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SYNTHESIS OF PHOSPHATES AND PHOSPHATE ISOSTERES OF FURANOSE SUGARS AS POTENTIAL ENZYME INHIBITORS#

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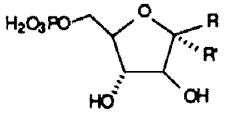
Abstract: Various D-furanose monosaccharides were synthesized as possible inhibitors of the gluconeogenic enzyme fructose 1,6-bisphosphatase. These included sulfamate, phosphoramidate, and epoxy analogues of the natural substrate, fructose 1,6-diphosphate (1), and arabinose and ribose analogues of a natural inhibitor, fructose 2,6-diphosphate (2). NMR studies were conducted to establish the stereochemistry of phosphate displacement at C1 in the synthesis of arabinose 1-phosphate derivatives. β-Ribose 1,5-diphosphate (35b) was prepared with >95% stereoselectivity.

In the absence of exogenous glucose, the life of animals and man is tightly linked to the maintenance of blood sugar through glucose biosynthesis from noncarbohydrate precursors. This process of "gluconeogenesis" is a major factor contributing to the increased glucose output in diabetes.[1] Consequently, specific inhibitors of gluconeogenesis could afford hypoglycemic agents useful for the treatment of this disease.[2] Of the four enzymes (pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase (FBPase), and glucose 6-phosphatase) that are unique to this pathway, compared to the glycolytic pathway,[3] FBPase (EC 3.1.3.11) particularly attracted our interest.[4] Thus, in 1978 we initiated a program to find inhibitors of this enzyme based on monosaccharide derivatives, in collaboration with Prof. Stephen Benkovic[5] of The Pennsylvania State University and Prof. Simon Pilkis of Vanderbilt University.[6] Various papers on chemical and biological results from our carbohydrate project have already been published.[7] In this paper, we discuss our undisclosed work on (1) the synthesis of substrate analogues of D-fructose 1,6-diphosphate, 1, containing sulfamate, phosphoramidate, and epoxy groups and (2) the synthesis of arabinose and ribose phosphates related to β-D-fructose 2,6-diphosphate,[7a,b] 2, a potent modulator of FBPase and 6-phosphofructo-1-kinase[3] (PFKase, EC 2.7.1.11).



1a R = $CH_2OPO_3H_2$, R' = OH
1b R = OH, R' = $CH_2OPO_3H_2$
2 R = OPO_3H_2, R' = CH_2OH
3a R = $CH_2OPO_3H_2$, R' = OMe
3b R = OMe, R' = $CH_2OPO_3H_2$
4a R = H, R' = $CH_2OPO_3H_2$
4b R = $CH_2OPO_3H_2$, R' = H
34a R = H, R' = OPO_3H_2
34b R = OPO_3H_2, R' = H

Results and Discussion

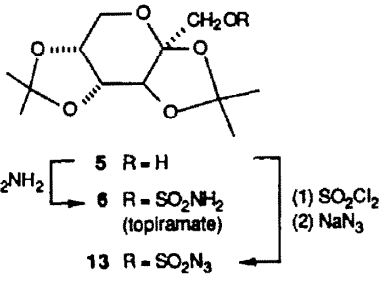
Compounds Related to 1. At the outset, we sought to synthesize substrate analogues of fructose 1,6-bisphosphate (1) as possible inhibitors. Benkovic et al. had reported that 3 and 4 are competitive inhibitors at concentrations < 1 mM by occupation of the active site,[8] which

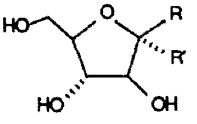
Dedicated to Professor Edward C. Taylor on the occasion of his 65th birthday.

indicated a tolerance for OMe and H in the place of OH at the anomeric position and a possible catalytic role for the C2 hydroxyl in the native substrate. Additionally, they suggested that FBPase inhibition was dependent on the furanose form of the sugar and on having OH groups at C3 and C4. This enzyme was also found to prefer the α form of the substrate (C2 α OH orientation and β $CH_2OPO_3^{-2}$; 1a), reflecting anomeric specificity.[9,10] Thus, we first examined substitution of the C1 phosphate group of 1 by a sulfamate (OSO_2NH_2) or phosphoramidate moiety, preferably in the orientation present in the natural substrate (viz. 1a). Then, we prepared potential irreversible inhibitors analogous to 1a, but bearing an oxirane functionality.

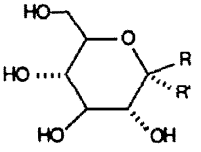
Diacetone fructose 5 was reacted with sulfamoyl chloride to give sulfamate 6, topiramate, which is undergoing clinical trials as an anticonvulsant agent (Scheme I).[7g] Treatment of 6 with methanolic HCl afforded a mixture of methyl glycosides comprised of 7a, 7b, 8a, and 8b in a 49:32:11:8 ratio.[7g] The presence of some pyranose forms did not pose a problem since phosphorylation of the primary hydroxyl was performed selectively. Unfortunately, various attempts to append a phosphoryl group to the oxygen at C6 in 7a/7b failed,[12] probably because of interference by the sulfamate NH_2 group (vide infra). A mixture of *N*,*N*-dimethylsulfamate derivatives, 9a:9b = 57:43 (no pyranose 10 was detected in the methanolysate), was successfully converted to corresponding phosphate diester 11 with diphenyl chlorophosphate and pyridine, suggesting that the SO_2NH_2 is incompatible with the phosphorylation procedures. Target 12 was then generated by hydrogenolysis (H_2/PtO_2).

Scheme I





7, 9, 14



8, 10, 15

7a, 8a R = $CH_2OSO_2NH_2$, R' = OMe

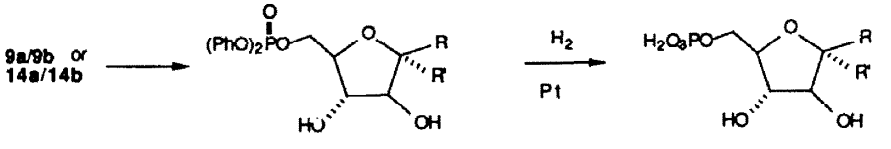
7b, 8b R = OMe, R' = $CH_2OSO_2NH_2$

9a, 10a R = $CH_2OSO_2NMe_2$, R' = OMe

9b, 10b R = OMe, R' = $CH_2OSO_2NMe_2$

14a, 15a R = $CH_2OSO_2N_3$, R' = OMe

14b, 15b R = OMe, R' = $CH_2OSO_2N_3$



11a R = $CH_2OSO_2NMe_2$, R' = OMe

11b R = OMe, R' = $CH_2OSO_2NMe_2$

16a R = $CH_2OSO_2N_3$, R' = OMe

16b R = OMe, R' = $CH_2OSO_2N_3$

12a R = $CH_2OSO_2NMe_2$, R' = OMe

12b R = OMe, R' = $CH_2OSO_2NMe_2$

17a R = $CH_2OSO_2NH_2$, R' = OMe

17b R = OMe, R' = $CH_2OSO_2NH_2$

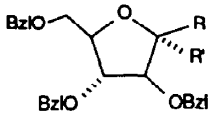
Given this outcome, we considered possible latent functionalities for the NH_2 unit, and choose to investigate the azido group. Azido sulfate **13** was synthesized by reacting **5** with sulfuryl chloride and pyridine, followed by sodium azide.[7g] Acid-catalyzed methanolysis of **13** afforded a mixture of methyl glycosides (**14a**:**14b**:**15a**:**15b** = 67:27:0:6), which was treated with diphenyl chlorophosphate/pyridine to give **16**. Compound **16** was deprotected by hydrogenolysis to give target **17**.

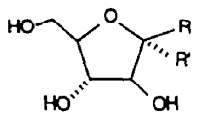
Known nitrile **18** (**18a**:**18b** = 1:5 ratio)[13] was reduced to amine **19** with lithium aluminum hydride (Scheme II). Desired amine **19** was reacted with phenyl dichlorophosphate in the presence of pyridine, and treated with methanol to form **20**. The benzyl groups were removed by transfer hydrogenation (cyclohexene and 10% Pd/C)[14] and the phenyl ester was hydrolyzed with calcium hydroxide to give monophosphoramidate **21** (**21a**:**21b** = 1:5).[15] Although **21** is formally an analogue of fructose 1-phosphate (and not fructose 1,6-diphosphate), it could be a substrate for hexokinase in vivo, and thereby be transformed into the 6-phosphate derivative.

