz (^{1}H) and 75 MHz (^{13}C) in D_2O unless otherwise indicated.

(3*R*,4*S*)-3-Hydroxy-4-methylthiomethylpyrrolidine Hydrochloride (2). Methanesulfonyl chloride (800 μL, 10 mmol) was added dropwise to a solution of Hunig's base (3.5 mL, 20 mmol) and (3*R*,4*R*)-1-*tert*-butoxycarbonyl-3-hydroxy-4-(hydroxymethyl)pyrrolidine (**1**) (2.2 g, 10 mmol) in CH_2Cl_2 (30 mL) at 0 °C, and the resulting solution allowed to warm to room temp. The reaction mixture was diluted with CH_2Cl_2, washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was purified by chromatography (5% MeOH in CH_2Cl_2) to afford, presumably, (3*R*,4*R*)-1-*tert*-butoxycarbonyl-3-hydroxy-4-(mesyloxymethyl)pyrrolidine (2.1 g) as an oil. Without further characterization, the product (900 mg) was dissolved in DMF (10 mL) and stirred with sodium thiomethoxide (400 mg, 5.7 mmol) at room temp overnight. The reaction was diluted with toluene, washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was purified by chromatography (ethyl acetate) to afford, presumably, (3*R*,4*S*)-1-*tert*-butoxycarbonyl-3-hydroxy-4-(methylthiomethyl)pyrrolidine (600 mg, 2.4 mmol) as a syrup, which was not further characterized but dissolved in MeOH (5.0 mL) and cHCl (1.0 mL) and concentrated in vacuo to afford (3*R*,4*S*)-3-hydroxy-4-(methylthiomethyl)pyrrolidine hydrochloride (**2**) (442 mg, 49% overall yield for three steps) as a syrup. ^{1}H NMR (D_2O) 4.40 (q, J = 3.1 Hz, 1H), 3.67 (dd, J = 12.0, 6.6 Hz, 1H), 3.50 (dd, J = 12.8, 5.1 Hz, 1H), 3.28 (dd, J = 12.8, 3.0 Hz, 1H), 3.22 (dd, J = 8.7, 3.4 Hz, 1H), 2.73−2.66 (m, 1H), 2.58−2.50 (m, 2H). ^{13}C NMR (D_2O) δ 73.5, 51.5, 48.6, 45.2, 34.3, 14.9. HRMS (MH^+) calcd for $C_6H_{14}NOS$: 148.0796. Found 148.0803.

(3*R*,4*S*)-1-[(7-Benzyloxymethyl-6-*tert*-butoxy-9-deazapurin-9-yl)methyl]-3-hydroxy-4-methylthiomethylpyrrolidine (4). Sodium cyanoborohydride (200 mg, 3.2 mmol) was added to a stirred solution of **3**[23] (800 mg, 2.32 mmol) and **2** (550 mg, 3.00 mmol) in methanol (10 mL), and the mixture was stirred overnight at room temp. The crude reaction mixture was absorbed onto silica and purified by chromatography (10% MeOH in CH_2Cl_2) to afford **4** (1.10 g, 78%) as a gum. ^{1}H NMR ($CDCl_3$) δ 8.48 (s, 1H), 7.54 (s, 1H), 7.33−7.23 (m, 5H), 5.75 (s, 2H), 4.50 (s, 2H), 4.12 (m, 1H), 4.02 (s, 2H), 3.30 (dd, J = 9.9, 7.5 Hz, 1H), 2.95 (m, 2H), 2.64 (dd, J = 12.7, 7.1 Hz, 1H), 2.52−2.38 (m, 3H), 2.07 (s, 3H), 1.70 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 156.4, 150.3, 150.3, 137.5, 133.3, 128.8, 128.2, 127.8, 117.1, 111.7, 83.5, 77.5, 76.1, 70.4, 61.4, 58.2, 48.8, 47.4, 37.3, 29.0, 16.0. HRMS (MH^+) calcd for $C_{34}H_{38}N_5O_3S$: 564.2975. Found 564.2976.

(3*R*,4*S*)-3-Acetoxy-1-[(7-benzyloxymethyl-9-deazahypoxanthin-9-yl)methyl]-4-methylthiomethylpyrrol-idine (5). Acetic anhydride (1 mL, excess) was added dropwise to a solution of compound **4** (1.1 g, 2.3 mmol), DMAP (30 mg, cat.), and Et_3N (2 mL, excess) in CH_2Cl_2 (20 mL) at room temp.. After 15 min, the reaction mixture was diluted with CH_2Cl_2, washed with satd $NaHCO_3$, water, and brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was purified by chromatography (5% MeOH in CH_2Cl_2) to afford a syrupy product (1.35 g), which was not further characterized. TFA (5 mL) was added dropwise to a solution of the syrup in CH_2Cl_2 (20 mL) at room temp and the mixture was then concentrated in vacuo. The resulting residue was dissolved in CH_2Cl_2 and washed with satd $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated in vacuo to afford **5** (800 mg, 76% for two steps) as a foam. ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H), 7.65 (s, 1H), 7.25−7.20 (m, 5H), 5.82 (s, 2H), 5.11 (brs, 1H), 4.56 (s, 2H), 4.47 (s, 2H), 3.80−3.59 (m, 3H), 3.32 (brs, 1H), 2.80−2.69 (m, 2H), 2.57 (dd, J = 13.0, 8.3 Hz, 1H), 2.07 (s, 3H), 2.05 (s, 3H).^{13}C NMR ($CDCl_3$) ?δ 170.7, 155.4, 145.8, 143.3, 137.2, 134.2, 128.8, 128.3, 128.1, 118.1, 106.9, 77.4, 75.7, 71.2, 57.0, 55.8, 48.1, 43.5, 35.0, 21.0, 16.1. HRMS (MH^+) calcd for $C_{23}H_{29}N_4O_4S$: 457.1910. Found 457.1915.

(3*R*,4*S*)-1-[(7-Benzyloxymethyl-9-deazaadenin-9-yl)-methyl]-3-hydroxy-4-methylthiomethylpyrrolidine (6).

Compound **5** (800 mg, 1.75 mmol) was dissolved in $POCl_3$ and heated under reflux for 1 h. The resulting solution was concentrated in vacuo and evaporated with toluene (×2) to afford a solid residue. Without further purification, the residue was dissolved in 7 N NH_3 in MeOH (15 mL) and heated in a sealed tube at 110 °C overnight. The reaction was concentrated in vacuo, and the resulting residue purified by chromatography (15% MeOH in CH_2Cl_2) to afford **6** (500 mg, 69%) as a syrup. ^{1}H NMR (d_4-MeOH) δ 8.24 (s, 1H), 7.85 (s, 1H), 7.29−7.26 (m, 5H), 5.74 (s, 2H), 4.63 (s, 2H), 4.43 (s, 2H), 4.26−4.22 (m, 1H), 3.70 (dd, J = 11.4, 6.9 Hz, 1H), 3.49 (dd, J = 12.1, 5.6 Hz, 1H), 3.29 (dd, J = 12.3, 3.3 Hz, 1H), 3.16 (dd, J = 11.4, 6.1 Hz, 1H), 2.76−2.67 (m, 1H), 2.50−2.44 (m, 2H), 2.07 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 153.4, 153.0, 149.9, 138.1, 137.0, 130.0, 129.7, 129.3, 116.5, 106.5, 79.5, 75.0, 72.3, 60.9, 57.8, 50.1, 47.6, 36.6, 16.0. HRMS (MH^+) calcd for $C_{21}H_{28}N_5O_2S$: 414.1964. Found 414.1970.

