Highly Potent Geminal Bisphosphonates. From Pamidronate Disodium (Aredia) to Zoledronic Acid (Zometa)

Leo Widler,* Knut A. Jaeggi, Markus Glatt, Klaus Müller, Rolf Bachmann, Michael Bisping, Anne-Ruth Born, Reto Cortesi, Gabriela Guiglia, Heidi Jeker, Rémy Klein, Ueli Ramseier, Johann Schmid, Gerard Schreiber, Yves Seltenmeyer, and Jonathan R. Green

Novartis Pharma Research, Arthritis and Bone Metabolism Therapeutic Area, CH-4002 Basel, Switzerland

Received January 14, 2002

Bisphosphonates (BPs) are pyrophosphate analogues in which the oxygen in P−O−P has been replaced by a carbon, resulting in a metabolically stable P−C−P structure. Pamidronate (**1b**, Novartis), a second-generation BP, was the starting point for extensive SAR studies. Small changes of the structure of pamidronate lead to marked improvements of the inhibition of osteoclastic resorption potency. Alendronate (**1c**, MSD), with an extra methylene group in the *N*-alkyl chain, and olpadronate (**1h**, Gador), the *N*,*N*-dimethyl analogue, are about 10 times more potent than pamidronate. Extending one of the *N*-methyl groups of olpadronate to a pentyl substituent leads to ibandronate (**1k**, Roche, Boehringer-Mannheim), which is the most potent close analogue of pamidronate. Even slightly better antiresorptive potency is achieved with derivatives having a phenyl group linked via a short aliphatic tether of three to four atoms to nitrogen, the second substituent being preferentially a methyl group (e.g., **4g**, **4j**, **5d**, or **5r**). The most potent BPs are found in the series containing a heteroaromatic moiety (with at least one nitrogen atom), which is linked via a single methylene group to the geminal bisphosphonate unit. Zoledronic acid (**6i**), the most potent derivative, has an ED_{50} of 0.07 mg/kg in the TPTX in vivo assay after sc administration. It not only shows by far the highest therapeutic ratio when comparing resorption inhibition with undesired inhibition of bone mineralization but also exhibits superior renal tolerability. Zoledronic acid (**6i**) has thus been selected for clinical development under the registered trade name Zometa. The results of the clinical trials indicate that low doses are both efficacious and safe for the treatment of tumor-induced hypercalcemia, Paget's disease of bone, osteolytic metastases, and postmenopausal osteoporosis.

Introduction

The first bisphosphonates (originally called diphosphonates) were synthesized in the 19th century.[1] They were initially used mainly as antiscaling and anticorrosive agents but also as complexing agents in the textile, fertilizer, and oil industries.[2] However, it was not until the late 1960s that their potential for the treatment of various diseases of bone mineral metabolism became evident. Geminal bisphosphonates (for simplicity usually just called bisphosphonates; BPs) are metabolically stable analogues of the naturally occurring inorganic pyrophosphate, which have been shown by Fleisch and co-workers to impair the formation and dissolution of calcium phosphate crystals in vitro.[3,4] Like pyrophosphate, bisphosphonates have high affinity for bone mineral and at high doses can modulate calcification both in vitro and in vivo. In contrast to pyrophosphate, BPs are also orally active, although their low bioavailability often limits the usefulness of oral administration. More importantly, the replacement of the oxygen atom between the two phosphonic acid moieties of pyrophosphate by a carbon atom opened up the possibility of attaching side chains (Figure 1). Preferably, R^1 is a hydroxyl group, which increases the affinity for calcium even further owing to the ability of such

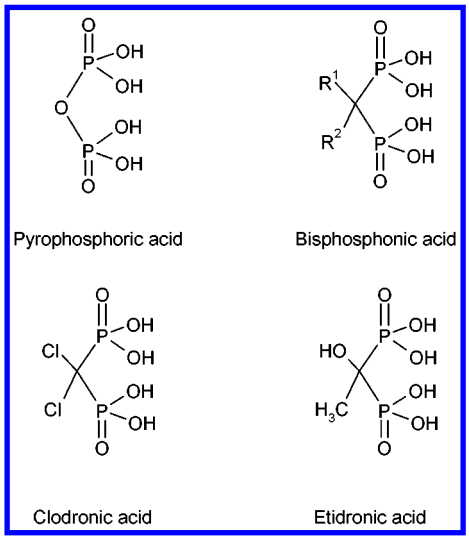


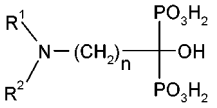
Figure 1. Bisphosphonic acids are stable analogues of pyrophosphoric acid. Clodronic and etidronic acid are the most important first-generation BPs.

derivatives to act as tridentate ligands. The nature of R^2 is key to the optimization of BPs as potent inhibitors of osteoclastic bone resorption. The first-generation BPs had either a single atom or a simple alkyl side chain at R^2 (e.g., clodronic and etidronic acid, Figure 1) and were relatively weak inhibitors of bone resorption. Antiresorptive potency was already markedly increased in pamidronate (**1b**; see Table 1), a second-generation

* To whom correspondence should be adddressed. Phone: +41 61 696 57 86. Fax: +41 61 696 60 71. E-mail: leo.widler@pharma.novartis.com.

10.1021/jm020819i CCC: $22.00
Published on Web 07/19/2002

Table 1. Dependence of Antiresorptive Potency of Pamidronate Analogues on N Substituents and on the Distance to the Phosphono Groups



compound[a]		R^1	R^2	*n*	ED_{50}[b] (μg/kg)
1a		H	H	1	150
1b	pamidronate	H	H	2	61
1c	alendronate	H	H	3	8
1d		H	H	4	20
1e	neridronate	H	H	5	60
1f		Ac	H	2	>1000
1g		Me	H	2	~15
1h	olpadronate	Me	Me	2	12
1i		propyl	Me	2	3
1j		Et	Et	2	3
1k	ibandronate	pentyl	Me	2	1.1

[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3.

compound and the first BP with a basic aminoalkyl group at R^2. Most of the highly potent third-generation BPs contain heteroaromatic rings with one or more nitrogen atoms as components of their side chains.

There has been a great deal of speculation about the exact mechanism of action of BPs. However, it is now clear that cellular effects on osteoclasts are involved rather than purely physicochemical effects on hydroxyapatite crystals as originally proposed.[4,5] Recently it has been found that the non-nitrogen-containing bisphosphonates with simple substituents (e.g., clodronate and etidronate) can be metabolically incorporated into non-hydrolyzable cytotoxic ATP analogues whereas the more potent nitrogen-containing bisphosphonates appear to inhibit the mevalonate pathway.[6−10] They may thus affect protein prenylation, thereby disrupting the intracellular trafficking of key regulatory proteins and inducing apoptosis (programmed cell death).[11] Effects of BPs on osteoclast recruitment[12] as well as indirect effects via stimulation of osteoblasts to produce an osteoclast inhibitory factor have also been described.[13−16]

The first BPs successfully used in the clinic were etidronate and clodronate in the 1970s and 1980s. The potency of these first-generation compounds was only moderate, and moreover, for etidronate, the therapeutic window between the inhibition of bone resorption and impaired bone mineralization was rather small. The search for more potent BPs by many pharmaceutical companies has resulted in a number of novel, highly potent derivatives.[18,19]

The first member of the second-generation BPs was pamidronate (**1b**, Table 1), originally synthesized by Henkel as an additive for detergents and subsequently licensed to Ciba-Geigy for development as a pharmaceutical agent (pamidronate disodium, Aredia). It was the first BP to contain a basic nitrogen atom in its alkyl side chain.[20] Pamidronate exhibits increased potency as an inhibitor of bone resorption and excellent tolerability, resulting in a wide therapeutic window. It has been extensively used clinically for the treatment of patients with tumour-induced hypercalcaemia and osteolytic

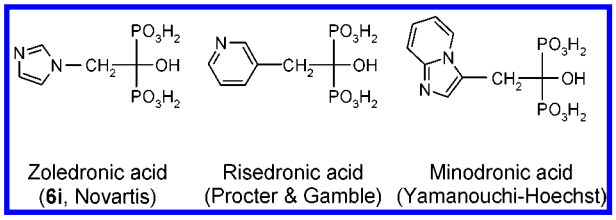


Figure 2. Important bisphosphonic acids with heteroaromatic substituents.

bone metastases arising from breast cancer or multiple myeloma and for Paget's disease of bone.[20] Subsequently, several other N-containing BPs of increasing potency have been developed for the treatment of both benign and malignant bone diseases: alendronate,[21] risedronate,[22] ibandronate,[23] and incadronate.[24]

In 1986, a chemistry program was initiated at Ciba-Geigy to find an even more potent BP as a follow-up to pamidronate for the treatment of osteoporosis and metastatic bone disease.[25] Our own results, as well as those of others,[9,26] clearly indicate that the ability of BPs to inhibit bone resorption is dependent on two separate properties of the BP structure. The phosphonate groups are essential for high affinity for bone mineral, which is further increased in compounds with a hydroxyl group at the R^1 position ("bone hook").[8,9,27−30] The nature of R^2 determines the biological activity of the molecule and influences the ability of the drug to interact with specific molecular targets. Because second-generation BPs exhibit physicochemical effects on mineralization similar to those of early BPs while possessing markedly increased potency as inhibitors of bone resorption, the mechanism of antiresorptive activity cannot be derived solely from direct effects on bone mineral.

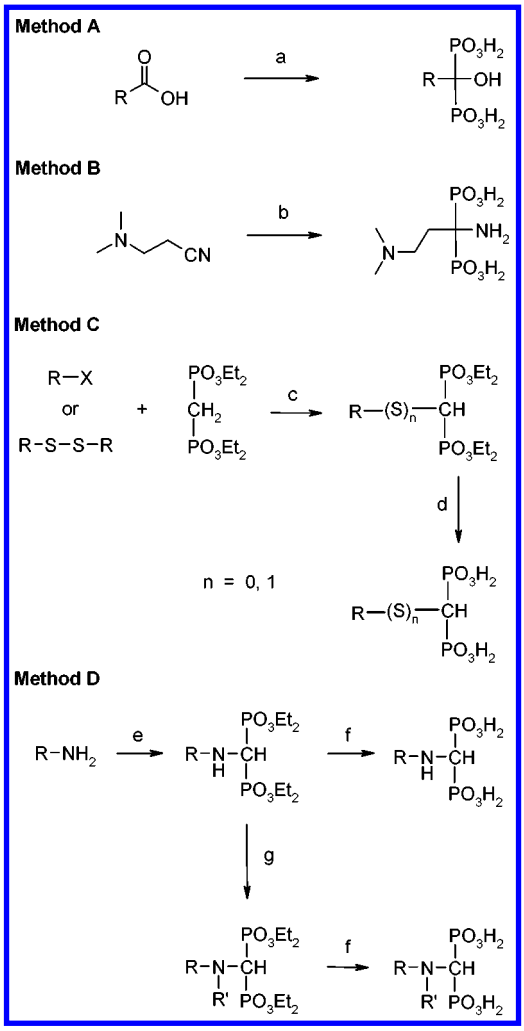
This paper describes our efforts to improve the potency and therapeutic window of pamidronate, the first second-generation bisphosphonate, toward zoledronic acid (Figure 2), which is so far the most potent agent of its class. Concurrent to our own efforts, similar SAR studies were undertaken in several laboratories. Published data are mainly available from work conducted at Procter & Gamble[27,31,32] and Yamanouchi.[33]

Chemistry

(a) Bisphosphonic and Bisphosphinic Acids. Scheme 1 summarizes the methods used for the preparation of different classes of bisphosphonates.

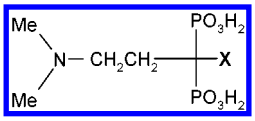
1-Hydroxybisphosphonate derivatives were prepared from the corresponding carboxylic acids by treatment with a mixture of phosphoric acid and phosphorus trichloride, followed by quenching with water (method A). While this reaction was usually performed in chlorobenzene at the laboratory scale, an improved process using methansulfonic acid as a solvent was preferred for large-scale preparation. Amino groups present in the substituent R were further modified as appropriate by reductive amination (**1h, 1i**, **1o, 4l**, **5r**; Tables 1, 3, 6, 7), acetylation (**1f**, Table 1), or hydrogenation (**1g**; Table 1, from the *N*-benzyl analogue). A similar approach afforded 1-aminobisphosphonates starting from nitriles (method B). This method was used to prepare **1m** (Table 2). Bisphosphonates in which the 1-hydroxy substituent is replaced by a hydrogen atom are accessible via alkylation of tetraethyl methylenebisphosphonate followed by acidic hydrolysis (method C; **1l**, Table 2; **9a**,

Scheme 1. General Methods for the Synthesis of Geminal Bisphosphonates[a]



[a] Reagents and conditions: (a) (i) H_3PO_4, PCl_3, chlorobenzene or MsOH, (ii) H_2O; (b) H_3PO_4, PBr_3; (c) NaH, THF; (d) 48% HBr or TMSBr; (e) $HC(OEt)_3$, HPO_3Et_2 (2 equiv); (f) 1 N HCl or TMSI; (g) R′−X, NaH, THF.

Table 2. Effect of the Substituent X on Antiresorptive Potency



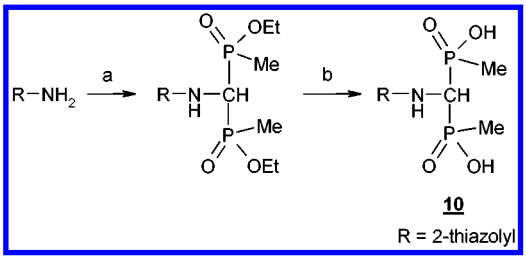
compound[a]		X	ED_{50}[b] (μg/kg)
1h	olpadronate	OH	12
1l		H	100
1m		NH_2	>200

[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3.

9b, Table 11). Finally, aminomethylene BPs were synthesized starting with a primary amine, which was treated with triethyl orthoformate and 2 equiv of diethyl phosphite. The phosphonate esters were hydrolyzed with 1 M HCl or trimethylsilyl iodide to give 1-amino-bisphosphonic acids (method D).

