Activity of Nitrogen-Containing and Non-Nitrogen-Containing Bisphosphonates on Tumor Cell Lines

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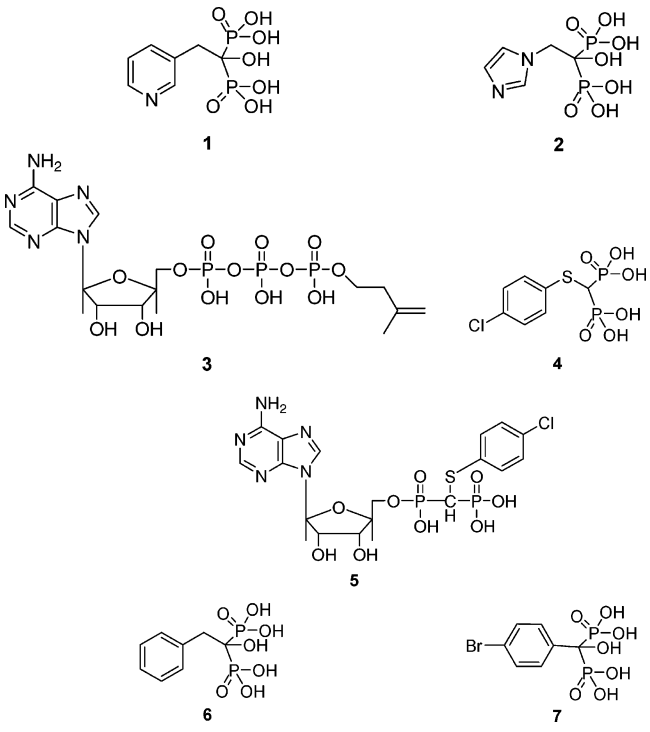
We synthesized and tested three series of bisphosphonates for their activity in inhibiting the growth of three human tumor cell lines: MCF-7 (breast), NCI−H460 (lung), and SF-268 (CNS). The first series of compounds consisted of 49 nitrogen-containing bisphosphonates, the most active species being a tetrakispivaloyloxymethyl (POM) ester, having an (average) IC_{50} of 6.8 μM. The second series of compounds consisted of nine terphenylbisphosphonates, the most active species also being a POM ester, having an IC_{50} of 2.2 μM. The third series of compounds consisted of seven halogen or cyanophenylbisphosphonates, the most active species again being a POM ester, having an IC_{50} of 500 nM. Taken together, these results are of interest because they show that bisphosphonate esters can have potent activity against a variety of tumor cell lines, with the most active terphenyl- and halophenyl-containing species having IC_{50} values $\sim$10−40$\times$ lower than the most potent commercially available bisphosphonates.

Introduction

Nitrogen-containing bisphosphonates (NBPs)[a] such as risedronate (**1**) and zoledronate (**2**) are used extensively in the treatment of osteoporosis, Paget's disease, and hypercalcemia due to malignancy.[1,2] They are also potent activators of human $\gamma\delta$ T cells,[3,4] as well as having in some cases direct anticancer[5−10] and antiparasitic[11−19] activity. They function by inhibiting the enzyme farnesyl diphosphate synthase (FPPS, EC 2.5.1.10), the enzyme responsible for the synthesis of the FPP used in protein prenylation, cholesterol, ergosterol, heme a, ubiquinone, and dolichol production. In addition, the isopentenyl diphosphate (IPP), which might accumulate on FPPS inhibition, can be converted to a "toxic ATP analog" (the isopentenyl ester of ATP) ApppI (**3**), which inhibits the mitochondrial adenine nucleotide translocase and is strongly pro-apoptotic.[20] A second class of bisphosphonates used clinically to treat osteoporosis are the non-nitrogen-containing bisphosphonates (NNBPs), such as clodronate, etidronate, and tiludronate (**4**). These compounds do not inhibit FPPS, rather, they also are converted to pro-apoptotic ATP analogues[21,22] in which the bisphosphonate condenses with AMP to form the β,γ-methylenetriphosphates AppCp (clodronate), AppEp (etidronate), and AppTp (**5**, tiludronate), which again all inhibit the mitochondrial ADP/ATP transporter and are pro-apoptotic. There are also a number of related NNBPs in development, such as the deaza-analogue of risedronate, **6**, which in early work[11] we found had activity against trypanosomatid parasites such as *Trypanosoma brucei*, the causative agent of African sleeping sickness, and *Plasmodium falciparum*, the causative agent of the most serious and prominent form of malaria. More recently, Lecouvey et al.[23,24] reported that **6** also had activity against a human tumor cell line and was anti-angiogenic, and in their latest work, this group reported that the *p*-bromophenyl bisphosphonate **7** had an IC_{50} of $\sim$95 μM against a human squamous cell carcinoma cell line.[25]



A problem with the bisphosphonates is, however, that they are poorly orally available and are rapidly adsorbed by bone. While the latter property is of course critical for their use in treating bone-related diseases, for further more general development as anticancer[26] or antiparasitic agents, it might be desirable to have more lipophilic species. Plus, it would be of interest to explore the activities of a wider range of bisphosphonates, both nitrogen-containing and non-nitrogen-containing (NBPs and NNBPs), against tumor cell lines. Here, we report the synthesis

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[a] Abbreviations: FPP, farnesyl diphosphate; FPPS, farnesyl diphosphate synthase; IPP, isopentenyl diphosphate; NBP, nitrogen-containing bisphosphonate; NNBP, non-nitrogen-containing bisphosphonate; POC, isopropyloxycarbonyloxymethyl; POM, pivaloyloxymethyl; QSAR, quantitative structure activity relationship.

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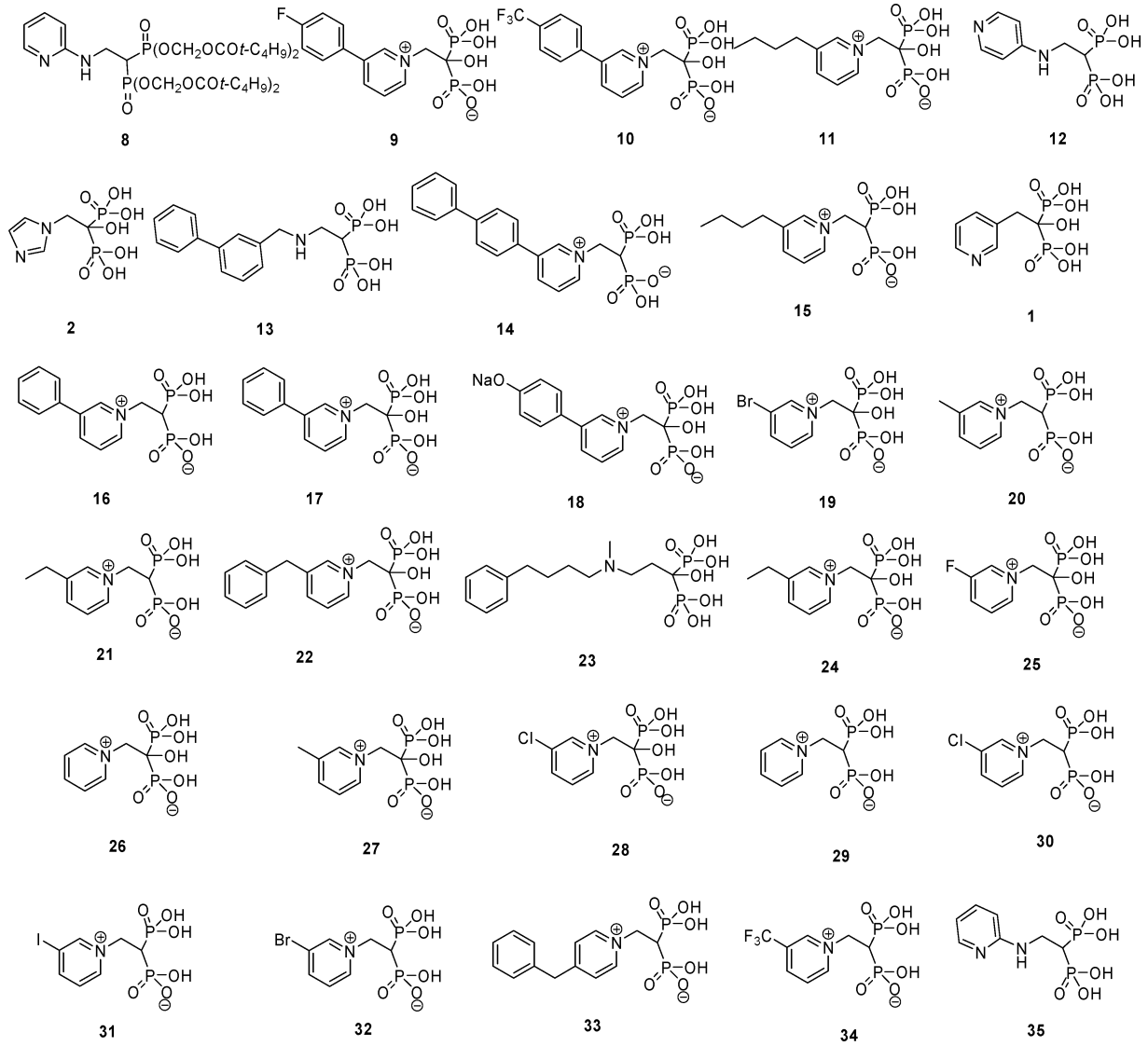


Figure 1. Structures of the most active nitrogen-containing bisphosphonates rank-ordered by activity from highest (**8**) to lowest (**35**).

and testing against three human tumor cell lines (MCF-7, NCI−H460, and SF-268) of three chemically quite distinct sets of bisphosphonates, including several which are very lipophilic.

General Synthetic Aspects. We show in Figures 1 and 2 (rank ordered in terms of decreasing activity) the structures of the 49 NBPs investigated. Most of these compounds are novel 2-(pyridinium-1-yl)ethylidene-1,1-bisphosphonates, among which those bearing a 1-hydroxyl group (**9**−**11**, **17**−**19**, **22**, **24**−**28**, **36**, **37**, **40**, **43**, **44**, **46**, and **49**) were made following the protocols described previously,[27] as shown in Scheme 1. Their synthesis involved the use (when necessary) of Suzuki coupling to produce a substituted pyridine, which was alkylated with bromoacetic acid, then phosphonylated with $H_3PO_3/POCl_3$, as shown in Scheme 1 (top).

The other class of pyridinium-1-yl compounds are those lacking the 1-hydroxyl group (**14**−**16**, **20**, **21**, **29**−**34**, **39**, **41**, **48**, and **50**−**54**), which were prepared by Michael addition of substituted pyridines (prepared according to Scheme 1) to vinylidene-1,1-bisphosphonic acid, as is also shown in Scheme 1 (bottom).[28]

The synthesis of **8**, a "pro-drug" of an NBP, containing four pivaloyloxymethyl (POM) ester groups, was prepared as shown in Scheme 2. Here tetramethyl vinylidene-1,1-bisphosphonate was converted to its tetrakis-pivaloyloxymethyl ester by treatment with chloromethyl pivalate and sodium iodide,[29] followed by Michael addition of 2-aminopyridine, to give **8**.

We also investigated 9 NNBPs containing terphenyl side chains (**55**−**63**), shown in Figure 3. The POM ester **55** was synthesized according to Scheme 3, while the free acids (**56**−**63**) of the terphenyl-containing NNBPs were synthesized basically as exemplified in Scheme 4 for the *meta*, *meta*-terphenyls (**56**, **58**, and **61**).

