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Supporting Information

Synthetic Lugdunin Analogues Reveal Essential Structural Motifs for Antimicrobial Action and Proton Translocation Capability

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SUPPORTING INFORMATION

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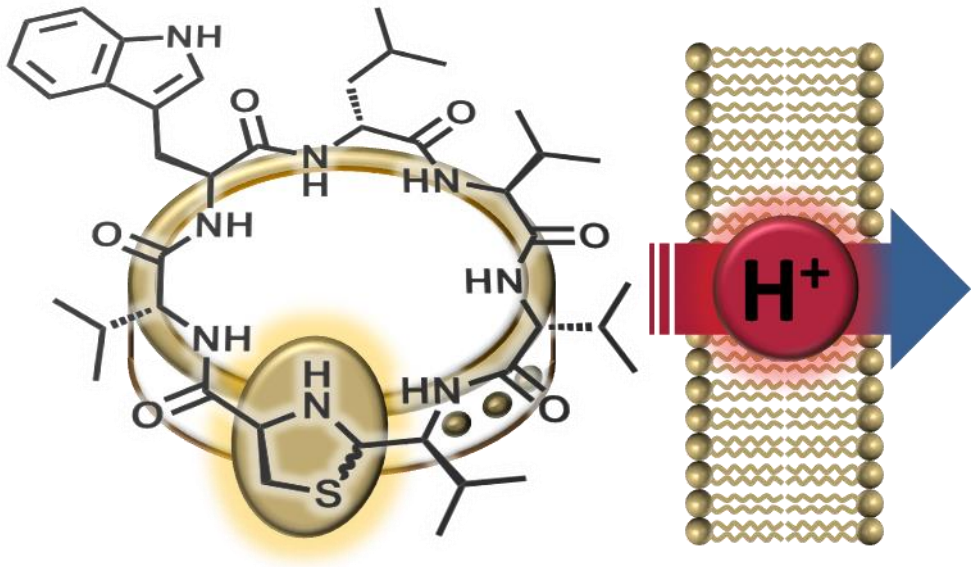
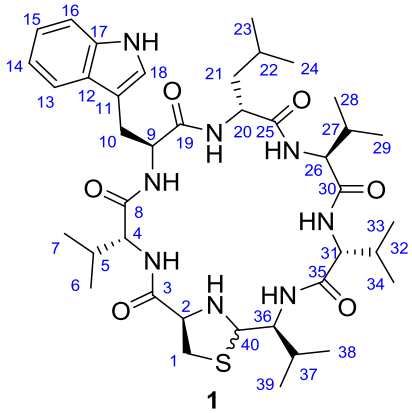
Graphical Abstract



Ornament clasp confers function: Synthetic analogues of lugdunin, the first antibiotic from the human nose, reveal essential motifs conferring antimicrobial activity against methicillin resistant *Staphylococcus aureus*. In addition to D-, L-configuration the thiazolidine heterocycle – for the first time decorating a cyclopeptide – is invaluable for the proton translocation mode of action.

SUPPORTING INFORMATION

Structures of peptides 1-24 and gramicidin S (25)



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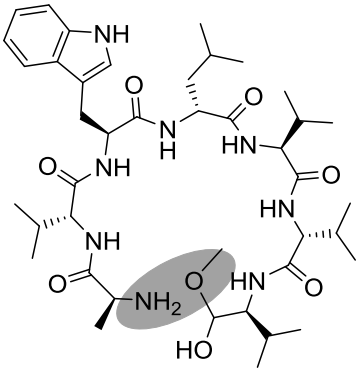

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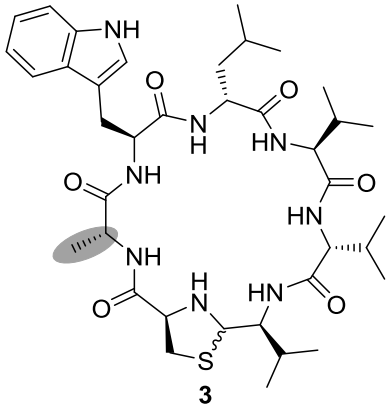

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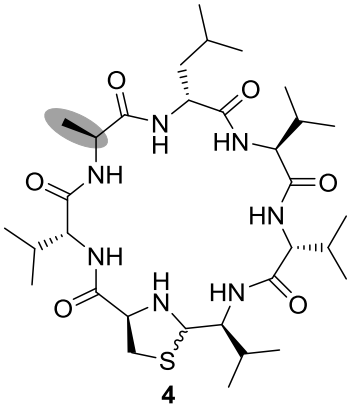

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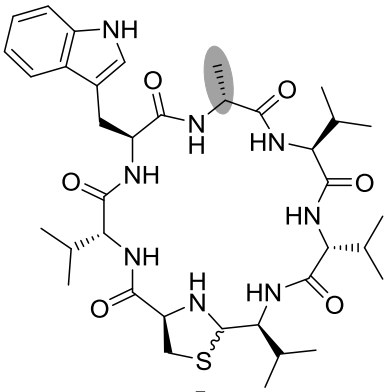

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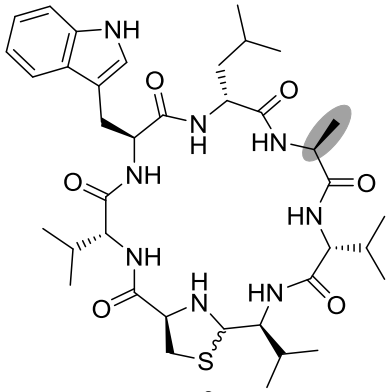

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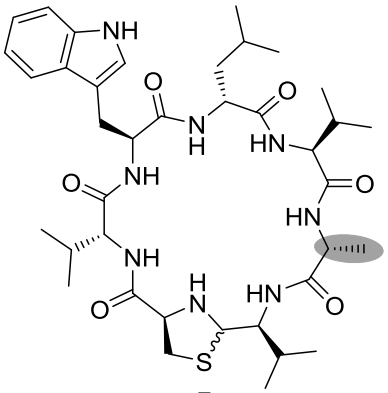

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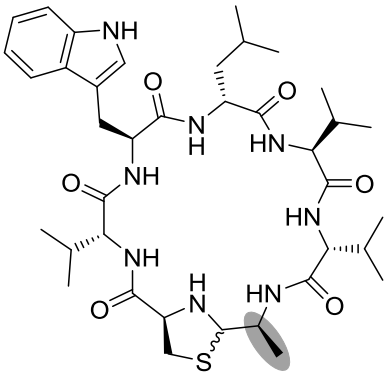

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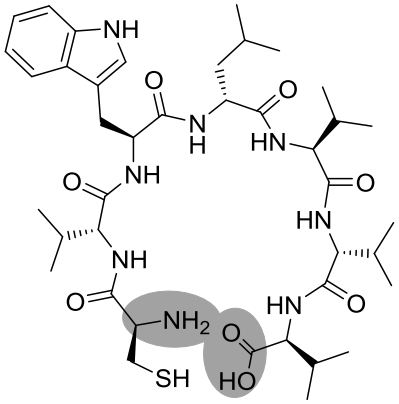

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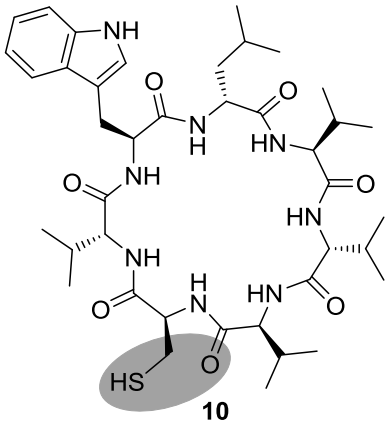

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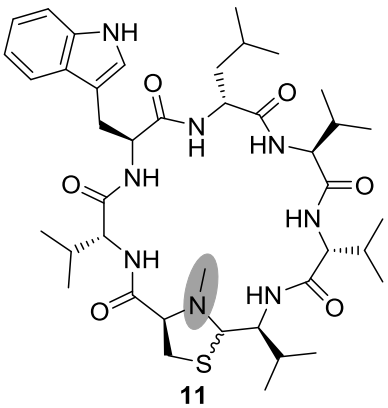

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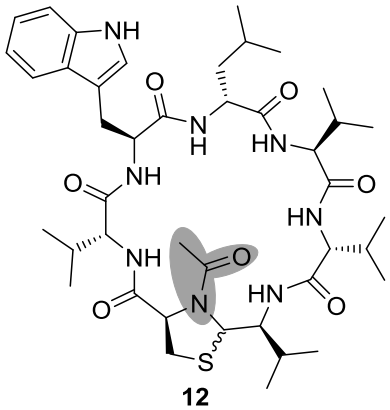

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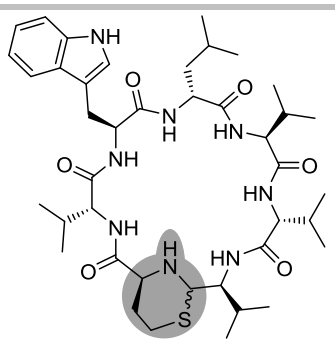

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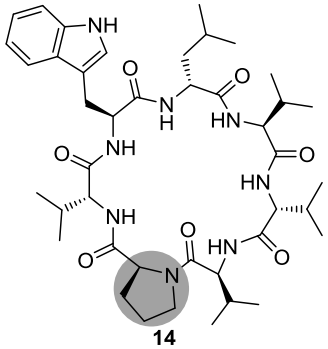

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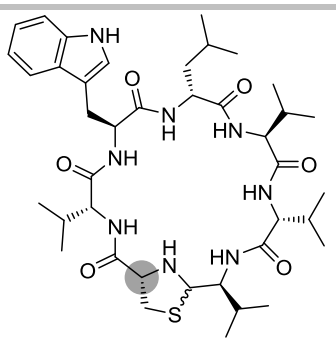

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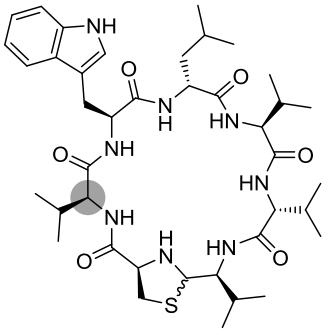

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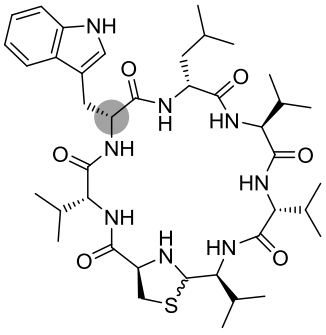

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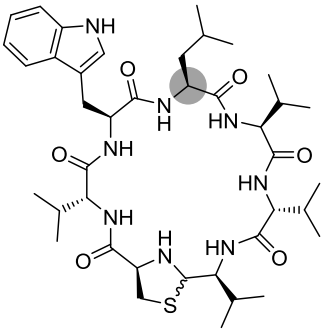

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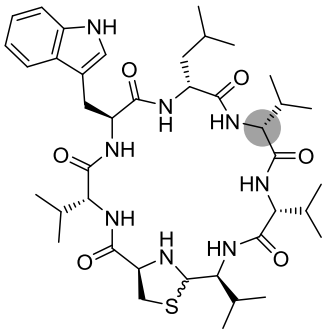

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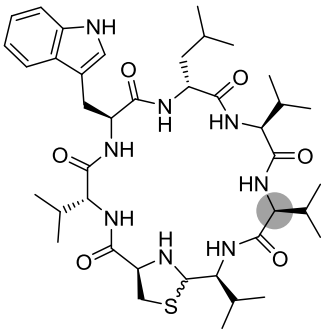

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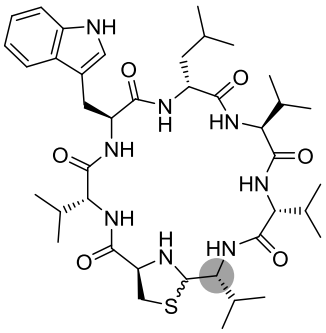

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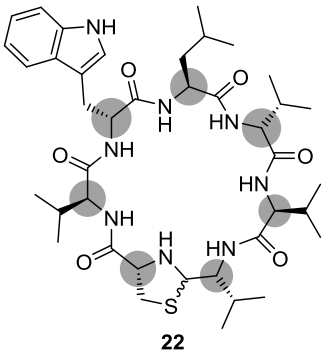

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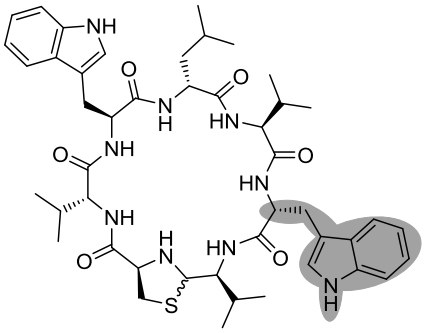

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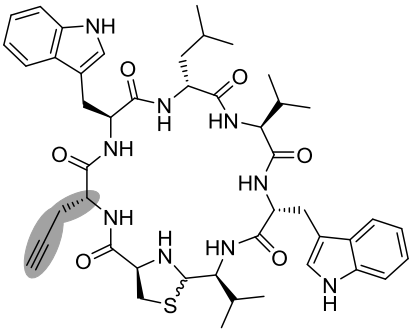

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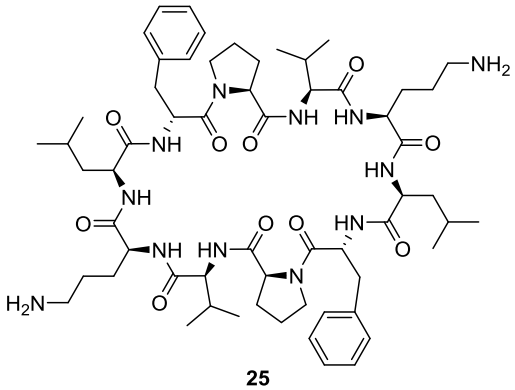

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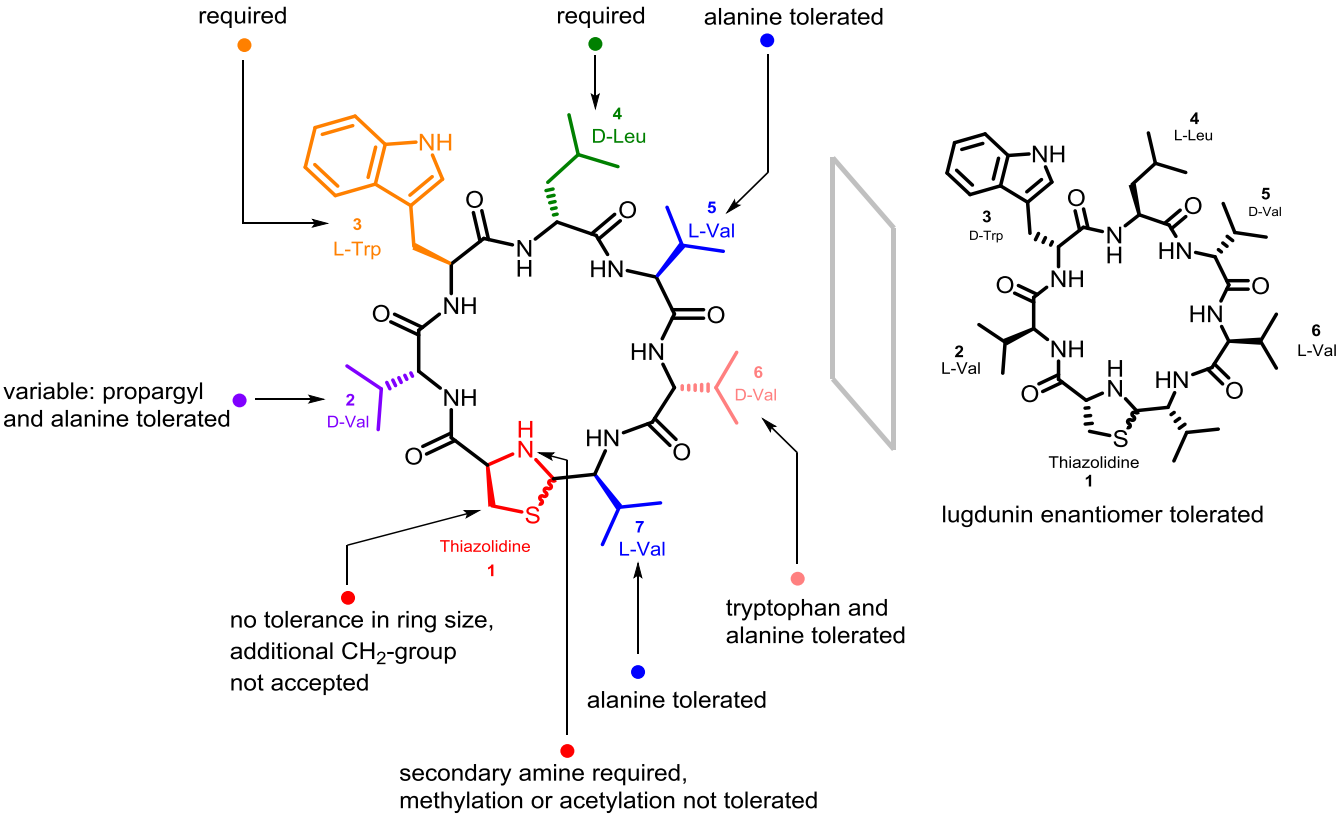
Results of the SAR-studies in one scheme



Strategy for designing fibupeptides 1-7, 11, 13, 15-20, 23 and 24

Fmoc-Based Solid-Phase Peptide Synthesis (SPPS)

Peptide aldehydes were prepared by manual SPPS using either a commercially available preloaded H-Val-H NovaSyn® TG resin (Novabiochem, Switzerland) or a H-Thr-Gly-NovaSyn® resin that is loaded with the desired amino acid (as described in the next paragraph). Peptide synthesis was performed following Fmoc-protection strategy in a fourfold excess of the protected amino acids with HATU/HOBt as coupling reagents, NMM as base and acetonitrile as solvent, as coupling efficiency of consecutive valine residues was higher in acetonitrile.

