Pyrazoline Bisphosphonate Esters as Novel Antiinflammatory and Antiarthritic Agents

Richard A. Nugent,*,† Megan Murphy,† Stephen T. Schlachter,† Colin J. Dunn,‡ Robert J. Smith,‡ Nigel D. Staite,‡ Louise A. Galinet,‡ Sharon K. Shields,‡ Danielle G. Aspar,‡ Karen A. Richard,‡ and Norman A. Rohloff‡

The Upjohn Company, Kalamazoo, Michigan 49001

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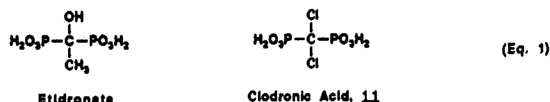
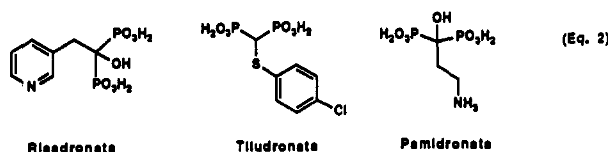
Vinylidenebisphosphonic acid tetraethyl ester (1) and diazo ketones 7a–l in ether at 22 °C yield pyrazoline bisphosphonate tetraethyl esters 8a–l in moderate to good yield. These compounds were evaluated in animal models of arthritis: rat adjuvant-induced polyarthritis (AIP) and murine antigen-induced arthritis (AIA) and a murine model of chronic inflammation, the delayed type hypersensitivity granuloma reaction (DTH-GRA). (5-Benzoyl-2,4-dihydro-3*H*-pyrazol-3-ylidene)-bisphosphonic acid tetraethyl ester (8a), and [5-(3-fluorobenzoyl)-2,4-dihydro-3*H*-pyrazol-3-ylidene]-bisphosphonic acid tetraethyl ester (8d) significantly inhibited the arthritis models, AIP (15 mg/kg) and AIA (25 mg/kg), as well as the DTH-GRA (25 mg/kg). Conversion of 8a to the corresponding bisphosphonic acid, 10a, resulted in loss of activity. Compounds with alkyl substituents on the pyrazoline nitrogen, 9a–d, were inactive in the DTH-GRA. These results show that 8a and 8d have novel antiinflammatory activity and are capable of inhibiting chronic arthritis and inflammation in animals. Such compounds might be useful in man for treating chronic tissue injury associated with arthropathies such as inflammatory joint disease as well as other chronic inflammatory diseases.

Methylenebisphosphonic acids are synthetic pyrophosphate mimics, which have been developed and used for the treatment of diseases of bone resorption.[1] However, studies in the rat adjuvant arthritis model with etidronate and clodronate[2,3] (eq 1) demonstrated significant sup-



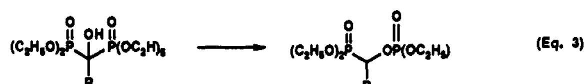
pression of bone erosion in the joint and consistently revealed significant inhibition of inflammation. Subsequent investigations with risedronate[4] and tiludronate[5] (eq 2) have confirmed and extended these initial observations. To our knowledge, the antiarthritic properties of



bisphosphonic acids (BPs) is poorly understood and has not been demonstrated in any other models of arthritis.[6] Furthermore, the antiinflammatory potential of bisphosphonic acids has been largely ignored.[7,8] We chose to examine the antiarthritic effects of several pyrazoline bisphosphonate tetraethyl esters (BPTEs) in the rat adjuvant-induced polyarthritis (AIP) and the mouse antigen-induced arthritis (AIA), and in a murine model of chronic inflammation, the delayed type hypersensitivity granuloma reaction (DTH-GRA).

Chemistry

In designing our molecules, our intent was to separate the bone resorptive effects of BPs from their antiinflammatory properties, by synthesizing novel BPTEs. Although relatively little is known about the metabolism of BPTEs, we anticipated little conversion to the corresponding BPs in vivo, based on analogy to the behavior of monophosphonates and alkyl phosphates.[9,10] Alkyl esters of hydroxymethylene BPs, such as risedronate and pamidronate, are prone to rearrange to phosphonate phosphates (eq 3) under mild conditions.[11] We felt that



joining the methylene BP to the primary amine of pamidronate to form a small heterocyclic ring would prevent the phosphonate shift.[12] Our strategy allowed us to employ an underutilized BPTE starting material, the vinylidene 1. Although 1 has been known for a number of years, there are few studies of its chemistry.[13] Our interests were in the possible dipolar cycloaddition reactions of the molecule. Although none have been reported with 1, [3 + 2] dipolar addition reactions of diazo species with vinylidenephosphonate esters,[14-16] and vinylidenebis(sulfones)[17] offered good precedent for the reaction.

