Communications to the Editor

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SYNTHESIS OF BIOLOGICALLY ACTIVE TETRAACETYL-3-DEOXY-D-MANNO-2-OCTULOSONIC ACID(KDO)-(α2→6)-D-GLUCOSAMINE ANALOGS OF LIPID A[1)]

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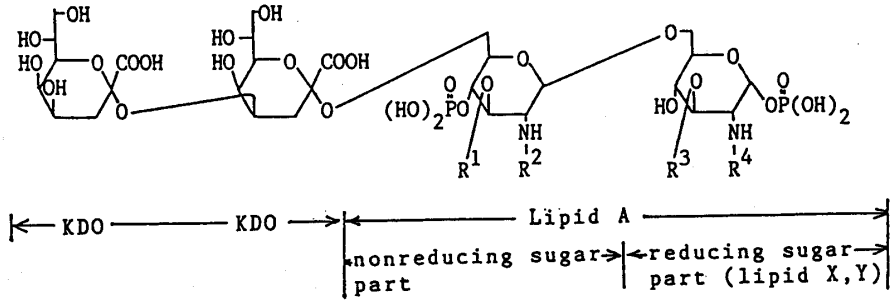
Novel synthesis of tetraacetyl-KDO-(α2→6)-monosaccharide analogs of lipid A is described. Also a preliminary analysis of their biological activity is presented.

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.[2)] Recently, two research groups disclosed the new structures of the KDO region of LPS from Salmonella minnesota (1a)[3)] and Esherichia coli (1b)[4)] where the KDO group was attached to lipid A with a (α2→6)linkage as shown below.



1a: R^1= C_{14}-O-C_{14}, R^2= C_{14}-O-C_{12}, R^3= C_{14}-OH, R^4= C_{14}-O-C_{16}
1b: R^1= C_{14}-O-C_{14}, R^2=C_{14}-O-C_{12}, R^3= R^4= C_{14}-OH
C_{14}-OH:(R)-3-hydroxytetradecanoyl, C_{14}-O-C_{12}:(R)-3-dodecanoyloxytetradecanoyl, C_{14}-O-C_{14}:(R)-3-tetradecanoyloxytetradecanoyl, C_{14}-O-C_{16}:(R)-3-hexadecanoyloxytetradecanoyl.

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.[5)]

We wish to describe here the synthesis of novel tetraacetyl-KDO-(α2→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)[5b] was acylated at the amino group with (R)-3-dodecanoyloxytetradecanoic acid in the presence of dicyclohexylcarbodiimide in CH_2Cl_2 at 0-5°C to yield 5a[6] [51%, mp 61-62°C, $[\alpha]_D^{24}$ -48.3° (c=1.15, $CHCl_3$)], then at the hydroxyl group with (R)-3-tetradecanoyloxytetradecanoic acid, dicyclohexylcarbodiimide and 4-dimethylaminopyridine in the same solvent to give 6a[6] [91%, mp 52-55°C, $[\alpha]_D^{23}$ -23.4° (c=0.82, $CHCl_3$)]. Subsequently the isopropylidene group of 6a was removed by hydrolysis with 90% aqueous acetic acid at 90°C for 15 min to yield 7a[6] [84%, mp 103-105°C, $[\alpha]_D^{23}$ -17.8° (c=0.92, $CHCl_3$)].

Glycosidation of 7a with 8 newly prepared from pentaacetyl-KDO[7] in the presence of $HgBr_2$-$Hg(CN)_2$ and Molecular sieves 4A in CH_2Cl_2 at room temperature for 24 h afforded 9αa[6] [31%, amorphous, $[\alpha]_D^{21}$ +9.2° (c=2.0, $CHCl_3$)] and 9βa[6] [6%, amorphous, $[\alpha]_D^{21}$ +19.2° (c=0.24, $CHCl_3$)], the glycosidic linkage structures of which were assigned from the evidences indicated in Chart 2.

