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Cyclohexenyl Nucleosides: Synthesis and Biological Activity of *trans*-3-(Purin-9-yl)-4-cyclohexenylcarbinols

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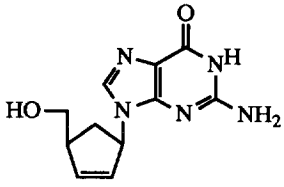
Abstract: The primary alcohol of *cis*-3-hydroxy-4-cyclohexenylcarbinol was protected with a silyl group and then the configuration of the secondary alcohol was inverted (using Mitsunobu conditions, followed by deacylation). The secondary alcohol was converted to a carbonate, which, in turn, was converted to 6-chloropurine derivatives, using palladium coupling conditions. A modified deprotection procedure gave the free alcohol of these acid / base sensitive nucleoside analogs. The resulting 6-chloropurine derivatives were treated with ammonia, giving the 6-aminopurine derivatives which were finally converted with adenosine deaminase to the guanosine and inosine analogs.

INTRODUCTION

Carbovir (carbocyclic 2',3'-didehydro-2',3'-dideoxyguanosine, **1**, Figure 1) has emerged as a promising new drug candidate for the treatment of AIDS due to its ability to selectively inhibit HIV-1 replication and infectivity in human T-cells below toxic concentrations.[1]

Modifications to the sugar portion of nucleosides has resulted in a number of compounds that display antiviral activity. A few examples include an acyclic nucleoside (acyclovir),[2] a four-member ring sugar nucleo-



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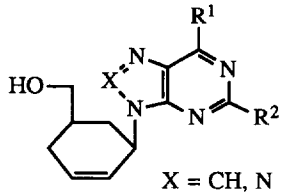

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X = CH, N
R^1 = NH_2, OH
R^2 = H, NH_2

Figure 1

side (oxetanocin),[3] and a series of six-member ring pyranosyl nucleosides.[4] These examples show that novel nucleosides with biological activity can be discovered when the sugar moieties of the nucleosides are significantly altered.

The biological activity of the pyranosyl nucleosides spurred our interest in synthesizing cyclohexyl nucleosides (of which only a few examples had been reported).[5] In our efforts to synthesize analogs of carbovir, we recently reported on the synthesis and biological activity of racemic *cis*-3-(purin-9-yl)-4-cyclohexenylcarbinols (**2**, Figure 1) as ring-expanded homologues of carbovir.[6] This report describes the synthesis and preliminary biological evaluation of novel cyclohexenyl nucleosides which are the racemic *trans* analogs of nucleosides **2**.

SYNTHESIS

The title compounds were synthesized as shown in the Scheme 1. Diol **3** was synthesized in three steps from 3-cyclohexenecarboxylic acid by literature procedures.[7] The primary alcohol of diol **3** was selectively protected with a *t*-butyldimethylsilyl group to give monoprotected alcohol **4** in 44 % yield. Disilylated alcohol **5** was also isolated from this reaction in 12 % yield. Diol **3** was partially immiscible with the solvent (CH_2Cl_2), however, the yield of monoprotected alcohol **4** was not improved (42%) by changing to DMF as the solvent.

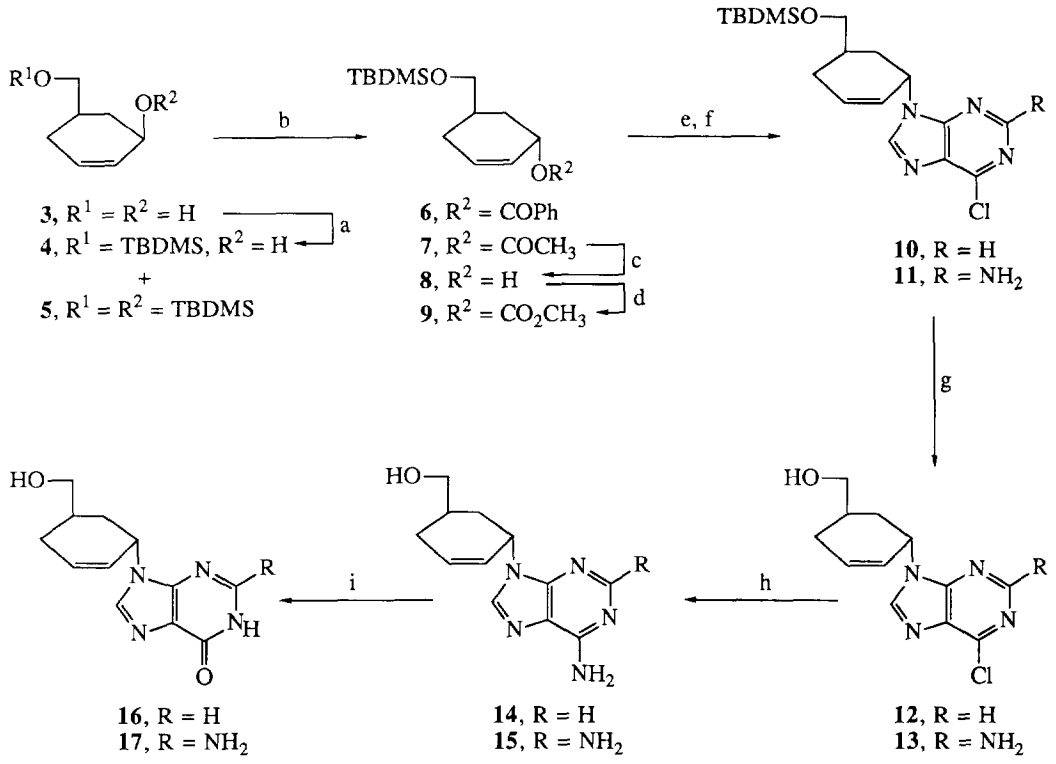
An attempt to directly displace the hydroxy group with 6-chloropurine, under Mitsunobu reaction conditions,[8] afforded the desired purine **10** in only 8 % yield (from alcohol **4**) with a *trans:cis* ratio of 2:1. Benzoic acid and acetic acid were used in reactions with alcohol **4** (under Mitsunobu reaction conditions[8]), giving esters **6** (6:1 ratio of isomers) and **7** (12:1 ratio of isomers) in yields of 88 and 93 %, respectively.

