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Novel Chemo-Enzymatic Synthesis of Optically Active Platelet Activating Factor[1]

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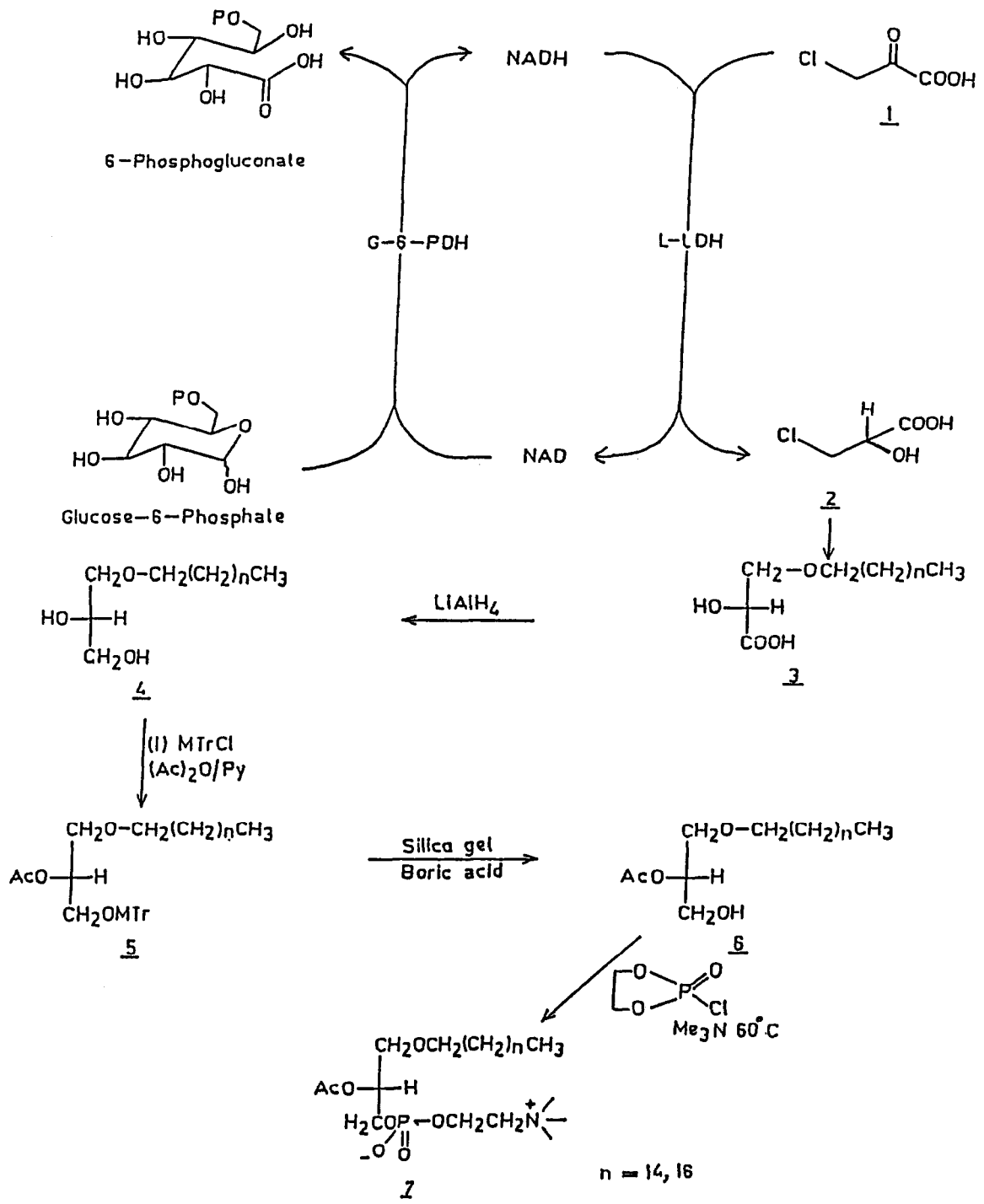
Abstract : This paper describes an enantioselective enzymatic synthesis of biologically active platelet activating factor (PAF) starting from chloropyruvic acid.

