Articles

Carbonyl-Containing Bisphosphonate Esters as Novel Antiinflammatory and Antiarthritic Agents

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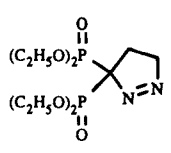
Received June 6, 1994®

A study of the decomposition of the pyrazoline bisphosphonate ester **2** identified **3** as the sole bisphosphonate component. Evaluation in a delayed-type hypersensitivity granuloma model of chronic inflammation in mice (DTH-GRA) showed **3** to be a potent inhibitor of granuloma formation (sc, 10 mg/kg, 45%), but in a murine model of antigen-induced arthritis (AIA), no significant inhibition was observed. As a result, new ketonic bisphosphonate tetraethyl esters were synthesized from vinylidenebisphosphonic acid tetraethyl ester **4** and activated carbonyl compounds in 13–84% yield. **6** significantly inhibited the pathology of both the DTH-GRA (sc, 25 mg/kg, 45%) and AIA models (sc, 25 mg/kg, 55%). Other compounds in the series were not as potent. Our results show that bisphosphonate ester **6** can inhibit the chronic inflammatory response associated with cutaneous granuloma formation and erosive arthritis.

Methylenebisphosphonic acids are synthetic pyrophosphate mimics, which are potent inhibitors of bone resorption,[1] and as such have found utility in the treatment of Paget's disease,[2,3] metastatic bone disease,[4] and recently osteoporosis.[5,6] These conditions are characterized by a progressive reduction in bone mineral density and subsequent loss of bone strength. Although rheumatoid arthritis also involves bone erosion, few clinical studies have been performed to evaluate the effectiveness of this class of compounds as antiarthritic agents.[7,8] Recently there has been renewed interest in the use of bisphosphonic acids for the treatment of this disease. We have shown that bisphosphonic esters can effectively reduce the inflammation associated with cutaneous granuloma formation and erosive arthritis.[9,10] In this report we will describe additional bisphosphonic acids esters which are potent antiinflammatory and antiarthritic agents.

Chemistry

In our previous report, we described the synthesis of pyrazoline bisphosphonic esters.[9] Pyrazoline-containing compounds such as **1**, however, proved unstable, but this problem was resolved by isomerizing the double bond as in **2**. Pyrazolines of this class were sufficiently



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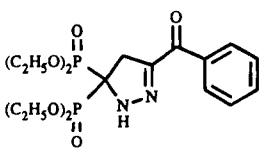
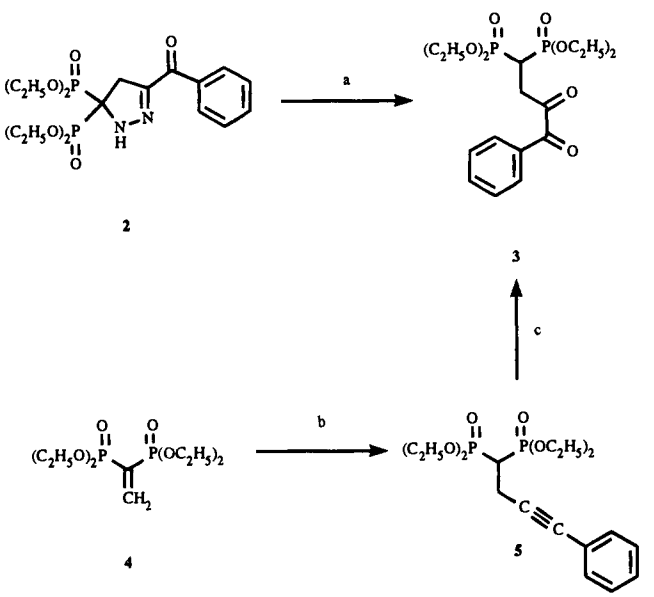


2

stable for characterization and biological testing, and many were stable for more than 1 year. However, a sample of **2**, which was left on the bench for several months, changed from a white solid to a yellow oil. Analysis of the product showed a complex mixture of products, but only a single bisphosphonic ester species appeared to be present. A close examination of the stability of **2** showed that heat and light had no effect but that water and oxygen dramatically increased the rate of decomposition. In the absence of either, there was no observed decomposition. On the basis of spectral evidence, we identified the new bisphosphonate as the diketone **3** (Scheme 1). An authentic sample was prepared by treating the vinylidenebisphosphonate ester **4** with the lithium anion of phenylacetylene to give **5**. This was then treated with potassium permanganate to give **3**, which was identical in all respects to the material previously isolated.

Scheme 1[a]



[a] (a) O_2, H_2O, 1 wk; (b) phenylacetylene, *n*-BuLi, −78–22 °C; (c) $KMnO_4$, $Bn(Et)_3N^+Cl^-$, CH_2Cl_2, HOAc/H_2O.

† Medicinal Chemistry Research.
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® Abstract published in *Advance ACS Abstracts*, November 15, 1994.

