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C-Glycosylphosphonyl Analogs of 5-Phosphorylribose 1-α-Diphosphate

II. The "2-Deoxy" Analog[1]

JOHN F. WITTE AND RONALD W. MCCLARD

Arthur F. Scott Laboratory of Chemistry, Reed College, Portland, Oregon 97202

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D-*ribo*-2,5-anhydro-1,3-dideoxy-1-(((dihydroxyphosphinyl)oxy)hydroxyphosphinyl)-6-phosphorylhexitol, compound **4,** along with 25% of its D-arabino epimer, was synthesized in 10 steps from 2-deoxyribonolactone. Compound **4** represents the 2-deoxy analog of the central metabolite 5-phosphorylribose 1-α-diphosphate (PRPP). The analog is a competitive inhibitor of yeast orotate phosphoribosyltransferase (PRTase) with $K_i/K_{m(\mathrm{PRPP})} = 24$ and is thus comparable to other *C*-glycosylphosphonyl PRPP analogs reported previously (R. W. McClard, and J. F. Witte (1990) *Bioorg. Chem.* **18,** 165–178). The analog is an even better inhibitor of both human hypoxanthine-guanine PRTase and glutamine : amido PRTase from *Escherichia coli* with $K_i/K_{m(\mathrm{PRPP})}$ values of 16 and 3, respectively. These results support the argument that the cis-diol grouping of nucleotides (or PRPP) plays a very minor role in binding of these substrates to PRTases. © 1996 Academic Press, Inc.

INTRODUCTION

Stable analogs of 5-phosphorylribose 1-α-diphosphate (PRPP, **1**) are important due to their applications in studying the mechanisms of phosphoribosyltransferases (PRTases). In a previous paper (*1*) we described two *C*-glycosylphosphonyl analogs that act as competent competitive inhibitors of orotate PRTase. We were unable to attain the "exact" analog, **2,** because upon acid hydrolysis of the intermediate **3** the generation of a free hydroxyl at C3 then allowed elimination of phosphate with the formation of a phostone with the phosphonate P which then hydrolyzed to the 1-phosphonate (*1*). Although the target (**2**) may still be attainable by another protection/deprotection scheme, we elected to pursue the synthesis of compound **4,** which we expected would evade the hydrolysis problem by virtue of its lack of a 3-OH group.

We reasoned that the lack of this 3-OH (comparable to the 2-OH of PRPP) should be a conservative change in the molecule's behavior since the *cis*-hydroxyls of PRPP (or conjugate nucleotide) seem to be of lower importance in the binding of these molecules in the active sites of certain PRTases. For example, nucleotide affinity columns constructed for the purposes of purification of bacterial orotate PRTase (*2*) and mammalian hypoxanthine-guanine PRTase (*3*) are connected to

[1] Part 1 of this series is MCCLARD, R. W., AND WITTE, J. F. (1990) *Bioorg. Chem.* **18,** 165–178.

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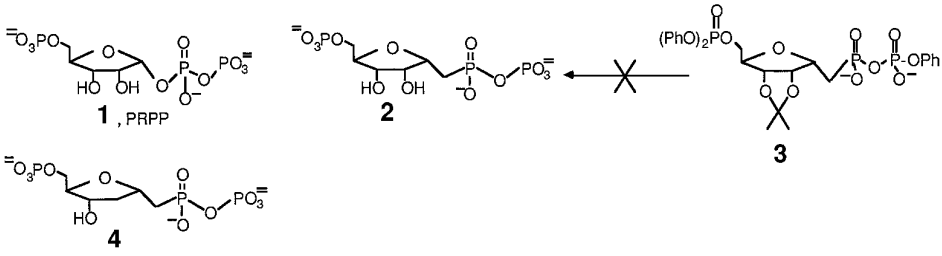
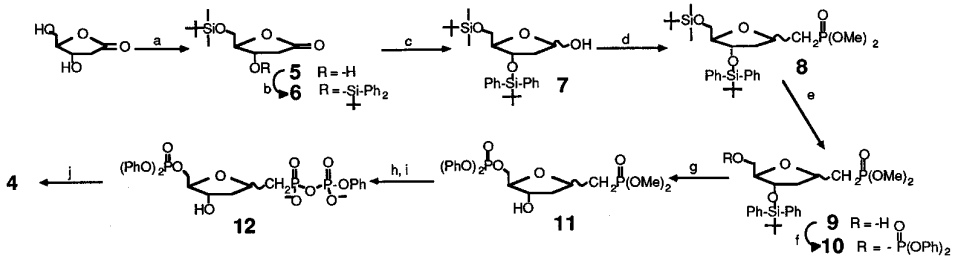


the resin through aldehydes generated from periodate-cleaved cis-diols of OMP and GMP, respectively. Similarly, hypoxanthine-guanine (HG) PRTase is effectively inhibited by acyclovir (*4*), which contains no ring hydroxyls at all. The most convincing rationale is provided by the recently published crystal structure of *Salmonella* orotate PRTase (*5*), which binds OMP with the 2′,3′-cis hydroxyls apparently pointing out into the solvent with little or no apparent interaction with active site residues. Here we describe the 10-step synthesis of **4,** as a mixture of its D-*ribo* and D-*arabino* epimers, from 2-deoxyribonolactone and demonstrate that this analog of PRPP is as good or, in some cases, better than previously published (*1*) *C*-glycosylphosphonyl analogs.