The synthesis consists of three parts: (a) Suzuki coupling of an methyl ester of a bromophenylalkanoic acid with a biphenyl boronic acid to form a methyl terphenylalkanoate; (b) hydrolysis of the ester so obtained to yield the corresponding carboxylic acid, followed by conversion to the acid chloride; and (c) reaction of the acid chloride with tris(trimethylsilyl) phosphite, followed by hydrolysis,[30] to afford the bisphosphonic acid.

Finally, we show in Figure 4 the structures of five pivaloyloxymethyl (POM) or isopropoxycarbonyloxymethyl (POC) esters (**64**−**68**) and two free acids (**69** and **70**) of halophenyl- or cyanophenyl-containing bisphosphonates (rank ordered in terms of activity, as described below). The POM esters were prepared by alkylation of tetramethyl methylenebisphosphonate with a benzyl bromide, followed by transesterification,[29] as shown in Scheme 5. This strategy was then extended to form the POC esters. The free acids (**69** and **70**) were prepared by

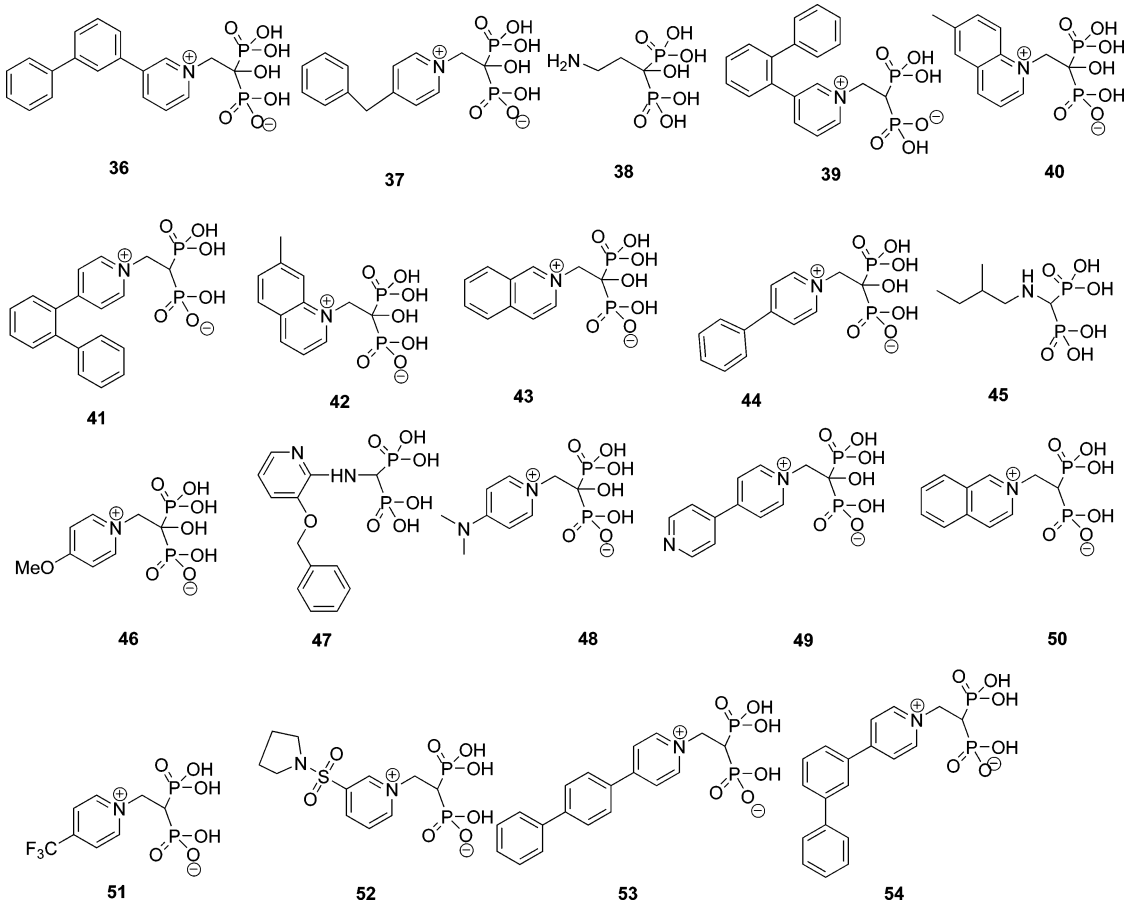
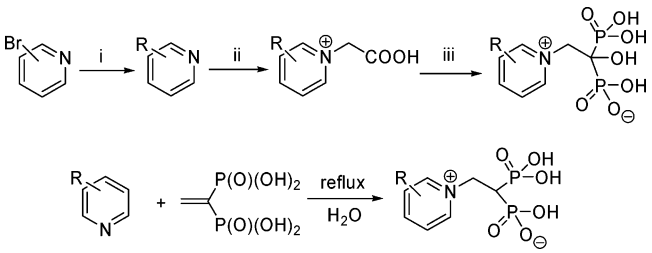


Figure 2. Structures of the less active nitrogen-containing bisphosphonates rank-ordered by activity as in Figure 1.

Scheme 1[a]



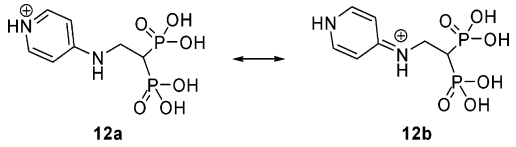
[a] Reagents: (i) $RB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3; (ii) $BrCH_2COOH$; (iii) H_3PO_3, $POCl_3$.

treatment of their tetramethyl esters with bromotrimethylsilane (TMSBr), followed by hydrolysis.

Full details of the synthesis of each novel compound are described later in the paper, and microanalytical results are provided in the Supporting Information.

Results and Discussion

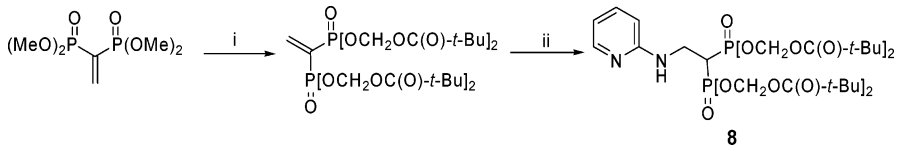
We first investigated the growth inhibition of the three human tumor cell lines MCF-7 (breast), NCI−H460 (lung), and SF-268 (CNS) by the nitrogen-containing bisphosphonates shown in Figures 1 and 2. The IC_{50} values so obtained are shown in Table 1, together with, for ease of comparison, the average IC_{50} values found, averaged across the three cell lines. The IC_{50} values for the free bisphosphonic acids (i.e., not including the POM ester, **8**) are all in the range of ~10 μM to >10 mM. Of the free acids investigated, the most active compounds are the three pyridinium-1-yl bisphosphonates (**9**−**11**), each containing a pyridinium-1-yl group and a hydrophobic side chain. Another particularly active compound (IC_{50} (avg) = 16.2 μM) is the 4-pyridyl-amino-containing bisphosphonate (**12**). This compound is known to be a potent FPPS inhibitor[31] whose activity can be related to the presence of charge delocalization as a result of resonance with the *p*-quinonoid species (**12a** and **12b**):



The next four most active compounds are zoledronate (**2**) and **13**−**15**, all of which have average IC_{50} values <30 μM (Table 1), followed by risedronate (**1**), with an average IC_{50} value of ~34 μM. All of the other free acid (or salt) compounds have IC_{50} values >40 μM, Table 1.

These results are all consistent with our previous pharmacophore modeling and QSAR (quantitative structure activity relationship) studies,[4,27,31] which indicated the importance of having a positive charge located either in an aromatic ring or in the β-position, two negative ionizable groups, as well as a hydrophobic feature for optimal activity, and the IC_{50} values found are not atypical of those reported for these and other bisphosphonates in other cell lines in the literature.[5−10] For example, Alvarez et al.[32] found IC_{50} values for alendronate, risedronate (**1**), and pamidronate (**38**) of >40, >40, and 33.6 μM, in the 13 762 rat mammary carcinoma cell line. Our results do, however, suggest that obtaining IC_{50} values of <10 μM for the NBPs may be difficult, presumably due to their high polarity. This can be countered to some extent by adding hydrophobic

Scheme 2[a]



[a] Reagents: (i) $ClCH_2OC(O)$-*t*-Bu, NaI, reflux; (ii) 2-aminopyridine, 20% overall yield.

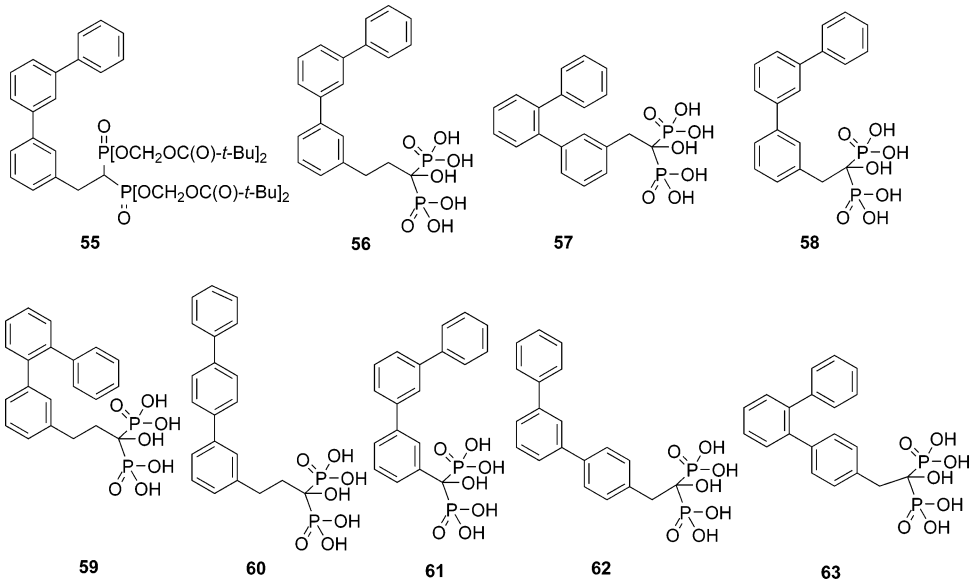
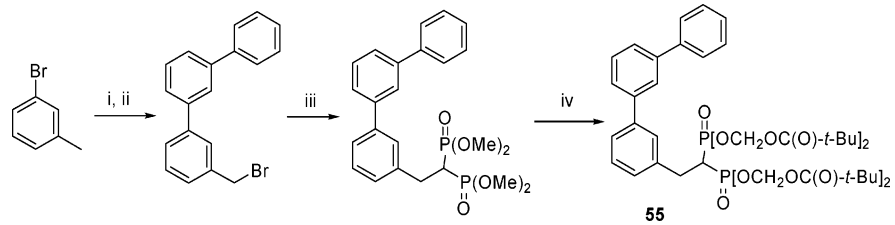


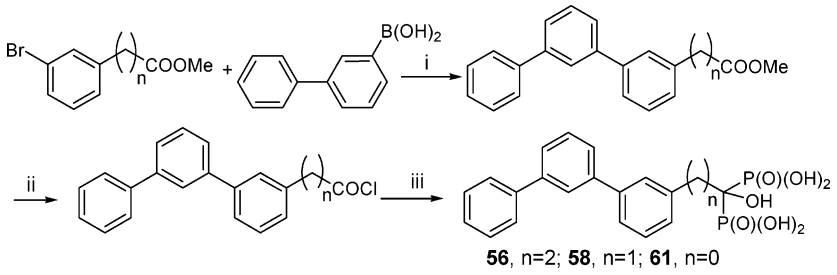
Figure 3. Structures of the terphenyl, non-nitrogen-containing bisphosphonates, rank-ordered by activity from highest (**55**) to lowest (**63**).

