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SYNTHESIS OF AZACYCLIC 2-DEOXY-KDO

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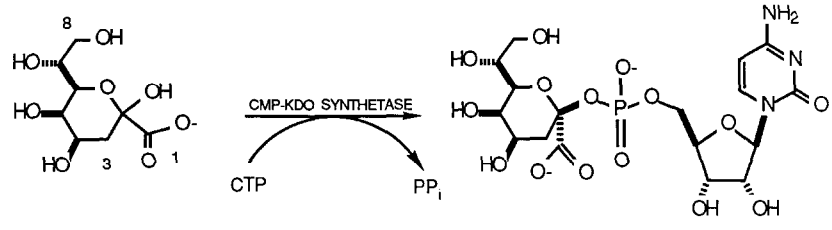
Abstract: Azacyclic analogues of 2-deoxy-KDO have been synthesized from KDO via reductive amination at C-2 and ring closure on C-6 with double inversion.

CMP-KDO synthetase[1] catalyzes a key step in the biosynthesis of bacterial lipopolysaccharide.[2] We and others[3] anticipate that a novel class of antibiotics will emerge from inhibitors of this enzyme. Kohlbrenner and Fesik[4] recently demonstrated that the glycosidic linkage in CMP-KDO is β.

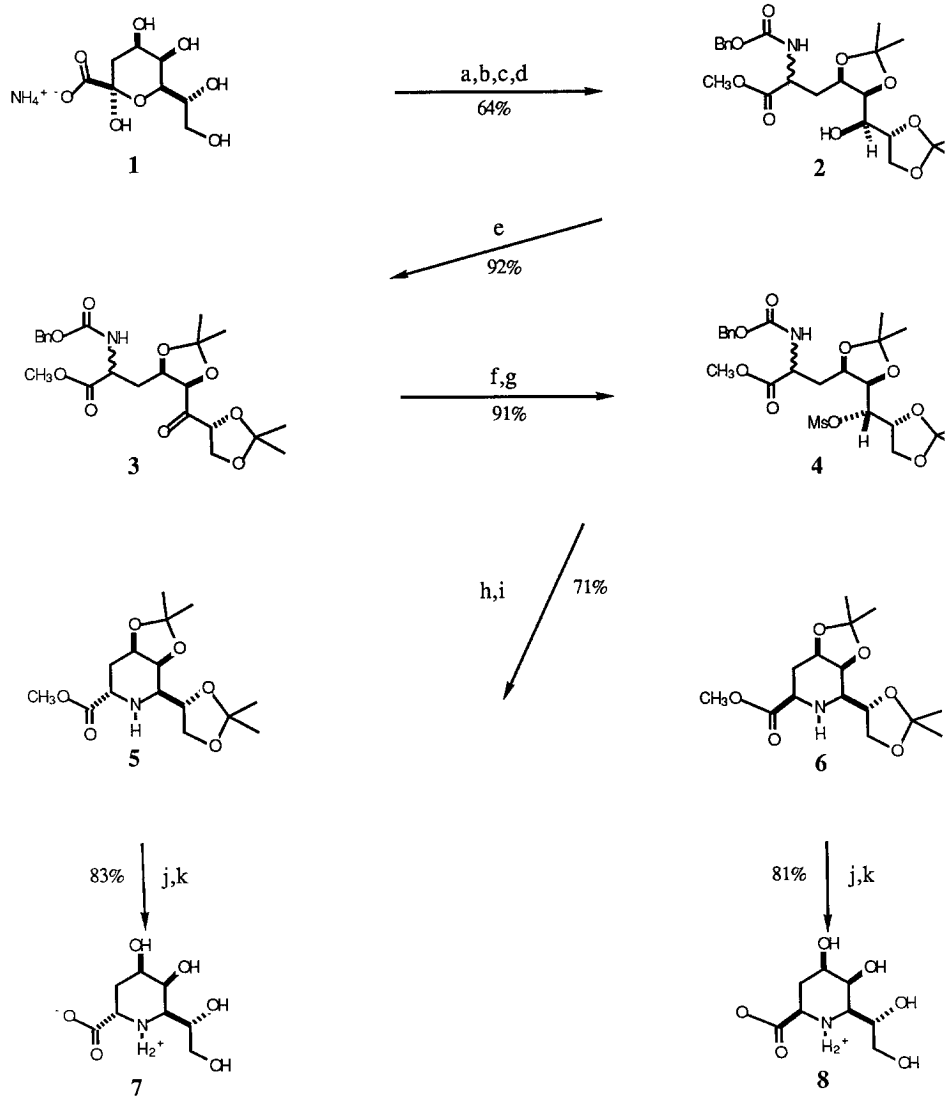


KDO CMP-KDO

Surprisingly, a carbocyclic analogue of KDO, which locks the carboxyl group in the required α orientation, is practically devoid of substrate or inhibitory activity.[5,6] This suggests that the ring oxygen accepts a critical hydrogen bond from the enzyme.[7] We reasoned that the azacyclic KDO derivative **7** could preserve the mandatory α stereochemistry of the carboxyl group and donate a hydrogen bond of increased strength[8] to the enzyme. As an L-α-amino acid, **7** or a peptide derivative might also utilize a bacterial transport system.[9]

Syntheses of several azacyclic glycosidase inhibitors have already been devised from carbohydrate starting materials.[10] Conceptually, the synthesis of **7** from the readily available (+)-KDO[11] (**1**) requires amination of the latent C-2 ketone followed by ring closure on C-6 with retention of configuration. Acid catalyzed isopropylidenation of **1** distinguished the C-6 hydroxyl group, and subsequent reductive amination[12] and protection furnished the alcohol **2** as an inseparable 2:1

SCHEME. SYNTHESIS OF AZACYCLIC 2-DEOXY-KDO



