Improved Synthesis of β-D-6-Methylpurine Riboside and Antitumor Effects of the β-D- and α-D-Anomers

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Abstract: 6-Methylpurine-β-D-riboside (β-D-MPR) has been synthesized by coupling 6-methylpurine and 1-O-acetyl-2,3,5-tri-O-benzoyl-D-ribose using conditions that produce the β-D-anomer exclusively. The *in vitro* antitumor effects of β-D-MPR and 6-methyl-purine-α-D-riboside (α-D-MPR) in five human tumor cell lines showed that β-D-MPR was highly active (IC_{50} values ranging from 6 to 34 nM). α-D-MPR, although less active than β-D-MPR, also exhibited significant antitumor effects (IC_{50} values ranging from 1.47 to 4.83 μM).

Keywords: 6-Methylpurine-β-D-riboside; 6-methylpurine-α-D-riboside; antitumor activity; synthesis

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Introduction

6-Methylpurine-β-D-riboside (**6**, β-D-MPR), an antibiotic isolated from culture broths of the basidiomycetes fungi *Collybia dryophilia* and *Collybia maculata*, has antifungal, antiviral and antitumor activity [1,2]. β-D-MPR is an excellent substrate of mammalian adenosine deaminase (ADA) and its mechanism of activation in tumor cells presumably relates to its interaction with this enzyme [3]. In contrast, 6-methylpurine-β-D-2'-deoxyriboside, a poor ADA substrate [3], is relatively nontoxic to human cells [2,4].

Protozoan parasites such as African trypanosomes and *Leishmania* cannot synthesize purines and must salvage them from their host organisms [5]. Consequently, they are less discriminate than mammalian cells in their enzymatic processing of preformed purines and purine nucleosides. For example, MPdR is cleaved by 5'-(methylthio)adenosine phosphorylase in African trypanosomes but not in human sarcoma 180 cells [6]. MPdR is selectively metabolized and growth inhibitory in *Leishmania* infected mouse macrophages [4]. Thus novel nucleoside analogs of 6-methylpurine (MP) may have potential as antiparasitic agents.

Methylpurine (MP) nucleoside analogs are often prepared by fusion of MP to an appropriate O-acylated sugar. This synthetic approach consistently produces product mixtures of α- and β-anomers [2,7]. For example, MPR, when prepared by fusion of MP with tetra-O-acetyl-β-D-ribofuranose gives a 10:1 mixture of β/α anomers [2] and requires a tedious chromatographic separation of the closely eluting α- and β-anomers to obtain pure the β-anomer. We have developed an improved synthesis of β-D-MPR that yields the β-anomer exclusively according to the reactions shown in Scheme 1.

