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Carbocyclic Analogues of D-Ribose-5-Phosphate: Synthesis and Behavior with 5-Phosphoribosyl α-1-Pyrophosphate Synthetases

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Abstract—The synthesis of cyclopentyl and cyclopentenyl analogues of the α-anomer of D-ribose-5-phosphate from D-ribonolactone and D-ribose is described. These analogues, which have the same absolute configuration as D-ribose-5-phosphate, were incubated with PRPP synthetases in an attempt to prepare the corresponding carbocyclic PRPP analogues. The carbocyclic ribose-5-phosphate analogues were found to be inhibitors, rather than substrates, for 5-phosphoribosyl α-1-pyrophosphate synthetases of both bacterial and human origin. The inhibitory behavior of the analogues is described. Copyright © 1996 Elsevier Science Ltd

Introduction

The PRPP[1] synthetases (ATP: D-ribose-5-phosphate pyrophosphotransferase, EC 2.7.6.1) catalyse the reaction of ribose-5-phosphate with ATP in the presence of magnesium ion to form 5-phosphoribosyl α-1-pyrophosphate (PRPP) and AMP. The enzymes are of interest for a number of reasons. First, the reaction catalysed by the enzymes is unusual in that it involves a nucleophilic attack at the β-phosphoryl group of the nucleoside triphosphate side chain.[1–3] Second, the product of the reaction, PRPP, is used in the biosynthesis of purine, pyrimidine, and pyridine nucleotides, as well as in the biosynthesis of histidine and tryptophan.[4,5] Third, an understanding of the regulation of PRPP synthesis is important since the reaction constitutes the branch point of a highly divergent network of biosynthetic pathways. Lastly, there is evidence that PRPP synthetase activity is elevated in tumors, and that inhibitors of PRPP synthetase possess antineoplastic activity.[6] For these reasons, a number of PRPP synthetases have been cloned and sequenced,[7–11] and some have been purified to homogeneity and characterized.[12–16]

Our interest in PRPP synthetases was stimulated by investigations of the biosynthesis of the carbocyclic nucleoside antibiotics aristeromycin[17] and neplanocin A.[18,19] On the basis of precursor incorporation and isotope dilution experiments,[20–22] it appeared that the biosynthesis of these nucleoside antibiotics might proceed via carbocyclic analogues of ribose-5-phosphate and PRPP. Consequently, a synthesis of the racemic form of the cyclopentyl analogue of PRPP (cPRPP) was devised.[23] This compound was subsequently found to be a useful tool for the investigation of glutamine phosphoribosylpyrophosphate amidotransferase.[24] It therefore appeared worthwhile to develop a simpler synthesis that would lead only to the correct enantiomeric form of cPRPP. The method that was first explored was to synthesize the correct enantiomer of the cyclopentyl analogue of ribose-5-phosphate and then attempt to convert it into cPRPP with PRPP synthetases. While this work was in progress, new insights into the aristeromycin biosynthetic pathway were obtained[25,26] that suggested the possible intermediacy of the cyclopentenyl analogue of PRPP in the formation of neplanocin A and aristeromycin. Accordingly, the correct enantiomer of the cyclopentenyl analogue of ribose-5-phosphate was also synthesized and its behavior with PRPP synthetases was investigated. We now report the synthesis of both of these analogues and describe their behavior with PRPP synthetases.

Abbreviations: AK, adenylate kinase; AMP, adenosine-5′-monophosphate; APP-MP, 4-amino-8-(β-D-ribofuranosylamino)-pyrimido [5,4-*d*]pyrimidine-5′-monophosphate; ATP, adenosine-5′-triphosphate; CI, chemical ionization; cPRPP, 1α-pyrophosphoryl-2α,3α-dihydroxy-4β-cyclopentanemethanol-5-phosphate; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DMAP, 4-dimethylaminopyridine; EDTA, ethylenediaminetetraacetic acid; ES, electrospray; LDA, lithium diisopropylamide; LDH, lactate dehydrogenase; NADH, nicotinamide adenine dinucleotide; PEP, phosphoenolpyruvate; PK pyruvate kinase; PRPP, 5-phospho-D-ribosyl-1α-pyrophosphate; R5P, D-ribose-5-phosphate; THP, tetrahydropyranyl; TMS, tetramethylsilane; TSP, sodium 3-(trimethylsilyl)propionate.

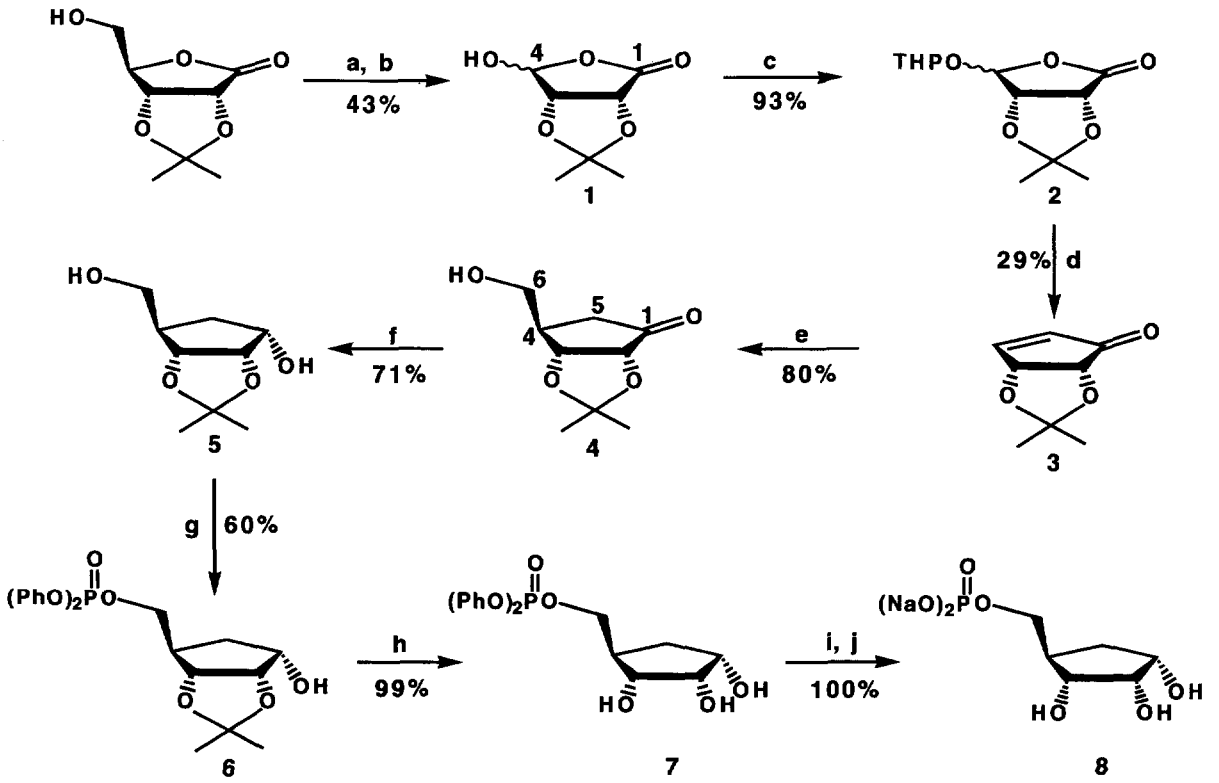
Results

Preparation of analogues

The correct enantiomer of the ribose-5-phosphate analogue **8** was synthesized from 2,3-*O*-isopropylidene-D-ribonolactone[27] in the manner outlined in Scheme 1. The first step involved the conversion of 2,3-*O*-isopropylidene-D-ribonolactone into the lactone **1** using a simplified version of the procedure of Beer et al.[28] Lactone **1** was then transformed into the cyclopentenone **3** by means of a Wittig reaction between dimethyl

methylphosphonate and the THP ether **2**. The use of the THP ether proved to be advantageous since it could be synthesized from **1** more readily and in higher yield (93%) than the isopropoxy ether that had been previously employed for the Wittig reaction.[29] The photochemical addition of methanol[21] to the cyclopentenone **3** then gave the saturated cyclopentanone **4** in 80% yield after chromatography. Reduction of the ketone **4** with sodium triacetoxyborohydride[30] gave exclusively the diol **5** with the alpha configuration at C-1. Selective phosphorylation of the primary hydroxyl group of **5** was then accomplished by treatment of **5** with diphenyl chlorophosphate in the presence of the hindered base diisopropylethylamine with the addition of DMAP after 48 h to drive the reaction to completion. The product **6** was obtained in 60% yield after chromatography. The final stages in the synthesis of **8** involved removal of the isopropylidene group from **6** by treatment with 1 N HCl to produce the triol **7** (99%) followed by deprotection of **7** by catalytic reduction with platinum and hydrogen to give **8**, which was converted into its sodium salt by passage down a column of the sodium form of Amberlite IR120 (plus), ca. 100% yield.

