A stereoselective process for the preparation of novel phosphonoalkylphosphinates

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Abstract

We have devised both a contiguous and a stepwise strategy for the synthesis of the pyridylaminomethane-based class of phosphonoalkylphosphinates (PAPs) that form via the intermediacy of the phosphonoethoxyaminomethane **XIa**. The PAPs result from condensation of picolines and phosphonoacetals in high chemoselective yield. Following reduction of aminopyridine **IIIb**, the unprecedented Pt(0)-catalyzed epimerization of the chelated amidine [(hydroxy)methylphosphinyl]-[(3-methyl-2-piperidinyl-idene)amino]methylphosphonic acid (**IIIa**) yielded a single racemic pair of PAPs (**IIIc**). The epimerization was found to occur more slowly than amidine formation itself.

Keywords: Phosphorus; Platinum; Palladium; Phosphoric acid

1. Introduction

The methylene bisphosphonic acid series (**I**) of compounds (Fig. 1) have generated a great deal of interest as mediators of bone metabolism [1]. On the contrary, the bisphosphinic acid analogues (**II**) of the above series have been shown to lack bone affinity [2]. The ongoing work in our laboratories to further elucidate structure activity relationships of the bisphosphonates (BPs) has recently led to a study of the requirements of the phosphorus–carbon–phosphorus moiety for bone activity. Due to their notable bone affinities which we have reported [2,3] (Table 1), we have completed the syntheses of a number of methylene phosphonoalkylphosphinate (PAP) hybrids of the BPs. A key analogue subset in this series has been the diastereomeric mixture of the piperidinylideneaminomethane–phosphonomethylphosphinates **IIIa**.

Here we report a unique condensation that was designed to produce **IIIb**, the aminopyridine precursor of **IIIa**, that minimizes the formation of BP and BP byproducts (Scheme 1). In the presence of both phosphonous and phosphinous acid esters (e.g. **IVa** and **Va**), the iminoether **XII** will incorporate two phosphorus-based

nucleophiles with only slight statistical preference for the phosphonous type. The antiresorptive activities of the BPs that we have studied have been reported to range from at least two to three orders of magnitude greater than their corresponding PAPs [4]. Thus, to study the effect of a PAP on the bone remodeling process, its BP contamination level needs to be significantly below 0.1%. Due to their negligible bone affinity, biphosphinic contaminants are not a concern. The processes that we present here have allowed us to produce pyridylaminomethane-derived PAPs that meet this criteria.

2. Results and discussion

2.1. Synthesis of PAPs

The synthesis of the corresponding diastereomeric mixture of PAP **IIIa** has previously been described [3]
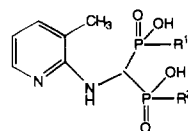
Ia: R^1,R^2 = OH Ib R^1,R^2 = OEt
II: R^1,R^2 = Alkyl
IIIb: R^1 = OH R^2 = Alkyl

Fig. 1.

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Table 1
Hydroxyapatite crystal growth inhibition

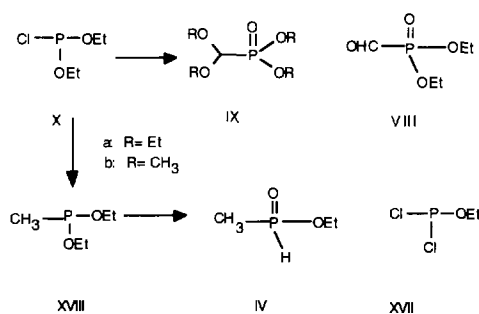| Compound	HAP-CGI [a]
Ethylidene-1,1-hydroxy bisphosphonic acid (EHDP)	
Ia	1.3
II	no activity
IIIa	46.6
IIIb	27.5

[a] HAP-CGI is the molar concentration required to inhibit crystal growth formation 50 min relative to EHDP.

Scheme 2.

by our laboratory. In a modification of a previously reported synthesis [5], equimolar portions of triethyl orthoformate, ethyl hydrogenmethylphosphinate (IVa), diethyl phosphite (Va), and 2-amino-3-methylpyridine (VI), were combined to give after chromatographic separation and subsequent hydrolysis, the desired PAP IIIb as its monohydrate in 17% yield. The deviation from the statistically predicted product distribution of Ib:II:IIIb = 1:1:2 is due to the increased reactivity of the phosphonous acid esters as compared to the phosphinous acid esters. Hydrogenation of IIIb over palladium on carbon provided the diastereomeric mixture of amidines IIIa.

For producing kilogram quantities of such amino methane PAPs that minimized contamination by their corresponding BPs, we designed an efficient synthetic strategy that allowed the selective combination of the phosphonate, and then the phosphinate moiety in a single step. A retrosynthetic analysis suggested that formylphosphonate VIII would be an appropriate precursor for the initial combination of the pyridylamino moieties, which would allow selective phosphinic acid moiety (Scheme 2). Because of the known difficulties in their preparation, and the instability of unprotected formylphosphonates, the corresponding diethoxy-methyl-phosphonic acid diethyl ester IXa was synthesized in high yield from triethyl orthoformate and diethyl chloro-phosphonite (X) [6]. Acetal IXa was then condensed with 2-amino-3-picoline in the presence of phosphinous acid ester IV. The three reactants were found to conveniently combine in one mixture on heat-

