Supporting Information

Development of Novel CXC Chemokine Receptor 7 (CXCR7) Ligands: Selectivity Switch from CXCR4 Antagonists with a Cyclic Pentapeptide Scaffold

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Table S1. Characterization data of synthetic peptides by MALDI TOF-MS

Peptide[a]	Sequence	Formula	*m*/*z* [MH^+]	
			Calcd.	Observed
1i	*cyclo*(-D-Tyr-L-Arg-L-Arg-L-Nal-L-MeAla-)	$C_{38}H_{51}N_{11}O_6$	758.41	758.39
1j	*cyclo*(-D-Tyr-L-Arg-L-Arg-L-Nal-D-MeAla-)	$C_{38}H_{51}N_{11}O_6$	758.41	758.42
2a	*cyclo*(-L-Tyr-L-Arg-L-Arg-L-Nal-L-Pro-)	$C_{39}H_{51}N_{11}O_6$	770.41	770.38
2b	*cyclo*(-D-Tyr-D-Arg-L-Arg-L-Nal-L-Pro-)	$C_{39}H_{51}N_{11}O_6$	770.41	770.46
2c	*cyclo*(-D-Tyr-L-Arg-D-Arg-L-Nal-L-Pro-)	$C_{39}H_{51}N_{11}O_6$	770.41	770.41
2d	*cyclo*(-D-Tyr-L-Arg-L-Arg-D-Nal-L-Pro-)	$C_{39}H_{51}N_{11}O_6$	770.41	770.44
3a	*cyclo*(-D-MeTyr-L-Arg-L-Arg-L-Nal-L-Pro-)	$C_{40}H_{53}N_{11}O_6$	784.43	784.36
3b	*cyclo*(-D-Tyr-L-MeArg-L-Arg-L-Nal-L-Pro-)	$C_{40}H_{53}N_{11}O_6$	784.43	784.41
3c	*cyclo*(-D-Tyr-L-Arg-L-MeArg-L-Nal-L-Pro-)	$C_{40}H_{53}N_{11}O_6$	784.43	784.37
3d	*cyclo*(-D-Tyr-L-Arg-L-Arg-L-MeNal-L-Pro-)	$C_{40}H_{53}N_{11}O_6$	784.43	784.43
4a	*cyclo*(-L-MeTyr-L-Arg-L-Arg-L-Nal-L-Pro-)	$C_{40}H_{53}N_{11}O_6$	784.43	784.41
4b	*cyclo*(-D-Tyr-D-MeArg-L-Arg-L-Nal-L-Pro-)	$C_{40}H_{53}N_{11}O_6$	784.43	784.39
4c	*cyclo*(-D-Tyr-L-Arg-D-MeArg-L-Nal-L-Pro-)	$C_{40}H_{53}N_{11}O_6$	784.43	784.42
4d	*cyclo*(-D-Tyr-L-Arg-L-Arg-D-MeNal-L-Pro-)	$C_{40}H_{53}N_{11}O_6$	784.43	784.39
5a	*cyclo*(-D-Ala-L-Arg-L-Arg-L-Nal-L-Pro-)	$C_{34}H_{49}N_{11}O_5$	692.40	692.44
5b	*cyclo*(-D-Tyr-L-Ala-L-Arg-L-Nal-L-Pro-)	$C_{37}H_{46}N_8O_6$	699.36	699.40
5c	*cyclo*(-D-Tyr-L-Arg-L-Ala-L-Nal-L-Pro-)	$C_{37}H_{46}N_8O_6$	699.36	699.45
5d	*cyclo*(-D-Tyr-L-Arg-L-Arg-L-Ala-L-Pro-)	$C_{30}H_{47}N_{11}O_6$	658.38	658.40

[a] Peptides **1a-h** were previously reported.[S1,S2]

Figure S1. The representative dose-response curves of binding competition assay for CXCR7.

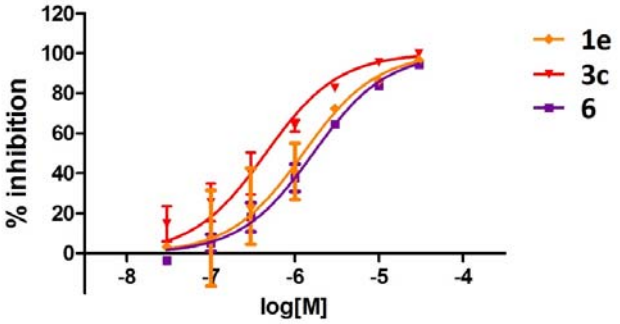


Figure S2. The representative dose-response curves of functional assays.