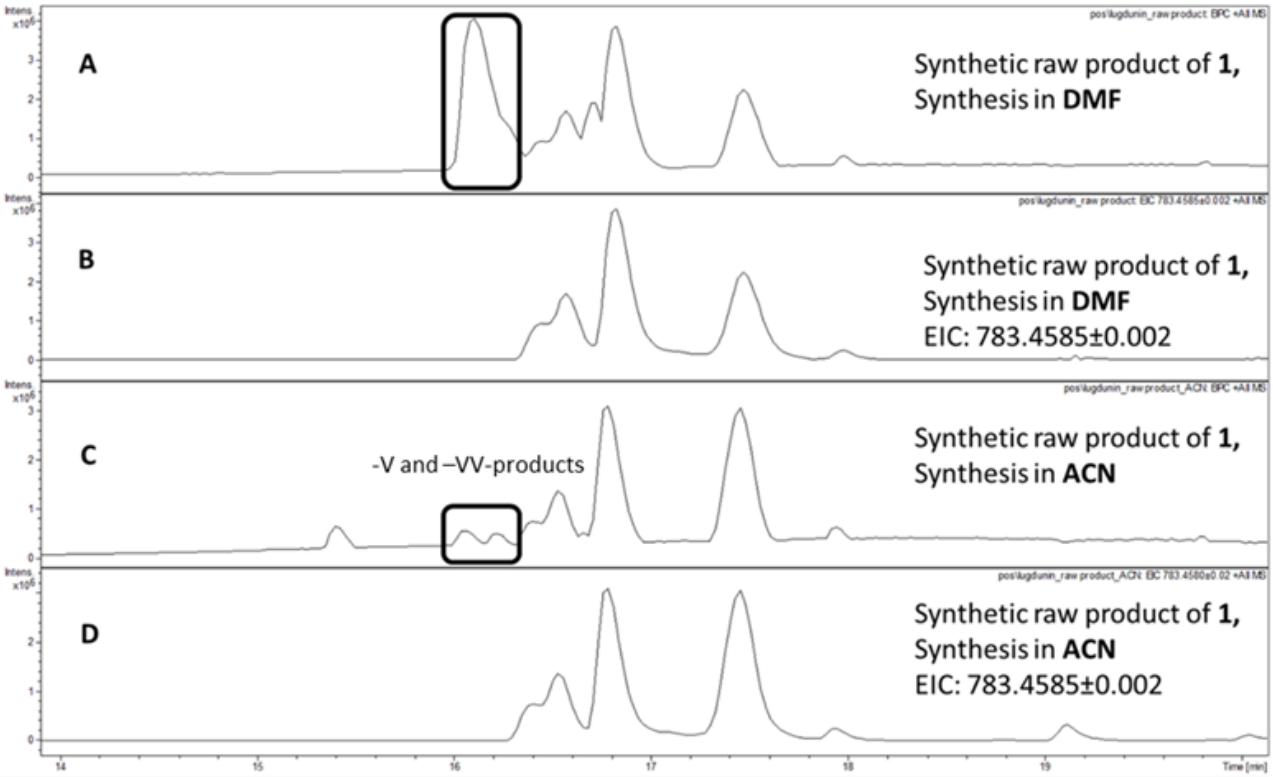


Figure S1: **A** LCMS-chromatogram of synthetic lugdunin (**1**) after cleavage from the resin. Synthesis was performed in DMF. **B** Extracted mass chromatogram (EIC) of the exact mass of **1** (783.4585±0.002) **C** LCMS-chromatogram of synthetic **1** after cleavage from the resin, synthesis was performed in ACN. The amount of "minus-valine-lugdunin" and "minus-two-valine-lugdunin" is clearly lower than in **A**. **D** Extracted mass chromatogram (EIC) of the exact mass of **1** (783.4585±0.002). (Nucleoshell RP18 (Macherey-Nagel), 0.3 mL min^{-1} flow rate, linear gradient from 10% to 100% methanol in 20 min).

Fmoc-D/L-Cys(Trt)-OH, Fmoc-HomoCys(Trt)-OH and Fmoc-*N*-Me-Cys(Trt)-OH were coupled in acetonitrile/dimethylformamide (50:50, v/v) because of low solubility in acetonitrile.

Standard operation for the coupling of the amino acids:

Step 0: 50 mg of the resin was left to swell in 1 mL acetonitrile for 30 minutes.

Step 1: The solution of amino acid, coupling reagents and base in 1 mL acetonitrile was added to the resin and incubated under rotation for 1 h.

Step 2: The resin in the syringe was washed with 1 mL acetonitrile (5 times).

Step 3: Fmoc-protecting group of the terminal amino acid was removed by treatment with 1 mL of a 40 % piperidine solution in DMF (2 times for 5 min).

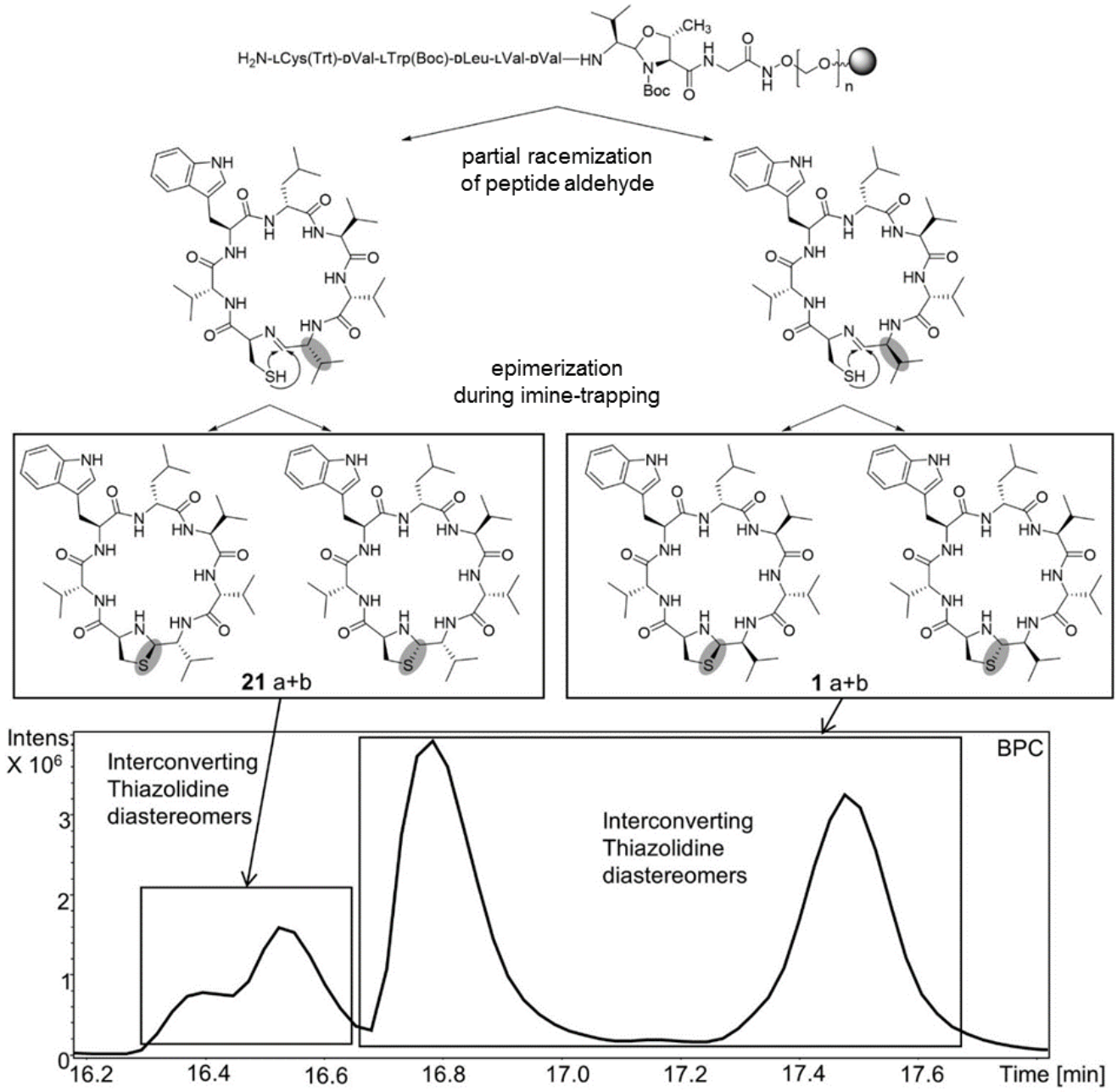
Step 4: The resin in the syringe was washed with 1 mL acetonitrile (5 times).

Steps 1-4 were repeated until the desired amino acid sequence was fully assembled on the resin. The Fmoc-group was removed and the resin was dried after washing with 1 mL

dimethylformamide (3 times), 1 mL isopropyl alcohol (3 times) and 1 mL diethyl ether (3 times). The side-chain protecting groups were removed by treatment with 0.5 mL trifluoro acetic acid for 20 min. The resin was washed intensively with 1 mL dichloromethane (5 times) and the product was cleaved from the resin by two treatments with 1 mL acetonitrile/water/trifluoro acetic acid (79.95:20:0.05, v/v/v) for 30 min and one time with 1 mL overnight to afford the product after lyophilisation.

Table S1: Amount of reagents for the synthesis of **1** on 50 mg H-Val-H NovaSyn® TG resin (loading 0.21 mmol/g)

reagent		excess	mmol	mg
amino acids	Fmoc-L-Cys(Trt)-OH	fourfold	0.042	24.6
	Fmoc-D/L-Val -OH	fourfold	0.042	14.3
	Fmoc-L-Trp(Boc)-OH	fourfold	0.042	22.1
	Fmoc-D-Leu-OH	fourfold	0.042	14.8
coupling reagents	HATU	fourfold	0.042	16.0
	HOBt	catalytic		
base	N-methylmorpholine	eightfold	0.084	8.5 (9.5 µl)



Scheme S1. The structure of the four lugdunin diastereomers after synthesis: Two because of the thiazolidine diastereomers due to epimerization and two because of the partial racemization of the aldehyde alpha-carbon during synthesis.

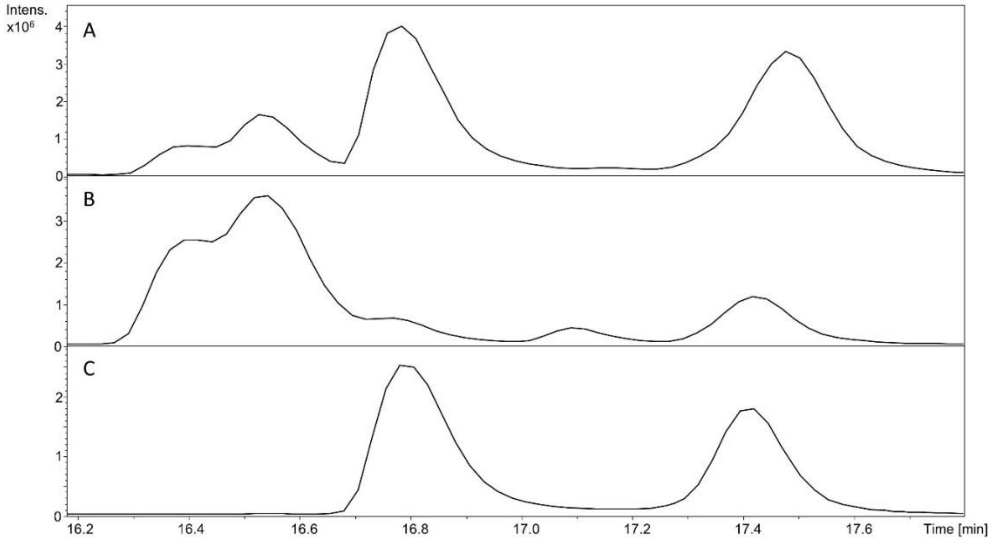


Figure S2. A LC-chromatogram of synthetic lugdunin (**1**) after cleavage from the resin. **B** LC-chromatogram of synthetic lugdunin analogue **21** (7-D-lugdunin). It can be seen, that the products of **1** from partial racemization in Val^7 are the 7-D-lugdunin-diastereomers. **C** LC-chromatogram of natural lugdunin for comparison. (Nucleoshell RP18 (Macherey-Nagel), 0.3 mL min^{-1} flow rate, linear gradient from 10% to 100% methanol in 20 min).

Preparation of amino acid-loaded H-Thr-Gly-NovaSyn®TG resin (Synthesis of 8, 21-22)

General procedure for the *N*-Fmoc protection of amino acid alcohol

The amino acid alcohol (S)-(+)-2-Amino-3-methyl-1-butanol for the synthesis of **21** and **22** and (S)-(+)-2-Amino-1-propanol for the synthesis of **8** was protected using Fmoc-OSu. For this purpose the amino acid alcohol (5 mmol) was dissolved in dd-H_2O (20 mL), containing $NaHCO_3$ (5 mmol). To this mixture Fmoc-OSu (5 mmol), dissolved in acetone (30 mL), was added and the reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, acetone was evaporated. The solution was acidified with citric acid and extracted with ethyl acetate (3 times). The combined organic phases were evaporated and the product was precipitated by adding cold diethyl ether.

The Fmoc-protected amino acid alcohols were then oxidized using IBX-PS resin and the obtained aldehydes were loaded onto H-Thr-Gly-NovaSyn®TG resin according to Novabiochem Tech Bulletin[1].

Synthesis of derivatives 9, 10 and 14

The synthesis of **9**, **10** and **14** was performed using standard Fmoc-chemistry in a fourfold excess of the protected amino acids with HATU/HOBt as coupling reagents and dimethylformamide as solvent on a Fmoc-L-Val-TCP-resin. To obtain peptide **9**, the cleavage from the resin was accomplished with 1 mL trifluoro acetic acid, containing 2.5 % dd-H_2O, 2.5 % thioanisole and 2.5 % ethanedithiole, for 3 h. After precipitation in cold dd-H_2O and centrifugation, **9** was obtained as crude product. To obtain peptides **10** and **14**, the cleavage from the resin was performed 5 times for 2 min with 1 mL 30 % hexafluoroisopropanol in dichloromethane. The fractions were combined and evaporated.

The fully assembled and side chain protected linear peptide precursors were then cyclized in solution. 0.1 mmol of the precursor were dissolved in dimethylformamide (20 mL). After addition of NMM (0.6 mmol) and HOBt (0.3 mmol), TBTU (0.3 mmol), dissolved in dimethylformamide (40 mL), was slowly dropped to the peptide containing solution during 2 h under fast stirring. After stirring overnight, the solvent was evaporated and the product was precipitated in cold diethyl ether. Deprotection of the amino acid side chains was performed as described above.

Acetylation of lugdunin (12)

1 mg of lugdunin (1.28 µmol, 1 eq.) was lyophilized into a reaction cup and supplemented with 200 µL H_2O and 380 µL DMSO. Acetylation was achieved by adding sodium carbonate (113.2 µmol, 88 eq.) and 1.2 mL acetic acid anhydride and agitation of the reaction over night at room temperature. Solvents were removed by lyophilization. The crude product was dissolved in methanol and subjected to preparative HPLC. Isocratic elution at 74.5 % methanol and a flow rate of 15 mL/min from a C4-RP column (Dr. Maisch, Reprosil-Gold 120, 5µm) afforded **12** in quantitative yields as a white solid.

Synthesis of gramicidin S (25)

2-chlorotrityl-resin (0.095 mmol) was swollen in dichloromethane for 10 min and then incubated with Fmoc-D-Phe-OH (0.38 mmol, 4 eq.) and DIPEA (0.38 mmol, 4 eq.) in dichloromethane/dimethylformamide (90/10, v/v) for 90 min. After the coupling, the resin was washed with dichloromethane and dimethylformamide. Coupling of the amino acid sequence was performed in dimethylformamide following the protocol described above, followed by

cleavage from the resin with trifluoro acetic acid/dd-H_2O/thioanisole/phenol (95/2.5/2.5/catalytic amount). After incubation for 2.5 h the peptide was precipitated in cold dd-H_2O and centrifuged. Subsequently, the linear peptide (13.7 µmol) was dissolved in dichloromethane (20 ml). HOBt (41.1 µmol, 3 eq.) and DIPEA (82.2 µmol, 6 eq.) were added to the solution. The reaction mixture was added dropwise to a solution of PyBOP (41.1 µmol, 3 eq.) in dichloromethane (40 ml) over 3 h. After being stirred for 20 h at RT, the solvent was evaporated. The Dde-protecting-group was removed with 2% hydrazine in THF for 16 h at RT. The reaction mixture was freeze-dried and the product was optained after HPLC.

Yield 43.4 mg (40 %).

$C_{60}H_{92}N_{12}O_{10}$ ($[M+H]^+$:$C_{60}H_{93}N_{12}O_{10}^+$, calc.: 1141.7132 m/z, meas.: 1141.7134 m/z, ∆ppm 0.18; $[M+2H]^{2+}$: $C_{60}H_{94}N_{12}O_{10}^{2+}$, calc.: 571.3602 m/z, meas.: 571.3610 m/z, ∆ppm 1.4).

HPLC analysis and semi-preparation

Purification was performed with system A (0.05% TFA in dd-H_2O) and system B (methanol) on a reverse-phase semi-preparative Kromasil 100 C_{18} column (5 µm, 250 × 8 mm, Dr. Maisch) and the corresponding pre-column Kromasil 100 C_{18} column (5 µm, 30 × 8 mm, Dr. Maisch). Flow rate was 1.5 mL/min. The elution was performed with a linear gradient of system A and sytem B (Table S2).

Table S2. Elution gradients during purification of peptides **1**-**11** and **13**-**25**

compound	gradient
1, **9** - **10**, **11**, **14**, **21** - **25**	30 % system A and 70 % sytem B to 10 % system A and 90 % system B in 30 min
15 - **20**	35 % system A and 65 % sytem B to 10 % system A and 90 % system B in 40 min
2 - **8**	30 % system A and 70 % sytem B to 15 % system A and 85 % system B in 40 min

Eluted products were monitored at 220 nm, collected and freeze-dried. Determination of the purity was done by HR-LCMS:

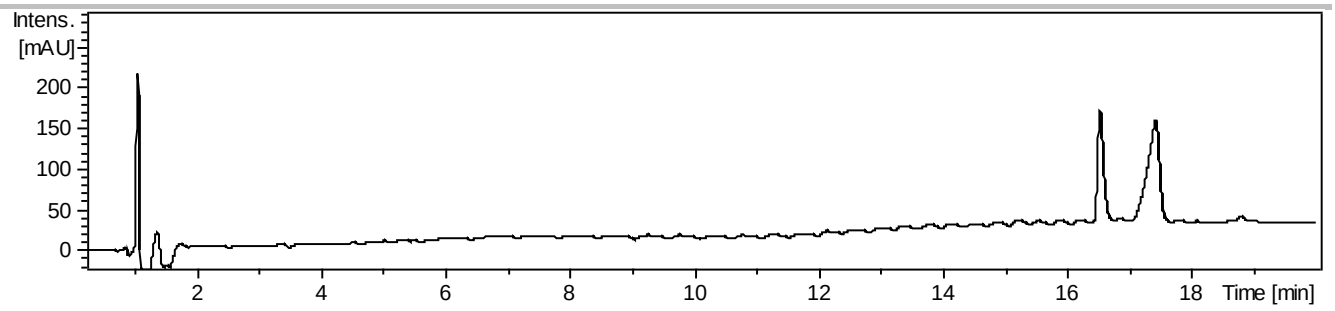


Figure S3. Analytical HPLC trace of **1** (Nucleoshell® EC RP-C18 column, 150 x 2 mm, 2.7µm, flow rate = 0.3 mL min^{-1} with a linear gradient from 90:10 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) to 0:100 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) over 20 minutes. Monitored at 220 nm.

