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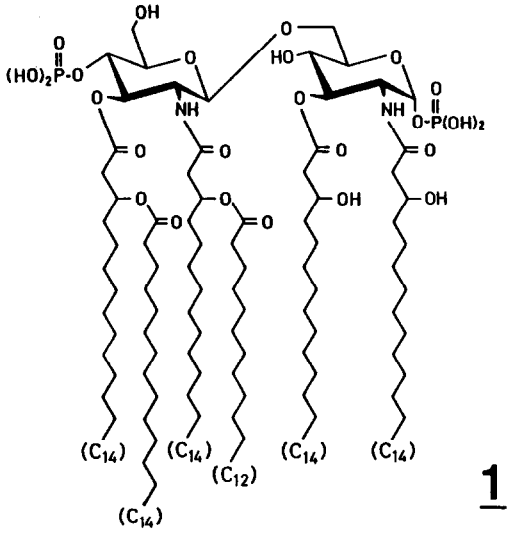
TOTAL SYNTHESIS OF ESCHERICHIA COLI LIPID A

Masahiro Imoto, Hiroyuki Yoshimura, Nobuki Sakaguchi, Shoichi Kusumoto, and Tetsuo Shiba*
Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

Summary: The first total synthesis of *E. coli* lipid A (1) is described. The synthetic compound was identical with a natural specimen and exhibited the full endotoxic activity. It was thus conclusively proved by this chemical synthesis that lipid A is the active principle of bacterial endotoxin.

Lipid A is the covalently bound lipid component of lipopolysaccharide (LPS) located on the cell surface of Gram negative bacteria. It was first isolated by Westphal and Lüderitz in 1954 through mild acid hydrolysis of LPS and claimed to be responsible for the endotoxic activities of LPS, e.g., lethal toxicity, pyrogenicity, tumor-necrotizing activity and so on.[1] Lipid A's from many *Enterobacteriaceae* were shown to have a common basic structure composed of a polyacylated β(1-6) disaccharide of D-glucosamine 1,4'-bisphosphate.[2,3] However, due to the inherent heterogeneity of a lipid A preparation even from a single bacterial species, the precise chemical structure was not elucidated until recently when homogeneous compounds were subjected to structural study.[4] Thus, we isolated the main component of *Escherichia coli* lipid A after removal of the glycosyl phosphate and methyl esterification. The chemical structure of this 1-dephospho derivative was then deduced by chemical and spectroscopic methods.[4a,5] The result was soon confirmed by a chemical synthesis.[6] From these works it could be unequivocally concluded that the whole structure of *E. coli* lipid A is represented as 1.[7] In this communication we describe a total synthesis of 1 which was expected to possess the full endotoxic activity.[8]



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The basic strategy for the synthesis of 1 was similar to that employed in our previous synthesis of the 1-dephospho derivative.[6] The relevant substituents on the disaccharide were introduced as far as possible at the stage of monosaccharide intermediates. Two exceptional substituents which were introduced after the formation of the disaccharide were the α-glycosyl phosphate and the (R)-3-dodecanoyloxytetradecanoyl group on the 2'-amino function. The former, the glycosyl phosphate, was introduced at the latest synthetic step by means of phosphorylation with butyllithium – dibenzyl phosphorochloridate followed by hydrogenolytic deprotection, because this was the sole procedure so far proved to be satisfactory to form an α-glycosyl phosphate in our previous work.[9,10] The reason was already discussed previously why 3-dodecanoyloxytetradecanoic acid had to be condensed at the 2'-amino group after the formation of the disaccharide.[6]

Two monosaccharide components which fit to the above strategy would be 2 and 3. The glycosyl bromide 2 was already prepared from the isopropylidene derivative of N-trichloroethoxycarbonyl glucosamine (4) and used as the glycosyl donor.[6] The glycosyl acceptor 3 was prepared from the same isopropylidene derivative (4) as shown in the scheme. Acylation of the free hydroxyl group of 4 with (R)-3-benzyloxytetradecanoic acid afforded 5 (64%, colorless syrup). After removal of its trichloroethoxycarbonyl group, the free amino group was acylated again with 3-benzyloxytetradecanoic acid. Hydrolysis of the isopropylidene ring gave the desired compound 3 (58% from 5, mp 80-82°C).[11]