Ester **6** reacted with 6-chloropurine, under palladium coupling conditions,[9] but gave chloropurine **10** (3.5:1 ratio of isomers) in only 16 % yield. Ester **6** was also recovered in this reaction in 74 % yield with the partial loss of the relative stereochemistry, starting with a 6:1 ratio of isomers and going to a 3.6:1 ratio of isomers. Under the same conditions, acetate **7** gave crude chloropurine **10** in only 7 % yield as a mixture of several isomers.

Acetate **7** was easily deprotected with potassium carbonate in MeOH, giving a mixture of alcohols in 93 % yield (12:1 ratio of isomers). A comparison of the ^1H NMR spectra of the mixture with alcohol **4** shows that the minor isomer is alcohol **4**. Therefore, the major isomer of the mixture is assigned the *trans* configuration of alcohol **8**. Alcohol **8** reacted with dimethylpyrocarbonate to give carbonate **9** in 78 % yield (*trans:cis* = 12:1), along with 5 % recovery of alcohol **8**.

Carbonate **9** reacted with 6-chloropurine, under palladium coupling conditions,[9] giving chloropurine **10** (4:1 ratio of isomers) in 57 % yield. Carbonate **9** reacted with 2-amino-6-chloropurine, under the same conditions, giving chloropurine **11** (6:1 ratio of isomers) in 62 % yield. All attempts to separate the isomers of purines **10** - **15** by thin-layer chromatography (TLC) failed. Purines **10** and **11** could be purified, however, by recrystallization from methanol / water. Three recrystallizations of each gave chloropurines **10** (52 %, 30 % overall from **9**) and **11** (66 %, 41 % overall from **9**) with 17:1 and 11:1 ratios of isomers, respectively. The recrystallized purines were then used in subsequent reactions.

The assignments of the relative stereochemistry of the major and minor isomers were made after the silyl



Scheme 1[a]

[a]*Reagents and conditions:* (a) TBDMSCl, imidazole, CH_2Cl_2, 0 °C, **4**: 44 %, **5**: 12 %; (b) Ph_3P, DEAD, THF, $PhCO_2H$ or AcOH, **6**: 88 %, **7**: 93 %; (c) K_2CO_3, MeOH, 93 %; (d) $(MeOCO_2)_2CO$, DMAP, THF, 78 %; (e) 6-chloropurine or 2-amino-6-chloropurine, NaH, $(Ph_3P)_4Pd$, DMF, 60 °C, **10**: 57 % (*trans:cis* = 4:1), **11**: 62 % (*trans:cis* = 6:1); (f) recrystallize 3 X from MeOH / H_2O, **10**: 30 % from **9** (*trans:cis* = 17:1), **11**: 41 % from **9** (*trans:cis* = 11:1); (g) TBAF, THF, AcOH, **12**: 94 % (*trans:cis* = 17:1), **13**: 100 % (*trans:cis* = 11:1); (h) NH_3, MeOH, **14**: 100 % (*trans:cis* = 17:1), **15**: 86 % (*trans:cis* = 11:1); (i) adenosine deaminase, buffer, **16**: 73 % (*trans:cis* = 17:1), **17**: 66 % (*trans:cis* = 11:1).

groups were removed from purines **10** and **11**. The ^1H NMR peaks of the minor isomers of purines **12** - **17** were found to agree with that of the *cis* isomers which had been previously synthesized by an unambiguous route.[6] Palladium-catalyzed coupling reactions of purines with carbonates and acetates have been shown to give 9-substituted purines in which the purine substitutes for the carbonate or acetate with retention of the relative stereochemistry.[9] Therefore, the *trans* stereochemistry was assigned to the major isomers of purines **10** - **17**.

