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An Improved Synthesis of 9-(3-Pyridylmethyl)-[2-^{14}C]-9-deazaguanine

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SUMMARY

Purine nucleoside phosphorylase (PNP) inhibitors may be useful in the treatment of a wide variety of disorders in which activated T-cells are pathogenic, such as rheumatoid arthritis and psoriasis, T-cell leukemia and lymphomas, and in the prevention of host-vs.graft rejection. A PNP inhibitor currently in clinical trials is 9-(3-pyridylmethyl)-9-deazaguanine (2-amino-7-(3-pyridylmethyl)-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidine or peldesine). We now report an improved route which gives 9-(3-pyridylmethyl)-[2-^{14}C]-9-deazaguanine **1** in high yield and avoids the formation of 2-methylthio-7-(3-pyridylmethyl)-4-oxo-3*H*,5*H*-[2-^{14}C]-pyrrolo[3,2-*d*]pyrimidine **1a**, the major byproduct of an earlier reported synthesis of **1**. In this new route, the source of the label was *N,N'*-bismethoxycarbonyl-*S*-methyl-[^{14}C]-isothiopseudourea **8**, prepared from 2-methyl-[^{14}C]-2-thiopseudourea in high yield by a phase transfer catalysis method. Condensation of **8** with methyl 3-amino-4-(3-pyridylmethyl)-1*H*-pyrrolo-2-carboxylate **4** in acetic acid and methanol gave adduct **9** which was cyclized by treatment with sodium methoxide to 2-amino-protected **10**. Deprotection of **10** by hydrolysis with 5% NaOH then gave target compound **1** in 82% yield (from **8**). The total yield of product **1** was 2.095 g or 459 mCi, based on a specific activity of 52.9 mCi/mmol.

KEY Words: 9-(3-pyridylmethyl)-9-deazaguanine, peldesine, carbon-14, methyl 3-amino-4-(3-pyridylmethyl)-1*H*-pyrrolo-2-carboxylate, phase transfer catalysis

INTRODUCTION

The essential purine salvage pathway enzyme purine nucleoside phosphorylase (PNP), catalyzes the reversible phosphorylation of purine ribonucleosides and 2'-deoxyribonucleosides (3). PNP is known to have a prominent role in the T-cell branch of the immune system, as illustrated by

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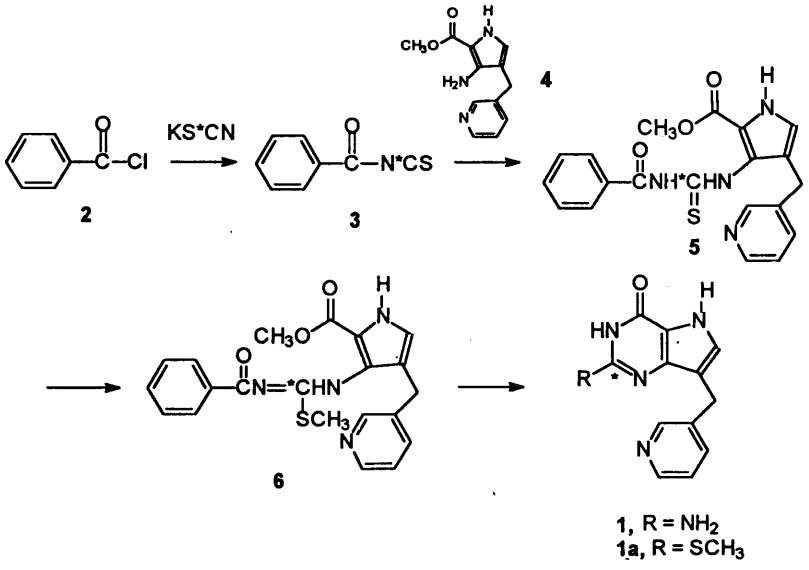
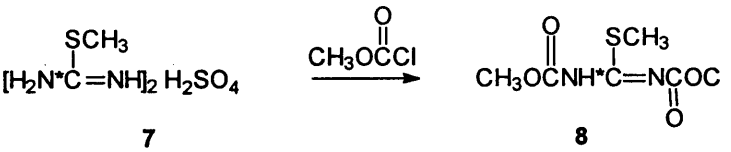
the observation of a unique and rare form of immune deficiency found in PNP-deficient children (4). These children exhibit severe T-cell immunodeficiency while maintaining normal or elevated B-cell function. This profile suggests that specific PNP inhibitors could selectively suppress T-cell function without affecting humoral immunity. Thus, PNP inhibitors are a class of selective immunosuppressive agents that may be useful in the treatment of a wide variety of disorders in which activated T-cells are pathogenic, such as rheumatoid arthritis and psoriasis, T-cell leukemia and lymphomas, and in the prevention of host-vs.graft rejection (5). One such PNP-inhibiting compound, 9-(3-pyridylmethyl)-9-deazaguanine (peldesine) **1**, is now in clinical trials.

