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CHEMICAL SYNTHESIS OF BISDEPHOSPHO LIPID A OF *Salmonella* ENDOTOXIN

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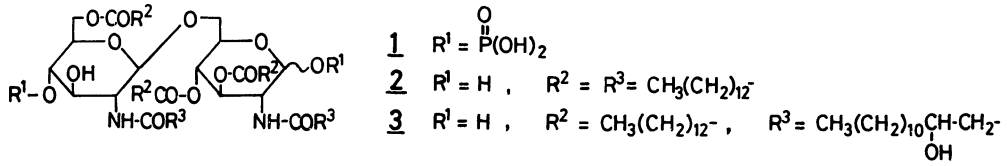
Synthesis is described on 6-*O*-[2-deoxy-2-((*R*)-3-hydroxytetradecanoylamino)-6-*O*-tetradecanoyl-β-D-glucopyranosyl]-2-deoxy-2-((*R*)-3-hydroxytetradecanoylamino)-3,4-di-*O*-tetradecanoyl-D-glucopyranose which corresponds to the phosphate-less lipid A structure of *Salmonella*-type bacterial endotoxin.

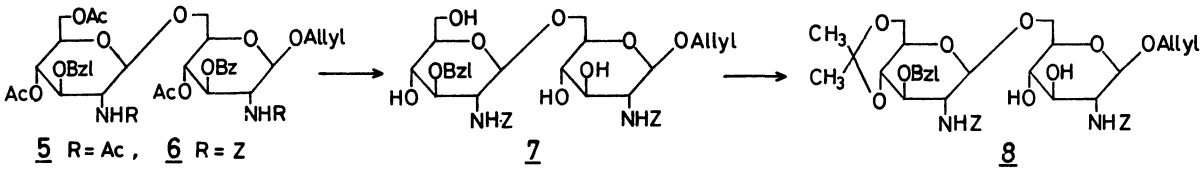
In view of the unique biological activities of lipid A moiety (1) in bacterial endotoxin, *e.g.*, lethal toxicity, pyrogenecity, adjuvant activity and so on,[1] we started synthetic study on this liposaccharide particularly for the purpose of elucidation of the relationship between chemical structure and biological activity. We have recently reported a synthesis of polyacyl disaccharide (2), which corresponds to the fundamental structure of *Salmonella*-type lipid A, using tetradecanoic (myristic) acid for both *O*- and *N*-acylations.[2] However, since optically active (*R*)-3-hydroxylated fatty acids are usually found as natural *N*-acyl function, synthesis of lipid A of this natural type seems to be important. Meanwhile, we succeeded to prepare the optically pure (*R*)-(-)-3-hydroxytetradecanoic acid by a simple asymmetric catalytic reduction of the corresponding keto ester,[3] now opening a way for the synthesis of natural lipid A. In this communication, preparation of a liposaccharide (3) using the synthetic hydroxy acid for *N*-acylation is described, thus being the first synthesis of bisdephospho structure of *Salmonella*-type lipid A.

Since initial attempts to protect the hydroxyl group in 3-hydroxytetradecanoic acid (4) had not been achieved successfully,[4] the synthetic route to 3

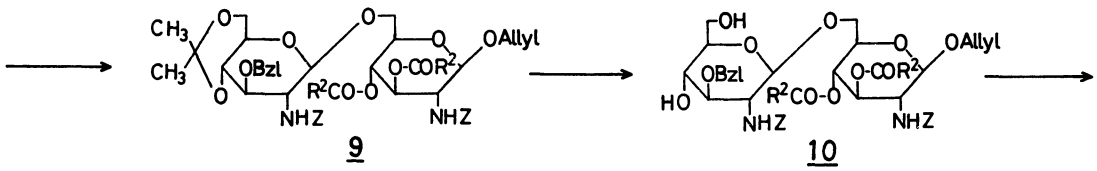
was constructed as that the hydroxy acid was introduced at the final stage of the synthesis where its protection is no more necessary. Thus, the disaccharide (5), allyl 6-*O*-(2-acetamido-4,6-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-2-acetamido-4-*O*-acetyl-3-*O*-benzoyl-2-deoxy-β-D-glucopyranoside, obtained in the previous work[2] was used as the starting material in this investigation. After removal of the two *N*-acetyl groups (reaction with Meerwein's reagent followed by mild acidic hydrolysis),[2] the free amino groups were protected by benzyloxycarbonylation (benzyloxycarbonyl chloride - pyridine in THF) against the successive *O*-acylation processes to afford 6 (61% from 5, mp 204-206°C, $[\alpha]_D^{28}$ +4.29°).[5] Removal of all *O*-acetyl and *O*-benzoyl groups in 6 was achieved with a mixture of conc. aqueous ammonia and ethanol (1:2, at 50°C for 4.5 hr)[6] to give 7 (75%, mp 213-215°C dec, $[\alpha]_D^{28}$ -16.9°).[5] It was then converted into 4',6'-*O*-isopropylidene derivative (8) (2,2-dimethoxypropane and *p*-toluenesulfonic acid)[7] (88%, mp 176-179°C, $[\alpha]_D^{28}$ -24.3°).[5] Acylation of 8 with tetradecanoyl chloride in pyridine (at 25°C for 1.5 hr) followed by hydrolysis of the isopropylidene group (90%