(3*R*,4*S*)-1-[(9-Deazaadenin-9-yl)methyl]-3-hydroxy-4-methylthiomethylpyrrolidine (7). Compound **6** (150 mg, 0.37 mmol) was dissolved cHCl (5 mL) and the resulting solution heated under reflux for 90 min.. The reaction was cooled to room temp, diluted with water (50 mL), and washed with $CHCl_3$ (×2), and the aqueous layer was concentrated in vacuo, followed by codistillation with water (×2). The resulting residue was redissolved in aq cNH_4OH and concentrated in vacuo and the residue purified by chromatography (12:4:0.5 CH_2Cl_2:MeOH:NH4OH v/v/v) to afford **7** (69 mg, 65%) as a solid. Mp 108−110 °C. ^{1}H NMR (D_2O) δ 7.96 (s, 1H), 7.31 (s, 1H), 4.00−3.95 (m, 1H), 3.74 (s, 2H), 3.05 (dd, J = 10.5, 7.9 Hz, 1H), 2.88 (dd, J = 11.1, 6.2 Hz, 1H), 2.71 (dd, J = 11.1, 4.0 Hz, 1H), 2.49 (dd, J = 13.0, 6.7 Hz, 1H), 2.40−2.24 (m, 2H), 2.16−2.13 (m, 1H), 1.93 (s, 3H). ^{13}C NMR (D_2O) δ 150.5, 150.1, 145.4, 130.4, 113.6, 108.33, 75.0, 59.8, 56.6, 47.4, 45.9, 35.9, 14.8. HRMS (MH^+) calcd for $C_{13}H_{20}N_5OS$: 294.1389. Found 294.1394. Anal. ($C_{13}H_{19}N_5OS\cdot 4/3H_2O$) C, H, N, S.

(3*R*,4*S*)-4-Benzylthiomethyl-3-hydroxypyrrolidine Hydrochloride (8). (3*R*,4*R*)-1-*tert*-Butoxycarbonyl-3-hydroxy-4-mesyloxymethylpyrrolidine (1.10 g, 3.7 mmol) was dissolved in DMF (2 mL) and added dropwise to a solution of benzyl mercaptan (870 μL, 7.4 mmol) and NaH (270 mg, 60% oil dispersion, 6.8 mmol) in DMF (10 mL) and stirred at room temp for 1 h. The reaction mixture was diluted with toluene, washed with water then brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was purified by chromatography (30% ethyl acetate in petroleum ether) to afford (3*R*,4*S*)-4-benzylthiomethyl-1-*tert*-butoxycarbonyl-3-hydroxypyrrolidine as a syrup. Without further characterization, the syrup was dissolved in MeOH (5.0 mL) and cHCl (1.0 mL) and concentrated in vacuo to afford **8** (730 mg, 76% overall yield for two steps). ^{1}H NMR (D_2O) δ 7.40−7.27 (m, 5H), 4.26−4.22 (m, 1H), 3.74 (s, 2H), 3.56 (dd, J = 12.4, 7.2 Hz, 1H), 3.37 (dd, J = 12.8, 5.2 Hz, 1H), 3.21(dd, J = 12.8, 3.0 Hz, 1H), 3.07 (dd, J = 12.4, 5.5 Hz, 1H), 2.61−2.52 (m, 1H), 2.47−2.34 (m, 2H). ^{13}C NMR (D_2O) δ 138.7, 129.5, 129.3, 127.9, 73.5, 51.5, 48.5, 45.4, 35.9, 31.8. (MH^+) calcd for $C_{12}H_{18}NOS$: 224.1109. Found 224.1102.

(3*R*,4*S*)-1-[(7-Benzyloxymethyl-6-*tert*-butoxy-9-deazapurin-9-yl)methyl]-4-benzylthiomethyl-3-hydroxypyrrolidine (9). Sodium cyanoborohydride (200 mg, 3.2 mmol) was added to a stirred solution of **3**[23] (800 mg, 2.32 mmol) and **8** (570 mg, 2.2 mmol) in methanol (10 mL), and the mixture was stirred overnight at room temp. The crude reaction mixture was absorbed onto silica and purified by chromatography (5% MeOH in CH_2Cl_2) to afford **9** (1.16 g, 96%) as a syrup. ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1H), 7.62 (s, 1H), 7.27−7.22 (m, 10H), 5.75 (s, 2H), 4.51 (s, 2H), 4.15 (s, 2H), 3.67 (s, 2H), 3.38 (dd, J = 10.7, 7.0 Hz, 1H), 3.12−3.02 (m, 2H), 2.69−2.63 (m, 1H), 2.54−2.49 (m, 1H), 2.44−2.39 (m, 2H), 1.70 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 156.6, 150.6, 150.0, 138.3, 137.5, 134.4, 129.3, 129.0, 128.8, 128.2, 127.8, 117.1, 109.0, 83.9, 77.9, 75.2, 70.7, 60.5, 57.7, 49.1, 47.0, 36.8, 33.7, 29.0. (MH^+) calcd for $C_{31}H_{49}N_4O_3S$: 547.2743. Found 547.2723.

(3*R*,4*S*)-3-Acetoxy-1-[(7-benzyloxymethyl-9-deazahypoxanthin-9-yl)methyl]-4-benzylthiomethylpyrrolidine (10). Acetic anhydride (1 mL, excess) was added dropwise to a solution of **9** (1.16 g, 2.12 mmol), DMAP (30 mg, cat.), and Et_3N (2 mL, excess) in CH_2Cl_2 (20 mL) at room temp. After 15 min, the reaction mixture was diluted with CH_2Cl_2, washed with satd aq $NaHCO_3$, water, brine, dried ($MgSO_4$), and concentrated in vacuo. The resulting residue was purified by chromatography (ethyl acetate) to afford a syrup (1.35 g). TFA (5 mL) was added dropwise to a solution of the syrup in CH_2Cl_2 (20 mL) at room temp and concentrated in vacuo. The resulting residue was redissolved in CH_2Cl_2 and washed with satd aq $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated in vacuo to afford **9** (900 mg, 80% for two steps) as a foam. ^{1}H NMR ($CDCl_3$) δ 7.90 (s, 1H), 7.33 (s, 1H), 7.28−7.17 (m, 10H), 5.91 (s, 2H), 4.85 (brs, 1H), 4.58 (s, 2H), 3.86−3.74 (m, 2H), 3.68 (s, 2H), 3.16−3.11 (m, 1H), 2.84−2.80 (m, 2H), 2.71 (dd, J = 11.4, 4.6 Hz, 1H), 2.50−2.36 (m, 2H), 2.27−2.21 (m, 1H), 2.00 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 171.3, 156.2, 145.8, 141.9, 138.6, 137.5, 131.4, 129.2, 128.8, 128.3, 128.2, 127.4, 117.9, 115.2, 78.9, 77.0, 70.9, 59.8, 58.7, 48.3, 45.1, 36.9, 34.4, 21.5. HRMS (MH^+) calcd for $C_{29}H_{33}N_4O_4S$: 533.2223. Found 533.2236.

(3*R*,4*S*)-1-[(7-Benzyloxymethyl-9-deazaadenin-9-yl)methyl]-4-benzylthiomethyl-3-hydroxypyrrolidine (11). Compound **10** (900 mg, 1.7 mmol) was dissolved in $POCl_3$ (15 mL) and heated at reflux for 1 h. The resulting solution was concentrated in vacuo and evaporated with toluene (×2) to afford a solid residue. Without further purification the residue was dissolved in 7 N NH_3 in MeOH (15 mL) and heated in a sealed tube at 130 °C overnight. The reaction mixture was cooled to room temp and concentrated in vacuo and the resulting residue purified by chromatography (10% MeOH in CH_2Cl_2) to afford **11** (720 mg, 87% yield for two steps) as a syrup. ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H), 7.67 (s, 1H), 7.35−7.25 (m, 10H), 5.52 (s, 2H), 4.56 (s, 2H), 4.23 (s, 2H), 3.68 (s, 2H), 3.54−3.48 (m, 1H), 3.22 (d, J = 3.4 Hz, 2H), 2.83 (brs, 1H), 2.63−2.45 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 152.2, 151.6, 149.5, 138.2, 135.7, 134.5, 129.2, 129.1, 128.9, 128.8, 128.2, 127.5, 115.2, 107.4, 77.6, 74.9, 70.7, 60.2, 57.3, 48.5, 46.9, 46.3, 36.7, 33.5, 23.1.

