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INHIBITION OF MEVALONATE 5-PYROPHOSPHATE DECARBOXYLASE BY A PROLINE-CONTAINING TRANSITION STATE ANALOG

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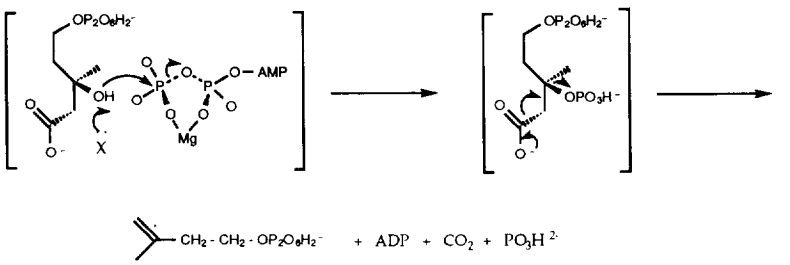
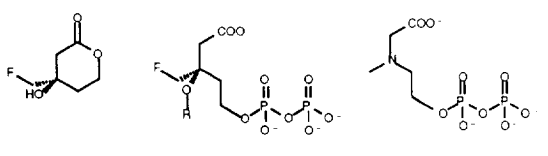
Abstract: Mevalonate 5-pyrophosphate decarboxylase is involved in the conversion of MevPP to IPP in the biosynthesis of steroids and terpenoids from mevalonate. Based on a hypothetical model of the transition state of this enzymatic transformation we have synthesized novel hydroxyacetyl proline analogs that are potent inhibitors of the enzyme.

Mevalonate 5-pyrophosphate (MevPP) decarboxylase (EC 4.1.1.33) is the enzyme involved in the conversion of MevPP to isopentenyl pyrophosphate (IPP) in the third step of the biosynthesis of steroids and terpenoids from mevalonate (Mev).[1,2] In a program aimed at the synthesis and biological evaluation of MevPP decarboxylase inhibitors with the goal of discovering novel agents for the treatment of hypercholesterolemia, we sought the design of analogs that would mimic MevPP at the transition state of its enzymatic transformation.[3] Investigations on the mechanism of action of the enzyme have been reviewed (Scheme 1).[4] Since the discovery of Mev as a key intermediate in the biosynthesis of sterols,[5] several investigators have attempted to inhibit cholesterol biosynthesis by the use of structural analogs of Mev as possible antimetabolites.[6] The most active antimetabolite, 6-fluoromevalonolactone (6-FMev), was found to inhibit the biosynthesis of cholesterol[7] and insect juvenile hormone[8] and the proliferation of Ras-transformed cells[9] by suppressing mevalonate metabolism.

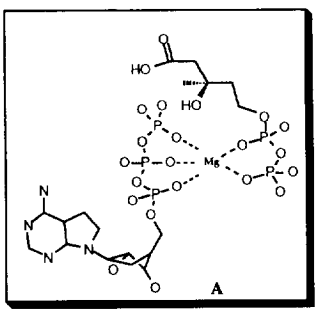
Scheme 1



The mechanism of action of 6-FMev was found to be exerted through its conversion to 6-fluoromevalonate pyrophosphate (6-FMevPP), which is a potent inhibitor of MevPP decarboxylase (K_i = 37 nM).[10] 6-FMevPPP has been recently reported to be a transition state metabolite of 6-FMevPP.[11]

