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THE SYNTHESIS OF PHOSPHONIC ACID AND PHOSPHATE ANALOGUES OF GLYCEROL-3-PHOSPHATE AND RELATED METABOLITES

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Abstract—A convenient route is described for the preparation of the isosteric phosphonic acid analogue of glycerol-3-phosphate, 3,4-dihydroxybutyl-1-phosphonic acid, in both enantiomeric forms and the racemic modification, starting with readily available materials. The (S)-enantiomer, that of absolute configuration corresponding to that of *sn*-glycerol-3-phosphate, has been found to be a growth inhibitor of several bacteria at low concentration. The synthetic route described is of particular value as it facilitates the preparation of a series of phosphonic acids and phosphates of related structure, in their enantiomeric forms, which are also of interest for metabolic regulation. These include the 3,4-epoxybutyl-1-phosphonate and a phosphate analogue of glycerol-3-phosphate with the related homodiglyceride. Investigations are continuing in the evaluation of the biological activity of the materials synthesized. A method is also described for the synthesis of the carbon-14 labelled 3-carboxy-3-hydroxybutyl-1-phosphonic acid, an analogue of phosphoglyceric acid known to serve as a substitute for the natural material in several biochemical processes.

The preparation of phosphonic acids having structures related to those of natural organic phosphates and their use as probes of biological mechanisms have been topics of interest for some time now.[1] Previous efforts of this laboratory have established the biological significance of phosphonic acids which are isosteric analogues of natural organic phosphates.[2–12] Of particular value for *in vivo* as well as *in vitro* investigations has been 3,4 - dihydroxybutyl - 1 - phosphonic acid (1), the isosteric analogue of glycerol-3-phosphate. This material has been found to be bacteriostatic at low concentrations to *Escherichia coli* and *Bacillus subtilus* 168 and bactericidal to *Bacillus subtilus* strain W23.[3,11]

$$H_2O_3PCH_2CH_2CH(OH)CH_2OH$$

1

$$H_2O_3PCH_2CH(OH)CH_2OH$$

2

While there are now noted in the literature four routes for the synthesis of 1[2,5,13,14] a further method was desired which would allow the preparation of the material as either of its pure enantiomers in sizable quantity and at the same time would provide convenient intermediates for the synthesis, in optically active form, of related analogues of interest. In the course of our biochemical investigations it has become of interest to examine analogues other than the phosphonic acids isosteric with the natural phosphates. In particular, it is desirable that functional groups other than the vicinal diol linkage be studied as well as variation in distance between hydroxyl and phosphoryl sites.

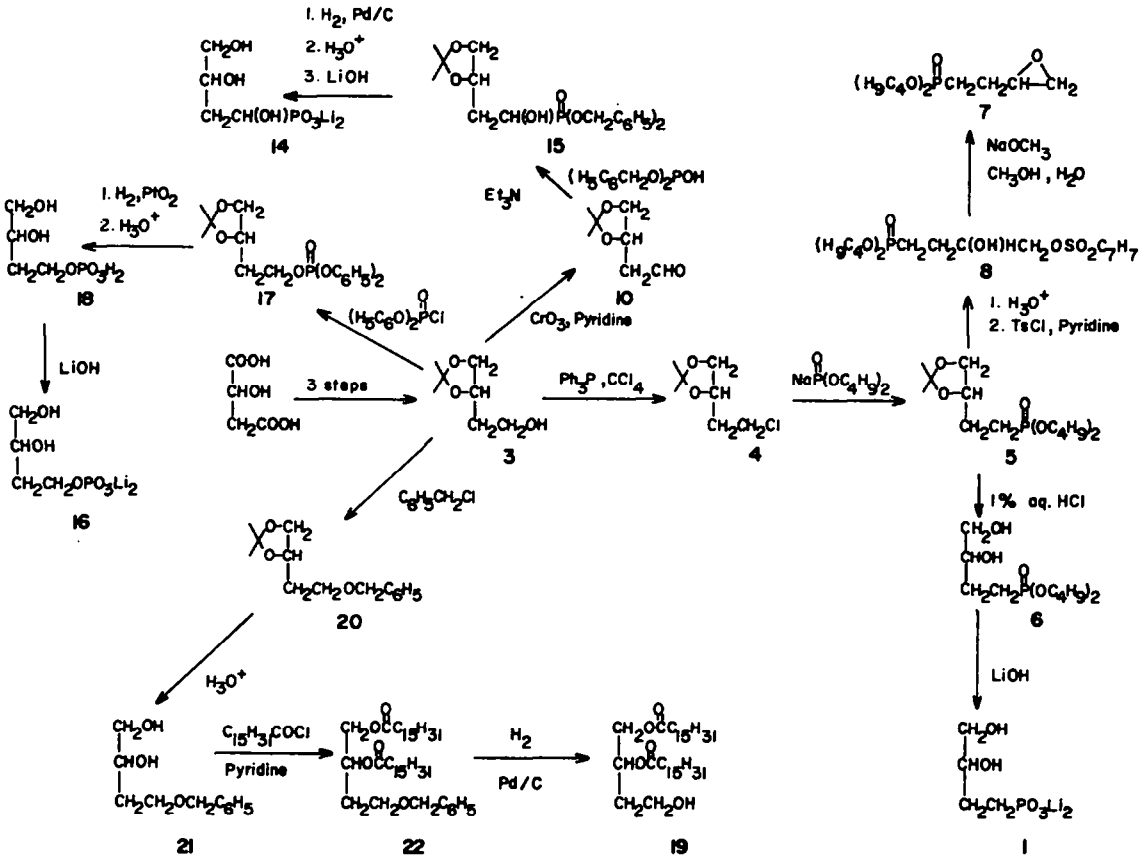
Prior efforts[3,4,15,16] indicated that the "shortened" non-isosteric phosphonic acid analogue of glycerol-3-phosphate, 2,3 - dihydroxypropyl - 1 - phosphonic acid (2), was not of significant value for *in vivo* studies, nor was it particularly efficient as studied by *in vitro* enzymatic reaction. Presumably its compacted structure hindered its interaction with the active sites of the enzymes involved. However, little is known about the geometric requirements in regard to analogues "lengthened" as compared to the natural material. It might be expected that the rotational flexibility of "lengthened" species would permit interaction in a manner similar to that of the natural material.