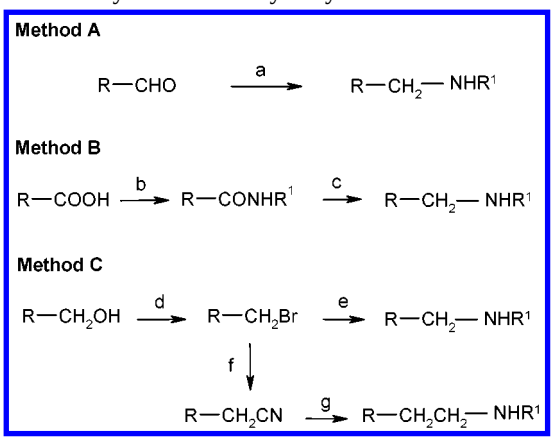
Aminomethylenebisphosphinic acids were prepared in an analogous way as the corresponding bisphosphospho-

Scheme 2. Synthesis of Aminomethylenebisphosphinic Acids[a]



[a] Reagents and conditions: (a) $HC(OEt)_3$, HP(O)MeOEt (2 equiv); (b) 1 N HCl or TMSBr.

Scheme 3. Synthesis of Arylalkylamines[a]



[a] Reagents and conditions: (a) (i) R^1NH_2, (ii) H_2, Pd/C or reductive amination with $NaBH_3CN$; (b) (i) $SOCl_2$, (ii) R^1NH_2; (c) $LiAlH_4$; (d) PBr_3 or HBr; (e) R^1NH_2; (f) NaCN; (g) Raney Ni.

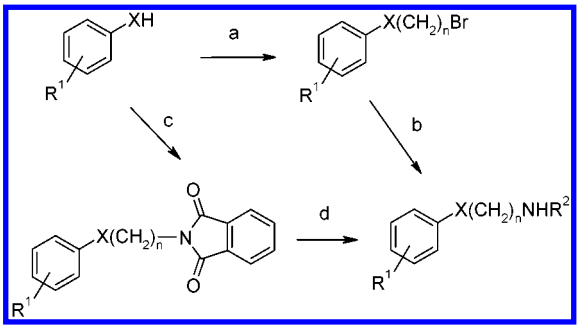
nic acids, by substitution of diethyl phosphite for ethyl methylphosphonite (Scheme 2).

(b) Synthesis of Precursor Amines and Acids. Arylalkylamines that are not commercially available can be prepared via several standard methods from carboxylic acids, aldehydes, or alcohols (Scheme 3). Arylalkylaldehydes can easily be transformed into secondary amines by reductive amination or by a two-step procedure via imine formation followed by reduction (Scheme 3, method A). Alternatively, the required amines may be synthesized starting with carboxylic acids, which are converted to amides followed by reduction with lithium aluminum hydride (Scheme 3, method B). Finally, a third method used was the substitution of bromine in arylalkyl bromides either directly with amines or with sodium cyanide followed by hydrogenation over Raney Ni to afford homologated primary amines (Scheme 3, method C). All primary amines were monobenzylated prior to their further transformation into carboxylic acids as outlined below (cf. Scheme 5).

Most of the phenoxy- and phenylsulfanylalkylamines were prepared by treatment of (substituted) phenols or thiophenols with α,ω-dibromoalkylenes under basic conditions. The reaction of the resulting bromides with primary amines afforded the desired secondary amines. Alternatively, alkylation of phenols and thiophenols with *N*-phthalimidoalkyl bromides followed by basic hydrolysis leads to primary amines (Scheme 4).

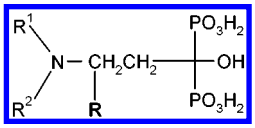
Analogues of pamidronate (**1b**, Table 1) have a two-carbon linker between the nitrogen of the side chain and the carbon between the phosphonic acid groups. The required carboxylic acid precursors for these compounds

Scheme 4. Synthesis of Phenoxy- and Phenylsulfanylalkylamines (X = O or S)[a]



[a] Reagents and conditions: (a) $Br(CH_2)_nBr$, NaOH, H_2O; (b) R^2NH_2, under pressure; (c) $Br(CH_2)_nNPhth$, NaOMe, EtOH; (d) 1 M KOH.

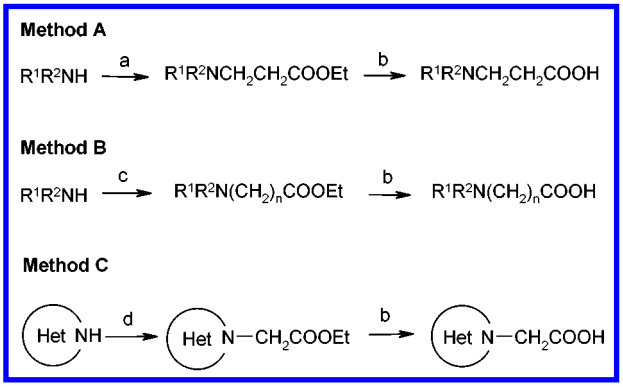
Table 3. Influence on Antiresorptive Potency by a Methyl Substituent α to the Amino Group



compound[a]		R^1	R^2	R	ED_{50}[b] (μg/kg)
1b	pamidronate	H	H	H	61
1n[c]		H	H	Me	3.4
1h	olpadronate	Me	Me	H	12
1o[c]		Me	Me	Me	18
1k	ibandronate	pentyl	Me	H	1.2
1p[c]		pentyl	Me	Me	65

[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3. [c] Racemate.

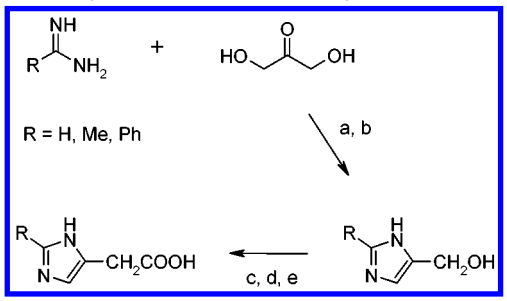
Scheme 5. Synthesis of carboxylic acids[a]



[a] Reagents and conditions: (a) ethyl acrylate; (b) 4 M HCl; (c) Hal−$(CH_2)_n$−COOEt, K_2CO_3, 2-butanone; (d) $BrCH_2COOEt$, NaOMe, MeOH.

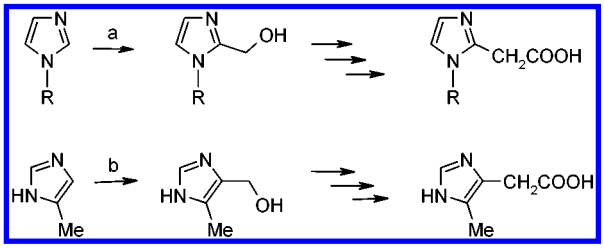
were conveniently obtained by conjugate addition of suitably substituted amines to ethyl acrylate (ethyl crotonate for **1p**, Table 3) and subsequent hydrolysis (Scheme 5, method A). Analogues with longer linkers were synthesized according to method B, Scheme 5. Bisphosphonates with heteroaromatic side chains preferably have a one-carbon atom linker. In cases where the heteroaromatic ring is linked via an aromatic ring nitrogen, the precursor acids were prepared by alkylation with ethyl chloro- or bromoacetate under basic conditions followed by acidic hydrolysis (Scheme 5, method C). Alkylation with ethyl 3-bromopropionate afforded the homologous acids.

Scheme 6. Synthesis of Imidazol-4-ylacetic Acids[a]



[a] Reagents and conditions: (a) NH_3(l), 70 °C, Δ*p*; (b) HCl; (c) $SOCl_2$; (d) NaCN, DMSO; (e) HCl, AcOH.

Scheme 7. Hydroxymethylation of Imidazole Derivatives[a]



[a] Reagents and conditions: (a) paraformaldehyde or 37% HCHO, 120−130 °C, Δ*p*; (b) 37% HCHO, 40 °C.

Imidazol-4-ylacetic acids were synthesized according to the literature.[34,35] Formamidine and dihydroxyacetone was condensed in liquid ammonia under pressure at 70 °C to give 4-hydroxymethylimidazole in moderate yield. By replacement of formamidine by acetamidine or benzamidine, the corresponding 2-methyl and 2-phenyl derivatives were obtained in good yield. The hydroxymethylimidazoles were then transformed into the corresponding chlorides with thionyl chloride followed by reaction with sodium cyanide/DMSO to form imidazolylacetonitriles, which were hydrolyzed with HCl/acetic acid (Scheme 6).

Imidazol-2-ylmethanol derivatives were prepared by heating N-substituted imidazoles with an aqueous formaldehyde solution to 120−130 °C under pressure according to literature procedures[36,37] (Scheme 7). By use of the same standard organic reactions as indicated in Scheme 6, these were homologated to the desired acetic acid analogues with 2-imidazolyl substituents. Two-carbon homologations using Wittig-type chemistry to afford after hydrogenation the corresponding propionic acid derivatives were achieved from the corresponding formyl derivatives.

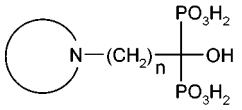
Rather surprisingly, hydroxymethylation of 4-methylimidazole at 40 °C affords the 5-hydroxymethyl derivative exclusively[38] (Scheme 7).

Results and Discussion

Antiresorptive potency of all compounds synthesized was directly assessed in vivo in thyroparathyroidectomized (TPTX) rats with hypercalcemia induced by 1,25-dihydroxyvitamin D_3.[39]

Since it was clear that the amino group was the key structural element for the high potency of pamidronate (**1b**, Table 1), the search for more potent derivatives concentrated on BPs containing at least one nitrogen in the side chain. Our SAR studies started with the synthesis of close analogues of pamidronate. The length

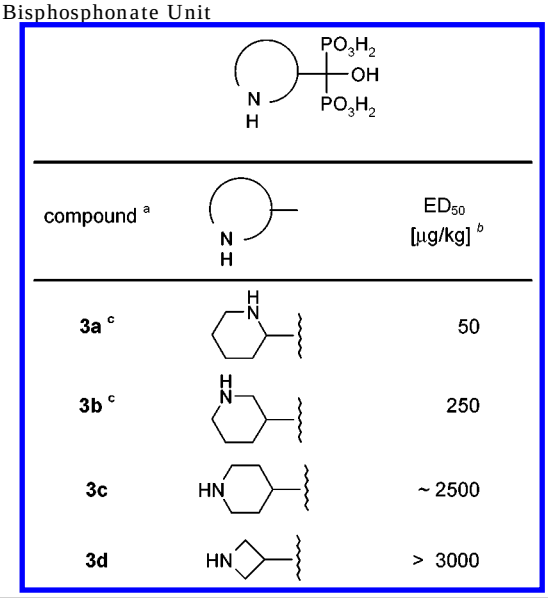
Table 4. Antiresorptive Potency of Bisphosphonates with a Cyclic Amino Substiutent



comp. [a]		R	n	ED_{50} [μg/kg] [b]
2a [c]		H	2	10
2b		H	3	25
2c		H	5	250
2d [d]		Ph	2	70
2e [d]		4-Cl-Ph	2	3.5
2f		H	2	5.6
2g		Ph	2	~ 11
2h		Ph	3	100
2i		Ph	5	> 300
2j		3-F-Ph	2	30
2k			2	25
2l			2	> 300
2m		Me	2	~ 400
2n		Ph	2	> 10000

[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3. [c] EB-1053. [d] Racemate.

Table 5. Antiresorptive Potency of Bisphosphonates with an Additional Bridge between the Amino Group and the Tether to the Bisphosphonate Unit



compound [a]		ED_{50} [μg/kg] [b]
3a [c]		50
3b [c]		250
3c		~ 2500
3d		> 3000

[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3. [c] Racemate.

of the tether between the methylenebisphosphonate unit and the amino group is important. Alendronate (**1c**, MSD/Gentili) with an additional methylene group is almost 10 times more potent than pamidronate and shows the best potency of all BPs with a primary amino group. Longer chains clearly diminish the activity. However, potency can also be increased by alkylation of the primary amine best exemplified by ibandronate (**1k**, Roche/Boehringer Mannheim). Acetylation on the other hand destroys the activity, indicating that a basic nitrogen functionality is of primary importance for high potency of BPs.

The importance of the substituent X, which may serve as an additional ligand and therefore may add to the strength of the bone "hook",[8,9,27−30] is highlighted in Table 2. Compound **1l**, which lacks the hydroxy group, is about 10-fold less active compared to **1h** (olpadronate, dimethyl pamidronate). The exchange of the hydroxy group by a primary amino group reduces the activity even further.

Interestingly the potency of pamidronate can be drastically increased by the addition of a methyl group to the 2-position of the ethylene bridge. The same modification, however, does not improve the potency of olpadronate and, in the case of ibandronate, leads to a significant loss of activity (cf. Table 3).

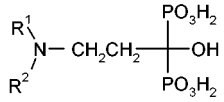
The nitrogen may be incorporated into a ring system, e.g., formally linking the methyl groups of **1h** (olpadronate) together. Such derivatives show similar activities as olpadronate itself for ring sizes 5−7. The eight-member ring derivative **2l** is clearly less active (Table 4). The size of the alkyl linker chain is very important; a chain length of 2 seems to be optimal (compare **2a** with **2b** and **2c** and compare **2g** with **2h** and **2i**), in contrast to the simple derivatives of Table 1, where alendronate with $n = 3$ is the most active analogue. Among the BPs with an additional phenyl substituent at the pyrrolidine (or piperidine ring), only **2e** exhibits increased potency compared to the unsubstituted ring analogue. Piperazine derivatives (**2m**, **2n**) show very low activity.

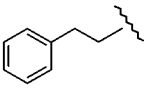
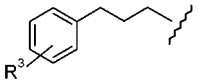
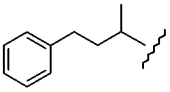
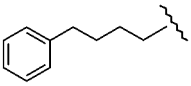
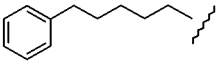
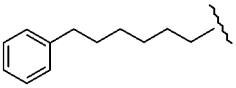
Another type of cyclic derivative is summarized in Table 5. The high dependence of antiresorptive potency on the optimal distance between the nitrogen atom and the bone "hook" is again nicely demonstrated by the series of regioisomers in the piperidine series. Rather unexpected is the higher activity of compound **3a** compared with **3b**, which has the same number of carbon atoms as pamidronate between C1 and the nitrogen.

