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# Synthesis and biological function of bacterial endotoxin

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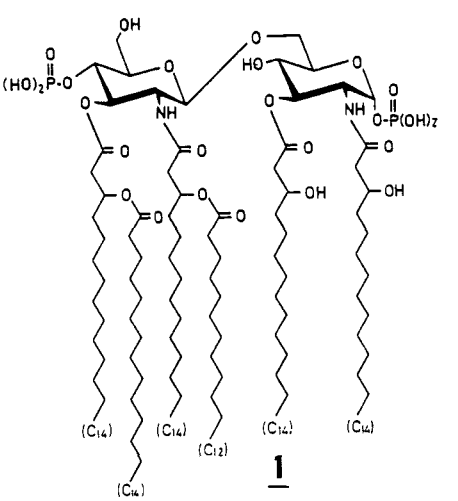
Abstract - Synthetic and biological studies continued in our laboratories contributed to elucidate the chemical structure and biological functions of endotoxin, which is lipopolysaccharide (LPS) located at the cell surface of gram-negative bacteria. In this paper the effect of structural variation on the biological activities is discussed of lipophilic part of LPS. The recent result of our synthetic approach toward the most simple LPS isolated and characterized from Escherichia coli Re mutant cells is also described.

## INTRODUCTION

Cell surface of gram-negative bacteria is covered by a characteristic amphiphilic component, lipopolysaccharide (LPS), which is composed of covalently bound polysaccharide and lipid part. LPS, being the chemical entity of bacterial endotoxin which was described already in 1982 by R. Pfeiffer, exhibits multiple biological activities against higher animals. These include toxic activities such as lethal toxicity, pyrogenicity, and tissue-necrotizing activity as well as many beneficial ones related to immunostimulation. We elucidated the chemical structure 1 of the lipid part (designated "lipid A") of Escherichia coli LPS (ref. 1) and confirmed the structure by a total synthesis (ref. 2). The synthetic 1 absolutely free from any contaminants from bacterial cells showed all of the biological activities of LPS mentioned above in identical potency with natural lipid A. Consequently, it was unequivocally proved that lipid A is the endotoxic principle of LPS.

## SYNTHESIS AND BIOLOGICAL ACTIVITIES OF LIPID A VARIANTS

Chemical structures of lipid A components isolated from many gram-negative bacterial species were elucidated in the meantime. They have the same basic structure of β(1-6)-linked glucosamine disaccharide 1,4'-bisphosphate as 1 but differ in numbers and locations of acyl groups. In view of the unique activities of lipid A, it seemed to be of great interest to synthesize them in order to confirm their proposed structures and particularly to study the effect of the structural variation on the biological activities of these glycolipids. Before our total synthesis, serious confusions were often encountered in the biological studies on lipid A owing to the intrinsic heterogeneity and difficulty of purification of the natural lipid A preparations used for the test. Fortunately, however, precise comparison of the biological activities of various analogs has now become possible by use of pure materials synthetically available.



