A novel approach to the synthesis of the purine anti-viral agent ganciclovir

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The aim of this research is to synthesize ganciclovir, a purine anti-viral drug in large scale via simple intermediates. Reaction of guanine 1 with acetic anhydride followed by the attachment of the 2-O-acetoxymethyl-1,3-di-O-benzyl glycerol 3 as a side chain, subsequently lead to the production of N2-acetyl-9-(1,3-dihydroxy-2-propoxymethyl)guanine 6 and N2, acetyl-9-(1,3-dihydroxy-2-propoxymethyl)guanine 7 and finally synthesizing ganciclovir 8. Among many pathways to the synthesis of purine derivatives, our four step procedure results in good yield and is proved to be an economic way of large scale synthesis.

Ganciclovir, 9-(1,3-dihydroxy-2-propoxymethyl)guanine 8, is a purine anti-viral drug which was first synthesized in 198214. Today, it is best known for the treatment of cytomegalovirus (CMV) in AIDS and is also used in the gene therapy of cancer. Its mechanism of action involves phosphorylation by cellular enzymes in infected cells. It has been shown that its concentration in infected cells is 10 times higher than in normal cells. It has been demonstrated that reduction in the level of phosphorylated ganciclovir, or mutation in DNA polymerase, may induce resistance of CMV to ganciclovir 8. Like many potent anti-viral drugs, ganciclovir exhibits adverse side effects such as retardation effects on bone marrow and central nervous system, severe headaches and anemia 7.

We required a concise, efficient and economical synthesis amenable to the large scale preparation of ganciclovir. The chemistry on the side chain prior to alkylation was investigated and this strategy allowed minimum consumption of formerly scarce purine reagents, and the least likelihood of side chain chemistry conflicting with functionality present on the purine nucleus.

The purine reagent for this synthesis was guanine 1, which was synthesized from guanidine nitrate in two steps (Scheme I), as described earlier 8. This modified procedure is based on a method reported in a patent for the synthesis of a pyrimidine derivative as the first step 9. Although purine derivatives are currently readily available, this two stage synthesis provided an economical, high yield synthesis where the yield was about 81%.

The general structure of the side chain 2 suggests that Y should be a good leaving group such as halogen or acetox, while the Xs are oxygen protecting groups that prevent potential reaction with the guanine ring. These hydroxyl protection groups must be fully removed in the last stage of the ganciclovir synthesis. In order to achieve these characteristics, we had used two methods in our earlier works.

In the first method, 2-O-acetoxymethyl-1,3-di-O-benzylglycerol 3 was synthesized via a three step procedure from epichlorohydrin (Scheme II)8, based on modification of two methods 10,11. As shown in Scheme II, epichlorohydrin was reacted with benzyl alcohol in the presence of a strong base (sodium hydride) at room temperature for 16 hr to give 1,3-dibenzy1 glycerol which was treated with paraformaldehyde in dichloroethane as a solvent at 0°C for 2 hr to yield, 1,3-di-O-benzyl-2-O-chloromethyl glycerol. It was kept at 40°C for at least 20 hr and then, sodium acetate in DMF was added in order to replace chlorine with acetox group (16 hr at room temperature).

The second method was similar to the first procedure described above. The only difference was that the first intermediate, 1,3-dibenzy1 glycerol, was formed in a more simple way. Benzyl alcohol was completely deprotonated in the presence of highly concentrated sodium hydroxide. Epichlorohydrin was then added slowly at low temperature. The mixture was vigorously stirred for 16 hr after which time 1,3-dibenzy1 glycerol was synthesized.
The reaction sequence for the total synthesis of gancyclovir 8 is schematically presented in Scheme III. The guanine 1 on reaction with acetic anhydride gave N₂, 9-diacetylguanine 4. Compound 4 was treated with 3 in the presence of toluenesulfonic acid to yield a mixture of two isomers, N₂-acetyl-7-(1,3-dibenzyloxy-2-propoxymethyl)guanine 5 and N₂-acetyl-9-(1,3-dibenzyloxy-2-propoxymethyl)guanine 6. The isomer 6 separated by toluene, was then reacted with 20% Pd-hydroxide/C under N₂ atm. to afford N₂-acetyl-9-(1,3-dihydroxy-2-propoxymethyl)guanine 7, which on reaction with 56% NH₄OH gave gancyclovir 8 (Scheme III).

**Experimental Section**

IH NMR spectra were recorded on a Bruker AMX 400 spectrometer at 400 MHz using DMSO-d₆ as solvent using TMS as internal standard (chemical shifts in δ, ppm), and IR spectra in KBr on a Perkin-Elmer model 457 infrared spectrophotometer.

