Synthesis of 9-(Hexane-1,6-diol-3-yl)purines, a Novel Series of Acyclic Nucleoside Analogs and Antiviral Evaluation

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New acyclic purine nucleoside analogs lacking the C(3')-C(4') bond, have been prepared from 4-aminocyclohexene and evaluated for antiviral activity. A new synthesis of 4-aminocyclohexene from cyclohex-3-ene-1-carbonyl chloride is also reported *via* a Curtius reaction. None of the two compounds exhibited any antiviral activity *in vitro* against HSV-1, HCMV and HIV-1.

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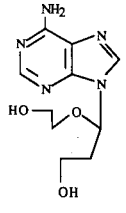
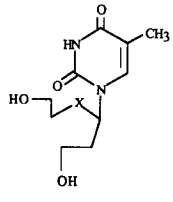
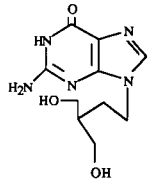
The discovery of acyclovir and of several other acyclic guanosine analogs as potent antiherpes drugs [1,2] has stimulated a great effort toward the synthesis of new acyclic nucleoside analogs as potential antiherpes (HSV) or anti-human cytomegalovirus (HCMV) agents [3] and, more recently, as anti human immunodeficiency virus type 1 agents [4] (HIV-1) the causative agent for acquired immunodeficiency syndrome (AIDS). Some acyclic nucleosides lacking the C(3')-C(4') bond, **1,2,** have been described previously [5,6]. Compounds **2** were tested for antiviral activity against HSV-1, HSV-2 and HIV-1 [6] and found much less active than acyclovir or AZT but the corresponding guanine nucleoside analog of **1** was not reported



1

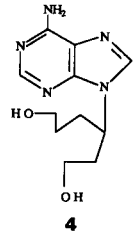


2 a : X=O
b : X=S



3 (BRL 39123)

up to date although it would be more related to potent antiherpes drugs such as acyclovir and ganciclovir [7]. On the other hand, potent antiviral activities have been found with a "carbo-acyclic" nucleoside analog such as BRL 39123 **3** [8] therefore we found of interest to synthesize the related structures **4** and **5** for the purpose of testing them against HSV, HCMV and HIV.



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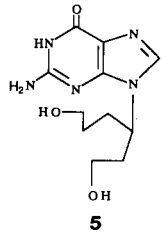

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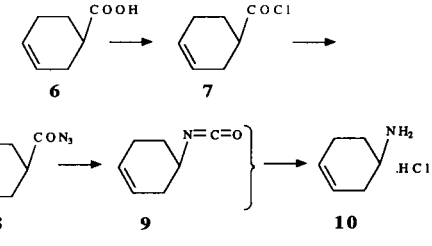
We report in the present paper the synthesis of the two new nucleoside analogs **4** and **5** from 4-aminocyclohexene whose a new synthesis is also reported. The antiviral activity of **4** and **5** is reported.

The preparation of 4-aminocyclohexene (**10**) was reported previously from 4-hydroxycyclohexene *via* the cyclohexenone oxime [9] or *via* the azide route [10] after a reduction step in 5.1% and 38% yield respectively. A shorter synthesis of **10** appeared recently [13] from 3-cyclohexene-1-carboxylic acid **6** which is readily available by silver oxide oxidation of commercial 3-cyclohexene-1-carboxaldehyde. A one step Hofmann type rearrangement of the corresponding primary amide led to **10** but the yield was not improved (37% yield from **6**). Therefore, we choose to perform a Curtius rearrangement of the acid azide **8**, which is easily obtained from 3-cyclohexene-1-carbonyl chloride **7**. The acid azide **8** and isocyanate **9** were not isolated and the overall process from **8** to **10** was carried out in one pot (Scheme I). Under these conditions, the yield of the sequence **6** → **10** was improved to a yield of 60%.

