

Medium optimization of *Streptomyces* sp. 17944 for tirandamycin B production and isolation and structural elucidation of tirandamycins H, I, and J

Mostafa E. Rateb,¹ Zhiguo Yu,¹ Yijun Yan,^{1,4} Dong Yang,¹ Tingting Huang,¹ Sanja Vodanovic-Jankovic,⁵ Michael A. Kron,⁵ and Ben Shen*^{1,2,3}

¹Department of Chemistry, ²Department of Molecular Therapeutics, and ³Natural Products Library Initiative at The Scripps Research Institute, The Scripps Research Institute, Jupiter, Florida 33458, United States; ⁴School of Life Science, Northeast Agricultural University, Harbin, Heilongjiang 150030, China; ⁵Bioengineering Center and Department of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, United States

*To whom correspondence should be addressed: Ben Shen, The Scripps Research Institute, 130 Scripps Way, #3A1, Jupiter, Florida 33458, United States. Tel: (561) 228-2456, Fax: (561) 228-2472, Email: shenb@scripps.edu

Running head: Tirandamycin B and congeners from *Streptomyces* sp. 17944

Keywords: AsnRS inhibitor, filariasis, medium optimization, *S. sp* 17944, tirandamycin

Supplementary Information

General experimental procedures	S2
References	S2
Figure S1. Sequence alignment of TamI from <i>S. sp.</i> 17944 and its homologues	S3
Figure S2. HRESIMS spectrum of TAM H (6)	S4
Figure S3. ¹ H NMR spectrum of TAM H (6)	S5
Figure S4. ¹³ C NMR spectrum of TAM H (6)	S6
Figure S5. HRESIMS spectrum of TAM I (7)	S7
Figure S6. ¹ H NMR spectrum of TAM I (7)	S8
Figure S7. ¹³ C NMR spectrum of TAM I (7)	S9
Figure S8. HRESIMS spectrum of TAM J (8)	S10
Figure S9. ¹ H NMR spectrum of TAM J (8)	S11
Figure S10. ¹³ C NMR spectrum of TAM J (8)	S12

General experimental procedures

Optical rotations were measured in methanol on a Perkin-Elmer 241 instrument at the sodium D line (589 nm). The UV spectra were acquired in methanol using Thermo Scientific NanoDrop 2000C UV-Vis spectrophotometer. ¹H and ¹³C NMR spectra were recorded at 25°C with a Bruker Avance III 700 MHz ULTRA Shield. HRESIMS were acquired with a Bruker Daltonics Ultra High Resolution TOF – Maxis spectrometer. Semi-preparative HPLC was performed on a Varian HPLC system with an Altima C-18 column (5 μ, 10.0 x 250 mm, Alltech, Deerfield, IL). Column chromatography was performed using SiO₂ (230-400 mesh, Natland International Corporation, Research Triangle Park, NC) and Sephadex LH-20 (Pharmacia, Kalamazoo, MI). All chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification.

S. sp. 17944,¹ *E. coli* ET12567,² pSET152,³ pKC1139,³ and ISP-2,² ISP-4,² TSB,² MS,² SLY^{4,5} were previously described. All common antibiotics are from standard commercial sources. DNA isolation, cloning, sequencing, PCR, and other standard microbiology and recombinant DNA experiments followed manufacture's protocols or literature procedures.^{2,6}

References

1. Yu, Z. *et al.* Tirandamycins from *Streptomyces sp.* 17944 inhibiting the parasite *Brugia malayi* asparagine tRNA synthetase, *Org. Lett.* **13**, 2034–7 (2011).
2. Kieser, T., M. J. Bibb, M. J. Buttner, K. F. Chater, & D. A. Hopwood. *Practical Streptomyces Genetics*. John Innes Foundation, Norwich, United Kingdom (2000).
3. Bierman, M., *et al.* Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene* **116**, 43-49 (1992).
4. Smanski, M. J., Peterson, R. M., Rajski, S. R. & Shen, B. Engineered *Streptomyces platensis* strains that overproduce antibiotics platensimycin and platencin. *Antimicrob. Agents Chemother.* **53**, 1299-1304 (2009).
5. Yu, Z., Rateb, M. E., Smanski, M. J., Peterson, R. M. & Shen, B. Isolation and structural elucidation of glucoside congeners of platencin from *Streptomyces platensis* SB12600. *J. Antibiot.* **66**, doi:10.1038/ja.2013.1.
6. Sambrook, J., Fritsch, E. F. & Maniatis, T. *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).

