SYNTHESIS OF LIPID A ANALOGS CONTAINING VARIOUS FATTY ACIDS

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ABSTRACT

SYNTHESIS OF LIPID A ANALOGS CONTAINING VARIOUS FATTY ACIDS. The lipid A analogs, namely 2-deoxy-6-*O*-{2-deoxy-3-*O*-[(R)-3-hydroxydecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl}-3-*O*-[(R)-3-hydroxydecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-α-D-glucopyranose 1,4'-bis(phosphate) and 2-deoxy-6-*O*-[2-deoxy-3-*O*-[(R)-3-hydroxytetradecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl]-3-*O*-[(R)-3-hydroxytetradecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-α-D-glucopyranose 1,4'-bis(phosphate) have been synthesized through the coupling reaction of intermediates allyl 3-*O*-[(R)-3-hydroxyalkanoyl]-2-deoxy-2-Troc-α-D-glucopyranose as glycosyl acceptor and glycosyl trichloroacetimidates as glycosyl donor in the presence of catalyst. The coupling reaction was performed at -20°C for 5 minute. After deprotection of Troc groups, the fatty acids were substituted to both N-amino groups followed by introduction of phosphate groups to 1-position, and then deprotection of protecting groups by catalytic hydrogenation. The products were purified using centrifugal partition chromatography (CPC) to get the white crystal in 15% and 17 % yield respectively.

ABSTRAK

SINTESIS ANALOG LIPID A MENGANDUNG BERBAGAI ASAM LEMAK. Analog lipid A, yaitu 2-deoksi-6-*O*-{2-deoksi-3-*O*-[(R)-3-hidroksidekanoil]-2-[(R)-3-hidroksitetradekanoilamino]-β-D-glukopiranosil}-3-*O*-[(R)-3-hidroksidekanoil]-2-[(R)-3-hidroksitetradekanoilamino]-α-D-glukopiranosa 1,4'-bis(fosfat) dan 2-deoksi-6-*O*-{2-deoksi-3-*O*-[(R)-3-hidroksitetradekanoil]-2-[(R)-3-hidroksitetradekanoilamino]-β-D-glukopiranosil}-3-*O*-[(R)-3-hidroksitetradekanoil]-2-[(R)-3-hidroksitetradekanoilamino]-α-D-glukopiranosa 1,4'-bis(fosfat) telah disintesis melalui reaksi kopling senyawa antara allil 3-*O*-[(R)-3-hidroksialkanoil]-2-deoksi-2-Troc-α-D-glukopiranosa sebagai glikosil akseptor dan glikosil trikloroasetimidat sebagai glikosil donor dengan adanya katalisator. Reaksi dilakukan pada suhu -20°C selama 5 menit. Setelah pelepasan gugus Troc, dilakukan substitusi asam lemak pada kedua gugus N-amino, dilanjutkan dengan pemasukan gugus fosfat pada posisi-1, dan kemudian pelepasan gugus pelindung secara hidrogenasi katalitik. Produk yang diperoleh dimurnikan dengan cara kromatografi partisi sentrifugal, diperoleh kristal berwarna putih dengan rendemen masing-masing 15% dan 17%.

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INTRODUCTION

Lipopolysaccharide (LPS), known as endotoxin is a very complex molecule noncovalently bound to and covers a cell surface of Gram-negative bacteria. It is known that LPS exerts toxic such as, induction of high fever, hypotension, lethal shock and so on. Paradoxically, the LPS threatening human health gives beneficial effects. LPS can enhance the body's overall immune response to bacterial or viral infections and to cancer [1]. The structure of LPS consists of three distinct parts. The outermost part is composed of a polysaccharide chain with repeating units of variable length and constitutes, which forms so called *O*-antigen. Next to the *O*-antigen polysaccharide is located a relatively invariable oligosaccharide, which is classified as core region. The core oligosaccharide is linked to a complex hydrophobic component, called lipid A, via unique 8-carbon sugar, 3-deoxy-D-manno-2-octulosonic acid (KDO) (Fig. 1) [2].

