# Synthesis of a carbohydrate-derived hydroxamic acid inhibitor of the bacterial enzyme (LpxC) involved in lipid A biosynthesis

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## SUPPORTING INFORMATION

**1, 6-Anhydro-4-O-benzyl-2-deoxy-2-C- (2-propenyl)-β-D-glucopyranose (2).**
To a solution of "Cerny's epoxide" **1** (1.01 g, 4.30 mmol) in THF (10 ml), allylmagnesium bromide (1.0 M in $Et_2O$, 12.8 ml, 12.8 mmol) was added slowly at room temperature and the reaction mixture was gently refluxed at 60 °C for 2 h. After cooling to room temperature, sat. $NH_4Cl$ (10 ml) was slowly added and the mixture was extracted with EtOAc (30 ml x 3). The combined organic layer was washed with brine (10 ml x 2), dried over $Na_2SO_4$ and concentrated. The residue was purified by chromatography (toluene /EtOAc, 8:1) to afford **2** (931 mg, 79%).
$^1$H NMR (300 MHz, CDCl3) δ 7.40 (5H, m), 5.79 (1H, m, H-2'), 5.45 (1H, br s, H-1), 5.12-5.05 (2H, m, H-3'), 4.62 (2H, q, *J* = 12.0 Hz, $OCH_2Ph$), 4.58 (1H, m, H-5), 4.05 (1H, dd, *J* = 0.7, 7.5 Hz, H-6), 3.70- 3.64 (2H, m, H-3 and H-6), 3.40 (1H, s, H-4), 2.30 (2H, m, H-1'), 1.79 (1H, br t, *J* = 7.0 Hz, H-2)
$^{13}$C NMR (100 MHz, CDCl3) δ 137.9, 135.8, 128.4, 127.8, 127.6, 117.2, 103.4, 78.9, 74.9, 71.4, 69.1, 65.5, 45.7, 33.5
HRMS calcd for $C_{16}H_{20}O_4Na$ 299.1259, found 299.1259

**3, 6-Di-O-acetyl-1, 5-anhydro-4-O-benzyl-2-deoxy-2-C-(2-propenyl)-D-glucitol (3).**
A solution of **2** (760 mg, 2.75 mmol) in dry $CH_2Cl_2$ (50 ml) was cooled to 0 °C under argon. $Et_3SiH$ (2.20 ml, 13.8 mmol) was added followed by $SnCl_4$ (0.49 ml, 4.13 mmol) at 0 °C and the mixture was stirred for 1 h at room temperature. The reaction mixture was poured into cold sat. $NaHCO_3$ (20 ml) and the aqueous layer was extracted with $CH_2Cl_2$ (20 ml x 3) at once. The combined organic layer was washed with brine (5 ml), dried over $Na_2SO_4$ and concentrated. The residue was directly acetylated using $Ac_2O$ (4 ml) and pyridine (5 ml) for overnight. After removing the solvent, the residue was purified by chromatography (toluene/EtOAc, 9:1) to afford **3** (877 mg, 88%).
$^1$H NMR (400 MHz, $CDCl_3$) δ 7.23 (5H, m), 5.64 (1H, m, H-2'), 4.95 (3H, m, H-3' and H-3), 4.51 (2H, q, *J* = 11.1 Hz, $OCH_2Ph$), 4.28 (1H, d, *J* = 11.9 Hz, H-6), 4.16 (1H, dd, *J* = 4.1, 11.9 Hz, H-6), 3.88 (1H, dd, *J* = 4.5, 11.5 Hz, H-1eq), 3.40 (2H, m, H-4 and H-5), 3.30 (1H, t, *J* = 11.5 Hz H-1ax), 2.10 (1H, m, H-1'), 2.00 (3H, s, OAc), 1.94 (3H, s, OAc), 1.88-1.80(2H, m, H-1' and H-2);
$^{13}$C NMR (100 MHz, $CDCl_3$) δ 170.5, 170.2, 137.6, 134.8, 128.4, 127.8, 127.8, 116.9, 77.7, 77.6, 76.9, 74.5, 69.8, 63.4, 41.0, 32.2, 20.9, 20.8
HRMS calcd for $C_{20}H_{26}O_6Na$ 385.1622, found 385.1613

