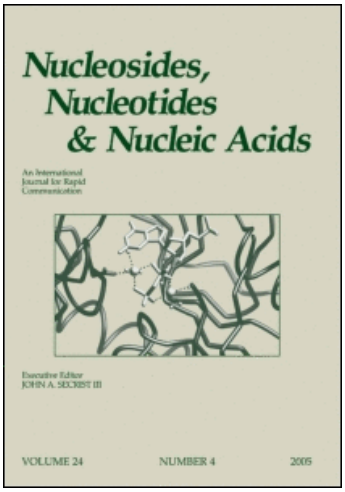

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The Synthesis of Aminopyri Dine-Based Phosphonoacetates as ADP Analogs

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THE SYNTHESIS OF AMINOPYRIDINE-BASED PHOSPHONOACETATES AS ADP ANALOGS

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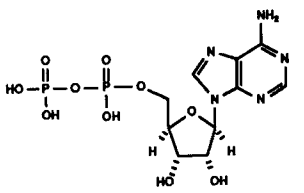
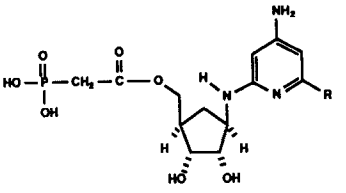
Abstract: The rationale for proposing the title compounds as potential adenosine diphosphate receptor antagonists is discussed. A convenient synthesis and formulation for the cyclopentenyl amino alcohol **8** is described.

Adenosine and its attendant phosphorylated derivatives play a central role in a number of physiological pathways. Specific to our interests is the fact that human platelets possess receptors for adenosine diphosphate, and that this nucleotide is a key stimulus for the shape-change and subsequent aggregation of these platelets.[1] It has been suggested that antagonism of platelet ADP-receptors might prove to be an interesting therapeutic approach to the inhibition of platelet aggregation and may lead to a useful antithrombotic treatment. Literally hundreds of analogs of ADP have been synthesized, and from these structures one may obtain clues as to new targets for potential antagonists. Based upon a number of observations in the literature, we have elected to synthesize the novel ADP-analogs represented by structure **1** shown in Fig. 1.

Several features of these novel structures are worth noting. We have simplified the purine base of ADP into the 2,4-diaminopyridine nucleus based mainly upon the systematic study of Antonini, et. al., who demonstrated that simplified heterocyclic ADP-analogs can retain bioactivity.[2] The substitution of a carbacyclic ring for the normally-occurring furan structure was designed to increase compound stability. Lastly, the diphosphate group has been replaced by a phosphonoacetate mimic. This group has recently been incorporated into other nucleotide structures to produce biologically active compounds,[3] and is hoped in our case to provide a metabolically stable alternative to the labile pyrophosphate function.



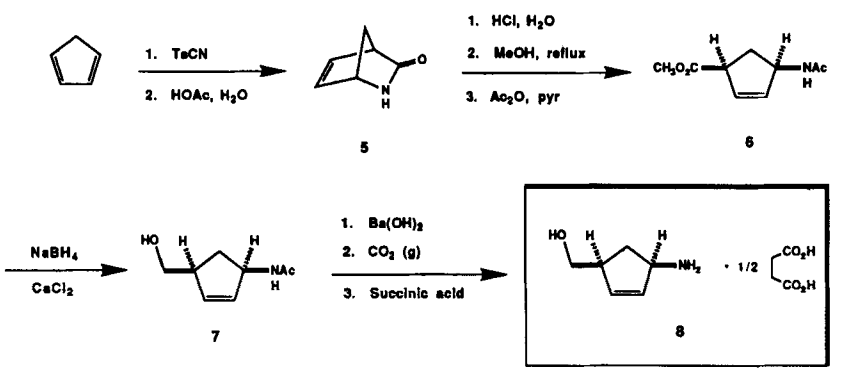
ADP



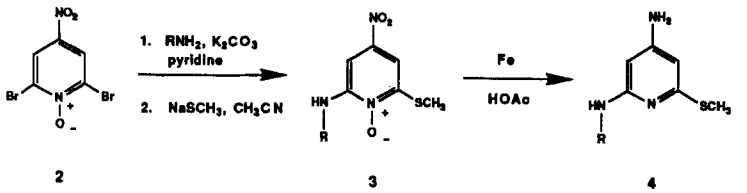
1a (R = Br)
b (R = SCH_3)

Figure 1: Adenosine diphosphate (ADP) and proposed antagonist (1)

