Synthesis of GLA-60 Positional Isomer as an LPS-Agonist and Its Activity

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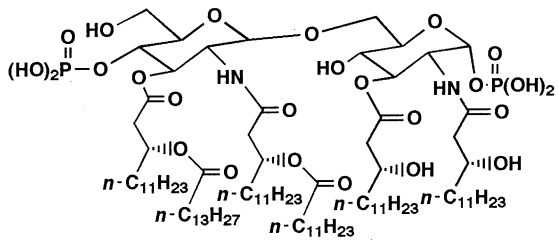
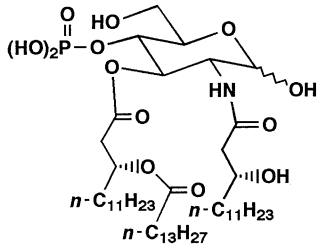
Compound **10** was synthesized from β-D-glucose pentaacetate in a stereocontrolled manner. It unexpectedly showed LPS-agonistic activity much stronger than that of GLA-60 toward human monoblastic U937 cells in the TNFα production.

In spite of the fact that lipopolysaccharide (LPS), an outer surface membrane component in Gram-negative bacteria, causes fever and lethal endotoxic shock in the septicemia of higher animals, it is also known as a highly potent stimulator of the immune system. Most of the biological activities of LPS reside in a relatively small portion of the molecule known as lipid A, a disaccharide unit bearing the constituent lipid moiety. Lipid A, which was first isolated by Westphal and Luderitz[1] and later chemically synthesized by Imoto[2] and Achiwa,[3] exists as a hydrophobic anchor substance holding an essentially linear polysaccharide chain to the cell wall.[4] In a series of structure-activity relationship studies on non-reducing subunit analogues of lipid A, Hasegawa and Kiso[5] have demonstrated that several LPS-agonistic activities are expressed by certain 4-*O*-phosphono-D-glucosamine derivatives pertaining to the structure of GLA-60.[5] Recently it has been shown that lipid A analogues also have LPS-antagonistic activity.[6] Therefore, we have been investigating the compounds related to GLA-60 for therapeutic use. In this paper, we describe the synthesis of GLA-60 positional isomer, *N*-[3-*O*-phosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-3-[(*R*)-hydroxy]tetradecanamide (**10**), which has exhibited LPS-agonistic activity.



Lipid A

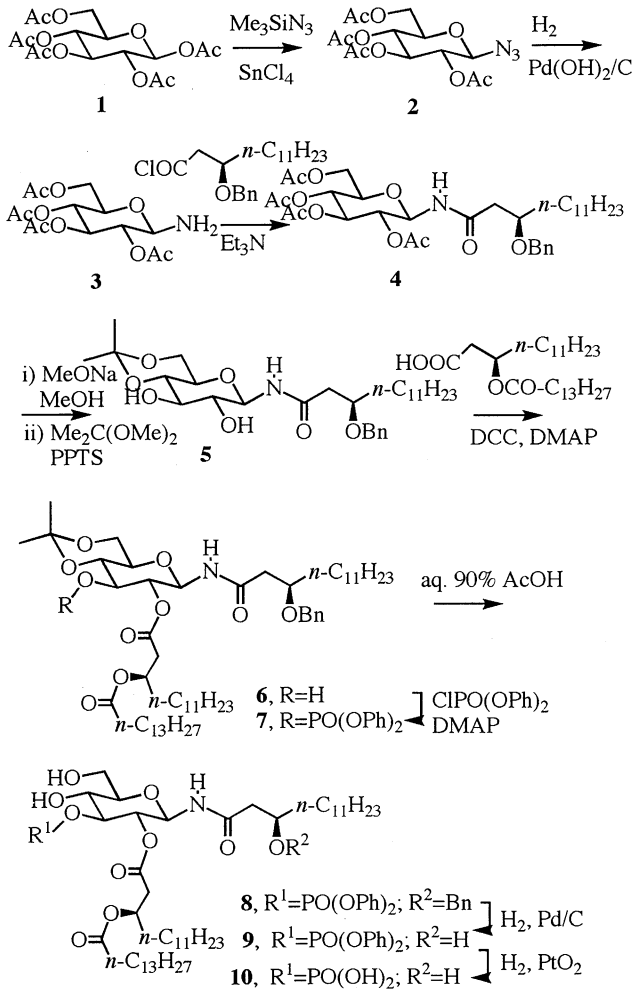


GLA-60

Figure 1. Structures of Lipid A and GLA-60.

