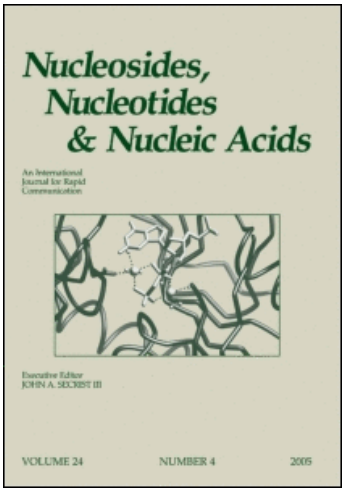

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AN EFFICIENT, SCALABLE SYNTHESIS OF THE HIV REVERSE TRANSCRIPTASE INHIBITOR ZIAGEN® (1592U89)

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Dedicated in memory of Gertrude B. Elion

ABSTRACT: Ziagen®, *(1S,cis)*-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol, was synthesized from *(1S,4R)*-azabicyclo[2.2.1]hept-5-en-3-one by efficient processes which bypass problematic steps in earlier routes. 2-Amino-4,6-dichloro-5-formamidopyrimidine is a key intermediate which makes possible an efficient construction of the purine from a chiral cyclopentenyl precursor.

INTRODUCTION

Carbocyclic nucleosides have inspired a great deal of innovative synthesis since Shealy and Clayton's first report of racemic carbocyclic adenosine.[1] This class of nucleosides has the potential of greater *in vivo* stability due to replacement of the hydrolytically and enzymatically labile glycosylic bond. Understandably, given the accompanying structural and stereoelectronic disturbances resulting from replacement of the sugar oxygen, the activities of most conventional carbocyclic nucleosides have been poorer than those of the corresponding ribosides.[2a] The challenges presented have generated an amazing variety of innovative structures and synthetic approaches, and a number of clinical candidates in the antiviral and anticancer areas have resulted.[2a-2c] To date, syntheses of carbocyclic nucleosides have rarely been required on a scale beyond the relatively small amounts required for research. The first regulatory agency approvals of a carbocyclic nucleoside drug were recently received for Ziagen® (1592U89, abacavir

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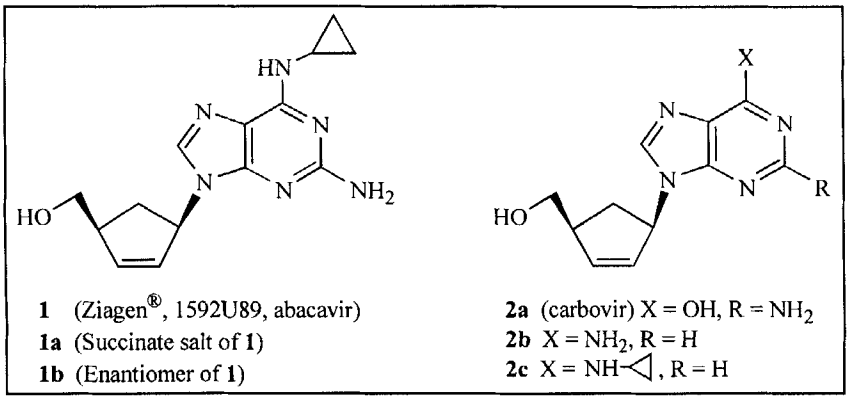


FIG. 1. Ziagen® and related cyclopentenyl nucleoside analogs.

sulfate, **1**) for the treatment of HIV infection in children and adults. Although the preclinical research that led to the selection of **1** has been published,[3] this is the first report preclinical research that led to the selection of **1** has been published,[3] this is the first report of some of the synthetic hurdles encountered in bringing the first carbocyclic nucleoside to the market.[4,5]

The *in vitro* anti-HIV activities of 2',3'-dideoxynucleosides led us and others to synthesize and evaluate many carbocyclic analogs in the late 1980s. Vince, Shannon, and co-workers first described potent *in vitro* anti-HIV activity for the cyclopentenylguanine "carbovir" (**2a**).[6] In preparation for proposed clinical studies, **2a** was evaluated in animals by Glaxo Inc. Toxicological responses in rats, dogs, and marmosets were noted[7] and Glaxo's development of **2a** was halted in 1991. At The Wellcome Research Laboratories, we were meanwhile extending our earlier discovery that cyclopentenyl and other purine nucleosides containing modifications at the purine 6-position retained the antiviral activities of the parent adenine and guanine nucleosides. Notably, some of these 6-modified purine nucleosides were not substrates for adenosine deaminase, a property that could result in enhanced *in vivo* stability. We studied **2a** in animals and found it to have low oral bioavailability and poor CNS penetration,[3] attributes undesirable for an anti-HIV candidate drug. Applying our growing knowledge of the existence of unappreciated anabolic pathways available to purine nucleosides, we extensively modified the 6-position of the cyclopentenylpurines, optimizing for *in vitro* anti-HIV

potency, oral bioavailability, and CNS penetration. We and others[6,8] had independently found the cyclopentenyladenine **2b** to have *in vitro* anti-HIV activity. Unfortunately, **2b** was extensively deaminated by adenosine deaminase to the inactive hypoxanthine analog in animals, and modifications to the 6-position (as in **2c**) destroyed activity.[3] In the 2-aminopurine series, however, we found numerous 6-modifications that retained the *in vitro* potency of **2a**. The 6-cyclopropylamino-analog **1** was selected for further development after confirmation in 90-day toxicology studies (monkeys and mice) that the maximal plasma concentrations at the "no observable toxic effect" doses were ca. 100-fold above the average clinical isolate IC_{50} values with no evidence of the cardiac, renal, neurological, or hematological toxicities that are observed with other nucleoside RT inhibitors.[3] The biochemical explanation for the different *in vivo* profiles of **1** and **2a** is that **1** is converted by a novel anabolic pathway to the triphosphate of **2a** in cells in a manner that bypasses **2a**.[9] The unique and unexpected intracellular activation of **1**, involving two new enzymes, was first described in full in 1994,[10] coincidental with the start of the clinical evaluation of **1**. In the phase 2 clinical studies in 1995, **1** proved to be significantly more potent than AZT and other approved nucleosides, resulting in durable viral load decreases of a magnitude observed only with the most potent HIV protease inhibitors.[11] Throughout the course of preclinical and phase 1/2 studies, there were synthetic problems that severely limited the supply of compound. A larger scale synthesis was urgently needed – for the first time, a carbocyclic nucleoside was required on metric ton scale. We now describe the evolution of a relatively simple, scalable route to **1** which enables the synthesis of the quantities now required for the new drug, Ziagen.®

RESULTS AND DISCUSSION

Synthetic options. The *in vitro* promise of carbovir (**2a**) has encouraged considerable research on synthetic routes to 2'3'-didehydro (i.e., cyclopentenyl) carbocyclic nucleosides since 1988. As late as 1994, however, we were forced to conclude that a robust commercial route to the class did not exist. Two general approaches are coupling of the intact purine to a carbocycle and construction of the purine from the cyclopentenylamine (FIG. 2).

Considerable attention was directed to the innovative palladium-catalyzed routes.[12-15] Although convergent coupling of an intact heterocyclic base to an allylically

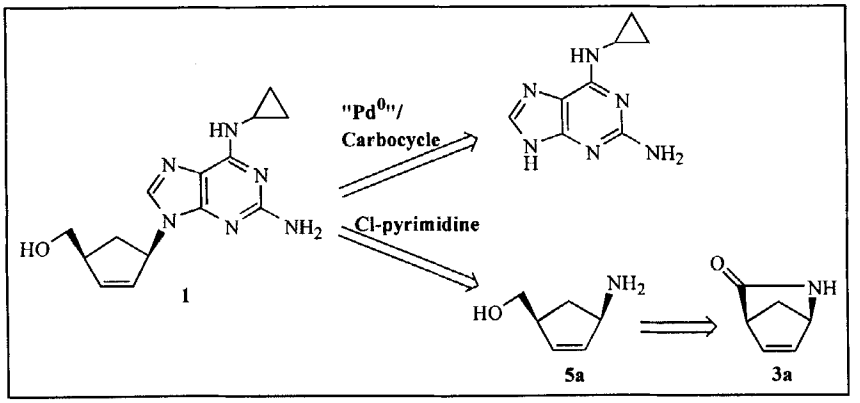
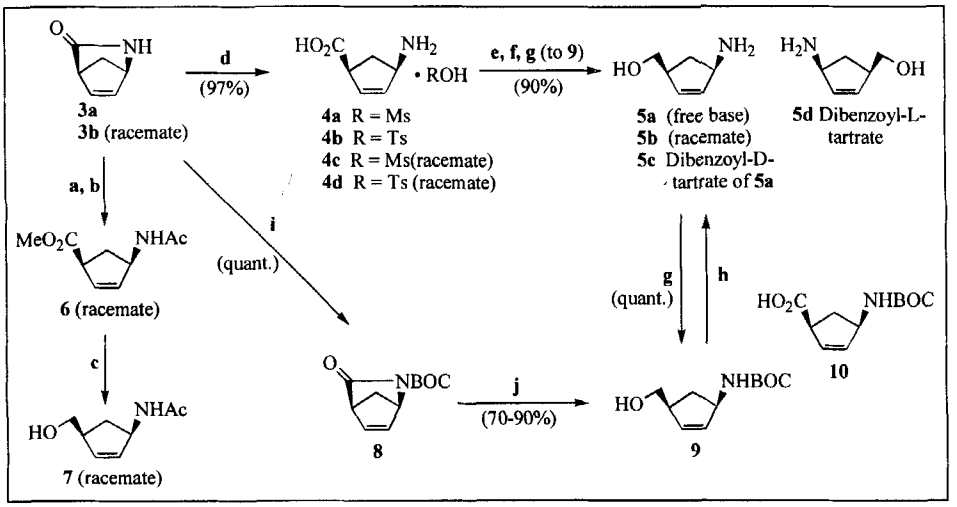


FIG. 2. Retrosynthetic analysis of routes to Ziagen®.

activated cyclopentene derivative appears more efficient than linear construction of the heterocycle from an amine substituent on the carbocycle, a number of practical issues became evident. The synthesis of 2-amino-6-cyclopropylaminopurine is not trivial on a large scale, due in part to the low solubility of 2-aminopurine precursors in suitable solvents. The lack of N9/N7 regioselectivity in the purine coupling is a major problem. The best N9/N7 ratio achieved to date using 2-amino-6-cyclopropylaminopurine is 95:5.[14] The 7-substituted isomer, in contrast to **1**, decomposes during mildly basic deblocking of the intermediate, producing the aglycone as a contaminant.[14,15] Since the aglycone, 2-amino-6-cyclopropylaminopurine, is relatively toxic,[16] no contamination by the 7-isomer was tolerated. Batch-wise chromatographic separations of N7 and N9 isomers were required to insure acceptable purity of the final drug prepared from such palladium-catalyzed couplings. In addition, production-scale synthesis of the required blocked cyclopentenol intermediates had not been developed at that time, despite notable efforts.[13] We continued to explore alternative routes to **1**.

