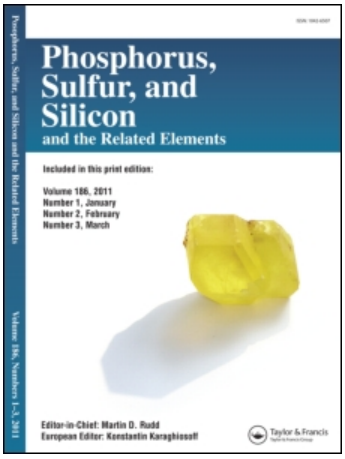

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Bisphosphonates: Molecular Modelling, Structure-Activity Relationships and the Rational Design of New Analogs

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BISPHOSPHONATES: MOLECULAR MODELLING, STRUCTURE-ACTIVITY RELATIONSHIPS AND THE RATIONAL DESIGN OF NEW ANALOGS

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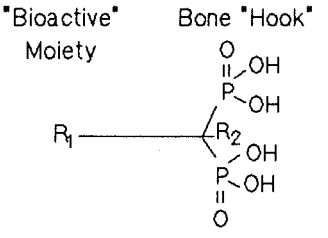
Abstract A combination of 2D NMR spectroscopy, x-ray crystallography and molecular mechanics have been used in the structure determination of a conformationally rigid antiresorptive active bisphosphonate. This rigid structure has provided a template from which other conformationally restricted actives have been successfully designed.

INTRODUCTION

A number of very potent antiresorptive bisphosphonates (BPs) are now known to be capable of altering abnormal bone metabolism in preclinical models[1] and in disease states such as osteoporosis and Paget's disease. The more highly potent antiresorptive BPs have evolved primarily in the past 5 to 10 years and are characterized by a basic nitrogen functionality, including a wide range of heterocyclic moieties. Varying the alkylamine moiety (R_1) or "bioactive tail" (Figure 1) can provide a wide range of antiresorptive potencies within a class of compounds with similar affinity for hydroxy apatite (HAP).[2]

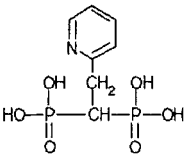
Figure 1



Over the past several years, we have developed a new strategy for the drug design of BPs based on the 3D visualization of low energy conformations of these compounds. In our laboratories, we optimize both the "bone hook" function (the moiety that is directly responsible for the primary HAP adsorption function) of the bisphosphonate molecule and the "bioactive" moiety (the appendage that includes the basic nitrogen functionality and imparts varying antiresorptive potency within a given affinity class). This paper discusses current advances in our use of molecular modelling to aid in the design of the "bioactive" moiety.

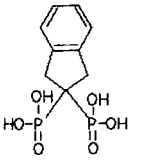
BIOACTIVE MOIETY

At the outset of these studies, a wide range of antiresorptive potent analogs were known. Many of the most potent BPs are characterized by a basic nitrogen functionality such as a heterocyclic substituent for the "bioactive moiety". Among these analogs are the pyridyl alkylidene bisphosphonates such as 1 and the cyclic bisphosphonates such as 2.[3] It had also been demonstrated that subtle changes in structure, such as a hydrocarbon substitution or a change in chain-length, produced wide variations in potency.[1] Since highly potent antiresorptive (LED <0.1 mg P/kg [TPTX[4], GR][1]) cyclic bisphosphonates were not known, we designed a series of nitrogen-substituted cyclic BPs to compliment our structure-activity learnings from the amino-substituted alkylidene BPs.



LED = 0.01 mg P/kg (GR)

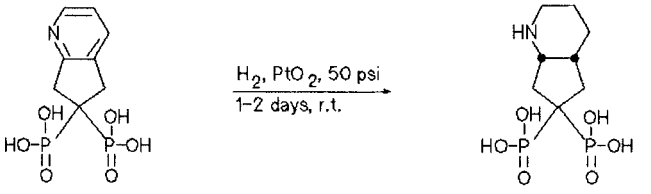
1



LED = 1.0 mg P/kg (GR)

2

Our SAR learnings were applied to the design of the pyrindine analogs 3 and 4. We discovered that although the unsaturated analog 3 was essentially inactive (LED >1.0 mg P/kg [TPTX]), hydrogenation to provide the fully saturated analog, 4, resulted in a highly potent antiresorptive agent (LED=0.01 mg P/kg [TPTX, GR]). The rigid conformation and opposing activities discovered with these two compounds provided a useful template for the beginning of our rational drug design strategy.[5] The rational drug design approach utilized in our laboratories is based on the conformational analysis of antiresorptive potent BPs in comparison with inactive BPs.

