Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Improved Diastereoselective Synthesis of Cyclic Tetrapeptide Pseudoxylallemycin A and Structural Analogues Illuminates the Key Role of the Base During Macrolactamization

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Supporting Information

Supporting Information Figures

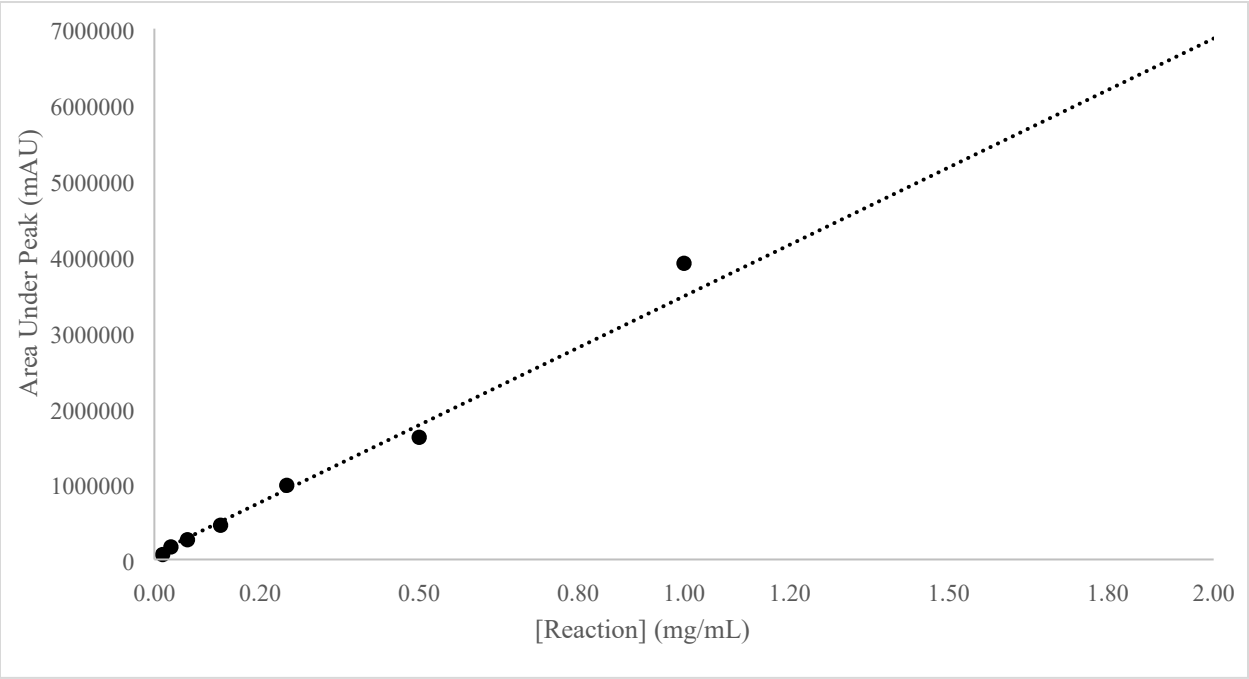
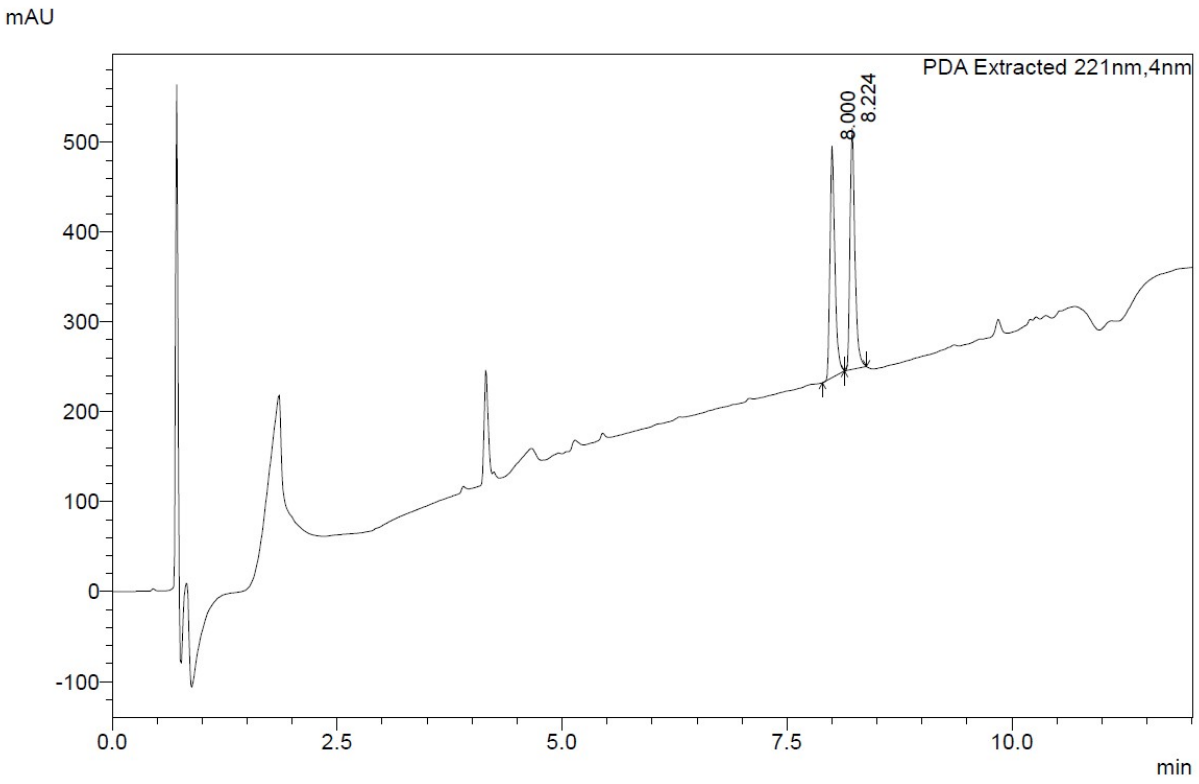


Figure S1: Standard curve relating HPLC peak area to sample concentration. A 1:1 serial dilution was performed on a 2.00 mg/mL solution of isolated epi-pseudoxylallemycin A, providing a concentration range of 0.0156 – 2.00 mg/mL. These samples were then diluted 1:1 with MeOH and run on a Shimadzu Prominence HPLC using a 0-100% gradient of solvent B over 12 minutes at 1 mL min^{-1} on a Phenomenex Luna C18 3 μM 50 mm x 4.60 mm column at room temperature. Solvent A = 100% H2O + 0.1% formic acid. Solvent B = 100% MeCN + 0.1% formic acid. Peak area was plotted against the original sample concentration prior to dilution with MeOH. Concentrations of reaction samples were interpolated from this plot and compared to theoretical concentrations to determine % yields.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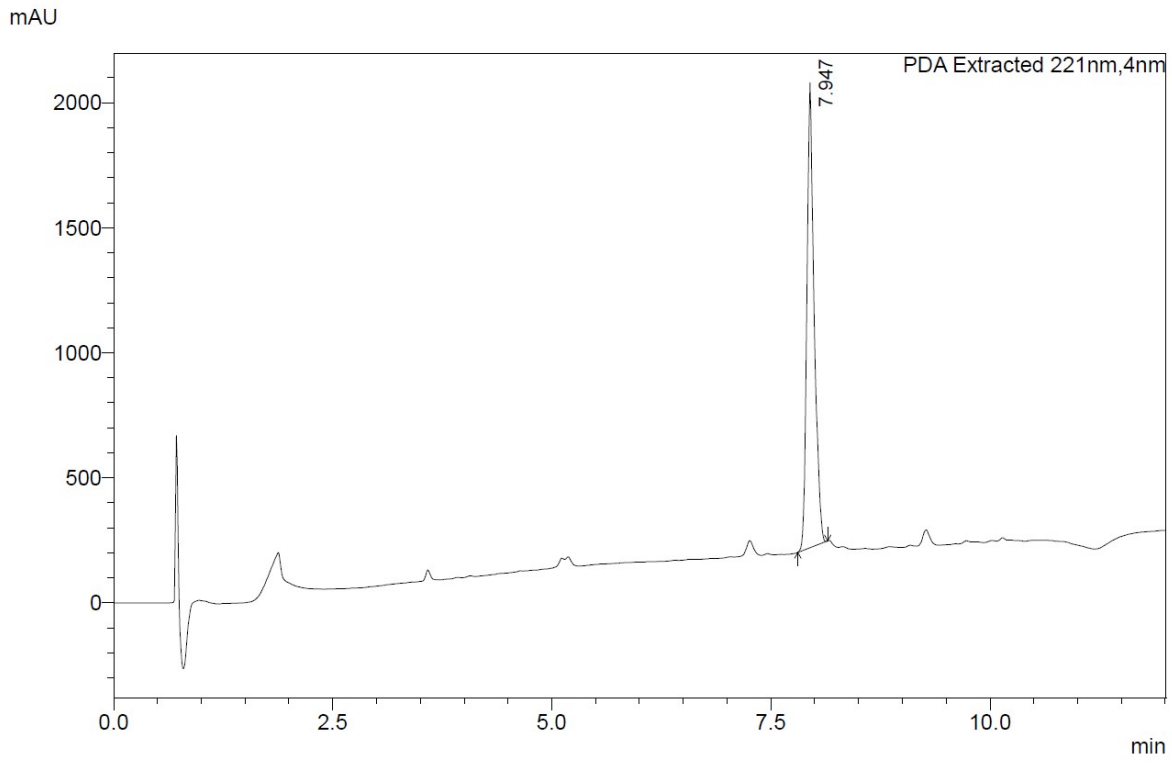
PDA Extracted 221nm,4nm
8.000
8.224
500
400
300
200
100
0
-100
0.0
2.5
5.0
7.5
10.0
min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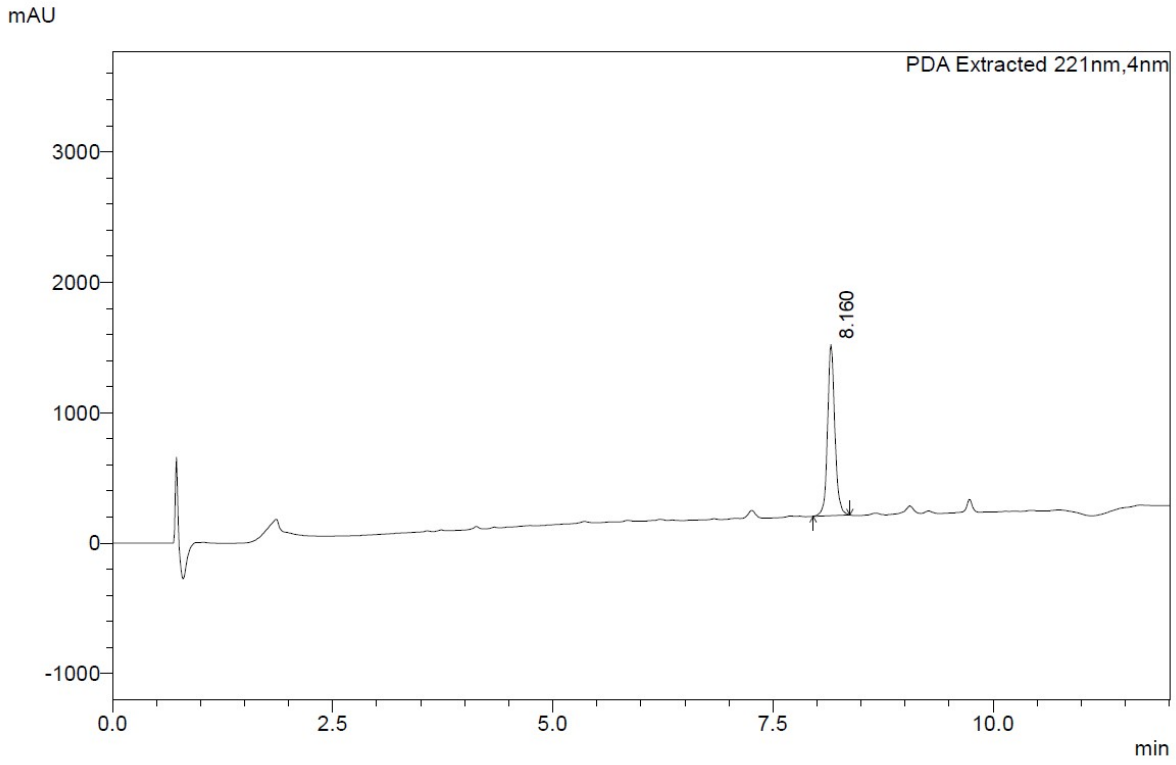
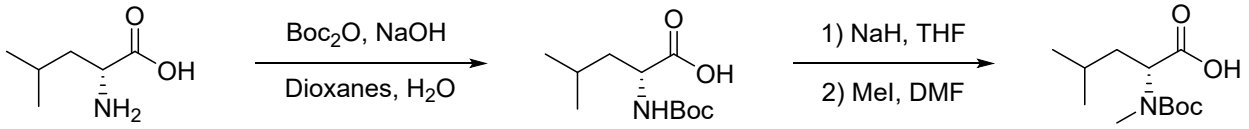


Figure S2: (a) HPLC trace showing cyclization of LTP **6** using T3P and collidine in DCE at 80°C after 48 hours. Sample was taken directly from reaction mixture and diluted 2:1 with MeOH. Epimer peak at 8.0 min. Natural product peak at 8.2 min. % area under the peaks provides DR of stereoisomers in the reaction mixture. (b) HPLC trace of isolated epimer. (c) HPLC trace of isolated natural product.