RESULTS AND DISCUSSION

The synthesis of **4** began with deoxyribose which was oxidized catalytically with $Pd/O_2/Mg(OH)_2$, by the method previously developed in this laboratory, to the corresponding lactone in almost quantitative yield (*6*). The resulting lactone was selectively protected at C5 with TBDMSi-Cl catalyzed by imidazole in DMF solution (*7*) to give **5** in 65% yield, the major by-product being the bis-TBDMSi derivative which was readily separated by chromatography. Introduction of the TBDPSi group at C3 was readily accomplished with TBDMSi-Cl in the presence of DMAP and CH_2Cl_2 to yield **6** in quantitative yield. Reduction of the protected lactone with DIBAL in toluene at −78°C during 1 h furnished the corresponding protected deoxyribose, **7,** in quantitative yield (*8*). Reaction of this material with the sodium salt of tetramethyl methylenebisphosphonate (from tetramethyl methylenebisphosphonate and NaH in glyme) during 24 h at room temperature under Horner–Emmons conditions (*9*) yielded the desired methylene phosphonate compound **8,** isolated as a mixture of epimers at C2. The TBDMSi group at C6 was selectively removed with 80% aqueous acetic acid at room temperature during 48 h to give the corresponding C6 hydroxy compound, **9,** in quantitative yield. Reaction of this material with diphenyl chlorophosphate in CH_2Cl_2 and DMAP introduced the desired diphenyl phosphoryl group to provide a quantitative yield of compound **10,** by analogy to previous work in this lab (*1*). Removal of the TBDPSi protecting group at C4 was accomplished with HF·Pyr in methylene chloride at 0°C to furnish compound **11** in 65% yield. Some substitution of -OPh by -F occurred at the C6



SCHEME 1. (a) TBDMSi-Cl/imidazole/DMF, 16 h. (b) TBDPSI-Cl/DMAP/CH_2Cl_2, 16 h. (c) DIBAL, toluene, −78°C, 1 h. (d) $[(MeO)_2P(O)]_2CH^-$, glyme. (e) aq. HOAc. (f) $ClP(O)(Oph)_2$/DMAP. (g) HF · pyridine/CH_2Cl_2. (h) TMS-Br. (i) 1. 1 eq. Bu_4H^+ OH^-/MeCN; 2. ImH^+ (PhO)P(O)(O^-)(Im), 65°C. (j) H_2/Pt on C/PtO_2, 80% aq. HOAc.

phosphorus under these conditions. The formation of the crucial phosphonylphosphate anhydride **12** was accomplished in 55% yield in two steps following our previous work (*1*). First the methyl ester groups were removed with TMSBr at 0°C and the resulting phosphonic acid was allowed to react with the imidazolide of monophenyl phosphate at 65°C in acetonitrile during 24 h to yield the product in 65% yield. Final deprotection was accomplished by hydrogenolysis (Pt/C/1 atm H_2, 80% aqueous acetic acid) to furnish the desired analog, **4,** in nearly quantitative yield from **12.**

From the point of the formation of compound **8,** the products of each successive reaction were carried through as a chromatographically inseparable mixture of epimers at C2. The mixture of isomers of the product compound **4** was determined by NMR spectral analysis (conducted by Michael Tears and John Grutzner at Purdue University) to be composed of approximately 75% of the desired "α anomer" and 25% of the "β anomer" (ratio based on integrations obtained from inverse-gated ^{31}P NMR spectrum).[2]

It is worth noting that the ready availability of aldonolactones, by a procedure developed in this laboratory (*6*), provides a convenient means for the protection/deprotection of the acetal/ketal functionality along the synthetic path involving a sugar derivative; this study is a modest example. The classical protection of the anomeric carbon via formation of an alkyl glycoside (*10*), a notable example being 2,3,5-tribenzylribose, proceeds with low yields and multiple byproducts. The synthe-

[2] Initial peak assignments were readily determined by H/H and P/H correlation spectroscopy and the respective orientation of the ring protons was determined through application of the Karplus relationship. After these assignments had been made it was possible to assign multiplicities and coupling constants to each of the protons of the major isomer. Thus the small coupling between H5 and H4 results from a *trans* relationship between these protons and establishes a *trans* configuration between the phosphoryl group at C6 and the hydroxyl at C4. The 10.7 and 6.5 Hz (coupling constants dimished by the presence of the hydroxyl at C4) couplings between H3β–H2 and H3β–H4, respectively, establishes a single *cis* relationship between these protons. These data translate into a 2,4-*cis* and a 4,5-*trans* relationship among the ring substituents and an overall configuration for the major isomer as depicted as compound **4**. In addition, the smaller *trans* couplings between H4–H3α(1.5 Hz) and H2–H3α(5.0 Hz) are also consistent with this assignment.

