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Synthesis of 9-[3-pyridylmethyl]-9-deazaguanine[2-^{14}Carbon]

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SUMMARY

^{14}C labeled 2-amino-7-(3-pyridylmethyl)-4-oxo-3*H*,5*H*-pyrrolo[3,2-*d*]pyrimidine(**6**) was synthesized starting from methyl 3-amino-4-(3-pyridylmethyl)-1*H* -pyrrolo-2-carboxylate. This pyrrole on condensation with ^{14}C labeled benzoylisothiocyanate followed by methylation gave methyl 3-[(N-benzoyl-S-methylisothiocarbamoyl)-amino]-4-(3-pyridylmethyl)-1*H*-pyrrole-2-carboxylate [3-^{14}C] (**5**), which on reaction with methanolic ammonia furnished the title compound(**6**).

Key Words: 9-[3-pyridylmethyl]-9-deazaguanine, Carbon-14

INTRODUCTION

Purine nucleoside phosphorylase (PNP) catalyzes the reversible phosphorylosis of purine ribonucleosides and 2'-deoxyribonucleosides. Therefore, PNP is an essential enzyme in the purine salvage pathway[1]. Interest in PNP as a drug target arises from its role in the T-cell branch of the immune system[2].

The importance of PNP for the integrity of the immune system became apparent with the observation of a unique and rare form of immune deficiency found in children who are deficient in the PNP enzyme. These children exhibit severe T-cell immunodeficiency while maintaining normal or elevated B-cell function[2]. This profile suggests that specific inhibitors of PNP would selectively suppress T-cell function without affecting humoral immunity. Thus, PNP inhibitors represent a new class of selective immunosuppressive agents that could be useful in the treatment

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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[3].

Our PNP inhibitor development program required a ^{14}C labelled 9-[3-pyridylmethyl]-9-deazaguanine. Since the position of the label was not critical, we chose C-2 of the 9-deazapurine ring to provide the highest possible radiochemical yield. This particular carbon comes from benzoylisothiocyanate in our synthetic scheme. Benzoyl isothiocyanate with a ^{14}C label was prepared by the reaction of potassium thiocyanate(^{14}C) with benzoyl chloride[4]. The condensation of methyl 3-amino-4-(3-pyridylmethyl)-1H-pyrrolo-2-carboxylate with benzoyl isothiocyanate followed by methylation gave 3-(N-Benzoyl-S-methylisothiocarbamoyl)-4-(3-pyridylmethyl)-1*H*-pyrrole-2-carboxylate (**5**).

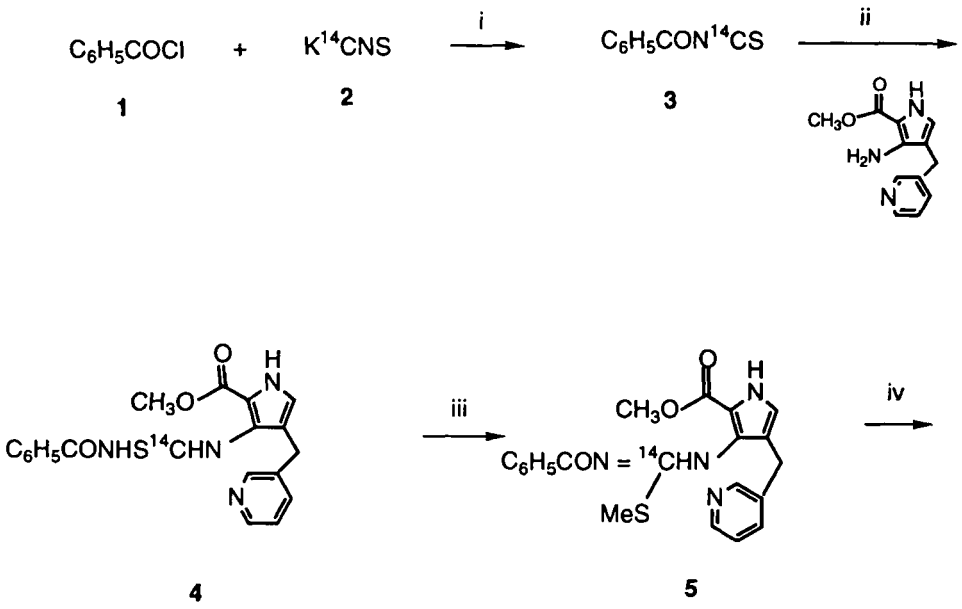
Compound **5** on reaction with methanolic ammonia at 95-100°C for 10 h in a steel bomb gave the desired 9-[3-pyridylmethyl]-9-deazaguanine (**6**) and the methylthio compound (**7**) in the ratio of 3.9:1 in 57 and 14% yields respectively.

