Vol. 1, No.3
Spring, 1990

J.Sci.I.R.Iran

SYNTHESIS OF PURINE ACYCLONUCLEOSIDES HAVING PRONOUNCED ANTIVIRAL ACTIVITY

G. H. Hakimelahi*, and F. Mohanazadeh

Department of Chemistry, Faculty of Science, University of Shiraz, Shiraz, Iran

H. Davary, and M. Zakerinia

School of Veterinary Medicine, University of Shiraz, Iran

Abstract

The synthesis of a series of purine analogues of the acyclonucleoside compound **14** (acyclovir) is described. Compounds in this series have been shown to have pronounced activity against herpes virus-type 1(HSV-1). The anti-viral activities of other analogs whose syntheses were recently reported [1] by us are also described.

Introduction

Much effort has been expended in the search for effective anti-viral agents, but relatively few effective agents have been found [2-6]. The continuing search for drugs to combat viral infections presents modern medicine with one of its greatest challenges. Much has been written about the potential of genetic engineering to provide abundant supplies of interferon and new vaccines. But the challenge is also being met in more conventional ways, and many chemicals look promising as potential anti-viral agents [7,8]. Viruses multiply inside cells by hijacking the cells own molecular machinary and forcing it into the service of the viral life-cycle. This insidious behavior means that many drugs which interfere with the life-cycle of the virus will do the same to normal host cells. The toxicity of such drugs (i.e. idoxuridine [6]) severely limits their use in anti-viral treatment. Drugs that are selectively toxic to only viruses or cells already infected with viruses, have proved extremely hard to find.

Viruses can contain from one to over 200 genes [9]. For their syntheses and replication certain nucleosides must be first phosphorylated to the corresponding triphosphates and then incorporated into the gene synthesis. The herpes group of viruses produce an enzyme called thymidine/or deoxycytidine kinase, which is responsible for adding a phosphate group to certain nucleoside precursors and then by the aid of certain cellular enzymes add two more phosphate groups to make the respective nucleoside triphosphates. Some viruses rely entirely on the enzymes of the infected cell to catalyse every step in the production of more viruses [9,10]. Other viruses make many of their own enzymes but still depend on cellular proteins to perform some essential task.

A compound which shares crucial features in common with the precursors of DNA/or RNA-nucleosides of viruses might be phosphorylated, similarly, by viral enzymes and enzymes native to the infected cells to make the corresponding triphosphate. This similarity allows drug triphosphate to bind to DNA/or RNA-polymerase enzyme that links the nucleosides together during the replication of viral DNA or RNA. This somehow inhibits copies of viral DNA or RNA from being made, and is the basis of the antiviral activity of some compounds (i.e. acyclovir [11] (**14**)).

Acyclovir [11-20] (zovirax) is specifically an antiviral drug because the enzymes of normal healthy cells can not efficiently perform the initial phosphate addition achieved by the viral enzyme. So its phosphate and triphosphate are not formed in healthy cells, whose DNA synthesis is therefore not affected. Also, the drug seems to inhibit the viral DNA

Key words: Secoribo-nucleosides, Antivirals

polymerase enzyme much more effectively than it does the DNA polymerase used by the cell to copy its own DNA.

We therefore began a program to synthesize a series of purin analogues based on the structure of acyclovir (14) and examine their antiviral activity.

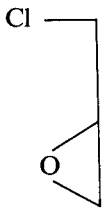
Discussion and Results

The general procedure for the synthesis of compounds **7** and **13** is outlined in Schemes 1 and 2. The first step in the synthesis of chloromethyl ether **4** involved reaction of epichlorohydrin (1) with benzoic acid in DMF/$NaHCO_3$ at 25° to afford **2** (95%). It should be noted that the above reaction at refluxed temperature gave compound **3** (80%). Treatment of **2** with CH_2O/HCl gave the desired chloromethyl ether **4** (50%). Compound **4** can be coupled directly to purines. For example, 6-chloropurine reacts with **4** in DMF/NEt_3 to produce the 9-alkylated product **5** and the N-7 isomer **6**. Treatment of **5** or **6** with MeOH/NH_3 results in adenine derivatives **7** or **8**.

