

Supporting Information

for

Stereocontrolled Total Synthesis of Muraymycin D₁ Having a Dual Mode of Action against *Mycobacterium tuberculosis*

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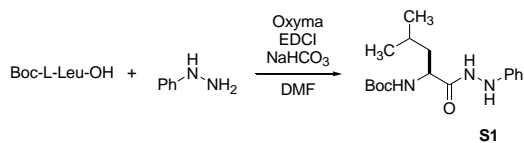
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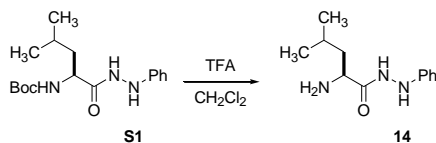
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General

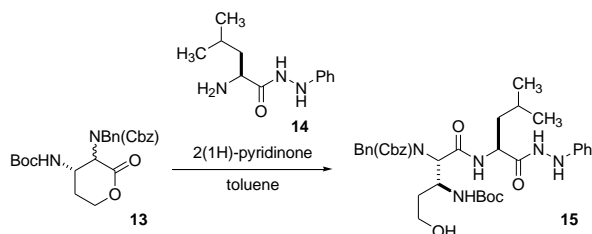
All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. Tetrahydrofuran (THF), methylene chloride (CH_2Cl_2), and DMF were purified via Innovative Technology's Pure-Solve System. All reactions were performed under an Argon atmosphere. All stirring was performed with an internal magnetic stirrer. Reactions were monitored by thin-layer chromatography (TLC) performed with 0.25 mm coated commercial silica gel plates (EMD, Silica Gel 60F₂₅₄) using UV light for visualization at 254 nm, or developed with ceric ammonium molybdate or anisaldehyde or copper sulfate or ninhydrin solutions by heating on a hot plate. Reactions were also monitored by using SHIMADZU LCMS-2020 with solvents: A: 0.1% formic acid in water, B: acetonitrile. And reactions were also monitored by SHIMADZU prominence HPLC using Phenomenex Kinetex 1.7 μ XB-C18 100A column (150 x 2.10 mm) and monitoring at 220, 254 nm with solvents: A: 0.05 M ammonium bicarbonate in water, B: methanol. Flash chromatography was performed with SiliCycle silica gel (Purasil 60 Å, 230-400 Mesh). Proton magnetic resonance (^1H -NMR) spectral data were recorded on 400, and 500 MHz instruments. Carbon magnetic resonance (^{13}C -NMR) spectral data were recorded on 100 and 125 MHz instruments. For all NMR spectra, chemical shifts (δH , δC) were quoted in parts per million (ppm), and J values were quoted in Hz. ^1H and ^{13}C NMR spectra were calibrated with residual undeuterated solvent (CDCl_3 : δH =7.26 ppm, δC =77.16ppm; CD_3CN : δH =1.94ppm, δC =1.32ppm; CD_3OD : δH =3.31ppm, δC =49.00 ppm; DMSO-d_6 : δH =2.50ppm, δC =39.52ppm; D_2O : δH =4.79 ppm) as an internal reference. The following abbreviations were used to designate the multiplicities: s=singlet, d=doublet, dd=double doublets, t=triplet, q=quartet, quin=quintet, hept=heptet, m=multiplet, br=broad. Infrared (IR) spectra were recorded on a Perkin-Elmer FT1600 spectrometer.



To a stirred solution of Boc-L-Leu-OH·H₂O (7.48 g, 30 mmol), phenylhydrazine (4.43 mL, 45 mmol), NaHCO₃ (25.2 g, 300 mmol) and oxyma (3.69 g, 45 mmol) in DMF (60 mL) was added EDCI (28.8 g, 150 mmol). The reaction mixture was stirred for 15 h at rt, quenched with aq. saturated NaHCO₃, extracted with EtOAc. The combined organic extracts were washed with 1M HCl, brine, dried over Na₂SO₄ and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (Hexanes/EtOAc 80:20 to 70:30) to afford **S1** (6.50 g, 20.2 mmol, 67%): TLC (hexanes/EtOAc 67:33) *R_f* = 0.40; [α]²²_D -0.528 (*c* = 0.95, CHCl₃); IR (thin film) *v*_{max} = 3278 (br), 2958, 2934, 2871, 1671, 1603, 1497, 1391, 1366, 1252, 1167, 1046, 1026, 750, 692 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.31 (brs, 1H), 7.20 (dd, *J* = 8.5, 7.3 Hz, 2H), 6.88 (t, *J* = 7.4 Hz, 1H), 6.82 – 6.78 (m, 2H), 6.12 (brs, 1H), 5.00 (d, *J* = 8.4 Hz, 1H), 4.21 (q, *J* = 7.9 Hz, 1H), 1.72 – 1.66 (m, 2H), 1.55 – 1.49 (m, 1H), 1.46 (s, 9H), 0.95 (d, *J* = 6.1 Hz, 3H), 0.93 (d, *J* = 6.1 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 172.54, 147.80, 129.13 (2C), 121.16, 113.55 (2C), 51.53, 40.42, 28.33 (3C), 24.72 (2C), 22.78, 22.10; HRMS (ESI+) *m/z* calcd for C₁₇H₂₇N₃O₃Na [M + Na] 344.1950, found 344.1952.

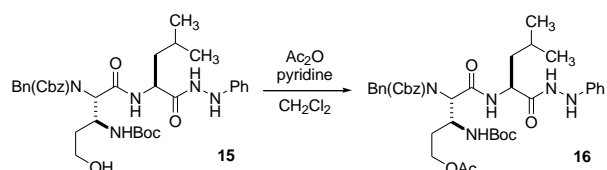


To a stirred solution of **S1** (18.8 g, 58.6 mmol) in CH₂Cl₂ (41 mL) was added TFA (18 mL). The reaction mixture was stirred for 2 h at rt, and all volatiles were evaporated in vacuo to provide **14** as an oil. The crude mixture was used for the next reaction without purification.

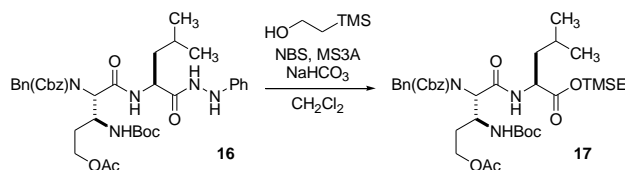


To a stirred solution of **14** and 2-hydroxypyridine (12.6 g, 133 mmol) in toluene (13.3 mL) was added **13**¹ (6.04 g, 13.3 mmol). The reaction mixture was stirred at 70 °C for 3 h and cooled to rt. Purification by silica gel column chromatography (hexanes/EtOAc 50:50 to 40:60) provided **15** (5.01 g, 7.41 mmol, 56%) as a red oil: TLC (hexanes/EtOAc 33:67) *R_f* = 0.40; [α]²²_D -0.081 (*c* = 0.79, CHCl₃); IR (thin film) *v*_{max} = 3292 (br), 3032, 2959, 2872, 1685, 1603, 1497, 1469, 1453, 1366, 1252, 1169, 1055, 752, 734, 695 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.62 (brs, 1H), 7.36 – 7.30 (m, 9H), 7.27 – 7.23 (m, 2H), 7.19 (dd, *J* = 8.5, 7.2 Hz, 4H), 6.86 (t, *J* = 7.3 Hz, 1H), 6.79 (d, *J* = 7.7 Hz, 1H), 6.15 (brs, 1H), 6.07 (brs, 1H), 5.56 (d,

$J = 9.5$ Hz, 1H), 5.18 (d, $J = 12.2$ Hz, 1H), 5.09 (d, $J = 12.2$ Hz, 1H), 4.89 (d, $J = 15.4$ Hz, 1H), 4.43 – 4.36 (m, 1H), 4.35 – 4.29 (m, 1H), 4.26 (d, $J = 15.6$ Hz, 1H), 4.10 (d, $J = 7.1$ Hz, 1H), 1.84 – 1.68 (m, 4H), 1.54 – 1.46 (m, 1H), 1.42 (s, 9H), 0.81 (d, $J = 6.3$ Hz, 3H), 0.76 (d, $J = 6.3$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.42, 169.00, 156.76, 147.98, 135.53, 129.15 (2C), 129.08 (2C), 128.61 (2C), 128.46 (2C), 128.16 (4C), 120.99, 113.61, 80.02, 68.47, 60.39, 58.75, 52.24, 50.77, 47.51, 41.29, 39.96, 34.66, 28.32 (3C), 25.27, 24.49, 22.87, 22.64, 21.40, 20.69, 14.09.; HRMS (ESI+) m/z calcd for $\text{C}_{37}\text{H}_{49}\text{N}_5\text{O}_7\text{Na}$ [$\text{M} + \text{Na}$] 698.3530, found 698.3544.

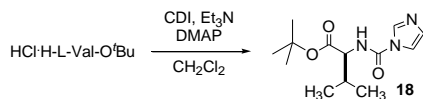


To a stirred solution of **15** (5.01 g, 7.41 mmol) in CH_2Cl_2 (15 mL) were added pyridine (3.0 mL, 37.0 mmol) and acetic anhydride (3.5 mL, 37.0 mmol). The reaction mixture was stirred for 3 h at rt, and all volatiles were evaporated in vacuo. Purification by silica gel column chromatography (hexanes/EtOAc 60:40 to 50:50) gave **16** (3.66 g, 5.10 mmol, 69%) as a brown foam: TLC (hexanes/EtOAc 50:50) $R_f = 0.50$; $[\alpha]_{\text{D}}^{22} +0.095$ ($c = 0.81$, CHCl_3); IR (thin film) $\nu_{\text{max}} = 3297$ (br), 2959, 1693, 1603, 1497, 1454, 1366, 1246, 1168, 1046, 753, 733, 695 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 8.51 (brs, 1H), 7.38 – 7.31 (m, 9H), 7.31 – 7.27 (m, 2H), 7.23 – 7.17 (m, 4H), 6.87 (t, $J = 7.4$ Hz, 1H), 6.79 (d, $J = 8.0$ Hz, 1H), 6.01 (brs, 1H), 5.80 (brs, 1H), 5.17 – 5.10 (m, 2H), 5.05 – 4.98 (m, 2H), 4.24 (d, $J = 15.4$ Hz, 1H), 4.14 – 4.10 (m, 2H), 4.06 (d, $J = 7.1$ Hz, 1H), 2.04 (s, 3H), 1.74 – 1.62 (m, 4H), 1.42 (s, 9H), 1.11 – 1.06 (m, 1H), 0.80 (d, $J = 6.2$ Hz, 3H), 0.75 (d, $J = 6.1$ Hz, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.27, 171.13, 168.90, 155.62, 148.06, 135.64, 129.25 (2C), 129.08 (2C), 128.64 (2C), 128.48 (2C), 128.28 (2C), 128.15 (2C), 121.00, 113.61, 79.75, 68.44, 63.17, 61.63, 60.41, 51.80, 50.66, 48.33, 41.30, 39.75, 30.01, 28.33 (3C), 24.48, 22.86, 21.30, 20.99; HRMS (ESI+) m/z calcd for $\text{C}_{39}\text{H}_{51}\text{N}_5\text{O}_8\text{Na}$ [$\text{M} + \text{Na}$] 740.3635, found 740.3566.

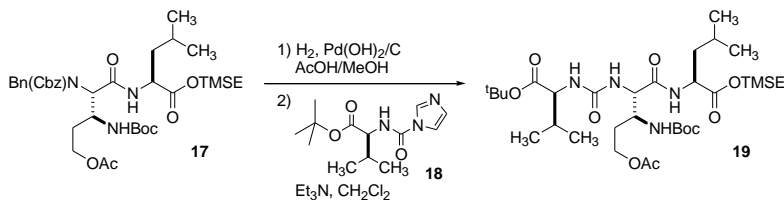


To a stirred suspension of **16** (3.42 g, 4.76 mmol), MS3A (6.84 g), NaHCO_3 (2.00 g, 23.8 mmol) and 2-(trimethylsilyl)ethanol (2.05 mL, 14.3 mmol) was added NBS (2.54 g, 14.3 mmol) at rt. The reaction mixture was stirred for 1 h, diluted with CH_2Cl_2 , filtered through Celite. The combined organic phase was washed with 1M HCl (aq), saturated NaHCO_3 (aq) and brine and dried over Na_2SO_4 , and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography to give **17** as white foam (2.75 g, 3.78 mmol, 79%): TLC (hexanes/EtOAc 67:33) $R_f = 0.40$; $[\alpha]_{\text{D}}^{20} +0.140$ ($c = 1.11$, CHCl_3); IR (thin film) $\nu_{\text{max}} = 3339$ (br), 2957, 1740, 1718, 1675, 1522, 1453, 1366, 1249, 1174, 1045, 861, 838, 696 cm^{-1} ; ^1H NMR (400 MHz, Chloroform- d) δ 7.48 – 7.35 (m, 2H), 7.35 – 7.27 (m, 4H), 7.22 (brs, 4H), 5.18 (brs, 2H), 4.66 – 4.59 (m, 1H), 4.53 (d, $J = 30.7$ Hz, 1H), 4.46 – 4.38 (m, 1H), 4.36 – 4.23 (m, 3H), 4.19 – 4.02 (m, 5H), 2.03 (s, 3H), 1.69 (brs, 4H), 1.57 – 1.46 (m, 1H),

1.39 (s, 9H), 1.00 – 0.92 (m, 3H), 0.85 – 0.76 (m, 3H), 0.04 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.32, 171.01, 168.25, 155.44, 135.94, 128.87 (2C), 128.49 (2C), 128.13 (2C), 127.84 (2C), 79.47, 68.06, 63.94, 63.55, 61.34, 50.79, 41.35, 30.76, 28.31 (3C), 24.77, 22.73, 21.91, 20.94, 17.35, -1.54 (3C); HRMS (ESI+) m/z calcd for $\text{C}_{38}\text{H}_{57}\text{N}_3\text{O}_9\text{NaSi}$ [$\text{M} + \text{Na}$] 750.3762, found 750.3814.

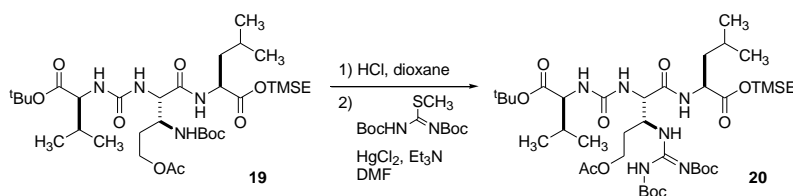


To a stirred suspension of the $\text{HCl}\cdot\text{H-L-Val-O}^t\text{Bu}$ (1.50 g, 7.15 mmol) in CH_2Cl_2 (21 mL) were added Et_3N (2.19 mL, 15.7 mmol) and DMAP (87.4 mg, 0.72 mmol), and N,N -carbonyldiimidazole (1.28 g, 7.87 mmol) was added at $0\text{ }^\circ\text{C}$. The reaction mixture was warmed to rt and stirred for 2 h. The reaction mixture was diluted with CH_2Cl_2 , and the combined organic phase was washed with H_2O and brine and dried over Na_2SO_4 . The crude material was purified by basic alumina column chromatography to give **18** as colorless oil (1.66 g, 6.22 mmol, 87%): TLC ($\text{CHCl}_3/\text{MeOH}$ 90:10) $R_f = 0.25$; ^1H NMR (500 MHz, CDCl_3) δ 8.19 (s, 1H), 7.43 (s, 1H), 6.75 (d, $J = 8.0$ Hz, 1H), 4.59 (m, 1H), 3.81 (s, 3H), 2.28 (m, 1H), 1.01 (m, 6H); ^{13}C NMR (125 MHz, CDCl_3) δ 172.1, 148.9, 136.1, 130.7, 115.9, 58.8, 52.6, 31.4, 18.9, 17.9; HRMS (ESI+) m/z calcd for $\text{C}_{13}\text{H}_{22}\text{N}_3\text{O}_3$ [$\text{M} + \text{H}$] 268.1661, found 268.1658.

