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ISOSTERES OF NATURAL PHOSPHATES. 10. SYNTHESIS OF ANALOGUES RELATED TO GLYCEROL 3-PHOSPHATE[1]

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Abstract - We have synthesized a pair of diastereoisomers of 1,3,4-trihydroxybutyl-1-phosphonic acid. Both bear the same absolute configuration as sn-glycerol 3-phosphate at the 3-position, but differ in configuration at the site adjacent to phosphorus. These have been synthesized by the regiospecific and stereospecific reaction of a chiral hydroborating agent with a β-substituted vinylphosphonate.

INTRODUCTION

We have for some time worked toward the synthesis of analogues of natural phosphate metabolites.[2] These analogues bear a carbon-phosphorus linkage in place of the normal phosphate ester linkage. The intent of such a replacement is to preclude phosphorus elision from the molecule while maintaining normal reactivity at other sites. The use of phosphonic acids isosteric with the natural phosphate metabolites has been successful for the regulation of metabolic processes in a number of instances.[2,3]

However, in some situations the simple isosteric phosphonic acid analogues of the natural phosphate metabolites were incapable of substituting for them in enzymatic processes. For example, (S)-3,4-dihydroxybutyl-1-phosphonic acid, the simple analogue of sn-glycerol 3-phosphate,[4] substitutes quite efficiently as a substrate for E. coli CDP-diacylglycerol:sn-glycerol 3-phosphate phosphatidyl transferase[5] and rabbit muscle NAD-linked glycerol 3-phosphate dehydrogenase,[6] and as an inhibitor of E. coli glycerol 3-phosphate:NAD(P) oxidoreductase,[7] but is not recognized by the acyl CoA:sn-glycerol 3-phosphate acyltransferase from E. coli.[7] This is intriguing in that the would-be product of the reaction, the lysophosphotidic acid, is a substrate for the E. coli acyl CoA:lysophosphatidate acyltransferase.[8] Different enzymes, catalyzing reactions involving the same substrate, recognize different sites on the substrate. For the design of drugs, the problem becomes one of deducing the point of recognition specificity and synthesizing the proper structural substitute.

Comparing characteristics of natural phosphate esters with their isosteric phosphonic acid analogues, one notes two points of difference. There is a difference in the second pK_a of the phosphorus acid site, about 0.5-1.5 pK_a unit, the phosphonic acid being the less acidic,[9] and there is a lack of non-bonded electrons at the site adjacent to phosphorus in the analogue.

Blackburn has noted recently[10] that the second pK_a of a phosphonic acid may be lowered by the introduction of an electronegative group at the site adjacent to phosphorus. Its solution dissociation characteristics can be made virtually identical with those of the corresponding phosphate. Work by Cooperman and Chiu[11] demonstrated that a hydroxyl function on the carbon of methylenediphosphonic acid, an analogue of pyrophosphate,

significantly changes its biological activity. The unsubstituted molecule is not recognized by a variety of pyrophosphatases, but the hydroxymethylenediphosphonic acid is a potent inhibitor of them.

These efforts suggest the hydroxyl group to be a prime candidate to overcome these difficulties, and that 1,3,4-trihydroxybutyl-1-phosphonic acid should be a suitable analogue for sn-glycerol 3-phosphate. Prior work in this laboratory[4] produced a mixture of diastereoisomers of this fundamental structure. These compounds could be separated partially by chromatography, and preliminary biological results were encouraging.[12] A more efficient and stereospecific route for the preparation of the 1,3,4-trihydroxybutyl-1-phosphonic acids was sought, along with routes for the preparation of other analogues.

EXPERIMENTAL

Reagents. All commercial chemicals were of reagent quality and used without further purification with the following exceptions: benzene, heptane, pentane and hexanes were dried over sodium ribbon; tetrahydrofuran (THF) was distilled over lithium aluminum hydride; dimethylformamide (DMF) and methylene chloride were distilled and stored over molecular sieves prior to use. The 1,2:5,6-di-O-isopropylidene-D-mannitol and the O-isopropylidene-D-glyceraldehyde were prepared according to the methods described by Baer and Fischer,[13] and the tetraisopropyl methylenediphosphonate was prepared according to the method described by Roy.[14]

Analytical. Thin layer chromatography (Tlc) was performed using Polygram Alox/N sheets purchased from Brinkamnn Instruments. Silica gel for preparative chromatography was from Baker (60-200 mesh). Infrared (IR) spectra were measured using Perkin-Elmer model 237-B and 598 spectrophotometers. Nuclear magnetic resonance (NMR) spectra were measured using a Varian model EM-360 instrument. Optical rotations were measured at 598 nm and 25° using a Perkin-Elmer model 141 polarimeter with a 1 dm cell. Elemental analyses were performed by Galbraith Laboratories of Knoxville, TN.

