Supporting Information

Lipidated peptidomimetics with improved antimicrobial activity

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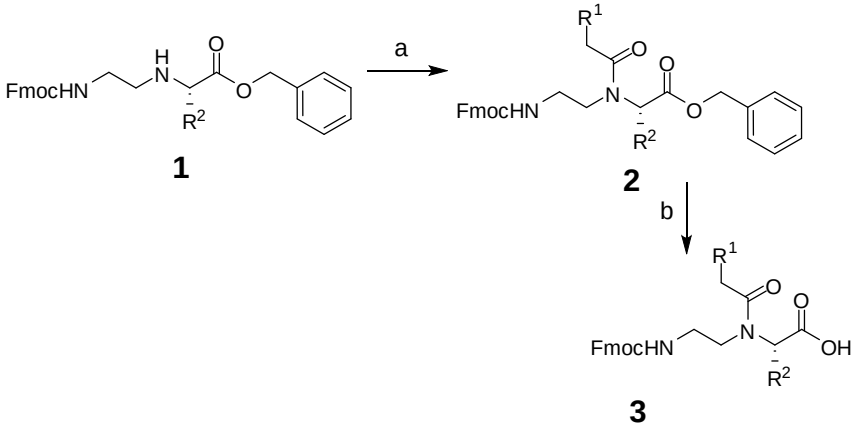
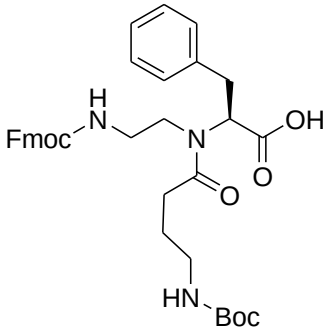
1. General experimental methods. α-amino acid esters and Rink amide resin (0.66 mmol/g, 200-400 mesh) were provided by Chem-Impex International, Inc. All other reagents and solvents were purchased from either Sigma-Aldrich or Fisher Scientific. The α-AApeptide building blocks were synthesized following previously reported procedure. [1-3] Lipidated α-AApeptides were prepared on a Rink amide resin in peptide synthesis vessels on a Burrell Wrist-Action shaker using α-AApeptide building blocks. The α-AApeptides were analyzed and purified on an analytical and a preparative Waters HPLC system, respectively, and then dried on a Labcono lyophilizer. Molecular weights of the lipidated α-AApeptides were identified on a Bruker AutoFlex MALDI-TOF mass spectrometer.

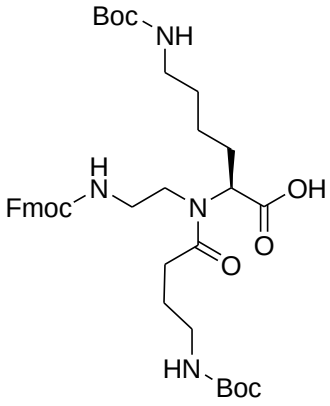
2. Synthesis of α-AApeptide building blocks [1-3]



Scheme S1. Synthesis of α-AApeptide building blocks. a) R_1CH_2COOH, DhBtOH/DIC, overnight. b) Pd/C, H_2, EtOAc, overnight. Please see ref [1-3] for detailed experimental procedure.



3a. 61% for two steps. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ = 7.84(m, 2H), 7.65-7.58(m, 2H), 7.36(m, 2H), 7.27(m, 2H),7.22(m, 2H), 7.16-7.10(m, 2H), 7.01(m, 1H), 6.77-6.72(s, 1H), 4.25-4.14(m, 4H), 3.16-3.06(m, 3H), 2.88-2.78(m, 4H), 2.57(m, 1H), 2.16(m, 2H), 1.53(m, 2H), 1.32(s, 9H). ^{13}C-NMR ($CDCl_3$, 125 MHz) 173.26, 156.49, 143.79, 141.27, 129.18, 128.72, 127.73, 126.86, 125.06, 124.35, 119.98, 79.22, 66.95, 63.66, 49.77, 47.06, 40.07, 39.24, 34.37, 30.35, 28.38, 25.11. HR-ESI: [M+H]+ cal 616.30191, found 616.30191.



3b. 67% for two steps. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ = 7.74(m, 2H), 7.57(m,2H), 7.37(m, 2H), 7.28(m,2H), 5.92(s, 1H), 4.99-4.63(m, 2H), 4.36-4.04(m, 4H), 3.61-3.07(m, 8H), 2.37-1.77(m,6H), 1.41-1.37(b,20), 0.82(m, 2H) ^{13}C-NMR ($CDCl_3$, 125 MHz) 173.92, 156.72, 156.27, 143.82, 141.26, 127.72, 127.07, 125.15, 119.98, 79.19, 67.07, 60.55, 48.19, 47.10, 40.11, 39.33, 31.93, 30.20, 29.70, 28.43, 25.40, 23.82, 14.12. HR-ESI: [M+H]+ cal 696.3734, found 697.3819.