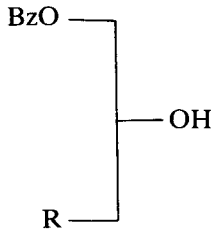
We have recently reported [21] that the preparation of nucleoside analogues by the fluoride methodology results in the formation of N-9 alkylated products. In a model reaction, the chloromethyl ether **9** was converted to adenine compound **20** by either of the following routes. In the first case **9** was reacted with 6-chloropurine in the presence of NEt_3 in DMF to afford **10**. Treatment of **10** with MeOH/NH_3 at 90°C gave **20** [1]. The alternative procedure used was condensation of persilylated adenine with **9** in THF using Bu_4NF as condensing agent to give **11** in good yield, treatment of **11** with MeOH/NH_3 gave **20** quantitatively [1]. Therefore for exclusive production of N-9 alkylated product **7** the fluoride route was examined and fortunately the desired product **7** was prepared in high yield.

Next, we turned our attention to the preparation of guanine compound **13**. The procedure was based on the $Hg(CN)_2$/silylated base method [22]. Thus, guanine was silylated with hexamethyl disilazane (HMDS) and coupled with **4** in refluxing benzene using $Hg(CN)_2$ and $(NH^+_4)_2SO_4$ as catalysts. The condensation was generally complete within 4 h to afford **12** (40%). The removal of the benzoyl group was easily accomplished by MeOH/NH_3 at 25°C to give **13**(97%).

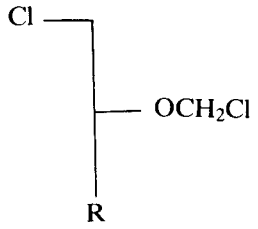
Scheme 1



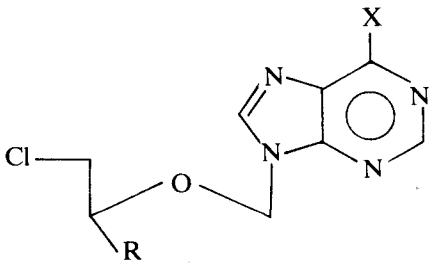
1



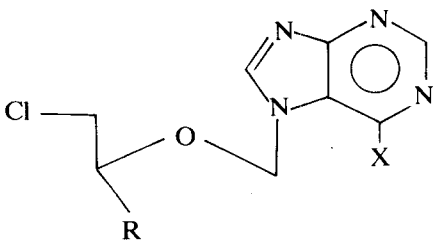
2 R = Cl
3 R = OBz



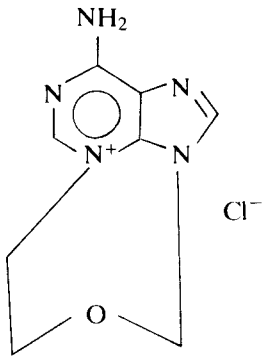
4 R = CH_2OBz
9 R = H



5 R = CH_2OBz, X = Cl
7 R = CH_2OH, X = NH_2
10 R = H , X = Cl
11 R = H , X = NH_2



6 R = CH_2OBz, X = Cl
8 R = CH_2OH, X = NH_2



20

Materials and Methods

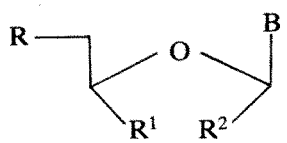
Compound solutions were prepared immediately before use in sterile phosphate buffer solution (PBS, PH 7.4) and filtered (0.45 m millipores filter). Virus [herpes simplex type 1 (HSV-1)], kindly provided by Dr. S. Saidi, University of Tehran, School of Hygine. They were passed three times in Hela cells and after each time were collected as described [23]. Titer of virus was determined by titration of serial 10-fold dilutions of the inolculum in confluent Hela cells in leighton tubes (4 tube per dilution). Titer, expressed as $TCID_{50}$ (50% cell culture infective dose), was determined by the method of Reed and Muench [24].

Hela cell monolayers in tissue culture tubes (16×150 mm) were used in all assays. Growth medium was basal medium Eagle (BME) consist of Earle's balanced salt solution (BSS) with 10% (V/V) inactivated new born calf serum (NCS), tryptose phosphate broth (TPB, 10 ml/100 ml) sodium bicarbonate (4.47 ml of 7.5% solution/1000 ml), glutamine (1 ml/100 ml), penicillin (100 units/ ml), streptomycin (100 g/ml) and mycostatin (50 units/ ml). For maintenance medium, serum was reduced to 2%, TPB and phenol red were excluded and sodium bicarbonate was replaced by N-2-hydroxy-ethylpiperazine-N' - 2-ethanesulfonic acid, 25 m M(HEPES) and sodium hydroxide (1.5 ml of 5% solution / 100 ml).

