SYNTHESIS OF BIOLOGICALLY ACTIVE N-ACYLATED L-SERINE-CONTAINING D-GLUCOSAMINE-4-PHOSPHATE DERIVATIVES OF LIPID A[1)]

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New N-acylated L-serine-containing non-phosphorylated and phosphorylated D-glucosamine derivatives structurally corresponding to lipid A disaccharide backbone were synthesized. Compound **4,5** showed mitogenic activity.

KEYWORDS N-acylated L-serine; D-glucosamine-4-phosphate; lipid A analog; lipoamino acid; mitogenic activity; key intermediate

Lipid A, the biologically active region of lipopolysaccharide (LPS),[2)] consists of a D-glucosaminyl-β(1-6)-D-glucosamine disaccharide carrying two phosphates and several fatty acids residues.[3)] Among the various synthetic lipid A analogs, D-glucosamine-4-phosphate analogs of the non-reducing unit of lipid A showed many of the biological activities of LPS.[4)] Recently, numerous acyclic analogs related to lipid A partial structure have been synthesized.[5)] We found that N-acylated L-serine-containing D-glucosamine analogs (**3**), prepared from lipoamino acid (**1**) and D-glucosamine derivative (**2**), in which the reducing D-glucosamine unit of lipid A was replaced by L-serine derivatives, possessed mitogenic activity (unpublished data), as shown in Chart 1. For clarifying the structure-activity relationships between the molecular structure and the biological activity of lipid A, we wish to report here the synthesis of N-acylated L-serine-containing D-glucosamine derivatives (**4** and **5**) structurally similar to lipid A disaccharide backbone