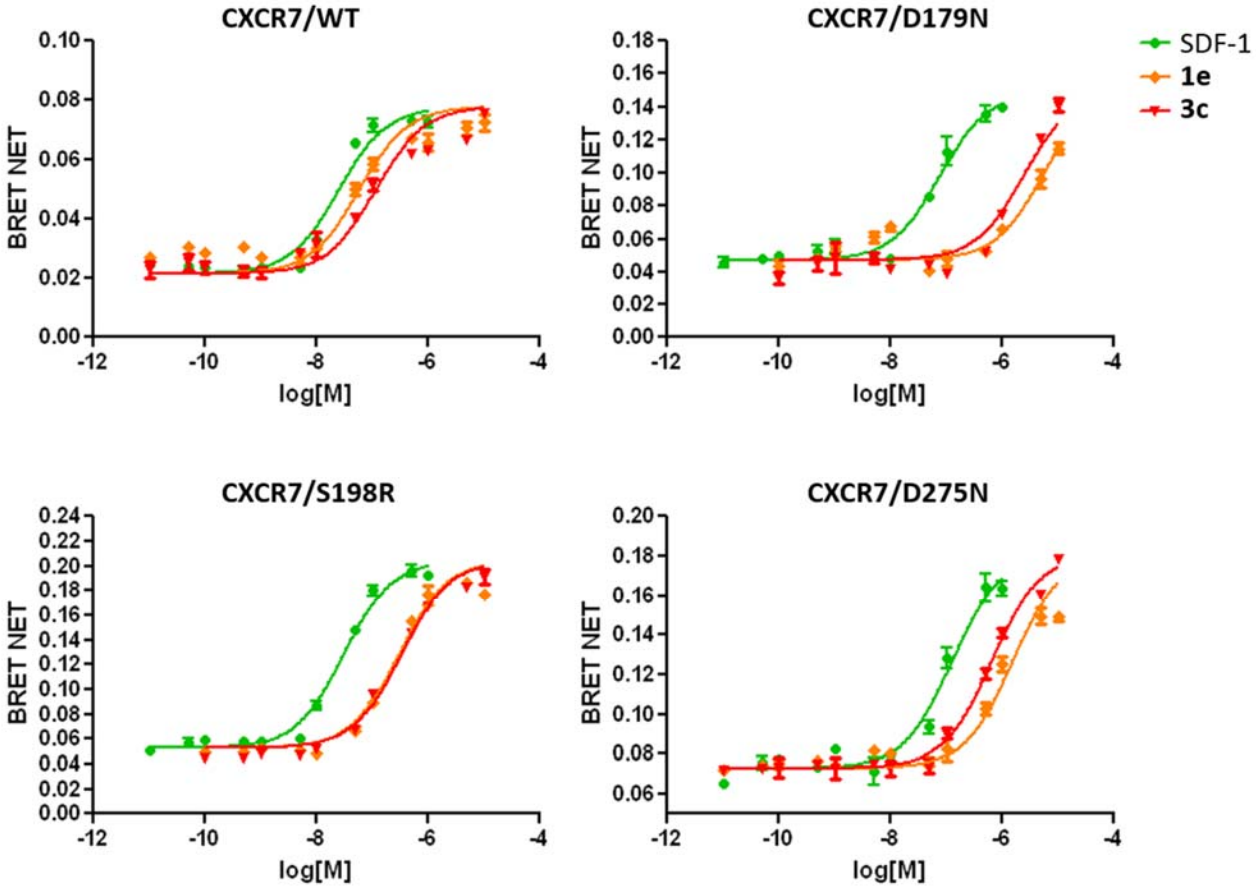


Figure S3. The binding pose of peptide **1e** for CXCR7. (A) Overall binding mode. (B) Interactions of L-Arg2. (C) Interactions of L-Arg3 and L-Nal4.

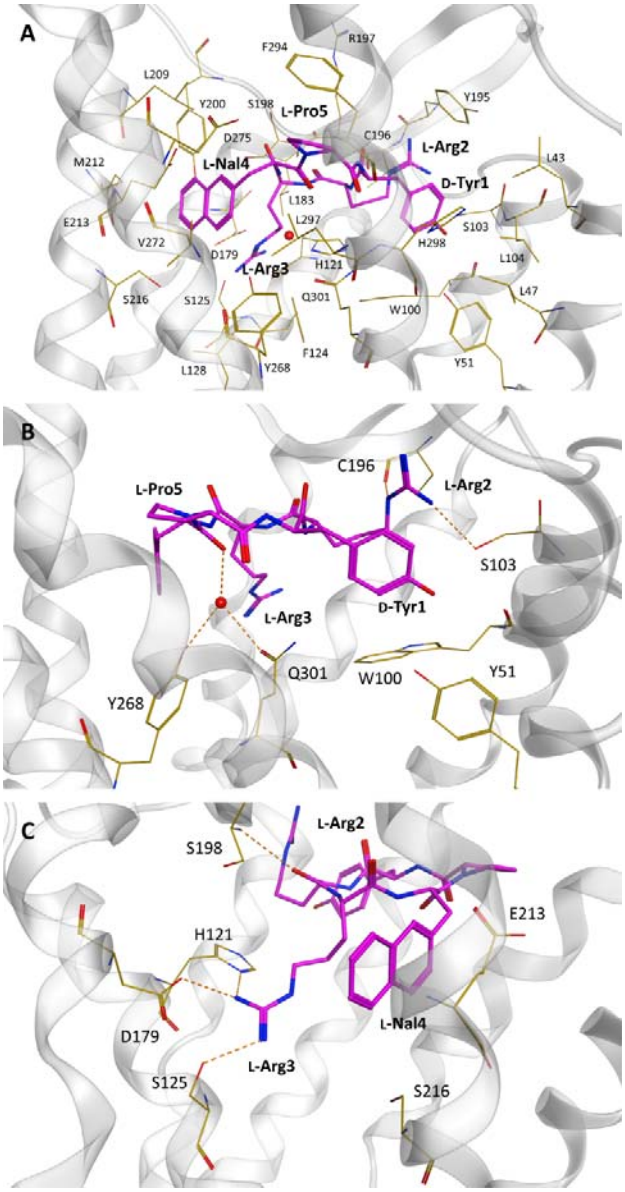
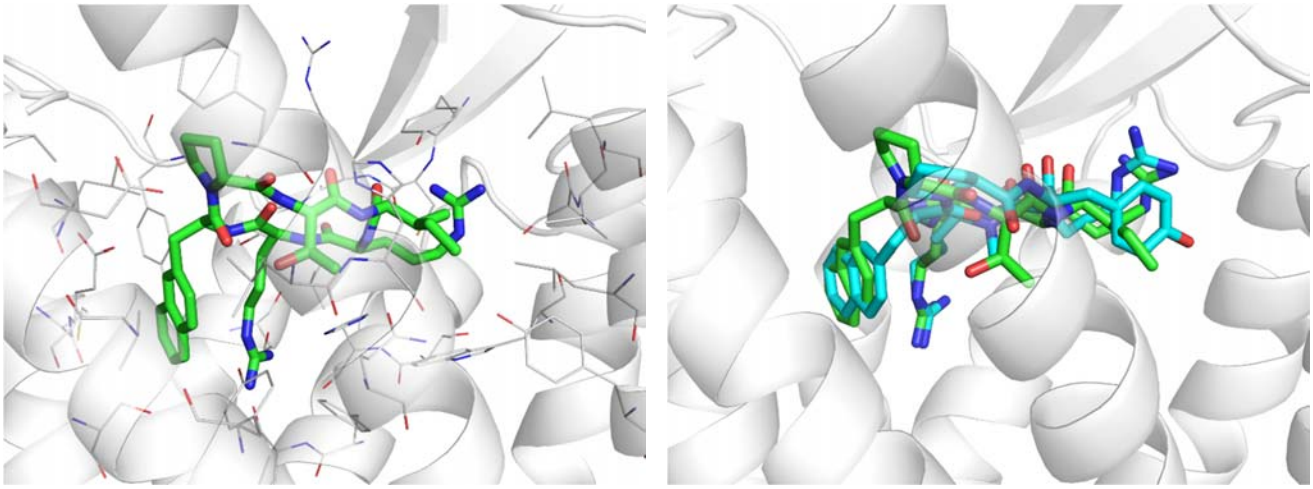