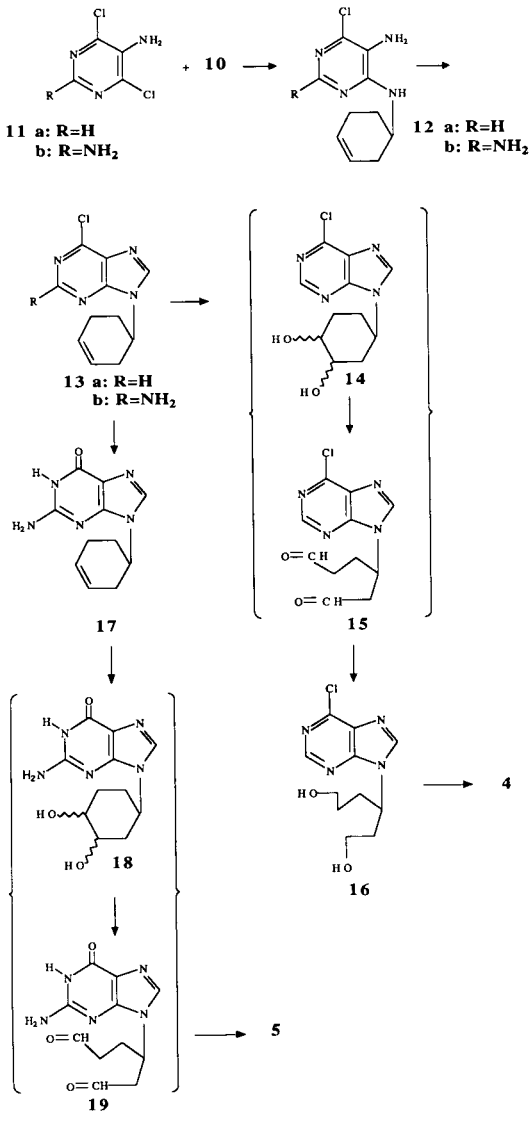
Scheme I



The synthesis of nucleoside analogs **4** and **5** was carried out as outlined in Scheme II. Reaction of **10** with 5-amino-4,6-dichloropyrimidine (**11a**) in butan-1-ol at reflux for 48 hours gave a 60% yield of **12a**. Acid catalyzed cyclisation of **12a** with triethyl orthoformate in dimethylacetamide furnished purine derivative **13a** in 80% yield. The guanine precursor **13b** was obtained according to the same sequence in 52% yield from **11b** [11,12].

Hydrolysis of the 6-chloro group of **13b** in 1*N* hydrochloric acid at reflux afforded the guanine derivative **17** in 80% yield. At this point both adenine precursor **13a** and guanine derivative **17** were treated in the same manner

Scheme II



and led to **16** and **5** respectively according to a one pot sequence of reactions including; a) *cis-trans* dihydroxylation of the double bond with osmium tetroxide; b) sodium periodate oxidation of the diol (**14** and **18**) in the dark; c) sodium borohydride reduction of the dialdehyde intermediate (**15** and **19**). The overall yield of **5** was 30% (from **17**) and the overall yield of **16** was 75% (from **13a**). Adenine nucleoside derivative **4** was obtained after treatment of **16** with liquid ammonia in a stainless steel bomb (39% yield). Both nucleoside analogs **4** and **5** have been tested for antiviral activity against HSV-1, HCMV and HIV-1 in cell culture. None of these compounds had significant activity against the three viruses studied.

EXPERIMENTAL

The melting points were taken on a Kofler hot stage apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian XL100 (^{1}H nmr, 100 MHz) and chemical shifts (δ) are reported in parts per million downfield from internal tetramethylsilane. Elemental analyses were performed by the "Service de Microanalyses", CNRS, ICSN, 91198 Gif sur Yvette, France. The preparative chromatographies were carried out in glass columns packed with 230-400 mesh silica gel (Kieselgel 60, Merck) under low pressure (1-10 bars).

Cyclohex-3-ene-1-carbonyl Chloride (**7**).

A solution of cyclohex-3-ene-1-carboxylic acid [13] (11 g, 87.19 mmoles) in thionyl chloride (8 ml) was stirred overnight at room temperature under exclusion of moisture. Excess thionyl chloride was removed by evaporation during 1 hour at 30° under reduced pressure (15-20 mm). Distillation of the residue gave 11 g (87%) of a colorless liquid bp 73-74°/19 mm; ^{1}H nmr (deuteriochloroform): δ 5.69 (degenerated AB system, 2H, 5.69), 2.99 (m, 1H, H1), 2.41-2.13 (m, 5H, 2 × H5, 1 × H6, 2 × H2).

Anal. Calcd. for C_7H_9OCl: C, 58.14; H, 6.27; Cl, 24.52. Found: C, 57.97; H, 6.44; Cl, 24.51.

4-Aminocyclohexene Hydrochloride **10**.