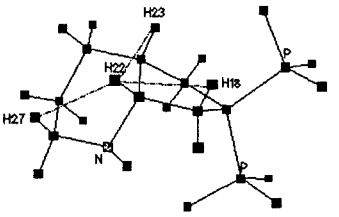


3

4

The conformational analysis of 4 depended on the use of MM2 calculations, single x-ray crystallography and NMR techniques. Initially, models of pyrindine 4 demonstrated that several unique ring-flip conformations needed to be considered. The final three-dimensional structure of 4 was then selected from the lowest energy conformer after MM2 energy minimization calculations.

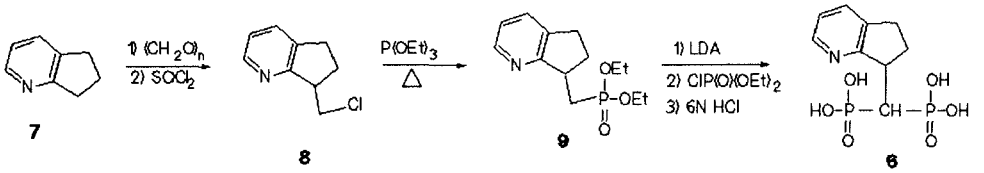
Figure 2



To further confirm the plausibility of this calculated conformation, a single crystal x-ray analysis was performed on the racemate 4. The results are shown in Figure 2 and are in excellent correlation to the calculated structure. To add to our hypothesis that Figure 2 was a reasonable representation *in vivo*, we then studied pyrindine 4 by NMR spectroscopy at an approximate physiological pH of 7.4. Finally, we used two-dimensional NMR techniques, in particular, the Nuclear Overhauser sensitive NOESY experiment, to calculate the same critical interproton distances (H_{22}-H_{23}, H_{22}-H_{18}, H_{22}-H_{27}).

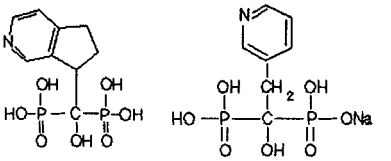
The potent pyrindine 4, therefore, appears to exist in a well-defined three-dimensional conformation that is markedly different than that of the relatively flat and inactive ring system of the dihydropyrindine BP.[5] We are now attempting to understand the importance of these molecular shape differences in hopes of determining optimum "bioactive" conformations for antiresorptive activity. For example, from studying how conformationally non-rigid potent analogue 1 fits the rigid analogue 4 we predicted that pyrindine **6** and pyrindine 10 would be active rigid analogs of 1 and 11 respectively.

The 1-pyrindine **6** can be prepared by the following synthetic sequence. Condensation of 2,3-cyclopentenopyridine **7** with paraformaldehyde in a bomb at 115°C for 12 hours provides the methyl alcohol in 71% yield.[6] The methyl alcohol is conveniently converted to the chloride **8** by treatment with thionyl chloride in benzene in 65% yield. The phosphonate **9** is then synthesized by employment of the Arbuzov reaction. The chloride **8** and triethyl phosphite are heated neat at 125°C under a stream of nitrogen for 48 hours. Excess triethyl phosphite is removed by Kugelrohr distillation and the phosphonate **9** is obtained in a 66% yield following prep HPLC. The bisphosphonate is then formed by the addition of phosphonate **9** to a solution of pregenerated LDA in THF at -78°C followed by the addition of diethyl chlorophosphite. Hydrolysis of the ethyl esters in refluxing 6N HCl provides the bisphosphonic acid **6** in good yield and high purity.



The 2-pyrindine **10** has been prepared by the photolytic ring contraction of a suitable substituted isoquinolinol. The details of the synthetic sequence will be discussed in a subsequent publication.

The pyrindines **6** and **10** have been tested in a number of preclinical models to assess their antiresorptive potency. Both pyrindines exhibit good antiresorptive potency in the growing rat model[1] although pyrindine **6** is not as active as the conformationally unrestricted analog **1**. Similarly, the conformationally restricted 2-pyrindine **10** is less potent than the nonrestricted hydroxy bisphosphonate **11**. These results may suggest that either our template structure **4** needs to be modified slightly and that it does not accurately depict the active conformation or that rigidity as in the conformationally restricted analogs **6** and **10** decreases the biological activity. Further studies in this area are ongoing in our laboratories.



10 **11**

	1	**6**		
Antiresorptive Potency Growing Rat Model (GR) Lowest Effective Dose (mg P/kg)	0.01	0.1	0.01	0.0003

CONCLUSIONS

A novel cyclic bisphosphonate, **4**, has been discovered with high potency, and it has been determined to have a definitive conformationally rigid structure. Our first attempt to utilize this information in drug design has also been described with the synthesis and testing of conformationally restricted analogs, pyrindine **6** and **10**. Thus, our evidence suggests that we have completed the initial steps necessary to implement a more rational design of "bioactive" moieties with potent bone activity.

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