For antiviral assay, after removal of growth medium from monolayers, tubes were washed by 37°C warmed PBC twice and cultures were inoculated with 100 $TCID_{50}$ of virus for 1 hr at 37°C.

Immediately the cultures were exposed to varying concentrations of test compounds (3 tubes per dilution) in maintenance medium. The viral CPE was recorded daily.

Scheme 2



12 B=Guanine, R=OBZ, $R^1=CH_2Cl$, $R^2=H$ (N-9)
13 B=Guanine, R=OH, $R^1=CH_2Cl$, $R^2=H$ (N-9)
14 B=Guanine, R=OH, $R^1=R^2=H$ (N-9)
15 B=Adenine, R=OH, $R^1=R^2=H$ (N-9)
16 B=Adenine, R=OH, $R^1=R^2=H$ (N-7)
17 B=Adenine, R=OH, $R^1=CH(OH)CH_2OH$, $R^2=H$ (N-9, 2R, 3R)
18 B=Adenine, R=OH, $R^1=CH(OH)CH_2OH$, $R^2=H$ (N-9, 2S, 3S)
19 B=Adenine, R=OH, $R^1=H$, $R^2=CH_2OH$ (N-9)

In parallel with the antiviral assay, confluent Hela cells (uninfected) were exposed to various concentrations of the test compounds in maintanance medium. After completion of CPE (100% cell destruction) in the control (virus infected), medium of tubes were removed aseptically, and cells were fixed by Boin's solution for 15 mins. at room temp. and stained by Heamatoxylin-Eosin staining method.

Antiviral activity was expressed as ED_{50} (the concentration of compound required to reduce the viral CPE by 50%).

Cytotoxicity was expressed as ED_{50} against cell morphology (the concentration of compound required to cause microscopically visible change or disruption in cell sheet in about half of the cells).

Biological Activity

The compounds described in this report and in the previous ones [1,11] were all tested for activity against HSV-1. Most of them did not show much toxicity towards cells. The results are summerized in Table 1, and show that the 50% inhibitory levels of the test compounds against HSV-1 ranged from 0.15 μg/ml for **14** to 17.72 μg/ml for **8**. The 50% inhibitory levels of the compounds against cell morphology is at least 97.5 μg/ml for vidarabin. This shows a broad safety margin of compounds.

Experimental Section

General Remarks. See Ref 1.

Preparation of 1-benzoyloxy - 3 - chloroprogan -2-01(2) and 1,3-dibenzoy loxypropan-2-01(3). Benzoic acid (2.44 g, 0.02 mol) and sodium bicarbonate (0.84 g, 0.01 mol) were dissolved in DMF (30 ml) containing epichlorohydrin (0.925 g, 0.01 mol).The reaction mixture was stirred at 25° for 12 h. The solution was partitioned between ether and water. The etherial layer was washed with water (2×100 ml), dried ($MgSO_4$), and evaporated to afford **2** (95%) as an oil. $R_f(CH_2Cl_2)$: 0.17; R_f (ether/hexane, 1:1): 0.33. NMR.(CCl_4):δ 3.32 (s,1H,OH), 3,67 (d,2H,CH_2Cl), 3.98-4.51 (m,1H,CHO), 4.60 (d,2H,CH_2O), 7.41-8.20 (m,5H,Ph). IR.(CCl_4): 3490 (OH), 1720 (ester) Cm^{-1}.

When benzoic acid (0.02 mol) and sodium bicarbonate (0.02 mol) was refluxed in DMF containing epichlorohydrin (0.01 mol), by similar work up, **3** was obtained (80%). $R_f(CH_2Cl_2)$: 0.06; R_f(ether/hexane, 1:1): 0.19. NMR(CCl_4:δ 3.40 (br., 1H,OH), 4.21-4.69 (br.s, 5H, CH_2CHCH_2), 7.29-8.19 (m, 10H, 2Ph). IR(CCl_4): 3480 (OH), 1735 (ester)Cm^{-1}.

Preparation of 1 - benzoyloxy - 3 - chloro - 2 -

Table 2. Antiviral Activity and Selectivity of Acyclonucleosides in Hela Cell Cultures.

