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Synthesis of Fluorinated Analogues of Lipid A

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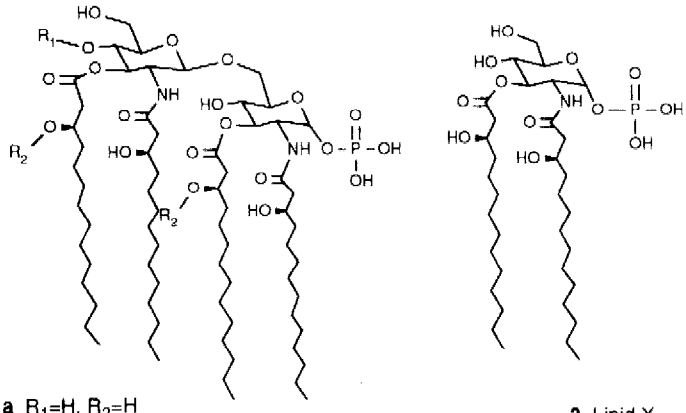
Keywords: lipid X; lipid A; fluorination; enzymatic synthesis; fluorinated disaccharides.

Abstract: *In order to study structure-activity relationships of lipid A derivatives, a series of fluorinated analogues of lipid X was synthesized. Subsequently, these were converted enzymatically into the corresponding disaccharidic lipid A analogues using lipid A synthase. This further demonstrates the low substrate specificity of this enzyme.*

Lipopolysaccharides (LPS) are constituents of the cell wall of *Gram* negative bacteria. They consist of an essentially linear polysaccharide chain, anchored to the cell wall by a hydrophobic disaccharide, lipid A (**1b**), which is responsible for most of the biological activities of LPS[1-3]. Lipid X is the reducing part of lipid A and the monosaccharide precursor in its biosynthesis[4-8]. Our search for LPS analogues with improved therapeutic index has led us to design synthetic routes for the preparation of lipid X derivatives and the corresponding disaccharides[8-11].



1a R_1=H, R_2=H
1b R_1=PO_3H_2, R_2=$CO(CH_2)_{12}CH_3$ (Lipid A)

2 Lipid X

Fig. 1.

A key step of lipid A biosynthesis in *Gram* negative bacteria is the coupling of lipid X to UDP-lipid X by means of lipid A synthase, leading to the 1-monophosphorylated lipid A precursor **1a**[4]. Crude preparations

of this enzyme from *E. coli* can be used for the synthesis of a variety of artificial lipid A precursor analogues on a preparative scale. The low substrate specificity allows broad structural variation of both the reducing and nonreducing sugar moieties[10,11].

Recently, we published the preparation of a series of *C*-glycosylated mono and disaccharide analogues of lipid A where the native 1-O-phosphate group has been replaced by a "bioisosteric" CH_2COOH substituent[10]. We now report the synthesis of a series of fluorinated analogues of lipid X and their enzymatic conversion to the corresponding disaccharides, in order to study structure activity relationships of synthetic lipid A derivatives and to probe the scope of the enzymatic disaccharide synthesis. The introduction of fluorine, the most electronegative of the elements[12], has strong effects on the chemical properties of a molecule and commonly causes profound changes in the pharmacological behavior of drug molecules[13-15].

Fluorination of position 2 of glucose can be easily performed by treating 3,4,6-tri-*O*-acetyl-glucal **3** with acetyl-hypofluorite[16-19] in glacial acetic acid. An apparatus for the generation and handling of acetyl-hypofluorite and the fluorination procedure have been described previously[18]. The resulting acetates **4a** and **4b** (70% total yield, α:β=7:1, chromatographic separation, n-hexane / ethyl acetate 4:1) were hydrolyzed quantitatively with CH_3ONa in CH_3OH. The crude gluco and manno product **5a** and **5b** were used without further purification.

