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Synthesis of a Carbohydrate-Derived Hydroxamic Acid Inhibitor of the Bacterial Enzyme (LpxC) Involved in Lipid A Biosynthesis

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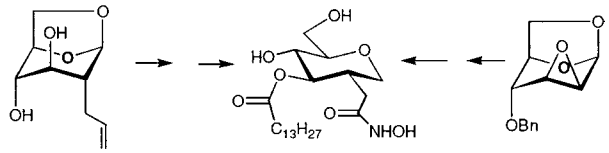
ABSTRACT



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The enzyme LpxC (UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-GlcNAc deacetylase) catalyzes the second step of lipid A biosynthesis and is essential for bacterial growth. A GlcNAc-derived hydroxamic acid inhibitor 8 of this enzyme was synthesized using two different routes. Compound 8 exhibits activity toward LpxC enzymes from a wider spectrum of bacterial species than any of the previously reported hydroxamic acid inhibitors.

The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant bacterial pathogens. There is an urgent need for compounds that act on novel molecular targets that circumvent the established resistance mechanisms. Gram-negative bacteria, which are responsible for a large number of infectious diseases, have unique outer membranes that contain lipopolysaccharides (LPS). Agents interfering with the biosynthesis of LPS are often bactericidal or bacteriostatic.[1]

The hydrophobic anchor part of the LPS molecule is called lipid A. Recent studies[2] have shown that the enzymes involved in the biosynthesis of lipid A are suitable pharmaceutical targets. Many of these enzymes are both unique and essential to Gram-negative bacteria. The enzyme LpxC is one example: it catalyzes the second step in lipid A biosynthesis (Scheme 1). LpxC has been identified in more than 40 Gram-negative species.[3]

In 1996, Merck scientists reported the discovery that hydroxamic acids, derivatized with hydrophobic aromatic moieties, could inhibit LpxC from *E. coli*. These inhibitors were shown to bind competitively to a zinc ion of the enzyme.[4,5] We now report the synthesis of compound **8** (Scheme 2), which is a carbohydrate-derived hydroxamic acid where the carbohydrate part resembles the natural substrate of the LpxC enzyme: UDP-3-*O*-[(*R*)-3-hydroxy-

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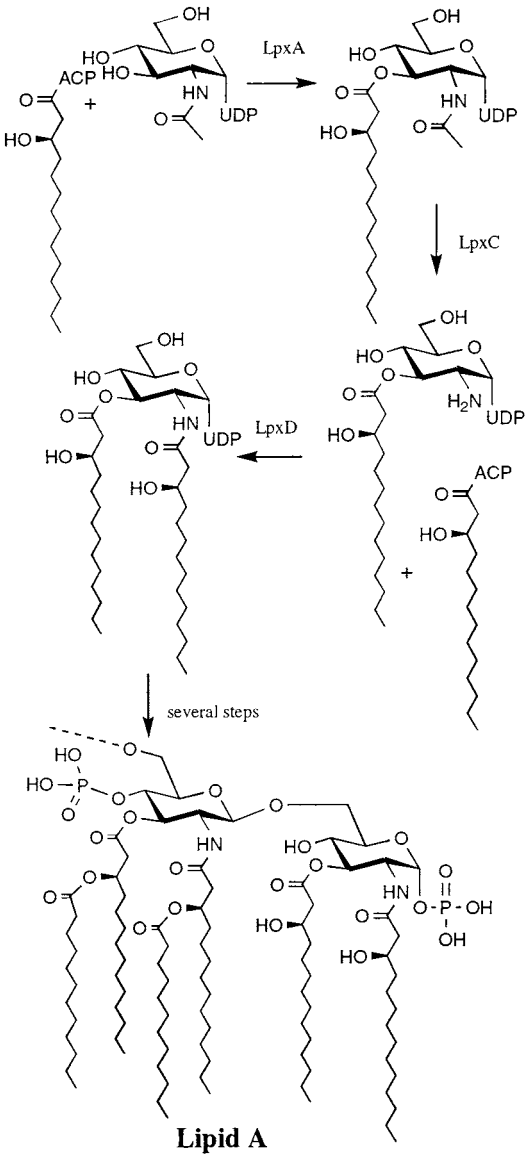
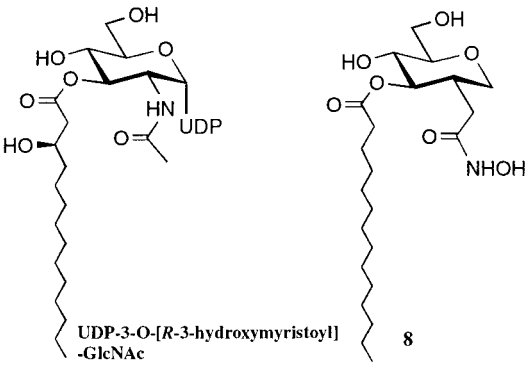
Scheme 1