A solution of cyclohexene **7** (55 g, 0.38 mole) in acetone (120 ml) was added dropwise at 0° to a stirred solution of sodium azide (38.2 g, 0.58 mole) in water (120 ml). The mixture was stirred for 1 hour at 0° and the aqueous phase was extracted twice with toluene (100 ml). The organic phase (acetone) was maintained at 0° during the extraction process and combined with the toluene washes, dried (magnesium sulfate), filtered and heated slowly to 60° and then to 80° until the evolution of nitrogen was complete (30-40 minutes). The solution was cooled again and concentrated hydrochloric acid (200 ml) was added. The mixture was stirred at room temperature until the vigor of evolution of carbon dioxide was much decreased (15 min) and heated under reflux with stirring for 10 minutes. The cooled mixture was then evaporated to dryness and co-evaporated several times with absolute ethanol. The resulting solid was stirred with acetone, collected by filtration and washed with acetone, yield 35 g (69%), mp 178-179°, lit [13] 178-179°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 5.6 (degen AB syst 2H, H1, H2), 3.16 (m, 1H, H4), 2.4-1.38 (m, 6H, 3 × CH_2).

Anal. Calcd. for $C_6H_{12}NCl$: C, 53.93; H, 9.05; N, 10.48. Found: C, 54.05; H, 8.91; N, 10.25.

5-Amino-6-chloro-4-[(cyclohex-3-en-1-yl)amino]pyrimidine (**12a**).

4-Aminocyclohexene (11 g) was prepared from the hydrochloride **10** which was treated with crushed potassium hydroxide and water in ice (the flask was equipped with a reflux condenser). The resulting mixture was distilled. Pellets of potassium hydroxide were added to the distillate and the two phases separated. The aqueous phase was discarded and the free amine was distilled again in the presence of potassium hydroxide pellets. Redistillation gave pure free 4-aminocyclohexene (bp = 136-138°) 87%. A solution of 5-amino-4,6-dichloropyrimidine (**11a**) (5.01 g, 30.88 mmoles) and 4-aminocyclohexene prepared above (3 g, 30.92 mmoles) in 1-butanol (50 ml) containing 5 ml of triethylamine was refluxed for 48 hours under nitrogen. The mixture was evaporated to dryness and co-evaporated several times with toluene to remove all the butanol. The title compound was purified by chromatography on a silica gel column (100 × 2.5 cm) eluting with dichloromethane-ethanol 95:5. The pure fractions were combined and evaporated to dryness. A solid was obtained which crystal-

lized in cyclohexane (4.16 g, 60%) mp = 167°; ^{1}H nmr (deuteriochloroform): δ 8.07 (1H, s, H2), 5.70 (2H, m, ethylenic), 4.97 (1H, broad signal, NH), 4.35 (1H, m, H1'), 3.22 (2H, s, NH_2), 2.7-1.5 (6H, m, 3 × CH_2).

Anal. Calcd. for $C_{10}H_{13}N_4Cl$: C, 53.40; H, 5.70; N, 24.90; Cl, 15.80. Found: C, 53.60; H, 5.55; N, 25.07; Cl, 15.93.

6-Chloro-9H-9-(cyclohex-3-en-1-yl)purine (**13a**).

To a solution of **12a** (150 mg, 0.668 mmole) in distilled dimethylacetamide (6 ml) and freshly distilled triethyl orthoformate (6 ml) was added, at 0° 0.1 ml of concentrated hydrochloric acid. This mixture was stirred at room temperature for 18 hours before it was evaporated to dryness with an oil pump (0.1 mm). Crystallization in ethanol yielded 126 mg (80%) of the title compound, mp 168-169°; ^{1}H nmr (deuteriochloroform): δ 8.75 (1H, s, H2), 8.21 (1H, s, H8), 5.85 (2H, m, ethylene), 4.94 (1H, m, H1'), 2.69-2.24 (6H, m, 3 × CH_2).

Anal. Calcd. for $C_{11}H_{11}N_4Cl$: C, 56.27; H, 4.69; N, 23.88; Cl, 15.13. Found: C, 56.37; H, 4.59; N, 24.12; Cl, 15.36.

3-(9H-6-Chloropurin-9-yl)hexane-1,6-diol (**16**).