Scheme 3[a]



[a] Reagents: (i) 3-biphenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3; (ii) NBS, AIBN; (iii) $CH_2(POOMe)_2$, NaH, 64% for three steps; (iv) $ClCH_2OC(O)$-*t*-Bu, NaI, reflux, 42% isolated yield.

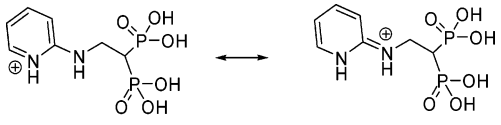
Scheme 4[a]



[a] Reagents: (i) $Pd(PPh_3)_4$, K_2CO_3; (ii) NaOH, THF−H_2O, and then $(COCl)_2$; (iii) $P(OTMS)_3$.

side chain features (**9**−**11**), but arguably, an even more pronounced effect on cell growth inhibition might be obtained by masking the bisphosphonate group by esterification. POM esters (prodrugs) of alendronate and pamidronate have been reported in the patent literature,[33] however, we were not able to obtain these products in high purity.

We therefore investigated the effects of esterification of the bisphosphonate groups of another bisphosphonate, **35**. This species is a very potent, low nM inhibitor of FPPS,[31,34] due we believe to its strong amidinium-like resonance stabilization



and, unlike other nitrogen-containing bisphonates, **35** can be

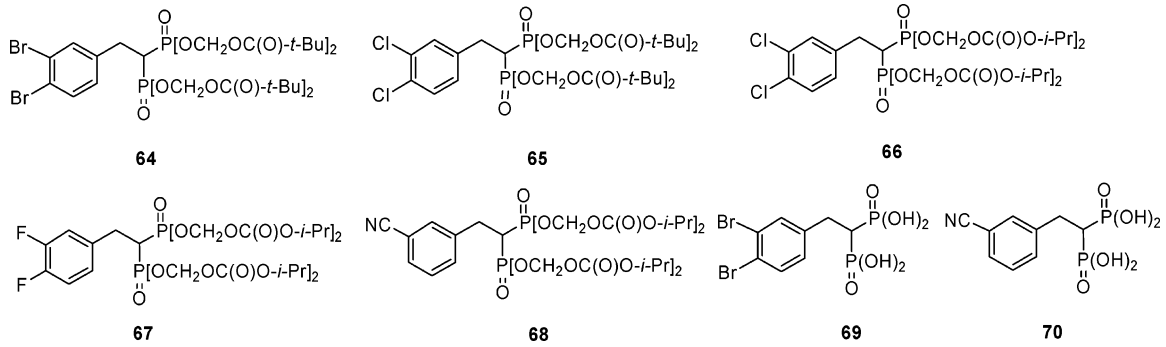
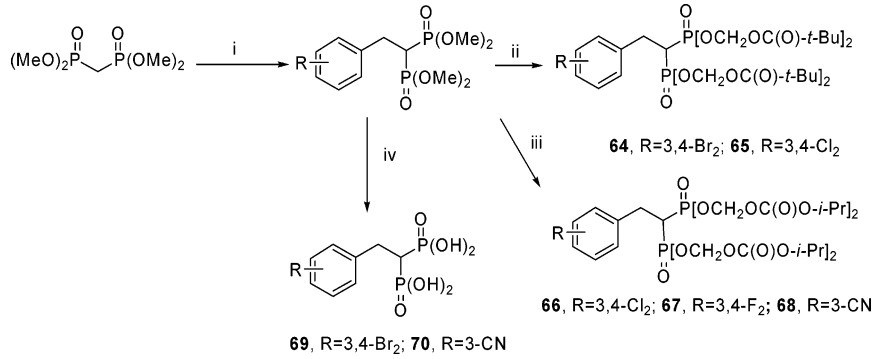


Figure 4. Structures of the benzylbisphosphonates investigated, rank-ordered by activity from highest (**64**) to lowest (**70**).

Scheme 5[a]



[a] Reagents: (i) NaH, $RC_6H_4CH_2Br$; (ii) $ClCH_2OC(O)$-*t*-Bu, NaI; (iii) $ClCH_2OC(O)O$-*i*-Pr, NaI; (iv) TMSBr.

readily converted to the tetrakis-POM ester via a mild Michael addition (Scheme 2).

The parent bisphosphonate, while a potent inhibitor of FPPS, has however only a 145 μM (average) IC_{50} value as the free acid against the three tumor cell lines, Table 1. However, on esterification, this IC_{50} value drops to 6.8 μM, Table 1, a factor of $\sim$20$\times$ improvement in potency due to masking of the highly polar bisphosphonate group. This is an interesting result because it implies that esterase activity in these tumor cell lines enables hydrolysis of the POM ester to the active free acid, opening up the possibility of developing other, even more potent, lipid soluble analogues using this approach.

The second series of bisphosphonates investigated were the nine terphenyl species shown in order of decreasing activity in Figure 3. The rationale for investigating these species in tumor cell growth inhibition was 4-fold: First, we previously found that the simple aryl bisphosphonate **6** had activity in several cell lines and others have found activity against tumor cell lines as well, so it seemed logical to investigate modified aryl side chains to try to improve cell uptake and, perhaps, target inhibition. Second, because these compounds lack the positive charge (or basic nitrogen site) found in the NBPs, they should present less of a challenge for conversion into lipophilic (POM and POC) esters. Third, in initial work we found that addition of a single phenyl group (to make *m*,*p*-biphenyl ethylidene bisphosphonates) did not result in active compounds (IC_{50} > 250 μM), necessitating incorporation of additional features: terphenyls, esters, and, as discussed below, halogen substitution. Fourth, we already had several of these compounds available in our laboratory.

Figure 3 shows the compounds investigated, and Table 2 shows the IC_{50} values determined. What can be seen immediately from the results presented in Table 2 is that all of the free acid NNBPs based on the terphenyl ring system are less active than are the most active free acid NBPs (Figures 1 and 2, Table 1). The most potent growth inhibitors (as the free acids) are **56** and **57**, which have average IC_{50} values of 45.1 and 78.9 μM, respectively. This is clearly a factor of $\sim$3$-$4$\times$ weaker than with the best NBPs. The most active species have a *meta* substitution on the ring closest to the bisphosphonate backbone, while the two *para*-substituted compounds (**62** and **63**) have very low (>480 μM) activity. The results shown in Table 2 indicate that a *meta*,*meta* (terphenyl, ring) substitution pattern with two side chain methylene groups results in the highest activity (45 μM) and that this activity decreases on removing these groups (141 μM, 1 CH_2 group, **58**; 299 μM, no CH_2 groups, **61**). Compounds with *para* substitutions on the ring attached to the bisphosphonate group are even less active (**62**, 482 μM; **63**, 622 μM). Interestingly, removal of the 1-OH group and esterification of the phosphonate groups to form the POM ester **55** results in a major increase in activity, with **55** having an IC_{50} of 2.2 μM (Table 2), a 60$\times$ increase in potency over the *m*,*m*-terphenyl analogue **58** (IC_{50} = 141 μM, Table 2). While at present we cannot be certain that this effect is exclusively due to esterification (since the absence of the 1-OH group could also in principle play a role), the enhanced activity of the POM ester NNBP compares favorably with the $\sim$20$\times$ enhancement on esterification seen with the NBPs (**35** $\rightarrow$ **8**) and suggests that investigation of other terphenyl POM esters may be worthwhile.

The final series of compounds investigated were another set of NNBPs, this time containing just a single ring, but with various electron-withdrawing substituents. The rationale for investigating these compounds is that the halogen-containing bisphosphonate (**4**) as well as the phenyl analogue **6** have activity in cells: tiludronate (**4**) against osteoclasts and **6** against *T. cruzi* and tumor cells. Because **4** is metabolized to **5** in cells and targets the mitochondrial adenine nucleotide translocase, we reasoned that **6** (and its halogenated analogues) might also kill tumor cells in the same way and that, perhaps, halogen

Table 1. IC_{50} Values for Tumor Cell Growth Inhibition by Nitrogen-Containing Bisphosphonates

compound	MCF-7 (μM)	NCI-H460 (μM)	SF-268 (μM)	mean IC_{50}[a] (μM)
8	6.44	5.95	7.89	6.76
9	17.3	5.60	6.50	9.80
10	14.5	23.2	10.4	16.0
11	21.4	14.8	13.1	16.4
12	22.7	12.6	14.4	16.6
2	27.7	11.7	14.3	17.9
13	46.0	5.73	6.18	19.3
14	25.1	21.5	23.2	23.3
15	26.5	33.0	24.1	27.9
1	35.7	43.7	23.3	34.2
16	34.6	41.3	43.6	39.8
17	44.2	52.2	27.2	41.2
18	37.2	74.4	30.7	47.4
19	33.5	54.5	55.7	47.9
20	38.1	62.0	44.6	48.2
21	42.6	62.0	44.8	49.8
22	69.2	18.5	67.0	51.6
23	82.5	22.9	49.2	51.6
24	50.7	71.7	45.8	56.1
25	58.1	65.8	49.3	57.7
26	79.4	69.9	46.8	65.4
27	98.1	66.8	52.6	72.5
28	121	58.1	58.3	79.1
29	72.3	109	65.1	82.4
30	132	59.0	72.7	87.8
31	123	53.7	89.3	88.6
32	143	92.9	109	115
33	125	141	133	133
34	129	142	132	134
35	223	63.7	147	145
36	171	117	174	154
37	191	211	111	171
38	141	71.8	408	207
39	236	208	232	225
40	260	279	261	267
41	280	284	243	269
42	289	270	257	272
43	288	297	260	282
44	304	312	290	302
45	447	469	301	406
46	409	644	436	496
47	339	3209	399	1316
48	>10 000	>10 000	>10 000	
49	>10 000	>10 000	>10 000	
50	>10 000	>10 000	>10 000	
51	>10 000	>10 000	>10 000	
52	>10 000	>10 000	>10 000	
53	>10 000	>10 000	>10 000	
54	>10 000	>10 000	>10 000	

[a] Mean values over the three cell lines.

Table 2. IC_{50} Values for Tumor Cell Growth Inhibition by Terphenyl-Containing Bisphosphonates

compound	MCF-7 (μM)	NCI-H460 (μM)	SF-268 (μM)	mean IC_{50}[a] (μM)
55	2.62	1.64	2.38	2.21
56	43.9	46.9	44.7	45.1
57	92.7	86.1	58.0	78.9
58	152	140	130	141
59	146	133	143	141
60	196	132	175	168
61	310	285	303	299
62	412	413	620	482
63	1063	329	475	622

[a] Mean values over the three cell lines.

substitution might result in enhanced activity (given that both tiludronate and **7** contain halogen groups). Likewise, halogenated aryl 1-amino bisphosphonates have been reported in the early literature[35] as herbicides and fungicides and might also have this mechanism of action.

Table 3. IC_{50} Values for Tumor Cell Line Growth Inhibition by Non-Nitrogen-Containing Benzyl Bisphosphonates

compound	MCF-7 (μM)	NCI-H460 (μM)	SF-268 (μM)	mean IC_{50}[a] (μM)	ring
64	0.22	0.62	0.65	0.50	3,4-Br_2-Ph
65	0.34	2.70	1.43	1.49	3,4-Cl_2-Ph
66	1.97	4.85	4.78	3.87	3,4-Cl_2-Ph
67	15.2	20.1	28.3	21.2	3,4-F_2-Ph
68	58.6	22.6	7.77	29.7	CN-Ph
69	533	446	349	442	3,4-Br_2-Ph
70	>1000	>1000	>1000		CN-Ph

[a] Mean values over the three cell lines.