Scheme I



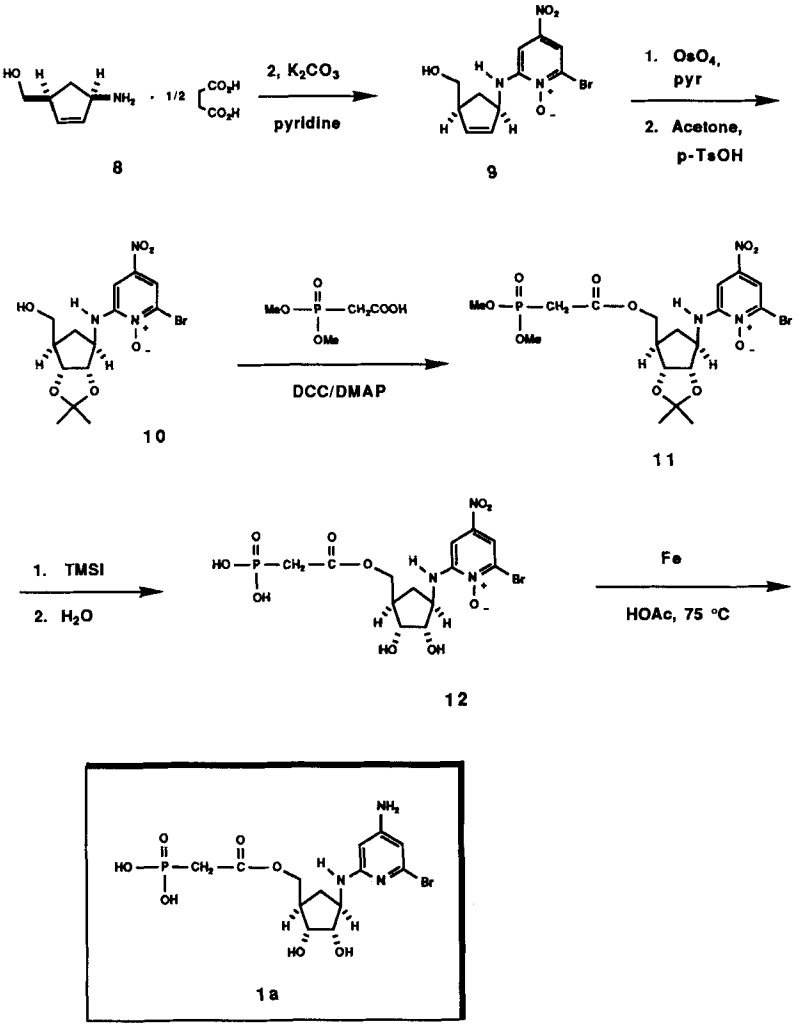
Our synthetic approach to the construction of targets such as **1** is illustrated in Eq. 1. Sequential displacement of the two halide atoms of 2,6-dibromo-4-nitropyridine-N-oxide **2** with amine and sulfur nucleophiles respectively gives good overall yields of the intermediate **3**. In preliminary model experiments, we demonstrated that the initial displacement could be successfully carried out with amines which bear an unprotected hydroxyl group. Bis-reduction of both the nitro group and the N-oxide functionality proceeds smoothly with iron in acetic acid to afford the desired 4-aminopyridines **4**.



Application of the strategy depicted in Eq. 1 toward the synthesis of structures such as **1** requires the *cis*-disubstituted cyclopentenyl amine **8**. This material was prepared by modification of the literature procedure (Scheme I).[4] Thus, cycloaddition of tosylcyanide with cyclopentadiene and subsequent hydrolysis of the resulting imino adduct afforded the bicyclic lactam **5** in 58% yield. Hydrolytic ring-opening of **5** generated the *cis*-1,3-cyclopentene system, which was esterified and acetylated to finally provide compound **6** in 72% overall yield. Ester reduction using *in-situ* generated calcium borohydride gave a 91% yield of the hydroxy-amide **7**. Anticipating a high degree of water solubility for the amino-alcohol to be obtained from the hydrolysis of **7**, we chose to carry out this reaction by employing aqueous barium hydroxide as the base. After refluxing for 4 h, the reaction mixture was cooled to RT and treated with gaseous carbon dioxide, effectively precipitating the barium as insoluble $BaCO_3$. Simple filtration and concentration then afforded the desired amino-alcohol, which was converted to the half-succinate salt **8** (90% from **7**). We found this salt to be a well-behaved, free-flowing powder which could be used directly in the subsequent coupling reaction.

Illustrated in Scheme II is the synthetic sequence leading to our proposed target compound **1a**. Admixture of the pyridine-N-oxide **2**, the half-succinate salt **8** (1 eq), and powdered K_2CO_3 (4 eq) in pyridine and stirring under N_2 afforded the desired adduct **9** as a yellow solid in yields ranging from 65-75%. Treatment

Scheme II



of a pyridine solution of **9** with a stoichiometric amount of osmium tetroxide gave rise to a 4:1 mixture of diastereomeric diols in good yield. The major isomer from this reaction could be obtained in pure form by careful fractional recrystallization, or alternatively one could convert the mixture of diol products into their respective acetonides (acetone, *p*-toluenesulfonic acid). The diastereomeric acetonides thus obtained are easily separable by flash chromatography.