Following the procedure of Degenhardt and Burdsall, tetraethyl vinylidenebisphosphonate (1) was prepared in 79% yield from the methylenebisphosphonate (2) (Scheme I).[18,19] Pyrazoline bisphosphonates were then synthesized through the reactions of vinylidene-BPTE (1) and an appropriate diazo compound. Addition of diazomethane to a 0 °C solution of 1 in ether gave, after concentration, the oily pyrazoline 3 in nearly quantitative yield. Upon standing for 1 week at room temperature or during attempted distillation, 3 was converted quantitatively into the propenylidene 4.[20]

Shifting the double bond from the $\Delta^{1,2}$ position to the $\Delta^{1,5}$ was envisioned to increase stability of the molecule. This migration was accomplished by introducing electron-withdrawing groups at the C-5 position. Ethyl diazoacetate and 1 in ether gave the unrearranged pyrazoline 5 as the crude product, but upon silica gel chromatography a rearrangement occurred which shifted the double bond

† Medicinal Chemistry Research.
‡ Hypersensitivity Diseases Research.

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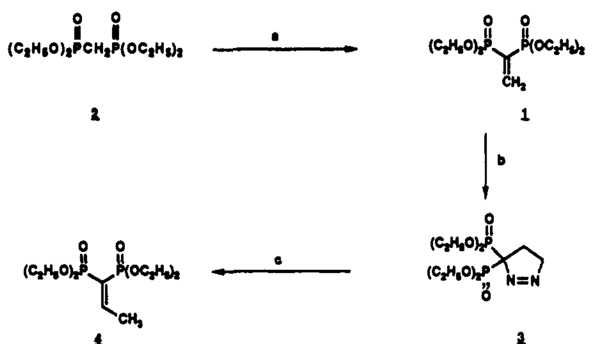
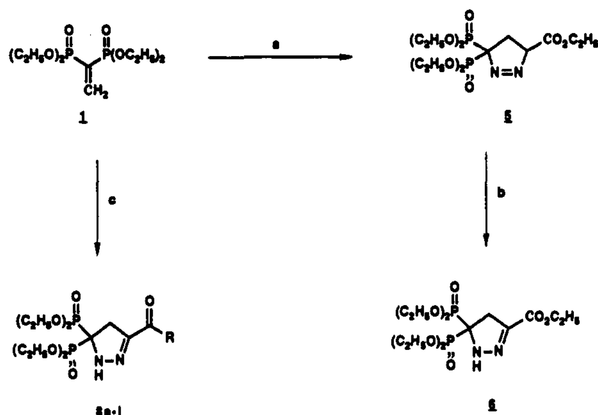
Scheme I [a]



[a] (a) CH_2O, $(C_2H_5)_2NH$, MeOH; tol, pTSA, $-H_2O$; (b) CH_2N_2, ether, 0 °C; (c) distillation

Scheme II [a]



[a] (a) $N_2CHCO_2C_2H_5$, ether, rt, 30 min; (b) silica gel; (c) N_2CHCOR (7a–l), ether, rt

into conjugation with the carbonyl (Scheme II). This transformation is obvious from the proton NMR, in which the C-4 multiplet of **5** is shifted from 2.5 ppm to 3.5 ppm in **6**, and simplified to a triplet. The large coupling constant of this triplet (26 Hz) indicates the presence of two adjacent phosphonates. **6** proved to be more unstable than **3**, and we were unable to identify the decomposition products.

However, 5-ketopyrazolines were found to be significantly more stable. Diazo ketones, **7a–l**, were prepared from the appropriate carboxylic acid chloride and diazomethane in the presence of triethylamine[21] or from a methyl ketone and ethyl formate, followed by treatment with tosyl azide.[22] The diazo ketones were then combined with **1** in ether at 22 °C and stirred overnight (Scheme II). The precipitate was collected and recrystallized from ethyl acetate to give the $\Delta^{1,5}$ pyrazolines **8a–l** (Table I). No trace of a $\Delta^{1,2}$ pyrazoline was ever observed.

Because of the electron-withdrawing nature of the C-5 carbonyl, the pyrazoline N-2 position is quite acidic and is easily alkylated with a variety of reagents (Scheme III). Thus, stirring pyrazoline **8a**, iodomethane, and DBU in THF under nitrogen at 22 °C gave, after column chromatography, the oily product **9a** in 81% yield. Using dihaloalkyl groups, it was possible to link two pyrazolines together, as in **9c** and **9d** (Table II).

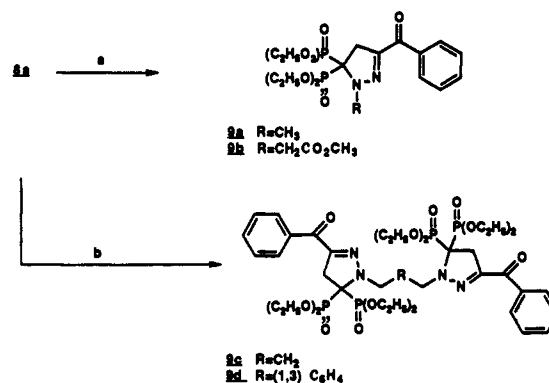
The BPTEs **8a** and **8g** underwent ester cleavage with TMS-Br in chloroform, followed by hydrolysis with water (Scheme IV). Because of the hydroscopic nature of both BPs, they were isolated as their disodium salt, **10a**, and dipotassium salt, **10b**, respectively (Table I).