TABLE 1
Inhibition of PRTases by the PRPP Analog Compound **4**

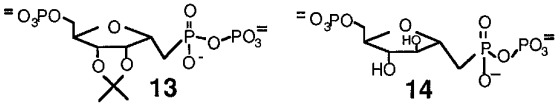
Enzyme	Observed $K_i/K_{m(PRPP)}$[a]	Corrected K_i/K_m[b]
yeast orotate PRTase	32	24
Human HG PRTase	20	16
E. coli Gln : amido PRTase[c]	4	3

[a] The $K_{m(PRPP)}$ for yeast orotate PRTase is equivalent to the K_{diss} in this case (11).
[b] Corrected for the fact that compound **4** is a 75 : 25 mixture of the $\alpha:\beta$ "Anomers," i.e., the D-*ribo* and D-*arabino* epimers.
[c] Performed by Dana Wolle and Howard Zalkin (Purdue University).

sis of 5-trityl-2,3-isopropylideneribose is also cumbersome. The scheme employed in this paper (i.e., oxidation to the lactone followed by reduction back to the aldose at the time the aldehyde oxidation state is required) involves a sequence of reactions that give distinct products in high yields.

Compound **4** acts as a respectable competitive inhibitor of three different PRTases from various sources as shown in Table 1.

By comparison, previously reported *C*-glycosylphosphonyl PRPP analogs **13** and **14** showed $K_i/K_{m(PRPP)}$ values of 16 and 54, respectively (*1*). Clearly, with regard to yeast orotate PRTase, removal of the inverted 3-OH from **14** results in tighter binding than for compound **4.** Interestingly, the latter is slightly weaker as an inhibitor than is **13** with its rather bulky acetonide functionality.



These results with compound **4,** in addition to other related observations (*1–4*), reveal the lack of relative importance of the sugar cis-diol in the nucleotide or PRPP in binding to orotate PRTase. This conclusion accords well with the recently published crystal structure of *Salmonella* orotate PRTase, which clearly reveals that the cis-diol grouping of bound OMP seems to point directly out into solvent. Compound **4** is an even better inhibitor (based on K_i/K_m values) of HG PRTase and glutamine : amido PRTase. It would appear that in these cases as well that the 2-OH of PRPP is not very crucial to binding.

EXPERIMENTAL

General

2-Deoxy-D-ribonolactone was synthesized from D-deoxyribose as described by Witte *et al.* (*6*). NMR spectra were obtained on a Bruker AC 300 instrument run at the following frequencies: ^{1}H, 300.13 MHz; ^{13}C, 75.47 MHz; ^{31}P, 121.50 MHz;

coupling constants are given in Hz. All spectra were performed in $CDCl_3$ except as noted. Routine assignments of structures were based on analyses of ^{13}C-DEPT, ^{1}H-^{1}H-COSY, and ^{13}C-^{1}H heteronuclear correlation (HETCOR) experiments. In the following "major" refers to the deduced major (D-*ribo* or "α") epimer, and "minor" refers to the minor (D-*arabino* or "β") epimer. Organic extracts obtained from aqueous mixtures were routinely dried with solid Na_2SO_4. Chromatography was performed using EM Science silica gel 60 (230–400 mesh) with solvents as indicated. TLC was performed on EM Science Kieselgel 60 F_{254} plates.

Syntheses

*5-(t-Butyldimethylsilyl)-2-deoxy-*D*-ribonolactone* (**5**). To a solution of 2-deoxyribonolactone (26.4 g, 0.2 mol), dried by evaporation with CH_3CN (3 × 30 ml) in dry DMF (300 ml) was added TBDMSi-Cl (31.4 g, 0.2 mol) and imidazole (13.7 g, 0.2 mol) at 0° with stirring under N_2. The resulting solution was allowed to stand for 16 h, whereupon water (200 ml) was added and the mixture extracted with CH_2Cl_2 (3 × 100 ml). The extracts were dried and all volatiles removed at reduced pressure. The resulting viscous semicrystalline material was stirred with MeOH (300 ml) and the crystalline material filtered off, washed with MeOH (50 ml) to give 7.26 g of the bis-TBDMSi derivative. The filtrates and washings were combined and evaporated under reduced pressure. The residue was chromatographed with hexane : EtOAc (2 : 3) to give the desired derivative (31.5 g, 65%). ^{1}H NMR: δ 0.09 (6H, 2s, -Si-C$\mathbf{H_3}$), 0.91 (9H, s, -Si-C(C$\mathbf{H_3}$)$_3$), 2.42 (1H, dd, $J_{2,2'}$ = 18.0, $J_{2,3}$ = 3.5, C2-H), 2.71 (1H, bs, -OH), 2.92 (1H, dd, $J_{2',2}$ = 18.0, $J_{2',3}$ = 9.0 Hz, C2-H′), 3.82 (2H, d, $J_{4,5}$ = 4.5, C5-H), 4.43 (1H, m, C4-H), 4.58 (1H, dt, J = 10.1, 1.5, C3-H). ^{13}C NMR: δ -5.81 and -5.75 (2C, -Si-**C**H$_3$), 18.04 (-Si-**C**(CH$_3$)$_3$), 25.63 (-Si-C(**C**H$_3$)$_3$) 38.80 (C2), 62.94 (C5), 69.37 (C3), 88.14 (C4), 177.10 (C=O).

