Supplementary information

Exploring the Impact of Amidation Status in *Meso*-Diaminopimelic Acid-Containing Disaccharide Peptidoglycan Fragments on Host Innate Immune Activation

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Biochemistry Materials and Methods

In vitro HEK-Blue™ mNOD1 and mNOD2 reporter assay

HEK-Blue™ cells expressing the mouse NOD1 or NOD2 receptor and carrying the NF-κB SEAP reporter gene (InvivoGen) were cultured in DMEM (Gibco) and supplemented with 10% FBS (Gibco). The reporter assays were conducted according to the manufacturer's instructions. Briefly, to the HEK-Blue NOD2, NOD1, Null2, and Null1 cells adhered in 96-well plates, 20 µL of the tested compounds at indicated concentrations were added and incubated for 16-18 h at 37 °C (total volume: 200 µL per well). Endotoxin-free deionized water was used as the negative control. For SEAP detection, 20 µL of supernatant from each well was collected and added to 180 µL of QUANTI-blue substrate (InvivoGen) in another 96-well microtiter plate. The mixture was incubated at 37 °C and SEAP activity was assessed by absorbance measurement at λ=650 nm by a Thermo Multi-Scanner Microplate reader. All compounds were reconstituted in endotoxin-free water. Results are representative of three independent biological triplicates as means ± standard error (SE).

In vitro RAW264.7 cell-based assay

RAW264.7 cells (American Type Culture Collection; ATCC, Manassas, VA, USA) were cultured in DMEM supplemented with 10% FBS and 1% Pen Strep antibiotics (Life Technologies Corporation) in 37 °C at 100% humidity in 5% CO_2. RAW264.7 WT cells (1.0×10^5 cells/well) were plated into 24-well plates for overnight attachment and treated with respective compounds at 20 µM in media supplemented with and without 10% FBS for 24hrs **(Figure S1).** Cell culture supernatants were collected for ELISA analysis.

RT-qPCR for cytokine gene expression in RAW264.7

RAW264.7 cells were seeded in complete DMEM at 1.0×10^5 cells/well in 24 well plate. The cells were treated with different compounds at 100 µM in serum-free media in 37 °C at 100% humidity in 5% CO_2 for 4 h. Total RNA was isolated using PrimWay Total RNA Extraction Kit according to the manufacturer's instruction. Total RNA was measured using Nanodrop™ (Thermo Fisher Scientific) for quality control. RNA was normalized to 1000 to 1000 ng and reversely transcribed to cDNA using HiScript III All-in-one RT SuperMix (Vazyme, China). The synthesized cDNA was used as the qPCR template to determine the expression levels of various genes against housekeeping gene *act-b* with the following primer pairs:

Actb_fw	CACTGTCGAGTCGCGTCC
Actb_rv	TCATCCATGGCGAACTGGTG
Tnfa_fw	GTCCCCAAAGGGATGAGAAGTT
Tnfa_rv	CTCCTCCACTTGGTGGTTTG
Il6_fw	CGGCCTTCCCTACTTCACAA
Il6_rv	TGCCATTGCACAACTCTTTTC

RT-PCR was performed using SsoAdvanced Universal SYBR green Supermix (Bio-Rad, US) according to the recommended protocol.Relative gene expression of target genes was determined utilizing $2^{-\Delta\Delta Ct}$ method and normalized to the housekeeping gene *act-b*. All primers used (customized design and ordered from Integrated DNA Technologies).

Bone marrow-derived macrophage culture and treatment

Bone marrow cells were obtained by flushing the bone marrow from the femur and tibia of euthanized SPF C57BL/6J female mice. Both ends of the bone were cut off and 10 mL complete media were used to flush a pair of femur and tibia through a 27G needle. Cells were spined at 300 ×g for 5min and then resuspended

in red blood cell lysis buffer (0.89% NH_4Cl, w/v, 10mM $KHCO_3$, 0.1mM EDTA) for 8 min. Lysis was quenched by complete media (IMDM+10% FBS+1% P/S). White blood cell pellets were resuspended in complete media followed by filtration and counting. Cells were seeded at 2.0×10^5 cells/well in 48-well plate in BMDM media (complete media supplemented with 20 ng/ml GM-CSF) for macrophage differentiation. Non-adherent cells were removed and supplemented with fresh complete BMDM media on day 3 for further differentiation. The cells were ready for treatment by day 6. For treatment, respective compounds (20 μM) were added to cells in serum-free media for 24 h. Cell supernatants were collected for ELISA analysis.

ELISA

All samples used for ELISA were centrifuged at 16,000 ×g for 5 min at 4 °C to remove any potential sediments or cell debris right before analysis. ELISA MAX™ standard sets (Biolegend) were utilized to evaluate cytokines in culture supernatants according to the recommended protocols from the manufacturer. Samples were prediluted with blocking buffer (1% BSA in PBS, pH 7.4), if required. BSA (Bovine Serum Albumin, powder, Sigma-Aldrich) stock solution (1%, w/v) was dissolved in sterile PBS (pH 7.4) followed by filtration. Color development was realized by substrate TMB set (Biolegend) and the reaction was stopped by 1 M sulfuric acid. The final absorbance at λ=450nm was recorded by Thermo Multi-Scanner Microplate and SkanIt software (Thermo Scientific).

Cellular uptake protocol and subsequent LC-MS analysis

HEK293T and RAW264.7 cells were seeded in complete DMEM at 2.0 × 105 cells/well, 1.5 ml per well in 12-well plates. The cells were treated with different compounds at 100 μM in serum-free media in 37 °C at 100% humidity in 5% CO_2 for 18 h, washed with cold PBS thrice, then stored at −80 °C. Cells were detached using 500 μl of cold 90% methanol repeatedly. Samples were collected and lysed in a heating block at 90 °C for 15 min. After centrifugation at 15,000 g for 15 min at 4 °C. the supernatant was collected and dried at room temperature in a SpeedVac concentrator. Dried samples were reconstituted in 50 μl, vortexed at 2,000 rpm for 15 min at 4 °C before centrifugation at 15,000*g* for 15 min at 4 °C. The soluble fraction was collected and subjected to LC-MS analysis in the Selective Ion Monitoring (SIM) mode.

LC-MS analysis was performed using Vanquish Core HPLC-Orbitrap Exploris 120 system, equipped with a Phenomenex Luna Omega Polar C18 column (100 x 4.6mm, 3 μm, 100 Å) with polar C18 SecurityGuard cartridges. Sample was separated in a 18-min gradient (buffer A: 0.05% formic acid in H2O, buffer B: 0.05% formic acid in acetonitrile, flow rate at 500 μL/min; 1% B in the first 0.5 min, 1-70% B from 0.5-16 min, 70% B maintained for 2 min, increased to 95% in 2 min and maintained for 2 min, then lowered to 1% in 0.5 min, equilibrated for 1.5 min).

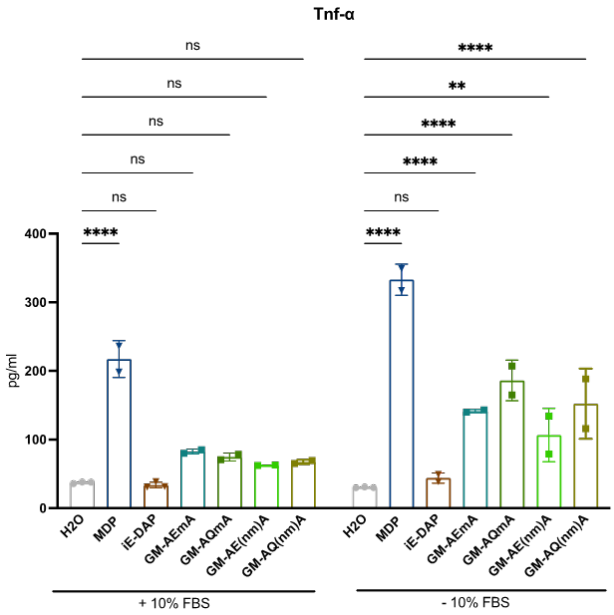


Figure S1. ***In vitro*** **cytokine production of the differentially amidated disaccharide PGNs synthesized in this study. (A)** Effect of FBS on the TNF-α production in RAW264.7 cells by ELISA after cells were treated with water or various compounds at 100 μM for 18h. Error bars represent replicated experiments with statistical analysis using ordinary one-way ANOVA (Dunnett's multiple comparison test) compared to the untreated sample (UT). Results are presented as mean ± standard error (SE). ****$p < 0.0001$, ***$p < 0.005$, **$p < 0.001$, * $p < 0.05$, ns not significant.

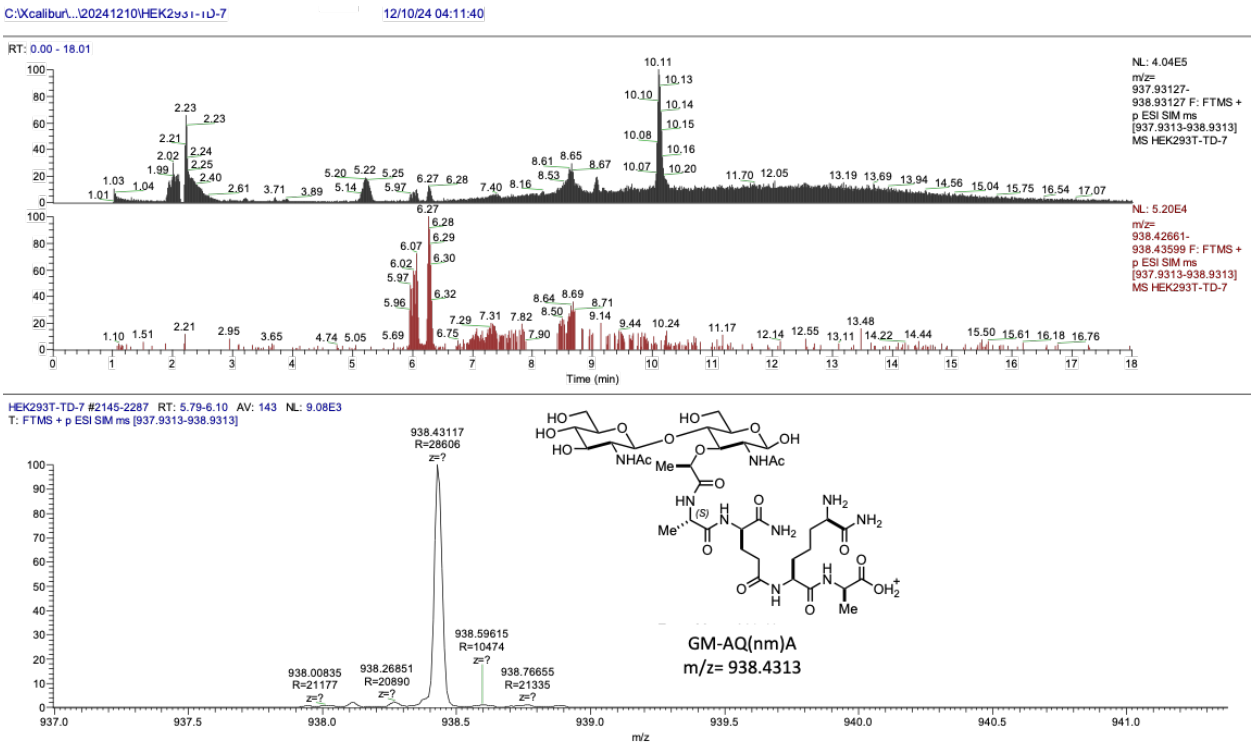
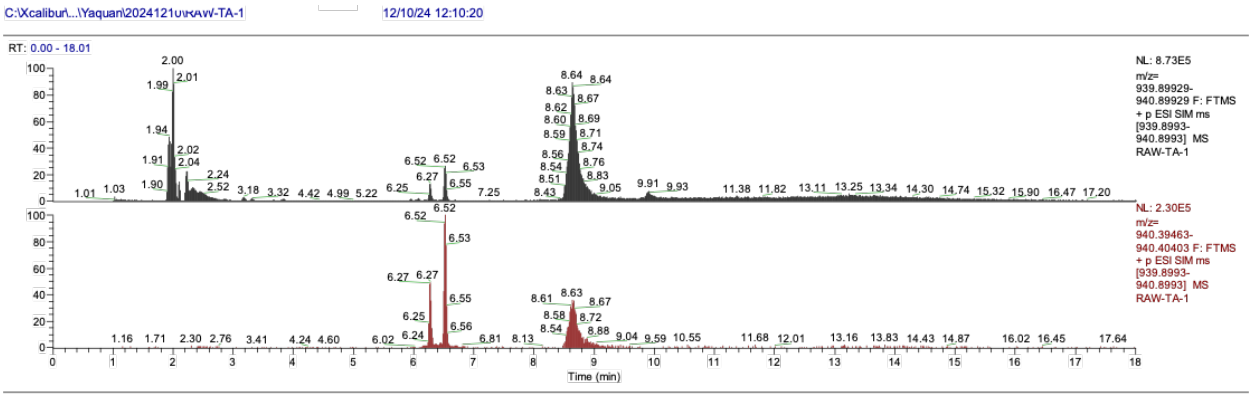
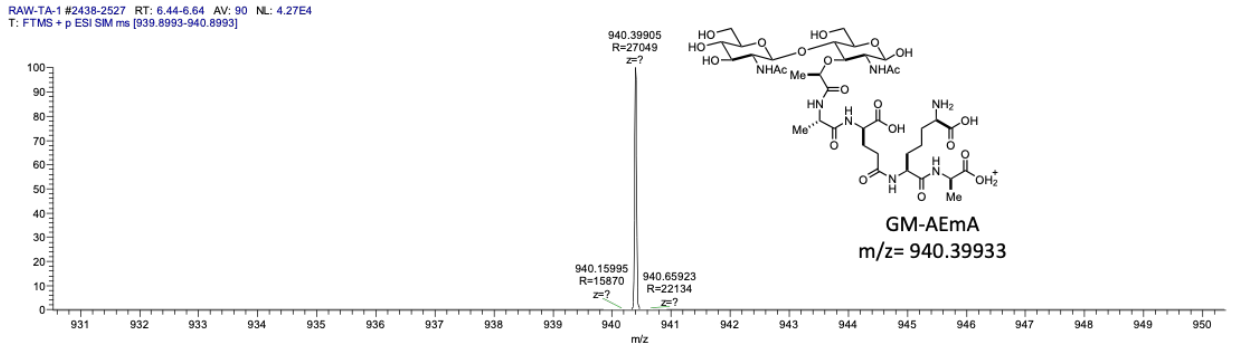
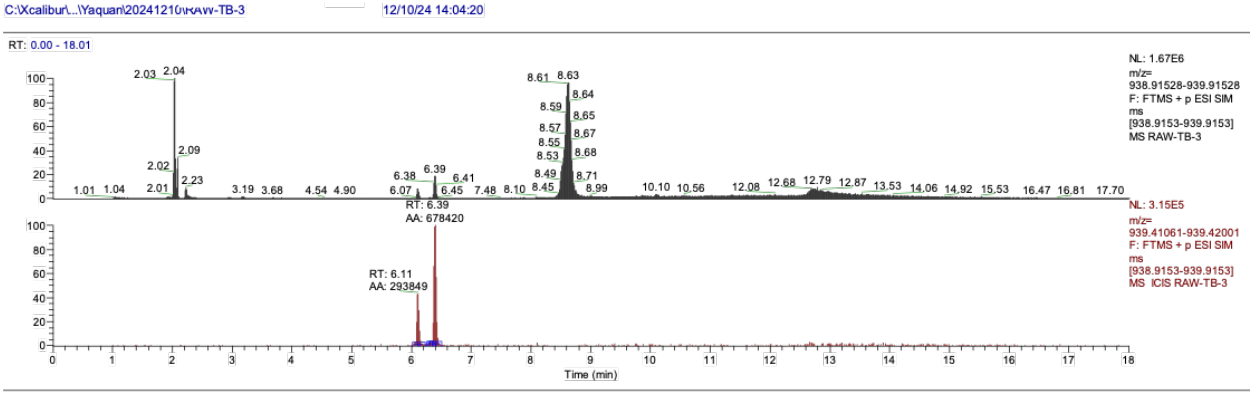
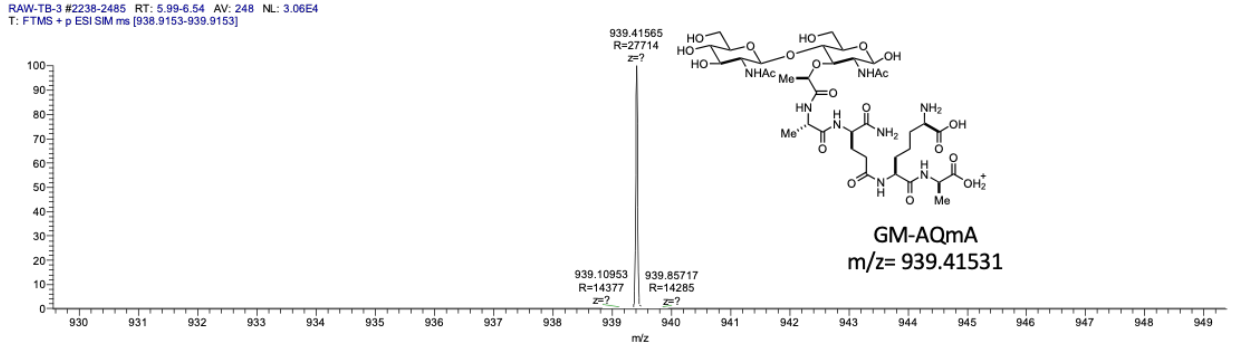


Figure S2. LC-MS analysis of cellular uptake of GM-AQ(nm)A in HEK293T cells.


C:\Xcalibur\...\Yaquan\20241210\RAW-TA-1
12/10/24 12:10:20
RT: 0.00 - 18.01
NL: 8.73E5
m/z= 939.89929-940.89929 F: FTMS + p ESI SIM ms [939.8993-940.8993] MS RAW-TA-1
NL: 2.30E5
m/z= 940.39463-940.40403 F: FTMS + p ESI SIM ms [939.8993-940.8993] MS RAW-TA-1
Time (min)


RAW-TA-1 #2438-2527 RT: 6.44-6.64 AV: 90 NL: 4.27E4
T: FTMS + p ESI SIM ms [939.8993-940.8993]
940.39905
R=27049
z=?
940.15995
R=15870
z=?
940.65923
R=22134
z=?
m/z
GM-AEmA
m/z= 940.39933


C:\Xcalibur\...\Yaquan\20241210\RAW-TB-3
12/10/24 14:04:20
RT: 0.00 - 18.01
NL: 1.67E6
m/z= 938.91528-939.91528 F: FTMS + p ESI SIM ms [938.9153-939.9153] MS RAW-TB-3
RT: 6.39
AA: 678420
RT: 6.11
AA: 293849
NL: 3.15E5
m/z= 939.41061-939.42001 F: FTMS + p ESI SIM ms [938.9153-939.9153] MS ICIS RAW-TB-3
Time (min)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T: FTMS + p ESI SIM ms [938.9153-939.9153]
939.41565
R=27714
z=?
939.10953
R=14377
z=?
939.85717
R=14285
z=?
m/z
GM-AQmA
m/z= 939.41531

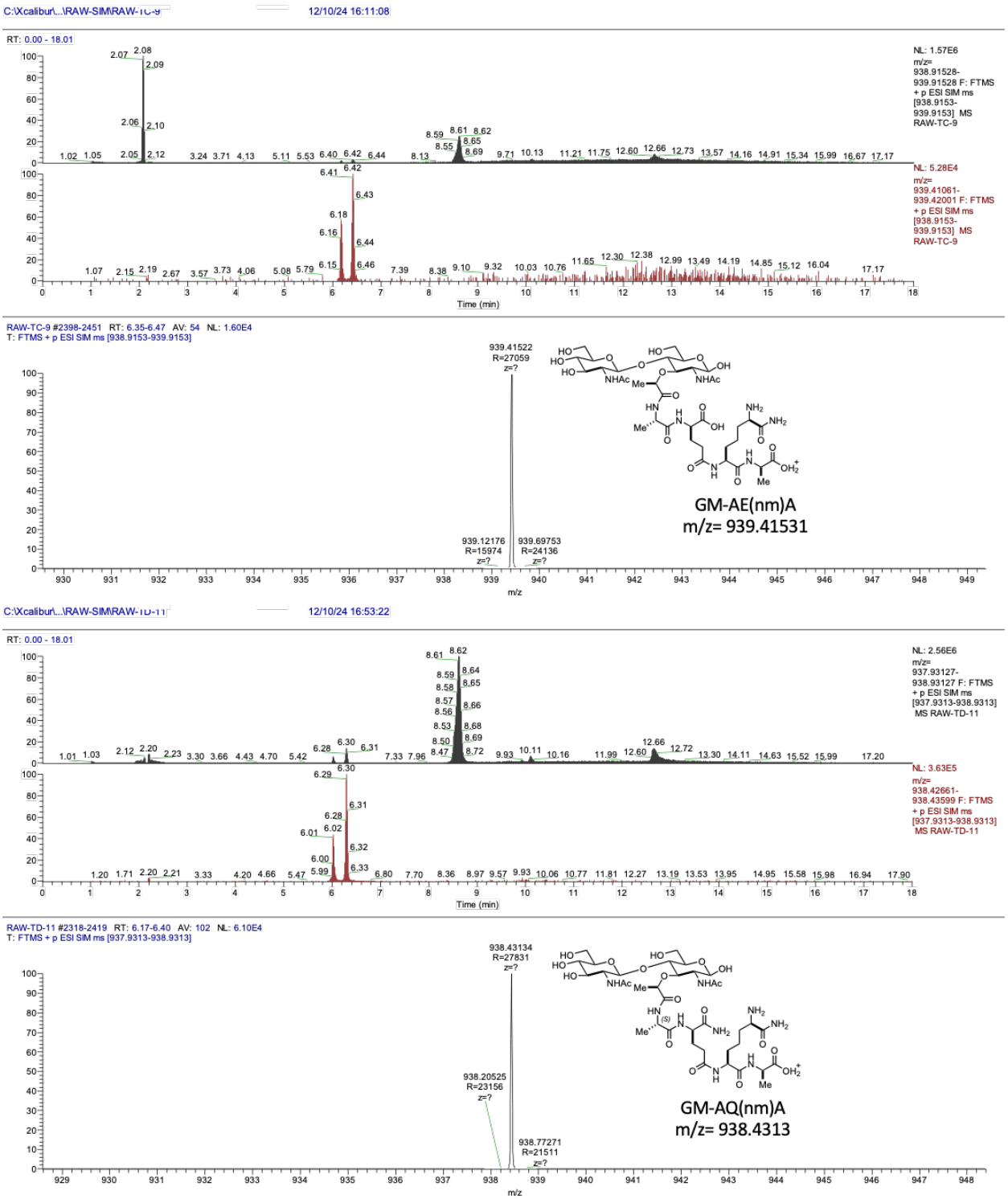


Figure S3. LC-MS analysis showing all four disaccharide-PGNs were detected intracellularly in RAW264.7 cells.

Chemical synthesis general procedures

Water was purified with a Millipore Milli-Q system (Merck K. Ga. Co., Darmstadt, Germany). Chemical reagents and solvents were obtained from commercial sources (Millipore-Sigma, TCI, Alfa-Aesar, BLDPharm, or Fluorochem). Normal phase column chromatography was carried out with Grace Davisil® LC60A 40-63 micron silica gel. Preparative HPLC was performed on an Agilent 1260 Infinity II equipped with a 21.2 x 250 mm C18 column using a 10 mL/min flow rate. A gradient of ACN (solvent B) and 0.1% aqueous TFA (solvent A) over 45 minutes was used in all cases, and the solvent B start and endpoints were indicated in each case. Removal of solvents was done with a rotary evaporator equipped with a diaphragm vacuum pump and chiller. Final muropeptide products were lyophilized from water/acetonitrile to ensure an accurate weight.

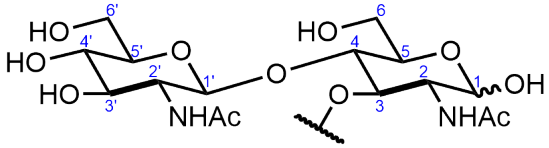
Unless otherwise stated, the yield of Boc deprotection reactions is assumed to be 100%. In our hands, many amine trifluoroacetate/hydrochloride salts are difficult to dry completely even with lyophilization. Further, these compounds sometimes give intractable NMR spectra due to peak broadening, and so we did not collect their NMR spectra.

High-resolution mass spectra were measured using a Themo-Fisher Vanquish Core HPLC-Orbitrap Exploris 120 system.

NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz for ^{1}H NMR, 101 MHz for ^{13}C NMR). Spectra of intermediates used $CDCl_3$, DMSO-d_6, or MeOH-d_4 as a solvent and were calibrated using the solvent peaks ($CDCl_3$: δ 7.26 for ^{1}H NMR and δ 77.16 for ^{13}C NMR; DMSO-d_6: δ 2.50 for ^{1}H NMR and δ 39.52 for ^{13}C NMR; MeOH-d_4: δ 3.31 for ^{1}H NMR and δ 49.00 for ^{13}C NMR). Spectra of final products were recorded using Shigemi BMS-005B NMR tube and D_2O was used as a solvent. To calibrate the spectra, about 0.2 µL DMSO was spiked in as an internal standard (δ 2.71 for ^{1}H NMR and δ 39.39 for ^{13}C NMR). Coupling constants (*J* values) are reported in Hertz (Hz). ^{1}H NMR spectral data are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constant, number of protons, assignment.

Regarding the annotation of NMR spectra of final compounds

The glycan rings are annotated as follows.

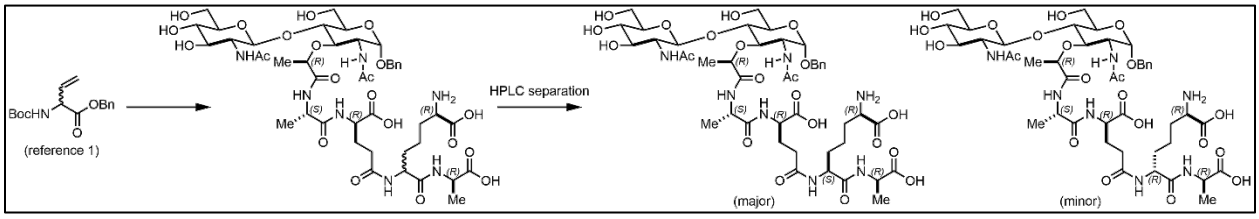


Integration of the H-1 protons indicated an α : β ratio of roughly 70: 30 in all cases. HSQC, COSY, and 2D-TOCSY measurements were used to assign the peaks. Since HMBC spectra were not collected for all the compounds, the *C*=O signals in the ^{13}C spectra are not assigned. In cases where corresponding signals of the alpha and beta isomers have very close chemical shifts, they are not assigned separately. For example, H-4α and H-4β are tabulated as H-4. It was not possible to assign H-2β in the proton NMR spectra since its HSQC cross-peak is invariably obscured by the H-2' cross-peak.

The muropeptides were isolated as mono-TFA salts after preparative HPLC, but the ^{13}C peaks of trifluoroacetate are not tabulated.

Regarding preparations of stereochemically pure mDAP-containing PGNs with different amidation status

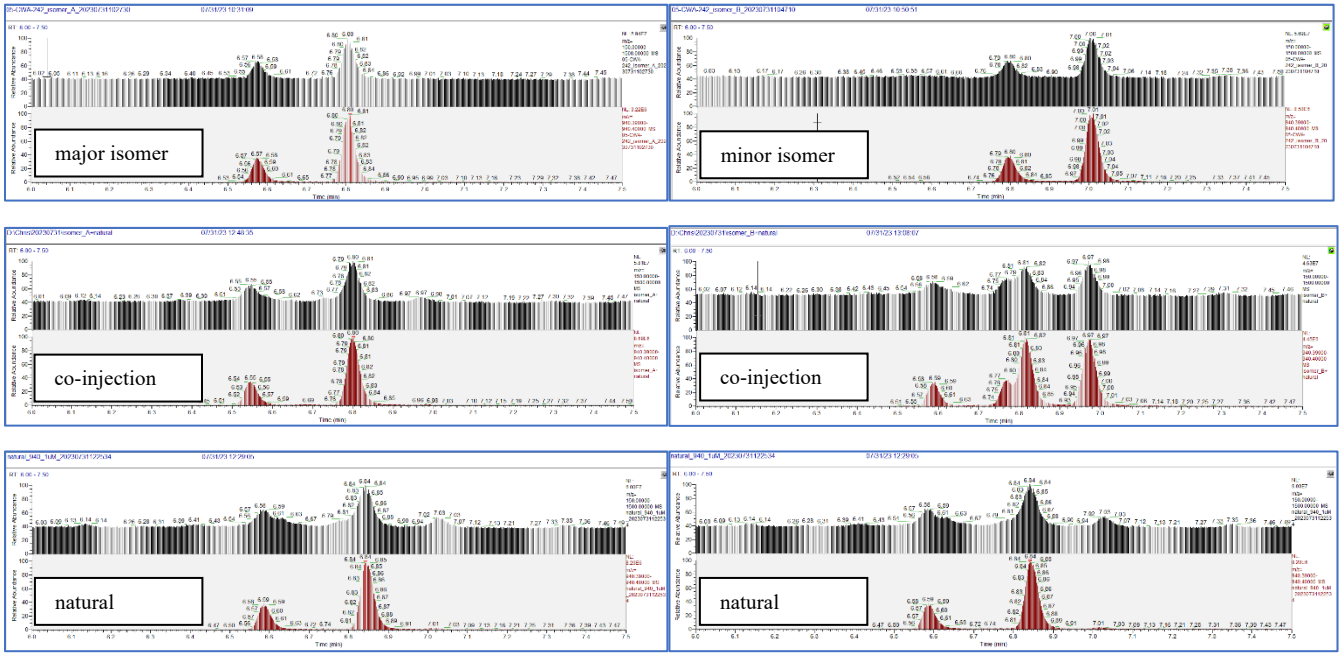
In our hands, the preparation of the mDAP precursor **SI-6** afforded 3 : 1 mixture of epimers. This issue became apparent in the ^{13}C NMR spectra of **SI-8**, **SI-19**, and downstream compounds. Epimeric impurities were carried forward, and only the major epimer is annotated in NMR spectra. We found that the impurity could be removed in all cases by incorporating a preparative HPLC purification in the second last step of the synthesis.



