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# SYNTHESIS AND BIOLOGICAL EVALUATION OF 1,1-DIFLUORO-2-(TETRAHYDRO-3-FURANYL)ETHYLPHOSPHONIC ACIDS POSSESSING A *N*9-PURINYLMETHYL FUNCTIONAL GROUP AT THE RING. A NEW CLASS OF INHIBITORS FOR PURINE NUCLEOSIDE PHOSPHORYLASES

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**Abstract**: 1,1-Difluoro-2-(tetrahydro-3-furanyl)ethylphosphonic acids *cis*-**3** and *trans*-**3** possessing a *N*9-purinylmethyl functionality at the ring were synthesized and tested as "multi-substrate analogue" inhibitors for purine nucleoside phosphorylases. Radical cyclization of allyic α,α-difluorophosphonate (*E*)-**7** was applied to construct the α,α-difluorophosphonate-functionalized tetrahydrofuranyl moiety. The $IC_{50}$ values of *cis*-**3** and *trans*-**3** for human erythrocyte PNP-catalyzed phosphorylation of inosine were determined to be 88 and 320 nM, respectively. The stereochemistry of the inhibitors was found to affect significantly the inhibitory potency. 

*Keywords:* enzyme inhibitors; phosphonic acids and derivatives; isosteres; mimetics; nucleotides

Purine nucleoside phosphorylase (PNP: EC. 2.4.2.1) is a ubiquitous enzyme of the purine salvage pathway. It catalyzes the reversible phosphorolysis of ribo- and 2′-deoxyribonucleosides of guanine and hypoxanthine in higher organisms, as well as of adenine in some prokaryotes.[1] Inhibitors of PNP have been suggested to have therapeutic value in the treatment of T-cell proliferative disease such as T-cell leukemia,[2] since individuals who genetically lack PNP suffer from impairment of their immune system but have normal B-cell function.[3] PNP inhibitors may also prolong the plasma half-lives of some chemotherapeutic agents such as 2′,3′-dideoxyinosine by preventing the PNP-catalyzed inactivation.[4] Consequently, extensive drug-discovery research has been devoted to the design and synthesis of inhibitors of PNP.

Since PNP accomplishes the reversible phosphorylation of the purine nucleosides *via* a ternary complex of enzyme, nucleoside, and orthophosphate, a number of metabolically stable acyclic nucleotide analogues containing a purine and a phosphate-like moiety have been synthesized and examined as "multi-substrate analogue" inhibitors of PNP.[5] However, there is no compound that has reached the stage of clinical trial. Of the PNP inhibitors reported, 9-(5′,5′-difluoro-5′-phosphonopentyl)guanine **1** developed by Halazy *et al.*,[5a] is one of the most potent and structurally simple inhibitors of PNP. We have planned to synthesize conformationally constrained molecules of **1**, which show significant inhibitory activity toward PNP (Fig. 1).[6] The synthesis and biological evaluation of a series of the analogous compounds constrained at the $\chi^2$-bond of **1** resulted in identification of novel nucleotide analogue (±)-**2b** which was a better inhibition motif for PNP isolated from *Cellulomonas* sp. rather than from human erythrocyte.[6a] The study also revealed that the hypoxanthine derivative (±)-**2b** (Ki = 8.8 nM) possesses a higher binding affinity for *Cellulomonas* sp. PNP than the corresponding guanine derivative (±)-**2a** (Ki = 28.2 nM).[6a] However, no significant difference in the binding affinities of the

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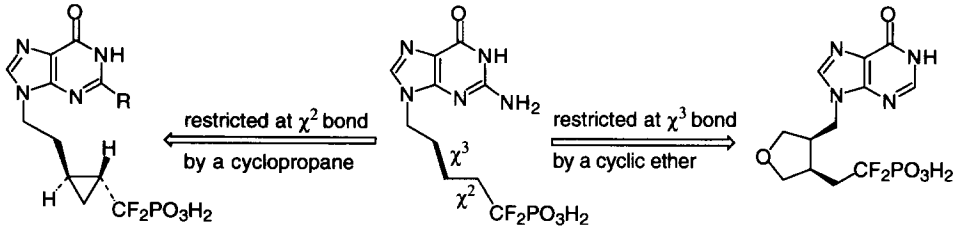
two inhibitors was observed for human erythrocyte-derived PNP.