Scheme 1. Synthesis of 6-methylpurine-β-D-riboside
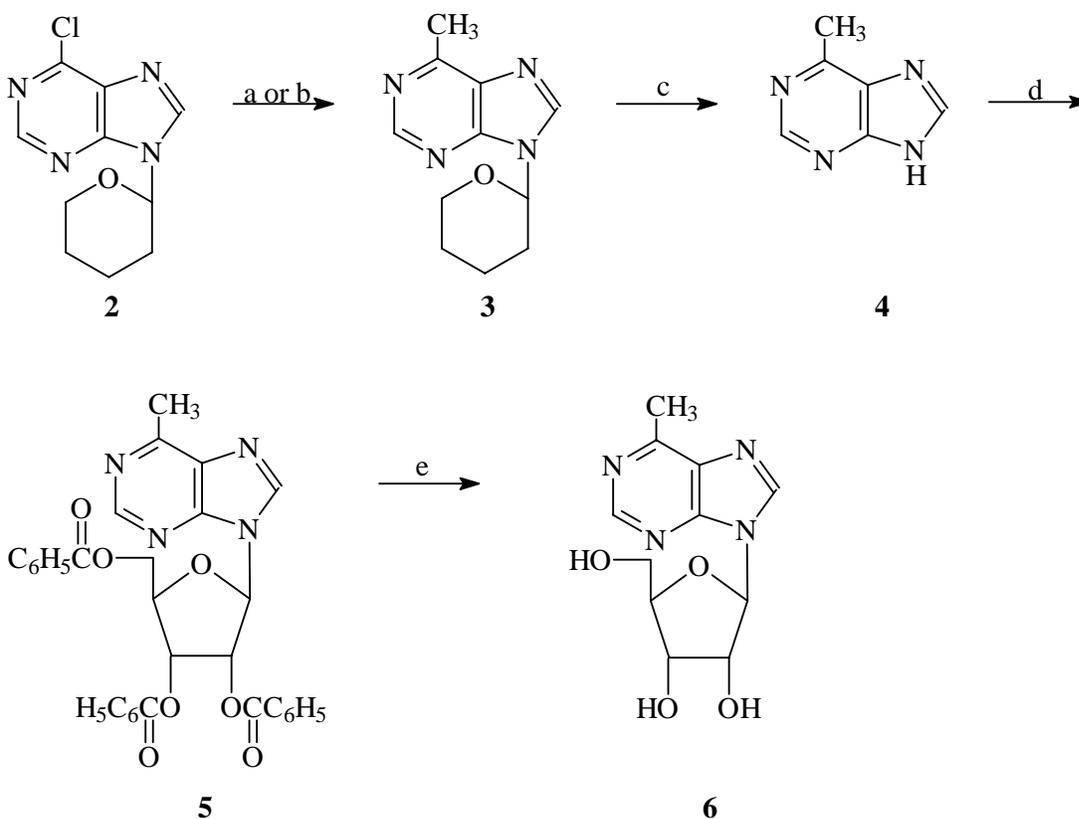
a. methyltriphenylphosphonium iodide / n-butyllithium, b. methylmagnesium iodide and cuprous iodide
c. Dowex 50W-X8 (H+) resin, d. 1-O-acetyl-2,3-5-tri-O-benzoyl-D-ribose / hydrobromic acid,
e. methanol/concentrated ammonium hydroxide (4:1)

Results and Discussion

Chemistry

Historically, a variety of nucleoside analogs have been prepared by the fusion of the appropriate nucleic acid with an O-acylated ribose in the presence of an acid catalyst. A drawback of this methodology is the generation of various product mixtures composed of both α- and β-anomers [2,7]. As stated previously, in the preparation of MPR via a fusion reaction a 10:1 mixture of β/α anomers was consistently produced [2]. Interestingly, when MPR was synthesized via the methodology developed by Laursen *et al.* [11], only the β-anomer was formed, yet when Laursen's procedure was developed to synthesize 3-β-D-ribofuranosyladenine (isoadenosine), only the N-9 β-anomer of methylpurine was formed under the conditions reported in this paper. Presumably, the difference in electronic contributions to the purine ring system between that of the amino group of adenine and the methyl group of methylpurine led to this discrepancy.

Biological Activity

Antiparasitic activity of β-D-MPR and α-D-MPR was examined previously in the EATRO 110 strain of *Trypanosoma brucei brucei* and two clinical isolates of *Trypanosoma brucei* rhodesiense: β-D-MPR was significantly active, with IC_{50} values in the range of 0.2- 2.0 μM, whereas α-D-MPR was less than 50% growth inhibitory at concentrations up to 100 μM [13]. β-D-MPR was also highly active in five human tumor cell lines, with IC_{50} values ranging from 6 to 34 nM (Table 1). β-D-MPR displayed an IC_{50} value of 20 nM against H.Ep. # 2 human epidermoid cells in a colony forming assay [14]. Data from our current studies provide further evidence of the highly toxic effects of this analog in mammalian cells and underscore its lack of selectivity as an antiparasitic agent. Unexpectedly, α-D-MPR exhibited significant antitumor effects in all five human tumor cell lines with IC_{50} values in the range of 1.47-4.83 μM. The possibility that the activity attributed to α-D-MPR might be due to presence of trace amounts of β-D-6MPR was ruled out by determining the purity of α-D-MPR (HPLC and NMR spectroscopy) and by evaluating the antitumor effects of α-D-6MPR in the presence of varying amounts of β-D-MPR using MCF7 breast carcinoma cells (data not shown).

