Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 949–952

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Investigation of New Potent KDO-8-Phosphate Synthetase Inhibitors

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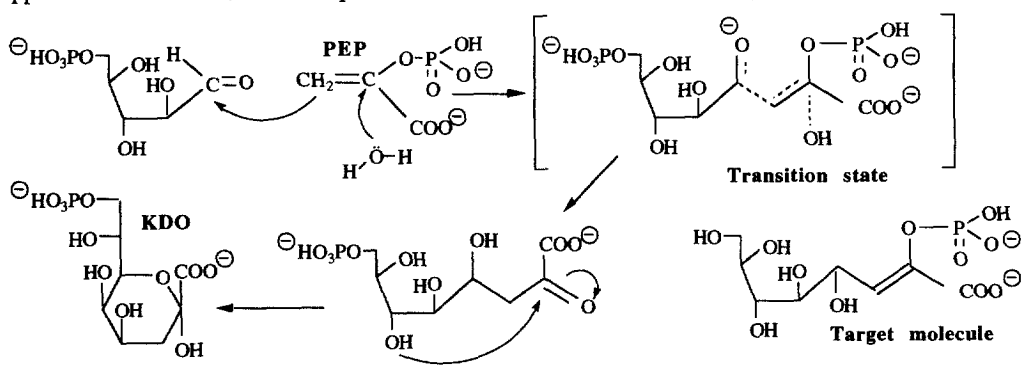
Received 17 December 1998; accepted 17 February 1999

Abstract : A total synthesis of (*Z,E*)-D-Glucophosphoenolpyruvate and its carboxylic ester derivatives is described in four or five steps from 2,3 : 5,6-Di-*O*-isopropylidene-4-*O*-*t*-butyldimethylsilyl-D-glucose

Incorporation of 3-deoxy-D-*manno*-2-octulosonic acid (KDO) to lipopolysaccharides appears to be a vital step in LPS biosynthesis and in growth of Gram-negative Bacteria.[1] As a result, the inhibition of KDO biosynthetic pathway has became an attractive goal in the research of new efficient antibacterial agents. Whereas most of works are relative to the inhibition of CMP-KDO synthetase that catalyses the fourth step of the biosynthetic pathway,[2] we have preferred to explore the possible inhibition of KDO-8-phosphate synthetase that catalyses the second step of the KDO biosynthetic route.[3]

In this step KDO-8-phosphate is formed in the reaction between D-arabinose-5-phosphate and phospho-enolpyruvate (PEP). It has been shown that the reaction mechanism implicates a cleavage of the C-O phosphate bond after an initial attack of water on the double bond and simultaneous reaction with the aldehyde (scheme 1).[4] That led us to consider a target molecule with a D-*gluco* configuration, analogous to the bisubstrate and close of the supposed transition state, that can represent an excellent mimic and therefore a potent competitive analog.



Scheme1

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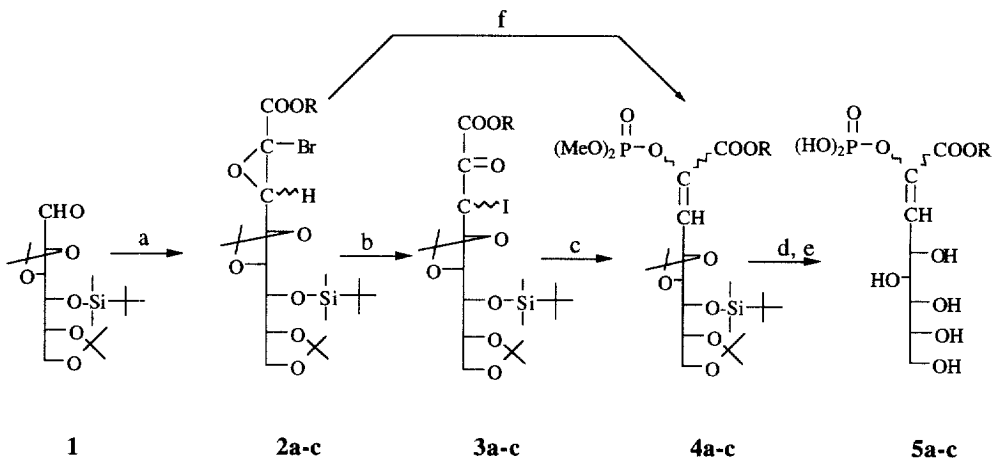
PII: S0960-894X(99)00111-0

