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Synthesis of (+)-(1*S*)-1-Pyrophosphoryl-(2*R*, 3*R*)-2,3-dihydroxy-(4*S*)-4-(phosphoryloxymethyl)cyclopentane, a Stable, Optically-Active Carbocyclic Analog of 5-Phosphoribosyl-1-pyrophosphate (PRPP)

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Abstract: A total synthesis of the cyclopentyl analog of the important biochemical intermediate 5-phosphoribosyl-1-pyrophosphate (PRPP) is reported. The synthesis proceeds from the benzylidene acetal of D-ribonolactone to the correct enantiomeric form of the cyclopentyl PRPP analog in ca. 4% overall yield. Because of the low reactivity of the carbocyclic analog compared to PRPP, the compound should be a highly useful tool for mechanistic and crystallographic investigations of the phosphoribosyltransferases, a family of enzymes that utilizes PRPP as a substrate.

The phosphoribosyltransferases are a group of enzymes that play a key role in the biosynthesis of pyridine, pyrimidine, and purine nucleosides as well as in the biosynthesis of the aromatic amino acids histidine and tryptophan.[1] The reaction catalyzed by these enzymes involves a formal S_N2 displacement of the pyrophosphate group of α-D-5-phosphoribosyl-1-pyrophosphate (PRPP, **1**, Scheme I) by a second substrate that contains nitrogen. The mechanism of these reactions has been postulated to involve an S_N1-like process,[1] but recent studies of orotate phosphoribosyltransferase suggest that a more associative transition-state may be involved.[2] Because of the essential role played by the phosphoribosyltransferases, mechanistic studies of these enzymes are currently a field of great interest.[2-8] An additional motivation for these studies is provided by the fact that inhibitors of some of these enzymes possess antiparasitic or anticancer activity.[9-11]

In 1993, we reported the first total synthesis of the carbocyclic analog **2** of PRPP in racemic form.[12a] The analog **2** was expected to be much less reactive than PRPP since no oxygen atom is present to stabilize the carbonium ion generated by departure of the pyrophosphate group. As a consequence, it appeared that **2** would be a useful tool for mechanistic and inhibitory studies of many of the phosphoribosyltransferases as well as providing a stable PRPP analog for X-ray crystallographic studies. Investigations of the behavior of racemic **2** with glutamine phosphoribosylpyrophosphate amidotransferase have since borne out the assumption that this analog can be a useful mechanistic tool.[13] Unfortunately, the original synthesis of **2** possessed two major deficiencies: it led to the racemic form of the compound, and the overall yield of **2** was very low. Furthermore, the synthesis of optically active **2** by this route appeared to be impractical because of the additional number of steps involved.[12a]

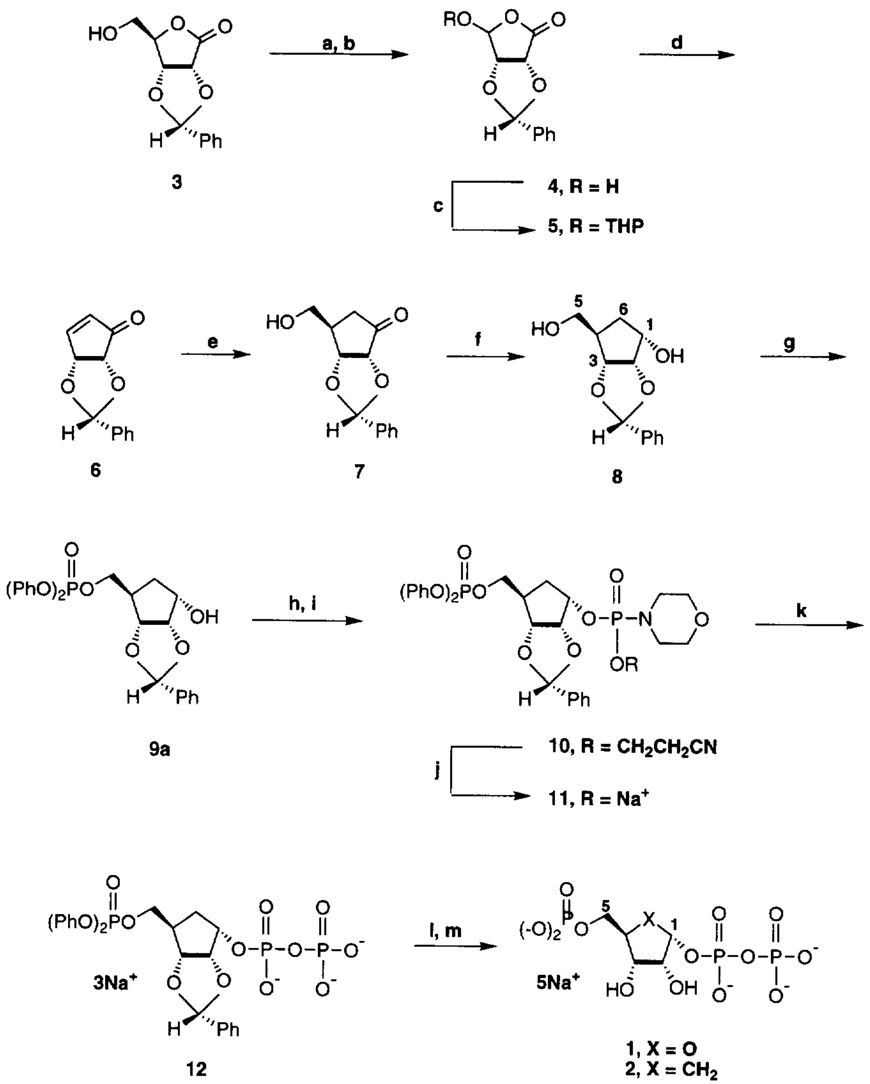
We would now like to report a new synthesis of the carbocyclic PRPP analog **2** that overcomes these deficiencies since it provides the compound in optically active form and proceeds in significantly higher overall yield. The synthesis should allow a more widespread use of this interesting PRPP analog for mechanistic and crystallographic studies of the phosphoribosyltransferase enzymes.[14]

