Pergamon

Tetrahedron Letters 41 (2000) 6843–6847

TETRAHEDRON LETTERS

Synthesis of *Helicobacter pylori* lipid A and its analogue using *p*-(trifluoromethyl)benzyl protecting group

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Received 12 June 2000; revised 3 July 2000; accepted 7 July 2000

Abstract

Synthesis of lipid A **1** isolated from *Helicobacter pylori* strain 206-1 has been achieved in 2.2% total yield through 14 steps from D-glucosamine by employing a *p*-(trifluoromethyl)benzyl group for protection of the hydroxy group on the 3-hydroxy fatty acid residue. The synthetic specimen was identical with the natural counterpart in chromatographic, spectroscopic, and biological aspects. A structural analogue **2** which lacks the ethanolamine residue of **1** was also synthesized, and **2** was found to exhibit less potent IL-1β-inducing activity than **1**.

Keywords: lipopolysaccharides; lipid A; *Helicobacter pylori*; IL-1β induction.

Helicobacter pylori is Gram-negative bacteria often observed in the stomach of patients with chronic gastritis. The bacteria are also considered to be a possible cause of gastric and duodenal ulcers. We have studied the chemical components of *H. pylori* strain 206-1 and succeeded in the isolation and structural determination of its characteristic lipid A **1** (Fig. 1),[1] which is the proximal partial structure of lipopolysaccharide constituting the outermost leaflet of the outer membrane bilayer. The chemical structure of *H. pylori* lipid A **1** is different from that of *Escherichia coli* as follows: (1) the presence of a smaller number of fatty acid residues but of longer chain lengths; (2) the absence of the phosphate group at the C4′-position; and (3) the presence of an ethanolamine group linked to the glycosyl phosphate functionality. This particular lipid A **1** was found to exhibit lower mitogenic activity than *E. coli* lipid A, whereas **1** induces the production of a comparable amount of interleukin-6 (IL-6) from human peripheral blood cells.[2]

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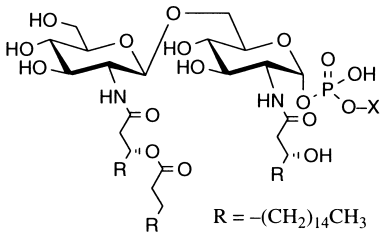
0040-4039/00/$ - see front matter
PII: S0040-4039(00)01158-8



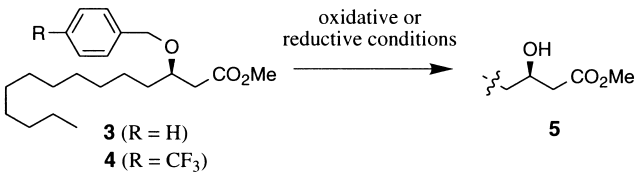
1 (X = -$(CH_2)_2NH_2$): *H. pylori* lipid A
2 (X = -H)

Figure 1.

In this letter, we report the synthesis of **1** and its analogue **2**, which lacks the ethanolamine group, by using novel methods for hydroxy group protection and phosphono group introduction. The IL-1β-inducing activity of the synthetic preparations is also described.

The synthesis was achieved using the benzyl-type protecting groups for all active functionalities to be protected until the final step. The parent benzyl (Bn) group used for the protection of the hydroxy groups on the 3-hydroxyacyl residue was, however, found in our previous study to be susceptible to oxidation: it is partly converted into a benzoyl group even by air oxidation during storage. To solve this problem, the *p*-(trifluoromethyl)benzyl group was newly developed in the present study.[3,4] Comparison of reactivities of unsubstituted and *p*-trifluoromethylated benzyl ethers is summarized in Table 1. Toward DDQ oxidation, the *p*-(trifluoromethyl)benzyl group was found to be stable under the conditions where the Bn group was nearly completely cleaved. For the reductive cleavage of the former, hydrogenolysis under higher hydrogen pressure was required than for the Bn group, but the cleavage yield was quantitative.