It is well-appreciated that assignment of relative stereochemistry in five-membered rings based upon NMR coupling constants is risky at best. Although the ^1H NMR of the major acetonide **10** displayed coupling patterns consistent with the illustrated *anti*-configuration, we sought more definitive evidence on which to base our assignments. NOE-difference spectroscopy provided that evidence. Detailed analysis of the minor

acetonide revealed a strong enhancement among all ring methine protons, confirming the *syn*-relationship between substituents and identifying the major isomer as the expected *anti*-product.[5]

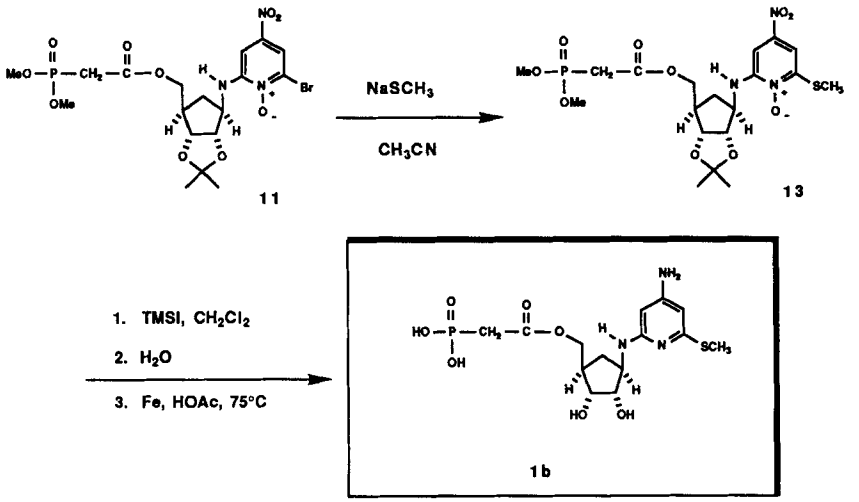
After some experimentation, we were able to find conditions under which this oxidation would proceed with catalytic amounts of osmium, a desirable result considering both the cost and toxicity of OsO_4. Although the catalytic reaction provided the mixture of diols in yield comparable to that of the stoichiometric reaction, the ratio of diastereomers produced was unfortunately much less favorable (ca. 2.5:1). Also, it was interesting to note that when the stoichiometric reaction was carried out in THF-water instead of pyridine, a much higher ratio of diastereomers obtained (ca. 8:1), but the unfortunate co-production of an inseparable contaminant rendered these conditions unusable.

With sufficient quantities of *anti*-acetonide **10** readily available, we were able to complete the synthesis of our proposed target as illustrated. Coupling of **10** with dimethylphosphonoacetic acid using DCC/DMAP provide the ester **11** in 94% yield after recrystallization. Nucleophilic demethylation of the phosphonate esters was accomplished with trimethylsilyl iodide in dichloromethane, generating the acetonide of **12**. When exposed to water, the phosphonic acid moiety of this compound autocatalyzed the hydrolysis of the acetonide, generating **12** in 81% overall yield. Finally, bis-reduction of **12** using iron fillings in acetic acid required elevated temperatures (75°C), but provided the desired target molecule **1a** in 66% yield.

The synthesis of the thiomethyl analog **1b** was straightforward and is illustrated in Scheme III. Treatment of the advanced intermediate **11** with sodium thiomethoxide in acetonitrile results in clean displacement to give compound **13** in 65% yield. Phosphate demethylation, acetonide hydrolysis, and bis-reduction finally affords the desired target **1b** in 63% overall yield.

The final compounds **1a** and **1b**, as well as all intermediates described herein, were evaluated for their ability to inhibit the ADP-induced aggregation of human platelets. Unfortunately, none of these compounds proved to be active in this assay.

Scheme III



EXPERIMENTAL

Proton and carbon magnetic resonance spectra were recorded on a Bruker Aspect 3000 Spectrometer and are reported in ppm on the δ scale from internal tetramethylsilane. Infrared spectra, combustion analyses, and mass spectra were determined by Physical and Analytical Chemistry (The Upjohn Company). All mass spectra were obtained on a Varian MAT CH5-DF spectrometer and are reported as $[M+H]^+$ ions peak-matched against 2-hydroxyethyl disulfide matrix clusters. Melting points were determined on a Thomas-Hoover Capillary Melting Point Apparatus and are uncorrected.

When necessary, solvents and reagents were dried prior to use. Anhydrous tetrahydrofuran refers to material that was distilled from sodium metal/benzophenone ketyl. Thin-layer chromatography was carried out using Analtech 250 micron silica gel GF plates. Flash chromatography was performed using EM Reagents Silica Gel 60 (230-400 mesh).

Unless otherwise noted, all non-aqueous reactions were carried out under an atmosphere of dry nitrogen using oven-dried glassware.

***cis*-4-Acetamido-1-hydroxymethylcyclopent-2-ene (7).** Powdered calcium chloride (16.65 g, 0.15 mol) and sodium borohydride (11.35 g, 0.30 mol) were combined in anhydrous THF (300 mL) and stirred at room temperature for 1 hr. A solution of **6** (18.22 g, 0.99 mol) in THF (250 mL) was then added in a single portion and the mixture was stirred overnight. After cooling to 0°C, the milky reaction mixture was carefully treated with ice-water (370 mL), followed by 6N HCl (58 mL). The now clear and colorless reaction was allowed to stir at room temperature for 1 h and then concentrated *in vacuo*. The residue thus obtained was azeotroped with methanol (4 x 260 mL) and pyridine (1 x 260 mL). To the resulting material was added pyridine (ca. 300 mL), producing a heterogeneous mixture which was filtered and concentrated. The concentrate was dissolved in methanol and decolorized using activated charcoal, which was removed by filtration through Celite. Concentration of the filtrate gave a yellow syrup which was chromatographed on 300 g silica gel using 10% methanol/ethyl acetate to give 14.11 g (91%) of **7** as white solid: R_f 0.35 (10% methanol/ethyl acetate); IR (mull) 3295, 3244, 1630, 1540, 1367, 1051, 1044, 1041, 770, 605 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 6.80 (br m, 1H, NH), 5.77 (d of m, 2H, J = 23 Hz, vinyl H's), 4.92 (br m, 1H, C_4-H), 3.89 (br m, 1H, C_1-H), 3.60 (q of d, 2H, J = 10.5 Hz, 4.0 Hz, CH_2O), 2.84 (br s, 1H, OH), 2.44 (d of t, 1H, J = 13.7, 8.7 Hz, C_5-H), 1.93 (s, 3H, CH_3), 1.43 (d of t, 1H, J = 13.7, 3.9 Hz, C_5-H); ^{13}C NMR (75.5 MHz, $CDCl_3$) 169.5, 134.8, 132.6, 64.1, 54.3, 46.7, 34.0, 32.2. Anal. Calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.03. Found: C, 61.72; H, 8.50; N, 9.03.

