and adjustable needle valve system, which permitted a continuous, sterile, pyrogen-free, 0.225% saline infusion. The animals were allowed to recover from anesthesia overnight before the start of drug treatments. Arterial pressure was recorded continuously through Statham P-23Gb transducers on a Honeywell 906C Visicorder. Mean arterial pressures were printed at $^1/_2$-h intervals through a data acquisition system (Data Graphics Corp., San Antonio, TX) by means of ASR-33 teletype units. The drug doses were computed on the basis of the base weight. Six rats were used in each treatment group.

Acknowledgment. The authors are indebted to B. Christensen for suggesting the use of (2-oxo-1,3-dioxol-4-yl)methyl esters as prodrugs of methyldopa. We also thank R. Brown for providing samples of 4-(bromomethyl)-5-methyl-1,3-dioxol-2-one, Y. Lee and J. Moreau for elemental analyses, J. Murphy for ^{1}H NMR spectra, and M. Banker for preparation of the manuscript. The authors are also grateful for the technical assistance of S. White in the bioavailability studies.

Registry No. **2** (R = *t*-Bu), 86005-11-0; **3a**, 80715-22-6; **3b**, 86005-12-1; **4**, 555-30-6; **5**, 62631-37-2; **6**, 86005-08-5; **7a** tartrate, 86005-10-9; **7b** tartrate, 86016-63-9; **8a**, 431-03-8; **8b**, 40898-19-9; di-*tert*-butyl dicarbonate, 24424-99-5; 4,4-dimethyl-2-hydroxy-3-pentanone, 52279-28-4.

Inhibition of Arabinose 5-Phosphate Isomerase. An Approach to the Inhibition of Bacterial Lipopolysaccharide Biosynthesis

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Arabinose 5-phosphate (A5P) isomerase is a key enzyme in the biosynthesis of lipopolysaccharide, an essential component of the outer membrane of Gram-negative bacteria. The mechanism of the isomerase is envisioned to involve an enediol intermediate. A series of compounds, which are analogues of the substrates or intermediate, were tested as inhibitors of A5P isomerase with the belief that a good inhibitor would stop bacterial growth or render the cells more susceptible to other antibiotics or natural defenses. In a series of phosphorylated sugars, the order of isomerase inhibitory activity was as follows: aldonic acids > alditols > aldoses. Nonphosphorylated sugars were much less inhibitory. The best inhibitor was erythronic acid 4-phosphate (**54**), which had $K_m/K_i = 29$. None of the compounds displayed antibacterial activity in vitro.

The rise of antibacterial resistance in pathogenic bacteria is a continuing medical problem. One approach to the attack of this problem is to develop a new antibacterial agent that has a completely novel mode of action with the expectation that no cross-resistance with older drugs would be found. Bacterial cell wall peptidoglycan has been the primary target for research of this type, but we report here a new approach to the inhibition of the formation of the outer membrane of Gram-negative bacteria.

The envelope of Gram-negative bacteria has three principal parts: the outer membrane (OM), peptidoglycan, and the cytoplasmic membrane (CM).[1] The functions of the outer membrane, in part, are to retain proteins and enzymes in the periplasmic space (between OM and CM), to protect the cell from undesirable enzymes and chemicals, to provide channels for nutrients and ions,[2] and to facilitate cell–cell interactions, such as adhesion, conjugation, and chemotaxis.

The primary components of the outer membrane are phospolipids, lipopolysaccharide, and proteins. The lipopolysaccharide (LPS) is found on the outside of the unsymmetrical lipid bilayer, with the polysaccharide chains exposed on the cell surface. The LPS determines the antigenicity, toxicity, adhesiveness, invasiveness, and penetrability of the cell. The structure of *Salmonella* LPS is shown in Figure 1. Much of the present knowledge of the structure and biosynthesis of LPS has come from the work of Osborn and co-workers.[3]

Lipopolysaccharide is an attractive target for chemotherapy because of three components unique to bacteria: the β-hydroxymyristoyl groups in lipid A, D-*manno*-3-deoxyoctulosonic acid (KDO), and L-*glycero*-D-*manno*-heptose. Mutants that are defective in LPS biosynthesis are usually less pathogenic and more susceptible to antibiotics.[4] Importantly, mutants that are defective in KDO biosynthesis are not viable. Hence, the inhibition of KDO biosynthesis became our primary target.

The biosynthesis of KDO has been discussed by Heath and co-workers,[5] and a schematic of KDO biosynthesis is shown in Figure 2. The biological significance of KDO was recognized by Unger and co-workers, who have thoroughly explored the chemistry of KDO. Their work has been summarized in a recent review.[6] All of the enzymes in this pathway have been isolated and characterized by Ray and co-workers,[7] and the isomerase was an early focal point.

The isomerase enzymes that interconvert aldoses and ketoses are found in both mammals and microorganisms. The function of the isomerases is to synthesize cell components rather than produce energy. The characteristics of a number of these enzymes were discussed by Topper[19] and later by Noltman.[20] More recently, Rose has discussed the mechanisms,[21] and, fortunately, much of this work has now been consolidated in the chapter by Walsh.[22]

[†] Department of Organic Chemistry.
[‡] Department of Microbiology.

(1) Inouye, M. "Bacterial Outer Membrane"; Wiley: New York, 1979; pp 1–12.
(2) Nikaido, H. *Biochim. Biophys. Acta* **1976**, *433*, 118.
(3) Osborn, M. J. In ref 1, pp 15–34 and references therein.
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(6) Unger, F. M. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 323–388.
(7) Ray, P. H. *J. Bacteriol.* **1980**, *141*, 635. Ray, P.; Benedict, C. *J. Bacteriol.* **1980**, *142*, 60. Ray, P.; Benedict, C.; Grasmuk, H. *J. Bacteriol.* **1981**, *145*, 1273.

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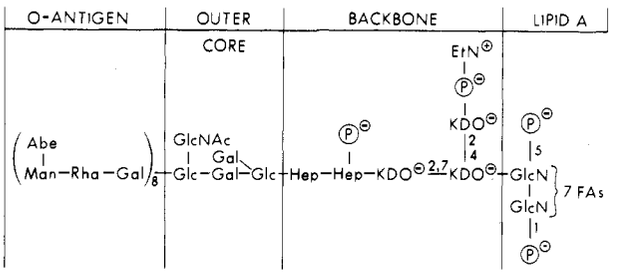


Figure 1. The structure of *Salmonella typhimurium* lipopolysaccharide.

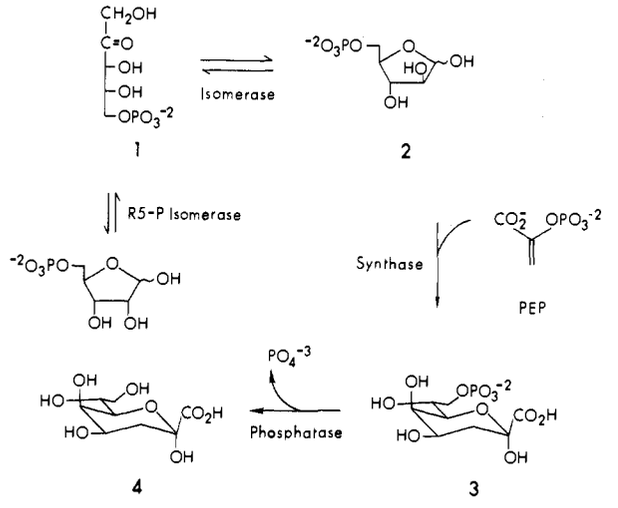


Figure 2. The biosynthetic pathway to KDO.

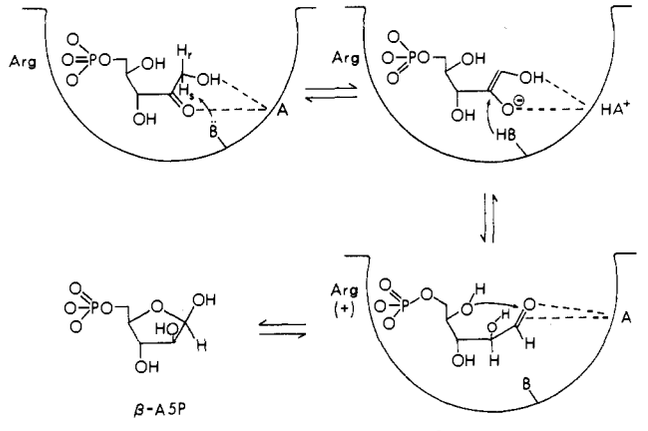
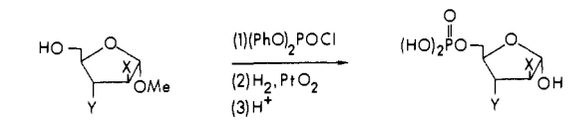


Figure 3. Plausible mechanism for arabinose 5-phosphate isomerase.

Based on this earlier work, we have formulated a plausible mechanism for A5P isomerase, which is shown in Figure 3, and have used this mechanism as a basis for the design of inhibitors. In short, this is a least-motion, 1,2-hydrogen shift mechanism that passes through a cis enediol(ate) intermediate. A basic group on the enzyme probably removes the *pro-S* proton on C-1 of ribulose 5-phosphate and reprotonates the substrate at C-2 on the same (si) face of the enediol. If the product cyclizes before departing from the enzyme, then β-D-arabinose 5-phosphate should be the kinetic product (or the kinetic substrate for the reverse reaction). This paper reports the preparation of aldose derivatives as substrate analogues and aldonic acids and alditols as mimics of the enediol intermediate. Within these classes, the effect of substitution of the hydroxyl groups and phosphate moiety on enzyme inhibition was studied.

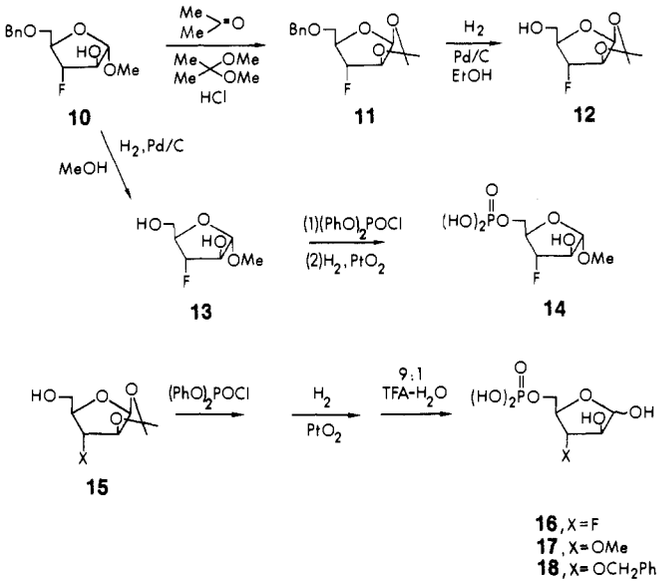
Chemistry. Aldose Phosphates. The synthesis portion of this program began with the preparation of ana-