(3*R*,4*S*)-4-Benzylthiomethyl-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxypyrrolidine (12). Compound **11** (330 mg, 0.7 mmol) was dissolved in a solution of MeOH (4 mL) and cHCl (4 mL) and heated under reflux for 90 min.. The reaction mixture was cooled to room temp, diluted with water (50 mL), and washed with $CHCl_3$ (×2) and the aqueous layer concentrated in vacuo, followed by codistillation with water (×2). The resulting residue was dissolved in aq cNH_4OH and concentrated in vacuo and the residue purified by chromatography to afford **12** (30 mg, 12%) as a solid. Mp 126−128 °C. ^{1}H NMR (d_4-MeOH) δ 8.17 (s, 1H), 7.46 (s, 1H), 7.26−7.16 (m, 5H), 3.93−3.90 (m, 1H), 3.83−3.74 (m, 2H), 3.68 (s, 2H), 3.03−2.97 (m, 1H), 2.80 (dd, J = 10.2, 6.4 Hz, 1H), 2.66−2.58 (m, 2H), 2.38 (dd, J = 12.5, 8.9 Hz, 1H), 2.30 (dd, J = 9.5, 7.2 Hz, 1H), 2.20−2.14 (m, 1H). ^{13}C NMR (d_4-MeOH) δ 152.5, 151.4, 147.4, 140.4, 130.4, 130.4, 129.8, 128.3, 115.5, 112.9, 77.3, 62.7, 59.2, 49.3, 48.6, 37.5, 35.6. HRMS (MH^+) calcd for $C_{19}H_{24}N_5OS$: 370.1702. Found 370.1694. Anal. ($C_{19}H_{23}N_5OS.2HCl\cdot H_2O$) C, H, N, Cl, S.

(3*R*,4*S*)-1-[(8-Aza-9-deazaadenin-9-yl)methyl]-4-benzylthiomethyl-3-hydroxypyrrolidine Hydrochloride (16). Sodium cyanoborohydride (20 mg, 0.32 mmol) was added to a stirred solution of **13** (180 mg, 0.52 mmol) and (3*R*,4*S*)-4-benzylthiomethyl-3-hydroxypyrrolidine hydrochloride (**8**) (95 mg, 0.37 mmol) in methanol (5 mL), and stirring was continued overnight at room temp. The crude reaction mixture was absorbed onto silica and purified by chromatography (12:4:0.5 CH_2Cl_2:MeOH:NH4OH v/v/v) to afford, presumably, (3*R*,4*S*)-1-[(8-aza-9-deazaadenin-9-yl)methyl]-4-benzylthiomethyl-3-hydroxypyrrolidine hydrochloride (**14**) as a foam (80 mg, 46%), which was committed to the next step without further characterization. Compound **14** was dissolved in 7 N NH_3 in MeOH (15 mL) and heated in a sealed tube at 110 °C overnight. The resulting solution was cooled to room temp and concentrated in vacuo and the resulting residue purified by chromatography

(5% MeOH in CH_2Cl_2) to afford, presumably, (3*R*,4*S*)-1-[8-aza-9-deaza-8-(tetrahydropyran-2-yl)-adenin-9-yl]methyl-3-hydroxy-4-benzylthiomethylpyrrolidine (**15**) (67 mg, 87%) as a foam. The product was not characterized further but dissolved in methanol (2.0 mL) and cHCl (2 mL) and concentrated in vacuo and the resulting residue triturated with 2-propanol to afford **16** (41 mg, 75%) as a white solid. ^{1}H NMR (d_4-MeOH) δ 8.35 (s, 1H), 7.29 (m, 5H), 4.75 (s, 2H), 4.22 (brs, 1H), 3.82 (m, 1H), 3.73 (s, 2H), 3.58 (m, 1H), 3.46 (m, 1H), 3.30 (m, 2H), 2.62 (dd, J = 12.2, 5.6 Hz, 1H), 2.48−2.31 (m, 2H). ^{13}C NMR (d_4-MeOH) δ 153.7, 152.0, 139.9, 138.8, 135.1, 130.4, 130.0, 128.5, 124.6, 74.6, 61.4, 58.4, 50.4, 47.7, 37.4, 33.4. (MH^+) calcd for $C_{18}H_{23}N_6OS$: 371.1654. Found 371.1670.

Procedures for the Synthesis of (3*R*,4*S*)-4-(Alkyl-, Aralkyl-, and Aryl-thiomethyl)-3-hydroxypyrrolidines. General Preparative Method. 4-Substituted-4-thiopyrrolidines were prepared from compound **1** essentially following the method detailed for the preparation of **2**, except where modifications are noted. In cases when the sodium thiolate was not directly available it was preformed by treating a stirred mixture of NaH (2.85 mmol) in DMF (5 mL) at 0 °C with the appropriate thiol (2.85 mmol). After stirring the mixture for 10 min, a solution of the intermediate mesylate (450 mg, 1.53 mmol) was added as a solution in DMF (5 mL) and the mixture was stirred at RT until the complete consumption of the mesylate was observed (0.5−4 h) by TLC. The *tert*-butoxycarbonyl-protected pyrrolidine intermediate was purified by chromatography (0−5% MeOH in CH_2Cl_2), and then a methanolic solution of the purified *tert*-butoxycarbonyl-protected pyrrolidine intermediate, following purification by chromatography, was treated with cHCl and concentrated in vacuo to afford the title compounds **17**−**26** (vide infra).

(3*R*,4*S*)-4-Ethylthiomethyl-3-hydroxypyrrolidine Hydrochloride (17). Following the general procedure (vide supra), mesylate (260 mg, 0.88 mmol) was processed to afford the title compound **17** (100 mg, 58%) as a syrup. ^{1}H NMR (D_2O) δ 4.30−4.24 (m, 1H), 3.53 (dd, J = 12.3, 7.2 Hz, 1H), 3.37 (dd, J = 12.8, 5.2 Hz, 1H), 3.14 (dd, J = 12.8, 3.1 Hz, 1H), 3.07 (dd, J = 12.2, 5.7 Hz, 1H), 2.65−2.55 (m, 1H), 2.46 (q, J = 7.4 Hz, 2H), 2.40−2.30 (m, 2H) 1.09 (t, J = 7.4 Hz, 3H). ^{13}C NMR (D_2O) δ 73.6, 51.5, 48.6, 45.6, 31.7, 26.0, 14.4. HRMS (MH^+) calcd for $C_7H_{16}NOS$: 162.0947. Found 162.0952.

(3*R*,4*S*)-3-Hydroxy-4-(1-propylthiomethyl)pyrrolidine Hydrochloride (18). Following the general procedure (vide supra), mesylate (264 mg, 0.89 mmol) was processed to afford the title compound **18** (139 mg, 0.66 mmol, 73%) as a syrup. ^{1}H NMR (D_2O) 4.41−4.37 (m, 1H), 3.67 (dd, J = 12.3, 7.2 Hz, 1H), 3.50 (dd, J = 12.8, 5.2 Hz, 1H), 3.27 (dd, J = 12.8, 3.1 Hz, 1H), 3.21 (dd, J = 12.2, 5.6 Hz, 1H), 2.76−2.71 (m, 1H), 2.61−2.50 (m, 4H), 1.59 (sextet, J = 7.3 Hz), 0.95 (t, J = 7.3 Hz, 3H). ^{13}C NMR (D_2O) 73.6, 51.5, 48.6, 45.7, 34.1, 32.1, 22.7, 13.1. HRMS (MH^+) calcd for $C_8H_{18}NOS$: 176.1104. Found 176.1106.

(3*R*,4*S*)-3-Hydroxy-4-(2-propylthiomethyl)pyrrolidine Hydrochloride (19). Following the general procedure (vide supra), mesylate (565 mg, 1.91 mmol) was processed to afford the intermediate compound (3*R*,4*S*)-1-*tert*-butoxycarbonyl-3-hydroxy-4-(2-propylthiomethyl)pyrrolidine (490 mg, 97%) as a syrup. ^{1}H NMR ($CDCl_3$): δ: 4.18−4.11 (m, 1H), 3.72−3.60 (m, 2H), 3.28−3.18 (m, 1H), 3.13 (dd, J = 11.1, 6.7 Hz, 1H), 2.95 (sept., J = 6.7 Hz, 1H), 2.69−2.50 (m, 2H), 2.33−2.22 (m, 1H), 1.46 (s, 9H), 1.29, 1.27 (s, 3H each). ^{13}C NMR ($CDCl_3$): δ: (note that some peaks are doubled due to slow conversion of rotamers) 154.93, 79.97, (75.48, 74.64), (52.81, 52.55), (49.69, 49.42), (46.13, 45.35), 35.71, 32.32, 28.87, 23.73, 23.69. The intermediate compound was converted to the title compound **19** as described in the general method (vide supra).