RESULTS AND DISCUSSION

Both enantiomers of 1 have been prepared starting with the commercially available optically active malic acids as illustrated in Scheme 1. For the (S)-enantiomer of 3,4 - dihydroxybutyl - 1 - phosphonic acid (S-1), the first three steps from *l*-malic acid yielding the (S) - 1,2 - *O* - isopropylidenebutane - 1,2,4 - triol (S-3) have been performed according to the previously reported procedure of Hayashi *et al.*[17] In a similar manner has now been prepared the (R)-enantiomer of 3,4-dihydroxybutyl-1-phosphonic acid (R-1); a synthesis of the racemic material, more convenient and proceeding in higher yields than those previously reported, begins with the commercially available butane-1,2,4-triol.

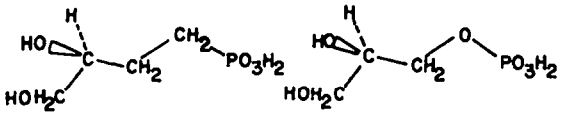
Attempts at the phosphonylation of 4 by Michaelis–Arbuzov reactions using either of several phosphite reagents gave results which were less than desirable. Purifications of the phosphonates (5) were hindered by their apparent partial decomposition upon attempts at distillation; as spectral data on the crude materials indicated them to be of *ca.* 95% purity, the reaction sequences were continued without further attempts at purification.

The compound S-1 obtained starting from *l*-malic acid is of the same absolute configuration about the internal OH as *sn*-glycerol-3-phosphate and would be expected to exhibit significantly different properties in biological systems as compared to its enantiomer or the racemic modification. This in fact has been observed. Compound S-1 exhibits activity in the inhibition of growth of



Scheme 1.

Escherichia coli strain 8 and *Bacillus subtilus* strains BD 170 and 1005 of twice the magnitude using racemic material.[18] It should be noted that the enantiomer (R-1) exhibits inhibition of about 8% the magnitude as (S-1); this is presumed to be due to the presence of approximately 8% (S-1) in the R-1 preparation as the *d*-malic acid used as starting material contains approximately 8% *l*-malic acid as an impurity.



S—1 sn-glycerol-3-phosphate

It has also been of interest to synthesize other phosphonates and phosphonic acids for use as analogues of glycerol-3-phosphate that the effect on metabolic processes of further structural variations might be observed. In light of the activity of (1R,2S)-1,2-epoxypropylphosphonic acid (phosphonomycin) as an antibacterial agent,[19] the introduction of an epoxide function in place of the vicinal diol of 1 appeared to be a reasonable structural choice for the generation of a new agent. The route for the generation of the phosphonate diester (7) is also illustrated in Scheme 1. This procedure as performed with racemic material is entirely applicable for the generation of optically active compounds.[20] For the initial investigations it was deemed most reasonable to prepare 7 as the racemate as it is not immediately obvious which enantiomer of 7 might be expected to exhibit biological activity.

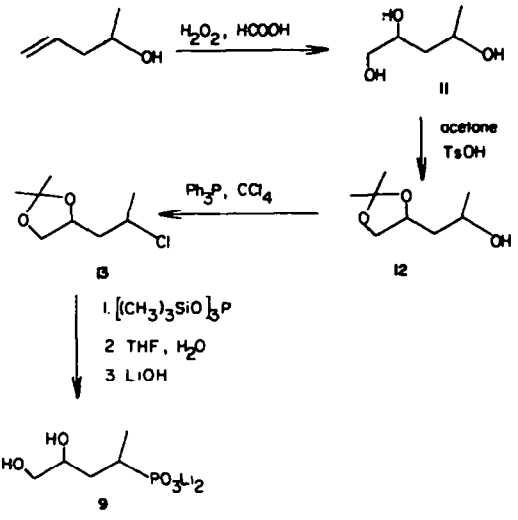
Three molecular systems have been prepared which are intended to accomplish metabolic regulation through the the introduction of structural entities capable of having selective steric interactions with the involved enzymes.

The general preparation of 1 - methyl - 3,4 - dihydroxybutyl - 1 - phosphonic acid, dilithium salt (9) is illustrated in Scheme 2 by a route which is fundamentally similar to that discussed above for the preparation of 1, although here the Michaelis–Arbuzov reaction of the alkyl chloride with tris(trimethylsilyl) phosphite[21–24] proceeded most favorably.

The second of these systems involves the introduction of a OH function α- to the phosphorus. The 1,3,4 - trihydroxybutyl - 1 - phosphonic acid, dilithium salt (14) is generated from S-10 as a pair of diastereoisomers as illustrated in Scheme 1.