RESULTS AND DISCUSSION

An important issue in devising a synthesis of the carbocyclic PRPP analog **2** was the need for protecting groups that can be removed under very mild conditions. The use of acidic conditions for removal of a diol protecting group was precluded due to the acid sensitivity of the pyrophosphate functionality.[15] The benzylidene acetal moiety, which can be removed by catalytic hydrogenolysis, was therefore exploited as a diol protecting group. Although the increased acid-sensitivity of this protecting group caused some difficulties due to the ease with which the group rearranged from the 2,3- to the 1,2-isomer in intermediates bearing a free C-1 hydroxyl group (see below), this was offset by the ease with which this group could be removed in the final stages of the synthesis. The benzylidene protecting group was introduced at the beginning of the synthesis by conversion of D-ribonic-γ-lactone into its benzylidene acetal **3** by the method of Chittenden and Regeling.[16] As previously reported, this method gave the desired 2',3'-*O*-benzylidene derivative as the endo stereoisomer. The presence of a single stereoisomer at the acetal carbon atom was confirmed by the presence of only one benzylidene signal in the ^{1}H NMR spectra of **3** and of all the subsequent intermediates.[17]

The synthesis of the chiral enone **6** utilized a carbocyclization reaction reported by Borchardt et. al.[18] and used by many others.[19] We devised a modified procedure to produce the substrate for this reaction. The lactone function of **3** was hydrolyzed with aqueous NaOH to expose a diol moiety which was then oxidatively cleaved in the same pot with $NaIO_4$ to give the desired cyclized lactol/lactone **4** in 81% yield. The protection of the lactol functionality of **4** as an isopropyl ether under acid-catalyzed conditions[19] was found to be problematic, presumably because of the difficulty of generating a carbonium ion at the lactol carbon atom. To circumvent this problem, the hydroxyl group of the lactol was converted to its THP ether under acidic conditions. This reaction proved to be far more efficient than isopropyl ether formation, and produced the desired THP ether **5** in 94% yield. The carbocyclization reaction leading from **5** to **6** was found to be somewhat capricious and very sensitive to temperature. The best conditions involved generation of the lithium salt of dimethyl methyl phosphonate at -78° C, followed by addition of a THF solution of the THP ether **5** precooled to -78° C. After 45 min at the same temperature, the reaction was quenched at -78° C. Under these conditions, the desired enone **6** could be obtained in 33% yield. While this yield is low, it is comparable to that reported by others for similar reactions.[19] Compound **6** proved to be highly susceptible to acidic conditions and decomposed upon attempted silica gel chromatography. Purification was accomplished by chromatography on Florisil. The photochemical 1,4-addition of methanol to the convex face of enone **6** proceeded in a completely stereospecific fashion to give

Scheme I[a]



[a] (a) NaOH, 45° C; (b) $NaIO_4$, 81%; (c) dihydropyran, PPTS, CH_2Cl_2, 94%; (d) n-BuLi, $(CH_3O)_2P(O)CH_3$, THF, -78° C, 33%; (e) CH_3OH, benzophenone, hν, 55%; (f) $NaBH(OAc)_3$, PhH, 94%; (g) $(PhO)_2POCl$, $(iPr)_2NEt$, DMAP, CH_2Cl_2, 67%; (h) 13, *1H*-tetrazole, THF; (i) mCPBA, 78%; (j) NH_3, CH_3OH; Amberlite IR-120 Plus (Na^+), 100%; (k) $(n\text{-}Bu)_3NH^+$ $H_2PO_4^-$, C_5H_5N; (l) $Pd(OH)_2$, H_2, C_2H_5OH, H_2O; (m) PtO_2, H_2, C_2H_5OH, H_2O; Amberlite IR-120 Plus (Na^+), 61%.