On a research scale, the synthesis of racemic cyclopentenyl nucleosides was especially facile starting from the racemic γ-lactam, 2-azabicyclo[2.2.1]hept-5-en-3-one (**3b**). This route was used for the earliest exploratory quantities of cyclopentenyl nucleosides in this family.[8,17,18] The lactam entry to carbocyclic nucleosides would appear to be ideal in certain respects, since the resulting cyclopentenyl sugar surrogate **5a** has the appropriate relative stereochemistry and unsaturation at the start.



Reagents and conditions: a) HCl, MeOH; b) Ac_2O, pyridine; c) $NaBH_4$, $CaCl_2$ or $NaBH_4$, MeOH (3 equiv), cat. AcOH, THF; d) MsOH or TsOH hydrate, H_2O (1-1.5 equiv), THF, 60 °C, 3 h; e) $LiAlH_4$, THF; f) NaF, 30 min, then H_2O; g) $(BOC)_2O$; h) conc. HCl (1.2 equiv), EtOH, reflux 1.5 h; i) $(BOC)_2O$, cat. DMAP, THF; j) $NaBH_4$ in THF-H_2O or in THF with MeOH (3 equiv) and cat. AcOH.

FIG. 3. Amino alcohol intermediates to cyclopentenyl nucleosides.

Cyclopentenylamine synthesis. Racemic lactam **3b** (FIG. 3) is efficiently prepared from a Diels-Alder reaction between a sulfonyl cyanide and cyclopentadiene.[19] This intermediate has been utilized for the synthesis of carbocyclic nucleosides for over 20 years, since the first reports of its conversion to amine-substituted carbocycles[20] that were elaborated to purine analogs by known methods.[1, 21, 22] The practical enzymatic resolution of lactam **3b**[23] provided enantiopure **3a** on a kilogram scale from an early point in our studies. Significant improvements in the Diels-Alder reaction suggested that industrial production of the precursor racemate **3b** would be feasible.[24] However, despite the encouragement provided by the potential availability of this enantiopure starting material, the practical utilization we describe was not immediately realized. A number of problems were encountered because of the unstable nature of the intermediates, especially in the linear construction of the purine from amine **5a**. Our discoveries related to utilization of the lactam to generate pure **5a** and, finally, construction of an acceptable pyrimidine intermediate with which it could be coupled came much later.

An efficient conversion of the lactam to amino alcohol **3b** was the highest priority initially. Evaluation of the 4-step literature conversion of **3b** to amide **7**[20] identified several problems. The clean reduction of ester **6** is critical to the final purity of drug substance. During the reduction step, any deprotonation α- to the ester group leads to isomeric and/or saturated byproducts (FIG. 4). Reduction of **6** with calcium borohydride (i.e. sodium borohydride and calcium chloride),[20] proved capricious on a large scale, with the reductions often stalling.[25] Difficult-to-separate impurities resulted in variable contamination of **1** by **11** and **12**. A sample of epimer **11**, which proved to be non-cytotoxic and inactive against HIV-1, was separated and characterized as the racemate from the epimeric cyclopentene precursor generated in a calcium borohydride reduction of **6**. The cytotoxicity of the saturated analog **12**,[26] synthesized in enantiopure form by hydrogenation of **1**, made the presence of this impurity particularly undesirable. This led to the observation that addition of three equivalents of methanol and catalytic acetic acid along with the sodium borohydride resulted in remarkable increases in the reduction rate, obviating the need for the supposedly more reactive calcium borohydride, without affecting the purity of the amino alcohol generated. It seems likely that the methanol added gives *in situ* formation of alkoxyborohydride species,[27] known to be stronger, more THF-soluble reducing agents.[28]

We chose instead to open the lactam to the amino acid and then reduce the amino acid with lithium aluminum hydride, thereby avoiding α-deprotonation and saving steps by eliminating the blocking and deblocking of the amino group. The lactam, racemic or enantiopure, was hydrolyzed efficiently to amino acid salts (**4a-4d**) in the presence of water and one equivalent of a strong acid, preferably a sulfonic acid. The resulting highly crystalline salts may be washed with THF as required, depending on the presence of impurities in the lactam. Amino alcohol **5** proved to be tightly chelated by aluminum salts, insoluble in anhydrous solvents such as THF, and relatively unstable in light and air when concentrated from solution. The isolation problem was addressed by adding the thermodynamically strongest ligand for aluminum that would be practical, i.e. fluoride, for which there is some literature precedent.[29] The reductions were quenched with water, and then sodium fluoride was added (6 equivalents per Al). The resulting sodium aluminum fluoride solids proved very insoluble and extractable by polar solvent

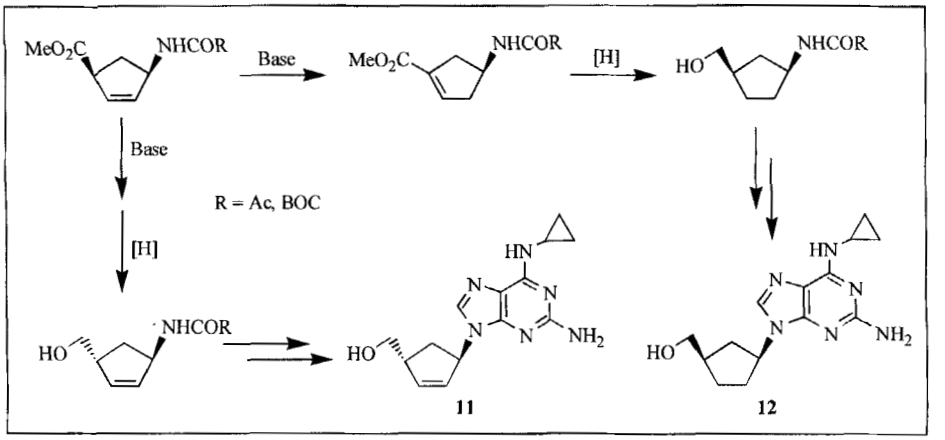


FIG. 4. Consequences of impurities generated in ester reductions.

systems such as aqueous or methanolic THF. THF with a small amount of water (5-10%) was an especially effective solvent. Using this "one-pot" sequence, the lactam was converted cleanly to the amino alcohol routinely on a kilogram scale.[30]

These conversions were also carried out starting with the more readily available racemic lactam **3b**. Enzymatic differentiation of the monophosphates[31] provided the first small enantiopure samples of **1** and its enantiomer **1b** for antiviral testing. As with carbovir, only the (–)-enantiomer had *in vitro* anti-HIV activity.[3] In order to assign the absolute configuration of the active (–)-enantiomer, the racemic amino alcohol **5b** was resolved efficiently by fractional crystallization of the diasteromeric dibenzoyl-D- and dibenzoyl-L-tartrates. The absolute configuration of the dibenzoyl-D-tartrate **5c** was determined by X-ray crystallographic analysis (FIG. 5) to be the same as that of D-ribosyl nucleosides.[32] Conversion of **5c** to **1** provided our first proof of absolute configuration. The enantiomeric dibenzoyl-L-tartrate **5e** was similarly converted to the HIV-inactive enantiomer **1b**.[3] This work was greatly facilitated by the development of a quantitative, enantiodifferentiating HPLC analysis for the cyclopentenylamines.[33] The enantiopurity of **5c** by HPLC was 99.9 %, comparable to that of **5a** later generated from **3a**.

Although salts of **5a** were stable crystalline solids, the free base was an air- and light-sensitive oil which darkened and polymerized during prolonged concentration of

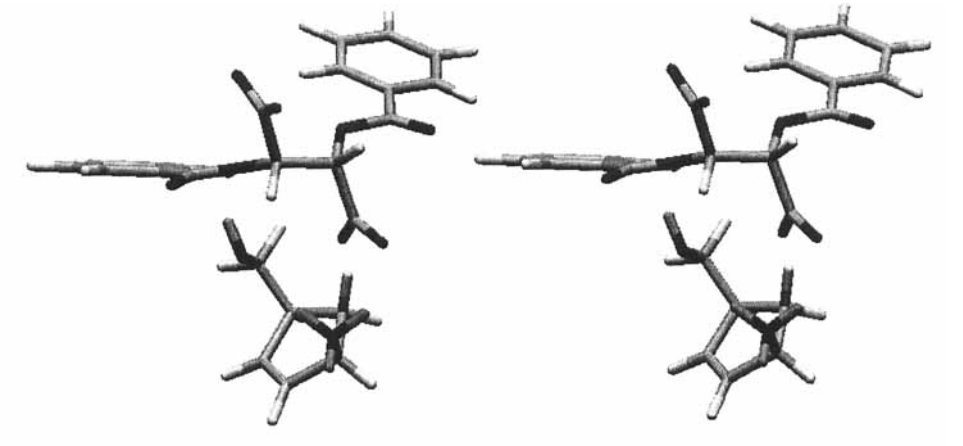


FIG. 5. Stereoview of X-ray structure of dibenzoyl-D-tartrate **5c**.