***cis*-4-Amino-1-hydroxymethylcyclopent-2-ene, half-succinate salt (8).** A solution of **7** (1.55 g, 10.0 mmol) in water (100 mL) was treated with barium hydroxide octahydrate (15.8 g, 50 mmol) and refluxed 4 hrs under nitrogen. After cooling to room temperature methanol was added (ca. 20 mL), and gaseous carbon dioxide was bubbled through the well-stirred reaction mixture overnight. The precipitated barium carbonate was removed by filtration through Celite (methanol washes). The filtrate was concentrated *in vacuo*, depositing methanol-insoluble solids which were removed by filtration. The crude product was concentrated to an oil and dissolved in ethanol (10 mL) and treated with a solution of succinic acid (1.18 g, 10.0 mmol) in methanol (ca. 7 mL). To the resulting solution was then added diethyl ether until clouding ceased (ca. 90 mL). The desired salt was induced to crystallize by scratching, and the product was collected by filtration and washed with ethanol to give 1.54 g (90%) of **8** as a white powder, mp 158-161°C: ^1H

NMR (300 MHz, $CDCl_3$ - for the free base) δ 7.28 (s, 3 H, NH_2 & O-H), 5.82 (s, 2 H, vinyl H's), 3.96 (broad d, 1 H, J = 6.9 Hz, N-CH), 3.59 (d, 2 H, J = 3.5 Hz, O-CH_2), 2.91 (m, 1 H, CH), 2.31 (m, 1 H, CH_{5a}), 1.37 (dt, 1 H, J = 13.4 Hz, J = 2.4 Hz, CH_{5b}); Anal. Calcd for $C_8H_{14}NO_2$: C, 55.80; H, 8.19; N, 8.14. Found: C, 55.84; H, 8.23; N, 8.21.

***cis*-1-Hydroxymethyl-4-(4-nitro-6-bromo-1-oxide-2-pyridinylamino)-2-cyclopentene (9).** A solution of **2**[5] (9.98 g, 33.5 mmol), **3** (5.77 g, 33.5 mmol), and potassium carbonate (18.5 g, 134 mmol) in pyridine (65 mL) was well-stirred for 5 days (twice as long as is actually necessary). The pyridine was removed *in vacuo* and the residue was partitioned between water (600 mL) and ethyl acetate (600 mL). The aqueous layer was extracted with ethyl acetate (2 x 500 mL), and the combined organic layers were washed with brine (100 mL), then dried over anhydrous magnesium sulfate. After filtration and concentration *in vacuo*, the residue was chromatographed on 700 g of 230-400 mesh silica gel using 2-3% methanol-chloroform to give 8.60g (78%) of **4** as an orange solid: R_f 0.50 (5% methanol-chloroform); IR (mull) 2955, 2925, 2870, 2855, 1625, 1525, 1335, 1305, 1210, 1195 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.73 (d, J = 2.9 Hz, 1 H, aromatic H), 7.56 (broad d, J = 8.6 Hz, 1 H, N-H), 7.48 (d, J = 2.9 Hz, 1 H, aromatic H), 6.02 (m, 1 H, vinylic H), 5.89 (m, 1 H, vinylic H), 4.67 (m, 1 H, N-CH), 3.67 (m, 2 H, O-CH_2), 3.01 (m, 2 H, CH & O-H), 2.62 (m, 1 H, CH_{2a}), 1.71 (m, 1 H, CH_{2b}); ^{13}C NMR (75.5 MHz, $CDCl_3$) 150.3, 143.8, 137.2, 131.4, 130.5, 109.4, 97.5, 64.3, 58.0, 47.2, 34.1; HRMS, m/e 329.0001 ($C_{11}H_{12}BrN_3O_4$ requires 329.0012); Anal. Calcd for $C_{11}H_{12}BrN_3O_4$: C, 40.02; H, 3.67; N, 12.73. Found: C, 40.35; H, 3.75; N, 12.58.