Scheme I

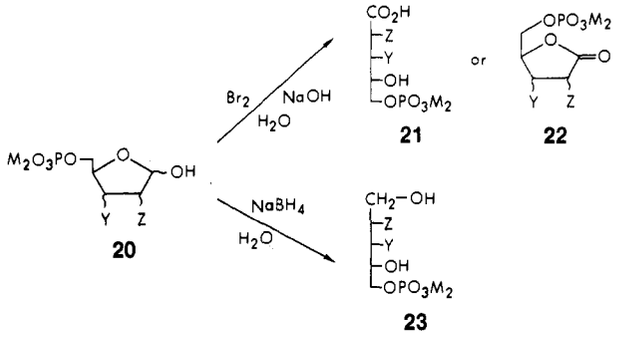


Starting No.	X	Y	Product No.
5	OH	OH	2
6	OH	H	7
8	F	OH	9

Scheme II



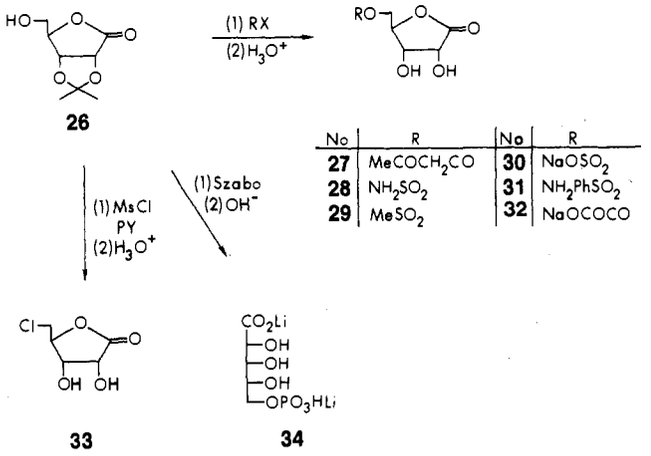
Scheme III



logues of the natural enzyme substrate D-arabinose 5-phosphate (A5P, **2**). Compound **2** and its 3-deoxy analogue **7** were made by the application of the Szabo phosphorylation sequence[8] to the appropriate methyl furanosides as shown in Scheme I.[9] The 2-fluoro analogue **9** was made by the same route from the furanoside 8, which had been made by the KHF_2 method of Wright, Taylor, and Fox.[10] The remaining compounds with substituents at the 3-position were prepared via isopropylidene-protected intermediates as shown in Scheme II. The methyl furanoside **10**[11] was converted to the 1,2-*O*-isopropylidene derivative **11** by the exchange method of Hirst, Jones, and Williams.[12,13] The primary hydroxyl was deprotected by

(8) Szabó, P.; Szabó, L. *J. Chem. Soc.* **1965**, 2944.
(9) Maehr, H.; Smallheer, J. *Carbohydr. Res.* **1978**, *62*, 178.
(10) Wright, J.; Taylor, N.; Fox, J. *J. Org. Chem.* **1969**, *34*, 2632.
(11) Wright, J.; Taylor, N. *Carbohydr. Res.* **1967**, *3*, 333.
(12) Hirst, E.; Jones, J.; Williams, E. *J. Chem. Soc.* **1947**, 1062.

Scheme IV



No	R	No	R
27	$MeCOCH_2CO$	**30**	$NaOSO_2$
28	NH_2SO_2	**31**	NH_2PhSO_2
29	$MeSO_2$	**32**	NaOCOCO

26 **33** **34**

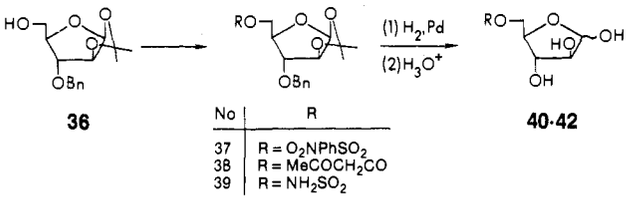
catalytic hydrogenolysis to produce the intermediate alcohol **12**. Protected furanose compounds **15** were phosphorylated by the Szabo method, and the final deprotection step was accomplished with 9:1 trifluoroacetic acid–H_2O[14] to produce the furanose 5-phosphates **16–18**. Debenzylation of **10** followed by phosphorylation of **13** according to Szabo[8] without acid hydrolysis gave **14**, the methyl furanoside of **16**.

Alterations at C-1 of the substrate analogues included substitution, oxidation, and reduction (Scheme III). Thus, A5P was treated with hydroxylamine in aqueous solution, and the product oxime **19** was precipitated from MeOH. This compound was found by NMR to be the open-chain oxime isomer rather than the arabinosyl hydroxylamine. The *E*/*Z* ratio was calculated to be 4.75 based on the peak integrations. Finally, the alditol phosphates (**23**) were prepared by reduction of the aldose phosphates with aqueous $NaBH_4$ according to Baddiley et al.[17]

Aldonic Acid Phosphate. Bromine oxidation of aldose derivatives by the Horecker method[15] (Scheme III) was used to prepare a series of aldonic acid phosphates (**21**). The corresponding lactones (**22**) were isolated when the final basic hydrolysis was omitted as in the case of 2-deoxyribono-1,4-lactone 5-phosphate (**24**). The presence of a lactone could be determined most easily by ^{13}C NMR because of the downfield shift of C-4 to about 90 ppm for the lactone vs. 70 ppm for the acid. The acids and lactones could be separated on a Bio-Gel P-2 column, as was done with the ribose 5-phosphate oxidation product, and TLC on PEI plates could be used to monitor the separation. In an alternative method, the oxygenation of fructose 6-phosphate according to Chirgwin and Noltman gave arabinonic acid 5-phosphate (**25**).[16]

Ribonolactones. Several new schemes were developed for the preparation of non-phosphate-containing analogs. A series of 5-*O*-substituted ribonolactones was prepared as shown in Scheme IV. The intermediate, protected lactone **26** was prepared in acetone catalyzed by concentrated HCl,[18] but the reaction had to be followed closely by TLC and stopped at the first sign of isomeric products. The intermediate **26** was allowed to react with a variety of reagents under basic conditions (pyridine or TEA/CH_2Cl_2) at or below 0 °C in the syntheses of **27–32**. Attempted sulfonylation in pyridine at room temperature gave the 5-chloro derivative **33** rather than 5-mesylate **29** or the 5-tosylate as expected. The facile displacement of 5-substituents on **26** led us to a new, useful method for the preparation of sugar phosphates. Thus, **26** was converted to the 5-bromo compound with Ph_3P/CBr_4, and this intermediate was treated with tetramethylammonium di-*tert*-butyl phosphate[39] in DME to produce **35**, the di-*tert*-butyl phosphate ester of **26**. This intermediate can be deprotected in one step with TFA to the lactone 5-phosphate (**22**, Z = Y = OH).

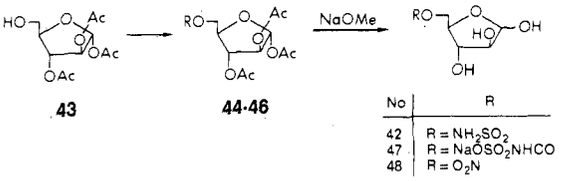
Scheme V



No	R
37	$R = O_2NPhSO_2$
38	$R = MeCOCH_2CO$
39	$R = NH_2SO_2$

36 **40-42**

Scheme VI



No	R
42	$R = NH_2SO_2$
47	$R = NaOSO_2NHCO$
48	$R = O_2N$

43 **44-46**

Arabinose Derivatives. The synthesis of 5-substituted arabinose analogues proved to be more difficult. Acid-stable functional groups were added to the 1,2-isopropylidene intermediate **36** as shown in Scheme V, but these compounds, the sulfamate (**42**, R = NH_2SO_2) especially, tended to decompose quickly when they were made by this route. The ester (**41**, R = acetoacetyl) could not be obtained in pure form. Alcohol **36** was converted to the primary bromide, but S_N2 reactions on this sterically hindered halide were not successful. The alternative route for base-stable products is shown in Scheme VI. The sulfamate made by this process was stable enough for isolation and purification. Apparently, the last traces of acid are difficult to remove after the TFA deprotection step in Scheme V, and great care must be used to avoid unwanted reactions.

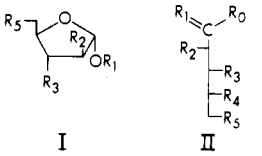
Arabinose 5-carbamate proved to be an elusive target. Initially, D-arabinose dipropyl dithioacetal (**49**) was treated with chlorosulfonyl isocyanate (CSI) at −70 °C, but no selectivity for the primary hydroxyl was observed. The dithioacetal **49** was also treated with phenyl chloroformate to give a cyclic carbonate, which was treated with NH_3/MeOH. A mixture of two carbamates, probably 4-O and 5-O, resulted. The dithioacetal **49** was tritylated, acetylated, and detritylated to obtain the protected intermediate **50**, which was carbamylated to give the 5-carbamate **51** (see Experimental Section). This intermediate was deacetylated with NaOMe/MeOH, but that product was not obtained in large enough quantity to be carried any further.

Furthermore, the triacetate **43** (Scheme VI) was converted to the 5-(phenyl carbonate), which was treated with NH_3/MeOH according to Hornemann,[37] but the product decomposed upon isolation. Finally, the triacetate **43** was treated with CSI under the same conditions used for **50**, but the initial product was the sulfonatocarbamate **45**, which we decided to keep rather than hydrolyze further to the carbamate because it was a novel compound and perhaps a good phosphate mimic.

Throughout this work, chromatography on Bio-Gel P-2 was found to be the best way of purifying the final arabinose products. In some cases this method was the only

(13) Levene, P.; Raymond, A. *J. Biol. Chem.* **1933**, *102*, 318.
(14) Christensen, J.; Goodman, L. *Carbohydr. Res.* **1968**, *7*, 510.
(15) Horecker, B. L. *Methods Enzymol.* **1957**, *3*, 172.
(16) Chirgwin, J.; Noltmann, E. *J. Biol. Chem.* **1975**, *250*, 7272.
(17) Baddiley, J.; Buchanan, J.; Carss, B.; Mathias, A. *J. Chem. Soc.* **1956**, 4583.
(18) Hough, L.; Jones, J.; Mitchell, D. *Can. J. Chem.* **1958**, *36*, 1720.

Table I. Inhibition of A5P Isomerase by Carbohydrate Phosphates



I II

Cmpd.	Structure				I_{50}[a] (mM)/[A5P[
	I, $R^5 = OPO_3$				
	R_1	R_2	R_3		
2	H	OH	OH		0.2 (Km)
45	H	αOH[b]	OH		80.0
7	H	OH	H		66.7
52	H	H	OH		NA @ 2
17	H	OH	OMe		NA @ 3
16	H	OH	F		61.3
9	H	F	OH		NA @ 5
14	Me	OH	F		91.3
18	H	OH	OCH_2Ph		NA @ 6
	II, R_4 = OH, $R_5 = OPO_3$				
	R_0	R_1	R_2	R_3	
25	OH	O	OH	OH	0.53
34	OH	O	αOH[b]	OH	1.6
55	OH	O	F	OH	4.7
56	OH	O	OH	F	62.0
24	OH[b]	O	H	OH	NA @ 6
54	–	–	=O,OH	OH	0.22
57	OH	H,H	OH	OH	19.3
58	OH	H,H	H	OH	NA @ 1.5
59	OH	H,H	αOH	OH	20.0
60	OH	H,H	OH	OH	0.7
61	OH	H,H	F	OH	5.3
62	OH	H,H	OH	F	100.0
63	OH	H,H	OH	OBn	233.3
19	H	NOH	OH	OH	8.7
64	glucitol		6-phosphate		NA @ 2.4
65	mannitol		6-phosphate		NA @ 6
66	OH	H,H	OH	OH $R_5 = CH_2PO_3$	86.7

[a] Competitive inhibition. NA, not active. [b] α implies a ribo configuration.

purification technique that had any beneficial effect.