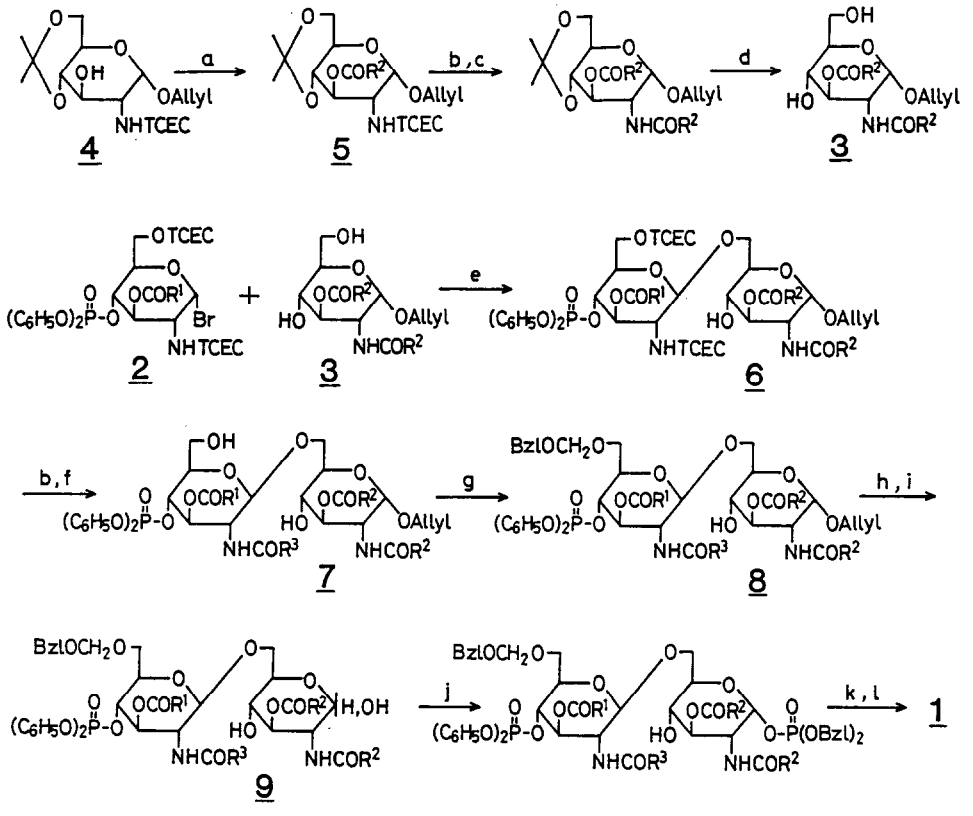
Condensation of the two components, 2 and 3, in the presence of mercuric(II) cyanide gave a single disaccharide 6 as the product (70%, resinous solid)[11], which was isolated after purification with a silica-gel column. The β(1-6) disaccharide structure of 6 could be assured by analogy with the previous work.[6] After the temporary trichloroethoxycarbonyl protection in 6 had been removed, the free 2'-amino group was now acylated with (R)-3-dodecanoyloxytetradecanoic acid to give 7 (71%, powder).[11]

Before the cleavage of the allyl glycoside, the 6'-hydroxyl group of 7 was again protected because this primary hydroxyl group could also react competitively under the reaction conditions employed for 1-O-phosphorylation. Thus, 7 was benzyloxymethylated to give 8 (74%, powder),[11] whose allyl group was in turn isomerized with an iridium complex[12] and the resultant 1-propenyl glycoside was cleaved with iodine[13] to give 9 (63%, powder).[11] The glycosidic hydroxyl group was then phosphorylated as described previously.[9,10] The reaction product was directly subjected to hydrogenolysis first with palladium to remove benzyl type protections and then with platinum to cleave phenyl esters of 4'-phosphate group.

The final product was isolated by means of a silica-gel column ($CHCl_3$-CH_3OH-H_2O-Et_3N 10:5:1:0.05). Contaminating cations and inorganic materials which originated from hydrogenolysis catalysts and silica gel were removed by combination of electrodialysis[14] and acidic precipitation[10] to afford the pure synthetic *E. coli* lipid A 1 as colorless powder (27% from 9, $[\alpha]_D^{15}$ + 11.3° (c 0.56, $CHCl_3$-CH_3OH 9:1)).[11] It was identified with the main component of *E. coli* lipid A on TLC.[15]

The biological activities of the synthetic 1 were tested by several collaborating groups and the details of the result will be soon published elsewhere. However, it can be already concluded that the synthetic 1 exhibits the activities identical with those of the natural *E. coli* lipid A in all test systems examined including pyrogenicity test and Shwartzman reaction.[8] Consequently, this work is the first successful chemical synthesis of a compound

which exhibits the full endotoxic activity. The structure responsible for the endotoxicity is thus finally established chemically.



