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CHEMICAL SYNTHESIS OF 1-DEPHOSPHO DERIVATIVE OF ESCHERICHIA COLI LIPID A

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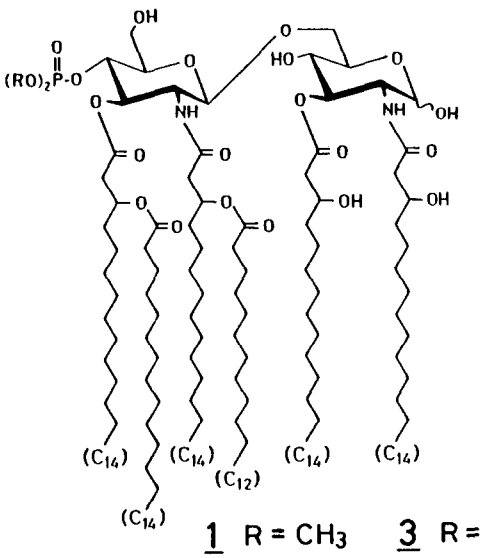
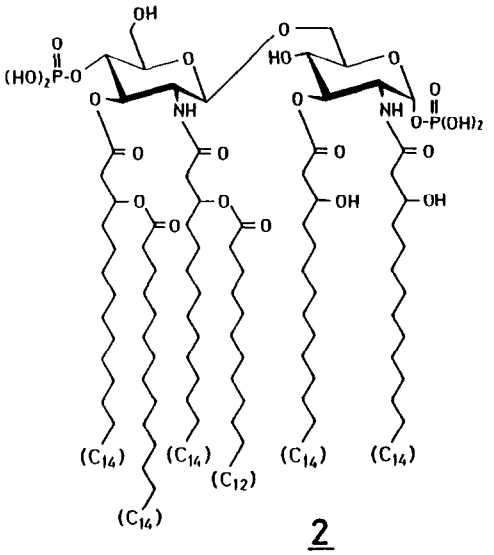
Summary: 1-Dephospho *E. coli* lipid A (3) was synthesized and shown to be identical with the corresponding derivative isolated from bacterial cells. This provides the unequivocal evidence supporting the structure of *E. coli* lipid A (2) recently proposed by us.

Lipid A, which is the covalently bound lipid component of cell surface lipopolysaccharide (LPS) of Gram negative bacteria, is known to be responsible for the endotoxic activities. In the previous papers, we described the elucidation of the chemical structure of *Escherichia coli* lipid A.[1] In those works the main component of lipid A was isolated in a pure state after removal of the glycosyl phosphate and dimethyl esterification of the remaining 4'-phosphate group. Chemical as well as spectroscopic studies revealed the structure of this 1-dephospho lipid A dimethyl ester and therefore that of the original lipid A to be 1 and 2, respectively. We now report a chemical synthesis of the 1-dephospho derivative (3) of lipid A and identification of its dimethyl ester with the corresponding natural specimen. This work provides an unequivocal evidence to confirm the structure of *E. coli* lipid A proposed by us.

In view of the structural feature of the present target molecule (3) that the two glucosamine residues are not equally acylated, it seemed to be advantageous to prepare monosaccharide intermediates which already had appropriate acyl groups at the correct positions



1 R = CH_3 3 R = H



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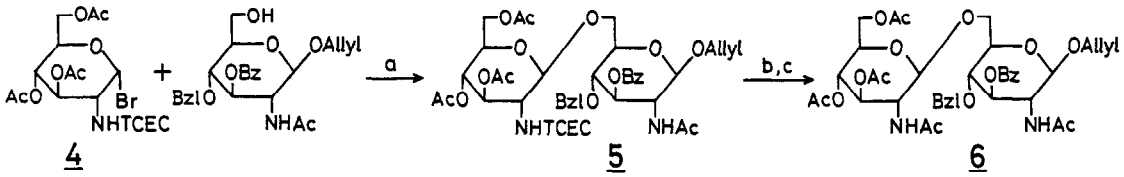
and to couple them together to form a disaccharide. Otherwise, tremendous manipulation of protecting groups would have been necessary in order to differentiate the two glucosamine residues of the disaccharide.[2] Only the (R)-3-dodecanoyloxytetradecanoyl group on the 2'-amino group was introduced exceptionally after the formation of the disaccharide linkage because a β-elimination of dodecanoic acid took place quite readily when the glycosidic position of an N-(3-dodecanoyloxytetradecanoyl)glucosamine derivative was activated for coupling.[3] For the temporary protection of that amino group during the glycoside formation, 2,2,2-trichloroethoxycarbonyl (TCEC) group proved to be suitable from the following experiment. Thus, a model glycosyl bromide (4) which could be prepared in a quantitative yield from the corresponding 1-O-acetate afforded exclusively the β-linked disaccharide (5) in a good yield. The β-configuration of the newly formed glycosidic bond was confirmed by converting 5 into the known disaccharide (6) obtained in our previous work.[2]



