ORGANIC LETTERS

2004
Vol. 6, No. 11
1753−1756

Synthesis of 4-Fluorinated UDP-MurNAc Pentapeptide as an Inhibitor of Bacterial Growth

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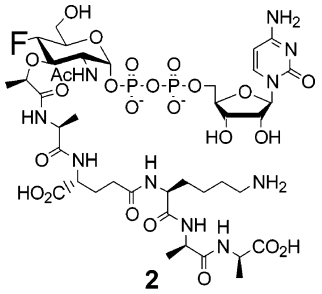
Received March 4, 2004

ABSTRACT



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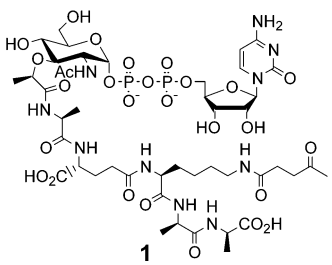
4-Fluorinated UDP-MurNAc pentapeptide, 2, has been synthesized. In our previous study, UDP-MurNAc pentapeptide analogue 1 was found to be incorporated into the bacterial cell wall through biosynthesis. Compound 2 showed growth-inhibition activity against Gram-positive bacteria when it was added to growth media at 0.01 mg/mL.

The bacterial cell wall is composed of sugar chains made up of *N*-acetyl glucosamine (GlcNAc) and *N*-acetyl muramic acid (MurNAc) connected by peptides, and the sugar chain structure is conserved in all bacteria (Figure 1). In our previous study,[1−3] UDP-MurNAc derivative **1** was found to be metabolically incorporated into the bacterial cell wall through its biosynthesis, allowing a fluorescent dye to be displayed on the surface of the bacteria. This finding has prompted us to create a novel inhibitor of cell wall synthesis.

In this paper, we synthesized a UDP-MurNAc analogue, 4-fluorinated UDP-MurNAc pentapeptide **2,** that cannot be incorporated into the cell wall, and investigated its inhibition effect on bacterial growth.



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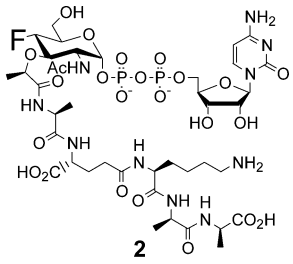

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10.1021/ol049598w CCC: $27.50
Published on Web 04/30/2004

