Hydrogenation Procedure. The requisite substrate (0.5–1 g) was accurately weighed into a 60-mL pressure bottle equipped with a magnetic stirrer. The rhodium/phosphine complex and solvent were then added. The flask was successively evacuated and filled with hydrogen. The solution was stirred until gas uptake ceased. The reduction solution was diluted to a known volume, and the optical yield was determined on a polarimeter by comparison with a standard.

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Synthesis of an Isosteric Phosphonate Analogue of Cytidine 5′-Monophospho-3-deoxy-D-*manno*-2-octulosonic Acid

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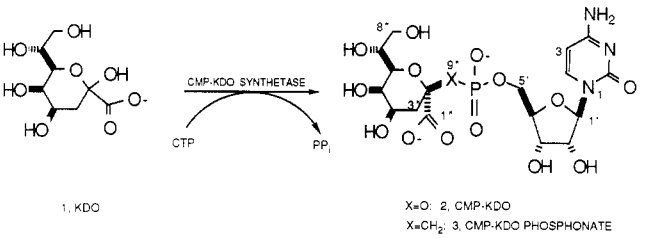
A stereoselective synthesis of **3**, a stable, isosteric phosphonate analogue of cytidine 5′-monophospho-3-deoxy-D-*manno*-2-octulosonic acid (CMP-KDO, **2**), is described. Key steps include alkylation of an ester enolate, Arbuzov reaction of a β-lactone, and esterification of a phosphonate under Mitsunobu conditions. Compound **3** is a modest inhibitor of CMP-KDO synthetase, a key enzyme in bacterial lipopolysaccharide biosynthesis.

3-Deoxy-D-*manno*-2-octulosonic acid (KDO, **1**; Scheme I) is a vital component of the outer membrane lipopolysaccharide (LPS) of all Gram-negative bacteria.[1] Several groups have pursued inhibition of KDO metabolism as a strategy for the development of novel antiinfective agents.[2] Condensation of KDO with CTP to form the extremely labile cytidine 5′-monophospho-3-deoxy-D-*manno*-2-octulosonic acid (**2**, CMP-KDO; Scheme I) may be the rate-limiting step in LPS biosynthesis.[3] The stereochemistry of this reaction was recently elucidated by Kohlbrenner and Fesik[4] and provides a basis for the rational design of enzyme inhibitors. A stable, β-linked CMP-KDO analogue could conceivably inhibit both the synthetase and the subsequent transferase[5] that catalyze displacement of CMP by a lipid A precursor.[6] Here we report the synthesis and preliminary biological evaluation of the CMP-KDO phosphonate **3**.[7]

Scheme I. Biosynthesis of CMP-KDO



Cornforth condensation[8] of D-arabinose and oxaloacetate provided multigram quantities of crystalline (+)-KDO (**1**) in a single step (Scheme II).[9] The desired pyranose ring isomer[10] was secured by acid-catalyzed isopropylidenation,

(1) (a) Unger, F. M. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 323–388. (b) *Bacterial Lipopolysaccharides: Structure, Synthesis, and Biological Activities*; Anderson, L., Unger, F. M., Eds.; ACS Symposium Series 231; American Chemical Society: Washington, DC, 1983. (c) *Bacterial Outer Membranes: Biogenesis and Functions*; Inouye, M., Ed.; Wiley: New York, 1979.

(2) (a) Bigham, E. C.; Gragg, C. E.; Hall, W. R.; Kelsey, J. E.; Mallory, W. R.; Richardson, D. C.; Benedict, C.; Ray, P. H. *J. Med. Chem.* **1984**, *27*, 717–726. (b) Ringrose, P. S. In *Medical Microbiology: Role of the Envelope in the Survival of Bacteria in Infection*; Easmon, C. S. F., Jeljaszewicz, J., Brown, M. R. W., Lambert, P. A., Eds.; Academic: New York, 1983; Vol. 3, Chapter 8. (c) Ray, P. H.; Kelsey, J. E.; Bigham, E. C.; Benedict, C. D.; Miller, T. A. In Reference 1b, pp 141–170. (d) Reference 1a, pp 387–388. (e) Molin, H.; Pring, B. G. *Tetrahedron Lett.* **1985**, 677–680.

(3) Ray, P. H.; Benedict, C. D.; Grasmuk, H. *J. Bacteriol.* **1981**, *145*, 1273–1280.

(4) Kohlbrenner, W. E.; Fesik, S. W. *J. Biol. Chem.* **1985**, *260*, 14695–14699.

(5) Munson, R. S.; Rasmussen, N. S.; Osborn, M. J. *J. Biol. Chem.* **1978**, *253*, 1503–1511.

(6) (a) Raetz, C. R. H.; Purcell, S.; Meyer, M. V.; Qureshi, N.; Takayama, U. *J. Biol. Chem.* **1985**, *260*, 16080–16088. (b) Raetz, C. R. H. *Rev. Infect. Dis.* **1984**, *6*, 463–471.

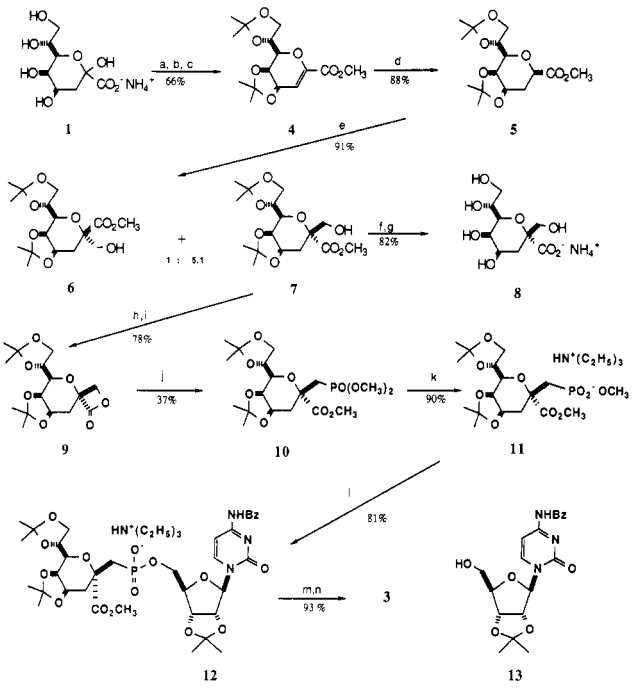
(7) (a) For a review of phosphonates as analogues of naturally occurring phosphates, see: Engel, R. *Chem. Rev.* **1977**, *77*, 349–367. For recent syntheses of isosteric phosphonate analogues of carbohydrate 1-phosphates, see: (b) Nicotra, F.; Ronchetti, F.; Russo, G. *J. Chem. Soc., Chem. Commun.* **1982**, 470–471. (c) Nicotra, F.; Ronchetti, F.; Russo, G. *J. Org. Chem.* **1982**, *47*, 4459–4462. (d) Nicotra, F.; Perego, R.; Ronchetti, F.; Russo, G.; Toma, L. *Carbohydr. Res.* **1984**, *131*, 180–184. (e) Tang, J.-C.; Tropp, B. E.; Engel, R. *Tetrahedron Lett.* **1978**, 723–726. (f) Chmielewski, M.; BeMiller, J. N.; Cerretti, D. P. *Carbohydr. Res.* **1981**, *97*, C1–C4. (g) McClard, R. W. *Tetrahedron Lett.* **1983**, 2631–2634. (h) Nicotra, F.; Panza, L.; Ronchetti, F.; Toma, L. *Tetrahedron Lett.* **1984**, 5937–5938. (i) McClard, R. W.; Fischer, A. C.; Mauldin, S. K.; Jones, M. E. *Bioorg. Chem.* **1984**, *12*, 339–348. (j) Julina, R.; Vasella, A. *Helv. Chim. Acta* **1985**, *68*, 819–830. (k) Meyer, R. B.; Stone, T. E.; Jesthi, P. K. *J. Med. Chem.* **1984**, *27*, 1095–1098.