ing with the removal of ethanol. Upon addition of water, complete ester hydrolysis resulted. After cooling, IIIb crystallized from the reaction mixture in 40% yield. At this stage, contamination due to the corresponding BP Ia, arising from the reaction of diethyl phosphite (DEP) with the transient phosphonimine VIIa, was determined to be less than 1% by ^{31}P NMR and HPLC. DEP is probably formed during the thermal disproportionation of the phosphonoacetal, with amounts up to 1% known to be introduced as a contaminant of IVa. The tetramethyl analogue IXb was synthesized from the corresponding methyl precursors. No advantage was found in using IXb during the attempts to synthesize the methyl analogue of IIIb.
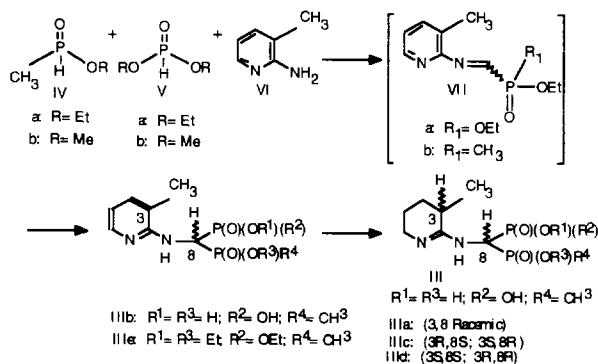
It was postulated that the condensation of 2-amino-3-picoline and phosphono-acetal IXa would yield the phosphonimine VIIa; however, none of this fully dehydrated product could be isolated by chromatography or distillation, or detected in situ (Scheme 3). Two independent routes to VIIa were investigated. Initial condensation of triethyl orthoformate and 2-aminopicoline yielded the ethoxyimine XII as predominantly the E isomer (9:1). Attempts to add one equivalent of DEP failed to yield any VIIa. Rather, the BP Ib was isolated in 44% yield, while the ethoxyimine and its v-formyl precursor XIII were recovered in a combined 50% yield. Furthermore, only diaddition was observed when
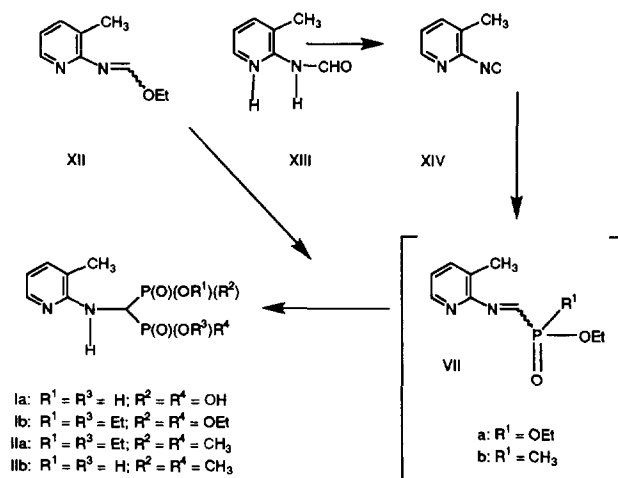
Scheme 1.
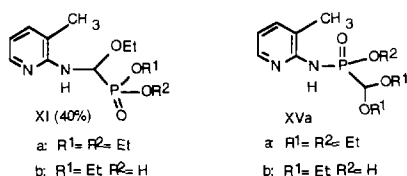
Scheme 3.

Scheme 4.

ethyl hydrogenmethylphosphinate was substituted for DEP, yielding the bisphosphinic acid diester **IIa**. None of iminophosphinate **VIIb** was detected. The attempted addition of either the phosphonate or the phosphinate to isonitrile **XIV** yielded the same diaddition products as before. In all cases studied, the phosphinate additions were more sluggish than the corresponding phosphonate additions. Although unsuccessful in isolating the phosphonoimine **VIIa** (Scheme 3), we had more success in synthesizing the corresponding ethanol adduct **XIa** (Scheme 4). It was found that the condensation of 2-amino-3-picoline and acetal **IXa** (Scheme 2) under similar conditions as those used during the one pot synthesis of **IIIb** (Scheme 1) yielded the mixture **XIa** and its half-acid ester **XIb** in 40% yield on a molar basis. Also formed in 40% yield was the half phosphonamide ester **XVa**, and the corresponding hydrolysis product, **XVb**. During the condensation process, a significant degree of partial phosphonate ester cleavage was observed, yielding **XIb** and **XVb**. The stability of **XIa** was poor, as it readily hydrolyzed to the half-acid ester **XIb**, but this pair could be enriched to about 90% by chromatographic means. Of these four compounds, only **XVb** proved too unstable to isolate in even an enriched form by chromatography. By treating the enriched mixture **XIa/XIb** with phosphinate **IV**, and then hydrolyzing the resultant mixture, a 90% yield of **IIIb** was obtained. The direct hydrolysis without isolation of the ethanol triester **IIIe** proved to be unavoidable. Although clearly observable by ^{31}P NMR, all attempts to isolate the corresponding triethyl ester **IIIe** failed, as it also exchanged ethanol in the presence of traces of water. A comparison of the yields achieved in our one pot route with this two stage process of isolation of **XIa/XIb** and its subsequent condensation show an excellent correlation between the production of **XIa/XIb** and the overall yield for the conversion of 2-amino-3-picoline into **IIIb** (35%). The value of the stepwise route, however, is that only one-third of the amount of valuable phosphinate **IV** is required.