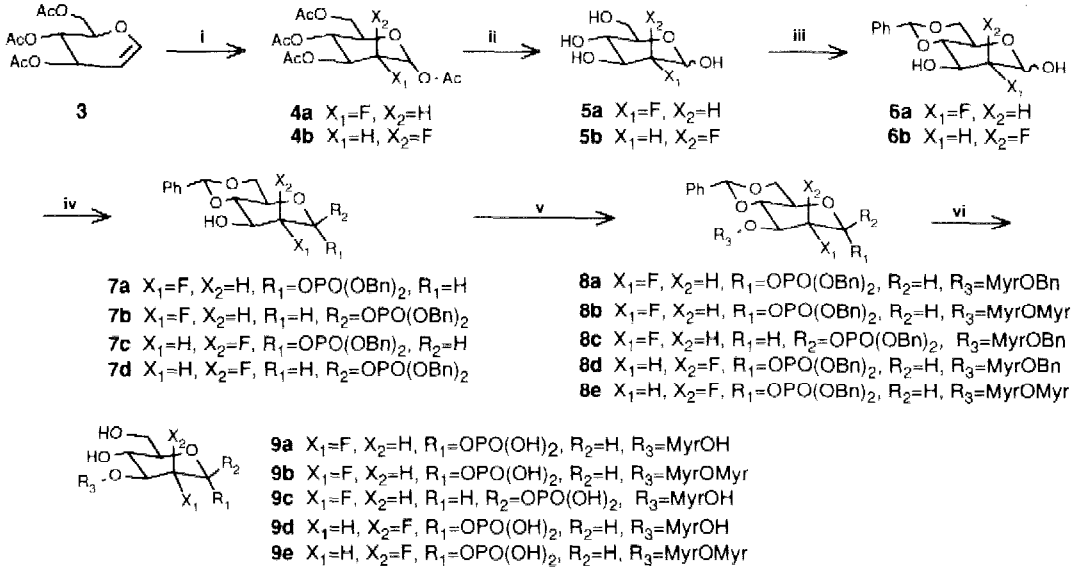


Fig. 2. **i**: AcOF, -70°; **ii**: CH_3ONa, MeOH; **iii**: $PhCH(OMe)_2$, p-TsOH, DMF; **iv**: n-BuLi, $ClPO(OBn)_2$, THF; **v**: RCOOH, DCCI, CH_2Cl_2; **vi**: H_2/Pd-C; THF-H_2O (9:1).

Benzylidenation of **5a** and **5b** (*p*-toluene sulfonic acid, benzaldehyde dimethylacetal, DMF) afforded **6a** and **6b** in about 80% yield each (chromatography: silicagel, toluene / ethyl acetate 2:1). Treatment of **6a** with n-butyl lithium and dibenzyl chlorophosphate in THF gave the 1-phosphates **7a** and **7b** selectively, while the 3-hydroxy group did not react under these conditions. Contrary to the glucosamine series, both α- and β-phosphate anomers of the fluoro-glucose derivative could be isolated and used for further reactions (α-phosphate **7a**: 46% yield, β-phosphate **7b**: 17.3% yield; chromatography on silicagel, toluene / ethyl acetate 4:1). Due to the instability of the β-phosphates in the glucosamine series during chromatography on

silicagel, only the α-anomeric phosphates could be isolated[9]. Using the same procedure for **6b**, again a mixture of both anomers was obtained, but only the manno α-phosphate **7c** could be isolated (yield 36.3 %, chromatography on silicagel, toluene / ethyl acetate 2:1), while the manno β-phosphate **7d** could not be separated and purified sufficiently by chromatography. Subsequently, position 3 was acylated with 3(R)-benzyloxy tetradecanoic acid or 3(R)-[3(R)-tetradecanoyloxy] tetradecanoic acid[20] in methylene chloride, using dicyclohexyl carbodiimide and catalytic amounts of 4-dimethylamino pyridine, affording **8a-e** (yields 70-80%, chromatography with toluene / ethyl acetate 15:1). Finally, all protective groups were removed in one step by catalytic hydrogenolysis (THF / water 9:1, Pd on charcoal, 10%) to afford the corresponding 1-phosphates of the monosaccharides **9a-e** (yields: **9a** 58%, **9b** 66%, **9c** 32%, **9d** 25%, **9e** 71%).