Antibacterial Microdilution Assay

The MIC determination assays were performed in a BSL-2 laboratory with appropriate security and biosafety measures. The MICs were determined using a broth microdilution assay closely following the guidelines of the Clinical and Laboratories Standards Institute (CLSI).[1] MICs were determined in duplicate for two biological replicates using 2-fold serial dilutions of test compounds or commercial antibiotics in cation-adjusted Mueller-Hinton broth. The central 60 wells of 96-well plates were prepared with the compound of interest at the indicated concentration, where 2 µL of the compound in DMSO were added prior to the addition of the culture. Overnight cultures of different bacterial strains were diluted to provide an initial inoculum of approximately 5 X 10^5 CFU/mL that was delivered to each well in 198 µL of culture (final DMSO concentration of ~1%). Initial inoculum concentrations were verified by plating to count CFU/mL. Each plate was equipped with experimental controls for full growth (6 wells) and full growth inhibition (6 wells), and all control wells were in the presence of 1% DMSO. The outer 36 wells were filled with 200 µL of culture each to prevent growth effects on the interior 60 wells. The plates were incubated at 37 °C for 18 hours, at which time they were removed from the incubator and were allowed to equilibrate to room temperature for 30 minutes. Absorbance of each well at 600 nm was then read using a microplate reader to determine the MIC, and visual confirmation of growth inhibition was also performed.

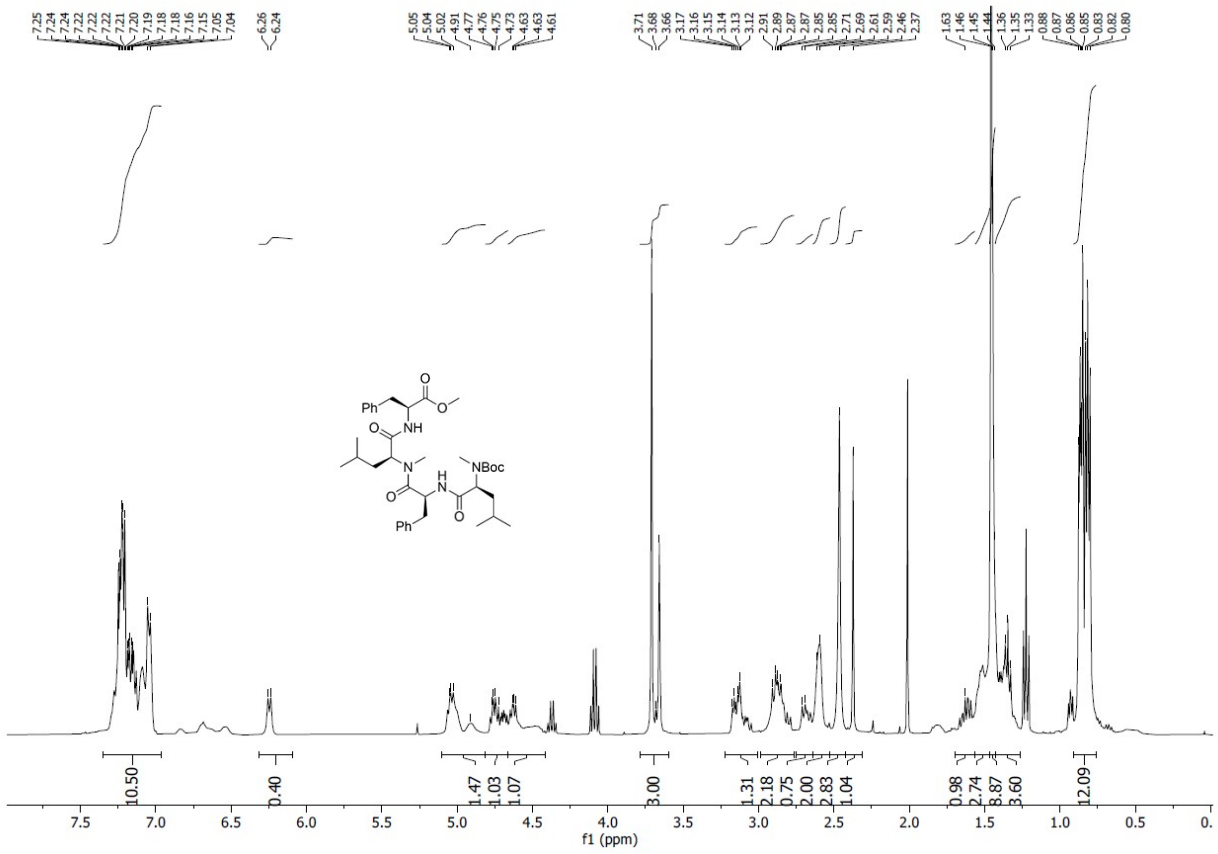
Preparation of Boc-*N*-Me-D-Leu-OH



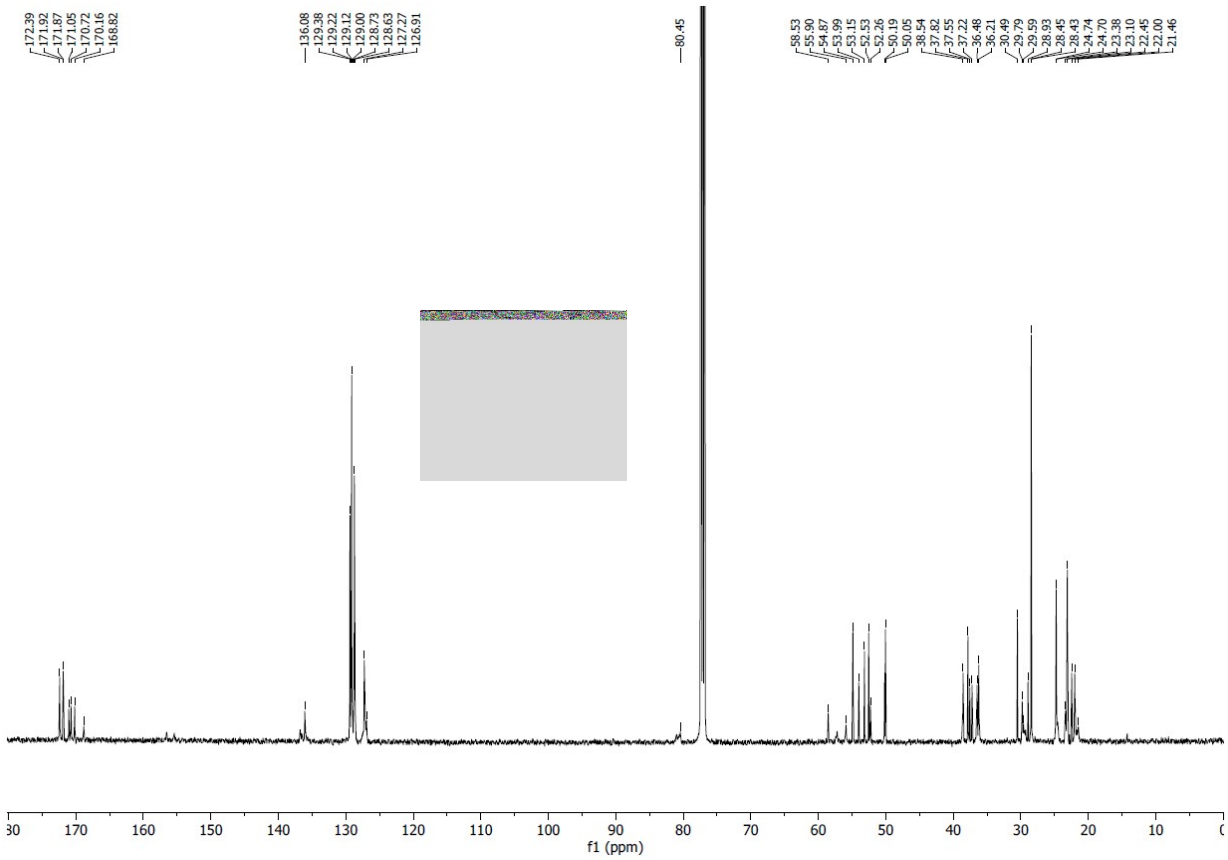
S3 **S4** **S5**

S3 (1.304 g, 9.94 mmol), dioxanes (40.0 mL), and 0.5 M NaOH (40.0 mL) were added to a round bottom flask. The mixture was cooled 0°C and Boc anhydride (2.74 mL, 11.9 mmol) was quickly added. The reaction was allowed to stir and warm gradually to rt for 3 h. The reaction mixture was diluted with EtOAc (20 mL) and the aqueous phase was acidified with 1M HCl. The organic was extracted from the aqueous using EtOAc (2 x 20 mL) and organic phases were combined, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford **S4** as a clear, colorless oil in quantitative yield and was used in subsequent reaction without purification. **S4** was dissolved in THF (33.1 mL) and cooled to 0°C. To the mixture was slowly added 60% NaH dispersed in mineral oil (4.771 g, 119.3 mmol), and the reaction was allowed to stir for 30 min. Temperature was maintained at 0°C and MeI (1.86 mL, 29.8 mmol) and DMF (1.71 mL) were added to the reaction mixture. The reaction was allowed to stir and warm gradually to rt for 4. The reaction was quenched with H_2O (10 mL) and transferred to a separatory funnel. The mixture was washed with hexanes (2 x 50 mL) and the aqueous phase was acidified with 1 M HCl. The product was extracted from the aqueous phase using EtOAc (4 x 50 mL). The organic phases were combined, washed with brine (50 mL), dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The methylated residue **S5** was afforded as an orange oil (2.373 g, 97%) and was used without purification. ^{1}H NMR (500 MHz, room temperature, $CDCl_3$) δ 9.43 (br, s, 1H), 4.90-4.55 (m, 1H), 2.81 (d, *J* = 12.9 Hz, 3H), 1.74 – 1.61 (m, 2H), 1.57 – 1.47 (m, 1H), 1.43 (d, *J* = 8.4 Hz, 9H), 0.95-0.87 (m, 6H). Spectra were in agreement with those of Boc-*N*-Me-L-Leu-OH in the literature.[2] $[\alpha]^{20}_D$ +22.7 (*c* = 0.05, EtOH).

NMR Spectra



^{1}H NMR of **6**.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171.87
171.05
170.72
170.16
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136.08
129.38
129.22
129.12
129.00
128.73
128.63
127.27
126.91
80.45
58.53
55.90
54.87
53.99
53.15
52.53
52.26
50.19
50.05
38.54
37.82
37.55
37.22
36.48
36.21
30.49
29.79
29.59
28.93
28.45
28.43
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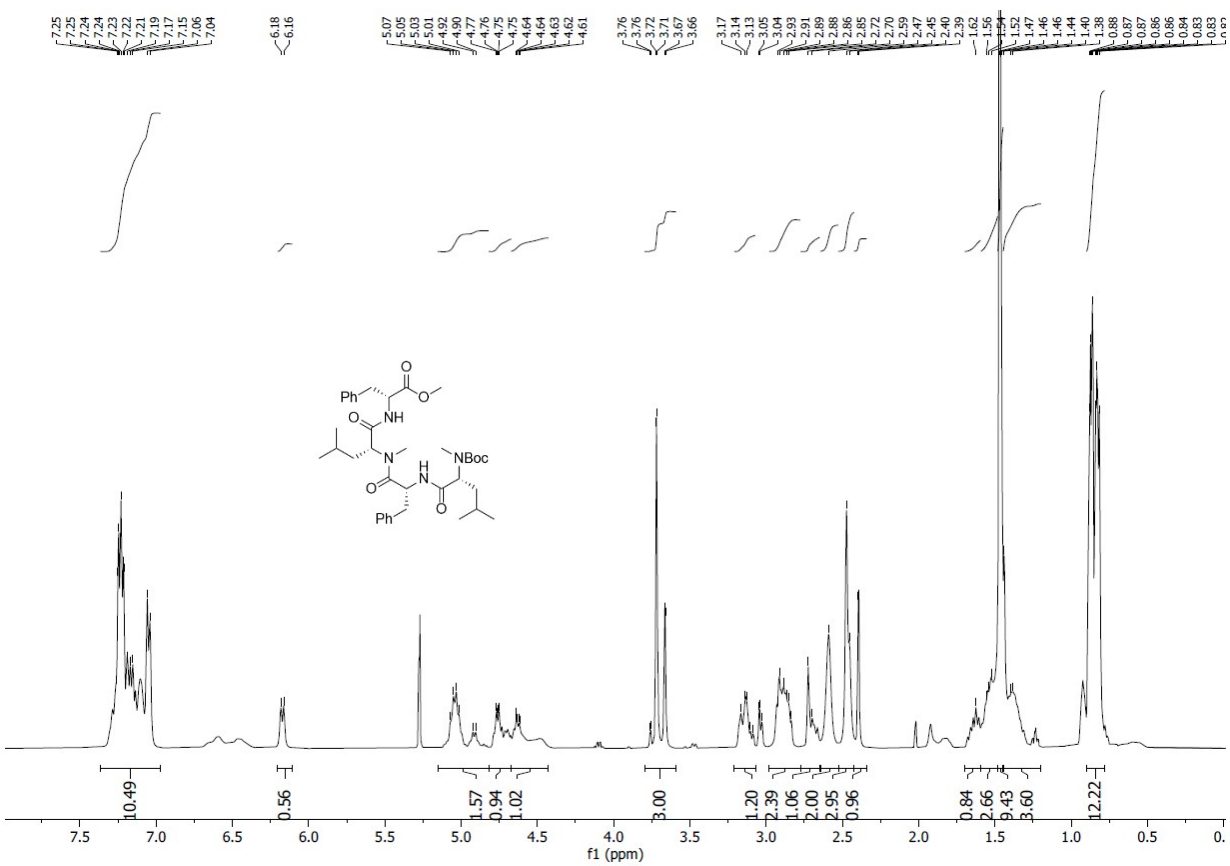
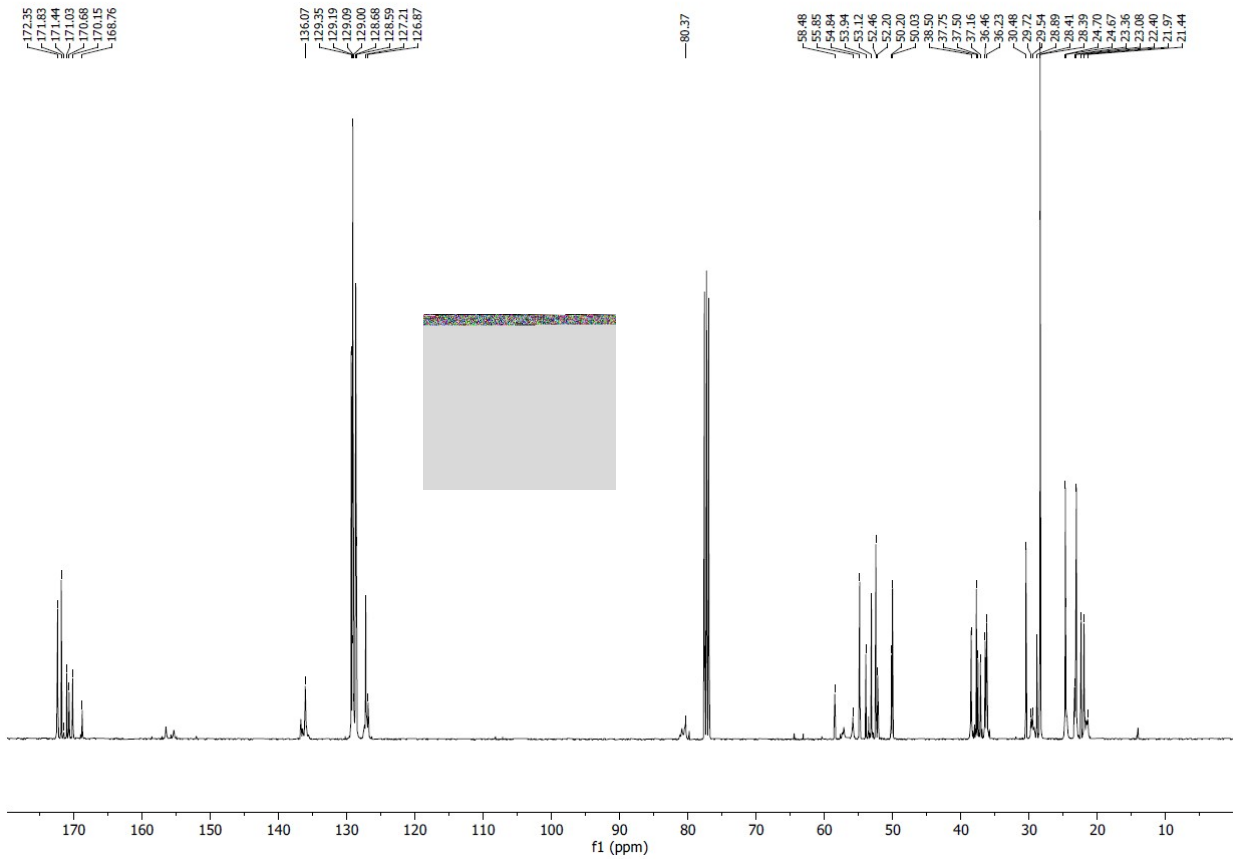
24.70
23.38
23.10
22.45
22.00
21.46
f1 (ppm)