The third of the systems incorporates steric variation from *sn*-glycerol-3-phosphate. The (S) - 3,4 - dihydroxybutyl - 1 - phosphate (S-16) is lengthened as regards the relationship between the phosphoryl and hydroxyl functions. The synthesis of S-16 from S-3 is illustrated in Scheme 1. An alternate route to the diester (S-17), displacement of chloride from S-4 using the silver salt of diphenyl phosphoric acid according to the method of Posternak,[26] gave results which were less than satisfactory.

Substitution of S-16 for *sn*-glycerol-3-phosphate in enzymatic processes for which the latter is a natural substrate would be expected to generate a variety of new compounds having their own significance. Of particular interest are a variety of lipids which could be generated.



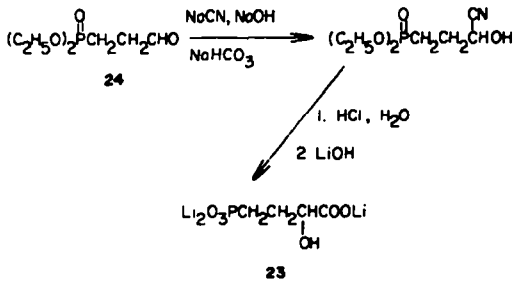
Scheme 2.

Unlike lipids related to the phosphonic acid analogue of glycerol-3-phosphate, previously synthesized in this laboratory,[27-29] phospholipids derived from S-16 would be expected to be susceptible to phosphate hydrolysis under non-enzymatic conditions if not enzymatic as well. Thus it was of interest to prepare the homodiglyceride (S-19) as illustrated in Scheme 1.

The isosteric phosphonic acid analogue of phosphoglyceric acid, 3 - carboxy - 3 - hydroxybutyl - 1 - phosphonic acid (23) has been found to interact with a variety of enzyme systems as a substitute for the natural substrate. These systems include the oxidation of NADH[30] and phosphorylation with phosphoglycerate kinase.[31] For the detailed investigation of the action of 23 with these and other enzyme systems, and *in vivo*, it has become necessary that 23 be available with a non-exchangeable isotopic label. The synthesis of 23 bearing a ^{14}C label at the carboxyl carbon can be accomplished using a modification of the approach of Pfeiffer *et al.*;[32] the variation necessarily introduced is a change from acidic to basic medium for addition as the major portion of label would be lost as gaseous material under the standard acidic conditions. Isolation of product can be performed by preparative paper chromatography. The route is outlined in Scheme 3.

EXPERIMENTAL

General. All chemicals were of reagent quality and used without further purification with the following exceptions: $CHCl_3$ and CH_2Cl_2 were distilled over P_2O_5; pyridine was dried over calcium hydride and distilled; palmitoyl chloride (commercial source) was distilled immediately prior to use; THF was distilled over LAH. Tlc was performed using Eastman Silica Gel Chromagram Sheet. Silica gel for preparative chromatography was from Baker (60–200 mesh). IR spectra were measured using a Perkin–Elmer 237-B spectrophotometer, and NMR spectra were measured with a Varian EM-360 instrument. Optical rotations were measured at 27° using a Rudolph polarimeter (Na-lamp) with a 1 dm cell.



Scheme 3.

Preparation of (S) - 1 - chloro - O - isopropylidenebutane - 3,4 - diol (S-4). Triphenylphosphine (2.08 g, 7.94 mmol) in 3.0 ml of CH_2Cl_2 was added dropwise over a period of 4 hr to a well stirred soln of 1.16 g (7.94 mmol) (S) - 1,2 - *O* - isopropylidenebutane - 1,2,4 - triol[17] and 1.85 g (12.0 mmol) CCl_4 in 2.0 ml CH_2Cl_2 at room temp. The mixture was stirred for 1 hr at which time there was added 60 ml of n-pentane and the resultant ppt of triphenylphosphine oxide was removed by filtration and washed with a further 60 ml n-pentane. The combined n-pentane solns were washed with sat. $NaHCO_3$aq, water, and brine, and dried over $MgSO_4$. After filtration to remove the drying agent the solvent was evaporated under reduced pressure and a further 60 ml of n-pentane were added to precipitate the last traces of triphenylphosphine oxide. This was filtered through glass wool and the solvent removed under reduced pressure to yield 1.12 g (85.7%) of pure S-4 as a light oil. Analysis indicated no further purification to be necessary; the material thus isolated exhibits a sharp single peak on glc using a 14 ft × 1/4 in. column of 20% HPL on Chromsorb W; NMR ($CDCl_3$) 1.37δ (3H, s), 1.40δ (3H, s), 1.97δ (2H, m), 3.63δ (3H, m), 4.09δ (2H, m); IR ($CHCl_3$, cm^{-1}) 3010–2780 (3 peaks), 1452, 1385, 1370, 1290, 1245, 1150, 1115, 1070; $[\alpha]_D^{27} = -14.3°$ (1 M, $CHCl_3$). (Found: C, 51.07; H, 7.87. Calc. for $C_7H_{13}O_2Cl$: C, 51.06; H, 7.90%).