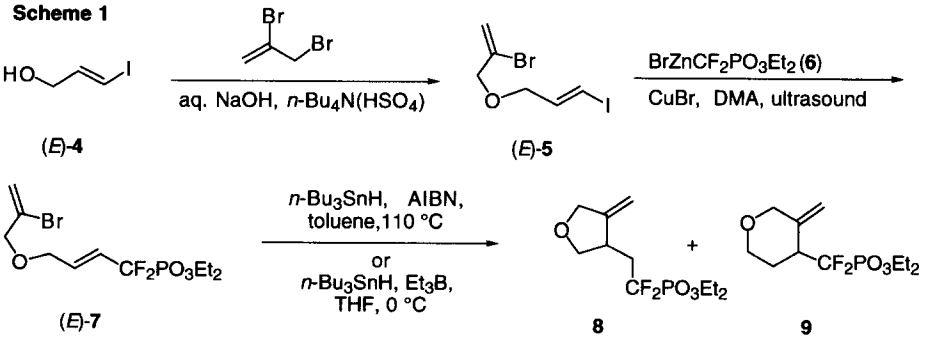
In the present study, to search for motifs effective to inhibit human PNP, we synthesized the novel nucleotide analogue *cis*-**3** and the corresponding *trans*-isomer *trans*-**3** as a new class of inhibitors of PNPs (Fig. 1). The design was based on the following hypotheses. First, the tetrahydrofuranyl moiety incorporated to the $\chi^3$-bond of **1** as a pharmacophore would be interactive with the hydrophobic patch of the ribose binding pocket in the active site of the human PNP in the desired manner.[5b] Second, the ring oxygen might serve as a hydrogen bond acceptor for Tyr-88 which exists in the pocket to assist the binding.[5b] Third, the hypoxanthine substitution for guanine might be an adequate modification when considering the results from our previous work.[6a]

**Fig. 1**




Radical cyclization of allylic α,α-difluorophosphonate (*E*)-**7** was applied to construct the suitably functionalized oxacycle **8**,[7] a key intermediate for the synthesis of the target nucleotide analogues *cis*-**3** and *trans*-**3** (Scheme 1). Treatment of iodoalkenol (*E*)-**4** with 2,3-dibromopropene in aqueous NaOH in the presence of a phase-transfer catalyst ($n$-$Bu_4N(HSO_4)$) gave the bis-functionalized ether (*E*)-**5** in 99% yield.[8] The cross-coupling reaction[9] of (*E*)-**5** with the zinc reagent **6** in dimethylacetamide (DMA) in the presence of CuBr under ultrasound irradiation proceeded selectively at the iodo-carbon to give the requisite (*E*)-**7** in 87% yield. The solvent and sonication were critical for inducing a good yield.[10] Radical cyclization of (*E*)-**7** was first conducted under the conventional conditions ($n$-$Bu_3SnH$ /AIBN / toluene / 110 °C) to give a mixture of **8** and **9** in 82% yield in a ratio of 88:12. The ratio was significantly improved when the reaction was carried out with $n$-$Bu_3SnH$ (1.1 equiv.) in THF (0.03 M solution) in the presence of a stoichiometric amount of $Et_3B$ at 0 °C. This reaction proceeded rapidly (10 min) to give exclusively 5-*exo*-*trig* cyclization product **8** in 91% yield.