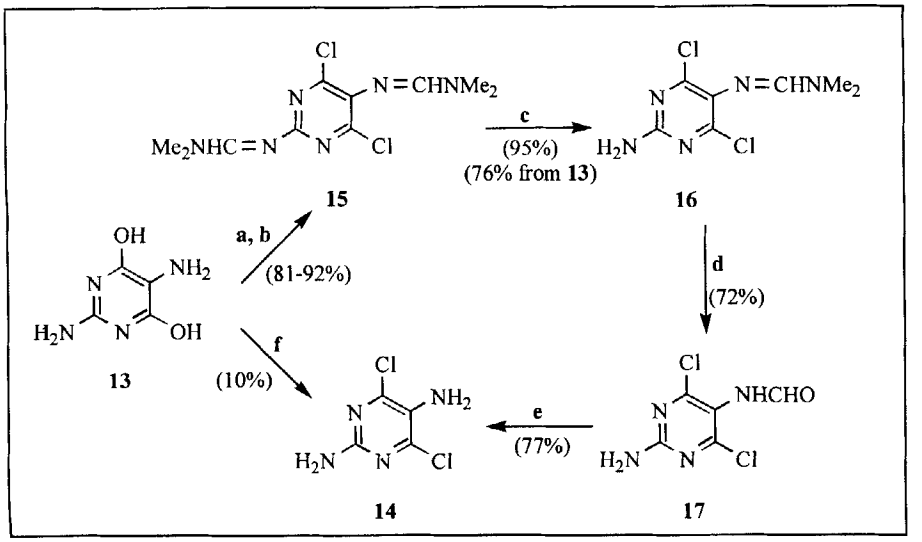
solutions. The BOC derivative was conveniently prepared in high yield by addition of di-*tert*-butyl dicarbonate after the addition of sodium fluoride in the LAH reduction workup.[30] The BOC amino alcohol **9** was a stable solid from which the amino alcohol was regenerated *in situ* using mild conditions, a slight excess of hydrochloric acid in ethanol, which is a suitable solvent for the direct condensation with an appropriate chloropyrimidine.

Although the most direct route to pure amino alcohol **5a** was *via* the hydrolysis / LAH reduction of **3a**, modification of the Katagiri sodium borohydride reduction of acyl derivatives of **3b**[34,35] provided another efficient option for the synthesis of the protected amino alcohol **9**. Quantitative derivitization of lactam **3a** with di-*tert*-butyl dicarbonate in the presence of catalytic DMAP provided THF solutions of BOC lactam **8** that were used directly in the reduction. We found **8** was stable at 25 °C in aqueous THF, although slow hydrolysis to **10** occurred on addition of one equivalent of sodium hydroxide. Addition of THF solutions of **8** to sodium borohydride in a minimum of water gave a 94% yield of high-purity **9** on a 100-gram scale. However, further scale-up resulted in slow, incomplete reductions and accompanying mechanical difficulties that were potentially hazardous. Most noteworthy was the tendency for sodium borohydride to precipitate from the THF/water mixture during the addition. As with

borohydride reductions of ester **6**, the addition of three equivalents of methanol with catalytic acetic acid allowed such reductions to be run efficiently in THF on a multi-kilogram scale. Indeed, the conversion of **3a** to **5a** was most efficiently performed using this procedure.

Chloropyrimidine intermediate. With potentially scalable procedures to generate enantiopure amino alcohol, we continued to search for an acceptable conversion to **1**. The original synthesis of carbocyclic 2-aminopurine nucleosides by Shealy and Clayton[22] coupled 2-amino-4,6-dichloropyrimidine with cyclopentylamines. Nitrogen functionality was then added at the 5-position of the resulting pyrimidine intermediates by a phenyldiazo insertion. Although a well-utilized research route, the potential hazards of large-scale diazotizations, combined with the properties of the triaminopyrimidine intermediate (**19** in FIG. 7) generated by zinc reduction of the 5-diazopyrimidine (it is sensitive to air and light, chelates metals, and requires chromatographic purification), considerably discouraged the use of this lactam-based entry to 2-aminopurine cyclopentenyl nucleosides.

Claimed improvements utilize 2,5-diamino-4,6-dichloropyrimidine (**14**)[36] or its bis-formamide.[37] The inefficient chlorination of readily accessible 2,5-diamino-4,6-dihydroxypyrimidine (**13**) to **14** (FIG. 6) with phosphoryl chloride in the presence of quaternary ammonium chlorides has been reported.[36,38] Such chlorinations were confirmed to result in extensive degradation of the starting pyrimidine to copious tars that coated equipment. Chromatographic purification was required to isolate **14** in ~10% yield on a several gram scale. Surprisingly, milder methods of chlorination of **13** do not appear to have been investigated. Novel dichloropyrimidine **15** was formed in high yield when **13** was refluxed in methylene chloride or chloroform with 6-8 equivalents of (chloromethylene)dimethylammonium chloride ("Vilsmeier reagent") – notably, with no tar formation. Commercially available Vilsmeier reagent effected both protection and solubilization of **13**, allowing efficient chlorination under mild conditions. Bis-(dimethylamino)methylene adduct **15** could be crystallized and stored as a stable white solid. Alternatively, **15** was conveniently converted to the solid mono-(dimethylamino)methylene adduct **16** (76% from **13**) by mild acidic hydrolysis of **15**, followed by neutralization and precipitation. The structure of **16** was assigned from the NMR coupling between the methylene CH and C5 and by subsequent reactions.



Reagents and conditions: a) $[Me_2NCHCl^+]Cl^-$ (6 equiv), $CHCl_3$, reflux 2 d; b) 5 N NaOH to pH 7; c) conc. HCl (2.6 equiv), EtOH-H_2O, 55 °C, 30 min; d) pH 3.2 phosphate buffer, reflux 4 h; e) HCl (0.2 equiv), EtOH-H_2O, reflux 6 h; f) $POCl_3$, PCl_5, CH_3CN, benzyltriethylammonium chloride.

FIG. 6. Chloropyrimidine intermediates to 2-aminopurines.

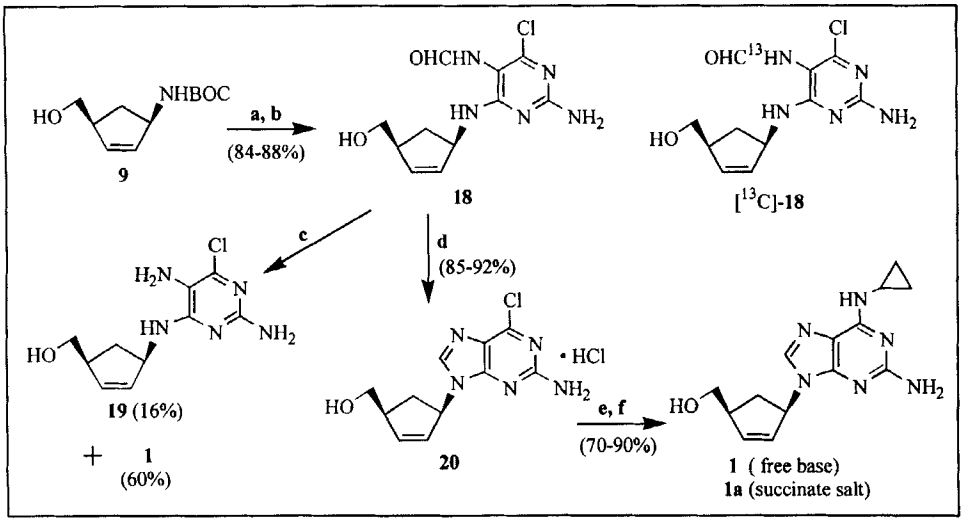
Attempts were made to utilize **16** without removal of the remaining (dimethylamino)methylene group. However, condensation of **16** with amino alcohol **5a** with or without base (K_2CO_3 or triethylamine) in refluxing n-butanol or DMF at 100 °C resulted in complex mixtures containing adducts of N,N-dimethylamine, amongst which were the 6-dimethylaminopurine analog of **1**, and several dimethylaminopyrimidines. Reasoning that N-methylaniline is not sufficiently nucleophilic to react with chloropurines and pyrimidines under these conditions, Vilsmeier chlorination of **13** was also carried out with the reagent prepared from N-methylformanilide. Unfortunately, the products from such chlorinations were dark oils, even as the HCl salts, and purification attempts resulted in decomposition. Since crystallinity is a highly desirable attribute for large-scale work, we searched for a way to utilize **16**.

Initial attempts to remove (dimethylamino)methylene groups from **15** or **16** in aqueous acid at pH 1 resulted in conversion to the oxopyrimidines. We searched for a pH that would result in selective hydrolysis of the (dimethylamino)methylene groups.

An upper limit to the pH range was set by the formation of N,N-dimethylamine pyrimidine adducts when the pH was allowed to rise above ~4. Buffering was desirable, since the dimethylamine generated raises the pH as hydrolysis progresses. An efficient conversion of **16** to **17** was achieved at pH 3 in a high ionic strength aqueous phosphate buffer chosen to limit the solubility of reactant and product. This solid-to-solid conversion resulted in average yields of about 70% of **17**, isolated in pure form by simple filtration. In large-scale preparations, the conversion of **13** to **17** is carried out efficiently without isolation of **15** or **16**. The pH is adjusted downward in steps to give **17** in overall yields of about 70% from **13**.

Although the production of **14** was of lesser importance for the synthesis of **1**, we found that efficient hydrolysis of **17** to **14** occurred if the pH of a slurry in aqueous phosphate buffer was dropped to 1-2 by the addition of sulfuric acid. Thus formamide **17** was hydrolyzed productively to **14** at pH 1, but **15** and **16** gave largely oxopyrimidines at this pH. This is understandable because the basic (dimethylamino)methylene groups are protonated in the pH range required for their efficient hydrolysis (pK_a values of **15** are 5.5 and 3.6; **16** has a pK_a of 6.0), and so inductively activate the dichloropyrimidine ring to attack by water. After their removal, the chloropyrimidines **14** and **17** are not as sensitive to acidic hydrolysis since their pK_a values for ring protonation are low (≤ 0.5). Thus, a yield of **14** (77%), much-improved over literature reports, was achieved by refluxing **17** with catalytic hydrochloric acid in aqueous ethanol.