Addition of (substituted) phenyl groups to one of the alkyl substituents at the amino group has resulted in some of the most potent BPs (**4g, 4j**, Table 6). The best potency is observed for derivatives with R^2 = methyl; ethyl seems to be less favorable (compare **4d** and **4e**). Also, the corresponding secondary amines are clearly less active in all cases investigated. There is a marked dependency of potency on the length of the linkers between the nitrogen and the phenyl ring; **4k** and especially **4l** with five and six methylene units, respectively, illustrate the rapid loss of antiresorptive potency for long-chain analogues.

The phenyl group may be linked via an oxygen or sulfur atom to the N-alkyl chain (Table 7). The second N-alkyl group R^1 should again be small, with methyl

Table 6. Antiresorptive Potency of Arylalkyl-Substituted Derivatives of Pamidronate



compound [a]	R^1	R^2	R^3	ED_{50} [μg/kg] [b]
4a		H		300
4b		Me		1.4
4c		H	H	20
4d		Me	H	1
4e		Et	H	15
4f		Me	3-Me	1.5
4g		Me	4-Cl	0.7
4h [c]		H		> 10'000
4i		H		1.0
4j		Me		0.4
4k		Me		20
4l		Me		1500

[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3. [c] Racemate.

Table 7. Antiresorptive Potency of Phenoxyalkyl- and Phenylsulfanylalkyl-Substitued Bisphosphonates

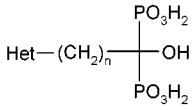
compound[a]	X	R^1	R^2	*m*	*n*	ED_{50}[b] (μg/kg)
5a	O	Me	H	2	2	1.5
5b	O	Me	4-Cl	2	2	1.7
5c	O	H	H	3	2	1.2
5d	O	Me	H	3	2	0.5
5e	O	Me	3-Me	3	2	1.7
5f	O	Me	4-F	3	2	0.6
5g	O	Me	4-Cl	3	2	1.3
5h	O	Me	4-MeO	3	2	1.2
5i	O	Et	H	3	2	20
5j	O	propyl	H	3	2	10
5k	O	butyl	H	3	2	500
5l	O	Me	H	4	2	4
5m	O	Me	H	6	2	7500
5n	O	Me	H	3	3	100
5o	S	H	H	2	2	>200
5p	S	Me	H	2	2	0.7
5q	S	H	H	3	2	7
5r	S	Me	H	3	2	0.33
5s	S	Me	4-Cl	3	2	7.8

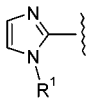
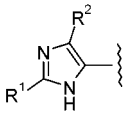
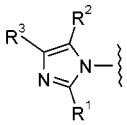
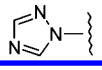
[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3.

being slightly better than hydrogen (cf. **5c**, **5d**, **5i**−**k**). The potency-enhancing effect of the additional methyl group becomes much more pronounced in the sulfur series (cf. **5o** vs **5p** and **5q** vs **5r**). Many of the compounds in Table 7 have ED_{50} values of 1 μg/kg or less. The exact position of the tertiary amino group is very important, exemplified by the reduction in activity of **5n** versus **5l**, which have the same number of atoms in the alkyl side chain between C1 and the phenyl ring. Their only difference is the position of the nitrogen atom. Very long distances between the bisphosphonate moiety and the phenyl ring are clearly detrimental to the activity of these derivatives (cf. **5m**), which is in accordance with the trends shown in Table 6. Small additional substituents on the phenyl ring have very little influence (cf. **5b**, **5e**−**h**).

The important basic nitrogen does not have to be an aliphatic amine. As was first shown by Procter & Gamble, it may be substituted by the nitrogen of a heteroaromatic ring system, and it has turned out that the most promising BPs belong to this class of compounds. The work by the Procter & Gamble group concentrated on six-membered ring systems. Their efforts culminated in the development of risedronate (Figure 2). At Ciba-Geigy, we focused on derivatives with five-membered heterocycles, of which the most attractive turned out to be the imidazole ring. There are three different ways to attach an imidazole to the carbon chain linker. In the series of 2-imidazolyl derivatives, the influence of different substituents at the nitrogen was investigated. The *N*-methyl derivative **6b** (Table 8) is about 10 times more active than the NH analogue **6a**. The larger *N*-benzyl group (**6c**), on the other hand, decreases activity. A drop in potency is found when the number of carbons in the linker chain

Table 8. Antiresorptive Potency of Bisphosphonates with Five-Membered Heterocyclic Aromatic Ring Systems



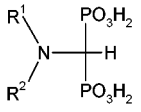
compound [a]	Het	R^1	R^2	R^3	n	ED_{50} [μg/kg] [b]
6a		H			1	5
6b		Me			1	0.6
6c		Bz			1	25
6d		H	H		1	0.3
6e		H	H		2	20
6f		Me	H		1	15
6g		Ph	H		1	> 3000
6h		H	Me		1	1.5
6i		H	H	H	1	0.07
6j		H	H	H	2	45
6k		Me	H	H	1	3
6l		H	Me	Me	1	1.5
6m		-			1	> 300
6n		-			1	600

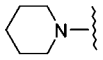
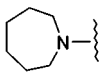
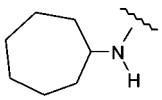
[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3.

is increased to two, as shown in the 4-imidazolyl series; **6e** has lost almost 2 orders of magnitude in activity compared to **6d**. A methyl and especially a phenyl substituent at the 2-position of these imidazoles also results in a marked drop in the ED_{50} value (cf. **6f**, **6g**). The most active BP known to date has been obtained by linking the imidazole ring via a nitrogen atom to the bone "hook" (**6i**). Extension of the linker chain leads again to a dramatic loss of activity (**6j**). Additional methyl substituents at the different ring carbon atoms do not greatly affect the potency of imidazolyl derivatives, but in all the cases investigated so far, there is a trend of a slight loss of activity (**6h**, **6k**, **6l**). Pyrazole and triazole proved to be less attractive heterocycles for BPs (**6m**, **6n**).

If the "bioactive moiety" R^1 (Figure 1) is linked via a nitrogen atom to the bone "hook", R^2 cannot be a hydroxyl group for stability reasons. Such BPs therefore are only bidentate ligands for calcium and thus have a lower affinity for bone. Nevertheless, several rather potent derivatives have been found among these derivatives. Table 9 shows some simple derivatives with aliphatic amino substituents. Whereas small amino groups lead to only marginally active BPs (**7a**,**7b**, Table 9), potency improves with increasing size and lipophilicity of the amino group (**7c**, **7d**). Takeuchi et al. (Yamanouchi) have reported extensively on secondary amine analogues.[33] Their finding that a sufficiently bulky substituent on the secondary nitrogen atom is necessary for potent antiresoptive activity corroborates our own data (cf. high potency of **7e**).

Table 9. Antiresorptive Potency of Derivatives of Aminomethanebisphosphonic Acid

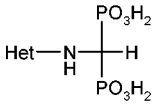


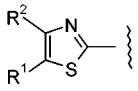
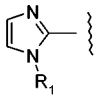
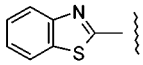
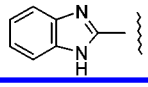
compound [a]	R^1R^2N	ED_{50} [μg/kg] [b]
7a	Me_2N	> 3000
7b		> 2000
7c		800
7d		40
7e [c]		7

[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3. [c] Incadronate (Yamanouchi).

The most attractive aminomethylenebisphosphonates are again those that have a heteroaromatic substituent. Of particular interest are compounds that bear thiazole

Table 10. Antiresorptive Potency of Heteroarylaminomethanebisphosphonic Acid



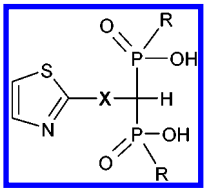
compound [a]	Het	R^1	R^2	ED_{50} [μg/kg] [b]
8a		H	H	5
8b		H	Me	100
8c		Me	H	1.5
8d		Et	H	1.5
8e		Pr	H	2
8f		Bu	H	0.9
8g		^{i}Pr	H	200
8h		$PhCH_2CH_2$	H	2.7
8i		Ph	H	> 1000
8j		H	-	~ 500
8k		Me	-	5
8l		$PhCH_2$	-	75
8m		Ph	-	200
8n		-	-	> 3000
8o		-	-	> 10'000

[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3.

substituents. The 2-thiazolyl derivative **8a** (Table 10) has a potency comparable to that of alendronate (**1c**) or at least 10 times that of pamidronate (**1b**, Table 1). Potency is strongly dependent on substituents in positions 4 and 5. A methyl group in position 4, i.e., next to the ring nitrogen, causes a 20-fold drop in activity (**8b**). Shifting the methyl group to the 5-position (**8c**) brings about a slight improvement in the ED_{50} value compared with that of the unsubstituted analogue. Larger substituents are also tolerated (**8d**−**f**); even the phenethyl derivative **8h** shows high potency. Only residues with high steric bulk at the point of attachment (cf. the isoproyl substituted derivative **8g** or **8i** with a directly linked phenyl group) are considerably less potent. These residues may prevent the important interaction of the basic amino functionality with the putative molecular target. Compound **8f** is the most potent in the series, equipotent to ibandronate (**1i**). Most of the aminomethylenebisphosphonates with an imidazolyl group are surprisingly less interesting. The best derivative **8k** is the one with an *N*-methyl group. Larger substituents diminish the activity (**8l**, **8m**) progressively, while the unsubstituted **8j** is even more than a 100 times less potent. The benzannulated derivatives have lost practically all activity.

Substitution of the nitrogen linker atom of the thiazole derivative **8a** by a methylene or a sulfur bridge led to **9a** and **9b**. Unexpectedly, these compounds are much less potent (Table 11). The poor potency of the bisphosphinic acid **10** on the other hand was expected, since the binding affinity of phosphinic acids is much reduced compared with that of bisphosphonates.[8,28,40]

Table 11. Comparison of Antiresorptive Potency of Bisphosphonic and Bisphosphinic Acids Containing a Thiazole Linked via Different Atoms X



compound[a]	X	R	ED_{50}[a] (μg/kg)
8a	NH	OH	5
9a	CH_2	OH	200
9b	S	OH	700
10	NH	Me	>10000

[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3.

Preclinical and Clinical Data of Zoledronic Acid

A selection of the most potent compounds of the different subclasses in the TPTX assay were further profiled in vitro in mouse calvaria cultures.[39] Good correlation was found between these data and the in vivo TPTX antiresorptive potency (cf. Table 12 and Figure 3). In the "Schenk model",[41] an acute in vivo model often used to compare the inhibitory potency of bisphosphonates on metaphyseal bone remodeling in intact, growing rats, zoledronate had a minimal effective dose of 0.3 μg/kg sc (equivalent to approximately 0.05 μg P/kg).[42] To assess the effect of BPs on bone miner-

Table 12. Inhibition of Bone Resorption vs Mineralization in the Calvaria Assay

compound[a]	TPTX vit D_3 ED_{50}[b] (μg/kg)	calvaria resorption inhib IC_{50} (μM)	calvaria mineralization inhib IC_{50} (μM)	ratio
pamidronate (**1b**)	61	0.2	100	500
alendronate (**1c**)	8	0.05	20	400
4b	1.4	0.02	30	1500
4d	1.0	0.05	4	80
5d	0.5	0.04	9	225
8c	1.5	0.04	15	375
zoledronic acid (**6i**)	0.07	0.002	30	15000

[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3.

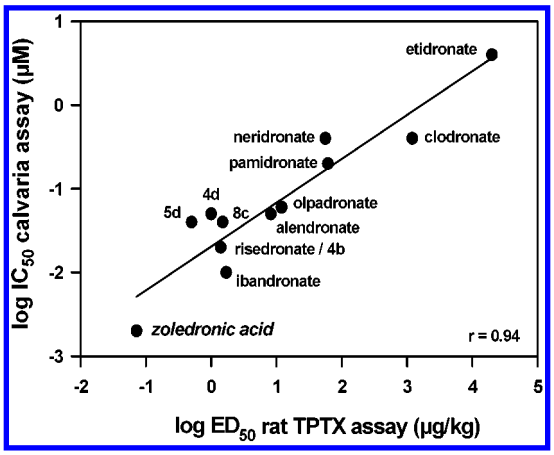


Figure 3. Comparison of in vitro (calvaria assay) and in vivo (TPTX) data.

alization, in vitro calcium incorporation into mouse calvaria was measured. In contrast to the resorption inhibition potency, effects on mineralization were very similar for most of the BPs investigated. Therefore, the increase in antiresorptive potency is not associated with a corresponding increase in inhibition of mineralization, and antiresorptive potency largely determines the ratio of mineralization inhibition versus resorption inhibition. Table 12 clearly shows the superior properties of **6i** (zoledronic acid, Figure 2) against all other compounds investigated.