The use of tetrabutylammonium fluoride (TBAF) at room temperature to deprotect purines **10** and **11** resulted in decomposition, and lowering the reaction temperature to 0 °C prohibitively slowed the reaction. The easy adaptation of adding a small amount of glacial acetic acid (2 equivalents) to the reaction mixture, however, gave clean cleavage of the silyl group in a reasonable length of time (overnight). Thus, chloropurines **12**

(*trans:cis* = 17:1) and **13** (*trans:cis* = 11:1) were obtained in yields of 94 and 100 %, respectively. This minor adaptation (which gives relatively neutral conditions for silyl deprotection) has not been previously reported as far we know, and might be of use for other substrates that are acid or base sensitive. Acetic acid in water and THF has been reported to remove a *t*-butyl-dimethylsilyl group in good yield from an acid / base sensitive compound.[10]

Chloropurines **12** and **13** were aminated with ammonia, giving adenosine analog **14** (*trans:cis* = 17:1) and diaminopurine **15** (*trans:cis* = 11:1) in yields of 100 and 86 %, respectively. Attempts to hydrolyze the chloropurines **12** and **13** in the usual manner with either hydrochloric acid (1 *N*) or sodium hydroxide (0.33 *N*) resulted only in decomposition. Apparently, unlike their *cis* analogs,[6] these *trans* cyclohexenyl nucleosides are sensitive to acid and base. Although the decomposition products were not characterized, we believe that the hydroxyl group was displacing the allylic purine in an intramolecular attack in these reactions and in the attempts to remove the silyl groups (with TBAF, without the presence of acetic acid) from purines **10** and **11**.

Adenosine analog **14** and diaminopurine **15** were converted with adenosine deaminase to inosine analog **16** and guanosine analog **17**. On microscale (~ 1 mg), these reactions appeared (by TLC) to proceed to half-completion in 2 - 3 days at room temperature and not proceed noticeably past this, even after one week. Upon warming to 36 °C and adding more enzyme, the reactions slowly continued to completion over 1 - 2 weeks. On larger scale, the reactions were warmed in a 36 °C bath throughout the reactions until complete. No attempts were made to separate the enantiomers. The adenosine analog **14** was converted slightly faster by the enzyme than diaminopurine **15**. Purification of these compounds was slightly hindered by the presence of glycerol (the commercial enzyme was in 50 % glycerol), resulting in yields of 73 and 66 % for purified **16** (*trans:cis* = 17:1) and **17** (*trans:cis* = 11:1), respectively.

Biological Results

Compounds **11** - **17** were tested for activity against HSV-1 and all were found, however, to be inactive. Compounds **14** - **17** were also tested against HIV-1 and found to be inactive. Compounds **11** - **17** were evaluated for cytotoxicity against P388 mouse leukemia cells. Only compounds **11** and **12** showed cytotoxicity (IC_{50}'s of 23 and 40 μg/mL, respectively) below 50 μg/mL. The cytotoxicity of this class of nucleosides is less than that of its *cis* analogs and the cytotoxicity that was observed may actually have been due to the minor amounts of the *cis* analogs contaminating compounds **11** and **12**. The *cis* analog of compound **12**, for comparison, has an IC_{50} of 4 μg/mL.[6] Further antiviral testing of this series of compounds is under way.

EXPERIMENTAL

General

Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Melting points were determined on a Mel-Temp II apparatus and are corrected. The NMR spectra were obtained on a Varian Unity 300 or 500 spectrometers and referenced to the solvent. Chemical shifts are expressed in ppm and coupling constants are in hertz. IR spectra were determined with KBr plates (oils) or pellets (solids) on a Nicolet 5DXC spectrometer and reported in cm^{-1}. Electron impact mass spectra (MS) were obtained with a Kratos/AEI MS-30 and chemical impact (CI) MS were obtained with a Finnigan 4000. Thin-layer chromatography (TLC) was

performed on EM Science silica gel 60 F_{254} (0.25 mm layer) and column chromatography was performed on EM Science silica gel 60 (230 - 400 mesh).