^{13}C NMR of **6**.



^{1}H NMR of methyl *N*-*N*-(*tert*-butoxycarbonyl)-*N*-methyl-*D*-leucyl-*D*-phenylalanyl-*N*-methyl-*D*-leucyl-*D*-phenylalaninate.



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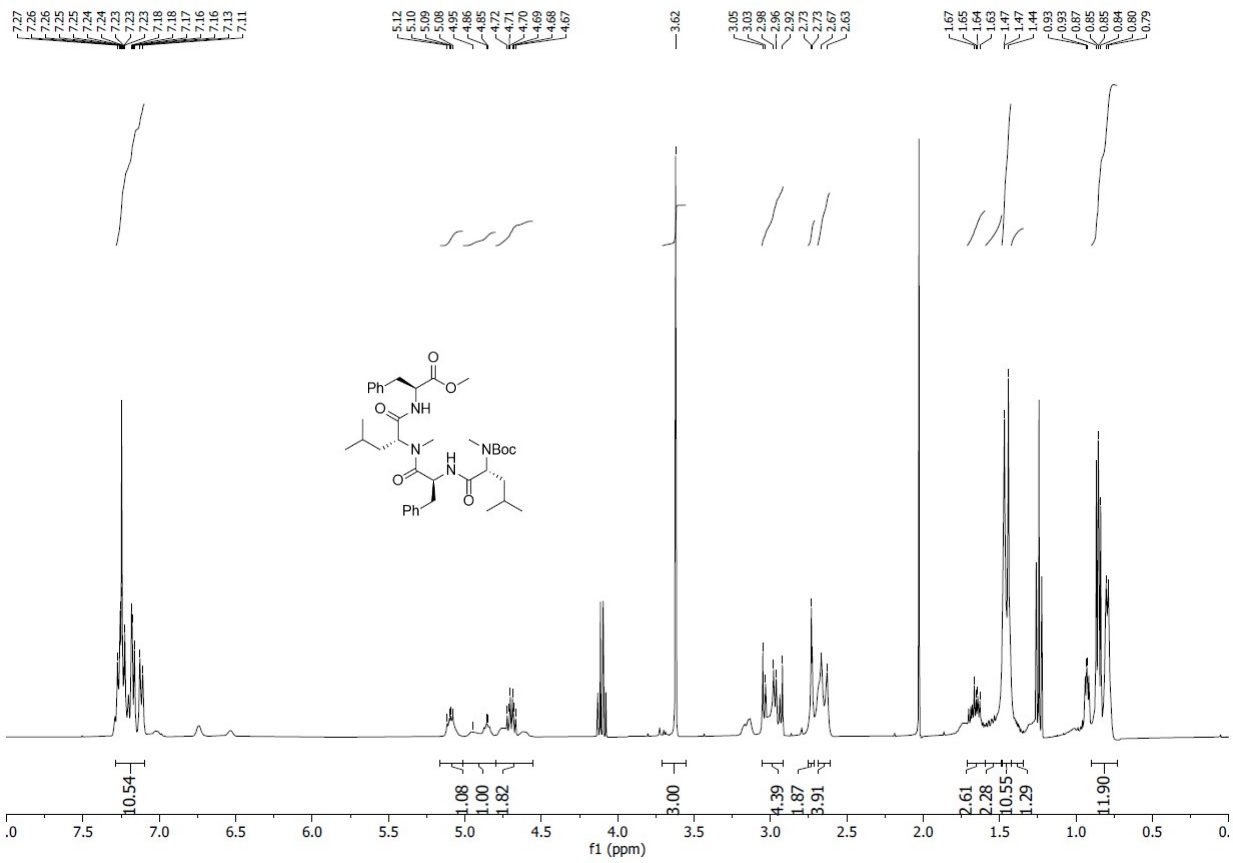

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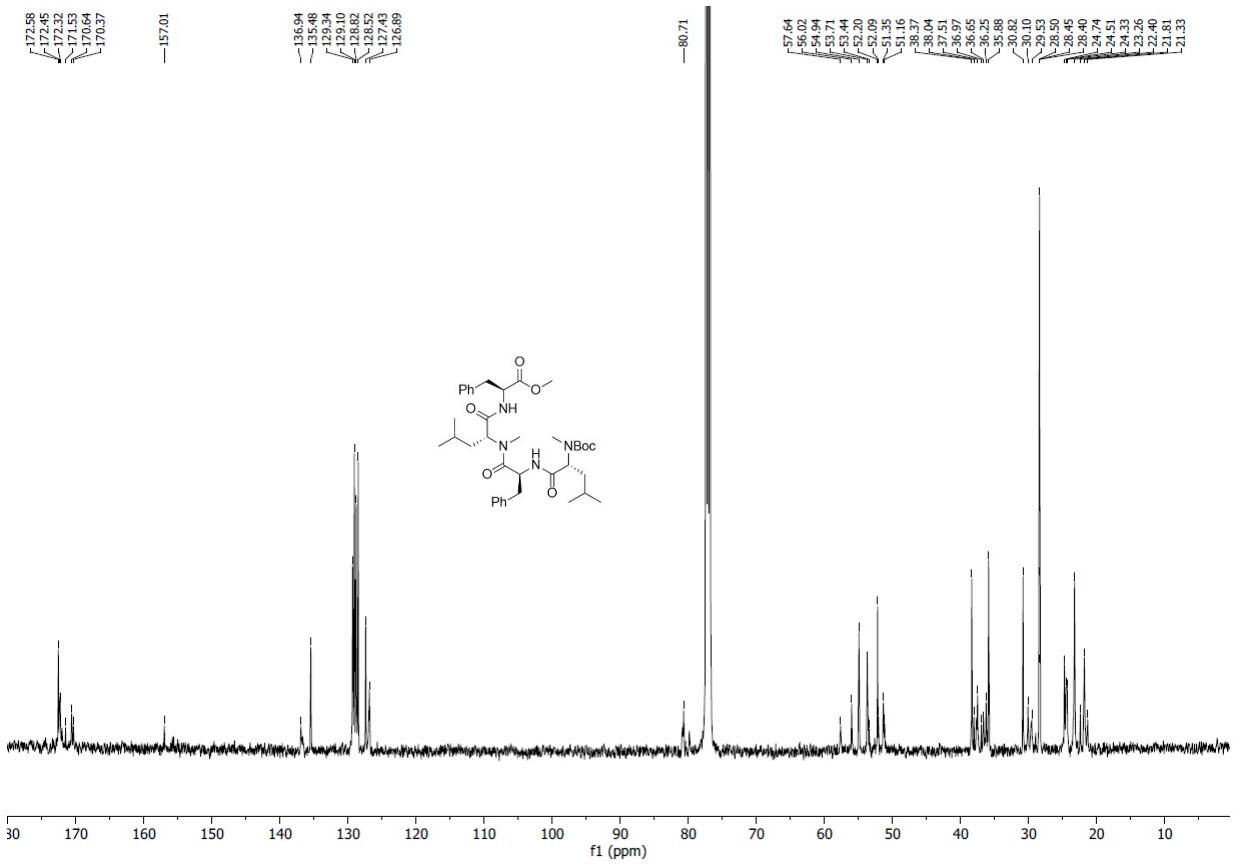

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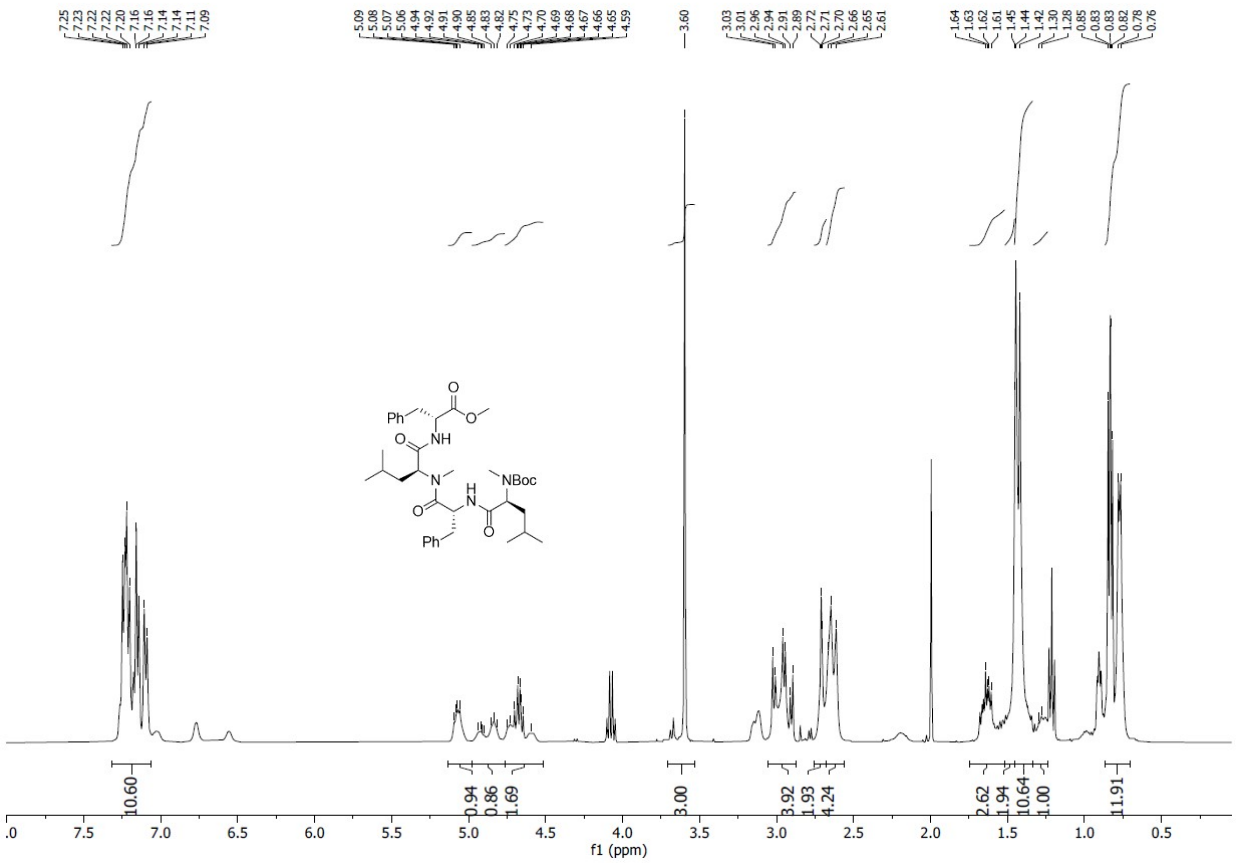

