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SYNTHETIC APPROACH TO BACTERIAL LIPOPOLYSACCHARIDE, PREPARATION OF TRISACCHARIDE PART STRUCTURES CONTAINING KDO AND 1-DEPHOSPHO LIPID A

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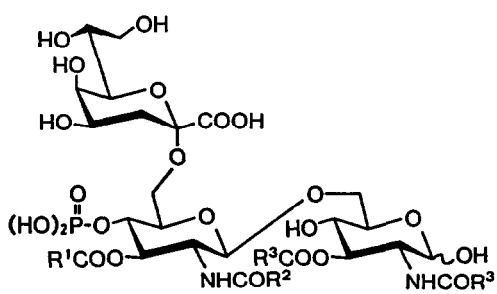
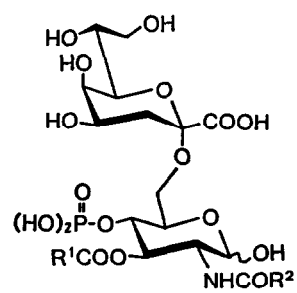
Summary: Title compounds (**1a,b**) and an analogous disaccharide (**2a**) were synthesized via a reaction of a ketosidic fluoride of 3-deoxy-D-manno-2-octulosonic acid (KDO) (**3**) with phosphorylated acyl glucosamine di- and monosaccharides.

Lipopolysaccharide (LPS), which is a cell surface amphiphile characteristic of gram-negative bacteria, consists of covalently bound polysaccharide part and lipid part called lipid A. LPS not only is a cell wall component indispensable for survival of the bacteria themselves but also exhibits against higher animals potent toxic and other various biological activities related to immunostimulation. One of the main themes of LPS research to identify the chemical entity of its biological activity was finally solved by our total chemical synthesis of lipid A.[1] Thus, polyacylated β(1-6) disaccharide of D-glucosamine 1,4'-bisphosphate synthesized according to the proposed structure of Escherichia coli lipid A showed all expected typical activity of LPS mentioned above.[1] Recently, however, new evidences are accumulated suggesting that a polysaccharide region of LPS linked close to lipid A influences the biological activity of the latter.[2,3] The unique sugar component, 3-deoxy-D-manno-2-octulosonic acid (previously called 2-keto-3-deoxy-D-manno-octonic acid, KDO), which locates at the innermost position of the polysaccharide and hence is directly bound to lipid A, is assumed to be particularly important. This situation prompted us to start a new synthetic work on LPS in order to study the significance of KDO moieties on the biological activity of LPS.



1a,b



2a

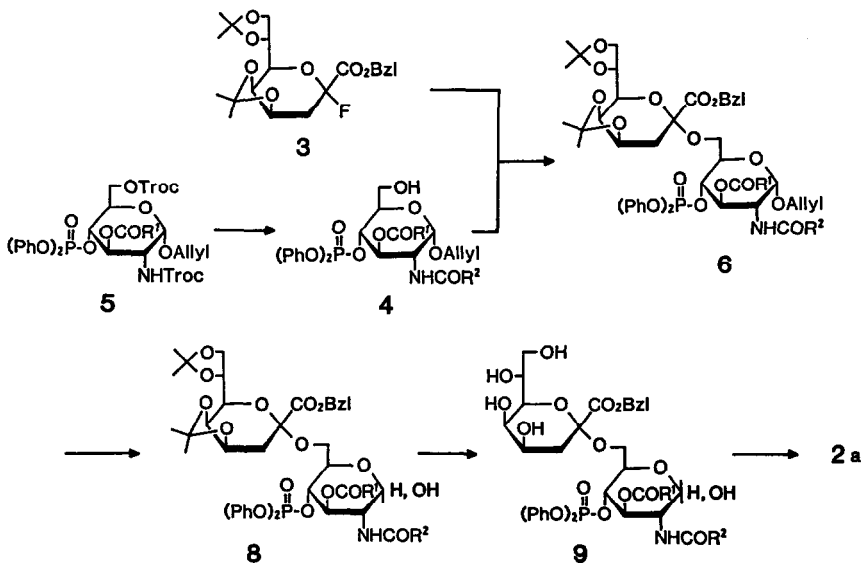
a : R^1CO = (R)-3-tetradecanoyloxytetradecanoyl
R^2CO = (R)-3-dodecanoyloxytetradecanoyl
R^3CO = (R)-3-hydroxytetradecanoyl