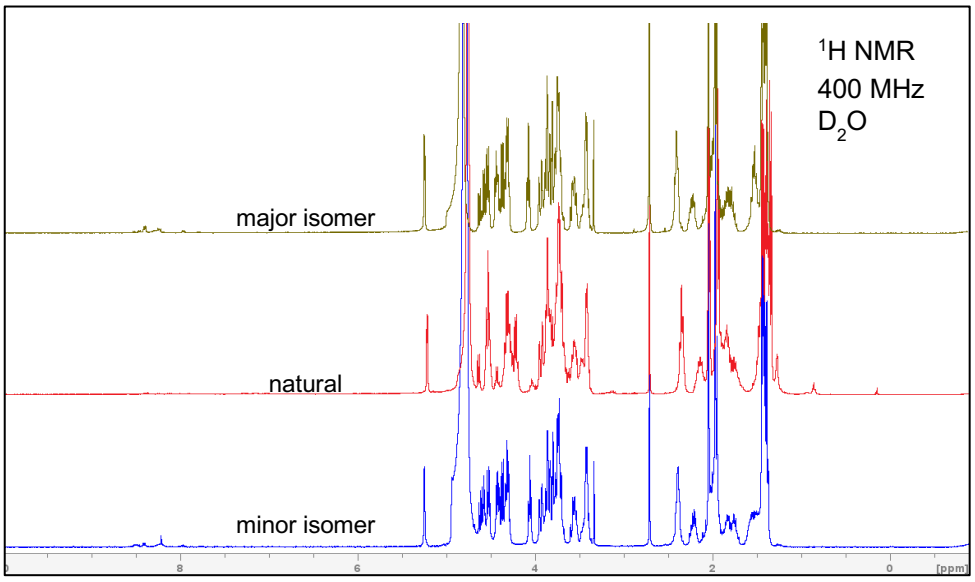
In the case of **GM-AEmA**, we subjected both major and minor isomers to benzyl ether deprotection and confirmed that the HPLC retention time of the major isomer matched that of an authentic sample, whereas the minor isomer was a mismatch. By analogy, we assumed the major isomer has the correct stereochemistry in all four final products.

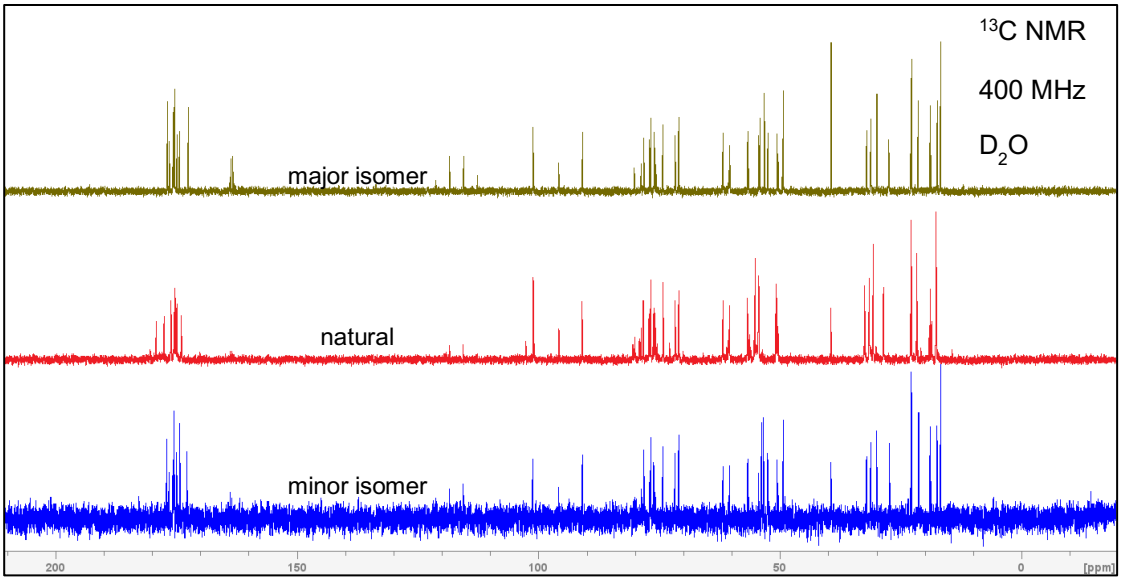
Chromatographic conditions:
injection: 10 µL of a 1 µM solution (ca. 10 pmol injection)
column: 4.6 x 100 mm Phenomex Luna Omega 3 µm Polar C18
flow rate: 0.5 mL/min
solvent A: H_2O with 0.05% formic acid
solvent B: ACN with 0.05% formic acid
detection: ESI-MS; target $[M + H]^+$ extraction in red

t (min)	% solvent B
0	1
0.5	1
10	40
10.1	99
12	99
12	1
16	1

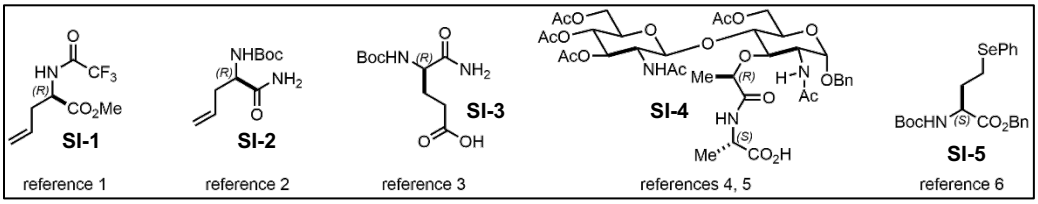


On the other hand, the ^{1}H and ^{13}C NMR spectra of the two **GM-AEmA** isomers did not exactly match the spectra from the natural product, so the analysis was not conclusive. Since the NMR measurements of muropeptides are highly sensitive to pH, analyte concentration, and impurities, we regard NMR as unsuitable for the assignment of stereoisomers.

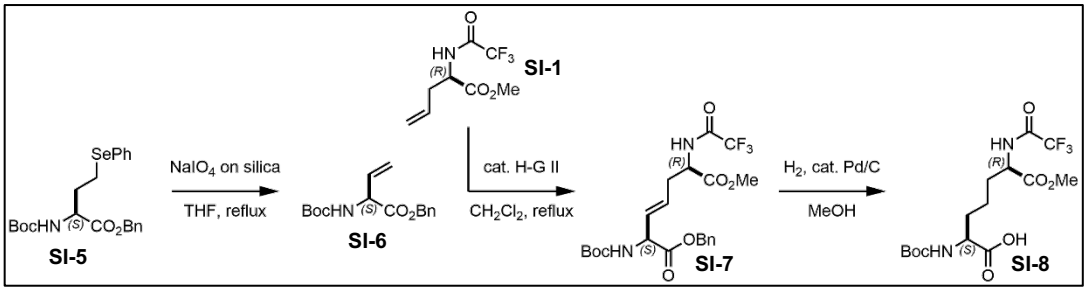




Starting materials for muropeptide synthesis



Synthesis of GM-AEmA



To a vigorously stirred solution of **SI-5** (2.56 g, 5.71 mmol, 1.0 eq) in 40 mL THF was charged 50% sodium periodate on silica gel (6.11 g, 14.3 mmol, 2.5 eq), and the mixture was refluxed. After 36 hours, the mixture was cooled to room temperature, filtered through a Celite pad, and rinsed forward with EtOAc. The filtrate was then concentrated to dryness. The crude residue was purified by column chromatography (35 g silica, 10 then 15% EtOAc in hexane) to afford **SI-6** (1.475 g, 5.06 mmol, 89% yield) as an oil.

^{1}H NMR (400 MHz, $CDCl_3$, 301 K, δ): 7.35 (m, 5H), 5.91 (ddd, J = 17.0, 10.1, 5.2 Hz, 1H), 5.34 (d, J = 17.0 Hz, 1H), 5.25 (d, J = 10.1 Hz, 1H), 5.21 (d, J = 12.2 Hz, 1H), 5.17 (d, J = 12.2 Hz, 1H), 4.92 (m, 1H), 1.44 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$, 301 K, δ): 170.7, 155.1, 135.3, 132.7, 128.7, 128.5, 128.3, 117.6, 80.2, 67.4, 56.0, 28.4.

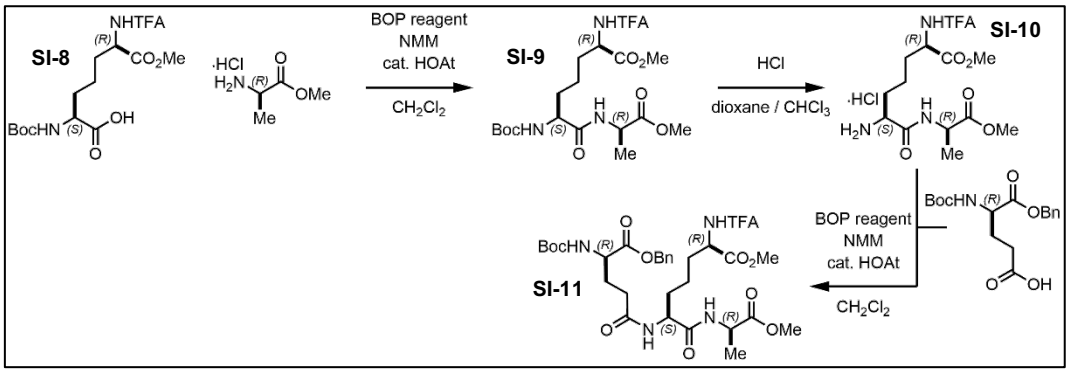
HRMS (ESI-TOF, m/z): calc'd for $C_{16}H_{21}NO_4Na^+$ ($[M + Na]^+$) 314.1363; found 314.1361. (*Note*: the dominant peak in the mass spectrum was 192.1017 arising from in-source fragmentation; the $[M + Na]^+$ peak was extremely weak).

To a rbf was charged **SI-1** (312 mg, 1.48 mmol, 1.0 eq), **SI-6** (648 mg, 2.22 mmol, 1.5 eq), and 15 mL CH_2Cl_2. The mixture was sparged with argon for 20 minutes. A reflux condenser was attached and the mixture was stirred at reflux. After 14.5 hours, the mixture was cooled to room temperature and concentrated to dryness. The crude residue was purified by column chromatography (20 to 30% EtOAc in hexane) to afford alkene **SI-7** (390 mg) as an oil which solidified on standing. This material was stirred with 8 mL EtOAc, 60 mg Pd/C was charged, and the suspension was placed under a hydrogen atmosphere. After 5 hours, the suspension was filtered through Celite and concentrated to dryness to afford **SI-8** (318 mg, 0.794 mmol, 54% over two steps) as a white solid.

^{1}H NMR (400 MHz, MeOH-d_4, 301 K, δ): 4.46 (m, 1H), 4.09 (m, 1H), 3.74 (s, 3H), 1.96 (m, 1H), 1.82 (m, 2H), 1.67 (m, 1H), 1.48 (m, 2H), 1.44 (s, 9H).

^{13}C NMR (101 MHz, MeOH-d_4, 301 K, δ): 175.9, 172.5, 159.0 (q, J = 37.0 Hz), 158.0, 117.3 (q, J = 286.8 Hz), 80.5, 54.4, 54.0, 53.0, 32.3, 31.2, 28.7, 23.3.

HRMS (ESI-TOF, m/z): calc'd for $C_{15}H_{24}N_2O_7F_3^+$ ([M + H]$^+$) 401.1530; found 401.1524.



To a stirred solution of **SI-8** (163 mg, 0.407 mmol, 1.0 eq) in 8.1 mL CH_2Cl_2 was charged H-(*R*)-Ala-OMe·HCl (99 mg, 0.713 mmol, 1.75 eq), *N*-methylmorpholine (134 µL, 1.2 mmol, 3.0 eq), HOAt (14 mg, 0.102 mmol, 0.25 eq), and finally BOP reagent (234 mg, 0.529 mmol, 3.0 eq). After 14 hours, the mixture was concentrated to dryness, taken up in EtOAc, and washed with 1% aqueous K_2CO_3 (x2), pH 2 buffer, and brine. The organic extract was dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by column chromatography (10 g silica, 4 to 5% MeOH in CH_2Cl_2) to afford **SI-9** (186 mg, 0.383 mmol, 94% yield) as an oil which solidified on standing.

^{1}H NMR (400 MHz, $CDCl_3$, 301 K, δ): 7.61 (d, *J* = 7.4 Hz, 1H), 6.97 (m, 1H), 5.32 (m, 1H), 4.53 (m, 2H), 4.12 (m, 1H), 3.74 (s, 3H), 3.70 (s, 3H), 1.91 (m, 1H), 1.79 (m, 2H), 1.61 (m, 1H), 1.403 (m, 2H), 1.402 (s, 9H). 1.37 (d, *J* = 7.2 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$, 301 K, δ): 173.3, 171.7, 171.4, 157.3 (q, *J* = 37.7 Hz), 155.9, 115.8 (q, *J* = 287.7 Hz), 80.4, 53.8, 52.9, 52.7, 52.5, 48.1, 32.0, 31.1, 28.3, 21.4, 18.0.

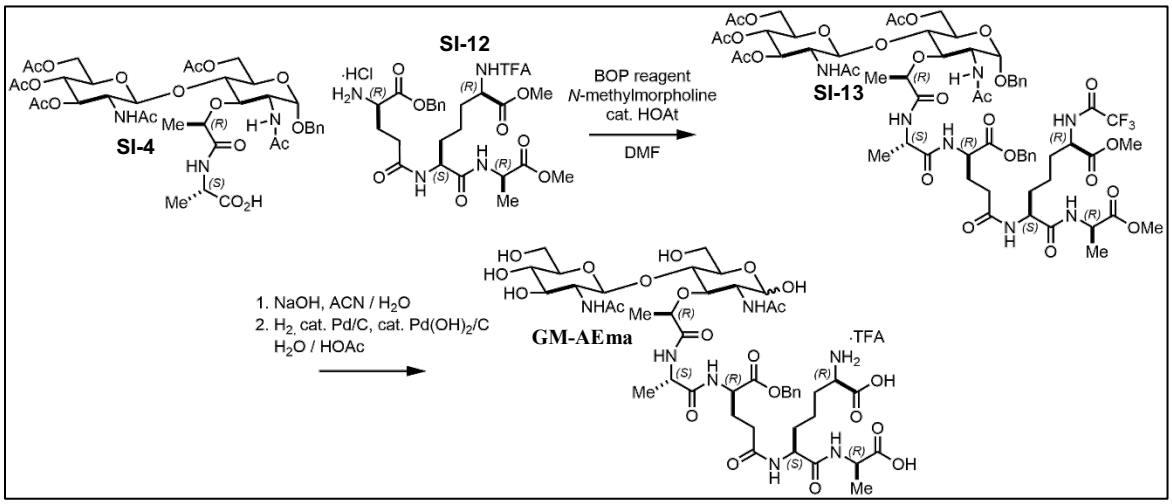
HRMS (ESI-TOF, m/z): calc'd for $C_{19}H_{31}F_3N_3O_8^+$ ([M + H]$^+$) 486.2058; found 486.2051.

SI-9 (186 mg, 0.383 mmol, 1.0 eq) was stirred with 1.0 mL $CHCl_3$, and then 4 M HCl in dioxane (1.0 mL, 4.0 mmol, 10.4 eq was charged). After 3.5 hours, the mixture was concentrated and co-evaporated several times with $CHCl_3$ to obtain **SI-10**. This material was stirred with 7.7 mL CH_2Cl_2 and then Boc-(*R*)-Glu(OH)-OBn (78 mg, 0.230 mmol, 1.2 eq), *N*-methylmorpholine (51 µL, 0.46 mmol, 2.4 eq), HOAt (6.5 mg, 0.048 mmol, 0.25 eq) and finally BOP reagent (98 mg, 0.221 mmol, 1.15 eq) were charged. After 14 hours, the mixture was concentrated, taken up in EtOAc, and washed with 1% aqueous K_2CO_3 (x3), pH 2 buffer, and brine. The organic extract was dried (Na_2SO_4), filtered, and concentrated. The crude residue was purified by column chromatography (15 g silica, 60 to 90% EtOAc in hexane) to afford **SI-11** (120 mg, 0.170 mmol, 89% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$, 301 K, δ): 7.81 (m, 1H), 7.41 (m, 1H), 7.32 (m, 5H), 6.72 (m, 1H), 5.45 (m, 1H), 5.14 (m, 2H), 4.48 (m, 3H), 4.27 (m, 1H), 3.72 (s, 3H), 3.65 (s, 3H), 2.28 (m, 2H), 2.25 (m, 1H), 1.97 to 1.74 (m, 4H), 1.67 (m, 1H), 1.391 (m, 2H), 1.389 (s, 9H), 1.34 (d, *J* = 7.2 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$, 301 K, δ): 173.2, 172.4, 172.1, 171.4, 171.1, 157.3 (q, *J* = 37.8 Hz), 155.9, 135.2, 128.6, 128.5, 128.3, 115.8 (q, *J* = 287.8 Hz), 80.3, 67.3, 52.76, 52.75, 52.66, 52.56, 52.4, 48.1, 32.1, 31.5, 30.9, 28.8, 28.2, 21.1, 17.4.

HRMS (ESI-TOF, m/z): calc'd for $C_{31}H_{44}F_3N_4O_{11}^+$ ([M + H]$^+$) 705.2953; found 705.2905.



SI-11 (120 mg, 0.170 mmol, 1.0 eq) was stirred with 1 mL CH_2Cl_2 and then 4 M HCl in dioxane (0.50 mL, 2.0 mmol, 11.8 eq) was charged. After 3 hours, the mixture was concentrated and co-evaporated several times with $CHCl_3$ to obtain **SI-12**. This material was stirred with 3.4 mL DMF and **SI-4** (147 mg, 0.179 mmol, 1.05 eq), *N*-methylmorpholine (47 µL, 0.425 mmol, 2.5 eq), HOAt (6.0 mg, 0.043 mmol, 0.25 eq) and finally BOP reagent (90 mg, 0.20 mmol, 1.2 eq) were charged. After 14 hours, the mixture was concentrated to dryness. The residue was stirred with 4 mL *i*-PrOH until a uniform slurry was obtained. The slurry was filtered and rinsed forward with 4 mL *i*-PrOH. The solids were dried under vacuum to obtain **SI-13** (144 mg, 0.102 mmol, 60% yield) as a white solid.

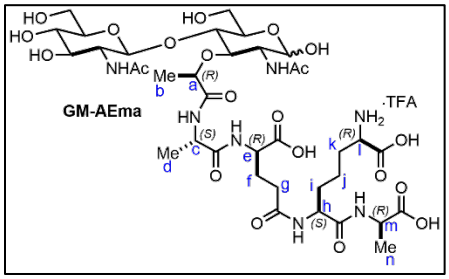
^{1}H NMR (400 MHz, DMSO-d_6, 301 K, δ): 9.81 (br s, 2H), 8.52 (d, J = 7.7 Hz, 1H), 8.41 (m, 2H), 8.08 (d, J = 8.6 Hz, 1H), 7.99 (m, 2H), 7.32 (m, 11H), 5.24 (t, J = 9.8 Hz, 1H), 5.09 (s, 2H), 4.88 (d, J = 3.1 Hz, 1H), 4.85 (t, J = 9.7 Hz, 1H), 4.73 (d, J = 8.1 Hz, 1H), 4.58 (d, J = 12.5 Hz, 1H), 4.41 (m, 4H), 4.26 (m, 5H), 4.04 (dd, J = 11.7, 4.2 Hz, 1H), 3.93 (d, J = 11.7 Hz, 1H), 3.70 (m, 3H), 3.65 (s, 3H), 3.59 (s, 3H), 3.44 (m, 1H), 2.17 (t, J = 7.2 Hz, 1H), 2.07 (s, 3H), 1.98 (m, 1H), 1.95 (s, 3H), 1.92 (s, 3H), 1.90 (s, 3H), 1.79 (m, 1H), 1.78 (s, 3H), 1.77 (m, 2H), 1.73 (s, 3H), 1.60 (m, 1H), 1.47 (m, 1H), 1.30 (m, 2H), 1.27 (d, J = 6.3 Hz, 3H), 1.26 (d, J = 7.2 Hz, 3H), 1.21 (d, J = 7.0 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6, 301 K, δ): 173.4, 173.0, 172.2, 171.54, 171.47, 171.2, 171.1, 170.2, 170.0, 169.79, 169.75, 169.67, 169.4, 156.8 (q, J = 36.8 Hz), 137.6, 135.9, 128.5, 128.3, 128.2, 127.9, 127.61, 127.55, 116.0 (q, J = 287.7), 99.3, 95.6, 76.33, 76.31, 75.5, 72.3, 70.5, 68.7, 68.5, 66.1, 63.6, 62.3, 61.6, 54.3, 53.6, 52.7, 52.4, 51.9, 51.8, 51.7, 47.8, 47.6, 31.8, 31.3, 29.5, 27.4, 22.70, 22.65, 21.7, 20.8, 20.5, 20.39, 20.38, 19.3, 18.8, 17.1.

HRMS (ESI-TOF, m/z): calc'd for $C_{63}H_{85}F_3N_7O_{26}^+$ ([M + H]$^+$) 1412.5491; found 1412.5483.

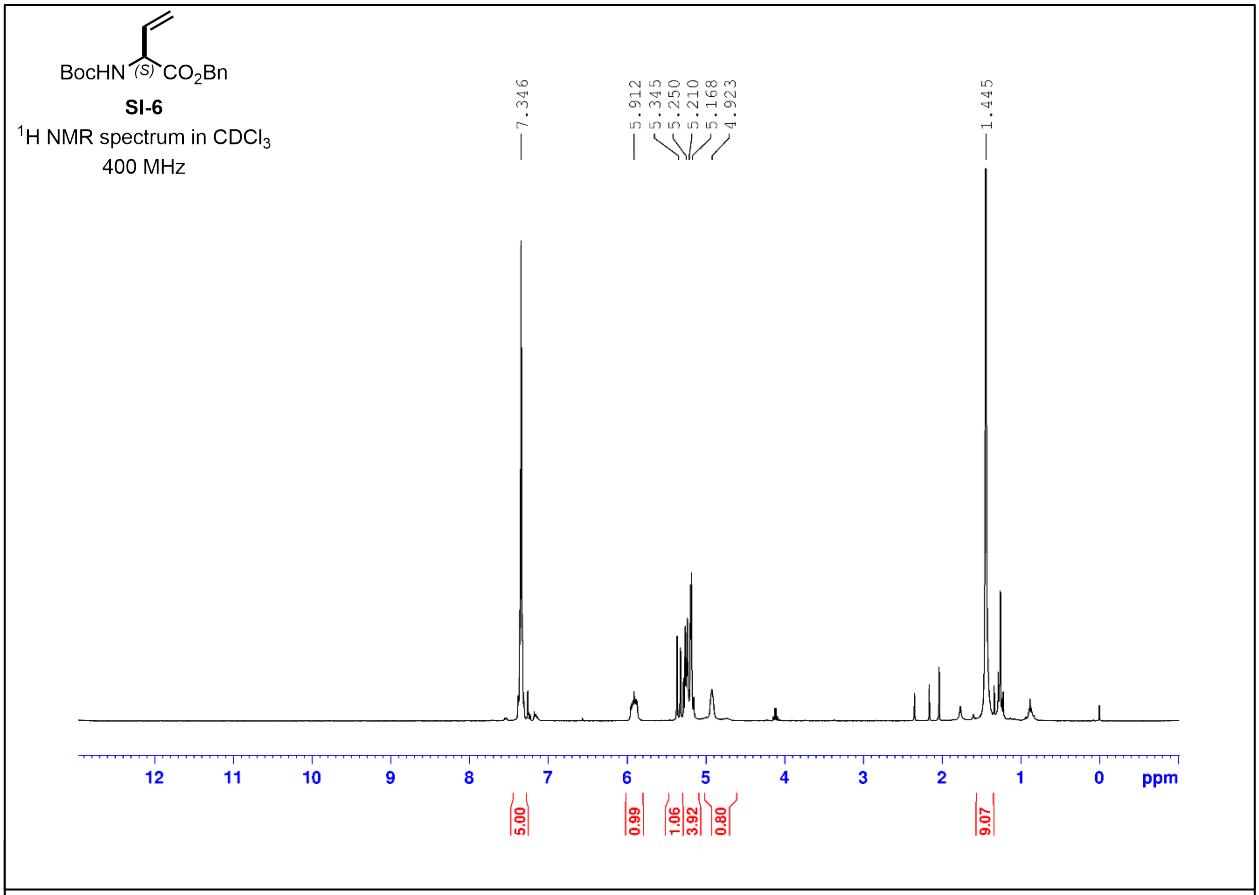
SI-13 (61.9 mg, 43.8 µmol, 1.0 eq) was stirred with 0.66 mL ACN and 1.31 mL H_2O, and 1 M aqueous NaOH (0.66 mL, 657 µmol, 15.0 eq) was charged. After 2 hours, the mixture was neutralized with Amberlite IR-120 H^+, the slurry was filtered through cotton, and the filtrate was concentrated. In three loads, this material was purified by preparative HPLC (0 to 40% ACN) to afford the benzyl ether intermediate (36 mg), which was a single epimer at C-h. This material was dissolved 1.6 mL H_2O and 0.4 mL AcOH, and the mixture was hydrogenated with 20 mg Pd/C, 20 mg $Pd(OH)_2$/C, and H_2 (balloon) under vigorous stirring. After 3 hours, the mixture was filtered through a syringe filter, rinsed forward with MeOH, and concentrated.

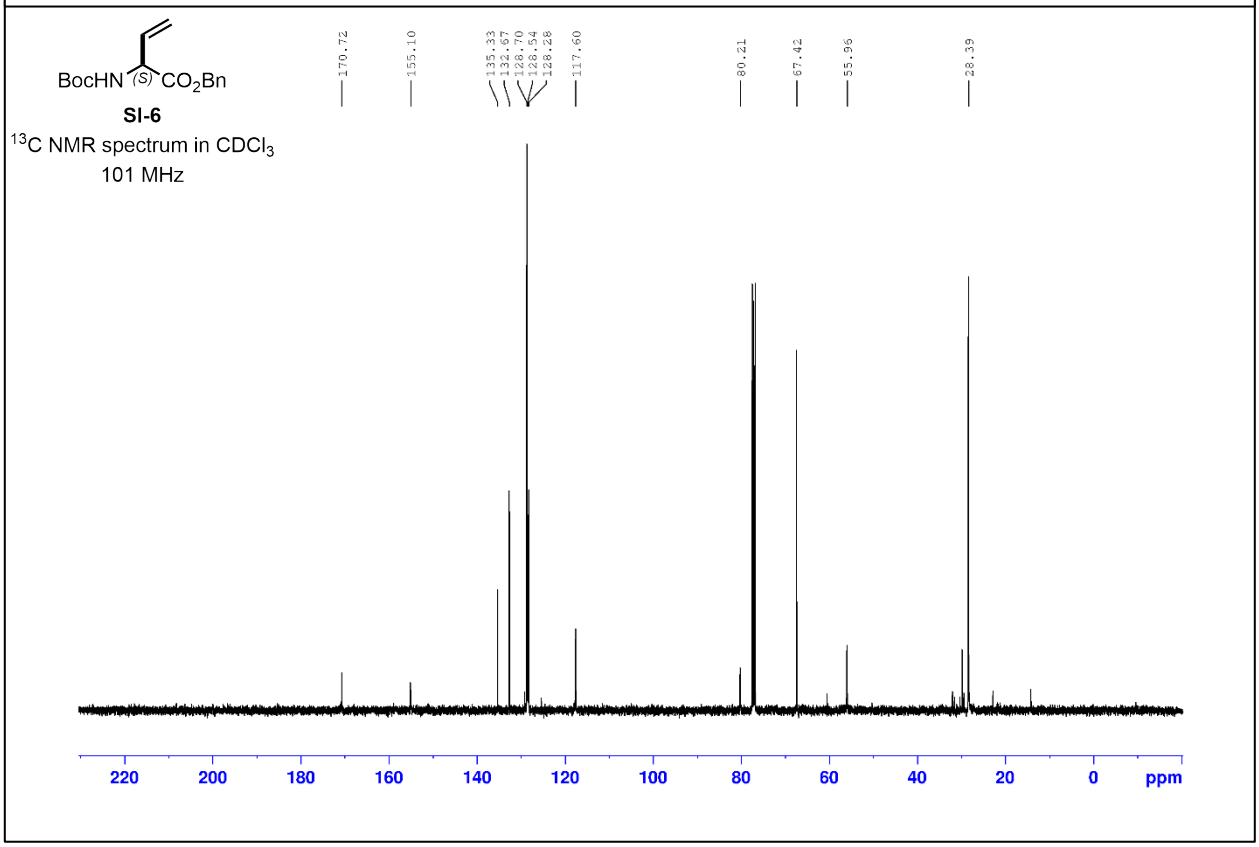
The crude material was purified by preparative HPLC (0 to 35% ACN) to afford **GM-AEmA** (23.1 mg, 21.9 µmol, 50% yield over two steps) as a white solid.