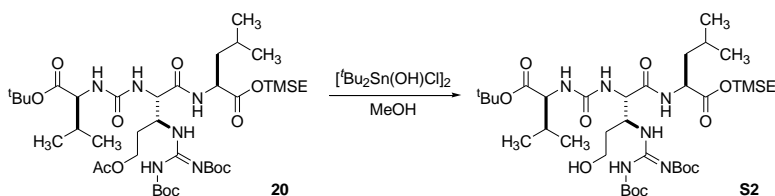


To a stirred solution of **17** (1.73 g, 2.38 mmol) in MeOH (100 mL) were added AcOH (0.2 mL) and $\text{Pd}(\text{OH})_2/\text{C}$ (20 wt % 0.17 g) under N_2 . H_2 gas was introduced via a double-folded balloon, and the reaction mixture was stirred for 21 h under H_2 . Upon completion, the solution was filtered through Celite and concentrated in vacuo to yield the desired primary amine. To a stirred solution of the primary amine and **18** (1.27 g, 4.76 mmol) in CH_2Cl_2 (2.4 mL) was added Et_3N (0.33 mL, 2.38 mmol) at rt. After 12 h, the reaction mixture was diluted with EtOAc , washed with NaHCO_3 (aq) and brine, and dried over Na_2SO_4 . The crude material was purified by silica gel column chromatography to give **19** as white foam (1.14 g, 1.62 mmol, 68% for 2 steps): TLC (hexanes/ EtOAc 33:67) $R_f = 0.30$; $[\alpha]_D^{22} -0.055$ ($c = 0.37$, CHCl_3); IR (thin film) $\nu_{\text{max}} = 3300$ (br), 2959, 2933, 2873, 1740, 1688, 1634, 1553, 1527, 1367, 1248, 1162, 1046, 861, 838 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 6.82 (d, $J = 8.1$ Hz, 1H), 6.21 (s, 1H), 5.06 (d, $J = 8.7$ Hz, 1H), 4.99 (d, $J = 8.4$ Hz, 1H), 4.52 (dd, $J = 7.8$, 5.2 Hz, 2H), 4.29 (ddd, $J = 22.3$, 9.9, 4.9 Hz, 2H), 4.19 (td, $J = 8.0$, 2.8 Hz, 2H), 4.08 (dt, $J = 11.9$, 6.3 Hz, 1H), 3.95 (quin, $J = 7.6$ Hz, 1H), 2.17 – 2.08 (m, 1H), 2.06 (s, 3H), 1.97 – 1.88 (m, 2H), 1.65 – 1.52 (m, 3H), 1.46 (s, 9H), 1.41 (s, 9H), 1.01 (dd, $J = 9.4$, 8.1 Hz, 2H), 0.95 (d, $J = 6.9$ Hz, 3H), 0.93 – 0.89 (m, 9H), 0.04 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.56, 171.72, 171.37, 170.36, 157.67, 81.78, 79.97, 63.60, 61.46, 58.57, 51.04, 41.37, 31.57, 28.30 (3C), 28.08

(3C), 24.79, 22.79, 21.94, 21.01, 18.93, 17.65, 17.39, -1.52 (3C); HRMS (ESI+) m/z calcd for $C_{33}H_{62}N_4O_{10}NaSi$ [$M + Na$] 725.4133, found 725.4094.

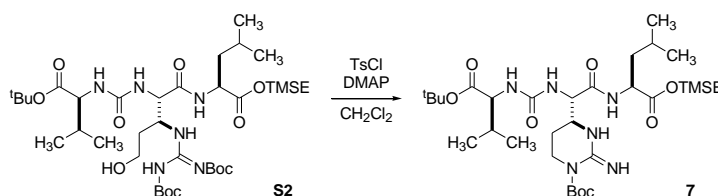


To a stirred solution of **19** (1.13 g, 1.61 mmol) was added a 4 M solution of HCl in dioxane (2.0 mL). The reaction mixture was stirred for 1 h at rt, and all volatiles were evaporated in vacuo to provide the free amine as an oil: TLC ($CHCl_3/MeOH$ 90:10) $R_f = 0.40$. To a stirred solution of the free amine in DMF (3.2 mL) were added *N,N'*-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (0.70 g, 2.42 mmol), Et_3N (1.12 mL, 8.06 mmol), and $HgCl_2$ (0.66 g, 2.42 mmol). The reaction mixture was stirred at 0 °C for 1.5 h. Upon completion, the reaction mixture was diluted with EtOAc and filtered through Celite. The combined organic phase was washed with brine, dried over Na_2SO_4 , and concentrated in vacuo. The crude material was purified by silica gel column chromatography (hexanes/EtOAc 80:20 to 67:33) to give **20** (0.66 g, 0.78 mmol, 49% for 2 steps): TLC (hexanes/EtOAc 67:33) $R_f = 0.30$; $[\alpha]_D^{22} -0.049$ ($c = 0.39$, $CHCl_3$); IR (thin film) $\nu_{max} = 3282$ (br), 2965, 1793, 1723, 1638, 1614, 1541, 1485, 1368, 1326, 1251, 1154, 1136, 1057, 1029, 860, 839, 810, 778 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ 11.36 (s, 1H), 8.65 (d, $J = 7.8$ Hz, 1H), 7.93 (br s, 1H), 6.89 (d, $J = 8.3$ Hz, 1H), 5.03 (d, $J = 8.9$ Hz, 1H), 4.60 (dd, $J = 7.4, 5.9$ Hz, 1H), 4.54 – 4.47 (m, 1H), 4.44 – 4.36 (m, 1H), 4.33 (dd, $J = 8.8, 4.7$ Hz, 1H), 4.23 – 4.06 (m, 5H), 2.25 – 1.94 (m, 5H), 2.06 (s, 3H), 1.65 – 1.54 (m, 4H), 1.52 (s, 9H), 1.48 (s, 9H), 1.46 (s, 9H), 1.01 – 0.96 (m, 2H), 0.94 (d, $J = 6.9$ Hz, 3H), 0.92 – 0.87 (m, 9H), 0.04 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.40, 171.81, 171.20, 170.34, 162.42, 157.90, 152.62, 83.43, 81.50, 79.76, 63.38, 61.24, 58.29, 57.71, 50.89, 41.14, 36.64, 31.67, 28.33 (3C), 28.06 (3C), 28.05 (3C), 24.79, 22.88, 21.82, 20.92, 19.05, 17.72, 17.31, -1.52 (3C); HRMS (ESI+) m/z calcd for $C_{39}H_{73}N_6O_{12}Si$ [$M + H$] 845.5056, found 845.4957.

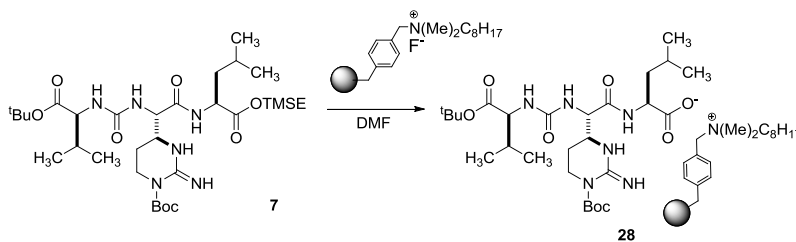


To a stirred solution of **20** (0.54 g, 0.64 mmol) in MeOH (1.3 mL) was added $[Bu_2Sn(OH)Cl]_2$ (0.18 g, 0.32 mmol). After 5 h at rt, all volatiles were evaporated in vacuo. The crude product was passed through a silica gel pad (hexanes/EtOAc 50:50) to provide the free alcohol **S2** (0.47 g, 0.58 mmol, 91%) as a white foam: TLC (hexanes/EtOAc 60:40) $R_f = 0.30$; $[\alpha]_D^{22} -0.082$ ($c = 0.67$, $CHCl_3$); IR (thin film) $\nu_{max} = 3318$ (br), 2961, 2873, 1730, 1648, 1611, 1543, 1412, 1392, 1368, 1307, 1251, 1227, 1155, 1132, 1058, 857, 839 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ 11.37 (s, 1H), 8.90 (d, $J = 8.4$ Hz, 1H), 6.63 (d, $J = 8.5$ Hz, 1H), 6.10 (d, $J = 6.2$ Hz, 1H), 5.18 (d, $J = 8.8$ Hz, 1H), 4.61 (td, $J = 8.7, 7.7, 3.5$ Hz, 2H),

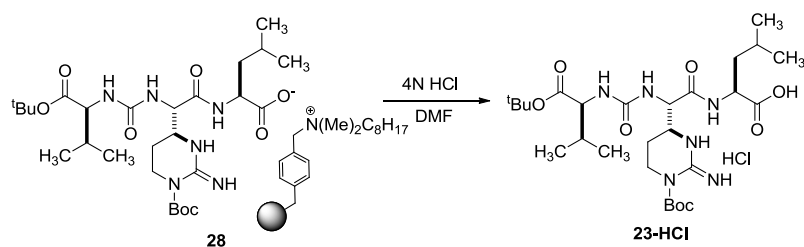
4.53 (ddd, $J = 11.3, 8.0, 3.9$ Hz, 1H), 4.26 (dd, $J = 8.7, 4.5$ Hz, 1H), 4.22 – 4.15 (m, 2H), 3.69 (dq, $J = 10.6, 3.0$ Hz, 1H), 3.54 (td, $J = 11.7, 2.6$ Hz, 1H), 2.18 – 2.08 (m, 1H), 1.97 – 1.78 (m, 3H), 1.70 – 1.54 (m, 2H), 1.50 (s, 9H), 1.46 (s, 18H), 1.02 – 0.98 (m, 2H), 0.96 (d, $J = 7.0$ Hz, 3H), 0.92 – 0.87 (m, 9H), 0.04 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.78, 171.78, 170.35, 162.59, 157.40, 156.52, 152.58, 83.49, 81.71, 79.53, 63.81, 58.63, 57.94, 56.37, 51.05, 50.84, 41.27, 34.77, 31.46, 28.21 (3C), 28.09 (3C), 28.07 (3C), 24.76, 22.99, 21.54, 18.94, 17.70, 17.38, -1.52 (3C); HRMS (ESI+) m/z calcd for $\text{C}_{37}\text{H}_{71}\text{N}_6\text{O}_{11}\text{Si}$ [$\text{M} + \text{H}$] 803.4950, found 803.5033.



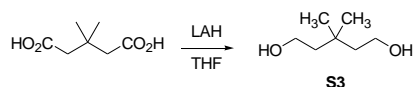
The alcohol **S2** (49.2 mg, 0.061 mmol) was dissolved in CH_2Cl_2 (0.6 mL), and TsCl (58.4 mg, 0.31 mmol) and DMAP (74.8 mg, 0.61 mmol) were added at 0 °C. The reaction mixture was warmed to rt and stirred for 1 h. Upon completion, the reaction mixture was quenched with 1N HCl (aq), extracted with EtOAc. The combined organic extracts were washed with saturated NaHCO_3 (aq), dried with Na_2SO_4 , concentrated in vacuo. The crude product was purified by silica gel column chromatography (hexanes/EtOAc 60:40 to 50:50) to yield **7** (29.0 mg, 0.037 mmol, 60%) as a colorless oil: TLC (hexanes/EtOAc 50:50) $R_f = 0.30$; $[\alpha]_D^{22} -0.115$ ($c = 0.75, \text{CHCl}_3$); IR (thin film) $\nu_{\text{max}} = 3283$ (br), 2960, 2933, 2873, 1733, 1639, 1542, 1367, 1337, 1250, 1148, 1056, 985, 936, 857, 838, 771 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3) δ 9.79 (brs, 1H), 8.15 (brs, 1H), 6.14 (brs, 1H), 5.11 (d, $J = 7.5$ Hz, 1H), 4.65 – 4.50 (m, 1H), 4.50 – 4.43 (m, 2H), 4.26 (dd, $J = 8.5, 4.2$ Hz, 1H), 4.19 – 4.15 (m, 2H), 4.06 (dt, $J = 11.5, 3.5$ Hz, 1H), 3.87 (dd, $J = 12.4, 3.5$ Hz, 1H), 3.33 (td, $J = 12.8, 4.2$ Hz, 1H), 2.17 – 2.07 (m, 1H), 2.07 – 2.00 (m, 1H), 1.71 (t, $J = 9.1$ Hz, 1H), 1.65 – 1.59 (m, 2H), 1.51 (s, 9H), 1.47 (s, 9H), 1.45 (s, 9H), 1.02 – 0.98 (m, 2H), 0.93 (d, $J = 6.9$ Hz, 3H), 0.91 – 0.87 (m, 9H), 0.02 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ 172.58, 171.66, 157.50, 153.20, 83.89, 81.38, 81.00, 63.21, 58.38, 57.95, 57.21, 54.17, 51.28, 50.83, 44.30, 40.19, 31.54, 28.27 (3C), 28.20, 28.08 (6C), 24.82, 23.02, 21.51, 18.92, 17.66, 17.32, -1.52 (3C); HRMS (ESI+) m/z calcd for $\text{C}_{37}\text{H}_{69}\text{N}_6\text{O}_{10}\text{Si}$ [$\text{M} + \text{H}$] 785.4844, found 785.4847.



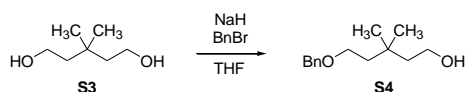
To a stirred solution of **7** (50.0 mg, 0.064 mmol) in DMF (0.6 mL) was added PS-F (1.1 mmol/g, 0.29 g, 0.32 mmol). After 2 h at 55 °C, the reaction was cooled to rt, TFA/DMF (1%, 0.6 mL) was added. The reaction mixture was stirred for 1.5 h and filtered. The filtered PS-F was washed with a solution of DMF/MeOH (10%, 1.8 mL) and combined organic solution was evaporated in vacuo. This was used in the following reactions.



The complex **28** was treated with 4N HC (in dioxane). The polymers were filtered off via a glass filter, and all volatiles were evaporated in vacuo to furnish **23-HCl** in quantitative yield: $[\alpha]_D^{20} +0.108$ ($c = 0.95$, MeOH); IR (thin film) $\nu_{\max} = 3282$ (br), 3069 (br), 2964, 2874, 1665, 1552, 1469, 1369, 1314, 1252, 1201, 1179, 1137, 837, 800, 721 cm^{-1} ; ^1H NMR (500 MHz, CD_3OD) δ 4.55 (d, $J = 5.5$ Hz, 1H), 4.44 (q, $J = 7.2$ Hz, 1H), 4.08 (d, $J = 4.9$ Hz, 1H), 3.90 (dt, $J = 9.6, 5.1$ Hz, 1H), 3.54 (dt, $J = 13.4, 5.0$ Hz, 1H), 3.45 (dt, $J = 8.4, 4.3$ Hz, 1H), 2.12 (tt, $J = 13.5, 5.3$ Hz, 2H), 1.87 (dtd, $J = 13.9, 9.0, 5.0$ Hz, 1H), 1.70 – 1.66 (m, 3H), 1.54 (s, 9H), 1.47 (s, 9H), 0.99 – 0.92 (m, 12H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.37, 171.98, 160.08, 156.15, 82.83, 69.13, 60.35, 57.19, 52.64, 41.45, 40.20, 37.97, 31.63, 30.14, 28.77, 28.46, 28.36 (3C), 28.11 (3C), 26.13, 24.97, 24.02, 23.41, 21.81, 19.57, 18.03, 14.37, 11.40; HRMS (ESI+) m/z calcd for $\text{C}_{27}\text{H}_{49}\text{N}_6\text{O}_8$ $[M + H]$ 585.3612, found 585.3630.

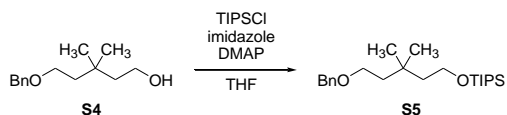


To a stirred suspension of LiAlH_4 (11 g, 295 mmol) in dry THF (200 mL) was added a solution of 3,3-dimethylglutaric acid (23.5 g, 147 mmol) in dry THF (100 mL) dropwise at 0°C under N_2 atmosphere, and the reaction mixture was warmed to rt. After 4h, the reaction was stirred at reflux for 4h and cooled to 0°C . The reaction was quenched by NaOH (aq). Extraction with EtOAc was conducted. The combined EtOAc phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by distillation to obtain pure **S3** (16.4 g, 124 mmol, 84 %): TLC (hexanes/EtOAc 20:80) $R_f = 0.20$; IR (thin film) $\nu_{\max} = 3317$ (br), 2955, 2934, 1676, 1469, 1366, 1030, 1006, 990 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 3.73 (t, $J = 7.0$ Hz, 4H), 2.04 (brs, 2H), 1.57 (t, $J = 7.0$ Hz, 4H), 0.94 (s, 6H); ^{13}C NMR (101 MHz, CDCl_3) δ 59.60 (2C), 44.06 (2C), 31.67, 28.08 (2C); HRMS (ESI+) m/z calcd for $\text{C}_7\text{H}_{16}\text{O}_2$ 132.1150, found 132.1144.

