Bioorganic Chemistry 65 (2016) 9–16

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

6-Methylpurine derived sugar modified nucleosides: Synthesis and evaluation of their substrate activity with purine nucleoside phosphorylases

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ARTICLE INFO

Article history:
Received 22 November 2015
Revised 22 December 2015
Accepted 23 December 2015
Available online 24 December 2015

Keywords:
6-Methylpurine
2-Fluoroadenine
Purine nucleosides
Vorbrüggen glycosylation
Purine nucleoside phosphorylase
Cancer gene therapy

ABSTRACT

6-Methylpurine (MeP) is cytotoxic adenine analog that does not exhibit selectivity when administered systemically, and could be very useful in a gene therapy approach to cancer treatment involving *Escherichia coli* PNP. The prototype MeP releasing prodrug, 9-(β-D-ribofuranosyl)-6-methylpurine, MeP-dR has demonstrated good activity against tumors expressing *E. coli* PNP, but its antitumor activity is limited due to toxicity resulting from the generation of MeP from gut bacteria. Therefore, we have embarked on a medicinal chemistry program to identify non-toxic MeP prodrugs that could be used in conjunction with *E. coli* PNP. In this work, we report on the synthesis of 9-(6-deoxy-β-D-allofuranosyl)-6-methylpurine (**3**) and 9-(6-deoxy-5-*C*-methyl-β-D-ribo-hexofuranosyl)-6-methylpurine (**4**), and the evaluation of their substrate activity with several phosphorylases. The glycosyl donors; 1,2-di-*O*-acetyl-3,5-di-*O*-benzyl-α-D-allofuranose (**10**) and 1-*O*-acetyl-3-*O*-benzyl-2,5-di-*O*-benzoyl-6-deoxy-5-*C*-methyl-β-D-ribohexofuran-ose (**15**) were prepared from 1,2:5,6-di-*O*-isopropylidine-α-D-glucofuranose in 9 and 11 steps, respectively. Coupling of **10** and **15** with silylated 6-methylpurine under Vorbrüggen glycosylation conditions followed conventional deprotection of the hydroxyl groups furnished 5′-*C*-methylated-6-methylpurine nucleosides **3** and **4**, respectively. Unlike 9-(6-deoxy-α-L-*talo*-furanosyl)-6-methylpurine, which showed good substrate activity with *E. coli* PNP mutant (M64V), the β-D-*allo*-furanosyl derivative **3** and the 5′-di-*C*-methyl derivative **4** were poor substrates for all tested glycosidic bond cleavage enzymes.

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1. Introduction

We have developed a suicide gene therapy approach for the treatment of solid tumors that is based on selective activation of none-toxic prodrugs of toxic adenine analogs such as 2-fluoroadenine and 6-methylpurine with *Escherichia coli* purine nucleoside phosphorylase (PNP) [1,2]. *E. coli* purine nucleoside phosphorylase (PNP) differs from human PNP in its ability to cleave adenosine and its analog [3,4] *E. coli* PNP is much more permissive about the substitution at the purine C-6 position [5] than mammalian enzyme, accepting amino, oxo and other substituents at that position while the human enzyme accepts only oxo (or thiooxo) group. *E. coli* PNP is not very tolerant of changes in the sugar moiety, strongly preferring a ribofuranosyl or 2′-deoxyribofuranosyl group in the β-configuration. 2-Fluoroadenine (F-Ade) and 6-methylpurine (6-MeP) were identified as adenine analogs with suitable toxicity profile, and could be incorporated into nucleosides that might sufficiently good substrates for *E. coli* PNP. These two adenine analogs demonstrate cytotoxicity with IC_{50}'s in the range of 0.1 and 1 μM, and are toxic to both proliferating and non-proliferating cells.[6] Recently, 7-deaza-6-MeP-riboside derivative was reported to show interesting cytotoxic activity [7]. Excellent *in vivo* antitumor activity has been demonstrated with strategy against human tumor xenografts in mice using a variety of prodrugs [8–11], including 9-[2-deoxy-β-D-ribofuranosyl]-6-methylpurine (MeP-dR; **1**), 9-[β-D-arabinofuranosyl]-2-F-adenine (F-araA; **2**), and 2-F-2′-deoxyadenosine. The best *in vivo* activity has been observed with F-araA [12], and a phase I clinical has been initiated