*5-(t-Butyldimethylsilyl)-3-(t-butyldiphenylsilyl)-2-deoxy-*D*-ribonolactone* (**6**). To a solution of **5** (23.5 g, 0.096 mol) in dry CH_2Cl_2 (250 ml) was added with stirring under N_2 TBDPSi-Cl (28.9 g, 27.3 ml, 0.11 mol) and DMAP (13.42 g, 0.11 mol) at 0°C. The resulting solution was allowed to stand at room temperature for 16 h. Water (200 ml) was added and the layers separated. The organic layer was dried, filtered, and volatiles removed at reduced pressure. The syrupy residue (53.4 g) chromatographed with hexane : EtOAc (5 : 1) gave 44.1 g (97%) of **6**, whose NMR spectra indicate the presence of two apparently rotationally constrained (at Si) conformers. ^{1}H NMR: δ -0.06 to 0.12 (6H, 4s, -Si-C$\mathbf{H_3}$), 0.77 to 1.10 (18H, 4s, -Si-C(C$\mathbf{H_3}$)$_3$), 2.37 to 2.84 (2H, m, C2-H and C2-H′), 3.13 (1H, dd, C5-H of major conformer), 3.58 (1H, dd, C5-H′ of major conformer), 3.72 to 3.83 (2H, m, C5-H and C5-H′ of minor conformer), 4.31 to 4.37 (1H, br m, C4-H), 4.45 (1H, dt, C3-H of major conformer), 4.51 (1H, dt, C3-H of minor conformer), 7.33 to 7.68 (10H, 2m, arom). ^{13}C NMR: δ -5.86, -5.68, -5.43, -0.01 (-Si-**C**H$_3$); 18.11, 19.00 (-Si-**C**(CH$_3$)$_3$); 25.65, 25.70, 25.78, 26.78 (-Si-C(**C**H$_3$)$_3$); 39.05 (C2); 62.45, 62.48 (C5); 69.60, 70.91 (C3); 88.10, 88.16 (C4); 127.96, 130.15, 132.85, 133.07, 135.62, 135.66 (arom); 176.12 (C=O).

*5-(t-butyldimethylsilyl)-3-(t-butyldiphenylsilyl)-2-deoxy-*D*-ribose* (**7**). To a solution of **6** (4.8 g, 0.01 mol) in dry toluene (25 ml) was added with stirring under N_2

DIBAL (20.2 ml, 1 M in cyclohexane) at −78°C for 60 min. The reaction was quenched with MeOH (5 ml) followed by saturated Na_2SO_4 (50 ml) and ether (100 ml) and allowed to warm to room temperature with vigorous stirring. The inorganic precipitate was filtered off. The filtrate was extracted with ether (2 × 100 ml) and the combined extracts were dried, filtered, and evaporated under reduced pressure to give 5.0 g of a colorless oil. The oil was chromatographed with hexane : EtOAc (5 : 1) to give 4.32 g of **7** (95%) as a mixture of anomers each with magnetically nonequivalent *t*-butyl groups. ^{1}H NMR: δ 0.05 to 0.18 (6H, 4s, -Si-C**H$_3$**), 0.88 to 1.20 (18H, 4s, -Si-C(C**H$_3$**)$_3$), 1.91 to 2.24 (4H, several m, -C2-H), 3.17 to 3.75 (2H, m, -C5-H), 3.91 to 4.12 (1H, m, -C4-H), 4.23 to 4.52 (1H, m, C3-H), 5.35 to 5.58 (1H, m, C1-H), 7.22 to 7.70 (10H, 2m, arom). ^{13}C NMR: δ -5.82, -5.76, -5.72, -5.56 (-Si-**C**H$_3$); 18.15, 18.18, 18.91 (-Si-**C**(CH$_3$)$_3$); 25.72, 25.77, 26.82, 26.91 (-Si-C(**C**H$_3$)$_3$); 41.98, 45.53 (C2); 63.32, 63.87 (C5); 73.82, 75.10 (C3); 87.30, 87.46 (C4); 99.32, 99.90 (C1); 127.71, 127.83, 129.99, 130.01, 132.82, 133.47, 133.63, 133.62, 135.68, 135.75 (arom).