Table I. Yields and Physical Properties of Pyrazoline Bisphosphonates Esters **8a–e** and Acids **10a–b**

no.	R	% yield	mp, °C	recryst solvent[a]	formula[b]
8a	C_6H_5	59	133.5–134	EA	$C_{18}H_{28}N_2O_7P_2$
8b	t-Bu	45	106–107	EA	$C_{16}H_{32}N_2O_7P_2$
8c	3-CH_3-C_6H_4	34	91–92	EA/Hex	$C_{19}H_{30}N_2O_7P_2$
8d	3-F-C_6H_4	33	104–105	EA	$C_{18}H_{27}FN_2O_7P_2$
8e	2-CH_3-C_6H_4	35	162–163	EA	$C_{19}H_{30}N_2O_7P_2$
8f	2,4-Cl_2-C_6H_3	39	155–156	EA	$C_{18}H_{26}Cl_2N_2O_7P_2$
8g	c-C_6H_{11}	47	165–166	EA	$C_{18}H_{34}N_2O_7P_2$
8h	4-CH_3O-C_6H_4	30	115–116	EA	$C_{19}H_{30}N_2O_8P_2$
8i	C_2H_5	28	96–98	MC/Hex	$C_{14}H_{28}N_2O_7P_2$
8j	2-F-C_6H_4	39	185–186	EA	$C_{18}H_{27}FN_2O_7P_2$
8k	4-Cl-C_6H_4	39	101–102	EA	$C_{18}H_{27}ClN_2O_7P_2$
8l	4-CH_3-C_6H_4	49	116–117	EA	$C_{19}H_{30}N_2O_7P_2$
10a	C_6H_5	70	>170 dec		$C_{10}H_{10}N_2Na_2O_7P_2$[c]
10b	c-C_6H_{11}	73	>150 dec		$C_{10}H_{16}K_2N_2O_7P_2$[d]

[a] Recrystallization solvents (EA) ethyl acetate, (Hex) hexane, (MC) methylene chloride. [b] Satisfactory elemental analyses (±0.4%) were obtained for all compounds unless otherwise noted. [c] ($\cdot 2.5\ H_2O$); N: calcd, 6.62; found, 6.10. [d] ($\cdot 0.5\ H_2O$).

Scheme III [a]



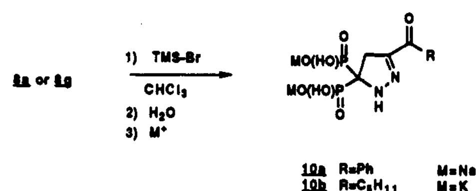
[a] (a) R-X, DBU, THF; (b) R-$(CH_2$-$X)_2$, DBU, THF

Table II. Yields and Physical Properties of N-Alkylated Pyrazoline Bisphosphonates **9a–c**

no.	R	% yield	formula[a]
9a	CH_3	81	$C_{19}H_{30}N_2O_7P_2$
9b	$CH_2CO_2CH_3$	75	$C_{21}H_{32}N_2O_7P_2$
9c	CH_2	39	$C_{39}H_{60}N_4O_{14}P_4$
9d	1,3-C_6H_4	17	$C_{44}H_{62}N_4O_{14}P_4$

[a] Satisfactory elemental analyses (±0.4%) were obtained for all compounds unless otherwise noted.

Scheme IV



Pharmacology

Similar to the histopathology in the human arthritic joint, the AIP model expresses synovitis, infiltration of the subsynovial tissue with numerous inflammatory cell types, and pannus formation leading to erosion of articular cartilage and bone. Perhaps the most distinctive aspect of the joint pathology in AIP is the marked resorption of bone that is caused by a granulomatous reaction and periosteal bone formation. The rapid onset (24 h) of arthritis in the injected paw is considered to be largely a nonimmune inflammatory response to complete Freund's adjuvant. In contrast, the subsequent arthritic reaction

Table III. Antiarthritic Activity of Pyrazoline Bisphosphonates[a]

	AIP (% inhibn) (ΔPV, 28 days)			antigen-induced arthritis	
no.	dose (mg/kg)	injected paw	noninjected paw	dose (mg/kg)	% inhibn
11	30	36	54*	200	52***
	15	33	70*	100	51***
	10	38	61*	50	46***
	5	21	51*	30	46***
				15	46***
				5	4
8a	100	13	50*	200	48***
	60	23	56*	100	48**
	15	25	31*	50	35*
	5	28	50*	25	16
8b	100	21	76**	100	16
	60	38	64**	50	28***
	15	21	55*		
	5	27	64*		
8c	100	3	50*	100	16
	15	6	54*		
8d	100	22	55*	100	55***
	60	8	46*	50	52***
	15	4	46*	25	42**
	5	0	0		
8e	100	9	66*	100	8
	15	18	49*		
8f	100	0	12	100	0
	15	3	38		
8g	100	16	29	200	11
	15	9	14	100	3
				50	30***
8h	100	0	16	100	23**
	15	12	17		

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

in the noninjected hindpaw and forepaws is delayed in onset and is mediated by immunological components. The suppression of bone destruction and periarticular inflammation in the noninjected paw in AIP, therefore, is considered to be an indication of potential antiarthritic activity in human rheumatoid arthritis.