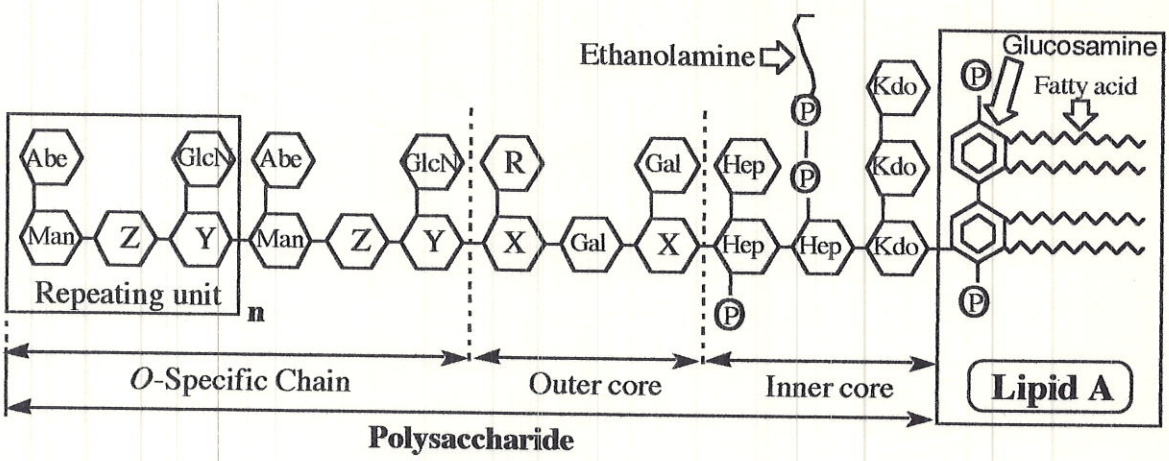


Figure 1. Chemical Structure of LPS from *Salmonlla typhymurium*
(⬡ : sugar; P : phosphate; Kdo : 3-deoxy-D-manno-2-octulosonic acid)

In 1954, WESTPHAL *etal.* isolated the lipid A by mild acid hydrolysis of LPS [3]. Since then, many works demonstrated that lipid A seemed to be **the active center** and **responsible** to **the endotoxic effect** of Gram-negative bacteria. Nevertheless, the structure of LPS had remained unknown for over 20 years, finally, it was determined by SHIBA *et al.* in 1980s through a synthetic approaches [4-7].

The chemical structure of lipid A's from various bacterial species are almost identical. The basic structure contains β-1,6-linked D-glucosamine disaccharide with phosphates at the positions 1 and 4' glucosamine residues and fatty acids at positions 2, 2'-N-amino groups and 3, 3' of the

glucosamine residues (Fig. 2) [8]. IMOTO *et al.* [9-11] reported that the structure of lipid A from *Eschericia coli* Re-mutant as 2-deoxy-6-*O*--{2-deoxy-2-[(R)-3-dodecanoyloxytetradecanoyl-amino]-3-*O*-[(R)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl}-3-*O*-[(R)-3-hydroxy-tetradecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-α-D-glucopyranose 1,4'-bis(phosphate) (compound **506**; Table 1, No.1), which was subsequently confirmed by a total synthesis. The synthetic **506** was then shown by GALANOS *et al.*[12] to exhibit biological activity with that of natural lipid A from *E. coli* . The same research group also prepared another analogs (Table 1, No. 2-10) which corresponds to a biosynthetic precursor of lipid A.

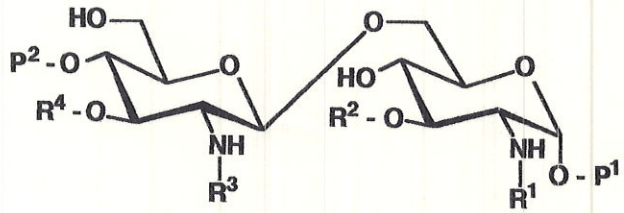


Figure 2. Chemical structure of disaccharide lipid A antigens.
(See also Table 1)

Table 1. Synthetic disaccharide lipid A antigens[a].

No.	Compound	R^4	R^3	R^2	R^1	P^1	P^2
1	506	C_{14}-O-C_{14}	C_{14}-O-C_{12}	C_{14}-OH	C_{14}-OH	$PO(OH)_2$	$PO(OH)_2$
2	505	C_{14}-O-C_{14}	C_{14}-O-C_{12}	C_{14}-OH	C_{14}-OH	$PO(OH)_2$	H
3	504	C_{14}-O-C_{14}	C_{14}-O-C_{12}	C_{14}-OH	C_{14}-OH	H	$PO(OH)_2$
4	503	C_{14}-O-C_{14}	C_{14}-O-C_{12}	C_{14}-OH	C_{14}-OH	H	H
5	LA-24-PP	C_{10}	C_{14}-OH	C_{14}-OH	C_{10}	$PO(OH)_2$	$PO(OH)_2$
6	LA-23-PP	C_{10}	C_{10}	C_{10}	C_{10}	$PO(OH)_2$	$PO(OH)_2$
7	403	C_{14}-OH	C_{14}-OH	C_{14}-OH	C_{14}-OH	H	H
8	404	C_{14}-OH	C_{14}-OH	C_{14}-OH	C_{14}-OH	H	$PO(OH)_2$
9	405	C_{14}-OH	C_{14}-OH	C_{14}-OH	C_{14}-OH	$PO(OH)_2$	H
(10)	**406**	**C_{14}-OH**	**C_{14}-OH**	**C_{14}-OH**	**C_{14}-OH**	**$PO(OH)_2$**	**$PO(OH)_2$**
(11)	**400**	**C_{10}-OH**	**C_{14}-OH**	**C_{10}-OH**	**C_{14}-OH**	**$PO(OH)_2$**	**$PO(OH)_2$**

[a] See structure in Fig. 2.
C_{10} : decanoyl
C_{10}-OH : (R)-3-hydroxydecanoyl
C_{14}-OH : (R)-3-hydroxytetradecanoyl
C_{14}-O-C_{14} : (R)-3-tetradecanoyloxytetradecanoyl