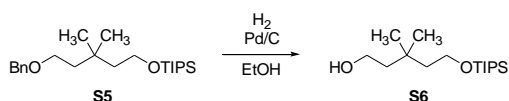


To a stirred suspension of NaH (2.27 g, 56.7 mmol) in dry THF (56.7 mL) was added **S3** (7.50 g, 56.7 mmol) at rt. After being stirred for 1 h, BnBr (6.75 mL, 56.7 mmol) was added to the reaction solution dropwisely at 0°C . The reaction mixture was warmed to rt and stirred for 18 h. The reaction was quenched with saturated NH_4Cl (aq.) at 0°C and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 80:20 to 50:50) to obtain **S4** (7.90 g, 35.5 mmol, 63%)

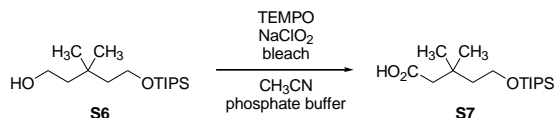
as a colorless oil: TLC (hexanes/EtOAc 80:20) $R_f = 0.20$; IR (thin film) $\nu_{\max} = 3357$ (br), 2955, 2930, 2869, 1719, 1469, 1453, 1365, 1274, 1203, 1098, 1070, 1045, 1027, 735, 696 cm^{-1} ; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.40 – 7.27 (m, 5H), 4.50 (s, 2H), 3.70 (t, $J = 7.2$ Hz, 2H), 3.55 (t, $J = 6.9$ Hz, 2H), 1.62 (t, $J = 6.9$ Hz, 2H), 1.56 (t, $J = 7.2$ Hz, 2H), 0.93 (s, 6H); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 138.29, 128.39 (2C), 127.66 (2C), 127.58, 73.12, 67.32, 59.72, 44.36, 41.10, 31.67, 28.03 (2C); HRMS (ESI+) m/z calcd for $\text{C}_{14}\text{H}_{22}\text{O}_2$ 222.1620, found 222.1622.



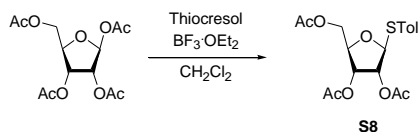
To a stirred solution of **S4** (7.90 g, 35.5 mmol) and imidazole (3.38 g, 49.7 mmol) in dry THF (125 mL) were added TIPSCl (11.4 mL, 53.3 mmol) and DMAP (0.43 g, 3.55 mmol) at 0 °C. The reaction mixture was warmed to rt and stirred for 16 h. The reaction was quenched with saturated NaHCO_3 (aq.) and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 97:3 to 80:20) to obtain **S5** (12.4 g, 32.7 mmol, 92%) as a colorless oil: TLC (hexanes) $R_f = 0.20$; IR (thin film) $\nu_{\max} = 2941, 2891, 2865, 1742, 1463, 1384, 1365, 1245, 1096, 1069, 1028, 1012, 996, 881, 732, 679$ cm^{-1} ; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.38 – 7.26 (m, 5H), 4.49 (s, 2H), 3.73 (dd, $J = 7.9, 7.1$ Hz, 2H), 3.54 (dd, $J = 7.9, 7.1$ Hz, 2H), 1.63 – 1.57 (m, 2H), 1.53 (dd, $J = 8.0, 7.1$ Hz, 2H), 1.13 – 1.00 (m, 21H), 0.93 (s, 6H); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 138.60, 128.33 (2C), 127.58 (2C), 127.45, 73.01, 67.46, 60.14, 44.83, 41.44, 31.44, 27.80 (2C), 18.05 (6C), 11.96 (3C); HRMS (ESI+) m/z calcd for $\text{C}_{23}\text{H}_{43}\text{O}_2\text{Si}$ [M + H] 379.3032, found 379.3062.



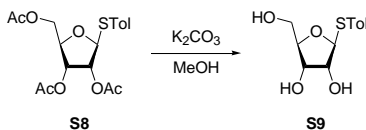
To a stirred solution of **S5** (12.4 g, 32.7 mmol) in EtOH (100 mL) was added Pd/C (10 wt % 200 mg). H_2 gas was introduced and the reaction mixture was stirred for 18 h under H_2 . The solution was filtered through Celite and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 95:5 to 80:20) to obtain **S6** (8.02 g, 27.8 mmol, 85%) as a colorless oil: TLC (hexanes/EtOAc 80:20) $R_f = 0.40$; IR (thin film) $\nu_{\max} = 3343$ (br), 2941, 2891, 2866, 1463, 1384, 1366, 1096, 1065, 1012, 995, 881, 745, 678, 656 cm^{-1} ; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 3.76 (t, $J = 6.9$ Hz, 2H), 3.72 (t, $J = 7.2$ Hz, 2H), 1.57 (td, $J = 7.1, 2.8$ Hz, 4H), 1.12 – 1.03 (m, 21H), 0.94 (s, 6H); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 60.30, 59.85, 44.31, 31.67, 28.14 (2C), 18.05 (6C), 11.95 (3C); HRMS (ESI+) m/z calcd for $\text{C}_{16}\text{H}_{36}\text{O}_2\text{Si}$ 288.2485, found 288.2473.



To a stirred solution of **S6** (8.02 g, 27.8 mmol) and TEMPO (0.22 g, 1.39 mmol) in MeCN (56 mL) an phosphate buffer (pH=6.8, 56 mL) were added NaClO₂ (3.02 g, 33.4 mmol) and bleach (8.25%, 14 mL) at 35 °C. After being stirred for 4 h, the reaction mixture was extracted with EtOAc and combined organic phase was dried over Na₂SO₄ and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 95:5 to 80:20) to give **S7** (8.13 g, 26.9 mmol, 97%) as an orange oil: TLC (hexanes/EtOAc 50:50) *R_f* = 0.50; IR (thin film) ν_{\max} = 2942, 2892, 2866, 1705, 1463, 1246, 1097, 1052, 996, 881, 738, 678, 656 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 3.88 (t, *J* = 5.8 Hz, 2H), 2.38 (s, 2H), 1.71 (t, *J* = 5.8 Hz, 2H), 1.20 – 1.11 (m, 3H), 1.09 (s, 12H), 1.08 (s, 6H), 1.07 (s, 6H); ¹³C NMR (101 MHz, CDCl₃) δ 173.89, 60.72, 46.82, 42.60, 32.40, 28.52 (2C), 17.91 (6C), 11.79 (3C); HRMS (ESI+) *m/z* calcd for C₁₆H₃₄O₃NaSi [M + Na] 325.2175, found 325.2171.

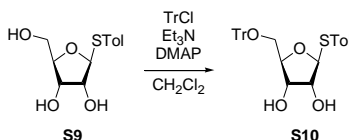


To a stirred solution of β -D-Ribofuranose 1,2,3,5-tetraacetate (25.3 g, 79.5 mmol) and thiocresol (10.9 g, 87.5 mmol) in CH₂Cl₂ (240 mL) was added BF₃·OEt₂ (30.2 mL, 238.6 mmol) dropwise at -78 °C. The reaction mixture was warmed to 0 °C and stirred for 30 min. The reaction was quenched with saturated NaHCO₃ (aq.) and extracted with CH₂Cl₂. The combined organic extracts were dried over Na₂SO₄ and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 90:10 to 70:30) to get **S8** (29.3 g, 76.6 mmol, 96%): TLC (hexanes/EtOAc 50:50) *R_f* = 0.60; $[\alpha]_{\text{D}}^{20}$ -0.411 (*c* = 0.51, CHCl₃); IR (thin film) ν_{\max} = 1742, 1371, 1214, 1091, 1045, 1017, 899, 810 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.41 (d, *J* = 8.1 Hz, 2H), 7.14 (d, *J* = 7.7 Hz, 2H), 5.25 – 5.22 (m, 1H), 5.21 – 5.17 (m, 2H), 4.26 – 4.20 (m, 2H), 4.07 (dd, *J* = 12.9, 5.5 Hz, 1H), 2.34 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 170.50, 169.63, 169.42, 138.80, 134.18 (2C), 129.81 (2C), 127.45, 87.95, 79.97, 73.67, 71.41, 63.46, 21.15, 20.75, 20.53 (2C); HRMS (ESI+) *m/z* calcd for C₁₈H₂₂O₇NaS [M + Na] 405.0984, found: 405.0970.

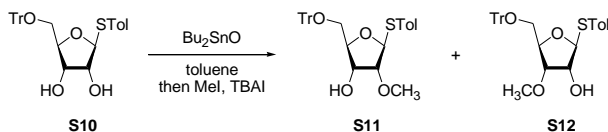


To a stirred solution of **S8** (24.7 g, 64.6 mmol) in MeOH (300 mL) was added K₂CO₃ (44.5 g, 322 mmol). After being stirred for 30 min, the reaction mixture filtered and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (CHCl₃/MeOH 75:25) to obtain **S9** (11.7 g, 45.6 mmol, 71%): TLC (EtOAc) *R_f* = 0.50; $[\alpha]_{\text{D}}^{20}$ -2.247 (*c* = 1.82, MeOH); IR (thin film) ν_{\max} = 3353 (br), 2921, 1492, 1399, 1331, 1199, 1034, 1015, 940, 806, 703 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.38 (d, *J* = 8.1 Hz, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 5.21 (d, *J* = 4.6 Hz, 1H), 4.15 (t, *J* = 5.1 Hz, 1H), 4.06 (t, *J* =

4.9 Hz, 1H), 4.01 (q, $J = 3.8$ Hz, 1H), 3.75 (dd, $J = 12.2, 3.5$ Hz, 1H), 3.62 (dd, $J = 12.2, 3.8$ Hz, 1H), 3.21 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 138.25, 132.73 (2C), 129.91 (2C), 128.78, 90.45, 84.81, 75.26, 71.42, 62.48, 21.11; HRMS (ESI+) m/z calcd for $\text{C}_{12}\text{H}_{16}\text{O}_4\text{NaS}$ [$\text{M} + \text{Na}$] 279.0667, found: 279.0670.

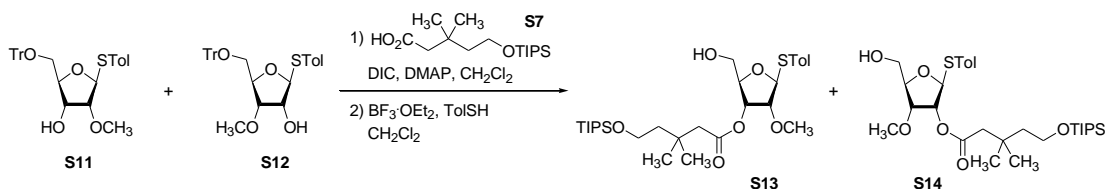


To a stirred solution of **S9** (11.7 g, 45.6 mmol) and trityl chloride (19.1 g, 68.5 mmol) in CH_2Cl_2 (230 mL) were added Et_3N (12.7 mL, 91.3 mmol) and DMAP (1.12 g, 9.13 mmol) at 0°C , and the reaction mixture was warmed to rt. After being stirred for 16 h, the reaction was quenched with water and extracted with CH_2Cl_2 . The combined organic solution was dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/ EtOAc 80:20 to 50:50) to get **S10** (17.3 g, 34.7 mmol, 76%): TLC (hexanes/ EtOAc 50:50) $R_f = 0.50$; $[\alpha]_D^{21} -1.029$ ($c = 1.42$, CHCl_3); IR (thin film) $\nu_{\text{max}} = 3395$ (br), 3058, 3021, 2921, 2870, 1596, 1491, 1448, 1399, 1319, 1216, 1184, 1154, 1075, 1031, 1012, 1001, 950, 900, 808, 745, 698, 666 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.46 (d, $J = 7.3$ Hz, 6H), 7.41 (d, $J = 7.9$ Hz, 2H), 7.32 – 7.21 (m, 9H), 7.07 (d, $J = 7.8$ Hz, 2H), 5.22 (d, $J = 4.9$ Hz, 1H), 4.16 (q, $J = 4.7$ Hz, 1H), 4.08 (dq, $J = 12.9, 4.7$ Hz, 2H), 3.26 (qd, $J = 10.0, 4.4$ Hz, 2H), 2.59 (d, $J = 4.7$ Hz, 1H), 2.41 (d, $J = 4.0$ Hz, 1H), 2.31 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 143.73 (2C), 137.84, 132.83 (3C), 129.73 (2C), 129.43, 128.74 (6C), 127.86 (6C), 127.07 (3C), 90.49, 86.96, 83.40, 75.27, 72.48, 64.20, 21.11; HRMS (ESI+) m/z calcd for $\text{C}_{31}\text{H}_{30}\text{O}_4\text{NaS}$ [$\text{M} + \text{Na}$] 521.1762, found: 521.1721.

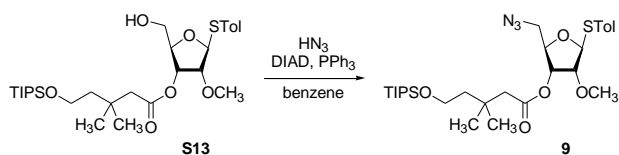


S10 (12.4 g, 24.8 mmol) and Dibutyltin(IV) oxide (9.25 g, 37.2 mmol) were dissolved in toluene (250 mL). The reaction mixture was stirred at reflux for 3 h and water was removed by Dean-Stark trap. After the reaction was cooled to rt, MeI (3.08 mL, 49.5 mmol) and $n\text{-Bu}_4\text{NI}$ were added. After being stirred for 16 h at reflux, the reaction was cooled to rt and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (hexanes/ EtOAc 80:20 to 67:33) to get **S11** and **S12** (9.03 g, 17.6 mmol, 71%) as diastereomeric mixture. Data for major diastereomer: TLC (hexanes/ EtOAc 67:33) $R_f = 0.40$; IR (thin film) $\nu_{\text{max}} = 3471$ (br), 3058, 3021, 2924, 1597, 1491, 1448, 1399, 1323, 1216, 1183, 1154, 1107, 1075, 1031, 1017, 982, 950, 899, 808, 745, 698, 666 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.51 – 7.39 (m, 8H), 7.32 – 7.21 (m, 9H), 7.07 (d, $J = 8.0$ Hz, 2H), 5.33 (d, $J = 4.2$ Hz, 1H), 4.09 – 4.03 (m, 2H), 3.79 – 3.76 (m, 1H), 3.48 (s, 3H), 3.28 (dd, $J = 10.2, 3.5$ Hz, 1H), 3.19 (q, $J = 5.4$ Hz, 1H), 2.50 (d, $J = 6.0$ Hz, 1H), 2.32 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 143.87 (2C), 137.78, 132.75 (3C), 129.73 (2C), 128.83 (6C), 127.78 (6C), 126.97 (3C), 90.75, 87.91, 86.77, 84.34, 80.87, 73.83, 71.08, 64.16, 58.41, 21.12; HRMS (ESI+) m/z calcd for $\text{C}_{32}\text{H}_{32}\text{O}_4\text{NaS}$ [$\text{M} + \text{Na}$] 535.1919, found: 535.1835. Data for minor

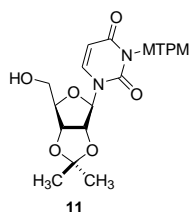
diastereomer: TLC (hexanes/EtOAc 67:33) $R_f = 0.40$; ^1H NMR (400 MHz, CDCl_3) δ 7.51 – 7.39 (m, 8H), 7.32 – 7.21 (m, 9H), 7.07 (d, $J = 7.7$ Hz, 2H), 5.17 (d, $J = 5.4$ Hz, 1H), 4.14 – 4.09 (m, 2H), 3.81 – 3.79 (m, 1H), 3.38 (s, 3H), 3.19 – 3.15 (m, 2H), 2.79 (d, $J = 6.8$ Hz, 1H), 2.31 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 143.78 (2C), 137.60, 132.75 (3C), 129.64 (2C), 128.73 (6C), 127.82 (6C), 127.04 (3C), 90.75, 87.91, 86.83, 84.10, 81.32, 73.83, 71.08, 63.81, 58.02, 21.12.



