Studies on Novel Bone Resorption Inhibitors. I. Synthesis and Pharmacological Activities of Aminomethylenebisphosphonate Derivatives[1)]

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Received June 24, 1992

A series of aminomethylenebisphosphonate derivatives was synthesized and evaluated for their antiresorptive activities using a parathyroid hormone (PTH)-induced hypercalcemia model in rats (PIH model). Among these compounds, (cycloheptylamino)methylenebis(phosphonic acid) (3j) was finally selected for further investigation, proving 10-fold as potent as pamidronate in the PIH model and an immobilization bone atrophy model in rats (DA model). The structure–activity relationships of this series of bisphosphonates are discussed.

Keywords bisphosphonate; YM175; bone resorption inhibitor; osteoporosis; pamidronate; etidronate

Osteoporosis is characterized by a decrease in bone mass and increase in susceptibility to fracture.[2)] Although the pathogenesis of osteoporosis is not well understood, the number of patients diagnosed as osteoporosis has been increasing relative to the increase in aged population. Currently, drugs such as estrogen,[3)] calcitonin[3)] and vitamin D derivatives[3)] are used for the treatment of osteoporosis. However, since these drugs are insufficiently effective clinically,[3)] a need exists for superior drugs. The bisphosphonates, which are carbon analogues of pyrophosphate, have been regarded as a potential new class of therapeutic agents for osteoporosis because of their antiresorptive activity.[4)] However, their side effects, including the inhibition of mineralization,[5)] limit their clinical use to hypercalcemia[6)] and Paget's disease.[6b,7)] Bisphosphonates without such side effects are potential therapeutic agents for osteoporosis. We therefore prepared a new series of bisphosphonate derivatives (**3**, **7**, **10**, **13**, **15**, **17** and **19**) and evaluated their antiresorptive activity with the aim of developing a potent bisphosphonate without the activity to inhibit mineralization. The new (alkylamino)methylenebisphosphonate derivatives, especially (cycloalkylamino)methylenebisphosphonate derivatives, have potent antiresorptive activity.

We describe here the synthesis and antiresorptive activities of (cycloalkylamino)methylenebisphosphonates and related derivatives.

Synthesis

As shown in Chart 1, the substituted aminomethylenebisphosphonates (**3**) listed in Table I were synthesized according to the method described in the literature.[8,9)] Namely, treatment of the amines (**1**) with triethyl orthoformate and diethyl phosphite gave the tetraethyl bisphosphonates (**2**). Subsequent hydrolysis of the bisphosphonate ester (**2**) under acidic conditions gave the desired bisphosphonates (**3**). Surprisingly, when 1-aminopiperidine (**4**) was used as an amine, deaminated tetraethyl piperidinomethylenebisphosphonate (**2q**) was obtained. Subsequent hydrolysis of **2q** with concentrated hydrochloric acid gave

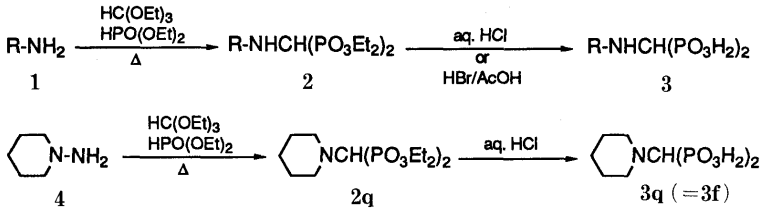


Chart 1

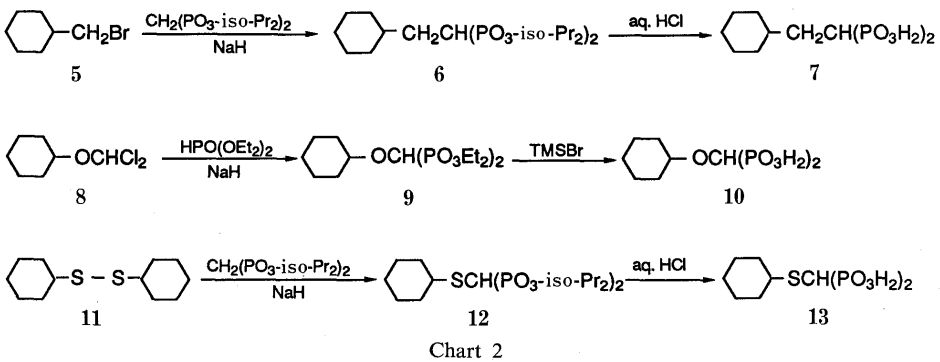
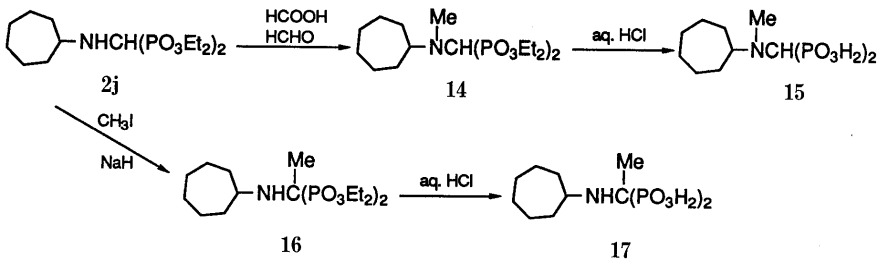
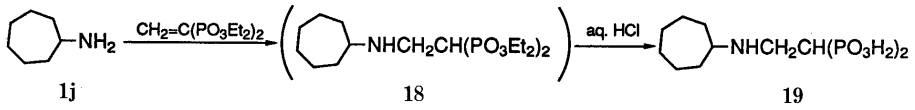