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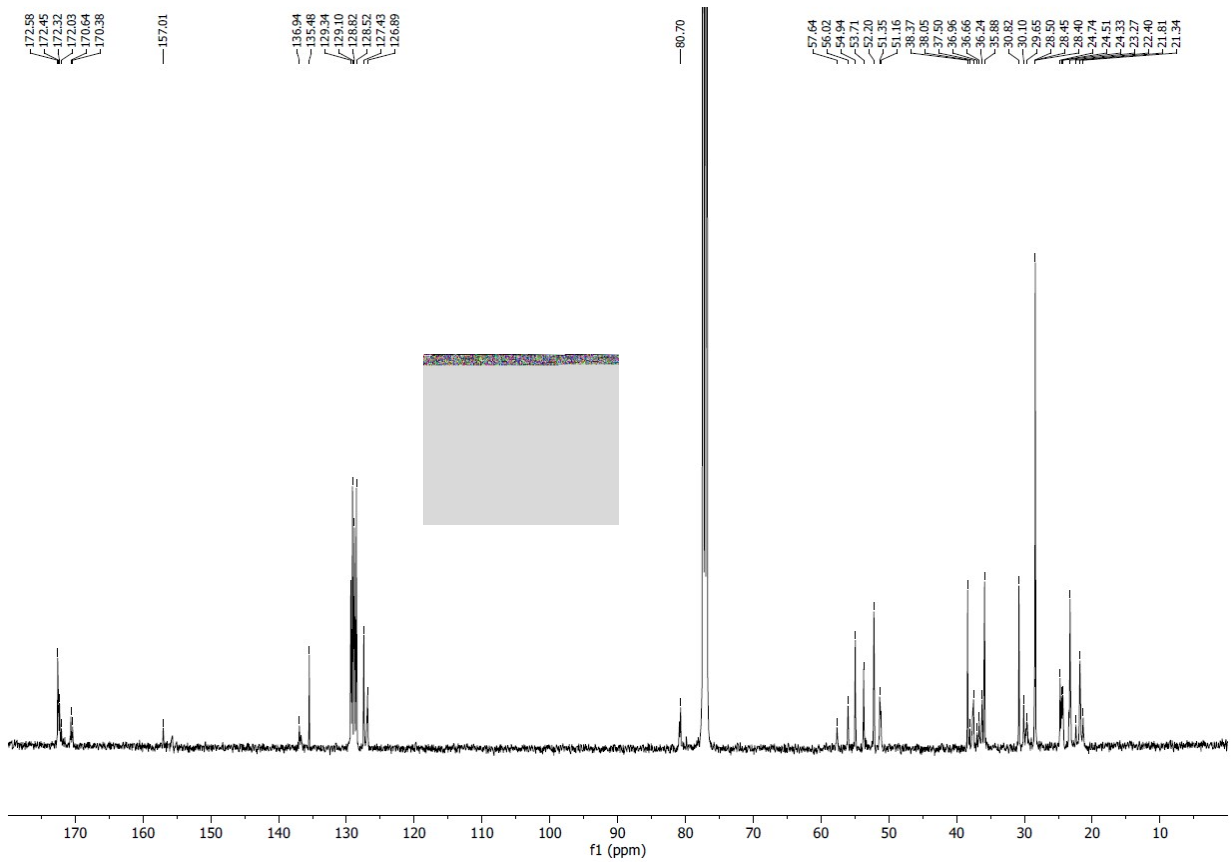

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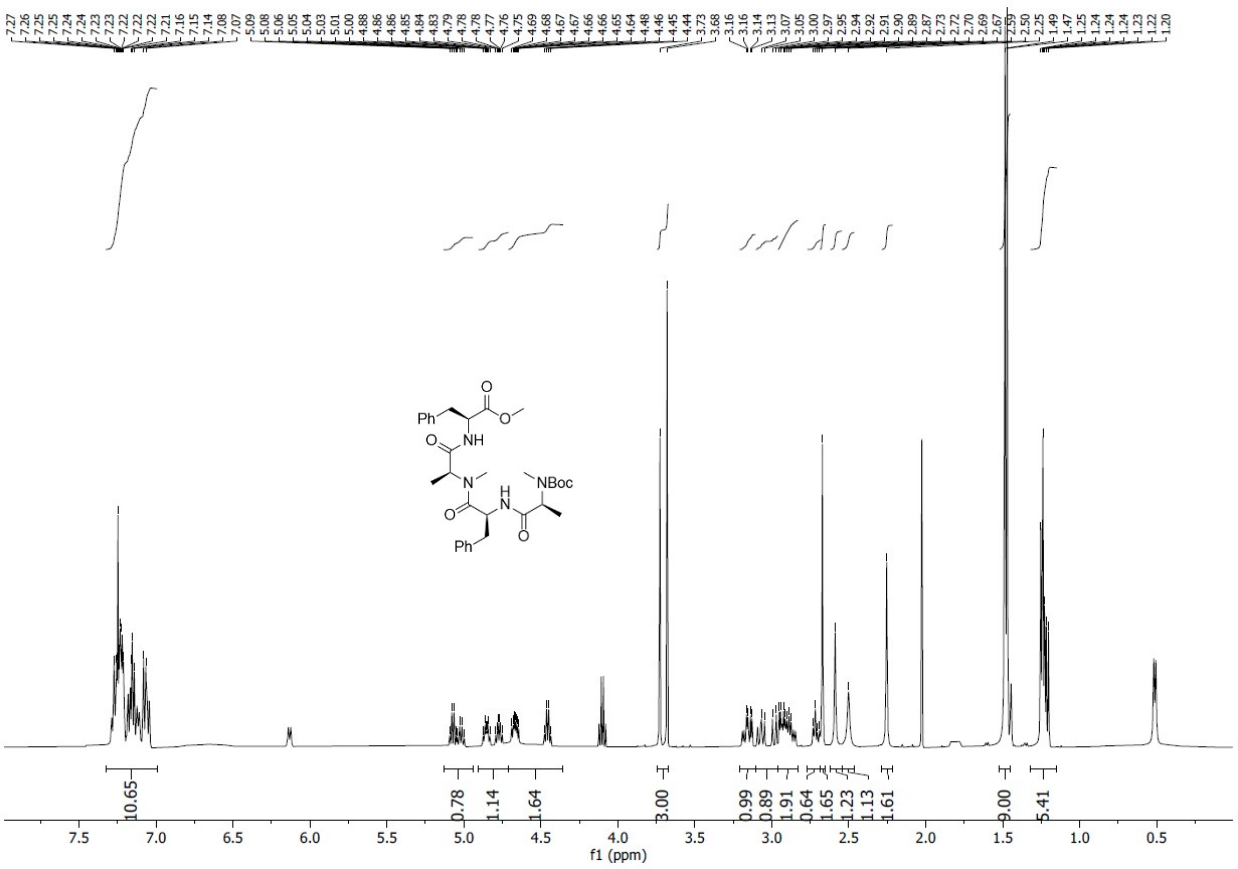

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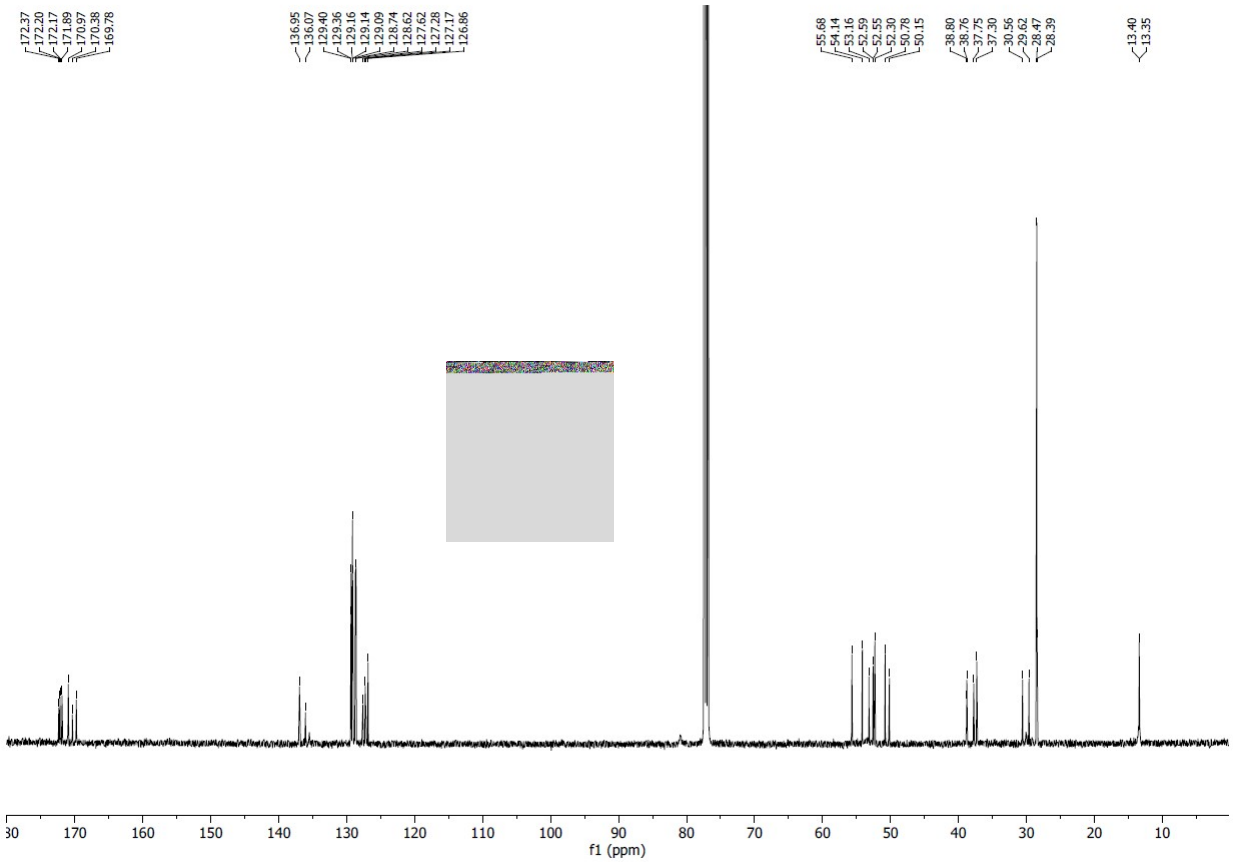

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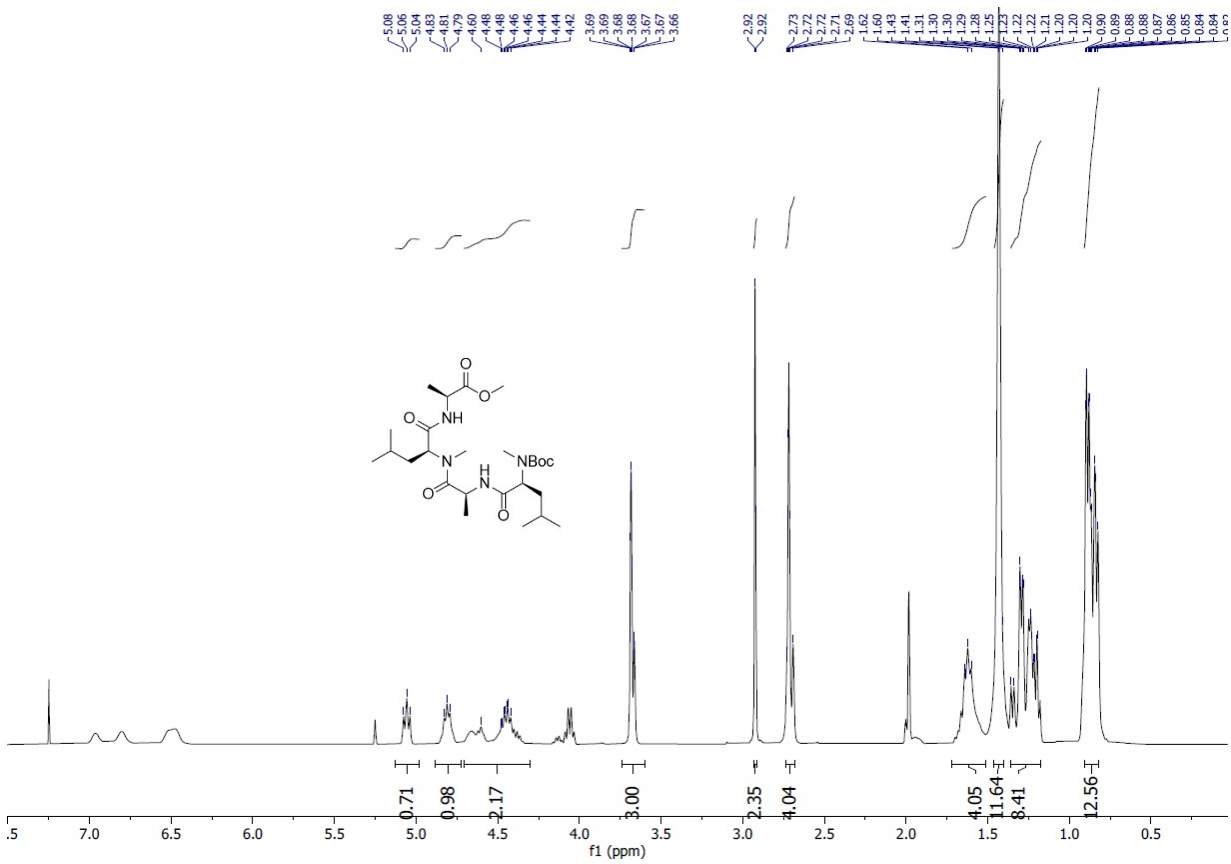