The correct enantiomer of the cyclopentenyl ribose-5-phosphate analogue **19** was synthesized as shown in Scheme 2. *p*-Methoxybenzyl alcohol was converted into p-methoxybenzyl methylthiomethylether (**9**) by alkylation with chloromethyl methyl sulfide (48% yield after chromatography). Treatment of **9** with sulfuryl chloride then gave chloromethyl *p*-methoxybenzyl ether,[31] which was used without further purification. A solution of tri-*n*-butylstannyl lithium in THF was generated by deprotonation of tri-*n*-butyltin hydride with LDA[32] and the anion was then alkylated with the chloromethyl *p*-methoxybenzyl ether to yield (*p*-methoxybenzyloxymethyl)tri-*n*-butylstannane (**10**), 76% after chromatography. For reasons that are unclear, **10** could not be prepared more directly from *p*-methoxybenzyl alcohol and iodomethyl tri-*n*-butylstannane using the method of Still.[32] Addition of *n*-butyllithium to a solution of **10** in THF generated *p*-methoxybenzyloxymethyllithium, which was condensed[33] with the (+)-cyclopentenone **11**[34] to yield the 1,2-addition product **12** in 81% yield after chromatography. The next stages in the synthesis involved acetylation of the allylic hydroxyl group of **12** to give the allylic acetate **13** (96% after chromatography) followed by the palladium-catalysed rearrangement[33] of **13** to produce the isomeric allylic acetate **14** (64% yield after chromatography). In preparation for the introduction of a phosphate group at C-5, the *p*-methoxybenzyl protecting group of **14** was removed with DDQ[35] to yield the allylic alcohol **15** (65% after chromatography). The choice of reagents for the introduction of a phosphate ester moiety at C-5 of **15** was governed by the requirement that the phosphate ester be deprotected without reduction of the double bond. It appeared that the trimethylsilylethyl group might satisfy this requirement.[36] Accordingly, bis(2-trimethylsilylethyl)-*N*,*N*-diethylphosphoramidite (**16**) was synthesized from diethyphosphoramidous dichloride[37] by treatment of the dichloride with trimethylsilylethanol in the presence of



Scheme 1. (a) NaOH; (b) $NaIO_4$; (c) dihydropyran, pyridinium *p*-toluenesulfonate; (d) $CH_3P(O)(OCH_3)_2$, Bu^nLi; (e) hv, CH_3OH, benzophenone; (f) $NaBH(OAc)_3$; (g) $(PhO)_2P(O)Cl$, $EtN(Pr^i)_2$, DMAP; (h) 1 N HCl; (i) PtO_2, H_2; (j) Amberlite IR120 (plus), Na form.

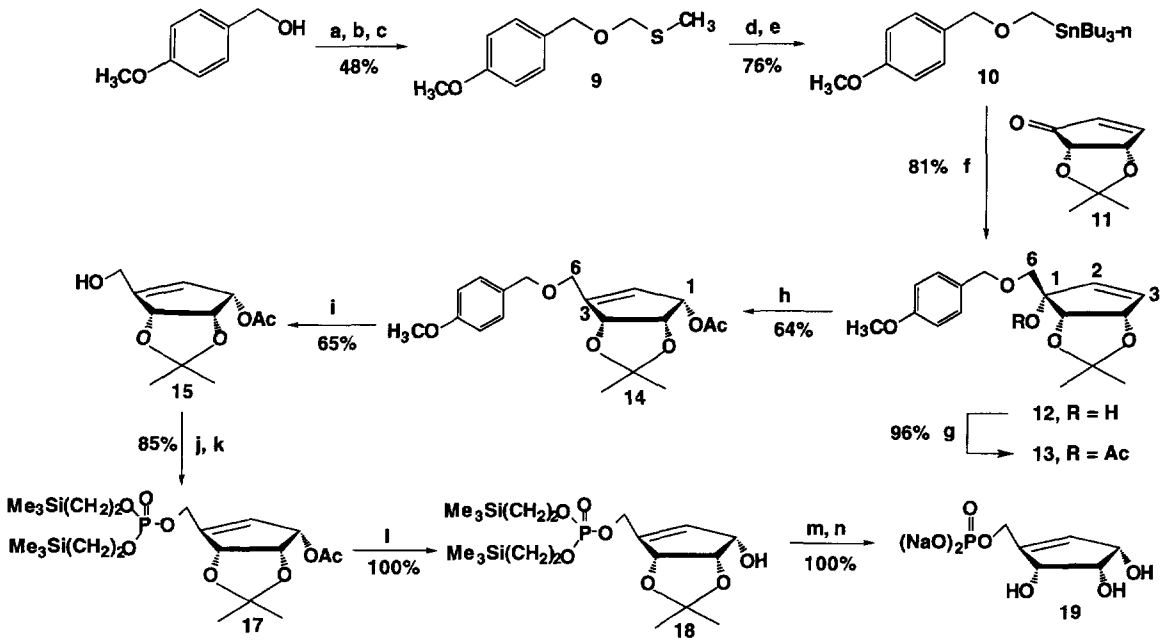
triethylamine (62% yield after distillation). While this work was in progress, a synthesis of the same reagent was reported by Ross et al.[38] Phosphorylation of the alcohol **15** with **16** was carried out in dry THF in the presence of 1*H*-tetrazole. The intermediate phosphite ester was oxidized without isolation by addition of an excess of *m*-chloroperbenzoic acid to the reaction mixture. After chromatographic purification, the desired trimethylsilyl ester **17** was obtained in 85% overall yield. The deprotection of **17** to yield the carbocyclic ribose-5-phosphate analogue **19** was accomplished in two steps. Exposure of **17** to ammonia-saturated methanol led to removal of the acetate ester and formation of the alcohol **18** in near quantitative yield. The crude alcohol **18** was then fully deprotected by using a modification of conditions reported by Swabe et al.[39] (i.e., treatment with a mixture of acetonitrile, water, and 48% HF). Attempts to utilize tetra-*n*-butylammonium fluoride for the desilylation step gave only partial deprotection. Finally, the product of the desilylation reaction was converted into its sodium salt by passage down a column of the sodium form of Amberlite IR120 (plus) to give **19** in quantitative yield.

Enzymatic studies with substrate analogues

The analogue **8** was assayed for substrate and inhibitory activity with *Salmonella typhimurium* PRPP synthetase using a minor modification of the ^{32}P transfer assay of Switzer[15] and the coupled spectrophotometric assay of Valentine and Kurschener[40] as modified by Braven et al.[41] A pH rate profile for the reactivity of **8** with the *S. typhimurium* enzyme was also determined using the ^{32}P transfer assay (Fig. 1). Analogue **8** was found to be an extremely poor substrate for the enzyme, even at its pH optimum (7.0) which was found to be one pH unit lower than that of R5P (8.0). At pH 7.5, analogue **8** exhibited competitive inhibition against R5P and uncompetitive inhibition against ATP with the *S. typhimurium* enzyme. The inhibition constants are shown in Table 1. Using the spectrophotometric assay at pH 7.5, analogue **8** was also found to inhibit both the human type I PRPP synthetase and *Bacillus subtilis* PRPP synthetase. The inhibition was competitive against R5P for the human enzyme, while the *B. subtilis* enzyme exhibited mixed-type inhibition against R5P. Analog **8** displayed noncompetitive inhibition against ATP with the human enzyme, but it did not exhibit inhibition against ATP with the *B. subtilis* enzyme within the usable range of ATP concentrations. The inhibition constants are also shown in Table 1.

Previous studies have shown that the degree of inhibition of human PRPP synthetase by the ATP analogue APP-MP is inversely related to the phosphate ion concentration, with the greatest inhibition being observed at an intracellular phosphate ion concentration (ca. 1 mM).[6] Therefore, the inhibition of type I human PRPP synthetase by analogue **8** was also examined at 1 mM phosphate ion concentration. Under these conditions, **8** was a more effective inhibitor of human PRPP synthetase with respect to both ribose-5-phosphate and ATP, and the inhibition with respect to both substrates was noncompetitive (Table 1).

The behavior exhibited by analogue **19** was more complex than that of analogue **8**. Although both **8** and **19** were very poor substrates for PRPP synthetases, the spectrophotometric assay indicated that **19** is a better



Scheme 2. (a) NaH; (b) NaI; (c) CH_3SCH_2Cl; (d) SO_2Cl_2; (e) Bu^n_3SnH, LDA; (f) Bu^nLi; (g) Ac_2OI, Et_3N, DMAP; (h) $Pd(CH_3CN)_2Cl_2$, *p*-benzoquinone; (i) DDQ; (j) $[(CH_3)_3CH_2CH_2SiO]_2PNEt_2$ (**16**), 1*H*-tetrazole; (k) MCPBA; (l) NH_3, CH_3OH; (m) HF, CH_3CN, H_2O; (n) Amberlite IR120 (plus), Na form.