The enzyme in our assay was isolated from *Escherichia coli* B cells and purified 50-fold.[23] The molecular weight is about 135 000, as estimated by Sephadex G-200 chromatography. The apparent K_m for ribulose 5-phosphate is 0.9–1.5 × 10^{-4} M, and the apparent K_m for A5P is 1–3 × 10^{-4} M. The enzyme is not inhibited by EDTA and has no cofactor metal requirement. Inhibitors were assayed by the method of Dische and Borenfreund.[24]

Discussion

The enzyme inhibition data are shown in Tables I and II. Our approach was to make a wide variety of changes on the substrate, A5P, and analogues of the enediol intermediate in order to elucidate the binding requirements of the enzyme.

Phosphorylated sugars bind better (low I_{50}) than nonphosphorylated sugars. A comparison of Tables I and II shows that the nonphosphates inhibit only at concentrations that are 10 to 20 times the substrate concentration. Among the phosphorylated analogues, the order of potency, aldonic acids > alditols > aldoses, reflects their ability to mimic the proposed enediol intermediate and supports the proposed mechanism.

The best inhibitor, erythronic acid 4-phosphate (**54**), has one less carbon atom than the substrate. This same relationship was found for inhibitors of glucose 6-phosphate isomerase and triose phosphate isomerase.[19-22] The K_m/K_i ratio (29) for this analogue is well below the ratio expected for a transition-state analogue.[35] The carboxylate group probably resembles the enediol intermediate and not the transition state. The 5-carbon analogues (**25** and **34**) are two to ten times less potent than **54**. In the 5-carbon series, substitution at C-2 with fluorine reduced potency but not as much as changes at C-3 did. The lactone **24**, surprisingly, has no activity.

Table II. Inhibition of A5P Isomerase by Nonphosphorylated Analogues

I III

Compd.	Structure				I_{50}[a]/[A5P]or % Inhibition (mM)
	I				
	R_1	R_2	R_3	R_5	
Ara	H	OH	OH	H	NA @ 5
42	H	OH	OH	OSO_2NH_2	19.2
47	H	OH	OH	$OC(O)NHSO_3Na$	19.2
48	H	OH	OH	ONO_2	Interfered
67	H	OH	OH	$OC(O)CH_2COMe$	Unstable
68	H	OH	OH	$OSO_2(4\text{-}NH_2Ph)$	Unstable
	III				
	R_2	R_3	R_5		
69	OH	OH	– OH		NA @ 6
27	OH	OH	$MeCOCH_2\text{-}CO_2$–		26.9
28	OH	OH	NH_2SO_3–		NA @ 12
29	OH	OH	$MeSO_3$		8.4
30	OH	OH	$NaOSO_3$–		35.9
31	OH	OH	$4\text{-}NH_2PhSO_3$-		8.4
32	OH	OH	$NaOCOCO_2$–		NA
33	OH	OH	Cl		12 @ 6

[a] See Table I. NA, not active.

The inhibition of spinach ribose 5-phosphate isomerase by erythronate 4-phosphate (**54**) has been reported by Wolfenden.[27] The R5P isomerase and the A5P isomerase presumably have similar mechanisms and differ only in the stereochemistry of the reaction. In contrast to our study, Wolfenden found a much larger K_m/K_i (3.3 × 10^{-3} M/4.4 × 10^{-6} M = 750). The simultaneous inhibition of both R5P isomerase and A5P isomerase is undesirable,

(19) Topper, Y. *Enzymes, 2nd Ed.* **1961**, *5*, 429–441.
(20) Noltmann, E. *Enzymes, 3rd Ed.* **1972**, *6*, 271–354.
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(28) Szabó, P.; Szabó, L. *J. Chem. Soc.* **1964**, 5139.
(29) The aldose was prepared according to: Unger, F.; Stix, D.; Moderndorfer, E.; Hammerschmid, F. *Carbohydr. Res.* **1978**, *67*, 349.
(30) Appel, R.; Berger, G. *Chem. Ber.* **1958**, *91*, 1333. With the modification that a CO_2/CCl_4 bath instead of an ice/water bath was used for cooling.
(31) Warren, C. B.; Malec, E. J. *J. Chromatogr.* **1972**, *64*, 219.
(32) Honeyman, J.; Morgan, J. W. W. *J. Chem. Soc.* **1955**, 3660.
(33) The triacetate was not dissolved initially in Ac_2O to eliminate the possibility of acetylation on the 5-OH group before fuming HNO_3 was added. Subsequently, alcohols were dissolved in cold HOAc before the $AcONO_2$ was added.
(34) Still, W. C.; Kahn, M.; Mitras, A. *J. Org. Chem.* **1978**, *43*, 2923.
(35) Wolfenden, R. *Biochemistry* **1970**, *9*, 3403; *Annu. Rev. Biophys. Bioeng.* **1976**, *5*, 271.
(36) The 5-chloro compound was also prepared by the action of $MeSO_2Cl$/pyridine or Ph_3P/CCl_4.
(37) El-Rahman, M.; Hornemann, U. *Carbohydr. Res.* **1974**, *38*, 355.
(38) Zinner, H.; Bradner, H.; Rembarz, G. *Chem. Ber.* **1956**, *89*, 800.
(39) Zwierzak, A.; Kluba, M. *Tetrahedron* **1971**, *27*, 3163.

since the intermediate, ribulose 5-phosphate, would accumulate and overcome the inhibition. Therefore, one needs a selective isomerase inhibitor.

The best alditol phosphate, **60**, was threefold less potent than **54**. The 5-carbon analogues **59–61** were much more potent than the 6-carbon analogues **64** and **65**. The effect of substitution was similar to that found with the aldonic acids. Fluorine and hydroxyl were acceptable at C-2, but fluorine and *O*-benzyl were not allowed at C-3. In contrast to the acids, the 2-deoxyalditol phosphate **58** was not active. Surprisingly, the phosphonate **66** was less active than its phosphate analogues. Apparently, substitution is needed at C-1 and C-2 to mimic the enediol, hydroxyl is needed specifically at C-3, and a terminal phosphate is needed to produce the best enzyme binding.

In contrast to the acids and alcohols, aldose phosphate analogues were not good enzyme inhibitors. The effect of substitution was different from that of the previous cases (**16** was active and **9** was not), and several compounds had no activity at the highest concentration used. The common sugar phosphates, glucose 6-phosphate, galactose 6-phosphate, mannose 6-phosphate, and erythrose 4-phosphate, were also not active. The oxime **19** was prepared as an acyclic analogue of the substrate **2**, but it, too, was only weakly inhibitory.

The nonphosphorylated compounds were prepared in the hope of finding a neutral or nonphosphorylated compound that could penetrate the bacterial cell. In addition, the phosphate group of the substrates and inhibitors could be binding to an arginine residue.[25,40] Polar and anionic groups other than phosphate were tried in an effort to find a group with good affinity for the hypothetical, isomerase active-site arginine. As mentioned above, the lactones in Table II inhibit, but only at high concentrations (about 10 times that of the substrate). The sulfonates **29** and **31** were the best inhibitors within this group, but, surprisingly, the sulfamate **28** and the sulfate **30** were very weak inhibitors. The arabinose sulfamate **42** was a better inhibitor but still too weak for any practical purpose. Unfortunately, the nitrate ester **48** interfered with the assay, and an I_{50} could not be determined. Lactone ester **27** was a weak inhibitor, but the corresponding arabinose derivative **67** was too unstable to test.

In summary, of the compounds tested, aldonic acid phosphates and alditol phosphates were the best inhibitors of arabinose 5-phosphate isomerase and resemble the enediol intermediate more than the aldose analogues do. An acidic proton is important for the best binding, but the diol geometry is also good. This result suggests that hydroxamic acids should be good isomerase enzyme inhibitors, a hypothesis that we are now testing. In spite of having $K_i << K_m$, these carbohydrate phosphates neither have antibacterial activity in vitro nor potentiate the activity of Crystal violet, presumably because they do not penetrate the bacterial cell. Our attempts to find good, nonphosphate inhibitors were not successful. Even though A5P isomerase remains an interesting biological target, substrate and enediol analogues of the type reported here are not likely to be therapeutically useful, and the search for LPS inhibitors must now include other enzymes and compounds with broader structural diversity.

Experimental Section

Melting points were run on a Thomas-Hoover capillary melting point apparatus and are corrected. Infrared spectra (Nujol, KBr) were recorded on a Perkin-Elmer 267 spectrophotometer. NMR spectra were determined with Varian T-60, XL-100, and CFT-20 spectrometers and Hitachi Perkin-Elmer R-24A and R-24B spectrometers with tetramethylsilane as the internal standard for spectra run in $CDCl_3$ or Me_2SO-d_6. Low-resolution mass spectra were obtained with a Varian MAT CH5 double-focusing mass spectrometer at 70 eV, and probe temperatures or amperages are noted; accurate masses were determined by peak matching at 10 000 resolution, 10% valley definition. Field-desorption data were determined with a Varian MAT 931 spectrometer. Microanalyses were performed by Atlantic Microlab, Inc., Atlanta, GA; Integral Microanalytical Laboratories, Raleigh, NC; and Galbraith Laboratories, Inc., Knoxville, TN. All C, H, and N analyses not reported here were acceptable (±0.4%) unless noted otherwise.

Column chromatography was performed on silica gel with both open columns (E. Merck, silica gel 60, 70–230 mesh) and a Waters Prep 500A instrument. TLC was done with silica gel (Whatman MK6F plates) and with anisaldehyde spray plus heat. P-2 column chromatography was performed with two 2.5 × 90 cm columns of Bio-Gel P-2 (200–400 mesh) and water at a flow rate of 30 mL/h. Concentrations were done with rotary evaporators at temperatures <40 °C.

The following compounds were purchased from Sigma Chemical Co.: D-arabinose 5-phosphate, 2-deoxy-D-ribose 5-phosphate (**52**), D-ribose 5-phosphate (**53**), D-mannitol 6-phosphate (**65**), D-galactose 6-phosphate, D-ribulose 5-phosphate, D-mannose 6-phosphate, erythrose 4-phosphate, D-ribono-γ-lactone (**69**), and sodium 2-deoxy-D-gluconate 6-(disodium phosphate).

3-Deoxy-D-*threo*-pentofuranose 5-(Disodium phosphate) (7). Methyl 3-deoxy-D-*threo*-pentofuranoside (**5**, Y = H) was phosphorylated and hydrogenolyzed under the conditions of Szabo[8] to obtain the methyl furanoside of **6** as the barium salt. This barium salt (2.1 g) was dissolved in 100 mL of H_2O and treated with AG 50W-X8 (H^+) until the pH reached 2.4. The resin was collected and washed, and the filtrate was heated on a steam bath for 15 min. The solution was cooled to 25 °C, and 1 N NaOH was added until pH 6.9. Water was evaporated under vacuum. The reaction was not complete (by NMR). The mixture was separated on a Bio-Gel P-2 column with H_2O. The fractions were evaporated, and the intermediate methyl furanoside (1.27 g) and hygroscopic product **7** were obtained: yield 0.2 g; GC/MS (per Me_3Si), *m/e* 487 (M − Me). The sample was homogeneous by Bio-Gel P-2 chromatography.