The articular pathology of antigen-induced arthritis (AIA) involves an initial intense inflammatory synovitis followed by chronic inflammation and severe erosion of articular cartilage and subchondral bone resembling human rheumatoid arthritis.[23] 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.[24,25]

The delayed type hypersensitivity granuloma assay is a model of chronic inflammation, in which mice were previously sensitized to methylated bovine serum albumin (mBSA) and surgically implanted with hydroxyapatite disks (two per mouse), soaked in mBSA, in order to generate granulomas. This model is unaffected by traditional nonsteroidal antiinflammatory drugs, such as aspirin, indomethacin, or ibuprofen.[26]

Results and Discussion

The data in Table III demonstrate the effects of subcutaneously administered pyrazoline BPTEs on a 28-day rat model of developing AIP. The changes in paw volume over time (ΔPV), which occurred in the injected and noninjected hindpaws of treated and control rats were quantitated by mercury displacement plethysmography. We chose to use the BP clodronate (11) as the relative control in our experiments because it has demonstrated activity in this model.[3] Clodronate, when administered at 5–30 mg/kg, exerted significant inhibitory effects ($p < 0.05$) on the noninjected hindpaw arthritis, whereas it was less effective (21–38% inhibition) against the swelling in the injected hindpaw. None of the new pyrazoline BPTEs significantly altered the arthritis in the 28-day injected paw; however, **8a,b** (5–60 mg/kg) caused a marginal suppression (23–38%) of this component of AIP and matched that observed with **11**. In contrast **8a–e** significantly inhibited ($p < 0.05$) noninjected paw arthritis when given at 15 and 100 mg/kg, whereas **8f** (15 mg/kg) exerted modest inhibition (38%) of noninjected hindpaw-associated arthritis, and **8g,h** showed only marginal (<20%) activity. The suppressive effects of **8a,b,d** and **11** on AIP were not dose-related. Nevertheless, **8a–b** (5–100 mg/kg) and **11** (5–30 mg/kg) exerted significant inhibitory effects (31–76%) on the noninjected hindpaw arthritis, and **8d** (15–100 mg/kg) caused a marked inhibition (44–55%) of this parameter.

Also included in Table III are the effects of subcutaneously administered pyrazoline BPTE's on AIA in mice after 28 days. The results of the assay were determined through histological scoring of sagittal joint sections to develop a global arthritis score consisting of periarticular inflammation, pannus formation, and cartilage and bone erosion. **11** (15–200 mg/kg) exerted significant inhibitory effects on the arthritis, and in general, the pyrazoline BPTEs did not display activity in the same dose range as **11**. Two exceptions were **8d**, which showed significant inhibition ($p < 0.01$) at 25–100 mg/kg, and **8a**, which also showed excellent inhibition (35–48%) although over a slightly higher range (50–200 mg/kg). Unexpectedly, **8b,g** demonstrated significant inhibition ($p < 0.001$) at 50 mg/kg, but only marginal activity at a higher dose (100 mg/kg). **8c,h** displayed marginal activity (100 mg/kg) which was not statistically significant, while **8e,f** were inactive in this model.

These nine compounds and others were profiled in a delayed-type hypersensitivity granuloma model. The BPTEs were administered subcutaneously, and the results are shown in Table IV. **11** reproducibly inhibited granuloma wet and dry weights and served as a positive control in our experiments. **8b,d** both significantly inhibited the granuloma in a dose-dependent manner at the doses examined (25–100 mg/kg), while **8a** exerted significant inhibition, which was not dose-related, over the same range. **8c,e,f,h** all displayed inhibitory activities which were equivalent to that of clodronate at 100 mg/kg. **8g,i–l** showed only moderate activity against the dry weight granuloma. In contrast to the BPTEs, neither of the two pyrazoline BPs **10a,b** gave significant inhibitions. This contrasts with the observed activity of the BP **11** and in the case of **10a**, with the corresponding BPTE **8a**. Replacement of the proton of the acidic N-2 nitrogen on **8a** with either a methyl, **9a**, or methyl acetate, **9b**, resulted in complete loss of activity, as did linking two pyrazolines together through either an alkyl or aryl bridge, **9c** and **9d**.

Conclusion

Several new pyrazoline BPTEs were studied for their antiinflammatory and antiarthritic activity in selected assays. The BP clodronate (**11**), administered subcutaneously, gave good suppression of both the rat adjuvant-

Table IV. Delayed-Type Hypersensitivity Granuloma Results of Pyrazoline Bisphosphonates[a]

no.	dose (mg/kg) sc	% inhibn of granuloma dry wt	wet wt
11	100	47***	45***
8a	100	51***	56***
	50	42***	33*
	25	42***	36*
8b	100	59***	65***
	50	48***	51***
	25	38**	46**
8c	100	41**	41**
8d	100	65***	58***
	50	51***	45***
	25	49***	42***
8e	100	48**	37**
8f	100	44**	55***
8g	100	31*	33*
8h	100	50***	35**
8i	200	35**	28*
8j	100	17	20
8k	100	33	28
8l	100	22	20
10a	100	19	18
10b	100	16	1
9a	100	8	11
9b	100	1	1
9c	100	5	10
9d	100	-31	-40

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

induced polyarthritis (AIP) and murine antigen-induced arthritis (AIA) models. **8a** and **8d** matched this compound for potency and/or efficacy in both models. Generally, there was only modest concordance between the activity of the pyrazoline BPTEs in the AIP and AIA models.

