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CHEMICAL SYNTHESES OF LIPID X AND LIPID Y, ACYL GLUCOSAMINE 1-PHOSPHATES ISOLATED FROM ESCHERICHIA COLI MUTANTS

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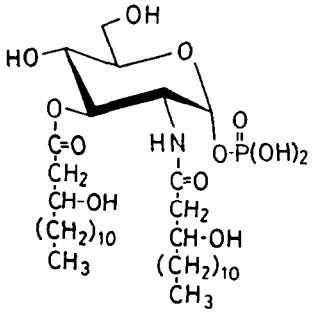
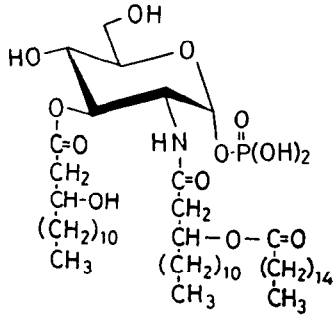
Abstract: Two new phospholipids, 2-N;3-O-bis(R)-3-hydroxytetradecanoyl-glucosamine 1-phosphate and 2-N-(R)-3-hexadecanoyloxytetradecanoyl-3-O-(R)-3-hydroxytetradecanoylglucosamine 1-phosphate, were synthesized and identified with natural lipid X and lipid Y respectively.

Recently, two new glucosamine-containing phospholipids (designated lipid X and lipid Y) were found by Nishijima and Raetz in certain mutants of *Escherichia coli* defective in phosphatidylglycerol synthesis.[1] Their chemical structures were then elucidated as depicted in the scheme below.[2,3] Lipid X (1) was thus shown to be a glucosamine 1-α-phosphate acylated at positions 2 and 3 with 3-hydroxytetradecanoic acid, while lipid Y (2) contains additional 1 mole of palmitic acid which is linked to the β-hydroxyl group of amide-bound hydroxytetradecanoic acid. Taking account of the structural similarity, it was suggested that these new phospholipids might be closely related to the well known cell-surface liposaccharide, lipid A,[4] whose structure was finally concluded by our recent analytical and synthetic studies[5,6] to be 1-α, 4'-diphosphate of β(1-6)glucosamine disaccharide acylated at positions 2,3,2', and 3' with (R)-3-hydroxy or (R)-3-acyloxytetradecanoic acids.[7] Lipid A is a lipophilic portion of the bacterial lipopolysaccharide (LPS) and possesses most of the characteristic endotoxic activities exhibited by whole LPS.



Lipid X (1)

