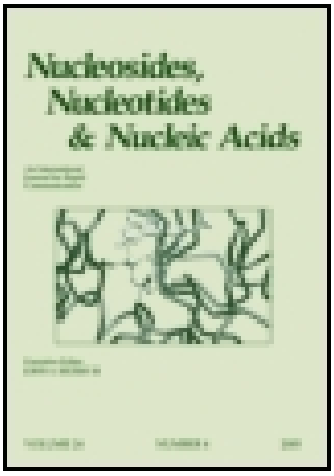

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6-Deoxycarbovir: A Xanthine Oxidase Activated Prodrug of Carbovir

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6-DEOXYCARBOVIR: A XANTHINE OXIDASE ACTIVATED PRODRUG OF CARBOVIR

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Abstract. (-)-(cis)-4-(2-Amino-9H-purin-9-yl)-2-cyclopentenyl carbinol (6-deoxycarbovir) was prepared in order to evaluate prodrug approaches to increased bioavailability of the anti-HIV agent, (-)-carbovir. Incubation experiments demonstrated that 6-deoxycarbovir was rapidly converted to (-)-carbovir by the enzyme, xanthine oxidase. Since xanthine oxidase activity is present in both the intestine and liver, a high first pass conversion to carbovir would be expected in vivo.

Introduction

Carbocyclic 2',3'-didehydro-2',3'-dideoxyguanosine (carbovir) has been identified as a potent and selective inhibitor of HIV-1 replication and cytopathic effects in a variety of human T-lymphoblastoid cell lines.[1,2] Racemic carbovir was subsequently separated into its D and L enantiomers which led to the observation that the antiviral activity resides in the (-) isomer that is analogous to the nucleoside, β-D-2',3'-didehydro-2',3'-dideoxyguanosine.[3] A comparison of myelotoxicities of the three antiviral agents, 3'-azido-3'-deoxythymidine (AZT), 2',3'-didehydro-2',3'-dideoxythymidine (D4T), and carbovir revealed that carbovir was the least toxic to human and murine heamatopoietic progenitor cells and AZT the most toxic.[4,5] Also, a specific difference between carbovir and other dideoxynucleosides, including AZT, is its relatively low inhibitory activity against DNA polymerase γ.[6,7] It has been suggested that the peripheral neuropathy associated with administration of dideoxynucleosides is due to their inhibition of DNA polymerase γ.[8] Thus, carbovir offers several potential advantages over the currently available nucleoside analogs. For this reason, carbovir and its prodrug forms are undergoing preclinical evaluation for the treatment of AIDS.

Previous studies have demonstrated that the oral bioavailability of the active enantiomer, (-) carbovir, is approximately 20%.[9] Several prodrugs of carbovir have been synthesized to improve its bioavailability. For example, the pharmacokinetic evaluation of (-) 6-aminocarbovir as an adenosine deaminase activated prodrug revealed an absolute bioavailability for (-) carbovir of 46.2%, a 2.5-fold increase compared to (-) carbovir.[10] In order to evaluate other prodrug approaches to increased bioavailability, we have prepared (-) 6-deoxycarbovir. Krenitsky and coworkers have reported that 9-substituted-2-aminopurines can be converted to 9-substituted guanines by the enzyme, xanthine oxidase.[11] Thus, increased oral absorption of (-) 6-

deoxycarbovir and subsequent enzymatic oxidation to (-) carbovir may provide increased bioavailability of the parent drug.

Chemistry

(-) Carbovir (**1**) was prepared from cis-[3-(2,6-diamino-9H-purin-9-yl)cyclopentenyl]carbinol (racemic diaminocarbovir) as described previously.[3] The alcohol moiety was subsequently blocked by esterification with acetic anhydride to give **2** (Scheme 1). The guanine moiety of **2** was converted to the 6-chloro-2-aminopurine intermediate **3** with phosphorous oxychloride in diethylaniline. The oily product was carried on to the next step without purification. Thus, treatment of **3** with methonolic ammonia provided the crystalline (-) 6-chlorocarbovir **4** as a pure product. The desired (-) 6-deoxycarbovir **5** was easily obtained by removal of the chloro group of **4** with zinc dust in ammonium hydroxide. The synthesis of (-) 6-aminocarbovir **6** was also prepared from the same intermediate with ammonia.

