Azetidine Based Transition State Analogue Inhibitors of *N*-Ribosyl Hydrolases and Phosphorylases

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N-Ribosyl phosphorylases and hydrolases catalyze nucleophilic displacement reactions by migration of the cationic ribooxacarbenium carbon from the fixed purine to phosphate and water nucleophiles, respectively. As the lysis reaction progresses along the reaction coordinate, the distance between the purine and carbocation increases and the distance between carbocation and nucleophile decreases. Immucillin-H and DADMe-immucillin-H have been shown previously to be potent inhibitors of purine nucleoside phosphorylases and lie more toward the reactant and products side of this reaction coordinate, respectively. Both these enzyme inhibitors, which are currently in human clinical trials for different indications, are chiral and expensive to manufacture. We now report the synthesis of azetidine analogues of the DADMe-immucillins, which, despite their lack of stereochemical complexity, remain potent inhibitors (equilibrium dissociation constants as low as 229 pM) of purine nucleoside phosphorylase (PNP), methylthioadenosine phosphorylase (MTAP), and methylthioadenosine nucleosidase (MTAN), with potential utility as drug candidates.

Introduction

N-Ribosyl transferases are a general class of enzymes that catalyze the transfer of ribose or deoxyribose moieties from nucleosides containing purine, pyrimidine, and related heterocycles, a common reaction in biology. Two major classes of these enzymes are hydrolases and phosphorylases. Hydrolases, which release the heterocycle to generate a free sugar ribosyl unit, include enzymes for DNA repair, RNA depurinations by plant toxins, and purine and pyrimidine nucleoside and nucleotide metabolism. These hydrolases provide heterocycles for subsequent salvage pathways, degrade molecules to inactivate function, or generate centers for the subsequent remodeling reactions, as in DNA repair. Phosphorylases, which transfer ribosyl groups to phosphate, are also involved in the pathway for nucleoside salvage, and genetic defects in this pathway prevent normal purine catabolism in humans.[1,2]

Robertson recently reported on the unique opportunities offered for drug design by enzymes, through the inhibition of their normal function, over other targets such as cell surface receptors and ion channels.[3] According to Robertson, of the 1278 unique chemical entities marketed as drugs in the U.S., some 317 target an enzyme as their primary mode of action. A growing class of these enzyme-targeted drugs, including pentostatin, captopril, saquinavir, oseltamivir, and zananivir, are tight-binding transition state analogue inhibitors. Transition state theory predicts that near-perfect mimics of the transition state will bind more tightly than substrate by the factor of enzymatic rate enhancement, typically $10^{10}-10^{15}$.[4] The five inhibitors above, which incorporate transition state features, have realized, in part, the increased binding affinity indicated by transition state theory as predicted by Pauling,[5] Lienhard,[6] and Wolfenden.[7,8]

Three enzymes representative of the hydrolase and phosphorylase classes of *N*-ribosyl transferases have been of particular interest to us over the previous decade: purine nucleoside phosphorylase (PNP[a]),[9–14] methylthioadenosine phosphorylase (MTAP),[15–17] and methylthioadenosine nucleosidase (MTAN).[18–22] Knowledge of the geometric and electronic features of the transition state structures of PNP,[23] MTAP,[15] and MTAN[18] has provided us with logical blueprints for the design of transition state analogues, and subsequently, we have synthesized inhibitors of these *N*-ribosyl transferases that incorporate transition state features and hence exhibit extraordinary affinities to their target enzymes.

Since the 1980s PNP has been a biological target chosen by a variety of pharmaceutical companies because it was postulated, as a result of the phenotype exhibited by patients who are unable to express PNP, that its inhibition should lead to the suppression of cellular and not humoral immunity.[24–26] We have reported the design and synthesis of a series of transition state analogues, two of which, immucillin-H (ImmH)[27] and DADMe-immucillin-H (DADMe-ImmH),[28–30] are currently in human clinical trials against T- and B-cell cancers and a variety of autoimmune diseases.[31–35]

MTAP functions solely in the polyamine pathway of mammals to remove the methylthioadenosine (MTA) product from both spermidine synthase and spermine synthase. Inhibition of polyamine synthesis is a validated anticancer target.[36–41] Cellular polyamines and the expression of polyamine pathway enzymes increase on rapid cell division. Depletion of the polyamines has been shown to be growth inhibitory and in some cases to restore transformed cells to the normal phenotype. We have reported the synthesis of a variety of transition state analogues of MTAP, one of which, MT-DADMe-ImmA, is currently in preclinical trials for the treatment of head and neck tumors.[16]

MTAN is found in bacteria but not mammals and functions in polyamine and quorum sensing pathways. MTAN catalyzes the hydrolysis of MTA to adenine and 5-methylthio-D-ribose[42–45]

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[a] Abbreviations: DADMe, 5′-deaza-1′-aza-2′-deoxy-1′-(9-methylene); ImmH, immucillin-H; ImmA, immucillin-A; MT, methylthio; PNP, purine nucleoside phosphorylase; MTAP, methylthioadenosine phosphorylase; MTAN, methylthioadenosine nucleosidase; MTA, methylthioadenosine; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine.

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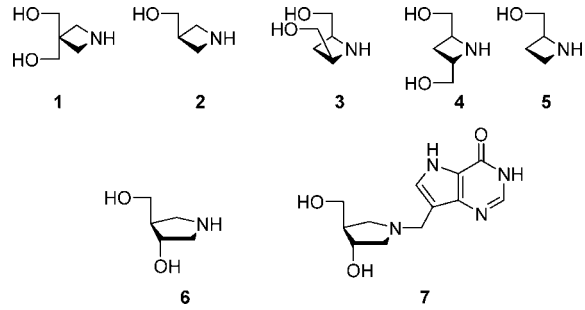


Figure 1. Target azetidines **1**–**5**, pyrrolidine subunit **6**, and DADMe-ImmH **7**.

and *S*-adenosylhomocysteine (SAH) to adenine and ribosylhomocysteine. MTA is the byproduct of the bacterial synthesis of acylated homoserine lactones from *S*-adenosylmethionine (SAM) and acyl−carrier proteins, in which the subsequent lactonization causes release of MTA and the acylated homoserine lactone. The acylated homoserine lactone is a bacterial quorum sensing molecule that is involved in bacterial virulence against human tissues. Inhibition of MTAN or MTAP in microbes will prevent MTA removal and subject the pathway to product inhibition, thereby inhibiting the quorum sensing pathway and decreasing the virulence of microbial infections. We have reported the synthesis of a variety of potent transition state analogue inhibitors of *Escherichia coli* MTAN, several of which have binding affinities in the femtomolar range and are hence some of the most powerful noncovalently bound enzyme inhibitors reported to date.[20]

In search of new and improved PNP,[9] MTAP, and MTAN inhibitors that are easier and more cost efficient to prepare, we have investigated the synthesis and bioactivity of compounds where the amine subunit is part of an azetidine. One of these azetidines can be easily prepared in as little as four steps from the commercially available diethyl bis(hydroxymethyl)malonate. We have found that the appropriate immucillins derived from these azetidine amine subunits are potent inhibitors of PNP, MTAP, and MTAN, with dissociation constants in the low nanomolar to picomolar range, and show potential as new drug candidates with lower costs than their counterparts currently in clinical trials.

Results and Discussion

Synthesis. A variety of methods have been previously published for the synthesis of a series of α- and *β*-amino acids containing an azetidine core.[46,47] Reduction of the carboxylic acid moieties of these amino acids or their ester precursors enabled ready access to five azetidines, compounds **1**–**5** (Figure 1). These compounds are close analogues of the pyrrolidine subunit **6** previously incorporated into the synthesis of DADMe-immucillin-H (**7**), a low picomolar inhibitor of PNP.

Synthesis of the analogous methylthio-containing azetidines, necessary for the synthesis of putative MTAP and MTAN inhibitors analogous to alkylthio- and arylthio-DADMe-immucillin-A series **8**, was only achieved in the series derived from *β*-amino acids (i.e., compounds **9** and **10**) (Figure 2).

