# Syntheses of 9-(2′-monoethylphosphonomethoxyethyl)-8-[$^{14}$C]guanine ([$^{14}$C]-EPMG) and 9-(2′-phosphonomethoxyethyl)-8-[$^{14}$C]guanine ([$^{14}$C]-PMEG)

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## SUMMARY

The synthesis of the title compounds [$^{14}$C]-PMEG (5) and [$^{14}$c]-EPMG (6) are described. Treatment of [$^{14}$C]guanine with acetic anhydride in 1-methyl-2-pyrrolidinone gave [$^{14}$C]N-acetylguanine (2). Reaction with 2-(diethylphosphonomethoxy) ethylmethanesulfonate in N,N-dimethylformamide in the presence of potassium carbonate yielded 2-N-acetyl-9(2′-diethylphosphonomethoxyethyl)-8-[$^{14}$C]-guanine (3). Removal of the acetyl group with aqueous methylamine gave 9-(2′-(diethylphosphonomethoxyethyl)-8-[$^{14}$C]-guanine (4). Treatment of the diethylphosphonate ester with sodium hydroxide and acidification with hydrochloric acid gave 9-(2′-(monoethylphosphonomethoxyethyl)-8-[$^{14}$C]-guanine (5). Silation of the diethylphosphonate ester with bromotrimethylsilane and treatment with water produced 9-(2′-phosphonomethyloxethyl)-8-[$^{14}$C]guanine (6).

## KEY WORDS

Antiviral, 9-(2-monoethylphosphonomethoxyethyl)-8-[$^{14}$C]-guanine, ([$^{14}$C]-EPMG), 9-(2-phosphonomethoxyethyl)-8-[$^{14}$C]-guanine, ([$^{14}$C]-PMEG).

## INTRODUCTION

Phosphonate analogues of nucleoside monophosphates were recently described as having potent, broad-spectrum antiviral activity[1]. The most

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potent overall activity in this series is displayed by 9-(2'-phosphonylmethoxyethyl)guanine (PMEG) which is highly active against herpes simplex virus (HSV) types 1 and 2, varicella zoster virus and cytomegalovirus (CMV) in vitro. The biological evaluation of 9-(2'-monoethylphosphonomethoxyethyl)guanine (EPMG) showed that is also had antiviral activity. Compared to PMEG, EPMG was much poorer against HSV-1 and 2, but was similar against CMV. Because these compounds are nucleotide analogs, their mechanism of action may require phosphorylation to a triphosphate analog which will exert its antiviral effect at the level of viral DNA polymerase. Diphosphoryl-PMEG (the triphosphate analog) has been prepared and shown to inhibit HSV-1 polymerase. It has also been shown that PMEG can be phosphorylated by mammalian GMP-kinase. Mechanism of action remains unanswered. These experiments need radiolabelled compounds to allow monitoring of the metabolism and cellular pharmacology. Additionally, the radiollabelled materials are needed to do pharmacokinetic studies at therapeutically relevant doses and to assess the extent of oral absorption. This paper describes the syntheses of 9-(2'monoethyl-phosphonomethoxyethyl)-8[$^{14}$C]guanine ([$^{14}$C]-EPMG) and 9-(2'-phosphonomethoxyethyl)-8-[$^{14}$C]guanine ([$^{14}$C]-PMEG).

## EXPERIMENTAL

[$^{14}$C]Guanine was purchased from NEN Research Corporation. All other reagents were ACS grade or the highest quality commercially available. NMR spectra were obtained on a Bruker Spectrospin 360 MHz instrument, using tetramethylsilane as an internal standard. Radioactivity was measured by a Beckman LS9000 liquid scintillator. TLC Plates: Silica gel, 250μ GF (Analtech). Method: Mobile phase, as indicated; visualization, UV 254 nm.

### [$^{14}$C]N-Acetylguanine (2)

Acetic anhydride (5 ml) was added to a stirred suspension of [$^{14}$C]guanine (503 mg, 200 mCi, 60 mCi/mmole) and unlabelled guanine (2.5 g, 20 mmole total) in 1-methyl-2-pyrrolidinone (30 ml), and the mixture was heated for 2 hr at 150°. The resulting dark brown solution was kept for 12 hr at room temperature to deposit fine crystals which were collected by filtration and dried in vacuo for 2 hr. The crude product was recrystallized from 50% aqueous ethanol (200 ml) to give white crystals of 2 (2.2 g, 57% yield).

