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THE PHOSPHONATE ANALOG OF 5-METHYLTHIORIBOSE 1-α-PHOSPHATE

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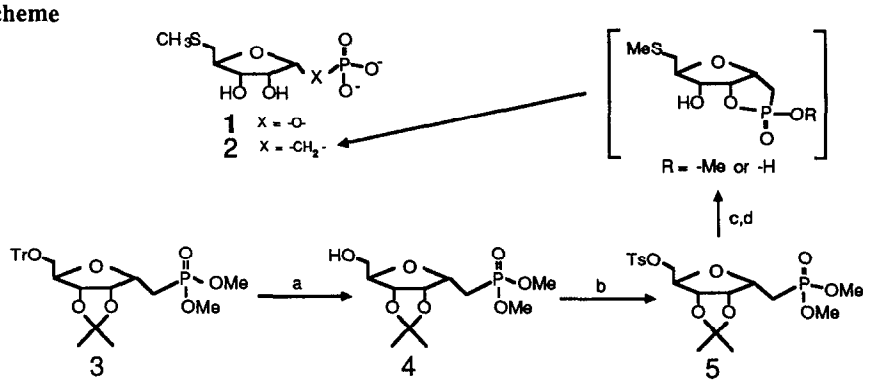
Abstract: The D-*altro* phosphonate analog, **2**, of 5-deoxy-5-methylthioribose 1-α-phosphate, **1**, was prepared in four steps from a known synthetic intermediate. The analog does not inhibit uptake of oxygen when allowed to compete with the natural metabolite, **1**, in a methionine-regeneration system.

The biosyntheses of spermine and spermidine are very important because of their potentially regulatory associations with nucleic acids. The rationale behind the design of specific inhibitors of this pathway has been presented.[1] The propylamino moieties of these products are donated by *S*-adenosylmethionine to leave 5'-deoxy-5'-methylthioadenosine, which, depending on the organism, is converted either via a phosphorylase or a hydrolase/kinase pair to 5-deoxy-5-methylthioribose 1-α-phosphate, **1**. In the case of *Klebsiella pneumoniae*, at least, it is known that **1** is converted to 1-phospho-5-deoxy-5-*S*-methylthioribulose by a specific isomerase and that the subsequent regeneration of methionine involves an oxidation by molecular oxygen.[2,3] It was thought that a phosphonate analog of **1** might then be a potential inhibitor of this important pathway. Here we describe the stereospecific synthesis of the *C*-glycosidic phosphonate analog **2**.

Scheme



a. H_2/Pd, MeOH; b. TsCl/pyridine, 20°; c. KSMe, MeOH; d. HCl, H_2O, 20°C.

Hydrogenolysis of the previously described compound **3**[4] yielded **4**,[5] which was tosylated[7] to give **5**. Substitution with MeS^- results in the desired introduction of the thiomethyl group at C-6 along with partial formation of a phosphonyl thioester (i.e., about 10% of the $-OCH_3$ groups replaced by $-SCH_3$).[8] All protecting groups were subsequently removed simultaneously with 1 M aqueous HCl at 20° to yield[8] the product, **2**. The facility with which this final deprotection was accomplished was probably due to the fact that upon hydrolysis of the isopropylidene moiety (known to occur with ease) the resulting free hydroxyl at C-3 is able to provide anchimeric assistance in the ester hydrolysis via formation of a labile phostone (shown in brackets in the Scheme). By analogy, when the C-6 diphenoxyphosphoryl derivative of **4** was treated with TsOH in aqueous methanol we had observed that the corresponding phostone methyl ester was formed.[9]

When **2** was included in an extract of *Klebsiella pneumoniae* along with the substrate, **1**, at the same concentration (0.8 mM) no inhibition of oxygen uptake was observed.[10] Although this compound does not apparently compete effectively with the subsequent isomerase for the substrate, **1**, one cannot rule out the

interesting possibility that the analog acts as a substrate for 5-deoxy-5-methylthioribose 1-α-phosphate isomerase, although that could not have occurred to a large extent since such competition would have resulted in a later intermediate which would have been unable to be oxidized, thus having decreased oxygen uptake as well. Compound **2** might then be useful in elucidating the mechanism of the ring-opening isomerase in the event it were a substrate.[11]

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5. Compound **4** was prepared as described by us[6] from the 6-triphenylmethyl ether, **3**.[4]