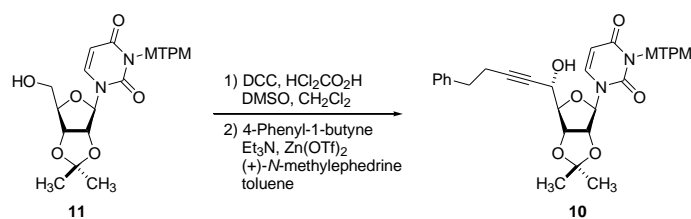
To a stirred solution of **S11** and **S12** (9.03 g, 17.6 mmol) and **S7** (6.39 g, 21.1 mmol) in CH_2Cl_2 (50 mL) were added DMAP (3.23 g, 26.4 mmol) and DIC (3.31 mL, 21.1 mmol). The reaction mixture was stirred for 20 h at rt and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 95:5 to 90:10) to afford desired ester (11.6 g, 14.5 mmol, 82%) as diastereomeric mixture. To a stirred solution of ester (11.6 g, 14.5 mmol) and thiocresol (3.60 g, 29.0 mmol) in CH_2Cl_2 was added $\text{BF}_3 \cdot \text{OEt}_2$ (0.73 mL, 5.80 mmol) at 0 °C. After being stirred for 1 h, the reaction was quenched with saturated NaHCO_3 (aq.) and extracted with EtOAc. The combined organic solution was dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 93:7 to 80:20) to afford desired **S13** (3.23 g, 5.82 mmol, 40%) and undesired **S14** (3.36 g, 6.05 mmol, 42%). Data for desired **S13**: TLC (hexanes/EtOAc 80:20) $R_f = 0.45$; $[\alpha]_D^{21} -0.378$ ($c = 0.82$, CHCl_3); IR (thin film) $\nu_{\text{max}} = 3475$ (br), 2940, 2865, 1735, 1493, 1462, 1389, 1367, 1223, 1189, 1093, 1051, 1017, 994, 882, 808, 743, 679, 657 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.43 (d, $J = 8.2$ Hz, 2H), 7.15 (d, $J = 7.9$ Hz, 2H), 5.20 (d, $J = 6.0$ Hz, 1H), 5.16 (dd, $J = 5.3, 3.9$ Hz, 1H), 4.13 (q, $J = 3.3$ Hz, 1H), 3.81 – 3.72 (m, 4H), 3.57 (dd, $J = 12.4, 3.2$ Hz, 1H), 3.40 (s, 3H), 2.34 (s, 3H), 2.32 (s, 2H), 1.62 (dd, $J = 7.5, 6.5$ Hz, 2H), 1.11 – 1.00 (m, 27H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.46, 138.51, 133.36 (2C), 129.87 (2C), 128.38, 88.22, 83.70, 82.51, 71.50, 62.29, 60.03, 58.81, 46.28, 44.47, 32.62, 27.48, 27.46, 21.15, 18.03 (6C), 11.89 (3C); HRMS (ESI+) m/z calcd for $\text{C}_{29}\text{H}_{50}\text{O}_6\text{NaSSi}$ [$\text{M} + \text{Na}$] 577.2995, found: 577.3037. Data for undesired **S14**: TLC (hexanes/EtOAc 80:20) $R_f = 0.40$; $[\alpha]_D^{22} -0.362$ ($c = 0.58$, CHCl_3); IR (thin film) $\nu_{\text{max}} = 3473$ (br), 2940, 2865, 1736, 1493, 1463, 1389, 1367, 1220, 1191, 1098, 1052, 1017, 996, 939, 882, 809, 742, 680, 657 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.38 (d, $J = 8.2$ Hz, 2H), 7.14 (d, $J = 8.0$ Hz, 2H), 5.35 (d, $J = 2.5$ Hz, 1H), 5.32 (dd, $J = 4.5, 2.5$ Hz, 1H), 4.05 (dt, $J = 7.2, 2.9$ Hz, 1H), 3.98 (dd, $J = 7.3, 4.6$ Hz, 1H), 3.82 (dd, $J = 12.3, 2.8$ Hz, 1H), 3.77 (t, $J = 7.0$ Hz, 2H), 3.60 (dd, $J = 12.3, 3.2$ Hz, 1H), 3.36 (s, 3H), 2.35 (s, 2H), 2.33 (s, 3H), 1.64 (td, $J = 6.9, 1.2$ Hz, 2H), 1.11 – 0.99 (m, 27H); ^{13}C NMR (101 MHz, CDCl_3) δ 170.99, 138.37, 132.76 (2C), 129.96 (2C), 128.73, 89.49, 82.60, 78.47, 74.69, 61.45, 60.03, 58.78, 46.41, 44.49, 32.66, 27.48 (2C), 21.13, 18.03 (6C), 11.89 (3C); HRMS (ESI+) m/z calcd for $\text{C}_{29}\text{H}_{50}\text{O}_6\text{NaSSi}$ [$\text{M} + \text{Na}$] 577.2995, found: 577.2998.



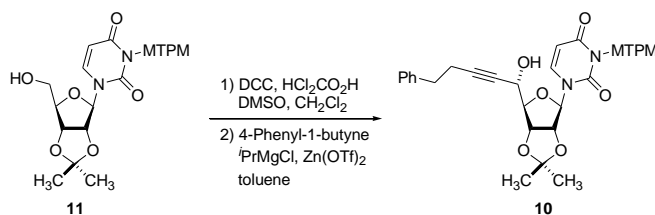
To a stirred solution of **S13** (2.50 g, 4.50 mmol) and PPh_3 (2.36 g, 9.01 mmol) in dry benzene (15 mL) were added HN_3 (0.6 M in benzene, 75 mL, 45.0 mmol) and DIAD (1.77 mL, 9.01 mmol). The reaction mixture was stirred for 12 h at rt and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 95:5) to afford **9** (2.29 g, 4.11 mmol, 91%): TLC (hexanes/EtOAc 80:20) $R_f = 0.70$; $[\alpha]_D^{22} -0.117$ ($c = 0.86$, CHCl_3); IR (thin film) $\nu_{\text{max}} = 2941, 2865, 2101, 1736, 1493, 1463, 1389, 1367, 1281, 1255, 1219, 1189, 1096, 1052, 1014, 995, 918, 882, 808, 754, 679, 657 \text{ cm}^{-1}$; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.45 (d, $J = 8.1 \text{ Hz}$, 2H), 7.14 (d, $J = 7.9 \text{ Hz}$, 2H), 5.20 (d, $J = 5.3 \text{ Hz}$, 1H), 4.99 (t, $J = 5.2 \text{ Hz}$, 1H), 4.16 (td, $J = 5.0, 4.0 \text{ Hz}$, 1H), 3.83 (t, $J = 5.4 \text{ Hz}$, 1H), 3.76 (t, $J = 7.0 \text{ Hz}$, 2H), 3.41 (t, $J = 4.6 \text{ Hz}$, 2H), 3.38 (s, 3H), 2.34 (s, 3H), 2.32 (s, 2H), 1.62 (td, $J = 6.9, 0.9 \text{ Hz}$, 2H), 1.11 – 1.01 (m, 27H); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 171.31, 138.31, 133.40 (2C), 129.77 (2C), 128.82, 88.87, 82.32, 81.05, 72.14, 60.08, 58.71, 52.73, 46.25, 44.51, 32.65, 27.49, 27.47, 21.14, 18.05 (6C), 11.98 (3C); HRMS (ESI+) m/z calcd for $\text{C}_{29}\text{H}_{49}\text{N}_3\text{O}_5\text{NaSi}$ [$\text{M} + \text{Na}$] 602.3060, found: 602.3016.



MTPM-protected uridine **11** was synthesized according to the reported procedure²: TLC (hexanes/EtOAc 33:67) $R_f = 0.50$; $[\alpha]_D^{22} -2.315$ ($c = 8.98$, CHCl_3); IR (thin film) $\nu_{\text{max}} = 3478$ (br), 3057, 2986, 2940, 1715, 1663, 1598, 1556, 1455, 1374, 1265, 1212, 1066, 1039, 853, 808, 787, 732, 702 cm^{-1} ; $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 7.51 (ddd, $J = 17.3, 8.5, 0.7 \text{ Hz}$, 1H), 7.33 – 7.27 (m, 2H), 7.18 (ddd, $J = 8.8, 6.9, 2.1 \text{ Hz}$, 1H), 6.85 (d, $J = 5.1 \text{ Hz}$, 2H), 6.51 (d, $J = 3.7 \text{ Hz}$, 1H), 5.72 (dd, $J = 8.1, 4.0 \text{ Hz}$, 1H), 5.60 – 5.51 (m, 2H), 5.48 (dd, $J = 12.6, 2.1 \text{ Hz}$, 1H), 4.95 – 4.93 (m, 1H), 4.92 (t, $J = 2.4 \text{ Hz}$, 1H), 4.30 – 4.25 (m, 1H), 3.89 (ddd, $J = 11.7, 9.1, 2.6 \text{ Hz}$, 1H), 3.82 – 3.73 (m, 4H), 1.57 (s, 3H), 1.36 (d, $J = 2.4 \text{ Hz}$, 3H); $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 162.10, 162.05, 159.51, 150.97, 150.91, 141.55, 141.31, 136.88, 135.28, 135.22, 134.02, 134.00, 133.84, 133.71, 131.17, 129.38, 126.22, 126.18, 125.44, 125.40, 115.33, 114.29, 114.26, 102.05, 102.01, 97.16, 96.82, 87.15, 87.06, 83.76, 83.65, 80.29, 69.50, 62.76, 62.72, 55.69, 27.25, 25.25; HRMS (ESI+) m/z calcd for $\text{C}_{27}\text{H}_{26}\text{N}_2\text{O}_8\text{NaCl}_4$ [$\text{M} + \text{Na}$] 669.0341, found: 669.0324.

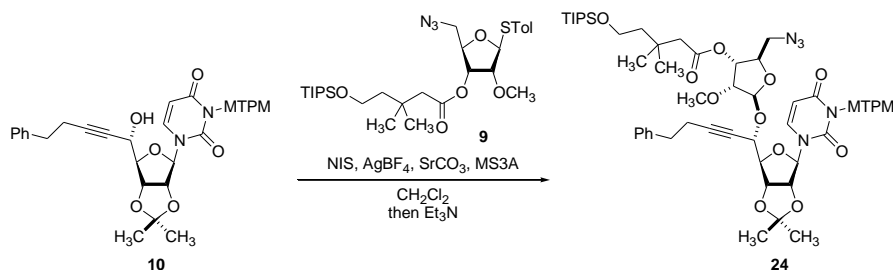


To a stirred solution of **11** (0.65 g, 1.0 mmol) and dichloroacetic acid (0.12 mL, 1.5 mmol) in CH_2Cl_2 (5.0 mL) and DMSO (1.0 mL) was added DIC (0.23 mL, 1.5 mmol) at 0 °C, and the reaction mixture was warmed to rt. After 8 h, the reaction was quenched with saturated NaHCO_3 (aq.) and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The precipitates were filtered, and the crude mixture was used for next reaction without purification. To a suspension of $\text{Zn}(\text{OTf})_2$ (1.45 g, 4.0 mmol) and (+)-*N*-methylephedrine (0.79 g, 4.8 mmol) in toluene (6 mL) was added Et_3N (0.61 mL, 4.8 mmol) at rt. After 2 h, 4-phenyl-1-butyne (0.62 mL, 4.8 mmol) was added. After 4 h, a solution of crude aldehyde (0.65 g, 1.0 mmol) in toluene (5 mL) was added. The reaction mixture was stirred for 16 h and quenched with saturated NaHCO_3 (aq.), extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 60:40) to afford desired **10** (0.62 g, 0.80 mmol, 80% for 2 steps). Data for desired **10**: TLC (hexanes/EtOAc 50:50) $R_f = 0.30$; $[\alpha]_D^{22} - 0.116$ ($c = 2.17$, CHCl_3); IR (thin film) $\nu_{\text{max}} = 3387$ (br), 3087, 2981, 2937, 1716, 1664, 1597, 1556, 1454, 1374, 1276, 1211, 1156, 1065, 1039, 916, 856, 807, 786, 733, 698 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.53 (ddd, $J = 20.4, 8.5, 0.7$ Hz, 1H), 7.35 – 7.27 (m, 4H), 7.24 – 7.15 (m, 4H), 6.85 (d, $J = 5.1$ Hz, 2H), 6.51 (d, $J = 5.4$ Hz, 1H), 5.68 (dd, $J = 8.1, 4.1$ Hz, 1H), 5.60 – 5.50 (m, 3H), 4.89 – 4.78 (m, 2H), 4.57 (ddt, $J = 12.0, 4.3, 2.0$ Hz, 1H), 4.24 (dd, $J = 4.4, 3.1$ Hz, 1H), 3.78 (d, $J = 3.3$ Hz, 3H), 2.83 (t, $J = 7.5$ Hz, 2H), 2.53 (td, $J = 7.4, 2.0$ Hz, 2H), 1.57 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ 162.11, 162.08, 159.50, 150.87, 150.85, 141.07, 140.84, 140.30, 140.27, 136.90, 135.36, 135.29, 133.99, 133.95, 133.79, 133.64, 131.21, 129.37, 129.34, 128.41, 128.39, 126.40, 126.21, 126.18, 125.49, 125.44, 115.34, 115.32, 114.28, 114.24, 101.79, 101.74, 96.69, 96.37, 89.23, 89.19, 86.83, 86.73, 84.09, 83.93, 80.91, 69.46, 63.02, 62.99, 55.68, 34.72, 34.70, 27.16, 25.29, 20.87, 20.85; HRMS (ESI+) m/z calcd for $\text{C}_{37}\text{H}_{34}\text{N}_2\text{O}_8\text{NaCl}_4$ [M + Na] 797.0967, found: 797.1004.



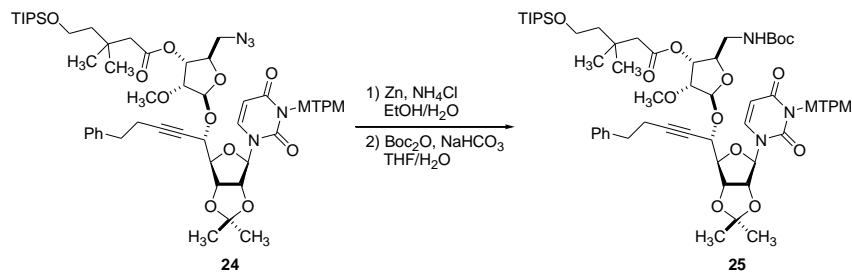
To a stirred solution of **11** (1.02 g, 1.58 mmol) and dichloroacetic acid (0.20 mL, 2.37 mmol) in CH_2Cl_2 (8 mL) and DMSO (1.58 mL) was added DIC (0.37 mL, 2.37 mmol) at 0 °C, and the reaction mixture was warmed to rt. After 16 h, the reaction was quenched with saturated NaHCO_3 (aq.) and extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The precipitates were filtered, and the crude mixture was used for next reaction without purification. To a stirred solution of

4-phenyl-1-butyne (1.1 mL, 7.90 mmol) in toluene (5 mL) was added i-PrMgCl (2.0M in THF, 4.0 mL, 7.90 mmol) at 0 °C. After 3 h, dry Zn(OTf)₂ was added and the reaction mixture was warmed up to rt. After 3 h, a solution of crude aldehyde (xxx g, 1.58 mmol) in toluene (5 mL) was added. The reaction mixture was stirred for 20 h and quenched with saturated NaHCO₃ (aq.), extracted with EtOAc. The combined organic extracts were dried over Na₂SO₄ and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 60:40) to afford desired **10** (0.64 g, 0.84 mmol, 53% for 2 steps) and undesired epimer (0.28 g, 0.35 mmol, 22% for 2 steps). Data for undesired epimer: TLC (hexanes/EtOAc 50:50) *R*_f = 0.40; [α]_D²² -0.851 (*c* = 2.80, CHCl₃); IR (thin film) *v*_{max} = 3429 (br), 3087, 2987, 2938, 1716, 1663, 1597, 1556, 1454, 1374, 1342, 1276, 1239, 1213, 1066, 1039, 917, 859, 807, 787, 750, 699 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.54 (dd, *J* = 14.6, 8.5 Hz, 1H), 7.37 – 7.27 (m, 4H), 7.25 – 7.15 (m, 4H), 6.85 (d, *J* = 3.4 Hz, 2H), 6.52 (d, *J* = 4.2 Hz, 1H), 5.70 (dd, *J* = 8.1, 2.3 Hz, 1H), 5.63 – 5.52 (m, 3H), 4.91 (ddd, *J* = 11.6, 6.4, 2.4 Hz, 1H), 4.78 (ddd, *J* = 18.8, 6.3, 3.7 Hz, 1H), 4.59 (ddt, *J* = 8.7, 3.0, 2.0 Hz, 1H), 4.28 (t, *J* = 2.7 Hz, 1H), 3.78 (d, *J* = 1.9 Hz, 3H), 2.84 (t, *J* = 7.4 Hz, 2H), 2.54 (td, *J* = 7.5, 2.0 Hz, 2H), 1.59 (s, 3H), 1.38 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 162.05, 159.48, 150.95, 140.65, 140.43, 140.27, 140.24, 136.92, 135.42, 133.91, 133.75, 133.64, 131.22, 129.34, 128.42 (4C), 126.42, 126.14, 126.12, 125.56, 115.31, 114.22, 114.19, 101.96, 101.92, 96.06, 95.70, 88.25, 88.20, 87.57, 87.51, 83.36, 83.22, 80.21, 80.15, 69.64, 69.57, 62.99, 55.68, 34.68, 34.66, 27.29, 25.32, 20.89, 20.88; HRMS (ESI+) *m/z* calcd for C₃₇H₃₄N₂O₈NaCl₄ [M + Na] 797.0967, found: 797.0894.