(±)-*cis*-5-(*tert*-Butyldimethylsilyloxymethyl)-2-cyclohexenol (4) and (±)-*cis*-1-(*tert*-Butyldimethylsilyloxy)-5-(*tert*-butyldimethylsilyloxymethyl)-2-cyclohexene (5). Diol **3** (4.01 g, 31.3 mmol) in CH_2Cl_2 (200 mL) was cooled in a -20 to -10 °C bath. Imidazole (2.34 g, 34.9 mmol) was added, followed by *tert*-butyldimethylsilylchloride (TBSCl, 5.19 g, 34.5 mmol). The solution was stirred for 2 hours, and then additional imidazole (0.23 g, 3.4 mmol) and TBSCl (0.50 g, 3.3 mmol) were added. Then the solution was stirred for an additional hour, and the solvent was removed under reduced pressure at room temperature, leaving an oil. The oil was adsorbed to silica gel, added to a column, and eluted with a gradient of hexane to EtOAc. The solvent was removed from the fractions with $R_f = 0.76$ (hexane : EtOAc, 1 :1), leaving a clear oil (1.36 g, 3.8 mmol, 12 %) which was identified as the disilylated alcohol **5**. The solvent was removed from the fractions with $R_f = 0.53$ (hexane : EtOAc, 1 :1), leaving monosilylated alcohol **4** as a clear oil (3.33 g, 13.8 mmol, 44 %). Monosilylated alcohol **4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.76 (d of m, 1H, J = 10.2), 5.68 (d, 1H, J = 10.2), 4.30 (m, 1H), 3.51 (d, 2H, J = 4.5), 2.16 - 2.04 (m, 2H), 1.90 - 1.70 (m, 3H, 1H is D_2O exchangeable), 1.21 (dd, 1H, J = 21.3, 11.7), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR / DEPT (75 MHz, $CDCl_3$) 131.2 (CH), 128.2 (CH), 67.6 (CH_2), 67.4 (CH), 35.7 (CH_2), 35.6 (CH), 28.3 (CH_2), 25.9 (3 CH_3), - 5.38 (C), -5.40 (2 CH_3); MS (CI, NH_3) *m / z* (intensity) 243 (MH^+, 5), 225 (M^+ - OH, 100). Anal. Calcd for $C_{13}H_{26}O_2Si$: C, 64.41; H, 10.81. Found: C, 64.25; H, 10.62. Disilylated alcohol **5**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.70 (d of m, 1H, J = 10.5), 5.58 (d, 1H, J = 10.5), 4.33 (m, 1H), 3.48 (dd, 2H, J = 6.3, 2.1), 2.04 - 1.99 (m, 2H), 1.90 - 1.65 (m, 2H), 1.22 (dd, 1H, J = 21.9, 12.0), 0.90 & 0.89 (2 s, 18H), 0.08 (s, 6H), 0.04 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) 132.2, 127.4, 68.5, 67.7, 36.0, 35.9, 28.2, 25.9 (2 s, 6 methyls), - 4.1, - 4.2, -5.4 (2); MS (CI, NH_3) *m / z* (intensity) 357 (MH^+, 3), 225 (M^+ - TBDMSO, 100). Anal. Calcd for $C_{19}H_{40}O_2Si_2$: C, 63.98; H, 11.30. Found: C, 64.08; H, 11.23.

(±)-*trans*-5-(*tert*-Butyldimethylsilyloxymethyl)-2-cyclohexenyl Benzoate (6). A solution of DEAD (0.40 g, 2.30 mmol) in anhydrous THF (10 mL) was added, *via* syringe, over 10 minutes to a solution of monosilylated alcohol **4** (0.50 g, 2.07 mmol), triphenylphosphine (0.60 g, 2.29 mmol), and benzoic acid (0.30 g, 2.46 mmol) in anhydrous THF (25 mL) in an oven-dried flask, protected with a drying tube. The resulting solution was stirred at room temperature for 3 hours and then the solvent was removed under reduced pressure. The resulting white solid was adsorbed to silica gel, and the silica gel was added to column and eluted with a gradient of hexane to hexane : EtOAc (5:1). The solvent was removed from the fractions with $R_f = 0.63$ (hexane : EtOAc, 4:1), leaving benzoate **6** (*trans:cis* = 6:1) as a clear oil (0.63 g, 1.82 mmol, 88 %). ^{1}H NMR (300 MHz, $CDCl_3$) (major isomer) δ 8.04 (d, 2H, J = 7.5), 7.55 (t, 1H, J = 7.5), 7.42 (t, 2H, J = 7.8), 6.06 (ddd, 1H, J = 9.9, 5.1, 2.4), 5.91 (d of m, 1H, J = 9.9), 5.53 (m, 1H), 3.56 (m, 2H), 2.24 (td, 1H, J = 17.7, 5.0), 2.12 (m, 1H), 2.02 (d of m, 1H, J = 11.7), 1.84 (M, 1H), 1.61 (ddd, 1H, J = 13.1, 4.2, 1.5), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) 133.3, 132.8, 129.6 (2C), 128.3 (2C), 124.4, 111.4, 67.6, 67.3, 32.1, 31.3, 28.4, 25.9 (2C), - 5.36, - 5.37; IR 1717; MS (CI, NH_3) *m / z* (intensity) 364 ($M + NH_4^+$, 18), 347 (MH^+, 0.5), 225 (M^+ - $PhCO_2$, 100). Anal. Calcd for $C_{20}H_{30}O_3Si$: C, 69.32; H, 8.73. Found: C, 69.50; H, 8.55.