(±)-[3aR-(3aα,4α,6α,6aα)]-6-[(6-Bromo-4-nitro-2-pyridinyl)amino]tetrahydro-2,2-dimethyl-4H-cyclopenta-1,3-dioxole-4-methanol, N-oxide (10). A solution of osmium tetroxide (924 mg, 3.63 mmol) in pyridine (25 mL) was cooled to 0°C and treated with **9** (1.19 g, 3.59 mmol) in a single portion. After stirring for 1 h at room temperature in the dark, the black reaction mixture was cooled to 0°C and treated in a slow stream with a solution of sodium bisulfite (1.65 g) in water (29 mL) to which was added pyridine (18 mL). After stirring for 30 min at room temperature, the mixture was diluted with saturated aqueous sodium chloride (100 mL) and extracted with ethyl acetate (8 x 200 mL). The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and concentrated *in vacuo* to a yellow solid. Recrystallization from ethanol (approx. 70 mL) gave the major diol isomer (690 mg, 53%) as an amorphous, canary-yellow solid.[6] The conversion of this material to the acetonide occurs under conditions equally applicable to the mixture of diastereomeric diols. Thus, a solution of the major diol isomer (2.12 g, 5.82 mmol) and *p*-toluenesulfonic acid monohydrate (0.5 g) in acetone (115 mL - containing 0.24% water) was stirred at room temperature for 2 h. The reaction mixture was then added in a slow stream to a well-stirred solution of saturated aqueous sodium bicarbonate (190 mL) at 0°C. The resulting mixture was partially concentrated *in vacuo* to remove the acetone, and the aqueous residue (which contained precipitated **10**) was extracted with dichloromethane (3 x 100 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to a yellow solid. Recrystallization from ethyl acetate/hexane gave **10** (2.06 g, 88%) as fine yellow crystals, mp 181.5-182.5°C: R_f 0.18 (80% ethyl acetate/hexane); IR (mull) 3298, 2953, 2925, 2869, 2855, 1625, 1531, 1215, 1062, 1048 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 8.13 (broad d, 1 H, J = 8.4, N-H), 7.76 (d, 1 H, J = 2.9 Hz, aromatic H), 7.64 (d, 1 H, J = 2.9 Hz, aromatic H), 4.68 (dd, 1 H, J = 6.1 Hz, J = 2.4 Hz, O-CH), 4.38 (dd, 1 H, J = 6.1 Hz, J = 2.4 Hz, O-CH), 4.0-3.85 (m, 2 H, N-CH & O-CH_{2a}), 3.74 (m, 1 H, O-CH_{2b}), 2.77 (m, 1 H, CH), 2.64 (m,

1 H, CH_{2a}), 2.45 (m, 1 H, O-H), 1.80 (m, 1 H, CH_{2b}), 1.55 (s, 3 H, CH_3), 1.29 (s, 3 H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) 150.4, 143.4, 131.5, 111.6, 109.5, 97.8, 86.8, 83.7, 63.7, 59.1, 46.2, 33.4, 26.7, 24.3; Anal. Calcd for $C_{14}H_{18}BrN_3O_6$: C, 41.60; H, 4.49; N, 10.40. Found: C, 41.65; H, 4.66; N, 10.33. HRMS (FAB) Calcd for $C_{14}H_{19}BrN_3O_6$: 404.0458. Found: 404.0472.

(±)-[3aR-(3aα,4α,6α,6aα)]-[6-[(6-Bromo-4-nitro-2-pyridinyl)amino]tetrahydro-2,2-dimethyl-4H-cyclopenta-1,3-dioxol-4-yl]methyl dimethyoxyphosphinylacetate, N-oxide (11). A solution of **10** (607 mg, 1.50 mmol), dimethyl phosphonoacetic acid (277 mg, 1.65 mmol), and 4-dimethylaminopyridine (18 mg, 0.150 mmol) in dry dichloromethane (15 mL) was treated with 1,3-dicyclohexylcarbodiimide (340 mg, 1.65 mmol) at room temperature. After 15 min, the reaction was filtered and the filtrate was concentrated *in vacuo*. The resulting residue was chromatographed on 50 g of 230-400 mesh silica gel using 100:2:1 ethyl acetate/methanol/triethylamine to give a yellow foam which yielded a yellow solid on trituration with hexane. The material was recrystallized from benzene/hexane to give **11** (778 mg, 94%) as tiny, yellow crystals, mp 137-139°C: R_f 0.11 (100% ethyl acetate); IR (mull) 2952, 2924, 2855, 1738, 1629, 1532, 1313, 1255, 1047, 1040 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.83 (d, 1 H, J = 2.9 Hz, aromatic H), 7.81 (d, 1 H, J = 2.9 Hz, aromatic H), 7.31 (broad d, 1 H, J = 7.2 Hz, N-H), 4.58 (dd, 1 H, J = 6.8 Hz, J = 3.5 Hz, O-CH), 4.49 (dd, 1 H, J = 6.8 Hz, J = 4.1 Hz, O-CH), 4.35 (dd, 1 H, J = 11.3 Hz, J = 4.9 Hz, O-CH_{2a}), 4.25 (dd, 1 H, J = 11.3 Hz, J = 4.6 Hz, O-CH_{2b}), 3.94 (m, 1 H, N-CH), 3.82 (d, 3 H, J = 11.3 Hz, O-CH_3), 3.81 (d, 3 H, J = 11.4 Hz, O-CH_3), 3.18 (dd, 1 H, J = 21.9 Hz, J = 14.3 Hz, P-CH_{2a}), 3.05 (dd, 1 H, J = 21.5 Hz, J = 14.3 Hz, P-CH_{2b}), 2.58 (m, 2 H, CH & CH_{2a}), 1.87 (m, 1 H, CH_{2b}), 1.61 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) 165.0 (d, J = 7 Hz), 150.6, 143.5, 130.9, 113.1, 110.4, 98.5, 86.2, 82.1, 65.8, 59.4, 53.1 (d, J = 6.4 Hz), 43.1, 33.7, 33.1 (d, J = 134 Hz), 27.1, 24.7; Anal. Calcd for $C_{18}H_{25}BrN_3O_{10}P$: C, 39.00; H, 4.55; N, 7.58. Found: C, 39.31; H, 4.65; N, 7.57; HRMS (FAB) Calcd for $C_{18}H_{26}BrN_3O_{10}P$: 554.0540. Found: 554.0570.