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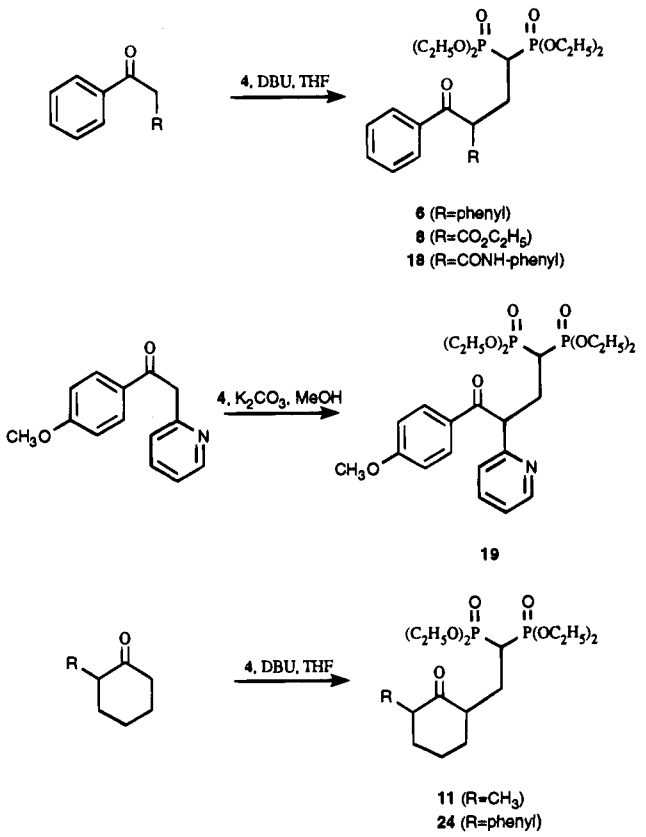
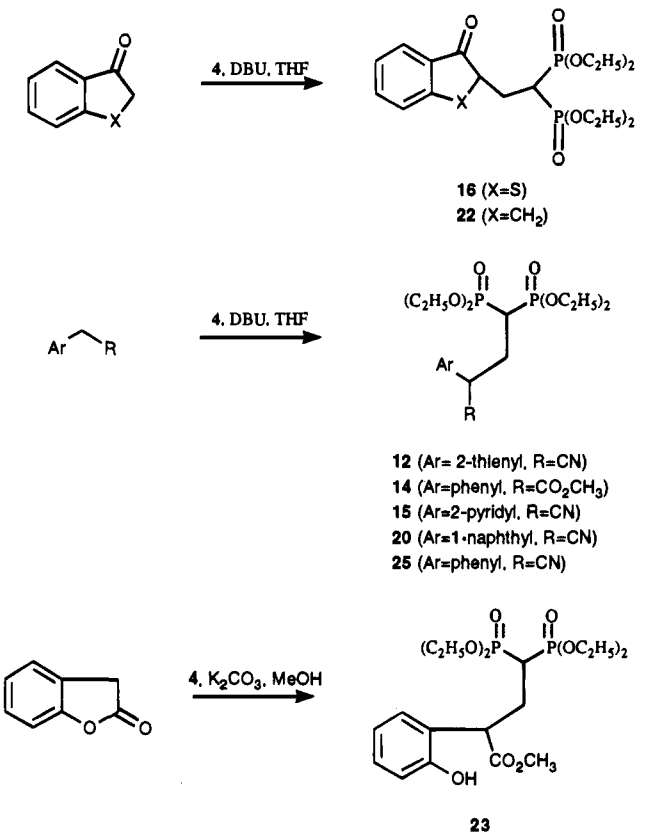
Scheme 2



Scheme 3

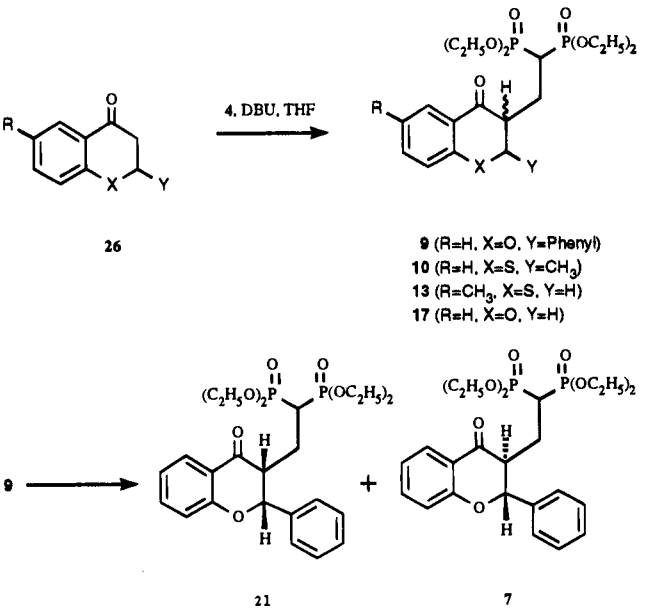


To synthesize additional ketonic bisphosphonic esters, we initially sought a general method for performing a Michael-like addition of activated carbonyl species to the vinylidene **4**.[11] Using the reaction of deoxybenzoin with **4** as a model, we examined several different bases and solvents. We first examined reaction conditions which utilized protic solvents and stoichiometric amounts of sodium alkoxide as base. Although the expected product **6** was isolated, we felt that the use of an organic base would permit a greater variety of solvents and substrates to be utilized and would improve the overall yield and quality of product. Triethylamine proved to be too weak a base to be effective, but DBU worked very well in protic and aprotic solvents. THF was later identified as the solvent of choice.

Although, in principle, only a catalytic amount of base was needed, we initially employed stoichiometric quantities of base. While these reactions were often complete within 30 min, they produced more side reactions than the corresponding catalytic ones. Via DBU, the reaction with deoxybenzoin and **4** proceeded very well with 10% base. However, the reaction rate slowed considerably and required heating to 50 °C in order to achieve an acceptable rate. These conditions were then applied to other carbonyl compounds to make **7**–**18**, **20**–**22**, and **24**–**25** (Schemes 2–4). For the synthesis of **19**, it was decided that an inorganic base would simplify the workup, and in this case K_2CO_3 in methanol was employed (Scheme 2). Under the same conditions, benzofuranone and **4** yielded the ester **23** in good yield (Scheme 3).