myristoyl]-GlcNAc. A study[6] previously showed that compound **8** exhibited inhibitory activity toward LpxC enzymes

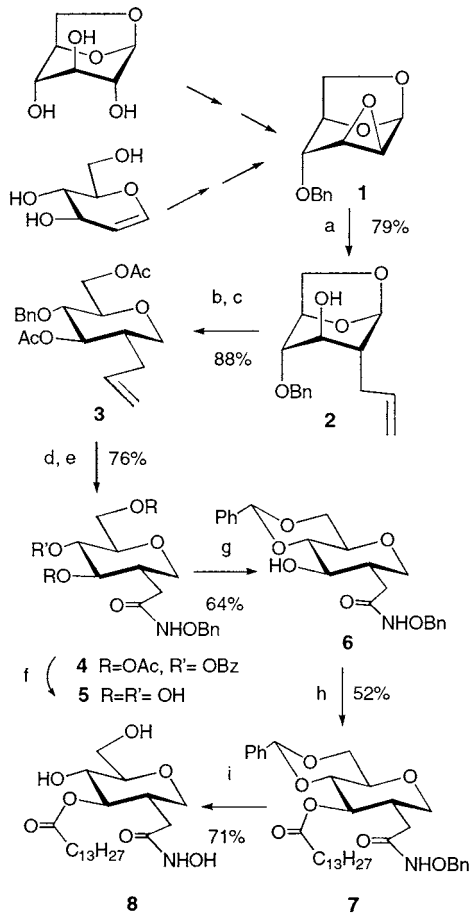
Scheme 2



from a broader range of bacterial species than the previously reported noncarbohydrate hydroxamic acid inhibitors.

Two synthetic routes were evaluated for the preparation of inhibitor **8**. In the first (Scheme 3), the epoxide **1**, available

Scheme 3 [a]



[a] Reaction conditions: (a) AllylMgBr, THF; (b) $SnCl_4$, Et_3SiH, CH_2Cl_2; (c) Ac_2O, pyridine; (d) $NaIO_4$, $RuCl_3 \cdot H_2O$, $CCl_4/CH_3CN/H_2O$; (e) $BnONH_2 \cdot HCl$, EDAC·HCl, TEA, CH_2Cl_2; (f) NaOMe, MeOH; (g) benzaldehyde dimethylacetal, $TsOH \cdot H_2O$, CH_3CN; (h) myristoyl chloride, DMAP, pyridine; (i) H_2, Pd/C, AcOH

from 1,6-anhydro-D-glucose[7] or D-glucal[8] in four or two steps, respectively, was opened with allylmagnesium bromide to give the expected *trans*-diaxial product **2** (79%). Reductive opening of the 1,6-anhydro ring with $SnCl_4$ and Et_3SiH followed by O-acetylation (Ac_2O/pyridine) gave **3** (88%). Oxidative cleavage of the double bond in **3** with ruthenium tetroxide (generated in situ from $RuCl_3$ and $NaIO_4$)[9] gave a carboxylic acid derivative, where the benzyl protecting group