Chart 2



2j 14 15 16 17

Chart 3

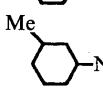
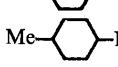


1j 18 19

Chart 4

TABLE I. Physical Data for Bisphosphonates (3)

R–CH(PO_3H_2)$_2$

Compd. No.	R	Yield (%)	mp (°C) (Recrys. solv.)[a]	Formula	Analysis (%) Calcd C	H	N	P	Found C	H	N	P
3a[b]	*n*-Pr–NH–	36.5[c]	228—230 (H–M)	$C_4H_{13}NO_6P_2 \cdot 1H_2O$	19.13	6.02	5.58	24.67	19.49	5.87	5.33	24.59
3b[b]	iso-Bu–NH–	68.3[c]	246—247 (H–M)	$C_5H_{15}NO_6P_2$	24.30	6.12	5.67	25.07	24.25	5.93	5.49	25.36
3c[d]	$(n\text{-Pr})_2$N–	51.8[c]	218—220 (H–A)	$C_7H_{19}NO_6P_2 \cdot 1/5H_2O$	30.16	7.01	5.02	22.22	30.55	6.94	4.90	21.74
3d	$(n\text{-Pr})_2$CHNH–	18.5[c]	248—250 (H–A)	$C_8H_{21}NO_6P_2$	33.22	7.32	4.84	21.42	33.20	7.17	4.92	21.43
3e[b]		45.0[e]	267—269 (L)	$C_7H_{17}NO_6P_2$	30.78	6.27	5.13	22.68	30.48	6.11	5.16	22.17
3f[f]		57.1[c]	248—250 (H)	$C_6H_{15}NO_6P_2$	27.81	5.83	5.41	23.91	27.60	5.75	5.38	23.84
3g		48.8[e]	214—216 (H–M)	$C_4H_{11}NO_6P_2 \cdot 1/5H_2O$	20.47	4.89	5.96	26.39	20.45	4.73	5.83	26.33
3h		63.1[e]	256—258 (M)	$C_5H_{13}NO_6P_2$	24.50	5.35	5.71	25.27	24.51	5.23	5.66	25.19
3i[b]		65.1[e]	228—229 (H–M)	$C_6H_{15}NO_6P_2 \cdot 1/10H_2O$	27.62	5.87	5.37	23.74	27.42	5.67	5.48	23.66
3j		63.1[e]	232—233 (H–M)	$C_8H_{19}NO_6P_2$	33.46	6.67	4.88	21.57	33.25	6.58	4.91	21.47
3k		64.8[e]	234—236 (H–M)	$C_9H_{21}NO_6P_2$	35.89	7.03	4.65	20.57	35.79	6.80	4.82	20.56
3l[g]		52.3[e]	238—240 (M–A)	$C_8H_{19}NO_6P_2$	33.46	6.67	4.88	21.57	33.07	6.39	4.86	21.83
3m[g]		78.6[e]	227—229 (H–M)	$C_8H_{19}NO_6P_2 \cdot 3/10H_2O$	32.84	6.75	4.79	21.17	32.63	6.61	4.78	21.41
3n[g]		66.2[e]	255—258 (H–M)	$C_8H_{19}NO_6P_2$	33.46	6.67	4.88	21.57	33.13	6.41	4.75	21.41
3o	 (*exo*)	85.1[e]	232—234 (H–M)	$C_8H_{17}NO_6P_2 \cdot 1/2H_2O$	32.66	6.17	4.76	21.06	32.65	6.03	4.77	20.89
3p	 (*endo*)	36.3[e]	233—234 (H–M)	$C_8H_{17}NO_6P_2 \cdot 1H_2O$	31.69	6.32	4.62	20.43	31.72	6.18	4.65	20.32

a) A, acetone; H, H_2O; L, aq. HCl; M, MeOH. b) See reference 13. c) Based on **1**. d) See reference 14. e) Based on **2**. f) See reference 15. g) Mixture of *cis* and *trans*.

piperidinomethylenebis(phosphonic acid) (**3q**). The structure of **3q** was identical with that of **3f**, which was derived from piperidine. This fact was confirmed by comparison of the physical and spectral data for **3q** and **3f**. Compound **6** was synthesized according to the method described in the literature.[10] Treatment of cyclohexylmethylbromide (**5**) with tetraisopropyl methylenebisphosphonate in the presence of NaH gave **6**. Hydrolysis of **6** with concentrated hydrochloric acid gave cyclohexylethylidenebisphosphonate (**7**) (Chart 2). Further, cyclohexyloxymethylenebisphosphonate (**10**) was synthesized according to the method described in the literature,[11] as shown in Chart 2. Namely,

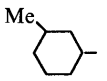
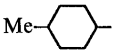
treatment of cyclohexyl dichloromethylether (**8**) with diethyl phosphite in the presence of NaH gave the tetraethyl phosphonate (**9**), and **9** was then hydrolyzed with bromotrimethylsilane to give **10**. Treatment of cyclohexyl disulfide (**11**) with tetraisopropyl methylenebisphosphonate in the presence of NaH afforded tetraisopropyl bisphosphonate (**12**). Subsequent hydrolysis of **12** with concentrated hydrochloric acid gave cyclohexylthiomethylenebisphosphonate (**13**). Methylation of **2j** was carried out as shown in Chart 3. Treatment of **2j** with formic acid and formaldehyde, followed by hydrolysis of **14** with concentrated hydrochloric acid gave *N*-methyl bisphosphonate (**15**), and treatment of **2j** with MeI in the presence of NaH, followed by hydrolysis of **16** with concentrated hydrochloric acid afforded 1-methyl bisphosphonate (**17**). Compound **19** was synthesized as shown in Chart 4. Treatment of cycloheptylamine (**1j**) with tetraethyl ethylidenebisphosphonate[12] followed by hydrolysis of **18** with concentrated hydrochloric acid gave (cycloheptylamino)ethylidenebisphosphonate (**19**).