the hydroxy ketone **7** in 55% yield.[12a,20,21] The use of substrate-guided chelation-controlled hydride reduction of **7** with $NaBH(OAc)_3$ then produced the diol **8** with the desired configuration at C-1.[12a,20,22,23] The diol **8** could be obtained in 94% yield after silica gel chromatography provided that triethylamine was added to the chromatographic solvents. In the absence of triethylamine, **8** tended to rearrange to the corresponding 1,2-*O*-benzylidene isomer. Selective phosphorylation of **8** at the primary hydroxyl group was accomplished by using diphenyl chlorophosphate in the presence of the hindered base diisopropylethylamine.[12a,20] After chromatography on Florisil, the desired phosphate ester **9a** was obtained in 67% yield. Extreme care was necessary during the isolation and purification of this compound due to the alacrity with which it rearranged to the 1,2-*O*-benzylidene isomer **9b**.

The introduction of the pyrophosphate moiety at the C-1 hydroxyl group of **9a** was facilitated by the novel use of a di-activated bis(morpholino)phosphite **13**.[24] The earlier synthesis of racemic **2** utilized 2,2,2-tribromoethyl phosphoromorpholinochloridate to functionalize the C-1 hydroxyl group of racemic **9a**.[12a,b] Because of the hindered nature of the C-1 hydroxyl group, this reaction proceeded in poor yield (35%), and required a period of four days for completion. In contrast, the *1H*-tetrazole mediated reaction of **9a** with **13** was complete after 3 h. In situ oxidation of the resulting phosphite with mCPBA then led to the desired phosphoromorpholidate **10** with an overall yield of 78%. Other advantages to this approach included the ease of purification of compound **10** by silica gel chromatography, and the simplicity with which it was converted into the phosphoromorpholidate **11**: deprotection of **10** with methanolic ammonia, followed by conversion of the resulting ammonium salt to the Na^+ salt by passage through a column of Amberlite IR-120 (Na^+ form), gave **11** in quantitative yield.

Treatment of **11** with an excess of the monotributylammonium salt of phosphoric acid in pyridine gave the desired pyrophosphate **12**.[25,26] Attempts to remove excess phosphate ion from **12** by chromatography on DEAE-Sephadex A-25 failed to give high yields. Side-products isolated after chromatography suggested that **12** undergoes partial decomposition under these conditions. Therefore **12** was deprotected in its unpurified state.

Initial attempts to deprotect **12** made use of PtO_2 and hydrogen,[27] since these conditions had previously been employed in the synthesis of racemic **2**.[12a] However, the deprotection of **12** in this manner resulted in the formation of aliphatic impurities which were evident in the ^1H NMR spectrum of the crude, deprotected product. These impurities could not be removed by extraction with non-polar organic solvents. Similar impurities had previously been encountered at this stage in the synthesis of racemic **2**, and were largely responsible for the low yield of the final deprotection step (23%).[12a] It was hypothesized that the PtO_2-catalyzed hydrogenation conditions probably led to some reduction of the benzene ring of the benzylidene acetal to produce a mixture of 2-*O* and 3-*O*-cyclohexylmethyl ethers and/or a 2,3-*O*-cyclohexylmethylidene acetal. This possibility is consistent with the ability of PtO_2 to catalyze the reduction of aromatic rings.[28] We therefore resorted to a two-step catalytic deprotection sequence to obtain **2** from **12** in higher yield.

$Pd(OH)_2$ was chosen as the initial catalyst since this compound is known to be selective for benzyl ethers.[29] Treatment of **12** with $Pd(OH)_2$ and hydrogen for 5 h at 1 atm pressure accomplished the complete removal of the acetal moiety and partial removal of one of the phenyl groups of the phosphate ester as judged by ^1H and ^{31}P NMR analysis. The partially deprotected product was then treated with PtO_2 and hydrogen to effect complete deprotection. Purification of the crude, deprotected product by chromatography over DEAE-Sephadex

A-25 using a gradient of triethylammonium bicarbonate then gave pure **2** as its triethylammonium salt. Product-containing fractions from the chromatography were most easily located by use of a phosphate ester detection reagent.[30] Attempts to convert **2** into the corresponding sodium salt using the method of Moffatt[25] were not entirely satisfactory as the procedure led to a mixture of sodium and triethylammonium salts. The triethylammonium salt was therefore converted to its Na^+ salt by passage through a column of Amberlite IR-120 (Na^+ form) cation exchange resin to give a 61% yield of **2** (based upon **11**). The final product was characterized by negative ion electrospray mass spectrometry and by proton, carbon-13, COSY, HETCOR, and phosphorus-31 NMR spectrometry. The mass spectral data and NMR spectra for the final product were in full agreement with the assigned structure. However, the NMR spectra indicated that small amounts of triethylammonium ions were still present.[31]

In summary, a carbocyclic analog of α-D-5-phosphoribosyl-1-pyrophosphate (PRPP) has been synthesized in the correct enantiomeric form by a route that should allow the preparation of sufficient quantities of the compound for mechanistic and structural investigations of the phosphoribosyltransferases. Investigations of the behavior of this analog with several phosphoribosyltransferases are in progress.