Compounds	ED_{50} (μg/ml)		Antiviral Index (B/A)
	HSV-1 (A)	Cell Morphology (B)	
7	11.36	132.00	11.61
8	17.72	369.00	20.82
13	0.17	155.60	915.29
14	0.15	245.00	1633.33
15	0.38	375.00	986.84
16	4.43	235.00	53.04
17	1.39	195.00	140.28
18	1.70	205.00	120.58
19	4.70	154.00	32.76
Vidarabine	14.80	97.50	6.58

chloromethoxy - propane (4) and 1- chloro - 2-chloromethoxyethane (9). Both compounds were prepared by an identical manner. Representative procedure: 1-Benzoyloxy-3-chloropropan-2.01 (0.20 mol) was added to dichloro-methane (50 ml) followed by paraformaldehyde (8g). The reaction mixture was cooled in an ice bath and dry HCL gas was bubbled through the stirred solution for 6 h. Anhydrous $CaCl_2$ was then added and after stirring for a few min. the solution was collected by filtration. The filtrate was evaporated at reduced pressure to yield compound **4** (50%). $R_f(CH_2Cl_2)$: 0.51. NMR (CCl_4):δ 3.71 (d, 2H, Ch_2Cl), 3.90-4.31 (m, 1H, CHO), 4.73 (d, 2H, CH_2O), 5.72 (s, 2H, OCH_2Cl), 7.37 (m, 5H, Ph). IR.(CCl_4): 1745 (ester).

Preparation of 9-[[2-benzoyloxy-(chloromethyl) ethoxy] methyl] -6-chloropurine (5) and 7-[[2-benzoyloxy-1-(chloromethy1) ethoxy] methyl] -6-chloropurine (6). 6-chloropurine (4.5g 29.25 m mol) was dissolved in DMF (50 ml) and triethylamine (4g) was added. The solution was cooled in an ice-salt bath and compound **4** (30 mmol) was added. After stirring for 1 h the mixture was removed from the ice bath and was allowed to stir at 25° for 12 h. The solution was partitioned between water and ethylacetate. The organic layer was washed with water (5×100 ml), dried ($MgSO_4$), and evaporated to yield 13 g of sirup. The residue was dissolved in a minimum of chloroform and applied to a silica gel column (24×6.3 Cm) eluted with 1% methanol in chloroform. The yield of compound **5** was 70% and that of compound **6** was 20%. Compound **5**: NMR ($CDCl_3$):δ 3.65 (d, 4H, CH_2Cl, CH_2OBZ), 4.15 (m, 1H, CHO), 5.90 (s, 2H, OCH_2N), 7.55 (m, 5H, Ph), 8.33 (s, 1H, H-C(2)), 8.79 (s, 1H, H-C(8)). UV. (MeOH): 264 nm. Compound **4**:The NMR spectra similar to that of the **5**. IR.(neat): 1725, 1600, 1569, 1500,1100 cm^{-1},similar to that of the **5**.UV.(MeOH): 268 nm.

Preparation of 9-[[2 - hydroxy - 1 - (chloromethyl)ethoxy] methyl] adenine (7). Comound **5** (0.05 mol) was dissolved in methanol (50 ml) and 60 ml of saturated MeOH/NH_3 was added. The solution was sealed in a pressure bottle which was then placed in an oil bath maintained at 90°for 36 h. On cooling, the mixture was removed from the bottle, concentrated to about 5 ml, and was precipitated by ether (50 ml). Crystalization from acetone gave pure **7** (95%). M.P. 192°. R_f(ether/MeOH, 4:1): 0.33. NMR (DMSO-d6):δ 3.55 (br., 1H, OH), 3.81 (d, 4H, CH_2O, CH_2Cl), 4.25 (m, 1H, CHO), 5.81 (s, 2H, OCH_2N), 7.45(br., s, 2H, NH_2), 8.31 (s, 1H, H-C (2)), 8.41 (s, 1H, H-C(8)). IR. (nujol): 3310, 3110, 1661, 1600, 1090 cm^{-1}. UV.(MeOH): 258 nm.

