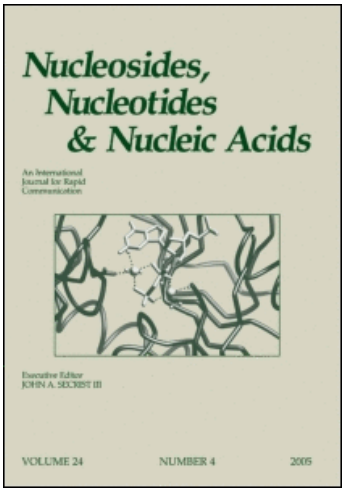

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PHOSPHONATE ANALOGS OF CARBOCYCLIC PHOSPHORIBOSYLAMINE AND CARBOCYCLIC GLYCINAMIDE RIBONUCLEOTIDE

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PHOSPHONATE ANALOGS OF CARBOCYCLIC PHOSPHORIBOSYLAMINE AND CARBOCYCLIC GLYCINAMIDE RIBONUCLEOTIDE

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Abstract. Analogs of intermediates in the *de novo* purine nucleotide biosynthetic pathway were synthesized to study the binding requirements of the corresponding enzymes. Because of the instability of the natural stubstrates, such as phosphoribosylamine, the use of the structurally stable phosphonate moiety and the carbocyclic ribose yields ideal analogs for these studies. In addition, these analogs can act as potential inhibitors of the *de novo* pathway leading to the design of anticancer agents. Enzyme studies with GAR synthetase and GAR transformylase reveal that the title compounds can act as substrates or inhibitors of the *de novo* enzymes.

Introduction

Glycinamide ribonucleotide (GAR) synthetase and glycinamide ribonucleotide transformylase catalyze the second and third steps in the de novo purine nucleotide biosynthetic pathway. Thus, they have been suggested as targets for the rational design of anticancer agents. [1-3] It has previously been shown that the carbocyclic analogs of phosphoribosylamine (PRA) and GAR, in which the ribose rings are replaced by cyclopentane rings, are substrates for GAR synthetase and GAR transformylase.[4-6]

In 1986, an acyclo phosphonate nucleoside analog, (S)-9-(3-hydroxy-2-phosphonomethoxy-propyl)adenine, was found to be active against a variety of viruses.[7] Since that time, the use of the membrane permeable phosphonate moiety has been intensively studied in the design of more useful antiviral agents. In fact, a related compound, 9-(2-phosphonylmethoxypropyl)adenine has recently

been reported to prevent SIV infection in macaques.[8] It therefore seemed reasonable that carbocyclic PRA and GAR analogs incorporating the phosphonate moiety may have the ability to enter cells and inhibit the corresponding enzymes. This report describes the synthesis of these analogs.

Chemistry

The carbocyclic PRA phosphonate analog **9** was prepared from the (-) lactam **1** as outlined in Scheme 1. A catalytic osmium tetroxide cis-dihydroxylation of (-) 2-azabicyclo[2.2.1]hep-5-ene-3-one using N-methylmorpholine N-oxide was employed as reported for the racemic lactam.[9] The oxidation product **2** was esterified (methanol/HCl) and gave **3** as the hydrochloride salt. The N-Boc blocked intermediate **5** was easily obtained from the corresponding 2,3-dimethylmethylene-dioxy derivative **4**. The reduction of **5** was achieved with calcium borohydride in THF, and the hydroxyl group of **6** was converted to the bromo group by treatment with triphenylphosphine and carbon tetrabromide to give **7**. The phosphonic acid diethyl ester **8** was subsequently formed from **7** with triethylphosphite. Hydrolysis of the ester and ketal blocking groups converted **8** to the desired carbocyclic ribofuranosylamine phosphonate **9**.