Table 1. Inhibition of PRPP synthetases by carbocyclic ribose-5-phosphate analogues[a]

Enzyme source	Analogue	K_i versus R5P μM	K_i versus ATP μM	Type of inhibition
Salmonella	**8**	551	3026	Competitive versus R5P
		950[b]		Uncompetitive versus ATP
Human Type I	**8**	186	465	Competitive versus R5P (70 mM P_i)
		71[c]	162[c]	Noncompetitive versus R5P (1 mM P_i)
				Noncompetitive versus ATP (70 mM, 1 mM P_i)
Bacillus	**8**	3811	NI[d]	Mixed-type versus R5P
Salmonella	**19**	490	710	Partial mixed-type versus R5P
				Partial mixed-type versus ATP
Human Type I	**19**	350	NI[d]	Partial noncompetitive versus R5P

[a]Measured by spectrophotometric assay at pH 7.5 with 70 mM P_i.
[b]Measured at pH 8.0 by radioassay with 100 mM P_i.
[c]Measured at 1 mM P_i concentration.
[d]No inhibition.

substrate for the *S. typhimurium* PRPP synthetase than analogue **8**: there was ca. 11% turnover with **19**, but only 3–4% turnover with **8**. Analogue **19** exhibited partial mixed-type inhibition against the *S. typhimurium* PRPP synthetase relative to both ribose-5-phosphate and ATP (Table 1). It showed partial noncompetitive inhibition against human type I PRPP synthetase relative to ribose-5-phosphate, but it did not show inhibition with respect to ATP within the range of usable ATP concentrations (Table 1). The partial inhibition observed in each case indicated that the enzyme–ATP–R5P–**19** complexes were also producing products, albeit less effectively than enzyme–ATP–ribose-5-phosphate complexes.

Michaelis constants for PRPP synthetases

The K_m values reported in the literature for the *Salmonella*, human, and *Bacillus* PRPP synthetases were obtained using the ^{32}P exchange assay method. These values were redetermined using the spectrophotometric assay in order to facilitate a comparison between the K_m values and the K_i values exhibited by the substrate analogues **8** and **19**. The K_m values determined by the spectrophotometric method are summarized in Table 2.

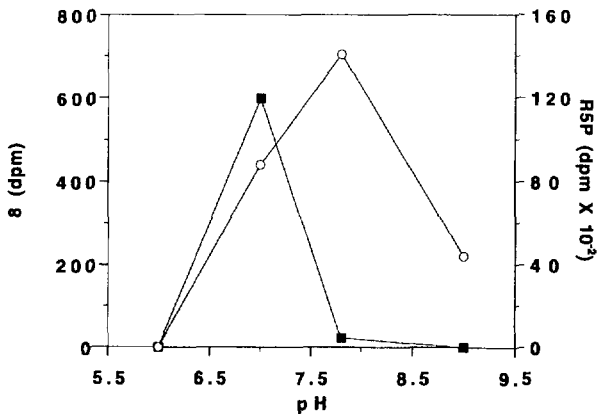


Figure 1. Plot of *S. typhimurium* PRPP synthetase activity versus pH for ribose-5-phosphate and carbocyclic analogue **8** (○, R5P; ■, **8**).

Table 2. Michaelis constants for PRPP synthetases[a]

Enzyme source	K_m for R5P (μM)	K_m for ATP (μM)
Salmonella	298	193
Human Type I	135	140
	92[b]	94[b]
Bacillus	791	1127

[a]Determined by spectrophotometric assay at pH 7.5, 70 mM P_i.
[b]Measured at 1 mM P_i.

Discussion

The investigations outlined in this article were initiated with the goal of preparing saturated and unsaturated carbocyclic analogues of PRPP. In order to accomplish this goal, syntheses of the correct enantiomeric forms of **8** and **19** from D-ribonolactone and D-ribose were devised. The syntheses were carried out with the expectation that the monophosphates **8** and **19** would serve as satisfactory substrates for PRPP synthetases. It was therefore something of a surprise to discover that neither analogue was an effective substrate for any of the PRPP synthetases that were examined. A thorough understanding of the reasons for this behavior will require that more insight be gained into the nature of the active sites of these enzymes. At the present time, very little is known about the types of amino acid residues that are located in the active sites and their roles in substrate binding and catalysis. Affinity labeling studies with 5′-(*p*-fluorosulfonylbenzoyl)adenosine[42] and the 2′,3′-dialdehyde derivative of ATP[43] have indicated that a histidyl residue and one or more lysine residues may be present at the ATP binding site, but similar information is not available with respect to the ribose-5-phosphate binding site. Nevertheless, it appears reasonable to postulate that the initial step in the pyrophosphorylation of the C-1 hydroxyl group of ribose-5-phosphate may involve deprotonation of the hydroxyl group by an active site base. If this hypothesis was correct, then the failure of **8** and **19** to serve as effective substrates might be due, at least in part, to the differences between the pK_a of the anomeric

hydroxyl group of ribose-5-phosphate (probably ca. 13–14)[44] and that of the corresponding hydroxyl group in analogues **8** and **19** (probably ca. 15).[45] However, it is clear that other factors are likely to be involved, since analogue **8** exhibits its maximum activity as a substrate for the *S. typhimurium* enzyme at a pH that is *lower* than the optimum pH for ribose-5-phosphate (7.0 versus 8.0; Fig. 1). In contrast to the behavior of **8** and **19**, carbocyclic analogues of phosphoribosylamine, glycinamide ribonucleotide, and formylglycinamide ribonucleotide have been found to serve as reasonable substrates for the enzymes of the de novo purine pathway.[46,47]

The carbocyclic ribose-5-phosphate analogues **8** and **19** display quite diverse inhibition behavior with the PRPP synthetases that were examined. The simplest behavior is exhibited with the analogue **8** and *S. typhimurium* PRPP synthetase. This analogue was found to be a competitive inhibitor against ribose-5-phosphate, and an uncompetitive inhibitor against ATP (Table 1). It exhibited K_i values that are two to three times larger than the corresponding K_m values (Tables 1 and 2). Since **8** displays uncompetitive inhibition against ATP with the *Salmonella* PRPP synthetase, this implies that **8** binds to the enzyme after ATP is bound, and that ribose-5-phosphate should also bind after ATP. Such an interpretation is consistent with the observations of Switzer,[48] who demonstrated that the *Salmonella* PRPP synthetase exhibits ordered binding in which ATP binds before ribose-5-phosphate.

With the human type I PRPP synthetase, analogue **8** was found to be competitive against ribose-5-phosphate, but there was some ambiguity in the interpretation of the double reciprocal plots observed with ATP as these gave nearly parallel lines (Fig. 2). Since it has been reported that the human PRPP synthetase exhibits an ordered Bi–Bi reaction with ribose-5-phosphate binding first,[49] it appears more likely that **8** exhibits noncompetitive rather than uncompetitive inhibition against ATP, and that the lines in the double-reciprocal plot are not truly parallel. The inhibition of the human PRPP synthetase by **8** was also examined at 1 mM phosphate ion concentration since this corresponds to the intracellular concentration of phosphate.[6] At low phosphate ion concentration, **8** exhibits noncompetitive inhibition with the human enzyme against both ribose-5-phosphate and ATP. This suggests that under these conditions the analogue is binding to other sites on the enzyme besides those occupied by ribose-5-phosphate and ATP. One possibility is that **8** is binding to the P_i binding site. Both the K_m values for ribose-5-phosphate and ATP and the K_i values for **8** with the human enzyme were significantly lower at 1 mM phosphate ion concentration than at 70 mM phosphate, and the K_i value for **8** versus ribose-5-phosphate was close to the K_m for this substrate under the same conditions (Tables 1 and 2). A similar effect of phosphate ion concentration on the inhibition of the human PRPP synthetases by the ATP analogue APP-MP has previously been reported.[6]

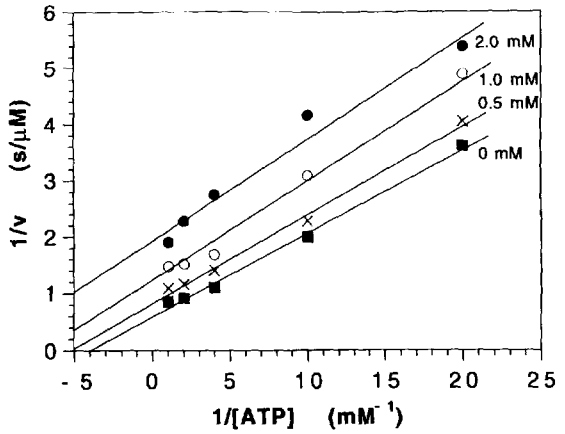


Figure 2. Double-reciprocal plots for the inhibition of human type I PRPP synthetase by analogue **8**. Concentrations of **8** ranged from 0 to 2 mM.