For synthesizing the fluorinated lipid A analogues, the required UDP-lipid X **10** was prepared according to the procedure described by *Raetz*[8] and was used without further purification. The preparation of lipid A synthase and the coupling of the fluorinated monosaccharides with UDP-lipid X were performed as described previously[10,11]. After dissolving the starting compounds in a buffer solution, a crude enzyme preparation was added and the mixture incubated at 30°C. After complete consumption of the UDP-lipid X (usually after 1 to 5 days), the reaction mixture was freeze dried and the product isolated after a series of chromatographic steps (on RP-18, solvent pyridine / acetic acid / water, gradient 39:1:60 → 70:1:29).

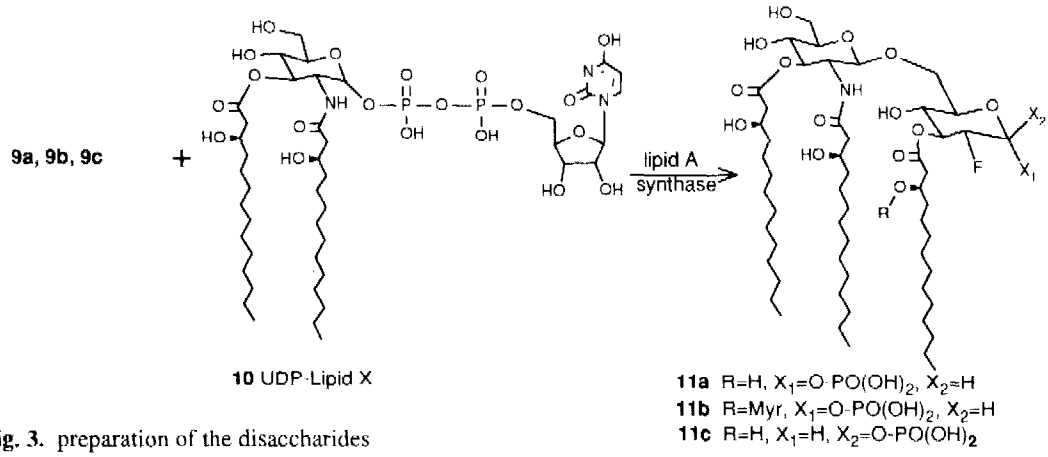


Fig. 3. preparation of the disaccharides

Using these conditions, only the gluco compounds afforded the corresponding disaccharidic α-phosphates **11a** (40% yield) and **11b** (20% yield) and the β-phosphate **11c** (20% yield). The manno lipid X analogues did not produce any isolable amounts of coupling products.

In summary, we could prepare a series of fluorinated analogues of lipid X and have been able to demonstrate further the low substrate specificity of lipid A synthase. The compounds have been tested in our biological assays (enhancement of nonspecific resistance to infections, endotoxicity). The results will be published elswhere.

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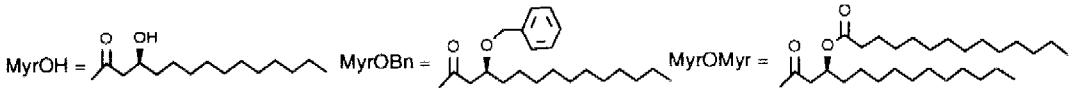
21. Abbreviations used in the figures:



