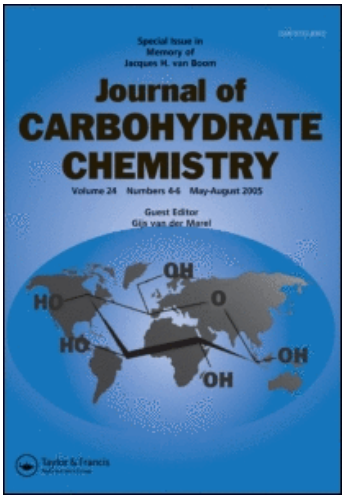

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Mapping the Active Site of the Bacterial Enzyme LpxC Using Novel Carbohydrate-Based Hydroxamic Acid Inhibitors

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Mapping the Active Site of the Bacterial Enzyme LpxC Using Novel Carbohydrate-Based Hydroxamic Acid Inhibitors

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LpxC (UDP-3-O-(*R*-3-hydroxymyristoyl)-GlcNAc deacetylase), an enzyme involved in the biosynthesis of lipid A, is crucial for the growth of Gram-negative bacteria. This enzyme has accordingly been identified as a potential target for the development of novel antibiotics against Gram-negative bacteria. The carbohydrate-derived hydroxamic acid **1** (1,5-anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-3-O-myristoyl-D-glucitol) was previously shown to exhibit a wide spectrum of inhibitory activity against LpxC enzymes. Here we describe the preparation of seven analogs of **1** and their enzymatic evaluation. Two of the hydroxyl groups (OH-3 and 6) of the GlcNAc residue were found to be involved in the binding interaction, and there is an important hydrophobic interaction in the vicinity O-3 position with the enzyme that recognizes aromatic as well as aliphatic substituents.

Keywords Carbohydrate analog, Enzyme inhibitor, Hydroxamic acid, Deacetylase

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In memory of Jacques H. van Boom.
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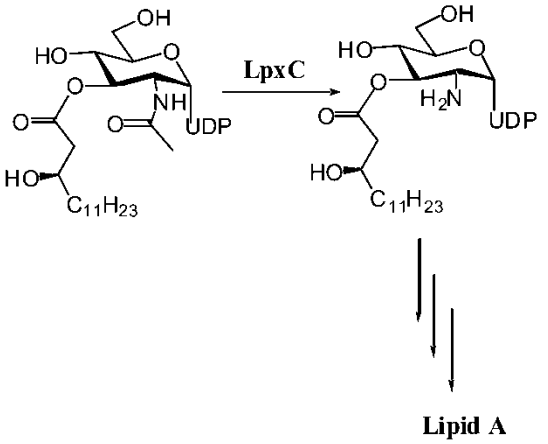
INTRODUCTION

The discovery of compounds with novel modes of antibacterial action is urgently needed to circumvent bacterial resistance. Lipopolysaccharide (LPS), also known as endotoxin,[1] is the major structural constituent of the outer membrane of Gram-negative bacteria protecting the sensitive inner membrane. LPS, released from the dying bacteria after treatment with conventional antibacterial agents, causes septic shock. The annual number of deaths from septic shock is about 70,000 in the United States alone,[2] a figure comparable to AIDS. Therefore, the inhibition of the biosynthesis of LPS would have multiple beneficial effects for the therapy of the Gram-negative infections by killing the bacteria and reducing the amount of endotoxin released when other antibiotics are administered simultaneously. Currently no marketed antibiotics are directed against the LPS biosynthetic pathway. LPS consists of lipid A, two KDO residues, the core oligosaccharide, and the O-antigen. The lipid A and two KDO units have been shown to be minimally required for the bacterial growth in general. Many Gram-negative bacteria, including most pathogens, synthesized lipid A species resembling the one found in *Escherichia coli.* Recently, lipid A has been recognized as a promising new target for the development of antibiotics against Gram-negative bacteria.[3]

Of many enzymes involved in lipid A biosynthesis, the N-deacetylase named LpxC, which catalyzes the first committed step in the pathway (Sch. 1),[4] has been suggested to be a good pharmaceutical target[5] and has attracted a lot of attention.[5–10] LpxC requires enzyme-bound Zn^{++} to effect amide bond cleavage. Several classes of hydroxamic acid derivatives have been reported to be potent inhibitors of LpxC (Fig. 1). Merck scientists first reported phenyloxazoline hydroxamic acid derivatives that were potent



Scheme 1

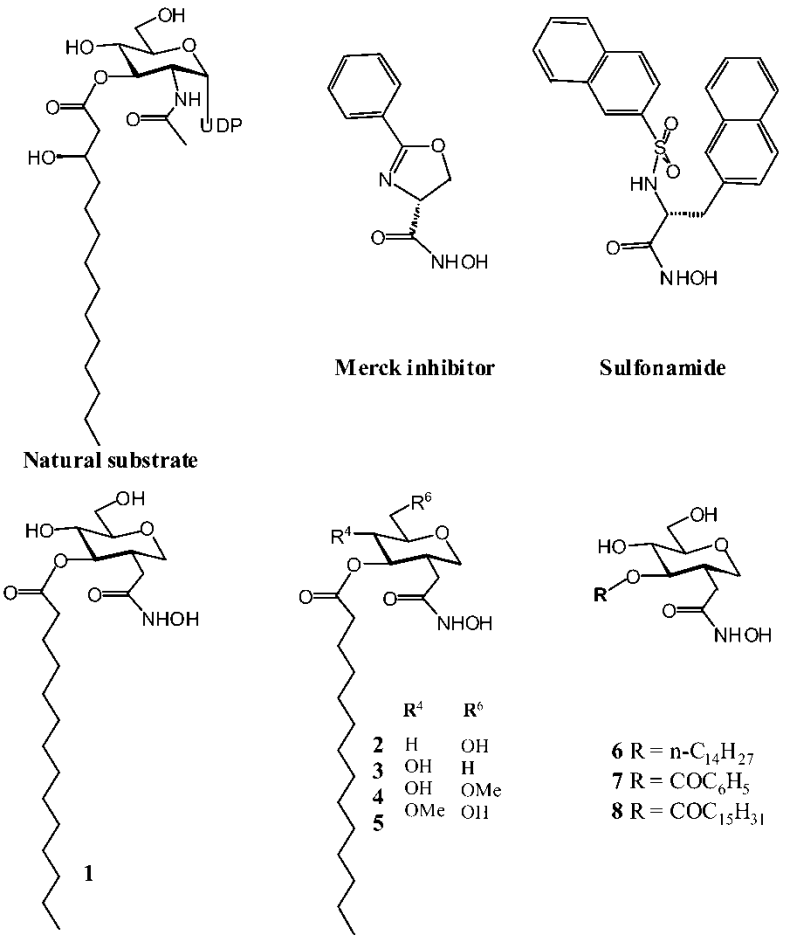


Figure 1: Structures of the substrate and inhibitors of LpxC.

inhibitors of LpxC.[5] Extensive series of analog failed to yield compounds that were broad-spectrum inhibitors.[6] More recently, sulfonamide derivatives of a-(R)-amino hydroxamic acid have been reported to show antibacterial activities against a wider panel of pathogens.[8]

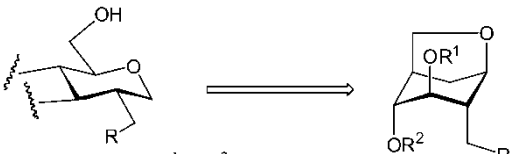
Our laboratory conceived of the idea of incorporating the hydroxamic acid directly into the carbohydrate substrate at the position of the acetamide group undergoing enzymatic hydrolysis.[6] The rationale was that all of the enzymes must recognize the natural substrate, so the more that an inhibitor had in common with this substrate, the more likely it would be a broad-spectrum inhibitor. Compound **1** (Fig. 1) was synthesized and indeed was shown to exhibit a wide spectrum of inhibitory activity with an $IC_{50} = 7.0 \pm 0.5\ \mu M$ (*A. aeolicus* LpxC); $7.2 \pm 1.9\ \mu M$ (*E. coli* LpxC).[6a] This class of LpxC inhibitor

contains the most potent compounds reported to date that inhibit the LpxCs of the most evolutionarily diverse Gram-negative bacteria. To explore structure-activity relationships, seven new analogs of **1**, namely **2**–**8**, have now been synthesized and evaluated (Fig. 1) These compounds contain simple modifications in structure: replacement of OH groups by H or OMe, and variations of the 14-carbon lipidic ester at O-3.

RESULTS AND DISCUSSION

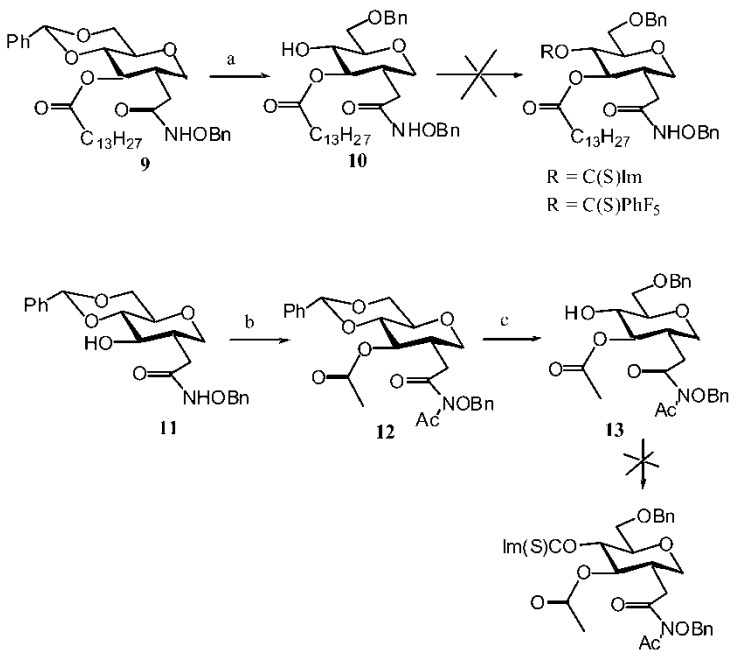
Compound **1** is a 1,2-dideoxy-2-C-branched-monosaccharide. To the best of our knowledge, there was no example reported in literature of the synthesis of this type of compound starting from a carbohydrate substrate. We recently, however, reported an efficient synthetic pathway for compound **1**.[6b] 1,6-Anhydro-β-D-glucopyranose derivatives underwent reduction with Et_3SiH in the presence of a Lewis acid, providing rapid access to carbohydrate synthons of this type. However, proper protecting groups were crucial for the transformation: some protecting groups, like alkyl and silyl ethers, proved effective, while the transformation was not successful with free hydroxyl or acetyl groups (Sch. 2).[6b]

Synthesis of analogs of 1. The first attempt to obtain the 4-deoxy analog **2** is shown in Scheme 3. Selective reductive ring opening of the benzylidene acetal in **9**[6b] using $NaCNBH_3$/HCl in THF gave the 6-O-benzyl ether **10** in 82% yield. However, attempted thiocarbonylation with either thiocarbonyldiimidazole or pentafluorophenyl chlorothionoformate under standard conditions failed to produce the desired products. It was assumed that the long chain at C-3 may have blocked the reaction and that thiocarbonylation at the hydroxamic ester nitrogen might be also occurring. To circumvent this problem, we *O*-acetylated **11**, the precursor to **9**,[6b] to produce **12**, where the long-chain fatty acid ester was no longer present. At the same time, the hydroxamic ester nitrogen was



$R^1 = R^2 = H$	No
$R^1 = R^2 = Ac$	No
$R^1 = H, R^2 = Bn$	Yes
$R^1 - H, R^2 - TBS$	Yes
$R^1 = H, R^2 = Me$	Yes

Scheme 2

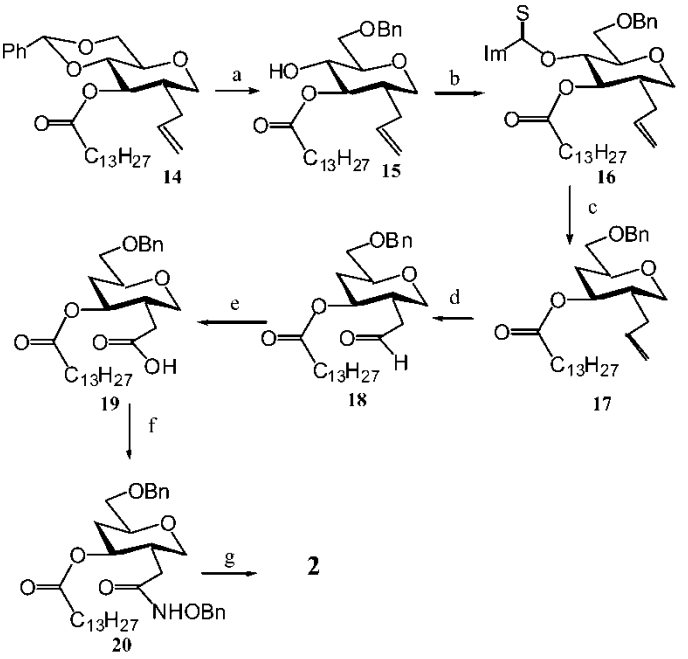


Scheme 3: [a]Reaction conditions: (a) $NaCNBH_3$/HCl, THF, 82%; (b) Ac_2O, pyridine; (c) $NaCNBH_3$/HCl, THF, 80%.

also protected with an acetyl group. Reductive ring opening of **12** now afforded **13** in good yield as expected (80%). Surprisingly, however, the attempted thiocarbonylation was still not successful. Therefore, we reasoned that the CONHOBn group may not be compatible with the thiocarbonylation conditions.

