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Synthesis of Novel Phosphonate Analogue of Kdo as a Tool for the Design of Potent Inhibitors for Lipopolysaccharide Biosynthesis

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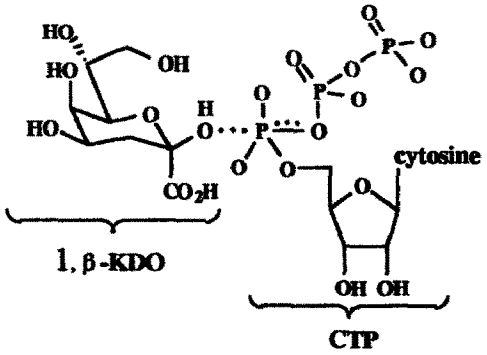
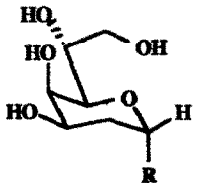
Abstract: The new phosphonate analogue 3 of 2-deoxy-β-Kdo (2) was prepared in a stereospecific manner via intramolecular C-C bond formation. X-Ray structure analysis and NMR studies of the cyclyzation product established the twist-boat conformation of the pyranose ring with α-anomeric configuration of the phosphonate linkage.

3-Deoxy-D-*manno*-2-octulosonic acid (Kdo, **1**) is a site-specific constituent of the lipopolysaccharide of most Gram-negative bacteria, and provides the link between lipid A and the growing polysaccharide chain.[1,2] Since bacterial mutants defective in Kdo biosynthesis are not viable,[3] the enzymes involved in the synthesis and incorporation of Kdo into lipopolysaccharide have been considered as attractive targets in the design of novel antibacterial drugs.[4] For this purpose, during the last decade most interest has been focused on the inhibition of CMP-Kdo synthetase [EC 2.7.7.38] which catalyzes the formation of the nucleoside derivative CMP-Kdo from Kdo and cytidine triphosphate (CTP).[5] Following the finding that β-Kdo, the minor form of Kdo in solution, is the actual substrate for this enzyme[6] and that the 2-deoxy-β-Kdo (2) is a potent inhibitor,[7] many groups have pursued the synthesis of various analogues of Kdo in order to discover more potent inhibitors than the analogue 2. Through these studies several analogues of Kdo modified at the carboxyl group,[8a] at the C-2,[8b] the C-8[8c] or in the ring,[8d] were synthesized and tested as inhibitors of CMP-Kdo synthetase. However, none were found to be significantly better than the parent 2. Although it is clear that the tightest binding inhibitor of this enzyme should be the stable analogue of the transition-state mimic (as illustrated below), the synthesis and commercial use of such a compound would be expensive and unrealistic.



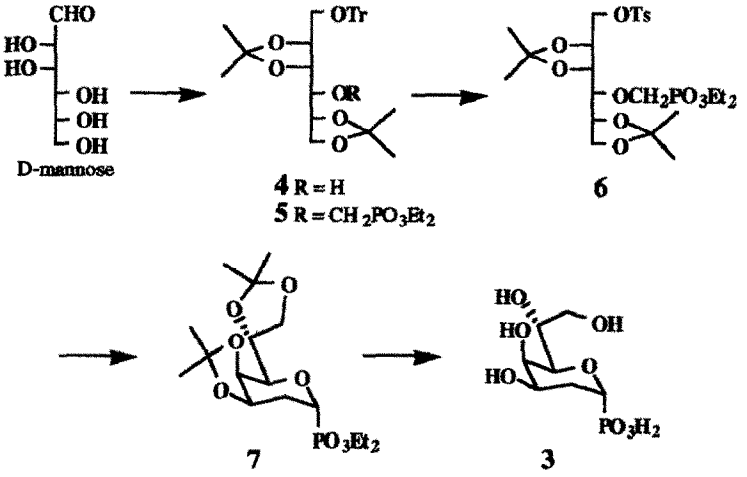


2 R = CO_2H
3 R = PO_3H_2

Therefore, based on the available data pertaining to this enzyme mechanism and its inhibition, we suggest the phosphonate analogue **3** as a possible potent inhibitor for several reasons: (i) analogue **3** is

isosteric to 2; (ii) in addition to closely mimicking the topological properties of 2, analogue 3 possesses electrostatic properties which are expected to allow it to bind more tightly; (iii) in many enzymes the carboxylate binding site is indistinct[9a,b] and may well be replaced by the isosteric phosphonate;[9a] (iv) since the enzyme requires Mg^{2+} for its catalytic activity,[5] the phosphonate moiety in 3 and α-phosphate of CTP can serve as a bidentate chelating ligand of a Mg^{2+} which might result in an extremely tight binding to the enzyme active site. This communication describes the first stereospecific synthesis of phosphonate 3, the new phosphonate analogue of 2-deoxy-β-Kdo (2) in which the carboxylic acid is replaced by phosphonic acid.