When flavanone **26a** (X = O, Y = phenyl) and **4** were reacted, the principal product was a 1:1 mixture of isomers, **9**, which could not be separated by chromatography (Scheme 4). Fortunately, upon standing, the

Scheme 4



cis isomer **21** precipitated and could be isolated by filtration, while the filtrate contained nearly pure trans isomer **7**. Under ambient conditions, **7** slowly equilibrated with **21** to yield a 1:1 mixture of isomers, **9**. The reaction of **4** and benzothiopyranone **26b** (X = S, Y = methyl) also produced a 1:1 mixture of isomers, **10**, but they were inseparable.

The reaction of 2-methylcyclohexanone with **4** was very sluggish, and attempts to use a catalytic amount of base failed (Scheme 2). Stoichiometric amounts of DBU were needed to drive the reaction to completion in 48–72 h, even at 50–60 °C in THF. The proton and carbon NMR spectra of **11** showed it to be a complex

mixture of isomers. In contrast, the reaction of 2-phenylcyclohexanone and **4** was successful using a catalytic amount of DBU. In this example, the carbon NMR of **24** shows the presence of only a small amount of a second epimer. Although it is impossible to draw conclusions from the proton spectrum, we suspect that the major product is the cis, diequatorial species.

Pharmacology

An initial evaluation of antiinflammatory activity was performed in the delayed-type hypersensitivity granuloma (DTH-GRA), a model of chronic, cutaneous inflammation. As we have previously shown, this model is unaffected by traditional nonsteroidal antiinflammatory drugs, such as indomethacin or ibuprofen,[12,13] but can be suppressed by bisphosphonic acids and esters.[9,10] In this assay, mice previously sensitized to methylated bovine serum albumin (mBSA) were surgically implanted with hydroxyapatite disks (two per mouse) soaked in mBSA, in order to generate delayed-type hypersensitivity granulomas. After 9 days, the granulomas were excised and the wet and dry weights were measured and compared to vehicle-dosed controls.

Selected compounds, which proved to be antiinflammatory in the DTH-GRA model, were tested in a murine model of antigen-induced arthritis (AIA). The articular pathology of AIA involves an initial intense inflammatory synovitis followed by chronic inflammation and severe erosion of articular cartilage and subchondral bone, resembling the pathology seen in human rheumatoid arthritis.[14] Both the synovitis and joint destruction are unaffected by nonsteroidal antiinflammatory drugs but can be suppressed by corticosteroids such as dexamethasone or cytotoxic agents such as azathioprine and methotrexate. However, the so-called "disease-modifying antirheumatic drugs", chloroquine, D-penicillamine, and sodium aurothiomalate, are without effect. Thus to date, this arthritis model has only been suppressed by potent immunosuppressive drugs and bisphosphonic acids and esters.[9,15,16]

Results and Discussion

The compounds described above were administered subcutaneously in the scruff of the neck to mice implanted with antigen-soaked hydroxyapatite disks and the granulomas examined after 9 days (Table 1). Clodronic acid and **2** reproducibly inhibited granuloma formation, as evidenced by decreased wet and dry tissue weights, and were positive controls in the assays. The decomposition product **3** was initially tested at 100 mg/kg but found to be toxic. At 10 mg/kg, **3** caused a 45% reduction in the dry weight of the granuloma, and testing at lower doses (Table 2) showed that the inhibition was still significant at 0.1 mg/kg.

The other drugs in this series were initially tested at 100 mg/kg, and the results are shown in Table 1. **6**, **8**, and **9** each inhibited granuloma dry weight greater than 50%. Interestingly, when the cis and trans isomers of **9** were isolated and tested separately, the trans isomer **7** produced significant inhibition (58%), but the cis isomer **21** had no activity (0%). Lower doses of **6** (Table 2) showed that it was not as potent as **3**, since it had modest, though statistically significant, activity at 25 mg/kg (38%).

A second group of compounds maintained significant inhibitory activity but were not as potent as those described above. The DTH-GRA model displays remarkable structural sensitivity for an *in vivo* assay. The second group of compounds contains the thioflavones **10** and **13** and the benzopyranone **17**, none of which were as active as the flavanone derivative **9**. Compound **14**, which differs from the potent **8** by a single carbonyl and a different ester, was only marginally active. Replacing the ester on **14** with a nitrile gave a toxic compound, **25**, but the thiophene analog **12** did show modest inhibitory activity at 100 mg/kg. Another analog, **15**, which was also toxic at 100 mg/kg was tested at 10 mg/kg and gave significant, although modest, inhibition of granuloma formation. Replacing the nitrile of **15** with a *p*-methoxybenzoyl group, which yields a deoxybenzoin-like analog of **6**, led to an inactive compound, **19**.