22. table of characteristic NMR and MS data of selected compounds (**7a-8e** in $CDCl_3$, **9** in $CDCl_3/CD_3OD$ 3:1, **11** in Pyridine-D_5; 250 MHz): **7a**: 5.21 (dq, 1H, $J_{1,F}$=4, $J_{1,2}$=7.5, $J_{1,P}$=7.5), 4.39 (dq, 1H, H_2, $J_{1,2}$=7.5, $J_{2,3}$=8.5, $J_{2,F}$=50), 4.06 (dt, 1H, H_3, $J_{3,2}$=8.5, $J_{3,4}$=14.5); **7b**: 5.94 (q, 1H, H_1, $J_{1,P}$=6.5, $J_{1,2}$=3.5), 4.49 (dq, 1H, H_2, $J_{2,1}$=3.5, $J_{2,3}$=9, $J_{2,F}$=50), 4.19 (dq, 1H, H_3, $J_{3,2}$=9, $J_{3,4}$=12); **7c**: 4.53 (dt, 1H, H_2, $J_{2,1}$=1.6, $J_{2,F}$=48); **8a**: 4.43 (dq, 1H, H_2, $J_{2,1}$=6, $J_{2,F}$=40); **8b**: 5.88 (dd, 1H, H_1, $J_{1,2}$=3.5, $J_{1,P}$=6.5), 5.61 (dt, 1H, H_3, $J_{3,2}$=9.5, $J_{3,4}$=9.5, $J_{3,F}$=11), 4.58 (dq, 1H, H_2, $J_{2,1}$=3.5, $J_{2,3}$=9.5, $J_{2,F}$=48); **8c**: 6.01 (dd, 1H, H_1, $J_{1,2}$=3.5, $J_{1,P}$=6.8), 5.64 (dt, 1H, H_3, $J_{3,2}$=9.5, $J_{3,4}$=9.5, $J_{3,F}$=11), 4.59 (dq, 1H, H_2, $J_{2,1}$=3.5, $J_{2,F}$=48); **8d**: 5.75 (dt, 1H, H_1, $J_{1,2}$=2, $J_{1,P}$=6.5, $J_{1,F}$=6.5), 5.22 (qd, 1H, H_3, $J_{3,2}$=2.5, $J_{3,4}$=10, $J_{3,F}$=26), 4.71 (dt, 1H, H_2, $J_{2,3}$=2.5, $J_{2,F}$=48.5); **8e**: 5.74 (dt, 1H, H_1, $J_{1,2}$=1.8, $J_{1,P}$=6, $J_{1,F}$=6), 5.24 (qd, 1H, H_3, $J_{3,4}$=10, $J_{3,2}$=2.5, $J_{3,F}$=22), 4.65 (dt, 1H, H_2, $H_{2,3}$=2.5, $J_{2,F}$=49); **9a**: 4.23 (dt, 1H, H_2, $J_{2,1}$=6, $J_{2,F}$=40), MS 511 $[MNa]^+$; **9b**: 5.45 (dq, 1H, H_1, $J_{3,2}$=9.5, $J_{3,4}$=9, $J_{3,F}$=40), 4.38 (dq, 1H, H_2, $J_{2,3}$=9.5, $J_{2,F}$=48), MS 601 $[MH^+\text{-}H_3PO_4]$; **9c**: 5.47 (dq, 1H, H_3, $J_{3,2}$=9.5, $J_{3,4}$=9, $J_{3,F}$=10), 4.43 (dq, 1H, H_2, $J_{2,3}$=9.5, $J_{2,F}$=40), MS 391 $[MH^+\text{-}H_3PO_4]$; **9d**: 5.18 (dq, 1H, H_3, $J_{3,2}$=2.5, $J_{3,F}$=23), 4.73 (dt, 1H, H_2, $J_{2,F}$=40), MS 511 $[MNa]^+$; **9e**: 5.22 (dq, 1H, H_3, $J_{3,4}$=10, $J_{3,2}$=2.5, $J_{3,F}$=20), 4.8 (dt, 1H, H_2, $J_{2,F}$=40), MS 601 $[MH^+\text{-}H_3PO_4]$; **11a**: 6.53 (dd, 1H, H_1, $J_{1,1}$=3.5, $J_{1,P}$=6.5), 6.22, (dt, 1H, H_3, $J_{3,2}$=$J_{3,4}$=9.5, $J_{3,F}$=11) 4.85 (m, 1H, H_2), FAB MS 1312 [M].

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