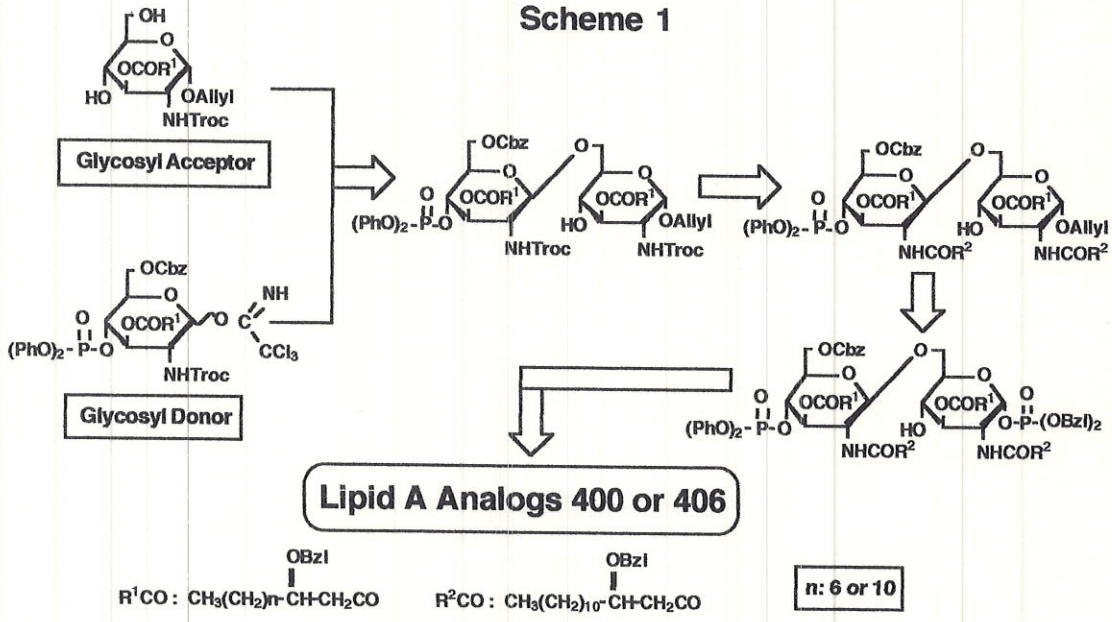
From the previous study on biological activities of many synthetic lipid A analogs containing different number and combination of fatty acids, it was shown that analog **506** was able to induce cytokine production from cultured human monocytes. The analog **406** did not induce cytokine, but on the contrary, suppressed their production from human monocytes. The other analogs

showed various activities depending on the number and kind of the fatty acids and phosphate moieties present in the molecules [13-15].

Those results stimulated to prepare another kind of lipid A analog, namely **400**, which contains only 4 moles of 3-hydroxy fatty acids like the biosynthetic precursor **406**. Two of the four fatty acids possesses shorter lipophilic chains with ten carbon atom, i.e., 2,2'-N-bis[(R)-3-hydroxydecanoyl]-3,3'-*O*- bis[(R)-3-hydroxytetradecanoyl]-2,2'-dideoxy β-(1→6) D-glucosamine disaccharide α-1,4'-bis(phosphate) (Fig. 2, No. 11). It was expected that the biological test of this new analog will give more information about **the effect of the fatty acid moiety** on the biological activities of lipid A. In addition, it has been decided to prepare analog **406** with a similar procedure, since more detailed study of its biological activity was required.

The synthetic plan shown in Scheme 1 includes the following steps: (i) Glycosidation to form the β(1→6) linked disaccharide; (ii) Introduction of (*R*)-3-benzyloxytetradecanoic acid to both amino groups in the disaccharide; (iii) Phosphorylation at 1-position of the reducing end glucosamine residues; (iv) The final hydrogenolytic deprotection.

Scheme 1



MATERIALS AND METHODS

Materials. The starting materials consisted of: allyl 3-*O*-[(R)-3-benzyloxydecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside and allyl 3-*O*-[(R)-3-

benzyloxytetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside as glycosyl acceptor which were prepared from D-glucosamine hydrochloride as reported previously [16], 6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-benzyloxydecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl trichloroacetimidate 4-(diphenyl phosphate) and 6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-benzyloxytetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxy-carbonylamino)-D-glucopyranosyl trichloroacetimidate 4-(diphenyl phosphate) which were prepared from glycosyl acceptor according to the reported procedure [17], (R)-3-benzyloxytetradecanoic acid which was prepared from β-ketoester according to the reported procedure [18], dibenzylphosphate, n-butyl lithium, tetramethylsillyl triflouromethnesulfonate, molecular sieves 4A, zinc dust, acetic acid, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), 1,5-cyclooctadiene bis(methyldiphenylphosphine) iridium hexafluorophosphate, iodine, hydrogen gas, palladium, platinum oxide, triethylamine, HPTLC Kieselgel 60 F_{254}, and Kieselgel 60 (ϕ 0,040-0,063 mm). The anhydrous solvents used were dichloromethane, dichloroethane, chloroform, tetrahydrofuran, toluene, methanol, water, and isobutyl alcohol.