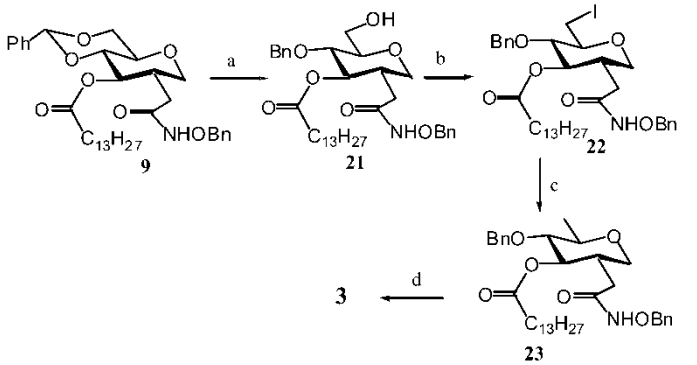
Finally, the synthesis of the required 4-deoxy analog **2** was accomplished as shown in Scheme 4, starting from **14**,[6b] which allowed us to remove O-4 before the labile CONHOBn group was introduced. Regioselective reductive ring opening of the benzylidene acetal in **14** gave **15**, which on thiocarbonylation using thiocarbonyldiimidazole in refluxing toluene overnight gave **16** in 64% over two steps. Treatment of **16** with Bu_3SnH and AIBN gave **17** in 85% yield. Ozonolysis of **17** followed by reduction with PPh_3 gave aldehyde **18** (87%). Further oxidation to the carboxylic acid derivative using $NaClO_2$ and NaH_2PO_4 in *t*-BuOH/2-methyl-2-butene/H_2O yielded **19** (100%). This product was coupled with *O*-benzylhydroxylamine using EDC to afford the protected hydroxamic acid derivative **20** (70%). After hydrogenation (H_2, Pd/C) in AcOH, compound **2** was obtained (70%).

The conversion of the 4,6-O-benzylidene acetal in **14** to the 4-benzyl ether was unsuccessful. This is because the standard conditions used in this



Scheme 4: [a]Reaction conditions: (a) $NaCNBH_3$/HCl, THF, 82%; (b) $Im_2C(S)$, toluene, 79%; (c) AIBN, Bu_3SnH, toluene, 85%; (d) O_3, Ph_3P, CH_2Cl_2, 87%; (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene/H_2O/*t*-BuOH, 100%; (f) $BnONH_2 \cdot HCl$, TEA, EDC, CH_2Cl_2, 70%; (g) H_2, Pd/C. AcOH, 70%.

transformation, such as the combinations of $LiAlH_4/ACl_3$ or $BH_3 \cdot THF$/(Lewis acid), were not compatible with the presence of an ester group or a double bond. Selective reductive ring opening of the benzylidene acetal could, however, be achieved with Bu_2BOTf and $BH3 \cdot THF$[11] from **9** (Sch. 5) to give **21** with

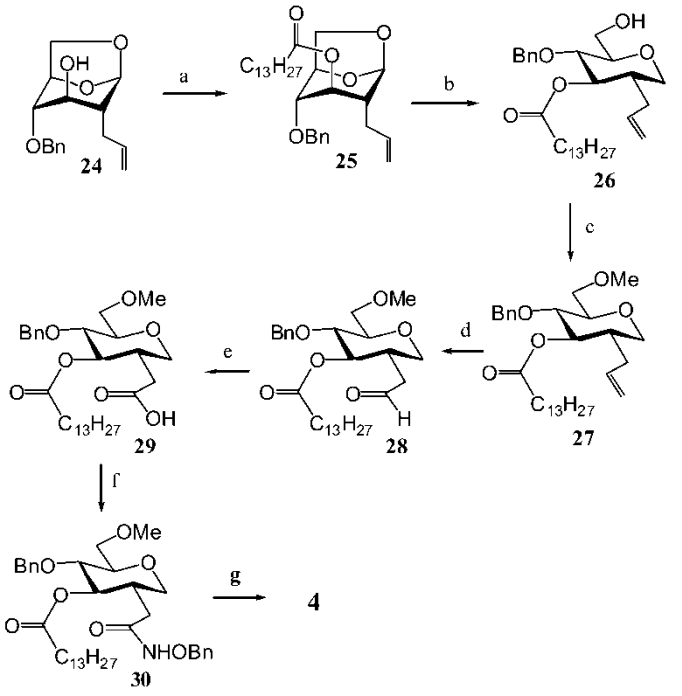


Scheme 5: [a]Reaction condition: (a) Bu_2BOTf, $BH_3 \cdot THF$, 71%; (b) I_2, Ph_3P, imidazole, toluene, 80%; (c) Bu_3SnH, AIBN, toluene, 82%; (d) H_2, Pd/C, AcOH, 70%.

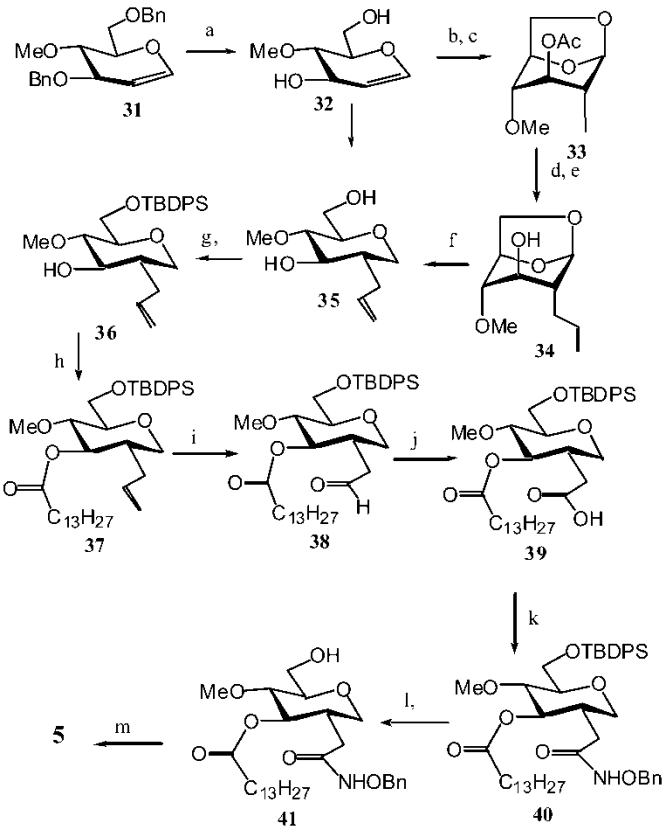
OH-6 free (71%). Treatment of **21** with I_2, Ph_3P and imidazole afforded **22** in 80% yield. Deiodination with Bu_3SnH and AIBN successfully furnished **23** (82%). Hydrogenation (H_2, Pd/C, AcOH) of **23** then gave **3** (70%).

The preparation of the 6-OMe derivative **4** would require an *O*-methylation reaction at some point. We therefore decided to install the methyl ether in the reaction sequence before introduction of the CONHOBn group to avoid possible side reactions (Sch. 6). *O*-Myristoylation of **24**[6b] gave **25** (84%), which on treatment with $SnCl_4$ and Et_3SiH afforded **26** (46%). Methylation of **26** was easily accomplished. Treatment with methyl trifluoromethanesulfonate and 2,6-di(tert-butyl-4-methyl) pyridine gave **27** (98%). Finally, as in Scheme 4, the reaction sequence was completed by ozonolysis, followed by reduction to the aldehyde **28** (80%) and further oxidation to carboxylic acid **29** (100%). Coupling with O-benzylhydroxylamine hydrochloride ($BnONH_2 \cdot HCl$) gave the hydroxamide **30** (71%), and upon hydrogenation (H_2, Pd/C, AcOH), compound **4** was obtained (69%).

The preparation of 4-*O*-methyl derivative **5** is shown in Scheme 7. Compound **31**, obtained from D-glucal according to the literature,[12] was subjected to Birch reduction to afford **32** (85%). Cyclization of **32** gave **33** (89%).



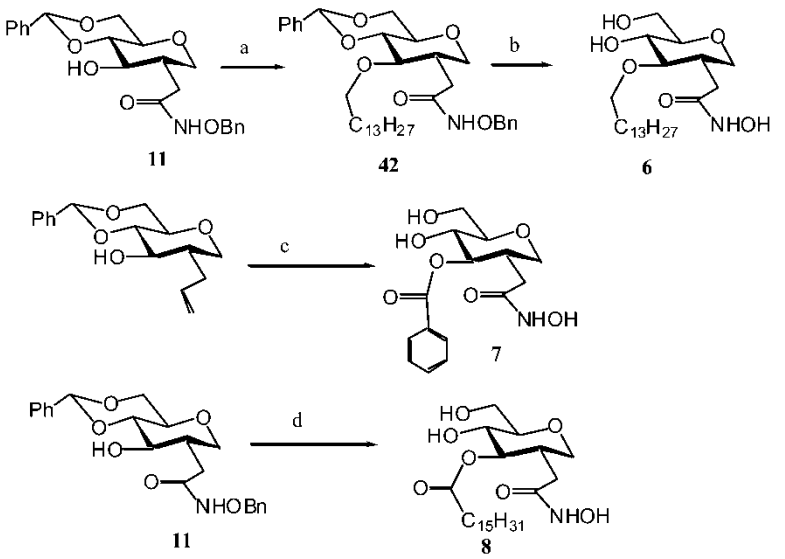
Scheme 6: [a]Reaction conditions: (a) myristoyl chloride, pyridine, 84%; (b) $SnCl_4$, Et_3SiH, CH_2Cl_2, 46%; (c) MeOTf, DBMP, CH_2Cl_2, 98%; (d) O_3, Ph_3P, CH_2Cl_2, 80%; (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene/H_2O/t-BuOH, 100%; (f) $BnONH_2 \cdot HCl$, TEA, EDC, CH_2Cl_2, 71%; (g) H_2, Pd/C, AcOH, 69%.



Scheme 7: [a]Reaction conditions: (a) Na, NH_3, THF, 85%; (b) $(Bu_3Sn)_2O$, I_2, CH_3CN, 89%; (c) Ac_2O, pyridine, 100%; (d) allyltributyltin, AIBN, toluene, 69%; (e) MeOH-H_2O-Et_3N, 100%; (f) Et_3SiH, $SnCl_4$, CH_2Cl_2, 66%; (g) *t*-TBDPSCl, imidazole, DMF, 97%; (h) myristoyl chloride, pyridine, 95%; (i) O_3, Ph_3P, CH_2Cl_2, 81%; (j) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene/H_2O/*t*-BuOH, 100%; (k) $BnONH_2 \cdot HCl$, TEA, EDC, CH_2Cl_2, 68%; (l) TBAF, THF, 91%; (m) H_2, Pd/C, AcOH, 64%.

