Targeting the Polyamine Pathway with Transition-State Analogue Inhibitors of 5′-Methylthioadenosine Phosphorylase

Gary B. Evans,[†] Richard H. Furneaux,[†] Vern L. Schramm,[‡] Vipender Singh,[‡] and Peter C. Tyler*[,†]

Carbohydrate Chemistry, Industrial Research Limited, P.O. Box 31310, Lower Hutt, New Zealand, and Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461

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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 latter stages of polyamine biosynthesis (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. Potent inhibitors of MTAP might allow the buildup of sufficient levels of MTA to generate feedback inhibition of polyamine biosynthesis. We have designed and synthesized a family of potential transition-state analogue inhibitors of MTAP on the basis of our knowledge of the transition-state structure of purine nucleoside phosphorylase and the assumption that it is likely the two enzymes share a common catalytic mechanism. Several of the inhibitors display slow-onset tight-binding properties, consistent with them being transition-state analogues, with the most potent having a dissociation constant of 166 pM.

Introduction

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, with their roles in growth-related processes being best defined.[1−3] Cellular proliferation requires an increased level of polyamine biosynthesis, and compared to quiescent cells, rapidly dividing cells have elevated polyamine pools. For this reason, the polyamine biosynthetic pathway has been identified as a therapeutic target for the treatment of proliferative diseases such as cancer and parasitic infections.[4−8] So far, the main focus has been on inhibitors of ornithine decarboxylase, an enzyme operating early in the polyamine biosynthetic pathway. However, rapid turnover of this target enzyme and dose-limiting ototoxicity with ornithine decarboxylase inhibitors such as difluromethylornithine have limited the applications of this otherwise promising drug.

The pathway for the latter stages of polyamine biosynthesis involves the action of spermidine and spermine synthases on decarboxylated *S*-adenosylmethionone to effect chain elongation through donation of propylamine residues in the synthesis of spermidine and spermine (Figure 1). A product of each of these steps is 5′-methylthioadenosine (MTA). In principle, therefore, accumulation of MTA could lead to feedback inhibition of spermidine and spermine synthases and thus down-regulation of polyamine biosynthesis. Elevated MTA in vitro is known to have a cytostatic effect and to have antiproliferative activity as well as being a product inhibitor of both spermidine and spermine synthases.[9−13] Additionally, MTA has been shown to be proapoptoptic in human hepatoma cells in vitro.[14] However, in vivo in humans, MTA is rapidly degraded by 5′-methylthioadenosine phosphorylase (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. Its phosphorolysis reaction produces 5-methylthio-D-ribose-1-phosphate and adenine (Figure 2).[15,16] Potent inhibitors of MTAP might be expected to raise the intracellular concentration of MTA sufficiently to achieve feedback inhibition of polyamine biosynthesis and have antiproliferative activity.

A report of the crystal structure of human MTAP at 1.7 Å has identified a high degree of structural homology with human purine nucleoside phosphorylase (PNP), and it is likely that these enzymes share common catalytic mechanisms.[17] Knowledge of the transition-state structure of PNP[18−20] has allowed us to design transition-state analogues that are extremely potent inhibitors of PNP.[21−31] The first-generation inhibitor, immucillin-H (**1**, Chart 1), has a $K_i^* = 56$ pM against human PNP. Assuming that the transition state for phosphorolysis of MTA by MTAP is similar to that of PNP, then 5′-methylthio-immucillin-A (**2**, Chart 1) becomes a desirable target as a potential transition-state analogue inhibitor of human MTAP. The immucillins are proving to be effective pharmacophores on the basis of their affinity for the target enzyme, chemical and metabolic stability, and biological availability.[32−34]

Here we report the synthesis of **2** and a family of its structural analogues as potential transition-state analogue inhibitors for MTAP. Their inhibitory properties against human MTAP extend to a dissociation constant of 166 pM and establish these compounds as powerful inhibitors for human MTAP while at the same time delineating some structure−activity relationships. We have described in an initial report the inhibitory properties of some of these compounds.[35,41]

* To whom correspondence should be addressed. Phone: 64-4-9313062. Fax: 64-4-9313055. E-mail: p.tyler@irl.cri.nz.
[†] Industrial Research Ltd.
[‡] Albert Einstein College of Medicine.

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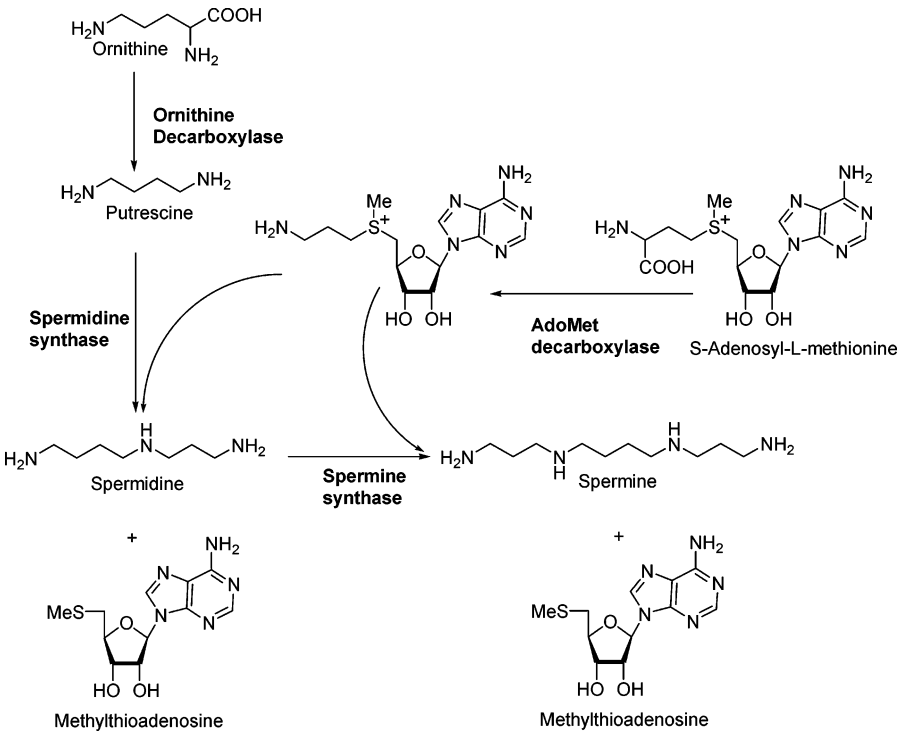


Figure 1. Polyamine biosynthesis from ornithine to spermine.