Our synthesis starts from D-mannose, continuing through the synthetic manipulations of its acyclic form, and finally using a direct stereospecific construction of the tetrahydropyran ring of 3 by intramolecular C-C bond formation.[10] D-Mannose was converted to the differentially protected 4 in three steps [(i) acetone, H_2SO_4; (ii) $NaBH_4$, MeOH; (iii) TrCl, Pyridine, 30°C)] with an overall 80% yield. The phosphonomethyl function was successfully incorporated at hydroxyl-4 by treatment of the sodium alkoxide of 4 (NaH, THF, 0°C, 30 min) with diethyl phosphonomethyltriflate[11] (0°C, 20 min and then 25°C for 10 min) to afford the phosphonate 5 in an 87% isolated yield. The next two steps aimed to introduce the leaving group at C-1 position. Hydrogenolysis of 5 (H_2, 4 atm, Pd/C, EtOH) was followed by tosylation (TsCl, pyridine, dimethylaminopyridine, 25°C) to attain the tosylate 6 in 85% isolated yield for two steps. The cyclyzation of 6 was successfully accomplished by treatment with 1.1 mol. equiv. LDA at -78°C in THF to afford a 50% isolated yield of the phosphonate 7 as a single stereoisomer, along with 41% of the starting tosylate 6. Attempts to improve the yield of 7 by adding excess LDA or using different reaction conditions (temperature and solvent) proved to be less satisfactory.



In order to determine the anomeric configuration of the phosphonate linkage, the ^1H, ^{13}C and ^{31}P-NMR spectra of the analytically pure 7 were recorded.[12] However, the relatively small $^3J_{P,H2}$ values suggested the absence of a 1,3-diaxial relation between phosphorus and C-2 protons.[13] Although these coupling constants are dependent on the dihedral angle,[14] and the observed spectral data could be reasonably explained by assuming the distortion of the pyranose ring, the opposite configuration of the anomeric phosphate could not be completely excluded. Therefore, single-crystal X-ray analysis of 7 was undertaken (Figure 1). This

analysis reveals a twist-boat conformation of the sugar ring, while the phosphonate moiety is oriented *trans* to the oxygen substitutions at carbons 3 and 4, indicating the α-anomeric configuration. Indeed, a similar distortion of the pyranose ring of protected Kdo has been reported and attributed to the presence of 4,5-*O*-isopropylidene[15a] and of 4,5-*O*-cyclohexylidene[15b] protecting groups.

Deprotection of the phosphonate 7 [trimethylsilyl bromide, methylene chloride, r.t.; Dowex H^+, H_2O; ion-exchange chromatography through AG1X8 (HCO_3^- form), eluted with the linear gradient of 0-0.6 M triethylammonium bicarbonate] provided the target phosphonate 3 (65%) as assessed from its spectral data.[16] Evaluation of 3 as an inhibitor of CMP-Kdo synthetase is underway and will be reported in due course.

The synthetic method described here, provides a new efficient approach for the construction of other glycopyranosyl and glycofuranosyl phosphonates as stable analogues to the biologically important glycosyl phosphates.[17] Synthesis is underway of other phosphonate analogues of Kdo and of Kdo-8-phosphate,[18] which might serve as potent inhibitors of other enzymes of Kdo biosynthesis.