Epoxyfructoside **22** (**22a**:**22b** = 3:1), obtained by dehydration of **23** with triphenylphosphine and diethyl azodicarboxylate,[16] was bisphosphorylated with diphenyl chlorophosphate in pyridine and hydrogenolyzed over platinum to furnish target **24** (**24a**:**24b** = 3:1; Scheme III). Reversal of the chemical sequence, i.e., phosphorylation of **23** followed by the dehydration procedure, was unsuccessful.

The synthesis of spiro-epoxide **25** was more involved (Scheme IV). Diacetone fructose **26**[7g] was oxidized to ketone **27** with oxalyl chloride and DMSO (85% yield, purified).[17] To obtain the desired β oxirane, **29**, olefin **30** was prepared by treatment of **27** with the lithium

Scheme II





18a R = H, R' = CN

18b R = CN, R' = H

19a R = H, R' = CH_2NH_2

19b R = CH_2NH_2, R' = H

20a R = H, R' = $CH_2NHP(O)(OMe)(OPh)$

20b R = $CH_2NHP(O)(OMe)(OPh)$, R' = H

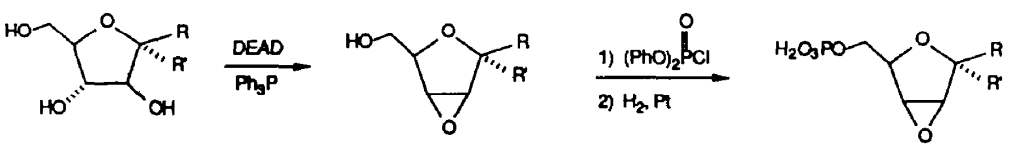
21a R = H, R' = $CH_2NHP(O)(OH)OMe$

21b R = $CH_2NHP(O)(OH)OMe$, R' = H

42a R = H, R' = OPO_3H_2

42b R = OPO_3H_2, R' = H

Scheme III



23a R = CH_2OH, R' = OMe

23b R = OMe, R' = CH_2OH

22a R = CH_2OH, R' = OMe

22b R = OMe, R' = CH_2OH

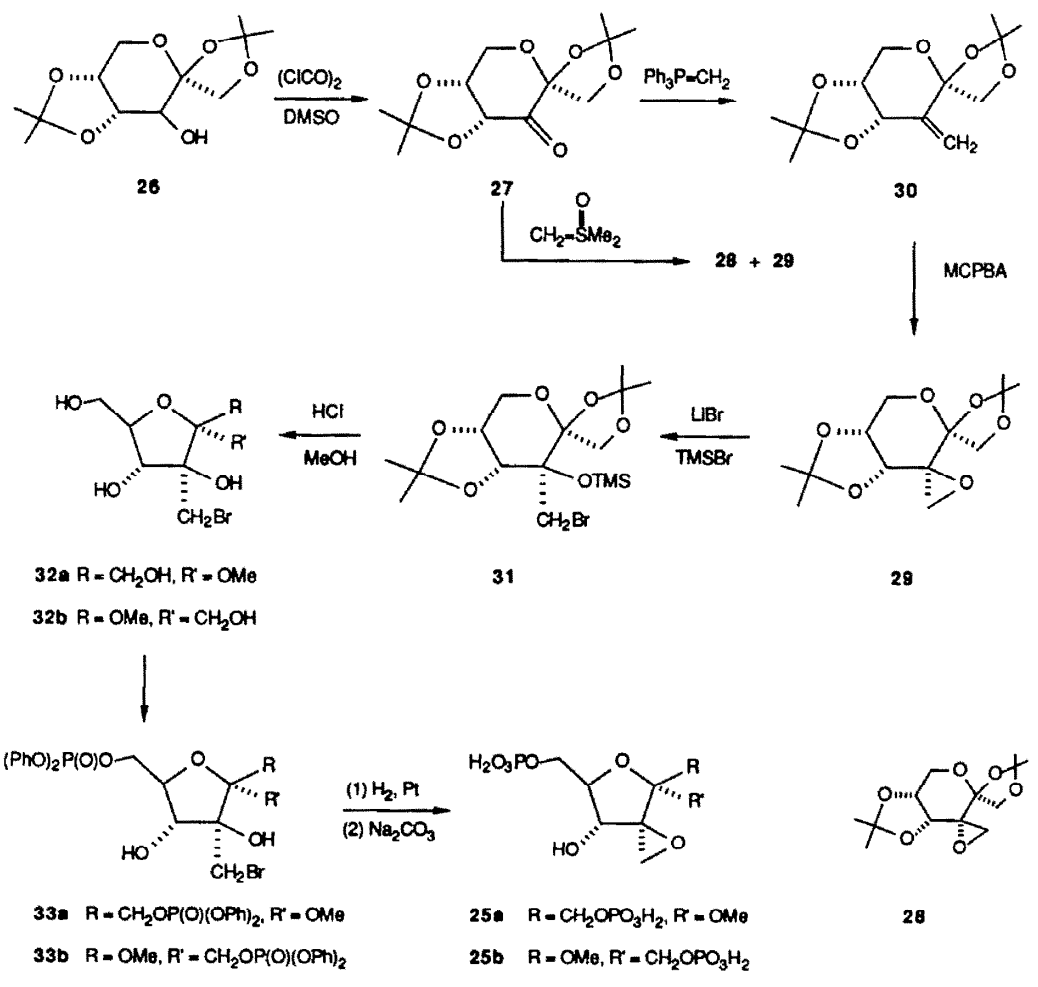
24a R = $CH_2OPO_3H_2$, R' = OMe

24b R = OMe, R' = $CH_2OPO_3H_2$

bromide adduct of methylenetriphenylphosphorane. Use of "salt-free" ylide, generated with sodium hexamethyldisilazide, resulted in destruction of the organic substrate and little formation of **30**, possibly because of an enolization-elimination process involving loss of the 4,5-acetonide moiety from **27** and subsequent fragmentation.[18] Epoxidation of **30** with MCPBA proceeded with exclusive (>95%) β facial selectivity, as anticipated from the stereochemistry of ketone-addition reactions,[19] to give **29**. The stereochemical assignment was tested by nucleophilic methylene transfer to ketone **27** by sulfur ylide reagents. Under kinetic control, we expected that addition would occur primarily from the β face of the molecule to afford the undesired α stereochemistry for the C3 oxygen.[19] Reaction of **27** with dimethylsulfonium methylide[20] in DMSO provided (in poor yield, presumably due to competing enolization-elimination) a single spiro-oxirane, assigned structure **28** (>95% addition to the β face). By contrast, reaction of **27** with dimethylsulfoxonium methylide,[20] a reagent known to favor thermodynamic control in addition reactions, furnished a mixture of **28** and **29** in a 3:2 ratio (40% yield).