The double reciprocal plots for *B. subtilis* PRPP synthetase were interesting in that the lines intersected on the vertical axis when 1/v was plotted as a function of 1/[ATP] and ribose-5-phosphate was fixed at different concentrations (Fig. 3). When 1/v was plotted against 1/[R5P], and ATP was fixed at several concentrations, the lines intersected close to the horizontal axis and to the left of the vertical axis (Fig. 4). Increasing ATP caused the apparent V_{max} to approach the actual V_{max}, while the apparent K_m for R5P approached zero (Fig. 4). These two types of behavior are indicative of a rapid equilibrium ordered bireactant system in which the substrates combine with the enzyme in an obligatory order.[50] In this case, it appears that ATP can only bind to the enzyme-R5P complex, i.e., the order of addition is R5P, then ATP. This binding order is the opposite of that reported for the *S. typhimurium* enzyme,[48] but it is the same as that reported for the human enzyme.[50]

The ribose-5-phosphate analogue **19** exhibited more complex inhibition behavior than the analogue **8**. With the *S. typhimurium* enzyme, **19** displayed partial mixed-type inhibition with respect to both ribose-5-phosphate

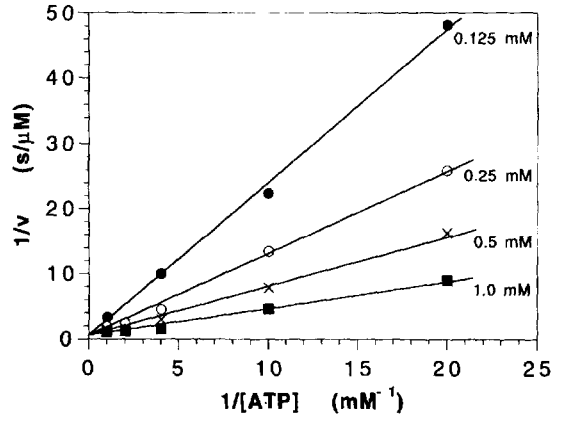


Figure 3. Double-reciprocal plots for *B. subtilis* PRPP synthetase with variable ATP concentrations and fixed R5P concentrations ranging from 0.125 mM to 1.0 mM.

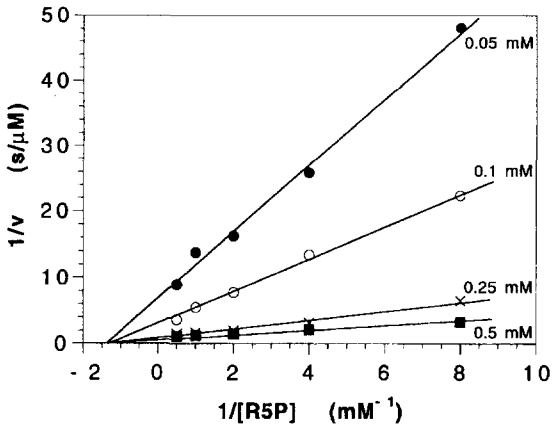


Figure 4. Double-reciprocal plots for *B. subtilis* PRPP synthetase with variable R5P concentrations and fixed ATP concentrations ranging from 0.05 mM to 0.5 mM. Plot for 1 mM ATP is the same as for 0.5 mM ATP.

and ATP. With the human enzyme, **19** showed partial noncompetitive inhibition with respect to ribose-5-phosphate, but no inhibition against ATP within the range of usable ATP concentrations. The equilibria describing these two situations are essentially the same, except that in the former analogue **19** reduces the affinity of the enzyme for both substrates, hence the mixed-type inhibition; in the latter, analogue **19** does not alter the affinity of the human enzyme for ribose-5-phosphate, hence the noncompetitive inhibition. In both cases, analogue **19** is probably binding to other sites on the enzyme besides those occupied by ribose-5-phosphate or ATP. The partial inhibition observed with both enzymes and **19** might be due to the occurrence of one or more of the following types of behavior: (1) analogue **19** may fail to bind correctly at the ribose-5-phosphate binding site, because its geometry differs significantly from that of the normal substrate, (2) analogue **19** may bind at another site and thereby cause a minor perturbation at the active site, or (3) analogue **19** may to a small extent be converted into product. In the first two cases, the inhibited enzyme is afforded some relief and is thus able to make products. In the third situation, the enzyme does not experience relief from inhibition, but some of the enzyme will be committed to a slower reaction pathway that leads to the formation of a carbocyclic PRPP analog. It appears that the third type of behavior is encountered with the *S. typhimurium* enzyme since we were able to observe some apparent turnover of **19** with this enzyme.

In summary, the correct enantiomeric forms of two carbocyclic analogues of ribose-5-phosphate have been synthesized and shown to act as inhibitors of PRPP synthetases. The fact that these analogues are inhibitors is of interest for two reasons. First, the results may provide new leads for the design of better inhibitors of human PRPP synthetase. Second, these compounds may prove to be useful in conjunction with the crystallographic analysis of the structures of PRPP synthetases.

Experimental

Materials and methods

Specialized organic reagents were purchased from Aldrich Chemical Co. and used without further purification unless otherwise indicated. Lactate dehydrogenase (LDH, 5 mg/mL, 5500 U/mL), adenylate kinase (AK) (1000 U/mL), pyruvate kinase (PK) (10 mg/mL, 5000 U/mL), and phosphoenolpyruvate (PEP) were purchased from Boeringer Mannheim Corp. D-Ribose-5-phosphate (R5P) and NADH were purchased from Sigma Chemical Co., and ATP was purchased from Aldrich Chemical Co. The carbocyclic ribose-5-phosphate analogues (+)-(1*S*,2*S*, 3*R*,4*R*)-1,2,3-trihydroxy-4-cyclopentanemethanol-6-phosphate (**8**) and (+)-(1*S*,4*R*,5*S*)-1,4,5-trihydroxy-3-cyclopent-2-enemethanol-6-phosphate (**19**) were synthesized as described below. Analytical TLC was carried out on Merck type 60 F-254 glass plates. Column chromatography utilized 230–400 mesh type 60A silica gel and Florisil purchased from Aldrich Chemical Co. Amberlite IR-120 (plus) ion-exchange resin was purchased from Aldrich, while AG 1×8 anion exchange resin was obtained from Bio-Rad.

Human PRPP synthetase isozyme I[15] was a gift from Dr Robert L. Switzer (University of Illinois, at Urbana) and Dr Jo Davisson (Purdue University). *B. subtilis* PRPP synthetase[12] was a gift from Dr Kenneth W. Harlow (University of Copenhagen, Denmark). *S. typhimurium* PRPP synthetase was purified from a genetically engineered *Escherichi coli* strain that overexpresses the *S. typhimurium* enzyme.[7] The overproducing construct was a gift from Dr Robert L. Switzer, and the enzyme was purified by his procedure.[15] Proton, ^{13}C, and ^{31}P NMR spectra were measured on a Bruker AC250 NMR spectrometer operating at 250, 62.89, and 101.25 MHz, respectively. Chemical shifts for protons are given in ppm downfield from tetramethylsilane (TMS, 0.0 ppm) for spectra measured in $CDCl_3$ and in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP, 0.0 ppm) for spectra taken in D_2O. Chemical shifts for ^{13}C are given in ppm downfield from TMS for spectra measured in $CDCl_3$ and in ppm downfield from TSP for spectra taken in D_2O. ^{31}P chemical shifts are given in ppm relative to 85% phosphoric acid (0.0 ppm) as an external standard. Optical rotations were measured on a Jasco DIP 370 digital polarimeter. Mass spectra were run on a Finnigan MAT 95 mass spectrometer. All melting points were measured on a Fisher–Johns melting point apparatus and are uncorrected.