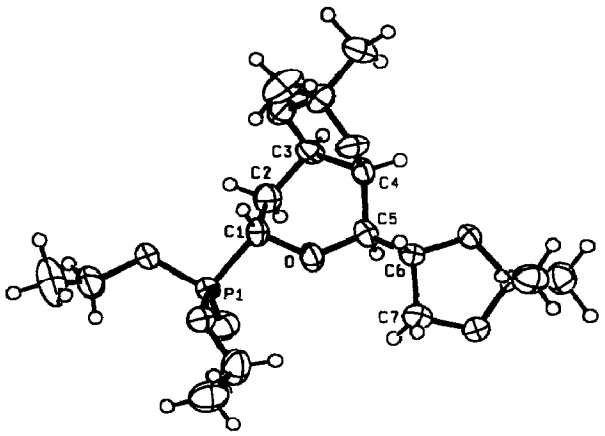


Figure 1. Computer drawing of 7 from single-crystal X-ray analysis. Crystallographic data: Orthorhombic $P2_12_12_1$, a=20.990(9)Å, b=10.963(5)Å, c=8.757(4)Å, V=2015.1Å^3, Z=4, R=0.060 for 1436 significant reflections. Radiation Mo Kα, λ=0.71069Å. Bond lengths (Å): C(1)-C(2)=1.506(8), C(2)-C(3)=1.506(8), C(3)-C(4)=1.542(8), C(4)-C(5)=1.516(9), C(5)-O=1.444(7), O-C(1)=1.436(7), C(5)-C(6)=1.519(8), C(6)-C(7)=1.511(1), C(1)-P1=1.806(6). Bond angles (°): C(2)-C(1)-P1=112.8(4), C(2)-C(1)-O=112.9(4), C(1)-C(2)-C(3)=110.8(5), C(3)-C(4)-C(5)=112.8(5), C(1)-O-C(5)=116.5(4), O-C(1)-P1=105.3(4), C(4)-C(5)-O=111.9(5), C(2)-C(3)-C(4)=111.5(5). Torsion angles (°): C(1)-C(2)-C(3)-C(4)=42.0(7), C(3)-C(4)-C(5)-C(6)=-171.8(5), C(4)-C(5)-O-C(1)=38.8(7), P1-C(1)-C(2)-C(3)=-179.1(4), C(3)-C(4)-C(5)-O=-54.2(6), O-C(1)-C(2)-C(3)=-59.8(6), C(2)-C(1)-O-C(5)=17.3(7), P1-C(1)-O-C(5)=140.8(4), C(2)-C(3)-C(4)-C(5)=12.2(6). The e.s.d. in parenthesis is in the unit of the least significant digit.

Acknowledgments: We thank Dr. M. Kapon for X-ray analysis of compound 7. This research was supported by the United States-Israel Binational Science Foundation (BSF), Jerusalem, Israel (grant 90-00478), and by the Center for Absorption in Science, Ministry of Immigrant Absorption and the Ministry of Science and Technology, State of Israel (grant 3611191).

REFERENCES AND NOTES

1. (a) Unger, F. M. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 323-388. (b) Ray, P. H.; Kelsey, J. E.; Bigham, E. C.; Benedict, C. D.; Miller, T. A. *Bacterial Lipopolysaccharides*; Anderson L.; Unger, F. M., Eds.; ACS Symp. Series 231; American Chemical Society: Washington, DC, 1983; pp. 141-170.
2. Inouye, M. *Bacterial Outer Membranes: Biogenesis and Functions*; Wiley: New York, 1979.