Coupling and purine formation. Given the simple nature of the reagents and workups involved (no chromatography) and the purity and stability of the solid intermediates generated, we felt it likely that the Vilsmeier chlorination chemistry and subsequent hydrolysis to provide **17** would prove to be scalable, and such is the case. Formamide **17** appeared to us to be the most attractive intermediate for the synthesis of **1** and related 9-substituted 2-aminopurines. Condensation of **17** with amino alcohol **5a**, which may be generated *in situ* from **9** in refluxing ethanolic HCl, provided **18** in >85% yields after crystallization (FIG. 7). The formation of **18** occurs under milder conditions than are required for the formation of triamopyrimidines (such as **19**) from **14**, due to the activation provided by formylation of the 5-amino group. Reaction between **5a** and **17** went to completion in 5-7 hours in refluxing ethanol, while several days in refluxing



Reagents and conditions: a) conc. HCl (1.2 equiv), EtOH, reflux 1.5 h; b) **17**, Et_3N, reflux 5-7 h; c) cyclopropylamine, n-BuOH, reflux 21 h; d) $CH(OEt)_3$, conc. HCl (4 equiv); e) cyclopropylamine, ethanol, reflux 3 h; f) salt formation.

FIG. 7. Synthesis of Ziagen® (**1**).

butanol gave incomplete reaction (~70%) and decomposition in condensations of **5a** and **14**. The 5-formamido intermediates such as **18** are crystalline solids that are stable to air and light, in contrast to triaminopyrimidines such as **19**. We have found **17** to be a generally useful reagent for the synthesis of other 2-aminopurines.[39]

Cyclization of **18** to chloropurine **20** occurred smoothly in triethylorthoformate with 4 equivalents of concentrated aqueous hydrochloric acid. Formamide **18** dissolved immediately on addition of acid, and the hydrochloride of **20** began to precipitate in a few minutes. The product was filtered off after several hours in ca. 85-90% yield, with no purification required. It is interesting that **18** could not be cyclized in triethylorthoformate with anhydrous acids, e.g. ethanesulfonic acid, concentrated sulfuric acid, or HCl (g). The rapid formation of **20** suggested that the conformation of the formamide of **18** may be unfavorable for cyclization and that the addition of water could disrupt internal H-bonds and facilitate cyclization. In order to better elucidate the cyclization mechanism by establishing the origin of the C-8 carbon of **18**, we synthesized **17** from Vilsmeier reagent prepared from [^{13}C]-DMF, converted it to [^{13}C]-

18, and cyclized this in triethylorthoformate / aqueous hydrochloric acid. The sample of **20** generated was not enriched in ^{13}C. Surprisingly, the C-8 of the purine originates entirely from triethylorthoformate, after hydrolysis of the formyl group from **18**.

Chloropurine **20** was utilized to prepare **1** (among a variety of other novel analogs).[40] Excess cyclopropylamine in refluxing ethanol provided **1** in excellent yield, after crystallization as a suitable salt. The free base of **1** is a glass or solid foam that traps solvents and thus a variety of salts were examined. The 1:1 succinate salt was used for early clinical work. Ziagen® (abacavir sulfate) is the 2:1 salt with sulfuric acid. This route was also used to prepare the HIV-inactive enantiomer **1b** from **5d**.

An alternative base-catalyzed cyclization of **18** to **1** was achieved in 60% yield in refluxing 1-butanol using cyclopropylamine as the base (FIG. 7). Due to partial hydrolysis to triamine **19** (16%), such base-catalyzed reactions required chromatographic purification, and thus were not useful for large-scale preparations. Since this conversion retains the formyl carbon at C-8, it has been useful for the synthesis of 8-[^{14}C]-**1** from [^{14}C]-DMF.

We have described early small-scale experiments and some of the first unoptimized larger scale (100 g to 1 kg) runs that indicated that the various steps we describe would be scalable and produce pure intermediates without chromatography or other operations that would be problematic in the manufacture of Ziagen®.

EXPERIMENTAL SECTION

General. NMR spectra were run in d_6-DMSO unless otherwise noted on a Varian Unity 400 or Varian XVR-300 spectrometer (^1H NMR, 300 or 400 MHz; ^{13}C NMR, 100 MHz). Chemical shift values are reported in parts per million. Elemental analyses were determined by Atlantic Microlabs, Atlanta, GA. Compounds analyzed for fractional amounts of solvent showed the appropriate solvent impurity signals in the ^1H NMR spectra. UV spectra were recorded on a Perkin-Elmer 751 spectrophotometer. Mass spectra were obtained from Oneida Research Services using a Finnigan 4500 TFQ mass spectrometer. FAB^+ mass spectra were obtained on a VG70SQ mass spectrometer (VG Ltd., Manchester, England) using a cesium ion source and a glycerol/hydrogen chloride matrix. Accurate mass measurements were performed on a Micromass LCT mass

spectrometer (Micromass Ltd., Manchester, UK) operating at 5000 resolution (measured at 50% peak height). Mass calculations were performed using Masslynx software. X-ray crystallography was carried out by Molecular Structure Corporation, The Woodlands, TX. Optical rotations were measured on a Perkin-Elmer-241 polarimeter in methanol, unless otherwise noted. Melting points were taken on a Mel-Temp capillary apparatus. Preparative flash column chromatography used silica gel 60 (40-63 μm, E. M. Science 9385-9). TLC was performed with silica gel 60A (250 μm) MKGF (Whatman) plates.

***(1S,cis)*-4-Amino-2-cyclopentene-1-carboxylic acid methanesulfonate (4a).** A solution of (–)-2-azabicyclo[2.2.1]hept-5-ene-3-one (**3a**) (97.45 g, 0.8929 mol, Chiroscience Ltd., Cambridge, England) in THF (500 mL) was filtered and warmed to 35 °C. A solution of methanesulfonic acid (63.7 mL, 0.982 mol) in water (24.1 mL, 1.34 mol) was added over 1.5 h such that the ensuing exotherm did not raise the temperature above 45 °C. The resulting slurry was heated at 60 °C for 3 h, then allowed to cool to room temperature over 15 h. The slurry was filtered and the cake washed twice with anhydrous THF (200 mL). An analytical sample of the wet cake was removed and dried to give **4a** as a white solid (1.264 g): m.p. 167-169 °C; $[\alpha]^{20}_{D} = -83.8$ (*c* 1.42); ^{1}H-NMR δ 12.6 (br s, 1H), 8.04 (br s, 3H), 6.10 (dt, 1H, J = 5.6, 2.0, 2.0 Hz), 5.85 (dt, 1H, J = 5.3, 2.3, 2.3 Hz), 4.19 (br s, 1H), 3.61 (m, 1H), 2.53 (quintet, J = 5.3 Hz overlapping DMSO peak), 2.39 (s, 3H), 1.93 (dt, 1H, J = 6.7, 6.7, 13.7 Hz); MS (CI, CH_4) *m/z* 128 $[M+1]^+$; MS (EI) *m/z* (rel intensity) 127 (11), 82 (100), 67 (14). Anal. Calcd for $C_7H_{13}NO_5S$: C, 37.66, H, 5.87; N, 6.27; S, 14.36. Found: C, 37.65; H, 5.88; N, 6.30; S, 14.44. The remaining wet cake was used directly in the preparation of **5a**.

***(1S,cis)*-4-Amino-2-cyclopentene-1-carboxylic acid 4-toluenesulfonate (4b).** Enantiopure salt **4b** was prepared from **3a** as for **4a**: white crystals (251 g, 97%); m.p. 166-168 °C; $[\alpha]^{20}_{D} = -53.1$ (*c* 0.54). Anal. Calcd for $C_{13}H_{17}NO_5S$: C, 52.16; H, 5.72; N, 4.68; S, 10.71. Found: C, 52.22; H, 5.72; N, 4.65; S, 10.70.

(±)-cis-4-Amino-2-cyclopentene-1-carboxylic acid methanesulfonate (4c). Racemic salt **4c** was prepared from **3b** as for **4a**: white crystals (10.27 g, 45.99 mmol, 98%); m.p. 137-139 °C. Anal. Calcd for $C_7H_{13}NO_5S$: C, 37.66; H, 5.87; N, 6.27; S, 14.36. Found: C, 37.60; H, 5.85; N, 6.25; S, 14.30.

(±)-cis-4-Amino-2-cyclopentene-1-carboxylic acid 4-toluenesulfonate (4d). Racemic salt **4d** was prepared from **3b** (48.66 g, 0.4459 mol) as for the enantiomer. The **4d** was used directly in the preparation of **5b** described below; an analytical sample of **4d** was prepared by drying a portion of the solid under vacuum; m.p. 191-193 °C; ^{1}H-NMR δ 12.62 (br s, 1H), 7.93 (br s, 3H), 7.47 and 7.11 (dd, 2H each, J = 8.0, 8.0 Hz), 6.11 (dt, 1H, J = 5.7, 1.9, 1.9 Hz), 5.82 (dt, 1H, J = 5.7, 2.8, 2.8 Hz), 4.20 (br m, 1H), 3.61 (br tt, 1H), 2.29 (s, 3H), 2.50 (dt overlapping DMSO peak, J = 5.8, 5.8, 11.5 Hz), 1.92 (dt, 1H, J = 6.7, 6.7, 13.4 Hz). Anal. Calcd for $C_{13}H_{17}NO_5S$: C, 52.16; H, 5.72; N, 4.68; S, 10.71. Found: C, 52.16; H, 5.76; N, 4.66; S, 10.62.

