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A New Synthesis for 2-Deoxy-KDO, a Potent Inhibitor of CMP-KDO Synthetase.

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Abstract : A new synthesis for the 2-deoxy KDO **2** by aldolic condensation of 2,3:5,6-di-O-isopropylidene-4-acetyl-D-manno aldehyde **4** with ethyl diazoacetate and conversion of the condensation product to the 3-deoxy-2-diazo ester **13** in four steps is reported. Rhodium (II) decomposition of the diazo compound leads to the α-anomer of the 2-deoxy pyranose **14** stereospecifically. Removal of isopropylidene groups and ester hydrolysis provides **2**, a potent inhibitor of CMP-KDO synthetase.

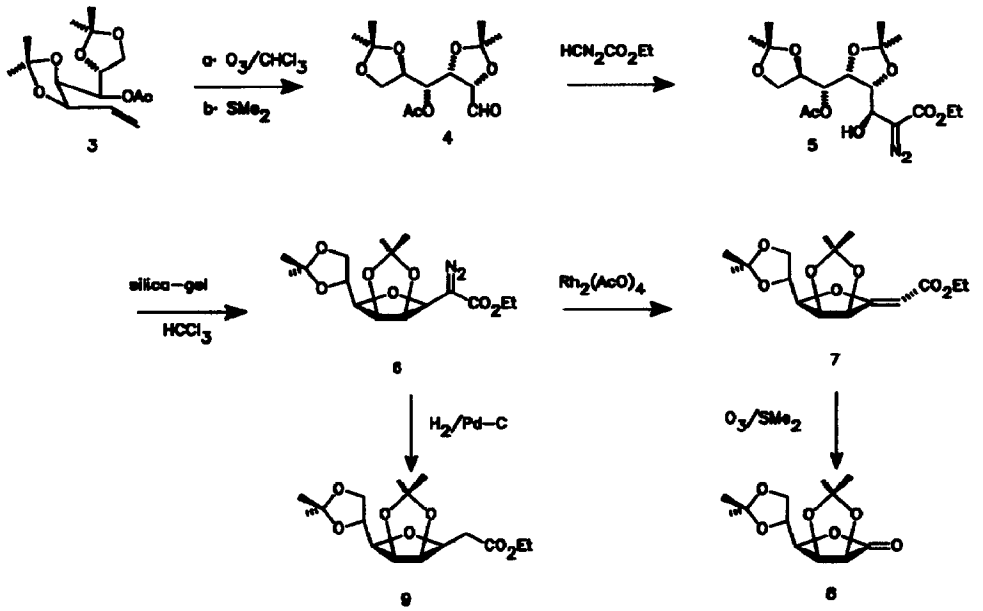
3-Deoxy-D-manno-2-octulosonic acid (KDO,**1**) is a higher monosaccharide present in lipopolysaccharides in the outer membrane of all Gram-negative bacteria[1]. KDO appears to be an essential component in the biosynthesis of LPS and hence, essential for Gram-negative bacteria to grow. The CMP-KDO synthetase is the enzyme responsible for incorporation of KDO to LPS, so, inhibitors of this enzyme would emerge as a new class of antibiotics against Gram-negative bacteria. These preliminary findings have fostered the search for KDO-analogue synthesis (Deoxy derivatives[2], Aza- or Carbo-cyclic analogues[3] and side or terminal chain modified derivatives[4]). Of all the analogues synthesized so far, the 2-deoxy KDO **2** is one of the most potent inhibitors of CMP-KDO synthetase[5]. In connection with previous studies on the chemistry of β-oxy-α-diazo carbonyl compounds carried out by our research group[6], we recently developed a synthesis for natural 3-deoxy-2-keto aldonic acids (KDO and DAH)[7] by aldolic condensation between monosaccharides in their aldehyde form and ethyl diazoacetate, and subsequent rhodium (II)-mediated rearrangement of the acetylated condensations products. Following these studies, in this work we developed an efficient synthesis for **2** via a novel route based on diazo chemistry methodology.

