

Supporting Information

for

Substrate Tolerance of Bacterial Glycosyltransferase MurG: Novel Fluorescence-based Assays

Katsuhiko Mitachi,^a Hyun Gi Yun,^b Cody D. Gillman,^b Karolina Skorupinska-Tudekc,^c
Ewa Swiezewskac,^c William M. Clemons Jr.,^b and Michio Kurosu*^a

^a Department of Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, 881 Madison Avenue, Memphis, TN 38163, USA

^b Division of Chemistry and Chemical Engineering, California Institute of Technology, 1200 E. California Bld. Pasadena, CA 91125, USA

^c Department of Lipid Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5a, 02-106 Warszawa, Poland

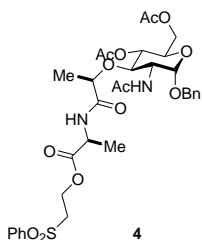
mkurosu@uthsc.edu

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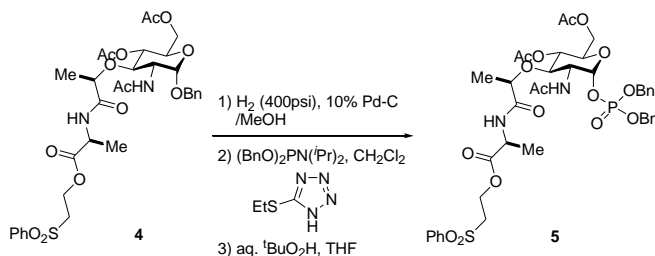
General

All chemicals were purchased from commercial sources and used without further purification unless otherwise noted. THF, CH₂Cl₂, 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 TLC using 0.25 mm coated commercial silica gel plates (EMD, Silica Gel 60F₂₅₄). TLC spots were visualized by UV light 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. 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 (¹³C-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 ¹³C NMR spectra were calibrated with residual undeuterated solvent (CDCl₃: δH = 7.26 ppm, δC = 77.16 ppm; CD₃CN: δH = 1.94 ppm, δC = 1.32 ppm; CD₃OD: δH = 3.31 ppm, δC = 49.00 ppm; DMSO-*d*₆: δH = 2.50 ppm, δC = 39.52 ppm; D₂O: 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. HPLC analyses were performed with a Shimadzu LC-20AD HPLC system. HR-MS data were obtained from a Waters Synapt G2-Si (ion mobility mass spectrometer with nanoelectrospray ionization).



2-(Phenylsulfonyl)ethyl ((R)-2-(((2S,3R,4R,5S,6R)-3-acetamido-5-acetoxy-6-(acetoxymethyl)-2-(benzyloxy)tetrahydro-2H-pyran-4-yl)oxy)propanoyl)-L-

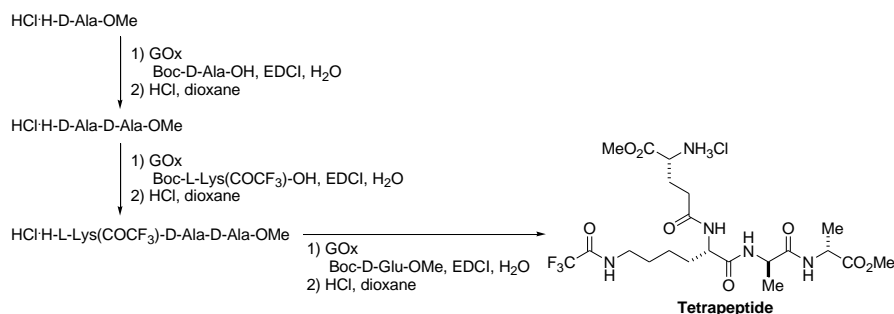
alaninate (4). The title compound was synthesized according to the reported procedure [1-3]: ¹H NMR (400 MHz, Chloroform-*d*) δ 7.92 (dd, *J* = 8.4, 1.2 Hz, 2H), 7.68 (tt, *J* = 7.4, 1.3 Hz, 1H), 7.61 – 7.56 (m, 2H), 7.41 – 7.34 (m, 3H), 7.34 – 7.30 (m, 2H), 6.86 (d, *J* = 6.9 Hz, 1H), 5.87 (d, *J* = 9.5 Hz, 1H), 5.06 (dd, *J* = 10.3, 9.2 Hz, 1H), 4.88 (d, *J* = 3.6 Hz, 1H), 4.69 (d, *J* = 11.7 Hz, 1H), 4.49 (d, *J* = 11.8 Hz, 1H), 4.43 (hex, *J* = 6.0 Hz, 2H), 4.36 (ddd, *J* = 10.3, 9.5, 3.7 Hz, 1H), 4.20 (dd, *J* = 12.3, 4.5 Hz, 1H), 4.13 (t, *J* = 7.1 Hz, 1H), 4.03 (dd, *J* = 12.3, 2.4 Hz, 1H), 3.95 (q, *J* = 7.0 Hz, 1H), 3.91 (ddd, *J* = 10.2, 4.6, 2.4 Hz, 1H), 3.64 (dd, *J* = 10.4, 9.2 Hz, 1H), 3.44 (qt, *J* = 6.3, 6.2 Hz, 2H), 2.11 (s, 3H), 2.07 (s, 3H), 1.88 (s, 3H), 1.30 (d, *J* = 6.8 Hz, 3H), 1.29 (d, *J* = 7.3 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 172.02, 171.60, 170.77, 170.22, 169.32, 139.08, 136.48, 134.07, 129.44 (2C), 128.74 (2C), 128.50, 128.33 (2C), 128.08 (2C), 97.02, 78.67, 78.45, 70.20, 69.30, 68.49, 62.06, 58.00, 54.87, 52.89, 47.95, 23.33, 20.85, 20.78, 18.56, 16.85; HRMS (ESI+) *m/z* calcd for C₃₃H₄₂N₂NaO₁₃S [M + Na] 729.2305, found: 729.2320.



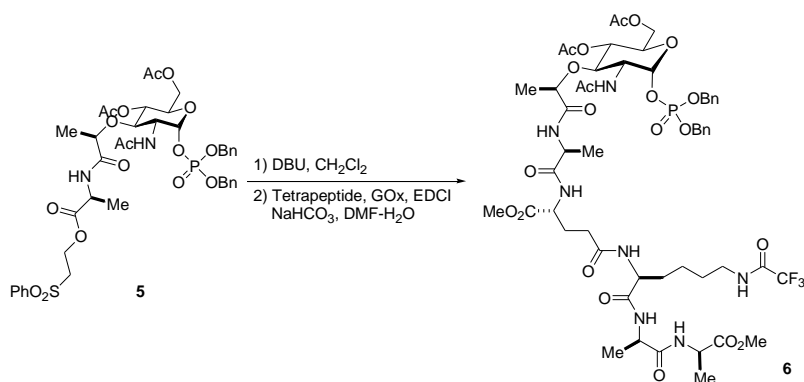
2-(Phenylsulfonyl)ethyl ((R)-2-(((2R,3R,4R,5S,6R)-3-acetamido-5-acetoxy-6-(bis(benzyloxy)phosphoryl)oxy)tetrahydro-2H-pyran-4-

yl)oxy)propanoyl)-L-alaninate (5). To a stirred solution of **4** (0.33 g, 0.46 mmol) in a 9:5:1 mixture of MeOH, formic acid and H₂O (15 mL) was added Pd-C (0.65 g). The reaction solution was stirred under hydrogen atmosphere (400 psi) for 17 h. The reaction mixture was filtrated and the residue was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (EtOAc/MeOH = 90/10) to afford the free-alcohol (0.26 g, 91%). To a stirred solution of the anomeric-free alcohol (0.21 g, 0.34 mmol) and 5-(ethylthio)-1*H*-tetrazole (0.13 g, 1.0 mmol) in CH₂Cl₂ (3.4 mL) was added dibenzyl *N,N*-diisopropylphosphoramidite (0.29 mL, 0.86 mmol) at 0 °C. After 2 h at 0 °C, the reaction was quenched with aq. saturated NaHCO₃ and extracted with CHCl₃. The combined organic solution was dried over Na₂SO₄ and concentrated *in vacuo*. To a stirred solution of the crude mixture and NaHCO₃ (58 mg, 0.69 mmol) in THF (3.4 mL), 70% aq. *tert*-butyl hydroperoxide (0.48 mL, 3.4 mmol) was added at 0 °C. After 30 min. at r.t., the reaction

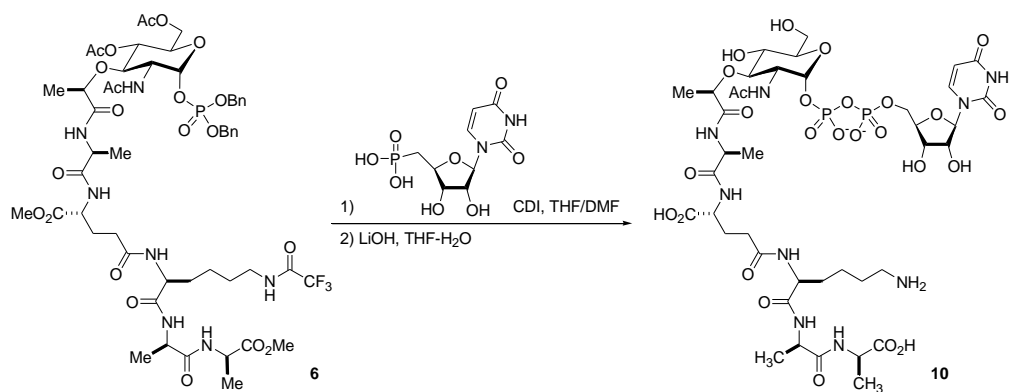
was quenched with aq. Na₂S₂O₃ and extracted with CHCl₃. The combined organic solution was dried over Na₂SO₄ and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (EtOAc/MeOH = 90/10) to afford 0.28 g (93% for 2 steps) of **5**: ¹H NMR (400 MHz, Chloroform-*d*) δ 7.92 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.68 (tt, *J* = 7.4, 1.3 Hz, 1H), 7.61 – 7.56 (m, 2H), 7.39 – 7.32 (m, 10H), 6.73 (d, *J* = 7.0 Hz, 1H), 6.12 (d, *J* = 9.0 Hz, 1H), 5.62 (dd, *J* = 5.8, 3.2 Hz, 1H), 5.14 – 4.97 (m, 6H), 4.45 (td, *J* = 6.2, 1.3 Hz, 2H), 4.33 (ddt, *J* = 10.6, 9.0, 3.1 Hz, 1H), 4.19 (t, *J* = 7.2 Hz, 1H), 4.10 (dd, *J* = 13.1, 4.6 Hz, 1H), 3.92 (d, *J* = 13.8 Hz, 1H), 3.89 (d, *J* = 9.8 Hz, 1H), 3.50 – 3.41 (m, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.75 (s, 3H), 1.30 (d, *J* = 6.9 Hz, 3H), 1.29 (d, *J* = 7.2 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 171.93, 171.51, 170.63, 170.60, 169.12, 139.05, 134.11 (2C), 129.47 (2C), 129.04 (2C), 128.80 (4C), 128.16 (2C), 128.08 (4C), 96.85, 96.78, 78.37, 70.15, 70.03, 69.98, 68.65, 61.45, 58.07, 54.84, 52.92, 52.85, 47.92, 22.97, 20.79, 20.65, 18.62, 17.00; ³¹P NMR (162 MHz, CDCl₃) δ -2.39 ; HRMS (ESI+) *m/z* calcd for C₄₀H₄₉N₂NaO₁₆PS [M + Na] 899.2438, found: 899.2412.



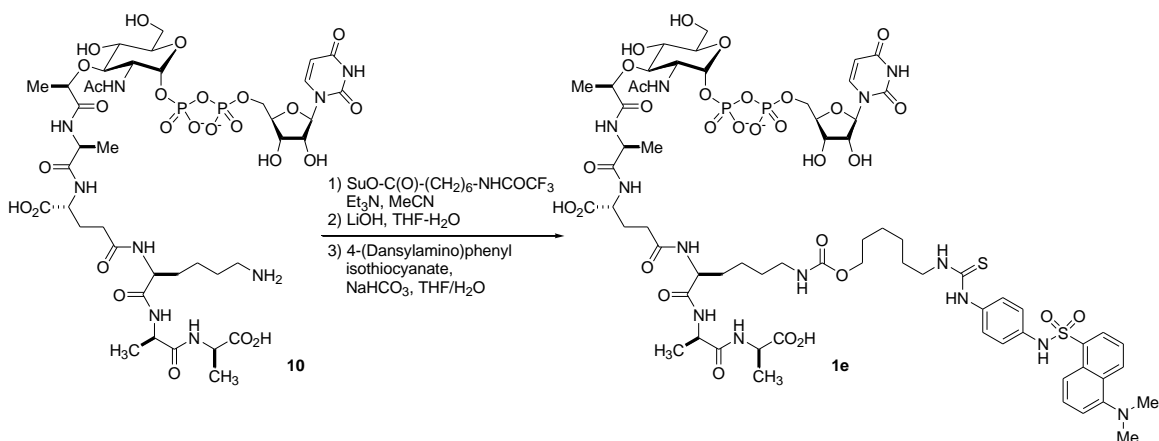
Tetrapeptide. Tetrapeptide was synthesized according to the reported procedure [4-6]: ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.45 (t, *J* = 5.8 Hz, 1H), 8.62 (s, 3H), 8.27 (d, *J* = 7.0 Hz, 1H), 8.23 (d, *J* = 8.1 Hz, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 4.34 – 4.25 (m, 1H), 4.26 – 4.18 (m, 2H), 3.99 (t, *J* = 6.7 Hz, 1H), 3.72 (s, 3H), 3.60 (s, 3H), 2.33 (ddt, *J* = 37.5, 15.2, 7.6 Hz, 2H), 2.05 – 1.94 (m, 2H), 1.64 – 1.40 (m, 6H), 1.29 (d, *J* = 7.2 Hz, 3H), 1.27 – 1.20 (m, 2H), 1.19 (d, *J* = 7.0 Hz, 3H); ¹³C NMR (101 MHz, DMSO) δ 173.87, 173.08, 172.26, 171.81, 170.74, 157.10 (q, *J* = 35.9 Hz), 116.96 (q, *J* = 288.3 Hz), 53.72, 53.62, 52.81, 52.45, 49.54, 48.58, 48.55, 32.49, 31.23, 28.89, 26.97, 23.51, 19.05, 17.72; HRMS (ESI+) *m/z* calcd for C₂₁H₃₅F₃N₅O₈ [M + H] 542.2438, found: 542.2457.



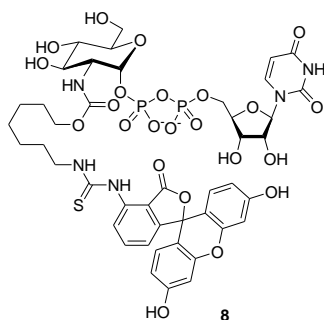
Methyl *N*²-(((*R*)-2-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-5-acetoxy-6-(acetoxymethyl)-2-((bis(benzyloxy)phosphoryl)oxy)tetrahydro-2*H*-pyran-4-yl)oxy)propanoyl)-*L*-alanyl)-*N*⁵-((*S*)-1-(((*R*)-1-(((*R*)-1-methoxy-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-1-oxo-6-(2,2,2-trifluoroacetamido)hexan-2-yl)-*D*-glutamate (6). To a stirred solution of **5** (78 mg, 0.089 mmol) in CH₂Cl₂ (0.45 mL) was added DBU (15 μL, 0.098 mmol). After being stirred for 1 h at r.t., the reaction was quenched with 1 M aq. HCl and extracted with EtOAc. The combined organic solution was dried over Na₂SO₄ and concentrated *in vacuo*. To a stirred solution of the crude mixture, tetrapeptide (0.10 g, 0.18 mmol) and NaHCO₃ (38 mg, 0.45 mmol) in 25:1 solution of DMF and H₂O (1.0 mL), GOx (41 mg, 0.18 mmol) and EDCI (34 mg, 0.18 mmol) were added. After being stirred for 2 h at r.t., the reaction was quenched with aq. saturated NaHCO₃ and extracted with EtOAc. The combined organic solution was dried over Na₂SO₄ and concentrated *in vacuo*. The crude mixture was purified by silica gel column chromatography (EtOAc/MeOH/Et₃N = 93/7/0.5 - 90/10/0.5) to afford 99 mg (90% for 2 steps) of **6**. ¹H NMR (400 MHz, Chloroform-*d*) δ 7.75 (t, *J* = 5.8 Hz, 1H), 7.60 (d, *J* = 7.7 Hz, 1H), 7.38 – 7.30 (m, 10H), 7.15 (d, *J* = 5.8 Hz, 1H), 7.10 (d, *J* = 8.5 Hz, 1H), 6.88 (d, *J* = 8.7 Hz, 1H), 5.64 (dd, *J* = 5.9, 3.2 Hz, 1H), 5.06 (ddd, *J* = 9.2, 8.4, 3.0 Hz, 6H), 4.54 – 4.30 (m, 7H), 4.24 (quin, *J* = 6.9 Hz, 1H), 4.15 – 4.09 (m, 2H), 3.91 (d, *J* = 10.6 Hz, 2H), 3.70 (s, 3H), 3.68 (s, 3H), 3.69 – 3.66 (m, 1H), 3.59 (t, *J* = 9.9 Hz, 1H), 3.31 (q, *J* = 6.5 Hz, 2H), 3.10 (q, *J* = 7.3 Hz, 1H), 2.38 – 2.13 (m, 4H), 2.07 (s, 3H), 1.99 (s, 3H), 1.78 (s, 3H), 1.58 (tt, *J* = 13.5, 6.2 Hz, 2H), 1.43 (d, *J* = 7.0 Hz, 3H), 1.38 (d, *J* = 7.4 Hz, 3H), 1.37 (d, *J* = 7.3 Hz, 3H), 1.29 (d, *J* = 6.6 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 173.32, 173.24, 172.73, 172.60, 172.18, 172.02, 171.94, 171.05, 170.57, 169.26, 157.45 (q, *J* = 36.8 Hz), 129.00 (2C), 128.76 (4C), 128.63, 128.56, 128.09 (4C), 70.02, 69.98, 68.63, 61.46, 53.82, 53.09, 53.01, 52.48, 52.29, 50.76, 50.27, 49.12, 48.03, 45.85, 39.42, 31.40, 31.16, 29.66, 28.16, 27.58, 22.78, 22.36, 20.79, 20.61, 18.54, 17.82, 17.64, 17.22, 8.57; ³¹P NMR (162 MHz, CDCl₃) δ -2.72; HRMS (ESI+) *m/z* calcd for C₅₃H₇₄F₃N₇O₂₁P [M + H] 1232.4628, found: 1232.4646.