(±)-*trans*-5-(*tert*-Butyldimethylsilyloxymethyl)-2-cyclohexen-1-yl Acetate (7). Prepared from monosilylated alcohol **4** (1.71 g, 7.07 mmol) and AcOH (1.00 mL, 1.05 g, 17.5 mmol) as described (except the reaction time was overnight for 17 hours) for benzoate **6**, giving acetate **7** (*trans:cis* = 12:1) as a clear oil (1.86 g, 6.55 mmol, 93 %). R_f = 0.61 (hexane : EtOAc, 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.02 (d of m, 1H, *J* = 9.9), 5.77 (d of m, 1H, *J* = 9.9), 5.27 (m, 1H), 3.52 (d, 2H, *J* = 6.0), 2.17 (dt, 1 H, *J* = 18.0, 5.0), 2.03 (s, 3H), 2.0 - 1.7 (m, 3H), 1.50 (td, 1H, *J* = 13.2, 5.0), 0.89 (s, 9H), 0.04 (6H); ^{13}C NMR (75 MHz, $CDCl_3$) 206.8, 133.1, 124.4, 67.2, 67.0, 31.8, 31.1, 28.2, 26.0 (3 CH_3), 21.4, - 5.36, - 5.40 (2 CH_3); IR 1734; MS (CI, NH_3) *m / z* (intensity) 302 (M + NH_4^+, 10), 285 (MH^+, 0.16), 225 (M^+ - AcO, 100). Anal. Calcd for $C_{15}H_{28}O_3Si$: C, 63.33; H, 9.92. Found: C, 63.10; H, 9.74.

(±)-*trans*-5-(*tert*-Butyldimethylsilyloxymethyl)-2-cyclohexen-1-ol (8). A solution of acetate **7** (1.86 g, 6.55 mmol) and K_2CO_3 (1.86 g, 13.48 mmol) in MeOH (85 mL) was stirred for 20 hours. Silica gel was added and the MeOH was removed under reduced pressure. The silica gel was added to a column of silica gel and eluted with a gradient of hexane to hexane : EtOAc (1:1). The solvent was removed under reduced pressure from fractions with R_f = 0.27 (hexane : EtOAc, 4:1), leaving alcohol **8** as a clear oil (1.47 g, 6.07 mmol, 93 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.91 (ddd, 1H, *J* = 9.9, 5.1, 2.1), 5.82 (d of m, 1H, *J* = 9.9), 4.22 (m, 1H), 3.58 (dd, 1H, *J* = 9.9, 5.7), 3.49 (dd, 1H, *J* = 9.9, 6.0), 2.15 (dt, 1H, *J* = 17.7, 4.7), 2.04 - 1.88 (m, 1H), 1.82 (d of m, 1H, *J* = 13.5), 1.76 - 1.67 (m, 1H), 1.56 (s, 1H), 1.48 (td, 1H, *J* = 12.9, 4.2), 0.89 (s, 9H), 0.04 (s, 6H); MS (CI, NH_3) *m / z* (intensity) 260 (M + NH_4^+, 16), 244 (M^+ + 2, 2), 243 (MH^+, 9), 242 (M^+, 32), 225 (M^+ - OH, 100). Anal. Calcd for $C_{13}H_{26}O_2Si$: C, 64.41; H, 10.81. Found: C, 64.23; H, 10.91.

(±)-*cis*-5-(*tert*-Butyldimethylsilyloxymethyl)-2-cyclohexen-1-ylcarbonic acid methyl ester (9). A solution of alcohol **8** (3.16 g, 13.06 mmol) and DMAP (1.08 g, 8.85 mmol) in anhydrous THF (90 mL) in an oven-dried flask was protected by a drying tube and cooled in an ice-bath. Dimethylpyrocarbonate (DMP, 15 mL) was added dropwise over 20 minutes. The solution was allowed to warm to room temperature and stir for 3.5 hours. The solution was reimmersed in the ice-bath and additional DMP (15 mL) was added slowly over 20 minutes. The resulting solution was removed from the ice-bath and allowed to stir for 2 days. Silica gel was added and the solvent was removed under reduced pressure. The silica gel was added to a column of silica gel and eluted with a gradient of hexane to hexane : EtOAc (4:1). The solvent was removed under reduced pressure from the fractions with R_f = 0.60 (hexane : EtOAc, 4:1), leaving carbonate **9** as a clear oil (3.05 g, 10.17 mmol, 78 %). Alcohol **8** was also recovered as a clear oil (0.15 g, 0.62 mmol, 5 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.05 (ddd, 1H, *J* = 9.9, 5.1, 2.4), 5.83 (d of m, 1H, *J* = 9.9), 5.13 (m, 1H), 3.76 (s, 3H), 3.54 (dd, 1H, *J* = 9.9, 5.1), 3.50 (dd, 1H, *J* = 9.9, 5.6), 2.17 (dt, 1 H, *J* = 18.0, 4.9), 2.05 - 1.90 (m, 2H), 1.85 - 1.75 (m, 1H), 1.65 - 1.50 (m, 1H), 0.88 (s, 9H), 0.03 (6H); IR 1747; MS (CI, NH_3) *m / z* (intensity) 318 (M + NH_4^+, 2), 301 (MH^+, 0.07), 225 (M^+ - $MeOCO_2$, 100). Anal. Calcd for $C_{15}H_{28}O_4Si$: C, 59.96; H, 9.39. Found: C, 60.17; H, 9.18.