Table 3 shows the effects of selected compounds on AIA in mice after 28 days. Mice were dosed subcutaneously, for 5 of every 7 days, for the duration of the study. The severity of the arthritis was determined from

Table 1. Delayed-Type Hypersensitivity Granuloma Results of Bisphosphonate Esters

no.	prep	yield, %	dose, mg/kg (sc)	percent inhibition[a] wet wt	percent inhibition[a] dry wt	formula[b]
clodronic acid			100	45 ± 8	50 ± 6 (n = 36)	
2			100	31 ± 9	35 ± 8 (n = 3)	
3[c]			10	49***	45***	$C_{18}H_{28}O_8P_2$
6	A	61	100	48***	58***	$C_{24}H_{24}O_7P_2$
7	A		100	67***	58***	$C_{25}H_{34}O_8P_2$
8	A	84	100	77***	54***	$C_{21}H_{34}O_9P_2$
9	A	75	100	46***	47***	$C_{25}H_{34}O_8P_2$
10	A	66	100	33**	42**	$C_{20}H_{32}O_7P_2S$
11	A	23	100	25*	38**	$C_{17}H_{34}O_7P_2$
12	A	26	100	40**	37**	$C_{16}H_{27}NO_6P_2S$
13	A	76	100	28*	35**	$C_{20}H_{32}O_7P_2S$
14	A	52	100	8	35*	$C_{19}H_{22}O_8P_2$
15[d]	A	48	10	36**	34**	$C_{17}H_{28}N_2O_6P_2$
16[e]	A	23	100	27*	28*	$C_{18}H_{28}O_7P_2S$
17	A	42	100	67	44	$C_{19}H_{30}O_8P_2$
18[f]	A	45	100	3	16	$C_{25}H_{35}NO_8P_2$
19	B	59	100	34*	14	$C_{24}H_{35}NO_8P_2$
20	A	41	100	0	0	$C_{22}H_{31}NO_6P_2$
21[g]	A		100	0	0	$C_{25}H_{34}O_8P_2$
22	A	25	100	0	0	$C_{19}H_{30}O_7P_2$
23[h]	B	46	100	5	0	$C_{19}H_{32}O_9P_2$
24	A	56	100	50	0	$C_{22}H_{36}O_7P_2$
25	A	13	100	tox		$C_{18}H_{29}NO_6P_2$

[a] (***) $p < 0.001$; (**) $p < 0.01$; (*) $p < 0.05$. Results for clodronic acid and **2** are from multiple experiments; otherwise the results expressed are from single experiments with 10 animals. [b] Satisfactory elemental analyses (0.4%) were obtained for all compounds unless otherwise noted. [c] C: calcd, 49.77; found, 49.14. [d] (0.25H_2O). [e] Mp 99–100 °C (Et_2O). [f] Mp 104–105 °C. [g] Mp 115 °C (Et_2O). [h] Mp 86–87 °C (methyl *tert*-butyl ether).

Table 2. Delayed-Type Hypersensitivity Granuloma Dose–Response Study of Selected Bisphosphonate Esters

compound	dose, mg/kg (sc)	percent inhibition[a] wet wt	percent inhibition[a] dry wt
clodronic acid	200	55 ± 3	59 ± 13 (n = 6)
	100	61 ± 3	63 ± 5
	50	48 ± 5	47 ± 10
	25	45 ± 8	45 ± 6
3	10	56 ± 6	54 ± 3 (n = 3)
	1	49 ± 8	49 ± 1
	0.1	43 ± 2	38 ± 11
	0.01	28 ± 1	27 ± 2
6	100	51 ± 7	57 ± 6 (n = 4)
	50	44 ± 2	48 ± 8
	25	35 ± 9	38 ± 11

[a] (***) $p < 0.001$; (**) $p < 0.01$; (*) $p < 0.05$.

Table 3. Antigen-Induced Arthritis Suppression by Bisphosphonate Esters

compound	dose, mg/kg (sc)	percent inhibition[a]
3	10	12
6	100	52***
	50	53***
	25	55***
9	100	21
11	100	46**
13	100	21**
14	100	38***
15	10	2

[a] (***) $p < 0.001$; (**) $p < 0.01$.

histological scoring of periarticular inflammation, pannus formation, and cartilage and bone erosion. The scores were then combined to give a global arthritis score of the affected joint. Clodronic acid exerted significant inhibitory effects on the arthritis and served as a potent control (Table 3). Unexpectedly, the most potent compound in the DTH-GRA, **3**, failed to significantly inhibit AIA at 10 mg/kg. A second compound, which inhibited granuloma formation at 10 mg/kg, **15**, also failed to inhibit the AIA model. In contrast, **6** did significantly reduce antigen-induced articular pathology at doses between 25 and 100 mg/kg ($p < 0.01$). At the lowest dose, which only marginally suppressed the DTH-GRA (29%), **6** still inhibited AIA by 55%. Both **11** and **14** also gave good inhibition at 100 mg/kg, but the racemic benzopyranone **9** and the benzothiopyranone **13** showed only marginal activity.

New ketonic bisphosphonate esters were also studied for their antiinflammatory/antiarthritic activity. The initial lead, **3**, showed excellent, dose–response inhibition of the DTH-GRA but failed to inhibit AIA. Surprisingly, merely replacing the carbonyl closest to the bisphosphonate esters with a phenyl group, **6**, produced a potent inhibitor of both inflammation and arthritis. We speculate that **3** selectively inhibits the development of the DTH-GRA lesion. The DTH-GRA represents a chronic inflammatory tissue response without the erosive component seen in the AIA (i.e., cartilage, bone resorption, and pannus formation). This is due to the different immunization protocols used for the two reactions, resulting in different inflammatory reactions.[17] Thus, it is conceivable that **3** suppresses inflammation but is limited to mechanisms involved in the generation of DTH-GRA lesions, while **6** exhibits additional antiinflammatory activity which extends to mechanisms associated with tissue damage in mBSA arthritic lesions which have both immediate hypersensitivity and delayed hypersensitivity components.[18]

Efforts to improve the activity of **6** failed to identify analogs which were more potent. **9** rigidifies the **6** structure and maintains the antiinflammatory activity, but the antiarthritic activity is lost. We were unable to test the potent isomer **7** because of its propensity to isomerize back to **9**. These results continue to prove that bisphosphonic esters represent a potent class of antiinflammatory and antiarthritic compounds and that changes to the moiety attached to the bisphosphonate ester can greatly affect the antiinflammatory and antiarthritic activity. Investigations in this area are continuing.

