RECENT WORK ON THE SYNTHESIS OF PHOSPHONATE-CONTAINING, BONE-ACTIVE HETEROCYCLES

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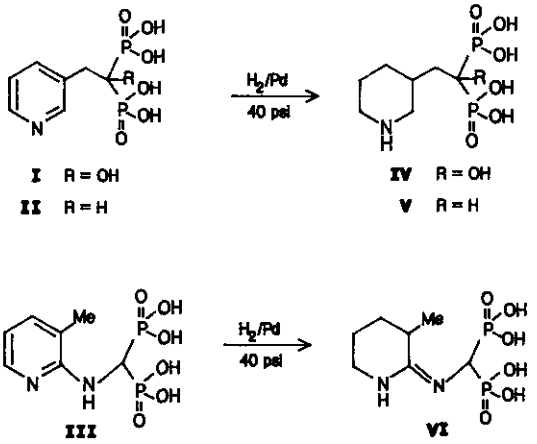
Abstract - New approaches to the design and synthesis of bone antiresorptive phosphonates have led to the discovery of the cyclic bisphosphonate series and the phosphonoalkylphosphinate heterocyclic series. The latter class, which offers substrates for interesting dianion chemistry, has been shown to include effective bone-active isosteres of the well known bisphosphonates.

Since the discovery of the adsorption function of 1-hydroxyethane-1,1-bisphosphonic acid (EHBP) to hydroxyapatite which led to the treatment of bone mineralization pathologies,[1] the synthetic chemistry in this field can be primarily characterized as a study of the structure-activity relationships of this bone affinity/antimineralization property. The 1980s, however, marked a distinct change of focus in bisphosphonate research toward the study of the structural characteristics responsible for new highly potent inhibitors of abnormal metabolic bone resorption.[2] During the past ten years, we and others have thus discovered multiple classes of potent antiresorptive phosphonate agents.[3] These phosphonates were characterized by their cyclic and heterocyclic substitution, the latter of which led to the more potent antiresorptive agents. In our laboratories, two approaches were envisioned at the outset: 1) to design analogues with high bone affinity, similar to EHBP, which could minimize the systemic doses needed for efficacy and 2) to design moieties with reduced bone affinity which could also widen the therapeutic index between antiresorptive and antimineralization activity.

The two major classes with high bone affinity that bore the most antiresorptive-active moieties were the hydroxyalkane (I) and the aminomethane (III) bisphosphonates. Convenient preparations of pyridyl analogues I, II, and III have been described elsewhere.[3] We have also found that novel heterocyclic-substituted bisphosphonates can be prepared through hydrogenation of I, II, and III. Thus, piperidine (IV, V) and amidine bisphosphonates (VI) were obtained and have been shown to also be highly potent antiresorptive agents.[4] To date, the most potent antiresorptive

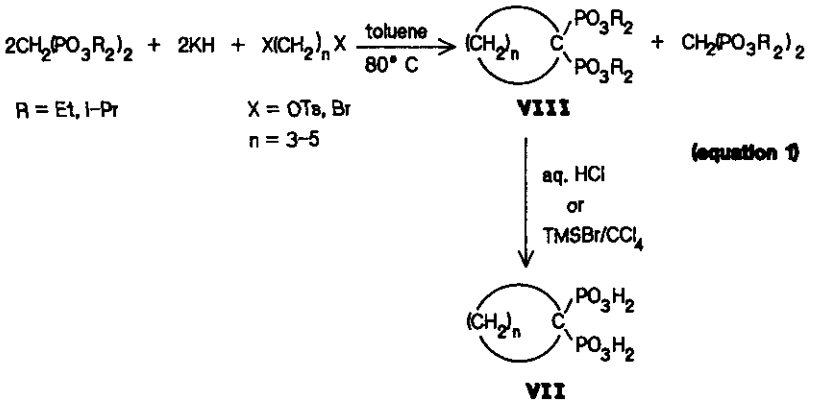
phosphonate known with clinical utility is 2-(3-pyridyl)-1-hydroxyethane bisphosphonic acid I (NE-58095, Norwich Eaton[4]) (LED = 0.0003 mg P/kg[5]).