All seven target azetidines, compounds **1**–**5**, **9**, and **10**, were incorporated into a series of putative enzyme inhibitors, analogues of the previously reported DADMe-immucillins, by two major chemical methods: the Mannich reaction and reductive amination.

The *tert*-butoxycarbonyl-protected derivatives of amines **1** and **2** were prepared via the previously reported intermediate azetidine **12**,[46] derived from the commercially available diethyl bis(hydroxymethyl)malonate (**11**) by bis-sulfonation and double displacement with benzylamine in one pot to afford **12** in moderate yield (Scheme 1). Reduction of the diethyl esters of azetidine **12** to the corresponding diol **14** using lithium aluminum hydride followed by hydrogenolysis of the benzyl group afforded azetidine **1**. Protection of the ring nitrogen of azetidine **1** could be readily achieved with di-*tert*-butyl dicarbonate under standard reaction conditions to afford compound **15** in good overall yield and with appropriate protection for synthesis of the methylthio analogue **9**.

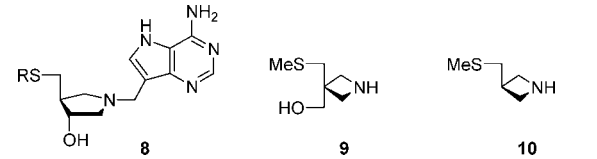
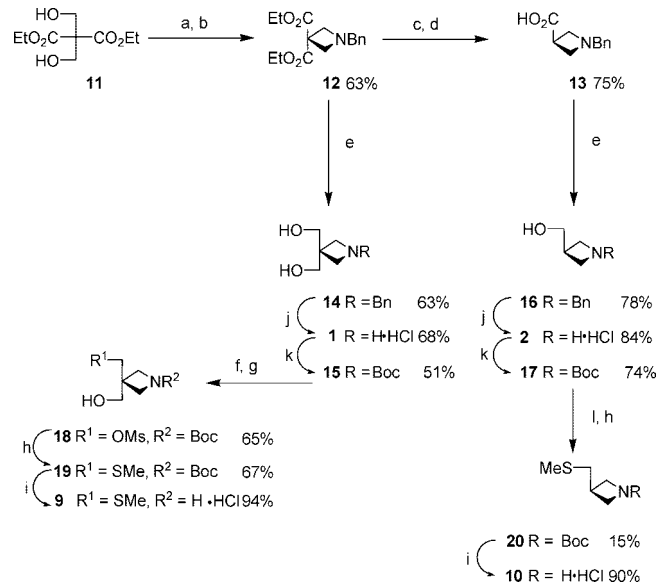


Figure 2. Alkyl/arylthio-DADMe-immucillin-A and azetidines **9** and **10**.

Scheme 1[a]



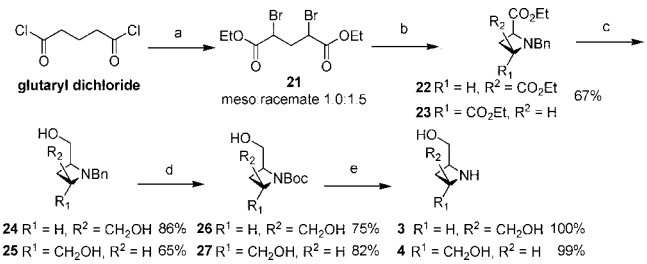
[a] Reagents: (a) Tf_2O, Hunig's base, acetonitrile −10 °C → 20 °C; (b) benzylamine, Hunig's base, acetonitrile, −10 °C → 70 °C; (c) NaOH, MeOH, 50 °C; (d) Water, reflux; (e) $LiAlH_4$, THF, room temperature; (f) dibutyltin oxide, toluene, reflux; (g) MsCl, toluene, room temperature; (h) NaSMe, DMF, room temperature; (i) HCl, MeOH, room temperature; (j) Pd/C, H_2(g), MeOH, room temperature and then HCl; (k) Boc_2O, Et_3N, MeOH, room temperature; (l) MsCl, Hunig's base, CH_2Cl_2.

Alternatively, saponification and decarboxylation of **12** afforded the carboxylic acid **13**, which could then be reduced to the alcohol **16**. Hydrogenolysis of compound **16** gave azetidine **2**, and analogous to compound **1**, the ring nitrogen could be protected under standard conditions with di-*tert*-butyl dicarbonate to afford **17** in good yield.

Compounds **1** and **2** could be separately transformed into the methylthioethers **9** and **10**. Compound **9** was prepared by the stannylation of **15**, followed by treatment with methanesulfonyl chloride to afford the monomesylate **18**. Subsequent displacement of the methanesulfonate by thiomethoxide yielded the Boc protected azetidine **19** in good yield. Deprotection of the azetidine **19** under standard conditions afforded, in excellent yield, the hydrochloride salt of compound **9** for use in the synthesis of putative MTAP/MTAN inhibitors.

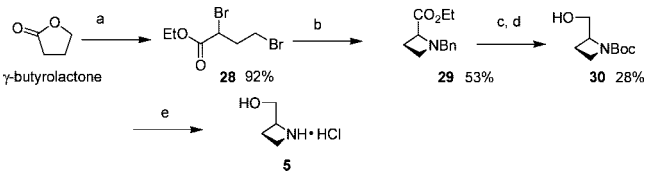
Similarly, azetidine **10** was prepared by mesylation of compound **17** and displacement of the resulting mesylate with

Scheme 2[a]



[a] Reagents: (a) (i) Br_2, *hν*, 85 °C; (ii) EtOH, 0 °C; (b) $BnNH_2$, C_6H_6, room temperature; (c) $LiAlH_4$, Et_2O, reflux; (d) H_2, $Pd(OH)_2/C$, Boc_2O, room temperature; (e) HCl, $MeOH/H_2O$, room temperature.

Scheme 3[a]



[a] Reagents: (a) Br_2, PBr_3, 110 °C, then EtOH, H_2SO_4, reflux; (b) $BnNH_2$, NEt_3, CH_3CN, room temperature; (c) $LiAlH_4$, Et_2O, room temperature; (d) H_2, $Pd(OH)_2/C$, Boc_2O, room temperature; (e) HCl MeOH/H_2O, room temperature.

thiomethoxide to afford compound **20**. Acid deprotection of **20** gave the hydrochloride salt of the thiomethyl azetidine **10** in good overall yield.

Target azetidines **3** and **4** were prepared from the previously reported cis and trans diols **24** and **25**, prepared from glutaryl chloride via the separable ethyl esters **22** and **23** (Scheme 2).[47]

Concomitant hydrogenolysis and Boc protection of the compounds **24** and **25** separately afforded azetidines **26** and **27**, respectively, in good yield. Incorporation of a methylthio group into compounds **26** and **27** by mesylation and thiomethoxide displacement was uneventful; however, elaboration of these modified subunits into putative MTAP and MTAN inhibitors was abandoned after numerous unsuccessful attempts. Azetidine derivatives **26** and **27** were readily converted into the corresponding target azetidines **3** and **4** as hydrochloride salts via acid deprotection, in quantitative yields, for use in the synthesis of putative PNP inhibitors.

The final target azetidine **5** was prepared by the previously reported method of Wasserman et al.,[48] whereby γ-butyrolactone was converted to ethyl 2,4-dibromobutyrate (**28**) via treatment with bromine and phosphorus tribromide (Scheme 3).

Reaction of the resulting dibromide **28** with benzylamine afforded compound **29**, incorporating the desired azetidine core. Reduction of compound **29** with lithium aluminum hydride, followed by hydrogenolysis and Boc protection afforded azetidine **30** in good yield. The incorporation of a methylthio group via standard procedures into the putative MTAP/MTAN inhibitors remained unsuccessful for this class of azetidines. The hydrochloride salt of azetidine **5** was readily available from **30** via acid deprotection of the Boc group in quantitative yield.