Inhibition of DTH GRA formation was not always predictive of the activity of a BPTE in the AIP or AIA models, suggesting that antiarthritic/antiinflammatory activity may involve more than one mechanism. With the exception of **8j** and **8l**, compounds which were unsubstituted at the pyrazoline N-2 position significantly inhibited granuloma formation, while those with substituted nitrogens **9a–d** were essentially inactive. This implies the need for this acidic functionality for antiinflammatory activity. The fact that BPTEs are active in all three assays suggests that bone binding is not a prerequisite for antiarthritic/antiinflammatory activity. We cannot exclude the possibility that small amounts BPTEs are hydrolyzed in vivo; however, the difference in activity between BPTE **8a** and the corresponding acid **10a** implies that **8a** is not converted to **10a** in significant quantities in vivo.

The biological activity of these pyrazoline BPTEs and clodronate in the AIA model represents the first reported example of a bisphosphonate derivative inhibiting a model of arthritis other than AIP and the first reported activity in a murine model of arthritis. The activity in the delayed type hypersensitivity granuloma is the first demonstration of the antiinflammatory activity of clodronate and BPTEs in a nonarthritis model. Thus BPTEs may represent a potentially useful and novel class of compounds for the treatment of human rheumatoid arthritis.

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 in $CDCl_3$ using tetramethylsilane as an internal standard unless stated otherwise. Melting points were measured on a Thomas-Hoover apparatus and are uncorrected. Thin-layer chromatography was conducted on Analtech GP silica gel plates. Column chromatography was conducted at medium pressure utilizing silica gel (E. Merck, 230–400 mesh). Diazo ketones (**7a–l**) were prepared from the appropriate carboxylic acid chloride and diazomethane in the presence of triethylamine[19] or from a methyl ketone and ethyl formate, followed by treatment with tosyl azide,[20] and were used without additional purification. **11** was synthesized according to the literature procedure.[27]

Ethenylidenebisphosphonic Acid Tetraethyl Ester (1). Paraformaldehyde (104.2 g, 3.47 mol) and Et_2NH (50.8 g, 0.69 mol) were combined in MeOH (2 L), warmed until clear, then treated with **2** (190.09 g, 0.659 mol), and refluxed for 18 h. The sample was concentrated in vacuo, then again from MeOH, and then once more from toluene. The residue was dissolved in toluene (1 L), treated with pTSA (0.5 g), and refluxed through a Dean–Stark trap for 18 h. The sample was concentrated in vacuo, dissolved in CH_2Cl_2, washed twice with H_2O, dried with $MgSO_4$, and concentrated in vacuo. The sample was purified by distillation at reduced pressure: 156.81 g (0.519 mol, 79%); $bp_{0.6}$ 140 °C (lit.[16] $bp_{0.05}$ 115–116 °C); ^{1}H NMR δ 7.1 (d, J = 39, 1 H), 6.7 (d, J = 39, 1 H), 4.1 (m, 8 H), 1.3 (t, J = 6, 12 H); IR (neat) 2984, 2934, 1651, 1580, 1479, 1444, 1392, 1254, 1166, 1098 cm^{-1}; MS m/e 300 (M^+).

(4,5-Dihydro-3*H*-pyrazol-3-ylidene)bisphosphonic Acid Tetraethyl Ester (3). Diazomethane was prepared in ether from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.[28] **1** (3.47 g, 11.6 mmol) in Et_2O (20 mL) was treated with a diazomethane solution until the yellow color was no longer discharged. The reaction was filtered through $MgSO_4$ and concentrated in vacuo to a yellow oil 3.65 g (10.7 mmol, 92%): ^{1}H NMR δ 4.77 (m, 2 H), 4.27 (m, 8 H), 2.17 (m, 2 H), 1.34 (m, 12 H); IR (neat) 2984, 2934, 2912, 1547, 1478, 1444, 1427, 1255, 1039, 1023 cm^{-1}; MS m/e 342 (M^+).

5,5-Bis(diethoxyphosphinyl)-4,5-dihydro-3*H*-pyrazole-3-acetic Acid Ethyl Ester (5) and 5,5-Bis(diethoxyphosphinyl)-4,5-dihydro-1*H*-pyrazole-3-acetic Acid Ethyl Ester (6). **1** (300 mg, 1.00 mmol) in Et_2O (2 mL) was treated at room temperature with a solution of ethyl diazoacetate (120 mg, 1.05 mmol) in Et_2O (1 mL). After stirring for 30 min, the reaction mixture was filtered through $MgSO_4$ and concentrated in vacuo: ^{1}H NMR δ 5.3 (m, 1 H), 4.3 (m, 10 H), 2.5 (m, 2 H), 1.4 (m, 15 H). The sample was then chromatographed on silica gel to remove traces of unreacted **1** (acetone/CH_2Cl_2 1:1): 218 mg (0.536 mmol, 54%); ^{1}H NMR δ 7.1 (brd s, 1 H), 4.2 (m, 10 H), 3.5 (t, J = 26, 2 H), 1.3 (m, 15 H).