D-*Ribo- and* D-*arabino-2,5-anhydro-6-*(*t-butyldimethylsilyl*)*-4-*(*t-butyldiphenylsilyl*)*-1,3-dideoxy-1-dimethoxyphosphinylhexitol* (**8**). To a suspension of NaH (0.33 g, 0.014 mol) in dry glyme (40 ml) was added dropwise, with stirring under N_2, a solution of tetramethyl methylenebisphosphonate (3.48 g, 0.015 mol in glyme, 10 ml) at 20°. After the evolution of H_2 had ceased, a solution of **7** (4.16 g, 8.7 mmol) in glyme (10 ml) was added rapidly. The resulting mixture was allowed to stand at room temperature for 16 h. During this time, a gummy material precipitated and TLC (hexane/EtOAc, 1 : 4) indicated that the reaction was complete. The reaction was quenched with 10% KH_2PO_4 (100 ml) and glyme was removed under reduced pressure. The aqueous mixture was extracted with CH_2Cl_2 (3 × 30 ml). The combined extracts were dried, filtered, and concentrated under reduced pressure to give a pale yellow oil. This material was chromatographed with EtOAc : hexane (1 : 1) to furnish 4.00 g (70% of theory) of **8** (mixture of the α and β "anomers") as a colorless viscous oil. ^{1}H NMR: δ -0.89 to 1.02 (6H, 3s, -Si-C**H$_3$**), 0.79 and 1.11 (18H, 2s, -Si-C(C**H$_3$**)$_3$), 1.48 to 2.43 (4H, several m, C1-H and C3-H), 3.15 to 3.42 (2H, 2m, C6-H), 3.68 to 3.79 (6H, 12s, -P(O)C**H$_3$**), 3.93 and 4.01 (1H, 2m, C5-H), 4.30 to 4.60 (2H, several m, C2-H and C4-H), 7.32 to 7.70 (10H, 2 m, arom). ^{13}C NMR: δ -5.70, -5.67, -5.52 (-Si-**C**H$_3$); 18.16, 18.97, 19.02 (-Si-**C**(CH$_3$)$_3$); 25.77, 26.98 (-Si-C(**C**H$_3$)$_3$); 31.37 (d, C1 of major, J_{CP} = 138), 32.3 (d, C1 of minor, $J_{CP} \sim 140$), 41.25 (d, C3 of minor, J_{CP} = 7.5); 42.56 (d, C3 of major, J_{CP} = 8.2); 63.55 (C6 of major); 63.64 (C6 of minor); 73.27 (C2 of major); 73.94 (C2 of minor); 74.96 (C4 of minor); 75.39 (C4 of major); 86.96 (C5 of minor); 88.02 (C5 of major); 127.60, 129.64, 129.72, 133.52, 133.67, 133.82, 135.67, 135.72 (arom). ^{31}P NMR (^{1}H-dec, relative to ext. 85% H_3PO_4): δ 31.22 (major), 31.43 (minor).

D-*Ribo- and* D-*arabino-2,5-anhydro-4-*(*t-butyldiphenylsilyl*)*-1,3-dideoxy-1-dimethoxyphosphinylhexitol* (**9**). A solution of **8** (4.00 g, 6.76 mmol) in 80% aqueous acetic acid was allowed to stand at 20°C for 2 days, at which time TLC indicated that starting material was no longer present. All volatiles were removed under reduced pressure, followed by high vacuum to give 3.20 g (94%) of pure **9** as a colorless viscous oil. ^{1}H NMR: δ 1.05 and 1.09 (-Si-C(C**H$_3$**)$_3$), 1.84 to 2.32 (4H, m, C1-H and C3-H), 3.04 to 3.52 (2H, m, C6-H), 3.66 to 3.78 (6H, 8s, -P(O)C**H$_3$**), 3.90

to 4.02 (1H, m, C5-H), 4.21 to 4.67 (2H, m, C2-H and C4-H), 7.32 to 7.70 (10H, 2m, arom). ^{13}C NMR: δ -0.12, -0.10 (-Si-**C**H_3); 18.88, 18.95 (-Si-**C**$(CH_3)_3$); 26.79, 26.82 (-Si-C(**C**$H_3)_3$); 29.68 (d, C1 of major, J_{CP} = 140); 32.04 (d, C1 of minor, J_{CP} = 136); 41.48 (d, C3 of minor, J_{CP} = 8.2); 42.33 (d, C3 of major, J_{CP} = 5.2); 51.73, 51.82, 51.90, 51.99, 52.50, 52.30 52.39, 52.59 (2C, split by P, -P(O)**C**H_3); 62.31 (C6 of minor); 62.61 (C6 of major); 73.12 (d, C2 of major, J_{CCP} = 6.0); 73.61 (C2 of minor); 74.47 (C4 of minor); 75.09 (C4 of major); 86.54 (C5 of minor); 88.82 (C5 of major); 127.64, 127.65, 127.69, 129.72, 129.78, 129.79, 129.87, 133.25, 133.55, 133.60, 135.61, 135.63, 135.65 (arom). ^{31}P NMR (^{1}H-dec, relative to ext. 85% H_3PO_4): δ 31.27 (major), 32.25 (minor).

