

Supporting Information

Design of a stable cyclic peptide analgesic derived from sunflower seeds that targets the κ -opioid receptor for the treatment of chronic abdominal pain

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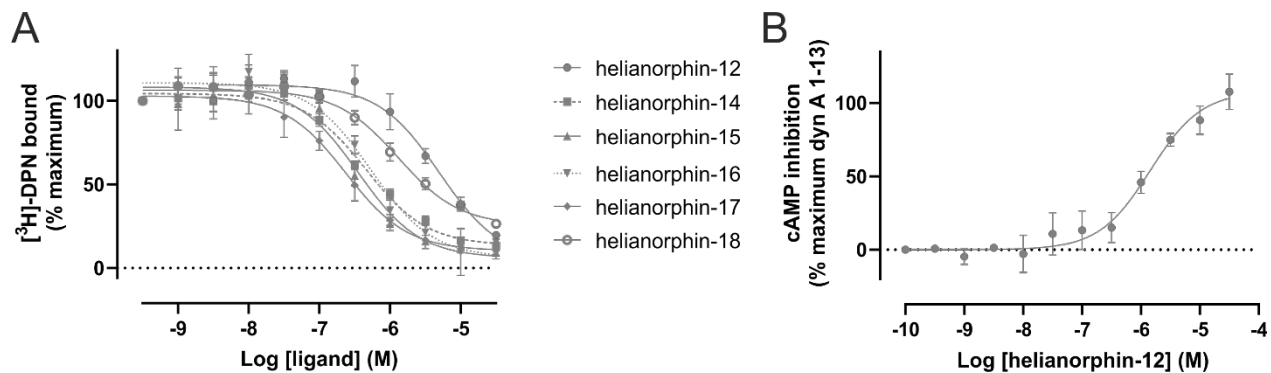


Figure S1. (A) Concentration-dependent displacement of [³H]-diprenorphine (1 nM; DPN) by helianorphin-12 and 14-18 in HEK293 cells stably expressing mouse KOR (n=3). To determine specific binding, nonspecific binding was subtracted from total binding. (B) cAMP inhibition was measured following mouse KOR activation by helianorphin-12 (n=4). Data are normalized to 100% and are mean \pm SD.

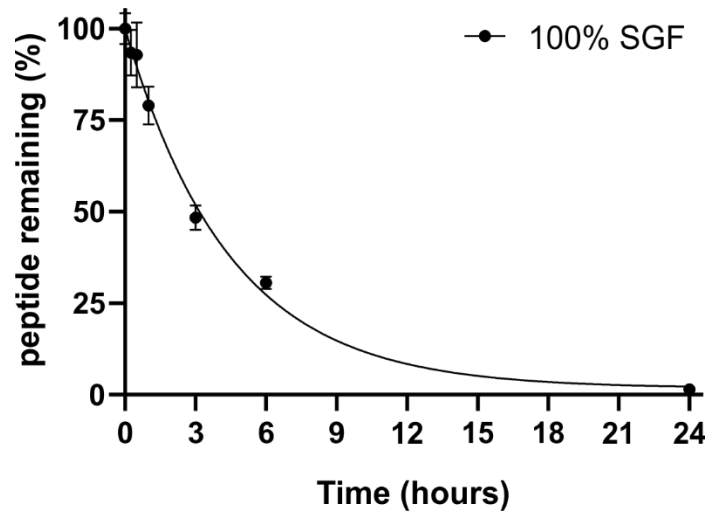


Figure S2. Stability of helianorphin-19 was determined by UPLC-MS after incubation in 100% simulated gastric fluid (SGF) (n=3).

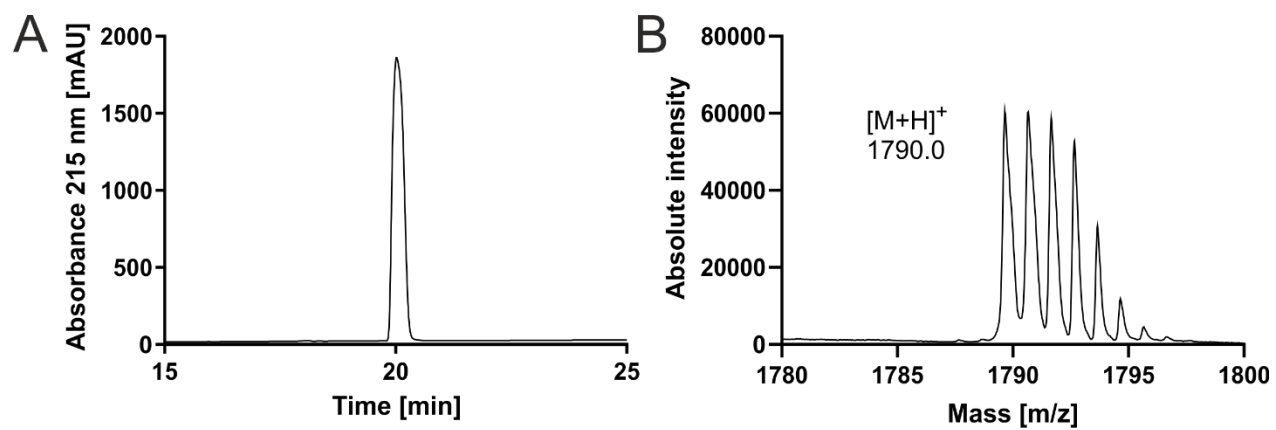


Figure S3. Quality control of helianorpin-19 was determined by (A) RP-HPLC and (B) MALDI mass spectrometry. The chromatogram at 215 nm indicates a purity >95% and the monoisotopic mass signal (1790.0 m/z) represents oxidized cyclic peptide.

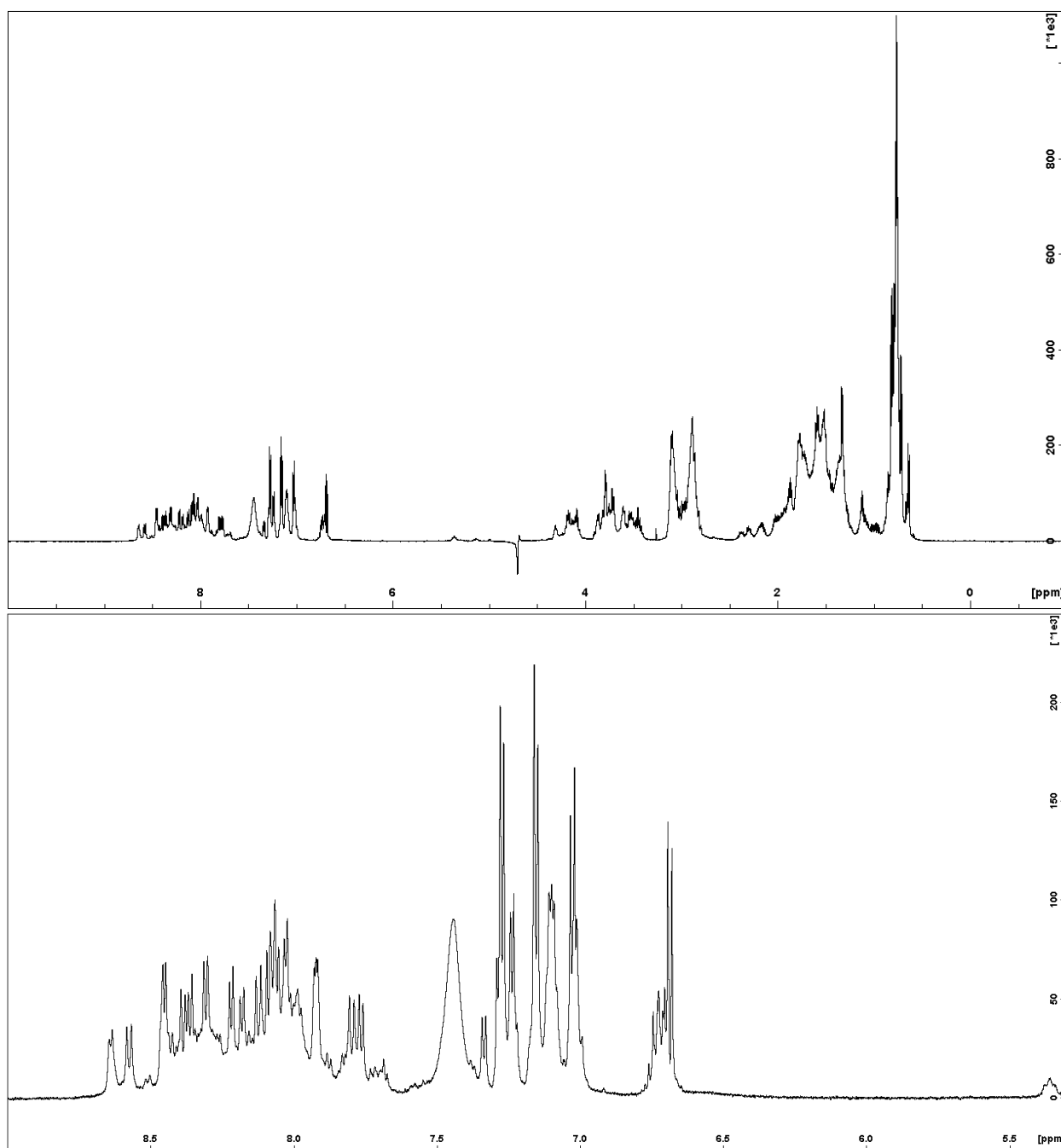


Figure S4. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-2.