General Procedure for the Synthesis of Pyrazoline Bisphosphonate Tetraethyl Esters. In a 50-mL round-bottom flask equipped with a magnetic stirrer and a nitrogen inlet, **1** (20 mmol) and a diazo ketone (20 mmol) were stirred in Et_2O (20 mL) at 22 °C for 20 h. The precipitate was filtered, washed with Et_2O (10 mL), and then recrystallized from the appropriate solvent.

(5-Benzoyl-2,4-dihydro-3*H*-pyrazol-3-ylidene)bisphosphonic Acid Tetraethyl Ester (8a). ^{1}H NMR δ 8.10 (m, 2 H), 7.50 (m, 3 H), 6.90 (t, J = 3, 1 H), 4.27 (m, 8 H), 3.69 (t, J = 24, 2 H), 1.34 (m, 12 H); ^{13}C NMR δ 187, 136, 132.7, 129.9, 128.1, 64.3, 63.8, 38, 16.5; IR (Nujol) 1631, 1600, 1578, 1548, 1433, 1260, 1242, 1161, 1067, 1043, 1020, 1001 cm^{-1}; MS m/e 446 (M^+).

[5-(2,2-Dimethyl-1-oxopropyl)-2,4-dihydro-3*H*-pyrazol-3-ylidene]bisphosphonic Acid Tetraethyl Ester (8b). ^{1}H NMR δ 6.69 (t, J = 3.95, 1 H), 4.24 (m, 8 H), 3.48 (t, J = 25.7, 2 H), 1.31 (m, 21 H); IR (Nujol) 3196, 1645, 1557, 1479, 1268, 1242, 1041 cm^{-1}; MS m/e 426 (M^+).

[2,4-Dihydro-5-(3-methylbenzoyl)-3*H*-pyrazol-3-ylidene]bisphosphonic Acid Tetraethyl Ester (8c). ^{1}H NMR δ 7.92 (m, 2 H), 7.32 (m, 2 H), 6.87 (brd s, 1 H), 4.25 (m, 8 H), 3.69 (t, J = 25.5, 2 H), 2.40 (s, 3 H), 1.34 (m, 12 H); IR (Nujol) 3108, 1634, 1603, 1586, 1552, 1262, 1052 cm^{-1}; MS m/e 460 (M^+).

[5-(3-Fluorobenzoyl)-2,4-dihydro-3*H*-pyrazol-3-ylidene]bisphosphonic Acid Tetraethyl Ester (8d). ^{1}H NMR δ 7.94 (m, 1 H), 7,84 (m, 1 H), 7.42 (m, 1 H), 7.25 (m, 1 H), 6.96 (brd s, 1 H), 4.26 (m, 8 H), 3.68 (t, J = 25.6, 2 H), 1.34 (m, 12 H); IR (Nujol) 1633, 1613, 1583, 1546, 1264, 1043, 1025 cm^{-1}; MS m/e 464 (M^+).

[2,4-Dihydro-5-(2-methylbenzoyl)-3*H*-pyrazol-3-ylidene]-bisphosphonic Acid Tetraethyl Ester (8e). ^{1}H NMR δ 7.49 (d, *J* = 7.5, 1 H), 7.35 (m, 1 H), 7.24 (m, 2 H), 6.82 (brd s, 1 H), 4.24 (m, 8 H), 3.67 (t, *J* = 25.6, 2 H), 2.37 (s, 3 H), 1.33 (m, 12 H); IR (Nujol) 3180, 1629, 1602, 1583, 1545, 1257, 1042, 1022 cm^{-1}; MS *m/e* 460 (M$^+$).

[5-(2,4-Dichlorobenzoyl)-2,4-dihydro-3*H*-pyrazol-3-ylidene]-bisphosphonic Acid Tetraethyl Ester (8f). ^{1}H NMR δ 7.42 (d, *J* = 7.8, 2 H), 7.30 (m, 2 H), 7.18 (brd s, 1 H), 4.24 (m, 8 H), 3.64 (t, *J* = 25.8, 2 H), 1.31 (m, 12 H); IR (Nujol) 3052, 1634, 1588, 1555, 1537, 1263, 1241, 1057 cm^{-1}; MS *m/e* 514 (M$^+$).

[5-(Cyclohexylcarbonyl)-2,4-dihydro-3*H*-pyrazol-3-ylidene]-bisphosphonic Acid Tetraethyl Ester (8g). ^{1}H NMR δ 6.77 (t, *J* = 3.67, 1 H), 4.24 (m, 8 H), 3.45 (t, *J* = 25.5, 2 H), 3.24 (dt, J_d = 2.8, J_t = 11.2, 1 H), 1.9–1.2 (m, 22 H); ^{13}C NMR δ 199, 148, 67, 65, 63.9, 63.4, 45.4, 36.3, 28.7, 25.5, 25.3, 16.1; IR (Nujol) 3186, 1656, 1552, 1449, 1267, 1253, 1241, 1162, 1041, 1019 cm^{-1}; MS *m/e* 452 (M$^+$).