Radical addition of allyltributylstannane with AIBN as the initiator followed by deacetylation gave **34** in 69% yield. Reductive opening of the 1,6-anhydro ring using our strategy gave **35** (66%). Selective *t*-butyldiphenylsilyation at the primary alcohol of **35** gave **36** (97%). *O*-Acylation with myristoyl chloride in pyridine then gave **37** (95%). Ozonolysis of **37** followed by reduction using PPh_3 gave aldehyde **38** (81%). Further oxidation using $NaClO_2$ and NaH_2PO_4 in *t*-BuOH/2-methyl-2-butene/H_2O yielded **39** quantitatively. This carboxylic acid coupled with *O*-benzylhydroxylamine using EDC to furnish **40** (68%). The *t*-butyldiphenylsilyl group was removed by TBAF to give **41** (91%). Hydrogenation of **41** gave **5** (64%).

Compounds **6**, **7**, and **8** were prepared according to the same synthetic strategy as the preparation of **1**,[6b] using 1-bromotetradecane, benzoyl chloride, and palmitoyl chloride instead of myristoyl chloride (Sch. 8).



Scheme 8: [a]Reaction conditions: (a) 1-bromotetradecane, NaH, DMF, 70%; (b) H_2, Pd/C, AcOH, 70%; (c) benzoyl chloride, pyridine, 95%; O3, Ph_3P, CH_2Cl_2, 87%; $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene/H_2O/*t*-BuOH, 100%; $BnONH_2 \cdot HCl$, TEA, EDC, CH_2Cl_2, 70%; H_2, Pd/C, AcOH, 62%; (d) palmitoyl chloride, pyridine, 55%; H_2, Pd/C, AcOH, 70%.

Inhibitor Evaluation. The seven new analogs of **1** were enzymatically evaluated as inhibitors of the *E. coli* LpxC in vitro. The results are presented in Figure 2. A detailed picture of the topography of a substrate-based inhibitor binding with the LpxC enzyme can be proposed based on these results. The two hydroxyl groups of the GlcNAc residue appear to be involved in the binding interaction, since replacement of either hydroxyl group with hydrogen caused a loss of the inhibitory activity. The enzyme cannot tolerate

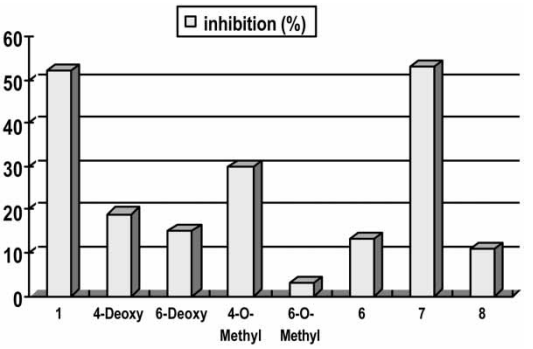


Figure 2: Inhibition of *E. coli* LpxC by compounds **1**–**8** (1 μg/mL).

increased steric bulk at OH-6, since the 6-O-methyl derivative had only barely detectable inhibitory activity (3%), while the enzyme can better tolerate the steric increase at OH-4 to some extent (30%). Our previous studies have shown that the inhibitor with or without a shorter O3 fatty acid constituent (C_6) lost inhibitory activity dramatically,[6a] suggesting the enzyme may have a narrow cleft exactly designed to fit the myristoyl chain of the substrate (UDP-3-O-(*R*-3-hydroxymyristoyl)-GlcNAc), which contributes importantly to binding interaction.[15]

Surprisingly, a decreased inhibitory activity was observed when adding two more methylene groups to the parent **1** to produce **8**. We reason that with the terminal methyl group the myristoyl chain packed against the enzyme, a longer acyl chain either cannot fit or is forced into solution that is unfavorable. The replacement of the acyl chain with a corresponding ether chain (**6**) resulted in the loss of some activity. The reason for this could be that the carbonyl group of the acyl chain is also involved in interaction with the protein. Therefore, certain long acyl chains, neither too short nor too long, are crucial for potent inhibition.

The inhibition data collected in Figure 2 independently suggest the binding mode presented schematically in Figure 3. This model is in complete agreement with that derived by solution NMR spectroscopy of the complex of LpxC with **1** and of the crystal structure of the native protein.[15] The most promising position for further elaboration to produce superior inhibitors should be at the 3-position, where clearly there is a hydrophobic interaction with the enzyme. Importantly, replacement of a myristoyl ester chain with a benzoyl group (**7**) did not diminish the inhibitory activity,

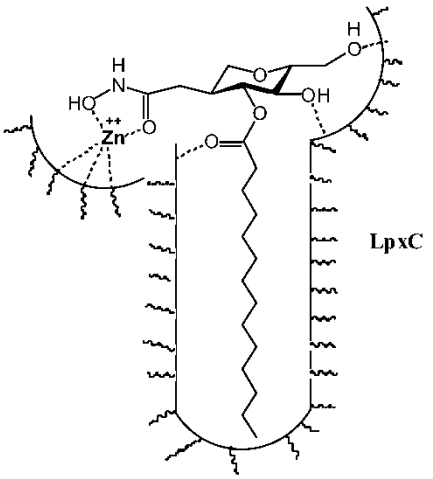


Figure 3: Schematic of proposed binding mode of **1** with LpxC.

and therefore this hydrophobic interaction is permissive to some structural variation. This may explain the success of many aromatic hydroxamic acids[5] in inhibiting the enzyme. We anticipate that this class of substrate-based designed inhibitors may lead to effective antibiotic against Gram-negative bacteria.

EXPERIMENTAL

General Procedure

Analytical TLC was performed on Silica Gel 60-F_{254} (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with 5% sulfuric acid in EtOH. All commercial reagents were used as supplied. Column chromatography was performed on Silica Gel 60 (E. Merck 40–60 μM, Darmstadt). Iatrobeads (beaded silica gel 6RS-8060) were from Iatron Laboratories (Tokyo). ^{1}H NMR spectra were recorded on 300 MHz (Varian Inova 300), 400 MHz (Varian Inova 400), or 500 MHz (Varian Inova 400). The first-order proton chemical shifts, δ_H, are referenced to either residual $CHCl_3$ (δ_H 7.24, $CDCl_3$) or CH_3OD (δ_H 3.30, CD_3OD). The ^{13}C chemical shifts, δ_C, are referenced to internal $CDCl_3$ (δ_C 77.00), or CD_3OD (δ_C 49.00). The diastereotopic methylene protons of benzyl groups are reported as ABd, meaning second-order doublets, with the chemical shifts reported as the midpoint between the peaks. Organic solutions were dried prior to concentration under vacuum at $<40^\circ$C (bath). Microanalyses and electrospray mass spectra were performed by the analytical services of this department.

1,5-Anhydro-6-O-benzyl-2-deoxy-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol (15). To a solution of **14**[6b] (160 mg, 0.33 mmol) and $NaCNBH_3$ (207 mg, 3.29 mmol) in dry THF (6 mL), HCl in Et_2O was added until bubbles ceased. The mixture was diluted with CH_2Cl_2 (20 mL) and water (5 mL) and poured into sat. $NaHCO_3$ (5 mL). The organic layer was washed with brine (5 mL), dried over Na_2SO_4 and then concentrated. The residue was purified by chromatography (EtOAc/hexane, 1 : 5) to yield **15** (131 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.25 (5H, m), 5.79 (1H, m, H-2′), 5.10–4.90 (2H, m, H-3′), 4.72 (1H, dd, $J = 9.0$, 10.6 Hz. H-3), 4.60 and 4.58 (2H, ABd, $J = 12.0$ Hz, OCH_2Ph), 3.96 (1H, dd, $J = 4.7$, 11.9 Hz, H-1q), 3.71 (2H, m, H-6), 3.55 (1H, m, H-4), 3.39 (1H, m, H-5), 3.15 (1H, t, $J = 11.9$ Hz, H-1 ax), 2.36 (2H, t, $J = 7.4$ Hz, $OC(O)CH_2$), 2.18 (1H, m, H-1′), 1.95 (1H, m, H-2), 1.86 (1H, m, H-1′), 1.63 (2H, m), 1.36–1.12 (20H, m), 0.90 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 137.8, 134.6, 128.4, 127.7, 117.1, 79.5, 78.4, 73.7, 71.7, 70.3, 69.8, 40.1, 34.4, 32.3, 31.9, 29.6, 29.4, 29.3, 29.2, 29.1, 24.9, 22.6, 14.1. HRMS calcd. for $C_{30}H_{48}O_4Na$ ($M + Na^+$) 511.3399; found 511.3401.

1,5-Anhydro-6-O-benzyl-2-deoxy-3-O-myristoyl-2-C-(2-propenyl)-4-O-(1-thiocarbonylimidazol)-D-glucitol(16). A solution of **15** (91 mg, 0.19 mmol) and N,N-thiocarbonyldiimidazole (110 mg, 0.62 mmol) in dry toluene (4 mL) was refluxed overnight. After cooling to rt, the mixture was concentrated and the residue was purified directly by chromatography (EtOAc/hexane, 1 : 4) to give **16** (85 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (1H, s), 7.50 (1H, s), 7.24–7.18 (5H, m), 7.00 (1H, s), 5.79 (1H, t, $J = 9.5$ Hz, H-4), 5.69 (1H, m, H-2′), 5.16 (1H, dd, $J = 9.2$, 10.7 Hz, H-3), 5.05–4.98 (2H, m, H-3′), 4.46 (2H, s, OCH_2Ph), 4.06 (1H, dd, $J = 4.6$, 11.5 Hz, H-1 eq), 3.70 (1H, m, H-5), 3.58 (1H, dd, $J = 3.2$, 10.7 Hz, H-6), 3.51 (1H, dd, $J = 5.1$, 10.7 Hz), 3.38 (1H, t, $J = 11.5$ Hz, H-1 ax), 2.24–2.10 (4H, m, H-2, H-1′, $OC(O)CH_2$), 1.90 (1H, m, H-1′), 1.40 (2H, m), 1.38–1.00 (20H, m), 0.88 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.5, 173.2, 134.0, 130.9, 128.2, 127.8, 127.7, 117.5, 79.3, 77.7, 74.4, 73.7, 69.9, 69.2, 40.6, 34.1, 32.1, 31.9, 29.6, 29.5, 29.3, 28.9, 24.7, 22.6, 14.1. HRMS calcd. for $C_{34}H_{50}N_2O_5SH$ ($M + H^+$) 599.3519; found 599.3518.

1,5-Anhydro-6-O-benzyl-2,4-dideoxy-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol (17). To a solution of **16** (63 mg, 0.11 mmol) and AIBN (5 mg, 0.04 mmol) in dry toluene (3 mL) was added Bu_3SnH (85 μL, 0.33 mmol) under argon. The mixture was refluxed for 3 hr. After cooling to rt, the mixture was concentrated. Column chromatography (EtOAc/hexane, 1 : 6) of the residue gave **17** (44 mg, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38–7.24 (5H, m), 5.70 (1H, m, H-2′), 5.05–4.95 (2H, m, H-3′), 4.70 (1H, m, H-3), 4.56 and 4.54 (2H, ABd, $J = 12.3$ Hz, OCH_2Ph), 4.04 (1H, dd, $J = 4.2$, 11.8 Hz, H-1 eq), 3.61 (1H, m, H- 5), 3.94–3.80 (2H, m, H-6), 3.38 (1H, t, $J = 11.8$ Hz, H-1 ax), 2.28 (2H, t, $J = 7.3$ Hz, $OC(O)CH_2$), 2.25 (1H, m, H-4), 1.98 (1H, m, H-1′), 1.86–1.78 (2H, m, H-2, H-1′), 1.60 (2H, m), 1.39 (1H, q, $J = 11.6$ Hz, H-4), 1.34–1.18 (20H, m), 0.90 (3H, t, $J = 7.0$ Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.2, 137.9, 135.0, 128.3, 127.6, 116.7, 75.5, 73.5,73.4, 72.8, 70.3, 40.7, 34.6, 34.1, 32.7, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 25.1, 22.8, 14.2. HRMS calcd. for $C_{30}H_{48}O_4Na$ ($M + Na^+$) 495.3450; found 495.3456.

