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Nitrogen-Containing Bisphosphonates as Carbocation Transition State Analogs for Isoprenoid Biosynthesis

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Nitrogen-containing bisphosphonates are potent bone antiresorptive agents as well as having herbicidal and antiparasitic activity, and are thought to act by inhibiting enzymes of the mevalonate pathway. Using molecular modeling and *ab initio* quantum chemical calculations, we show that bisphosphonates can act as aza-isoprenoid transition state analogs, thereby inhibiting isoprenoid biosynthesis. The two phosphonate groups of the 1,1-bisphosphonates readily dock into the diphosphate-Mg^{2+} binding site in farnesyl diphosphate synthase, while the charged ammonium (or pyridinium or imidazolium) groups act as carbocation transition state analogs, whose binding is stabilized by a cluster of oxygen atoms in the active site cleft, and an overall negative electrostatic potential in this region. Enhanced activity is shown to correlate with increasing van der Waals stabilization due to N-alkylation, or the presence of a charged, planar (sp^2-hybridized) aromatic residue in the carbocation binding site. These results are of general interest since they suggest a rational approach to bisphosphonate drug design.

Bisphosphonates have been employed as therapeutic agents for more than twenty years and presently comprise nearly $1 billion of the global pharmaceutical market (1). However, little has been known about their mode of action in treating disorders such as osteoporosis, Paget's disease, and disorders associated with the hypercalcemia caused by malignancy (2). In recent work, it has been suggested that in bone the potent nitrogen-containing bisphosphonates act as inhibitors of protein prenylation, thereby disrupting signaling and ultimately inducing apoptosis (3). In addition, it has been shown that N-containing bisphosphonates inhibit isopentenyl pyrophosphate (IPP) isomerase/farnesyl pyrophosphate (FPP) synthase activity *in vitro* in a dose-dependent manner which mimics their *in vivo* bone anti-resorptive properties (4). Similarly, N-containing bisphosphonates have been shown to inhibit farnesyl pyrophosphate/geranylgeranyl pyrophosphate (GGPP) synthase activity in plants (5, 6). In this Communication, we explore the structural origins of the activity of several N-containing bisphosphonates. We propose, based on quantum chemical calculations and molecular modeling studies and structural analogies with known N-containing isomerase/synthase inhibitors, that N-containing bisphosphonates act as isoprenoid pyrophosphate carbocation transition state analogs. These fit into the FPP/GGPP synthase active site, and, potentially, dimethylallyl pyrophosphate (DMAPP) isomerase binding sites.

EXPERIMENTAL

Computational aspects. We carried out *ab initio* Hartree-Fock calculations of the molecular electrostatic potential $\Phi(\mathbf{r})$ and the charge density $\rho(\mathbf{r})$ in the geranyl pyrophosphate (GPP) carbocation **7,** the known isoprenoid diphosphate synthase inhibitor **8a,** truncated for calculational purposes to **8b,** and the bisphosphonate **9** using the Gaussian-94 program (7). The calculations were performed using the x-ray geometry of **7** (8), which was modified using standard bond lengths and angles (9) to create **8b** and **9.** The bond torsions for the GPP carbocation were based on the x-ray structure of GPP bound to farnesyl synthase (8), while the structures of **8b** and **9** were based on superimposing these analogues onto the transition state **7** in such a way that there was a minimum in the rms deviation of the coordinates of the anionic (P_1, P_2) and cationic (C^+, N^+) sites between the substrate/transition state **7** and each inhibitor.

We used a uniform 6-31G(d,p) basis set for these calculations, and displayed the electrostatic potentials $\Phi(\mathbf{r})$ mapped onto a constant charge density ($\rho(\mathbf{r}) = 0.05$ e/a_o^3) surface using the Cerius^2 (Molecular Simulations, Inc., San Diego, CA) program (9). We also evaluated the electrostatic potential in the ligand binding site using the Delphi program (10).

These and other related calculations were carried out using Silicon Graphics (Mountain View, CA) O-200 and O-2 computers in this laboratory, and on an SGI O-2000 cluster in the National Center for Supercomputing Application, located in Urbana, IL.