Enzyme assays

PRPP synthetase activity was measured by a minor modification of the ^{32}P transfer assay of Switzer[15] and the coupled spectrophotometric assay of Valentine and Kurschener[40] as modified by Braven et al.[41] In the former assay, the assay buffer consisted of 100 mM potassium phosphate, 50 mM triethanolamine, 0.75 mM Na_2EDTA, pH 8.0. Each assay contained 0.5 mM

ATP, 0.34 μCi [γ-^{32}P]ATP, 5 mM $MgCl_2$, 0.075–0.40 mM ribose-5-phosphate, and 0–1.08 mM of **8** in a 290 μL volume. The reaction was initiated by addition of 10 μL of *S. typhimurium* PRPP synthetase (0.38 μg of protein) and incubation was carried out for 5 min at 37 °C. The reaction was terminated by addition of 300 μL of cold 5% (v/v) perchloric acid followed by incubation on ice for 8 min. Acid-washed Norit charcoal (180 μL, 20% v/v) was then added with vigorous mixing. After incubation on ice for 10 min, 120 μL of 50 mM tetrasodium pyrophosphate, pH 7.0, containing 5 mg/mL of bovine serum albumin was added. After additional incubation on ice for 10 min, the charcoal was removed by centifugation, and the radioactivity in the supernatant was measured by liquid scintillation counting. In the spectrophotometric assay, the amount of AMP produced was measured indirectly by following the decrease in absorbance of NADH at 340 nm. The sequence of reactions is: (1) R5P + ATP → PRPP + AMP, catalysed by PRPP synthetase; (2) AMP + ATP → 2 ADP, catalysed by AK; (3) 2 ADP + 2 PEP → 2 ATP + 2 pyruvate, catalysed by PK; and (4) pyruvate + NADH + H^+ → lactate + NAD^+, catalysed by LDH. The rate of the reaction was calculated using the formula $(\Delta A.s^{-1})/2 \times 6220\ M^{-1}$, where ΔA is change in absorbance, 6220 M^{-1} is the extinction coefficient of NADH at 340 nm, and 2 is the stoichiometric factor. The assay buffer for this procedure consisted of 100 mM potassium phosphate, 0.75 mM Na_2EDTA, pH 7.5. Each assay contained 1.0 mM R5P, 1.0 mM ATP, 3 mM $MgCl_2$, 15 mM KCl, 25 mM NaF, 0.8 mM PEP, 0.2 mM NADH, variable amounts of **8** or **19**, 0.002 units/mL each of AK, PK, and LDH, and a given amount of PRPP synthetase in a total volume of 1 mL. The amounts of PRPP synthetase used per reaction were 4.1 μg (human PRPP synthetase), 25.5 μg (*B. subtilis* PRPP synthetase), and 25.5 μg (*S. typhimurium* PRPP synthetase). Stock solutions of ATP, R5P, PEP, NADH, AK, PK, LDH, and PRPP synthetases were all prepared in 50 mM potassium phosphate buffer, pH 7.5. Except where stated differently, the final concentration of inorganic phosphate (P_i) was about 70 mM. To carry out the assay, the reaction mixture was first allowed to equilibrate at 37 °C for 3 min, and then the reaction was started by adding R5P. The assay reaction was performed at 37 °C and was monitored for 2 min using a Hewlett Packard Diode Array spectrophotometer model 8452A and Hewlett Packard 89531A MS-DOS UV–vis operating software.

Inhibition of PRPP synthetases by 8 and 19

Carbocyclic ribose-5-phosphate analogues **8** and **19** were examined as inhibitors of the PRPP synthetases mentioned above. For the inhibition experiments, the inhibitor was fixed at several concentrations, one substrate was kept at a saturating level, and the second substrate was varied systematically. In initial velocity experiments, the concentration of one substrate was fixed at different concentrations, while the concentration of the second substrate was varied systematically. Initial velocity experiments were performed primarily for the determination of K_m values for comparison with respective K_i values. Experimental data was processed as Lineweaver–Burk (double-reciprocal) functions, and data points were fit to plots using the least-square errors method. Kinetic constants were derived from secondary plots of the double reciprocal plots. The K_i for the inhibition of *Salmonella* PRPP synthetase by **8** was measured by both assay methods. A K_i was derived from the radioassay data using a Dixon plot, while a second value for the K_i was derived from the coupled assay data using a Lineweaver–Burk plot.

pH Optimum for 8 with *Salmonella* PRPP synthetase

The pH optima for ribose-5-phosphate and **8** with the *Salmonella* enzyme were determined by the radioactive assay method described above. The buffers used were 100 mM potassium phosphate at pH 6.2, 7.2, and 8.2, while 100 mM potassium phosphate containing 100 mM glycine was used at pH 9.2. In the actual assay mixtures, these buffers gave pH values of 6.0, 7.0, 8.0, and 9.0. The assay time was 15 min in each experiment.

Synthetic procedures

Synthesis of (+)-(1*S*,2*S*,3*R*,4*R*)-1,2,3-trihydroxy-4-cyclopentanemethanol-6-phosphate (8).

2,3-*O*-Isopropylidene-L-erythuronolactone (1). To a solution of 14.41 g (76.6 mmol) of 2,3-*O*-isopropylidene-D-ribonolactone[27] in 300 mL of H_2O was added 3.06 g (76.6 mmol) of NaOH as a solid. The resulting solution was heated to 40 °C for 0.5 h, cooled to 0 °C, and 17.53 g (82 mmol, 1.2 equiv) of $NaIO_4$ was added as a solid. The mixture was then stirred in the dark for 0.5 h, after which time 15 g of Amberlite IR-120 (H^+ form) resin was added with ice cooling to lower the pH to 3.0. The resin and aqueous mixture were then washed five times with 200 mL portions of EtOAc. The EtOAc layers were combined, washed with H_2O, and dried by evaporation to give 5.72 g (43%) of a white powder. This crude material was used in the next reaction. A pure sample was obtained by crystallization (EtOAc:hexane 1:1); mp 105–106 °C (lit. mp[28] 103–104 °C) MS (EI) *m/e* 124 (M^+); ^{1}H NMR (acetone-d_6): 5.75 (bd, 1 H, H-4), 4.96 (d, 1 H, H-2), 4.65 (d, 1 H, H-3), 3.10 (bs, 1 H, -OH), 1.39 and 1.36 ppm [2s, 6 H, $C(CH_3)_2$]; ^{13}C NMR (acetone-d_6, ppm): 114.4, 100.1, 81.4, 77.6, 75.6, 26.9, 25.7 ppm.

4-*O*-(2′-Tetrahydropyranyl)-2,3-*O*-isopropylidene-L-erythuronolactone (2). Dihydropyran (18 mL) was added to a solution of pyridinium *p*-toluenesulfonate (1.04 g, 4.1 mmol, 0.1 equiv) and 7.2 g (41.3 mmol) of crude **1** in 50 mL of dry CH_2Cl_2 at room temperature. The reaction mixture was stirred for 2.5 h, at which point TLC analysis (silica gel, hexane:EtOAc 85:15) showed that the reaction was complete. The reaction was quenched by the addition of 25 mL of saturated $NaHCO_3$, followed by dilution with H_2O and CH_2Cl_2. The CH_2Cl_2 layer was removed and the aqueous layer was reextracted with CH_2Cl_2. The organic layers were combined, washed with H_2O and brine, and dried by

evaporation to give 10.4 g (99%) of a yellow oil. The yellow oil was purified by column chromatography (silica gel, hexane:EtOAc 85:15) to give 9.91 g (93%) of a colorless oil: MS (positive CI with CH_4) *m/e* 259 $(M+1)^+$; ^1H NMR ($CDCl_3$): 5.82, 5.67 [2s, 1 H, $CH(O)_2$], 5.02 (m, 1 H, H-4), 4.85 (t, 1 H, H-2), 4.62 (dd, 1 H, H-3), 3.84, 3.62 (2m, 2 H, CH_2O), 1.79–1.51 [m, 6 H, $(CH_2)_3$], 1.47 and 1.39 ppm [2s, 6 H, $C(CH_3)_2$].

(−)-(2*R*,3*R*)-2,3-Dihydroxy-2,3-*O*-isopropylidene-4-cyclopenten-1-one (3). To a solution of 2.54 mL (23.5 mmol, 1.2 equiv) of dimethyl methylphosphonate in 100 mL of dry THF was added 15.2 mL (23.5 mmol) of 1.54 M Bu^{n}Li dropwise at −78 °C. The resulting mixture was stirred at −78 °C for 0.5 h, after which time 5.05 g (19.6 mmol) of lactone **2** in 40 mL of dry THF was added quickly through a canulla. The reaction was stirred at −78 °C for 2 h, and then allowed to warm to −20 °C over 0.5 h. The reaction mixture was quenched by the addition 200 mL of 5% aqueous NH_4Cl. The resulting mixture was extracted with 250 mL of EtOAc, and then with 250 mL of CH_2Cl_2. The organic layers were combined, dried and evaporated to give 2.18 g (72%) of crude yellow oil. Purification of the oil by column chromatography (silica gel, hexane:EtOAc, 85:15) gave 870 mg (29%) of a white solid: $[\alpha]_D$ −65.5° (*c* 1.02, $CHCl_3$), lit: $[\alpha]_D$ −70.7° (*c* 1, $CHCl_3$),[34] $[\alpha]_D$ −71.8° (*c* 0.925, $CHCl_3$);[52] mp 58–60 °C (lit. mp 66–67 °C);[34] ^1H NMR ($CDCl_3$): 7.60 (dd, 1 H, =C**H**CO), 6.22 (d, 1 H, C**H**=CHCO), 5.26 (dd, 1 H, H-2), 4.46 (d, 1 H, H-3), 1.42 ppm [s, 6 H, $C(CH_3)_2$]; ^{13}C NMR ($CDCl_3$): 202.8, 159.5, 134.0, 115.2, 78.4, 76.3, 27.2, 25.9 ppm.