We show, therefore, in Figure 4 and Table 3 the structures and IC_{50} values for a series of such bisphosphonates containing electron-withdrawing groups. For the free acid/salt species (**69** and **70**), the IC_{50} values, not surprisingly, are very high, >400 μM, Table 3. However, on conversion to the POM and POC esters, there are major increases in potency. Indeed, each of the five esters investigated have considerable activity against each cell line, with (average) IC_{50} values in the range of 500 nM to 30 μM, with the most active compound having the lowest IC_{50} value (500 nM) of any of the compounds investigated in this study. There are also some clear structural trends in activity. For the POM, the activity is 3,4-Br_2Ph (**64**) > 3,4-Cl_2Ph (**65**); the activity of the 3,4-Cl_2Ph POM (**65**) is greater than that of the 3,4-Cl_2Ph POC (**66**); and the activities of each of these compounds are much greater than those of the 3,4-F_2Ph, CN−Ph POC (**67**, **68**), Table 3. Thus, conversion of the free acids into more lipid soluble forms appears to result in enhanced uptake into the tumor cells because, in the case of the free acids, the IC_{50} values are much higher (a factor of >800 for the most active compound, **64**, versus its free acid, **69**).

Mechanistically, based on what is known about the mode of action of the NBPs, it seems likely that many of the most active NBPs target primarily FPPS, resulting in inhibition of protein prenylation as well as production of the pro-apoptotic species ApppI (**3**). On the other hand, the NNBPs clodronate, etidronate, and tiludronate are thought to act by forming only toxic ATP analogues (such as **5**), and it is possible that this mechanism also operates with species such as **6**, **7**, and the benzylbisphosphonates shown in Figure 4 because these compounds have essentially no inhibitory effects on FPPS (data not shown). Although we cannot rule out other possible mechanisms for these and indeed the terphenyl bisphosphonates, the observation that substitution of the bisphosphonate's methylene group by Cl_2, OH/Me, or a chlorophenylthio group (clodronate, etidronate, tiludronate) leads in every case to incorporation of these NNBPs into AppXp analogues, clearly suggests a similar mechanism with related species (such as **64**), with inhibition of the mitochondrial adenine nucleotide translocase leading to apoptosis.

However, independent of mechanism, the results presented above show that NNBPs clearly have activity against these three human tumor cell lines, with the most active compounds being considerably more potent than the most active NBPs, due, we believe at least in part, to their enhanced lipophilicity. For the NBPs **8** and **35**, esterification results in a factor of 20 increase in potency (6.8 μM versus 145 μM); in the case of the terphenyl NNBPs, esterification (combined perhaps with removal of the 1-OH group) gives a factor of ~60× increase in potency, while for the dibromophenyl-containing bisphosphonate, POM ester formation results in a factor of ~800× increase in activity, with IC_{50} values of 500 nM (**64**) versus 442 μM (**69**).

Conclusions. The results we have shown above are of interest for a number of reasons. First, we have investigated the growth

inhibition behavior of 49 nitrogen-containing bisphosphonates (NBPs) against three human tumor cell lines: MCF-7 (breast), NCI−H460 (lung), and SF-268 (CNS). The IC_{50} values of the most potent NBPs are in the range 10−20 μM. However, formation of a POM ester of one NBP resulted in a decrease in IC_{50} from 145 to 6.8 μM, suggesting that related ester analogues may be worth investigating in these and other tumor cell lines. Second, we synthesized and tested a series of nine novel bisphosphonates containing the very hydrophobic, terphenyl side chain. Broadly speaking, these compounds had a similar range in activity as did the NBPs. However, the most active compound was again an ester, which was found to have an IC_{50} value of 2.2 μM. Third, we synthesized a series of bisphosphonates containing electron-withdrawing (halo, cyano) phenyl groups. The free acids had poor activity (>400 μM), but the POM and POC esters were much more active, with IC_{50} values as low as 500 nM, >800 times the activity of the free acid form and likewise more active than the most active NBPs. Overall, these results are of interest because they represent the first report of the activity of a wide range of novel bisphosphonates against tumor cell growth. The IC_{50} values found for the NNBPs (in their ester forms) are as low as 500 nM (dibromophenyl) or 2.2 μM (terphenyl), considerably lower than the values found for the free acid NBPs in the same assays. Given the extensive literature on the effects of NBPs on tumor cell growth inhibition, further work on the lipid soluble NNBPs may be of interest, including, for example, their potential use in topical applications in vivo, as well as their activity against parasitic protozoa.

Experimental Section

All reagents used were purchased from Aldrich (Milwaukee, WI). The purities of all compounds were routinely monitored by using ^{1}H and ^{31}P NMR spectroscopy at 400 or 500 MHz on Varian (Palo Alto, CA) Unity spectrometers using, in some instances, absolute spin-count quantitative analyses. The elemental analysis results for all new compounds are provided in the Supporting Information (Table S1).

The synthesis of **1**, **2**, **11**−**13**, **17**, **18**, **22**−**27**, **35**−**38**, **43**, **45**, **47**, and **48** have been described previously,[11,13,16,27] and the samples used here were from the previous batches. The five following general methods detailed below were used to make all new compounds:

General Method A (Suzuki Coupling; Schemes 1, 3, and 4): An aryl boronic acid or its ester (6 mmol), a bromo-substituted aromatic compound (5 mmol), K_2CO_3 (15 mmol), and $Pd(PPh_3)_4$ (50 mg) in toluene (10 mL) and H_2O (3 mL) were refluxed under N_2 overnight. Upon extraction with diethyl ether, the product was purified by column chromatgraphy.

General Method B (Synthesis of 2-(Pyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid): A substituted pyridine (1.2 mmol) and vinylidene-1,1-bisphosphonic acid[28] (1 mmol) in water (1 mL) was refluxed for 2 h. Upon removal of solvent, the residue was triturated with ethanol (3 mL), and the resulting white suspension was filtered and washed with ethanol (2 × 2 mL), affording pure 2-(pyridinium-1-yl)ethylidene-1,1-bisphosphonic acid as a white powder.

General Method C (Alkylation of Tetramethyl Methylenebisphosphonate; Schemes 3 and 5): Tetramethyl methylenebisphosphonate (2 mmol) in dry DMF (2 mL) was treated with NaH (2.2 mmol) in an ice bath. A benzyl bromide (2 mmol) was added to the resulting solution. The reaction mixture was stirred at room temperature for 1 h before being quenched with saturated NH_4Cl. The product was extracted with diethyl ether and purified by column chromatography.

General Method D (Transesterification; Schemes 3 and 5): The tetramethyl ester of a bisphosphonic acid (1 mmol), NaI (4 mmol), and chloromethyl pivalate (5 mmol; or chloromethyl isopropyl carbonate, when making POC esters) were refluxed overnight under N_2 in dry acetonitrile (5 mL).[29] Upon removal of solvent, the residue was partitioned between water and diethyl ether, and the organic layer was washed with water and concentrated. The product was purified by using flash column chromatography (silica gel; hexane/ethyl acetate (10/1), then ethyl acetate).

General Method E (Synthesis of Terphenylbisphosphonates; Scheme 4): The methyl ester of a carboxylic acid (1 mmol) was hydrolyzed with 3 N NaOH (1 mL) in methanol (5 mL) at room temperature for 1 h. After acidification with 2 N HCl, methanol was removed, and the resulting carboxylic acid was filtered and then washed with water. The dried acid was dissolved in benzene (5 mL) and oxalyl chloride (2 mmol) was added, followed by one drop of DMF. The reaction mixture was then stirred for 1 h. Upon removal of solvent, the crude acid chloride so obtained was dissolved in dry THF (5 mL) and $P(OTMS)_3$ (2 mmol) was added. After 3 h at room temperature, the solvent was removed, methanol−H_2O (2 mL, 1:1) was added, and the mixture was stirred for 30 min. Concentrated aqueous NaOH was then added to precipitate the target compound, which was washed thoroughly with methanol and then ether and dried to afford the bisphosphonic acids as their sodium salts.

Tetrakis-pivaloyloxymethyl 2-(Pyridin-2-ylamino)ethylidene-1,1-bisphosphonate (8). The tetrakis-pivaloyloxymethyl ester of vinylidene-1,1-bisphosphonic acid was made from tetramethyl vinylidene-1,1-bisphosphonate (488 mg, 2 mmol), following General Method D but without chromatographic purification. The product was then reacted with 2-aminopyridine (141 mg, 1.5 mmol) in $CHCl_3$ (2 mL) at room temperature overnight. The reaction mixture was subjected to flash chromatography (silica gel; hexane/ethyl acetate (10/1), then ethyl acetate) to afford **8** (295 mg, 20% overall yield). Quantitative ^{1}H NMR indicated 95% purity. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30−1.40 (m, 36H, CH_3), 2.08 (tt, J = 24.4, 6.4 Hz, 1H, $NHCH_2CH$), 3.90−4.02 (m, 2H, $NHCH_2$), 5.60−5.70 (m, 8H, OCH_2O), 6.47 (d, J = 8.8 Hz, 1H, aromatic), 6.55−6.58 (m, 1H, aromatic), 7.35−7.40 (m, 1H, aromatic), 8.01 (dm, J = 8.8 Hz, 1H, aromatic). ^{31}P NMR ($CDCl_3$): δ 19.38.

1-Hydroxy-2-[3-(4-fluorophenyl)pyridinium-1-yl]ethylidene-1,1-bisphosphonic Acid Monosodium Salt (9). Compound **9** was prepared from 3-bromopyridine (2 mmol) and 4-fluorophenyl boronic acid (2.4 mmol) following a published procedure[27] (303 mg, 38% overall yield). Anal. ($C_{13}H_{13}FNNaO_7P_2$) C, H, N. ^{1}H NMR (400 MHz, D_2O): δ 4.90 (t, J = 9.6 Hz, 2H, CH_2), 7.10−7.15 (m, 2H, aromatic), 7.55−7.60 (m, 2H, aromatic), 7.80−7.85 (m, 1H, aromatic), 8.54 (d, J = 8.4 Hz, 1H, aromatic), 8.64 (d, J = 6.4 Hz, 1H, aromatic), 8.94 (s, 1H, aromatic). ^{31}P NMR (D_2O): δ 14.06. ^{19}F NMR (D_2O): δ −112.46.

1-Hydroxy-2-[3-(4-trifluoromethylphenyl)pyridinium-1-yl]-ethylidene-1,1-bisphosphonic Acid Disodium Salt (10). Compound **10** was prepared from 3-bromopyridine (2 mmol) and 4-trifluoromethylphenyl boronic acid (2.4 mmol) following a published procedure[27] (358 mg, 36% overall yield). Anal. ($C_{14}H_{12}F_3NNa_2O_7P_2\cdot1.5H_2O$) C, H, N. ^{1}H NMR (400 MHz, D_2O): δ 4.93 (t, J = 9.6 Hz, 2H, CH_2), 7.70−7.75 (m, 4H, aromatic), 7.90−7.95 (m, 1H, aromatic), 8.63 (d, J = 8.4 Hz, 1H, aromatic), 8.72 (d, J = 6.4 Hz, 1H, aromatic), 9.04 (s, 1H, aromatic). ^{31}P NMR (D_2O): δ 14.02. ^{19}F NMR (D_2O): δ −63.14.

