Large Scale Synthesis of Cyclodiphospho-D-glycerate

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Received January 23, 1996

Introduction

Thermophilic microorganisms require significant adaptations of their cellular constituents and biochemistry to live at high temperatures. Thermostabilizing features in thermophiles include changes in their protein and nucleic acid stability as well as chemical differences in their intracellular environments. In archaeal (formerly archaebacterial) thermophilic species that produce methane, such as the methanogen species *Methanothermus fervidus* (optimal growth at 83 °C) and *Methanopyrus kandleri* (optimal growth at 98 °C), very high concentrations of the potassium salt of cyclo-2,3-diphospho-D-glycerate (cDPG) appear to be important for *in vivo* enzyme thermostability.[1−4] In methanogen species, cDPG is widely distributed and has been shown *in vitro* to increase the half life of thermal inactivation of enzymes purified from thermophilic species by greater than 100-fold.[2−4] Since cDPG is present at concentrations of *ca.* 0.3 M in *M. fervidus* and *ca.* 1.0 M in *M. kandleri* this compound is likely to play a major role in the thermoadaptation of thermophilic methanogen species.[4]

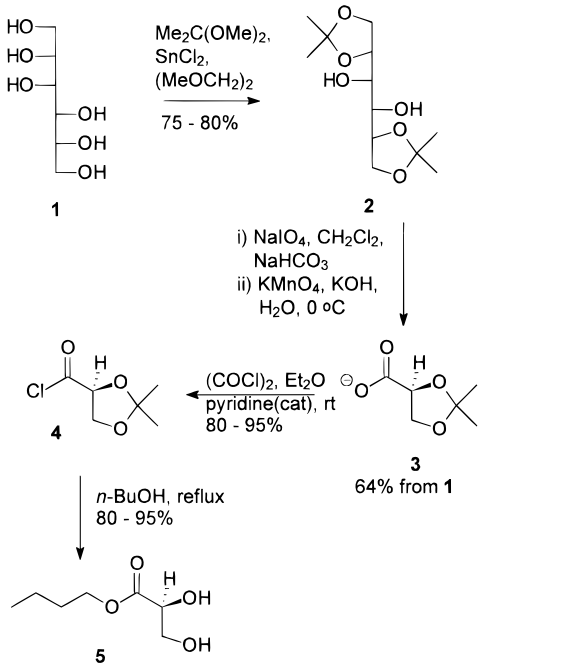
cyclo-2,3-Diphospho-D-glycerate can be extracted and purified from *M. fervidus* or *Methanobacterium thermoautotrophicum*.[2,5] Unfortunately, fermentative production of cDPG is not practical as the organisms are not easy to culture on a large scale (high temperature, growth on H_2) and limited amounts of the material can be obtained. Previous synthetic approaches to cDPG are also unsuitable because of low yield and expense of starting material.[6,7] For a complete series of biophysical experiments, a synthesis capable of producing multigram quantities of the target compound was required.

We report here an inexpensive, relatively straightforward synthesis that can be applied to the large scale production of cDPG. We have routinely been able to make cDPG in greater than 30% overall yield from mannitol.

Results and Discussion

We originally set ourselves the task of developing a synthesis of cDPG that could be done on a large scale (*ca.* 100 g of target compound) and that was relatively inexpensive and simple to carry out. The main constraint imposed was to devise a synthetic scheme which minimized the effort spent in purification at the intermediate stages and avoided, as much as possible, the need for chromatography.

Scheme 1. Synthesis of *n*-Butyl D-Glycerate (5) from Mannitol (1)



Previously, cDPG has been synthesized from 2,3-bisphosphoglycerate in aqueous media using a water soluble carbodiimide reagent.[6] The yield of the process was only 10% and a mere 50 μmol of product was obtained. Considering the expense of 2,3-bisphosphoglycerate this approach was not feasible. Nobel and Potter have recently reported a synthesis of cyclic pyrophosphate compounds based on desulfurization of vicinal bisphosphothioates.[8] The process was shown to work well in model systems although the high yields of the bisphosphate make it less suitable in the present work.

