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Synthesis of an Analog of Biosynthetic Precursor Ia of Lipid A by an Improved Method: A Novel Antagonist Containing Four (*S*)-3-Hydroxy Fatty Acids

Koichi Fukase[a], Wen-Chi Liu[a], Yasuo Suda[a], Masato Oikawa[a], Akira Wada[a], Saeko Mori[a], Arthur J. Ulmer[b], Ernst Th. Rietschel[b], and Shoichi Kusumoto[a]*

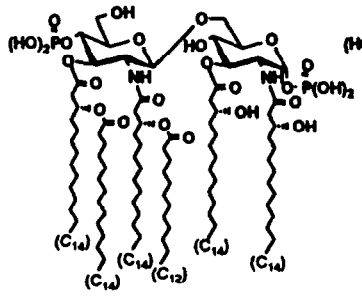
[a]Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

[b]Forschungsinstitut Borstel, Parkallee 22, 23845, Borstel, Germany

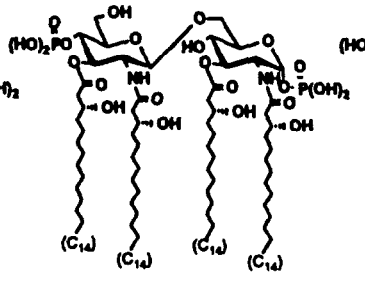
Abstract: Synthesis of an analog of a biosynthetic precursor of lipid A containing four (*S*)-3-hydroxytetradecanoic acids was effected via an improved synthetic route to investigate the relationship between the bioactivity and the chirality of the 3-hydroxy fatty acid residues in lipid A. The *S*-analog inhibited endotoxin-induced interleukin-6 (IL-6) production from human peripheral whole blood cells more strongly than the natural-type biosynthetic precursor with (*R*)-hydroxy acids, a known antagonist of LPS-induced cytokine release in human peripheral blood mononuclear cells.

Lipopolysaccharide (LPS), also termed endotoxin, is a cell surface amphiphile characteristic of Gram-negative bacteria. LPS induces various cytokines to exhibit potent toxic activity such as pyrogenicity or lethality as well as beneficial activities such as antitumor activity.[1] LPS consists of a glycolipid component called lipid A covalently bound to a polysaccharide portion. Our previous total synthesis of *Escherichia coli* lipid A **1** unequivocally demonstrated that lipid A is the chemical entity responsible for the typical biological activities of LPS.[2,3] Although the number and the structure of fatty acids in lipid A strongly influence its biological activities,[1,4,5] the relationship between the bioactivity and the chirality of 3-hydroxy fatty acids has not been studied yet. We, therefore, initiated the synthesis of unnatural-type lipid A analogs containing (*S*)-3-hydroxy fatty acids.

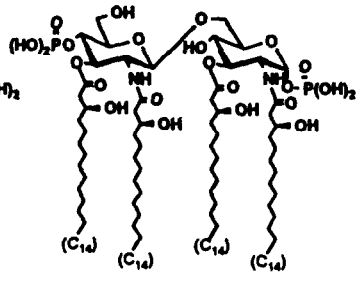
We have previously found that a biological precursor ("precursor Ia") (**2**) of lipid A inhibited the LPS-induced production of various cytokines from human peripheral blood mononuclear cells.[4] This compound contains four moles of (*R*)-3-hydroxytetradecanoic acid but lacks non-hydroxylated acids present in **1**. Endotoxin antagonists are important as a tool to investigate the mechanism of LPS action and as pharmaceutical agents for treatment of endotoxin-related diseases such as endotoxin-shock.[1,5,6] We synthesized in the present study a new analog **3** of precursor Ia containing four (*S*)-3-hydroxytetradecanoic acids.



Escherichia coli lipid A **1**

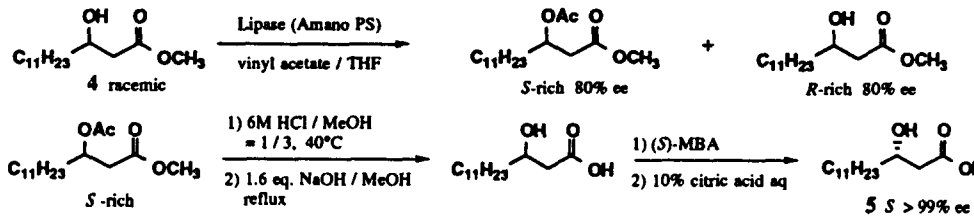


precursor Ia **2**



S-analog **3**

(*S*)-3-Hydroxytetradecanoic acid (**5**) was prepared through Lipase-catalyzed transesterification[7] of racemic methyl 3-hydroxytetradecanoate (**4**).[8] The enzyme reaction was stopped when the 50% of the starting racemic **4** was transformed, and then the *S*-rich acetylated product was separated by silica-gel column chromatography. The *O*-acetyl group and methyl ester were then removed by acidic and alkaline hydrolysis, respectively. Fractional crystallization of the resulting *S*-rich hydroxy fatty acid as (*S*)-(-)-α-methylbenzyl amine (MBA) salt for two times gave the optically pure (*S*)-3-hydroxytetradecanoic acid (**5**) [$[\alpha]_D^{24}$ = +15.4 (c 1.00, $CHCl_3$)] in 25% yield from racemic **4**.