The hydrochloride salts of compounds **1**–**5** were now used for the preparation of the target immucillins **34**, **35**, **40**, **41**, and **42** (Scheme 4) as transition state analogue inhibitors of PNP. Initially, we investigated the more elegant approach to the immucillins via the Mannich reaction, which we have previously utilized with considerable success.[30] This approach proved to be unsuccessful, presumably because of the reduced stability of the intermediate iminium ion that needs to be formed between the azetidine and formaldehyde, as a result of the lower Lewis basicity of an azetidine nitrogen relative to a pyrrolidine nitrogen.[49] We therefore reverted to our original approach to the DADMe-immucillins, that is, the reductive amination of the previously reported aldehyde **31** with azetidines **1** and **2** using $NaCNBH_3$ to afford the protected immucillins **32** and **33** in good and moderate yields, respectively. Similarly, reductive amination of aldehyde **36** with azetidines **3**–**5** afforded the protected immucillins **37**−**39**, respectively, in good to moderate yields. Global deprotection of compounds **32**, **33**, **37**, **38**, and **39** afforded the putative PNP inhibitors **34**, **35**, **40**, **41**, and **42** in good yields.

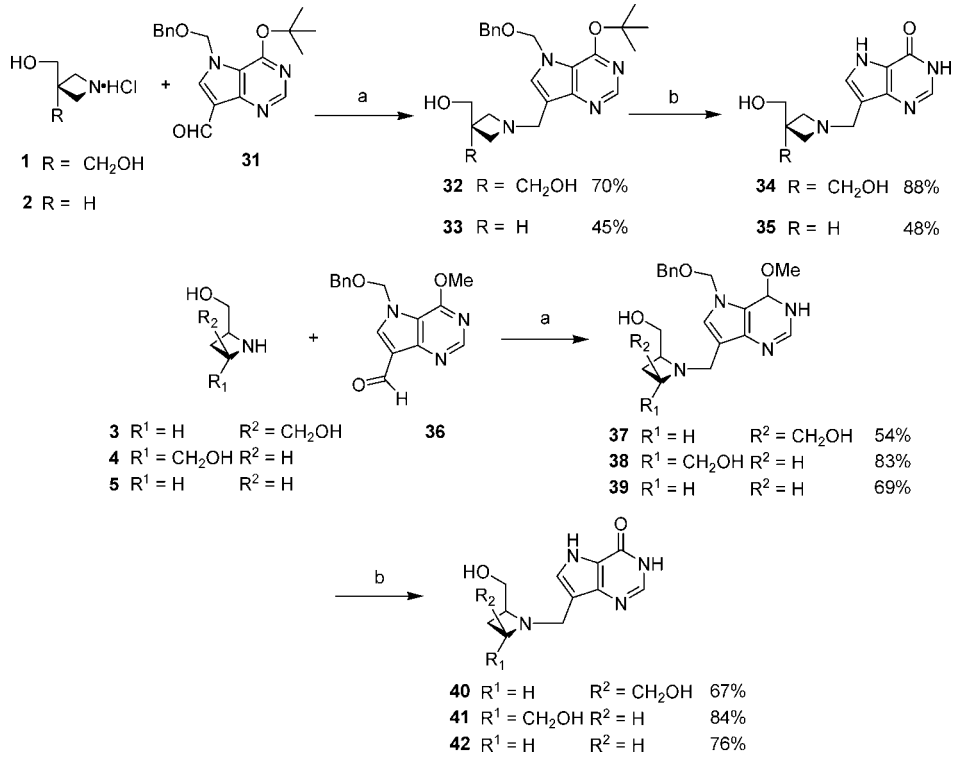
Next, we investigated the incorporation of the methylthioazetidine hydrochlorides **9** and **10** into the putative MTAP/MTAN inhibitors **43** and **44** (Scheme 5).

Unlike the unsuccessful Mannich reactions with amine hydrochlorides **1**−**5** where deazahypoxanthine was used as the nucleophile, the Mannich reaction of compounds **9** and **10** with 9-deazaadenine afforded immucillins **43** and **44** in moderate yield. 9-Deazaadenine has been previously shown to be considerably more reactive than deazahypoxanthine,[30] and hence, despite the apparently transient nature of the intermediate iminium ion (vide supra), it was gratifying to isolate Mannich products even in moderate yield.

Inhibition Studies. The inhibition of human, bovine, and *Plasmodium falciparum* (malarial) PNPs was evaluated with the azetidines **34**, **35**, and **40**−**42** (Table 1). Though few of these exhibited slow-onset inhibition, all bound reasonably tightly to the mammalian enzymes, with dissociation constants ranging from 10^{-7} to 10^{-10} M. The malarial enzyme, however, did not bind these compounds as well, yielding the best dissociation constant of 191 nM, in the case of **42**. This finding is not surprising, as *Plasmodium falciparum* PNP has repeatedly shown poor selectivity for immucillin transition state analogue inhibitors relative to its mammalian counterparts.[50] None of the azetidine inhibitors approached the potency of DADMe-ImmH (**7**), which, with a final dissociation constant of 11 pM, is one of the most effective inhibitors of human PNP. It is likely that the more constrained four-membered azetidine ring does not enable the inhibitors to adopt as favorable a conformation as with the five-membered pyrrolidine ring found in the immucillins. Despite this geometric challenge, however, most of the azetidines exhibited substantial inhibition of the mammalian enzymes, resulting in inhibition constants as low as 200 pM for human PNP in the cases of **34** and **42**. The mammalian enzymes reveal a degree of discrimination with the 2′-substituted series of azetidines **40**−**42**, favoring monosubstitution as in **42** and better tolerating the cis configuration **40** over trans **42** in the case of disubstitution. It is worth noting that, as has previously been shown for DADMe-ImmH (**7**),[13] one of the enantiomers of **42** may actually be significantly more potent than is revealed by the 260 pM dissociation constant for the racemate.

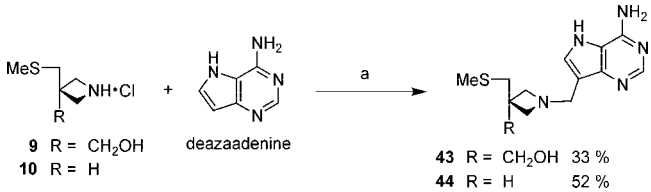
The two methylthio-substituted azetidines **43** and **44** were similarly examined as inhibitors of human MTAP and *Escherichia coli* and *Streptococcus pneumoniae* MTANs (Table 2). Whereas the analogously substituted azetidine **34** inhibited the mammalian PNPs with 200 pM affinity, compound **43** only approached this level of inhibition with *Escherichia coli* MTAN. In contrast, the monosubstituted azetidine **44** was bound significantly more tightly by all three enzymes, lowering the dissociation constants by 2- to 70-fold. Although neither azetidine was as effective as the most potent members of the series of alkylthio- and arylthioimmucillins **8**, such as MT-

Scheme 4[a]



[a] Reagents: (a) $NaCNBH_3$, MeOH, room temperature; (b) HCl, MeOH, reflux.

Scheme 5[a]



[a] Reagents: (a) 37% aqueous HCHO, H_2O/1,4-dioxane, 95 °C.

DADMe-ImmA, these compounds are nevertheless good inhibitors of MTAP and MTANs.

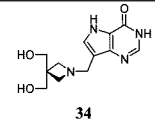
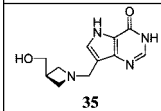
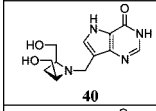
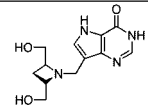
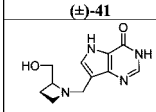
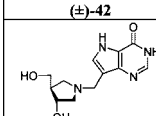
Conclusions

A series of azetidine based transition state analogue inhibitors of purine nucleoside phosphorylase, methylthioadenosine phosphorylase, and methylthioadenosine nucleosidase have been designed and synthesized on the basis of a dissociative transition state structure. Considerable effort has been invested in the simplification of the overall structures of these inhibitors (cf. first and second generation inhibitors) in order to afford "lower cost of goods" drug candidates. Because of their relatively tight binding to their target enzymes, several of these candidates may warrant further investigations in a preclinical setting.