Equipment. The equipment used were reaction flask equipped with magnetic stirrer and cooler connected to a balloon containing nitrogen, rotary evaporator with vacuum line, column chromatography equipped with fraction collector, polarimeter Perkin-Elmer 241 model, recycling preparative HPLC model LC-908 with JAIGEL-2H column, elemental analyzer, JEOL JNM-EX270 and JNM-GX500 NMR spectrometer, and JMS-SX102 mass spectrometer.

Procedures.

1). Preparation of **allyl 3-*O*-[(R)-3-Benzyloxydecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-6-*O*-{6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-bezyloxydecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-α-D-glucopyranoside 4'-(diphenyl phosphate) (3a)**

To a solution of allyl 3-*O*-[(R)-3-benzyloxydecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxy-carbonylamino)-α-D-glucopyranoside **1a** (600 mg, 916 μmol) in absolute dichloroethane (10.0 ml) was added molecular sieves 4A and stirred at -20°C for 5 min. To this solution were added 6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-benzyloxydecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl-amino)-D-glucopyranosyl trichloroacetimidate 4-(diphenyl phosphate) **2a** (860 mg, 764 μmol)

and trimethylsilyl trifluoromethanesulfonate (9 µl, 46 µmol), and the reaction was performed for 5 min with stirring at -20°C. After removal of molecular sieves by filtration, the reaction solution was neutralized with triethylamine, and concentrated under reduced pressure. Purification of the product by recycling preparative HPLC, model LC-908 (column: JAIGEL-2H, 2.3 x 60 cm; solvent: chloroform; flow rate: 3.5 ml/min; retention time: 24.8 min).

2). Preparation of **allyl 3-*O*-[(R)-3-benzyloxytetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-6-*O*-{6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-bezyloxytetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-α-D-glucopyranoside 4'-(diphenyl phosphate) (3b)**

To a solution of allyl 3-*O*-[(R)-3-Benzyloxytetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside **1b** (2.4 g, 2.9 mmol) in absolute dichloroethane (50 ml) was added molecular sieves 4A and stirred at -20°C for 5 min. To this solution were added 6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-benzyloxytetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl trichloroacetimidate 4-(diphenyl phosphate) **2b** (3.5 g, 3.0 mmol) and trimethylsilyl trifluoromethanesulfonate (50 µl, 0.2 mmol), and the reaction was performed for 5 min with stirring at -20°C. After removal of molecular sieves by filtration, the reaction solution was neutralized with triethylamine, and concentrated under reduced pressure. Purification of the product by recycling preparative HPLC, model LC-908 (column: JAIGEL-2H, 2.3 x 60 cm; solvent: chloroform; flow rate: 3.5 ml/min; retention time: 24.6 min).

3). Preparation of **allyl 3-*O*-[(R)-3-Benzyloxydecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino]-2-deoxy-6-*O*-[6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-benzyloxydecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino]-2-deoxy-β-D-glucopyranosyl]-α-D-glucopyranoside 4'-(diphenyl phosphate) (4a)**

To the compound **3a** (350 mg, 217 µmol) in acetic acid (5 ml) was added zinc dust and the mixture was stirred at room temperature for 30 min. The catalyst was filtered off, followed by removing of the solvent by coevaporation with toluene three times. The crude product was dissolved in chloroform and washed with 1M HCl. The organic layer was dried and concentrated under reduced pressure, then obtained product (275 mg) was dissolved in anhydrous dichloromethane (5 ml) and stirred with triethylamine (63 µl, 452 µmol) for 30 min. To this reaction mixture were then added EDC·HCl (96 mg, 615 µmol) and (R)-3-

benzyloxytetradecanoic acid (206 mg, 616 μmol). The mixture was stirred at room temperature for 20 h and then work up as usual. The obtained crude product then purified by recycling preparative HPLC, model LC-908 (column: JAIGEL-2H, 2.3x60 cm, solvent: chloroform, flow rate: 3.5 ml/min).

4). Preparation of **allyl 3-*O*-[(R)-3-benzyloxytetradecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino]-2-deoxy-6-*O*-{6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-benzyloxytetradecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino]-2-deoxy-β-D-glucopyranosyl}-α-D-glucopyranoside 4'-(diphenyl phosphate) (4b)**

To the compound **3b** (1.5 g, 0.87 mmol) in acetic acid (15 ml) was added zinc dust (3.5 g, 53 mmol) and the mixture was stirred at room temperature for 30 min. The catalyst was filtered off, followed by removing of the solvent by coevaporation with toluene three times. The crude product was dissolved in chloroform and washed with 1M HCl. The organic layer was dried and concentrated under reduced pressure, then obtained product (1.1 g) was dissolved in anhydrous dichloromethane (10 ml) and stirred with triethylamine (200 μl, 1.6 mmol) for 30 min. To this reaction mixture were then added EDC·HCl (295 mg, 1.9 mmol) and (R)-3-benzyloxytetradecanoic acid (0.61 g,1.8 mmol). The mixture was stirred at room temperature for 20 h and then work up as usual. The obtained crude product then purified by recycling preparative HPLC, model LC-908 (column: JAIGEL-2H, 2.3x60 cm, solvent: chloroform, flow rate; 3.5 ml/min).