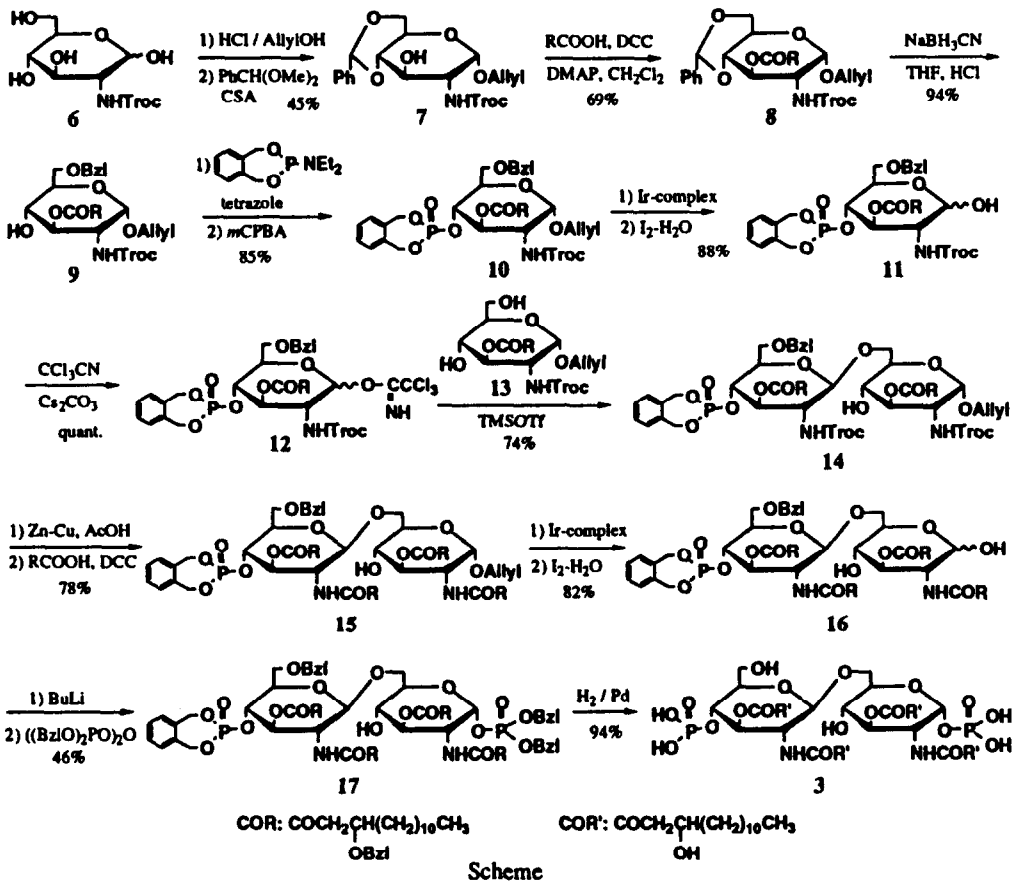
The synthesis of *S*-analog **3** was carried out via an improved synthetic route as follows. The coupling of the two monosaccharides was accomplished by use of a glycosyl trichloroacetimidate as a donor to give the desired β(1→6) disaccharide in a higher yield than the Königs-Knorr and oxazoline methods in our previous works.[2,3,9] The *o*-xylidene group was employed in place of the phenyl group in the previous work for protection of the 4'-phosphate.[10] The *o*-xylidene group is stable enough through the sequence of transformation and can be readily removed by catalytic hydrogenolysis simultaneously with the benzyl groups. The removal of all the protecting groups at the final step was thus effected in one single step of hydrogenolysis.