RESULTS AND DISCUSSION

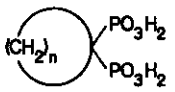
The alternative drug design approach was to study congeners with reduced bone affinity, a property which has been shown to translate directly to reduced antimineralization potency, while maintaining antiresorptive potency similar to or better than that of EHBP. The first attempts at this involved studying the cyclic bisphosphonates, in which the geminal substituted carbon of the P-C-P moiety is a member of the ring. This was found to reduce the bone affinity, and a series of these compounds was therefore studied.

The synthesis of the cyclic bisphosphonates (VII) was accomplished through a dialkylation of tetraalkylmethane bisphosphonate with an alkyl ditosylate or dihalide, followed by deesterification of VIII with either acid or bromotrimethylsilane.[6] In most cases examined, optimum yields for the cyclization process were achieved with the stoichiometry indicated in

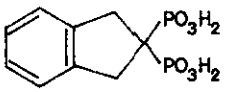


(equation 1)

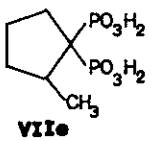
equation 1. This procedure appears to be quite general, allowing for the preparation of a wide range of cyclic bisphosphonates with a variety of ring sizes and substituents in good yields (e.g., VIIa-f). Attempts to prepare cyclic bisphosphonates with ring sizes larger than cyclohexane produced substantial amounts of monosubstituted methane bisphosphonates (IX) and tetraphosphonates (X).



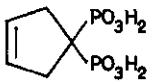
VIIa, n = 3
VIIb, n = 4
VIIc, n = 5



VIId

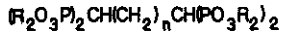


VIIe



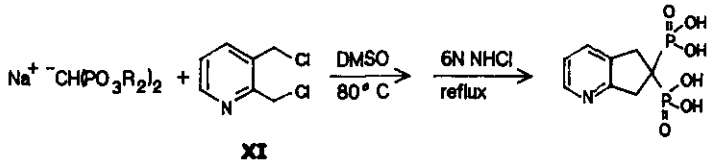
VIIf

IX

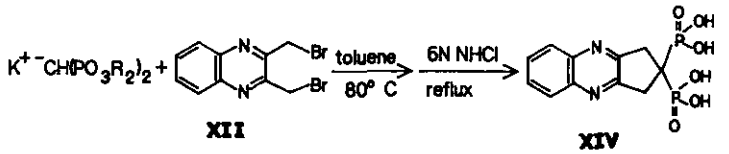


X

Finally, because a wider range between the bone-affinity properties and the antiresorptive potency was desired in this bisphosphonate class, attempts to find more potent inhibitors of resorption were made with the use of heterocylic functionality. With the discovery of high potency with pyridine substituents in the acyclic classes, the nitrogen-containing cyclic bisphosphonates were then designed. Thus, as with other cyclic bisphosphonates, bishalomethyl precursors **XI** and **XII** were sought to prepare dihydropyrindine **XIII** and quinoxalinecyclopentane **XIV**. We found either the NCS halogenation of lutidine or the thionyl chloride conversion of bishydroxymethylpyridine provided, in low yields, a suitable intermediate (**XI**) for methane-1,1-bisphosphonic acid (MBP) dialkylation and subsequent acid hydrolysis (6N HCl) to **XIII**.