5). Preparation of **3-*O*-[(R)-3-benzyloxydecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino]-6-*O*-{6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-benzyloxydecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino]-2-deoxy-β-D-glucopyranosyl}-2-deoxy-α-D-glucopyranose 4'-(diphenyl phosphate) (5a)**

To a degassed solution of **4a** (115 mg, 61 μmol) in THF (5 ml) was added 1,5-cyclooctadiene bis(methyldiphenylphosphine) iridium hexaflurophosphate (10 mg). After activation of the catalyst with hydrogen, the mixture was stirred for 15 min, then iodine (31 mg, 122 μmol, dissolved in 2.0 ml of THF) and water (0.5 ml) were added and the reaction mixture was stirred for additional 20 min. To the mixture was added 5% aqueous sodium thiosulfate. After extraction with chloroform, the solution was washed with water, then dried over $MgSO_4$.

After removal of the solvent, the crude product obtained was purified by silica gel column chromatography (15 g, chloroform/acetone : 20:1).

6). Preparation of **3-*O*-[(R)-3-benzyloxytetradecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino]-6-*O*-{6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-benzyloxytetradecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino]-2-deoxy-β-D-glucopyranosyl}-2-deoxy-α-D-glucopyranoside 4'-(diphenyl phosphate) (5b)**

To a degassed solution of **4b** (1.1 g, 0.56 mmol) in THF (15 ml) was added 1,5-cyclooctadiene bis(methyldiphenylphosphine) iridium hexaflurophosphate (100 mg). After activation of the catalyst with hydrogen, the mixture was stirred for 15 min, then iodine (283 mg, 1.2 mmol, dissolved in 4.0 ml of THF) and water (2.0 ml) were added and the reaction mixture was done under the same conditions as described in the preparation of **5a**. The residue obtained was purified by silica gel column chromatography (50 g, chloroform/acetone : 20:1).

7). Preparation of **3-*O*-[(R)-3-benzyloxydecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino] -6-*O*-{6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-benzyloxydecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino]-2-deoxy-β-D-glucopyranosyl}-2-deoxy-α-D-glucopyranoside 1-(dibenzyl phosphate) 4'-(diphenyl phosphate) (6a)**

To the solution of **5a** (60 mg, 32.3 μmol) in anhydrous THF (4 ml) was added 15% n-buthyllithium in hexane (27 μl, 41.5 μmol) at -72°C. The mixture was stirred for 5 min then the solution of tetrabenzyl pyrophosphate in 2 ml of THF (23 mg, 42.6 μmol) was added and stirred for additional 5 min. The mixture was allowed gradually to room temperature, then neutralized with saturated $NaHCO_3$ and extracted with chloroform. After dried and evaporation of the solvent, the product was purified by recycling preparative HPLC, model LC-908 (column: JAIGEL-2H, 2.3 x 60 cm; solvent: chloroform; flow rate: 3.5 ml/min).

8). Preparation of **3-*O*-[(R)-3-benzyloxytetradecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino]-6-*O*-{6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-benzyloxytetradecanoyl]-2-[(R)-3-benzyloxytetradecanoylamino]-2-deoxy-β-D-glucopyranosyl}-2-deoxy-α-D-glucopyranoside 1-(dibenzyl phosphate) 4'-(diphenyl phosphate) (6b)**

To the solution of **5b** (100 mg, 51 μmol) in anhydrous THF (4 ml) was added 15% n-BuLi in hexane (43 μl, 66 μmol) at -72°C. The mixture was stirred for 5 min then the solution of tetrabenzyl pyrophosphate in 2 ml of THF (41mg, 76 μmol) was added and stirred for additional 5

min. The mixture was allowed gradually to room temperature, then neutralized with saturated $NaHCO_3$ and extracted with chloroform. After dried and evaporation of the solvent, the product was purified by recycling preparative HPLC, model LC-908 (column: JAIGEL-2H, 2.3 x 60 cm; solvent: chloroform; flow rate: 3.5 ml/min).

9). Preparation of **2-deoxy-6-*O*-{2-deoxy-3-*O*-[(R)-3-hydroxydecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl}-3-*O*-[(R)-3-hydroxydecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-α- D - g l u c o p y r a n o s e 1,4'-bis(phosphate) (400)**

To a solution of **6a** (60 mg, 28.3 μmol) in THF (10 ml) was added palladium black (70 mg) and stirred under 7 kg/cm^2 of hydrogen at room temperature for 24 h, then palladium black (70 mg) was added and stirred for additional 24 h. The palladium catalyst was removed by filtration, then the organic layer was concentrated under reduced pressure. The residue was dissolved in THF-H_2O (20:1, 15 ml), then platinum oxide (50 mg) was added and the mixture was stirred under hydrogen atmosphere at room temperature. After 15 h, the platinum oxide (30 mg) was added again, then stirred for additional 8 h. The platinum catalyst removal by filtration, then concentrated under reduced pressure, and lyophilized. Purification of the product was done by centrifugal partition chromatography (solvent : water / methanol / hexane / triethanolamine = 1 / 20 / 20 / 0,02).