Platelet activating factor (PAF), 2-acetyl glyceryl ether phosphorycholines are chemically members of phospholipid family. They have attracted considerable interest principally owing to their pronounced biologically responses[2-5].

Present report describes a straight forward and shortest synthesis of PAF starting from chloropyruvic acid 1. Chloropyruvic acid on L-lactic dehydrogenase catalyzed reduction with NADH yields (L)-chlorolactic acid 2.

The conditions used to synthesize 2 have the following constrains : firstly, the reducing agent in the system NADH is very expensive to be used in stoichiometric radio. Secondly, the halopyruvic acid deactivates lactic dehydrogenase (presumably by alkylation). In order to overcome these constrains we have used a recylling system (glucose-6-phosphate/glucose-6-phosphate dehydrogenase). The deactivation of lactatedehydrogenase was minimized by using less reactive chlorpyruvic acid. The concentration of chloropyruvic acid was maintained close to K_m by slow addition[6].

L-chlorolactic acid was synthesized from chloropyruvic acid by the following method : A mixture of dithiothretol (3.6 mM), EDTA (1.4 mM), $MgCl_2$ (7.0 mM) and glucose-6-phosphate (0.51 mol) was taken and its pH adjusted at 7.6 with KOH. The solution was degassed and to it was added NAD (0.53 mmol) and aqueous suspension of (L)-LDH (2300 units) and G-6 PDH (280 units). The pH of the reaction was maintained in between 7.4-8 by addition of 1.0 M KOH solution. An aqueous


6-Phosphogluconate
NADH
G-6-PDH
L-LDH
1
Glucose-6-Phosphate
NAD
2
LiAlH4
3
4
(I) MTrCl
(Ac)2O/Py
5
Silica gel
Boric acid
6
Me3N 60°C
7
n = 14, 16

solution of chloropyruvic acid (1.0 M, maintained at 5°C) was added dropwise. A total of (0.5 mol) of chloropyruvic acid and (1.27 mmol) of NAD were added over the course of reaction. After 72 hrs the reaction mixture was washed with 0.05 M HEPES buffer (pH 7.5) separated by centrifugation and resuspended in fresh buffer. To this solution $BaCl_2$ was added followed by addition of 2 ml of ethanol. The precipitate formed (which is L-chlorolactic acid was filtered, washed and dried[6] mp 88-89°C $[\alpha]_D$ +4.14 (c, 10 g/100 H_2O).

L-chlorolactic acid on reaction with hexadecanol or octadecanol in presence of NaH gives 3[7] which on reduction with lithium aluminum hydride afforded diol 4. Diol on reaction with 4-methoxy tritylchloride (MTr-Cl) followed by acetylation of the free hydroxy group with acetic anhydride and pyridine gave 5. The selective removal of MTr-group (without migration of aceltyl group taking place) was done by passing a solution of 5 in pet ether through a short silica/boric acid column to yield 6. Phosphorylation of 6 with 2-chloro-2-oxo 1,3,2 dioxaphospholane in presence of trimethylamine in benzene[8] finally yielded the desired L-PAF 7[9-12].

References and Notes

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11. M.S.: n=14 M^+ 532; n=16 M^+ 567; m.p. : n=14, 248°C; n=16,265°C; $[\alpha]_D$ n=14 +3.50 (methanol); $[\alpha]_D$ n=16 +2.35 (methanol); IR (KBr) 1730, 1240, 1210 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.84 (t, 3H, ($\underline{CH_3}$), 1.20-1.50 (m 28H, ($\underline{CH_2}$)$_n$), 3.46 (t, 2H, O-$\underline{CH_2}$), 3.59 (d, 2H, $\underline{CH_2}$-O-CO) 2.05 (s, 3H, O-CO-$\underline{CH_2}$), 5.18 (q, 1H, $\underline{CH}$-oAc) 4.01 (m, 2H, $\underline{CH_2}$-O-P), 4.30 (bm, 2H, P-O-$\underline{CH_2}$), 3.75 (t, 2H, $\underline{CH_2}$-$\overset{+}{N}$-

$(CH_3)_3$, 3.36 (s, 9H, $-\overset{+}{N}(\underline{CH_3})_3$).

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