Preparation of Diisopropyl (S)-(E)-3,4-O-Isopropylidene-3,4-dihydroxybut-1-enyl-1-phosphonate (I). To tetraisopropyl methylenediphosphonate (17.94g, 0.057mol) in dry heptane (200mL) under a nitrogen atmospehere was added at room temperature butyllithium in hexane (38.5mL of 1.5M solution, 0.052mol) and stirred for 2h. The reaction was cooled to 0° and O-isopropylidene-D-glyceraldehyde (6.78g, 0.052mol) in dry heptane (25mL) was added. After completion of the addition the reaction was heated at reflux for 2h. It was quenched by the addition of water (500mL), the organic layer separated, and the aqueous layer was extracted with heptane (2x100mL). The combined organic solutions were dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was vacuum distilled (110-115°/0.025 Torr) to yield 14.57g (96%) of the desired product as a colorless liquid. Analytical data: NMR (δ) ($CDCl_3$) 1.04(3H,s), 1.15(12H,d,J=6Hz), 1.18 (3H,s), 3.44(1H,m), 3.93(1H,m), 4.39(1H,m), 4.43(2H,d-sept,J=6Hz,J'=5Hz), 5.60(1H,d-d, J=18Hz,J'=2Hz), 6.01-6.88(1H,m); IR (cm^{-1}) (CCl_4) 3030-2800, 1670, 1450, 1375, 1245, 1150, 1105, 990; $[\alpha]_D^{25}$= +15.48° (0.32M, EtOH); Found: C, 53.58; H, 8.19. Calc. for $C_{13}H_{25}O_5P$: C, 53.42; H, 8.56%.

Preparation of Diisopropyl (S)-3,4-O-Isopropylidene-3,4-dihydroxybutyl-1-phosphonate (II). The olefin I (30.00g, 0.103mol) was hydrogenated over platinum oxide catalyst in absolute ethanol (200mL) at atmospheric pressure until no more hydrogen consumption could be observed. The material was filtered through Celite and the solvent was evaporated under reduced pressure to give in quantitative yield the desired material as a pale oil which required no further purification. Analytical data: Tlc (CH_2Cl_2) R_f=0.55; NMR (δ) ($CDCl_3$) 1.20-1.58 (18H,complex), 1.74(4H,m), 3.59(1H,m), 4.04 (1H,m), 4.50(1H,m), 4.60(2H,d-sept,J=6Hz, J'=5Hz); IR (cm^{-1}) (CCl_4) 3025-2800, 1450, 1375, 1250, 1150, 1065, 975; $[\alpha]_D^{25}$= -1.15° (0.5M, EtOH); Found: C, 52.91; H, 9.37. Calc. for $C_{13}H_{27}O_5P$: C, 53.05; H, 9.25%.

Preparation of Diisopropyl (1R,3S)-3,4-O-Isopropylidene-1,3,4-trihydroxybutyl-1-phosphonate (IIIa). To a cooled (-25°) solution of (-)-diisopinocampheylborane[15] in anhydrous ether (50mL) was added dropwise the olefin I (6.00g, 0.0205mol) in anhydrous ether (20mL). The reaction was stirred overnight at room temperature Water (10mL) was added to the cooled (0°) mixture followed by the addition of 30% NaOH solution (9mL) and 30% hydrogen peroxide (18mL). The mixture was stirred 2h below 40°. A further addition of 30% NaOH (9mL) was made and the aqueous layer was saturated with potassium carbonate. The layers were separated and the aqueous portion was extracted with ether (4x50mL). The combined organic solutions were washed with brine (2x20mL), dried over magnesium sulfate and concentrated under reduced pressure. The crude product was vacuum distilled (110-120°/0.0025Torr) to give 3.05g (48.0%) of the desired product as a colorless liquid. Analytical data: Tlc (CH_2Cl_2) R_f=0.23; NMR (δ) ($CDCl_3$) 1.12-1.62(18H,complex), 1.90 (2H,m), 2.43(1H,m), 2.82(1H,br s), 3.60(1H,m), 4.15(2H,m), 4.77(2H,d-sept,J=6Hz,J'=5Hz); IR (cm^{-1}) (CCl_4) 3550-3110, 3020-2800, 1455, 1375, 1245, 1105, 990; $[\alpha]_D^{25}$= -2.43° (0.32M, EtOH); Found: C, 50.62; H, 9.07. Calc. for $C_{13}H_{27}O_6P$: C, 50.32; H, 8.77%.