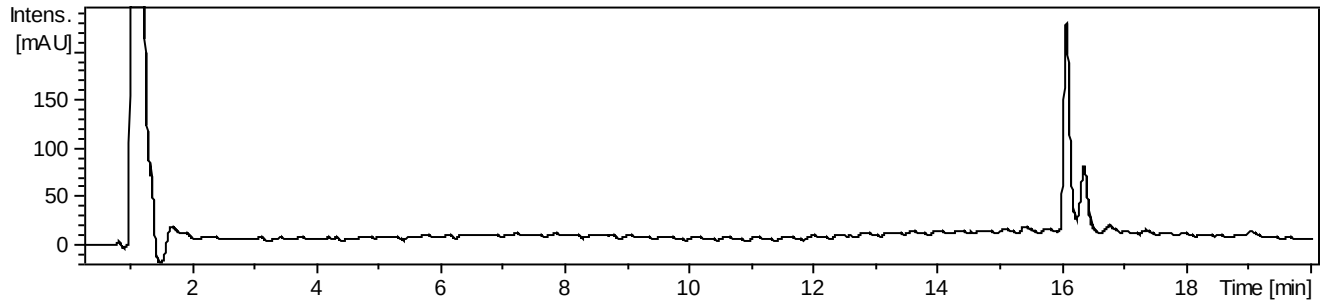


Figure S4. Analytical HPLC trace of **3** (Nucleoshell® EC RP-C18 column, 150 x 2 mm, 2.7µm, flow rate = 0.3 mL min^{-1} with a linear gradient from 90:10 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) to 0:100 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) over 20 minutes. Monitored at 220 nm.

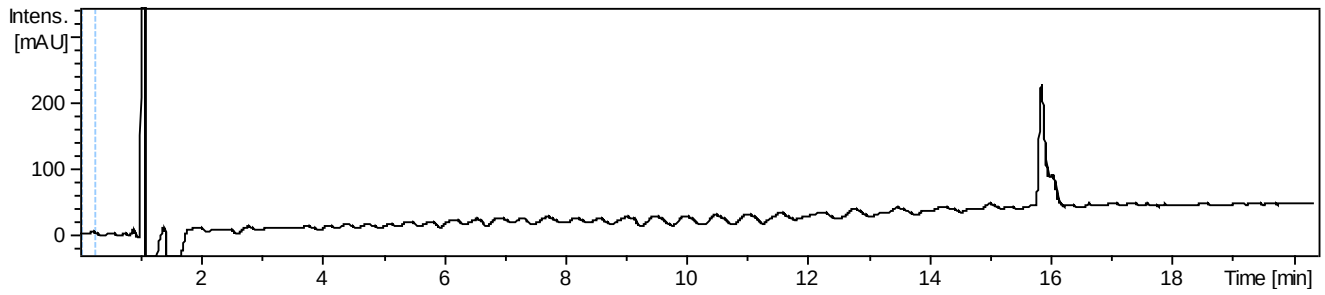


Figure S5. Analytical HPLC trace of **6** (Nucleoshell® EC RP-C18 column, 150 x 2 mm, 2.7µm, flow rate = 0.3 mL min^{-1} with a linear gradient from 90:10 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) to 0:100 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) over 20 minutes. Monitored at 220 nm.

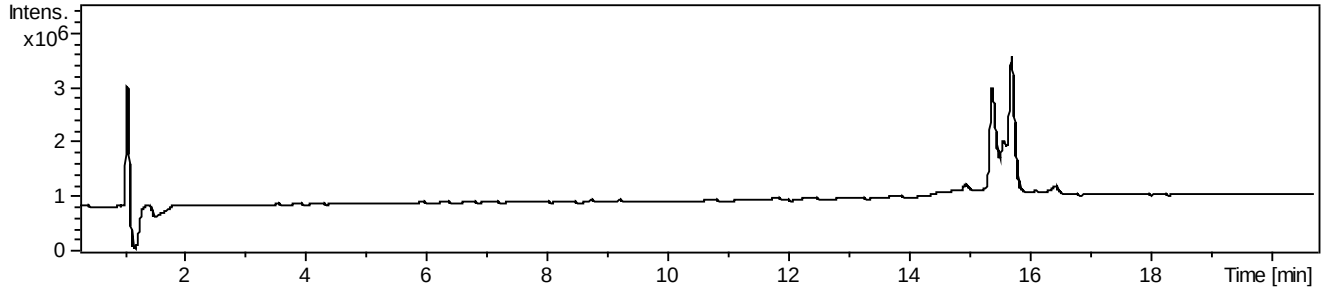


Figure S6. Analytical HPLC trace of **7** (Nucleoshell® EC RP-C18 column, 150 x 2 mm, 2.7µm, flow rate = 0.3 mL min^{-1} with a linear gradient from 90:10 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) to 0:100 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) over 20 minutes. Monitored at 220 nm.

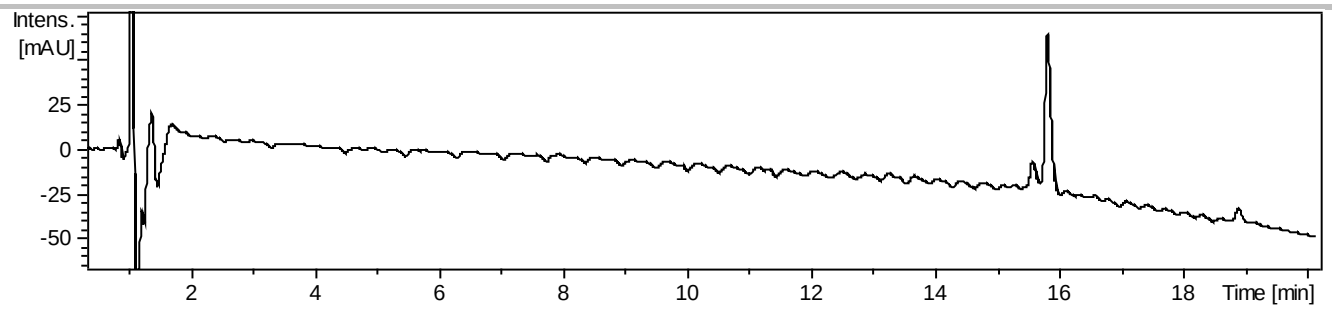


Figure S7. Analytical HPLC trace of **8** (Nucleoshell® EC RP-C18 column, 150 x 2 mm, 2.7µm,flow rate = 0.3 mL min^{-1} with a linear gradient from 90:10 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) to 0:100 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) over 20 minutes. Monitored at 220 nm.

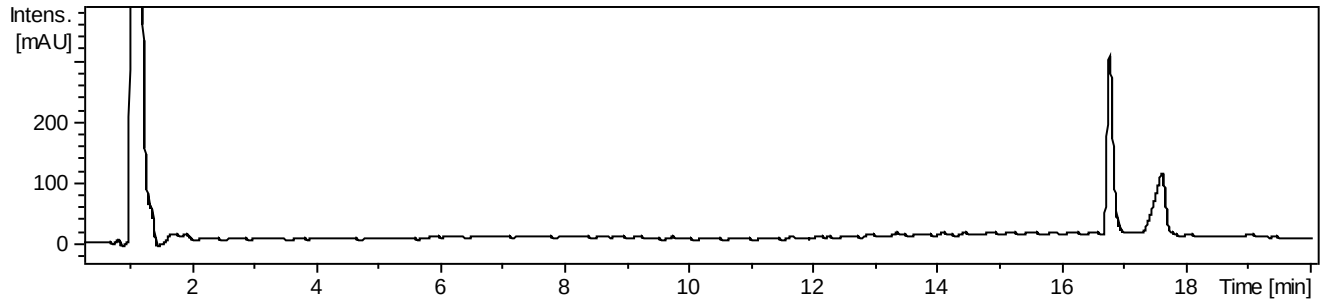


Figure S8. Analytical HPLC trace of **22** (Nucleoshell® EC RP-C18 column, 150 x 2 mm, 2.7µm, flow rate = 0.3 mL min^{-1} with a linear gradient from 90:10 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) to 0:100 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) over 20 minutes. Monitored at 220 nm.

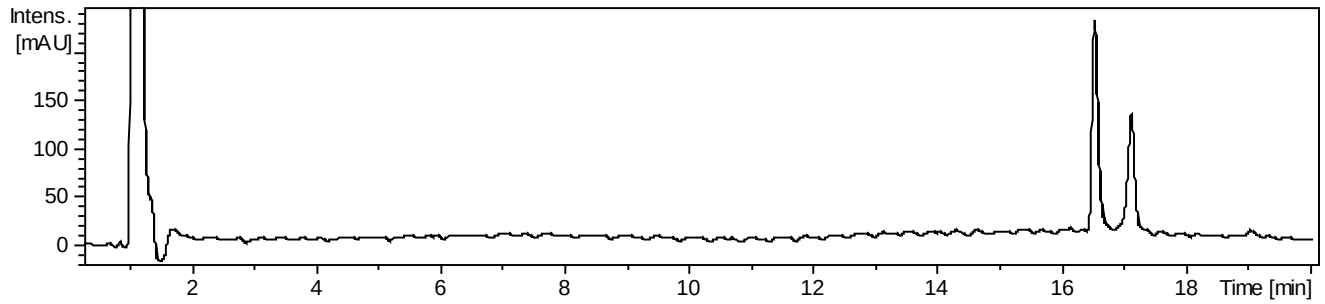


Figure S9. Analytical HPLC trace of **23** (Nucleoshell® EC RP-C18 column, 150 x 2 mm, 2.7µm, flow rate = 0.3 mL min^{-1} with a linear gradient from 90:10 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) to 0:100 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) over 20 minutes. Monitored at 220 nm.

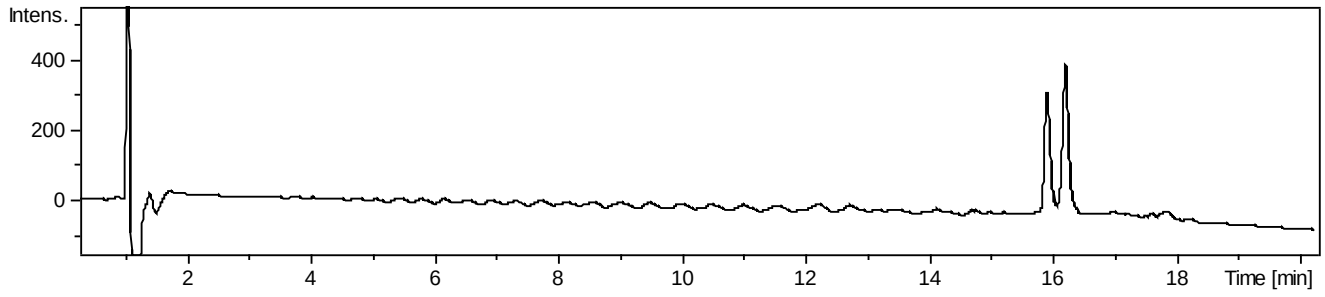


Figure S10. Analytical HPLC trace of **24** (Nucleoshell® EC RP-C18 column, 150 x 2 mm, 2.7µm, flow rate = 0.3 mL min^{-1} with a linear gradient from 90:10 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) to 0:100 (0.1% FA in H_2O: 0.06% FA in MeOH, v:v) over 20 minutes. Monitored at 220 nm.

Antimicrobial activity assay (MIC-assay)

Antibacterial activities of **1** and its analogues were measured using a dilution method as previously described.[2] Shortly, varying concentrations of lugdunin and its derivatives in Mueller Hinton Broth (MHB) were inoculated with bacteria from an overnight culture to a final density of 1×10^6 colony forming units (cfu) per mL in microtiter plates and incubated at 37 °C for 21 h under continuous shaking. The OD_{600} of each well was measured with a microtiterplate reader, and the lowest peptide concentrations, which displayed no bacterial growth, were defined as the MIC.

Growth control	100 % DMSO
	culture without any additives
Positive controls	Daptomycin (10 mg/mL in DMSO, MIC: 6.25 µg/mL)
	Vancomycin (10 mg/mL in MilliQ-H_2O, MIC: 0.78 µg/mL)
	Natural lugdunin (10 mg/mL in DMSO, MIC: 3.13 µg/mL)
	Gramicidin S (10 mg/mL in DMSO, MIC: 6.25 µg/mL)

Membrane Potential Assay

S. aureus NCTC8325 was grown to the exponential phase in lysogeny broth (LB) + 0.1% glucose, harvested and resuspended to an optical density at 600 nm (OD_{600}) of 0.5 in phosphate buffered saline (PBS) + 0.1% glucose. Cells were incubated with 30 µM 3,3'- diethyloxacarbocyanine iodide ($DiOC_2(3)$, Molecular Probes) for 15 min in the dark and subsequently treated with a concentration series of lugdunin and its derivatives for 30 and 60 minutes. The protonophore carbonyl cyanide m-chlorophenyl hydrazone (CCCP, Sigma Aldrich) at a concentration of 5 µM (MIC CCCP 12.5 µM) was used as a positive control and DMSO as a negative control. Fluorescence was measured at an excitation wavelength of 485 nm and two emission wavelengths, 530 nm (green) and 630 nm (red), using a microplate reader (TECAN Infinite M200). Experiments were performed as set of 2 biological replicates with 2 technical replicates each.

SUPPORTING INFORMATION

Table S3. MIC-values against *S. aureus* NCTC8325 of peptides included in the membrane potential assay.

	AA sequence	highlighted differences	*S. aureus* NCTC8325 MIC in µg mL^{-1}
1	(C**V**W**L**V**V**V)	lugdunin	3.1
3	(C**A**W**L**V**V**V)	**2-Ala**-lugdunin	12.5
5	(C**V**W**A**V**V**V)	**4-Ala**-lugdunin	100
11	(Me-C**V**W**L**V**V**V)	***N*-methyl**thiazolidine-lugdunin	> 100
21	(C**V**W**L**V**VV**)	**7**-ᴅ-lugdunin	50
22	(**C**V**W**L**V**V**V**)	**enantio**-lugdunin	1.6
23	(C**V**W**L**V**W**V)	**6**-**Trp**-lugdunin	3.1
25	VOLF**P**VOLF**P**$_{cycl.}$	gramicidin S	6.2
	(C**V**W**L**V**V**V)	natural lugdunin	3.1

Single-letter codes for amino acids, the brackets in the sequence indicate cyclic structure (cyclisation via thiazolidine), *cycl.* indicates cyclisation via peptide bond, bold letters represent ᴅ-amino-acids. Ala = alanine, Trp = tryptophan

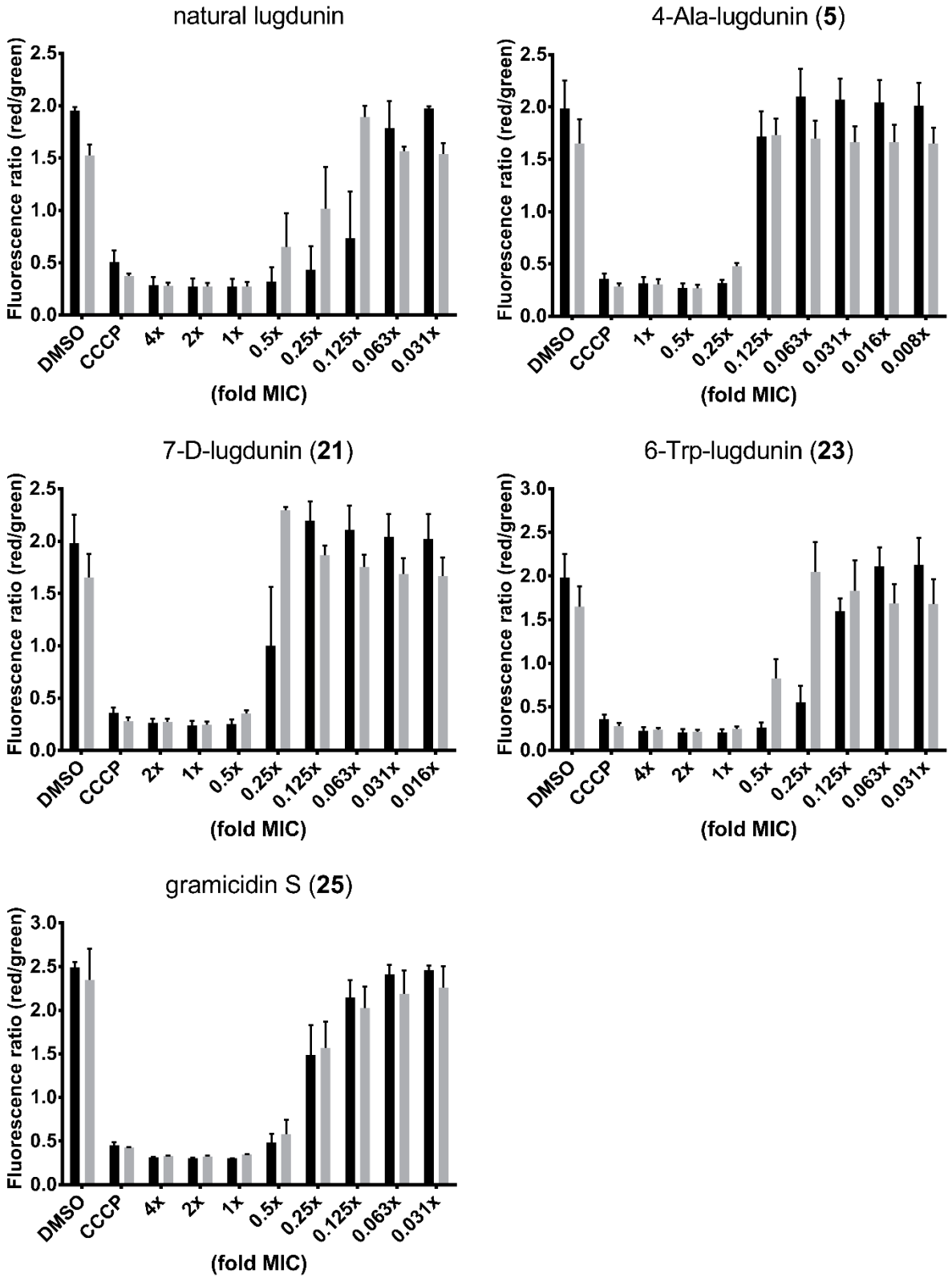


Figure S11. Effect of natural lugdunin, fibupeptides **5**, **21** and **23** as well as gramicidin S (**25**) on the *S. aureus* NCTC8325 membrane potential measured after 30 (black bars) and 60 (grey bars) minutes of treatment in PBS + 0.1% glucose. The protonophore CCCP (5 µM) was used as a positive and DMSO a negative control. Error bars represent the SD of 2 biological replicates including 2 technical replicates each.