Experimental Section

Materials and Methods. Mass spectra, infrared spectra, and combustion analysis were obtained by the Physical and Analytical Chemistry Unit of The Upjohn Co. ^{1}H NMR and ^{13}C NMR spectra were obtained at 300 MHz on a Bruker AM 300 spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard unless stated otherwise. J values are reported in hertz (Hz). Melting points were measured on a Thomas/Hoover apparatus and are uncorrected. Thin-layer chromatography was conducted on Analtech GF silica gel plates. Column chromatography was conducted at medium pressure utilizing silica gel (E. Merck, 70–230 mesh). **4** was prepared by methods previously described.[9,19]

(4-Phenyl-3-butynylidene)bisphosphonic Acid, Tetraethyl Ester (5). Phenylacetylene (2.4 mL, 22 mmol) in THF (20 mL), at −78 °C, was treated with *n*-BuLi (13.8 mL of 1.6 M in hexane, 22 mmol) and then stirred for 30 min. The solution was treated with **4** (6.00 g, 20 mmol) in THF (20 mL) and stirred for 5 min and then warmed to 22 °C for 1 h. It was diluted with EtOAc, washed with H_2O, 1 N HCl, saturated $NaHCO_3$, and brine, and then dried with $MgSO_4$ and concentrated *in vacuo*. The yellow liquid was purified by chromatography (EtOAc, EtOAc/acetone, 1:1): 4.43 g (11.0 mmol, 55%). ^{1}H NMR: δ 7.40 (m, 2H), 7.27 (m, 3H), 4.23 (m, 8H), 3.05 (dt, $J_d = 6.1$, $J_t = 16.5$, 2H), 2.66 (tt, $J_{t1} = 6.2$, $J_{t2} = 23.3$, 1H), 1.34 (m, 12H). IR (neat): 2981, 1703, 1599, 1491, 1442, 1392, 1251 cm^{-1}. MS: m/e 402 (M^+).

(3,4-Dioxo-4-phenylbutylidene)bisphosphonic Acid, Tetraethyl Ester (3). **5** (3.01 g,7.48 mmol) dissolved in CH_2Cl_2 (75 mL) and acetic acid (4.1 mL) was treated with benzyltriethylammonium chloride (0.42 g, 1.9 mmol), and the solution was heated to reflux. A solution of potassium permanganate (4.83 g, 31 mmol) in H_2O (80 mL) was added, and the solution was stirred at reflux for 4–6 h while monitored by TLC. When complete, the mixture was cooled, acidified with 10% HCl, and treated with $NaHSO_3$ to obtain a colorless solution. The layers were separated, and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were washed thrice with saturated $NaHCO_3$ and brine, dried with $MgSO_4$, and then concentrated *in vacuo* to a yellow oil. The crude was purified by chromatography (EtOAc): 2.8 g (6.4 mmol, 86%). ^{1}H NMR: δ 8.08 (d, 2H), 7.64 (t, 1H), 7.50 (t, 2H), 4.19 (m, 8H), 3.43 (dt, $J_{t1} = 6.2$, $J_{t2} = 15$, 2H), 3.34 (tt, $J_{t1} = 6.3$, $J_{t2} = 24$, 1H), 1.33 (m, 12H). ^{13}C NMR: δ 197.3, 190.4, 134.4, 131.7, 130.3, 128.6, 62.7, 34.5, 30.7 (t, $J = 136$), 16.1. IR (neat): 1720, 1673, 1597, 1580, 1450, 1392, 1166 cm^{-1}. MS: m/e 434 (M^+).

Procedure A: (4-Oxo-3,4-diphenylbutylidene)bisphosphonic Acid, Tetraethyl Ester (6). **4** (2.70 g, 9 mmol), deoxybenzoin (1.96 g, 10 mmol), and DBU (0.15 mL) were heated to 50 °C in THF (10 mL) for 40 h. The reaction mixture was cooled, diluted with CH_2Cl_2, washed with H_2O, dried with $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed (EtOAc, EtOAc/acetone, 1:1): 3.02 g (6.08 mmol, 61%). ^{1}H NMR: δ 7.98 (d, $J = 7$, 2H), 7.45 (m, 1H), 7.32 (m, 6H), 7.23 (m, 1H), 5.28 (t, $J = 7$, 1H), 4.1 (m, 7H), 3.9 (m, 1H), 2.6 (m, 1H), 2.4 (m, 2H), 1.35 (m, 6H), 1.25 (t, $J = 7$, 3H), 1.18 (m, 3H). ^{13}C NMR: δ 199, 138, 136, 133, 128.9, 128.6, 128.4, 128.3, 127, 62, 51, 34, 30, 16. IR (neat): 2983, 1681, 1253, 1065, 1042, 1028, 972 cm^{-1}. MS: m/e 496 (M^+).