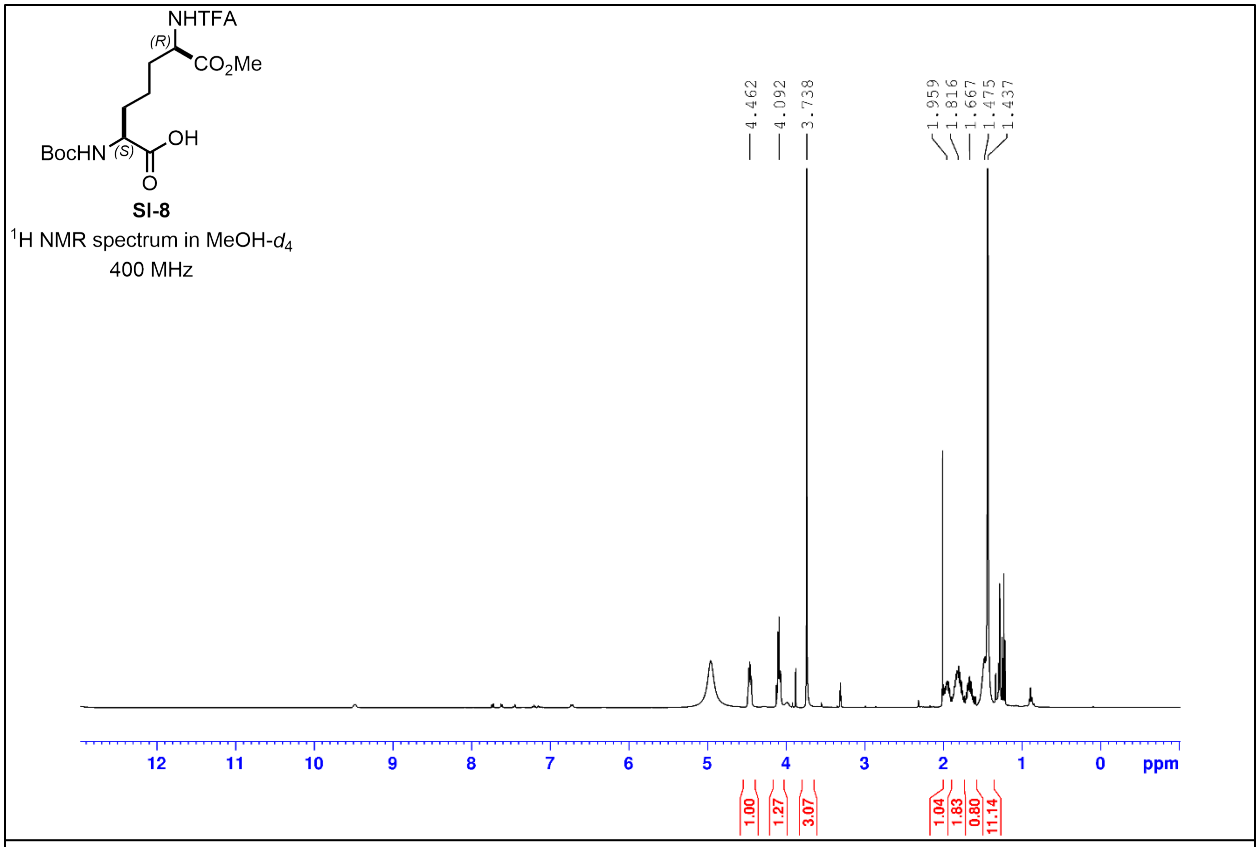


^{1}H NMR (400 MHz, D_2O, 301 K, δ): 5.25 (d, *J* = 3.3 Hz, 1H; H-1α), 4.63 (d, *J* = 8.3 Hz, 1H; H-1β), 4.58 (q, *J* = 6.5 Hz, 1H; H-aα), 4.53 (d, *J* = 8.3 Hz, 1H; H-1'α), 4.52 (d, *J* = 8.2 Hz, 1H; H-1'β), 4.434 (m, 1H; H-aβ), 4.433 (dd, *J* = 9.1, 5.1 Hz, 1H; H-e), 4.37 (q, *J* = 7.3 Hz, 1H; H-c), 4.32 (m, 1H; H-h), 4.31 (q, *J* = 6.8 Hz, 1H; H-m), 4.07 (t, *J* = 6.3 Hz, 1H; H-l), 3.94 (d, *J* = 12.1 Hz, 1H; H-6'), 3.87 (H-4), 3.864 (H-6β), 3.863 (H-5α), 3.82 (H-6α), 3.80 (H-2α), 3.76 (H-6'), 3.75 (H-2'), 3.722 (H-3α), 3.716 (H-6), 3.59 (H-3β), 3.56 (H-3'), 3.47 (H-5β), 3.43 (H-5'), 3.42 (H-4'), 2.40 (m, 2H; H-g), 2.23 (m, 1H; H-f), 2.04 (s, 3H; acetamide), 2.01 (m, 1H; H-f), 1.98 (m, 2H; H-k), 1.97 & 1.95 (s & s, 3H; acetamide), 1.81 (m, 2H; H-i), 1.53 (m, 2H; H-j), 1.44 (d, *J* = 7.3 Hz, 3H; H-d), 1.41 (d, *J* = 6.8 Hz, 3H; H-n), 1.40 (d, *J* = 6.5 Hz; H-b).

^{13}C NMR (101 MHz, D_2O, 301 K, δ): 176.8, 176.3, 175.7, 175.59, 175.58, 175.49, 175.46, 175.33, 175.31, 175.2, 174.8, 174.29, 174.25, 172.4, 101.10 C-1'α), 101.06 (C-1'β), 95.8 (C-1β), 90.9 (C-1α), 80.1 (C-3β), 78.7 (C-aβ), 78.2 (C-aα), 77.0 (C-3α), 76.74 (C-5'α), 76.71 (C-5'β), 76.0 (C-4α), 75.9 (C-5β), 75.6 (C-4β), 74.2 (C-3'), 71.7 (C-5α), 70.9 (C-4'), 61.8 (C-6'), 60.6 (C-6β), 60.4 (C-6α), 56.7 (C-2'α), 56.6 (C-2'β), 56.5 (C-2β), 54.4 (C-2α), 54.2 (C-hα), 54.1 (C-hβ), 53.2 (C-l), 52.5 (C-eβ), 52.4 (C-eα), 50.5 (C-cα), 50.3 (C-cβ), 49.3 (C-m), 32.0 (C-g), 31.2 (C-i), 29.9 (C-k), 27.5 (C-fα), 27.4 (C-fβ), 23.0, 22.8 & 22.7 (acetamide), 21.4 (C-j), 18.9 (C-bα), 18.7 (C-bβ), 17.5 (C-dβ), 17.4 (C-dα), 16.8 (C-n).


BocHN (S) CO2Bn
SI-6
1H NMR spectrum in CDCl3
400 MHz
7.346
5.912
5.345
5.250
5.210
5.168
4.923
1.445
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
5.00
0.99
1.06
3.92
0.80
9.07

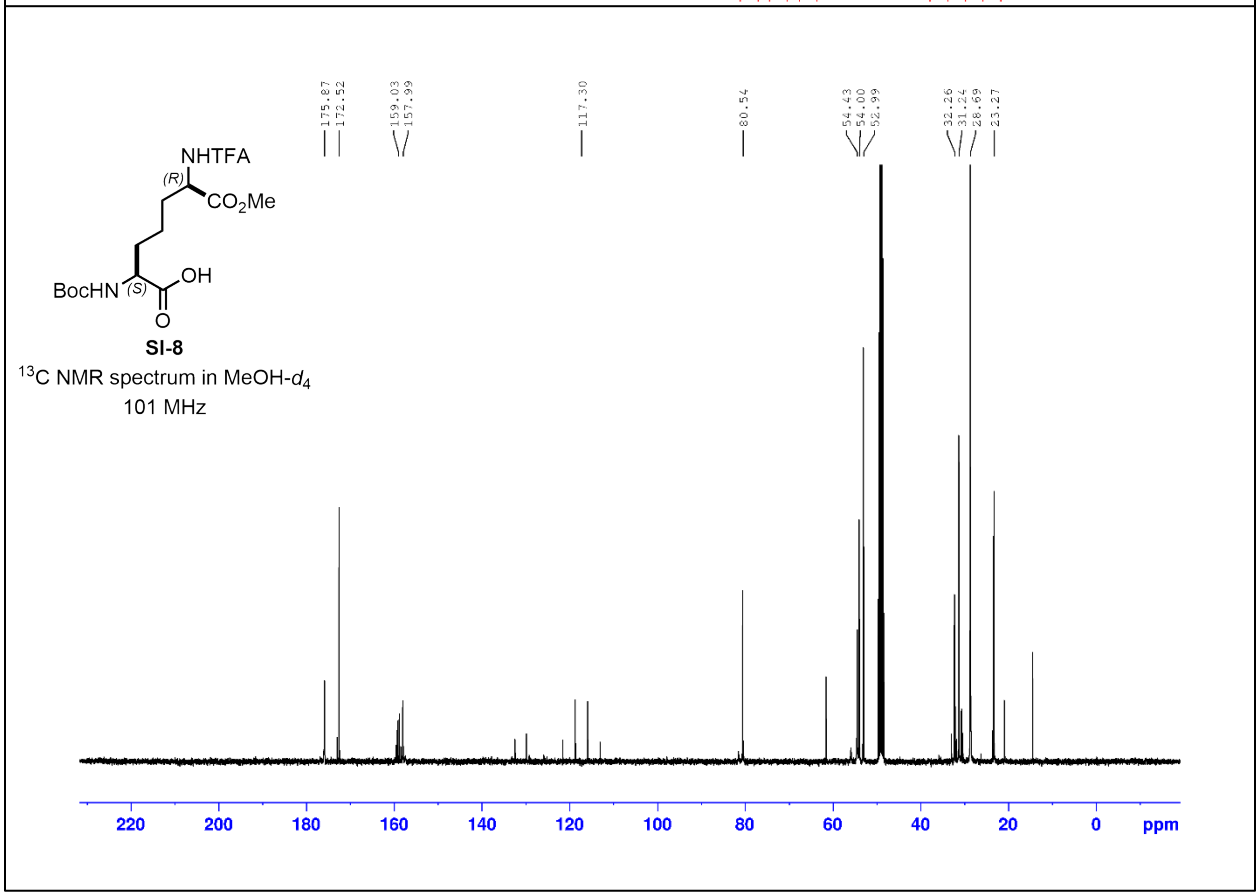

BocHN (S) CO2Bn
SI-6
13C NMR spectrum in CDCl3
101 MHz
170.72
155.10
135.33
132.67
128.70
128.54
128.28
117.60
80.21
67.42
55.96
28.39
220 200 180 160 140 120 100 80 60 40 20 0 ppm



SI-8

^{1}H NMR spectrum in MeOH-d_4

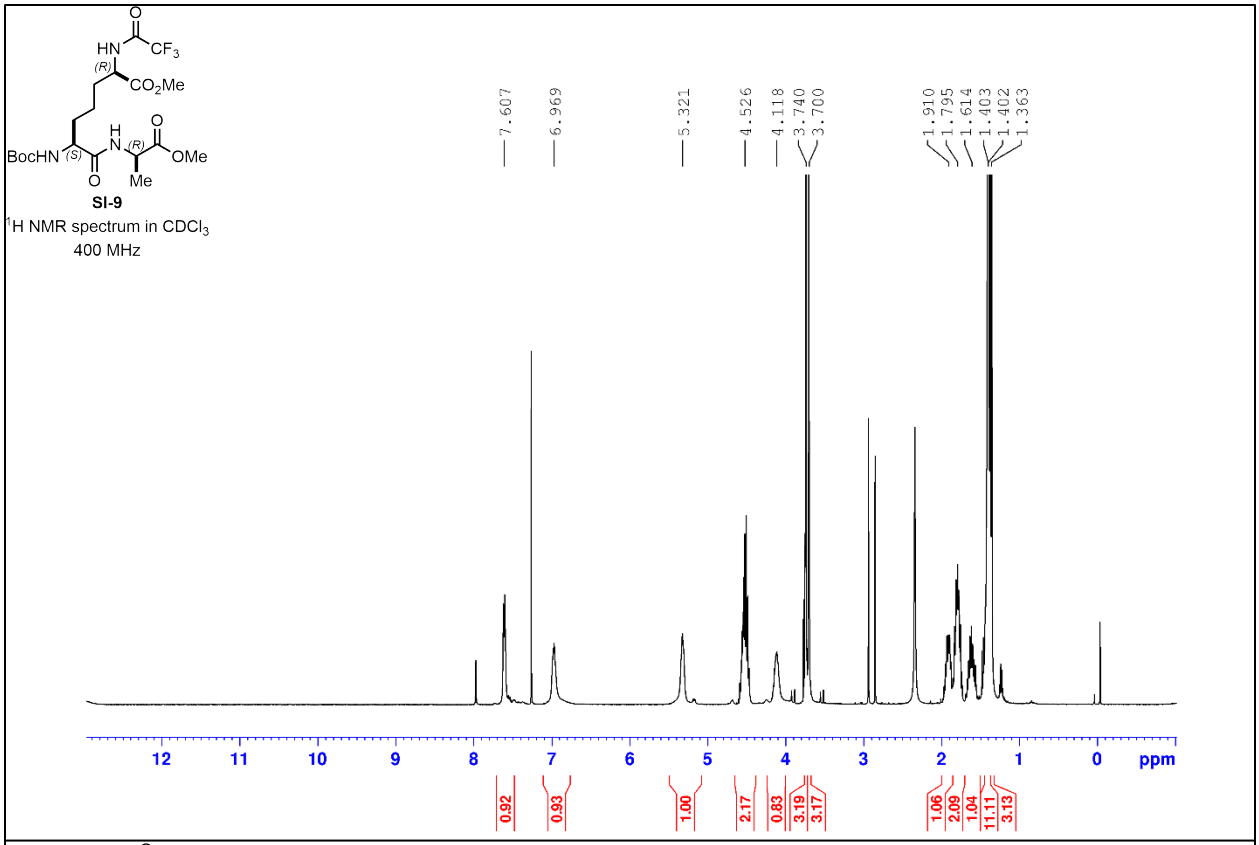
400 MHz

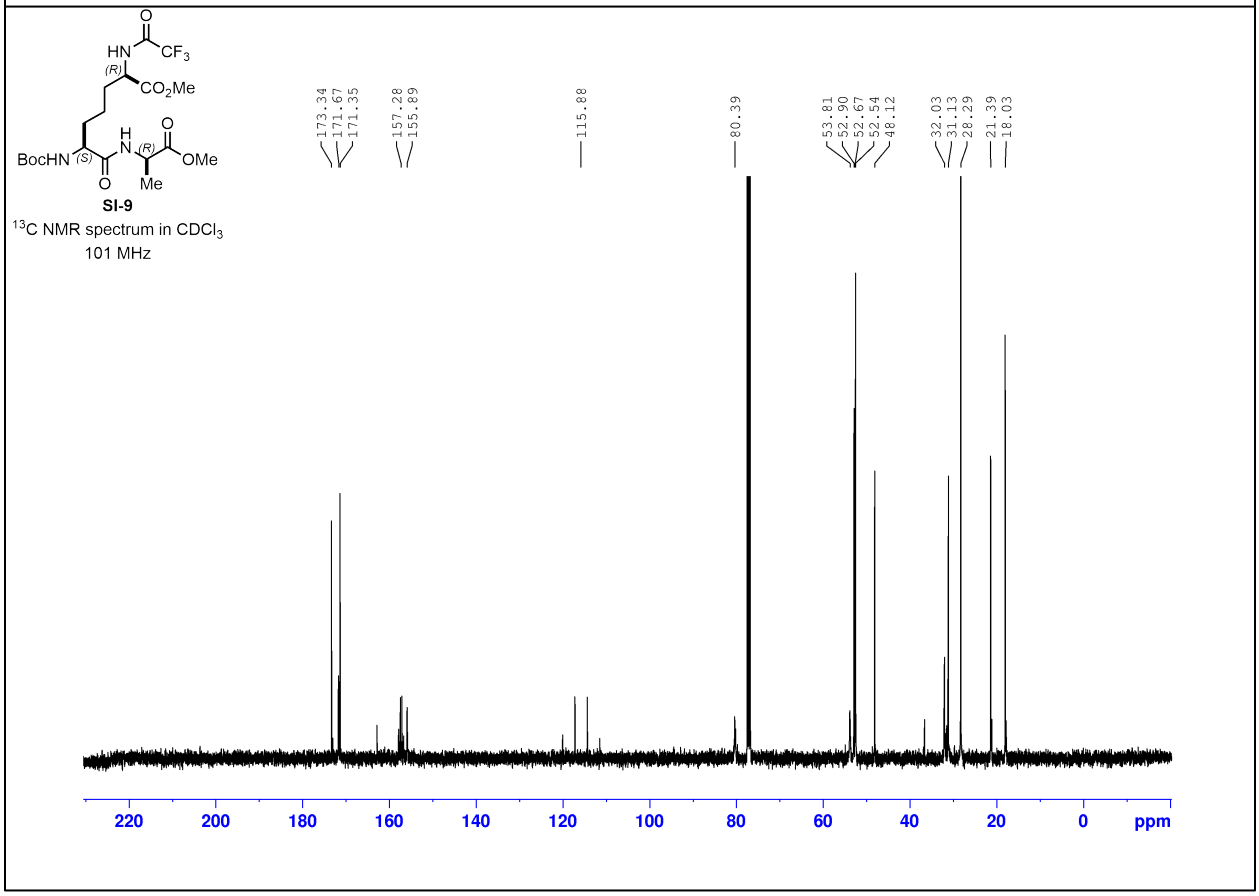


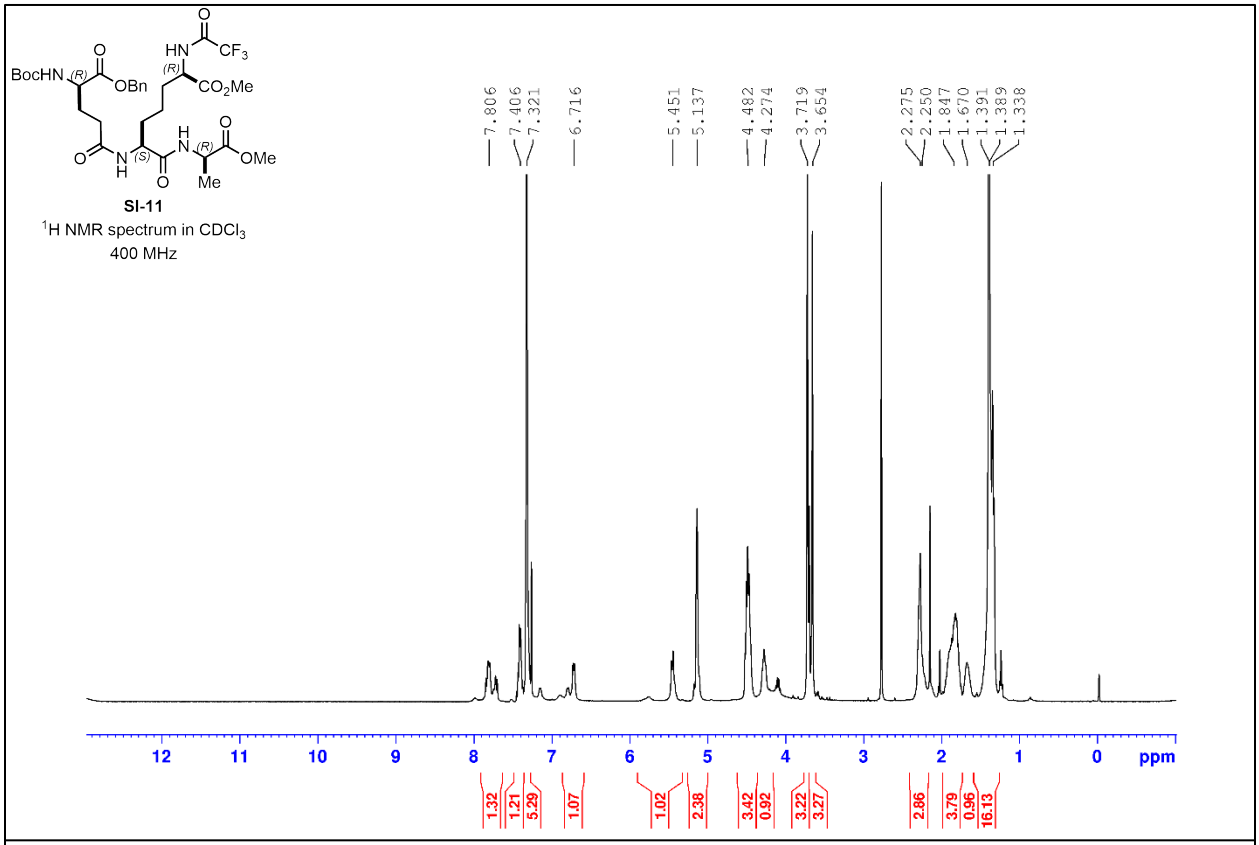
SI-8

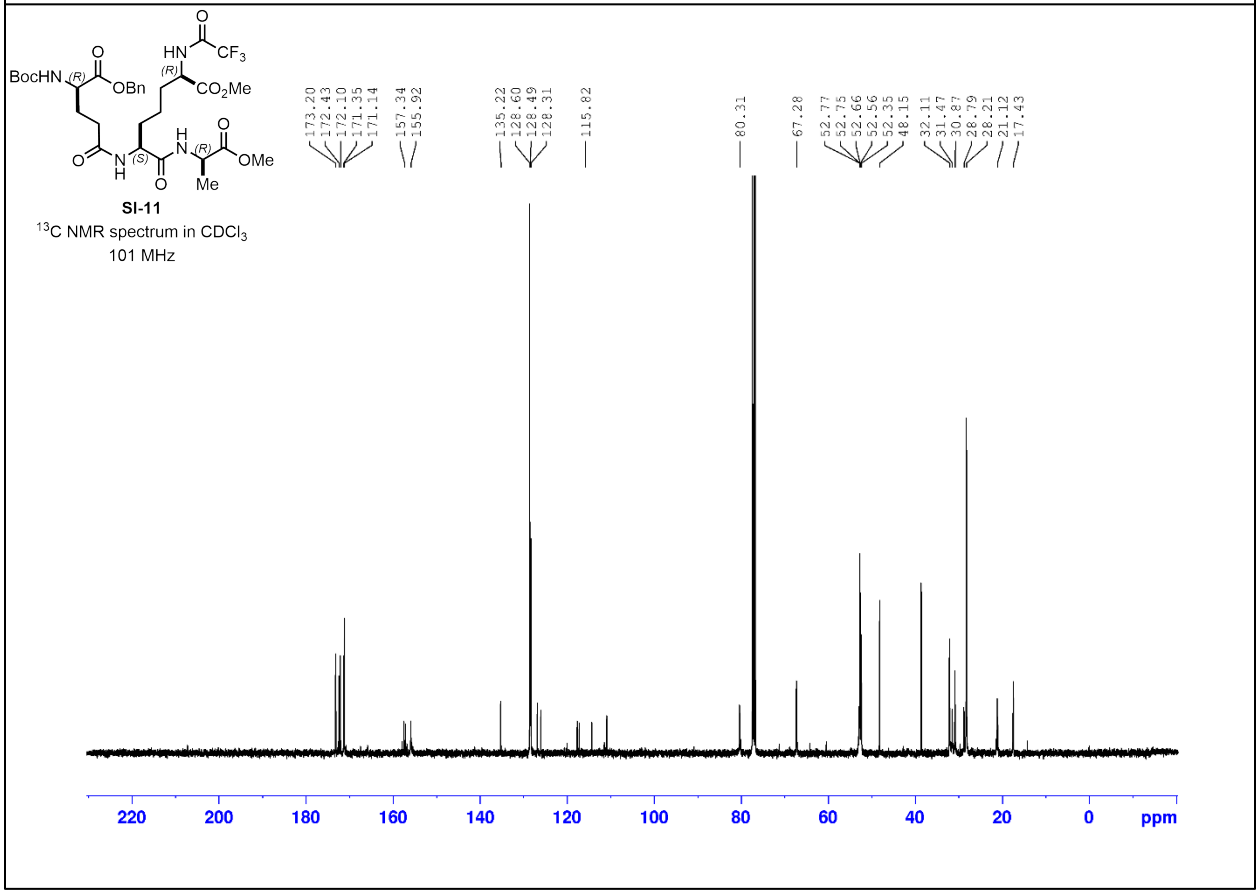
^{13}C NMR spectrum in MeOH-d_4

101 MHz


SI-9
1H NMR spectrum in CDCl3
400 MHz
7.607
6.969
5.321
4.526
4.118
3.740
3.700
1.910
1.795
1.614
1.403
1.402
1.363
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.92
0.93
1.00
2.17
0.83
3.19
3.17
1.06
2.09
1.04
11.11
3.13


SI-9
13C NMR spectrum in CDCl3
101 MHz
173.34
171.67
171.35
157.28
155.89
115.88
80.39
53.81
52.90
52.67
52.54
48.12
32.03
31.13
28.29
21.39
18.03
220 200 180 160 140 120 100 80 60 40 20 0 ppm


BocHN
OBn
HN
CF3
CO2Me
OMe
Me
SI-11
1H NMR spectrum in CDCl3
400 MHz
7.806
7.406
7.321
6.716
5.451
5.137
4.482
4.274
3.719
3.654
2.275
2.250
1.847
1.670
1.391
1.389
1.338
ppm
1.32
1.21
5.29
1.07
1.02
2.38
3.42
0.92
3.22
3.27
2.86
3.79
0.96
16.13


