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Synthesis of 2,6-Anhydro-3-deoxy-5-*O*-phosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid as a New Potent Endotoxin Antagonist and its Dimeric Analogue

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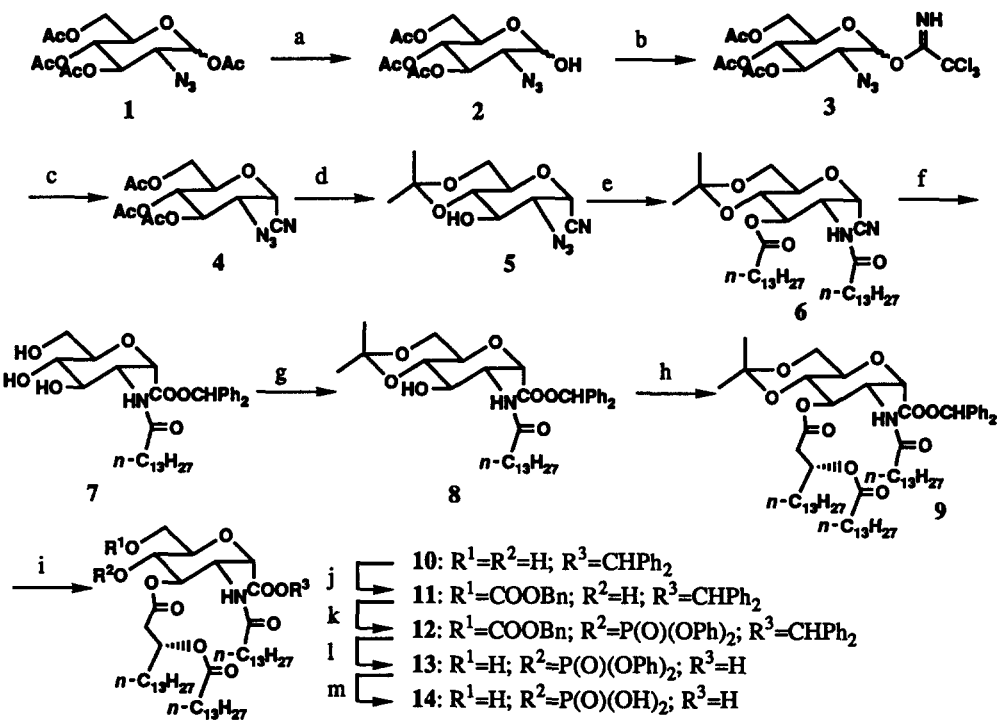
Abstract: *A pyran carboxylic acid analogue of GLA-60 (**14**) and its dimeric analogue **18** were synthesized in a stereocontrolled manner. Compound **14** showed strong LPS-antagonistic activity.*

Lipopolysaccharides (LPS) cover the outer surface membrane of such Gram-negative bacteria as *Salmonella minnesota*, *Salmonella typhirium*, and *Escherichia coli*, and are highly potent stimulators of the immune system. A variety of responses, both beneficial and harmful, can be elicited by LPS. One of these harmful responses is fatal endotoxic shock (bacterial sepsis) caused as a consequence of acute inflammatory response, a fact which has precluded the clinical use of LPS. Most of the biological activities of LPS reside in a relatively small portion of the molecule, that is, the terminal disaccharide phospholipid subunit known as lipid A,[1] which is a hydrophobic anchor substance holding an essentially linear polysaccharide chain to the cell wall. Lipid A was chemically synthesized by both Shiba's and Achiwa's groups.[2]

In a series of investigations by Hasegawa and Kiso[3] on the relationship between the molecular structure and biological activity of non-reducing sugar subunit analogues of lipid A, it has been demonstrated that several kinds of the biological activities of LPS can be expressed by certain 4-*O*-phosphono-D-glucosamine derivatives such as GLA-60.[3] Recently, Qureshi's group[4] has isolated a lipid A-related compound from *Rhodobacter sphaeroides* as an inseparable mixture of three compounds, which showed potent LPS antagonist activity. Furthermore, an Eizai group has developed a related compound, E5531,[5] as a highly potent anti-septicemia drug.