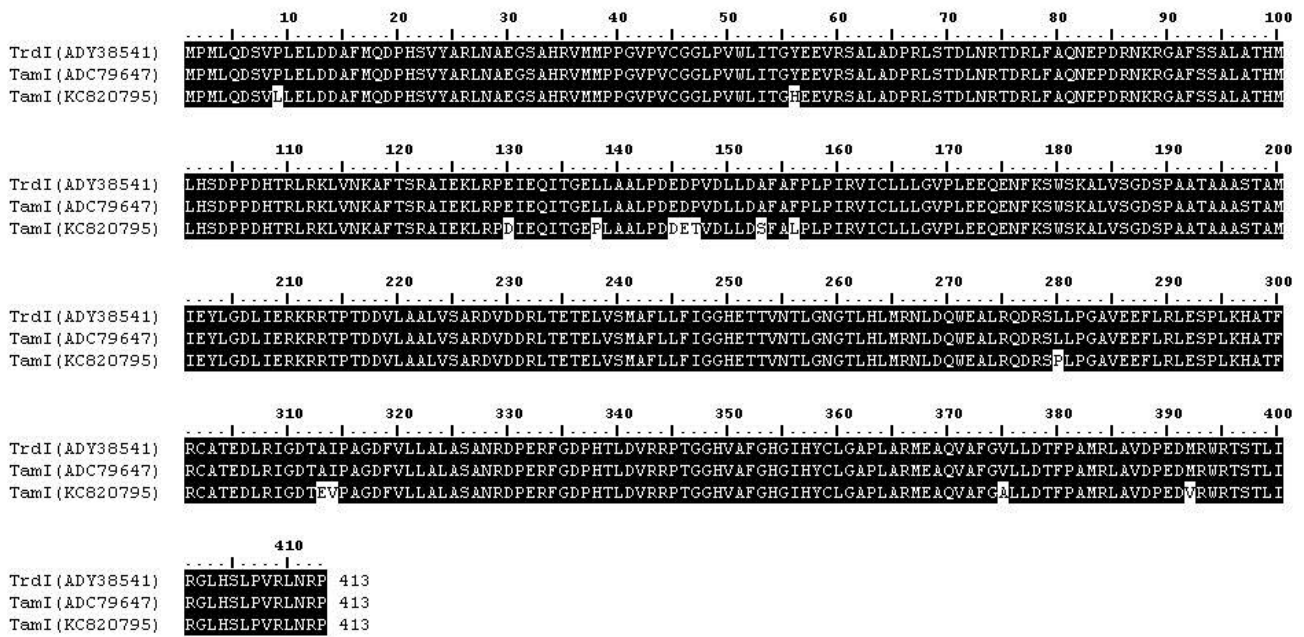


Figure S1. Sequence alignment of TamI from *S. sp.* 17944 and its homologues TamI from *S. sp.* 307-9 and TrdI from *S. sp.* SCSIO 1666. TrdI (ADY38541) and TamI (ADC79647) are protein accession numbers and TamI (KC820795) is the nucleotide accession number at GenBank, and multiple sequence alignments were performed by using the CLUSTAL X program.

435 #55-59 RT: 1.55-1.66 AV: 5 NL: 3.68E6
T: FTMS + p ESI Full ms [150.00-2000.00]

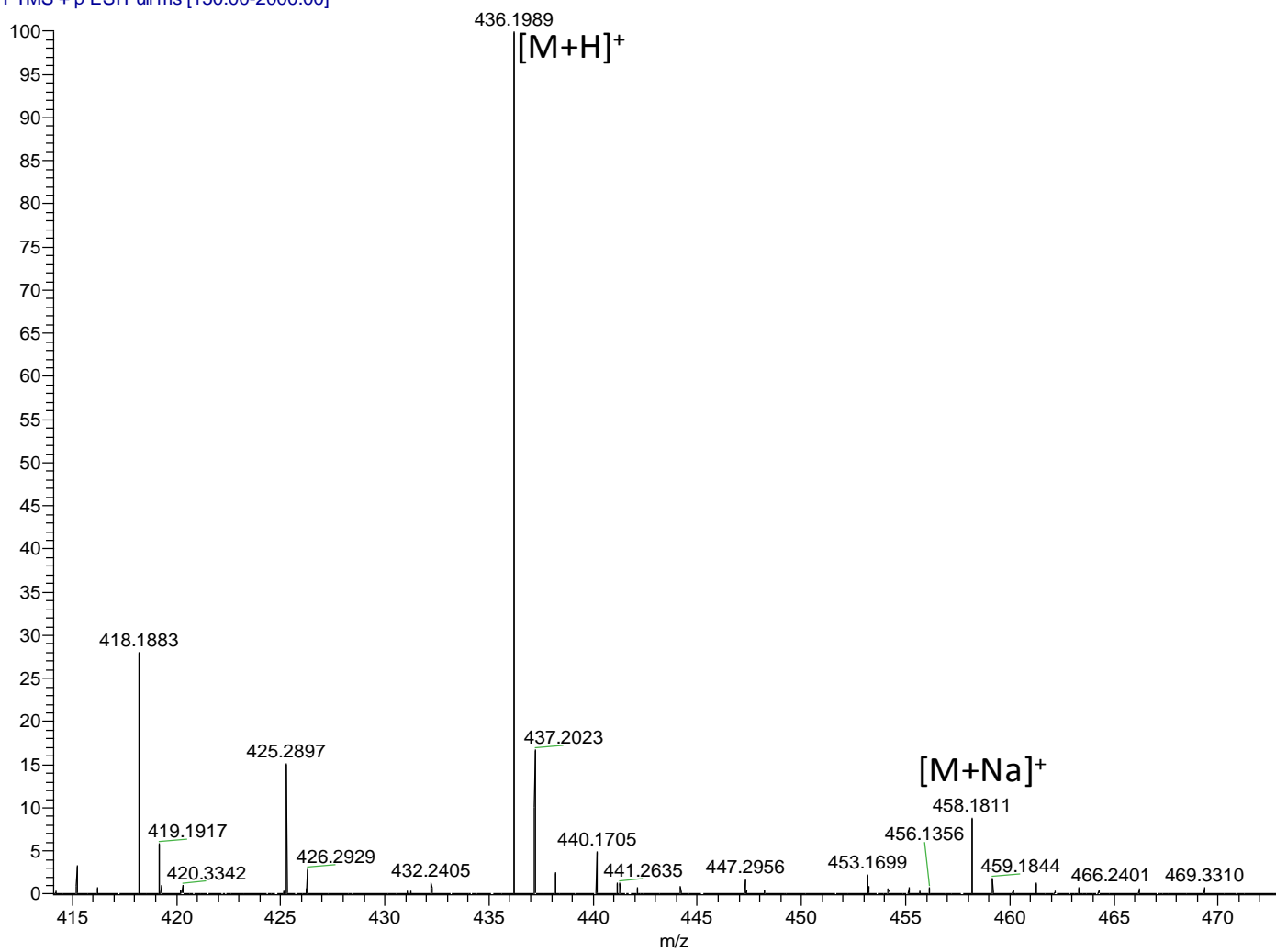


Figure S2. HRESIMS spectrum of TAM H (6)