Since the epoxide functionality would be sensitive to subsequent chemistry, we temporarily masked it as bromohydrin **31** by S_N2 ring-opening with LiBr in the presence of bromotrimethylsilane. Bromohydrin **31** was methanolyzed and the resulting mixture of methyl glycosides (**32a** and **32b**) was bisphosphorylated in the usual way to supply **33**, which was greatly enriched in **33a**. The four phenyl groups were then removed by hydrogenation over platinum, and the epoxide was regenerated with sodium carbonate to furnish **25a**.[21,22]

Scheme IV



26 **27** **30**

32a R = CH_2OH, R' = OMe

32b R = OMe, R' = CH_2OH

31 **29**

33a R = $CH_2OP(O)(OPh)_2$, R' = OMe

33b R = OMe, R' = $CH_2OP(O)(OPh)_2$

25a R = $CH_2OPO_3H_2$, R' = OMe

25b R = OMe, R' = $CH_2OPO_3H_2$

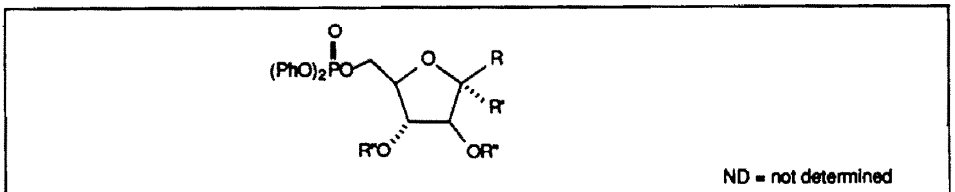
28

Compounds Related to 2. In 1980, **2** was recognized as a potent endogenous inhibitor of FBPase and activator of PFKase.[7a,b] Since this profile of activity was precisely the one that we desired, analogues of this agent were prepared. The tertiary anomeric phosphate of **2** is very hydrolytically[23] and enzymatically unstable.[7a,b,11] As an initial structural modification, we sought to replace the CH_2OH group with H and assay the biological activity. We synthesized arabinose[7a] (**34**; Scheme V) and ribose 1,5-diphosphates (**35**; Scheme VI), and a phosphonate isostere of β-D-arabinose 1,5-diphosphate (**34b**).[7c,d,24]

Highly stereoselective routes to the arabinose derivatives (**34**) originated with starting material **36** (Scheme V).[7a] There were two key aspects in these syntheses: (1) high stereocontrol in phosphate displacement at the anomeric center and (2) the judicious choice of protecting groups and their removal in a single step by Li-ammonia reduction, allowing the sensitive products to be isolated from the crude reaction mixtures without resort to chromatography. Specifics of the synthesis of **34a** and **34b** have been published,[7a] but we have now investigated the stereochemical course of the reaction sequence in detail by 360-MHz ^1H NMR analysis of the intermediates.

In the synthesis of arabinose 1,5-diphosphate (**34**), the anomeric phosphate group was introduced by dibenzyl phosphate displacement of an anomeric halide,[23b] and stereocontrol was determined by the nature of the protective group on C2.[7a] In the preparation of **34b** (**34a**:**34b** = 8:92), acetate **36** (**36a**:**36b** = 80:20) was converted in two steps to acetate **37** (**37a**:**37b** > 95:5), which was transformed into bromide **38** with HBr in methylene chloride [**38a**:**38b** = 82:18; 360-MHz ^1H NMR (C_6D_6) δ 3.90-4.55 (m, 9H), 6.21 (d, 0.18H, H_1 of **38b**, J = 3.3 Hz), 6.33 (br s,

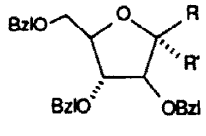
Scheme V



ND = not determined

a R = H, R' = OAc; b R = OAc, R' = H → a R = H, R' = Br; b R = Br, R' = H → a R = H, R' = OP(O)(OBzl)2; b R = OP(O)(OBzl)2, R' = H → (Li, NH_3) → **34**

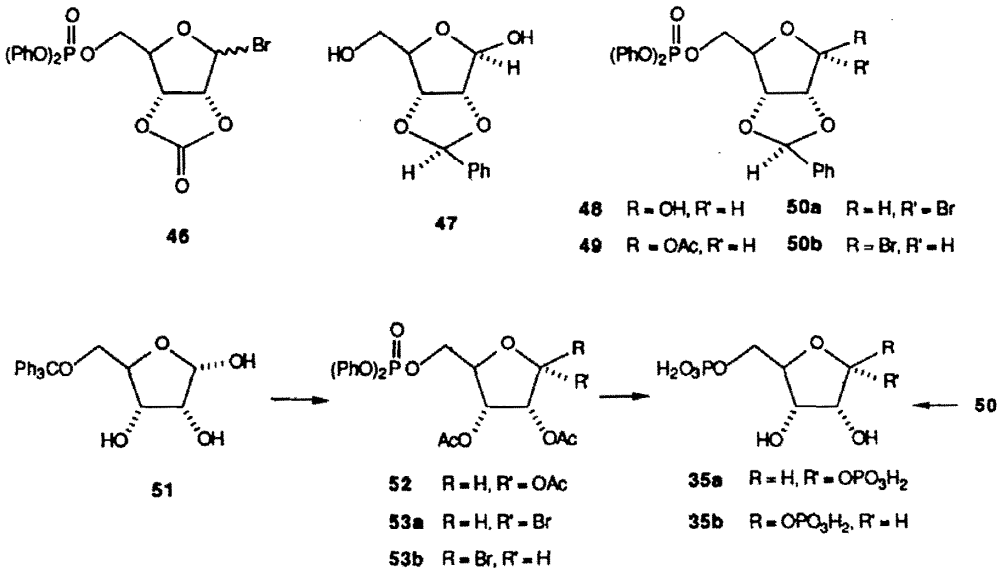
R" = Bzl	**37a**:**37b** > 95:5	**38a**:**38b** = 82:18	**39a**:**39b** = 16:84	**34a**:**34b** = 8:92
R" = Ac	**40a**:**40b** = 90:10	**41a**:**41b** > 95:5	**43a**:**43b** = ND	**34a**:**34b** = 86:14



a R = H, R' = OAc; b R = OAc, R' = H → a R = H, R' = Br; b R = Br, R' = H → a R = H, R' = OP(O)(OBzl)2; b R = OP(O)(OBzl)2, R' = H → **42**

36a:**36b** = 80:20	**44a**:**44b** = 87:13	**45a**:**45b** = 19:81	**42a**:**42b** = 14:86

Scheme VI



46 **47**

48 R = OH, R' = H **50a** R = H, R' = Br

49 R = OAc, R' = H **50b** R = Br, R' = H

51 **52** R = H, R' = OAc **35a** R = H, R' = OPO_3H_2

53a R = H, R' = Br **35b** R = OPO_3H_2, R' = H

53b R = Br, R' = H **50**

0.82H, H_1 of **38a**)]. The bromide had clearly equilibrated to a mixture of anomers enriched in the β isomer.[25] Displacement by dibenzyl phosphate proceeded with inversion of configuration at C1 to give a 16:84 ratio of **39a**:**39b** [360-MHz ^1H NMR (C_6D_6) δ 6.14 (dd, 0.84H, H1 in **39b**, J = 4.0 Hz, J_{PH} = 5.7 Hz), 6.19 (d, 0.16H, H1 in **39a**, J_{PH} = 4.7 Hz)], indicating that the stereochemistry of **39** had been established at the stage of bromide **38**. The extent of bromide equilibration could impart variability to the final product ratios in synthetic preparations, which was probably experienced when we obtained the 8:92 ratio of **34a**:**34b**.[7a] The synthesis of **34a** (**34a**:**34b** = 86:14) started with **40**, also prepared from **36**.[7a] Tetraacetate **40** (**40a**:**40b** = 90:10) was converted into bromide **41** [**41a**:**41b** = >95:5; 360-MHz ^1H NMR (C_6D_6) δ 6.10 (d, 0.02H, H1 in **41b**, J = ca. 3 Hz), 6.18 (s, 0.98H, H1 in **41a**)]. We relied on participation of the C2 acyl group to direct the phosphate displacement to the α face of the molecule (opposite to the β acyl group at C2). In a similar vein, Khorana had prepared arabinose 1-phosphate (**42**) by treatment of 2,3,5-tri-O-benzoyl-D-arabinofuranosyl bromide with triethylammonium dibenzyl phosphate, followed by hydrogenolysis and saponification.[23b] These researchers had similarly expected α phosphate addition due to anchimeric assistance of the C2 acyl group and, although they suspected the presence of some β isomer,[23b] they assigned their product as 90% or better **42a**. Later work by Aspinall and co-workers,[26] in which **42a** was prepared by the phosphoric acid fusion method, brought the reported high level of stereocontrol into question. Both of these earlier efforts relied heavily on optical rotation measurements to determine stereochemistry,[23b,26] whereas we have used ^{13}C and ^1H NMR to arrive at unambiguous isomer assignment and quantitation. Only a 43:57 ratio of **34a**:**34b** was obtained on treatment of **41** with dibenzyl phosphate (to give **43**), followed by deprotection with Li-ammonia.[7a] However, when **41** was treated with silver tetrafluoroborate prior to phosphate displacement, an 86:14 ratio of **34a**:**34b** was obtained. Although we were unsuccessful in monitoring the silver-assisted phosphate displacement by NMR, it is clear that some retention of configuration occurred in the reactions that produce **34a**, as **41** was almost exclusively the α isomer (**41a**); more retention was realized in the silver-assisted displacement reaction. The inability of the C2 acyl group to induce more backside attack in the absence of silver ion is surprising, but the result agrees with Aspinall's observations in the preparation of **42**.