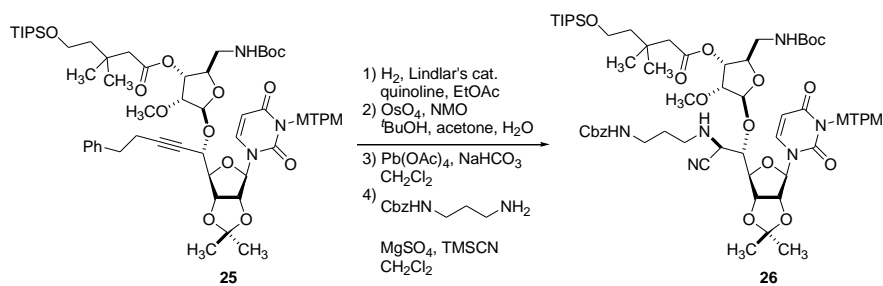


To a stirred suspension of **10** (0.42 g, 0.54 mmol), **9** (0.63 g, 1.08 mmol), MS3A (1.70 g) and SrCO₃ (0.80 g, 5.40 mmol) in CH₂Cl₂ (22 mL) were added AgBF₄ (53 mg, 0.27 mmol) and NIS (0.24 g, 1.08 mmol) at 0 °C. After 8 h, the reaction mixture was added Et₃N (2 mL) and passed through a silica gel pad (hexanes/EtOAc 1:1). The combined organic phase was concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 90:10 to 80:20 to 70:30) to afford **24** (0.60 g, 0.49 mmol, 91%): TLC (hexanes/EtOAc 70:30) *R*_f = 0.40; [α]_D²³ +0.020 (*c* = 0.48, CHCl₃); IR (thin film) *v*_{max} = 2940, 2866, 2101, 1723, 1675, 1600, 1556, 1455, 1374, 1278, 1214, 1099, 1070, 882, 747, 681 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.54 (dd, *J* = 20.0, 8.5 Hz, 1H), 7.35 – 7.27 (m, 4H), 7.24 – 7.16 (m, 4H), 6.85 (d, *J* = 6.0 Hz, 2H), 6.51 (d, *J* = 2.4 Hz, 1H), 5.71 – 5.65 (m, 2H), 5.60 – 5.49 (m, 2H), 5.22 (d, *J* = 4.5 Hz, 1H), 4.95 (ddd, *J* = 6.7, 4.9, 3.0 Hz, 1H), 4.79 (td, *J* = 6.9, 3.2 Hz, 1H), 4.60 (dddd, *J* = 16.8, 10.2, 5.2, 2.3 Hz, 2H), 4.29 (ddd, *J* = 6.5, 3.2, 1.6 Hz, 1H), 4.22 (tdd, *J* = 7.0, 3.6, 1.6 Hz, 1H), 3.88 (d, *J* = 4.9 Hz, 1H), 3.79 – 3.74 (m, 5H), 3.50 – 3.42 (m, 1H), 3.38 – 3.34 (m, 1H), 3.33 (d, *J* = 1.4 Hz, 3H), 2.84 (t, *J* = 7.3 Hz, 2H), 2.56 (tt, *J* = 7.7, 2.0 Hz, 2H), 2.33 (s, 2H), 1.62 (t, *J* = 6.9 Hz, 2H), 1.57 (s, 3H), 1.36 (s, 3H), 1.11 – 1.01 (m, 27H); ¹³C NMR (101 MHz, CDCl₃) δ 171.42, 162.11, 162.10, 159.48, 150.72, 150.67, 140.23, 140.15, 140.12, 140.00, 136.92, 135.43, 135.31, 133.93, 133.88, 133.76, 133.61, 131.24, 129.36,

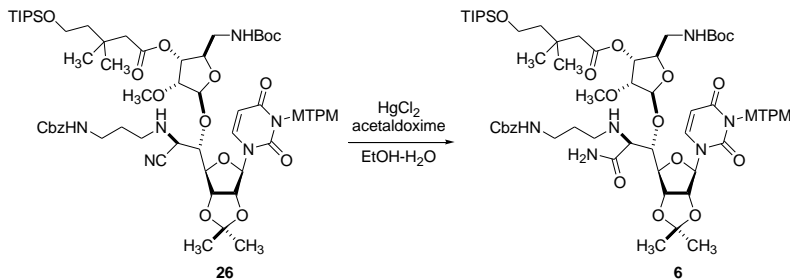
129.30, 128.47 (2C), 128.46 (2C), 128.39 (2C), 126.47, 126.45, 126.17, 126.12, 125.55, 125.44, 115.31, 115.28, 114.28, 104.40, 104.36, 101.81, 101.78, 94.95, 94.67, 88.70, 88.21, 88.01, 84.38, 84.30, 82.15, 81.37, 81.28, 80.02, 73.26, 73.20, 69.54, 69.41, 68.58, 68.49, 60.03, 58.51, 58.50, 55.66, 53.60, 53.55, 46.18, 44.51, 36.62, 34.49, 34.47, 32.64, 27.41, 27.39, 27.10, 25.38, 20.84, 18.03 (6C), 11.93 (3C) ; HRMS (ESI+) m/z calcd for $C_{59}H_{75}N_5O_{13}NaSiCl_4$ [M + Na] 1252.3782, found: 1252.3826.



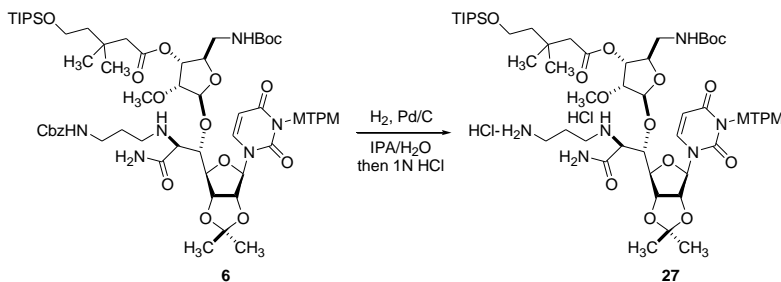
A suspended solution of **24** (0.60 g, 0.49 mmol), NH_4Cl (0.52 g, 9.8 mmol) and Zn (0.64 g, 9.8 mmol) in EtOH/ H_2O (9:1, 9.8 mL) was stirred at 80 °C for 8 h and cooled to rt. The precipitates were filtered and the combined organic solution was concentrated in vacuo. The crude mixture was used for the next reaction without purification. To a stirred solution of crude mixture in THF (9.8 mL) were added saturated NaHCO_3 (aq., 9.8 mL) and Boc_2O (0.32 g, 1.47 mmol). The reaction mixture was stirred for 6 h at rt, and the aqueous layer was extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography (hexanes/EtOAc 80:20 to 67:33) to afford **25** (0.57 g, 0.44 mmol, 90% for 2 steps): TLC (hexanes/EtOAc 67:33) R_f = 0.20; $[\alpha]_D^{22}$ -0.060 (c = 2.93, CHCl_3); IR (thin film) ν_{max} = 3387 (br), 2939, 2866, 1717, 1672, 1598, 1556, 1512, 1454, 1365, 1276, 1247, 1212, 1160, 1096, 1067, 1045, 881, 786, 680 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.53 (dd, J = 18.8, 8.8 Hz, 1H), 7.34 – 7.26 (m, 4H), 7.23 – 7.16 (m, 4H), 6.84 (d, J = 6.4 Hz, 2H), 6.50 (d, J = 3.7 Hz, 1H), 5.71 – 5.63 (m, 2H), 5.59 – 5.48 (m, 2H), 5.41 – 5.31 (m, 1H), 5.29 – 5.20 (m, 1H), 4.99 – 4.89 (m, 1H), 4.84 – 4.75 (m, 1H), 4.67 – 4.59 (m, 1H), 4.58 – 4.42 (m, 1H), 4.33 – 4.27 (m, 1H), 4.22 – 4.15 (m, 1H), 3.91 – 3.86 (m, 1H), 3.79 – 3.74 (m, 5H), 3.49 – 3.39 (m, 1H), 3.36 – 3.32 (m, 1H), 3.31 (d, J = 0.9 Hz, 2H), 3.22 (dt, J = 13.2, 6.1 Hz, 1H), 2.83 (t, J = 7.3 Hz, 2H), 2.57 (tt, J = 7.6, 2.2 Hz, 2H), 2.31 (s, 2H), 1.62 (t, J = 6.9 Hz, 2H), 1.57 (s, 3H), 1.41 (s, 9H), 1.37 (s, 3H), 1.10 – 1.01 (m, 27H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.39, 159.49, 156.27, 150.73, 140.02, 136.91, 135.37, 135.28, 133.96, 133.91, 131.23, 129.36, 129.32, 128.48 (2C), 128.34 (2C), 126.48, 126.13, 125.38, 115.32, 115.28, 114.17, 104.40, 101.84, 84.39, 84.31, 79.65, 69.37, 60.05, 58.50, 55.66, 46.19, 44.66, 34.49, 32.60, 28.40, 28.21 (3C), 27.40, 27.31, 27.12, 25.40, 20.81, 18.03 (6C), 11.94 (3C); HRMS (ESI+) m/z calcd for $C_{64}H_{85}N_3O_{15}NaSiCl_4$ [M + Na] 1326.4402, found: 1326.4432.



To a stirred solution of **25** (0.26 g, 0.20 mmol) and quinoline (46.4 μL , 0.39 mmol) in EtOAc (100 mL) was added Lindlar catalyst (100 mg). H_2 gas was introduced and the reaction mixture was stirred for 10 h under H_2 atmosphere at rt. The solution was filtered through Celite and washed with 1N HCl (aq.). The combined organic solution was dried over Na_2SO_4 , concentrated in vacuo. The crude mixture was used for the next reaction without purification. To a stirred solution of the crude mixture and NMO (0.23 g, 2.0 mmol) in t-BuOH/acetone (1:1, 5.0 mL) was added OsO_4 (4% in water, 2.5 mL, 0.39 mmol) at rt. The reaction mixture was stirred for 8 h, diluted with EtOAc and quenched with saturated NaHCO_3 aq./ saturated Na_2SO_3 aq. (2:1). The heterogeneous mixture was stirred for 30 min, extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was passed through a silica gel pad (hexanes/EtOAc 33:67) to afford diol. To a stirred solution of diol (0.21 g, 0.16 mmol) and NaHCO_3 (0.13 g, 1.56 mmol) in CH_2Cl_2 (1.6 mL) was added Pb(OAc)_4 (0.12 g, 0.28 mmol) at 0 $^\circ\text{C}$. The reaction mixture was stirred for 1 h at 0 $^\circ\text{C}$ and quenched with saturated NaHCO_3 aq., extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was used for the next reaction without purification. A CH_2Cl_2 (1.0 mL) solution of the crude mixture of **8**, Cbz-protected 1,3-diaminopropane (0.11 g, 0.47 mmol) and MgSO_4 (0.19 g, 1.56 mmol) were stirred at rt. After 3 h, the reaction was added TMSCN (58.4 μL , 0.47 mmol) and stirred for 20 h at rt. After completion, the reaction mixture was quenched with saturated NaHCO_3 aq., extracted with EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography (hexanes/EtOAc 67:33 to 50:50) to afford **26** (0.17 g, 0.12 mmol, 60% for 4 steps): TLC (hexanes/EtOAc 50:50) $R_f = 0.40$; $[\alpha]_D^{22} -0.034$ ($c = 0.17$, CHCl_3); IR (thin film) $\nu_{\text{max}} = 3359$ (br), 2927, 2865, 1716, 1678, 1600, 1525, 1456, 1367, 1251, 1070 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.45 (dd, $J = 17.6, 8.4$ Hz, 1H), 7.41 – 7.28 (m, 5H), 7.24 – 7.16 (m, 4H), 6.88 (d, $J = 5.3$ Hz, 2H), 6.46 (d, $J = 12.9$ Hz, 1H), 6.03 – 5.96 (m, 1H), 5.74 – 5.68 (m, 1H), 5.58 – 5.48 (m, 2H), 5.43 (d, $J = 8.7$ Hz, 2H), 5.36 – 5.16 (m, 3H), 5.07 (dd, $J = 11.9, 6.7$ Hz, 3H), 5.00 (d, $J = 2.9$ Hz, 1H), 4.93 (t, $J = 6.6$ Hz, 1H), 4.86 (d, $J = 15.2$ Hz, 1H), 4.22 (d, $J = 4.2$ Hz, 2H), 4.18 (s, 2H), 4.03 (dd, $J = 7.8, 4.9$ Hz, 1H), 3.84 – 3.74 (m, 3H), 3.46 – 3.16 (m, 5H), 3.04 (q, $J = 5.7$ Hz, 1H), 2.99 – 2.88 (m, 1H), 2.88 – 2.73 (m, 1H), 2.73 – 2.47 (m, 2H), 2.38 – 2.25 (m, 2H), 1.69 (t, $J = 5.8$ Hz, 2H), 1.59 (s, 3H), 1.47 – 1.37 (m, 12H), 1.11 – 1.02 (m, 27H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.11, 159.62, 156.44, 156.22, 142.48, 136.86, 136.81, 134.66, 134.30, 134.06, 131.10, 129.55, 128.68 (2C), 128.50 (2C), 128.44, 128.35, 128.17, 128.12, 128.08, 126.45, 125.01, 115.44, 114.18, 108.03, 101.87, 98.74, 84.87, 82.92, 82.30, 79.06, 66.57, 60.37, 60.09, 58.48, 55.72, 51.72, 46.22, 45.29, 44.71, 41.29, 32.61, 29.68, 28.47 (3C), 28.45, 28.37, 28.35, 27.37, 27.29, 26.95, 25.19, 21.02, 18.06 (6C), 14.19, 14.09, 11.96 (3C); HRMS (ESI+) m/z calcd for $\text{C}_{67}\text{H}_{93}\text{N}_6\text{O}_{17}\text{SiCl}_4$ [M + H] 1421.5121, found: 1421.5131.