1 $R^1 = P(O)(OH)_2$
2 $R^1 = H$, $R^2 = R^3 = CH_3(CH_2)_{12}$-
3 $R^1 = H$, $R^2 = CH_3(CH_2)_{12}$-, $R^3 = CH_3(CH_2)_{10}CH(OH)CH_2$-

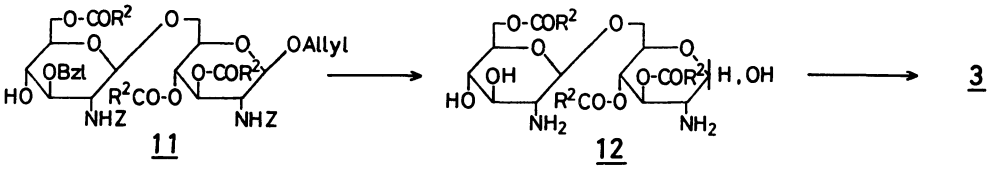


5 R = Ac, 6 R = Z



7 8

9 10



11 12 3

Ac = CH_3CO-, Allyl = CH_2=$CHCH_2$-, Bz = C_6H_5CO-, Bzl = $C_6H_5CH_2$-, Z = $C_6H_5CH_2OCO$-

aqueous acetic acid at 90°C for 40 min) gave 10 (mp 159-160°C, $[\alpha]_D^{28}$ -1.60°).[5,8] 6'-*O*-Monoacylation of 10 was readily accomplished again with tetradecanoyl chloride in pyridine (at 7-10°C for 2 hr) to give tri-*O*-tetradecanoyl disaccharide (11) (94%, mp 156-158°C, $[\alpha]_D^{28}$ -2.12°).[5] After the glycosidic allyl group had been removed (isomerization with $RhCl(PPh_3)_3$ followed by cleavage with HgO - $HgCl_2$),[2,9] one *O*-benzyl and two *N*-benzyloxycarbonyl groups were simultaneously hydrogenolyzed to give 12 having free amino groups. It was then subjected to *N*-acylation reaction with (*R*)-(-)-4 and dicyclohexylcarbodiimide (in THF - $CHCl_3$ at room temperature). The reaction proceeded very slowly and required 5 days for completion even by use of 10 equivalents amount of each reagent. The main product (3) (mp 192-195°C dec, $[\alpha]_D^{28}$ +0.41°)[5] was isolated by silica gel column chromatography. Gas chromatographic analysis indicated that no *O*-acylation had occurred with the hydroxy acid even in the long reaction period mentioned above.[10] Thus, synthesis of 6-*O*-[2-deoxy-2((*R*)-3-hydroxytetradecanoylamino)-6-*O*-tetradecanoyl-β-D-glucopyranosyl]-2-deoxy-2-((*R*)-3-hydroxy-tetradecanoylamino)-3,4-di-*O*-tetradecanoyl-D-glucopyranose (3) was achieved, which corresponds to the fundamental structure of natural *Salmonella*-type lipid A lacking phosphate moiety at 1 and 4' positions.

The new synthetic route described above is of a great value not only for the preparation of compounds having *N*-hydroxyacyl function such as 3 but also for the syntheses of those with other *N*-acyl groups in further studies for structure-activity relationship, because various *N*-acyl analogs can be readily prepared *via* the common synthetic intermediate 12 in this synthesis. Moreover, in view of experimental readiness,this synthetic strategy is very favorable, since the *N*,*N'*-bisbenzyloxycarbonyl intermediates are more easily handled than the corresponding *N*,*N'*-diacyl compounds in the previous approach[2] due to the higher solubility of the former.[8]

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References and Notes

1) C. Galanos, O. Lüderitz, E. T. Rietschel, and O. Westphal, International Review of Biochemistry, Biochemistry of Lipids II, Vol.14, p.239, University Park Press, Baltimore, 1977.

2) M. Inage, H. Chaki, S. Kusumoto, and T. Shiba, Tetrahedron Lett., 21 (1980) in press.

3) A. Tai, M. Nakahata, T. Harada, Y. Izumi, S. Kusumoto, M. Inage, and T. Shiba, Chem. Lett., 1980, in press.

4) All common *O*-protecting groups examined so far caused considerable side reactions at either introduction or deprotection step.

5) Satisfactory elemental analysis was obtained for the compound. Optical rotation was measured for a solution (*c* 0.5) in chloroform - methanol (5:1).

6) Sodium alkoxide was not used to avoid the formation of oxazolidone ring from the *N*-benzyloxycarbonyl and the vicinal hydroxyl groups.

7) M. Kiso and A. Hasegawa, Carbohyd. Res., 52, 87 (1976).

8) In contrast to the extremely low solubility of the *N*,*N'*-ditetradecanoyl derivative in the previous work,[2] no problem was encountered in the solubility of 10.

9) P. A. Gent and R. Gigg, J. Chem. Soc., Chem. Commun., 1974, 277. R. Gigg and C. D. Warren, J. Chem. Soc. (C), 1968, 1903.

10) The fatty acid of ester form involved in 3 was analyzed as methyl ester after cleavage with sodium methoxide. No peak was detected other than methyl tetradecanoate.

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