Pathak and Montgomery previously reported a synthesis for 9-(3-pyridylmethyl)-[2-^{14}C]-9-deazaguanine **1** in which the label was introduced with benzoyl-[^{14}C]-isothiocyanate **3** (1). Benzoyl-[^{14}C]-isothiocyanate **3**, prepared by reacting potassium [^{14}C]-thiocyanate and benzoyl chloride **2** (6), was condensed with methyl 3-amino-4-(3-pyridylmethyl)-1*H*-pyrrolo-2-carboxylate **4**. Adduct **5** was methylated, and the resulting **6** was then treated with methanolic ammonia at 95-100°C to give target compound **1** in 57% yield, plus a 14% yield of 2-methylthio analog **1a**. The present report describes a synthetic approach that gives **1** in higher yield, without the formation of **1a**.



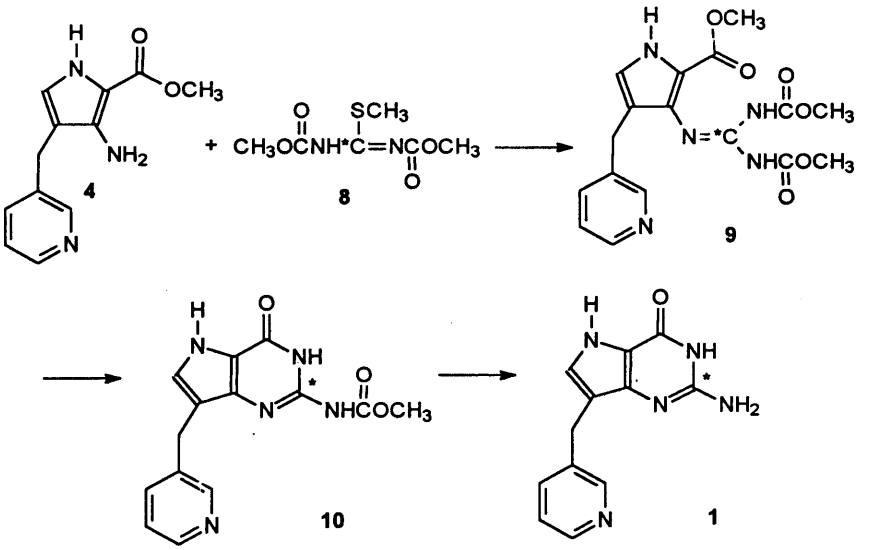
RESULTS AND DISCUSSION

Our new synthesis introduced the label with *N,N'*-bismethoxycarbonyl-*S*-methyl-[^{14}C]-isothiopseudourea **8**, prepared in high yield and high purity by the phase transfer catalysis method of Skiblinski, Stec, Januchowski, and Parys (2). 2-Methyl-[^{14}C]-2-thiopseudourea sulfate **7**, tetrabutylammonium bromide, and methyl chloroformate were added to a solution of methylene chloride and aqueous sodium carbonate to give *N,N'*-bismethoxycarbonyl-*S*-methyl-[^{14}C]-isothiopseudourea **8**.



7 **8**

Bismethoxycarbonyl starting material **8** was then condensed with methyl 3-amino-4-(3-pyridylmethyl)-1*H*-pyrrolo-2-carboxylate **4** in acetic acid and methanol to give adduct **9**. Cyclization of **9** by treatment with sodium methoxide followed by neutralization of the reaction mixture with acetic acid gave **10**, the 2-amino-protected direct precursor to the target compound **1**. Deprotection of **10** by hydrolysis with 5% NaOH at 50°C then gave 2-amino-7-(3-pyridylmethyl)-4-oxo-3*H*,5*H*-[2-^{14}C]-pyrrolo[3,2-*d*]pyrimidine **1** in 98% yield from the limiting reagent **4** (for an overall yield of 80% from **8** or 65.6 % from **7**), with none of the major byproduct **1a** that also formed in the previously reported synthesis.