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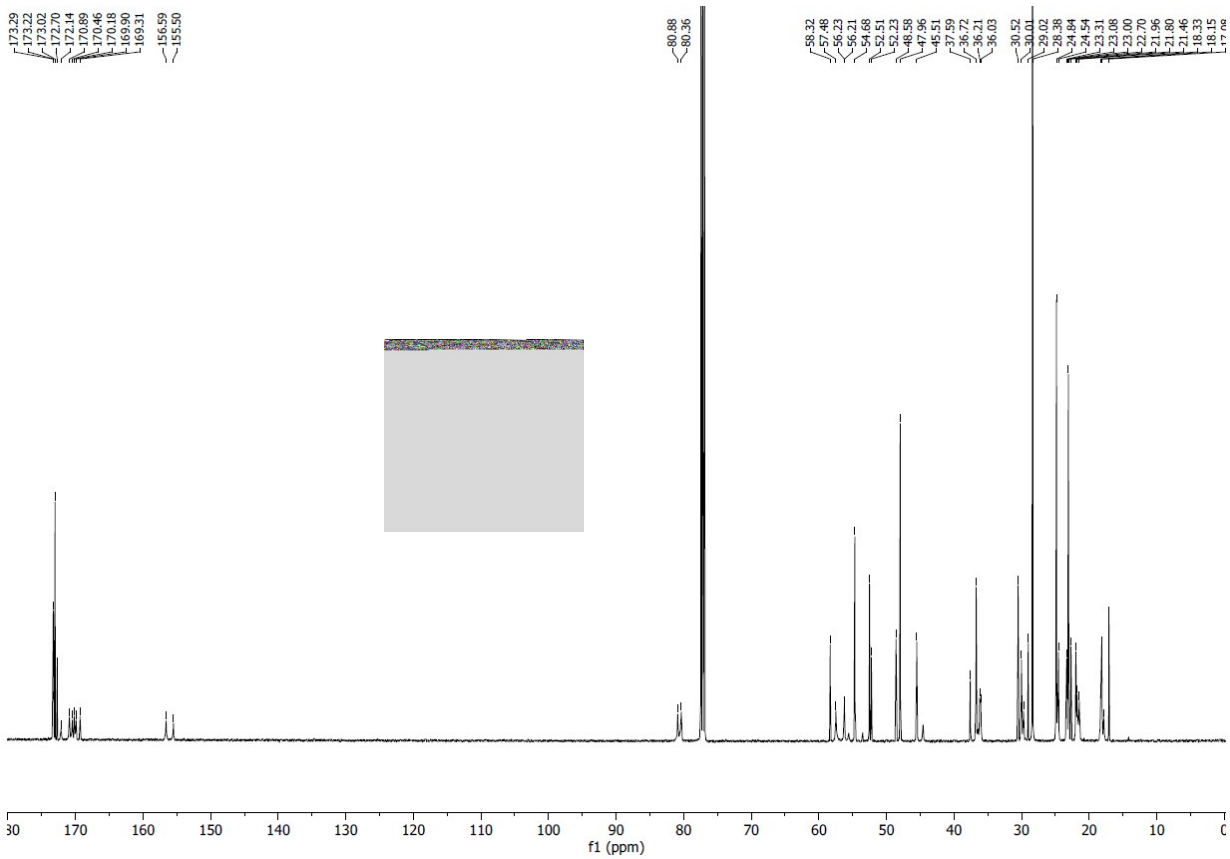

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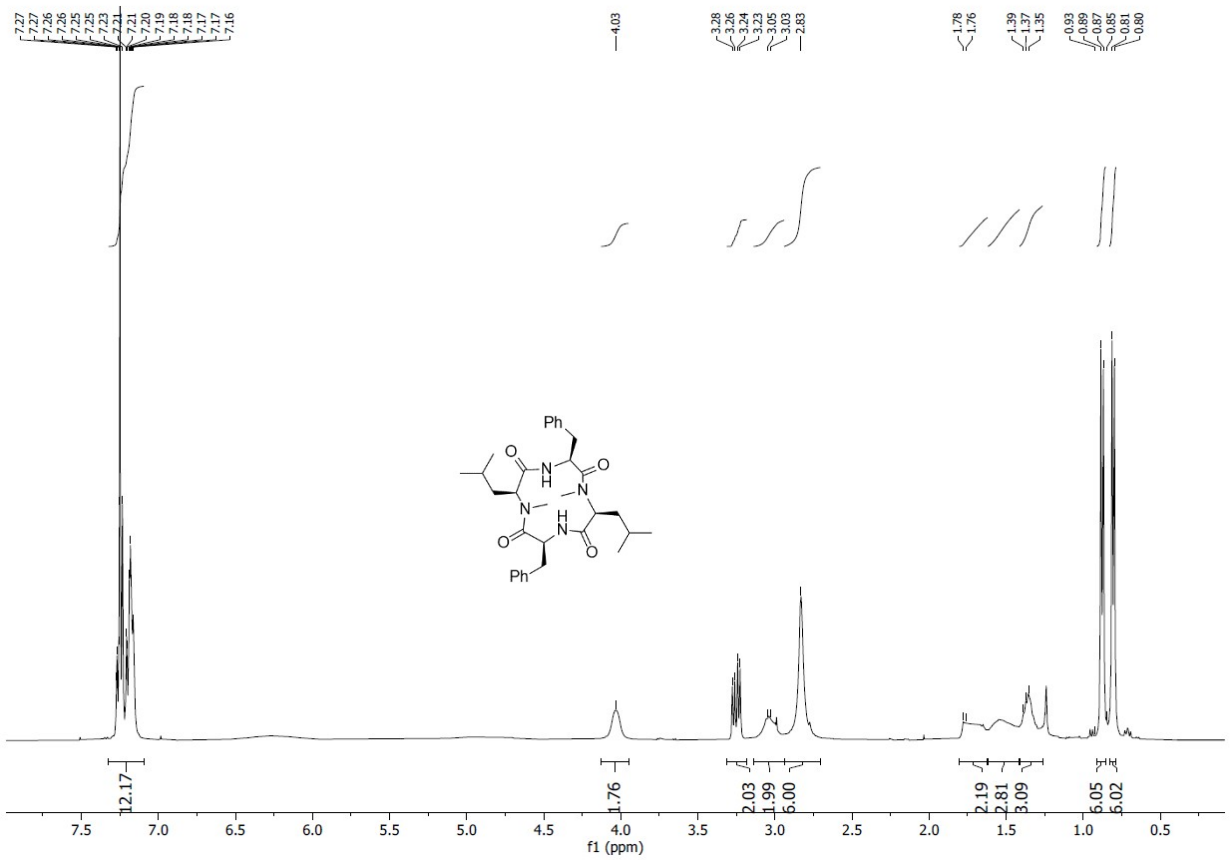
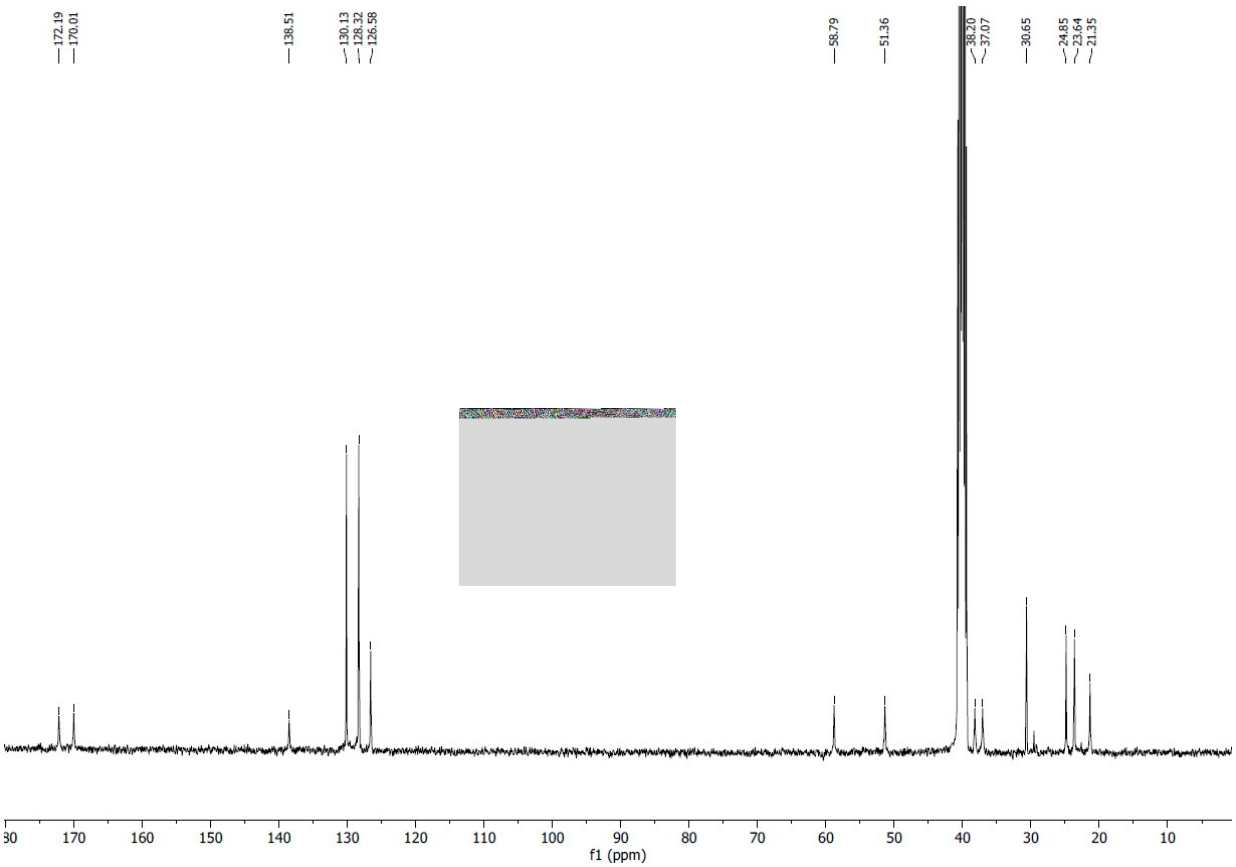


^{1}H NMR of pseudoxylallemycin A.



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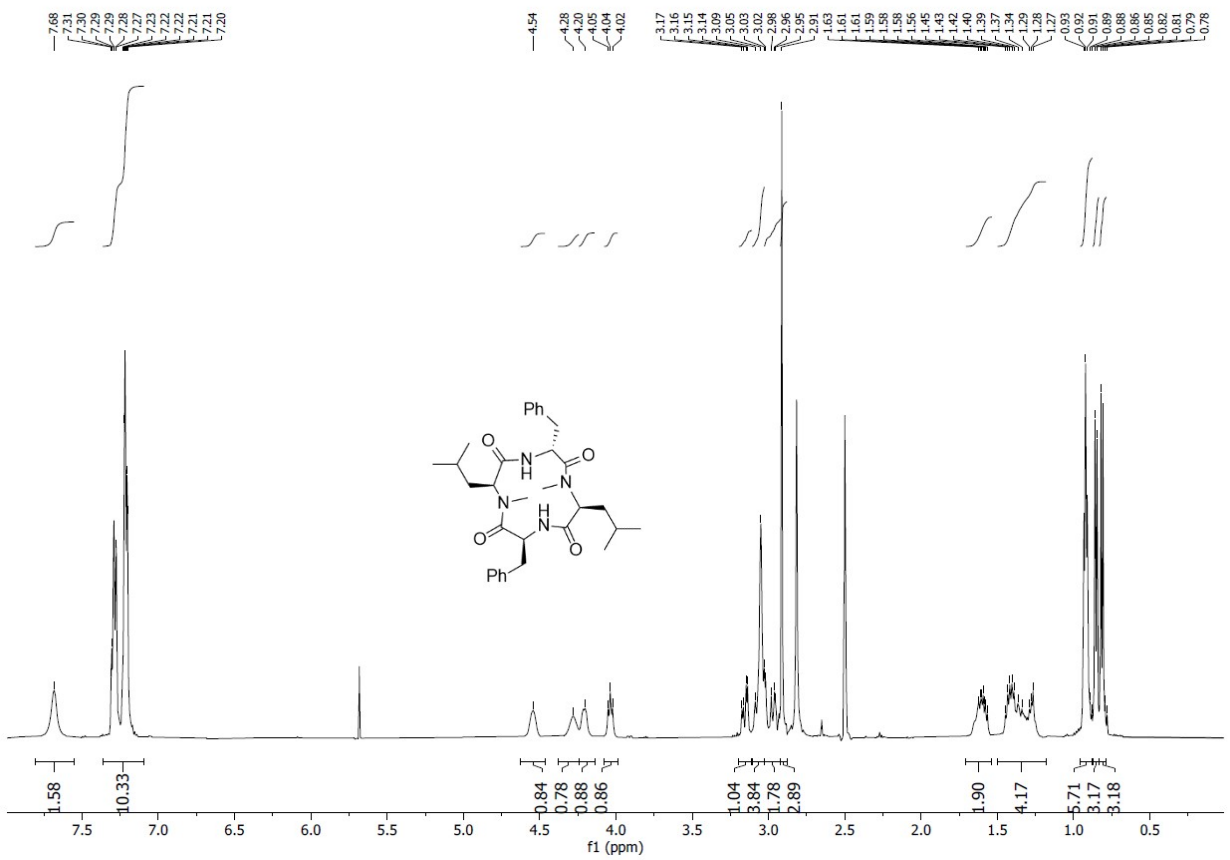

