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# Tight binding enantiomers of pre-clinical drug candidates

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ABSTRACT

MTDIA is a picomolar transition state analogue inhibitor of human methylthioadenosine phosphorylase and a femtomolar inhibitor of *Escherichia coli* methylthioadenosine nucleosidase. MTDIA has proven to be a non-toxic, orally available pre-clinical drug candidate with remarkable anti-tumour activity against a variety of human cancers in mouse xenografts. The structurally similar compound MTDIH is a potent inhibitor of human and malarial purine nucleoside phosphorylase (PNP) as well as the newly discovered enzyme, methylthioinosine phosphorylase, isolated from *Pseudomonas aeruginosa*. Since the enantiomers of some pharmaceuticals have revealed surprising biological activities, the enantiomers of MTDIH and MTDIA, compounds **1** and **2**, respectively, were prepared and their enzyme binding properties studied. Despite binding less tightly to their target enzymes than their enantiomers compounds **1** and **2** are nanomolar inhibitors.



## 1. Introduction

Transition state analogues,[1] of enzyme catalysed reactions, are often potent inhibitors of the target enzyme.[2–4] Both the enzyme, and more often than not, the inhibitors are chiral. With regards to α-amino acids and sugars this chirality is often manifested in the form of L- and D-modifications, respectively. Surprisingly, given the chirality of the enzyme target, and biological targets in general, the D form of the α-amino acids and the L-form of the sugars often retain or offer improved biological activity when compared with their enantiomers.[5–10] Coupled with this improved biological activity, enantiomers may exhibit useful pharmacological activities in terms of improved solubility, bioavailability, stability, and reduced toxicity in vivo as well as extending the patent life of existing drugs.[11] In particular the study of the biological activity of a variety of L-nucleosides has often revealed surprising activity[8,9] and our laboratories have demonstrated the biological activity of the L-nucleoside analogues[12,13] of the immucillins.[14]

Immucillins, based on transition state analogue design of purine nucleoside phosphorylase (PNP), have been described.[14] Syntheses of the enantiomers of the two clinical leads, Forodesine[15–18] and Ulodesine,[19–21] have been reported together with their biological activity (Fig. 1). (3*R*,4*S*)–MT-DADMe-ImmH (MTDIH, *ent*-**1**) is a related compound and its activity as a PNP inhibitor has also been described.[22] Recently the activity of MTDIH against the newly described enzyme methylthioinosine phosphorylase (MTIP) has also been published and the potential of inhibitors of MTIP as antibiotics discussed.[23]

Another immucillin with considerable potential as a drug candidate is (3*R*,4*S*)-5′-methylthio-DADMe-ImmA (MTDIA, *ent*-**2**)[24] (Fig. 1) and hence we are interested in the synthesis of its enantiomer. In particular, where whole-body methylthioadenosine phosphorylase (MTAP) is blocked by MTDIA,[24] dramatic effects are observed in mouse xenografts of a variety of human tumours.[25,26] Also, MTDIA is a femtomolar inhibitor of the dual-substrate enzyme methylthioadenosine/*S*-adenosylhomocysteine nucleosidase (MTAN)[27] from *Escherichia coli* and MTDIA and its analogues may act as antibiotics that do not induce resistance.[28,29]

Therefore, given the biological activity of MTDIH and MTDIA, and the surprising activity of the enantiomers of Forodesine and Ulodesine, we investigated the synthesis and enzyme inhibitory activity of their enantiomers, (3*S*,4*R*)-MTDIH **1** and (3*S*,4*R*)-MTDIA **2** (Fig. 2). In the case of **2** we also investigated its binding to *Ec*MTAN both in terms of the thermodynamics of binding and through the crystal structure of *Ec*MTAN with **2** in the active site.

## 2. Results and discussion

### 2.1. Chemistry

Previously, Clinch et al. described the large scale synthesis of (3*R*,4*R*)-hydroxymethylpyrrolidin-3-ol from diethyl maleate via

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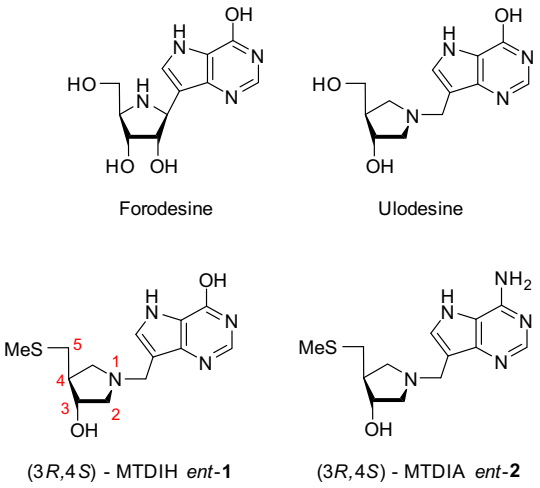