A solution of 6-chloro-9-(3-cyclohexen-1-yl)purine (346 mg, 147 mmole) in 2-methyl-2-propanol (10 ml) and water (5 ml) was treated with 1 ml of an osmium tetroxide solution (2.5% in 2-methyl-2-propanol) and *N*-methylmorpholine *N*-oxide (250 mg) at 85-90° under a nitrogen atmosphere. After one hour, the mixture was evaporated to dryness and azeotroped with toluene (3 times) to remove the morpholine. The residue was redissolved in water (5 ml) and subjected to a short column of Dowex 1 × 8-50 (OH^-) which retained the osmium esters. The column was washed with water. Washings were evaporated to dryness to a solid which was treated as follows: the residue was redissolved in water (30 ml) while sodium metaperiodate (6 ml of a 0.5 *M* solution) was added and stirring was continued for 2 hours in the dark.

An aqueous solution of sodium borohydride (10 ml, 9 mmoles) was then added at 0° and stirred at room temperature for 4 hours. The mixture was neutralized with acetic acid (10% aqueous solution) and evaporated to dryness. The residue was co-evaporated with toluene and ethanol several times to remove the acetic acid. The residue was then subjected to a silica gel column eluting with dichloromethane-ethanol 9:1. The pure fractions were evaporated to dryness and the residue triturated with diethyl ether to an amorphous solid (300 mg, 75%) mp 97-98°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.26 (1H, s, H2), 8.18 (1H, s, H8), 4.62 (1H, m, H1'), 4.40 (1H, t, OH), 4.25 (1H, t, OH), 3.40-3.08 (4H, m, 2CH_2, OH), 2.6-1.8 (4H, m, 2CH_2), 1.5-0.95 (2H, m, CH_2).

Anal. Calcd. for $C_{11}H_{15}N_4O_2Cl$: C, 48.79; H, 5.54; N, 20.70. Found: C, 49.10; H, 5.60; N, 21.10.

3-(9H-6-Aminopurin-9-yl)hexane-1,6-diol (**4**).

A solution of 3-(9H-6-chloropurin-9-yl)hexane-1,6-diol (250 mg, 1.16 mmoles) in ethanol (5 ml) was stirred in a stainless steel bomb in the presence of liquid ammonia (300 ml) at room temperature for 24 hours. The excess of ammonia was evaporated and the residue redissolved in methanol (20 ml) and stirred with hydrochloric acid 1*N* (40 ml) for 45 minutes. The mixture was evaporated to dryness and subjected to a column of IRA 400 (OH^-) prepared in methanol. The washing (methanol) was evaporated and purified on a column (75 × 2.5 cm) of sephadex G10 prepared in water. The pure fractions were combined and evaporated to dryness to an oil which could not be crystallized and which was dried several days in vacuum over phosphorus pentoxide (120 mg, 40%); ^{1}H nmr (dimethyl sulfoxide-d_6): δ 8.19 (1H, s, H2), 8.15 (1H, s, H8), 7.14 (2H, s, NH_2), 4.60 (1H, m, H1'), 4.38 (2H, unresolved m, 2OH), 3.30 (4H, m, 2 × CH_2 OH), 2.05 (4H, m, 2 × CH_2), 1.24 (2H, m, CH_2).

Anal. Calcd. for $C_{11}H_{17}N_5O_2$, ½ H_2O: C, 50.76; H, 6.92; N, 26.92. Found: C, 50.95; H, 6.62; N, 27.16.

2,5-Diamino-6-chloro-4-[(cyclohex-3-en-1-yl)amino]pyrimidine (**12b**).

A solution of 2,5-diamino-4,6-dichloropyrimidine (**11b**) [11,12] (3.3 g, 18.5 mmoles), 4-aminocyclohexene (1.5 g, 15.4 mmoles) and triethylamine (10 ml) in butan-1-ol (50 ml) was stirred at 100° for 48 hours under argon. The mixture was evaporated to dryness and the residue was redissolved in dichloromethane (100 ml). The organic phase was washed with water (3 × 20 ml), dried (magnesium sulfate) and evaporated to a yellow oil which was subjected to column chromatography eluting with dichloromethane ethanol 95:5. A slightly colored syrup was obtained (2.6 g, 70%) which was sufficiently pure for the next step; ^{1}H nmr (deuteriochloroform): δ 5.70 (d, 2H, H3', H4'), 5.55 (bd, 1H, NH), 4.37 (bs, 2H, NH2), 4.22 (m, 1H, H1'), 1.92-2.52 (m, 8H, 2H2', 2H5', 2H6', NH2).