We also investigated the analogous conversion of **36** to **42** by 360-MHz ^{1}H NMR, looking at the stereochemistry of each intermediate in the synthesis. Acetate **36** was converted into bromide **44** (**44a**:**44b** = 87:13), which was then displaced with dibenzyl phosphate to give **45** (**45a**:**45b** = 19:81). The nearly correspondent but opposite ratio of the stereoisomers of **44** and **45** argues for inversion of configuration at C1 in the phosphate displacement, in the same manner as for **39** (above). A small amount of unreacted **44** remaining had an unchanged ratio of 89:11 (**44a**:**44b**). This suggests that the bromide isomers were not being equilibrated and selectively siphoned off during the reaction course. Deprotection of **45** with Li-ammonia generated **42** (**42a**:**42b** = 14:86).

Ribose 1,5-diphosphate (**35**) was prepared to examine the effect of varying the configuration of the C2 hydroxyl on the biological activity (Scheme VI). Khorana reported the preparation of α-ribose 1,5-diphosphate (**35a**),[27] in which the key step was the dibenzyl phosphate displacement of bromide **46** to give mainly the α isomer after deprotection. Preparation of α-ribose 1,5-diphosphate originated with 2,3-*O*-benzylidene-D-ribose (**47**). The primary hydroxyl group was phosphorylated yielding **48**, and the anomeric hydroxyl was converted to the acetate (**49**). Acetate **49** was transformed into bromide **50** (undetermined stereochemistry), reacted with dibenzyl phosphate, and deprotected with Li-ammonia to give a mixture of **35a**:**35b** in a 3:1 ratio. Although the benzylidene group on C2 was not expected to participate in the reaction in the manner of an acyl group,[29] a considerable amount of **35b** was formed. We suppose that the 2,3-benzylidene group imposed a conformational constraint on bromide **50** to influence the stereochemistry.[30]

β-Ribose 1,5-diphosphate (**35b**) was prepared starting from readily obtained 5-*O*-trityl-D-ribose (**51**).[31] Compound **51** was peracetylated, the trityl group was removed, and the resulting free hydroxyl was phosphorylated to give **52**, which was then converted to bromide **53** as before. Treatment of **53** with silver tetrafluoroborate followed by dibenzyl phosphate displacement and Li-ammonia deprotection gave **35b**. No **35a** could be detected in this preparation by 360-MHz ^{1}H and 15.1-MHz ^{13}C NMR, showing the efficiency of the silver-assisted phosphorylation method in promoting anchimeric assistance of the C2 acyl group.

Biological Results. The phosphates and phosphate isosteres described here were tested for their biological activity in *in vitro* assays. Compounds **12**, **17**, and **21** did not inhibit FBPase (K_i > 1 mM), although **12** was a weak inhibitor showing 25% inhibition at 1.5 mM, in the presence of 0.2 mM of fructose 1,6-diphosphate (**1**). The potential irreversible inhibitors **24** and **26** were incubated in the presence of Mg^{+2}, Mn^{+2}, or Zn^{+2} and FBPase for up to one hour prior to the addition of the substrate (**1**); however, no inhibition was obtained above that seen within two minutes after addition of the inhibitor to a running system. Compound **24** inhibited FBPase with approximately 50% activity at a concentration of 5.9 x 10^{-4} M, in the presence of 1.4 to 3.5 x 10^{-5} M of **1**. Both isomers of **34** were active in inhibiting FBPase and activating PFKase, as has been reported.[7a,b] They also inhibited 2,6-FBPase/PFKase, the bifunctional enzyme[11] that controls the levels of **2** *in vivo*. At a concentration of **2** and inorganic phosphate of 20 mM, **34a** (2 mM) inhibited 2,6-FBPase 28% and **34b** (2.5 mM) inhibited the enzyme 65%. At a concentration of fructose 6-phosphate of 0.2 M, **34a** (2.0 mM) inhibited 2,6-PFKase 28% and **34b** (2.5 mM) inhibited the enzyme 54%. Neither ribose compound, **35b** nor the preparation of **35** enriched in **35a**, inhibited FBPase, highlighting the importance of the C2 hydroxy configuration for the biological effect.[33]

Compounds **34a** and **34b** had many of the characteristics that we sought initially. They increased glycolysis by stimulation of PFKase and inhibited FBPase. Additionally, **34b** potentiated the AMP-induced inhibition of FBPase,[7b] and **34b** and **34a** were not hydrolyzed readily by either of the phosphatase enzymes that could degrade them. However, neither these, nor any of the other compounds prepared herein, were active in blocking gluconeogenesis in isolated hepatocytes. Presumably, such compounds with anionic phosphate groups are unable to cross cell membranes and gain access to the intracellular milieu.

Experimental Section

General Procedures. Proton NMR spectra were recorded on a Varian EM-390 (90 MHz) or Bruker AM-360 spectrometer (the latter so designated) with $CDCl_3$ as solvent, except as indicated, and Me_4Si as an internal reference. Compounds run in D_2O were referenced externally to dioxane (δ 3.53). NMR studies of the isomeric ratios of intermediates were conducted in 5-mm NMR tubes at 360 MHz at ambient probe temperature. NMR spectral data on **35** and **42** were accumulated in aqueous solutions containing a trace of added cyclohexylamine (pH 8) to prevent hydrolysis of the anomeric phosphates. ^{13}C NMR spectra were recorded on a JEOL FX60Q spectrometer in $CDCl_3$ at 15.1 MHz unless indicated otherwise; only proton-decoupled ^{13}C data are reported, along with multiplicities. For phosphate salts, the amount of accompanying amine was corroborated by NMR integration. Optical rotation measurements were conducted on a Perkin-Elmer Model 241 polarimeter. Elemental analysis were performed by Atlantic Microlab, Inc., Atlanta, GA, or Galbraith Laboratories, Knoxville, TN. TLC analysis was conducted on silica gel plates (0.8-μ thickness), which were visualized by UV, if appropriate, and by H_2SO_4 charring. All melting points are corrected.

Methyl 1-Dimethylsulfamoyl-D-fructofuranoside (9). The dimethylsulfamoyl analogue of **6**[7g] (15.0 g, 60 mmol) was treated with 7.9% methanolic hydrogen chloride (900 mL) at ambient temperature. After 4 d, the reaction mixture was cooled to 0 °C and treated with lead carbonate until basic. The mixture was filtered and concentrated in vacuo to yield a light tan syrup (11.9 g, 66%), $[\alpha]_D^{23}$ +10.6° (c 0.50, water). IR (neat) ν_{max} 1370, 1180 (OSO_2) cm^{-1}. ^1H NMR δ 2.85 (s, 6H, CH_3), 3.32 (s, 1.3H, β-OMe), 3.40 (s, 1.7H, α-OMe), 3.6-4.8 (m, 10H). ^{13}C NMR (D_2O) δ 40.1 (NMe), 41.0 (NMe, pyr), 50.9 (OMe), 51.4 (OMe), 63.5, 64.5, 66.4 (pyr), 68.8, 69.5, 70.3 (pyr), 70.4 (pyr), 71.1 (pyr), 71.6 (pyr), 76.7, 79.4, 79.7, 82.2, 83.6, 86.2, 103.6 (s, C2 of **9b**), 108.7 (s, C2 of **9a**); integration of the C2 signals indicated a 57:43 ratio of **9a** and **9b**; only a small amount of **10** was present. Anal. Calcd for $C_9H_{19}NO_8S \cdot 0.5H_2O$: C, 34.83; H, 6.50; H_2O, 2.90. Found: C, 35.14; H, 6.50; H_2O, 3.27.