(−)-(2*R*,3*R*,4*R*)-2,3-Dihydroxy-2,3-*O*-isopropylidene-4-hydroxymethylcyclopentan-1-one (4). A solution of 830 mg (5.39 mmol) of (−)-enone **3** and 160 mg of benzophenone in 350 mL of MeOH was degassed by flushing with argon for 1 h. The solution was then irradiated at >350 nm using a medium pressure Hanovia Hg lamp (450 watts) with a Pyrex filter for 1 h. The MeOH was evaporated to give 1.03 g (100%) of an orange oil. The reaction was repeated on the same scale and the crude product from both reactions combined and purified by column chromatography (Florisil, 10–100% EtOAc in hexane) to give 1.61 g (80%) of a colorless oil: $[\alpha]_D$ −148.9° (*c* 1, MeOH); ^1H NMR ($CDCl_3$): 4.72 (d, 1 H, H-2), 4.31 (d, 1 H, H-3), 3.89–3.68 (m, 2 H, CH_2OH), 2.78 (m, 1 H, H-5), 2.56 (m, 1 H, H-5), 2.20 (m, 1 H, H-4), 1.44 and 1.35 ppm [2s, 6 H, $C(CH_3)_2$]; ^{13}C NMR ($CDCl_3$): 214.2, 112.5, 81.3, 78.8, 63.9, 38.7, 37.0, 26.6, 24.5 ppm.

(−)-(1*S*,2*S*,3*R*,4*R*)-2,3-Dihydroxy-2,3-*O*-isopropylidene-4-hydroxymethylcyclopentan-1-ol (5). Glacial acetic acid (1.05 mL, 6 equiv) was added to a suspension of 232 mg (6.12 mmol, 2 equiv) of $NaBH_4$ in 500 mL of dry benzene at room temperature. The mixture was heated to reflux for 15 min and then cooled to 10 °C. A solution of 570 mg of ketone **4** in 20 mL of benzene was added. The reaction was allowed to warm to room temperature and then stirred for 48 h at which time TLC analysis (silica gel, EtOAc) showed the reaction was complete. The product was isolated by the addition of 3.0 mL of saturated NH_4Cl and 150 mL of H_2O followed by extraction twice with $CHCl_3$, and then once with EtOAc. The organic layers were combined, dried and evaporated to give 410 mg (71%) of an orange oil. A pure sample (120 mg, 21%) was obtained by crystallization from EtOAc:petroleum ether. Additional product was recovered from the mother liquors by chromatography (silica gel, ethyl acetate): $[\alpha]_D$ −18.4° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): 4.54 (m, 2 H, H-2, H-3), 4.14 (m, 1 H, H-1), 3.68–3.48 (m, 2 H, CH_2OH), 2.26 (m, 1 H, H-4), 1.80 (m, 2 H, H-5), 1.50 and 1.36 [2s, 6 H, $C(CH_3)_2$]; ^{13}C NMR ($CDCl_3$): 111.9, 82.6, 79.7, 71.3, 64.0, 44.39, 34.9, 26.2, 24.4 ppm.

(−)-(1*S*,2*S*,3*R*,4*S*)-2,3-Dihydroxy-2,3-*O*-isopropylidene-4-diphenylphosphoryloxymethylcyclopentan-1-ol (6). Diphenyl chlorophosphate (0.26 mL, 1.27 mmol, 1.2 equiv) was added to a solution of 200 mg (1.06 mmol) of diol **5** and 0.925 mL (5.3 mmol, 5 equiv) of diisopropylethylamine in 10 mL of dry CH_2Cl_2 at room temperature. The mixture was stirred for 48 h, 2 mg of DMAP was then added, and stirring was continued, with periodic monitoring by TLC (silica gel, EtOAc:hexane:MeOH 80:18:2). The product was isolated after a total of 72 h by addition of 1 mL of Et_3N followed by 25 mL of H_2O. The mixture was extracted twice with CH_2Cl_2, and the combined CH_2Cl_2 layers were washed with brine, dried, and evaporated to give 560 mg of a red oil. Column chromatography (silica gel, EtOAc:hexane 1:1, followed by EtOAc) gave 270 mg (60%) of **6** as a colorless oil: $[\alpha]_D$ −3.4° (*c* 0.72, $CHCl_3$); HRMS (positive CI with CH_4) *m/e* 421.1411, calcd for $C_{21}H_{26}O_7P$ $(M+H)^+$, 421.1416; ^1H NMR ($CDCl_3$): 7.39–7.18 (m, 10 H, ArH), 4.44–4.03 (m, 5 H, H-1, H-2, H-3, H-6), 2.46 (m, 1 H, H-4), 1.97–1.88 (m, 2 H, H-5), 1.49 and 1.30 ppm [2s, 6 H, $C(CH_3)_2$]; ^{13}C NMR ($CDCl_3$): 150.4, 129.8, 125.4, 119.9, 112.1, 81.6, 79.6, 70.9, 69.7 (d, J_{CP}=5.6 Hz), 42.5 (d, J_{CP}=6.9 Hz), 34.6, 26.0, 24.2 ppm; ^{31}P NMR ($CDCl_3$) −14.2 ppm.

(+)-(1*S*,2*S*,3*R*,4*S*)- 2,3-Dihydroxy-4-diphenylphosphoryloxymethylcyclopentan-1-ol (7). A solution of 90 mg (0.214 mmol) of hydroxy phosphate **6** in 1.8 mL of THF was cooled to 0 °C and treated with 1.1 mL of 1 N HCl. The mixture was allowed to warm to room temperature and stirred for 21 h, at which point TLC analysis (silica gel, EtOAc) showed that the reaction was complete. The reaction was quenched by the addition of AG-1-X8 (—OH form) anion exchange resin until the mixture was neutral. The resin was removed by filtration and thoroughly washed with THF and deionized H_2O. The filtrate was evaporated to give 94 mg of a colorless gum. Column chromatography (silica gel, EtOAc) of the crude product gave 80.1 mg (99%) of **7** as a colorless gum: $[\alpha]_D$ +21.5° (*c* 1, MeOH); HRMS (positive CI with CH_4) *m/e* 381.1108, calcd for $C_{18}H_{22}O_7P$ $(M+H)^+$ 381.1103; ^1H NMR ($CDCl_3$): 7.37–7.14 (m, 10 H, ArH), 4.26 (m, 2 H, H-6), 4.00 (m, 1 H, H-1), 3.72 (m, 2 H, H-2, H-3), 3.60

(bs, 3 H, —OH), 2.48 (m, 1 H, H-4), 1.89 (m, 1 H, H-5), 1.52 (m, 1 H, H-5); ^{13}C NMR (CD_3OD): 150.3, 129.9, 125.5, 120.0, 73.3, 71.8, 70.3, 44.4, 32.1 ppm; ^{31}P NMR (CD_3OD): −13.9 ppm.

Disodium (+)-(1*S*,2*S*,3*R*,4*R*)-1,2,3-trihydroxy-4-cyclopentanemethanol-6-phosphate (8). A mixture of 170 mg (0.45 mmol) of phosphate **7** and 80 mg of PtO_2 in 40 mL of absolute EtOH was hydrogenated at 40 psi H_2 for 18 h. The mixture was filtered through Celite and evaporated to give 130 mg of a yellow oil. The sample was dissolved in 5 mL of deionized H_2O with the aid of a small amount of Amberlite IR-120 (plus) (Na^+ form) ion exchange resin. The resulting clear solution was then passed through a column (0.5 × 10 cm) of the same resin followed by 50 mL of deionized H_2O. The eluent was evaporated to give 122 mg (100%) of **8** as a white foam: $[\alpha]_D$ +25.1° (*c* 1, MeOH); MS (ES) *m/e* 229 $(M+1)^+$, 251 $(M+Na)^+$, 273 $(M+2Na)^+$; ^{1}H NMR (D_2O): 3.96 (m, 1 H, H-1), 3.77–3.65 (m, 4 H, H-2, H-3, H-6), 2.18 (m, 1 H, H-4), 1.75–1.57 (m, 2 H, H-5) ppm; ^{13}C NMR (D_2O): 74.6, 73.4, 71.3, 67.0, 43.6, 31.6 ppm; ^{31}P NMR (D_2O) +0.9 ppm.