Table 1. Effects of α-D-MPR and β-D-MPR on *in vitro g*rowth of human tumor cells at 72 hours.

Cell Line	IC_{50} (μM)	
	β-D-MPR	**α-D-MPR**
A121 (ovarian)	0.017	2.65
A549 (non-small cell lung)	0.006	1.47
HT-29 (colon)	0.034	4.83
MCF7-S (breast)	0.012	1.75
MCF7-R (breast)	0.026	4.08

Conclusions

Cellular DNA and RNA are comprised exclusively of β-D-nucleosides and consequently, nucleoside antimetabolites used clinically for cancer treatment are β-anomeric structures. Scant attention has been given to the antitumor effects of α-D-nucleoside structures (α-nucleosides) [15]. The significant antitumor activity of α-D-MPR, an α-nucleoside, is an unusual finding, which warrants further biochemical characterization.

Acknowledgements

Experimental

General.

^{1}H-NMR spectra were recorded on either a Bruker 400-MHz or Varian EM 390 spectrometer. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. Column chromatography was performed using silica gel 60 (230-400 mesh), TLC was done using EM Industries Aluminum sheets (precoated with silica gel 60 F_{254}) and preparative TLC using Analtech Uniplates (silica gel GF, 20 x 20 cm and 1000 microns thick). Most common reagents and solvents were purchased from Sigma-Aldrich Inc., St. Louis MO. The purity of all final compounds was determined by HPLC (McPherson FL75, C18 column, mobile phase CH_3CN/10mM ammonium phosphate, pH 4.4) and verified by elemental analysis (Robertson Microlab. Inc. of Madison, NJ).

6-Methyl-9-(tetrahydro-2-pyranyl)purine (**3**). 6-Chloro-9-(tetrahydro-2-pyranyl)purine (**2**) was prepared from 6-chloropurine (**1**) in 72% yield as described [8]. Compound **2** was converted to **3** by Method A or Method B. Although the yield of **3** using Method A far exceeds that obtained by Method B, the purified product was isolated more easily by Method B.

Method A: The general procedure of Taylor and Martin [9] was followed. A mixture of **2** (5.45 g, 23 mmol), methyltriphenylphosphonium iodide (20.75 g, 51 mmol) and n-butyllithium (1.6 M in tetrahydrofuran, 30 mL) was refluxed under argon for 2 h. Sodium carbonate (1 equiv, 2.46 g) in water (10 mL) was added and refluxing continued an additional 3 h. The reaction mixture was concentrated *in vacuo*, dissolved in methylene chloride, washed with water (2 X 250 mL), dried over magnesium sulfate, filtered, and then evaporated *in vacuo*. The crude product was applied to a silica gel column [packed in ethyl acetate/methanol (99:1)] and was eluted isocratically with the same solvent system. Fractions containing product were pooled, concentrated *in vacuo*, applied to a silica gel column packed in ethyl acetate and eluted isocratically with ethyl acetate. Fractions containing product were pooled to give 3.92

g (79%) of **3**: ^{1}H NMR (CDCl$_3$) δ 1.6-2.2 (m, 6H, 2', 3', 4'-CH$_2$), 2.80 (s, 3H, CH$_3$), 3.80 (m, 2H, 5'-CH$_2$), 5.65 (m, 1H, 1'-CH), 8.15 (s, 1H, arom H), 8.70 (s, 1H, arom H).

<u>Method B</u>: The general procedure described by Dvorakova *et al* [10] was followed. Cuprous iodide (15.91 g, 83.6 mmol) and methylmagnesium iodide [3M in tetrahydrofuran (THF), 55 mL) in anhydrous THF (380 mL) was stirred under argon at -78° C for 30 min. A solution of **2** (5.05 g, 21 mmol) in 250 ml THF was added and stirring was continued at -78°C for an additional 2 hr. The reaction mixture was stirred overnight at room temperature and quenched as described [10]. The crude product was applied to a silica gel column (packed in methylene chloride) and eluted with a gradient of methylene chloride-methanol (100:0, 250 mL; 99:1, 500 mL; 98:2 >> 2 L). Fractions containing product were pooled and evaporated *in vacuo* to give 1.308 g (29%) of **3**.

