tracted once again with EtOAc. The solvent was removed *in vacuo*, and the oily residue was treated with ethereal HCl. After two crystallizations from EtOH–Et_2O, there was obtained 3.66 g of IVa·HCl, mp 288–289° dec, $[\alpha]^{25}D$ −63.6° (*c* 1, H_2O). Employing (*S*)-VI in an identical procedure afforded 3.8 g of IVb·HCl, mp 287–288° dec, $[\alpha]^{25}D$ +66.9° (*c* 1, H_2O). The infrared spectra of both anitpodes were identical with the optically active salts obtained by optical resolution.[24] The nmr spectrum exhibited multiplet resonances at 2 (6 H, $(CH_2)_3$), 3.5 (2 H, NCH_2), 4.4 (1 H, NCHCO), and 7.7 (*W* = 16 cps, 10 H, Ar) ppm.

The bases were generated from the purified hydrochloride salts by treatment with 1 *N* NaOH and were recrystallized from Skelly B to give crystals, mp 97–98°, $[\alpha]^{25}D$ +59.8° (IVa) and −57.9° (IVb) (*c* 2, MeOH). The high-resolution infrared spectrum of IV at 0.5 and 0.005 *M* concentrations showed bands at 3450 (O–H···N) and 3315 cm^{-1} (NH).

Acknowledgment.—The authors are grateful to Dr. H. H. Wolf of the College of Pharmacy, Ohio State University, who informed us of the results of his studies on the effect of α-methyltyrosine on CNS activity of pipradrol and ephedrine isomers.

(24) (+)-Pipradrol dibenzoyl-(+)-tartrate neutral salt monohydrate was obtained from a MeOH solution of 2 equiv of pipradrol[6] and 1 equiv of resolving acid; mp 138–142°, $[\alpha]^{25}D$ −69° (*c* 2, MeOH). (−)-Pipradrol dibenzoyl-(+)-tartrate neutral salt was crystallized from either MeOH (10–15 ml/g) or 95% EtOH (20–25 ml/g); mp 170–172°, $[\alpha]^{25}D$ −36° (*c* 2, MeOH). The two salts were converted to the optically active bases which were recrystallized from 75–90° petroleum ether to give (+)-pipradrol (IVa), mp 98–100°, $[\alpha]^{25}D$ +58.5° (*c* 2, MeOH), and (−)-pipradrol (IVb), mp 95–97°, $[\alpha]^{25}D$ −62.5° (*c* 2, MeOH). The two bases were converted to the HCl salts which were purified by recrystallization from either 95% EtOH or a mixture of MeCOEt–MeOH; IVa·HCl, mp 303–305° dec, $[\alpha]^{25}D$ −68.8°, and IVb·HCl, mp 305–307° dec, $[\alpha]^{25}D$ +68.5° (*c* 2, H_2O). The melting points of the HCl salts, determined by the customary capillary tube method, are decomposition points which are quite variable and depend upon the rate of heating. The (*R*) and (*S*) enantiomers employed in the pharmacological studies were obtained by the above procedure (private communication from E. R. Andrews and P. L. Tiernan, The Wm. S. Merrell Company, and J. L. Schaar, currently at Monsanto Research Corp., Dayton, Ohio).

Enzyme Inhibitors. XIX. The Synthesis of Some 1-Hydroxy-2-hydroxymethyl-4-(6-substituted-9-purinyl)cyclohexanes as Nucleoside Analogs[1a]

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The syntheses of some 1-hydroxy-2-hydroxymethyl-4-(6-substituted-9-purinyl)cyclohexanes were accomplished by the following procedure. Diethyl 4,4-ethylenedioxypimelate on Dieckmann cyclization gave 2-carbethoxy-4,4-ethylenedioxycyclohexanone. Catalytic hydrogenation of the ketone followed by $LiAlH_4$ reduction of the ester gave 2-hydroxymethyl-4,4-ethylenedioxycyclohexanol which after several additional reactions was separated into *trans*- and *cis*-3-acetoxymethyl-4-acetoxycyclohexanes (**5a** and **5b**). Hydrogenation of the ketone group of **5a** and **5b** gave the alcohols which were converted into tosylates. Displacement of the tosylate with azide followed by catalytic hydrogenation of the azides gave the amines. The major product from **5a** was 1α-amino-3α-hydroxymethyl-4β-hydroxycyclohexane, whereas **5b** gave nearly an equal mixture of 1α- and 1β-amino-3α-hydroxymethyl-4α-hydroxycyclohexanes. The stereochemistry of these trisubstituted cyclohexanes was deduced from nmr studies. Finally two of these amines were converted into some 1β-hydroxy-2α-hydroxymethyl-4α-(6-substituted-9-purinyl)cyclohexanes and 1α-hydroxy-2α-hydroxymethyl-4α-(6-amino-9-purinyl)-cyclohexane. These compounds were not potent inhibitors of adenosine deaminase, possibly because they are repelled by the hydrophobic region of this enzyme.

In several previous studies, it was found that certain 9-(substituted cyclopentyl)- and 9-(substituted cyclohexyl)adenines were capable of inhibiting the enzyme, adenosine deaminase. For example, if an OH group were located on the 2 position of the cyclopentyl or cyclohexyl group, it was found that this substitution increased inhibition relative to the unsubstituted cycloalkyl group.[2] However, if an OH group were located at position 3 of the cyclohexyl group or if a hydroxymethyl group were located at position 4 of the cycloalkyl group, little change in inhibition of adenosine deaminase was noted relative to 9-cyclopentyladenine.[3,4] Based on this and other data,[5] it was concluded that the 2-OH group of these inhibitors makes a contribution to binding to adenosine deaminase. In order to determine the effect on inhibition of adenosine deaminase by certain 9-cyclohexyl-6-substituted purines that contain both an OH group and a hydroxymethyl group on the cyclohexyl moiety, we decided to prepare some 1-hydroxy-2-hydroxymethyl-4-(6-substituted-9-purinyl)cyclohexanes. This paper describes the synthesis, stereochemistry and enzymatic evaluation of these compounds.