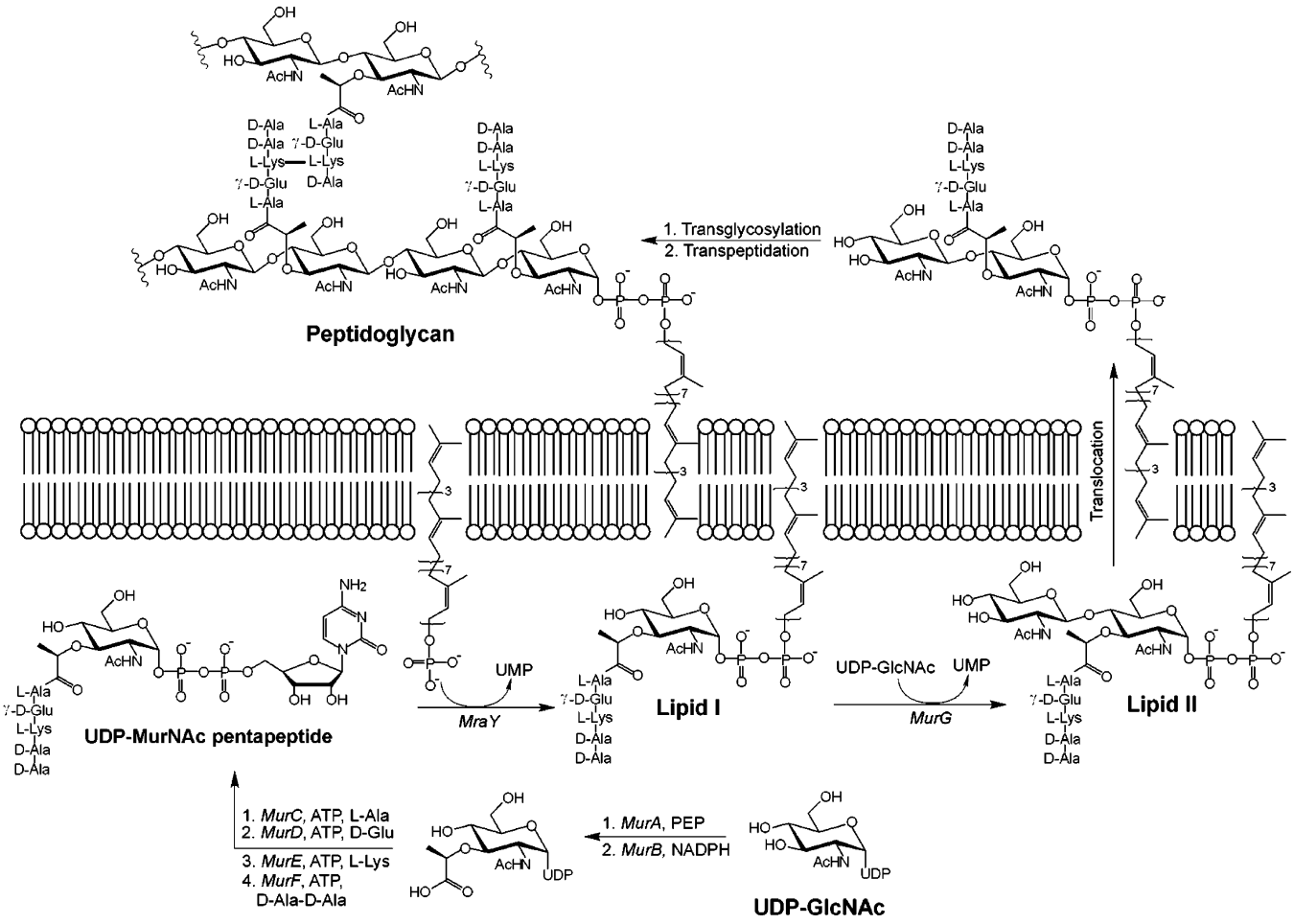

Figure 1. Biosynthetic route of the bacterial cell wall.

Fluorine-substituted analogues of biologically active organic compounds have been widely studied.[4,5] Fluorinated carbohydrate derivatives have widespread medical applications as substrate mimics for the inhibition of enzymatic processes because of the inability of enzymes to differentiate between the fluorinated and original compounds. Some fluorinated glycosyl donors are recognized as artificial substrates of glycosyltransferases.[6−8] Recently, Walker et al. reported that fluorinated lipid I analogues can be a potent inhibitor of MurG, which catalyzes the transfer of *N*-acetyl glucosamine from UDP to the C-4 hydroxyl residue of lipid I.[9] As muramic acid is a specific component in all bacteria, a fluorinated muramic acid derivative would make a promising candidate for a new type of antibiotic.

As illustrated in Scheme 1, we have established a synthetic route to the key intermediate 4-F-MurNAc-1-phosphate **13** from the less expensive *N*-acetyl galactosamine (GalNAc) rather than MurNAc. The anomeric position of GalNAc was protected by the allyl group. Using Lewis acid $BF_3{\cdot}Et_2O$ as a catalyst, heating the commercially available D-GalNAc at 70 °C for 2 h gave the desired α-anomer **3** in 80% yield. Subsequent regioselective benzylidenation of the C-4 and C-6 positions of the obtained allyl glycoside was performed using benzaldehyde dimethyl acetal and a catalytic amount of camphor-10-sulfonic acid (CSA) in dimethylformamide (DMF) to produce the partially unprotected saccharide **4** with a free 3-hydroxyl group. The compound **4** was converted into the fully protected glycoside **5** by reaction with 4-methoxybenzyl chloride in the presence of sodium hydrate and tetrabutylammonium iodide in tetrahydrofuran (THF)[10] at 40 °C. This reaction could not proceed at all at room temperature. The benzylidene acetal was reductively opened with sodium cyanotrihydroborate ($NaBH_3CN$) and hydrogen chloride in THF to provide the required 6-*O*-benzyl ether **6**