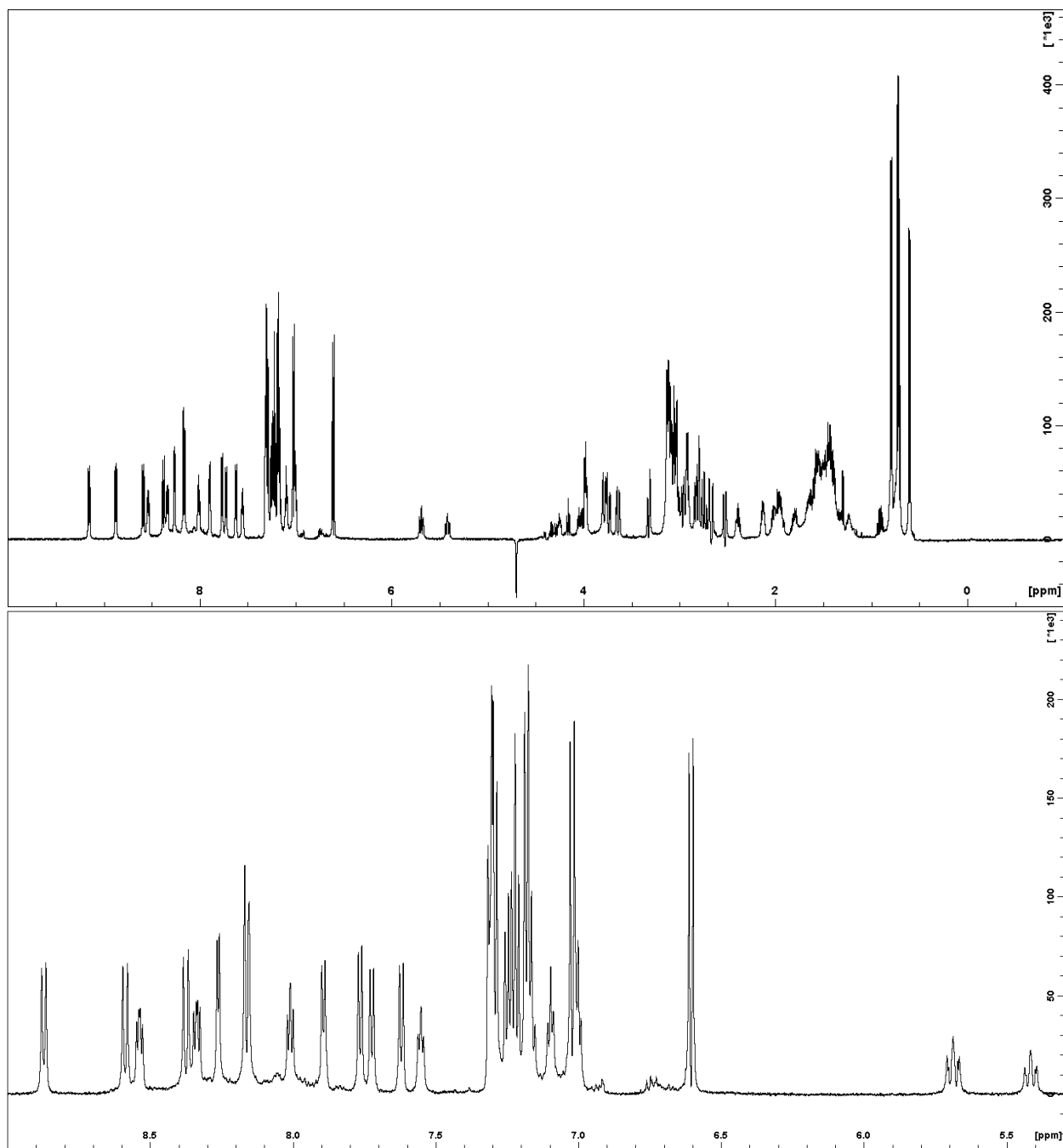


Figure S5. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-3.

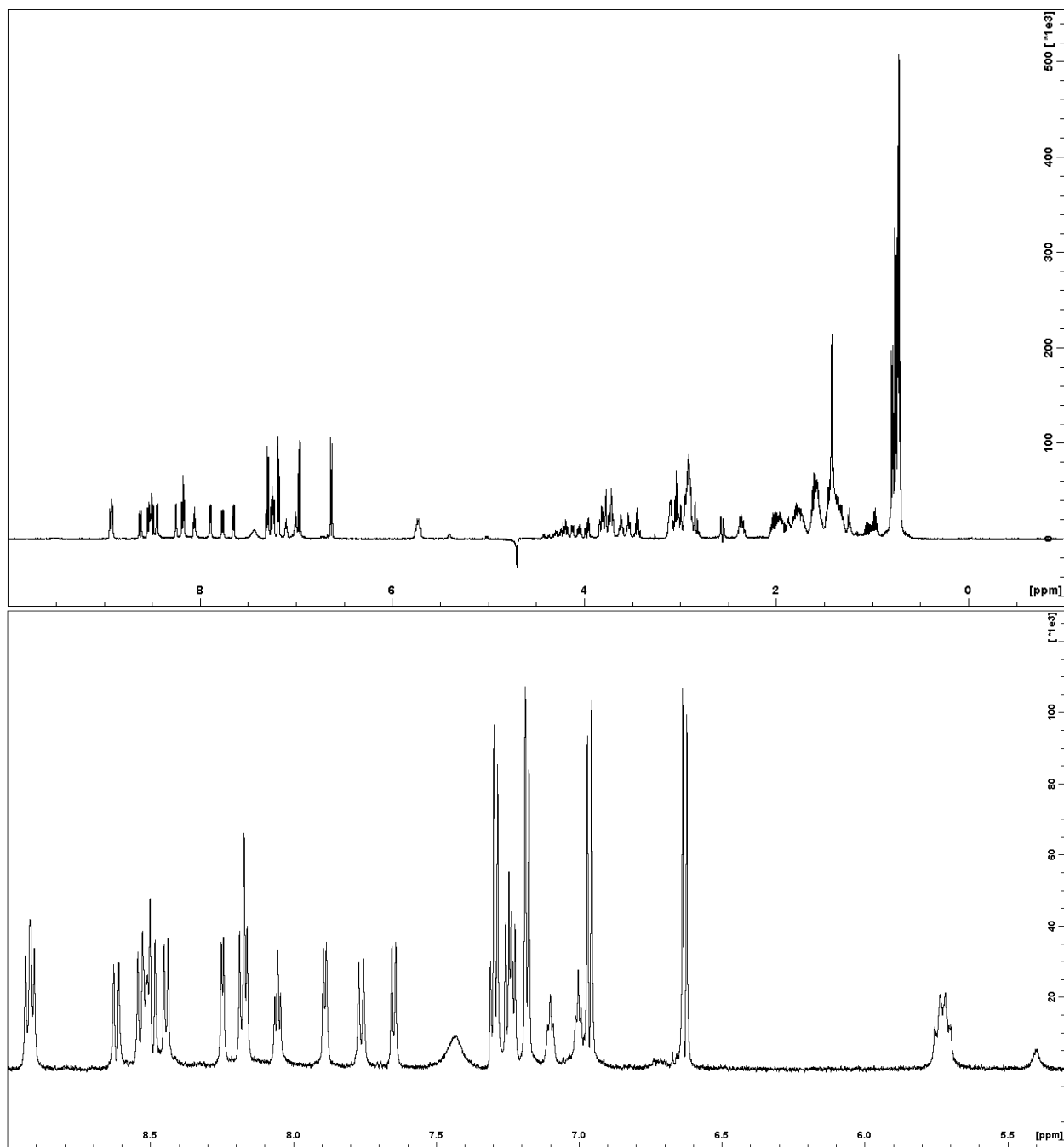


Figure S6. Full one-dimensional ¹H NMR spectrum (0-10 ppm) of helianorpin-4.

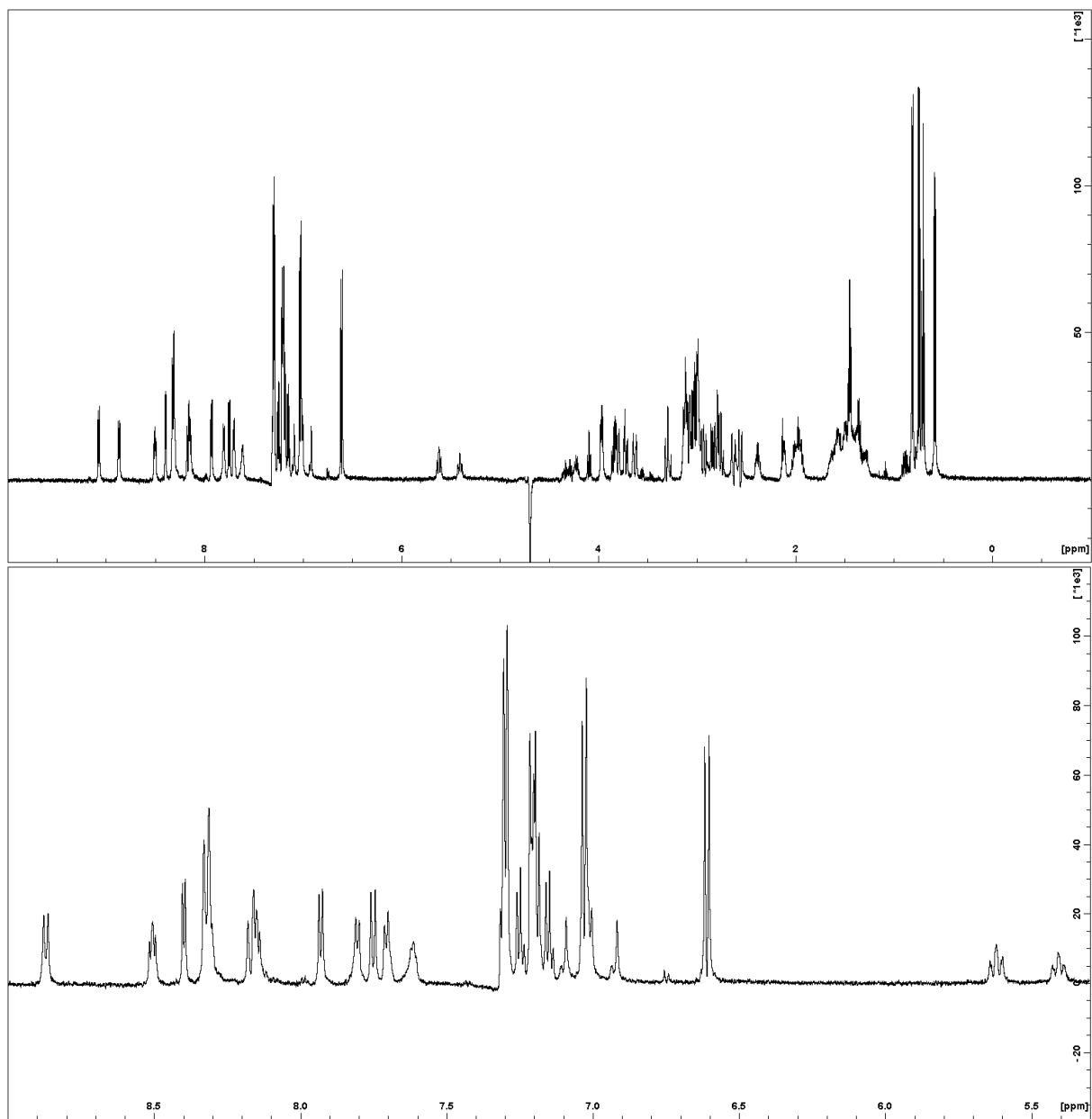


Figure S7. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-5.