Pharmacological Results and Discussion

The antiresorptive activities of the bisphosphonates thus obtained were evaluated on the basis of ability to reduce serum Ca^{2+} concentration in the parathyroid hormone (PTH)-induced hypercalcemia model in rats (PIH model), as described in Experimental. The results are listed in Table II. First of all, we focused our efforts on investigating the effect of the substituent on the nitrogen and the nitrogen functionality on the antiresorptive activity. Compounds **3b** and **3e**, which have bulkier substituents than compound **3a**, showed more potent antiresorptive activity than **3a**. On the other hand, the activity of **3d** was slightly decreased. Further, the antiresorptive activity of the tertiary amino derivatives **3c** and **3f** was remarkably decreased by comparison with that of **3e**. Similarly, compound **15** showed less potent activity than **3j**. These results suggest that a sufficiently bulky substituent such as a cycloalkyl ring on the secondary nitrogen atom is necessary for potent antiresorptive activity. Further, we investigated the indispensability of the aminomethylene group between the cycloalkyl ring and bisphosphonic acid. A remarkable decrease in potency was observed upon replacing the amino moiety of **3e** with other functional groups, such as methylene (**7**), oxygen (**10**) and sulfur (**13**). Moreover, introducing a methyl group at the C-1 atom of compound **3j** or elongating the aminomethylene moiety of **3j** to an aminoethylene moiety resulted in a decreased antiresorptive activity (compounds **17** and **19**). These results suggest that the amino moiety is indispensable and an unsubstituted methylene moiety is desirable as a link chain for potent antiresorptive activity. Subsequently, the substituent effect of the cycloalkyl ring on the nitrogen atom was examined by replacing the cyclohexyl ring of **3e** with other cycloalkyl rings (**3g—k**). As the size of cycloalkyl ring was expanded from a three-(**3g**) to six-(**3e**) or seven-membered ring (**3j**), antiresorptive activity was increased, but a remarkable decrease in potency was observed in the compound with the eight-membered ring (**3k**). These results suggest that antiresorptive activity is greatly affected by the lipophilicity of the cycloalkyl ring, in other words, the activity progressively increases with increase in the lipophilicity of the cycloalkyl ring, but only up to the seven-membered ring (**3j**). However, differences in potency were observed between compounds **3l**, **3m** and **3n**, which all contain a methyl group on the cyclohexyl ring of compound **3e**. The 2-methyl derivative (**3l**) and 4-methyl derivative (**3n**) showed more potent activities than the 3-methyl derivative (**3m**). Therefore, it seems that not only the lipophilicity of the substituent on the nitrogen atom but also the conformation of the compound is important to optimize the activity, because these compounds (**3l**, **3m** and **3n**) may have almost the same lipophilicity. To confirm the inference that the conformation of the compound is important to optimize the activity, we compared the potencies of two compounds (**3o** and **3p**) which have the same lipophilicity and basicity as each other. As anticipated, a large difference in potency was observed between the *exo*-norbornane derivative (**3o**) and its *endo*-isomer (**3p**), **3o** being 10-fold as potent as **3p**.

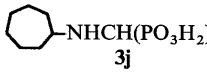
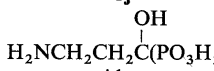
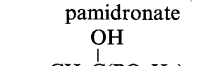
Table II. Effects of Bisphosphonates in PIH Model

R–X–Y–$(PO_3H_2)_2$

Compd. No.	R	X	Y	MED[a]
3a	*n*-Pr–	–NH–	–CH–	0.3
3b	iso-Bu–	–NH–	–CH–	0.1
3c	$(n\text{-Pr})_2$–	–N–	–CH–	>1.0
3d	$(n\text{-Pr})_2$CH–	–NH–	–CH–	1.0
3e		–NH–	–CH–	0.03
3f			–CH–	>1.0
3g		–NH–	–CH–	1.0
3h		–NH–	–CH–	0.3
3i		–NH–	–CH–	≤0.1
3j		–NH–	–CH–	0.03
3k		–NH–	–CH–	0.3
3l[b]		–NH–	–CH–	≤0.1
3m[b]		–NH–	–CH–	0.3
3n[b]		–NH–	–CH–	0.1
3o		–NH–	–CH–	0.03
3p		–NH–	–CH–	0.3
7		$-CH_2-$	–CH–	>0.3
10		–O–	–CH–	>0.3
13		–S–	–CH–	>0.3
15		Me –N–	–CH–	0.1
17		–NH–	Me –C–	0.1
19		–NH–	$-CH_2CH-$	1.0

a) Minimum effective dose, mg/kg, s.c. *b*) Mixture of *cis* and *trans*.

TABLE III. Comparison of Potencies of Bisphosphonates

Structure	PIH model (MED,[a] mg/kg) s.c.	PIH model (MED,[a] mg/kg) *p.o.*	DA model (MED,[a] mg/kg) *p.o.*
 **3j**	0.03	30	10
 pamidronate	0.3	300	100
 etidronate	30	>300	N.T.[b]

a) Minimum effective dose. *b*) Not tested.