Table 1
Removal of Bn and *p*-(trifluoromethyl)benzyl groups under oxidative or reductive conditions



3 (R = H)
4 (R = CF_3)

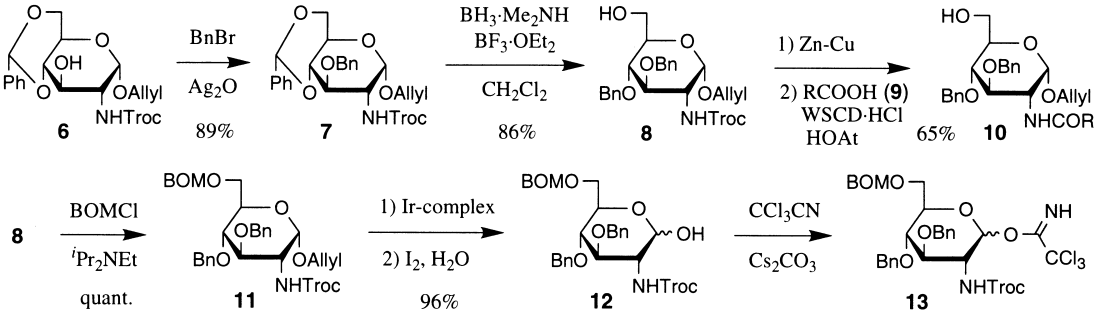
5

starting compound	yield of **5** DDQ CH_2Cl_2–H_2O, 10 h	H_2, Pd black THF, 24 h
3	100%	100% (2 kg cm^{-2})
4	7%	100% (5 kg cm^{-2})

* in parentheses are pressures of H_2

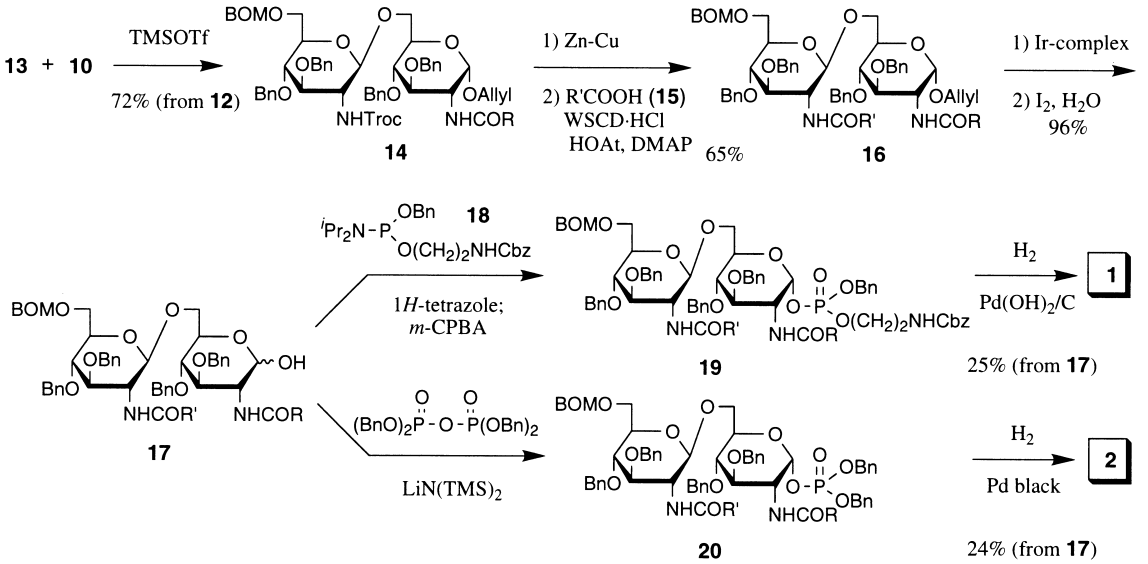
The known protected glucosamine **6**,[5] prepared from D-glucosamine hydrochloride in three steps, was benzylated to give **7** in 89% yield (Scheme 1). Reductive opening of the benzylidene group in **7** was then effected by our protocol (BH_3·Me_2NH, BF_3·OEt_2, CH_2Cl_2)[6] in 86% yield, providing exclusively the 4-*O*-Bn ether **8** which is a common intermediate for the synthesis of both glucosamine components (**10**, **13**) for the subsequent disaccharide formation. The glycosyl

acceptor **10** was prepared by the reductive deprotection of the *N*-Troc group in **8** followed by acylation of the generated amino group with (*R*)-3-(*p*-(trifluoromethyl)benzyloxy)octadecanoic acid (RCOOH, **9**).†



Scheme 1.