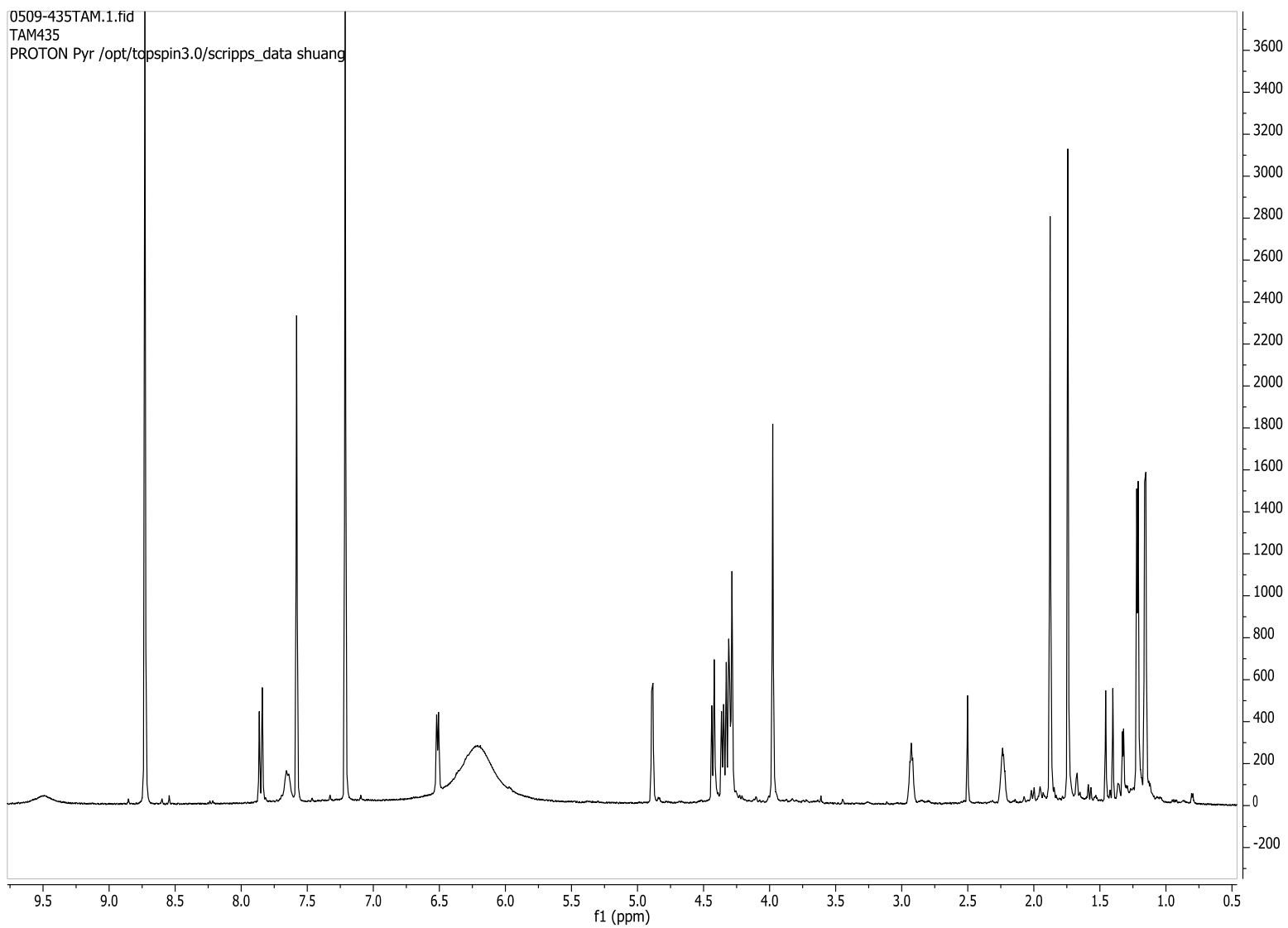


Figure S3. ^1H NMR spectrum of TAM H (6)