(3*R*,4*S*)-4-Butylthiomethyl-3-hydroxypyrrolidine Hydrochloride (20). Following the general procedure (vide supra), mesylate (438 mg, 1.48 mmol) was processed to afford **20** (284 mg, 1.25 mmol, 84%) as a syrup. ^{1}H NMR (D_2O) δ 4.40−4.36 (m, 1H), 3.66 (dd, J = 12.3, 7.2 Hz, 1H), 3.48 (dd, J = 12.8, 5.2 Hz, 1H), 3.26 (dd, J = 12.8, 3.1 Hz, 1H), 3.21 (dd, J = 12.2, 5.6 Hz, 1H), 2.76−2.70 (m, 1H), 2.62−2.50 (m, 4H), 1.61−1.51 (m, 2H), 1.40−1.33 (m, 2H), 0.86 (t, J = 7.3 Hz, 3H). ^{13}C NMR (D_2O) δ 73.5, 51.5, 48.6, 45.7, 32.2, 31.7, 31.3, 21.7, 13.3. HRMS (MH^+) calcd for $C_9H_{20}NOS$: 190.1260. Found 190.1257.

(3*R*,4*S*)-4-Cyclohexylthiomethyl-3-hydroxypyrrolidine Hydrochloride (21). Following the general procedure (vide supra), mesylate (565 mg, 1.91 mmol) was processed to afford the intermediate (3*R*,4*S*)-1-*tert*-butoxycarbonyl-4-cyclohexylthiomethyl-3-hydroxypyrrolidine (428 mg, 71%) as a syrup.. NMR ($CDCl_3$): δ: 4.17−4.09 (m, 1H), 3.72−3.60 (m, 2H), 3.28−3.18 (m, 1H), 3.15−3.09 (m, 1H), 2.74−2.64 (m, 2H), 2.60−2.53 (m, 1H), 2.32−2.23 (m, 1H), 1.96 (m, 2H), 1.81−1.77 (m, 2H), 1.66−1.61 (m, 1H), 1.45 (s, 9H), 1.35−1.24 (m, 5H). ^{13}C NMR ($CDCl_3$): δ: (note that some peaks are doubled due to slow conversion of rotamers) 154.91, 79.94, (75.57, 74.72), (52.80, 52.55), (49.70, 49.43), (46.24, 45.41), 44.26, 33.99, 33.93, 31.90, 28.87, 26.43, 26.12. The intermediate compound was converted to the title compound **21** as described in the general method (vide supra).

(3*R*,4*S*)-3-Hydroxy-4-phenylthiomethylpyrrolidine Hydrochloride (22). Following the general procedure (vide supra), mesylate (300 mg, 1.00 mmol) was processed to afford **22** (692 mg, 0.69 mmol) as a syrup. ^{1}H NMR (D_2O) δ 7.51−7.24 (m, 5H), 4.38−4.34 (m, 1H), 3.56 (dd, J = 12.2, 7.7 Hz, 1H), 3.45 (dd, J = 12.6, 5.2 Hz, 1H), 3.26−3.00 (m, 3H), 2.88 (dd, J = 13.7, 8.3 Hz, 1H), 2.48−2.37 (m, 1H). ^{13}C NMR (D_2O) δ 134.5, 130.5, 129.9, 127.6, 73.4, 51.5, 48.4, 45.5, 34.3. HRMS (MH^+) calcd for $C_{11}H_{16}NOS$: 210.0953. Found 210.0948.

(3*R*,4*S*)-4-(4-Chlorophenylthiomethyl)-3-hydroxypyrrolidine Hydrochloride (23). Following the general procedure (vide supra), mesylate (245 mg, 0.83 mmol) was processed to afford **23** (212 mg, 0.76 mmol, 91%) as a syrup. 1H NMR (d_4-MeOH) 7.51−7.39 (m, 2H), 7.35−7.31 (m, 2H), 4.38−4.33 (m, 1H), 3.59 (dd, J = 12.0, 7.6 Hz, 1H), 3.47 (dd, J = 12.4, 4.9 Hz, 1H), 3.28−3.18 (m, 3H), 2.93 (dd, J = 13.6, 9.0 Hz, 1H), 2.49−2.38 (m, 1H). ^{13}C NMR (D_2O) 135.7, 134.1, 132.9, 130.8, 74.8, 52.9, 49.6, 47.5, 35.8. HRMS (MH^+) calcd for $C_{11}H_{15}NOSCl$: 244.0557. Found 244.0546.

(3*R*,4*S*)-4-(3-Chlorophenylthiomethyl)-3-hydroxypyrrolidine Hydrochloride (24). Following the general procedure (vide supra), mesylate (300 mg, 1.02 mmol) was processed to afford **24** (192 mg, 0.685 mmol, 67%) as a syrup. ^{1}H NMR (D_2O) 7.33−7.15 (m, 4H), 4.39−4.35 (m, 1H), 3.59 (dd, J = 12.2, 7.7 Hz, 1H), 3.47 (dd, J = 12.7, 5.1 Hz, 1H), 3.28−3.04 (m, 3H), 2.89 (dd, J = 13.7, 8.3 Hz, 1H), 2.49−2.41 (m, 1H). ^{13}C NMR (D_2O) 136.9, 134.7, 131.0, 129.3, 128.1, 127.2, 73.4, 51.5, 48.4, 45.3, 34.0. HRMS (MH^+) calcd for $C_{11}H_{15}NOSCl$: 244.0557. Found 244.0556.

(3*R*,4*S*)-4-(4-Fluorophenylthiomethyl)-3-hydroxypyrrolidine Hydrochloride (25). Following the general procedure (vide supra), mesylate (281 mg, 0.951 mmol) was processed to afford **25** (160 mg, 0.607 mmol, 64%) as a syrup. ^{1}H NMR (D_2O) 7.49−7.42 (m, 2H), 7.19−7.06 (m, 2H), 4.41−4.36 (m, 1H), 3.60 (dd, J = 12.3, 7.7 Hz, 1H), 3.48 (dd, J = 12.8, 5.2 Hz, 1H), 3.27−3.16 (m, 2H), 3.07 (dd, J = 13.8, 6.9 Hz, 1H), 2.88 (dd, J = 13.8, 8.3 Hz, 1H), 2.45−2.38 (m, 1H). ^{13}C NMR (D_2O) 164.2, 160.9, 133.7, 133.6, 129.4, 116.9, 116.6, 73.3, 51.5, 48.4, 45.5, 35.5. HRMS (MH^+) calcd for $C_{11}H_{15}NOSF$: 228.0853. Found 228.0856.

(3*R*,4*S*)-3-Hydroxy-4-(4-pyridylthiomethyl)pyrrolidine Hydrochloride (26). Following the general procedure (vide supra), mesylate (348 mg, 1.18 mmol) was processed to afford **26** (105 mg, 0.426 mmol, 36%) as a syrup. ^{1}H NMR (D_2O) 8.42 (d, J = 7.2 Hz, 2H), 7.82 (d, J = 7.2 Hz, 2H), 4.51−4.46 (m, 1H), 3.74 (dd, J = 12.4, 7.8 Hz, 1H), 3.57 (dd, J = 12.8, 5.5 Hz, 1H), 3.44 (dd, J = 13.6, 7.3 Hz, 1H) 3.34−3.22 (m, 3H), 2.78−2.60 (m, 1H). ^{13}C NMR (D_2O) 164.1, 139.4, 122.9, 73.4, 51.4, 48.4, 44.3, 31.3. HRMS (MH^+) calcd for $C_{10}H_{15}N_2OS$: 211.0900. Found 211.0908.

(3*R*,4*R*)-1-*tert*-Butoxycarbonyl-4-[(*tert*-butyldiphenylsilyloxy)methyl]-3-hydroxypyrrolidine (45). *tert*-Butyldiphenylsilyl chloride (0.740 mL, 2.85 mmol) was added dropwise to a stirred solution of the diol **1** (560 mg, 2.58 mmol) and imidazole (270 mg, 3.97 mmol) in DMF (12 mL) cooled to

0 °C. After stirring for 2 h, the reaction mixture was diluted with toluene (100 mL) and washed with water (25 mL) and brine (25 mL), and the organic layer was dried ($MgSO_4$) and concentrated in vacuo to yield the crude product. Purification by chromatography (20−50% ethyl acetate in petroleum ether) afforded **45** (837 mg, 1.84 mmol, 71%) as an oil. ^{1}H NMR ($CDCl_3$) 7.70−7.62 (m, 4H), 7.48−7.38 (m, 6H), 4.30−4.20 (m, 1H), 3.75 − 3.45 (m, 4H), 3.30−3.05 (m, 2H), 2.32−2.25 (m, 2H), 1.44 (s, 9H), 1.06 (s, 9H). ^{13}C NMR ($CDCl_3$) 154.9, 135.9, 133.4, 130.3, 128.2, 79.8, 73.9, 73.0, 64.7, 53.1, 52.8, 48.7, 48.0, 46.8, 46.4, 28.9, 27.3, 19.6. HRMS (MH^+) calcd for $C_{26}H_{38}NO_4Si$: 456.2565. Found 456.2531.