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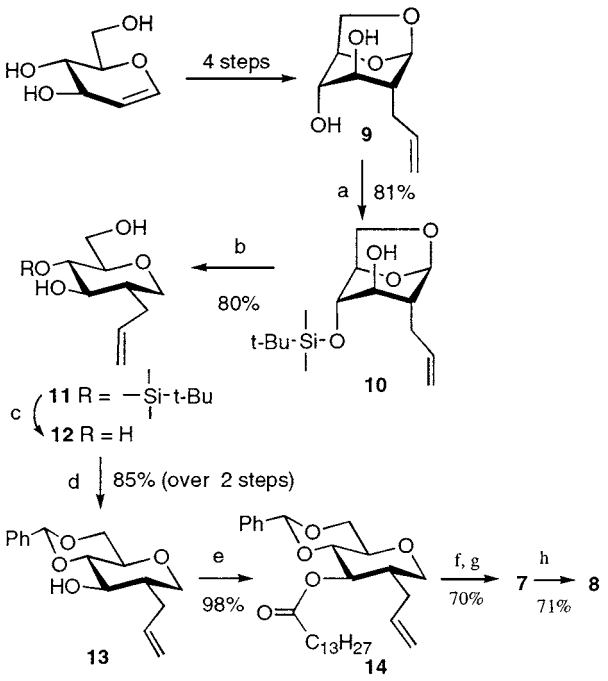
Scheme 4 [a]



[a] Reaction conditions: (a) *tert*-butyldimethylsilyl chloride, imidazole, DMF; (b) $TiCl_4$, Et_3SiH, CH_2Cl_2; (c) TBAF, THF; (d) benzaldehyde dimethylacetal, $TsOH·H_2O$, CH_3CN; (e) myristoyl chloride, pyridine; (f) $NaIO_4$, $RuCl_3·H_2O$, $CCl_4/CH_3CN/H_2O$; (g) $BnONH_2·HCl$, EDAC·HCl, TEA, CH_2Cl_2; (h) H_2, Pd/C, AcOH

at OH-4 had been oxidized to a benzoate group. The crude product was reacted with *O*-benzylhydroxylamine and a condensing reagent (EDAC) to give the protected hydroxamic acid derivative **4** (76%). Deacylation (NaOMe) of **4** and subsequent reaction with benzaldehyde dimethylacetal gave **6** (64%). O-Acylation with myristoyl chloride then gave **7** (52%). The low yield of **7** in the O-acylation step was attributed to concomitant N-acylation. Deprotection of **7** (H_2/Pd/C) gave the final product **8** (71%).

Some drawbacks with this first synthetic scheme were identified. The synthesis is not practical on a large scale, and the early introduction of the −CONHOBn group creates problems with side reactions at the O-acylation stage. Therefore, a second synthetic pathway to **8** was devised (Scheme 4), starting with derivative **9** (available[10] in four steps from D-glucal). Surprisingly, both **9** and its 3,4-di-*O*-acetyl derivative failed to give significant quantities of reductive ring-opening product when treated with $SnCl_4$ and Et_3SiH. However, selective *tert*-butyldimethylsilyation of **9** gave **10** (81%), which on treatment with $SnCl_4$ and Et_3SiH afforded **11** (67%) in 10 min. Use of $TiCl_4$ instead of $SnCl_4$ raised the yield of **11** to 80% but required 2 h.

Desilylation of **11** with TBAF and subsequent reaction with benzaldehyde dimethylacetal gave **13** (85%), O-myristoylation of which gave **14** (98%). Oxidative cleavage of the double bond in **14** with ruthenium tetroxide, using the same conditions as in the first pathway, gave the carboxylic acid derivative where hydroxamic acid function could be introduced near the end of the synthetic route using *O*-benzylhydroxylamine/EDAC to give the protected hydroxamic acid derivative **7** (70%). Finally, as in the first pathway, catalytic hydrogenation of **7** gave **8** (71%). This second synthetic pathway gave a 26.8% total yield of **8** (from **9**), as compared to 12.5% (from **1**) for the first pathway.

In summary, the second synthetic pathway (Scheme 4) to the inhibitor **8** is the more efficient of the two. Synthesis of analogues of **8** and further biological evaluation of the synthesized derivatives is currently ongoing in our laboratory.

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Supporting Information Available: Experimental procedures and spectroscopic data for compounds **2**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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