Pergamon

Tetrahedron Letters, Vol. 37, No. 40, pp. 7271-7274, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01646-2

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

Masao Shiozaki,*[a] Takashi Mochizuki,[a] Takanori Wakabayashi,[a]
Shin-ichi Kurakata,[b] Tohru Tatsuta,[b] and Masahiro Nishijima[c]
[a] *Exploratory Chemistry Research Laboratories, Sankyo Co. Ltd.*
[b] *Biological Research Laboratories, Sankyo Co. Ltd.*
Hiromachi 1-2-58, Shinagawa-ku, Tokyo 140, Japan
[c] *Department of Biochemistry and Cell Biology, National Institute of Health, Toyama 1-23-1, Shinjuku-ku, Tokyo 162, Japan*

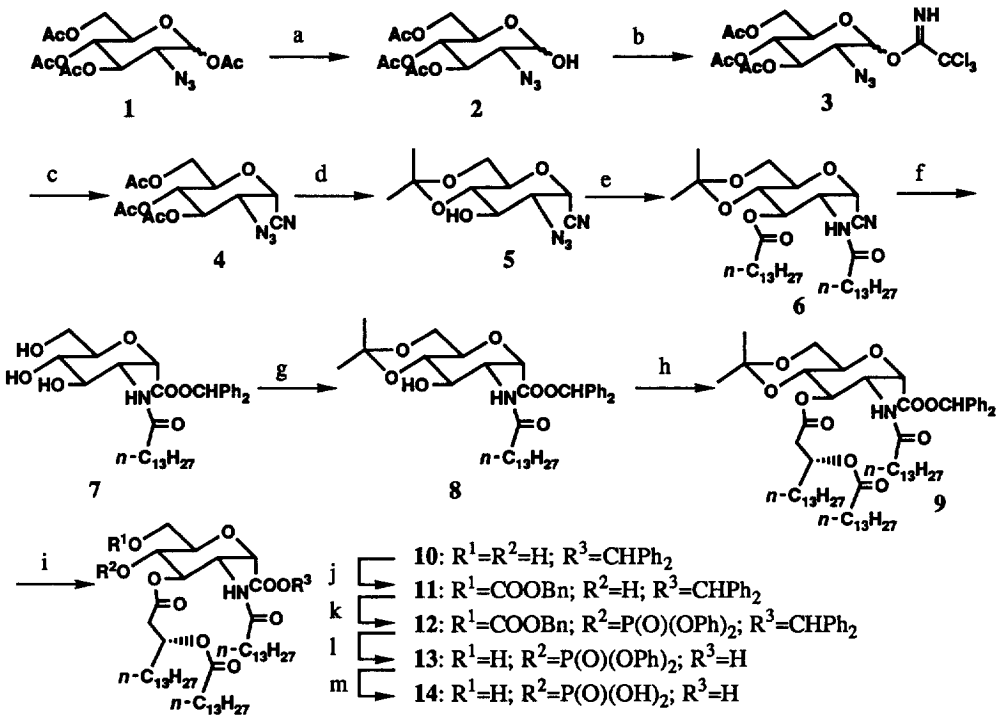
Abstract: *A pyran carboxylic acid analogue of GLA-60 (**14**) and its dimeric analogue **18** were synthesized in a stereocontrolled manner. Compound **14** showed strong LPS-antagonistic activity.*

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



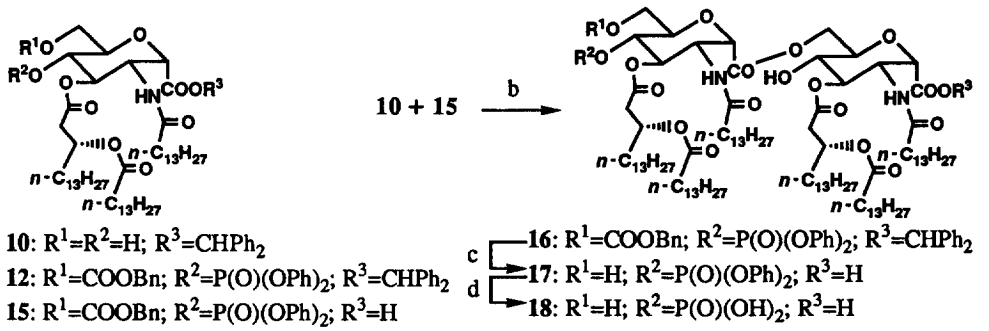
Reagents and Conditions: a) H_2NNH_2-AcOH, 50 °C, 2 min, DMF, 92%; b) CCl_3CN, $LiN(TMS)_2$, 0 °C, then 24 °C, 1 h, THF, 85%; c) TMSCN, cat. TMSOTf, 24 °C, 15 h, CH_2Cl_2, quantitative; d) (i) cat. KOH, EtOH, 24 °C, 30 min; (ii) $Me_2C(OMe)_2$, cat. *p*-TsOH·H_2O, 24 °C, 16 h, DMF, 65%; e) (i) Ph_3P, 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.