Experimental Section

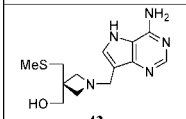
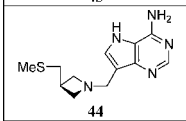
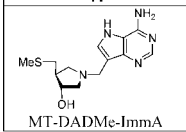
NMR spectra were recorded on a Bruker AC-300 instrument at 300 MHz (^{1}H) or 75 MHz (^{13}C). Normally, spectra were measured in $CDCl_3$ with Me_4Si as internal reference. When D_2O was the solvent, acetone (δ ^{1}H, 2.20; ^{13}C, 33.2) was used as an internal reference. High-resolution accurate mass determinations were performed by Hort Research Ltd., Palmerston North, New Zealand, on a VG70-250S double focusing, magnetic sector mass spectrometer under chemical ionization conditions using isobutane or ammonia as the ionizing gas or under high-resolution FAB conditions in a glycerol or nitrobenzyl alcohol matrix. Melting points were determined on a Reichert hot stage microscope and are uncorrected. Aluminum-backed silica gel sheets (Merck or

Table 1. Inhibition Constants for the Interaction of Immucillins with a Variety of PNPs[a]

Compound	Human PNP (nM)	Bovine PNP (nM)	*P. falciparum* PNP (nM)
 **34**	$K_i = 0.229 \pm 0.015$	$K_i = 0.665 \pm 0.06$ $K_i^* = 0.236 \pm 0.003$	$K_i > 10{,}000$
 **35**	$K_i = 6.3 \pm 1.1$	$K_i = 4.8 \pm 0.3$	$K_i > 10{,}000$
 **40**	$K_i = 12.9 \pm 0.3$	$K_i = 16 \pm 3$	$K_i = 1{,}290 \pm 30$
 **(±)-41**	$K_i = 280 \pm 40$	$K_i = 360 \pm 40$	$K_i = 580 \pm 30$
 **(±)-42**	$K_i = 1.8 \pm 0.3$ $K_i^* = 0.260 \pm 0.02$	$K_i = 1.8 \pm 0.2$	$K_i = 191 \pm 11$
 DADMe-ImmH (7)	$K_i = 1.1 \pm 0.12$ $K_i^* = 0.0107 \pm 0.0011$[b]	$K_i = 1.0 \pm 0.2$ $K_i^* = 0.10 \pm 0.02$	$K_i = 0.500 \pm 0.04$[c]

[a] K_i is the dissociation constant for the first step in E + I ↔ EI ↔ EI*, the two-step binding characteristic of slow-onset tight-binding inhibition. K_i* is the overall dissociation constant for E + I ↔ EI*. In cases where only K_i is reported, no slow-onset inhibition was observed. [b] Reference 11. [c] Reference 50.

Table 2. Inhibition Constants for the Interaction of Azetidine Immucillins with MTAP and MTANs[a]

Compound	Human MTAP (nM)	*E. coli* MTAN (nM)	*S. pneumoniae* MTAN (nM)
 43	$K_i = 140 \pm 7$	$K_i = 0.84 \pm 0.09$	$K_i = 150 \pm 12$
 44	$K_i = 2.0 \pm 0.1$	$K_i = 0.45 \pm 0.05$	$K_i = 84 \pm 6$
 MT-DADMe-ImmA	$K_i = 1.7 \pm 0.2$ $K_i^* = 0.090 \pm 0.010$[b]	$K_i = 0.048 \pm 0.003$ $K_i^* = 0.0020 \pm 0.0002$[c]	$K_i = 24$[d]

[a] K_i is the dissociation constant for the first step in E + I ↔ EI ↔ EI*, the two-step binding characteristic of slow-onset tight-binding inhibition. K_i^* is the overall dissociation constant for E + I ↔ EI*. In cases where only K_i is reported, no slow-onset inhibition was observed. [b] Reference 17. [c] Reference 20. [d] Reference 19.

Reidel de Haen) were used for thin layer chromatography. Column chromatography was performed on silica gel (230–400 mesh, Merck). Chromatography solvents were distilled prior to use.

Chemistry. 1-Benzylazetidine-3,3-dimethanol (14). $LiAlH_4$ (1.0 M in THF, 65 mL, 65 mmol) was added dropwise to a solution of diethyl 1-benzylazetidine-3,3-dicarboxylate (1.0 g, 3.43 mmol) (**12**) in THF (20 mL). The resulting suspension was stirred overnight at room temperature, quenched with water (0.25 mL), 15% aqueous NaOH (0.25 mL), and water (0.75 mL), filtered through Celite, and concentrated in vacuo. Chromatography (7 N NH_3 in MeOH/CH_2Cl_2 = 5:95 → 10:90) of the resulting residue afforded **14** (450 mg, 63%) as an oil. ^{1}H NMR ($CDCl_3$): δ 7.33−7.20 (m, 5H), 3.74 (s, 4H), 3.65 (s, 2H), 3.12 (s, 4H). ^{13}C NMR ($CDCl_3$): δ 137.4, 129.02, 128.8, 127.7, 66.8, 63.3, 58.7, 41.0. HRMS for $C_{12}H_{17}NO_2$ [M^+] calcd, 207.1259; found, 207.1259.

Azetidine-3,3-dimethanol Hydrochloride (1). $Pd(OH)_2$ (20% on C, 150 mg, 1.9 mmol) was added to a solution of **14** (400 mg, 1.9 mmol) in MeOH (4 mL) and left to stir under an atmosphere of hydrogen overnight at room temperature. The reaction was filtered through Celite and concentrated in vacuo. Chromatography (1,4-dioxane/NH_4OH = 50:50) of the resulting residue afforded **1** as a colorless oil, which was converted to its HCl salt (200 mg, 68%) for characterization. ^{1}H NMR (D_2O): δ 3.97 (s, 4H), 3.69 (s, 4H). ^{13}C NMR (D_2O): δ 62.4, 49.8.

***tert*-Butyl 3,3-Bis(hydroxymethyl)azetidine-1-carboxylate (15).** Di-*tert*-butyl dicarbonate (2.9 g, 16.40 mmol) was added portionwise to a solution of **1** (961 mg, 8.2 mmol) in MeOH (20 mL) at room temperature. After 1 h, the reaction was concentrated in vacuo. Chromatography (MeOH/CH_2Cl_2 = 5:95 → 10:90) of the resulting residue afforded **15** (900 mg, 51%) as a syrup. ^{1}H NMR ($CDCl_3$): δ 3.81 (s, 4H), 3.67 (s, 4H), 1.43 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 157.2, 80.3, 66.2, 54.1, 39.8, 28.8. HRMS for $C_{10}H_{19}NO_4$ [MH^+] calcd, 218.1392; found, 218.1391.

1-Benzylazetidine-3-methanol (16). $LiAlH_4$ (2.3 M in THF, 10 mL, 23 mmol) was added dropwise to a suspension of **13** (2.2 g, 11.50 mmol) in THF (30 mL) at room temperature, and the resulting mixture was left to stir for 16 h. The reaction was quenched with water (0.7 mL), 15% aqueous NaOH (0.7 mL), and water (2.1 mL), and the mixture was stirred for 30 min, filtered through Celite, and concentrated in vacuo. Chromatography (7 N NH_3 in MeOH/CH_2Cl_2 = 5:95 → 10:90) of the resulting residue afforded **16** (1.6 g, 78%). ^{1}H NMR ($CDCl_3$): δ 7.30–7.17 (m, 5H), 3.63 (d, J = 6.2 Hz, 2H), 3.55 (s, 2H), 3.31 (t, J = 7.7 Hz, 2H), 3.00 (t, J = 6.1 Hz, 2H), 2.56 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 138.2, 128.9, 128.7, 127.5, 64.6, 63.9, 57.3, 33.1. HRMS for $C_{11}H_{15}NO$ [M^+] calcd, 177.1154; found, 177.1150.

Azetidine-3-methanol Hydrochloride (2). $Pd(OH)_2$ (20% on C, 600 mg, 7.90 mmol) was added portionwise to a stirred suspension of **16** (1.4 g, 7.90 mmol) in MeOH (20 mL, 494 mmol) under an atmosphere of hydrogen. After 24 h, the mixture was filtered through Celite and concentrated in vacuo. The resulting residue was converted to the HCl salt to afford **2** (820 mg, 84%) as a syrup, which was characterized without additional purification. ^{1}H NMR (D_2O): δ 4.20 (t, J = 9.8 Hz, 2H), 3.98 (m, 2H), 3.75 (d, J = 5.4 Hz, 2H), 3.11 (m, 1H). ^{13}C NMR (D_2O): δ 61.7, 48.8, 48.8, 33.6. HRMS for C_4H_9NO [M^+] calcd, 87.0684; found, 87.0683.