In clinical studies, renal problems have been observed following intravenous infusions of several bisphosphonates. Therefore, in the profiling of new, potent BPs, there was clearly a need to investigate renal tolerability.[43] Urinary excretion of the cytosolic enzyme malate dehydrogenase (MDH) is a sensitive early marker of renal toxicity[34] and was used in a rat renal tolerability screening assay. MDH excretion values for pamidronate were identical to control levels, whereas those for alendronate were slightly increased. The three compounds **4d**, **5d**, and **6i** (zoledronic acid, Figure 2) showed a similar minor, nonsignificant trend toward elevated MDH excretion. By contrast, **8c**, which had previoulsy been shown to be nephrotoxic in rats in vivo, produced markedly elevated MDH levels, whereas **4b** showed a similar but less pronounced profile. To rank compounds, a therapeutic ratio was calculated by dividing the reciprocal of the bone antiresoptive ED_{50} value from the TPTX assay in mg/kg by the total units of MDH activity

Table 13. Short-Term Renal Tolerability and Therapeutic Ratio of Selected BP after Repeated Subcutaneous Injections

compound[a]	TPTX vit D_3 ED_{50}[b] (μg/kg)	total MDH[c] (U)	therapeutic ratio[d]
pamidronate (**1b**)	61	13.4	1.2
alendronate (**1c**)	8	18.9	6.6
4b	1.4	52.5	19
4d	1.0	17.4	120
5d	0.5	23.7	84
8c	1.5	112.4	4.5
zoledronic acid (**6i**)	0.07	17.6	790

[a] Bisphosphonates were synthesized as free bisphosphonic acids; the generic names given refer, however, to their salt forms. [b] The ED_{50} is the dose of compound administered sc, which results in a 50% reduction of the hypercalcemia induced in TPTX rats by 1,25-dihydroxyvitamin D_3. [c] Total units of malate dehydrogenase (MDH) excreted in eight 24-h urine samples collected after initiating treatment with the test compound. The corresponding value for control animals injected with saline was 12.8 units. [d] Therapeutic ratio comparing nephrotoxic potential versus bone antiresorptive potency, calculated by dividing the reciprocal of the ED_{50} value in the TPTX assay (mg/kg) by the total urinary malate dehydrogenase excretion.

excreted (Table 13). Once again, these data demonstrate that zoledronic acid (**6i**) is far superior to the other compounds by a wide margin. It was therefore selected for additional preclinical and clinical studies.

Overall, in a range of pharmacological assays, zoledronic acid was 100−850 times more active than pamidronate as an inhibitor of osteoclastic bone resorption (Table 14).[39] Short-term treatment of rats with zoledronic acid prevented the bone loss associated with estrogen deficiency following ovariectomy[45] and reduced the systemic osteopenia occurring in rats and rabbits with experimental arthritis.[45,46] Long-term treatment of ovariectomized rats[47] and adult monkeys[48] with low doses of zoledronic acid maintained the bone mass and strength at levels similar to those of intact control animals without any deleterious effects on bone architecture or mineralization. Renal and intestinal tolerability studies indicated that at identical doses the tolerability profile of zoledronic acid is similar to, or better than, that of pamidronate.[43] Thus, when its high antiresorptive potency is taken into consideration, zoledronic acid has a much greater therapeutic ratio than pamidronate (Table 14).

Recently, the molecular target of the nitrogen-containing bisphosphonates has been identified as an enzyme in the mevalonate pathway: farnesyl diphosphate synthase. Inhibition of this enzyme prevents the prenylation of intracellular signaling proteins, thereby impairing many cellular functions, which in turn induces apoptosis and finally cell death. Among the bisphosphonates, zoledronic acid is the most potent inhibitor of farnesyl diphosphate synthase and inducer of osteoclastic apoptosis identified to date.[49,50]

The results of the clinical trials indicate that low doses of zoledronic acid (Zometa) are both efficacious and safe for the treatment of tumor-induced hypercalcemia,[51] Paget's disease of bone,[52] osteolytic metastases,[53] and postmenopausal osteoporosis.[54]

The bone mass and biochemical marker data from the phase II osteoporosis trial[54] indicate that an annual intravenous infusion of 4 mg of zoledronic acid may be an effective treatment for postmenopausal osteoporosis. In the only publication to date on a large phase III trial

Table 14. Comparison of the Relative Potencies between Zoledronic Acid and Pamidronate in Various Efficacy and Tolerability Models in Rats

	treatment period	zoled (*a*)	pamid (*b*)	ratio (*b*/*a*)
efficacy models				
inhibition of $1,25(OH)_2D_3$-induced hypercalcemia in TPTX rats, ED_{50} μg/kg	4 d	0.072	61	850
increased radiographic bone density in young intact rats, ED_{50} μg/kg	10 d	1.7	390	230
increased cancellous bone area in young intact rats, ED_{50} μg/kg	10 d	0.071	7.1	100
increased cancellous bone calcium in young intact rats, ED_{50} μg/kg	10 d	0.17	22.7	130
increased cancellous bone hydroxyproline in young intact rats, ED_{50} μg/kg	10 d	1.1	210	190
renal and intestinal tolerability models				
renal: increased serum urea level in rats, ED_{100}, mg/kg	1 h	38	11	0.3
renal: increased urinary excretion of MDH in rats, NOL mg/kg	14 d	>1.0	0.1	<0.1
intestinal: increased mucosal permeability of perfused ileal loop in rats, NOL mM	2 h	10	10	1

with zoledronic acid, the compound proved to be superior to pamidronate for the treatment of tumor-induced hypercalcemia.[55]

Experimental Section

Reagents and solvents were used as purchased without further purification. Column flash chromatography was performed on silica gel 60 (230−400 mesh ASTM, E. Merck). Melting points were determined in an open capillary and are not corrected. Thin-layer chromatography (TLC) was performed on precoated silica gel plates (E. Merck, silica gel 60 F_{254}, 0.25 mm). Compounds were visualized by UV light or iodine vapor. For TLC of bisphosphonates, cellulose plates without fluorescence indicator (0.1 mm thick, E. Merck) were used. The most useful solvent system for these very polar compounds was a five-component mixture consisting of 30 parts of water, 30 parts of pyridine, 25 parts of *n*-butanol, 12 parts of 2-butanone, and 3.5 parts of formic acid. For detection a solution of a Mo/MoO_3 staining reagent[56] was used. Too heavy staining should be avoided; the spots develop best without any heating of the plates. ^{1}H NMR spectra were recorded on Varian-60, Varian Gemini 200/300, and Bruker AM 250/300/360 spectrometers. Chemical shifts of signals are expressed in parts per million (ppm) relative to tetramethylsilane as internal standard or to the deuterated solvents used. Coupling constants *J* are reported in hertz (Hz). For ^{13}C NMR spectra, a Bruker XL-300 instrument was used, and for ^{31}P NMR, a Varian Mercury 300 instrument was used. Elemental analyses were within ±0.4% of the calculated values unless indicated otherwise. Generally the bisphosphonates were dried for several hours at 80−120 °C. Even so, many of them retain residual solvents, especially water. On prolonged exposure to air, stable hydrates can be obtained, which however was not done for most of the compounds synthesized. Chemical yields were not optimized and are highly dependable on the ease of crystallization of the crude reaction product. They range from a few percent up to 80%, typically being around 40−60% (see details below).

Hypercalcemia Assay in the TPTX Rat. As a primary screen to assess the effects of bisphosphonates on mineral metabolism, the thyroparathyroidectomised (TPTX) rat with hypercalcemia induced by 1,25-dihydroxyvitamin D_3 was used. Removal of the thyroid and parathyroid glands results in a test animal with a low serum calcium concentration that can be modulated by various calciotropic agents. In the routine assay, repeated injections of $1,25(OH)_2D_3$ were used to stimulate bone resorption and to produce acute hypercalcemia. A reduction in the hypercalcemia following bisphosphonate treatment was taken as indirect evidence for the inhibition of osteoclastic activity. In the standard assay protocol, young male rats (130−150 g body weight) were thyroparathyroidectomized and then allowed a 4-day recovery period before a fasting serum sample was collected. Animals with a serum calcium concentration above 1.88 mM were discarded, the remaining animals were randomly divided into groups of 5. On postoperative days 5−8, $1,25(OH)_2D_3$ was administered (125 pmol/kg sc) together with various doses of the test bisphosphonate (sc). Total serum calcium was determined in a fasting blood sample collected on day 9. In each experiment, the upper and lower limits of hypercalcemia (100% and 0%, respectively) were established with control TPTX animals, which had only received $1,25(OH)_2D_3$ or saline. Pamidronate was used as a positive control and caused a clear dose-dependent inhibition of hypercalcemia, achieving 100% efficacy at a dose of approximately 500 μg/kg and a mean ED_{50} value of 61 μg/kg sc. The presented TPTX data are the means of at least three independent experiments.

Calvaria Assay in Vitro. The effect of various bisphosphonates on osteoclastic bone resorption in vitro was determined in murine calvarial cultures.[39] Pairs of hemicalvaria from neonatal mice (4−7 days old) were first preincubated for 24 h in the presence or absence of test compound so that each treated hemicalvaria had a corresponding untreated control from the same animal. Then, a stimulator of calcium release (20 nM $1,25(OH)_2D_3$) was added and the incubation was continued for a further 72 h. At the end of the incubation period, the calcium content of the medium and that of the calvaria were determined to permit calculation of the amount of calcium released from the calvaria as a percentage of the its calcium content. Bisphosphonate treatment resulted in a dose-dependent inhibition of calcium release, with full efficacy at the higher concentrations. Pamidronate was again used as the reference compound, showing a mean IC_{50} of 0.2 μM. The presented calvaria data are the means of two to six independent experiments with five to seven calvaria in each treatment group.

A modification of the calvarial assay was used to assess the possible interference of BPs with bone mineralization in vitro.[39] Calvaria were cultured in a manner similar to that described above but with the following changes: calcium incorporation was stimulated by replacing the $1,25(OH)_2D_3$ with 2 mM calcium 1,2-glycerophosphate, and the incubation period was reduced to 48 h, but the 24 h preincubation with bisphosphonate remained unchanged. The amount of calcium and inorganic phosphate incorporated into the calvaria was calculated as the percent increase relative to the initial calcium content. Bisphosphonate treatment dose-dependently inhibited the incorporation of calcium and inorganic phosphate into the calvaria, yet the ratio of these two ions remained close to 1.66, the value for hydroxyapatite.

Renal Tolerability Assay. Renal tolerability of bisphosphonates was investigated in rats by monitoring the urinary excretion of the cytosolic marker enzyme malate dehydrogenase as a parameter of nephrotoxicity.[43] Test compound was repeatedly administered to male rats (180−230 g body weight, three animals per treatment group) as nine subcutaneous injections (0.01, 0.1, and 1.0 mg/kg) at 1−2 day intervals over a 2 week period. Malate dehydrogenase activity was measured colorimetrically in eight 24-h urine collections and calculated as total units of excreted enzymatic activity. The presented data are from a single experiment.

Preparation of Starting Materials. The following heterocyclic amines were prepared according to the literature: 2-amino-1-methylimidazole and 2-amino-1-benzylimidazole,[57] 2-amino-1-phenylimidazole,[58] 1,2,4-thiadiazol-5-amine,[59] and 5-alkyl-substituted 2-aminothiazoles.[60] 4,5-Dimethylimidazole

was obtained by reduction of 2-hydroxymethyl-3-methylimidazole with red phosphorus in hydroiodic acid at 160 °C in a sealed tube in analogy to a literature procedure.[61]

A three-step procedure afforded 4-phenylpiperidines starting with 1-benzylpiperidin-4-one. After Grignard addition of (substituted) phenylmagnesium bromide, water was eliminated (concentrated HCl/AcOH) followed by catalytic reduction (H_2, Pd/C) of the double bond and concurrent cleavage of the benzyl protecting group.

Reduction of ethyl 4-nitro-3-phenylbutyrate[62] led directly to the 3-phenyl-γ-lactam, which was reduced with lithium aluminum hydride to give 3-phenylpyrrolidine. 3-(4-Chlorophenyl)pyrrolidine was prepared accordingly.

All other precursors were synthesized following the outlines given in the chemistry section above.

Abbreviations. h, hour; rt, room temperature; bp, boiling point; mp, melting point; equiv, equivalent; iV, in vacuo; HV, high vacuum; br, broad; dec, decomposing; BP, bisphosphonate; sc, subcutaneous.

Preparation of Bisphosphonates. 1-Hydroxy-2-aminoethylidene-1,1-bisphosphonic acid (**1a**) was prepared as described in the literature.[63] Pamidronate (**1b**) was obtained from Henkel. Compounds **3a** and **7a**−**d** were prepared by the Agro Chemical Division of Ciba-Geigy, following a procedure described in a Henkel patent.[64]

The other bisphosphonates were prepared according to Scheme 1, methods A−D.

Method A: Synthesis of 1-Hydroxy-1,1-bisphosphonates. General Procedure for the Synthesis of 1-Hydroxy-1,1-bisphosphonates. One equivalent of the corresponding carboxylic acid is dissolved in 3 equiv of phosphoric acid (85%) and chlorobenzene (ca. 500 mL/mol starting material) by heating to 100−110 °C. The hot solution is treated dropwise with 2 equiv of phosphorus trichloride, resulting in a vigorous exothermic reaction and gas evolution. Within 15 min to 1 h, a thick precipitate forms, which eventually prevents stirring. Heating is continued for another 3 h without stirring. Then the bulk of the chlorobenzene is decanted followed by removal of the remaining solvent under reduced pressure. The white or yellow residue is taken up in water (1000 mL/mol substrate) and heated to reflux for approximately 1 h. If necessary, the mixture is treated with charcoal before filtration and concentration iV. Crystallization of the resulting slightly yellow oil may be tedious. In most cases, it can be induced by the careful addition of small amounts of ethanol to an aqueous solution at room temperature. Sometimes it may be advantageous to wash the crude oil first with acetone, which is decanted after phase separation.

In an alternative workup procedure, concentrated HCl is added to the crude reaction mixture and heating continued for several hours until gas formation gradually subsides. Charcoal is added, and stirring is continued for a few minutes. After filtration, the solution is concentrated iV to leave in most cases a syrupy mass, which is crystallized by careful addition of water. Similar to the first workup procedure described above, the oily crude bisphosphonate may first be washed with acetone to facilitate crystallization.

According to this general method, the following derivatives were synthesized.

1-Hydroxy-4-aminobutylidene-1,1-bisphosphonic Acid (Alendronate, 1c). Yield: 18%; mp 235 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.26 (t, 2H), 1.32−1.62 (m, 4H). ^{31}P NMR (5% NaOD/D_2O): δ 19.51. Anal. ($C_4H_{13}NO_7P_2$) C, H, N, P.

1-Hydroxy-5-aminopentylidene-1,1-bisphosphonic Acid (1d). Yield: 42%; mp 208−212 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.85 (t, 2H), 1.83−1.98 (m, 2H), 1.52−1.72 (m, 4H), 19.51. Anal. ($C_5H_{15}NO_7P_2$) C, H, N, P.