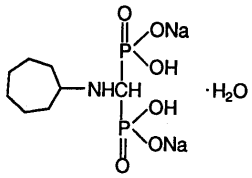


Fig. 1. Structure of YM175

This result suggests that the conformation of the compound is an important factor determining the antiresorptive activity, and the lipophilicity of the substituent on the nitrogen atom is another.

The structure–activity relationships described above demonstrated that the secondary nitrogen atom bearing a sufficiently bulky substituent, such as a cycloalkyl ring, is necessary for potent antiresorptive activity, and that the lipophilicity of the substituent on the nitrogen atom and the conformation of the compound are also important determinants of the antiresorptive activity. Among the potent compounds (**3e**, **3j** and **3o**), compound **3j** was selected for further development on the basis of the results of preliminary toxicological studies on these compounds (data not shown). The potencies of compound **3j**, pamidronate[6b] and etidronate[16] were compared in two animal models, the PIH model and the immobilization bone atrophy model in rats (DA model), as described in Experimental. The results are listed in Table III. Compound **3j** was 10-fold and 1000-fold as potent as pamidronate and etidronate, respectively. Compound **3j** was therefore selected for clinical investigation in the form of disodium dihydrogen (cycloheptylamino)methylenebis(phosphonic acid)monohydrate (YM175, Fig. 1).

We found no unfavorable effects of YM175 on bone metabolism under the conditions employed. YM175 possessed activity to increase bone strength in osteoporotic rats,[17] and did not induce osteomalacia even at a toxic dose.[18] YM175 is currently under phase II clinical study.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra were obtained with a JEOL JNM-EX90, JNM-FX100 or JMN-FX270 spectrometer using $CDCl_3$, DMSO-d_6 or D_2O as a solvent. Chemical shifts are given in δ values (ppm) using tetramethylsilane as an internal reference, and coupling constants are given in Hz. Mass spectra (MS) were obtained with a JEOL JMS-DX300 or Hitachi M-80 mass spectrometer. Infrared (IR) spectra were obtained with a Hitachi 270-30 infrared spectrophotometer. Elementary analyses were carried out on a Yanaco MT-3 or MT-5 CHN analyzer. Column chromatography was performed using silica gel (Wakogel C-200). Yields were not optimized. Spectral data for **2** and **3** are shown in Tables IV and V, respectively. Elemental analyses of **3** are shown in Table I.

TABLE IV. Yields and Spectral Data for Tetraethyl Bisphosphonates (**2**)

Compd. No.	Yield (%)	^{1}H-NMR ($CDCl_3$), δ (ppm)	FAB-MS *m/z*
2e	36.4	0.88—2.00 (11H, m, CH_2, NH) 1.32 (12H, t, J=7 Hz, CH_3) 2.60—2.96 (1H, m, CH) 3.42 (1H, t, J=22 Hz, CH) 4.00—4.42 (8H, m, CH_2)	386 (M^++H) 248
2g	44.7	0.42 (4H, d, J=5 Hz, CH_2) 1.33 (12H, t, J=8 Hz, CH_3) 1.94 (1H, br s, NH) 2.66 (1H, t, J=5 Hz, CH) 3.40 (1H, t, J=22 Hz, CH) 3.96—4.16 (8H, m, CH_2)	344 (M^++H) 206
2h	51.0	1.32 (12H, t, J=7 Hz, CH_3) 1.54—2.56 (7H, m, CH_2, NH) 3.22 (1H, t, J=22 Hz, CH) 3.42—3.72 (1H, m, CH) 4.02—4.42 (8H, m, CH_2)	358 (M^++H) 220
2i	82.0	1.32 (12H, t, J=7 Hz, CH_3) 1.44—2.00 (9H, m, CH_2, NH) 3.30 (1H, t, J=22 Hz, CH) 3.40—3.60 (1H, m, CH) 4.00—4.36 (8H, m, CH_2)	372 (M^++H) 234
2j	64.2	1.22—2.04 (13H, m, CH_2, NH) 1.34 (12H, t, J=7 Hz, CH_3) 2.78—3.16 (1H, m, CH) 3.36 (1H, t, J=23 Hz, CH) 3.98—4.36 (8H, m, CH_2)	400 (M^++H) 262
2k	79.6	1.32—1.96 (15H, m, CH_2, NH) 1.36 (12H, t, J=8 Hz, CH_3) 2.84—3.20 (1H, m, CH) 3.36 (1H, t, J=22 Hz, CH) 4.02—4.44 (8H, m, CH_2)	414 (M^++H) 276
2l[a]	49.7	0.76—2.60 (13H, m, CH_3, CH_2, CH, NH) 1.36 (12H, t, J=7 Hz, CH_3) 2.76—3.08 (1H, m, CH) 3.40 (1H, t, J=22 Hz, CH) 3.92—4.44 (8H, m, CH_2)	400 (M^++H) 262
2m[a]	49.3	0.64—2.40 (12H, m, CH_3, CH_2, CH) 1.36 (12H, t, J=7 Hz, CH_3) 2.20 (1H, br s, NH) 2.60—3.20 (1H, m, CH) 4.00—4.42 (8H, m, CH_2)	400 (M^++H) 262
2n[a]	56.6	0.80—2.08 (12H, m, CH_3, CH_2, CH) 1.34 (12H, t, J=7 Hz, CH_3) 1.98 (1H, br s, NH) 2.48—3.24 (1H, m, CH) 3.36, 4.02 (1H, each t, J=22 Hz, CH) 4.00—4.40 (8H, m, CH_2)	400 (M^++H) 262
2o	87.4	0.82—2.52 (11H, m, CH_2, CH, NH) 1.34 (12H, t, J=7 Hz, CH_3) 2.86—3.20 (1H, m, CH) 3.32 (1H, t, J=22 Hz, CH) 3.98—4.40 (8H, m, CH_2)	398 (M^++H) 260
2p	73.9	1.04—2.46 (11H, m, CH_2, CH, NH) 1.36 (12H, t, J=7 Hz, CH_3) 3.28 (1H, t, J=22 Hz, CH) 3.34—3.60 (1H, m, CH) 4.04—4.44 (8H, m, CH_2)	398 (M^++H) 260