2-C-(Aldehydomethyl)-1,5-anhydro-6-O-benzyl-2,4-dideoxy-3-O-myristoyl-D-glucitol (18). Ozone was passed through a solution of **17** (48 mg, 0.10 mmol) in CH_2Cl_2 (10 mL) at −78°C until the solution turned blue. The excess ozone was removed with a stream of oxygen for 10 min, followed by addition of Ph_3P (53 mg, 0.20 mmol). The mixture was allowed to warm to rt for 2 hr and then concentrated. The residue was purified by chromatography (EtOAc/hexane, 1 : 3) to afford **18** (42 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (1H, s), 7.38–7.20 (5H, m), 4.67 (1H, m, H-3), 4.56 and 4.52 (2H, ABd, $J = 12.3$ Hz, OCH_2Ph), 4.04 (1H, dd, $J = 4.5$, 11.8 Hz, H-1 eq), 3.63 (1H, m, H-5), 3.50–3.40 (2H, m, 2H-6), 3.19 (1H, t, $J = 11.8$ Hz, H-1 ax), 2.45–2.32 (2H, m, H-1′, H-2), 2.23 (2H, t, $J = 7.4$ Hz, $OC(O)CH_2$), 2.14 (1H, m, H-4), 2.02 (1H, m, H-1′), 1.60 (2H, m), 1.44 (1H, q, $J = 11.7$ Hz, H-4), 1.34–1.18

(20H, m), 0.86 (3H, t, $J = 7.0$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.9, 173.1, 137.8, 128.3, 127.6, 75.5, 73.5, 73.4,72.6, 70.0, 42.9, 36.4, 34.4, 33.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.2, 24.9, 22.8, 14.2.

2-C-(Carboxymethyl)-1,5-anhydro-6-O-benzyl-2,4-dideoxy-3-O-myristoyl-D-glucitol (19). To a solution of **18** (38 mg, 0.08 mmol) in *t*-BuOH (2 mL) and 2-methyl-2-butene (0.6 mL) was added a solution of $NaClO_2$ (91 mg, 0.80 mmol) and NaH_2PO_4 (138 mg, 0.80 mmol) in water (1.5 mL) over 5 min. The mixture was stirred for 1 hr, then diluted with ice water and extracted with EtOAc. The combined organic extracts were washed with brine (5 mL), dried over Na_2SO_4, evaporated, and further dried under oil vacuum overnight to afford **19**. The acid was used without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.20 (5H, m), 4.67 (1H, m, H-3), 4.56 and 4.52 (2H, ABd, $J = 12.3$ Hz, OCH_2Ph), 4.04 (1H, dd, $J = 4.5$, 11.8 Hz, H-1 eq), 3.63 (1H, m, H-5), 3.50–3.40 (2H, m, 2H-6), 3.19 (1H, t, $J = 11.8$ Hz, H-1 ax), 2.45–2.32 (1H, m, H-1′), 2.23 (3H, m, $OC(O)CH_2$,H-2), 2.10–2.00 (2H, m, H-4, H-1′), 1.60 (2H, m), 1.44 (1H, q, $J = 11.7$ Hz, H-4), 1.34–1.18 (20H, m), 0.86 (3H, t, $J = 7.0$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 173.4, 137.8, 129.7, 128.4, 127.7, 127.6, 75.5, 73.4, 73.0, 72.5, 69.9, 38.0, 34.3, 33.8, 32.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.2, 24.9, 22.8, 14.2.

1, 5-Anhydro-6-O-benzyl-2-C-(carboxymethyl N-benzyloxyamide)-2,4-dideoxy-3-O-myristoyl-D-glucitol (20). To a solution of the acid **19** (40 mg, 0.08 mmol) and $BnONH_2 \cdot HCl$ (13 mg, 0.08 mmol) in CH_2Cl_2 (0.5 mL) were added successively EDC (19 mg, 0.10 mmol) and triethylamine (13 μL, 0.10 mmol) at room temperature. The reaction mixture was stirred for 2 hr. CH_2Cl_2 (10 mL) was then added and the solution was washed with water (5 mL) and brine (5 mL), then dried over Na_2SO_4. The residue after concentration was purified by chromatography (toluene/EtOAc, 4 : 1) to afford **20** (34 mg, 70%). ^{1}H NMR (400 MHz, CD3OD) δ 7.40–7.20 (10H, m), 4.84 and 4.80 (2H, ABd, $J = 11.2$ Hz, OCH_2Ph), 4.70 (1H, m, H-3), 4.53 (2H, s, OCH_2Ph), 3.93 (1H, dd, $J = 4.3$, 11.5 Hz, H-1 eq), 3.62 (1H, m H-5), 3.52–3.42 (2H, m, H-6), 3.18 (1H, t, $J = 11.3$ Hz, H-1 ax), 2.30 (2H, m, $OC(O)CH_2$), 2.20–2.10 (2H, m, H-2, H-1′), 2.02 (1H, m, H-4), 1.80 (1H, dd, $J = 7.2$, 13.8 Hz, H-1′), 1.58 (2H, m), 1.35 (1H, q, $J = 11.5$ Hz, H-4), 1.33–1.20 (20H, m), 0.88 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 174.9, 170.7, 139.5, 136.9, 130.3, 129.7, 129.5, 129.4, 128.9, 128.7, 79.0, 76.7, 74.8, 74.4, 73.9, 70.8, 39.7, 35.2, 33.1, 32.9, 30.8, 30.7, 30.6, 30.5, 30.4, 30.2, 25.9, 23.8, 14.5. HRMS calcd. for $C_{36}H_{53}NO_6Na$ ($M + Na^+$) 618.3765; found 618.3762.

1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2,4-dideoxy-3-O-myristoyl-D-glucitol (2). Pd/C (8 mg) was added to a solution of **20** (12 mg, 20 μmol) in AcOH (2 mL). The reaction mixture was stirred for 4 hr under a balloon of H_2. After filtration through Celite, the Celite pad was washed well

with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15 : 1) to give **2** (5.9 mg, 70%). ^{1}H NMR (400 MHz, CD_3OD) δ 4.71 (1H, m, H-3), 4.00 (1H, dd, $J = 4.3$, 11.5 Hz, H-1 eq), 3.52–3.44 (3H, m, 2H-6, H-5), 3.22 (1H, t, $J = 11.5$ Hz, H-1 ax), 2.33 (2H, $OC(O)CH_2$), 2.22–2.15 (2H, H-2, H-1′), 2.02 (1H, m, H-4), 1.83 (1H, dd, $J = 9.7$, 15.7 Hz, H-1′), 1.61 (2H, m), 1.38–1.26 (21H, m, H-4, $C_{10}H_{20}$), 0.90 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.0, 170.7, 78.3, 75.1, 70.9, 65.9, 39.8, 35.1, 34.8, 33.1, 32.9, 30.8, 30.7, 30.6, 30.5, 30.4, 30.2, 25.9, 23.7, 14.4. HRMS calcd. for $C_{22}H_{41}NO_6Na$ ($M + Na^+$) 438.2832; found 438.2829.

1,5-Anhydro-4-O-benzyl-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-3-O-myristoyl-D-glucitol (21). A solution of 1M BH_3 in THF (0.2 mL) was added to **9** (12 mg, 20 μmol) at 0°C, and then a solution of 1M Bu_2BOTf in CH_2Cl_2 (0.02 mL, 20 μmol) was added to the reaction slowly. After 1 hr at 0°C, triethylamine (0.1 mL) was then added, followed by careful addition of methanol until the evolution of H_2 ceased. The reaction mixture was then concentrated and purified by chromatography (toluene/EtOAc, 2 : 1) to give **21** (8.5 mg, 71%). ^{1}H NMR (500 MHz, CD_3OD: $CDCl_3 = 4 : 1$) δ 7.40–7.20 (10H, m), 4.92 (1H, dd, $J = 9.1$, 10.9 Hz, H-3), 4.80 (2H, overlapped with H_2O, OCH_2Ph), 4.64 and 4.58 (2H, 2d, $J = 11.3$ Hz, OCH_2Ph), 3.89 (1H, dd, $J = 4.8$, 11.6 Hz, H-1 eq), 3.80 (1H, dd, $J = 2.0$, 12.0 Hz, H-6), 3.67 (1H, dd, $J = 4.6$, 12.0 Hz, H-6), 3.54 (1H, t, $J = 9.1$ Hz, H-4), 3.36 (1H, m, H-5), 3.19 (1H, t, $J = 11.6$ Hz, H-1 ax), 2.28 (2H, m, $OC(O)CH_2$), 2.16 (1H, m, H-2), 2.06 (1H, dd, $J = 5.0$, 14.8 Hz, H-1′), 1.81 (1H, dd, $J = 8.7$, 14.8 Hz, H-1′), 1.52 (2H, m), 1.34–1.18 (20H, m), 0.88 (1H, t, $J = 6.8$ Hz); ^{13}C NMR (125 MHz, CD_3OD: $CDCl_3 = 4 : 1$) δ 175.4, 170.2, 139.5, 136.8, 130.3, 129.7, 129.5, 129.4, 128.8, 128.7, 81.9, 79.4, 79.1, 78.5, 78.4, 75.7, 70.4, 62.4, 40.0, 35.2, 33.1, 32.5, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 25.8, 23.8, 14.6. HRMS calcd. for $C_{36}H_{53}NO_7Na$ ($M + Na^+$) 634.3720; found 634.3721.

1,5-Anhydro-4-O-benzyl-2-C-(carboxymethyl N-benzyloxyamide)-2,6-dideoxy-6-iodo-3-O-myristoyl-D-glucitol (22). To a solution of **21** (12 mg, 20 μmol) in toluene (1 mL) were added Ph_3P (10.3 mg, 40 μmol), I_2 (10 mg, 0.040 mmol) and imidazole (5.3 mg, 78 μmol) successively. The mixture was stirred at rt for 4 hr and was then directly purified by chromatography (EtOAc/toluene, 1 : 2) to give **22** (11 mg, 80%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.40–7.20 (10H, m), 4.99 (1H, dd, $J = 8.9$, 10.9 Hz, H-3), 4.83 (2H, overlapped with H_2O, OCH_2Ph), 4.72 and 4.65 (2H, ABd, $J = 11.3$ Hz, OCH_2Ph), 3.86 (1H, dd, $J = 4.8$, 11.7 Hz, H-1 eq), 3.50 (1H, dd, $J = 2.8$, 10.9 Hz, H-6), 3.42 (1H, t, $J = 8.9$ Hz, H-4), 3.40 (1H, dd, $J = 10.9$, 5.2 Hz, H-6), 3.27 (1H, t, $J = 11.7$ Hz, H-1 ax), 3.01 (1H, m, H-5), 2.28 (2H, m, $OC(O)CH_2$), 2.19 (1H, m, H-2), 2.06 (1H, dd, $J = 5.1$, 14.8 Hz, H-1′), 1.81 (1H, dd, $J = 8.5$, 14.8 Hz, H-1′), 1.52 (2H, m), 1.35–1.18 (20H, m), 0.90 (1H, t, $J = 6.8$ Hz); ^{13}C NMR (100 MHz,

CD_3OD) δ 175.1, 170.1, 139.5, 130.3, 129.7, 129.5, 129.4, 128.9, 128.7, 82.5, 79.5, 78.9, 77.9, 76.1, 70.1, 40.1, 35.1, 33.1, 32.3, 30.7, 30.5, 30.4, 30.2, 25.7, 23.7, 7.6. HRMS calcd. for $C_{36}H_{52}NO_6INa$ ($M + Na^+$) 744.2737; found 744.2730.