2-Deoxy-2-fluoro-D-arabinofuranose 5-(Dipotassium phosphate) (9). Methyl 2-deoxy-2-fluoro-α-D-arabinofuranoside[10] (**8**; 5 g) was phosphorylated by the Szabo method[8] to obtain the diphenyl ester as a syrup (11.3 g, 94%). The crude product was dissolved in 400 mL of dry acetone containing 1% HCl by weight, and the solution was allowed to stand at room temperature for 96 h to convert any 3-fluoro contaminant to the 1,2-*O*-isopropylidene derivative. The solution was neutralized with solid $NaHCO_3$ and filtered, and the filtrate was evaporated to a syrup. The syrup was chromaographed on silica gel with $CHCl_3$ to obtain 5.45 g (45%) of pure **70** as a syrup: NMR ($CDCl_3$) δ 3.32 (s, OMe), 3.8–4.4 (m, 5 H, H-3, H-4, H-5, OH), 4.9 (dd, J_{FH} = 50 Hz, J_{23} = 1.6 Hz, 1 H, H-2), 5.0 (d, J_{FH} = 10 Hz, H-1), 7.2 (br s, 10 H, Ar H).

The diphenyl phosphate **70** (5.45 g) was hydrogenolyzed and neutralized with KOH to obtain **71** as a syrup: yield 3.34 g (99%); NMR (D_2O) δ 3.4 (s, OMe), 3.9–4.3 (m, H-3, H-4, H-5), 4.85 (d, J_{HF} = 50 Hz, H-2), 5.0 (d, J_{HF} = 10 Hz, H-1), 5.3 (d, J = 4 Hz, H-1 of β anomer).

The methyl furanoside **71** (3.34 g) was swirled with 25 mL of 9:1 (v/v) trifluoroacetic acid/H_2O for 15 min. Water (2 mL) was added, and the solution was evaporated. The addition of water and evaporation were repeated after chromatography on Bio-Gel P-2 with H_2O: MS (per Me_3Si), *m/e* 505 (M − 15), 431 (M − Me_3SiO).

3-Deoxy-3-fluoro-D-arabinose 5-(Disodium phosphate) (16). Methyl 5-*O*-benzyl-3-fluoro-D-arabinofuranoside (**10**)[11] was converted to the 1,2-*O*-isopropylidene derivative under the conditions of Hirst, Jones, and Williams.[12] The crude product was chromatographed on silica gel with $CHCl_3$ to obtain **11** as a yellow oil: TLC ($CHCl_3$) R_f 0.7; NMR ($CDCl_3$) δ 1.3 (s, Me), 1.4 (s, Me), 3.65 (d, J = 7 Hz, H-5), 4.25 (m, H-4), 4.6 (s, OCH_2), 4.85 (d, J = 4 Hz, H-2), 5.1 (dd, J_{FH} = 52 Hz, J_{34} = 1 Hz, H-3), 5.95 (d, J = 4 Hz, H-1), 7.3 (s, Ar H).

(40) Schneider, F. *Naturwissenschaften* 1978, *65*, 376.

A sample of 11 (0.2 g) in 30 mL of EtOH containing 50 mg of 5% Pd/C was hydrogenolyzed at 40 psi for 3 h. The catalyst was filtered, and a TLC indicated incomplete conversion. The sample was hydrogenolyzed over fresh catalyst for 2 h. The catalyst was filtered, and the solvent was evaporated to give 0.11 g of a colorless oil **12**. The crude product was used in the next step without additional purification.

The 5-OH intermediate **12** (0.11 g) was treated under Szabo conditions[8] to obtain the diphenyl phosphate as a colorless oil: yield 0.21 g. TLC (silical gel, $CHCl_3$) showed one majo. spot at R_f 0.3 and phenol at R_f 0.6. The NMR spectrum was consistent with the structure. This 5-(diphenyl phosphate) (0.2 g) was hydrogenolyzed over 60 mg of PtO_2 at 50 psi in a Parr apparatus. The water-soluble fraction was stirred for 15 min with 9:1 (v/v) trifluoroacetic acid/H_2O. The acid was evaporated, and the residue was coevaporated three times with H_2O and three times with 95% EtOH. (The EtOH evaporation can produce glycosides if traces of acid remain!) This product was chromatographed on Bio-Gel P-2 with water to obtain **16** as a single compound: MS (per Me_3Si), m/e 505 (M − Me), 431 (M − Me_3SiO).

3-*O*-Methyl-D-arabinose 5-(Barium phosphate) (17). The method of Hirst[12] for the L isomer was used to prepare 1,2-*O*-isopropylidene-3-*O*-methyl-D-arabinofuranose (**15**, Y = OMe). This substance (1.7 g) was phosphorylated on the 5-position by the method of Szabo[8] to obtain the diphenyl phosphate: yield 1.1 g. The protected phosphate (1.0 g) was hydrogenolyzed over PtO_2 on a Parr shaker. The product was treated with 10 mL of 9:1 (v/v) trifluoroacetic acid/H_2O for 15 min, and solvent was evaporated. The residue was dissolved in H_2O, which was evaporated. The crude product was passed through a Bio-Gel P-2 column. The product was dissolved in 40 mL of H_2O, and the solution was adjusted to pH 1.2 with Dowex 50 (H^+) and filtered. The filtrate was treated with 7.4 mL of 0.45 N $Ba(OH)_2$ solution and diluted with 150 mL of EtOH, but no precipitate formed. The solvent was evaporated, and the residue was dissolved in EtOH and diluted with Et_2O. A white precipitate was isolated by centrifugation: yield 0.43 g; GC/MS (per Me_3Si), m/e 517 (M − Me, 2.92).

3-*O*-Benzyl-D-arabinose 5-(Dihydrogen phosphate) (18). A solution of 3-*O*-benzyl-1,2-*O*-isopropylidene-D-arabinofuranose[26] (**36**; 1.0 g) in 11 mL of dry pyridine was added to $POCl_3$ (0.6 g) in 2.6 mL of pyridine at −40 °C. This mixture was cooled with an ice–salt bath, stirred 2 h, and then added to 5 mL of 90% aqueous pyridine, 50 g of ice, and saturated $Ba(OH)_2$ until alkaline (ca. 50 mL). The solvent was evaporated, and the residue was diluted to 50 mL with H_2O, which was evaporated. The residue was diluted to 50 mL with water, and the solution was frozen overnight, thawed, adjusted to pH 1 with concentrated H_2SO_4, and heated on a steam bath for 2 h. The cooled suspension was treated with 0.85 g of $AgCO_3$ and filtered. The filtrate was treated again with 0.85 g of $AgCO_3$, but no precipitate formed. The solution was filtered, treated with H_2S, filtered, purged with N_2, and adjusted to pH 10 with saturated $Ba(OH)_2$ (ca. 10 mL). The suspension was centrifuged, and the supernatant was decanted and condensed to 25 mL. The condensed solution was poured into 75 mL of absolute EtOH, but no precipitate formed. The solution was evaporated to dryness. The product was passed through a Dowex 50W (H^+) column and then chromatographed on Bio-Gel P-2 with H_2O. The fractions were evaporated to obtain the title compound **18**: yield 0.8 g (69%); MS (per Me_3Si), m/e 593.2007 (M − 15, $C_{23}H_{46}O_8PSi_4$).

(*E*,*Z*)-D-Arabinose 5-(Dilithium phosphate) Oxime (19). A solution of D-arabinose 5-(dilithium phosphate)[9] (1.02 g, 0.0041 mol) in 5 mL of H_2O was treated with NH_2OH·HCl (0.31 g, 0.0045 mol). The pH was adjusted to 7.7 with 1 M LiOH, and a precipitate formed. The mixture was treated with charcoal and filtered. The filtrate was condensed to ~2 mL and stored at −20 °C overnight. This residue was diluted with 1 mL of H_2O and added dropwise to 75 mL of MeOH with rapid stirring. The white precipitate was collected and washed with MeOH and dry Et_2O. The product was reprecipitated from 1:1 MeOH–acetone, washed, and dried under high vacuum to give **19**: yield 0.5 g (44%); mp 170 °C dec; IR (KBr) 3300, 1620, 1400, 1300, 1110, 1080, 1000 cm^{-1}; ^{13}C NMR (D_2O) δ 67.1 (C-5, *Z*), 67.6 (d, J_{PC} = 4.4 Hz, C-5, *E*), 71.3 (C-2, *E*), 72.83 (d, J_{PC} = 6 Hz, C-4, *E*), 73.6 (*Z*), 74.3 (C-3, *E*), 155.6 (C-1, *E*), 156.4 (C-1, *Z*); ^{31}P NMR (D_2O + EDTA) 3.67 ppm from H_3PO_4 (t, *E*), 2.84 ppm (t, *Z*); ^1H NMR (D_2O) δ 7.0 (d, J_{12} = 5 Hz, *Z*), 7.6 (d, J_{12} = 5 Hz, *E*), *E*/*Z* = 4.75; $[\alpha]^{25}_D$ −9.9° (*c* 1.0, H_2O). Anal. ($C_5H_{12}Li_2NO_8P·H_2O$) C, N; H: calcd, 4.40; found, 3.95.

5-*O*-Acetoacetyl-D-ribono-1,4-lactone (27). To a stirred solution of 2.35 g (0.0125 mol) of 2,3-*O*-isopropylidene-D-ribono-1,4-lactone[18] (**26**) in 50 mL of dry pyridine chilled to 0–5 °C and protected by a drying tube was added dropwise over a 1-min period 1.095 g (0.0130 mol) of distilled diketene. After 11 min the solution was removed from the cooling bath and was allowed to warm to room temperature and stand unstirred for 3 days. The solution was concentrated under vacuum at 50 °C to 3.40 g of orange oil, which was chromatographed on 110 g of silica gel with 1:1 hexanes/EtOAc. Fractions containing clean, desired product [TLC (1:1 hexanes/EtOAc, R_f 0.3] were combined and concentrated under vacuum to a yellow oil, **72**, which eventually solidified: yield 1.66 g (49%); NMR ($CDCl_3$) was consistent with the structure of the product.

To 1.35 g (0.00496 mol) of **72** was added 13.5 mL of 9:1 (v/v) trifluoroacetic acid/H_2O. After 10 min the slightly cloudy solution was filtered, and the clear filtrate was concentrated under vacuum to a pale yellow solid, which was washed with 2 × 10 mL of ether in a funnel and dried under vacuum at room temperature: yield 1.11 g. A 0.97-g sample of solid was dissolved in 200 mL of boiling ethyl acetate, and to this solution was added 1000 mL of boiling benzene. The solution was concentrated by boiling to 400–500 mL and was allowed to cool slowly to room temperature. White needles of **27** were collected by filtration, washed with 2 × 25 mL of benzene, and dried under vacuum at 45 °C: yield 0.78 g (78%); mp 137–138 °C; IR (Nujol) 1772 (C-1 CO), 1740 (ester CO) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.17 (s, 3 H), 3.66 (s, 2 H), 4.0–4.6 (m, 5 H), 5.50 (d, *J* = 4 Hz, 1 H), 5.79 (d, *J* = 7 Hz, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 29.95 (CH_3), 49.26 ($COCH_2CO$), 63.48 (C-5), 68.17 and 68.61 (C-2, C-3), 81.81 (C-4), 166.82 (ester CO), 175.67 (C-1 CO), 201.31 (ketone CO); $[\alpha]^{20}_D$ +5.2° (*c* 0.5, H_2O). Anal. ($C_9H_{12}O_7$) C, H.