a) Mixture of *cis* and *trans*.

Synthesis of 2. Tetraethyl (Cyclopentylamino)methylenebis(phosphonate) (2i) A typical example is given to illustrate the general procedure.

TABLE V. Spectral Data for Bisphosphonates (3)

Compd. No.	^{1}H-NMR (D_2O), δ (ppm)		FAB-MS m/z
3a	0.98	(3H, t, J=8 Hz, CH_3)	234 (M^++H)
	1.54—1.96	(2H, m, CH_2)	
	3.32	(2H, t, J=8 Hz, CH_2)	
	3.50	(1H, t, J=18 Hz, CH)	
3b	1.02	(6H, d, J=8 Hz, CH_3)	248 (M^++H)
	1.84—2.28	(1H, m, CH)	
	3.20	(2H, d, J=8 Hz, CH_2)	
	3.26	(1H, t, J=17 Hz, CH)	
3c	0.96	(6H, t, J=7.2 Hz, CH_3)	276 (M^++H)
	1.80	(4H, m, CH_2)	
	3.24—3.72	(4H, m, CH_2)	
	3.76	(1H, t, J=19.5 Hz, CH)	
3d	0.96	(6H, t, J=7 Hz, CH_3)	290 (M^++H)
	1.16—1.88	(8H, m, CH_2)	
	3.16	(1H, t, J=17 Hz, CH)	
	3.48—3.74	(1H, m, CH)	
3e	1.04—2.36	(10H, m, CH_2)	274 (M^++H)
	3.34	(1H, t, J=17 Hz, CH)	
	3.40—3.68	(1H, m, CH)	
3f[a]	1.32—2.35	(6H, m, CH_2)	260 (M^++H)
	3.27	(1H, t, J=17.5 Hz, CH)	
	3.43—3.87	(4H, m, CH_2)	
3g	0.84	(4H, d, J=5.5 Hz, CH_2)	463 ($2M^+$+H)
	3.10	(1H, t, J=5.5 Hz, CH)	232 (M^++H)
	3.14	(1H, t, J=17.5 Hz)	
3h	1.68—2.64	(6H, m, CH_2)	246 (M^++H)
	3.08	(1H, t, J=17 Hz, CH)	
	4.20	(1H, t, J=4 Hz, CH)	
3i	1.48—2.48	(8H, m, CH_2)	260 (M^++H)
	3.56	(1H, t, J=19 Hz, CH)	
	3.84—4.32	(1H, m, CH)	
3j	1.32—2.44	(12H, m, CH_2)	288 (M^++H)
	3.14	(1H, t, J=17 Hz, CH)	
	3.56—4.02	(1H, m, CH)	
3k	1.20—2.48	(14H, m, CH_2)	603 ($2M^+$+H)
	3.34	(1H, t, J=17 Hz, CH)	302 (M^++H)
	3.60—4.00	(1H, m, CH)	
3l[b]	0.76—2.40	(9H, m, CH_2, CH)[c]	575 ($2M^+$+H)
	1.08	(3H, d, J=6 Hz, CH_3)	288 (M^++H)
	2.76—3.14	(1H, m, CH)	
	3.44	(1H, t, J=17 Hz, CH)	
3m[b]	0.70—2.36	(9H, m, CH_2, CH)	575 ($2M^+$+H)
	0.96	(3H, d, J=6 Hz, CH_3)	288 (M^++H)
	3.36—3.96	(2H, m, CH)	
3n[b]	0.64—2.28	(12H, m, CH_3, CH_2, CH)[c]	288 (M^++H)
	3.08—3.60	(1H, m, CH)	
	3.36	(1H, t, J=17 Hz, CH)	
3o	1.10—2.04	(8H, m, CH_2)	571 ($2M^+$+H)
	2.36—2.66	(2H, m, CH)	286 (M^++H)
	3.18	(1H, t, J=17 Hz, CH)	
	3.50—3.70	(1H, m, CH)	
3p	1.02—1.86	(7H, m, CH_2, CH)	571 ($2M^+$+H)
	1.94—2.46	(2H, m, CH)	286 (M^++H)
	2.56—2.76	(1H, m, CH)	
	3.20	(1H, t, J=16 Hz, CH)	
	3.80—4.16	(1H, m, CH)	

a) IR (KBr): 1220, 1132 (P–O) cm^{-1}. *b*) Mixture of *cis* and *trans*. *c*) Using DMSO-d_6 as a solvent.