Procedure B: [4-(4-Methoxyphenyl)-4-oxo-3-(2-pyridinyl)butylidene]bisphosphonic Acid, Tetraethyl Ester (19). *n*-BuLi (1.6 M in hexane, 62.5 mL, 0.10 mol) was added slowly to a solution of 2-picoline (9.9 mL, 0.10 mol) in ether (100 mL). The reaction mixture was warmed to reflux for 30 min, and then a solution of methyl 4-methoxybenzoate (8.31 g, 50 mmol) in ether (50 mL) was added slowly so that the reflux was maintained. After reflux was continued for an additional 30 min, the reaction was poured onto ice/HCl, diluted with EtOAc, and extracted (3 × 10% HCl). The acidic fractions were washed with EtOAc and then neutralized with sodium hydroxide and $NaHCO_3$ until pH 7.5. The product was extracted into EtOAc, dried with $MgSO_4$, and concentrated *in vacuo*. The product was distilled [bp 175–185 °C (0.2 mmHg)], and then the solidified product was recrystallized from cyclohexane: 4.856 g (21.4 mmol, 42%).[20]

The pyridine (2.50 g, 11 mmol), **4** (3.00 g, 10 mmol), and K_2CO_3 (2.07 g, 15 mmol) were stirred in methanol (20 mL) overnight. The reaction mixture was concentrated *in vacuo*, taken up in EtOAc, washed with NaCl, dried with $MgSO_4$, and

concentrated *in vacuo*. The product was chromatographed (EtOAc, EtOAc/acetone, 1:1, acetone): 3.093 g (5.86 mmol, 59%). ^{1}H NMR: δ 8.53 (d, J = 4.93, 1H), 8.02 (m, 2H), 7.61 (m, 1H), 7.35 (d, J = 7.8, 1H), 7.12 (m, 1H), 6.85 (m, 2H), 5.41 (t, J = 7.2, 1H), 4.16 (m, 8H), 3.81 (s, 3H), 2.77 (m, 1H), 2.50 (m, 2H), 1.38 (m, 6H), 1.26 (t, J = 7.1, 3H), 1.17 (t, J = 7.1, 3H). ^{13}C NMR: δ 196.5, 163.2, 158.8, 149.7, 136.7, 131.1, 129.3, 122.8, 121.9, 113.5, 62.8, 62.7, 62.4, 62.3, 62.2, 55.2, 53.5, 33.9 (t), 28.4, 16.3, 16.2, 16.1, 16.0. IR (neat): 2982, 1674, 1499, 1475, 1470, 1435, 1321, 1392, 1253 cm^{-1}. MS: m/e 527 (M^+).

[2-(3,4-Dihydro-4-oxo-2-phenyl-2*H*-1-benzopyran-3-yl)-ethylidene]bisphosphonic Acid, Tetraethyl Ester (9). IR (neat): 2983, 1683, 1607, 1580, 1499, 1474, 1464, 1257 cm^{-1}. MS: m/e 524 (M^+).

***cis*-[2-(3,4-Dihydro-4-oxo-2-phenyl-2*H*-1-benzopyran-3-yl)ethylidene]bisphosphonic Acid, Tetraethyl Ester (21).** ^{1}H NMR: δ 7.93 (dd, J_1 = 7.86, J_2 = 1.6, 1H), 7.45 (m, 6H), 7.08 (m, 2H), 5.65 (s, 1H), 4.00 (m, 8H), 3.32 (dt, J_d = 11.1, J_t = 3.9, 1H), 2.59 (m, 1H), 2.02 (m, 2H), 1.20 (m, 6H), 1.09 (m, 3H), 1.03 (m, 3H).

***trans*-[2-(3,4-Dihydro-4-oxo-2-phenyl-2*H*-1-benzopyran-3-yl)ethylidene]bisphosphonic Acid, Tetraethyl Ester (7).** ^{1}H NMR: δ 7.77 (dd, J_1 = 6.2, J_2 = 1.6, 1H), 7.35 (m, 6H), 6.91 (t, J = 8.6, 2H), 5.09 (d, J = 11.0, 1H), 3.95 (m, 8H), 2.90 (m, 1H), 2.26 (m, 1H), 1.61 (m, 1H).

(3-Benzoyl-4-ethoxy-4-oxobutylidene)bisphosphonic Acid, Tetraethyl Ester (8). ^{1}H NMR: δ 8.08 (d, J = 8.3, 2H), 7.61 (m, 1H), 7.49 (m, 2H), 5.10 (t, J = 7.3, 1H), 4.17 (m, 8H), 2.58 (m, 3H), 1.35 (m, 9H), 1.17 (dt, J_t = 7.1, J_d = 1.1, 3H). IR (neat): 2983, 1738, 1685, 1597, 1581, 1448, 1392, 1369, 1251 cm^{-1}. MS: m/e 492 (M^+).

[2-(3,4-Dihydro-2-methyl-4-oxo-2*H*-1-benzothiopyran-3-yl)ethylidene]bisphosphonic Acid, Tetraethyl Ester (10). ^{1}H NMR: δ 8.04 (m, 1H), 7.40 (m, 1H), 7.20 (m, 2H), 4.17 (m, 8H), 3.55 (m, 1H), 3.37 (m, 0.5H), 3.10 (m, 0.5H), 2.7–1.9 (m, 3H), 1.51 (d, J = 6.9, 3H), 1.34 (m, 12H). IR (neat): 2981, 1675, 1590, 1560, 1478, 1459, 1437, 1391, 1256 cm^{-1}. MS: m/e 478 (M^+).

