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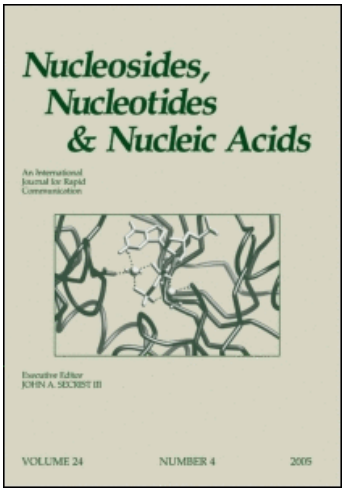

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Synthesis and Biological Activity of 2-Fluoro Adenine and 6-Methyl Purine Nucleoside Analogs as Prodrugs for Suicide Gene Therapy of Cancer

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 2-FLUORO ADENINE AND 6-METHYL PURINE NUCLEOSIDE ANALOGS AS PRODRUGS FOR SUICIDE GENE THERAPY OF CANCER

A. V. Silamkoti, P. W. Allan, A. E. A. Hassan, A. T. Fowler, E. J. Sorscher, W. B. Parker, and J. A. Secrist III □ *Southern Research Institute, Birmingham, Alabama, USA*

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□ *A novel series of 6-methylpurine nucleoside derivatives with substitutions at 5′-position have been synthesised. These compounds bear a 5′-heterocycle such as triazole or a imidazole with a two carbon chain, and an ether, thio ether or amine. To extend the SAR study of 2-fluoroadenine and 6-methyl purine nucleosides, their corresponding α—linker nucleosides with* L*-xylose and* L*-lyxose were also synthesized. All of these compounds have been evaluated for their substrate activity with E. coli PNP.*

INTRODUCTION

Suicide gene therapy is a novel approach among those explored for selectively killing tumor cells without harming normal cells. A strategy has been developed earlier based on the selective expression of *E. coli* purine nucleoside phosphorylase (*E. coli* PNP) in tumor cells, making these cells sensitive to an otherwise nontoxic or weakly toxic agent. *E. coli* PNP selectively cleaves certain weakly toxic purine nucleosides, such as certain 6-methylpurine and 2-fluoroadenine nucleosides to their toxic purine or purine analogs.[1]

Several analogs of 6-methylpurine and 2-fluoroadenine nucleosides have been prepared earlier for their substrate activity to *E. coli* PNP. Recently, we have been examining a variety of mutant enzymes of *E. coli* PNP. These have included mutation of Met 64 and His 4. Thus, our synthetic efforts have focused on finding prodrugs specifically tailored to these mutant enzymes, thereby providing additional specificity to the cleavage process. To that end, we have recently synthesized 6-methyl-9-[5-O-[2-(1H-1,2,4-triazol-l-yl)ethyl]-β-D-ribofuranosyl]-9H (**4**)

This investigation was supported by the National Cancer Institute, National Cooperative Drug Discovery Grant (U19CA67763). We thank Dr. J.M. Riordan, Mr. Mark Richardson, Mrs. J.C. Bearden, and Mrs. S. Campbell for spectral and HPLC analyses. Ms. Stephanie Brannon for help in preparation of this poster.

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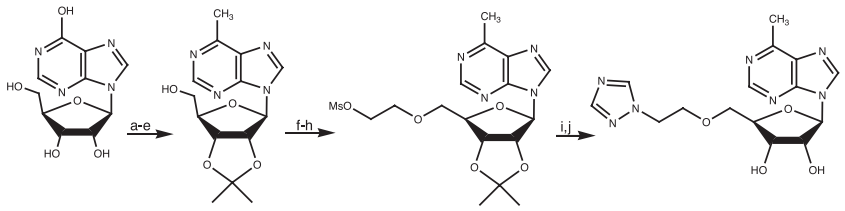
9-[[5-deoxy-5-[2-(1H-imidazol-4-yl)ethyl]-thio]-β-D-ribofuranosyl]-6-methyl-9H-purine (**10**), 2-fluoro-9-α-L-xylofuranosyl-9-H-purin-6-amine (**14**), 9-α-L-lyxofuranosyl-6-methyl-9-H-purine (**16**), 2-fluoro-9-α-L-lyxofuranosyl-9-H-purine-6-amine (**17**) and 9-(2-deoxy-α-L-lyxofuranosyl)-2-fluoro-9-H-purine-6-amine (**18**). Compounds 4, 7, and 10 are designed to provide 5-membered ring replacements for the imidazole that is listd in various His4 mutations. The other compounds focus on Met64 mutations. All of these newly synthesized compounds were evaluated for their substrate activity to *E. coli* PNP and several mutant forms of *E. coli* PNP. The detailed synthesis of these compounds is presented.