A mixture of cyclopentylamine (3.00 g, 35.3 mmol), triethyl orthoformate (6.25 g, 42.2 mmol) and diethyl phosphite (19.45 g, 141 mmol) was stirred at 150 °C for 1.5 h with continuous removal of the ethanol formed. After cooling, the volatiles were removed *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (49 : 1) as an eluent to give **2i** (10.74 g, 82.0%) as a pale yellow oil. The other compounds (**2a**—**p**) were prepared similarly. The crude tetraesters (**2a**—**d** and **2f**) were used without purification in the next reaction.

Synthesis of 3. (Cyclopentylamino)methylenebis(phosphonic acid) (3i) A typical example is given to illustrate the general procedure.

A solution of **2i** (7.97 g, 21.5 mmol) in concentrated hydrochloric acid (80 ml) was stirred under reflux for 2.5 h, and then the solvent was evaporated off *in vacuo*. The residue was dissolved in a mixture of MeOH (80 ml) and acetone (160 ml), and the solution was stirred below 5 °C overnight. The precipitates were collected by filtration and washed with acetone. Recrystallization from a mixture of H_2O and MeOH gave **3i** (3.62 g, 65.1%) as colorless crystals. The other compounds except **3g** were prepared similarly.

(Cyclopropylamino)methylenebis(phosphonic acid) (3g) A mixture of **2g** (1.28 g, 3.73 mmol) and 25% HBr–AcOH (13 ml) was stirred at 45 °C for 2 h, then evaporated *in vacuo*. The residue was dissolved in a mixture of MeOH (15 ml) and acetone (20 ml), and the solution was stirred below 5 °C overnight. The precipitates were collected by filtration and washed with acetone. Recrystallization from a mixture of H_2O and MeOH gave **3g** (0.42 g, 48.8%) as colorless crystals.

Tetraethyl Piperidinomethylenebis(phosphonate) (2q)[19] A mixture of 1-aminopiperidine (3.50 g, 35 mmol), triethyl orthoformate (6.00 g, 40.5 mmol) and diethyl phosphite (21.20 g, 154 mmol) was stirred at 150 °C for 2 h with continuous removal of the ethanol formed. After cooling, the volatiles were removed *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (49 : 1) as an eluent to give **2q** (3.10 g, 24.4%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): 1.36 (12H, t, J=8 Hz, CH_3), 1.44—1.72 (6H, m, CH_2), 2.82—3.06 (4H, m, CH_2), 3.32 (1H, t, J=23 Hz, CH), 4.02—4.38 (8H, m, CH_2). FAB-MS m/z: 372 (M^++H), 234.

Piperidinomethylenebis(phosphonic acid) (3q) A solution of **2q** (1.10 g, 3.0 mmol) in concentrated hydrochloric acid (11 ml) was stirred under reflux for 4 h, and then the solvent was evaporated off *in vacuo*. The residue was dissolved in MeOH (30 ml), and the solution was stirred at room temperature for 2 h. The precipitates were collected by filtration and washed with MeOH and acetone. Recrystallization from H_2O gave **3q** (0.48 g, 62.5%) as colorless needles, mp 247—250 °C. ^{1}H-NMR (D_2O): 1.40—2.14 (6H, m, CH_2), 3.22 (1H, t, J=18 Hz, CH), 3.46—3.82 (4H, m, CH_2). FAB-MS m/z: 260 (M^++H). IR ν (KBr): 1220, 1132 cm^{-1}. *Anal.* Calcd for $C_6H_{15}NO_6P_2$: C, 27.81; H, 5.83; N, 5.41; P, 23.91. Found: C, 27.66; H, 5.80; N, 5.41; P, 23.78.

Tetraisopropyl 2-Cyclohexylethylidene-1,1-bis(phosphonate) (6) NaH (60% dispersion in mineral oil, 0.44 g, 11.0 mmol) was added to a solution of tetraisopropyl methylenebis(phosphonate) (3.44 g, 10.0 mmol) in *N*,*N*-dimethylformamide (DMF) (7 ml) at room temperature and the resulting mixture was stirred for 1 h. Cyclohexylmethyl bromide **5** (1.78 g, 10.0 mmol) was added dropwise and the resulting mixture was stirred at 100 °C overnight. After removal of the solvent *in vacuo*, the residue was dissolved in AcOEt, washed with brine, and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the residue was chromatographed on silica gel using $CHCl_3$–MeOH–28% NH_3 (100 : 1 : 0.1) as an eluent to give **6** (1.30 g, 29.5%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): 0.64—2.06 (13H, m, CH_2, CH), 1.36 (24H, d, J=7 Hz, CH_3), 2.28 (1H, tt, J=7, 24 Hz, CH), 4.56—5.02 (4H, m, CH). FAB-MS m/z: 441 (M^++H), 273.

2-Cyclohexylethylidene-1,1-bis(phosphonic acid) (7) A solution of **6** (1.20 g, 2.72 mmol) in concentrated hydrochloric acid (15 ml) was stirred under reflux for 3.5 h, and then the solvent was evaporated off *in vacuo*. The residual oil was crystallized from a mixture of MeOH and acetone to give **7** (0.57 g, 76.8%) as colorless crystals, mp 225—226 °C. ^{1}H-NMR (D_2O): 0.64—2.04 (13H, m, CH_2, CH), 2.40 (1H, tt, J=7, 23.5 Hz, CH). FAB-MS m/z: 545 ($2M^+$+H), 273 (M^++H). *Anal.* Calcd for $C_8H_{18}O_6P_2$: C, 35.30; H, 6.67; P, 22.76. Found: C, 35.26; H, 6.49; P, 22.83.