2. Solid phase synthesis, purification and characterization of lipidated α-AApeptides. [1-3]

Lipidated α-AApeptides were prepared on a Rink amide resin in peptide synthesis vessels on a Burrell Wrist-Action shaker following standard Fmoc chemistry protocol of solid phase peptide synthesis using synthesized α-AApeptide building blocks. [1-3] Each coupling cycle included an Fmoc deprotection using 20% piperidine in DMF, and 8 h coupling of 1.5 equiv of α-AApeptide building blocks onto resin in the presence 4 equiv of DIC (diisopropylcarbodiimide) / DhbtOH (3-4-Dihydro-3-hydroxy-4-oxo-1-2-3-benzotriazine) in DMF. The lipidation was achieved on resin by capping the N-terminus of last α-

AApeptide building block with palmitic acid (hexadecanoic acid). Next, the resin was transferred into 4 mL vials and the lipidated α-AApeptides were cleaved from solid support in 50:48:2 TFA/CH_2Cl_2/triisopropylsilane overnight. Then solvent was evaporated and the residues were analyzed and purified on an analytical (1 mL/min) and a preparative Waters (20 mL/min) HPLC systems, respectively, using 5% to 100% linear gradient of solvent B (0.1% TFA in acetonitrile) in A (0.1% TFA in water) over 40 min, followed by 100% solvent B over 10 min. The HPLC traces were detected at 215 nm. The desired fractions were collected and lyophilized. The molecular weights of lipidated α-AApeptides (Figure S1 and Table S1) were obtained on Bruker AutoFlex MALDI-TOF mass spectrometer using α–cyano-4-hydroxy-cinnamic acid.

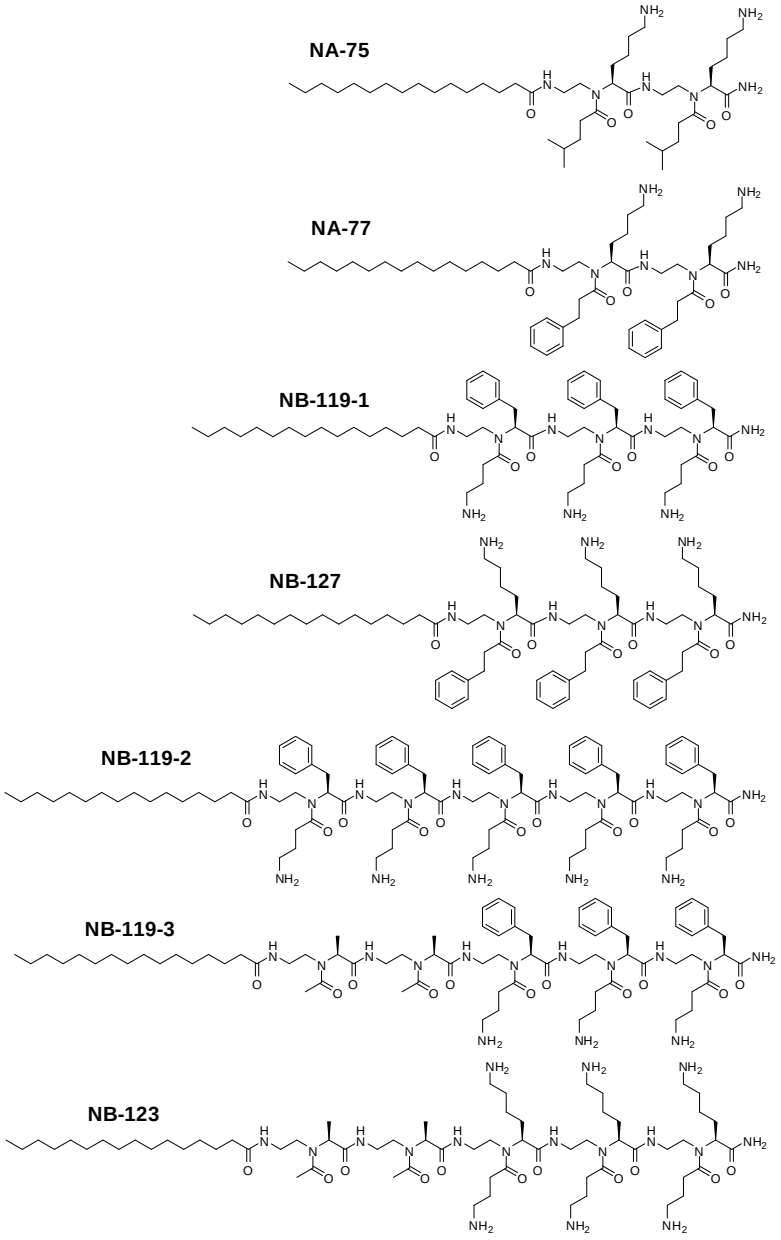


Figure S1. The structures of synthesized lipidated α-AApeptides.

