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BISPHOSPHONATE PRODRUGS. SYNTHESIS AND IDENTIFICATION OF (1-HYDROXYETHYLIDENE)-1,1-BISPHOSPHONIC ACID TETRAESTERS BY MASS SPECTROMETRY, NMR SPECTROSCOPY AND X-RAY CRYSTALLOGRAPHY

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BISPHOSPHONATE PRODRUGS. SYNTHESIS AND IDENTIFICATION OF (1-HYDROXYETHYLIDENE)-1,1-BISPHOSPHONIC ACID TETRAESTERS BY MASS SPECTROMETRY, NMR SPECTROSCOPY AND X-RAY CRYSTALLOGRAPHY

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The preparation and identification of symmetric, $H_3CC(OH)[P(O)(OR)_2]_2$, where R=Me, Et, Pr^i, Ph, and non-symmetric, $H_3CC(OH)[P(O)(OR^1)(OR^2)][P(O)(OR^3)(OR^4)]$, where R^1=Me, R^2=R^3=R^4=Ph; R^1=R^2=R^3=Ph, R^4=Me; R^1=R^3=Me, R^2=R^4=Ph; R^1=R^2=Et, Pr^i, Ph and R^3=R^4=Me; tetraester derivatives of etidronate have been studied. Compounds were prepared from $HP(O)(OR^1)(OR^2)$ and $AcP(O)(OR^3)(OR^4)$ species under reflux. Mechanism studies have been made using $HP(O)(OCD_3)(OPh)$ and AcP(O)(OMe)(OPh) as starting materials. ^1H, ^{13}C, ^{31}P NMR data and the MS fragmentation data in the gas phase are reported. The solid-state structures are given for three of the compounds, where R=Et, Ph and R^1=R^2=Ph, R^3=R^4=Me.

Keywords: etidronate; esters; NMR; MS; X-ray

INTRODUCTION

Chemically and enzymatically stable bisphosphonate compounds, characterised by a P-C-P bridge, are commonly used in treatment of various diseases of bone and calcium metabolism[1]. These compounds bind strongly

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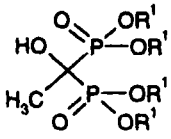
to calcium phosphate and inhibit its formation, aggregation and dissolution. The affinity for the bone mineral is the basis for their use as skeletal markers and inhibitors of bone resorption. Etidronate, like other bisphosphonates, is a highly polar tetra acidic compound with a low bioavailability, only 3–7% of the drug dose[2]. One way to decrease the polarity and to increase the absorption is to prepare derivatives like tetraesters which are non-ionic compounds. Tetraesters can be synthesised by esterification of etidronic acid or using esterified monophosphorous compounds as starting material[3,4]. Previously some studies have been made from tetramethyl and tetraethyl esters of etidronate[3,4,5,6], but this is the first systematic study of symmetric and non-symmetric tetraester derivatives of etidronate. The prepared "simple" tetraesters **1–4** were used as model compounds to study new prodrug approaches for etidronate.



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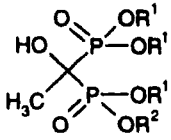

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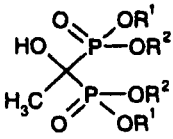

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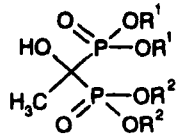


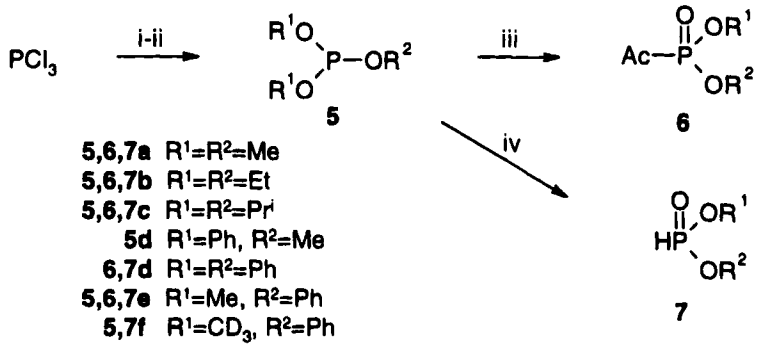
4

Tetramethyl, tetraethyl and some cyclic esters of etidronate have been synthesised earlier and also studied with NMR and IR spectroscopy[3,4]. The crystal structure is determined only for tetramethyl ester[6]. No mass spectra information from tetraesters of etidronate were found in the literature.

RESULTS AND DISCUSSION

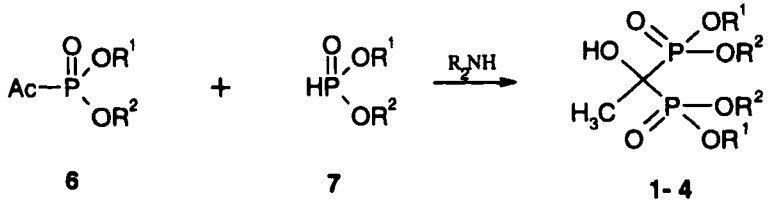
Synthesis

Monophosphorous starting materials **6** and **7**, were prepared from phosphorous trichloride by known methods[7,8] by first changing clorine atoms with selected R groups followed by adding either acetyl chloride or water (see Scheme 1). Deuterated derivatives **5f** and **7f** were prepared using the above approach by using CD_3OD as the alcohol species.



SCHEME 1 i) R^1OH; ii) R^2OH/pyridine; iii) acetyl chloride; iv) H_2O

Symmetric and unsymmetric tetraesters of (1-hydroxyethylidene)-1,1-bisphosphonates were prepared from dialkyl acetylphosphonate **6** and dialkylphosphite **7**, in the presence of catalytic amount of dialkylamine (see Scheme 2). The synthesised tetraester derivatives with yields and ^{31}P NMR chemical shifts are listed in Table I. The yields for the symmetric tetraesters **1a-c** were rather high, 81–92%, but the tetraphenyl derivative **1d** was obtained only with 38% yield due to a formation of the acetylated tetraphenyl derivative $H_3CC(AcO)[P(O)(OPh)_2]_2$.