Figure S4. The plausible binding mode of peptide **6** for CXCR7. (A) Overall binding mode of **6**. (B) Superimposed structures of CXCR7-**3c** (cyan) and CXCR7-**6** (green) complexes.



Method: The three-dimensional structure of peptide **6** was built based on the FC131 structure in CXCR4-FC131 complex.[S3] The heavy atom coordinates of Arg3 and Nal4 in peptide **6** were identical to those of FC131. Subsequently, 10 ns molecular dynamics simulation in aqueous solution was carried out using Desmond. During the calculation, positional restraints (1 kcal·mol^{-1}·Å^{-2}) were applied to the heavy atoms of guanidine group in Arg3 and naphthalene in Nal4. The 2001 ligand structures were extracted from the MD trajectory and were clustered using the coordinates. The representative structure in the largest cluster of **6** was placed into the receptor structure of CXCR7-**3c** complex model. After the side chain conformations of CXCR7 and peptide **6** were manually changed to avoid bumps and distortions, the CXCR7-**6** complex structure was energy minimized using MacroModel with OPLS 2005 forcefield, in which the backbone structures of CXCR7 were fixed. In the energy-minimized model, two guanidine groups of Arg and naphthalene group in peptide **6** occupy the identical binding pockets of peptide **3c**.

References:

(S1) Fujii, N.; Oishi, S.; Hiramatsu, K.; Araki, T.; Ueda, S.; Tamamura, H.; Otaka, A.; Kusano, S.; Terakubo, S.; Nakashima, H.; Broach, J. A.; Trent, J. O.; Wang, Z.; Peiper, S. C. Molecular-size reduction of a potent CXCR4-chemokine antagonist using orthogonal combination of conformation- and sequence-based libraries. *Angew. Chem., Int. Ed.* **2003**, *42*, 3251-3253.

(S2) Ueda, S.; Oishi, S.; Wang, Z.; Araki, T.; Tamamura, H.; Cluzeau, J.; Ohno, H.; Kusano, S.; Nakashima, H.; Trent, J.O.; Peiper, S.C.; Fujii, N. Structure-activity relationships of cyclic peptide-based chemokine receptor CXCR4 antagonists: disclosing the importance of side-chain and backbone functionalities. *J. Med. Chem.* **2007**, *50*, 192-198.

(S3) Yoshikawa, Y.; Kobayashi, K.; Oishi, S.; Fujii, N.; Furuya, T. Molecular modeling study of cyclic pentapeptide CXCR4 antagonists: new insight into CXCR4-FC131 interactions. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2146-2150.