Table S1. MALDI analysis of lipidated α-AApeptides.

Lipidated α-AApeptides	Yield (based on loading of the resin)	molecular weight (Actual)	molecular weight (found)
NA-75	17.8%	794.2	795.9 ($M+H^+$)
NA-77	22.7%	862.2	863.8 ($M+H^+$)
NB-119-1	13.5%	1081.4	1081.7 ($M+H^+$)
NB-127	6.8%	1165.6	1167.0 ($M+H^+$)
NB-119-2	11.2%	1632.1	1632.2 ($M+H^+$)
NB-119-3	8.3%	1393.8	1394.0 ($M+H^+$)
NB-123	6.2%	1336.8	1137.0 ($M+H^+$)

3. Antimicrobial assays

The microbial organisms used were *E. coli* (JM109), *B. subtilis* (BR151), multi-drug resistant *S. epidermidis* (RP62A), *C. albicans* (ATCC 10231), Vancomycin-resistant *E. faecalis* (ATCC 700802), Methicillin-resistant *S. aureus* (ATCC 33592), *K. pneumoniae* (ATCC 13383), multi-drug resistant *P. aeruginosa* ATCC 27853. The minimum inhibitory concentration (MIC) is the lowest concentration that completely inhibits the growth of bacteria in 24 h. The highest concentration tested for antimicrobial activity was 50 μg/mL. The antimicrobial activities of the lipidated α-AApeptides were determined in a sterile 96 -well plates by broth micro-dilution method. Bacterial cells [2, 4, 5] and fungi [2, 4, 6] were grown overnight at 37 ℃ in 5 mL medium, after which a bacterial suspension (approximately 10^6 CFU/mL) or fungal suspension Candida albicans (ATCC 10231) (approximately 10^3 CFU/mL) in Luria broth or trypticase soy was prepared. Aliquots of 50 μL bacterial or fungal suspension were added to 50 μL of medium containing the α-AApeptides for a total volume of 100 μL in each well. The α-AApeptides were prepared in PBS buffer in 2 –fold serial dilutions, with the final concentration range of 0.5 to 50 μg/mL. Plates were then incubated at 37 ℃ for 24 h (for bacteria) or 48 h (for *Candida albicans* (ATCC 10231). The lowest concentration at which complete inhibition of bacterial growth (determined by a lack of turbidity) is observed throughout the incubation time is defined as the minimum inhibitory concentration (MIC). The experiments were carried out independently three times in duplicates.

4. Fluorescence microscopy

A double staining method with DAPI (4',6-Diamidino-2-phenylindole dihydrochloride,
Sigma, >98%) and PI (Propidium iodide, Sigma) as fluorophores was used to visualize and differentiate the viable from the dead *E. coli* or *B. subtilis* cells. DAPI as a double stranded DNA binding dye, stains all bacterial cells irrespective of their viability. Whereas Ethidium derivatives such as propidium iodide (PI) is capable of passing through only damaged cell membranes and intercalates with the nucleic acids of injured and dead cells to form a bright red fluorescent complex. [7] The cells were first stained with PI and then with DAPI. Bacterial cells were grown until they reached mid-logarithmic phase and then they (~2×10^6 cells) were incubated with the lipo-α-AApeptide at the concentration of 2× MIC (10 μg/ml) for 2 h. Then the cells were pelleted by centrifugation at 3000g for 15 min in an eppendorf microcentrifuge. The supernatant was then decanted and the cells were washed with 1× PBS several times and then incubated with PI (5 μg/ml) in dark for 15 min at 0 ^oC. The excess PI was removed by washing the cells with 1× PBS several times. Then the cells were incubated with DAPI (10 μg/ml in water) for 15 mins in dark at 0 ^oC. The DAPI solution was removed and cells were washed with 1× PBS several times. Controls were performed following the exact same procedure for bacteria without the addition of lipo-α-

AApeptides. The bacterial cells were then visualized by using the Zeiss Axio Imager Z1optical microscope with an oil-immersion objective (100×). [8]