b : $R^1CO = R^2CO = R^3CO$ = (R)-3-hydroxytetradecanoyl

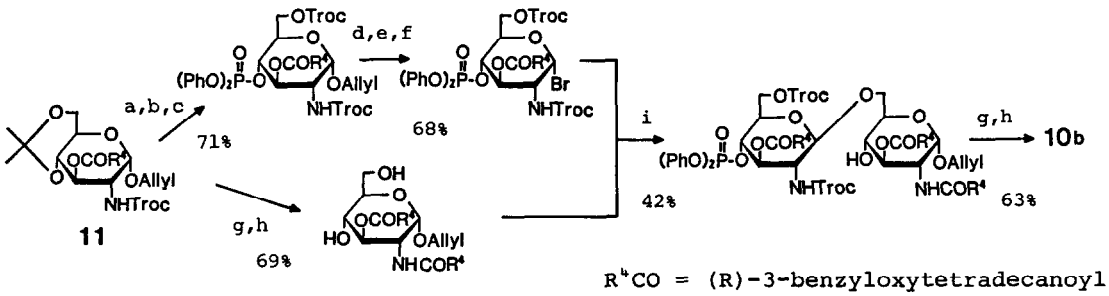
According to our previous structural determination of LPS of *E. coli* Re mutant,[4] KDO residues in LPS exist in the α-ketopyranosidic form and the first one is bound to the 6'-hydroxyl group of lipid A. In this communication we describe a synthesis of trisaccharide part structures of LPS (**1a** and **b**) containing one KDO and 1-dephospho lipid A.[5] Its disaccharide analog **2a** lacking the reducing glucosamine residue of **1a** was also prepared.

For the construction of the structure of **1** and **2**, a protected KDO fluoride **3** prepared previously was employed.[6] Coupling conditions of **3** and the subsequent deprotection procedures of the product were first examined using a monosaccharide acceptor **4**. This compound **4** corresponding to the non-reducing half of lipid A was prepared from a known synthetic intermediate **5**[1] by conventional procedures [i) Zn powder - AcOH, ii) Et_3N, iii) (R)-3-dodecanoyloxytetradecanoic acid - DCC, 59% as syrup].[7] The fluoride **3** and the acceptor **4** were treated with boron trifluoride etherate in the presence of ethyldiisopropylamine (in CH_2Cl_2 at 0°C),[6,8,9] to give the desired **6** (68%, syrup),[7,10] whose disaccharide structure and the α-ketosidic configuration were confirmed by ^1H NMR.[11] TLC analysis of the reaction mixture indicated that the undesired corresponding β-anomer was formed only in a trace amount.[6] Therefore it could not be characterized.

Deprotection of **6** was carried out in the following sequence. The allyl group was removed in two steps [i) isomerization to 1-propenyl group with an Ir complex, $[Ir(COD)(PCH_3(C_6H_5)_2)_2]PF_6$, ii) cleavage with I_2 in aqueous THF][1,12,13] to give **8** (93%, syrup).[7] The isopropylidene groups were then cleaved by treating **8** with a mixture (1:30) of 95% aqueous trifluoroacetic acid (TFA) and CH_2Cl_2 at room temperature for 10 min. Deisopropylidenation proceeded smoothly without effecting the acid-labile ketosidic linkage to afford **9** in a quantitative yield.[7] The benzyl and phenyl groups in **9** were then removed by stepwise hydrogenolysis in THF first with palladium and then with platinum, respectively. The final product was purified successively by electrodialysis and precipitation with cold hydrochloric acid from an aqueous solution of the triethylammonium salt.[14] Lyophilization from water afforded the free acid form of **2a** as colorless powder.[15] The structure of **2a** was confirmed by ^1H NMR after conversion into the trimethyl ester with diazomethane.[16]