***(1S,cis)*-4-Amino-2-cyclopentene-1-methanol (5a).** The THF-wet cake of methanesulfonate **4a** was suspended in dry THF (400 mL) and transferred via cannula to a rapidly stirring solution of lithium aluminum hydride in THF (1.0 M, 1.6 L, 1.6 mol, Aldrich) cooled in an ice/acetone bath. The rate of transfer was limited to control the rate of gas evolution and to keep the temperature between 0 ° and 10 °C (total time of addition 1.5 h). The resulting mixture was warmed to reflux over the course of two hours, then refluxed for 16 h. Approximately 1.6 L of solvent was removed by distillation, the resulting slurry was cooled in an ice-acetone bath, then treated with diethyl ether (dry, 1L) and NaF (403.3 g, 9.605 mol, Aldrich). Water (86 mL, 4.8 mol) was added slowly at such a rate (3 h) that the temperature was kept below 5 °C and the hydrogen evolution was moderated. The resulting slurry was filtered and the cake washed with THF (200 mL), then 7% water-THF (500 mL). Quantitative HPLC analysis (see below) of the filtrate showed it to contain 60.04 g of the title compound. The cake was reslurried in 7% water-THF (1L) for a half hour, filtered, and washed with 7% water-THF (400 mL), then 10% water-THF (300 mL). Quantitative HPLC analysis

of the filtrate showed it to contain 26.70 g of the title compound. The cake was reslurried in MeOH (1L) for 16 h, filtered, and washed with MeOH (500 mL). Quantitative HPLC analysis showed it to contain 4.09 g of the title compound. The total yield of **5a** was 90.83 g (90.5% from **4a**, corrected for the analytical sample of **4a** removed).

Dilute solutions of **5a** remained colorless when stored in the dark under nitrogen. Portions of **5a** were further characterized after evaporation of the water-THF. Initially colorless residual oils darkened rapidly, even when stored in the dark under inert atmosphere at 30 °C. Such samples were generally used immediately or stored as stable salts or derivatives. Black syrups with the odor of ammonia resulted after storage of **5a** or **5b** at 25 °C in the light. A freshly prepared sample of **5a** was a colorless oil: $[\alpha]^{20}_{D}$ = –54.9 (*c* 0.24, EtOH); ^{1}H-NMR δ 5.67 (s, 2H), 3.75 (m, 1H), 3.33 (d, 2H, J = 6.05 Hz), 2.63 (m, 1H), 2.21 (dt, 1H, J = 8.0, 8.0, 13.0 Hz), 1.04 (dt, 1H, J = 5.6, 5.6, 13.0 Hz). Anal. Calcd for $C_6H_{11}NO{\cdot}0.50\ H_2O$: C, 58.99, H, 9.90; N, 11.47. Found: C, 59.17; H, 9.74; N, 11.51. Such oils (**5a** or racemate **5b**) isolated from LAH reductions of the corresponding amino acids were identical by elemental analysis (fit for hemihydrate) and ^{1}H-NMR to samples prepared from salts **5c** or **5d** by treatment of a solution in 95% EtOH with an excess of Amberlite IRA-400 (hydroxide form) anion exchange resin.

(±)-*cis*-4-Amino-2-cyclopentene-1-methanol (5b). The slurry of **4d** in THF was reduced with LAH as in the preparation of **5a**; combined filtrates were concentrated to a pale yellow oil under vacuum (0.397 mol, 89% by HPLC analysis as described below). By the same method but on about twice the scale, 97.40 g (0.8924mol) of **3b** yielded extracts containing **5b** (0.793 mol, 89%).

Analysis of amine intermediates: *(1S,cis)*-4-amino-2-cyclopentene-1-methanol (5a) and racemate (5b). Samples of amino alcohols and their salts were analyzed by an adaptation of the method of Brückner, et al.[33] for the determination of the enantiopurity of amino acids. Amines were derivatized using o-phthaldialdehyde and N-acetyl-L-cysteine and the resulting UV-absorbing diastereomers analyzed by HPLC. The

chromatographic separation of the derivatives used an Optima II ODS 100 x 4.5 mm, 3 μm column (III Supplies Co., Meriden, CT) and gradient elution at 0.9 mL/min using initially 100% sodium acetate buffer, 40 mM, pH 6.5, with a linear ramp to 18% acetonitrile over 15 min and a subsequent hold at 18% acetonitrile for 15 minutes. Detection was at 338 nm. Samples were dissolved in 0.1 M borate buffer, pH 10.4. The identity and purity of the samples were established by comparison with derivatives prepared from dibenzoyl tartrate salts **5c** and **5e**, which had retention times of about 21 and 22 minutes, respectively.

***(1S,cis)*-4-Amino-2-cyclopentene-1-methanol dibenzoyl-D-tartrate (5c).** To a solution of **5b** (2.26 g, 20.0 mmol) and dibenzoyl-D-tartaric acid (3.62 g, 10.0 mmol) in refluxing absolute EtOH (35 mL) was added refluxing acetonitrile (ca. 150 mL). The resulting slightly cloudy solution cooled to room temperature over 2 h. The precipitated white needles were recrystallized from EtOH-acetonitrile three times, providing **5c** as white plates (1.07 g, 37%); 99.99 % enantiopurity by HPLC analysis, as described above; m.p. 160-162 °C; $[\alpha]^{20}{}_{D}$ = +66.9 (*c* 0.28); Anal. Calcd for $C_6H_{11}NO{\cdot}0.50$ ($C_{18}H_{14}O_8$): C, 61.63; H, 6.21; N, 4.79. Found: C, 61.56; H, 6.24; N, 4.74. Further crystallization of **5c** gave large, colorless needles which were used for X-ray crystallographic analysis. The salt crystallized in the space group C2 with one $C_6H_{11}NO$ cation and one-half $C_{18}H_{14}O_8$ dianion as the asymmetric unit. The absolute configuration of **5c** was established from the known absolute configuration of the dibenzoyl-D-tartrate dianion.

***(1R,cis)*-4-Amino-2-cyclopentene-1-methanol dibenzoyl-L-tartrate (5d).** Starting with racemate **5b** and dibenzoyl-L-tartaric acid, **5d** was precipitated and recrystallized as for **5c** to give white plates (1.00 g, 34%); 99.98 % enantiopurity by HPLC assay; m.p. 160-162 °C; $[\alpha]^{20}{}_{D}$ = –68.2 (*c* 0.24); Anal. Calcd for $C_6H_{11}NO{\cdot}0.50\ C_{18}H_{14}O_8$: C, 61.63; H, 6.21; N, 4.79. Found: C, 61.59; H, 6.21; N, 4.76.

***(1R,cis)-tert*-Butyl N-[4-(hydroxymethyl)-2-cyclopenten-1-yl] carbamate (9) from 5a.** The combined THF extracts containing **5a** from the above LAH reduction were

cooled in an ice-acetone bath and treated with di-*tert*-butyl dicarbonate (199.42 g, 0.9265 mol, Aldrich). The mixture was allowed to stir and warm to room temperature over 18 h, during which time gas evolved and a clear solution formed. The resulting solution was evaporated under vacuum to an oil. The oil was partitioned between EtOAc (300 mL) and phosphate buffer (100 mL of 1.5 M potassium dihydrogen phosphate adjusted to pH 7.0 with 50% sodium hydroxide-water). The phases were separated, the aqueous phase was re-extracted twice with EtOAc (200 mL). The organic phases were filtered through silica gel (50 g.). Evaporation gave an oil (220.78 g) which solidified from hexanes-EtOAc to colorless crystals of **9** (156.1 g, 83%):[30] TLC (10% MeOH-$CHCl_3$, iodine visualization) $R_f = 0.51$; m.p. 73-73.7 °C; $[\alpha]^{20}_D = -2.78$ (*c* 5.07); ^{1}H-NMR δ 6.72 (d, 1H, J = 7.9 Hz), 5.80 and 5.60 (two m, 2H), 4.59 (t, 1H, J = 5.2 Hz), 4.45 (m, 1H), 3.35 (m, overlapping H_2O), 2.60 (m, 1H), 2.30 (m, 1H), 1.40 (s, 9H), 1.2 (m, 1H); MS (CI, CH_4) 214 $[M+1]^+$. Anal. Calcd for $C_{11}H_{19}O_3N$: C, 61.95; H, 8.98, N, 6.57. Found: C, 61.87; H, 8.96; N, 6.59. An additional 10.14 g of crystalline **9** was recovered from the mother liquor, bringing the total yield to 166.24 g (88% from **3a**).

When starting with **3a** of high purity, filtration and washing of **4a** was not necessary. In addition, it was found convenient to reverse the addition of the LAH solution to the THF slurry of **4a**. When **9** rather than **2a** was desired, it was also found efficient to add di-*tert*-butyl dicarbonate before extraction from the aluminum salts. A solution of **3a** (6.00 g, 55.0 mmol) in anhydrous THF (30 mL) was warmed to 34°C and stirred while methanesulfonic acid (3.6 mL, 55 mmol) in water (0.99 mL, 55 mmol) was added over 10 min. An exotherm of 10 °C was observed within 5 min and a crystalline solid began to precipitate. The mixture was refluxed (oil bath at 74 °C) for 2.5 h, cooled to -10 °C, and a solution of LAH (1.0 M in THF, 100 mL) added. The first 15 mL was added over 10 min and an exotherm of 7 °C noted. The remaining 85 mL was added rapidly with no further exotherm noted. The mixture was brought to reflux over 30 min and reflux continued for 18 h. The mixture was cooled to 25 °C and NaF (25.2 g, 0.600 mole) was added. After stirring for 30 minutes, water (5.3 mL) was added over 10 min to the cooled (ice bath) mixture. The mixture was stirred for 30 min at 25 °C and di-*tert*-butyl dicarbonate (12.6 mL, 55.0 mmol) was then added. This mixture was stirred

for 16 h, filtered, and the cake triturated with ethyl acetate (2 x 50 mL). The combined filtrate-wash was washed with water (20 mL) and evaporated. The residual syrup crystallized from EtOAc-hexanes to colorless crystals of **9** (10.32 g, 88%), identical in properties to the above-described sample.