1-Hydroxy-6-aminohexylidene-1,1-bisphosphonic Acid (Neridronate, 1e). Yield: 19%; mp 247−249 °C. ^{1}H NMR (NaO/D_2O, 300 MHz): δ 2.27 (t, 2H), 1.45−1.63 (m, 2H), 1.18−1.30 (m, 2H), 1.14 (5-line system, 2H), 0.90−1.03 (m, 2H). ^{13}C NMR (NaOD/D_2O): δ 75.5 (t, J_{C-P} = 129 Hz), 40.2, 35.0, 27.8, 27.2, 24.1. ^{31}P NMR (5% NaOD/D_2O): δ 19.69. Anal. ($C_6H_{17}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-diethylaminopropylidene-1,1-bisphosphonic Acid (1j). Yield: 56%; mp 220 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.42−2.62 (m, 2H), 1.24 (t, 4H), 1.63−1.80 (m, 2H), 0.70 (t, 6H). ^{31}P NMR (5% NaOD/D_2O): δ 19.15. Anal. ($C_7H_{19}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-(methylpentylamino)propylidene-1,1-bisphosphonic Acid (Ibandronate, 1k). Yield: 6%; mp 126−133 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.52−2.61 (m, 2H), 2.20−2.29 (m, 2H), 2.03 (s, 3H), 1.81−1.94 (m, 2H), 1.24−1.37 (m, 2H), 1.03−1.18 (m, 4H), 0.68 (t, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 19.24. Anal. ($C_9H_{23}NO_7P_2 \cdot 0.25H_2O$) C, H, N, P.

1-Hydroxy-3-aminobutylidene-1,1-bisphosphonic Acid (1n). Yield: 41%; mp 145−146 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 3.79 (m, 1H), 2.00−2.33 (m, 2H), 1.28 (d, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 19.25 (AB system). Anal. ($C_4H_{13}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-methyl-3-(methylpentylamino)butylidene-1,1-bisphosphonic Acid (1p). Yield: 24%; mp 135−136 °C (dec). ^{1}H NMR (NaOD/D_2O, 200 MHz): δ 3.54−3.67 (m, 1H), 2.64−2.76 (m, 1H), 2.45−2.58 (m, 1H), 2.30 (s, 3H), 1.98−2.18 (m, 1H), 1.76 (q, 1H), 1.35−1.49 (m, 2H), 1.04−1.18 (m, 4H), 0.92 (d, 3H), 0.66 (t, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 18.73 (AB system). Anal. ($C_{10}H_{25}NO_7P_2 \cdot 0.5H_2O$) C, H, N, P. C: calcd, 35.09; found, 34.6.

1-Hydroxy-3-(pyrrolidine-1yl)propylidene-1,1-bisphosphonic Acid (2a). Yield: 44%; mp 239−242 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.74−2.53 (m, 2H), 2.25 (br s, 4H), 1.67−1.85 (m, 4H), 1.42 (br s, 4H). ^{31}P NMR (5% NaOD/D_2O): δ 19.07. Anal. ($C_7H_{17}NO_7P_2$) C, H, N, P.

1-Hydroxy-4-(pyrrolidine-1yl)butylidene-1,1-bisphosphonic Acid (2b). Yield: 75%; mp 249−250 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.22 (br s, 2H), 2.21 (br t, 2H), 1.35−1.60 (m, 8H). ^{31}P NMR (5% NaOD/D_2O): δ 19.49. Anal. ($C_8H_{19}NO_7P_2$) C, H, N, P.

1-Hydroxy-6-(pyrrolidine-1yl)hexylidene-1,1-bisphosphonic Acid (2c). Yield: 62%; mp 239−240 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.08−2.25 (m, 6H), 1.4−1.65 (m, 2H), 1.41 (br s, 4H), 1.14−1.32 (m, 4H), 0.88−1.03 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.70. Anal. ($C_{10}H_{23}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-(3-phenylpyrrolidine-1yl)propylidene-1,1-bisphosphonic Acid (2d). Yield: 18%; mp 221 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.02−7.12 (m, 4H), 6.93−7.02 (m, 1H), 3.06 (5-line system, 1H), 2.87 (t, 1H), 2.48−2.68 (m, 3H), 2.36−2.48 (m, 1H), 2.23 (t, 1H), 1.89−2.04 (m, 1H), 1.70−1.89 (m, 2H), 1.46−1.62 (m,1H). ^{31}P NMR (5% NaOD/D_2O): δ 19.09. Anal. ($C_{13}H_{21}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-[3-(4-chlorophenyl)pyrrolidine-1yl]propylidene-1,1-bisphosphonic Acid (2e). Yield: 20%; mp 219 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.04 (d, 2H), 6.99 (d, 2H), 3.04 (5-line system, 1H), 2.85 (t, 1H), 2.47−2.67 (m, 3H), 2.33−2.47 (m, 1H), 2.20 (t, 1H), 1.87−2.03 (m, 1H), 1.69−1.87 (m, 2H), 1.42−1.68 (m, 1H). ^{31}P NMR (5% NaOD/D_2O): δ 19.06. Anal. ($C_{13}H_{20}ClNO_7P_2 \cdot 0.5H_2O$) C, H, N, P.

1-Hydroxy-3-(piperidine-1yl)propylidene-1,1-bisphosphonic Acid (2f). Yield: 82%; mp 234 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.27−2.40 (m, 2H), 2.14 (br s, 4H), 1.64−2.84 (m, 2H), 1.22 (br s, 4H), 1.13 (br s, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.13. Anal. ($C_8H_{19}NO_7P_2$) C, H, N.

1-Hydroxy-3-(4-phenylpiperidine-1yl)propylidene-1,1-bisphosphonic Acid (2g). Yield: 67%; mp 243−245 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.92−7.13 (m, 5H), 2.79 (br d, 2H), 2.38−2.49 (m, 2H), 2.80 (br t, 1H), 1.70−1.95 (m, 4H), 1.53 (br d, 2H), 1.27−1.46 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.15. Anal. ($C_{14}H_{23}NO_7P_2 \cdot H_2O$) C, H, N, P.

1-Hydroxy-4-(4-phenylpiperidine-1yl)butylidene-1,1-bisphosphonic Acid (2h). Yield: 75%; mp 230 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.82−7.12 (m, 5H), 2.77 (br d, 2H), 2.28 (br t, 1H), 2.00−2.09 (br, 2H), 1.81 (t, 2H), 1.44−1.60 (m, 6H), 1.27−1.44 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.48. Anal. ($C_{15}H_{25}NO_7P_2$) C, H, N, P.

1-Hydroxy-6-(4-phenylpiperidine-1-yl)hexylidene-1,1-bisphosphonic Acid (2i). Yield: 35%; mp 236−237 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.91−7.13 (m, 5H), 2.74 (d, 2H), 2.30 (br t, 1H), 2.02−2.13 (m, 2H), 1.81 (t, 2H), 1.46−1.66 (m, 4H), 1.39 (t, 2H), 1.16−1.33 (m,4 H), 0.90−1.05 (m,2 H). ^{31}P NMR (5% NaOD/D_2O): δ 19.70. Anal. ($C_{17}H_{29}NO_7P_2\cdot 0.25H_2O$) C, H, N, P.

1-Hydroxy-3-[4-(3-fluorophenyl)piperidine-1yl]propylidene-1,1-bisphosphonic Acid (2j). Yield: 11%; mp 247−248 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.98−7.10 (m, 1H), 6.62−6.86 (m, 3H), 2.77 (d, 2H), 2.41 (br s, 2H), 2.31 (br t, 1H), 1.70−1.95 (m, 4H), 1.63 (d, 2H), 1.24−1.42 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.13. Anal. ($C_{14}H_{22}FNO_7P_2\cdot 0.5H_2O$) C, H, N, P.

1-Hydroxy-3-(azepan-1yl)propylidene-1,1-bisphosphonic Acid (2k). Yield: 32%; mp 230−232 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.45−2.65 (m, 2H), 2.32−2.40 (m, 4H), 1.66−1.84 (m, 2H), 1.20−1.40 (m, 8 H). ^{31}P NMR (5% NaOD/D_2O): δ 19.15. Anal. ($C_9H_{21}NO_7P_2$) C, H, N, P.

1-Hydroxy-4-(azocan-1yl)butylidene-1,1-bisphosphonic Acid (2l). Yield: 81%; mp 248−249 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.34−2.45 (m, 2H), 2.04−2.14 (m,2 H), 1.37−1.55 (m, 4H), 1.17−1.37 (m, 10H). ^{31}P NMR (5% NaOD/D_2O): δ 19.51. Anal. ($C_{11}H_{25}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-(4-methylpiperazin-1yl)propylidene-1,1-bisphosphonic Acid (2m). Yield: 50%; mp 340 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 1.8−2. 9 (br, 8H, piperazine), 2.44−2.55 (m, 2H), 1.99 (s, 3H), 1.75−1.95 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 18.92. For combustion analysis, this compound was crystallized in the form of its Na_3 salt by the addition of dilute sodium hydroxide solution. The solid contains a small amount of phosphoric acid plus 2 mol of crystal water. Anal. ($C_8H_{17}N_2O_7P_2Na_3\cdot 2H_2O\cdot 0.15H_3PO_4$) C, H, N, Na, P.

1-Hydroxy-3-(4-phenylpiperazin-1yl)propylidene-1,1-bisphosphonic Acid (2n). Yield: 17%; mp 234 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.08 (t, 2H), 6.82 (d, 2H), 6.75 (t, 1H), 2.72−2.97 (br s, 4H), 2.32−2.56 (m, 6H), 1.68−1.90 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.05. Anal. ($C_{13}H_{22}N_2O_7P_2$) C, H, N.

1-Hydroxy-1-(piperidin-3-yl)methylidenebisphosphonic Acid (3b). Yield: 52%; mp 244−246 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.90 (d, 1H), 2.56 (d, 1H), 2.48 (t, 1H), 2.14 (td, 1H), 1.65−1.80 (m, 2H), 1.30−1.48 (m,2 H), 0.95−1.15 (m,1 H). ^{31}P NMR (5% NaOD/D_2O): δ 18.81. Anal. ($C_6H_{15}NO_7P_2$) C, H, N, P.

1-Hydroxy-1-(piperidin-4-yl)methylidenebisphosphonic Acid (3c). Yield: 47%; mp 227−230 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.68 (d, 2H), 2.12 (t, 2H), 1.62−1.80 (m, 1H), 1.59 (d, 2H), 1.30−1.47 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 18.97. Anal. ($C_6H_{15}NO_7P_2\cdot 0.25H_2O$) C, H, N, P.

1-Hydroxy-1-(azetidin-3-yl)methylidenebisphosphonic Acid (3d). Yield: 16%; mp 242−243 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 3.65−3.80 (m, 2 H), 3.15−3.30 (m, 3 H). ^{13}C NMR (NaOD/D_2O, XL-300): δ 76.3 (t, J_{C-P} = 133 Hz), 49.1 (t, J_{C-P} = 10 Hz), 40.4. ^{31}P NMR (5% NaOD/D_2O): δ 18.09. Anal. ($C_4H_{11}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-phenethylaminopropylidene-1,1-bisphosphonic Acid (4a). Yield: 50%; mp 205−206 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 6.97−7.14 (m, 5 H), 2.46−2.64 (m, 6H), 1.60−1.82 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.02. Anal. ($C_{11}H_{19}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-(methylphenethylamino)propylidene-1,1-bisphosphonic Acid (4b). Yield: 48%; mp 187−188 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 6.97−7.14 (m, 5H), 2.45−2.60 (m, 4H), 2.53−2.45 (m, 2H), 2.01 (s, 3H), 1.68−1.90 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.02. Anal. ($C_{12}H_{21}NO_7P_2\cdot 0.25H_2O$) C, H, N, P.

1-Hydroxy-3-(3-phenylpropylamino)propylidene-1,1-bisphosphonic Acid (4c). Yield: 37%; mp 219 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.91−7.09 (m, 5H), 2.46−2.56 (m, 2H), 2.35 (t, 2H), 2.27 (t, 2H), 1.62−1.80 (m, 2H), 1.47 (5-line system, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.17. Anal. ($C_{12}H_{21}NO_7P_2\cdot H_2O$) C, H, N, P.

1-Hydroxy-3-[methyl-(3-phenylpropyl)amino]propylidene-1,1-bisphosphonic Acid (4d). Yield: 55%; mp 176 °C (dec). ^{1}H NMR (D_2O, 300 MHz): δ 7.00−7.20 (m, 5H), 2.48−2.59 (m, 2H), 2.44 (t, 2H), 2.20−2.30 (m, 2H), 2.00 (s, 3H), 1.77−1.94 (m, 2H), 1.55−1.68 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.00. Anal. ($C_{13}H_{23}NO_7P_2\cdot H_2O$) C, H, N, P.

1-Hydroxy-4-[ethyl-(3-phenylpropyl)amino]propylidene-1,1-bisphosphonic Acid (4e). Yield: 25%; mp 195−197 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 6.88−7.08 (m, 5 H), 2.44−2.53 (m, 2H), 2.14−2.33 (m, 6H), 1.63−1.84 (m, 2H), 1.39−1.56 (m, 2H), 0.67 (t, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 19.03. Anal. ($C_{14}H_{25}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-[methyl-(3-tolylpropyl)amino]propylidene-1,1-bisphosphonic Acid (4f). Yield: 34%; mp 193−195 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 6.94−7.04 (m, 1H), 6.79−6.91 (m, 3H), 2.38−2.42 (m, 2H), 2.31 (t, 2H), 2.11−2.24 (m, 2H), 2.05 (s, 3H), 1.94 (s, 3H), 1.66−1.88 (m, 2H), 1.42−1.62 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.00. Anal. ($C_{14}H_{25}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-[(3-(4-chlorophenyl)propyl)methylamino]-propylidene-1,1-bisphosphonic Acid (4g). Yield: 21%; mp 162−166 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.04 (d, 2H), 6.95 (d, 2H), 2.38−2.50 (m, 2H), 2.30 (t, 2H), 2.08−2.19 (m,2 H), 1.89 (s, 3H), 1.65−1.88 (m,2 H), 1.40−1.60 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.02. Anal. ($C_{13}H_{22}ClNO_7P_2\cdot H_2O$) C, H, N. C: calcd, 37.20; found, 36.60.