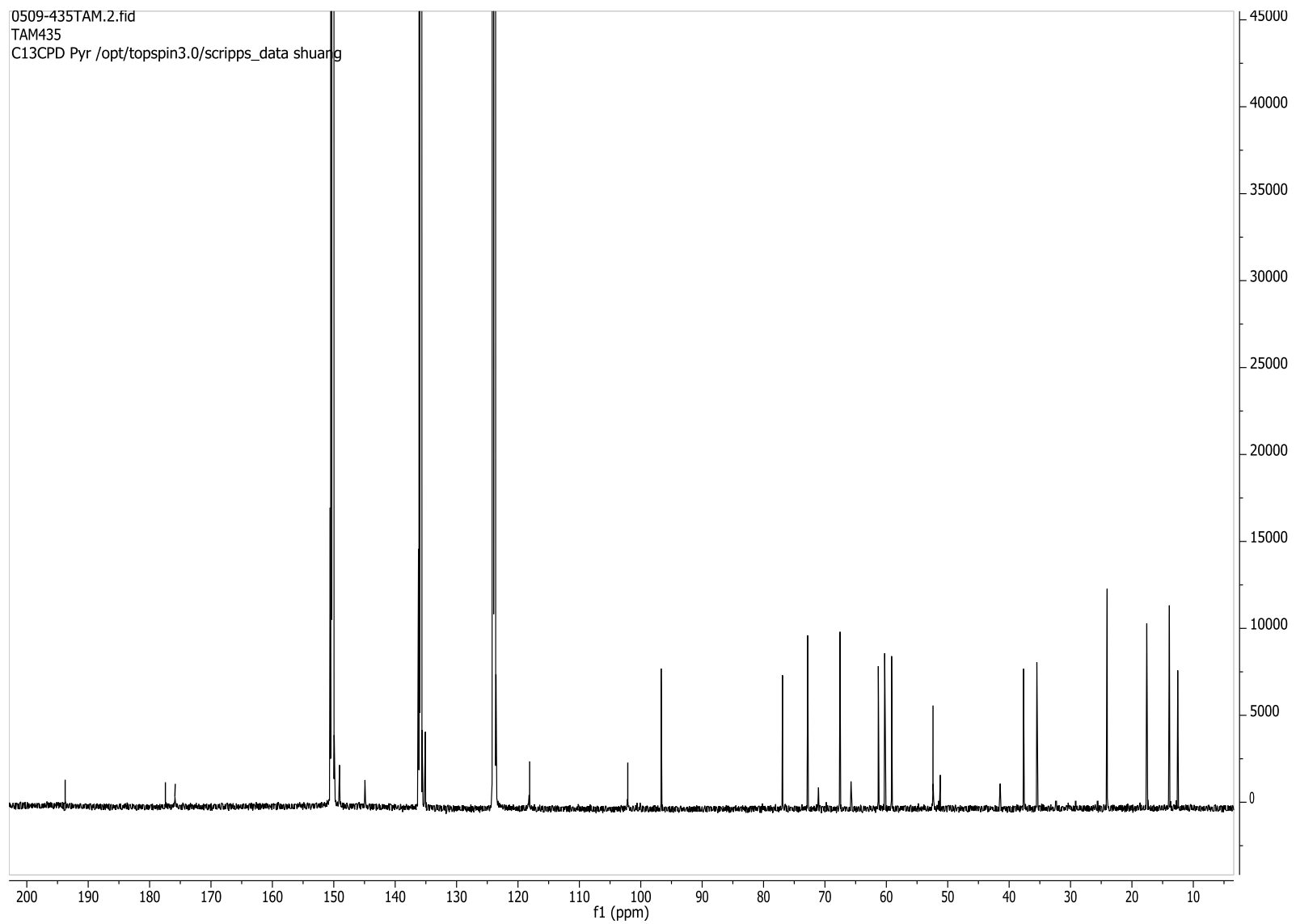


Figure S4. ^{13}C NMR spectrum of TAM H (6)

581 #45-48 RT: 1.25-1.33 AV: 4 NL: 2.78E6
T: FTMS + p ESI Full ms [150.00-2000.00]