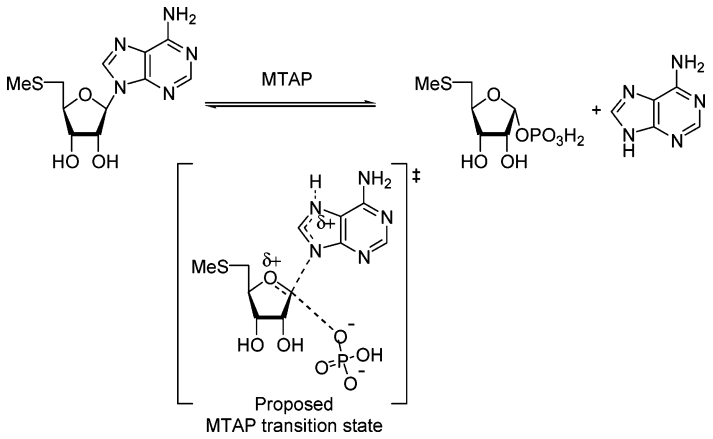
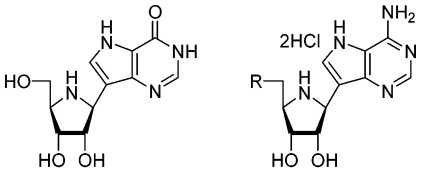
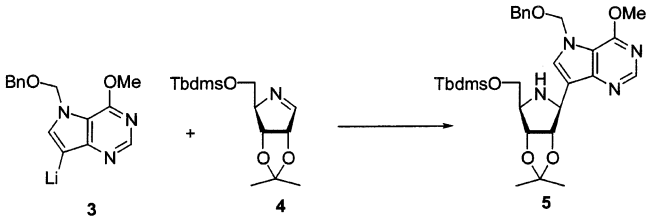


Figure 2. Phosphorolysis of methylthioadenosine catalyzed by MTAP.

Chart 1



Scheme 1

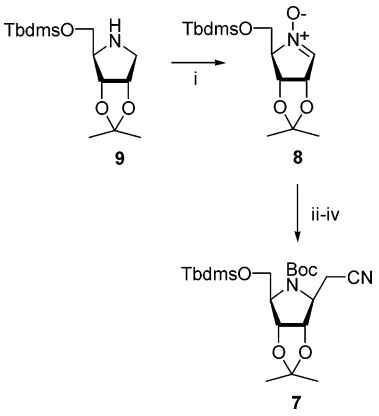


Results and Discussion

Synthesis of Inhibitors. Immucillin-H (**1**) is most conveniently prepared by a convergent route in which the lithiated deazapurine derivative **3** is added to the imine **4**, affording adduct **5** in high yield (Scheme 1).[23] The corresponding deazaadenine compound, immucillin-A (**6**, Chart 1), is unavailable by this method, as 9-lithio-9-deazaadenine derivatives are not stable enough under the conditions required for addition to this imine, even when the reaction is promoted by Lewis acids.[36] Immucillin-A can be prepared by elaboration of the 2,5-iminoheptononitrile (**7**), whereby the deazaadenine moiety is built up in a standard manner.[22] This heptononitrile **7** can be synthesized in good yield from the nitrone **8**,[36] which is generated from the readily available iminoribitol derivative **9** (Scheme 2).[22,37]

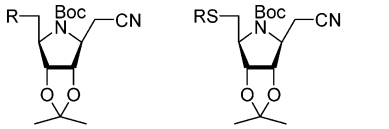
Our previous attempts to effect nucleophilic displacements at the 5′-position of imino-C-nucleosides had resulted in participation of the deazapurine moiety without formation of the desired product. Consequently, we decided to prepare the 5′-methylthio-immucillin-A (**2**) and other 5′-substituted thio-immucillins by synthesizing the corresponding 7-substituted thioheptononi-

Scheme 2[a]



[a] Reagents: (i) SeO_2, H_2O_2; (ii) $LiCH_2CN$, THF, −70 °C; (iii) Zn, HOAc; (iv) $(Boc)_2O$.

Chart 2



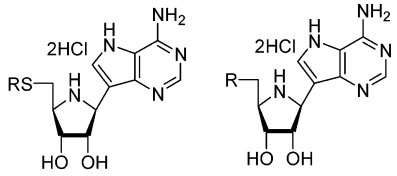
10 R = OH
11 R = OMs
25 R = OMe
26 R = H
27 R = CH_2CH_3

12 R = Me
13 R = Et
14 R = Pr
15 R = 2-hydroxyethyl
16 R = phenyl
17 R = 4-fluorophenyl
18 R = 4-chlorophenyl
19 R = 4-methylphenyl
20 R = 3-chlorophenyl
21 R = 3-methylphenyl
22 R = benzyl
23 R = 1-naphthyl
24 R = 2-fluoroethyl

triles and then elaborating them into the desired imino-C-nucleosides. Thus, desilylation of **7** afforded alcohol **10** that on mesylation gave **11** and, following displacement reactions using the sodium salts of the appropriate thiols, gave the 7-thioheptononitrile derivatives **12**−**23** (Chart 2). Treatment of the hydroxyethyl compound **15** with DAST afforded the 2-fluoroethyl derivative **24**, while methylation of the alcohol **10** generated the *O*-methyl ether **25** (Chart 2). The 7-deoxyheptononitrile **26** (Chart 2) was prepared by displacement of mesylate **11** with sodium iodide, followed by treatment with tributyltin hydride. Oxidation of alcohol **10** with the Dess−Martin periodinane generated the corresponding aldehyde, which was treated with ethylene triphenylphosphorane followed by catalytic hydrogenation to afford the nonononitrile **27** (Chart 2).