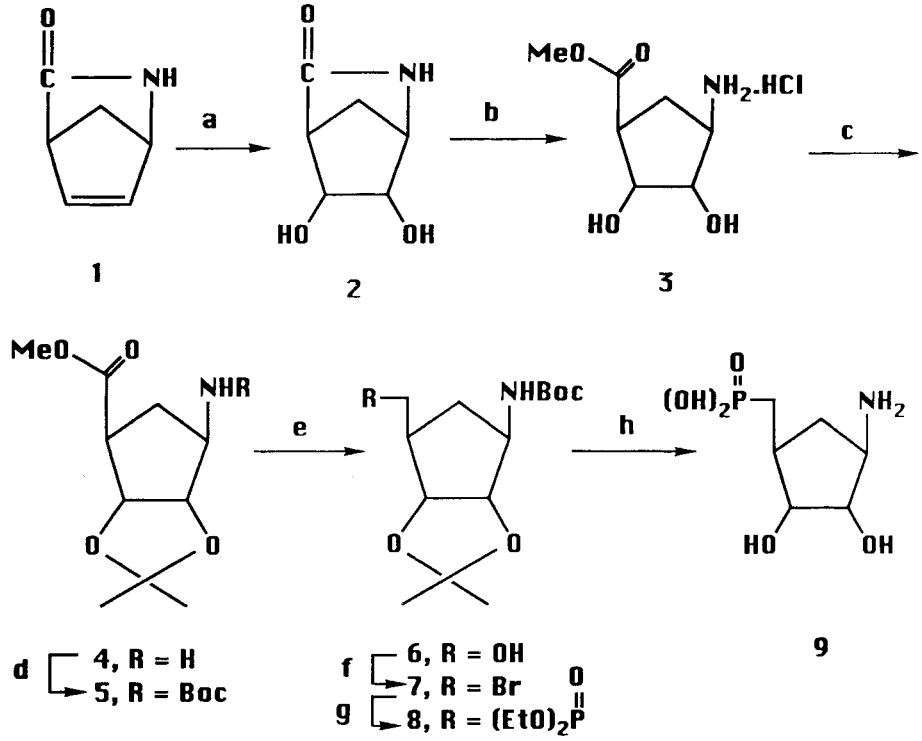
The corresponding GAR analog **12** was prepared from **8** (Scheme 2). Thus, removal of the tert-butoxycarbonyl from **8** and subsequent coupling of the amine **10** with Boc-glycine gave the fully blocked derivative **11**. Final removal of all blocking groups in the presence of BTMS gave the desired product, 1S-(1β,2α,3α,4β)-4-amino-2,3-dihydroxycyclopentanemethylphosphonic acid **12** which was isolated as the free acid, the barium salt, and the ammonium salt.

Results

Preliminary studies show that **9** was accepted as a substrate in the forward reaction with GAR synthetase. Phosphonate **12** was accepted as a substrate for GAR synthetase in the reverse reaction, and was a competitive inhibitor of GAR transformylase. Detailed presentations of the enzyme kinetics of these compounds and related analogs have been reported.[10,11]