References

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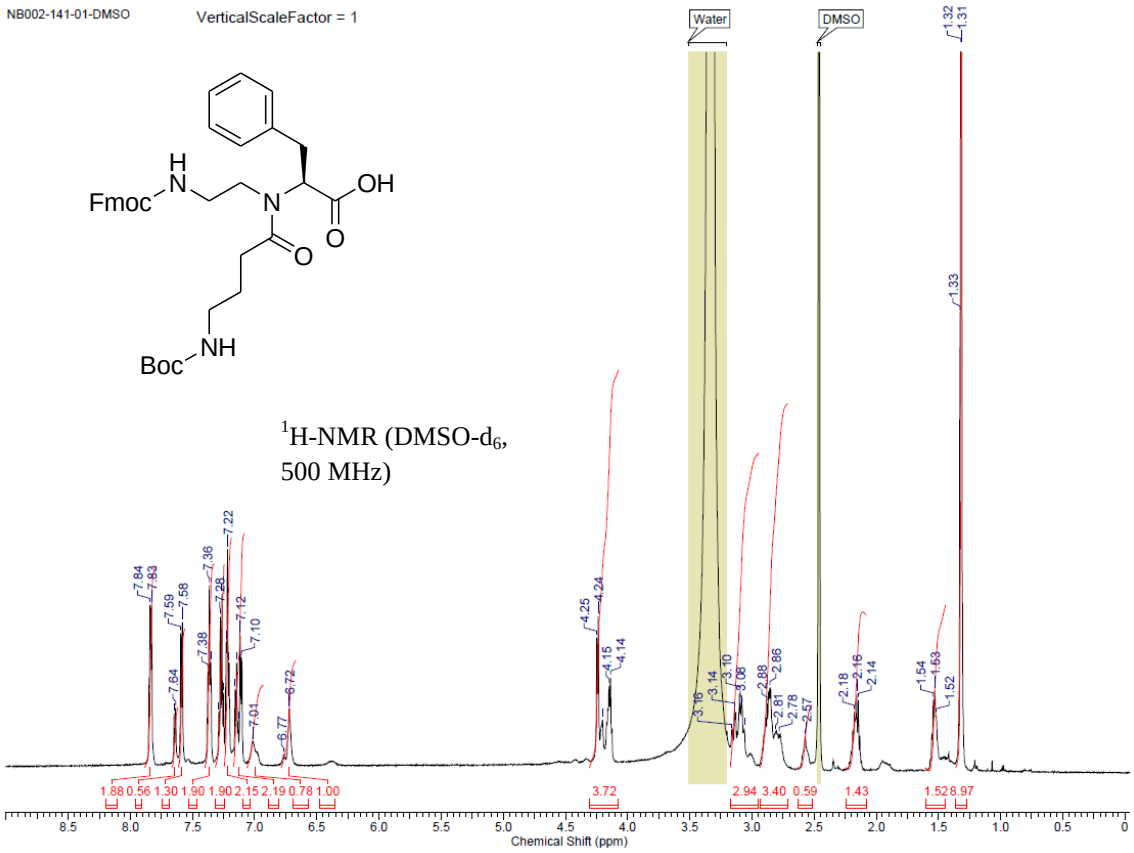