Mannitol was the obvious choice of starting material because it is readily available and possesses the absolute stereochemistry at C-2 and C-5 required to give an enantiospecific synthesis of the target (Schemes 1 and 2). The enantiomer, cyclodiphospho-L-glycerate, can also be prepared as the conversion of ascorbic acid into the acetonide of L-glyceraldehyde is a known procedure.[9]

D-Glyceraldehyde acetonide was prepared from mannitol essentially by the procedures of Schmid and Bryant[10] and Chittenden.[11] In our hands, **2** of greater than 95% purity could be obtained in 75−80% yield representing an improvement over the published procedure: the only change being that the 2,2-dimethoxypropane was freshly distilled before use to ensure removal of acetone and methanol. Traces of the starting material, the mono-, and tris-acetonide were removed from the product **2** by virtue of their differential solubility in hexanes or acetone. Periodate oxidation[10] followed by direct permanganate oxidation,[12] without purification of the alde-

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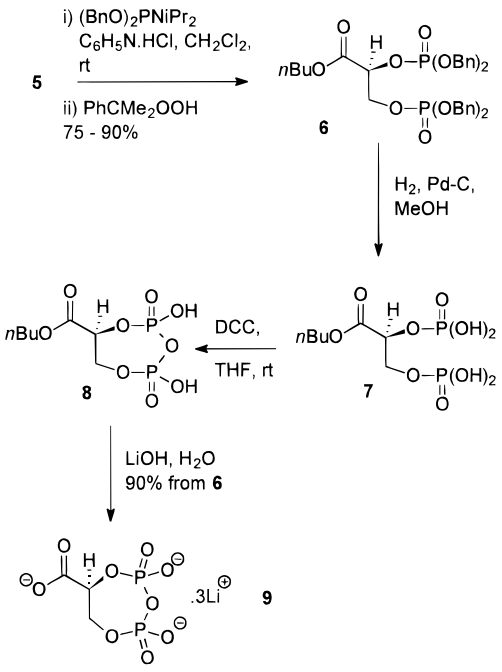
† This paper is dedicated to the memory of Conor R. O'Hanlon, friend, colleague, graduate student.

(1) Seeley, R. J.; Fahrney, D. E. *Curr. Microbiol.* **1984,** *10*, 85.
(2) Hensel, R.; König, H. *FEMS Microbiol Lett.* **1988**, *49*, 75.
(3) Hensel, R.; Jacob, I. *Syst. Appl. Microbiol.* **1994**, *16*, 242.
(4) Lehmacher, A.; Hensel, R. *Mol. Gen. Genet.* **1994**, *242*, 163.
(5) Kanodia, S.; Roberts, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5217.
(6) Gorris, L. G. M.; Korteland, J.; Derksen, R. J. A. M.; Van Der Drift, C.; Vogels, G. D. *J. Chromatogr.* **1990**, *504*, 421.
(7) Tohidi, M.; Orgal, L. E. *J. Mol. Evol.* **1990**, *30*, 97.
(8) Noble, N. J.; Potter, B. V. L. *J. Chem. Soc., Chem. Commun.* **1989**, 1194.
(9) Jung, M. E.; Shaw, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 6304; Takano, S.; Numata, H.; Ogasawara, K. *Heterocycles* **1982**, *19*, 327.
(10) Schmid, C. R.; Bryant, J. D. *Org. Synth.* **1993**, *68*, 6.
(11) Chittenden, G. J. F. *Carbohydr. Res.* **1980**, *87*, 219.
(12) Tankaka, A.; Yamashita, K. *Agric. Biol. Chem.* **1980**, *44*, 199.

S0022-3263(96)00147-8 CCC: $12.00

Scheme 2. Synthesis of cDPG (9) from *n*-Butyl D-Glycerate (5)



hyde, gave the known potassium D-glycerate in 64% overall yield from mannitol. Overall yields were substantially lower when purification of the intermediate aldehyde was attempted. Purification of **3** could be conveniently achieved at this time by recrystallization from ethanol.

The chloride **4** was obtained from **3** by the procedure of Tanaka and Yamashita.[12] The acyl chloride, itself, or esters readily derived from it, represent chiral synthons comparable in utility to D-glyceraldehyde acetonide. Purification of the chloride was done by Kügelrohr distillation. As with glyceraldehyde acetonide, however, higher overall yields were achieved when **4** was used directly in the next step without purification.