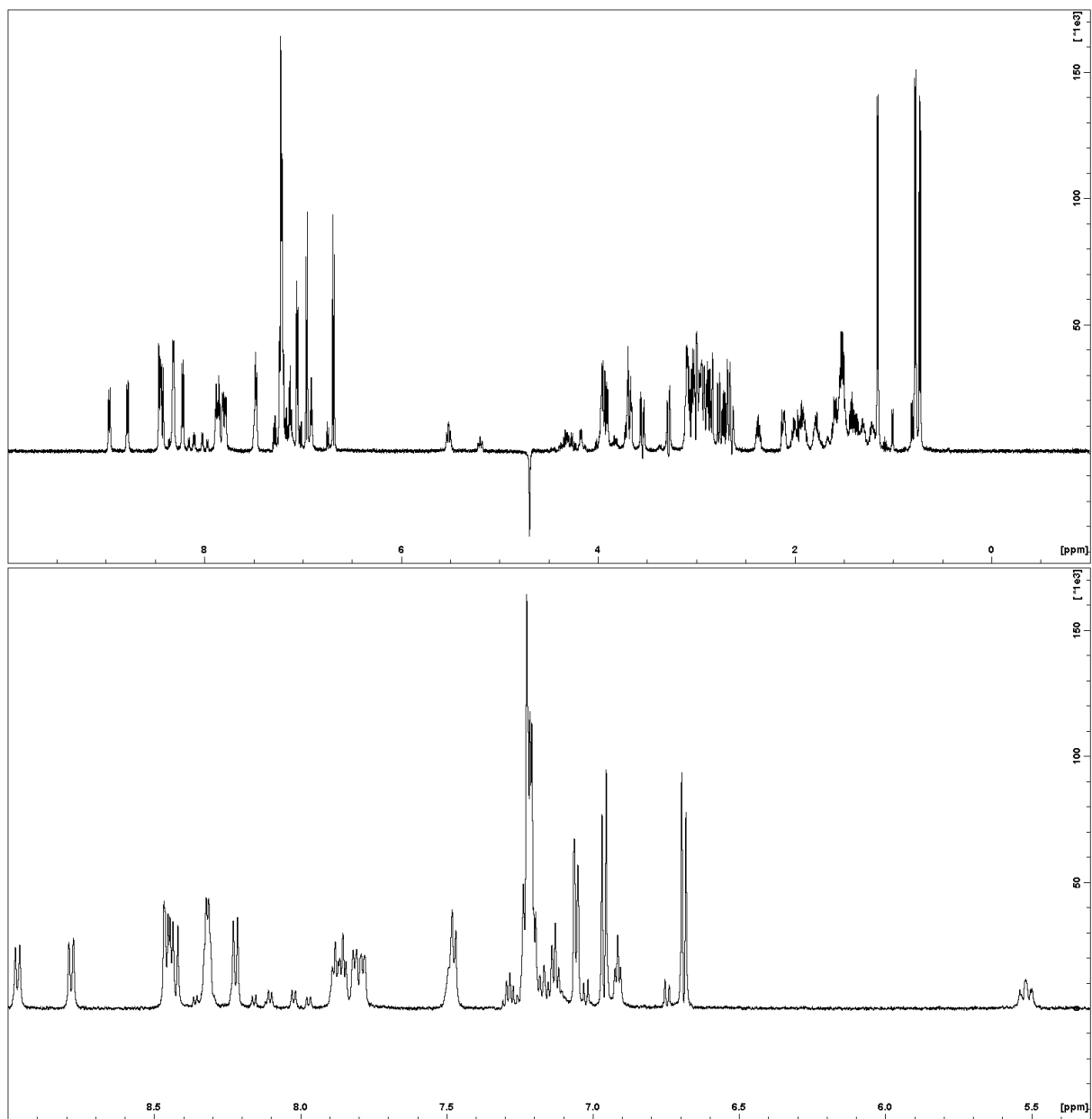


Figure S8. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-6.

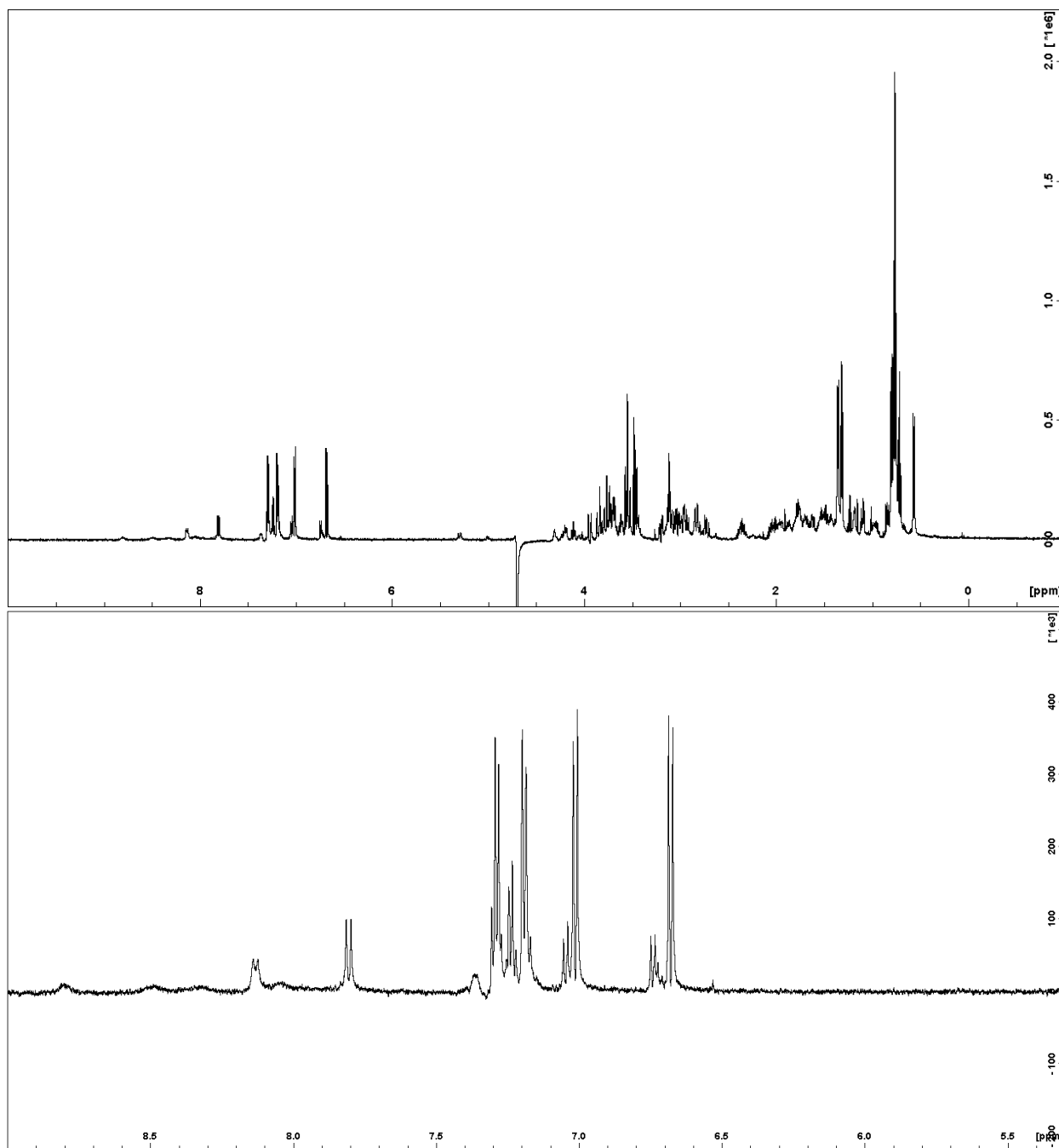


Figure S9. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-7.