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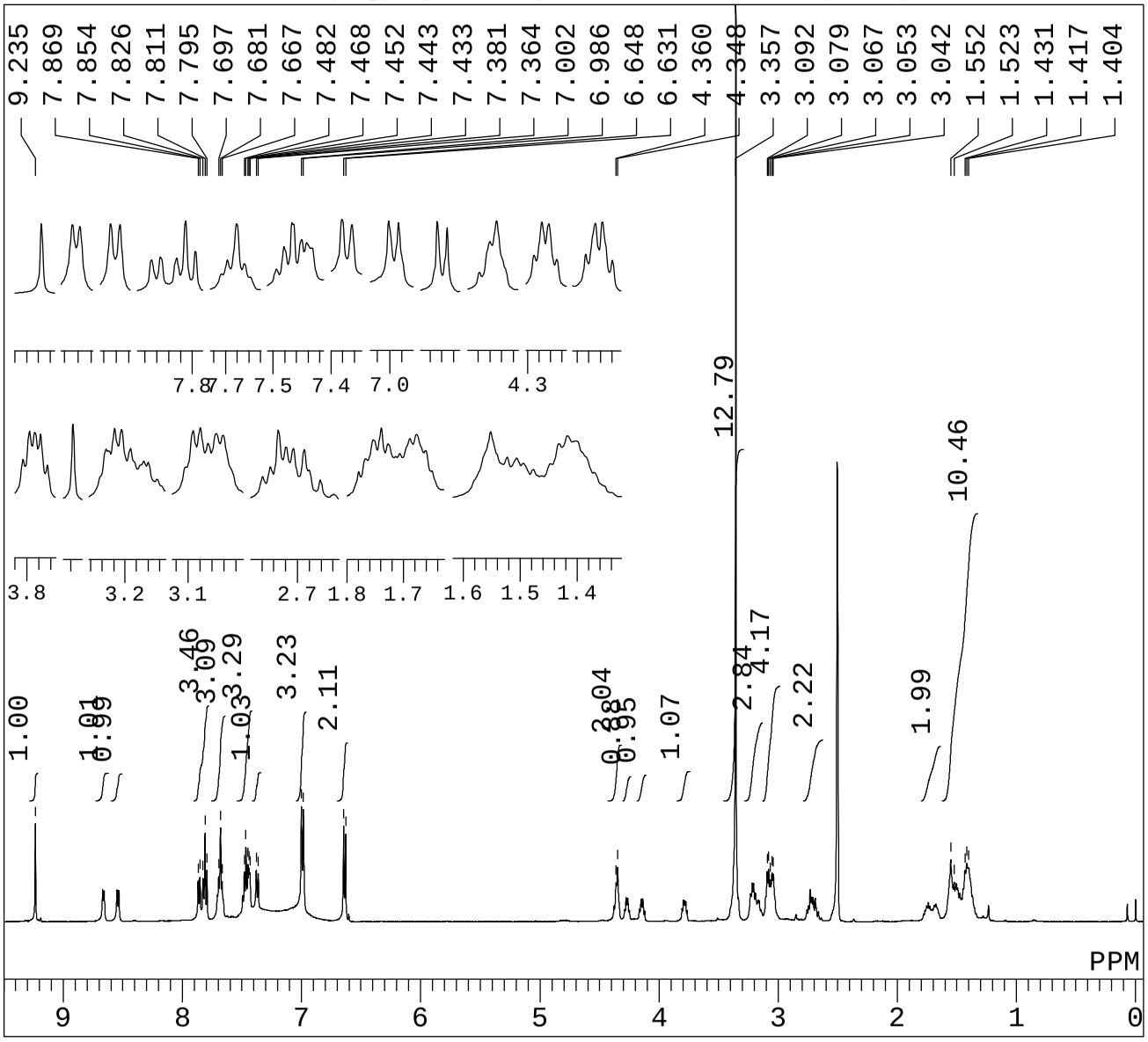