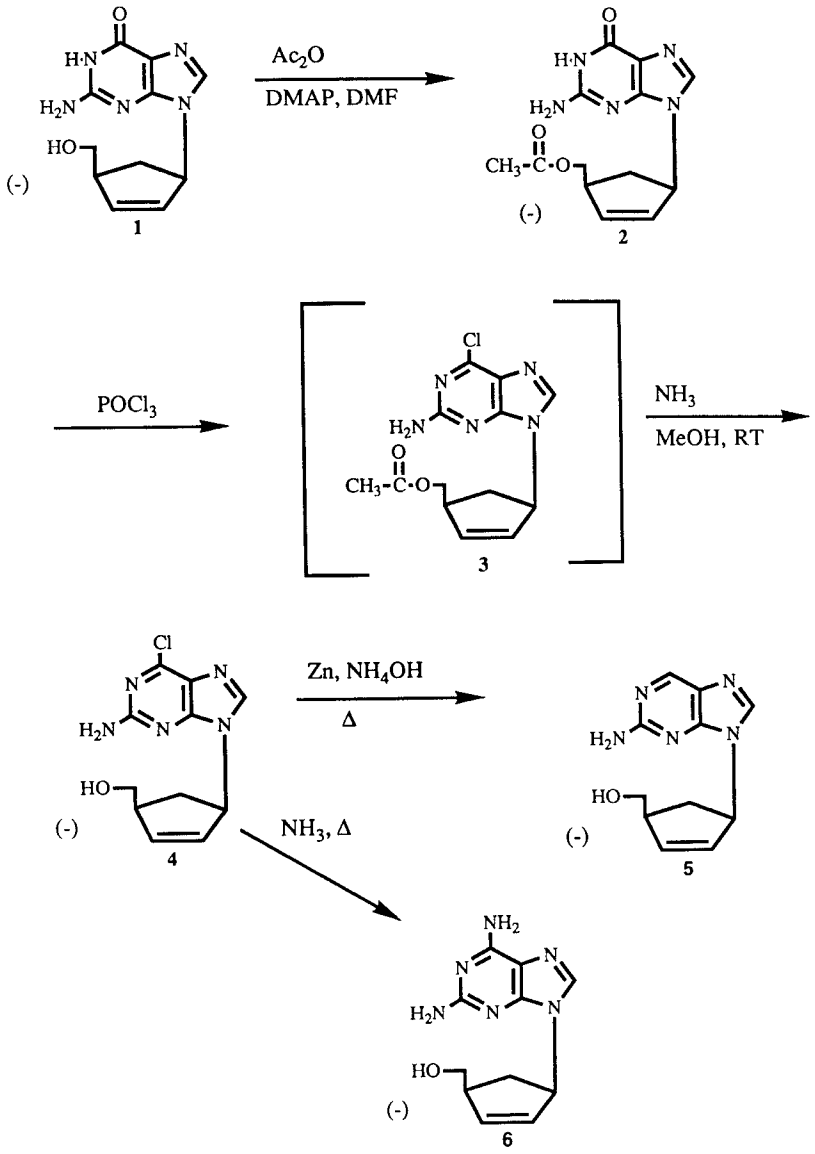
Results

The substrate activity of (-) 6-deoxycarbovir was evaluated using bovine milk xanthine oxidase (EC 1.2.3.2). The conversion of 5 to (-) carbovir was followed spectrophotometrically as illustrated in Figure 1. Thus, at pH 7.2, the initial λmax for (-) deoxycarbovir (223 nm) slowly decreased with a concomitant increase in absorption at 250 nm corresponding to that of (-) carbovir. In a second experiment, 50 mg of (-) 6-deoxycarbovir was incubated with xanthine oxidase and yielded a pure sample of (-) carbovir as the sole product.