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http://dx.doi.org/10.1016/j.bioorg.2015.12.006
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to evaluate its safety and efficacy [13]. However, the antitumor activity of this approach is still limited by the inherent toxicity of the prodrugs. Although MeP-dR is much less toxic than F-araA *in vitro*, it is much more toxic to mice, due to its cleavage by PNP that is expressed in intestinal bacteria [14], which results in the systemic liberation of 6-methylpurine (MeP), which is known not to have selective *in vivo* antitumor activity [15]. In spite of this toxicity good *in vivo* antitumor activity has been observed with MeP-dR, and it is possible that MeP prodrugs could be effectively used in a gene therapy system utilizing *E. coli* PNP, if they were not cleaved by intestinal bacteria. Therefore, we have initiated a program to change the substrate specificity of *E. coli* PNP to identify an enzyme/prodrug combination that can cleave MeP containing prodrugs that are poor substrates for the endogenous bacterial phosphorylases.

Our studies have led to the identification of an *E. coli* PNP mutant (sM64V) that is able to cleave numerous 5′-modified nucleoside analogs with much greater efficiency than the wild-type enzyme [10,16,17]. The replacement of the M64 with V64 of the wild *E. coli PNP* enzyme enabled the formation of a hydrophobic pocket in the vicinity of the 5′-postion. This in turn, enabled the fitting of 6-MeP-riboside with a small substituent at the 5′-position, into the active site of the enzyme [16]. 9-(6-deoxy-α-L-talofuranosyl)-6-methylpurine [methyl(talo)-MeP-R; **2**] was identified as the best M64V MeP-based substrate (Fig. 1) [18]. Methyl (talo)-MeP-R at its maximally tolerated dose (MTD) demonstrated antitumor activity against D54 tumors that express M64V. However, methyl(talo)-MeP-R did not demonstrate better antitumor activity than did the original prodrugs with the wild-type enzyme. In an effort to identify better combination of enzyme/prodrug combination, herein we report on the detailed synthesis and the substrate activity of 9-(6-deoxy-β-D-allofuranosyl)-6-methylpurine (**3**) [methyl(allo)-MeP-R], the diastereomer of methyl(talo)-MeP-R, and 9-(6-deoxy-5-*C*-methyl-β-D-ribo-hexofuranosyl)-6-methyl purine (**4**) [5′-*C*-dimethyl-MeP-R] with wild type *E. coli* PNP, its mutant M64V as well as other several enzymes known to cleave the glycosidic bond of adenosine (Fig. 1).