SCHEME 2

During the synthesis of the mixed etidronate tetraesters **2–4** rather unexpected phenomena were observed indicating that the reaction mechanism is more complex than proposed. It is known that acetylated monophosphonates **6** can react to the corresponding acetylated tetraalkyl etidronate

derivatives at high temperature[5] but in our experiments at 50°C this by-product was observed only for **6d**. When diphenyl acetylphosphonate (**6d**), the starting material for **1d**, was synthesized, *ca.* 42% of the acetylated tetraphenyl derivative was obtained indicating the presence of the $AcOP(OPh)_2$ intermediate, which is the key reagent to acetylated etidronate derivatives. In this case the rearrangement process from the five coordinated phosphorus to the three coordinated one is probably promoted by conjugation effect since the aromatic rings can delocalise and stabilize the lone pair electrons from the three coordinated phosphorus atom rather easily. On the other hand, when amines have been used as catalyst, trace amounts of the corresponding etidronate tetraesters with free hydroxyl group have been found[9].

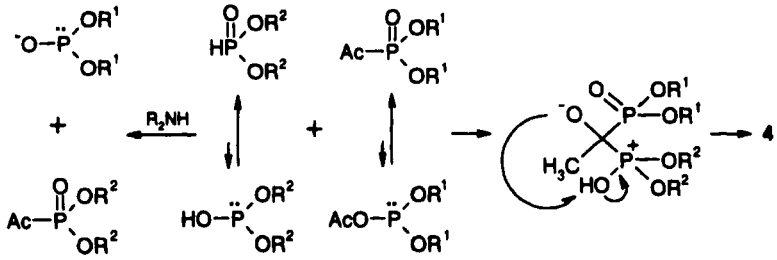
TABLE I ^{31}P NMR shifts and yields for tetraesters of etidronate. Yields were not optimised

			Starting material		*Yield*	*^{31}P*	
Product	*R^1*	*R^2*	*6*	*7*	*(%)*	*P*	*P'*
1a	Me	Me	a	a	89	22.92	22.92
1b	Et	Et	b	b	92	21.02	21.02
1c	Pr^i	Pr^i	c	c	81	19.40	19.40
1d	Ph	Ph	d	d	38	12.56	12.56
2a	Me	Ph	e	a	48[a]	22.57, 22.55	18.34, 18.56
2b	Ph	Me	e	d	72	17.71, 17.22	12.86, 12.82
3	Me	Ph	e	e	16[b]	18.09[c], 1811[d]	17.82[d]
4a	Me	Et	a	b	38[e]	23.37	20.60
4b	Me	Pr^i	a	c	43[f]	23.75	18.72
4c	Me	Ph	a	d	55	22.12	13.43

a. 20% of 3 as by-product.
b. 50% of 2a as by-product.
c. Meso form.
d. Preparation and analysis of the spectrum was made with PERCH software[10].
e. 10% of 1a as by-product.
f. 20% of 1a as by-product.

However, these findings do not explain the observed by-products **1a** and **3** from our experiments where mixed etidronate tetraesters were prepared. The reaction mixture of **2a**, as based on the ^{31}P NMR spectrum, contained

also **1a** (~3%) and **3** (~20%). In the case of **4a** and **4b, 1a** was detected with 10% and 20% yields, respectively. The only rational explanation to this is that the acetyl group in dialkyl acetylphosphonate is broken down and reacts with dialkyl phosphite as described on Scheme 3.



SCHEME 3

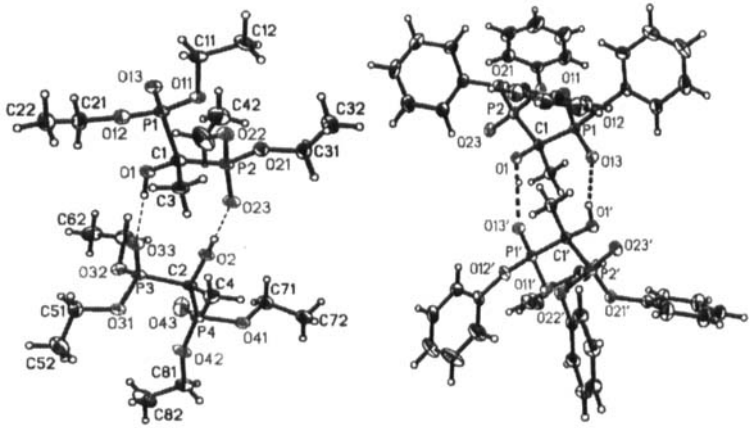
A special case was the synthesis of the compound **3**, because the reaction mixture contained not only **1a** (~2%) but **2a** about three times more than **3**. According to our experiments with the deuterated phosphite **7f** [HP(O)(OCD_3)(OPh)] used to prepare the compound **3**, two molecules of **7f** have to react with each other to give -$P(OCD_3)_2$ as intermediate since $H_3CC(OH)[P(O)(OCD_3)_2][P(O)(OMe)(OPh)]$ was found to be the main product over the expected product, according to the ^{13}C and ^{31}P NMR spectra. From the ^{31}P spectrum the numbers of OCD_3 and OCH_3 groups were detected from the proton irradiated spectrum.

Another unexpected product was obtained when diphenyl acetylphosphonate (**6d**), the starting material for **1d**, was synthesized. In this case about 42% of the tetraphenyl ester of etidronate was formed which hydroxyl group was acetylated. We tried to purify **6d** by a vacum distillation but that was not successful and the synthesis was continued without further purification. **1d** and the acetylated tetraphenyl ester of etidronate were then separated by column chromatography using dichloromethane/ethylacetate (85:15) as the eluent.

Crystal structures

The crystal structures were determined for the compounds **1b, 1d** and **4c**. In **1b** the asymmetric unit contains two independent molecules which are

hydrogen bonded together to form dimeric units shown in Fig. 1. The O1...O13' and O1'...O13 distances of 2.669(3) and 2.691(3) Å indicate medium strong intermolecular hydrogen bonds while the other contacts are normal van der Waals interactions. The hydrogen bonded dimeric unit posesses pseudo two fold rotation symmetry and the independent molecules are in the same conformation. The dihedral angles O(13)-P(1)-C(1)-P(2) and O(23)-P(2)-C(1)-P(2) are −170.4(2) and 62.3(2)° in molecule A and −171.6(2) and 57.1(2)° in molecule molecule B, respectively.