**3, 6-Di-O-acetyl-1, 5-anhydro-4-O-benzoyl-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-D-glucitol (4).**

To a solution of **3** (150 mg, 0.41 mmol) in $CCl_4/CH_3CN/H_2O$ (1:1:1, 15 ml), $NaIO_4$ (443 mg, 0.41 mmol) and $RuCl_3 \cdot H_2O$ (10 mg, 0.05 mmol) were successively added at room temperature. The reaction mixture was stirred vigorously for 2 h and then extracted with $CH_2Cl_2$ (15 ml x 4). The combined organic layers were dried over $Na_2SO_4$ and concentrated to afford crude acid (140 mg, 86%). This crude product was used directly in the next step. To a solution of the crude product and $BnONH_2 \cdot HCl$ (57 mg, 0.36 mmol) in $CH_2Cl_2$ (4 ml), EDAC·HCl (82 mg, 0.43 mmol) and triethylamine (60 μl, 0.43 mmol) were successively added at room temperature and stirred for 3 h. $CH_2Cl_2$ (15 ml) was added and the solution was washed with 1N HCl (5 ml), $H_2O$ (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, 3:2) to afford **4** (157 mg, 76%).

$^1$H NMR (400 MHz, $CD_3OD$) δ 7.99-7.25 (10H, m), 5.12 (2H, m, H-3 and H-4), 4.80 (2H, m, $OCH_2Ph$), 4.15 (1H, dd, *J* = 4.9, 12.2 Hz, H-6), 4.08 (1H, dd, *J* = 2.8, 12.2 Hz, H-6), 3.96 (1H, dd, *J* = 4.8, 11.5 Hz, H-1eq), 3,74 (1H, m, H-5), 3.14 (1H, t, *J* = 11.5 Hz, H-1ax), 2.40 (1H, m, H-2), 2.18 (1H, dd, *J* = 5.0, 14.8 Hz, H-1'), 1.98-1.96 (4H, s, OAc and H-1'), 1.82 (3H, s, OAc)

$^{13}$C NMR (100 MHz, $CDCl_3$) δ 171. 1, 170.7, 167.8, 165.4, 135.3, 133.5, 129.3, 128.8, 125.2, 78.1, 76.5, 74.9, 70.5, 69.4, 63.1, 38.0, 31.4, 21.4, 20.6, 20.6

HRMS calcd for $C_{26}H_{29}NO_9Na$ 522.1740, found 522.1745

**1, 5-Anhydro-4, 6-O-benzylidene-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-D-glucitol (6).**

To a solution of **4** (120 mg, 0.24 mmol) in MeOH (5 ml), NaOMe (catalytic) was added at room temperature and stirred for 3 h. The reaction mixture was then neutralized using Amberlite IR-120 ($H^+$), filtered and concentrated. The solid residue was directly used in the next step. To a suspension of the crude product in $CH_3CN$ (30 ml), benzaldehyde dimethylacetal (54 μl, 0.36 mmol) and $TsOH \cdot H_2O$ (7 mg, 0.04 mmol) were successively added at room temperature. The suspension could become clear within 15 minutes. After 3 h, triethylamine (100 μl) was added to the reaction mixture at 0 °C and the solvent was removed by evaporation. The residue was purified by chromatography (toluene/EtOAc, 1:1) to give **6** (72 mg, 64%).