10). Preparation of **2-deoxy-6-*O*-{2-deoxy-3-*O*-[(R)-3-hydroxytetradecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl}-3-*O*-[(R)-3-hydroxytetradecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-α-D-glucopyranose 1,4'-bis(phosphate) (406)**

To a solution of **6b** (110 mg, 49 μmol) in THF (10 ml) was added palladium black (100 mg) and stirred under 7 kg/cm^2 of hydrogen at room temperature for 24 h, then palladium black (100 mg) was added and stirred for additional 24 h. The palladium catalyst was removed by filtration, then the organic layer was concentrated under reduced pressure. The residue was dissolved in THF-H_2O (20:1, 15 ml), then platinum oxide (75 mg) was added and the mixture was stirred under hydrogen atmosphere at room temperature. After 15 h, the platinum oxide (50 mg) was added again, then stirred for additional 8 h. The platinum catalyst removal by filtration, then concentrated under reduced pressure, and lyophilized. Purification of the product was done

by centrifugal partition chromatography (solvent : water / methanol / hexane / triethanolamine = 1 / 20 / 20 / 0,02).

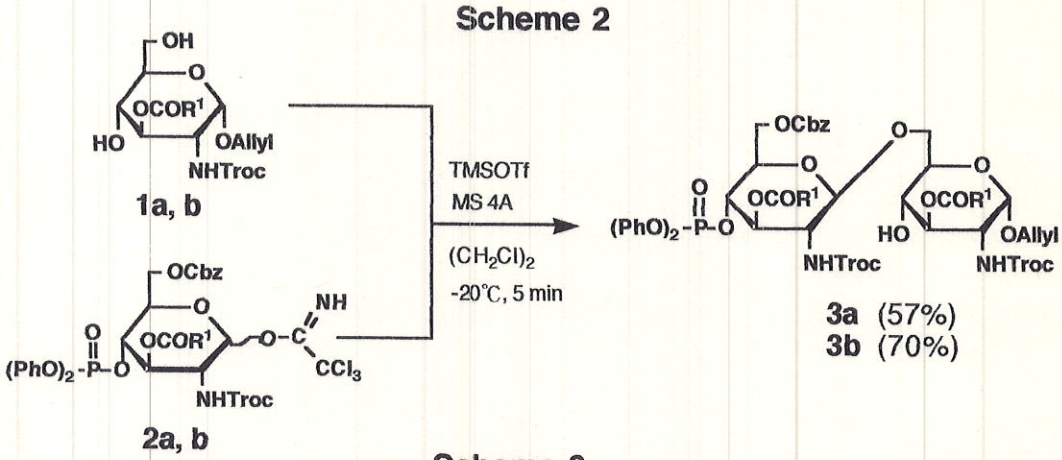
Methods. The reaction kinetic was monitored using thin layer chromatography (TLC) on Kieselgel 60 F_{254} . Purification of the products **5a** and **5b** were carried out using column chromatography on kieselgel 60 (ϕ 0.040-0.063 mm), eluent : chloroform/acetone = 20/1, and the products **3a, 3b, 4a, 4b, 6a, 6b, 400,** and **406** were purified by recycling preparative high performance chromatography model LC-908 using gel permeation column, eluent : chloroform. The products were confirmed by ^1H NMR and ^1H-^1H COSY NMR spectrum, FAB-mass spectrum, optical rotation, and elemental analysis.

RESULTS AND DISCUSSIONS

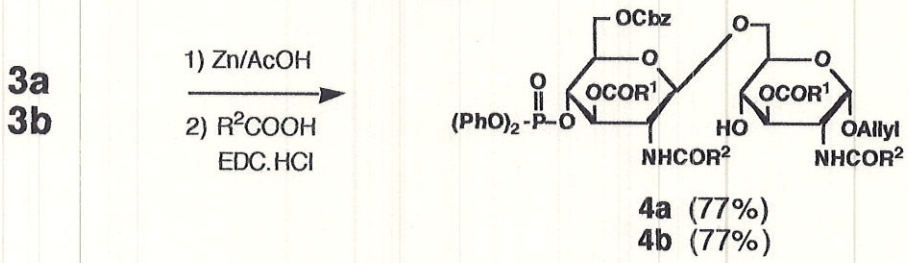
Disaccharides derivative **3a** and **3b** were prepared from the coupling reaction of glycosyl acceptor **1a,b** with glycosyl donors **2a,b** as described in Scheme 2. The reaction were carried out in dichloromethane in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst and molecular sieves 4A at -20°C. The reaction time was very fast (< 5 min), even through the chemical yield of **3a** (57%) and **3b** (70%) was not very good. In case of preparation of **3a**, the starting material **1a** was also recovered in 42 %. The trichloroethoxycarbonyl (Troc) group on the 2-amino function of glycosyl donor ensures the formation of β-linkage as confirmed in the previous work. Because the primary 6-hydroxyl group has much higher reactivity than secondary one at 4-position in a glycosyl acceptor **2a**, **2b**, the β(1→ 6) linked glycoside was formed without protection of the 4-hydroxyl function in the glycosyl acceptor [11]. In fact, ^1H NMR data of H-1' (d = 4.03 ppm, d, $J_{1'-2'}$ = 9.5 Hz), clearly showed the formation of a β(1→ 6) linkage. After deprotection of the Troc group at 2-N-amino group by heating of the **3a, 3b** with Zn/acetic acid, the (R)-3-benzyloxytetradecanoic acid were introduced to both amino group by use of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) as catalyst in dichloromethane. Purification of the product using recycling preparative HPLC gave **4a** and **4b** in 77% yield, respectively.