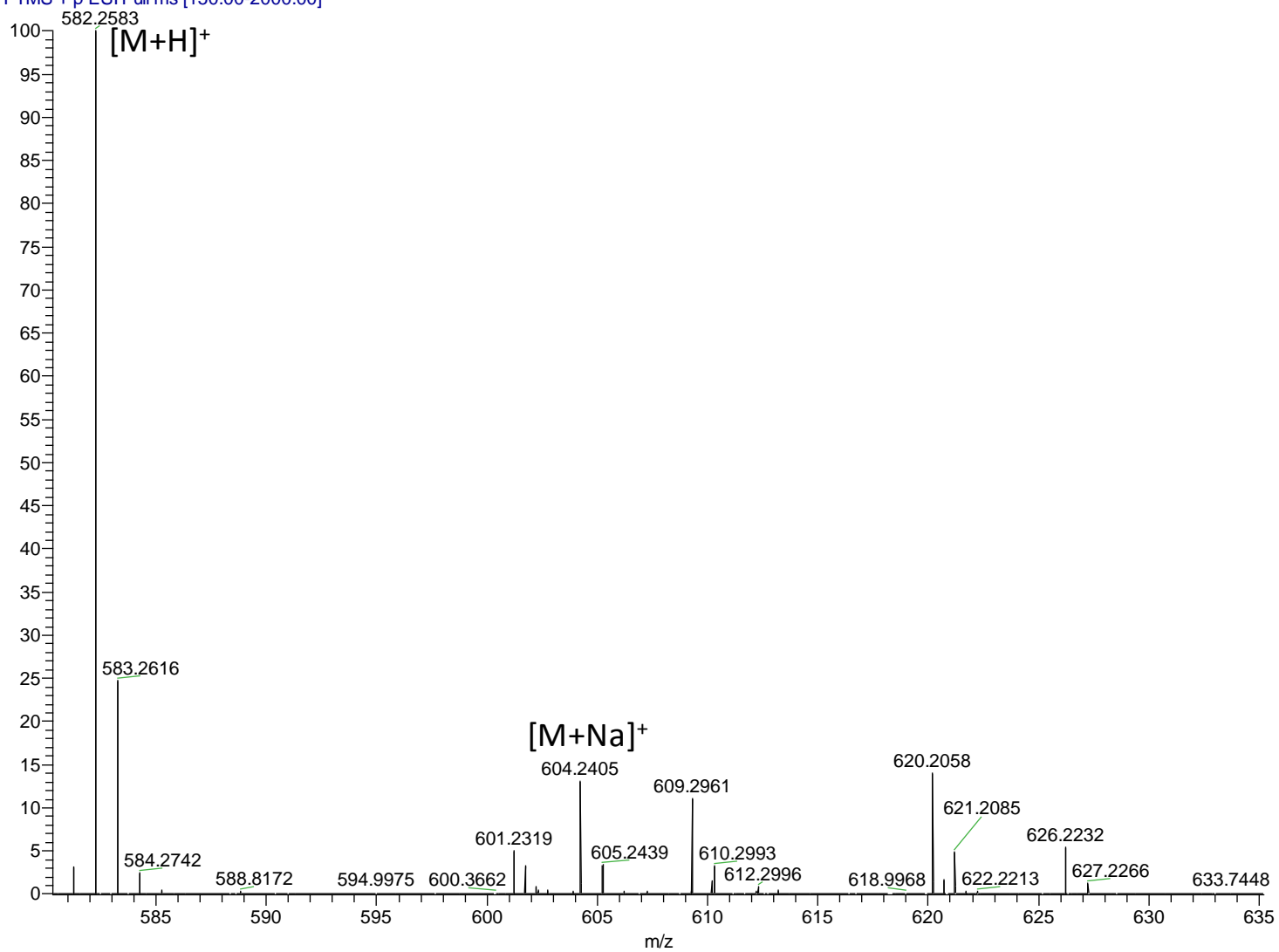


Figure S5. HRESIMS spectrum of TAM I (7)

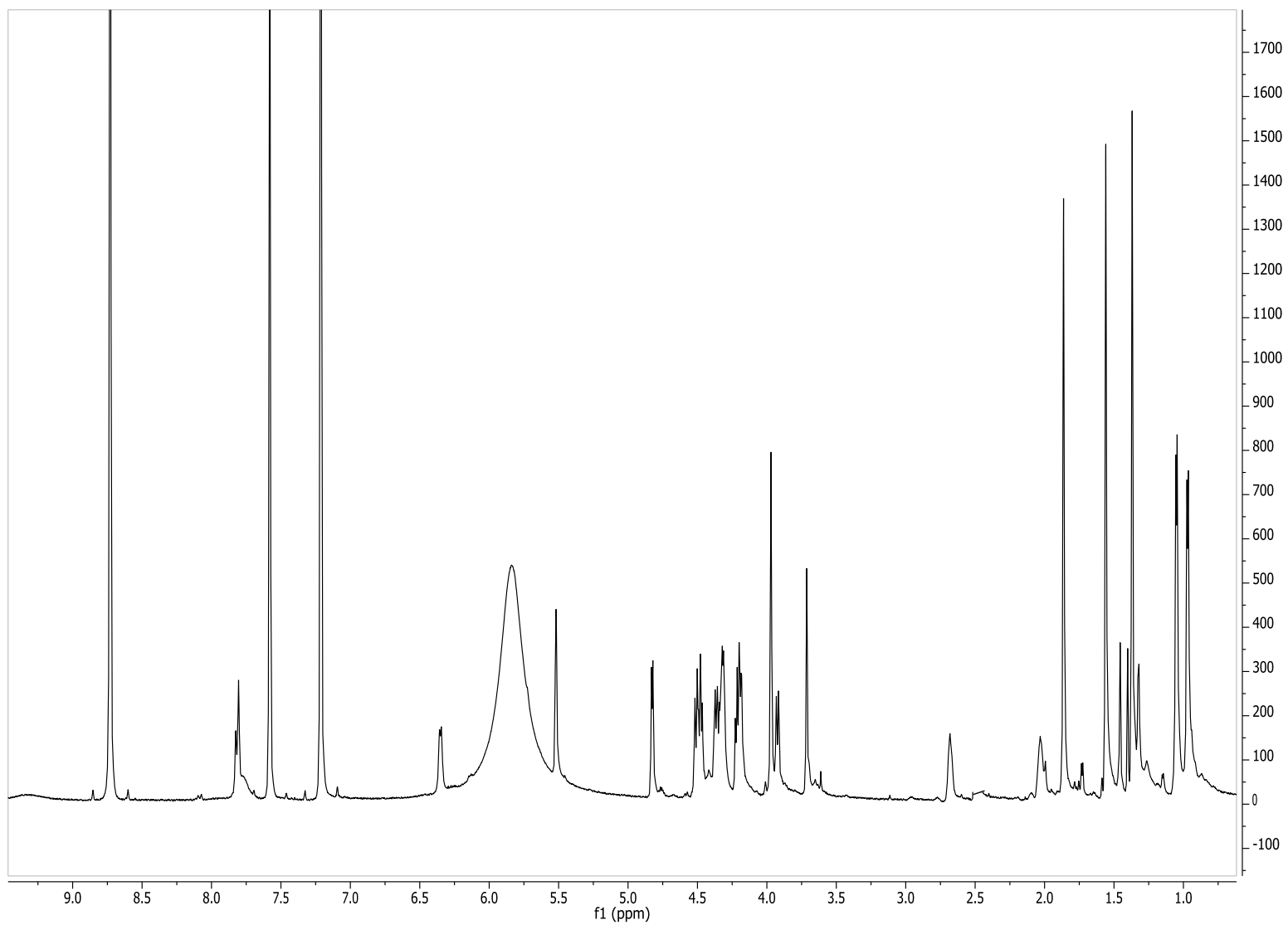


Figure S6. ^1H NMR spectrum of TAM I (7)