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

R^1CO : $CH_3(CH_2)_{10}\underset{CH_3(CH_2)_{12}COO}{\overset{|}{C}}HCH_2CO$, R^2CO : $CH_3(CH_2)_{10}\underset{BzlO}{\overset{|}{C}}HCH_2CO$, R^3CO : $CH_3(CH_2)_{10}\underset{CH_3(CH_2)_{10}COO}{\overset{|}{C}}HCH_2CO$

a) R^2CO_2H/4-dimethylaminopyridine/dicyclohexylcarbodiimide(DCC) in $CHCl_3$, r.t., 1 hr;
b) Zn/AcOH, r.t.; c) R^2CO_2H/DCC in $CHCl_3$, r.t., 30 min; d) 90% AcOH, 90°C, 30 min;
e) $Hg(CN)_2/CaSO_4$ in $CHCl_3$, reflux, 33 hr; f) R^3CO_2H/DCC in $CHCl_3$, r.t., 17 hr;
g) $BzlOCH_2Cl/(iPr)_2EtN$ in CH_2Cl_2, r.t., 48 hr; h) $[Ir(COD)(PCH_3(C_6H_5)_2)_2]PF_6$ in THF;
i) I_2 in aq. THF; j) $(BzlO)_2POCl$/BuLi in THF; k) H_2/Pd-black; l) H_2/PtO_2

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References and Notes

1) O. Westphal and O. Lüderitz, Angew. Chem., 66, 407 (1954).
2) E. Th. Rietschel et al., "Bacterial Lipopolysaccharide", ACS Symposium Series 231, Ed. by L. Anderson and F. M. Unger, p. 195, American Chemical Society, Washington D.C. (1983). See also the references cited in our previous papers.
3) Though slight variations in the structure of lipid A were observed from species to species, for example, the presence of polar substituents on phosphate groups or the presence of additional non-hydroxylated acyl groups, the biological activities are practically in the same amplitude.
4) a) M. Imoto, S. Kusumoto, T. Shiba, H. Naoki, T. Iwashita, E. Th. Rietschel, H.-W. Wollenweber, C. Galanos, and O. Lüderitz, Tetrahedron Lett., 24, 4017 (1983).
b) N. Qureshi, K. Takayama, D. Heller, and C. Fenselau, J. Biol. Chem., 258, 12947 (1983).
5) M. Imoto, S. Kusumoto, T. Shiba, E. Th. Rietschel, C. Galanos, and O. Lüderitz, Tetrahedron Lett., in press.
6) S. Kusumoto, H. Yoshimura, M. Imoto, T. Shimamoto, and T. Shiba, Tetrahedron Lett., in press.
7) The α-configuration of the glycosidic phosphate in natural lipid A was determined: S. M. Strain, S. W. Fesik, and J. M. Armitrage, J. Biol. Chem., 258, 2906 (1983). The chemical structures of lipid A's from several enterobacteria were also deduced by a German group: U. Seydel, B. Lindner, H.-W. Wollenweber, and E. Th. Rietschel, Eur. J. Biochem., in press.
8) Many of the endotoxic activities were known to be expressed by a biosynthetic precursor of lipid A whose structure corresponds to that lacking both non-hydroxylated acyl groups of 1. This was confirmed by our recent chemical synthesis of the precursor molecule. However, both synthetic and natural precursors do not represent the full endotoxicity. For example, it neither acts as potent pyrogen nor induces Shwartzman reaction. See M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto, and T. Shiba, Tetrahedron Lett., 25, 2667 (1984) and references in it. Therefore, the presence of the non-hydroxylated acyl groups in 1 is expected to be very critical for the exhibition of biological activity.
9) M. Inage, H. Chaki, S. Kusumoto, and T. Shiba, Chem. Lett., 1982, 1281.
10) See our previous work cited in 8).
11) The structures of the all intermediates and the final product were confirmed by NMR spectra and elemental analyses.
12) J. J. Oltvoort, C. A. A. van Boeckel, J. H. de Koning, and J. H. van Boom, Synthesis 1981, 305.
13) M. A. Nashed and L. Anderson, J. Chem. Soc., Chem. Commun., 1982, 1274.
14) C. Galanos and O. Lüderitz, Eur. J. Biochem., 54, 603 (1975).
15) For further identification with the natural lipid A, synthetic 1 was heated in aqueous acetic acid and then treated with diazomethane. The product was indistinguishable on TLC from 1-dephospho lipid A dimethyl ester isolated from natural lipid A. See ref. 4a).

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