1-Hydroxy-3-[(1-methyl-3-phenylpropyl)amino]propylidene-1,1-bisphosphonic Acid (4h). Yield: 28%; mp 142−150 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 6.97−7.18 (m, 5H), 2.57−2.76 (m, 2H), 2.32−2.57 (m, 3H), 1.70−1.90 (m, 2H), 1.52−1.70 (m, 1H), 1.26−1.44 (m, 1H), 0.89 (d, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 19.05. Anal. ($C_{13}H_{23}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-[(4-phenylbutyl)amino]propylidene-1,1-bisphosphonic Acid (4i). Yield: 70%; mp 191−193 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 6.94−7.14 (m, 5H), 2.50−2.62 (m, 2H), 2.27−2.40 (m,4 H), 1.66−1.87 (m, 2H), 1.30−1.46 (m, 2H), 1.14−1.30 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 18.99. Anal. ($C_{13}H_{23}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-[methyl-(4-phenylbutyl)amino]propylidene-1,1-bisphosphonic Acid (4j). Yield: 30%; mp 128−132 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 6.91−7.12 (m, 5H), 2.28−2.49 (m, 4H), 2.09−2.18 (m, 2H), 1.88 (s, 3H), 1.65−1.88 (m, 2H), 1.12−1.40 (m, 4H). ^{31}P NMR (5% NaOD/D_2O): δ 19.01. Anal. ($C_{14}H_{25}NO_7P_2$) C, H, N, P. P: calcd, 16.25; found, 15.3.

1-Hydroxy-3-[(5-phenylpentyl)amino]propylidene-1,1-bisphosphonic Acid (4k). Yield: 30%; mp 124−127 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 6.93−7.13 (m, 5H), 2.40−2.49 (m, 2H), 2.35 (t, 2H), 2.06−2.16 (m, 2 H), 1.91 (s, 3H), 1.64−1.90 (m, 2H), 1.34 (5-line system, 2 H), 1.15−1.27 (m, 2H), 0.93−1.08 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.00. Anal. ($C_{14}H_{25}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-[methyl-(2-phenoxyethyl)amino]propylidene-1,1-bisphosphonic Acid (5a). Yield: 63%; mp 125−130 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.06 (t, 2 H), 6.70−6.77 (m, 3 H), 3.86 (t, 2 H), 2.42−2.56 (m, 4 H), 1.97 (s, 3 H), 1.64−1.86 (m, 2 H). ^{31}P NMR (5% NaOD/D_2O): δ 19.00. Anal. ($C_{12}H_{21}NO_8P_2\cdot 0.5H_2O$) C, H, N, P.

1-Hydroxy-3-[methyl-(2-(4-chlorophenoxy)ethyl)amino]propylidene-1,1-bisphosphonic Acid (5b). Yield: 14%; mp 216−218 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.36 (d, 2H), 7.01 (d, 2H), 4.18 (t, 2H), 2.80−2.90 (m, 4H), 2.32 (s, 3H), 2.04−2.20 (br m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 18.99. Anal. ($C_{12}H_{20}ClNO_8P_2\cdot 0.5H_2O$) C, H, N, P.

1-Hydroxy-3-[(3-phenoxypropyl)amino]propylidene-1,1-bisphosphonic Acid (5c). Yield: 23%; mp 148−155 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.05 (t, 2H), 6.64−6.76 (m, 3H), 3.78 (t, 2H), 2.52 (br t, 2H), 2.38 (t, 2 H), 1.50−1.80 (m, 4H). ^{31}P NMR (5% NaOD/D_2O): δ 19.03. Anal. ($C_{12}H_{21}NO_8P_2$) C, H, N, P.

1-Hydroxy-3-[methyl-(3-phenoxypropyl)amino]propylidene-1,1-bisphosphonic Acid (5d). Yield: 72%; mp 198−200 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.36 (t, 2H),

6.99−7.06 (m, 3H), 4.15 (t, 2H), 3.24 (br t, 2H), 3.10−3.20 (m, 2H), 2.61 (s, 3H), 2.10−2.33 (m, 4H). ^{31}P NMR (5% NaOD/D_2O): δ 19.00. Anal. ($C_{13}H_{23}NO_8P_2$) C, H, N, P.

1-Hydroxy-3-[methyl-(3-(3-methylphenoxy)propyl)amino]propylidene-1,1-bisphosphonic Acid (5e). Yield: 27%; mp 152−158 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.27 (t, 1H), 6.82−6.85 (m, 3H), 4.12 (t,2 H), 2.83−2.90 (m, 2H), 2.64−2.72 (m, 2H), 2.35 (s, 3H), 2.32 (s, 3H), 2.06−2.21 (m, 2H), 1.96−2.06 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.00. Anal. ($C_{14}H_{25}NO_8P_2$) C, H, N, P.

1-Hydroxy-3-[methyl-(3-(4-fluorophenoxy)propyl)amino]propylidene-1,1-bisphosphonic Acid (5f). Yield: 35%; mp 165−172 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.12 (t, 2H), 6.96−7.05 (m, 2H), 4.10 (t, 2H), 2.83−2.90 (m, 2H), 2.64−2.73 (m, 2H), 2.31 (s, 3H), 2.07−2.17 (m, 2H), 1.95−2.07 (m,2 H). ^{31}P NMR (5% NaOD/D_2O): δ 19.00. Anal. ($C_{13}H_{22}FNO_8P_2\cdot 0.5H_2O$) C, H, N, F, P.

1-Hydroxy-3-[methyl-(3-(4-chlorophenoxy)propyl)amino]propylidene-1,1-bisphosphonic Acid (5g). Yield: 71%; mp 155−162 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.00 (d, 2H), 6.62 (d, 2H), 3.72 (t, 2H), 2.33−2.46 (m, 2H), 2.16−2.27 (m, 2H), 1.88 (s, 3H), 1.50−1.80 (m, 4H). ^{31}P NMR (5% NaOD/D_2O): δ 19.00. Anal. ($C_{13}H_{21}ClNO_8P_2$) C, H, N, Cl, P.

1-Hydroxy-3-[methyl-(3-(4-methoxyphenoxy)propyl)-amino]propylidene-1,1-bisphosphonic Acid (5h). Yield: 23%; mp 127−133 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.78 (br s, 4H), 3.86 (t, 2H), 3.69 (s, 3H), 2.62−2.62 (m, 2H), 2.33−2.46 (m,2 H), 2.03 (s, 3H), 1.69−1.98 (m, 4H). ^{31}P NMR (5% NaOD/D_2O): δ 19.00. Anal. ($C_{14}H_{25}NO_9P_2\cdot 0.5H_2O$) C, H, N, P.

1-Hydroxy-3-[ethyl-(3-phenoxypropyl)amino]propylidene-1,1-bisphosphonic Acid (5i). Yield: 61%; mp 195−197 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.07 (t, 2H), 6.74 (t, 1H), 6.71 (d, 2H), 3.78 (t, 2H), 2.46−2.57 (m, 2H), 2.30−2.40 (m, 2H), 2.27 (q, 2H), 1.59−1.84 (m, 4H), 0.71 (t, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 19.16. Anal. ($C_{14}H_{25}NO_8P_2$) C, H, N, P.

1-Hydroxy-3-[propyl-(3-phenoxypropyl)amino]propylidene-1,1-bisphosphonic Acid (5j). Yield: 50%; mp 197−199 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.06 (t, 2H), 6.73 (t, 1H), 6.70 (d, 2H), 3.77 (t, 2H), 2.46−2.58 (m,2 H), 2.30−2.40 (m,2 H), 2.08−2.19 (m, 2H), 1.58−1.84 (m, 4H), 1.16 (6-line system, 2H), 0.52 (t, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 19.16. Anal. ($C_{15}H_{27}NO_8P_2\cdot 0.5H_2O$) C, H, N, P.

1-Hydroxy-3-[butyl-(3-phenoxypropyl)amino]propylidene-1,1-bisphosphonic Acid (5k). Yield: 45%; mp 181−183 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.07 (t, 2H), 6.74 (t, 1H), 6.71 (d, 2H), 3.79 (t, 2H), 2.46−2.57 (m,2 H), 2.30−2.40 (m, 2H), 2.13−2.21 (m, 2H), 1.58−1.83 (m, 4H), 1.05−1.19 (m, 2H), 0.94 (6-line system, 2H), 0.55 (t, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 19.16. Anal. ($C_{16}H_{29}NO_8P_2$) C, H, N, P.

1-Hydroxy-3-[methyl-(4-phenoxybutyl)amino]propylidene-1,1-bisphosphonic Acid (5l). Yield: 45%; mp 153−156 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.15 (t, 2H), 6.76−6.88 (m, 3H), 3.85 (t, 2H), 2.42−2.55 (m, 2H), 2.16−2.27 (br t, 2H), 1.96 (s, 3H), 1.70−1.90 (m, 2H), 1.30−1.60 (m, 4H). ^{31}P NMR (5% NaOD/D_2O): δ 19.01. Anal. ($C_{14}H_{25}NO_8P_2$) C, H, N, P. P: calcd, 15.59; found, 14.9.

1-Hydroxy-3-[methyl-(6-phenoxyhexyl)amino]propylidene-1,1-bisphosphonic Acid (5m). Yield: 55%; mp 183−187 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.07 (t, 2H), 6.74 (t, 1H), 6.71 (d, 2H), 3.77 (t, 2H), 2.34−2.44 (m, 2H), 2.04−2.14 (m, 2H), 1.87 (s, 3H), 1.64−1.84 (m, 2H), 1.46 (5-line system, 3H), 1.09−1.26 (m, 4H), 0.97−1.09 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.14. Anal. ($C_{16}H_{29}NO_8P_2$) C, H, N, P.

1-Hydroxy-3-[methyl-(3-phenoxypropyl)amino]butylidene-1,1-bisphosphonic Acid (5n). Yield: 72%; mp 221−223 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.37 (t, 2H), 6.99−7.08 (m, 3H), 4.11 (t, 2H), 2.72−2.80 (m, 2H), 2.55−2.63 (m, 2H), 2.38 (s, 3H), 1.96−2.09 (m, 2H), 1.76−1.93 (m, 4H). ^{31}P NMR (5% NaOD/D_2O): δ 19.37. Anal. ($C_{14}H_{25}NO_8P_2\cdot 0.5H_2O$) C, H, N, P.

1-Hydroxy-3-[(2-phenylsulfanylethyl)amino]propylidene-1,1-bisphosphonic Acid (5o). Yield: 71%; mp 108−116 °C. ^{1}H NMR (NaOD/D_2O, 200 MHz): δ 7.21−7.44 (m, 5H), 3.08 (t, 2H), 2.70−2.88 (m, 4H), 1.83−2.09 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.01. Anal. ($C_{11}H_{19}NO_7P_2S\cdot 0.25H_2O$) C, H, N, P, S.

1-Hydroxy-3-[methyl-(2-phenylsulfanylethyl)amino]-propylidene-1,1-bisphosphonic Acid (5p). Yield: 30%; mp 170−172 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.48 (d, 2H), 7.39 (t, 2H), 7.30 (t, 1H), 3.18−3.27 (m, 2H), 2.95−3.08 (m, 4H), 2.51 (s, 3H), 2.17 (7-line system, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 18.97. Anal. ($C_{12}H_{21}NO_7P_2S$) C, H, N, P, S.

1-Hydroxy-3-[(3-phenylsulfanylpropyl)amino]propylidene-1,1-bisphosphonic Acid (5q). Yield: 63%; mp 154−156 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.32−7.36 (m, 4H), 7.23−7.30 (m, 1H), 3.03 (t, 2H), 2.96 (t, 2H), 2.78 (t, 2H), 2.07 (7-line system, 2H), 1.83 (5-line system, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.02. Anal. ($C_{12}H_{21}NO_7P_2S$) C, H, N, P, S.

1-Hydroxy-3-[methyl-(2-(4-chlorophenylsulfanylethyl))amino]propylidene-1,1-bisphosphonic Acid (5s). Yield: 31%; mp 125−130 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.07 (s, 4H), 2.67 (t, 2H), 2.34−2.46 (m, 2H), 2.21 (t, 2H), 1.84 (s, 3H), 1.64−1.80 (m, 2H), 1.48 (5-line system, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.11 Anal. ($C_{13}H_{22}ClNO_7P_2S$) C, H, Cl, N, P, S.

1-Hydroxy-2-(imidazol-2-yl)ethylidene-1,1-bisphosphonic Acid (6a). Yield: 30%; mp 235 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.66 (s, 2H), 3.09 (t, J_{H-P} = 13 Hz, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 18.78. Anal. ($C_5H_{10}N_2O_7P_2\cdot 0.5H_2O$) C, H, N, P. P: calcd, 22.04; found, 22.5.

1-Hydroxy-2-(1-methylimidazol-2-yl)ethylidene-1,1-bisphosphonic Acid (6b). Yield: 46%; mp 261 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.66 (s, 1H), 6.57 (s, 1H), 3.41 (s, 3H), 2.99 (t, J_{H-P} = 11 Hz, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 17.96. Anal. ($C_6H_{12}N_2O_7P_2\cdot H_2O$) C, H, N, P.

1-Hydroxy-2-(1-benzylimidazol-2-yl)ethylidene-1,1-bisphosphonic Acid (6c). Yield: 52%; mp 171 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.3−7.5 (m, 5H), 7.24 (br s, 1H), 7.19 (br s, 1H), 5.43 (s, 2H), 3.52 (t, J_{H-P} = 12 Hz, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 17.81.

1-Hydroxy-2-(imidazol-4-yl)ethylidene-1,1-bisphosphonic Acid (6d). Yield: 41%; mp 238−240 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.49 (s, 1H), 6.70 (s, 1H), 3.16 (t, J_{H-P} = 12 Hz, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 18.98. Anal. ($C_5H_{10}N_2O_7P_2$) C, H, N, P.

1-Hydroxy-3-(imidazol-4-yl)propylidene-1,1-bisphosphonic Acid (6e). Yield: 38%; mp 249−252 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.25 (s, 1H), 6.53 (s, 1H), 2.52−2.63 (m, 2H), 1.77−1.95 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.31. Anal. ($C_6H_{12}N_2O_7P_2\cdot 0.75H_2O$) C, H, N, P.