(±)-(1α,2β,3β,4α)-1-[4-[(6-Bromo-4-nitro-2-pyridinyl)amino]-2,3-dihydroxycyclopentyl]methyl phosphonoacetate, N-oxide (12). Iodotrimethylsilane (0.16 mL, 1.10 mmol) was added to a solution of **11** (261 mg, 0.471 mmol) in dry dichloromethane (5 mL) at -78°C. The cooling bath was removed and the reaction mixture allowed to warm to room temperature. After stirring for 30 min, water (approx. 1.5 mL) was added and stirring was continued for 10 min. The heterogeneous mixture was then directly concentrated *in vacuo*, dissolved in acetone containing a small amount of methanol, and treated with cyclohexylamine (0.13 mL). The resulting precipitate was filtered, washed with acetone, and dried *in vacuo* to give an orange-yellow solid.[7] Finally, this material was dissolved in water and allowed to flow slowly through a column (1 cm dia.) of 200-400 mesh Dowex 50W-X8 (hydrogen form) resin (3 g) to exchange hydrogen for cyclohexylammonium and simultaneously remove the acetonide functionality. The column was washed with water until the effluent was near colorless (total volume of 40 mL), giving **12** (238 mg, 81%) as a clean orange solid upon lyophilization from a minimum amount of water: R_f 0.25 (4:1:1 acetonitrile/water/concentrated aqueous ammonium hydroxide); IR (mull) 2953, 2924, 2868, 2855, 1731, 1624, 1538, 1343, 1277, 1207 cm^{-1}; ^1H NMR (300 MHz, D_2O) 7.69 (d, 1 H, J = 2.9 Hz, aromatic H), 7.56 (d, 1 H, J = 2.9 Hz, aromatic H), 4.03 (m, 2 H, O-CH_2), 3.84 (m, 3 H, O-CH's & N-CH), 2.87 (d, 2 H, J = 21.0 Hz, P-CH_2), 2.35-2.10 (m, 2 H, CH_{2a} & CH), 1.26 (m, 1 H, CH_{2b}); ^{13}C NMR (75.5 MHz, D_2O) 147.8 (d, J = 6.0 Hz), 129.4, 124.5, 110.5, 88.2, 78.7, 54.7, 50.5, 44.6, 35.3, 19.7, 14.1 (d, J = 124 Hz),

7.2; Anal. Calcd for $C_{13}H_{17}BrN_3O_{10}P \times 1H_2O$: C, 30.97; H, 3.80; N, 8.34. Found: 31.20; H, 3.80; N, 8.22; HRMS (FAB) Calcd for $C_{13}H_{18}BrN_3O_{10}P$: 485.9914. Found: 485.9916.

(±)-(1α,2β,3β,4α)-1-[4-[(4-Amino-6-bromo-2-pyridinyl)amino]-2,3-dihydroxycyclopentyl]methyl phosphonoacetate (1a). A solution of **12** (131 mg, 0.260 mmol) in glacial acetic acid (3 mL) was stirred with 40 mesh iron filings (161 mg, 2.89 mmol) under nitrogen at 75°C for 1.25 h. The dark mixture was cooled to room temperature and diluted with water. A considerable amount of a dark-olive precipitate was filtered off and washed with water. The filtrate was applied to a column (1.4 cm dia.) of 200-400 mesh Dowex 50W-X8 (hydrogen form) resin (6 g), and the column was washed with water (several vol.'s). The product-containing material was eluted with 1:10 concentrated aqueous ammonium hydroxide/water (25 mL) followed by 1:1 concentrated aqueous ammonium hydroxide/water (200 mL). Concentration *in vacuo* of the combined aqueous ammonium hydroxide effluent gave 93 mg of a burgundy glass. This material was dissolved in water and passed through a column (1.4 cm dia.) of 200-400 mesh Dowex 2-X8 (formate form) resin (6 g). The column was washed with water (several vol.'s), then the crude product was eluted with 4 N aqueous formic acid (200 mL). The residue obtained upon removal of the solvents *in vacuo* was chromatographed on 7 g of 230-400 mesh silica gel using 6:1:1 acetonitrile/water/concentrated aqueous ammonium hydroxide to give **1a** (45 mg, 39%) as a faint pink solid after lyophilization : R_f 0.21 (4:1:1 acetonitrile/water/concentrated aqueous ammonium hydroxide); ^{1}H NMR (300 MHz, D_2O) 6.22 (d, 1 H, J = 1.3 Hz, aromatic H), 5.71 (d, 1 H, J = 1.5 Hz, exchangeable aromatic H), 4.16 (m, 2 H, O-CH_2), 3.97 (t, 1 H, J = 5.5 Hz, O-CH), 3.85 (t, 1 H, J = 5.2 Hz, O-CH), 3.71 (quart, 1 H, J = 7.1 Hz, N-CH), 2.82 (d, 2 H, J = 20.3 Hz, P-CH_2), 2.5-2.2 (m, 2 H, CH_{2a} & CH), 1.24 (dt, 1 H, J = 12.8 Hz, J = 8.2 Hz, CH_{2b}); ^{13}C NMR (75.5 MHz, D_2O) 149.6 (d, J = 6.5 Hz), 136.0, 113.9, 83.1, 66.7, 54.8, 51.1, 44.2, 34.6, 19.5, 15.3 (d, J = 117 Hz), 7.7; HRMS (FAB) Calcd for $C_{13}H_{20}BrN_3O_7P$: 440.0223. Found: 440.0225.