[2-(3-Methyl-2-oxocyclohexyl)ethylidene]bisphosphonic Acid, Tetraethyl Ester (11). ^{1}H NMR: δ 4.19 (m, 8H), 3.0–1.5 (m, 11H), 1.35 (m, 12H), 1.0 (d, J = 6.3, 3H). IR (neat): 2981, 1706, 1477, 1446, 1392, 1368, 1251 cm^{-1}. MS: m/e 412 (M^+).

[3-Cyano-3-(2-thienyl)propylidene]bisphosphonic Acid, Tetraethyl Ester (12). ^{1}H NMR: δ 7.32 (m, 1H), 7.11 (m, 1H), 6.99 (m, 1H), 4.77 (t, J = 7.3, 1H), 4.21 (m, 8H), 2.54 (m, 3H), 1.36 (m, 12H). IR (neat): 2983, 2242, 1478, 1442, 1258 cm^{-1}. MS: m/e 467 (M^+).

[2-(3,4-Dihydro-6-methyl-4-oxo-2H-1-benzothiopyran-3-yl)ethylidene]bisphosphonic Acid, Tetraethyl Ester (13). ^{1}H NMR: δ 7.82 (s, 1H), 7.13 (m, 2H), 4.13 (m, 8H), 3.3–3.0 (m, 3H), 2.67 (m, 2H), 2.26 (s, 3H), 1.96 (m, 1H), 1.27 (m, 12H). IR (neat): 2981, 1674, 1602, 1471, 1443, 1395, 1253 cm^{-1}. MS: m/e 478 (M^+).

α-[2, 2-Bis(ethoxyphosphinyl)ethyl]benzeneacetic Acid, Methyl Ester (14). ^{1}H NMR: δ 7.31 (m, 5H), 4.01 (m, 9H), 3.57 (s, 3H), 2.40 (m, 3H), 1.27 (m, 12H). IR (neat): 2983, 1735, 1601, 1584, 1494, 1478, 1255 cm^{-1}. MS: m/e 450 (M^+).

[3-Cyano-3-(2-pyridinyl)propylidene]bisphosphonic Acid, Tetraethyl Ester (15). ^{1}H NMR: δ 8.59 (m, 1H), 7.72 (dt, J_t = 7.7, J_d = 1.8, 1H), 7.40 (d, J = 7.8, 1H), 7.26 (m, 1H), 4.63 (t, J = 7.7, 1H), 4.15 (m, 8H), 2.56 (m, 3H), 1.39 (m, 12H). MS: m/e 539 (M^+).

[2-(3-Oxo-2,3-dihydrothianaphthen-2-yl)ethylidene]-bisphosphonic Acid, Tetraethyl Ester (16). ^{1}H NMR: δ enol 7.8 (m, 1H), 7.6 (m, 1H), 7.3 (m, 2H), 4.3–3.9 (m, 8H), 3.41 (dt, J_t = 16.2, J_d = 5.8, 2H), 2.62 (tt, J_1 = 23.7, J_2 = 5.8, 1H), 1.3 (m, 12H); ketone 7.77 (m, 1H), 7.5 (m, 1H), 7.3 (m, 2H), 4.46 (m, 1H), 2.9 (m, 2H), 2.2 (m, 1H), 1.3 (m, 12H). IR (Nujol): 3191, 1605, 1546, 1250 cm^{-1}. MS: m/e 450 (M^+).

[2-(4-Oxo-3,4-dihydro-2*H*-1-benzopyran-3-yl)ethylidene]-bisphosphonic Acid, Tetraethyl Ester (17). ^{1}H NMR: δ 7.81 (dd, J_1 = 7.8, J_2 = 1.7, 1H), 7.42 (m, 1H), 6.93 (m, 2H), 4.50 (dd, J_1 = 11.4, J_2 = 4.6, 1H), 4.10 (m, 8H), 3.20 (m, 1H), 2.76 (m, 1H), 2.40 (m, 1H), 1.87 (m, 1H), 1.28 (m, 12H). IR (neat): 2983, 1689, 1606, 1580, 1480, 1466, 1459, 1251 cm^{-1}. MS: m/e 448 (M^+).

(4-Anilino-3-benzoyl-4-oxobutylidene)bisphosphonic Acid, Tetraethyl Ester (18). ^{1}H NMR: δ 9.55 (s, 1H), 8.08 (d, J = 7.4, 2H), 7.57 (m, 3H), 7.46 (t, J = 7.8, 2H), 7.30 (t, J = 7.6, 2H), 7.09 (t, J = 7.4, 1H), 4.96 (t, J = 5.6, 1H), 4.17 (m, 8H), 2.58 (m, 3H), 1.38 (m, 6H), 1.28 (m, 6H). ^{13}C NMR: δ 212.4, 195.5, 167.1, 138.0, 136.2, 133.7, 128.9, 128.8, 128.5, 124.3, 119.6, 63.1 (m), 54.0, 34.5, 25.9, 16.3 (m). IR (Nujol): 1699, 1682, 1606, 1599, 1589, 1549, 1442, 1241 cm^{-1}. MS: m/e 539 (M^+).

[3-(1-Naphthyl)-4-nitrilobutylidene]bisphosphonic Acid, Tetraethyl Ester (20). ^{1}H NMR: δ 8.24 (d, J = 8.4, 1H), 7.88 (m, 2H), 7.74 (d, J = 7.1, 1H), 7.57 (m, 3H), 5.30 (dd, J_1 = 10.5, J_2 = 5.6, 1H), 4.20 (m, 8H), 2.6 (m, 3H), 1.36 (m, 12H). IR (neat): 2983, 2242, 1599, 1541, 1478, 1443, 1254 cm^{-1}. MS: m/e 467 (M^+).