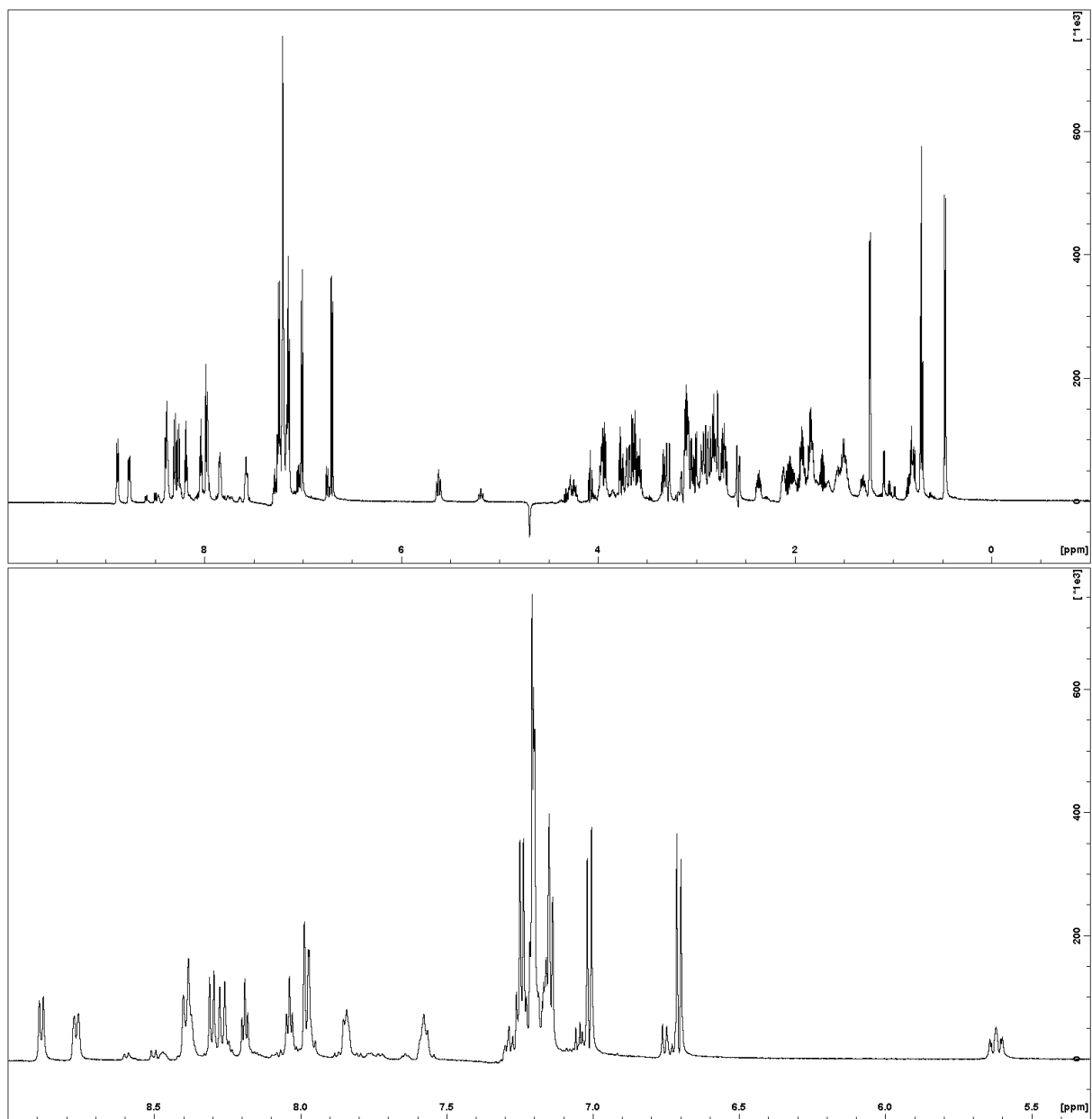


Figure S10. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-8.

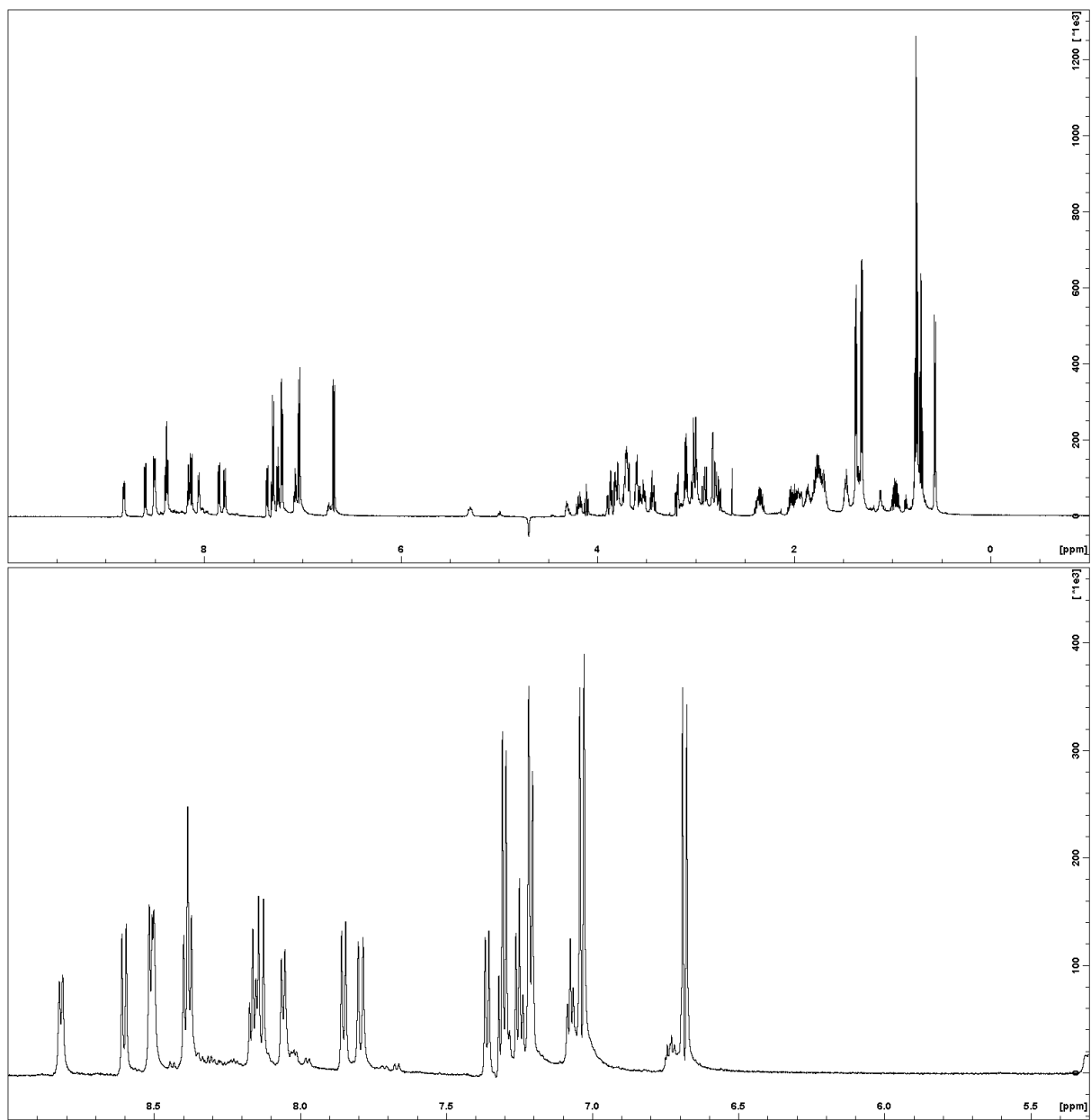


Figure S11. Full one-dimensional ¹H NMR spectrum (0-10 ppm) of helianorpin-9.

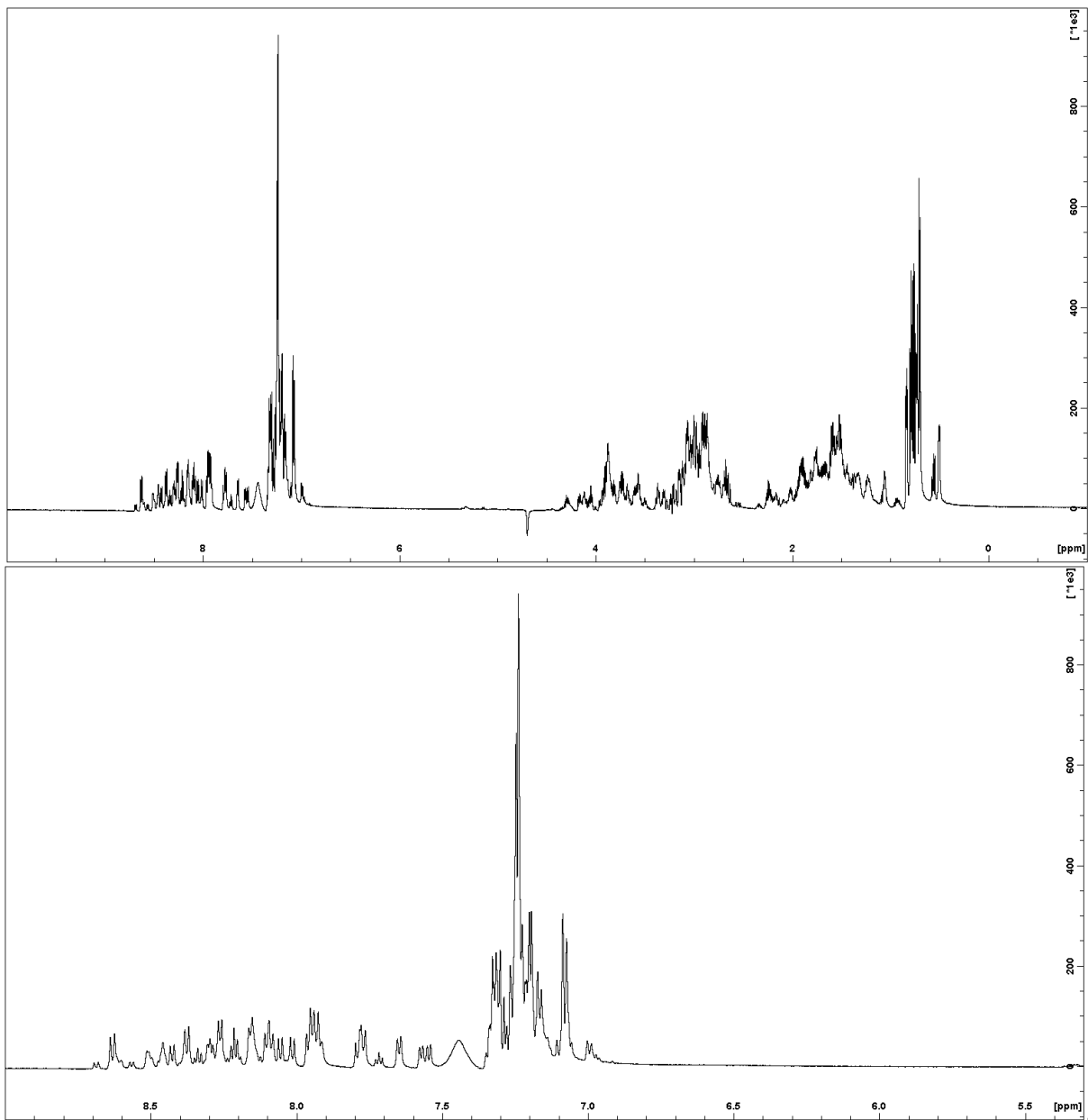


Figure S12. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-10.

