Tetrahedron Letters Vol. 21, pp 3889 - 3892 0040-4039/80/1001-3889$02.00/0

SYNTHESIS OF LYPOSACCHARIDE CORRESPONDING TO FUNDAMENTAL STRUCTURE OF *Salmonella*-TYPE LIPID A

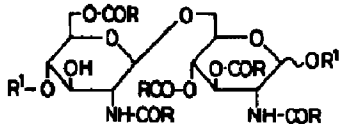
Masaru Inage, Haruyuki Chaki, Shoichi Kusumoto, and Tetsuo Shiba*
Department of Chemistry, Faculty of Science, Osaka University
Toyonaka, Osaka 560, Japan

Summary: 6-*O*-(2-Deoxy-2-myristoylamino-6-*O*-myristoyl-β-D-glucopyranosyl)-2-deoxy-2-myristoylamino-3,4-di-*O*-myristoyl-D-glucopyranose (2) was prepared in a synthetic approach to lipid A which is the active center of bacterial endotoxin.

"Lipid A" moiety, which is the common structural component in cell wall lipopolysaccharide, was shown to possess most of the important biological activities described for bacterial endotoxin, *e.g.*, lethal toxicity, pyrogenecity, adjuvant activity and so on.[1] The basic structure of lipid A has been recently established as 1 mainly by Westphal's group[1] and others.[2] However, natural lipid A from bacterial cell wall has never been obtained as a chemically pure substance. It always exists as a complex mixture of congeners or analogs.[3] Even a contamination of impurities in natural materials may not be excluded. In this situation, synthetic approach to this natural liposaccharide seemed to be very urgent and important particularly from the view point of clarification of the true active structural unit and also elucidation of the relationship between chemical structure and biological activity. In addition, lipid A has a common feature to the muramyl peptide, for which we have already piled many synthetic studies,[4] as that both are of bacterial cell wall origin, showing many similar biological activities to mammals though different in structures. In this regards, we attempted now the chemical construction of lipid A molecule by total synthesis. In the present communication, the synthesis of the fundamental structure of lipid A devoid of phosphate moiety is reported.

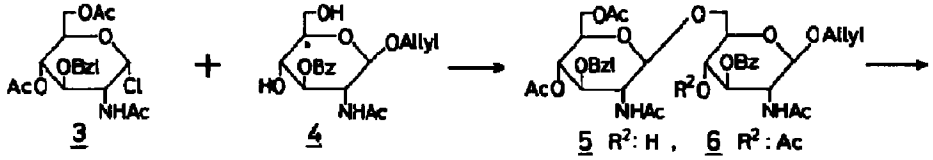
As the first approach in the series of this synthetic study, we employed

myristic acid for both *O*- and *N*-acylation of the disaccharide, though 3-hydroxylated fatty acid is known as *N*-acyl function in the natural Lipid A. As shown in the scheme, the introduction of acyl groups into glucosamine disacchaide backbone was performed stepwise. Two monosaccharide components, 3 and 4, were prepared from D-glucosamine respectively. In the coupling reaction, the primary hydroxyl group in 4 showed much greater reactivity than the secondary one as expected. Thus, König-Knorr condensation ($Hg(CN)_2$ in CH_2Cl_2) afforded the β(1-6) disaccharide, *i.e.*, allyl 6-*O*-(2-acetamido-4,6-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-2-acetamido-3-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (5), as a sole