Pore Formation

Pore formation was monitored using the Live/Dead BacLight bacterial viability kit (Molecular Probes). *S. aureus* NCTC8325 was grown in LB at 37 °C to the exponential phase. Aliquots (100 µl) of the cells were treated with either 10 µg/ml or 30 µg/ml of lugdunin (approx. 3 x or 10 x MIC), 100 µg/ml of crude nisin (2.5% nisin, Sigma-Aldrich) corresponding to 1-2 x MIC or left untreated as control. Samples were taken after 15 min of antibiotic treatment, then 0.2 µl of a 1:1 mixture of SYTO9 and propidium iodide were added per 100 µl of culture and further incubated for 15 min at room temperature in the dark before microscopic analysis. Samples were visualized by phase contrast and fluorescence microscopy on microscope slides covered with a thin film of 1% agarose using a Zeiss Axio Observer Z1 automated microscope. Images were acquired with an Orca Flash 4.0 V2 camera (Hamamatsu) and a C Plan-Apo 63x/1.4 Oil DIC objective (Zeiss). Images were processed using the Zen software package (Zeiss).

Preparation of unilamellar vesicles

Large unilamellar vesicles (LUVs) filled with the fluorescent dyes pyranine (8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt) and 5(6)-Carboxyfluorescein were prepared by the extrusion method. A lipid film was prepared by drying a lipid solution (2 mg lipid in 150 µL chloroform) in a small test tube under a nitrogen stream and further for 3 h in vacuum. The film was incubated in 1 mL buffer (100 mM carboxyfluorescein, 20 mM HEPES, pH 7.4 or 0.5 mM pyranine, 100 mM KCl, 5 mM HEPES, pH 7.4) for 30 min. The suspension was thoroughly mixed for 90 s and extruded 31 times through a polycarbonate membrane with a nominal pore diameter of 200 nm. The external dye was removed via size exclusion chromatography (illustra NAP-25 G25 column, GE Healthcare) in buffer containing 275 mM KCl, 20 mM HEPES, pH 7.4 for carboxyfluorescein or 100 mM KCl, 5 mM HEPES, pH 7.4 for pyranine. Phospholipid concentration of the vesicle suspensions was quantified by determining inorganic phosphate. For this, vesicle samples of 30 µL as well as calibration samples of known NaH_2PO_4 concentration were heated with 200 µL perchloric acid (70%) to 220 °C for 1 h. The residues were dissolved in 700 µL 12.6% (w/v) $HClO_4$ containing 0.45% (w/v) NH_4MoO_4 and 700 µL of a 1.7% (w/v) ascorbic acid solution were added. After incubation for 5 min at 80 °C, the absorption of the samples at 820 nm was measured and phospholipid concentration was calculated from the calibration curve.

Carboxyfluorescein dequenching assay

All measurements were performed with a Jasco FP-6500 spectrofluorometer at 20 °C. Carboxyfluorescein filled POPC vesicles were suspended in buffer (275 mM KCl, 20 mM HEPES, pH 7.4) to a final volume of 800 µL and a concentration of 50 µM phospholipid. After 100 s, 8 µL of peptide solution in DMSO was added (final concentration 1 or 5 µM). Dye leakage was monitored as an increase in fluorescence at λ_{ex}=480 nm and λ_{em}=520 nm. After 15 min, the vesicles were lysed by addition of 16 µL 10% (w/v) Triton X-100. The fluorescence signal was normalized to the intensities directly before addition of peptide and after lysis.

Proton transport assay

To generate a proton gradient, vesicles composed of POPC and filled with the pH-sensitive fluorescent dye pyranine at pH 7.4 were diluted in buffer with pH 6.4 or 8.4 (100 mM KCl, 5 mM HEPES) to a final lipid concentration of 50 µM and a volume of 800 µL. Pyranine fluorescence was monitored at λ_{ex}=458 nm and λ_{em}=512 nm. After acquisition of a baseline for 250 s, 8 µL of a 5 to 500 µM solution of peptide in DMSO was added. At the end of each measurement, the vesicles were lysed by addition of 16 µL 10% (w/v) Triton X-100. The fluorescence intensity was normalized to the values directly before peptide addition and after lysis. To show the correlation between proton and cation transport in this assay, both the protonophore CCCP (50 nM) and the potassium carrier valinomycin (25 nM) were added consecutively in otherwise identical experiments (Figure S12A). To determine the amount of pyranine leakage, vesicles were suspended in buffer at pH 8.4 and instead of Triton X-100 the membrane impermeable quencher p-xylene-bis-pyridinium bromide (DPX, 16 µL, 0.2 M in DMSO) was added 300 s after peptide addition. This results in a drop in fluorescence intensity representing the absolute amount of free dye in bulk solution (Figure S11B). All quenching measurements were conducted in quick succession to give comparable absolute intensities, which were normalized to the maximum reached after addition of lugdunin at a peptide to lipid ratio of 1:10. Significant pyranine leakage was only observed at high lugdunin concentrations (peptide to lipid ratio <100), comparable to the results from the carboxyfluorescein leakage assay.

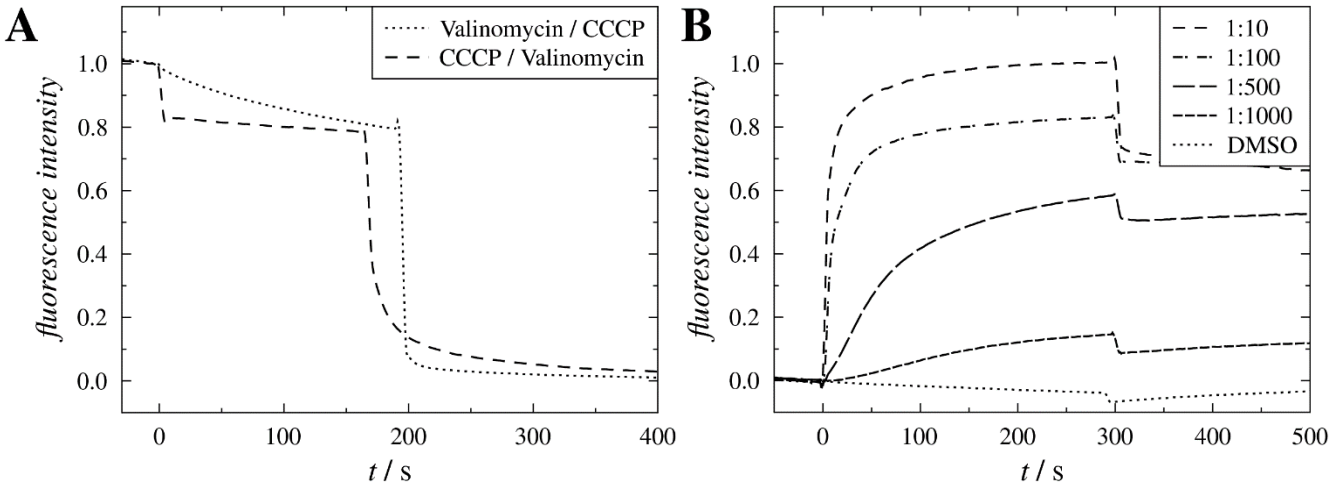


Figure S12. Time course of normalized pyranine fluorescence **A:** after addition of CCCP and valinomycin in different order and **B:** after addition of lugdunin (**1**) showing proton efflux and quenching of free pyranine with DPX. Vesicles composed of POPC, containing 100 mM KCl, 5 mM HEPES, 0.5 mM pyranine, pH 7.4 in 100 mM KCl, 5 mM HEPES, total lipid concentration 50 µM.

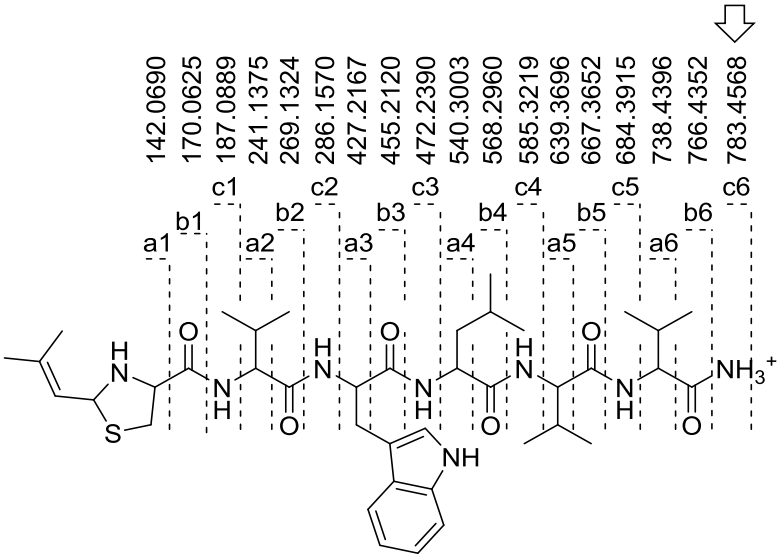
Characterization of lugdunin and its analogues

Mass spectra were recorded on a HPLC-UV-HR mass spectrometer (MaXis 4G with Performance Upgrade kit with ESI-Interface, Bruker Daltonics). In order to obtain high resolution mass spectrometry (HR-MS) data, peptides were applied to a Dionex Ultimate 3000 HPLC system (Thermo Fisher Scientific), coupled to the MaXis 4G ESI-QTOF mass spectrometer (Bruker Daltonics). The ESI source was operated at a nebulizer pressure of 2.0 bar, and dry gas was set to 8.0 L min^{-1} at 200 °C. MS/MS spectra were recorded in auto MS/MS mode with collision energy stepping enabled. Sodium formiate was used as internal calibrant in each analysis. The routine gradient was 10 % system B (MilliQ-H_2O with 0.1 % formic acid) and 90 % system A (methanol with 0.06 % formic acid) to 100 % system B in 20 min with a flow rate of 0.3 mL/min on a Nucleoshell® EC RP-C_{18} (150 x 2 mm, 2.7µm) from Macherey-Nagel.

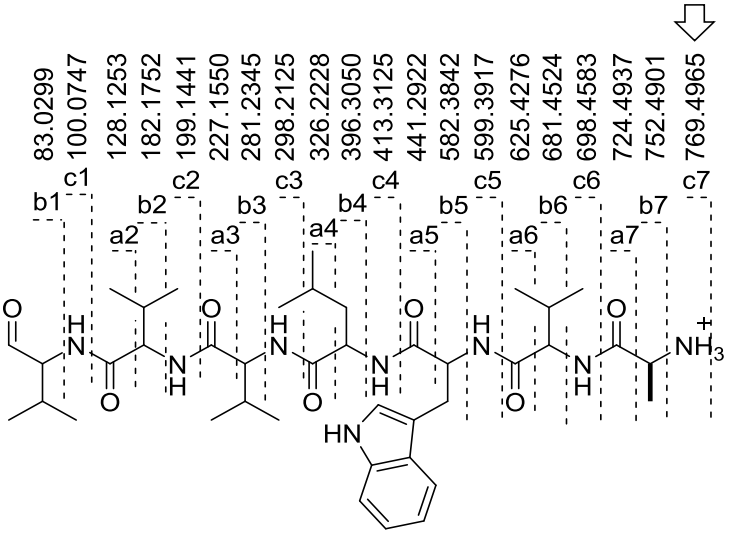
Fragment ion analysis by LC-HRMS for fibupeptides 1 - 24

Fragmentation route molecules are shown in linearized form.

Lugdunin (1)
[M+H]+ calculated for $C_{40}H_{63}N_8O_6S$, 783.4586; found 783.4596 (1.4 ppm err; 9.2 mSigma)

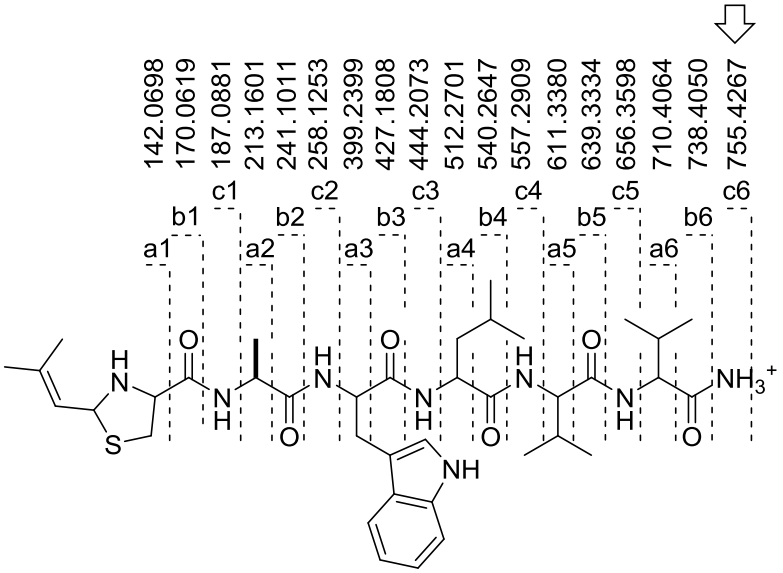


1-Alanine-lugdunin (2)
[M+H]+ calculated for $C_{40}H_{65}N_8O_7S$, 769.4971; found 769.4965 (0.7 ppm err; 2.5 mSigma)



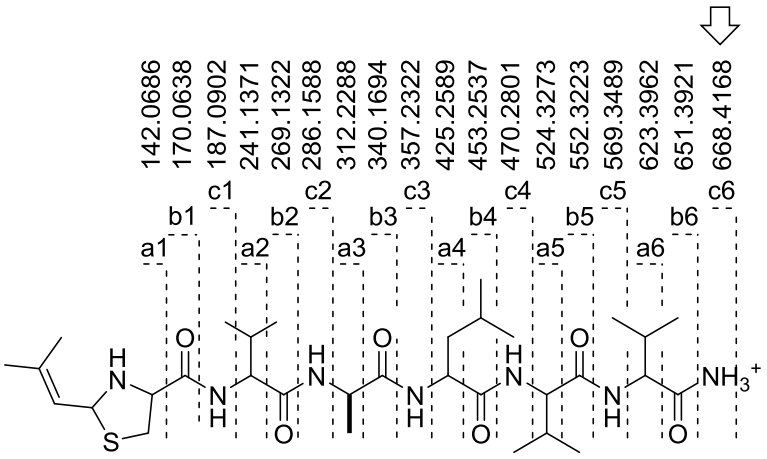
2-Alanine-lugdunin (3)

[M+H]+ calculated for $C_{38}H_{59}N_8O_6S$, 755.4273; found 755.4267 (0.7 ppm err; 10.3 mSigma)



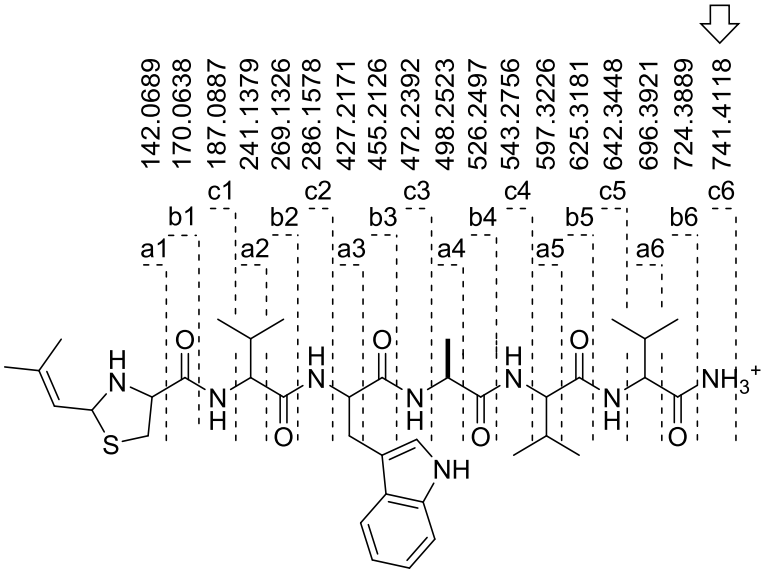
3-Alanine-lugdunin (4)

[M+H]+ calculated for $C_{32}H_{58}N_7O_6S$, 668.4164; found 668.4168 (0.6 ppm err; 25.4 mSigma)



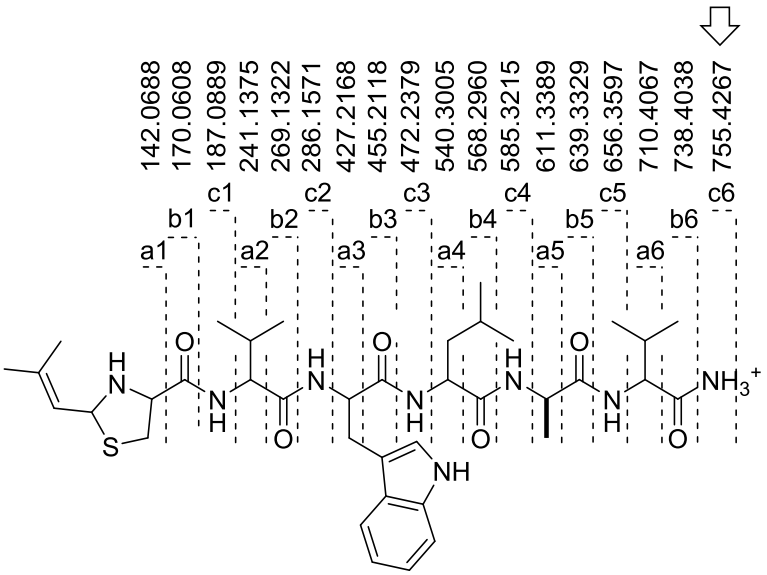
4-Alanine-lugdunin (5)

[M+H]+ calculated for $C_{37}H_{57}N_8O_6S$, 741.4116; found 741.4118 (0.2 ppm err; 3.3 mSigma)



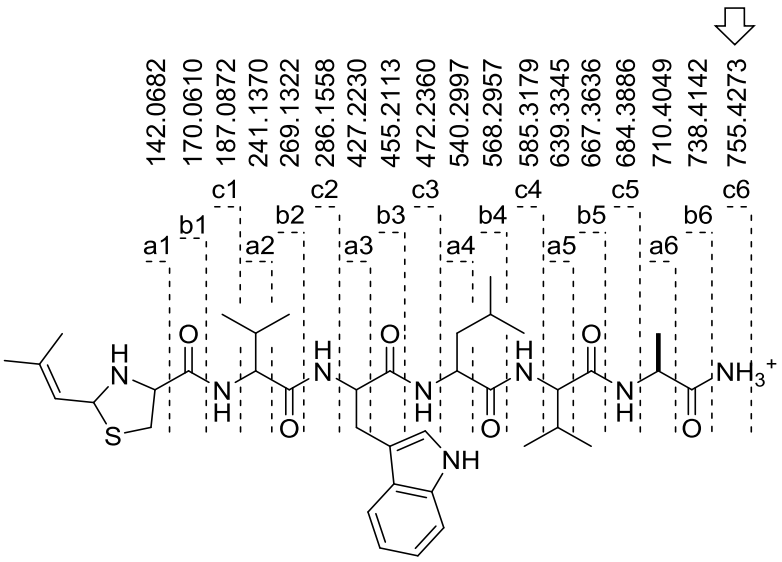
5-Alanine-lugdunin (6)