The heptononitriles **12**−**26** and nonononitrile **27** were individually converted into the imino-C-nucleosides **2** and **28**−**42** (Chart 3) by way of a standard sequence of reactions used for the construction of the 9-deazaadenine moiety.[22,38] For example, treatment of the methylthio derivative **12** with sodium hydride and ethyl formate effected functionalization of the active methylene to give the enol **43** (Scheme 3). Exposure of this material to aminoacetonitrile led to the enamine **44**, and subsequent treatment with methylchloroformate and DBU caused pyrrole formation with N1 protected as the carbamate. Addition of methanol to the reaction solution provided the unprotected pyrrole **45**. Finally, reaction with formamidine generated the deazaadenine moiety,

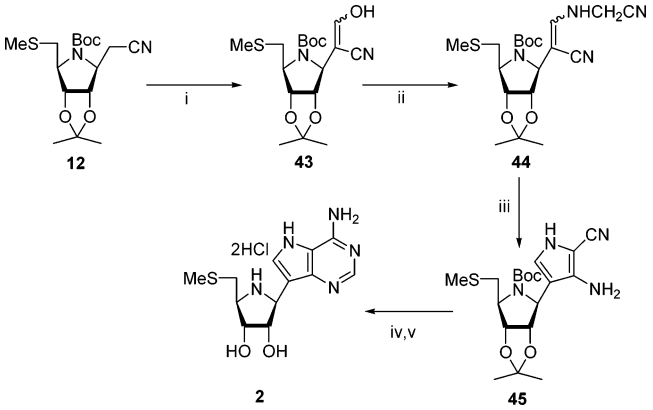
Chart 3



2 R = Me
28 R = Et
29 R = Pr
30 R = 2-hydroxyethyl
31 R = phenyl
32 R = 4-fluorophenyl
33 R = 4-chlorophenyl
34 R = 4-methylphenyl
35 R = 3-chlorophenyl
36 R = 3-methylphenyl
37 R = benzyl
38 R = 1-naphthyl
39 R = 2-fluoroethyl

40 R = OMe
41 R = H
42 R = CH_2CH_3

Scheme 3[a]



[a] Reagents: (i) NaH, EtOCHO, DMF; (ii) H_2NCH_2CN; (iii) MeOCOCl, DBU, CH_2Cl_2, then add MeOH; (iv) formamidine, EtOH, reflux; (v) aqueous HCl, MeOH.

and acid deprotection gave rise to the 5′-methylthioimmucillin-A (**2**).

Biological Results. (i) Enzymatic Assays. Reaction mixtures (1.0 mL) containing 100 mM potassium phosphate, pH 7.4, 50 mM KCl, 0.1−2 mM dithiothreitol, methylthioadenosine at 0.2 or 0.25 mM, and variable concentrations of inhibitor were warmed to 25 °C in a temperature-controlled spectrophotometer, and reactions were initiated with 1−10 nM purified human MTAP. Activity was monitored by the decrease in absorbance at 274 nm equivalent to 1.6 mM^{-1} cm^{-1}. The equilibrium constant for this reaction is unfavorable ($K_{eq} \approx 0.001$) for phosphorolysis, so care must be taken to accurately estimate intital rates, even with the high phosphate concentration.

(ii) Inhibition Studies. Inhibition by transition-state analogue inhibitors often 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: $E + I \leftrightarrow E^*I$. Experimentally, inhibition constants were estimated by two different

methods. 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 times the enzyme concentration.[24] Under conditions of high inhibitor concentration, initial rates are difficult to measure because the tight-binding phase occurs rapidly. Inhibition curves were also fitted to the general integrated rate equation:

$$P = v_s t + (v_o - v_s)(1 - e^{-kt})/k$$

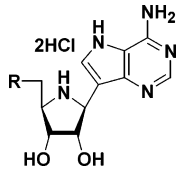
where v_o, v_s, and k represent respectively initial rate, final steady-state rate, and first-order rate constant for attainment of the final rate. The value of k increases hyperbolically as a function of inhibitor concentration, such that

$$k = k_6 \frac{1 + (I/(K_i^*(1 + A/K_a))}{1 + (I/(K_i(1 + A/K_a))}$$

where k_6 is the rate of conversion of E*I to EI, and I and A are inhibitor and substrate concentrations.[39]