The chloride **4** was derivatized using (*R*)-α-methylbenzylamine. The diastereomeric amide product was analyzed by ^{1}H NMR. No resonances corresponding to the alternate diastereoisomer were observed showing that no racemization of the α-carbon had occurred up to this point.[13]

Formation of the butyl ester **5** was carried out without addition of any base to neutralize the acid produced in the reaction. In this way esterification and deprotection of the acetonide could be conveniently carried out in one step. The butyl ester, **5**, was purified by short-path distillation.

The butyl ester, rather than, for example, the methyl or benzyl ester, was used in this sequence as it represented the best compromise between hydrophobicity and stability. Use of a benzyl ester would conflict with later protection of the phosphate groups. Use of a methyl ester gave intermediate compounds that were very hydrophilic and, therefore, difficult to analyze by TLC and in the latter stages of the synthesis almost impossible to recover from aqueous solution.

The critical procedure in the proposed synthesis was the method chosen to introduce protected forms of phosphate into the sequence. Various strategies were attempted based on the use of either phosphorus(III) or phosphorus(V) chemistry and either acid- or base-catalyzed reactions. All trials involving either pyrophosphoryl chloride ($Cl_2P(O)OP(O)Cl_2$), $POCl_3$, tetrabenzyl pyrophosphate, or dibenzyl chlorophosphonate with **5** met with the same fate: destruction of the carbon skeleton of **5**. With hindsight, the plethora of alternate leaving groups and the ease with which elimination reactions could occur doomed this particular strategy.

The same fate befell similar reactions of 1-alkyl and 1-acyl substituted glycerol derivatives.

Initially, benzyl H-phosphonate[14] was evaluated as a P(III) phosphorylation reagent; however, these efforts met with little success for either **5** or 1-alkyl and acyl glycerol derivatives. After much trial, dibenzyl diisopropylphosphoramidite[15] emerged as the reagent of choice for phosphorylation of **5**. Dibenzyl diisopropylphosphoramidite can be made from PCl_3 in two steps conveniently on a molar scale.[15−17]

Optimization of the coupling procedure was done to determine both the choice of acid catalyst and method of oxidation to the corresponding phosphorus(V) compound. Pyridinium hydrochloride was most effective as an acid catalyst giving the derivative **6** in 75−90% yield as compared to ethereal HCl (<20%) or trichloroacetic acid (50−75%). 1*H*-Tetrazole was not investigated due to its relative expense and the observation that the use of pyridinium hydrochloride already gave acceptable yields. Early investigations of this reaction employed *tert*-butyl hydroperoxide as an oxidant. We were, however, uncomfortable with the use of *tert*-butyl hydroperoxide on the scale required to produce significant quantities of our target compound. Cumene hydroperoxide was investigated as an alternate oxidizing agent. The reaction proceeds more cleanly and in higher yield. Currently, yields of 90−95% are consistently achieved when the phosphorylation is done on batches of 40−50 mmol of **5**. Other products of this reaction include tribenzyl phosphite, tribenzyl phosphate, and dibenzyl phosphite. This is the only stage of the synthetic sequence where significant chromatography is required. Intermediate **6** must be free of all P(III) species or the efficiency of the subsequent catalytic hydrogenation is severely compromised.

The last stages of the synthesis can be done without purification of the intermediates although some care must be exercised. The hydrogenation proceeds smoothly in methanol to give the free acid form of the phosphate. The reaction is best followed by ^{1}H NMR, observing the loss of the benzylic proton resonances at 5.15 ppm. If the product is left too long, hydrolysis and transesterification of the butyl ester occurs. Attempts to do the reaction under neutral conditions or in 1-butanol met with limited success. The butyl ester obtained after the hydrogenation is soluble in THF. The coupling to give the cyclic pyrophosphate was successfully carried out using 1,3-dicyclohexylcarbodiimide in THF. The conversion was quantitative and no bisphosphate species could be detected by ^{31}P NMR. On completion of the coupling

(13) To determine if resonances from the minor diastereoisomer could be resolved and detectable in such an NMR experiment, a sample of the authentic diastereoisomer was mixed with the amide derived from **4** and (*S*)-α-methylbenzylamine and analyzed. The minor diastereoisomer was clearly detectable.