1,5-Anhydro-4-O-benzyl-2-C-(carboxymethyl N-benzyloxyamide)-2,6-dideoxy-3-O-myristoyl-D-glucitol (23). A solution of **22** (17 mg, 24 μmol) and AIBN (1.5 mg, 9.0 μmol) in dry toluene (0.5 mL) was degassed with argon for 10 min. Bu_3SnH (25 μL, 96 μmol) was then added under argon. The mixture was heated to 90°C for 30 min and, after cooling to rt, was directly purified by chromatography (EtOAc/toluene, 1 : 2) to give **23** (11.5 mg, 82%). ^1H NMR (400 MHz, CD_3OD) δ 7.40–7.20 (10H, m), 4.89 (1H, dd, $J = 9.0$, 10.9 Hz, H-3), 4.82 (2H, overlapped with H_2O, OCH_2Ph), 4.62 and 4.60 (2H, ABd, $J = 11.4$ Hz, OCH_2Ph), 3.79 (1H, dd, $J = 4.8$, 11.7 Hz, H-1 eq), 3.36–3.30 (2H, m, H-4, H-5), 3.17 (1H, t, $J = 11.7$ Hz, H-1 ax), 2.28 (2H, m, $OC(O)CH_2$), 2.18 (1H, m, H-2), 2.06 (1H, dd, $J = 5.1$, 14.8 Hz, H-1′), 1.81 (1H, dd, $J = 8.7$, 14.8 Hz, H-1′), 1.52 (2H, m), 1.35–1.18 (23H, m, H-6 and $C_{10}H_{20}$), 0.89 (1H, t, $J = 6.8$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 170.2, 139.6, 136.9, 130.8, 130.2, 129.6, 129.4, 129.3, 128.7, 128.6, 84.5, 79.0, 78.2, 77.6, 75.9, 70.3, 40.4, 35.2, 33.2, 32.6, 30.9, 30.8, 30.6, 30.5, 30.3, 25.9, 23.9, 18.9, 14.6. HRMS calcd. for $C_{36}H_{53}NO_6Na$ ($M + Na^+$) 618.3765; found 618.3761.

1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2,6-dideoxy-3-O-myristoyl-D-glucitol (3). Pd/C (7 mg) was added to a solution of **23** (12 mg, 20 μmol) in AcOH (2 mL). The reaction mixture was stirred for 4 hr under a balloon of H_2. After filtration through Celite, the Celite pad was washed well with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15 : 1) to give **3** (5.9 mg, 70%). ^1H NMR (400 MHz, CD_3OD) δ 4.70 (1H, dd, $J = 9.0$, 10.9 Hz, H-3), 4.00 (1H, dd, $J = 4.7$, 11.6 Hz, H-1 eq), 3.25 (1H, m, H-5), 3.20 (1H, t, $J = 11.6$ Hz, H-1 ax), 3.09 (1H, t, $J = 9.0$ Hz, H-4), 2.39 (2H, t, $J = 7.4$ Hz, $OC(O)CH_2$), 2.25 (H, m, H-2), 2.11 (1H, dd, $J = 4.9$, 10.0 Hz H-1′), 1.82 (1H, dd, $J = 10.0$, 15.7 Hz, H-1′) 1.65 (2H, m), 1.40–1.25 (20H, m, $C_{10}H_{20}$), 1.24 (3H, d, $J = 6.1$ Hz, 2H-6), 0.90 (3H, t, $J = 6.8$ Hz).

After sitting overnight in the NMR machine, myristoyl migration was observed: ^1H NMR (400 MHz, CD_3OD) δ 4.70 (1H, dd, $J = 9.0$, 10.9 Hz, H-3), 4.54 (0.4 H, t, $J = 9.0$ Hz, H-4), 4.00 (1H, dd, $J = 4.7$, 11.6 Hz, H-1 eq), 3.25 (1H, m, H-5), 3.20 (1H, t, $J = 11.6$ Hz, H-1 ax), 3.09 (1H, t, $J = 9.0$ Hz, H-4), 2.39 (2H, t, $J = 7.4$ Hz, $OC(O)CH_2$), 2.25 (H, m, H-2), 2.11 (1H, dd, $J = 4.9$, 10.0 Hz H-1′), 1.82 (1H, dd, $J = 10.0$, 15.7 Hz, H-1′) 1.65 (2H, m), 1.40–1.25 (20H, m, $C_{10}H_{20}$), 1.24 (3H, d, $J = 6.1$ Hz, 2H-6), 1.10 (d, $J = 6.1$ Hz, 2H-6), 0.90 (3H, t, $J = 6.8$ Hz); HRMS calcd. for $C_{22}H_{41}NO_6Na$ ($M + Na^+$) 438.2832; found 438.2829.

1,6-Anhydro-4-O-benzyl-2-deoxy-3-O-myristoyl-2-C-(2-propenyl)-β-D-glucopyranose (25). To a solution of **24** (340 mg, 1.23 mmol) in pyridine (8 mL), DMAP (70 mg, 0.57 mmol) and myristoyl chloride (0.86 mL, 3.16 mmol) were successively added at 0°C and then stirred at rt for 20 hr. EtOAc (20 mL) was added to the reaction mixture and the organic layer was washed with 10% citric acid (5 mL), H_2O (5 mL × 2), sat. $NaHCO_3$ (5 mL), and brine (5 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (toluene/EtOAc, 9 : 1) to afford **25** (503 mg, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.22 (5H, m), 5.80 (1H, m, H-2′), 5.49 (1H, s, H-1), 5.14–5.08 (2H, m, H-3′), 4.90 (1H, s, H-3), 4.80 (1H, d, $J = 12.3$ Hz, OCH_2Ph), 4.65 (1H, d, $J = 12.3$ Hz, OCH_2Ph), 4.54 (1H, br d, $J = 5.6$ Hz, H-5), 3.96 (1H, d, $J = 7.0$ Hz, H-6), 3.70 (1H, dd, $J = 5.6$, 7.0 H, H-6), 3.22 (1H, s, H-4), 2.40–2.26 (4H, m, 2H-1′ and $OC(O)CH_2$), 1.79 (1H, br t, $J = 8.0$ Hz, H-2), 1.38–1.20 (20H, m), 0.82 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 137.7, 135.6, 128.4, 127.8, 127.7, 117.4, 102.4, 75.5, 74.7, 71.1, 69.1, 64.8, 42.6, 34.5, 33.9, 31.9, 29.6, 29.4, 29.3, 29.2, 24.8, 22.6, 14.1. HRMS calcd. for $C_{30}H_{46}O_5Na$ ($M + Na^+$) 509.3243; found 509.3243.

1,5-Anhydro-4-O-benzyl-2-deoxy-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol (26). To a solution of **25** (100 mg, 0.21 mmol) in CH_2Cl_2 (20 mL), Et_3SiH (0.16 mL, 1.02 mmol) and $SnCl_4$ (36 μL, 0.31 mmol) were successively added at 0°C. After stirring at rt for 1 hr, the reaction mixture was poured into cold sat. $NaHCO_3$ (5 mL) and the aqueous layer was extracted with CH_2Cl_2 (10 mL × 2) at once. The combined organic layers were washed with brine (5 mL) and dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (toluene/EtOAc, 6 : 1) to give **26** (46 mg, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.20 (5H, m), 5.70 (1H, m, H-2′), 5.06–4.95 (3H, m, H-3, H-3′), 4.61 and 4.59 (2H, ABd, $J = 11.3$ Hz, OCH_2Ph), 3.94 (1H, dd, $J = 4.3$, 11.6 Hz, H-1 eq), 3.85 (1H, dd, $J = 2.6$, 11.9 Hz, H-6), 3.70 (1H, dd, $J = 4.0$, 11.9 Hz, H-6), 3.52 (1H, t, $J = 9.4$ Hz, H-4), 3.30 (1H, m, H-5), 3.18 (1H, t, $J = 11.6$ Hz, H-1 ax), 2.31–2.10 (3H, m, H-1′, $OC(O)CH_2$), 1.95–1.80 (2H, m, H-2, H-1′), 2.50 (2H, m), 1.38–1.20 (20H, m), 0.84 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 137.9, 134.8, 128.4, 127.7, 127.6, 116.9, 79.8, 77.1, 74.5, 69.7, 62.1, 41.2, 34.4, 32.2, 31.8, 29.6, 29.5, 29.4, 29.3, 29.2, 24.8, 22.6, 14.1. HRMS calcd. for $C_{30}H_{48}O_5Na$ ($M + Na^+$) 511.3399; found 511.3399.

1,5-Anhydro-4-O-benzyl-2-deoxy-6-O-methyl-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol (27). To a solution of **26** (70 mg, 0.14 mmol) and 2,6-di(*tert*-butyl-4-methyl) pyridine (882 mg, 4.30 mmol) was added methyl trifluoromethanesulfonate (0.32 mL, 2.84 mmol). The mixture was stirred overnight and quenched by an aqueous ammonia solution (5 mL) and extracted with CH_2Cl_2 (10 mL × 3). The extracts were washed with 10% HCl (5 mL), and brine (5 mL), dried over Na_2SO_4, and concentrated. The residue was purified by chromatography (toluene/EtOAc, 8 : 1) to afford **27** (71 mg, 98%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.20 (5H, m), 5.70 (1H, m, H-2′), 5.06–4.95 (3H, m, H-3, H-3′), 4.61 and 4.59 (2H, ABd, $J = 11.3$ Hz, OCH_2Ph), 3.94 (1H, dd, $J = 4.7$, 11.7 Hz, H-1 eq), 3.58–3.53 (3H, m, 2H-6, H-4), 3.40–3.35 (4H, m, H-5, OMe), 3.25 (1H, t, $J = 11.7$ Hz, H-1 ax), 2.31–2.10 (3H, m, H-1′, $OC(O)CH_2$), 2.00–1.80 (2H, m, H-2, H-1′), 2.50 (2H, m), 1.38–1.20 (20H, m), 0.84 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 138.1, 134.9, 128.3, 127.6, 127.5, 116.8, 79.2, 77.2, 74.5, 71.4, 69.9. 59.2, 41.1, 34.4, 32.2, 31.8, 29.6, 29.4, 29.3, 29.2, 24.8, 22.6, 14.1. HRMS calcd. for $C_{31}H_{50}O_5Na$ ($M + Na^+$) 525.3556; found 525.3554.

2-C-(Aldehydomethyl)-1,5-anhydro-4-O-benzyl-2-deoxy-6-O-methyl-3-O-myristoyl-D-glucitol (28). Ozone was passed through a solution of **27** (30 mg, 60 μmol) in CH_2Cl_2 at −78°C until the solution turned blue. The excess ozone was removed with a stream of oxygen for 10 min, followed by addition of Ph_3P (23 mg, 88 μmol). The mixture was allowed to warm to rt for 2 hr, concentrated, and purified by chromatography (EtOAc/hexane, 1 : 3) to afford **28** (24 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (1H, s), 7.38–7.20 (5H, m), 5.00 (1H, dd, $J = 9.1$, 10.7 Hz, H-3), 4.61 and 4.59 (2H, ABd, $J = 14.4$ Hz, OCH_2Ph), 3.98 (1H, dd, $J = 4.5$, 11.4 Hz, H-1 eq), 3.64–3.56 (3H, m, 2H-6, H-4), 3.44–3.34 (4H, m, H-5, OMe), 3.21 (1H, t, $J = 11.4$ Hz, H-1 ax), 2.56–2.40 (2H, m, H-2, H-1′), 2.31–2.10 (3H, m, H-1′, $OC(O)CH_2$), 2.00–1.80 (2H, m, H-2,), 2.50 (2H, m), 1.38–1.20 (20H, m), 0.84 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.4, 173.4, 137.9, 128.4, 127.7, 127.5, 79.4, 76.9, 74.6, 71.3, 69.6, 59.2, 42.2, 36.4, 34.2, 31.9, 29.6, 29.5, 29.3, 29.2, 29.1, 24.7, 22.6, 14.1. HRMS calcd. for $C_{30}H_{48}O_6Na$ ($M + Na^+$) 527.3349; found 527.3349.

