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Carbohydrate Research 324 (2000) 225–230

CARBOHYDRATE RESEARCH

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Synthesis and biological activities of lipid A-type pyrancarboxylic acid derivatives

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Received 17 September 1999; accepted 7 December 1999

Abstract

The synthesis of lipid A-type pyrancarboxylic acid derivatives, which have a carboxylic acid group in the anomeric position of the reducing part of the disaccharide instead of the phosphate group in lipid A, is described. One of the compounds thus synthesized, which has an acyl substitution pattern similar to that of *Escherichia coli* lipid A, showed lipopolysaccharide (LPS)-agonistic activity. The other, which contains four lipid chains in the molecule, exhibited strong LPS-antagonistic activity toward human monoblastic U937 cells.

Keywords: Lipid A; Pyrancarboxylic acid; LPS antagonist; TNFα production

Lipopolysaccharide (LPS) [1] is a component of the outer surface membrane of Gram-negative bacteria. LPS is known to stimulate the immune system, resulting in many pathophysiological events such as fever, depression of blood pressure, platelet aggregation, shock, and organ failure leading to bacterial sepsis [2]. 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 [3,4], which is a hydrophobic anchor substance that links an essentially linear polysaccharide chain to the cell wall. Many synthetic and natural lipid A analogues have already been investigated, and it has been determined which parts of the structure of the molecules are important for their biological activity by changing the number and length of lipid chains and by replacing the anomeric phosphate group with other acidic moieties. In fact, lipid A **1** from *Escherichia coli*, which contains six lipid chains in the molecule, shows LPS-agonistic activity, but its biosynthetic precursor lipid IVa (**2**), which lacks the dodecanoyl and tetradecanoyl groups of **1** and thus contains only four lipid chains, exhibits LPS-antagonistic activity in human systems [5] (Fig. 1).

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0008-6215/00/$ - see front matter
PII: S0008-6215(99)00326-2

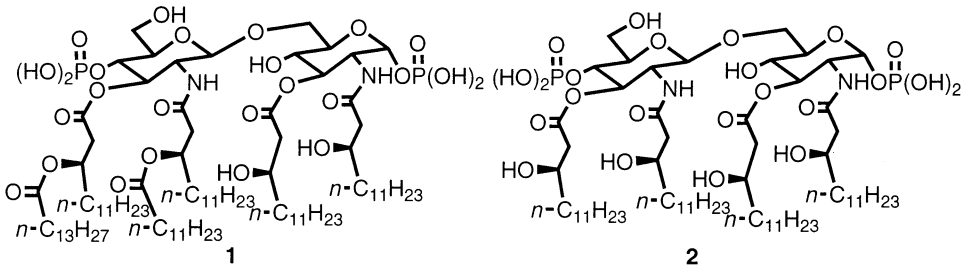


Fig. 1. The structures of *E. coli* lipid A (**1**) and lipid IVa (**2**).

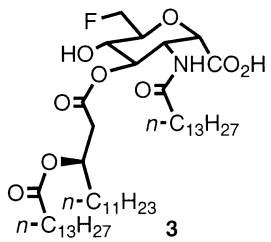


Fig. 2. The structure of pyrancarboxylic acid **3**.

We also investigated the biological activities of GLA-60 [6] related compounds to seek an LPS antagonist as a potential antisepticemia drug and found that the pyrancarboxylic acid derivative **3** showed LPS-antagonistic activity toward human monoblastic U937 cells [7] (Fig. 2).

For developing further potent LPS antagonists according to the design discussed above, we planned the synthesis of two lipid A-type pyrancarboxylic acid derivatives. One is a 6′-*O*-methyl-lipid A-type pyrancarboxylic acid **4**, which has a similar structure to that of *E. coli* lipid A except that its anomeric phosphate group and 6′-hydroxy group are changed to a carboxylic acid group and a methoxy group, respectively (Fig. 3). The 6′-hydroxy group is methylated because a free hydroxy group might cause instability of the compounds according to the report on the LPS antagonist E-5531 [8]. The other is a 6′-*O*-methyl-2′-acetamidolipid A-type pyrancarboxylic acid **5**, in which the (*R*)-3-dodecanoyloxytetradecanamido group in the 2′ position of **4** has been replaced with an acetamido group in **5** as shown in Fig. 3. Thus it contains four lipid chains in the molecule, but in a different fashion from that of the LPS antagonist **2**. With such synthesized compounds, we first would like to investigate whether the carboxy group directly attached to the pyran in the molecule is crucial for its LPS antagonism, and if not, we would determine whether the more stable carboxy group could be exchanged for an anomeric phosphate group without loss of biological activity. Secondly, we wanted to examine the effect of the number of lipid chains in our compounds on their biological activities. In this paper, we describe their syntheses and biological activities[1].