**Synthesis of N₂, 9-diacetylguanine 4.** Guanine 1 (20 g, 0.132 moles) in acetic anhydride (300 mL) was refluxed for 16 h. on an oil bath. The excess acetic anhydride was removed by distillation under reduced pressure. The resulting solid N₂, 9-diacetylguanine 4 was washed 3 times with 15 ml aliquots of toluene, yield: 95%, mp > 300°C; IR (KBr, cm⁻¹): 3150, 1720, 1705, 1685, 1605, 1528, 1220/619; ¹H NMR: δ 2.2 (s, 3H, 9-COCH₃), 2.58 (s, 3H, 2-COCH₃), 7.8 (s, 1H, N-H), 8.1 (s, 1H, H-8).

**Synthesis of N₂-acetyl-9-(1,3-dibenzyloxy-2-propoxymethyl)guanine 6.** In a two-necked flask equipped with a condenser a mixture of 3 (650 g, 1.88 moles), 4 (423 g, 1.88 moles), p-toluenesulfonic acid (4 g, 0.02 moles) and sulfalene (500 mL) was heated at 95°C under a N₂ atmosphere for 13 hr. The reaction was allowed to proceed after addition of p-toluenesulfonic acid (4 g, 0.02 moles) for 72 hr under the same conditions. The reaction mixture was cooled to room temperature and toluene (4 mL) was added. The mixture was transferred to a chromatographic column containing silica gel after filtration. The column was washed with toluene, dichloromethane and a 20% solution of dichloromethane in methanol. The oily mixture contained two isomers, N₂-acetyl-7-(1,3-dibenzyloxy-2-propoxymethyl)guanine 5 and N₂-acetyl-9-(1,3-dibenzyloxy-2-propoxymethyl)guanine 6. Addition of toluene to this oily mixture induced separation of N₂-acetyl-9-(1,3-dibenzyloxy-2-propoxymethyl)guanine isomer 6 as a white precipitate which was recrystallized from ethyl acetate, yield 262g (31%), mp 145-46°C; IR (KBr, cm⁻¹): 3320, 3150m, 3050m, 1720s, 1600s, 1570s, 1550s, 1280s, 1120s, 780s; ¹H NMR: 8.13 (s, H, H-8), 7.82 (s, 1H, N-H), 7.35 (m, 10H, ArH), 5.95 (s, 2H, CH₂), 4.41(s, 4H), 4.05 (m, 1H, H-4'), 3.4 (m, 4H, H-3', H-5'), 2.18 (s, 3H, CH₃).

**Synthesis of N₂-acetyl-9-(1,3-dihydroxy-2-propoxymethyl)guanine 7.** To 2100 mL of cyclohexane was added 20% paladium hydroxide in carbon (6.6 g,
0.08 moles), ethanol (940 mL) and compound 6 (90 g, 0.2 moles). The mixture was refluxed under a nitrogen atmosphere for 8 hr. The additional catalyst (1.3 g) was then added and the refluxing of mixture was continued for 32 hr. The mixture was cooled to room temperature, filtered and the filtrate was added
to boiling water (500 mL). The resulting solid thus obtained was filtered and washed with boiling water (250 mL). Addition of methanol to the solid, precipitated product 7 as a white solid which was recrystallized from methanol/ethyl acetate (1:1), yield 90%, mp 205-08°C; IR (KBr, cm⁻¹): 3460, 3320, 3210, 2890, 1700, 1680, 1460, 1360s, 1180, 780; ¹H NMR: 7.86 (s, 1H, H-8), 7.6 (s, 1H, N-H), 6.52, (s, 2H, NH₂), 5.38, (s, 2H, CH₂), 4.71 (m, 1H, H-4'), 3.52 (m, 4H, H-3', H-5'), 2.62 (s, 3H, COCH₃).

Synthesis of 9-(1,3-dihydroxy-2-propoxy-methyl)guanine, Gancyclovir 8. To a 56% solution of ammonium hydroxide (800 mL) was added 7 (105 g, 0.36 moles) and methanol (800 mL). The mixture was stirred at room temperature for 17 hr. Methanol and water were distilled off and the white precipitate of gancyclovir 8, was recrystallised from 700 mL distilled water, yield 94%, mp 250°C, IR (KBr, cm⁻¹): 3480, 3300, 3210, 2880, 2830, 1740, 1680, 1640, 1480, 1360, 1100s, 760, 701; ¹H NMR: 10.64 (bs, 1H, NH, NH), 7.81 (s, 1H, H-8), 6.5 (s, 2H, NH₂), 5.44 (s, 2H, H-2, H-1'), 4.63(m, 1H, H-4'), 3.55(m, 4H, H-3', H-5').

References