BocHN
OBn
HN
CF3
CO2Me
OMe
Me
SI-11
13C NMR spectrum in CDCl3
101 MHz
173.20
172.43
172.10
171.35
171.14
157.34
155.92
135.22
128.60
128.49
128.31
115.82
80.31
67.28
52.77
52.75
52.66
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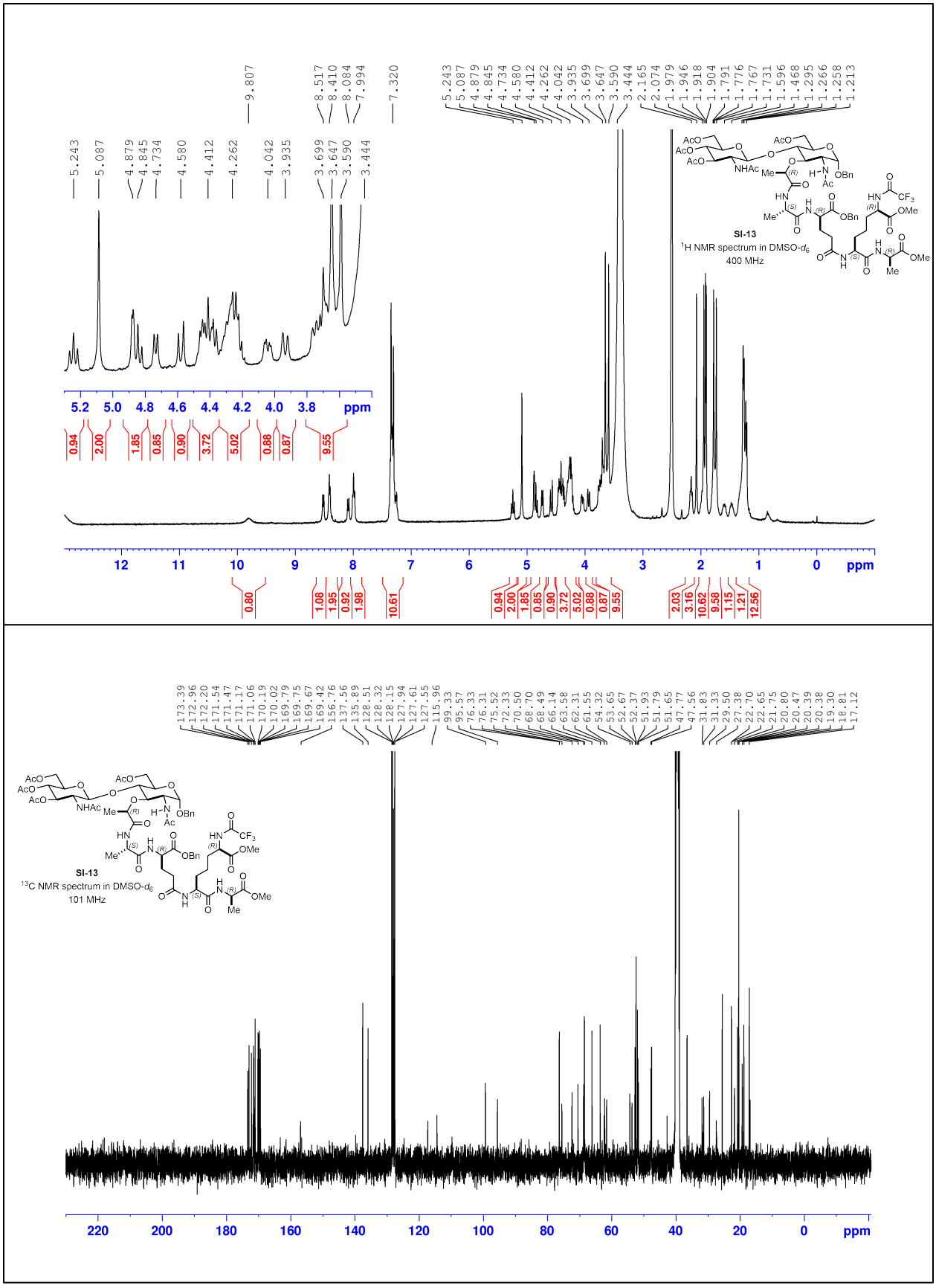
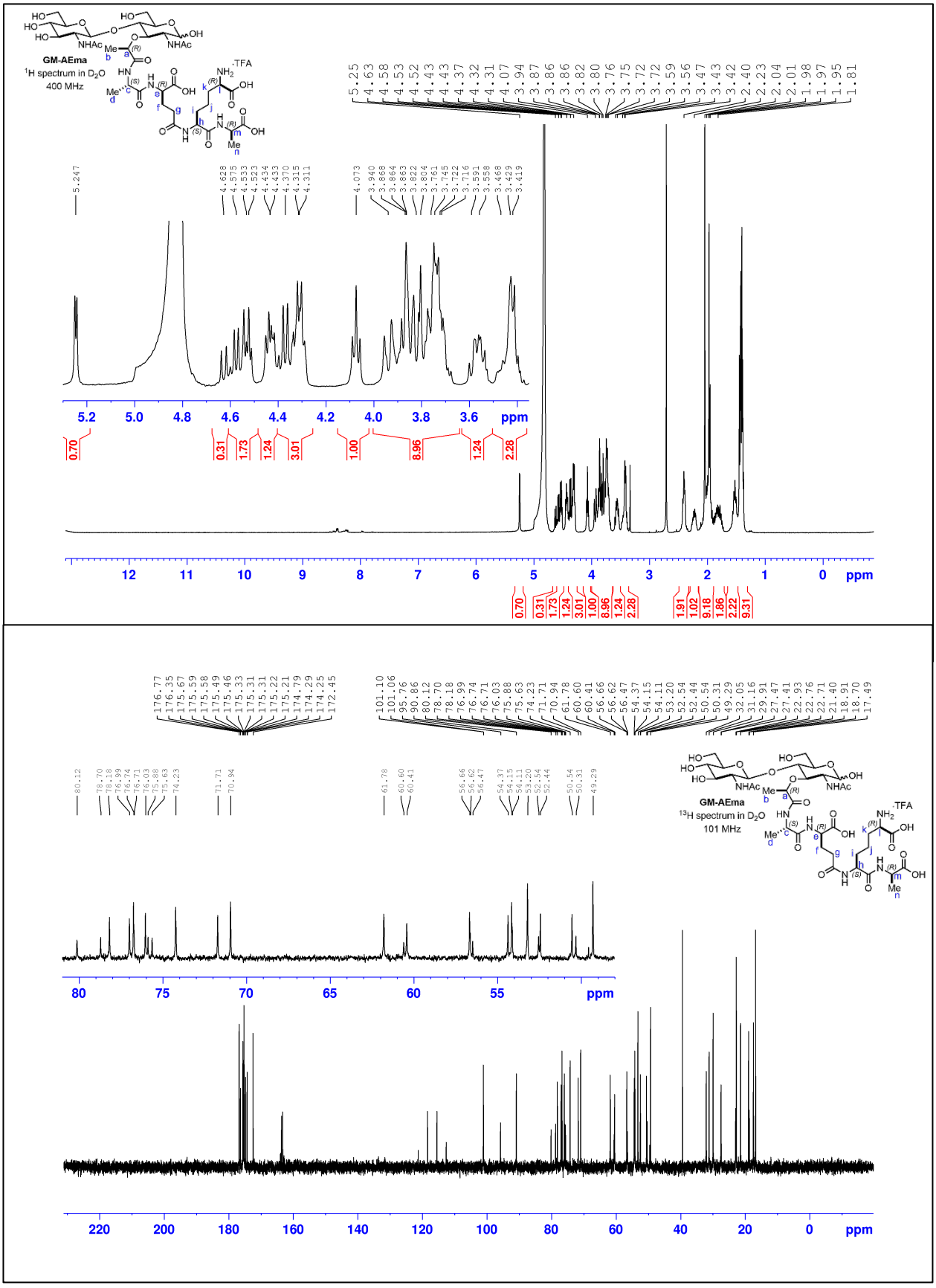
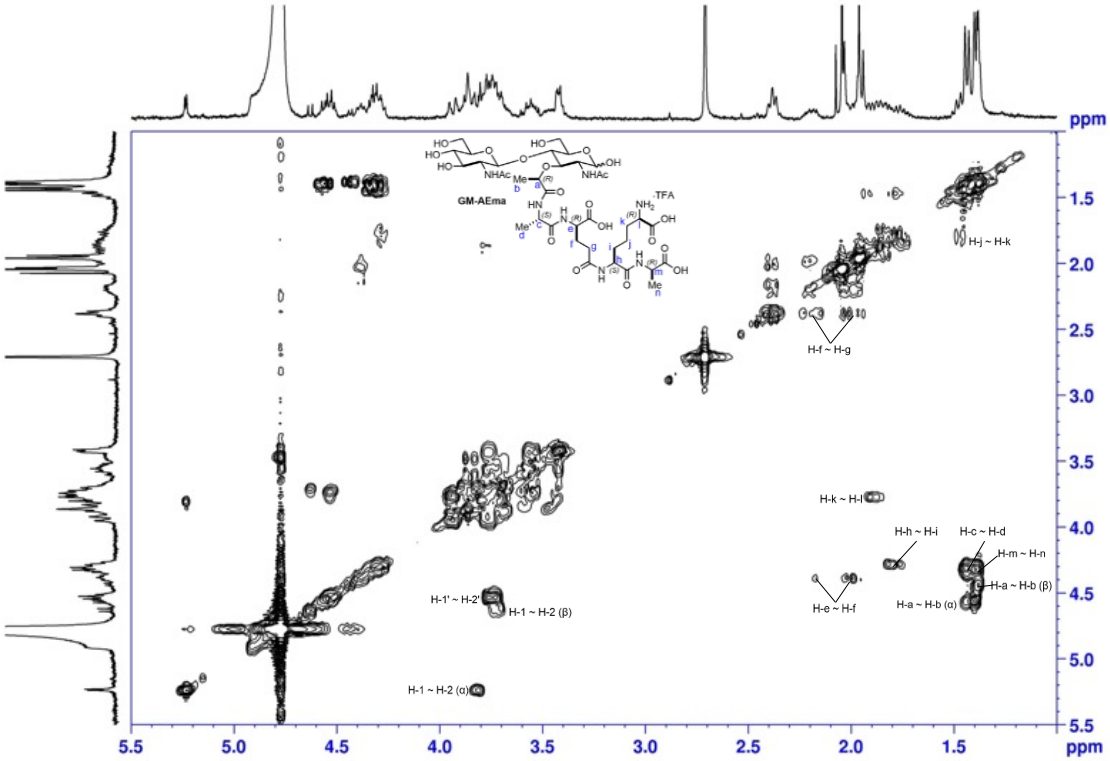
32.11
31.47
30.87
28.79
28.21
21.12
17.43
ppm

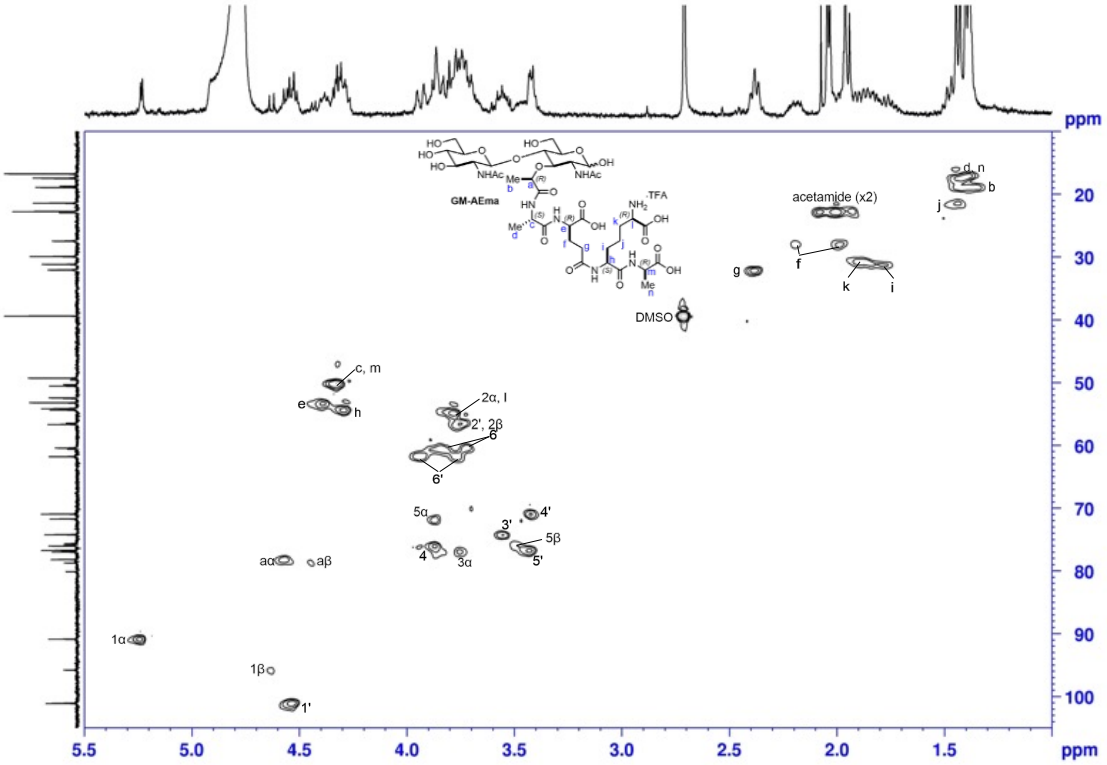

SI-13
^{1}H NMR spectrum in DMSO-d_6
400 MHz
SI-13
^{13}C NMR spectrum in DMSO-d_6
101 MHz


GM-AEma
^{1}H spectrum in D_2O
400 MHz
^{13}H spectrum in D_2O
101 MHz
·TFA
ppm

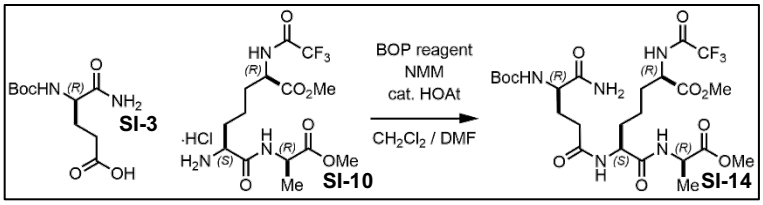
COSY of **GM-AEmA**



HSQC of **GM-AEmA**



Synthesis of GM-AQmA

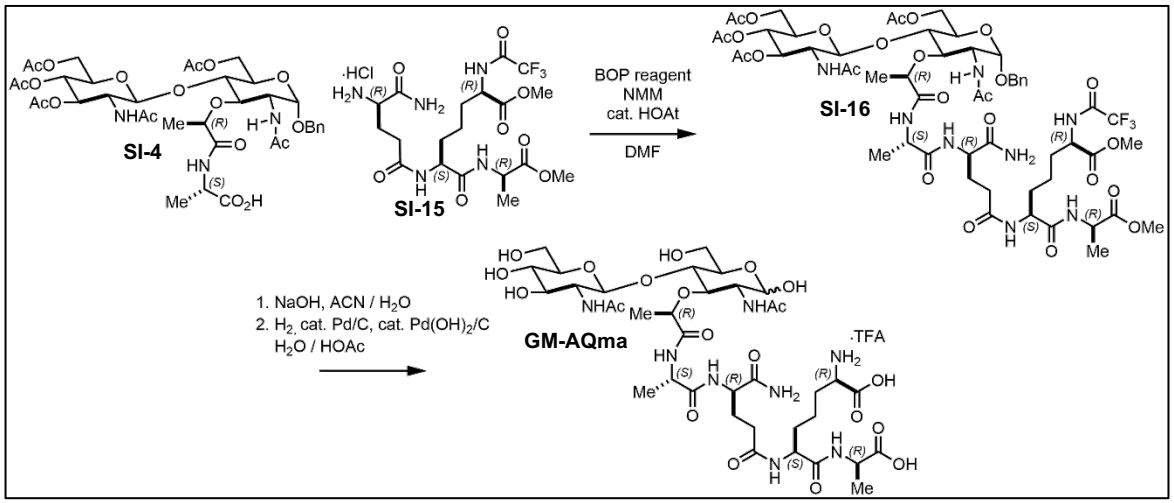


To a stirred solution of **SI-3** (57 mg, 0.230 mmol, 1.2 eq), **SI-10** (81.0 mg, 0.192 mmol, 1.0 eq), *N*-methylmorpholine (51 µL, 0.461 mmol, 2.4 eq) and HOAt (6.5 mg, 0.048 mmol, 0.25 eq) in 3.8 mL CH_2Cl_2 was charged BOP reagent (98 mg, 0.221 mmol, 1.15 eq). After 36 hours, the mixture was concentrated on the rotovap. The residue was diluted with EtOAc and washed with 2% aqueous K_2CO_3 (x3), pH 2 buffer (x2), and brine. The organic extract was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by column chromatography (15 g silica, 2 to 10% *i*-PrOH in EtOAc to afford **SI-14** (58 mg, 0.0945 mmol, 49% yield) as a white solid.

^{1}H NMR (400 MHz, MeOH-d_4, 301 K, δ): 4.43 (m, 2H), 4.34 (m, 1H), 4.05 (m, 1H), 3.74 (s, 3H), 3.70 (s, 3H), 2.36 (m, 2H), 2.09 (m, 1H), 1.95 (m, 1H), 1.81 (m, 3H), 1.70 (m, 1H), 1.449 (m, 2H), 1.446 (s, 9H), 1.38 (d, J = 7.3 Hz, 1H).

^{13}C NMR (101 MHz, MeOH-d_4, 301 K, δ): 175.2, 174.9, 174.4, 174.0, 173.9, 159.1 (q, J = 37.1 Hz), 157.8, 131.3 (q, J = 256.6 Hz), 80.8, 54.9, 54.5, 54.1, 53.0, 52.8, 49.5, 32.8, 32.4, 31.4, 29.7, 28.7, 23.1, 17.3.

HRMS (ESI-TOF, m/z): calc'd for $C_{24}H_{39}F_3N_5O_{10}^+$ ([M + H]$^+$) 614.2644; found 614.2641.



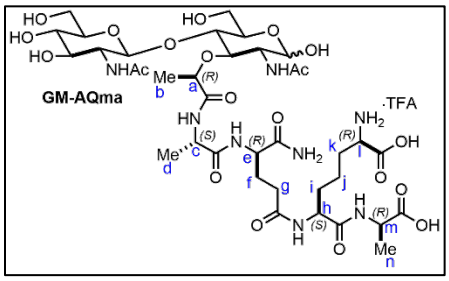
SI-14 (58 mg, 94.5 µmol, 1.0 eq) was stirred with 1.0 mL CH_2Cl_2 and then 0.5 mL 4M HCl in dioxane was charged. After 7 hours, the mixture was concentrated to dryness and co-evaporated several times with $CHCl_3$ to afford **SI-15**. This material was stirred with 1.9 mL DMF and then **SI-4** (82 mg, 99.2 µmol, 1.05 eq), *N*-methylmorpholine (26 µL, 236 µmol, 2.5 eq), HOAt (3.2 mg, 23.6 µmol, 0.25 eq) and finally BOP reagent (50 mg, 113 µmol, 1.2 eq) were charged. After 13 hours, the mixture was concentrated on the rotovap. The resulting semisolid residue was triturated with 1.0 mL CH_2Cl_2 (x6) to afford **SI-16** (67 mg, 50.7 µmol, 54% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6, 301 K, δ): 9.80 (t, J = 6.3 Hz, 1H), 8.39 (d, J = 6.0 Hz, 1H), 8.34 (d, J = 7.4 Hz, 1H), 8.25 (d, J = 7.9 Hz, 1H), 8.09 (d, J = 8.8 Hz, 1H), 7.96 (m, 2H), 7.31 (m, 2H), 7.03 (br s, 1H), 5.26 (t, J = 9.9 Hz, 1H), 4.878 (d, J = 3.2 Hz, 1H), 4.877 (t, J = 9.6 Hz, 1H), 4.74 (d, J = 8.2 Hz, 1H), 4.60 (d, J = 12.5 Hz, 1H), 4.47 to 4.34 (m, 4H) 4.27 (m, 3H), 4.19 (dd, J = 12.0, 3.4 Hz, 1H), 4.15 (m, 1H), 4.03 (dd, J = 11.7, 3.8 Hz, 1H), 3.96 (d, J = 12.0 Hz, 1H), 3.77 (dt, J = 9.9, 2.4 Hz, 1H), 3.72 to 3.63 (m, 3H), 3.65 (s, 3H), 3.61 (s, 3H), 3.44 (m, 1H), 2.14 (m, 2H), 2.07 (s, 3H), 1.954 (s, 3H), 1.951 (m, 1H), 1.94 (s, 3H9, 1.91 (s, 3H), 1.80 (s, 3H), 1.76 (m, 1H), 1.74 (s, 3H), 1.70 (m, 2H), 1.60 (m, 1H), 1.48 (m, 1H), 1.30 (m, 2H), 1.27 (d, J = 7.0 Hz, 3H), 1.25 (d, J = 7.2 Hz, 3H), 1.22 (d, J = 6.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6, 301 K, δ): 173.4, 173.2, 172.9, 172.0, 171.54, 171.48, 171.0, 170.2, 170.1, 169.74, 169.67, 169.6, 169.4, 156.7 (q, J = 36.7 Hz), 137.5, 128.3, 127.62, 127.55, 115.9 (q, J = 288.0 Hz), 99.3, 95.6, 76.4, 76.2, 75.4, 72.2, 70.4, 68.7, 68.50, 68.47, 62.3, 61.7, 54.3, 53.6, 52.7, 52.34, 52.25, 51.9, 51.8, 48.1, 47.5, 31.8, 31.6, 29.5, 28.0, 22.67, 22.65, 21.8, 20.8, 20.45, 20.41, 20.3, 18.9, 18.6, 17.1.

HRMS (ESI-TOF, m/z): calc'd for $C_{56}H_{80}F_3N_8O_{25}^+$ ([M + H]$^+$) 1321.5181; found 1321.5160.

SI-16 (36 mg, 27.2 µmol, 1.0 eq) was stirred with 544 µL ACN and 272 µL H_2O. To this suspension was added 1.0 M aqueous NaOH (272 µL, 272 µmol, 15 eq). A clear solution was observed soon thereafter. After 1 hour, the pH was adjusted to 4-5 with Amberlite IR-120 H^+, the suspension was filtered, and the filtrate was concentrated. The crude residue was purified by preparative HPLC (0 to 35% ACN) to afford the intermediate benzyl ether as a single epimer at C-h. To the intermediate was added 1.6 mL H_2O, 0.4 mL AcOH, 20 mg Pd/C, and 20 mg $Pd(OH)_2$/C. The mixture was vigorously stirred under H_2 atmosphere (balloon). After 1 hour 40 minutes, the mixture was filtered through a syringe filter and the filtrate was concentrated. The crude material was purified by preparative HPLC (0 to 20% ACN) to afford **GM-AQmA** (10.1 mg, 9.6 µmol, 35% yield over two steps) as a white solid.

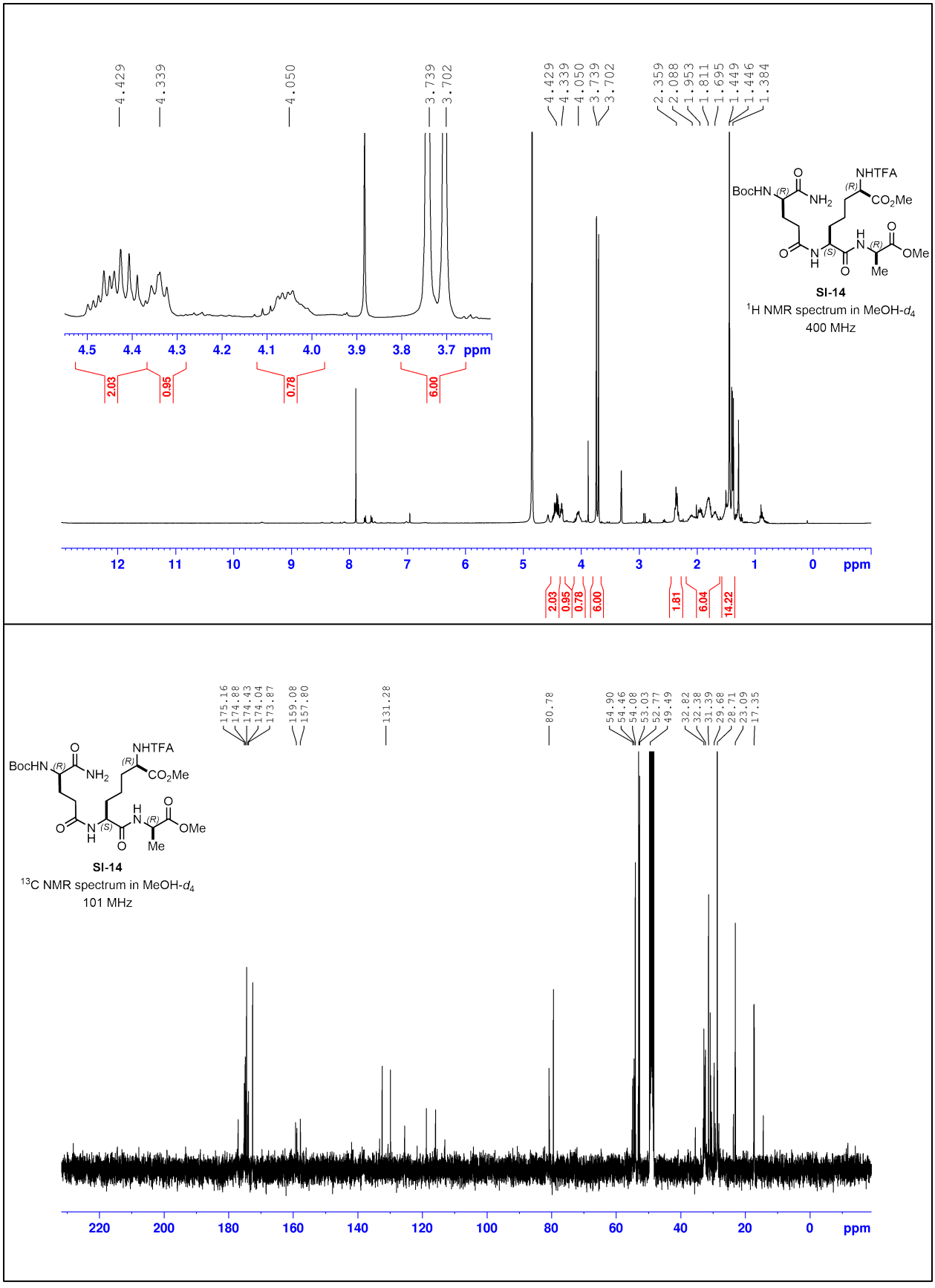


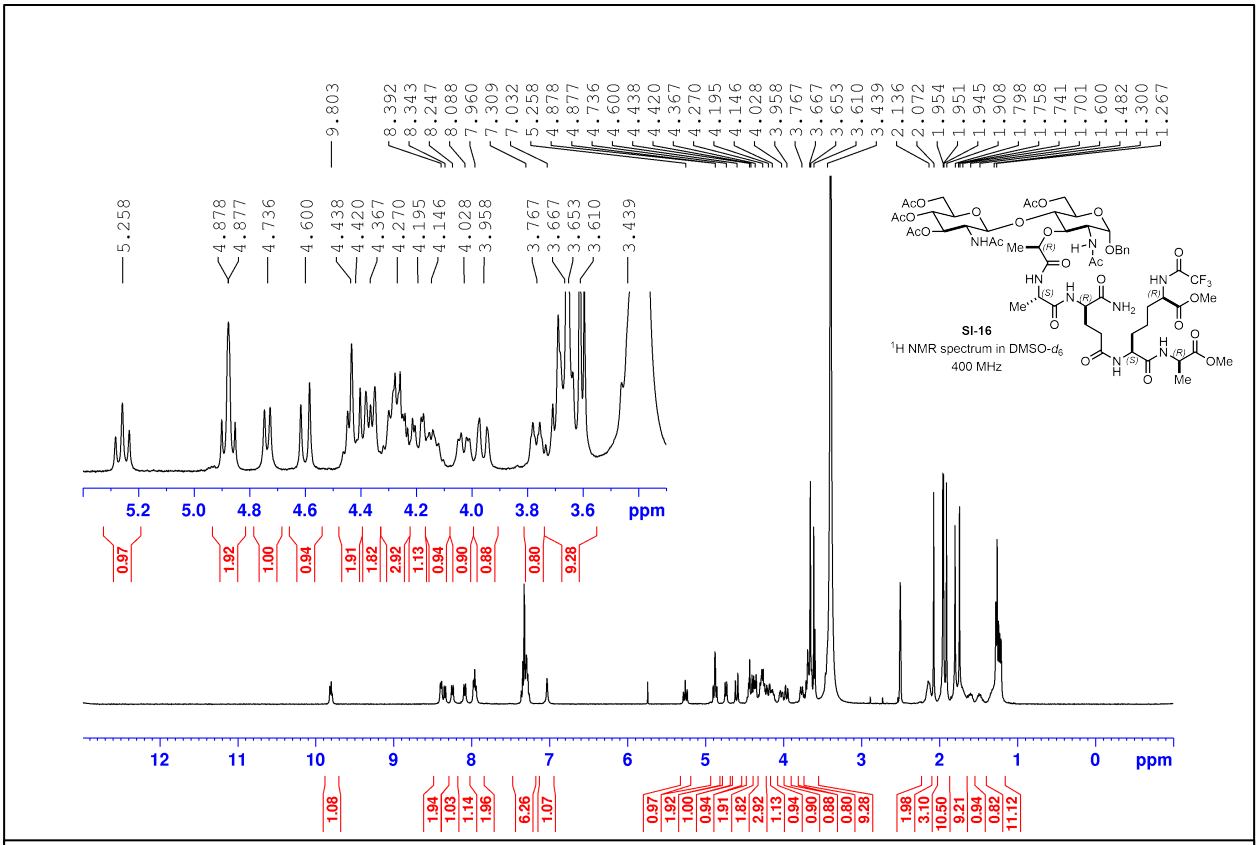
^{1}H NMR (400 MHz, D_2O, 301 K, δ): 5.24 (d, J = 3.2 Hz, 1H; H-1α), 4.63 (d, J = 8.3 Hz, 1H; H-1β), 4.56 (q, J = 6.8 Hz, 1H; H-aα), 4.54 (d, J = 8.4 Hz, 1H; H-1'α), 4.52 (d, J = 8.1 Hz, 1H; H-1'β), 4.41 (q, J = 6.8 Hz, 1H; H-aβ), 4.37 (m, 1H; H-m), 4.36 (m, 1H; H-e), 4.31 (m, 1H; H-h), 4.29 (m, 1H; H-c), 4.07 (t, J = 6.4 Hz, 1H; H-l), 3.94 (d, J = 12.2 Hz, 1H; H-6'a), 3.864 (m, 1H; H-4), 3.862 (m, 1H; H-5α), 3.82 (m, 1H; H-2α), 3.80 (m, 1H; H-6a), 3.77 (m, 1H; H-6'b), 3.743 (m, 1H; H-2'), 3.741 (m, 1H; H-3α), 3.72 (m, 1H; H-6b), 3.59 (m, 1H; H-3β), 3.56 (m, 1H; H-3'), 3.47 (m, 1H; H-5β), 3.425 (m, 1H; H-5'), 3.417 (m, 1H; H-4'), 2.40 (m, 2H; H-g), 2.17 (m, 1H; H-f), 2.05 (s, 3H; acetamide), 1.98 (m, 1H; H-g), 1.974 (m, 2H; H-k), 1.968 & 1.95 (s, 3H & s, 3H; acetamide), 1.81 (m, 2H; H-i), 1.52 (m, 2H; H-j), 1.44 (d, J = 7.3 Hz, 3H; H-d), 1.41 (d, J = 7.3 Hz, 3H; H-n), 1.40 (d, J = 6.8 Hz, 3H; H-b).

