

## Communications to the Editor

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**SYNTHESIS OF BIOLOGICALLY ACTIVE TETRAACETYL-3-DEOXY-D-MANNO-2-OCTULOSONIC ACID(KDO)-( $\alpha$ 2 $\rightarrow$ 6)-D-GLUCOSAMINE ANALOGS OF LIPID A<sup>1)</sup>**

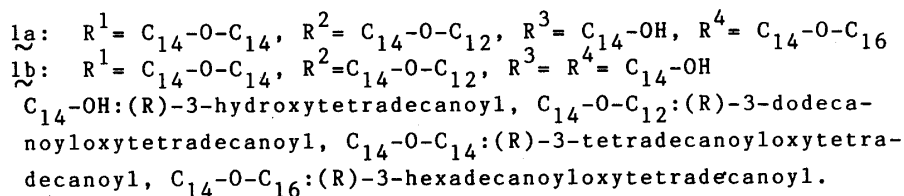
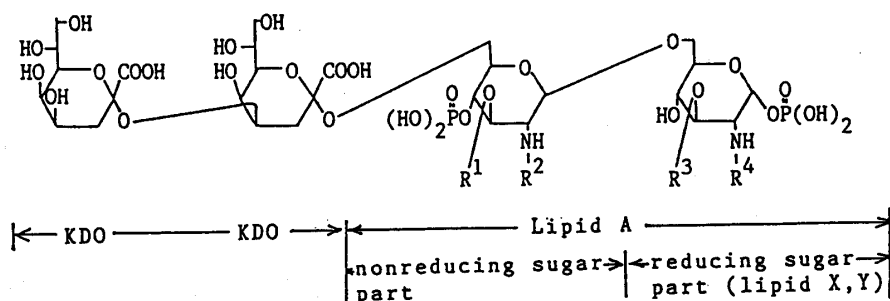
Shin-ichi Nakamoto and Kazuo Achiwa\*

Shizuoka College of Pharmacy, 2-2-1 Oshika, Shizuoka 422, Japan

Novel synthesis of tetraacetyl-KDO-( $\alpha$ 2 $\rightarrow$ 6)-monosaccharide analogs of lipid A is described. Also a preliminary analysis of their biological activity is presented.

**KEYWORDS**———lipid A analog; KDO; KDO linked glucosamine derivative; 4-phosphorylated glucosamine derivative; mitogenic activity

3-Deoxy-D-manno-2-octulosonic acid (2-keto-3-deoxyoctonic acid: KDO) occurs as a ketosidic component in all lipopolysaccharide (LPS) of Gram-negative bacteria and seems to play a biologically important role in being mitogenic and in amplifying the antitumor activity of lipid A, a biologically active site fragment of LPS.<sup>2)</sup> Recently, two research groups disclosed the new structures of the KDO region of LPS from *Salmonella minnesota* (1a)<sup>3)</sup> and *Escherichia coli* (1b)<sup>4)</sup> where the KDO group was attached to lipid A with a ( $\alpha$ 2 $\rightarrow$ 6)linkage as shown below.



Besides, we and the other groups have found that the nonreducing sugar moiety of lipid A is more important than the other part (cf. lipid X and Y) for expressing the biological activities of LPS.<sup>5)</sup>

We wish to describe here the synthesis of novel tetraacetyl-KDO-( $\alpha$ 2 $\rightarrow$ 6)-D-glucosamine analogs (nonreducing sugar part) (3a-c) of lipid As as shown in Chart 1 and 2, and to present preliminary results of studies of their biological activity.