The starting β-D-glucose pentaacetate (**1**) was employed for the synthesis of the title compounds. Treatment of **1** in CH_2Cl_2 with trimethylsilyl azide using tin(IV) chloride as a Lewis acid for 30 min at 25 °C gave an azide **2** (94%) stereoselectively,[7] and the azido group was hydrogenolyzed in THF-H_2O (2:1) at 25 °C to an amine **3** (44%, after silica gel chromatography) using $Pd(OH)_2$ on carbon as a catalyst. Silica gel chromatography of the amine **3** may cause the yield low by absorption. Compound **3** was treated with (*R*)-3-(benzyloxy)tetradecanoyl chloride and triethylamine in dichloromethane for 30 min at 25 °C to yield an amide **4** (84%). The coupling constant between C1-H and C2-H of **4** was *J*=9.2 Hz. This reveals that C1-H and C2-H are oriented to α-axial and β-axial, respectively. Compound **4** was converted to acetonide **5** (72%) by treatment with sodium methoxide in methanol for 30 min at 25 °C, and then with 2,2-dimethoxypropane for 3 h at 25 °C using pyridium *p*-toluenesulphonate (PPTS) as a catalyst in *N*,*N*-dimethylformamide (DMF) in succession. This 2,3-diol compound **5** was treated with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid,[8] 4-dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide (DCC) in DMF for 1 h at 25 °C to give 2-*O*-monoacylated compound **6** (67%) regioselectively. Verification that acylation has occurred at the 2-position of this 2,3-diol compound came from ^1H NMR data of compounds **5** and **6**, showing the C2 proton of compound **6** had shifted to lower magnetic fields than that of compound **5** (from δ 3.20 to 4.70). The remaining 3-hydroxy group of **6** was phosphorylated with diphenyl chlorophosphate and DMAP in CH_2Cl_2 for 30 min at 25 °C to give **7** (94%). The acetonide group of compound **7** was cleaved by treatment with aqueous 90% acetic acid for 5 h at 60 °C to give 4,6-diol **8** (76%). Compound **8** in turn was debenzylated using 10% palladium on carbon in THF for 3 h at 25 °C to give **9** (77%). Finally, hydrogenolysis of phosphate ester **9** in THF for 3-16 h at 25 °C using platinum(IV) oxide as a catalyst yielded the phosphono compound **10** (99%).[9] Thus compound **10** was synthesized in a stereocontrolled manner.

Compound **10** unexpectedly showed LPS-agonistic activity much stronger than that of GLA-60 toward human monoblastic U937 cells. The TNFα production (% of control; 10 ng/ml of LPS=100) of lipid A, GLA-60, and compound **10** in the concentrations of 0.01 μM, 0.1 μM, 1 μM, and 10 μM were as follows. Lipid A was 21.0, 136.0, 385.0, and 651.0; GLA-60



Scheme 1.

was 12.0, 12.0, 7.0, and 13.0; and compound **10** was 14.0, 20.0, 57.0, and 611.0, respectively. Comparing the structure of **10** with GLA-60 or lipid A, the positions of the substituents were each shifted one position. Moreover, the substitutions at the C1 (anomeric amido), C2 (ester), and C3 (phosphono) positions of compound **10** are aligned with C1-β, C2-α, and C3-β, respectively, and those of GLA-60 and lipid A are aligned with C2-α (amido), C3-β (ester), and C4-α (phosphono), respectively. Nevertheless, U937 cell recognized **10** as an LPS-agonist just like GLA-60 and lipid A. We cannot provide a reasonable explanation for this phenomenon. This finding may offer a new concept for less toxic anticancer drugs relating to GLA-60 and lipid A.

References and Notes

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9 ^{1}H NMR (pyridine-d$_5$) of **10**: δ 0.89 (9H, t, *J*=6.6 Hz), 1.05-1.60 (56H, m), 1.60-1.82 (6H, m), 2.51 (2H, t, *J*=7.3 Hz), 2.77 (1H, dd, *J*=4.4, 14.7 Hz), 2.84 (1H, dd, *J*=6.6, 14.7 Hz), 2.95 (1H, dd, *J*=7.3, 16.1 Hz), 3.05 (1H, dd, *J*=7.3, 16.1 Hz), 3.93-4.00 (1H, m, C_5-*H*), 4.20-4.52 [4H, m, C_4-*H*, C_6-H_2, C*H*-(OH)-$C_{11}H_{23}$], 5.12 (1H, q, $J_{2,3}$=$J_{3,4}$=J_{HCOP}=8.8 Hz, C_3-*H*), 5.57-5.68 [2H, m, C_2-*H*, C*H*-(OCO$C_{13}H_{27}$)-$C_{11}H_{23}$], 6.10 (1H, t, *J*=8.8-9.4 Hz, changed to a doublet, *J*=9.4 Hz, on addition of D_2O, C_1-*H*), 6.21 (5H, broad, OH x 5), 9.81 (1H, d, *J*=8.8 Hz, C_1-N*H*).