Preparation of dibutyl (S) - O - isopropylidene - 3,4 - dihydroxybutyl - 1 - phosphonate (S-5). To metallic Na (1.26 g, 0.055 g-atom) in a dried flask under an anhyd. atmosphere was added 150 ml hexane (dried over Na). The hexane was heated to a gentle reflux, at which time there was added dibutyl phosphite (10.6 g, 55.0 mmol) dropwise over a period of 25 min. The heating was continued until all of the Na metal had dissolved. At this time there was added (S) - 1 - chloro - *O* - isopropylidenebutane - 3,4 - diol (9.06 g, 55.0 mmol) and the mixture continued to be heated at reflux for 48 hr. After cooling, the mixture was washed with water, the organic layer being separated, dried over $MgSO_4$, filtered, and evaporated under reduced pressure leaving an oil which was vacuum distilled (134°/0.05 Torr) to yield 7.65 g (45.0%) of S-5 as a colorless liquid. The material as isolated exhibited spectra in accord with the proposed structure although elemental analysis indicated the presence of a small impurity (*ca.* 5%) which could not be removed by distillation or chromatography. This possibly arises by cleavage of the dioxolane ring. The distilled material was deemed to be of sufficient purity to be used in the following step. Analytical data: NMR ($CDCl_3$) 0.98δ (3H, s), 1.44δ (3H, s), 1.21–2.56δ (12H, complex), 3.54δ (1H, m), 4.03δ (6H, complex); IR (between salts, cm^{-1}) 3025–2805, 1562, 1375, 1250, 1150, 1065, 1025, 975; $[\alpha]_D^{27} = -2.2°$ (1 M, MeOH).

Preparation of dilithium (S) - 3,4 - dihydroxybutyl - 1 - phosphonic acid (S-1). The dibutyl (S) - *O* - isopropylidene - 3,4 - dihydroxybutyl - 1 - phosphonate (5.49 g, 17.9 mmol) was stirred overnight with 120 ml 1% HCl after which the solvent was removed under reduced pressure without heating. The residual oil was refluxed with 100 ml 1.5 M LiOH for 48 hr during which time a white ppt began to form. To the mixture was added 100 ml of abs EtOH to complete the precipitation; the ppt was filtered off, washed with anhyd. ether, and dried under vacuum to yield 2.48 g (76.0%) of pure S-1. Spectral data (NMR, IR) of S-1 thus isolated corresponded with that for previous preparations of racemic material.[5] The optical rotation was measured by dissolving 0.95 g of S-1 in 4 ml of conc. HCl and diluting with abs EtOH to a total volume of 10 ml: $[\alpha]_D^{27} = +14.6°$; the salt was insufficiently soluble in non-acidic media for measurements to be made. (Found: C, 26.42; H, 5.12. Calc. for $C_4H_9PO_5Li_2$: C, 26.40; H, 4.99%).

Preparation of dilithium (R) - 3,4 - dihydroxybutyl - 1 - phosphonic acid (R-1). Commercial *d*-malic acid was converted to the dimethyl ester in 86.7% yield by the procedure previously described[17] for the (S)-enantiomer and exhibited $[\alpha]_D^{27} = +6.5°$.[33] The diester (26.2 g, 162 mmol) thus prepared was dissolved in

32 ml THF and added dropwise to 22.0 g (579 mmol) LAH in 1000 ml THF and heated at reflux for 18 hr. The excess hydride was decomposed by the addition of 180 ml of water. The resultant mixture was filtered and the filtrate concentrated to a thick oil under reduced pressure. This concentrate was purified by subjection to chromatography on a column of 60 g of silica gel being eluted with 660 ml (3:1, V:V) and 780 ml (2:1, V:V) $CHCl_3$–EtOH. The combined eluents were concentrated under reduced pressure and the residual oil vacuum distilled (135°/0.025 Torr) to yield 5.7 g (33.3%) of (R)-1,2,4-butanetriol which exhibited NMR and IR spectra identical to that for the (S)-enantiomer and $[\alpha]_D^{27} = +24.6°$ (2.6 M in MeOH) which compared with a value of $-25.0°$ as measured for the (S)-enantiomer under identical conditions. The triol (5.7 g, 54.0 mmol) was stirred in 450 ml of acetone in the presence of 3 g *p*-toluenesulfonic acid monohydrate at room temp. for 3.5 hr after which anhyd. $NaHCO_3$ was added and the stirring continued. The mixture was filtered and the solvent evaporated under reduced pressure to yield an oily residue which was dissolved in EtOAc, washed successively with $NaHCO_3$aq and brine and dried over $MgSO_4$. After filtration and removal of the solvent under reduced pressure the residue was vacuum distilled (83°/3.2 Torr) to yield 6.12 g (77.6%) of R-3 which exhibited NMR and IR spectra identical to those of the (S)-enantiomer. Of this material 4.64 g (31.8 mmol) were immediately treated in the same manner as described above for the (S)-enantiomer for conversion to R-4. There was thus obtained 3.0 g (57%) of R-4 as a light oil which exhibited chromatographic and spectral properties identical to those of the (S)-enantiomer and $[\alpha]_D^{27} = +15.1°$ (1 M, $CHCl_3$). For generation of the phosphonic acid, R-1, (3.00 g, 18.0 mmol) of R-4 were treated as described above for the (S)-enantiomer to yield on distillation (125°/0.05 Torr) 2.50 g (45.0%) of dibutyl (R) - *O* - isopropylidene - 3,4 - dihydroxybutyl - 1 - phosphonate (R-5) which exhibited IR and NMR spectra identical to those as observed for the (S)-enantiomer. The dioxolane (R-5) combined from two preparations (5.74 g, 18.7 mmol) was deprotected and hydrolyzed as already described for the (S)-enantiomer to yield 3.00 g (88.2%) of R-1 which exhibited NMR and IR spectra identical to those of the (S)-enantiomer. The optical rotation, measured in the same manner as for the (S)-enantiomer, exhibited $[\alpha]_D^{27} = -13.5°$. (Found: C, 26.38; H, 5.23. Calc. for $C_4H_9PO_5Li_2$: C, 26.40; H, 4.99%).

