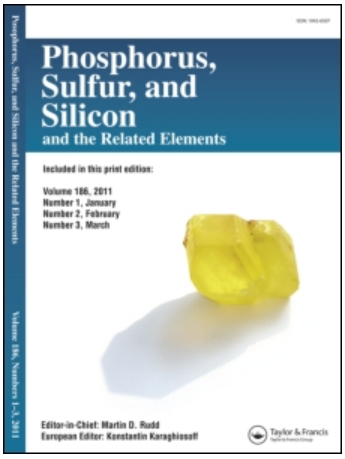


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# The Design and Synthesis of Bone-Active Phosphinic Acid Analogues: 1. The Pyridylaminomethane Phosphonoalkylphosphinates

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# THE DESIGN AND SYNTHESIS OF BONE-ACTIVE PHOSPHINIC ACID ANALOGUES: I. THE PYRIDYLAMINOMETHANE PHOSPHONOALKYLPHOSPHINATES

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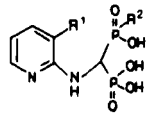
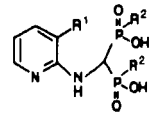
Abstract The bisphosphonic acids and their salts, the bisphosphonates, have been the subject of study by a number of research groups for their marked ability to modulate bone metabolism. From a recent study of phosphinic acid isosteres, we have designed a novel related class of bone active agents, the pyridylaminomethane phosphonoalkylphosphinates I.

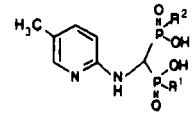


For over 20 years, the bisphosphonic acids and their salts, the bisphosphonates, have been known to be effective inhibitors of bone resorption and bone mineralization.[1] This report summarizes a study resulting in the discovery of the first bone resorption-inhibiting phosphinic acid analogues. Compounds exhibiting these properties may potentially be used for the treatment of diseases characterized by excessive resorption such as Paget's disease and osteoporosis. The objectives of this work were: 1) to study the effects on inhibition of bone resorption after structural modifications on the geminal bisphosphonate moiety through phosphinic acid substitution and 2) to assess the synthetic feasibility of the resulting bone-active pyridylaminomethane class of phosphonoalkylphosphinates I.

Initially, very little was known about the preparative feasibility and biological activity of these phosphinic acid synthetic targets.[2] Therefore, it was useful to study the scope of these bisphosphonate isosteres as a synthetic challenge and for their potential biological benefits relative to the bisphosphonates (II). As the project progressed, it became increasingly plausible that, with phosphinic acid modification, the physical affinity to bone could be decreased, and therefore, the potential pharmacological advantages could be studied.







**Ia** $R^1 = CH_3, R^2 = CH_3$ **IIIa** $R^1 = CH_3, R^2 = CH_3$ **IVa** $R^2 = CH_3$, $R^1 = OH$
**Ib** $R^1 = H$, $R^2 = CH_3$ **IIIb** $R^1 = CH_3, R^2 = n\text{-butyl}$ **IVb** $R^2 = n\text{-hexyl}$, $R^1 = OH$
**Ic** $R^1 = CH_3, R^2 = n\text{-butyl}$ **IIIc** $R^1 = H$, $R^2 = CH_3$ **V** $R^2 = R^1 = CH_3$
**II** $R^1 = CH_3, R^2 = OH$ **IIId** $R^1 = H$, $R^2 = n\text{-butyl}$

## RESULTS AND DISCUSSION

### Pyridylaminomethane Bis(alkylphosphinic acids)

We first prepared a series of bis(alkylphosphinic acids) according to Maier's procedure[2] (procedure A), and we have extended this work to the preparation of a wider range of bis(alkylphosphinic acid) analogues (**IIIa-IIId,V**). Briefly, after heating a mixture of triethylorthoformate, an alkyl hydrogen alkylphosphinate, and an aminopyridine and subsequent aqueous hydrolysis, pyridylaminomethane bis(alkylphosphinic acids) III are conveniently isolated. The testing of **IIIa** in a hydroxyapatite seeded crystal growth model[3] suggested a marked decrease in bone affinity as implied by no detectable

inhibition of crystal growth. This finding held true with several additional members of this class of bisphosphinates (IIIb-IIId, V). Furthermore, no antiresorptive activity was detected with IIIa *in vivo* in the thyroparathyroidectomized (TPTX) rat[4] (a parathyroid hormone stimulated bone resorption model) and Schenk models[5] (a measure of resorption activity in the growing rat long bone).

After assessment of these biological results, we sought to reinstate bone affinity in our synthetic drug targets by designing related phosphinic acid analogues. Included in this effort was a plan to synthesize members of the geminally substituted phosphonoalkylphosphinate (I) class of compounds[6], hybrids of the very active bisphosphonic acids (II) and the inactive bisphosphinic (III) acids.