(a) H_2SO_4, Me_2CO (b) $NaCNBH_3$, NH_4OAc, MeOH; (c) ZNOS, MeOH, H_2O, pH 9; (d) CH_2N_2, Et_2O; (e) $(COCl)_2$, DMSO, TEA; (f) $NaBH_4$, MeOH; (g) MsCl, TEA, CH_2Cl_2; (h) Pd, cyclohexadiene, E tOH; (i) $(i\text{-}Pr)_2NEt$, MeCN; (j) TFA, H_2O, THF; (k) TEA, H_2O

mixture of diastereomers. Swern oxidation[13] of **2** followed by $NaBH_4$ reduction of the resultant ketone at -78°C produced another inseparable 2:1 mixture of alcohols. Remarkably, careful scrutiny of the 300 MHz ^1H NMR spectrum revealed less than 5% of **2**. While mesylation of the inverted C-6 alcohol proceeded smoothly, the benzyl carbamate protecting group resisted hydrogenolysis except under "transfer" conditions.[14] Ring closure to the azacycles **5** and **6** required heating the free seco-amines in a sealed tube for four hours at 100°C in acetonitrile containing 4 equivalents of diisopropylethylamine. Since the conformationally restrictive 4,5 acetonide is expected to promote cyclization, the relative difficulty of this process probably reflects the reduced rates of S_N2 displacements alpha to oxygen.[15] Although **5**[16] and **6**[17] were separated by chromatography, distortion of the piperidine ring by the acetonide postponed the unambiguous assignment of the C-2 stereochemistry until deprotection to the amino acids **7**[18] and **8**[19] was completed. In the major epimer (**7**), $^3J_{H-2,3a}$=6Hz and $^3J_{H-2,3e}$=2Hz; in the minor epimer (**8**), $^3J_{H-2,3a}$=12Hz and $^3J_{H-2,3e}$=3Hz.

Disappointingly, **7** was only a modest inhibitor of CMP-KDO synthetase, with an I_{50}=250 μM.[20] As expected, the I_{50} of **8** was greater than 5 mM. Since the oxacyclic analogue of **7** is an excellent inhibitor,[21] the poor binding of **7** can be attributed to replacement of the ring oxygen with nitrogen rather than to deletion of the C-2 hydroxyl group.

