GlcNAc-1-P-transferase-tunicamycin complex structure reveals basis for inhibition of N-glycosylation

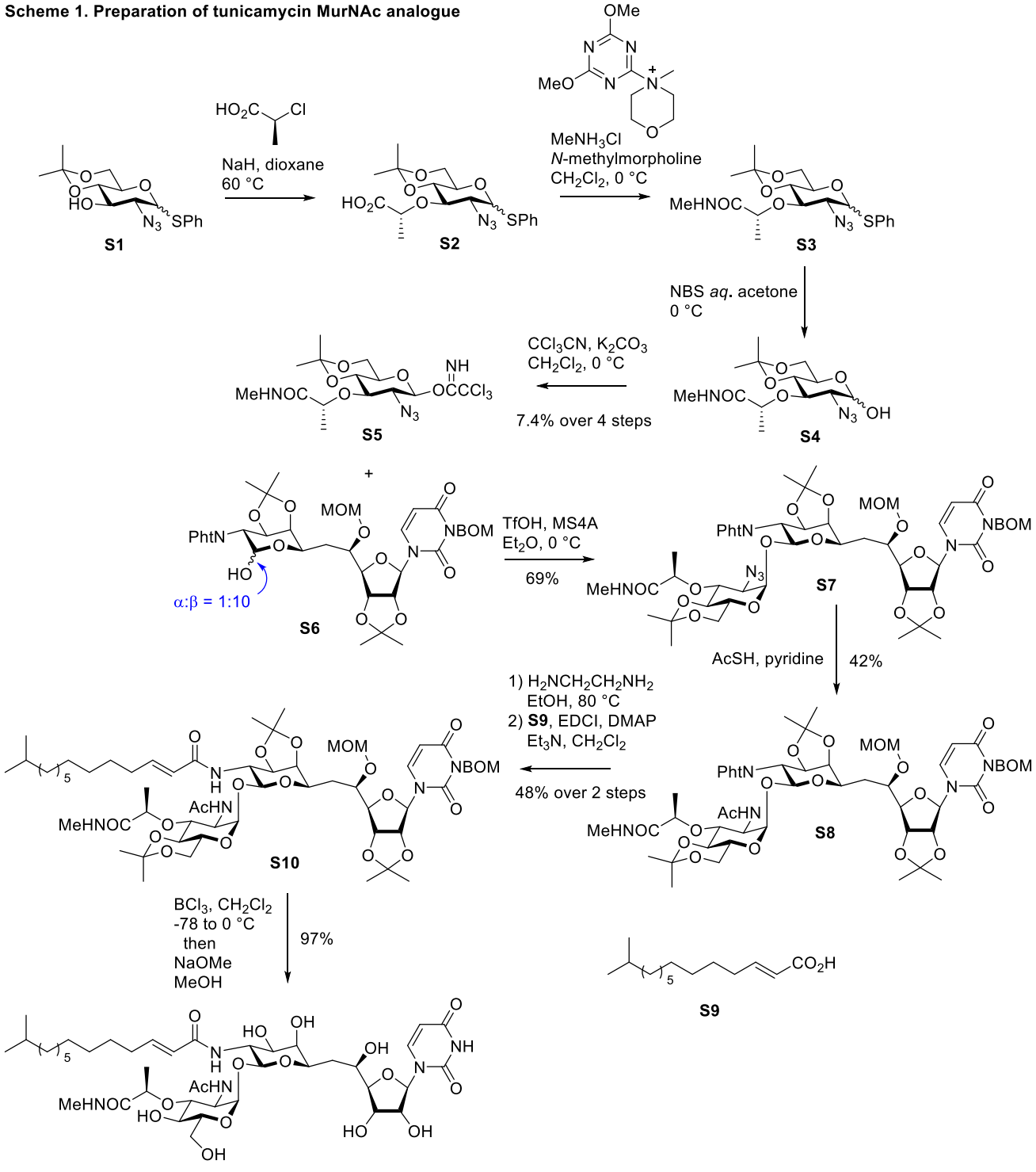
Supplementary Note 1: Chemical synthesis of Tunicamycin-MurNAc

Jiho Yoo[1*], Ellene H. Mashalidis[1*], Alvin C. Y. Kuk[1*], Kazuki Yamamoto[2], Benjamin Kaeser[1], Satoshi Ichikawa[2], Seok-Yong Lee[1†]

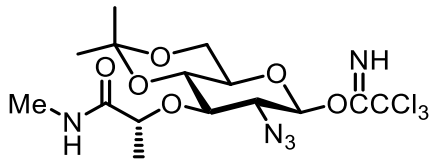
General experimental methods

All reactions except those carried out in aqueous phase were performed under argon atmosphere, unless otherwise noted. Materials were purchased from commercial suppliers and used without further purification, unless otherwise noted. Solvents were distilled according to the standard protocol. Isolated yields were calculated by weighting products. The weight of the starting materials and the products were not calibrated. Analytical thin layer chromatography (TLC) was performed on Merck silica gel $60F_{254}$ plates. Normal-phase column chromatography was performed on Merck silica gel 5715 or Wakogel 60N. Flash column chromatography was performed on Kanto Chemical Silica Gel 60N (spherical, neutral. 40-50 μm). ^{1}H NMR were measured in $CDCl_3$, DMSO-d_6 and methalnol-d_4 solution, and reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as internal standard using JEOL ECS400, ECX400, ECA500, unless otherwise noted. ^{13}C NMR were measured in $CDCl_3$ or methanol-d_4 solution, and referenced to residual solvent peaks of $CDCl_3$ (77.16 ppm) or methanol-d_4 (49.00 ppm) using ECS400, ECX400, ECA500. Coupling constant (*J*) was reported in hertz (Hz). Abbreviations of multiplicity were as follows; s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad. Data were presented as follows; chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^{1}H-^{1}H COSY spectra. Mass spectra were obtained on Waters MICRO MASS LCT-premier and mass analyzer type used for the HRMS measurements was TOF. Optical rotation was measured on a Rudolph Research Analytical Autopol IV automatic polarimeter.

Scheme 1. Preparation of tunicamycin MurNAc analogue



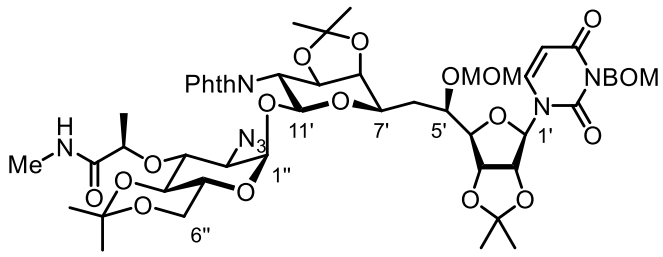
*2-Azido-2-deoxy-3-O-[(R)-1-(methylaminocarbonyl)ethyl]-4,6-isopropylidene-β-D-glucopysanosyl trichloroacetimidate (**S5**)*