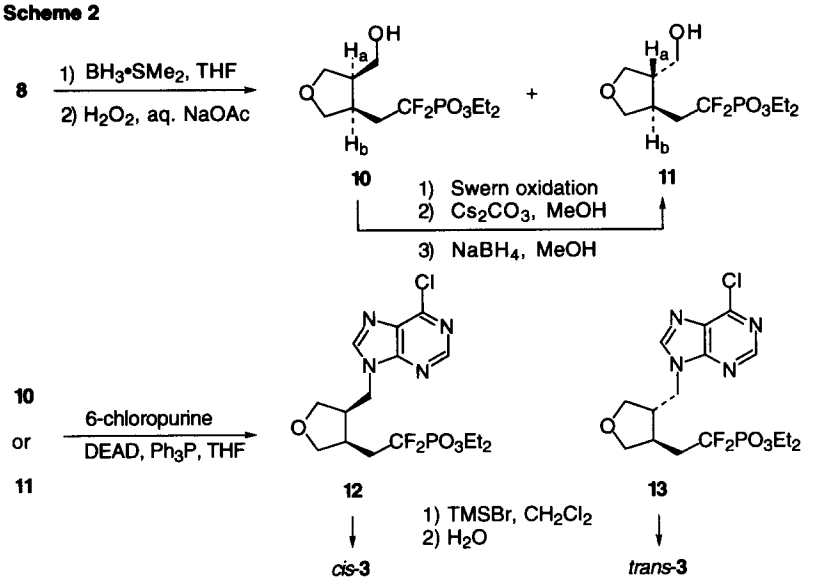
**Scheme 1**




Conversion of **8** to the target nucleotide analogues *cis*-**3** and *trans*-**3** was accomplished as shown in Scheme 2. Hydroboration of **8** with a borane-$SMe_2$ complex in THF at 0 °C, followed by oxidative work-up ($H_2O_2$, aqueous NaOAc) gave a mixture of alcohols **10** and **11** in a ratio of 72:28 in 62% yield.[11] The relative stereochemistry of **10** and **11** was confirmed by NOESY (500 MHz, $CDCl_3$) experiments. In the NOESY spectrum of **10** a correlation between the methine protons ($H_a$ and $H_b$) was observed, whereas there was no correlation from $H_b$ to

the α methylene protons of the hydroxy group. On the other hand, the NOESY spectrum of **11** revealed that there were correlations between $H_b$ and the methylene protons α to the hydroxy group. These results show the stereochemistry of the major isomer **10** should be cis. The *cis*-alcohol **10** was transformed to the *trans*-isomer **11** in 30% overall yield, *via* the corresponding aldehyde by the oxidation-reduction sequence, including base-catalyzed epimerization of the aldehyde. The Mitsunobu coupling of **10** and **11** with 6-chloropurine under the standard conditions (DEAD, $Ph_3P$, THF) gave the corresponding *N*9-alkylated purine derivatives **12** and **13** in 58% and 56% yield, respectively, along with the *N*7-alkylated purine derivatives. Removal of the ethyl protecting group and hydrolysis of a 6-chloropurine for **12** and **13** were performed by treatment with TMSBr in $CH_2Cl_2$, followed by hydrolysis with $H_2O$ in one-pot, to give *cis*-**3** and *trans*-**3** as amorphous powders in 93% and 97% yield, respectively.[12]

**Scheme 2**




The inhibitory potencies of *cis*-**3** and *trans*-**3** were assessed with the $IC_{50}$ values for phosphorylations of inosine catalyzed by PNPs purified from *Cellulomonas* sp. and human erythrocyte.[13] Table shows the $IC_{50}$ values of *cis*-**3** and *trans*-**3** in comparison with those of the nucleotide analogues **1** and (±)-**2a,b**.[6a] The results indicate that both *cis*-**3** and *trans*-**3** are strong inhibitors of *Cellulomonas* sp. PNP with $IC_{50}$ values of 35 and 37 nM, respectively, and that the two compounds are approximately 15-fold more potent than the reference compound **1**. On the other hand, the assessment with human erythrocyte PNP revealed that, while the inhibitory potency of *trans*-**3** is similar to those of **1** and **2a,b**, *cis*-**3** more strongly inhibited the enzyme activity with an $IC_{50}$ value of 88 nM. Dixon plot analysis indicated that *cis*-**3** had a Ki value of 15.2 nM against human erythrocyte PNP (data not shown).[14] The Ki value of the reference compound **1**

**Table.** Inhibition of PNPs by nucleotide analogues **1**, (±)-**2a,b**, *cis*-**3** and *trans*-**3**.

| Compound | $IC_{50}$ (nM)[a] for human erythrocyte PNP[b] | for *Cellulomonas* sp. PNP[c] |
|---|---|---|
| **1** | 380[d] | 540[d] |
| (±)-**2a** | 330[d] | 390[d] |
| (±)-**2b** | 340[d] | 70[d] |
| *cis*-**3** | 88 | 35 |
| *trans*-**3** | 320 | 37 |