### 2.2. Development of chemistry to a phosphonic acid source

Diethyl chlorophosphonite **X** and ethyl dichlorophosphonite **XVII** were obtained from the disproportionation of a 1:1 mixture of triethyl phosphite and phosphorus trichloride (Scheme 2). These compounds were easily separated by distillation. A twofold excess of triethyl

phosphite gave **X** in 90% yield. Treatment of **X** with triethyl orthoformate provided an excellent yield of phosphonoacetal **IXa**. Finally, methyl Grignard addition to **X** yielded diethyl methylphosphonite **XVIII**. The reaction was quite exothermic, and the order of addition impacted on our ability to moderate the process. While almost uncontrollable when adding the chlorophosphonite to the Grignard, the slow reverse addition of Grignard to chlorophosphonite was safely handled on the 100 g scale. Oxidative substitution of ethanol by water yielded phosphinate **IV**. [2]

2.3. Stereoselective amidine synthesis

The amidine mixture **IIIa** was obtained in high yield after palladium(0)-catalyzed hydrogenation of **IIIb**. The amidines are diastereotopic mixtures that can be separated by the uncommon chromatographic conditions of water/ammonium hydroxide/methanol on silica. The erythro $(3S,8R)$, $(3R,8S)$ isomeric pair **IIIc** was found to predominate only slightly over the threo $(3R,8R/3S,8S)$ pair **IIId** in a ratio of 55:45 (Scheme 1). This ratio showed a variance of 10% between many runs. The latter pair was less stable, and under aqueous alcoholic conditions, more facilely underwent ring opening to give the racemic amino amide (Scheme 5). The more stable pair of amidines **IIIc** could be hydrolyzed to racemic amino amide **XVIa** within minutes under aqueous basic conditions. Most significant to note, however, is that only very minor isomerization of the $(R,R/S,S)$ amidine **IIId** into the $(R,S/S,R)$ amidine **IIIc** was observed under the palladium reduction conditions. In an attempt to reduce the reaction time for the palladium-catalyzed reduction, even in the presence of almost stoichiometric quantities of palladium catalyst, Adams catalyst was investigated. Not surprisingly, the reaction rate was accelerated tremendously. However, we noted that after the hydrogenation had proceeded to about 80% conversion, there was a clear increase in the ratio of the erythro isomers at the expense of the threo ones. Once all of the starting aminopyridine **IIIb** was consumed, the isomerization proceeded to completion (> 95% by ^{1}H and ^{31}P NMR), yielding pure **IIIc**.

The typical reduction time for the platinum-catalyzed runs was around 30 h on the 250 g scale. Epimerization was readily followed by ^{31}P NMR. It was found that in 100 h, greater than 95% epimerization was effected. In

XVI
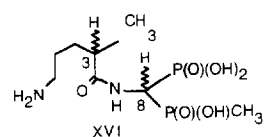
a: (3R, 8R;3S,8R)
b: (3R, 8R;3S,8S)

Scheme 5.

comparison, the original palladium-catalyzed reduction was only 20% completed in the same period, and took 10 days for complete conversion to the amidine systems IIIa. Here, even 2 weeks of reaction time failed to yield any noticeable change in the isomer ratio of amidines.

Attempts to epimerize the mixture isolated from the palladium reduction (IIIa) with PtO$_2$ in the absence of a hydrogen atmosphere failed to cause any isomerization. In the presence of the reduced metal, however, isomerization proceeded as before. In order to determine whether the hydrogen at C-3 or C-8 was epimerizing, the mixture (IIIa) was treated under reducing platinum conditions with deuterium in the presence of D$_2$O. Incorporation of deuterium was complete, and only observed at C-3 by NMR. No exchange at C-8 was detected. We believe that the amidines are better ligands for platinum(0) than for palladium(0). In the pH range (5–7) for the reductions, the acidity of the amidine-platinate complex is sufficiently high for equilibration to occur.

3. Structural assignments

A crystallographic analysis to confirm these stereochemical assignments has been discussed in a preliminary report [7].

4. Conclusion

After the discovery of a novel class of bone-active agents, the pyridyl amino methane PAPs, new synthetic methodology has been developed for facile synthesis. Methodology suitable for large-scale, as well as useful small-scale synthesis has been developed. Furthermore, an unusual Pt(0)-catalyzed epimerization was found to afford one single racemic pair of phosphonomethylphosphinate isomers.

5. Experimental section

5.1. 3-Methylpyridyl-2-aminomethylene-bisphosphonic acid (Ia)

The synthesis has been reported by Benedict and Perkins [8].

5.2. 3-Methylpyridyl-2-aminomethylene-bis(phosphonic acid, diethyl ester) (Ib)

5.2.1. From iminoether XII with base catalysis

To a solution of iminoether XII (2.20 g, 0.0144 mol) and DEP Va (2.00 g, 0.0144 mol) in absolute ethanol (25 ml) was added at 23 °C potassium t-butoxide (1.65 g,

0.0144 mol). After stirring for 50 min, the reaction was complete. No iminophosphonate VII was detected in the ^{1}H or ^{31}P spectra of the crude mixture. The mixture was purified by chromatography (SiO$_2$; EtOAc; R_f = 0.4), yielding BP Ib (1.95 g, 0.0050 mol; 69% based upon Va). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.96 (d, 1H), 7.24 (d, 1H), 6.56 (t, 1H), 5.73 (dt, 1H; methine), 4.62 (d, 1H; N–H), 4.18 (m, 8H), 2.17 (s, 3H), 1.19 (t, 6H), 1.16 (t, 6H). ^{31}P NMR 121.48 MHz, CDCl$_3$): δ 18.526. ^{13}C NMR (75.46 MHz, CDCl$_3$, proton and phosphorus coupled): δ 154.27 (C$_2$), 146.16, 143.80 (C$_6$), 138.62, 136.53 (C$_4$), 117.37 (C$_3$), 115.36, 113.19 (C$_5$), 65.37, 63.40, 61.41(O–CH$_2$), 47.59, 45.80, 43.86, 41.91 (C$_8$; P–C–P), 18.86, 17.58, 15.92, 13.81 (C$_7$), 18.82, 17.15, 15.45, 13.76 (OCH$_2$–CH$_3$). MS (EI; 70 eV): m/z 394(M +), 348(M − EtOH), 257(M − P(O)(OEt)$_2$). Anal. Calc. for C$_{15}$H$_{28}$N$_2$O$_6$P$_2$: C, 45.69; H, 7.16; N, 7.10. Found: C, 45.43; H, 7.31; N, 6.97.