***(1R,4S)-tert*-Butyl 3-oxo-2-azabicyclo[2.2.1]hept-5-ene 2-carboxylate (8).** To a solution of di-*tert*-butyl dicarbonate (550 g, 2.52 mol) and **3a** (250 g, 2.29 mol) in THF (1.5 L) was added a catalytic amount of 4-dimethylaminopyridine (2.8 g, 0.02 mol). The solution was stirred at 25-30 °C until the evolution of carbon dioxide had ceased (ca. 2 h). Such THF solutions of **8** were generally used directly in the reduction to **9** (see below). Isolation of **8** was possible by evaporation of THF and crystallization of the residual oil from hexanes to give a near-quantitative yield of colorless crystals, m.p. 85-86 °C [lit. m.p. 84-86 °C (EtOAc-hexanes)[35]; 55-57 °C (pentane)[41]]; ^{1}H-NMR ($CDCl_3$) identical to lit.[41] Anal. Calcd for $C_{11}H_{15}NO_3$: C, 63.14; H, 7.23; N, 6.69. Found: C, 63.23; H, 7.20; N, 6.73.

Stability of 8: Solutions of **8** were stable in 10% aqueous THF, showing no evidence of hydrolysis after 21 h at 25 °C. Addition of 1 equivalent of 1N sodium hydroxide resulted in conversion over 2 h to acid **10**, isolated as the sodium salt after evaporation and lyophilization from water; hygroscopic white powder; m.p. 185-186 °C; $[\alpha]^{20}_D = -64.3$ (*c* 0.32); NMR δ 7.18 (d, 1H, J = 8.6 Hz), 5.88 (m, 1H), 5.62 (m, 1H), 4.35-4.43 (m, 1H), 3.05 (m, 1H), 1.90-2.04 (m, 1H), 1.60-1.80 (m, 1H), 1.37 (s, 9H). Anal. Calcd for $C_{11}H_{18}NO_4Na{\cdot}0.5\ H_2O$: C, 51.16; H, 6.63; N, 5.42. Found: C, 51.03; H, 6.46; N, 5.42.

***(1R,cis)-tert*-Butyl N-[(4-hydroxymethyl)-2-cyclopenten-1-yl] carbamate (9) from 8.**

(a) In aqueous THF: A solution of **8** (95.50 g, 0.456 mol) in THF (500 mL)-water (50 mL) was added over 10 minutes to a well-stirred, cooled (<30 °C) slurry of sodium borohydride (Aldrich, 99%, 21.96 g, 0.580 mol) in water (100 mL). TLC (silica gel, 2:1 hexane-EtOAc, potassium permanganate visualization) showed complete conversion after 2 h. The pH was adjusted, with cooling, to ~3 by addition of

concentrated HCl (ca. 50 mL). The aqueous mixture was diluted with toluene (750 mL), and the two phases were separated. The cloudy aqueous layer was extracted with 1:1 toluene-THF (2 x 300 mL). The combined organic layers were washed with a solution of 9:1 saturated sodium chloride-saturated sodium bicarbonate, dried (sodium sulfate), and concentrated under vacuum to a colorless oil. Evaporation of hexanes (200 mL) left a crystalline solid, which was dried to provide **9** (91.3 g, 94%); m.p. 72-74 °C, ^{1}H-NMR identical with that of the above sample. Anal. Calcd for $C_{11}H_{19}NO_3$: C, 61.95; H, 8.98; N, 6.57. Found: C, 61.66; H, 8.91; N, 6.52.

(b) In THF with methanol. To a solution of **8** (2.29 mol) in THF (1.5L) was added methanol (440 g, 13.75 mol) and a catalytic amount of glacial acetic acid (3.6 g, 0.06 mol). This solution of **8** was added to a slurry of sodium borohydride (174 g, 4.58 mol) in THF (1.75 L) under controlled conditions maintaining the temperature between 20 and 25 °C (approximately 1.5 h). The reaction was quenched by the addition of toluene (2.3 L) and the controlled addition of 2N hydrochloric acid (2.3 L, 4.6 mol), maintaining the temperature below 30 °C. After phase separation, the aqueous layer was extracted with toluene (1.15 L), and the combined organic phases were washed with water (2.3 L), filtered and concentrated to about ¼ the original volume. Hexane was added to crystallize **9** (379 g, 77.5 %); m.p. 72-74 °C; ^{1}H-NMR identical with that of the above sample. Anal. Calcd for $C_{11}H_{19}NO_3$: C, 61.95; H, 8.98; N, 6.57. Found: C, 61.87; H, 8.92; N, 6.57.

2,5-Bis-{[(dimethylamino)methylene]amino}-4,6-dichloropyrimidine (15). 2,5-Diamino-4,6-dihydroxypyrimidine hemisulfate (**13**) (25.0 g, 0.131 mole) was stirred in $CHCl_3$ (400 mL) while a slurry of solid (chloromethylene)dimethylammonium chloride (88.0 g, 0.651 mole as 95%, Aldrich) in $CHCl_3$ (400 mL) was added. The reaction mixture was brought cautiously to reflux with nitrogen sweeping the HCl evolved into a trap. When the evolution of HCl slowed after about 1 h, the sweep was stopped and the reaction kept under a gentle positive pressure of nitrogen. After 24 h of gentle reflux, TLC (silica gel, 5% MeOH-CHCl3) showed a mixture of **15** and the lower R_f monochloro intermediate. Additional (chloromethylene)dimethylammonium chloride (50.0 g, 0.370 mole) was added and reflux continued for an additional 20 h. The

mixture was cooled (ice bath) and diluted with sufficient ice water to dissolve solids (ca. 300 mL); 5N NaOH was added slowly with vigorous stirring until the aqueous layer was neutral. The $CHCl_3$ layer was washed with water (3 x 400 mL), dried (Na_2SO_4), and concentrated to a yellow solid (36 g). A solution of the solid in EtOAc (~300 mL) was washed through a silica gel pad to give, after evaporation of solvent, pure **15** as an off-white solid (30.75 g, 81%). Such a sample was recrystallized from EtOAc to white granules of **15**; m.p. 123-125 °C; ^{1}H-NMR δ 8.49 and 7.69 (both s, each 1H), 3.16 (s, 3H), 3.03 (s, 6H), 2.97 (s, 3H); MS (CI, CH_4) 291, 289[M+1]$^+$; UV (pH 7 phosphate buffer) λ_{max} 296 nM (ε33,300); pK_a (spectrophotometric, 25 °C) 5.50 and 3.57. Anal. Calcd for $C_{10}H_{14}N_6Cl_2$: C, 41.54; H, 4.88: N, 29.06; Cl, 24.52. Found: C, 41.59; H, 4.91; N, 29.01; Cl, 24.47.

2-Amino-5-{[(dimethylamino)methylene]amino}-4,6-dichloropyrimidine (16). 2,5-Diamino-4,6-dihydroxypyrimidine hemisulfate (**13**) (48.0 g, 0.251 mole) was reacted as above. One portion of (chloromethylene)dimethylammonium chloride (170 g, 1.25 mole as 95%) was added initially, followed by additional 50.0 g portions added at 24 and 48 h during a total reflux period of 72 h. Crude **15** was isolated as above (66.7 g, 92%) and dissolved in 95% EtOH (900 mL) and 6 N HCl (100 mL). The solution was heated to 55 °C over 0.5 h and copious precipitate formed. The mixture was cooled (ice bath) and the pH adjusted to 8 with concd NH_4OH. The resulting mixture was evaporated to tan solid. The solid was slurried in cold water (100 mL), filtered, washed with cold water (3 x 30 mL), then cold MeOH (2 x 20 mL), and dried to give **16** as white powder (44.21 g, 76% from **13**); m.p. > 250 °C ; ^{1}H-NMR δ 7.59 (s, 1H), 6.90 (br s, 2H), 3.00 and 2.94 (both s, 3H each). The regiochemistry of the hydrolysis was confirmed using 2D-HMBC NMR. The spectrum shows clear correlations between the NH_2 and the guanidine carbon as well as clear correlation between the methylene proton and the C-5 carbon of the pyrimidine ring. MS (CI, CH_4) 236, 234 [M+1]$^+$; UV (pH 7 phosphate buffer) λ_{max} 328 nM (ε 4500), 255 (ε 15,800); pK_a (spectrophotometric, 25 °C) 6.02 and <0.5. Anal. Calcd for $C_7H_9N_5Cl_2$: C, 35.92; H, 3.88: N, 29.92; Cl, 30.29. Found: C, 35.66; H, 3.86; N, 29.74; Cl, 30.54.

When **15** was isolated and purified as above (5.87 g, 20.3 mmol) and then subjected to the same hydrolysis conditions, filtration and washing with water gave **16** (4.50 g, 95%) of comparable purity.

2-Amino-4,6-dichloro-5-formamidopyrimidine (17). A slurry of **16** (25.0 g, 0.107 mol) in 1.5 M aqueous potassium phosphate buffer (300 mL, prepared by adjusting the pH of a 1.5 M solution of KH_2PO_4 to 3.2 by addition of 85% phosphoric acid) was gently refluxed (oil bath 130 °C) for 4 h. The pH was maintained at ~3.2 by addition of 85% phosphoric acid, as needed, throughout this period. Lower pH resulted in over-hydrolysis to **14**. Precipitated **17** was filtered, washed with water (3 x 10 mL) and MeOH (2 x 10 mL), and dried to off-white powder (16.0 g, 72%); m.p. >250 °C; ^{1}H-NMR shows major and minor amide rotamers: δ 9.81 and 9.46 (s, 0.8H, and d, J = 11 Hz, 0.2H), 8.25 and 8.00 (s, 0.8H, and d, J = 11 Hz, 0.2H), 7.69 and 7.63 (overlapping br s, 0.4H and 1.6H); MS (CI,CH_4) 209, 207 $[M+1]^+$; pK_a (spectrophotometric, 25 °C) <0.5. Anal. Calcd for $C_5H_4N_4OCl_2$: C, 29.01; H, 1.95; N, 27.06; Cl, 34.25. Found: C, 29.17; H, 2.00; N, 27.01; Cl, 34.33.