Methyl 1-Dimethylsulfamoyl-D-fructofuranoside 6-Diphenylphosphate (11). The preparation of **9** above (7.6 g, 25 mmol) was dissolved in 150 mL of pyridine and treated with a solution of diphenyl chlorophosphate (7.6 g, 28 mmol) in 35 mL of toluene at -20 °C. The mixture was stirred at -5 °C for 2 h and then concentrated under vacuum to an oil, which was treated with water and extracted into chloroform. The organic extract was washed with 5% H_2SO_4, 2.5% $NaHCO_3$, and brine; dried (Na_2SO_4), filtered, and concentrated to give a syrup. This substance was purified on a dry silica gel column (EtOAc/hexane, 4:1) twice to give a pale yellow syrup (2.31 g, 17%), $[\alpha]_D^{23}$ +16.5° (c 0.33, MeOH). IR (neat) ν_{max} 1370, 1280 (P=O), 1180 (OSO_2), 960 cm^{-1}. ^1H NMR δ 2.85 (s, 6H, NMe), 3.32, (s, 1.4H, β-OMe), 3.40 (s, 1.6H, α-OMe), 3.9-4.3 (m, 9H), 7.3 (m, 10H). ^{13}C NMR δ 38.5 (q, NMe), 48.9 (q), 49.5 (q), 63.1 (t), 66.4 (t), 68.3 (dt, C6, J_{PC} = 5.9 Hz), 75.7 (d), 78.3 (d), 78.8 (d), 79.6 (d), 79.8 (d), 84.7 (dd, C5, J_{PC} = 7.8 Hz), 101.6 (s, C2 of **11b**), 108.1 (s, C2 of **11a**), 119.9 (d), 120.3 (d), 125.7 (d), 129.9 (d), 150.4 (ds, 2C, J_{PC} = 6.8 Hz); integration indicated a mixture of **11a** and **11b** in a ratio of 53:47. Anal. Calcd for $C_{21}H_{28}NO_{11}PS \cdot 0.6H_2O$: C, 46.34; H, 5.41; N, 2.57; H_2O, 1.99. Found: C, 46.56; H, 5.45; N, 2.63; H_2O, 2.14.

Methyl 1-Dimethylsulfamoyl-D-fructofuranoside 6-Phosphate (12). A mixture of **11** (2.48 g, 4.7 mmol, α/β = 3:1) in 100 mL of MeOH was shaken with 2.46 g, of platinum oxide under 40 psig of hydrogen for 18 h. The solution was filtered and concentrated to give **12** as a syrup (1.48 g, 83.5%), $[\alpha]_D^{23}$ +28.7° (c 0.60, MeOH). IR (neat) ν_{max} 1380, 1180 (OSO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ 2.8 (s, 3H, NMe), 3.25 (s, 1H, β-OMe), 3.30 (s, 2H, α-OMe), 3.5-4.1 (m, 7H), 5.0 (br s, 4H, OH). ^{13}C NMR (D_2O) δ 38.7 (NMe), 49.7 (q), 50.3 (q), 65.9 (t), 66.3 (t), 68.2 (d), 74.9 (d), 77.8 (d), 78.2 (d), 80.8 (d), 82.7 (d), 83.2 (d), 102.4 (C2 of **12b**), 107.3 (C2 of **12a**); integration of C2 signals gave a 74:26 ratio of **12a**:**12b**. Anal. Calcd for $C_9H_{20}NO_{11}S \cdot 1.1H_2O$: C, 27.07; H, 5.55; N, 3.51; H_2O, 4.51. Found: C, 26.95; H, 5.59; N, 3.46; H_2O, 4.94.

Methyl D-Fructofuranoside 1-Azidosulfate (14). Azide **13** (15.39 g, 42 mmol) was

methanolyzed as described above for the preparation of 9, and the resulting crude material was purified on a dry silica gel column (EtOAc/MeOH, 4:1) to give a pale yellow oil (6.68 g, 44%), $[\alpha]_D^{23}$ +11.8° (c 0.40, water). IR (neat) ν_{max} 2142 (azide), 1400, 1190 (OSO_2) cm^{-1}. ^1H NMR (DMSO-d_6/$CDCl_3$) δ 3.2 (s, 0.8H, β-OMe), 3.3 (s, 2.0H, α-OMe), 3.3-4.2 (m, 7H), 4.4 (br s, 3H). ^{13}C NMR (D_2O) δ 49.7 (q), 50.1 (q), 62.0 (t), 63.0 (t), 67.4 (t), 69.4 (t), 70.1 (d), 71.4 (d), 72.1 (d), 75.1,(d) 77.7 (d), 78.4 (d), 81.0 (d), 82.4 (d), 84.7 (d), 101.3 (s, C2 of 15a), 103.4 (s, C2 of 14b), 108.5 (s, C2 of 14a); integration of the ^{13}C NMR signals for C2 indicated a 67:27:0:6 ratio of 14a:14b:15a:15b. Anal. Calcd for $C_7H_{13}N_3O_8S \cdot 0.1H_2O \cdot 0.1CH_4O$: C, 28.85; H, 5.20; H_2O, 0.54. Found: C, 29.07; H, 4.33; H_2O, 1.21.

Methyl D-Fructofuranoside 1-Azidosulfate 6-Diphenylphosphate (16). The mixture of 14 and 15 was phosphorylated in the general way described above, and the product was purified on a dry column (EtOAc/hexane, 4:1) to give 16 (3.0 g, 25%), $[\alpha]_D^{23}$ +21.0° (c 0.20, MeOH). IR (neat) ν_{max} 2143 (azide), 1408, 1274 (P=O), 1190 (OSO_2), 968 cm^{-1}. ^1H NMR δ 3.3 (br s, 3H, OMe), 3.5-4.6 (m, 7H), 7.3 (m, 10H). Anal. Calcd for $C_{19}H_{22}N_3O_{11}PS \cdot 0.1H_2O \cdot 0.1CH_3OH$: C, 42.77; H, 4.25; H_2O, 0.34. Found: C, 43.15; H, 4.40; H_2O, 0.95.

Methyl 1-Sulfamoyl-D-fructofuranoside 6-Phosphate (17), Cyclohexylamine Salt (2:3). Compound 16 (4.30 g, 8.0 mmol) was deprotected under the same conditions as described for 12, to give a nearly colorless resin. A solution of this material in deionized water was treated with cyclohexylamine until the solution was moderately alkaline (pH 10). Removal of solvent by lyophilization afforded a gum, which was triturated several times with dry ether, filtered, and dried for 2 d under vacuum to give the salt of 17 as a white foam (3.38 g, 77%), $[\alpha]_D^{23}$ +8.5° (c 0.07, water). IR (neat) ν_{max} 3360 (NH_2), 1360, 1180 (OSO_2) cm^{-1}. ^1H NMR (DMSO-d_6) δ 1.0-2.0 (m, 17H), 2.8, (br s, 1.7H), 3.2 (br s, 3H, OMe), 3.5-4.1 (m, 7H), 6.4 (br s, 9.4H). The resonance at δ 6.4 disappeared on exchange with D_2O. ^{13}C NMR (D_2O) δ 24.7 (t), 25.2 (t), 31.2 (t), 49.6 (q), 50.3 (q), 51.2 (d), 65.7 (t), 67.9 (t), 75.3 (d), 77.7 (d), 78.1 (d), 81.7 (d), 102.2 (s, C2 of 17b), 107.4 (s, C2 of 17a); integration of the resonances at δ 102.2 and 107.4 gave a 57:43 ratio of 17a to 17b. Anal. Calcd for $C_7H_{16}NO_{11}PS \cdot 1.7C_6H_{13}N \cdot 1.5H_2O$: C, 37.64; H, 7.56; N, 6.89; H_2O, 4.93. Found: C, 38.01; H, 7.47; N, 6.49; H_2O, 3.93.

