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SYNTHESIS OF [3H]-DESCICLOVIR, PRODRUG OF THE ANTIVIRAL ACYCLOVIR

Allan R. Moorman* and John A. Hill†
Department of Experimental Therapy* and Chemical Development Laboratories†
Wellcome Research Laboratories
Research Triangle Park, North Carolina 27709

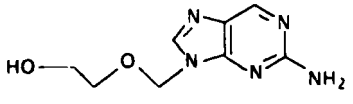
SUMMARY

The title compound was prepared by direct radiochemical synthesis from 2-acetylamino-9-[(2-hydroxyethoxy)methyl]-9*H*-purine. The product had a specific activity of 21.5 Ci mmol^{-1} and a radiochemical purity of 99.2%. The general approach described may be applicable to other "acyclic" nucleosides.

Key Words: [3H]-Desciclovir, Radioimmunoassay, Acyclovir, Prodrug

INTRODUCTION

Acyclovir {9-[(2-hydroxyethoxy)methyl]guanine, ZOVIRAX®} is an antiherpetic agent (1,2) currently in clinical use. It has recently been reported that desciclovir, **1**, {2-[(2-amino-9*H*-purin-9-yl)methoxy]ethanol, 6-deoxyacyclovir} is a prodrug of acyclovir with improved bioavailability (3). The mechanism of activation has been shown to involve oxidation at the 6-position of the purine moiety by xanthine oxidase (3). To facilitate the clinical evaluation of this drug, a radioimmunoassay (RIA) specific for desciclovir has been developed (4,5,6). Here we wish to report the synthesis of tritiated desciclovir **5**, by a method which may prove generally useful for other "acyclic" nucleoside analogues.



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*To whom correspondence should be directed.

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EXPERIMENTAL

Materials and Methods -- 2-Acetylamino-9-[(hydroxyethoxy)methyl]-9*H*-purine was obtained from Burroughs Wellcome Co., Chemical Development Laboratories, Greenville, NC. [3H]-Sodium borohydride (~86.0 Ci mmol^{-1}) was purchased from ICN Radiochemicals, Irvine, CA. All other chemicals were purchased from Aldrich Chemical Company, Milwaukee, WI, and were of the highest purity available.

Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H-nmr were recorded on a Varian XL-200 (200.057 MHz) in $CDCl_3$ and are reported relative to tetramethylsilane. Elemental analysis was carried out by Atlantic Microlabs, Atlanta, GA. Mass spectral analyses were carried out by Oneida Research Services, Whitesboro, NY. Radiochemical purity was determined using a Bioscan System 200 Imaging Scanner following thin layer chromatography on silica gel 60 with 4:1 $CHCl_3$:MeOH.

2-Acetylamino-9-[[(1,3-diphenylimidazolidin-2-yl)methoxy]methyl]-9*H*-purine -- (3)

A 250 ml three-necked flask was charged with 5.025 g (0.020 mole) of 2-acetylamino-9-[(hydroxyethoxy)methyl]-9*H*-purine, 2, and 45 ml of anhydrous DMSO. Dicyclohexylcarbodiimide (12.38 g, 0.060 mole) was added to the mixture, under argon. The mixture was stirred until homogeneous, cooled to 10°C, and 0.875 ml of dichloroacetic acid (0.011 mole) added over 10 minutes. After warming to room temperature, the mixture was allowed to stir under argon overnight.

The reaction was quenched by the slow, careful addition of a solution of 5.00 g oxalic acid dihydrate (0.040 mole) in methanol (20 ml). After stirring for 20 minutes, the mixture was filtered, the cake washed with methanol (4 x 20 ml), and the combined filtrates treated with 5.2 g of 1,2-dianilinoethane (85%, 0.021 mole). The solution was stirred for two hours at room temperature, during which time a precipitate formed. After collecting the precipitate, water was added to the filtrate to constant cloudiness, and the mixture chilled at 4°C overnight. The solid was collected, partitioned