A solution of **S1** (200 mg, 0.593 mmol) in 1,4-dioxane (12 mL) was treated with NaH (60% dispersion in mineral oil, 47.4 mg) at room temperature, and the mixture was heated to 60 °C for 30 min. To the mixture was added (*S*)-2-chloropropionic acid (204 μL, 2.37 mmol) and additional NaH (119 mg) at 60 °C, and the resulting mixture was heated to 70 °C for 3 h. The reaction mixture was cooled and partitioned between hexane and H_2O. The aqueous layer was acidified with *conc.* HCl at a cooling-bath. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. A solution of **S2**, $MeNH_2$·HCl (94.5 mg, 1.40 mmol) and *N*-methylmorpholine (154 μL, 1.40 mmol) in CH_2Cl_2 (2 mL) was treated with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM, 438 mg, 1.40 mmol) at 0 °C and stirred at room temperature for 3 h. The reaction mixture was partitioned between EtOAc and 1 M *aq.* HCl, and the organic layer was washed with *sat. aq.* $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. A solution of **S3** in acetone/H_2O (v/v 5/1, 3 mL) was treated with *N*-bromosuccinimide (NBS, 211 mg, 1.19 mmol) at 0 °C for 20 min. The reaction was quenched with *sat. aq.* $Na_2S_2O_3$/*sat. aq.* $NaHCO_3$ (v/v 1/1), and the resulting mixture was extracted with CH_2Cl_2. The organic layer was washed with 0.1 M *aq.* NaOH (three times) and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. A solution of **S4** in CH_2Cl_2/CCl_3CN (v/v 4/1, 1.25mL) was treated with K_2CO_3 powder (9.9 mg, 71.7 μmol) at 0 °C and stirred at room temperature for 17 h. The insoluble solid was filtered off through a Celite pad and washed with CH_2Cl_2. The filtrate was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (ϕ0.8×8 cm, treated with Et_3N; hexane/EtOAc = 3/2 → 1/1) to afford **S5** (20.9 mg, 7.4% over 4 steps, α/β = 1/20) as a white foam.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.84 (s, 1H, imidate NH), 7.20 (br s, 1H, amide NH), 5.77 (d, 1H, H-1, $J_{1,2}$ = 8.3 Hz), 4.41 (q, 1H, C*H*, $J_{CH,Me}$ = 6.9 Hz), 3.98 (dd, 1H, H-6, J_{gem} = 10.8, $J_{6,5}$ = 5.7 Hz), 3.79 (t, 1H, H-6, *J* = 10.3 Hz), 3.75 (t, 1H, H-4, *J* = 9.2 Hz), 3.67 (dd, 1H, H-2, $J_{2,3}$ = 9.7, $J_{2,1}$ = 8.3 Hz), 3.41-3.35 (m, 2H, H-3, H-5), 2.86 (d, 3H, N-CH_3, *J* = 5.2 Hz), 1.51 (s, 3H, isopropylidene), 1.39 (s, 3H, isopropylidene), 1.41 (d, 3H, CH-CH_3, $J_{CH3,CH}$ = 6.9 Hz).

*3-(Benzyloxymethyl)-1-((11S)-6,10-dideoxy-11-O-{2-azido-2-deoxy-4,6-O-isopropylidene-3-O-[(R)-1-(methylaminocarbonyl)ethyl]-α-D-gluco-hexopyranosyl}-2,3:8,9-di-O-isopropylidene-5-O-(methoxymethyl)-10-phthalimido-L-galacto-β-D-allo-undecodialdo-1,4-furanose-11,7-pyranos-1-yl)uracil (**S7**)*

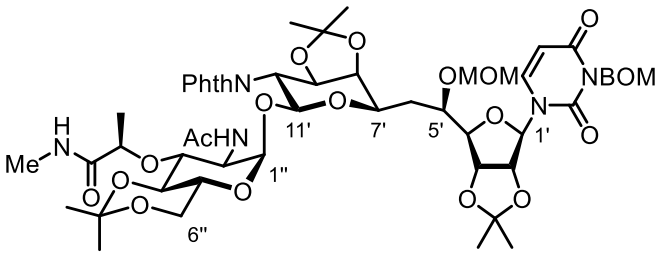


A solution of **S5** (20.5 mg, 43.2 μmol), **S6** (20.0 mg, 25.6 μmol) and molecular sieves 4A (50 mg) in Et_2O (1 mL) was treated with TfOH (0.1 M in Et_2O, 102 μL, 40 mol%) at 0 °C for 2 h. To the reaction mixture was added CH_2Cl_2 (0.2 mL) and stirred at 0 °C for 7.5 h. The reaction was quenched with Et_3N (50 μL) and the mixture was warmed to room temperature. The insoluble solid was filtered off through a Celite pad and washed with EtOAc. The filtrate was washed with *sat. aq.* $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in*

vacuo. The residue was purified by preparative TLC (hexane/EtOAc = 2/3 twice) to afford **S7** (19.3 mg, 69%) as a white powder.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.85 (br s, 2H, Phth), 7.74 (br s, 2H, Phth), 7.38-7.27 (m, 6H, Ph, H-6), 6.61 (q, 1H, N*H*-Me, J = 4.6 Hz), 5.83 (d, 1H, H-1', $J_{1',2'}$ = 3.4 Hz), 5.75 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.51 (d, 1H, N-CH_2-OBn, J_{gem} = 9.7 Hz), 5.49 (d, 1H, N-CH_2-OBn, J_{gem} = 9.7 Hz), 5.26 (d, 1H, H-11', $J_{11',10'}$ = 8.6 Hz), 5.01 (d, 1H, H-1", $J_{1",2"}$ = 3.4 Hz), 4.93 (dd, 1H, H-3', $J_{3',2'}$ = 6.9, $J_{3',4'}$ = 4.9 Hz), 4.77-4.74 (m, 2H, H-2', H-9'), 4.71-4.67 (m, 4H, Bn, O-CH_2-OMe), 4.32 (t, 1H, H-10', J = 9.2 Hz), 4.16-4.11 (m, 2H, C*H*-CH_3, H-5'), 4.09 (dd, 1H, H-8', $J_{8',9'}$ = 4.6, $J_{8',7'}$ = 1.7 Hz), 4.03-3.99 (m, 2H, H-4', H-7'), 3.91-3.84 (m, 2H, H-5", H-6"), 3.66 (t, 1H, H-6", J = 10.0 Hz), 3.51 (t, 1H, H-4", J = 9.2 Hz), 3.46 (t, 1H, H-3", J = 9.2 Hz), 3.43 (s, 3H, OMe), 3.24 (dd, 1H, H-2", $J_{2",3"}$ = 9.2, $J_{2",1"}$ = 3.4 Hz), 2.64 (d, 3H, N-CH_3, J = 4.6 Hz), 2.20 (m, 1H, H-6'), 1.77 (m, 1H, H-6'), 1.68 (s, 3H, isopropylidene), 1.60 (s, 3H, isopropylidene), 1.43 (s, 3H, isopropylidene), 1.37 (s, 3H, isopropylidene), 1.36 (s, 3H, isopropylidene), 1.33 (s, 3H, isopropylidene), 1.30 (d, 3H, CH-CH_3, $J_{CH3,CH}$ = 6.9 Hz); ESIMS-HR calcd for $C_{52}H_{66}N_7O_{19}$ 1092.4413, found 1092.4406.

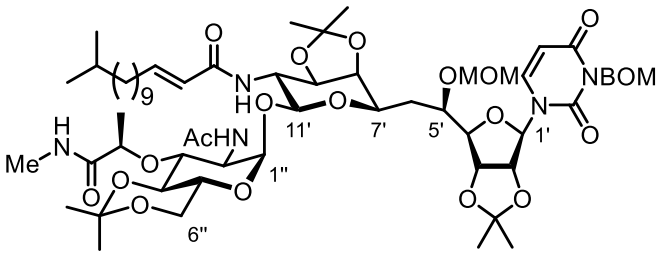
*3-(Benzyloxymethyl)-1-((11S)-6,10-dideoxy-11-O-{2-acetamide-2-deoxy-4,6-O-isopropylidene-3-O-[(R)-1-(methylaminocarbonyl)ethyl]-α-D-gluco-hexopyranosyl}-2,3:8,9-di-O-isopropylidene-5-O-(methoxymethyl)-10-phthalimido-L-galacto-β-D-allo-undecodialdo-1,4-furanose-11,7-pyranos-1-yl)uracil (**S8**)*



