SYNTHESIS OF CARBON-14 LABELLED *CIS*-2-AMINO-1,9-DIHYDRO-9-[4-(HYDROXYMETHYL)-2-CYCLOPENTEN-1-YL]-6H-PURINE-6-ONE; [8-^{14}C]CARBOVIR: A PROMISING ANTI-AIDS DRUG

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SUMMARY

The synthesis of the title compound (3) is described. Treatment of a solution of triethyl [^{14}C]orthoformate in dry chloroform with *cis*-[4-(2,5-diamino-6-chloro-4-pyrimidinyl)-amino]-2-cyclopentenyl]carbinol (1) afforded crude 2. Hydrolysis of crude 2 with 2 N sodium hydroxide gave 3 in 34% overall radiochemical yield with specific activity of 19.6 mCi/mmol.

Key Words: AIDS, HIV, Carbovir, carbon-14, triethyl [^{14}C]orthoformate, *cis*-2-amino-1,9-dihydro-9-[4-(hydroxymethyl)-2-cyclopenten-1-yl]-[8-^{14}C]-6H-purine-6-one

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a devastating disease that results from infection by human immunodeficiency virus (HIV) (1). Although a number of nucleosides have been tested as anti-HIV agents (2), only 3'-azido-3'-deoxythymidine (AZT) has been approved for the treatment of AIDS. However, the hematologic toxicity of AZT (3) and the emergence of AZT resistant HIV variants (4) provide powerful incentives for the discovery of superior therapeutic agents. In this regard, Vince et al. (5) have tested a series of carbocyclic nucleoside analogs as potential anti-HIV drugs. They identified carbocyclic 2',3'-didehydro-2',3'-dideoxyguanosine (3) (Carbovir: NSC-614846) as a potential antiretroviral agent which inhibited the infectivity and replication of HIV in T-cells at a concentration of approximately 200-400 fold below toxic concentrations. A recent paper (6) describes the synthesis and anti-HIV activity of carbovir (3) and related nucleosides.

0362-4803/91/060645-05$05.00

Received 13 October, 1990
Revised 7 January, 1991

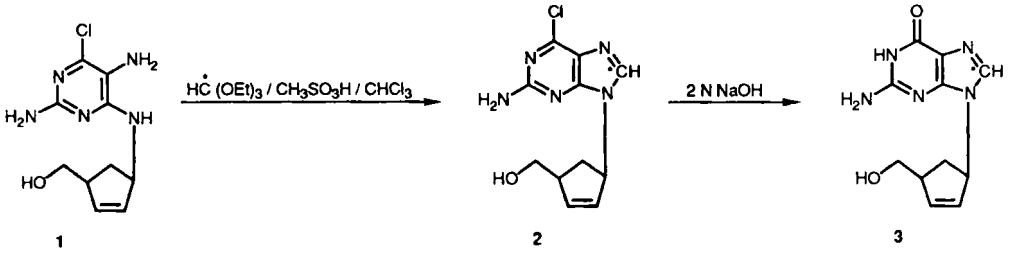
This document describes the synthesis of [8-^{14}C]carbovir (3) which was carried out as an aid to the biological evaluation of this new potential anti-AIDS compound.

RESULTS AND DISCUSSION

The synthesis of [8-^{14}C]-carbovir (3) is summarized in Scheme I. A solution of triethyl [^{14}C]orthoformate in dry chloroform, prepared by modification of a literature (7) procedure (Scheme II), was reacted with 1 (6) in the presence of methanesulfonic acid as catalyst to afford crude chloropurine 2. Hydrochloric acid was used as catalyst for this ring closure in the literature procedure(6), but proved inferior to methanesulfonic acid when triethyl orthoformate was used as limiting reagent. Hydrolysis of 2 with 2 N sodium hydroxide followed by recrystallization from methanol afforded [8-^{14}C]-carbovir as yellowish crystals in 34% radiochemical yield (based on the amount of potassium [^{14}C]cyanide used).

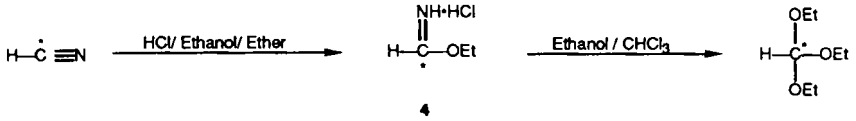
[8-^{14}C]Carbovir, thus prepared, had a specific activity of 19.6 mCi/mmol. HPLC analysis of the product indicated that it was 99% chemically pure (UV_{254} detection) and 97% radiochemically pure (radioactivity detection). The UV spectrum of [8-^{14}C]-3 [λ_{max} (0.1 N NaOH) 271 (ϵ 11200)] compared well with that of an analytically pure sample [λ_{max} (0.1 N NaOH) 271 (ϵ 11250)].