1-Hydroxy-2-(2-methylimidazol-4-yl)ethylidene-1,1-bisphosphonic Acid (6f). Yield: 43%; mp 261−262 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 6.38 (s, 1H), 2.96 (t, J_{H-P} = 12 Hz, 2H), 2.01 (s, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 19.02. Anal. ($C_6H_{12}N_2O_7P_2\cdot 0.5H_2O$) C, H, N.

1-Hydroxy-2-(2-phenylimidazol-4-yl)ethylidene-1,1-bisphosphonic Acid (6g). Yield: 30%; mp 223−224 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.45 (d, 2H), 7.10 (t, 2H), 7.02 (t, 1H), 6.58 (s, 1H), 3.05 (t, J_{H-P} = 12 Hz, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.12. Anal. ($C_{11}H_{14}N_2O_7P_2\cdot 1.5H_2O$) C, H, N.

1-Hydroxy-2-(5-methylimidazol-4-yl)ethylidene-1,1-bisphosphonic Acid (6h). Yield: 51%; mp 217−218 °C. ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.26 (s, 1H), 2.99 (t, J_{H-P} = 12 Hz, 2H), 1.88 (s, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 19.12. Anal. ($C_6H_{12}N_2O_7P_2\cdot 0.25H_2O$) C, H, N, P.

1-Hydroxy-2-(imidazol-1-yl-amino)ethylidene-1,1-bisphosphonic Acid (6i). Yield: 67%; mp 242−244 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.80 (s, 1H), 7.30 (s, 1H), 6.95 (s, 1H), 4.50 (t, J_{H-P} = 10 Hz, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 16.18. Anal. ($C_5H_{10}N_2O_7P_2\cdot H_2O$) C, H, N, P.

1-Hydroxy-3-(imidazol-1-yl-amino)propylidene-1,1-bisphosphonic Acid (6j). Yield: 63%; mp 246−247 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.50 (s, 1H), 7.02 (s, 1H), 6.66 (s, 1H), 4.08−4.18 (m, 2H), 2.00−2.20 (m, 2H). ^{31}P NMR (5%

NaOD/D_2O): δ 18.35. Anal. ($C_6H_{12}N_2O_7P_2$) C, H, N, P. P: calcd, 21.65; found, 22.5.

1-Hydroxy-2-(2-methylimidazol-1-yl)ethylidene-1,1-bisphosphonic Acid (6k). Yield: 2.3%; mp 245−246 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 6.97 (s, 1H), 6.41 (s, 1H), 4.06 (t, J_{H-P} = 10 Hz, 2H), 2.04 (s, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 16.40. Anal. ($C_6H_{12}N_2O_7P_2$) C, H, N, P. P: calcd, 21.65; found, 21.0.

1-Hydroxy-2-(4,5-dimethylimidazol-1-yl)ethylidene-1,1-bisphosphonic Acid (6l). Yield: 30%; mp 251−252 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.56 (s, 1H), 4.17 (t, J_{H-P} =10 Hz, 2H), 1.92 (s, 3H), 1.81 (s, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 16.55. Anal. ($C_7H_{14}N_2O_7P_2$) C, H, N, P.

1-Hydroxy-2-(pyrrazol-1-yl)ethylidene-1,1-bisphosphonic Acid (6m). Yield: 23%; mp 234 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.55 (s, 1H), 7.22 (s, 1H), 7.22 (s. 1H), 6.00 (s, 1H), 4.36 (t, J_{H-P} = 9 Hz, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 16.15. Anal. ($C_5H_8N_2O_7P_2 \cdot 2H_2O$) C, H, N, P.

1-Hydroxy-2-([1,2,4]triazol-1-yl)ethylidene-1,1-bisphosphonic Acid (6n). Yield: 32%; mp 255 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 8.23 (s, 1H), 7.66 (s, 1H), 4.39 (t, J_{H-P} = 9 Hz, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 16.61. Anal. ($C_4H_9N_3O_7P_2$) C, H, N, P.

Method B: Synthesis of 1-Amino-1,1-bisphosphonates. 1-Amino-3-(dimethylamino)propylidene-1,1-bisphosphonic Acid (1m). 1m was synthesized as indicated in method B of Scheme 1, according to a literature procedure,[65] in 56% yield: mp 275−278 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.22−2.33 (m,2 H), 1.89 (s, 6H), 1.63−1.72 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 21.48. Anal. ($C_5H_{16}N_2O_6P_2 \cdot 0.25H_2O$) C, H, N, P.

Method C: Synthesis of 1-Aminoalkyl-1,1-bisphosphonates. 3-(Dimethylamino)propylidene]-1,1-bisphosphonic Acid (1l). A suspension of 2.18 g (50 mmol) of sodium hydride (ca. 55%), which had been washed with several portions of petroleum ether to remove the mineral oil, in 45 mL of THF was treated at 0 °C dropwise with 14.4 g (50 mmol) of tetraethyl methylenebisphosphonate. In an exothermic reaction, a gray-yellow solution formed, which was stirred for 1.5 h at 0 °C. Meanwhile, 5.9 g (55 mmol) of 2-chloroethyldimethylamine hydrochloride was partitioned between diethyl ether and 6 N NaOH. The organic layer was concentrated to give the free base. This was taken up in 15 mL of THF and added dropwise to the reaction mixture, maintaining 0 °C. The mixture was allowed to reach room temperature, and stirring was continued overnight. Because there was still some starting material left, another equivalent of the alkylating agent was added and the reaction mixture was kept for 2 weeks at room temperature. At this point, only little starting material remained. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. Purification by flash chromatography (toluene/ethanol initially 4:1, then 7:3 containing 1% of aqueous ammonia solution) afforded 3.5 g (20% yield) of tetraethyl 3-(dimethylamino)propylidene]bisphosphonate (bp 120 °C, 30 mbar). ^{1}H NMR ($CDCl_3$, 250 MHz): δ 4.12 (m, 8H), 2.61 (tt, 1H), 2.50 (t, 2H), 2.22 (s, 6H), 1.91−2.17 (m, 2H), 1.30 (t, 12H).

A mixture of 3.6 g (10.0 mmol) of tetraethyl 3-(dimethylamino)propylidenebisphosphonate in 30 mL of 48% HBr solution was heated to reflux overnight. After cooling to room temperature, the mixture was then diluted with water and charcoal was added. Filtration and concentration gave a yellow-brown oil, which was thoroughly dried under HV. The residue was treated with 10 mL of MeOH and then with the same amount of acetone to induce crystallization. After drying several hours at 80 °C under HV, the mixture yielded 1.7 g (51% yield) of 3-(dimethylamino)propylidene-1,1-bisphosphonic acid in form of the hydrobromide: mp 197−200 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.15−2.27 (m, 2H), 1.89 (s, 6H), 1.45−1.7 (m, 2H), 1.15 (tt, 1H). ^{31}P NMR (5% NaOD/D_2O): δ 21.48. Anal. ($C_5H_{15}NO_6P_2 \cdot HBr$) C, H, N, P, Br.

2-(Thiazol-2-yl)ethylidene-1,1-bisphosphonic Acid (9a). 9a was synthesized starting with 2-chloromethylthiazole:[66] mp 259 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.32 (d, 1H), 7.08 (d, 1H), 3.15 (td, 2H, J_{H-P} = 15 Hz), 1.97 (tt, 1H, J_{H-P} = 21 Hz). ^{31}P NMR (5% NaOD/D_2O): δ 19.88. Anal. ($C_5H_9NO_6P_2S$) C, H, N, P, S.

(Thiazol-2-ylsulfanyl)methylenebisphosphonic Acid (9b). Tetraethyl methylenebisphosphonate (12.2 g, 42.6 mmol) was dissolved in 100 mL of anhydrous THF, cooled to − 20 °C, and deprotonated under argon with sodium hydride (4.22 g, ca. 55% dispersion in mineral oil). After the mixture was stirred for 5 min at this temperature, the temperature was raised to 0 °C for another $^1/_2$ h. After the mixture was cooled to −30 °C, a solution of 9.00 g (38.76 mmol) of di-(2-thiazolyl)-disulfide[67] in 50 mL of THF was added dropwise within 10 min. A slightly yellow suspension formed. After being stirred for 1 h at room temperature, the mixture was filtered and the filtrate was concentrated. The residue was taken up in 1 N HCl, and the resulting solution was adjusted to pH 2−3. The aqueous layer was extracted with several portions of ethyl acetate, which were combined and washed with a small amount of aqueous HCl, followed by a wash with saturated sodium carbonate solution (pH 9−10). Drying and concentration iV afforded 13.6 g of crude product, which was chromatographed (ethyl acetate/acetone 4:1). Yield: 10.2 g (65%). ^{13}C NMR (NaOD/D_2O, 300 MHz): δ 161.7, 142.0, 119.8, 63.8−64.1 (m), 38.4 (t, J_{C-P} = 137 Hz), 16.3. Anal. ($C_{12}H_{23}NO_6P_2S_2$) C, H, N, P, S.

An amount of 4.03 g (10.0 mmol) of the tetraester prepared in the previous step was heated to reflux in 30 mL of concentrated HCl overnight. After cooling to room temperature, the mixture was diluted with 20 mL of water and filtered. After complete removal of the water, the solid residue was twice dissolved in freshwater and concentrated again to remove traces of Hl. Recrystallization from 20 mL of hot EtOH and washing with acetone gave colorless crystals, which were dried under HV. Yield: 2.64 g (91%), mp 255−258 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.29 (d, 1H), 7.09 (d, 1H), 3.10 (t, J_{H-P} = 18 Hz, 1H). ^{31}P NMR (5% NaOD/D_2O): δ 14.20. Anal. ($C_4H_7NO_6P_2S_2$) C, H, N, P, S.

Method D: Synthesis of 1-Alkyl- and Arylamino-1,1-bisphosponates. (5-Methylthiazol-2-yl)aminiomethylenebisphosphonic Acid (8c). In a flask fitted with a distillation head, a mixture of 114.2 g (1.00 mol) of 2-amino-5-methylthiazole,[50] 200 mL (1.20 mol) of triethyl orthoformate, and 260 mL (2.02 mol) of diethyl phosphite was heated to 120−125 °C. Within 3 h, 155 mL (about 90% of the calculated amount) of EtOH was distilled off. The residual 437 g of a viscous yellow oil was submitted to hydrolysis in the next step without further purification. ^{1}H NMR ($CDCl_3$, 60 MHz): δ 6.85 (s, 1H), 5.05 (t, J_{H-P} = 22 Hz, 1H), 4.32 (m, 8H), 2.37 (s, 3H), 1.39 (td, 12H).

The crude tetraester (437 g) obtained in the previous step was heated to reflux in 4 L of 1 N HCl for 24 h. The resulting slightly yellow solution was filtered and then diluted with 1.25 L of acetone. The product crystallized in the form of a very fine precipitate. After the mixture was stirred under reflux for 1 h, the white solid could be filtered and washed with 1.25 L of hot acetone. Yield (after drying under HV at 120 °C): 194.5 g (67%); mp 225 °C. ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.31 (s, 1H), 3.13 (t, J_{H-P} = 20 Hz, 1H), 1.90 (s, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 15.13. Anal. ($C_5H_{10}N_2O_6P_2 \cdot 0.25H_2O$) C, H, N, P, S.

(Benzylimidazol-2-yl)aminomethylenebisphosphonic Acid (8o). A solution of 5.02 g (8.75 mmol) of tetraethyl (1-tosylbenzylimidazol-2-yl)aminomethylenebisphosphonate in 60 mL of 1 N HCl was heated for 100 °C for 16 h. Because some partially hydrolyzed phoshonate was still visible by TLC, the temperature was increased to 120 °C for another 4 days. The white solid that had formed was filtered off and washed with a small amount of water. The crude product was taken up in a mixture of water and 1% acetone. The insoluble part was removed by filtration, and the filtrate was concentrated. The residue was recrystallized from water, followed by washing with hot water and acetone to give 1.16 g (43%) of product; mp 300 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.72−6.81 (m, 2H), 6.46−6.52 (m, 2H), 3.51 (t, J_{H-P} = 20 Hz, 1H). Anal. ($C_8H_{11}N_3O_6P_2 \cdot 1.5H_2O$) C, H, N.

In analogy to the examples described above under method D, the following aminomethylenebisphosphonic acids were prepared (yields given are those over two steps).

Cycloheptylaminomethylenebisphosphonic Acid (7e). Yield: 33%; mp 250−251 °C (dec). ^{1}H NMR (NaOD/D_2O, 360 MHz): δ 3.72−3.80 (m, 1H), 3.14 (t, J_{H-P} = 16 Hz, 1H), 2.08−2.19 (m, 2H), 1.63−1.77 (m, 4H), 1.45−1.63 (m, 6H). ^{31}P NMR (5% NaOD/D_2O): δ 15.47. Anal. ($C_8H_{19}NO_6P_2$) C, H, N, P.

(Thiazol-2-yl)aminomethylenebisphosphonic Acid (8a). Yield: 19%; mp 292 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.63 (d, 1H), 6.17 (d, 1H), 3.12 (t, J_{H-P} = 18 Hz, 1H). ^{13}C NMR (NaOD/D_2O): δ 174.0, 138.4, 106.3, 59.4 (t, J_{C-P} = 126 Hz). ^{31}P NMR (5% NaOD/D_2O): δ 14.94. Anal. ($C_4H_8N_2O_6P_2S$) C, H, N, P, S.

(4-Methylthiazol-2-yl)aminomethylenebisphosphonic Acid (8b). Yield: 11%; mp 294 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 5.80 (s, 1H), 3.09 (t, J_{H-P} = 19 Hz, 1H), 1.81 (s, 3H). ^{13}C NMR (NaOD/D_2O): δ 173.6, 148.2, 59.2 (t, J_{C-P} = 126 Hz), 17.1. ^{31}P NMR (5% NaOD/D_2O): δ 14.90. Anal. ($C_5H_{10}N_2O_6P_2S$) C, H, N, S. P: calcd, 21.50; found, 20.89.