(14) Larson, E.; Lüning, B. *Tetrahedron Lett.* **1994**, *35*, 2737.
(15) Yu, K.-L.; Fraser-Reid, B. *Tetrahedron Lett.* **1988**, *29*, 979.
(16) Uhlmann, E.; Engels, J. *Tetrahedron Lett.* **1986**, *27*, 1023.
(17) Tanaka, T.; Tamatsukuri, S.; Ikehara, T. *Tetrahedron Lett.* **1983**, *24*, 3171.

reaction, water was added and the solution neutralized to pH 4 by the addition of lithium hydroxide. Organic impurities were removed at this stage by filtration and washing with dichloromethane. Hydrolysis at pH 12 using aqueous lithium hydroxide gave the trilithium salt of the target compound in approximately 90% yield from **6**.

The potassium salt of cDPG is required for biophysical studies yet the synthesis has been developed to make the lithium salt. The final cleavage reaction, salt formation, and crystallization of the cDPG proceed more reliably with the lithium salt than the potassium salt. Once final purification has been secured, conversion of the cDPG to its potassium salt by cation exchange chromatography is a simple matter.

The cDPG made in this study has been characterized fully and is identical to material isolated from the natural source. Higher quality spectra are obtainable for the synthesized material than isolated material largely due to sample availability and these should prove to be useful standards for biophysical studies. It is difficult to record good ^{13}C spectra with the small amounts of cDPG that can be recovered from fermentation sources. Not only are all the carbon resonances split into doublets, the presence of phosphorus prevents the usual NOE derived signal enhancement caused by ^{1}H-decoupling.

In conclusion, cDPG can be made on a multigram scale in 30% overall yield from mannitol. The compound will find wide use in studies of the thermal stability of proteins and nucleic acids from methanogen species as well as more general applications to protein thermostability. One distinct advantage to the synthesis procedure is in its reliance on well developed and tested chemistry, a feature crucial to the successful repetition of this work. This route also allows the for large scale production of 2,3-bisphosphoglycerate *via* LiOH hydrolysis of the butyl ester **7**.

Experimental Section

General. The solvents CH_2Cl_2 (CaH_2), Et_2O (Na/K−benzophenone), *n*-BuOH (CaH_2), and THF (Na/K−benzophenone) were dried and distilled prior to use. Solutions were concentrated by evaporation *in vacuo*.

1,2:5,6-Diisopropylidenemannitol (2) Mannitol (200 g, 1.1 mol) was placed in a 2 L three-neck flask equipped with a mechanical stirrer. Anhydrous dimethoxyethane (500 mL) and freshly distilled, methanol free 2,2-dimethoxypropane (320 mL, 2.6 mol) were added and the slurry stirred vigorously. $SnCl_2$ (0.2 g, 1 mmol) was added and the slurry was heated at reflux. After 16 h the resulting clear solution was left to cool to rt, and then pyridine (1.0 mL) was added. The solvent was removed by evaporation *in vacuo* (80 °C, 20 Torr). Upon cooling, a white solid formed. The solid was suspended in hexanes (1 L) and the insoluble **2** collected by filtration. **2** was dissolved in acetone (1.5 L), and the residual solid was removed by filtration. The acetone extract was evaporated *in vacuo*, and the resulting crystals of **2** (227 g, 75%) were dried under vacuum (0.1 Torr, 16 h). The crude product was identified by comparison with spectral data reported for the known compound.[12]

Potassium (2*S*)-2,3-Isopropylideneglycerate (3). In a 3 L, two neck flask, equipped with a mechanical stirrer and reflux condenser, **2** (227 g, 0.85 mol) was dissolved in a mixture of CH_2Cl_2 (2 L) and a saturated aqueous solution of $NaHCO_3$ (100 mL). $NaIO_4$ (280 g, 1.3 mol) was added over 20 min without cooling. After 4 h, anhydrous $MgSO_4$ (400 g) was added in several small portions with vigorous stirring. The resulting suspension was filtered and the filter cake washed with CH_2Cl_2 (2 L). The combined organic fractions were evaporated *in vacuo* at less than 30 °C to give crude 2,3-isopropylideneglyceraldehyde which was used immediately in the next reaction.