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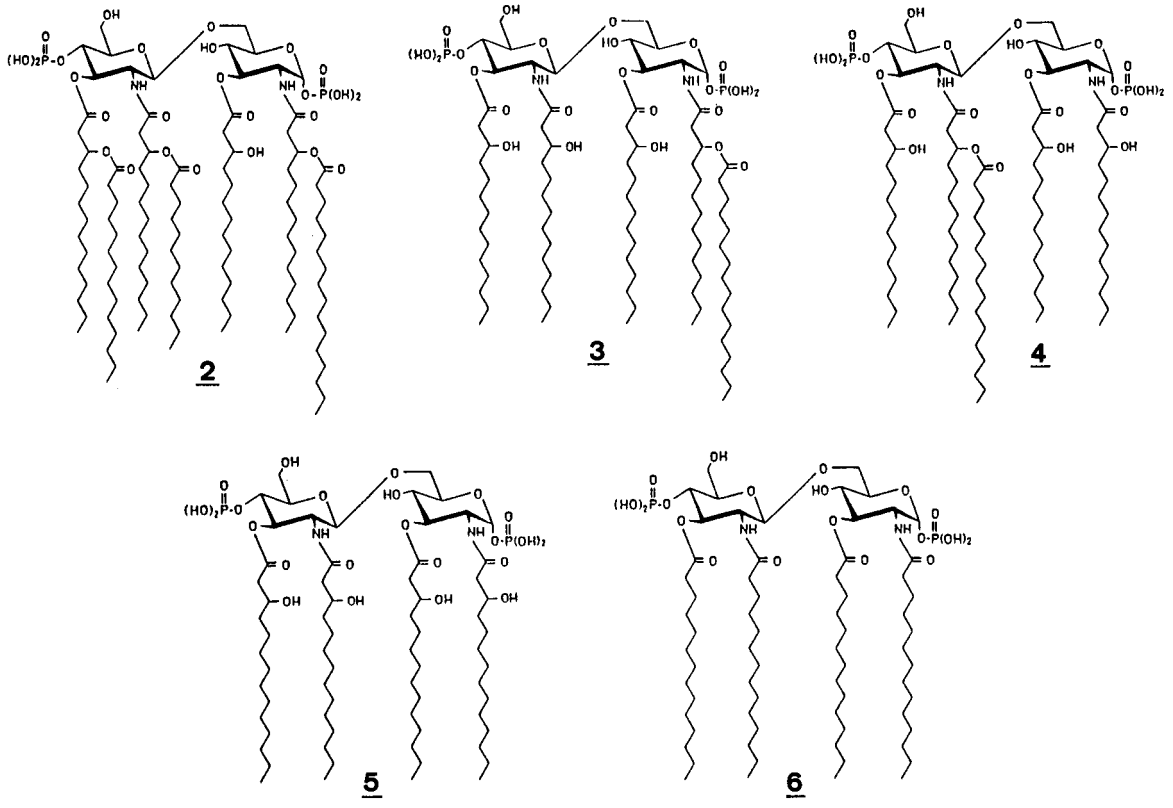
The chemical structures of some of the lipid A analogs (2 - 6) synthesized are shown below. Compound 2 is the main component of Salmonella lipid A which contains an additional acyl group as compared to E. coli-type lipid A (1). Compounds 3 and 5 corresponds to the biosynthetic precursors of lipid A isolated from certain Salmonella-species. They are designated precursor Ib and Ia, respectively. Compound 4 is an artificial structural isomer of the former. These compounds could be prepared as exemplified in Fig. 1 by adequate modification of the synthetic route established for 1 previously.

Some of the biological activities of the synthetic compounds are summarized in Table 1 (ref.




3, 4 & 5). Lethal toxicity, pyrogenicity, and Shwartzman reaction, which is an expression of harmful tissue-necrotizing activity, represent typical toxic effect of LPS. E. coli-type lipid A (1) showed the highest toxicity among the compounds tested. The potency decreased remarkably by either increasing or reducing the number of acyl groups in structure 1. This indicates the importance of the adequate balance of the lipophilic and hydrophilic character for the expression of toxicity. Precursor Ia (5) contains only four moles of 3-hydroxytetradecanoic acid and hence is devoid of 3-acyloxyacyl structure which is present in 1. The much weaker toxicity observed in this compound (5) seems to suggests the significance of the acyloxyacyl moieties for these biological activities. Precursor Ib and its isomer (3 and 4) had intermediate toxicity, corresponding to the number of the acyloxyacyl group. In spite of different toxicity, these synthetic compounds showed comparable potencies of beneficial activity as represented by immunoadjuvant activity (Table 1). Particularly worth to note is one of the