### 2-N-Acetyl-9-(2'-diethylphosphonomethoxyethyl)-8-[$^{14}$C]-guanine (3)

A mixture of 2-(diethylphosphonomethoxy)ethylmethanesulfonate (3.31 g, 11.3 mmole), [$^{14}$C]N-acetylguanine (2) (2.2 g, 11.3 mmole) and potassium

carbonate (3.12 g, 22.6 mmole) in dry N,N-dimethylformamide (120 ml) was heated at 100°C for 4 hr. The reaction mixture was allowed to cool to room temperature and the insoluble material was removed by filtration. The filtrate was concentrated *in vacuo* to give a viscous yellow oil, which was purified by column chromatography on silica gel using 5%-10% methanol-methylene chloride. Fractions containing desired product (Rf = 0.5) were combined and concentrated to a white solid (3) (500 mg, yield = 12%).

9-(2'-diethylphosphonomethoxyethyl)-8[$^{14}$C]guanine (4)

2-N-Acetyl-9-(2'diethylphosphonomethoxyethyl)-8-[$^{14}$C]-guanine (3) (500 mg, 1.29 mmole) was dissolved in 40% aqueous methylamine (18 ml) and the solution was stirred at room temperature for 45 min. The reaction mixture was then concentrated *in vacuo* and evaporated three times with toluene. The residual gummy white solid was stirred in hot ethyl acetate for 1 hr to remove N-methylacetamide which was generated in the reaction. The resulting white slurry was cooled to room temperature and the product was collected by filtration to provide 4 as a white solid (300 mg, yield = 67%). TLC in 15% methanol-methylene chloride (Rf = 0.2).

9-(2'-monoethylphosphonomethoxyethyl)-8-[$^{14}$C]guanine (5)

9-(2'-diethylphosphonomethoxyethyl)-8-[$^{14}$C]-guanine (4) (120 mg, 0.34 mmole) was dissolved in 1N sodium hydroxide solution (3.9 ml) and the solution was stirred for 1 hr at room temperature. 10% aqueous hydrochloric acid (3.9 ml) was added to the solution and then concentrated *in vacuo*. The remaining white solid was purified by column chromatography on C18 adsorbent in water. Product was eluted with 5% methanol-water and concentrated to a white solid (5) (55.7 mg, yield = 52%) having a radiochemical purity of 98.6% and specific activity of 22.2 μCi/mg.