[2,4-Dihydro-5-(4-methoxybenzoyl)-3*H*-pyrazol-3-ylidene]-bisphosphonic Acid Tetraethyl Ester (8h). ^{1}H NMR δ 8.19 (m, 2 H), 6.94 (m, 2 H), 6.87 (t, *J* = 3.94, 1 H), 4.25 (m, 8 H), 3.87 (s, 3 H), 3.69 (t, *J* = 25.5, 2 H), 1.35 (m, 12 H); IR (Nujol) 1626, 1594, 1575, 1563, 1513, 1262, 1241, 1059, 1022 cm^{-1}; MS *m/e* 476 (M$^+$).

[2,4-Dihydro-5-(1-oxopropyl)-3*H*-pyrazol-3-ylidene]-bisphosphonic Acid Tetraethyl Ester (8i). ^{1}H NMR δ 6.92 (t, *J* = 3.5, 1 H), 4.14–4.33 (m, 8 H), 3.48 (t, *J* = 25.6, 2 H), 2.82 (q, *J* = 7.4, 2 H), 1.30–1.36 (m, 12 H), 1.12 (t, *J* = 7.5, 3 H); ^{13}C NMR δ 196, 149, 66 (t), 64 (d), 36, 31, 8; IR (Nujol) 3210, 1664, 1265, 1245, 1046, 1024 cm^{-1}; MS *m/e* 398 (M$^+$).

[5-(2-Fluorobenzoyl)-2,4-dihydro-3*H*-pyrazol-3-ylidene]-bisphosphonic Acid Tetraethyl Ester (8j). ^{1}H NMR δ 8.20 (m, 2 H), 6.93 (m, 3 H), 4.24 (m, 8 H), 3.69 (t, *J* = 25.5, 2 H), 1.34 (m, 12 H); IR (Nujol) 1632, 1614, 1545, 1488, 1263, 1241, 1042 cm^{-1}; MS *m/e* 464 (M$^+$).

[5-(4-Chlorobenzoyl)-2,4-dihydro-3*H*-pyrazol-3-ylidene]-bisphosphonic Acid Tetraethyl Ester (8k). ^{1}H NMR δ 8.11 (m, 2 H), 7.41 (m, 2 H), 7.04 (t, *J* = 3, 1 H), 4.24 (m, 8 H), 3.68 (t, *J* = 25.6, 2 H), 1.34 (m, 12 H); IR (Nujol) 3202, 1630, 1588, 1572, 1560, 1259, 1240, 1091, 1021, 980, 919 cm^{-1}; MS *m/e* 480 (M$^+$).

[2,4-Dihydro-5-(4-methylbenzoyl)-3*H*-pyrazol-3-ylidene]-bisphosphonic Acid Tetraethyl Ester (8l). ^{1}H NMR δ 8.03 (d, *J* = 8.0, 2 H), 7.24 (d, *J* = 8.0, 2 H), 6.90 (brd s, 1 H), 4.23 (m, 8 H), 3.69 (t, *J* = 25.6, 2 H), 2.40 (s, 3 H), 1.32 (m, 12 H); IR (Nujol) 3205, 1719, 1628, 1604, 1574, 1557, 1509, 1260, 1058, 1022 cm^{-1}; MS *m/e* 460 (M$^+$).

General Procedure for Bisphosphonic Acids. In a 50-mL round-bottom flask equipped with a magnetic stirrer and nitrogen inlet, the bisphosphonate tetraester (4.6 mmol) was dissolved in chloroform (10 mL), treated with TMS-Br (27.6 mmol), and then heated to 40 °C for 5 h. After concentrating in vacuo, the crude material was diluted with EtOAc and water and then stirred for 30 min. The layers were separated, and the aqueous layer was treated with Darco, filtered through Celite, and lyophilized to give the crude, hydroscopic tetraacid. The acid was converted to the appropriate salt form in methanol and the precipitate filtered and air-dried.

(5-Benzoyl-2,4-dihydro-3*H*-pyrazol-3-ylidene)bisphosphonic Acid Disodium Salt (10a). ^{1}H NMR (D_2O) δ 7.9–7.45 (m, 5 H), 3.53 (t, *J* = 24, 2 H); IR (Nujol) 3209, 1599, 1572, 1267, 1178, 1098, 1029, 1002, 914 cm^{-1}; MS *m/e* 379 (M$^+$).

[5-(Cyclohexylcarbonyl)-2,4-dihydro-3*H*-pyrazol-3-ylidene]-bisphosphonic Acid Dipotassium Salt (10b). ^{1}H NMR (D_2O) δ 3.31 (t, *J* = 25, 2 H), 3.11 (m, 1 H), 1.69 (m, 5 H), 1.30 (m, 5 H); IR (Nujol) 3177, 1630, 1547, 1350, 1166, 1138, 1076, 1058, 972 cm^{-1}; MS *m/e* 495 (M+K_2).