Preparation of Diisopropyl (1S,3S)-3,4-O-Isopropylidene-1,3,4-trihydroxybutyl-1-phosphonate (IIIb). The reaction was performed as noted for IIIa starting with I (6.00g, 0.0205mol) and (+)-diisopinocampheylborane (0.08mol).[15] The crude product was vacuum distilled (120-130°/0.0025Torr) to give 3.30g (52.0%) of the desired product as a colorless liquid. Analytical data: Tlc (CH_2Cl_2) R_f=0.28; NMR and IR ($CDCl_3$ and CCl_4 respectively) are virtually identical with those for IIIa; $[\alpha]_D^{25}$= +2.82° (0.32M, EtOH); Found: C, 50.45; H, 9.12. Calc. for $C_{13}H_{27}O_6P$: C, 50.32; H, 8.77%.

Preparation of (1R,3S)-1,3,4-Trihydroxybutyl-1-phosphonic Acid (IVa). The ester IIIa (2.00g, 6.45mmol) was dissolved in trimethylbromosilane (10.0g, 64.5mmol) under nitrogen.

The reaction mixture was stirred at room temperature overnight. Excess trimethylbromosilane and other volatiles were removed under reduced pressure and the residual oil was treated with 95% EtOH (10mL). Volatile materials were removed under reduced pressure to give 1.04g (86.7%) of pure IVa which exhibited a single spot on Tlc. Analytical data: NMR (δ) (D_2O) 1.35-2.40(2H,br), 3.28-4.40(4H,br); KBr (cm^{-1}) (KBr) 3750-3040, 3000-2850, 1650, 1435; $[\alpha]_D^{25}$= +2.65° (0.24M, EtOH); Found: C, 25.61; H, 6.02. Calc. for $C_4H_{11}O_6P$: C, 25.80; H, 5.91%.

Preparation of (1S,3S)-1,3,4-Trihydroxybutyl-1-phosphonic Acid (IVb). The reaction was performed as noted for IVa starting with IIIb (2.00g, 6.45mmol) and trimethylbromosilane (10.0g, 64.5mmol). There resulted 0.96g (79.9%) of the pure IVb which exhibited a single spot on Tlc. Analytical data: NMR (D_2O) and IR (KBr) are virtually identical with those for IVa; $[\alpha]_D^{25}$= -10.23° (0.24M, EtOH); Found: C, 25.80; H, 5.87. Calc. for $C_4H_{11}O_6P$: C, 25.80; H, 5.91%.

Preparation of (S)-(E)-3,4-Dihydroxybut-1-enyl-1-phosphonic Acid (V). The reaction was performed as noted for IVa starting with I (6.12g, 21.0mmol) and trimethylbromosilane (32.0g, 210mmol), to give 3.01g (85.0%) of the pure V which exhibited a single spot on Tlc. Analytical data: NMR (δ) (D_2O) 3.64(2H,br), 4.36 (1H,br), 5.67-6.99(2H,complex); IR (cm^{-1}) (KBr) 3800-3050, 3040-2850, 1640; $[\alpha]_D^{25}$= +7.2° (1M, H_2O). The free acid was hygroscopic and therefore was converted to the dicyclohexylammonium salt by dissolution in a minimum of a 1:1:1 toluene:acetone:methanol mixture and the addition of a four-fold molar amount of dicyclohexylamine. After stirring for 10h the fine crystals were filtered, washed with ether and recrystallized from 1:1 methanol:ether to give the pure mono(dicyclohexylammonium) salt of V. Found: C, 54.64; H, 9.52. Calc. for $C_{16}H_{32}NO_5P$: C, 55.00; H, 9.23%.