[2-(2,3-Dihydro-1-oxo-1*H*-inden-2-yl)ethylidene]bisphosphonic Acid, Tetraethyl Ester (22). ^{1}H NMR: δ 7.73 (d, J = 7.6, 1H), 7.59 (t, J = 7.4, 1H), 7.45 (d, J = 7.6, 1H), 7.37 (t, J = 7.4, 1H), 4.21 (m, 8H), 3.40 (m, 1H), 3.22 (m, 1H), 3.02 (tt, J_1 = 23.9, J_2 = 6.2, 1H), 2.80 (dd, J_1 = 16.9, J_2 = 4.0, 1H), 2.48 (m, 1H), 2.11 (m, 1H), 1.36 (m, 12H). IR (neat): 2983, 1709, 1610, 1588, 1476, 1464, 1392, 1368, 1251 cm^{-1}. MS: m/e 432 (M^+).

α--[2,2-Bis(ethoxyphosphinyl)ethyl]-2-hydroxybenzeneacetic Acid, Methyl **Ester (23).** ^{1}H NMR: δ 7.15 (dt, J_t = 6.7, J_d = 1.6, 1H), 7.06 (dd, J = 7.7, J = 1.6, 1H), 6.96 (dd, J = 8.1, J = 1.6, 1H), 6.85 (t, J = 7.4, 1H), 4.49 (t, J = 7.4, 1H), 4.15 (m, 8H), 3.69 (s, 3H), 2.6 (m, 1H), 2.31 (m, 2H), 1.31 (m, 12H). IR (neat): 2984, 1734, 1642, 1598, 1508, 1475, 1443, 1251 cm^{-1}. MS: m/e 466 (M^+).

[2-(3-Phenyl-2-oxocyclohexyl)ethylidene]bisphosphonic Acid, Tetraethyl Ester (24). ^{1}H NMR: δ 7.4–7.1 (m, 5H), 4.19 (m, 4H), 3.85 (m, 3H), 3.55 (m, 1H), 2.9–1.7 (m, 11H), 1.35 (t, J = 7.7, 6H), 1.83 (t, J = 7.7, 3H), 1.11 (t, J = 7.7, 3H). ^{13}C NMR: δ 212.2, 138.5, 128.7, 127.4, 126.6, 62 (m), 57.1, 39.8, 34.5, 33.2, 31.7 (t), 29.1, 25.4, 16.1 (m). IR (neat): 2981, 1708, 1599, 1581, 1491, 1447, 1250 cm^{-1}. MS: m/e 474 (M^+).

(3-Cyano-3-phenylpropylidene)bisphosphonic Acid, Tetraethyl Ester (25). ^{1}H NMR: δ 7.38 (s, 5H), 4.45 (t, J = 6.9, 1H), 4.15 (m, 8H), 2.45 (m, 3H), 1.34 (m, 12H). IR (neat): 2984, 2242, 1601, 1587, 1494, 1478, 1456, 1444, 1256 cm^{-1}. MS: m/e 417 (M^+).

Biological Procedures. Delayed-Type Hypersensitivity Granuloma. Groups of 10 female CF-1 mice (25 g) were sensitized with an emulsion of methylated bovine serum albumin (mBSA) in saline with Freund's incomplete adjuvant and dextran by sc injection over the inguinal lymph node.[10] Three weeks later, hydroxyapatite (HA) discs (6 mm diameter) soaked in mBSA solution (30 mg/mL of saline) were implanted sc in the dorsum of the mice (two discs, bilaterally). All drugs were prepared as solutions, suspensions, or emulsions. Each mouse received compound in a volume of 0.1 mL/10 g of body weight, sc in the scruff of the neck. Dosing commenced on the day of implantation of the mBSA-soaked discs and was continued thereafter on a daily basis until day 9, when the mice were euthanized. The granulomatous lesions were then excised and both wet and dry tissue weights measured. Results were analyzed by Student's paired t-test.

Antigen-Induced Arthritis. Groups of 10 female C57Bl/6 mice, 14–16 weeks of age, were immunized sc with an emulsion of mBSA and Freund's complete adjuvant supplemented with extra heat-killed *M. tuberculosis*.[10] Secondary immunizations were performed after 7 days, and after a further 14 days, the animals were challenged intraarticularly with 200 μg of mBSA in saline in the left rear stifle joint. The experimental compounds were dissolved, suspended, or emulsified in sterile saline, sonicated where appropriate to homogeneous doses, and stored frozen in aliquots. Fresh aliquots were used for each day of dosing. Mice were dosed sc in the scruff of the neck from the day of intraarticular mBSA challenge (day 0) using a 5 of 7 day dosing regimen until the conclusion of the study on day 28. The mBSA-injected stifle joint was then skinned, removed, and fixed in phosphate-

buffered formaldehyde solution prior to decalcification and histological preparation. The assessment of arthritis was performed on sagittal joint sections stained with hematoxylin and eosin. Sections were graded from 1 (mild) to 5 (severe) for soft tissue inflammation, pannus formation, and extent of cartilage and bone erosion. The component scores were summed to give a global arthritis score (maximum = 20). Results were analyzed by one-way analysis of variances, compared to controls.

Acknowledgment. We are grateful to Katherine A. Franz for technical help in preparing histological specimens and Charles E. Fultz for synthetic assistance. The support of the Physical and Analytical Chemistry Unit of Upjohn Laboratories is also appreciated.

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