(±)-*tert*-Butyl[[*trans*-3-(6-chloro-9*H*-purin-9-yl)-4-cyclohexen-1-yl]methoxy]dimethylsilane (10). 6-Chloropurine (736 mg, 4.76 mmol) was added to a solution of NaH (95 %, 109 mg, 4.31 mmol) in anhydrous DMF (50 mL) in an oven-dried flask. A drying tube was added and the solution was heated in a 60 °C oil-bath for 30 minutes. After cooling to room temperature, a solution of carbonate **9** (1.30 g, 4.33 mmol) in anhydrous DMF (10 mL), and tetrakis(triphenylphosphine)palladium(0) (900 mg, 0.78 mmol) was added. The flask was covered with foil, placed in a 60 °C oil-bath for 4 - 5 hours, and then allowed to cool overnight. The solvent was removed *in vacuo* and the resulting residue was dissolved in EtOAc : MeOH (1:1). The solution was filtered through a silica gel plug and rinsed with EtOAc : MeOH (1:1). Silica gel was added to the filtrate and the solvent was removed under reduced pressure. The silica gel was added to a column of silica gel and eluted with a gradient of hexane to hexane : EtOAc (2:1). The solvent was removed under reduced pressure from the fractions with R_f = 0.45 (hexane : EtOAc, 1:1), giving an analytically pure chloropurine **10** as a white solid (0.93 g, 2.45 mmol, 57 %, mp 100-103 °C, *trans:cis* = 4:1). All attempts to separate the isomers by TLC failed. A portion of the white solid (0.83 g, 2.19 mmol) was recrystallized three times from MeOH / H_2O, giving white flaky crystals (0.43 g, 1.13 mmol, *trans:cis* = 17:1). ^{1}H NMR (500 MHz, $CDCl_3$, major isomer) δ 8.76 (s, 1H), 8.18 (s, 1H), 6.38 (ddd, 1H, *J* = 10.0, 5.0, 2.5), 5.89 (d of m, 1H, *J* = 9.9), 5.36 (m, 1H), 3.47 (d, 2H, *J* = 5.5), 2.15 (dt, 1H, *J* = 18.0, 4.8), 2.11 (br d, 1H, *J* = 14.0), 1.82 (d of m, 1H, *J* = 13.5), 2.00 (m, 1H), 1.48 (tdd, 1H, *J* = 13.0, 5.0, 1.5), 1.76 (m, 1H), 0.80 (s, 9H), - 0.02 (s, 3H), - 0.04 (s, 3H); MS (CI, NH_3) *m / z* (intensity) 381 (MH^+ + 2, 4), 380 (M^+ + 2, 2), 379 (MH^+, 10), 265 (100). Anal. Calcd for $C_{18}H_{27}N_4OClSi$: C, 57.05; H, 7.18; N, 14.78. Found: C, 57.15; H, 6.98; N, 14.66.

(±)-[[*trans*-3-(2-Amino-6-chloro-9*H*-purin-9-yl)-4-cyclohexen-1-yl]methoxy]*tert*-butyldimethylsilane (11). Prepared from 2-amino-6-chloropurine (807 mg, 4.76 mmol) and carbonate **9** (1.35 g, 4.50 mmol) as described for chloropurine **10**, giving analytically pure chloropurine **11** as a white solid (1.10 g, 2.79 mmol, 62 %, mp 133.5-135 °C). R_f = 0.47 (hexane : EtOAc, 1:1, *trans:cis* = 6:1) All attempts to separate the isomers by TLC failed. A portion of the white solid (0.99 g, 2.51 mmol) was recrystallized three times from MeOH / H_2O, giving white flaky crystals (0.65 g, 1.65 mmol, *trans:cis* = 11:1). ^{1}H NMR (300 MHz, $CDCl_3$, major isomer) δ 7.85 (s, 1H), 6.31 (m, 1H), 5.89 (d of m, 1H, *J* = 9.9), 5.23 - 5.03 (m, 3H, 2H are D_2O exchangeable), 3.50 (dd, 1H, *J* = 9.9, 5.4), 3.46 (dd, 1H, *J* = 9.9, 5.4), 2.31 (dt, 1H, *J* = 18.0, 4.8), 2.02 (br dd, 1H, *J* = 11.6, 2.0), 1.95 (td, 1H, *J* = 9.2, 2.4), 1.82 (td, 1H, *J* = 12.8, 5.1), 1.75 (m, 1H), 0.82 (s, 9H), 0.00 (s, 3H), - 0.02 (s, 3H); MS (EI) *m / z* (intensity) 396 (MH^+ + 2, 2), 395 (M^+ + 2, 6), 394 (MH^+, 4), 393 (M^+, 18), 336 (100). Anal. Calcd for $C_{18}H_{28}N_5OClSi$: C, 54.87; H, 7.16; N, 17.78. Found: C, 54.63; H, 6.92; N, 17.59.