1b **1d**

FIGURE 1 X-ray structures of the compounds **1b** and **1d**

The asymmetric unit in **1d** contains two independent molecules as in **1b** but the space group is now P1 while it was P-1 in **1b**. The independent molecules form similar hydrogen bonded dimeric units as **1b** the O(1)...O(13)' and O(1)'...O(13) interactions being 2.661(2) and 2.652(2) Å, respectively. The molecules are not in the same conformation compared to **1b** as shown by Fig. 1. The dihedral angles O(13)-P(1)-C(1)-P(2) and O(23)-P(2)-C(1)-P(1) are 174.61(8) and 171.14(8)° in the molecule A, respectively. The corresponding angles in the molecule B are −174.96(8) and −170.93(8)°. The conformations of the molecules A and B differ only

in the orientation of the phenyl groups, Fig. 1. Based on our literature search this is the first P-C-P ester structure in which the P=O bonds are cis in the plane defined by P-C-P. In the molecule A O13 and O23 deviate −0.128(2) and 0.207(2) Å from the above mentioned plane and the corresponding atoms in the molecule B 0.120(2) and −0.213(2) Å, respectively.

The asymmetric unit of **4c** contains only one molecule (Fig. 2), the space group being P-1. Sentrosymmetrically related molecules form similar hydrogen bonded dimeric units as **1b** and **1d** the O(1)...O23(1-x, -y, 1-z) distance being 2.643(2) Å. The conformation of the molecule is comparable to **1b** with dihedral angles O(13)-P(1)-C(1)-P(2) and O(23)-P(2)-C(1)-P(1) being −59.11(9) and −171.20(7)°, respectively. In the determined compounds the corresponding bond lengths and angles are equal and quite comparable to other similar compounds.

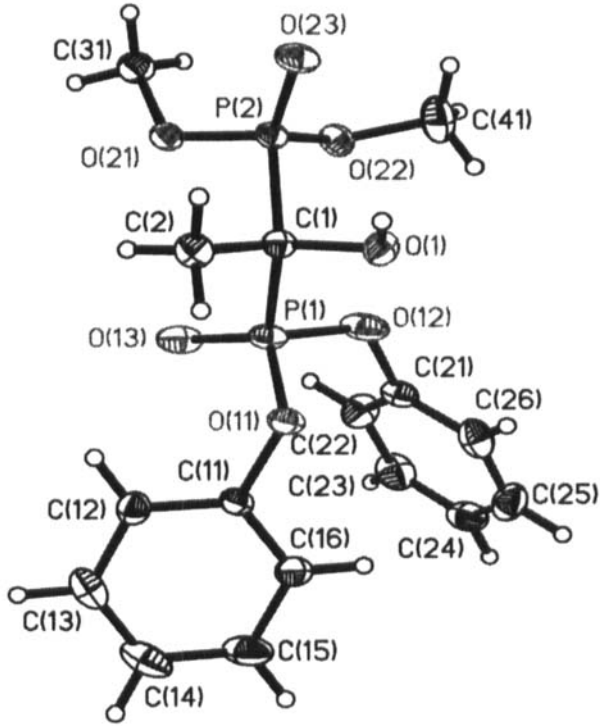


FIGURE 2 X-ray structure of the compound **4c**

Identification of the compounds

Formation of the correct products during the reactions were easily detected from ^1H spectra since the methyl protons signals at P-C-P backbone give rise to a characteristic triplet ($^3J_{HP}$= ~17 Hz) at 1.60–2.01 ppm due to the phosphorus couplings. The lowest value, 1.60 ppm, for the tetraisopropyl derivative **1c** and the highest, 2.01 ppm, for the tetraphenyl compound **1d** can be explained due to the shielding and anisotropic effects, respectively. The prochirality of the symmetric ester system is clearly seen from the ^1H spectrum of **1b**, since two CH_3 signals from the OEt moieties differ *ca.* 0.01 ppm from each other. Rather unexpected was the triplet observed for the tetraester compounds after crystallization or chromatography at *ca.* 5 ppm (e.g. for **1a** at 5.23 ppm) belonging to the O<u>H</u>-proton although measurements were performed in normal d-chloroform. This indicates a strong hydrogen bond from the O<u>H</u>-proton to the phosphorus double bonded oxygen. Compairing this result to the X-ray studies it is possible that instead of intramolecular hydrogen bonding two etidronate esters can form a dimeric unit like carboxylic acids in nonpolar solvents. On the other hand, a large $^2J_{PP}$ coupling constant (see later) compared to P-CH_2-P and P-CCl_2-P structures is an evidence of an intramolecular hydrogen bonding.

^{13}C chemical shifts were typical and characteristic for the prepared tetraester systems. In the case of symmetric esters the α-carbons appear as two separate lines (e.g. **1b** OCH_2 at 63.68 and 63.53ppm) due to prochirality of the molecule. J_{CP} coupling constants were at the expected range except the $^2J_{CP}$ coupling for the CH_3-group at the bridging carbon being only *ca.* 0–2 Hz while a typical value for a P-O-C case is *ca.* 7 Hz. The appearance of P-O-<u>C</u>-<u>C</u> carbon signals for the symmetric structures (**1a-d, 3**) were similar to the X_2MBP compound, being a virtual quintet due to AA'X spinsystem. However, in the spectra only a triplet was observed since the satellite signals for quintet were relatively small due to large $^2J_{PP}$ coupling (e.g. for **2a** 42 Hz). This coupling constant was also the most significant difference between previously studied X_2MBP derivatives ($^2J_{PP}$ ~ 10–20 Hz) and the compounds studied here. The large $^2J_{pp}$value can be explained based on the substituent effect to the hydroxyl group which is hydrogen bonded to the P=O group changing its polarity and hindering its rotation.

