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A new motif for inhibitors of geranylgeranyl diphosphate synthase

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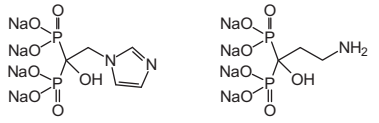
ABSTRACT

The enzyme geranylgeranyl diphosphate synthase (GGDPS) is believed to receive the substrate farnesyl diphosphate through one lipophilic channel and release the product geranylgeranyl diphosphate through another. Bisphosphonates with two isoprenoid chains positioned on the α-carbon have proven to be effective inhibitors of this enzyme. Now a new motif has been prepared with one isoprenoid chain on the α-carbon, a second included as a phosphonate ester, and the potential for a third at the α-carbon. The pivaloyloxymethyl prodrugs of several compounds based on this motif have been prepared and the resulting compounds have been tested for their ability to disrupt protein geranylgeranylation and induce cytotoxicity in myeloma cells. The initial biological studies reveal activity consistent with GGDPS inhibition, and demonstrate a structure–function relationship which is dependent on the nature of the alkyl group at the α-carbon.

1. Introduction

Geranylgeranyl diphosphate synthase (GGDPS) is a key enzyme in the later stages of the mevalonate pathway in humans[1,2] and other organisms.[3] Among other applications, the linear C_{20} diterpenoid that it affords (geranylgeranyl diphosphate or GGDP) is utilized to convert a variety of proteins to lipoproteins through post-translational modification, and the resulting proteins are central to important signaling and protein transport processes. Clinical drugs such as zoledronate (**1**) and pamidronate (**2**, Fig. 1)[4] that inhibit the upstream enzyme farnesyl diphosphate synthase (FDPS) are used for treatment of osteoporosis, Paget's disease, and malignant bone disease including myeloma.[5] However, there is evidence that suggests that these drugs exert their effect through depletion of GGDP,[2] which increases interest in identification of compounds that inhibit GGDPS directly.

One compound that has been found to inhibit GGDPS selectively is digeranyl bisphosphonate (**3**, Fig. 2).[6] The bisphosphonate head group is important to binding with the enzyme through coordination with magnesium cations in the active site. However, early investigation into the nature of substrate binding elucidated the importance of the two nonpolar side chains as well, because they can occupy hydrophobic channels within the enzyme. This led to the hypothesis that binding would be enhanced with a 'V-shaped' inhibitor.[1] Early inhibitors attained a V-shape through placement of two isoprenoid chains on the α-carbon of the bisphosphonate,[6,7] but some linear compounds also show significant activity. For example, we recently reported that a linear triazole bisphosphonate is also a potent inhibitor of this enzyme (IC_{50} = 45 nM).[8] This clearly supports the view that still other strategies for including isoprenoid chains on a methylene bisphosphonate may offer opportunities for potent inhibitors. In this paper, we report the synthesis and initial bioassays of a family of compounds that can achieve a shape similar to digeranyl bisphosphonate by placement of one isoprenoid chain on the α-carbon and a second isoprenoid chain as a phosphonate ester (e.g., **4**). Among other attractive features, this structure could more closely mimic the degree of negative charge found in a diphosphate monoester, and this general structure still allows for incorporation of a third substituent on the α-carbon.



1 (zoledronate) **2** (pamidronate)

Figure 1. Some bisphosphonates in current clinical use.

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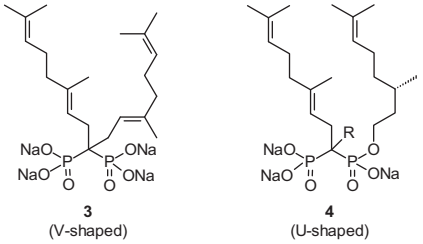


Figure 2. V- and U-shaped isoprenoid bisphosphonates.