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7. *Synthesis of* **5**, *D*-altro-*2,5-anhydro-1-deoxy-1-dimethoxyphosphinyl-3,4-isopropylidene--6-toluenesulfonylhexitol.* Compound **4** (3.07 g, 10.3 mmole) was tosylated by standard procedure (recrystallized toluenesulfonyl chloride, 2.72 g, 14.2 mmole, and dimethylaminopyridine, 1.84 g, 15.3 mmole, in CH_2Cl_2 at 20° for 24 hr. This yielded 4.81 g of crude product (TLC: ethyl acetate, Rf ~ 0.3) which was purified by silica gel chromatography (ethyl acetate) to give 3.1 g (66%) of needle crystals upon evaporation of the solvent. This material was recrystallized from methylcyclohexane/toluene (M.p. = 103 - 104.5°). Anal. Calcd. for $C_{18}H_{27}O_9PS$: 48.00 C, 6.04 H, 6.88 P. Found 48.37 C, 6.00 H, 6.47 P. ^1H NMR: δ 1.25 and 1.39 (6H, s, acetonide), 2.09 (1H, ddd, -CHH-P), 2.16 (1H, ddd, -CHH-P), 2.40 (3H, s, CH_3-Ph-), 3.68 (6H, d, P-OCH_3), 3.91 (1H, dd, -CHH-OTs), 3.99 (1H, dd, -CHH-OTs), 4.16 (2H, m, C(2)H and C(5)H), 4.62 (2H, m, C(3)H and C(4)H), 7.30 and 7.72 (4H, d, -SO_2-Ph-CH_3).

8. *Synthesis of* **2**, *D*-altro-*1,6-dideoxy-1-dihydroxyphosphinyl-6-methylthiohexitol.* The above tosylate (2.18 g, 4.84 mmole) was dissolved in 50 mL of anhydrous methanol and 1 g (~ 24 mmole) of potassium metal was added in small pieces. Methanethiol (2.4 g, 2.8 mL, 50 mmole) was then added and the mixture was stirred at 20° for 18 hr. The mixture was evaporated to a syrup which was dissolved in about 50 mL CH_2Cl_2 to which was added 25 mmole of glacial acetic acid. This was evaporated until no odor of methanethiol could be detected. The residue was shown by GC/MS to be made up of a mixture of the dimethyl ester of isopropylidene-**2** plus about 10% of the monomethyl monothiomethyl phosphonyl ester. The residue was then dissolved in 50 mL 1 M aqueous HCl and left to stand at 20° for 36 hr, at which time NMR showed deprotection to be complete. Volatiles were removed by repeated evaporation. The solution was then passed over a column of Dowex 50 (H^+) to remove K^+. The eluate was evaporated, isopropanol was added and evaporated three times, followed by one cycle with toluene to give 1.3 g of crude **2**. This residue was dissolved in water and redistilled cyclohexylamine was added until the pH reached ~ 10. The water was evaporated to give a solid that was dissolved in ethanol to which was added about 0.5 vol acetone. Precipitation of a solid then commenced slowly. The highly hygroscopic solid was collected by filtration and the filtrate and washings were evaporated, redissolved in a small amount of ethanol and reprecipitated to yield a small amount of additional product. The solid was dried over P_2O_5 (total = 0.85 g, 50 % yield from **4**). This solid proved to be the monocyclohexylammonium salt of **2**. Further fractionation yielded only cyclohexylammonium tosylate. A small amount of the cyclohexylammonium salt of **2** was recrystallized from methanol/ether and dried *in vacuo* over P_2O_5 to yield an analytical sample (M.p. 180°-182°). Anal. Calcd. for the monocyclohexylammonium salt, $C_{13}H_{28}NO_6PS$: 43.69 C, 7.90 H, 8.67 P, 8.97 S. Found: 43.31 C, 7.82 H, 8.90 P, 8.99 S. The Na salt (eluted from Dowex-50, Na^+ form) was used to obtain the following NMR data. ^1H NMR: δ 1.93 (1H, ddd, -CHH-P), 2.08 (1H, ddd, -CHH-P), 2.19 (3H, s, -CH_3), 2.77 (1H, dd, -CHH-OP), 2.90 (1H, dd, -CHH-OP), 4.03 (1H, dt, C(5)H), 4.27 (2H, m, C(4)H and C(3)H), 4.34 (1H, m, C(2)H). ^{13}C NMR: δ 17.96 (CH_3-), 31.77 (d, -CH_2-P, J_{CP} = 128.5 Hz), 38.32 (-CH_2-OP), 75.19 (C4), 78.11 (C3), 80.30 (C2), 81.55 (C5). ^{31}P NMR (relative to 85 % H_3PO_4): δ 18.82 (dt, J^2_{PH} = 18 Hz, J^3_{PH} = 6 Hz).

9. This intermediate has a downfield ^{31}P shift (relative to the open-chain counterpart) of about 20 ppm typical of 5-membered ring phosphonates and phosphates; our observation in the current case is in keeping with results previously published from this laboratory.[6]

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