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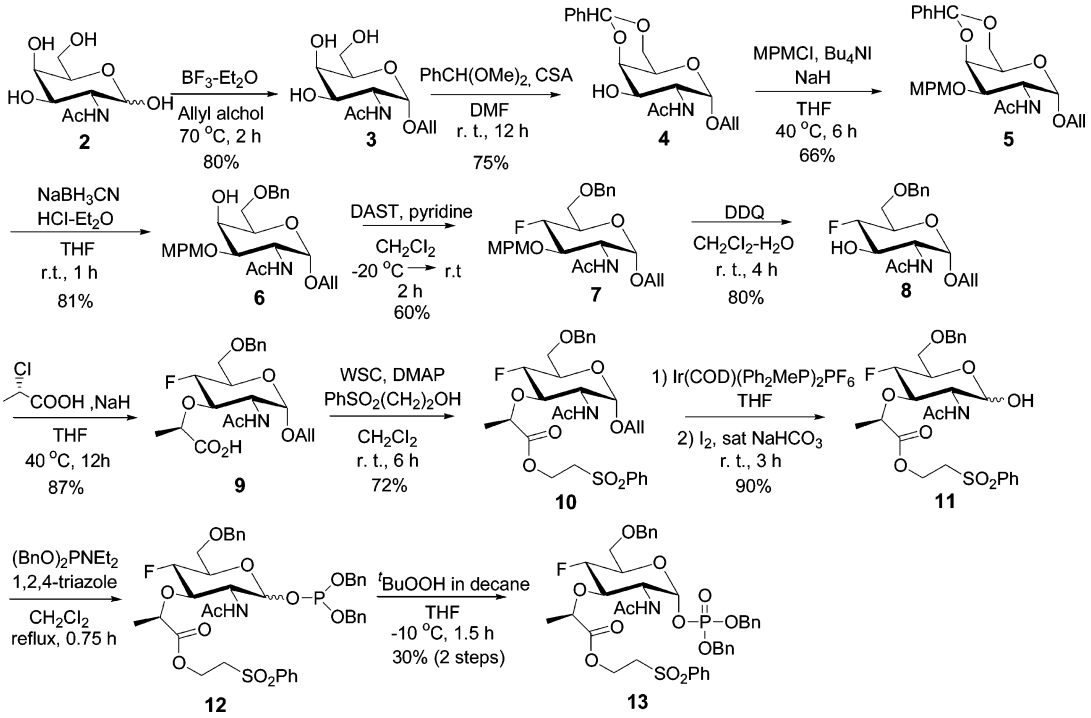
Scheme 1



without the formation of the isomeric 4-*O*-benzyl ether.[11] We then focused our attention on the fluorination reaction to obtain the key intermediate **7** by treating **6** with (diethylamino)sulfur trifluoride (DAST)[12] under nucleophilic displacement conditions as shown in the scheme to give the desired 4-fluoroglucosaminide **7** in 60%yield. The *p*-methoxyphenylmethyl (MPM) group of **7** was successfully removed using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane (DCM) to afford the intermediate **8**, which was reacted with (*S*)-(−)-2-chloropropionic acid to give the compound **9** in 80% yield. The protection of the carbonyl group of **9** with 2-phenylsulfonyl ethanol also led to the formation of the compound **10**. The anomeric position protected by the allyl moiety could be deprotected using (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexanefluorophosphate reagent[13,14] to generate the intermediate **11** as an α−*β* mixture. This deallylation was also attempted using palladium chloride in acetic acid, but the yield was much lower. Phosphorylation of **11** was carried out with dibenzyl diethylphosphoramidite (DDP)[15] and triazole in DCM to give dibenzyl phosphite **12** as a mixture of α and *β*. The stereo mixture of **12** was oxidized with *tert*-butylperoxide (TBHP) in THF at −10 °C to generate the single α-isomer **13** (the coupling constant between the anomeric and C-2 proton was 3.05 Hz) in an isolated yield of 30% after purification by silica gel column chromatography.[16] This oxidation reaction was unsuccessful when performed with hydrogen peroxide because of a break in the P−O bond.

Prior to the coupling reaction with a peptide moiety, deprotection of the carboxyl group with 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) in CH_2Cl_2 was carried out.[17] Pentapeptide **15** was synthesized from Boc-D-Ala-D-Ala-OMe by using Boc general protocol. Coupling pentapeptide **15** with the muramyl carboxyl group of **14** gave the glycopeptide **16** in 66% yield for the two steps. The benzyl-protecting groups of the phosphate moiety in **16** were removed in the presence of trioctylamine. The hydrophilic cationic counterion was used to increase solubility in the organic solvent in the next step. Subsequent reaction of the trioctylammonium phosphate salt with uridine 5′-monophosphomorpholidate and 1H-tetrazole in pyridine for 3 days produced the protected UDP-4-F-MurNAc pentapeptide.[18] Removal of the protecting groups was accomplished by rapid treatment with aqueous sodium hydroxide. The mixture was purified by column chromatography, and the final compound was confirmed by NMR and ESI mass spectroscopy (see details in Supporting Information).