Chemistry.—Our main goal in this area was to develop a general route for the preparation of 1-hydroxy-2-hydroxymethyl-4-(6-substituted-9-purinyl)-cyclohexanes which could later be applied to each of the four possible isomers. For the sake of simplicity, no consideration will be given to the stereochemistry of the intermediates in the procedure outlined in Chart I. However, the separation of isomers and their identification will be described in the following section. Dieckmann cyclization of diethyl 4,4-ethylenedioxypimelate (1) by a modification of a previously reported

(1) (a) This investigation was supported by Grant T-337A from the American Cancer Society, by research Grant 5-R01-GM-09775-05 from the Public Health Service, by research career program award 5-K3-CA-18718-05 from the National Cancer Institute, and training Grant 5-T1-GM-555-05 from the Division of Medical Sciences, U. S. Public Health Services, Bethesda, Md. (b) National Merit Winner, Lunsford Richardson Award.

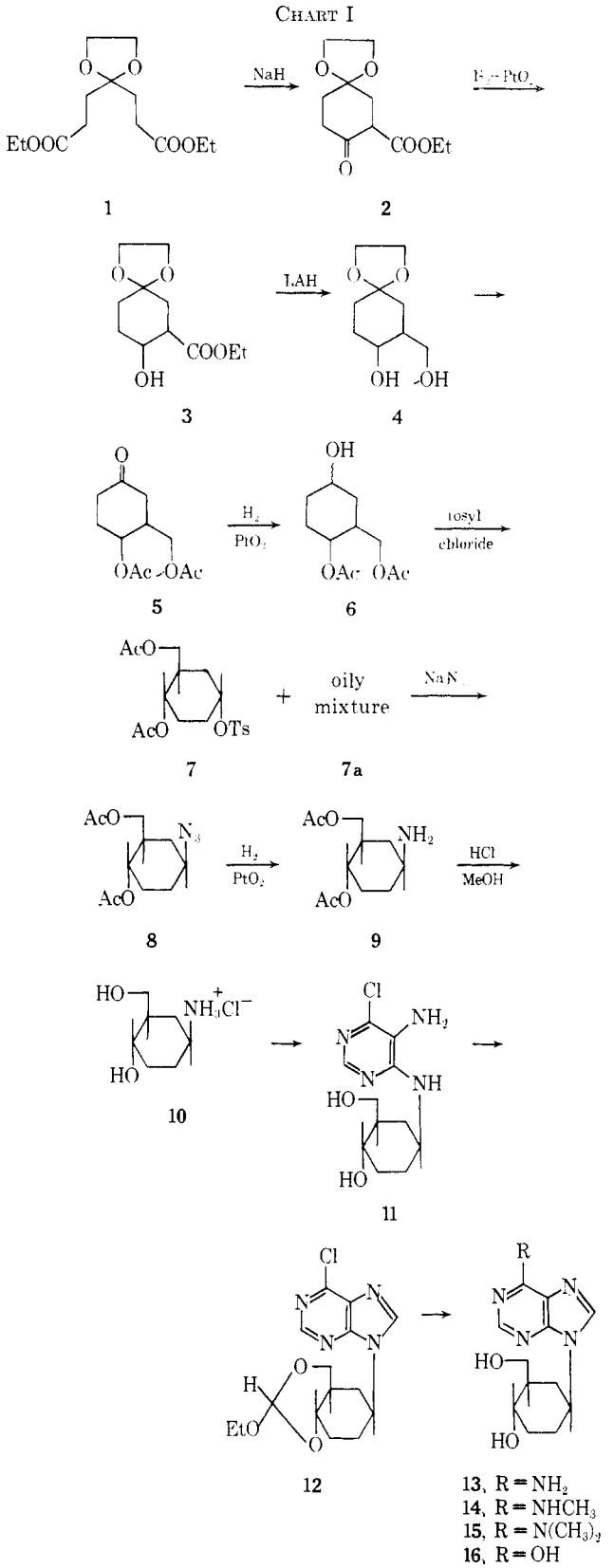
(2) H. J. Schaeffer, S. Marathe, and V. Alks, *J. Pharm. Sci.*, **53**, 1368 (1964).

(3) H. J. Schaeffer, K. K. Kaistha, and S. K. Chakraborti, *ibid.*, **53**, 1371 (1964).

(4) H. J. Schaeffer, D. D. Godse, and G. Liu, *ibid.*, **53**, 1510 (1964).

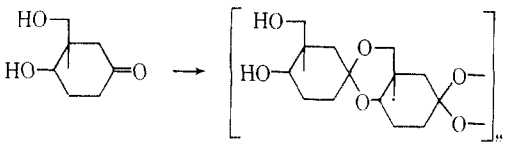
(5) H. J. Schaeffer, D. Vogel, and R. Vince, *J. Med. Chem.*, **8**, 502 (1965).