Tetraethyl Cyclohexyloxymethylenebis(phosphonate) (9)[11] NaH (60% dispersion in mineral oil, 6.0 g, 150 mmol) was added to a solution of diethyl phosphite (20.0 g, 150 mmol) in tetrahydrofuran (THF) (100 ml) with ice-bath cooling. The mixture was stirred for 2.5 h at room temperature, then cyclohexyl dichloromethylether **8** (10.0 g, 55 mmol) was added with ice-bath cooling and the whole was stirred at room temperature overnight. After removal of the solvent *in vacuo*, the residue was dissolved in AcOEt. This solution was washed with saturated $NaHCO_3$ solution and brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* and the residue was chromatographed on silica gel using $CHCl_3$–MeOH–28% NH_3 (50 : 1 : 0.1) as an eluent to give **9** (4.5 g, 21.3%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): 1.04—2.20 (10H, m, CH_2), 1.34 (12H, t, J=7 Hz, CH_3), 3.50—3.88 (1H, m, CH), 3.92—4.44 (9H, m, CH_2, CH). FAB-MS m/z: 387 (M^++H).

Cyclohexyloxymethylenebis(phosphonic acid) (10)[11] Bromotrimethylsilane (10.0 g, 65.3 mmol) was added dropwise to a solution of **9** (2.30 g, 5.96 mmol) in CH_2Cl_2 (12 ml) with ice-bath cooling. The mixture was stirred for 5 h at room temperature, then the solvent was evaporated off *in vacuo*. The residual oil was crystallized from a mixture of CH_3CN and acetone to give **10** (1.10 g, 67.4%) as colorless crystals, mp 145—146 °C. ^{1}H-NMR (D_2O): 1.00—2.16 (10H, m, CH_2), 3.44—3.78 (1H, m, CH), 4.14 (1H, t, J=18 Hz, CH). FAB-MS m/z: 549 ($2M^+$+H), 275 (M^++H).

Anal. Calcd for $C_7H_{16}O_7P_2$: C, 30.67; H, 5.88; P, 22.60. Found: C, 30.53; H, 5.67; P, 22.67.

Tetraisopropyl (Cyclohexylthio)methylenebis(phosphonate) (12) Tetraisopropyl methylenebis(phosphonate) (4.0 g, 11.6 mmol) was added to a suspension of NaH (60% dispersion in mineral oil, 0.5 g, 12.5 mmol) in DMF (18 ml) at room temperature and the mixture was stirred for 1 h. Dicyclohexyl disulfide (**11**) (3.0 g, 13.0 mmol) was added and the resulting mixture was stirred at 80 °C overnight and then at 150 °C for 7 h. After removal of the solvent *in vacuo*, the residue was dissolved in AcOEt. This solution was washed with brine, and dried over $MgSO_4$. The solvent was evaporated off *in vacuo* and the residue was chromatographed on silica gel using $CHCl_3$–MeOH (49 : 1) as an eluent to give **12** (1.0 g, 16.7%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): 1.08—2.24 (10H, m, CH_2), 1.38 (24H, d, J=7 Hz, CH_3), 2.84—3.32 (1H, m, CH), 2.96 (1H, t, J=22 Hz, CH), 4.64—5.12 (4H, m, CH). FAB-MS *m/z*: 459 (M^++H).

(Cyclohexylthio)methylenebis(phosphonic acid) (13) A solution of **12** (1.0 g, 2.18 mmol) in concentrated hydrochloric acid (10 ml) was stirred under reflux for 3 h, and then the solvent was evaporated off *in vacuo*. The residual oil was crystallized from a mixture of CH_3CN and acetone to give **13** (0.4 g, 63.5%) as colorless crystals, mp 234—236 °C. ^{1}H-NMR (D_2O): 1.00—2.24 (10H, m, CH_2), 2.76—3.12 (1H, m, CH), 3.16 (1H, t, J=22 Hz, CH). FAB-MS *m/z*: 291 (M^++H). *Anal.* Calcd for $C_7H_{16}O_6P_2S$: C, 28.97; H, 5.56; P, 21.35. Found: C, 28.73; H, 5.42; P, 21.49.

Tetraethyl [(*N*-Cycloheptyl-*N*-methyl)amino]methylenebis(phosphonate) (14) A mixture of **2j** (2.00 g, 5.0 mmol), 88% formic acid (1.57 g, 30.0 mmol) and 37%formaldehyde in water (0.81 g, 10.0 mmol) was stirred at 80 °C for 3.5 h. The resulting mixture was poured into ice-cold water and adjusted to pH 8 with 10% aqueous NaOH, then extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (49 : 1) as an eluent to give **14** (1.25 g, 60.4%) as a colorless oil. ^{1}H-NMR ($CDCl_3$): 1.22—2.16 (12H, m, CH_2), 1.34 (12H, t, J=7 Hz, CH_3), 2.64 (3H, t, J=2.5 Hz, CH_3), 2.74—3.10 (1H, m, CH), 3.62 (1H, t, J=25 Hz, CH), 4.00—4.26 (8H, m, CH_2). FAB-MS *m/z*: 414 (M^++H), 276.