During our investigation of the biological activity of compounds related to GLA-60, we have also found that carboxymethyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-phosphono-3-*O*-

Scheme 1



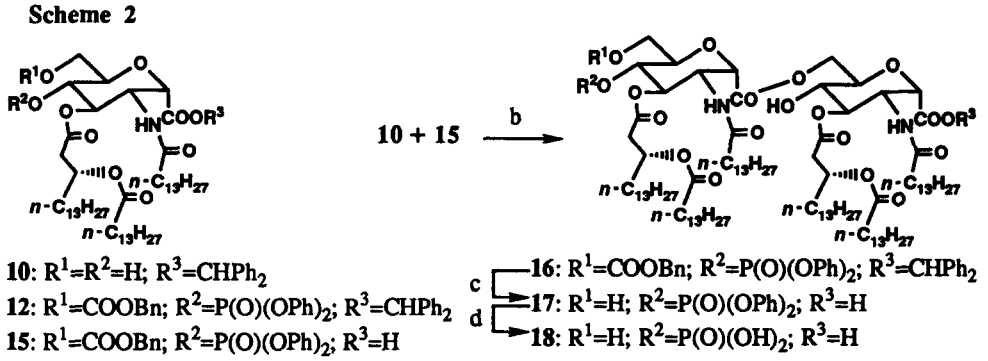
Reagents and Conditions: a) H_2NNH_2-AcOH, 50 °C, 2 min, DMF, 92%; b) CCl_3CN, $LiN(TMS)_2$, 0 °C, then 24 °C, 1 h, THF, 85%; c) TMSCN, cat. TMSOTf, 24 °C, 15 h, CH_2Cl_2, quantitative; d) (i) cat. KOH, EtOH, 24 °C, 30 min; (ii) $Me_2C(OMe)_2$, cat. *p*-TsOH·H_2O, 24 °C, 16 h, DMF, 65%; e) (i) Ph_3P, THF-H_2O, 24 °C, 16 h; (ii) tetradecanoic acid, DCC, DMAP, 24 °C, 3 h, THF; (iii) tetradecanoyl chloride, Et_3N, 24 °C, 16 h, THF, 43%; f) (i) 4M HCl in dioxane-H_2O (10:1), 55-60 °C, 4 h; (ii) Ph_2CN_2, 55-60 °C, 1.5 h, DMF, 53%; g) $Me_2C(OMe)_2$, cat. *p*-TsOH·H_2O, 25 °C, 16 h, DMF, 56%; h) (*R*)-3-(tetradecanoyloxy)tetradecanoic acid, DCC, DMAP, 24 °C, 16 h, CH_2Cl_2, 96%; i) aq. 85% AcOH, 70-75 °C, 1 h, 58%; j) ClCOOBn, pyridine, 0-5 °C, 30 min, CH_2Cl_2, 97%; k) $ClP(O)(OPh)_2$, DMAP, 24 °C, 4 h, CH_2Cl_2, 87%; l) H_2, Pd/C, 25 °C, 10 h, THF, 89%; m) H_2, PtO_2, 25 °C, 3-10 h, THF, 92%.

[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside exhibited fairly strong LPS antagonistic activity.[6] By analogy from this result, we designed a pyran carboxylic acid (**14**) as a related compound. In this paper, we describe the synthesis of **14** and its dimeric analogue **18**, and the LPS-antagonistic activity of **14**.

The starting 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-D-glucopyranoside (**1**), obtained from D-glucosamine hydrochloride using the method reported by Vasella,[7] was converted to 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-glucopyranoside (**2**) according to Excoffier's procedure.[8] Treatment of **2** with trichloroacetonitrile in THF at 0 °C using $LiN(TMS)_2$ as a base gave a mixture of α- and β-imidates. The mixture was partly separated by silica gel chromatography to **3**-α (mp.

