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SYNTHESIS AND EVALUATION OF 3-DEOXY-D-MANNO-2-OCTULOSONATE-8-PHOSPHATE (KDO8P) SYNTHASE INHIBITORS

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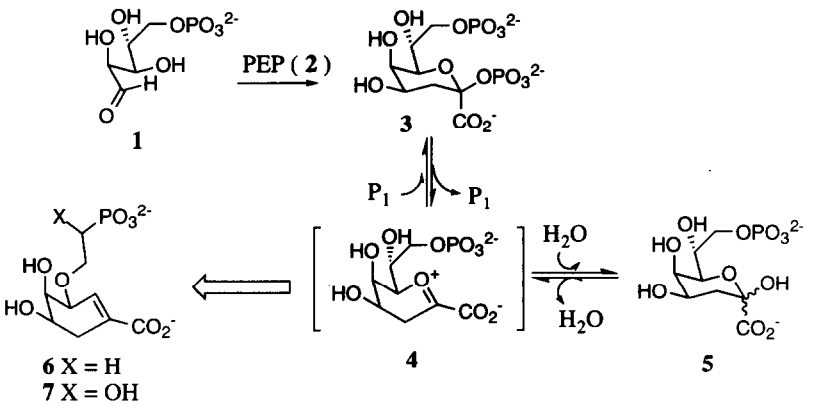
Abstract: Carbocyclic analogues as transition state mechanism based inhibitors of 3-deoxy-D-manno-2-octulosonate-8-phosphate synthase were designed, synthesized, and evaluated for their biological activity.

The biosynthesis of lipopolysaccharide (LPS) is unique to Gram-negative bacteria and has been an attractive target in the search of new antibacterial agents.[1] The biosynthesis of LPS involves a large number of enzymatic steps, of which the synthesis of 3-deoxy-D-manno-2-octulosonate-8-phosphate (KDO8P, **5**) is crucial.[2] KDO8P is formed by the KDO8P synthase catalyzed condensation of D-arabinose 5-phosphate (Ara5P) **1** with phosphoenolpyruvate (PEP) **2**. Baasov and coworkers proposed a mechanistic pathway in which PEP condenses with **1** to form cyclic bisphosphate intermediate **3**, followed by the loss of inorganic phosphate to generate oxocarbenium ion **4** (Scheme 1).[3] The reactive oxocarbenium ion **4** then undergoes hydrolysis to form **5**. Their results also suggests that KDO8P synthase not only actively catalyzes the formation of bisphosphate **3** but also catalyzes its decomposition to generate oxocarbenium ion **4**.

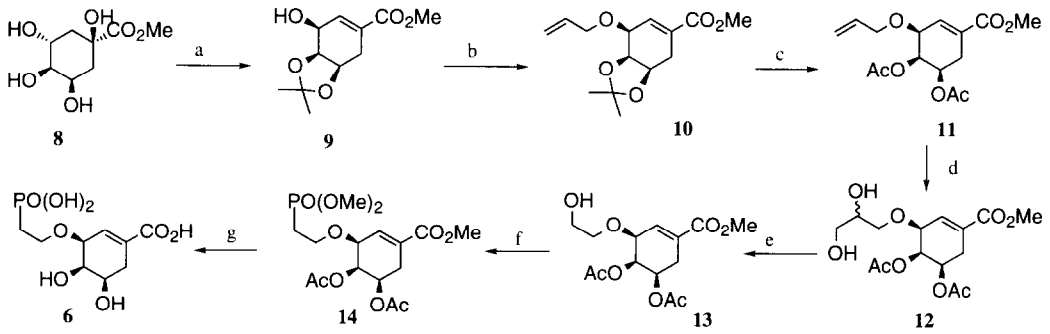
Scheme 1



Our efforts have been directed toward identifying KDO8P synthase inhibitors such as **6** and **7**, which mimic the transition state **4**. The cyclohexene scaffold was selected as a replacement for the oxonium ring of **4**.[4] The cyclohexene ring would keep the conformation of **6** and **7** similar to **4** and should be chemically stable. In structures **6** and **7**, the phosphate in **4** was also replaced with a chemically and biologically more stable phosphonate functionality.[5] The hydroxy group α to the phosphonate in **7** was designed to mimic the 7-hydroxy moiety in **4** and should also decrease the second pk_a of the phosphonate to be close to that of the phosphate in **4**. To simplify the chemistries for **6** and **7**, we use ether linkages between the side chains and the cyclohexene rings instead of carbon linkages.

The synthesis of compound **6** is illustrated in Scheme 2. Allylic alcohol **9** was prepared by literature procedures from quinic acid **8**.[6] The alkylation of allylic alcohol **9** was difficult due to aromatization: however, the combination of allyl bromide and silver oxide gave allyl ether **10** in moderate yield. Deprotection of the isopropylidene group, followed by acetylation gave triester **11**. The selective oxidation of the terminal double bond of **11** with m-CPBA generated an intermediate epoxide which was hydrolyzed to diol **12**. Cleavage of diol **12**, followed by reduction afforded primary alcohol **13**. Alcohol **13** was converted to the corresponding bromide which was then heated with trimethyl phosphite to produce phosphonate **14**. Deprotection of the dimethyl phosphonate with trimethylsilyl bromide, followed by hydrolysis and acidification gave compound **6**.

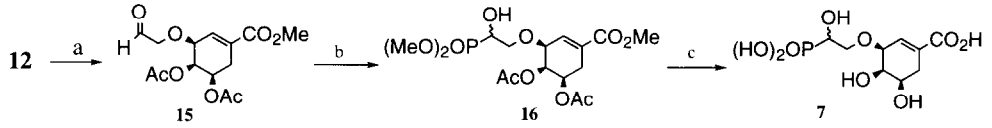
Scheme 2[a]



[a]Key: (a) ref 5; (b) allyl bromide/Ag_2O/DMF(56%); (c) (i) MeOH/TsOH; (ii) Ac_2O/pyridine (75%); (d) (i) m-CPBA; (ii) Dowex-H^+/THF/H_2O (82%); (e) (i) $NaIO_4/SiO_2/H_2O/CH_2Cl_2$; (ii) $NaBH_4$/MeOH (30%); (f) (i) CBr_4/PPh_3;(ii) $P(OMe)_3$/135 °C (81%); (g) (i) TMSBr; (ii) KOH/MeOH/H_2O; (iii) Dowex-H^+ (100%)

The preparation of compound **7** is shown in Scheme 3. Aldehyde **15**, prepared from diol **12** was treated with dimethyl phosphite in the presence of potassium fluoride to give hydroxy phosphonate **16**, as a 1:1 mixture of diastereomers. The diastereomeric mixture **16** was deprotected to generate compound **7**.

Scheme 3[a]



[a]Key: (a) NaIO4; (b) $P(OH)(OMe)_2$/KF (50%); (c) (i) TMSBr; (ii) KOH; (iii) Dowex-H^+ (95%)

Analogues **6** and **7** were tested by using a KDO8P synthase enzymatic assay.[7] Their inhibitory activities are summarized in Table 1. As shown, compound **7** is at least sixfold more active than compound **6**. This indicates that the hydroxy group α to the phosphonate in **7** plays an important role and possibly mimics the 7-hydroxy moiety in **4**.

Table 1

Compound	IC_{50} (mM)
R5P[a]	4.3
6	>25
7	3.8

[a]D-ribose-5-phosphate (R5P) was used as a positive control.[7]

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