The 5′-methylthio-immucillin-A (**2**) is a powerful inhibitor of MTAP and, after slow onset, has $K_i^* = 1.03$ nM. The K_m/K_i^* value of 4800, 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. When the methyl group is extended to ethyl (as in **28**), the binding affinity increases to give $K_i^* = 266$ pM and a K_m/K_i^* value of 18 800, while the *n*-propyl analogue **29** has a binding affinity similar to that of **28**. 2-Hydroxyethyl (**30**) and 2-fluoroethyl (**39**) compounds are weaker inhibitors at 14 and 3.2 nM, respectively, establishing the hydrophobic character of the methyl/ethyl binding region on the enzyme. Replacement of the sulfur of **2** with oxygen (**40**) or a methylene group (**42**) resulted in a considerable decrease in inhibitory potency to 134 and 44 nM, respectively, and no slow-onset phase of inhibition that characterizes the more powerful of the MTAP inhibitors was observed. In view of these results, it was surprising that the 5′-phenylthio (**31**) and 5′-(4-chlorophenyl)thio (**33**) derivatives displayed affinity equal or superior to that of the parent compound **2**, with K_i^* values of 1.0 nM and 166 pM, respectively. The 5′-(4-fluorophenyl)thio (**32**) and 5′-(4-methylphenyl)thio (**34**) derivatives are both within a factor of 2 in affinity relative to the 5′-phenylthio (**31**) derivative, with K_i^* values of 2 nM and 640 pM, respectively. The 5′-(3-methylphenyl)thio compound **36** shows affinity similar to that of this group, with $K_i^* = 628$ pM. In contrast, the 5′-(3-chlorophenyl)thio **(35)**, the 5′-benzylthio (**37**), and the 5′-naphthylthio (**38**) derivatives were weaker inhibitors that did not cause slow-onset inhibition and gave K_i values of 6.4, 26, and 90 nM, respectively. Even though we classify these as "weak" inhibitors, it should be noted that these compounds bind 56−780-fold more tightly than substrate, and the MTA substrate for this enzyme binds relatively tightly, with $K_m = 5.0$ μM. An interesting compound (**41**) in this series lacks the 5′-methylthio group that distinguishes the reaction of MTAP from other purine nucleoside phosphorylases and is key for recognition of its biological substrate. For **41**, without the 5′-methylthio, the K_i value of 360 nM is only 7-fold less than the K_m value and does not cause a slow-onset inhibition phase.

Table 1. Inhibition of Human MTAP by Immucillin-A Series Analogues[a]



		inhibition constants	
compd	R	K_i (nM)	K_i^* (nM)
2	methylthio	26 ± 0.8	1.0 ± 0.5
28	ethylthio	9.2 ± 1.0	0.266 ± 0.03
29	*n*-propylthio	4.0 ± 0.6	0.214 ± 0.01
30	(2-hydroxyethyl)thio	56 ± 4.0	14 ± 1.2
31	phenylthio	3.6 ± 0.4	1.0 ± 0.1
32	(4-fluorophenyl)thio	6.4 ± 0.6	2.0 ± 0.2
33	(4-chlorophenyl)thio	0.576 ± 0.076	0.166 ± 0.024
34	(4-methylphenyl)thio	4.4 ± 1.2	0.640 ± 0.018
35	(3-chlorophenyl)thio	6.4 ± 0.4	nd
36	(3-methylphenyl)thio	1.39 ± 0.14	0.628 ± 0.034
37	benzylthio	26 ± 2.0	nd
38	1-naphthylthio	90 ± 16.0	nd
39	(2-fluoroethyl)thio	20 ± 2.0	3.2 ± 0.3
40	methoxy	134 ± 10	nd
41	H	720 ± 360	nd
42	ethyl	44 ± 4.0	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.

The K_i^* values for the 5′-arylthio- and 5′-alkylthio-9-deazaadenyliminoribitols identify a new class of powerful inhibitors for this central enzyme of polyamine metabolism. Kinetic isotope effect measurements to characterize the transition-state structure of MTAP are in progress (V. Singh and V. L. Schramm) and confirm that MTAP is similar to all other known purine nucleoside *N*-ribosyl transferases in being characterized by ribooxacarbenium character at the transition state.[40] The compounds listed in Table 1 resemble the transition state by having the positive charge on the ribosyl analogue and the elevated pK_a at N7. These features have been demonstrated to be important transition-state mimics of the closely related purine nucleoside phosphorylases.[25] The structures of the most powerful inhibitors (**28**, **29**, and **33**) are of interest in terms of the X-ray crystal structure of human MTAP.[35] The 5′-methylthio region is surrounded by the side chains from five hydrophobic amino acids (Thr18, Phe177, Val233, Val236, and Leu279B, from the adjacent subunit) and His137B from a loop on the adjacent subunit. We hypothesize that the ethyl and propyl groups of **28** and **29** show improved binding by more favorable interactions with these hydrophobic groups. However, the presence of the single His in this cluster also provides the environment for a favorable H-bond interaction. The enhanced binding of the 5′-(4-chlorophenyl)thio (**33**) relative to 5′-(3-chlorophenyl)thio (**35**) derivative suggests a specific orientation for this interaction, which remains for future structural exploration.

Conclusions

Using the knowledge gained from our work on the transition state of purine nucleoside phosphorylase, we have designed and synthesized putative transition-state analogues of human 5′-methylthioadenosine phosphorylase. Some of these compounds are extremely potent inhibitors and display slow-onset tight-binding characteristics that are consistent with them being 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.

Inhibition Assays. The enzymatic activity and inhibition assays for MTAP have been described recently.[35]