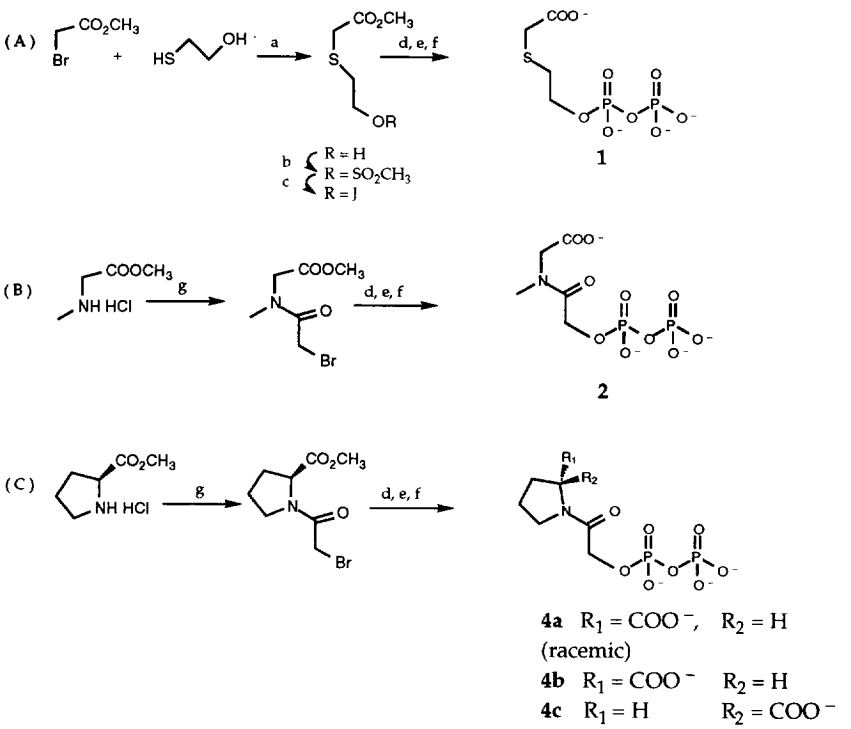


6 - FMev **R= H** **6 - FMevPP** **3**
R= PO_3H_2 **6 - FmevPPP**



In this communication, we postulate a hypothetical model **A** of the transition state of the MevPP decarboxylation reaction where the Mg^{2+} serves as a linker between MevPP and ATP by forming a pentacoordinated ligation between the pyrophosphate and triphosphate groups, respectively. This MevPP-Mg-ATP complex was modeled after the crystal structure of the $(ATP)_2$-Mg complex,[12] available from the Cambridge Data Bank. In this model, one of the ATP molecules in the crystal structure was replaced by a MevPP molecule in such a way that a pentacoordinate Mg^{2+} now ligates to three oxygen atoms of the triphosphate and two oxygen atoms of the pyrophosphate groups, while the C-3 hydroxyl group of MevPP lies in bond-forming proximity to the g-phosphate group of ATP. A molecular modeling program, TFIT,[13] which combines conformational searching and template superposition, identified two low energy conformations of MevPP in which the pyrophosphate, C-3 hydroxyl and carboxyl groups are in a *syn-syn* conformational relationship.

Scheme 2



Reagents and conditions: (a) K_2CO_3, DMF; (b) MsCl, TEA, CH_2Cl_2; (c) NaI, acetone; (d) $P_2O_6H_3$–.(n-$Bu_4N)_3$, CH_3CN; (e) DOWEX - 50W, NH_4+ form, cellulose column purification; (f) LiOH, H_2O; (g) $BrCH_2COCl$, $NaHCO_3$, H_2O-CH_2Cl_2.

As a first step in testing this hypothetical transition state model, we designed MevPP analog **1** in which the C-3 carbon was replaced by a sulfur atom mimicking the electronegative environment of the C-3 hydroxyl of the parent substrate. The synthesis of **1** is depicted in Scheme 2A.[14,15]

The thio analog **1** was found to be a potent inhibitor of MevPP decarboxylase with an IC_{50} = 150 nM.[16] It is important to note that compound **1** is five times more potent than the 3-aza analog **3** (Ki = 750 nM), which was recently reported by Abeles and co-workers as a mimic of a carbocationic transition state of the decarboxylation reaction.[11]

The encouraging activity found with the thio analog **1** led us to synthesize the amide analog **2,** which in addition to the 3-methyl group possesses the carbonyl oxygen at the approximate locus of the C-3 hydroxyl

group of the parent substrate. The synthesis of compound **2** is depicted in Scheme 2 B.[14,15] Unexpectedly, compound **2** (IC_{50} = 300 nM)[16,17] was two times less potent than the thio analog **1**. With the consideration of the transition state model, we rationalized that the weaker activity of **2** might be due to an unfavorable mixture of a *cis* and *trans* amide conformers of which only the *trans* isomer is a transition state analog of MevPP. The knowledge that proline plays an important role in directing the secondary structure of polypeptides and frequently occurs in the i+1 position of reverse turn motifs,[18] prompted us to synthesize the glycolyl-(dl)-proline pyrophosphate **4a** as a *syn* transition state analog of MevPP. The dipeptide-like compound **4a** was found to be a remarkably potent competitive inhibitor of MevPP decarboxylase with IC_{50} = 15 nM.[16] Starting with racemic, d- and l-proline methyl ester the racemic **4a** and its (**S**)-**4b** and (**R**)-**4c** enantiomers were synthesized as depicted in Scheme 2 C.[14,15] As expected, the (**R**)-**4c**, having a configuration compatible with the transition state model, was proven to be an extremely potent inhibitor of the enzyme (IC_{50} = 9 nM),[16,17] while the (**S**)-**4b** enantiomer was only weakly active (IC_{50} = 5 mM). Double reciprocal plots indicate that compound **4c** is a competitive inhibitor of the enzyme with respect to MevPP and non-competitive with respect to ATP (Figure, A and B respectively).