5-*O*-Sulfamyl-D-ribono-1,4-lactone (28). To a stirred mixture of 2.35 g (0.0125 mol) of **23** in 50 mL of CH_2Cl_2 chilled to 0–5 °C and protected by a drying tube was added 2.54 g (0.0251 mol) of triethylamine and then 2.89 g (0.0250 mol) of sulfamyl chloride.[30] A complete solution resulted. After 18 min the solution was removed from the cooling bath and was allowed to stand overnight at room temperature. After 20 h, the solution with a small amount of precipitated white solid was concentrated under vacuum to a paste/solid mixture to which was added 50 mL of EtOAc. Undissolved white solid was collected by filtration. The cloudy filtrate was concentrated under vacuum to a yellow paste, which was chromatographed on 100 g of silica gel with EtOAc: yield 2.22 g (67%) of a pale yellow oil (**73**), which eventually solidified: TLC (EtOAc) R_f 0.7; NMR (Me_2SO-d_6).

To 2.20 g (0.0823 mol) of **73** was added 22 mL of 9:1 (v/v) trifluoroacetic acid/H_2O. The mixture was swirled for 12 min, and a solution resulted. After another 37 min, the solution was concentrated under vacuum at 45 °C to an oil, which was coevaporated once with H_2O and twice with CH_2Cl_2. The oil was subjected to high vacuum at 40 °C, and an off-white, crystalline solid resulted: yield 1.86 g. The solid was washed on the filter with 2 × 10 mL of ether and was dried overnight at 40 °C: yield 1.60 g of white solid. A 1.49-g sample of solid was recrystallized from EtOAc/toluene by a procedure similar to the one used for **27**. White, crystalline solid **28** was collected by filtration, washed with 2 × 25 mL of toluene, and dried under vacuum at 45 °C: yield 1.36 g (78%); mp 141–141.5 °C; IR (Nujol) 1782 (C-1 C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.1–4.6 (m, 5 H), 5.59 (d, *J* = 3 Hz, 1 H), 5.92 (d, *J* = 7 Hz, 1 H), 7.69 (br s, 2 H); ^{13}C NMR (Me_2SO-d_6) δ 67.6 (C-5), 68.3 and 68.7 (C-2, C-3; or C-3, C-2), 81.7 (C-4), 175.6 (C-1); $[\alpha]^{25}_D$ +26.4° (*c* 1.015, H_2O). Anal. ($C_5H_9NO_7S$) C, H, N.

5-*O*-(Methylsulfonyl)-D-ribono-1,4-lactone (29). To a stirred solution of 1.00 g (0.0053 mol) of **26** and 1.1 mL of triethylamine in 30 mL of dry CH_2Cl_2 chilled to 0–5 °C was added 0.610 g (0.0053 mol) of methanesulfonyl chloride (MsCl).[36] After 1 h and 50 min, another 0.102 g (0.0009 mol) of MsCl was added. After 55 min since the second addition of MsCl, the solution was diluted to 100 mL with CH_2Cl_2 and washed with 2 × 5 mL of water. The aqueous layer was backwashed with 50 mL of CH_2Cl_2, the combined CH_2Cl_2 solution was dried over Na_2SO_4 and filtered,

and the filtrate was concentrated under vacuum at 40 °C to a yellow to brownish-yellow, viscous oil **74**: yield 1.47 g (quantitative); NMR (Me_2SO-d_6).

To 1.44 g (0.00575 mol) of **74** was added 14 mL of 9:1 (v/v) trifluoroacetic acid/H_2O. After 8 min, the slightly cloudy solution was filtered, and the filtrate was concentrated under vacuum at 45 °C to an off-white solid, which was washed with 2 × 10 mL of ether to remove a brownish contaminant: yield of white solid 1.14 g. White crystals of **29** were obtained after two recrystallizations from EtOAc: yield 0.624 g (51%); mp 120–121.5 °C; IR (Nujol) 1779 (CO), 1460 and 1170 (SO_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.26 (s, 3 H), 4.15 (d, J = 6 Hz, 1 H), 4.25–4.60 (m, 4 H), 5.65 (br s, 2 H); ^{13}C NMR (Me_2SO-d_6) δ 36.67 (CH_3), 68.14, 68.36, 68.51 (C-2, C-3, C-5, not necessarily respectively), 81.55 (C-4), 175.53 (C-1); $[\alpha]^{20}_D$ +28.2° (*c* 1.00, H_2O). Anal. ($C_6H_{10}O_7S$) C, H.

D-Ribono-1,4-lactone 5-(Sodium sulfate) (30). A solution of chlorosulfuric acid (1.35 mL) in 8 mL of dry CH_2Cl_2 was added dropwise over 15 min to a cold (−8 °C) solution of **26** (1.9 g, 0.01 mol) in 50 mL of dry pyridine. This solution was stirred overnight at room temperature, cooled to 6 °C, and treated with a solution of H_2O (3 mL) and pyridine (5 mL). This solution was stirred for 1 h, diluted with H_2O, and evaporated. The yellow residue was dissolved in 10 mL of H_2O, the solution was adjusted to pH 7 with 1 N NaOH and filtered, and the filtrate was evaporated. The crude product was chromatographed on Bio-Gel P-2 in H_2O. The anisaldehyde-positive fractions were combined and lyophilized. TLC on C-18 reversed-phase plates with 9:1 PrOH/0.5% aqueous Et_4NCl revealed one major spot at R_f 0.22.

The combined product of several runs (1.1 g, 0.0038 mol) was treated with 10 mL of 9:1 (v/v) trifluoroacetic acid/H_2O for 10 min and evaporated at ≤33 °C. The residue was evaporated with CH_2Cl_2 several times and dissolved in 10 mL of H_2O. The solution was adjusted to pH 8 with saturated $Ba(OH)_2$. The suspension was purged with CO_2, and the precipitated barium salts were collected by filtration. The filtrate was condensed to 15 mL, diluted with 20 mL of 95% EtOH, chilled, and filtered to remove insolubles. The filtrate was condensed to 10 mL, diluted with 40 mL of 3:1 acetone/EtOH, chilled, and filtered. The filtrate was condensed to 5 mL and diluted to 50 mL with acetone. Cooling overnight produced large, colorless crystals of **30**. The combined yield of the two crops was 0.44 g (45%): mp 192–195 °C; IR (KBr) 1780 (C=O), 1360 and 1200 ($ROSO_3$) cm^{-1}; NMR (Me_2SO-d_6) δ 3.31 (H_2O), 3.9 (d, J_{45} = 3.81 Hz, 2 H, H-5), 4.15 (A of ABMX, J_{23} = 5.34 Hz, J_{34} = 0.7 Hz, J_{OH} = 3.9 Hz, 1 H, H-3), 4.39 (br t, 1 H, H-4), 4.41 (B of ABMX, J_{23} = 5.34 Hz, J_{OH} = 7.5 Hz, 1 H, H-2), 5.15 (d, J = 3.82 Hz, 1 H, 3-OH), 5.77 (d, J = 7.5 Hz, 1 H, 2-OH); $[\alpha]^{25}_D$ +115.2° (*c* 0.5, H_2O). Anal. ($C_5H_7NaO_8S{\cdot}0.5H_2O$) C, H, Na.

5-O-[(*p*-Aminophenyl)sulfonyl]-D-ribono-1,4-lactone (31). To a stirred solution of 3.00 g (0.0159 mol) of **26**[18] in 30 mL of dry pyridine, chilled in a CCl_4/CO_2 bath (−23 °C) and protected by a drying tube, was added 3.89 g (0.0175 mol) of *p*-nitrobenzenesulfonyl chloride at one time. After 2 h and 20 min, to the solution was added, at 5-min intervals, 0.3, 0.3, 0.3, 0.6, and 1.5 mL of H_2O. Cloudiness developed after the first addition of 0.3 mL. After 5 min, an additional 30 mL of H_2O was added, and after another 5 min, the mixture was removed from the cooling bath and allowed to warm to room temperature. White crystals of **75** were collected by filtration, washed well with 2 × 15 mL and 2 × 30 mL of H_2O, and dried under vacuum at 50 °C: yield 2.67 g (45%); NMR (Me_2SO-d_6).

To 0.420 g (0.0112 mol) of **75** was added 4.2 mL of 9:1 (v/v) trifluoroacetic acid/H_2O. After 38 min the mixture, including precipitated white solid, was concentrated under vacuum at 45 °C to a white solid **76**, which was washed with 2 × 10 mL of ether and dried under vacuum at 50 °C: yield 0.360 g (96%); NMR (Me_2SO-d_6).

Lactone **76** (0.355 g, 0.00107 mol) in 20 mL of DMF was hydrogenated on a Parr shaker over 0.100 g of 10% Pd/C under 37–31 psi of H_2. After 2 h, the mixture was filtered through Celite. The filtrate was concentrated under vacuum at 40 °C to a pale yellow oil: yield 0.33 g. The combined oil from two batches (0.43 g) was chromatographed twice on silica gel with EtOAc. Fractions containing only desired product [TLC (EtOAc) R_f 0.5] were combined and partially concentrated under vacuum. A small amount of solid was removed by filtration. The filtrate was concentrated under vacuum at 40 °C to an oil that became an off-white solid **31**: yield 0.161 g; mp 107–109 °C; IR (Nujol) 1775 (C-1 CO), 1374 and 1166 (SO_2) cm^{-1}; NMR (Me_2SO-d_6) δ 3–7 (br, NH_2, OH, H_2O), 3.95–4.45 (m, 5 H), 6.67 and 7.50 (AA'BB', 4 H); $[\alpha]^{20}_D$ +1.4° (*c* 0.475, 1:7 Me_2SO/DMF); MS (field desorption, 14 mA), *m/e* 303 (M). Anal. ($C_{11}H_{13}NO_7S$) C, H, N.

5-O-Oxalo-D-ribono-1,4-lactone Sodium Salt (32). To a stirred solution of 3.00 g (0.0159 mol) of **26** in 100 mL of dry pyridine chilled to 0–5 °C was added, over a 3-min period, 3.17 g (0.0160 mol) of benzyloxalyl chloride.[31] A viscous, yellowish oil precipitated during the addition, and stirring was stopped. After 10 min, the mixture was removed from the cooling bath, and the remaining oil was dissolved. After 21 h, another 1.59 g (0.00801 mol) of benzyloxalyl chloride was added over a 1-min period. A viscous oil precipitated initially during the addition but redissolved. After 39 min, another 1.58 g (0.00796 mol) of benzyloxalyl chloride was added over a 1- to 2-min period. TLC after 35 min indicated a small amount of starting lactone. After 1 h and 15 min, the dark solution was concentrated under vacuum at 35 °C to a dark brownish-red paste, which was partitioned between 100 mL of H_2O and 100 mL of EtOAc, the EtOAc layer was separated, dried over Na_2SO_4, and filtered, and the filtrate was concentrated under vacuum at 35 °C to 6.3 g of dark oil, which was chromatographed twice on silica gel in a Waters Prep 500 A apparatus with 2:1 hexanes/EtOAc: yield of colorless oil **77** 2.55 g (46%); NMR (Me_2SO-d_6).

To 2.49 g (0.00711 mol) of **77** was added 25 mL of 9:1 (v/v) trifluoroacetic acid/H_2O. After 30 min, the slightly cloudy solution was filtered and then concentrated under vacuum at 40 °C to a white solid, which was coevaporated with CH_2Cl_2: yield of white to off-white solid **78** 2.24 g (quantitative); NMR (Me_2SO-d_6).