EXPERIMENTAL

Materials and Methods. Specialized organic reagents were purchased from Aldrich Chemical Co. and used without further purification unless otherwise indicated. Analytical thin-layer chromatography was carried out on Merck type 60 F-254 glass plates. Column chromatography utilized 230-400 mesh type 60A silica gel and 200 mesh Florisil purchased from Aldrich Chemical Co. Amberlite IR-120 (plus) ion-exchange resin was purchased from Aldrich, while AG 1 x 8 anion exchange resin was obtained from Bio-Rad. D-(+)-Ribonic-γ-lactone was obtained from Acros Organics. Proton, carbon-13, and phosphorus-31 NMR spectra were measured on a Bruker AC250 NMR spectrometer operating at 250 MHz, 62.89 MHz, and 101.25 MHz, respectively. Chemical shifts for protons are given in parts per million (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 carbon-13 are given in parts per million downfield from TMS for spectra measured in $CDCl_3$ and in ppm downfield from TSP for spectra taken in D_2O. Phosphorus-31 chemical shifts are given in parts per million 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. The ionization modes used for mass spectral analysis were: electron impact (EI), chemical ionization (CI), fast atom bombardment (FAB), and electrospray (ES). All melting points were measured on a Fisher-Johns melting point apparatus and are uncorrected.

(2*R*, 3*S*)-*O*-Benzylidene L-erythuronolactone (4). A mixture of 23.6 g (0.1 mol) of (2*R*, 3*R*)-*O*-benzylideneribonolactone (**3**)[16] in 600 mL of H_2O was treated with 4 g of NaOH and heated to 45° C for 30 min. After 5 min a clear solution had formed. The reaction solution was cooled to 0° C and 25.7 g (0.12 mol) of $NaIO_4$ was added as a powder while protecting the reaction mixture from light. The heterogeneous mixture was stirred at 0° C for 45 min, and the pH of the solution was then adjusted to 3 by addition of prewashed Amberlite IR-120 PLUS (H^+ form) resin at 0° C. The clear reaction solution was extracted with EtOAc (2 x 500 mL). The aqueous layer was saturated with NaCl and reextracted once with EtOAc (500 mL) and then with

$CHCl_3$ (500 mL). The combined organic layers were washed with H_2O and brine, dried and evaporated to give 17.9 g (81%) white solid. This crude product was used directly in the next step. An analytical sample was obtained by recrystallization from 2:1 $CHCl_3$-hexane. **4:** m.p. 109-111° C; $[\alpha]^{25}_D$ -9.7° (c=1, MeOH); ^1H NMR ($CDCl_3$) δ 4.32 (bs, 1 H), 4.76 (d, 1 H), 5.00 (2s, 1 H), 5.90 (s, 1 H), 6.03 (s, 1 H), 7.41 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 75.3, 80.6, 99.4, 107.6, 126.7, 128.8, 130.2, 134.7, 173.2. HRMS (EI) calcd for $C_{11}H_{10}O_5$ 222.0528, found 222.0518. Anal. Calcd for $C_{11}H_{10}O_5 \cdot 0.33\ H_2O$, C, 57.91; H, 4.71. Found: C, 57.84; H, 4.60.

(2*R*, 3*S*)-*O*-Benzylidene-4-*O*-tetrahydropyranyl-L-erythuronolactone (5). To the clear solution of 17.7 g (79.6 mmol) of **4** and 8.72 mL (95.5 mmol) of dihydropyran in 625 mL of dry CH_2Cl_2 was added 2.0 g (7.96 mmol) of pyridinium p-toluenesulfonate as a solid. The reaction was stirred under argon for 5 h and 80 mL of satd. $NaHCO_3$ was then added to quench the reaction. The reaction mixture was diluted with H_2O (250 mL) and extracted with CH_2Cl_2 (2 x 300 mL). The combined organic layers were washed with 1 M $NaHSO_3$ (3 x 250 mL), H_2O (1 x 250 mL) and brine (1 x 250 mL). Drying and evaporation gave 22.9 g (94%) cloudy white oil. This compound was very acid sensitive and decomposed upon silica gel chromatography. ^1H NMR showed it was sufficiently pure for use in the next step. **5:** ^1H NMR ($CDCl_3$) δ 1.52-1.78 (m, 6 H), 3.58-3.90 (m, 2 H), 4.80 (t, 1 H), 5.00 (m, 2 H), 5.78 and 5.94 (2s, 1 H), 6.04 (d, 1 H), 7.40 (m, 5 H).