2-[3-(4-Biphenyl)pyridinium-1-yl)]ethylidene-1,1-bisphosphonic Acid (14). Compound **14** was prepared from 3-(4-biphenyl)-pyridine (1.2 mmol), following General Method B as a white powder (210 mg, 45%). Anal. ($C_{19}H_{19}NO_6P_2$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.20 (tt, J = 21, 6.5 Hz, 1H, CH), 4.80−4.85 (m, 2H, CH_2), 7.22−7.43 (m, 3H, aromatic), 7.52−7.76 (m, 7H, aromatic), 8.26−8.42 (m, 1H, aromatic), 8.67 (s, 1H, aromatic), 9.00 (m, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.25.

2-(3-Butylpyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid (15). Compound **15** was prepared from 3-butylpyridine (1.2 mmol) following General Method B as a white powder (240 mg, 72%). Anal. ($C_{11}H_{19}NO_6P_2\cdot0.5H_2O$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 0.72 (t, J = 6.5 Hz, 3H, CH_3), 1.10−1.15 (m, 2H, CH_2), 1.45−1.50 (m, 2H, CH_2), 2.60−2.80 (m, 3H, CH and CH_2), 4.65−4.85 (m, 2H, NCH_2), 7.69 (t, J = 6 Hz, 1H, aromatic), 8.20 (d, J

= 8 Hz, 1H, aromatic), 8.50−8.60 (m, 2H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 14.80.

2-(3-Phenylpyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid (16). Compound **16** was prepared from 3-phenylpyridine (1.2 mmol) following General Method B as a white powder (274 mg, 80%). Anal. ($C_{13}H_{15}NO_6P_2$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.48 (tt, J = 21, 6.5 Hz, 1H, CH), 4.80−5.00 (m, 2H, NCH_2), 7.40−7.60 (m, 5H, aromatic), 7.80−7.90 (m, 1H, aromatic), 8.58 (d, J = 8 Hz, 1H, aromatic), 8.66 (d, J = 6 Hz, 1H, aromatic), 8.97 (s, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 14.72.

1-Hydroxy-2-[(3-bromoropyridinium-1-yl)]ethylidene-1,1-bisphosphonic Acid Monosodium Salt (19). Compound **19** was prepared from 3-bromopyridine (1.2 mmol) following a published procedure[27] (168 mg, 42%). Anal. ($C_7H_9BrNNaO_7P_2\cdot H_2O$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 4.67 (t, J = 8 Hz, 2H, CH_2), 7.59 (t, J = 7 Hz, 1H, aromatic), 8.60 (d, J = 8 Hz, 1H, aromatic), 8.87 (d, J = 6 Hz, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 14.75.

2-(3-Methylpyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid (20). Compound **20** was prepared from 3-methylpyridine (1.2 mmol) following General Method B as a white powder (230 mg, 78%). Anal. ($C_8H_{13}NO_6P_2\cdot 0.75H_2O$) C, H, N. ^{1}H NMR (400 MHz, D_2O): δ 2.36 (s, 3H, CH_3), 2.68 (tt, J = 25.2, 7.2 Hz, 1H, CH), 4.70−4.90 (m, 2H, NCH_2), 7.70−7.75 (m, 1H, aromatic), 8.17 (d, J = 8 Hz, 1H, aromatic), 8.54 (d, J = 6 Hz, 1H, aromatic), 8.59 (s, 1H, aromatic). ^{31}P NMR (162 MHz, D_2O): δ 14.85.

2-(3-Ethylpyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid Monosodium Salt (21). Compound **21** was prepared from 3-ethylpyridine (1.2 mmol) following General Method B as a white powder (238 mg, 75%). Anal. ($C_9H_{14}NNaO_6P_2$) C, H, N. ^{1}H NMR (400 MHz, D_2O): δ 1.11 (t, J = 6.4 Hz, 3H, CH_3), 2.60−2.80 (m, 3H, CH and CH_2), 4.70−4.90 (m, 2H, NCH_2), 7.70−7.75 (m, 1H, aromatic), 8.17 (d, J = 8 Hz, 1H, aromatic), 8.54 (d, J = 6 Hz, 1H, aromatic), 8.58 (s, 1H, aromatic). ^{31}P NMR (162 MHz, D_2O): δ 14.80.

1-Hydroxy-2-[(3-chloropyridinium-1-yl)]ethylidene-1,1-bisphosphonic Acid (28). Compound **28** was prepared from 3-chloropyridine (1 mmol) following a published procedure[27] (83 mg, 25.4%). Anal. ($C_7H_{10}ClNO_7P_2\cdot 0.5H_2O$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 4.77 (t, J = 8 Hz, 2H, CH_2), 7.76 (t, J = 6 Hz, 1H, aromatic), 8.27 (d, J = 8 Hz, 1H, aromatic), 8.68 (d, J = 6 Hz, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.17.

2-(Pyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid (29). Compound **29** was prepared from pyridine (1.2 mmol) following General Method B as a white powder (228 mg, 85%). Anal. ($C_7H_{11}NO_6P_2\cdot 0.25H_2O$) C, H, N. ^{1}H NMR (400 MHz, D_2O): δ 2.71 (tt, J = 25.2, 7.2 Hz, 1H, CH), 4.70−4.90 (m, 2H, NCH_2), 7.87 (t, J = 6.4 Hz, 2H, aromatic), 8.37 (t, J = 8 Hz, 1H, aromatic), 8.75 (d, J = 6 Hz, 2H, aromatic). ^{31}P NMR (162 MHz, D_2O): δ 14.78.

2-[(3-Chloropyridinium-1-yl)]ethylidene-1,1-bisphosphonic Acid (30). Compound **30** was prepared from 3-chloropyridine (1.2 mmol) following General Procedure B as a white powder (205 mg, 68%). Anal. ($C_7H_{10}ClNO_6P_2\cdot 0.5H_2O$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.15 (tt, J = 21, 6.5 Hz, 1H, CH), 4.53−4.78 (m, 2H, CH_2), 7.76 (t, J = 6 Hz, 1H, aromatic), 8.30 (d, J = 8 Hz, 1H, aromatic), 8.74 (d, J = 6 Hz, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.03.

2-(3-Iodopyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid (31). Compound **31** was prepared from 3-iodopyridine (1.2 mmol) following General Method B as a white powder (284 mg, 75%). Anal. ($C_7H_{10}INO_6P_2$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.15 (tt, J = 21, 6.5 Hz, 1H, CH), 4.67−4.78 (m, 2H, CH_2), 7.52 (t, J = 7 Hz, 1H, aromatic), 8.58 (d, J = 8 Hz, 1H, aromatic), 8.77 (d, J = 6 Hz, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.32.

2-(3-Bromopyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid (32). Compound **32** was prepared from 3-bromopyridine (1.2 mmol) following General Method B as a white powder (241 mg, 70%). Anal. ($C_7H_{10}BrNO_6P_2$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.17 (tt, J = 21, 6.5 Hz, 1H, CH), 4.52−4.77 (m, 2H, CH_2), 7.53 (t, J = 7 Hz, 1H, aromatic), 8.57 (d, J = 8 Hz, 1H, aromatic), 8.77 (d, J = 6 Hz, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 14.98.

2-[4-Benzylpyridinium-1-yl]ethylidene-1,1-bisphosphonic Acid (33). Compound **33** was prepared from 4-benzylpyridine (1.2 mmol) following General Procedure B as a white powder (202 mg, 52%). Anal. ($C_{14}H_{16.25}NNa_{0.75}P_2O_6$) C, H, N. ^{1}H NMR (400 MHz, D_2O): δ 2.20 (tt, J = 21, 6.5 Hz, 1H, CH), 4.08 (s, 2H, $PhCH_2$), 4.70−4.80 (m, 2H, CH_2CH), 7.10−7.30 (m, 5H, aromatic), 7.61 (d, J = 6.8 Hz, 2H, aromatic), 8.46 (d, J = 6.8 Hz, 2H, aromatic). ^{31}P NMR (162 MHz, D_2O): δ 14.5.

2-(3-Trifluoromethylpyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid (34). Compound **34** was prepared from 3-trifluoromethylpyridine (1.2 mmol) following General Method B as a white powder (231 mg, 69%). Anal. ($C_8H_{10}F_3NO_6P_2$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.84 (tt, J = 21, 6.5 Hz, 1H, CH), 4.95−5.00 (m, 2H, CH_2), 8.12 (t, J = 6 Hz, 1H, aromatic), 8.77 (d, J = 8 Hz, 1H, aromatic), 9.07 (s, 1H, aromatic), 9.40 (s, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 14.98.

2-[3-(2-Phenylphenyl)pyridinium-1-yl]ethylidene-1,1-bisphosphonic Acid (39). Compound **39** was prepared from 3-(2-phenylphenyl)pyridine (1.2 mmol) following General Method B as a white powder (200 mg, 48%). Anal. ($C_{19}H_{19}NO_6P_2$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.15 (tt, J = 21, 6.5 Hz, 1H, CH), 4.61−4.70 (m, 2H, CH_2), 6.88−7.03 (m, 2H, aromatic), 7.06−7.16 (m, 3H, aromatic), 7.30−7.50 (m, 5H, aromatic), 7.68−7.73 (m, 1H, aromatic), 8.56 (s, 1H, aromatic), 8.78 (m, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.31.

1-Hydroxy-2-[(6-methylquinolinium-1-yl)]ethylidene-1,1-bisphosphonic Acid (40). Compound **40** was prepared from 6-methylquinoline (1 mmol) following a published procedure[27] (66 mg, 19%). Anal. ($C_{12}H_{15}NO_6P_2\cdot H_2O$) C, H, N. ^{1}H NMR (500 MHz): δ 2.44 (s, 3H, CH_3), 5.28 (t, J = 8 Hz, 2H, CH_2), 7.41−7.92 (m, 2H, aromatic), 8.33 (s, 1H, aromatic), 8.70−8.90 (m, 2H, aromatic), 9.08 (d, J = 6.0 Hz, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.15.

2-[4-(2-Phenylphenyl)pyridinium-1-yl]ethylidene-1,1-bisphosphonic Acid (41). Compound **41** was prepared from 4-(2-phenylphenyl)pyridine (1.2 mmol) following General Method B as a white powder (187 mg, 44%). Anal. ($C_{19}H_{19}NO_6P_2\cdot 0.3H_2O$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.10 (tt, J = 21, 6.5 Hz, 1H, CH), 4.61−4.70 (m, 2H, CH_2), 6.88−7.03 (m, 2H, aromatic), 7.04−7.06 (m, 2H, aromatic), 7.06−7.21 (m, 3H, aromatic), 7.37−7.51 (m, 4H, aromatic), 8.52 (d, J = 7 Hz, 2H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.31.

1-Hydroxy-2-(7-methylquinolinium-1-yl)ethylidene-1,1-bisphosphonic Acid (42). Compound **42** was prepared from 7-methylquinoline (1 mmol) following a published procedure[27] (88 mg, 24%). Anal. ($C_{12}H_{17}NO_8P_2$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.45 (s, 3H, CH_3), 5.31 (t, 2H, J = 10 Hz, CH_2), 7.64 (d, J = 8.5 Hz, 1H, aromatic), 7.74 (t, J = 7.5 Hz, 1H, aromatic), 8.00 (t, J = 8.5 Hz, 1H, aromatic), 8.30 (s, 1H, aromatic), 8.84 (d, J = 7.5 Hz, 1H, aromatic), 9.10 (d, J = 6.0 Hz, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.15.