The glycosyl donor **12** was prepared from *N*-(2,2,2-trichloroethoxycarbonyl)-D-glucosamine (*N*-Troc-D-glucosamine, **6**). Allyl glycosidation and subsequent benzylidenation gave a 4,6-*O*-protected α-allyl glycoside **7**. After 3-*O*-acylation of **7** with (*S*)-3-benzyloxytetradecanoic acid prepared from **5** in a manner similar to the preparation of the corresponding (*R*)-isomer,[2,3] regioselective reductive cleavage of the benzylidene group in **8** gave 6-*O*-protected **9**. Phosphitylation of the 4'-hydroxyl group in **9** with 2-diethylamino-1,3,2-benzodioxaphosphepane proceeded smoothly in the presence of tetrazole.[10] The resulting phosphite was oxidized with *m*CPBA to give the phosphate **10** in good yield. The allyl group in **10** was cleaved to give **11**.[11,12] Compound **11** was allowed to react with CCl_3CN in the presence of Cs_2CO_3[13] as a catalyst to give glycosyl trichloroacetimidate **12** to be used as the donor. The glycosyl acceptor **13** was prepared from 4,6-*O*-isopropylidenated allyl glycoside of *N*-Troc-D-glucosamine via 3-*O*-acylation followed by removal of the isopropylidene group. Coupling of **12** with **13** was effected by use of trimethylsilyl triflate (TMSOTf) in 1,2-dichloroethane to give the disaccharide **14** in good yield: the β(1→6) structure of **14** was confirmed by ^1H-^1H COSY and HMBC spectra. After cleavage of the Troc groups of **14**, two moles of (*S*)-benzyloxytetradecanoic acid were introduced to the 2- and 2'-amino groups of the product. The allyl group in fully acylated **15** was then cleaved to give **16** which possesses a free 1-hydroxyl group. 1α-*O*-Phosphorylation of **16** was carried out via 1-*O*-lithiation with BuLi and subsequent treatment with tetrabenzyl diphosphate as in our previous synthetic study.[2,3,9,14] The protected 1,4'-bisphosphate **17** was purified by silica-gel chromatography. Finally, one-step hydrogenolytic deprotection (7 kg/cm^2 of H_2) with Pd-black gave the desired free *S*-analog **3** in good yield, which was effectively purified by centrifugal partition chromatography (CPC) (butanol-THF-H_2O = 45 : 35 :100)

on a Model LLB-M apparatus (Sanki Engineering LTD, Kyoto, Japan).[15] The structure of **3** was confirmed by negative FAB-MS [m/z 1403.9 [(M-H)$^-$]].

In preliminary experiments, the activities of *S*-analog **3** and synthetic precursor Ia **2** to inhibit the induction of interleukin 6 (IL-6) by LPS was determined.[16] A mixture consisting of test sample, LPS (*E. coli* 0111:B4; Sigma Chemicals Co.), and heparinized human peripheral whole-blood collected from an adult volunteer in RPMI 1640 medium (Flow Laboratories, Irvine, Scotland) was incubated at 37°C in 5% CO_2 for 24 h. The levels of induced IL-6 were measured by means of enzyme-linked immunosorbent assay (ELISA). Both **3** and **2** effectively inhibited the IL-6 production induced by LPS as shown in Figure 1. Quite interestingly, the inhibitory activity of *S*-analog **3** was obviously stronger than that of *R*-form **2**.

S-Analog **3** of precursor Ia was successfully synthesized as described in 4.5% yield from *N*-Troc-D-glucosamine (**6**). Further detailed study on the bioactivity of **3** is currently under progress.



Scheme

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phosphepane. This work was supported in part by the Grant-in-Aid for Scientific Research on Priority Areas No. 06240105 from the Ministry of Education, Science and Culture, Japan.

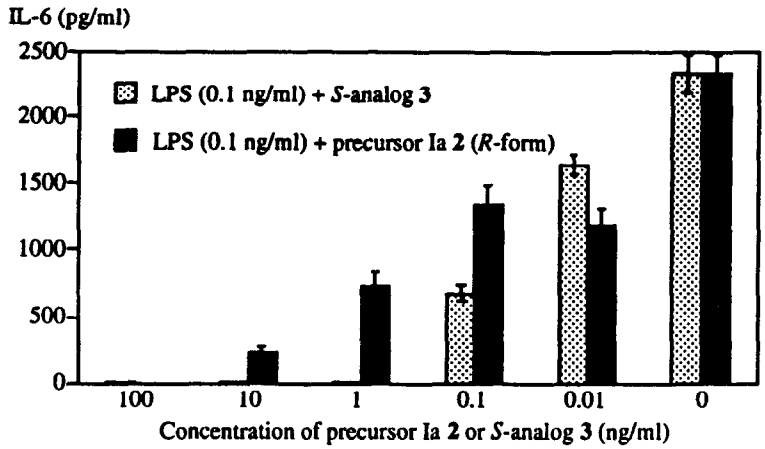

Figure 1. Inhibitory activity of (*R*)-fatty acid containing lipid A precursor Ia and its *S*-analog on the induction of interleukin-6 (IL-6) in human peripheral whole blood cells.

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