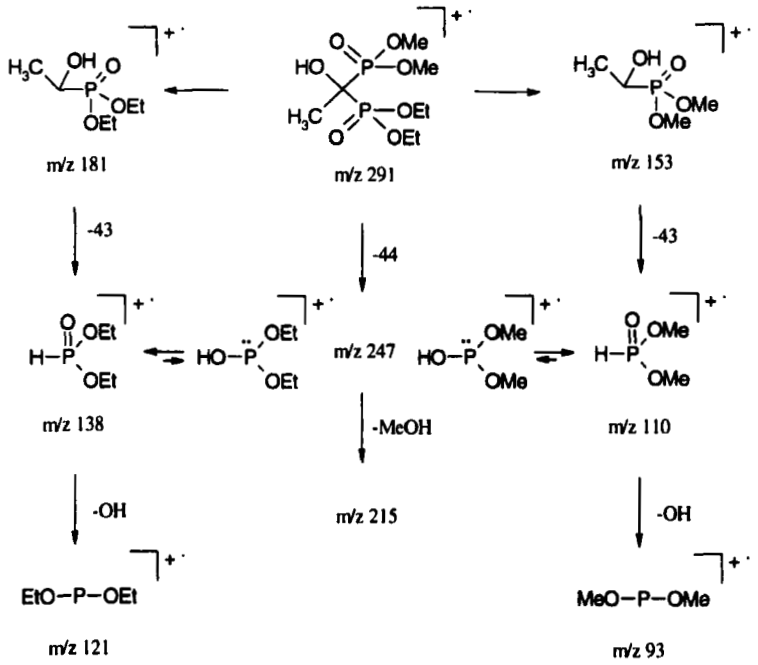
This is the first systematic study of the MS fragmentation pathways for tetraalkyl derivatives of etidronate. Previously tetraalkyl methylenebisphosphonate (MBP) derivatives, $X_2[P(O)(OR)_2]_2$ (X=H or Cl), have been

studied[8] in details but the fragmentation routes for the studied etidronate tetraesters are significantly different.

The mass spectra of the compounds **1a-c, 2a-b, 3, 4a-c** were studied systematically with electron ionization (EI at 70 eV) and chemical ionization (CI), and the fragmentation pathways were confirmed by accurate mass measurements of each fragment. The relative abundances (≥ 5%) of the ions formed in the fragmentation process are shown in Table II. The molecular ion peak was rather strong only for compound **4a** (9%), although clearly detected also for the rest of the compounds. Self-chemical ionization, fragmentation of the $[M+H]^+$ ions, is favourable for all the compounds except for **4c**. Two typical fragmentation examples, compounds **4a** and **2a**; are presented in Scheme 4 and 5.

TABLE II The 70 eV mass spectra of the compounds **1a-c, 2a-b, 3, 4a-c**. Peaks with relative intensities **(RI)** greater than 5% of the intensity of the base peak above m/z 50 are included

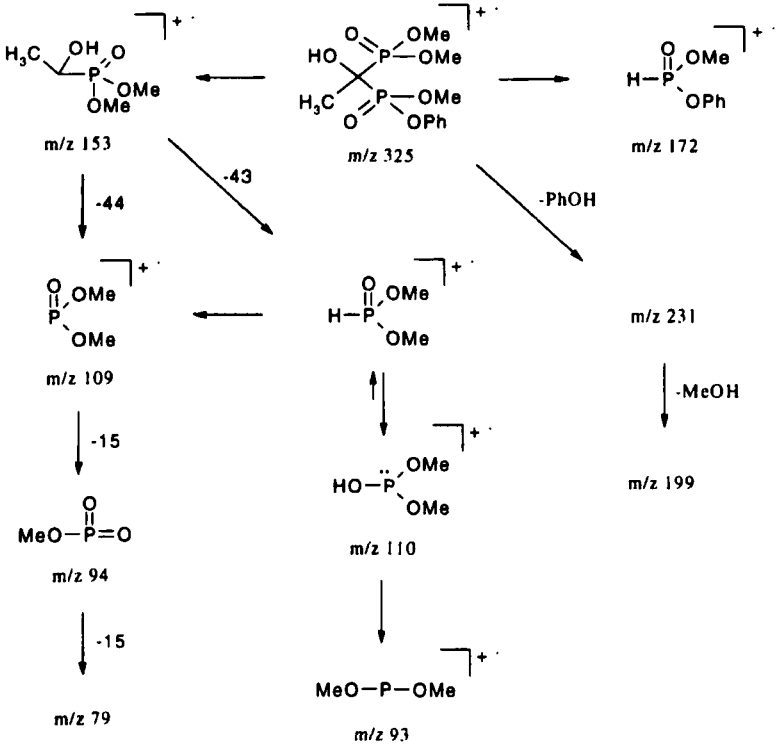
Comp.	*m/z (RI %)*
1a	187 (7), 153 (41), 127 (5), 124 (5), 111 (10), 110 (29), 109 (22), 95 (15), 93 (100), 80 (40), 79 (66), 75 (67), 69 (7), 63 (5), 57 (5).
1b	275 (5), 229 (7), 182 (13), 181 (100), 155 (18), 153 (14), 139 (8), 138 (20), 135 (5), 127 (9), 125 (7), 121 (63), 111 (39), 110 (9), 109 (14), 99 (13), 93 (64), 91 (7), 89 (54), 83 (32), 82 (33), 81 (19), 65 (43), 61 (11).
1c	233 (6), 209 (10), 207 (5), 206 (20), 189 (8), 168 (5), 167 (35), 163 (10), 126 (15), 125 (100), 124 (20), 123 (6), 109 (11), 107 (7), 99 (12), 83 (17), 82 (11), 61 (18).
2a	293 (9), 232 (5), 231 (100), 215 (5), 199 (10), 187 (13), 173 (5), 172 (18), 155 (5), 153 (9), 127 (5), 124 (8), 110 (25), 109 (33), 105 (11), 95 (10), 94 (94), 93 (42), 80 (12), 79 (32), 77 (29), 75 (5), 69 (9), 66 (28), 65 (25), 59 (10).
2b	356 (8), 355 (51), 234 (17), 155 (10), 140 (7), 136 (12), 95 (7), 94 (100), 93 (9), 77 (27), 66 (10), 65 (17).
3	294 (9), 293 (85), 215 (5), 199 (5), 172 (14), 171 (5), 155 (14), 136 (11), 105 (9), 95 (7), 94 (100), 93 (8), 79 (9), 77 (27), 66 (16), 65 (22).
4a	291 ($[M + H]^+$, 9), 247 (6), 215 (7), 181 (47), 154 (9), 153 (43), 138 (13) 127 (15), 121 (23), 111 (26), 110 (16), 109 (17), 95 (5), 93 (100), 89 (19), 83 (10), 82 (13), 81 (7), 80 (7), 79 (23), 75 (38), 65 (19), 61 (7).
4b	235 (7), 234 (8), 217 (11), 209 (7), 191 (27), 167 (18), 166 (8), 154 (25), 153 (37), 137 (7), 127 (23), 126 (5), 125 (90), 124 (59), 123 (20), 111 (15), 110 (36), 109 (42), 107 (13), 99 (8), 95 (9), 93 (100), 83 (12), 82 (18), 80 (16), 79 (39), 75 (37), 65 (7), 61 (15), 58 (5).
4c	293 (10), 234 (23), 233 (5), 170 (7), 153 (5), 149 (6), 140 (17), 124 (9), 111 (8), 110 (30), 109 (16), 97 (8), 95 (15), 94 (100), 93 (22), 85 (6), 84 (5), 83 (10), 82 (5), 81 (20), 80 (18), 79 (22), 77 (22), 73 (8), 71 (10), 69 (34), 66 (19), 65 (20), 60 (7), 57 (15), 55 (9).