***N*-*tert*-Butoxycarbonyl-3,6-imino-4,5-*O*-isopropylidene-7-*O*-methanesulfonyl-2,3,6-trideoxy-D-*allo*-heptononitrile (11).** Tetrabutylammonium fluoride (1 M in THF, 75 mL) was added to a solution of **7**[22] (19.1 g, 44.8 mmol) in THF (50 mL), and the resulting solution was allowed to stand for 0.5 h. Chloroform (350 mL) was added, and the solution was washed with water (×2), dried, and concentrated to dryness. Chromatography afforded *N*-*tert*-butoxycarbonyl-3,6-imino-4,5-*O*-isopropylidene-2,3,6-trideoxy-D-*allo*-heptononitrile (**10**) (13.6 g, 43.6 mmol, 97%) as a syrup. A solution of some of this material (1.0 g, 3.2 mmol) in dichloromethane (15 mL) containing diisopropylethylamine (1.12 mL, 6.3 mmol) was treated with methanesulfonyl chloride (0.37 mL, 4.8 mmol) at 0 °C, and then the solution was stirred at room temperature for 1 h. Normal processing and chromatography afforded syrupy title compound **11** (1.12 g, 2.87 mmol, 90%). ^{1}H NMR ($CDCl_3$): δ 4.75 (dd, J = 5.7, 1.4 Hz, 1H), 4.61 (brs, 1H), 4.38 (brd, J = 5.7 Hz, 2H), 4.19 (m, 2H), 3.07 (s, 3H), 2.81 (m, 2H), 1.50 (s, 12H), 1.35 (s, 3H). ^{13}C NMR: δ 154.0, 117.5, 113.3, 83.3, 82.4, 81.3, 68.6, 64.2, 61.5, 60.7, 37.7, 28.6, 27.6, 25.6, 21.8. HRMS (MH^+): calcd for $C_{16}H_{27}N_2O_7S$, 391.1539; found, 391.1539.

General Procedure for the Synthesis of 5′-Substituted Thio-immucillin-A Compounds 2 and 28−39. A solution of *N*-*tert*-butoxycarbonyl-3,6-imino-4,5-*O*-isopropylidene-7-*O*-methanesulfonyl-2,3,6-trideoxy-D-*allo*-heptononitrile (x mmol) in DMF ($2x$ mL) was added to a solution of the appropriate sodium thiolate [prepared by adding sodium hydride ($1.8x$ mmol) to a solution of the thiol ($2x$ mmol) in DMF ($4x$ mL)], and the resulting solution was stirred for 3 h. Toluene was added, and the mixture was washed twice with water, dried, and concentrated to dryness. Chromatography afforded the corresponding 7-thioheptononitriles **12**−**24**. This material in THF ($8x$ mL) and ethyl formate (x mL) was treated with sodium hydride ($4x$ mmol), and the mixture was stirred for 2 h. Acetic acid ($0.5x$ mL) and chloroform ($20x$ mL) were added, and the mixture was washed with water, dried, and concentrated to dryness. Sodium acetate ($8x$ mmol) and aminoacetonitrile bisulfate ($4x$ mmol) were added to a solution of the crude product in methanol ($8x$ mL), and the mixture was stirred at room temperature for 2 d (or heated under reflux for 2−3 h) and then was concentrated to dryness. Chloroform ($20x$ mL) was added, and the mixture was washed with water, dried, and concentrated to dryness. The crude residue in dichloromethane ($12x$ mL) was treated with DBU (x mL) and methyl chloroformate ($0.25x$ mL), and the solution was stirred at room temperature for 18 h. Methanol ($4x$ mL) was then added, and after 1 h the solution was washed with 2 M HCl and aqueous sodium bicarbonate, dried, and concentrated to dryness. Chromatography then afforded the corresponding pyrroles. This material in ethanol ($8x$ mL) containing formamidine acetate ($2x$ mmol) was heated under reflux for 4 h. The solution was concentrated to dryness, and the crude material was purified by chromatography. A solution of the product in methanol ($4x$ mL) and 4 M HCl ($4x$ mL) was allowed to stand for 4 h and then concentrated to dryness. Trituration with 2-propanol gave solid product as a bishydrochloride in ∼50% overall yield.