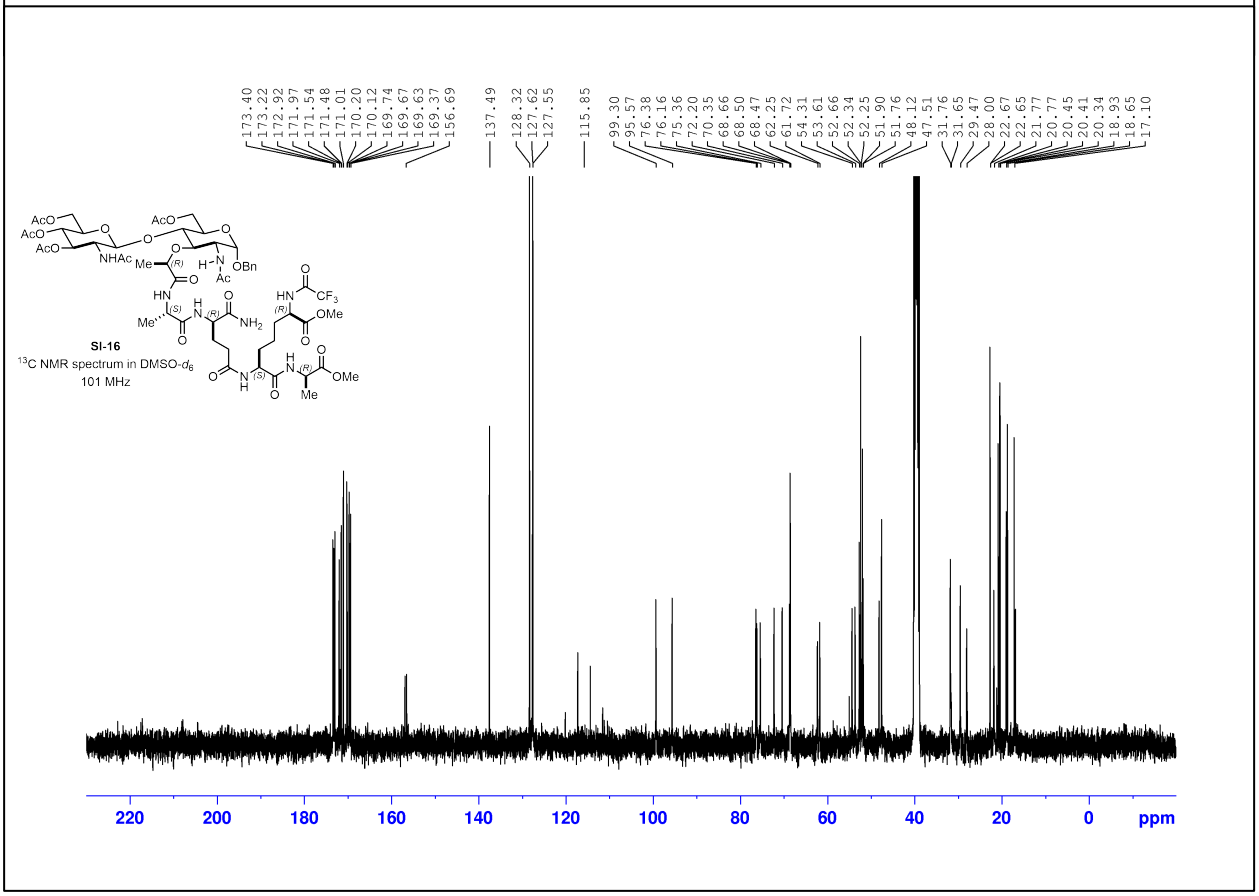
^{13}C NMR (101 MHz, D_2O, 301 K, δ): 176.8, 176.5, 176.4, 175.8, 175.74, 175.65, 175.6, 175.5, 175.3, 175.2, 174.8, 174.28, 174.25, 172.5, 101.10 (C-1'α), 101.07 (C-1'β), 95.7 (C-1β), 90.9 (C-1α), 80.2 (C-3β), 78.7 (C-aβ), 78.2 (C-aα), 77.0 (C-3α), 76.75 (C-5'α), 76.71 (C-5'β), 76.0 (C-4α), 75.9 (C-5β), 75.7 (C-4β), 74.2 (C-3'), 71.7 (C-5α), 71.0 (C-4'), 61.8 (C-6'), 60.6 (C-6β), 60.4 (C-6α), 56.7 (C-2'α), 56.6 (C-2'β), 56.5 (C-2β), 54.4 (C-2α), 54.1 (C-h), 53.4 (C-eβ), 53.3 (C-eα), 53.2 (C-l), 50.7 (C-cα), 50.5 (C-

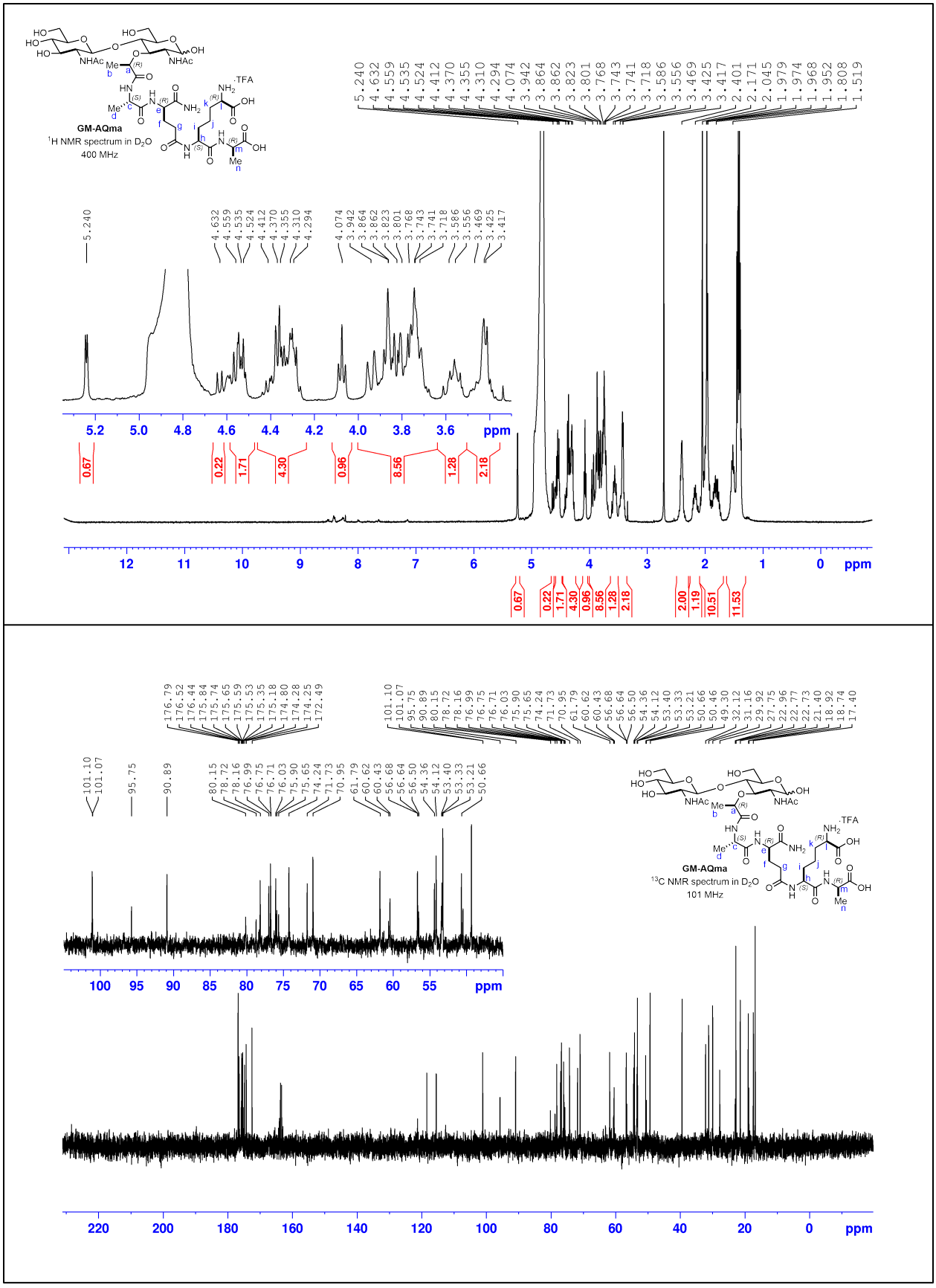
cβ), 49.3 (C-m), 32.1 (C-g), 31.2 (C-i), 29.9 (C-k), 27.7 (C-f), 23.0, 22.8 & 22.7 (acetamide), 21.4 (C-j), 18.9 (C-bα), 18.7 (C-bβ), 17.4 (C-dβ), 17.3 (C-dα), 16.8 (C-n).

HRMS (ESI-TOF, m/z): calc'd for $C_{37}H_{63}N_8O_{20}^+$ ($[M + H]^+$) 939.4159; found 939.4135.

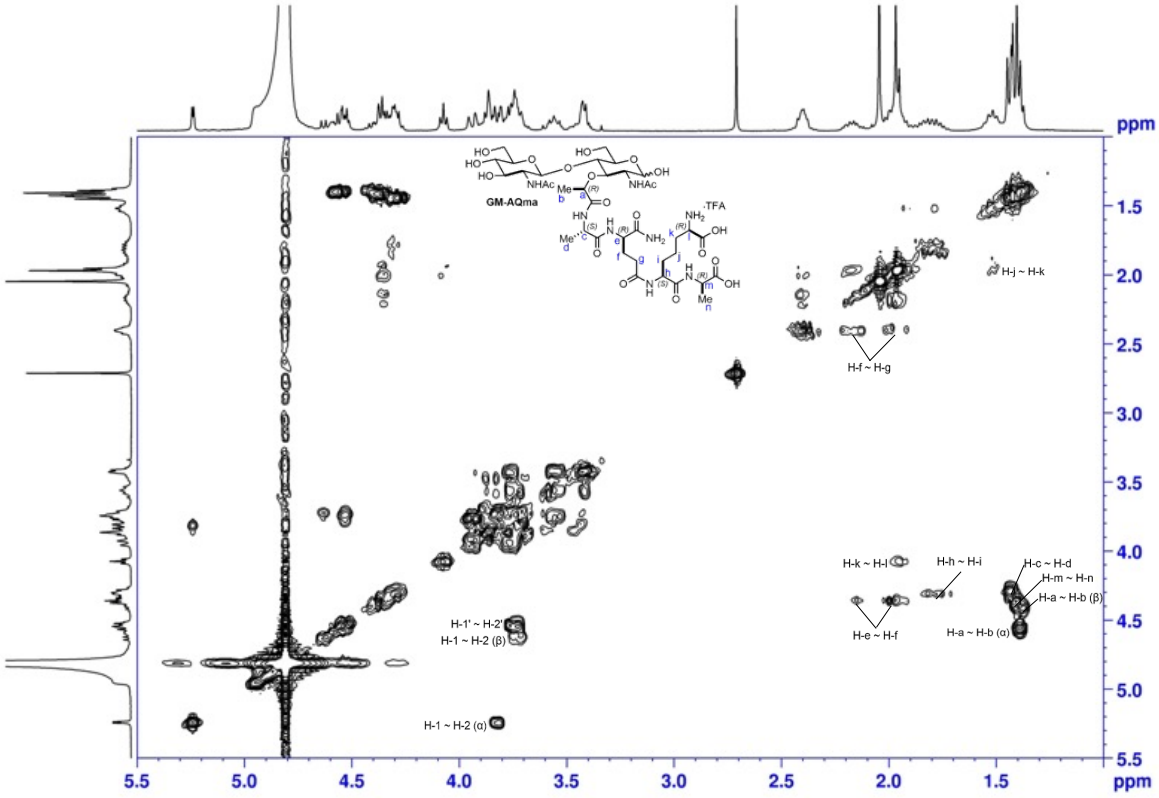

4.429
4.339
4.050
3.739
3.702
2.359
2.088
1.953
1.811
1.695
1.449
1.446
1.384
4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 ppm
2.03
0.95
0.78
6.00
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
2.03
0.95
0.78
6.00
1.81
6.04
14.22
BocHN
NHTFA
NH2
CO2Me
OMe
Me
SI-14
1H NMR spectrum in MeOH-d4
400 MHz
175.16
174.88
174.43
174.04
173.87
159.08
157.80
131.28
80.78
54.90
54.46
54.08
53.03
52.77
49.49
32.82
32.38
31.39
29.68
28.71
23.09
17.35
220 200 180 160 140 120 100 80 60 40 20 0 ppm
SI-14
13C NMR spectrum in MeOH-d4
101 MHz


SI-16
^{1}H NMR spectrum in DMSO-d_6
400 MHz

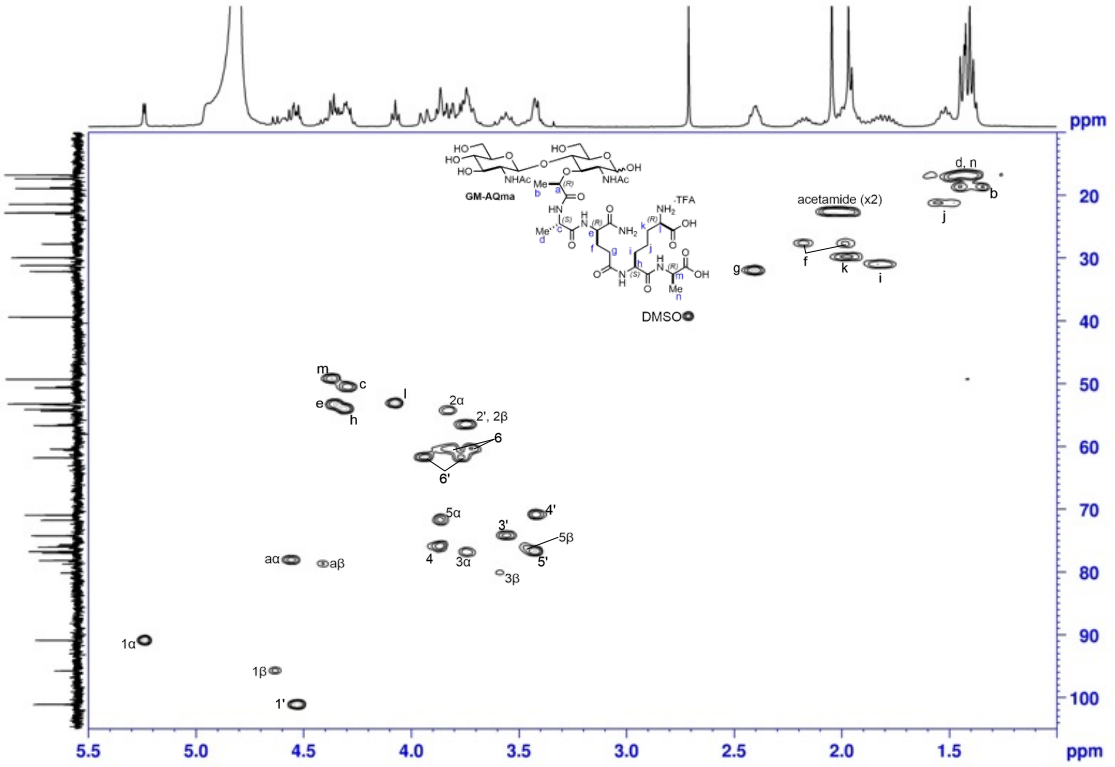

SI-16
^{13}C NMR spectrum in DMSO-d_6
101 MHz


GM-AQma
^{1}H NMR spectrum in D_2O
400 MHz
GM-AQma
^{13}C NMR spectrum in D_2O
101 MHz

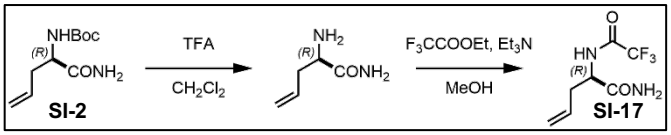
COSY of **GM-AQmA**



HSQC of **GM-AQmA**



Synthesis of GM-AE(m-NH_2)A (i.e. GM-AE(nm)A)

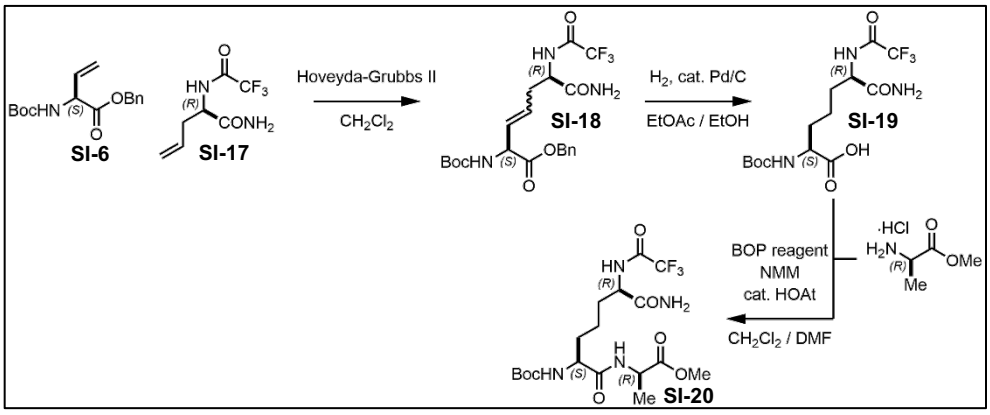


To a stirred solution of **SI-2** (381 mg, 1.78 mmol, 1.0 eq) in 3 mL CH_2Cl_2 was charged 3 mL TFA. After 2 hours, the mixture was concentrated to dryness and co-evaporated several times with *i*-PrOH. The residue was stirred with 15 mL MeOH. Et_3N (620 µL, 4.45 mmol, 2.5 eq) was charged, and the mixture was cooled in an ice bath. Ethyl trifluoroacetate (373 µL, 3.12 mmol, 1.75 eq) was charged in portions over 5 minutes, and the cooling bath was removed. After 45 minutes additional ethyl trifluoroacetate (200 µL, 1.68 mmol, 0.94 eq) was added. After another 2.5 hours the mixture was concentrated to dryness and co-evaporated with PhMe. The crude residue was purified by column chromatography (20 g silica, 40 then 60% EtOAc in hexane) to afford **SI-17** (282 mg, 1.34 mmol, 75% yield) as a white solid.

^{1}H NMR (400 MHz, MeOH-d_4, 301 K, δ): 5.77 (dddd, J = 17.1, 10.1, 7.3, 6.7 Hz, 1H), 5.16 (dq, J = 17.1, 1.5 Hz, 1H), 5.11 (dp, J = 10.1, 0.9 Hz, 1H), 4.48 (dd, J = 8.9, 5.3 Hz, 1H), 2.64 (m, 1H), 2.48 (m, 1H).

^{13}C NMR (101 MHz, MeOH-d_4, 301 K, δ): 174.6, 158.8 (q, J = 37.5 Hz), 134.1, 119.0, 117.4 (q, J = 286.9), 54.3, 37.0.

HRMS (ESI-TOF, m/z): calc'd for $C_7H_{10}F_3N_2O_2^+$ ([M + H]$^+$) 211.0689; found 211.0687.



A stirred suspension of **SI-6** (478 mg, 1.64 mmol, 1.5 eq), **SI-17** (230 mg, 1.09 mmol, 1.0 eq) and 11 mL CH_2Cl_2 was sparged with argon for 30 minutes (*note*: undissolved **SI-17** can easily cause clogs, so it is best to use a thick sparge needle). Second generation Hoveyda-Grubbs catalyst (28 mg, 0.040 mmol, 0.04 eq) was charged, and the mixture was then heated to a gentle reflux. After 20 hours, the mixture was cooled to room temperature and concentrated. The residue was purified by column chromatography (25 g silica, 15 to 70% EtOAc in hexane) to afford the cross-metathesis product **SI-18** (228 mg, 0.482 mmol, 44% yield) as an oil. This material was stirred with 4 mL EtOAc and 1 mL EtOH. 53 mg Pd/C was charged, and the mixture was stirred under H_2 (balloon) atmosphere. After 12 hours, the mixture was filtered through Celite and rinsed forward with EtOAc. The filtrate was concentrated to dryness, and the residue was purified by column chromatography (10 g silica, 5 to 15% MeOH in CH_2Cl_2). As residual methanol was difficult to remove, the purified material was taken up in 1 : 1 / ACN : H_2O and lyophilized to afford **SI-19** (156 mg, 0.404 mmol, 37% yield over 2 steps) as a white solid.

^{1}H NMR (400 MHz, MeOH-d_4, 301 K, δ): 4.41 (m, 1H), 4.08 (m, 1H), 1.84 (m, 3H), 1.68 (m, 1H), 1.48 (m, 2H), 1.44 (s, 9H).

^{13}C NMR (101 MHz, MeOH-d_4, 301 K, δ): 176.1, 175.2, 159.0 (q, J = 37.4 Hz), 158.1, 117.5 (q, J = 286.5), 80.6, 54.74, 54.69, 32.4, 32.3, 28.7, 23.3.

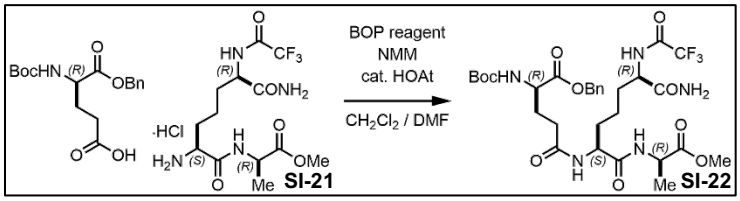
HRMS (ESI-TOF, m/z): calc'd for $C_{14}H_{23}F_3N_3O_6^+$ ([M + H]$^+$) 386.1533; found 386.1530.

To a stirred solution of **SI-19** (134 mg, 0.348 mmol, 1.0 eq), H-D-Ala-OMe·HCl (85 mg, 0.609 mmol, 1.75 eq), *N*- methylmorpholine (115 µL, 1.04 mmol, 3.0 eq) and HOAt 12 mg, (0.087 mmol, 0.25 eq) in 5 mL CH_2Cl_2 and 2 mL DMF was charged BOP reagent (200 mg, 0.452 mmol, 1.3 eq). After 2 hours, the mixture was partly concentrated to remove most of the CH_2Cl_2 and then diluted with EtOAc. This material was washed with 2% aqueous K_2CO_3 (x3), pH 2 buffer, and brine. The organic extract was dried (Na_2SO_4), filtered, and concentrated. The crude material was purified by column chromatography (20 g silica, 2 : 1 : 0.1 then 2 : 1 : 0.2 hexane : EtOAc : *i*-PrOH) to afford **SI-20** (147 mg, 0.312 mmol, 90% yield) as a white solid.

^{1}H NMR (400 MHz, MeOH-d_4, 301 K, δ): 4.41 (m, 2H), 4.07 (m, 1H), 3.71 (s, 3H), 1.89 (m, 1H), 1.77 (m, 2H), 1.63 (m, 1H), 1.49 (m, 2H), 1.44 (s, 9H), 1.38 (q, J = 7.3 Hz, 3H).

^{13}C NMR (101 MHz, MeOH-d_4, 301 K, δ): 175.1, 174.6, 174.4, 158.9 (q, J = 37.3 Hz), 157.7, 117.3 (q, J = 286.5 Hz), 80.8, 55.6, 54.7, 52.8, 49.4, 33.0, 32.3, 28.6, 23.1, 17.5.

HRMS (ESI-TOF, m/z): calc'd for $C_{18}H_{30}F_3N_4O_7^+$ ([M + H]$^+$) 471.2061; found 471.2052.

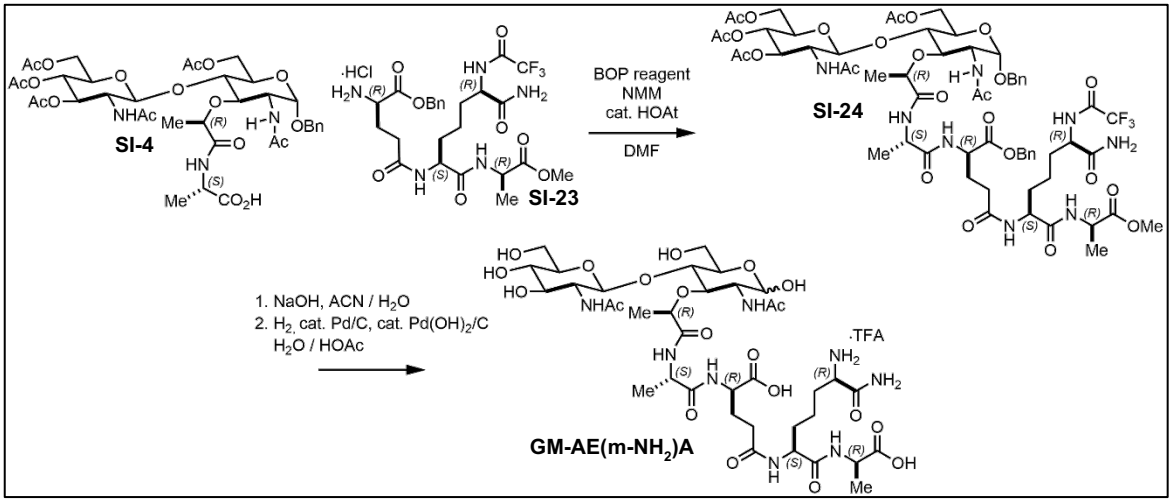


SI-20 was stirred with 2 mL dioxane and then 1 mL 4M HCl in dioxane was charged. After 5 hours, the mixture was concentrated and co-evaporated several times with *i*-PrOH to afford hydrochloride salt **SI-21** (105 mg). To a stirred solution of **SI-21** (45 mg, 111 µmol, 1.0 eq), Boc-(*R*)-Glu(OH)-OBn (41 mg, 122 µmol, 1.1 eq), *N*-methylmorpholine (31 µL, 278 µmol, 2.5 eq), and HOAt (4 mg, 28 µmol, 0.25 eq) in 2 mL CH_2Cl_2 and 1 mL DMF was charged BOP reagent (59 mg, 133 µmol, 1.2 eq). After 16 hours, the mixture was concentrated to dryness and co-evaporated several times with *i*-PrOH. The residue was diluted with EtOAc, and this material was washed with 2% aqueous K_2CO_3 (x3) and brine. The organic extract was dried (Na_2SO_4), filtered, and concentrated. The crude material was purified by column chromatography (12 g silica, 90% EtOAc in hexane, EtOAc, then 5% *i*-PrOH in EtOAc) to afford **SI-22** (48 mg, 70 µmol, 63% yield) as a white solid.

^{1}H NMR (400 MHz, MeOH-d_4, 301 K, δ): 7.35 (m, 5H), 5.16 (m, 2H), 4.37 (m, 3H), 4.15 (m, 1H), 3.69 (s, 3H), 2.35 (m, 2H), 2.15 (m, 1H), 1.88 (m, 2H), 1.79 (m, 2H), 1.67 (m, 1H), 1.45 (m, 2H), 1.43 (s, 3H), 1.36 (d, J = 7.4 Hz, 3H).

^{13}C NMR (101 MHz, MeOH-d_4, 301 K, δ): 175.2, 174.8, 174.6, 174.41, 173.99, 158.9, 158.1, 137.3, 131.4, 129.6, 129.3, 129.2, 80.8, 67.9, 54.8, 54.6, 54.1, 52.8, 49.4, 32.8, 32.6, 32.3, 28.7, 28.5, 23.0, 17.2.

HRMS (ESI-TOF, m/z): calc'd for $C_{30}H_{43}F_3N_5O_{10}^+$ ([M + H]$^+$) 690.2957; found 690.2942.

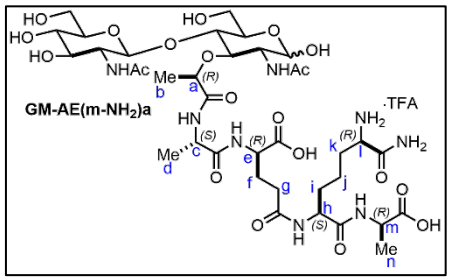


SI-22 (48 mg, 70 µmol, 1.0 eq) was stirred with 1 mL dioxane, 0.5 mL CH_2Cl_2 and 0.5 mL 4 M HCl in dioxane. After 5 hours, the mixture was concentrated and co-evaporated several times with *i*-PrOH to afford **SI-23**. This material was stirred in 1.4 mL DMF, and **SI-4** (61 mg, 74 µmol, 1.05 eq), *N*-methylmorpholine (19 µL, 175 µmol, 2.5 eq), HOAt (2.4 mg, 18 µmol, 0.25 eq) and finally BOP reagent (39 mg, 87.5 µmol, 1.25 eq) was charged. After 17 hours, the mixture was concentrated to dryness and then co-evaporated several times with *i*-PrOH. The crude residue was triturated with 1 mL CH_2Cl_2 (x4) to afford **SI-24** (75 mg, 53.7 µmol, 77% yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6, 301 K, δ): 9.39 (d, J = 7.9 Hz, 1H), 8.50 (d, J = 7.3 Hz, 1H), 8.40 (m, 1H), 8.35 (d, J = 7.5 Hz, 1H), 8.08 (d, J = 8.7 Hz, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.54 (br s, 1H), 7.32 (m, 10H), 7.13 (br s, 1H), 5.24 (t, J = 9.7 Hz, 1H), 5.09 (s, 2H), 4.88 (d, J = 3.2 Hz, 1H), 4.85 (t, J = 9.6 Hz, 1H), 4.73 (d, J = 7.4 Hz, 1H), 4.58 (d, J = 12.6 Hz, 1H), 4.41 (m, 4H), 4.27 (m, 5H), 4.05 (d, J = 11.2 Hz, 1H), 3.94 (d, J = 11.6 Hz, 1H), 3.76 (d, J = 9.4 Hz, 1H), 3.68 (m, 3H), 3.60 (s, 3H), 3.44 (m, 1H), 2.18 (t, J = 6.8 Hz, 1H), 2.07 (s, 3H), 1.98 (m, 1H), 1.95 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.78 (s, 3H), 1.77 (m, 1H), 1.74 (s, 3H), 1.69 (m, 2H), 1.61 (m, 1H), 1.46 (m, 1H), 1.29 (m, 2H), 1.27 (d, J = 7.0 Hz, 3H), 1.24 (d, J = 7.3 Hz, 3H), 1.22 (d, J = 6.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6, 301 K, δ): 173.4, 172.9, 172.2, 172.1, 171.52, 171.47, 171.0, 170.1, 170.0, 169.72, 169.70, 169.6, 169.4, 156.4 (q, J = 36.3 Hz), 137.5, 135.9, 128.5, 128.3, 128.1, 127.9, 127.6, 127.5, 116.1 (q, J = 287.6 Hz), 99.3, 95.6, 76.31, 76.29, 75.5, 72.3, 70.5, 68.7, 68.48, 68.47, 66.1, 62.3, 61.5, 54.3, 53.6, 53.2, 51.9, 51.7, 47.7, 47.5, 32.0, 31.3, 30.7, 27.4, 22.7, 22.6, 21.8, 20.8, 20.44, 20.36, 20.35, 19.3, 18.8, 17.1, 16.9.

HRMS (ESI-TOF, m/z): calc'd for $C_{62}H_{84}F_3N_8O_{25}^+$ ([M + H]$^+$) 1397.5500; found 1397.5470.