SCHEME 4 The fragmentation of **4a**

The main fragmentation routes for the studied compounds were the fragmentation of P-C-P backbone and a loss of maximum two alkyls from P-C-P phosphorus ends. However, the main difference between X_2MBP and the etidronate tetraester derivatives is the base peak, which for the former is achieved either after loss of all alkyls from phosphorus ends or fragmentation of the P-C-P backbone of either side[8]. In the etidronate case the base peak is either fragment m/z 93 (**1a, 4a, 4b**) or m/z 94 (**2b, 3, 4c**). The phenyl containing derivative **2a** and tetraethyl derivative **1b** are special cases leading to base peaks at m/z 231 and 181 indicating loss of the phenol for **2a** as described in Scheme 4 and the fragmentation of the P-C-P backbone for **1b**.

The fragmentation of all alkyls leading to the etidronic acid, m/z 207 (20%), is observed only for the branched tetraisopropyl derivative **1c**. The



SCHEME 5 The fragmentation of **2a**

intensive peak at m/z 94 is typical only for phenyl containing derivatives. This is due to fragmentation of the P-C-P backbone followed by loss of CH_3CHOH (43) and Me units leading to the $MeOPO_2$ ion at 93.9333. On the other hand, compounds without phenyl lead to a peak at m/z 92.9157, which belongs to the $P(OMe)_2$fragment.

CONCLUSIONS

The products studied were easily prepared with good purity and reasonable yields using the approach developed. The structures of the compounds were identified by NMR and mass spectrometric methods. Mechanism of

the reaction was studied using deuterium derivative indicating transesterification processes between used monophosphorous starting materials. In the NMR studies rather large $^2J_{PP}$ (*ca.* 40 Hz) coupling was characteristic for P-C-P bridge and small, only 2 Hz $^2J_{CP}$ coupling to the bridge methyl. In the solid state the studied molecules exist as dimeric units bound together with -O-H···O=P hydrogen bonds. In the mass spectrometric studies two main fragmentation routes were observed: fragmentation of the P-C-P backbone leading to the base peak at m/z 94 or 93 and loss of two alkyls from the P-C-P phosphorus ends.

EXPERIMENTAL

General

All reagents used in reactions were dried and distilled before use. Trialkyl phosphites, dimethyl (**8a**), diethyl (**8b**) and diphenyl phosphite (**8d**) were purchased from Aldrich. Yields were not optimised. The purity of the compounds were ≥ 95%, unless stated otherwise. The ^1H, ^{31}P and ^{13}C NMR spectra were recorded on a Bruker AM 400 spectrometer operating at 400.1, 172.0 and 100.7 MHz, respectively. TMS was used as an internal standard for ^1H and ^{13}C measurements and 85% H_3PO_4 was as an external standard for ^{31}P measurements. $^3J_{HH}$ couplings are indicated by the letter "J" and all the values are given in Hz. Number of protons on each carbon were detected from DEPT-135 experiment and are marked after each carbon using letters d, t or q. $^nJ_{CP}$ couplings were calculated from carbon spectra and are given in Hertz in parenthesis. In the case of symmetric structures only the sums of the J_{CP} couplings (ΣJ_{CP}, the width of the virtual triplet) are given, since the satellite lines were unambiguous to detect from the backround. Mass spectra were obtained on a Varian VG 70–250SE spectrometer.

Details of the crystal parameters and structure determinations of compounds **1b**, **1d** and **4c** are summarised in Table III. The structures were solved by direct methods using SHELXS-97[11] and refined using SHELXL-97[12]. Nonhydrogen atoms were refined anisotropically. The hydrogen atoms were placed at calculated positions and not refined except for the -OH hydrogen atoms which were located from difference Fourier

maps and not refined for **1b** but refined for **1d** and **4c** with a fixed isotropic thermal parameters (U_{iso}=0.05 Å^2).