2,5-Diamino-4,6-dichloropyrimidine (14). A mixture of **17** (7.50 g, 36.2 mmol), 0.1 N hydrochloric acid (75 mL), and EtOH (75 mL) was refluxed 6 h. Solvents were evaporated and the residual solid partitioned between water (300 mL) adjusted to pH 8 with $NaHCO_3$ and EtOAc (3 x 100 mL). The combined EtOAc layers were dried (Na_2SO_4), diluted with hexanes (300 mL), and washed through a silica gel pad to remove pink color. Evaporation of solvent left **14** as white powder (5.00 g, 77%); recrystallization of such a sample from 95% EtOH gave **14** as white needles; m.p.185-187 °C dec [lit. m.p. 198 °C (EtOH)[37]; 188-191 °C[42]]; ^{1}H-NMR δ 6.50 (br s, 2H), 4.73 (br s, 2H); MS (CI, CH_4) 181, 179 $[M+1]^+$; pK_a (spectrophotometric, 25 °C) 0.496. Anal. Calcd for $C_4H_4N_4Cl_2$: C, 26.83; H, 2.25; N, 31.30; Cl, 39.61. Found: C, 26.95; H, 2.24; N, 31.19; Cl, 39.53.

***(1S,cis)*-4-[(2-Amino-6-chloro-5-formamido-4-pyrimidinyl)amino]-2-cyclopentene-1-methanol (18) from 5a.** A mixture of **17** (140 g, 0.68 mole), **5a** (76.5 g, 0.68 mol),

and triethylamine (207 mL) in ethanol (1.4 L) was refluxed for 7 h. After cooling to room temperature, 1 N NaOH (700 mL) was added with stirring. The mixture was cooled to ~5 °C and filtered. The filtrate was concentrated and then portions of ethanol (2 x 700 mL) were added and evaporated. Water (700 mL) was added and the solution was concentrated to half-volume. After stirring overnight, the precipitated **18** was filtered and washed with cold water (700 mL). The off-white solid was dried at 40 °C to give 168.8 g (88%), m.p. 95-98 °C. Crystallization of such a sample from MeOH-EtOAc gave **18** as white powder (83%); m.p. 170-171 °C; $[\alpha]^{20}{}_{D}$ = +21.2 (*c* 0.50); ^{1}H-NMR δ 8.99 and 8.58 (s and d, J = 11.1 Hz, total 1H), 8.11 and 7.80 (s and d, J = 11.1 Hz, total 1H), 6.77 and 6.61 (two d, J = 8.0, 8.0 Hz) overlapping 6.60 and 6.48 (two br s, total 3H), 5.85 (m, 1H), 5.70 (m, 1H), 5.00-5.15 (m, 1H), 4.71 (t, J = 5.1 Hz, 1H), 3.30-3.45 (m overlapping H_2O), 2.72 (m, 1H), 2.45-2.25 (m, 1H), 1.37 (m, 1H); MS (CI, CH_4) 286, 284 $[M+1]^+$, 190, 188 $[B+2H]^+$; pK_a (spectrophotometric, 25 °C) 2.63. Anal. Calcd for $C_{11}H_{14}N_5O_2Cl$: C, 46.57; H, 4.97; N, 24.69; Cl, 12.50. Found: C, 46.63; H, 4.99; N, 24.58; Cl, 12.59.

***(1S,cis)*-4-[(2-Amino-6-chloro-5-formamido-4-pyrimidinyl)amino]-2-cyclopentene-1-methanol (18) from 9.** To a solution of **9** (16.0 g, 75 mmol) in EtOH (38 mL) was added concentrated hydrochloric acid (8.8 mL). The solution was refluxed for 1.5 h, cooled, and added to a mixture of **17** (15.52 g, 75 mmol), triethylamine (45.8 mL, 0.32 mole), and EtOH (38 mL). After reflux for 6 h, 5N sodium hydroxide (36 mL) was added to the cooled reaction mixture. Evaporation left brown glass which was dissolved in hot isopropanol. The solution was filtered through a pad of silica gel / Celite / charcoal and concentrated to 100 mL. Product precipitated as white powder (18.8 g, 84%) on concentration of the isopropanol solution; m.p. 169-170 °C; ^{1}H-NMR identical with that of the above sample of **18**. Chromatography of the mother liquor contents (silica gel, elution with 10% MeOH-$CHCl_3$) gave a higher R_f contaminant which was 6-chloropurine **20** (0.83 g, 4%).

[Formyl-^{13}C]-(*1S,cis*)-4-[(2-amino-6-chloro-5-formamido-4-pyrimidinyl)amino]-2-cyclopentene-1-methanol ([^{13}C]-18). A solution of [carbonyl-^{13}C]-N,N-

dimethylformamide (99 atom %, Aldrich, 4.00 g, 54.0 mmol) and oxalyl chloride (4.66 mL, 54.0 mmol) in diethyl ether (150 mL) was stirred at 0 °C for 25 minutes. The precipitated [^{13}C]-(chloromethylene)dimethylammonium chloride was filtered, washed with diethyl ether (2x50 mL), and dissolved in anhydrous choroform (15 mL). This solution of [^{13}C]-Vilsmeier reagent was used to prepare **16** (400 mg, 25%) as above; ^{1}H-NMR δ 7.54 (d, $^1J_{C,H}$ = 177.2 Hz, 1H), 6.86 (s, 2H, NH_2), 2.95 (d, $^3J_{C,H}$ = 4.1 Hz, 3H), 2.89 (d, $^3J_{C,H}$ = 2.4 Hz, 3H). Hydrolysis of this sample of **16** as above gave **17** (215 mg, 61%); ^{1}H-NMR shows major and minor amide rotamers: δ 9.78 and 9.42 (s, 0.8H, and br s, 0.2H), 8.21 and 7.96 (d, $^1J_{C,H}$ =198.6Hz, 0.8H, and br s, J = 196.8 Hz, 0.2H), 7.67 and 7.61 (overlapping br s, 0.4H and 1.6H). This sample of **17** was reacted with **5a** as above to give [^{13}C]-**18**, isolated as a white solid (185 mg, 65%), after elution from a silica gel column with 5% methanol-ethyl acetate and crystallization from methanol-ethyl acetate; ^{1}H-NMR differs from that of **18** for the 5-HN^{13}CHO couplings; δ 9.03 and 8.62 (d, d, $^2J_{C,H}$ = 2.9, 11.4 Hz, total 1H, NH), 8.15 and 7.82 (d, $^1J_{C,H}$ = 195.8 Hz and dd, $^1J_{C,H}$ = 150 Hz, $^3J_{H,NH}$ = 11.3 Hz, total 1H, CHO); HRMS consistent with greater than 95% ^{13}C-enrichment: obsd *m/z* 307.0771; calcd for $^{12}C_{10}{}^{13}C_1H_{14}N_5O_2{}^{35}ClNa$ *m/z*, 307.0767 $[M+Na]^+$; *m/z* 306 peak (all ^{12}C formula) was less than 2% the intensity of the 307 peak.

***(1S,cis)*-4-(2-Amino-6-chloro-9-H-purin-9-yl)-2-cyclopentene-1-methanol Hydrochloride (20).** A slurry of **18** (1.00 g, 3.50 mmol) and triethylorthoformate (18 mL) was stirred while concentrated hydrochloric acid (37%, 1.25 mL, 15 mequiv) was added in one portion. A clear, colorless solution resulted within one minute. After 4 h, the precipitate was collected and washed with t-butyl methyl ether (15 mL) to give the hydrochloride salt of **20** as a white powder (975 mg, 92%), m.p. >250 °C; $[\alpha]^{20}{}_D$ = –116 (*c* 0.27); ^{1}H-NMR δ 8.18 (s, 1H), 6.90 (br s, 4H), 6.18 (m, 1H), 5.93 (m, 1H), 5.48 (m, 1H), 3.47 (d, J = 5.7 Hz, 2H), 2.90 (m, 1H), 2.63 (m, 1H), 1.67 (m, 1H); MS (CI, CH_4) *m/z* 268, 266 $[M+1]^+$, 172, 170 $[B+2H]^+$. Anal. Calcd. for $C_{11}H_{12}N_5OCl{\cdot}HCl$: C, 43.73; H, 4.34; N, 23.18; Cl, 23.47. Found: C, 43.62; H, 4.34; N, 23.07; Cl, 23.53.

On a larger scale starting with 80 g (0.28 mol) of **18** in triethylorthoformate (1.2 L), concentrated hydrochloric acid (96 mL, 1.2 mol) was added with cooling to maintain

the temperature below 25 °C and stirred for 18 h. The hydrochloride of **20** was filtered, washed with *tert*-butyl methyl ether (125 mL) and dried to give 74 g (85 % of theory). The crude hydrochloride of **20** was generally used directly to prepare **1**.

In the same manner, starting with [^{13}C]-**18** (100 mg, 0.35 mmol), triethylorthoformate (1.8 mL), and concentrated hydrochloric acid (37%, 0.125 mL), a sample of the hydrochloride of **20** was isolated as white powder (74 mg, 70%); ^{1}H-NMR spectrum identical to above spectrum of un-enriched sample, i.e. no couplings to ^{13}C observed; HRMS consistent with an abundance of greater than 95% of the expected natural abundance of carbon 12: obsd *m/z* 288.0627; calcd for $^{12}C_{11}H_{12}N_5O^{35}ClNa$ *m/z*, 288.0628 $[M+Na]^+$; *m/z* 289 peak was 17% the intensity of the 288 peak (14% expected for natural abundance of carbon 13 (accuracy of peak height measurements estimated to be ± 5%).

***(1S,cis)*-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol (1) from 20.** A solution of **20** (3.19 g, 12.0 mmol) and cyclopropylamine (10 mL) in methanol (50 mL) was stirred at 70 °C in a glass-lined Parr bomb for 12 h. After cooling, 1 N sodium hydroxide (12 mL) was added and volatiles evaporated. Chromatography on a silica gel column eluted with 5% methanol-chloroform gave **1** as a white solid foam (3.09 g, 90%); trituration of such a sample with wet acetonitrile gave an amorphous white solid which did not melt sharply (shrinks at 87 °C, fluid by 160 °C); $[\alpha]^{20}_D$ = –59.7 (*c* 0.15); pK_a (spectrophotometric, 25 °C) 5.06, 0.41; UV $\lambda_{max}(\varepsilon)$:(pH 7.0, phosphate buffer) 284 (15500), 259 (8960); ^{1}H-NMR δ 7.60 (s, 1H), 7.26 (d, J = 4.1 Hz, 1H), 6.10, 5.87 (m, m, 2H), 5.82 (s, 2H), 5.40 (m, 1H), 4.75 (t, J = 5.2 Hz, 1H), 3.45 (m, 2H), 3.01 (m, 1H), 2.85 (m, 1H), 2.60 and 1.58 (m, m, 2H), 0.65, 0.59 (m, m, 4H); MS (CI/CH_4) *m/z* 287 $[M+1]^+$, 191 $[B+2H]^+$. Anal. Calcd. for $C_{14}H_{18}N_6O{\cdot}0.75\ H_2O$: C, 56.08; H, 6.55; N, 28.03. Found: C, 56.05; H, 6.65; N, 27.88.