$^1$H NMR (500 MHz, $CDCl_3$: $CD_3OD$ = 2:1) δ 7.38-7.10 (10H, m), 5.40 (1H, s, CHPh), 4.68 (2H, s, $CH_2Ph$), 4.08 (1H, dd, *J* = 4.9, 10.3 Hz, H-6), 3.78 (1H, dd, *J* = 4.8, 11.5 Hz, H-1eq), 3.59 (1H, t, *J* = 10.3 Hz, H-6), 3.28 (2H, m, H-3 and H-4), 3.16 (1H, m, H-5), 3.10 (1H, t, *J* =11.5 Hz, H-1ax), 2.28 (1H, dd, *J* = 4.5, 12.7 Hz, H-1'), 1.98 (1H, m, H-2), 1.72 (1H, dd, *J* = 8.2, 12.7 Hz, H-1')

$^{13}$C NMR (125 MHz, $CDCl_3$: $CD_3OD$ = 2:1) δ 169.3, 137.0, 135.1, 128.9, 128. 8, 128.4, 128.2, 125.9, 101.7, 82.9, 77.8, 72.6, 71.5, 70.1, 68.6, 40.4, 31.6

HRMS calcd for $C_{22}H_{25}NO_6Na$ 422.1580, found 422.1581

**1, 5-Anhydro-4, 6-O-benzylidene-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-3-O-myristoyl-D-glucitol (7).**

To a solution of **6** (20 mg, 0.05 mmol) in pyridine (0.5 ml), DMAP (3 mg, 0.03 mmol) and myristoyl chloride (27 μl, 0.10 mmol) were successively added at 0 °C and stirred for 18 h at room temperature. EtOAc (30 ml) was then added to the reaction mixture and the solution was washed with 10% citric acid (5 ml x 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 **7** (16 mg, 52%).
$^1$H NMR (400 MHz, $CD_3OD$: $CDCl_3$ = 2:1) δ 7.40-7.24 (10H, m), 5.58 (1H, s, CHPh), 5.01 (1H, dd, *J* = 9.4, 10.6 Hz, H-3), 4.82 (2H, m, $OCH_2Ph$), 4.21 (1H, dd, *J* = 4.9, 10.4 Hz, H-6), 3.92 (1H, dd, *J* = 4.9, 11.5 Hz, H-1eq), 3.72 (1H, t, *J* = 10.4 Hz, H-6), 3.62 (1H, t, *J* = 9.4 Hz, H-4), 3.41 (1H, m, H-5), 3.32 (1H, t, *J* = 11.5 Hz, H-1ax), 2.40 (1H, m, H-2), 2.33 (2H, t, *J* = 7.3 Hz, $OC(O)CH_2$), 2.14 (1H, dd, *J* = 4.8, 14.7 Hz, H-1'), 1.88 (1H, dd, *J* = 14.7, 9.0 Hz, H-1'), 1.56 (2H, m), 1.24 (20H, m), 0.89 (3H, t, *J* = 6.8 Hz)
$^{13}$C NMR (100 MHz, $CD_3OD$: $CDCl_3$ = 2:1) δ 175.3, 170.1, 138.9, 136.9, 130.4, 129.9, 129.8, 129.6, 129.1, 127.3, 102.7, 81.9, 74.8, 73.1, 71.1, 69.8, 39.9, 35.3, 33.1, 32.5, 30.8, 30.8, 30.6, 30.5, 30.1, 26.2, 23.8, 14.6
HRMS calcd for $C_{36}H_{51}NO_7Na$ 632.3563, found 632.3568