A solution of **S7** (19.1 mg, 17.5 μmol) in pyridine (1.2 mL) was treated with thioacetic acid (0.6 mL) at room temperature for 59 h under the dark condition. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene. The residue was purified by silica gel column chromatography (ϕ0.8×8 cm; $CHCl_3$/MeOH = 0% → 3% gradually) to afford **S8** (8.2 mg, 42%) as a white powder.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.88 (br s, 2H, Phth), 7.77 (m, 2H, Phth), 7.38-7.27 (m, 6H, Ph, H-6), 6.44 (q, 1H, N*H*-Me, J = 5.2 Hz), 5.93 (d, 1H, AcN*H*, J = 8.6 Hz), 5.88 (1H, H-1', $J_{1',2'}$ = 3.4 Hz), 5.76 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.52 (d, 1H, N-CH_2-OBn, J_{gem} = 9.7 Hz), 5.48 (d, 1H, N-CH_2-OBn, J_{gem} = 9.7 Hz), 5.16 (d, 1H, H-11', $J_{11',10'}$ = 8.6 Hz), 4.93 (d, 1H, H-1", $J_{1",2"}$ = 4.0 Hz), 4.91 (dd, 1H, H-3', $J_{3',2'}$ = 6.9, $J_{3',4'}$ = 4.6 Hz), 4.78 (dd, 1H, H-9', $J_{9',10'}$ = 8.6, $J_{9',8'}$ = 5.2 Hz), 4.74 (dd, 1H, H-2', $J_{2',3'}$ = 6.9, $J_{2',1'}$ = 3.4 Hz), 4.71-4.67 (m, 4H, Bn, O-CH_2-OMe), 4.30 (t, 1H, H-10', J = 8.6 Hz), 4.14 (br d, 1H, H-5', J = 10.3 Hz), 4.10 (dd, 1H, H-8', $J_{8',9'}$ = 5.2, $J_{8',7'}$ = 2.0 Hz), 4.03-3.97 (m, 3H, H-4', H-7', H-2"), 3.94 (q, 1H, C*H*-CH_3, J = 6.9 Hz), 3.88 (td, 1H, H-5", J = 10.3, $J_{5",6"}$ = 5.2 Hz), 3.82 (dd, 1H, H-6", J_{gem} = 10.3, $J_{6",5"}$ = 5.2 Hz), 3.67 (t, 1H, H-6", J = 10.6 Hz), 3.56 (t, 1H, H-4", J = 9.5 Hz), 3.41 (s, 3H, OMe), 3.35 (t, 1H, H-3", J = 9.7 Hz), 2.76 (d, 3H, N-CH_3, J = 5.2 Hz), 2.19 (ddd, 1H, H-6', J_{gem} = 14.9, J = 10.9, J = 2.0 Hz), 1.81 (ddd, 1H, H-6', J_{gem} = 14.9, J = 9.7, J = 2.3 Hz), 1.65, 1.60, 1.44, 1.37, 1.35, 1.33, 1.32 (each s, each 3H, isopropylidene×3, Ac), 1.32 (d, 3H, CH-CH_3, J = 6.9 Hz); ESIMS-HR calcd for $C_{54}H_{70}N_5O_{20}$ 1108.4614, found 1108.4617.

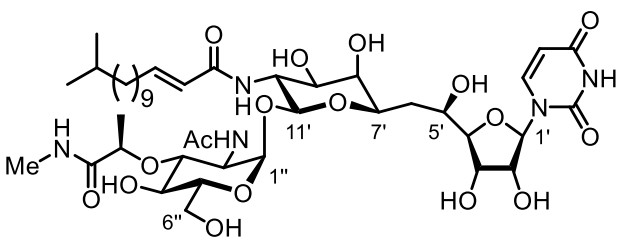
*3-(Benzyloxymethyl)-1-((11S)-6,10-dideoxy-11-O-{2-acetamide-2-deoxy-4,6-O-isopropylidene-3-O-[(R)-1-(methylaminocarbonyl)ethyl]-α-D-gluco-hexopyranosyl}-2,3:8,9-di-O-isopropylidene-5-O-(methoxymethyl)-10-(13-methyltetradec-2-enamido)-L-galacto-β-D-allo-undecodialdo-1,4-furanose-11,7-pyranos-1-yl)uracil (**S10**)*



A solution of **S8** (8.1 mg, 7.31 μmol) in EtOH (0.5 mL) was treated with ethylenediamine (50 μL) and the mixture was heated at 80 °C for 9 h. The reaction mixture was cooled to room temperature, concentrated *in vacuo* and co-evaporated with toluene. The residue was purified by silica gel column chromatography (ϕ0.5×3 cm, treated with 0.1% Et_3N; $CHCl_3$/MeOH = 0% → 5% → 10%) to afford the crude amine. A solution of the amine in CH_2Cl_2 (0.5 mL) was treated with Et_3N (3.04 μL, 21.9 μmol), HOAt (1.5 mg, 11.0 μmol), **S9** (2.6 mg, 11.0 μmol) and EDCI (2.2 mg, 11.7 μmol) sequentially at room temperature for 6 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (ϕ0.5×4 cm; hexane/EtOAc = 1/1 → $CHCl_3$/MeOH = 0% → 2%) to afford **S10** (4.2 mg, 48%) as a white powder.

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.38-7.27 (m, 6H, Ph, H-6), 6.89-6.83 (m, 2H, CH=CHβ, N*H*-Me), 6.52 (d, 1H, AcN*H*, J = 9.7 Hz), 6.05 (br d, 1H, N*H*-10', J = 5.7 Hz), 5.86-5.82 (m, 2H, H-1', CH=CHα), 5.74 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 5.50 (d, 1H, N-CH_2-OBn, J_{gem} = 9.7 Hz), 5.47 (d, 1H, N-CH_2-OBn, J_{gem} = 9.7 Hz), 4.94-4.92 (m, 2H, H-11', H-1"), 4.89 (dd, 1H, H-3', $J_{3',2'}$ = 6.9, $J_{3',4'}$ = 4.3 Hz), 4.73 (dd, 1H, H-2', $J_{2',3'}$ = 6.9, $J_{2',1'}$ = 2.9 Hz), 4.70 (s, 2H, Bn), 4.66 (s, 2H, O-CH_2-OMe), 4.40 (dd, 1H, H-9', $J_{9',10'}$ = 8.6, $J_{9',8'}$ = 5.2 Hz), 4.24 (td, 1H, H-2", J = 9.7, $J_{2",1"}$ = 3.4 Hz), 4.04-3.98 (m, 4H, H-4'. H-5', H-7', H-8'), 3.92 (td, 1H, H-5", J = 10.3, $J_{5",6"}$ = 5.2 Hz), 3.88 (q, 1H, C*H*-CH_3, J = 6.9 Hz), 3.85 (dd, 1H, H-6", J_{gem} = 10.3, $J_{6",5"}$ = 5.2 Hz), 3.69 (t, 1H, H-6", J = 10.5 Hz), 3.60 (t, 1H, H-4", J = 9.5 Hz), 3.46-3.39 (m, 2H, H-10', H-3"), 3.37 (s, 3H, OMe), 2.78 (d, 3H, N-CH_3, J = 5.2 Hz), 2.17-2.09 (m, 3H, H-6', side chain CH_2γ), 1.90 (s, 3H, Ac), 1.76 (m, 1H, H-6'), 1.58-1.12 (m, 38H, isopropylidene×3, CH_2×8, C*H*×2), 0.86 (d, 6H, CH-(CH_3)$_2$, J = 6.3 Hz); ESIMS-HR calcd for $C_{61}H_{94}N_5O_{19}$ 1200.6543, found 1200.6523.