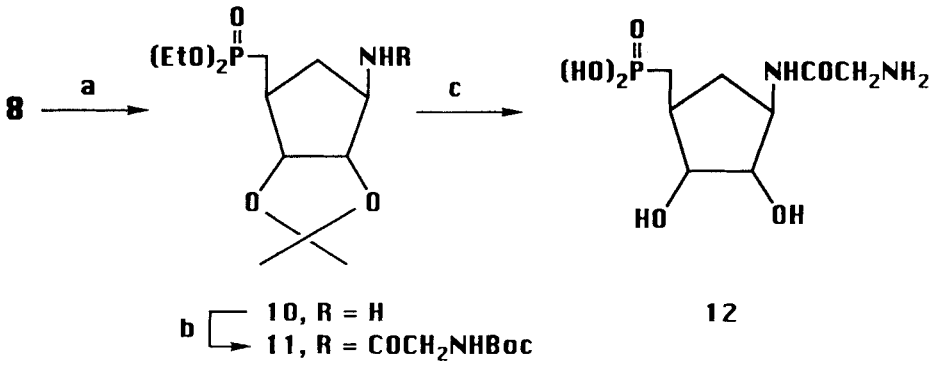
Experimental

Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Melting points were determined on a Mel-Temp II apparatus and are corrected. The NMR spectra were obtained on a Bruker AC-200, Varian Unity 300 or Varian Unity 500 spectrometers and referenced to the solvent. Chemical shifts are expressed in ppm. IR spectra were determined with KBr pellets (solids) or plates (oils) on a Nicolet 5DXC spectrometer and given in cm^{-1}. Electron impact (EI) mass spectra (MS) were obtained with a Kratos/AEI MS-30 spectrometer, chemical impact (CI) MS were obtained with a Finnigan 4000 spectrometer, and fast-atom bombardment (FAB) MS were obtained with a VG 7070E-HF spectrometer, ISP MS were obtained with SCIEX API III spectrometer. Column chromatography was performed on EM Science silica gel 60 (230 - 400 mesh), and preparative thin-layer chromatography was performed on EM Science silica gel 60 F_{254} (1.0 mm layer). DMF was dried over molecular sieves. All other solvents and chemicals are



Reaction conditions: a, OsO_4, N-methylmorpholine N-oxide; b, MeOH, HCl; c, $Me_2C(OMe)_2$, TsOH, DMF; d, $(t\text{-}BuO_2C)_2O$, $NaHCO_3$; e, $Ca(BH_4)_2$, THF; f, PPh_3, CBr_4, THF; g, $P(OEt)_3$; h, BTMS.

Scheme 1



Reaction conditions: a, CF_3COOH; b, Boc-glycine, hydroxybenozotriazole, 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide, 4-methylmorpholine; c, BTMS.

Scheme 2

reagent grade unless specified otherwise. 2-Azabicyclo[2.2.1]hept-5-ene-3-one (Vince's lactam)[12] was obtained from Acros Organics, Pittsburgh PA, and from Chiroscience Limited, Cambridge , UK.

(-)exo-*cis*-5,6-Dihydroxy-2-azabicyclo[2.2.1]heptane-3-one (2): (-)2-Azabicyclo-[2.2.1]hept-5-ene-3-one (12.5 g, 114 mmoles) and N-methylmorpholine N-oxide (17.5 g, 149 mmoles) were dissolved in water (37.5 mL) and t-butanol (50 mL). To the mixture was added osmium tetroxide solution (2.5% w/vol, 0.137g in 5.5 mL, 0.54 mmoles) dropwise under nitrogen over a ten minute period. The reaction mixture became dark brown and temperature rose to 69°C, then returned to room temperature slowly. After 2h stirring, the solvent was evaporated and the residue was dried by azeotrope distillation with abs. EtOH and toluene and crystallized from methanol to give the solid product **2**. Yield 12.94 g (79%), mp 173-175 °C, $[\alpha]^{25}_D$ = -104.9 ° (c=1, MeOH); $[\alpha]^{25}_D$ = -98.4 ° (c=1, H_2O). IR (KBr) cm^{-1}: 3500-3100, 3050, 2950, 1716, 1654. MS (CI): 144 (M^++1). ^1H NMR (DMSO-d_6): δ 7.54 (s,1H, NH), 5.00 (m, 2H, 2OH), 3.74 (m, 2H, H-5, H-6), 3.43 (m, 1H, H-1), 2.25 (m, 1H, H-4), 1.81 (m, 2H, CH_2). *Anal.* Calcd for $C_6H_9NO_3$: C, 50.34; H, 6.34; N, 9.79. Found: C, 50.20; H, 6.59; N, 9.94.

