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Preparation of the Phosphonic Acid Analogue of 3-Deoxy-D-Manno-2-Octulosonic Acid (KDO)

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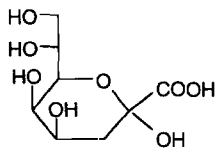
Abstract : Synthesis of phosphonic acid analogue of KDO has been obtained by an efficient coupling of 2,3:5,6-di-O-isopropylidene-D-mannitol triflate with lithiated diethyl formyl phosphonate anion equivalent followed by subsequent deprotecting steps.

In a preceding paper we described a new efficient synthesis for 3-deoxy-D-manno-2-octulosonic acid (KDO) which is an integral component of lipopolysaccharides (LPS) from cell walls of Gram-negative bacteria.[1] In LPS, a KDO moiety connects the lipophile lipid A to the antigenic inner-core saccharide region via a ketosidic bond.

The rate limiting step in the biosynthetic incorporation of KDO to lipid A is catalysed by CMP-KDO synthetase and appears to be a vital step in LPS biosynthesis, and then, in growth of Gram-negative bacteria.[2] As a result, the preparation of KDO analogues as potent inhibitors of this enzyme represents an important target for the design of successful new antibacterial agents.[3]

In an attempt to prepare such a compound we described here a synthesis of the phosphonic acid analogue **2** of KDO **1**.



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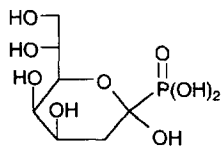


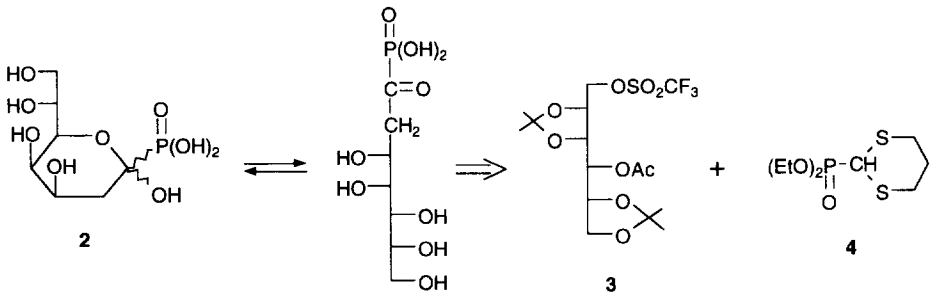
2

The preparation of this phosphonic acid **2** seems interesting for the following reasons :
- the replacement of the carboxylic acid function of **1** by a most hydrophilic phosphonic acid moiety has to enhance uptake of the hydrophilic outer cell membrane of Gram-negative bacteria
- on the other side, activation of KDO with cytidine triphosphate catalysed by CMP-KDO synthetase needs a divalent cation as Mg^{2+}.[2] A strong chelating α-hydroxyphosphonic acid such as **2** has to compete with cytidine triphosphate towards Mg^{2+} in this activation step.

The dihalogeno acetate anions Darzens methodology previously used to install an α-ketoester moiety onto a suitable protected D-mannose in the key step of our synthesis of KDO cannot be transposed to the preparation of the phosphonic acid analogue **2**.[1] Diethyl dihalogenomethylphosphonate anions are known,