**1, 6-Anhydro-4-O-(*tert*-butyldimethyl) silyl-2-deoxy-2-C-(2-propenyl)-β-D-glucopyranose (10).**
To a solution of **9** (670 mg, 3.6 mmol) and imidazole (980 mg, 14.4 mmol) at 0 °C in DMF (12 ml) was added *tert*-butyldimethylsiyl chloride (651 mg, 4.34 mmol). The solution was allowed to slowly warm to room temperature overnight (11h). Additional *tert*-butyldimethylsily chloride (100 mg) was then added. After being stirred for an additional 1 h, the solution was poured into an Erlenmeyer flask, diluted with 25 ml saturated $NaHCO_3$, 25 ml water, and 50 ml $Et_2O$ and then stirred vigorously for 15 min. The layers were separated and the aqueous layer was extracted with $Et_2O$ (30 ml x 3). The combined organic layers were washed with brine (10 ml), dried over $Na_2SO_4$, filtered, concentrated and purified by chromatography (EtOAc/hexane, 1:10) to yield **10** (875 mg, 81%).
$^1$H NMR (400 MHz, $CDCl_3$) δ 5.80 (1H, m, H-2'), 5.45 (1H, br s, H-1), 5.13-5.05(2H, m, H-3'), 4.36 (1H, d, *J* = 5.3 Hz, H-5), 4.12 (1H, dd, *J* = 0.5, 7.5 Hz, H-6), 3.70-3.65 (2H, m, H-6 and H-4), 3.51 (1H, m, H-3), 2.30 (2H, m, H-1'), 1.78 (1H, br t, *J* = 7.0 Hz, H-2), 0.92 (9H, s), 0.10 (6H, s)
$^{13}$C NMR (100 MHz, $CDCl_3$) δ 136.0, 116. 8, 103.5, 77.4, 73.0, 72.3, 65.2, 45.8, 33.4, 25.7, 18.0, -4.9, -5.0
HRMS calcd for $C_{15}H_{28}O_4SiNa$ 323.1655, found 323.1653

**1, 5-Anhydro-4-O-(*tert*-butyldimethyl) silyl-2-deoxy-2-C-(2-propenyl)-D-glucitol(11).**
To a solution of **10** (1.00 g, 3.33 mmol) and $Et_3SiH$ (2.10 ml, 13.2 mmol) in $CH_2Cl_2$ (50 ml), $TiCl_4$ (3.95 ml, 1.0 M in $CH_2Cl_2$, 3.95 mmol) was added dropwise at – 78 °C, allowing the reaction to warm to – 15 °C over 2 h. The reaction was poured into cold sat. $NaHCO_3$ (20 ml) and extracted with $CH_2Cl_2$ (20 ml x 2). The combined organic layers were washed with brine (10 ml), and dried with $Na_2SO_4$. The residue was purified by chromatography (EtOAc/hexane, 1:7) to afford **11** (800 mg, 80 %).

$^1$H NMR (400 MHz, $CDCl_3$) δ 5.78 (1H, m, H-2'), 5.09-4.99 (2H, m, H-3'), 3.91 (1H, dd, *J* = 4.8, 11.7 Hz, H-1eq), 3.82 (1H, dd, *J* = 2.7, 11.7 Hz, H-6), 3.63 (1H, dd, *J* = 5.6, 11.7 Hz, H-6), 3.49 (1H, t, *J* = 9.0 Hz, H-4), 3.27 (1H, *J* = 9.0 Hz, H-3), 3.17 (2H, m, H-5 and H-1 ax), 2.54 (1H, m, H-1'), 1.96 (1H, m, H-2), 1.76 (1H, m, H-1'), 0.90 (9H, s), 0.06 (6H, s)
$^{13}$C NMR (100 MHz, $CDCl_3$) δ 135.7, 116.7, 80.6, 77.6, 73.3, 69.9, 62.3, 41.9, 32.6, 25.9, 18. 2, -3.9, - 4.8
HRMS calcd for $C_{15}H_{30}O_4SiNa$ 325.1811, found 325.1816