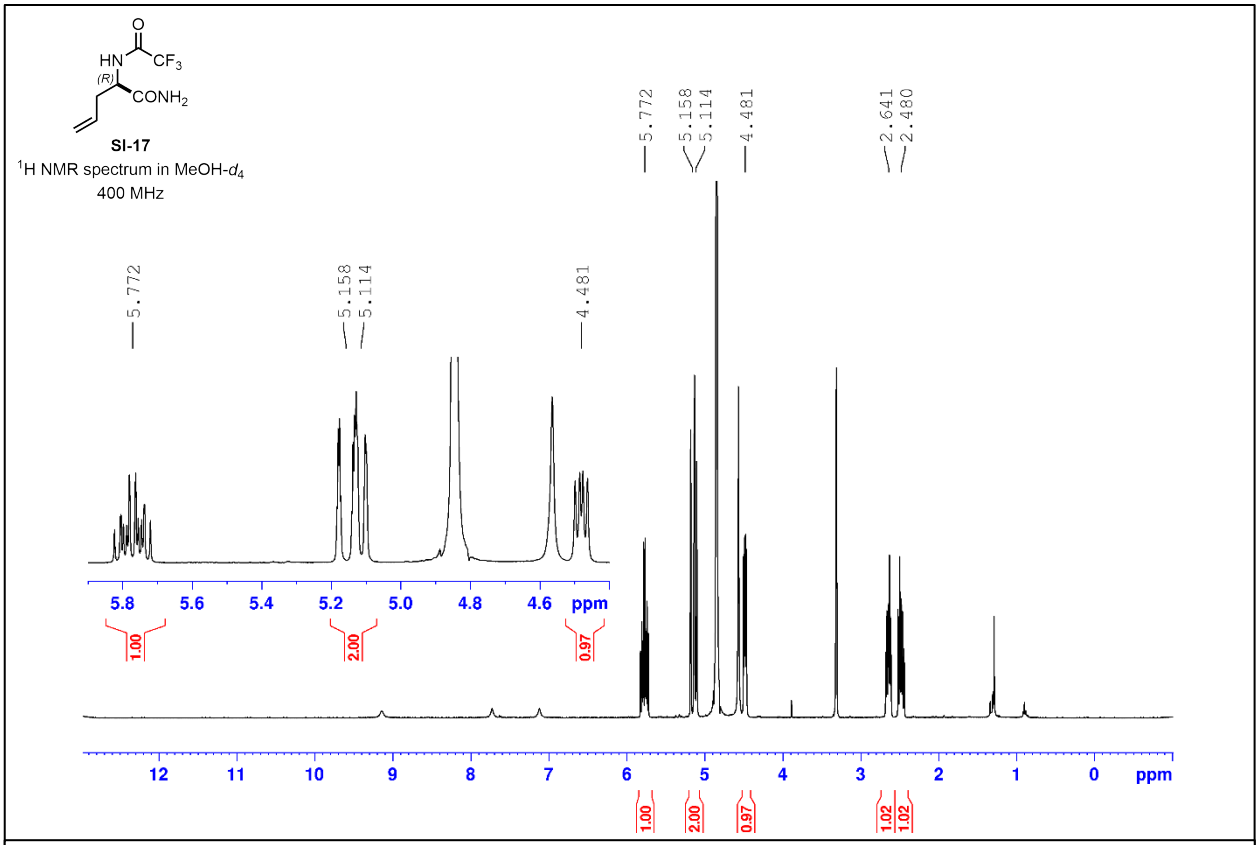
SI-24 (26.4 mg, 18.9 µmol, 1.0 eq) was stirred with 190 µL H_2O and 190 µL ACN in an ice bath. To this suspension was charged 1 M aqueous NaOH (190 µL, 190 µmol, 10 eq). A clear solution was obtained soon thereafter. After 1 hour the cooling bath was removed. After a further 3.5 hours, the pH was adjusted to ~3.5 by charged 95 µL 2 M aqueous TFA, and the mixture was concentrated. The residue was purified by preparative HPLC (0 to 40% ACN) to afford the intermediate benzyl ether as a single

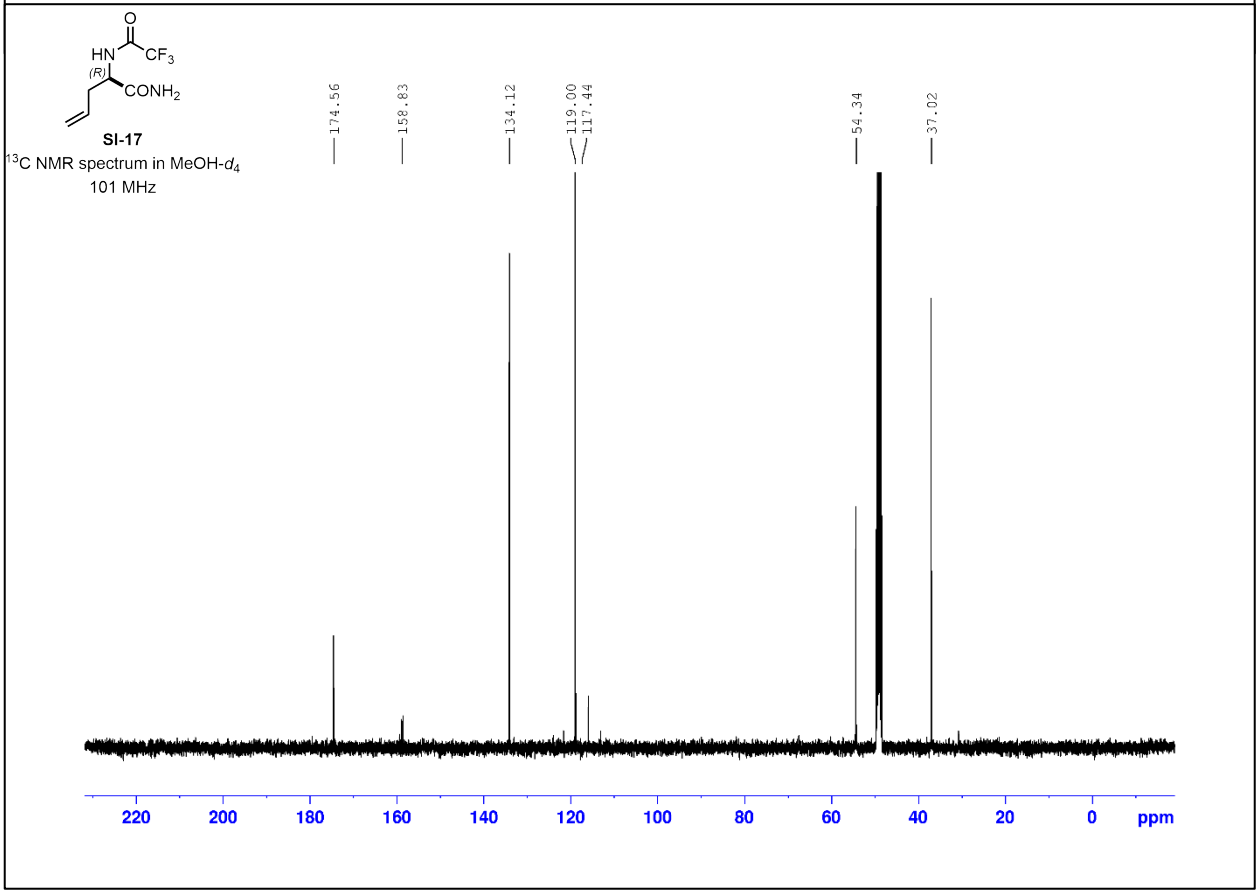
epimer at C-h. This material was stirred with 1.6 mL H_2O and 0.4 mL HOAc. 25 mg Pd/C and 25 mg $Pd(OH)_2/C$ were charged, and the suspension was vigorously stirred under an atmosphere of H_2 (balloon). After 5 hours, the mixture was filtered through a syringe filter and the filtrate was concentrated. The residue was purified by preparative HPLC (0 to 20% ACN) to afford **GM-AE(m-NH_2)A** (6.8 mg, 6.5 μmol, 34% yield over two steps) as a white solid.

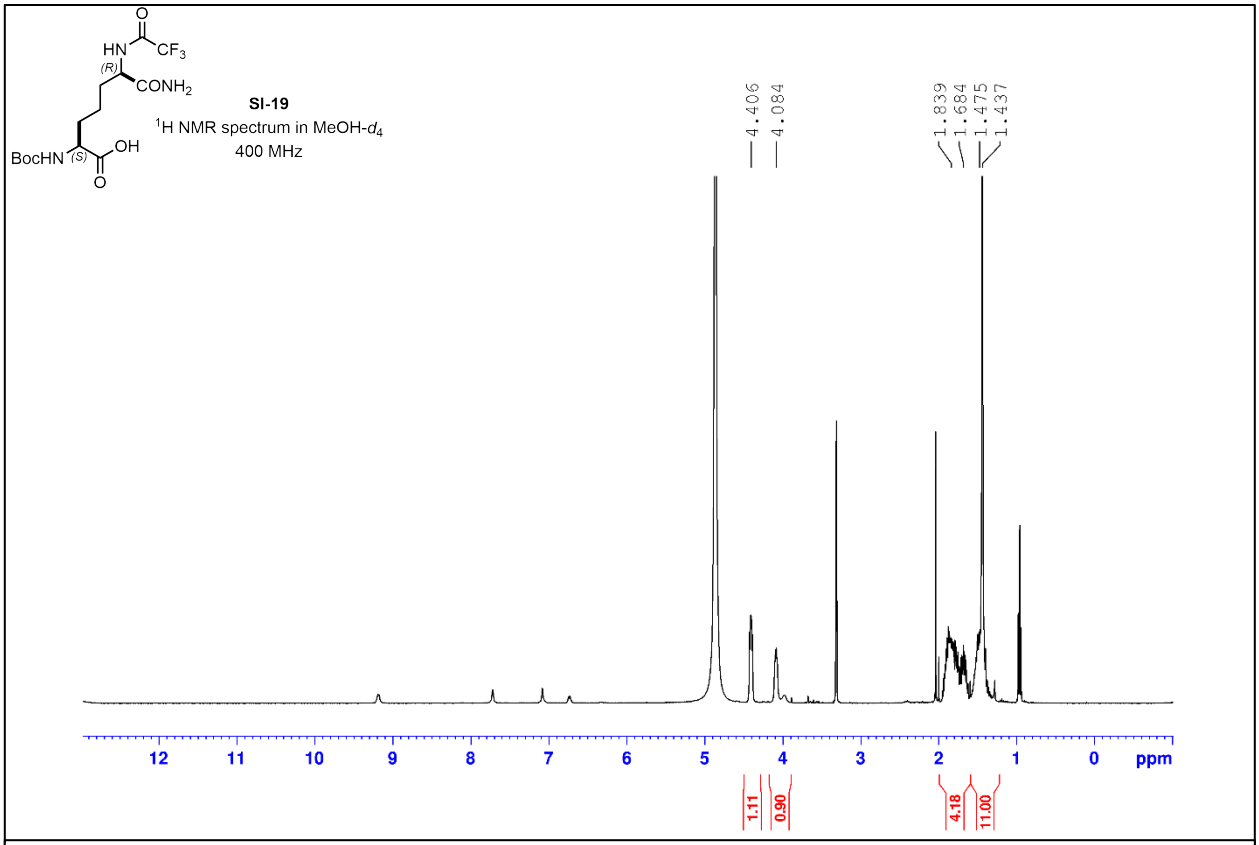
^{1}H NMR (400 MHz, D_2O, 301 K, δ): 5.25 (d, *J* = 3.2 Hz, 1H; H-1α), 4.62 (d, *J* = 8.3 Hz, 1H; H-1β), 4.58 (q, *J* = 6.7 Hz, 1H; H-aα), 4.53 (d, *J* = 8.2 Hz, 1H; H-1'α), 4.52 (d, *J* = 8.0 Hz, 1H; H-1'β), 4.43 (m, 1H; H-e), 4.42 (m, 1H; H-aβ), 4.37 (q, *J* = 7.1 Hz, 1H; H-m), 4.31 (q, *J* = 7.2 Hz, 1H; H-c), 4.30 (m, 1H; H-h), 4.01 (t, *J* = 6.5 Hz, 1H; H-l), 3.94 (d, *J* = 12.2 Hz, 1H; H-6'a), 3.87 (m, 1H; H-6aβ), 3.865 (m, 1H; H-4), 3.860 (m, 1H; H-5α), 3.82 (m, 1H; H-2α), 3.81 (m, 1H; H-6aα), 3.76 (m, 1H; H-6'b), 3.74 (m, 1H; H-2'), 3.72 (m, 1H; H-3α), 3.71 (m, 1H; H-6b), 3.57 (m, 1H; H-3β), 3.55 (m, 1H; H-3'), 3.46 (m, 1H; H-5β), 3.423 (m, 1H; H-5'), 3.417 (m, 1H; H-4'), 2.40 (m, 2H; H-g), 2.22 (m, 1H; H-f), 2.04 (s, 3H; acetamide), 2.01 (m, 1H; H-f), 1.97 & 1.95 (s & s, 3H; acetamide), 1.90 (m, 2H; H-k), 1.80 (m, 2H; H-i), 1.47 (m, 2H; H-j), 1.44 (d, *J* = 7.2 Hz, 3H; H-d), 1.41 (d, *J* = 7.1 Hz, 3H; H-n), 1.40 (d, *J* = 6.7 Hz, 3H; H-b).

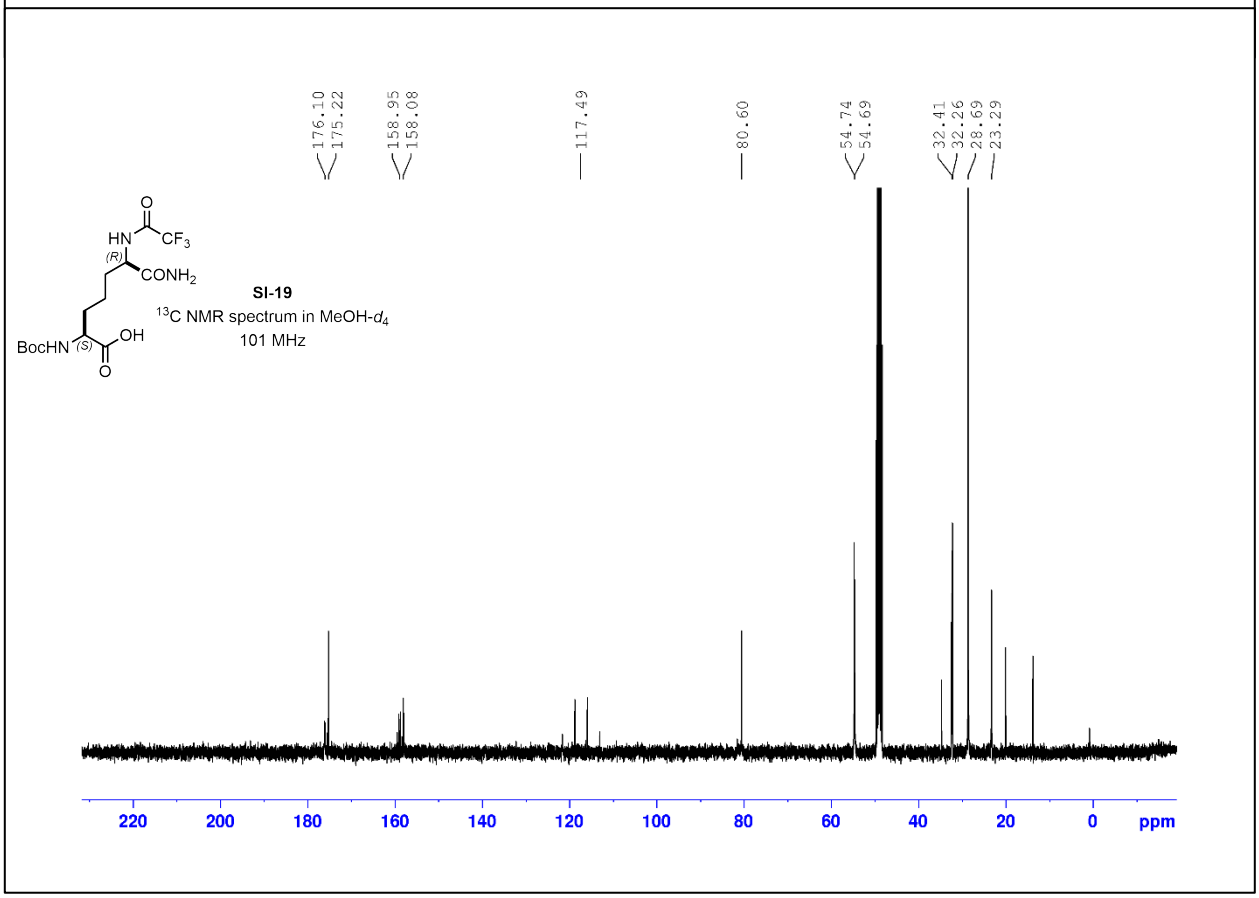
^{13}C NMR (101 MHz, D_2O, 301 K, δ): 176.8, 176.4, 175.7, 175.6, 175.52, 175.48, 175.4, 175.34, 175.32, 175.26, 175.2, 174.8, 174.29, 174.26, 172.5, 101.12 (C-1'α), 101.08 (C-1'β), 95.8 (C-1β), 90.9 (C-1α), 80.1 (C-3β), 78.7 (C-aβ), 78.2 (C-aα), 77.0 (C-3α), 76.8 (C-5'α), 76.7 (C-5'β), 76.1 (C-4α), 75.9 (C-5β), 75.7 (C-4β), 74.2 (C-3'), 71.7 (C-5α), 70.9 (C-4'), 61.8 (C-6'), 60.6 (C-6β), 60.4 (C-6α), 56.7 (C-2'α), 56.6 (C-2'β), 56.5 (C-2β), 54.4 (C-2α), 54.2 (C-h), 53.4 (C-l), 52.6 (C-eβ), 52.5 (C-eα), 50.6 (C-cα), 50.3 (C-cβ), 49.3 (C-m), 32.0 (C-g), 31.2 (C-i), 30.9 (C-k), 27.5 (C-f), 22.9, 22.8 & 22.7 (acetamide), 21.2 (C-j), 18.9 (C-bα), 18.7 (C-bβ), 17.5 (C-dβ), 17.4 (C-dα), 16.8 (C-n).

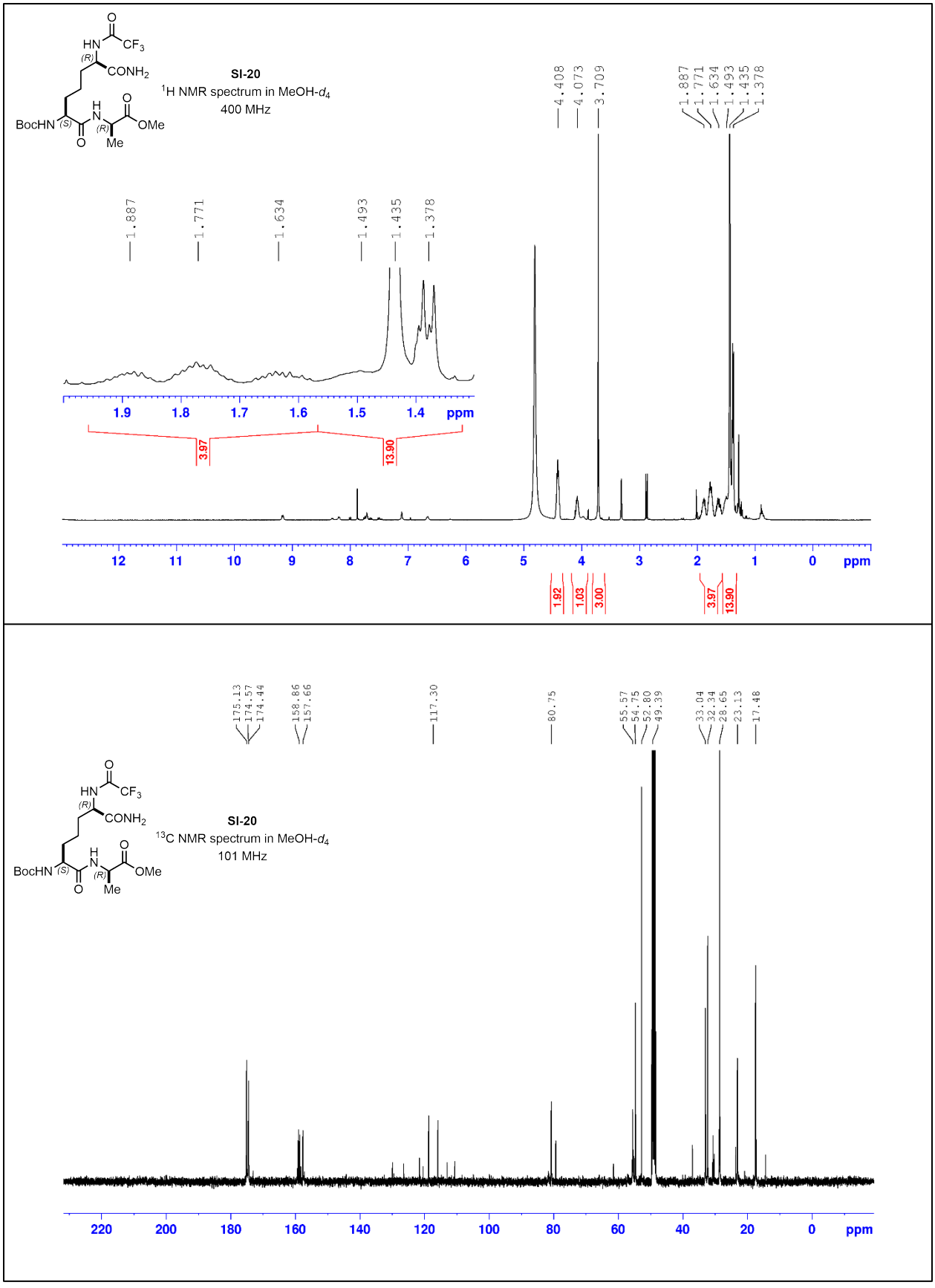
HRMS (ESI-TOF, m/z): calc'd for $C_{37}H_{63}N_8O_{20}^+$ ($[M + H]^+$) 939.4153; found 939.4141.

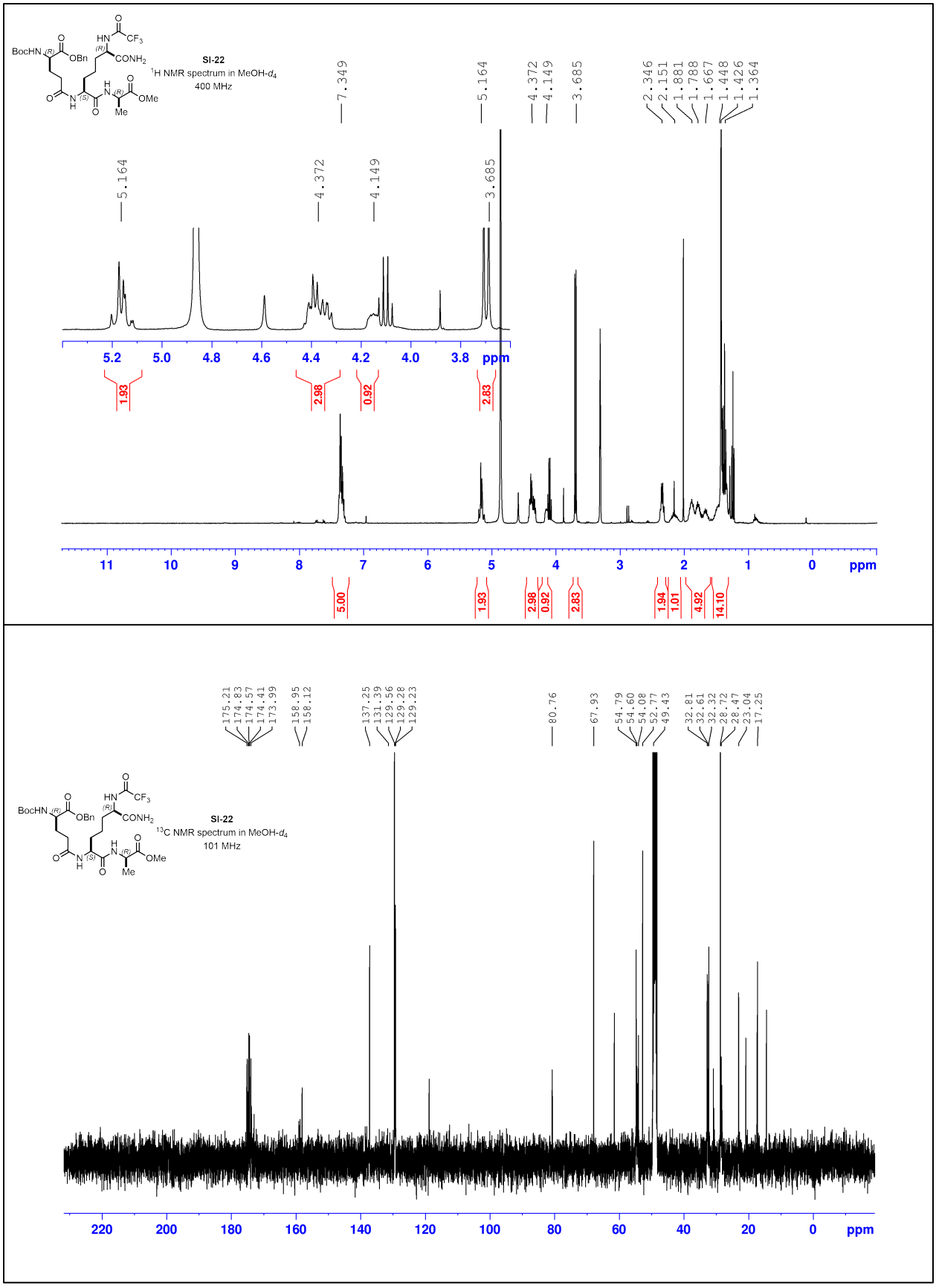

SI-17
^{1}H NMR spectrum in MeOH-d_4
400 MHz
5.772
5.158
5.114
4.481
2.641
2.480
5.8
5.6
5.4
5.2
5.0
4.8
4.6
ppm
1.00
2.00
0.97
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
2.00
0.97
1.02
1.02

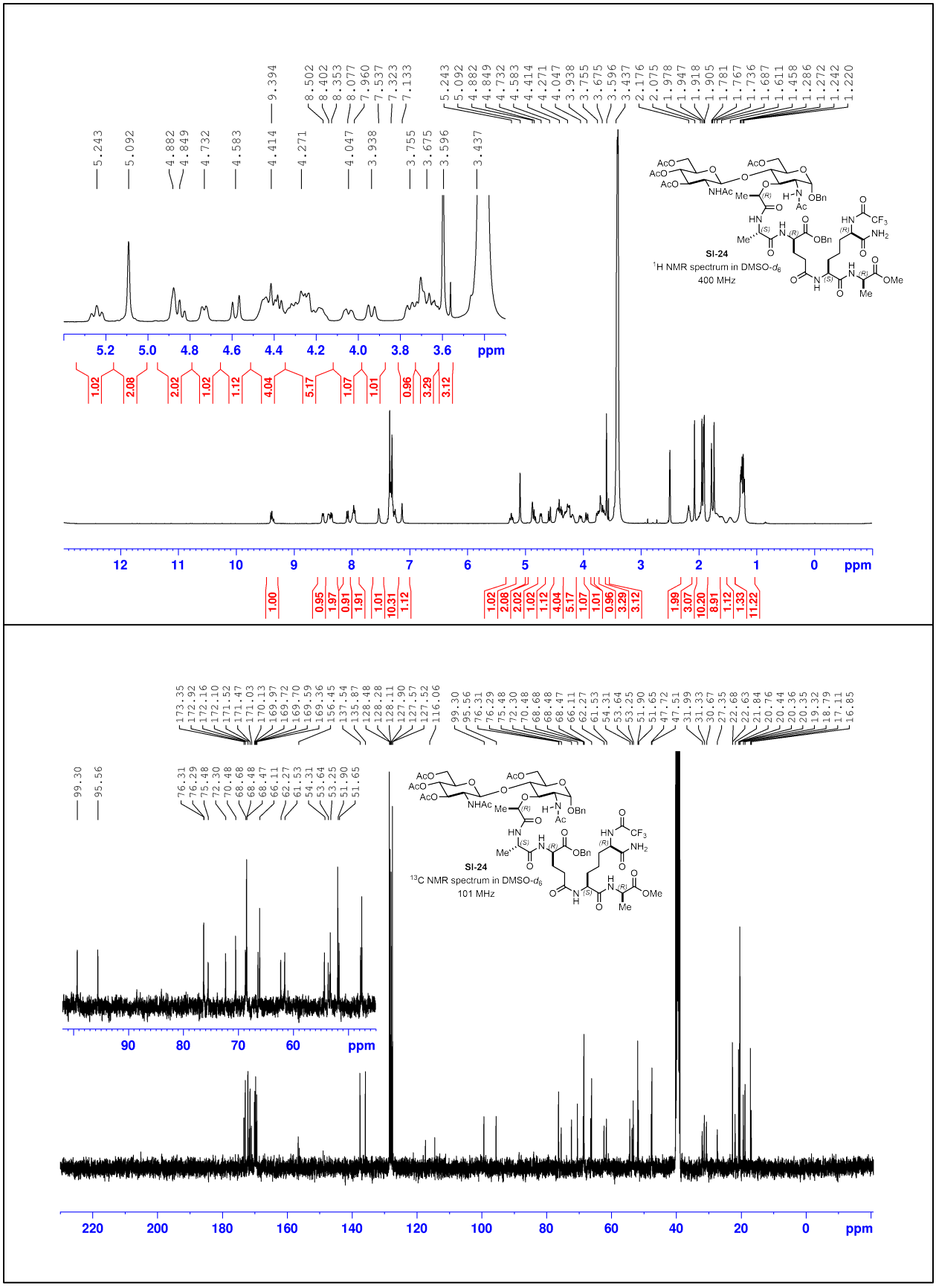

SI-17
^{13}C NMR spectrum in MeOH-d_4
101 MHz
174.56
158.83
134.12
119.00
117.44
54.34
37.02
220
200
180
160
140
120
100
80
60
40
20
0
ppm