***tert*-Butyl 3-(Hydroxymethyl)azetidine-1-carboxylate (17).** Et_3N (1 mL, 7.1 mmol) was added dropwise to a stirred solution of **2** (500 mg, 4.0 mmol) in MeOH (5 mL). After 5 min, di-*tert*-butyl dicarbonate (846 mg, 5.0 mmol) was added and the mixture was stirred for 16 h and then concentrated in vacuo. Chromatography (MeOH/CH_2Cl_2 = 5:95 → 10:80) of the resulting residue afforded **17** as a colorless oil (560 mg, 74%). ^{1}H NMR ($CDCl_3$): δ 3.97 (t, J = 8.5 Hz, 2H), 3.71 (m, 4H), 2.69 (m, 1H), 1.43 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 156.9, 79.8, 64.5, 51.7, 30.9, 28.7. HRMS for $C_{19}H_{17}NO_3$ [M^+] calcd, 187.1208; found, 187.1207.

***tert*-Butyl 3-(Hydroxymethyl)-3-[(methanesulfonyloxy)methyl] azetidine-1-carboxylate (18).** Dibutyltin oxide (1.24 g, 5.0 mmol) was added to a stirred suspension of **15** (900 mg, 4.1 mmol) in toluene (10 mL) and heated to reflux for 1 h. The mixture was cooled to room temperature. Then methanesulfonyl chloride (0.39 mL, 5.0 mmol) was added dropwise to the clear solution and the resulting reaction allowed to stand for 16 h. Chromatography (MeOH/CH_2Cl_2 = 5:95) of the crude solution afforded **18** as an oil (800 mg, 2709 μmol, 65%). ^{1}H NMR ($CDCl_3$): δ 4.40 (s, 2H), 3.78 (s, 2H), 3.73 (s, 4H), 3.07 (s, 3H), 1.44 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 156.8, 80.4, 70.5, 63.6, 53.6, 38.9, 37.6, 28.7. HRMS for $C_{11}H_{21}NO_6S$ [MH^+] calcd, 207.1259; found, 207.1259.

***tert*-Butyl 3-(Hydroxymethyl)-3-[(methanesulfonyloxy)methyl] azetidine-1-carboxylate (19).** Sodium thiomethoxide (285 mg, 4.1 mmol) was added portionwise to a stirred solution of **18** (800 mg, 2.7 mmol) in DMF (5 mL) at room temperature. After 3 h, the mixture was diluted with toluene (100 mL), washed with water (25 mL) and brine (25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (MeOH/CH_2Cl_2 = 5:95) of the crude residue afforded **19** as an oil (450 mg, 67%). ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 2H), 3.74 (d, J = 8.8 Hz, 2H), 3.66 (d, J = 8.8 Hz, 2H), 2.87 (s, 2H), 2.16 (s, 3H), 1.44 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 156.9, 80.0, 65.8, 56.1, 40.1, 39.9, 28.7, 17.5. HRMS for $C_{11}H_{21}NO_3S$ [MH^+] calcd, 247.1242; found, 247.1246.

3-(Methylthiomethyl)azetidin-3-methanol Hydrochloride (9). HCl (30% aqueous, 1.5 mL, 49 mmol) was added dropwise to a solution of **19** (430 mg, 17 mmol) in MeOH (4.5 mL). The resulting solution was left at room temperature for 1 h and concentrated in vacuo to afford **9** as a syrup (300 mg, 94%), which was used in the next step without purification or characterization.

***tert*-Butyl 3-(Methylthiomethyl)azetidine-1-carboxylate (20).** Methanesulfonyl chloride (0.53 mL, 6.8 mmol) was added dropwise to a stirred solution of **17** (530 mg, 2.8 mmol) and Hunig's base (0.986 mL, 5.6 mmol) in CH_2Cl_2 (10 mL) and left overnight at room temperature. The mixture was then diluted with CH_2Cl_2 (100 mL) and washed with water (25 mL) and brine (25 mL), dried ($MgSO_4$), and concentrated in vacuo. Sodium thiomethoxide (218 mg, 3109 μmol) was added portionwise to a solution of the residue, presumably *tert*-butyl 3-(methanesulfonyloxymethyl)azetidine-1-carboxylate (550 mg, 73%), in DMF (5 mL) and stirred at room temperature overnight. The mixture was diluted with toluene (100 mL) and washed with water (25 mL) and brine (25 mL), dried ($MgSO_4$), and concentrated in vacuo. Chromatography (MeOH/CH_2Cl_2 = 5:95) of the resulting residue afforded **20** as an oil (120 mg, 27%). ^{1}H NMR ($CDCl_3$): δ 3.98 (m, 2H), 3.54 (m, 2H), 2.65 (brs, 3H), 2.03 (s, 3H), 1.37 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 155.3, 78.3, 53.1, 37.4, 27.4, 14.5.

3-(Methylthiomethyl)azetidine Hydrochloride (10). HCl (30% aqueous, 1.5 mL, 49 mmol) was added dropwise to a solution of **20** (120 mg, 0.55 mmol) in MeOH (4.5 mL). The resulting solution

was left at room temperature for 1 h and concentrated in vacuo to afford **10** (76 mg, 90%) as a syrup, which was used in the next step without purification or characterization.

***meso-tert*-Butyl 2,4-Bis(hydroxymethyl)azetidine-1-carboxylate (26).** 1-Benzyl 2,4-bis(hydroxymethyl)azetidine (**24**) (1.16 g, 5.60 mmol) was dissolved in EtOH (10 mL) and di-*tert*-butyl dicarbonate (2.44 g, 11.2 mmol) added followed by 20% $Pd(OH)_2$/C (200 mg). The atmosphere was replaced with hydrogen by the successive application of vacuum, and then a balloon of hydrogen was fitted to the reaction vessel. The reaction mixture was allowed to stir overnight, and then the suspension was filtered through Celite, the volatiles were removed under reduced pressure, and the residue purified by flash chromatography on silica (60:40 to 100:0 EtOAc/hexane) to give **26** as a colorless oil (915 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.27–4.16 (m, 2H), 4.20–3.05 (br s, 2H), 3.77 (br d, J = 11.4 Hz, 2H), 3.61 (br dd, J = 11.4, 5.4 Hz, 2H), 2.18 (ddd, J = 11.4, 8.7, 8.7 Hz, 1H), 1.98 (ddd, J = 11.4 6.7, 6.7 Hz, 1H), 1.43 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.4, 80.8, 64.5, 60.3, 28.2, 19.7. ESI-HRMS for $C_{10}H_{19}N_1O_4Na_1$ [M + Na^+] calcd, 240.1212; found, 240.1218. Anal. ($C_{10}H_{19}N_1O_4 \cdot 0.2H_2O$) C, H, N.

***meso*-2,4-Bis(hydroxymethyl)azetidine Hydrochloride (3).** A solution of **26** (480 mg, 2.20 mmol) in 2:1 MeOH/concentrated HCl (10 mL) was stirred for 20 min and then concentrated under reduced pressure. The product was azeotropically dried by the addition and evaporation of acetonitrile several times giving **3** as a colorless hygroscopic solid after drying under high vacuum (344 mg, 100%). ^{1}H NMR (300 MHz, D_2O): δ 4.62–4.50 (m, 2H), 3.83 (d, J = 4.8 Hz, 4H), 2.50 (dt, J = 12.0, 9.0 Hz, 1H), 2.37 (dt, J = 12.0, 9.0 Hz, 1H). ^{13}C NMR (75 MHz, D_2O): δ 60.9, 58.2, 22.5.