**1, 5-Anhydro-2-deoxy-2-C-(2-propenyl)-D-glucitol (12).**
A solution of **11** (1.00 g, 3.31 mmol) at room temperature in THF (40 ml) was treated with TBAF (1.0 M solution in THF, 8.50 ml, 8.50 mmol) under Argon. After 2.5 h, the reaction was concentrated and purified directly by chromatography ($CH_2Cl_2$ /MeOH, 15:1).
Note: it is difficult to remove the residue of TBAF from the product, so it was acetylated with $Ac_2O$ and pyridine and purified by chromatography (E tOAc /hexane, 1:2). Deacetylation with NaOMe in MeOH then gave pure **12** (623 mg, 94 %).
$^1$H NMR (400 MHz, $CD_3OD$) δ 5.80 (1H, m, H-2'), 5.05-4.95 (2H, m, H-3'), 3.87 (1H, dd, *J* = 4.7, 11.7 Hz, H-1eq), 3.80 (1H, dd, *J* = 2.3, 12.5 Hz, H-6), 3.58 (1H, dd, *J* = 5.7, 12.5 Hz, H-6), 3.21-3.05 (4H, m, H-3, H-4, H-5, and H-1ax), 2.51 (1H, m, H-1'), 1.85 (1H, m, H-2), 1.65 (1H, m, H-1')
$^{13}$C NMR (100 MHz, $CD_3OD$) δ 137.3, 116.9, 82.6, 77.9, 73.3, 70.8, 43.3, 33.4
HRMS calcd for $C_9H_{16}O_4Na$ 211.0946, found 211.0944

**1, 5-Anhydro-4, 6-O-benzylidene-2-deoxy-2-C-(2-propenyl)-D-glucitol (13).**
To a solution of **12** (580 mg, 3.09 mmol) in dry $CH_3CN$ (40 ml), benzaldehyde dimethylacetal (0.70 ml, 4.67 mmol) and $TsOH{\cdot}H_2O$ (118 mg, 0.62 mmol) were successively added at room temperature. After 3 h, triethylamine (2.0 ml) was added to the reaction mixture at 0 °C and the solvent was removed by evaporation. The residue was purified by chromatography (EtOAc/hexane, 1:5) to afford **13** (770 mg, 90%).
$^1$H NMR (400 MHz, $CDCl_3$) δ 7.42-7.38 (5H, m), 5.82 (1H, m, H-2'), 5.52 (1H, s), 5.14-5.00 (2H, m, H-3'), 4.27 (1H, dd, *J*= 5.0, 10.3 Hz, H-6), 3.95 (1H, dd, *J* = 4.6, 11.4 Hz, H-1eq), 3.69 (1H, t, *J* =10.3 Hz, H-6), 3.55 (1H, t, *J* = 9.0, Hz, H-3), 3.46 (1H, t, *J* = 9.0 Hz, H-4), 3.34 (1H, m, H-5), 3.23 (1H, t, *J* = 11.4 Hz, H-1ax), 2.59-2.50 (1H, m, H-1'), 2.00-1.85 (2H, m, H-2 and H-1')
$^{13}$C NMR (100 MHz, $CDCl_3$) δ 137.3, 135.3, 129.2, 128.3, 128. 3, 126.2, 126.2, 117.0, 101.9, 83.3, 73.0, 71.2, 70.5, 68.9, 42.1, 32.3
HRMS calcd for $C_{16}H_{20}O_4Na$ 299.1259, found 299.1259

**1, 5-Anhydro-4, 6-O-benzylidene-2-deoxy-2-C-(2-propenyl)-3-O-myristoyl-D-glucitol (14).**
To a solution of **13** (760 mg, 2.75 mmol) in pyridine (9.0 ml), myristoyl chloride (1.85 ml, 6.81 mmol) was added at 0 °C, and the mixture was stirred for 3 h at room temperature. EtOAc (30 ml) was added to the reaction mixture and the solution washed