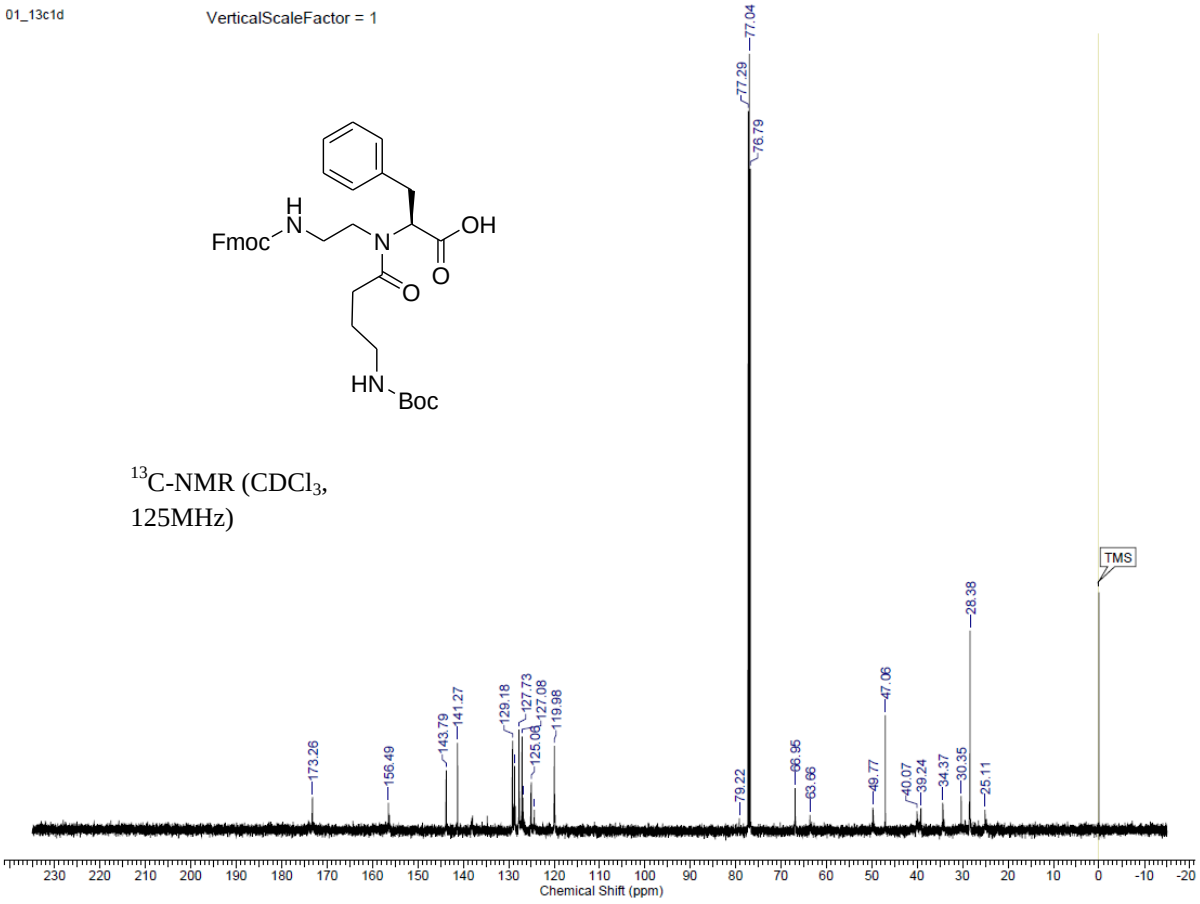
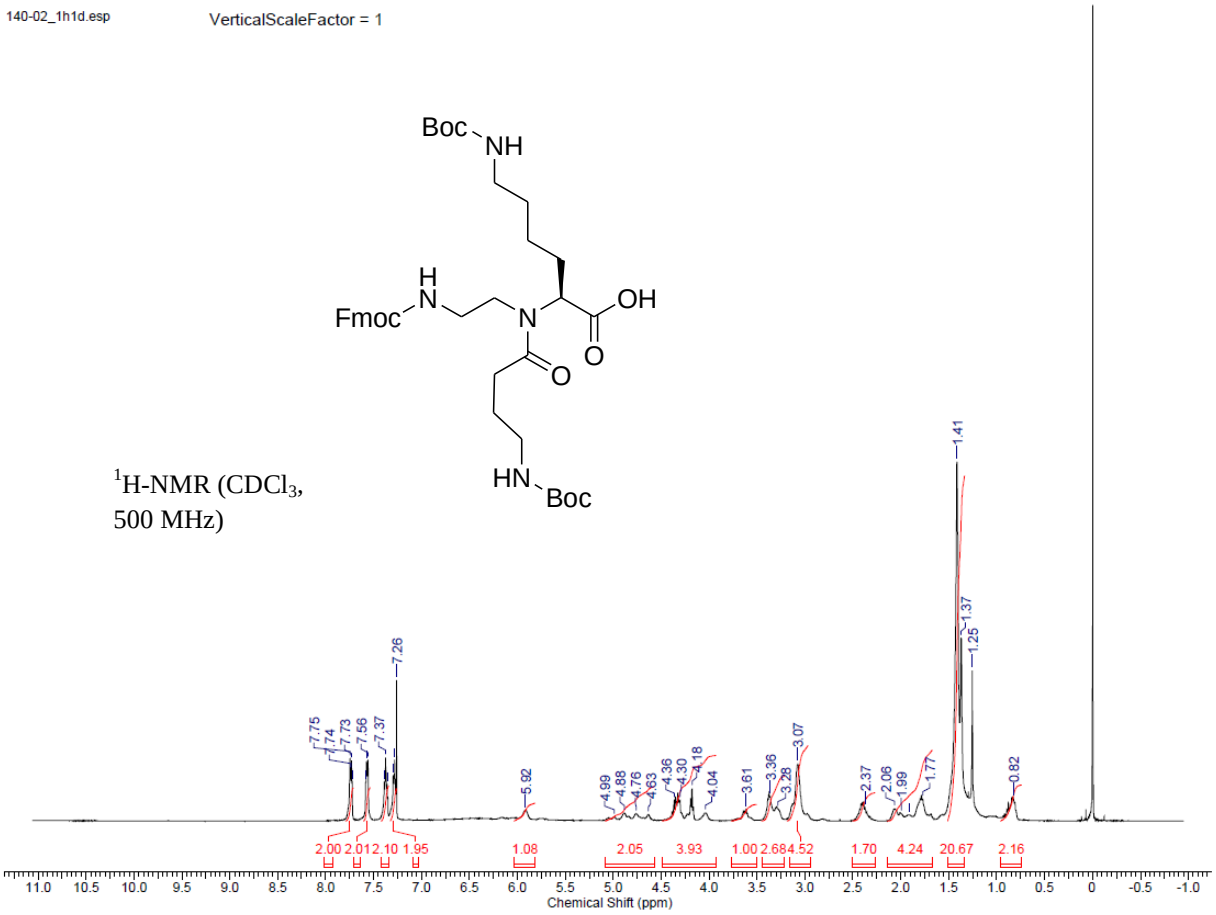