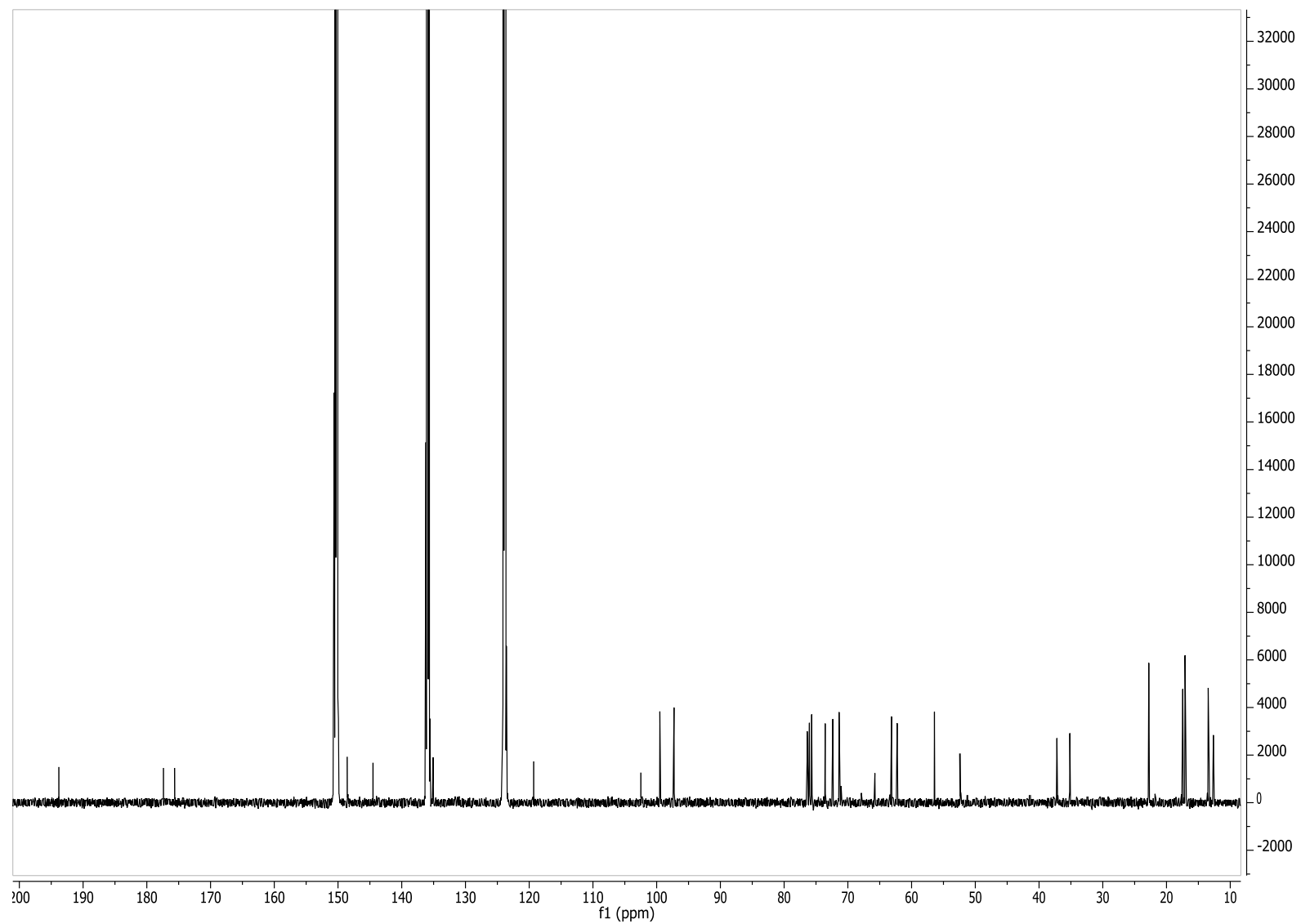


Figure S7. ^{13}C NMR spectrum of TAM I (7)

595 #60-70 RT: 1.68-1.96 AV: 11 NL: 8.58E5
T: FTMS + p ESI Full ms [150.00-2000.00]