Phosphorylation of 9αa was carried out with diphenylphosphorochloridate, pyridine and 4-dimethylaminopyridine in CH_2Cl_2. The reaction was complete in 4 h at room temperature to give 10αa[6] [92%, syrup, $[\alpha]_D^{22}$ +17.6° (c=1.42, $CHCl_3$)]. The protective benzyl and phenyl groups of 10αa[6] were then removed stepwise by hydrogenolysis catalyzed with 10% Pd-on-carbon at 35°C for 5 h and PtO_2 at room temperature for 24 h in methanol to give 3αa[6] [54%, mp 123-125°C, $[\alpha]_D^{22}$ +19.4° (c=0.32, $CHCl_3$)].

The compounds, 3αb[6,8] and 3αc,[6,9] 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 CH_2Cl_2 at room temperature for 16 h.

To establish the stereochemistry of the glycosidation products (9α and 9β) by means of ^{1}H-NMR spectroscopy,[10] the compounds, 13α[6] and 13β,[6] 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,[5d] was carried out by the procedure described above to yield 12α [46%, amorphous, $[\alpha]_D^{25}$ +11.3° (c=3.28, $CHCl_3$)], and 12β [3.7%, amorphous, $[\alpha]_D^{22}$ +18.4° (c=1.1, $CHCl_3$)]. Each product was then phosphorylated by the above mentioned treatment to yield 13α[6] [77%, amorphous, $[\alpha]_D^{20}$ +22.7° (c=0.8, $CHCl_3$)], ^{1}H-NMR($CDCl_3$)δ: 2.27 (dd, J=13.4, 5.9Hz, 3-Heq of KDO], and 13β[6] [68%, amorphous, $[\alpha]_D^{20}$ +31.4° (c=0.68, $CHCl_3$), ^{1}H-NMR($CDCl_3$)δ: 2.47 (dd, J=11.7, 4.6Hz, 3-Heq of KDO)]. ^{1}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 α-ketoside.[4]

Subsequent deprotection of the TCEC group at the 2- and 3-positions of the glucosamine skeleton of 13α with zinc powder in acetic acid at room temperatue for 5 h afforded 14α [41%, amorphous, $[\alpha]_D^{24}$ +29.0° (c=2.12, $CHCl_3$)]. The simultaneous acylation of the amino and hydroxyl groups of 14α with (R)-3-tetradecanoyloxytetradecanoic acid gave 10αb[6] [40%, syrup, $[\alpha]_D^{24}$ +10.4° (c=1.35, $CHCl_3$)]. The NMR and IR spectra and the values of the optical rotation of 10αb thus obtained were identical with those of 10αb as indicated in Chart 1.