First, the amino-hydroxyl-compound (4)<sup>5b)</sup> was acylated at the amino group with (R)-3-dodecanoyloxytetradecanoic acid in the presence of dicyclohexylcarbodiimide in  $\text{CH}_2\text{Cl}_2$  at 0-5°C to yield 5a<sup>6)</sup> [51%, mp 61-62°C,  $[\alpha]_D^{24}$  -48.3° (c=1.15,  $\text{CHCl}_3$ )], then at the hydroxyl group with (R)-3-tetradecanoyloxytetradecanoic acid, dicyclohexylcarbodiimide and 4-dimethylaminopyridine in the same solvent to give 6a<sup>6)</sup> [91%, mp 52-55°C,  $[\alpha]_D^{23}$  -23.4° (c=0.82,  $\text{CHCl}_3$ )]. Subsequently the isopropylidene group of 6a was removed by hydrolysis with 90% aqueous acetic acid at 90°C for 15 min to yield 7a<sup>6)</sup> [84%, mp 103-105°C,  $[\alpha]_D^{23}$  -17.8° (c=0.92,  $\text{CHCl}_3$ )].

Glycosidation of 7a with 8 newly prepared from pentaacetyl-KDO<sup>7)</sup> in the presence of  $\text{HgBr}_2\text{-Hg}(\text{CN})_2$  and Molecular sieves 4A in  $\text{CH}_2\text{Cl}_2$  at room temperature for 24 h afforded 9a<sup>6)</sup> [31%, amorphous,  $[\alpha]_D^{21}$  +9.2° (c=2.0,  $\text{CHCl}_3$ )] and 9b<sup>6)</sup> [6%, amorphous,  $[\alpha]_D^{21}$  +19.2° (c=0.24,  $\text{CHCl}_3$ )], the glycosidic linkage structures of which were assigned from the evidences indicated in Chart 2.

Phosphorylation of 9a was carried out with diphenylphosphorochloridate, pyridine and 4-dimethylaminopyridine in  $\text{CH}_2\text{Cl}_2$ . The reaction was complete in 4 h at room temperature to give 10a<sup>6)</sup> [92%, syrup,  $[\alpha]_D^{22}$  +17.6° (c=1.42,  $\text{CHCl}_3$ )]. The protective benzyl and phenyl groups of 10a<sup>6)</sup> were then removed stepwise by hydrogenolysis catalyzed with 10% Pd-on-carbon at 35°C for 5 h and  $\text{PtO}_2$  at room temperature for 24 h in methanol to give 3a<sup>6)</sup> [54%, mp 123-125°C,  $[\alpha]_D^{22}$  +19.4° (c=0.32,  $\text{CHCl}_3$ )].

The compounds, 3ab<sup>6,8)</sup> and 3ac<sup>6,9)</sup> were similarly synthesized from 7b and 7c as described above. The starting compounds, 7b and 7c, were also prepared by simultaneous acylation of the amino and hydroxyl groups of 4 with the corresponding fatty acids in the presence of dicyclohexylcarbodiimide and 4-dimethylaminopyridine, respectively, in  $\text{CH}_2\text{Cl}_2$  at room temperature for 16 h.

To establish the stereochemistry of the glycosidation products (9a and 9b) by means of  $^1\text{H-NMR}$  spectroscopy,<sup>10)</sup> the compounds, 13a<sup>6)</sup> and 13b<sup>6)</sup> bearing two 2,2,2-trichloroethoxycarbonyl (TCEC) substituents instead of the fatty acids on the 2-amino and 3-hydroxyl groups of 10 were synthesized. Thus, glycosidation of the two components, 8 and 11,<sup>5d)</sup> was carried out by the procedure described above to yield 12a [46%, amorphous,  $[\alpha]_D^{25}$  +11.3° (c=3.28,  $\text{CHCl}_3$ )], and 12b [3.7%, amorphous,  $[\alpha]_D^{22}$  +18.4° (c=1.1,  $\text{CHCl}_3$ )]. Each product was then phosphorylated by the above mentioned treatment to yield 13a<sup>6)</sup> [77%, amorphous,  $[\alpha]_D^{20}$  +22.7° (c=0.8,  $\text{CHCl}_3$ )],  $^1\text{H-NMR}(\text{CDCl}_3)\delta$ : 2.27 (dd,  $J=13.4$ , 5.9Hz, 3-Heq of KDO), and 13b<sup>6)</sup> [68%, amorphous,  $[\alpha]_D^{20}$  +31.4° (c=0.68,  $\text{CHCl}_3$ )],  $^1\text{H-NMR}(\text{CDCl}_3)\delta$ : 2.47 (dd,  $J=11.7$ , 4.6Hz, 3-Heq of KDO).  $^1\text{H-NMR}$  spectroscopic analysis of the former indicated the smaller chemical shift value of 3-Heq proton in the KDO residue, which is characteristic of  $\alpha$ -ketoside.<sup>4)</sup>