Lactone **78** (2.22 g, 0.00716 mol) in 75 mL of EtOAc was hydrogenolyzed on a Parr shaker over 0.100 g of 5% Pd/C under 39–33 psi of H_2. After 15 min, H_2 consumption was complete. After 1 h and 20 min, the mixture was filtered through Celite. The filtrate was concentrated under vacuum at 30 °C to a white solid: yield 1.46 g. A 1.40-g sample of solid was recrystallized from EtOAc/CCl_4 by the procedure used for **27**. White, crystalline solid **79** was collected, washed with 50 mL of CCl_4, and dried under vacuum at room temperature: yield 1.16 g (77%). NMR (Me_2SO-d_6) of this compound indicated mainly product with approximately $^1/_8$ mol of D-ribono-1,4-lactone. Therefore, the sodium salt **32** was made.

To a solution of 1.03 g (0.00468 mol) of **79** in 20 mL of H_2O was added 0.393 g (0.00468 mol) of $NaHCO_3$. After effervescence had stopped (pH was neutral), the water was removed under vacuum at room temperature: yield of a solid/paste mixture 1.21 g. The mixture was dissolved in 4 mL of H_2O, and a small amount of undissolved solid was removed by suction filtration. The filtrate was chromatographed on Bio-Gel P-2. Fractions containing desired product (RI, TLC) were combined and concentrated to 0.9 g of white solid, which was dissolved in 4 mL of H_2O. The solution was filtered, the filtrate was diluted with 50 mL of acetone, and the resulting mixture was refrigerated for 3 days. White solid **32** was collected by filtration, washed with 2 × 5 mL of a cold 4:50 H_2O/acetone mixture, and dried under vacuum at room temperature: yield 0.500 g (44%); mp 199.5–201 °C dec; IR (Nujol) 1789 (C-1 CO), 1743 (ester CO), 1661 (carboxylate CO) cm^{-1}; ^{13}C NMR (D_2O) δ 66.63 (C-5), 71.31 and 71.87 (C-2, C-3; or C-3, C-2), 86.05 (C-4), 165.68 and 166.34 (ester CO and carboxylate CO, not necessarily respectively), 180.74 (C-1) (weak peaks at δ 69.93, 71.60, and 75.49 indicated a small amount of open-chain ribonic acid analogue); $[\alpha]^{23}_D$ +24.7° (*c* 0.505, H_2O). Anal. ($C_7H_7NaO_8{\cdot}0.25H_2O$) C, H, Na.

5-Chloro-5-deoxy-D-ribono-1,4-lactone (33). To a stirred solution of 1.175 g (0.00624 mol) of **26** in 20 mL of dry pyridine chilled to 0–5 °C and protected by a drying tube was added 2.77 g (0.0125 mol) of *p*-nitrobenzenesulfonyl chloride. After 13 min, the solution was removed from the cooling bath and then allowed to warm to room temperature and stand overnight. After 17 h, precipitated solid was removed by filtration. The filtrate was concentrated under vacuum to a dark, partially solidified paste, which was triturated with 20 mL of EtOAc, and undissolved solid was removed by filtration. The clear filtrate was concentrated under vacuum at 40 °C to a yellow liquid: yield 0.88 g. A 0.83-g sample of liquid was chromatographed on 25 g of silica gel with

EtOAc: yield 0.75 g (62%) of an oil (**80**),[36] which quickly solidified; TLC (EtOAc, R_f 0.8); NMR (Me_2SO-d_6) δ 1.34 overlapping 1.36 (2 s, 6 H), 4.00 (d, 2 H), 4.75–5.05 (m, 3 H). Anal. ($C_8H_{11}ClO_4$) C, H. The product, therefore, is the 5-chloro derivative and not the 5-*p*-nitrobenzenesulfonyl compound that was expected.

To 0.69 g (0.00334 mol) of **80** was added 6.9 mL of 9:1 (v/v) trifluoroacetic acid/H_2O. After 45 min, the solution was filtered, and the filtrate was concentrated at 40 °C under vacuum to an oil, which was coevaporated twice with CH_2Cl_2. The residual oil solidified, and off-white solid was dried under vacuum at 50 °C: yield 0.56 g. The solid was recrystallized from hexanes/EtOAc. White needles of **33** were collected by filtration, washed with 2 × 10 mL of hexanes, and dried under vacuum at 50 °C: yield 0.42 g (76%); mp 86.5–87 °C; IR (Nujol) 1769 (C-1 CO) cm^{-1}; NMR (Me_2SO-d_6) δ 3.8–4.0 (m, 2 H), 4.0–4.25 (m, 1 H), 4.25–4.65 (m, 2 H), 5.62 (br d, J = 4 Hz, 1 H), 5.91 (br d, J = 7 Hz, 1 H); $[\alpha]^{25}_D$ +17.9° (*c* 0.96, H_2O). Anal. ($C_5H_7ClO_4$) C, H, Cl.

Lithium D-Ribonate 5-(Lithium hydrogen phosphate) (34). A sample of **26** (1.03 g, 0.00547 mol) was phosphorylated by the Szabo method.[8] The crude **81** was recrystallized from 95% ethanol/water to afford 1.44 g (63%) of needles: mp 106–107 °C; IR ($CHCl_3$) 1800, 1593, 1200, 975 cm^{-1}; NMR ($CDCl_3$) δ 1.35 (s, 3 H, Me), 1.47 (s, 3 H, Me), 4.35–4.77 (m, 5 H, C-2, C-3, C-4, and C-5 H's), 7.27 (m, 10 H, aromatic). Anal. ($C_{20}H_{21}O_8P$) C, H.

A solution of **81** (1.30 g, 0.0031 mol) was hydrogenolyzed[8] over PtO_2 to yield 1.03 g of crude intermediate 2,3-*O*-isopropylidene-D-ribono-1,4-lactone 5-(dihydrogen phosphate). This intermediate was treated with water (10 mL), warmed at 40 °C for 1 h, adjusted to pH 7.6 with 1 M LiOH, and concentrated to a small volume (~4 mL) under vacuum. This solution was added dropwise to 1:1 acetone/MeOH (~50 mL), affording a white precipitate, which was collected by suction filtration: yield 0.72 g (90%) of hydrated white powder **34**; mp >300 °C; IR (Nujol) 3500–3000 (OH), 1605, 1150–1000 (phosphate) cm^{-1}; ^{13}C NMR (D_2O) δ 68.10 (d, J_{PC} = 4.8 Hz, C-5), 73.35 (d, J_{PC} = 6.4 Hz, C-4), 75.55 (C-3), 76.20 (C-2), 180.25 (C-1). Anal. ($C_5H_9Li_2O_9P$) C, H.

3-*O*-Benzyl-1,2-*O*-isopropylidene-5-*O*-[(*p*-nitrophenyl)-sulfonyl]-β-D-arabinofuranose (37). The furanose **36** was treated with *p*-nitrobenzenesulfonyl chloride at −20 °C by the procedure described for **38**. After chromatography (silica, CH_2Cl_2), a pale yellow solid was obtained: yield 3.3 g (66%); NMR ($CDCl_3$) δ 1.28 (s, 3 H, Me), 1.37 (s, 3 H, Me), 3.9 (br s, 1 H, H-3), 4.25 (s, 3 H, H-4 and H-5), 4.57 (s, 2 H, OCH_2), 4.6 (d, J = 4 Hz, 1 H, H-2), 5.85 (d, J = 4 Hz, 1 H, H-1), 7.31 (br s, 5 H, Ph), 8.0–8.5 (AA'BB', 4 H, Ar).

5-*O*-Acetoacetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-β-D-arabinofuranose (38). A stirred solution of 3.00 g (0.0107 mol) of 3-*O*-benzyl-1,2-*O*-isopropylidene-β-D-arabinofuranose (**36**)[28] and 40 mL of dry pyridine in a dry 100-mL round-bottom flask protected by a drying tube was chilled with an ice/water bath and was treated over a 20-s period with 0.900 g (0.0107 mol) of diketene (distilled). After 2 h and 20 min, another 0.450 g (0.00535 mol) of diketene was added. After an additional 2 h and 30 min, the orange solution was concentrated under high vacuum at 50 °C to a dark orange paste: yield 4.32 g. The paste was chromatographed on silica gel in a Waters Prep 500 A apparatus with 2:1 hexanes/EtOAc: yield 2.90 g. A 0.500-g sample was recrystallized from hexanes: yield 0.275 g (41%); mp 74–74.5 °C; IR (KBr) 1728 (ester CO), 1747 (lactone CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (s, 3 H, Me), 1.52 (s, 3 H, Me), 1.96 and 2.24 (2 s, 3 H, Me), 3.44 (s, 2 H, CH_2), 3.96 (br s, 1 H, H-3), 4.29 (s, 3 H, H-4 and H-5), 4.59 (s, 2 H, OCH_2), 4.64 (d, J = 4 Hz, 1 H, H-2), 5.90 (d, J = 4 Hz, 1 H, H-1), 7.33 (s, 5 H, Ph); $[\alpha]^{25}_D$ +25.9° (*c* 0.49, $CHCl_3$). Anal. ($C_{19}H_{24}O_7$) C, H.

3-*O*-Benzyl-1,2-*O*-isopropylidene-5-*O*-sulfamyl-β-D-arabinofuranose (39). The intermediate **36** (0.011 mol) in 40 mL of dry CH_2Cl_2 at 0 °C was treated with 2.2 g of triethylamine and 2.5 g of sulfamyl chloride[30] (0.021 mol). After 2 h, the reaction was worked up as in **38**. The yield of pale yellow oil was 0.75 g (19%): NMR ($CDCl_3$) δ 1.34 (s, 3 H, Me), 1.58 (s, 3 H, Me), 3.97 (br s, 1 H, H-3), 4.3–4.6 (m, 3 H, H-4 and H-5), 4.59 (s, 2 H, OBzl), 4.67 (d, J = 4 Hz, 1 H, H-2), 5.02 (br s, 2 H), 5.93 (d, J = 4 Hz, 1 H, H-1), 7.31 (s, 5 H, Ar); TLC (1:1 hexanes/EtOAc) R_f 0.55.

Methyl 3-Deoxy-3-fluoro-α-D-arabinofuranoside 5-(Disodium phosphate) (14). Methyl 5-*O*-benzyl-3-deoxy-3-fluoro-α-D-arabinofuranoside[11] was deprotected over Pd/C according to Wright, Taylor, and Fox.[10] From 2 g of starting material was obtained 0.76 g of **82** as a syrup: NMR ($CDCl_3$) δ 3.48 (s, OMe), 3.55 (m, 5-CH_2), 3.9 (m, H-2), 4.15 (m, H-3), 4.5–4.7 (m, H-4), 4.95 (s, H-1).

Methyl 3-deoxy-3-fluoro-α-D-arabinofuranoside (**82**; 0.76 g) was phosphorylated[8] to **83** as a light yellow oil: yield 1.54 g; TLC (85:15 EtOAc/$CHCl_3$) R_f 0.47; NMR ($CDCl_3$) δ 3.35 (s, OMe), 4–4.7 (m, H-2, H-3, H-4, and H-5), 4.9 and 5.0 (s, H-1), 7.3 (br s, Ar H).

The diphenyl ester (**83**; 1.54 g) was hydrogenolyzed over PtO_2 and neutralized with NaOH to obtain **14** as a syrup: yield 1.13 g. NMR showed no phenyl absorptions. The product was converted to the dihydrogen form by ion exchange: MS (field desorption), *m/e* 247 (M + H, 100), 269 (M + Na, 26) 215 (M + 7 H − MeOH, 29), 135 ($C_5H_8FO_3$, 16), 167 (M + H − H_2PO_3, 17).