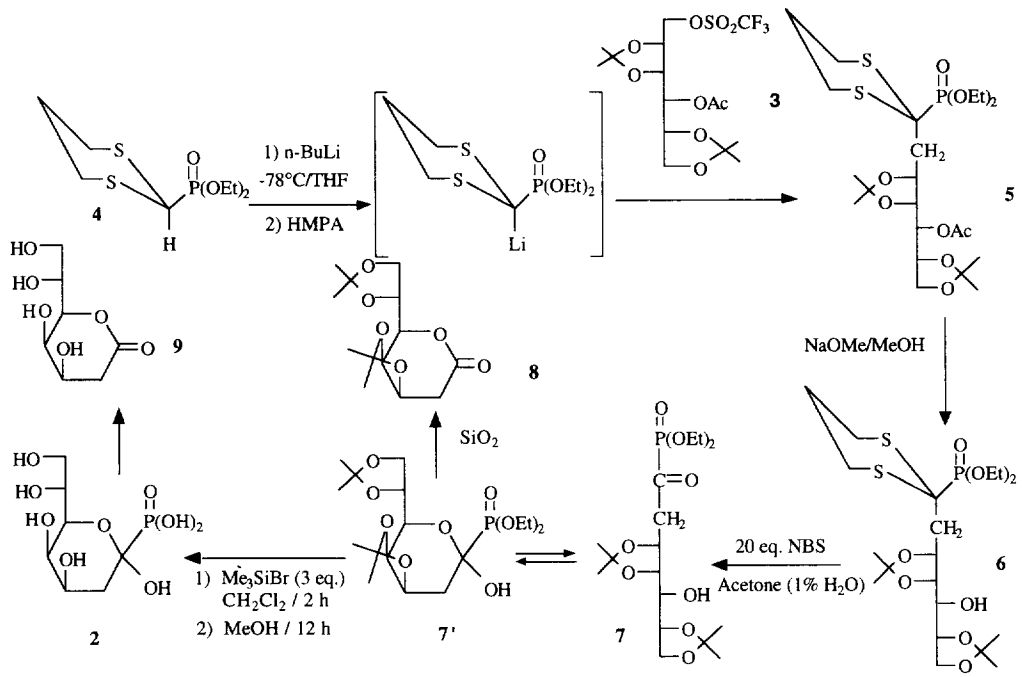
indeed, only as Horner reagents and lead to gem dihalogeno olefins when they react with carbonyl compounds.[4]

To overcome the problem we studied another approach based on the previous synthesis of KDO using a nucleophilic displacement of a triflate[5] at C-1 of the D-mannitol derivative **3** by the diethyl formylphosphonate anion equivalent derived from **4**. The advantage of such a strategy should be the exclusive formation of the required D-manno configuration without the epimerisation which is observed in routes to KDO via an aldol [1,6] or Wittig-type connection,[7] leading to the formation of D-gluco configuration as a by-product.



The dithianephosphonate **4** was obtained in two steps from 1,3-dithiacyclohexane by monochlorination with N-chlorosuccinimide (24h stirring in benzene at room temperature) followed by reaction with triethylphosphite during 4h at 60°C (67%, $Eb_{0.01}$=120-125°C).[8] Triflate **3** was obtained in five steps from 2,3 : 5,6-di-O-isopropylidene-D-mannose according to the Shiba *et al* synthetic route (76% overall yield).[5] It was used immediately for the next reaction with the anion derived from **4**.

The lithiated anion derived from **4** is known and has been used for the synthesis of ketene S,S-thioacetals.[9] After metalation of **4** (1mmol in 2 ml of THF) with n-butyllithium (0.8 ml, 1.6 M in hexane) at -78°C during 30 mn, HMPA (0.8 ml) was added followed by addition of triflate **3** (1mmol in 3 ml of THF).The reaction medium became blood-red. It was stirred for 1h at -78°C and then allowed to warm to 0°C before hydrolysis (10 ml H_2O). A classical work up afforded crude dithioketalphosphonate **5** as an oil, which was purified by silica gel column chromatography (ethyl acetate/petroleum ether 1/1, Rf = 0.45) (65% yield). Removal of the acetoxy protecting group (0.1 M NaOMe/MeOH at room temperature for 15h) gave **6** (84% yield after purification on silicagel column chromatography, ethyl acetate/petroleum ether 1/1, Rf = 0.38). As a consequence of the great sensitivity towards nucleophiles of the C-P bond in α-ketophosphonate **7**, subsequent cleavage with NBS of the dithioketal **6** into **7** was a crucial step that needed adapted conditions. It was absolutely necessary to minimise the amount of water in the reaction medium whereas a large excess of NBS (20 eq.) in wet acetone (1% H_2O) was used. The α-ketophosphonate **7** so-formed immediately cyclised *in situ.* into **7'** and was isolated as a crude colourless oil. Phosphonate **7'**, indeed, degraded during purification on silica gel column chromatography and gave lactone **8** accompanied of diethyl phosphite as a result of the lability of the C-P bond.