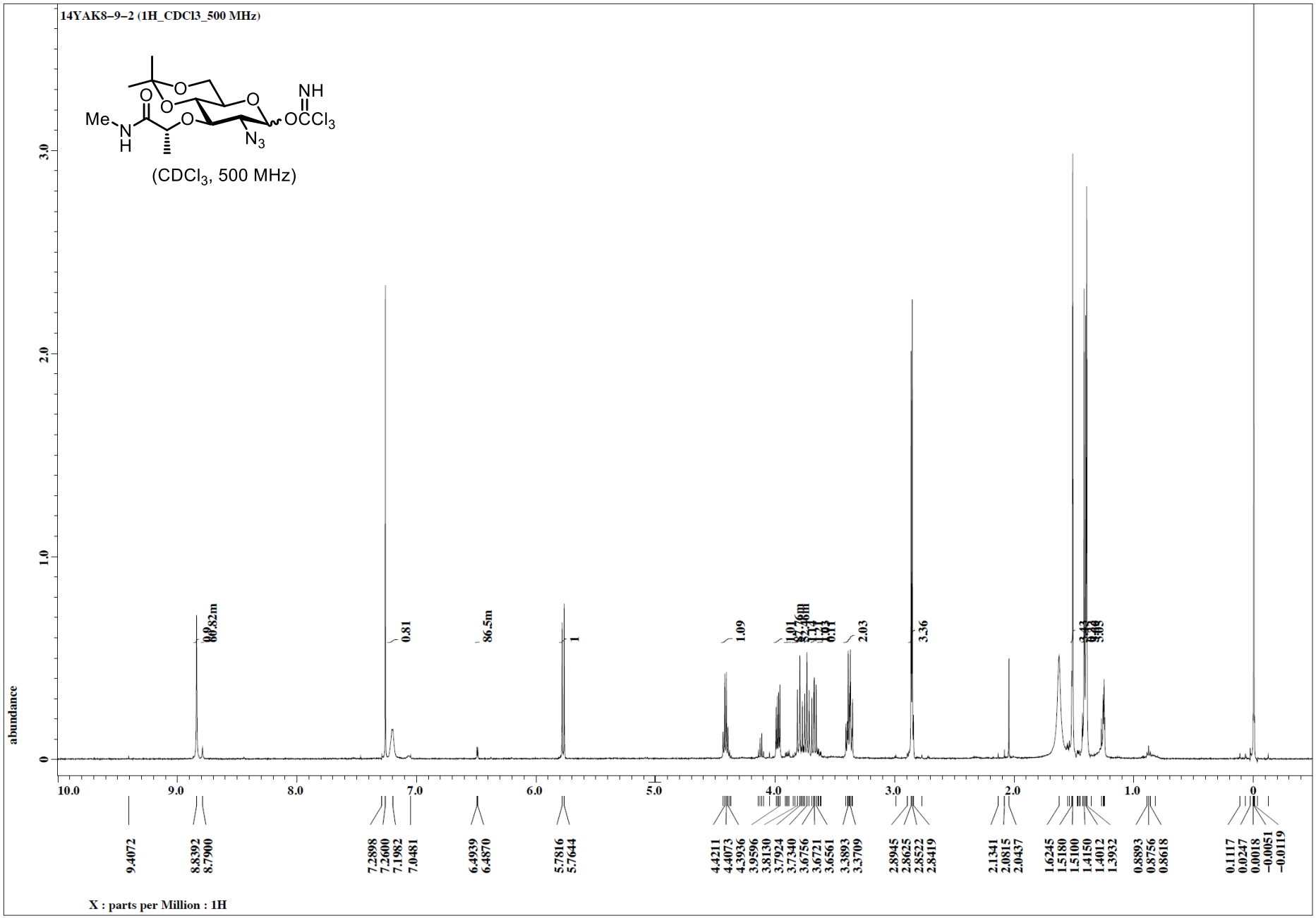
tunicamycin MurNAc analogue

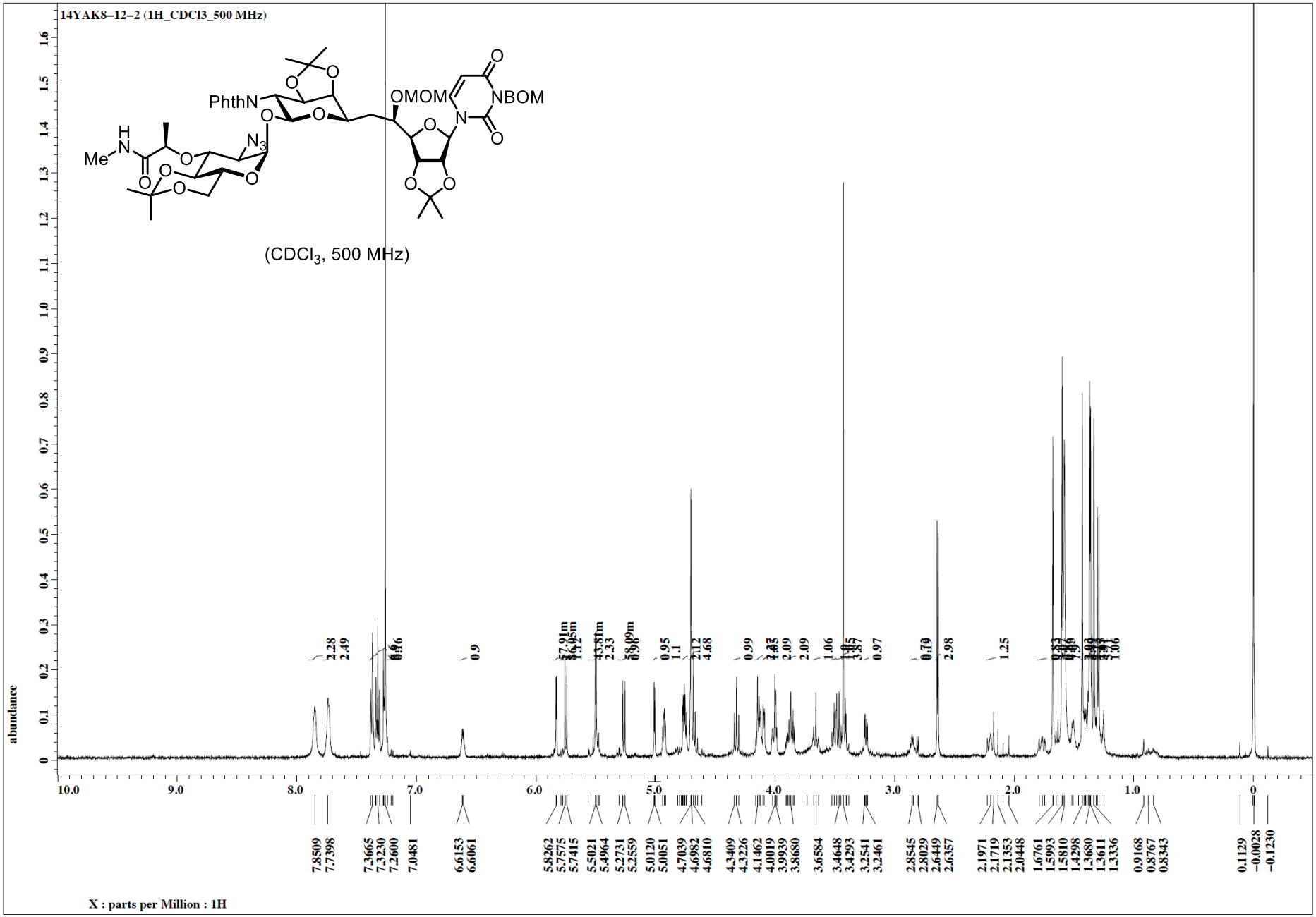


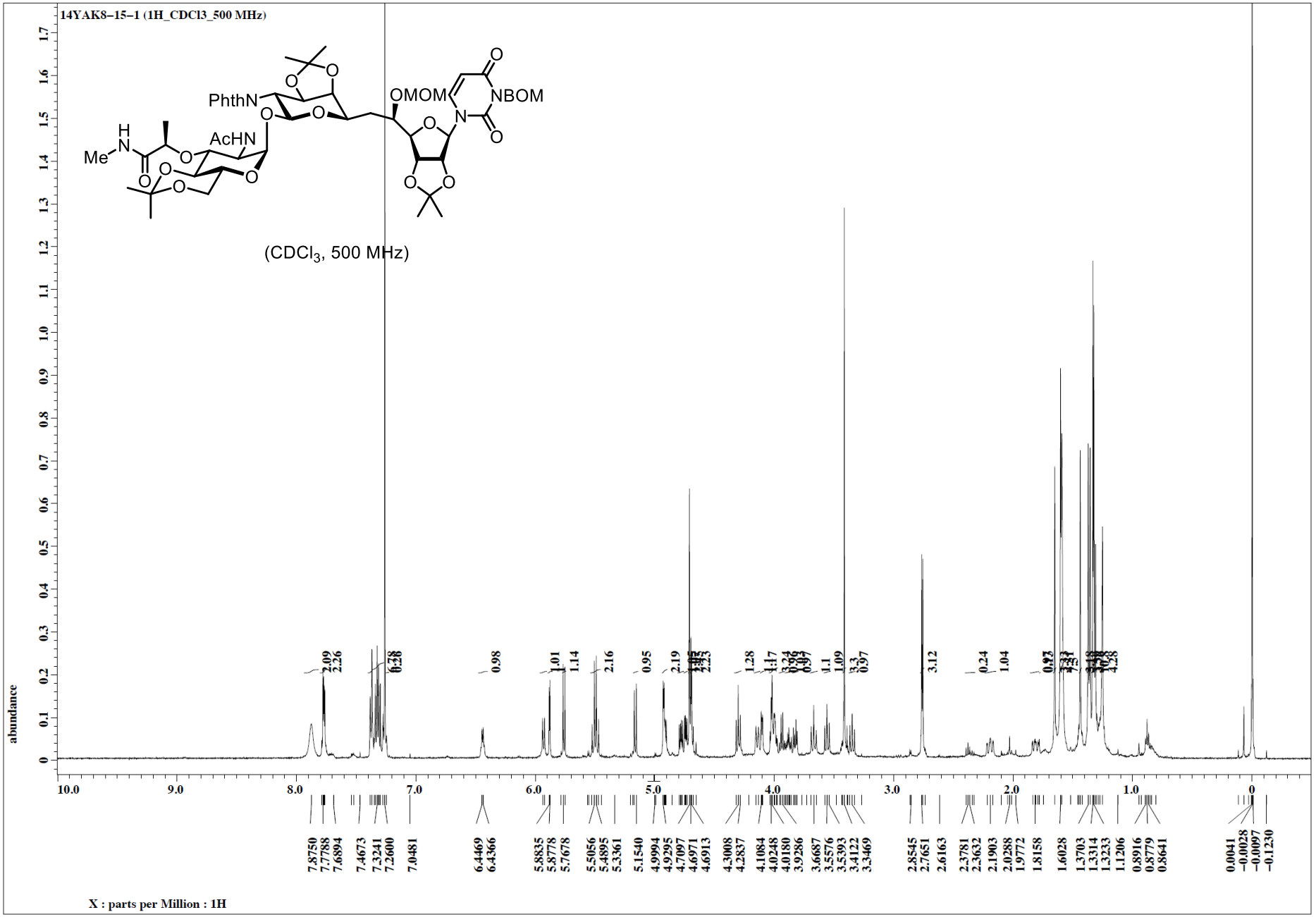
A solution of **S10** (4.2 mg, 3.50 μmol) in CH_2Cl_2 (0.5 mL) was treated with BCl_3 (1 M in CH_2Cl_2, 105 μL, 105 μmol) at -78 °C for 15 min, and the mixture was stirred at 0 °C for 30 min. Sodium methoxide (5 M in MeOH, 105 μL, 525 μmol) was added to the mixture at 0 °C, and the resulting mixture was stirred at room temperature for 10 min. The mixture was neutralized with Dowex 50W×4 and the insoluble solid was filtered off. The filtrate was