(±)-(1α,2β,3β,4α)-1-[2,3-dihydroxy-4-[[6-(methylthio)-4-nitro-2-pyridinyl]amino]cyclopentyl]methyl phosphonoacetate, N-oxide (13). Sodium thiomethoxide (58 mg, 0.823 mmol) was added in a single portion to a solution of **11** (433 mg, 0.784 mmol) in dry acetonitrile (10 mL) at room temperature. After stirring for 30 min, the dark mixture was diluted with dichloromethane and filtered. The filtrate was concentrated *in vacuo* and the residue was chromatographed on 100 g of 230-400 mesh silica gel using 3% methanol/ethyl acetate + 0.2% added triethylamine to give **13** (264 mg, 65%) as a yellow solid, mp 132-134°C: R_f 0.17 (5% methanol/ethyl acetate); IR (mull) 2955, 2926, 2868, 2855, 1532, 1336, 1286, 1061, 1034, 869 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) 7.58 (d, 1 H, J = 2.8 Hz, aromatic H), 7.26 (d, 1 H, J = 2.7 Hz, aromatic H), 7.16 (broad d, 1 H, J = 6.7 Hz, N-H), 4.57 (dd, 1 H, J = 6.8 Hz, J = 3.5 Hz, O-CH), 4.49 (dd, 1 H, J = 6.8 Hz, J = 4.0 Hz, O-CH), 4.33 (dd, 1 H, J = 11.3 Hz, J = 5.0 Hz, O-CH_{2a}), 4.25 (dd, 1 H, J = 11.3 Hz, J = 4.9 Hz, O-CH_{2b}), 3.95 (m, 1 H, N-CH), 3.82 (d, 6 H, J = 11.1 Hz, O-CH_3's), 3.18 (dd, 1 H, J = 21.8 Hz, J = 14.4 Hz, P-CH_{2a}), 3.06 (dd, 1 H, J = 21.5 Hz, J = 14.4 Hz, P-CH_{2b}), 2.56 (m, 2 H, CH & CH_{2a}), 2.53 (s, 3 H, S-CH_3), 1.85 (m, 1 H, CH_{2b}), 1.61 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) 165.5 (d, J = 6.3 Hz), 152.9, 149.4, 144.7, 113.0, 100.7, 95.0, 86.3, 82.1, 65.9, 59.0, 53.1 (d, J = 6.0 Hz), 43.1, 33.7, 33.1 (d, J = 134 Hz), 27.1, 24.7, 14.2; Anal. Calcd for $C_{19}H_{28}N_3O_{10}PS$: C, 43.76; H, 5.41; N, 8.06. Found: C, 43.75; H, 5.47; N, 8.03. HRMS (FAB) Calcd for $C_{19}H_{29}N_3O_{10}PS$: 522.1311. Found: 522.1284.