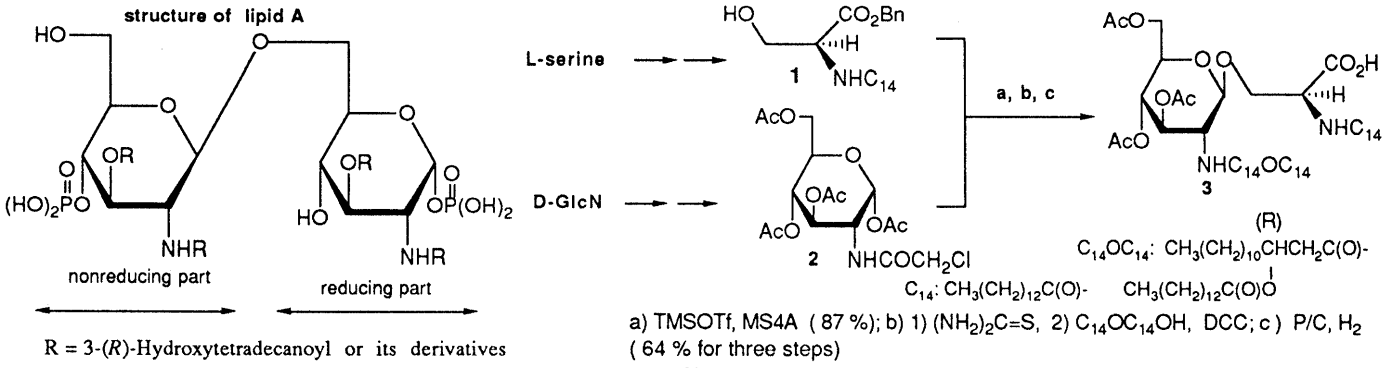
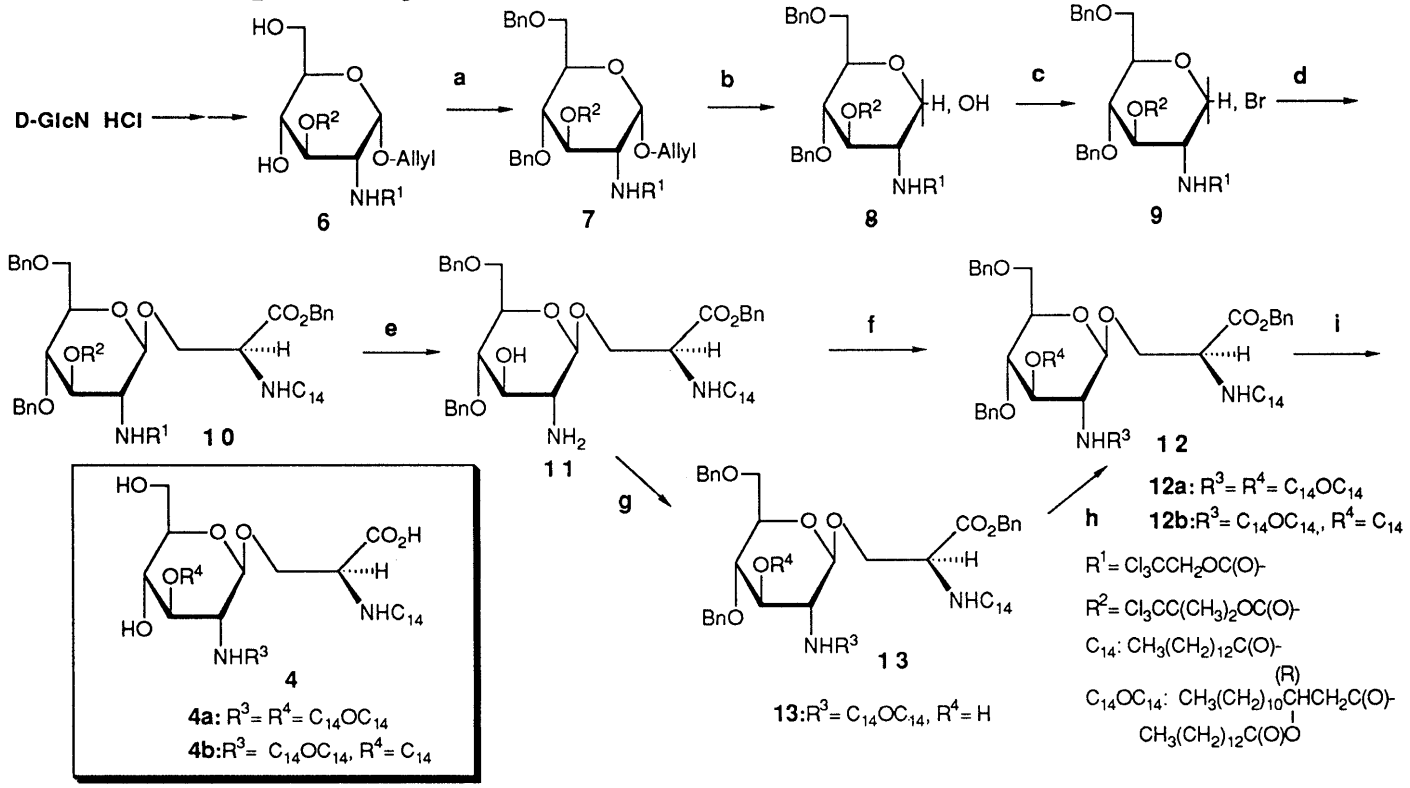


Chart 1

As our synthesis strategy to prepare **4** and **5**, we designed the suitably functionalized key intermediate (**11** and **19**) carrying one amino and one hydroxy group at the C-2 and C-3 positions of D-glucosamine skeleton, respectively.

First, we have synthesized the non-phosphorylated D-glucosamine-derived lipid A analogs to examine whether a phosphate group is required or not in lipid A analogs for biological activity, as indicated in Chart 2. The diol **6**[6)] was benzylated with benzyl trichloroacetimidate in the presence of catalytic amount of trifluoromethanesulfonic acid to give the dibenzyl compound **7** in 62 % yield. Removal of the O-allyl group with iridium catalyst, followed by hydrolysis with I_2-H_2O-pyridine gave the alcohol **8** in 60 % yield. Bromination of **8** with the Vilsmeier reagent, generated *in situ* by the use of thionyl bromide and DMF,[7)] gave the bromide **9** in quantitative yield. Condensation of **9** and lipoamino acid (**1**) with $HgBr_2$ as the promoter gave the β-glycoside **10** in 60 % yield; the configuration of the glycosidic linkage was assigned as β form from ^{1}H-NMR data ($J_{1,2}$=8.1Hz). Treatment of **10** with zinc-dust in acetic acid gave the amino alcohol compound **11** in 94 % yield. The key intermediate **11** thus obtained was acylated with