2-C-(Carboxymethyl)-1,5-anhydro-4-O-benzyl-2-deoxy-6-O-methyl-3-O-myristoyl-D-glucitol (29). To a solution of **52** (24 mg, 48 μmol) in *t*-BuOH (1.5 mL) and 2-methyl-2-butene (0.5 mL) was added a solution of $NaClO_2$ (43 mg, 0.48 mmol) and NaH_2PO_4 (66 mg, 0.48 mmol) in water (1 mL). The mixture was stirred for 1 hr, then diluted with ice-water (5 mL), and extracted with EtOAc (10 mL × 3). The combined organic extracts were washed with brine (5 mL), dried over Na_2SO_4, concentrated, and dried under high vacuum overnight to give 28. The acid was used without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.20 (5H, m), 4.94 (1H, dd, $J = 9.0$, 10.6 Hz, H-3), 4.61 and 4.59 (2H, ABd, $J = 14.4$ Hz, OCH_2Ph), 3.98 (1H, dd, $J = 4.7$, 11.5 Hz, H-1 eq), 3.64–3.56 (3H, m, 2H-6, H-4), 3.40–3.32 (4H, m, H-5, OMe), 3.21 (1H, t, $J = 11.5$ Hz), 2.56–2.40 (2H, m, H-2, H-1′), 2.31–2.10 (3H, m, H-1′, $OC(O)CH_2$), 2.50 (2H, m), 1.38–1.20 (20H, m), 0.84 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 173.5, 137.9, 128.5, 128.4, 127.7, 127.6, 79.3, 76.9, 76.5, 74.6, 71.3, 69.6, 59.2, 38.4, 34.3, 32.4, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 24.7, 22.6, 14.1.

1,5-Anhydro-4-O-benzyl-2-deoxy-2-C-(carboxymethyl N-hydroxyamide)-6-O-methyl-3-O-myristoyl-D-glucitol (30). To a solution of the acid **29** (24 mg, 50 μmol) and $BnONH_2 \cdot HCl$ (7.6 mg, 50 μmol) in CH_2Cl_2 (0.3 mL) were added successively EDC (12.8 mg, 70 μmol) and triethylamine (9 μL, 70 μmol) at rt. The reaction mixture was stirred for 2 hr. CH_2Cl_2 (10 mL) was then added and the solution was washed with water (5 mL) and brine (5 mL), then dried over Na_2SO_4. After concentration, the residue was purified by chromatography (toluene/EtOAc, 4 : 1) to afford **30** (21 mg, 71%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.40–7.20 (10H, m), 4.92 (1H, dd, $J = 9.0$, 10.9 Hz, H-3), 4.93 and 4.80 (2H, ABd, $J = 11.3$ Hz, OCH_2Ph), 4.60 and 4.58 (1H, dd, $J = 4.8$, 11.5 Hz, H-1 eq), 3.62–3.54 (2H, m, 2H-6), 3.53 (1H, t, $J = 9.0$ Hz, H-4), 3.36 (1H, m, H-5), 3.34 (3H, s, OMe), 3.29 (1H, t, $J = 11.5$ Hz, H-1 ax), 2.28 (2H, m, $OC(O)CH_2$), 2.17 (1H, m, H-2), 2.06 (1H, dd, $J = 5.1$, 14.8 Hz, H-1′), 1.82 (1H, dd, $J = 8.6$, 14.8 Hz, H-1′), 1.51 (2H, m), 1.34–1.18 (20H, m), 0.89 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.2, 170.2, 139.6, 136.9, 130.3, 129.7, 129.5, 129.4, 126.7, 80.8, 79.0, 78.6, 78.3, 75.7, 72.7, 70.4, 59.5, 40.0, 35.1, 33.1, 32.4, 30.8, 30.7, 30.6, 30.5, 30.4, 30.2, 25.7, 23.7, 14.1. HRMS calcd. for $C_{37}H_{55}NO_7Na$ ($M + Na^+$) 648.3876; found 648.3874.

1,5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-6-O-methyl-3-O-myristoyl-D-glucitol (4). Pd/C (8 mg) was added to a solution of **30** (12 mg, 19 μmol) in AcOH (2 mL), The reaction mixture was stirred for 3 hr under a balloon of H_2. After filtration through Celite, the Celite pad was washed well with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15 : 1) to give **4** (5.9 mg, 69%). ^{1}H NMR (400 MHz, CD_3OD) δ 4.73 (1H, dd, $J = 8.7$, 10.9 Hz, H-3), 3.91 (1H, dd, $J = 4.8$, 11.7 Hz, H-1 eq), 3.64 (1H, dd, $J = 2.0$, 10.8 Hz, H-6), 3.53 (1H, dd, $J = 5.4$, 10.8 Hz, H-6), 3.40–3.30 (2H, m, H-4, H-5), 3.20 (1H, t, $J = 11.7$ Hz, H-1 ax), 2.38 (2H, t, $J = 7.3$, $OC(O)CH_2$), 2.24 (1H, m, H-2), 2.09 (1H, dd, $J = 4.9$, 14.5 Hz, H-1′), 1.81 (1H, dd, $J = 9.0$, 14.5 Hz H-1′), 1.51 (2H, m), 1.40–1.20 (20H, m), 0.9 (3H, t, $J = 7.4$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.7, 170.3, 81.4, 78.9, 73.3, 70.7, 70.4, 59.5, 39.8, 35.1, 33.1, 32.6, 30.8, 30.4, 30.6, 30.4, 30.2, 25.9, 23.7, 14.4. HRMS calcd. for $C_{23}H_{43}NO_7$ ($M + Na^+$) 468.2937; found 468.2936.

4-O-Methyl-D-glucal (32). A solution of sodium metal in 500 mL ammonia was stirred for 1 hr at −78°C and then distilled to another three-neck round bottom flask. Small pieces of sodium metal were added until a deep blue color persisted at −78°C. A solution of **31** (1.16 g, 3.40 mmol) and MeOH (0.276 mL, 6.80 mmol) in THF (50 mL) was added dropwise to the above solution. The reaction mixture was stirred for 2 hr, and then quenched with MeOH until the solution turned clear. Ammonia was then evaporated and coevaporated with MeOH and AcOH. The residue was purified by chromatography (EtOAc/acetone, 1 : 1) to give **32** (464 mg, 85%). The NMR data were the same as those described in the literature.[13]

3-O-Acetyl-1,6-anhydro-2-deoxy-2-iodo-4-O-methy-β-D-glucopyranose (33). The glucal **32** (60 mg, 0.38 mmol) was treated with *bis*-(tributyltin)oxide (0.15 mL, 0.29 mmol) and actived powdered 3Å molecular sieves (200 mg) in refluxing dry acetonitrile (5 mL) for 3 hr. The mixture was cooled to 0°C under argon, and iodine (143 mg, 0.563 mmol) was added in one portion. The dark-brown mixture was stirred at 0°C for 15 min, then 3 hr at rt. The mixture was filtered through Celite and concentrated. To the residue was added aq. sodium thiosulfate (10 mL) and it was extracted with EtOAc (20 mL × 3). The combined organic layers were dried over Na_2SO_4 and concentrated, and further dried under oil vacuum overnight. The residue was then treated with Ac_2O (3 mL) and pyridine (3 mL) overnight. After removing the solvent, the residue was purified by chromatography (EtOAc/hexane, 1 : 3) to afford **33** (110 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.65 (1H, s, H-1), 5.39 (1H, s, H-3), 4.54 (1H, d, $J = 5.8$ Hz, H-5), 4.08 (1H, d, $J = 7.6$ Hz, H-6), 3.90 (1H, s, H-2), 3.76 (1H, dd, $J = 5.8$, 7.6 Hz, H-6), 3.50 (3H, s, OMe), 3.16 (1H, s, H-4), 2.08 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 102.5, 78.2, 74.1, 71.3, 65.5, 57.4, 21.5, 21.0. HRMS calcd. for $C_9H_{13}O_5INa$ ($M + Na^+$) 350.9700; found 350.9697.

1,6-Anhydro-2-deoxy-4-O-methyl -2-C- (2-propenyl)-β-D-glucopyranose (34). A solution of **33** (110 mg, 0.34 mmol) and AIBN (20 mg, 0.12 mmol) in dry toluene (6 mL) was degassed with argon for 15 min. Allyltributylstannane (0.21 mL, 0.68 mmol) was added under argon, and the mixture was heated to 80°C for 3 hr. TLC (EtOAc/hexane, 1 : 1) showed complete conversion. The solvent was evaporated and a solution of the residue in acetonitrile (10 mL) was washed with hexane (10 mL), and then concentrated. Chromatography of the residue gave 56 mg (69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.78 (1H, m, H-2′), 5.36 (1H, s, H-1), 5.18–5.04 (2H, m, H-3′), 4.80 (1H, s, H-3), 4.55 (1H, d, $J = 5.8$ Hz, H-5), 4.00 (1H, d, $J = 8.2$ Hz, H-6), 3.75 (1H, dd, $J = 5.9$, 7.5 Hz, H-6), 3.46 (3H, s, OMe), 3.08 (1H, br s, H-4), 2.28 (2H, t, $J = 7.8$ Hz, H-1′), 2.05 (3H, s, OAc), 1.76 (1H, br t, $J = 8.0$ Hz, H-2).

A solution of above compound in 10 : 10 : 1 MeOH-H_2O-Et3 N (5 mL) was left overnight at rt and then concentrated, dried under oil vacuum overnight to give **34**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (1H, m, H-2′), 5.42 (1H, s, H-1), 5.10–5.06 (2H, m, H-3′), 4.60 (1H, d, $J = 5.3$ Hz, H-5), 4.10 (1H, d, $J = 7.5$ Hz, H-6), 3.72 (1H, dd, $J = 5.3$, 7.5 Hz, H-6), 3.66 (1H, br s, H-3), 3.44 (3H, s, OMe), 3.20 (1H, br s, H-4), 2.28–2.20 (2H, m, H-1′), 1.78 (1H, br t, $J = 8.4$, H-2); ^{13}C NMR(100 MHz, $CDCl_3$) δ 135.8, 117.2, 103.4, 81.5, 74.4, 68.5, 65.4, 57.4, 45.7, 33.5. HRMS calcd. for $C_{10}H_{16}O_4Na$ ($M + Na^+$) 223.0946; found 223.0947.

1,5-Anhydro-2-deoxy-4-O-methyl-2-C-(2-propenyl)-D-glucitol (35). To a solution of **34** (196 mg, 0.98 mmol) and Et_3SiH (0.82 mL, 5.15 mmol) in CH_2Cl_2 (50 mL), $SnCl_4$ (1.55 mL, 1M in CH_2Cl_2, 1.55 mmol) was added

dropwise at 0°C. The reaction mixture was stirred at rt for 40 min. The reaction was then poured into cold sat. $NaHCO_3$ (5 mL) and extracted with CH_2Cl_2 (20 mL × 2). The combined organic layers were washed with brine (5 mL) and dried with Na_2SO_4. The residue was purified by chromatography (acetone/toluene, 1 : 6) to give **35** (130 mg, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81–5.68 (1H, m, H-2′), 5.05–4.98 (2H, m, H-3′), 3.90–3.80 (2H, m, H-6, H-1 eq), 3.68 (1H, dd, $J = 4.4$, 11.8 Hz, H-6), 3.54 (2H, s, OMe), 3.34 (1H, dd, $J = 8.7$, 9.9 Hz, H-3), 3.15 (1H, m, H-5), 3.10–3.02 (2H, m, H-1 ax, H-4), 2.48 (1H, m, H-1′), 1.89 (1H, m, H-2), 1.78 (1H, m, H-1′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.5, 116.9, 81.6, 79.7, 76.5, 69.7, 62.3, 60.8, 42.1, 32.4. HRMS calcd. for $C_{10}H_{18}O_4Na$ ($M + Na^+$) 225.1103, found 225.1103.