5.2.2. From iminoether XII with base catalysis

To a solution of iminoether XII (2.20 g, 0.0144 mol) and DEP Va (2.0 g, 0.0144 mol) in absolute ethanol (25 ml) at 25 °C was added two drops of BF$_3$/Et$_2$O. After 48 h, the reaction had gone to completion, with BP Ib the sole phosphorus-containing product. The mixture was concentrated and purified as above, yielding 1.71 g of Ib.

5.2.3. From isonitrile XIV

A solution of isonitrile XIV (1.00 g, 0.0085 mol), DEP Va (1.05 g, 0.0076 mol) and Cu$_2$O (0.1 g, 0.0007 mol) in tetrahydrofuran (10 ml) was stirred at 23 °C for 12 h. Purification as before yielded only the diaddition product Ib.

5.3. 3-Methylpyridyl-2-aminomethylene-bis(methylphosphinic acid, diethyl ester) (IIa)

5.3.1. From isonitrile XIV

A solution of isonitrile XIV (1.00 g, 0.0085 mol), ethyl hydrogenmethylphosphinate IVa (0.827 g, 0.0077 mol) and Cu$_2$O (0.01 g, 7.0 × 10^{-5} mol) were heated for 6 h at 50 °C. The mixture was concentrated and purified by column chromatography (SiO$_2$; EtOAc; R_f = 0.4). The bisphosphinate esters IIa were isolated as a mixture of diastereomers; spectrally identical to that reported by Maier [5].

5.3.2. From iminoether XII

A mixture of iminoether XII (2.75 g, 0.0180 mol), ethyl hydrogenmethylphosphinate (1.00 g, 0.0139 mol), and potassium t-butoxide (0.224 g, 0.002 mol) in ethanol (25 ml) was heated at reflux for 2 h. The mixture was concentrated and purified as before. The isomer ratio of diastereomers IIa remained essentially constant by the two synthetic methods.

5.4. 3-Methylpyridyl-2-aminomethylene-bisphosphinic acid (IIb)

The synthesis and characterization of **IIb** has been described [5].

5.5. (3S,R,8S,R)-[(Hydroxy)methylphosphinyl][(3-methyl-2-piperidinylidene)amino]methylphosphonic acid (IIIa)

A suspension of 10.0 g (0.0357 mol) of aminopyridine **IIIb** in 150 ml of deionized H_2O was adjusted to pH 7.2 by the addition of a solution of saturated NaOH at 0 °C. After dissolution, 14.0 g of 5% palladium(0) on carbon (0.0066 mol) was added. The mixture was placed on a Parr shaker and thoroughly degassed with N_2. The mixture was then shaken under an H_2 atmosphere at between 35–45 psi and at ambient temperature until H_2 uptake ceased (120 h). After degassing with N_2, the mixture was filtered through diatomaceous earth above a Troyfelt filtering pad. The solvent was removed by rotary evaporation. Amidine **IIIa** consisting of a 52:48 mixture of **IIIc:IIId** was isolated as a white solid. The product was dried at 80 °C to a constant weight of 9.7 g. M.p.: degr. upon loss of H_2O; 100–120 °C. ^{1}H NMR (300 MHz, D_2O, pH = 1.5, HDO decoupled): δ 3.94–3.82 (2d, 1H; methine; $j = 15.6$, $j = 15.7$), 3.48–3.41 (m, 2H), 2.92 (q, 1H), 1.96 (m, 2H), 1.82 (m, 1H), 1.66 (m, 1H), 1.46 (d, 3H; $j = 14.54$ Hz), 1.44 (d, 3H; $j = 13.89$ Hz), 1.40 (d, 3H; $j = 6.9$ Hz), 1.38 (d, 3H; $j = 6.8$ Hz). ^{31}P NMR (121.48 MHz, D_2O,NaOD): δ 33.92, 33.81 (d, 1P; $j = 13.66$ Hz), 33.49, 33.38 (d, 1P; $j = 13.24$ Hz), 10.50, 10.39 (d, 1P; $j = 13.24$ Hz), 10.22, 10.10 (d, 1P; $j = 13.66$ Hz). ^{13}C NMR (75.46 MHz,D_2O, NaOD, proton decoupled): δ 169.72, 169.65, 169.61, 169.54, 57.14, 57.00, 56.10, 55.95, 55.49, 55.33, 54.40, 54.27, 44.05, 33.35, 33.29, 27.73, 20.67, 20.58, 19.49, 18.28, 16.98. MS (FAB, −VE): m/z 851.1(3M − 1), 567.0(2M − 1), 283.0(M − 1); MS (FAB, +VE): m/z 853.3(3M + 1), 569.1(2M + 1), 285.1(M + 1). Anal. Calc. for $C_8H_{18}N_2O_5P_2 \cdot H_2O$: C, 31.78; H, 6.67; N, 9.27. Found: C, 31.66; H, 6.73; N, 9.06.

5.6. [(Hydroxy)methylphosphinyl][3-methyl-2-pyridylamino]methylphosphonic acid (IIIb)

5.6.1. One pot synthesis from phosphonoacetal IXa

A 5 l three-neck round bottom flask equipped with a mechanical stirrer was charged with diethyl methylphosphonite (1094 g, 1200 ml, 8.04 mol). After deoxygenating with N_2, the mixture was cooled to 0 °C. To this mixture was added H_2O (144 ml, 8.00 mol) over the course of 2 h. This was stirred at 5–20 °C for 16 h. To the resultant equimolar solution of ethyl hydrogenmethylphosphinate **IVa** and ethanol was added phos-