Potassium D-Arabinonate 5-(Dipotassium phosphate) (25). Fructose 6-(disodium phosphate) (5 g, 0.013 mol) was oxidized by O_2 according to the method of Chirgwin and Noltman.[16] The yield of barium salt was 2.3 g. This material was converted to the acid form with Dowex 50 (H^+) and chromatographed on Dowex 1 (Cl^-) to obtain the tripotassium salt: ^{13}C NMR (D_2O) δ 67.7 (C-2 and C-5), 71 (C-4), 72 (C-3), and 202 (C-1); MS (per Me_3Si), *m/e* 663 (M − 15, 6.7).

Aldonic Acid Phosphates. In general, these compounds were prepared from the aldose phosphates by bromine oxidation under conditions similar to those described by Horecker.[15] The initial products were passed through a Bio-Gel P-2 column, and the purified products were precipitated from a concentrated aqueous solution by the addition of methanol, ethanol, or acetone. The following compounds were made by this method.

2-Deoxy-D-ribono-1,4-lactone 5-(Disodium phosphate) (24).[17] From 0.18 g (0.0007 mol) of 2-deoxy-D-ribose 5-phosphate was obtained 0.07 g (37%) of **24** as a colorless, hygroscopic powder: ^{13}C NMR (D_2O) δ 40.32 (C-2), 65.91 (d, J_{PC} = 4.3 Hz, C-5), 71.37 (C-3), 90.79 (d, J_{PC} = 8.4 Hz, C-4), 182.61 (C-1). Anal. ($C_5H_7Na_2O_7P{\cdot}H_2O$) C, H.

Sodium D-Erythronate 4-(Sodium phosphate) (54).[27] From 0.1 g (0.00052 mol) of D-erythrose 4-phosphate was obtained 0.05 g (32%) of an equimolar mixture of di- and trisodium salts as a white powder: mp 198 °C dec; ^{13}C NMR (D_2O; downfield from TSP) δ 67.34 (d, J_{PC} = 4.5 Hz, C-4), 75.56 (d, J_{PC} = 6.2 Hz, C-3), 76.09 (C-2), 181.27 (C-1). Anal. ($C_4H_{6.5}Na_{2.5}O_8P{\cdot}1.5H_2O$) C, H.

Sodium 2-Deoxy-2-fluoro-D-arabinonate 5-(Disodium phosphate) (55). From 0.12 g of **9** was obtained 0.019 g of **55**: MS (per Me_3Si), *m/e* 608 (M), 593 (M − Me).

Sodium 3-Deoxy-3-fluoro-D-arabinonate 5-(Disodium phosphate) (56). From a solution of **16** (0.1 g in 0.5 mL of H_2O) was obtained 0.055 g of **56** as a lyophilized solid: MS (per Me_3Si) was consistent with the structure.

Alditol Phosphates.[17] To a solution of aldose 5-phosphate (2 mmol) in H_2O (10 mL) was added a solution of 2 mmol of $NaBH_4$ in 5 mL of H_2O. Hydrogen was evolved but subsided quickly. This solution was stirred for 12 h at room temperature. The excess borohydride was destroyed with HOAc (0.8 mL). The solution was evaporated, and the residue was suspended in methanol and evaporated three times to remove the borate. The product was dissolved in 5 mL of H_2O, the pH was adjusted to 7 with 1 N NaOH, and the solution was chromatographed on Bio-Gel P-2.

2-Deoxy-D-*erythro*-pentitol 5-(disodium phosphate) (58)[28] was obtained as a white powder: yield 52%; ^{13}C NMR (D_2O) δ 35.3 (C-2), 59.5 (C-1), 65.8 (d, J = 4.5 Hz, C-5), 69.4 (d, J = 5.8 Hz, C-4), 75.03 (C-3); MS (per Me_3Si), *m/e* 576 (M), 561 (M − Me).

D-Ribitol 5-(disodium phosphate) (59)[17] was obtained as a white powder: yield 80%; ^{13}C NMR (D_2O) δ 62.99 (s, C-1), 65.49 (d, J = 4.7 Hz, C-5), 71.97 (d, J = 6 Hz, C-4), 72.50 and 72.77 (2 s, C-2 and C-3); MS (per Me_3Si), *m/e* 649 (M − Me, 1.33).

D-Arabinitol 5-(disodium phosphate) (60) was obtained as a white powder: yield 43%; ^{13}C NMR (D_2O) δ 63.5 (s, C-1), 65.7 (d, J = 4.9 Hz, C-5), 70.4 and 70.55 (2 s, C-2 and C-3), 70.7 (d, J = 7.2 Hz, C-4); MS (per Me_3Si), *m/e* 649 (M − Me, 0.8).

2-Deoxy-2-fluoro-D-arabinitol 5-(dihydrogen phosphate) (61): yield 90%; MS (per Me_3Si) *m/e* 579 (M − Me, 4.2), 447 (M − 2 Me_3Si − H, 86), 431 (M − OMe_3Si − Me_3Si − H, 15), 415 (M − 2 OMe_3Si − H, 2.4).

3-Deoxy-3-fluoro-D-arabinitol 5-(dihydrogen phosphate) (62) was obtained as a white powder: yield 29%; MS (per Me_3Si), m/e 608 (M − Me).

3-*O*-Benzyl-D-arabinitol 5-(disodium phosphate) (63) was obtained as a white powder: yield 80%; MS (per Me_3Si), m/e 682 (M, 1), 667 (M − Me, 1.8); exact mass of M was 682.2780 for $C_{27}H_{59}O_8PSi_5$.

5-Deoxy-5-(phosphonomethyl)-D-*arabino*-pentitol (66) was obtained as a white powder: yield 77%; MS (per Me_3Si), m/e 647 (M − Me, 2.6).[29]

D-Arabinofuranose 5-Sulfamate (42). A stirred solution of 2.50 g (0.00905 mol) of D-arabinofuranose-1,2,3-triacetate (**43**)[37] and 1.84 g (0.0182 mol) of triethylamine in 60 mL of dry CH_2Cl_2 protected with a drying tube was chilled with an ice/water bath. To this solution was added 2.09 g (0.0189 mol) of sulfamyl chloride[30] over a 1.5-min period, and a solution quickly resulted. After 2 h, the solution was removed from the cooling bath and was allowed to warm to room temperature and stand for 2 days. The solution was concentrated under vacuum at 35 °C to a residue to which was added 25 mL of EtOAc and ~1.0 g of triethylamine. To this milky suspension was added 25 mL of EtOAc and 50 mL of H_2O. The two-phase mixture was shaken, and the separated H_2O layer was extracted with 50 mL of EtOAc. The combined EtOAc extract was dried (Na_2SO_4) and filtered, and the filtrate was concentrated to a yellowish to brownish-yellow paste: yield 2.62 g. The paste was chromatographed on silica gel in a Waters Prep 500 A apparatus with 1:1 hexanes/EtOAc: yield of colorless paste **44** 1.60 g (50%); NMR ($CDCl_3$).

To a stirred solution of 1.59 g (0.00447 mol) of **44** in 25 mL of dry MeOH protected by a positive N_2 pressure was added by pipet a solution of 0.045 g (0.00196 mol) of Na in 25 mL of dry MeOH. After 2 h and 30 min, the solution was neutralized with 12 drops of glacial AcOH and concentrated to a mixture of white solid and colorless, hard paste: yield 1.22 g. A 1.20-g sample was chromatographed on Bio-Gel P-2. Fractions containing desired product (RI, TLC) were combined and concentrated to a colorless paste **42**: yield 0.75 g (73%); IR (neat) 1363 and 1182 (SO_2) cm^{-1}; ^{13}C NMR (Me_2SO-d_6) δ 69.08 (α C-5), 76.78 and 79.39 (α C-2 and C-3 or α C-3 and C-2), 82.41 (α C-4), 102.05 (α C-1); peaks due to the β anomer and some isomer(s) were present also: $[\alpha]^{20}_D$ +18.2° (c 1.05, H_2O); MS (field desorption, 20 mA), m/e 252 (M + Na). Anal. ($C_5H_{11}NO_7S$) C, H, N, S.

5-*O*-(Sulfonatocarbamyl)-D-arabinofuranose Sodium Salt (47). A stirred solution of 2.50 g (0.00905 mol) of **43**[37] in 300 mL of dry ether, under N_2, was chilled to −75 °C with a dry ice/2-propanol bath. To the solution was added over a 2-min period from a syringe 0.85 mL (1.38 g, 0.00976 mol) of chlorosulfonyl isocyanate. After 41 min, another 0.10 mL (0.163 g, 0.00115 mol) of chlorosulfonyl isocyanate was added. The solution was stirred overnight under N_2 as the cooling bath was allowed to warm gradually to room temperature and then stirred at room temperature for 6–7 h. After 3 days in a refrigerator, the solution was pipetted into 225 mL of water that had been chilled with an ice/water bath. The mixture was stirred vigorously during warming to room temperature. After 10 min, 0.92 g (0.011 mol) of $NaHCO_3$ was added to the mixture, and it still was strongly acidic. Another 0.92 g (0.011 mol) of $NaHCO_3$ was added after 43 min, and the pH was about 4. Another 0.10 g (0.0012 mol) of $NaHCO_3$ was added after 3.5 h, and the pH was about 5. The ether was removed under vacuum, and the mixture was washed with 200 mL of CH_2Cl_2. The pH of the aqueous layer at 4–5 did not change overnight (meaning no further hydrolysis to H_2SO_4). The aqueous solution was concentrated under high vacuum at 40 °C to a white solid, which was suspended in 100 mL of EtOAc. The mixture was filtered, and the filtrate was concentrated to a white solid: yield 3.67 g. NMR (Me_2SO-d_6) IR, and a microanalysis indicated mainly Na salt of intermediate N-sulfonic acid. A 3.21-g sample of solid was chromatographed on Bio-Gel P-2 with H_2O: yield of a white solid **46**, 2.48 g (74%); NMR (Me_2SO-d_6).

To a solution of 1.10 g (0.00261 mol) of sulfonatocarbamate **46** in 20 mL of dry MeOH under N_2 was added at one time by pipet a solution of 0.035 g (0.00152 mol) of Na in 20 mL of dry MeOH. After 6.5 min, precipitated white solid was present (not present after 5 min). After 1 h and 49 min a solution of 0.005–0.010 g of Na in 3 mL of dry MeOH was added. After 2 h, white solid was collected by filtration, washed with 3 mL and then 5 mL of dry MeOH, and dried overnight under vacuum at 35 °C: yield 0.617 g. The filtrate was neutralized with ten drops of glacial AcOH, was allowed to stand overnight, and was concentrated under vacuum at 40 °C to a white solid: yield 0.32 g.