(8) (a) Cornforth, J. W.; Firth, M. E.; Gottschalk, A. *Biochem. J.* **1958**, *68*, 57–61. (b) Hershberger, C. S.; Davis, M.; Binkley, S. B. *J. Biol. Chem.* **1968**, *1585*–1588.

(9) For other syntheses of KDO see: (a) Danishefsky, S. J.; Pearson, W. J.; Segmuller, B. E. *J. Am. Chem. Soc.* **1985**, *107*, 1280–1285. (b) Perry, M. B.; Williams, D. T. In *Methods in Carbohydrate Chemistry*; Whistler, R. L., BeMiller, J. N., Eds.; Academic: New York, 1976; Vol. 7, pp 44–48. (c) Reference 1a, pp 365–373. (d) Paquet, F.; Sinay, P. *J. Am. Chem.. Soc.* **1984**, *106*, 8313–8315. (e) Schmidt, R. R.; Betz, R. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 430–431. (f) Collins, P. M.; Overend, W. G.; Shing, T. *J. Chem. Soc., Chem. Commun.* **1981**, 1139–1140.

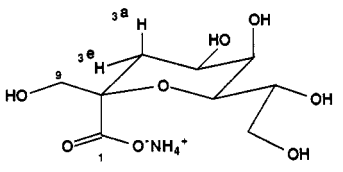
0022-3263/87/1952-2174$01.50/0

Scheme II. Synthesis of the CMP-KDO Phosphonate 3[a]



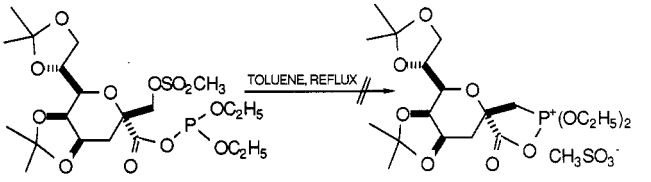
[a] Key: (a) $(CH_3)_2CO$, H_2SO_4; (b) CH_2N_2, Et_2O; (c) CH_3SO_2Cl, Et_3N, CH_2Cl_2; (d) W-2 Ra-Ni, H_2, EtOH; (e) LDA, CH_2O, THF; (f) LiOH, H_2O; (g) CF_3CO_2H, H_2O, NH_4OH; (h) LiOH, MeOH, H_2O, H_3O^+; (i) Et_3N, $C_6H_5SO_2Cl$, CH_2Cl_2; (j) $P(OCH_3)_3$; (k) Et_3N, C_6H_5SH, THF; (l) $(C_6H_5)_3P$, $[(CH_3)_2CHO_2CN]_2$, **13**, THF, Et_3N, C_6H_5SH, THF; (m) CF_3CO_2H, H_2O, CH_2Cl_2; (n) NaOH, MeOH, H_2O, H^+ resin, NH_4OH.

esterified with diazomethane, and then dehydrated to the glycal **4** in an overall yield of 66%. Hydrogenation over W-2 Raney nickel proceeded exclusively from the unencumbered α face.[11] In contrast, addition of gaseous formaldehyde to the lithium enolate of **5** occurred predominantly (5.1:1)[12] from the β face to yield the chromatographically separable C glycosides **6** and **7**. Confirmation of the anticipated[13] equatorial selectivity was derived from the 7.4-Hz C-1,H-3a heteronuclear coupling constant[14] observed in the KDO homologue **8**.[15]



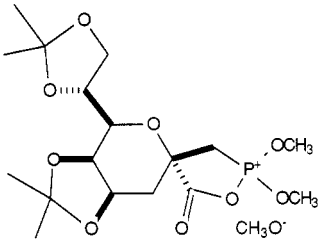
8

Displacement of the alcohol moiety in **7** by a phosphorus nucleophile proved challenging. The triflate ester[16] was inert to refluxing trimethyl phosphite,[17,18] and attempted intramolecular delivery using the carboxylate as a phosphorus ligand returned starting material.[19] However,



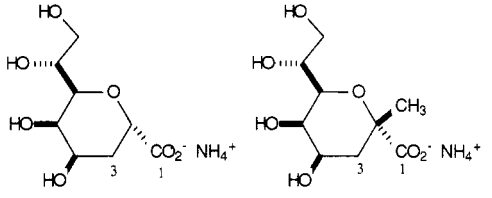
14 **15**

utilization of the carboxyl residue as a leaving group divested the S_N2 transition state of its neopentyl character. Thus, heating the β-lactone **9** in neat trimethyl phosphite reproducibly delivered the phosphonate **10** in 37% yield.[20] Intermediacy of the phosphonium salt **16** would account for the formation of the trimethyl ester.



16

With the critical carbon–phosphorus bond finally in place, selective demethylation with thiophenoxide[21] set the stage for esterification of the phosphonate **11** to the known cytidine derivative **13**.[22] Surprisingly, phosphonic acid



i **ii**

(10) In aqueous solution, KDO exists as a 70:30 pyranose–furanose mixture. Cherniak, R.; Jones, R. G.; Gupta, D. S. *Carbohydr. Res.* **1979**, *75*, 39–49.

(11) Initial experiments in this area were carried out by David Riley and Robert Hallas. ^1H NMR analysis of the corresponding tetrol methyl ester indicates a $^2C_5$ conformation with $^3J_{H\text{-}2,3e}$ = 2 Hz and $^3J_{H\text{-}2,3a}$ = 12 Hz.

(12) This diastereomeric ratio was determined by capillary GC analysis of the crude reaction mixture.

(13) (a) Evans, D. A. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: New York, 1983; Vol. 3. (b) Petragnani, N.; Yonashiro, M. *Synthesis* **1982**, 521–578. (c) Beau, J.-M.; Sinay, P. *Tetrahedron Lett.* **1985**, 6193–6196.

(14) (a) The H9 and H9′ protons were selectively decoupled in this experiment. $^3J_{C\text{-}1,H\text{-}3}$ values ≤1 Hz would be expected if the carbomethoxy group were equatorially disposed: (a) Unger, F. M.; Stix, D.; Schulz, G. *Carbohydr. Res.* **1980**, 191–195. (b) Reference 4. (c) Haverkamp, J.; Spoormaker, T.; Dorland, L.; Vliegenthart, J. F. G.; Schaver, R. *J. Am. Chem. Soc.* **1979**, *101*, 4851–4853.

(15) Other electrophiles displayed similar equatorial selectivity at −78 °C: protonation with citric acid, 4.6:1;[12] methylation with methyl iodide, >95:5 by ^1H NMR. Stereochemical assignments were made as for **8**, with $^3J_{C\text{-}1,H\text{-}3a}$ = 8.1 Hz, $^3J_{H\text{-}2,H\text{-}3a}$ = 6.5 Hz, and $^3J_{H\text{-}2,H\text{-}3e} \leq 1$ Hz for **i** and $^3J_{C\text{-}1,H\text{-}3a}$ = 7.1 Hz for **ii**. Attempted alkylation with diethyl iodomethyl phosphonate resulted in proton transfer.

(16) Binkley, R. W.; Ambrose, M. G.; Hehemann, D. G. *J. Org. Chem.* **1980**, *45*, 4387–4391.

(17) For a review of the Arbuzov reaction see: Bhattacharya, A. K.; Thyagarajan, G. *Chem. Rev.* **1981**, *81*, 415–430.

(18) Sulfonates are known to undergo the Arbuzov reaction: (a) Myers, T. C.; Preis, S.; Jensen, E. V. *J. Am. Chem. Soc.* **1954**, *76*, 4172–4173. (b) Colle, K. S.; Lewis, E. S. *J. Org. Chem.* **1978**, *43*, 571–574.

(19) The acyl phosphite **14**, prepared in situ from the triethylammonium carboxylate and diethyl chlorophosphite, may rearrange thermally to the acyl phosphonate, which would hydrolyze to the acid on workup. Edmunson, R. S. In *Comprehensive Organic Chemistry*; Sutherland, I. O., Ed.; Pergammon: New York, 1979; Vol. 2, p 1214.