The following compounds were prepared in the same manner.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-dideoxy-1,4-imino-5-methylthio-D-ribitol Bishydrochloride (2). The solid decomposed at 223−225 °C without melting. ^{1}H NMR: δ 8.33 (s, 1H), 7.97 (s, 1H), 4.90 (d, J = 8.4 Hz, 1H), 4.75 (m, 1H), 4.38 (t, J = 4.2 Hz, 1H), 3.87 (m, 1H), 3.05 (dd, J = 14.4, 5.7 Hz, 1H), 2.91 (dd, J = 14.4, 9.3 Hz, 1H), 2.11 (s, 3H). ^{13}C NMR: δ 149.4 (C), 143.6 (CH), 139.1 (C), 133.0 (CH), 113.0, 105.6 (C), 73.2, 72.5 63.6, 56.3 (CH), 33.5 (CH_2), 14.6 (CH_3). Anal. ($C_{12}H_{19}Cl_2N_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-dideoxy-5-ethylthio-1,4-imino-D-ribitol Bishydrochloride (28). Mp: 204−212 °C dec. ^{1}H NMR: δ 8.37 (s, 1H), 8.00 (s, 1H), 4.94 (d, J = 8.6 Hz, 1H), 4.78 (dd, J = 5.2, 8.6 Hz, 1H), 4.42 (t, J = 4.8 Hz, 1H), 3.91−3.84 (m, 1H), 3.15 (dd, J = 6.0, 14.5 Hz, 1H), 2.96 (dd, J = 9.3, 14.5 Hz, 1H), 2.65 (d, J = 7.4 Hz, 1H), 2.60 (d, J = 7.4 Hz, 1H), 1.22 (t, J = 7.4 Hz, 3H). ^{13}C NMR: δ 149.4 (C), 143.6 (CH), 139.4 (C), 133.0 (CH), 113.1, 105.8 (C), 73.2, 72.6, 64.1, 56.4 (CH), 30.9, 25.7 (CH_2), 14.2 (CH_3). Anal. ($C_{13}H_{21}Cl_2N_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-dideoxy-5-*n*-propylthio-1,4-imino-D-ribitol Bishydrochloride (29). Mp: 213−215 °C dec. ^{1}H NMR: δ 8.37 (s, 1H), 8.00 (s, 1H), 4.94 (d, J = 8.6 Hz, 1H), 4.78 (dd, J = 5.2, 8.6 Hz, 1H), 4.42 (t, J = 4.7 Hz, 1H), 3.91−3.84 (m, 1H), 3.12 (dd, J = 6.0, 14.5 Hz, 1H), 2.96 (dd, J = 9.2, 14.5 Hz, 1H), 2.60 (t, J = 7.2 Hz, 2H), 1.64−1.52 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H). ^{13}C NMR: δ 149.4 (C), 143.6 (CH), 139.4 (C), 133.0 (CH), 113.1, 105.8 (C), 73.2, 72.6, 64.2, 56.4 (CH), 33.7, 31.3, 22.6 (CH_2), 13.0 (CH_3). Anal. ($C_{14}H_{23}Cl_2N_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-dideoxy-5-(2-hydroxyethyl)thio-1,4-imino-D-ribitol Bishydrochloride (30). The solid charred at ∼220 °C without melting. ^{1}H NMR: δ 8.38 (s, 1H), 8.00 (s, 1H), 4.95 (d, J = 8.5 Hz, 1H), 4.79 (dd, J = 5.2, 8.5 Hz, 1H), 4.43 (t, J = 4.6 Hz, 1H), 3.94−3.87 (m, 1H), 3.74 (t, J = 6.0 Hz, 2H), 3.18 (dd, J = 5.8, 14.5 Hz, 1H), 3.01 (dd, J = 9.2, 14.5 Hz, 1H), 2.81−2.76 (m, 2H). ^{13}C NMR: δ 149.4 (C), 143.6 (CH), 139.4 (C), 133.0 (CH), 113.1, 105.8 (C), 73.2, 72.6, 64.3 (CH), 60.8 (CH_2), 56.5 (CH), 34.2, 31.7 (CH_2). Anal. ($C_{13}H_{21}Cl_2N_5O_3S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-dideoxy-1,4-imino-5-phenylthio-D-ribitol Bishydrochloride (31). Mp: 180−182 °C. ^{1}H NMR: δ 8.30 (s, 1H), 7.96 (s, 1H), 7.44−7.25 (m, 5H), 4.88 (d, J = 8.3 Hz, 1H), 4.76 (dd, J = 5.0, 8.3 Hz, 1H), 4.41 (t, J = 4.6 Hz, 1H), 3.75 (m, 1H), 3.54 (dd, J = 5.7, 14.8 Hz, 1H), 3.35 (dd, J = 9.2, 14.8 Hz, 1H). ^{13}C NMR (D_2O): δ 149.1 (C), 143.4 (CH), 139.5 (C), 133.0 (CH), 132.9 (C), 130.9, 130.0, 128.1 (CH), 113.0, 105.9 (C), 73.2, 72.6, 63.8, 56.7 (CH), 33.8 (CH_2). Anal. ($C_{17}H_{21}Cl_2N_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-dideoxy-5-(4-fluorophenyl)thio-1,4-imino-D-ribitol Bishydrochloride (32). The solid charred at 240 °C without melting. ^{1}H NMR: δ 8.32 (s, 1H), 7.97 (s, 1H), 7.50−7.43 (m, 2H), 7.12−7.04 (m, 2H), 4.89 (d, J = 8.5 Hz, 1H), 4.76 (dd, J = 5.0, 8.5 Hz, 1H), 4.40 (t, J = 4.5 Hz, 1H), 3.74−3.67 (m, 1H), 3.47 (dd, J = 5.9, 14.8 Hz, 1H), 3.30 (dd, J = 9.2, 14.8 Hz, 1H). ^{13}C NMR: δ 162.8 (d, $J_{C,F}$ = 245 Hz, C), 149.1 (C), 143.4 (CH), 139.6 (C), 134.1 (d, $J_{C,F}$ = 8.4 Hz, CH), 133.0 (CH), 127.9 (C), 116.9 (d, $J_{C,F}$ = 22 Hz, CH), 113.0, 105.8 (C), 73.2, 72.5, 63.7, 56.6 (CH, 35.0 (CH_2). Anal. ($C_{17}H_{20}Cl_2FN_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-5-(4-chlorophenyl)thio-1,4-dideoxy-1,4-imino-D-ribitol Bishydrochloride (33). The solid charred at 260−270 °C without melting. ^{1}H NMR: δ 8.32 (s, 1H), 7.97 (s, 1H), 7.40 (d, J = 8.7 Hz, 2H), 7.34 (d, J = 8.7 Hz, 2H), 4.89 (d, J = 8.3 Hz, 1H), 4.77 (dd, J = 5.0, 8.3 Hz, 1H), 4.42 (t, J = 4.7 Hz, 1H), 3.75 (m, 1H), 3.54 (dd, J = 5.7, 14.8 Hz, 1H), 3.35 (dd, J = 9.2, 14.8 Hz, 1H). ^{13}C NMR: δ 149.0 (C), 143.3 (CH), 133.6 (C), 133.0, 132.4 (CH), 131.6 (C),