This new synthesis starts with condensation of the D-manno aldehyde **4** (synthesized from 5-O-acetyl-1,2-dideoxy-3,4:6,7-di-O-isopropylidene-D-manno-hept-1-enitol **3**[8] by ozonolysis) with ethyl diazoacetate in the absence of solvent and catalyst to yield the β-hydroxy-α-diazo ester **5** in a high yield and complete stereoselectivity. Further purification of **5** on silica gel converts the product into the furanoside derivative **6**, as a single isomer[9]. The structure of **6** was checked by rhodium-catalysed decomposition to give the Z olefin **7**[10], and subsequent ozonolysis of **7** to the known manno lactone **8**[11]. In order to elucidate the absolute configuration of the *anomeric* centre of **6**, this compound was hydrogenated (Pd-C) yielding quantitatively the known β-furanose **9**[12] (Scheme 2). To prevent cyclization to **6**, the crude product **5** was directly acetylated to give **10**[13] (93% from **4**). Although the absolute configuration of **5** at C-3 is irrelevant since this chiral centre is lost in the subsequent steps, the synthesis of **6** allowed us to establish the absolute configuration of this chiral centre. The retention of the manno configuration may be assigned to acetyl migration of the hydroxyl group at C-6 to free hydroxyl at C-3 and subsequent nucleophilic displacement of the acetate by the free hydroxyl at C-6. This intramolecular

reaction is possible if the diastereoisomer formed in the aldol condensation possesses a 3-(S) configuration. The results of the conformational analysis of the starting aldehyde by Chem-X calculations were consistent with the complete stereoselectivity and the stereochemistry observed in the addition step.