1-Deoxy-1-[(methoxyphenoxyphosphonyl)amino]-3,4,6-tri-O-benzyl-2,5-anhydro-D-glucitol (20b). Nitrile 18 was reduced to amine 19 with lithium aluminum hydride in THF at 0 °C the normal manner. Amine 19 (9.73 g, 20 mmol) dissolved in 60 mL of THF was added dropwise to a solution of phenyl dichlorophosphate in 70 mL of THF at 0 °C under argon. After 2 h, the suspension was filtered, 15 mL of MeOH was added, and the mixture was heated at reflux for 21 h. The solution was concentrated and redissolved in methylene chloride; the solution was washed with water, 2.5% H_2SO_4, 2.5% $NaHCO_3$, and brine; dried (Na_2SO_4) and concentrated. The crude reaction was purified on a dry silica gel column (EtOAc/hexane, 4:1) to give pure 20 as a syrup (8.12 g, 50%), $[\alpha]_D^{23}$ +14.6° (c 0.33, MeOH). IR (neat) ν_{max} 2950 (NH), 1260 (P=O), 1048, 1026 cm^{-1}. ^1H NMR δ 3.0-3.5 (m, 5H), 3.5-3.8 (d, 3H, $^3J_{CP}$ = 11 Hz), 4.2-4.55 (m, 6H), 7.0-7.3 (m, 20H). ^{13}C NMR δ 41.1 (t, C1 of 20b), 42.9 (t, C1 of 20a), 53.4 (dq, OMe, J_{PC} = 5.9 Hz), 70.3 (t), 71.5 (t), 71.7 (t), 73.4 (t), 82.4 (d), 83.0 (d), 83.2 (d), 120.1 (d), 120.4 (d), 124.7 (d), 127.7 (d), 128.4 (d), 129.6 (d), 137.4 (s), 137.7 (s), 138.0 (s), 149.9 (s, 4' of OPh); the ratio of 20a to 20b was determined to be ca. 1:5 by measurement of the signals for C1. Different batches of product varied in α/β anomeric ratio from 1:2.5 to 1:5, presumably due to fractionation during chromatography. Anal. Calcd for $C_{34}H_{38}NO_7P \cdot 0.1H_2O$: C, 67.45; H, 6.36; N, 2.31; H_2O, 0.30. Found: C, 67.27; H, 6.41; N, 2.39; H_2O, 0.84.

1-Deoxy-1-[(hydroxymethoxyphosphonyl)amino]-2,5-anhydro-D-glucitol (21b), Methylamine Salt (1:1). A solution of 20 (12.8 g, 20 mmol) in 104 mL of cyclohexene and 208 mL of EtOH was treated with 10% Pd/C (7.9 g) and refluxed for 1.5 d, filtered, and concentrated to a syrup. A solution of this material in 100 mL of water was treated with $Ca(OH)_2$ (13 g), and the mixture was stirred for 3 d, filtered, and lyophilized to a foam. One mol-equiv of Na_2CO_3 (20 mmol) was added to a solution of this foam in 150 mL of water. The solution was stirred for 15 min, filtered; the filtrate was treated with Dowex 50W-X8 resin until it became acidic.

It was washed with ether, and the aqueous layer was treated with methylamine for 30 min with stirring. The mixture was lyophilyzed to give a white foam (2.7 g, 43%), $[\alpha]_D^{23}$ +10.0° (c 0.30, water). IR (neat) ν_{max} 3200 (NH_2), 1050 cm^{-1}. ^1H NMR (D_2O) δ 2.8 (s, 3H, NMe), 3.2-3.5 (m, 2H, CH_2N), 3.65 (d, 2.25H, β-OMe, $^3J_{PH}$ = 12 Hz), 3.68 (d, 0.75H, α-OMe, $^3J_{PH}$ = 12 Hz), 3.9-4.5 (m, 7H); α/β = 1:3. ^{13}C NMR (D_2O) δ 22.1 (NMe), 36-38 (m, C1), 49.3 (m, OMe), 59.1 (m, C6), 73-77 (complex m), 79-83 (complex m). Anal. Calcd for $C_7H_{16}NO_7P \cdot CH_5N \cdot 1.7H_2O$: C, 30.13; H, 7.71; N, 8.79; H_2O, 9.60. Found: C, 30.40; H, 7.62; N, 8.41; H_2O, 6.44.

Methyl 3,4-Anhydro-D-tagatofuranoside 1,6-Diphosphate (24), Methylamine Salt (1:3). Compound **22**[16] (17.0 g, 0.10 mol, α/β ratio = 2:1) was phosphorylated as in the preparation of **11**. After purification of the resulting tetraphenyl phosphate compound, 8.7 g of it (14 mmol) was shaken with a mixture of platinum oxide (3.5 g) in EtOH (225 mL) at 42 psig for 5 h. The solution was filtered, added to an equal volume of MeOH, and treated with methylamine (45 mL). The solvent was evaporated to give a foam, which was triturated with cold EtOH, filtered, and dried in vacuo for 3 d to give the salt of **24** (2.44 g, 37%) as a white solid, $[\alpha]_D^{23}$ +6.6° (c 0.35, water). IR (KBr) ν_{max} 1291 (P=O), 968 cm^{-1}. ^1H NMR (D_2O) δ 2.8 (s, 10H, 3.3 NMe), 3.61 (s, 2H, α-OMe), 3.71 (s, 1H, β-OMe), 4.0-4.8 (m, 7H); integration of the methoxy singlets indicated a 2:1 ratio of **24a** and **24b**. Anal. Calcd for $C_7H_{14}O_{11}P_2 \cdot 3.3CH_5N \cdot 1.5H_2O$: C, 26.57; H, 7.25; N, 9.93. Found: C, 26.37; H, 7.51; N, 10.23.

1,2:4,5-Bis-*O*-(1-methylethylidene)-3,3'-anhydro-3-*C*-(hydroxymethyl)-β-D-fructopyranose (29). To a solution of lithium diisopropylamide (90.16 g, 0.84 mol) in 450 mL of THF, under nitrogen, was added methyltriphenylphosphonium bromide (316.2 g, 0.90 mol) with stirring at 0 °C. After 45 min, compound **27**[18] (105.6 g, 0.41 mol), prepared by Swern oxidation of **26**,[7g] was added. After 30 min, the solution was poured into ice water and the mixture was extracted twice with ether. The combined extracts were washed with 3% H_2O_2 (3 x 150 mL), brine, dried (Na_2SO_4), filtered, and concentrated to about 50 mL. The precipitated triphenylphosphine oxide was filtered off and washed with cold ether, and the remaining organic solvent was removed. To crude syrup **30** (69.7 g, 66%) was added 1,2-dichloroethane (1 L) and *m*-chloroperbenzoic acid (78.3 g, 0.39 mmol, 85% assay), and the resulting solution was refluxed for 4.5 h, cooled, and treated with hexane (700 mL). After filtration, the filtrate was washed with 8% Na_2SO_3 (2 x 500 mL), 5% NaOH (2 x 500 mL), water, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated to give **29** as a white solid (68.7 g, 93%). A sample was rigorously purified on a dry silica gel column (EtOAc/hexane, 1:3), mp 85-86 °C, $[\alpha]_D^{23}$ -119° (c 1.41, MeOH). ^1H NMR δ 1.3-1.6 (m, 12), 2.9 (s, 2H), 3.8-4.5 (m, 6H). ^{13}C NMR δ 24.8 (q), 25.7 (q), 26.1 (q), 26.2 (q), 47.2 (t, oxirane), 56.2 (s, C3), 63.2 (t, C6), 73.5 (t, C1), 73.9 (d), 74.3 (d), 102.9 (s, C2), 110.5 (s), 110.7 (s). Anal. Calcd for $C_{13}H_{20}O_6$: C, 57.34; H, 7.40. Found: C, 57.43; H, 7.29.