129.8 (CH), 113.0, 105.9 (C), 73.3, 72.6, 63.6, 56.7 (CH), 34.0 (CH_2). Anal. ($C_{17}H_{20}Cl_3N_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-dideoxy-1,4-imino-5-(4-methylphenyl)thio-D-ribitol Bishydrochloride (34). The solid charred at 250 °C without melting. ^{1}H NMR: δ 8.31 (s, 1H), 7.96 (s, 1H), 7.33 (d, J = 8.2 Hz, 2H), 7.17 (d, J = 8.2 Hz, 2H), 4.88 (d, J = 8.4 Hz, 1H), 4.77 (dd, J = 5.0, 8.4 Hz, 1H), 4.40 (t, J = 4.5 Hz, 1H), 3.73 (m, 1H), 3.48 (dd, J = 5.8, 14.8 Hz, 1H), 3.30 (dd, J = 9.1, 14.8 Hz, 1H). ^{13}C NMR: δ 149.0 (C), 143.3 (CH), 139.6, 139.0 (C), 133.0, 131.6, 130.6 (CH), 129.0, 113.0, 105.9 (C), 73.3, 72.6, 63.9, 56.8 (CH), 34.4 (CH_2), 20.5 (CH_3). Anal. ($C_{18}H_{23}Cl_2N_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-5-(3-chlorophenyl)thio-1,4-dideoxy-1,4-imino-D-ribitol Bishydrochloride (35). The solid charred at 230 °C without melting. ^{1}H NMR: δ 8.30 (s, 1H), 7.96 (s, 1H), 7.41 (d, J = 0.5 Hz, 1H), 7.34−7.22 (m, 3H), 4.89 (d, J = 8.2 Hz, 1H), 4.77 (dd, J = 5.0, 8.1 Hz, 1H), 4.42 (t, J = 4.8 Hz, 1H), 3.80−3.73 (m, 1H), 3.57 (dd, J = 5.7, 14.8 Hz, 1H), 3.36 (dd, J = 9.2, 14.8 Hz, 1H). ^{13}C NMR: δ 148.9 (C), 143.3 (CH), 139.6, 135.1, 134.8 (C), 133.1, 131.1, 129.9, 128.8, 128.0 (CH), 113.0, 105.9 (C), 73.3, 72.6, 63.6, 56.8 (CH), 33.6 (CH_2). Anal. ($C_{17}H_{20}Cl_3N_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-dideoxy-1,4-imino-5-(3-methylphenyl)thio-D-ribitol Bishydrochloride (36). The solid charred at 250−260 °C without melting. ^{1}H NMR: δ 8.30 (s, 1H), 7.97 (s, 1H), 7.24 (m, 3H), 7.12 (m, 1H), 4.89 (d, J = 8.3 Hz, 1H), 4.77 (dd, J = 5.0, 8.3 Hz, 1H), 4.41 (t, J = 4.5 Hz, 1H), 3.76 (m, 1H), 3.53 (dd, J = 5.7, 14.8 Hz, 1H), 3.33 (dd, J = 9.1, 14.8 Hz, 1H), 2.26 (s, 3H). ^{13}C NMR: δ 149.0 (C), 143.3 (CH), 140.4, 139.6 (C), 133.0 (CH), 132.7 (C), 131.2, 129.8, 128.8, 127.8 (CH), 113.0, 105.9 (C), 73.3, 72.6, 63.8, 56.7 (CH), 33.8 (CH_2), 20.8 (CH_3). Anal. ($C_{18}H_{23}Cl_2N_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-5-benzylthio-1,4-dideoxy-1,4-imino-D-ribitol Bishydrochloride (37). The solid charred at 235 °C without melting. ^{1}H NMR: δ 8.34 (s, 1H), 7.94 (s, 1H), 7.37−7.24 (m, 5H), 4.89 (d, J = 8.4 Hz, 1H), 4.31 (t, J = 4.7 Hz, 1H), 3.83 (s, 2H), 3.78−3.71 (m, 1H), 2.98 (dd, J = 6.3, 14.6 Hz, 1H), 2.90 (dd, J = 8.8, 14.6 Hz, 1H). ^{13}C NMR: δ 149.2 (C), 143.5 (CH), 138.1 (C), 133.0, 129.4, 128.0 (CH), 113.0, 105.9 (C), 73.2, 72.6, 63.9, 56.6 (CH), 35.6, 30.8 (CH_2). Anal. ($C_{18}H_{23}Cl_2N_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-dideoxy-1,4-imino-5-(1-naphthyl)thio-D-ribitol Bishydrochloride (38). The solid charred at 270 °C without melting. ^{1}H NMR (MeOH-d_4/DMSO-d_6): δ 7.83 (s, 1H), 7.68 (d, J = 7.9 Hz, 1H), 7.45 (s, 1H), 7.27−7.14 (m, 3H), 6.96−6.80 (m, 3H), 4.19 (d, J = 8.6 Hz, 1H), 3.96 (dd, J = 5.1, 8.6 Hz, 1H), 2.96−2.74 (m, 3H). ^{13}C NMR (MeOH-d_4/DMSO-d_6): δ 143.4, 133.5 (C), 131.9 (CH), 131.6, 129.9 (C), 128.9, 128.1, 127.6, 126.3, 125.9, 125.1, 123.8 (CH), 111.8 (C), 72.1, 71.7, 62.7, 55.3 (CH), 32.8 (CH_2). Anal. ($C_{21}H_{23}Cl_2N_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-dideoxy-5-(2-fluoroethyl)thio-1,4-imino-D-ribitol Bishydrochloride (39). A solution of **15** (1.0 g) in dry chloroform (10 mL) was treated with DAST (0.71 mL). The solution was allowed to stand for 16 h and then was washed with water and aqueous $NaHCO_3$, dried, and concentrated to dryness. Chromatography afforded syrupy **24** (0.558 g). This material was converted into the title compound by the same sequence of reactions described above in the preparation of **2** to give a solid **39** (0.307 g). The solid charred at 210−220 °C without melting. ^{1}H NMR: δ 8.38 (s, 1H), 8.00 (s, 1H), 4.95 (d, J = 8.5 Hz, 1H), 4.79 (dd, J = 5.2, 8.5 Hz, 1H), 4.66 (dt, J = 5.6, 46.9 Hz, 2H), 4.43 (t, J = 4.6 Hz, 1H), 3.94−3.88 (m, 1H), 3.22 (dd, J = 5.9, 14.5 Hz, 1H), 3.09−2.86 (m, 3H). ^{13}C NMR: δ 149.4 (C), 143.6 (CH), 139.5 (C), 133.1 (CH), 113.1, 105.8 (C), 84.2 (d, $J_{C,F}$ = 164 Hz, CH_2), 73.2, 72.6, 64.2, 56.5 (CH), 31.9 (CH_2), 31.9 (d, $J_{C,F}$ = 20 Hz, CH_2). Anal. ($C_{13}H_{20}Cl_2FN_5O_2S$): C, H, Cl, N, S.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-dideoxy-1,4-imino-5-*O*-methyl-D-ribitol Bishydrochloride (40). The silyl ether **7** (0.5 g, 1.17 mmol) was desilylated as described above in the preparation of **11**. A solution of the crude product in THF was treated with methyl iodide (0.125 mL, 2 mmol) and sodium hydride (0.10 g, 60%, 2.5 mmol). The mixture was stirred for 2 h and then quenched with ethanol. Chloroform was added, and normal processing afforded a syrup. This material was treated as described above in the preparation of **2** to give title compound **40**. Mp: 175−180 °C. ^{1}H NMR: δ 8.44 (s, 1H), 8.05 (s, 1H), 5.01 (d, J = 8.9 Hz, 1H), 4.81 (dd, J = 4.8, 8.9 Hz, 1H), 4.48 (dd, J = 3.4, 4.8 Hz, 1H), 4.00 (m, 1H), 3.85 (dd, J = 5.4, 11.2 Hz, 1H), 3.79 (dd, J = 3.9, 11.2 Hz, 1H), 3.43 (s, 3H). ^{13}C NMR: δ 149.8 (C), 143.9 (CH), 138.6 (C), 132.9 (CH), 113.0, 105.5 (C), 73.8, 71.2 (CH), 68.9 (CH_2), 64.3 (CH), 59.1, (CH_3), 55.9 (CH). Anal. ($C_{12}H_{19}Cl_2N_5O_3$): C, H, Cl, N.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-imino-1,4,5-trideoxy-D-ribitol Bishydrochloride (41). Sodium iodide (1.3 g, 8.6 mmol) was added to a solution of **11** (0.50 g, 1.28 mmol) in acetone (10 mL), and the mixture was heated under reflux for 24 h and then concentrated to dryness. The residue was partitioned between chloroform and water, and the organic phase was dried and concentrated to dryness. A solution of the residue in benzene (10 mL) was treated with tributyltin hydride (1.0 mL, 3.7 mmol), and the solution was heated under reflux for 2 h and then concentrated to dryness. The residue in ether was stirred for 1 h with 10% aqueous KF, and the organic layer was dried and concentrated to dryness. Chromatography afforded a syrup (0.25 g) of, presumably, *N*-*tert*-butoxycarbonyl-3,6-imino-4,5-*O*-isopropylidene-2,3,6,7-tetradeoxy-D-*allo*-heptononitrile. This material was treated as described above in the preparation of **2** to give **41**. Mp: 198−200 °C. ^{1}H NMR: δ 8.41 (s, 1H), 8.04 (s, 1H), 4.96 (d, J = 8.5 Hz, 1H), 4.88 (dd, J = 4.8, 8.5 Hz, 1H), 4.31 (t, J = 4.5 Hz, 1H), 3.87 (dq, J = 4.2, 7.1 Hz, 1H), 1.54 (d, J = 7.1 Hz, 3H). ^{13}C NMR: δ 149.5 (C), 143.7 (CH), 139.2 (C), 132.8 (CH), 113.1, 106.2 (C), 74.5, 73.2, 60.8, 56.2 (CH), 16.0 (CH_3). Anal. ($C_{11}H_{17}Cl_2N_5O_2$): C, H, Cl, N.