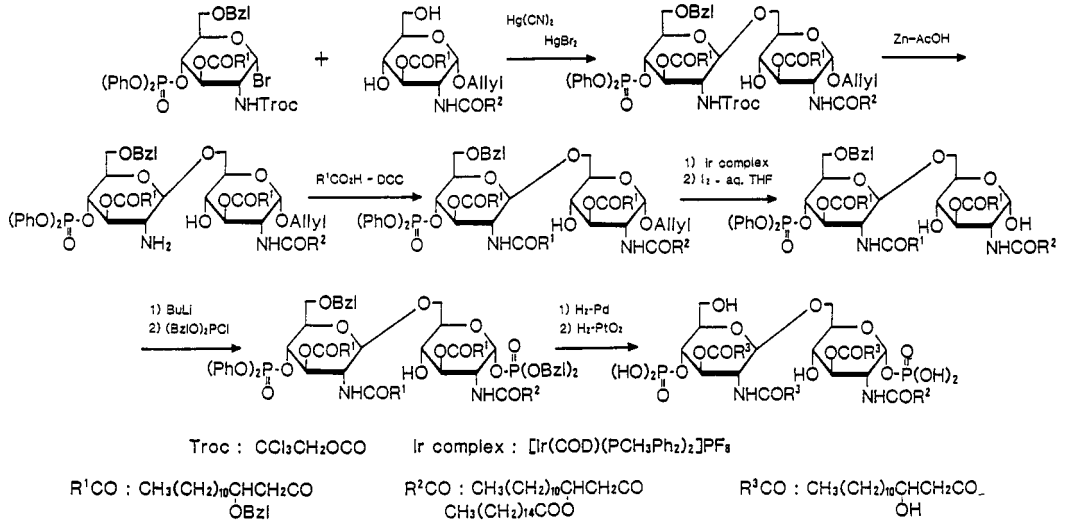



Fig. 1. Synthetic scheme of lipid A analogs.

Table 1. Biological activity of synthetic lipid A variants.

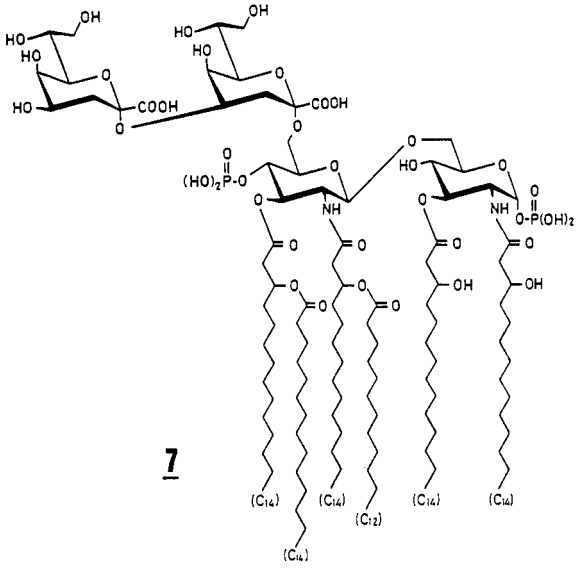
| Compound | | Schematic[a] structure | Lethal toxicity: Chicken embryos | Lethal toxicity: $GalNH_2$-sensitized mice | Pyrogenicity | Shwartzman reaction | Immunoadjuvant activity (humoral) |
|---|---|---|---|---|---|---|---|
| Salmonella lipid A | 2 | P—[4]—[3]—P | - | +++ | ++ | - | +++ |
| E. coli lipid A | 1 | P—[4]—[2]—P | +++ | +++ | +++ | +++ | +++ |
| Precursor Ib | 3 | P—[2]—[3]—P | | | ++ | ++ | |
| Isomer of Ib | 4 | P—[3]—[2]—P | | | ++ | ++ | |
| Precursor Ia | 5 | P—[2]—[2]—P | - | +++ | + | - | +++ |
| | 6 | P—[2*]—[2*]—P | - | + | ± | - | +++ |

a) The arabic numeral in the square represents the number of acyl groups bound to each glucosamine residue. The mark * indicates that the acyl groups are not hydroxylated.

artificial analogs 6 obtained by synthetically replacing 3-hydroxytetradecanoic acid, the typical fatty acid component of natural lipid A, with non-hydroxylated acid. This compound showed very low toxicity but still retained immunostimulating and other beneficial activities. These results lead to the conclusion that the harmful and favorable activities of lipid A could be separated depending on the chemical structures. One could thus expect a future possibility to suppress the toxic action of bacterial endotoxin by adequate modification of the structure and to utilize its favorable activities such as tumor-necrotizing or immunostimulating activity to preserve health of human being.