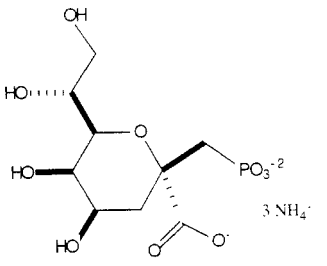
(20) The major byproduct of this reaction was the methyl ester **7**. The analogous reaction with β-propiolactone is known: (a) McConnel, R. L.; Coover, H. W. *J. Am. Chem. Soc.* **1956**, *78*, 4453–4455. (b) Kreutzkamp, N. *Naturwissenschaften* **1956**, *43*, 81–82. This transformation was suggested to us by Drs. John Tadanier and Joseph Dellaria.

(21) Daub, G. W.; Van Tamelen, E. E. *J. Am. Chem. Soc.* **1977**, *99*, 3526–3528.

(22) (a) Sung, W. L.; Narang, S. A. *Can. J. Chem.* **1982**, *60*, 111–120. (b) Jones, G. H.; Taniguchi, M.; Tegg, D.; Moffatt, J. G. *J. Org. Chem.* **1979**, *44*, 1309–1317.

activation using reagents employed in the routine synthesis of oligonucleotides[23] was completely ineffective. (Mesitylenesulfonyl)tetrazole,[24] for instance, led to slow 5′-sulfonylation of the alcohol **13**, even with the unhindered model compound, triethylammonium methyl benzylphosphonate. Although deficient as an electrophile, the phosphonate **11** reacted readily as a nucleophile toward the activated alcohol under modified Mitsunobu conditions.[25] Demethylation[21] then produced the stereochemically homogeneous monoester **12**, and sequential treatment with aqueous acid and base smoothly removed the remaining protecting groups. Pure CMP-KDO phosphonate **3** precipitated from water as a white powder on the addition of methanol. All spectroscopic data are in full accord with the proposed structure. In particular $^1J_{P,C\text{-}9'}$ = 133.4, $^2J_{P,C\text{-}5'}$ = 4.5, and $^3J_{P,C\text{-}4'}$ = 6.8 Hz are observed in the ^{13}C NMR spectrum.

In a purified CMP-KDO synthetase assay[26] ([KDO] = 1 mM, [CTP] = 0.5 mM), the phosphonate **3** was a modest inhibitor, with I_{50} = 4.1 mM. To assess the contribution of the cytidyl residue to the binding affinity, the phosphonate **17** was also prepared from the diester **11**.[27] Since the I_{50}'s of CMP and **17** are both greater than 10 mM, the



17

relative affinity of the product analogue **3** may be due to the entropic advantage of a "bidentate" ligand.[28] In a permeabilized whole cell assay,[29] neither phosphonate **3** nor **17** significantly inhibited transfer of KDO from CMP to lipid A precursor.

Recent reports underscore the unpredictable behavior of phosphonate analogues with enzymes described in earlier reviews.[7] Level and co-workers[32] compared the isosteric 4,5-dideoxy-5-phosphono-D-*erythro*-pentose and the nonisosteric 4-deoxy-4-phosphono-D-*erythro*-tetrose to D-erythrose 4-phosphate. While the V_{max} of the isosteric and nonisosteric analogues were 29% and 5%, respectively, of the V_{max} for the natural substrate with *Escherichia coli* 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthetase, the V_{max} was the same for all compounds with yeast transaldolase. The same authors[33] found *E. coli* 3-dehydroquinate synthetase to bind 100 times more strongly to the nonisosteric 3,7-dideoxy-7-phosphono-D-*arabino*-heptulosonic acid than to the isosteric 3,7,8-trideoxy-8-phosphono-D-*arabino*-octulosonic acid and 20 times better than to the natural substrate, 3-deoxy-D-*arabino*-heptulosonate 7-phosphate. Surprisingly, the enzyme recognized neither analogue as a substrate. Unger,[34] on the other had, probably synthesized 9-phosphono-3,8,9-trideoxy-D-*manno*-nonulosonate from the arabinose 5-phosphate analogue 5,6-dideoxy-6-phosphono-D-*arabino*-hexose using *E. coli* KDO 8-phosphate synthetase. Finally, Meyer[7k] found that 2,5-anhydro-1-deoxy-1-phosphono-D-altritol, an isosteric analogue of α-D-ribofuranose 1-phosphate, failed to inhibit purine nucleoside phosphorylase.

In general, enzymes bind isosteric phosphonate analogues several times more weakly than the naturally occurring phosphates. This has been attributed to small differences in bonding geometries, steric interactions with the methylene hydrogens, deletion of a hydrogen bond to the phosphonate oxygen, and incomplete ionization of phosphonate diacids. Any but the last of the above factors could explain why **3** as a substrate analogue fails to inhibit transfer of KDO to lipid A precursor. Unfortunately, direct evaluation of **3** as a product analogue with CMP-KDO synthetase is precluded by the instability of CMP-KDO. Although it is not catalytically advantageous for the enzyme to bind its substrates or products strongly,[35] the K_m's for KDO and CTP, 31 and 11 μM, respectively,[26] suggest that the synthetase binds the CMP-KDO phosphonate **3** relatively weakly.

Remarkably, 2-deoxy-KDO[36] (structure i, footnote 15) has I_{50} = 13 μM,[26] yet labeled material shows no binding to the synthetase in the absence of CTP. This substrate-induced inhibition implies a binding synergy that would not be available to **3** or other compounds that must occupy the CTP binding site. These observations and the fact that pyrophosphate itself has I_{50} = 5 mM[3] suggest that the design and synthesis of a CTP-KDO bisubstrate analogue[37] might lead to a potent inhibitor of CMP-KDO synthetase.

Experimental Section

^1H NMR spectra were recorded at 300 MHZ. Chemical shifts are reported as δ values relative to tetramethylsilane (δ 0.0) as an internal standard. Data are reported as follows: chemical shift (multiplicity, integrated intensity, coupling constants, assignment). ^{13}C NMR spectra were recorded at 75.48 MHz. Optical rotations were measured in 1-dm cells of 1-mL capacity on a digital polarimeter. Analytical thin-layer chromatography was conducted

(23) For reviews, see: (a) Amarnath, V.; Broom, A. D. *Chem. Rev.* **1977**, *77*, 183–217. (b) Reese, C. B. *Tetrahedron* **1978**, *34*, 3143–3179. (c) Ikehara, M.; Ohtsuka, E.; Markham, A. F. *Adv. Carbohydr. Chem. Biochem.* **1978**, *36*, 135.

(24) Stawinski, J.; Hozumi, T.; Narang, S. A. *Can. J. Chem.* **1976**, *54*, 670–672.

(25) (a) Mitsunobu, O. *Synthesis* **1981**, 1–28. (b) Kimura, J.; Fujisawa, Y.; Yoshizawa, T.; Fukuda, K.; Mitsunobu, O. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1191–1196.

(26) Kohlbrenner, W. E.; Wideburg, N., to be submitted for publication.

(27) Aqueous base effected hydrolysis of only the carbomethoxy group. Hydrolysis of the phosphonate monomethyl ester under mildly acidic conditions (pH 2, room temperature, 38 h) is noteworthy and presumably involved anchimeric assistance from the carboxyl group: Benkovic, S. J.; Schray, K. J. In *Transition States of Biochemical Processes*; Gandour, R. D., Schowen, R. L., Eds. Plenum: New York, 1978; Chapter 13, pp 508–512.

(28) Fersht, A. *Enzyme Structure and Mechanism*; W. H. Freeman: New York, 1985; pp 307–308, 322.

(29) Darveau, R.; Capobianco, J., to be submitted for publication.

(30) Adam, W.; Baeza, J.; Lin, J. C. *J. Am. Chem. Soc.* **1972**, *94*, 2000–2006.