References and Notes

1. O. Westphal and O. Luderitz, *Angew. Chem.*, **66**, 407-417 (1954).
2. a) M. Imoto, H. Yoshimura, N. Sakaguchi, S. Kusumoto, and T. Shiba, *Tetrahedron Lett.*, **26**, 1545-1548 (1985). b) S. Takahashi, S. Nakamoto, K. Ikeda, and K. Achiwa, *Tetrahedron Lett.*, **27**, 1819-1822 (1986).
3. a) M. Matsuura, Y. Kojima, J. Y. Homma, Y. Kubota, A. Yamamoto, M. Kiso, and A. Hasegawa, *FEBS Lett.*, **167**, 226-230 (1984). b) M. Kiso, H. Ishida, and A. Hasegawa, *Agric. Biol. Chem.*, **48**, 251-252 (1984). c) M. Kiso, S. Tanaka, M. Fujita, Y. Fujishima, Y. Ogawa, H. Ishida, and A. Hasegawa, *Carbohydr. Res.*, **162**, 127-140 (1987). d) M. Kiso, Y. Ogawa, S. Tanaka, Y. Fujishima, M. Fujita, S. Tanaka, and A. Hasegawa, *J. Carbohydr. Chem.*, **6**, 625-638 (1987).
4. a) N. Qureshi, J. P. Honovich, H. Hara, R. J. Cotter, and K. Takayama, *J. Biol. Chem.*, **263**, 5502-5504 (1988). b) N. Qureshi, K. Takayama, and R. Kurtz, *Infect. Immunol.*, **59**, 441-444 (1991). c) cf. W. J. Christ, P. D. McGuinness, O. Asano, Y. Wang, M. A. Mullarkey, M. Perez, L. D. Hawkins, T. A. Blythe, G. R. Dubuc, and A. L. Robidoux, *J. Am. Chem. Soc.*, **116**, 3637-3638 (1994).
5. W. J. Christ, O. Asano, A. L. C. Robidoux, M. Perez, Y. Wang, G. R. Dubuc, W. E. Gavin, L. D. Hawkins, P. D. McGuinness, M. A. Mullarkey, M. D. Lewis, Y. Kishi, T. Kawata, J. R. Bristol, J. R. Rose, D. P. Rossignol, S. Kobayashi, I. Hishinuma, A. Kimura, N. Asakawa, K. Katayama, and I. Yamatsu, *Science*, **268**, 80-83 (1995).
6. M. Shiozaki, N. Deguchi, W. M. Macindoe, M. Arai, H. Miyazaki, T. Mochizuki, T. Tatsuta, J. Ogawa, H. Maeda, and S. Kurakata, *Carbohydr Res.*, **283**, 27-51 (1996).
7. A. Vasella, C. Witzig, J-L. Chiara, and M. Martin-Romas, *Helv. Chim. Acta*, **74**, 2073-2077 (1991).
8. G. Excoffier, D. Gagnaire, J-P. Utille, *Carbohydr. Res.*, **39**, 368-373 (1975).
9. M. G. Hoffmann and R. R. Schmidt, *Liebigs Ann. Chem.*, 2403-2419 (1985).
10. Physical data of **14**: 270 MHz ^1H 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).
12. S. Heidenreich, D. Lang, M. Tepel, and K. M. Rahn, *Transpl. Immunol.*, **2**, 35-40 (1994).

(*Received in Japan* 3 *July* 1996; *revised* 13 *August* 1996; *accepted* 20 *August* 1996)