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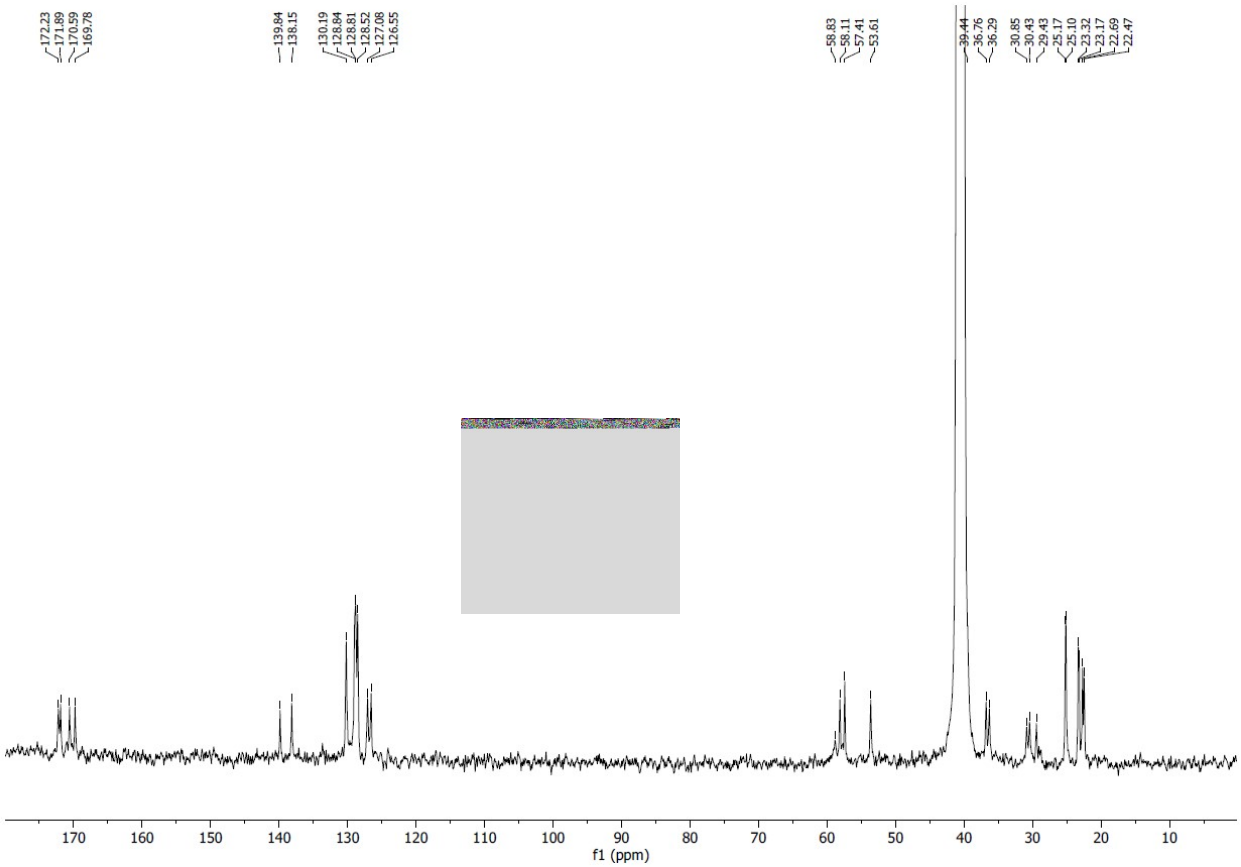

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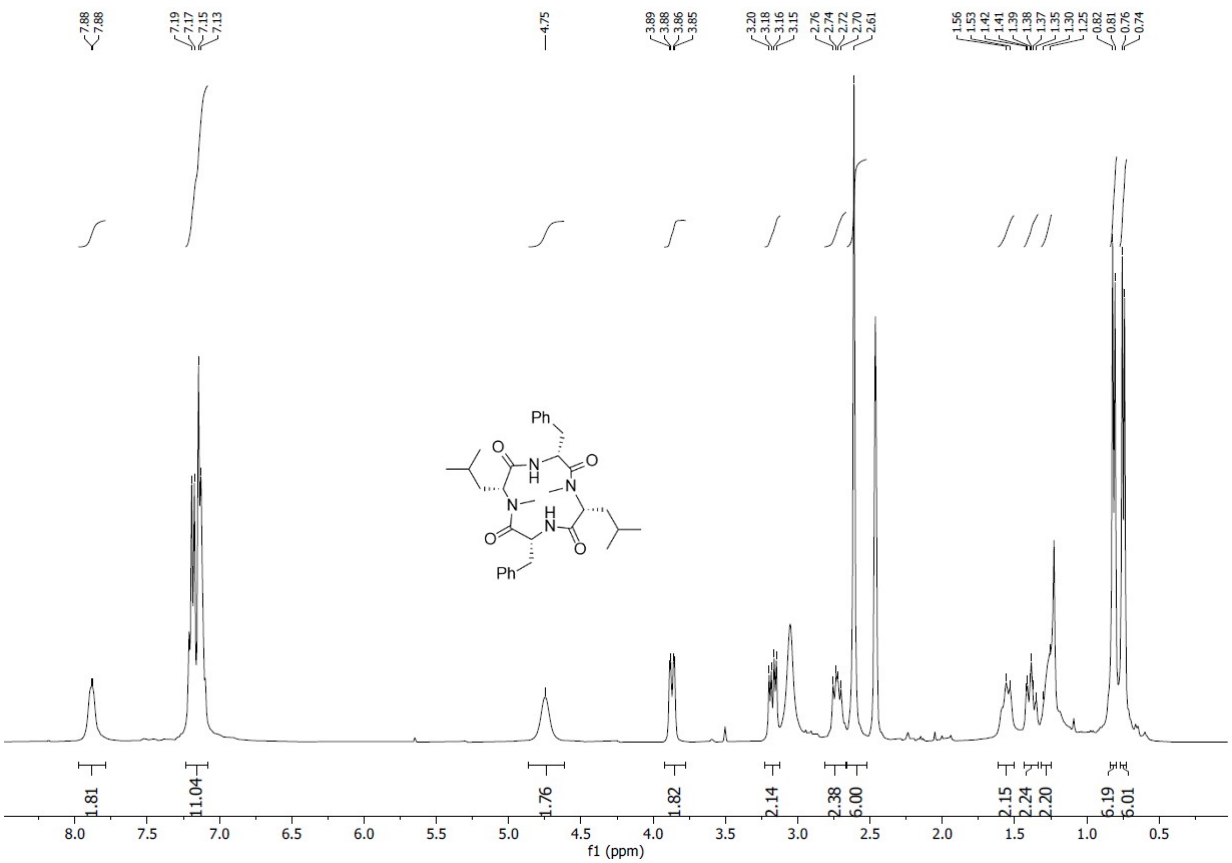
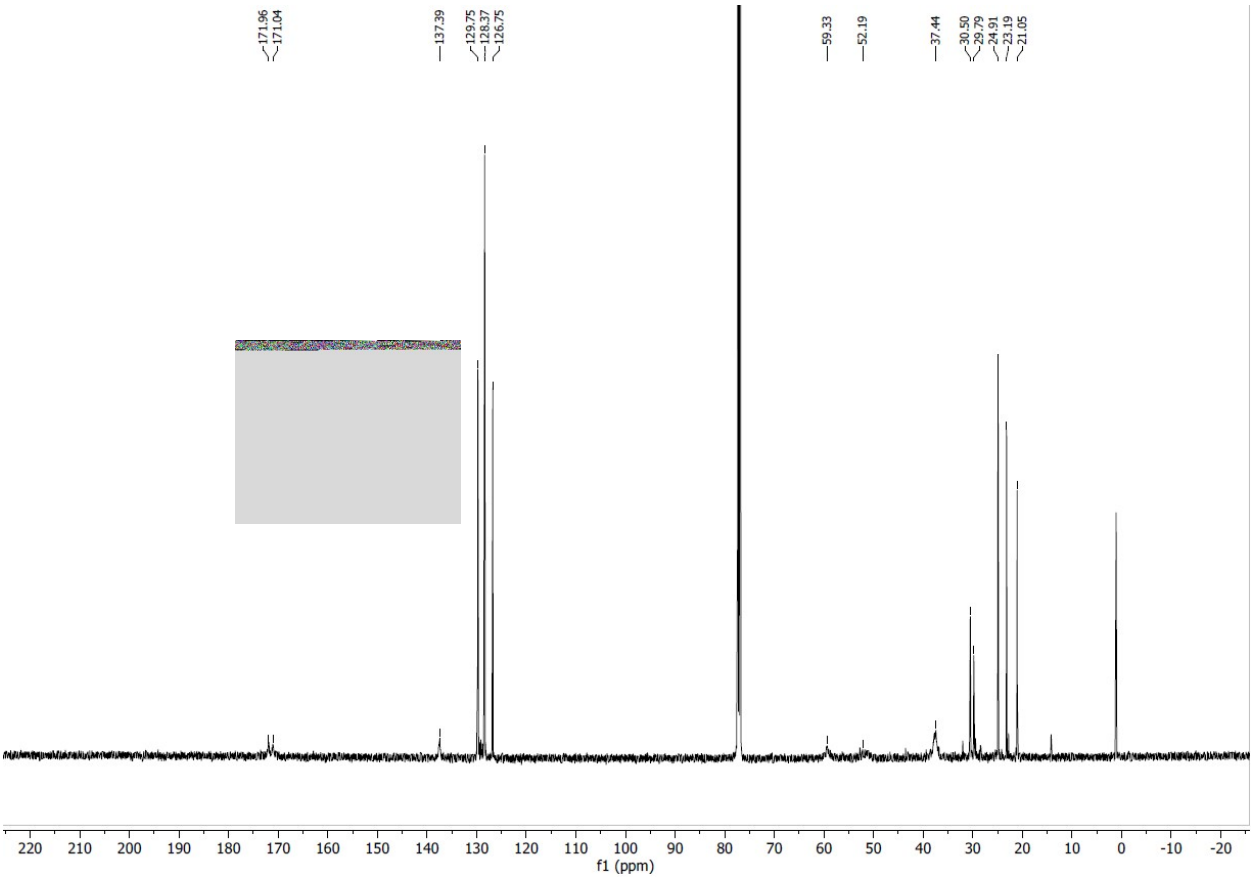


^{1}H NMR of (3*R*,6*R*,9*R*,12*R*)-3,9-dibenzyl-6,12-diisobutyl-1,7-dimethyl-1,4,7,10-tetraazacyclododecane-2,5,8,11-tetraone.



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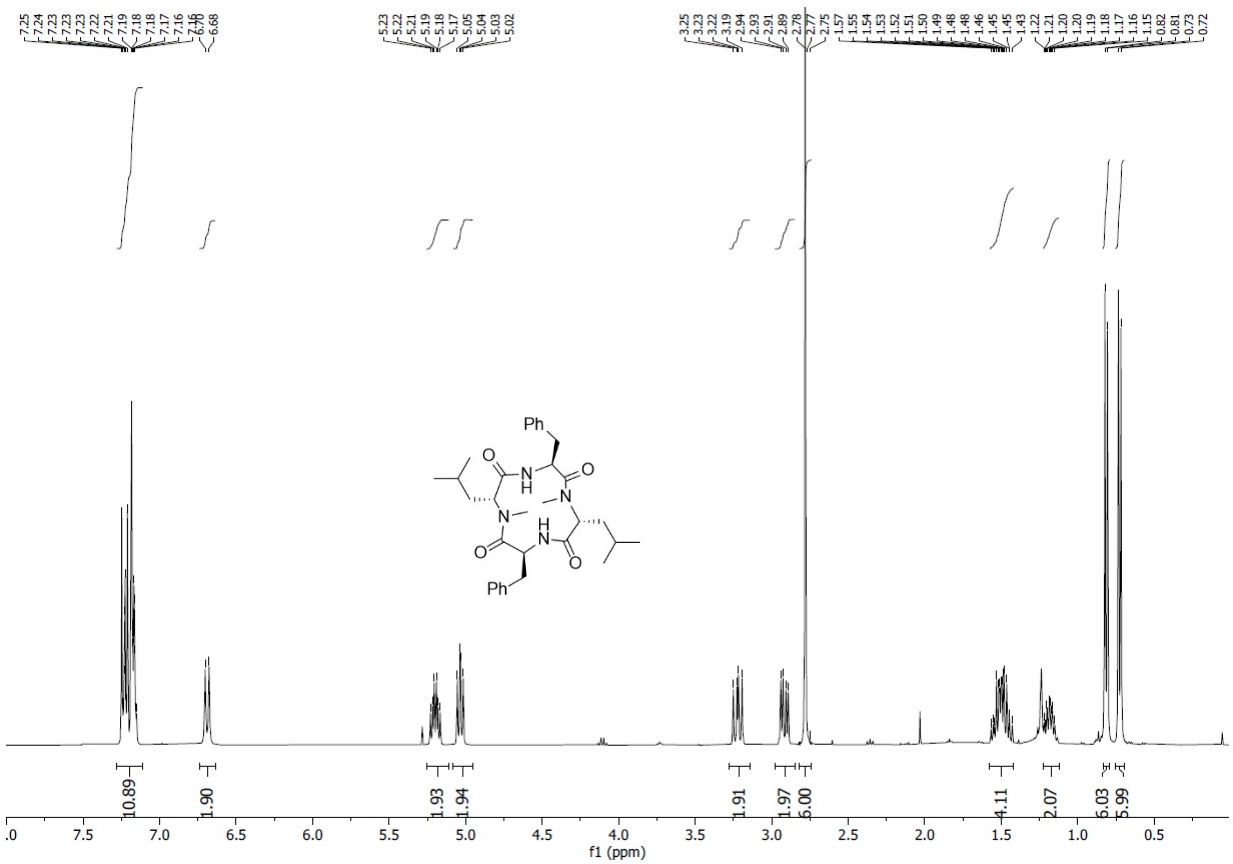