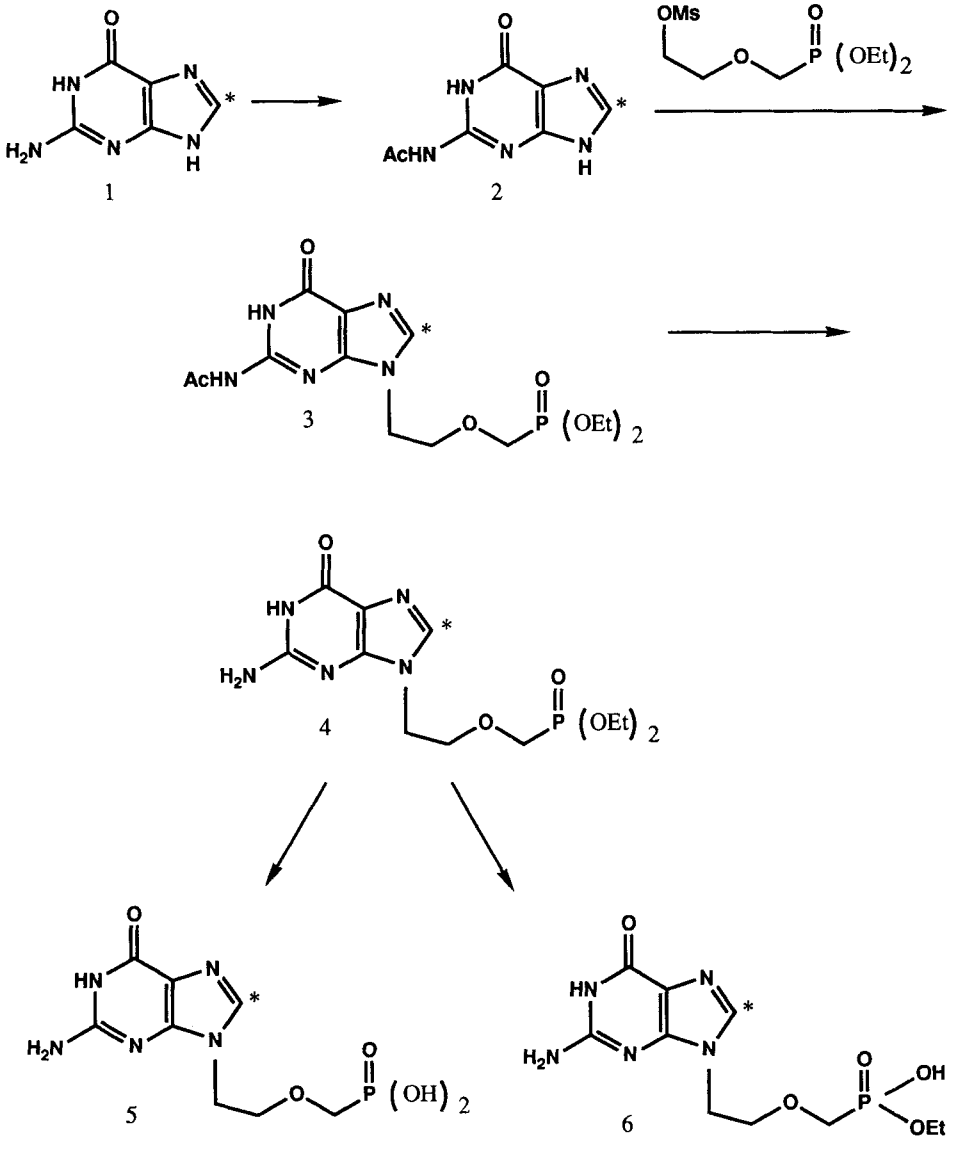
9-(2'-phosphonomethoxyethyl)-8-[$^{14}$C] guanine (6)

Bromotrimethylsilane (0.5 ml, 3.4 mmole) was added dropwise over 2 min to a solution of 9-(2'-diethylphosphonomethoxy ethyl)-8-[$^{14}$C]guanine (4) (120 mg, 0.34 mmole) in dry N,N-dimethylformamide (distilled over phosphorus pentoxide, 3 ml) at room temperature under argon in a foil covered flask. The pale orange solution was stirred at room temperature for 4 hr and was concentrated *in vacuo* to give a viscous yellow oil. After drying in high vacuum for 1 hr the oil was dissolved in water (250 μl) and acetone (8 ml) was added. The cloudy solution was cooled for 20 hr at 0°C. The resulting white precipitate was removed by filtration and dried *in vacuo* to give a white crystalline solid (6) (77 mg, yield = 78%) having a radiochemical purity of 97.3% and specific activity of 22.1 μCi/mg.

RESULTS AND DISCUSSION

[$^{14}$C]-N-Acetylguanine (2) was obtained by treatment of [$^{14}$C]guanine with acetic anhydride in 1-methyl-2-pyrrolidinone. Reaction of (2) with 2-(diethylphosphonomethoxy)ethylmethanesulfonate in N,N-dimethylformamide in the presence of potassium carbonate at high temperature (100°C) for

SYNTHETIC PATHWAY



several hours gave a mixture of 7 and 9 substituted product. Due to their chemical similarity, separation by column chromatography was very difficult and resulted in a low yield of 3. Deprotection of 3 with aqueous methylamine produced 4 in good yield without purification. N-methylacetamide, which was generated in the reaction, was removed with hot ethyl acetate extraction. At this point the diethylphosphonate ester was divided into 2 equal parts. Hydrolysis of 4 to the monoethylphosphonate ester was achieved with 1 N sodium hydroxide solution and acidification with 10% hydrochloric acid. Purification by column chromatography on C18 adsorbent removed the generated sodium chloride from the desired product (5) which had a radiochemical purity of 98.6% and specific activity of 22.2 μCi/mg. Reaction of the second part of 4 with excess bromotrimethylsilane in freshly distilled N,N-dimethylformamide under argon and subsequent treatment with water produced the phosphonate diacid 6 having a radiochemical purity of 97.3% and specific activity of 22.1 μCi/mg. All experimental conditions were optimized using non-radiolabelled materials.

REFERENCES

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