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Synthesis of the Carbon Pseudosugar Analog of Lipid X

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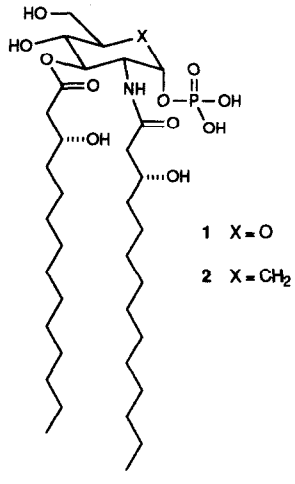
Abstract: *A carbocyclic analog (2) with a deactivated pseudo-anomeric phosphate of the lipopolysaccharide biosynthetic intermediate Lipid X (1) was prepared in order to better understand the biological behavior of the parent compound. Two related synthetic routes to the carbocyclic analog 2 starting from vinyl ether 3 proceeding in 12 and 13 steps are reported.*

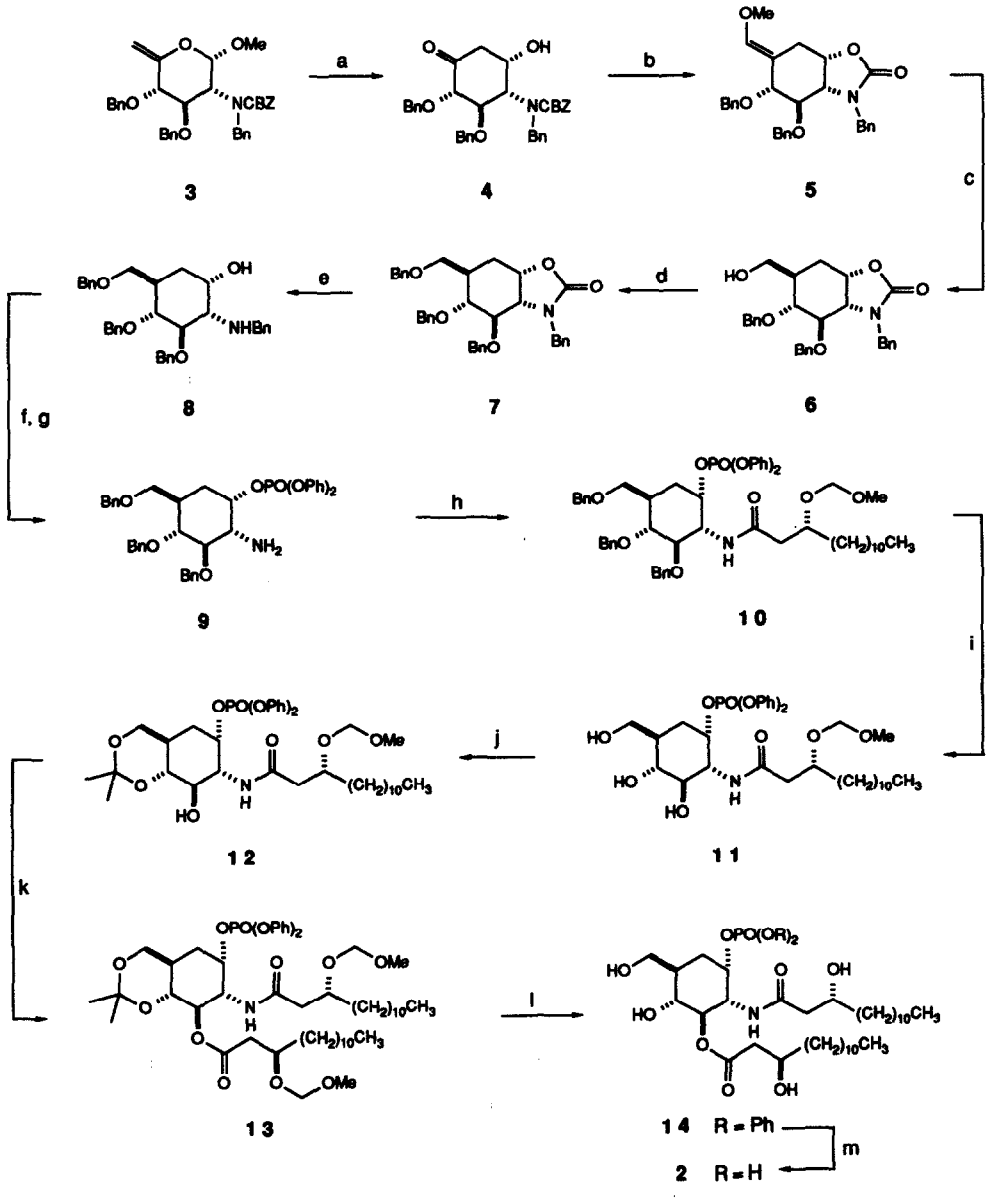
Lipid X is an intermediate in the biosynthesis of lipopolysaccharide from gram-negative bacteria, first isolated and characterized from a phosphatidylglycerol deficient mutant strain of *E. coli* defective in the pgsB gene by Raetz and coworkers in 1983.[1] Several chemical syntheses have appeared, the most efficient being a route reported by Macher that assembles the molecule in only five steps.[2] Although numerous pharmacological activities have been attributed to it, research into the biological properties of Lipid X has been complicated by the fact that small quantities of potent bioactive immunostimulatory and inhibitory byproducts have been reported to contaminate some preparations.[3] These byproducts apparently arise from glycosidation reactions that occur at the somewhat labile C-1 phosphate of this molecule and its protected precursors.