Methyl 3-*C*-Bromomethyl-D-fructofuranoside 1,6-Bis(diphenylphosphate) (33). A solution of **29** (11.9 g, 40 mmol) in THF (190 mL) was treated with lithium bromide (34.3 g, 0.39 mol), pyridine (11.9 g), and bromotrimethylsilane (2.6 g, 45 mmol) at 8 °C.[34] After stirring for 2 h at ambient temperature, ether (100 mL) was added and the solution was washed with 0.1 N HCl and concentrated in vacuo to give **31** as a yellow syrup: MS (CI-CH_4) m/e 353 (MH^+). This material was immediately methanolyzed, as in preparation of **9**, to give **32** as a yellow semi-solid: MS (CI-NH_3) m/e 287 (MH^+); ^1H NMR indicated one major isomer. Compound **32** (11.4 g, 39 mmol) was phosphorylated in the usual manner to give **33**, which was purified on a dry silica gel column (EtOAc/hexane, 2:1). This resulted in 19.2 g (66%) of a syrup (**33**), $[\alpha]_D^{25}$ +35.0° (c 0.33, MeOH). MS (CI-CH_4) m/e 751 (MH^+). IR (KBr) ν_{max} 1248 (P=O), 1046 cm^{-1}. ^1H NMR δ 3.1 (m, 1H), 3.2 (br s, 3H), 3.3-4.6 (m, 9H), 7.0-7.6 (m, 20H). ^{13}C NMR δ 35.4 (t, CH_2Br), 48.9 (q, OMe), 61.8 (dt, C6, J_{PC} = 5.9 Hz), 68.4 (dt, C1, J_{PC} = 5.9 Hz), 79.5 (d, C4), 82.6 (s, C3), 84.6 (dd, C5, J_{PC} = 8.0 Hz), 107.6 (dq, C2 of **33a**, J_{PC} = 9.8 Hz), 119.9 (d), 120.3 (d), 125.6 (d), 129.9 (d), 150.3 (ds, 2C, J_{PC} = 7.8 Hz); only **33a** was observed (possible enrichment on chromatography). Anal. Calcd for $C_{32}H_{33}BrO_{12}P_2 \cdot 0.5H_2O$: C, 50.54; H, 4.51; Br, 10.51; water, 1.18. Found: C, 50.59; H, 4.54; Br, 10.37; water, 0.92.

Methyl 3,3'-Anhydro-3-C-(hydroxymethyl)-α-D-fructofuranoside 1,6-Biphosphate (25a), Cyclohexylamine Salt (2:5). A solution of **33a** (6.89 g, 9.0 mmol) in 300 mL of MeOH was shaken with platinum oxide (2.0 g) under 40 psig of hydrogen for 18 h. The mixture was filtered and concentrated to give a foam, which was treated with water (20 mL) and Na_2CO_3 (5.21 g) and stirred for 18 h to form a sodium salt of **25a**.[35] The solution was neutralized with Dowex 50W-X8 (cation resin, pH 7), filtered through Amberlite IR-45 (anion resin, pH 8-10) and concentrated. This foam was dissolved in MeOH, treated with Dowex 50W-X8 resin in the cyclohexylamine form, filtered, and concentrated. This material was precipitated twice from MeOH with EtOAc to give a white powder (3.50 g, 58%), $[\alpha]_D^{23}$ +13.1° (c 0.79, water). IR (KBr) ν_{max} 1235, 1083 cm^{-1}. ^1H NMR (D_2O) δ 0.9-1.7 (m, 27H), 2.8 (m, 2.6H), 3.1 (s, 3H, OMe), 3.2 (s, 2H), 3.7-4.0 (m, 6H). ^{13}C NMR (D_2O) δ 23.6, 24.1, 30.1, 50.1 (cyclohexylamine); 45.0 (t, oxirane), 48.1 (q, OMe), 58.1 (d, C5), 63.5 (pair of t, C1 and C6), 78.1 (d, C4), 81.4 (s, C3), 84.0 (d, C5), 108.5 (ds, C2, J_{PC} = 8.8 Hz); only **25a** was observed. Calcd for $C_8H_{16}O_{12}P_2 \cdot 2.5C_6H_{13}N \cdot 3.0H_2O$: C, 41.36; H, 8.19; N, 5.24; H_2O, 8.09. Found: C, 41.14; H, 7.99; N, 5.72; H_2O, 6.49 (Br, 0.85).

D-Arabinose 1-Phosphate (42), Cyclohexylamine Salt (4:7). Acetate **36** (5.0 g, 10.8 mmol) was converted into bromide **44**, which was reacted with dibenzyl phosphate to give **45**, and the resulting material was deprotected with Li-ammonia by the procedure we described earlier.[7a] This gave the cyclohexylamine salt of **42** (0.25 g, 5.4%) as a white powder. IR (KBr) ν_{max} 2940, 1644, 1633, 1450, 1393, 1080 cm^{-1}. 360-MHz ^1H NMR (D_2O) δ 0.8-1.8 (m, 19.5H), 2.82-2.95 (m, 1.8H), 3.3-3.95 (m, 5H), 5.19 (d, ca. 0.15H, H1 of **42a**, J = 7 Hz), 5.22 (t, ca. 0.85H, H1 of **42b**, J = 5 Hz). ^{13}C NMR (D_2O) δ 23.7, 24.2, 30.3, 50.2 (cyclohexylamine); 57.3 (C5 of **42a**); 61.6 (C5 of **42b**); 73.5 (C3 of **42b**); 76.4 (C2 of **42b**); 77.2 (C3 of **42a**); 81.5, 81.9 (C4 of **42b**, C-2 of **42a**); 84.0 (C4 of **42a**); 95.9 (d, J_{CP} = 4.89 Hz, C1 of **42b**), 102.5 (d, J_{CP} = 3.91 Hz, C1 of **42a**); integration of the peaks for **42a** and **42b** revealed a 1:5 ratio. Anal. Calcd for $C_5H_{11}O_8P \cdot 1.8C_6H_{13}N \cdot H_2O$: C, 44.48; H, 8.60; N, 5.91; P, 7.26; H_2O, 4.22. Found: C, 44.51; H, 8.92; N, 5.60; P, 6.98; H_2O, 4.29.

α-D-Ribose 1,5-Diphosphate (35a), Cyclohexylamine Salt (1:4). 2,3-O-Benzylidene-β-D-ribofuranose was prepared from D-ribose, benzaldehyde, and freshly fused zinc chloride according to the literature,[36] except that only 1.5 mol-equiv of benzaldehyde was employed. The yield of first-isolated product was 55% (mp 108-110 °C). [A sample of **47** was recrystallized from EtOAc/hexane: mp 123-124 °C (lit.[36a] mp 123-124 °C); $[\alpha]_D^{23}$ -26.8° (c 0.94, MeOH).] Phosphorylation of **47** (6.4 g, 0.027 mol) in the usual way gave crude **48**, a sample of which was chromatographed on a dry column of silica gel to yield an off-white powder (4.1 g, 33%); mp 90-95 °C; $[\alpha]_D^{23}$ 0.0° (c 0.23, MeOH). ^1H NMR δ 3.6-4.0 (br s, 1H, OH), 4.2-4.8 (m, 5H), 5.5 (s, 1H), 5.8 (m, 1H), 7.1-7.6 (m, 15H). A solution of unchromatographed material (28 g, 0.06 mol) in pyridine (150 mL) was treated with acetic anhydride (12.2 g, 0.12 mol). The mixture was stirred for 3.5 h, poured into ice-water, and extracted with ethyl acetate twice. The combined extracts were washed with 1N HCl, 2.5% $NaHCO_3$, and brine, then dried and concentrated in vacuo to a solid. Recrystallization from ethyl acetate/hexane furnished a white solid (**49**, 17.5 g, 58%), mp 94-96 °C, $[\alpha]_D^{23}$ -19.9° (c 0.48, MeOH). IR (KBr) ν_{max} 1757 (C=O), 1298 (P=O) cm^{-1}. ^1H NMR δ 2.0 (s, 3H, Me), 4.3 (m, 2H), 4.6 (t, 1H), 4.8 (2 d, 2H), 5.8 (s, 1H, β-H1), 6.4 (s, 1H), 7.2-7.5 (m, 15H). ^{13}C NMR δ 21.0 (q), 67.6 (t), 68.0 (t), 81.7 (d), 84.9 (d), 85.6 (d), 101.4 (d, β-C1), 106.6 (d), 119.8 (d), 120.2 (d), 125.5, (d), 126.8 (d), 128.4 (d), 129.9 (d), 135.3 (s), 150.0 (s), 150.5 (s), 169.2 (s, C=O); the compound appeared to be >95% β-furanose. Anal. Calcd for $C_{26}H_{25}O_9P$: C, 60.94; H, 4.92. Found: C, 61.13; H, 4.98.