CHART I



procedure gave a good yield of 2-carbethoxy-4,4-ethylenedioxycyclohexanone (**2**).[6]

Catalytic reduction (PtO_2) of **2** gave **3** which was subsequently converted to the corresponding diol **4** with $LiAlH_4$. Attempts to isolate the corresponding ketone by acid-catalyzed removal of the ketal blocking group from **4** were unsuccessful. The infrared spectrum of aliquots from the reaction mixture exhibited a carbonyl peak at 1725 cm^{-1} and disappearance of the ketal peak at 1180 cm^{-1}; however, complete removal of the H_2O from this mixture gave a resinous product which no longer exhibited the carbonyl absorption. This resinous material could be rehydrolyzed to the ketone with dilute acid. The results indicate that the ketone diol (**17**) is formed, but polymerized to a polyketal (**18**) when the H_2O is removed from the reaction mixture. In order to overcome this difficulty, the ketal (**4**) was hydrolyzed in the presence of Dowex 50W-X8 resin.

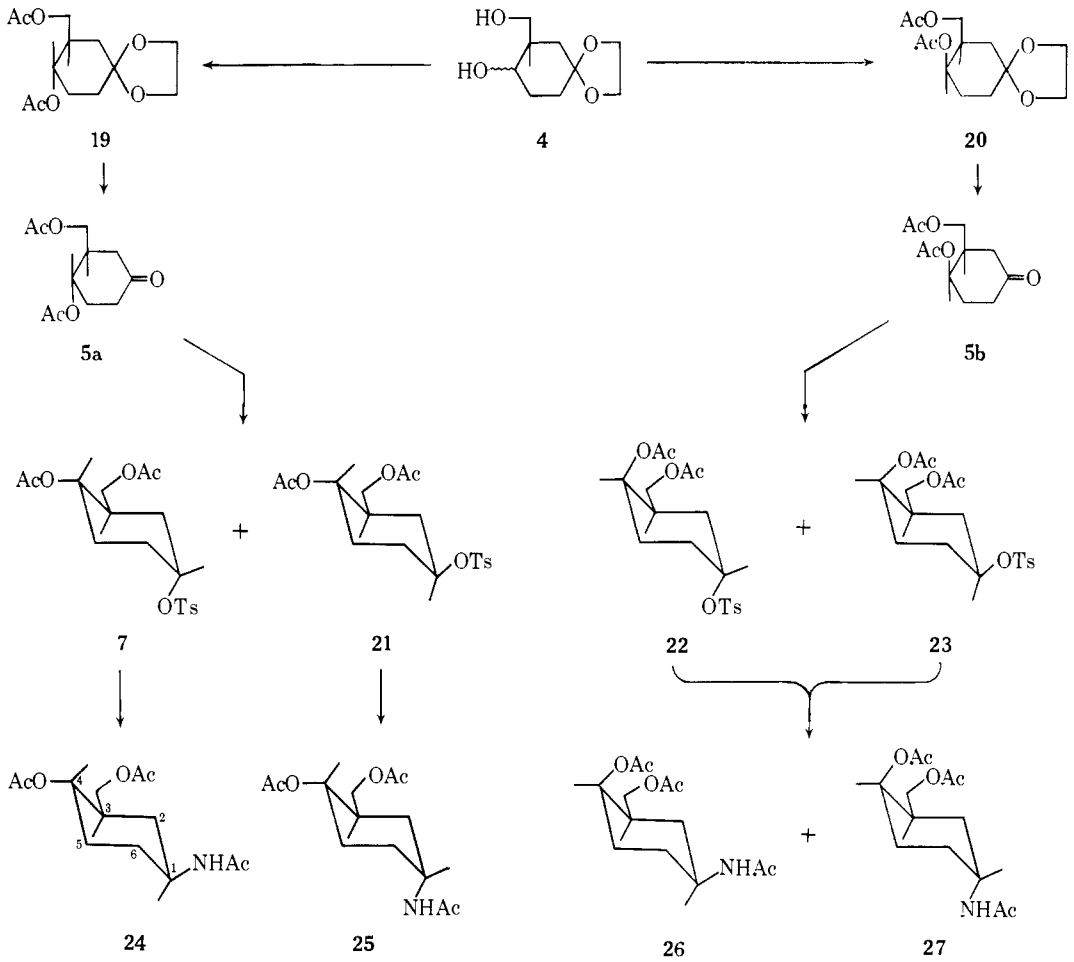


The neutral filtrate was evaporated so as to remove only about 95% of the H_2O. The resulting diol (**17**) and ethylene glycol were immediately dissolved in pyridine and fully acetylated with excess Ac_2O. The diacetate (**5**) was then removed from the ethylene glycol diacetate by distillation *in vacuo*. Preliminary attempts to convert **5** to the corresponding amine (**9**) by first converting **5** to an oxime followed by catalytic reduction were unsuccessful. Catalytic reduction of the ketone (**5**) resulted in the formation of **6** in quantitative yield. Treatment of **6** with *p*-toluenesulfonyl chloride gave two tosylate fractions: a solid fraction (27%) and an oily fraction (51%). The solid isomer (**7**) was treated with NaN_3 in DMF and gave the azide (**8**) as an oil which was not purified. Hydrogenation (PtO_2) of the azide gave a good yield of 3-acetoxymethyl-4-acetoxycyclohexylamine (**9**). The acetate esters of **9** were removed with 1% HCl in MeOH and a crystalline hydrochloride salt (**10**) was obtained. Condensation of **10** with 5-amino-4,6-dichloropyrimidine gave **11**. Cyclization of **11** with ethyl orthoformate produced a partially solidified material which exhibited ultraviolet absorption characteristic of a 6-chloropurine (265 mμ) but did not exhibit OH absorption in the infrared spectrum. This product was assigned the orthoester structure (**12**) based on the previous observation that 3-(5-amino-6-chloro-4-pyrimidinylamino)-1,2-propanediol was converted to the orthoester under similar conditions.[5] Attempts to purify **12** by recrystallization gave a mixture of formate esters. Reaction of **12** with the appropriate nucleophilic reagent followed by acid hydrolysis gave the desired 1-hydroxy-2-hydroxymethyl-4-(6-substituted-9-purinyl)-cyclohexanes (**13**-**16**).

