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Bone Active Bisphosphonate Mechanistic Studies: Synthesis of a 2-PyrindinylmethyleneBisphosphonic Acid *via* a Photolytic Ring Contraction

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ABSTRACT: *Recent research on the bone active bisphosphonate class of drugs has led to highly potent nitrogen-containing analogs. The involvement of specific cellular targets and therefore a critical bisphosphonate pharmacophore required in the mechanism of inhibition of bone resorption is suggested. To further test this concept, the successful design of a potent conformationally restricted analog, based on the structure-activity relationships deduced from this research, has been accomplished via the preparation of a 2-pyrindinylmethylene bisphosphonate NE-10501 (***3***). An interesting photolytic ring contraction was utilized as the key step in the synthesis of this novel bone active compound.* © 2000 John Wiley & Sons, Inc. Heteroatom Chem 11:442–448, 2000

INTRODUCTION

Bisphosphonate drugs have been used successfully for the treatment of bone disorders such as Paget's disease of bone, cancer-related hypercalcemia, and postmenopausal osteoporosis. The early compounds in this class were designed many years ago as metabolically stable analogs of the endogenous bone-mediating agent, pyrophosphate. Bisphosphonic acids, such as etidronate, are metabolically stable, and remarkably, maintain many of the favorable bone-modulating effects of pyrophosphate [1].

Subsequently, bisphosphonates were discovered that exhibit significantly more potency than etidronate in their ability to inhibit bone resorption. For example, in acute growing rat models, newer generation bisphosphonates exhibited over 1000-fold increases in such antiresorptive potency [2]. The antiresorptive mechanism of these new bisphosphonates, until recently, was not known, but speculation implicating a variety of biochemical targets was clearly triggered with these new findings. In our research laboratories, we have been studying the structure-activity relationships of these newer potent antiresorptive bisphosphonic acids. This research produced libraries of bisphosphonates that included useful diagnostic tools that guided key biochemical studies [3]. Ultimately, these studies have led to an understanding that these modern bisphosphonates initially target bone, desorb from the mineral surface, concentrate in the osteoclast cytosol, and interrupt cell signaling via inhibition of G-protein prenylation. Enzymes of the mevalonic acid metabolism pathway, such as farnesyl pyrophosphate synthase have been implicated, and such specific enzyme targets, and the primary functional events leading to antiresorptive activity, continue to be further validated [4].

A variety of interesting potent heterocyclic bisphosphonates were designed in the course of this re-

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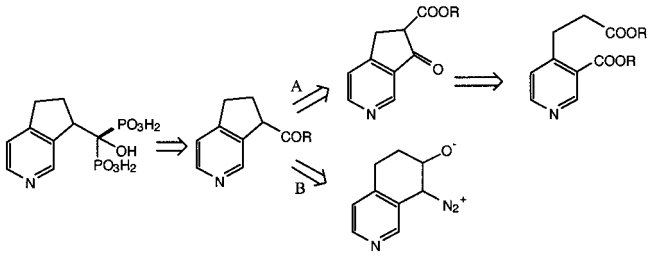
search. The design of conformationally restricted analogs based on less constrained bisphosphonates has also been a goal in our research to provide highly specific benchmarks for these biochemical studies, and to further demonstrate the selectivity of the putative biochemical targets. One such study that will be described in this report led to the design and synthesis of a unique pyrindine-containing bisphosphonate.

Key to these highly potent newer generation bisphosphonate compounds is their basic nitrogen moiety. The discovery of potent antiresorptive activity with a *cis*-octahydropyrindine bisphosphonate **1**, in light of the inactivity of the tetrahydro analog **2**, led to the formulation of these compounds as active and inactive conformational templates [5]. Following are depictions of minimized conformations of these two compounds (a and b) and an overlap (c) holding the phosphonates (yellow/red) in a similar orientation and demonstrating the different nitrogen orientations (blue) (Figure 1). We therefore have continued to test the validity of the active conformational template by the design of new analogs.