TCEC : CCl_3CH_2OCO, Bz : C_6H_5CO, Bzl : $C_6H_5CH_2$, Allyl : $CH_2=CHCH_2$

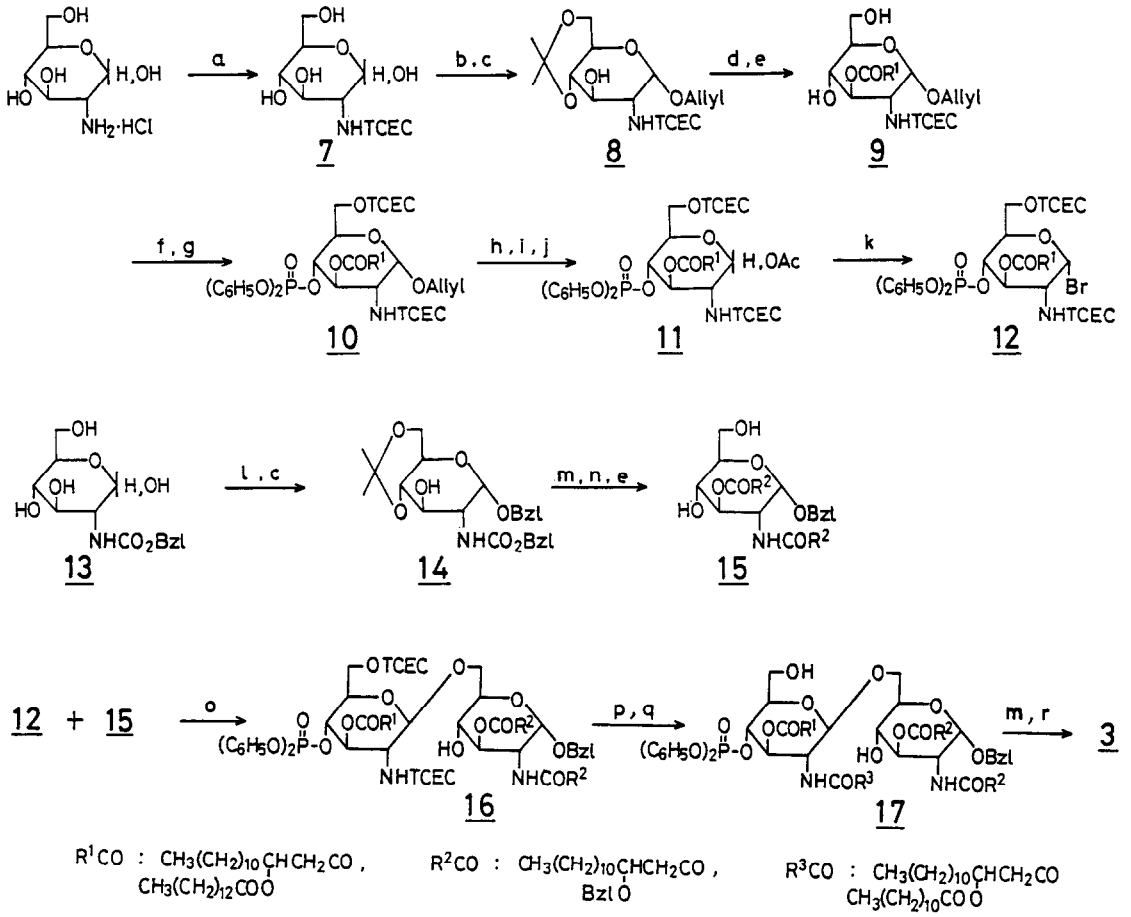
a) $Hg(CN)_2$ in $CHCl_3$, reflux, 40 min; b) Zn/AcOH, 45°C; c) Ac_2O/Et_3N

According to the above strategy, the glycosyl bromide (12) seemed to be the appropriate candidate for the non-reducing glucosamine component in the disaccharide synthesis. It was prepared from glucosamine as shown in the scheme. Acid-catalyzed allyl glycosidation of N-TCEC-glucosamine (7) (mp 183-184°C dec) followed by reaction with 2,2-dimethoxypropane afforded the isopropylidene derivative (8) (41% from 7, mp 185-187°C).[4] Acylation of the 3-hydroxyl group of 8 with (R)-3-tetradecanoyloxytetradecanoic acid and subsequent hydrolysis of the isopropylidene group gave 9 (66% from 8, mp 68-70°C). After selective protection of the 6-hydroxyl group by trichloroethoxycarbonylation, the phosphate moiety was introduced by use of diphenylphosphorochloridate[5] to give a syrupy product (10) (54% from 9), whose allyl group was isomerized with an iridium complex[6] and then cleaved with iodine.[7] Subsequent acetylation afforded a crystalline 1-O-acetate (11) (66% from 10, mp 77-80°C), which was nearly quantitatively converted into the desired bromide (12).[8]

The component corresponding to the reducing glucosamine residue was prepared from N-benzyloxycarbonylglucosamine (13). Acid-catalyzed glycosidation followed by acetonide formation afforded 14.[4] After selective hydrogenolysis of the N-protecting benzyloxycarbonyl group, both 2-amino and 3-hydroxyl groups were acylated with (R)-3-benzyloxytetradecanoic acid to give 15 (33% from 13, mp 88-89°C).