TABLE III Crystallographic data for the compounds **1b, d** and **4c**

	1b	*1d*	*4c*
Formula	$C_{10}H_{24}O_7P_2$	$C_{27}H_{24}O_7P_2$	$C_{17}H_{20}O_7P_2$
M	318.23	510.39	386.26
Crystal system	Triclinic	Triclinic	Triclinic
Space group	P-1	P1	P-1
a/Å	8.3102(9)	8.2307(2)	9.4998(3)
b/Å	14.1387(11)	11.1691(2)	10.1739(3)
c/Å	15.0087(12)	15.0551(2)	11.2174(4)
α/°	110.062(2)	110.307(1)	76.796(2)
β/°	104.948(3)	102.085(1)	67.864(2)
γ/°	92.519(3)	97.401(1)	65.978(2)
V/Å^3	1583.3(2)	1238.21(4)	913.59(5)
T/K	120(2)	120(2)	120(2)
Z	4	2	2
μ (Mo-Kα)/mm^{-1}	0.30	0.22	0.27
D_c/Mg m^{-3}	1.335	1.369	1.404
θ Range	3.09–26.00	2.59–27.49	2.63–27.49
Unique reflections	5780 (R_{int}=0.0626)	10072 (R_{int}=0.0149)	3862 (R_{int}=0.0179)
R [I>2σ(I)]	0.0540	0.0267	0.0304
wR (F^2, all data)	0.1481	0.0725	0.0835
GooF	1.007	1.013	1.082
$\Delta\rho_{max}/\Delta\rho_{min}$(e Å^{-3})	0.53/-0.49	0.28/-0.30	0.034/-0.035

Diphenyl methyl phosphite (5d)

Prepared from PCl_3 (60 g, 0.44 mol) and phenol (32 g, 0.34 mol), to give $(PhO)_2PCl$ (14.08 g, 33% b.p. 85–87°C/12 mbar) which was then treated with methanol (1.79 g, 0.057 mol) and pyridine (4.43 g, 0.056 mol) to give

5d (11.1 g, 80%, b.p. 117°C/0.3 mbar). NMR ($CDCl_3$): δ_H 7.33–7.29 (4H, m), 7.12–7.08 (6H, m), 3.79 (3H, d, $^3J_{HP}$=8.9); δ_P 6.18.

Dimethyl phenyl phosphite (5e)

Prepared from PCl_3 (60 g, 0.44 mol) and phenol (16 g, 0.17 mol), to give $PhOPCl_2$ (14.68 g, 44%, b.p. 85–87°C/12 mbar) which was then treated with methanol (4.8 g, 0.15 mol) and pyridine (11.78 g, 0.15 mol) to give **5e** (10.13 g, 73%, b.p. 45°C/0.3 mbar). NMR ($CDCl_3$): δ_H 7.32–7.28 (2H, m), 7.10–7.05 (3H, m), 3.65 (6H, d, $^3J_{HP}$=10.2); δ_P 11.14.

Dimethyl acetylphosphonate (6a)

Prepared by the known method[2] from trimethyl phosphite (33.2 g, 0.27 mol) and acetyl chloride (21.1 g, 0.27 mol) to give **6a** (36.7 g, 89%, b.p. 86–87°C/11 mbar). NMR ($CDCl_3$): δ_H 3.88 (6H, d, $^3J_{HP}$=10.7), 2.50 (3H, d, $^3J_{HP}$=5.3); δ_P −0.40.

Diethyl acetylphosphonate (6b)

Prepared from triethyl phosphite (9.2 g, 0.055 mol) and acetyl chloride (4.4 g, 0.056 mol) to give **6b** (8.0 g, 81%, b.p. 96–98°C/13 mbar). NMR ($CDCl_3$): δ_H 4.24 (4H, m), 2.49 (3H, d, $^3J_{HP}$=5.1), 1.39 (6H, J=7.1).

Diisopropyl acetylphosphonate (6c)

Prepared from triisopropyl phosphite (7.9 g, 0.038 mol) and acetyl chloride (3.0 g, 0.038 mol) to give **6c** (3.9 g, 49%, b.p. 86–87°C/11 mbar). NMR ($CDCl_3$): δ_H 4.79 (2H, m), 2.47 (3H, d, $^3J_{HP}$=5.0), 1.39 (12H, d, J=6.2); δ_P −3.84.

Diphenyl acetylphosphonate (6d)

Prepared from **5d** (2.58 g, 0.010 mol) and acetyl chloride (5 ml) to give **6d** with 56% purity which was used to prepare **1c** without further purification. NMR ($CDCl_3$): δ_H 7.37–7.09 (10H, m), 2.61 (3H, d, $^3J_{HP}$=5.7).

Methyl phenyl acetylphosphonate (6e)

Prepared from **5e** (2.55 g, 0.014 mol) and acetyl chloride (1.08 g, 0.014 mol) to give **6e** (2.0 g, 68%, b.p. 75–78°C/0.2 mbar). NMR ($CDCl_3$): δ_H 7.37–7.33 (2H, m), 7.24–7.18 (3H, m), 3.95 (3H, d, $^3J_{HP}$=10.9), 2.53 (3H, d, $^3J_{HP}$=5.5); δ_P – 5.63.

Diisopropyl phosphite (7c)

Prepared by known method[6] from triisopropyl phosphite (21 g, 0.1 mol) and water (1.8 g, 0.1 mol) to give **7c** (12.4 g, 75%, b.p. 70–78°C/ 5 mbar). NMR ($CDCl_3$): δ_P 4.97.

Methyl phenyl phosphite (7e)

Prepared from **5e** (3.45 g, 0.019 mol) and water (0.33 g, 0.018) to give **7e** (2.43 g, 76%, b.p. 35°C/0.8 mbar). NMR ($CDCl_3$): δ_H 7.24–7.19 (2H, m), 6.90–6.86 (3H, m), 6.79 (1H, d, $^1J_{HP}$=704.0), 3.79 (3H, d, $^3J_{HP}$=11.9); δ_P 11.23.

(1-Hydroxyethylidene)-1,1-bisphosphonic acid tetramethyl ester (1a)

Prepared following the procedure developed by Nicholson et al[3]. Dimethyl phosphite (**7a**) (26.6 g, 0.24 mol) and dibutylamine (3.1 g, 0.024 mol) in ether (350 ml) were cooled to 0°C; **6a** (36.7 g, 0.24 mol) was slowly introduced at this temperature with stirring to the mixture and it was allowed to warm to room temperature. Stirring was continued for 3 hours, the product was filtered and re-crystallised from dry toluene, to give **1a** as colourless crystals (56.2 g, 89%). NMR ($CDCl_3$): δ_H 5.23 (-O<u>H</u>, t, $^3J_{HP}$=9.6), 3.89 (12H, m, $^3J_{HP}$=10.7), 1.72 (3H, t, $^3J_{HP}$=16.4); δ_P 22.92; δ_C 71.81 t ($^1J_{CP}$=157.4), 54.47 qt (ΣJ_{CP}=6.8), 54.25 qt (ΣJ_{CP}=6.9), 20.13 q. (Found: $[M + H]^+$ 263.0538. Calc. For $C_6H_{17}O_7P_2$: $[M + H]^+$ 263.0450).