EXPERIMENTAL

The potassium thiocyanate[^{14}C] was purchased from Morvek Biochemicals Inc.. NMR spectra were recorded at 400 MHz using a Brucker WH-400 instrument and chemical shifts are reported in parts per million. IR spectra were recorded using a Nicolet MX-1 FT-IR spectrometer. MS spectra were taken using a Varian MAT-311A instrument. Radiochemical Purity (RCP) of the final product was measured using HPLC connected to radiometric detector FLO-one A-500 series and TLC scan using Packard model 7220/21 radiochromatogram scanner. HPLC of the final product was done using ISRP column, UV detector at 237 nm with 100mM ammonium formate:acetonitrile 85:15 as eluent. Rf values are from softlayer silica gel plates of 250 micron with 1 micro gm of compound or mixture applied.

Benzoyl isothiocyanate[^{14}C], **3:**

Potassium thiocyanate[^{14}C] (37 mg, 0.38 mmol) was dried under a stream of nitrogen at 110° for 10 h in a 25 ml round bottom flask. Anhydrous toluene (20 ml) was added and the



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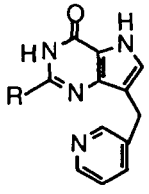

6, R=NH_2

7, R=SCH_3

Reagents: i. Reflux in anhydrous toluene, -H_2O, 8 hr, 110^{0}C, ii. dichloromethane, room temperature, 15 minutes, iii. DBN, CH_3I, 0-5^{0}C, iv. saturated methanolic ammonia in a steel bomb, 95-100^{0}C, 10 hr.

potassium thiocyanate was further dried overnight through the azeotropic separation of water. To this suspension was added benzoyl chloride (58 mg, 0.41 mmol) through a syringe and the reaction mixture was refluxed for 8 h. The toluene was evaporated and the residue, **3** was used in the next step.

Methyl 3-(N-benzoyl-S-methylisothiocarbamoyl)amino-4-[3-pyridylmethyl]-1*H*-pyrrole-2-carboxy late-[3-^{14}C], **5**:

To the benzoyl isothiocyanate[^{14}C] in 5 ml anhydrous dichloromethane was added methyl 3-amino-4-(3-pyridylmethyl)-1H-pyrrolo-2-carboxylate (61.5 mg, 0.26 mmol). After 15 min. TLC showed the absence of benzoyl isothiocyanate and the solvent was evaporated by blowing nitrogen through the solution. The residue was applied to a prep TLC plate and developed in chloroform-methanol 95:5. The band at Rf 0.48 was scraped from the plate and eluted with the same solvent to furnish pure **4** (Rf 0.54 in $CHCl_3$-methanol 94:6; Rf 0.38 in ethyl acetate). $M^+ + 1$ - 395. IR(nujol, cm^{-1}): 1710, 1680, 1580, 1525, 1435. NMR($CDCl_3$): δ 3.82(s, 3H), 3.94(s, 2H), 6.62(d, 3.1 Hz, 1H), 7.18(dd, 4.8 & 7.7 Hz, 1H), 7.55(m, 3H), 7.65(t, 7.2 Hz, 1H), 7.89(d, 7.5 Hz, 2H), 8.43(dd, 1.4 & 4.7 Hz, 1H), 8.52(d, 2.1 Hz, 1H), 12.02(s, 1H). The compound **4** was suspended in 5 ml of anhydrous dichloromethane and cooled in an ice-water bath for 10 min. To it was added DBN (38 mg, 0.3 mmol) using a dry syringe followed by methyl iodide (43 mg, 0.3 mmol). The light yellow color disappeared instantly, but the stirring was continued for 30 minutes when TLC (ethyl acetate) showed a complete reaction. The dichloromethane was evaporated and the residue was purified by prep TLC to furnish **5**, 49 mg, m.p. 187-189^0 (Rf 0.32 in ethyl acetate).

9-[3-pyridylmethyl]-9-deazaguanine[2-^{14}C], **6**:

Compound **5** (49 mg, 0.12 mmol) in 15 ml of methanol presaturated with ammonia was heated in a steel bomb at 95-100^{0}C for 10 h. The bomb was cooled in ice-water, opened and the methanol evaporated. The residue was boiled with methanol (3 ml) and the solid was filtered and crystallized from methanol to give **6**, 16.6 mg, (Rf 0.26 in chloroform-methanol 85:15). m.p. 327-329^{0}C; $M^+ + 1$ at 242, ^{1}H-NMR(DMSO-d_6): δ 3.8(s, 2H), 5.84(br s, 2H, NH_2), 6.96(s, H-8), 7.24(dd, 4.7 & 7.7 Hz, H-5'), 7.59(d, 7.8 Hz, H-4'), 8.34(d, 4.5 Hz, H-6'), 8.46(s, H-2'), 10.34 and 11.27(br s, N-H). This material showed 100% radiochemical purity by HPLC.

The methanol soluble fraction furnished **7**, 4.5 mg, m.p. 241-243^0, $M^+ + 1$ at 273, ^{1}H-NMR(DMSO-d_6): δ 2.53(s, 3H), 3.9(s, 2H), 7.19(d, 2.7 Hz, 1H), 7.25(dd, 4.7 & 7.7Hz, 1H), 7.67(dt, 4.7 & 7.7Hz, 1H), 8.34(dd, 1.3 & 4.7Hz, 1H), 8.55(d, 1.6Hz, 1H), 11.76 and 12.07(br s, N-H)

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