(±)-*trans*-[3-(6-Chloro-9*H*-purin-9-yl]-4-cyclohexenyl)carbinol (12). Tetrabutylammonium fluoride [1*M* in anhydrous THF, 1.21 mL, 1.21 mmol] was added to a solution of chloropurine **10** (152.0 mg, 0.401 mmol) and glacial acetic acid (0.15 mL, 2 equivalents) in anhydrous THF (5.5 mL) in an oven-dried flask protected with a drying tube. The solution was stirred overnight and then poured through a silica gel plug and rinsed with EtOAc (5 X 15 mL). Silica gel was added to the filtrate and the solvent was removed under reduced pressure. The silica gel was placed on a column and eluted with a gradient of hexane to EtOAc and then EtOAc to EtOAc : MeOH (4:1). The solvent was removed under reduced pressure from the fractions with R_f = 0.18

(EtOAc), giving chloropurine **12** as a white powder (100.1 mg, 0.378 mmol, 94 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.19 (s, 1H), 6.39 (ddd, 1H, J = 9.9, 4.8, 2.4), 5.89 (d of m, 1H, J = 9.9), 5.39 (m, 1H), 3.55 (t, 2H, J = 5.3), 2.42 (dt, 1H, J = 18.3, 4.8), 2.17 (br d, 1H, J = 13.8), 2.04 - 1.90 (m, 2H), 1.86 (d, 1H, J = 4.5), 1.80 (m, 1H); MS (EI) m/z (intensity) 267 (MH^+ + 2, 2), 266 (M^+ + 2, 9), 265 (MH^+, 4), 264 (M^+, 31), 155 (100). Anal. Calcd for $C_{12}H_{13}N_4OCl$: C, 54.45; H, 4.95; N, 21.17. Found: C, 54.20; H, 4.76; N, 20.97.

(±)-*trans*-[3-(2-Amino-6-chloro-9*H*-purin-9-yl]-4-cyclohexenyl)carbinol (13). Prepared from chloropurine **11** (226.9 mg, 0.58 mmol) as described (with molar scaling) for chloropurine **12**, giving chloropurine **13** as a white powder (162.1 mg, 0.58 mmol, 100 %). R_f = 0.71 ($CHCl_3$: MeOH, 4:1); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.94 (s, 1H), 6.94 (s, 2H, D_2O exchangeable), 6.20 (m, 1H), 5.81 (m, 1H), 5.00 (m, 1H), 4.47 (br s, 1H, D_2O exchangeable), 3.28 (m, 1H, becomes dd upon D_2O exchange, J = 10.0, 4.5), 3.23 (m, 1H, becomes dd upon D_2O exchange, J = 10.0, 5.0), 2.20 (br d, 1H, J = 17.5), 2.02 (br d, 1H, J = 8.5), 1.95 (br dd, 1H, J = 18.0, 7.0), 1.64 (m, 2H); MS (EI) m/z (intensity) 282 (MH^+ + 2, 2), 281 (M^+ + 2, 10), 280 (MH^+, 5), 170 (100). Anal. Calcd for $C_{12}H_{14}N_5OCl$: C, 51.53; H, 5.04; N, 25.04. Found: C, 51.50; H, 5.11; N, 24.95.

(±)-*trans*-[3-(6-Amino-9*H*-purin-9-yl]-4-cyclohexenyl)carbinol (14). Chloropurine **12** (100.1 mg, 0.378 mmol) and a magnetic stir bar in MeOH (15 mL) was cooled in a dry ice - acetone bath and then liquid NH_3 was added (~ 50 mL). The bomb was sealed and then heated in a 60 °C oil-bath for 30 - 40 hours with stirring. The bomb and contents were cooled to room temperature and the NH_3 was vented. Silica gel was added and the solvent was removed under reduced pressure. The silica gel was added to a column and eluted with $CHCl_3$: MeOH (4:1). The solvent was removed under reduced pressure from fractions with R_f = 0.51 ($CHCl_3$: MeOH, 4:1), giving adenosine analog **14** as a white foam (92.5 mg, 0.378 mmol, 100 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.94 (s, 1H), 7.24 (s, 2H, D_2O exchangeable), 6.20 (m, 1H), 5.84 (m, 1H), 5.13 (m, 1H), 4.49 (br s, 1H, D_2O exchangeable), 3.30 (m, 1H), 3.24 (m, 1H), 2.42 (d of m, 1H, J = 15.3), 1.99 (d of m, 1H, J = 10.8), 1.80 - 1.50 (m, 3H); MS (EI) m/z (intensity) 246 (MH^+, 3), 245 (M^+, 16). Anal. Calcd for $C_{12}H_{15}N_5O$: C, 58.76; H, 6.16; N, 28.55. Found: C, 58.55; H, 6.20; N, 28.36.

(±)-*trans*-[3-(2,6-Diamino-9*H*-purin-9-yl]-4-cyclohexenyl)carbinol (15). Prepared from chloropurine **13** (161.1 mg, 0.575 mmol) as described for adenosine analog **14**, giving diaminopurine **15** as a white powder (128.4 mg, 0.494 mmol, 86 %). R_f = 0.35 ($CHCl_3$: MeOH, 4:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.51 (s, 1H), 6.67 (s, 2H, D_2O exchangeable), 6.16 (m, 1H), 5.81 (m, 3H, 2H are D_2O exchangeable), 4.91 (m, 1H), 4.49 (br s, 1H, D_2O exchangeable), 3.23 (m, 2H), 2.22 (br d, 1H, J = 16.5), 1.96 (br d, 1H, J = 11.7), 1.74 (d of m, 1H, J = 17.1), 1.60 (m, 2H); MS (EI) m/z (intensity) 261 (MH^+, 4), 260 (M^+, 22), 150 (100). Anal. Calcd for $C_{12}H_{16}N_6O \cdot 1/4\ H_2O$: C, 54.43; H, 6.28; N, 31.74. Found: C, 54.32; H, 6.48; N, 31.75.