(3*R*,4*R*)-1-*tert*-Butoxycarbonyl-4-[(*tert*-butyldiphenylsilyloxy)methyl]-3-methoxymethoxypyrrolidine (46). Bromomethyl methyl ether (0.420 mL, 5.2 mmol) was added dropwise to a stirred solution of **45** (787 mg, 1.73 mmol) and diisopropylethylamine (1.50 mL, 8.47 mmol) in toluene (50 mL). The reaction mixture was heated to 70 °C for 3 h. After cooling to room temp, the reaction mixture was subjected to standard aqueous workup to yield the crude product which was purified by chromatography (20−30% ethyl acetate in petroleum ether) to afford **46** (841 mg, 1.68 mmol, 97%) as an oil. ^{1}H NMR ($CDCl_3$) 7.70−7.62 (m, 4H), 7.48−7.38 (m, 6H), 4.61 (s, 2H), 4.20−4.15 (m, 1H) 3.60−3.20 (m, 6H), 3.30 (s, 3H), 2.43−2.38 (m, 1H), 1.45 (s, 9H), 1.06 (s, 9H). ^{13}C NMR ($CDCl_3$) 154.9, 135.9, 133.7, 130.2, 128.5, 128.1, 96.0, 95.9, 79.6, 76.7, 63.5, 55.8, 51.5, 50.9, 47.3, 46.8, 46.6, 46.4, 28.9, 27.2, 19.6, 14.6. HRMS (MH^+) calcd for $C_{28}H_{42}NO_5Si$: 500.2827. Found 500.2821.

(3*R*,4*R*)-1-*tert*-Butoxycarbonyl-4-hydroxymethyl-3-methoxymethoxypyrrolidine (47). Tetrabutylammonium fluoride (1 M in THF, 1.90 mL, 1.90 mmol) was added dropwise to a stirred solution of **46** (779 mg, 1.56 mmol) in THF (20 mL). After 2 h at room temp, the solvent was removed in vacuo to yield a residue which was purified by chromatography (70% ethyl acetate in petroleum ether) to afford **47** (264 mg, 1.01 mmol, 65%) as a thin film. ^{1}H NMR ($CDCl_3$) 4.66 (s, 2H), 4.15−4.05 (m, 1H) 3.60−3.48 (m, 4H), 3.37 (s, 3H), 3.37−3.10 (m, 2H), 2.78−2.60 (m, 1H), 2.45−2.35 (m, 1H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$) 155.0, 96.2, 79.9, 78.1, 77.2, 62.6, 55.9, 51.5, 50.9, 47.1, 46.9, 46.5, 46.3, 28.8. HRMS (MH^+) calcd for $C_{12}H_{24}NO_5$: 262.1654. Found 262.1796.

(3*R*,4*R*)-1-*tert*-Butoxycarbonyl-3-methoxymethoxy-4-methoxymethylpyrrolidine (48). Sodium hydride (60% dispersion in mineral oil, 42 mg, 1.05 mmol) was added to a stirred solution of **47** (173 mg, 0.66 mmol) and iodomethane (0.100 mL, 1.59 mmol) in DMF (10 mL) cooled to 0 °C. After 1 h at room temp, the reaction mixture was subjected to a standard aqueous workup and purified by chromatography (20−35% ethyl acetate in petroleum ether) to afford **48** (147 mg, 0.534 mmol, 81%) as an oil. ^{1}H NMR ($CDCl_3$) 4.65 (s, 2H), 4.12−4.02 (m, 1H), 3.63−3.52 (m, 2H), 3.37 (s, 3H), 3.34 (s, 3H), 3.37−3.10 (m, 4H), 2.52−2.41 (m, 1H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$) 154.9, 96.1, 79.7, 77.9, 72.7, 59.3, 55.9, 51.4, 50.9, 47.4, 46.9, 45.1, 44.2, 28.9. HRMS (MH^+) calcd for $C_{13}H_{26}NO_5$: 276.1806. Found 276.1813.

(3*R*,4*R*)-3-Hydroxy-4-methoxymethylpyrrolidine Hydrochloride (27). cHCl (1 mL) was added to a stirred solution of **48** (152 mg, 0.552 mmol) in MeOH (3 mL). After 4 h at room temp, the solvent was removed in vacuo to yield the crude product which was azetroped with D_2O (×2) to afford **27** (87 mg, 0.552 mmol, 100%) as a hygroscopic glass. ^{1}H NMR (D_2O) 4.30−4.26 (m, 1H), 3.52−3.28 (m, 4H), 3.22 (s, 3H), 3.15−3.00 (m, 2H), 2.48−2.37 (m, 1H). ^{13}C NMR (D_2O) 72.1, 71.6, 58.8, 52.0, 46.7, 45.7. HRMS (MH^+) calcd for $C_6H_{14}NO_2$: 132.1019. Found 132.1012.

(3*R*,4*R*)-4-Benzyloxymethyl-1-*tert*-butoxycarbony-3-methoxymethoxypyrrolidine (49). Sodium hydride (60% dispersion in mineral oil, 28 mg, 0.70 mmol) was added to a stirred solution of **47** (91 mg, 0.35 mmol) and benzyl bromide (0.10 mL, 0.84 mmol) in DMF (5 mL) cooled to 0 °C. After 1 h at room temp, the reaction mixture was subjected to a standard aqueous workup to yield the crude product which was purified by chromatography (20−40% ethyl acetate in petroleum ether) to afford **49** (108 mg, 0.31 mmol, 88%) as an oil. ^{1}H NMR ($CDCl_3$) 7.40−7.20 (m, 5H) 4.64 (s, 2H), 4.51 (s, 2H), 4.15−4.05 (m, 1H) 3.61−3.15 (m, 6H), 3.34 (s, 3H), 2.58−2.46 (m, 1H), 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$) 155.0, 138.4, 128.8, 128.1, 128.1, 128.0, 96.1, 79.7, 77.9, 76.9, 73.6, 69.9, 55.9, 51.5, 50.9, 47.4, 46.9, 45.2, 44.3, 28.9. HRMS (MH^+) calcd for $C_{19}H_{30}NO_5$: 352.2119. Found 352.2132.

(3R,4R)-4-Benzyloxymethyl-3-hydroxypyrrolidine Hydrochloride (28). HCl (4 M in dioxane, 2.5 mL) was added to a stirred solution of **49** (107 mg, 0.304 mmol) in MeOH. After 14 h at room temp, the solvent was removed in vacuo to yield the crude product which was purified by chromatography (20−35% 7 N NH_3/MeOH in CH_2Cl_2) to afford **28** (51 mg, 0.25 mmol, 82%) as a hygroscopic glass. ^{1}H NMR (D_2O, free base) 7.32−7.15 (m, 5H), 4.36 (s, 2H), 3.92−3.85 (m, 1H), 3.35 (dd, J = 9.8, 7.0 Hz, 1H), 3.24 (dd, J = 9.8, 7.8 Hz, 1H), 2.97 (dd, J = 11.8, 7.9 Hz, 1H), 2.75 (dd, J = 12.4, 5.5 Hz, 1H), 2.57 (dd, J = 12.4, 3.4 Hz, 1H), 2.36 (dd, J = 11.8, 5.7 Hz, 1H), 2.15−2.05 (m, 1H). ^{13}C NMR (D_2O) 137.6, 129.0, 128.8, 128.6, 74.7, 73.1, 71.0, 53.2, 48.0, 47.6. HRMS (MH^+) calcd for $C_{12}H_{18}NO_2$: 208.1332. Found 208.1329.