KOH (128 g, 2.0 mol) was dissolved in water (3 L) in a 10 L beaker cooled in a large ice-salt bath. Crude 2,3-isopropylideneglyceraldehyde and ice (1.0 kg) were added with manual stirring. $KMnO_4$ (230 g, 1.5 mol) in water (2 L) was added cautiously, with constant manual stirring. The reaction temperature was kept below 10 °C by the addition of ice to the reaction vessel. The brown sludge obtained was neutralized to pH 8 by the cautious addition of 50% (v/v) aqueous H_2SO_4. The water was removed by evaporation *in vacuo* (60 °C, 20 Torr) to give, on cooling, a white solid. The solid was extracted with boiling ethanol (2 × 750 mL) and separated from the inorganic potassium salts by filtration. The solution was concentrated and the crude **3** (199 g, 63%) dried (70 °C at 0.1 Torr). **3** was readily recrystallized from ethanol:[13] ^{1}H NMR (D_2O) δ 1.23 (s, 3H), 1.29 (s, 3H), 3.75 (dd, 1H, J = 6.9, 8.3 Hz), 4.12 (dd, 1H, J = 7.6, 8.3 Hz), 4.35 (dd, 1H, J = 6.9, 7.6 Hz); ^{13}C NMR (D_2O) δ 23.3, 23.7, 65.8, 74.1, 109.1, 176.2; IR (KI disc) 1597 s cm^{-1}; HRMS calcd for $C_6H_9O_4$ (M^+) 145.0523, found 145.0501.

(2*S*)-2,3-Isopropylideneglyceryl Chloride (4). **3** (50 g, 0.27 mol) was suspended in anhydrous Et_2O (500 mL) and anhydrous pyridine (0.5 mL). The mixture was cooled with an ice bath, and then oxalyl chloride (49.5 mL, 0.34 mol) was added dropwise by syringe over a period of 30 min. The ice bath was removed, and the contents of the flask were stirred for 20 h at room temperature. The residue of KCl was removed by filtration and the solvent removed (35 °C, 20 Torr) to give **4** (38.2 g, 85%). Further purification was achieved by Kügelrohr distillation (bp 70−75 °C, 20 Torr). ^{1}H NMR δ 1.36 (s, 3H), 1.44 (s, 3H), 4.25 (m, 2H), 4.77 (t, 1H, J = 5.7 Hz); ^{13}C NMR δ 25.2, 25.5, 66.8, 81.1, 112.9, 173.1; IR (liquid film) 1772 cm^{-1}; HRMS calcd for $C_5H_9O_2$ (M − COCl) 101.0586, found 101.0603.

***n*-Butyl (2*S*)-Glycerate (5).** 1-Butanol (900 mL) was placed in a 2 L round bottom flask, equipped with a reflux condenser and magnetic stirrer. **4** (50.0 g, 0.27 mol) was added, and the contents of the flask were heated at reflux for 1 h. The flask was cooled and the butanol removed (60 °C, 20 Torr). The residue was distilled (90 °C, 0.1 Torr) to give **5** (46.7 g, 95%). ^{1}H NMR δ 0.94 (t, 3H, J = 7.5 Hz), 1.44 (m, 2H, J = 7.5 Hz), 1.66 (quin, 2H, J = 7.5 Hz), 3.19 (br m, 2H), 3.87 (dq, 2H, J = 3.8, 8.9 Hz), 4.22 (t, 2H, J = 6.7 Hz), 4.27 (t, 1H, J = 3.8 Hz); ^{13}C NMR δ 3.6, 19.1, 30.6, 64.3, 65.7, 72.0, 173.1; IR (liquid film) 3402 br s and 1739 cm^{-1}; HRMS calcd for $C_7H_{14}O_4$ (M^+) 162.0892, found 162.0894.