(1-Hydroxyethylidene)-1,1-bisphosphonic acid tetraethyl ester (1b)

Prepared similarly to **1a**, from **6b** and **7b**. The solids after evaporation of ether and freezing were washed with hexane to give **1b** as colourless crys-

tals with 92% yield. NMR ($CDCl_3$): δ_H 4.77 (-OH, t, $^3J_{HP}$=9.3), 4.23 (8H, m), 1.70 (3H, t, $^3J_{HP}$=16.3), 1.36 (6H, t, J=7.1), 1.35 (6H, t, J=7.1). δ_P 21.02. δ_C 71.47 t ($^1J_{CP}$=156.3), 63.68 tt (ΣJ_{CP}=6.9), 63.53 tt (ΣJ_{CP}=6.9), 20.15 q, 16.50 q. (Found: $[M + H]^+$ 319.1108. Calc. For $C_{10}H_{25}O_7P_2$: $[M + H]^+$ 319.1076).

(1-Hydroxyethylidene)-1,1-bisphosphonic acid tetraisopropyl ester (1c)

Prepared similarly to **1a**, from **6c** and **7c** in toluene without cooling and the reaction mixture was stirred for 5 hours at 50°C. Toluene was removed and the residue was dissolved in water and extracted three times with hexane followed by evaporation to give **1c** as a yellow oil with 81% yield. NMR ($CDCl_3$): δ_H 4.82 (4H, m), 1.60 (3H, t, $^3J_{HP}$=16.0), 1.37 (24H, m); δ_P 19.40; δ_C 72.29 dt (ΣJ_{CP}=7.0), 72.04 dt (ΣJ_{CP}=7.6), 71.18 t ($^1J_{CP}$=155.6), 24.45–23.65 m, 19.94 qt ($^2J_{CP}$=2.2). (Found: $[M + H]^+$ 375.1757. Calc. For $C_{14}H_{33}O_7P_2$: $[M + H]^+$ 375.1702).

(1-Hydroxyethylidene)-1,1-bisphosphonic acid tetraphenyl ester (1d)

Prepared similarly to **1a** from **6d** and **7d** but without cooling and diethylamine was used as a catalyst. The reaction mixture was refluxed for 30 hours, the solvent was removed and the product was purified by column chromatography using dichloromethane/ethylacetate (85:15) as eluent to give **1d** as a white solid with 38% yield. NMR ($CDCl_3$): δ_H 7.30–7.24 (8H, m) 7.21–7.18 (8H, m), 7.15–7.11 (4H, m), 2.01 (3H, t, $^3J_{HP}$=17.5). δ_P 12.56. δ_C 150.53 t, 129.69 d, 125.37 d, 120.77 t, 72.04 t ($^1J_{CP}$=161.1), 20.14 q. Crystallised from toluene for x-ray analysis.

[1-(Dimethoxyphosphonyl)-1-hydroxyethyl]-1-phosphonic acid methyl phenyl ester (2a)

Prepared similarly to **1c**, from **6e** and **7a**. After evaporation the residue was purified by column chromatography using ethylacetate as eluent to give **2a** as a colourless syrup with 48% yield, containing a pair of diastereomers (ratio 60:40). NMR ($CDCl_3$): δ_H 7.33–7.24 (4H, m), 7.17–7.14 (1H, m), 5.25 (-OH, t, $^3J_{HP}$=9.6), 5.16 (-OH, dd, $^3J_{HP}$=8.5, $^3J_{HP}$=10.2), 3.92–3.85 (9H, m), 1.802 and 1.799 (3H, t, $^3J_{HP}$=16.7); δ_P 22.57 d ($^2J_{PP}$=42.1)

18.34 d and 22.55 d ($^2J_{PP}$=40.6) 18.56 d; δ_C 150.65 d ($^2J_{CP}$=10.0), 150.57 d ($^2J_{CP}$=10.1), 129.63 d, 125.06 d, 120.58 dd ($^3J_{CP}$=4.3), 71.93 dd ($^1J_{CP}$=157.6, $^1J_{CP}$=160.1) and 71.90 dd ($^1J_{CP}$=155.4, $^1J_{CP}$=159.0), 55.14 q, 54.44 q, 20.11 q. (Found: $[M + H]^+$ 325.0671. Calc. For $C_{11}H_{19}O_7P_2$: $[M + H]^+$ 325.0606).

[1-(Diphenoxyphosphonyl)-1-hydroxyethyl]-1-phosphonic acid methyl phenyl ester (2b)

Prepared similarly to **1d**, from **6e** and **7d**, but the reaction mixture was refluxed for 5 hours. After evaporation the residue was purified by column chromatography using ethylacetate as eluent to give **2b** as a slightly yellow syrup with 72% yield and 85% purity. Pair of diastereomers (ratio 60:40). NMR ($CDCl_3$): δ_H 7.31–7.16 (15H, m), 4.59 (-OH, dd, $^3J_{HP}$=8.9, $^3J_{HP}$=10.4), 4.41 (-OH, dd, $^3J_{HP}$=7.6, $^3J_{HP}$=9.7), 3.91 (3H, d, $^3J_{HP}$=10.8), 1.964 and 1.967 (3H, t, $^3J_{HP}$=17.1); δ_P 17.71 d ($^2J_{PP}$=42.5) 12.86 d and 17.22 d ($^2J_{PP}$=45.2) 12.82 d. At the ^{13}C NMR spectrum signals from diastereomeric forms can not be separated from each other due to overlapping peaks: δ_C 150.50 s, 129.69 d, 125.38 d, 125.25 d, 120.78 d, 120.61 d, 120.20 d, 72.01 dd ($^1J_{CP}$=155.7, $^1J_{CP}$=157.6), 71.98 t ($^1J_{CP}$=162.7), 55.53 qd ($^2J_{CP}$=7.2), 55.31 qd ($^2J_{CP}$=7.3), 20.18 qt ($^2J_{CP}$=1.9), 20.09 qt ($^2J_{CP}$=2.2). (Found: $[M + H]^+$ 449.0990. Calc. For $C_{21}H_{23}O_7P_2$: $[M + H]^+$ 449.0919).