1-Hydroxy-2-(4-phenylpyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid (44). Compound **44** was prepared from 4-phenylpyridine (1 mmol) following a published procedure[27] (158 mg, 41% overall yield). Anal. ($C_{13}H_{18}NO_7P_2\cdot 1.5H_2O$) C, H, N. ^{1}H NMR (400 MHz, D_2O): δ 4.70−4.85 (m, 2H, CH_2CH), 7.10−7.30 (m, 5H, aromatic), 7.71 (d, J = 6.8 Hz, 2H, aromatic), 8.50 (d, J = 6.8 Hz, 2H, aromatic). ^{31}P NMR (162 MHz, D2O): δ 14.7.

1-Hydroxy-2-(4-methoxypyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid (46). Compound **46** was prepared from 4-methoxypyridine (1 mmol) following a published procedure[27] (88 mg, 28%). Anal. ($C_8H_{13}NO_8P_2$) C, H, N. ^{1}H NMR (500 MHz): δ 3.94 (s, 3H, OMe), 5.22 (t, J = 10 Hz, 2H, CH_2), 7.22 (d, J = 6 Hz, 2H, aromatic), 8.44 (d, J = 6 Hz, 2H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.15.

1-Hydroxy-2-[4-(pyridine-4-yl)pyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid Disodium Salt (49). Compound **49** was prepared from 4,4′-bipyridyl (1 mmol) following a published procedure[27] (113 mg, 25%). Anal. ($C_{12}H_{13}N_2Na_2O_7P_2\cdot 2.5H_2O$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 4.93 (t, J = 10 Hz, 2H, CH_2), 8.27 (d, J = 6.5 Hz, 2H, aromatic), 8.36 (d, J = 6.5 Hz, 2H,

aromatic), 8.85 (d, $J = 6.5$ Hz, 2H, aromatic), 8.94 (d, $J = 6.5$ Hz, 2H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 13.68.

2-(Isoquinolinium-2-yl)ethylidene-1,1-bisphosphonic Acid (50). Compound **50** was prepared from isoquinoline (1.2 mmol) following General Method B as a white powder (262 mg, 82%). Anal. ($C_{11}H_{14}NO_6P_2 \cdot 0.5H_2O$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.79 (tt, $J = 21$, 6.5 Hz, 1H, CH), 4.80−5.00 (m, 2H, NCH_2), 7.80−8.40 (m, 6H, aromatic), 9.61 (s, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.01.

2-(4-Trifluoromethylpyridinium-1-yl)ethylidene-1,1-bisphosphonic Acid (51). Compound **51** was prepared from 4-trifluoromethylpyridine (1.2 mmol) following General Method B as a white powder (213 mg, 62%). Anal. ($C_8H_{10}F_3NO_6P_2 \cdot 0.5H_2O$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.17 (tt, $J = 21$, 6.5 Hz, 1H, CH), 4.94−5.01 (m, 2H, CH_2), 8.27 (d, $J = 6.5$ Hz, 2H, aromatic), 9.10 (d, $J = 6.5$ Hz, 2H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 14.75.

2-[3-(Pyrollidin-1-ylsulfonyl)pyridinium-1-yl]ethylidene-1,1-bisphosphonic Acid (52). Compound **52** was prepared from 3-(pyrollidin-1-ylsulfonyl)pyridine[36] (1.2 mmol) following General Method B as a white powder (270 mg, 66%). Anal. ($C_{11}H_{18}N_2O_8P_2S \cdot 0.5H_2O$) C, H, N. ^{1}H NMR (500 MHz): δ 1.60−1.65 (m, 4H, $2CH_2$), 2.73 (tt, $J = 21$, 6.5 Hz, 1H, CH), 3.10−3.20 (m, 4H, $2CH_2$-NS), 4.94−5.01 (m, 2H, NCH_2), 8.10−8.15 (m, 1H, aromatic), 8.80 (d, $J = 6.5$ Hz, 1H, aromatic), 9.06 (d, $J = 6.5$ Hz, 1H, aromatic), 9.43 (s, 1H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 14.19.

2-[4-(4-Phenylphenyl)pyridinium-1-yl)]ethylidene-1,1-bisphosphonic Acid (53). Compound **53** was prepared from 4-(4-phenylphenyl)pyridine (1.2 mmol) following General Method B as a white powder (222 mg, 53%). Anal. ($C_{19}H_{19}NO_6P_2$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.19 (tt, $J = 21$, 6.5 Hz, 1H, CH), 4.51−4.60 (m, 2H, CH_2), 7.31 (t, $J = 6$ Hz, 1H, aromatic), 7.38 (t, $J = 6$ Hz, 2H, aromatic), 7.57 (d, $J = 7.5$ Hz, 2H, aromatic), 7.66 (d, $J = 8.5$ Hz, 2H, aromatic), 7.80 (d, $J = 8.5$ Hz, 2H, aromatic), 8.00 (d, $J = 7$ Hz, 2H, aromatic), 8.68 (d, $J = 7$ Hz, 2H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.31.

2-[4-(3-Phenylphenyl)pyridinium-1-yl)]ethylidene-1,1-bisphosphonic Acid Monosodium Salt (54). Compound **54** was prepared from 4-(3-phenylphenyl)pyridine (1.2 mmol) following General Method B as a white powder (306 mg, 68%). Anal. ($C_{19}H_{18}NO_6P_2Na \cdot 0.5H_2O$) C, H, N. ^{1}H NMR (500 MHz, D_2O): δ 2.19 (tt, $J = 21$, 6.5 Hz, 1H, CH), 4.49−4.61 (m, 2H, CH_2), 7.26 (t, $J = 15$ Hz, 2H, aromatic), 7.32 (t, $J = 15$ Hz, 2H, aromatic), 7.47 (d, $J = 7.5$ Hz, 2H, aromatic), 7.66 (m, 2H, aromatic), 7.80 (s, 1H, aromatic), 7.95 (d, $J = 7$ Hz, 2H, aromatic), 8.68 (d, $J = 7$ Hz, 2H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 15.24.

Tetrakis-pivaloyloxymethyl 2-[3-(3-Phenylphenyl)phenyl]ethylidene-1,1-bisphosphonate (55). 3-Biphenyl boronic acid (2.0 g, 10 mmol), 3-bromotoluene (1.7 g, 10 mmol), K_2CO_3 (3.0 g, 21.7 mmol), and $Pd(PPh_3)_4$ (100 mg) were refluxed in toluene−H_2O (50 mL, 5/1) overnight under N_2. Upon extraction with diethyl ether, the crude product was then refluxed overnight with *N*-bromosuccimide (1.95 g, 11 mmol) and AIBN (100 mg) in anhydrous CCl_4 (30 mL). After washing successively with 5% HCl then 10% $NaHCO_3$, the organic layer was dried and concentrated to give crude 3-(3-phenylphenyl)benzyl bromide as a white powder. This was then reacted following General Method C, followed by General Method D, affording compound **55** as a pale yellow powder (472 mg, 27% overall yield). Quantative ^{1}H NMR indicated 94% purity. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.2−1.30 (m, 36H, CH_3), 2.80 (tt, $J = 24.8$, 6.8 Hz, 1H, $ArCH_2CH$), 3.15 (td, $J = 17.2$, 6.8 Hz, 2H, $ArCH_2$), 5.62−5.69 (m, 8H, $POCH_2$), 7.23−7.85 (m, 13H, aromatic). ^{31}P NMR (162 MHz, CDCl3): δ 20.52.

1-Hydroxy-3-[3-(3-phenylphenyl)phenyl]propylidene-1,1-bisphosphonic Acid Trisodium Salt (56). Compound **56** was prepared from methyl 3-(3-phenylphenyl)phenylpropionate (1 mmol) following General Method E as a white powder (324 mg, 61%). Anal. ($C_{21}H_{19}O_7P_2Na_3 \cdot H_2O$) C, H. ^{1}H NMR (400 Hz, D_2O): δ 2.01−2.12 (m, 2H, CH_2), 2.80−2.85 (m, 2H, $ArCH_2$), 7.23−7.57 (m, 12H, aromatic), 7.77 (s, 1H, aromatic). ^{31}P NMR (162 Hz, D_2O): δ 19.41.

1-Hydroxy-2-[3-(2-phenylphenyl)phenyl]ethylidene-1,1-bisphosphonic Acid Disodium Salt (57). Compound **57** was prepared from methyl 3-(2-phenylphenyl)phenylacetate (1 mmol) following General Method E as a white powder (213 mg, 43%). Anal. ($C_{20}H_{18}O_7P_2Na_2 \cdot H_2O$) C, H. ^{1}H NMR (400 MHz, D_2O): δ 3.10 (t, $J = 12$ Hz, 2H, CH_2), 6.73−7.40 (m, 13H, aromatic). ^{31}P NMR (162 Hz, D_2O): δ 19.23.

1-Hydroxy-2-[3-(3-phenylphenyl)phenyl]ethylidene-1,1-bisphosphonic Acid Monosodium Salt (58). Compound **58** was prepared from methyl 3-(3-phenylphenyl)phenylacetate (1 mmol) following General Method E as a white powder (265 mg, 56%). Anal. ($C_{20}H_{19}NaO_7P_2 \cdot H_2O$) C, H. ^{1}H NMR (400 MHz, D_2O): δ 3.23 (t, $J = 12$ Hz, 2H, CH_2), 7.20−7.80 (m, 13H, aromatic). ^{31}P NMR (162 Hz, D_2O): δ 19.20.

1-Hydroxy-3-[3-(2-phenylphenyl)phenyl]propylidene-1,1-bisphosphonic Acid Trisodium Salt (59). Compound **59** was prepared from methyl 3-(2-phenylphenyl)phenylpropionate (1 mmol) following General Method E as a white powder (271 mg, 51%). Anal. ($C_{21}H_{19}O_7P_2Na_3 \cdot H_2O$) C, H. ^{1}H NMR (500 MHz, D_2O): δ 1.98−2.10 (m, 2H, CH_2), 2.69−2.72 (m, 2H, $ArCH_2$), 6.70 (d, $J = 6.5$ Hz, 1H, aromatic), 6.97 (t, $J = 7.5$ Hz, 1H, aromatic), 7.04−7.17 (m, 7H, aromatic), 7.32−7.45 (m, 4H, aromatic). ^{31}P NMR (202 MHz, D_2O): δ 19.38.

1-Hydroxy-3-[3-(4-phenylphenyl)phenyl]propylidene-1,1-bisphosphonic Acid Disodium Salt (60). Compound **60** was prepared from methyl 3-(4-phenylphenyl)phenylpropionate (1 mmol) following General Method E as a white powder (270 mg, 55%). Anal. ($C_{21}H_{20}O_7P_2Na_2$) C, H. ^{1}H NMR (400 MHz, D_2O): δ 2.05−2.10 (m, 2H, CH_2), 2.80−2.85 (m, 2H, $ArCH_2$), 7.22−7.32 (m, 6H, aromatic), 7.35−7.64 (m, 7H, aromatic). ^{31}P NMR (162 MHz, D_2O): δ 19.08.

1-Hydroxy-[3-(3-phenylphenyl)phenyl]methylene-1,1-bisphosphonic Acid Monosodium Salt (61). Compound **61** was prepared from methyl 3-(3-phenylphenyl)benzoate (1 mmol) following General Method E as a white powder (174 mg, 40%). Anal. ($C_{19}H_{17}O_7P_2Na \cdot 0.25H_2O$) C, H. ^{1}H NMR (400 MHz, D_2O): δ 7.17−7.25 (m, 2H, aromatic), 7.33 (t, $J_{H-H} = 7.2$ Hz, 2H, aromatic), 7.40 (t, $J = 8$ Hz, 1H, aromatic), 7.47 (d, $J = 7.8$ Hz, 1H, aromatic), 7.56−7.58 (m, 4H, aromatic), 7.65 (d, $J = 8$ Hz, 1H, aromatic), 7.83 (s, 2H, aromatic). ^{31}P NMR (162 MHz, D_2O): δ 17.59.