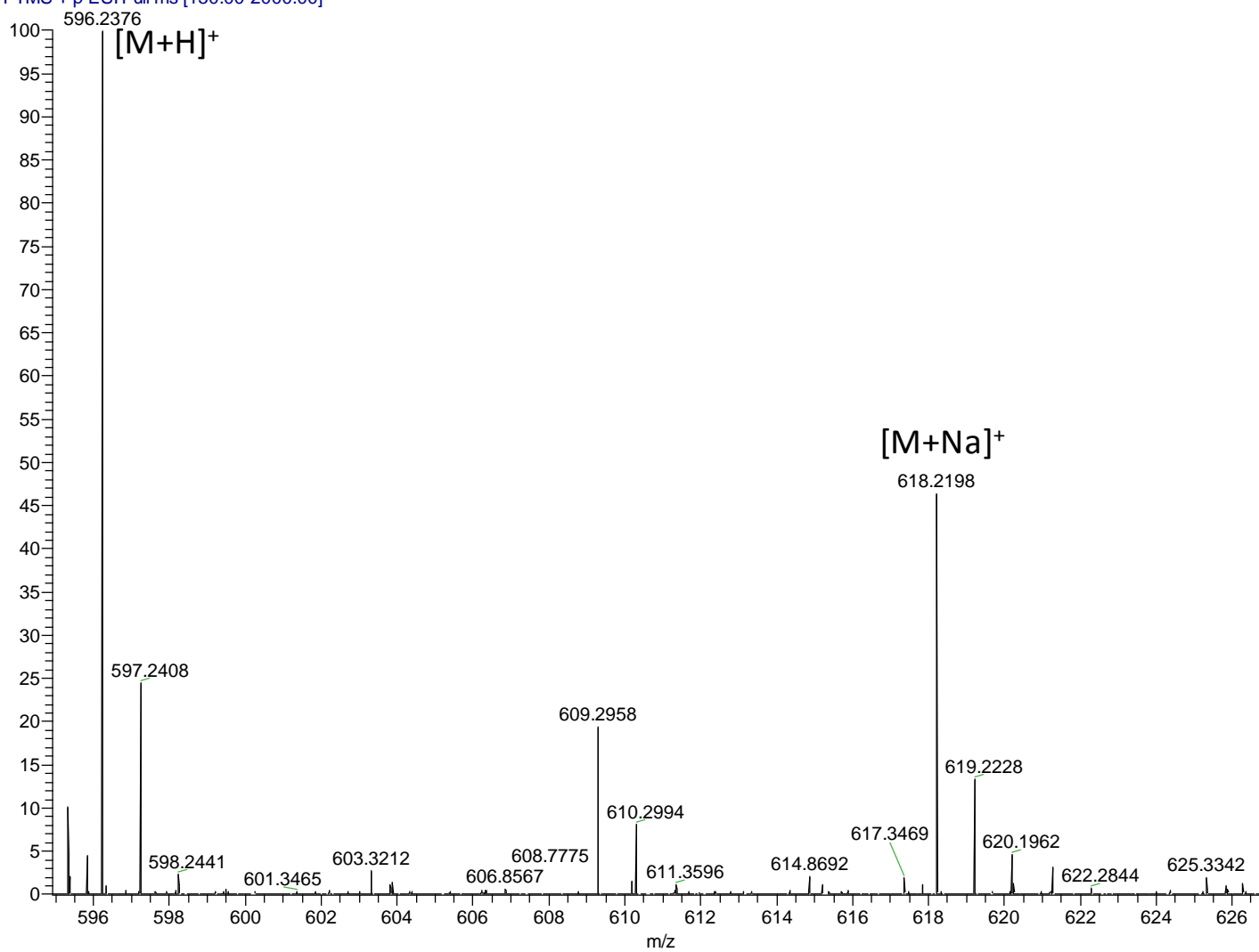


Figure S8. HRESIMS spectrum of TAM J (8)