2-Amino-6-chloro-9H-9-(cyclohex-3-en-1-yl)purine (**13b**).

A solution of **12b** (150 mg, 0.62 mmole) in redistilled *N,N*-dimethylacetamide (10 ml) was cooled to 0°. A mixture of redistilled triethyl orthoformate (10.4 ml) and aqueous 12*N* hydrochloric acid (0.5 ml) was added with stirring. The stoppered flask was stirred overnight at room temperature and evaporated to dryness. The residue was dissolved in 50% acetic acid (30 ml) and stirred at room temperature for 4 hours. The mixture was evaporated to dryness, co-evaporated three times with methanol (50 ml), redissolved in ammonia (5% in methanol) (30 ml) and stirred overnight at room temperature. The residue of evaporation was adsorbed on silica gel and chromatographed on a silica gel column eluting with dichloromethane-ethanol 95:5. The pure fractions were combined and concentrated to a colorless syrup 117 mg (75%); ^{1}H nmr (deuteriochloroform): δ 7.86 (s, 1H, H8), 5.79 (A B syst, 2H, H3', H4'), 5.18 (br s, 2H, NH_2), 4.63 (m, 1H, H1'), 2.58-2.14 (m, 6H, H2', H5', H6').

Anal. Calcd. for $C_{11}H_{12}N_5Cl$: C, 53.01; H, 4.81; N, 28.11. Found: C, 52.92; H, 4.98; N, 28.05.

9-(Cyclohex-3-en-1-yl)guanine (**17**).

A solution of **13b** (150 mg, 0.6 mmole) in 1*M* hydrochloric acid (15 ml) was heated under reflux for 6 hours. The solution was evaporated to dryness and co-evaporated several times with ethanol. The residue was dissolved in water (2-3 ml) and neutralized with 6 *M* sodium hydroxide. The title compound **17** crystallized in the cold (+ 5°). It was then filtered and washed with cooled water, 110 mg, yield 80%, mp 143°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 10.52 (br s, 1H, NH), 7.77 (s, 1H, H8), 6.41 (br s, 2H, NH_2), 5.76 (br s, 2H, H3', H4'), 4.41 (m, 1H, H1'), 2.22-2.00 (m, 6H, H2', H5', H6').

Anal. Calcd. for $C_{11}H_{13}N_5O$: C, 57.14; H, 5.62; N, 30.30. Found: C, 57.02; H, 5.71; N, 30.30.

9-(Dihydroxy-1,6-hex-3-yl)guanine (**5**).

A solution of **17** (60 mg, 0.25 mmole) in methanol (10 ml) was added dropwise to a mixture of *N*-methylmorpholine *N*-oxide (40 mg), water (5 ml) and 1*M* osmium tetroxide in *t*-butyl alcohol (0.04 ml). The mixture was heated at 90° for 45 minutes, evaporated to dryness, redissolved in methanol (5 ml) and subjected to a column a Dowex 50 1×8 (OH^- form). The cyclohexenediol intermediate **18** was purified on a silica gel column eluting with dichloromethane-ethanol 8:2 and immediately used in the next reaction. A solution of **18** (60 mg, 0.22 mmole), water (10 ml) and sodium periodate (96 mg, 0.44 mmole) was stirred at room temperature, in the dark for 3 hours. The solution was cooled to 0° and sodium borohydride (50 mg, 1.25 mmoles) in water (5 ml) was then added and the resulting mixture was stirred for 2 hours. An excess of acetone was added and the mixture was neutralized with acetic acid. The title compound **5** was obtained after chromatography on silica gel column (dichloromethane-ethanol 8:2). An analytical sample was obtained by crystallization in ethanol 18 mg (30%) mp >260°; ^1H nmr (dimethyl sulfoxide-d_6): δ 9.35 (s, 1H, NH), 7.64 (s, 1H, H8), 6.35 (s, 2H, NH_2), 4.41 (q, 2H, H3', OH), 3.34-3.30 (m, 5H, 2H1', 2H6', OH), 2.01-1.93 (m, 4H, 2H4', 2H5'), 1.27-1.19 (m, 2H, H2').

Anal. Calcd. for $C_{11}H_{17}N_5O_3$, ¼ H_2O: C, 48.61; H, 6.44; N, 25.78. Found: C, 48.86; H, 6.47; N, 26.18.

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