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

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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), consists of a D-glucosaminyl-β(1-6)-D-glucosamine disaccharide carrying two phosphates and several fatty acids residues. 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. Recently, numerous acyclic analogs related to lipid A partial structure have been synthesized. We found that N-acylated L-serine-containing D-glucosamine analogs, 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in which the reducing D-glucosamine unit of lipid A was replaced by L-serine derivatives, possessed mitogenic activity (unpublished data), as shown in Chart 1.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.

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 was benzylated with benzyl trichloracetimidate 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 I₂-H₂O-pyridine gave the alcohol 8 in 60% yield. Bromination of 8 with the Vilsmeyer reagent, generated in situ by the use of thionyl bromide and DMF, gave the bromide 9 in quantitative yield. Condensation of 9 and lipoamino acid (1) with HgBr₂ as the promoter gave the β-glycoside 10 in 60% yield; the configuration of the glycosidic linkage was assigned as β form from 1H-NMR data (J₁,₂=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

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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, \([\alpha]_D -9.3^\circ\) (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, \([\alpha]_D -8.2^\circ\) (CHCl\(_3\)-MeOH).

![Diagram of chemical structures](image)

\(\text{Chart 2}\)

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 benzylxoxymethyl chloride and tetramethyleurea 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 TBCOC 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, \([\alpha]_D -2.5^\circ\) (CHCl\(_3\)).
As a 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.

REFERENCES


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