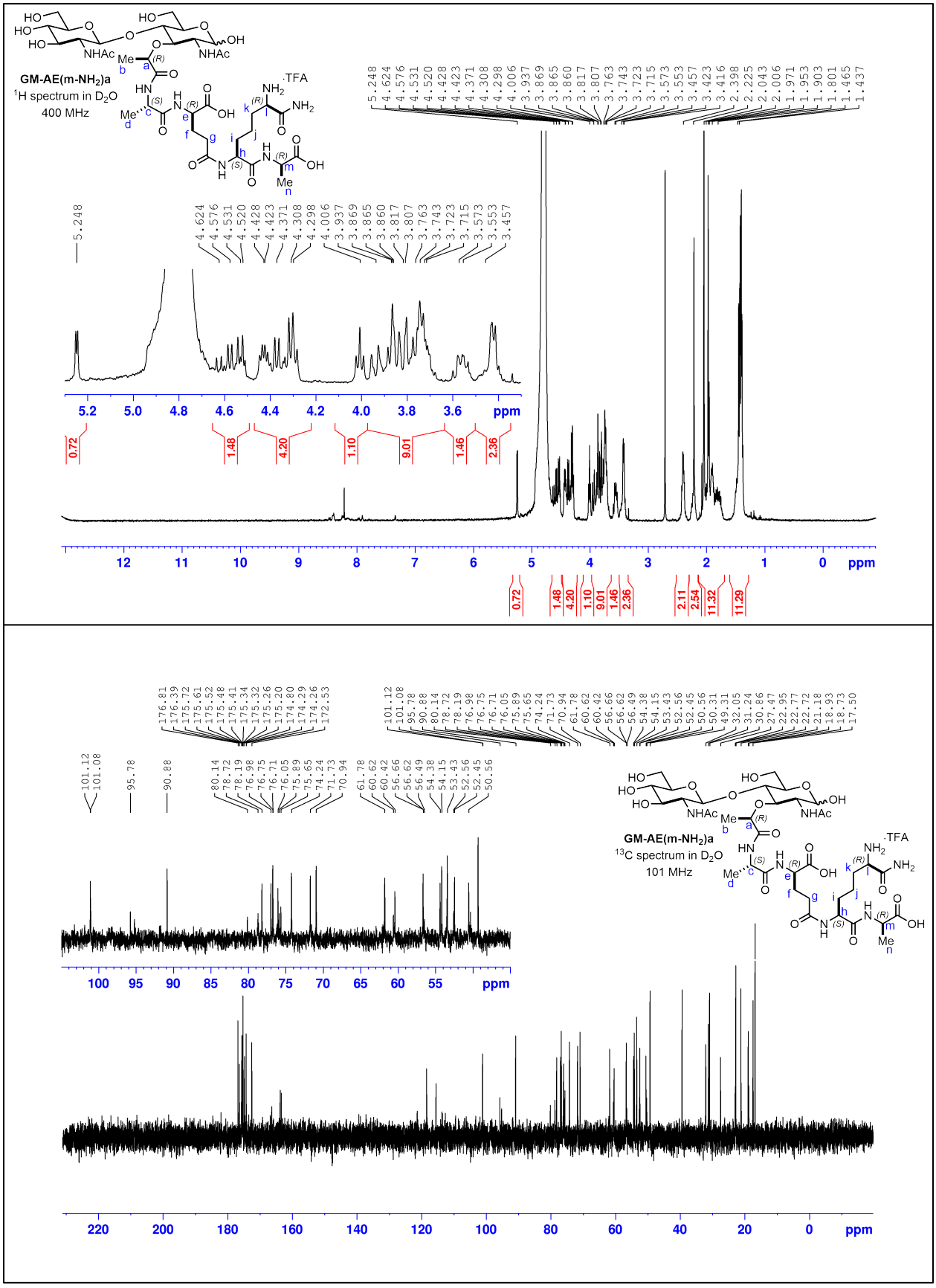

SI-19
^{1}H NMR spectrum in MeOH-d_4
400 MHz
4.406
4.084
1.839
1.684
1.475
1.437
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.11
0.90
4.18
11.00


SI-19
^{13}C NMR spectrum in MeOH-d_4
101 MHz
176.10
175.22
158.95
158.08
117.49
80.60
54.74
54.69
32.41
32.26
28.69
23.29
220
200
180
160
140
120
100
80
60
40
20
0
ppm

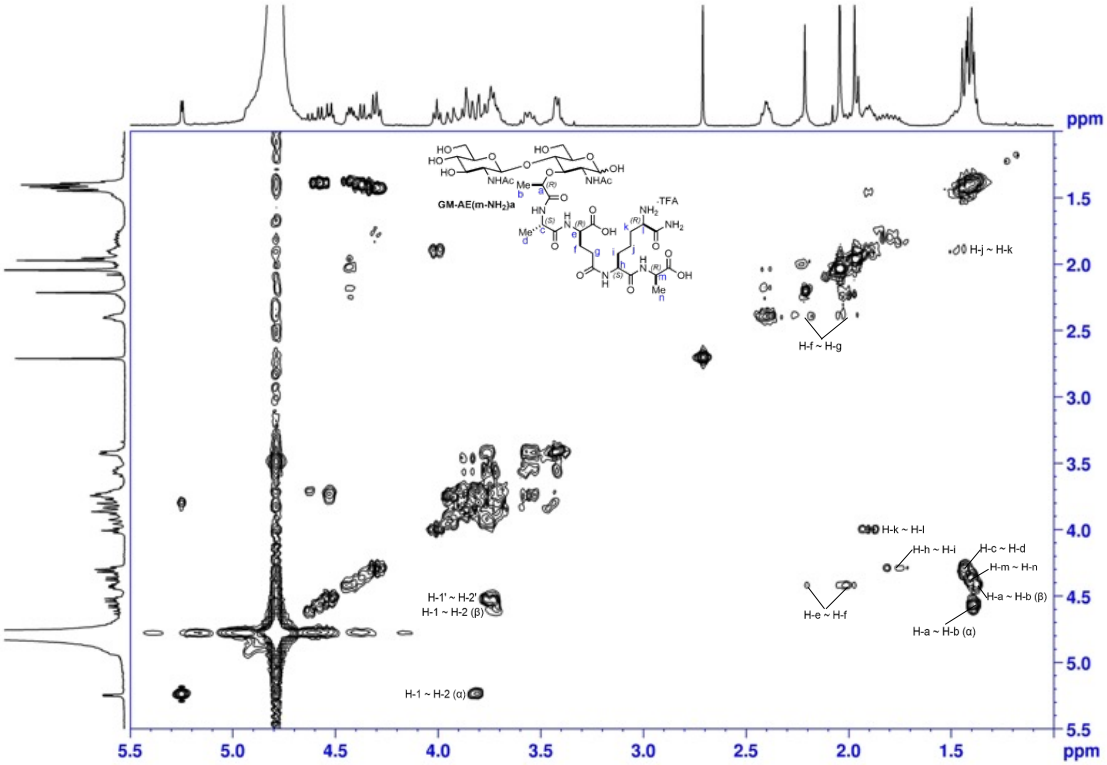

SI-20
^{1}H NMR spectrum in MeOH-d_4
400 MHz
4.408
4.073
3.709
1.887
1.771
1.634
1.493
1.435
1.378
1.9
1.8
1.7
1.6
1.5
1.4
ppm
3.97
13.90
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.92
1.03
3.00
3.97
13.90
SI-20
^{13}C NMR spectrum in MeOH-d_4
101 MHz
175.13
174.57
174.44
158.86
157.66
117.30
80.75
55.57
54.75
52.80
49.39
33.04
32.34
28.65
23.13
17.48
220
200
180
160
140
120
100
80
60
40
20
0
ppm


SI-22
^{1}H NMR spectrum in MeOH-d_4
400 MHz
7.349
5.164
4.372
4.149
3.685
2.346
2.151
1.881
1.788
1.667
1.448
1.426
1.364
ppm
SI-22
^{13}C NMR spectrum in MeOH-d_4
101 MHz
175.21
174.83
174.57
174.41
173.99
158.95
158.12
137.25
131.39
129.56
129.28
129.23
80.76
67.93
54.79
54.60
54.08
52.77
49.43
32.81
32.61
32.32
28.72
28.47
23.04
17.25
ppm

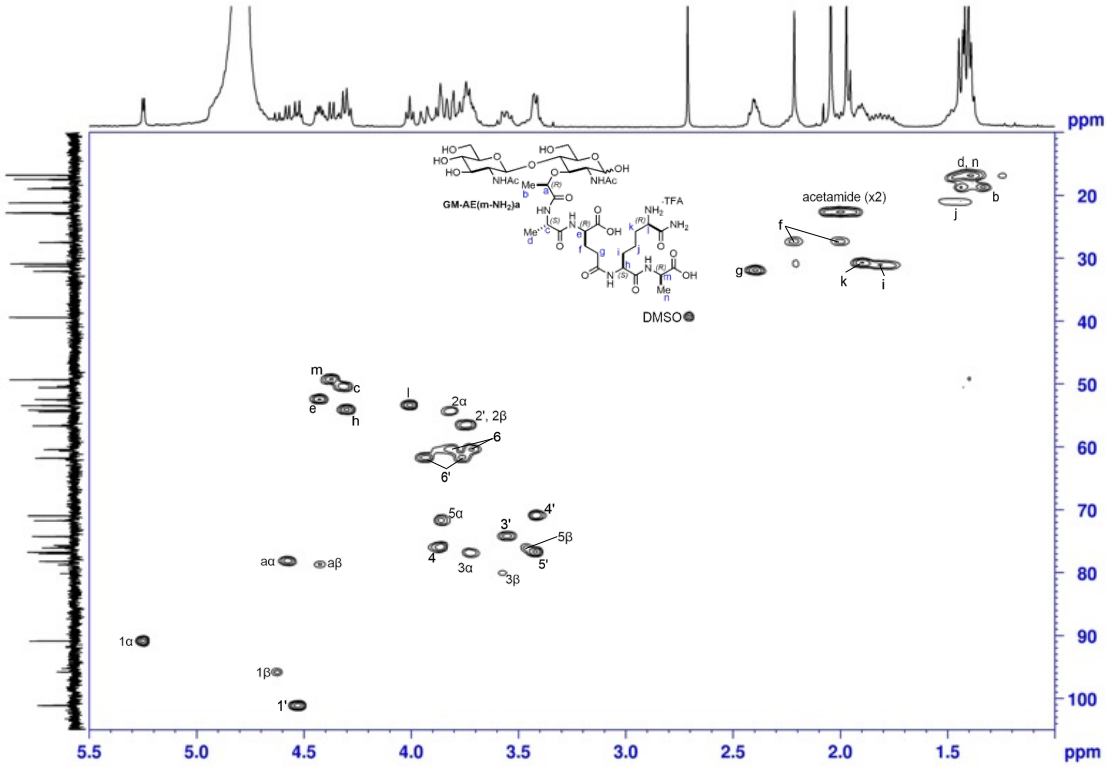

SI-24
^{1}H NMR spectrum in DMSO-d_6
400 MHz
SI-24
^{13}C NMR spectrum in DMSO-d_6
101 MHz


GM-AE(m-NH2)a
1H spectrum in D2O
400 MHz
.TFA
5.248
4.624
4.576
4.531
4.520
4.428
4.423
4.371
4.308
4.298
4.006
3.937
3.869
3.865
3.860
3.817
3.807
3.763
3.743
3.723
3.715
3.573
3.553
3.457
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
ppm
0.72
1.48
4.20
1.10
9.01
1.46
2.36
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.72
1.48
4.20
1.10
9.01
1.46
2.36
2.11
2.54
11.32
11.29
GM-AE(m-NH2)a
13C spectrum in D2O
101 MHz
.TFA
176.81
176.39
175.72
175.61
175.52
175.48
175.41
175.34
175.32
175.26
175.20
174.80
174.29
174.26
172.53
101.12
101.08
95.78
90.88
80.14
78.72
78.19
76.98
76.75
76.71
76.05
75.89
75.65
74.24
71.73
70.94
61.78
60.62
60.42
56.66
56.62
56.49
54.38
54.15
53.43
52.56
52.45
50.56
50.31
49.31
32.05
31.24
30.86
27.47
22.95
22.77
22.72
21.18
18.93
18.73
17.50
100
95
90
85
80
75
70
65
60
55
ppm
220
200
180
160
140
120
100
80
60
40
20
0
ppm

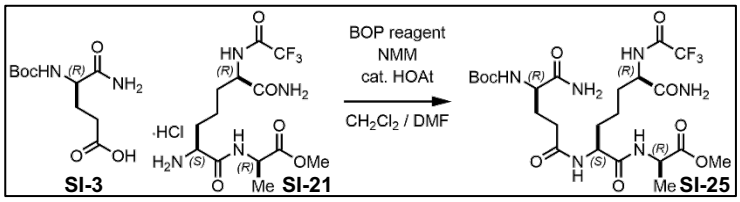
COSY of **GM-AE(m-NH$_2$)A**



HSQC of **GM-AE(m-NH₂)A**



Synthesis of GM-AQ(m-NH$_2$)A (i.e. GM-AQ(nm)A)

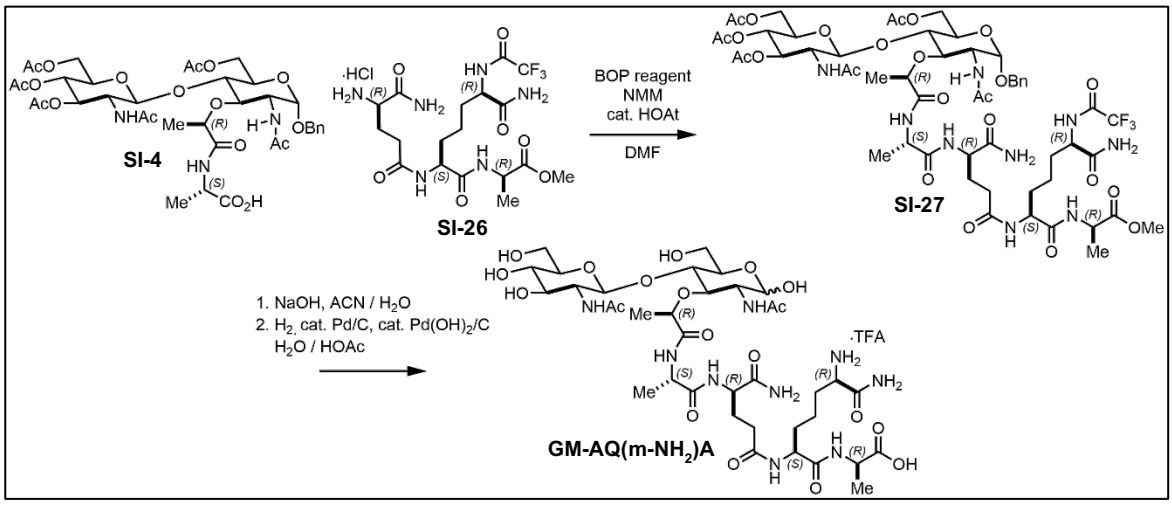


To a stirred solution of **SI-21** (60 mg, 147 µmol, 1.0 eq) in 3 mL DMF was charged **SI-3** (40 mg, 162 µmol, 1.1 eq), *N*-methylmorpholine (40 µL, 368 µmol, 2.5 eq), HOAt (5 mg, 37 µmol, 0.25 eq) and finally BOP reagent (78 mg, 176 µmol, 1.2 eq). After 14 hours, the mixture was concentrated to dryness and co-evaporated several times with *i*-PrOH. The residue was diluted with EtOAc and 2% aqueous K_2CO_3. The product was partitioned almost entirely into the aqueous layer and could not be significantly back-extracted into chloroform. Thus, the aqueous material was adsorbed onto 2.5 g silica gel and purified by column chromatography (30 g silica, 10 to 25% *i*-PrOH in EtOAc) to afford **SI-25** (41 mg, 69 µmol, 47% yield) as a semisolid.

^{1}H NMR (400 MHz, MeOH-d_4, 301 K, δ): 4.39 (m, 3H), 4.05 (m, 1H), 3.70 (s, 3H), 2.36 (m, 2H), 2.08 (m, 1H), 1.84 (m, 4H), 1.71 (m, 1H), 1.49 (m 2H), 1.44 (s, 9H), 1.38 (d, J = 7.3 Hz, 1H).

^{13}C NMR (101 MHz, MeOH-d_4, 301 K, δ): 177.2, 175.2, 174.6, 173.9, 173.7, 158.9 (q, J = 37.0 Hz), 157.8, 131.1 (q, J = 262.5), 80.9, 54.9, 54.8, 54.4, 52.9, 49.5, 32.8, 32.4, 32.2, 29.5, 28.7, 23.0, 17.3.

HRMS (ESI-TOF, m/z): calc'd for $C_{23}H_{38}F_3N_9O_9^+$ ([M + H]$^+$) 599.2647; found 599.2642.



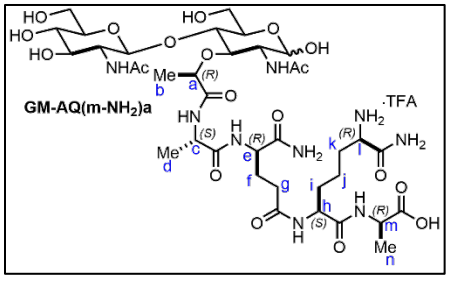
SI-25 (41 mg, 69 µmol, 1.0 eq) was stirred with 1 mL dioxane and then 0.5 mL 4 M HCl in dioxane was charged. After 5 hours, the mixture was concentrated to dryness, and the solid residue was triturated with 2 mL Et_2O (x2) to afford hydrochloride salt **SI-26**. This material was stirred with 1.4 mL DMF and **SI-4** (59 mg, 71 µmol, 1.05 eq), *N*-methylmorpholine (19 µL, 170 µmol, 2.5 eq), HOAt (2.3 mg, 17 µmol, 0.25 eq) and finally BOP reagent (38 mg, 85 µmol, 1.25 eq) were charged. After 15 hours, the mixture was concentrated to dryness, and the crude residue was triturated with 1 mL CH_2Cl_2 to afford **SI-27** (52 mg, 39.8 µmol, 59% yield) as a faintly pink solid.

^{1}H NMR (400 MHz, DMSO-d_6, 301 K, δ): 9.40 (d, J = 7.4 Hz, 1H), 8.39 (d, J = 5.6 Hz, 1H), 8.35 (d, J = 7.2 Hz, 1H), 8.25 (d, J = 7.7 Hz, 1H), 8.08 (d, J = 8.2 Hz, 1H), 7.96 (m, 2H), 7.54 (br s, 1H), 7.32 (m, 6H), 7.13 (br s, 1H), 7.03 (br s, 1H), 5.26 (t, J = 9.7 Hz, 1H), 4.89 (d, J = 9.8 Hz, 1H), 4.87 (d, J = 9.8 Hz, 1H), 4.74 (d, J = 7.9 Hz, 1H), 4.60 (d, J = 12.5 Hz, 1H), 4.39 (m, 4H), 4.21 (m, 5H), 4.03 (dd, J = 11.5, 3.4 Hz, 1H), 3.96 (d, J = 11.8 Hz, 1H), 3.77 (d, J = 9.3 Hz, 1H), 3.67 (m, 3H), 3.61 (s, 3H), 3.43 (m, 1H), 2.13 (m, 2H), 2.07 (s, 3H), 1.954 (s, 3H), 1.950 (m, 1H), 1.945 (s, 3H), 1.91 (s, 3H), 1.80 (s, 3H), 1.74 (s, 3H), 1.71 (m, 2H), 1.69 (m, 1H), 1.61 (m, 1H), 1.47 (m, 1H), 1.274 (m, 2H), 1.266 (d, J = 7.0 Hz, 3H), 1.25 (d, J = 7.5 Hz, 3H), 1.22 (d, J = 6.8 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6, 301 K, δ): 173.4, 173.2, 172.9, 172.1, 172.0, 171.5, 171.3, 170.2, 170.1, 169.72, 169.65, 169.61, 169.4, 156.4 (q, J = 36.2 Hz), 137.5, 128.3, 127.6, 127.5, 116.1 (q, J = 287.5 Hz), 99.3, 95.6, 76.4, 76.1, 75.4, 72.2, 70.3, 68.7, 68.49, 68.46, 62.2, 61.7, 54.3, 53.6, 53.2, 52.2, 51.9, 48.1, 47.5, 31.9, 31.8, 30.7, 28.0, 22.66, 22.65, 21.9, 20.8, 20.44, 20.40, 20.3, 19.0, 18.6, 17.1 16.8.

HRMS (ESI-TOF, m/z): calc'd for $C_{55}H_{79}F_3N_9O_{24}^+$ ($[M + H]^+$) 1306.5185; found 1306.5182.

SI-27 (19 mg, 15.3 µmol, 1.0 eq) was stirred with 150 µL H_2O and 150 µL ACN and cooled in an ice bath. To this suspension was charged 1 M aqueous NaOH (153 µL, 153 µmol, 10.0 eq), and the cooling bath was removed. A clear solution was observed soon thereafter. After 2 hours, the pH was adjusted to ~4 by charging 73 µL 2 M aqueous TFA, and the mixture was concentrated. The crude material was purified by preparative HPLC (0 to 35% ACN) to afford the intermediate benzyl ether as a single epimer at C-h. This material was taken up in 1.6 mL H_2O and 0.4 mL HOAc and 25 mg Pd/C and 25 mg $Pd(OH)_2$/C were charged. This suspension stirred vigorously under an atmosphere of H_2 (balloon). After 3 hours, the mixture was filtered through a syringe filter and concentrated. The crude material was purified by preparative HPLC (0 to 20% ACN) to afford **GM-AQ(m-NH$_2$)A** (3.2 mg, 3.0 µmol, 20% yield over 2 steps) as a white solid.

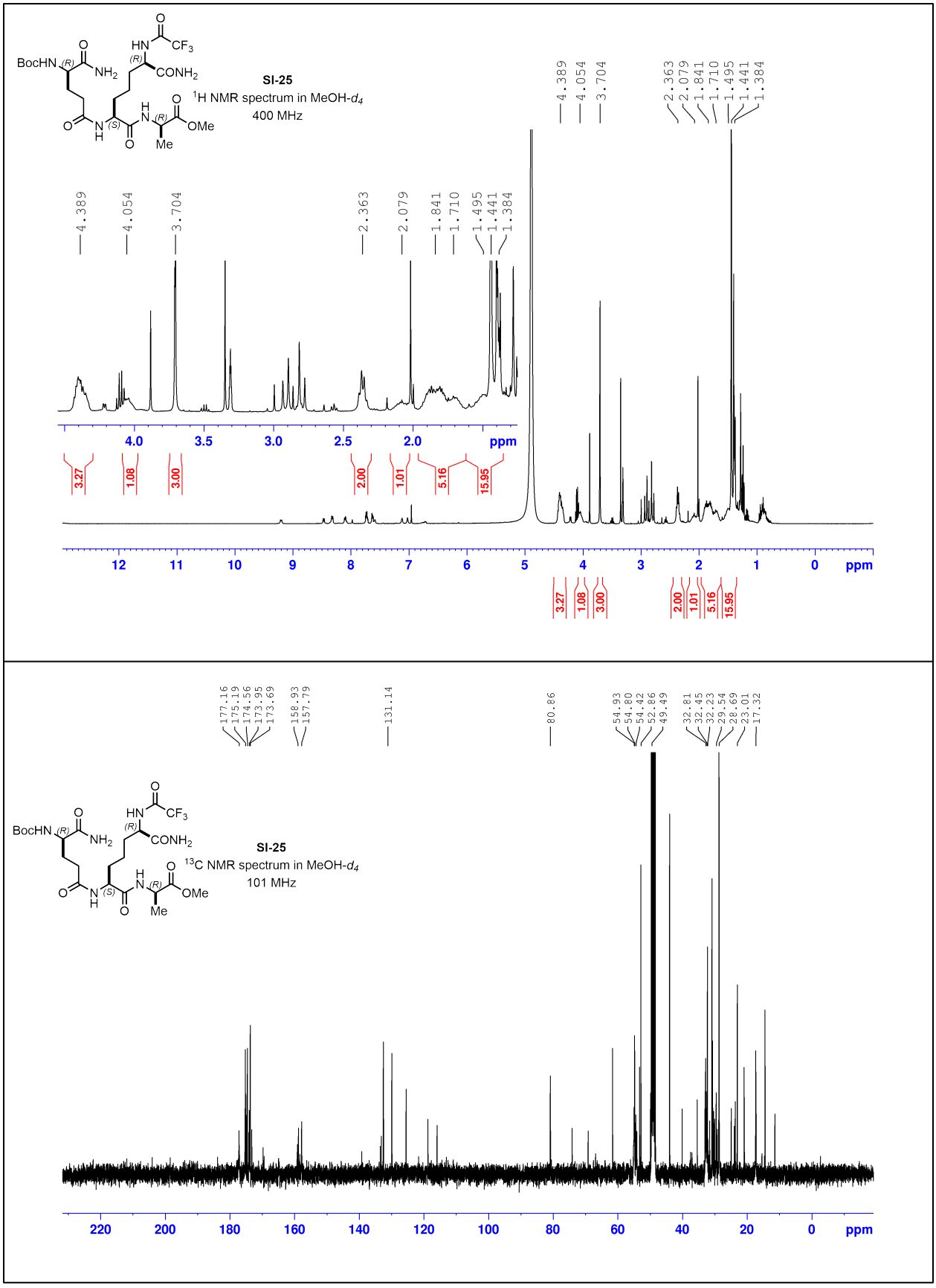


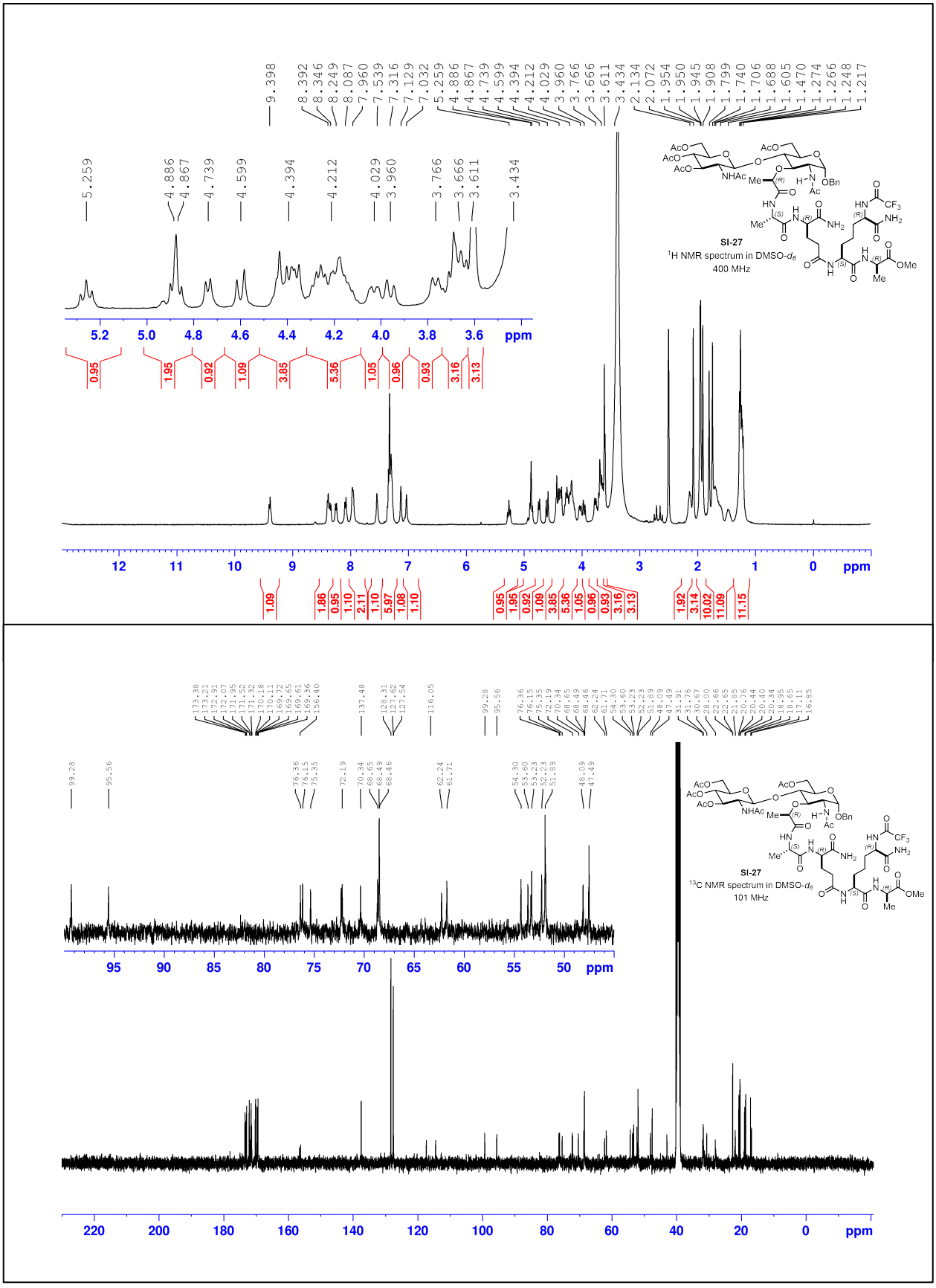
^{1}H NMR (400 MHz, D_2O, 301 K, δ): 5.24 (d, J = 3.3 Hz, 1H; H-1α), 4.63 (d, J = 8.3 Hz, 1H; H-aβ), 4.56 (q, J = 7.0 Hz, 1H; H-aα), 4.53 (d, J = 8.3 Hz, 1H; H-1'α), 4.52 (d, J = 8.3 Hz, 1H; H-1'β), 4.41 (q, J = 7.0 Hz, 1H; H-aβ), 4.37 (m, 1H; H-m), 4.35 (m, 1H; H-e), 4.301 (m, 1H; H-h), 4.295 (m, 1H; H-c), 4.01 (t, J = 6.5 Hz, 1H; H-l), 3.94 (d, J = 12.1 Hz, 1H; H-6'a), 3.87 (m, 1H; H-4), 3.86 (m, 1H; H-5α), 3.83 (m, 1H; H-2α), 3.81 (m, 1H; H-6a), 3.77 (m, 1H; H-6'b), 3.744 (m, 1H; H-2'), 3.740 (m, 1H; H-3α), 3.72 (m, 1H; H-6b), 3.59 (m, 1H; H-3β), 3.56 (m, 1H; H-3'), 3.47 (m, 1H; H-5β), 3.43 (m, 1H; H-5'), 3.42 (m, 1H; H-4'), 2.40 (m, 2H; H-g), 2.17 (m, 1H; H-f), 2.04 (s, 3H; acetamide), 1.98 (m, 1H; H-f), 1.97 & 1.95 (s, 3H & s, 3H; acetamide), 1.91 (m, 2H; H-k), 1.81 (m, 2H; H-i), 1.47 (m, 2H; H-j), 1.44 (t, J = 7.2 Hz, 3H, H-d), 1.41 (t, J = 7.1 Hz, 3H; H-n), 1.40 (t, J = 7.0 Hz, 3H; H-b).