[a] Determined in the presence of 0.1 mM inosine and 100 mM Pi (pH 7.5).[13] [b] Purchased from Sigma. [c] Purchased from Toyobo biochemicals. [d] The data were taken from the previous reports.[6a]

for human erythrocyte PNP was determined to be 53.0 nM under the same conditions.[5a,14] Therefore, the present study demonstrates that such structural modification of **1** with a tetrahydrofuranyl ring and a hypoxanthine nucleobase is a useful method for increasing both the binding affinity and inhibition potency to PNP. Additionally, the stereochemistry of the compounds **3** greatly affects the inhibitory potency for human erythrocyte PNP, but not for *Cellulomonas* sp.-derived PNP. The observed difference may result from a difference in the structure of the hydrophobic patch of the ribose binding pocket in the active site between the two species of PNP.[5b,15] Further studies addressing how *cis*-**3** but not *trans*-**3** preferably interacts with the active site of human erythrocyte PNP will be required. Clarification of these questions may lead to a possible creation of a specific inhibitor of human PNP.

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7. The radical cyclization of allylic α,α-difluorophosphonates with various radicals has been investigated for the synthesis of biologically interesting α,α-difluoromethylenephosphonates of carbocyclic and heterocyclic ring systems. The general synthetic aspects of the radical cyclization will be reported elsewhere.
8. All new compounds were fully characterized by $^{1}H$-, $^{13}C$-, $^{31}P$-, $^{19}F$-NMR, IR and MS analyses.
9. For coupling reactions of the zinc reagent **6** with iodoalkene and iodobenzene derivatives: a) Yokomatsu, T.; Suemune, K.; Murano, T.; Shibuya, S. *J. Org. Chem.* **1996**, *61*, 7207. b) Yokomatsu, T.; Murano, T. Suemune, K.; Shibuya, S. *Tetrahedron* **1997**, *53*, 815. c) Yokomatsu, T.; Minowa, T.; Murano, T.; Shibuya, S. *Tetrahedron* **1998**, *54*, 9341. d) Yokomatsu, T.; Murano T.; Umesue, I.; Soeda, S.; Shimeno, H.; Shibuya, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 529.
10. A very low yield (15%) was obtained when the coupling reaction was carried out in DMF without ultrasound irradiation according to our protocol for the coupling reaction with iodoalkenes.[9a]
11. Diastereomerically pure alcohols **10** and **11** were obtained through preparative HPLC separation [Inertsil (GL-science), EtOAc, flow rate: 5 mL/min, UV-detector (254 nm)] of the corresponding trityl ethers, followed by deprotection [Amberlyst 15E®, MeOH].
12. Selected spectroscopic data of *cis*-**3**: $^{13}C$ NMR ($CD_3OD$, 100 MHz) δ 154.79, 150.46, 148.81, 140.83, 122.62 (dt, $J_{CP}$ = 258.5 Hz, $J_{CF}$= 210.0 Hz), 117.69, 73.12, 71.36, 62.10, 61.85, 47.06, 46.96, 45.95, 43.44, 36.13, 33.62, 32.67 (dt, $J_{CP}$ = 15.2 Hz, $J_{CF}$ =21.0 Hz); $^{31}P$ NMR ($CD_3OD$, 162 MHz) δ 4.10 (t, $J_{PF}$= 104.1 Hz), $^{19}F$ NMR ($CD_3OD$, 376 MHz, BTF) δ -47.8 (1F, dddd, $J_{FF}$ = 286.2 Hz, $J_{FP}$ = 104.1 Hz, $J_{FH}$ = 28.9, 9.3 Hz), –50.91 (1F, dddd, $J_{FF}$ = 286.2 Hz, $J_{FP}$ = 104.1 Hz, $J_{FH}$ = 27.3, 12.8 Hz); IR (KBr) 2341, 1710, 1571, 1029 $cm^{-1}$: UV ($H_2O$) $\lambda_{max}$ 250.7 nm (ε=11854); FABMS *m/z* 365($MH^+$). FABHRMS *m/z* calcd for $C_{12}H_{16}F_2N_4O_5P$ ($MH^+$):365.0826. Observed: 365.0801.
13. The $IC_{50}$ values were determined by a xanthine oxidase-coupled assay as previously described.[6] The concentration of Pi used for measurement of inhibition was previously reported to be 100 nM.[6] However, this is an error and should read 100 mM.
14. The Ki value was determined at pH 7.5.[6a]
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