[M+H]+ calculated for $C_{38}H_{59}N_8O_6S$, 755.4273; found 755.4276 (0.4 ppm err; 3.2 mSigma)



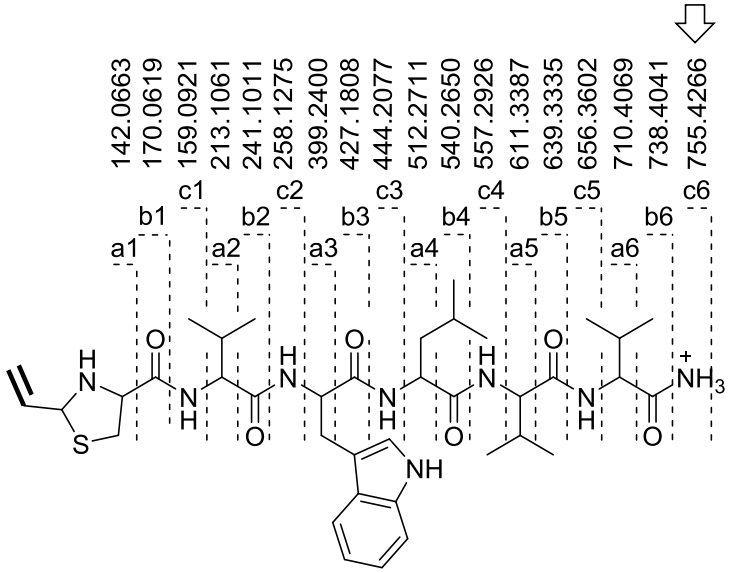
6-Alanine-lugdunin (7)

[M+H]+ calculated for $C_{38}H_{59}N_8O_6S$, 755.4273; found 755.4273 (0.2 ppm err; 13.9 mSigma)



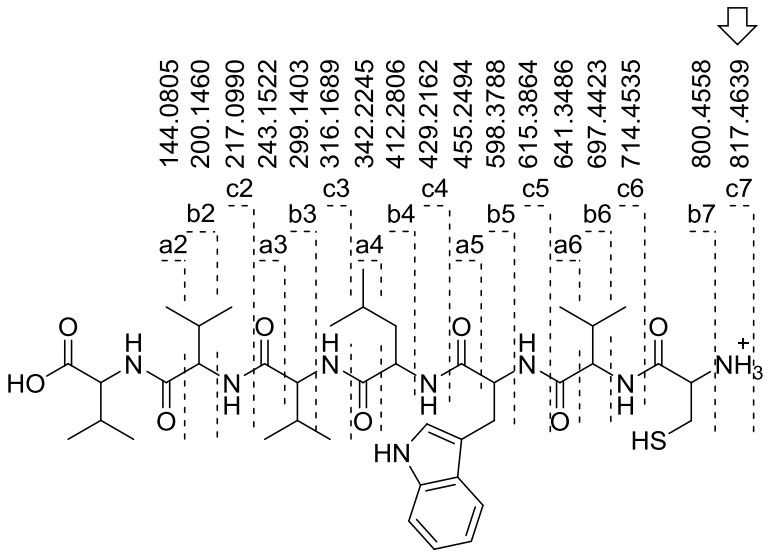
7-Alanine-lugdunin (8)

[M+H]+ calculated for $C_{38}H_{59}N_8O_6S$, 755.4273; found 755.4266 (0.9 ppm err; 5.8 mSigma)



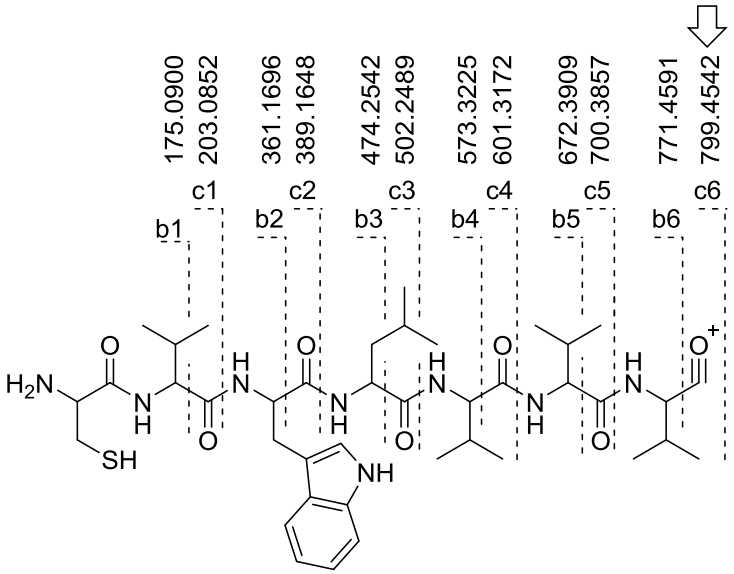
Linear lugdunin (9)

[M+H]+ calculated for $C_{40}H_{65}N_8O_8S$, 817.4641; found 817.4637 (0.4 ppm err; 123.9 mSigma)



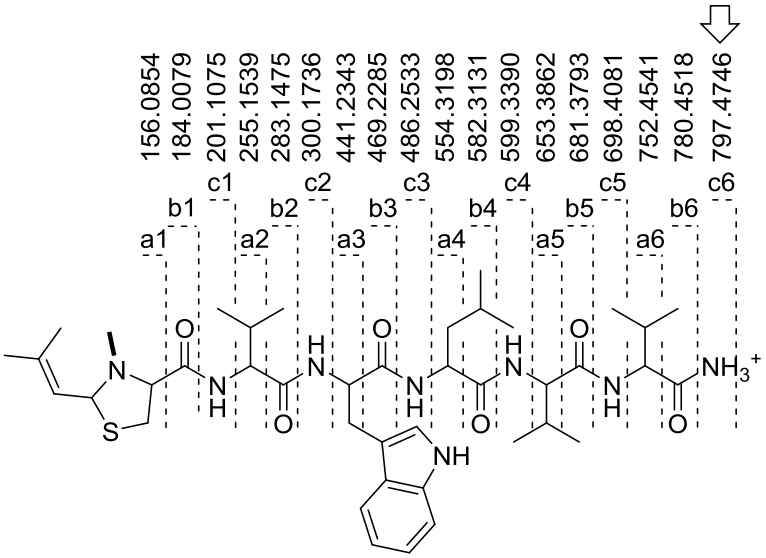
Cyclized homodetic lugdunin (10)

[M+H]+ calculated for $C_{40}H_{63}N_8O_7S$, 799.4535; found 799.4525 (1.2 ppm err; 3.0 mSigma)



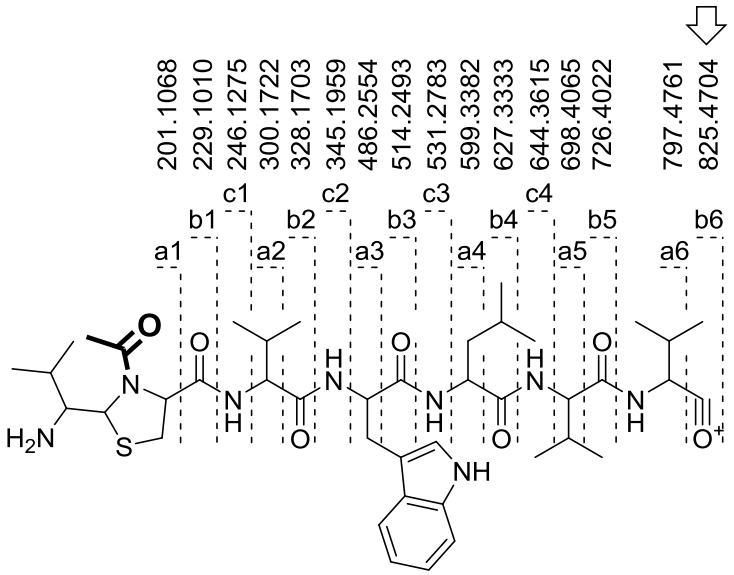
***N*-methylthiazolidine-lugdunin (11)**

[M+H]+ calculated for $C_{41}H_{65}N_8O_6S$, 797.4742; found 797.4746 (0.4 ppm err; 4.3 mSigma)



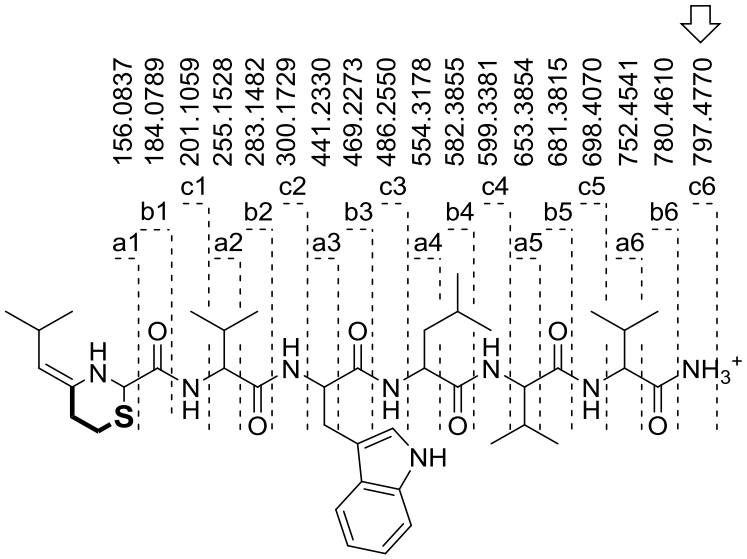
***N*-acetylthiazolidine-lugdunin (12)**

[M+H]+ calculated for $C_{32}H_{65}N_8O_7S$, 825.4691; found 825.4675 (1.9 ppm err; 9.5 mSigma)



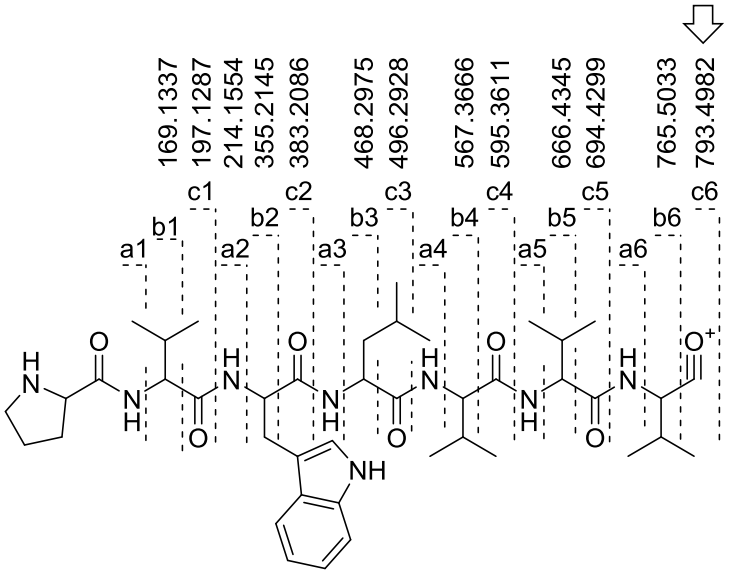
1,3-thiazinan-lugdunin (13)

[M+H]+ calculated for $C_{41}H_{65}N_8O_6S$, 797.4742; found 797.4770 (3.5 ppm err; 255.7 mSigma)



1-Pro-lugdunin (14)

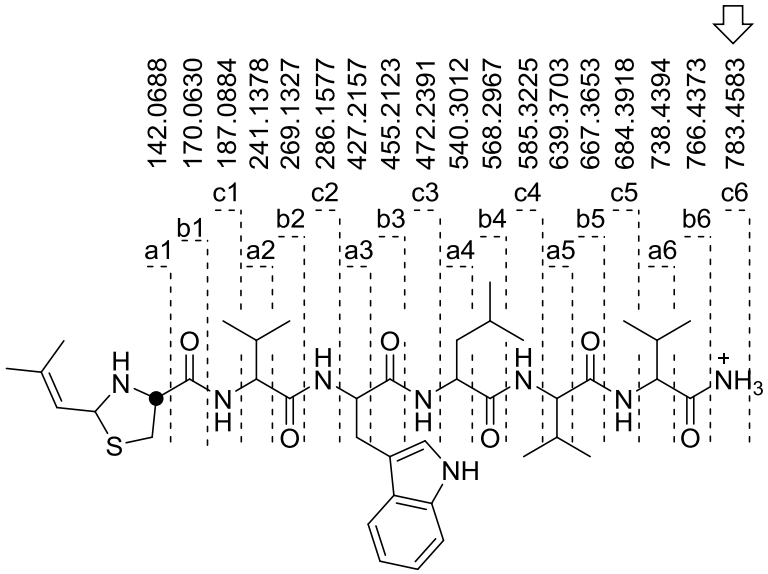
[M+H]+ calculated for $C_{42}H_{65}N_8O_7$, 793.4971; found 793.4982 (1.1 ppm err; 2.7 mSigma)



SUPPORTING INFORMATION

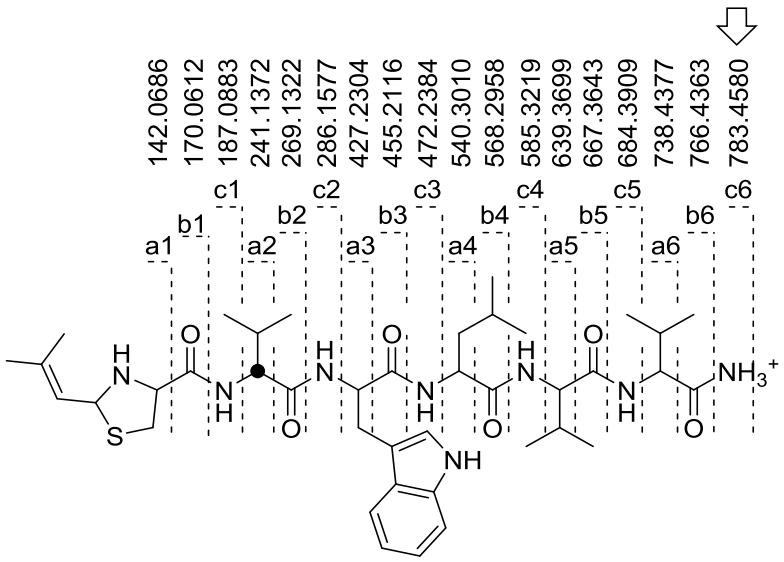
1-D-lugdunin (15)

[M+H]+ calculated for $C_{40}H_{63}N_8O_6S$, 783.4586; found 783.4583 (0.4 ppm err; 5.0 mSigma)



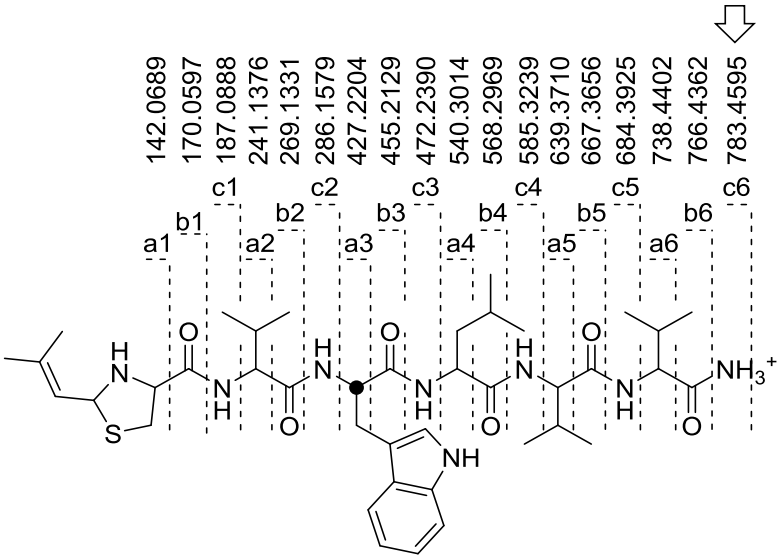
2-L-lugdunin (16)

[M+H]+ calculated for $C_{40}H_{63}N_8O_6S$, 783.4586; found 783.4580 (0.7 ppm err; 4.3 mSigma)



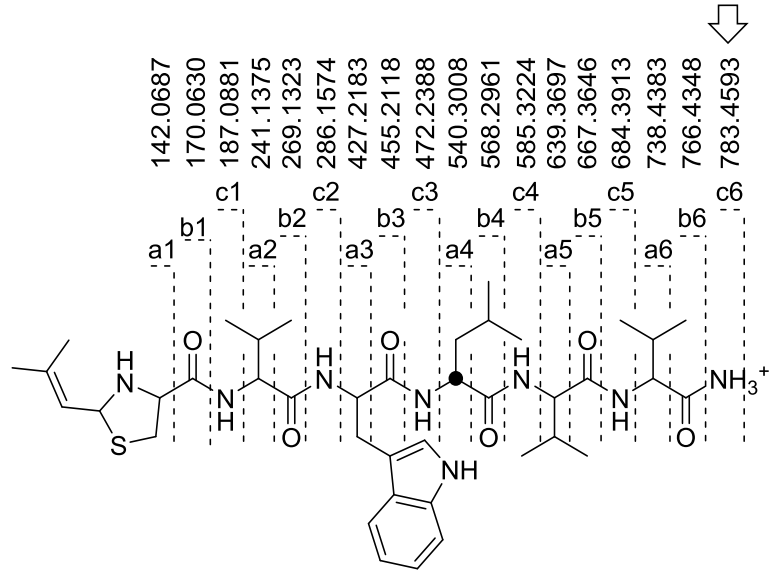
3-D-lugdunin (17)

[M+H]+ calculated for $C_{40}H_{63}N_8O_6S$, 783.4586; found 783.4595 (1.2 ppm err; 5.6 mSigma)



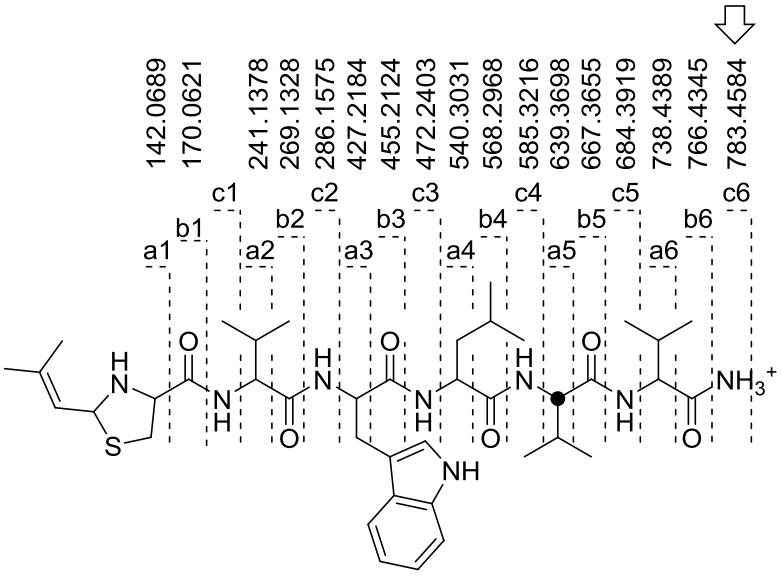
4-L-lugdunin (18)