## Pyridylaminomethane Phosphonoalkylphosphinates

The general procedure A was mimicked to initially prepare small quantities of the desired pyridylaminomethane phosphonomethylphosphinate (Ia) (see procedure B). One molar equivalent each of phosphite precursors to phosphonic and phosphinic acids[7] were used. Thus, diethyl or diisopropyl phosphite and ethyl hydrogen methylphosphinate in combination with triethylorthoformate and 2-amino-3-picoline were heated to yield a mixture of the desired phosphonomethylphosphinate and the corresponding bisphosphinate and bisphosphonate esters. After a difficult chromatographic separation and ester hydrolysis, the desired product Ia was isolated in very low yield (12%).

Aminomethanephosphonomethylphosphinate Ia was then screened *in vitro* and *in vivo* for bone activity. Interestingly, it was first noted that this analogue demonstrated hydroxyapatite affinity as measured by the crystal growth inhibition model and verified by HAP uptake studies.[8] However, this congener and other phosphonoalkylphosphinates tested to date appeared to be more than an order of magnitude less potent in hydroxyapatite seeded crystal growth inhibition (Table I) than the corresponding bisphosphonates. This feature suggested that novel pharmacologic activity would be possible if a congener could be designed and synthesized with appropriate antiresorptive potency; for example, where diphosphonates owe their toxicity to high affinity to bone and other potentially strong chelation effects, this property might be minimized with the phosphonophosphinates.

This analogue Ia was next screened in the TPTX[4] and Schenk[5] rat models. Activity was noted at 1.0 mg P/kg. Also, the latter studies demonstrated no inhibition of bone mineralization in contradistinction to the activity of the corresponding bisphosphonate at 10 mg P/kg.

With this exciting new discovery, two goals became obvious. First, the scope of this class of compounds needed exploration via broader analogue preparation. Secondly, a more feasible synthetic procedure to obtain the aminomethane phosphonomethylphosphinates needed to be developed to facilitate further testing of Ia and to permit the desired analogue preparation.

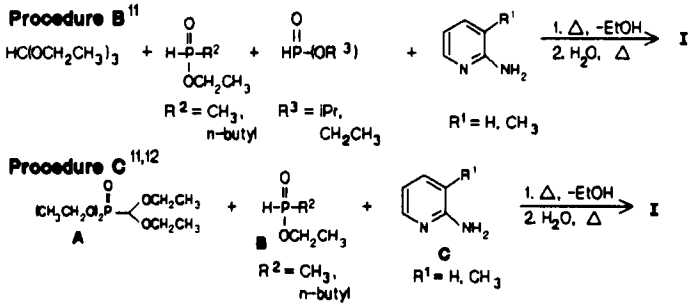
## Synthetic Improvements

An improved synthesis was then designed to allow stepwise introduction of the desired precursors that lead specifically to the phosphonate and phosphinate moieties. The phosphonic acid center was set first by the preparation of diethoxymethylphosphonic acid diethyl ester A[9] which can be prepared in high yield in one step from triethylorthoformate and diethylchlorophosphite. This precursor was initially chosen because of the known difficulties of preparation and stability of unprotected formylphosphonates.[10]

We then postulated that the reaction of aminopyridine C with A would yield an imino phosphonate moiety suitable for completing a selective phosphonophosphinate synthesis. Thus, introduction of the final alkyl hydrogen alkylphosphinate moiety B would complete a stepwise synthesis without the production of any bisphosphonic or bisphosphinic acid impurities.

Several stepwise attempts with varying combinations of these precursors were initially studied. We then found equimolar quantities of A, B and C could be combined in one mixture and heated while driving off ethanol, to yield (~35%) the desired pyridylaminomethane phosphonomethylphosphinate Ia, following water hydrolysis.

In fact, as can be seen in procedure C, the desired product crystallizes from the reaction mixture in analytical purity! HPLC analysis exhibited less than 0.02% of other bisphosphonate impurities in the desired phosphonomethylphosphinate product prepared in this one pot procedure. Furthermore, this key, one pot, solventless condensation reaction is now proving its utility in large scale synthesis of 100-1000 g quantities.




The synthetic work described herein has led to a valuable series of bone-active analogues (Table I). Through the two procedures described herein, we have varied the phosphinate alkyl moiety and the substituents on the pyridyl ring (Ia-Ic, and IVa-b, respectively). Our work has led to a novel synthetic procedure suitable for kilogram synthesis. Although this new selective procedure may still require further optimization, the 35% yields attained to date with analogue Ia are suitable for preclinical scale-up, and the difficult purification steps have been avoided as a result of the high analytical and regiochemical purity of the process. Manuscripts describing related synthetic work and the biological and toxicological properties of the phosphonoalkylphosphinate series are in preparation.