(31) (a) Yount, R. G. In *Advances in Enzymology*; Meister, A., Ed.; Wiley: New York, 1975; Vol. 43, pp 1–56. (b) Scheit, K. H. *Nucleotide Analogs: Synthesis and Biological Function*; Iley: New York, 1980; Chapter 4. (c) Webster, D.; Jondorf, W. R.; Dixon, H. B. F. *Biochem. J.* **1976**, *155*, 433–441.

(32) Le Mare'chal, P.; Froussios, C.; Level, M.; Azerad, R. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 1097–1103.

(33) (a) Le Mare'chal, P.; Froussius, C.; Level, M.; Azerad, R. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 1104–1109. (b) Le Mare'chal, P.; Froussius, C.; Level, M.; Azerad, R. *Carbohydr. Res.* **1981**, *94*, 1–10.

(34) Unger, F. M.; Stix, P.; Mödernodofer, E.; Hammerschmid, F. *Carbohydr. Res.* **1978**, *67*, 349–356.

(35) Reference 28, pp 324–331.

(36) Further details on this inhibitor will be published elsewhere.

(37) The bisubstrate analogue approach led to a powerful inhibitor of gentamicin acetyltransferase I. (a) Williams, J. W.; Northrop, D. B. *J. Antibiot.* **1979**, *32*, 1147–1154. For additional examples and discussion, see: (b) Wolfenden, R. In Reference 27, Chapter 15, pp 555–578. (c) Jencks, W. P. *Adv. Enzymol.* **1975**, 220–410. (d) Lindquest, R. N. In *Drug Design*; Ariëns, E. J., Ed.; Academic: New York, 1975; Chapter 2, pp 56–68.

on precoated glass plates: silica gel 60 F-254, layer thickness 0.25 mm, E. Merck and Co. Silica gel chromatography utilized E. Merck silica gel 60 (70–230-mesh ASTM). All reaction solvents were dried and distilled before use, and all reactions were conducted under an inert atmosphere.

Glycal 4. To a vigorously stirred suspension of 10.0 g (36.6 mmol) of KDO ammonium salt monohydrate[8] in 500 mL of dry acetone at 25 °C was added 2.1 mL (38 mmol) of concentrated H_2SO_4. After 3 h, the solution was neutralized with concentrated NH_4OH, filtered, and then concentrated under reduced pressure. The residue was extracted with 4 × 100 mL of chloroform from 100 mL of saturated aqueous NaCl acidified to pH 3 with H_2SO_4. The combined organic extracts were dried ($MgSO_4$), concentrated under reduced pressure, redissolved in ether, treated with excess ethereal diazomethane, and then concentrated under reduced pressure to afford 10.3 g (85%) of the methyl ester diacetonide as an oil. Chromatography of a portion of this material on silica gel with 1:1 ethyl acetate–hexane provided the analytical sample: R_f 0.19 (silica gel, 1:1 ethyl acetate–hexane); $[\alpha]^{22}_D$ +27.3° (*c* 1.37, $CHCl_3$, equilibrated 8 h); IR ($CDCl_3$) 3530, 3000, 2960, 2940, 1745, 1455, 1440, 1385, 1375, 1220, 1150, 1070 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36, 1.38, 1.43, 1.47 (4 s, 12 H, 2 (CH_3)$_2$C), 1.91 (dd, 1 H, J = 5 Hz, J' = 15 Hz, CC*H*HC), 2.51 (dd, 1 H, J = 6 Hz, J' = 15 Hz, CCH*H*C), 3.57 (br s, 1 H, O*H*), 3.83 (s, 3 H, OCH_3), 3.89 (dd, 2 H, J = 2 Hz, J' = 8 Hz, $OCH_2CHCHCH$), 3.99 (dd, 1 H, J = 5 Hz, J' = 9 HZ, OC*H*HCH), 4.08 (dd, 1 H, J = 6 Hz, J' = 9 Hz, OCH*H*CH), 4.26 (dd, 1 H, J = 2 Hz, J' = 6 Hz, CH_2CHCH), 4.35 (ddd, 1 H, J = 8 Hz, J' = 6 Hz, J'' = 5 Hz, OCH_2CHCH), 4.52 (ddd, 1 H, J = 6 Hz, J' = 6 Hz, J'' = 5 Hz, CH_2CHCH); EI MS, m/z 317 $(M - CH_3)^+$, 299, 273; exact mass calcd for $C_{14}H_{21}O_8$ $(M - CH_3)^+$ 317.1236, found 317.1244.

To a stirred solution of 5.30 g (15.9 mmol) of the above methyl ester hemiketal in 160 mL of dichloromethane at room temperature were added 5.55 mL (39.8 mmol) of triethylamine and 1.85 mL (23.9 mmol) of methanesulfonyl chloride. After 3 h the reaction was poured into 100 mL of 10% aqueous $NaHCO_3$, the phases separated, and the aqueous phase was extracted with 2 × 50 mL of dichloromethane. The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography of the residue on 250 g of silica gel with 1:3 ethyl acetate–hexane afforded 3.90 g (78%) of the glycal **4** as a colorless oil: R_f 0.51 (silica gel, 1:1 ethyl acetate–hexane); $[\alpha]^{22}_D$ +36.8° (*c* 1.12, $CHCl_3$); IR ($CDCl_3$) 2990, 2955, 2935, 1750, 1455, 1440, 1380, 1370, 1250, 1220, 1160, 1075 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H, $^3/_2$ (CH_3)$_2$C), 1.45 (s, 3 H, $^1/_2$ (CH_3)$_2$C), 3.79 (s, 3 H, OCH_3), 3.85 (dd, 1 H, J = 8 Hz, J' = 1 Hz, $OCH_2CHCHCH$), 4.17 (dd, 1 H, J = 9 Hz, J' = 6 Hz, OC*H*HCH), 4.21 (dd, 1 H, J = 9 Hz, J' = 4 Hz, OCH*H*CH), 4.46 (ddd, 1 H, J = 8 Hz, J' = 6 Hz, J'' = 4 Hz, OCH_2CHCH), 4.47 (ddd, 1 H, J = 6 Hz, J' = 1.5 Hz, J'' = 1 Hz, OCH*C*HCHCH=C), 4.78 (dd, 1 H, J = 6 Hz, J' = 3 Hz, C=CHC*H*CH), 6.00 (dd, 1 H, J = 3 Hz, J' = 1.5 Hz); EI MS, m/z 299 $(M - CH_3)^+$; FAB MS, exact mass calcd for $C_{15}H_{23}O_7$ $(M + H)^+$ 315.1444, found 315.1449.

Methyl Ester 5. To a solution of 910 mg (2.90 mmol) of the above glycal in 30 mL of absolute ethanol was added 1.1 g of W-2 Raney nickel, and this mixture was stirred vigorously at room temperature under 1 atm of hydrogen for 30 min. The catalyst was then removed by filtration and washed with 5 × 10 mL of ethanol, and the combined filtrates were concentrated under reduced pressure. Chromatography of the residue on 90 g of silica gel with 1:4 ethyl acetate–pentane afforded 811 mg (88%) of the methyl ester **5** as a colorless oil: R_f 0.23 (silica gel, 1:1 ethyl acetate–hexane); $[\alpha]^{22}_D$ +28.9° (*c* 1.53, $CHCl_3$); IR ($CDCl_3$) 2995, 2960, 2940, 2895, 1755, 1740, 1455, 1440, 1385, 1375, 1245, 1215, 1145, 1060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36, 1.38, 1.43, 1.49 (4 s, 12 H, 2 (CH_3)$_2$C), 2.03 (ddd, 1 H, J = 14 Hz, J' = J'' = 8 Hz, CHC*H*HCH), 2.18 (ddd, 1 H, J = 14 Hz, J' = J'' = 5 Hz, CHCH*H*CH), 3.51 (dd, 1 H, J = 8 Hz, J' = 2 Hz, $OCH_2CHCHCH$), 3.75 (s, 3 H, OCH_3), 4.06–4.14 (m, 3 H, OCH_2C*H*), 4.21 (dd, 1 H, J = 6 Hz, J' = 2 Hz, $CH_2CHCHCH$), 4.37 (dd, 1 H, J = 8 Hz, J = 5 Hz, O_2CCHCH_2), 4.38 (ddd, J = 8 Hz, J' = 6 Hz, J'' = 5 Hz, CH_2CHCH); EI MS, m/z 301 $(M - CH_3)^+$; exact mass calcd for $C_{14}H_{21}O_7$ $(M - CH_3)^+$ 301.1287, found 301.1291. Anal. Calcd for $C_{15}H_{24}O_7$: C, 56.95; H, 7.65. Found: C, 56.92; H, 7.90.