between water and $CHCl_3$, and the organic layer dried ($MgSO_4$). After evaporation, the residue was combined with the initial precipitate and purified by flash chromatography (silica gel 60, 230-400 mesh, E. Merck, 2.5 x 22.0 cm, 3:2 $CHCl_3$:Acetone). Yield: 5.02 g (56%). The compound was sufficiently pure at this point to be used for the following steps. An analytical sample was prepared by recrystallization from MeOH/H_2O. mp: 155-56°C; ^{1}H-nmr ($CDCl_3$): δ 8.79 (s, 1H, H-6), 8.21 (br s, 1H, NH), 7.80 (s, 1H, H-8), 7.18 (dd, 4H, H-3 and H-5 of phenyl rings, J = 7.4, 7.9 Hz), 6.73 (t, 2H, H-4 of phenyl rings, J = 7.4 Hz), 6.57 (d, 4H, H-2 and H-6 of phenyl rings, J = 7.9 Hz), 5.42 (s, 2H, N-CH_2-O), 5.36 (t, 1H, N-CH-N, J = 2.6 Hz), 4.01 (d, 2H, C-CH_2-O, J = 2.6 Hz), 3.67-3.49 (ddd, 4H, N-CH_2CH_2-N), 2.50 (s, 3H, CH_3); EI-MS: 223 (N,N'-Diphenylimidazolidinyl); Cl-MS (CH_4): 472 (M+29), 444 (M+1), 267 (444 - 2-acetamidopurine), 223 (N,N'-Diphenylimidazolidinyl).

Analysis calculated for: $C_{24}H_{25}N_7O_2 \cdot 0.50\ H_2O$: C, 63 70: H, 5.79; N, 21.67. Found: C, 63.60; H, 579; 21.62.

2-O-[2-(Acetylamino)-purin-9-yl)-methyl]glycol aldehyde -- (4)

Compound 3 (0.887 g, 2.00 mmol) was added to a mixture of trifluoroacetic acid (5.00 ml) and water (1.00 ml), chilled to 10°C. The mixture was allowed to warm to room temperature, stirred one hour, then quenched with water (10.00 ml). The 1,2-dianilinoethane which had formed was removed by extraction with ethyl acetate (5 x 10 ml), the combined organic phases re-extracted with water (5.00 ml), and the combined aqueous portions adjusted to pH 7.00 with solid $NaHCO_3$. This was then diluted to 20.00 ml to afford a solution with a 0.1 molar theoretical concentration of 4 and used directly.

2-[(2-Amino-9*H*-purin-9-yl)methoxyl-1-[^{3}H]-ethanol -- (5)

The aqueous solution of 4 (3.15 ml, 78.8 mg theoretical content, 0.316 mmol) was added dropwise over approximately 60 seconds to a 10 ml long-neck round-bottom flask equipped with a magnetic stirring bar containing 6.3 mg of [^{3}H]-$NaBH_4$ (86 Ci mmol^{-1}) in 2.0 ml of distilled H_2O. After stirring under argon for 17 hours at room temperature, the mixture was treated with

acetone (1.00 ml), stirred for 10 minutes, and evaporated to dryness on a rotary evaporator. The residue was applied to a column of silica gel (2.0 g, 240-400 mesh) in 3:1 acetone: n-propanol and eluted with the same solvent, collecting eight 2 ml fractions and eight 5 ml fractions. Those fractions containing product (3-15) were combined and evaporated to dryness, affording 45.3 mg (56.6%) of the intermediate [^{3}H]-2 as a white solid.

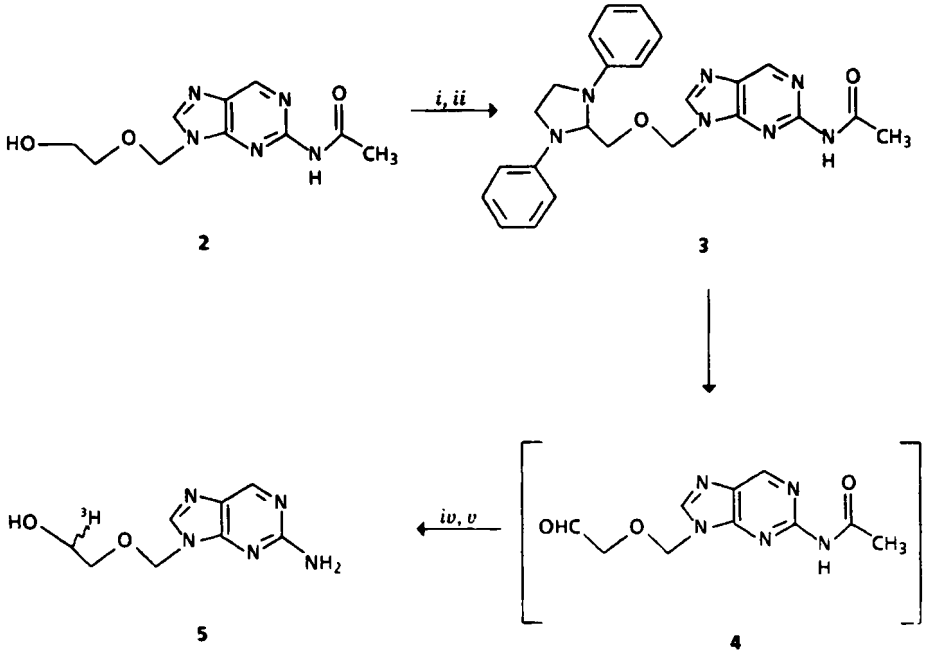
The solid was treated with 40% aqueous methylamine (10.00 ml) for 60 minutes at room temperature and evaporated to dryness. Acetone (3.50 ml) was added, the solid triturated thoroughly, and collected on a Hirsch funnel. After washing with fresh acetone (1.5 ml), the solid was dried *in vacuo* for 10 hours at 42°C, providing 10.3 mg of the desired 5 which comigrated on TLC (R_f = 0.22) with authentic desciclovir and was found to have a radiochemical purity of 99.2% and a specific activity of 21.5 Ci mmol^{-1}. The combined filtrate and washings could be recycled to yield additional compound 5.