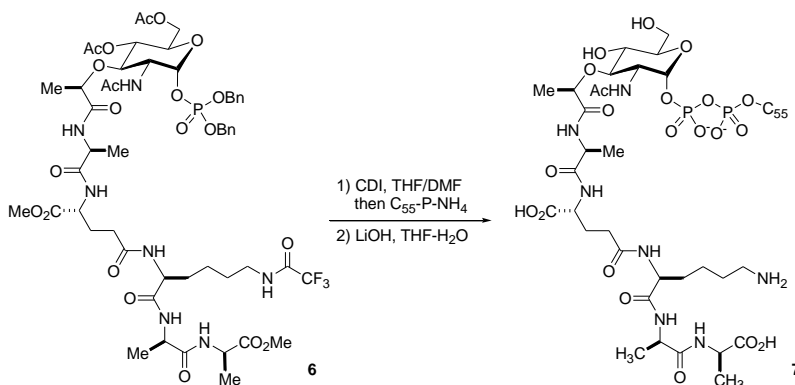
Park's nucleotide (10). To a stirred solution of **6** (7.5 mg, 6.1 μmol) in MeOH (10 mL) was added Pd-C (15 mg). After being stirred under hydrogen atmosphere (1 atm) for 1 h at r.t., Et₃N (0.5 mL) was added to the reaction mixture. After being stirred for 1 h at r.t., the catalyst was filtered through a pad of Celite. The filtrate solution of pentapeptide was concentrated *in vacuo* and the resulting product was dried in high vacuum. To a stirred solution of uridyl phosphate (62 mg, 0.15 mmol) in 1:1 solution of THF and DMF (2.0 mL) was added CDI (74 mg, 0.46 mmol). After being stirred for 14 h at r.t., MeOH (0.2 mL) was added to the reaction mixture. After being stirred for 1 h, the solution was concentrated *in vacuo* and the resulting product was dried in high vacuum. To a stirred solution of the crude product of pentapeptide in 4:1 solution of THF and DMF (2.0 mL) was added the crude product of uridyl phosphate. After being stirred for 48 h at r.t., the reaction mixture was filtered and concentrated *in vacuo*. To a stirred solution of the crude product in THF (1.7 mL) was added 1.7 mL of aq. LiOH (43.2 mg, 1.03 mmol). After being stirred for 2.5 h at r.t., the reaction mixture was filtered and concentrated *in vacuo*. The filtrate was purified by reverse phase HPLC [column: HYPERSIL GOLD™ (175 Å, 12 μm , 150 x 20 mm), solvents: 0 : 100 CH₃CN : 0.05 M aq. NH₄HCO₃ for 5 min then 5 : 95 CH₃CN : 0.05 M aq. NH₄HCO₃ for 10 min then 10 : 90 CH₃CN : 0.05 M aq. NH₄HCO₃ for 10 min, flow rate: 4.0 mL/min, UV: 254nm] to afford **10** (4.7 mg, 68% overall, retention time: 18.7 min): ¹H NMR (400 MHz, Deuterium Oxide) δ 7.95 (d, *J* = 8.1 Hz, 1H), 5.99 – 5.94 (m, 2H), 5.46 (dd, *J* = 7.2, 3.2 Hz, 1H), 4.37 – 4.34 (m, 2H), 4.31 (d, *J* = 7.2 Hz, 1H), 4.29 – 4.09 (m, 11H), 3.94 (ddd, *J* = 10.1, 2.3, 2.1 Hz, 1H), 3.89 – 3.81 (m, 2H), 3.78 (dd, *J* = 10.2, 9.0 Hz, 1H), 3.63 (dd, *J* = 10.2, 9.0 Hz, 1H), 2.99 (t, *J* = 7.5 Hz, 2H), 2.30 (t, *J* = 7.6 Hz, 2H), 2.16 (ddd, *J* = 12.8, 7.7, 4.8 Hz, 1H), 2.00 (s, 3H), 1.94 – 1.84 (m, 1H), 1.83 – 1.75 (m, 2H), 1.68 (dd, *J* = 8.6, 6.1 Hz, 2H), 1.43 (d, *J* = 7.3 Hz, 3H), 1.39 (d, *J* = 6.9 Hz, 3H), 1.36 (d, *J* = 6.9 Hz, 3H), 1.33 (d, *J* = 7.1 Hz, 3H); HRMS (ESI+) *m/z* calcd for C₄₀H₆₆N₉O₂₆P₂ [M + H] 1150.3594, found: 1150.3581.



Park's nucleotide-*N*^ε-C₆-dansyl (1e**).** To a stirred solution of **10** (6.3 mg, 5.5 μmol) and SuO-C(O)-(CH₂)₆-NHCOCF₃ (5.8 mg, 0.017 mmol) in MeCN (0.5 mL) was added Et₃N (3.9 μL, 0.028 mmol). After being stirred for 12 h at r.t., the reaction was concentrated *in vacuo*. To a stirred solution of the crude product in THF (0.5 mL) was added 0.2 mL of aq. LiOH (2.3 mg, 0.055 mmol). After being stirred for 3 h at r.t., the reaction mixture was filtered and concentrated *in vacuo*. The crude product was purified by reverse phase HPLC [column: HYPERSIL GOLD™ (175 Å, 12 μm, 150 x 20 mm), solvents: 0 : 100 CH₃CN : 0.05 M aq. NH₄HCO₃ for 5 min then 5 : 95 CH₃CN : 0.05 M aq. NH₄HCO₃ for 10 min then 10 : 90 CH₃CN : 0.05 M aq. NH₄HCO₃ for 10 min, flow rate: 4.0 mL/min, UV: 254nm]. To a stirred solution of the product and NaHCO₃ (4.6 mg, 0.055 mmol) in a 4 : 1 solution of THF and H₂O (0.5 mL) was added 4-(dansylamino)phenyl isothiocyanate (10.5 mg, 0.028 mmol). After being stirred for 4 h at r.t., the reaction mixture was filtered and concentrated *in vacuo*. The filtrate was purified by reverse phase HPLC [column: Phenomenex Luna (100 Å, 10 μm, C18, 250 x 10 mm), solvents: 10 : 90 CH₃CN : 0.05 M aq. NH₄HCO₃ for 5 min then 20 : 80 CH₃CN : 0.05 M aq. NH₄HCO₃ for 10 min then 30 : 70 CH₃CN : 0.05 M aq. NH₄HCO₃ for 10 min, flow rate: 3.0 mL/min, UV: 350nm] to afford **1e** (6.4 mg, 70% overall, retention time: 24.7 min): ¹H NMR (400 MHz, Deuterium Oxide) δ 8.55 (d, *J* = 8.2 Hz, 1H), 8.30 (d, *J* = 8.2 Hz, 1H), 8.15 (d, *J* = 7.4 Hz, 1H), 7.87 (d, *J* = 8.1 Hz, 1H), 7.32 (d, *J* = 7.2 Hz, 1H), 7.07 (s, 1H), 6.87 (s, 4H), 5.93 (ddd, *J* = 3.8, 1.6, 0.9 Hz, 1H), 5.89 (d, *J* = 8.0 Hz, 1H), 5.41 (dd, *J* = 7.7, 3.2 Hz, 1H), 4.30 (d, *J* = 3.3 Hz, 3H), 4.28 – 4.01 (m, 10H), 3.92 – 3.87 (m, 1H), 3.82 – 3.77 (m, 2H), 3.74 – 3.70 (m, 1H), 3.61 – 3.57 (m, 1H), 3.39 – 3.32 (m, 2H), 2.98 – 2.90 (m, 2H), 2.80 (s, 6H), 2.24 – 2.18 (m, 4H), 2.14 – 2.09 (m, 3H), 1.95 (s, 3H), 1.80 – 1.76 (m, 1H), 1.62 – 1.43 (m, 4H), 1.37 (d, *J* = 7.5 Hz, 3H), 1.34 (d, *J* = 7.1 Hz, 3H), 1.28 (d, *J* = 7.0 Hz, 3H), 1.26 (d, *J* = 6.9 Hz, 3H), 1.17 – 1.11 (m, 4H), 0.86 – 0.78 (m, 4H); HRMS (ESI+) *m/z* calcd for C₆₆H₉₆N₁₃O₃₀P₂S₂ [M + H] 1676.5303, found: 1676.5322.

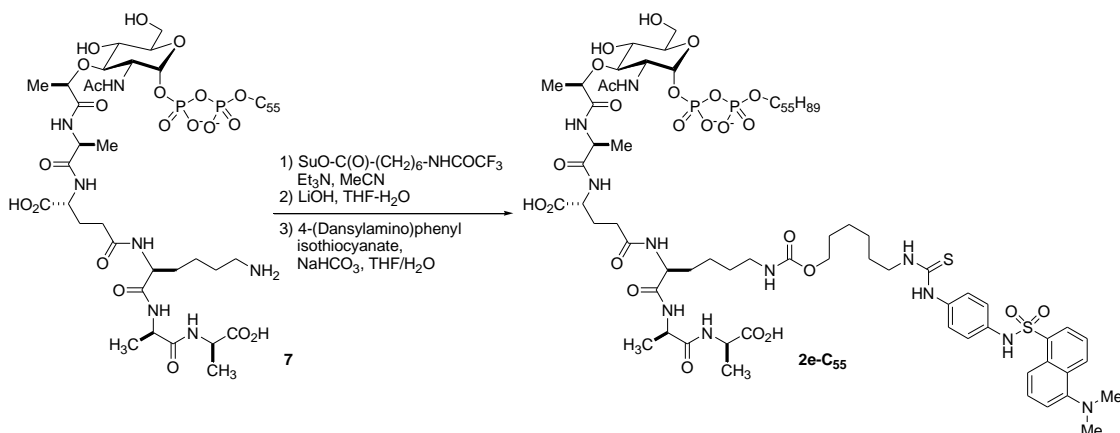


UDP-GlcN-C₆-FITC (8). The title compound was synthesized according to the reported procedure ^[7]: ¹H NMR (400 MHz, Deuterium Oxide) δ 7.90 (d, $J = 8.1$ Hz, 1H), 7.71 – 7.63 (m, 1H), 7.61 – 7.51 (m, 1H), 7.37 – 7.30 (m, 3H), 7.30 – 7.20 (m, 4H), 5.99 – 5.85 (m, 2H), 5.52 (d, $J = 6.3$ Hz, 1H), 4.35 – 4.27 (m, 3H), 4.26 – 4.13 (m, 2H), 4.12 – 4.00 (m, 2H), 3.92 – 3.84 (m, 1H), 3.80 (dd, $J = 16.2, 3.2$ Hz, 1H), 3.76 – 3.69 (m, 2H), 3.62 – 3.56 (m, 2H), 3.54 – 3.47 (m, 1H), 3.45 – 3.37 (m, 1H), 1.70 – 1.57 (m, 4H), 1.46 – 1.33 (m, 4H); HRMS (ESI⁺) m/z calcd for C₄₄H₅₁N₅NaO₂₃P₂S [M + H] 1134.2069, found: 1134.2084.



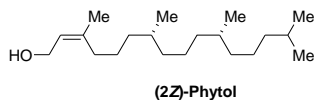
Lipid I (7). To a stirred solution of **6** (9.2 mg, 7.5 μ mol) in MeOH (10 mL) was added Pd-C (18 mg). After being stirred under hydrogen atmosphere (1 atm) for 1 h at r.t., Et₃N (0.5 mL) was added to the reaction mixture. After being stirred for 1 h at r.t., the catalyst was filtered through a pad of Celite. The filtrate solution was concentrated *in vacuo* and the resulting product was dried in high vacuum. To a stirred solution of the crude product in 3:1 solution of THF and DMF (0.5 mL) was added CDI (3.6 mg, 0.023 mmol). After being stirred for 3 h at r.t., MeOH (20 μ L) was added to the reaction mixture. After being stirred for 30 min, the solution was concentrated *in vacuo* and the resulting product was dried in high vacuum. To a stirred solution of the crude product in 3:1 solution of THF and DMF (0.5 mL) was added C₅₅-P-NH₄ (5.3 mg, 6.0 μ mol). After being stirred for 48 h at r.t., the reaction mixture was filtered and concentrated *in vacuo*. To a solution of the crude product in THF (0.5 mL) was added 0.05 mL of aq. LiOH (3.2 mg, 0.075 mmol). After being stirred for 3 h at r.t., the reaction mixture was filtered and concentrated *in vacuo*. The filtrate was purified by reverse phase HPLC [column: Phenomenex Luna (100 Å, 10 μ m, C18, 250 x 10 mm), solvents: a gradient elution of 85 : 15 to 100 : 0 MeOH : 0.05 M aqueous

NH₄HCO₃ over 30 min, flow rate: 3.0 mL/min, UV: 220nm] to afford **7** (retention time: 24.3 min). ¹H NMR (400 MHz, Methanol-*d*₄) δ 5.57 – 5.51 (m, 1H), 5.48 – 5.42 (m, 1H), 5.18 – 5.06 (m, 11H), 4.55 – 4.48 (m, 2H), 4.41 – 4.10 (m, 6H), 4.03 – 3.96 (m, 1H), 3.88 (q, *J* = 10.3 Hz, 2H), 3.74 (dd, *J* = 12.3, 5.1 Hz, 1H), 3.53 (t, *J* = 9.5 Hz, 1H), 2.99 – 2.88 (m, 2H), 2.79 (s, 1H), 2.31 (s, 3H), 2.14 – 2.03 (m, 31H), 1.99 (q, *J* = 7.8, 7.1 Hz, 8H), 1.90 – 1.78 (m, 2H), 1.74 (s, 3H), 1.68 (s, 21H), 1.63 – 1.58 (m, 10H), 1.47 (d, *J* = 7.2 Hz, 3H), 1.41 (t, *J* = 6.7 Hz, 6H), 1.36 (d, *J* = 7.2 Hz, 3H), 1.30 (d, *J* = 7.6 Hz, 8H), 0.89 (d, *J* = 8.1 Hz, 2H); HRMS (EI) calcd for C₈₆H₁₄₄N₇O₂₁P₂ ([M + H]⁺): 1672.9891, found: 1672.9908.

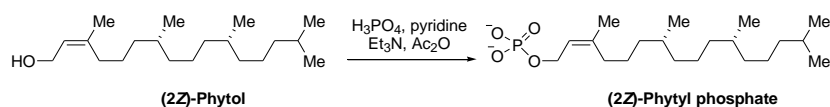


C₅₅-Lipid I-N^ε-C₆-dansyl (2e-C₅₅). To a stirred solution of **7** (4.4 mg, 2.6 μmol) and SuO-C(O)O-(CH₂)₆-NHCOCF₃ (4.6 mg, 0.013 mmol) in MeCN (0.5 mL) was added Et₃N (3.6 μL, 0.026 mmol). After being stirred for 12 h at r.t., the solution was concentrated *in vacuo*. To a stirred solution of the crude product in THF (0.5 mL) was added 0.05 mL of aq. LiOH (1.1 mg, 0.026 mmol). After being stirred for 1 h at r.t., the reaction mixture was filtered and concentrated *in vacuo*. The crude product was purified by reverse phase HPLC [column: Phenomenex Luna (100 Å, 10 μm, C18, 250 x 10 mm), solvents: a gradient elution of 85 : 15 to 100 : 0 MeOH : 0.05 M aqueous NH₄HCO₃ over 30 min, flow rate: 3.0 mL/min, UV: 220nm]. To a stirred solution of the product and NaHCO₃ (2.2 mg, 0.026 mmol) in 4 : 1 solution of THF and H₂O (0.5 mL) was added 4-(dansylamino)phenyl isothiocyanate (5.0 mg, 0.013 mmol). After being stirred for 4 h at r.t., the reaction mixture was filtered and concentrated *in vacuo*. The filtrate was purified by reverse phase HPLC [column: Phenomenex Luna (100 Å, 10 μm, C18, 250 x 10 mm), solvents: a gradient elution of 85 : 15 to 100 : 0 MeOH : 0.05 M aqueous NH₄HCO₃ over 30 min, flow rate: 3.0 mL/min, UV: 350nm] to afford **2e-C₅₅** (4.6 mg, 80% overall, retention time: 23.4 min): ¹H NMR (400 MHz, Methanol-*d*₄) δ 7.97 (s, 1H), 7.76 – 7.71 (m, 1H), 7.58 (d, *J* = 8.9 Hz, 1H), 7.32 (t, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 8.3 Hz, 1H), 7.02 (d, *J* = 7.5 Hz, 1H), 5.49 – 5.42 (m, 1H), 5.39 (t, *J* = 5.7 Hz, 1H), 5.31 – 5.25 (m, 1H), 5.12 – 4.99 (m, 11H), 4.46 (t, *J* = 6.4 Hz, 2H), 4.34 – 4.05 (m, 6H), 4.00 – 3.88 (m, 3H), 3.82 (d, *J* = 11.8 Hz, 1H), 3.61 (td, *J* = 14.4, 13.4, 6.5 Hz, 3H), 3.54 (s, 1H), 3.50 – 3.37 (m, 2H), 3.12 (q, *J* = 7.3 Hz, 2H), 3.04 – 2.94 (m, 1H), 2.29 – 2.15 (m, 4H), 2.07 – 1.88 (m, *J* = 8.8, 7.9 Hz, 41H), 1.66 (s,

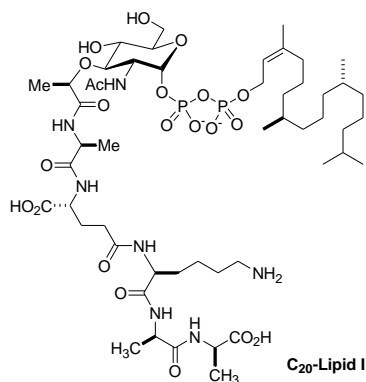
3H), 1.61 (s, 21H), 1.55 – 1.51 (m, 10H), 1.46 – 1.40 (m, 3H), 1.33 (dt, $J = 18.1, 7.1$ Hz, 12H), 1.22 (d, $J = 5.0$ Hz, 18H).



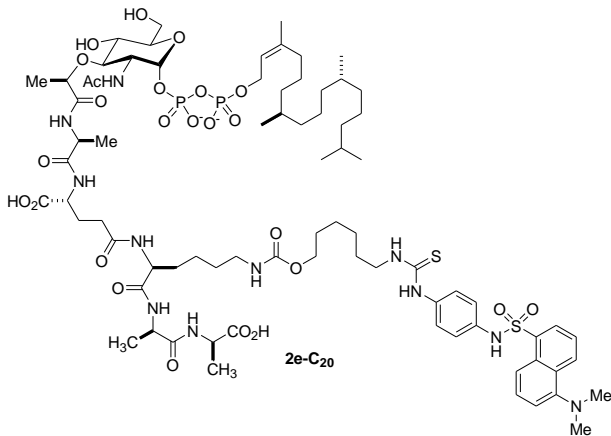
(2Z)-Phytol was purchased as an *E/Z* mixture, and used after purification by silica gel column chromatography (hexanes/EtOAc = 95/5): ^1H NMR (400 MHz, Chloroform-*d*) δ 5.41 (td, $J = 7.1, 1.4$ Hz, 1H), 4.13 (t, $J = 3.4$ Hz, 2H), 2.09 – 2.01 (m, 2H), 1.74 (d, $J = 1.1$ Hz, 3H), 1.56 – 1.46 (m, 1H), 1.45 – 1.21 (m, 14H), 1.16 – 1.01 (m, 4H), 0.89 – 0.82 (m, 12H); ^{13}C NMR (101 MHz, CDCl_3) δ 140.66, 123.97, 59.16, 39.39, 37.44, 37.37, 37.30, 36.86, 32.80, 32.70, 32.21, 27.99, 25.71, 24.80, 24.47, 23.45, 22.72, 22.63, 19.75, 19.69; HRMS (EI) calcd for $\text{C}_{20}\text{H}_{41}\text{O}$ ($[\text{M} + \text{H}]^+$): 297.3157, found: 297.3172.



(2Z)-Phytyl phosphate. To a stirred solution of phosphoric acid (9.80 mg, 0.10 mmol), pyridine (40.4 μL , 0.50 mmol), and (2Z)-phytol **S1** (126 mg, 0.42 mmol) was added Et_3N (27.9 μL , 0.20 mmol). After being stirred for 30 min, Ac_2O (18.9 μL , 0.20 mmol) was added to the reaction mixture. After being stirred for 24 h at 80 $^\circ\text{C}$, the reaction mixture was cooled to r.t., and the reaction was quenched with water (1 mL). After being stirred for 1 h at 80 $^\circ\text{C}$, the reaction mixture was cooled to r.t., and the aqueous solution was washed with ether. The aqueous solution was lyophilized, and the crude mixture was purified by DOWEX 50WX8 afforded **(2Z)-phytyl phosphate** (24.5 mg, 65%): ^1H NMR (400 MHz, Methanol-*d*₄) δ 5.41 (td, $J = 7.0, 1.5$ Hz, 1H), 4.42 (td, $J = 6.7, 1.2$ Hz, 2H), 3.20 (q, $J = 7.3$ Hz, 2H), 2.09 (ddd, $J = 8.3, 6.5, 3.4$ Hz, 2H), 1.73 (d, $J = 1.3$ Hz, 3H), 1.54 (dp, $J = 13.1, 6.6$ Hz, 1H), 1.46 – 1.01 (m, 16H), 0.95 – 0.78 (m, 12H); ^{13}C NMR (101 MHz, MeOD) δ 141.20, 123.10, 123.02, 63.10, 63.05, 40.54, 38.52, 38.50, 38.39, 37.95, 33.94, 33.22, 29.14, 26.70, 25.88, 25.51, 23.55, 23.08, 22.99, 20.18, 20.11, 9.19; ^{31}P NMR (162 MHz, MeOD) δ 1.08; HRMS (EI) calcd for $\text{C}_{20}\text{H}_{42}\text{O}_4\text{P}$ ($[\text{M} + \text{H}]^+$): 377.2821, found: 377.2838.