Hydroxymethyl Methyl Esters 6 and 7. To a stirred solution of 19.5 mmol of lithium diisopropylamide in 98 mL of THF at −78 °C was added a solution of 5.15 g (16.3 mmol) of the above methyl ester **5** in 20 mL of THF over 10 min. After 5 min, 5 g (166 mmol) of dry, gaseous paraformaldehyde was introduced just above the reaction surface in a stream of argon over a period of 45 min. Saturated, aqueous NH_4Cl/NH_4OH pH 8.5 buffer (30 mL) was added and the reaction poured into 100 mL of water and then extracted with 3 × 100 mL of dichloromethane. The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography of the residue on 400 g of silica gel with 4:6 ethyl acetate–hexane afforded first 4.25 g (76%) of the hydroxymethyl methyl ester **7** as a colorless oil: R_f 0.22 (silica gel, 1:1 ethyl acetate–hexane); $[\alpha]^{22}_D$ −37.8° (*c* 0.55, $CHCl_3$); IR ($CDCl_3$) 3590, 3420, 2990, 2950, 2930, 2890, 1745, 1730, 1455, 1435, 1380, 1370, 1245, 1210, 1170, 1150, 1120, 1075 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37, 1.37, 1.43, 1.51 (4 s, 12 H, 2 (CH_3)$_2$C), 1.98 (dd, 1 H, J = 16 Hz, J' = 3.5 Hz, CC*H*HCH), 2.23 (dd, 1 H, J = 16 Hz, J' = 3.5 Hz, CCH*H*CH), 2.61 (dd, 1 H, J = 9 Hz, J' = 3.5 Hz, O*H*), 3.44 (dd, 1 H, J = 8.5 Hz, J' = 2 Hz, $OCH_2CHCHCH$), 3.74 (dd, 1 H, J = 11 Hz, J' = 9 Hz, HOC*H*HC), 3.96 (dd, 1 H, J = 11 Hz, J' = 3.5 Hz, HOCH*H*C), 4.11 (dd, 1 H, J = 8.5 Hz, J' = 6 Hz, OC*H*HCH), 4.27 (ddd, 1 H, J = 8.5 Hz, J' = 6 Hz, J'' = 3.5 Hz, OCH_2CHCH), 4.31 (dd, 1 H, J = 8 Hz, J' = 2 Hz, $CH_2CHCHCH$), 4.37 (dd, 1 H, J = 8.5 Hz, J' = 3.5 Hz, OCH*H*CH), 4.54 (ddd, 1 H, J = 8 Hz, J' = J'' = 3.5 Hz, CH_2CHCH); EI MS, m/z 331 $(M - CH_3)^+$, 316, 273; exact mass calcd for $C_{15}H_{23}O_8$ $(M - CH_3)^+$ 331.1393, found 331.1385.

There was then eluted 833 mg (15%) of the hydroxymethyl methyl ester **6** as a colorless oil: R_f 0.11 (silica gel, 1:1 ethyl acetate–hexane); IR ($CDCl_3$) 3570, 2985, 2950, 2930, 1755, 1730, 1455, 1440, 1380, 1370, 1250, 1210, 1165, 1115, 1070, 1050 cm^{-1}; ^1H NMR ($CDCl_3$) 1.32, 1.37, 1.44, 1.44 (4 s, 12 H, 2 (CH_3)$_2$C), 1.79 (dd, 1 H, J = 15 Hz, J' = 2 Hz, CC*H*HCH), 2.13 (dd, 1 H, J = J' = 6 Hz, O*H*), 2.67 (dd, 1 H, J = 15 Hz, J' = 3 Hz, CCH*H*CH), 3.50 (dd, 1 H, J = 7 Hz, J' = 2 Hz, $OCH_2CHCHCH$), 3.66 (d, 2 H, J = 6 Hz, $HOCH_2C$), 3.75 (s, 3 H, OCH_3), 4.06 (dd, 1 H, J = 9 Hz, J' = 5 Hz, OC*H*HCH), 4.15 (dd, 1 H, J = 9 Hz, J' = 6 Hz, OCH*H*CH), 4.27 (dd, 1 H, J = 8 Hz, J' = 2 Hz, $CH_2CHCHCH$), 4.35 (ddd, 1 H, J = 7 Hz, J' = 6 Hz, J'' = 5 Hz, OCH_2CHCH), 4.56 (ddd, 1 H, J = 8 Hz, J' = 3 Hz, J'' = 2 Hz, CH_2CHCH); EI MS, m/z 331 $(M - CH_3)^+$.

Hydroxymethyl KDO 8. To a stirred solution of 31.2 mg (0.090 mmol) of the above methyl ester **7** in methanol at room temperature was added 220 μL (0.110 mmol) of 0.5 N aqueous LiOH. After 1.5 h, the reaction was diluted with 2 mL of water, neutralized with Dowex HCR-S resin, and filtered. To the filtrate was added 350 μL (4.54 mmol) of trifluoroacetic acid. After 3 h at room temperature, the reaction was concentrated under reduced pressure and then coevaporated with several portions of 1-propanol. Chromatography of the residue on 5 g of silica gel with 50:50:2:0.1 chloroform–methanol–water–ammonium hydroxide followed by lyophilization afforded 19 mg (82%) of hydroxymethyl KDO **8** as a white granular solid: R_f 0.19 (silica gel, 5:5:0.8 chloroform–methanol–water); $[\alpha]^{26}_D$ +62.9° (*c* 1.25, H_2O); ^1H NMR (D_2O, HOD = 4.80 ppm) 1.96 (dd, 1 H, J = J' = 13 Hz, CC*H*HCH), 2.40 (dd, 1 H, J = 13 Hz, J' = 5 Hz, CHC*H*CH), 3.78 (d, 1 H, J = 8 Hz, $OCH_2CHCHCH$), 3.82 (d, 1 H, J = 12 Hz, OC*H*HC), 3.88 (d, 1 H, J = 12 Hz, OCH*H*C), 3.92–4.05 (m, 2 H, OCH_2CHCH, CH_2CHCH), 4.05–4.15 (m, 2 H, OCH_2CH), 4.19 (d, 1 H, J = 3 Hz, $CH_2CHCHCH$); ^{13}C NMR (D_2O, CH_3CN = 1.30 ppm) 178.16, 83.21, 74.50, 69.51, 67.93, 67.80, 66.34, 64.41, 30.77; FAB MS (m/z) exact mass calcd for $C_9H_{17}O_8$ $(M + H - NH_3)^+$ 253.0923, found 253.0926.