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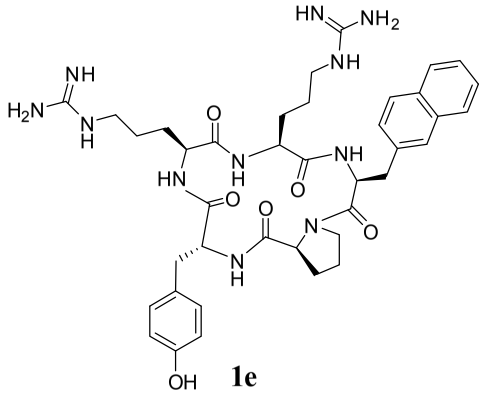
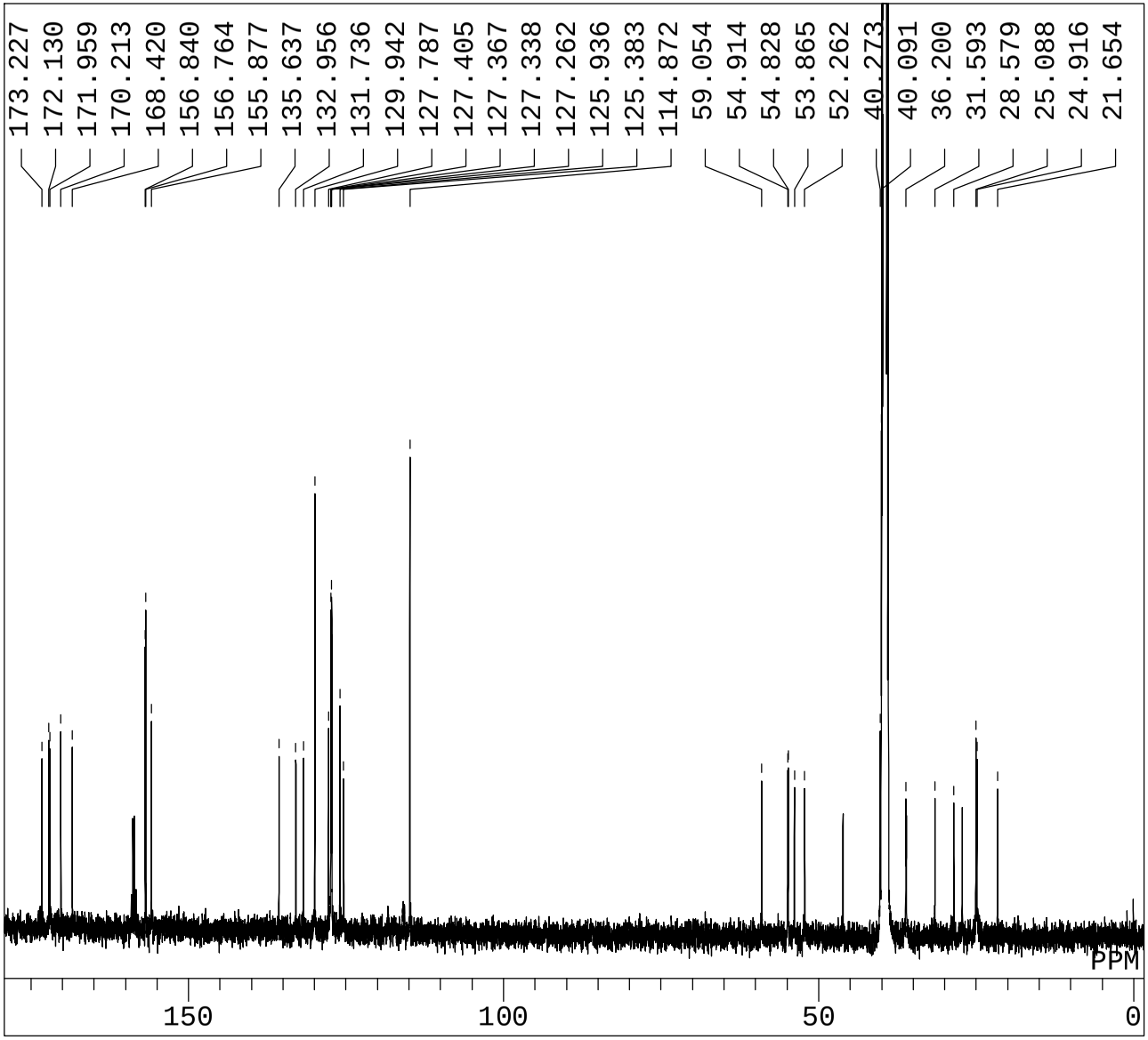


1e

\\10.242.96.55\‹¤—Lƒf[ƒ^(nmr400)\data\seizo\kuroyanagi\L39-C-1.als



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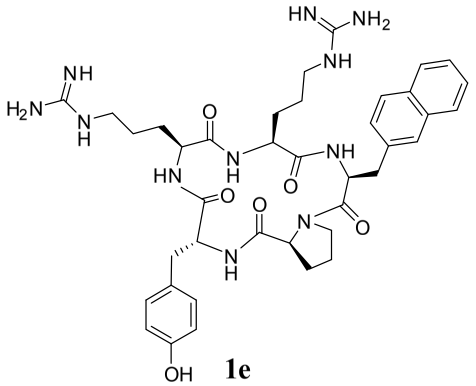

1e

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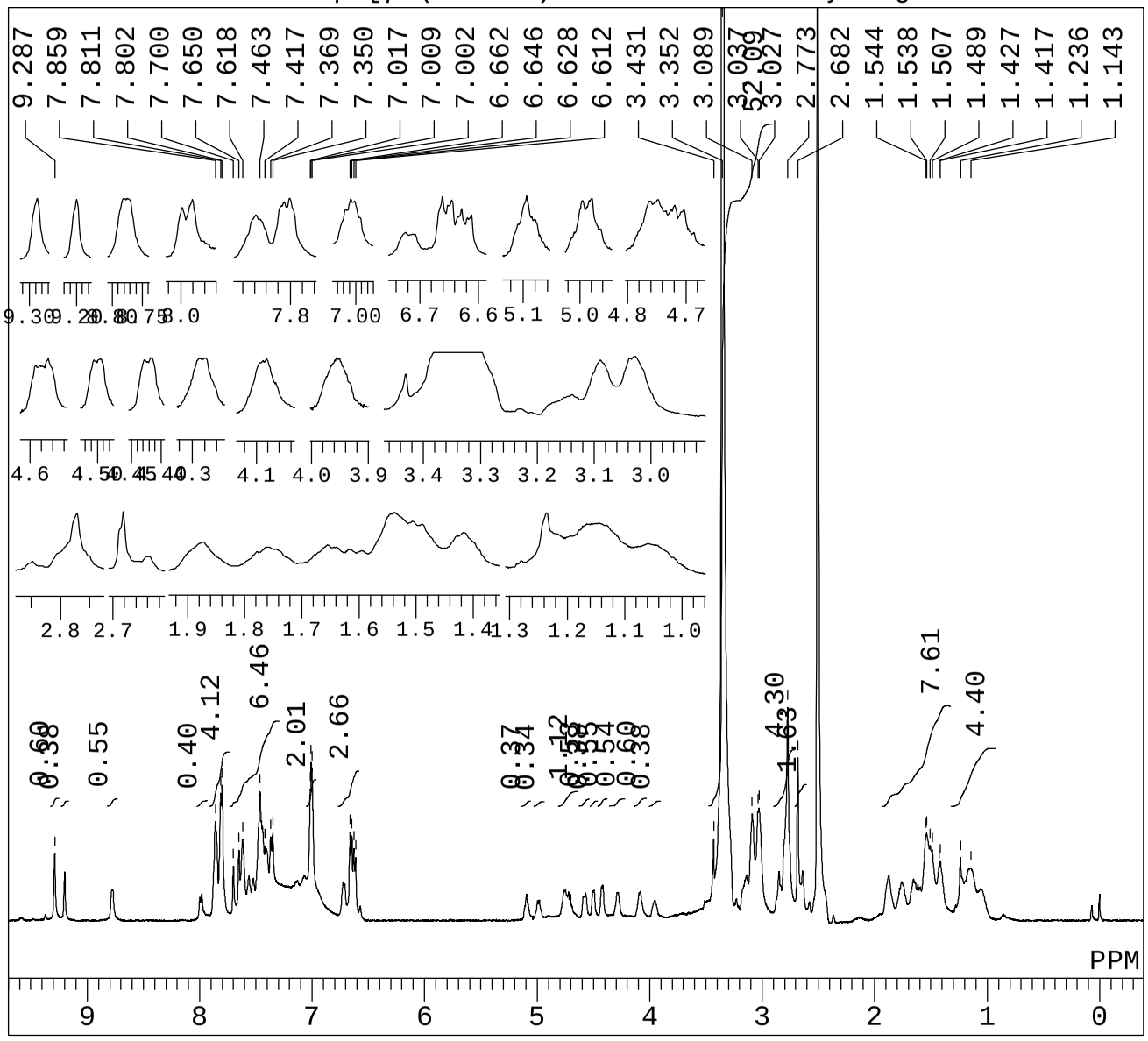