(±)-*trans*-1,9-Dihydro-9-[5-(hydroxymethyl)-2-cyclohexen-1-yl]-6*H*-purin-6-one (16). Adenosine analog **14** (92.5 mg, 0.377 mmol) was dissolved in pH 6.8 buffer (0.05 *M* K_2PO_4, 10 mL).

Adenosine deaminase (AD, 7 X 36.7 units then 7 X 71.4 units) was added once each day for 14 days. At that time, adenosine analog **16** could no longer be detected by TLC. The H_2O was removed under reduced pressure and the resulting white powder was adsorbed to silica gel which was added to a column and eluted with a gradient of $CHCl_3$ to $CHCl_3$: MeOH (3:1). The solvent was removed under reduced pressure from the fractions with R_f = 0.44 ($CHCl_3$: MeOH, 4:1), giving a wet white solid. The solid was recrystallized from H_2O, giving inosine analog **16** in three crops of white to pale yellow crystals (total: 67.4 mg, 0.274 mmol, 73 %). Mp of first crop 267-267.5 °C. ^1H NMR (500 MHz, DMSO-d_6) δ 12.29 (s, 1H, D_2O exchangeable), 8.04 (s, 1H), 7.89 (s, 1H), 6.20 (ddd, 1H J = 9.8, 4.5, 1.8), 5.82 (d of m, 1H J = 9.8), 5.12 (m, 1H), 4.47 (br s, 1H, D_2O exchangeable), 3.30 (t, 2H, J = 5.0), 2.22 (d of m, 1H, J = 18.0), 1.97 (d of m, 1H, J = 11.5), 1.77 (d of m, 1H, J = 18.5), 1.67 (m, 2H); MS (CI, NH_3) m / z (intensity) 248 (MH^+ + 1, 12), 247 (MH^+, 100). Anal. Calcd for $C_{12}H_{14}N_4O_2$: C, 58.53; H, 5.73; N, 22.75. Found: C, 58.38; H, 5.86; N, 22.67.

(±)-*trans*-2-Amino-1,9-dihydro-9-[5-(hydroxymethyl)-2-cyclohexen-1-yl]-6*H*-purin-6-one (17). Diaminopurine **15** (123.4 mg, 0.474 mmol) was dissolved in pH 6.8 buffer (0.05 *M* K_2PO_4, 25 mL) and warmed in a 36 °C bath. Adenosine deaminase (AD, 46.7, 11 X 93.4, and then 9 X 186.8 units) was added each day for 21 days. The solution was sonicated on day 13 to break up the bacteria that was starting to form. After 19 days, the reaction appeared to have stopped as determined by TLC (85 - 90 % complete at this point). The H_2O was removed under reduced pressure and the resulting pale yellow oil was adsorbed to silica gel which was added to a column and eluted with a gradient of $CHCl_3$ to $CHCl_3$: MeOH (3:1). The solvent was removed under reduced pressure from the fractions with R_f = 0.44 ($CHCl_3$: MeOH, 4:1), R_f = 0.33, and a fraction with a mixture of the two. The R_f = 0.33 fractions gave a wet white solid which was triturated with H_2O and filtered, giving guanosine analog **17** as a white solid (41.6 mg, mp 174 - 177 °C). The filtrate was then developed by preparative TLC [5 X EtOAc : MeOH (4:1)]. The darkest band (as viewed under UV light) was extracted with $CHCl_3$: MeOH (3:1), giving additional guanosine analog **17** as a white solid (12.5 mg); The middle fraction from the column was developed by preparative TLC [2 X EtOAc : MeOH (9:1), 2 X EtOAc : MeOH (4:1)], giving additional guanosine analog **17** as a white solid (27.7 mg); total: 81.8 mg, 0.313 mmol, 66 %). An analytical sample was prepared by recrystallization from H_2O (mp 177 - 180 °C). ^1H NMR (300 MHz, DMSO-d_6) δ 10.57 (s, 1H, D_2O exchangeable), 7.55 (s, 1H), 7.89 (s, 1H), 6.49 (s, 1H, D_2O exchangeable), 6.17 (m, 1H), 5.78 (m, 1H), 4.88 (m, 1H), 4.49 (br s, 1H, D_2O exchangeable), 3.28 (m, 1H), 3.23 (m, 1H), 2.21 (d of m, 1H, J = 16.8), 1.94 (d of m, 1H, J = 12.0), 1.73 (m, 1H), 1.61 (m, 2H); MS (CI, NH_3) m / z (intensity) 279 (M + NH_4^+ or M^+ + H_2O, 5), 90 (100), 60 (100). Anal. Calcd for $C_{12}H_{15}N_5O_2 \cdot H_2O$: C, 51.60; H, 6.13; N, 25.07. Found: C, 51.69; H, 6.24; N, 25.09.

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