C₂₀-Lipid I. The title compound was synthesized according to the procedure described for Lipid I (**7**): ¹H NMR (400 MHz, Methanol-*d*₄) δ 5.54 (d, *J* = 7.3 Hz, 1H), 5.43 (q, *J* = 6.6 Hz, 1H), 4.54 (t, *J* = 6.6 Hz, 2H), 4.41 – 4.09 (m, 6H), 4.02 – 3.95 (m, 1H), 3.92 – 3.79 (m, 2H), 3.74 (dd, *J* = 12.1, 5.1 Hz, 1H), 3.53 (t, *J* = 9.5 Hz, 1H), 2.98 – 2.89 (m, 2H), 2.32 (s, 3H), 2.13 – 2.05 (m, 1H), 2.05 – 1.97 (m, 5H), 1.91 – 1.77 (m, 2H), 1.69 (s, 3H), 1.60 – 1.50 (m, 4H), 1.47 (d, *J* = 7.2 Hz, 4H), 1.45 – 1.35 (m, 14H), 1.35 – 1.22 (m, 10H), 1.19 – 1.04 (m, 4H), 0.88 (t, *J* = 6.4 Hz, 12H); HRMS (EI) calcd for C₅₁H₉₄N₇O₂₁P₂ ([M + H]⁺): 1202.5978, found: 1202.5995.



C₂₀-Lipid I-*N*^ε-C₆-dansyl (2e-C₂₀**).** The title compound was synthesized according to the procedure described for C₅₅-Lipid I-*N*^ε-C₆-dansyl (**2e-C₅₅**): ¹H NMR (400 MHz, Methanol-*d*₄) δ 8.55 (d, *J* = 8.2 Hz, 1H), 8.36 (d, *J* = 8.7 Hz, 1H), 8.19 (d, *J* = 7.4 Hz, 1H), 7.76 (d, *J* = 8.2 Hz, 1H), 7.57 (q, *J* = 7.5 Hz, 1H), 7.27 (d, *J* = 7.2 Hz, 1H), 6.96 (s, 4H), 5.41 – 5.21 (m, 2H), 4.88 – 4.81 (m, 2H), 4.38 – 4.29 (m, 2H), 4.12 (tdd, *J* = 19.8, 11.3, 5.6 Hz, 6H), 4.01 (dd, *J* = 10.9, 5.4 Hz, 1H), 3.91 (t, *J* = 6.5 Hz, 2H), 3.85 – 3.78 (m, 2H), 3.74 – 3.68 (m, 1H), 3.54 (dt, *J* = 10.4, 7.1 Hz, 2H), 3.50 – 3.46 (m, 1H), 3.35 (d, *J* = 1.2 Hz, 2H), 3.27 – 3.21 (m, 1H), 3.15 – 3.11 (m, 1H), 2.90 – 2.77 (m, 8H), 2.61 – 2.53 (m, 2H), 2.39 – 2.29 (m, 4H), 2.26 – 2.22 (m, 2H), 2.15 (t, *J* = 7.6 Hz, 2H), 2.06 (d, *J* = 5.5 Hz, 3H), 2.02 (d, *J* = 1.2 Hz, 3H), 1.89 (d, *J* = 1.2 Hz, 4H), 1.61 (q, *J* = 7.9 Hz, 2H), 1.53 (q, *J* = 6.4, 5.9 Hz, 2H), 1.41 (d, *J* = 1.2 Hz, 2H), 1.31 (dd, *J* = 9.1, 5.0 Hz, 26H), 0.90 (t, *J* = 6.6 Hz, 12H); HRMS (ESI⁺) *m/z* calcd for C₇₇H₁₂₄N₁₁O₂₅P₂S₂ [M + H] 1728.7687, found: 1728.7669.

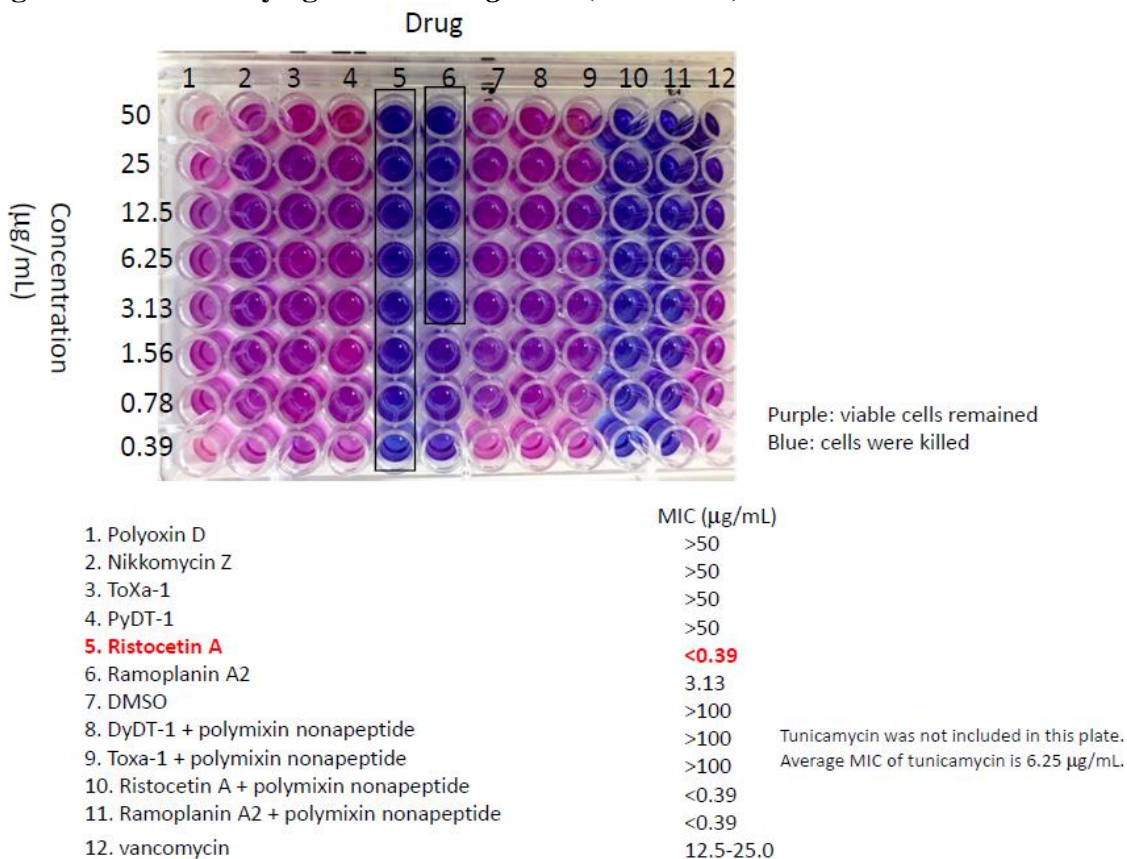
Bacterial strains and growth of bacteria

Mycobacterium smegmatis (ATCC 607), *Staphylococcus aureus* (BAA-1683), *Bacillus subtilis* (ATCC 6051), and *E. coli* (ATCC 10798) were obtained from American Type Culture Collection (ATCC). A single colony of *Mycobacterium smegmatis* was obtained on Difco Middlebrook 7H10 nutrient agar enriched with with albumin, dextrose, and catalase (ADC). Single colonies of *S.aureus* 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 0.2-0.5. The OD was monitored at 600 and 570 nm using a 96-well microplate reader. A single colony of *C. difficile* was obtained on a BHI agar plate and incubated at 37 °C under anaerobic conditions for 48 h (Gas: 10% hydrogen, 5% carbon dioxide and 85% nitrogen mixture was used. Chambers: Plas Labs™ Model 855 Anaerobic Chambers was used.). The other bacteria were cultured in the recommended conditions by ATCC.

MIC assays

Minimum inhibitory concentrations were determined by broth dilution microplate alamar blue assay or by OD measurement. All compounds were stored in DMSO or water (1 mg/100 µL concentration). This concentration was used as the stock solution for all MIC studies. Each compound 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 10 µL). The bacterial suspension at log phase (190 µL) was added to each well (total volume of 200 µL). *M. smegmatis*, *S. aureus*, *E. coli*, and the aerobic bacteria were incubated for 24 h (48-72 h for *M. smegmatis*) at 37 °C. 20 µL of resazurin (0.02%) was added to each well and incubated for 4 h for *Mycobacterium spp.*, and 1 h for the other bacteria (National Committee for Clinical Laboratory Standards (NCCLS) method (pink = growth, blue = no visible growth)). The OD measurements were performed for all experiments prior to colorimetric. The anaerobic bacteria were applied to a microplate reader. The MIC values were determined according to the colorimetric assays using resazurin. The absorbance of each well was also measured at 570 and 600 nm via UV-Vis. If necessary, CFU method was applied to confirm bactericidal activity. MIC against *M. tuberculosis* was performed at UTHSC RBL. Due to the compliance, images acquired in the BSL-3 in RBL are prohibited to show. The MIC data were recorded on laminated paper.

Figure S1. MIC assay against *M. smegmatis* (ATCC607).



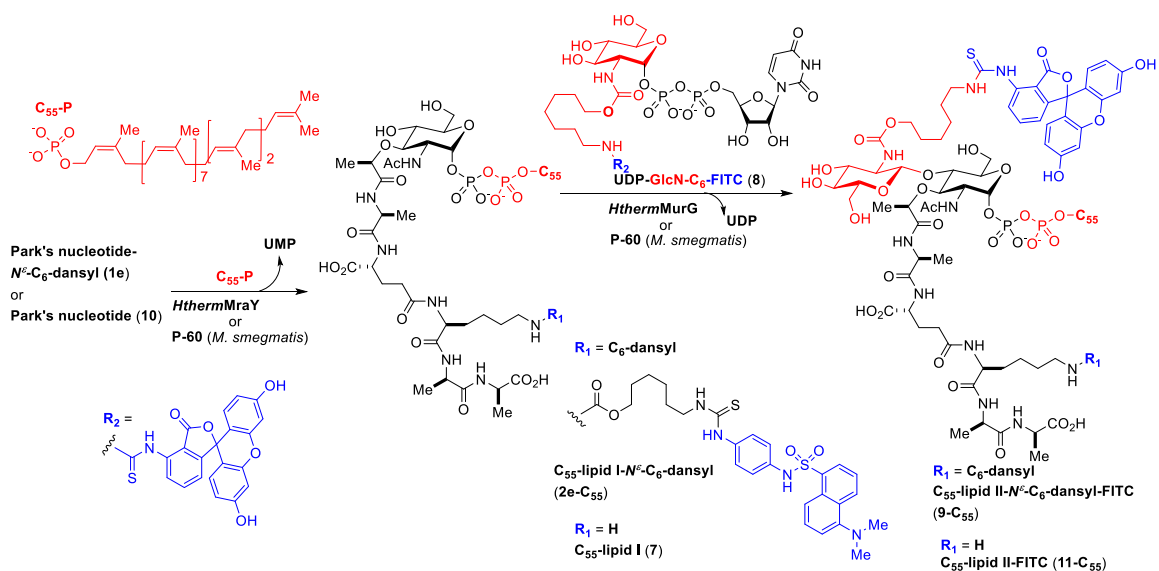
Preparation of membrane fraction P-60 containing *MraY* and *MurG*

M. smegmatis cells were harvested by centrifugation (4700 rpm) at 4 °C followed by washing with 0.9% saline solution (thrice). The washed cell pellets were washed with homogenization buffer (containing 50 mM K₂HPO₄, 5 mM MgCl₂, 5 mM 1,4-dithio-DL-threitol, and 10% glycerol, pH = 7.2) (thrice), and approximately 5 g of pellet (wet weight) was collected. The washed cell pellets were suspended in homogenization buffer and disrupted by probe sonication on ice (5 s on and 2 s off for 1 min, then cool down for 1.5 min, 5 cycles, cool down for 15 min, and then, 5 s on and 2 s off for 1 min, cool down for 1.5 min, 5 cycles). The resulting suspension was centrifuged at 4,700 x g for 15 min at 4 °C to remove unbroken cells. The lysate was centrifuged at 25,000 x g for 20 min at 4 °C. The supernatant was subjected to ultracentrifugation at 60,000 x g for 1 h at 4 °C. The supernatant was discarded, and the membrane fraction containing *MurG* enzyme (P-60) was suspended in the Tris-HCl buffer (pH = 7.5). Total protein concentrations were approximately 8 to 10 mg/mL. Aliquots were stored in Eppendorf tubes at -80 °C. Similarly, the membrane fractions containing *WecA* enzyme (P-60) were prepared from *E. coli*.

MurG assay.

MurG assay substrates, Park's nucleotide-*N*^ε-C₆-dansyl, UDP-GlcN- *N*^ε-C₆-FITC, were

chemically synthesized according to the reported procedures.



Protocol A:

Park's nucleotide- N^6 - C_6 -dansylthiourea (**1e**) (2 mM stock solution, 1.88 μ L), CHAPS (20%, 1.25 μ L), β -mercaptoethanol (50 mM, 5 μ L), $MgCl_2$ (0.5 M, 5 μ L), KCl (2 M, 5 μ L), and C_{55} -phosphate dissolved in $NaHCO_3$ (50 mM) : DMSO (1 : 4) (4 mM, 2.81 μ L) were placed in a 1.5 mL Eppendorf tube. To a stirred reaction mixture, *Hy*MraY (4.18 mg/mL, 1 μ L) was added (total volume of reaction mixture: 50 μ L adjust with Tris buffer (50 mM, pH = 8.0)). The reaction mixture was incubated for 1 h at 37 $^{\circ}C$. To a reaction mixture, inhibitor molecule (0 - 100 μ g/mL in Tris buffer), UDP-GlcNAc (10 mM stock solution, 1.88 μ L), and P60 (1 mg/ μ L, 30 μ L) or *Hy*MurG (5.2 mg/mL, 5 μ L) were added. The reaction mixture was incubated for 1 h at 37 $^{\circ}C$, and quenched with water saturated n-butanol (150 μ L). Two phases were mixed via vortex for 2 min and centrifuged at 25,000 $\times g$ for 10 min. The upper n-butanol phase was assayed via reverse-phase HPLC. The n-butanol phase (30 μ L) was injected into HPLC (solvent: a gradient elution of $CH_3OH/0.05$ M aq. $NH_4HCO_3 = 70 : 30$ to 100 : 0 over 30 min; UV: 350 nm; flow rate: 1.0 mL/min; column: Luna 5 μ m C_8 , 100 A, 250 \times 4.60 mm), and the area of the peak for C_{55} -lipid II- N^6 - C_6 -dansyl-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. All inhibition curves, Michaelis-Menten plots and Lineweaver-Burk plots were obtained by using GraphPad Prism 7.04.

Protocol B:

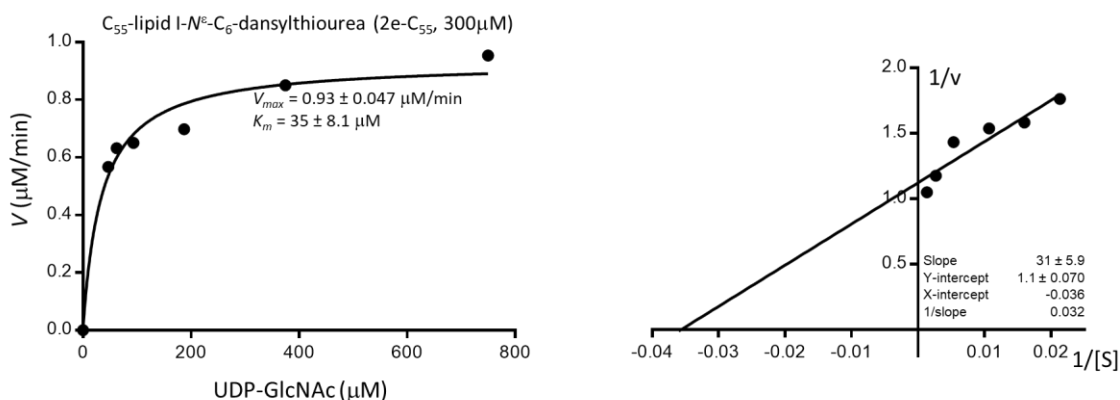
Park's nucleotide- N^6 - C_6 -dansylthiourea (**1e**) (2 mM stock solution, 1.88 μ L), CHAPS (20%, 1.25 μ L), β -mercaptoethanol (50 mM, 5 μ L), $MgCl_2$ (0.5 M, 5 μ L), KCl (2 M, 5 μ L), and C_{55} -phosphate dissolved in $NaHCO_3$ (50 mM) : DMSO (1 : 4) (4 mM, 2.81 μ L) were placed in a 1.5 mL Eppendorf tube. To a stirred reaction mixture, P60 (1 mg/ μ L, 30 μ L) was added (total volume of reaction mixture: 60 μ L adjust with Tris buffer (50 mM, pH = 8.0)). The reaction mixture was incubated for 1 h at 37 $^{\circ}C$. To a reaction mixture, tunicamycin (10 mg/mL stock solution, 0.25 μ L) was added, then inhibitor molecule (0 - 100 μ g/mL in Tris buffer) and UDP-GlcNAc (10 mM stock solution, 1.88 μ L) were added. The reaction

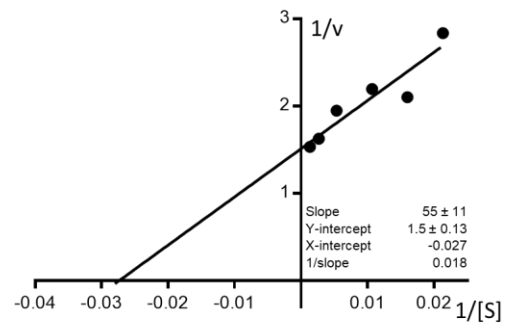
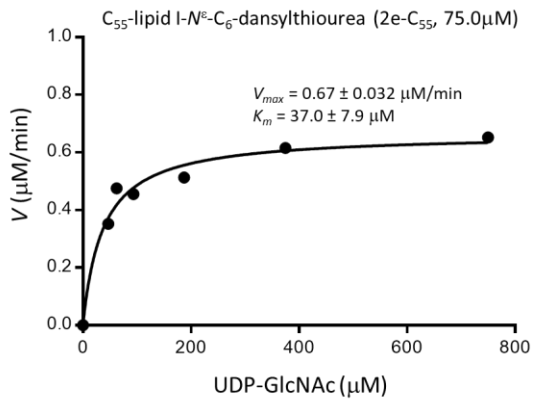
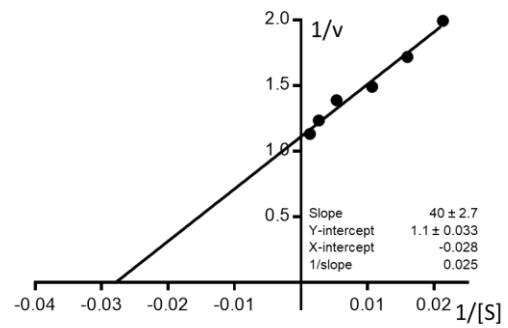
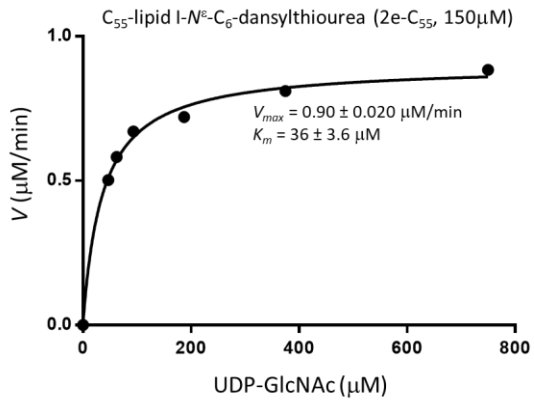
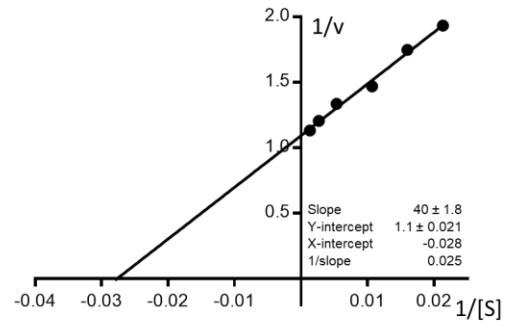
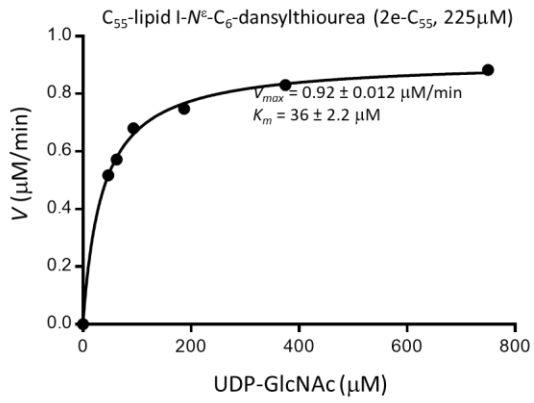
mixture was incubated for 1 h at 37 °C, and quenched with water saturated n-butanol (150 μ L). Two phases were mixed via vortex for 2 min and centrifuged at 25,000 xg for 10 min. The upper n-butanol phase was assayed via reverse-phase HPLC. The n-butanol phase (30 μ L) was injected into HPLC (solvent: a gradient elution of CH₃OH/0.05 M aq. NH₄HCO₃ = 70 : 30 to 100 : 0 over 30 min; UV: 350 nm; flow rate: 1.0 mL/min; column: Luna 5 μ m C₈, 100 A, 250 x 4.60 mm), and the area of the peak for C₅₅-lipid II-*N*^E-C₆-dansyl-FITC was quantified to obtain the IC₅₀ value. The IC₅₀ values were calculated from plots of the percentage product inhibition versus the inhibitor concentration. All inhibition curves, Michaelis-Menten plots and Lineveaver-Burk plots were obtained by using GraphPad Prism 7.04.