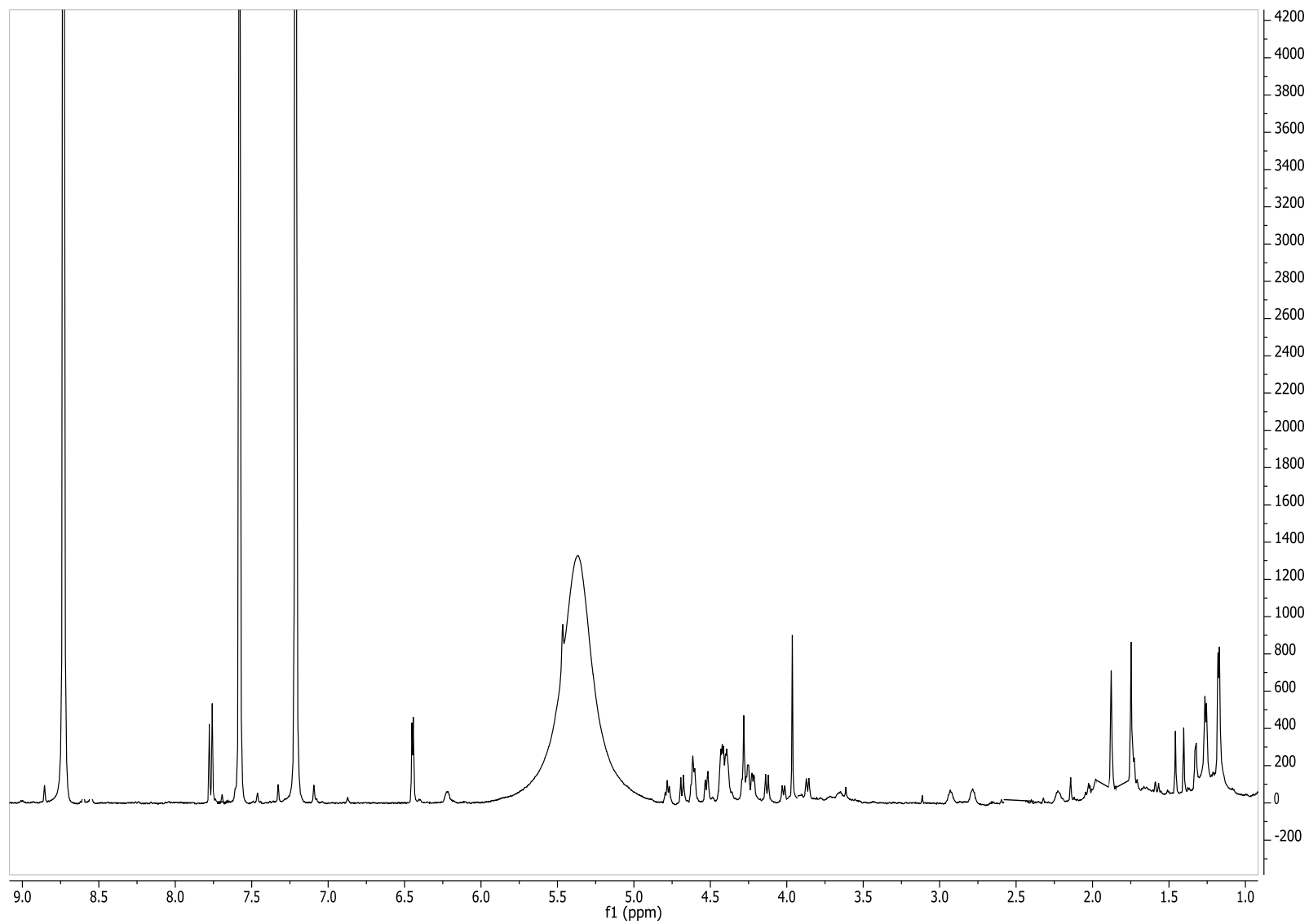


Figure S9. ¹H NMR spectrum of TAM J (8)

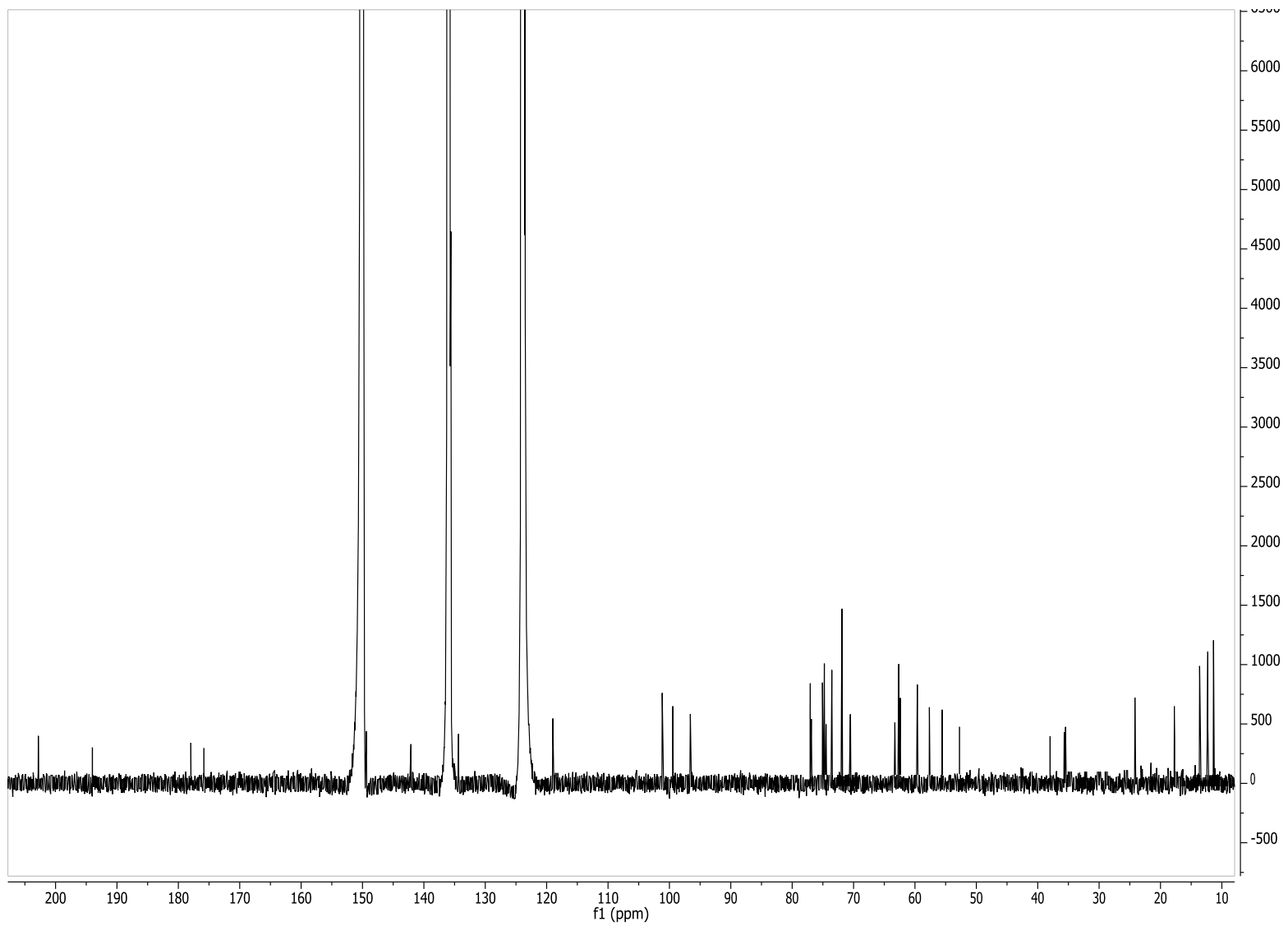


Figure S10. ^{13}C NMR spectrum of TAM J (8)