phonoacetal **IXa** (2000 g, 2200 ml, 8.33 mol) and 2-amino-3-picoline (1019 g, 1000 ml, 9.44 mol). The vessel was fitted with a distillation head and then heated to 150 °C for 1 h, 160 °C for 9 h, and then 180 °C for 3 h. During the course of heating, an expected volume of 1400 ml of ethanol was recovered. The pot temperature was allowed to drop to 100 °C before 1500 ml of H_2O was added. After distilling an additional 1400 ml of ethanol, a second portion of 2000 ml of H_2O was added to the reaction mixture. After 8 h, heating was stopped, and the mixture was allowed to cool to 20 °C and stir for 12 h. A first crop of 770 g of **IIIb** was triturated in 1300 ml of H_2O and 300 ml of methanol. The triturant was combined with the original filtrate and placed in the freezer for 2 days. An additional 130 g of **IIIb** precipitated. The chromatographic system of SiO_2: 85/10/5 $CH_3OH/H_2O/NH_4OH$ was used to purify **IIIb**. ^{1}H NMR (300 MHz, D_2O, NaOD, HDO decoupled): δ 7.69 (d, 1H; $j = 7.2$ Hz), 7.65 (d, 1H; $j = 6.3$ Hz), 6.80 (dd, 1H), 4.00 (dd, 1H; $j = 20.1$, 15.6 Hz), 2.23 (s, 3H), 1.42 (d, 3H; $j = 14.4$ Hz). ^{31}P NMR (121.48 MHz, D_2O, NaOD): δ 35.11 (d, 1P; $j = 15$ Hz), 11.54 (d, 1P; $j = 15$ Hz). MS (FAB, −VE): 279(M − 1), 261(−H_2O). Anal. Calc. for $C_8H_{14}N_2O_5P_2$: C, 34.30; H, 5.04; N, 10.00. Found: C, 34.54; H, 5.30; N, 9.79.

5.6.2. From a mixture of aminoether phosphonates XIa and XIb

A mixture of diester **XIa** (0.31 g, 0.001 mol), half-acid ester **XIb** (0.65 g, 0.0024 mol), and ethyl hydrogenmethylphosphinate (0.479 g, 0.0044 mol) was heated at 170 °C for 8 h. Ethanol was removed by distillation as it formed. The temperature was lowered to 110 °C and H_2O (50 ml) was added to the mixture. After 8 h, the mixture was chilled at −8 °C. After 20 h, the tri-acid **IIIb** was collected via filtration as an off-white solid. After trituration with hot methanol, **IIIb** was isolated and dried at 60 °C for 30 h; yielding a white solid weighing 0.81 g. The product was identical to that reported above.

5.7. (3R,8S;3S,8R)-[(Hydroxy)methylphosphinyl][(3-methyl-2-piperidinylidene)amino]methylphosphonic acid (IIIc)

A 2 l Parr vessel was charged with H_2O (1000 ml) and aminopyridine **IIIb** (242 g, 0.864 mol). The mixture was adjusted to pH 5.0 by the addition of 6 M NaOH. The aminopyridine dissolved upon addition of the base. Into the mixture was suspended PtO_2 (5.0 g, 0.022 mol). The vessel was alternately flushed with N_2 and then with H_2 for three cycles. The H_2 pressure was maintained between 25–40 psi and the temperature remained constant at 23 °C during the course of the reaction. ^{1}H and ^{31}P NMR of the mixture after 24 h showed the

reduction to be 85% complete, and the isomer ratio **IIIc:IIId** due to the start of epimerization to be 70:30. After 48 h, less than 5% of the aminopyridine **IIIb** remained by NMR. After 104 h, the isomer ratio **IIIc:IIId** was > 98:2. The mixture was filtered through diatomaceous earth and then concentrated to a volume of 600 ml. The pH was lowered to 2.4 with conc. HCl, and CH_3CN (350 ml) was added. The mixture was placed in the freezer for 6 days. After a slow crystallization, **IIIb** (258.4 g, 0.855 mol) was dried to a constant weight at 65 °C, existing as its monohydrate. M.p.: 100–120 °C (degr. upon loss of H_2O). ^{1}H NMR (300 MHz, D_2O, NaOD, HDO decoupled): δ 4.19–4.02 (2d, 1H; methine; $j = 15.8$ Hz, $j = 15.7$ Hz), 3.57–3.39 (m, 2H), 2.92 (q, 1H), 1.96 (m, 2H), 1.82 (m, 1H), 1.66 (m, 1H), 1.56 (d, 3H; $j = 14.6$ Hz), 1.36 (d, 3H; $j = 7.2$ Hz). ^{31}P NMR (121.48 MHz, D_2O, NaOD): δ 33.92, 33.81 (d, 1P; $j = 13.66$ Hz), 10.22, 10.10 (d, 1P; $j = 13.66$ Hz). ^{13}C NMR (75.46 MHz, D_2O, NaOD, proton decoupled): δ 170.72, 56.28, 55.41, 54.53, 53.47, (previous four values; P–C–P) 44.61, 33.64, 27.90, 21.11, 19.57, 17.50, 16.24. MS (FAB, −VE): m/z 851.1(3M − 1), 567.0(2M − 1), 283.0(M − 1); MS (FAB, +VE): m/z 853.3(3M + 1), 569.1(2M + 1), 285.1(M + 1). Anal. Calc. for $C_8H_{18}N_2O_5P_2 \cdot H_2O$: C, 31.78; H, 6.67; N, 9.27. Found: C, 31.72; H, 6.97; N, 9.18.

5.8. (3S,8R)-[(Hydroxy)methylphosphinyl][(3-methyl-2-piperidinylidene)amino]methylphosphonic acid (IIIc)

A 0.37 g sample of racemate **IIIc** was added to 25 ml of hot acetone. A 30 ml aliquot of hot distilled water was added, one drop at a time. The solution was brought to a boil for 10 min. A 15 ml aliquot of the hot solution was filtered into a container which was sealed and placed in a refrigerator. Single crystals of sufficient size were obtained after 1 week.