General Procedure for Alkylation of Pyrazoline Bisphosphonic Esters. In a 25-mL round-bottom flask, **8a** (2.50 mmol) was dissolved in THF (5 mL) and treated with DBU (0.75 mL, 5.0 mmol) and alkylating agent (**9a**, **9b**: 5 mmol; **9c**, **9d**: 1.25 mmol). The reaction was stirred at 22 °C for 30 min, and then it was diluted with EtOAc, washed with 1 N HCl and saturated $NaHCO_3$, dried with $MgSO_4$, and concentrated in vacuo. The crude products were chromatographed on silica gel.

(5-Benzoyl-2,4-dihydro-2-methyl-3*H*-pyrazol-3-ylidene)-bisphosphonic Acid Tetraethyl Ester (9a): sample chromatographed with EtOAc and then EtOAc/acetone (7:3); ^{1}H NMR δ 8.10 (m, 2 H), 7.52 (m, 1 H), 7.42 (m, 2 H), 4.24 (m, 8 H), 3.82 (t, *J* = 27.9, 2 H), 3.59 (s, 3 H), 1.32 (m, 12 H); IR (neat) 2983, 1624, 1617, 1599, 1576, 1539, 1255, 1062, 1024 cm^{-1}; MS *m/e* 460 (M$^+$).

3-Benzoyl-5,5-bis(diethoxyphosphinyl)-4,5-dihydro-1*H*-pyrazole-1-acetic Acid Methyl Ester (9b): sample chromatographed with EtOAc and then EtOAc/acetone (7:3); ^{1}H NMR δ 8.1 (m, 2 H), 7.53 (m, 1 H), 7.42 (m, 2 H), 4.59 (s, 2 H), 4.24 (m, 8 H), 3.83 (t, *J* = 27.0, 2 H), 3.79 (s, 3 H), 1.34 (m, 12 H); IR (neat) 1762, 1743, 1627, 1599, 1577, 1546, 1254, 1060, 1035 cm^{-1}; MS *m/e* 518 (M$^+$).

[1,3-Propanediylbis(3-benzoyl-1*H*-pyrazol-5(4*H*)-ylidene)]tetrakisphosphonic Acid Octaethyl Ester (9c): sample chromatographed with EtOAc/acetone (6:2); ^{1}H NMR δ 8.1 (m, 4 H), 7.48 (m, 2 H), 7.34 (m, 6 H), 4.20 (m, 16 H), 3.83 (t, *J* = 7.56, 4 H), 3.80 (t, *J* = 27.7, 4 H), 2.43 (m, 2 H), 1.28 (m, 24 H); IR (neat) 1624, 1617, 1599, 1576, 1538, 1253, 1060, 1026 cm^{-1}; MS *m/e* 933 (M$^+$).

[1,3-Phenylenebis(3-benzoyl-1*H*-pyrazol-5(4*H*)-ylidene)]-tetrakisphosphonic Acid Octaethyl Ester (9d): sample chromatographed with EtOAc/acetone (1:1); ^{1}H NMR δ 8.0 (m, 4 H), 7.37 (m, 10 H), 4.91 (s, 4 H), 4.22 (m, 16 H), 3.83 (t, *J* = 27.3, 4 H), 1.28 (m, 24 H); IR (neat) 1737, 1703, 1626, 1619, 1598, 1576, 1545, 1255, 1028, 1023 cm^{-1}; MS *m/e* 995 (M$^+$).

Biological Procedures. Adjuvant-Induced Polyarthritis. Groups of 10 male Wistar rats (200 g) were challenged with an intradermal injection of complete Freund's adjuvant (*M. tuberculosis* in mineral oil) in the left hindpaw on day 0. Test compounds were dissolved or suspended in sterile saline and sonicated where appropriate to homogeneous doses. All compounds were then adjusted to pH 7.0 with 1 N NaOH and stored frozen in aliquots. Fresh aliquots were used for each day of dosing. Rats were dosed once daily (sc) for 28 days on a milligram/kilogram of body weight basis. The normal rat control group received vehicle po and the arthritic rat control group received vehicle sc. Changes in paw volume over time (ΔPV) which occurred in the arthritic control and treated rats (injected and noninjected hindpaws) were quantitated on day 28 by mercury-displacement plethysmography. Results were analyzed by one way analysis of variants then Student's unpaired *t* test.

Antigen-Induced Arthritis. Groups of 10 female C57Bl/6 mice, 6–8 weeks of age, were immunized sc with an emulsion of methylated bovine serum albumin (mBSA) and Freund's complete adjuvant supplemented with extra heat killed *M. tuberculosis*. 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 into the left rear stifle joint. The compounds were dissolved, suspended, or emulsified in sterile saline and sonicated where appropriate to homogeneous doses. All compounds were then adjusted to pH 7.0 with 1 N NaOH 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 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). Statistical comparisons were performed by one-way analysis of variance (ANOVA), compared to vehicle-treated controls.

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. Three weeks later, hydroxyapatite (HA) discs (6-mm diameter) soaked in mBSA solution (30 mg/mL saline) were implanted sc in the dorsum of the mice (two discs, bilaterally). All drugs were prepared as solutions, suspensions, or emulsions, and the pH was adjusted to 7.4 with 0.1 M NaOH. Each mouse

received compound in a volume of 0.1 mL/10 g 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 nine, 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.

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