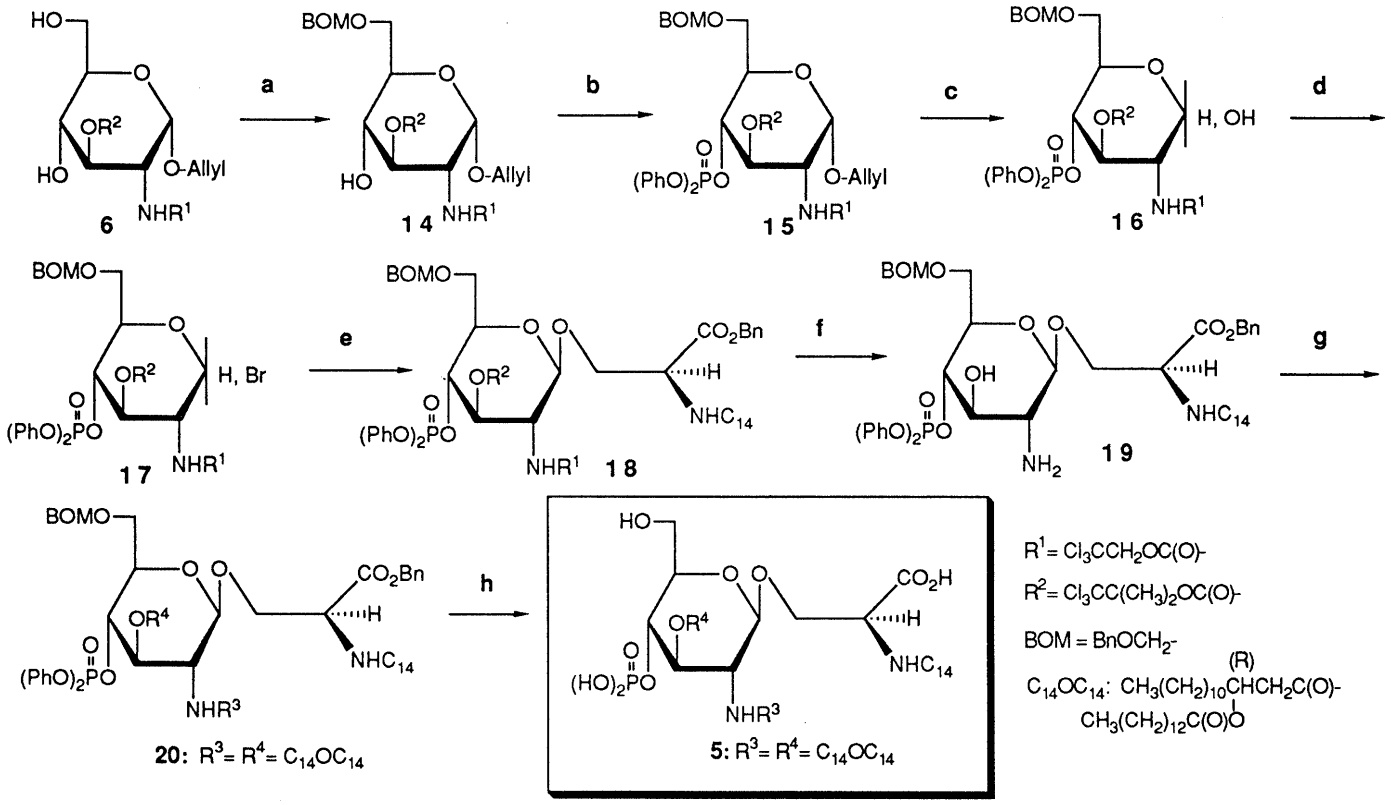
optically active (*R*)-3-tetradecanoyloxytetradecanoic acid[8] in the presence of dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-dimethylaminopyridine (DMAP) to give **12a** in 48 % yield. Finally, catalytic hydrogenolysis using palladium-black in methanol-THF gave the desired compound **4a** in 66 % yield, white powder, $[\alpha]_D$ -9.3°($CHCl_3$), after purification followed by lyophilization from dioxane. Similarly, the compound **4b**, bearing the (*R*)-3-tetradecanoyloxytetradecanoyl group at N-2 and the tetradecanoyl group at O-3 of D-glucosamine skeleton of the GLA-27 type,[9] was synthesized stepwise by successive acylation of the amino and hydroxy groups of **11**. Compound **11** was first acylated at the amino group with (*R*)-3-tetradecanoyloxytetradecanoic acid and DCC to give **13** in 59 % yield. The remaining hydroxy group of **13** was acylated with tetradecanoyl chloride, pyridine-DMAP to give **12b** in 57 % yield. Finally, deprotection of **12b** as described for the preparation of **4a** gave the desired product **4b** in 68 % yield, white powder, $[\alpha]_D$ -8.2°($CHCl_3$-MeOH).