(+)Methyl[1S-(1β, 2α, 3α, 4β)]-4-amino-2,3-dihydroxycyclopentane carboxylate Hydrochloride (3): Dry HCl gas (18.3 g) was dissolved in ice cooled MeOH (150 Ml). To this solution was then added exo-*cis*-5,6-Dihydroxy-2-azabicyclo[2.2.1]heptane-3-one, **2**, (23.3 g, 0.163 moles), and the mixture was refluxed under nitrogen for 2 h. The solvent was evaporated and the crude product was triturated with ether and collected by filtration. Yield 33.1 g (95%), mp 157-158 °C, $[\alpha]^{25}_D$ = +7.6 ° (c=1, MeOH); $[\alpha]^{25}_D$ = +19.3 ° (c=1, H_2O). IR (KBr) cm^{-1}: 3500-2700, 2650, 1723, 1379. MS (FAB): 176 (M^+-Cl). ^1H NMR (DMSO-d_6): δ 8.29 (s, 3H, NH_3^+), 5.27 (m, 2H, 2OH), 4.04 (m, 1H, H-3), 3.76 (m, 1H, H-2), 3.64 (s, 3H, CO_2Me), 2.24 (m,1H, CHH), 1.67 (m,1H, CHH). *Anal.* Calcd for $C_7H_{14}ClNO_{14}$: C, 39.72; H, 6.67; N, 6.62. Found: C, 39.70; H, 6.62; N, 6.59.

(+)Methyl[1S(1β, 2α, 3α, 4β)]-4-amino2,3-(dimethylmethylenedioxy)cyclopentane-carboxylate Hydrochloride(4): The diol **3** (16.9 g, 80 mmoles) was dissolved in DMF (50 mL). To the solution was added p-toluenesulfonic acid monohydrate (0.14 g) and 2,2-dimethoxy-propane (60 mL, 480 mmoles) and the mixture was stirred for 48 h. Anhydrous ether (200 mL) and acetone (200 mL) were added to precipitate the product. Yield 18.7 g (93%) of white crystals; mp 188-190°C. Recrystalization from methanol- ethyl acetate gave the pure product **4**, mp 190-191 °C, $[\alpha]^{25}_D$ = +31 ° (c=1, H_2O); $[\alpha]^{25}_D$ = +22 ° (c=1, MeOH). IR (KBr) cm^{-1}: 3600-2500, 1719, 1620. MS (FAB): 216 (M^+-Cl)· ^1H NMR (DMSO-d_6): δ 8.35 (m, 3H, NH_3^+, D_2O exchangeable), 4.77 (m, 1H, H-3), 4.59 (m, 1H, H-2), 3.66 (s, 3H, CO_2Me), 3.42 (m, 1H, H-4), 2.99 (m, 1H, H-1), 2.40 (m, 1H, CHH), 1.99 (m, 1H, CHH), 1.44 (s, 3H, Me-c-Me), 1.25 (s, 3H, Me-C-Me). *Anal*. Calcd for $C_{10}H_{18}ClNO_4$: C, 47.71; H, 7.21; N, 5.57. Found: C, 47.90; H, 6.98; N, 5.60.

(+)Methyl[1S-(1β, 2α, 3α, 4β)]-4-N(tert-butyloxycarbonyl)amino-2,3-(dimethylmethylenedioxy)cyclopentanecarboxylate (5): The amine salt **4** (7.55 g, 30 mmoles) and $NaHCO_3$ (2.52 g, 30 mmol) were dissolved in water (38 mL). To the mixture was added a solution of di-t-butyldicarbonate (7.20 g, 33 mmoles) in 1,4-dioxane (120 mL). The clear solution was stirred overnight. Solvent was removed, the residue was partitioned between $CHCl_3$ (200 mL) and H_2O (50 mL). The organic layer was concentrated to yield **5** as a solid product; 9.30 g (98%), mp 122-123°C, $[\alpha]^{25}_D$ = +4.4 ° (c=1, MeOH). IR (KBr) cm^{-1}: 3381, 3050, 2985, 1730, 1685, 1526. MS (FAB): 316 (M+1). ^1H NMR ($CDCl_3$): δ 5.29 (m, 1H, NH), 4.61 (m, 1H, H-3), 4.45 (m, 1H, H-2), 4.05 (m, 1H, H-4), 3.72 (s, 3H, CO_2Me), 2.96 (m, 1H, CH-CO_2Me), 2.37 (m, 1H, CHH), 1.80 (m, 1H, CHH), 1.42 (s, 12H, CMe_3, Me-C-Me), 1.27 (s, 3H, Me-C-Me). *Anal.*. Calcd. for $C_{15}H_{25}NO_6$: C, 57.13; H, 7.99; N, 4.44. Found: C, 57.05; H, 7.78; N, 4.50.