(3*R*,4*S*)-3-Hydroxy-4-(1-propyl)pyrrolidine Hydrochloride (29). To a suspension of ethyltriphenylphosphonium bromide (2.9 g, 6.93 mmol) in dry THF (15 mL) under argon at 0 °C was added *n*-BuLi in THF (1.6 M, 4.0 mL, 6.40 mmol) and the deep red solution left stirring without cooling for 10 min. After re-cooling to 0 °C, the aldehyde **50** (580 mg, 2.7 mmol) in THF (10 mL) was added and the mixture stirred at room temp for 12 h. The reaction was then quenched with water (1 mL) and extracted with dichloromethane (100 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ (15 mL) then water (15 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was subjected to chromatography (30% ethyl acetate in petroleum ether) to afford, presumably, (3*R*,4*S*)-*N*-*tert*-butoxycarbonyl-3-hydroxy-4-propenyl-pyrrolidine (**51**) (165 mg, 27%) as a syrup. Without further characterization, the alkene (165 mg, 0.73 mmol) was dissolved in ethanol (10 mL), 10% Pd/C (60 mg) was added, and the suspension was stirred under an atmosphere of hydrogen for 3 h. After filtration, the solvent was removed in vacuo to afford, presumably, (3*R*,4*S*)-*N*-*tert*-butoxycarbonyl-3-hydroxy-4-(1-propyl)pyrrolidine as a syrup (172 mg, 100%), which was committed to the next step without further purification. To a solution of the intermediate *tert*-butoxycarbonyl-protected amine (172 mg, 0.75 mmol) in methanol (10 mL) was added cHCl (4 mL) and the solution stirred at 40 °C for 30 min. After removal of the solvent in vacuo and azeotroping with toluene, the title compound **29** was obtained as a syrup (138 mg, 100%). ^{1}H NMR (d_4-MeOH): δ: 4.20−4.16 (m, 1H), 3.59−3.52 (m, 1H), 3.44−3.39 (m, 1H), 3.19−3.14 (m, 1H), 3.05−2.99 (m, 1H), 2.23−2.17 (m, 1H), 1.55−1.28 (m, 4H), 0.98−0.94 (m, 3H). ^{13}C NMR (d_4-MeOH): δ: 75.65, 52.82, 50.30, 47.45, 34.69, 22.30, 14.78.

(3*R*,4*S*)-3-Hydroxy-4-phenylethylpyrrolidine Hydrochloride (30). To a suspension of benzyltriphenylphosphonium bromide (1.75 g, 4.97 mmol) in dry THF (10 mL) under argon at 0 °C was added *n*-BuLi in THF (1.6 M, 2.33 mL, 3.73 mmol), and the deep red solution was stirred without cooling for 10 min. After recooling to 0 °C, the aldehyde **50** (335 mg, 1.56 mmol) in THF (5 mL) was added and the mixture stirred at room temp for 12 h. The reaction was then quenched with water (1 mL) and extracted with dichloromethane (100 mL). The organic phase was washed with saturated aqueous $NaHCO_3$ (15 mL) and water (15 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was subjected to chromatography (30% ethyl acetate in petroleum ether) to afford, presumably, compound **52** as a 1:3 *E*/*Z* mixture of stereoisomers (290 mg, 64%), which was committed to the next step without further characterization. To **52** (290 mg, 1.00 mmol) in ethanol (20 mL) was added 10% Pd/C (250 mg), and the suspension was stirred under an atmosphere of hydrogen for 12 h. After filtration, the solvent was removed in vacuo to afford, presumably, (3*R*,4*S*)-*N*-*tert*-butoxycarbonyl-3-hydroxy-4-(2-phenylethyl)pyrrolidine (254 mg 87%), which was com-

mitted to the next step without further characterization. To a solution of the *tert*-butoxycarbonyl-protected amine (254 mg, 0.87 mmol) in methanol (10 mL) was added cHCl (4 mL) and the solution stirred at 40 °C for 30 min. After removal of the solvent in vacuo and azeotroping with toluene, the title compound **30** was obtained as a solid (202 mg, quantitative). ^{1}H NMR (d_4-MeOH): δ: 7.14 (m, 5H), 4.22 (m, 1H), 3.52 (dd, J = 11.8, 7.4 Hz, 1H), 3.39 (dd, J = 12.3, 4.9 Hz, 1H), 3.14 (dd, J = 12.3, 2.8 Hz, 1H), 3.02 (dd, J = 11.8 Hz, 1H), 2.71 (m, 2H), 2.20 (m, 1H), 1.84 (m, 1H), 1.62 (m, 1H). ^{13}C NMR (d_4-MeOH): δ: 142.94, 129.93, 129.89, 127.56, 75.56, 52.90, 48.55, 47.28, 35.18, 34.44.

General Procedure for the Preparation of Compounds (31−44) Using the Mannich Reaction. Pyrrolidine hydrochloride (**17**−**30**) (1.0 mol equiv) and sodium acetate (1.0 mol equiv) were dissolved in water and 1,4-dioxane (4:1 v/v, 2 mL per mmol), and to the solution was added aqueous formaldehyde (1.0−1.5 mol equiv) and deazaadenine (0.8−1.5 mol equiv). The reaction was stirred at 95 °C for 1 h and then cooled to room temp. Silica gel (1.0 g per mmol of pyrrolidine) was added, and the mixture was evaporated to dryness. Purification by chromatography on silica gel, using gradient elution with CH_2Cl_2:MeOH:NH_4OH (95:5:1→80:20:1 v/v/v) as the eluent, afforded the compounds **31**−**44** as the free base or partial acetic acid salt, which was converted to the HCl salt by addition and evaporation of excess concentrated HCl, unless stated otherwise.[25]

(3*R*,4*S*)-1-[(9-Deaza-adenin-9-yl)methyl]-4-ethylthiomethyl-3-hydroxypyrrolidine (31). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride **17** (99 mg, 0.50 mmol) was processed to afford **31** (92 mg, 60%) as the syrupy free base. ^{1}H NMR (d_4-MeOH) 8.16 (s, 1H), 7.52 (s, 1H), 4.00−3.82 (m, 3H), 3.12 (dd, J = 9.9, 7.9 Hz, 1H), 2.92 (dd, J = 10.5, 6.3 Hz, 1H), 2.76−2.68 (m, 2H), 2.55−2.41 (m, 4H), 2.25−2.15 (m, 1H), 1.21 (t, J = 7.4 Hz, 3H). ^{13}C NMR (d_4-MeOH) δ 152.5, 151.5, 147.3, 130.7, 115.6, 112.1, 77.0, 62.4, 59.1, 49.4, 48.8, 35.5, 27.2, 15.5. HRMS (MH^+) calcd for $C_{14}H_{21}N_5OS$: 308.1540. Found 308.1535. Anal. ($C_{14}H_{21}N_5OS\cdot{}^1/_2H_2O$) C, H, N, S.

(3*R*,4*S*)-1-[(9-Deaza-adenin-9-yl)methyl]-3-hydroxy-4-(1-propylthiomethyl)pyrrolidine (32). Following the general procedure for the Mannich reaction (vide supra) pyrrolidine hydrochloride **18** (70 mg, 0.33 mmol) was processed to afford **32** (62 mg, 58%) as the syrupy free base. ^{1}H NMR (d_4-MeOH) δ 8.17 (s, 1H), 7.50 (s, 1H), 4.00−3.79 (m, 3H), 3.08 (dd, J = 9.8, 7.9 Hz, 1H), 2.86 (dd, J = 10.3, 6.4 Hz, 1H), 2.72−2.62 (m, 2H), 2.50−2.38 (m, 4H), 2.22−2.12 (m, 1H), 1.55 (sextet, J = 7.3 Hz, 2H), 0.95 (t, J = 7.3 Hz, 3H). ^{13}C NMR (d_4-MeOH) δ 152.5, 151.4, 147.4, 130.5, 115.6, 112.7, 77.2, 62.6, 59.2, 49.4, 49.0, 36.1, 35.6, 24.3, 14.1. HRMS (MH^+) calcd for $C_{15}H_{23}N_5OS$: 322.1696. Found 322.1709. Anal. ($C_{15}H_{23}N5OS\cdot{}^1/_2H_2O$) C, H, N, S.

(3*R*,4*S*)-1-[(9-Deaza-adenin-9-yl)methyl]-3-hydroxy-4-(2-propylthiomethyl)pyrrolidine (33). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride **19** was processed to afford **33** (183 mg, 38%) as the hydrochloride salt. ^{1}H NMR (MeOH-d_4): δ: 8.16 (s, 1H), 7.49 (s, 1H), 3.99−3.94 (m, 1H), 3.82 (dd, J = 18.7, 13.4 Hz, 1H), 3.04 (dd, J = 9.7, 7.9 Hz, 1H), 2.95−2.82 (m, 2H), 2.75 (dd, J = 12.5, 6.0 Hz, 1H), 2.66 (dd, J = 10.3, 4.2 Hz, 1H), 2.50 (dd, J = 12.5, 9.1 Hz, 1H), 2.38 (dd, J = 9.7, 7.1 Hz, 1H), 2.21−2.10 (m, 1H), 1.23, 1.22 (2s, 3H each). ^{13}C NMR (MeOH-d_4): δ: 152.48, 151.38, 147.40, 130.45, 115.54, 112.90, 77.29, 62.66, 59.26, 49.32, 49.09, 36.49, 34.66, 24.19. HRMS (MH^+) calcd for $C_{15}H_{23}N_5OS$: 322.1696:. Found 322.1701. Anal. ($C_{15}H_{23}N_5OS\cdot2HCl\cdot2H_2O$) C, H, N, Cl, S.