a) $Cl_3CC(OBn)=NH$, CF_3SO_3H, CH_2Cl_2-cyclohexane (1: 2), rt, 15 h ; b) 1) $[CODIr(PMePh_2)_2]PF_6$, THF, 50°C, 2 h, 2) I_2, pyridine, THF-H_2O, rt, 15 min; c) $SOBr_2$, CH_2Cl_2-DMF (10:1), 0-5°C, 2h; d) **1**, $HgBr_2$, CH_2Cl_2, rt, 48 h; e) Zn, HOAc, 40-50°C, 48 h; f) $C_{14}OC_{14}OH$, DCC-DMAP, CH_2Cl_2, rt, 15 h; g) $C_{14}OC_{14}OH$, DCC, CH_2Cl_2, rt, 20 h; h) $C_{14}Cl$, pyridine-DMAP, CH_2Cl_2, rt, 15 h; i) Pd-black, H_2, MeOH-THF (2:1), rt, 24 h .

Chart 2

Next, the synthesis of phosphorylated D-glucosamine-derived lipid A analogs **5** was carried out as follows. The 6-*O*-hydroxy group of **6** was selectively protected with benzyloxymethyl chloride and tetramethylurea to give **14** in 66 % yield. The phosphorylation of **14** with diphenyl phosphorochloridate in the presence of pyridine-DMAP gave compound **15** in 89 % yield. Deprotection of allyl group of **15** as described for the preparation of **8** gave compound **16** in 81 % yield. Condensation of **1** and the bromide **17**, newly prepared from **16** and Vilsmeier reagent ($SOBr_2$-DMF), in the presence of $HgBr_2$ afforded coupling compound **18** in 33 % yield. Deprotection of TCEC and TCBOC groups of **18** with zinc powder in acetic acid gave the key intermediate **19** in almost quantitative yield. The simultaneous acylation of the amino and hydroxy groups of **19** with (*R*)-3-tetradecanoyloxytetradecanoic acid and DCC-DMAP gave **20** in 56 % yield. Finally, the protective benzyl and phenyl groups of **20** were removed by stepwise hydrogenolysis catalyzed by Pd-black and then platinum oxide in methanol to give the expected compound **5** in 44 % yield, white powder, $[\alpha]_D$-2.5° ($CHCl_3$).



a) BOMCl, TMU, CH_2Cl_2, rt, 24 h; b) $(PhO)_2P(O)Cl$, pyridine-DMAP, CH_2Cl_2, rt, 16 h; c) 1) $[CODIr(PMePh_2)_2]PF_6$, 50°C, 2 h; 2) I_2, pyridine, THF-H_2O, rt, 15 min; d) $SOBr_2$, CH_2Cl_2-DMF (10:1), 0-5°C, 2 h; e) **1**, $HgBr_2$, CH_2Cl_2, rt, 24 h; f) Zn, HOAc, 40-50°C, 48 h, g) $C_{14}OC_{14}OH$, DCC-DMAP, CH_2Cl_2, rt, 24 h; h) 1) Pd/C, H_2, MeOH, 40°C, 6 h, 2) PtO_2, H_2, MeOH, 40°C, 24 h.

Chart 3

As preliminary examination of the biological activity, the mitogenicity of compound **4b** showed about twice the activity on the splenocytes of C3H/He mice, while **4a,5** exhibited the same level in comparison with the original acyl-derivatives of D-glucosamine-4-phosphate.

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