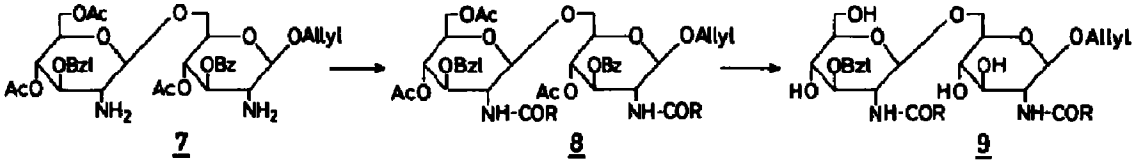


1 R^1 : $P(OH)_2$ (O)

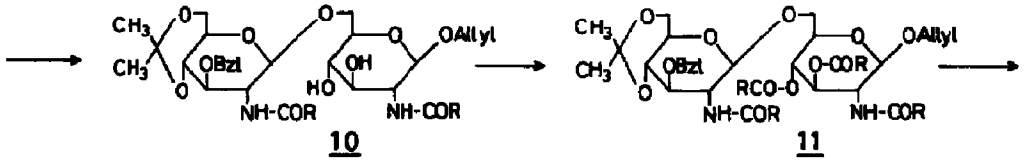
2 R^1 : H , R : $CH_3(CH_2)_{12}$



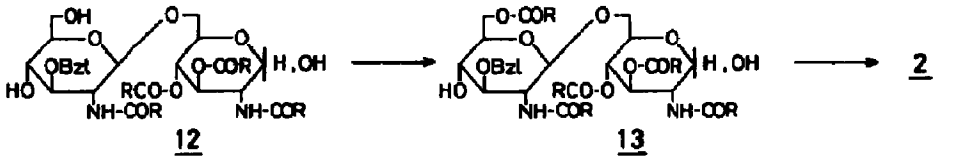
3 + 4 → 5 R^2: H , 6 R^2: Ac



7 → 8 → 9



10 → 11



12 → 13 → 2

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

product (56%, mp 248-250°C dec, $[\alpha]_D^{28}$ -9.97°).[5,6] After acetylation of the 4-hydroxyl group, both *N*-acyl groups were exchanged as follows. By treatment of 5 with triethyloxonium fluoroborate (in CH_2Cl_2 in the presence of K_2CO_3)[7] followed by mild acidic hydrolysis (1 equivalent of 1N aqueous HCl in THF at room temperature for 1 hr), only *N*-acetyl groups were removed resulting in the formation of the disaccharide with two free amino groups (7), which was then acylated with myristoyl chloride in pyridine to give *N*,*N*'-dimyristoyl disaccharide (8) (63%, mp 206-210°C, $[\alpha]_{365}^{28}$ +12.1°).[5] The all *O*-acyl groups in 8 were then removed (0.1N KOH in ethanol at 50°C for 4 hr), and the product (9) was treated with 2,2-dimethoxypropane (in DMF in the presence of *p*-toluenesulfonic acid at room temperature for 2 hr)[8] to afford 4',6'-*O*-isopropylidene derivative (10) (83%, mp 235-238°C, $[\alpha]_{365}^{28}$ +61.4°).[5] The remaining two free hydroxyl groups in 10 were acylated with myristoyl chloride in pyridine. Although rather long reaction period (at 25°C for 5 hr) was required for completion of the reaction, tetramyristoyl disaccharide (11) was obtained in a good yield (71%, mp 215-217°C, $[\alpha]_{365}^{28}$ -17.0°).[5] Synthesis of 2 would be completed by hydrolysis of the isopropylidene group in 11 followed by 6'-*O*-monoacylation and then deprotection. However, at the step of removal of the isopropylidene group, the product formed was found to be sparingly soluble in common organic solvents. Low solubility of the intermediate was unfavorable for the subsequent reaction procedures. However, this difficulty could be in fact avoided by removing the allyl glycoside prior to the above procedure. Thus, the allyl group was isomerized with $RhCl(PPh_3)_3$ (in ethanol-benzene-water at 90°C for 16 hr)[9] and then cleaved with HgO-$HgCl_2$ (in acetone-water at room temperature for 15 min).[10] Successive acid hydrolysis (90% acetic acid at 90°C for 15 min) afforded 12, which was easily soluble in pyridine and chloroform-methanol. Selective 6'-*O*-acylation of 12 proceeded again with myristoyl chloride in pyridine satisfactorily (at 8°C for 1.5 hr) to give the pentamyristoyl β(1-6) glucosamine disaccharide derivative (13) (81%, mp 149-151°C, $[\alpha]_{365}^{28}$ +26.3°).[5] Hydrogenolytic removal of the remaining benzyl group in 13 gave the desired product of the fundamental structure without phosphate moiety of *Salmonella*-type lipid A, *i.e.*, 6-*O*-(2-deoxy-2-myristoylamino-6-*O*-myristoyl-β-

D-glucopyranosyl)-2-deoxy-2-myristoylamino-3,4-di-*O*-myristoyl-D-glucopyranose (2) (97%, mp 186-188°C, $[\alpha]_{365}^{28}$ +11.4°).[5] Test of this synthetic liposaccharide for various biological activities of lipid A are in progress.

This stepwise acylation method is advantageous particularly for the purpose of the selective acylation of amino and hydroxyl functions with different species of fatty acids as seen in most of the natural lipid A.[1]

References and Footnotes

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) H. G. Khorana *et al.*, *J. Biol. Chem.*, 254, 5906, 5918, 5926 (1979).
3) Various *O*-acyl groups (such as C_{12}-C_{16}) are found simultaneously in any lipid A samples. Furthermore, the compounds lacking some of the *O*-acyl moieties are usually mixed in the natural specimen.
4) S. Kusumoto, M. Inage, T. Shiba, I. Azuma, and Y. Yamamura, *Tetrahedron Lett.*, 1978, 4899 and references cited therein.
5) Satisfactory elemental analysis was obtained for this compound. Optical rotation was measured for a solution (*c* 0.5) in chloroform-methanol (5 : 1).
6) Removal of all *O*-protections from 5 afforded *N*,*N*'-diacetyldisaccharide (14), whose NMR spectrum indicated the β-configuration of the glycosidic linkage (H-1': δ 4.52ppm, d, *J*=8.0Hz). Moreover, 14 was clearly distinguished from authentic *N*,*N*'-diacetylchitobiose on TLC. These facts undoubtedly assure the β(1-6) disaccharide structure in 5.
7) Y. Kishi, T. Fukuyama, M. Aratani, F. Nakatsubo, T. Goto, S. Inoue, H. Tanino, S. Sugiura, and H. Kakoi, *J. Am. Chem. Soc.*, 94, 9219 (1972).
8) M. Kiso and A. Hasegawa, *Carbohyd. Res.*, 52, 87 (1976).
9) P. A. Gent and R. Gigg, *J. Chem. Soc., Chem. Commun.*, 1974, 277.
10) R. Gigg and C. D. Warren, *J. Chem. Soc. (C)*, 1968, 1903.

(Received in Japan 2 July 1980)