**Table I**

| | HAP-CGI[a] | TPTX[b] (LED)[d] | Schenk[c] (LED) | Microanalysis C, H | $^{31}$P NMR (ppm) | Procedure |
|---|---|---|---|---|---|---|
| **Ia** | 27.5 | 1.0 | 1.0 | 34.54, 5.30 | 34.6, 10.8[f] | B & C |
| **Ib** | 30.5 | - | - | 31.25, 4.60 | 39.3, 12.4[f] | B & C |
| **Ic** | 26.9 | - | 1.0 | 41.46, 6.58 | 42.8, 14.3[f] | B & C |
| **II** | 1.3 | 0.001 | 0.001 | 29.70, 4.32 | 16.1 | A |
| **IIIa** | N.A. (100)[e] | N.A. (10) | N.A. (10) | 38.69, 5.55 | 38.2 | A |
| **IIIb** | N.A. (100) | - | - | 49.62, 7.97 | 40.4 | A |
| **IIIc** | N.A. (100) | - | - | 35.85, 5.81 | 38.1 | A |
| **IIId** | N.A. (100) | - | - | 47.14, 8.17[g(1)] | 40.9 | A |
| **IVa** | 7.3 | 0.1 | 1.0 | 33.55, 5.23[g(1)] | 39.5, 12.5[f] | B & C |
| **IVb** | 4.6 | - | - | 41.05, 7.01[g(3)] | 42.4, 13.1[f] | B |
| **V** | N.A. (100) | - | N.A. (10) | 36.58, 5.87[g(2)] | 37.7 | A |

a HAP-CGI - hydroxyapatite-crystal growth inhibition. The data reported are representative of the concentration x $10^{-6}$ M required to inhibit hydroxyapatite seeded crystal growth formation for 50 minutes in a supersaturated medium. These data are also normalized to a value of 1.0 for 1-hydroxyethane-1,1-bisphosphonic acid as a reference standard.

b TPTX - thyroparathyroidectomized rat model

c Schenk - a measure of resorption activity in the growing rat long bone

d LED - lowest effective dose (mg P/kg)

e N.A. (x) - no activity (highest concentration)

f pD >11

g(n) 0.5 x (n) molar equivalents of water contained in product

## EXPERIMENTAL SECTION

NMR spectra were obtained on either a JEOL FX90Q or GE GN-300MHz instrument.

### Procedure A

See Maier[2] for general experimental procedures.

### Procedure B[11]

*[(Hydroxy)methylphosphinyl][(3-methyl-2-pyridinyl)amino]methylphosphinic Acid.* Ia. Ethyl hydrogen methylphosphinate[7] (49 mmol) was combined with diisopropyl phosphite (8.1 ml; 49 mmol), 2-amino-3-methylpyridine (5.3 g; 49 mmol), and triethylorthoformate (10 ml; 49 mmol) in a flask equipped with a magnetic stirring bar, a distillation head, and an argon atmosphere. The reaction mixture was then brought slowly to 150-160°C and maintained at this temperature for 1 h while ethanol distilled from the reaction flask. The reaction mixture was then concentrated and purified on medium-pressure chromatography apparatus with a gradient of 5-15% ethanol/methylene chloride to yield 3.0 g of the desired phosphonomethylphosphinate triester (a mixture of isopropyl and ethyl esters are isolated as a result of transesterification). $^{31}P$ NMR ($CDCl_3$): 45.4 (m); 17.5 (m). The ester mixture (0.9 g) was refluxed in 4 ml of water for 1 hr, and the resulting white precipitate was filtered and dried to yield 0.5 g (12% overall yield for the two step process) of desired product (m.p. 273-274°, uncorrected). The spectral data were found identical to that reported in procedure C.

### Procedure C[11,12]

*[(Hydroxy)methylphosphinyl][(3-methyl-2-pyridinyl)amino]methylphosphinic Acid.* Ia. Diethoxymethylphosphonic acid diethyl ester A[9] (15 g, 62.5 mmol), ethyl hydrogen methylphosphinate B (9.6 g; 62.5 mmol), and 2-amino-3-picoline C (6.75, 62.5 mmol) were combined in a flask equipped with a short path distillation head, magnetic stirrer, and a nitrogen atmosphere. The flask was placed in an oil bath, and the temperature was slowly brought to 170°C and the reaction mixture was kept at this temperature until no further ethanol distilled (total reaction time approximately 4 to 5 h). After this time, the bath temperature was cooled to 110°C, 75 ml of water were added, and this reaction mixture was allowed to reflux for 1 h. The resulting white precipitate was collected, rinsed with hot water (2 x 10 ml), and dried to yield 5.7 g (32%) of desired product.

$^1H$ NMR ($D_2O$; 1 equivalent NaOD): 7.69 (1H, d, J=7.2 Hz); 7.65 (1H, d, J=6.3 Hz); 6.8 (1H, dd); 4.0 (1H, dd, J=20.1 Hz, J=15.6 Hz); 2.23 (3H, s); 1.42 (3H, d, J=14.4 Hz). $^{31}P$ NMR $D_2O$; 1 equivalent NaOH: 35.11 (d, J=15 Hz); 11.54 (d, J=15 Hz). Anal. Calc'd. 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.



## ACKNOWLEDGMENTS

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