5.9. (3R,8R;3S,8S)-[(Hydroxy)methylphosphinyl][(3-methyl-2-piperidinylidene)amino]methylphosphonic acid (IIId)

Amidine **IIIa** consisting of a 60:40 mixture of **IIIc:IIId** was enriched by chromatography (SiO_2: 85/10/5 $CH_3OH/H_2O/NH_4OH$) to yield a mixture of **IIIc:IIId** = 25:75. This mixture (5.0 g) was suspended in acetonitrile (70 ml) and heated at reflux. To the suspension was added H_2O (40 ml). Heating continued until the white solid dissolved. The solution was filtered while still hot. The filtrate was allowed to cool slowly until it reached 22 °C. Acetonitrile (30 ml) was added dropwise, slowly with stirring, until the mixture just turned turbid. The mixture was then placed in the freezer at −12 °C for 6 days. Upon isolation by filtration, the resultant product had an isomer ratio of **IIIc:IIId** = 10:90. The above procedure was repeated,

resulting in a second crop of material with an isomer ratio of **IIIc:IIId** = < 5: > 95. A final recrystallization yielded **IIIe** (1.5 g) with an isomeric purity of > 98%. M.p.: degr. upon loss of H_2O; 100–120 °C. ^{1}H NMR (300 MHz, D_2O, pH = 1.5, HDO decoupled): δ 3.94–3.82 (2d, 1H; methine; $j = 15.6$ Hz, $j = 15.7$ Hz), 3.48–3.41 (m, 2H), 2.92 (q, 1H), 1.96 (m, 2H), 1.82 (m, 1H), 1.66 (m, 1H), 1.46 (d, 3H; $j = 14.54$ Hz), 1.44 (d, 3H; $j = 13.89$ Hz), 1.40 (d, 3H; $j = 6.9$ Hz), 1.38 (d, 3H; $j = 6.8$ Hz). ^{31}P NMR (121.48 MHz, D_2O, NaOD): δ 33.49, 33.38 (d, 1P; $j = 13.2$ Hz), 10.50, 10.39 (d, 1P; $j = 13.2$ Hz). ^{13}C NMR (75.46 MHz, D_2O, NaOD, proton decoupled): δ 169.72, 169.65, 169.61, 169.54, 57.00, 55.95, 55.33, 54.27, 44.05, 33.35, 33.29, 27.73, 20.67, 20.58, 19.49, 18.28, 16.98. MS (FAB, −VE): m/z 851.1(3M − 1), 567.0(2M − 1), 283.0(M − 1); MS (FAB, +VE): m/z 853.3(3M + 1), 569.1(2M + 1), 285.1(M + 1). Anal. Calc. for $C_8H_{18}N_2O_5P_2 \cdot H_2O$: C, 31.78; H, 6.67; N, 9.27. Found: C, 32.22; H, 6.74; N, 9.33.

5.10. Ethyl hydrogenmethylphosphinate (IVa)

Preparation with 1 equiv. of ethanol described for **IIIb** (Section 5.6). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07, 6.27 (d, 1H; $j_{P,H} = 534$ Hz), 4.14–4.06 (m, 1H), 4.05–3.99 (m, 1H), 1.49 (d, 3H; $j = 126$ Hz). ^{31}P NMR (300 MHz, $CDCl_3$): δ 34.70, 32.99 (d, 1P; $j_{P,H} = 514$ Hz). IR (neat): ν 2390 (P–H) cm^{-1}.

5.11. Diethyl methylphosphonite, or Phosphonous acid, methyl-, diethylester (XVIII)

A 1 l, three-neck flask was equipped with a pressure-equalized dropping funnel, mechanical stirrer and thermometer. Under N_2, it was charged with ether (200 ml) and diethyl chlorophosphonite **X** (46.98 g, 43.42 ml, 0.300 mol). The mixture was cooled to −12 °C. The dropping funnel was charged with methylmagnesiumbromide (100 ml of 3 M, 0.300 mol). The Grignard reagent was added dropwise and at such a rate that the internal pot temperature was maintained below −8 °C. This took 120 min. After the addition was complete, the mixture was allowed to warm to 0 °C, and stir for an additional 14 h. The mixture was vacuum distilled from the magnesium salts at below 0.01 atm and trapped with a cold finger at −78 °C. Then the phosphonite was fractionally distilled from the ether at 0.065 atm, between 45–48 °C. The isolated product weighed 30.4 g and displayed a single ^{31}P NMR resonance (121.48 MHz, $CDCl_3$) at δ 177.96.

5.12. Diethoxymethyl phosphonic acid, diethyl ester (IXa) [6]

To a solution of triethyl orthoformate (2371 g, 16.0 mol) at 20 °C was added over 45 min, diethyl

chlorophosphonite **X** (2506 g, 16.0 mol). The mixture was warmed to 80 °C and maintained for 1 h. The product was isolated via vacuum distillation at 83–87 °C/0.0003 atm. The yield was 3410 g. ^{1}H NMR (300 MHz, CDCl$_3$): δ 4.74 (d, 1H), 4.16 (m, 4H), 3.81 (m, 2H), 3.64 (m, 2H), 1.29 (t, 6H), 1.17 (t, 6). ^{31}P NMR (121.48 MHz, CDCl$_3$): δ 14.36. IR (Neat): ν 2948, 1469, 1438, 1381, 1358, 1243, 1018 cm^{-1}. MS (CI/CH$_4$): m/z 241(M + 1). Anal. Calc. for C$_9$H$_{21}$O$_5$P$_1$: C, 45.00; H, 8.81. Found: C, 44.89; H, 8.72.