(2*R*, 3*R*)-*O*-Benzylidene-2,3-dihydroxycyclopent-4-enone (6). To the solution of 4.80 mL (44.3 mmol) of dimethyl methyl phosphonate in 140 mL of dry THF was added 27.7 mL (44.3 mmol) of 1.6 M n-BuLi at -78° C. The reaction was stirred at this temperature for 30 min during which time a large amount of white precipitate formed. A clear solution of 11.31 g (36.9 mmol) of **5** in 30 mL of dry THF was cooled to -78° C in a dry ice-acetone bath and added quickly through a canula to the flask containing the Wittig reagent. TLC analysis of the resulting reaction mixture (hexane-EtOAc, 7:3) indicated that the lactone **5** had been completely consumed after 30 min. Workup after 45 min was performed by quenching the mixture with satd. NH_4Cl (200 mL) at -78° C and allowing the quenched reaction to warm to room temperature. This was followed by extraction of the mixture with EtOAc (3 x 250 mL). The combined organic layers were washed with brine, dried and evaporated to give 5.38 g of the crude product. The reaction was repeated in the same manner using an additional 11.66 g (38.1 mmol) of **5**. The combined crude products were then purified over Florisil (stepwise gradient with hexane-EtOAc ranging from 93:7 to 1:1) to give 4.97 g (33%) of enone **6**. An analytical sample was crystallized from 1:1 pet. ether-EtOAc. **6:** m. p. 104-106° C; $[\alpha]^{25}_D$ -205° (c=1.05, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.55 (d, 1 H), 5.39 (dd, 1 H), 6.16 (s, 1 H), 6.18 (d, 1 H), 7.38 (m, 5 H), 7.59 (dd, 1 H); ^{13}C NMR ($CDCl_3$) δ 76.6, 78.8, 108.5, 126.4, 128.7, 129.4, 134.1, 136.0, 158.1, 201.2. MS (FAB) $[M+H]^+$ at 203. Anal. Calcd for $C_{12}H_{10}O_3 \cdot 0.10H_2O$, C, 70.65; H, 5.04. Found: C, 70.45; H, 5.17.

(2*R*, 3*R*)-*O*-Benzylidene-2,3-dihydroxy-(4*R*)-4-(hydroxymethylene)cyclopentanone (7). A solution containing 410 mg (2.03 mmol) of enone **6** and 60 mg of benzophenone in 350 mL of MeOH was degassed for 1 h with a stream of argon. The solution was then photolyzed through a Pyrex filter using a 450 watt Hanovia mercury lamp in an Ace glass photolysis apparatus. After 1.5 h the slightly yellow reaction solution was evaporated to give 520 mg of the crude product as a yellow oil. The reaction was run three additional times on the same scale and the crude products combined to give a total of 2.2 g. Chromatography on Florisil (pet. ether-EtOAc 1:1 followed by EtOAc) gave 1.04 g (55%) of pure product as a clear oil. **7:** $[\alpha]^{25}_D$

-164.2° (c=1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.61 (m, 1 H), 2.77 (m, 1 H), 3.24 (bs, 1 H), 3.56 and 3.75 (2m, 2 H), 4.38 (d, 1 H), 4.72 (d, 1 H), 5.77 (s, 1 H), 7.38 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 37.1, 38.3, 63.5, 78.7, 83.2, 104.9, 126.2, 127.9, 129.3, 135.5, 213.4. HRMS (CI with CH_4) calcd for $C_{13}H_{15}O_4$ 235.0970, found 235.0971.

(2*S*, 3*R*)-*O*-Benzylidene-2,3-dihydroxy-(4*R*)-4-(hydroxymethyl)-(1*S*)-cyclopentanol (8). Glacial acetic acid (0.90 mL, 15.7 mmol) was added to a suspension of 200 mg (5.24 mmol) of $NaBH_4$ in 500 mL of dry benzene. The resulting suspension was refluxed for 30 min to form a clear solution. To this solution was added 615 mg (2.62 mmol) of ketone **7** in 30 mL of dry benzene at 10° C. The mixture was allowed to warm to room temperature and then stirred for 48 h, at which point TLC analysis indicated that the reaction was complete. Workup was by evaporation of most of the benzene and addition of 150mL of satd. NH_4Cl. The crude reaction mixture was extracted with $CHCl_3$ (2 x 150 mL) followed by EtOAc (1 x 150 mL). The aqueous layer was then acidified with 2N HCl and extracted first with $CHCl_3$ (1 x 150 mL) and then with EtOAc (1 x 150 mL). The combined organic layers were washed with brine, dried and evaporated to give 640 mg of an off-white solid. Column chromatography over silica gel (hexane-EtOAc-Et_3N 80:19:1 followed by EtOAc-MeOH-Et_3N 90:9:1) gave 583 mg (94%) of **8** as a white solid. **8**: m.p. 122-123° C; $[\alpha]^{25}_D$ -23.2° (c=1, MeOH); ^1H NMR ($CDCl_3$) δ 1.72 (t, 1 H), 1.85 and 2.03 (2m, 2 H), 2.46 (m, 2 H), 3.52 and 3.66 (2m, 2 H), 4.22 (m, 1 H), 4.57 (m, 2 H), 5.85 (s, 1 H), 7.38-7.54 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 30.3, 43.9, 63.6, 76.1, 79.0, 79.7, 104.1, 126.7, 128.4, 129.8, 137.2. HRMS (EI) calcd. for $C_{13}H_{16}O_4$ 236.1048, found 236.1044. Anal. Calcd for $C_{13}H_{16}O_4$, C, 66.09; H, 6.83. Found: C, 66.04; H, 6.85.

