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SYNTHESIS OF PEPTIDYLGLYCOPHOSPHOLIPIDS, NOVEL DERIVATIVES OF MURAMYL-DIPEPTIDE[1]

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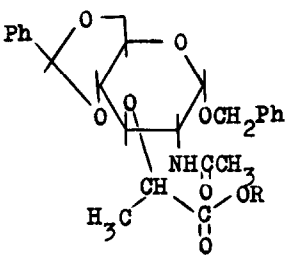
Abstract Synthesis of 6-deoxy-6-L-α-dipalmitoylphosphatidylethanolamine-N-acetylmuramyl-L-alanyl-D-isoglutamine-γ-methyl ester is described.

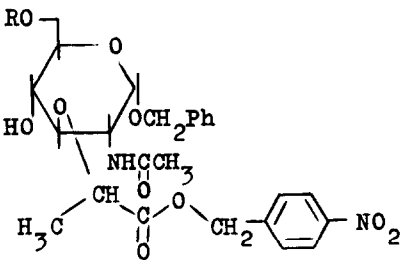
N-Acetylmuramyl-L-alanyl-D-isoglutamine (MDP) has been shown to be the minimal adjuvant-active structure capable of replacing whole mycobacterial cells in Freund's complete adjuvant (FCA) for increasing levels of humoral antibodies against a given antigen and for inducing delayed hypersensitivity[2,3]. It has been reported that owl monkeys are effectively protected against a human parasite after immunization with an appropriate antigen in liposomes containing 6-O-stearoyl-MDP, in a way similar to that obtained with FCA[4]. These observations prompted us to undertake the synthesis of MDP derivatives whose adjuvant activity may not require any oily vehicle, such as liposomes or mineral oil. This report describes the synthesis of one of such novel MDP derivatives in which phosphatidylethanolamine has been linked to C-6 of the 6-deoxy muramyl residue via C-N linkage (7).

1-O-Benzyl-4,6-di-O-benzylidine-N-acetylmuramic acid (1) was prepared following the published procedure[5]. Reaction of 1 with p-nitrobenzyltosylate[6] in anhydrous acetone, under reflux for 2 h, gave the nitrobenzyl ester 2 (m.p.162-163°C from $CHCl_3$-hexane; $[\alpha]_D^{25}$ + 13.1 (C 1.2, $CHCl_3$); ν_{max}(KBr): 1750 and 1640 (C=O), 1520 and 1340 cm^{-1} (NO_2); δ ($CDCl_3$)[7]: 1.35 (d, J=7Hz, 3H), 1.95 (s, 3H), 5.15 (s, 2H), 5.27 (d, J=2.5Hz, 1H), 5.5 (s, 1H) in 75% yield[8]. Heating of 2 in 60% aqueous acetic acid on a boiling water bath for about 1 h yielded 3. The latter, without purification, was reacted with p-tosyl chloride (5 fold excess) in dry pyridine at 0°C for 1 h. The mixture obtained after removal of the solvent was chromatographed over silicic acid column. Elution of the column with 5% CH_3OH in $CHCl_3$ furnished the monotosyl derivative[9] 4 (m.p.76-79°C; $[\alpha]_D^{25}$ + 7.6 (C 2.5, $CHCl_3$); ν_{max}(KBr): 1750 and 1640 (C=O),