D-*Ribo- and* D-*arabino-2,5-anhydro-4-*(*t-butyldiphenylsilyl*)*-1,3-dideoxy-1-dimethoxyphosphinyl-6-diphenoxyphosphinylhexitol* (**10**). To a solution of **9** (3.25 g, 6.80 mmol) in dry CH_2Cl_2 (100 ml) was added with stirring under N_2 diphenyl chlorophosphate (2.19 g, 1.92 ml, 8.16 mmol) and DMAP (1.00 g, 8.16 mmol). The resulting solution was allowed to stand at room temperature for 36 h. Water (100 ml) was added and the layers were separated. The organic layer was washed successively with 1 M HCl (25 ml), 10% $NaHCO_3$ (100 ml), and water (100 ml) and dried. CH_2Cl_2 was removed under reduced pressure to give 5.50 g of a pale yellow viscous oil which was chromatographed (EtOAc) to furnish 4.35 g (92%) of **10** as a colorless viscous oil. ^{1}H NMR: δ 1.05 and 1.09 (9H, 2s, -Si-C(C**H$_3$**$)_3$), 1.41 to 2.39 (4H, m, C1-H and C3-H), 3.58 to 3.67 and 3.90 to 3.96 (2H, 2m, C6-H), 3.67 to 3.73 (6H, 6s, -P(O)C**H$_3$**), 3.97 to 4.02 (1H, m, C5-H), 4.24 to 4.37 (1H, m, C4-H), 4.46 to 4.58 (1H, m, C2-H), 7.08 to 7.64 (20H, 4m, arom). ^{13}C NMR (major): δ 18.89, (-Si-**C**$(CH_3)_3$); 26.78, (-Si-C(**C**$H_3)_3$); 30.85 (d, C1, J_{CP} = 139); 42.58 (d, C3, J_{CP} = 8.2); 51.96, 52.04, 52.36, 52.45, (2C, split by P, -P(O)**C**H_3); 68.24 (C6, J_{COP} = 6.8); 73.69 (C2); 74.76 (C4); 85.61 (C5, J_{CCOP} = 8.2); 119.82, 119.89, 125.25, 127.74, 127.80, 127.84, 129.63, 129.66, 129.84, 129.94, 130.02, 132.91, 133.00, 133.16, 133.25, 135.60, 150.24, 150.33 (arom). ^{31}P NMR (^{1}H-dec, relative to ext. 85% H_3PO_4): δ -11.76 (phosphate, major), -11.72 (phosphate, minor), 30.66 (phosphonate, major), 30.72 (phosphonate, minor).

D-*Ribo- and* D-*arabino-2,5-anhydro-1,3-dideoxy-1-*(*dimethoxyphosphinyl*)*-6-*(*diphenoxyphosphinyl*)*hexitol* (**11**). To a solution of **10** (0.86 g, 1.23 mmol) in CH_2Cl_2 (10 ml) in a Teflon tube was added with stirring 70% HF/pyridine (1 ml) at 0°C. The reaction was allowed to proceed for 1 h at 0°C and then quenched with water (5 ml). The mixture was extracted with CH_2Cl_2 (3 × 10 ml). The extracts were dried, filtered, and evaporated under reduced pressure to a viscous oil. Chromatography with EtOAc : IPA (4 : 1) yielded 0.36 g (62%) of pure **11**. ^{1}H NMR: δ 1.62 to 2.39 (4H, m, C1-H and C3-H), 3.65 to 3.72 (6H, 6s, -P(O)C**H$_3$**), 3.58 to 3.67 and 3.90 to 3.96 (2H, 2m, C6-H), 4.00 (1H, br m, C5-H of major), 4.10 (1H, m, presumed C5-H of minor), 4.20 to 4.30 (3H, m, C4-H and C6-H's), 4.38 to 4.48 (1H, m, C2-H), 7.12 to 7.40 (10H, 2m, arom). ^{13}C NMR: δ 30.77 (d, C1, major, J_{CP} = 139), 31.51 (d, C1, minor, J_{CP} = 134); 40.50 (d, C3, minor, J_{CCCP} = 6.8); 41.78 (d, C3, major, J_{CCCP} = 6.8); 51.97, 52.06, 52.14, 52.21, 52.36, 52.45 (2C, split by P, -P(O)**C**H_3); 68.58, 68.67, 68.76 (C6, J_{COP} unclear); 72.34 (C4, minor); 72.52 (C4, major), 73.18 (C2, major); 73.34 (C2, minor); 83.72 (C5, d, minor, J_{CCOP} = 7.5); 84.70 (C5, d, major, J_{CCOP} = 7.5); 119.78, 119.80, 119.86, 125.32, 129.64, 150.13, 150.23 (arom).