Stereochemistry.—During the course of this work the intermediate **4** was converted to 2-hydroxymethyl-4,4-ethylenedioxycyclohexanol diacetate which partially crystallized and was separated into a solid fraction (**19**) (Chart II) and an oil (**20**). The assignment of stereochemistry of these two isomers was done by nmr spectroscopy. Several investigators found that the nmr signal of the hydrogen on the carbon atom bearing a hydroxy or acetoxy group in a cyclohexanol exhibits a half-width of 7 cps if the hydrogen is equatorial, but

(6) P. D. Gardner, L. Rand, and R. Haynes, *J. Am. Chem. Soc.*, **78**, 3425 (1956).

CHART II



exhibits a half-width of 22 cps if the hydrogen is axial,[7-9] and that the signal from the equatorial hydrogen is approximately 30 cps downfield from the axial hydrogen.[10,11] Table I lists the nmr signals for the compounds illustrated in Chart II in which H_a represents an axial hydrogen on the carbon bearing the indicated substituent and H_e represents an equatorial hydrogen of the same carbon of the cyclohexane ring. Since it is reasonable to assume that the *trans* isomer will have its substituents diequatorial, a signal at 275 cps with a half-width of 22 cps for **19** established that this compound has an axial hydrogen on the carbon bearing the acetoxy group and is therefore the *trans* isomer. In the case of **20**, the less bulky acetoxy group will occupy the axial position. Thus, the signal at 301 cps with a half-width of 7 cps for the corresponding equatorial hydrogen established that **20** is the *cis* isomer. The *trans*-diacetate (**19**) was converted to the tosylates **7** and **21** as described for the conversion of **4** to **7**. Tosylate **7**, which accounted for 48% of the mixture, was separated from the oily isomer **21** by crystallization. This solid isomer **7**, which was identical in all respects with the solid isomer **7** in Chart I, was assigned the stereochemistry indicated in Chart II by nmr analysis. Eliel has demonstrated that cyclohexyl tosylates in which the tosylate group is in the axial position exhibit a signal with a chemical shift of 273 cps for the equatorial hydrogen on the carbon bearing the tosylate group.[10] In the case where the tosylate group is purely equatorial, the corresponding axial proton exhibits a signal at 243 cps. Eliel also observed that in the acetate esters the equatorial proton absorbs at 299 cps, whereas the axial proton has a signal at 268 cps. Compound **7** exhibited a broad signal at 273 cps which integrated for two protons. This spectrum is in agreement with the

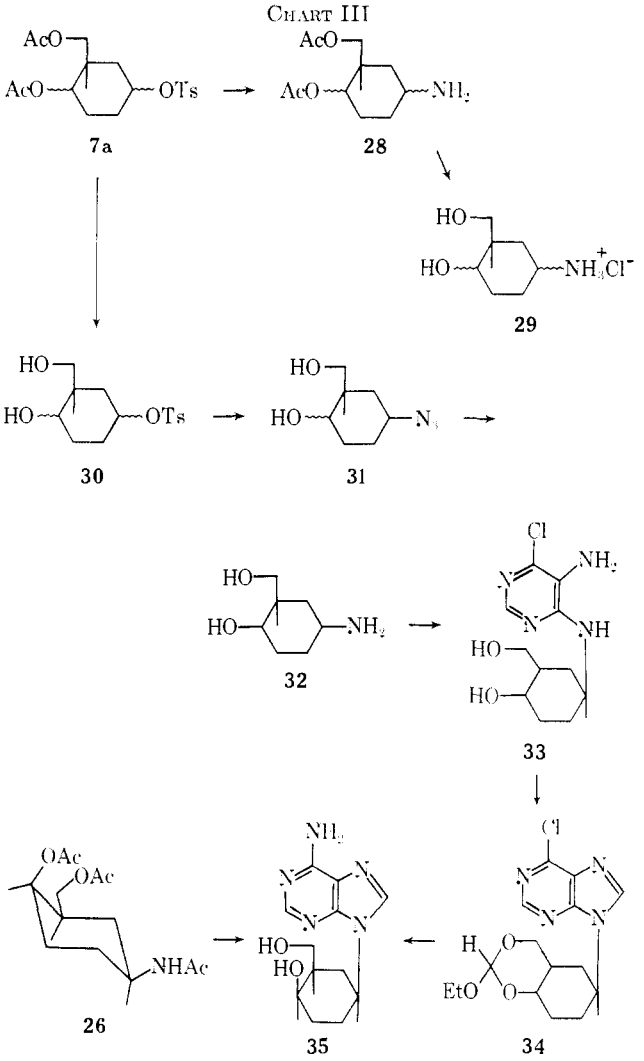
TABLE I[a]
SUMMARY OF THE NMR SPECTRUM OF SOME TRISUBSTITUTED CYCLOHEXANES

Compd	Substituent	H_a	H_e
19[b]	OAc	275	
20[b]	OAc	...	301
7[b]	OAc	273	...
	OTs	...	273
24[c]	OAc	283	...
	NHAc	246[d]	...
26[c]	OAc	...	302
	NHAc	245[d]	...

[a] Values are given in cps downfield from Me_4Si where H_a indicates an axial proton and H_e indicates an equatorial proton on the carbon of the cyclohexane ring bearing the substituent indicated. [b] Spectrum in CCl_4. [c] Spectrum in $CDCl_3$. [d] The signal for this proton coincides with the signal for the acetoxymethyl protons. The value reported is the approximate center of the total peak.