2. Results and discussions

Vorbrüggen glycosylation of 1,2,3,5-tetra-*O*-acylribofuranosides with silylated 6-alkyl/aryl-purines is been reported by us and others to selectively produce the corresponding 9-(β-D-ribofurano syl)-6-alkyl/arylpurine nucleosides [17,19–21] in high yields. The anomeric configuration is predominantly *trans* with respect to the 1′-(6-alkyl/arylpurine) moiety and 2′-*O*-acyl group [17,19–22]. Procedures reported in the literature for the synthesis of the 6-deoxy-D-allofuranose include: (i) Treatment of 2,3-*O*-isopropylidine-5-*O*-tosyl-L-rhamnofuranose with sodium methoxide provides the corresponding 2,3-*O*-isopropylidine-6-deoxyallo furanose derivative [23–25]; (ii) Treatment of suitably protected D-ribopento-dialdofuranoside (e.g. 2,3-*O*-isopropylidine-β-D-ribopento-dialdofuranoside) derivative with methyl organometallics gives a mixture of the D-allofuranoside and L-talofuranoside derivatives with tedious column separation [26–31]; (iii) Ring opening of the 5,6-anhydro-1,2-*O*-isopropylidine-α-D-allofuranose under reducing conditions [32]. We chose to implement the later approach for the synthesis of the key intermediate **10**, starting with the commercially available 1,2:5,6-di-*O*-isopropylidine-α-D-glucofuranose (Scheme 1). Inversion of the configuration of the 3-hydroxyl group of the glucofuranose derivative **4** was attained by sequential oxidation ($RuO_2{\cdot}xH_2O$)/KIO_4/$BnEt_3NBr/CHCl_3$) and reduction of the produced 3-keto derivative with ($NaBH_4$/EtOH), benzylation of the 3-OH group of the resultant 1,2:5,6-Di-*O*-isopropylidine-α-D-allofuranose with (BnBr/DMF) followed by selective deprotection of the 5,6-*O*-isopropylidine group gave the diol derivative **6** in 63% overall yield [33–36]. Treatment of **6** with (Bu_2SnO in $CHCl_3$/MeOH; 10:1) gave a cyclic stannylene intermediate, which was mono-tosylated at the 6-position by the treatment with $TsCl/CH_2Cl_2$. Treatment of the later intermediate with K_2CO_3 in dry MeOH at 0 °C gave the corresponding epoxide in **7** in 84% yield [37–40]. It is worth noting that performing the reaction at room temperature resulted in the generation of a substantial amount of 6-*O*-methyl derivative on the expense on the 5,6-anhydro derivative **7**. Regioselective ring opening of the epoxide **7** with $LiAlH_4$ in dry THF at −5 °C gave the corresponding 6-deoxy derivative **8** [41,42] in 98% yield. ^{1}H NMR spectrum of **8** showed a doublet signal (δ: 1.22 ppm; J = 6.4 Hz) corresponding to the 5-methyl group. Treatment of **8** with BnBr in the presence of NaH in DMF gave the 3,5-di-*O*-dibenzyl derivative **9** in 82% yield. Acetolysis of **9** gave the corresponding 1,2-di-*O*-diacetyl derivative **10** in 75% yield as a mixture of anomers. Coupling the silylated 6-methylpurine with the 6-deoxyallofuranose derivative **10** in the presence of $SnCl_4$ in dry CH_3CN, followed by treatment with NH_3/MeOH gave the corresponding 9-(2,3,5-tri-*O*-benzoyl-β-D-allofuranosyl)-6-methylpurine derivative (**11**) in 84% yield. The β-configuration of **11** was confirmed by NOE correlation between H-8 and H-3′. Irradiation at the 8-H proton, a 1% NOE enhancement was observed at the H-3′ proton. Treatment of **11** with BCl_3 in dry CH_2Cl_2 at − 78 °C gave the deprotected 9-(β-D-allofuranosyl)-6-methylpurine (**3**) in 78% yield.

The synthesis of 9-(6-doxy-5′-*C*-methyl-β-D-allofuranosyl)-6-methylpurine (**4**) was achieved by coupling of the glycosyl donor **15** with silylated 6-MeP (Scheme 2) under Vorbrüggen glycosylation conditions. Sequential oxidation of the diol derivative **6** with KIO_4 and PDC to the corresponding carboxylic acid derivative, then followed by esterification gave the 4-methyl ester derivative **12** in 72% yield. Treatment of **12** with MeMgBr in THF gave the 6-doxy-5-*C*-methyl derivative **13** in 89% yield. ^{1}H NMR of **13** showed the 5-*C*-dimethyl signals resonating at 1.27 and 1.21 ppm, respectively. Sequential benzoylation of the 5-hydroxyl group of **13**, methanolysis

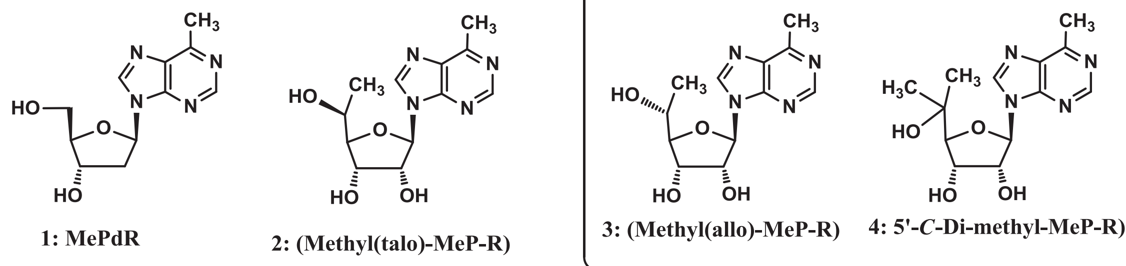

Fig. 1. Structures of 5′-modified 6-methylpurine ribonucleosides. Compounds **3** and **4** are described in this paper, compound **2** has been described elsewhere (**18**).

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