Preparation of dilithium (R,S) - 3,4 - dihydroxybutyl - 1 - phosphonic acid (1). Racemic 1,2,4-butanetriol (63.6 g, 600 mmol) was treated in an identical manner as described above for the (R)-enantiomer to yield on distillation (76°/1 Torr) 60.0 g (68.5%) of 3 which corresponded in IR and NMR spectra with the enantiomeric forms described previously. Conversion of 40.6 g (278 mmol) of racemic dioxolane (3) to the chloride was preformed in an identical manner to that as described for the enantiomeric forms to yield 32.5 g (71.1%) of 4. The chloride 4, (23.0 g, 140 mmol), was then phosphonylated using a soln of sodium dibutyl phosphite in hexane as described above for the (S)-enantiomer. In this way was obtained 25.6 g (59.7%) of 5 upon vacuum distillation (130°/0.1 Torr). The diester (5) was converted in the manner described for the enantiomeric forms in 71% yield to the racemic 1 which corresponds in all spectral and chromatographic properties with that as prepared by other routes.[2,5]

Preparation of dibutyl (R,S) - 4 - tosyloxy - 3 - hydroxybutyl - 1 - phosphonate (8). The racemic dioxolane (5), 15.0 g (49.0 mmol), was stirred with 330 ml 1% HCl, after which the solvent was removed under reduced pressure. The residual oil was dissolved in 400 ml $CHCl_3$, washed with water (3 × 100 ml) and dried over $MgSO_4$. After filtration the solvent was evaporated under reduced pressure to yield 11.0 g of a yellow oil which was dissolved in 13 ml pyridine, cooled to 0°, and added dropwise to a cooled (0°) soln of 7.90 g (41.4 mmol) *p*-toluenesulfonyl chloride in 96 ml benzene. The mixture was allowed to come to room temp. and was stirred for 48 hr after which there was added a further 120 ml benzene and the mixture washed successively with 2 N HCl (3 × 130 ml), sat $NaHCO_3$aq (3 × 60 ml), water (4 × 140 ml) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the residual oil taken up in a minimum volume of 1:1 EtOAc–$CHCl_3$ and passed through a short (20 g) column of silica gel. After removal of the solvent under reduced pressure there was isolated 2.24 g (12.6%) of 8 as an oil which exhibited a single spot of $R_f = 0.34$ upon tlc developed with 1:1 EtOAc–$CHCl_3$. Spectral analysis: NMR ($CDCl_3$) 0.61–1.92δ (18H, complex, $CH_3CH_2CH_2$– and PCH_2CH_2–), 2.15δ (3H, s), 3.30–4.02δ (7H, complex, OCH– and OCH_2), 4.39δ (1H, s), 6.86–7.60δ (4H, AA'BB'); IR (between salts, cm^{-1}) 3130–3750, 2825–3090, 1600, 1472, 1370, 1248, 1200, 1185, 1105.

Preparation of dibutyl (R,S) - 3,4 - epoxybutyl - 1 - phosphonate (7). To a soln of 8 (2.24 g, 5.34 mmol) freshly prepared in 8 ml MeOH was added a soln of 0.28 g (5.35 mmol) of NaOMe in 1.1 ml water. The mixture was refluxed for 3 hr, cooled, and the solvent removed under reduced pressure. To the residue was added 100 ml ether and the resultant ppt of sodium tosylate removed by filtration under suction. The soln was washed with water (3 × 25 ml), dried over $MgSO_4$, and the solvent removed under reduced pressure to yield an oil which was vacuum distilled (108°/0.05 Torr) giving 0.90 g (63.7%) of pure 7 which exhibited a single spot of $R_f = 0.46$ upon tlc with 1:1 EtOAc–$CHCl_3$ and spectral data in accord with that for a terminal epoxide.[34] Spectral analysis: NMR ($CDCl_3$) 0.76–1.80δ (18H, complex, $CH_3CH_2CH_2$– and PCH_2CH_2–), 2.02δ (1H, t), 2.27δ (1H, t), 2.55δ (1H, m), 3.59δ (4H, dt); IR (between salts, cm^{-1}) 2815–3050, 1470, 1252, 1155, 1122, 1070, 1030, 980. (Found: C, 54.68; H, 9.37. Calc. for $C_{12}H_{25}PO_4$: C, 54.53; H, 9.53%).