For the synthesis of **4**, the 6-hydroxy group of allyl 2-deoxy-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl] - 2 - (2,2,2 - trichloroethoxycarbonylamino)-α-D-glucopyranoside (**6**) [3b] was silylated with *tert*-butyldimethylsilyl chloride and imidazole in *N*,*N*-dimethylformamide (DMF) to give the 6-*O*-monosilylated compound **7**. Subsequently, 4-*O*-phosphorylation of **7** with diphenyl chlorophosphate and *N*,*N*-dimethylaminopyridine (DMAP) and successive deprotection of the 6-*O*-silyl group with 3 M aqueous hydrochloric acid in tetrahydrofuran (THF) provided the alcohol **8**. After methylation of the 6-hydroxy group of **8** with trimethyloxonium tetrafluoroborate and 2,6-di(*tert*-butyl)-4-methylpyridine, deprotection of the anomeric allyl group of the compound thus obtained (**9**) was accomplished by treatment with (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate in THF and successive treatment with I_2–H_2O, affording hemiacetal **10** [9], which was converted into the trichloroacetoimidate **11** by the reaction of **10** with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) [10] (Scheme 1).

[1] Compounds were characterized by identity and purity by ^{1}H NMR spectroscopy, IR spectroscopy, mass spectrometry and by elemental analysis or high-resolution mass spectrometry.

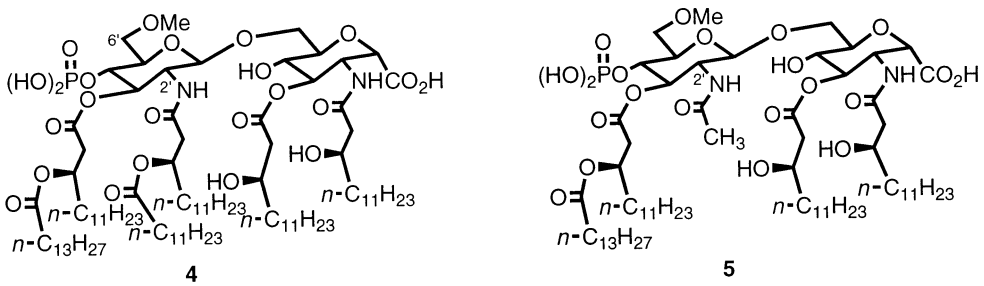


Fig. 3. 6′-*O*-Methyl-lipid A type pyrancarboxylic acid **4** and its 2′-acetamido derivative **5**.

Glycosylation of **11** with glycosyl acceptor **12**, which was prepared using a previously reported procedure [7], was conducted using trimethylsilyl trifluoromethanesulfonate (Me_3-SiOTf) as a catalyst according to the known method [3b], to give the β-oriented pseudodisaccharide **13**. After deprotection of the trichloroethoxycarbonyl group of **13** with zinc dust in acetic acid, condensation of the resulting amine with (*R*)-3-dodecanoyloxytetradecanoic acid was performed with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI), providing **14**. Deprotection of the two benzyl ether groups and the diphenylmethyl ester group of **14** by hydrogenolysis using $Pd(OH)_2$/C as a catalyst gave **15**. Finally, hydrogenolytic deprotection of the diphenyl phosphate ester group of **15** using PtO_2 as a catalyst furnished **4**[2] (Scheme 2).

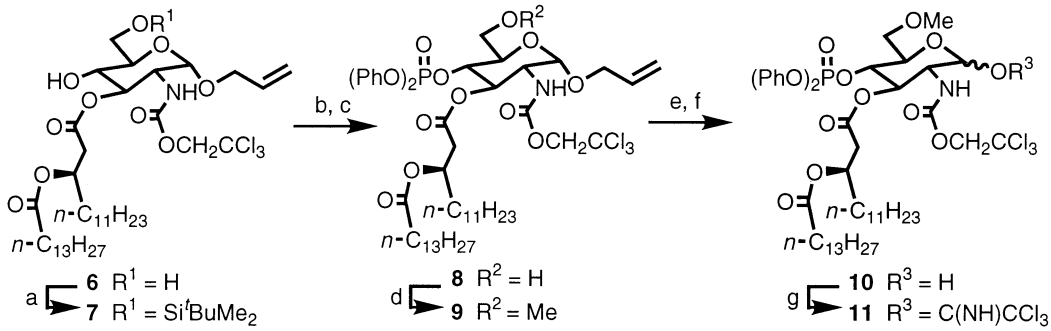
The 2′-acetamido derivative **5** was synthesized from the aforementioned intermediate **13**. After the generation of the amine from **13** by zinc dust in acetic acid, acetylation of the amine with acetic acid and EDCI yielded **16**. By the same procedure as that for the synthesis of **4**, the two benzyl ether groups and the diphenylmethyl ester group of **16** were deprotected and then the diphenyl phosphate ester group of the compound thus obtained (**17**) was deprotected to yield **5**[3] (Scheme 3).