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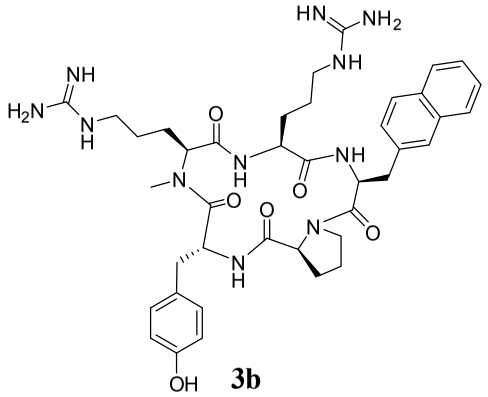

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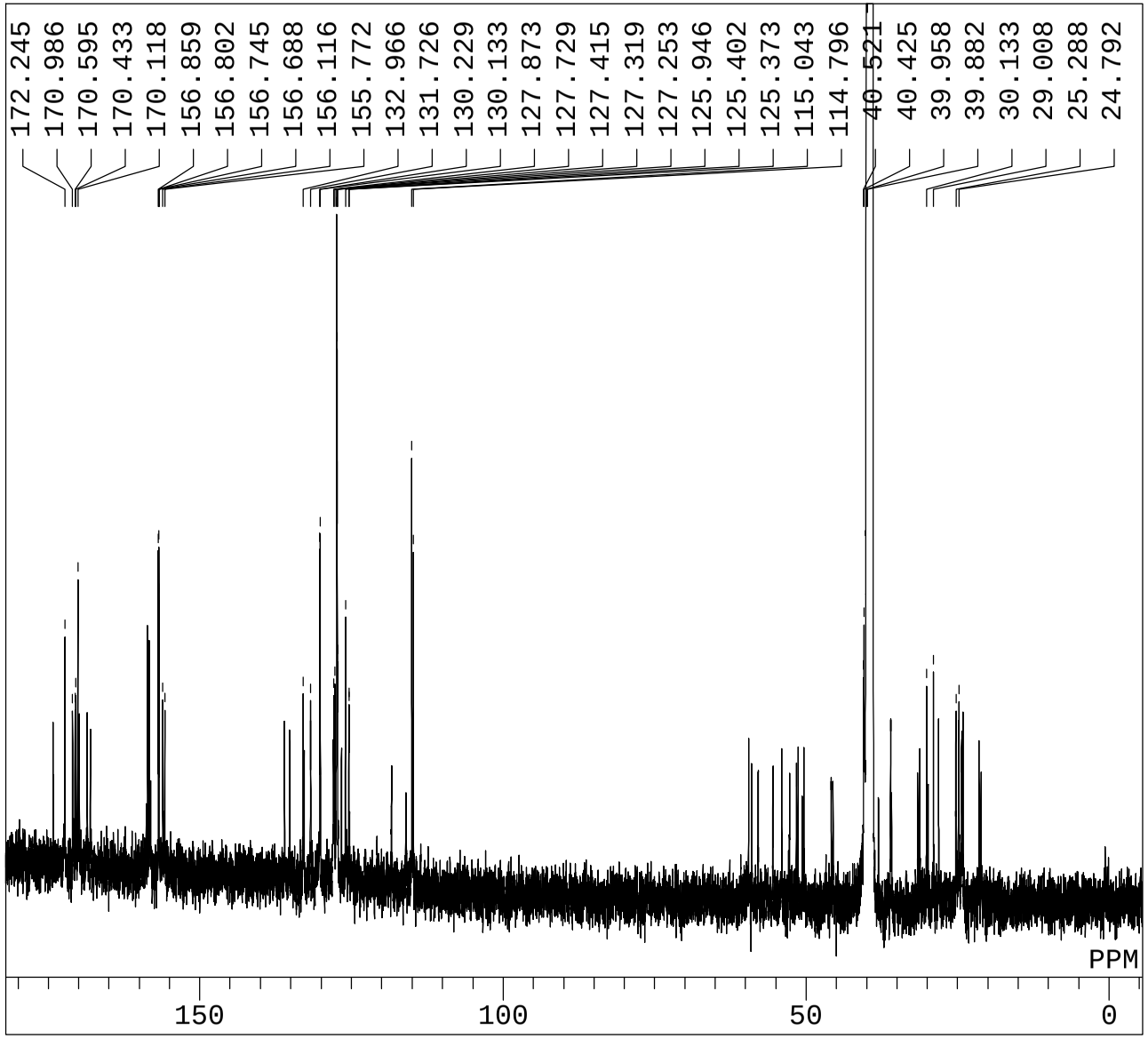