5.13. Dimethoxymethyl phosphonic acid, dimethyl ester (IXb)

To trimethyl phosphite (62.0 g, 0.500 mol) at 25 °C was added phosphorus trichloride (40.5 g, 0.295 mol). The mixture was stirred at room temperature for 1 h, and then heated at 80 °C for 1 h. The mixture was cooled to 40 °C and trimethyl orthoformate (84.3 g, 0.794 mol) was added. The mixture was heated for 1 h, cooled and concentrated. HPLC purification (SiO$_2$; dichloromethane:IPA 97:3; R_f = 0.7) yielded 104 g of **IXb**. ^{1}H NMR (300 MHz, CDCl$_3$): δ 4.63 (d, 1H), 3.84 (d, 6H; P–O–CH_3), 3.51 (s, 6H). ^{31}P NMR (121.48 MHz, CDCl$_3$): δ 16.10. MS (CI/CH$_4$): m/z 185.1(M + 1). Anal. Calc. for C$_9$H$_{21}$O$_5$P$_1$: C, 32.62; H, 7.12. Found: C, 33.04; H, 7.46.

5.14. Diethyl chlorophosphonite (X)

A 1 l flask equipped with a mechanical stirrer and a reflux condenser was charged with phosphorus trichloride (140.1 g, 89 ml, 1.02 mol). To this was added in a single portion, triethyl phosphite (336.8 g, 346 ml, 2.03 mol). The exotherm was gently achieved; the pot temperature rose to 70 °C and no cooling other than the condenser was warranted. The mixture was allowed to cool over a 1 h period to 29 °C, and then was reheated to 80 °C for an additional 3 h. The mixture was once again cooled, and then distilled at 0.017 atm and 42–44 °C to yield 380 g of **X**. ^{31}P NMR (121.48 MHz, CDCl$_3$): δ 166.90.

5.15. Ethoxy[2-(3-methylpyridyl)amino]methylphosphonic acid, diethyl ester (XIa)

A mixture of 2-amino-3-methylpyridine **VI** (1.08 g, 0.01 mol) and phosphonoacetal **IXa** (2.40 g, 0.01 mol) were heated at 165 °C for 3 h. The ethanol that formed was recondensed into the reaction vessel. The mixture was then allowed to stir at 100 °C for 14 h. The viscous mixture was diluted with 2 ml of CHCl$_3$, and then subjected to gradient HPLC (SiO$_2$, pore size = 20 μm; CHCl$_3$:CH$_3$OH 9:1 to 100% CH$_3$OH). The early phosphorus-containing fractions (R_f = 0.8; CHCl$_3$:CH$_3$OH

9:1) weighed 0.89 g and were identified as **IXa** by ^{31}P and ^{1}H NMR. Fractions containing **XIa** weighed 340 mg and eluted shortly after **IXa**. They had the following spectral properties. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.93 (d, 1H), 7.17 (d, 1H), 6.52 (t, 1H), 5.81 (dd, 1H; methine), 4.94 (dd, 1H; N–H), 4.11 (m, 4H; P(O)–OCH_2), 3.44 (m, 2H), 2.15 (s, 3H), 1.24 (t, 6H; PO$_2$CH$_2$CH_3), 1.12 (t, 3H). ^{31}P NMR (121.48 MHz, CDCl$_3$): δ 23.74. The next fraction isolated (R_f = 0.8; CH$_3$OH) weighed 0.6 g and was identified as **XVa**. ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.36 (bs, 1H), 7.57 (d, 1H), 7.43 (d, 1H), 6.52 (t, 1H), 4.68 (d, 1H; j = 1.4 Hz), 4.05 (m, 2H; phosphonamide ethoxy), 3.85 (m, 2H), 3.71 (m, 2H), 2.39 (s, 3H), 1.28 (t, 3H), 1.18 (t, 6H). ^{31}P NMR (121.48 MHz, CDCl$_3$): δ 10.59.

5.16. Ethoxy[2-(3-methylpyridyl)amino]methyl-phosphonic acid, ester XIb

The half-acid ester was extremely polar (R_f = 0.05; CH$_3$OH) and streaked during the column wash as it diluted on the column. Upon concentration of the wash, 750 mg of **XIb** was obtained. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.99 (s, 1H), 7.70 (d, 1H), 7.02 (d, 1H), 6.39 (t, 1H; methine), 5.46 (t, 2H; N–H, P–OH), 3.92 (m, 2H; P(O)–OCH_2), 3.82 (m, 2H), 2.09 (s, 3H), 1.24 (t, 3H; PO$_2$CH$_2$CH_3), 0.99 (t, 3H). ^{31}P NMR (121.48 MHz, CDCl$_3$): δ 14.54.

5.17. (E,Z)-Ethyl-N-[2(3-methylpyridyl)]iminomethyl ether (XII)

A solution of 2-amino-3-methylpyridine **VI** (108 g, 1.00 mol), triethyl orthoformate (200 g, 1.35 mol) and p-toluenesulfonic acid (1.0 g, 0.005 mol) was heated at 120 °C for 5 h. Ethanol was removed continuously during heating. The mixture was cooled to 50 °C and then subjected to vacuum distillation at 0.0007 atm. After a forerun of triethyl orthoformate was recovered at 53 °C, the product distilled at 64–66 °C. The yield of **XII** was 114 g (70%). ^{1}H NMR showed the product mixture to contain a 9:1 ratio of E:Z isomers.

E isomer. ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.22 (s, 1H), 8.15 (d, 1H), 7.45 (d, 1H), 6.97 (t, 1H), 4.37 (q, 2H), 2.27 (s, 3H), 1.41 (t, 3H). Z isomer. ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.26 (s, 1H), 8.14 (d, 1H), 7.43 (d, 1H), 6.93 (t, 1H), 3.59 (q, 2H), 2.22 (s, 3H), 1.21 (t, 3H). E:Z mixture MS (EI/70 eV): m/z 164.1, 119. Anal. Calc. for C$_9$H$_{12}$N$_2$O: C, 65.83; H, 7.37; N, 17.06. Found: C, 65.22; H, 7.71; N, 16.78.