The biological activities of compounds **4** and **5**, thus synthesized, were investigated by measuring TNFα production in human monoblastic U937 cells. U937 cells cultured in the presence of TPA for 72 h at 37 °C were incubated in RPMI-1640 medium with 10% newborn calf serum and graded concentrations of the compounds in the absence (LPS agonism) or presence (LPS antagonism) of LPS (30 ng/mL) in a humidified atmosphere of 5% CO_2 for 4.5 h at 37 °C. After incubation, the amounts of TNFα produced in the culture supernatants were determined by means of enzyme-linked immunosorbent assay (ELISA).

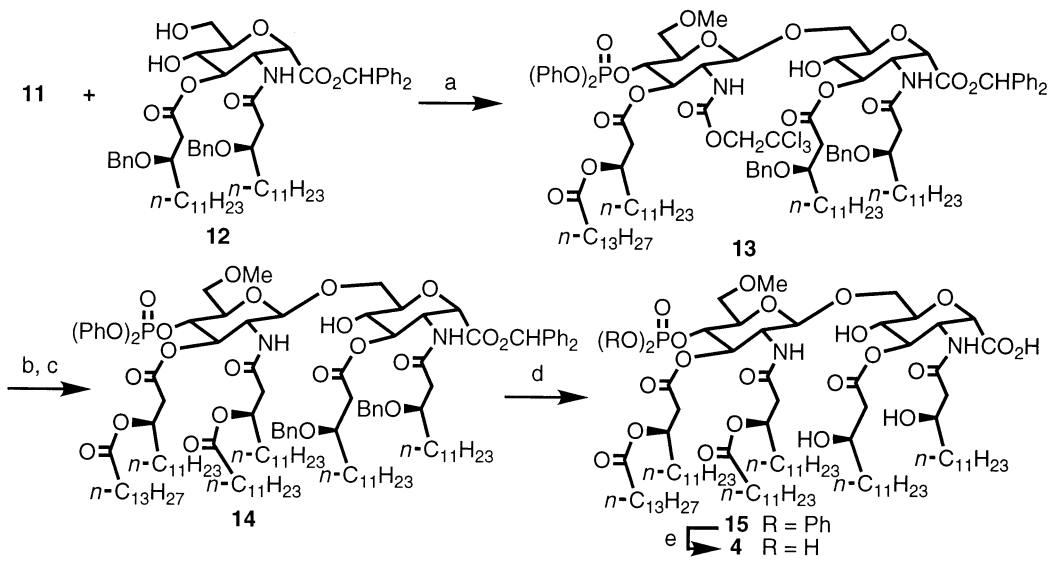
As a result, in the absence of LPS, compound **4** produced TNFα in a dose-dependent manner, showing LPS-agonistic activity, but compound **5** did not produce TNFα even at a

[2] Physicochemical data for 2,6-anhydro-3-deoxy-7-*O*-[2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanamido]-6-*O*-methyl-4-*O*-phosphono-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-[(*R*)-3-hydroxytetradecanamido]-D-*glycero*-D-*ido*-heptonic acid (**4**): $[\alpha]_D^{26}$ −4.0° (*c* 0.20, $CDCl_3$). IR: ν_{max} (KBr) 3351 (broad), 1734, 1664 cm^{-1}; ^1H NMR (400 MHz, 5:1 CD_3OD–$CDCl_3$): δ 5.30–5.20 (4 H, m), 4.67 (1 H, d, *J* 8.8 Hz), 4.48 (1 H, d, *J* 5.1 Hz), 4.37–4.33 (1 H, m), 4.14–4.05 (2 H, m), 4.01–3.96 (1 H, m), 3.92–3.87 (2 H, m), 3.82–3.76 (3 H, m), 3.64–3.55 (3 H, m), 3.42 (3 H, s), 2.78–2.21 (12 H, m), 1.67–1.23 (120 H, m), 0.90 (18 H, t, *J* 6.6 Hz); HRFABMS (positive ion): Calcd for $C_{96}H_{179}N_2O_{23}PNa$ $[M+Na]^+$ 1782.2534. Found: *m*/*z* 1782.2542.