UV/VIS spectroscopy-based assay

Park's nucleotide-*N*^E-C₆-dansylthiourea (**1e**) (2 mM stock solution, 1.88 μ L), CHAPS (20%, 1.25 μ L), β -mercaptoethanol (50 mM, 5 μ L), MgCl₂ (0.5 M, 5 μ L), KCl (2 M, 5 μ L), and C₅₅-phosphate dissolved in NaHCO₃ (50 mM) : DMSO (1 : 4) (4 mM, 2.81 μ L) were placed in a 1.5 mL Eppendorf tube. To a stirred reaction mixture, HyMraY (4.18 mg/mL, 1 μ L) was added (total volume of reaction mixture: 50 μ L adjust with Tris buffer (50 mM, pH = 8.0)). The reaction mixture was incubated for 1 h at 37 °C. To a reaction mixture, inhibitor molecule (0 - 100 μ g/mL in Tris buffer), UDP-GlcN-C₆-FITC (10 mM stock solution, 1.88 μ L), and P60 (1 mg/ μ L, 30 μ L) or HyMurG (5.2 mg/mL, 5 μ L) were added. The reaction mixture was incubated for 1 h at 37 °C, and quenched with water saturated n-butanol (150 μ L). Two phases were mixed via vortex for 2 min and centrifuged at 25,000 xg for 10 min. The upper n-butanol phase was washed with n-butanol saturated 1 : 1 mixture of saline/0.2 M mannitol (50 μ L, thrice) and then, the washed n-butanol phase (20 μ L) was transferred to a 384 well black plate and fluorescence was measured at an excitation of 485 nm and emission of 528 nm. The IC₅₀ values were calculated from plots of the percentage product inhibition versus the inhibitor concentration. All inhibition curves, Michaelis-Menten plots and Lineveaver-Burk plots were obtained by using GraphPad Prism 7.04.

Figure S2. Michaelis-Menten plots and Lineweaver-Burk plots for 2e-C₅₅ at the different concentrations of UDP-GlcNAc.





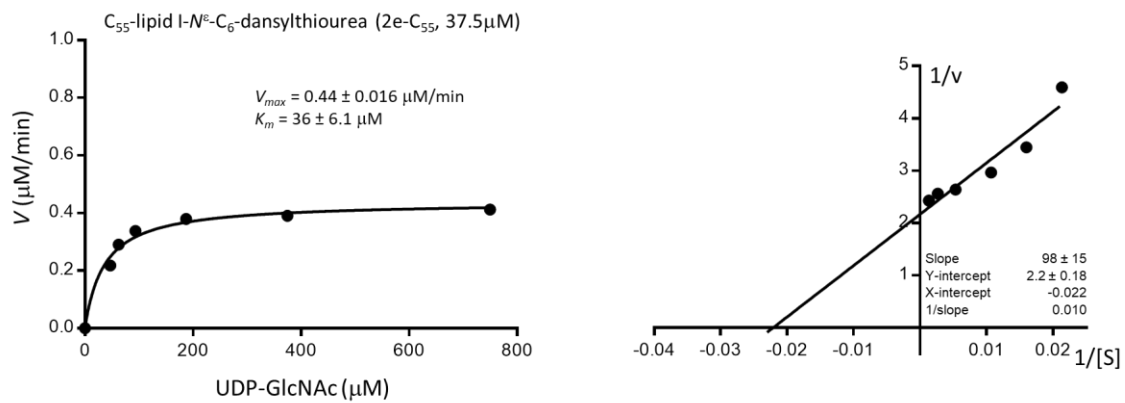


Figure S3. Superimposed Lineweaver-Burk plots for 2e-C₅₅ at the different concentrations of UDP-GlcNAc.

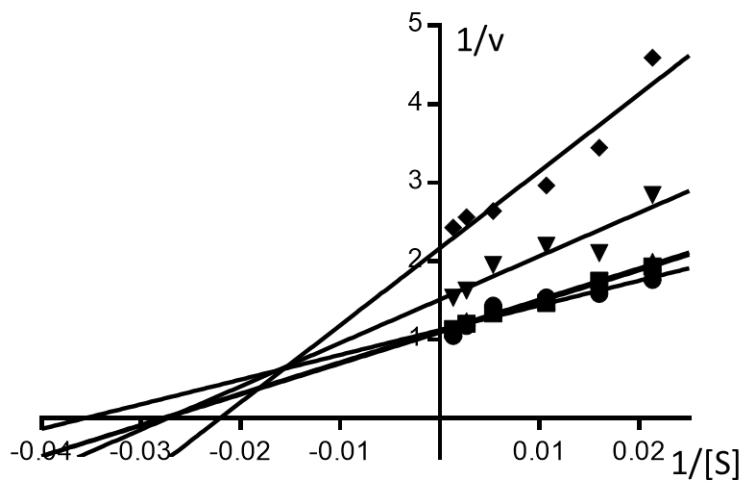
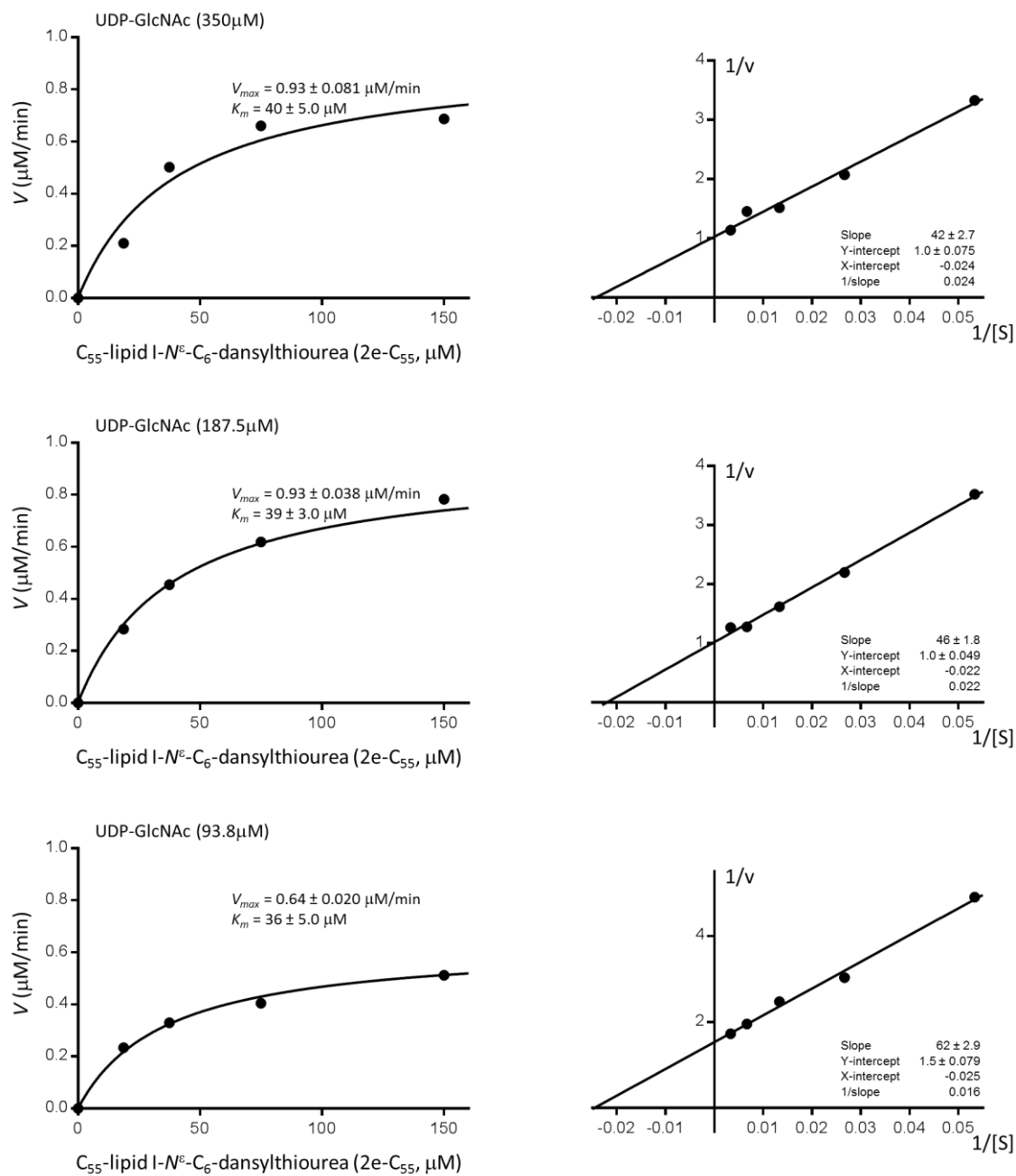


Figure S4. Michaelis-Menten plots and Lineweaver-Burk plots for UDP-GlcNAc at the different concentrations of 2e-C55.



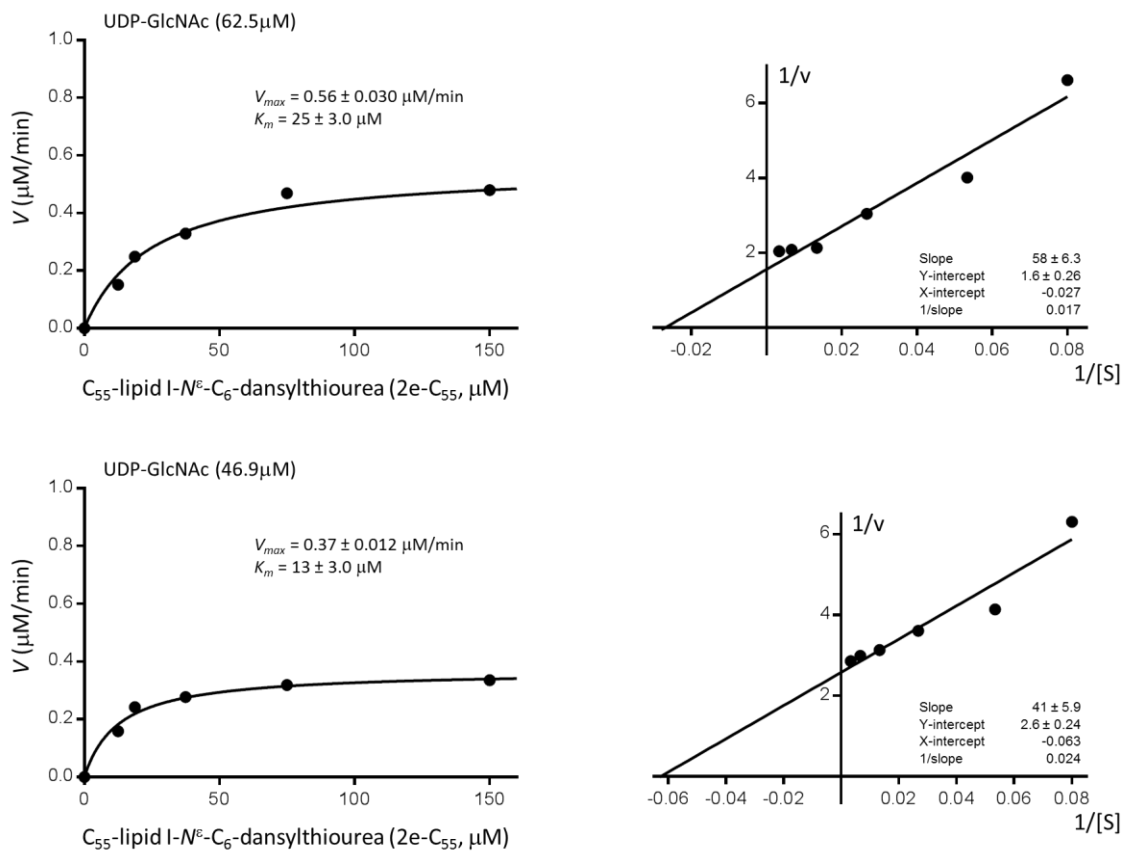


Figure S5. Superimposed Lineweaver-Burk plots for UDP-GlcNAc at the different concentrations of 2e-C₅₅ (93.8-350 μM)

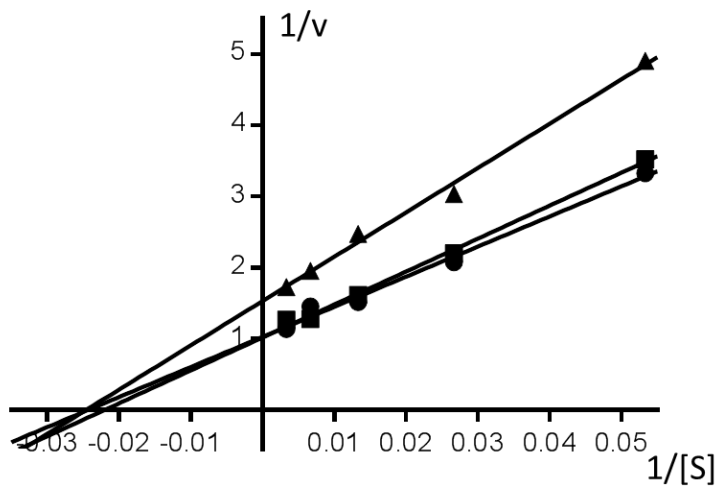


Figure S6. Inhibition curve for 2e-C55 by restocetin A against MurG.

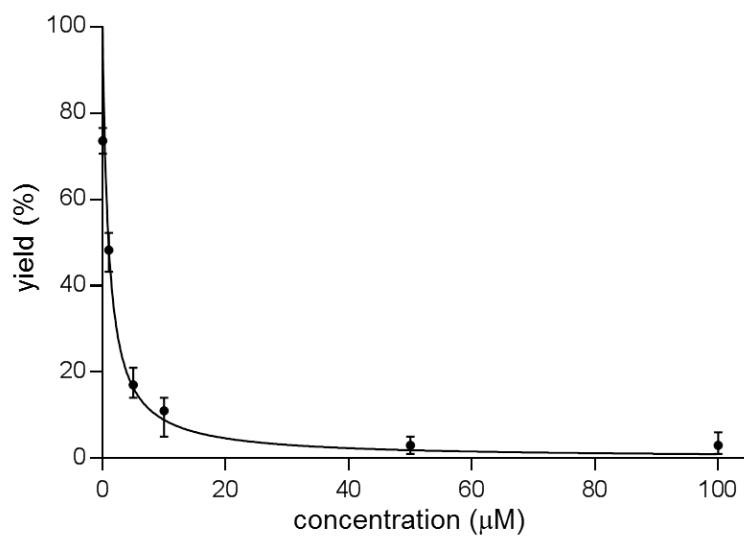


Figure S7. Inhibition curve for 1e by restocetin A against MraY (IC₅₀ 0.81 μM against *Msmeg*MraY).

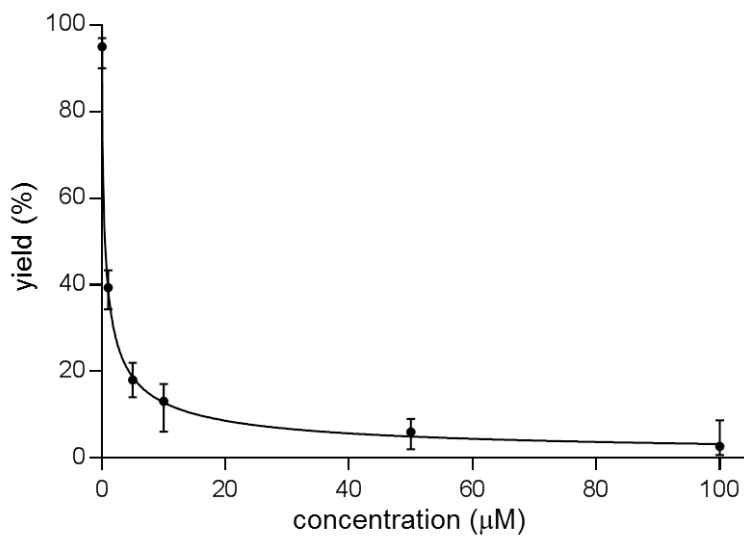


Figure S8. *MsmegMraY* kinetics in the presence of ristocetin A at different concentrations of 1e.

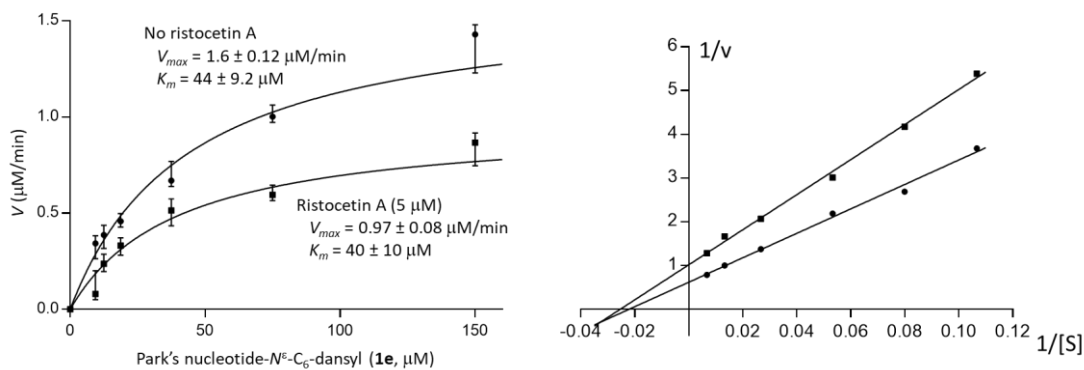


Figure S9. *MsmegMraY* kinetics in the presence of ristocetin A at different concentrations of C₅₅-P.