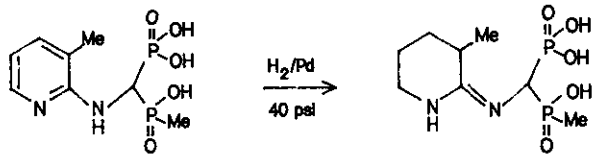
XI **XIII**



XII **XIV**

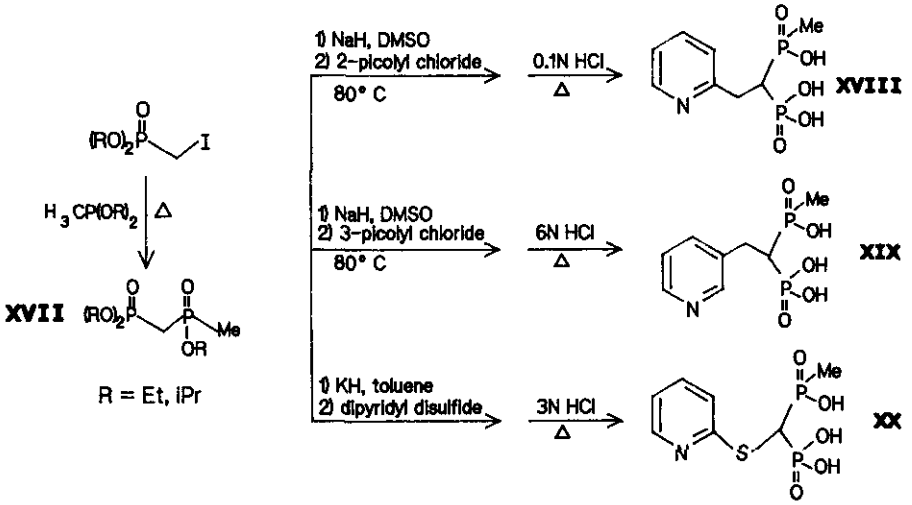
Likewise, the bisbromomethylquinoxaline XII was dialkylated with the MBP potassium anion as described above. Final conversion to XIV was also accomplished via hydrolysis with 6N HCl. Unfortunately, potency better than other cyclic bisphosphonates was not observed with these analogues. Efforts have continued in this line of drug design and will be discussed in more detail in future reports.

We have also undertaken studies to alter bone affinity through P-C-P phosphonate moiety modification. As a result of this work, we were gratified to find that the phosphonic-carbon-phosphinic acid system offers antiresorptive classes of compounds although reduced bone affinity is observed.[7] We have thus been successful in our attempts to widen the therapeutic index between antiresorptive and antimineralization activity with this novel class. The first of this series to be prepared was the pyridyl aminomethane phosphonoalkylphosphinate class, of which phosphonomethylphosphinate (PMP) XV is a member which has been reviewed previously.[8] Also the corresponding amidines such as XVI can be conveniently prepared in quantitative yields as was done with the bisphosphonates.

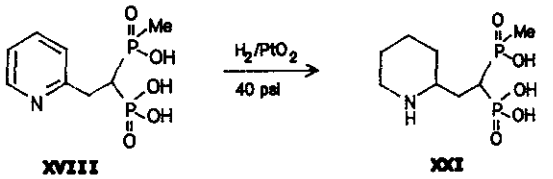


XV XVI

We have studied the alkylation chemistry within this class as well. The Arbuzov product, methylene phosphonomethylphosphinate ester (MPMP) XVII,[9] can be alkylated and hydrolyzed as *can the corresponding MBP to yield heterocyclic systems such as* the 2- and 3-pyridylethane PMP adducts, XVIII and XIX, respectively. Also dipyridyl disulfide can be conveniently alkylated to prepare pyridylthiomethane PMP (XX). All three products exhibited antiresorptive potency.



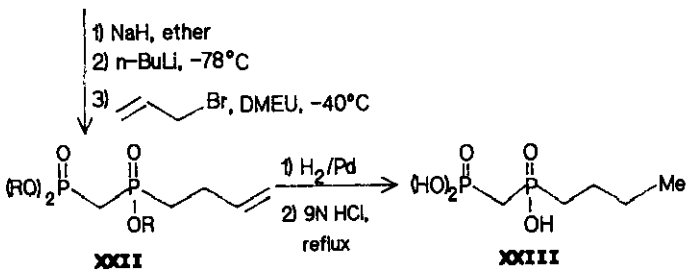
Piperidylethane PMP **XXI**, another antiresorptive active analogue in this series, was also prepared on hydrogenation of XVIII over PtO_2 at 40 psi in quantitative yield.



XVIII **XXI**

Finally, we and another group have looked at the utility of dianion chemistry with this interesting MPMP (**XVII**) building block.[7,10] For example, allyl bromide adds at -40°C to the dianion generated with 1 eq. of NaH (room temperature) and 1 eq. of nBuLi (-78°C) to yield methylene phosphonobutenylphosphinate ester **XXII** in moderate yields (30-50%). After silica gel chromatography, this can be hydrogenated to the corresponding butane congener and finally hydrolyzed with aqueous HCl to the free acid **XXIII**.[11] We are currently considering the utility of this synthetic route in the production of heterocyclic PMP dianion products.