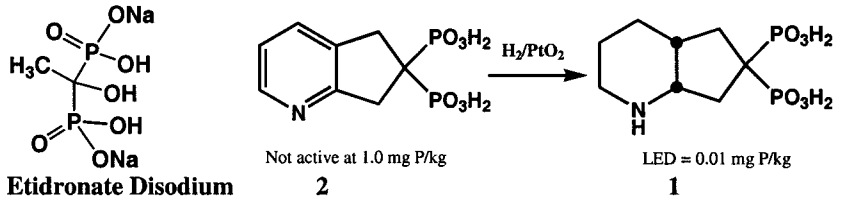
In this effort, our structure activity learnings have been applied to the design of a 2-pyrindinylmethylene bisphosphonic acid **3**. This compound was designed as a conformationally restricted active analog of the osteoporosis therapeutic agent, Risedronate, marketed under the tradename Actonel. Thus, the 2-pyrindine ring system holds the basic nitrogen functionality and the bisphosphonic acid moieties in what we have proposed to be the biologically active conformation. Below is a three-dimensional depiction of the subject compounds demonstrating the potential biologically active conformation of risedronate (e) as derived from its overlap with **1** (d) and the overlap of **3** with **1** (f) to demonstrate its preference for the predicted active conformation (Figure 2).

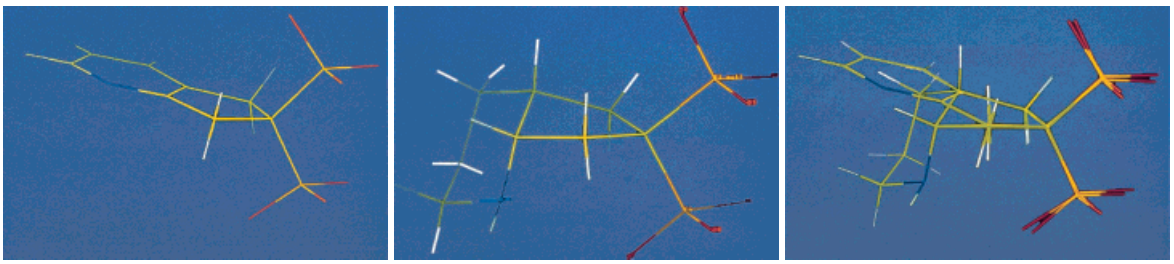
SYNTHESIS

Retrosynthetic analysis of the desired 2-pyrindine ring system of **3** revealed two entries into this relatively unknown bicycle. First, a Dieckmann condensation of a properly 3,4-disubstituted pyridine would afford a pyrindine (path A), and second, we proposed the ring contraction of an isoquinolinol could also afford the desired ring system (path B).



We first briefly explored routes for the synthesis of 3,4-disubstituted pyridines. It has been reported that 3,4-pyridine dicarboxylic anhydride can be taken to the desired disubstituted pyridine **5** but we chose to avoid this lengthy procedure [6]. A shorter



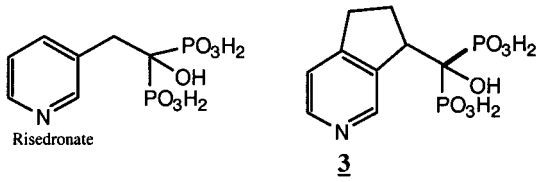


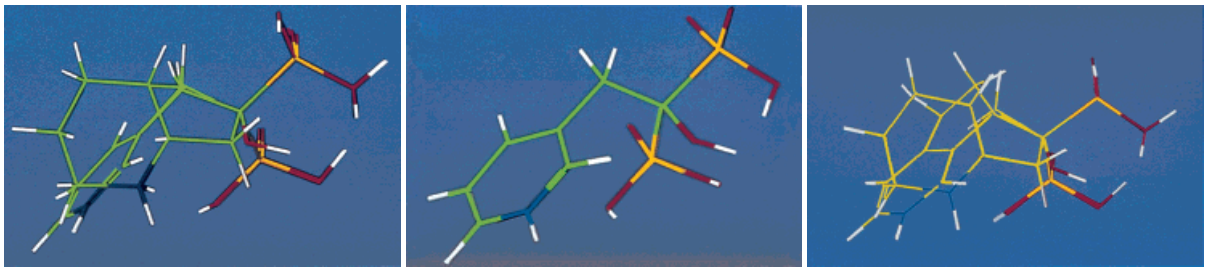
a.
Dihydropyrindine BP **2**
LED=NA (1 mg P/kg)