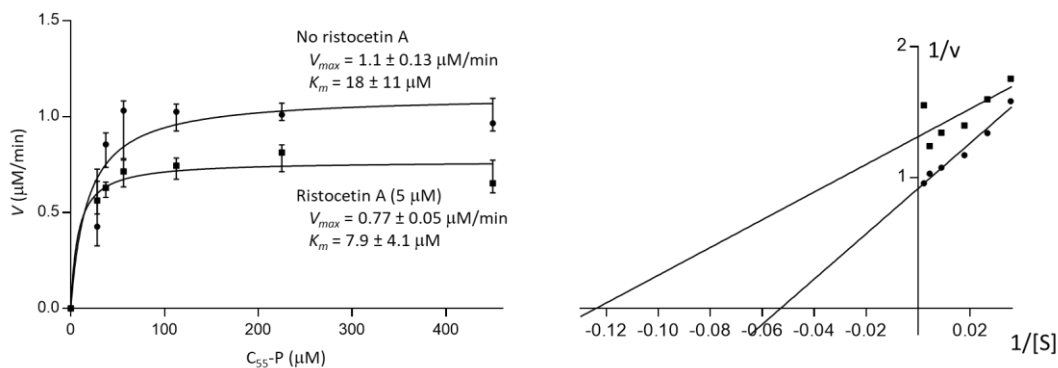


Figure S10. Product-time curve for the MurG-catalyzed reactions (2e-C₅₅ (75 μM) UDP-GlcNAc (375 μM)).

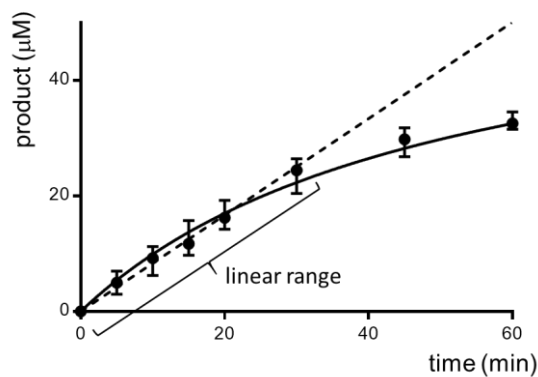


Figure S11. Effect of DMSO on the MurG activity.

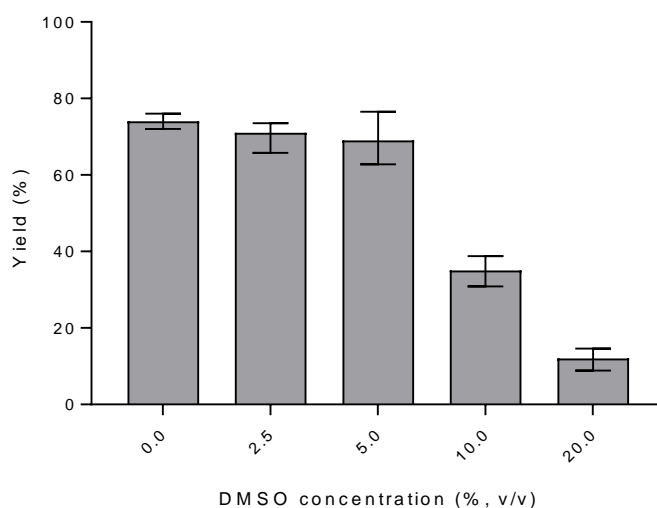


Table S1. MraY, WecA and AgIH assays [7], [8] against 15 and 16.

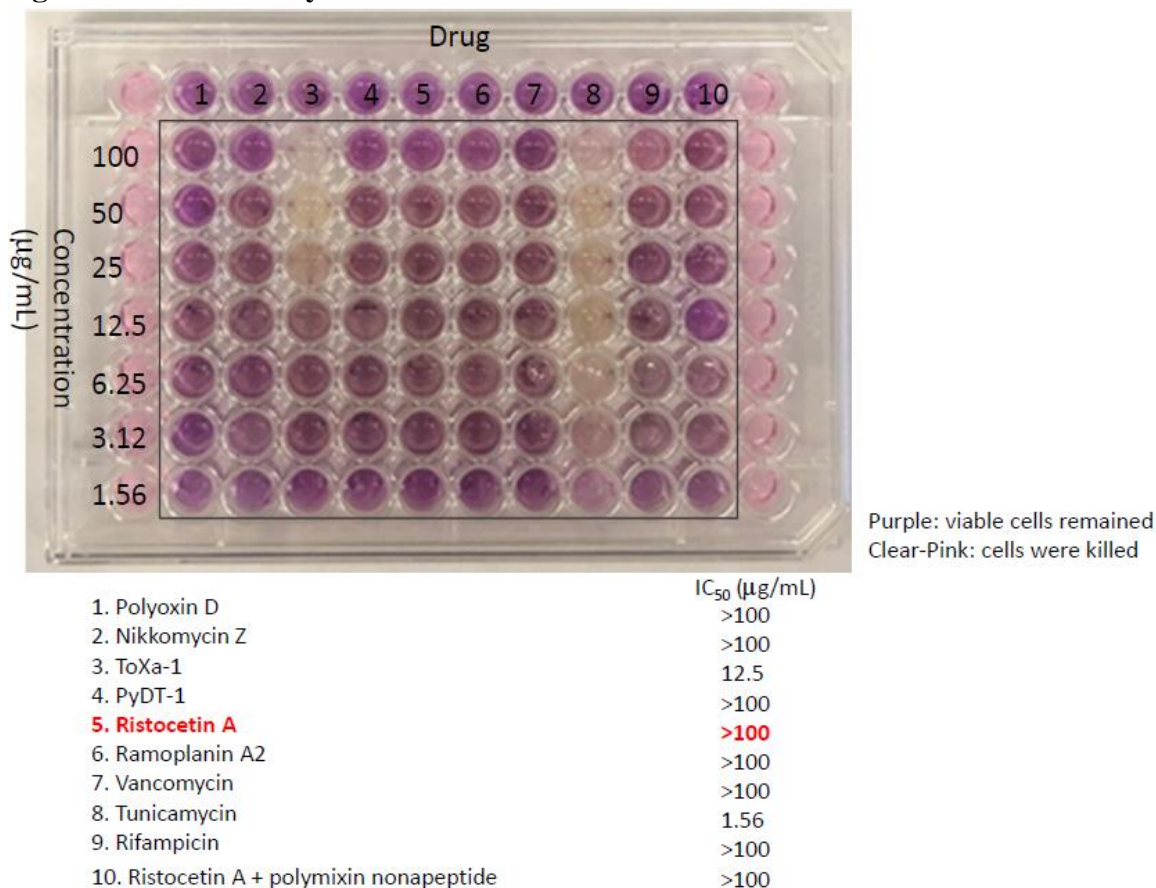
entry	Compound	HyMraY inhibition (%)			WecA inhibition (%)			MjAgIH inhibition (%)		
		1 μ M	10 μ M	100 μ M	1 μ M	10 μ M	100 μ M	1 μ M	10 μ M	100 μ M
1	ToXa-1 (15)	0	0	0	0	2	3	0	0	2
2	PyDT-1 (16)	0	3	5	0	5	13	0	4	9

Each experiment was performed two-three times. Analyses: HPLC-based method

Determination of cytotoxicity in Vero Cells

Selected molecules were tested for cytotoxicity (IC₅₀) in Vero cells via 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. Inoculating number of cells were 400,000 cells/mL and a final 40,000 cells/well. After 48h of exposure of molecules to this cell line at concentrations ranging from 0.78 to 100 μ g/mL, 10 μ L of MTT, yellow tetrazolium dye; was added to each well and the assay was terminated by removing the media and addition of 100 μ L of DMSO. The 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 570 nm by BioTek Synergy HT Spectrophotometer. Linearity of the MTT response to the cell number was determined.

Figure S12. MTT assay in Vero cells.



Structural comparison between MurG proteins.