concentrated *in vacuo*, the residue was purified by HPLC (YMC-Pack R&D ODS D-ODS-5-A, 250 × 20 mm, 0.1% TFA 60% MeCN/H_2O) to afford tunicamycin MurNAc analogue (3.1 mg, 97%) as a white solid.

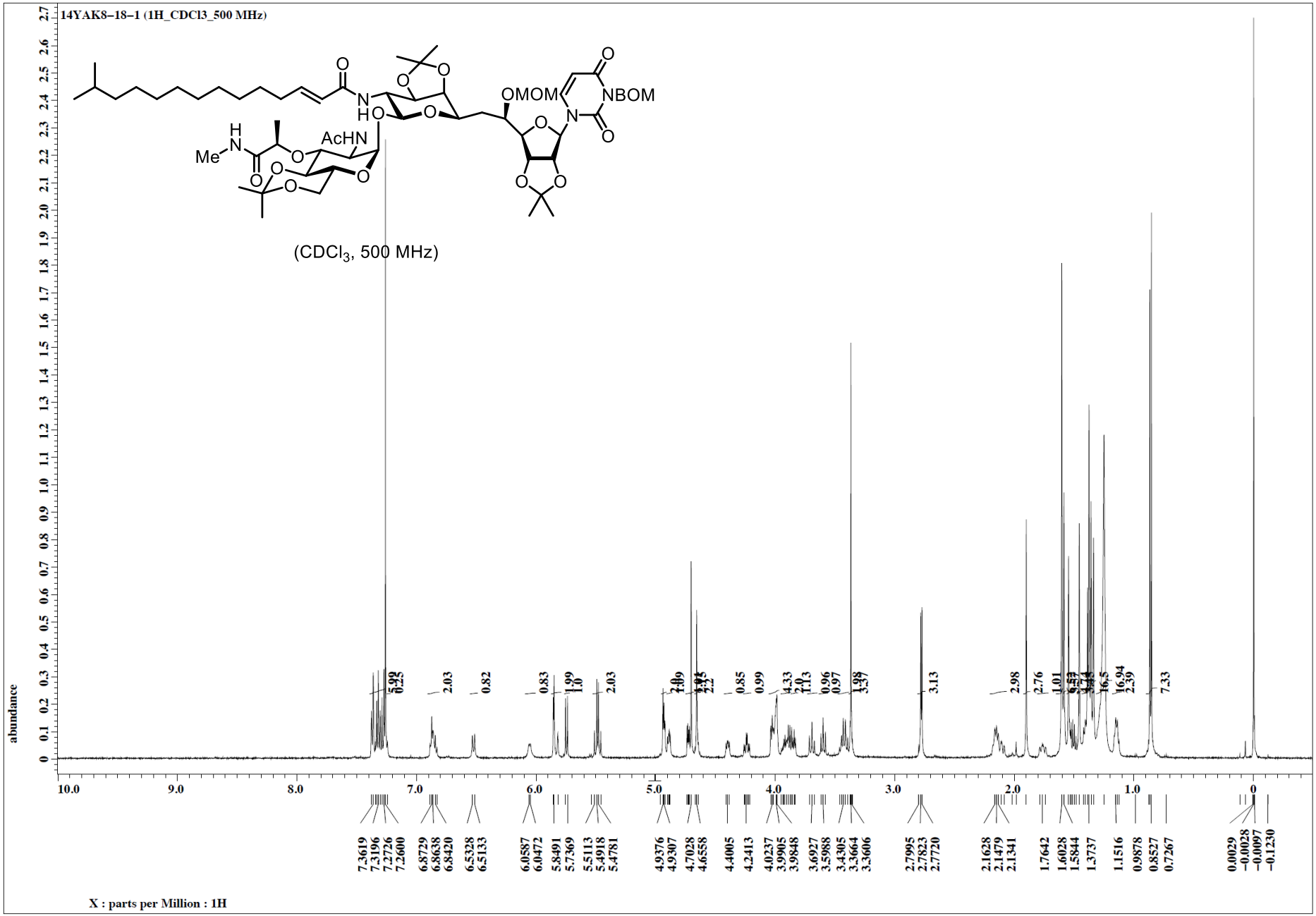
^{1}H NMR (CD_3OD, 500 MHz) δ 7.92 (d, 1H, H-6, $J_{6,5}$ = 8.0 Hz), 6.81 (dt, 1, CH=C*H*β, J = 14.9, J = 7.2 Hz), 5.95-5.92 (m, 2H, H-1', CH=C*H*α), 5.75 (d, 1H, H-5, $J_{5,6}$ = 8.0 Hz), 4.93 (d, 1H, H-1", $J_{1",2"}$ = 3.4 Hz), 4.61 (d, 1H, H-11', $J_{11',10'}$ = 8.6 Hz), 4.22-4.16 (m, 3H, H-2', H-3', C*H*-CH_3), 4.06-3.99 (m, 3H, H-5', H-10', H-5"), 3.97 (dd, 1H, H-2",