A 0.600-g sample of the first solid and a 0.31-g sample of the second solid were combined and chromatographed with H_2O on Bio-Gel P-2. Fractions containing the cleanest desired product (RI, TLC) were combined and concentrated under high vacuum at room temperature to a colorless paste: yield 0.39 g. Trituration with 20 mL of ether produced several batches of solid **47**, which were dried under vacuum at 40 °C: mp 80–120 °C dec; ^1H NMR (Me_2SO-d_6) δ 3.5–4.1 (m, 5 H) (C-5 CH_2, C-4 H, C-3 H, C-2 H), 4.7–5.1 (m) and 5.1–5.3 (m) (3 H) (C-1 H, C-3 OH, C-2 OH), 6.07 (d, J = 7.1 Hz, C-1 β-OH) and 6.22 (d, J = 5.3 Hz, C-1 α OH) (1 H), 9.20 (s, β NH), and 9.24 (s, α NH) (1 H); ^{13}C NMR (Me_2SO-d_6) δ 64.49 (α C-5), 65.90 (β C-5), 75.27 (β C-2), 76.39 (β C-3), 77.14 (α C-2), 79.02 (β C-4), 79.56 (α C-3), 82.32 (α C-4), 95.85 (β C-1), 101.77 (α C-1), 152.93 (α and β C=O); $[\alpha]^{20}_D$ +11.3° (c 0.485, H_2O). Anal. ($C_6H_{10}NNaO_9S$) C, H, N, S, Na.

D-Arabinofuranose 5-Nitrate (48). To 2.02 g (0.00729 mol) of **43**[37] in a flask chilled with an ice/water bath was added intermittently over a 7-min period a chilled solution of 1.00 mL of fuming HNO_3 in 10 mL of Ac_2O. Stirring began about halfway through the addition of the HNO_3 solution as the viscous triacetate dissolved. The yellow solution was removed from the cooling bath 9 min after the addition of HNO_3 had been completed. After another 25 min, solid K_2CO_3 was added until fizzing could not be seen (some solid K_2CO_3 was present). The mixture was poured onto 200 mL of ice, and yellow, oily drops were present in a rather strongly acidic mixture. K_2CO_3 was added until all fizzing ceased (excess K_2CO_3 was present). The mixture (~160 mL) was extracted with 160 mL of CH_2Cl_2, the aqueous layer was washed again with 150 mL of CH_2Cl_2, and the combined CH_2Cl_2 solution was dried over Na_2SO_4. The solution was filtered and concentrated to a paste: yield 2.24 g. The paste was purified by flash chromatography[34] on silica gel with CH_2Cl_2 to remove Ac_2O: yield of pale yellow paste **46** 1.84 g (78%); NMR ($CDCl_3$).

To a stirred solution of 1.95 g (0.00607 mol) of **46** in 80 mL of dry CH_3OH protected with a drying tube was added 0.014 g (0.00026 mol) of $NaOCH_3$. After 1 h and 5 min, two drops (~0.03 g, ~0.0005 mol) of glacial AcOH was added to the pale yellow solution, and the resulting nearly colorless solution was concentrated to a pale yellow solid: yield 1.21 g. The solid was recrystallized from EtOAc/toluene by the procedure used for **27**. White needles of **48** were collected, washed with 2 × 12 mL of toluene, and dried under vacuum at 50 °C: yield 1.05 g (89%); mp 133–134 °C; IR (Nujol) 1630 and 1284 (NO_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.6–4.1 (m, 3 H, C-4 H and C-5 CH_2), 4.4–5.2 (m, 4 H, C-1 H, C-2 H, C-3 H, C-3 OH), 5.40 (d, J = 4.8 Hz, 1 H, C-2 OH), 6.33 (d, J = 6.5 Hz, 1 H, C-1 OH) (a probable anomeric mixture was indicated by weak peaks overlapping most of the peaks reported above): $[\alpha]^{20}_D$ +39.1° (c 1.075, H_2O). Anal. ($C_5H_9NO_7$) C, H, N.

D-Arabinose 2,3,4-Triacetate 5-Carbamate Di-*n*-propyl Dithioacetal (51). A mixture of 20.00 g (0.0306 mol) of 5-*O*-trityl-D-arabinose 2,3,4-triacetate di-*n*-propyl dithioacetal[38] in 200 mL of 80% AcOH was heated on a steam bath with intermittent swirling. After 11–12 min, a complete solution was present. After 46 min, the solution was removed from the steam bath, and solid precipitated as cooling to room temperature occurred. To the mixture was added 200 mL of H_2O, and more solid precipitated. The mixture was refrigerated for 3 days and was filtered while still cold to remove trityl alcohol. The filtrate was saturated with NaCl and washed with 2 × 400 mL of CH_2Cl_2. The combined CH_2Cl_2 solution was washed with 2 × 400 mL of 5% $NaHCO_3$ and 200 mL of H_2O and was dried over Na_2SO_4. The solution was filtered, and the filtrate was concentrated to a colorless paste of D-arabinose 2,3-4-triacetate di-*n*-propyl dithioacetal, **50**: yield 12.24 g (97%); NMR ($CDCl_3$).

To a stirred solution of 10.0 g (0.0244 mol) of **50** in 800 mL of anhydrous ether, chilled to −72 °C under N_2, was added, over 2.5 min, 2.7 mL (4.39 g, 0.0310 mol) of chlorosulfonyl isocyanate from a syringe. The reaction solution was allowed to warm as the cooling bath warmed. After 19.5 h, the temperature of the solution was 3 °C. When the temperature was 15 °C, the solution

was added to 600 mL of cold H_2O. The mixture was stirred vigorously as it was allowed to warm to room temperature. After 25 min, to the mixture was added 2.60 g (0.0310 mol) of $NaHCO_3$ (pH was still strongly acidic). After 1 h and 10 min, another 2.60 g (0.0310 mol) of $NaHCO_3$ was added (pH still was strongly acidic). After 1 h and 25 min, another 1.86 g (0.0221 mol) of $NaHCO_3$ was added, and the pH of the mixture was 4-5. After 1 h and 40 min, the ether was removed under vacuum at 30 °C. The remaining mixture was adjusted to pH 5-6 with 0.41 g (0.00488 mol) of $NaHCO_3$ and was extracted with 2 × 50 mL of CH_2Cl_2. The combined CH_2Cl_2 extract was washed with 250 mL of water, dried over Na_2SO_4, and filtered. The filtrate was concentrated to a pale yellow oil:. yield 11.0 g. The oil was chromatographed on silica gel in a Waters Prep 500 A apparatus with 3:2 hexanes/EtOAc: yield 7.58 g (69%) of a colorless oil **51**, which solidified; mp 44-47 °C; IR (Nujol) 1745 (CO), 3428 and 3540 (NH) cm^{-1}; NMR ($CDCl_3$) δ 0.98 (2 t's, 6 H, propyl CH_3), 1.2-1.9 (m, 4 H, propyl C-2 CH_2), 2.05 (s) and 2.12 (s) (9 H, acetyl CH_3), 2.63 (2 t's, 4 H, propyl C-1 CH_2), 3.8-4.4 (m, 3 H, C-1 H and C-5 CH_2), 4.84 (br s, 2 H, NH_2), 4.9-5.2 (m, 1 H, C-4 H), 5.27 (dd, J = 7.5 and 2.5 Hz, 1 H, C-2 H), 5.73 (dd, J = 7.5 and 2.5 Hz, 1 H, C-3 H); $[\alpha]^{20}_D$ +24.6° (c 1.05, $CHCl_3$). Anal. ($C_{18}H_{31}NO_8S_2$) C, H, N, S.

2,3-*O*-Isopropylidene-D-ribono-1,4-lactone 5-(Di-*tert*-butyl phosphate) (49). The lactone **26** was treated with Ph_3P in CBr_4 to obtain the 5-bromo derivative. The bromo compound (1.2 g, 0.0047 mol) in 5 mL of dry DME was added dropwise to a refluxing solution of tetramethylammonium di-*tert*-butyl phosphate (1.3 g, 0.0047 mol) in 19 mL of dry DME under N_2.[39] This mixture was refluxed for 1 h, cooled, and filtered. The filtrate was evaporated to an oil, which was dissolved in EtOAc and washed with H_2O. The EtOAc solution was dried (Na_2SO_4) and evaporated to a pale yellow oil **49**: yield 1.7 g (94%); NMR (Me_2SO-d_6) δ 1.3-1.5 (several s, 24 H), 4.1 (dd, J_{PH} = 6 Hz, J_{45} = 3 Hz, 2 H, H-5), 4.75 (s and m, 3 H, H-2, H-3, H-4).

Isomerase Assay.[24] A mixture containing 170 μL of 0.1 M histidine buffer at pH 7.5, 30 μL of inhibitor solution, and 50 μL of enzyme preparation was preincubated for 2 min at 37 °C. The reaction was initiated by the addition of 50 μL of 10 mM D-arabinose 5-phosphate or D-ribulose 5-phosphate and was terminated at 0, 3, 6, 9, and 12 min by the addition of 50 μL of 1.5% cysteine hydrochloride solution, followed immediately by 1.5 mL of 25 N H_2SO_4. After being mixed, the samples were treated with 50 μL of 0.12% carbazole in 95% EtOH and incubated at 37 °C for 30 min. The absorbance was read at 540 nm. The conversion of 1 μmol of D-A5P to 1 μmol of D-ribulose 5-phosphate gave a ΔOD of 8.2 ± 0.34.

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Supplementary Material Available: NMR data for compounds **44-46**, **50**, and **72-78** (2 pages). Ordering information is given on any current masthead page.

Oligonucleotide Structural Parameters That Influence Binding of 5′-*O*-Triphosphoadenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine to the 5′-*O*-Triphosphoadenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine Dependent Endoribonuclease: Chain Length, Phosphorylation State, and Heterocyclic Base

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A number of 2′,5′-linked oligoadenylates and their analogues were prepared and evaluated for their ability to interact with the 5′-*O*-triphosphoadenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine (2-5A) dependent endoribonuclease of mouse L cells. The oligonucleotides were assayed for their ability to antagonize the action of 2-5A, to displace a radiolabeled probe from the 2-5A-dependent nuclease, or to inhibit translation in a cell-free system. These experiments demonstrated the following: (1) Three AMP residues in a 5′-phosphorylated oligonucleotide were needed for maximum interaction with the endonuclease, and higher oligomers (≥4 AMP residues) did not show significantly higher binding. (2) The third (2′-terminal) adenosine residue was required for optimal binding activity. (3) 5′-Phosphorylation of the oligonucleotide was necessary for maximum binding to the endonuclease, but the first (from the 5′ terminus) internucleotide phosphate of higher unphosphorylated or core oligomers, such as A2′p5′A2′p5′A2′p5′A, may partly replace the requirement for a 5′-monophosphate moiety; in agreement with this, the 5′-methyl ester of 5′pA2′p5′A2′p5′A, i.e., Me-p5′A2′p5′A2′p5′A, was bound to the endonuclease as well as or better than the higher core oligomers but approximately 100 times more effectively than the trimer core, A2′p5′A2′p5′A. (4) Base-modified analogues, such as p5′C2′p5′C2′p5′C, p5′U2′p5′U2′p5′U, or p5′I2′p5′I2′p5′I, were at least 2000 times less effectively bound to the endonuclease than p5′A2′p5′A2′p5′A. (5) The triphosphate ppp5′I2′p5′I2′p5′I was 10 000 times less active than 2-5A as an inhibitor of translation. These latter two points implied the critical role of the adenine N^1-nitrogen and/or exocyclic amino group in the binding of 2-5A to the endonuclease.

The 2′,5′-phosphodiester bond linked oligoriboadenylate 5′-*O*-triphosphoadenylyl-(2′→5′)-adenylyl-(2′→5′)-adenosine (2-5A) is believed to be a mediator of interferon action (for reviews, see ref 1-4). Although it is probably not involved in all the biological effects of interferon, there is strong evidence that 2-5A may be responsible for the inhibition of encephalomyocarditis virus and reovirus replication by interferon.[5,6] Other data have suggested a possible role for the 2-5A system in the normal regulation

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