Hydrogenivirga sp. (128-5-R1-1) and *M. tuberculosis* (H37Rv): 26% identity /42% similarity

```

Query 37  SVLAGGGTAGHVEPAMAVADALVALDPR-VRITALGTLRGLLETRLVPQRGYHLELITAV 95
Sbjct 4   K.FI.....G..FY..LS..KF.---.KNGYE.CYF..KN.I.SEKDFLGKKY.FD.KG. 60

Query 96  PMPRKPGDLARLPSRVWRVREARDVLDDVDAD---VVVGFGGYVALP---AYLAARG 148
Sbjct 61  R-----KGILNK..SIYKLLKT.FKIKKIIKKEKPLFSIC....TSV.LGIASW.SG-- 113

Query 149 LPLPRRRRRRIPVVIHEANARAGLANRVGAHTADRVL SAVPDSGL----RRAEVVGVVPR 204
Sbjct 114 -----DLY...Q.SIPSYS.ILLSKF.KKIFITFEYTKKYFPEEKTHLT.L.I. 163

Query 205  ASIAALDRAVL-RAEARAHFGFPDDARVLLVFGGSQGAVSLNRAVSGAAADLAAAGVCVL 263
Sbjct 164  K.L--.K.LS.TKE...KILNINAKEK.V.I.....KK..EICKLKL.E--KHKD.IFI 219

Query 264  HAHGPQNVLELRRRAQGDPPYVAVPYLDRMELAYAAAADLVICRAGAMTVAEVS AVGLPAI 323
Sbjct 220  NIQ.KSKLGNISKNI-----I.FD.FED.G.L.KVS.....S..N.ILTF.KY.. 273

Query 324  YVPLPI-GNGEQRNALPVVNAGGGMVVADAALTPELVARQVAGLLT-----DPARLA 375
Sbjct 274  FI.Y.YAASNH.YY.VKWLEEKNLCK.ITEENISQ--IYKEFEN..NIDFSKFE.KI.EL 331