(-)[1R-(1β, 2α, 3α, 4β)-4-N(tert-butyloxycarbonyl)amino-2,3-(dimethylmethylenedioxy)cyclopentanecarbinol (6): A mixture of ground $CaCl_2$ (6.66 g, 60 mmoles), $NaBH_4$ (3.42 g, 90 mmoles) and THF (150 mL) was stirred for 1.5h under nitrogen. A solution of the methyl ester **5** (9.46 g, 30 mmoles) in THF (120 mL) was added to the mixture and stirred overnight. Water (110 mL) was added dropwise into the cooled reaction mixture. Glacial acetic acid was added to adjust the solution to pH 5 and the THF was removed under reduced pressure. The remaining aqueous solution was extracted with CH_2Cl_2. The organic layer was dried and evaporated in vacuo and gave **6** as a solid product; 8.20 g (94%), mp 78-80 °C. Recrystalization from EtOAc / hexane gave pure **6**; mp 80-81 °C, $[\alpha]^{25}_D$ = -15.4 ° (c=1, MeOH). IR (KBr) cm^{-1}: 3600-3100, 3000, 2900, 1670, 1542. MS (EI): 288 (M^++1). ^1H NMR (DMSO-d_6): δ 6.93 (m, 1H, NH, D_2O exchangeable), 4.78 (m, 1H, OH, D_2O exchangeable), 4.29 (m, 2H, CH_2OH), 3.70 (m, 1H, H-3), 3.39 (m, 2H, H-2, H-4), 2.02 (m, 2H, H-1, H-5), 1.41(m, 1H, H-5),1.38 (s, 12H, CMe_3, Me-C-Me), 1.20 (s, 3H, Me-C-Me). *Anal.* Calcd. for $C_{14}H_{25}NO_5$: C, 58.51; H, 8.77; N, 4.88. Found: C, 58.38; H, 8.60; N, 4.90.

(-)[1S-(1β, 2α, 3α, 4β)]-4-N(tert-butyloxycarbonyl)amino-2,3-(dimethylmethylenedioxy)cyclopentanemethylbromide (7): To a solution of the hydroxy compound **6** (5.78 g, 20 mmoles) in freshly distilled dry THF (110 mL) was added CBr_4 (13.28 g, 40 mmoles) and PPh_3 (10.49 g, 40 mmoles), and the mixture was stirred overnight. Solid was removed from the reaction mixture and the filtrate was concentrated and purified on silica gel (AcOEt/ hexane) to yield 5.07 g (72%) of bromide **7**; mp 122-124 °C, [a]$^{25}_D$ = -26.8° (c=1, MeOH). IR (KBr) cm^{-1}: 3400, 3000, 2900, 1717, 1638, 1540. MS (EI): 352 (M^++1), 3(36 (M^+-Me). ^1H NMR (DMSO-d_6): δ 7.08 (m, 1H, NH), 4.35 (m, 2H, CH_2Br), 3.56 (m, 2H, H-2, H-3), 3.34 (m,1H, H-4), 2.12 (m, 2H, H-1, H-5),1.50 (m, 1H, H-5), 1.39 (s, 12H, CMe_3, Me-C-Me), 1.21 (s, 3H, Me-C-Me). *Anal*. Calcd for $C_{14}H_{24}BrNO_4$: C, 48.00; H,6.91; N, 4.00. Found: C, 47.98; H, 7.06; N, 3.93.