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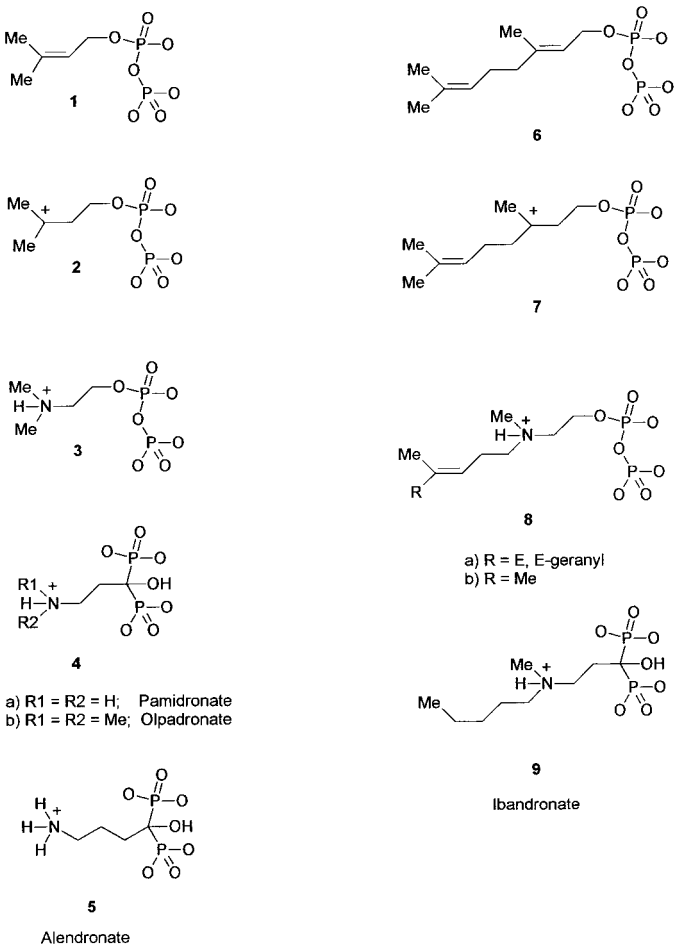


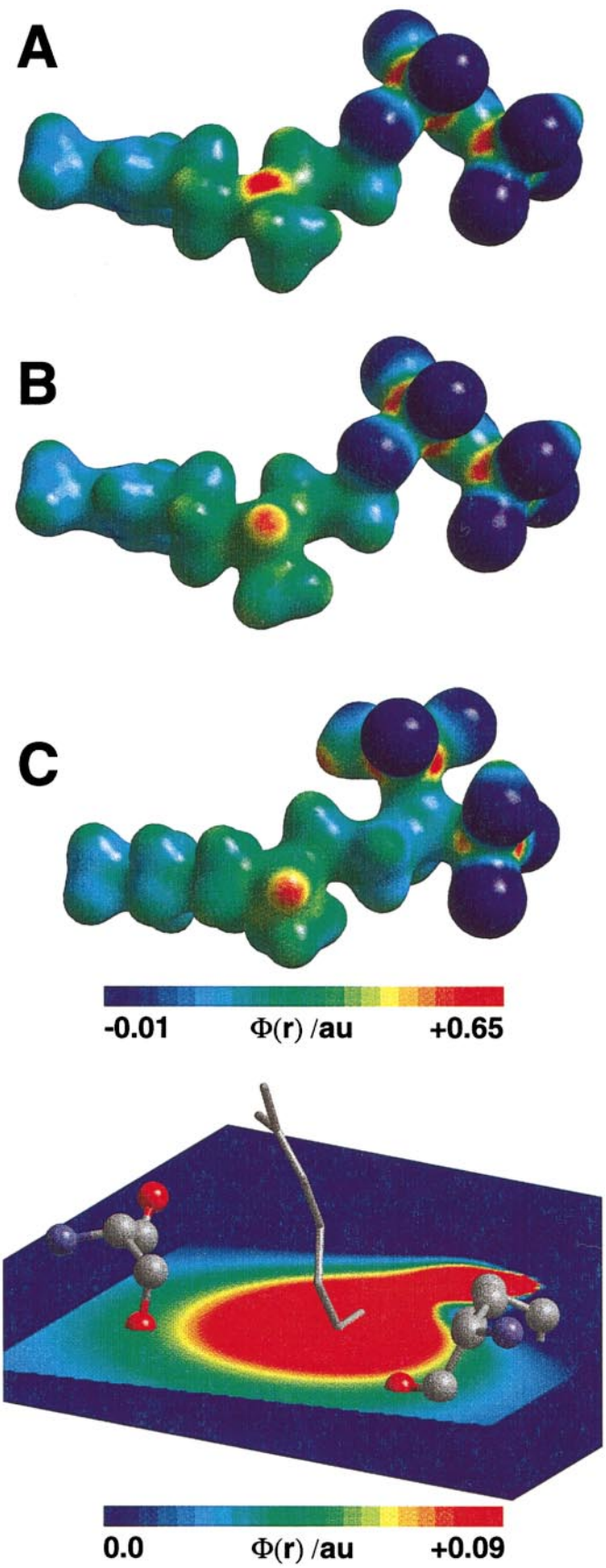
FIG. 1. Structure of compounds discussed in the text; not all hydrogens are shown.