[3] Physicochemical data for 2,6-anhydro-7-*O*-[2-acetamido-2-deoxy-6-*O*-methyl-4-*O*-phosphono-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-deoxy-3-[(*R*)-3-hydroxytetradecanamido]-4-*O*-[(*R*)-3-hydroxytetradecanoyl]-D-*glycero*-D-*ido*-heptonic acid (**5**): $[\alpha]_D^{26}$ −6.6° (*c* 0.20, $CDCl_3$). IR: ν_{max} (KBr) 3362, 1737, 1665 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 5.30 (1 H, dd, *J* 10.7, 8.8 Hz), 5.23–5.15 (2 H, m), 4.66 (1 H, d *J* 8.5 Hz), 4.50 (1 H, d, *J* 5.7 Hz), 4.34–4.29 (1 H, m), 4.22–4.19 (1 H, m), 4.10 (1 H, d, *J* 1.4 Hz), 4.00–3.94 (1 H, m), 3.88–3.73 (5 H, m), 3.62–3.57 (2 H, m), 3.54 (1 H, t, *J* 9.1 Hz), 3.41 (3 H, s), 2.72–2.22 (8 H, m), 1.94 (3 H, s), 1.62–1.56 (4 H, m), 1.48–1.40 (6 H, m), 1.39–1.23 (72 H, m), 0.91 (12 H, t, *J* 7.0–6.6 Hz); HRFABMS (positive ion): Calcd for $C_{72}H_{133}N_2O_{21}PNa$ $[M+Na]^+$ 1415.9036. Found: *m*/*z* 1415.9034. Anal. Calcd for $C_{72}H_{133}N_2O_{21}P{\cdot}H_2O$: C, 61.25; H, 9.64; N, 1.98; P, 2.19. Found: C, 61.53; H, 9.50; N, 1.99; P, 2.23.



Scheme 1. Reagents and conditions : (a) *t*-$BuMe_2SiCl$, imidazole, DMF, rt, 1 h, 98%; (b) $ClP(O)(OPh)_2$, DMAP, CH_2Cl_2, rt, 2 h; (c) aq 3 M HCl, THF, 50 °C, 1 h, 96% for 2 steps from 7; (d) Me_3OBF_4, 2,6-di(*tert*-butyl)-4-methylpyridine, CH_2Cl_2, rt, 24 h, 68%; (e) $[Ir(COD)PMePh_2)_2]PF_6$, THF, rt, 1 h; (f) I_2–H_2O, 60 °C, 5 h, 73% for 2 steps from 9; (g) Cl_3CCN, cat. DBU, CH_2Cl_2, 0 °C, 30 min.

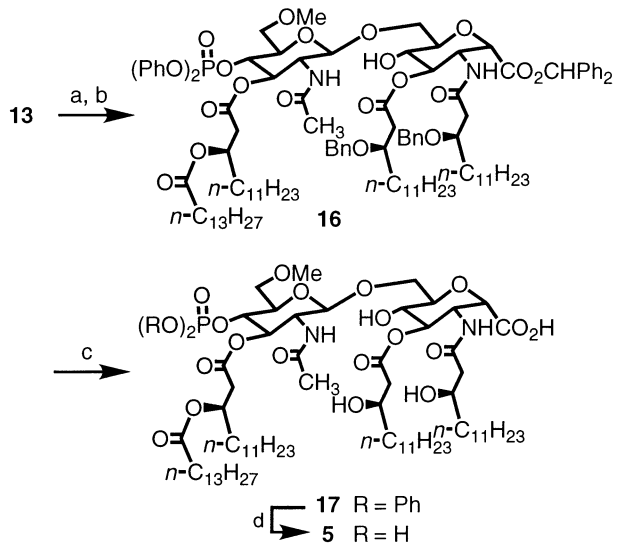


Scheme 2. Reagents and conditions (a) cat. Me_3SiOTf, 4 Å MS, CH_2Cl_2, −78 °C, 1 h, 57% for 2 steps from 10; (b) Zn, AcOH, rt, 3 h; (c) (*R*)-3-dodecanoyloxytetradecanoic acid, EDCI, CH_2Cl_2, rt, 16 h, 59% for 2 steps from 13; (d) H_2, 20% $Pd(OH)_2$/C, AcOEt, rt 2 h, 57%; (e) H_2, PtO_2, THF, rt, 16 h, 49%.

concentration of 50 μM, thus showing no LPS-agonistic activity (Fig. 4).

On the other hand, in the presence of 30 ng/mL of LPS, compound **5** inhibited, in a dose-dependent manner, the production of TNFα induced by LPS, and its IC_{50} value was 0.6 nM (Fig. 5).