(2*S*, 3*R*)-*O*-Benzylidene-2,3-dihydroxy-(4*R*)-4-(diphenylphosphoryloxymethyl)-(1*S*)-cyclopentanol (9a). To the clear solution of 498 mg (2.11 mmol) of diol **8** in 30 mL of dry CH_2Cl_2 was added 1.85 mL (10.6 mmol) of dry diisopropylethylamine at room temperature. The resulting solution was stirred for 30 min and 0.48 mL (2.32 mmol) of diphenylphosphoryl chloride was then added dropwise. After 44 h, 2 mg of 4-dimethylaminopyridine was added as a solid and the stirring was continued. After 68 h, 3 mL of Et_3N was added followed by 3 mL of H_2O. Dilution with CH_2Cl_2 and H_2O (100 mL each) was followed by removal of the aqueous layer. This layer was re-extracted with CH_2Cl_2 (100 mL) and the organic layers were combined and washed with brine, dried and evaporated to give 980 mg of an orange oil. Column chromatography of the oil on Florisil (hexane-EtOAc 8:2, followed by hexane-EtOAc 1:1, followed by EtOAc) gave 659 mg (67%) of the desired monophosphorylated product **9a** as a clear oil . Attempted chromatography of **9a** on silica gel led to its rearrangement to the 1,2-*O*-benzylidene isomer **9b**. **9a**: m.p. 83-85° C; $[\alpha]^{25}_D$ +10.3° (c=1, $CHCl_3$); ^1H NMR ($CDCl_3$) d 1.78-1.88 and 2.01-2.12 (2m, 2 H), 2.50 (bs, 1 H), 2.66 (m, 1 H), 4.06-4.52 (m, 5 H), 5.79 (s, 1 H), 7.16-7.50 (m, 15 H); ^{13}C NMR ($CDCl_3$) d 34.9, 42.1 (d, J_{CP} = 7.9 Hz), 69.6 (d, J_{CP} = 5.9 Hz), 71.3, 80.6, 82.8, 105.8, 119.9, 125.4, 126.6, 128.4, 129.8, 150.4; ^{31}P NMR ($CDCl_3$) d -14.3. MS (CI with CH_4): $[M+1]^+$ at 469. Anal. Calcd for $C_{25}H_{25}PO_7$. $0.5H_2O$, C, 62.89; H, 5.49. Found: C, 63.26; H, 5.46. **9b**: ^1H NMR ($CDCl_3$) δ 1.36-1.49 (m, 1H), 2.00 (dd, 1H), 2.34 (m, 1H), 2.67 (d, 1H), 3.64 (m, 1H), 4.32-4.57 (m, 4 H), 5.70 (s, 1H), 7.16-7.52 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 30.3, 42.8 (d, J_{CP} = 7.1 Hz), 68.4 (d, J_{CP} = 6.2 Hz), 73.0, 78.1, 79.2, 103.9, 119.9, 125.3, 126.6, 128.2, 129.7, 135.6, 150.3; ^{31}P NMR ($CDCl_3$) δ -14.0.

Bis(morpholino)-2-cyanoethylphosphite (13).[24] A solution of dry 2-cyanoethanol (2.40 mL, 35 mmol) in 12 mL of dry acetonitrile was added dropwise through a canula over 15 min to a solution of 21.0 mL (240 mmol) of freshly distilled PCl_3 in 15 mL of dry acetonitrile cooled in ice. The ice bath was then removed and the clear solution stirred at room temperature for 45 min. The acetonitrile and excess PCl_3 were removed in vacuo to give a milky white residue. This residue was dissolved in 200 mL of dry ether and the solution cooled in an ice bath. A solution of 6.9 mL (80 mmol) of dry morpholine and 11.2 mL of dry triethylamine in 100 mL of dry ether was then added dropwise with stirring over a 30 min period to the ethereal solution of the white residue. The resulting white suspension was allowed to warm to room temperature and the mixture was then stirred for 20 h. The white precipitate was filtered off and washed with dry ether (2 x 100 mL). The combined filtrates were mixed with 200 mL of water and the organic layer was separated. The aqueous layer was extracted with 200 ml of ether and the combined organic layers were dried and evaporated to give 8.7 g (91%) of **13** as a white solid. **13**: m.p. 34-35° C; ^1H NMR ($CDCl_3$) δ 2.67 (t, 2 H), 3.04 (m, 8 H), 3.60 (m, 8 H), 3.87 (q, 2 H); ^{13}C NMR ($CDCl_3$) δ 20.2 (d, J_{CP} = 7.5 Hz), 45.0 (d, J_{CP} = 14.9 Hz), 59.6 (d, J_{CP} = 20.7 Hz), 67.8 (d, J_{CP} = 6.4 Hz), 117.5; ^{31}P NMR ($CDCl_3$) δ +128.3. HRMS (EI) calcd. for $C_{11}H_{20}N_3O_3P$ 273.1242, found 273.1244.