RESULTS AND DISCUSSION

We show in Fig. 1 the structures of: DMAPP, **1,** and GPP, **6;** their putative carbocation intermediates, **2, 7;** known isomerase/synthase inhibitors **3, 8a,** and several important N-containing bisphosphonates, **4a, 4b, 5, 9.** Evidence for a carbocation intermediate in the DMAPP isomerase reaction has accumulated over the years (11–15), and as can be seen from Fig. 1, structure **3,** 2-(dimethylamino)ethyl diphosphate, in the N-protonated form, has a noticeable structural resemblance to the proposed carbocation transition state/reactive intermediate, **2,** since both contain two phosphoryl groups and a cationic center in approximately the same spatial orientation. And, from the perspective of the mode of action of the potent N-containing bisphosphonates, the drugs pamidronate **4a** and olpadronate **4b** also have strong structural similarities to the transition state species, **2,** as can be seen in Fig. 1. That is, the protonated dimethylamino species, **4,** both contain an (aza)isoprenoid-like sidechain and two phosphorus sites. Further evidence for the carbocationic mechanism of DMAPP isomerase comes from the observation that 2-(trimethylammonio)ethyl pyrophosphate is also a potent inhibitor of isopentenyl pyrophosphate isomerase, since once again it is thought to act as a transition state analog (11–13). Similarly, alendronate, **5,** is a potent inhibitor of bone resorption, and of the growth of the protozoan *Dictyostelium discoideum* (14), with the only difference from pamidronate, **4a,** being the addition of an extra CH_2 group. As discussed in more detail below, alendronate may even more closely resemble **2,** due to the increased spatial separation between the cationic and anionic sites due to the additional CH_2 group. In addition to potentially inhibiting DMAPP isomerase, pamidronate, olpadronate and alendronate are expected to inhibit the FPP/GGPP synthase, the structure of which has recently been reported (8, 16). This enzyme has a catalytic site which consists of a large central cavity surrounded by ten alpha helices. The isoprenyl diphosphates (DMAPP, GPP, FPP) bind to the enzyme and are positioned with their hydrophobic chains towards the protein's interior, while their diphosphate groups are bent with respect to the long axis of the molecule, and are stabilized by Coulombic interactions with one or two Mg^{2+} bridges to a DDXXD motif (8, 16) which is highly conserved amongst the isoprenyl synthases (16).

Now, in order to act as transition state analogs/inhibitors of the prenyl synthase enzymes, the N-containing bisphosphonates need to be able to fit into the active site of the enzyme, and undergo similar steric (hard-core repulsive) and attractive (Coulombic, van der Waals dispersive) interactions. To investigate potential electrostatic interactions, we therefore carried out *ab initio* calculations of the molecular electrostatic potential ($\Phi(\mathbf{r})$) for the putative GPP carbocation, **7,** the aza-isoprenoid GPP analogue **8b,** and the potent N-containing bisphosphonate, ibandronate **9,** and mapped $\Phi(\mathbf{r})$ onto a charge density ($\rho(\mathbf{r})$) isosurface, as shown in Figs. 2A–2C. There are strong similarities between the GPP transition state, GPP+ (Fig. 2A), the aza-analog (Fig. 2B) and the bisphosphonate, ibandronate (Fig. 2C), with a large positive charge buildup (red) in the center of the molecule, located ~5.5 Å from P_1 and P_2. While the positive charge is expected, what is of more interest is the observation that the rms deviations between the coordinates of the two phosphorus atoms and the cationic center positions in GPP (or its carbocation transition state), and in the known GPP synthase inhibitor **8a** and the bisphosphonate ibandronate, **9,** are ~0 and ~0.6 Å, respectively, which strongly suggests that both **8a** and **9** act by docking into the GPP (and FPP, GGPP) synthase binding site, causing enzyme inhibition.