SCHEME I

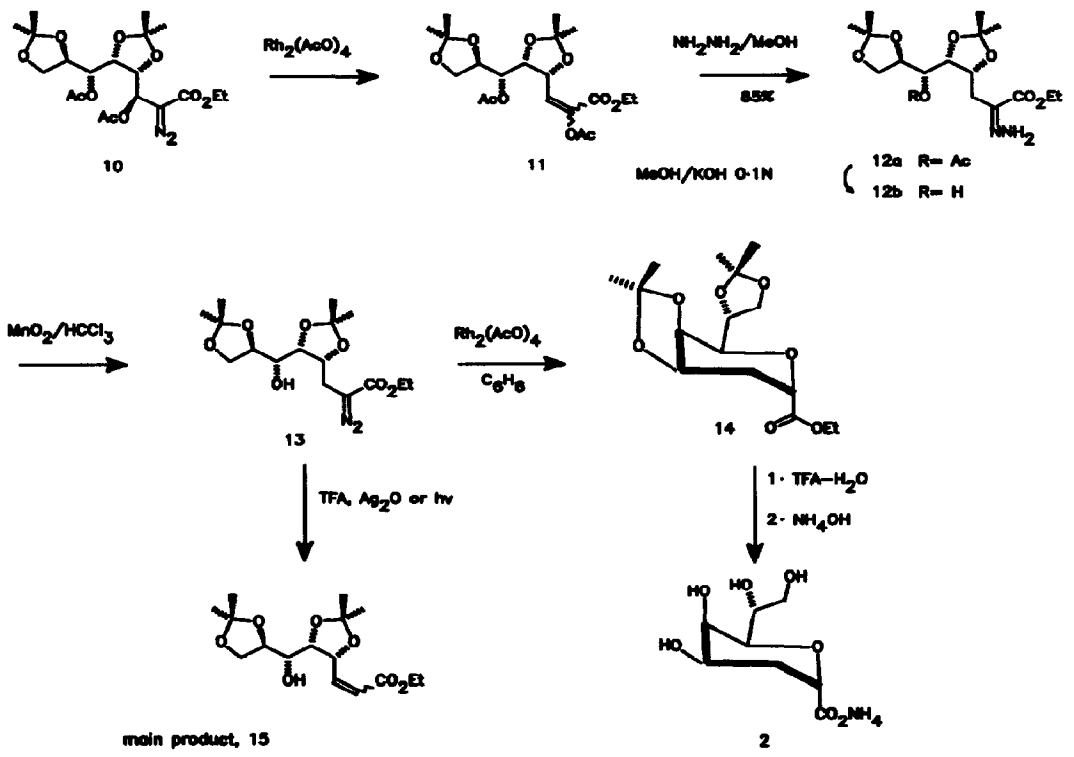
SCHEME II



Treatment of the acetylated product **10** with dirhodium tetraacetate in chloroform yielded the enol-acetate ester **11** quantitatively as both Z:E olefins. Treatment of **11** with a 1M methanol solution of hydrazine provided the hydrazone **12a** in high yield (84%) and subsequent acetate hydrolysis (MeOH-KOH 1N) gave the hydrazone **12b** quantitatively. **12b** was obtained directly from **11** in a similar yield by reaction with excess 1M hydrazine. Finally, oxidation of the hydrazone **12b** with manganese dioxide in chloroform yielded the diazo compound **13**[14] in a 98% yield. Similarly, **13** was obtained in a quantitative yield from **12a** by the oxidation-hydrolysis sequence. In previous work we used the reaction of **13** with m-CPBA to obtain the di-O-isopropylidene KDO derivative in a 92%yield. Likewise, **13** is a key product in the synthesis of the 2-deoxy KDO by intramolecular OH trapping of the carbenoid species produced from the diazo carbon. Rhodium (II) appears to be the most efficient catalyst for this type of reactions[15]. Thus, treating **13** with a catalytic amount of dirhodium tetraacetate in benzene effected its decomposition in a few minutes, with N_2 release. As expected, a single product was obtained that was

characterized as the 2-deoxy KDO **14** by comparison with NMR data and specific rotation given in the literature[2e]. Decomposition of this diazo compound **13** by use of other reagents (UV light, silver dioxide, acids) provided the 1,2-hydrogen shifted derivative **15** as the main product, together with a small amount of the cyclic compound **9**. The insertion product **14** or its β-anomer were detected in a low yield when **13** was decomposed by acids (acetic acid, TFA), but not in the silver oxide decomposition or photolysis. Finally, acetal hydrolysis of **14** and saponification of the ester group with ammonia provided the ammonium salt of the α-2-Deoxy-KDO, **2**[16] in quantitative yield (Scheme 3).

SCHEME III



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REFERENCES AND NOTES

1. Unger, F. M. *Adv. Carbohydr. Chem. Biochem.*, **1981**, *38*, 323.

2. [a] Luthman, K.; Orbe, M.; Waglund, T. and Claesson, A. *J. Org. Chem.*, **1987**, *52*, 3777; [b] Claesson, A. *J. Org. Chem.*, **1987**, 52, 4414; [c] Luthman, K. and Claesson, A. *Carbohydr. Res.*, **1987**, *166*, 233; [d] Shing, T. K. M. *Tetrahedron Lett.*, **1992**, *33*, 1307; [e] Boons, G. J. P. H.; van Delft, F. L.; van der Klein, P. A. M.; van der Marel, G. A. and van Boom, J. H. *Tetrahedron*;

1992, *48*, 885.