β-Lactone 9. To a stirred solution of 4.00 g (1.5 mmol) of the above methyl ester **7** in 40 mL of methanol was added 27.8 mL (13.9 mmol) of 0.5 N aqueous LiOH. After 3 h at room temperature, the reaction mixture was poured into 200 mL of dichloromethane and washed with 50 mL of saturated aqueous NaCl acidified to pH 3 with 10% H_2SO_4. The aqueous phase was extracted with 3 × 50 mL of dichloromethane, and the combined extracts were dried ($MgSO_4$), treated with 8 mL (57.4 mol) of triethylamine, and concentrated under reduced pressure to afford 4.86 g (97%) of a white solid. To a stirred solution of 4.40 g (10.1 mmol) of this crude triethylammonium carboxylate in 200 mL of dichloromethane were added 7.6 mL (54 mmol) of triethylamine and then, dropwise over 10 min, 4.5 mL (35 mmol) of benzenesulfonyl chloride.[30] After 1 h, 1.30 mL (10.1 mmol) of benzene-

sulfonyl chloride was added, and after 30 m, the reaction was cooled to 0 °C and then poured into 100 mL of saturated aqueous NaCl acidified to pH 3 with 10% H_2SO_4. The aqueous phase was extracted with 100 mL of dichloromethane, and the combined organic phases were washed with 75 mL of saturated aqueous NaCl, dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography of the residue on 100 g of silica gel with 1:4 ethyl acetate–hexane afforded 2.55 g (80%) of the β-lactone **9** as a colorless oil: R_f 0.34 (silica gel, 1:1 ethyl acetate–hexane); $[\alpha]^{26}_D$ +28.6° (*c* 1.67, $CHCl_3$); IR ($CDCl_3$) 2985, 2935, 1835, 1380, 1370, 1250, 1210, 1170, 1135, 1120, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37, 1.37, 1.42, 1.44 (4 s, 12 H, 2 $(CH_3)_2C$), 2.25 (dd, 1 H, J = 15 Hz, J' = 2 Hz, CH*H*HCH), 2.36 (ddd, 1 H, J = 15 Hz, J' = 3 Hz, J'' = 1 Hz, CCH*H*CH), 3.62 (dd, 1 H, J = 9 Hz, J' = 2 Hz, $OCH_2CHCHCH$), 4.04 (dd, 1 H, J = 9 Hz, J' = 4 Hz, OC*H*HCH), 4.10 (dd, 1 H, J = 9 Hz, J' = 6 Hz, OCH*H*CH), 4.21 (ddd, 1 H, J = 9 Hz, J' = 6 Hz, J'' = 4 Hz, OCH_2CHCH), 4.31 (dd, 1 H, J = 6 Hz, J' = 1 Hz, OC*H*HC), 4.39 (d, 1 H, J = 6 Hz, OCH*H*C), 4.40 (dd, 1 H, J = 8 Hz, J' = 2 Hz, $CH_2CHCHCH$), 4.66 (ddd, 1 H, J = 8 Hz, J' = J'' = 3 Hz, CH_2CHCH); EI MS, m/z 299 (M − CH_3)$^+$, 255 (M − CH_3 − CO_2)$^+$, 241; exact mass calcd for $C_{14}H_{19}O_7$ (M − CH_3)$^+$ 299.1131, found 299.1124.

Dimethyl Phosphonate 10. A glass pressure tube was rinsed with NH_4OH, dried at 120 °C, and then charged with 820 mg (2.62 mmol) of the β-lactone **9**. Trimethyl phosphite (24.6 mL) freshly distilled from CaH_2 was added, and the solution was purged with argon. The tube was sealed with a Teflon screw cap and immersed for 24 h in an oil bath at 100 °C. The excess trimethyl phosphite was removed under vacuum at 50 °C. Chromatography of the residue on 40 g of silica gel with ethyl acetate afforded 424 mg (37%) of the phosphonate **10** as a colorless oil: R_f 0.14 (silica gel, ethyl acetate); $[\alpha]^{22}_D$ −32.2° (*c* 0.86, $CHCl_3$); IR ($CDCl_3$) 2995, 2960, 2940, 2890, 2855, 1750, 1735, 1450, 1435, 1385, 1375, 1245, 1210, 1200, 1170, 1080, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) 1.36, 1.37, 1.43, 1.52 (4 s, 12 H, 2 $(CH_3)_2C$), 1.98 (ddd, 1 H, J = 15 Hz, J' = 4.5 Hz, J'' = 3 Hz, CC*H*HCH), 2.28 (ddd, 1 H, J = 15 Hz, J' = 3.5 Hz, J'' = 1 Hz, CCH*H*CH), 2.50 (dd, 1 H, J = 19 Hz, J' = 15 Hz, PC*H*HC), 2.87 (dd, 1 H, J = 18 Hz, J' = 15 Hz), 3.53 (dd, 1 H, J = 9 Hz, J' = 2 Hz, $OCH_2CHCHCH$), 3.67 (d, 3 H, J = 11 Hz, $POCH_3$), 3.69 (d, 3 H, J = 11 Hz, $POCH_3$), 3.80 (s, 3 H, OCH_3), 4.07 (dd, 1 H, J = 9 Hz, J' = 6 Hz, OC*H*HCH), 4.25 (ddd, 1 H, J = 9 Hz, J' = 6 Hz, J'' = 4 Hz, OCH_2CHCH), 4.31 (dd, 1 H, J = 8 Hz, J' = 2 Hz, $CH_2CHCHCH$), 4.37 (dd, 1 H, J = 9 Hz, J' = 4 Hz, OCH*H*CH), 4.60 (dddd, 1 H, J = 8 Hz, J' = 3.5 Hz, J'' = 3 Hz, J''' = 1 Hz); EI MS, m/z 423 (M − CH_3)$^+$, 365 (M − C_3H_6O); exact mass calcd for $C_{17}H_{28}O_{10}P$ (M − CH_3)$^+$ 423.1420, found 423.1412.

Monomethyl Phosphonate 11. To a solution of 389 mg (0.887 mmol) of the above dimethyl phosphonate **10** in 1.5 mL of THF were added 740 μL (5.31 mmol) of triethylamine and 364 μL (3.55 mmol) of thiophenol. After 20 h at room temperature, the reaction mixture was concentrated under reduced pressure. Chromatography of the residue on 150 g of silica with 190:60:1 and then 120:80:1 chloroform–methanol–triethylamine afforded 419 mg (90%) of **11** as a white solid: R_f 0.33 (silica gel, 120:80:1 chloroform–methanol–triethylamine); $[\alpha]^{22}_D$ +0.53° (*c* 1.48, $CHCl_3$); IR ($CDCl_3$) 2990, 2955, 1740, 1455, 1380, 1370, 1245, 1210, 1175, 1060, 1055 cm^{-1}; ^1H NMR ($CDCl_3$) 1.30 (t, 9 H, J = 8 Hz, CH_3CH_2N), 1.35, 1.35, 1.39, 1.50 (4 s, 12 H, 2 $(CH_3)_2C$), 2.30 (dd, 1 H, J = 18 Hz, J' = 15 Hz, PC*H*HC), 2.35 (br d, 2 H, J = 5 Hz, CCH_2CH), 2.47 (dd, 1 H, J = 17 Hz, J' = 15 Hz, PCH*H*C), 3.02 (q, 6 H, J = 8 Hz, CH_3CH_2N), 3.29 (dd, 1 H, J = 9 Hz, J' = 2 Hz, $OCH_2CHCHCH$), 3.53 (d, 3 H, J = 11 Hz, $POCH_3$), 3.75 (s, 3 H, $COCH_3$), 4.07 (dd, 1 H, J = 9 Hz, J' = 6 Hz, OCH*H*CH), 4.12 (dd, 1 H, J = 9 Hz, J' = 4 Hz, OCH*H*CH), 4.21 (dd, 1 H, J = 7 Hz, J' = 2 Hz, $CH_2CHCHCH$), 4.24 (ddd, 1 H, J = 9 Hz, J' = 6 Hz, J'' = 4 Hz, OCH_2CHCH), 4.55 (ddd, 1 H, J = 7 Hz, J' = J'' = 5 Hz, CH_2CHCH); FAB MS, m/z 526 (M + H + Et_3N)$^+$, 425 (M + H)$^+$; exact mass calcd for $C_{23}H_{45}NO_{10}P$ 526.2781, found 526.2785.