(1*S*)-1-(9-Deazaadenin-9-yl)-1,4-imino-1,4,5,6,7-pentadeoxy-D-*ribo*-heptitol Bishydrochloride (42). Dess−Martin periodinane (1.42 g, 3.35 mmol) was added to a stirred solution of **10** (0.7 g, 2.24 mmol) in dichloromethane (20 mL). After 0.5 h, the mixture was concentrated to dryness. Ether (20 mL) was added, and the mixture was washed twice with 1:1 saturated aqueous $NaHCO_3$/10% aqueous $Na_2S_2O_3$, dried, and concentrated to dryness. A solution of the crude product in dry THF (8 mL) was added to the red solution obtained after adding *n*-butyllithium (3.4 mL, 1.6M, 5.44 mmol) to a suspension of ethyl triphenylphosphonium iodide (2.44 g, 5.84 mmol) in THF (25 mL) and stirring for 0.5 h. The resulting dark solution was stirred for 0.5 h and then diluted with petroleum ether (100 mL) and washed with water. Normal processing afforded, after chromatography, syrupy material which was stirred in ethanol with 10% Pd/C under hydrogen for 2.5 h. Removal of the solids and solvent and chromatography afforded a syrup (0.28 g), which was treated as above in the preparation of **2** to give **42**. Mp: 206−215 °C dec. ^{1}H NMR: δ 8.33 (s, 1H), 7.95 (s, 1H), 4.84 (d, J = 8.9 Hz, 1H), 4.28 (t, J = 4.7 Hz, 1H), 3.67−3.56 (m, 1H), 1.87−1.66 (m, 2H), 1.46−1.33 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H). ^{13}C NMR: δ 149.7 (C), 143.8 (CH), 138.8 (C), 132.9 (CH), 113.1, 105.6 (C), 73.0, 72.9, 65.1, 55.9 (CH), 32.8, 19.4 (CH_2), 13.2 (CH_3). Anal. ($C_{13}H_{21}Cl_2N_5O_2$): C, H, Cl, N.

Supporting Information Available: Elemental analyses for target compounds **2** and **28**−**42**. This material is available free of charge via the Internet at http://pubs.acs.org.

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