Also of interest is the observation that the positive charge distribution in the carbocation and aza-analogs interacts with five oxygen atoms. We show a two-dimensional contour of the electrostatic potential in a

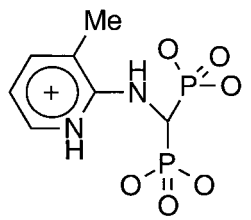


plane defined by two of these oxygens and the tertiary carbocation center of the putative GPP transition state, Fig. 2D, and similar results are obtained for all of the aza-analogs as well, with five oxygen atoms (16, 17) being within a 5.3 Å radius of the cationic center. In addition, computations of the electrostatic potential of the protein itself, using the DelPhi program (10), reveals several patches of negative $\Phi(\mathbf{r})$ adjacent the carbocation center.

GPP, its transition state, the known aza-analog inhibitors **3** and **8a,** and the bisphosphonates **4a, 4b, 5,** and **9,** may all be readily docked into the substrate binding site with little or no steric hindrance from the protein. In addition, in each case the 1,1-bisphosphonate groups appear to readily mimic the "bent" diphosphate binding geometry, interacting with one or more Mg^{2+} ions. Some typical results, for alendronate, ibandronate, and risedronate, are shown together with the GPP carbocation structure in Fig. 3.

With alendronate (Fig. 3B), there is only a 0.3 Å rms deviation between the P_1, P_2 and N positional coordinates of alendronate and those of the bound GPP (Fig. 3A), which may help explain the known enhanced activity of alendronate **5** over pamidronate **4a,** which has a larger (0.6 Å) rms deviation. The higher activity of N,N-dimethylpamidronate, i.e. olpadronate (4), is naturally attributed to enhanced van der Waals attractive interaction in the hydrophobic pocket, with the Me_2N group more closely resembling the dimethylallyl moiety of the carbocation. With ibandronate, (Fig. 3C), the C_5 sidechain simply confers additional van der Waals dispersive attraction.

The transition state model may also be extended to the aromatic N-containing bisphosphonates. Shown below are the potent anti-resporptive N-(2-(3-methylpyridyl)) aminomethylene bisphosphonate, **10** (18), which is also known to strongly inhibit FPP synthase (5), and risedronate, **11,** another potent bone anti-resorptive agent (18) as well as an inhibitor of *D. discoideum* growth (19) and the most potent growth inhibitor of *Entamoeba histolytica* (20).



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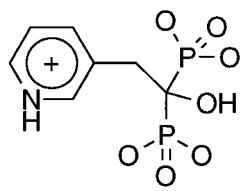

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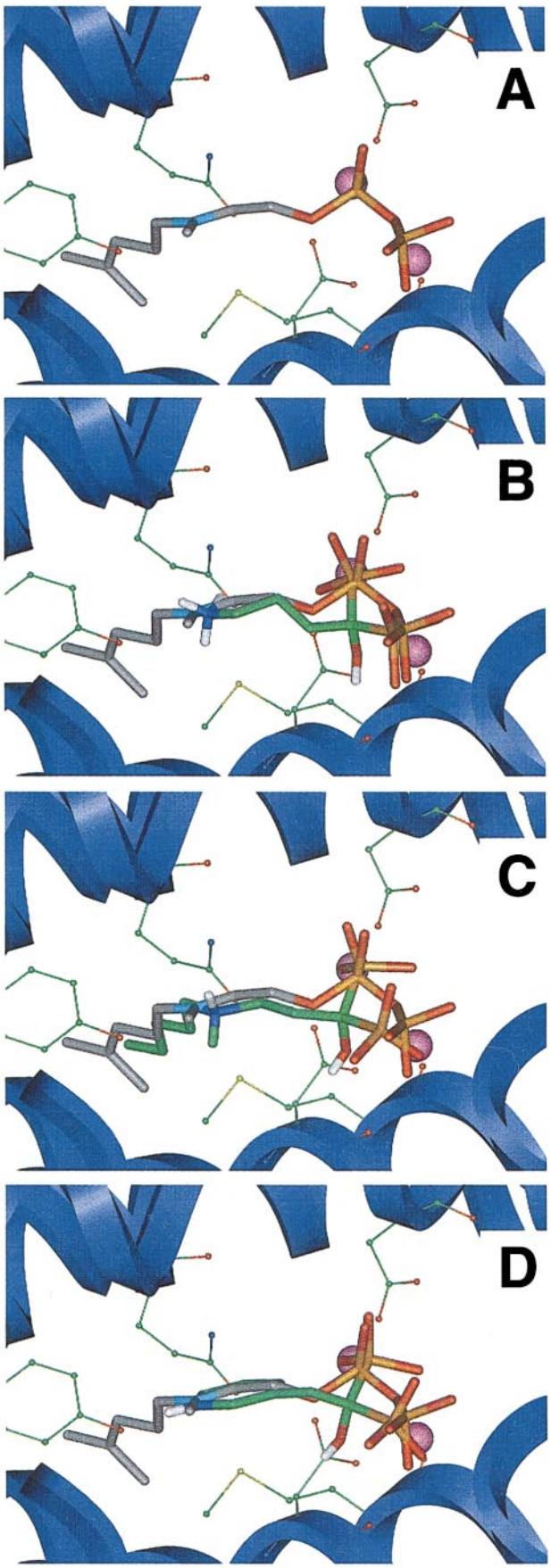
FIG. 2. Electrostatics results for prenyldiphosphates and aza-analogs. A-C, electrostatic potential $\Phi(\mathbf{r})$ mapped onto 0.05 e/a_o^3 charge density ($\rho(\mathbf{r})$) isosurfaces for: A, GPP transition state **7;** B, aza-geranyl diphosphate **8b;** and C, ibandronate **9.** D, Slice through the $\Phi(\mathbf{r})$ surface defined by the OH group of serine 113, the O of LYS 214, and the tertiary carbocation center of the GPP transition state in FPP synthase.