[M+H]+ calculated for $C_{40}H_{63}N_8O_6S$, 783.4586; found 783.4593 (0.9 ppm err; 4.4 mSigma)



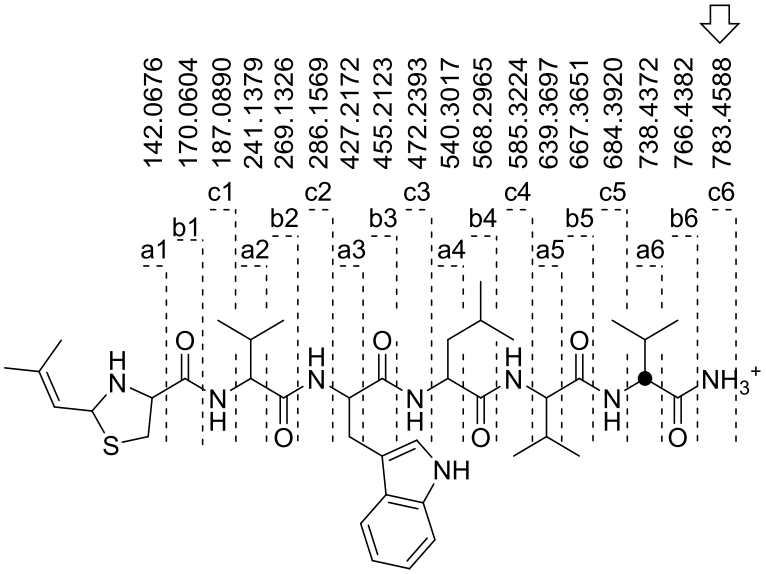
5-D-lugdunin (19)

[M+H]+ calculated for $C_{40}H_{63}N_8O_6S$, 783.4586; found 783.4584 (0.2 ppm err; 4.3 mSigma)



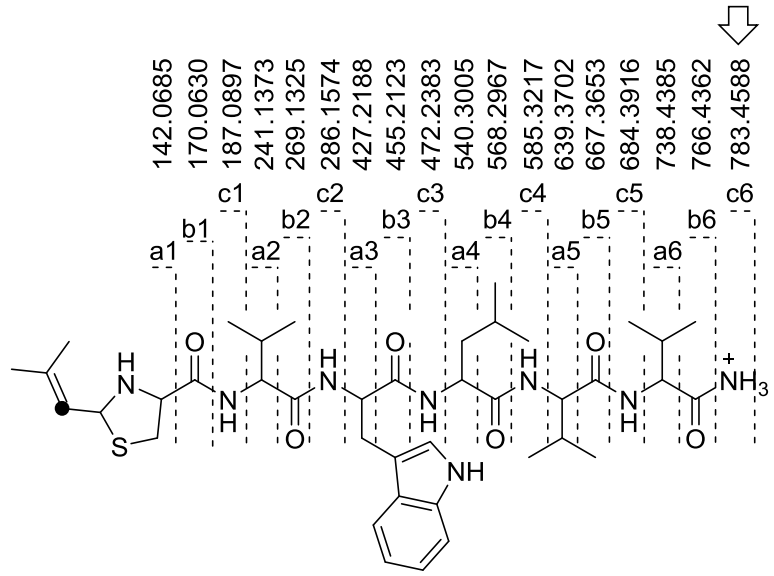
6-L-lugdunin (20)

[M+H]+ calculated for $C_{40}H_{63}N_8O_6S$, 783.4586; found 783.4588 (0.3 ppm err; 3.3 mSigma)



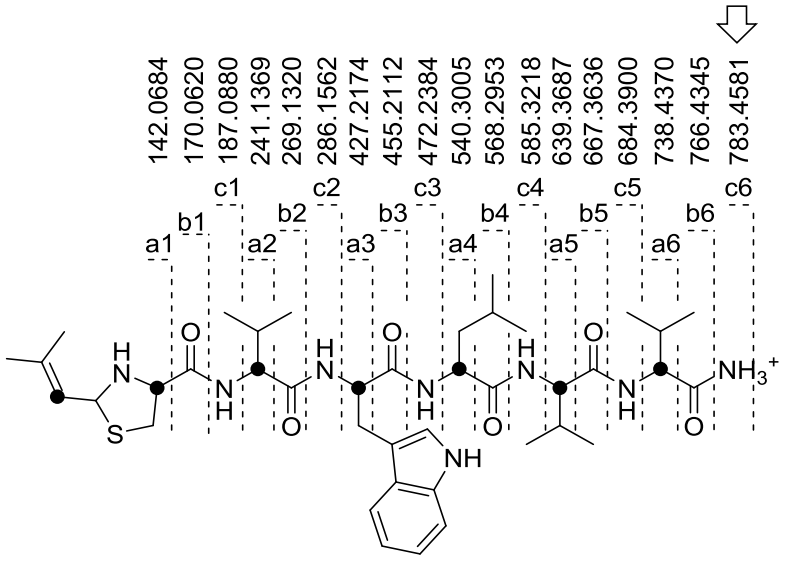
7-D-lugdunin (21)

[M+H]+ calculated for C40H63N8O6S, 783.4586; found 783.4585 (0.1 ppm err; 4.4 mSigma)



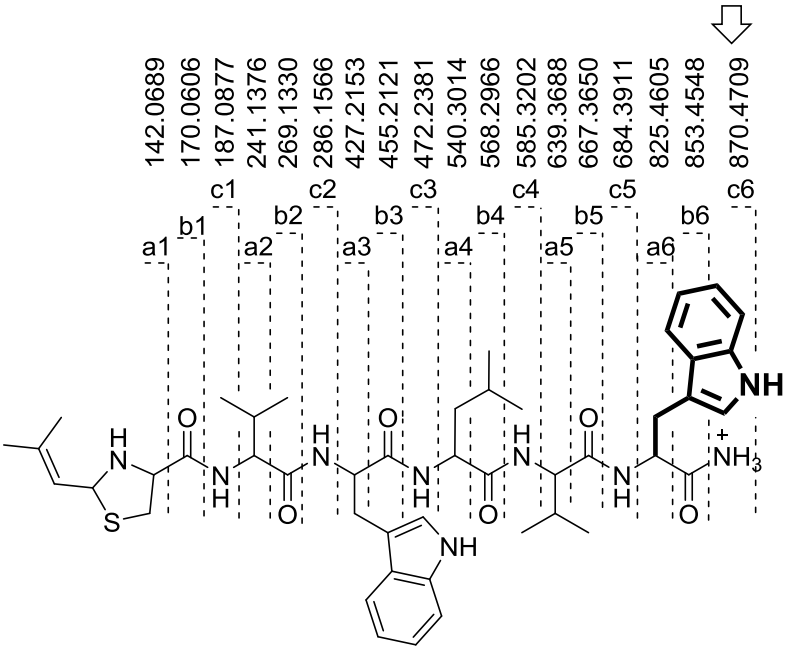
Enantio-lugdunin (22)

[M+H]+ calculated for $C_{40}H_{63}N_8O_6S$, 783.4586; found 783.4581 (0.6 ppm err; 77.0 mSigma)



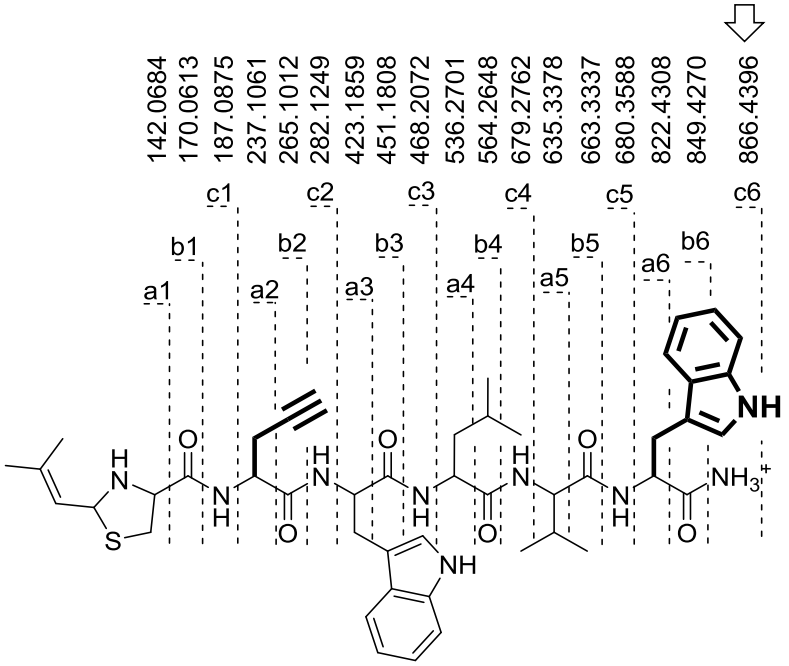
6-Trp-Lugdunin (23)

[M+H]+ calculated for $C_{46}H_{64}N_9O_6S$, 870.4695; found 870.4709 (1.6 ppm err; 13.7 mSigma)



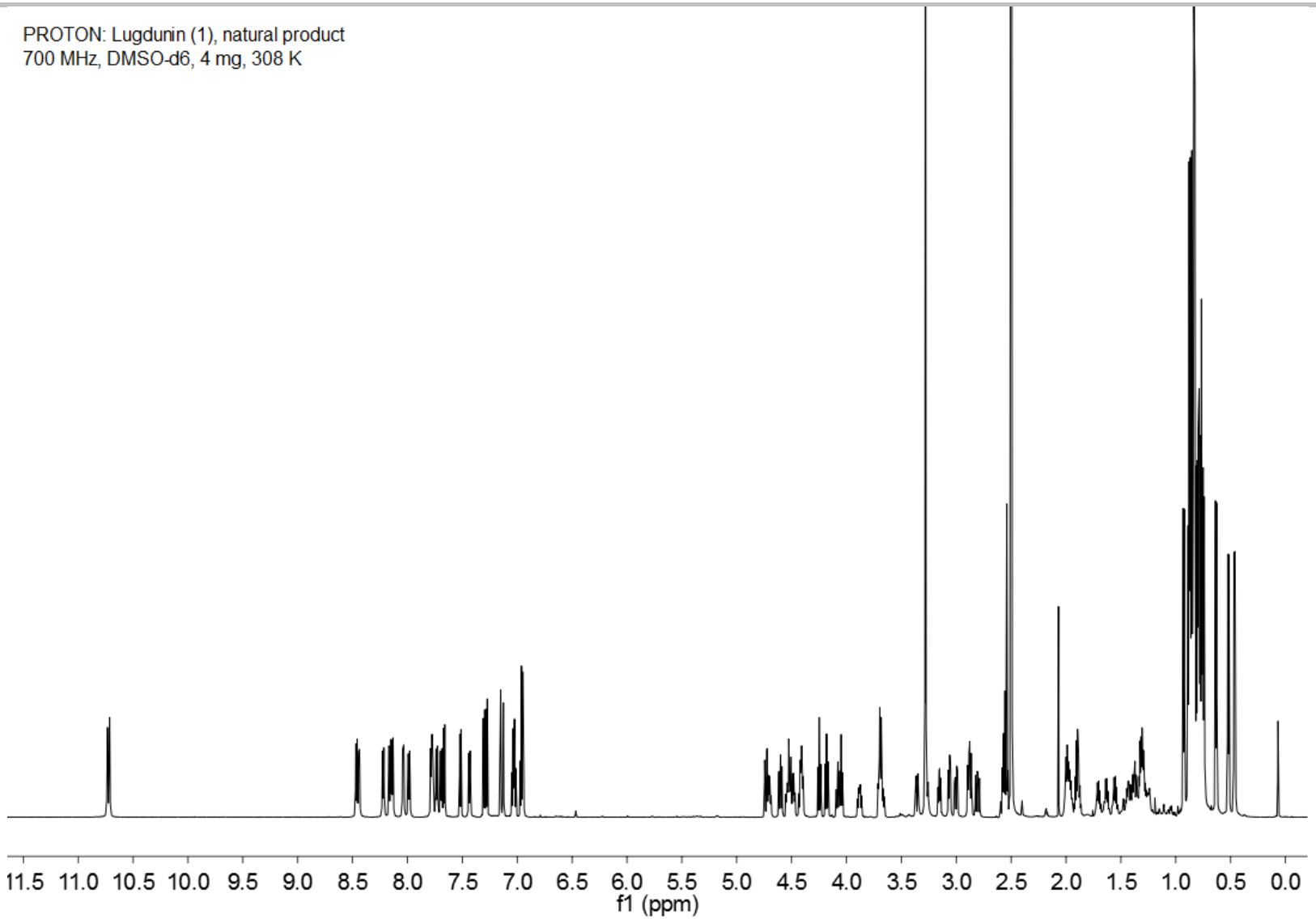
2-Pra-6-Trp-Lugdunin (24)

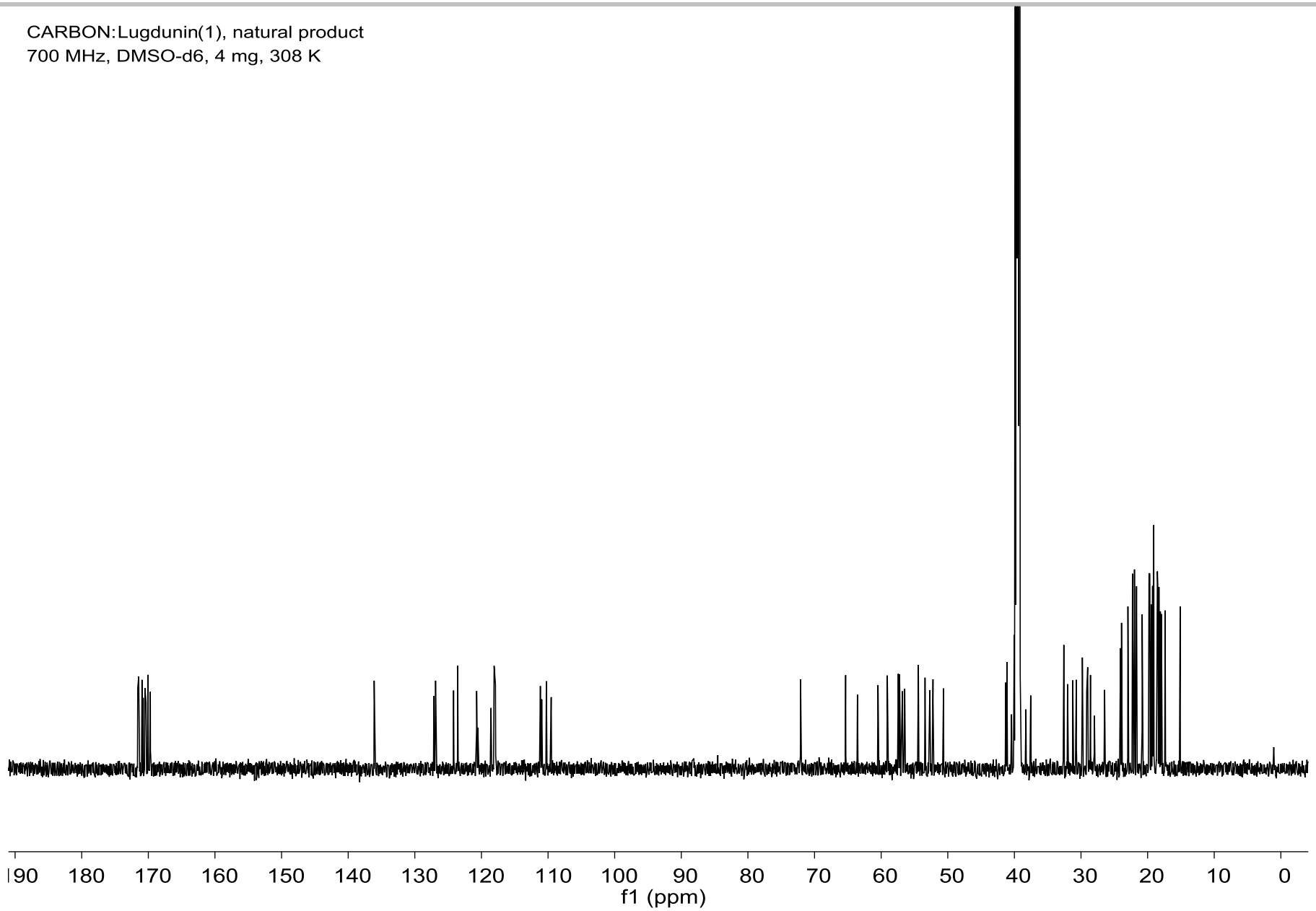
[M+H]+ calculated for $C_{46}H_{60}N_9O_6S$, 866.4382; found 866.4378 (0.2 ppm err; 9.7 mSigma)