Thus, phosphonate **7'** was characterized on the crude product. One single stereomer was formed and assigned to α-configuration on the basis of the ^{1}J C-P and ^{3}J C-P value coupling constants[10] (91% yield, Rf = 0.32 ethyl acetate / petroleum ether : 1/1. NMR ^{1}H $CDCl_3$ 4.81-4.70 (m, 1 H, H5) ; 4.45-4.36 (m, 1 H, H3) ; 4.32-4.25 (m, 1 H, H6) ; 4.21-4.06 (m, 5 H, H4, OCH_2) ; 4.05-3.97 (m, 2 H, H7) ; 2.10-1.80 (m, 3 H, H2, OH) ; 1.43 ; 1.36 (s, s ; 6 H ; $-C(CH_3)_2$) ; 1.30 ; 1.27 (s , s, 6 H ; $-C(CH_3)_2$) ; 1.30 (t, 6 H, ^{3}J = 7Hz ; OCH_2CH_3). NMR ^{13}C ($CDCl_3$) 109.5 ; 109.3 ($C(CH_3)_2$) ; 96.0 (d, ^{1}J C-P = 210 Hz, C1) ; 74.6 (C4) ; 71.0 (C6) ; 69.6 (d, ^{3}J C-P = 9 Hz, C5) ; 68.9 (d, ^{3}J C-P = 11 Hz, C3) ; 67.0 (C7) ; 64.3 (d, ^{2}J C-P = 6 Hz, OCH_2) ; 63.6 (d, ^{2}J C-P = 6 Hz, OCH_2) ; 32.8 (d, ^{2}J C-P = 7 Hz, C2) ; 28.2 ; 27.1 ($-C(CH_3)_2$)) ; 26.6 ; 26.1 ($-C(CH_3)_2$) ; 16.7 (OCH_2CH_3) ; 16.6 (OCH_2CH_3) . NMR ^{31}P ($CDCl_3$) 13.6.

Finally, simultaneous removal of the isopropylidene and diethyl phosphonic ester groups were carried out in a one step sequence involving the reaction between **7'** and bromotrimethylsilane (3 eq., 2 h in dichloroethane) followed by methanolysis with a small amount of methanol to prevent the break of the C-P bond (7 eq. ; 12 h at room temperature).[11] In these conditions, and after vacuum evaporation, the phosphonic acid **2**, analogue of KDO, was obtained as a white pasty solid, which was purified by washings with ether (60% yield). Only one stereomer was also observed as indicated by a singlet in ^{31}P NMR spectrum ; NMR^{31}P (acetone-d^6) 16.3 ; NMR ^{1}H (acetone-d^6) 4.85-4.75 (m, 1 H, H5) ; 4.40-4.30 (m, 1 H, H3) ; 4.20-4.00 (m,2H, H4, H6) ; 3.98-3.90 (m, 2 H, H7) ; 2.12-1.98 (m, 2 H, H2). NMR ^{13}C (acetone-d^6) 79.5 (C5) ; 76.5 (C6) ; 71.8 (C3) ; 69.2 (C4) ; 66.0 (C7) ; 38.1 (^{2}J C2-P= 5Hz, C2).

It should be noted that phosphonic acid **2**, analogue of KDO, was fragile and slowly degraded, even at -15°C, to yield lactone **9** and H_3PO_3 (50% degraded after three weeks at -15°C).

The synthesis of the phosphonic acid **2**, analogue of KDO, can be thus obtained by an efficient coupling of diisopropylidene mannitol triflate with lithiated formyl phosphonate anion equivalent followed by subsequent deprotecting steps. However this compound **2** and its precursor diisopropylidene α-ketophosphonate **7'** are relatively less stable than analogues of the KDO series as a consequence of the particular lability of the C-P bond in the α-ketophosphorylated species compared to the α-ketocarboxylic acid derivatives.

References and Notes

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[11] The silylation step was monitored by ^{31}P NMR spectroscopy. After 2h, the characteristic singlet of **7'** at 13.6 ppm has disappeared on behalf of a singlet at 4.6 ppm. This last signal proves the transformation of diethyl phosphonate **7'** into ditrimethylsilyl phosphonate. However it has not been possible to state at this stage of the reaction if the isopropylidene groups are still presents.

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