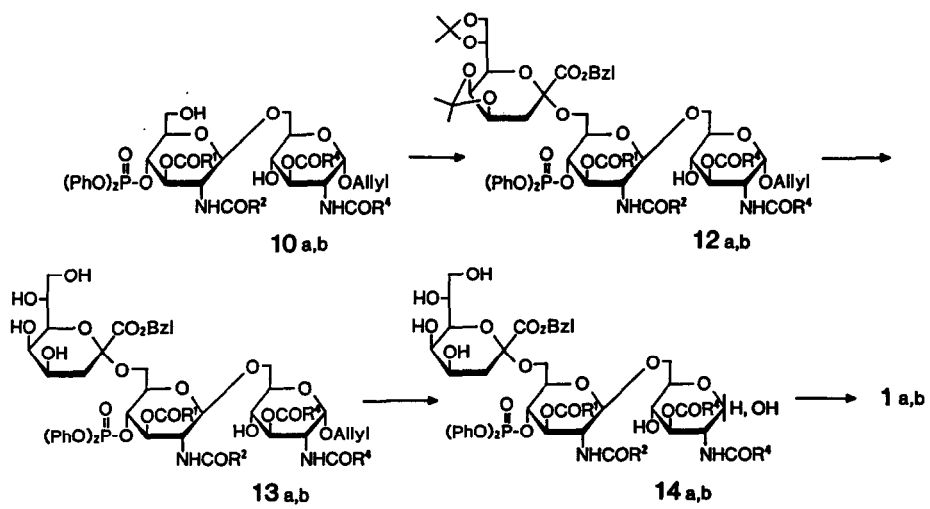


On the basis of above successful preparation of **2a**, the synthesis of **1a** and **b** was then undertaken by using two disaccharide acceptors **10a** and **b** for the coupling with the KDO fluoride **3**. The acylation patterns of these disaccharides correspond to those of E. coli-type lipid A and a biosynthetic precursor of lipid A (precursor Ia)[17] respectively.[18] The former acceptor (**10a**) was already available as an intermediate of our total synthesis of lipid A.[1] The latter (**10b**) was obtained from another early intermediate **11** in a manner similar to the preparation of **10a** as illustrated in the scheme.[7]



a) 90% AcOH, b) 2,2,2-trichloroethoxycarbonyl chloride (TrocCl) - pyridine,
c) $(PhO)_2POCl$ - DMAP, d) $[Ir(COD)(PCH_3Ph_2)_2]PF_6$, d) I_2 - aq.THF,
f) $SOBr_2$ - DMF, g) Zn - AcOH, h) R^4CO_2H - dicyclohexylcarbodiimide

Condensation of **3** with **10a** was effected as above (with ethyldiisopropylamine and boron trifluoride etherate in CH_2Cl_2 under Ar at 0°C). Purification by silica-gel column chromatography ($CHCl_3$-MeOH, 20:1) after removal of the isopropylidene groups gave the trisaccharide **13a** (56%, syrup).[7] Cleavage of the allyl protecting group as above followed by chromatographic purification (silica gel, $CHCl_3$-acetone 1:1) gave **14a**,[7] which was then hydrogenolyzed in two steps to afford **1a**. It was purified by precipitation with cold hydrochloric acid from an aqueous solution of the triethylammonium salt (51% from **14a**, colorless powder after lyophilization from water).[19] Compound **1b** was prepared similarly as shown in the scheme. Purification as above gave **1b** as colorless powder.[20]



The compounds **1a,b** and **2a** obtained by the present work are being tested for their antigenic and other biological activities by our collaborative group in order to elucidate the role of KDO moiety. The result will be published separately elsewhere.