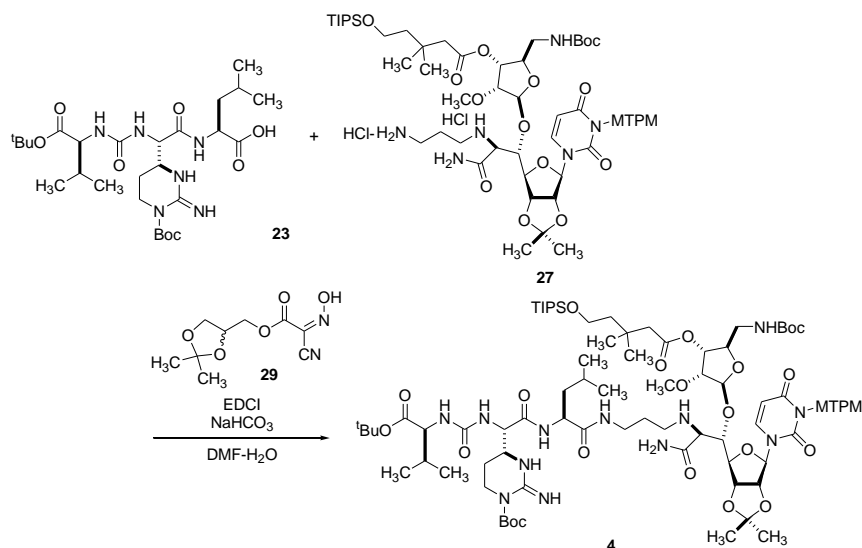


To a stirred solution of **26** (0.17 g, 0.12 mmol) in EtOH/H₂O (9:1, 2.4 mL) were added HgCl₂ (64.1 mg, 0.24 mmol) and acetaldoxime (72.0 μL, 1.18 mmol) at rt. The reaction mixture was stirred for 6 h at rt, added E₃N (49.4 μL, 0.35 mmol) and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (CHCl₃/MeOH 98:2 to 95:5) to afford **6** (0.12 g, 0.082 mmol, 69%): TLC (CHCl₃/MeOH 95:5) *R_f* = 0.40; [α]_D²¹ +0.198 (*c* = 0.32, MeOH); IR (thin film) ν_{max} = 3334 (br), 2928, 2865, 1715, 1701, 1677, 1524, 1456, 1367, 1250, 1100, 1071, 1047, 882 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) δ 7.64 (d, *J* = 8.5 Hz, 1H), 7.56 – 7.50 (m, 1H), 7.40 (dt, *J* = 6.0, 3.0 Hz, 1H), 7.37 – 7.31 (m, 5H), 7.28 (ddd, *J* = 8.4, 5.3, 2.6 Hz, 2H), 6.96 (d, *J* = 23.0 Hz, 2H), 6.55 (d, *J* = 69.8 Hz, 1H), 5.75 – 5.68 (m, 1H), 5.64 (dd, *J* = 15.7, 1.8 Hz, 1H), 5.61 – 5.52 (m, 2H), 5.28 (ddd, *J* = 27.8, 6.7, 3.4 Hz, 1H), 5.13 – 5.01 (m, 4H), 4.94 – 4.81 (m, 1H), 4.35 (dd, *J* = 21.6, 9.5 Hz, 1H), 4.24 (d, *J* = 7.6 Hz, 1H), 4.14 (tq, *J* = 5.6, 2.7 Hz, 1H), 3.91 (dd, *J* = 7.0, 4.9 Hz, 1H), 3.83 (td, *J* = 6.8, 1.5 Hz, 2H), 3.80 (d, *J* = 13.5 Hz, 3H), 3.43 – 3.38 (m, 1H), 3.36 (d, *J* = 3.5 Hz, 3H), 3.22 (t, *J* = 6.8 Hz, 2H), 3.18 – 3.10 (m, 1H), 2.72 – 2.59 (m, 1H), 2.58 – 2.46 (m, 1H), 2.35 (dd, *J* = 13.5, 1.1 Hz, 1H), 2.32 – 2.27 (m, 1H), 1.69 – 1.60 (m, 4H), 1.58 (s, 3H), 1.45 (d, *J* = 4.7 Hz, 2H), 1.42 (d, *J* = 2.8 Hz, 9H), 1.40 (d, *J* = 8.4 Hz, 3H), 1.13 – 1.05 (m, 27H); ¹³C NMR (101 MHz, CD₃OD) δ 172.91, 164.65, 161.41, 158.91, 158.26, 152.32, 138.54, 138.13, 135.21, 134.97, 132.64, 130.31, 129.51 (2C), 128.98, 128.81 (2C), 127.40, 126.71, 116.49, 115.31, 108.56, 102.45, 98.20, 85.76, 84.69, 84.24, 84.06, 81.34, 80.39, 79.47, 78.22, 77.69, 73.60, 70.88, 67.37, 63.12, 61.50, 58.75, 56.53, 47.09, 46.85, 45.39, 39.70, 35.70, 33.59, 32.75, 31.11, 28.92 (3C), 28.27, 28.22, 27.51, 25.76, 25.70, 23.70, 18.60 (6C), 13.22 (3C); HRMS (ESI+) *m/z* calcd for C₆₇H₉₅N₆O₁₈SiCl₄ [M + H] 1439.5226, found: 1439.5168.

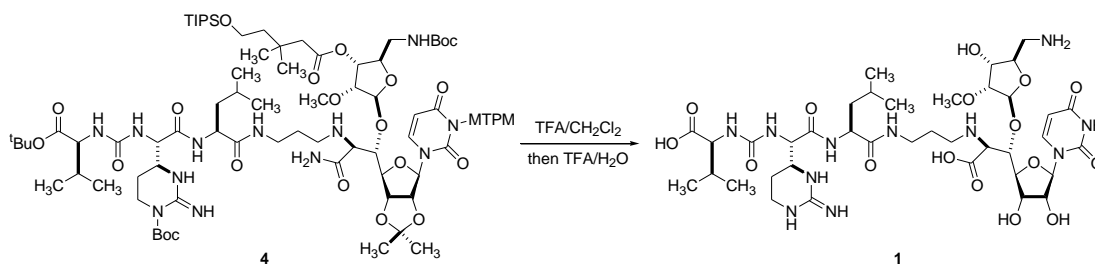


To a stirred solution of **6** (12.0 mg, 8.37 μmol) in IPA/H₂O/AcOH (95:5:1, 6.0 mL) was added Pd/C (10wt % 6.0 mg) under N₂ atmosphere. H₂ gas was introduced and the reaction mixture was stirred for 3 h under H₂. The solution was filtered through Celite and concentrated in vacuo. The crude mixture was used for the next reaction without purification: ¹H NMR (500 MHz, CD₃OD) δ 7.64 (d, *J* = 8.5 Hz, 1H), 7.56 – 7.50 (m, 1H), 7.40 (dd, *J* = 6.3, 2.1 Hz, 1H), 7.28 (ddd, *J* = 8.4, 5.3, 2.5 Hz, 2H), 6.96 (d, *J* = 23.0 Hz, 2H), 6.55 (d, *J*

= 69.8 Hz, 1H), 5.71 (dd, J = 8.0, 3.6 Hz, 1H), 5.64 (dd, J = 15.7, 1.8 Hz, 1H), 5.28 (ddd, J = 27.8, 6.7, 3.3 Hz, 1H), 5.14 – 5.01 (m, 1H), 4.35 (dd, J = 21.6, 9.5 Hz, 1H), 4.24 (q, J = 3.4 Hz, 1H), 4.19 – 4.09 (m, 1H), 3.91 (dd, J = 7.0, 4.9 Hz, 1H), 3.87 – 3.76 (m, 6H), 3.44 – 3.34 (m, 1H), 3.25 – 3.09 (m, 1H), 2.65 (tq, J = 16.8, 9.8, 8.3 Hz, 1H), 2.52 (ddd, J = 27.7, 12.2, 6.7 Hz, 1H), 2.39 – 2.25 (m, 2H), 1.64 (ddt, J = 14.2, 7.6, 4.1 Hz, 2H), 1.58 (s, 3H), 1.46 – 1.35 (m, 12H), 1.15 – 1.03 (m, 21H); HRMS (ESI+) m/z calcd for $C_{59}H_{89}N_6O_{16}SiCl_4$ [$M + H$] 1305.4858, found: 1305.4887.

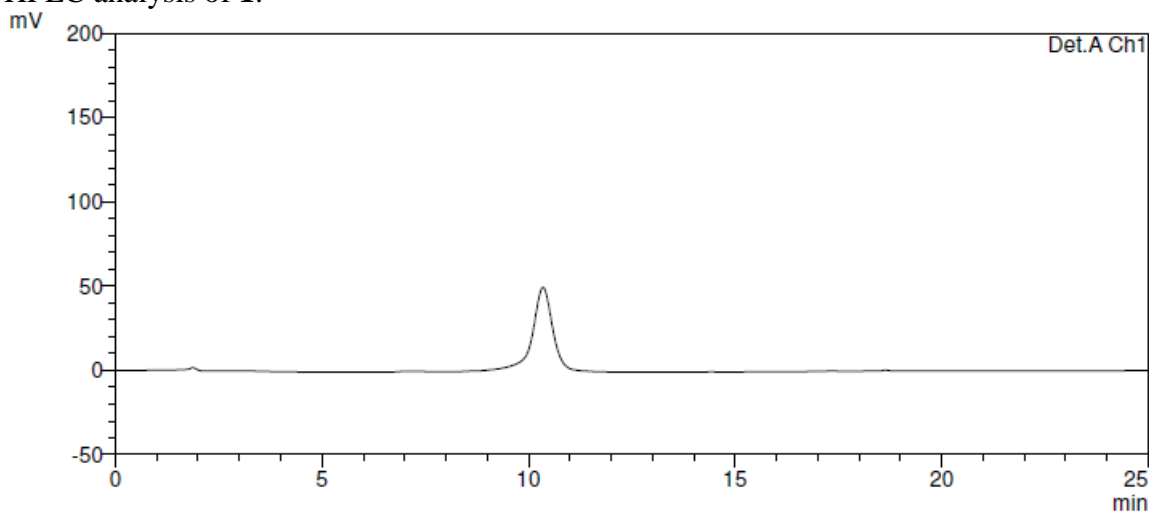


To a stirred solution of **27** (10.5 mg, 8.05 μ mol), **23** (9.4 mg, 0.016 mmol), $NaHCO_3$ (13.5 mg, 0.16 mmol) and **29** (9.1 mg, 0.040 mmol) in DMF/H_2O (25:1, 0.3 mL) was added EDCI (7.7 mg, 0.040 mmol). The reaction mixture was stirred for 3 h at rt, quenched with saturated $NaHCO_3$ (aq.), extracted with $CHCl_3$. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by silica gel column chromatography ($CHCl_3/MeOH$ 95:5 to 90:10) to afford desired **4** (13.8 mg, 7.33 μ mol, 91%): 1H NMR (500 MHz, CD_3OD) δ 7.74 – 7.46 (m, 2H), 7.41 (t, J = 2.9 Hz, 1H), 7.38 – 7.22 (m, 2H), 6.97 (d, J = 15.2 Hz, 2H), 6.57 (d, J = 62.4 Hz, 1H), 5.76 (t, J = 6.7 Hz, 1H), 5.70 (d, J = 15.1 Hz, 1H), 5.58 (q, J = 10.3 Hz, 2H), 5.28 (d, J = 27.5 Hz, 1H), 5.04 (d, J = 8.3 Hz, 1H), 4.92 (dd, J = 10.0, 4.3 Hz, 1H), 4.74 (dd, J = 23.4, 5.7 Hz, 1H), 4.51 (d, J = 4.8 Hz, 2H), 4.42 – 4.36 (m, 1H), 4.33 (q, J = 7.1 Hz, 2H), 4.24 (s, 1H), 4.17 – 4.07 (m, 2H), 4.04 (d, J = 4.0 Hz, 1H), 3.91 (d, J = 5.1 Hz, 1H), 3.88 – 3.78 (m, 6H), 3.72 – 3.62 (m, 1H), 3.54 – 3.43 (m, 2H), 3.43 – 3.34 (m, 2H), 2.76 – 2.49 (m, 2H), 2.38 – 2.28 (m, 2H), 2.22 – 2.07 (m, 2H), 2.04 (dq, J = 13.7, 4.5 Hz, 2H), 1.82 (dtd, J = 14.0, 9.3, 4.9 Hz, 4H), 1.70 – 1.56 (m, 10H), 1.48 (d, J = 3.1 Hz, 9H), 1.46 (d, J = 3.9 Hz, 19H), 1.45 – 1.38 (m, 4H), 1.09 (d, J = 4.4 Hz, 21H), 0.99 – 0.89 (m, 12H); HRMS (ESI+) m/z calcd for $C_{86}H_{135}N_{12}O_{23}SiCl_4$ [$M + H$] 1871.8286, found: 1871.8354.



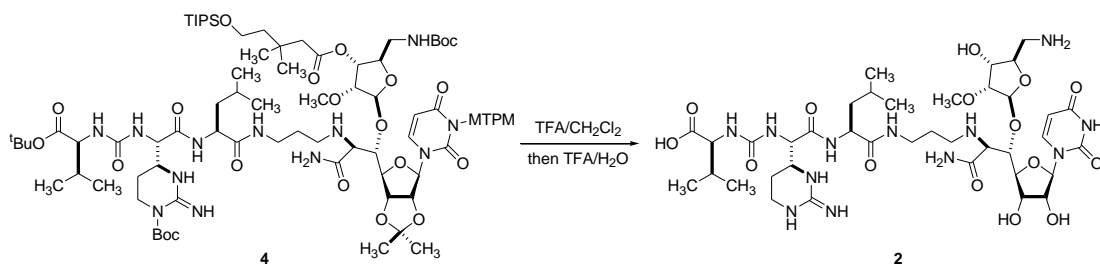
To a stirred solution of **4** (4.1 mg, 2.17 μmol) in CH_2Cl_2 (0.30 mL) was added TFA (0.20 mL). The reaction mixture was stirred for 5 h at rt, and all volatile were evaporated in vacuo. To a stirred solution of the crude mixture in H_2O (0.2 mL) was added TFA (0.3 mL). The reaction mixture was stirred for 20 h at 60 $^\circ\text{C}$, and all volatile were evaporated in vacuo. The crude mixture was purified by C18 reverse-phase HPLC [column: HYPERSIL GOLDTM (175 \AA , 12 μm , 250 x 10 mm), solvents: a gradient elution of 15:85 to 25:75 MeOH:0.1% TFA in H_2O over 20 min, flow rate: 2.0 mL/min, UV: 254 nm] to afford **1** (1.7 mg, 0.0018 mmol, 84%, retention time: 10 min): ^1H NMR (500 MHz, CD_3OD) δ 7.69 (d, J = 8.0 Hz, 1H), 5.78 (d, J = 8.1 Hz, 1H), 5.68 (d, J = 3.4 Hz, 1H), 5.25 (d, J = 2.1 Hz, 1H), 4.49 (s, 1H), 4.46 (t, J = 4.4 Hz, 1H), 4.38 (d, J = 5.0 Hz, 1H), 4.36 (d, J = 5.6 Hz, 1H), 4.34 (d, J = 3.9 Hz, 1H), 4.32 (d, J = 7.1 Hz, 1H), 4.30 (s, 2H), 4.23 (t, J = 5.3 Hz, 1H), 4.19 (d, J = 5.0 Hz, 2H), 4.13 – 4.07 (m, 2H), 3.85 – 3.80 (m, 2H), 3.50 (s, 3H), 3.46 – 3.41 (m, 4H), 2.91 – 2.87 (m, 2H), 2.21 – 2.13 (m, 2H), 2.07 (dt, J = 13.9, 5.9 Hz, 2H), 1.87 (s, 2H), 1.64 (t, J = 7.2 Hz, 2H), 1.02 – 0.85 (m, 12H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.76, 173.14, 171.45, 170.55, 159.89, 82.72, 79.49, 61.53, 60.15, 56.84, 52.91, 41.77, 38.25, 37.62, 36.24, 32.20, 28.90, 28.57, 28.50, 28.46, 28.40, 28.36, 28.33, 28.26, 28.21, 26.06, 26.03, 23.60, 21.92, 19.63, 18.59, 18.03, 17.27, 17.08, 14.57, 13.22; HRMS (ESI⁺) m/z calcd for $\text{C}_{38}\text{H}_{64}\text{N}_{11}\text{O}_{16}$ [$\text{M} + \text{H}$] 930.4533, found 930.4516.

HPLC analysis of **1**.