The l-allyl group was then removed through two-step process, namely isomerization of the allyl ether to the 1-propenyl ether using the hydrogen-activated cationic iridium complex [1,5-cyclooctadiene bis(methyldiphenylphosphine) iridium hexaflurophosphate], and conversion of the propenyl ether into the free alcoholic group using iodine/H_20 [19] (Scheme 4). Furification of the product by silica gel column chromatography gave disaccharide **5a** and **5b** with free hydroxyl groups in 74% and 77% yield, respectively.

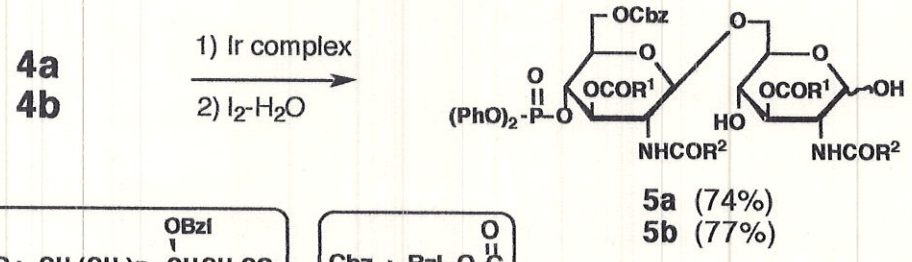
Scheme 2

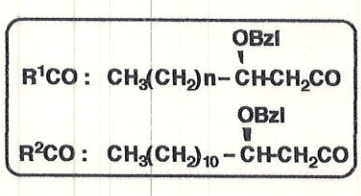


Scheme 3



Scheme 4





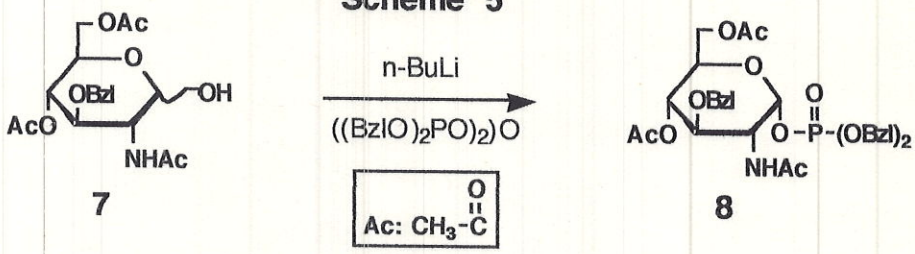
a, n=6
b, n=10

Ir complex : $[Ir(COD)(PMePh_2)_2]PF_6$

The next step was phosphorylation of the disaccharide **5a,b** at the 1- position. The glycosyl dibenzylphosphate was know to be unstable, especially in the acidic conditions. But,

MACHER [20] reported to use acetic acid for neutralization of the reaction mixture after phosphorylation. They purified the product of the glycosyl dibenzyl phosphate moiety by silica gel column chromatography without any problems. KUROSAWA reported in his thesis that a glycosyl dibenzyl phosphate was partially cleaved by treatment with an aqueous saturated $NaHCO_3$ solution after the phosphorylation reaction [21]. From those cases, it has been tried to find a better work-up procedure for the phosphorylation using a model compound **7** as a model substrate (Scheme 5). Phosphorylation was performed by using dibenzyl pyrophosphate and n-butyl lithium in THF at -72°C. The starting material **7** (Rf = 0.10) was disappeared within 5 min, as monitored by HPTLC (chloroform/acetone, 4/1), and the formation of the dibenzyl phosphate derivative **8** (Rf = 0.33) was observed. The reaction mixture was then gradually allowed to come up to room temperature overnight. After neutralization of the reaction mixture with an aqueous saturated $NaHCO_3$ solution, the product was extracted with chloroform. The crude product obtained after removal of the solvent was then purified by recycling preparative HPLC (column: JAIGEL-2H; solvent: chloroform; flow rate: 3.5 ml/min) to give **8**.