The synthesis of pseudosugars, carbocycles in which the saccharide ring oxygen is replaced by a methylene group, is of interest as such compounds may be isosteres for pharmacologically active sugars and may possess interesting biological activities.[4] As these systems lack the problems associated with a reactive anomeric center, a glucosamine pseudosugar analog might serve as a useful probe for the elucidation of biological properties intrinsic to pure Lipid X. Two slightly different synthetic approaches were followed to accomplish this goal.

The construction of the glucosamine pseudosugar ring system followed that of earlier reports[5] (Scheme 1). Ferrier rearrangement of vinyl ether **3** was carried out in 73% yield using mercuric sulfate catalysis, affording a 9:1 α:β ratio at pseudo C-1 of cyclohexanone **4**. This diastereomeric ratio was found to decrease at lower temperatures and with shorter reaction times, suggesting that some degree of equilibration occurred during this process. The use of Pd^{II} catalysts under similar conditions furnished cyclohexanone **4** in rather low yields with a reversed diastereomeric ratio.[5] Olefination of cyclohexanone **4** was accompanied by cyclization of the amine CBZ protecting group onto the C-1 hydroxyl with concomitant loss of benzyl alcohol to furnish enol ether-carbamate **5**. This compound was subsequently subjected to mercuric acetate hydrolysis, reduction, and benzylation to yield the protected carbocyclic glucosamine **7**.