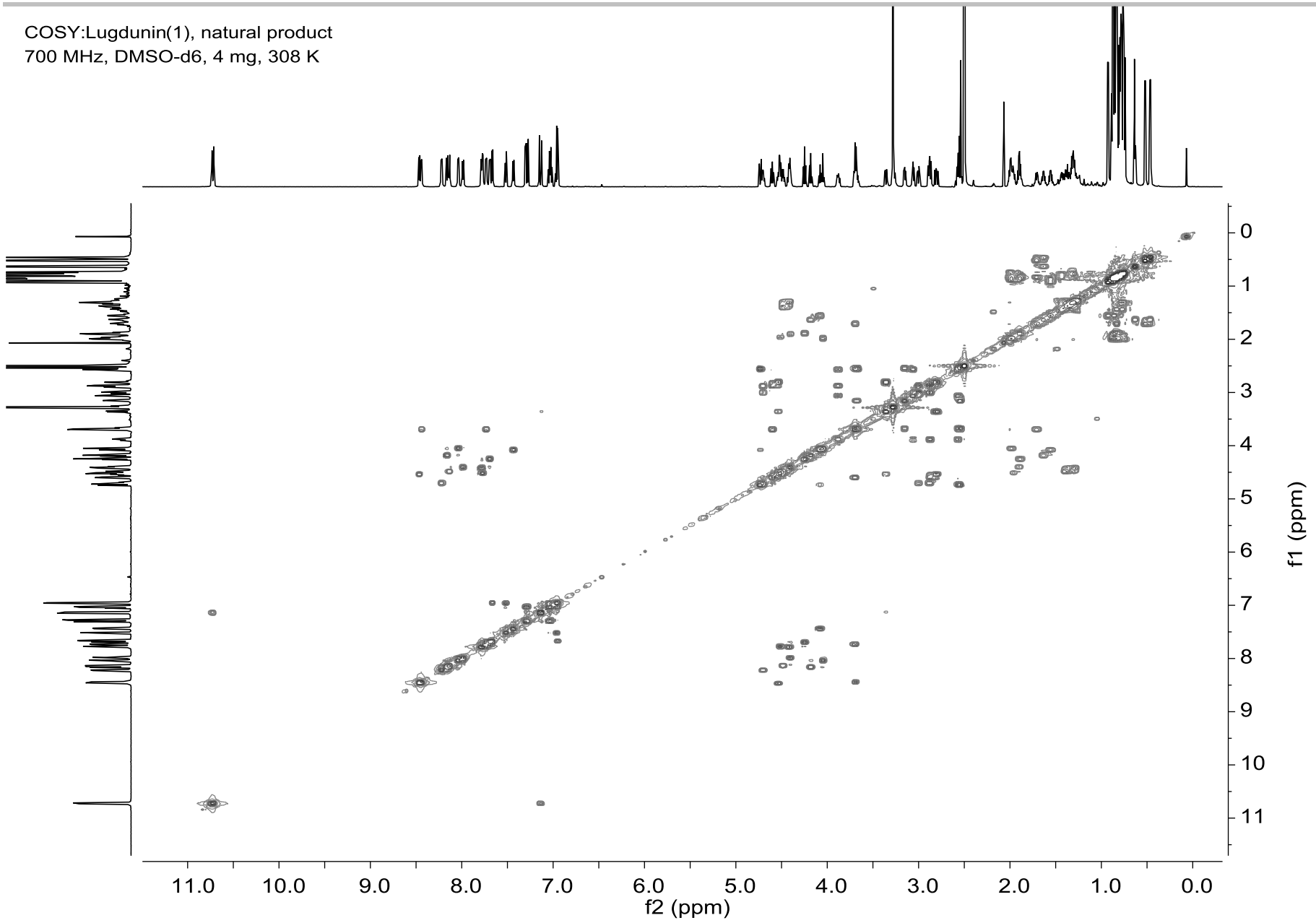


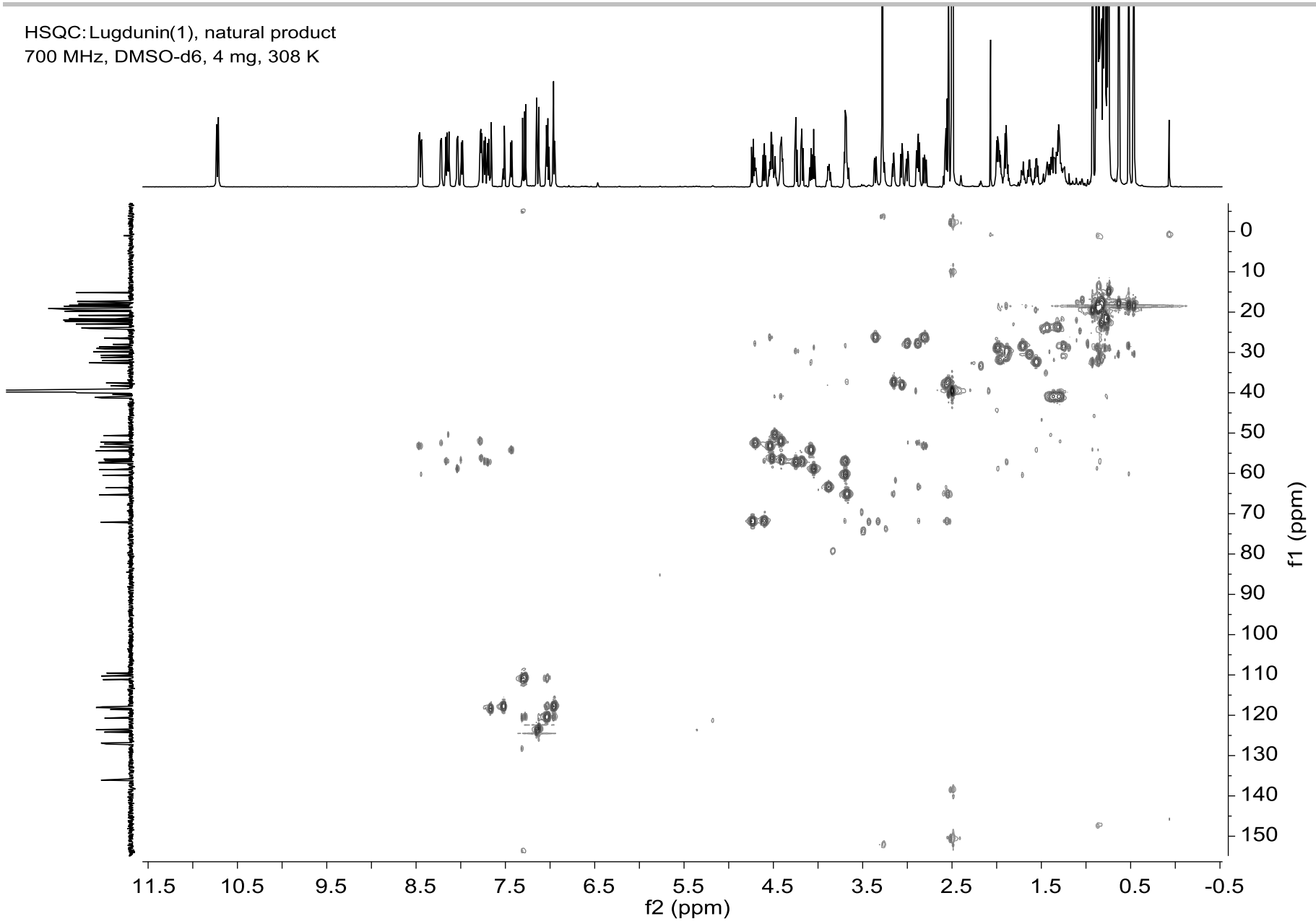
NMR-Spectra of lugdunin (1) and analogues 2-24 and gramicidin S (25)

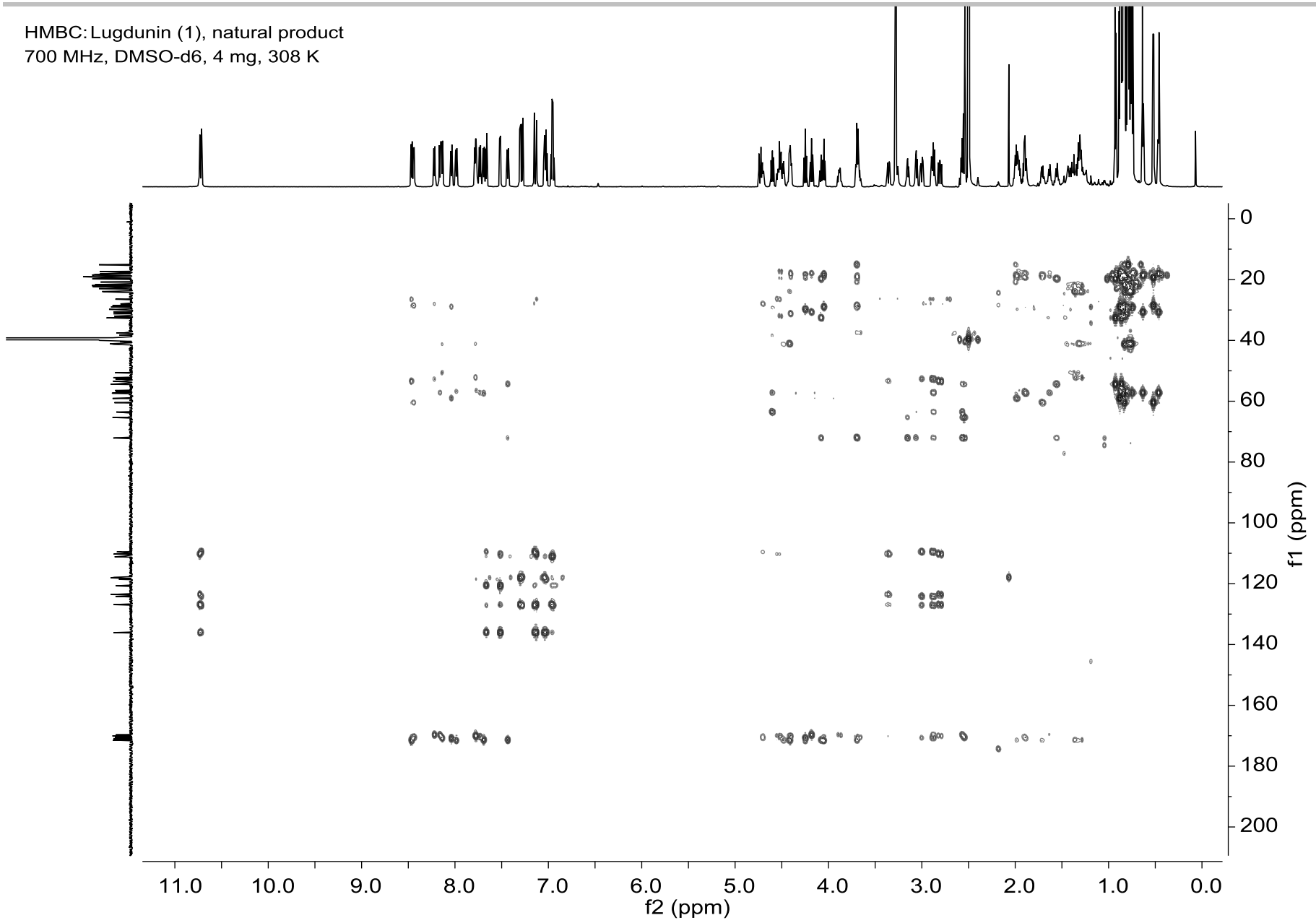
NMR spectra were recorded on a Bruker AMX-600 (^{1}H: 600 MHz, ^{13}C: 150 MHz) and Bruker AvanceIII-700 (^{1}H: 700 MHz, ^{13}C: 175 MHz).

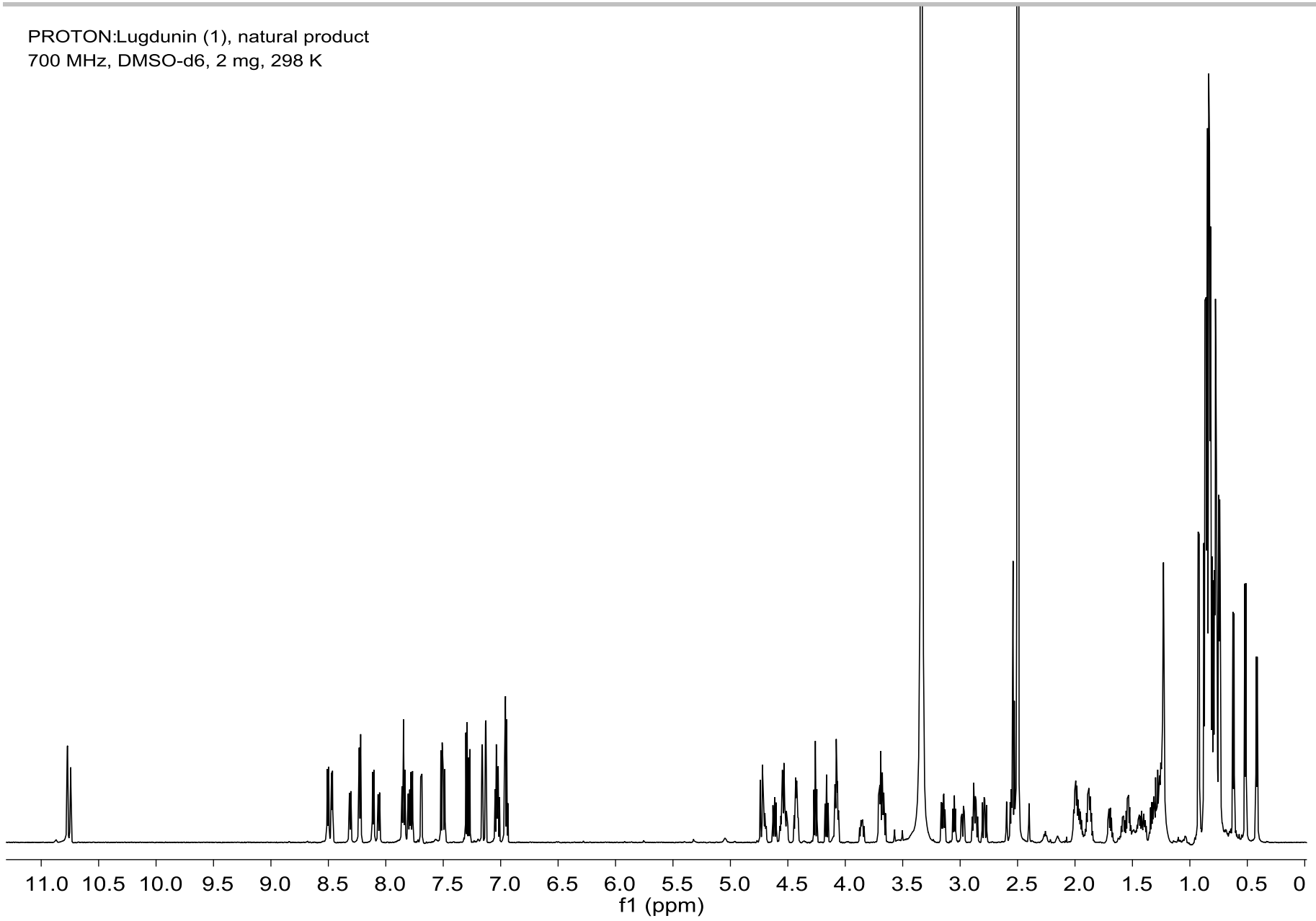

PROTON: Lugdunin (1), natural product
700 MHz, DMSO-d6, 4 mg, 308 K
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

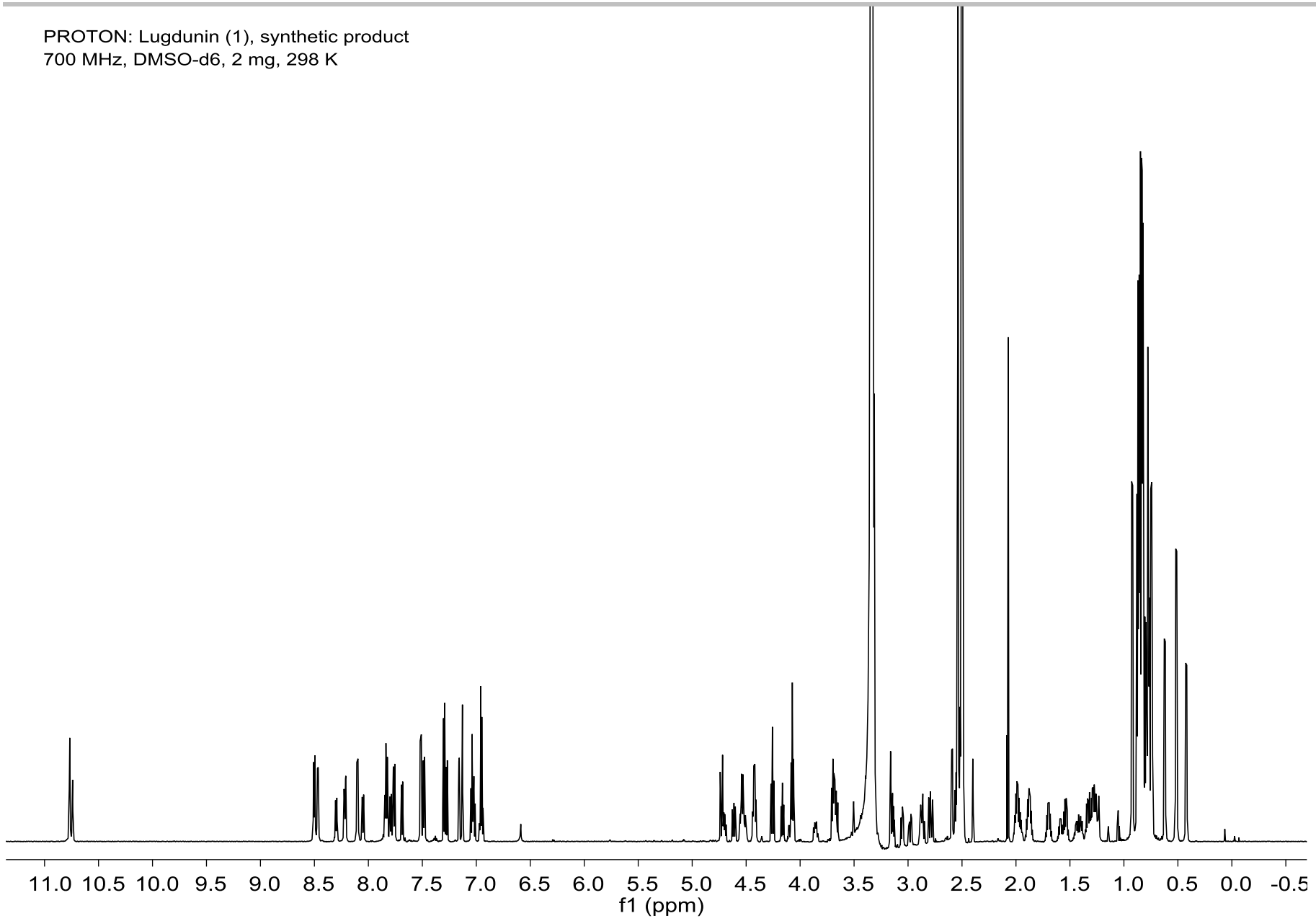

CARBON:Lugdunin(1), natural product
700 MHz, DMSO-d6, 4 mg, 308 K
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