Preparation of (S) - 1 - oxo - O - isopropylidenebutane - 2,3 - diol (S-10). The primary alcohol S-3 (8.76 g, 60.0 mmol) dissolved in 30 ml CH_2Cl_2, was added in a single portion to a soln of CrO_3–pyridine complex, prepared by the addition of 36.0 g (360 mmol) CrO_3 to 58.2 ml pyridine with vigorous stirring at 0°, and allowed to come to room temp. with stirring over a 12 hr period. The solvent was evaporated under reduced pressure and 1400 ml ether were added to the residue. The insoluble salts were removed by filtration and the filtrate was washed successively with 5% $NaHCO_3$aq, $CuSO_4$aq, brine, and water and dried over $NaSO_4$. The ether was removed under reduced pressure and the residual liquid vacuum distilled (74°/4.7 Torr) to yield 2.26 g (26.2%) of pure S-10. Spectral analysis: NMR ($CDCl_3$) 1.39δ (3H, s), 1.42δ (3H, s), 2.77δ (2H, dd, $J_A = 6$ Hz, $J_B = 1.5$ Hz), 3.60δ (1H, dd, $J_C = 8$ Hz, $J_D = 8$ Hz), 4.20δ (1H, dd, $J_C = 8$ Hz, $J_B = 6$ Hz), 4.59δ (1H, m), 9.80δ (1H, d, $J_B = 1.5$ Hz); IR ($CHCl_3$, cm^{-1}) 2840–3080, 2765, 1725, 1452, 1385, 1370, 1345, 1325, 1250, 1152, 1065; $[\alpha]_D^{27} = +13.3°$ (1 M in $CHCl_3$). (Found: C, 57.95; H, 8.46. Calc. for $C_7H_{12}O_3$: C, 58.32; H, 8.39%).

Preparation of dibenzyl (1RS,3S) - 3,4 - O - isopropylidene - 1,3,4 - trihydroxybutyl - 1 - phosphonate (15). A soln of 6.54 g (26.6 mmol) dibenzyl hydrogen phosphite[35] with 3.02 g (20.1 mmol) of S-10 and 1 ml triethylamine in 60 ml dry benzene was heated at 60–80° for 4 hr. The mixture was diluted with 400 ml benzene and washed successively with sat $NaHCO_3$aq (2 × 120 ml), 1 N HCl (2 × 120 ml), water (2 × 120 ml) and dried over $MgSO_4$. Upon evaporation of the solvent there was obtained 5.72 g (69.8%) of 15 as an oil which exhibited a single spot of $R_f = 0.36$ on tlc with 5:5:1 benzene–ethyl ether–ethanol. From the two sets of doublets for benzylic hydrogens in the NMR it is estimated that the two diastereoisomers are present in a 4:1 ratio. Spectral analysis: NMR ($CDCl_3$) 1.30δ (6H, broad singlet), 2.00δ (2H, m), 3.51δ (1H, t), 3.84–4.49δ (3H, complex, OCH_2, OCH), 4.55δ (1H, s), 4.85–5.18δ (4H, pair of doublets for benzylic hydrogens), 7.30δ (10H, s); IR (between salts, cm^{-1}) 3160–3730, 3790–3110, 1505, 1460, 1375, 1370, 1225, 1160, 1035; $[\alpha]_D^{27} = +0.7°$ (0.4 M in $CHCl_3$). (Found: C, 62.33; H, 6.45. Calc. for $C_{21}H_{27}PO_6$: C, 62.06; H, 6.09%).

Preparation of (1RS,3S) - 1,3,4 - trihydroxybutylphosphonic acid (14). The dibenzyl phosphonate (15) (5.0 g; 12.3 mmol), was dissolved in 130 ml abs EtOH and hydrogenated on a Parr apparatus over 200 mg 10% Pd/C at 40 psig H_2 at room temp. until no more H_2 was absorbed. The catalyst was removed by filtration through Celite and filtrate was evaporated under reduced pressure to yield 2.0 g (87%) of the free acid (14) which exhibited a single spot on tlc and no benzylic or Me protons in the NMR. For analysis the free acid was converted to the dilithium salt by being dissolved in EtOH and treated with

ethanolic LiOH until basic. The resultant ppt was filtered off, washed several times with EtOH and ether, and dried under vacuum. Spectral analysis: NMR ($D_2O + CD_3COOD$) 2.65δ (2H, broad), 3.85–5.10δ (4H, complex); IR (KBr, cm^{-1}) 3040–3750, 2850–3000, 1650, 1435; $[\alpha]_D^{27} = -6.92°$ (0.52 g in 2 ml conc. HCl diluted to 10 ml). (Found: C, 24.34; H, 4.55. Calc. for $C_4H_9PO_6Li_2$: C, 24.27; H, 4.58%).

Preparation of 1,2,4-*pentanetriol* (11). The racemic triol was prepared in 62% yield using a modification of the method described by Birkofer and Beckmann.[36]

Preparation of 1,2 - O - *isopropylidenepentane* - 1,2,4 - *triol* (12). The triol (11), 20.4 g (169 mmol), was stirred in 1000 ml acetone with 2 g *p*-toluenesulfonic acid monohydrate at room temp. for 14 hr. $NaHCO_3$ was suspended in the mixture, stirred for 2 hr, and filtered. The acetone was removed under reduced pressure and the residue dissolved in 150 ml EtOAc which was then washed successively with sat $NaHCO_3$aq, brine, and water and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure the residual liquid was vacuum distilled (86°/1.7 Torr) to yield 21.3 g (78.7%) pure 12. Spectral analysis: NMR ($CDCl_3$) 1.20δ (3H, d, $J_A = 6$ Hz), 1.35δ (3H, s), 1.41δ (3H, s), 1.69δ (2H, dd, $J_B = J_C = 5.5$ Hz), 3.16δ (1H, s), 3.39–4.55δ (4H, m). (Found: C, 59.89; H, 10.06. Calc. for $C_8H_{16}O_3$: C, 59.97; H, 10.10%).