5.18. 2-Formamidenyl-3-methylpyridine (XIII)

A solution of 2-amino-3-methylpyridine (25.0 g, 0.23 mol) and butyl formate (35.7 g, 0.35 mol) were heated at reflux (107 °C) for 16 h. By ^{1}H NMR, only

50% of the amine was consumed, but the excess butyl formate was removed by rotary evaporation at this point. The crude mixture was recrystallized from ether–hexanes, yielding 13.9 g of pure formamide XIII (44%). For XIII: m.p. = 138–139 °C. ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.52 (d, 1H), 8.47 (bs, 1H), 8.14 (d, 1H), 7.47 (d, 1H), 6.92 (t, 1H), 2.28 (s, 3H). IR(Nujol): ν 1702, 1596 cm^{-1}. MS (EI/70 eV): m/z 136.2, 109. Anal. Calc. for C$_7$H$_8$N$_2$O: C, 61.84; H, 5.93; N, 20.58. Found: C, 61.46; H, 6.17; N, 20.25.

5.19. [2-(3-Methylpyridyl)]isonitrile (XIV)

To t-butyl alcohol (150 ml) was added at 23 °C potassium t-butoxide (31.6 g, 0.282 mol) and then formamide XIII (14.0 g, 0.103 mol). The temperature rose to 42 °C. The flask was cooled to 20 °C, and maintained at that temperature as phosphorus oxychloride (9.87 g, 0.064 mol) was added dropwise. The mixture was then heated gently at 35 °C for 2.5 h. The mixture was poured into a 1% solution of sodium bicarbonate (500 ml), and extracted with petroleum ether (3 × 250 ml). The organic phase was dried and concentrated. The product was recrystallized twice from hexanes, yielding 8.6 g of XIV. The product sublimed readily under aspirator pressure and was not stable at room temperature.

5.20. N-(1-(5-Amino-2-methyl-1-oxo)-pentyl)-aminomethane phosphonomethylphosphinic acid (XVIa / XVIb)

A 10% aqueous solution of amidine IIIa (10.0 g, 0.033 mol) at pH = 12 was heated at reflux for 72 h. The hydrolysis was calculated to be 75% complete by ^{1}H and ^{31}P NMR. The diastereomeric mixture of open chained isomers XVIa/XVIb was isolated as such by preparative HPLC (SiO$_2$, pore size = 20 μm; CH$_3$OH:H$_2$O 9:1). ^{1}H NMR (300 MHz, D$_2$O): δ 4.35 (m, 1H), 3.00 (m, 2H), 2.55 (m, 1H), 1.58(m,2H), 1.53(m,1H), 1.39(d,1.5H;j = 14.0Hz), 1.36 (d, 1.5H; j = 14.0Hz), 1.17 (d, 1.5H; j = 6.8Hz), 1.16 (d, 1.5H; j = 6.8Hz). ^{31}P NMR (121.48 MHz, D$_2$O, NaOD): δ 35, 18. ^{13}C NMR (75.46 MHz, D$_2$O, NaOD): δ 180.4, 52.2 (two overlapping dd), 42.5, 41.4, 32.4, 26.9, 19.2, 17.6 (d; j = 96 Hz).

5.21. Ethyl dichlorophosphonite (XVII)

A 1 l flask equipped with a mechanical stirrer and a reflux condenser was charged with phosphorus trichloride (140.1 g, 89 ml, 1.02 mol). To this was added in a single portion, triethyl phosphite (169.5 g, 175 ml, 1.01 mol). The pot temperature rose to 70 °C and no cooling other than the condenser was employed. The mixture was allowed to cool over a 1 h period to 29 °C, and then was reheated to 80 °C for an additional 3 h. The mixture was once again cooled, and then distilled at 41–42 °C/0.02 atm to yield 135 g of XVII. ^{31}P NMR (300 MHz, CDCl$_3$): δ 177.31. MS (EI/70 eV): m/z 146.9. Anal. Calc. for C$_2$H$_5$Cl$_2$OP: C, 16.35; H, 3.43; Cl, 48.25. Found: C, 16.21; H, 3.08; Cl, 48.77.

References

[1] (a) A.D. Geddes, S.M. D'Souza, F.H. Ebetino, K.J. Ibbotson, in J.N.M. Heersche and J.A. Kanis (eds.), Bone and Mineral Research/8, Elsevier, London, 1994, pp. 265–306. (b) W.K. Sietsema and F.H. Ebetino, Exp. Opin. Invest. Drugs, 3 (12) (1994) 1255.
[2] (a) F.H. Ebetino and L.A. Jamison, Phosphorus Sulfur Silicon, 51–52 (1990) 23. (b) R.J. Sunberg, F.H. Ebetino, C.T. Mosher and C.F. Roof, Chemtech, (1991) 55.
[3] (a) F.H. Ebetino, C.R. Degenhardt, C.M. Perkins, K.Y. Johnson, J.A. Bevan and H.M. Olson, Calcif. Tissue Int., 38 (suppl.) (1985) S31. (b) F.H. Ebetino, inventor, European Patent Publication #298553, 1989. (c) F.H. Ebetino, inventor, European Patent Publication #298555, 1989.
[4] F.H. Ebetino and J.E. McOsker, J. Bone Miner. Res., 4 (suppl. 1) (1989) S165.
[5] L. Maier, Phosphorus Sulfur Silicon, 11 (1981) 311.
[6] M. Mikolajczyk, B. Costisella and Z. Grzejszczak, Tetrahedron Lett., 6 (1976) 477.
[7] F.H. Ebetino, J.D. Berk and T. Emge, National ACS Convention, poster #42, Organic Chemistry Division, Washington, DC, 1990.
[8] J.J. Benedict and C.M. Perkins, inventors, US Patent 4902679, 1990.