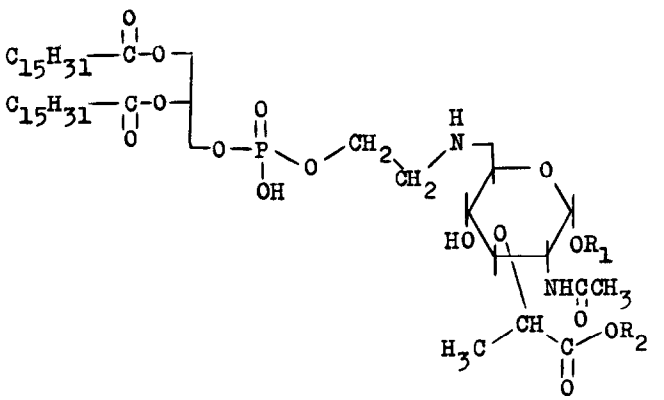
(1) R = H

(2) R = p-$O_2NC_6H_4CH_2$



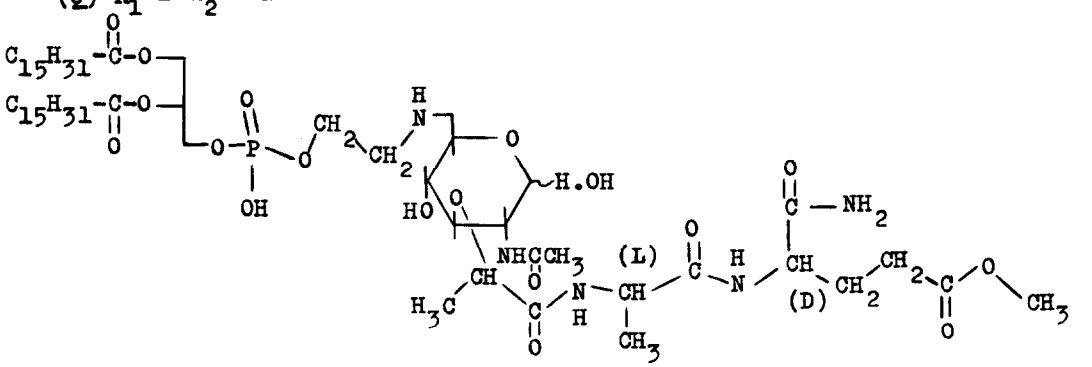
(3) R = H

(4) R = p-$H_3CC_6H_4SO_2$



(5) R_1 = $PhCH_2$, R_2 = p-$O_2NC_6H_4CH_2$

(6) R_1 = R_2 = H



(7)

1520 and 1340 (NO_2), 1360 and 1170 cm^{-1} (SO_3); δ ($CDCl_3$): 1.35 (d, J=7Hz, 3H), 1.87 (s, 3H), 2.30 (s, 3H), 5.02 (d, J=2.5Hz, 1H), 5.13 (s, 2H) in 50% overall yield from 2.

A mixture of the monotosylate 4, L-α-dipalmitoylphosphatidylethanolamine[10], and triethylamine in anhydrous $CHCl_3$ was heated at 80-90°C in a sealed tube for 12-15 h. Chromatography of the reaction mixture, after removal of the solvent, on a silicic acid column afforded 5, which was slightly contaminated with some non-phosphorus impurity. An analytically pure sample of 5 was obtained after further chromatography of the impure material over Sephadex LH-20 column[11]. Yield: 55% (m.p.54-56°C; $[\alpha]_D^{25}$ + 3.8 (C 0.8, $CHCl_3$); ν_{max}(KBr): 1750, 1740 and 1640 (C=O), 1520 and 1340 cm^{-1} (NO_2)). Catalytic hydrogenation of 5 over Pd-black, in glacial acetic acid, gave 6 (m.p.165-167°C; ν_{max}(KBr): 1740 and 1645 cm^{-1} (C=O)) in over 80% yield. The acid 6 was coupled to L-alanyl-D-isoglutamine-γ-methyl ester in THF/DMF (4:1) mixture using DCC/N-hydroxysuccinimide procedure[12]. The peptidylglycophospholipid 7 (m.p.144-146°C; $[\alpha]_D^{25}$ -0.8 (C 1.1, CH_3OH)) was isolated in nearly 58% yield after chromatography of the reaction mixture over Sephadex LH-20 column.

Having obtained the peptidylglycophospholipid 7 by this method, we are currently preparing analogs of 7, with unsaturated fatty acyl chains in the phospholipid moiety. Attempts are also being made to convert 7 into the corresponding phosphatidylcholine analog.

The adjuvant activity of 7 has been tested in guinea pigs using egg albumin as antigen. The new MDP derivative when administered with the antigen in saline induced more delayed type hypersensitivity as compared to MDP given in water-in-oil emulsion. The details of biological activity of 7 and its analogs will be published in due course of time.

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References & Notes

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7. ^{1}H-nmr spectra were recorded in a Perkin-Elmer R-32 instrument using tetramethylsilane as internal standard.
8. Yields refer to analytically pure material. The satisfactory data from elemental analysis have been obtained for all the compounds.
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10. L-α-Dipalmitoylphosphatidylethanolamine was purchased from Sigma Chemical Company.
11. Sephadex LH-20 (25-100 μm beads) chromatography was performed on a 2.5x100 cm column with $CHCl_3/CH_3OH$ (1:1) as the eluant.
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