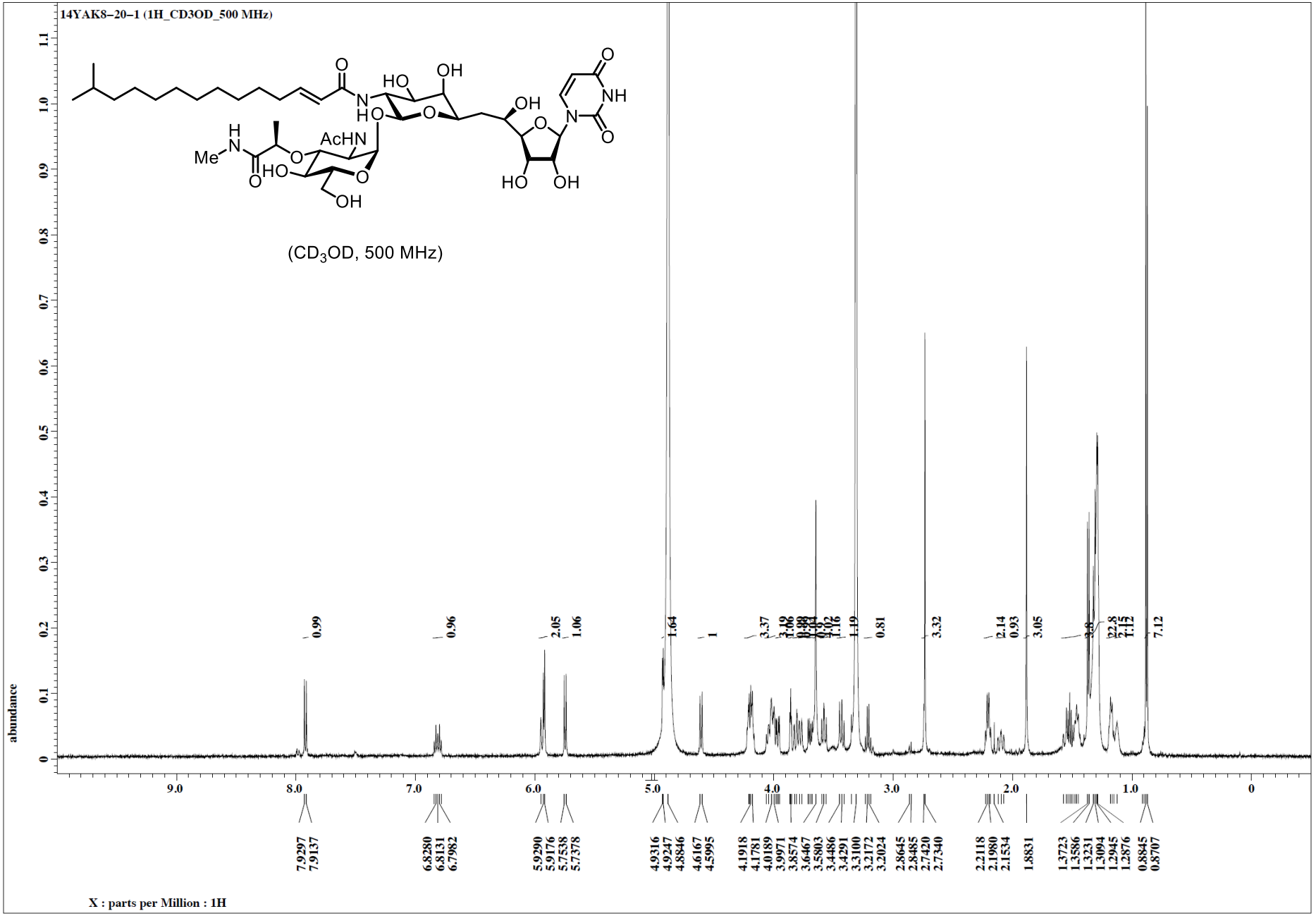
$J_{2",3"}$ = 10.3, $J_{2",1"}$ = 3.4 Hz), 3.86 (t, 1H, H-4', J = 3.3 Hz), 3.82 (dd, 1H, H-6", J_{gem} = 12.0, $J_{6",5"}$ = 2.3 Hz), 3.78 (br d, 1H, H-7', J = 10.0 Hz), 3.69 (dd, 1H, H-6", J_{gem} = 12.0, $J_{6",5"}$ = 5.7 Hz), 3.65 (m, 2H, H-8', H-9'), 3.58 (t, 1H, H-3", J = 9.5 Hz), 3.43 (t, 1H, H-4", J = 9.5 Hz), 2.73 (s, 3H, OMe), 2.20 (q, 2H, side chain CH_2γ, J = 7.1 Hz), 2.10 (m, 1H, H-6'), 1.88 (s, 3H, Ac), 1.57-1.43 (m, 4H, H-6', side chain CH, CH_2), 1.37 (d, 3H, CH-CH_3, J = 6.9 Hz), 1.37-1.15 (m, 14H, CH_2×7), 0.88 (d, 6H, side chain CH-(CH_3)$_2$, J = 7.1 Hz); ESIMS-HR calcd for $C_{42}H_{70}N_5O_{17}$ 916.4767, found 916.4741.


14YAK8--9--2 (1H_CDCl3_500 MHz)
Me
N
H
O
O
O
O
O
O
NH
OCCl3
N3
(CDCl3, 500 MHz)
abundance
X : parts per Million : 1H


14YAK8--12--2 (1H_CDCl3_500 MHz)
PhthN
OMOM
NBOM
Me
N3
(CDCl3, 500 MHz)
abundance
X : parts per Million : 1H


14YAK8-15-1 (1H_CDCl3_500 MHz)
PhthN
AcHN
OMOM
NBOM
Me
(CDCl3, 500 MHz)
abundance
X : parts per Million : 1H


14YAK8–18–1 (1H_CDCl3_500 MHz)
OMOM
NBOM
AcHN
Me
(CDCl3, 500 MHz)
abundance
X : parts per Million : 1H


14YAK8–20–1 (1H_CD3OD_500 MHz)
(CD3OD, 500 MHz)
abundance
X : parts per Million : 1H
0.99
0.96
2.05
1.06
1.64
1
3.37
0.81
3.32
2.14
0.93
3.05
7.12
7.9297
7.9137
6.8280
6.8131
6.7982
5.9290
5.9176
5.7538
5.7378
4.9316
4.9247
4.8846
4.6167
4.5995
4.1918
4.1781
4.0189
3.9971
3.8574
3.6467
3.5803
3.4486
3.4291
3.3100
3.2172
3.2024
2.8645
2.8485
2.7420
2.7340
2.2118
2.1980
2.1534
1.8831
1.3723
1.3586
1.3231
1.3094
1.2945
1.2876
0.8845
0.8707

Elemental Composition Report

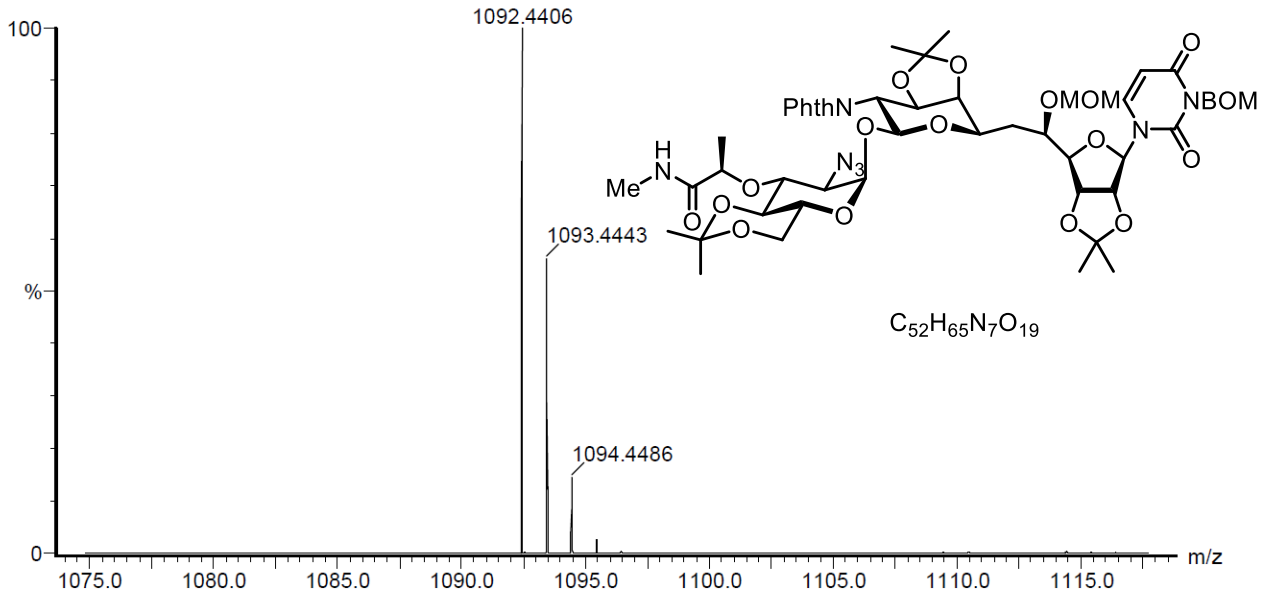
Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off

Monoisotopic Mass, Even Electron Ions
2786 formula(e) evaluated with 15 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 5-60 H: 10-100 N: 0-10 O: 0-20 Na: 0-1 S: 0-1