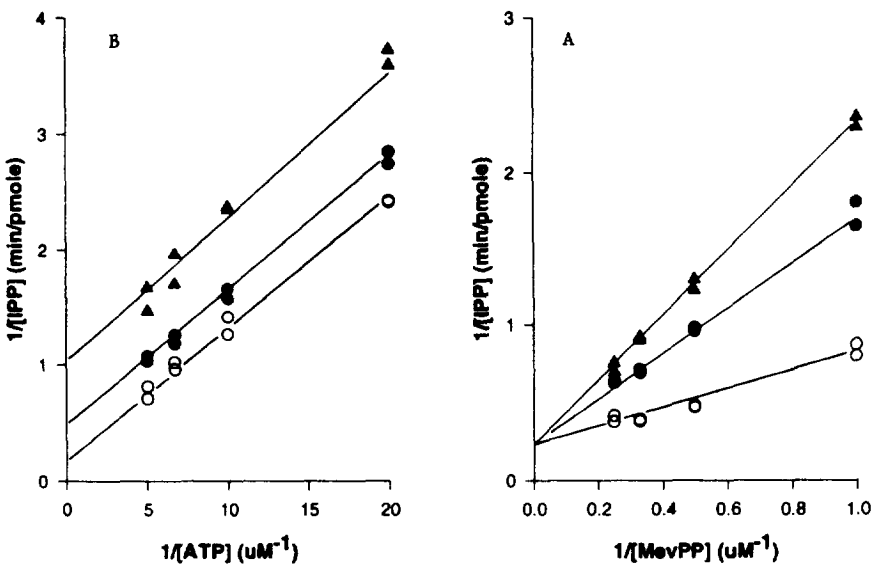


Figure . Double-reciprocal plots showing the effects on the formation of IPP of variable concentrations of ATP (B) and MevPP (A) in the absence (o) and at two fixed concentrations of 5μM (●) and 10μM (▲) of the inhibitor **4c**.

Based on recent reports that proline-containing short linear peptides show significant secondary structure in water,[19] we examined the NMR spectra to see whether a similar secondary structure exists for compound **4c**. NMR studies of compound **4c** indicated the presence of two different forms in water at 30°C, which gave rise to two sets of resonances in its ^{1}H, ^{13}C and ^{31}P NMR spectra.[20] The rotating frame NOESY (ROESY)[21] experiment determined that the glycolyl methylene group is in close proximity to proline $C^{d}H_2$ in both forms. The ^{1}H-^{13}C, HMQC spectrum on the other hand showed two sets of signals for both the carbon and proton resonances corresponding to the *cis* and *trans* conformers in a ratio of 1:2.5.[22] The ROE signals between the glycolyl methylene and the proline $C^{d}H$ in the ^{1}H NMR spectrum for the *cis* conformer may be attributed to transfer of magnetization upon interconversion between the two forms. We have subsequently examined the NMR spectra in the presence of Mg^{2+}. Distinct ROE cross peaks between the glycolyl methylene and the proline $C^{d}H$ for the *trans* isomer and between the glycolyl methylene and the $C^{a}H$ for the *cis* isomer were observed. The latter experiment indicated that the two comformers were present in the same 1:2.5 ratio but their interconversion was impeded in the presence of Mg^{2+}.

Presently, our work is focused on verification of the mode of action of this potent inhibitor of MevPP decarboxylase and on the discovery of pyrophosphoric acid mimics that are suitable for overcoming problems associated with stability and bioavailability of this functionality.

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20. Chemical shifts of ^{1}H, ^{13}C, and ^{31}P NMR defining *cis* and *trans* conformers of **4c**; ^{1}H NMR: (*trans*) δ, 1.92 ($C^{g}H_2$), 1.88 ($C^{b'}H$), 2.16 ($C^{b}H$), 3.5 ($C^{d}H_2$), 4.24 ($C^{a}H$), 4.62 and 4.58 (CH_2CO); (*cis*) δ, 1.77 ($C^{g'}H$), 1.86($C^{g}H$) 2.05 ($C^{b'}H$), 2.25 ($C^{b}H$), 3.5 ($C^{d}H$), 4.23($C^{a}H$), 4.54 and 4.65 (CH_2CO); ^{13}C NMR: (*trans*) δ, 27.5 (C^{g}), 32.5 (C^{b}), 49.4 (C^{d}), 65.2 (C^{a}); (*cis*) δ, 25.2 (C^{g}), 34.9 (C^{b}), 50.6 (C^{d}), 64.5 (C^{a}); ^{31}P NMR: (*trans*) δ, -4.2691(d, $^2J_{pp}$ = 20.39 Hz, α-P), -8.8848 (d, $^2J_{pp}$ = 20.43 Hz, β-P); (*cis*) δ, -4.1743 (d, $^2J_{pp}$ = 19.959 Hz, α-P), -8.8419 (d, $^2J_{pp}$ = 20.002, β-P).
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