Scheme 1

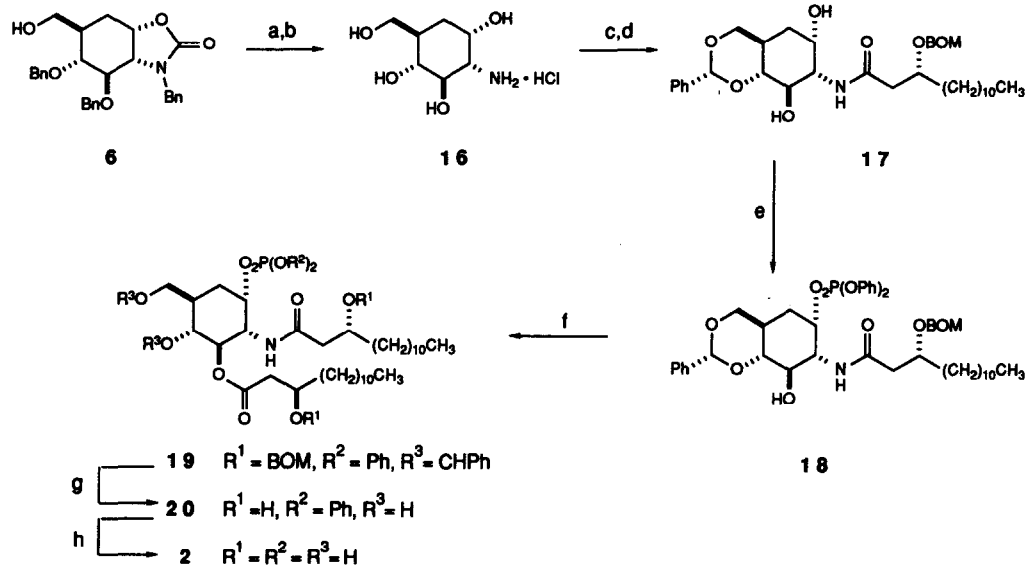


Reaction conditions: a) $HgSO_4$, H_2SO_4, dioxane, H_2O, 9:1 α:β pseudo C-1, 73%. b) $Ph_3PCH_2OCH_3Cl$, nBuLi, DME, 0° to 10°C, 60%. c)i. $Hg(OAc)_2$, MeCN, H_2O, 0°C; ii. $NaBH_4$, 68% (~1:5 α:β C-5). d) NaH, BnBr, DMF, 0°C to RT, 67%. e) NaOH, EtOH, reflux, 90%. f)i. nBuLi, THF, -78°C; ii. $(PhO)_2POCl$, -78° to -40°C, 80%. g) H_2, 20% $Pd(OH)_2$, MeOH, 61%. h) (R)-3-methoxymethyloxytetradecanoic acid (15), DCC, CH_2Cl_2, 92%. i) H_2, 20% $Pd(OH)_2$, THF, MeOH, 98% crude. j) DMP, CSA, DMF, RT, 69%. k) 15, DCC, DMAP, CH_2Cl_2, RT, 64%. l) HCl, MeOH, 50°C, 97%. m) H_2, PtO_2, H_2O, EtOH, 90%

After urethane removal, all attempts to effect acylation of the secondary amine at pseudo C-2 of **8** failed. Instead, when n-butyl lithium was used as base, clean acylation at the pseudo C-1 hydroxyl group was observed. Consequently, the hydroxyl group at C-1 of **8** was phosphorylated by treatment with n-butyl lithium and diphenyl phosphoryl chloride. When this tetrabenzylated product was subjected to catalytic hydrogenation, it was found difficult to effect complete debenzylation. While the factors that render the N-benzyl group unexpectedly more susceptible than the O-benzyl groups to hydrogenolytic cleavage are unclear, it is probably amine poisoning of the catalyst that is responsible for this reaction halting at the stage of **9**, since these conditions do not include any additional acid. Although complete hydrogenolytic deblock may be possible under different conditions, this unexpected result was exploited to purify the relatively non-polar intermediate amine **9** from a small amount of readily removable cyclic phosphoramide. Prompt N-acylation with sidechain (**R**)-3-methoxymethyloxytetradecanoic acid (**15**)[6] was followed by complete hydrogenolytic debenzylation and the resulting triol **11** was selectively diprotected as the acetonide **12**. The pseudo C-3 hydroxyl was then acylated with sidechain acid **15** and this was followed by stepwise removal of the blocking groups. After first treatment with hydrochloric acid in warm methanol and then catalytic hydrogenation, carbocyclic Lipid X (**2**) was isolated as the free acid. NMR data acquired for this compound were found to be only somewhat similar to those of Lipid X (**1**).[7]

Analogs of Lipid X bearing acyloxyacyl sidechains may possess interesting biological activities.[8] It was expected that this type of functionality might not be compatible with the hydrochloric acid used to deblock the 3-hydroxytetradecanoyl sidechains in the previous synthesis. Therefore, an alternative route to carbocyclic

Scheme 2



Reaction conditions: a) NaOH, EtOH, reflux, 62%. b) H_2, 20% $Pd(OH_2)$, MeOH, HCl, quant. crude. c) N-hydroxy succinyl (**R**)-3-benzyloxymethyloxytetradecanoate (**21**), DIEA, DMF, 62%, 2 steps from **16**. d) $PhCH(OMe_2)$, *p*-TsOH, DMF, 50%. e)i. 2.1 equivalents nBuLi, THF, -78°C; ii. 1.1 equivalents $(PhO)_2POCl$, 36% (based on consumed starting material). f) (**R**)-3-benzyloxymethyloxytetradecanoic acid (**22**), $EtNCN(CH_2)_3NMe_2$ • HCl, DMAP, CH_2Cl_2, 74%. g) H_2, 20% $Pd(OH)_2$, RT, 200 PSI, EtOAc, MeOH, 88%. h) H_2, PtO_2, H_2O, EtOH quant.

Lipid X was developed that might be more suitable for the incorporation of such acid sensitive sidechains (Scheme 2). This route parallels the Macher Lipid X route[2] and was formulated based on the expected selectivity of the phosphorylation of diol **17**.

Carbamate hydrolysis of **6** was followed by complete hydrogenolytic deprotection in acidic methanol. Selective acylation of crude **16** using the N-hydroxysuccinyl sidechain ester **21**[9], followed by 4,6 benzylidene formation afforded diol **17** in an analogous fashion to the published Lipid X synthesis.[2] For the synthesis of Lipid X, the more acidic nature of the anomeric hydroxyl group relative to the C-3 hydroxyl group was utilized to selectively phosphorylate C-1. Even though for **17** differentiation on the basis of relative acidity is not possible, some selectivity might be expected for the C-1 over C-3 hydroxyl solely on the basis of steric environment. Unfortunately, although selective C-1 phosphorylation of this diol was observed, it was difficult to force this reaction to completion and in practice it was necessary to recycle unreacted starting material. After acylation at C-3 with the second sidechain **22**[9], sequential mild hydrogenolyses afforded Carbocyclic Lipid X (**2**), which was identical to that produced by the previous route.