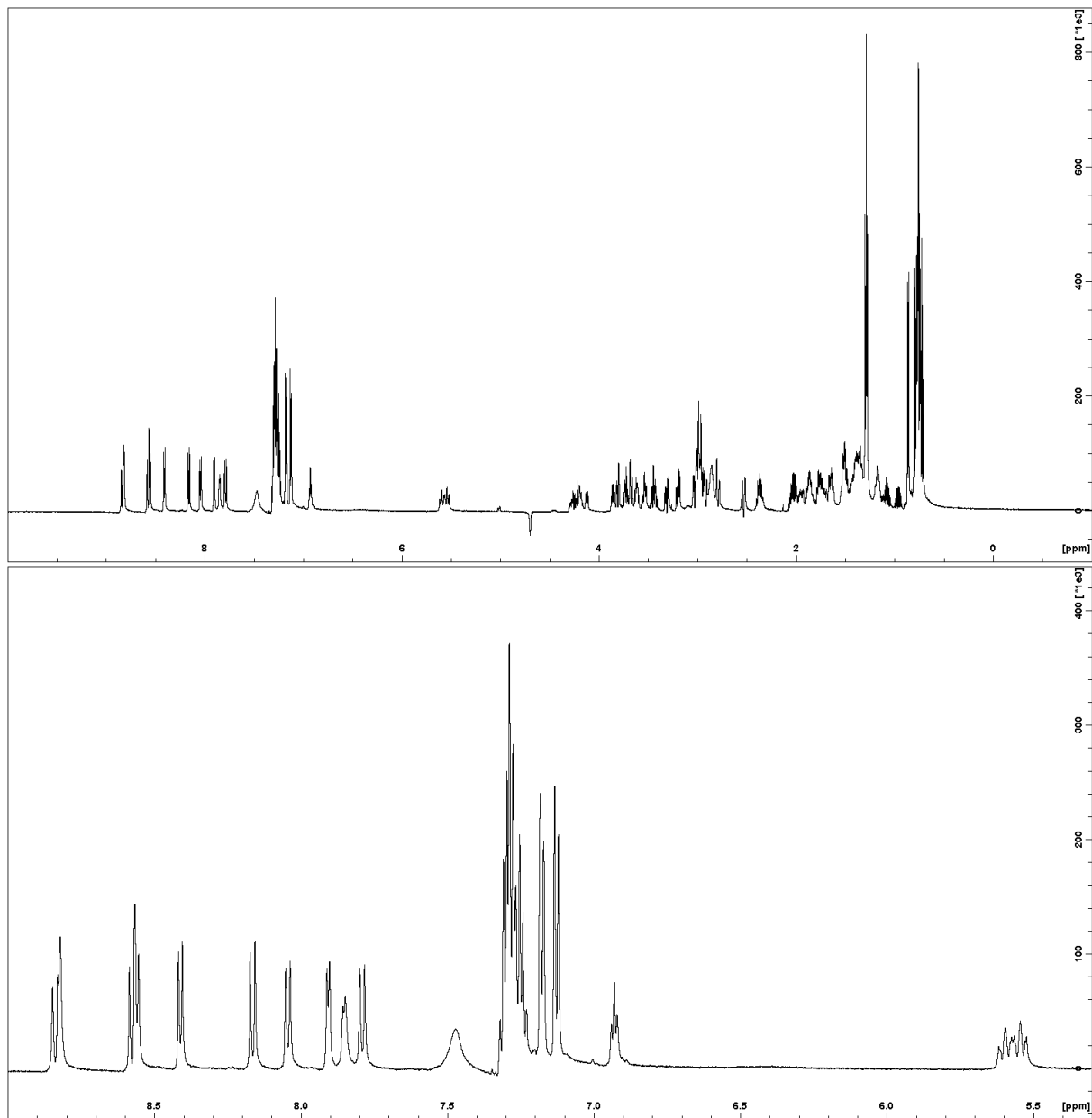


Figure S13. Full one-dimensional ¹H NMR spectrum (0-10 ppm) of helianorpin-11.

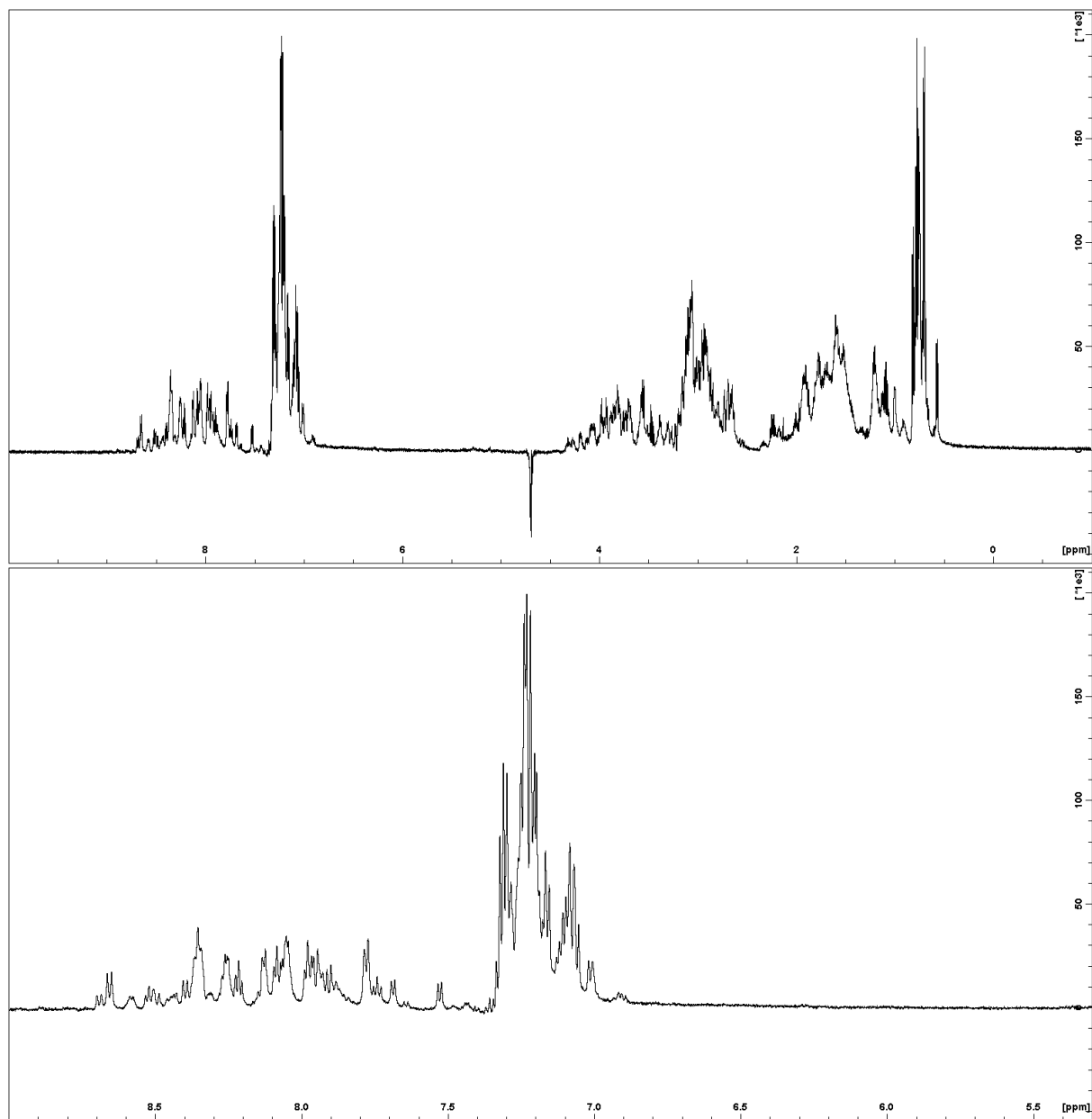


Figure S14. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-12.