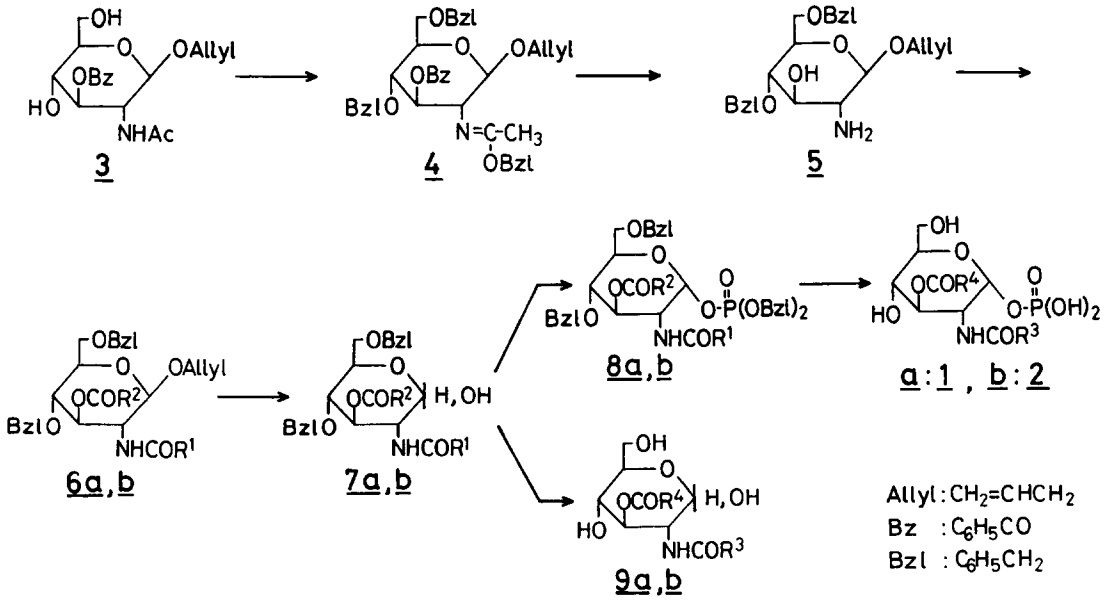


Lipid Y (2)

As a part of our chemical studies on these unique cell-surface liposaccharides,[8] we now describe a synthesis of lipid X (1) and lipid Y (2). These structures were constructed from a common glucosamine derivative in the following principles which were shown to be successful in our previous synthesis of lipid A analogs. First, benzyl group was used as the sole persistent protecting group. Secondly, the α-glycosyl phosphate moiety was introduced by means of the butyllithium - phosphorochloridate procedure immediately before the final hydrogenolytic deprotection.

Allyl 2-acetamido-3-O-benzoyl-2-deoxy-β-D-glucopyranoside (3) prepared via a 4,6-isopropylidene derivative was used as the starting material. This compound was subjected to acid catalyzed benzylation[9] (benzyl trichloroacetimidate - trifluoromethanesulfonic acid in CH_2Cl_2-cyclohexane (1:1) first at -40°C then at 0°C for 6 hr). Under these conditions complete benzylation occurred both on hydroxyl groups and the amide function to give di-O-benzylated benzyl imino ether (4). Acidic hydrolysis (aqueous 1N HCl in THF at room temperature) of 4 followed by removal of the benzoyl group (0.1N NaOMe at room temperature for 15 min) afforded 5 (43% from 3, syrup), which was used as a common synthetic intermediate for lipid X and lipid Y.

Simultaneous acylation of the amino and 3-hydroxyl groups of 5 with optically pure (R)-3-benzyloxytetradecanoic acid (dicyclohexylcarbodiimide -



a : $R^1CO = R^2CO = CH_3(CH_2)_{10}\underset{\displaystyle OBzl}{\underset{|}{C}}HCH_2CO$, $R^3CO = R^4CO = CH_3(CH_2)_{10}\underset{\displaystyle OH}{\underset{|}{C}}HCH_2CO$

b : $R^1CO = R^3CO = CH_3(CH_2)_{10}\underset{\displaystyle CH_3(CH_2)_4COO}{\underset{|}{C}}HCH_2CO$, $R^2CO = CH_3(CH_2)_{10}\underset{\displaystyle OBzl}{\underset{|}{C}}HCH_2CO$, $R^4CO = CH_3(CH_2)_{10}\underset{\displaystyle OH}{\underset{|}{C}}HCH_2CO$

dimethylaminopyridine (DCC-DMAP) in CH_2Cl_2 at room temperature for 1 hr) gave 6a (56%, mp 82-84°C). Its allyl group was then isomerized into 1-propenyl group with an iridium complex ($[Ir(COD)(PMePh_2)_2]PF_6$ in THF at 50°C for 1 hr)[10] and then cleaved off (I_2 in aqueous THF)[11] to give 7a (63%, mp 124-125°C). NMR spectrum and TLC behavior of 7a indicated that this compound existed as a single anomer which we assumed to be α in analogy to many other N-acyl glucosamine derivatives with free reducing end.[12] In our experience only the corresponding α-anomer was usually obtained after cleavage of glycosidic bond and purification by silica gel column chromatography even when the starting glycoside had β-configuration. Phosphorylation of the α-glycosidic hydroxyl group of 7a was effected as described previously (i) butyllithium in THF at -70°C, ii) dibenzylphosphorochloridate at the same temperature).[12] The reaction mixture was immediately subjected to hydrogenolysis.