Both compounds possess significant structural similarities to the proposed transition states, having planar sp^2-hybridized carbocations and 1,1-bisphosphonate groups which can be readily mapped into the binding site in FPP synthase, as shown for example for risedronate in Fig. 3D, which has only a 0.35 Å rms deviation in the coordinates of P_1, P_2 and N^+ with respect to the corresponding allylic carbocation structure of GPP, Fig. 3A.

These results all strongly support the hypothesis that those N-containing bisphosphonates which have strong bone anti-resorptive, herbicidal, and anti-parasitic activity act as aza-isoprenoid carbocation transition state analogs. Their action is most likely the same as that of the *known* aza-analogs **3** and **8a,** which inhibit DMAPP isomerase and GPP/FPP synthase, respectively, with the longer isoprenoid analog also active in inhibiting GGPP synthesis as well (21). Our results also give a simple explanation of the known reactivity profiles: alendronate > pamidronate, and ibandronate > olpadronate > pamidronate, in terms of enhanced Coulombic and van der Waals attraction, while the high activity of risedronate is attributable to its small rms deviation from the GPP^+ structure and its planar, sp^2-hybridized pyridinium ring, which mimics the sp^2-hybridized isoprenoid carbocation. These results are of interest since they may lead to many novel approaches to osteoporosis drug and herbicide design, such as the use of prenyl side-chain substitutions, the use of other cationic centers (e.g. sulfonium, amidinium, guanidinium), as well as the use of (aza) phosphonyl phosphinyl (CPCP) analogs (22) which our molecular modeling studies indicate can have <0.06 Å charge center rms deviations from the native prenyldiphosphates. And, since it has recently been found that the bisphosphonates pamidronate and alendronate are active against trypanosomatid (*Trypanosoma cruzi*) and apicomplexan (*Toxoplasma gondii*) parasites as well (23, 24) exhibiting similar reactivity profiles to those seen in bone resorption and in *D. discoideum* growth (alendronate > pamidronate), it appears that additional drug-design efforts in these areas may also be worthwhile, based on the transition state model described above.

FIG. 3. Diagrams showing the spatial location of the GPP/GPP^+ transition state in FPP synthase, Ref. (16), together with (superimposed) the structures of alendronate, ibandronate and risedronate. The GPP^+ carbocation center is in light blue, the nitrogen sites in the bisphosphonates are dark blue, the phosphate groups are pink/red, the Mg^{2+} ions are orange. A, GPP/GPP^+ (drawn with its putative carbocation transition state in light blue); B, GPP^+ plus alendronate; C, GPP^+ plus ibandronate; D, GPP^+ plus risedronate (the benzene ring is seen edge on, and lies atop the sp^2-hybridized carbocation).

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