b.
Octahydropyrindine BP **1**
LED= 0.01 mg P/kg

c.
Overlap of **2** and **1**

FIGURE 1





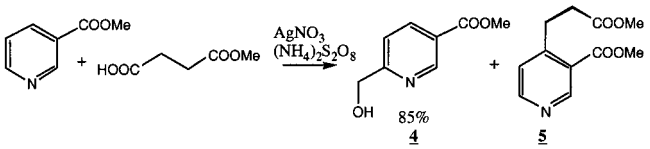
d. Overlap of **risedronate** and **2**

e. Predicted active conformation of **risedronate**

f. Overlap of **3** and **1**

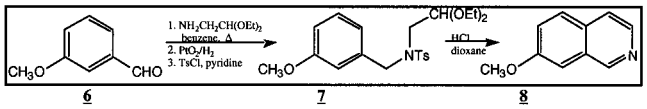
FIGURE 2

route appeared to be the direct silver catalyzed radical alkylation of methyl nicotinate [7]. Unfortunately, in our hands this procedure failed to provide sufficient quantities of the desired adduct **5** due to the production of **4** in a competing side reaction.

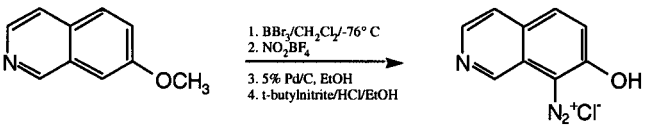


We next turned our attention to the development of novel methodology for the synthesis of the 2-pyrindine ring system via a proposed ring contraction of an isoquinolinol. The photochemical decomposition or Wolff rearrangement of *o*-quinone diazides is known as the Sus reaction and has been used to prepare five-membered rings as well a number of fused ring systems [8]. It has not previously been employed for the preparation of 2-pyrindines.

Synthesis of 7-hydroxy isoquinoline was accomplished using a modification of the Pomeranz-Fritsch reaction [9]. Namely, *m*-methoxy benzaldehyde is condensed with aminoacetaldehyde diethyl acetal in refluxing benzene to provide the imine quantitatively. Hydrogenation of the imine over Adam's catalyst provides the amine which is then tosylated with tosyl chloride in pyridine. These three steps can be performed on a large scale without purification to provide the tosylate **7** in a 93% yield. Cyclodehydration is then effected by HCl in dioxane to provide the requisite isoquinoline **8** in a 67% yield.

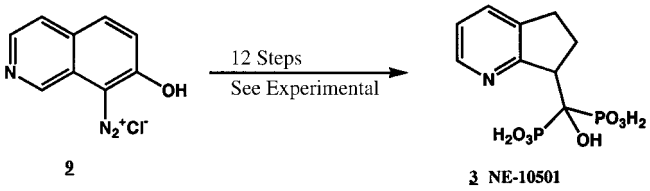


The isoquinoline was further functionalized to provide the ring contraction precursor, a diazo oxide **9**. Treatment of 7-methoxy isoquinoline with boron tribromide in methylene chloride at −76°C cleanly affords 7-hydroxy isoquinoline. The 8-nitro analog is obtained exclusively upon treatment of the isoquinolinol with nitronium tetrafluoroborate in tetramethylene sulfone. Finally, the diazo salt is prepared following hydrogenation to afford the amine followed by diazotization with *t*-butyl nitrite in ethanolic HCl.



Irradiation of the diazo oxide **9** with a 275 watt sun lamp following neutralization of the HCl salt with sodium bicarbonate provided the desired ring contracted product in a 50% yield. This reaction is complicated by the diazo coupling of product and starting material. Note it is this exact side reaction that is exploited in the diazo-type offset photocopying process. The desired bisphosphonic acid is then obtained as follows. Hydrogenolysis of the pyrindine ring system provides the dihydropyrindine carbox-

ylic acid, methyl ester. Saponification of the ester affords the acid that is then subjected to normal phosphorylation conditions (PCl_3/H_3PO_3 then aqueous HCl). This 12-step synthetic sequence provided the desired bisphosphonic acid **3** (NE-10501).



2 **3** NE-10501

EVALUATION OF BIOLOGICAL ACTIVITY

The bone-mediating properties of the conformationally restricted predicted active, NE-10501, were directly compared to the more flexible pyridine analog, risedronate, in several relevant models. In the Schenk growing rat model [10] the antiresorptive lowest effective dose (LED) for NE-10501 was found to be 0.01 mg P/kg. This is less potent than risedronate with LED = 0.0003 mgP/kg. We also found the conformationally restricted pyrindine to be less active than risedronate in the rat adjuvant arthritis model. In this model, we have demonstrated that risedronate provides a dramatic decrease in inflammation and in bone resorption at the 0.03 mg P/kg dose level [11]. The pyrindine was found to be ineffective at this dose level. Similar in vitro potency rankings have now also been reported in several cell-based assays shown to track these antiresorptive structure-activity relationships [12].