130-131 °C) and **3**-β (mp. 136-137 °C). The next stage is the most critical step in this series of synthesis, because α-oriented carboxylic acid equivalent is needed. Schmidt's group[9] has already reported that treatment of 3,4,6-tri-*O*-benzyl-2-azido-2-deoxy-α-D-glucopyranosyl trichloroacetimidate with trimethylsilyl cyanide using trimethylsilyl trifluoromethanesulfonate as a catalyst yielded a corresponding α-cyanide ($J_{1,2}$=5.4 Hz). Application of this reaction to the compound **3**-α gave an α-cyanide **4** as expected. Moreover, application of this reaction to **3**-β exclusively formed **4**. Also the mixture of **3**-α and **3**-β gave **4** stereospecifically in quantitative yield. Deacetylation of **4** with a catalytic amount of KOH in EtOH, and acetonide formation between C4-OH and C6-OH with 2,2-dimethoxypropane using *p*-TsOH as a catalyst formed **5** (mp 172-173 °C). The NMR coupling constant between C1-H and C2-H of **4** was J=6.0 Hz which was a little bit larger than that of tri-benzyl analogue. However, the α-cyano configuration of **5** was confirmed from observation of the NOE effect between C1-H and C2-H of **5**. Treatment of **5** in THF with (i) PPh_3 and H_2O, (ii) tetradecanoic acid, DCC and DMAP, and (iii) tetradecanoyl chloride and Et_3N yielded **6** (mp. 59-61 °C). Hydrolysis of nitrile **6** with 4M HCl in dioxane-H_2O (v/v, 10:1), and esterification of the resulting carboxylic acid with Ph_2CN_2 gave a diphenylmethyl ester **7** (mp. 176-179 °C). Acetonide formation between C4-OH and C6-OH of **7** with 2,2-dimethoxypropane using *p*-TsOH as a catalyst gave **8** (mp. 113-115 °C). Esterification of **8** with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid, DCC and DMAP formed **9**. Deprotection of acetonide **9** with aqueous 85% AcOH gave **10** (mp. 105-106 °C). Treatment of **10** with benzyl chloroformate and pyridine yielded **11**. Treatment of **11** with diphenyl chlorophosphate and DMAP formed **12**. Hydrogenolysis of **12** using 10% Pd/C as a catalyst gave a carboxylic acid **13**, which was further converted to **14**[10] using PtO_2 as a catalyst.

Scheme 2



Reagents and Conditions: a) CF_3COOH, CH_2Cl_2, 24 °C, 1 h, 56%; b) DCC, DMAP, 24 °C, 16 h, CH_2Cl_2, THF, 11%;[10] c) H_2, Pd/C, THF, 24 °C, 6 h, quantitative; d) H_2, PtO_2, THF, 24 °C, 16 h, 80%.

The dimeric analogue **18** was synthesized as follows. Deprotection of diphenylmethyl ester of **12** with CF_3COOH in CH_2Cl_2 gave **15**. Esterification of **10** and **15** with DCC and DMAP in CH_2Cl_2 formed **16**.[11] Hydrogenolysis of **16** using 10% Pd/C gave **17** which was further

hydrogenolized to **18** by using PtO_2 as a catalyst. Thus compound **14** and **18** were synthesized in a stereo- and regiocontrolled manner.

Biological activity: Compound **14** as well as carboxymethyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside showed endotoxin antagonistic activity toward human monoblastic U937 cells as an index which indicated the inhibition of LPS-induced TNFα production.[6] In the presence of 10 ng/ml LPS (obtained from *E. coli* serotype 026:B6), the IC_{50} values of carboxymethyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside,[6] compound **14** and prednisolone (an antiinflammatory steroid known to have a potent inhibitory activity on TNFα production by stimulated monocytes)[12] were 0.005, 0.017 and 0.014 μM, respectively. However compound **18** did not show any effective activities. Compound **14** may be potent for treatment of Gram-negative septic shock.

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10. Physical data of **14**: 270 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.0-6.8 Hz), 1.26 (58H, broad s), 1.57 (6H, broad s), 2.10-2.80 (6H, m), 3.60-4.10 (3H, m), 4.20-4.70 (3H, m), 5.06-5.40 (2H, m), 7.00 (1H, broad s, NH), FAB MS; 935 $(M+H)^+$.
11. Carboxylic acid **15** reacted with DCC mainly to yield α-CON(cyclohexyl)CONH(cyclohexyl).
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