Preparation of 4 - *chloro* - O - *isopropylidenepentane* - 1,2 - *diol* (13). To a well stirred soln of 12 (16.0 g, 100 mmol) with 23.3 g (151 mmol) CCl_4 in 25 ml CH_2Cl_2 was added dropwise at room temp. over a period of 4 hr a soln of 26.3 g (100 mmol) triphenylphosphine in 38 ml CH_2Cl_2. The mixture was allowed to stir for 6 days after which 720 ml n-pentane were added and the resultant ppt of triphenylphosphine oxide was filtered. The filtrate was washed successively with 300 ml each of sat $NaHCO_3$aq, brine, and water and then dried over $MgSO_4$. The solvent was removed under reduced pressure to yield 12.6 g (70.6%) of pure 13. Spectral analysis: NMR ($CDCl_3$) 1.00–2.38δ (11H, complex, $-CH_2-$, $-C(CH_3)_2$, $-CH_3$), 3.38–4.56δ (4H, complex, OCH, OCH_2, ClCH);[37] IR (between salts, cm^{-1}) 2735–3010, 1430, 1370, 1250, 1195, 1052. (Found: C, 53.93; H, 8.20. Calc. for $C_8H_{15}O_2Cl$: C, 53.78; H, 8.46%).

Preparation of dilithium 1 - *methyl* - 3,4 - *dihydroxybutyl* - 1 - *phosphonic acid* (9). A mixture of 13 (8.3, 35 mmol) and 42 g (140 mmol) tris(trimethylsilyl) phosphite[21–24] was stirred under a N_2 at 170° for 3 days. At this time the excess phosphite was removed under reduced pressure and a mixture of 40 ml water and 230 ml THF was added and heated at reflux for 16 hr. After cooling, the mixture was decolorized with charcoal and filtered. The filtrate was concentrated under reduced pressure and ethanolic LiOHaq was added to pH = 8. The resultant ppt was filtered off, washed with EtOH and ether, and dried under vacuum to give 2.83 g of crude 9. The crude material was reprecipitated from aqueous soln by addition of EtOH to yield 1.24 g (18.1%) of 9 which exhibited a single spot of $R_f = 0.62$ on tlc with 10:1 MeOH–0.1 N HCl and gave acceptable analytical data. Spectral analysis: NMR (5% CD_3COOD in D_2O) 1.32δ (3H, broadened singlet), 1.42–2.29δ (3H, m), 3.41–4.48δ (3H, m); IR (KBr, cm^{-1}) 3200–3800, 2750–3080, 1650, 1430, 1320. (Found: C, 30.58; H, 5.64. Calc. for $C_5H_{11}PO_5Li_2$: C, 30.64; H, 5.66%).

Preparation of dilithium (*S*) - 3,4 - *dihydroxybutyl* - 1 - *phosphate* (*S*-16). To S-3 (2.92, 22.0 mmol) in 12 ml pyridine cooled to 0° was added dropwise 7.14 g (25.0 mmol) diphenyl phosphorochloridate. After standing for 12 hr at 0°, 1.2 ml water was added and the mixture concentrated under reduced pressure. The residual solid was dissolved in 150 ml benzene and washed successively with 100 ml each water, cold 1 N HCl, cold 1 N $KHCO_3$, and water and dried over $MgSO_4$. After evaporation of the solvent under reduced pressure the residual solid was dissolved in 150 ml abs EtOH and hydrogenated over 100 mg PtO_2 in a Parr apparatus at 52 psig of H_2 until no more H_2 was taken up. The catalyst was removed by filtration through celite and the solvent evaporated under reduced pressure to yield the free acid as a viscous oil. For analysis, the free acid was converted to its dilithium salt (S-16) by dissolution in 50 ml of water and the addition of a saturated ethanolic soln of $LiOH \cdot H_2O$ to pH = 8. The resultant ppt was filtered off, washed with EtOH and ether, and dried under vacuum to yield 1.53 g (33.6%) of S-16 as a crystalline material which analyzed as the hemihydrate; this water is firmly bound and could not be removed by heating the material under vacuum. The material exhibited a single spot of $R_f = 0.50$ on tlc using 10:1 MeOH–0.01 N HCl. Spectral analysis: NMR (5% CD_3COOD in D_2O) 1.67δ (2H, m), 3.15–4.30δ (5H, complex); IR (KBr, cm^{-1}) 3050–3850, 2760–3015, 1650, 1455, 1540, 1390, 1340, 1260, 1210; $[\alpha]_D^{27} = -20.4$ (free acid, 0.7 M in EtOH). (Found: C, 23.35; H, 4.46. Calc. for $C_4H_9PO_6Li_2 \cdot 1/2\,H_2O$: C, 23.19; H, 4.34%).