Subsequent deprotection of the TCEC group at the 2- and 3-positions of the glucosamine skeleton of 13a with zinc powder in acetic acid at room temperature for 5 h afforded 14a [41%, amorphous,  $[\alpha]_D^{24}$  +29.0° (c=2.12,  $\text{CHCl}_3$ )]. The simultaneous acylation of the amino and hydroxyl groups of 14a with (R)-3-tetradecanoyloxytetradecanoic acid gave 10ab<sup>6)</sup> [40%, syrup,  $[\alpha]_D^{24}$  +10.4° (c=1.35,  $\text{CHCl}_3$ )]. The NMR and IR spectra and the values of the optical rotation of 10ab thus obtained were identical with those of 10ab as indicated in Chart 1.

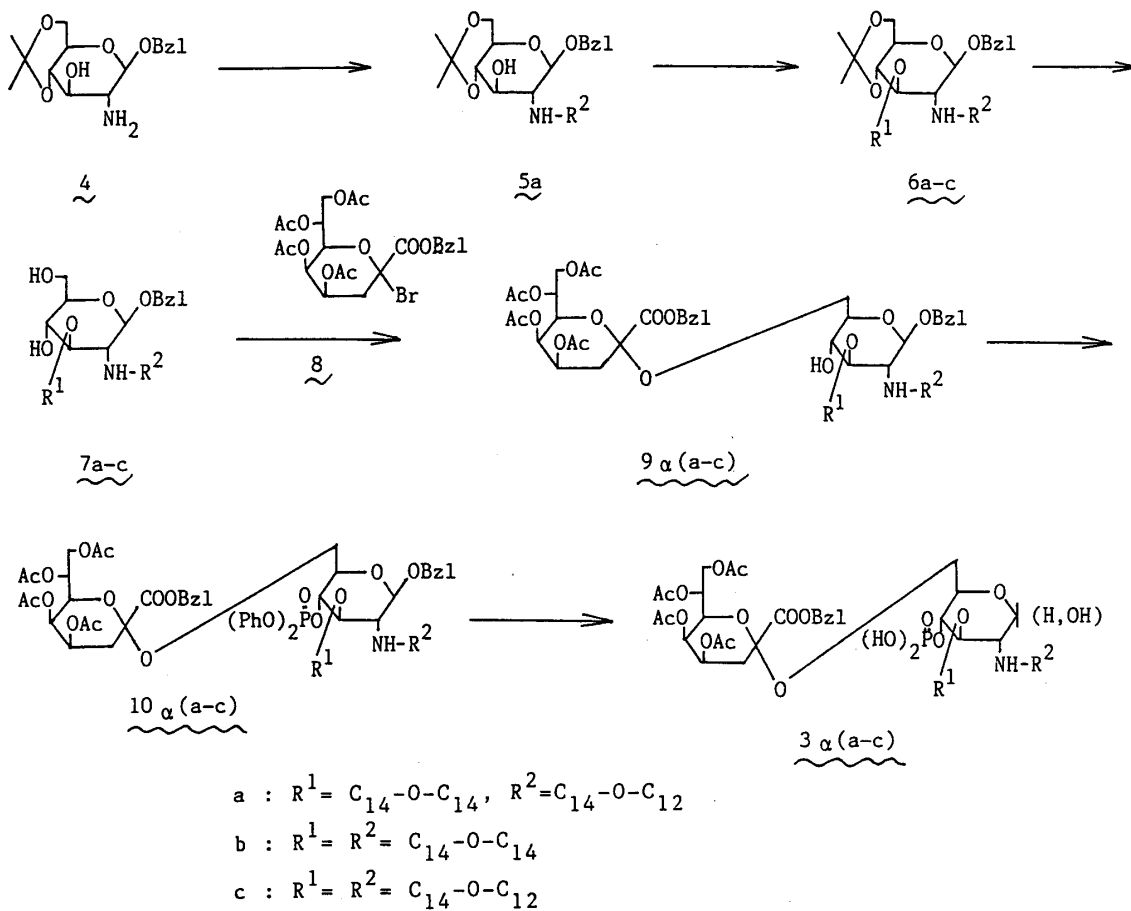