***n*-Butyl (2*S*)-2,3-Bis(dibenzylphospho)glycerate (6).** A solution of pyridinium hydrochloride (19.75 g, 155 mmol) in CH_2Cl_2 (750 mL) was added to a solution of **5** (8.1 g, 50 mmol) and dibenzyl diisopropylphosphoramidate (51.9 g, 150 mmol) in CH_2Cl_2 (500 mL). The solution was stirred at room temperature for 1 h and then cooled to 0 °C. Cumene hydroperoxide (80%)-(28.8 mL, 155 mmol) was cautiously added to the cooled solution. After 1 h at rt the solvent was concentrated. Chromatography on silica gel, eluting with EtOAc−hexanes (1:2), gave **6** (32.5 g, 95.3%). ^{1}H NMR δ 0.85 (t, 3H, J = 7.3 Hz), 1.25 (sextet, 2H, J = 7.3 Hz), 1.53 (quin, 2H, J = 7.3 Hz), 4.10 (t, 2H, J = 6.7 Hz), 4.32 (m,), 4.95−5.15 (m, 8H), 7.22−7.34 (m, 20H); ^{13}C NMR δ 13.6, 19.0, 30.5, 66.1, 67.1 (br t, J 5.7), 69.7 (m), 128.0, 128.2, 128.2, 128.4, 128.6, 135.6 (t, J = 7.0 Hz), 166.9 (d, J = 3.6 Hz); ^{31}P NMR δ −1.32 (s) and −1.80 (s); IR (liquid film) 1759 cm^{-1}; HRMS calcd for $C_{35}H_{40}O_{10}P_2$ (M^+) 682.2098, found 682.2110.

***Cyclo*-(2*S*)-2,3-diphosphoglycerate, Trilithium Salt (9)** *Note*: ***6** must be free of phosphines or the catalyst will be poisoned. The rate of this reaction varies considerably and must be monitored by TLC or NMR.* Palladium on carbon (1.0 g, 5% Pd) was added to a solution of **6** (10.0 g, 14.7 mmol) in MeOH (200 mL) and stirred under an atmosphere of H_2 for between 1.5 and 10 h. The course of the reaction was followed by ^{1}H NMR. On completion, the suspension was filtered through Celite and the solvent removed to give crude **7** (4.6 g, 95% approximately): ^{1}H NMR (D_2O−CD_3OD) 0.88 (t, 3H, J = 6.6 Hz), 1.32 (sextet, 2H, J = 7.3 Hz), 1.56 (pent, 2H, J = 7.3 Hz), 4.05−4.40 (m, 3H), 4.19 (t, 2H, J = 6.6 Hz) and 4.96 (br s, 4H); ^{13}C NMR (D_2O) 13.51, 18.98, 30.39, 66.50 (t, J = 4.9 Hz), 67.10, 73.94 (dd, J = 4.6, 8.2 Hz), and 172.85 (d, J = 4.2 Hz); ^{31}P NMR 3.06 (s) and 3.51 (s);IR (liquid film) 3650−2100 br s and 1736 s cm^{-1}. Residual methanol in the crude **7** was removed by azeotropic evaporation with anhydrous THF (2 × 100 mL). The crude acid was dissolved in THF (200 mL), and a solution of DCC (3.64 g, 17.6 mmol) in THF (20 mL) was added. The

suspension was stirred for 16 h at 22 °C. Water (300 mL), followed by LiOH (sufficient to raise the pH to 4.0), was added and the mixture filtered. The aqueous solution was washed with CH_2Cl_2 (2 × 200 mL). The aqueous phase was evaporated to dryness (50 °C, 20 Torr) to give **8** as a hydrate: ^{1}H NMR (D_2O) 0.85 (t, 3H, J = 7.3 Hz), 1.32 (sextet, 2H, J = 7.3 Hz), 1.60 (pent, 2H, J = 7.3 Hz), 4.18 (t, 2H, J = 6.5 Hz), 4.15−4.45 (m, 3H); ^{13}C NMR (D_2O) 13.3, 18.7, 68.0 (d, J = 6.6 Hz), 75.6 (d, J = 5.0 Hz), and 169.5 (d, J = 10.3 Hz; ^{31}P NMR (D_2O-H_2O) −11.13 (d, J = 17.2 Hz), −9.85 (d, J = 17.2 Hz); IR (KI disc) 3467 bs s, 1749 s, and 1644 s cm^{-1}; HRMS calcd for $C_7H_{11}O_9P_2$ (M^+ − 2 Li) 300.9878, found 300.9884. LiOH monohydrate was added portionwise to a solution of **8** in water (20 mL) until a pH of 12 was maintained, and the solution was stirred at rt for 30 min. The pH was adjusted to 7.0 by addition of 1.0 M H_2SO_4. The solvent was removed. The product obtained was digested with water−methanol (1:9), and the residual solid removed by filtration. The solvent was again removed to give the desired product (3.5 g, 90%): $[\alpha]_D$ +7.7° (*c* 4.49, water); ^{1}H NMR (D_2O) δ 4.10−4.40 (m, 3H); ^{13}C NMR (D_2O) δ 70.0 (d, J = 6.75 Hz), 78.6 (d, J = 7.4 Hz), 174.6 (d, J = 6.7 Hz); ^{31}P NMR (D_2O-H_2O) δ −11.05 (d, J = 17.5 Hz), −9.80 (d, J = 17.6 Hz).