The strategy we propose to obtain such a target molecule with a phosphoenolpyruvate moiety linked to five consecutive hydroxylated carbon atoms of D-*gluco* configuration is based on a Perkow reaction using a suitable β-halogeno-α-ketoacid derivative precursor and trialkylphosphite.[5]

Previously, we have described the usefulness of the potassium dihalogenoacetate anion methodology combined with the mild opening of α-halogenoglycidic esters with Lewis acids to introduce directly a β-halogeno-α-keto ester moiety on a carbonyl compound.[6] In the present case the application of this strategy to the suitable protected D-glucose **1** has to lead to the required β-iodo-α-ketoester precursor **3**. Subsequent reaction of this last compound with a trialkylphosphite has to give the protected target molecule **4**.



a) $CHBr_2COOR$, 2 equiv., ROK, 2 equiv. (R=Me, ROH/Et_2O : 1/20; **2a,** 70% yield, diastereomeric ratio : 47/53 ; R=iPr, ROH/Et_2O : 1.5/1; **2b,** 83% yield, diastereomeric ratio: 48/52 ; R=tBu, ROH/Et_2O : 5/3, **2c**, 92% yield, diastereomeric ratio : 45/55) b) MgI_2, 2 equiv., -15°C (R=Me, Et_2O/Toluene : 3/2; R=iPr, Et_2O/Toluene : 6/1; R=tBu, Et_2O/Toluene : 3/1) c) MeO_3P, 1 equiv., Et_2O, 20°C, 15mn, **4a**, 49% yield, E/Z : 73/27 ; **4b**, 58% yield, E/Z : 98/2 ; **4c**, 45% yield, E/Z : 92/8 d) Me_3SiBr, 2 equiv./dichloroethane (R=Me, 20°C, 2h, R=iPr, 20°C, 2h, R=tBu, 20°C, 4h) e) MeOH, 20°C, 48h, **5a**, R=Me, 100% yield, E/Z : 73/27 ; MeOH, 20°C, 72h, **5b**, R=iPr, 100% yield, E/Z : 98/2 ; **5c**, R=H, 100% yield, E/Z : 98/2 f) MeO_3P, 1 equiv., Pyridine, 1 equiv./Toluene, 15mn, **4a**, 82% yield, E/Z : 52/48, **4b**, 74% yield, E/Z : 66/34

The aldehyde precursor **1** derived from D-glucose with suitable protections compatible with the Darzens reaction conditions was previously prepared in four steps.[7] Darzens reaction was then realized, with the anion derived from alkyl dibromoacetate (alkyl = Me, iPr, tBu) using ROK as base, in ROH-ether as solvent (R=Me, iPr, tBu) leading to α-bromoglycidic ester **2** (**2a**, R=Me, 70% yield, diastereomeric ratio : 47/53; **2b**, R=iPr, 83% yield, diastereomeric ratio : 48/52; **2c**, R=tBu, 92% yield, diastereomeric ratio : 45/55). It was noteworthy that these reactions were not stereoselective with the aldehyde **1** derived from D-glucose whereas in analog experimental conditions a better stereoselectivity was observed with the epimer derived from D-mannose.[6b] Subsequent treatment of the diastereomeric mixture **2** with magnesium iodide led to the opening of the bromoepoxy ring and gave α-iodoketoester diastereomers **3** which were then treated with trimethylphosphite in

ether at room temperature. The reaction was immediate and the trimethylphosphite was entirely consumed after 15 min, leading to the expected phosphoenolpyruvate derivative **4** (**4a**, 49% yield, *E/Z* : 73/27; **4b**, 58% yield, *E/Z* : 98/2; **4c**, 45% yield, *E/Z* : 92/8). The *E* and *Z* stereomers were assigned on the basis of the greater *trans* ^4J P-H value coupling than *cis* value coupling.[8] The observed strong *E* stereoselectivity was in agreement with the Borowitz model suggested for the Perkow reaction[9] with a marked influence of the steric hindrance of the ester. The relatively modest observed yields for the two steps transformation of **2** into **4** that needed the passing across the unstable α-iodoketoester **3** led us to investigate a more direct route. We found that the reaction between the bromoglycidic ester **2a** and trimethylphosphite, in the presence of pyridine,[10] was very convenient and directly afforded the phosphoenolpyruvate **4a** in a good yield, but, however, without stereoselectivity (82% yield, *E/Z* : 52/48). Similarly this reaction applied to **2b** gave **4b** also with a decrease in the stereoselectivity (74% yield, *E/Z* : 66/34).