Chart 1

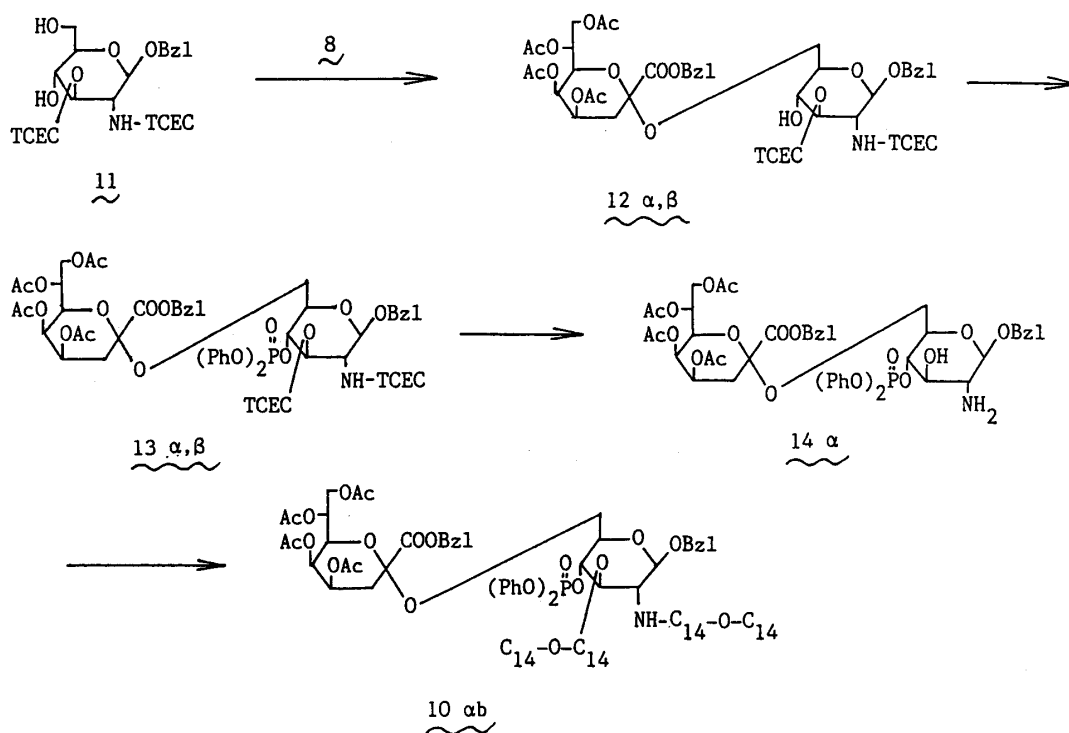


Chart 2

Preliminary examination of the biological activity revealed that these compounds (3a-c) possess the mitogenic activity comparable with that of lipid A.<sup>11)</sup>

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- 6) Satisfactory analytical and spectral data were obtained for these compounds.
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- 8) mp 129-132°C,  $[\alpha]_D^{24} +14.2^\circ$  (c=0.5, CHCl<sub>3</sub>).
- 9) mp 122-124°C,  $[\alpha]_D^{22} +24.6^\circ$  (c=1.05, CHCl<sub>3</sub>).
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