1-Hydroxy-2-[4-(3-phenylphenyl)phenyl]ethylidene-1,1-bisphosphonic Acid Monosodium Salt (62). Compound **62** was prepared from methyl 4-(3-phenylphenyl)phenylacetate (1 mmol) following General Method E as a white powder (201 mg, 44%). Anal. ($C_{20}H_{19}O_7P_2Na$) C, H. ^{1}H NMR (400 MHz, D_2O): δ 3.21 (t, $J = 12.4$ Hz, CH_2), 7.27 (t, $J = 7.2$ Hz, 1H, aromatic), 7.34−7.60 (m, 11H, aromatic), 7.80 (s, 1H, aromatic). ^{31}P NMR (162 MHz, D_2O): δ 19.11.

1-Hydroxy-2-[4-(2-phenylphenyl)phenyl]ethylidene-1,1-bisphosphonic Acid Disodium Salt (63). Compound **63** was prepared from methyl 4-(2-phenylphenyl)phenylacetate (1 mmol) following General Method E as a white powder (232 mg, 45%). Anal. ($C_{20}H_{18}O_7P_2Na_2 \cdot 2H_2O$) C, H. ^{1}H NMR (400 MHz, D_2O): δ 3.06 (t, $J = 12.4$ Hz, CH_2), 6.94 (d, $J = 8$ Hz, 2H, aromatic), 7.01−7.07 (m, 2H, aromatic), 7.11−7.17 (m, 4H, aromatic), 7.30−7.39 (m, 5H, aromatic). ^{31}P NMR (162 MHz, D_2O): δ 18.97.

Tetrakis-pivaloyloxymethyl 2-(3,4-dibromophenyl)ethylidene-1,1-bisphosphonate (64). Compound **64** was prepared from 3,4-dibromobenzyl bromide (1 mmol) following General Method C, followed by General Method D, as a pale yellow powder (159 mg, 18% overall yield). Anal. ($C_{32}H_{50}Br_2O_{14}P_2$) C, H. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20−1.30 (m, 36H, CH_3), 2.79 (tt, $J = 24.8$, 6.8 Hz, 1H, $ArCH_2CH$), 3.08 (td, $J = 17.2$, 6.8 Hz, 2H, $ArCH_2$), 5.62−5.69 (m, 8H, $POCH_2$), 7.10 (d, $J = 8.4$ Hz, 1H, aromatic), 7.43 (d, $J = 8.4$ Hz, 1H, aromatic), 7.56 (s, 1H, aromatic). ^{31}P NMR (162 MHz, $CDCl_3$): δ 20.35.

Tetrakis-pivaloyloxymethyl 2-(3,4-dichlorophenyl)ethylidene-1,1-bisphosphonate (65). Compound **65** was prepared from 3,4-dichlorobenzyl bromide (1 mmol) following General Method C, followed by General Method D, as a pale yellow powder (153 mg, 21%). Anal. ($C_{32}H_{50}Cl_2O_{14}P_2$) C, H. ^{1}H NMR (400 MHz, $CDCl_3$):

δ 1.20−1.30 (m, 36H, CH_3), 2.80 (tt, J = 24.8, 6.8 Hz, 1H, $ArCH_2CH$), 3.15 (td, J = 17.2, 6.8 Hz, 2H, $ArCH_2$), 5.62−5.69 (m, 8H, $POCH_2$), 7.10 (d, J = 8.4 Hz, 1H, aromatic), 7.33−7.35 (m, 2H, aromatic). ^{31}P NMR (162 MHz, $CDCl_3$): δ 20.41.

Tetrakis-isopropoxycarbonyloxymethyl 2-(3,4-dichlorophenyl)ethylidene-1,1-bisphosphonate (66). Compound **66** was prepared from 3,4-dichlorobenzyl bromide (1 mmol) following General Method C, followed by General Method D, as a pale yellow powder (136 mg, 17%). Anal. ($C_{28}H_{42}Cl_2O_{18}P_2$) C, H. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.32 (d, J = 6.4 Hz, 24H, CH_3), 2.75 (tt, J = 24.4, 6.4 Hz, 1H, $ArCH_2CH$), 3.47 (td, J = 17.2, 6.8 Hz, 2H, $ArCH_2$), 4.89−4.95 (m, 4H, $CHMe_2$), 5.60−5.70 (m, 8H, OCH_2O), 7.13 (d, J = 6.8 Hz, 1H, aromatic), 7.35 (d, J = 6.8 Hz, 1H, aromatic), 7.38 (s, 1H, aromatic). ^{31}P NMR (162 MHz, $CDCl_3$): δ 21.85.

Tetrakis-isopropoxycarbonyloxymethyl 2-(3,4-difluorophenyl)ethylidene-1,1-bisphosphonate (67). Compound **67** was prepared from 3,4-difluorobenzyl bromide (1 mmol) following General Method C, followed by General Method D, as a pale yellow powder (107 mg, 14%). Anal. ($C_{28}H_{42}F_2O_{18}P_2$) C, H. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33 (d, J = 6.4 Hz, 24H, CH_3), 2.88 (tt, J = 24.4, J = 6.4 Hz, 1H, $ArCH_2CH$), 3.40 (td, J = 17.2, 6.8 Hz, 2H, $ArCH_2$), 4.89−4.95 (m, 4H, $CHMe_2$), 5.60−5.72 (m, 8H, OCH_2O), 6.99−7.13 (m, 3H, aromatic). ^{31}P NMR (162 MHz, $CDCl_3$): δ 21.94. ^{19}F NMR (376 MHz, $CDCl_3$): −140.79∼ −140.67 (m, 1F), −138.08∼ −137.97 (m, 1F).

Tetrakis-isopropoxycarbonyloxymethyl 2-(3-cyanophenyl)-ethylidene-1,1-bisphosphonate (68). Compound **68** was prepared from 3-cyanobenzyl bromide (1 mmol) following General Method C, followed by General Method D, as a pale yellow powder (91 mg, 12%). Anal. ($C_{29}H_{43}NO_{18}P_2$) C, H, N. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (d, J = 6.4 Hz, 24H, CH_3), 2.79 (tt, J = 20.8, J = 6.8 Hz, 1H, $ArCH_2CH$), 3.40 (td, J = 16.8, 6.8 Hz, 2H, $ArCH_2$), 4.89−4.96 (m, 4H, $CHMe_2$), 5.60−5.70 (m, 8H, OCH_2O), 7.36 (t, J = 8 Hz, 1H, aromatic), 7.50−7.54 (m, 2H, aromatic), 7.57 (s, 1H, aromatic). ^{31}P NMR (162 MHz, $CDCl_3$): δ 21.70.

2-(3,4-Dibromophenyl)ethylidene-1,1-bisphosphonic Acid (69). Compound **69** was prepared from 3,4-dibromobenzyl bromide (1 mmol) following General Method C, followed by hydrolysis with bromotrimethylsilane, as a white powder (275 mg, 65% overall yield). Anal. ($C_8H_{10}Br_2O_6P_2$) C, H. ^{1}H NMR (400 MHz, D_2O): δ 2.78 (tt, J = 20.8, 6.8 Hz, 1H, $ArCH_2CH$), 3.12 (td, J = 17.2, 6.8 Hz, 2H, $ArCH_2$), 7.10 (d, J = 8.4 Hz, 1H, aromatic), 7.43 (d, J = 8.4 Hz, 1H, aromatic), 7.56 (s, 1H, aromatic). ^{31}P NMR (162 MHz, $CDCl_3$): δ 19.87.

2-(3-Cyanophenyl)ethylidene-1,1-bisphosphonic Acid Trisodium Salt (70). Compound **70** was prepared from 3-cyanobenzyl bromide (1 mmol) following General Method C, followed by hydrolysis with bromotrimethylsilane, as a white powder (29%). Anal. ($C_9H_8NNa_3O_6P_2 \cdot H_2O$) C, H, N. ^{1}H NMR (400 MHz, D_2O): δ 2.79 (tt, J = 20.8, 6.8 Hz, 1H, $ArCH_2CH$), 3.40 (td, J = 16.8, 6.8 Hz, 2H, $ArCH_2$), 4.89−4.96 (m, 4H, $CHMe_2$), 5.60−5.70 (m, 8H, OCH_2O), 7.39 (t, J = 8 Hz, 1H, aromatic), 7.45−7.52 (m, 2H, aromatic), 7.59 (s, 1H, aromatic). ^{31}P NMR (162 MHz, D_2O): δ 19.81.

Cell Growth Inhibition Assays. The human tumor cell lines MCF-7 (breast adenocarcinoma), NCI−H460 (lung large cell), and SF-268 (central nervous system glioblastoma) were obtained from the National Cancer Institute. All lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 2 mM L-glutamine at 37 °C in a 5% CO_2 atmosphere with 100% humidity. A broth microdilution method was used to determine IC_{50} values for growth inhibition by each bisphosphonate. Cells were inoculated at a density of 5000 cells/well into 96-well flat bottom culture plates containing 10 μL of the test compound, previously half-log serial diluted (from 0.316 mM to 0.1 pM), for a final volume of 100 μL. NBPs were typically initially dissolved in H_2O (0.01 M), while NNBPs were typically dissolved in DMSO (0.01 M). Plates were then incubated for 4 days at 37 °C in a 5% CO_2 atmosphere at 100% humidity after which an MTT ((3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation assay (ATCC, Manassas, VA)[37] was used to obtain dose−response curves. The DMSO carrier had no effect on cell proliferation. GraphPad PRISM version 4.0 software for windows (GraphPad Software, Inc., San Diego, CA, www.graphpad.com) was used to fit the data to a rectangular hyperbolic function:

$$I = \frac{I_{max} C}{IC_{50} + C}$$

where I is the percent inhibition, I_{max} = 100% inhibition, C is the concentration of the inhibitor, and IC_{50} is the concentration for 50% growth inhibition. Typical dose−response curves for 2 NBPs, 2 terphenyl, and 2 halophenyl NBPs are shown in the Supporting Information (Figure S1).