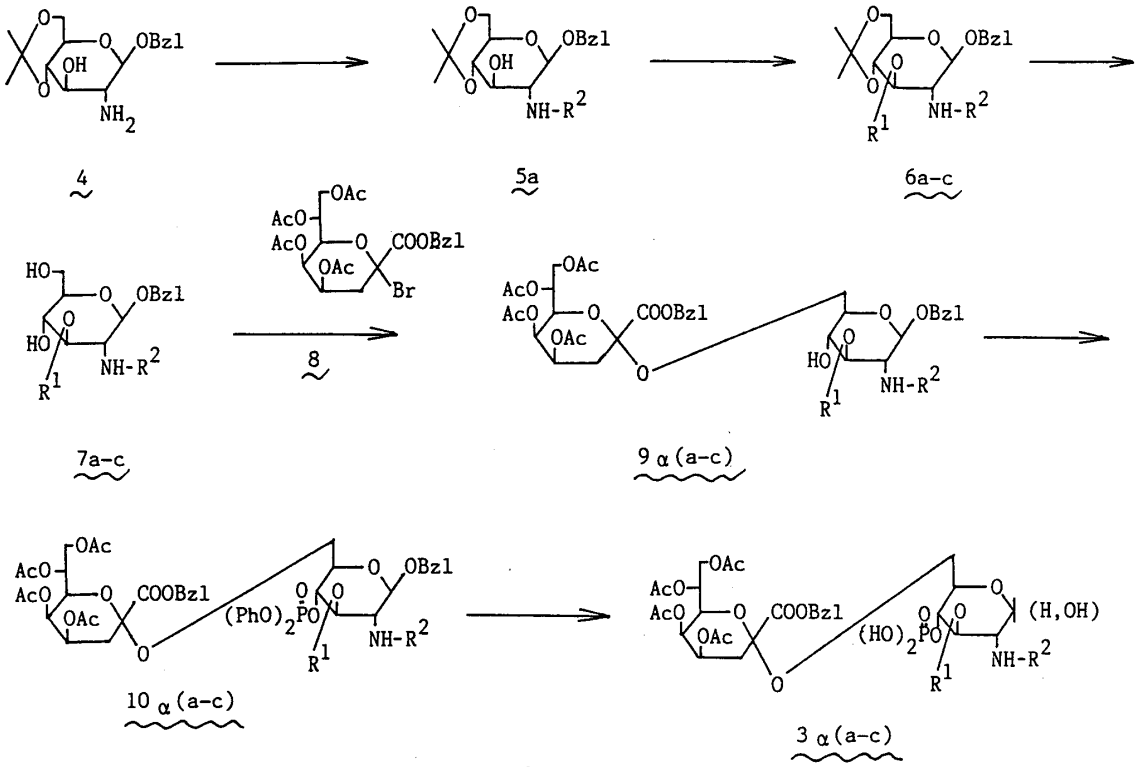


Chart 1

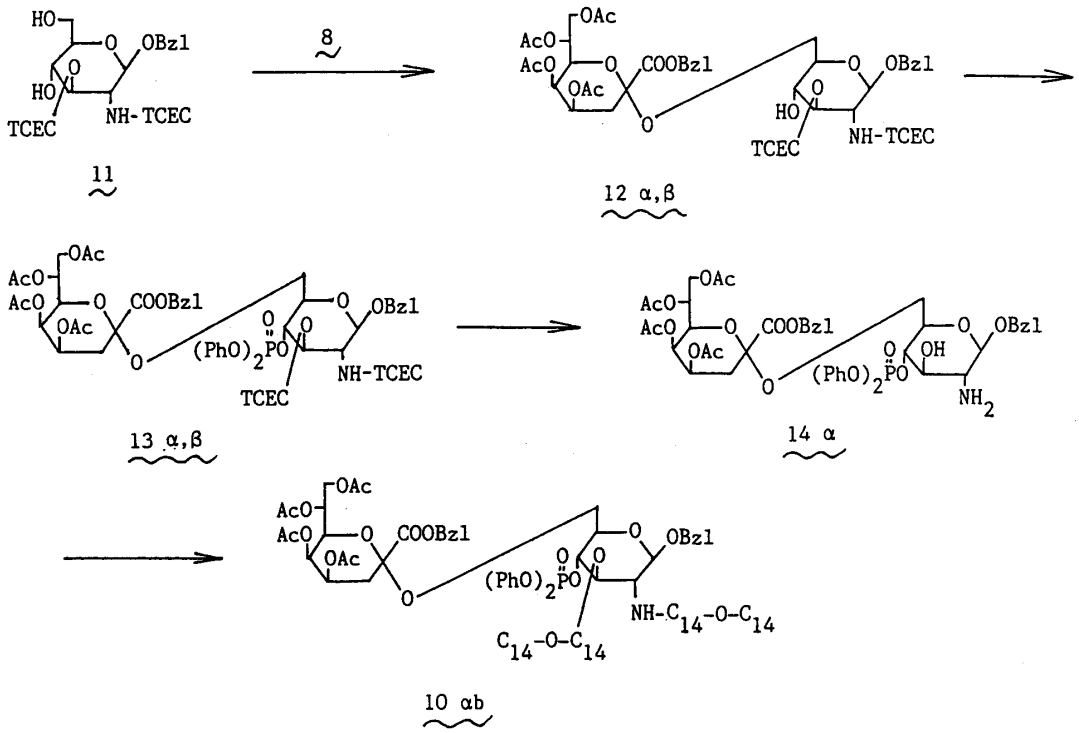


Chart 2

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

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