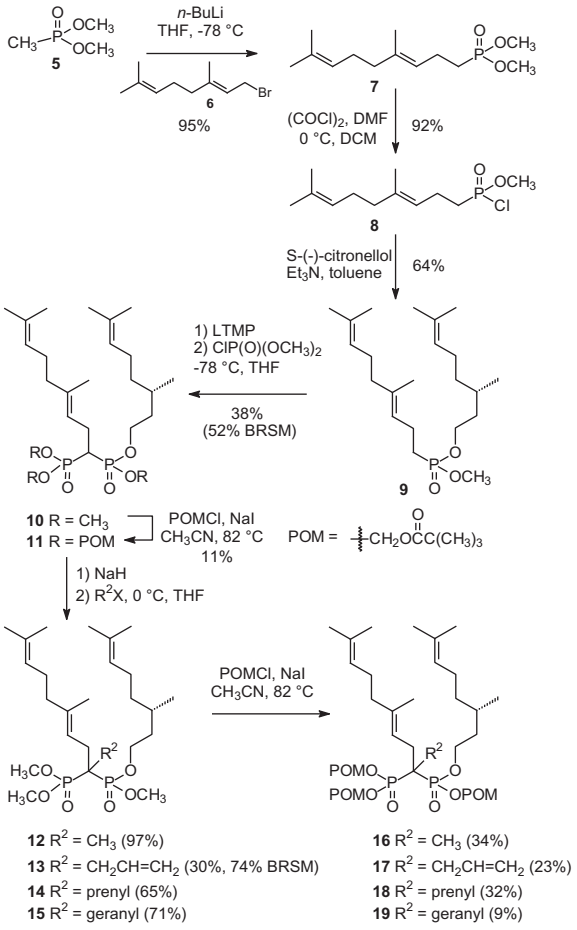
2. Synthesis

At the outset of our work we decided to target a prodrug of structures like compound **4** rather than the salts themselves for a variety of reasons. Highly charged phosphonate salts may have trouble crossing the cell membrane,[9,10] while more lipophilic prodrugs can penetrate rapidly by simple diffusion and liberate the charged phosphonate once inside the cell.[11] Various analyses of cell lysate easily can distinguish between compounds that inhibit GGDPS and those that inhibit geranylgeranyl transferase I and/or II (GGTase I and GGTase II), thus providing information on both activity and selectivity from one assay. The specific prodrug form selected was the pivaloyloxymethyl or POM group, in part because of its long history[12] and in part because it already is in clinical use in both antibiotics[13] and especially in antiviral agents.[14] The POM group is cleaved by nonspecific esterases within the cell, which liberates pivalic acid, formaldehyde, and the phosphonic acid, while simple alkyl esters of phosphonic acids are far more stable to metabolism.[11]

The synthetic sequence began with commercial dimethyl methylphosphonate (**5**, Scheme 1). After treatment of this phosphonate with *n*-BuLi, reaction with freshly prepared geranyl bromide (**6**) gave the alkyl phosphonate **7**. The mono methyl ester mono acid chloride **8** then could be obtained by reaction with oxalyl chloride in DMF.[15] In principle, reaction of this acid chloride with any alcohol should afford a mixed diester. However, out of concern for the lability of an allylic ester like that derived from geraniol,[16] *S*-(−)-citronellol was employed to provide the mixed ester **9**. Phosphonylation of compound **9** could be accomplished by treatment with strong base and dimethyl chlorophosphate.[17–19] This gave the key intermediate **10** in modest yield.

Compound **10** served as the point of divergence to obtain several products of similar structure. The direct reaction of this trimethyl ester with POMCl in the presence of sodium iodide gave the triPOM compound **11** in low yield,[16] and the larger citronellyl ester appeared to be replaced more slowly than the methyl groups under these conditions. To introduce an additional substituent at the α-carbon, treatment of ester **10** with sodium hydride followed by reaction with an alkylating agent (methyl iodide, or allyl, prenyl, or geranyl bromide) gave the expected products **12**–**15**. With each of these four compounds, reaction with POMCl and sodium iodide converted the methyl esters to POM groups while preserving the isoprenoid ester, albeit in low yields. Nevertheless, through these parallel reactions the desired products **16**–**19** were obtained in quantities sufficient for bioassay.

It should be noted that compounds **11** and **16**–**19** were obtained as mixtures of stereoisomers. While the (*S*)-citronellol employed was a single enantiomer, no effort was expended at this stage to control the stereochemistry at the adjacent phosphorus or at the α-carbon. This is readily apparent in the ^{31}P NMR spectra of these compounds (cf. the Supporting information). In the phosphorus spectrum of compound **9**, two resonances of equal intensity are observed reflecting two stereoisomers at phosphorus. Introduction of the second phosphorus through formation of compound **10** results in a more complex ^{31}P NMR spectrum, with two clusters of peaks reflecting both formation of the new stereocenter at the α-carbon and phosphorus–phosphorus coupling. Similar clusters are observed in most of the subsequent products, although introduction of the second geranyl chain in compounds **15** and **19** eliminates the stereocenter at the α-carbon, diminishes the number of diastereomers, and simplifies those phosphorus spectra.



Scheme 1. Synthesis of bisphosphonate mixed esters.

Assuming that the POM groups function as prodrugs as intended,[11] the phosphorus stereocenter will be destroyed once the POM group of the mixed phosphonate ester is hydrolyzed within the cell. The stereochemistry at the α-carbon is more permanent, at least in compounds **16**–**18** and probably in compound **10** at physiological pH, so even after POM hydrolysis the materials tested would be a mixture of two diastereomers. In theory, these diastereomers can be separated but because such separations can prove challenging it was decided to postpone any efforts along these lines until the biological activity of the new structures were determined. While there may be some risks associated with this approach a similar strategy has proven useful with two olefin stereoisomers, where the material first demonstrated activity as a mixture[20] and subsequent synthesis of the individual stereoisomers allowed identification of the more active isomer.[21]

After significant activity was observed in the first representatives of this U-shaped series (vide infra), we began to investigate the importance of stereochemistry through preparation of an *R*-(+)-citronellol ester of the most active inhibitor, compound **16**. Accordingly, the acid chloride **8** was allowed to react with the *R*-(+)-citronellol enantiomer to afford the mixed ester **20** (Scheme 2). Phosphonylation to the bisphosphonate **21** was achieved under conditions parallel to those used to obtain compound **10**, and