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Supporting Information Available: Microchemical analysis results for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Reszka, A. A.; Rodan, G. A. Nitrogen-containing bisphosphonate mechanism of action. *Mini-Rev. Med. Chem.* **2004**, *4*, 711−719.
(2) Green, J. R. Bisphosphonates: preclinical review. *Oncologist* **2004**, *9* (Suppl. 4), 3−13.
(3) Wilhelm, M.; Kunzmann, V.; Eckstein, S.; Reimer, P.; Weissinger, F.; Ruediger, T.; Tony, H. P. $\gamma\delta$ T cells for immune therapy of patients with lymphoid malignancies. *Blood* **2003**, *102*, 200−206.
(4) Sanders, J. M.; Ghosh, S.; Chan, J. M.; Meints, G.; Wang, H.; Raker, A. M.; Song, Y.; Colantino, A.; Burzynska, A.; Kafarski, P.; Morita, C. T.; Oldfield, E. Quantitative structure−activity relationships for $\gamma\delta$ T cell activation by bisphosphonates. *J. Med. Chem.* **2004**, *47*, 375−384.
(5) Riebeling, C.; Forsea, A. M.; Raisova, M.; Orfanos, C. E.; Geilen, C. C. The bisphosphonate pamidronate induces apoptosis in human melanoma cells in vitro. *Br. J. Cancer* **2002**, *87* (3), 366−371.
(6) Evdokiou, A.; Labrinidis, A.; Bouralexis, S.; Hay, S.; Findlay, D. M. Induction of cell death of human osteogenic sarcoma cells by zoledronic acid resembles anoikis. *Bone* **2003**, *33* (2), 216−228.
(7) Iguchi, T.; Miyakawa, Y.; Yamamoto, K.; Kizaki, M.; Ikeda, Y. Nitrogen-containing bisphosphonates induce S-phase cell cycle arrest and apoptosis of myeloma cells by activating MAPK pathway and inhibiting mevalonate pathway. *Cell. Signalling* **2003**, *15*, 719−727.
(8) Forsea, A. M.; Muller, C.; Riebeling, C.; Orfanos, C. E.; Geilen, C. C. Nitrogen-containing bisphosphonates inhibit cell cycle progression in human melanoma cells. *Br. J. Cancer* **2004**, *91* (4), 803−810.
(9) Kitagawa, Y.; Hiraga, T.; Yura, Y.; Yoneda, T. Suppression by incadronate of invasion and growth of A-375 human melanoma in mandible in nude mice. *Oncol. Rep.* **2005**, *13* (2), 211−216.
(10) Chuah, C.; Barnes, D. J.; Kwok, M.; Corbin, A.; Deininger, M. W.; Druker, B. J.; Melo, J. V. Zoledronate inhibits proliferation and induces apoptosis of imatinib-resistant chronic myeloid leukaemia cells. *Leukemia* **2005**, *19* (11), 1896−1904.
(11) Martin, M. B.; Grimley, J. S.; Lewis, J. C.; Heath, H. T., III; Bailey, B. N.; Kendrick, H.; Yardley, V.; Caldera, A.; Lira, R.; Urbina, J. A.; Moreno, S. N.; Docampo, R.; Croft, S. L.; Oldfield, E. Bisphosphonates inhibit the growth of *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, *Toxoplasma gondii*, and *Plasmodium falciparum*: a potential route to chemotherapy. *J. Med. Chem.* **2001**, *44* (6), 909−916.
(12) Yardley, V.; Khan, A. A.; Martin, M. B.; Slifer, T. R.; Araujo, F. G.; Moreno, S. N.; Docampo, R.; Croft, S. L.; Oldfield, E. In vivo activities of farnesyl pyrophosphate synthase inhibitors against *Leishmania donovani* and *Toxoplasma gondii*. *Antimicrob. Agents Chemother.* **2002**, *46* (3), 929−931.
(13) Martin, M. B.; Sanders, J. M.; Kendrick, H.; de Luca-Fradley, K.; Lewis, J. C.; Grimley, J. S.; Van Brussel, E. M.; Olsen, J. R.; Meints, G. A.; Burzynska, A.; Kafarski, P.; Croft, S. L.; Oldfield, E. Activity of bisphosphonates against *Trypanosoma brucei rhodesiense*. *J. Med. Chem.* **2002**, *45* (14), 2904−2914.

(14) Rodriguez, N.; Bailey, B. N.; Martin, M. B.; Oldfield, E.; Urbina, J. A.; Docampo, R. Radical cure of experimental cutaneous leishmaniasis by the bisphosphonate pamidronate. *J. Infect. Dis.* **2002**, *186* (1), 138−140.

(15) Montalvetti, A.; Fernandez, A.; Sanders, J. M.; Ghosh, S.; Van Brussel, E.; Oldfield, E.; Docampo, R. Farnesyl pyrophosphate synthase is an essential enzyme in *Trypanosoma brucei*: in vitro RNA interference and in vivo inhibition studies. *J. Biol. Chem.* **2003**, *278* (19), 17075−17083.

(16) Ghosh, S.; Chan, J. M. W.; Lea, C. R.; Meints, G. A.; Lewis, J. C.; Tovian, Z. S.; Flessner, R. M.; Loftus, T. C.; Bruchhaus, I.; Kendrick, H.; Croft, S. L.; Kemp, R. G.; Kobayashi, S.; Nozaki, T.; Oldfield, E. Effects of bisphosphonates on the growth of *Entamoeba histolytica* and *Plasmodium* species in vitro and in vivo. *J. Med. Chem.* **2004**, *47* (1), 175−187.

(17) Garzoni, L. R.; Caldera, A.; Meirelles, M. D. L.; de Castro, S. L.; Docampo, R.; Meints, G. A.; Oldfield, E.; Urbina, J. A. Selective in vitro effects of the farnesyl pyrophosphate synthase inhibitor risedronate on *Trypanosoma cruzi*. *Int. J. Antimicrob. Agents* **2004**, *23* (3), 273−285.

(18) Garzoni, L. R.; Waghabi, M. C.; Baptista, M. A.; de Castro, S. L.; Meirelles, M. D. L.; Britto, C. C.; Docampo, R.; Oldfield, E.; Urbina, J. A. Antiparasitic activity of risedronate in a murine model of acute Chagas' disease. *Int. J. Antimicrob. Agents* **2004**, *23* (3), 286−290.

(19) Ling, Y.; Sahota, G.; Odeh, S.; Chan, J. M.; Araujo, F. G.; Moreno, S. N.; Oldfield, E. Bisphosphonate inhibitors of *Toxoplasma gondi* growth: in vitro, QSAR, and in vivo investigations. *J. Med. Chem.* **2005**, *48* (9), 3130−3140.

(20) Moenkkoenen, H.; Auriola, S.; Lehenkari, P.; Kellinsalmi, M.; Hassinen, I. E.; Vepsaelaeinen, J.; Moenkkoenen, J. A new endogenous ATP analogue (ApppI) inhibits the mitochondrial adenine nucleotide translocase (ANT) and is responsible for the apoptosis induced by nitrogen-containing bisphosphonates. *Br. J. Pharmacol.* **2006**, *147* (4), 437−445.

(21) Rogers, M. J.; Brown, R. J.; Hodkin, V.; Blackburn, G. M.; Russell, R. G.; Watts, D. J. Bisphosphonates are incorporated into adenine nucleotides by human aminoacyl-tRNA synthetase enzymes. *Biochem. Biophys. Res. Commun.* **1996**, *224* (3), 863−869.

(22) Benford, H. L.; Frith, J. C.; Auriola, S.; Monkkonen, J.; Rogers, M. J. Farnesol and geranylgeraniol prevent activation of caspases by aminobisphosphonates: biochemical evidence for two distinct pharmacological classes of bisphosphonate drugs. *Mol. Pharmacol.* **1999**, *56* (1), 131−140.

(23) Hamma-Kourbali, Y.; Di Benedetto, M.; Ledoux, D.; Oudar, O.; Leroux, Y.; Lecouvey, M.; Kraemer, M. A novel non-nitrogen containing bisphosphonate inhibits both in vitro and in vivo angiogenesis. *Biochem. Biophys. Res. Commun.* **2003**, *310* (3), 816−823.

(24) Lecouvey, M.; Leroux, Y.; Kraemer, M.; Crepin, M.; Manouni, D. E.; Louriki, M. Hydroxymethylene bisphosphonic acid salts and partial esters with improved bioavailability, their preparation methods and uses against cancerous tumors and other maladies. *PCT Int. Appl.* **2003**, 66. CODEN: PIXXD2 WO 2003008425 A1 20030130.

(25) Guenin, E.; Ledoux, D.; Oudar, O.; Lecouvey, M.; Kraemer, M. Structure−activity relationships of a new class of aromatic bisphosphonates that inhibit tumor cell proliferation in vitro. *Anticancer Res.* **2005**, *25* (2A), 1139−1145.

(26) Rondeau, J.-M.; Bitsch, F.; Bourgier, E.; Geiser, M.; Hemmig, R.; Kroemer, M.; Lehmann, S.; Ramage, P.; Rieffel, S.; Strauss, A.; Green, J. R.; Jahnke, W. Structural basis for the exceptional in vivo efficacy of bisphosphonate drugs. *ChemMedChem* **2006**, *1*, 267−273.

(27) Sanders, J. M.; Song, Y.; Chan, J. M. W.; Zhang, Y.; Jennings, S.; Kosztowski, T.; Odeh, S.; Flessner, R.; Schwerdtfeger, C.; Kotsikorou, E.; Meints, G. A.; Gómez, A. O.; González-Pacanowska, D.; Raker, A. M.; Wang, H.; Morita, C. T.; Oldfield, E. Pyridium-1-yl bisphosphonates are potent inhibitors of farnesyl diphosphate synthase and bone resorption. *J. Med. Chem.* **2005**, *48*, 2957−2963.

(28) Af'erev, I. S.; Mikhalin, N. V. Reactions of vinylidenediphosphonic acid with nucleophiles. 5. Addition of heterocyclic amines and trimethylamine to vinylidenediphosphonic acid. *Izv. Akad. Nauk, Ser. Khim.* **1995**, *8*, 1590−1592.

(29) Vepsalainen, J. J. Bisphosphonate prodrugs: a new synthetic strategy to tetraacyloxymethyl esters of methylenebisphosphonates. *Tetrahedron Lett.* **1999**, *40*, 8491−8493.

(30) Lecouvey, M.; Mallard, I.; Bailly, T.; Burgada, R.; Leroux, Y. A mild and efficient one-pot synthesis of 1-hydroxymethylene-1,1-bisphosphonic acids. Preparation of new tripod ligands. *Tetrahedron Lett.* **2001**, *42*, 8475−8478.

(31) Sanders, J. M.; Gomez, A. O.; Mao, J.; Meints, G. A.; Van Brussel, E. M.; Burzynska, A.; Kafarski, P.; Gonzalez-Pacanowska, D.; Oldfield, E. 3-D QSAR investigations of the inhibition of *Leishmania* major farnesyl pyrophosphate synthase by bisphosphonates. *J. Med. Chem.* **2003**, *46*, 5171−5183.

(32) Alvarez, E.; Westmore, M.; Galvin, R. J. S.; Clapp, C. L.; Considine, E. L.; Smith, S. J.; Keyes, K.; Iversen, P. W.; Delafuente, D. M.; Sulaimon, S.; Zambrano, C.; Ma, L.; Sato, M.; Martin, T. J.; Teicher, B. A.; Galbreath, E. J. Properties of bisphosphonates in the 13762 rat mammary carcinoma model of tumor-induced bone resorption. *Clin. Cancer Res.* **2003**, *9* (15), 5705−5713.

(33) Saari, W. S.; Anderson, P. S. Acyloxymethyl esters of bisphosphonic acids as bone resorption inhibitors. U.S. Patent 5,227,506, July 13, 1993.

(34) Dunford, J. E.; Thompson, K.; Coxon, F. P.; Luckman, S. P.; Hahn, F. M.; Poulter, C. D.; Ebetino, F. H.; Rogers, M. J. Structure−activity relationships for inhibition of farnesyl diphosphate synthase in vitro and inhibition of bone resorption in vivo by nitrogen-containing bisphosphonates. *J. Pharmacol. Exp. Ther.* **2001**, *296* (2), 235−242.

(35) Devlin, B. R. J. Biologically active compositions. Br. GB 1508772, 1978.

(36) Breant, P.; Marsais, F.; Queguiner, G. Regioselective lithiation of 3-pyridylsulfonic acid derivatives: a convenient route to various new 4-substituted 3-pyridylsulfonamides. *Synthesis* **1983**, (10), 822−824.

(37) MTT cell proliferation assay. ATCC: Manassas, VA, 2001; http://www.atcc.org/documents/pdf/30-1010k.pdf.

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