(±)-(2*R*,4*R*)-*tert*-Butyl 2,4-Bis(hydroxymethyl)azetidine-1-carboxylate (27). To a stirred solution of (±)-(2*R*,4*R*)-*N*-benzyl-2,4-bis(hydroxymethyl)azetidine (**25**) (570 mg, 2.75 mmol) in EtOH (10 mL) was added di-*tert*-butyl dicarbonate (1.2 g, 5.5 mmol) and then 20% $Pd(OH)_2$/C (400 mg). The atmosphere was replaced with hydrogen by successive applications of vacuum, and a hydrogen balloon was fitted to the reaction vessel. The reaction mixture was stirred overnight and then filtered through Celite. The mixture was concentrated under reduced pressure and the product purified by flash chromatography on silica (EtOAc) to give **27** as a colorless oil (490 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.58–4.23 (m, 3H), 3.93–3.62 (m, 4H), 2.32 (br s, 1H), 2.15–1.85 (br m, 2H), 1.47 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.5, 81.4, 67.0, 64.8, 61.7, 61.5, 28.3, 20.8. ESI-HRMS for $C_{10}H_{19}N_1O_4Na_1$ [M + Na] calcd, 240.1212; found, 240.1213.

(±)-(2*R*,4*R*)-2,4-Bis(hydroxymethyl)azetidine Hydrochloride (4). A solution of **27** (480 mg, 2.20 mmol) in 2:1 MeOH/concentrated HCl (10 mL) was stirred for 20 min and then concentrated under reduced pressure. The product was azeotropically dried by the addition and evaporation of acetonitrile several times, giving **4** as a colorless hygroscopic solid (339 mg, 99%). ^{1}H NMR (300 MHz, D_2O): δ 4.50–4.39 (m, 2H), 3.91–3.87 (m, 4H), 2.44 (t, J = 8.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 61.0, 59.0, 22.3.

(±)-Ethyl 1-Benzylazetidine-2-carboxylate (29). A mixture of (±)-ethyl 2,4-dibromobutanoate (**28**) (15 g, 54.8 mmol), Et_3N (16.6 g, 164 mmol), and benzylamine (5.87 g, 54.8 mmol) was heated to reflux for 3 h and then concentrated under reduced pressure to give a solid suspension. Water (150 mL) was then added and the mixture extracted with ether (2 × 100 mL). The organic phase was dried and then concentrated under reduced pressure and the residue purified by dry flash chromatography on silica (hexanes and then 1:3 ethyl acetate/hexanes) to give **29** as a pale-yellow oil (6.3 g, 53%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.27 (m, 5H), 4.16–4.03 (m, 2H), 3.82 (d, J = 12.6 Hz, 1H), 3.73 (dd, J = 8.4, 8.4 Hz, 1H), 3.61 (d, J = 12.8 Hz, 1H), 3.34 (ddd, J = 7.4, 7.4, 2.0 Hz, 1H), 2.95 (ddd, J = 7.4, 7.4, 7.4 Hz, 1H), 2.44–2.31 (m, 1H), 2.27–2.16 (m, 1H), 1.20 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.5, 137.1, 129.0, 128.2, 127.1, 64.5, 62.4, 60.5, 50.8, 21.5, 14.0.

(±)-*tert*-Butyl 2-Hydroxymethylazetidine-1-carboxylate (30). To a stirred solution of **29** (3.67 g, 16.7 mmol) in dry diethyl ether (50 mL) cooled to 4 °C was slowly added a solution of $LiAlH_4$ in diethyl ether (1.0 M, 16 mL, 16.0 mmol). The mixture was allowed to stir at room temperature for 1 h and then carefully quenched with EtOAc followed by 2 M NaOH (4 mL). The reaction mixture was allowed to stir for 1 h, and then the aluminates were removed by filtration and the filtrate was concentrated under reduced pressure to give a colorless oil. The oil was dissolved in ethanol (20 mL), and then di-*tert*-butyl dicarbonate (5.24 g, 24 mmol) and 20% $Pd(OH)_2$/C (500 mg) were added. The atmosphere was replaced by hydrogen by the successive applications of vacuum, and then a hydrogen balloon was fitted to the mixture, which was allowed to stir overnight. The hydrogen atmosphere was replaced with Ar, and then the suspension was filtered through Celite. The filtrate was concentrated under reduced pressure and the residue purified by flash chromatography to give **30** as a colorless oil (850 mg, 28%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.52–4.38 (m, 1H), 3.94–3.63 (m, 4H), 2.25–2.12 (m, 1H), 2.02–1.87 (m, 1H), 1.46 (s, 9H).

1-[(7-Benzyloxymethyl-4-*tert*-butoxy-9-deazapurin-9-yl)methyl]azetidine-3,3-dimethanol (32). 7-Benzyloxymethyl-6-*tert*-butoxy-9-deazapurine-9-carbaldehyde (**31**) (219 mg, 645 μmol) was added to a suspension of **1**·HCl (90 mg, 586 μmol) in methanol (5 mL), and the resulting suspension was stirred for 5 min. $NaBH_3CN$ (55.2 mg, 879 μmol) was then added, and the resulting mixture was stirred overnight at room temperature. The crude mixture was absorbed onto silica and concentrated in vacuo. Chromatography ($MeOH/CH_2Cl_2$ = 10:90 → 20:80) of the resulting residue afforded **32** as a syrup (180 mg, 70%). ^{1}H NMR ($CDCl_3$): δ 8.42 (s, 1H), 7.81 (s, 1H), 7.23–7.14 (m, 5H), 5.74 (s, 2H), 4.54 (brs, 2H), 4.51 (s, 2H), 4.16 (brs, 4H), 3.67 (brs, 4H), 1.66 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 156.8, 150.8, 149.4, 137.5, 135.8, 128.7, 128.1, 127.8, 117.2, 104.6, 84.3, 78.1, 70.0, 62.4, 57.2, 48.5, 42.5, 28.9. HRMS for $C_{24}H_{32}N_4O_4$ [MH^+] calcd, 441.2502; found, 441.2509.

1-[(9-Deazahypoxanthin-9-yl)methyl]azetidine-3,3dimethanol (34). Concentrated HCl (1.5 mL, 49 mmol) was added to a solution of **32** (98 mg, 222 μmol) in MeOH (1.5 mL), and the resulting solution was heated at reflux for 2.5 h. The mixture was cooled to room temperature and concentrated in vacuo. Chromatography (CH_2Cl_2/MeOH/NH_4OH = 50:40:10) afforded **34** as a syrup (52 mg, 88% yield), which was converted to the HCl salt for characterization. ^{1}H NMR (D_2O): δ 8.00 (s, 1H), 7.70 (s, 1H), 4.41 (s, 2H), 4.04 (q, J = 10.9 Hz, 4H), 3.68 (s, 2H), 3.50 (s, 2H). ^{13}C NMR (D_2O): δ 155.3, 114.3, 143.4, 131.7, 118.1, 105.02, 62.3, 61.6, 55.8, 47.4, 41.3. HRMS for $C_{12}H_{16}N_4O_3$ [MH^+] calcd, 265.1301; found, 265.1308. Anal. ($C_{12}H_{16}N_4O_3 \cdot 3HCl$) C, H, N.

1-[(7-Benzyloxymethyl-6-*tert*-butoxy-9-deazapurin-9-yl)methyl]azetidine-3-methanol (33). 7-Benzyloxymethyl-6-*tert*-butoxy-9-deazapurine-9-carbaldehyde (**31**) (272 mg, 0.80 mmol) was added to a stirred suspension of **2** (90 mg, 0.73 mmol) in MeOH (5 mL) and stirred for 5 min. $NaBH_3CN$ (68.6 mg, 1.1 mmol) was then added and the resulting mixture stirred overnight at room temperature. The crude mixture was absorbed onto silica and concentrated in vacuo. Chromatography ($MeOH/CH_2Cl_2$ = 5:95 → 20:80) of the resulting residue afforded **33** as a syrup (135 mg, 45%). ^{1}H NMR ($CDCl_3$): δ 8.35 (s, 1H), 7.72 (s, 1H), 7.20–7.08 (m, 5H), 5.68 (s, 2H), 4.44 (s, 2H), 4.43 (s, 2H), 4.17 (t, J = 10.0 Hz, 2H), 4.06 (t, J = 6.3 Hz, 2H), 3.64 (d, J = 2.9 Hz, 2H), 2.90 (m, 1H), 1.60 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 156.7, 150.9, 149.7, 137.4, 135.5, 128.8, 128.1, 127.8, 117.2, 104.8, 84.2, 78.1, 70.8, 60.4, 55.4, 48.2, 31.4, 28.9. HRMS for $C_{23}H_{30}N_4O_3$ [MH^+] calcd, 411.2396; found, 411.2409.