(3*R*,4*S*)-4-(1-Butylthiomethyl)-1-[(9-deaza-adenin-9-yl)-methyl]-3-hydroxypyrrolidine (34). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride **20** (125 mg, 0.55 mmol) was processed to afford **34** (97 mg, 52%) as the syrupy free base. ^{1}H NMR (d_4-MeOH) δ 8.16 (s, 1H), 7.50 (s, 1H), 3.99−3.79 (m, 3H), 3.08 (dd, J = 9.7, 7.9 Hz, 1H), 2.87 (dd, J = 10.3, 6.4 Hz, 1H), 2.75−2.69 (m, 2H), 2.51−2.38 (m, 4H), 2.22−2.12 (m, 1H), 1.55−1.32 (m, 4H), 0.90 (t, J = 7.3 Hz, 3H). ^{13}C NMR (d_4-MeOH) δ 152.5, 151.4, 147.4, 130.5, 115.6, 112.6, 77.1, 62.6, 59.2, 49.4, 49.0, 36.1, 33.3, 33.2, 23.3, 14.4. HRMS (MH^+) calcd for $C_{16}H_{25}N_5OS$: 336.1853. Found 336.1850. Anal. ($C_{16}H_{25}N_5OS$) C, H, N, S.

(3*R*,4*S*)-4-Cyclohexylthiomethyl-1-[(9-deaza-adenin-9-yl)methyl]-3-hydroxypyrrolidine (35). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride **21** (192 mg, 0.685 mmol) was processed to afford **35** (144 mg, 34%) as the hydrochloride salt. ^{1}H NMR (MeOH-d_4): δ: 8.15 (s, 1H), 7.50 (s, 1H), 3.97−3.92 (m, 1H), 3.82 (dd, J = 19.1, 13.4 Hz, 2H), 3.06−3.00 (m, 1H), 2.84 (dd, J = 10.3, 6.4 Hz, 1H), 2.75 (dd, J = 12.5, 5.9 Hz, 1H), 2.67−2.58 (m, 2H), 2.48 (dd, J = 12.5, 9.3 Hz, 1H), 2.37 (dd, J = 9.8, 7.2 Hz, 1H), 2.20−2.08 (m, 1H), 1.94−1.92 (m, 2H), 1.74−1.72 (m, 2H), 1.60−1.58 (m, 1H), 1.36−1.19 (m, 5H). ^{13}C NMR (MeOH-d_4): δ: 152.48, 151.35, 147.32, 130.50, 115.48, 112.74, 77.21, 62.62, 59.18, 49.38, 49.26, 45.10, 35.27, 35.20, 34.18, 27.48, 27.39. HRMS (MH^+) calcd for $C_{18}H_{27}N_5OS$: 362.2009. Found 362.2016. Anal. ($C_{18}H_{27}N_5OS\cdot2HCl\cdot H_2O$) C, H, N, S, Cl.

(3*R*,4*S*)-1-[(9-Deaza-adenin-9-yl)methyl]-3-hydroxy-4-phenylthiomethylpyrrolidine (36). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride **22** (210 mg, 0.86 mmol) was processed to afford **36** (175 mg, 57%) as the hydrochloride salt. ^{1}H NMR (d_4-MeOH) δ 8.22 (s, 1H), 7.74 (s, 1H), 7.33−7.15 (m, 2H), 4.43 (s, 2H), 4.26 (m, 1H), 3.62 (dd, J = 11.7, 7.9 Hz, 2H), 3.48 (dd, J = 12.0, 5.6 Hz, 1H), 3.25 (t, dd, J = 12.0, 3.3 Hz, 1H), 3.15 (m, 2H), 2.85 (dd, J = 13.5, 9.1 Hz, 1H), 2.43 (m, 1H). ^{13}C NMR (d_4-MeOH) δ 152.9, 152.1, 146.8, 136.8, 132.7, 131.4, 130.6, 128.1, 115.8, 106.3, 74.9, 60.6, 57.4, 49.7, 47.7, 36.3. HRMS (MH^+) calcd for $C_{18}H_{21}N_5OS$: 356.1545. Found 356.1542. Anal. ($C_{18}H_{21}N_5OS\cdot3HCl\cdot2H_2O$) C, H, N, S, Cl.

(3*R*,4*S*)-4-(4-Chlorophenylthiomethyl)-1-[(9-deaza-adenin-9-yl)methyl]-3-hydroxypyrrolidine (37). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride 23 (180 mg, 0.64 mmol) was processed to afford 37 (180 mg, 72%) as the hydrochloride salt. ^{1}H NMR (d_4-MeOH) δ 8.25 (s, 1H), 7.84 (s, 1H), 7.35−7.23 (m, 5H), 4.54 (s, 2H), 4.30 (m, 1H), 3.74 (dd, J = 11.9, 7.9 Hz, 1H), 3.59 (dd, J = 12.2, 5.6 Hz, 1H), 3.40−3.15 (m, 4H), 2.89 (dd, J = 13.5, 9.1 Hz, 1H), 2.47 (brs, 1H), 1.98 (s, 3H). ^{13}C NMR (d_4-MeOH) δ 153.0, 151.8, 146.1, 135.7, 134.0, 133.2, 132.2, 130.7, 115.7, 105.5, 74.6, 60.4, 57.3, 49.2, 47.7, 36.1, 23.0. HRMS (MH^+) calcd for $C_{18}H_{20}ClN_5OS$: 390.1155. Found 390.1264. Anal. ($C_{18}H_{20}N_5ClOS\cdot2H_2O\cdot2HCl$) C, H, N, Cl, S.

(3*R*,4*S*)-4-(3-Chlorophenylthiomethyl)-1-[(9-deaza-adenin-9-yl)methyl]-3-hydroxypyrrolidine (38). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride **24** (192 mg, 0.69 mmol) was processed to afford **38** (139 mg, 0.357 mmol, 52%) as the hydrochloride salt. ^{1}H NMR (d_4-MeOH) δ 8.16 (s, 1H), 7.46 (s, 1H), 7.25−7.05 (m, 4H), 4.01−3.97 (m, 1H), 3.87−3.76 (m, 2H), 3.18 (dd, J = 12.9, 5.9 Hz, 1H), 2.99 (dd, J = 9.8, 7.9 Hz, 1H), 2.94−2.86 (m, 2H), 2.64 (dd, J = 10.2, 4.3 1H), 2.41 (dd, J = 9.9, 7.0 Hz, 1H), 2.26 − 2.15 (m, 1H). ^{13}C NMR (d_4-MeOH) δ 152.5, 151.4, 147.4, 140.7, 136.1, 131.6, 130.4, 129.8, 128.6, 127.4, 115.5, 112.8, 77.1, 62.6, 58.9, 49.3, 48.7, 37.4. HRMS (MH^+) calcd for $C_{18}H_{20}N_5OClS$: 390.1150. Found 390.1142. Anal. ($C_{18}H_{20}ClN_5OS\cdot2HCl\cdot{}^1/_2H_2O$) C, H, N, S, Cl.

(3*R*,4*S*)-1-[(9-Deaza-adenin-9-yl)methyl]-4-(4-fluorophenylthiomethyl)-3-hydroxypyrrolidine (39). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride **25** (149 mg, 0.57 mmol) was processed to afford **39** (55 mg, 0.15 mmol, 27%) as the hydrochloride salt. ^{1}H NMR (d_4-MeOH) δ 8.16 (s, 1H), 7.46 (s, 1H), 7.40−7.30 (m, 2H), 7.00−6.90 (m, 2H), 4.02−3.97 (m, 1H), 3.86−3.75 (m, 2H), 3.11 (dd, J = 12.9, 5.9 Hz, 1H), 3.00 (t, J = 8.7 Hz, 1H), 2.90−2.75 (m, 2H), 2.65−2.59 (m, 1H), 2.41−2.32 (m, 1H), 2.20−2.10 (m, 1H). ^{13}C NMR (d_4-MeOH) δ 165.5, 162.0, 152.5, 151.4, 147.4, 134.1, 134.0, 133.1, 130.4, 117.4, 117.1, 115.5, 112.9, 77.2, 62.7, 59.0, 49.3, 48.8, 39.1. HRMS (MH^+) calcd for $C_{18}H_{20}N_5OFS$: 374.1445. Found 374.1438. Anal. ($C_{18}H_{20}FN_5OS\cdot2HCl\cdot2H_2O$) C, H, N, S.