(2*S*)-2,3-Bisphosphoglycerate, Dilithium Salt. This compound was made from **6** in the same way that **9** was made from **6** (see above) with the exception that after the hydrogenation step, water and $LiOH\cdot H_2O$ were added directly to saponify the butyl ester. The dilithium salt was obtained as a glassy solid in yields averaging 90%. The product was shown to be identical to a commercially obtained sample of (2*S*)-2,3-bisphosphoglycerate.

Acknowledgment. We wish to thank The College of Pharmacy, The Ohio State University for support of this work. We also thank Professor John Reeve and Dr. Rowan A. Grayling (Department of Microbiology, The Ohio State University) for providing a sample of the naturally occurring cyclo-D-diphosphoglycerate isolated from *M. fervidus*. Advice concerning the synthesis of H-phosphonates and their use, from Dr. Elizabeth Larson and Professor Björn Lüning, is gratefully acknowledged.

Supporting Information Available: ^{13}C and ^{31}P NMR spectra of the synthesized cDPG and ^{1}H and ^{13}C NMR spectra of **6** (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Note Added in Proof. Shortly after original submission of this manuscript a synthesis of cDPG from D-mannitol was published by the group of Berkessel. They achieved the gram-scale conversion of D-mannitol into the methyl ester of the acyclic bisphosphoglycerate in 9% overall yield. Subsequently, cDPG which was 80% pure was obtained in 20% yield from the methyl ester. Berkessel, A.; Geisel, U.; Hérault, D. A. *Tetrahedron Lett.* **1996**, *37*, 355.

JO9601472

Additions and Corrections

Vol. 60, 1995

Fang-Jie Zhang, Guo-Qiang Lin,* and Qi-Chen Huang . Synthesis, Resolution, and Absolute Configuration of Optically Pure 5,5″-Dihydroxy-4′,4″,7,7″-tetramethoxy-8,8″-biflavone and Its Derivatives.

Page 6428, Scheme 2, first line, (+) should be (±).

Page 6429, Figure 2, left side, first line, should read "The dashed lines refer to (−)-**1**, (−)-**5**, and (−)-**6**. The solid lines refer to (+)-**1**, (+)-**5**, and (+)-**6**."

JO964006B

S0022-3263(96)04006-6

Takanori Yamazaki, Aleksandr Kasatkin, Yasufumi Kawanaka, and Fumie Sato*. Allyl as Protective Group for the Acidic Hydrogen of Malonic Ester.

Page 2266. The reference given below refers to some earlier work on the use of the alkoxycarbonyl moiety as a protective ("blocking") group in the malonic ester synthesis and was inadvertently omitted from our paper. Padgett, H. C.; Csendes, I. G.; Rapoport, H. *J. Org. Chem.* **1979**, *44*, 3492.

JO964009O

S0022-3263(96)04009-1

Andrew Streitwieser,* Faraj Abu-Hasanayn, Arndt Neuhaus, and Frank Brown. An *ab Initio* Study of Some Phenyl- and (Halophenyl)alkali Compounds.

Page 3151. The correct spelling of second author's name is **Faraj Abu-Hasanayn.**

JO9640130

S0022-3263(96)04013-3