Judging from the biological activities of compounds **4** and **5**, we determine the following: (1) The carboxy group attached to the pyran is exchangeable with the anomeric phosphate group in lipid A. (2) The number of lipid chains in lipid A-type pyrancarboxylic acid derivatives also plays a decisive role for LPS agonism and antagonism as for other types of lipid A-related compounds [5]. The acyl substitution pattern in compound **5**,



Scheme 3. Reagents and conditions: (a) Zn, AcOH, rt, 3 h; (b) AcOH, EDCI, CH_2Cl_2, rt, 16 h, 77% for 2 steps from 13; (c) H_2, 20% $Pd(OH)_2$/C, AcOEt, rt, 16 h, 63%; (d) H_2, PtO_2, THF, rt, 16 h, 99%.

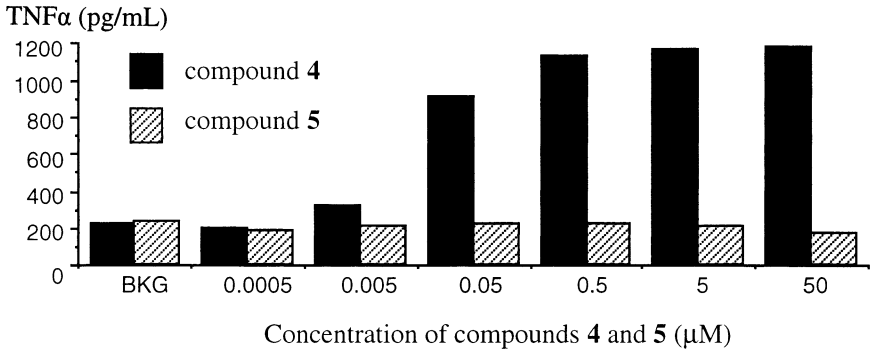


Fig. 4. Effects of compounds **4** and **5** on TNFα production of U937 cells.

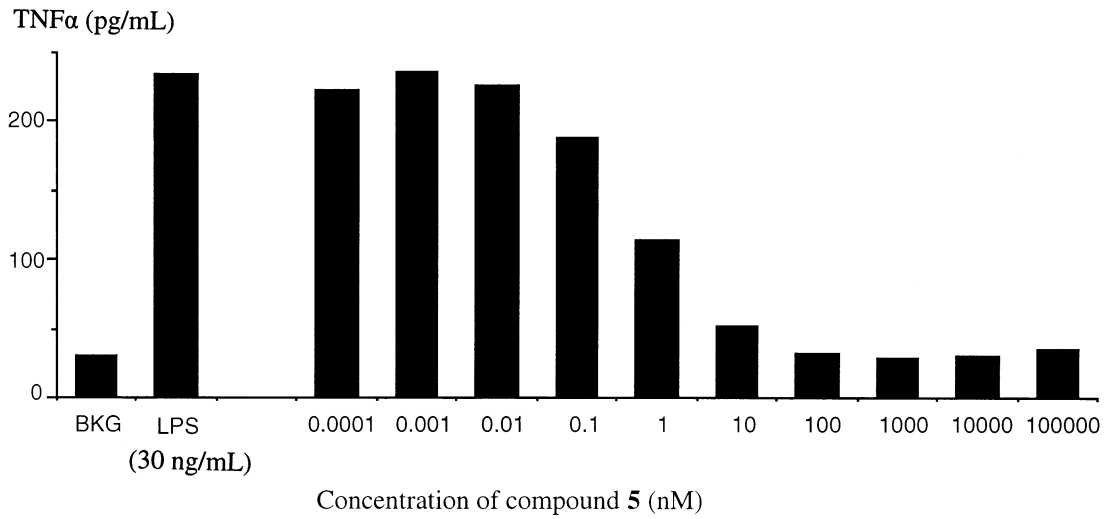


Fig. 5. Effect of compound **5** on TNFα production of U937 cells in the presence of LPS.

which has a 2′-acetamido group in place of dodecanoyloxytetradecanamido group in *E. coli* lipid A, is also effective for showing LPS-antagonistic activity as well as the pattern in lipid IVa (**2**).

In conclusion, we have synthesized lipid A-type pyrancarboxylic acid derivatives. One of the compounds has a similar acyl substitution pattern to *E. coli* lipid A, and showed LPS-agonistic activity. The other, which has four lipid chains in the molecule, but in a different fashion from lipid IVa, exhibited strong LPS-antagonistic activity.

Acknowledgements

This study was supported, in part, by the Social Insurance Agency Fund commissioned by the Japan Health Sciences Foundation.

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