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References and Notes

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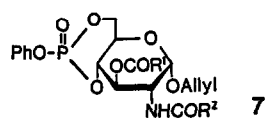
2) E. Th. Rietschel, L. Brade, U. Schade, U. Seydel, U. Zähringer, S. Kusumoto, and H. Brade, "Bacterial Endotoxins: Properties and Structure of Biologically Active Domains" in "Surface Structure of Microorganisms and Their Interaction with Mammalian Host" ed. by U. Schwartz and M. Richmond, Verlag Chemie, Weinheim (1988).
3) Until recently the polysaccharide part was assumed to represent only the serological specificity of the individual bacterial species.
4) LPS of Re mutant has a very simple structure lacking most of the polysaccharide part and comprises only two moles of KDO and lipid A. U. Zähringer, B. Lindner, U. Seydel, E. Th. Rietschel, H. Naoki, F. M. Unger, M. Imoto, S. Kusumoto, and T. Shiba, Tetrahedron Lett., **26**, 6321 (1985).
5) Paulsen and Schüller recently reported synthesis of carbohydrate backbone of Re LPS which contains a disaccharide of KDO, i.e., α-KDO-(2→4)-α-KDO-(2→6)-β-D-GlcN-(1→6)-D-GlcN, and its N,N'-diacyl derivative. However, these compounds lack the phosphate and all O-acyl groups which are essential for the expression of biological activity. H. Paulsen and M. Schüller, Liebigs Ann. Chem. **1987**, 249. See also H. Paulsen, M. Stiem, and F. Unger, ibid., **1987**, 273.
6) The fluoride **3** was shown to give preferentially the desired α-ketosides of KDO. M. Imoto, N. Kusunose, Y. Matsuura, S. Kusumoto, and T. Shiba, Tetrahedron Lett., **28**, 6277 (1987).
7) Most of the intermediates were obtained as syrup or amorphous solid after purification by silica-gel column chromatography ($CHCl_3$-MeOH or $CHCl_3$-acetone). The purity of each product was checked by TLC analysis and the structure confirmed by ^1H NMR and elemental analysis.
8) H. Kunz and W. Sager, Helv. Chim. Acta, **68**, 283 (1985).
9) The molar ratio of the reactants are as follows: **3**:**4**:boron trifluoride:amine, 1:0.65:1.5:1.
10) When condensation of **3** and **4** was carried out in the presence of triethylamine in place of ethyldiisopropylamine as in the previous works (ref. 6 and 8), no KDO glycoside **6** was formed because base induced cyclization of **4** to form **7** occurred much faster than the desired glycosidation reaction. This side reaction could be suppressed by using more bulky ethyldiisopropylamine.



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11) ^1H NMR of **6** (100MHz, $CDCl_3$): δ 1.84 (1H, dd, J=15.9, 3.1Hz, H_{3ax}) and 2.70 (1H, dd, J=15.9, 3.9Hz, H_{3eq}). These values assures the α-ketosidic configuration (see ref. 6). Intensities of other signals are also in good agreement with those expected.
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) Conditions for the acid precipitation and electrodialysis were the same as described previously in ref. 1).
15) Compound **2a**: $[\alpha]_D^{21}$ +13.7° (c 0.50, $CHCl_3$-MeOH 9:1). Elemental analysis, found: C, 61.37; H, 9.41; N, 1.12%. Calcd for $C_{68}H_{126}NO_{21}P$: C, 61.65; H, 9.59; N, 1.06%.
16) ^1H NMR of **2** trimethyl ester (500MHz, $CDCl_3$-DMSO-d_6 95:5): δ 0.85 (12H, t, CH_3 of acyl groups), 3.31 (3H, s, CO_2CH_3) and 3.74 (6H,d, $PO(OCH_3)_2$).
17) M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto, and T. Shiba, Bull. Chem. Soc. Jpn., **60**, 2197 (1987) and references in it.
18) Comparison of biological activity of **1a** and **b** is expected to be of interest because the corresponding two lipid A's exhibit distinctly different activity from each other. See ref. 1).
19) Compound **1a**: $[\alpha]_D^{18}$ +9.0° (c 0.50, $CHCl_3$-MeOH 9:1). Elemental analysis, found: C, 63.30; H,10.02; N, 1.31%. Calcd for $C_{102}H_{189}N_2O_{29}P$: C, 63.20; H, 9.83; N, 1.45%.
20) Compound **1b**: $[\alpha]_D^{21}$ -2.6° (c 0.50, $CHCl_3$-MeOH 9:1). Elemental analysis, found: C, 58.82; H, 9.03; N, 1.69%. Calcd for $C_{76}H_{141}N_2O_{27}P$: C, 59.05; H, 9.19; N, 1.81%.

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