Morpholidate (10). To a solution of 338 mg (0.72 mmol) of alcohol **9a** and 237 mg (0.864 mmol) of phosphite **13** in 10 mL of dry THF was added 152 mg (2.16 mmol) of *1H*-tetrazole as a solid. The resulting solution was initially clear, but formed a white precipitate after 30 min. The solution was stirred for 3 h total, after which time it was cooled to -78°C in a dry ice-acetone bath and 166 mg (0.96 mmol) of 85% mCPBA in 10 mL of dry CH_2Cl_2 was added. The resulting solution was allowed to warm to room temperature over 15 min and 30 mL of a 1 M $NaHSO_3$ solution was then added. The resulting mixture was stirred vigorously for 10 min and then extracted with ether (2 x 150 mL). The ether layers were combined, washed twice with 1 M $NaHSO_3$, once with 0.25 M $NaHCO_3$, and then dried and evaporated to give 490 mg of a cloudy oil. Column chromatography of the oil on silica gel (hexane-EtOAc 1:1, followed by EtOAc, and then by EtOAc-MeOH 9:1) gave 378 mg (78%) of **10** as a clear oil. **10**: $[\alpha]^{25}_D$ -26.5° (c=1, MeOH); ^1H NMR ($CDCl_3$) δ 2.00 (m, 1 H), 2.34-2.71 (m, 4 H), 3.12 (m, 4 H), 3.57 (m, 4 H), 4.07-4.28 and 4.50-4.75 (m, 7 H), 5.77 (d, 1 H), 7.18-7.51 (m, 15 H); ^{13}C NMR ($CDCl_3$) δ 19.3, 32.2, 41.7, 44.3, 60.6, 66.6, 69.3, 75.5, 79.6, 82.2, 105.1, 105.5, 116.6, 119.8, 125.4, 126.5, 128.2, 129.8, 135.9, 150.1; ^{31}P NMR ($CDCl_3$) δ +5.0 and -14.3. MS (FAB) $[M+1]^+$ at 671.

Deblocked Morpholidate (11). A solution of 290 mg (0.43 mmol) of morpholidate **10** in 20 mL of a 1:1 mixture of satd. NH_3-MeOH and MeOH was stirred for 45 min, at the end of which time TLC analysis (EtOAc-MeOH 6:4, R_f of product = 0.23) showed that the reaction was complete. The solvents were evaporated to give 280 mg of the crude NH_4^+ salt. ^1H NMR and ^{31}P NMR analysis of the residue confirmed that the desired product had been formed. An aqueous solution (10 mL) of the crude product was converted to its Na^+ salt by passage through a column (1.5 x 16 cm) of Amberlite IR-120 PLUS (Na^+ form) resin. The resin was washed with 100 mL of H_2O and the combined eluants were evaporated (<40°C) to give 290 mg (105%) of **11** as a white foam. The material appeared to be pure as judged by TLC, ^1H NMR, and ^{31}P NMR and was used directly in the next reaction. **11**: ^1H NMR ($CDCl_3$) δ 1.83 (m, 1 H), 2.10 (m, 1 H), 2.38 (m, 1 H), 2.89 (bs, 4 H), 3.42 (bs, 4 H), 4.03-4.17 (m, 2 H), 4.32 (d, 1 H), 4.57 (m, 2 H), 5.59 (s, 1 H), 7.12-7.45 (m, 15 H);

^{13}C NMR (CD_3CN:D_2O, 1:1) δ 32.1, 43.3, 45.9, 67.9, 70.9, 74.6, 81.9, 82.5, 105.8, 121.0, 126.8, 128.0, 129.5, 130.9, 131.2, 137.1, 151.0; ^{31}P NMR ($CDCl_3$) δ +5.2 and -14.4. MS (FAB) $[M+1]^+$ at 640.