Preparation of (*S*) - 4 - *benzyloxybutane* - 1,2 - *diol* (*S*-21). To S-3 (5.25 g, 36.6 mmol) in 20 ml dry DMF was added 2.47 g finely powdered KOH with stirring and cooling to 0°. Benzyl chloride, 5.80 g (45.9 mmol), was added and the mixture allowed to warm, ultimately being heated at 70° for 6 hr. After cooling, 50 ml water were added and the mixture extracted with 4 × 35 ml $CHCl_3$. The combined extracts were washed with water, dried over $MgSO_4$, and evaporated under reduced pressure to give a yellow oil which was treated with 2 ml 2 N HCl and sufficient acetone to effect soln. After heating at reflux for 1.5 hr the mixture was cooled, 20 ml EtOH were added, and the solvent removed under reduced pressure. The residue was dissolved in 55 ml $CHCl_3$, washed with 3 × 12 ml water, dried over Na_2SO_4, and the solvent removed under reduced pressure to yield 2.53 g (35.3%) of pure S-21 as an oil. Spectral analysis: NMR ($CDCl_3$) 1.69δ (2H, dt, $J_A = 6$ Hz, $J_B = 5.5$ Hz), 3.18–3.87δ (5H, complex), 4.03δ (2H, broad singlet), 4.41δ (2H, s) 7.24δ (5H, s); IR (between salts, cm^{-1}) 3160–3740, 2755–3120, 1500, 1460, 1365, 1320, 1215, 1095, 1035, 910, 870, 740, 698; $[\alpha]_D^{27} = -15.6°$ (0.3 M in MeOH). (Found: C, 67.16; H, 8.32. Calc. for $C_{11}H_{16}O_3$: C, 67.32; H, 8.22%).

Preparation of (*S*) - 4 - *benzyloxy* - 1,2 - *dipalmitoyloxybutane* (*S*-22). To a soln of S-21 (1.00 g, 5.10 mmol) in 2 ml pyridine, cooled to 0°, was added dropwise 3.30 g (12.0 mmol) palmitoyl chloride in 24 ml benzene. The mixture was allowed to come to room temp. and continue stirring for 24 hr at which time a further 25 ml benzene were added and the mixture washed successively with 3 × 7 ml 2 N HCl and 4 × 7 ml water and dried over $MgSO_4$. Upon evaporation of the solvent under reduced pressure there was obtained a white solid which was twice recrystallized from 95% EtOH to yield 1.54 g (44.9%) pure S-22 m.p. 40–41°. Spectral analysis: NMR ($CDCl_3$) 0.59–2.04δ (60H, complex), 2.08–2.59δ (4H, complex), 3.57δ (2H, t, J = 6 Hz), 4.25δ (2H, m), 4.51δ (2H, s), 5.29δ (1H, m), 7.32δ (5H, s); IR (KBr, cm^{-1}) 2830–3075, 1745, 1470, 1422, 1405, 1365, 1285, 1251, 1230, 1205, 1185, 1080, 1030, 975, 943; $[\alpha]_D^{27} = -9.4°$ (0.074 M in $CHCl_3$). (Found: C, 77.00; H, 11.17. Calc. for $C_{43}H_{76}O_5$: C, 76.73; H, 11.38%).

Preparation of (*S*) - 4 - *hydroxy* - 1,2 - *dipalmitoyloxybutane* (*S*-19). A soln of S-22 (0.503 g, 0.747 mmol) in 50 ml 1:1 MeOH–EtOAc with 100 mg 10% Pd–C was hydrogenated in a Parr apparatus at 40 psig of H_2 until no more H_2 was taken up. The catalyst was removed by filtration through Celite and the solvent evaporated under reduced pressure to yield a solid material which upon recrystallization from MeOH yielded 0.405 g (93.0%) of pure S-19 m.p. 58–60°. Spectral analysis: NMR ($CDCl_3$) 0.39–1.98δ (60H, complex), 2.03–2.70δ (4H, complex), 3.63δ (2H, m), 4.18δ (2H, m), 4.70δ(1H, s), 5.17δ (1H, m); IR ($CHCl_3$, cm^{-1}) 3240–3700, 3190, 2860–3050, 1740, 1472, 1425, 1385, 1320, 1280, 1185, 1065; $[\alpha]_D^{27} = -13.0°$ (0.05 M in $CHCl_3$). (Found: C, 73.93; H, 12.27. Calc. for $C_{36}H_{70}O_5$: C, 74.17; H, 12.10%).

Preparation of trilithium 3 - *carboxy* - 3 - *hydroxypropyl* - 1 - *phosphonic acid* (23). A soln of 142.5 mg (2.908 mmol) NaCN and 116.4 mg (2.908 mmol) NaOH in 5 ml water was frozen in a 15 ml round bottom flask and to it was added 564.2 mg (2.908 mmol) of 24[38] dissolved in 5 ml 0.5 M $NaHCO_3$aq. The flask was loosely stoppered, the frozen soln allowed to melt, after which time the flask is sealed and kept at room temp. for 4 days. After this time the mixture was heated at 80° for 5 hr and evaporated to near dryness under reduced pressure. To the residue was added 5 ml conc. HCl and the mixture heated at 130° for 16 hr after which it was again concentrated to near dryness. To the residue was added 10 ml abs EtOH, the mixture filtered, and saturated ethanolic LiOH added until precipitation was complete. The ppt was filtered, washed with EtOH and ether, and dried under

vacuum to yield 405.0 mg (53.5%) of 23 which exhibited spectral and chromatographic properties identical to those of previous preparations.[8,32] This method has proven entirely suitable for the preparation of trilithium 3 - [^{14}C] - carboxy - 3 - hydroxypropyl - 1 - phosphonic acid; the conditions preclude the loss of radioactive cyanide in the vapor form and allow the use of very small quantities necessary for preparations of high specific activity. For isolation of the labelled acid, paper chromatography (What No. 1) was used, eluting the labelled material beside a sample of unlabelled. The pertinent elution data is as follows: System No. 1; 2-butanol:ammonia:water 6:3:1, $R_f = 0.11$. System No. 2; 0.1 N HCl:MeOH 1:10, $R_f = 0.78$.

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