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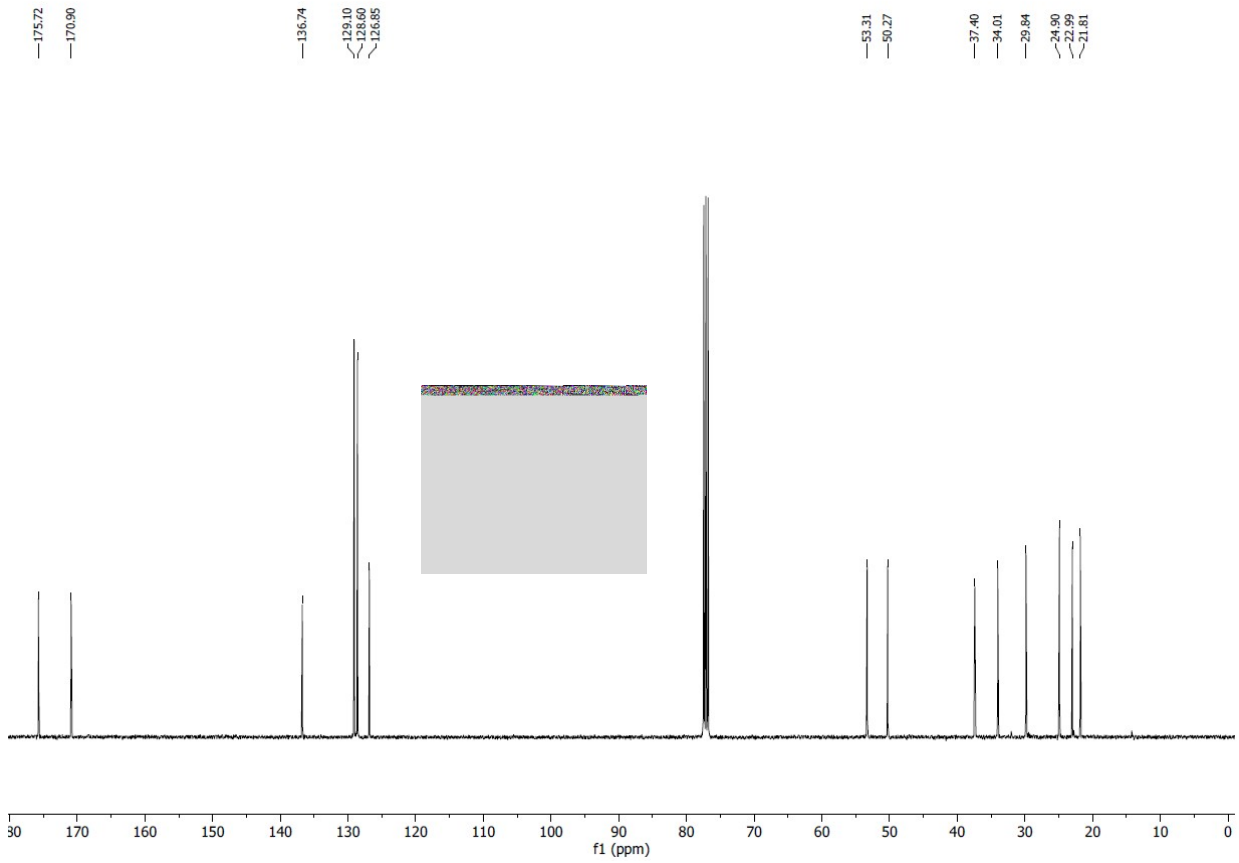

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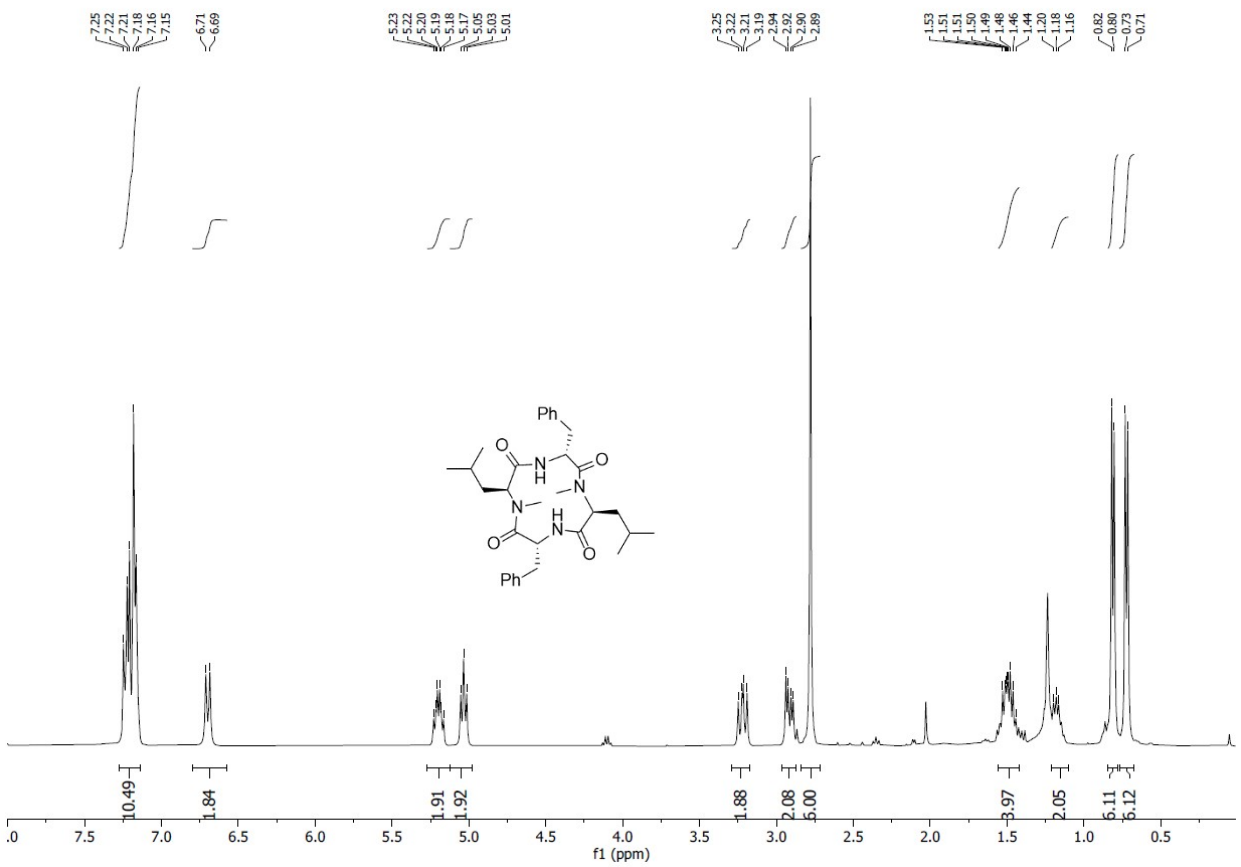

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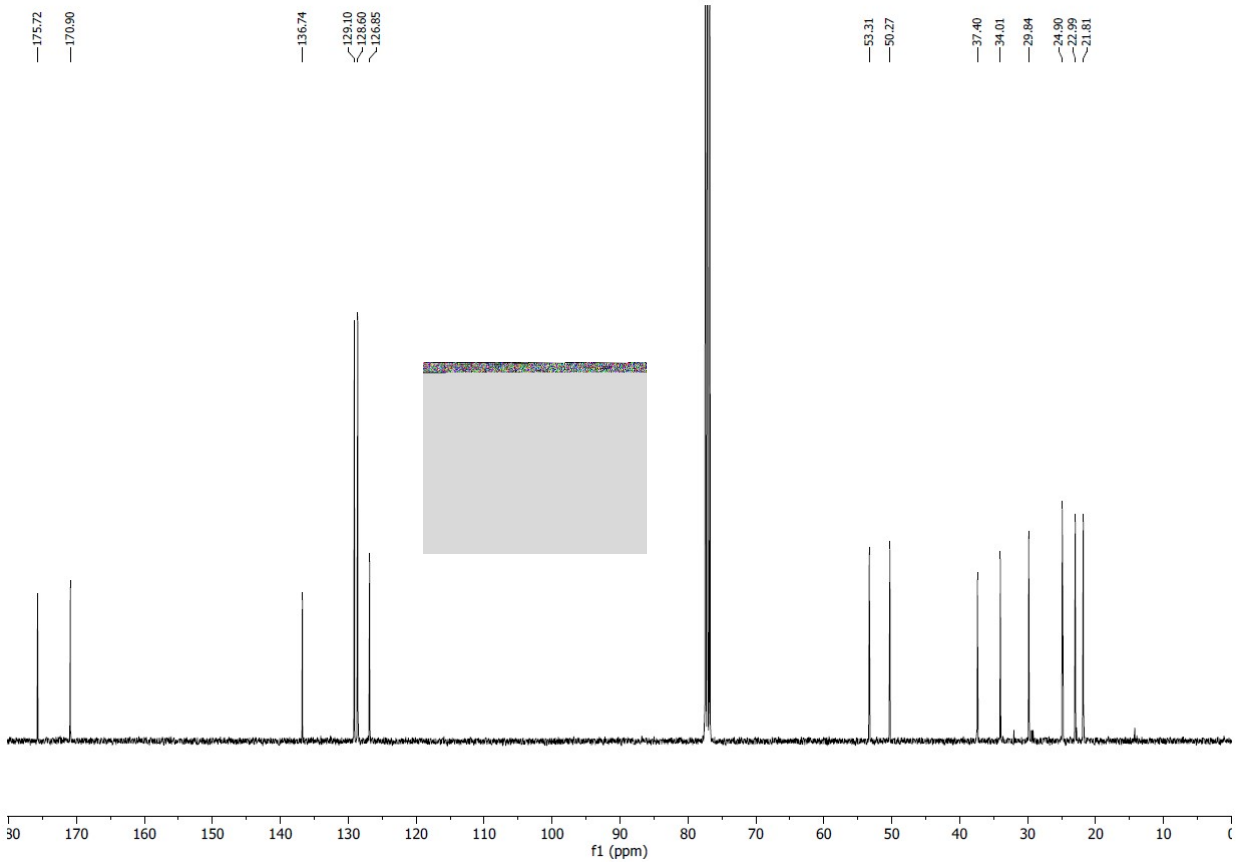

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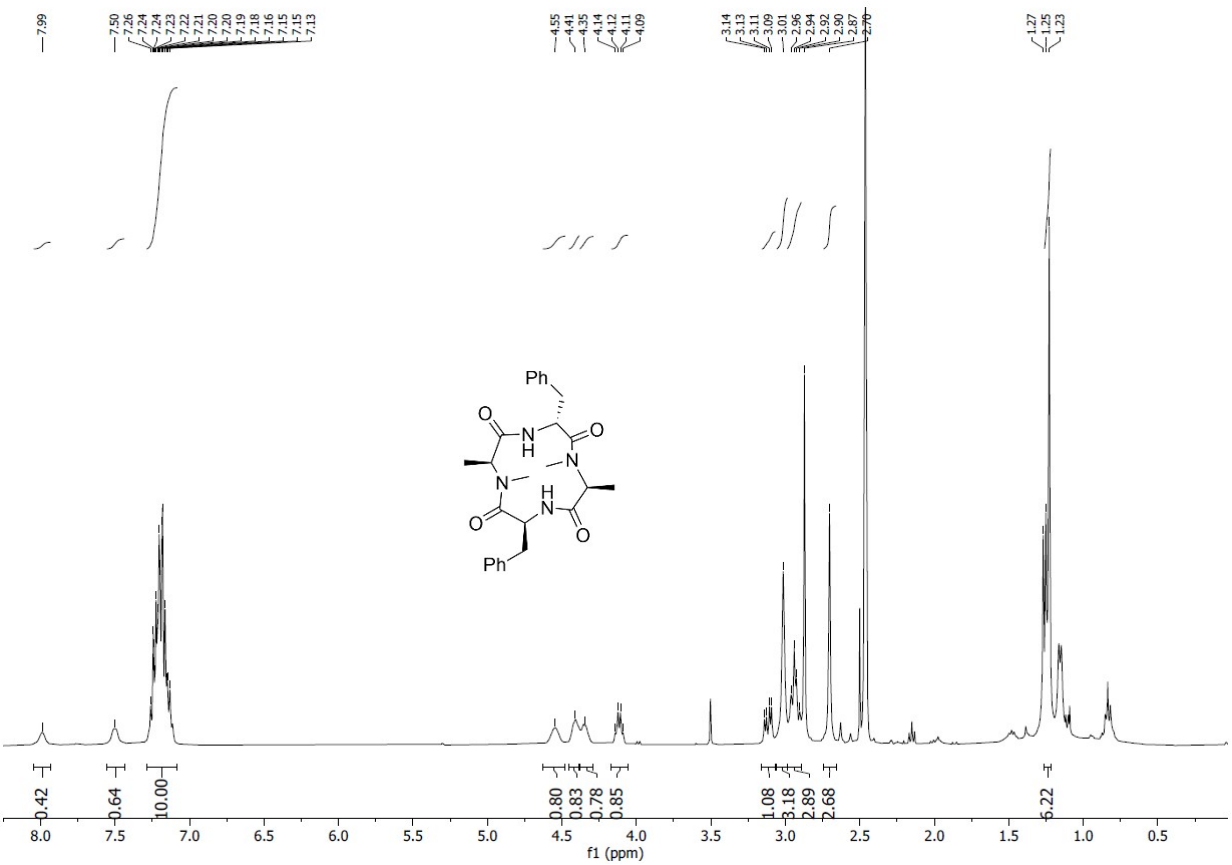