On a larger scale, a solution of **20** (1061 g, 3.51 mol) and cyclopropylamine (1180 g, 20.67 mol) in absolute ethanol (8.0 L) was refluxed for 3 h. The solution was cooled to 30 °C and sodium hydroxide solution (265g, 6.63 mol in 550 mL of water) was added. Precipitated sodium chloride was filtered. The filtrate was concentrated *in vacuo*, diluted with acetone (5.5 L), and chilled to 10 °C. Precipitated **1** was filtered and

dried. The assayed weight of the product (wt/wt assay by HPLC and UV) was 703 g (69.9 % of theory). This material was used directly to form the succinate salt **1a**.

***(1S,cis)*-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol Succinate (1a).** A solution of succinic acid (12.15 g, 102 mmol) in ethanol (140 mL) was added to a solution of the free base of **1** (30.93 g, 102 mmol) in ethanol (140 mL). The ethanol was evaporated and the residual solid crystallized from water (500 mL) to give the succinate salt of **1** as a white solid (30.7 g, 73%), m.p. 168-169 °C; $[\alpha]^{20}_{D} = -33.4$ (*c* 0.52); ^{1}H-NMR δ 12.2 (br s, 2H, 2 CO_2H), 7.61 (s, 1H, purine H-8), 7.30 (s, 1H, NH), 6.08 (ddd, 1H, H-2), 5.86 (s, 2H, NH_2), 5.85 (ddd, 1H, H-3), 5.38 (m, 1H, H-4), 3.43 (d, J = 5.9 Hz, 2H, CH_2O), 3.01 (m, 1H, cyclopropyl CH), 2.85 (dd, 1H, H-1), 2.44 (s, 4H, 2 succinate CH_2), 1.57 and 2.58 (ddd, ddd, 2H, CH_2), 0.56, 0.63 (m, m, 4H, 2 cyclopropyl CH_2); an HMQC spectrum confirms proton assignments and permits assignment of protonated carbons, as well as identifying protons attached to heteroatoms; HMBC correlations from purine H-8 assigns unprotonated purine carbons C-4 and C-5; purine C-6 and C-5 correlate with the NH; cyclopentene H-4 correlates to both purine C-8 and purine C-4, confirming that the cyclopentene ring is attached at N-9; selective irradiation of the CH_2O protons at 3.43 gave a slight enhancement of purine H-8 in the NOE difference spectrum, confirming the cis substitution of the cyclopentene ring; ^{13}C-NMR δ 173.8 (succinate C=O), 160.1 (purine 2), 155.9 (purine 6), 150.9 (purine 4), 138.1 (C-2), 135.0 (purine 8), 130.0 (C-3), 113.5 (purine 5), 64.1 (CH_2OH), 58.2 (C-4), 47.7 (C-1), 34.3 (C-5), 28.9 (succinate CH_2), 23.7 (cyclopropyl CH), 6.5 (cyclopropyl CH_2); (MS (EI) *m/z*: 286 $[M]^+$, 271 $[M\text{-}CH_3]^+$, 256 $[M\text{-}HCHO]^+$, 189$[B]^+$. Anal. Calcd. for $C_{14}H_{18}N_6O{\cdot}C_4H_6O_4{\cdot}0.5\ H_2O$: C, 52.29; H, 6.09; N, 20.33. Found: C, 52.27; H, 6.10; N, 20.30. A second crop (6.20 g) of comparable purity was collected on concentration of the mother liquor, bringing the yield to 88%.

***(1S,cis)*-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol (1) and (1S, 4R)-4-[(2,5-diamino-6-chloro-4-pyrimidinyl)amino]-2-cyclopentene-1-methanol (19) from 18.** A solution of **18** (250 mg, 0.883 mmole) was

refluxed gently (oil bath 130 °C) in dry n-butanol (5 mL) under nitrogen with cyclopropylamine (0.30 mL, 4.4 mmol) for 16 h. A second portion of cyclopropylamine (0.15 mL) was added and reflux continued for another 5 h. Volatiles were evaporated and the residual oil dissolved in ethanol-water (1:1) with 1 N sodium hydroxide (0.5 mL). Volatiles were again evaporated and the residue chromatographed on a silica gel flash column (1x10"). Elution with 5% MeOH-EtOAc gave fractions containing **22** as a pink solid (35 mg, 16%), m.p. 159.5-161.0 °C; $[\alpha]^{20}_{D}$ = +0.37 (*c* 0.54); ^{1}H-NMR δ 6.41 (d, J = 7.6 Hz, 1H), 5.90 (m, 1H), 5.75(m, 1H), 5.62 (br s, 2H), 5.05 (m, 1H), 4.67 (t, J = 5.0 Hz, 1H), 3.96 (br s, 2H), 3.40 (m, 2H), 2.70 (m, 1H), 2.40 (m, 1H), 1.30 (m, 1H); mass spectrum (CI, CH_4): *m/z* 256, 258 $[M+1]^+$, 160, 162 $[B+2H]^+$. Anal. Calcd. for $C_{10}H_{14}N_5OCl$: C, 46.97; H, 5.52; N, 27.39. Found: C, 47.03; H, 5.54; N, 27.45. Continued elution with 10% methanol-ethyl acetate gave **1** as an off-white solid foam (160 mg, 60%); mass spectrum and ^{1}H-NMR same as above sample of free base. Anal. Calcd. for $C_{14}H_{18}N_6O \cdot 0.15\ H_2O \cdot 0.40$ MeOH: C, 57.30; H, 6.64; N, 27.84. Found: C, 57.59; H, 6.48; N, 27.70.

(±)-*(trans)*-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol Hydrochloride (11). Samples of racemic **7** resulting from reductions of **6** with calcium borohydride in THF contained variable levels of impurities from over-reduction and epimerization. After conversion to chloropurine, lower R_f impurities were separated and further purified by chromatography on silica gel. Several columns, eluting with 10% methanol-ethyl acetate and with 2% methanol-chloroform, followed by crystallizations from methanol-acetonitrile, gave a sample of (±)-*trans*-4-(2-amino-6-chloro-9-H-purin-9-yl)-2-cyclopentene-1-methanol containing a few percent of the reduced analog from ^{1}H-NMR. Such a sample (2.00g, 7.5 mmol) was reacted with cyclopropylamine as for **1** and further purified on a silica gel column eluted with 2-5% methanol-chloroform. A solid hydrochloride salt was then prepared by addition of 1N HCl, evaporation, and slurrying with ethyl acetate to give an **11** as off-white powder (1.26 g, 48%), m.p.125-145 °C with efferves.; mass spectrum (CI, CH_4): *m/z* 287 $[M+1]^+$, 191 [B+2H]; ^{1}H-NMR δ 12.4, 9.9, 7.85 (all br, total 2H), 8.23 (s, 0.1H), 4.64

(m, 0.1H), 7.97 (s, 1H), 6.22 and 5.94 (ddd, ddd, 2H), 5.49 (m, 1H), 3.44 (d, J = 6.1 Hz, overlapping H_2O), 3.16 (m, 1H), 2.95 (m, 1H), 2.20, 2.00 (m, m, 2H), 0.97, 0.81 (m, m , 4H); resonances at 8.23 and 4.64 allowed estimation of ~10% contamination by the saturated analog (HCl salt of racemate of **12** prepared separately). Anal. Calcd. for $C_{14}H_{18}N_6O \cdot 1.00$ HCl·0.85 H_2O·0.20 EtOAc: C, 49.97; H, 6.32; N, 23.62; Cl, 9.97. Found: C, 49.95; H, 6.36; N, 23.76; Cl, 9.96.

***(1R,cis)*-3-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-1-cyclopentane-1-methanol Succinate (12).** A solution of **1** (85.00 g, 0.297 mol) in absolute ethanol (700 mL) was shaken with 10 % palladium on carbon (800 mg) under hydrogen (50 psi) for 1.5 h. Filtration through Celite and concentration gave **12** as a white solid foam (84.17 g, 98 %). The succinate salt was prepared as for **1** and crystallized from water to give white crystals, m.p. 170-175 °C; $[\alpha]^{20}{}_D = -7.8$ (*c* 0.29); ^{1}H-NMR δ 12.0 (br s, 2H), 7.82 (s, 1H), 7.27 (d, J = 4.3 Hz, 1H), 5.80 (s, 2H), 4.60 (m, 2H), 3.42 (m, 2), 3.07 (m, 1H), 2.43 (s, 4H), 1.50-2.30 (m, 7H), 0.62 (m, 4H). Anal. Calcd. for $C_{14}H_{20}N_6O \cdot 1.00$ $C_4H_6O_4 \cdot 1.05$ H_2O: C, 50.83; H, 6.66; N, 19.76. Found: C, 50.83; H, 6.66; N, 19.79.

***(1R,cis)*-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol (1b).** Amino alcohol **5d** was converted to **1b** by procedures used for **1**. A sample of **1b** was characterized as the free base after final purification on silica gel. Solidification from methanol-acetonitrile gave **1b** as an amorphous white solid; $[\alpha]^{20}{}_D$ = +58.1 (*c* 0.45); ^{1}H-NMR identical with that of **1**. Anal. Calcd. for $C_{14}H_{18}N_6O \cdot 0.29$ H_2O: C, 57.67; H, 6.42; N, 28.82. Found: C, 57.66; H, 6.42; N, 28.83.

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