1-[(9-Deazahypoxanthin-9-yl)methyl]azetindine-3-methanol (35). Compound **33** (95 mg, 231 μmol) was dissolved in concentrated HCl (5 mL, 1.63 mmol) and heated at reflux for 2 h, and the mixture was then concentrated in vacuo. Chromatography (CH_2Cl_2/MeOH/NH_4OH = 5:4:1) of the resulting residue afforded **35** as a white solid (28 mg, 48%). ^{1}H NMR (D_2O): δ 7.82 (1H, s), 7.28 (2H, s), 4.70 (1H, s), 3.71 (2H, s), 3.54 (d, J = 6.3 Hz, 2H), 3.48 (t, J = 8.5 Hz, 2H), 3.17 (t, J = 7.8 Hz, 2H), 2.61 (septet, J = 7.1 Hz, 1H). ^{13}C NMR (D_2O): δ 157.4, 144.7, 144.06, 129.1, 117.8, 109.52, 63.2, 55.3, 55.3, 49.6, 31.3. HRMS for $C_{11}H_{16}N_4O_3$ [MH^+] calcd, 235.1196; found, 235.1194. Anal. ($C_{11}H_{16}N_4O_3$) C, H, N.

***meso*-1-[(7-Benzyloxymethyl-9-deaza-6-methoxypurin-9-yl)methyl]azetidine-2,4-dimethanol Hydrochloride (37).** To a stirred solution of aldehyde **36** (277 mg, 0.93 mmol) in EtOH (3 mL) at ambient temperature was added **3**·HCl (143 mg, 0.93 mmol) followed after 5 min by $NaBH_3CN$ (88 mg, 0.48 mmol). The mixture was left to stir overnight, after which time all of the starting aldehyde had dissolved. The reaction mixture was absorbed onto silica gel, the volatiles were removed under reduced pressure, and the product was purified by flash chromatography ($CHCl_3$/MeOH = 95:5 to 80:20) to give colorless crystals, which were taken up in water. Concentrated HCl was added and the mixture was then concentrated under reduced pressure to dryness to afford **37** (70 mg, 54%). ^{1}H NMR (300 MHz, D_2O): δ 8.61 (s, 1H), 8.02 (s, 1H), 7.25–7.07 (m, 5H), 5.90 (s, 2H), 4.68 (s, 2H), 4.58 (s, 2H), 4.54–4.43 (m, 2H), 4.24 (s, 3H), 3.72 (dd, J = 13.2, 5.7 Hz, 2H), 3.61 (dd, J = 13.2, 3.2 Hz, 2H), 2.47 (dt, J = 12.1, 9.0 Hz, 1H), 2.28 (dt, J = 9.6, 9.0 Hz, 1H). ^{13}C NMR (75 MHz, D_2O): δ 159.4, 148.4, 142.5, 139.9, 137.0, 128.8, 128.6, 128.2, 116.7, 102.6, 78.7, 72.0, 66.6, 60.2, 56.5, 47.3, 20.4. ESI-HRMS for $C_{21}H_{27}N_4O_4$ [M + H^+] calcd, 399.2032; found, 399.2046.

***meso*-[(9-Deazahypoxanthin-9-yl)methyl]azetidine-2,4-dimethanol Hydrochloride (40).** A solution of **37** (114 mg, 0.26 mmol) in concentrated HCl (3 mL) was heated under reflux for 3 h and then cooled to room temperature. The mixture was evaporated to dryness under reduced pressure, and residual HCl was removed by the addition and evaporation of acetonitrile several times. The residue was absorbed onto silica and purified by flash chromatography (2-propanol/H_2O/NH_4OH = 9:1:1) to give a colorless gum. This was converted to its hydrochloride salt for characterization by the addition and evaporation of concentrated HCl, yielding **40** as a colorless solid (53 mg, 67%) after trituration with 2-propanol. HPLC purity: 99.5% (220 nm). ^{1}H NMR (300 MHz, D_2O): δ 8.21–8.15 (m, 1H), 7.75–7.72 (m, 1H), 4.57 (s, 2H), 4.50 (dddd, J = 9.0, 9.0, 5.5, 3.6 Hz, 2H), 3.69 (13.3, 5.5 Hz, 2H), 3.58 (dd, J = 13.3, 3.6 Hz, 2H), 2.48–2.36 (m,1H), 2.28 (dt, J = 12.1, 9.0, Hz, 1H). ^{13}C NMR (75 MHz, D_2O, freebase): δ 155.9, 144.2, 142.9, 130.2, 117.5, 111.5, 64.5, 62.7, 49.1, 24.0. ESI-HRMS for $C_{12}H_{17}N_4O_3$ [M + H^+] calcd, 265.1301; found, 265.1316. Anal. ($C_{12}H_{16}N_4O_3$·2.6H_2O) C, H, N.

(±)-(2*R*,4*R*)-1-[(7-Benzyloxymethyl-9-deaza-6-methoxypurin-9-yl)methyl]azetidine-2,4-dimethanol Hydrochloride (38). To a stirred solution of aldehyde **36** (210 mg, 0.70 mmol) in EtOH (7 mL) at ambient temperature was added **4**·HCl (100 mg, 0.65 mmol) followed after 5 min by $NaBH_3CN$ (67 mg, 1.0 mmol). The mixture was left to stir overnight, after which time most of the starting aldehyde had dissolved. The reaction mixture was absorbed onto silica gel under reduced pressure and the product purified by flash chromatography ($CHCl_3$/MeOH = 95:5 to 80:20) to give colorless crystals, which were taken up in water. Concentrated HCl was added, and then the mixture was concentrated under reduced pressure to afford **38** as a colorless hygroscopic solid (235 mg, 83%). ^{1}H NMR (300 MHz, D_2O): δ 8.78 (s, 1H), 8.13 (s, 1H), 7.20–7.04 (m, 5H), 5.86 (s, 2H), 4.62 (s, 2H), 4.62–4.47 (m, 3H), 4.27 (s, 3H), 4.26–4.04 (m, 2H), 3.57 (br d, J = 10.5 Hz, 1H), 3.30 (br d, J = 10.5 Hz, 1H), 2.46 (t, J = 8.1 Hz, 2H). ^{13}C NMR (75 MHz, D_2O): δ 160.0, 147.4, 140.2, 140.1, 136.9, 128.9, 128.7, 128.3, 116.8, 102.3, 78.8, 72.1, 68.4, 65.0, 60.2, 58.8, 57.0, 42.2, 20.8. ESI-HRMS for $C_{21}H_{27}N_4O_4$ [M + H^+] calcd, 399.2032; found, 399.2014.

(±)-(2*R*,4*R*)-[(9-Deazahypoxanthin-9-yl)methyl]azetidine-2,4-dimethanol Hydrochloride (41). A solution of **38** (60 mg, 0.13 mmol) was heated to reflux in concentrated HCl (5 mL). After 3 h, the mixture was concentrated under reduced pressure and the residue purified by successive flash chromatography on silica (9:1:1 2-propanol/H_2O/NH_4OH and then 65:35:7:1 $CHCl_3$/MeOH/H_2O/NH_4OH). The isolated product was dissolved in 1 M HCl (2 mL) and again concentrated in vacuo to give **41** as a hygroscopic colorless gum (35 mg, 84%). HPLC purity: 96% (290 nm). ^{1}H NMR (300 MHz, D_2O): δ 8.57 (s, 1H), 7.72 (s, 1H), 4.65 (d, J = 6.9 Hz, 2H), 4.60–4.48 (m, 2H), 4.21 (dd, J = 14.2, 6.4 Hz, 1H), 14.2, 3.0 Hz, 1H), 3.52 (dd, J = 13.2, 4.6 Hz, 1H), 3.22 (dd, J = 13.2, 3.4 Hz, 1H), 2.54–2.37 (m, 2H). ^{13}C NMR (75 MHz, D_2O): δ 154.2, 144.7, 137.7, 132.4, 118.6, 104.1, 67.8, 64.7, 60.0, 58.8, 42.5, 20.6. ESI-HRMS for $C_{12}H_{17}N_4O_3$ [M + H^+] calcd, 265.1301; found, 265.1316.