3. [a] Molin, H and Pring, B. G. *Tetrahedron Lett.*, **1985**, *26*, 677; [b] Norbeck, D. W. and Kramer, J. B. *Tetrahedron Lett.*, **1987**, *28*, 773.
4. [a] Norbeck, D. W.; Kramer, J. B. and Lartey, P. A. *J. Org. Chem.* **1987**, *52*, 2174; [b] Tadanier, J.; Lee, C. M.; Whittern, D. and Wideburg, N. *Carbohydr. Res.*, **1990**, *201*, 185; [c] Tadanier, J.; Lee, C. M.; Hengeveld, J.; Rosenbrook Jr., W.; Whittern, D. and Wideburg, N. *Carbohydr. Res.*, **1990**, *201*, 209.
5. [a] Claesson, A.; Luthman, K; Gustafsson, K. and Bondesson, G. *Biochem. Biophys.Res. Commun.*, **1987**, *143*, 1063; [b] Goldman, R.; Kohlbrenner, W.; Lartey, P. and Pernet, A. *Nature*; **1987**, *329*, 162.
6. [a] López-Herrera, F. J. and Sarabia-García, F. *Tetrahedron Lett.*, **1993**, *34*, 3467; [b] López-Herrera, F. J. and Sarabia-García, F. *Tetrahedron Lett.*, **1994**, *35*, 2929.
7. López-Herrera, F. J. and Sarabia-García, F. *Tetrahedron Lett*, precedent paper.
8. Manno-aldehyde **4** was synthesized from 2,3:5,6-di-O-isopropylidene-D-mannofuranose in three steps: Wittig reaction with methyltriphenylphosphorane and acetylation to compound **3**, reported by Haudrechy, A. and Sinaÿ, P. *J. Org. Chem.*, **1992**, *57*, 4142, and ozonolysis of **3**. This avoids the time-consuming synthesis of the 4-O-tButyldimethylsilyl analogue (the aldehyde employed in the previous work), which involves 6 steps from 2,3:5,6-di-O-isopropylidene-D-mannofuranose. Moreover, **4** was more reactive than the 4-O-silyl analogue and exhibited a high stereoinduction in the condensation step.
9. Product **6**: $[\alpha]_D^{20}$ +32.51° ($CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ ppm; 4.72 (m, 2H, H-4, H-5); 4.39 (d, 1H, $J_{3,4}$ = 2.4 Hz, H-3); 4.33 (ddd, 1H, $J_{7,8}$= 5.1Hz, $J_{7,8}$= 5.5 Hz and $J_{7,6}$= 7.5 Hz, H-7); 4.16 (q, 2H, J = 7.2 Hz, -OCH_2-); 4.03 (dd, 1H, $J_{8,7}$= 5.5 Hz, and $J_{8,8'}$ = 8.7 Hz, H-8); 3.97 (dd, 1H, $J_{7,8'}$= 5.1 Hz and $J_{8,8'}$= 8.7 Hz, H-8'); 3.47 (dd, 1H, $J_{6,5}$ = 2.4 Hz and $J_{6,7}$= 7.5 Hz, H-6); 1.38 (s, 6H, CMe_2); 1.31 and 1.27 (2s, 6H, CMe_2); 1.20 (t, 3H, J = 7.2 Hz, -$CO_2CH_2\underline{CH_3}$). Elemental Analysis: Calcd for $C_{16}H_{24}O_7N_2$, 53.93% C, 6.79% H, 7.86% N; found: 53.58% C, 6.96% H, 8.09% N.
10. Hürzeler, M.; Bernet, B.; Mäder, T. and Vasella, A. *Helv. Chim. Acta*, **1993**, *76*, 1779.
11. Horton, D. and Jewell, J. S. *Carbohydr. Res.*, **1966**, *2*, 251.
12. Ohrui, H.; Jones, G. H.; Moffat, J. G.; Maddox, M. L.; Christensen, A. T. and Byram, S. K. *J. Am. Chem. Soc.*., **1975**, *97*, 4602.