^{1}H-NMR (DMSO-d_6, 500 MHz)



^{13}C-NMR ($CDCl_3$, 125MHz)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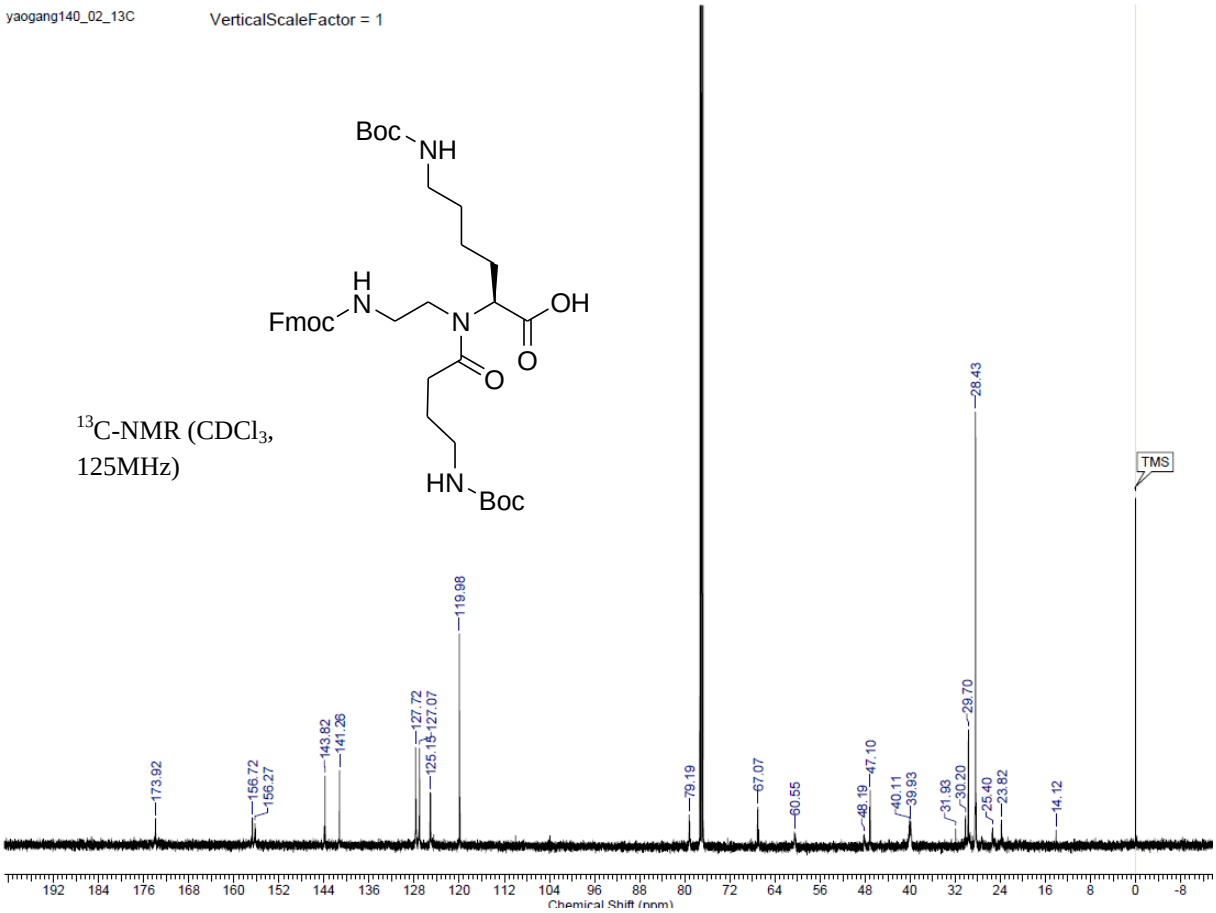
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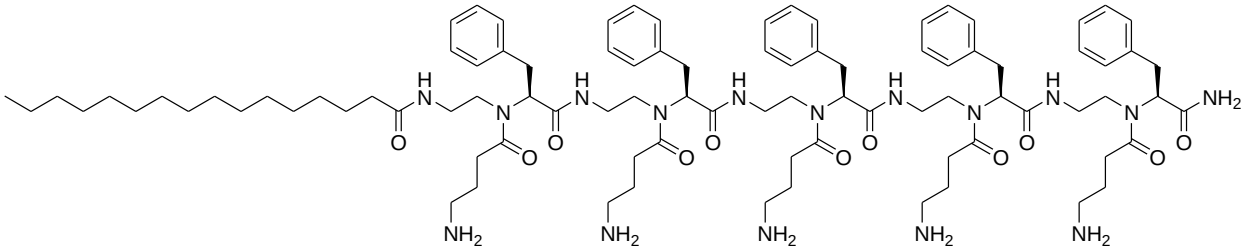
Chemical Shift (ppm)