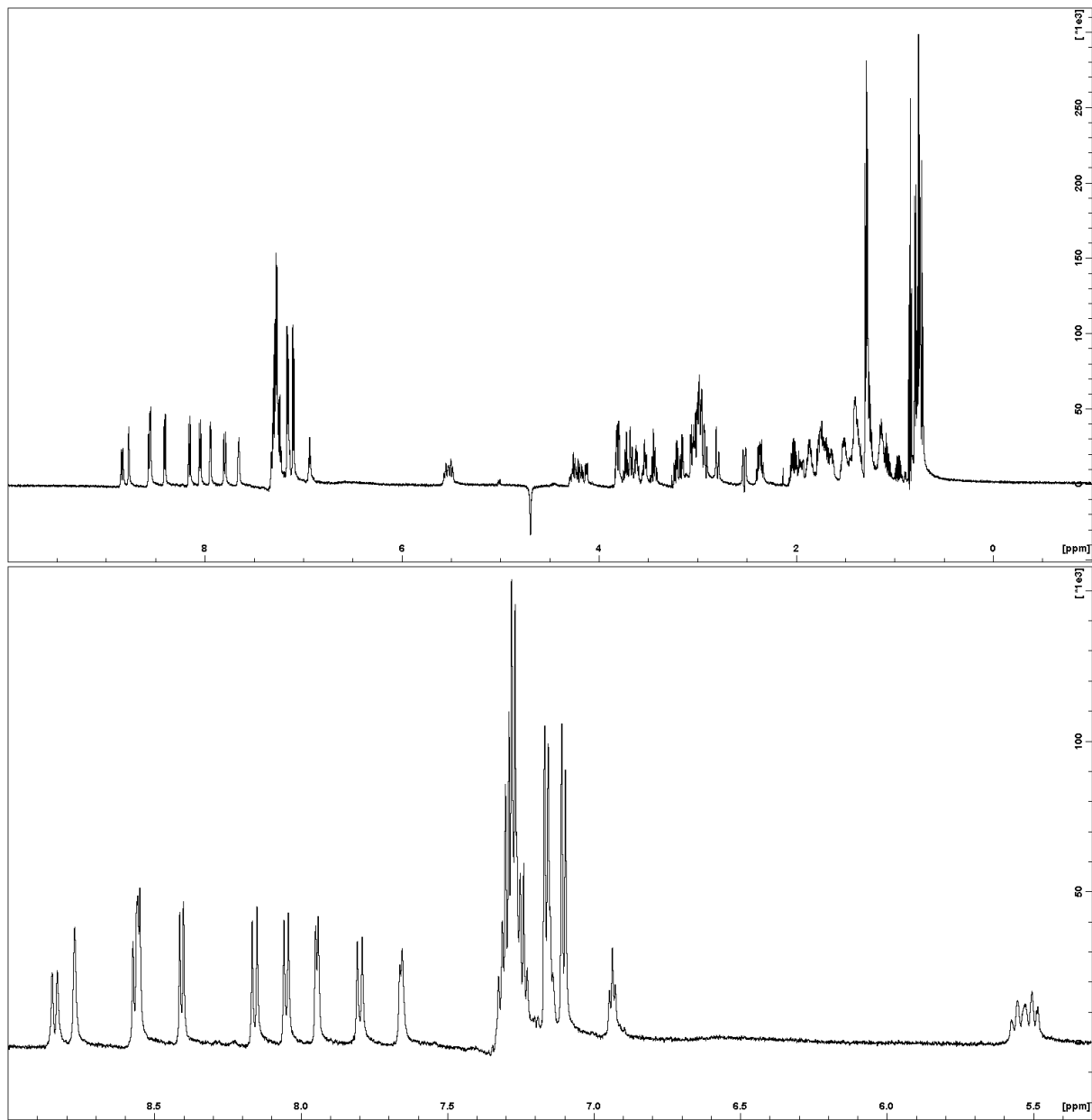


Figure S15. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorphin-13.

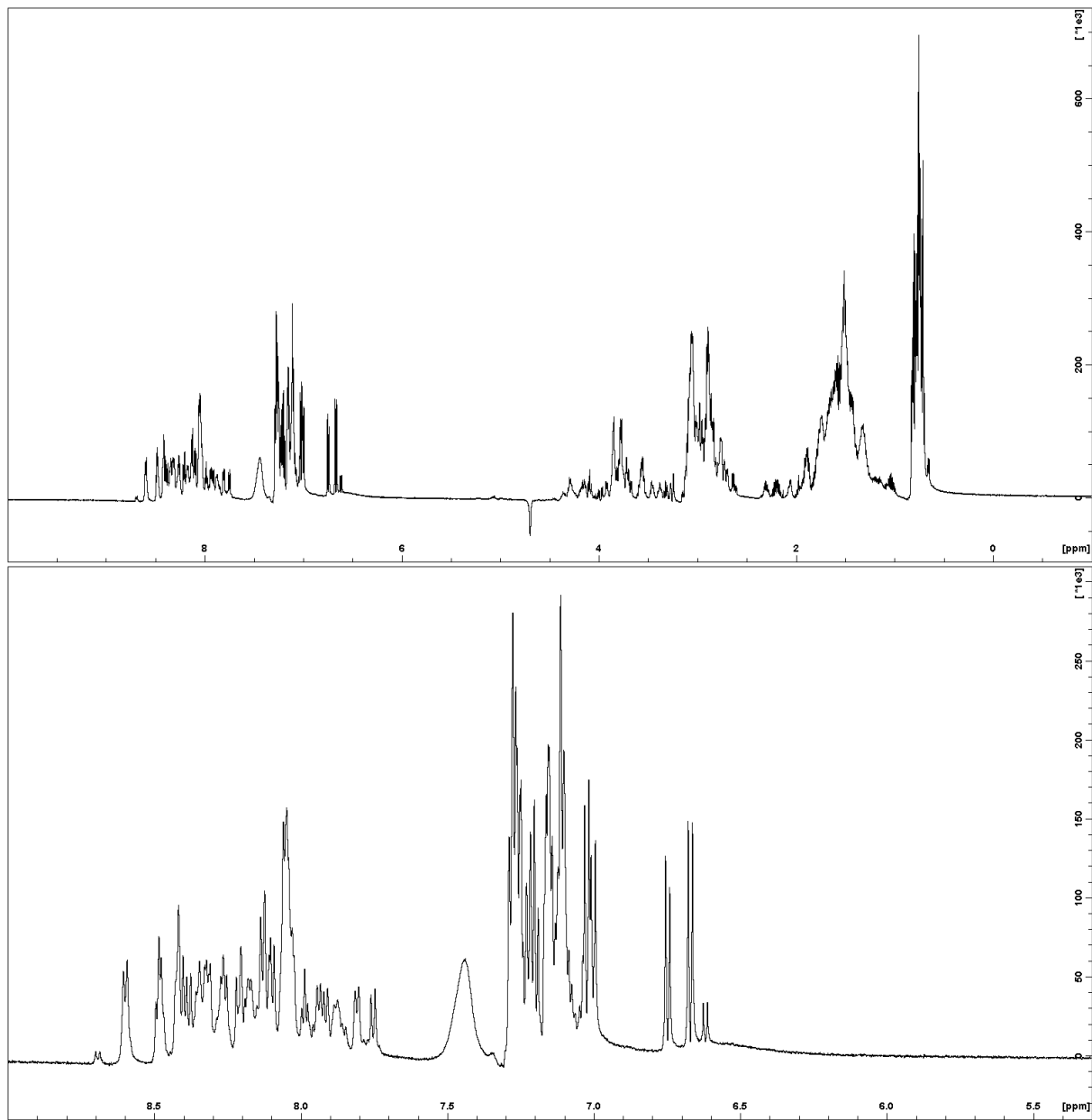


Figure S16. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-14.

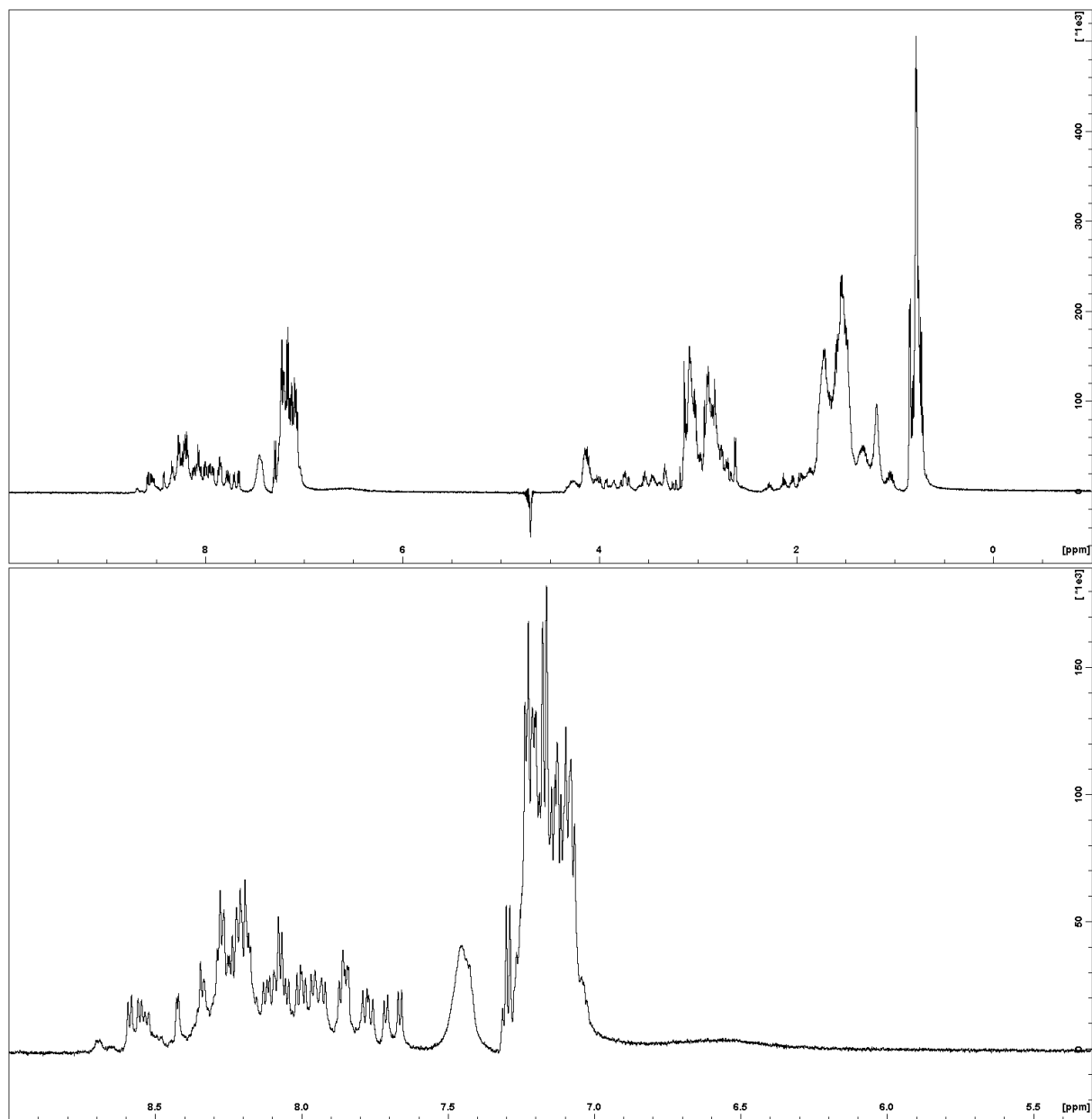


Figure S17. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-15.