Preparation of 7 - [[2 - hydorxy - 1 - (chloromethyl)ethoxy] methyl] adenine (8). Compound **6** (0.05 mol) was treated as above for **5**. The product **8** was obtained in 90% yield. M.P. 209°. R_f(ether/ MeOH, 4:1): 0.11. NMR. (DMSO-d6):δ 3.41 (br., 1H, OH), 3.72 (br., 5H, CH_2O, CHO CH_2Cl), 5.71 (s, 1H, OCH_2N), 7.40 (br., s, 2H, NH_2), 8.29 (s, 1H, H-C (2)), 8.40 (s, 1H, H-C (8)). IR. (nujol): 3100-3290, 1711, 1620, 1580, 1510, 1115 cm^{-1}. UV.(MeOH): 257 nm.

Preparation of 3,9-(Ethanoxymethano) adenine-3-ium Chloride (20). Compound **20** was obtained (80%) by treatment of 9 with 6-chloropurine to afford 10. Reaction of **10** with MeOH/NH_3 gave **20**. The procedures were identical to those explained for the preparation of **7** from **4**. Compound **20** was found to be identical with an authentic sample [1].

Preparation of 9-[(2-benzoyloxy-1-(chloromethyl) ethoxy] methyl] guanine(12) and 9-[(2hydroxy-1-(chloromethyl) ethoxy] methyl] guanine (13).

Guanine (0.09 mol) was suspended in HMDS. Ammonium sulfate (1g)was added and the mixture was heated at reflux with exclusion of moisture for 25 h. The, by now clear, solution was concentrated at reduced pressure to leave a pale yellow solid. Mercuric cyanide (24 g, 0.095 mol) and benzene 250 ml were added and the system was heated to reflux. To the refluxing solution was added compound **4** (0.093 mol). The system was heated at reflux for 10 h. The benzene was removed at reduced pressure and the residue was stirred with CH_2Cl_2 (800 ml). The solution was washed successively with K_2CO_3 (10% in H_2O, 200 ml) and water (200 ml). The solution was then evaporated to small volume. Methanol (50 ml) was added and the solvents were removed at reduced pressure. The residue was applied to a silica gel column (8.5×30 Cm, 200 g). Elution with AcOEt/MeOH 4:1 gave **12** (40%). M.P. 230 dec. R_f(iPrOH/NH_4OH/H_2O, 7:1:2) 0.89. NMR.(DMSO-d6/D_2O):δ 3.71 (d, 4H, CH_2Cl, CH_2OBz) 3.99 (m, 1H, CHO), 5.88 (s, 2H, OCH_2N), 7.61 (m, 5H, Ph), 8.20 (br.s, 1H, H-C(8)). UV. (MeOH): 253, 270 (sh) nm.

Treatment of **12** with MeOH/NH_3, after 20h at 25°, gave **13** (97%). M.P. 270 dec. R_f(iPrOH/NH_4OH/H_2O, 7:1:2) 0.41. NMR.(DMSO-d6/D_2O): 3.81 (m, 5H, OCH_2CHCH_2Cl), 5.89 (s, 2H, OCH_2N), 8.31 (s, 1H, H-C(8)). UV. (MeOH):253, 270 (sh) nm.

Preparation of Compounds 7 and 11 by Fluoride Method. Both compounds were prepared by identical procedure which will be described for **11** only.

Adenine (0.01 mol) was suspended in HMDS (50 ml). Ammounium sulfate (0.3 g) was added and the mixture was heated at reflux temperature until a clear solution was obtained (10 h). The solvent was evaporated and the residue was dissolved in dry THF. Chloromethyl ether **9** (0.015 mol) was added and the solution was refluxed. Tetra-n-Butylammonium fluoride (0.01 mol) in dry benzene (5 ml) was added within 1h. After 3h, the solution was partitioned between EtOAc and water. The organic layer was evaporated and the residue was dissolved in MeOH. Evaporation of MeOH and purification of residue on silica gel using $CHCL_3$ as solvent gave **11** (80%). M.P. 110-111. R_f(AcOEt/MeOH 9.5:0.5) 0.46. NMR ($CDCl_3$):δ 3.80 (m, 4H, $ClCH_2CH_2O$), 5.69 (s, 2H, OCH_2N), 7.30 (br. 2H, NH_2), 8.21 (s, 1H, H-C (2)), 8.41 (s, 1H, H-C (8)). UV.(MeOH): 260 nm.

Treatment of **11** with MeOH/NH_3 at 90° gave 3,9-(ethanoxy-methano)adenin-3-ium chloride[1](20)in quantitative yield, m.p. 225°.

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