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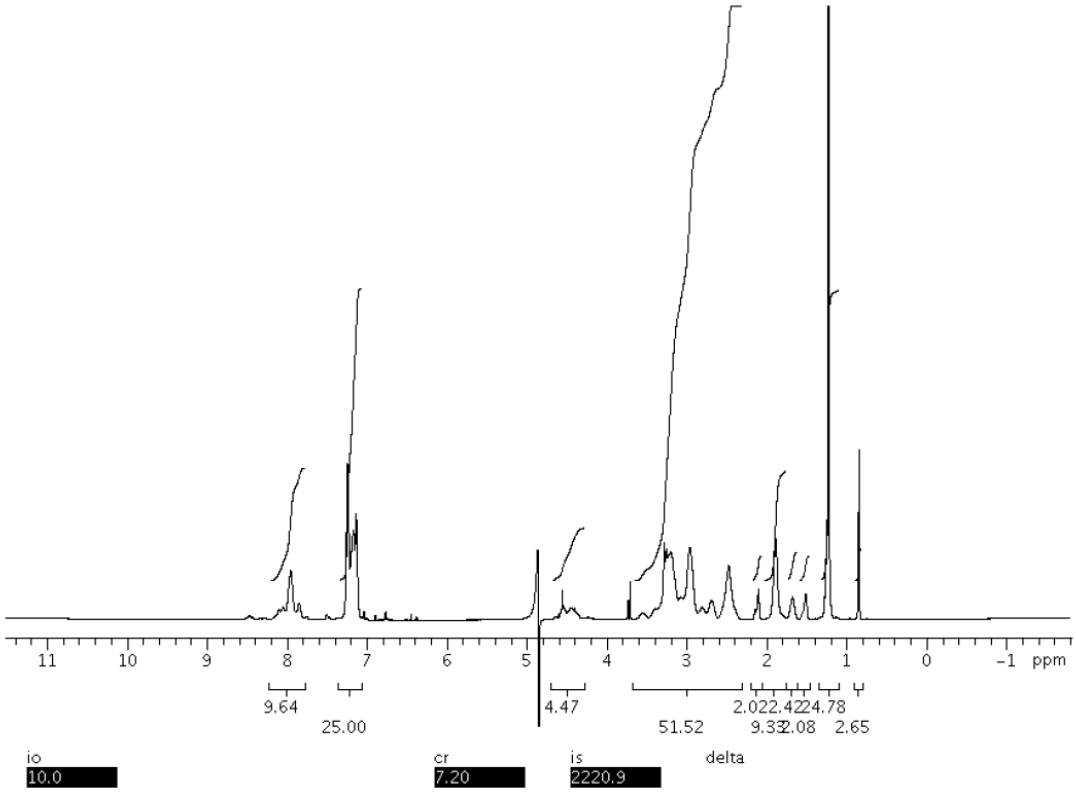
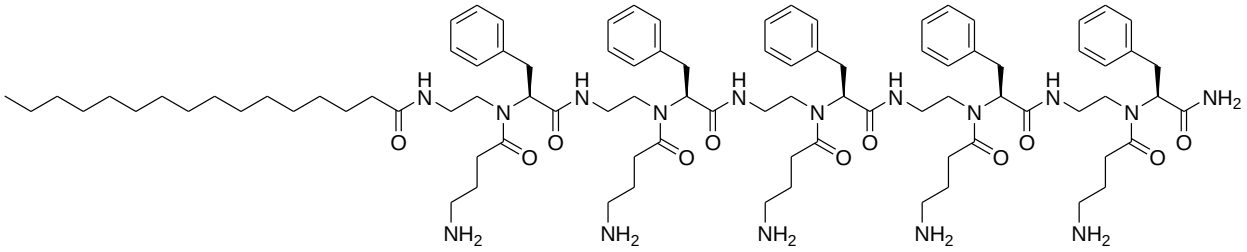


^{1}H-NMR (CD_3OH, 600MHz) δ = 8.05-7.85 (17H), 7.24-7.13 (25H), 4.60-4.31 (5H), 3.62-2.32 (50H), 2.10-1.51 (14H), 1.23 (24H), 0.85 (3H).