1,5-Anhydro-6-O-*tert*-butyldiphenylsily-2-deoxy-4-O-methyl-2-C-(2-propenyl)-D-glucitol (36). To a solution of **35** (72 mg, 0.36 mmol) and imidazole (48 mg, 0.71 mmol) in DMF (2 mL) was added *tert*-butyldiphenylsilyl chloride (0.14 mL, 0.54 mmol). The mixture was stirred at rt for 3 hr, then diluted with Et_2O (30 mL), washed with H_2O (5 mL × 2) and brine (5 mL), dried over Na_2SO_4, and concentrated, and the residue was purified by chromatography (EtOAc/hexane, 1 : 5) to afford **36** (152 mg, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.34 (10H, m), 5.80 (1H, m, H-2′), 5.10–5.00 (2H, m, H-3′), 3.90 (1H, dd, $J = 4.7$, 11.7 Hz, H-1 eq), 3.88–3.82 (2H, m, H-6), 3.54 (3H, s, OMe), 3.38–3.26 (2H, m, H-3, H-4), 3.09 (1H, m, H-5), 3.04 (1H, t, $J = 11.7$ Hz, H-1 ax), 2.48 (1H, m, H-1′), 1.89 (1H, m, H-2), 1.78 (1H, m, H-1′), 1.04 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.8, 135.5, 133.8, 133.3, 129.5, 129.4, 127.5, 127.4, 116.6, 81.2, 80.4, 77.3, 69.8, 63.5, 60.7, 42.2, 32.7, 26.9, 19.5. HRMS calcd. for $C_{26}H_{36}O_4SiNa$ ($M + Na^+$) 463.2281; found 463.2281.

1,5-Anhydro-6-O-tert-butyldiphenylsily-2-deoxy-4-O-methyl-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol (37). To a solution of **36** (147 mg, 0.33 mmol) in pyridine (5 mL), myristoyl chloride (0.27 mL, 0.99 mmol) was added at 0°C, and the mixture was stirred at rt for 3 hr. EtOAc (20 mL) was added to the reaction mixture. The mixture was washed with H_2O (5 mL × 2) and brine (5 mL) and dried over Na_2SO_4. The residue was purified by chromatography to give **37** (206 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78–7.34 (10H, m), 5.74 (1H, m, H-2′), 5.02–4.98 (2H, m, H-3′), 4.80 (1H, dd, $J = 9.2$, 10.4 Hz, H-3), 3.93 (1H, dd, $J = 4.3$, 11.6 Hz, H-1 eq), 3.88–3.84 (2H, m, H-6), 3.41 (3H, s, OMe), 3.09 (1H, t, $J = 9.2$ Hz, H-4), 3.20 (1H, m, H-5), 3.10 (1H, t, $J = 11.6$ Hz, H-1 ax), 2.38 (2H, t, $J = 7.9$ Hz, $OC(O)CH_2$), 2.35 (1H, m, H-2), 1.88 (2H, m, H-1′), 1.66 (2H, m), 1.40–1.20 (20H, m), 1.06 (9H, s), 0.88 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 135.8, 135.6, 135.2, 133.3, 129.5, 127.6, 127.4, 80.6, 78.6, 77.3, 69.6, 63.3, 60.1, 41.2, 34.6, 32.4, 31.9, 29.6, 29.5, 29.3, 29.2, 26.8, 24.9, 22.4, 19.4, 14.1. HRMS calcd. for $C_{40}H_{62}O_5Si$ ($M + Na^+$) 673.4262; found 673.4262.

2-C-(Aldehydomethyl)-1,5-anhydro-6-O-*tert*-butyldiphenylsily-2-deoxy-4-O-methyl-3-O-myristoyl-2-C-(2-propenyl)-D-glucitol (38). Ozone was passed through a solution of **37** (150 mg, 0.23 mmol) in CH_2Cl_2 (20 mL) at -78°C until the solution turned blue. The excess ozone was removed with a stream of oxygen for 10 min, followed by addition of Ph_3P (121 mg, 0.46 mmol). The mixture was allowed to warm to rt for 3 hr, and then concentrated and purified by chromatography (EtOAc/hexane, 1 : 9) to afford **38** (122 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.70 (1H, s), 7.78–7.34 (10H, m), 4.87 (1H, dd, $J = 9.2$, 10.4 Hz, H-3), 3.96 (1H, dd, $J = 4.5$, 11.5 Hz, H-1 eq), 3.90–3.84 (2H, m, H-6), 3.44 (1H, t, $J = 9.2$ Hz, H-4), 3.42 (3H, s, OMe), 3.20 (1H, m, H-5), 3.14 (1H, t, $J = 11.5$ Hz, H-1 ax), 2.50–2.40 (2H, m, H-2, H-1′), 2.38 (2H, t, $J = 7.4$ Hz, $OC(O)CH_2$), 2.20 (1H, m, H-1′), 1.66 (2H, m), 1.40–1.20 (20H, m), 1.06 (9H, s), 0.88 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.7, 173.5, 135.8, 135.6, 133.7, 133.2, 129.6, 127.6, 127.5, 80.6, 78.2, 77.1, 69.0, 60.2, 42.4, 36.4, 34.4, 31.9, 29.6, 29.4, 29.3, 29.2, 26.8, 24.9, 22.6, 19.4, 14.1.

2-C-(Carboxymethyl)-1, 5-anhydro-6-O-tert-butyldiphenylsily-2-deoxy-4-O-methyl-3-O-myristoyl- 2-C-(2-propenyl)-D-glucitol (39). To a solution of **38** (80 mg, 0.12 mmol) in *t*-BuOH (3 mL) and 2-methyl-2-butene (1 mL) was added a solution of $NaClO_2$ (110 mg, 1.23 mmol) and NaH_2PO_4 (169 mg, 1.23 mmol) in water (2 mL) slowly. The mixture was stirred for 1 hr, then diluted with ice water (5 mL) and extracted with EtOAc (10 mL × 3). The combined organic extracts were washed with brine (5 mL), dried over Na_2SO_4, evaporated, and further dried under high vacuum overnight to afford **39**. The acid was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78–7.34 (10H, m), 4.90 (1H, dd, $J = 9.2$, 10.6 Hz, H-3), 4.05 (1H, dd, $J = 4.6$, 11.5 Hz, H-1 eq), 3.90–3.86 (2H, m, H-6), 3.44 (1H, t, $J = 9.2$ Hz, H-4), 3.42 (3H, s, OMe), 3.22 (1H, m, H-5), 3.18 (1H, t, $J = 11.5$ Hz, H-1 ax), 2.40–2.30 (4H, m, H-2, H-1′, $OC(O)CH_2$), 2.18 (1H, m, H-1′), 1.66 (2H, m), 1.40–1.20 (20H, m), 1.06 (9H, s), 0.88 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 173.5, 135.7, 135.5, 133.6, 133.2, 129.5, 127.6, 127.5, 80.6, 78.3, 76.8, 69.3, 63.1, 60.3, 38.6, 34.5, 32.7, 31.9, 29.7, 29.6, 29.4, 29.3, 26.9, 25.0, 22.8, 19.5, 14.2. HRMS calcd. $C_{39}H_{60}O_7SiNa$ ($M + Na^+$) 691.4006; found 691.4002.

1,5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-6-O-tert-butyldiphenylsily-2-deoxy-4-O-methyl-3-O-myristoyl-D-glucitol (40). To a solution of the acid **39** (25 mg, 37 μmol) and $BnONH_2 \cdot HCl$ (7.2 mg, 0.04 mmol) in CH_2Cl_2 (0.3 mL) were added successively EDC (9.5 mg, 0.05 mmol) and triethylamine (10 μL, 0.08 mmol) at rt. The reaction mixture was stirred for 2 hr. CH_2Cl_2 (10 mL) was then added and the solution was washed with water (5 mL) and brine (5 mL), and then dried over Na_2SO_4. The residue after concentration was purified by chromatography

(toluene/EtOAc, 4 : 1) to afford **65** (19 mg, 68%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.78–7.34 (10H, m), 4.80 (3H, overlapped with H_2O, H-3, OCH_2Ph), 3.86 (2H, m, H-6), 3.84 (1H, dd, $J = 4.5$, 11.5 Hz, H-1 eq), 3.43 (1H, t, $J = 9.4$ Hz, H-4), 3.40 (3H, s, OMe), 3.20 (1H, m, H-5), 3.14 (1H, t, $J = 11.5$ Hz, H-1 ax), 2.42–2.34 (2H, m, $OC(O)CH_2$), 2.25 (1H, m, H-2), 2.06 (1H, dd, $J = 5.1$, 14.8 Hz, H-1′), 1.82 (1H, dd, $J = 8.7$, 14.8 Hz, H-1′), 1.63 (2H, m), 1.40–1.20 (20H, m), 1.06 (9H, s), 0.88 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 170.2, 136.9, 136.7, 134.4, 130.8, 130.3, 129.6, 129.4, 128.7, 128.6, 81.9, 79.8, 79.0, 78.6, 70.2, 64.4, 60.8, 40.2, 35.3, 33.2, 32.6, 30.9, 30.8, 30.7, 30.6, 30.5, 30.4, 27.5, 26.0, 23.9, 20.3, 14.6. HRMS calcd. $C_{46}H_{67}NO_7SiNa$ ($M + Na^+$) 796.4584; found 796.4583.

1,5-Anhydro-2-deoxy-2-C-(carboxymethyl N-hydroxyamide)-4-O-methyl-3-O-myristoyl-D-glucitol (41). A solution of **40** (16 mg, 21 μmol) in THF (0.3 mL) was treated with TBAF (1.0 M solution in THF, 0.10 mL, 0.10 mmol) at rt under argon. After 2.5 hr, the reaction was concentrated. The residue was dissolved in EtOAc (10 mL) and then washed with water (5 mL × 2) and brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography (toluene/EtOAc, 1 : 1) to afford **41** (10 mg, 91%). ^{1}H NMR (400 MHz, CD_3OD: $CDCl_3 = 3:1$) δ 7.40–7.34 (5H, m), 4.80 (3H, overlapped with H_2O, H-3, OCH_2Ph), 3.86 (1H, dd, $J = 4.8$, 11.7 Hz, H-1 eq), 3.77 (1H, dd, $J = 1.8$, 11.9 Hz, H-6), 3.63 (1H, dd, $J = 4.3$, 11.9 Hz, H-6), 3.41 (3H, s, OMe), 3.25–3.11 (3H, m, H-5, H-4, H-1 ax), 2.42-2.34 (2H, m, $OC(O)CH_2$), 2.26 (1H, m, H-2), 2.05 (1H, dd, $J = 4.9$, 14.8 Hz, H-1′), 1.80 (1H, dd, $J = 8.9$, 14.8 Hz, H-1′), 1.63 (2H, m), 1.40–1.20 (20H, m), 0.88 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, CD_3OD: $CDCl_3 = 3:1$) δ 175.3, 170.2, 136.8, 136.0, 130.5, 130.3, 129.9, 129.7, 129.5, 128.6, 81.8, 79.9, 79.1, 78.5, 70.3, 62.4, 60.9, 39.9, 35.4, 33.1, 32.5, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 27.3, 25.9, 23.8, 14.6. HRMS calcd. for $C_{30}H_{49}NO_7Na$ ($M + Na^+$) 558.3407; found 558.3408.

1,5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-4-O-methyl-3-O-myristoyl-D-glucitol (5). Pd/C (8 mg) was added to a solution of **41** (10 mg, 19 μmol) in AcOH (2 mL). The reaction mixture was stirred for 2.5 hr under a balloon of H_2. The reaction mixture was then filtered through Celite and the Celite pad was washed well with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15 : 1) to give **5** (5.3 mg, 64%). ^{1}H NMR (400 MHz, CD_3OD) δ 4.87 (1H, dd, $J = 8.7$, 10.8 Hz, H-3), 3.93 (1H, dd, $J = 4.8$, 11.6 Hz, H-1 eq), 3.79 (1H, dd, $J = 2.0$, 11.9 Hz, H-6), 3.65 (1H, dd, $J = 4.2$, 11.9 Hz, H-6), 3.41 (3H, s, OMe), 3.28 (3H, m, H-4, H-5, H-1 ax), 2.48–2.36 (2H, m, $OC(O)CH_2$), 2.28 (1H, m, H-2), 2.10 (1H, $J = 5.0$, 14.5 Hz, H-1′), 1.84 (1H, dd, $J = 9.0$, 14.5 Hz, H-1′), 1.70 (2H, m), 1.40–1.30 (20H, m), 0.90 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.3, 170.3, 81.9, 79.9, 78.5,

70.3, 62.3, 60.7, 40.0, 35.2, 33.1, 32.5, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 25.9, 23.7, 14.4. HRMS calcd. for $C_{23}H_{43}NO_7Na$ $(M + Na^+)$ 468.2937; found 468.2934.