[(*N*-Cycloheptyl-*N*-methyl)amino]methylenebis(phosphonic acid) (15) A solution of **14** (1.20 g, 2.90 mmol) in concentrated hydrochloric acid (15 ml) was stirred under reflux for 3 h, and then the solvent was evaporated off *in vacuo*. The residual oil was crystallized from a mixture of MeOH and CH_3CN to give **15** (0.20 g, 22.9%) as colorless crystals, mp 205—207 °C. ^{1}H-NMR (D_2O): 1.42—2.42 (12H, m, CH_2), 3.10 (3H, s, CH_3), 3.50 (1H, t, J=18 Hz, CH), 3.76—4.12 (1H, m, CH). FAB-MS *m/z*: 302 (M^++H). *Anal.* Calcd for $C_9H_{21}NO_6P_2 \cdot 1/2H_2O$: C, 34.84; H, 7.14; N, 4.51; P, 19.97. Found: C, 34.87; H, 6.91; N, 4.58; P, 19.87.

Tetraethyl 1-(Cycloheptylamino)ethylidene-1,1-bis(phosphonate) (16) Compound **2j** (1.50 g, 3.76 mmol) was added to a suspension of NaH (60% dispersion in mineral oil, 0.17 g, 4.25 mmol) in DMF (15 ml) with ice-cooling and the mixture was stirred for 0.5 h. MeI (0.57 g, 4.02 mmol) was added and the resulting mixture was stirred at room temperature for 4 h and then at 50 °C for 1.5 h. After removal of the solvent *in vacuo*, the residue was suspended in ice-cold water and extracted with $CHCl_3$. The extract was washed with brine, dried over $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (99 : 1) as an eluent to give **16** (1.05 g, 67.7%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$): 1.12—2.06 (16H, m, CH_3, CH_2, NH), 1.32 (12H, t, J=7 Hz, CH_3), 3.02—3.30 (1H, m, CH), 4.02—4.40 (8H, m, CH_2). FAB-MS *m/z*: 414 (M^++H), 276.

1-(Cycloheptylamino)ethylidene-1,1-bis(phosphonic acid) (17) A solution of **16** (1.00 g, 2.42 mmol) in concentrated hydrochloric acid (15 ml) was stirred under reflux for 4 h, and then the solvent was evaporated off *in vacuo*. The residue was dissolved in MeOH (20 ml), and the solution was stirred at room temperature for 3 h. The precipitates were collected by filtration and washed with acetone. Recrystallization from a mixture of H_2O and MeOH gave **17** (0.28 g, 38.5%) as colorless crystals, mp 214—215 °C. ^{1}H-NMR (D_2O): 1.34—2.36 (12H, m, CH_2), 1.60 (3H, t, J=13 Hz, CH_3), 3.72—4.04 (1H, m, CH). Negative FAB-MS *m/z*: 300 (M^+−H). *Anal.* Calcd for $C_9H_{21}NO_6P_2 \cdot 1/5H_2O$: C, 35.46; H, 7.08; N, 4.60; P, 20.32. Found: C, 35.41; H, 6.92; N, 4.71; P, 20.13.

2-(Cycloheptylamino)ethylidene-1,1-bis(phosphonic acid) (19) A mixture of cycloheptylamine (2.00 g, 17.7 mmol) and tetraethyl ethylidenebis(phosphonate) (6.40 g, 21.3 mmol) in THF (15 ml) was stirred at 60 °C for 5 h. After removal of the solvent *in vacuo*, the residue was refluxed in concentrated hydrochloric acid (80 ml) for 2 h, and then the solvent was evaporated off *in vacuo*. The residue was dissolved in a mixture of MeOH (5 ml) and acetone (10 ml), and the solution was stirred at room temperature for 3 h. The precipitates were collected by filtration and washed with acetone. Recrystallization from a mixture of H_2O and aceton gave **19** (0.40 g, 7.50%) as colorless crystals, mp 225—227 °C. ^{1}H-NMR (D_2O): 1.24—2.24 (12H, m, CH_2), 2.30 (1H, tt, J=7, 20 Hz, CH), 3.14—3.72 (3H, m, CH_2, CH). FAB-MS *m/z*: 302 (M^++H). *Anal.* Calcd for $C_9H_{21}NO_6P_2$: C, 35.89; H, 7.03; N, 4.65; P, 20.57. Found: C, 35.68; H, 6.80; N, 4.40; P, 20.29.

Antiresorptive Activity Reduction in Serum Ca^{2+} Concentration in Rats (PIH model): Test compound was administered subcutaneously or orally to rats (Wistar, male, 5-week-old, N=5). After three days, PTH (synthetic human 1—34, 30 μ/kg) was given intravenously. A blood sample was then collected with a vacuum tube 45 min after PTH injection. Free Ca(Ca^{2+}) concentration in serum was measured with an electrolyte analyzer (sera 252, Horiba Manufacturing Co., Ltd.). Results are expressed as minimum effective dose (MED, mg/kg). Statistical significance of differences was analyzed by using the one-way ANOVA test (p<0.05).

Inhibition of Decrease in Dry Weight of the Bone in Rats (DA model): In rats (Wistar, male, 5-week-old, N=5), a 4 mm or longer section of the left brachial plexus was excited to immobilize the left forelimb. Test compound was administered orally every day for two weeks after immobilization and then the left humerus was removed. After the bone had been dehydrated and defatted with alcohol and acetone, its dry weight was measured. Results are expressed as MED (mg/kg). Statistical significance of differences was analyzed by using the one-way ANOVA test (p<0.05).

Acknowledgment We are grateful to Dr. Murase for his valuable suggestions concerning structure–activity relationships and to the staff of the pharmacological department for biological evaluations. Our thanks are also due to the staff of the analysis center for measurement of ^{1}H-NMR and mass spectra and elemental analysis.

References and Notes

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