RESULTS AND DISCUSSION

Acyclovir is a useful antiviral agent (1,2), shown to be clinically effective against herpes simplex infections (7), although only 15-20% of the oral dose is typically absorbed in humans (8). For less sensitive viruses, such as varicella zoster virus, increased oral bioavailability might be important (9). Considerable effort has been expended toward the development of a suitable prodrug which is well absorbed orally and then converted to acyclovir (3,10,11). One of the more promising approaches has been to utilize a congener of acyclovir lacking the 6-hydroxy group (3). This compound, desciclovir (1), is converted to acyclovir through the action of xanthine oxidase (3).

To facilitate the analysis of clinical trial samples, radiolabeled desciclovir was required for the development of a radioimmunoassay. Radioiodinated desciclovir was found to be unacceptable for this purpose, limited by the short half-life of ^{125}I and a narrow concentration range in which the assay was linear (5,12). We undertook the synthesis of tritiated desciclovir as shown in Scheme 1 to overcome these problems.

Earlier studies with acyclovir had suggested that the 2-amino-moiety on the purine base might lead to difficulties in preparing the imidazolidine-adduct of the intermediate aldehyde (13). To avoid these potential problems, we initiated our synthesis with 2-acetylamino-9-[(2-hydroxyethoxy)-methyl)-9*H*-purine, 2. Pfitzner-Moffatt oxidation (14) of 2 using pyridinium trifluoro-acetate as the acid catalyst led to extensive decomposition from which the imidazolidine adduct, 3, could be isolated in poor yield only after extensive column chromatography.



SCHEME 1

Synthetic Approach to [3H]-Desciclovir. *i.* DMSO, dicyclohexylcarbodiimide, dichloroacetic acid. *ii.* $(CO_2H)_2$, 1,2-dianilinoethane. *iii.* CF_3CO_2H:H_2O (5:1), $NaHCO_3$. *iv.* [3H]-$NaBH_4$. *v.* CH_3NH_2.

Substituting dichloroacetic acid for pyridinium trifluoroacetate and treatment of the intermediate aldehyde solution with 1,2-dianilinoethane allowed the desired compound, 3, to be readily isolated following flash chromatography.

Regeneration of the free aldehyde (4) was effected by treatment of 3 with trifluoroacetic acid in water. A 5:1 ratio was found to be optimal for the subsequent workup and reduction. The 1,2-dianilinoethane formed was readily

extracted with ethyl acetate to afford an acidic aqueous solution of the desired aldehyde. All attempts to isolate and characterize the aldehyde, 4, led to extensive decomposition. Following neutralization with solid $NaHCO_3$ and dilution to a more easily manipulated volume, we incorporated the desired label by reduction with [^{3}H]-sodium borohydride. The [^{3}H]-2 was purified by column chromatography and treated with aqueous methylamine to afford the desired compound, 5, with a specific activity of 21.5 Ci mmol^{-1} and a radiochemical purity of 99.2%.

The utility of the title compound in analyzing clinical trial samples of desciclovir has been previously described (4,5). The synthetic approach described may find wide application to the incorporation of tritium in a variety of "acyclic" nucleosides for use in developing radioimmunoassays and in the study of their metabolism.

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