Benzyloxymethylation of the free hydroxy group of **8** gave a fully protected glucosamine derivative **11** quantitatively. After cleavage of the allyl group in **11** by successive treatments with an activated cationic iridium complex and aqueous iodine,[7] the product **12** was treated with CCl_3CN in the presence of Cs_2CO_3 as a catalyst to give the glycosyl trichloroacetimidate **13**. Coupling of the two glucosamine derivatives (**10**, **13**) proceeded smoothly by the action of a catalytic amount of TMSOTf to afford the β(1,6)-disaccharide **14** in a stereoselective manner by neighboring carbamate group participation (Scheme 2). *N*-acylation with (*R*)-3-(octadecanoyloxy)-octadecanoic acid (R′COOH, **15**) followed by allyl group removal was then carried out by standard reaction procedures to furnish **17** ready for the next phosphorylation.



Scheme 2.

† The carboxylic acids **9** and **15** were synthesized according to our published procedure.[10]

For α-selective introduction of the dihydrogen phosphate group at the glycosidic position of the disaccharide backbone of lipid A, we have already established an efficient method by using tetrabenzyl diphosphate.[8] In the case of an asymmetric phosphodiester-type structure, like the present 2-aminoethyl hydrogen phosphate linked at the same position, no promising route to its stereoselective formation was known. The typical phosphoramidite procedure was, therefore, first examined and it turned out to give the desired α-anomer exclusively, though the yield was not very high. Thus, successive phosphitylation of **17** with the phosphoramidite **18**[9] in the presence of 1*H*-tetrazole followed by oxidation with *m*-CPBA gave the fully protected lipid A **19** as a diastereomeric mixture at the phosphorus atom. No trace of the corresponding β-phosphate isomer was detected after the next hydrogenolytic deprotection though the starting **17** was used as an anomeric mixture. This exclusive stereoselectivity would be due to the instability of the β-phosphate moiety that might readily isomerize to the α-configuration or be cleaved spontaneously. The final one-step hydrogenolytic deprotection was performed by 9 kg cm^{-2} of H_2 over $Pd(OH)_2$/C to provide the desired lipid A **1** as a white powder in 25% yield after silica gel chromatography. The synthetic lipid A proved to be identical with the natural counterpart[1] in chromatographic aspects. This was also confirmed by comparison of their NMR (^1H, ^1H–^1H COSY) and ESI-MS (negative mode) spectra. By the use of the *p*-(trifluoromethyl)benzyl group for protection of the 3-hydroxy fatty acid, oxidative formation of the corresponding benzoyl derivative was completely avoided, which certainly made the final purification easier.

An analogue **2** that lacks the ethanolamine residue of **1** was also synthesized by treating **17** with tetrabenzyl diphosphate and $LiN(TMS)_2$[8,10] followed by hydrogenolytic deprotection (8 kg cm^{-2} of H_2, Pd black) of **20**. The crude material was purified by liquid–liquid partition chromatography[5] on Sephadex® LH-20 gel furnishing the analogue **2**.

The synthetic lipid A was found to show identical biological activities (mitogenic effect and TNF-α production) with the natural product whose activity is known to be weaker than that of *Escherichia coli* lipid A.[2] The amounts of interleukin-1β, induced in human peripheral blood mononuclear cells by 0.1 ng/well of **1** and **2**, were determined to be 2125 pg/mL and 982 pg/mL, respectively. The analogue **2** thus showed lower activity in interleukin-1β production at this concentration than *H. pylori* lipid A **1**, suggesting the positive effect of the ethanolamine residue in the cytokine-inducing activity. Further studies on the bioactivity and other physical properties of **1** and **2** are currently under way.

Acknowledgements

The authors thank Mr. Seiji Adachi of Osaka University for his skillful measurement of NMR spectra.

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