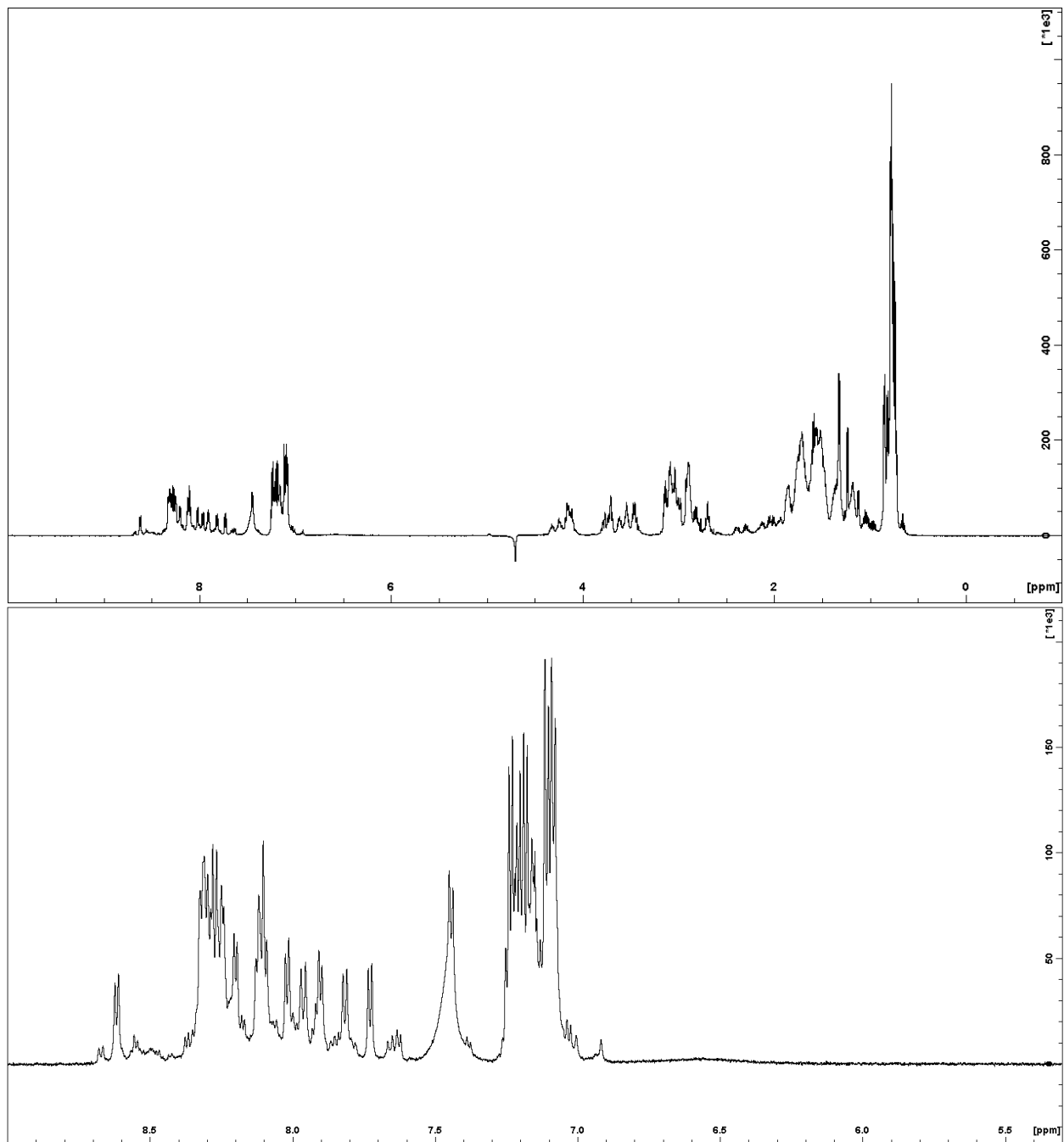


Figure S18. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-16.

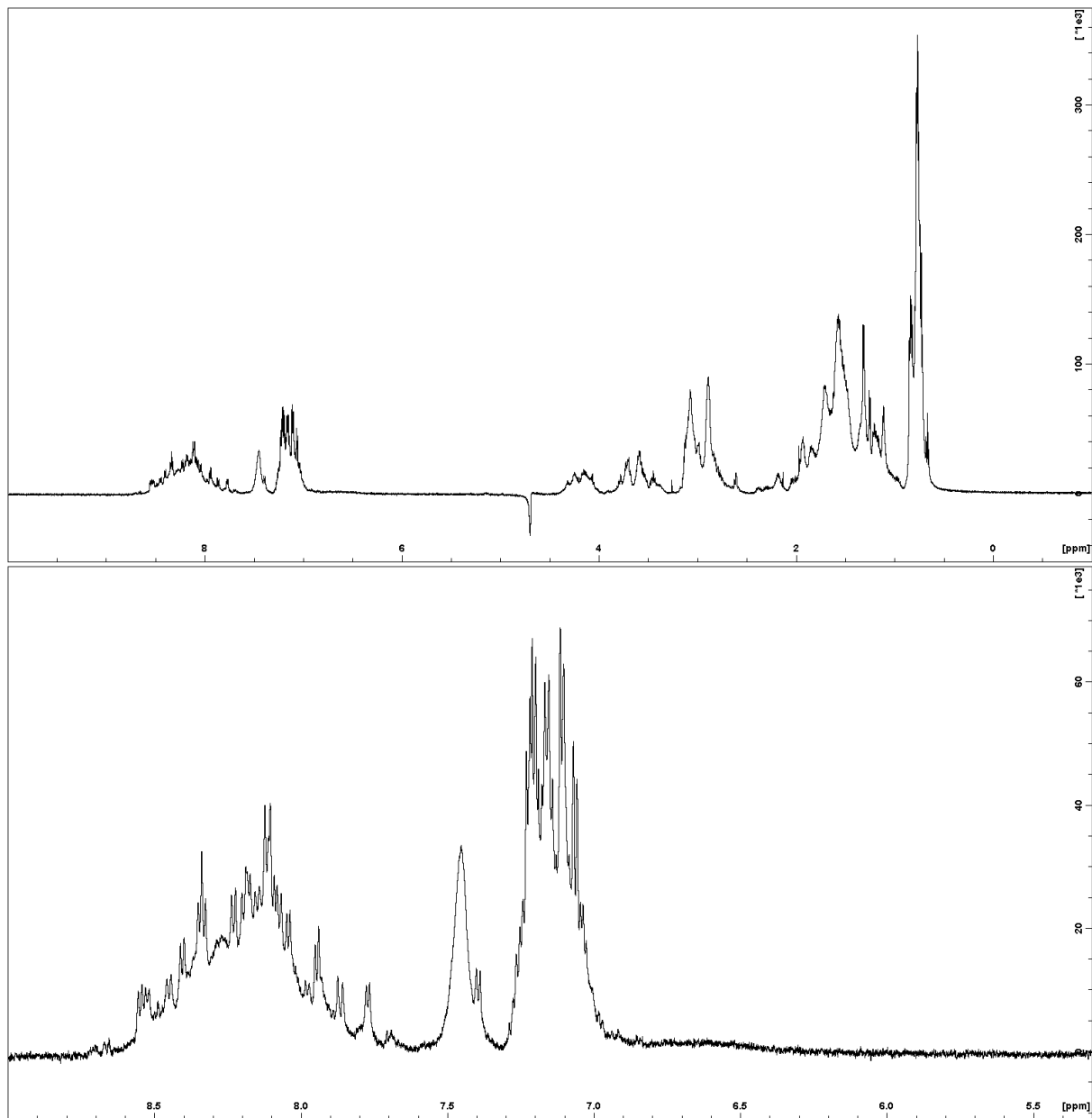


Figure S19. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-17.

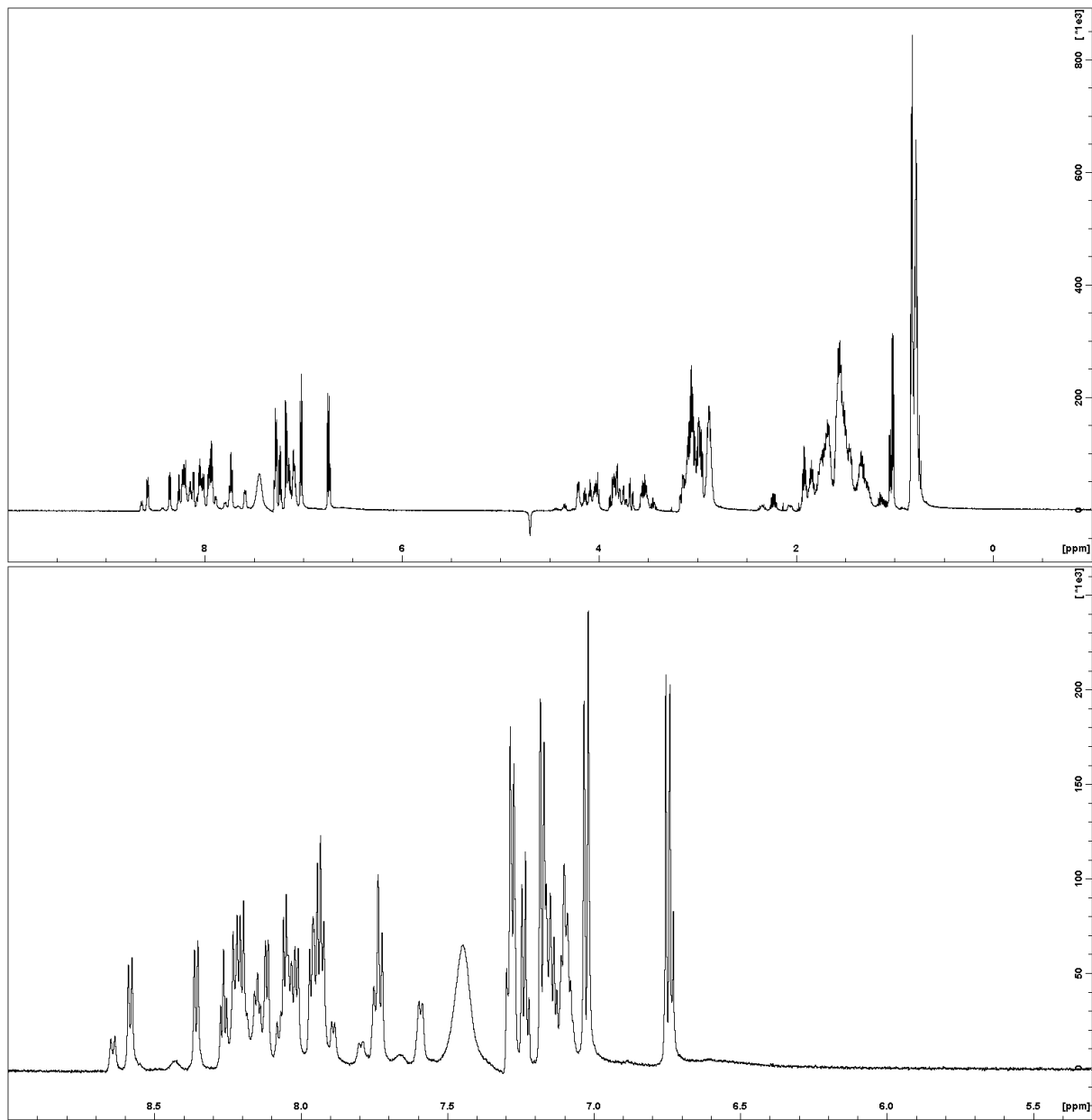


Figure S20. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-18.