RESULTS AND DISCUSSION

Two diastereoisomers of the 1,3,4-trihydroxybutyl-1-phosphonic acid structure have been synthesized as shown in Scheme I. Both bear the same configuration at carbon-3 as sn-glycerol 3-phosphate, but differ in configuration at the carbon directly attached to phosphorus. In the synthetic scheme producing these species the olefin I is involved which is hydrated through a hydroboration-oxidation scheme using a chiral hydroborating reagent.[15] Thin-layer-chromatography of the products IIIa and IIIb indicate no detectable amounts of the "improper" stereoisomer in either preparation. Prior efforts[16] have noted hydroboration-oxidation of β-substituted vinylphosphonates to place the hydroxyl α- to phosphorus. The regiospecificity of the hydroboration here is confirmed by other chemical evidence. The reversion under basic conditions of dialkyl α-hydroxyphosphonates to the corresponding dialkyl phosphites and aldehyde has been known for quite some time. Heating a mixture of IIIa and IIIb with aqueous potassium hydroxide liberated (S)-1-oxo-O-isopropylidenebutane-2,3-diol.[4] This reversion under basic conditions, well known from other studies,[17] confirms the orientation.

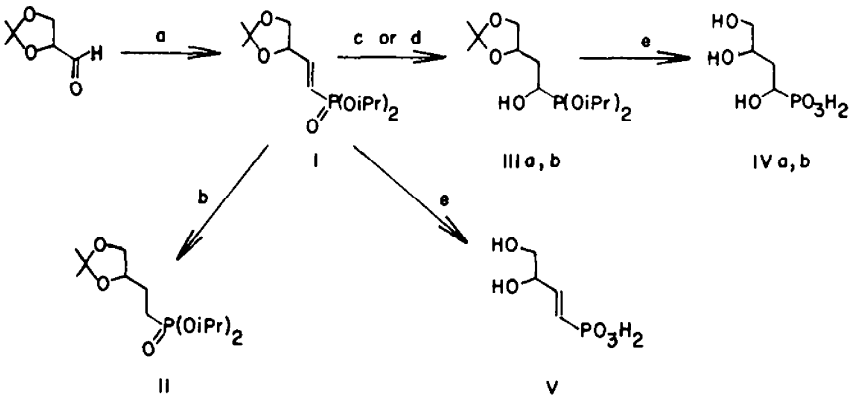
The assignment of stereochemistry for the 1-position in IIIa and IIIb is made using the reaction dynamic analysis as previously described.[18] It is assumed that the bulky diisopropoxyphosphoryl group would lie preferentially toward the "small" substituent rather than toward the "medium" substituent of the chiral borane during the approach. On this basis it is expected that the (-)-diisopinocampheylborane would generate the product alcohol of R absolute configuration at the 1-position and (+)-diisopinocampheylborane the product of S absolute configuration at the same site. Inspection of models for these systems supports this proposal, even with the presence of a chiral site already in the olefin substrate. Although no further confirming studies of this assignment have been done, it should be noted that Tlc data indicate that the reactions proceed stereospecifically.

Generation of the free phosphonic acids has been performed using trimethylbromosilane followed by hydrolysis with 95% ethanol.[20-22] Trimethyliodosilane gave rapid reaction but also numerous side products which hindered purification.

The introduction of the hydroxyl group α- to the phosphonic acid function has accomplished the desired goal. Both IVa and IVb block bacterial growth and serve as inhibitors for the acyltransferase reaction. Details of the biochemical activities of these materials will be presented in a separate report.

The intermediate I which served as a precursor to IVa and IVb has also been of use for several other purposes. The use of I allows to be prepared 3,4-dihydroxybutyl-1-phosphonic acid of the same configuration as sn-glycerol 3-phosphate and at the same time to bear a radioactive label at a non-labile position. Reduction of the olefinic linkage is accomplished using tritium gas. On ester cleavage there is generated (S)-3,4-dihydroxybutyl-1-phosphonic acid bearing tritium at the

Scheme I



a: BuLi, Tetraisopropyl methylenebisphosphonate
b: H_2, PtO_2
c: (for IIIa) 1.(-)-diisopinocampheylborane, 2. H_2O_2, KOH, H_2O
d: (for IIIb) 1.(+)-diisopinocampheylborane, 2. H_2O_2, KOH, H_2O
e: 1.$(CH_3)_3SiBr$, 2. 95% Ethanol

1- and 2- positions.

Cleavage of the ester linkages of I generates the substituted vinylphosphonic acid V. This material has been found to act as a weak antibacterial agent.

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