Gram-positive bacteria (*L. plantarum* JCM1149) were incubated at 37 °C in lactobacilli MRS broth (Difco Laboratories) in the presence of **2** at a concentration of 0.01 mg/mL. Compound **2** was dissolved in a buffered solution and sterilized before addition to the bacteria. After 3, 5, 7, and 10 h, the number of bacteria in the culture was counted

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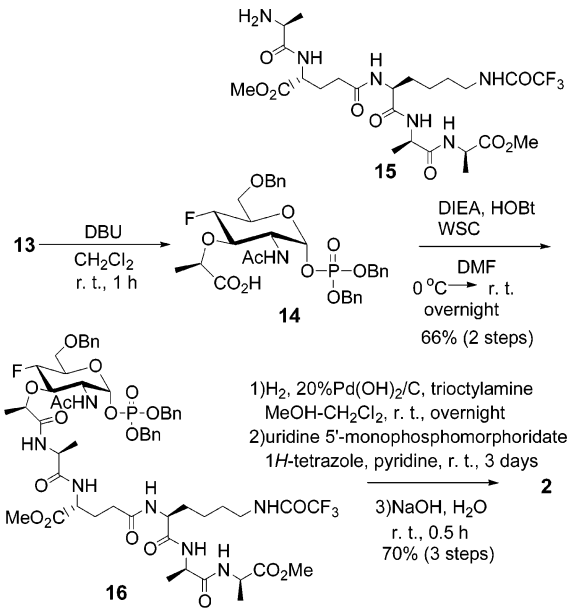
Scheme 2



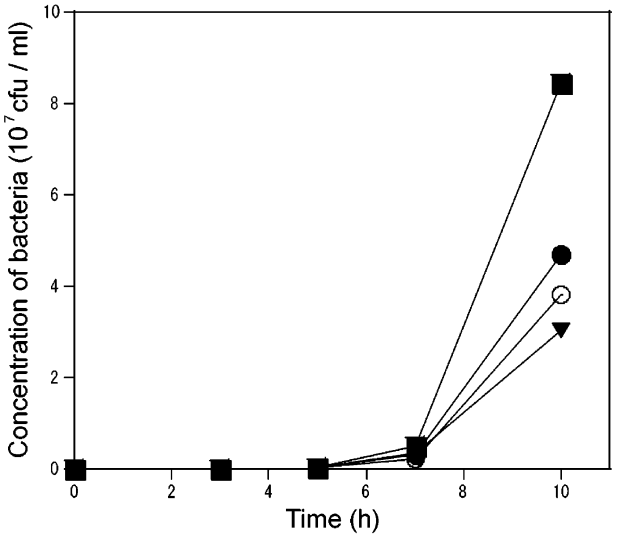


Figure 2. Changes in bacterial proliferation in the presence of compound **2**, fosfomycin, and compound **2** with fosfomycin: (■) without adding any antibiotics (negative control); (●) compound **2**; (○) fosfomycin; (▼) compound **2** with fosfomycin.

by the conventional plate counting method. Figure 2 shows the time course of the growth of the bacteria in the presence of **2**. Bacterial growth was significantly inhibited as compared to the negative control, which was cultured without the addition of any antibiotics.

In our previous study,[3] enhanced incorporation of UDP-MurNAc derivative **1** in the bacterial cell wall was observed by the addition of fosfomycin with **1** to the medium. Fosfomycin is an antibiotic that inhibits the early stage of cell wall biosynthesis; that is, the transformation from UDP-GlcNAc to UDP-MurNAc. We consider that the added UDP-MurNAc derivative **1** was used preferentially as a substrate for cell wall biosynthesis because the production of natural UDP-MurNAc in bacteria was depressed by fosfomycin. The inhibition of bacterial growth was greater in the presence of **2** with fosfomycin than in the presence of **2** or fosfomycin alone. Compound **2** could be utilized as a substrate mimic by MraY and then block peptidoglycan synthesis at the MurG step, or it could inhibit the MraY step directly.

In conclusion, we synthesized a UDP-4-F-MurNAc pentapeptide toward a novel inhibitor of bacterial growth from GalNAc as a starting material. Fluorine was introduced through an interconversion reaction from a galacto- to a gluco-configuration at the C-4 position. The UDP-4-F-MurNAc pentapeptide showed growth inhibition activity against Gram-positive bacteria. We demonstrated that UDP-MurNAc derivatives are useful not only as precursors to modify the bacterial cell wall but also as inhibitors of bacterial growth. The kinetic details of inhibitor **2** will be reported soon using recombinant MraY and MurG.

Acknowledgment. We thank Ms. S. Oka (Hokkaido University) for ESI mass spectrometry measurements. This work was partly supported by a grant for the "Glycocluster Project" from NEDO and a Grant-in-Aid for Exploratory Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Supporting Information Available: Experimental procedures, spectroscopic data for all new molecules, and procedures used for the inhibition studies of bacterial growth. This material is available free of charge via the Internet at http://pubs.acs.org.

OL049598W

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