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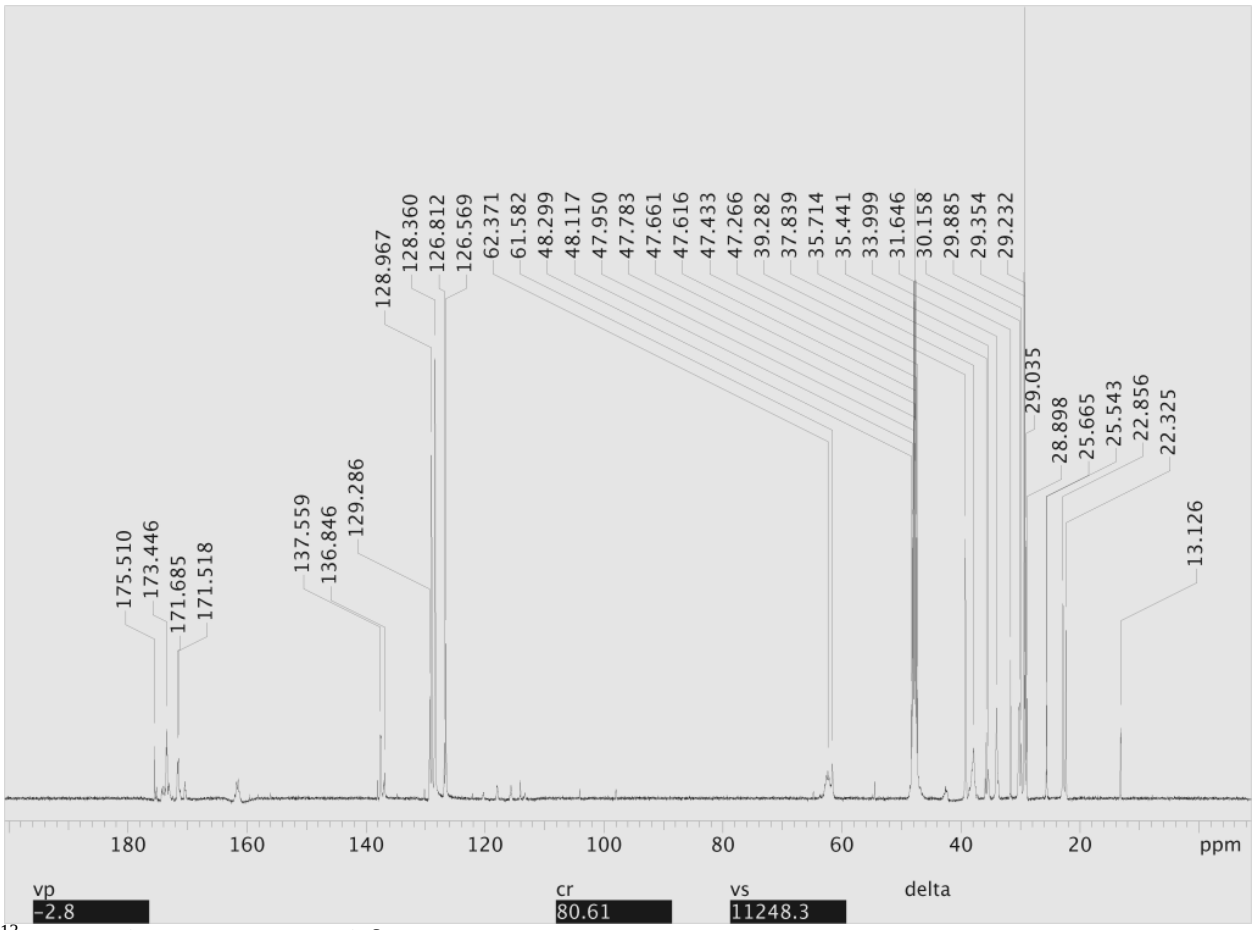

^{13}C-NMR (CD_3OH, 125 MHz) δ = 75.51, 173.44, 171.68, 170.14, 161.68, 137.60, 136.85, 129.29, 128.97, 128.36, 126.81, 126.57, 62.37, 61.58, 39.28, 37.84, 35.71, 35.44, 33.99, 31.65, 30.16, 29.88, 29.35, 29.23, 29.04, 28.90, 25.67, 25.54, 22.86, 22.33, 13.13.