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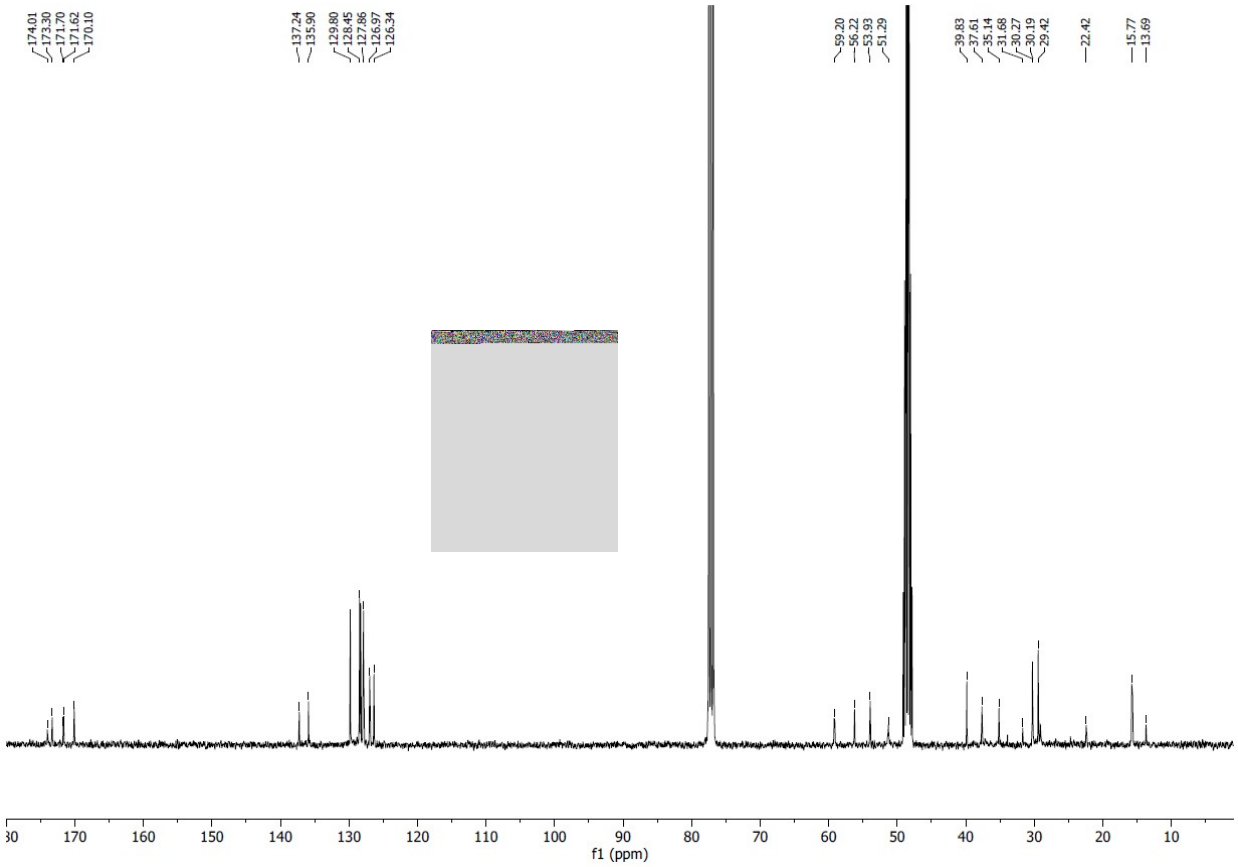

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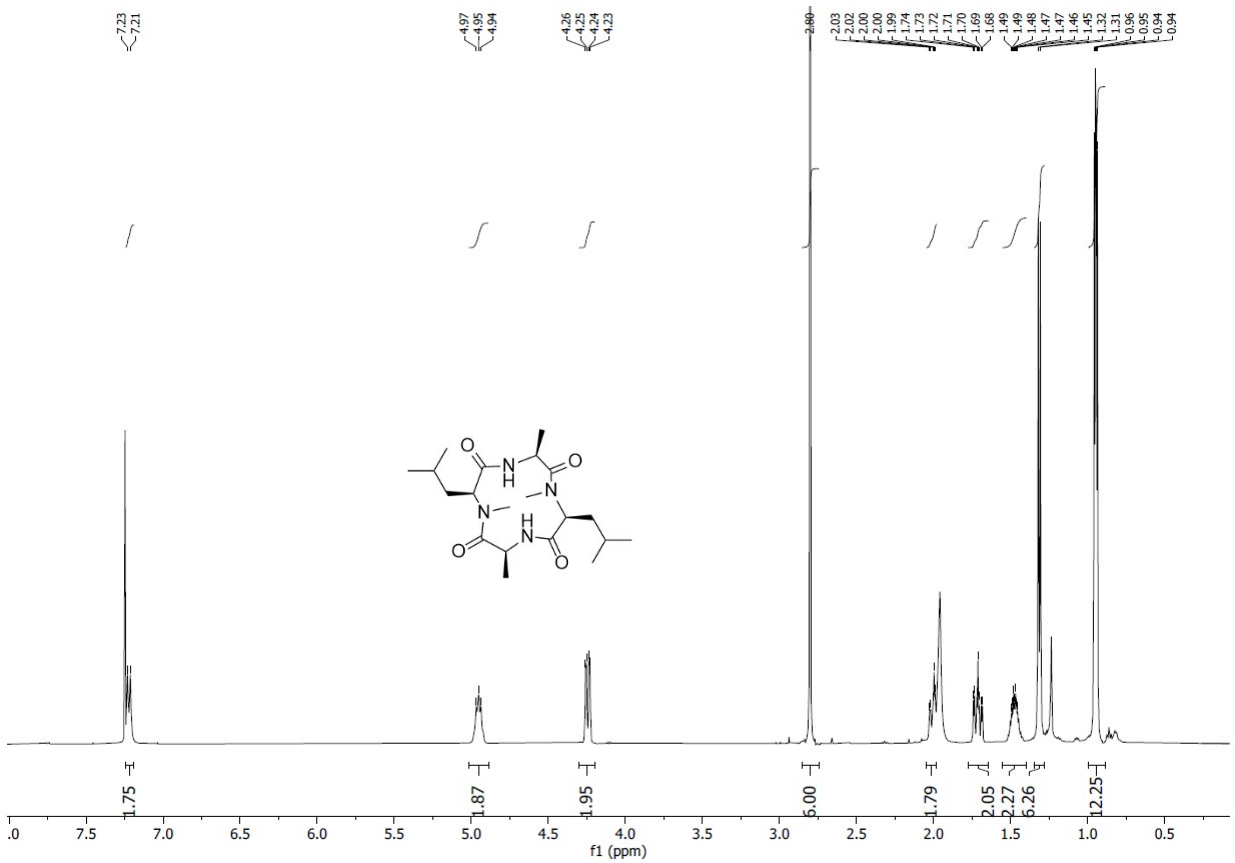

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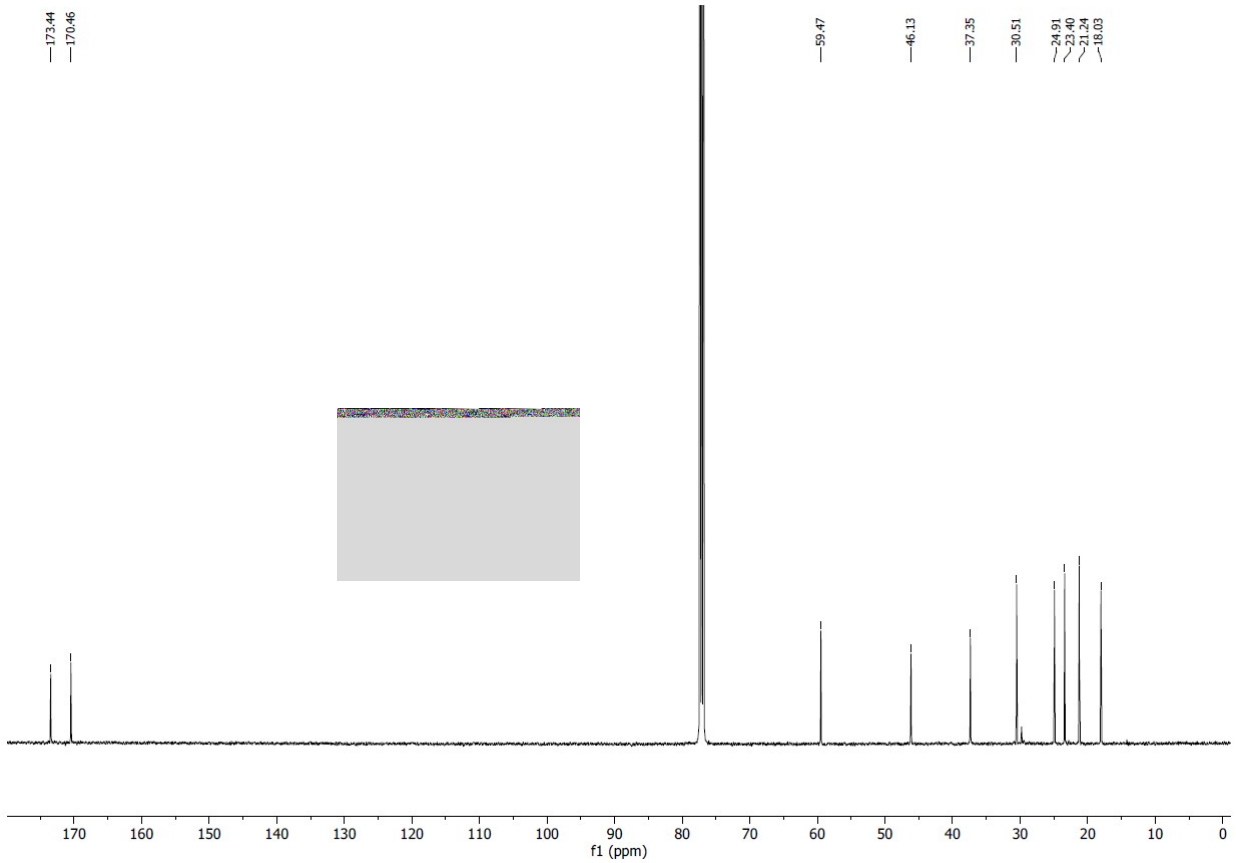

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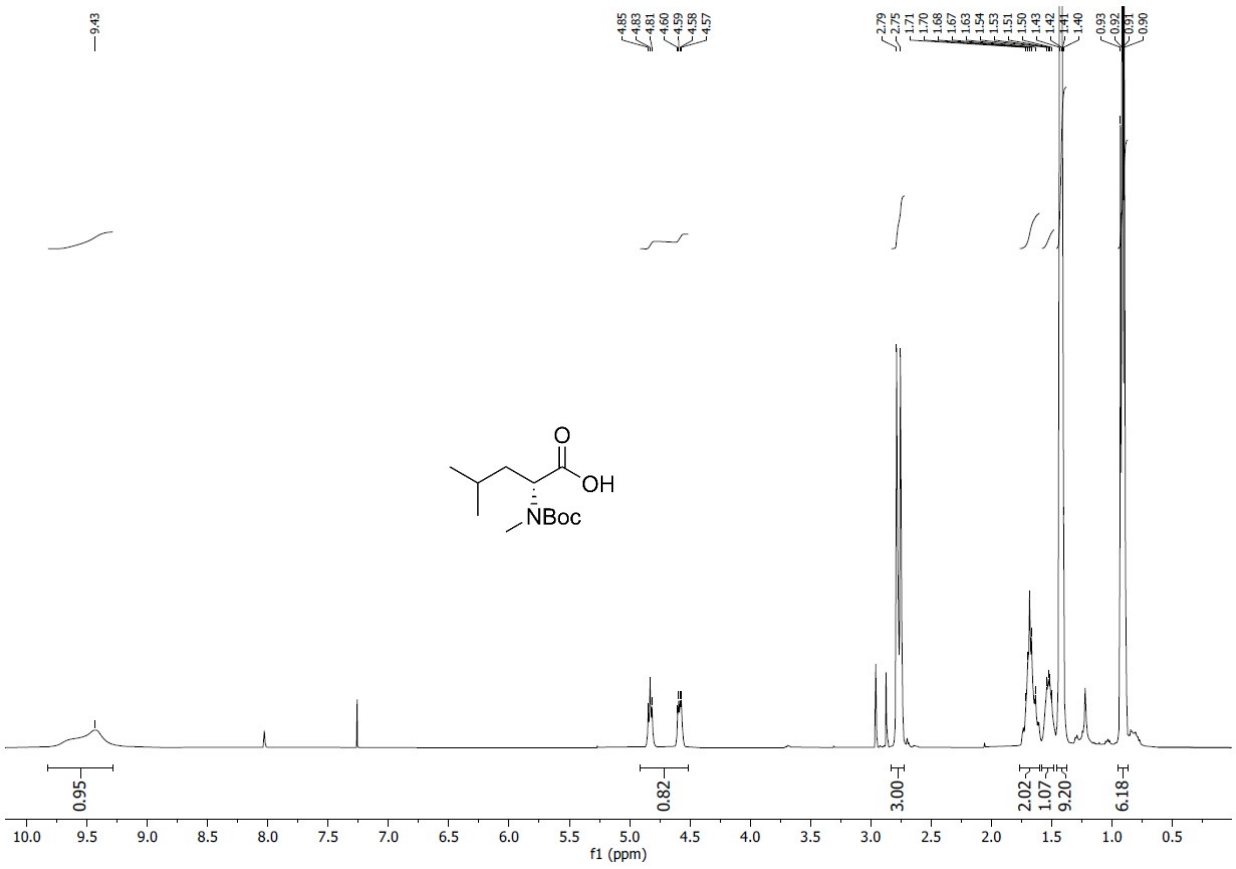
9.43
4.85
4.83
4.81
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4.59
4.58
4.57
2.79
2.75
1.71
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1.67
1.63
1.54
1.53
1.51
1.50
1.43
1.42
1.41
1.40
0.93
0.92
0.91
0.90
O
OH
NBoc
0.95
0.82
3.00
2.02
1.07
9.20
6.18
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

^{1}H NMR of **S5**.

References

(1) *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*, 11th ed.; Wayne, **2018**.

(2) Bossler, H.G.; Seebach, D. Peptide Enolates. *C*-Alkylation of Glycine Residues in linear tri-, tetra-, and pentapeptides *via* dilithium azadienediolates. *Helv. Chim. Acta* **1994**, *77*, 1124—1165. https://doi.org/10.1002/hlca.19940770424.