**Figure 1.**

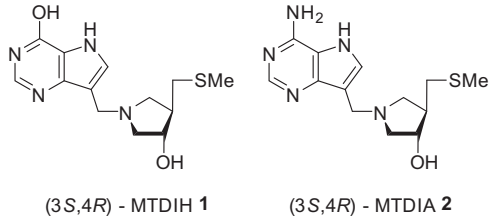



**Figure 2.**

isoxazolidine **3** (Scheme 1).[30] The key step in the synthesis involves an enantioselective enzymatic resolution of compound **4**, using the lipase Novozyme 435, to afford the carboxylic acid (3*S*,4*R*)-**5** and the ethyl ester (3*R*,4*S*)-**6**. Compound (3*S*,4*R*)-**5** has been converted to *ent*-**7** and then to (3*R*,4*R*)-hydroxymethylpyrrolidin-3-ol *ent*-**9**. The other product from the enantioselective resolution process, compound (3*R*,4*S*)-**6** can be used to synthesise the desired enantiomers of MTDIA and MTDIH. Therefore reduction of **6** was achieved with borane, generated in situ from $BF_3{\cdot}OEt_2$ and $NaBH_4$, to afford diol 7 in good yield which could be readily separated from a minor contaminant, the ethyl ether **8** (8%). Hydrogenolysis of the *N*-benzyl protecting group was readily achieved using Pearlman's catalyst where the reaction solvent, methanol, was doped with 1% aqueous ammonia (v:v). Boc protection of amine **9** could be achieved in situ but we preferred a stepwise process to afford carbamate **10** in excellent overall yield for the two steps. Methanesulfonation of compound **10** was carried out at −60 °C and although the reaction was not entirely regioselective, the over-reacted side-product could be carried through to the next step along with the desired product **11**, and the resulting impurity removed later by chromatography. Dibutyltin oxide can also be used to achieve regioselective mesylation, however due to the inherent toxicity of organotin compounds we were not prepared to use this method. Treatment of the crude product from the mesylation step with sodium thiomethoxide at room temperature afforded the Boc protected 5-methylthiopyrrolidine **12** in moderate yield for the two steps. Removal of the Boc protecting group was achieved using concentrated HCl in methanol to afford the desired amine hydrochloride **13**, the right-hand side of the two target molecules, in quantitative yield.

Synthesis of the 9-deazapurine component of the target molecule **1**, 9-deazahypoxanthine, has been well described and it was prepared using a method reported previously.[31,32]

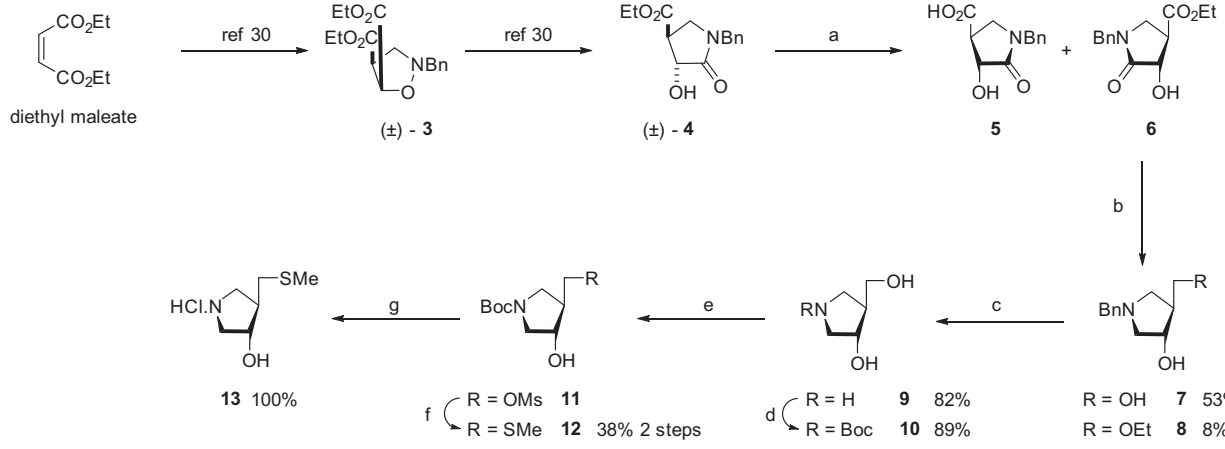
The synthesis of 9-deazaadenine is less well described[33–35] and although our preferred route has been previously outlined[36] the synthetic details of this robust and scalable process have not been previously revealed (Scheme 2).

Ethyl(ethoxymethylene)cyano acetate (**14**) was treated with aminoacetonitrile to afford enamine **15** in excellent yield. The enamine nitrogen of compound **15** was protected using methylchloroformate, again in excellent yield, to afford **16** and this was then cyclised using DBU to provide pyrrole **17** in good yield. Reaction of pyrrole **17** with formamidine acetate in ethanol gave ester **18** which was efficiently decarboxylated to afford 9-deazaadenine as a crystalline solid in 25% overall yield for the 5 steps without chromatography.

Target compounds **1** and **2** were synthesized in a similar fashion to that previously described for their enantiomers[24] utilizing Mannich chemistry.[37] The amine hydrochloride **13** was dissolved in a 1,4-dioxane:water mixture which was buffered with sodium acetate. Formaldehyde was added followed by either 9-deazahypoxanthine or 9-deazaadenine and the resulting mixtures heated to 95 °C to afford the target compounds **1** and **2** in good to moderate yields, respectively, (Scheme 3).

## 2.2. Inhibition studies

The inhibition of human and *Plasmodium falciparum* PNP, *Pseudomonas aeruginosa* MTIP, human MTAP and *Escherichia coli*




**Scheme 1.** (a) Novozyme 435, $H_2O$, acetone, pH 7.5, 27 °C, reflux; (b) $BF_3{\cdot}OEt_2$, $NaBH_4$, THF, 0 °C → room temperature; (c) $Pd(OH)_2$, $H_2$, conc $NH_4OH$, MeOH, room temperature; (d) $Boc_2O$, MeOH, room temperature; (e) MsCl, Hunig's base, $CH_2Cl_2$, −60 °C; (f) NaSMe, DMF, room temperature; (g) MeOH; conc HCl, room temperature.