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6. An independent synthesis of carbocyclic KDO from quinic acid was completed in this laboratory just prior to the publication of ref. 5. In our assay (ref. 20), the I_{50} of carbocyclic KDO was 3 mM. No substrate activity was observed at 10 mM.
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16. Physical data for **5**: R_f=0.27 (silica gel, 7:3/ether:petroleum ether); ^{1}H NMR ($CDCl_3$, TMS=0.0 ppm) δ 1.37, 1.38, 1.43, 1.50 (4s, 12H, 2(CH_3)$_2$C), 1.68 (bs, 1H, NH), 1.79 (ddd, 1H, J=15Hz, J'=11Hz, J"=3Hz, H-3), 2.16 (ddd, 1H, J=15Hz, J'=6Hz, J"=3Hz, H-3), 2.78 (dd, 1H, J=8Hz, J'=2Hz, H-6), 3.73 (s, 3H, OCH_3), 3.83 (dd, 1H, J=11Hz, J'=6Hz, H-2), 4.00 (ddd, 1H, J=8Hz, J'=7Hz, J"=5Hz, H-7), 4.06 (dd, 1H, J=9Hz, J'=5Hz, H-8), 4.11 (dd, 1H, J=9Hz, J'=7Hz, H-8'), 4.35 (dd, 1H, J=8Hz, J'=2Hz, H-5), 4.53 (ddd, 1H, J=8 Hz, J'=J"=3Hz, H-4); EI MS, m/z 316 $(M+H)^+$, 300 $(M-CH_3)^+$.
17. Physical data for **6**: R_f=0.22 (silica gel, 7:3/ether:petroleum ether); ^{1}H NMR ($CDCl_3$, TMS=0.0 ppm) 1.34, 1.37, 1.44, 1.47 (4s, 12H, 2 (CH_3)$_2$C), 2.04 (dd, 2H, J=6Hz, J'=5Hz, H-3a, H-3e), 2.70 (dd, 1H, J=8Hz, J'=2Hz, H-6), 3.58 (dd, 1H, J=J'=6Hz, H-2), 3.75 (s, 3H, OCH_3), 4.03-4.14 (m, 3H, H-7, H-8, H-8'), 4.26 (dd, 1H, J=7Hz, J'=2Hz, H-5), 4.36 (ddd, 1H, J=7Hz, J=J'=5Hz, H-4); EI MS, m/z 316 $(M+H)^+$, 300$(M-CH_3)^+$.
18. Physical data for **7**: R_f=0.12 (silica gel, 4:3:1/$CHCl_3$:MeOH:15M NH_4OH); ^{1}H NMR (D_2O, HOD=4.80 ppm) δ 2.22 (ddd, 1H, J=13Hz, J'=13Hz, J"=6Hz, H-3a), 2.33 (ddd, 1H, J=13Hz, J'=5Hz, J"=2Hz, H-3e), 3.61 (dd, 1H, J=6Hz, J'=0.5Hz, H-6), 3.73 (ddd, 1H, J=13Hz, J'=5Hz, J"=3Hz, H-4), 3.80 (d, 2H, J=6Hz, H-8, H-8'), 4.02 (ddd, 1H, J=J'=J"=6Hz, H-7), 4.12 (dd, 1H, J=6Hz, J'=2Hz, H-2), 4.23 (bs, 1H, H-5); DCI/NH_3 MS, m/z 239 $(M+NH_4)^+$, 222 $(M+H)^+$.
19. Physical data for **8**: R_f=0.14 (silica gel, 4:3:1/$CHCl_3$:MeOH:15M NH_4OH); ^{1}H NMR (D_2O, HOD=4.80 ppm) δ 1.96 (ddd, 1H, J=J'=J"=12Hz, H-3a), 2.27 (ddd, 1H, J=12Hz, J'=5Hz, J"=3Hz, H-3e), 3.32 (dd, 1H, J=7Hz, J'=0.5Hz, H-6), 3.71 (dd, 1H, J=12Hz, J'=3Hz, H-2), 3.80 (d, 2H, J=6Hz, H-8, H-8'), 3.93 (ddd, 1H, J=12Hz, J'=5Hz, J"=3Hz, H-4), 4.05 (ddd, 1H, J=J'=J"=6Hz, H-7), 4.25 (bs, 1H, H-5); FAB ms, m/z 244 $(M+Na)^+$, 222 $(M+H)^+$.
20. Kohlbrenner, W.E.; Wideburg, N. To be published elsewhere. This assay employed purified enzyme with [KDO]=1mM and [CTP]=0.5mM.
21. Details on the synthesis and biological evaluation of this compound will be published elsewhere.

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