13. Product **10**: $[\alpha]_D^{20}$ +2.81° ($CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ ppm: 5.43 (dd, 1H, $J_{6,5}$ = 1.9 Hz and $J_{6,7}$= 5.9 Hz, H-6); 4.57 (d, 1H, $J_{3,4}$= 10 Hz, H-3); 4.43 (dd, 1H, $J_{5,6}$= 1.9 Hz, and $J_{5,4}$= 6.1 Hz, H-5); 4.27-4.14 (m, 4H, H-6, H-4, -$CO_2\underline{CH_2}CH_3$); 3.99 (dd, 1H, $J_{8,8'}$= 8.7 Hz and $J_{8,7}$= 6.2 Hz, H-8); 3.90 (dd, 1H, $J_{8,8'}$= 8.7 Hz and $J_{8',7}$= 6.7 Hz, H-8'); 2.10 (s, 3H, -OCOMe); 1.45 and 1.36 (2s, 6H, CMe_2); 1.33 (s, 6H, CMe_2); 1.25 (t, 3H, J = 7.1 Hz, -CO2$CH_2\underline{CH_3}$). Elemental Analysis: Calcd for $C_{20}H_{30}O_{10}N_2$, 52.38% C, 6.60% H, 6.11% N; found: 52.11% C, 6.54% H, 6.10% N.
14. Product **13**: $[\alpha]_D^{20}$ +30.55° ($CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ ppm: 4.39 (ddd, 1H, $J_{4,3'}$ = 3.3 Hz, $J_{4,5}$= 6.9 Hz and $J_{4,3}$= 9.2 Hz, H-4); 4.30 (dd, 1H, $J_{5,6}$ = 1.7 Hz and $J_{5,4}$ = 6.9 Hz, H-5); 4.20 (q, 2H, J = 7.2 Hz, -OCH_2-); 4.09 (dd, 1H, $J_{8,7}$ = 8.6 Hz and $J_{8,8'}$= 10.5 Hz, H-8); 3.98 (dt, 1H, $J_{7,8}$ and $J_{6,7}$ = 8.6 Hz and $J_{7,8'}$ = 2.5 Hz, H-7); 3.96 (dd, 1H, $J_{8',7}$ = 2.5 Hz and $J_{8',8}$= 10.5 Hz, H-8'); 3.50 (dt, 1H, $J_{6,7}$ and $J_{6,\text{-OH}}$ = 8.6 Hz and $J_{6,5}$ = 1.7 Hz, H-6); 2.72 (dd, 1H, $J_{3,4}$ = 9.2 Hz and $J_{3,3'}$ = 14.9 Hz, H-3); 2.59 (dd, 1H, $J_{3',4}$ = 3.3 Hz and $J_{3,3'}$ = 14.9 Hz, H-3'); 2.24 (d, 1H, $J_{6,\text{-OH}}$ = 8.6 Hz, -OH); 1.43, 1.38, 1.33 and 1.31 (4s, 12H, 2 CMe_2); 1.24 (t, 3H, J = 7.2 Hz, -$CO_2CH_2\underline{CH_3}$). Exact mass calcd for $C_{16}H_{26}O_7N_2$ -15: 343.1505; found: 343.1526.
15. Adams, J. and Spero, D. *Tetrahedron*; **1991**, *47*, 1765 and references therein.
16. ^{1}H-NMR data of **2** (D_2O) δ ppm: 4.46 (dd, 1H, $J_{2,3}$ = 1.1 Hz and $J_{2,3'}$ = 6.5Hz, H-2); 4.05 (d, 1H, $J_{4,5}$ = 2.8 Hz, H-5); 3.94 -3.84 (m, 3H, H-7, H-8, H-8'); 3.78 (ddd, 1H, $J_{4,5}$ = 2.9 Hz , $J_{3,4}$ = 5.2Hz, $J_{4,3'}$ = 12.3 Hz, H-4); 3.61 (d, 1H, $J_{6,7}$ = 8.3 Hz, H-6); 2.27 (ddd,1H, $J_{2,3}$ = 1.1 Hz , $J_{3,4}$ = 5.2Hz, $J_{3,3'}$ = 12.3 Hz, H-3); 2.10 (ddd, 1H, $J_{2,3'}$ = 6.5 Hz , $J_{3,3'}$ = $J_{3,4}$ = 12.3 Hz, H-3').^{13}C-NMR (D_2O) δ ppm: 179.5 (C-1);75.91, 75.65, 70.88, 68. 44, 68.06, 65.59 (C-4, 5, 6,7, 8) and 30.0 (C-3). m. p. = 179 °C, $[\alpha]_D^{20}$ +68.6° (c 1.02, H_2O); lit[5a] m. p. = 181-187 °C, $[\alpha]_D^{20}$ +70.0° (c 1.68, H_2O).

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