Synthesis of (+)-(1*S*,4*R*,5*S*)-1,4,5-trihydroxy-3-cyclopent-2-enemethanol-6-phosphate (19). *p*-Methoxybenzyl methylthiomethyl ether (**9**). A 2.15 g (89.8 mmol, 2 equiv) portion of 50% NaH in oil dispersion was weighed into a dried flask and washed under argon with 50 mL of dry petroleum ether. The resulting solid was resuspended in 50 mL of 1,2-dimethoxyethane and 5.6 mL (44.9 mmol) of 4-methoxybenzyl alcohol was added dropwise at 0 °C. The still heterogeneous suspension was stirred at 0 °C for 45 min and 6.72 g (44.9 mmol, 1 equiv) of NaI was then added as a solid followed by 3.75 mL (44.9 mmol, 1 equiv) of chloromethyl methyl sulfide. The reaction mixture was stirred at 0 °C for 1 h and allowed to warm to room temperature over the next 4 h. The reaction mixture was quenched by addition of 100 mL of H_2O, and it was then extracted twice with 200 mL portions of ether. The ether layers were combined, washed with brine, dried, and evaporated to give 16.95 g of a yellow oil. Purification of the crude product by column chromatography (silica, hexane:EtOAc, 95:5, followed by hexane:EtOAc 90:10) gave 4.24 g (48%) of **9** as a colorless oil: ^{1}H NMR ($CDCl_3$): 7.28 (d, 2 H, ArH), 6.88 (d, 2 H, ArH), 4.66 (s, 2 H, $PhCH_2$), 4.55 (s, 2 H, SCH_2), 3.81 (s, 3 H, OCH_3), 2.18 ppm (s, 3 H, SCH_3).

(*p*-Methoxybenzyloxymethyl)tri-*n*-butylstannane (10). To a solution of 4.24 g (21.4 mmol) of thioether **9** in 35 mL of dry CH_2Cl_2 at −78 °C was added dropwise over 15 min a solution of 1.72 mL (21.4 mmol, 1 equiv) of SO_2Cl_2 in 15 mL of dry CH_2Cl_2. The resulting orange solution was stirred at this temperature for 30 min, after which time the solvents were evaporated to give 4.03 g (100%) of the crude chloromethyl ether as a brown oil: ^{1}H NMR ($CDCl_3$): 7.28 (d, 2 H, ArH), 6.90 (d, 2 H, ArH), 5.50 (s, 2 H, CH_2Cl), 4.68 (s, 2 H, $PhCH_2$), 3.81 ppm (s, 3 H, OCH_3). The crude product was used directly in the next reaction.

Tri-*n*-butyltin hydride (5.72 mL, 21.3 mmol) was added dropwise to a solution of 1.2 equiv (30.6 mmol) of LDA in 50 mL of dry THF at 0 °C. The yellow solution was stirred for 20 min, and 4.0 g (25.5 mmol) of the above chloromethyl ether was then added as a solution in dry THF (20 mL). The reaction was stirred for 1 h 45 min at 0 °C and poured into 350 mL of EtOAc. This solution was diluted with 250 mL of H_2O and the organic layer was removed. The aqueous portion was reextracted with 150 mL of EtOAc and the combined organic layers were washed with brine, dried, and evaporated to give 10.07 g of a brown oil. Purification of the crude product by column chromatography (silica, hexane:EtOAc 97:3) then gave 7.14 g (76%) of **10** as a colorless oil: ^{1}H NMR ($CDCl_3$): 7.25 (d, 2 H, ArH), 6.86 (d, 2 H, ArH), 4.34 (s, 2 H, $PhCH_2$), 3.80 (s, 3 H, OCH_3), 3.71 (s, 2 H, $SnCH_2$), 1.57–0.80 ppm (m, 27 H, $SnBu_3$).

(+)-(1*S*,4*S*,5*S*)-4,5-Dihydroxy-4,5-*O*-isopropylidene-1-(*p*-methoxybenzyloxy)methylcyclopent-2-en-1-ol (12). To a solution of 1.53 g of the stannane **10** (3.47 mmol) in 20 mL of dry THF was added 2.2 mL (3.33 mmol, 0.96 equiv) of 1.54 M Bu^nLi at −78 °C. The resulting greenish-yellow solution was stirred at −78 °C for 5 min and a solution of 534 mg (3.47 mmol) of the (+)-enone **11**[34] in 10 mL of THF was added. The resulting solution was stirred at −78 °C for 15 min. The reaction was then quenched by the addition of 20 mL of saturated NH_4Cl, and extracted twice with EtOAc. The combined organic layers were washed with H_2O, brine, and then dried and evaporated to give 2.17 g of a yellow oil. Chromatography of the oil (silica, hexane:EtOAc 85:15) yielded 860 mg (81%) of **12** as a colorless oil: $[\alpha]_D$ +68.7° (*c* 1.2, CH_2Cl_2); MS (positive CI with CH_4) gave $[M-H]^+$ at 305; ^{1}H NMR ($CDCl_3$): 7.22 (d, 2 H, ArH), 6.87 (d, 2 H, ArH), 5.89 and 5.73 (2d, 2 H, H-2 and H-3), 5.01 (d, 1 H, H-4), 4.41 (m, 3 H, H-5 and $PhCH_2$), 3.78 (s, 3 H, OCH_3), 3.54–3.38 (q, 2 H, H-6), 3.24 (s, 1 H, OH), 1.43 and 1.38 ppm [2s, 6 H, $C(CH_3)_2$]; ^{13}C NMR ($CDCl_3$): 159.0, 136.8, 132.7, 129.8, 129.1, 113.6, 112.3, 83.7, 81.5, 80.3, 73.4, 73.0, 55.0, 27.5, 26.4 ppm.

(+)-(1*S*,4*S*,5*S*)-4,5-Dihydroxy-4,5-*O*-isopropylidene-1-(*p*-methoxybenzyloxy)methylcyclopent-2-en-1-ol acetate (13). To a solution of 860 mg (2.81 mmol) of the tertiary alcohol **12**, 7.8 mL (20 equiv) of Et_3N and 17 mg (0.05 equiv) of DMAP in 15 mL of CH_2Cl_2 was added 5.3 mL (20 equiv) of acetic anhydride at room temperature. The solution was stirred for 48 h at which point TLC analysis (silica, hexane:EtOAc 7:3) showed that the reaction was complete. The solvents were evaporated and the residue was purified by column chromatography on silica gel (hexane:EtOAc 90:10, followed by hexane:EtOAc 80:20) to give 940 mg (96%) of **13** as a colorless oil; $[\alpha]_D$ +94.2° (*c* 1, CH_2Cl_2); MS (negative CI with NH_3) gave $[M-1]^-$ at 347; ^{1}H NMR ($CDCl_3$): 7.18 (d, 2 H, ArH), 6.86 (d, 2

H, ArH), 6.00 (s, 1 H, H-2, H-3), 5.01 (d, 1 H, H-4), 4.75 (d, 1 H, H-5), 4.43 (s, 2 H, $PhCH_2$), 3.84–3.73 (m, 5 H, OCH_3, H-6), 2.06 (s, 3 H, $OCOCH_3$), 1.37 and 1.36 ppm [2 s, 6 H, $C(CH_3)_2$]; ^{13}C NMR ($CDCl_3$): 170.0, 159.2, 134.2, 133.5, 129.8, 129.1, 113.7, 112.0, 88.8, 83.8, 80.7, 73.1, 71.0, 55.2, 27.6, 27.0, 21.6 ppm.

(−)-(1*S*,4*R*,5*S*)-4,5-Dihydroxy-4,5-*O*-isopropylidene-3-(*p*-methoxybenzyloxy)methylcyclopent-2-en-1-ol acetate (14). A solution containing 875 mg (2.51 mmol) of acetate **13**, 136 mg (0.5 equiv) of benzoquinone and 65 mg (0.10 equiv) of $Pd(CH_3CN)_2Cl_2$ in 50 mL of dry THF was refluxed for 3.5 h until TLC analysis (silica, hexane:EtOAc 85:15) showed that the reaction was complete. The cooled reaction mixture was evaporated and the residue was dissolved in EtOAc. The resulting solution was washed with H_2O and brine, then dried, and evaporated to give 930 mg of a brown oil. Purification by column chromatography (silica, hexane:EtOAc 80:20) gave 410 mg (64%) of **14** as a colorless oil; $[\alpha]_D$ −31.9° (*c* 1.2, CH_2Cl_2); MS (positive CI with CH_4) showed $[M\text{-}H]^+$ at 347; ^1H NMR ($CDCl_3$): 7.27 (d, 2 H, ArH), 6.88 (d, 2 H, ArH), 5.78 (s, 1 H, H-2), 5.36 (m, 1 H, H-1), 4.92 (m, 2 H, H-4, H-5), 4.52 (s, 2 H, $PhCH_2$), 4.17 (m, 2 H, H-6), 3.81 (s, 3 H, OCH_3), 2.11 (s, 3 H, $OCOCH_3$), 1.39 and 1.37 ppm [2s, 6 H, $C(CH_3)_2$]; ^{13}C NMR ($CDCl_3$): 170.5, 159.2, 145.7, 129.9, 129.3, 126.3, 113.8, 112.8, 82.8, 77.2, 75.2, 72.6, 66.1, 55.2, 27.2, 26.6, 20.8 ppm.