4 **8** **9**

10 **1**

EXPERIMENTAL

The 2-methyl-[^{14}C]-2-thiopseudourea sulfate (700 mCi, sp. act. = 106.54 mCi/mmol) was purchased from Amersham Life Science. The identities of all reaction products were confirmed by TLC comparison with authenticated unlabeled reference samples from BioCryst Pharmaceuticals, using Analtech Silica Gel GF 250μ chromatography plates. Radiochemical purity (RCP) and chemical purity of the final product were determined by HPLC using a Sphereclone 5μ ODS-1 column, UV detector at 254 nm, eluting with a 20-80% MeOH in 100mM ammonium formate gradient, flow rate = 1.5 mL/min. For RCP determination, the HPLC effluent was collected in scintillation vials as a series of 35 one-minute samples. Each sample was counted for ocpm or dpm with a Packard TriCarb Liquid Scintillation Spectrometer, and percent purity was determined by dividing the total radioactivity of the samples corresponding to **1** by the total radioactivity eluted from the column.

N,N'-Bismethoxycarbonyl-*S*-methyl-[^{14}C]-isothiopseudourea **8**

To a magnetically stirred solution of water (8 mL) and sodium carbonate (1.48 g, 0.014 mol) in a 25 ml round-bottomed flask was added methylene chloride (7 mL). The mixture was then cooled to 0-5°C, and while maintaining this temperature range, the 2-methyl-[^{14}C]-2-thiopseudourea sulfate (7, 1.83 g, 0.0066 mol, 700 mCi,), tetrabutylammonium bromide (0.093 g, 0.00028 mol), and methyl chloroformate (4 g, 0.042 mol) were added with stirring. Then, NaOH (0.787 g, 3.15 mL of a 25% solution, 0.0197 mol) was slowly added over a 3-5 minute span, and the reaction mixture was allowed to slowly warm to 15°C over 1 h. The temperature was maintained at 15-20°C for another two hours. These conditions were then maintained and the reaction intermittently checked by TLC (3:1 cyclohexane/EtOAc, silica gel plates) until the reaction had gone to completion. The reaction was worked up by adding another 25 mL CH_2Cl_2 and 15 mL water and isolating the organic layer. Evaporation of the solvent gave 2.53 g of crude product that was recrystallized in MeOH (25 mL) to give 2.18 g of the desired *N,N'*-bismethoxycarbonyl-*S*-methyl-[^{14}C]-isothiopseudourea **2** as needles (80.1% yield).

2-Amino-7-(3-pyridylmethyl)-4-oxo-3*H*,5*H*-[2-^{14}C]-pyrrolo[3,2-*d*]pyrimidine **1**

Methyl 3-amino-4-(3-pyridylmethyl)-1*H*-pyrrolo-2-carboxylate (**4**, 2.02 g, 0.0088 mol) was added to a stirred solution of MeOH (18 mL) and acetic acid (2.64 g, 0.044 mol). *N,N'*-Bismethoxy-carbonyl-*S*-methyl-[^{14}C]-isothiopseudourea (**8**, 2.18 g, 0.0106 mol,) was then added, and the mixture was stirred overnight. After monitoring of the reaction by TLC (9:1 $CHCl_3$ /MeOH) showed the complete conversion of **4** to adduct **9**, sodium methoxide (25% in MeOH, 12.1 mL, 0.053 mol) was added. The mixture was stirred until TLC monitoring showed that **9** had been converted to 2-amino-protected **10**, the direct precursor to 2-amino-7-(3-pyridylmethyl)-4-oxo-3*H*,5*H*-[2-^{14}C]-pyrrolo[3,2-*d*]-pyrimidine **1**. Acetic acid (0.55 g, 0.0092 mol) was added to neutralize the reaction mixture, and the resulting solid was collected by filtration, washed with MeOH (5 mL), and dried. This isolated solid (comprised of **10** as well as some sodium acetate) was then added to 5% NaOH (18.0 g, 0.022 mol), and this mixture was heated at 50°C until TLC showed that the conversion of **10** to **1** was complete. The reaction mixture was cooled to room temperature, and acetic acid (1.34 g, 0.023 mol) was added to give a pH of 6-7. The resulting off-white solid was filtered and dried, giving 2.095 g of **1** in 98% yield from **4** (or 82 % yield from **8**). The TLCs (silica gel, 5:1 $CHCl_3$:MeOH, R_fs for both = ~0.4) and HPLCs of the product and an unlabeled reference sample of **1** (retention times: ^{14}C-labeled **1**, ~19.88 min; unlabeled reference sample of **1**, 19.92 min) were identical. HPLC analysis showed that the product was >99% pure chemically and radiochemically. The radiochemical yield was 459 mCi, based on a specific activity of 52.85 mCi/mmol that was determined from a serially diluted sample of an exactly weighed aliquot of **1**.

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