The reduction in bone antiresorptive activity observed with the pyrindine, NE-10501, compared to the pyridyl analog, risedronate, can at least be partially explained by a reduction in binding to the surface of hydroxyapatite, the main calcium-phosphate mineral component of bone. With typical adsorption experiments, we have recently demonstrated that the pyrindine analog is adsorbed onto the surface of hydroxyapatite much more slowly than risedronate, and as well, a much higher solution concentration is required than for Risedronate to reach an equilibrium concentration with the conformationally restricted analog. Thus, lower affinity for bone mineral is predicted for NE-10501 from this in vitro assessment [13].

CONCLUSION

We have designed a conformationally restricted bone active analog from the predicted active conformation of the osteoporosis drug, risedronate. This novel 2-pyrindinylmethylene bisphosphonic acid **3** has been synthesized in 12 steps. The key reaction in the synthetic sequence is a photolytic ring contraction of an isoquinolinol to provide the 2-pyrindine ring system. The pyrindine bisphosphonic acid has been found to possess potent but less antiresorptive activity (LED = 0.01 mgP/kg) compared to the nonconformationally restricted parent compound risedronate (LED = 0.0003 mgP/kg) in an acute bone model.

This work is consistent with the structure-activity relationships already established with the bisphosphonic acid class of compounds. The modest reduction in antiresorptive potency obtained upon conformationally restricting the basic nitrogen functionality relative to the phosphonate residues in this series is likely to be at least in part, due to the physical chemistry of these compounds, that is, the physical chemistry of the hydroxyapatite adsorption isotherm. It is also important to note that the pyrindine analog was synthesized and tested as a racemic mixture. A reduction in biological activity with the racemic mixture could also suggest that a stereochemical recognition event is associated with antiresorptive activity. Current understanding of the biochemical target of bisphosphonates is consistent with this hypothesis. The close tracking of the structure-activity relationships of this compound and other heterocyclic series of bisphosphonates has been useful in identifying key targets in their antiresorptive mechanism of action [14]. Thus enzyme targets in the mevalonic acid metabolic pathway have now been identified as relevant to bone activity, and these findings offer researchers new understandings from which to capitalize on the biomedical utility of this important class of compounds.

EXPERIMENTAL

7: *N-(2,2-Diethoxyethyl)-N-[(3-methoxyphenyl)methyl]-4-methylbenzenesulfonamide ($C_{21}N_{29}NO_3S$)*

To a 5 L round-bottom flask equipped with a magnetic stir bar, condenser, and heating mantle was added 111.9 g, 100 mL (0.822 mole) of *m*-anisaldehyde **6**, 114.9 g, 125.5 mL (0.863 mol, 1.05 equiv.) of aminoacetaldehyde diethyl acetal, and 2600 mL of benzene. This yellow solution was stirred and when heated to reflux for 3.5 hours 800 mL of benzene was evaporated, and the remaining solution was placed into a hydrogenator, and 2.0 g of PtO_2 was added under a $N_2\uparrow$ stream. This slurry was then placed onto a hydrogenator at 58 lb. The catalyst was filtered through Celite, rinsed well with benzene, and evaporated to dryness to yield 208 g of a brown oil. TLC-

R_f = 0.75 (5% IPA/CH_2Cl_2). This oil was then dissolved in one liter of pyridine (dry) and a solution of 172.4 g (0.9 mol, 1.1 equiv) of *p*-methylbenzenesulfonyl chloride dissolved in 600 mL of (dry) pyridine was added while the solution was stirring. The resulting solution was allowed to stir at room temperature for 3 days. The pyridine was then removed by evaporation to yield an oil. This oil was poured into one liter of ice water and stirred at 0°C for 1 hour, then extracted with 6 × 500 mL of ether. The combined extracts were washed with 3 × 500 mL of brine dried over $MgSO_4$, filtered, and evaporated to yield an orange oil, 312 g, 93%, TLC-R_f − 0.3 (5 drops of IPA in 10 mL of CH_2Cl_2); ^{1}H-NMR ($CDCl_3$) -δ 7.72 (d, 2H, aromatic), 7.29 (d, 2H, aromatic), 7.16 (t, 1H, aromatic), 6.77 (d, 2H, aromatic), 6.66 (s, 1H, aromatic), 4.54 (t, 1H, $CH_2\underline{CH}$), 4.5 (s, 2H, N$\underline{CH}$), 3.6 (m, 2H, $\underline{CH_2}CH_3$), 3.36 (m, 2H, $\underline{CH_2}CH_3$), 3.22 (d, 2H, $\underline{CH_2}CH$), 3.7 (s, 3H, $\underline{CH_3}O$), 2.43 (s, 2H, CH_3) abd 1,13 ppm (t, 6H, $CH_2\underline{CH_3}$).