XVII



XXII **XXIII**

CONCLUSION

We and others have demonstrated the vital utility of heterocyclic chemistry in the design of potent new bone antiresorptive compounds. The approach of designing new cyclic bisphosphonic acids was examined, and diminished antimineralization side effects were discovered. This same tack was shown to be useful in the design of the new phosphonoalkylphosphinate class where potent antiresorptive agents were discovered despite ~10-fold reduction in bone affinity. Future discussions of the biological activity of this series are forthcoming.[12] The alkylation chemistry in this class closely resembles that discovered for the bisphosphonic acids with the exception that interesting dianion chemistry has now been demonstrated.

EXPERIMENTAL

Nmr spectra were recorded on a JEOL FX90Q or a GE GN300 instrument with Me_4Si or sodium 3-(trimethylsilyl)propionate as a ^1H internal standard. For ^{31}P nmr spectra 85% H_3PO_4 was used as an external standard. Chemical ionization mass-spectral data was obtained on a Hewlett Packard 5985B GC-MS by means of direct insertion. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Typical procedures for the syntheses of the recent examples described herein are as follows.[6-8]

Tetraisopropyl Cyclobutane-1,1-bisphosphonate, **VIIIa**

To a stirred, ice-cold suspension of KH (1.66 g of a 35% mineral oil dispersion, 14.5 mmol) in 20 ml of dry toluene was added dropwise a solution of tetraisopropyl methanediphosphonate (5.00 g, 14.5 mmol) in 50 ml of toluene. Upon completion of addition, the ice bath was removed and the clear yellow solution was stirred at room temperature for 1 h. 1,3-Propanediol ditosylate (2.79 g, 7.33 mmol) was dissolved in 10 ml of toluene and added to the reaction mixture. The mixture was heated at 80°C for 1 h, then cooled in an ice bath, filtered, and concentrated. The concentrate was then chromatographed (1:1 hexane:THF eluent) on silica gel to afford 1.89 g (70% based on the ditosylate) of the desired ester as a white crystalline material (mp 39.5-42°C). ^1H Nmr ($CDCl_3$): 4.91-4.69 (m, 4H, OCH), 2.76-2.11 (overlapping m, 6H total, $-CH_2-$), 1.35 (d, 24H, J=6.3 Hz, CH_3). ^{31}P Nmr ($CDCl_3$): 24.3 ppm. Isobutane CI mass spectrum m/e 385 $(M+H)^+$. Anal. Calcd for $C_{16}H_{34}O_6P_2$: C, 49.99; H, 8.92; P, 16.12. Found: C, 49.65; H, 8.83; P, 16.25.

Cyclobutane-1,1-bisphosphonic Acid **VIIa**

A solution of the BP ester **VIIIa** (2.0 g, 5.4 mmol) in 10 ml of 12N HCl was refluxed for 2 h. The solution was then concentrated under vacuum with additional H_2O to remove the final traces of HCl. The residue was then dissolved in H_2O and treated with activated charcoal. The charcoal was removed by filtration and the filtrate was concentrated using ether to remove the last traces of H_2O. The product was then dried under vacuum at 50°C for 24 h to afford 0.99 g of a white solid (mp 214-216°C) (85%). ^1H Nmr (D_2O): 2.62-2.12 (overlapping m, ring CH_2). ^{31}P Nmr (D_2O): 25.0 ppm. Anal. Calcd for $C_4H_{10}O_6P_2$: C, 22.23; H, 4.67; P, 28.67. Found: C, 22.04; H, 4.42; P, 28.54.