CHEMISTRY

The target compound 9H-purine-9-[5-O-[2-(1H-1,2,4-triazol-1-yl)ethyl]-β-D-ribofuranosyl]-6-methyl (**4**) was synthesized as depicted in Scheme 1. Inosine was converted to **2** following the usual method.[2] The two carbon ether linkage was generated by reacting **2** with ethyl bromoacetate under basic conditions to yield the corresponding ester, 9H-purine-9-[5-O-[ethyl-2-acetate]-2,3-isopropylidene-β-D-ribofuranosyl]-6-methyl. The ester functional group was reduced to alcohol with $LiBH_4$ in the presence of MeOH to afford the hydroxyl compound 9H-purine-9-[5-O-[ethanol-2-yl]-2,3-isopropylidene-β-D-ribofuranosyl]-6-methyl. This hydroxyl group was then converted to the mesityl derivate 9H-purine-9-[5-O-[2-(O-methanesulphonyl)-ethyl]-2,3-isopropylidene-β-D-ribofuranosyl]-6-methyl (**3**). **3** was coupled with triazole under basic conditions to give 9H-purine-9-[5-O-[2-(1H-1,2,4-triazol-1-yl)ethyl]-2,3-isopropylidene-β-D-ribofuranosyl]-6-methyl this on acidic deprotection of isopropylidene group afforded **4**.

The synthesis of 9H-purine-9-[[5-deoxy-5-[2-(1H-imidazol-4-yl)ethyl]-thio]-β-D-ribofuranosyl]-6-methyl (**7**) is outlined in Scheme 2. **2** was converted to the thio ester 9H-purine-9-[[5-deoxy-5-(acetyl)-thio]-β-D-ribofuranosyl]-6-methyl by reacting with thiol acetic acid, under Mitsunobu conditions. Followed by the condensation with 4-(2-methanesulphonylethyl)-N-(t-butyloxycarbonyl)-1H-imidazole (**6**) in



SCHEME 1 ***Reagents and conditions:*** a) acetone, $HC(OEt)_3$, TsOH, RT, 4 hrs; b) Ac_2O-pyridine, 40°C, 2 hrs; c) $POCl_3$, DMF, 1,2-Dichloroethane, reflux, 3 hrs; d) MeZnCl, Tetrakis, THF, RT–reflux, 2 hrs; e) MeONa-MeOH, RT, 1 hr; f) $NaNH_2$, 1,4-dioxane, $BrCH_2CO_2Et$, reflux, 2 hrs; g) $LiBH_4$, Et_2O-MeOH, 0°C, 5–10 mins; h) MsCl,TEA, DCM, 0°C, 1 hr; i) NaH, Triazole, 1,4-dioxane, reflux, 2 hrs; j) 1N HCl, THF, RT, overnight.