(±)-1-[(7-Benzyloxymethyl-9-deaza-6-methoxypurin-9-yl)methyl]azetidine-2-methanol (39). To a stirred solution of **30** (162 mg, 0.86 mmol) dissolved in MeOH (2 mL) was added concentrated HCl (1 mL). The reaction mixture was stirred for 20 min and then concentrated under reduced pressure. Residual HCl was removed by the addition and evaporation of acetonitrile several times to afford **5** as the HCl salt, which was committed to the next step without purification or characterization. The gumlike hydrochloride salt **5** was taken up in EtOH (10 mL) and aldehyde **36** (197 mg, 0.66 mmol) followed by the addition of $NaBH_3CN$ (63 mg, 0.99 mmol). The reaction mixture was allowed to stir overnight and then acidified to pH 1 using concentrated HCl. A small amount of HCN was evolved at this point. The reaction mixture was absorbed onto silica under reduced pressure and the product purified by flash chromatography ($CHCl_3$/MeOH/NEt_3 = 90:10:0.5) to give **39** as a colorless solid (170 mg, 69%), mp 214–216 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (s, 1H), 7.33 (s, 1H), 7.31–7.20 (m, 5H), 5.70 (s, 2H), 4.45 (s, 2H), 4.09 (s, 3H), 3.97 (d, J = 13.5 Hz, 1H), 3.80 (d, J = 13.5 Hz, 1H), 3.68 (br s, 1H), 3.55–3.46 (m, 1H), 3.45–3.42 (m, 2H), 3.34 (ddd, J = 8.8, 6.9, 2.5 Hz, 1H), 3.01 (ddd, J = 8.7, 8.7, 7.3 Hz, 1H), 2.14–2.00 (m, 1H), 1.90 (dddd, J = 10.1, 8.1, 8.1, 2.4 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.2, 149.9, 149.8, 136.7, 131.5, 128.3, 127.8, 127.5, 115.8, 114.0, 76.8, 70.0, 66.6, 64.0, 53.5, 51.3, 50.6, 18.7. ESI-HRMS for $C_{20}H_{25}N_4O_3$ [M + H^+] calcd, 369.1927; found, 369.1948.

(±)-1-[(9-Deazahypoxanthin-9-yl)methyl]azetidine-2-methanol (42). A solution of **39** (68 mg, 0.18 mmol) was heated to reflux in concentrated HCl (3 mL) for 2 h. The mixture was concentrated under reduced pressure and then azeotropically dried by the addition and evaporation of acetonitrile. The residue was purified by flash chromatography on silica ($CHCl_3$/MeOH/H_2O/NH_4OH = 65:35:7:1) to give **42** as an amorphous white solid (33 mg, 76%), mp 213–216 °C. HPLC purity: 98.9% (220 nm). ^{1}H NMR (300 MHz, 60:40 CD_4OD/D_2O): δ 8.03 (s, 1H), 7.60 (s, 1H), 4.23 (d, J = 13.8 Hz, 1H), 4.08 (d, J = 13.5 Hz, 1H), 4.10–3.98 (m, 1H), 3.65–3.49 (m, 4H), 2.27–2.11 (m, 2H). ^{13}C NMR (75 MHz, 60:40 CD_4OD/D_2O): δ 156.1, 144.9, 143.6, 130.8, 118.7, 109.9, 68.3, 63.4, 51.1, 49.4, 20.0; ESI-HRMS for $C_{11}H_{15}N_4O_2$ [M + H] calcd, 235.1195; found, 235.1196.

1-[(9-Deazaadenin-9-yl)methyl]-3-methylthiomethylazetidine-3-methanol Hydrochloride (43). NaOAc (134 mg, 1633 μmol) was added to a solution of **9**·HCl (300 mg, 1.6 mmol) in water (4 mL) and 1,4-dioxane (2 mL), and the resulting suspension was stirred at room temperature for 5 min. Formaldehyde solution (0.131 mL, 1.6 mmol) was then added dropwise followed by 9-deazaadenine (241 mg, 1.8 mmol), and the resulting suspension was heated to 95 °C (bath temperature). After 2 h, the crude reaction was absorbed onto silica and concentrated in vacuo. Chromatography (NH_4OH/MeOH/CH_2Cl_2 = 2:48:50) of the resulting residue afforded **43** as a syrup (180 mg, 33.4%). ^{1}H NMR (D_2O): δ 7.88 (brs, 1H), 7.29 (brs, 1H), 3.81 (s, 2H), 3.46 (s, 2H), 3.37 (dd, J = 17.5, 9.8 Hz, 4H), 2.46 (s, 2H), 2.55 (m, 2H), 1.83 (s, 3H). ^{13}C NMR (D_2O): δ 150.5, 150.2, 145.2, 130.5, 113.8, 106.2, 64.2, 57.8, 48.3, 39.8, 38.6, 16.5. HRMS for $C_{13}H_{19}N_5OS$ [MH^+] calcd, 294.1388; found, 294.1388. Anal. ($C_{13}H_{19}N_5OS$) C, H, N.

1-[(9-Deazaadenin-9-yl)methyl]-3-methylthiomethylazetidine (44). NaOAc (0.048 g, 0.586 mmol) was added to a solution of **10**·HCl (0.09 g, 0.586 mmol) in water (2 mL) and stirred for 15 min. Formaldehyde solution (0.047 mL, 0.586 mmol), 9-deazaadenine (86 mg, 0.644 mmol), and 1,4-dioxane (1 mL) were added consecutively, and the resulting suspension was stirred at 95 °C for 3 h. The crude reaction was absorbed onto silica and concentrated in vacuo. Chromatography (NH_4OH/MeOH/CH_2Cl_2 = 2:48:50) of the resulting residue afforded product contaminated with ammonium acetate. Further chromatography using Amberlyst 15 (H_2O → 2% aqueous NH_4OH) afforded **44** as a syrup (80 mg, 52%). ^{1}H NMR (D_2O): δ 8.06 (s, 1H), 7.34 (s, 1H), 3.71 (s, 2H),

3.40 (m, 2H), 2.95 (m, 2H), 2.55 (m, 3H), 1.93 (s, 3H). ^{13}C NMR (D_2O): δ 152.5, 151.4, 147.2, 129.8, 115.6, 112.4, 60.2, 60.2, 52.4, 39.1, 31.7, 15.7. HRMS for $C_{12}H_{17}N_5S$ [MH^+] calcd, 264.1283; found, 264.1288. Anal. ($C_{12}H_{17}N_5S \cdot {}^2/_3H_2O$) C, H, N.

Inhibition Assays. For PNP assays, inosine and inhibitor concentrations were determined spectrophotometrically using an ϵ_{260} of 7.1 mM^{-1} cm^{-1} (pH 6)[51] and an ϵ_{261} of 9.54 mM^{-1} cm^{-1} (pH 7),[52] respectively. For MTAN/MTAP assays, methylthioadenosine and inhibitor concentrations were determined using an ϵ_{260} of 14.9 mM^{-1} cm^{-1} (pH 6)[51] and an ϵ_{275} of 8.5 mM^{-1} cm^{-1} (pH 7),[52] respectively. PNP and MTAN/MTAP activities were monitored by xanthine oxidase coupled assays, as previously described.[19,53] In all cases, the inhibitor concentration was at least 10-fold greater than the enzyme concentration, as required for simple analysis of slow-onset tight-binding inhibition.[54] Michaelis constants used in data fitting were as follows: 40, 34, and 5 μM for inosine with human, bovine, and *P. falciparum* PNPs, respectively; 5, 0.43, and 23 μM for MTA with human MTAP, *E. coli* MTAN, and *S. pneumoniae* MTAN, respectively.

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Supporting Information Available: Analytical data, HPLC traces, and NMR spectra of target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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