(7) R. U. Lemieux, R. K. Kulling, J. H. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958).
(8) J. I. Musher, *ibid.*, **83**, 1146 (1961).
(9) K. L. Williamson and W. S. Johnson, *ibid.*, **83**, 4623 (1961).
(10) E. L. Eliel and M. H. Gianni, *Tetrahedron Letters*, 97 (1962).
(11) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1955, p 473.

CHART III



assignment of **7** as illustrated in Chart II. Displacement of the tosylate ester with sodium azide causes an inversion at the center of reaction thus giving rise to the azide which was subsequently reduced to the amine and acetylated to give the amide **24**. The nmr spectrum of **24** is in agreement with the all-equatorial isomer. A small quantity of a second product was obtained from **5a**, and it is suggested that this material has the stereochemistry as shown in **25**. By analogy, the stereochemistry of **26** was assigned from the nmr data of Table I. Similarly, a second product was obtained from **5b**, and it is proposed that this product has the stereochemistry as shown in **27**.

These results establish that the oily tosylate ester (**7a**) from Chart I consists of a mixture of isomers **21**–**23** (Chart II). The conversion of **7a** to the amine hydrochloride **28**, as illustrated in Chart III, was carried out in the same manner as described for the corresponding isomer (**7**) on Chart I. However, when **28** was deacetylated (HCl–MeOH), the resulting hydrochloride (**29**) would not crystallize and, consequently, could not be purified. Therefore, distillable amounts of the free amine (**32**) were obtained by an alternate procedure outlined in Chart III. In this sequence, **7a** was deacetylated (HCl–MeOH) and gave the diol (**30**) which was converted to the free amine (**32**) by catalytic reduction of the crude azide (**31**). Distillation of the crude mixture gave a pure compound in a 44% yield which as shown below proved to be **32**. The pyrimidine **33** was prepared by the condensation of **32** with 5-amino-4,6-dichloropyrimidine. Cyclization of the crude pyrimidine with triethyl orthoformate produced a purine which did not exhibit OH absorption in the infrared spectrum. This product was assigned the orthoester structure **34** based on previous observations on the corresponding isomer. An analytical sample of the orthoester (**34**) was obtained by recrystallization; however, the major fraction was obtained as a mixture of formate esters. Reaction of **34** with NH_3–MeOH followed by acid hydrolysis gave the 6-amino derivative (**35**) as the main product. When the all-*cis* triacetate (**26**) was deacetylated by acid hydrolysis and converted to the purine by the same sequence of reactions employed for the conversion of **32** to the purine, the product obtained was identical in uv, ir, melting point, and tlc R_f values with **35** and the mixture melting point was not lowered. Thus, the stereochemistry of isomer **35** was assigned to an all-*cis* configuration as indicated in Chart III.

A second purine was also isolated in small amount when **7a** was carried through the series of reactions outlined in Chart III. The stereochemistry of this purine (mp 205–208°) has not been assigned due to the small amount of material isolated. However, the stereochemistry could be either that of **25** or **27**. The isolation of larger amounts of this material and its stereochemical assignment will be carried out at a later date.

Enzymic Evaluation.—When this group of compounds was tested as inhibitors of adenosine deaminase, it was found that they were either weakly inhibitory or noninhibitory at 0.12 m*M*. An evaluation of **13** and **35** revealed that both of these compounds were only weakly inhibitory of this enzyme since the $([I]/[S])_{0.5}$ for these compounds was greater than 7. Recently, it has been shown that adenosine deaminase has both a hydrophobic binding region and a region for binding an OH group if the OH group is attached to the 2′-carbon of a 9-alkyladenine.[5,12] It is possible that when the adenine moiety of **13** and **35** complex with the enzyme, the substituted cyclohexyl group is positioned in or near the hydrophobic region of adenosine deaminase. Consequently, these compounds are weak inhibitors because the hydroxyl and hydroxymethyl groups on the cyclohexyl nucleus of **13** and **35** cause repulsion of the inhibitor from the hydrophobic region of the enzyme. Currently, we are attempting to synthesize compounds that can bridge to both the hydrophobic and hydroxyl binding regions of adenosine deaminase.

Experimental Section[13]

Method A. 2-Carbethoxy-4,4-ethylenedioxycyclohexanol (3).—A mixture of 7.00 g (30.6 mmoles) of **2**, 200 ml of 95% EtOH, and 200 mg of PtO_2 was hydrogenated at room temperature at an initial pressure of 4.2 kg/cm² until the theoretical amount of H_2 had been absorbed. After filtration of the reaction mixture, the volatile materials were removed *in vacuo* and gave 7.05 g of a

(12) H. J. Schaeffer and D. Vogel, *J. Mol. Chem.*, **8**, 507 (1965).

(13) The infrared spectra were determined on a Perkin-Elmer Model 137 spectrophotometer, the ultraviolet spectra were determined on a Perkin-Elmer Model 4000A spectrophotometer, the enzyme studies were done on a Gilford Model 2000 spectrophotometer. Where analyses are indicated only by symbols of the elements, analytical results for those elements were within ±0.4% of the theoretical values. The melting points unless otherwise noted were taken in open capillary tubes on a Mel-Temp apparatus and are corrected.