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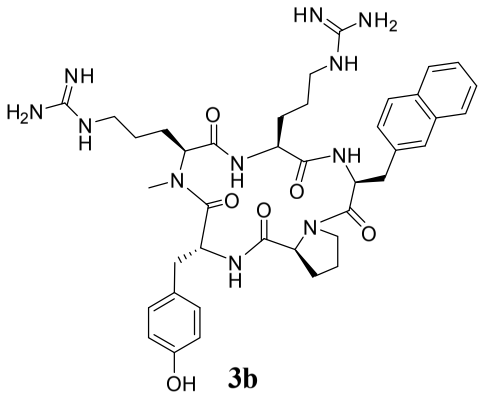

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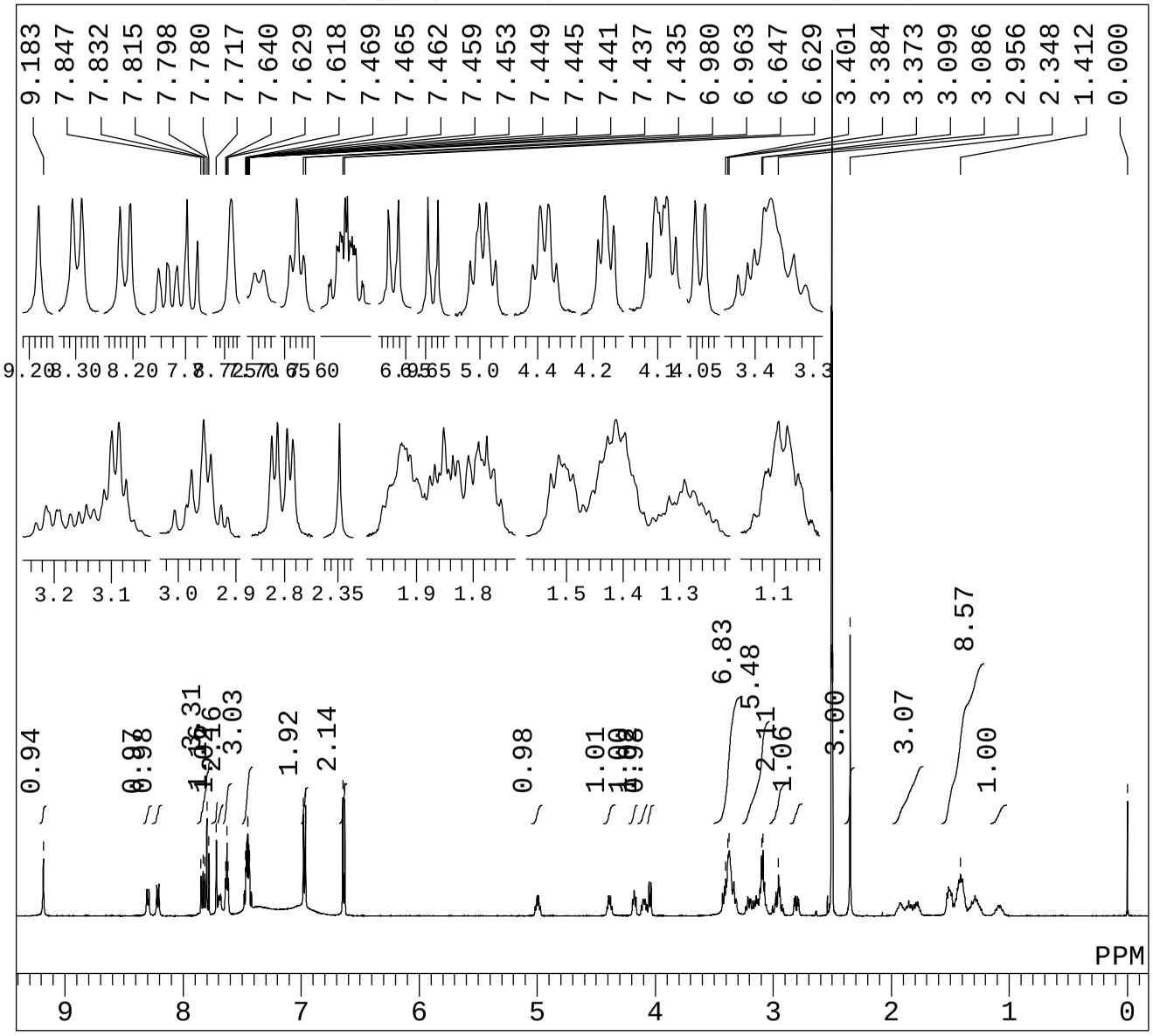