[(Hydroxy)methylphosphinyl][(3-methyl-2-piperidinylidene)amino]methylphosphonic Acid Monohydrate, **XVI**

[(Hydroxy)methylphosphinyl][(3-methyl-2-pyridinyl)amino]methylphosphonic acid **XV** (1.7 g, 6.07 mmol), 100 ml of distilled H_2O and Pd/C (0.5 g) were placed in a 500 ml Parr hydrogenation

bottle. The mixture was hydrogenated at room temperature (40 psi) for 2 days. The solution was filtered and washed with distilled H_2O. The filtrate was then concentrated under vacuum to yield 1.67 g **XVI**. An analytical sample was purified from H_2O/ethanol. ^{31}P Nmr (D_2O): 9.45 ppm (d, J=13 Hz); 39.39 ppm (d, J=14 Hz). ^{1}H Nmr (D_2O): 1.43 (d, J=5 Hz, $C\underline{H}_3$); 1.67 (d, J=14 Hz, $C\underline{H}_3$); 1.93 (m, 2 H's, $C\underline{H}_2$); 2.96 (m, $C\underline{H}$); 3.48 (m, $C\underline{H}_2$-NH); 4.17 (dd, J_1=34 Hz, J_2=17 Hz, $C\underline{H}$). ^{13}C Nmr (D_2O): 17.23 (d, J=97 Hz, $\underline{C}H_3$-P); 19.72 (s); 21.05 (s); 28.00 (s); 33.71 (s); 44.59 (s); 55.32 (dd, J_1=212, J_2=106, P-$\underline{C}$H-P); 170.61 (s). Anal. Calcd for $C_8H_{18}N_2O_5P_2 \cdot H_2O$: C, 31.80; H, 6.67; N, 9.27. Found: C, 31.50; H, 6.52; N, 9.22.

[(Hydroxy)methylphosphinyl][2-pyridinylthio]methylphosphonic Acid Monohydrate, **XX**

Potassium hydride (35%; 1.36 g; 0.01 mol) was placed in a two-neck, round-bottom flask with 50 ml of distilled toluene. The flask was then cooled in an ice bath, and diethyl [(isopropyloxy)methylphosphinyl]methylphosphonate **XVII** (2.72 g; 0.01 mol) was added dropwise over a 15 min period. The ice bath was removed after the addition, and the solution was then stirred at room temperature for 1 h. The round bottom flask was again cooled to 0°C in an ice bath and 2,2´-dipyridyl disulfide (2.20 g; 0.01 mol) dissolved in 30 ml of dry toluene was quickly added to the reaction flask. This solution was stirred at 0°C for 1 h and then a second equivalent of potassium hydride (35%; 1.36 g; 0.01 mol) was added to the reaction flask. The solution was allowed to gradually come to room temperature, and was stirred overnight under ambient conditions. The solution was then filtered through Celite and the filtrate was evaporated to yield a yellow oil. Purification was accomplished by silica gel preparative hplc with 75:25 acetone:hexanes to yield 1.91 g (52%) of pure product. ^{31}P Nmr ($CDCl_3$): 19.16, 44.91 ppm.

The pure ester (1.49 g, 4.0 mmol) was refluxed overnight in 3N HCl (50 ml). The solution was concentrated, and the product was re-evaporated with isopropanol several times. The resulting solid was recrystallized with acetone/H_2O to yield 0.80 g (71%) **XX**. ^{31}P Nmr (D_2O): 10.67, 40.57 ppm. ^{1}H Nmr (D_2O; pD=11): 1.51 (3H, d, J=14 Hz); 3.75 (1H, dd, J=19 Hz; J=20 Hz); 7.13-7.80 (3H, m); 8.39 ppm (1H, d, J=7 Hz). Anal. Calcd for $C_7H_{11}NO_5P_2S \cdot H_2O$: C, 27.91; H, 4.35; N, 4.65. Found: C, 28.27; H, 4.08; N, 4.65.

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11. [(Hydroxy)butylphosphinyl]methylphosphonic Acid **XXIII**: ^{31}P Nmr (D_2O): 17.08 (s), 49.92 (s). ^{13}C Nmr (D_2O): 15.33 (s), 25.60 (s), 25.72 (d, J=17 Hz), 31.19 (dd, J=78 Hz, 127 Hz), 31.37 (d, J=95 Hz). ^{1}H Nmr (D_2O) (IBM WP100SY): 0.87 (t, J=7.5 Hz, 3H, CH_3); 1.13-2.13 (overlapping m, 6H, CH_2); 2.52 (q, J=16 Hz, 20 Hz, 2H, PCH_2P).

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