## SYNTHETIC STUDY ON RE TYPE LIPOPOLYSACCHARIDE

Although lipid A is now recognized to be the active principle responsible to the multiple biological functions of LPS as already described above, recent observations indicate that the polysaccharide part in LPS plays a significant role to modify the biological activity (ref. 6). The unique eight-carbon acidic sugar component, 3-deoxy-D-manno-2-octulosonic acid (formerly called 2-keto-3-deoxyoctonic acid, KDO) which is directly bound to lipid A is assumed to be particularly important, for example, for the antigenicity of the molecule. In order to analyze the significance of KDO moieties for the biological function, we thus started a new synthetic approach toward Re-type LPS of E. coli whose structure 7 had been deduced previously by us (ref. 7). It represents the most simple natural LPS so far known and is composed of only two moles of KDO and lipid A, lacking most of the polysaccharide part.

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For the chemical construction of the tetrasaccharide structure of 7, stepwise condensation of two KDO moieties with a lipid A part was attempted. A novel, glycosyl donor 8 of pyranosidic KDO feasible for this synthesis was first prepared from D-mannose and glyoxylic acid (ref. 8 & 9). The fluoride 8 proved to give predominantly the desired α-ketoside of KDO by condensation with an suitable glycosyl acceptor in the presence of boron trifluoride etherate in a satisfactory yield (ref. 9). Thus, coupling of the fluoride 8 with a synthetic intermediate of lipid A 9 afforded a trisaccharide 10. It was then coupled via the corresponding monoacetonide 12 with the same fluoride to give the desired tetrasaccharide 12. The position of the newly formed KDO linkage was reasonably assumed to be at 4 by considering the known higher reactivity of the equatorial 4-hydroxyl group than that of axially oriented one on C-5 of KDO. By usual sequence of deprotection reactions (ref. 2), 12 was converted into the 1-dephospho derivative 13 of E. coli Re LPS.

Tetramethyl ester obtained from 13 by action of diazomethane was identified by $^1$H-NMR with the corresponding natural product prepared and purified from crude Re LPS of bacterial origin. This was the first direct comparison and identification of a synthetic and natural LPS derivative. The chemical structure 7 of Re LPS was thus confirmed by this work. Synthetic 13 and the corresponding trisaccharide derivative obtained by deprotection of 10 are being sub-