To summarize, (-) 6-deoxycarbovir is rapidly converted to (-) carbovir by xanthine oxidase. Xanthine oxidase activity is present in high amounts in both the intestine and liver, so a high first pass conversion to carbovir would be expected in vivo. Animal studies designed to confirm this hypothesis are underway and will be presented elsewhere.

Experimental. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Melting points were determined on a Mel-Temp apparatus and are corrected. Nuclear magnetic resonance spectra were obtained on a General Electric 300. IR spectra were determined with KBr pellets on a Perkin-Elmer 281 spectrometer, and UV spectra were determined on a Beckman DU-70-spectrophotometer. Thin-layer chromatography (TLC) was performed on 0.25-mm layers of Merck silica gel 60F-254 and column chromatography was done on Merck 60 (230-400 mesh). Mass spectra were obtained with an AEI Scientific Apparatus Limited MS-30 mass spectrometer.

(-)-(1R,4S)-2-Amino-1,9-dihydro-9-(4-hydroxymethylacetyl-2-cyclopenten-1-yl)-6H-purin-6-one (2). (-)-Carbovir (**1**) (500 mg, 2.02 mmol) was suspended in DMF (15 ml), and to this suspension was added triethylamine (0.50 ml, 3.59 mmol), 4-dimethylaminopyridine (20 mg), and acetic anhydride (0.20 ml, 2.07 mmol). This was stirred at ambient temperature for 75 minutes, after which time methanol was added to quench the reaction and the solvents were removed in vacuo. The crude product was purified on a silica gel column. Elution with chloroform-methanol 9:1 v/v gave 524 mg (90%) of **2**. An analytical sample was obtained by recrystallization from methanol-water; mp 238-242° C; MS (EI, 30 eV) 289 (M^+), 151 (B^+); IR (KBr) 3395, 3332 (OH, NH), 2896 (aliphatic C-H), 1736 (ester), 1694 (amide), 1630, 1602



Scheme 1

(C=C, C=N) cm^{-1}; NMR (DMSO d_6) δ 7.58 (s, 1H, 8-H), 6.44 (s, 2H, NH_2, exch), 6.10 (m, 1H, vinyl C-H), 5.95 (m, 1H, vinyl C-H), 5.36 (m, 1H, 1'-H), 4.07 (d, 2H, OCH_2), 3.08 (m, 1H, 4'-H), 2.65 (dt, 1H, CHH), 2.01 (s, 3H, CH_3), 1.55 (dt, 1H, CHH); Optical rotation $[\alpha]_D^{23}$, -99.6° (c, 0.15 methanol). Anal. Calc'd for $C_{13}H_{15}N_5O_3$: C, 53.98; H, 5.19; N, 24.22; Found: C, 54.04; H, 5.35; N, 24.04.

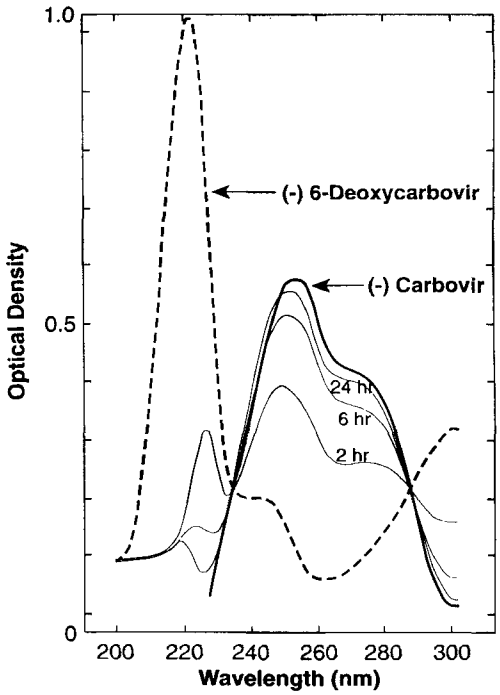


Figure 1. UV spectra of (-) 6-deoxy-carbovir, (-) carbovir, and the xanthine oxidase conversion of (-) 6-deoxy-carbovir to (-) carbovir at various time intervals.