TABLE II
PHYSICAL CONSTANTS AND ANALYTICAL DATA FOR SOME PURINES AND THEIR INTERMEDIATES

Compd	Recrystn solvent[a]	Mp, °C	Bp, °C (mm)	Yield, %	Formula	Analyses
3			85–86 (0.03)	78.5	$C_{11}H_{18}O_5$	C, H
4			119–120 (0.05)	62.5	$C_9H_{16}O_4$	C, H
5			123 (0.05)	82.5	$C_{11}H_{16}O_5$	C, H
6			136–139 (0.20)	93.3	$C_{11}H_{18}O_5$	C, H
7	A	94–96		48.9	$C_{18}H_{24}SO_7$	C, H, S
7a			*b*	50.8	$C_{18}H_{24}SO_7$	C, H
9			100–103 (0.09)	62.0	$C_{11}H_{19}NO_4$	C, H, N
10	B	160–164		66.9	$C_7H_{16}ClNO_2$	C, H, N
11		*c*		77.8	$C_{11}H_{17}ClN_4O_2$	C, H, N
13	C	233–235		50.2	$C_{12}H_{17}N_5O_2$	C, H, N
14	D	170–175		75.9	$C_{13}H_{21}Cl_2N_5O$	C, H, N, Cl
15	D	202–204		79.8	$C_{14}H_{23}Cl_2N_2O_2 \cdot 0.5H_2O$	C, H, N, Cl
19	E	47–48		35.2	$C_{13}H_{20}O_6$	C, H
20			103 (0.05)	42.5	$C_{13}H_{20}O_6$	C, H
5a			108–109 (0.05)	82.5	$C_{11}H_{16}O_5$	C, H
5b			93–96 (0.05)	83.9	$C_{11}H_{16}O_5$	C, H
21			*b*	37.8	$C_{18}H_{24}SO_7$	C, H, S
22, 23[d]			*b*	69.3	$C_{18}H_{24}SO_7$	C,[d] H, S
24	F	113–115		73.2	$C_{13}H_{21}NO_5$	C, H, N
25			*b*	49.8	$C_{13}H_{21}NO_5$	C, H, N
26	F	109–112		55.1	$C_{13}H_{21}NO_5$	C, H, N
27			*b*	42.8	$C_{13}H_{21}NO_5$	C, H, N
28			90–94 (0.07)	60.0	$C_{11}H_{19}NO_4$	C, H, N
32			120–125 (0.10)	44.3	$C_7H_{15}NO_2$	C, H, N
34	G	115–118		22.2	$C_{15}H_{19}ClN_4O_3$	C, H, N
35	C	230–233		22.4	$C_{12}H_{17}N_5O_2$	C, H, N

[a] A, EtOAc–hexane; B, MeOH–Et_2O; C, MeOH; D, HCl–MeOH/Et_2O; E, hexane; F, CCl_4; G, C_6H_6–hexane. [b] Could not be distilled; purified by column chromatography. [c] Mp 218°; resolidifies and remelts at 223°. [d] Mixture of isomers. *Anal.* C: calcd, 56.23; found, 56.66.

liquid which was purified by distillation (See Table II for analyses[14] and physical constants).

2-Hydroxymethyl-4-ethylenedioxycyclohexanol (4).—To an ice-cold mixture of 990 mg (26.1 mmoles) of $LiAlH_4$ in 50.0 ml of anhydrous Et_2O was added a solution of 4.00 g (17.4 mmoles) of **3** in 50.0 ml of anhydrous Et_2O. The mixture was heated under reflux for 8 hr. The chilled reaction mixture was titrated with H_2O, and the white precipitate was removed by filtration. The filtrate, after being dried ($MgSO_4$), was evaporated *in vacuo* and gave 2.81 g (86%) of the diol. The liquid product was dissolved in 25.0 ml of H_2O and washed with Et_2O (two 20-ml portions).[15] Removal of the H_2O *in vacuo* gave 2.48 g of diol.

Method B. 3-Acetoxymethyl-4-acetoxycyclohexanone (5).—A solution of 53.0 g (0.282 mole) of **4** in 350 ml of H_2O was stirred with 100 g of Dowex 50W-X8 (H^+) resin at 50° for 2 hr. The resin was removed by filtration and washed with 100 ml of H_2O. The filtrate was evaporated to 65 g[16] and dissolved in 200 ml of pyridine. Ac_2O (215 g, 2.09 moles) was added, and the mixture was stirred overnight at room temperature and then at 60° for 20 min. MeOH (50 ml) was added, and the mixture was evaporated *in vacuo* to constant weight. Distillation of the crude mixture gave **5**.

3-Acetoxymethyl-4-acetoxycyclohexanol (6) was prepared from **5** by method A.

Method C. 3-Acetoxymethyl-4-acetoxycyclohexyl Tosylate (7 and 7a).—A mixture of 766 mg (3.33 mmoles) of **6** and 955 mg (5.00 mmoles) of *p*-toluenesulfonyl chloride in 2.5 ml of pyridine was stirred at room temperature for 18 hr. The reaction mixture was chilled and acidified with concentrated HCl. H_2O (15 ml) and $CHCl_3$ (15 ml) were added, and the phases were separated. The aqueous phase was extracted ($CHCl_3$, two 15-ml portions). The combined extracts were washed [H_2O (20 ml), 5% $NaHCO_3$ (two 20-ml portions), H_2O (20 ml)]. After being dried ($MgSO_4$), $CHCl_3$ was removed *in vacuo* and gave 1.25 g (99%) of an oil which partially crystallized. The solid isomer (**7**) was obtained pure by two recrystallizations from EtOAc–hexane. The filtrates were combined and gave a mixture of isomers which could not be crystallized. The oil was passed through a neutral alumina column (4.0 g) using a solvent of EtOAc–hexane (1:1). Collection of the first 45 ml and evaporation of the solvent gave the analytical product (**7a**).