Condensation of the two components, 12 and 15 (used in 2:1 molar ratio), proceeded smoothly in the presence of $Hg(CN)_2$ to give a single disaccharide (16) (80% on the basis of 15, syrup).[9] There seemed to be no doubt on the β(1-6) linkage in 16 as judged from the model experiment described above and the known great difference of the reactivity between the

6- and 4-hydroxyl groups.[10] Both TCEC-groups in 16 were removed and (R)-3-dodecanoyloxytetradecanoyl group was now introduced on the resultant free 2'-amino group of the disaccharide to give 17 (49% from 16, colorless powder) which contains the all requisite structural elements for the target molecule. The final hydrogenolytic deprotection of 17 afforded the desired 1-dephospho lipid A (3) (94% from 16). In order to remove the traces of contaminating cations which originated from the hydrogenolysis catalysts and formed salts with the phosphate groups of 3, the product was dissolved in $CHCl_3$-CH_3OH (9:1), treated with 1N HCl at 0°C and washed



a) TCEC-Cl/$NaHCO_3$ in H_2O; b) 2% dry HCl in AllylOH, 100°C, 20 min; c) $(CH_3O)_2C(CH_3)_2$/TsOH/$CaSO_4$ in acetone; d) R^1CO_2H/4-dimethylaminopyridine (DMAP)/dicyclohexylcarbodiimide (DCC) in CH_2Cl_2, r.t., 1 hr; e) 90% AcOH, 90°C, 10 min; f) TCEC-Cl/Pyr, 0°C, 20 min; g) $(C_6H_5O)_2POCl$/DMAP/Pyr in CH_2Cl_2, r.t., 2 hr; h) $[Ir(COD)(PCH_3(C_6H_5)_2)_2]PF_6$ in THF; i) I_2 in aq.THF; j) Ac_2O/Pyr in $CHCl_3$; k) dry HBr in CH_2Cl_2, r.t., overnight; l) 2.5% dry HCl in BzlOH, 100°C, 20 min; m) H_2/Pd-black; n) R^2CO_2H/DMAP/DCC, r.t., 30 min; o) $Hg(CN)_2$ in $CHCl_3$, reflux, 22 hr; p) Zn/AcOH, r.t.; q) R^3CO_2H/DCC in CH_2Cl_2, r.t.; r) H_2/PtO_2

with water. Lyophilization from dioxane afforded 3 free from cations as colorless powder.[9)]

On treatment with diazomethane, 3 afforded the dimethyl ester 1 which was identified with the corresponding compound from *E. coli* lipid A by means of TLC ($CHCl_3$-CH_3OH 9:1) as well as 360 MHz ^{1}H-NMR. The identity was further confirmed after acetylation. ^{1}H-NMR spectrum of the synthetic pentaacetate ($[\alpha]_D^{30}$ + 17.9°)[9)] of 3 was again completely superimposable with that of the peracetate of natural origin ($[\alpha]_D^{30}$ + 19.0°). This was the first direct identification of a synthetic compound with a single natural lipid A derivative, and the result gives the final evidence for the correctness of the chemical structure of lipid A proposed by us.

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References and Notes

1) The preceding communication and the reference cited in it.
2) Therefore, the basic strategy was different from that employed in our recent synthesis of a biosynthetic precursor of lipid A. See M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto, and T. Shiba, Tetrahedron Lett., 25, 2667 (1984).
3) When 1-propenyl β-glycoside of an N-(3-dodecanoyloxytetradecanoyl)glucosamine derivative was subjected to a standard condition of oxazoline preparation ($HgCl_2$-HgO in CH_3CN), simultaneous elimination of dodecanoic acid occurred to give an oxazoline with a conjugated double bond.
4) Contrary to the Hg ion promoted glycosidation of the glycosyl bromide described above, glucosamine with a urethane-type N-protecting group afforded on acid-catalyzed glycosidation predominantly the α-glycoside which can be isolated in an acceptable yield. Therefore, it provides a convenient and practical way to prepare a single anomer of a simple glycoside of a glucosamine derivative.
5) P. Szabó, S. R. Sarfati, C. Diolez, and L. Szabó, Carbohydr. Res., 111, C9 (1983).
6) J. J. Oltvoort, C. A. A. van Boeckel, J. H. de Koning, and J. H. van Boom, Synthesis, 1981, 305.
7) M. A. Nashed and L. Anderson, J. Chem. Soc., Chem. Commun., 1982, 1274.
8) The syrupy residue remained after evaporation of the reaction mixtures in vacuo showed almost a single spot of the bromide on TLC. It was used for the next coupling reaction after coevaporation from benzene and drying over KOH in vacuo. The bromide was considerably more stable than those of usual N-acylglucosamine derivatives.
9) The structures of the all important intermediates and the final product were confirmed with NMR spectra and elemental analyses.
10) M. Inage, H. Chaki, S. Kusumoto, and T. Shiba, Tetrahedron Lett., 21, 3889 (1980).

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