(-)-(1S,4R)-4-(2-Amino-6-chloro-9-H-purin-9-yl)-2-cyclopentenylacetylcarbinol (3). Compound **2** (202 mg, 0.699 mmol) was placed into a 100 ml round bottom flask containing $POCl_3$ (8 ml) and N,N-diethylaniline (0.11 ml, 0.699 mmole). The flask was placed into an oil bath at 125° C for seven minutes. The excess phosphorylchloride was then removed in vacuo and ice water (15 ml) was added. This solution was stirred for 5 minutes before being extracted with chloroform (3 X 15 ml). The combined organic layers were washed with sat'd aqueous K_2CO_3 then water, and dried over magnesium sulfate. Evaporation of the chloroform gave **3** as an oil which was carried on to the next step without further purification. MS (EI, 30 EV), 307 (M^+), 169 (B^+); NMR ($CDCl_3$) δ 7.80 (s, 1H, 8-H), 6.10 (m, 1H, vinyl C-H), 5.95 (m,1H, vinyl C-H) 5.60 (m, 1H, 1'H), 5.37 (s, 2H, NH_2, exhc), 4.16 (t, 2H, $0CH_2$), 3.16 (m, 1H, 4'H), 2.85 (dt, 1H, CHH), 2.04 (s, 3H, CH_3), 1.68 (dt, 1H, CHH).

(-)-(1S,4R)-4-(2-Amino-6-chloro-9H-purin-9-yl)-2-cyclopentenylcarbinol (4). The ester **3** was dissolved in saturated menthanolic ammonia and allowed to stand at ambient temperature overnight. The solvents were removed in vacuo and the crude product was

chromatographed on a silica gel column. Elution with chloroform-methanol 19:1 gave **4** as an oil which was crystallized from methanol-water and yielded 78 mg (42% from **2**), mp 154-157° C; MS (EI, 30EV), 265 (M^+), 169 (B^+); IR (KBr) 3325, 3205 (OH, NH), 1609, 1560 (C=C, C=N); NMR (DMSO d_6) δ 8.03 (s, 1H, 8-H), 6.88 (s, 2H, NH_2) 6.17 (m, 1H, vinyl C-H), 5.92 (m, 1H, vinyl C-H), 5.45 (m, 1H, 1'H), 4.70 (t, 1H, OH), 3.45 (m, 2H, OCH_2), 2.90 (m,1H, 4'H), 2.65 (dt, 1H, CHH), 1.65 (dt, 1H, CHH); Optical rotation $[\alpha]_D^{23}$ -101.6°(c, 0.15, methanol). Anal. Calc'd for $C_{11}H_{12}N_5ClO.1/4\ H_20$: C, 48.89; H, 4.63; N, 25.92. Found: C,48.66; H, 4.66; N, 25.8.