Cytidyl Phosphonate 12. To a stirred solution of 2.10 g (8.00 mmol) of triphenylphosphine in 20 mL of THF at 0 °C was added 1.57 mL (8.00 mmol) of diisopropyl azodicarboxylate over 4 min. After 30 min, 0.73 mL (~0.27 mmol) of the resulting fine, white slurry was added to a mixture of 114 mg (0.293 mmol) of the alcohol **13** and 119 mg (0.226 mmol) of the above phosphonate **11** in 2.0 mL of THF at 0 °C. Within 5 min the resulting mixture became a clear solution, and after 30 min at 0 °C, the cooling bath was removed. After 24 h at room temperature, 1.0 mL of methanol was added and the reaction mixture was concentrated under reduced pressure. Chromatography of the residue on 30 g of silica gel with ethyl acetate and then 95:5 ethyl acetate–methanol afforded 145 mg (81% based on **11**) of the cytidyl methyl phosphonate as a 1:1 mixture of diastereomers: R_f 0.10 (silica gel, ethyl acetate); IR ($CDCl_3$) 3410, 3000, 2960, 2940, 1755, 1735, 1705, 1670, 1630, 1555, 1480, 1385, 1375, 1325, 1300, 1250, 1215, 1070 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29, 1.35, 1.41, 1.50, 1.54, 1.59 (6 s, 18 H, 3 $(CH_3)_2C$), 1.90–2.10, 2.20–2.35 (2 br m, 2 H, CCH_2CH), 2.45 (dd, "$^1/_2$ H", J = 19 Hz, J' = 15 Hz, PC*H*HC), 2.51 (dd, "$^1/_2$ H", J = 19 Hz, J' = 15 Hz, PC*H*HC), 2.90 (dd, "$^1/_2$ H", J = 17 Hz, J' = 15 Hz, PCH*H*C), 2.94 (dd, "$^1/_2$ H", J = 17 Hz, J' = 15 Hz, PCH*H*C), 3.53 (dd, "$^1/_2$ H", J = 9 Hz, J' = 2 Hz, $COCH_2CHCHCH$), 3.55 (dd, "$^1/_2$ H", J = 9 Hz, J' = 2 Hz, $COCH_2CHCHCH$), 3.67 (d, "$^3/_2$ H", J = 9 Hz, $POCH_3$), 3.71 (d, "$^3/_2$ H", J = 9 Hz, $POCH_3$), 3.79 (s, 3 H, OCH_3), 4.03–4.12 (m, 1 H), 4.17–4.48 (m, 7 H), 4.53–4.63 (br m, 1 H), 4.82 (dd, "$^1/_2$ H", J = 6 Hz, J' = 4 Hz, NCHCHCHCH), 4.88 (dd, "$^1/_2$ H", J = 6 Hz, J' = 3 Hz, NCHCHCHCH), 4.98 (dd, "$^1/_2$ H", J = 6 Hz, J' = 2 Hz, NCHCHCH), 5.00 (dd, "$^1/_2$ H", J = 6 Hz, J'' = 2 Hz, NCHCHCH), 5.82 (d, "$^1/_2$ H", J = 2 Hz, NCHCH), 5.83 (d, "$^1/_2$ H", J = 2 Hz, NCHCH), 7.52 (dd, 2 H, J = J' = 7 Hz, m-$C_6H_2H_3$), 7.54 (d, 1 H, J = 7 Hz, NCH=CH), 7.63 (t, 1 H, J = 7 Hz, p-C_6HH_4), 7.90 (d, 2 H, J = 7 Hz, o-$C_6H_2H_3$), 7.90 (d, 1 H, J = 7 Hz, NCH=CH), 8.83 (br s, 1 H, NH); FAB MS, exact mass calcd for $C_{36}H_{49}O_{15}N_3P$ (M + H)$^+$ 794.2901, found 794.2926.

Further elution with 60:40:4:0.2 chloroform–methanol–water–triethylamine afforded 13.0 mg (11%) of the starting phosphonate **11**.

To a solution of 116 mg (0.146 mmol) of the above cytidyl methyl phosphonate in 0.4 mL of THF were added 122 μL (0.877 mmol) of triethylamine and 60 μL (0.585 mmol) of thiophenol. After 24 h at room temperature the reaction mixture was concentrated under reduced pressure. Chromatography of the residue on 20 g of silica gel with chloroform and then 75:25:1 chloroform–methanol–triethylamine afforded 96 mg (75%) of the cytidyl phosphonate **12**: R_f 0.25 (silica gel, 4:1 chloroform–methanol); $[\alpha]^{22}_D$ +49.4 (*c* 0.64, $CHCl_3$); IR ($CDCl_3$) 3680, 3415, 3000, 2955, 2440(b), 1740, 1710, 1670, 1625, 1560, 1485, 1390, 1380, 1250, 1220, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.17 (t, 9 H, J = 7 Hz, NCH_2CH_3), 1.32, 1.32, 1.32, 1.37, 1.49, 1.58 (6 s, 18 H, 3 $(CH_3)_2C$), 2.28 (dd, 1 H, J = 17 Hz, J'' = 15 Hz, PC*H*HC), 2.26 (dd, 1 H, J = 15 Hz, J' = 3 Hz, CC*H*HCH), 2.35 (dd, 1 H, J = 15 Hz, J' = 4 Hz, CCH*H*CH), 2.50 (dd, 1 H, J = 17 Hz, J' = 15 Hz, PCH*H*C), 3.29 (dd, 1 H, J = 9 Hz, J' = 2 Hz, $COCH_2CHCHCH$), 3.73 (s, 3 H, OCH_3), 4.03 (dd, 1 H, J = 8 Hz, J' = 6 Hz, COC*H*HCH), 4.12 (dd, 1 H, J = 8 Hz, J' = 4 Hz, COCH*H*CH), 4.00–4.15 (m, 2 H, $POCH_2CH$), 4.21 (dd, 1 H, J = 7 Hz, J' = 2 Hz, $CCH_2CHCHCH$), 4.21 (ddd, 1 H, J = 9 Hz, J' = 7 Hz, J'' = 4 Hz, $COCH_2CHCH$), 4.45 (ddd, 1 H, J = J' = J'' = 3 Hz, $POCH_2CHCH$), 4.55 (ddd, 1 H, J = 7 Hz, J' = 4 Hz, J'' = 3 Hz, CCH_2CHCH), 4.79 (dd, 1 H, J = 6 Hz, J' = 2 Hz, NCHCHCH), 4.86 (dd, 1 H, J = 6 Hz, J' = 3 Hz, NCHCHCHCH), 6.03 (d, 1 H, J = 2 Hz, NCHCH), 7.45 (d, 1 H, J = 7 Hz, NCH=CH) 7.49 (dd, 2 H, J = J' = 7 Hz, m-$C_6H_2H_3$), 7.59 (t, 1 H, J = 7 Hz, p-C_6HH_4), 7.86 (d, 2 H, J = 7 Hz, o-$C_6H_2H_3$), 8.34 (d, 1 H, J = 7 Hz, NCH=CH); FAB MS, m/z 780 (M + H)$^+$ 802 (M + Na)$^+$, 818 (M + K)$^+$; exact mass calcd for $C_{35}H_{47}N_3O_{15}P$ (M + H)$^+$ 780.2745, found 780.2763.