Method D. 1α-Amino-3α-acetoxymethyl-4β-acetoxycyclohexane (9).—A solution of 10.0 g (26.0 mmoles) of **7**, 5.09 g (78.0 mmoles) of NaN_3, and 3.28 g (78.0 mmoles) of LiCl in 100 ml of DMF was heated at 50° with stirring for 48 hr. The reaction mixture was filtered to remove the inorganic salts, and the filtrate was evaporated *in vacuo* to remove the solvent. H_2O (50 ml) was added to the residue, and the mixture was extracted ($CHCl_3$, three 50-ml portions). The combined $CHCl_3$ extracts were washed (H_2O, three 50-ml portions) and dried ($MgSO_4$). Removal of the $CHCl_3$ *in vacuo* gave the crude azide (**8**) which was not further purified; yield 6.49 g (97.8%); ν (cm^{-1}) (film) 2100 (N_3), 1730 (C=O), 1240 (C–O–C). The crude azide was dissolved in 125 ml of absolute EtOH and hydrogenated over 200 mg of PtO_2 at an initial pressure of 4.2 kg/cm^2 for 2 hr at room temperature. Removal of the catalyst by filtration and evaporation of the filtrate *in vacuo* gave a dark oil, yield 5.61 g. The crude product was fractionated *in vacuo*.

1α-Amino-3α-hydroxymethyl-4β-hydroxycyclohexane Hydrochloride (10).—A solution of 500 mg (2.18 mmoles) of **9** in 10 ml of 2% HCl–MeOH was left at room temperature for 24 hr. Removal of the volatile material *in vacuo* gave a colorless oil which was crystallized from MeOH–Et_2O.

1β-Hydroxy-2α-hydroxymethyl-4α-(5-amino-4-chloro-6-pyrimidinylamino)cyclohexane (11).—A solution of 1.00 g (5.50 mmoles) of **10**, 1.80 g (11.0 mmoles) of 5-amino-4,6-dichloropyrimidine, and 1.66 g (16.5 mmoles) of Et_3N in 15 ml of 1-butanol was heated under reflux under N_2 for 48 hr and then chilled in ice. After removal of the insoluble $Et_3N \cdot HCl$ by filtration, the filtrate was evaporated *in vacuo* to a yellow semisolid which solidified on trituration with C_6H_6. The C_6H_6-insoluble fraction was removed by filtration and gave 1.93 g of a mixture of **11** contaminated with $Et_3N \cdot HCl$. The crude mixture was dissolved in H_2O (25 ml) and MeOH (1 ml) and decolorized with Norit. The filtrate was evaporated to 10 ml, chilled, and filtered to remove the pure product.

1β-Hydroxy-2α-hydroxymethyl-4α-(6-amino-9-purinyl)cyclohexane (13).—A suspension of 1.00 g (3.66 mmoles) of **11** and 40 mg of ethanesulfonic acid in 15 ml of triethyl orthoformate was

(14) The analyses reported in this paper were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

(15) The diol decomposes on distillation when this step is omitted.

(16) Complete removal of the water results in polymerization.

stirred at room temperature until the uv spectrum had completely shifted to 265 mμ at pH 1 (24 hr). The volatile materials were removed *in vacuo* at 40–45° to an oil which partially solidified on standing. The crude 6-chloropurine (**12**) was transferred to a steel bomb and heated in liquid NH_3 (15 ml) at 60° for 16 hr. Removal of the volatile materials by evaporation, followed by drying *in vacuo* gave a solid residue, yield 1.17 g. A solution of 500 mg of the crude mixture in H_2O (5 ml) was passed through a Dowex 50W-X8 column (5 g). The column was washed with H_2O until the washings were free of Cl^-. After the material had remained on the column for 45 min, the column was eluted with 10% NH_4OH (100 ml). Evaporation of the eluate *in vacuo* gave 170 mg of **13**.

Method E. 1β-Hydroxy-2α-hydroxymethyl-4α-(6-methylamino-9-purinyl)cyclohexane Dihydrochloride (14).—A solution of 383 mg (1.13 mmoles) of crude **12** in 15 ml of 40% aqueous $MeNH_2$ was heated under reflux for 5 hr. The volatile materials were removed *in vacuo* and gave a light yellow oil. MeOH (5 ml) was added and the $MeNH_2$·HCl was precipitated by the addition of ether (15 ml). The salt was removed from the chilled solution by filtration, and the filtrate was evaporated *in vacuo* to a viscous oil. The oil was dissolved in 1 *N* HCl (10 ml) and allowed to stand at room temperature for 1 hr. After the volatile constituents had been removed *in vacuo*, the crude material was crystallized from MeOH–HCl/Et_2O.

1β-Hydroxy-2α-hydroxymethyl-4α-(6-dimethylamino-9-purinyl)cyclohexane dihydrochloride (15) was prepared from crude **12** and 25% aqueous Me_2NH by method E.

Method F. *cis*- and *trans*-Hydroxymethyl-4,4-ethylenedioxycyclohexanol Diacetate (19 and 20).—A solution of 12.8 g (68.5 mmoles) of **4** and 21.0 g (0.205 mole) of Ac_2O in 55 ml of pyridine was allowed to stand at room temperature for 22 hr. MeOH (5 ml) was added and the volatile materials were removed *in vacuo* and gave an oily product, yield 17.6 g (94.8%). Crystallization from hexane gave 9.44 g of the *trans* isomer (**19**).

The filtrate from the first crystallization was evaporated *in vacuo* and gave 8.20 g of the *cis* isomer (**20**) as an oil. Distillation *in vacuo* gave the pure product.

***trans*-3-Acetoxymethyl-4-acetoxycyclohexanone (5a)** was prepared from **19** by method B. ***cis*-3-Acetoxymethyl-4-acetoxycyclohexanone (5b)** was prepared from **20** by method B.