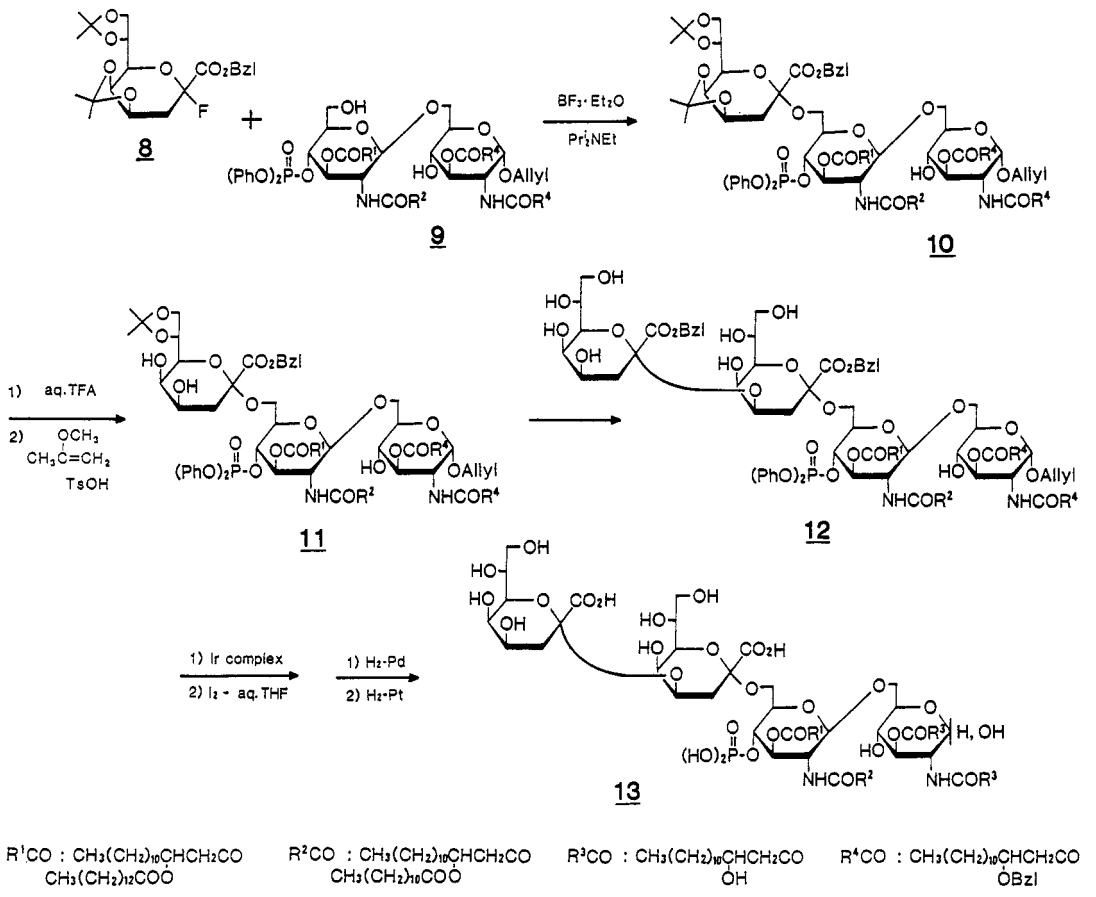


Fig. 2. Synthetic scheme of 1-dephospho derivative of E. coli Re LPS.

jected to biological test and expected to give clear informations concerning the role of KDO moieties. Further effort is being undertaken to introduce the glycosyl phosphate in order to finish the chemical synthesis of complete Re LPS (7).

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## REFERENCES

1. M. Imoto, S. Kusumoto, T. Shiba, E.T. Rietschel, C. Galanos and O. Lüderitz, Tetrahedron Lett. 26, 907-908 (1985).
2. M. Imoto, H. Yoshimura, T. Shimamoto, N. Sakaguchi, S. Kusumoto and T. Shiba, Tetrahedron Lett. 26, 1545-1548 (1985) and Bull. Chem. Soc. Jpn. 60, 2205-2214 (1987).
3. C. Galanos, O. Lüderitz, E.T. Rietschel, O. Westphal, H. Brade, L. Brade, M. Freudenberg, U. Schade, M. Imoto, H. Yoshimura, S. Kusumoto and T. Shiba, Eur. J. Biochem. 148, 1-5 (1985).
4. S. Kotani, H. Takada, I. Takahashi, M. Tsujimoto, T. Ogawa, T. Ikeda, K. Harada, H. Okamura, T. Tamura, S. Tanaka, T. Shiba, S. Kusumoto, M. Imoto, H. Yoshimura and N. Kasai, Infect. Immun., 52, 872-884 (1986).
5. E.T. Rietschel, L. Brade, U. Schade, C. Galanos, M. Freudenberg, O. Lüderitz, S. Kusumoto and T. Shiba, Eur. J. Biochem., 169, 27-31 (1987).
6. E.T. Rietschel, L. Brade, U. Schade, U. Seydel, U. Zähringer, S. Kusumoto and H. Brade, Surface Structure of Microorganisms and Their Interaction with Mammalian Host, Verlag Chemie, Weinheim (1988).
7. U. Zähringer, B. Lindner, U. Seydel, E.T. Rietschel, H. Naoki, F.M. Unger, M. Imoto, S. Kusumoto and T. Shiba, Tetrahedron Lett. 26 6321-6324 (1985).
8. M. Imoto, S. Kusumoto and T. Shiba, Tetrahedron Lett., 28, 6235-6238 (1987).
9. M. Imoto, N. Kusunose, Y. Matsuura, S. Kusumoto and T. Shiba, Tetrahedron Lett., 28, 6277-6280 (1987).