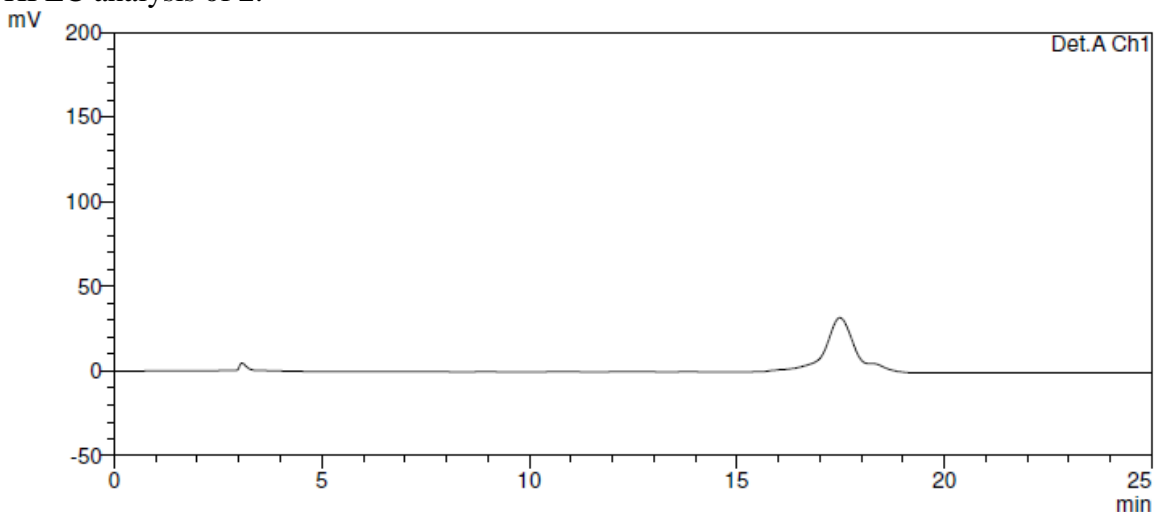
Conditions:

column: Phenomenex Kinetex 1.7 μ XB-C18 100 \AA 150 x 2.10 mm column, solvents: 25 : 75 MeOH : 0.1% TFA in water, UV: 254 nm



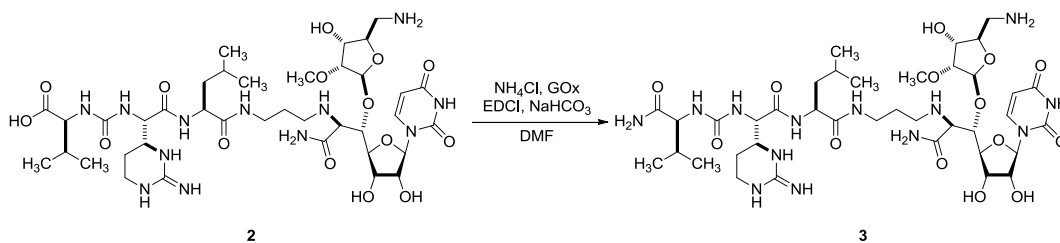
To a stirred solution of **4** (7.6 mg, 4.03 μmol) in CH_2Cl_2 (0.30 mL) was added TFA (0.20 mL). The reaction mixture was stirred for 6 h at rt, and all volatile were evaporated in vacuo. To a stirred solution of the crude mixture in H_2O (0.3 mL) was added TFA (0.2 mL). The reaction mixture was stirred for 4 h at rt, and all volatile were evaporated in vacuo. The crude mixture was purified by C18 reverse-phase HPLC [column: HYPERSIL GOLD™ (175 \AA , 12 μm , 250 x 10 mm), solvents: a gradient elution of 15 : 85 to 25 : 75 MeOH : 0.1% TFA in H_2O over 20 min, flow rate: 2.0 mL/min, UV: 254 nm], to afford **2** (3.3 mg, 0.0035 mmol, 88% retention time: 17.5 min): ^1H NMR (400 MHz, CD_3OD) δ 7.69 (d, $J = 8.0$ Hz, 1H), 5.78 (d, $J = 8.0$ Hz, 1H), 5.68 (d, $J = 3.4$ Hz, 1H), 5.27 – 5.24 (m, 1H), 4.50 – 4.48 (m, 1H), 4.46 (dd, $J = 5.0, 3.5$ Hz, 1H), 4.38 (d, $J = 4.4$ Hz, 1H), 4.36 (d, $J = 5.4$ Hz, 1H), 4.34 (d, $J = 3.5$ Hz, 1H), 4.33 – 4.31 (m, 1H), 4.30 – 4.29 (m, 2H), 4.25 – 4.22 (m, 1H), 4.19 (d, $J = 5.1$ Hz, 2H), 4.10 (d, $J = 7.1$ Hz, 2H), 3.84 – 3.81 (m, 2H), 3.50 (s, 3H), 3.46 – 3.42 (m, 4H), 2.93 – 2.91 (m, 2H), 2.20 – 2.13 (m, 2H), 2.09 – 2.02 (m, 2H), 1.92 – 1.83 (m, 2H), 1.68 – 1.60 (m, 2H), 1.02 – 0.88 (m, 12H); HRMS (ESI+) m/z calcd for $\text{C}_{38}\text{H}_{65}\text{N}_{12}\text{O}_{15}$ [M + H] 929.4692, found 929.4710.

HPLC analysis of **2**.



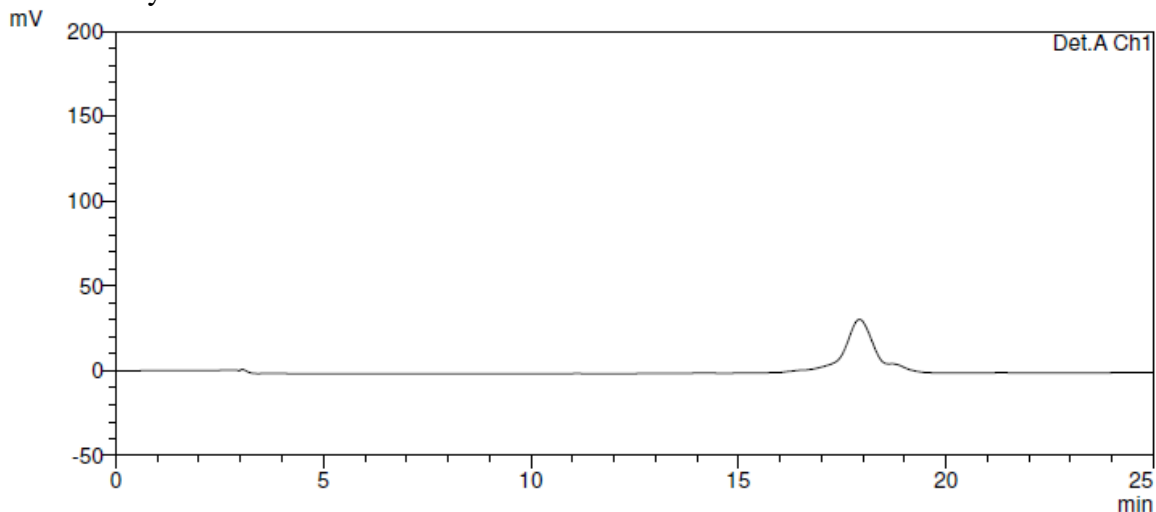
Conditions:

column: Phenomenex Kinetex 1.7 μ XB-C18 100 \AA 150 x 2.10 mm column, solvents: 25 : 75 MeOH : 0.1% TFA in water, UV: 254 nm



To a stirred solution of **2** (1.9 mg, 2.04 μmol), NH_4Cl (5.4 mg, 0.10 mmol), NaHCO_3 (2.6 mg, 0.030 mmol) and GOx (1.4 mg, 0.010 mmol) in DMF (0.2 mL) was added EDCI (1.9 mg, 0.010 mmol). The reaction mixture was stirred for 6 h at rt, filtered and concentrated in vacuo. The crude mixture was purified by C18 reverse-phase HPLC [column: HYPERSIL GOLD™ (175 \AA , 12 μm , 250 x 10 mm), solvents: a gradient elution of 15 : 85 to 25 : 75 MeOH : 0.1% TFA in H_2O over 20 min, flow rate: 2.0 mL/min, UV: 254 nm] to afford **3** (1.40 mg, 1.51 μmol , 75%, retention time: 18 min): ^1H NMR (500 MHz, CD_3OD) δ 7.70 (d, $J = 8.0$ Hz, 1H), 5.77 (d, $J = 8.0$ Hz, 1H), 5.27 – 5.23 (m, 1H), 4.48 (s, 1H), 4.44 (t, $J = 4.2$ Hz, 1H), 4.38 (d, $J = 4.9$ Hz, 1H), 4.37 (d, $J = 6.3$ Hz, 1H), 4.34 (d, $J = 6.3$ Hz, 1H), 4.32 (d, $J = 5.8$ Hz, 1H), 4.31 – 4.28 (m, 2H), 4.26 – 4.22 (m, 2H), 4.21 – 4.19 (m, 1H), 4.19 – 4.16 (m, 2H), 4.09 (dd, $J = 12.2, 6.1$ Hz, 2H), 3.85 – 3.78 (m, 1H), 3.49 (s, 3H), 3.46 – 3.41 (m, 4H), 2.92 – 2.89 (m, 2H), 2.16 (p, $J = 7.5, 6.5$ Hz, 2H), 2.10 – 1.99 (m, 2H), 1.91 – 1.81 (m, 2H), 1.67 – 1.57 (m, 2H), 1.02 – 0.85 (m, 12H); HRMS (ESI+) m/z calcd for $\text{C}_{38}\text{H}_{66}\text{N}_{13}\text{O}_{14}$ [$\text{M} + \text{H}$] 928.4852, found 928.4864.

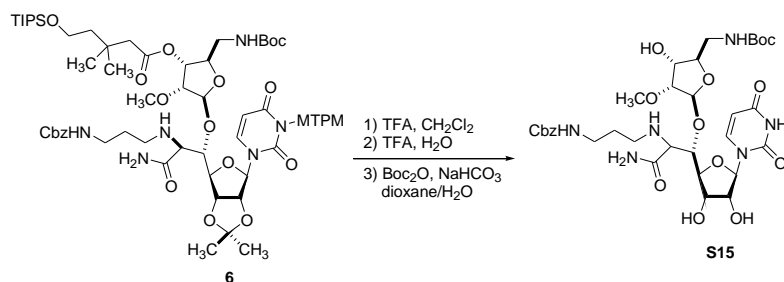
HPLC analysis of **3**.



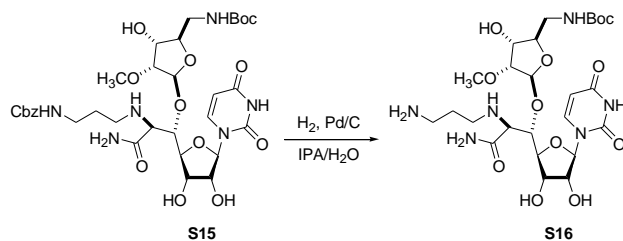
Conditions:

column: Phenomenex Kinetex 1.7 μ XB-C18 100 \AA 150 x 2.10 mm column, solvents: 25 : 75 MeOH : 0.1% TFA in water, UV: 254 nm

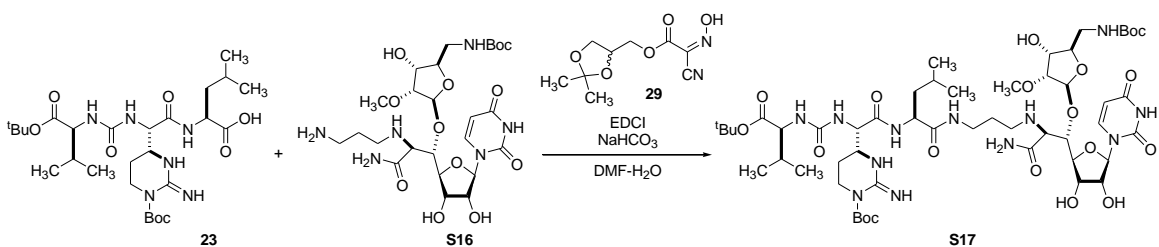
The following experiments were performed to characterize the key intermediates via ^1H -NMR and ^{13}C -NMR. **S15**, **S16**, **S17** do not show diastereomers caused by the MTPM group.



To a stirred solution of **6** (79.3 mg, 0.055 mmol) in CH₂Cl₂ (0.60 mL) was added TFA (0.40 mL). The reaction mixture was stirred for 3 h at rt, and all volatile were evaporated in vacuo. To a stirred solution of the crude mixture in H₂O (0.6 mL) was added TFA (0.4 mL). The reaction mixture was stirred for 3 h at rt, and all volatile were evaporated in vacuo. To a stirred solution of the crude mixture and NaHCO₃ (23.1 mg, 0.27 mmol) in dioxane/H₂O (5:1, 1.2 mL) was added Boc₂O (60.0 mg, 0.27 mmol). The reaction mixture was stirred for 2 h at rt, filtered and concentrated in vacuo. The crude product was purified by silica gel column chromatography (CHCl₃/MeOH 95:5 to 90:10 to 80:20) to afford **S15** (32.6 mg, 0.043 mmol, 79%): TLC (CHCl₃/MeOH 70:30) *R_f* = 0.30; [α]_D²¹ +0.137 (*c* = 0.53, MeOH); IR (thin film) ν_{\max} = 3336 (br), 2934, 1690, 1525, 1455, 1366, 1253, 1167 cm⁻¹; ¹H NMR (500 MHz, CD₃OD) δ 7.90 (d, *J* = 8.1 Hz, 1H), 7.39 – 7.32 (m, 5H), 7.31 – 7.26 (m, 1H), 5.81 (d, *J* = 2.7 Hz, 1H), 5.77 (d, *J* = 8.1 Hz, 1H), 5.16 (d, *J* = 2.7 Hz, 1H), 5.07 (s, 2H), 4.21 – 4.13 (m, 3H), 4.08 (t, *J* = 5.0 Hz, 1H), 4.06 – 4.01 (m, 1H), 3.93 (q, *J* = 5.2 Hz, 1H), 3.68 – 3.60 (m, 1H), 3.43 (d, *J* = 5.9 Hz, 2H), 3.40 (s, 3H), 3.35 (d, *J* = 0.9 Hz, 1H), 3.28 (d, *J* = 5.6 Hz, 1H), 3.21 (td, *J* = 6.7, 2.8 Hz, 2H), 2.64 (hept, *J* = 6.1, 5.1 Hz, 2H), 1.69 (p, *J* = 6.8 Hz, 2H), 1.44 (s, 9H); ¹³C NMR (101 MHz, CD₃OD) δ 142.36, 129.46 (2C), 128.93, 128.79 (2C), 108.22, 102.74, 91.85, 85.90, 85.28, 84.48, 81.23, 80.36, 75.39, 71.95, 71.32, 67.38, 65.09, 58.56, 46.58, 43.84, 39.73, 31.08, 28.87 (3C); HRMS (ESI+) *m/z* calcd for C₃₃H₄₉N₆O₁₄ [M + H] 753.3307, found: 753.3340.

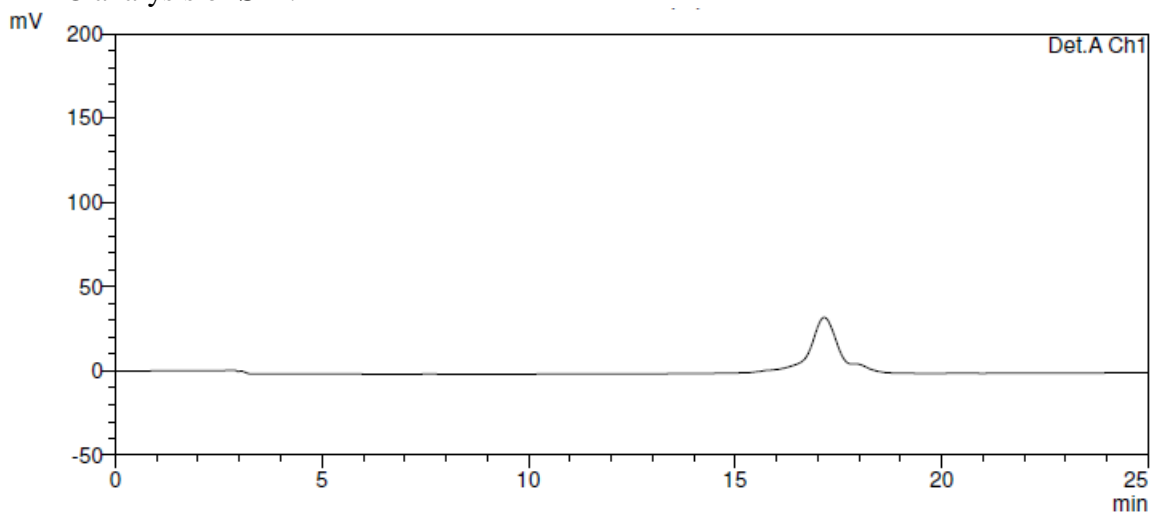


To a stirred solution of **S15** (19.0 mg, 0.025 mmol) in IPA/H₂O (9:1, 1.0 mL) was added Pd/C (10wt % 9.5 mg) under N₂ atmosphere. H₂ gas was introduced and the reaction mixture was stirred for 2 h under H₂. The solution was filtered through Celite and concentrated in vacuo. The crude mixture was use for the next reaction without purification.