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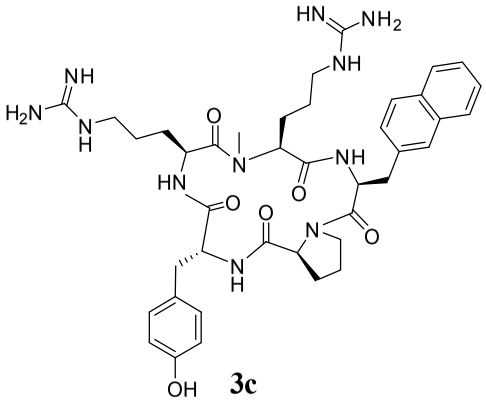

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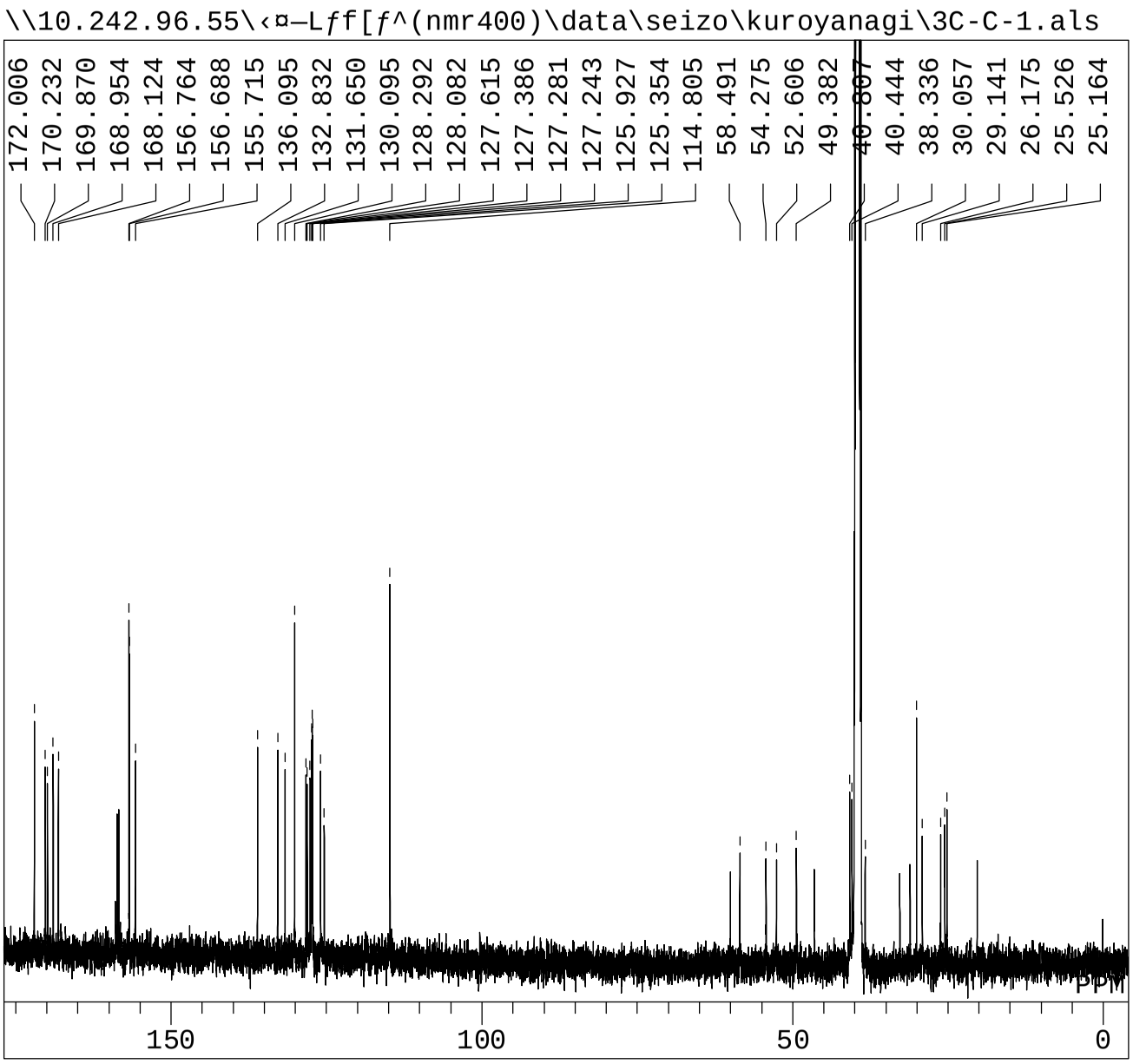

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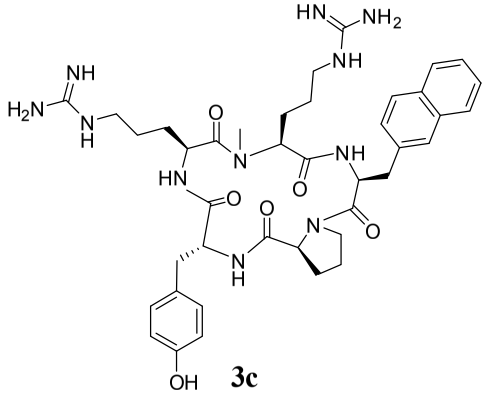

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