(1-Hydroxyethylidene)-1,1-bisphosphonic acid P,P'-dimethyl diphenyl ester (3)

Prepared similarly to **1d** from **6e** and **7e**, but the reaction mixture was refluxed for 10 hours. After evaporation the residue was purified by column chromatography using ethylacetate as eluent to give **3** as a colourless syrup with 16% yield. NMR ($CDCl_3$): δ_H 7.32–7.14 (10H, m), 5.42 (-OH, t, $^3J_{HP}$=10.0), 5.25 (-OH, dd, $^3J_{HP}$=8.8, $^3J_{HP}$=9.7), 3.91 (6H, m), 1.89 (3H, t, $^3J_{HP}$=16.9); δ_P 18.09 (meso form), 17.82 d ($^2J_{PP}$=44.3) 18.11 d; Signals from meso and diastereomeric forms can not be separated from each other due to overlapping peaks: δ_C 150.53 t, 129.68 d, 129.66 d, 125.13 d, 125.10 d, 120.60 d, 120.57 d, 72.02 t ($^1J_{CP}$=159.8), 71.96 t ($^1J_{CP}$=158.9), 55.29 q, 20.10 q. (Found: $[M + H]^+$ 387.0799. Calc. For $C_{16}H_{21}O_7P_2$: $[M + H]^+$ 387.0763).

[1-(Dimethoxyphosphonyl)-1-hydroxyethyl]-1-phosphonic acid diethyl ester (4a)

Prepared similarly to **1a**, from **6a** and **7b**, but the reaction mixture was stirred for 16 hours. After evaporation the residue was washed with hexane, evaporated and extracted four times with pentane. Pentane was removed and **4a** was obtained as a colorless oil with 38% yield. NMR ($CDCl_3$): δ_H 4.71 (-O<u>H</u>), 4.24 (4H, m), 3.88 (3H, d, $^3J_{HP}$=10.5), 3.87 (3H, d, $^3J_{HP}$=10.5), 1.70 (3H, t, $^3J_{HP}$=16.3), 1.36 (6H, t, J=6.8); δ_P 23.37 d ($^2J_{PP}$=39.3), 20.60 d; δ_C 71.71 t ($^1J_{CP}$=155.9), 63.81 dd ($^2J_{CP}$=7.4), 63.74 dd ($^2J_{CP}$=7.2), 54.32 qd ($^2J_{CP}$=7.0), 54.18 qd ($^2J_{CP}$=7.1), 20.09 qt ($^2J_{CP}$=1.9), 16.49 qd ($^3J_{CP}$=5.6), 16.47 qd ($^3J_{CP}$=5.7). (Found: $[M + H]^+$ 291.0810. Calc. For $C_8H_{21}O_7P_2$: $[M + H]^+$ 291.0763).

[1-(Dimethoxyphosphonyl)-1-hydroxyethyl]-1-phosphonic acid diisopropyl ester (4b)

Prepared similarly to **1c**, from **6a** and **7c**, but the reaction mixture was stirred for 24 hours. Solvent was removed and the product was purified by column chromatography using ethylacetate/methanol (90:10) as eluent to give **4b** as a colourless syrup with 43% yield. NMR ($CDCl_3$): δ_H 4.83 (2H, m), 3.87 (3H, d, $^3J_{HP}$=10.6), 3.87 (3H, d, $^3J_{HP}$=10.5), 1.66 (3H, t, $^3J_{HP}$=16.1), 1.37 (6H, d, J=6.2), 1.37 (6H, d, J=6.2); δ_P 23.75 d ($^2J_{PP}$=40.7) 18.72 d; δ_C 72.59 dd ($^2J_{CP}$=7.4), 72.46 dd ($^2J_{CP}$=7.6), 71.57 dd ($^1J_{CP}$=153.7, $^1J_{CP}$=157.3), 54.16 qd ($^2J_{CP}$=6.6), 54.10 qd ($^2J_{CP}$=6.3), 24.38 qd ($^3J_{CP}$=2.9), 24.25 qd ($^3J_{CP}$=2.9). 23.76 qd ($^3J_{CP}$=2.7), 23.70 qd ($^3J_{CP}$=2.3), 19.99 qt ($^2J_{CP}$=1.9). (Found: $[M + H]^+$ 319.1078. Calc. For $C_{10}H_{25}O_7P_2$: $[M + H]^+$ 319.1076).

[1-(Diphenoxyphosphonyl)-1-hydroxyethyl]-1-phosphonic acid dimethyl ester (4c)

Prepared similarly to **1d**, from **6a** and **7d**, but the reaction mixture was refluxed for 2 hours. Two layers were formed during this time and the lower layer was separated and dissolved in toluene and placed in the freezer overnight. The formed solid was filtered and re-crystallised from dry toluene to give **4c** as colourless crystals with 55% yield. NMR ($CDCl_3$): δ_H 7.31–7.26 (4H, m), 7.21–7.14 (6H, m), 4.66 (-O<u>H</u>, t,

$^3J_{HP}$=8.8), 3.88 (3H, d, $^3J_{HP}$=10.6), 3.87 (3H, d, $^3J_{HP}$=10.6), 1.87 (3H, dd, $^3J_{HP}$=16.3, $^3J_{HP}$=17.6); δ_P 22.12 d ($^2J_{PP}$=42.1), 13.43 d; δ_C 150.62 d ($^2J_{CP}$=10.3), 150.55 d ($^2J_{CP}$=10.4), 129.64 d, 129.56 d, 125.26 d, 120.77 dd ($^3J_{CP}$=4.3), 120.76 dd ($^3J_{CP}$=4.3), 71.92 dd ($^1J_{CP}$=157.1, $^1J_{CP}$=160.2), 54.68 qd ($^2J_{CP}$=7.2), 54.39 qd ($^2J_{CP}$=7.3), 20.13 q. (Found: $[M]^+$ 386.0828 Calc. For $C_{16}H_{20}O_7P_2$: $[M]^+$ 386.0684).

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