To a stirred solution of **S16** (3.7 mg, 0.006 mmol), **23** (14.7 mg, 0.025 mmol), NaHCO₃ (10.6 mg, 0.13 mmol) and **29** (5.8 mg, 0.025 mmol) in DMF/H₂O (25:1, 0.4 mL) was added EDCI (14.5 mg, 0.076 mmol). The reaction mixture was stirred for 4 h at rt, filtered and concentrated in vacuo. The crude mixture was purified by C18 reverse-phase HPLC [column: HYPERSIL GOLD™ (175 Å, 12 μm, 250 x 10 mm), solvents: elution of 70 : 30 MeOH : 0.05M NH₄HCO₃ in H₂O, flow rate: 2.0 mL/min, UV: 254 nm] to afford **S17** (4.8 mg, 0.004 mmol, 68%, retention time: 17 min): ¹H NMR (500 MHz, CD₃OD) δ 7.93 (d, *J* = 8.1 Hz, 1H), 5.82 (d, *J* = 3.1 Hz, 1H), 5.76 (d, *J* = 8.1 Hz, 1H), 5.15 (d, *J* = 2.3 Hz, 1H), 4.42 (d, *J* = 4.5 Hz, 1H), 4.33 – 4.28 (m, 1H), 4.20 – 4.13 (m, 5H), 4.09 (tt, *J* = 10.1, 5.1 Hz, 5H), 4.01 – 3.92 (m, 1H), 3.83 – 3.74 (m, 1H), 3.67 – 3.62 (m, 1H), 3.46 – 3.44 (m, 4H), 3.41 (s, 3H), 3.19 – 3.15 (m, 2H), 2.36 – 2.28 (m, 1H), 2.18 – 2.08 (m, 2H), 2.02 – 1.95 (m, 2H), 1.72 – 1.65 (m, 1H), 1.65 – 1.57 (m, 2H), 1.48 (s, 9H), 1.47 (s, 9H), 1.44 (s, 9H), 1.00 – 0.88 (m, 12H); HRMS (ESI⁺) *m/z* calcd for C₅₂H₈₉N₁₂O₁₉ [M + H] 1185.6367, found 1185.6391.

HPLC analysis of **S17**.



Conditions:

column: Phenomenex Kinetex 1.7 μ XB-C18 100 Å 150 x 2.10 mm column, solvents: 25 : 75 MeOH : 0.1% TFA in water, UV: 254 nm

Bacterial strains and growth of bacteria

Mycobacterium tuberculosis (H₃₇Rv) was obtained through BEI Resources, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH). *Mycobacterium smegmatis* (ATCC 607), *Klebsiella pneumoniae* (ATCC 8047), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter baumannii* (ATCC 19606), *Staphylococcus aureus* (ATCC 6538D-5), *Clostridium difficile* (ATCC 700057), *Enterococcus faecium* (ATCC 349) and *E. coli* (ATCC 29425) were obtained from

American Type Culture Collection (ATCC). A single colony of *Mycobacterium* was obtained on Difco Middlebrook 7H10 nutrient agar enriched with 10% oleic acid, albumin, dextrose, and catalase (OADC) for *M. tuberculosis* by incubating for 15 days, and with albumin, dextrose, and catalase (ADC) for *M. smegmatis* by incubating for 48 h at 37°C in a static incubator. Seed cultures and larger cultures were obtained using Middlebrook 7H9 broth enriched with OADC (for *M. tuberculosis*) by incubating for 15 days and ADC (for *M. smegmatis*) by incubating for 48h at 37°C in a shaking incubator (200rpm). Single colonies of *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, *S.aureus*, *E. faecium* and *E. coli* were grown on tryptic soy agar for 24 h at 37°C in a static incubator and cultured in tryptic soy broth until log phase to be an optical density (OD) of 0.4-0.5. The OD was monitored at 600 nm using a 96-well microplate reader. A single colony of *C. difficile* was obtained on ATCC medium:

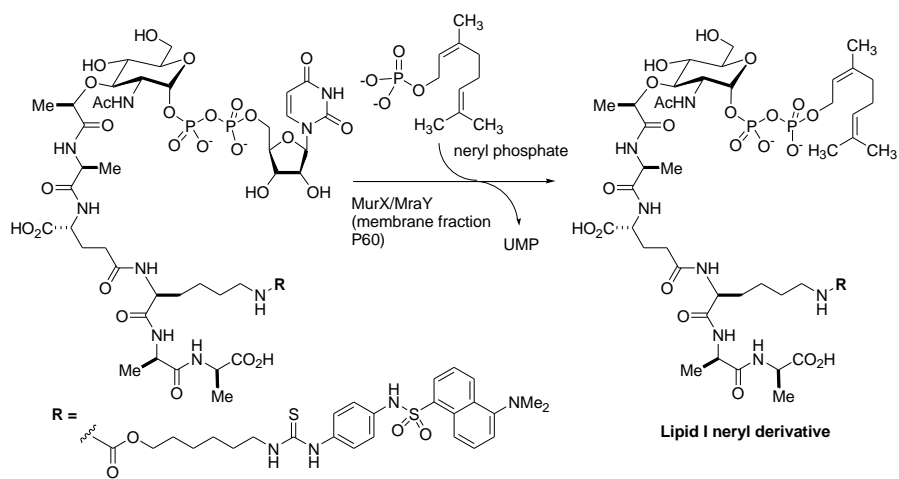
2107 Modified reinforced clostridial agar (pre-reduced), and liquid cultures were obtained in ATCC medium: 2107 Modified reinforced clostridial broth (pre-reduced) under anaerobic conditions.

Preparation of membrane fraction P-60 containing MurX/MraY and WecA

M. tuberculosis cells were harvested by centrifugation (4700 rpm) at 4 °C followed by washing with 0.9% saline solution (thrice), and approximately 5 g of pellet (wet weight) was collected. The washed cell pellets were suspended in homogenization buffer (containing 50 mM MOPS [pH = 8.0], 0.25 M sucrose, 10 mM MgCl₂, and 5 mM 2-mercaptoethanol) and disrupted by probe sonication on ice (10 cycles of 60 s on and 90 s off). The resulting suspension was centrifuged at 1,000 xg for 10 min at 4 °C to remove unbroken cells. The supernatant was centrifuged at 25,000 xg for 40 min at 4 °C (3 or 4 times). All pellets in each tube were pooled, and a second sonication was performed (10 cycles of 60 s on and 90 s off). The lysate was centrifuged once at 25,000 xg for 1 h, and the supernatant was subjected to ultracentrifugation at 60,000 xg for 1 h at 4 °C. The supernatant was discarded, and the membrane fraction containing MurX enzyme (P-60) was suspended in the Tris-HCl buffer (pH = 7.5) containing 2-mercaptoethanol. Total protein concentrations were approximately 8 to 10 mg/mL. Aliquots were stored in Eppendorf tubes at -80 °C. Similarly, the membrane fractions containing MraY and WecA enzyme (P-60) were prepared from *M. smegmatis* and *E. coli*, respectively.

MurX/MraY assay

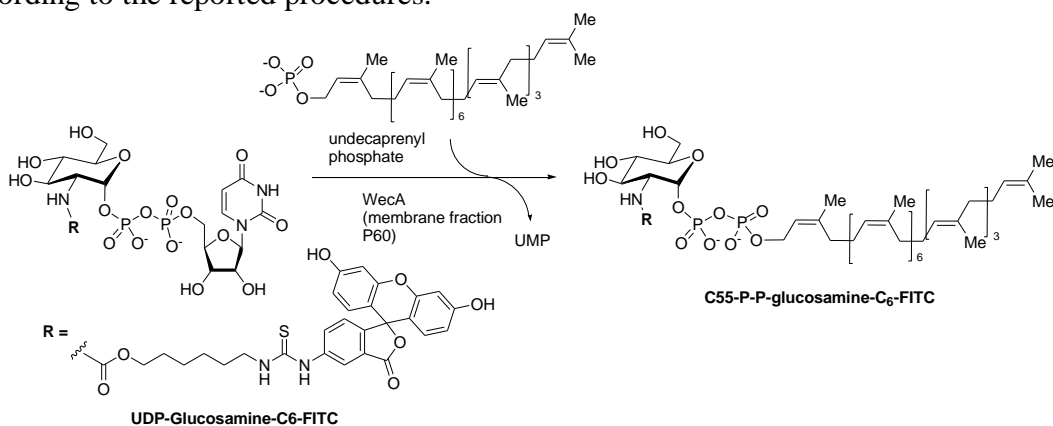
MurX/MraY assay substrates, Park's nucleotide-*N*⁶-C6-dansyl, neryl phosphate, were chemically synthesized according to the reported procedures.³



Park's nucleotide- N^6 -C6-dansyl (2 mM stock solution, 3.75 μ L [75 μ M]), $MgCl_2$ (0.5 M, 10 μ L [50mM]), KCl (2 M, 10 μ L [200mM]), Triton X-100 (0.5%, 11.25 μ L), Tris buffer (pH 8.0, 50 mM, 2.5 μ L), neryl phosphate (6, 10 mM, 45 μ L), and inhibitor (0 - 100 μ M, in DMSO, [2.5 μ L]) were placed in a 500 μ L Eppendorf tube. To a stirred reaction mixture, P-60 (15 μ L) was added (total volume of reaction mixture: 100 μ L). The reaction mixture was incubated for 1 h at room temperature (26 $^{\circ}C$) and quenched with $CHCl_3$ (200 μ L). Two phases were mixed via vortex and centrifuged at 25,000 xg for 10 min. The upper aqueous phase was assayed via reverse-phase HPLC. The water phase (10 μ L) was injected into HPLC (solvent: $CH_3CN/0.05$ M aq. $NH_4HCO_3 = 25:75$; UV: 350 nm; flow rate: 0.5 mL/min; column: Kinetex 5 μ m C8, 100 A, 150 x 4.60 mm), and the area of the peak for lipid I-neryl derivative was quantified to obtain the IC_{50} value. The IC_{50} values were calculated from plots of the percentage product inhibition versus the inhibitor concentration.

WecA assay

WecA assay substrate, UDP-Glucosamine-C6-FITC was chemically synthesized according to the reported procedures.⁴



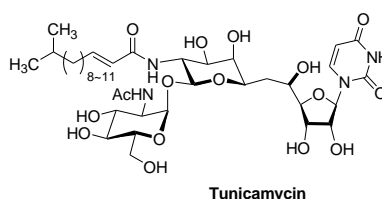
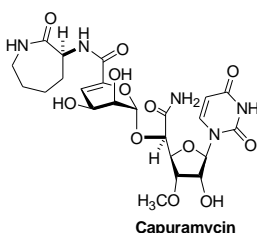
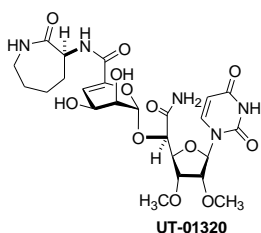
UDP-Glucosamine-C6-FITC (2 mM stock solution, 0.56 μ L), $MgCl_2$ (0.5 M, 4 μ L), β -mercaptoethanol (50 mM, 5 μ L), CHAPS (5%, 11.25 μ L), Tris buffer (pH 8.0, 50 mM, 6.19 μ L), undecaprenyl phosphate (4 mM, 2.5 μ L), and synthesized compounds (10 μ M,

in DMSO, 0.5 μL) were placed in a 500 μL Eppendorf tube. To a stirred reaction mixture, P-60 (20 μL) was added (total volume of reaction mixture: 50 μL). The reaction mixture was incubated for 2 h at 37 $^{\circ}\text{C}$ and quenched with n-butanol (150 μL). Two phases were mixed via vortex and centrifuged at 10,000 $\times g$ for 3 min. The upper organic phase was assayed via reverse-phase HPLC. The organic phase (25 μL) was injected into HPLC (solvent: gradient elution of 85:15 to 95:5 MeOH/0.05 M aq. NH_4HCO_3 ; UV: 485 nm; flow rate: 0.5 ml/ min; column: Kinetex 5 μm C8, 100 \AA , 150 x 4.60 mm), and the area of the peak for C55-P-P-glucosamine-C₆-FITC was quantified to obtain the IC_{50} value. The IC_{50} values were calculated from plots of the percentage product inhibition versus the inhibitor concentration.

MIC assays

Minimum inhibitory concentrations were determined by broth dilution microplate alamar blue assay. All compounds were stored in DMSO (1 mg/100 μL). This concentration was used as the stock solution for all MIC studies. Each compound (8 μL) from stock solution was placed in the first well of a sterile 96 well plate and a serial dilution was conducted with the culturing broth (total volume of 100 μL). The bacterial suspension at log phase (100 μL) was added to each well (total volume of 200 μL). *M. tuberculosis* plates were incubated for 15 days at 37 $^{\circ}\text{C}$ in a static incubator. for *M. smegmatis*, *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *E. faecium*, *C. difficile* and *E. coli* were incubated for 48hrs at 37 $^{\circ}\text{C}$. 20 μL of resazurin (0.02%) was added to each well and incubated in a shaking incubator (120 rpm) for 4 h for *M. tuberculosis*, 2hrs for *M. smegmatis*, and 1hr for *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *E. faecium*, *C. difficile* and *E. coli*. The MIC values were determined according to the National Committee for Clinical Laboratory Standards (NCCLS) method (pink = growth, blue = no visible growth). The absorbance of each well was also measured at 570 and 600 nm via a microplate reader.

| Compound | growth inhibition [MIC_{50} ($\mu\text{g/mL}$)] | | | | | | | | |
|--|---|---------------------|----------------------|----------------------|---------------------|------------------|-------------------|---------------------|----------------|
| | <i>M. tuberculosis</i> | <i>M. smegmatis</i> | <i>P. aeruginosa</i> | <i>K. pneumoniae</i> | <i>A. baumannii</i> | <i>S. aureus</i> | <i>E. faecium</i> | <i>C. difficile</i> | <i>E. coli</i> |
| Muraymycin D ₁ (1) | 1.56 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| Muraymycin D ₁ -amide (2) | 1.56 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| Muraymycin D ₁ -diamide (3) | 6.25 | >100 | >100 | >100 | >100 | >100 | >100 | >100 | >100 |
| Tunicamycin | 12.5 | - | - | - | - | - | - | - | - |
| Capuramycin | 6.25 | - | - | - | - | - | - | - | - |
| UT-01320 ⁵ | 1.25 | - | - | - | - | - | - | - | - |



Determination of cytotoxicity in Vero Cells

Selected molecules were tested for cytotoxicity (IC₅₀) in Vero cells via a MTT colorimetric assay. Vero cell line was cultured in Complete eagle's minimum essential growth medium (EMEM) containing L-glutamine, sodium pyruvate, minimum essential amino acids, penicillin-streptomycin and 10% fetal bovine serum. After 72h of exposure of molecules to this cell line at concentrations ranging from 0.4 to 400mg/mL, the culture medium was changed to complete EMEM without phenol red before addition of yellow tetrazolium dye; MTT. Viability was assessed on the basis of cellular conversion of MTT into a purple formazan product. The absorbance of the colored formazan product was measured at 600nm by BioTek Synergy HT Spectrophotometer.

| Compound | IC ₅₀ (μg/mL) |
|--|--------------------------|
| Muraymycin D ₁ (1) | >300 |
| Muraymycin D ₁ -amide (2) | >300 |
| Muraymycin D ₁ -diamide (3) | >300 |
| Tunicamycin | 0.62 |
| Capuramycin | >300 |
| UT-01320 | >300 |

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- (1) Aleiwi, B. A.; Schneider, C. M.; Kurosu, M. *J. Org. Chem.* **2012**, *77*, 3859-3867.
- (2) Wang, Y.; Kurosu, M. *Tetrahedron* **2012**, *68*,4797-4803.
- (3) (a) Siricilla, S.; Mitachi, K.; Skorupinska-Tudek, K.; Swiezewska, E.; Kurosu, M. *Anal. Biochem.* **2014**, *461*, 36-45.
- (4) (ref19b in the manuscript) Mitachi, K.; Siricilla, S.; Yang, D.; Kong, Y.; Skorupinska-Tudek, K.; Swiezewska, E.; Kurosu, M. *Anal. Biochem.* **2016**, accepted for publication.
- (5) Siricilla, S.; Mitachi, K.; Wan, B.; Franzblau, S. G.; Kurosu, M. *J. Antibiot.* **2015**, *68*, 271-278.