8: *7-Methoxyisoquinoline ($C_{10}H_9NO$)*

To a 2 L round-bottom flask, equipped with a magnetic stir bar, condenser, and $N_2\uparrow$ inlet, was added 75 g (0 184 mol) of N-(2,2-diethoxyethyl)-N-[3-methoxyphenyl) methyl]-4-methyl-benzenesulfonamide, 1.0 liter of dioxane, and 200 mL of 6$\underline{N}$ HCl. This slurry was stirred and heated to reflux with an oil bath under $N_2\uparrow$ for 18 hr. The reaction solution was slowly poured into 1 L of distilled H_2O, stirred for .5 hr, then extracted with ether (2 × 500 mL). The pH of the aqueous layer was adjusted to 8 with ammonium hydroxide and extracted with 4 × 500 mL of dichloromethane. The combined extracts were dried over $MgSO_4$, filtered, and evaporated to yield 30 g of an oil. The crude product was purified on a Prep 500A using 12.5% acetone in dichloromethane. Fractions were collected, combined, and evaporated to yield 19.7 g of a brown oil, 67%.

TLC-R_f = 0.23 (40% EtOAc/hexanes).

^{1}H-NMR ($CDCl_3$)-δ 9.14 (s, 1H, H^1), 8.40 (d, 1H, H^2, J = 5 Hz), 7.71 (d, 1H, H^4, J = 9 Hz), 7.57 (d, 1H, H^3, J = 5 Hz), 7.33 (d, 1H, H^5, 9 Hz), 7.20 (s, 1H, H^6) and 3.94 ppm (s, 3H, $\underline{CH_3}O$).

9*: 7-Hydroxy-8-isoquinolinediazonium Chloride, ($C_9H_6ClN_3O$)*

7-Hydroxyisoquinoline (C_9H_7NO). To a 2 L, three-necked round-bottom flask equipped with a magnetic stir bar, $N_2\uparrow$ inlet, addition funnel (500 mL), and drying tube, all flame-dried under $N_2\uparrow$, was added 19.7 g (0.124 mol) 7-methoxyisoquinoline **8** and 800 mL of dry dichloromethane. This solution was stirred and cooled to −76°C and 628 mL (0.628 mol) of 1.0 M boron tribromide in dichloromethane was added dropwise while maintaining this temperature. Thereafter, the slurry was stirred for 18 hours, allowing the temperature to rise to room temperature. (A white solid precipitated from the reaction.) The reaction slurry was poured into 1 L of ice water and stirred for 1 hour. The layers were separated, and the aqueous layer was then adjusted to pH ~7 with 1N NaOH. A yellow solid precipitated, was filtered off, and air dried to yield 14.5 g of a yellow solid, 81%.

TLC-R_f − 0.1 (36% EtOAc/hexanes).

^{1}H-NMR (DMSO-d_6) δ 10.13 (s, 1H, OH), 9.07 (s, 1H, H^1_3), 8.25 (d, 1H, H^2, J = 6 Hz), 7.80 (d, 1H, H^5, J = 9 Hz), 7.65 (d, 1H, H^3, J = 6 Hz), 7.32 (d, 1H, H^4, J = 9 Hz) and 7.24 ppm (s, 1H, H^6).

7-Hydroxy-8-nitroisoquinoline ($C_9H_6N_2O_3$). To a 300 mL round-bottom flask equipped with a magnetic stir bar and $N^2\uparrow$ inlet was added 14.5 g (0.1 mol) of 7-hydroxyisoquinoline and 100 mL of warmed tetramethylene sulfone. The brown slurry was stirred, and 18.6 g (0.14 mol) of nitronium tetrafluoroborate was added portionwise with cooling (ice bath). The reaction mixture was stirred for 3 hours, and the reaction was quenched with 100 mL of methanol. Evaporation to dryness and trituration twice with ether yielded a dark solid precipitate that was filtered off and dried to yield 19.0 g, 100% of product.