Finally, simultaneous removal of the isopropylidene and ester groups was attempted in a two steps sequence involving the reaction between **4** and bromotrimethylsilane followed by methanolysis, as we have previously described.[2a] In these conditions, **4c** directly afforded the target molecule **5c** entirely deprotected (quantitative yield, *E/Z* : 92/8). In the same conditions, only the methyl carboxylic ester group into **4a** and the isopropyl carboxylic ester group into **4b** subsisted, whereas all other protected groups were removed, leading respectively to **5a** (quantitative yield, *E/Z* : 73/27) and **5b** (quantitative yield, *E/Z* : 98/2) . Subsequent alkaline hydrolysis with KOH/MeOH 1M was only possible with **5a** and gave the free target molecule **5c** whereas various attempts to hydrolyse the isopropyl ester in **5b** did not succeed and led to degraded products.

Since the molecules **5a-c** represented the target PEP analogues and appeared to be set for an intramolecular enzymatic cyclisation it was been verified that these molecules were chemically stable as well in acidic medium (they were unchanged after 48h to 72h in methanol at pH 2) as basic conditions (no significative degradation was observed after heating at 60°C at pH 10 during 3h). Moreover, they could be stored during several months at -30°C. Biological tests were effected with compounds **5a**, **5b**, and **5c** on three Gram-negative Bacteria, *Escherichia coli, Yersinia enterocolitica and Pseudomonas aeruginosa* and on Gram-positive *Staphylococus aureus*. Neither inhibitor effect was observed on the bacterial growth at the maximal tested concentration (500μg/ml). However, in the presence of **5b**, Gram-negative strains presented a marked mucous aspect whereas without **5b** the same strains presented a smooth aspect that was not observed with *Staphylococus aureus*. Otherwise, *Pseudomonas aeruginosa* did no more synthesize pyoverdine in the presence of **5b**. Neither cytotoxic activity[11] on human fibroblastic cells of **5b** was observed, up to 500μg/ml. All these observations seem indicate a difficult membrane uptake with **5a** and **5c** with a slight better membrane penetration in the case of **5b**[12] which was the most lipophilic compound among the three tested. As a consequence, the observed mucous aspect could be the result of polysaccharide accumulation caused by KDO inhibition. The oligosaccharidic core of the LPS could not be linked to lipid A and therefore could not participated to a correct LPS biosynthesis. Further biological studies are under investigation to precise these first observations and hypotheses.

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7. **1** was prepared from 2,3 : 5,6-di-*O*-isopropylidene-D-glucose propane-1,3-diyl dithioacetal according to : Kölln,O.; Redlich, H.; Frank, H. *Synthesis* **1995**, 1383. Protection of the 4-hydroxyl with *t*-butyldimethylsilyl chloride and subsequent cleavage of the dithioacetal with NBS gave **1**.

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12. Analytical data of **5b** : Major (*E*) : ^{1}H NMR (250 MHZ, D_2O), δ (ppm) = 6.4 (dd, 1H, *J* =2.0 Hz, *J* =7.5 Hz) ; 5.1 (m, 1H, *J* =6.0 Hz) ; 4.9 (m, 1H) ; 4.4.3-3.5 (m, 5H) ; 1.3 (d, 6H, *J* =6.0 Hz) ^{13} C NMR (62.5 MHZ, C_3D_6O), δ (ppm) = 161.0, 138.2, 127.5, 80.2, 76.7, 75.6, 68.1, 67.1, 64.2, 24.7, 24.0 ^{31} P NMR (101.25 MHz, C_3D_6O), δ (ppm) = –2.41. Minor (*Z*) : ^{1}H NMR (250 MHZ, D_2O), δ (ppm) = 6.2 (dd, 1H, *J* =2.5 Hz, *J* =7.5 Hz) ; 5.6-5.5 (m, 1H). Other signals were situated under those of major (*E*). ^{13} C NMR (62.5 MHZ, C_3D_6O), δ (ppm) = 160.8, 140.7, 124.7, 78.6, 76.9, 76.3, 69.1, 66.9, 64.3, 23.2, 22.4 ^{31} P NMR (101.25 MHz, C_3D_6O), δ (ppm) = –2.1. MS (ESI) : *m/z:* 359 (M-H)$^-$.