(−)-(1*S*,4*R*,5*S*)-4,5-Dihydroxy-4,5-*O*-isopropylidene-3-hydroxymethylcyclopent-2-en-1-ol acetate (15). DDQ (391 mg, 1.72 mmol, 1.5 equiv) was added to a solution of 400 mg (1.15 mmol) of benzyl ether **14** in 25 mL of CH_2Cl_2:H_2O 20:1 at 0 °C. The dark-green solution with a pink precipitate was allowed to warm to room temperature, whereupon TLC analysis (silica, hexane:EtOAc 1:1) showed that the reaction was complete after 1.5 h. Work up was accomplished by the addition of 25 mL of saturated $NaHCO_3$ followed by extraction twice with CH_2Cl_2. The organic layers were combined, washed successively with H_2O and brine, dried, and evaporated to give 320 mg of the crude product. Purification by column chromatography (silica, hexane:EtOAc 60:40) gave 170 mg (65%) of **15** as a colorless oil: $[\alpha]_D$ −55.5° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$): 5.75 (d, 1 H, H-2), 5.37 (m, 1 H, H-1), 4.95 (m, 2 H, H-4, H-5), 4.37 (m, 2 H, H-6), 2.12 (s, 3 H, $OCOCH_3$), 1.42 and 1.38 ppm [2s, 6 H, $C(CH_3)_2$]; ^{13}C NMR ($CDCl_3$): 170.6, 147.8, 125.1, 112.8, 82.7, 77.2, 75.0, 59.6, 27.1, 26.4, 20.7 ppm.

Bis(2-trimethylsilylethyl)-*N*,*N*-diethylphosphoramidite (16). Diethylamine (20.65 mL, 0.2 mol, 2 equiv) was added dropwise to a solution containing 8.74 mL (0.1 mol) of freshly distilled PCl_3 in 60 mL of dry THF at <0 °C (dry ice/acetone). The reaction was allowed to warm to room temperature over 4 h. The heterogenous mixture was filtered through a pre-dried sintered glass funnel and the filter cake was washed twice with 50 mL of dry THF. The solvent was evaporated and the crude intermediate was fractionally distilled to give 12.4 g (71%) of diethylphosphoramidous dichloride bp 48–50 °C (5 mm Hg) (lit. bp 62 °C at 8 mm Hg).[36] A solution of 6.06 mL (42 mmol, 2 equiv) of 2-trimethylsilylethanol and 6.5 mL (46.4 mmol, 2.2 cquiv) of Et_3N in 15 mL of dry THF at 0 °C was added to a solution of 3.68 g (21.1 mmol) of diethyl phosphoramidous dichloride in 10 mL of THF. The resulting heterogenous mixture was allowed to warm to room temperature and then stirred for 3.5 h. The reaction was worked up by the addition of 50 mL of 5% $NaHCO_3$ and 100 mL of EtOAc. The organic layer was removed and washed twice with 5% $NaHCO_3$, brine, then dried, and evaporated to give 6.72 g (97%) of crude **16.** Vacuum distillation gave 4.30 g (62%) of **16** as a colorless oil, bp 119–122 °C (3 mm Hg). The compound was stable for at least 6 months at freezer temperature: ^1H NMR ($CDCl_3$): 4.15 (q, 1 H, CH_2O), 3.83 (q, 1 H, CH_2O), 3.64 (m, 2 H, CH_2O), 3.00 (m, 4 H, CH_2N), 0.95 (m, 10 H, $SiCH_2$ and CH_3), −0.50 ppm (d, 18 H, $SiCH_3$); ^{13}C NMR ($CDCl_3$): 64.2, 60.4, 37.2, 20.01, 15.0, −1.47 ppm; ^{31}P NMR ($CDCl_3$): +142.6 ppm.

(1*S*,4*R*,5*S*)-4,5-Dihydroxy-4,5-*O*-isopropylidene-3-bis(2-trimethylsilylethyl) phosphoryloxymethylcyclopent-2-en-1-ol acetate (17). To a solution of 125 mg (0.55 mmol) of alcohol **15** and 198 mg (0.60 mmol, 1.1 equiv) of the phosphoramidous ester **16** in 4 mL of dry THF was added at room temperature 116 mg (1.65 mmol, 3 equiv) of 1*H*-tetrazole. The resulting slightly yellow solution was stirred for 1 h and the temperature was then lowered to −50 °C in a dry ice/acetone bath. A solution containing 126 mg (0.73 mmol, 1.33 equiv) of 85% *m*-chloroperbenzoic acid in 8 mL of dry CH_2Cl_2 was added. The resulting mixture was allowed to warm to room temperature over 10 min, and then stirred for an additional 10 min before the addition of 50 mL of 10% $NaHSO_3$. The resulting mixture was stirred vigorously for 10 min, and then extracted twice with 50 mL of ether. The ether layers were combined, washed twice with 20 mL of 10% $NaHSO_3$, and then twice with 20 mL of 5% $NaHCO_3$ before being dried and evaporated to give 480 mg of **17** as a yellow oil. Purification by column chromatography (silica, $CHCl_3$:MeOH 99:1) gave 236 mg (85%) of **17** as a colorless oil: MS (positive CI with CH_4) gave $[M+1]^+$ at 509: ^1H NMR ($CDCl_3$): 5.82 (m, 1 H, H-2), 5.40 (m, 1 H, H-1), 5.37 (m, 1 H, H-4), 4.95 (m, 1 H, H-5), 4.71 (m, 2 H, H-6), 4.17 (m, 4 H, CH_2OP), 2.10 (s, 3 H, $OCOCH_3$), 1.39 and 1.37 [2s, 6 H, $C(CH_3)_2$], 1.06 (m, 4 H, CH_2Si), 0.04 ppm (s, 18 H, $SiCH_3$); ^{13}C NMR ($CDCl_3$): 171.2, 144.1, 126.8, 113.5, 83.0, 77.4, 75.1, 66.5, 63.3, 27.3, 26.7, 20.6, 19.5, −1.5 ppm; ^{31}P NMR ($CDCl_3$) −3.6 ppm.

(1*S*,4*R*,5*S*)-4,5-Dihydroxy-4,5-*O*-isopropylidene-3-bis(2-trimethylsilylethyl) phosphoryloxymethylcyclopent-2-en-1-ol (18). A solution of 48.4 mg (0.095 mmol) of acetate **17** in 3 mL of NH_3 saturated MeOH was stirred for 7 h and the solvents were then evaporated to give 44 mg of **18** (100%) as a colorless oil: ^1H NMR ($CDCl_3$): 5.82 (s, 1 H, H-2), 4.93 (d, 1 H, H-4), 4.74 (t, 1 H, H-5), 4.64–4.54 (m, 3 H, H-1, H-6), 4.12 (m, 4 H, CH_2OP), 1.38 and 1.35 [2s, 6 H, $C(CH_3)_2$], 1.06 (m, 4

H, CH_2Si), −0.03 ppm (s, 18 H, $SiCH_3$); ^{13}C NMR ($CDCl_3$): 140.7, 133.1, 112.7, 82.4, 77.9, 73.2, 66.3, 63.0, 27.6, 26.6, 19.5, −1.2 ppm; ^{31}P NMR ($CDCl_3$): −3.6 ppm.

Disodium (+)-(1*S*,4*R*,5*S*)-1,4,5-trihydroxy-3-cyclopent-2-enemethanol-6-phosphate (19). A solution of 44 mg (0.094 mmol) of alcohol **18** in 9.4 mL (10 mM) of $CH_3CN:H_2O$, 48% HF, 85:15:5, was stirred for 5 h at room temperature. Most of the solvents were evaporated (ca. 0.50 mL remained) and 2 mL of methoxytrimethylsilane was then added. The mixture was then evaporated to dryness. This addition–evaporation process was repeated with an additional 2 mL of methoxytrimethylsilane. The resulting white solid was dissolved in 5 mL of H_2O with the aid of ca. 2 g of washed Amberlite IR-120 (plus), Na^+ form, cation exchange resin. The solution was then passed through a column (0.5 × 10 cm) of the same resin. The column was washed with 50 mL of H_2O and the eluant was evaporated to give 21 mg (ca. 100%) of **19** as a white solid: $[\alpha]_D$ +10.1° (*c* 1.1, H_2O); MS (ES) 271 (diNa^+ salt); ^1H NMR (D_2O): 6.02 (bs, 1 H, H-2), 4.35 (m, 4 H, H-4, H-5, H-6), 4.06 ppm (m, 1 H, H-1); ^{13}C NMR (D_2O): 143.7, 130.6, 73.0 (2C), 72.2, 63.1 ppm; ^{31}P NMR (D_2O) +1.6 ppm.

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