The desired product 1 was purified first with silica gel column chromatography ($CHCl_3$-MeOH-H_2O-Et_3N 15:10:2:0.2) in order to remove the sole sugar-containing by-product (9a) which corresponds to the dephospho derivative of 1. Further purification was effected by electrodialysis[13] (in water at 50V) which was continued for 6 hr with occasional addition of triethylammonium acetate. After this procedure the product became water-soluble on neutralization with triethylamine. From the resultant aqueous solution, pure 1 of free acid form could be precipitated on addition of cold 0.1N HCl (24% from 7a).[14]

Treatment of this free-acid (1) with diazomethane afforded dimethyl ester whose ^{1}H-NMR spectrum (200MHz, in $CDCl_3$-DMSO-d_6) was superimposable with that of dimethyl ester derived from the natural specimen presented in the literature.[2] Pure dephospho derivative (9a) (55%, mp 192-193°C)[14] was also prepared by direct hydrogenolysis of 7a.

Synthesis of lipid Y was performed in a similar way except that two different fatty acids were used for the acylation of 5. Thus, 5 was first acylated at the amino group with (R)-3-hexadecanoyloxytetradecanoic acid (DCC in CH_2Cl_2 at room temperature for 1 hr) then at the hydroxyl group with (R)-3-benzyloxytetradecanoic acid (DCC-DMAP in CH_2Cl_2 at room temperature for 1 hr) to give 6b (86%, mp 80-82°C). Removal of the allyl group was effected as above to yield 7b (73%, mp 122-124°C). In this case, a mixture of both anomers was obtained even after purification with a silica gel column (see above) as shown in TLC and NMR. However, an anomerization to α- from β-anomer could be accelerated by dissolving the mixture in THF-acetic acid (3:1) at room temperature. The α-anomer obtained from the solution by evaporation of the solvent was subjected to the next transformation. Phosphorylation, deprotection and successive purification were carried out in the same manner as described for lipid X. Thus, lipid Y was isolated after electrodialysis and acidic precipitation (34%, from 7b).[14] ^{1}H-NMR spectrum of its dimethyl ester was also identical with that of natural lipid Y dimethyl ester given in the literature.[3] Dephospho derivative (9b) (81%, mp 135-140°C)[14] of lipid Y was also prepared by hydrogenolysis of 7b.

This work gave not only an evidence supporting the proposed structures of lipid X and Y but also a possibility to investigate biological activities of these liposaccharides by use of pure materials. This is one of the obvious advantages of a synthetic approach because some heterogeneities, e.g., in fatty acid composition, still existing even in a purified preparation of natural source can be avoided.[3] In fact, in collaboration with other research groups, some clear-cut results in biological tests were already obtained for these synthetic compounds.

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14) Some selected physical data of products are given below.
1: $[\alpha]_D^{15}$ + 14.0° (c 0.2, $CHCl_3$); elemental analysis: found: C, 55.72; H, 9.43; N, 1.97%; calcd for $C_{34}H_{66}NO_{12}P\cdot H_2O$: C, 55.95; H, 9.11; N, 1.92%.
9a: $[\alpha]_D^{17}$ + 13.0° (c 0.16, $CHCl_3$-MeOH 1:1); found: C, 64.42; H, 10.41; N, 2.13%; calcd for $C_{34}H_{65}NO_9$: C, 64.63; H, 10.37; N, 2.22%.
2: $[\alpha]_D^{13}$ + 10.0° (c 0.53, $CHCl_3$); found: C, 60.88; H, 10.19; N, 1.55%; calcd for $C_{50}H_{96}NO_{13}P\cdot 2H_2O$: C, 60.89; H, 10.22; N, 1.42%.
9b: $[\alpha]_D^{13}$ + 218.3° (c 0.71, C_6H_6); found: C, 69.01; H, 11.01; N, 1.56%; calcd for $C_{50}H_{95}NO_{10}$: C, 69.00; H, 11.00; N, 1.61%.
The structures of all synthetic intermediates were confirmed by NMR. All crystalline compounds gave satisfactory results in elemental analyses.

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