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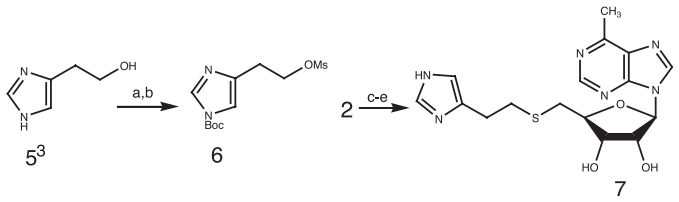


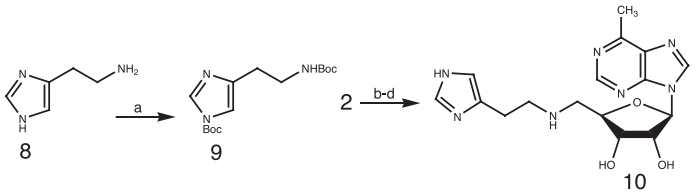
SCHEME 2 ***Reagents and conditions:*** a) Boc_2O, MeOH, TEA, RT, overnight; b) MsCl, TEA DCM, RT, 1 hr; c) CH_3COSH, Ph_3P, DEAD, overnight; d) NaOMe, comp-**4**, MeOH, 0°C-RT, overnight; e)1N HCL, THF, RT, overnight.

MeOH in the presence of NaOMe to give 9H-purine-9-[[5-deoxy-5-[2-(1H-imidazol-4-yl)ethyl]-thio]-2,3-isopropylidene-β-D-ribofuranosyl]-6-methyl. This on hydrolysis of isopropylidene group afforded the target molecule **7**.

Preparation of 9H-purine-9-[[5-deoxy-5-[2-(1H-imidazol-4-yl)ethyl]-amino]-β-D-ribofuranosyl]-6-methyl (**10**) was accomplished by following the steps as described in Scheme 3. The mesityl analogue of **2** was condensed with N-1-bis[t-butoxycarbonyl]-histamine (**9**), in DMF and NaH as base, the resulting compound 9H-purine-9-[[5-deoxy-5-[2-(1H-imidazol-4-yl)ethyl]-amino-5-N-(t-butyloxycarbonyl)]-β-D-ribofuranosyl]-6-methyl was deprotected to afford **10**.

Scheme 4 describes the synthetic pathway of the target molecule 9-H-purin-6-amine-2-fluoro-9-α-L-xylofuranosyl (**14**). The conversion of L-xylose to 1-chloro-2,3,5-tri-O-benzyl-L-xylofuranoside,[4,5] (**11**), was accomplished by the usual method. The silylated derivative of 2-fluoroadenine (**13**) was coupled with **11** under neutral condition, using powdered molecular sieves, in 1,2-dichloroethane at reflux temperature. The resulting nucleoside 9-H-purin-6-amine-2-fluoro-9-α-L-2,3,5-tri-O-benzyl-xylofuranosyl was deblocked with BCl_3 to give **14**.

9-H-Purine-9-α-L-lyxofuranosyl-6-methyl (**16**), 9-H-purin-6-amine-2-fluoro-9-α-L-lyxofuranosyl (**17**) and 9-H-purin-6-amine-9-(2-deoxy-α-L-lyxofuranosyl)-2-fluoro (**18**) were synthesized following the steps illustrated in Scheme 5. L-lyxose was converted to 1-O-acetyl-2,3,5-tri-O-benzoyl-L-lyxofuranose (**12**),[6] following the reported procedure. **12** was coupled with the respective silylated derivatives of **13** & **15** using $SnCl_4$ as the coupling agent. Saponification of the resulting nucleosides led to compounds **17** & **16,** respectively. The 2′-hydroxy of **17** was deoxygenated following the reported procedures[7] with some modifications to afford **18**.



SCHEME 3 ***Reagents and conditions:*** a) Boc_2O, MeOH, TEA, RT, overnight; b) MsCl, TEA, DCM, RT, 1 hr; c) Comp-9, NaH, DMF, 0°C–100°C, 2 hrs; d) 1N HCl, THF, RT, overnight.