TLC-R_f = 0.23, 50% IPA/CH_2CL_2 + (NH_4OH, 3 drops per 10 mL).

^{1}H-NMR (DMSO-d_6) δ 9.23 (s, 1H, H^1), 8.55 (d, 1H, H^2, J = 6 Hz), 8.21 (d, 1H, H^5 J = 9 Hz), 8.11 (d, 1H, H^3, J = 6 Hz), and 7.70 ppm (d, 1H, H^4, J = 9 Hz).

8-Amino-7-hydroxyisoquinoline HCl Salt ($C_9H_8N_2O$ HCl). A hydrogenation can was charged with 28.5 g (0.15 mol) of 7-hydroxy-8-nitroisoquinoline, 6.0 g 5% Pd/C (wet), and 725 mL of ethanol. The slurry was then hydrogenated until $H_2\uparrow$ uptake had stopped and was then removed, filtered through Celite, and rinsed well with ethanol. The crude product was evaporated to dryness, dissolved in methanol, and the HCl salt was precipitated with etheric/HCl. The resulting orange solid was filtered off, triturated with ether, and filtered again to yield 19 g (65%) of product after drying.

TLC-R_f = 0.28, 10% MeOH/CH_2Cl_2 + (NH_4OH, 3 drops per 10 mL).

^{1}H-NMR (DMSO-d_6) δ 9.80 (s, 1H, H^1), 8.15 (d, 1H, H^2, J = 6.6 Hz), 8.08 (d, 1H, H^3, J = 6.6 Hz), 7.66 (d, 1H, H^5, J = 8.4 Hz), 7.28 (d, 1H, H^4, J = 8.4 Hz), and 6.0 ppm (bs, 3H, NH_2, OH).

7-Hydroxy-8-isoquinolinediazonium Chloride-$C_9H_6ClN_3O$ To a 2 L round-bottom flask equipped with a magnetic stir bar and 500 mL addition funnel was added 4.94 g (0.0252 mol) of 8-amino-7-hydroxyisoquinoline hydrochloride and 70 mL of ethanolic/HCl. This slurry was stirred and cooled with an ice bath to −0°C, a solution of 17.46 mL of *t*-butylnitrite, 790 mL of ethanol and 58 mL of distilled H_2O was then added dropwise over an hour. Stirring was continued for 2 hours. A solid was removed by filtration, and the desired product was precipitated with 2 L of ether. It was then filtered off rinsed with ether, and dried to yield 2.6 g, 50% of a yellow solid.

TLCR_f = 0.42, 7% MeOH/CH_2Cl_2 + (NH_4OH, 3 drops per 10 mL).

^{1}H-NMR (DMSO-d_6) δ 9.13 (s, 1H, H^1), 8.51 (d, 1H, H^2, J = 5.7 Hz), 7.98 (d, 1H, H^5, J = 9.9 Hz), 7.94 (d, 1H, H^3, J = 5.7 Hz), and 7.00 ppm (d, 1H, H^4, J = 9.9 Hz).

3*: NE-10501*

7H-2-Pyrindine-7-carboxylic methyl Ester ($C_{10}H_9NO_2$). To a 1 L round-bottom flask equipped with a magnetic stir bar, drying tube and ice bath was added 0.5 g (0.0024 mol) of 2-hydroxy-8-isoquinolinediazonium chloride, 650 mL of anhydrous methanol, and 302 mg (0.036 mol, 1.5 equiv.) of sodium bicarbonate. After cooling to 0°C, the reaction mixture was irradiated with a 275 watt sun lamp while maintaining the temperature at ~0°C. After 3 hours, the reaction mixture was evaporated to dryness under vacuum to yield a dark brown solid. The product was extracted from the crude mixture with 3 × 150 mL of dichloromethane. The combined extracts were dried over $MgSO_4$, filtered, and evaporated to dryness to yield an orange solid 210 mg, 50%.

TLC-R_f = 0.68, 10% IPA/CH_2Cl_2 + (NH_4OH, 3 drops per 10 mL).