(1*S*)-Pyrophosphoryl-(2*R*, 3*R*)-*O*-Benzylidene-2,3-dihydroxy-(4*R*)-4-(diphenylphosphoryloxymethyl)cyclopentane (12). A mixture of 280 mg (0.44 mmol) of **11** and 372 mg (1.32 mmole, 3.0 equiv) of tributylammonium phosphate was dried by suspension in 5 mL of dry benzene and removal of the benzene with a vacuum pump. This process was repeated three times. The resulting mixture was then kept at a pressure of 0.33 mm for 2 h to remove residual traces of benzene. The mixture was then dissolved in 15 mL of dry pyridine to form an off-white solution. After 22 h a 0.33 mL aliqout of the reaction mixture was removed and diluted with 0.33 mL of D_2O. ^{31}P NMR analysis of the diluted aliquot indicated that the reaction was nearly complete. After 45 h of reaction time, the pyridine was evaporated (<40°C) to give an oily residue. This was dissolved in a 1:1 solution of H_2O and MeOH and passed through a column (1.5 x 16 cm) of Amberlite IR-120 PLUS (Na^+ form) resin. The column was washed with 150 mL of H_2O, and the combined eluants were evaporated to give 500 mg (> 100%) of an off-white solid. TLC (isopropanol:conc. NH_4OH:H_2O 65:25:10) revealed the presence of one UV active spot with an $R_f = 0.50$ (the R_f of **11** in this solvent system is 0.67). Attempts to purify the compound by anion exchange chromatography led to partial decomposition. Therefore, the crude reaction product was utilized for the deprotection reactions. **12**: ^1H NMR (D_2O) δ 1.95 (m, 1 H), 2.27 (m, 1 H), 2.49 (m, 1 H), 4.10 (m, 1 H), 4.25 (m, 1 H), 4.36 (d, 1 H), 4.64 (m, 2 H), 5.64 (s, 1 H), 7.08-7.47 (m 15 H); ^{31}P NMR (D_2O) δ +1.23 (excess $Na^+H_2PO_4^-$), -8.1 (d, $J_{PP} = 20$ Hz), -11.1 (d, $J_{PP} = 20$ Hz), -11.9 (s). MS (negative ES) $[M-2\ Na^+]$ at 649.

(+)-(1*S*)-1-Pyrophosphoryl-(2*R*,3*R*)-2,3-dihydroxy-(4*S*)-4-(phosphoryloxymethyl)-cyclopentane (2). A 100 mg portion of crude **12** from the previous step was dissolved in 20 mL of 1:1 EtOH-H_2O and treated with 125 mg of $Pd(OH)_2$ and 1 atm of H_2. After 5 h, TLC (isopropanol:conc. NH_4OH:H_2O 65:25:10) showed complete conversion of **12** (R_f 0.50) to a product with a lower R_f (0.12). The mixture was filtered through a pad of Celite and the pad was washed twice with 20 mL 1:1 EtOH-H_2O. The combined filtrates were evaporated to give 90 mg of a clear oil. Examination of this oil in D_2O by ^1H NMR revealed that complete loss of the benzylidene group and partial loss of the phenyl groups had taken place.

The crude product from $Pd(OH)_2$ treatment was dissolved in 40 mL of 1:1 EtOH-H_2O and 35 mg of PtO_2 was added. This mixture was hydrogenated using a Parr apparatus at 50 psi of H_2 for 42 h at room temperature. The crude product was filtered through a pad of Celite and this was washed as above. The solvents were evaporated to give 90 mg of a clear oil. ^1H and ^{31}P NMR analysis showed that only a trace (<5%) of the phenyl ester moiety remained. The crude product from the Pt catalyzed reduction was dissolved in 5 mL of H_2O to give a solution whose pH was 8.0, and loaded onto a column (1.5 x 20 cm) of DEAE-Sephadex A-25 resin. A linear gradient of 0 to 0.9 M $Et_3NH^+HCO_3^-$ (pH 7.6) buffer was used to elute the product. Product-containing fractions eluted at a buffer concentration of ca. 0.23 M and were located by measuring the UV absorbance at 800 nm after treatment of 0.50 mL portions of the fractions with 0.50 mL of phosphate assay solution.[30] The product-containing fractions were evaporated in the presence of MeOH to aid in the removal of the buffer to give 40 mg of white solid. This material was dissolved in 5.0 mL of H_2O and loaded onto a column (1.5 x 16 cm) of Amberlite IR-120 PLUS (Na^+ form) resin. Elution with 150 mL of H_2O followed by evaporation gave 26.7 mg (61% based upon **11**) of **2** as a white solid. **2**: $[\alpha]^{25}_D$ +11.9° (c = 1, H_2O); ^1H NMR (D_2O) δ 1.77 (m, 1H, H-6), 2.12 (m, 1 H, H-6), 2.45 (m, 1 H, H-4), 3.79 (m, 2 H, H-5), 3.98 (t, 1 H, H-3), 4.15 (t, 1 H, H-2), 4.63 (m, 1 H, H-1); ^{13}C NMR (D_2O) δ 34.3 (d, $^3J_{CP}$ = 3.8 Hz) (C-6), 46.9 (d, $^3J_{CP}$ = 7.6 Hz) (C-4), 69.6 (d, $^2J_{CP}$ = 5.5 Hz) (C-5), 76.4 (C-3), 76.9 (d, $^3J_{CP}$ = 3.4 Hz) (C-2), 79.6 (d, $^2J_{CP}$ = 6.4 Hz) (C-1); ^{31}P NMR (D_2O) δ 1.2 (s), -9.2 (d, J_{PP} = 21 Hz), -11.1 (d, J_{PP} = 21 Hz). MS (negative ES): [M· 5 Na$^+$] at 498; [M· 4 Na$^+$] at 475; [M· 3 Na$^+$ + H] at 453; [M· 2 Na$^+$ + 2 H] at 431; [M· Na$^+$ + 3 H] at 409 (base-peak); [M + 4 H] at 387.

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