Query 376  AMTAAARV 384
Sbjct 332  SI.D.EK.I 340

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E. coli (K-12) and *M. tuberculosis* (H37Rv): 39% identity /53% similarity

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Query 92  ITAVPMPRKPGGDLARLPSRVWRVREARDVLDVDDADVVVGFGGYVALPAYLAARGLPL 151
Sbjct 60  .RISGLRG.GIKA.IAA.L.IFN.W.Q..AIMKAYKP...L.M....SG.GG...WS.G- 118

Query 152 PPRRRRRIPVVIHEANARAGLANRVGAHTADRVL SAVPDSGLRRAEVVGVPRASIAALD 211
Sbjct 119 -----...L..Q.GI...T.KWL.KI.TK.MQ.F.GA-FPN.....N...TDVL..P 170

Query 212  RAVLRAEARAHFGFPDDARVLLVFGGSGAVSLNRAVSGAAADLAAAGVCVLH--AHGPO 269
Sbjct 171  LPQQ.LAG.-----EGPV...V.-.....RI..QTMPQV..K.GDS-.TIW.QSGK.S. 223

Query 270  NVLELRRAAQGDPPYVAVPYLDRMELAYAAADLVICRAGAMTVAEVS AVGLPAIYVPLPI 329
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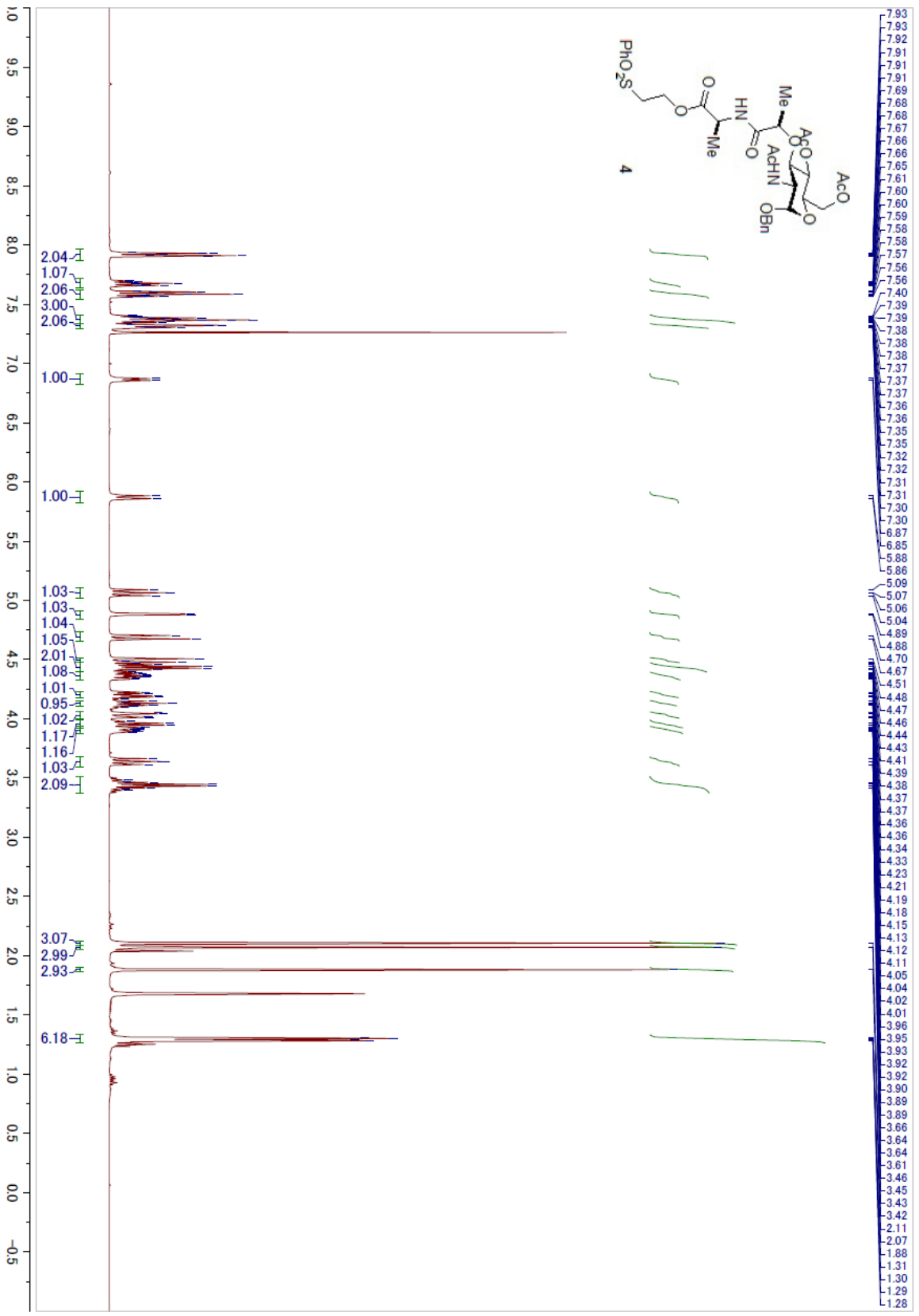
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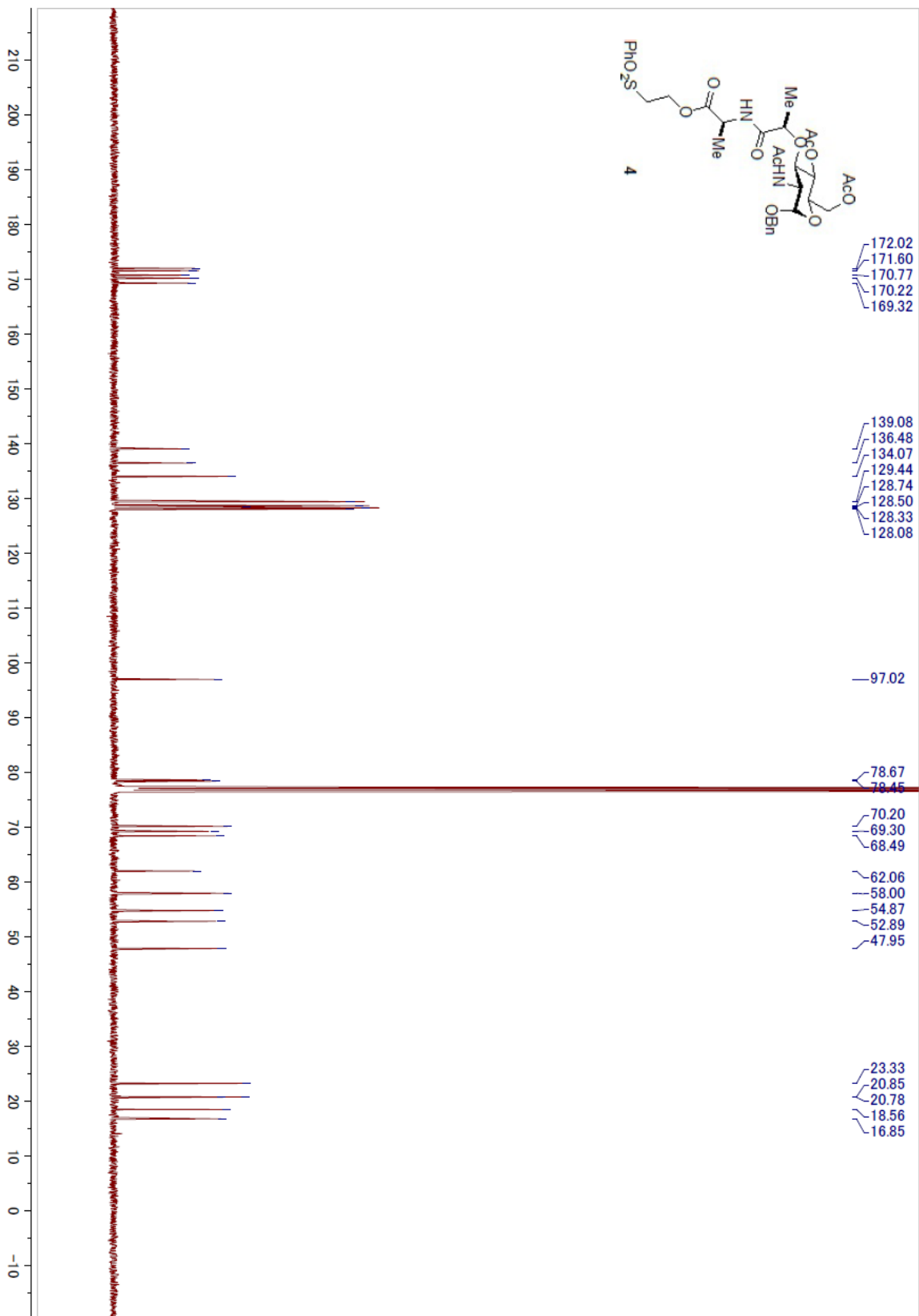