CMP-KDO Phosphonate 3. To a stirred solution of 42 mg (0.047 mmol) of the above phosphonate **12** in 3 mL of CH_2Cl_2 was added 1.5 mL of 90% aqueous trifluoroacetic acid. After 2 h at room temperature, the reaction was concentrated and codistilled with five portions of toluene at reduced pressure. The residue was dissolved in 1 mL of methanol, diluted with 4 mL of water, and brought to pH 14 with 1 M aqueous NaOH. After 3 h at room temperature, it was adjusted to pH 3 with a minimum amount of Dowex HCR-S resin and filtered. The resin was washed with 10 × 5 mL of 7 M NH_4OH, and the combined filtrates were concentrated under reduced pressure. The residue was dissolved in 0.5 mL of water. Addition of 3 mL of methanol caused 8.5 mg of **3** to precipitate as a white powder. The mother liquors were concentrated under reduced pressure, dissolved in water, and passed through an aqueous 1.3 × 25 cm G-10 Sephadex column to remove ammonium benzoate. Lyophilization afforded an ad-

ditional 16.3 mg of **3** as a white powder for a combined yield of 93%: R_f 0.05 (silica gel, 40:60:4:0.2 chloroform–methanol–water–15 M ammonium hydroxide); $[\alpha]^{22}_D$ +44.6° (*c* 0.505, H_2O); ^1H NMR (D_2O, HOD = 4.80 ppm) δ 2.09 (dd, 1 H, *J* = 17 Hz, *J'* = 15 Hz, PC*H*HC), 2.12 (dd, 1 H, *J* = *J'* = 13 Hz, CC*H*HCH), 2.15 (dd, 1 H, *J* = 17 Hz, *J'* = 15 Hz, PC*H*HCH), 2.39 (dd, 1 H, *J* = 13 Hz, *J'* = 5 Hz, CCH*H*CH), 3.37 (d, 1 H, *J* = 8 Hz, $HOCH_2CHCHCH$), 3.70 (dd, 1 H, *J* = 9 Hz, *J'* = 4 Hz, HOC*H*HCH), 3.75 (ddd, 1 H, *J* = 13 Hz, *J'* = 5 Hz, *J''* = 3 Hz, CCH_2CHCH), 3.80–3.90 (m, 3 H, HOCH*H*CH, $HOCH_2CHCH$, $POCH_2CHCH$), 4.04 (ddd, 1 H, *J* = 12 Hz, *J'* = 6 Hz, *J''* = 2 Hz, POC*H*HCH), 4.17 (ddd, 1 H, *J* = 12 Hz, *J'* = 4 Hz, *J''* = 2 Hz, POCH*H*CH), 4.25 (br s, 1 H, $CCH_2CHCHCH$), 4.26–4.34 (m, 2 H, NC*H*CHCH), 5.93 (d, 1 H, *J* = 3 Hz, NC*H*CH), 6.17 (d, 1 H, *J* = 7 Hz, NCH=C*H*), 8.09 (d, 1 H, *J* = 7 Hz, NC*H*=CH); ^{13}C NMR (D_2O, 125.76 MHz) δ 33.75, 36.67 (d, *J* = 133.4 Hz), 62.41 (d, *J* = 4.5 Hz), 63.96, 66.40, 67.70, 68.96, 69.05, 74.22, 74.38, 79.41 (br s), 82.82 (d, *J* = 6.8 Hz), 89.48, 95.98, 142.32, 154.46, 163.72, 178.98 (br s); FAB MS, *m/z* 542 $(M + H)^+$, 564 $(M + Na)^+$; exact mass calcd for $C_{18}H_{28}N_3O_{14}PNa$ $(M + Na)^+$ 564.1207, found 564.1224.

Phosphonate 17. To a stirred solution of 55 mg (0.105 mmol) of the phosphonate **11** in 3 mL of methanol was added 0.6 mL of aqueous 1 N NaOH. After 5 h at 50 °C, the reaction was cooled, diluted with 3 mL of water, brought to pH 2 with Dowex HCR-S resin, filtered, and concentrated under reduced pressure. The residue was dissolved in 3.0 mL of water and allowed to stand at room temperature for 38 h. TLC (silica gel, 4:3:1 chloroform–methanol–15 M ammonium hydroxide) indicated conversion of a diacid (R_f 0.11) to the triacid **17** (R_f 0.01). The reaction mixture was concentrated under reduced pressure. Chromatography of the residue on 5 g of Baker (aminopropyl)silane-bonded silica gel with 60:40:2 methanol–water–15 M ammonium hydroxide and lyophilization afforded 30.1 mg (78%) of the phosphonate **17** as a white solid: R_f 0.08 (EM Science, HPTLC-NH_2, F-254, 60:40:2 methanol–water–15 M ammonium hydroxide); $[\alpha]^{22}_D$ 49.4° (*c* 0.64, H_2O); ^1H NMR (D_2O, HOD = 4.80 ppm) δ 2.04 (dd, 1 H, *J* = 21 Hz, *J'* = 15 Hz, PC*H*HC), 2.08 (dd, 1 H, *J* = *J'* = 13 Hz, CC*H*CH), 2.10 (dd, 1 H, *J* = 21 Hz, *J'* = 15 Hz, PCH*H*C), 2.35 (dd, 1 H, *J* = 13 Hz, *J'* = 5 Hz, CCH*H*CH), 3.38 (d, 1 H, *J* = 9 Hz, $OCH_2CHCHCH$), 3.68–3.90 (m, 5 H); ^{13}C NMR (D_2O, CH_3CN = 1.40 ppm) δ 34.49 (d, *J* = 3.8 Hz), 38.82 (d, *J* = 129.8 Hz), 64.58, 66.89, 68.25, 69.65, 75.17, 80.06 (d, *J* = 4.5 Hz), 179.96 (d, *J* = 9 Hz); FAB MS, *m/z* 317 $(M + H)^+$; exact mass calcd for $C_9H_{18}O_{10}P$ $(M + H)^+$ 317.0638, found 317.0645.

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A 1,3-Dipolar Cycloaddition Route to the 3(*R*)- and 3(*S*)-Hydroxy-(2*S*)-arginines

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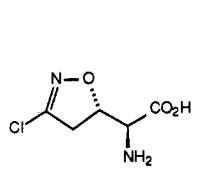
The 3(*R*)- and 3(*S*)-hydroxy-(2*S*)-arginines (**4a** and **4b**) were prepared from the corresponding β-hydroxyornithines (**3a** and **3b**) via the methyl 2(*S*)-[2-benzyl-(5(*R*,*S*)-isoxazolidinyl][*N*-((benzyloxy)carbonyl)amino]acetates (**5a** and **5b**). The isoxazolidines **5** were prepared from the 1,3-dipolar cycloaddition of 2(*S*)-vinylglycine derivative **6** with a nitrone generated in situ from *N*-benzylhydroxylamine and paraformaldehyde.

Our studies on the biosynthesis of acivicin (**1**)[2] and streptothricin F (**2**)[3] required the synthesis of the (2*S*,3*R*)- and (2*S*,3*S*)-β-hydroxyornithines (**3a** and **3b**) and the corresponding β-hydroxyarginines (**4a** and **4b**), respectively. Syntheses of the *threo*- and *erythro*-β-hydroxy-



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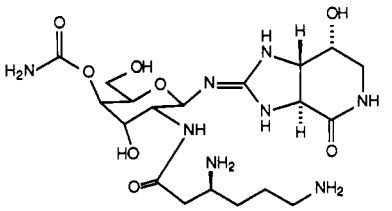

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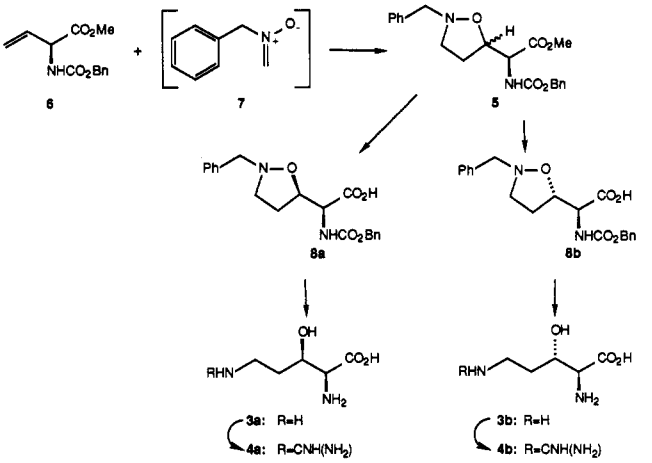
ornithines have previously been reported,[4] although only in racemic form; furthermore, these syntheses were either lengthy or not amenable to the introduction of isotope labels.

Noting that isoxazolines can be reduced to afford β-amino alcohols,[5] we found that **3b** could be obtained in 78% yield from a catalytic reduction of **1**. Since acivicin and its C3 diastereomer have been synthesized from 2-(*S*)-(*N*-phthalimidovinyl)glycine,[6] this would constitute a formal synthesis of **3a** and **3b**. Removing what would be extraneous functionality for our purpose, we focused on

Scheme I



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