Synthesis of 6-Aryloxy- and 6-Arylalkoxy-2-chloropurines and Their Interactions with Purine Nucleoside Phosphorylase from *Escherichia coli*

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Dedicated to Professor Frank Seela on the occasion of his 60th birthday

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The phase transfer method was applied to perform the nucleophilic substitution of 2,6-dichloropurines by modified arylalkyl alcohol or phenols. Since under these conditions only the 6-halogen is exchanged, this method gives 2-chloro-6-aryloxy- and 2-chloro-6-arylalkoxy-purines. 2-Chloro-6-benzylthiopurine was synthesized by alkylation of 2-chloro-6-thiopurine with benzyl bromide. The stereoisomers of 2-chloro-6-(1-phenyl-1-ethoxy)purine were obtained from *R*- and *S*-enantiomers of *sec.*-phenylethylalcohol and 2,6-dichloropurine.

All derivatives were tested for inhibition with purified hexameric *E. coli* purine nucleoside phosphorylase (PNP). For analogues showing $IC_{50} < 10$ μM, the type of inhibition and inhibition constants were determined. In all cases the experimental data were best described by the mixed-type inhibition model and the uncompetitive inhibition constant, K_{iu}, was found to be several-fold lower than the competitive inhibition constant, K_{ic}. This effect seems to be due to the 6-aryloxy- or 6-arylalkoxy substituent, because a natural PNP substrate adenine, as well as 2-chloroadenine, show mixed type inhibition with almost the same inhibition constants K_{iu} and K_{ic}.

The most potent inhibition was observed for 6-benzylthio-2-chloro-, 6-benzyloxy-2-chloro-, 2-chloro-6-(2-phenyl-1-ethoxy), 2-chloro-6-(3-phenyl-1-propoxy)- and 2-chloro-6-ethoxypurines (K_{iu} = 0.4, 0.6, 1.4, 1.4 and 2.2 μM, respectively). The *R*-stereoisomer of 2-chloro-6-(1-pheny-1-ethoxy)purine has K_{iu} = 2.0 μM, whereas inhibition of its *S* counterpart is rather weak ($IC_{50} > 12$ μM). More rigid (e.g. phenoxy-), non-planar (cyclohexyloxy-), or more bulky (2,4,6-trimethylphenoxy-) substituents at position 6 of the purine base gave less potent inhibitors (IC_{50} = 26, 56 and >100 μM, respectively). The derivatives are selective inhibitors of hexameric "high-molecular mass" PNPs because no inhibitory activity *vs.* trimeric *Cellulomonas sp.* PNP was detected.

By establishing the ligand-dependent stabilization pattern of the *E. coli* PNP it was shown that the new derivatives, similarly as the natural purine bases, are able to form a dead-end ternary complex with the enzyme and orthophosphate. It was also shown that the derivatives are substrates in the reverse synthetic direction catalyzed by *E. coli* PNP.