Method G. 3α-Acetoxymethyl-4β-acetoxycyclohexyl 1α-*p*-toluenesulfonate (21).—A solution of 7.00 g (30.6 mmoles) of **5a** in 100 ml of EtOH was hydrogenated over 100 mg of PtO_2 at 4.1 kg/cm² for 16 hr. After removal of the catalyst by filtration, evaporation of the filtrate *in vacuo* gave the corresponding alcohol as a clear oil in quantitative yield. Then method C was used. Crystallization of the crude product from EtOAc–hexane gave a solid tosylate (**7**) in two fractions, yield 5.58 g (48.9%), mp 94–96°. When further addition of hexane no longer gave a solid precipitate, removal of the solvent *in vacuo* gave 4.48 g (39.9%) of the oily isomer **21**. The oil was placed on a silica gel column (120 g) and eluted ($CHCl_3$), and 70-ml fractions were collected. The first eight fractions were evaporated and gave the pure product as an oil.

3α-Acetoxymethyl-4α-acetoxycyclohexyl tosylates (22 and 23) were prepared from **5b** by method G. The crude product (5.00 g) was placed on a silica gel column (120 g) and eluted with 2% MeOH–$CHCl_3$. Twelve 70-ml fractions were collected and evaporated *in vacuo*. Fractions 9 and 10 gave 3.60 g of a mixture of pure tosylates (**22** and **23**) as an oil.

1α-Acetamido-3α-acetoxymethyl-4β-acetoxycyclohexane (24) was prepared by method F from **10**.

1α-Acetamido-3β-acetoxymethyl-4α-acetoxycyclohexane (25) was prepared from **21** by method D. The crude amine could not be purified by distillation. Therefore, the amine was dissolved in 3 ml of pyridine and 0.5 ml of Ac_2O. After the reaction mixture had been left at 50° for 18 hr, the volatile materials were removed *in vacuo* and gave the amide as an oil, yield 1.22 g (82% over-all). A sample of the oily product (370 mg) was placed on a 17.5-cm column containing 9 g of silica gel. The column was eluted with 100 ml of $CHCl_3$. A 2% MeOH–$CHCl_3$ solution was then passed through the column and 30-ml fractions were collected. Fractions 8 and 9 were evaporated *in vacuo* and gave 225 mg of the pure **25** as an oil.

1-Acetamido-3α-acetoxymethyl-4α-acetoxycyclohexanes (26 and 27) were prepared from **22** and **23** by method D. A solution of 297 mg (1.30 mmoles) of the amine and 265 mg (2.60 mmoles) of Ac_2O in 3.0 ml of pyridine was heated overnight at 45–50°. MeOH (1 ml) was added and the mixture was evaporated *in vacuo* to 394 mg of oily product which partially crystallized. The mixture was separated by filtration. Three recrystallizations of the crude product from CCl_4 gave the all-*cis* isomer (**26**) as a solid material.

The filtrate from which **26** had been separated was passed through a column of neutral alumina (2.0 g) with CCl_4. Evaporation of the eluent *in vacuo* gave a second isomer (**27**) as an oil.

3-Acetoxymethyl-4-acetoxycyclohexylamine (28) was prepared from **7a** by method D.

3-Hydroxymethyl-4-hydroxycyclohexylamine (32).—A solution of 10.1 g (26.1 mmoles) of **7a** in 100 ml of 1% MeOH–HCl was allowed to stand at room temperature for 18 hr. Removal of the volatile constituents *in vacuo* gave **30**. The crude diol **30** was utilized to prepare **32** by method D.

Ethyl 4-(6-chloro-9-purinyl)-*cis*-2-hydroxymethyl-1-hydroxycyclohexylorthoformate (34) was prepared from **32** and 5-amino-4,6-dichloropyrimidine by a modification of the procedure used to prepare **12**. The filtrate from the recrystallization was evaporated and gave a mixture of formate esters as shown by its infrared spectrum.

1α-Hydroxy-2α-hydroxymethyl-4α-(6-amino-9-purinyl)cyclohexane (35) was prepared from **34** and liquid NH_3 by method E. After isolation of **35** by crystallization of the crude product from MeOH, the filtrate gave a second isomer on the addition of Et_2O: yield 84.0 mg; mp 205–208°; ν (cm⁻¹) (KBr) 3400 and 3200 (OH and NH_2), 1650 (NH_2), 1600 and 1560 (C=N and C=C); λ_{max} [mμ (ε × 10⁻³)] pH 1, 260 (1.50).

Anal. Calcd for $C_{12}H_{17}N_5O_2$: C, 54.73; H, 6.50; N, 26.60. Found: C, 54.90; H, 6.30; N, 26.47.

***cis,cis*-2-Hydroxymethyl-4-(6-amino-9-purinyl)cyclohexanol (35) from 26.**—A solution of 7.15 mg (2.63 mmoles) of **26** in 3 ml of MeOH and 5 ml of 4 *N* HCl was heated under reflux for 2 hr. Removal of the volatile materials *in vacuo* gave a white foam which softened on exposure to air. To the foam was added 10 ml of 1-butanol, 433 mg (2.63 mmoles) of 5-amino-4,6-dichloropyrimidine, and 800 mg (7.90 mmoles) of Et_3N and the solution was treated as previously described; yield of **35**, 40 mg, mp 230–232°. Ir and uv spectra, melting point, and mixture melting point with **35** were identical with those of **35** obtained by the previous procedure.

Reagents and Assay Procedure.—Adenosine and adenosine deaminase (Type I, calf intestinal mucosa) were purchased from the Sigma Chemical Co. The assay procedure for reversible inhibitors has previously been described[5,12] and is a modification of the general procedure described by Kaplan.[13] The measurements of the initial rates of the enzymic reactions were performed at 25° in 0.05 *M* phosphate buffer at pH 7.6.

(13) N. O. Kaplan, *Methods Enzymol.*, **2**, 473 (1955).