20171024_4 no acid
14YAK8_12_2 1112 (4.083)

1: TOF MS ES+
2.54e+005



Minimum:				-1.5	
Maximum:		3.0	5.0	50.0	
Mass	**Calc. Mass**	**mDa**	**PPM**	**DBE**	**Formula**
1092.4406	1092.4405	0.1	0.1	28.5	C59 H67 N5 O12 Na S
	1092.4413	-0.7	-0.6	23.5	C52 H66 N7 O19
	1092.4415	-0.9	-0.8	26.5	C60 H70 N O16 S
	1092.4418	-1.2	-1.1	33.5	C60 H63 N9 O8 Na S
	1092.4391	1.5	1.4	23.5	C58 H71 N O16 Na S
	1092.4423	-1.7	-1.6	15.5	C47 H71 N7 O19 Na S
	1092.4389	1.7	1.6	20.5	C50 H67 N7 O19 Na
	1092.4388	1.8	1.6	27.5	C56 H66 N7 O14 S
	1092.4430	-2.4	-2.2	24.5	C55 H67 N5 O17 Na
	1092.4375	3.1	2.8	22.5	C55 H70 N3 O18 S
	1092.4443	-3.7	-3.4	29.5	C56 H63 N9 O13 Na
	1092.4447	-4.1	-3.8	18.5	C49 H70 N7 O19 S
	1092.4364	4.2	3.8	24.5	C54 H67 N7 O14 Na S
	1092.4454	-4.8	-4.4	27.5	C57 H66 N5 O17
	1092.4355	5.1	4.7	32.5	C59 H62 N7 O14

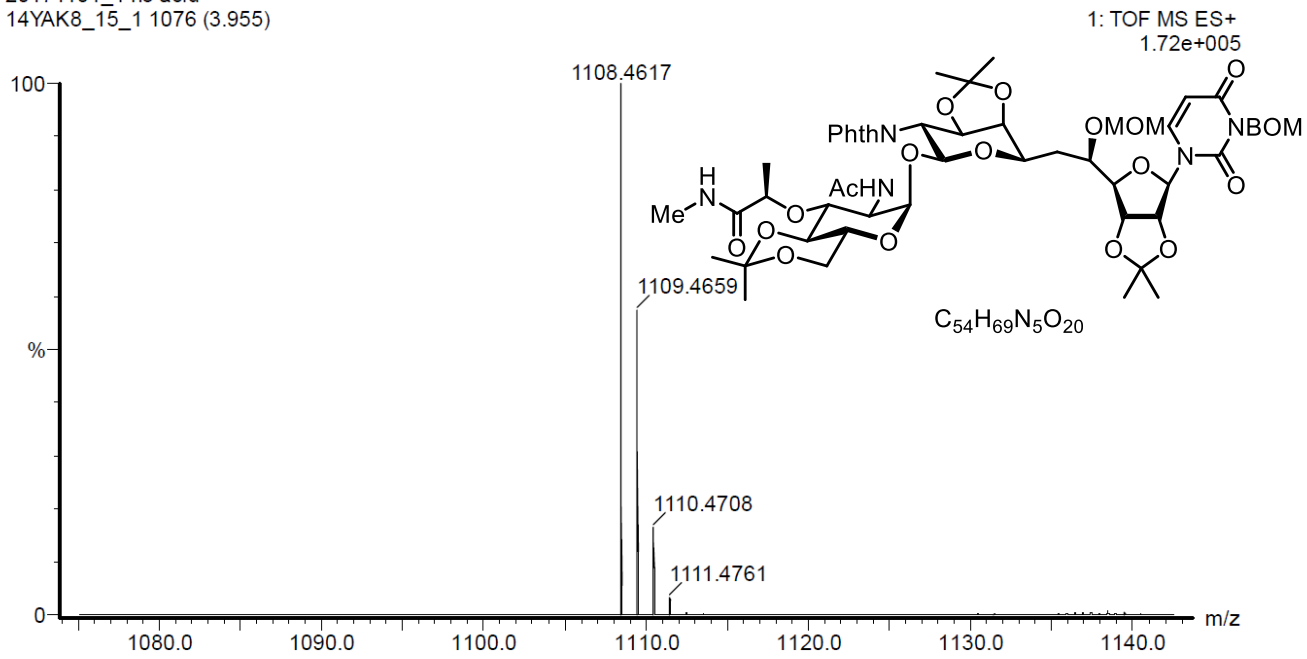
Elemental Composition Report

Single Mass Analysis

Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off

Monoisotopic Mass, Even Electron Ions
4032 formula(e) evaluated with 25 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 5-80 H: 10-100 N: 0-10 O: 0-25 Na: 0-1

20171101_1 no acid
14YAK8_15_1 1076 (3.955)



Minimum:				-1.5	
Maximum:		5.0	5.0	50.0	
Mass	Calc. Mass	mDa	PPM	DBE	Formula
1108.4617	1108.4614	0.3	0.3	22.5	C54 H70 N5 O20
	1108.4612	0.5	0.5	36.5	C69 H67 N O11 Na
	1108.4609	0.8	0.7	40.5	C67 H62 N7 O9
	1108.4625	-0.8	-0.7	41.5	C70 H63 N5 O7 Na
	1108.4628	-1.1	-1.0	27.5	C55 H66 N9 O16
	1108.4630	-1.3	-1.2	23.5	C57 H71 N3 O18 Na
	1108.4603	1.4	1.3	24.5	C53 H67 N9 O16 Na
	1108.4601	1.6	1.4	17.5	C53 H74 N O24
	1108.4636	-1.9	-1.7	39.5	C71 H66 N O11
	1108.4596	2.1	1.9	35.5	C66 H66 N3 O13
	1108.4639	-2.2	-2.0	46.5	C71 H59 N9 O3 Na
	1108.4644	-2.7	-2.4	28.5	C58 H67 N7 O14 Na
	1108.4590	2.7	2.4	19.5	C52 H71 N5 O20 Na
	1108.4585	3.2	2.9	37.5	C65 H63 N7 O9 Na
	1108.4649	-3.2	-2.9	44.5	C72 H62 N5 O7
	1108.4649	-3.2	-2.9	10.5	C45 H75 N5 O25 Na
	1108.4654	-3.7	-3.3	26.5	C59 H70 N3 O18
	1108.4577	4.0	3.6	14.5	C51 H75 N O24 Na
	1108.4577	4.0	3.6	48.5	C78 H62 N O6
	1108.4574	4.3	3.9	18.5	C49 H70 N7 O22
	1108.4572	4.5	4.1	32.5	C64 H67 N3 O13 Na
	1108.4662	-4.5	-4.1	15.5	C46 H71 N9 O21 Na
	1108.4663	-4.6	-4.1	49.5	C73 H58 N9 O3
	1108.4569	4.8	4.3	36.5	C62 H62 N9 O11
	1108.4665	-4.8	-4.3	45.5	C75 H63 N3 O5 Na