(±)-(1α,2β,3β,4α)-1-[4-[[4-Amino-6-(methylthio)-2-pyridinyl]amino]-2,3-dihydroxycyclopentyl]methyl phosphonoacetate (1b). Iodotrimethylsilane (0.093 mL, 0.656 mmol) was added to a solution of **13** (155 mg, 0.297 mmol) in dry dichloromethane (3 mL) at -78°C. The cooling bath was removed and the red solution was allowed to warm to room temperature. Water (approx. 1 mL) was added and stirring was continued for 15 min. The reaction mixture was directly concentrated *in vacuo*. The residue was allowed to stand for a time with acetone (to regenerate some acetonide) then reconcentrated. The residue was taken up in acetone (10 mL) and treated with cyclohexylamine (0.075). The yellow cyclohexylammonium salt, which precipitated quantitatively, was filtered and washed with acetone. This material was dissolved in water (approx. 50 mL) and allowed to flow slowly through a short column of 200-400 mesh Dowex 50W-X8 (hydrogen form) resin (3 g) where both cyclohexylamine and the acetonide functionality were removed. The column was washed with water until the effluent was colorless. The effluent was concentrated *in vacuo* to a small volume then lyophilized to give 135 mg (96%) of an orange solid.[8] The majority of this material (132 mg, 0.291 mmol) was dissolved in glacial acetic acid (3 mL) was stirred with 40 mesh iron filings (163 mg, 2.91 mmol) at 75°C under nitrogen for 1 h. The reaction mixture was diluted with water and passed through a column (1.4 cm dia.) of 200-400 mesh Dowex 50W-X8 (hydrogen form) resin (6 g). The column was washed with water (several vol.'s), then the product was eluted with 1:9 concentrated aqueous ammonium hydroxide/water (25 mL - to prevent overheating of the column) followed by 1:1 concentrated aqueous ammonium hydroxide/water (200 mL). The effluent was concentrated *in vacuo* to a small volume then lyophilized to give an off-white solid (90 mg). This material was chromatographed on 7 g of 230-400 mesh silica gel (the sample was adsorbed onto 0.5 g of silica gel which was then applied to the head of the column) using 4:1:1 acetonitrile/water/concentrated aqueous ammonium hydroxide to give **1b** (79 mg, 66%) as a pale pink solid after concentration of the appropriate fractions to a small volume followed by lyophilization: R_f 0.18 (4:1:1 acetonitrile/water/concentrated aqueous ammonium hydroxide); ^{1}H NMR (300 MHz, D_2O) 5.98 (s, 1 H, aromatic H), 5.63 (s, 1 H, exchangeable aromatic H), 4.24 (d, 2 H, J = 3.3 Hz, O-CH_2), 4.06 (t, 1 H, J = 5.0 Hz, O-CH), 3.94 (m, 1 H, O-CH), 3.71 (m, 1 H, N-CH), 2.85 (d, 2 H, J = 20.2 Hz, P-CH_2), 2.50 (s, 3 H, S-CH_3), 2.49 (m, 1 H, CH), 2.33 (m, 1 H, CH_{2a}), 1.42 (m, 1 H, CH_{2b}); ^{13}H NMR (75.5 MHz, D_2O) 169.0, 155.2, 149.8, 147.0, 94.4, 80.9, 72.8, 69.5, 61.7, 53.0, 37.9, 34.6 (d, J = 112 Hz), 26.1, 10.8; HRMS (FAB) Calcd for $C_{14}H_{23}N_3O_7PS$: 408.0994. Found: 408.0996.

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5. For example, irradiating the proton resonance of the nitrogen-bearing C-4 carbon results in approximately a 7% enhancement of the adjacent C-3 methine signal.

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7. For the major diol isomer: mp 180-182°C: R_f 0.15 (10% methanol/chloroform- the *syn* isomer has R_f 0.18); IR (mull) 3511, 2954, 2925, 2870, 2855, 1624, 1546, 1336, 1190, 1100 cm^{-1}; ^1H NMR (300 MHz, pyridine-d_5) 8.42 (broad d, 1 H, J = 7.6 Hz, N-H), 7.93 (d, 1 H, J = 3.0 Hz, aromatic H), 7.90 (d, 1 H, J = 2.9 Hz, aromatic H), 7.03 (broad s, 1 H, O-H), 6.70 (broad s, 1 H, O-H), 6.60 (broad s, 1 H, O-H), 4.64 (t, 1 H, J = 4.7 Hz, O-CH), 4.48 (t, 1H, J = 5.7 Hz, O-CH), 4.39 (m, 1 H, N-CH), 4.10 (dd, 1 H, J = 10.7 Hz, J = 4.4 Hz, O-CH_{2a}), 4.05 (dd, 1 H, J = 10.4 Hz, J = 4.3 Hz, O-CH_{2b}), 2.85-2.65 (m, 2 H, CH & CH_{2a}), 1.94 (m, 1H, CH_{2b}); ^{13}C NMR(75.5 MHz, pyridine-d_5) 149.5, 141.1, 129.0, 107.7, 96.4, 76.2, 72.1, 61.2, 57.0, 44.5, 28.8; Anal. Calcd for $C_{11}H_{14}BrN_3O_6$: C, 36.28; H, 3.87; N, 11.54. Found: C, 36.50; H, 3.78; N, 11.37. HRMS (FAB) Calcd for $C_{11}H_{15}BrN_3O_6$: 364.0145. Found: 364.0141.

8. This material is the monocyclohexylammonium salt of the acetonide of **12**.

9. For the diol-phosphonate derived from **13**: R_f 0.24 (4:1:1 acetonitrile/water/concentrated aqueous ammonium hydroxide); ^1H NMR (300 MHz, D_2O) 7.42 (d, 1 H, J = 2.7 Hz, aromatic H), 7.14 (d, 1 H, J = 2.7 Hz, aromatic H), 4.21 (m, 1 H, O-CH_2), 4.1-3.9 (m, 3 H, O-CH's & N-CH), 3.02 (d, 2 H, J = 21.1 Hz, P-CH_2), 2.5-2.3 (m, 2 H, CH_{2a} & CH), 2.48 (s, 3 H, S-CH_3), 1.42 (dt, 1 H, J = 13.1 Hz, J = 8.8 Hz, CH_{2b}); ^{13}C NMR (75.5 MHz, D_2O) 169.4 (d, J = 6.0 Hz), 154.9, 150.0, 147.0, 100.4, 95.9, 76.4, 72.2, 66.3, 56.8, 41.3, 35.7 (d, J = 125 Hz), 29.1, 13.5; Anal. Calcd for $C_{14}H_{20}N_3O_{10}PS$: 454.0685. Found: 454.0698.