In preliminary biological evaluations, carbocyclic Lipid X (**2**) did not demonstrate any immunostimulatory activities. Insofar as the pseudosugar-sugar comparison is valid, this observation lends support to the idea that immunostimulation observed with Lipid X preparations may be due to contaminants.

References and notes

1. See Raetz, C. R. H. *Annu. Rev. Biochem.* **1990**, *59*, 129 and references contained therein.
2. Macher, I. *Carbohydrate Res* **1987**, *162*, 79-84
3. Aschauer, H.; Grob, A.; Hildebrandt, J.; Schüetze, E.; Stüetz, P. *J. Biol. Chem.* **1990**, *265*, 9159; Lam, C.; Hildebrandt, J.; Schütze, E.; Rosenwirth, B.; Proctor, R. A.; Liehl, E.; Stütz, P. *Infect. and Immun.* **1991**, *59*, 2351.
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6. Sidechain acid **15** was synthesized by protection of phenacyl (**R**)-3-hydroxytetradecanoate with methoxymethyl bromide and diisopropylethylamine followed by ester deprotection with zinc dust in acetic acid. See reference 2 and references contained therein for the preparation of a similar compound.
7. All NMR chemical shifts reported relative to TMS (δ). Selected 400 MHz ^{1}H data for **2** (CD_3OD): 0.90 (6H, t, J = 7 Hz, 2 x $C\underline{H}_3$), 1.60 (1H, t, J = 14.7 Hz, C-0 α H), 1.99 (1H, m, C-5 H), 2.18 (1H, dt, J = 14.7, 3.2 Hz, C-0 β H), 2.27 (1H, dd, J = 8.4, 14.7 Hz, α $C\underline{H}_2$), 2.34 (1H, dd, J = 4.2, 14.7 Hz, α $C\underline{H}_2$), 2.43 (1H, dd, J = 8.4, 14.7 Hz, α $C\underline{H}_2$), 2.50 (1H, dd, J = 4.2, 14.7 Hz, α $C\underline{H}_2$), 3.54 (1H, dd, J = 9.5, 10.5 Hz, C-4 H), 3.68 (1H, dd, J = 4.2, 10.5 Hz, α C-6 H), 3.73 (1H, dd, J = 5.3, 10.5 Hz, β C-6 H), 3.94 (2H, m, 2 x β $C\underline{H}$OH), 4.05 (1H, dt, J = 10.5, 3.2 Hz, C-2 H), 4.61 (1H, m, C-1 H), 5.09 (1H, dd, J = 9.5, 10.5 Hz, C-3 H). Selected 100 MHz ^{13}C data for **2** (CD_3OD): 39.10, 39.16 (2 x γ CH_2), 40.63 (C-5), 44.38, 45.67 (2 x α CH_2), 55.09 ($J_{C2\text{-}P}$ = 7.3 Hz, C-2), 63.97 (C-6), 70.20, 70.47 (2 x β CHOH), 73.62 (C-4), 77.05 (C-3), 77.18 ($J_{C1\text{-}P}$ = 5.8 Hz, C-1), 174.50, 175, 07 (CON and COO). Selected 400 MHz ^{1}H data for **2** (as monotris salt in CD_3OD): 0.90 (6H, t, J = ~7 Hz), 2.08 (1H, m), 2.21 (1H, d, J = ~14 Hz), 2.30 (2H, m), 2.44 (2H, m), 3.52 (1H, t, J = ~10 Hz), 3.75 (1H, dd, J = ~7, 12 Hz), 4.46 (1H, bd, J = ~5 Hz), 5.15 (1H, t, J = ~10 Hz). Selected 400 MHz ^{1}H data for **1** (as monotris salt in CD_3OD): 0.89 (6H, t, J = ~7 Hz), 2.30 (2H, m), 2.46 (2H, m), 3.58 δ, (1H, t, J = ~10 Hz), 3.70 (1H, dd, J = ~8, 10 Hz), 3.83 (1H, d, J = ~12 Hz), 4.15 (1H, dt, J = ~14 Hz), 5.22 (1H, t, J = ~10 Hz), 5.47 (1H, dd, J = ~5, 8 Hz).
8. See for example Macher, I.; Unger, F. M.; Raetz, C. R. H. UK patent application GB2179945 A **1987**; Charon, D.; Chaby, R.; Malinvaud, A.; Mondange, M.; Szabó, L. *Biochemistry* **1985**, *24*, 2736.
9. See reference 2 and references contained therein for the preparation of a similar compound.

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