^{31}P NMR (^{1}H-dec, relative to ext. 85% H_3PO_4): δ -11.51 (phosphate, major), -11.43 (phosphate, minor), 30.88 (phosphonate, major), 31.30 (phosphonate, minor).

D-*ribo- and* D-*arabino-2,5-anhydro-1,3-dideoxy-6-*(*diphenoxyphosphinyl*)*-1-*((((*phenoxy*)*hydroxyphosphinyl*)*oxy*)*hydroxyphosphinyl*)*hexitol, ammonium salt* (**12**). To a solution of **11** (1.34 g, 2.87 mmol) in CH_2Cl_2 (40 ml) was added TMS-Br (1.58 g, 1.34 ml, 10.3 mmol) with stirring under N_2 at 0°C. The reaction was allowed to warm to room temperature and after 3 h all volatiles were removed under high vacuum. The resulting viscous mass was dissolved in anh. MeOH (10 ml) and evaporated *in vacuo* (<1 mm). This material was dissolved in 50% aqueous MeOH and taken to pH 4.5 with aqueous 0.4 M tetrabutylammonium hydroxide. Volatiles were removed (<1 mm) and the residue was dried by azeotropic distillation with anh. MeCN (3 × 10 ml). An aqueous solution of disodium phenyl phosphate dihydrate (0.82 g, 3.16 mmol) was passed over a column of Dowex-50 (H^+ form). Effluent was collected until the pH reached 4.5. The effluent was evaporated *in vacuo* and dried by evaporation with MeCN (3 × 20 ml). The resulting solid was dissolved in anh. MeCN (10 ml) and carbonyl diimidazole (0.52 g, 3.16 mmol) was added with stirring under N_2. CO_2 was evolved as stirring was continued at 20°C for 2 h, whereupon a solution of the mono(tetrabutylammonium) phosphonate (described above) in anh. MeCN (10 ml) was added. The mixture was stirred at 50°C for 16 h. All volatiles were removed at 1 mm. The resulting gum was dissolved in water (20 ml) and passed over a Dowex-50 column (NH_4^+ form). The column was eluted with water until the pH of the effluent reached 5.0. The effluent was evaporated under reduced pressure to a gummy residue. The residue was chromatographed with $MeCN:H_2O:NH_3$ (90:10:1), followed by the same mixture at 80:10:1. The fractions containing the desired phosphonylphosphate were combined and concentrated under reduced pressure to yield 1.23 g (65%) of a gum. This material was taken directly to the next step.

Deprotection of ***12*** *to* D-*ribo- and* D-*arabino-2,5-anhydro-1,3-dideoxy-1-*(((*dihydroxyphosphinyl*)*oxy*)*hydroxyphosphinyl*)*-6-phosphorylhexitol,* (***4***). The gum from above was dissolved (in 50% aqueous acetic acid (40 ml). PtO_2 (1.00 g) and 10% Pt/C (1.00 g) were added and the resulting suspension was stirred rapidly under H_2 at 1 atm until the uptake of H_2 ceased at 105% of theory. The reaction mixture was filtered and the filtrate was evaporated at 0.1 mm to yield a colorless gum which was found to be the desired **4** as the free acid. ^{1}H NMR (D_2O; referenced to D_4-TMS-propionate at $\delta = 0$): δ 1.89 (m, C3-H_A, minor), 1.93 (m, C3-H_A, major), 1.98 (m, C1-H_A, major + minor), 2.1 (m, C1H_B, minor), 2.20 (ddd, C3-H_B, major), 2.31 (m, C1-H_B, major), 2.58 (m, C3-H_B, minor), 3.75 to 3.87 (m, C6-H_A and C6-H_B, major + minor), 3.97 (dt, C5-H, major), 4.02 (m, C5-H, minor), 4.36 (m, C4-H, major + minor), 4.44 (apparent nonet, C2-H, major + minor). ^{1}H coupling constants: Major isomer, H1$_A$ to (H1$_B$, -14.5; H2, 9.3; P, 19.2), H1$_B$ to (H2, 5.0; P, 19.2), H2 to (H3$_A$, 10.7; H3$_B$, 5.0; P, 5.2), H3$_A$ to (H3$_B$, -13.5; H4, 6.5), H3$_B$ to (H4, 1.5), H4 to (H5, 2.6), H5 to (H6$_A$, 6.0; H6$_B$, 6.0), H6$_A$ to (H6$_B$, -14.5; P, 5.5), H6$_B$ to (P, 5.5). Minor isomer, H2 to (H3$_A$, 8.5; H3$_B$, 7.0), H3$_A$ to (H3$_B$, -13.5; H4, 6.5), H3$_B$ to (H4, 7.0), H4 to (H5, 10.6), H5 to (H6$_A$, 6.3; H6$_B$, 4.6), H6 to (P, 5.5). ^{13}C NMR (D_2O; referenced to D_4-TMS-propionate at $\delta = 0$): δ 36.67 (d, C1, major, $J_{CP} = 134$), 37.34 (d, C1, minor, $J_{CP} = 134$); 42.45 (d, C3, minor, $J_{CCCP} \sim 4$); 42.98