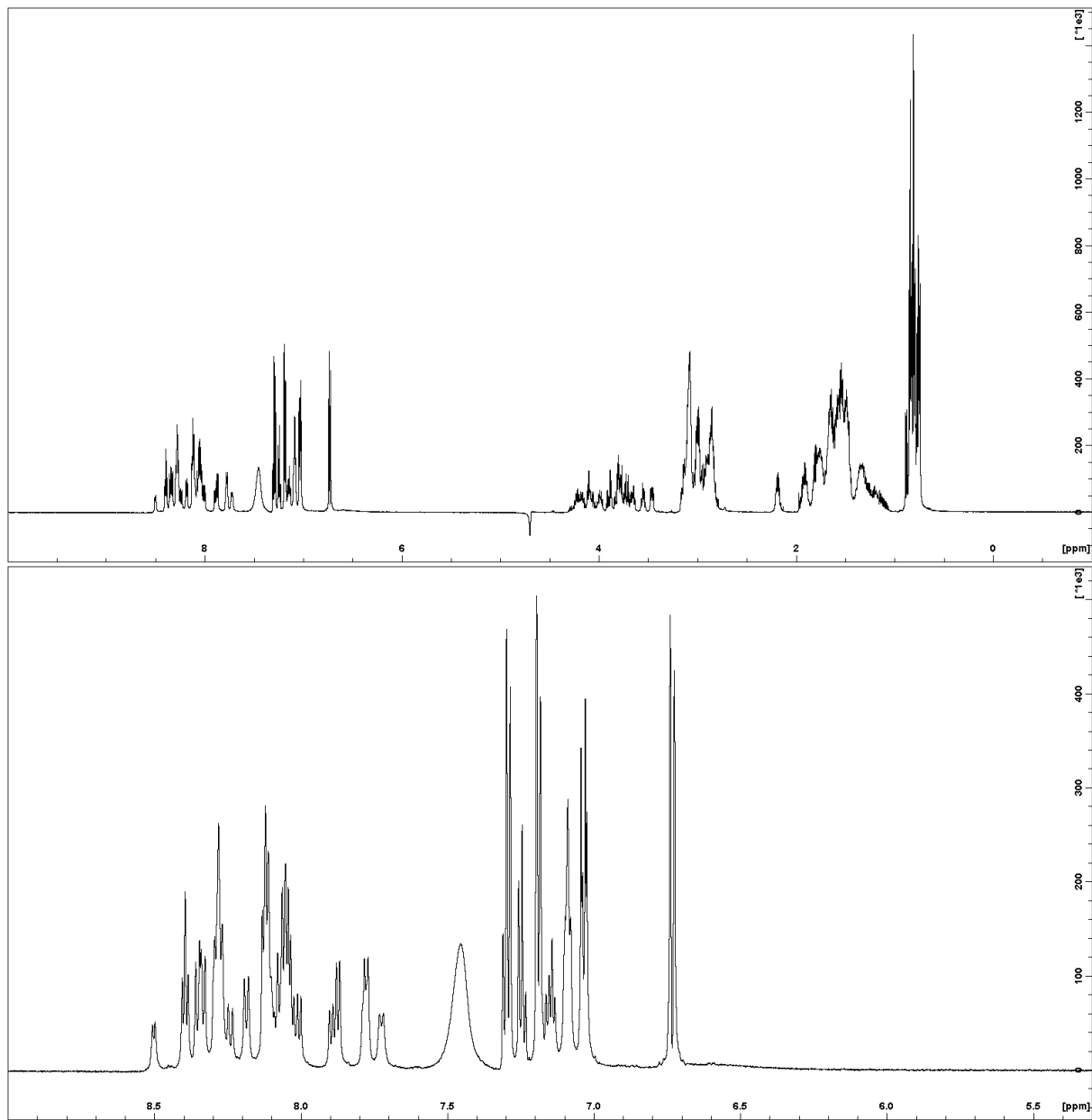


Figure S21. Full one-dimensional ^1H NMR spectrum (0-10 ppm) of helianorpin-19.

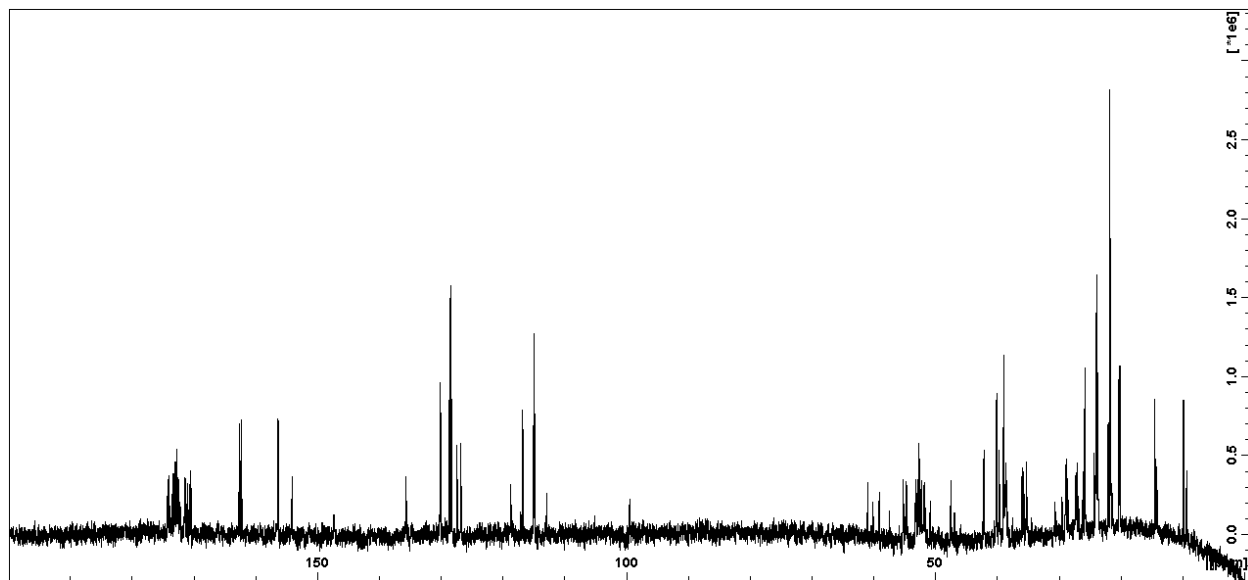


Figure S22. Full one-dimensional ¹³C NMR spectrum (0-200 ppm) of helianorpin-19.

Table S1. Analytical data of synthesized peptides.

Peptide	HPLC purity (%)*	HPLC retention times (min)	Calculated mass (m/z)	Observed mass (m/z)#
SFTI-1	100.0	36.9	1513.8	1513.6
helianorphan-1	99.8	33.4	2362.3	2362.0
helianorphan-2	99.7	37.0	2526.5	2526.1
helianorphan-3	99.9	38.5	1739.9	1739.6
helianorphan-4	96.0	37.6	1904.0	1903.8
helianorphan-5	99.3	10.5	1583.8	1583.8
helianorphan-6	99.3	7.6	1571.7	1571.7
helianorphan-7	100.0	10.1	1512.7	1512.7
helianorphan-8	99.5	9.2	1623.8	1623.8
helianorphan-9	98.3	8.8	1651.8	1651.9
helianorphan-10	94.2	10.0	1577.8	1577.8
helianorphan-11	93.5	10.2	1464.7	1464.7
helianorphan-12	100.0	9.8	1575.8	1575.8
helianorphan-13	100.0	10.5	1603.8	1603.8
helianorphan-14	98.9	7.2	2362.3	2362.2
helianorphan-15	99.5	7.8	2501.4	2501.4
helianorphan-16	100.0	9.6	2608.5	2608.5
helianorphan-17	100.0	9.4	2608.5	2608.9
helianorphan-18	99.1	7.3	1891.1	1891.4
helianorphan-19	99.9	20.0	1790.0	1790.0

*Purity of SFTI-1 and helianorphan 1-4 and 19 (calculated by automatic peak integration from 5-45 min) was determined by RP-HPLC using a Phenomenex Jupiter C₁₈ column (5 μ M, 300 Å, 150 x 2 mm) and a linear gradient of 5-65% solvent B in 60 min at a flow rate of 1 mL/min. Purity of helianorphan 5-18 was assessed by RP-UPLC using a Phenomenex Luna Omega column (1.6 μ m C₁₈ 100 Å, 50 x 2.1 mm) and a linear gradient of 1-61% of solvent B in 15 min at a flowrate of 0.6 mL/min was applied. Purity was calculated by automatic peak integration from 3-15 min.

#Monoisotopic masses of peptides obtained by ESI- and/or MALDI-MS are shown.