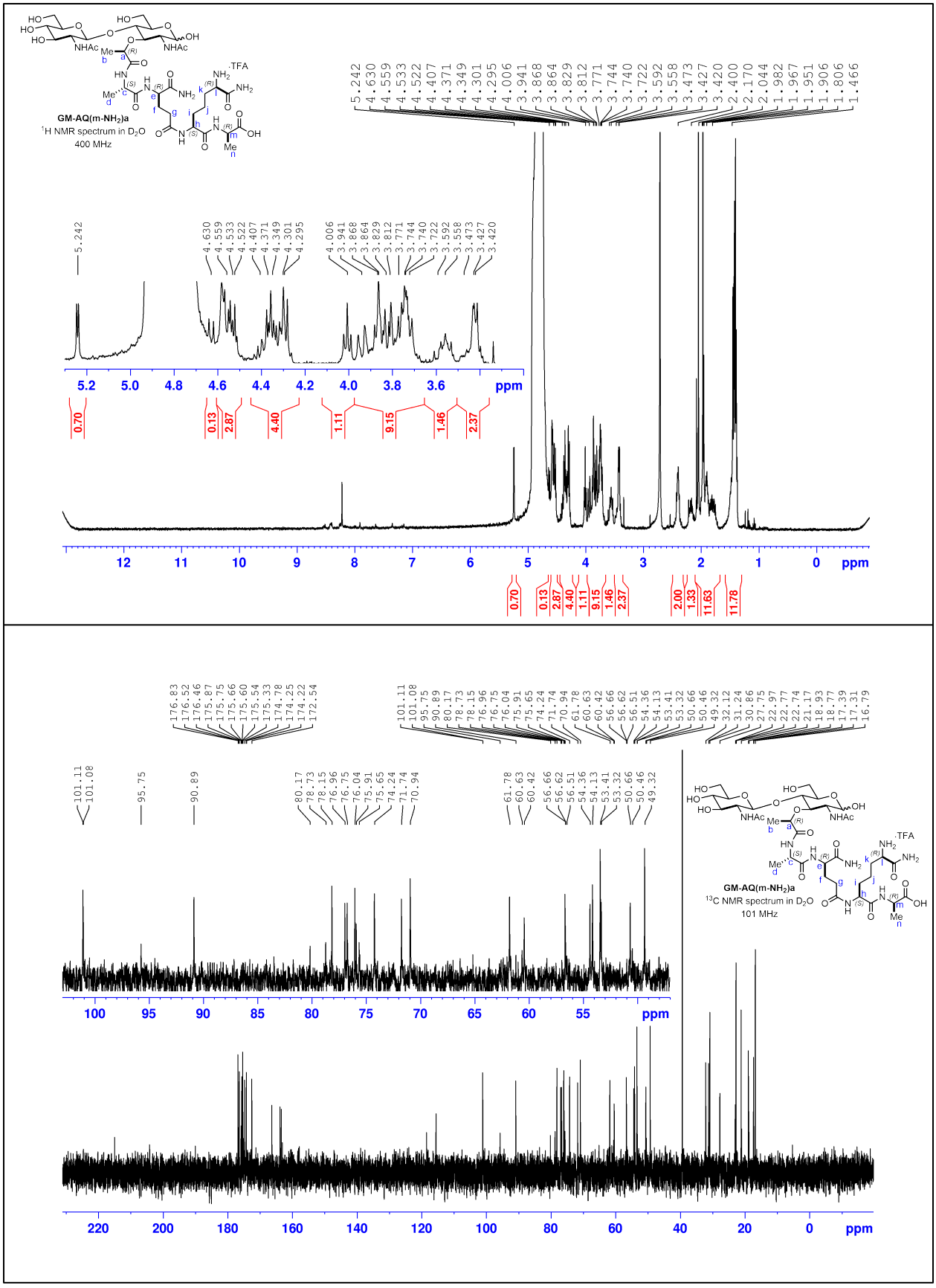
^{13}C NMR (101 MHz, D_2O, 301 K, δ): 176.8, 176.52, 176.46, 175.9, 175.75, 175.66, 175.6, 175.5, 175.3, 174.8, 174.25, 174.22, 172.5, 101.11 (C-1'α), 101.08 (C-1'β), 95.8 (C-1β), 90.9 (C-1α), 80.2 (C-3β), 78.7

(C-aβ), 78.2 (C-aα), 77.0 (C-3α), 76.7 (C-5'), 76.0 (C-4α), 75.9 (C-5β), 75.65 (C-4β), 74.2 (C-3'), 71.7 (C-5α), 70.9 (C-4'), 61.8 (C-6'), 60.6 (C-6β), 60.4 (C-6α), 56.7 (C-2'α), 56.6 (C-2'β), 56.5 (C-2β), 54.4 (C-2α), 54.1 (C-h), 53.4 (C-l), 53.3 (C-e), 50.7 (C-cα), 50.5 (C-cβ), 49.3 (C-m), 32.1 (C-g), 31.2 (C-i), 30.9 (C-k), 27.7 (C-f), 23.0, 22.8 & 22.7 (acetamide), 21.2 (C-j), 18.9 (C-bα), 18.8 (C-bβ), 17.4 (C-dα), 17.3 (C-dβ), 16.8 (C-n).

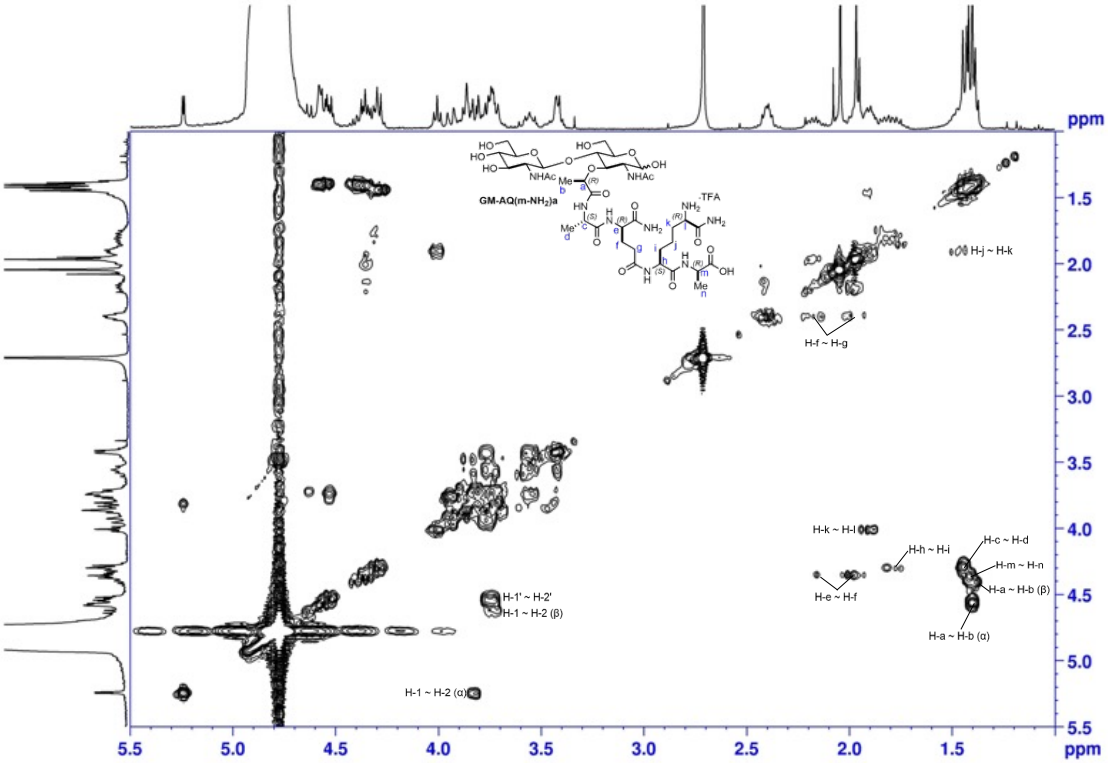
HRMS (ESI-TOF, m/z): calc'd for $C_{37}H_{64}N_9O_{19}^+$ ($[M + H]^+$) 938.4313; found 938.4306.


SI-25
^{1}H NMR spectrum in MeOH-d_4
400 MHz
4.389
4.054
3.704
2.363
2.079
1.841
1.710
1.495
1.441
1.384
ppm
3.27
1.08
3.00
2.00
1.01
5.16
15.95
SI-25
^{13}C NMR spectrum in MeOH-d_4
101 MHz
177.16
175.19
174.56
173.95
173.69
158.93
157.79
131.14
80.86
54.93
54.80
54.42
52.86
49.49
32.81
32.45
32.23
29.54
28.69
23.01
17.32
ppm

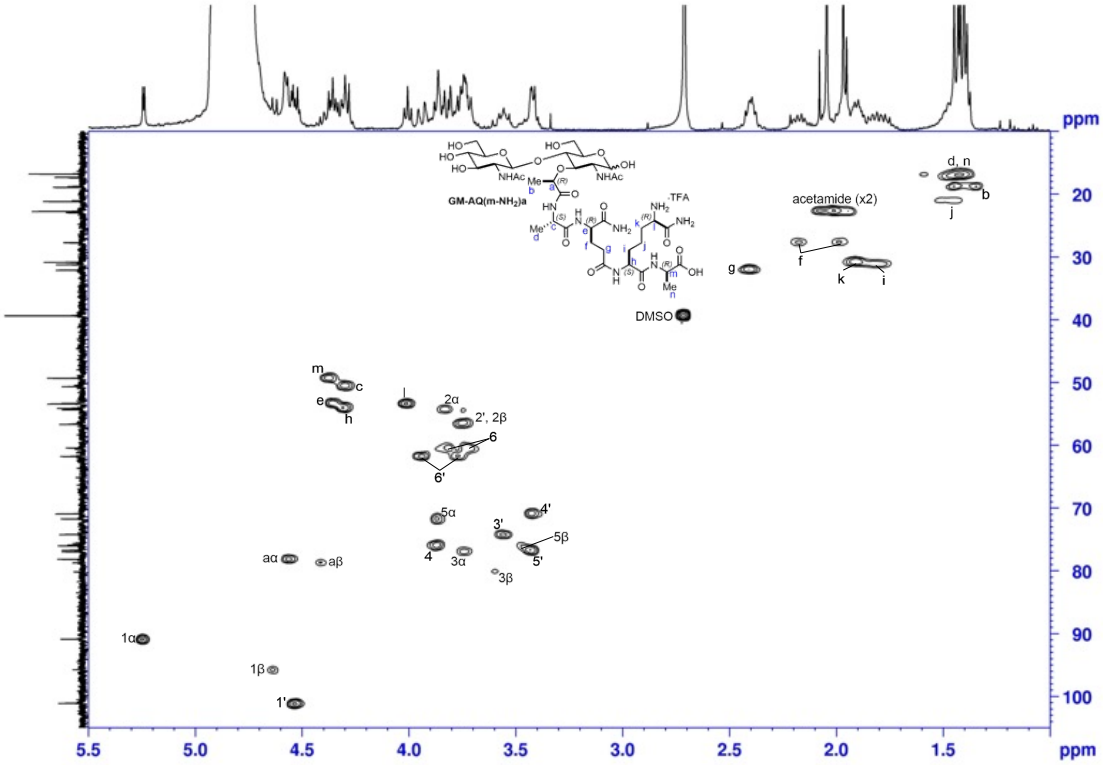

SI-27
^{1}H NMR spectrum in DMSO-d_6
400 MHz
SI-27
^{13}C NMR spectrum in DMSO-d_6
101 MHz


GM-AQ(m-NH2)a
1H NMR spectrum in D2O
400 MHz
GM-AQ(m-NH2)a
13C NMR spectrum in D2O
101 MHz
ppm

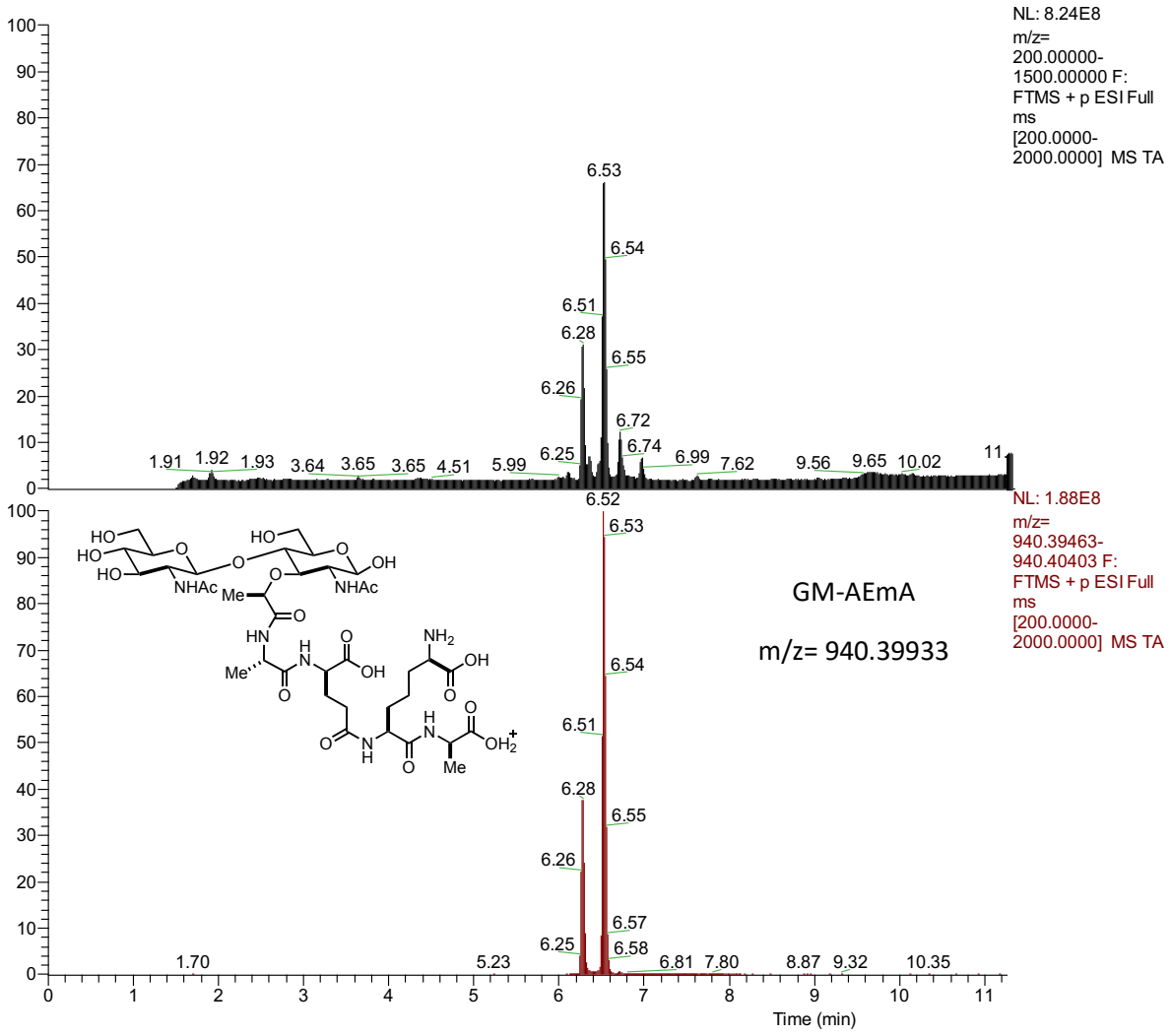
COSY of **GM-AQ(m-NH_2)A**

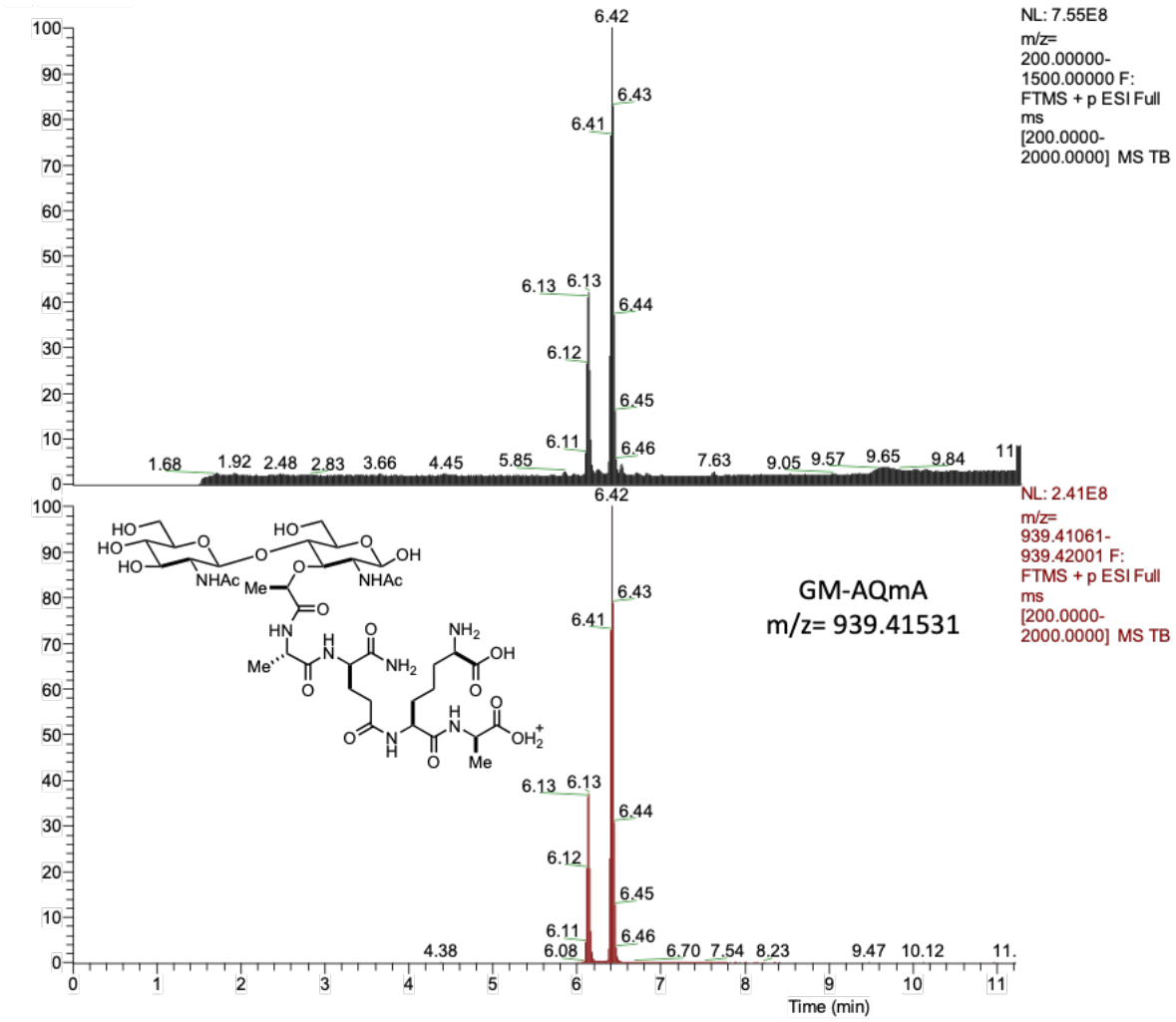


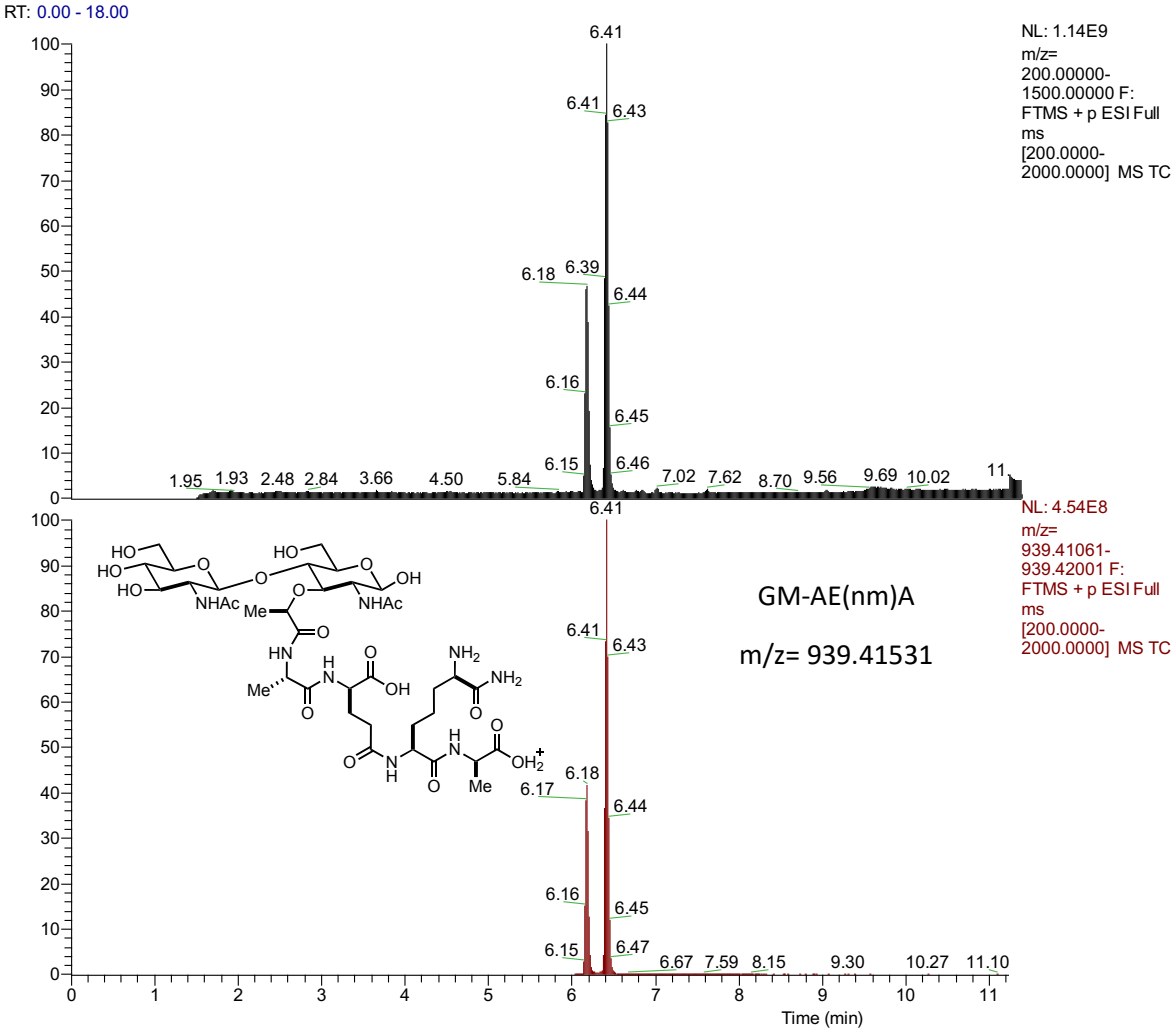
HSQC of **GM-AQ(m-NH$_2$)A**

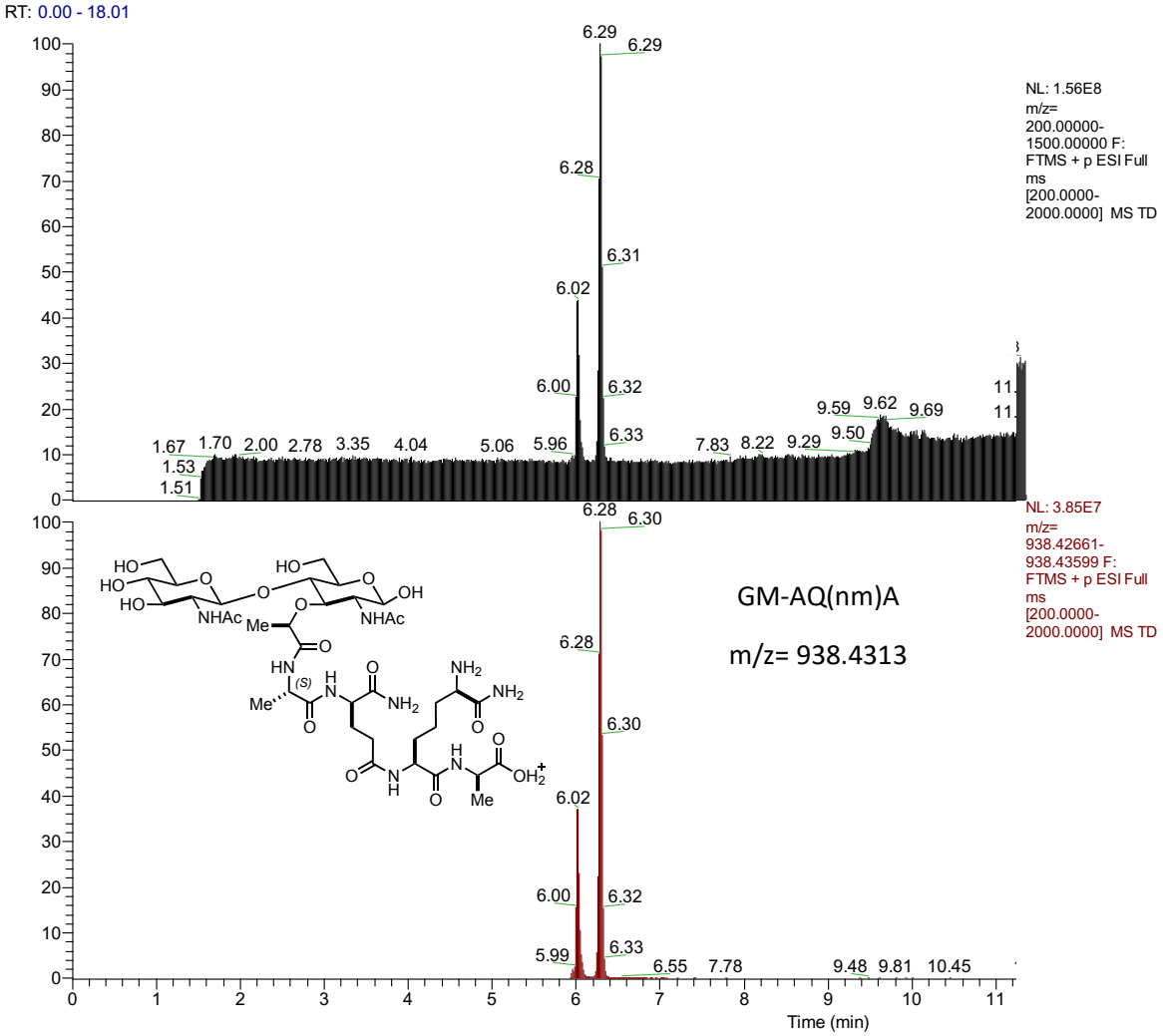


HPLC-MS spectra of disaccharide tetrapeptides synthesized in this study




NL: 7.55E8
m/z= 200.00000-1500.00000 F: FTMS + p ESI Full ms [200.0000-2000.0000] MS TB
NL: 2.41E8
m/z= 939.41061-939.42001 F: FTMS + p ESI Full ms [200.0000-2000.0000] MS TB
GM-AQmA
m/z= 939.41531
Time (min)


RT: 0.00 - 18.00
NL: 1.14E9
m/z= 200.00000-1500.00000 F: FTMS + p ESI Full ms [200.0000-2000.0000] MS TC
NL: 4.54E8
m/z= 939.41061-939.42001 F: FTMS + p ESI Full ms [200.0000-2000.0000] MS TC
GM-AE(nm)A
m/z= 939.41531
Time (min)


RT: 0.00 - 18.01
NL: 1.56E8
m/z=
200.00000-
1500.00000 F:
FTMS + p ESI Full
ms
[200.0000-
2000.0000] MS TD
NL: 3.85E7
m/z=
938.42661-
938.43599 F:
FTMS + p ESI Full
ms
[200.0000-
2000.0000] MS TD
GM-AQ(nm)A
m/z= 938.4313
Time (min)

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