(-)[1S-(1b,2a,3a,4b)]-4-N(tert-butyloxycarbonyl)amino-2,3-(dimethylmethylenedioxy)cyclopentanemethylphosphonic acid diethyl ester (8) The bromide **7** (3.52 g, 10 mmoles) was dissolved in $P(OEt)_3$ (51 ml, 300 mmoles) and refluxed at 175 °C for 30 h. under nitrogen. After removing solvent, the residue was purified on a slica gel column ($CHCl_3$/MeOH) to yield 5.29g (39%) of phosphonate **8**; mp 106-108 °C, $[a]^{25}_D$ = -2.1° (c=1, MeOH). IR (KBr) cm^{-1}: 3299, 2978, 1700, 1541. MS (CI): 408 (M^++1). ^1H NMR ($CDCl_3$): δ 4.92,(m, 1H, NH), 4.45 (m, 1H, H-3), 4.31 (m, 1H, H-2), 4.08 (m, 4H, 2 $CH_3\underline{CH_2}O$), 3.78 (m, 1H, H-4), 2.36 (m, 2H, PCH_2), 2.03 (m, 1H, H-1), 1.76 (m, 2H, H-5), 1.40 (s, 3H, Me-C-Me), 1.36 (s, 9H, CMe_3), 1.32 (t, 6H, 2 $\underline{CH_3}CH_2O$), 1.24 (s, 3H, Me-C-Me) *Anal.* Cacld for $C_{18}H_{34}NO_7P$: C, 53.06; H, 8.49; N, 3.44. Found: C, 53.10; H, 8.37; N, 3.31.

(+) [1S-(1β, 2α, 3α, 4β)]-4-Amino-2,3-dihydroxy-1-cyclopentanemethyl phosphonic acid (9): To the phosphonate **8** (0.41 g, 1.0 mmole) in anh. DMF (5.0 mL) was added BTMS (4.59 g, 4.0 mL, 30 mmoles) and the mixture was stirred overnight under nitrogen. The solvent was removed under reduced pressure at 35 °C, and the residue was dissolved in water (1.0 mL). To this solution was added $Ba(OAc)_2$ (1 M solution, 2.0 mL) and the pH was adjusted to 8.5 by adding 0.5 N NaOH. The mixture was kept at 4 °C overnight and the solids were removed by filtration. The filtrate was diluted with abs. EtOH and cooled at -18 °C and the barium salt was collected by filtration to give 0.34 g (97%). $[\alpha]25_D$ = +1.5 ° (c=1, H_2O). MS (FAB): 347 (M+). IR (KBr) cm-1: 3600-2400, 1557, 1548, 1409, 1055. ^{31}P NMR (D_2O): single peak at 15.62. ^1H NMR (D_2O): δ 3.87 (m, 1H, H-3), 3.61 (m, 1H, H-2), 3.25 (m, 1H, H-4), 2.28 (m, 1H, H-1), 2.05 (m, 1H,PC<u>H</u>H), 1.42 (m, 2H, H-5 , PCH<u>H</u>), 1.13 (m, 1H, H-5). To prepare the free phosphonic acid, the barium salt was dissolved in water, and the solution was passed through an Amberlite CG-50 column. The acidic eluate was collected and treated with acetone. The solid product **9** was collected by filtration , mp 267 °C (dec). MS (ISP): 212 (M^++1). *Anal.* Calcd for $C_6H_{14}NO_5P$: C, 34.13; H, 6.68; N, 6.64. Found: C, 34.04; H, 6.90; N, 6.37.

(-)[1S-(1β, 2α, 3α, 4β)]-4-N(Glycyl)amino-2,3-dihydroxycyclopentanemethylphosphonic acid diethyl ester (11): The phosphonate **9** (0.41 g, 1.0 mmoles) was dissolved in 99 % CF_3CO_2H (10mL) and stirred for 1h. The volatile material was removed under reduced pressure, the residue was dissolved in MeOH, and the solution was passed through an IRA-400 (OH^-) column. Evaporation of the methanolic eluate gave the amino compound **10**. ^1H NMR ($CDCl_3$): δ 4.40 (d, 2H, H-2, H-3), 4.06 (m, 5H, 2 $CH_3\underline{CH_2}O$, H-4), 3.60 (broad, 2H, NH_2), 3.42 (m, 1H, H-1), 2.32 (m, 2H, PCH_2), 2.17-1.56 (m, 2H, H-5), 1,43 (s, 3H, Me-C-Me).1.32 (t, 6H, 2 $\underline{CH_3}CH_2O$), 1.29 (s, 3H, Me-C-Me). N-Boc-glycine (0.19 g, 1.1 mmoles), hydroxybenzotriazole hydrate (0.16 g, 1.2 mmoles), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.23 g, 1.2 mmoles) and 4-methylmorpholine (0.11 mL, 1.0 mmoles) were added to **10** in anh. DMF (5.0 mL). The mixture was stirred overnight, the solvent