(5-Ethylthiazol-2-yl)aminomethylenebisphosphonic Acid (8d). Yield: 50%; mp 243 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.70 (s, 1H), 3.49 (t, J_{H-P} = 18 Hz, 1H), 2.64 (q, 2H), 1.20 (t, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 15.11. Anal. ($C_6H_{12}N_2O_6P_2S\cdot0.5H_2O$) C, H, N, P, S.

(5-Propylthiazol-2-yl)aminomethylenebisphosphonic Acid (8e). Yield: 52%; mp 275 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.85 (s, 1H), 3.82 (t, J_{H-P} = 19 Hz, 1H), 2.62 (t, 2H), 1.61 (6-line system, 2H), 0.93 (t, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 15.14. Anal. ($C_7H_{14}N_2O_6P_2S\cdot0.5H_2O$) C, H, N, P, S.

(5-Butylthiazol-2-yl)aminomethylenebisphosphonic Acid (8f). Yield: 43%; mp 267 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.67 (s, 1H), 3.50 (t, J_{H-P} = 19 Hz, 1H), 2.62 (t, 2H), 2.55 (5-line system, 2H), 2.35 (6-line system, 2H), 0.90 (t, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 15.14. Anal. ($C_8H_{16}N_2O_6P_2S$) C, H, N, P, S.

(5-Isopropylthiazol-2-yl)aminomethylenebisphosphonic acid (8g). Yield: 46%; mp 255 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.72 (s, 1H), 3.50 (t, J_{H-P} = 18 Hz, 1H), 2.98 (7-line system, 1H), 1.23 (d, 6H). ^{31}P NMR (5% NaOD/D_2O): δ 15.11. Anal. ($C_7H_{14}N_2O_6P_2S$) C, H, N. P: calcd, 19.59; found, 18.77. S: calcd, 10.14; found, 9.22.

(5-Phenethylthiazol-2-yl)aminomethylenebisphosphonic Acid (8h). Yield: 15%; mp 293 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.23−7.42 (m, 5H), 6.61 (s, 1H), 3.49 (t, J_{H-P} = 18 Hz, 1H), 2.84−3.00 (m, 4H). ^{31}P NMR (5% NaOD/D_2O): δ 15.10. Anal. ($C_{12}H_{16}N_2O_6P_2S$) C, H, N, P, S.

(5-Phenylthiazol-2-yl)aminomethylenebisphosphonic Acid (8i). Yield: 3%; mp 298−302 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.36 (d, 2H), 6.92−7.12 (m, 3H), 6.44 (s, 1H), 3.04 (t, J_{H-P} = 19 Hz, 1H). Anal. ($C_{10}H_{12}N_2O_6P_2S\cdot0.25H_2O$) C, H, N, P, S.

(Imidazol-2-yl)aminomethylenebisphosphonic Acid (8j). 8j was prepared as described in the general method for **8c**, using 2-amino-1-benzylimidazole as the starting material. Then the benzyl group was cleaved by hydrogenolysis over 5% Pd/C, followed by P ester cleavage with HCl as usual. Yield (over three steps): 5%; mp 297 °C (dec). ^{1}H NMR (NaOD/D_2O, 360 MHz): δ 6.42 (br s, 2H), 3.24 (t, J_{H-P} = 18 Hz, 1H).

(1-Methylimidazol-2-yl)aminomethylenebisphosphonic Acid (8k). Yield: 5%; mp 323−326 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.21 (d, 1H), 6.15 (d, 1H), 3.45 (t, J_{H-P} = 20 Hz, 1H), 3.01 (s, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 16.17.

(1-Benzylimidazol-2-yl)aminomethylenebisphosphonic Acid (8l). Yield: 3%; mp 261−265 °C (dec). ^{1}H NMR (NaOD/D_2O, 360 MHz): δ 7.36−7.48 (m, 5H), 6.61 (d, 1H), 6.57 (d, 1H), 5.03 (s, 2H), 3.91 (t, J_{H-P} = 20 Hz, 1H). ^{31}P NMR (5% NaOD/D_2O): δ 16.26. Anal. ($C_{11}H_{15}N_3O_6P_2\cdot1.33HCl\cdot0.25H_2O$) C, N, P. H: calcd, 4.22; found, 3.77.

(1-Phenylimidazol-2-yl)aminomethylenebisphosphonic Acid (8m). Yield: 25%; mp 275 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.28 (d, 4H), 7.16 (6-line system, 1H), 6.52 (s, 1H), 6.41 (s, 1H), 3.60 (t, J_{H-P} = 20 Hz, 1H). ^{13}C NMR (NaOD/D_2O): δ 151.1, 137.1, 130.8 (2 carbons), 128.7, 126.0 (2 carbons), 123.7, 117.1, 54.4 (t, J_{P-C} = 126 Hz). ^{31}P NMR (5% NaOD/D_2O): δ 15.95. Anal. ($C_{10}H_{13}N_3O_6P_2$) C, H, N, P.

(Benzthiazol-2-yl)aminomethylenebisphosphonic Acid (8n). Yield: 59%; mp 290 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.38 (d, 1H), 7.09 (d, 1H), 7.03 (t, 1H), 6.81 (t, 1H), ca. 3.5 (br, 1H). ^{13}C NMR (NaOD/D_2O): δ 152.2.0, 130.3, 127.0, 122.3, 122.2, 117.7. P−C−P not visible. ^{31}P NMR (5% NaOD/D_2O): δ 14.44. Anal. ($C_8H_{10}N_2O_6P_2S\cdot0.25H_2O$) C, H, N, P, S.

Transformation Reactions of Bisphosphonates. N-Methylation: 1-Hydroxy-3-[methyl-(6-phenylhexyl)amino]propylidenebisphosponic Acid (4l). A mixture of 1.58 g (4.0 mmol) of 1-hydroxy-3-[(6-phenylhexyl)amino]propylidene-1,1-bisphosphonic acid (prepared according to method A), 3.6 mL of 85% formic acid, and 0.9 mL of formaldehyde solution (37% soluble in water, 12 mmol) was heated under reflux for 17 h. The residue remaining after concentration iV was treated with EtOH to yield, after drying under HV at 110 °C for 4 h, the product in the form of a white solid. Yield: 70%; mp 149−152 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 7.00−7.18 (m, 5H), 2.36−2.52 (m, 4H), 2.08−2.20 (m, 2H), 1.96 (s, 3H), 1.70−1.85 (m, 2H), 1.30−1.46 (m, 2H), 1.16−1.29 (m, 2H), 1.08−1.13 (m,4 H). ^{31}P NMR (5% NaOD/D_2O): δ 19.14. Anal. ($C_{16}H_{29}NO_7P_2$) C, H, N, P.

In an analogous way, the following N-methylated bisphosphonates were synthesized:

1-Hydroxy-3-(dimethylamino)propylidene-1,1-bisphosphonic Acid (1h, Olpadronate). Starting with 1-hydroxy-3-aminopropylidenebisphosponic acid (pamidronic acid), the title compound was prepared by double methylation in 92% yield; mp 210−212 °C (dec).^{1}H NMR (D_2O, 250 MHz): δ 3.26 (t, 2H), 2.70 (s, 6H), 2.19 (7-line system, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 18.96. Anal. ($C_5H_{15}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-(methylpropylamino)propylidenebisphosponic Acid (1i). This compound was obtained by methylation of 1-hydroxy-3-[*N*-propylamino]propylidenebisphosphonate (prepared in 67% yield following the general procedure of method A, starting with 3-(*N*-propylamino)propanoic acid): 28% yield; mp 140−145 °C. ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 2.30−2.41 (m, 2H), 1.95−2.05 (m, 2H), 1.83 (s, 3H), 1.60−1.67 (m, 2H), 1.11 (6-line system, 2H), 0.51 (t, 3H). Anal. ($C_7H_{19}NO_7P_2$) C, H, N, P. P: calcd, 21.27; found, 20.7.

1-Hydroxy-3-(dimethylamino)butylidene-1,1-bisphosphonic Acid (1o). Yield: 47%; mp 229−230 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 3.82−3.95 (m, 1H), 2.70 (br s, 6H), 2.16−2.38 (m, 1H), 1.95−2.12 (m, 1H), 1.20 (d, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 18.73. Anal. ($C_6H_{17}NO_7P_2$) C, H, N, P.

1-Hydroxy-3-[methyl-(4-phenylbutyl)amino]propylidene-1,1-bisphosphonic Acid (4j). Yield: 30%; mp 128−132 °C (dec). ^{1}H NMR (NaOD/D_2O, 250 MHz): δ 6.91−7.12 (m, 5H), 2.28−2.49 (m, 4H), 2.09−2.18 (m, 2H), 1.88 (s, 3H), 1.65−1.88 (m, 2H), 1.12−1.40 (m, 4H). ^{31}P NMR (5% NaOD/D_2O): δ 19.01. Anal. ($C_{14}H_{25}NO_7P_2$) C, H, N, P. P: calcd, 16.25; found, 15.3.

1-Hydroxy-3-[(methyl-3-phenylsulfanylpropyl)amino]-propylidene-1,1-bisphosphonic Acid (5r). A suspension of 3.08 g (8.0 mmol) of 1-hydroxy-3-[(3-phenylsulfanylpropyl)-amino]propylidene-1,1-bisphosphonic acid (**5q**) in 90 mL of acetonitrile was treated dropwise at room temperature with 16 mL of 1 N NaOH under vigorous stirring. After complete addition, stirring was continued for another 15 min at 30 °C. To the resulting white suspension, 3.0 mL of formaldehyde solution (37% soluble in water, 40 mmol) was added, followed by 0.8 g (12.8 mmol) of sodium cyanoborohydride after an additional 5 min. The temperature rose to approximately 30 °C, and the pH increased from 7 to 9. The pH was adjusted to 7 with a small amount of acetic acid. After sitting several days at room temperature, the white suspension was filtered and the white solid was washed with EtOH. Recrystallization from water/EtOH gave 2.7 g (63%) of the bisphosphonate in the form of its disodium salt; mp 245−247 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 7.25−7.50 (m, 5H), 3.15−3.40 (m, br, 4H), 3.05 (t, 2H), 2.73 (s, 3H), 2.29 (7-line system, 2H), 2.01 (5-line

system, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.11. Anal. ($C_{13}H_{21}NO_7P_2SNa_2 \cdot 5H_2O$) C, H, N, P, S.

N-Acetylation: 1-Hydroxy-3-acetylaminopropylidene-1,1-bisphosphonic Acid (1f). After cooling to −5 °C, a solution of 3.5 g (15 mmol) of 1-hydroxy-3-aminopropylidene-1,1-bisphosphonic acid (pamidronic acid) in 120 mL of 0.5 M NaOH was treated within 1 h with 1.7 mL (18 mmol) of acetanhydride with vigorous stirring. Stirring was continued for another 4 h at 0 °C followed by slow warming to room temperature. Concentration iV yielded a colorless oil, which started to crystallize after treatment with a 2-propanol/acetone mixture. Recrystallization from a small amount of water afforded 3.9 g (76%) of product in the form of its 2.75 sodium salt containing also 0.5 equiv of water (after drying overnight at 100 °C under HV); mp 300 °C (dec). ^{1}H NMR (D_2O, 100 MHz): δ 3.40 (t, 2H), 1.85−2.30 (m, 2H), 1.92 (s, 3H). ^{31}P NMR (5% NaOD/D_2O): δ 18.93. Anal. ($C_5H_{10.25}NO_8P_2Na_{2.75} \cdot 0.5H_2O$) C, H, N, Na, P.

N-Debenzylation: 1-Hydroxy-3-methylaminopropylidene-1,1-bisphosphonic Acid (1g). A mixture of 25.0 g (ca. 90%, 66 mmol) of 1-hydroxy-3-(benzylmethylamino)propylidene-1,1-bisphosphonic acid (prepared according to the general procedure of method A, starting with benzylmethylaminopropionic acid) in 500 mL of water/methanol 7:3 was hydrogenated over Pearlman's catalyst (2.5 g). After 1 day at room temperature, more catalyst was added (1 g), and once again after 1 more day (1.2 g). The mixture was filtered after a total of 5 days (total time). The catalyst had to be extracted several times with water (50 °C). The product could be crystallized from 10% MeOH/water. Yield: 12.9 g (78%) after drying at 80 °C under HV; mp 148 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 2.49 (t, 2H), 1.98 (s, 3H), 1.6−1.8 (m, 2H). ^{31}P NMR (5% NaOD/D_2O): δ 19.13. Anal. ($C_4H_{13}NO_7P_2 \cdot H_2O$) C, H, N, P.

Preparation of Bisphosphinates. (Thiazol-2-yl)aminomethylene-1,1-bis(methylphosphinic Acid) (10). In a flask fitted with a distillation head, a mixture of 2.00 g (20 mmol) of 2-aminothiazole,[50] 4.0 mL (24 mmol) of triethyl orthoformate, and 4.37 g (40.4 mmol) of methylphosphinic acid methyl ester was heated to 120−125 °C. Within 24 h, 2.8 mL (about 80% of the calculated amount) of EtOH was distilled off. The residual 7.0 g of a viscous yellow oil was purified by flash chromatography (10% MeOH/ethyl acetate). Yield: 3.00 g (46%) as a mixture of diastereomers; mp 111−113 °C. Anal. ($C_{10}H_{20}N_2O_4P_2S$) C, H, N, P, S.

The diester (1.63 g, 5.00 mmol) obtained in the previous step was heated to reflux in 20 mL of 1 N HCl for 6 h. The resulting clear and slightly yellow solution was diluted with 40 mL of acetone. Within 15 min, the product crystallized in the form of a white precipitate. It was filtered and washed sequentially with acetone/water 3:1, twice with acetone, and finally with petroleum ether. Yield (after drying under HV): 1.02 g (76%); mp 270 °C (dec). ^{1}H NMR (NaOD/D_2O, 300 MHz): δ 6.65 (d, 1H), 6.25 (d, 1H), 3.53 (t, $J_{H-P} = 16$ Hz, 1H), 0.95 (A_6XX' spin system, 6H). ^{31}P NMR (5% NaOD/D_2O): δ 35.49. Anal. ($C_6H_{12}N_2O_4P_2S \cdot 0.25H_2O$) C, H, N, P, S.

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JM020819I