(3*R*,4*S*)-1-[(9-Deaza-adenin-9-yl)methyl]-3-hydroxy-4-(4-pyridylthiomethyl)pyrrolidine (40). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride 26 (81 mg, 0.33 mmol) was processed to afford 40 (53 mg, 0.15 mmol, 45%) as the hydrochloride salt. ^{1}H NMR (D_2O), free base, 8.43 (d, J = 7.2 Hz, 1H), 8.42 (s, 1H), 8.00 (s, 1H), 7.77 (d, J = 7.2 Hz, 1H), 4.64 (s, 2H), 4.52−4.47 (m, 1H), 3.94 (dd, J = 12.1, 8.0 Hz, 1H), 3.67 (dd, J = 12.6, 5.7 Hz, 1H), 3.50−3.15 (m, 4H), 2.78−2.64 (m, 1H). ^{13}C NMR (D_2O) 163.9, 150.2, 144.6, 139.5, 135.4, 122.8, 113.2, 102.7, 73.0, 59.0, 55.9, 48.1, 44.4, 31.5. HRMS (MH^+) calcd for $C_{17}H_{20}N_6OS$: 357.1492. Found 357.1509. Anal. ($C_{17}H_{20}N_6OS\cdot3HCl\cdot2H_2O$) C, H, N, S, Cl.

(3*R*,4*R*)-1-[(9-Deaza-adenin-9-yl)methyl]-3-hydroxy-4-methoxymethylpyrrolidine (41). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride **27** (87 mg, 0.52 mmol) was processed to afford **41** (85 mg, 60%) as the hydrochloride salt. ^{1}H NMR (d_4-MeOH) δ 8.19 (s, 1H), 7.63 (s, 1H), 4.18−4.05 (m, 3H), 3.40−2.28 (m, 3H), 3.30 (s, 3H), 3.10 (dd, J = 11.0, 5.7 Hz, 1H), 2.95 (dd, J = 11.0, 3.3 Hz, 1H), 2.77 (dd, J = 10.8, 6.7 Hz, 1H), 2.41−2.29 (m, 1H). ^{13}C NMR (d_4-MeOH) δ 152.7, 151.8, 147.2, 131.6, 115.7, 109.5, 74.3, 74.1, 62.2, 59.6, 56.5, 49.4, 49.0. HRMS (MH^+) calcd for $C_{13}H_{19}N_5O_2$: 278.1612. Found 278.1626. Anal. ($C_{13}H_{19}N_5O_2\cdot2HCl\cdot0.5H_2O$) C, H, N, Cl.

(3*R*,4*R*)-4-(Benzyloxymethyl)-1-[(9-deaza-adenin-9-yl)-methyl]-3-hydroxypyrrolidine (42). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride **28** (55 mg, 0.23 mmol), was processed to afford **42** (18 mg, 22%) as the hydrochloride salt. ^{1}H NMR (d_4-MeOH) δ 8.17 (s, 1H), 7.55 (s, 1H), 7.30−7.20 (m, 5H), 4.46 (bs, 2H), 4.10−4.00 (m, 3H), 3.55−3.38 (m, 2H), 3.23−3.18 (m, 1H), 2.98 (dd, J = 10.7, 5.8 Hz, 1H), 2.85 (dd, J = 10.7, 3.4 Hz, 1H), 2.68 (dd, J = 10.4, 6.9 Hz, 1H), 2.38−2.30 (m, 1H). ^{13}C NMR (d_4-MeOH) δ 152.6, 151.7, 147.2, 139.9, 131.3,129.8, 129.3, 129.1, 115.6, 110.4, 74.5, 74.3, 71.9, 62.3, 56.6, 49.4, 49.0. Anal. ($C_{19}H_{23}N_5O_2\cdot2HCl\cdot0.75H_2O$) C, H, N, Cl.

(3*R*,4*S*)-1-[(9-Deazaadenin-9-yl)methyl]-3-hydroxy-4-(1-propyl)pyrrolidine (43). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride **29** was processed to afford **43** (58 mg, 31%) as the hydrochloride salt. ^{1}H NMR (MeOH-d_4): δ: 8.18 (s,1H), 7.51 (s,1H), 3.91−3.85 (m, 3H), 3.10 (dd, J = 9.6, 8.0 Hz, 1H), 2.82−2.72 (m, 2H), 2.22 (dd, J = 9.6, 8.0 Hz, 1H), 2.04−1.95 (m, 1H), 1.56−1.44 (m, 1H), 1.39−1.21 (m, 3H), 0.92−0.87 (m, 3H). ^{13}C NMR (MeOH-d_4): δ: 152.52, 151.45, 147.37, 130.59, 115.55, 112.50, 77.94, 62.63, 59.94, 49.55, 48.59, 36.89, 22.67, 14.91. HRMS (MH^+) calcd for $C_{14}H_{21}N_5O$: 276.1819. Found 276.1822. Anal. ($C_{14}H_{21}N_5O\cdot2HCl\cdot2H_2O$) C, H, N, Cl.

(3*R*,4*S*)-1-[(9-Deazaadenin-9-yl)methyl]-3-hydroxy-4-(2-phenylethyl)pyrrolidine (44). Following the general procedure for the Mannich reaction (vide supra), pyrrolidine hydrochloride **30** was processed to afford, presumably, (3*R*,4*S*)-1-[(6-chloro-9-Deazapurin-9-yl)methyl]-3-hydroxy-4-(2-phenylethyl)pyrrolidine (70 mg, 38%), Without further characterization this was dissolved in 7 N ammonia in methanol (4 mL) and heated in a sealed tube at 130 °C for 3 h at which point the reaction was cooled to room temp and concentrated in vacuo. Chromatography (CH_2Cl_2:MeOH:NH_4OH 80:20:1 v/v/v) of the resulting residue afforded **44** as a syrup (29 mg, 44%). ^{1}H NMR (D_2O): δ ppm: 8.40 (s, 1H), 7.83 (s, 1H), 7.26 (m, 5H), 4.33 (m, 4H), 4.07 (m, 1H), 3.80 (m, 2H), 2.75 (m, 2H), 2.37 (m, 1H), 1.90 (m, 1H), 1.66 (m, 1H). ^{13}C NMR (D_2O): δ 149.54, 144.47, 142.40, 133.05, 129.05, 128.85, 126.51, 113.64, 103.48, (74.87, 72.96), (55.34, 54.87), (52.59, 52.09), (45.96, 43.65), 33.30, 32.94, 32.33. $C_{19}H_{23}N_5O$: (MH^+): calc.: 338.19754, found:338.19794.

Biology. Protein Preparation. Human MTAP was expressed in *E. coli* BL21(DE3) using the pQE32 expression vector (provided by Drs. Carson and Rosenbech of Scripps Institute La Jolla, CA). Protein with a His-6-N-terminal label was purified by Ni−NTA (Qiagen) chromatography, concentrated, and stored at −80 °C. Details are provided in ref 1.

Enzymatic Assays. Reaction mixtures of 1.0 mL contained 100 mM potassium phosphate (Sigma) pH 7.4, 50 mM KCl (Sigma), 0.1 to 2 mM dithiothreitol (Sigma), 5'-methylthioadenosine (Sigma) at 0.25 or 0.30 mM, and 0.5 units of xanthine oxidase (Sigma) and variable concentrations of inhibitor. After warming to 25 °C in a temperature-controlled spectrophotometer (Cary 300 Bio spectrophotometer from Varian), reactions were initiated with 1 to 10 nM of purified human MTAP. Activity was monitored by the increase in absorbance at 294 nm equivalent to 15.2 mM^{-1} cm^{-1}. The coupled reaction is irreversible and measures the formation of 2,8-dihydroxyadenine from adenine.

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Supporting Information Available: Analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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