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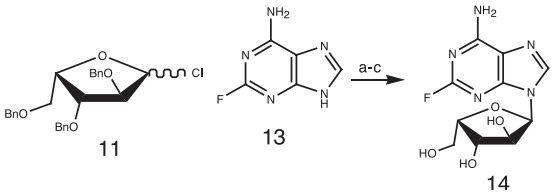
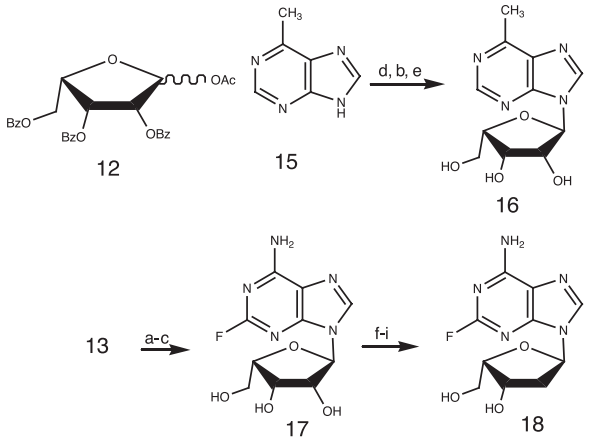


SCHEME 4 ***Reagents and conditions:*** a) N,O-*bis*(trimethylsilyl)acetamide, AcCN, reflux one hour; b) Comp-**11** molecular sieves, 1,2-dichloroethane, reflux; c) BCl_3 DCM, 0°C-RT, overnight.

The structures of all the target molecules were confirmed by MS, ^{1}H-NMR, HPLC, and CHN analysis.

RESULTS

Compounds **4**, **7**, and **10** were incubated with crude extracts (100 μg/mL) of *E. coli* that were transfected with *E. coli* PNP or the various *E. coli* PNP mutants (H5A, H5I, H5L, H5M, H5V, and M64V). Compound **10** was not cleaved by any of the extracts. Compound **7** was readily cleaved by non-transfected cells and its cleavage has not enhanced in any of the bacterial extracts that had been transfected. These results indicated that **7** was a good substrate for an enzyme other than PNP that was endogenously expressed in the bacterium. Compound **4** was not cleaved by purified *E. coli* PNP or the M64V mutant. However, it was cleaved at a rate of 3 nmoles/mg/hr in non-transfected cells. Greater cleavage was detected in *E. coli* that had been transfected with H5A (9 nmoles/mg-hr), H5I (15 nmoles/mg-hr), H5L



SCHEME 5 ***Reagents and conditions:*** a) HMDS, $(NH_4)_2$ SO_4, reflux, 31 h; b) Comp-**12** $SnCl_4$, AcCN, −10°C to RT, 3–5 h; c) 0.5 M KOH, EtOH, RT, 5 h; d) HMDS, TMSCl, 1,2-dichloroethane, reflux, 2 h; e) NaOMe, MeOH, 5°C, 2 h; f) $TIPDSCl_2$, pyridine, RT, overnight; g) ImC(S)Im, DMAP, AcCN, 80°C, overnight; h) TTMSS, Bu^tOOBu^t, toluene, reflux, 2 h; i) Et_4NFxH_2O, AcCN, RT, 1 h.

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TABLE 1 *E. coli* PNP and M64V Incubated with 100 μM of Each Compound Shown

Compound	*E. coli* PNP	M64V	M64V/WT
MeP-dR#	528,000	593,000	1
17	8,000	244,000	30
14	<1	4	>4
16	218	10,000	50
18	2,600	32,000	12

The rate of cleavage was determined by HPLC separation of base from nucleoside. Each number is the average of at least 2 determinations and is represented in nmol/mg/hr.

(21 nmoles/mg-hr), and H5V (12 nmoles/mg-hr). Since these values were significantly above the rate of cleavage in non-transfected cells, these enzymes had increased ability to cleave this compound over wild-type enzyme, although we believe that the rate cleavage is too low to be useful in a gene therapy approach for the treatment of cancer. F-araA, which is a poor substrate for *E. coli* PNP, is cleaved at a rate of 1000 nmoles/mg/hr in cells transfected with *E. coli* PNP (Table 1).

Since 9-H-purin-6-amine-2-fluoro-9-α-L-lyxofuranosyl (**17**) was a good substrate for the M64V mutant, we made other analogs with similar structure to explore the SAR of this enzyme (**14**, **16**, **18**).

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