(d, C3, major, J_{CCCP} = 4.1); 66.53 (d, C6, minor, $J_{COP} \sim 5$); 67.17 (d, C6, major, J_{COP} = 4.9); 74.74 (C4, minor); 75.42 (C4, major), 77.13 (C2, minor); 77.72 (C2, major); 85.78 (d, C5, minor, $J_{CCOP} \sim 8$); 87.60 (C5, d, major, J_{CCOP} = 8.1). ^{31}P NMR (D_2O; ^{1}H-dec; relative to ext. 85% H_3PO_4): δ -7.58 (phosphate anhydride, major and minor epimers superimposed, J_{POP} = 24.3), 2.71 (6-phosphate, major), 2.78 (6-phosphate, minor), 11.79 (phosphonate anhydride, major, J_{POP} = 24.4), 11.84 (phosphonate, minor, J_{POP} = 24.4).

Enzymes and Assays

Orotate PRTase was purified to homogeneity from baker's yeast by a combination of literature methods. Initial purification was carried out according to the procedure of Umezu *et al.* (12) through the DEAE cellulose step with the omission of the ethanol fractionation. The ammonium sulfate step was changed to a fractionation of 0–55% of saturation at 4°C. Enzyme that eluted in the second peak of activity from the DEAE column was then applied to OMP-Sepharose and eluted with OMP as described by Dodin (2). The enzyme routinely had specific activities >50 unit/mg at 25°C and was homogeneous as determined by SDS gel electrophoresis. It was assayed by observing the decrease in absorbance at 295 nm due to the conversion of orotic acid to OMP (molar absorptivity $\Delta\varepsilon$ = 3.35 mM^{-1}). Human HG PRTase was obtained from Professor C. C. Wang (University of California at San Francisco). It was assayed by the spectrophotometric method of Giacomello and Salerno (13). The activity of glutamine : amido PRTase from *Eschenichia coli* was determined by measuring the PRPP-dependent formation of glutamate from glutamine according to Messenger and Zalkin (14); these assays were conducted by Dana Wolle and Howard Zalkin at Purdue University.

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REFERENCES

1. McClard, R. W., and Witte, J. F. (1990) *Bioorg. Chem.* **18,** 165–178.
2. Dodin, G. (1981) *FEBS Lett.* **134,** 20–24.
3. Gutensohn, W. (1977) *Adv. Exp. Med. Biol.* **76A,** 586–590.
4. Tuttle, J. V., Krenitsky, T. A., and Elion, G. B. (1983) *Biochem. Pharmacol.* **32,** 3011–3015.
5. Scapin, G., Grubmeyer, C., and Sacchettini, J. C. (1994) *Biochemistry* **33,** 1287–1294.
6. Witte, J. F., Firth, R., and McClard, R. W. (1995) *Carbohydr. Lett.* **1,** 123–124.
7. Atwood, S. V., and Barrett, A. G. M. (1984) *J. Chem. Soc. Perkin Trans. I*, 1315–1322.
8. Okabe, M., Sun, R.-C., Tam, S. K.-Y., Todaro, L. J., and Coffen, D. L. (1988) *J. Org. Chem.* **53,** 4780–4786.

9. McClard, R. W., and Witte, J. F. (1992) *in* Handbook of Organophosphorus Chemistry (Engel, R., Ed.), pp. 655–681. Marcel Dekker, New York.
10. Barker, R., and Fletcher, H. G. (1961) *J. Org. Chem.* **26,** 4605–4611.
11. Victor, J., Greenberg, L. B., and Sloan, D. L. (1979) *J. Biol. Chem.* **254,** 2647–2655.
12. Umezu, K., Amaya, T., Yoshimoto, A., and Tomita, K. (1971) *J. Biochem.* **70,** 249–267.
13. Giacomello, A., and Salerno, C. (1978) *J. Biol. Chem.* **253,** 6038–6044.
14. Messenger, L. J., and Zalkin, H. (1979) *J. Biol. Chem.* **254,** 3382–3392.