Scheme I



1 2 3

Scheme II



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EXPERIMENTAL

Potassium [^{14}C]cyanide was purchased from New England Nuclear Corporation. E. Merck silica gel 60 F-254 analytical plates were used for analytical TLC. Developed TLC plates were scanned on a Berthold Model LB283 Linear Analyzer system. HPLC was done using a Waters Assoc. Model 6000A dual pump system with a Model V6K septumless injector and a Berthold Model LB503-HDS radioactivity monitor as detector. Ultraviolet spectra were recorded on a Varian Model 2290 spectrophotometer. ^{1}H NMR was measured on a Bruker WM-250 spectrometer using tetramethyl silane as internal standard. Radioactivity was measured by a Packard Tri-Carb 4000 liquid scintillation counter.

cis-2-Amino-1,9-dihydro-9-[4-(hydroxymethyl)-2-cyclopenten-1-yl]-6H-purine-6-one; [8-^{14}C]Carbovir (3)

Triethyl [^{14}C]orthoformate was prepared by modifying the literature procedure (7). Dry hydrogen [^{14}C]cyanide (generated by the addition of 2.5 mL of 85% phosphoric acid to potassium [^{14}C]cyanide, 98.9 mCi, 5 mmol) was vacuum transferred at water aspirator pressure through a dry calcium chloride tube into a 10 mL flask fitted with a vacuum stopcock adapter, containing dry hydrogen chloride [generated from sodium chloride (10 mmol) and 10 mL of conc. sulfuric acid], dry ether (1.1 mL) and dry ethanol (0.5 mL). After reacting the mixture at -12°C for 72 h, volatiles were removed and the crystalline iminoester hydrochloride 4 (427 mg, 78%) was obtained. Dry chloroform (5 mL) and anhydrous ethanol (0.460 mL, 7.8 mmol) were added to the flask and the mixture was stirred and refluxed under argon for 1 h. (It had been determined previously that under these conditions, complete conversion of 4 into triethyl orthoformate occurred). After cooling to room temperature diamine 1 (993 mg, 3.9 mmol), methanesulfonic acid (35 μL) and dry chloroform (5 mL) were added and the mixture was stirred and refluxed under argon for an additional 2 h and 10 min. The mixture was transferred into a 100-mL flask and the volatiles were removed. Hot 2 N NaOH solution (50 mL) was added to the residue and the mixture was stirred and refluxed in an oil bath (130-140°C) under argon for 15 min. Norit (1.5 g) was added to the mixture and refluxing was continued for 15 min. After cooling to room temperature, the mixture was filtered. The

filtrate, after extraction with ethyl acetate (50 mL), was neutralized with ~ 3.9 N HCl and cooled in an ice bath for 2 h. The precipitate was collected, washed with ice water (20 mL) and dried in vacuum to afford crude 3 (680 mg, 55%) as an off-white solid. This material was dissolved in hot methanol (70 mL), cooled and filtered. The filtrate was concentrated to 20 mL and refrigerated for 40 h. The yellowish crystals that deposited were filtered and dried in vacuum for 1 h to afford 3 (425 mg, radiochemical yield 34%); UV_{max} (0.1 N NaOH) 271 nm (ϵ 11200); TLC Silica n-butanol-acetic acid-water (5:3:2) R_f 0.7. The specific activity was 19.6 mCi/mmol. The radiochemical purity was 97% (radioactivity detection) and chemical purity was 99% (UV_{254} detection) by HPLC [25 x 0.46 cm Dynamax-60A 8 μ C18 column, void-volume 2.0 mL, 75:25 (v/v) water-acetonitrile, 1.5 mL/min, t_R 1.7 min for [8-^{14}C]-carbovir and 2.5 min for the impurity, recovery of radioactivity > 99%]. ^{1}H NMR of an analytically pure nonlabeled sample prepared by the same procedure was identical to that of an authentic sample of carbovir[6]: ^{1}H NMR (DMSO-d_6): δ 1.57 (ddd, 1, J = 5.7, 5.8, 13.7 Hz, -CH_2), 2.59 (ddd, 1, J = 8.7, 4.9, 13.7 Hz, -CH_2), 2.86 (m, 1, C<u>H</u>CH_2OH), 3.44 (t, 2, J = 5.4 Hz, C<u>H</u>$_2$OH), 4.72 (t, 1, J = 5.2 Hz, OH), 5.34 (m, 1, C<u>H</u>N), 5.86 (m, 1, =C<u>H</u>-CH-CH_2OH), 6.11 (m, 1, =C<u>H</u>-CH-N), 6.44 (s, 2, NH_2), 7.59 (s, 1, CH=N), 10.58 (s, 1, NH or OH).

ACKNOWLEDGEMENT

This work was supported by the National Cancer Institute under Contract NO1-CM-87227. We wish to thank Dr. R. D. Haugwitz for helpful discussions and the authentic sample of carbovir.

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