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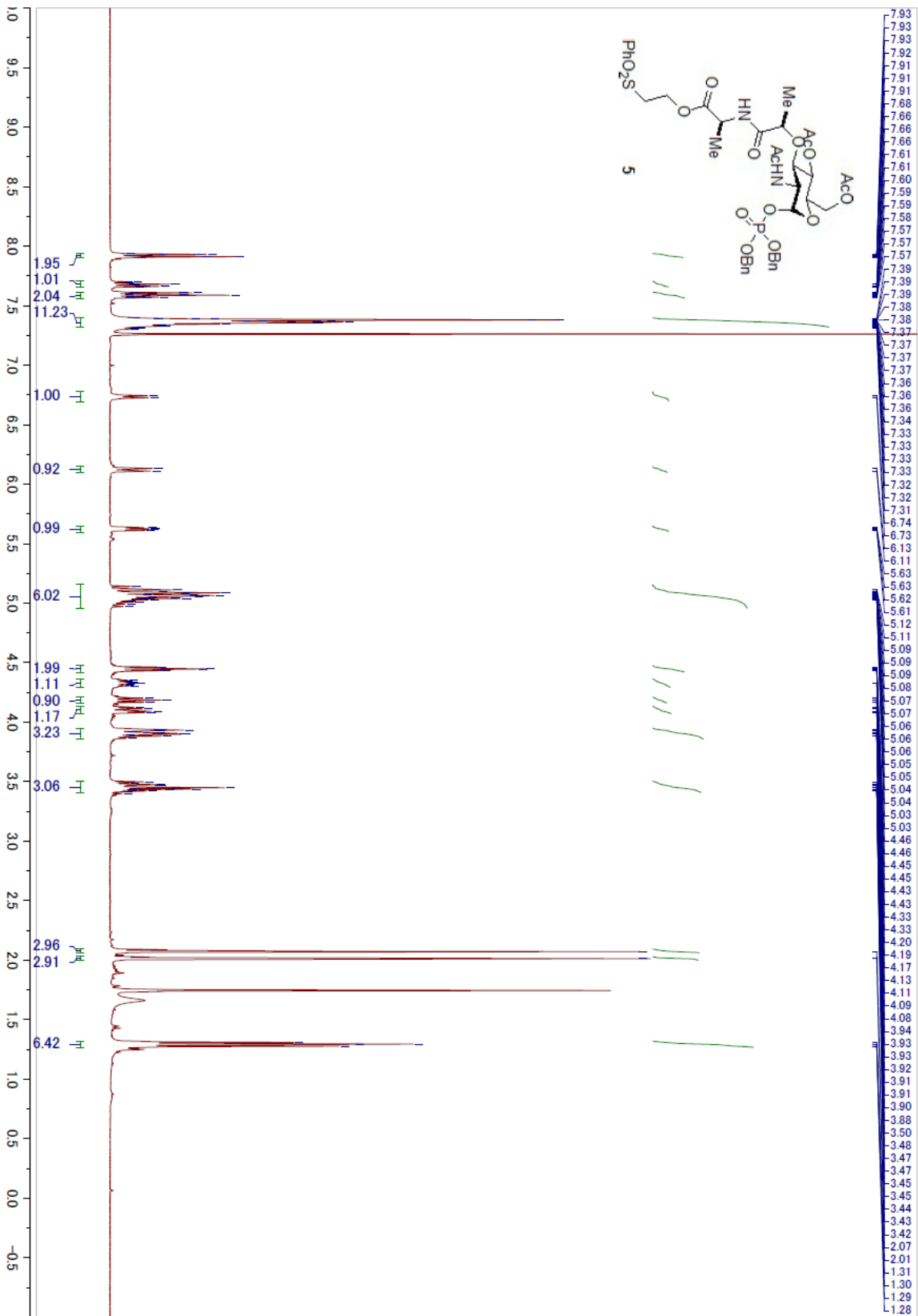
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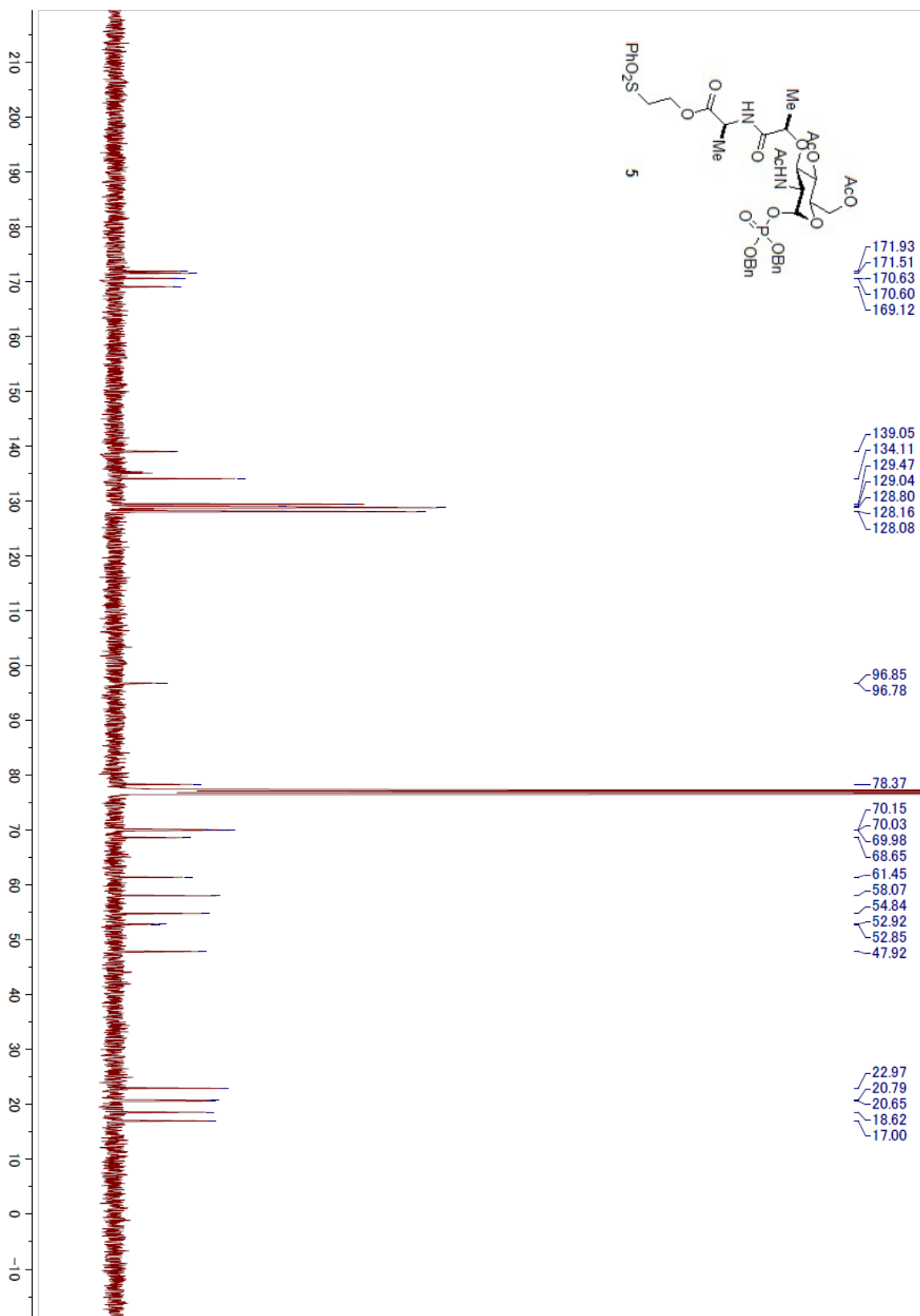
References

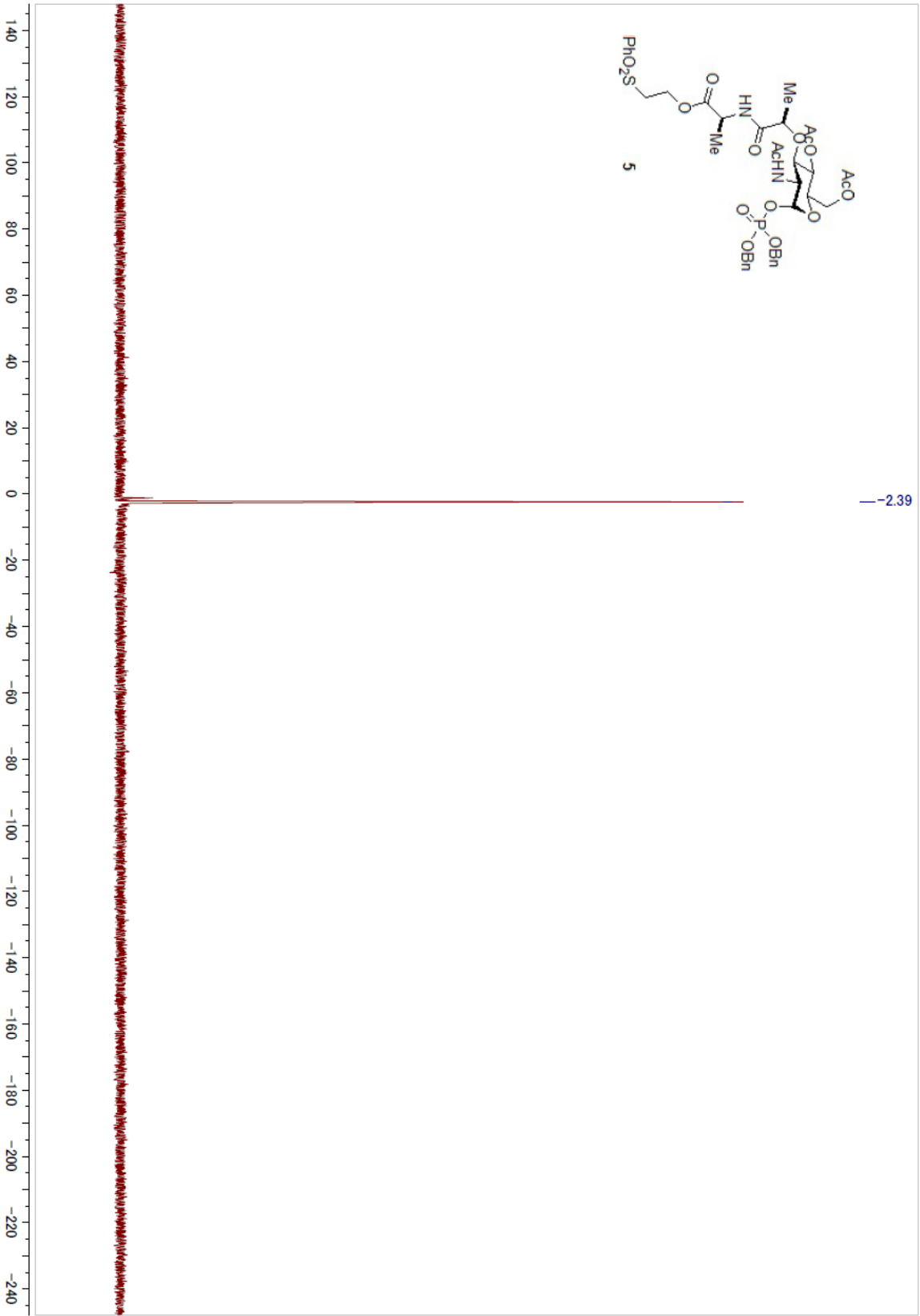
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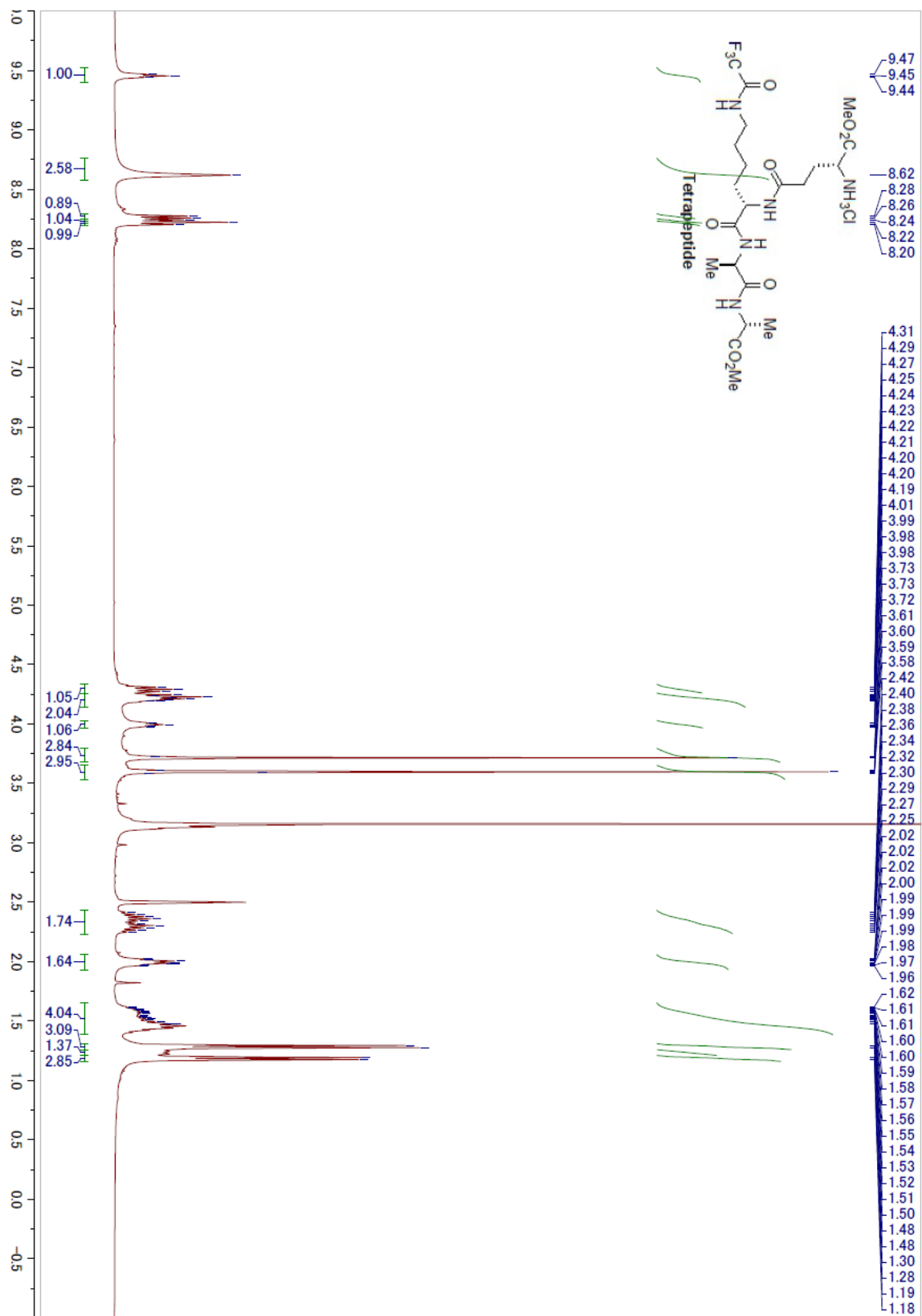


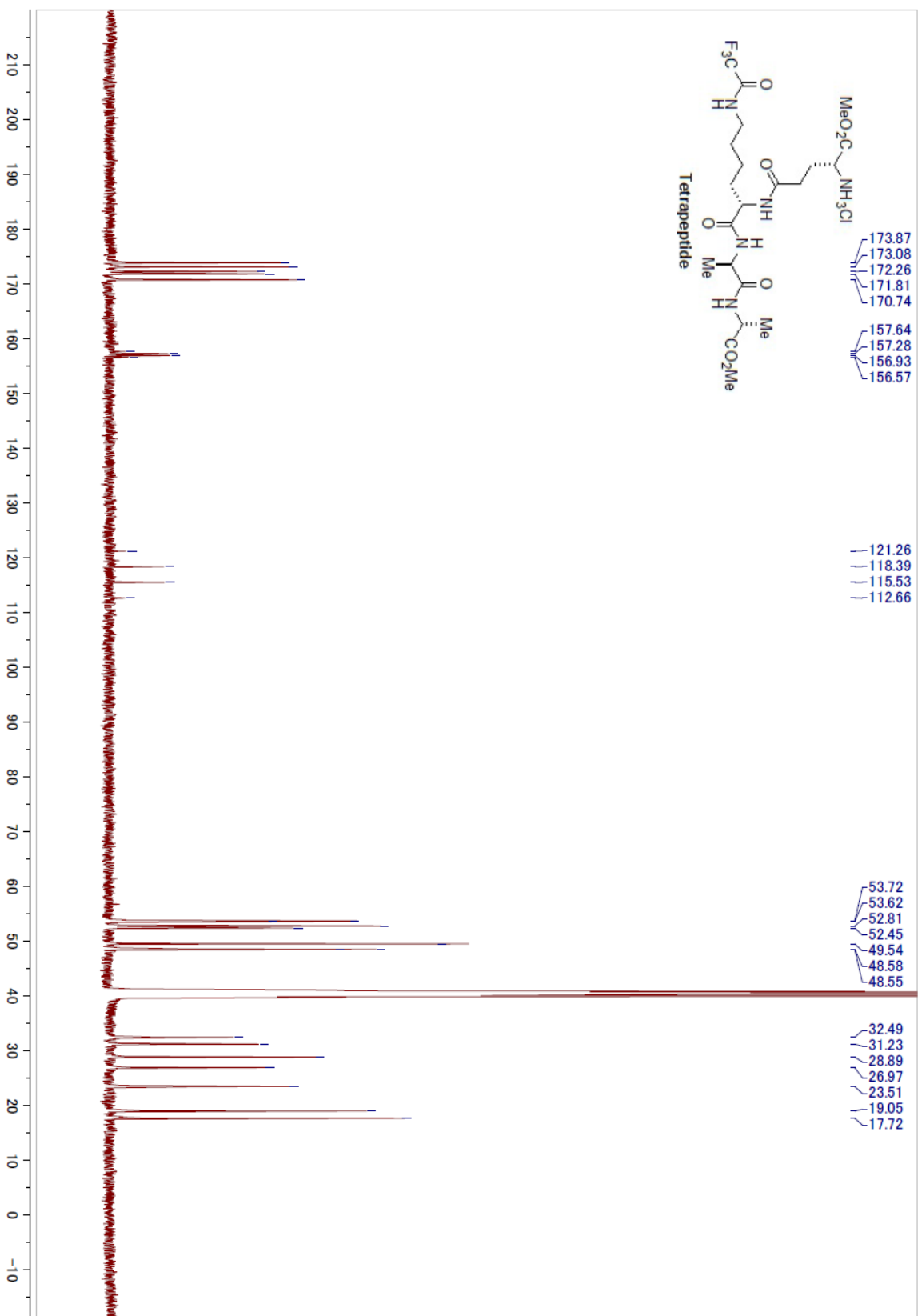


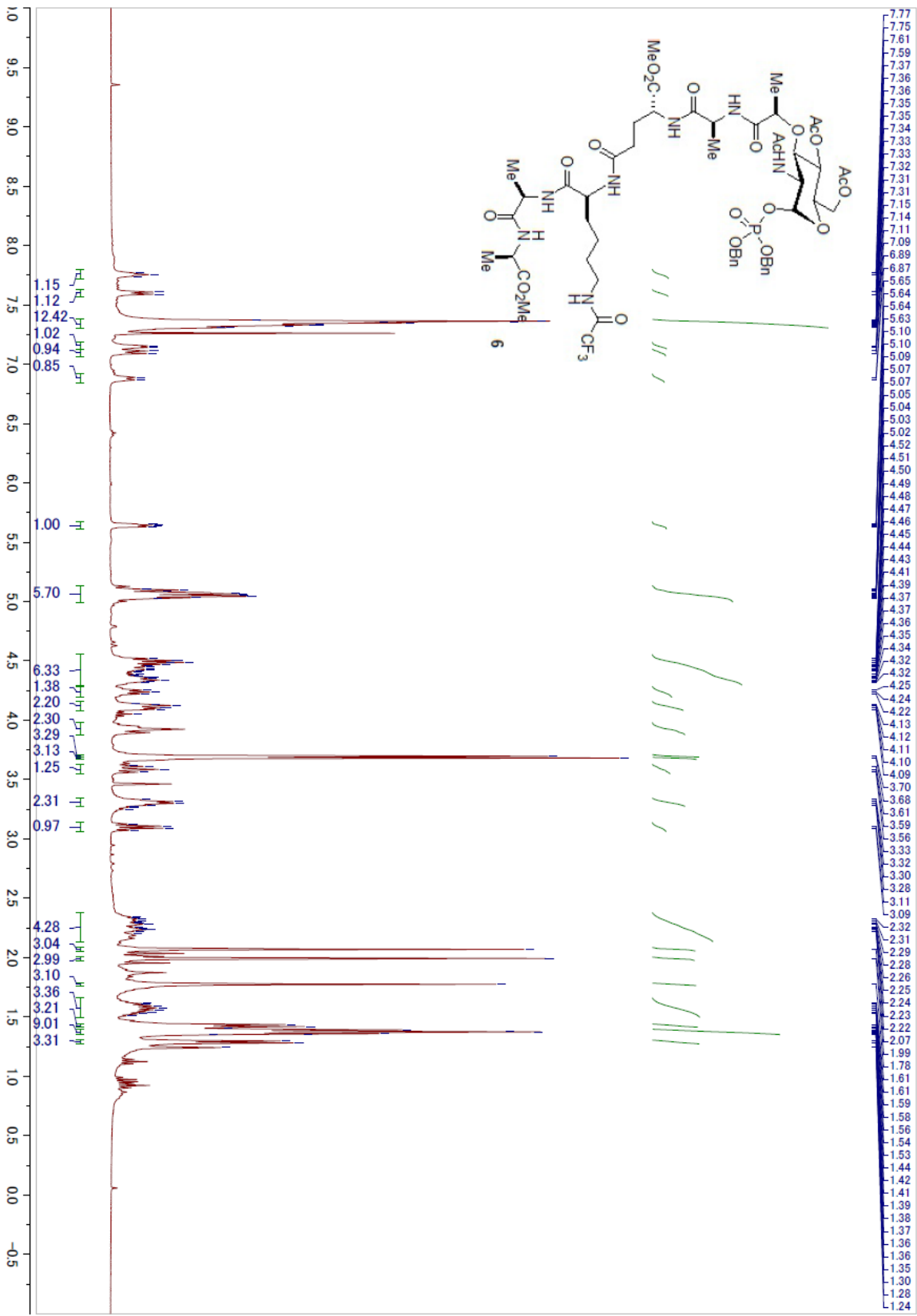


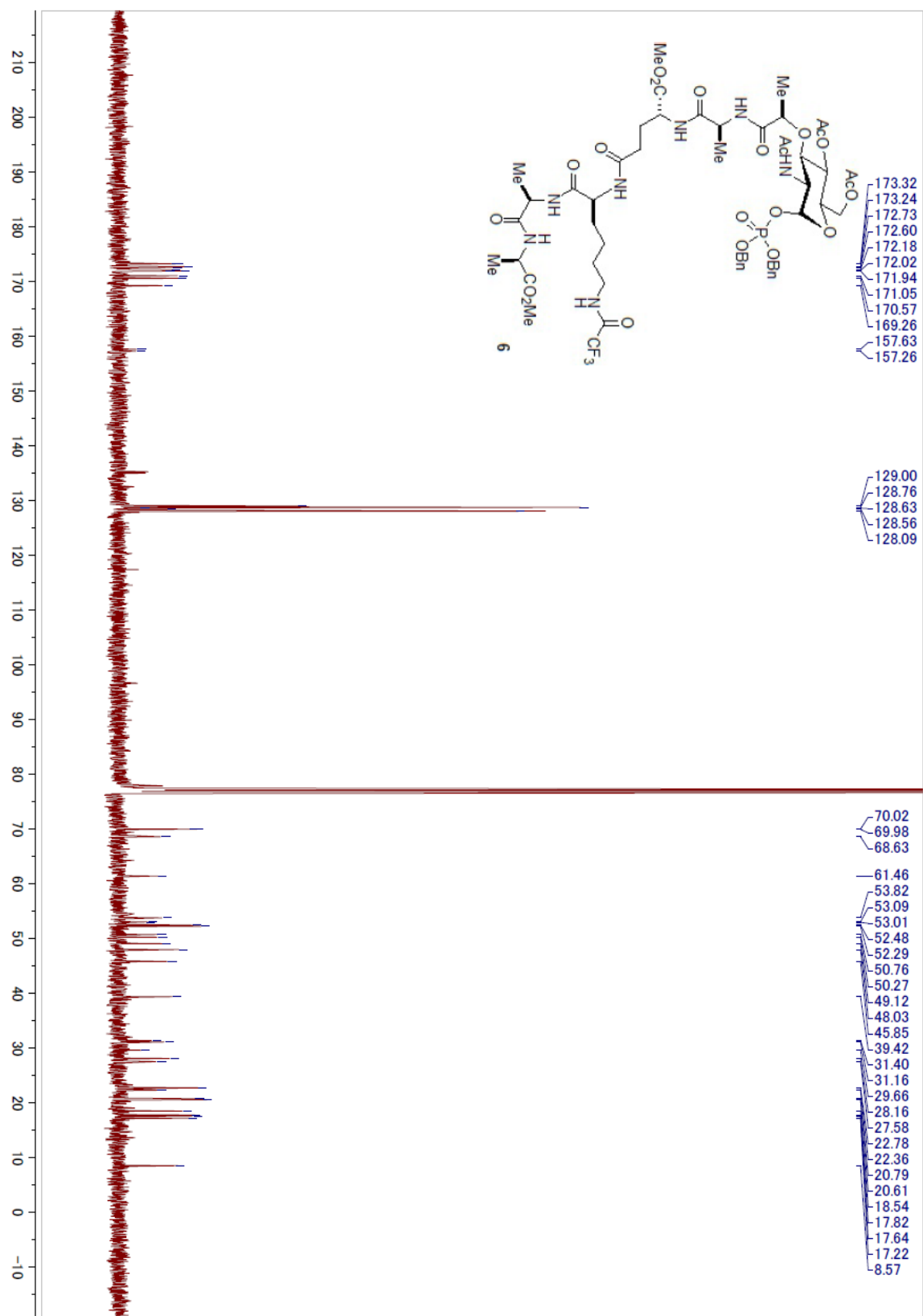


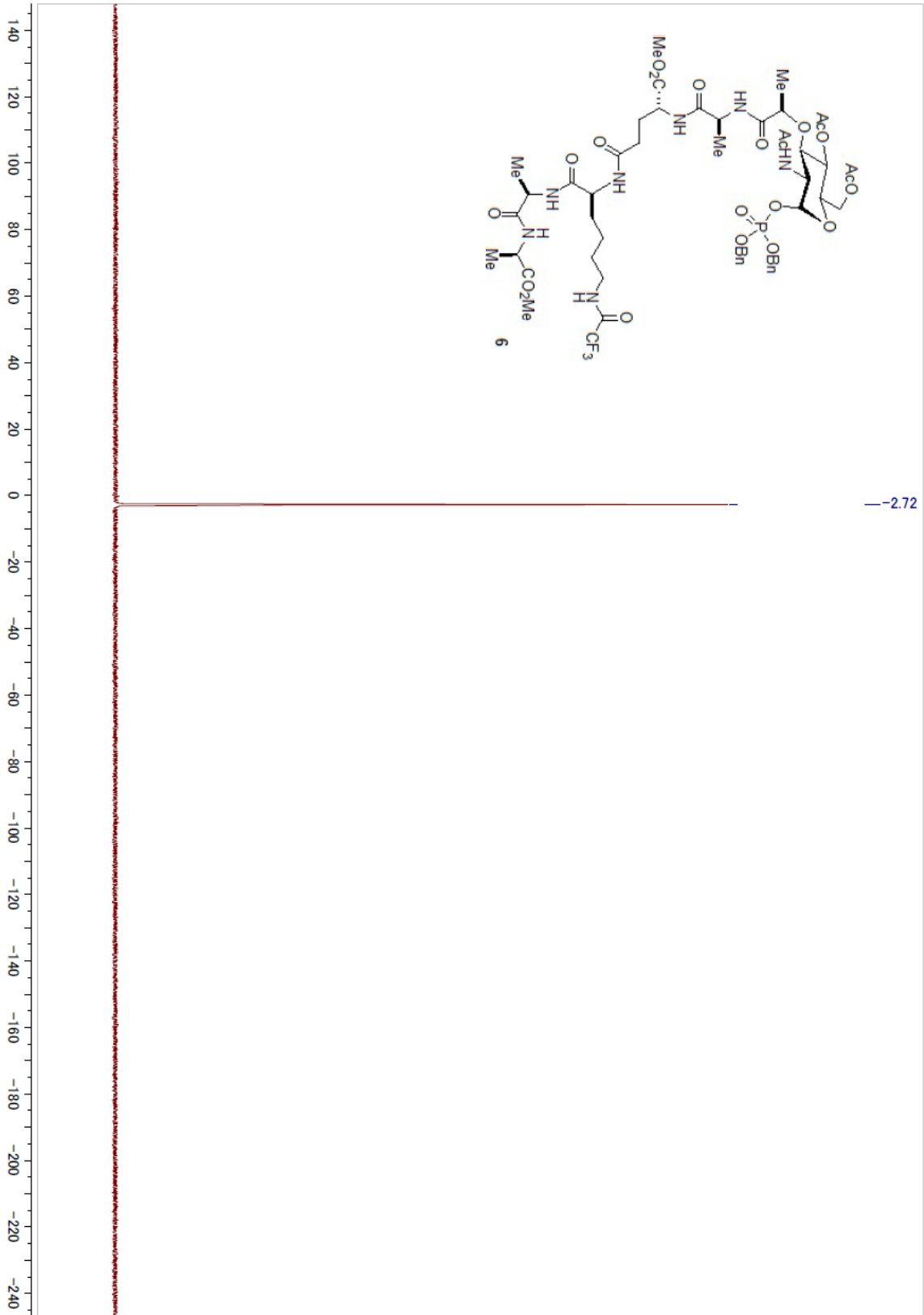


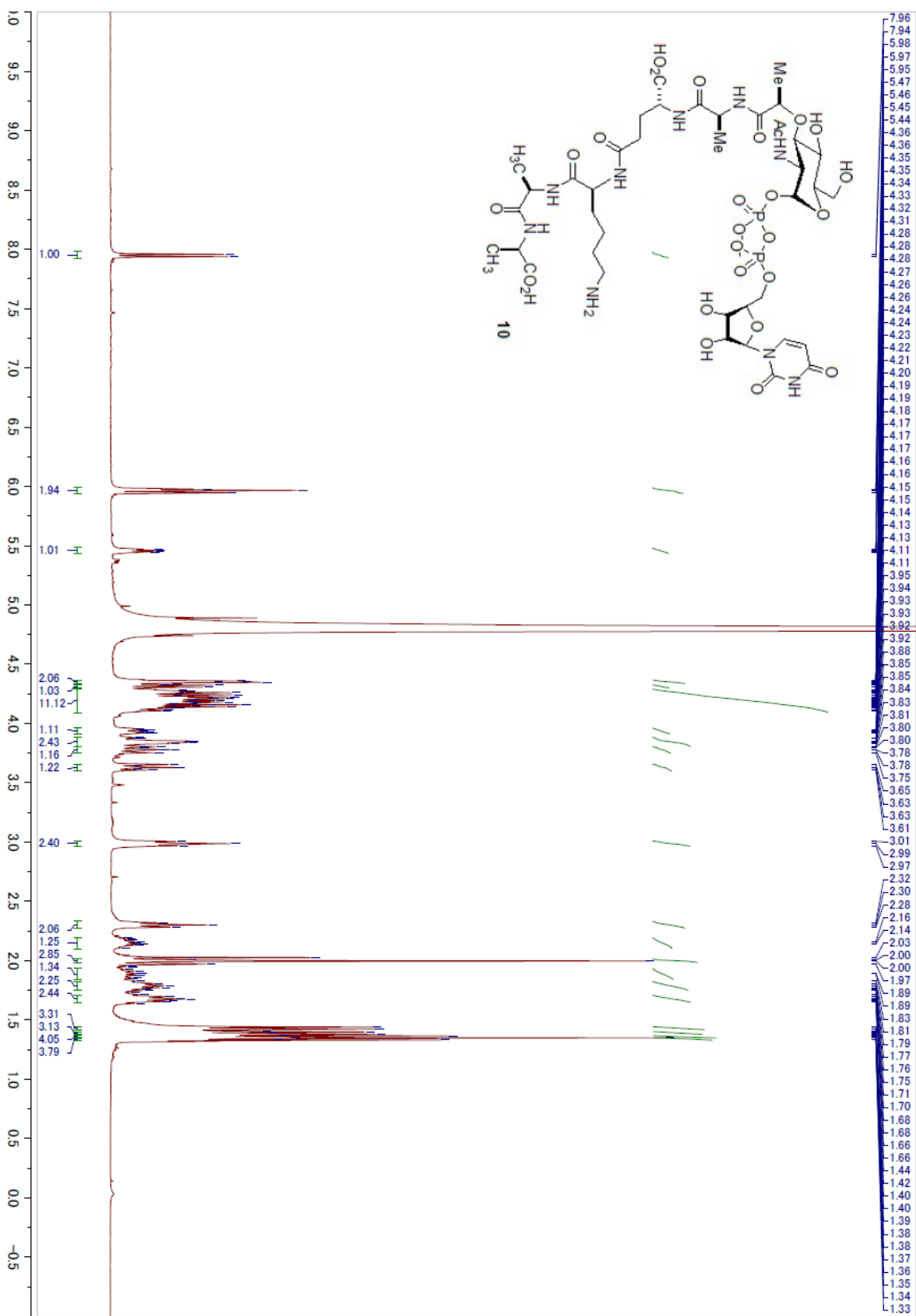


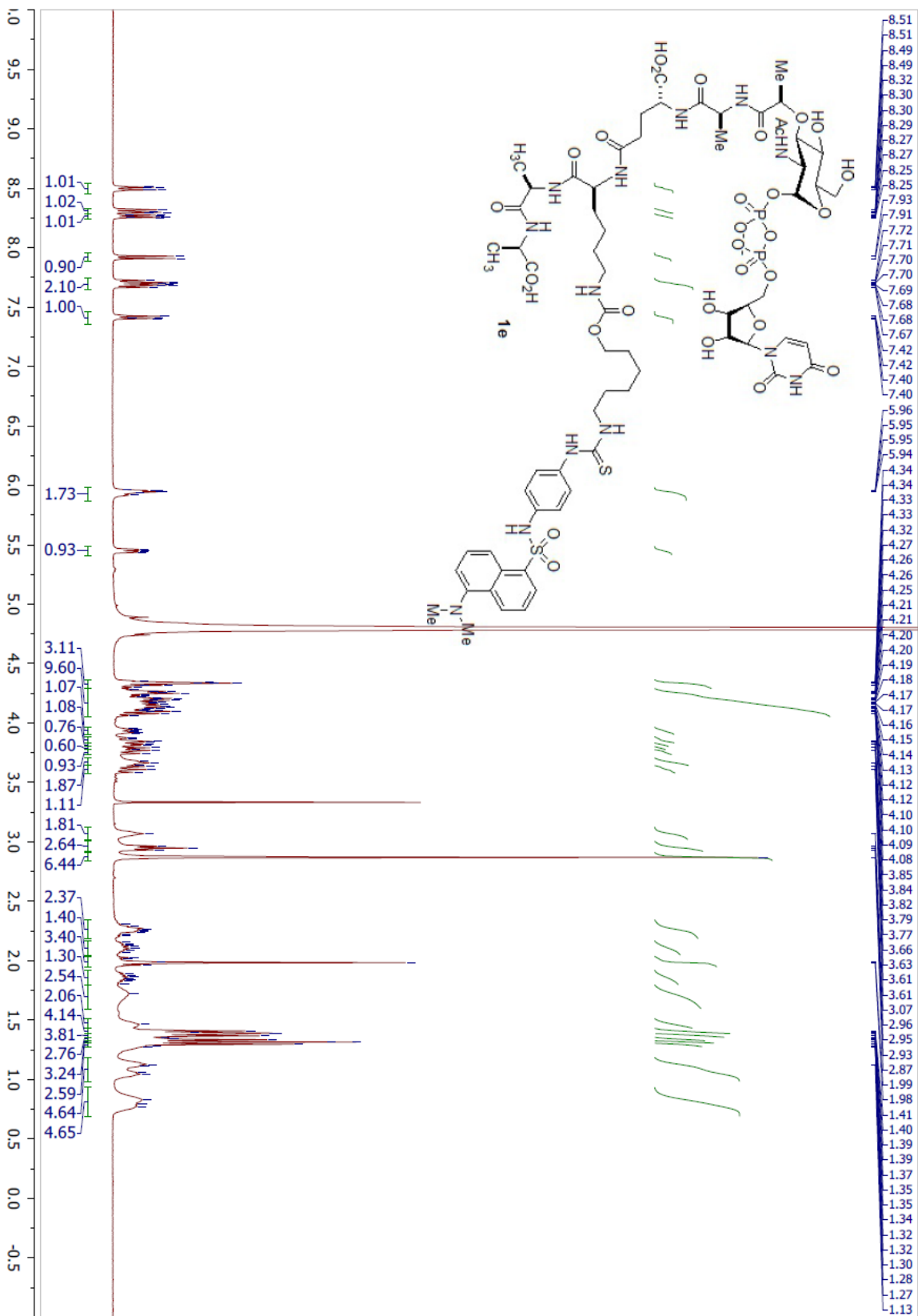


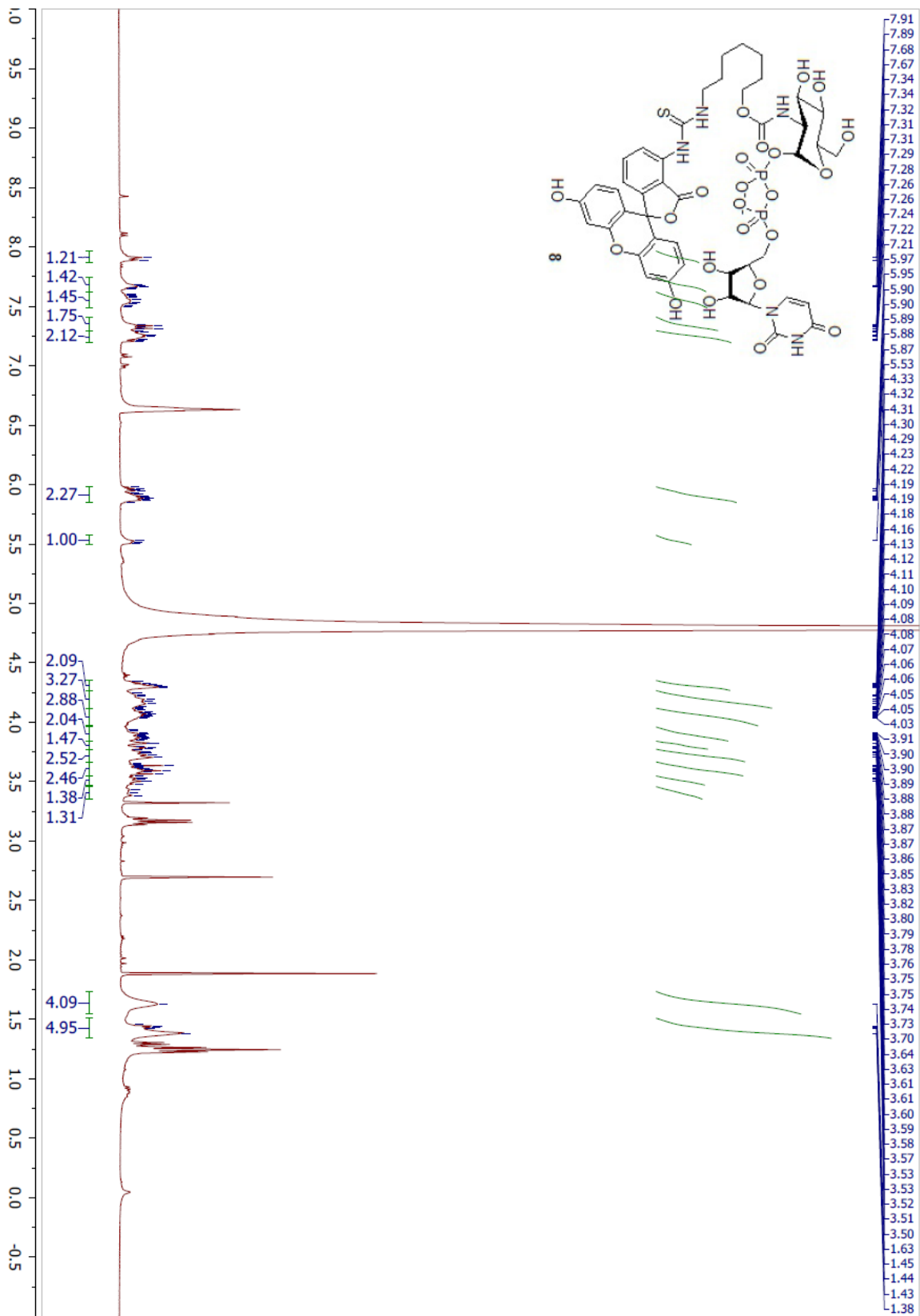


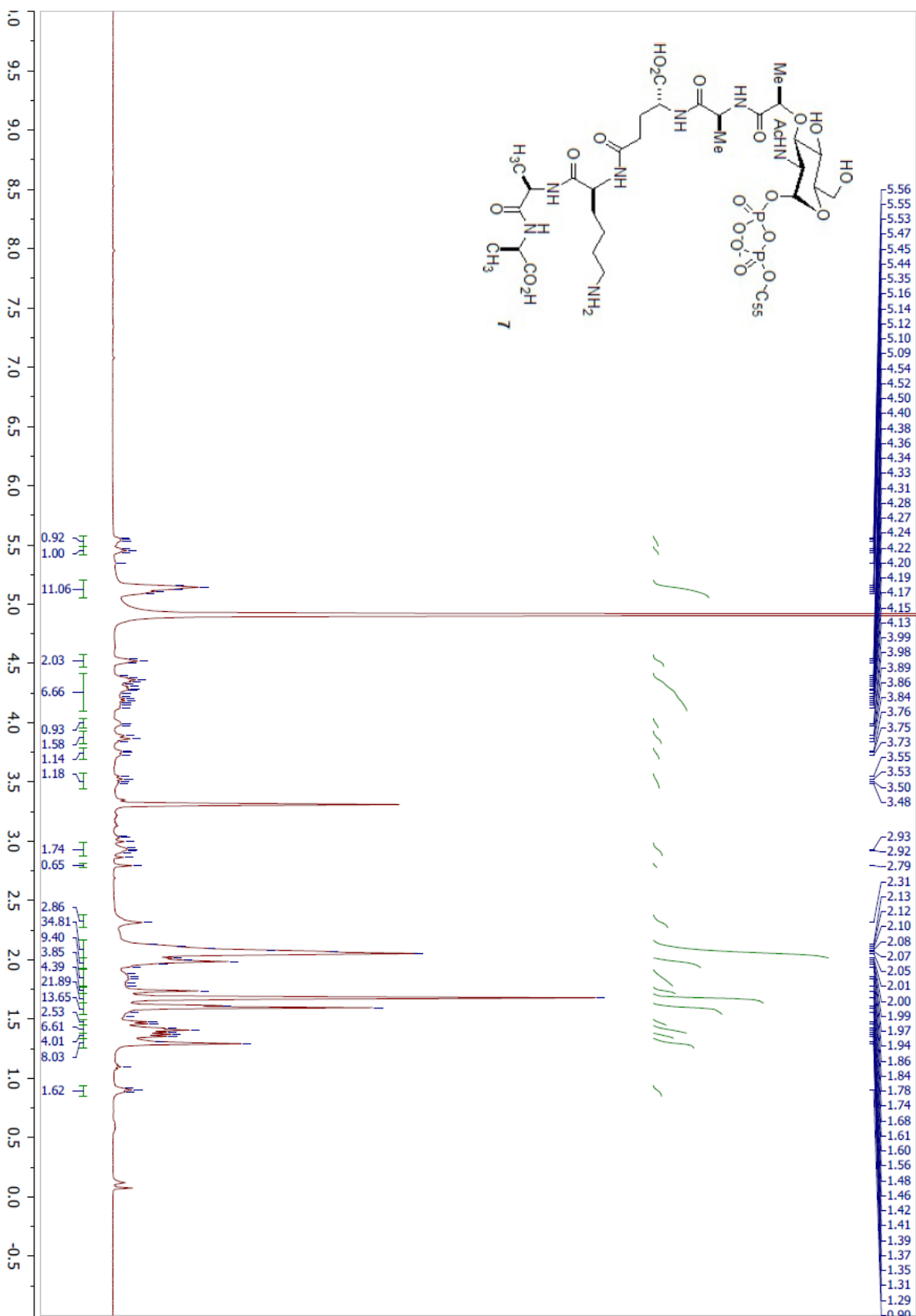


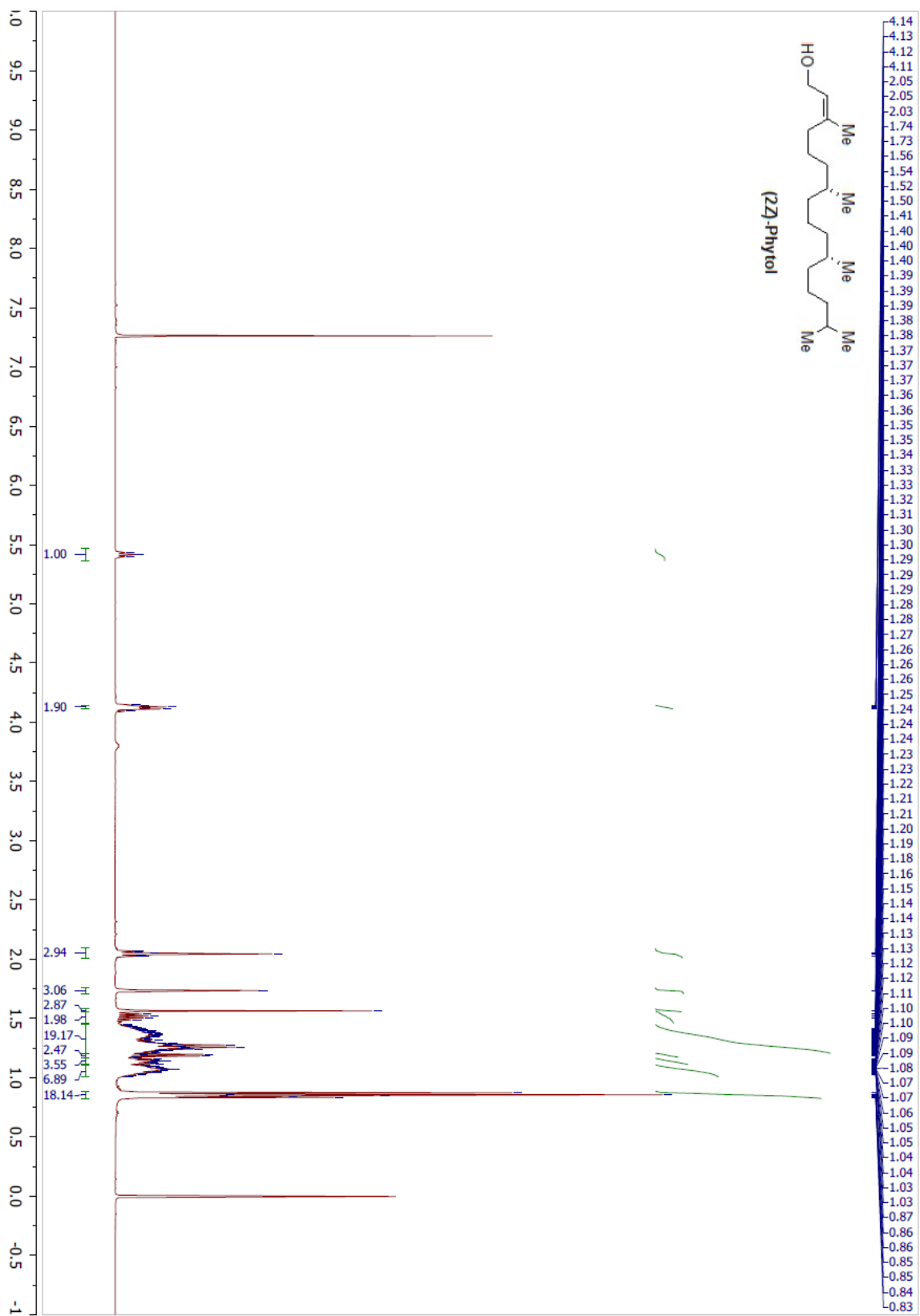


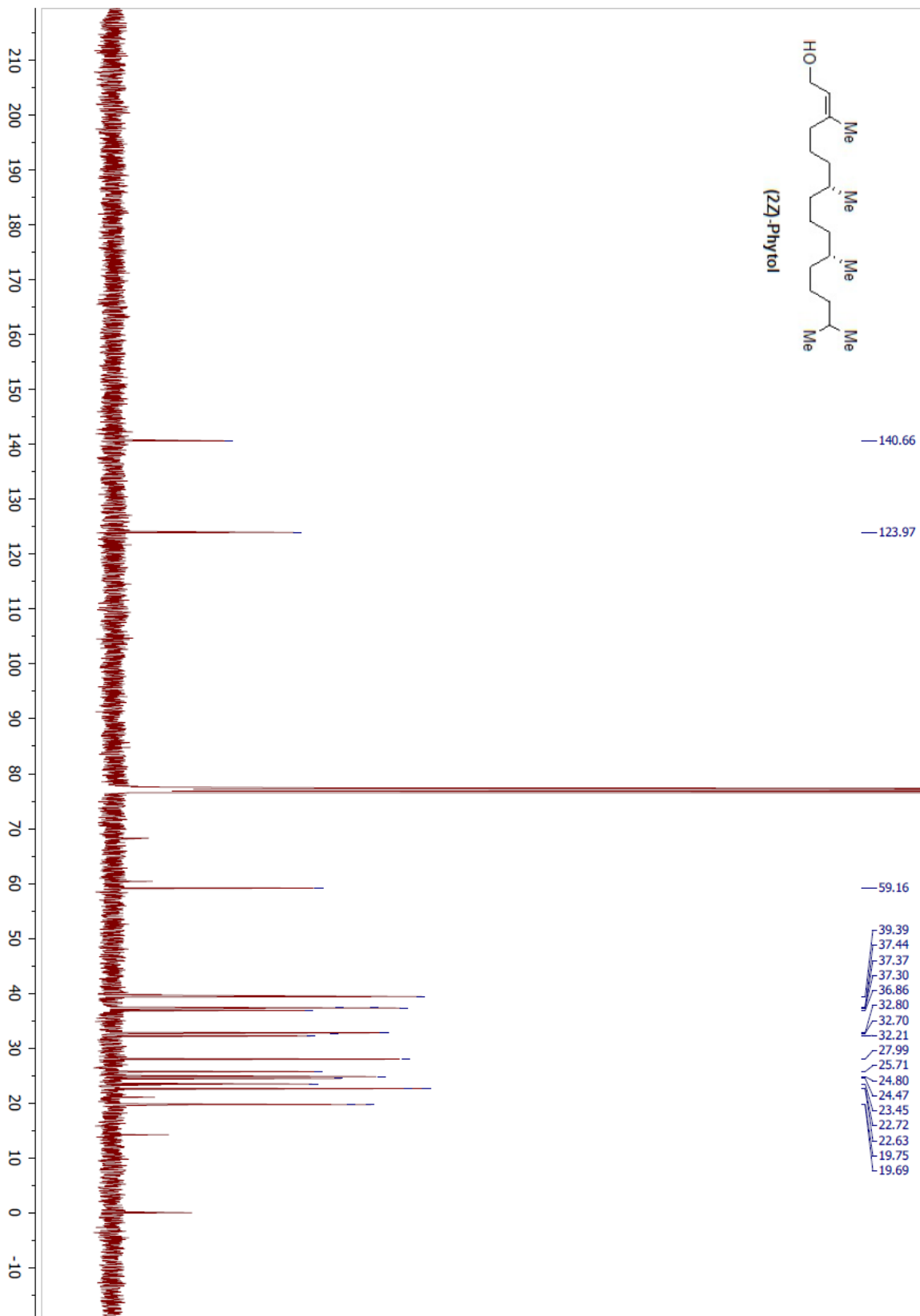


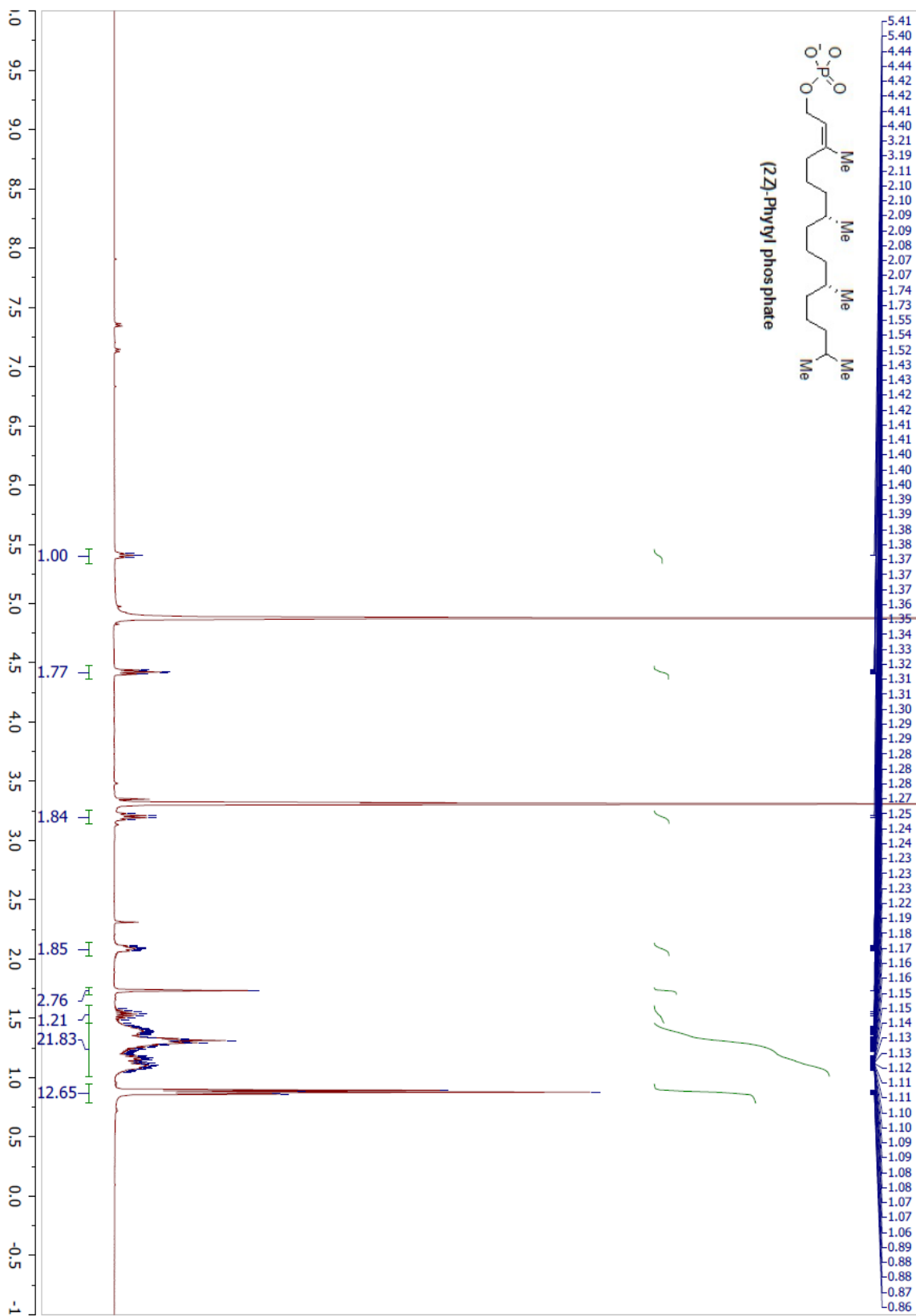


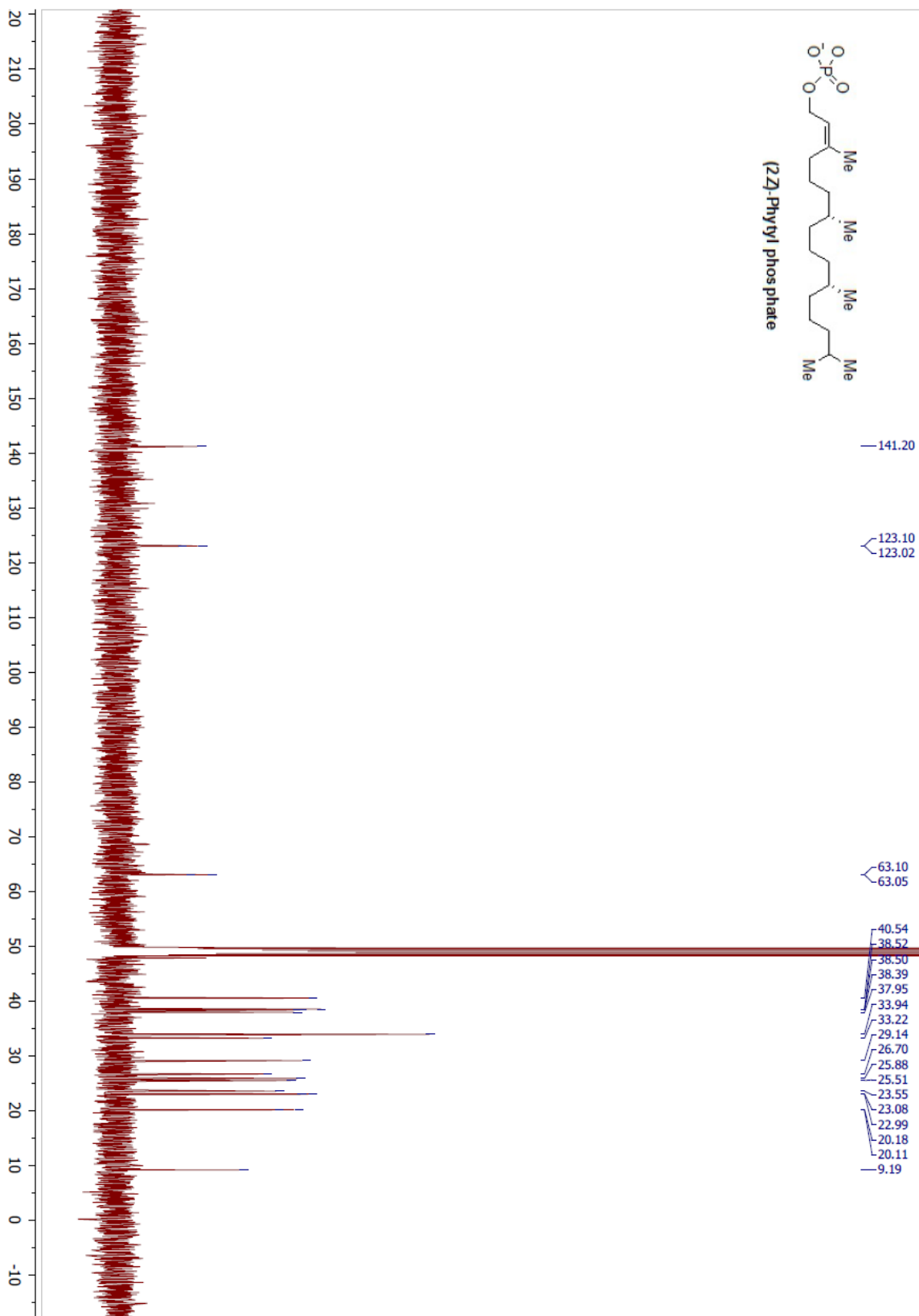


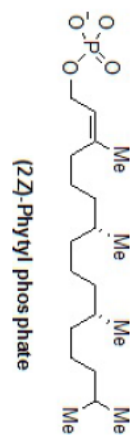












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