Acetate **49** (4.22 g, 8.0 mmol) was converted to the bromide at 0 °C in 500 mL of methylene chloride that was saturated with HBr. The bromide was reacted with dibenzyl phosphate (2.51 g, 9.0 mmol) and triethylamine (0.91 g, 9.0 mmol), then lithium (1.0 g) and ammonia (200 mL) in THF (100 mL) at -78 °C, in the usual fashion. After 15 min, the deep blue solution was quenched with crushed ice until the blue color no longer remained. The solvent was blown off by a stream of nitrogen and the residue was taken up into distilled water. The

mixture was filtered, treated with a pyridinium-Dowex 50W-X8 resin (to pH 7), filtered, and treated with saturated aqueous barium hydroxide (to pH 10). The precipitate was collected and dried to give 4.21 g of gummy glass. This was dissolved in water and treated with pyridinium resin (pH > 7), and converted in the usual way to the cyclohexylamine salt,[7a] isolated as a white powder (1.38, 23.4%), mp 154-159 °C; $[\alpha]_D^{23}$ +12.7° (c 0.12, water). IR (KBr) ν_{max} 1241, 1071 cm^{-1}. ^1H NMR (D_2O) δ 0.9-1.9 (m, 40H), 2.9 (m, 4H), 3.6-4.1 (m, 5H), 5.1-5.4 (m, 1H). ^{13}C NMR (D_2O) δ 23.7, 24.2, 30.3, 50.1 (cyclohexylamine); 63.6 (t, α-C6, J_{PC} = 3.9 Hz), 65.4 (t, β-C6, J_{PC} = 4.9 Hz), 69-75 (complex m), 81-82 (m), 83.6 (dt, α-C4, J_{PC} = 8.8 Hz), 97.1 (dd, α-C1, J_{PC} = 3.9 Hz), 101.9 (dd, β-C1, J_{PC} = 2.9 Hz); the α/β ratio was 3:1 from integration of the C1 resonances. Anal. Calcd for $C_5H_{12}O_{11}P_2 \cdot 4.0C_6H_{13}N \cdot 1.6H_2O$: C, 47.35; H, 9.21; N, 7.62; P, 8.42; H_2O, 3.92. Found: C, 48.42; H, 9.08; N, 7.30; P, 7.85; H_2O, 3.59.

1,2,3-Tri-O-acetyl-α-D-ribofuranose 5-Diphenylphosphate (52). To a solution of 5-O-trityl-D-ribose[32] (51; 16.9 g, 48.1 mmol) in pyridine (50 mL) was added 50 mL of acetic anhydride, and the mixture was heated at 95 °C for 1.5 h under nitrogen. The solvent was evaporated, and the residue was dissolved in ether and washed with water (3 times), dried ($MgSO_4$), filtered, and concentrated to give a foam. This material was treated with 150 mL of 80% aqueous HOAc and heated at 95 °C for 1.5 h. After cooling, 150 mL of water was added to the solution and triphenylmethanol was filtered off. The filtrate was treated with ca. 10 g of NaCl and the mixture was extracted with $CHCl_3$ (4 times). The combined extracts were dried ($MgSO_4$), filtered, and concentrated to yield a pale yellow oil (7.7 g, 65%). This material was dissolved in 50 mL of pyridine and treated with diphenyl chlorophosphate (5.93 mL, 28.6 mmol) at 0 °C. Cooling was ceased and the reaction was stirred at ambient temperature for 2 h. Water was added and the mixture was extracted with ether, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by HPLC (Waters Prep 500; EtOAc/hexane, 35:65) to give 52, pure by TLC (5.05 g, 23% from 51). IR (neat) ν_{max} 1759, 1491, 1221, 1190 cm^{-1}. ^1H NMR δ 0.98 (s, 3H), 1.04 (s, 3H), 1.12 (s, 3H), 4.4 (m, 3H), 5.35 (m, 2H), 6.12 (s, 1H), 7.2 (m, 10H). ^{13}C NMR δ 20.3 (q, 2C), 20.8 (q), 67.3 (dt, J_{CP} = 5.9 Hz), 70.0 (d), 74.0 (d), 79.9 (d), 97.9 (d, C1), 119.9 (d, 2C), 120.1 (d, 2C), 125.4 (d, 2C), 129.3 (d, 4C), 150.2 (ds, J_{CP} = 6.8 Hz), 169.0 (s), 169.3 (s), 169.5 (s). Only the α isomer could be detected by ^{13}C NMR. Anal. Calcd for $C_{23}H_{25}O_{11}P$: C, 54.34; H, 4.96; P, 6.00. Found: C, 54.54; H, 5.10; P, 6.17.

β-D-Ribose 1,5-diphosphate (35b), Cyclohexylamine Salt (3:10). Acetate 52 (5.0 g, 10.1 mmol) was converted into 35b by the same procedure as described for the preparation of 34a,[7a] with the addition of silver tetrafluoroborate to 53 prior to the addition of dibenzyl phosphate. This yielded the cyclohexylamine salt of 35b as a white powder (0.60 g, 8.8%). IR (KBr) ν_{max} 2950, 1629, 1621, 1564, 1554, 1453, 1276, 1246 cm^{-1}. 360-MHz ^1H NMR (D_2O) δ 0.8-0.9 (m, 3.5 H), 0.9-1.1 (m, 13.9H), 1.27-1.38 (m, 3.5H), 1.4-1.5 (m, 6.9H), 1.60-1.72 (m, 6.9H), 2.75-2.9 (m, 3.3H), 3.61-3.85 (m, 3H, H4 and H5), 3.87 (d, 1H, H3, $J_{2,3}$ = 4.7 Hz), 4.56 (dd, 1H, H2, $J_{2,3}$ = 4.6 Hz, J_{CP} = 7.2 Hz), 5.20 (d, 1H, H1, J_{PH} = 6.6 Hz). ^{13}C NMR (D_2O) δ 23.7, 24.2, 30.2, 50.1 (cyclohexylamine); 65.4 (d, C5, J_{CP} = 4.9 Hz); 70.5 (C3); 75.1 (d, J_{CP} = 5.9 Hz); 81.3 (d, C4, J_{CP} = 6.8 Hz); 102.0 (d, C1, J_{CP} = 3.9 Hz). Anal. Calcd for $C_5H_{12}O_{11}P_2 \cdot 3.3C_6H_{13}N \cdot 2.4H_2O$: C, 43.77; H, 8.84; N, 6.79; P, 9.10; H_2O, 6.35. Found: C, 43.92; H, 8.57; N, 6.69; P, 9.92; H_2O, 6.64.

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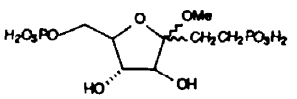
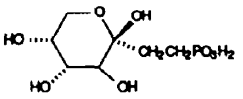
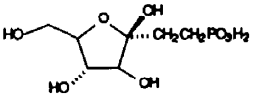


A



B



B'

ably possess the β C2 configuration, as depicted in **B** and **B'**(Que, L., Jr.; Gray, G. R. Biochemistry **1974**, 13, 146 and Koerner, T. A., Jr.; Voll, R. J.; Cary, L. W.; Younathan, E. S. Ibid. **1980**, 19, 2795). ^{1}H NMR (D_2O) δ 0.9-2.0 (m, ca. 25H), 3.2-3.9 (m, 9H). Anal. Calcd for $C_7H_{15}O_8P \cdot 1.6(C_6H_{13}N) \cdot 1.2(C_2H_6O) \cdot 0.7(H_2O)$: C, 47.08; H, 9.23; N, 4.62; P, 6.39; H_2O, 2.60. Found: C, 47.27; H, 8.85; N, 4.15; P, 7.47; H_2O, 2.51. MS (CI-NH_3) of persilylated material: m/e 691 (MH^+).

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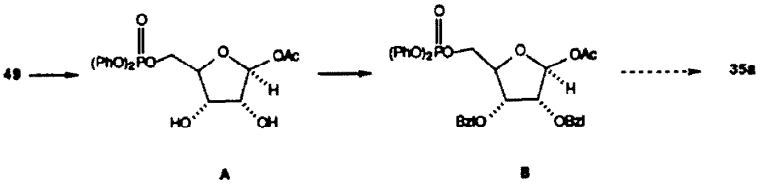

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