(-)-(1S, 4R)-4-(2-Amino-9H-purin-9-yl)-2-cycopentenylcarbinol (5). A mixture of **4** (150 mg, 0.565 mmol), zinc dust (0.50 g), and concentrated ammonium hydroxide (20 ml) was heated under reflux for four hours. The reaction mixture was filtered and the filtrate was evaporated in vacuo. Purification of the final product was acheived on a silica gel column. Elution with chlorororm-methanol (9:1) yielded compound **5** as an oil. The oil was dissovled in abs ethanol/ether (1:1), and HCl gas was slowly passed through the solution. A dihydrochloride salt of **5** was formed and the white salt was removed by filtration; yield, 89 mg (51.7%); mp 150-155° C; MS (EI) 231 (M^+ freebase), 135 B^+; IR (KBr) 3367, 3304 (OH, NH), 1680, 1652 (C=C, C=N); NMR (DMSO), d_6) δ 9.0 (s, 1H, 8-H), 8.52 (s, 1H, 6-H), 8.15 (m, exch), 6.20 (m, 1H, vinyl C-H), 5.93 (m, 1H, vinyl C-H), 5.50 (m, 1H, 1-H'), 3.48 (m, 2H, OCH_2), 2.90 (1H, 4'-H), 2.65 (dt, 1H, CHH), 1.70 (dt, 1H, CHH); UV λmax 223 nm (0.1N HCl), Optical rotation $[\alpha]_D^{23}$ -124.7° (c, 0.15, methanol); Anal. Calc'd for $C_{11}H_{15}N_50\cdot 2HCl$: C,43.42; H, 4.93; N, 23.03. Found: C, 43.35; H, 4.99; N, 22.86.

(-)-(1S, 4R)-4-(2,6-Diamino-9H-purin-9-yl)-2-cyclopentenylcarbinol (6). The 6-chloro derivative **4** (300 mg, 1.13 mmol) was placed in a sealed stainless steel bomb with liquid ammonia and the bomb was heated in an oil bath at 70° C for 64 hours. The bomb was cooled and opened and the ammonia was allowed to evaporate. The remaining crude solid residue was purified on a silica gel column using chloroform-methanol (9:1) as the eluent and pure **6** was obtained from recrystallization from ethanol-ether; yield, 202 mg (75.2%); mp 106-108° C; MS (EI, 30EV) 246 (M^+), 150 (B^+); IR (KBr) 3466, 3395, 3332, 3184 (OH, NH), 1666, 1668, 1595 (C=C, C=N); NMR (DMSO, d_6) δ 7.60 (s, 1H, 8-H), 6.62 (s, 2H, NH_2), 6.10 (m, 1H, vinyl C-H), 5.86 (m, 1H, vinyl CH), 5.85 (s, 2H, NH_2), 5.40 (m, 1H, 1'-H), 4.73 (t, 1H, OH), 3.50 (m, 2H, OCH_2), 2.88 (m, 1H, 4'-H), 2.60 (dt, 1H, CHH), 1.60 (dt, 1H, CHH); Optical rotation $[\alpha]_D^{23}$ -78.7° (c, 0.15, methanol); Anal. Calc'd for $C_{11}H_{14}N_6O$: C,53.66; H, 5.87; N, 34.15. Found: C, 53.54; H, 5.69; N, 33.88.

Xanthine Oxidase Conversion of (-) 6-Deoxycarbovir to Carbovir. A solution of 50 mg of (-) 6-deoxycarbovir (**5**) dihydrochloride salt (0.164 mmole) and an eqivalent amount of NaOH was added to 2 ml of buffer (0.1M potassium phosphate, 0.1 mM EDTA, pH 7.2). To this solution was added 2 units of milk xanthine oxidase (EC 1.2.3.2) (Boehringer-Mannheim) and the solution was incubated at rt for 96 hr with the addition of additional 2 unit aliquots of enzyme at 24, 48, and 72 hr intervals. The solution was heated in boiling water for 3 minutes and filtered

through Celite to remove precipitated protein. The filtrate was concentrated and (-) carbovir was obtained by flash chromatography on silica gel using 5% methanol/chloroform followed by 10% methanol/chloroform as eluent. Fractions containing product were concentrated to a white solid which was subsequently recrystallized from water and gave 31.6 mg (75%) of pure (-) carbovir, mp 250-252° C; optical rotation $[\alpha]_D^{23}$ -62.6 (c, 0.3, methanol). This material was identical in all respects to an authentic sample of (-) carbovir.

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