with diluted HCl (5 ml), sat. $NaHCO_3$ (5 ml), and brine (5 ml), dried over $Na_2SO_4$ and the residue was purified by chromatography (EtOAc/hexane, 1:7) to give **14** (1.30 g, 98%).
$^1$H NMR (400 MHz, $CDCl_3$) δ 7.42-7.38 (5H, m), 5.72 (1H, m, H-2'), 5.50 (1H, s), 5.14-5.00 (3H, m, H-3' and H-3), 4.30 (1H, dd, *J* = 4.9, 10.2 Hz, H-6), 3.99 (1H, dd, *J* = 4.9, 11.6 Hz, H-1eq), 3.70 (1H, t, *J* = 10.2 Hz, H-6), 3.56 (1H, t, *J* = 9.3 Hz, H-4), 3.42 (1H, m, H-5), 3.31 (1H, t, *J* = 11.6 Hz, H-1ax), 2.32 (2H, t, *J* = 7.4 Hz, $OC(O)CH_2$), 2.22 (1H, m, H-1'), 2.05 (1H, m, H-2), 1.92 (1H, m, H-1'), 1.60 (2H, m), 1.36-1.12 (20H, m), 0.90 (3H, t, J = 6.7 Hz)
$^{13}$C NMR (100 MHz, $CDCl_3$) δ 173.2, 137.3, 134.6, 128.8, 128.1, 128.1, 126.0, 117.2, 101.3, 80.8, 73.2, 71.8, 70.6, 68.9, 41.0, 34.4, 32.4, 31.9, 29.6, 29.6, 29.6, 29.6, 29.4, 29.3, 29.2, 29.0, 25.1, 22.6, 14.1
HRMS calcd for $C_{30}H_{46}O_5Na$ 509.3243, found 509.3244

**1, 5-Anhydro-4, 6-O-benzylidene-2-C-(carboxymethyl N-benzyloxyamide)-2-deoxy-3-O-myristoyl-D-glucitol (7).**
To a solution of **14** (200mg, 0.41 mmol) in $CCl_4/CH_3CN/H_2O$ (2:2:3, 7ml), $NaIO_4$ (396 mg, 1.85 mmol) and $RuCl_3 \cdot H_2O$ (8 mg, 0.04 mmol) were successively added at room temperature. The reaction mixture was stirred vigorously for 1 h and extracted with $CH_2Cl_2$ (20 ml x 2). The combined organic layers was dried over $Na_2SO_4$ and concentrated to afford the acid. This crude product was directly in the next step. To a solution of the crude acid and $BnONH_2 \cdot HCl$ (56mg, 0.35 mmol) in $CH_2Cl_2$ (3 ml) were added successively EDAC·HCl (87 mg, 0.45 mmol) and triethylamine (63 µl, 0.45 mmol) at room temperature. The reaction mixture was stirred for 2 h. $CH_2Cl_2$ (20 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 to afford **7** (175 mg, 70%.)

**1, 5-Anhydro-2-C-(carboxymethyl N-hydroxyamide)-2-deoxy-3-O-myristoyl-D-glucitol (8).**
Pd-C (20 mg) was added to a solution of **7** (30 mg) in AcOH (2 ml). The reaction mixture was stirred for 6 h 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 **8** (15 mg, 71%).
$^1$H NMR (400 MHz, $CD_3OD$) δ 4.74 (1H, dd, *J* = 8.9, 10.0 Hz, H-3), 3.94 (1H, dd, *J* = 4.7, 11.6 Hz, H-6), 3.81 (1H, dd, *J* = 2.32, 11.8 Hz, H-1eq), 3.62 (1H, dd, *J* = 5.7, 11.6 Hz, H-6), 3.38 (1H, t, *J* = 10.0 Hz, H-4), 3.20 (2H, m, H-5 and H-1ax), 2.38 (1H, t, *J* = 7.4 Hz, $OC(O)CH_2$), 2.24 (1H, m, H-2), 2.10 (1H, dd, *J* = 4.9, 14.5 Hz, H-1'), 1.72 (1H, dd, *J* = 9.1, 14.5 Hz, H-1'), 1.60 (2H, m), 1.30 (20H, 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, 33.0, 32.7, 30.8, 30.7, 30.4, 30.2, 25.9, 23.7, 14.4
HRMS: calcd for $C_{22}H_{41}NO_7Na$ 454. 2780; found 454.2780