Elemental Composition Report

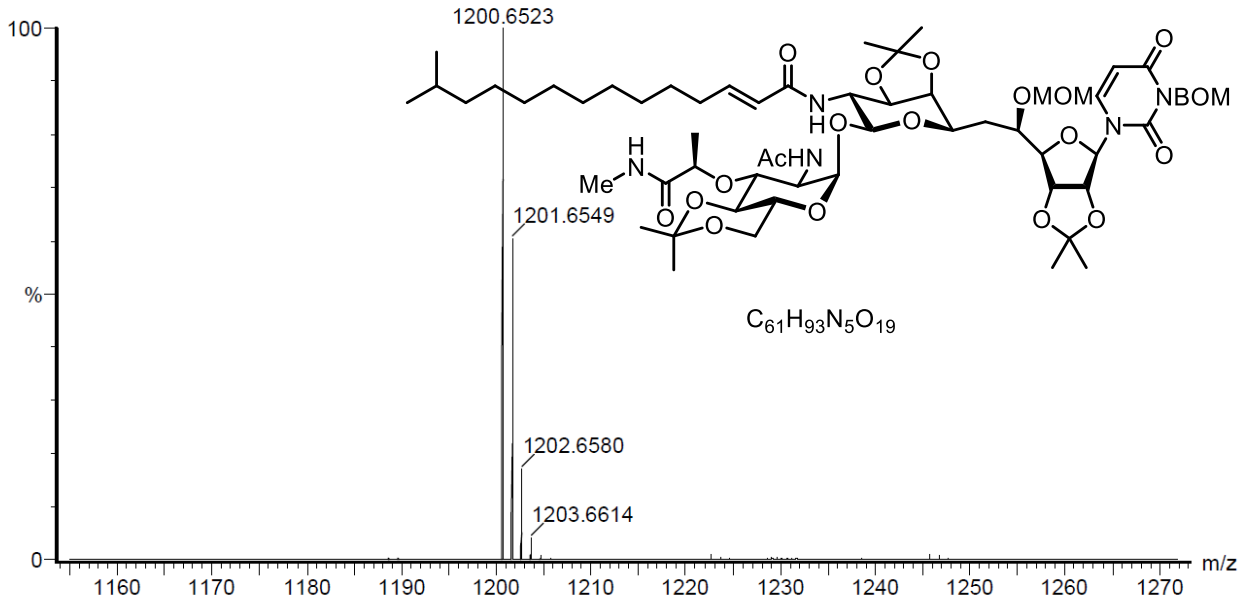
Single Mass Analysis

Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off

Monoisotopic Mass, Even Electron Ions
3287 formula(e) evaluated with 22 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 5-80 H: 10-100 N: 0-10 O: 0-25 Na: 0-1

20171101_4 no acid
14YAK8_18_1 1391 (5.115)

1: TOF MS ES+
1.33e+005



Minimum:				-1.5	
Maximum:		5.0	5.0	50.0	
Mass	Calc. Mass	mDa	PPM	DBE	Formula
1200.6523	1200.6525	-0.2	-0.2	30.5	C73 H90 N3 O12
	1200.6519	0.4	0.3	14.5	C59 H95 N5 O19 Na
	1200.6530	-0.7	-0.6	12.5	C60 H98 N O23
	1200.6514	0.9	0.7	32.5	C72 H87 N7 O8 Na
	1200.6532	-0.9	-0.7	19.5	C60 H91 N9 O15 Na
	1200.6538	-1.5	-1.2	35.5	C74 H86 N7 O8
	1200.6506	1.7	1.4	9.5	C58 H99 N O23 Na
	1200.6541	-1.8	-1.5	31.5	C76 H91 N O10 Na
	1200.6503	2.0	1.7	13.5	C56 H94 N7 O21
	1200.6543	-2.0	-1.7	17.5	C61 H94 N5 O19
	1200.6500	2.3	1.9	27.5	C71 H91 N3 O12 Na
	1200.6498	2.5	2.1	31.5	C69 H86 N9 O10
	1200.6554	-3.1	-2.6	36.5	C77 H87 N5 O6 Na
	1200.6556	-3.3	-2.7	22.5	C62 H90 N9 O15
	1200.6489	3.4	2.8	8.5	C55 H98 N3 O25
	1200.6559	-3.6	-3.0	18.5	C64 H95 N3 O17 Na
	1200.6484	3.9	3.2	26.5	C68 H90 N5 O14
	1200.6565	-4.2	-3.5	34.5	C78 H90 N O10
	1200.6567	-4.4	-3.7	41.5	C78 H83 N9 O2 Na
	1200.6479	4.4	3.7	10.5	C54 H95 N7 O21 Na
	1200.6474	4.9	4.1	28.5	C67 H87 N9 O10 Na
	1200.6573	-5.0	-4.2	23.5	C65 H91 N7 O13 Na

Elemental Composition Report

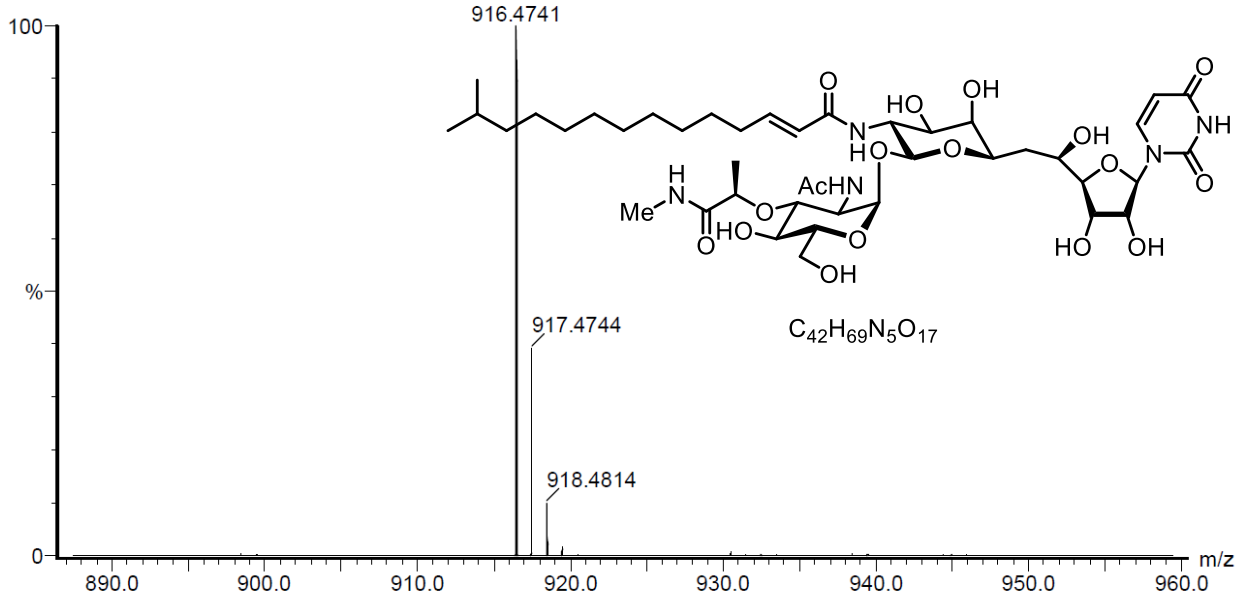
Single Mass Analysis

Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off

Monoisotopic Mass, Even Electron Ions
4104 formula(e) evaluated with 24 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 5-80 H: 10-100 N: 0-10 O: 0-25 Na: 0-1

20171101_5 no acid
14YAK8_20_1 959 (3.525)

1: TOF MS ES+
1.09e+005



Mass	Calc. Mass	mDa	PPM	DBE	Formula
Minimum:				-1.5	
Maximum:		5.0	5.0	50.0	
916.4741	916.4743	-0.2	-0.2	7.5	C40 H71 N5 O17 Na
	916.4738	0.3	0.3	25.5	C53 H63 N7 O6 Na
	916.4748	-0.7	-0.8	23.5	C54 H66 N3 O10
	916.4730	1.1	1.2	36.5	C66 H62 N O3
	916.4729	1.2	1.3	2.5	C39 H75 N O21 Na
	916.4753	-1.2	-1.3	5.5	C41 H74 N O21
	916.4756	-1.5	-1.6	12.5	C41 H67 N9 O13 Na
	916.4726	1.5	1.6	6.5	C37 H70 N7 O19
	916.4724	1.7	1.9	20.5	C52 H67 N3 O10 Na
	916.4721	2.0	2.2	24.5	C50 H62 N9 O8
	916.4762	-2.1	-2.3	28.5	C55 H62 N7 O6
	916.4764	-2.3	-2.5	24.5	C57 H67 N O8 Na
	916.4767	-2.6	-2.8	10.5	C42 H70 N5 O17
	916.4713	2.8	3.1	1.5	C36 H74 N3 O23
	916.4708	3.3	3.6	19.5	C49 H66 N5 O12
	916.4706	3.5	3.8	33.5	C64 H63 N O3 Na
	916.4778	-3.7	-4.0	29.5	C58 H63 N5 O4 Na
	916.4703	3.8	4.1	37.5	C62 H58 N7 O
	916.4702	3.9	4.3	3.5	C35 H71 N7 O19 Na
	916.4780	-3.9	-4.3	15.5	C43 H66 N9 O13
	916.4783	-4.2	-4.6	11.5	C45 H71 N3 O15 Na
	916.4697	4.4	4.8	21.5	C48 H63 N9 O8 Na
	916.4695	4.6	5.0	14.5	C48 H70 N O16
	916.4788	-4.7	-5.1	27.5	C59 H66 N O8