3. Rick, P. D. and Osborn, M. J. *Proc. Natl. Acad. Sci. USA* **1972**, *69*, 3756-3760.
4. (a) Hammond, S. M.; Claesson, A.; Jansson, A. M.; Larsson, L. G.; Brian, G. P.; Town, C. M.; Ekstrom, B. *Nature* **1987**, *327*, 730-732. (b) Goldman, R.; Kohlbrenner, W.; Lartey, P.; Pernet, A. *Nature* **1987**, *329*, 162-164.
5. Ray, P. H., Benedict, C. D., and Grasmuk, H. *J. Bacteriol.* **1981**, *145*, 1273-1280.
6. Kohlbrenner, W. E. & Fesik, S. W. *J. Biol. Chem.* **1985**, *260*, 14695-14700.
7. Claesson, A., Luthman, K., Gustafsson, K., and Bondesson, G. *Biochem. Biophys. Res. Commun.* **1987**, *143*, 1063-1068.
8. (a) Luthman, K., Claesson, A., Jansson, A. M., and Pring, B. G. *Carbohyd. Res.* **1987**, *166*, 233-251; (b) Luthman, K., Orbe, M., Waglund, T., Claesson, A. *J. Org. Chem.* **1987**, *52*, 3777-3784; Waglund, T., Luthman, K., Orbe, M. *Carbohyd. Res.* **1990**, *206*, 269-276; (c) Pring, B. G., Jansson, M., Persson, K., Andersson, I., Gagner-Milchert, I., Gustafsson, K., and Claesson, A. *J. Med. Chem.* **1989**, *32*, 1069-1074; (d) Molin, H. and Pring, B. G. *Tetrahedron Lett.* **1985**, *26*, 677-680; Norbeck, D. W. and Kramer, J. B. *Tetrahedron Lett.* **1987**, *28*, 773-776.
9. (a) Alberg, D. G., Lauhon, C. T., Nyfeler, R., Fassler, A. and Bartlett, P. A. *J. Am. Chem. Soc.* **1992**, *114*, 3535-3546; (b) Baasov, T., Sheffer-Dee-Noor, S., Kohen, A., Jakob, A. and Belakhov, V. *Eur. J. Biochem.* **1993**, *217*, 991-999.
10. A similar method for the synthesis of compound 2 was recently published: Ohrui, H., Morita, M., and Meguro, H. *Carbohyd. Res.* **1992**, *224*, 319-325.
11. Phillon, D. P. and Andrew, S. S. *Tetrahedron Lett.* **1986**, *27*, 1477-1480.
12. ^{1}H-NMR (500 MHz, MeOH-d_4) δ 1.33 (6H, dt, *J*=7.1 and 6.0 Hz), 1.31 (3H, s), 1.33 (3H, s), 1.34 (3H, s), 1.37 (3H, s), 2.01 (1H, dddd, $J_{2e\text{-}2a}$=15.0 Hz, $J_{2e\text{-}1}$=4.8 Hz, $J_{2e\text{-}P}$=1.9 Hz, $J_{2e\text{-}3}$=2.9 Hz, 2-H_e), 2.13 (1H, dddd, $J_{2a\text{-}2e}$=14.9 Hz, $J_{2a\text{-}3}$=2.9 Hz, $J_{2a\text{-}1}$=12.6 Hz, $J_{2a\text{-}P}$=13.9 Hz, 2-H_a), 3.66 (1H, ddd, *J*=7.6, 1.5 and 1.5 Hz, 5-H), 3.99 (1H, dd, *J*=8.4 and 5.5 Hz, 7-H), 4.06 (1H, dd, *J*=8.4 and 6.2 Hz, 7'-H), 4.15 (1H, m, 6-H), 4.18 (4H, dq, *J*=7.8 and 7.05 Hz, OCH_2), 4.29 (1H, ddd, *J*=12.7, 4.8 and 2.3 Hz, 1-H), 4.34 (1H, ddd, *J*=8.2, 1.5 and 1.2 Hz, 4-H), 4.62 (1H, dddd, *J*=8.2, 4.3, 2.8 and 2.8 Hz, 3-H); ^{13}C-NMR δ 16.75 (d, *J*=2.4 Hz), 16.90 (d, *J*=2.4 Hz), 24.60, 25.30, 25.60 (d, *J*=3.5 Hz), 26.42, 27.02, 63.20 (d, *J*=7.5 Hz), 64.40 (d, *J*=6.8 Hz), 66.20 (d, *J*=171. 4 Hz), 67.92, 70.65 (d, *J*=13.4 Hz), 73.45 (d, *J*=1.9 Hz), 73.60 (d, *J*=0.8 Hz), 75.46, 110.04, 110.32; proton coupled ^{31}P-NMR (80.2 MHz, referenced to D_3PO_4 at 0 ppm) δ 22.3 (dq, *J*=13.9 and 7.8 Hz).
13. Although many reports suggest the '*Karplus-like*' dependence of $^3J_{P,H}$ of phosphonates, the exact anomeric configuration of the glycosylphosphonates could not be unequivocally determined without having all the spectral data of the opposite anomer as well: Meuwly, R. and Vasella, A. *Helv. Chim. Acta* **1986**, *69*, 25-34.
14. Neeser, J. R., Tronchet, J. M. J., Charollias, E. J. *Can. J. Chem.* **1983**, *61*, 2112-2120.
15. (a) Imoto, M., Kusunose, N., Matsuura, Y., Kusumoto, S., and Shiba, T. *Tetrahedron Lett.* **1987**, *28*, 6277-6280; (b) Schmidt, R. R. and Esswein, A. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1178-1180.
16. ^{1}H-NMR (400 MHz, D_2O) δ 1.78-1.89 (2H, m, 2-H), 3.50-3.55 (2H, m, 6-H + 7-H), 3.60 (1H, dd, *J*=12.1 and 4.5 Hz, 7'-H), 3.68 (1H, d, *J*=9.3 Hz, 5-H), 3.79 (1H, d, *J*=2.9 Hz, 4-H), 3.83 (1H, ddd, *J*=8.0, 5.1 and 4.3 Hz, 1-H), 4.12 (1H, ddd, *J*=11.0, 6.0 and 2.9 Hz, 3-H); ^{13}C-NMR δ 30.0 (d, *J*=3.5 Hz), 66.20, 68.81, 69.52, 72.25, 74.61 (d, *J*=147.8 Hz), 76.18; proton coupled ^{31}P-NMR (80.2 MHz, D_2O, referenced to D_3PO_4 at 0 ppm) δ 14.4 (dm, *J*=23.6 Hz).
17. Vaghefi, M. M., Bernacki, R. J., Dalley, N. K., Wilson, B. E., and Robins, R. K. *J. Med. Chem.* **1987**, *30*, 1383-1391. For the synthetic scheme to glycosyl phosphonates which is based on the reaction of 1-*O*-acetyl-glycoses with trialkylphosphite in the presence of trimethylsilyl trifluoromethanesulfonate see reference in note 13.
18. a) Baasov, T.; Jacob, A. *J. Am. Chem. Soc.* **1990**, *112*, 4972-4974; (b) Sheffer-Dee-Noor, S., Belakhov, V. & Baasov, T. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1583-1588.

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