1,5-Anhydro-4,6-O-benzylidene-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-3-O-tetradecane-D-glucitol (42). Compound **11** (40 mg, 0.10 mmol) was dissolved in DMF (1 mL); sodium hydride (60% in oil, 10 mg, 0.25 mmol) and 1-bromotetradecane (89 μL, 0.30 mmol) were then added. The reaction mixture was stirred at rt for 5 hr. (TLC: EtOAc/toluene, 1:1 showed some starting material was still left). The mixture was diluted with CH_2Cl_2 (10 mL), washed with diluted HCl (5 mL), and sat. $NaHCO_3$, dried over Na_2SO_4, and concentrated, and the residue was purified by chromatography (EtOA/toluene, 3:1) to give **42** (40 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (1H, br s, NH), 7.50–7.30 (10H, m), 5.54 (1H, s, CHPh), 4.88 (2H, br s, OCH_2Ph), 4.27 (1H, dd, $J = 4.9$, 10.4 Hz, H-6), 4.01 (1H, dd, $J = 4.9$, 11.5 Hz, H-1 eq), 3.90 (1H, dt, $J = 6.7$, 9.3 Hz, OCH_2), 3.70 (1H, t, $J = 10.4$ Hz, H-6), 3.56 (1H, t, $J = 8.9$ Hz, H-4), 3.48 (1H, m, OCH_2), 3.36–3.30 (2H, m, H-5, H-1 ax), 3.26 (1H, dd, $J = 8.9$, 10.3 Hz, H-3), 2.40 (1H, br s, H-1′), 2.18 (1H, br s, H-2), 1.95 (1H, br s, H-1′), 1.50 (2H, m), 1.33–1.18 (20H, m), 0.89 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 137.5, 129.1, 128.8, 128.6, 128.1, 125.9, 101.1, 83.8, 80.5, 72.9, 71.7, 70.5, 68.9, 31.9, 30.2, 29.7, 29.6, 29.4, 29.3, 26.1, 22.6, 14.1. HRMS calcd. for $C_{36}H_{54}NO_6$ $(M + H^+)$ 596.3951; found 596.3953.

1,5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-3-O-tetradecane-D-glucitol (6). Pd-C (20 mg) was added to a solution of **42** (30 mg, 52 μmol) in AcOH (2 mL). The reaction mixture was stirred for 6 hr under a balloon of H_2. After the filtration, the solvent was evaporated and the residue was purified on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15:1) to give **6** (13 mg, 70%). ^{1}H NMR (400 MHz, CD_3OD) δ 3.92–3.85 (2H, m, H-1 eq, OCH_2), 3.80 (1H, dd, $J = 2.3$, 11.8 Hz, H-6), 3.59 (1H, dd, $J = 6.1$, 11.8 Hz, H-6), 3.53 (1H, m, OCH_2), 3.33 (1H, d, $J = 10.0$ Hz, H-4), 3.14 (1H, t, $J = 11.4$ H-1 ax), 3.12 (1H, m, H-5), 3.00 (1H, dd, $J = 8.7$, 10.0 Hz, H-3), 2.46 (1H, dd, $J = 4.0$, 14.3 Hz, H-1′), 2.05 (1H, m, H-2), 1.84 (H, dd, $J = 9.5$, 14.3 Hz, H-1′), 1.56 (2H, m), 1.40–1.20 (20H, m), 0.85 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 171.2, 85.9, 82.9, 73.8, 73.1, 70.5, 63.1, 33.1, 32.6, 31.4, 30.8, 30.7, 30.5, 27.2, 23.7, 14.4. HRMS calcd. for $C_{22}H_{43}NO_6Na$ $(M + Na^+)$ 440.2988; found 440.2990.

1,5-Anhydro-3-O-benzyol-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-D-glucitol (7). To a solution of 1,5-Anhydro-4, 6-O-benzylidene-2-deoxy-2-C-(2-propenyl)-D-glucitol[6b] (Sch. 8, 50 mg, 0.18 mmol) in pyridine (2 mL), benzoyl chloride (0.13 mL, 1.08 mmol) was added at 0°C, and the mixture was stirred for 3 hr at rt. EtOAc (30 mL) was added to the reaction mixture and the solution was washed with diluted HCl (5 mL), sat. $NaHCO_3$ (5 mL), and brine (5 mL), and

dried over Na_2SO_4 and the residue was purified by chromatography (EtOAc/hexane, 1 : 7) to give 63 mg (95%).

Ozone was passed through a solution of above compound (30 mg, 82 μmol) in CH_2Cl_2 (10 mL) at −78°C until the solution turned blue. The excess ozone was removed with a stream of oxygen for 10 min, followed by addition of Ph_3P (53 mg, 0.20 mmol). The mixture was allowed to warm to rt for 2 hr, and then concentrated. The residue was purified by chromatography (EtOAc/hexane, 1 : 3) to afford the aldehyde (26 mg, 87%).

To a solution of above compound (26 mg, 70 μmol) in *t*-BuOH (2 mL) and 2-methyl-2-butene (0.6 mL) was added a solution of $NaClO_2$ (91 mg, 0.80 mmol) and NaH_2PO_4 (138 mg, 0.80 mmol) in water (1.5 mL) over 5 min. The mixture was stirred for 1 hr, then diluted with ice water and extracted with EtOAc. The combined organic extracts were washed with brine (5 mL), dried over Na_2SO_4, evaporated, and further dried under high vacuum overnight to afford the acid (27 mg, 100%).

To a solution of the acid (27 mg, 70 μmol) and $BnONH_2 \cdot HCl$ (13 mg, 80 μmol) in CH_2Cl_2 (0.5 mL) were added successively EDC (19 mg, 0.10 mmol) and triethylamine (13 μL, 0.10 mmol) at rt. The reaction mixture was stirred for 2 hr. CH_2Cl_2 (10 mL) was then added and the solution was washed with water (5 mL) and brine (5 mL), and then dried over Na_2SO_4. The residue after concentration was purified by chromatography (toluene/EtOAc, 4 : 1) to afford hydroxamide 24 mg (70%).

Pd-C (10 mg) was added to a solution of the above hydroxyamide (10 mg, 52 μmol) in AcOH (2 mL). The reaction mixture was stirred for 6 hr under a balloon of H_2. After the filtration, the solvent was evaporated and the residue was purified on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15 : 1) to give **7** (4 mg, 62%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.10–7.48 (5H, COC_6H_5), 5.02 (1H, dd, $J = 9.0$, 10.8 Hz, H-3), 4.03 (1H, dd, $J = 4.7$, 11.6 Hz, H-1 eq), 3.85 (1H, dd, $J = 2.4$, 11.9 Hz, H-6), 3.67 (1H, dd, $J = 5.7$, 11.9 Hz, H-6), 3.58 (1H, t, $J = 9.0$ Hz, H-4), 3.30 (2H, m, H-5 and H-1 ax), 2.42 (1H, m, H-2), 2.20 (1H, dd, $J = 4.2$, 14.6 Hz, H-1′), 1.91 (1H, dd, $J = 9.8$, 14.6 Hz, H-1′). HRMS calcd. for $C_{15}H_{19}NO_7Na$ 348.1059; found 348.1057.

1,5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-3-O-palmitoyl-D-glucitol (8). To a solution of **11** (20 mg, 50 μmol) in pyridine (0.5 mL), DMAP (3 mg, 30 μmol) and palmitoyl chloride (30 μL, 0.10 mmol) were successively added at 0°C and stirred for 20 hr at rt. EtOAc (30 mL) was then added to the reaction mixture and the solution was washed with 10% citric acid (5 mL × 3), H_2O (5 mL), sat. $NaHCO_3$ (5 mL), and brine (5 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by chromatography (toluene/EtOAc, 4 : 1) to afford 18 mg (55%).

Pd-C (10 mg) was added to a solution of above compound (10 mg, 16 μmol) in AcOH (2 mL). The reaction mixture was stirred for 6 hr under a balloon of

H_2. After the filtration through Celite, the Celite pad was washed well with MeOH. The solvent was evaporated and the residue was purified by gravity chromatography on an Iatrobeads SiO_2 column ($CHCl_3$/MeOH, 15 : 1) to give **8** (5 mg, 70%). ^{1}H NMR (400 MHz, CD_3OD) δ 4.75 (1H, dd, $J = 9.0$, 10.8 Hz, H-3), 3.94 (1H, dd, $J = 4.7$, 11.6 Hz, H-1 eq), 3.80 (1H, dd, $J = 2.3$, 11.8 Hz, H-6), 3.62 (1H, dd, $J = 5.7$, 11.8 Hz, H-6), 3.38 (1H, t, $J = 9.0$ Hz, H-4), 3.25–3.15 (2H, m, H-5 and H-1 ax), 2.38 (1H, t, $J = 7.5$ Hz, $OC(O)CH_2$), 2.25 (1H, m, H-2), 2.10 (1H, dd, $J = 4.9$, 14.5 Hz, H-1′), 1.82 (1H, dd, $J = 9.1$, 14.5 Hz, H-1′), 1.60 (2H, m), 1.40–1.20 (24H, m), 0.88 (3H, t, $J = 6.7$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 175.7, 170.3, 82.7, 79.1, 70.7, 70.3, 62.9, 39.8, 35.1, 33.1, 32.7, 30.8, 30.7, 30.6, 30.4, 30.2, 25.9, 23.7, 14.4. HRMS calcd. for $C_{24}H_{45}NO_7Na$ 482.3094; found 482.3097.

Inhibitor Assays

LpxC activity assay. The *E. coli* LpxC substrate, [a-^{32}P]-UDP-3-O-(*R*-3-hydroxymyristoyl)-GlcNAc was prepared and purified as described previously by acylation of [a-^{32}P] UDP-GlcNAc using purified *E. coli* LpxA (provided by T. J. O. Wyckoff, Duke University).[14] The assays were performed at 30°C and contained 4 μM UDP-3-O-(*R*-3-hydroxymyristoyl)GlcNAc, 1 mg/mL BSA in 25 mM sodium phosphate, pH 7.4, and 0.1 nM purified *E. coil* LpxC. The activity assays were performed in plastic microcentrifuge tubes in a reaction volume of 20 μL. At each time point (chosen so that the total conversion to product was less than 10%), 5 μL portions of each reaction mixture were removed and added to 1 μL of 1.25 M NaOH to stop the reaction. The alkaline samples were incubated for an additional 10 min at 30°C to ensure complete hydrolysis of the ester-linked acyl chains from the LpxC substrate and product, and then were neutralized by the addition of 1 μL of 1.25 M acetic acid and 1 μL of 5% trichloroacetic acid. The neutralized samples were incubated on ice for 5 min and centrifuged for 2 min in a microcentrifuge. Portions of the supernatants (1 μL) were spotted onto PEI-cellulose TLC plates for separation of the remaining substrate (detected as [a-^{32}P]-UDP-GlcNAc) from the product (detected as [a-^{32}P]-UDP-GlcN). After air drying, the plates were soaked for 10 min in methanol to improve resolution before chromatography. The plates were developed with 0.2 M aqueous guanidine-HCl as the solvent system. The radioactive spots on the plates were analyzed using a PhosphorImager equipped with ImageQuant software (Molecular Dynamics, Inc.) to determine the yields of product produced in each reaction mixture.

Inhibition of LpxC activity. Stock solutions (10 mg/mL) and any further dilutions of each inhibitor were made in 100% dimethylsulfoxide (DMSO). Compounds were added to a final concentration of 1 μg/mL to an assay mixture containing 4 μM UDP-3-O-(*R*-3-hydroxymyristoyl)GlcNAc in 25 mM

sodium phosphate buffer, pH 7.4. Additional DMSO was added to maintain compound solubility at a final assay concentration of 10% in DMSO. Purified *E. coli* LpxC was incubated with 1 mg/mL BSA in buffer and diluted into the final assay mixture to 0.1 nM at 30°C. The initial velocities were plotted as a function of inhibitor concentration for each compound. The percent inhibition of each compound at 1 μg/mL was calculated based on comparison of the specific activity of a reaction containing inhibitor to a wild-type reaction containing no inhibitor and substituting DMSO instead.

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