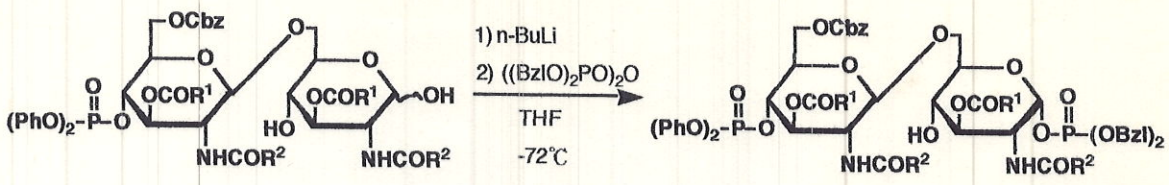
Scheme 5



Since the yield of **8** was over 90% according to this procedure, the phosphorylation of **5a,b** were performed in a similar manner to give the desired product **6a,b** with the glycosyl dibenzyl phosphate moiety after HPLC purification in 97% (Scheme 6).

The benzyloxycarbonyl (Cbz) and benzyl groups in **6a,b** were then deprotected by catalytic hydrogenolysis using palladium black in THF at the pressure of 7 kg/cm^2 for 2 days. Finally, the phenyl groups of 4'-phosphate were deprotected with platinum at room temperature for 1 day (Scheme 7). The negative mode FAB-MS of the products showed an ion at *m/z* 1291.8 and *m/z* 404.2, which correspondent to the pseudo molecular ion [(M-H)$^-$] of the desired product **400** and **406**. Purification of **400** and **406** using centrifugal partition chromatography gave a white crystal of triethylamine salt form in 40% and 50% yields respectively.

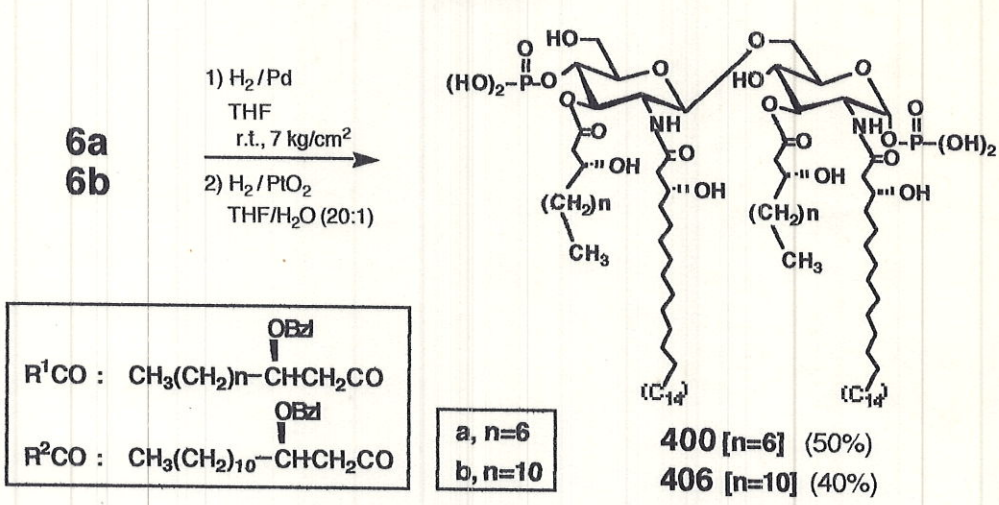
Scheme 6



5a
5b

6a (97%)
6b (97%)

Scheme 7



400 [n=6] (50%)
406 [n=10] (40%)

CONCLUSIONS

The lipid A analogs 2-deoxy-6-*O*-{2-deoxy-3-*O*-[(R)-3-hydroxydecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl}-3-*O*-[(R)-3-hydroxydecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-α-D-glucopyranose 1,4'-bis(phosphate) (**400**) and 2-deoxy-6-*O*-{2-deoxy-3-*O*-[(R)-3-hydroxytetradecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl}-3-*O*-[(R)-3-hydroxytetradecanoyl]-2-[(R)-3-hydroxytetradecanoylamino]-α-D-glucopyranose 1,4'-bis(phosphate) (**406**) could be synthesized through the coupling reaction of allyl 3-*O*-[(R)-3-benzyloxyalkanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (**1a** and **1b**) as glycosyl acceptors and 6-*O*-benzyloxycarbonyl-3-*O*-[(R)-3-benzyloxyalkanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl trichloroacetimidate 4-(diphenyl phosphate) (**2a** and **2b**) as glycosyl donors in the presence of TMSOTf as catalyst followed by introduction of fatty acid to both amino groups and phosphorylation at 1-position of the reducing glucosamine residues. The coupling reaction time was very fast (within 5 minutes), even through the chemical yield was not high.

The lipid A analogs **400** and **406** were found as a white crystals with 15% and 17% overall yields respectively. The negative mode FAB-MS of **400** was *m/z* 1291.8 [(M-H)$^-$] and **406** was *m/z* 1404.2[(M-H)$^-$].

The problem of the phosphorylation reaction at glycosidic position could be overcome by gradually allowed the mixture reaction to come up to room temperature overnight followed by neutralization with an aqueous saturated $NaHCO_3$ solution, and then purified by recycling preparative HPLC using gel permeation column.

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