was evaporated and the residue was redissolved in AcOEt. This solution was washed with 10 % citric acid, then saturated $NaHCO_3$, and dried over $MgSO_4$. The solvent was evaporated and the residue, after pre-adsorption on a small amount of silica gel, was purified through a silica gel column (2 x 20 cm) using $CHCl_3$/MeOH as the eluent. The pure product was obtained as a syrup; 0.42 g (90%), $[a]^{25}_D$ = -4.4 ° (c=1, MeOH). IR(neat) cm^{-1}: 3350, 3010, 2950, 2380, 2350, 1760, 1660. MS(EI): 465 (M^++1). ^1H NMR ($CDCl_3$): δ 6.96 (d, 1H, NHCO), 5.18 (broad, 1H, $NHCO_2$), 4.52 (m, 1H, H-3), 4.35 (m, 1H, H-2), 4,10 (m, 4H, 2 $CH_3\underline{CH_2}O$), 3.79 (m, 2H, CH_2N), 2.41 (m, 2H, PCH_2), 2.07 (m, 1H, H-5), 1.76 (m, 1H, H-1), 1.65 (m, 1H, H-5). 1,47 (s, 3H, Me-C-Me), 1.43 (s, 9H, CMe_3), 1.32 (t, 6H, 2 $\underline{CH_3}CH_2O$), 1.26 (s, 3H, Me-C-Me). *Anal.* Calcd for $C_{20}H_{37}N_2O_8P$: C, 51.71; H, 8.03; N, 6.03. Found: C, 51.44; H, 7.99; N, 5.93.

(-)[1S-(1β, 2α, 3α, 4β)]-4-N(Glycyl)amino-2,3-dihydroxycyclopentanemethylphosphonic acid (12): Compound **11** (0.46 g, 1.0 mmoles)was dissolved in anh. DMF (5.0 mL). To the solution was added BTMS (4.59 g, 4.0 ml, 30 mmoles) and the reaction mixture was stirred overnight under nitrogen. The solvent was evaporated and the residue was dissolved in water (1.0 mL), mixed with $Ba(OAc)_2$ solution (1 M, 1.2 mL) and was processed as described above for compound **9**. Yield of the barium salt was 0.24 g (60%). $[a]^{25}_D$ = -1.8° (c=1, H_2O). IR (KBr) cm^{-1}: 3600-2400, 1653, 1559, 1541, 1409, 1052 (P-O-C). MS (ISP): 404 (M^+). ^{31}P NMR (D_2O): δ single peak at 20.51. ^1H NMR (D2O): δ 4.02 (m, 1H, H-3), 3.90 (m, 1H, H-2), 3.75 (s, 2H, COCH2N), 3.70 (m, 1H, H-4), 2.42 (m, 1H, H-1), 2.15 (m, 1H, PCHH), 1.90 (m, 1H, PCH H), 1.60 (m, 1H, CHH), 1.54 (m, 1H, CHH).

The free phosphonic acid was obtained by passing an aqueous solution of the barium salt through an Amberlite CG-50 column. The acidic eluate was collected and concentrated, then triturated with acetone and ethanol to obtain a white powder, m.p. 136°C (effervescence), MS (ISP); 268 (M^+). The ammonium salt was obtained by adjusting an aqueous solution of the free acid to pH 8 with an ammonium hydroxide solution followed by lyophylization. A fluffy white crystalline product was obtained, mp 110°C (effervescence), MS (IPS): 286 (M^++1).

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