^{1}H-NMR (DMSO-d_6) δ 8.78 (s, 1H, H^1), 7.63 (d, 1H, H^4, J = 3.9 Hz), 7.58 (d, 1H, H^2, J = 6 Hz), 7.51 (d, 1H, H^3, J = 6 Hz), 6.36 (d, 1H, H^5, J = 3.9 Hz) and 3.71 ppm (s, 3H, OCH_3).

6,7-Dihydro-5H-2-pyrindine-7-carboxylic Acid, methyl Ester ($C_{10}H_{11}NO_2$) A hydrogenation jar was charged with 0.8 g (0.00457 mol) of 7H-2-pyrindine-7-carboxylic acid, methyl ester, 2.0 g of 5% Pd/C (wet) and 125 mL of anhydrous methanol. This slurry was then hydrogenated until $H_2\uparrow$ uptake ceased, then filtered through Celite, and rinsed well with methanol. Evaporation to dryness yield 430 mg, 53% of an oil.

TLC-R_f = 0.79, 10% IPA/CH_2Cl_2 (NH_4OH, 3 drops per 10 mL).

^{1}H-NMR (DMSO-d_6) δ 8.50 (s, 1H, H^1), 8.4 (d, 1H, H^2), 7.35 (d, 1H, H^3), 4.22 (t, 1H, H^6), 3.68 (s, 3H, OCH_3), 2.95 (m, 2H, H^4) and 2.60 ppm (m, 2H, H^5).

6,7-Dihydro-5H-2-pyrindine-7-carboxylic Acid HCl Salt ($C_9H_9NO_2HC$). To a round bottom flask, equipped with stir bar, condenser, and oil bath, was added 0.53 g (0.003 mol) of 6,7-dihydro-5H-2-pyrindine-7-carboxylic acid methyl ester, 3.1 mL of 1N NaOH, and 30 mL of anhydrous methanol. This solution was stirred and heated at 58°C for 2.5 hours. Evaporation to dryness under vacuum yielded a tan solid. This solid was then stirred with 20 mL of ethanolic/HCl for an hour. A tan solid precipitated and was filtered off, rinsed with ether, and dried to yield 256 mg.

TLC-R_f = 0.17, 30% MeOH/CH_2Cl_2 + (NH_4OH, 3 drops per 10 mL).

^{1}H-NMR (DMSO-d_6) δ 8.78 (s, 1H, H^1), 8.70 (d, 1H, H^2), 7.98 (d, 1H, H), 4.3 (t, 1H, H^6), 3.15 (m, 2H), and 2.25 ppm (m, 2H).

[1-Hydroxy-(6-7-dihydro-5H-2-pyrind-7-yl)methylene]bis[phosphonic Acid] ($C_9H_{13}NO_7P_2$). To a two-necked, round-bottom flask equipped with a stir bar, condenser, and $N_2\uparrow$ inlet was added 0.540 g (0.0028 mol) of 6,7-dihydro-5H-2-pyrindine-7-carboxylic acid, HCl salt, 708 mg (0.00863 mol, 3 equiv.) of phosphorous acid and 10 mL of chlorobenzene. This slurry was stirred while adding 1.19 g, 0.75 mL (0.00863 mol, 3.0 equiv.) of phosphorus trichloride with a syringe under $N_2\uparrow$. Stirring was continued, and the reaction mixture was heated to 105°C for 4 hours. During this time, the slurry was cooled to room temperature. The mixture was decanted to remove the chlorobenzene solvent, then 10 mL of 1N HCl was added to the residue followed by stirring and heating at reflux overnight. Evaporation to dryness and trituration with acetone (3 × 50 mL) yielded 107 mg of a solid.

Fast atom bombardment mass spectrum calculated for $C_9H_{13}NO_7P_2$ ([M-H]$^-$) = 308, found 308.

^{31}P-NMR (D_2O)-δ 17.24 (d, $J_{p\text{-}p}$ = 35 Hz) and 15.91 (d, $J_{p\text{-}p}$ = 35 Hz) ^{1}H-NMR (D_2O)-δ 8.85 (s, 1H,

H^1), 8.40 (d, 1H, H^2), 7.77 (d, 1H, H^3), 4.10 (m, 1H, Ch_2CH), 3.1 (m, 2H), and 2.5 ppm (m, 2H).

Anal. Calcd. For $C_9H_{13}NO_7P_2$ + 1/3 H_2O: C, 34.28; H, 4.37; N, 4.44.

Found C, 34.55; H, 4/58; N, 4.11

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