6-Methylpurine (**4**). Compound **3** (1.31 g) was dissolved in water/methanol and applied to a column of Dowex 50W-X8 (H$^+$) resin. The column was washed thoroughly with water and eluted with 0.1 M ammonium hydroxide. All UV positive fractions were pooled and concentrated *in vacuo* to give 0.75 g (96%) of **4**: ^{1}H-NMR (DMSO-d$_6$) δ 2.75 (s, 3H, CH$_3$) 8.45 (s, 1H, arom H), 8.75 (s, 1H, arom H).

Tri-O-benzoyl-6-methylpurine-β-D-riboside (**5**). Coupling of 6-methylpurine to 2,3-5-tri-O-benzoyl-D-ribose was accomplished using reaction conditions that Laursen *et al* reported for preparation of 3-isoadenosine. [11]. Hydrobromic acid (30% in acetic acid, 35.2 mL) was added to a solution of 1-O-acetyl-2,3-5-tri-O-benzoyl-D-ribose (6.602 g, 12 mmol) in methylene chloride (12 mL). The solution was stirred at room temperature for 75 min under nitrogen and then concentrated *in vacuo* below 35°C. The resulting oil was azeotroped 5 times with toluene and dissolved in acetonitrile (80 ml). 6-Methylpurine (1.632 g, 12 mmol) was added and the reaction mixture was refluxed for 40 hr. After cooling to room temperature, concentrated ammonium hydroxide (1.8 mL) was added and the solution was concentrated *in vacuo*. The resulting oil was triturated with ether (2 x 200 mL). The ether extracts were combined and concentrated *in vacuo*. The crude product was dissolved in methylene chloride and applied to a silica gel column (22 X 350 mm) packed in methylene chloride. The column was eluted with a gradient of methylene chloride/methanol (100:0, 250 mL; 99:1, 250 mL; 98:2 >> 250 mL). Appropriate fractions were pooled and concentrated *in vacuo* to give the desired product (1.905 g, 27%) as an oil: ^{1}H-NMR (CDCl$_3$) δ 2.75 (s, 3H, CH$_3$), 4.80 (m, 3H, 4'-CH and 5'-CH$_2$), 6.40, (m, 3H, 1'-CH, 2'-CH, 3'-CH), 7.20-8.30 (br m, 16 H, arom H), 8.75 (s, 1H, arom H).

6-Methylpurine-β-D-riboside (**6**). Tri-O-benzoyl-6-methylpurine-β-D-riboside (1.882 g, 3.3 mmol) was dissolved in 4:1 methanol/concentrated ammonium hydroxide (50 mL) and stirred at room temperature for 18 hr. The reaction mixture was concentrated *in vacuo* and azeotroped 3 times with ethanol. The crude product was dissolved in water (50 mL), extracted with methylene chloride (3 x 50 mL) and the aqueous layer concentrated *in vacuo* and further dried under vacuum to give **6** (0.82 g, 93%): ^{1}H-NMR (DMSO-d$_6$) δ 2.75 (s, 3H, CH$_3$), 3.55 (m, 2H, 5'-CH), 3.95 (m, 1H, 4'-CH), 4.0 (m, 1H, 3'-CH), 4.20 (m, 1H, 2'-CH), 5.10-5.50 (br m, 2H, 2'-OH and 3'-OH), 6.00 (m, 1H, 1'-CH), 8.75 (2 overlapping s, 2H, arom H); UV λ$_{max}$ 260.3 nm.

6-Methylpurine-α-D-riboside. The synthetic procedure of Montgomery and Hewson was used [2].

Biology

The *in vitro* antitumor activity of β-D-MPR and α-D-MPR was evaluated in a panel of five human tumor cell lines (A121 ovarian carcinoma, A549 non-small cell lung carcinoma, HT-29 colon adenocarcinoma, MCF7-S breast adenocarcinoma and MCF7-R adriamycin resistant breast adenocarcinoma) as described [12].

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Sample availability: Not available.