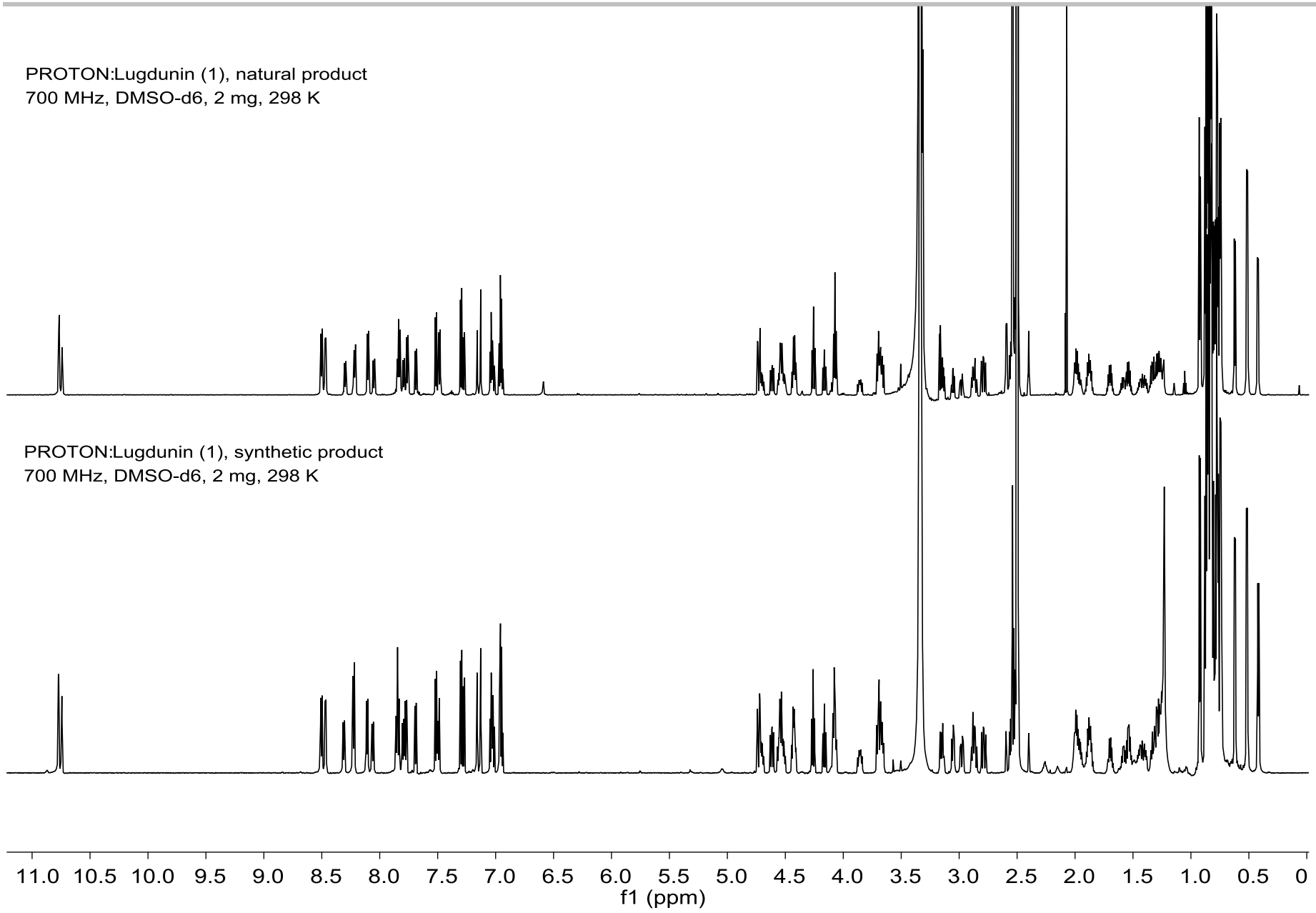

COSY:Lugdunin(1), natural product
700 MHz, DMSO-d6, 4 mg, 308 K
f2 (ppm)
f1 (ppm)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
1
2
3
4
5
6
7
8
9
10
11


HSQC: Lugdunin(1), natural product
700 MHz, DMSO-d6, 4 mg, 308 K
f2 (ppm)
f1 (ppm)


HMBC: Lugdunin (1), natural product
700 MHz, DMSO-d6, 4 mg, 308 K
0
20
40
60
80
100
120
140
160
180
200
f1 (ppm)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)

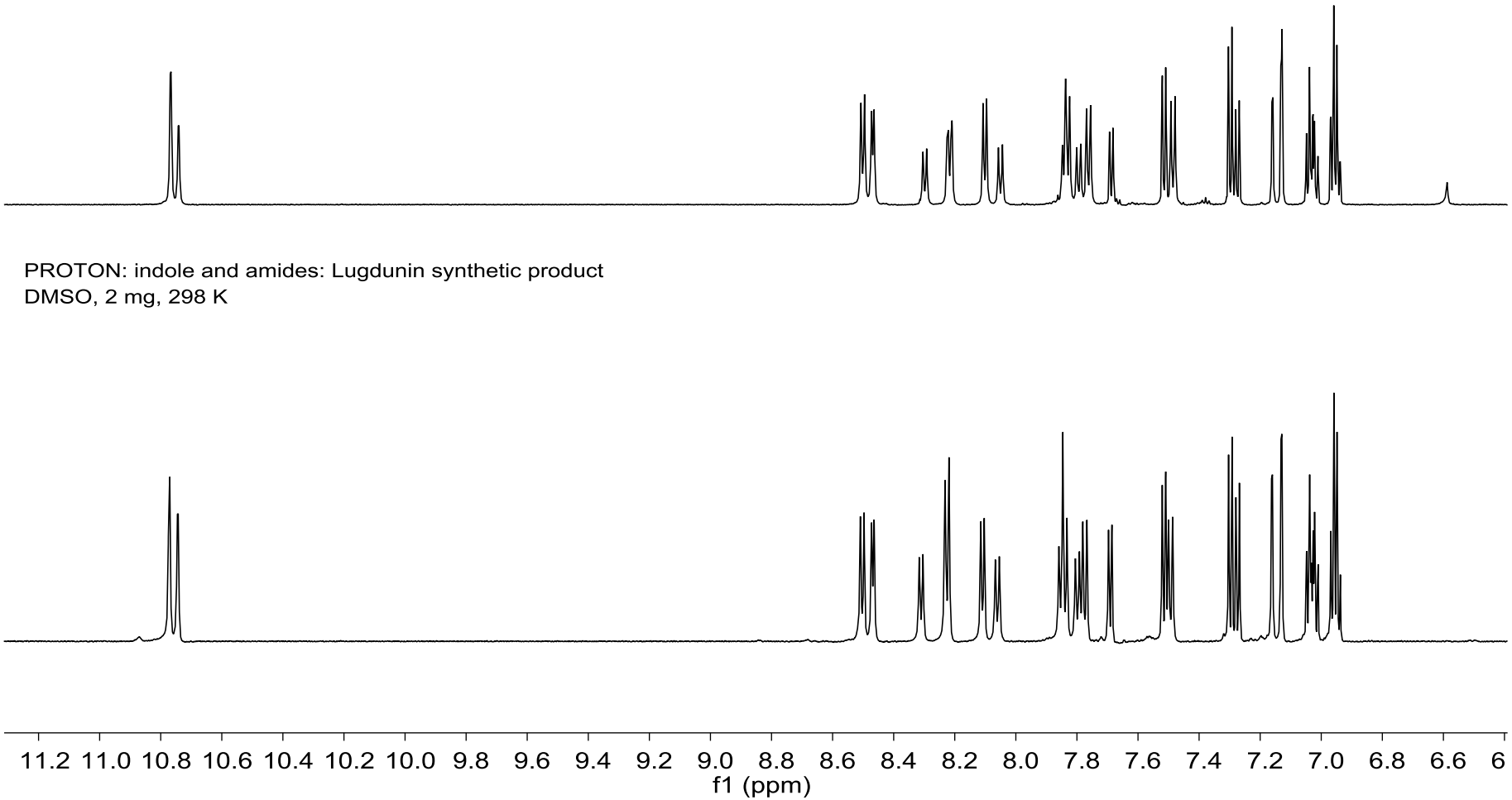

PROTON:Lugdunin (1), natural product
700 MHz, DMSO-d6, 2 mg, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)


PROTON: Lugdunin (1), synthetic product
700 MHz, DMSO-d6, 2 mg, 298 K
11.0 10.5 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 0.0 -0.5
f1 (ppm)


PROTON:Lugdunin (1), natural product
700 MHz, DMSO-d6, 2 mg, 298 K
PROTON:Lugdunin (1), synthetic product
700 MHz, DMSO-d6, 2 mg, 298 K
11.0 10.5 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 0
f1 (ppm)

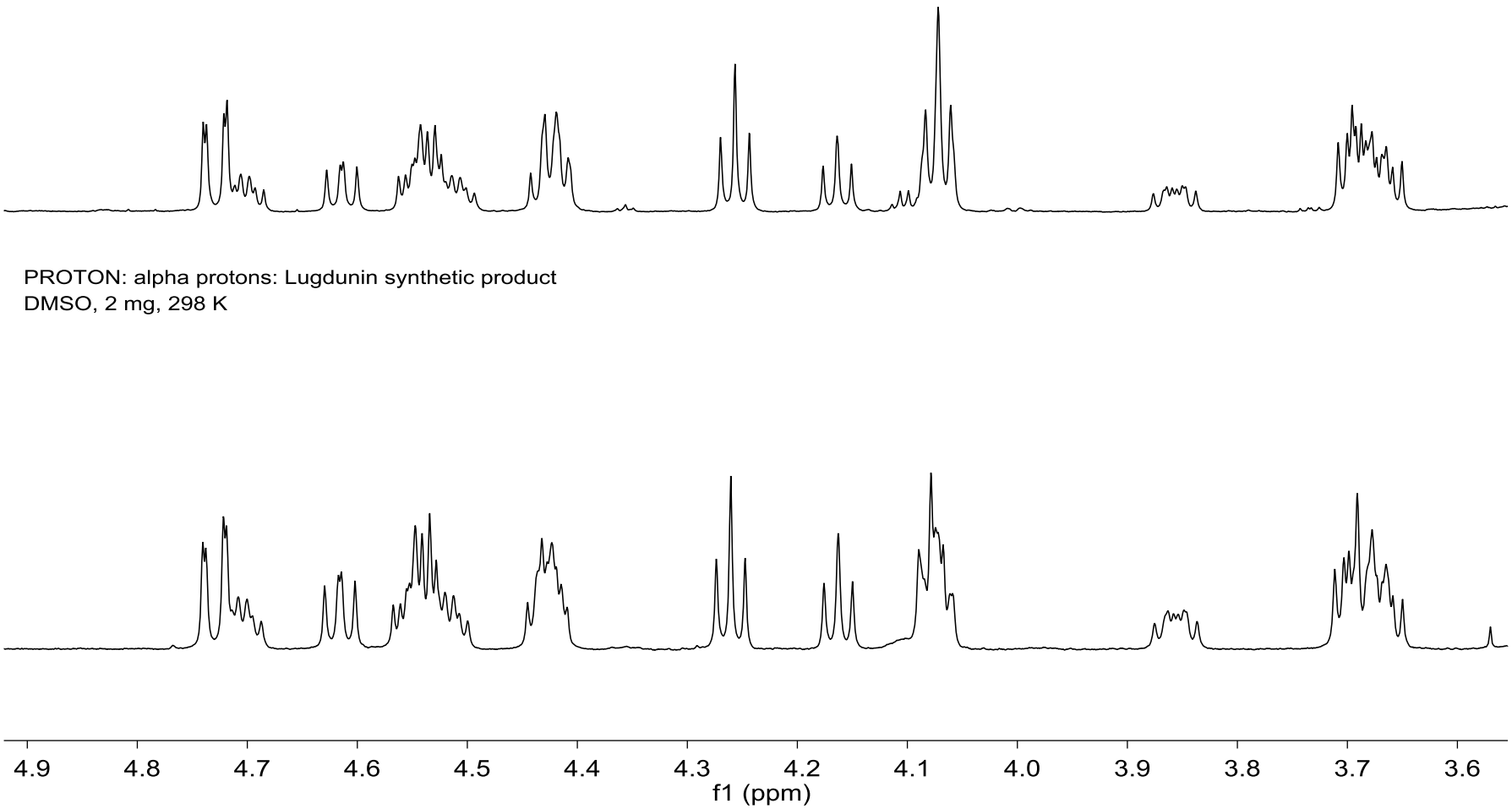
PROTON: indole and amides: Lugdunin natural product
DMSO, 2 mg, 298 K

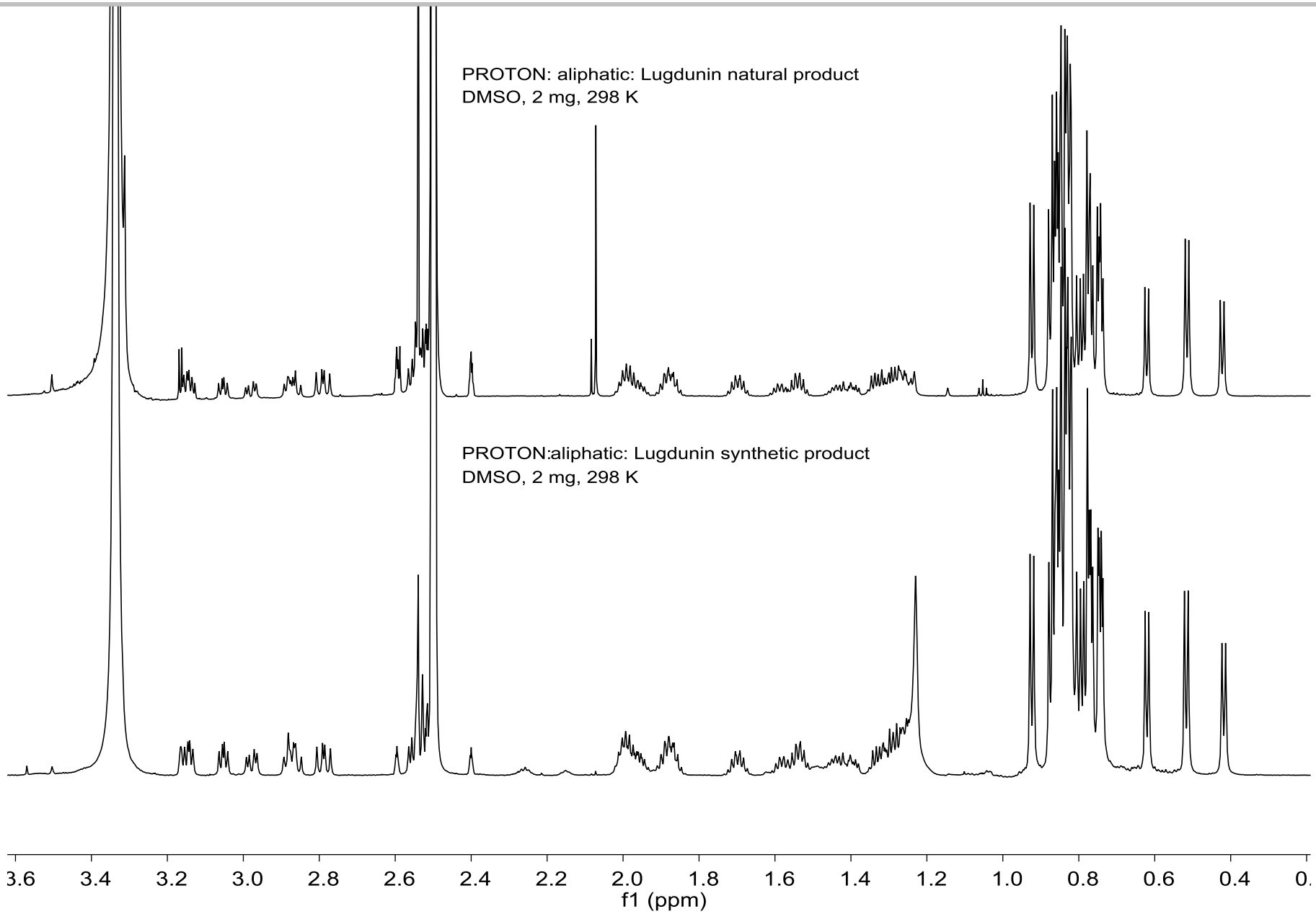
PROTON: indole and amides: Lugdunin synthetic product
DMSO, 2 mg, 298 K

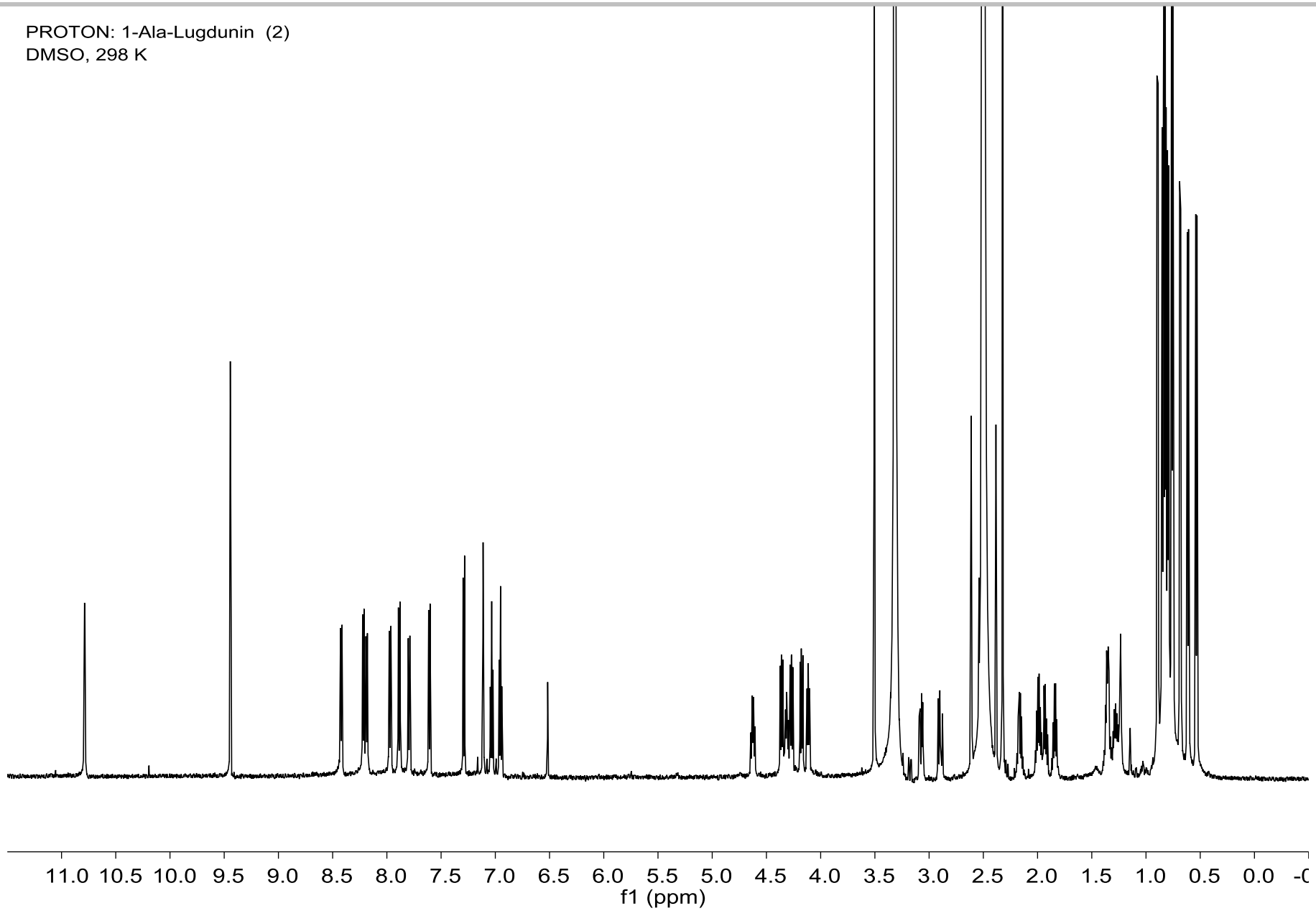


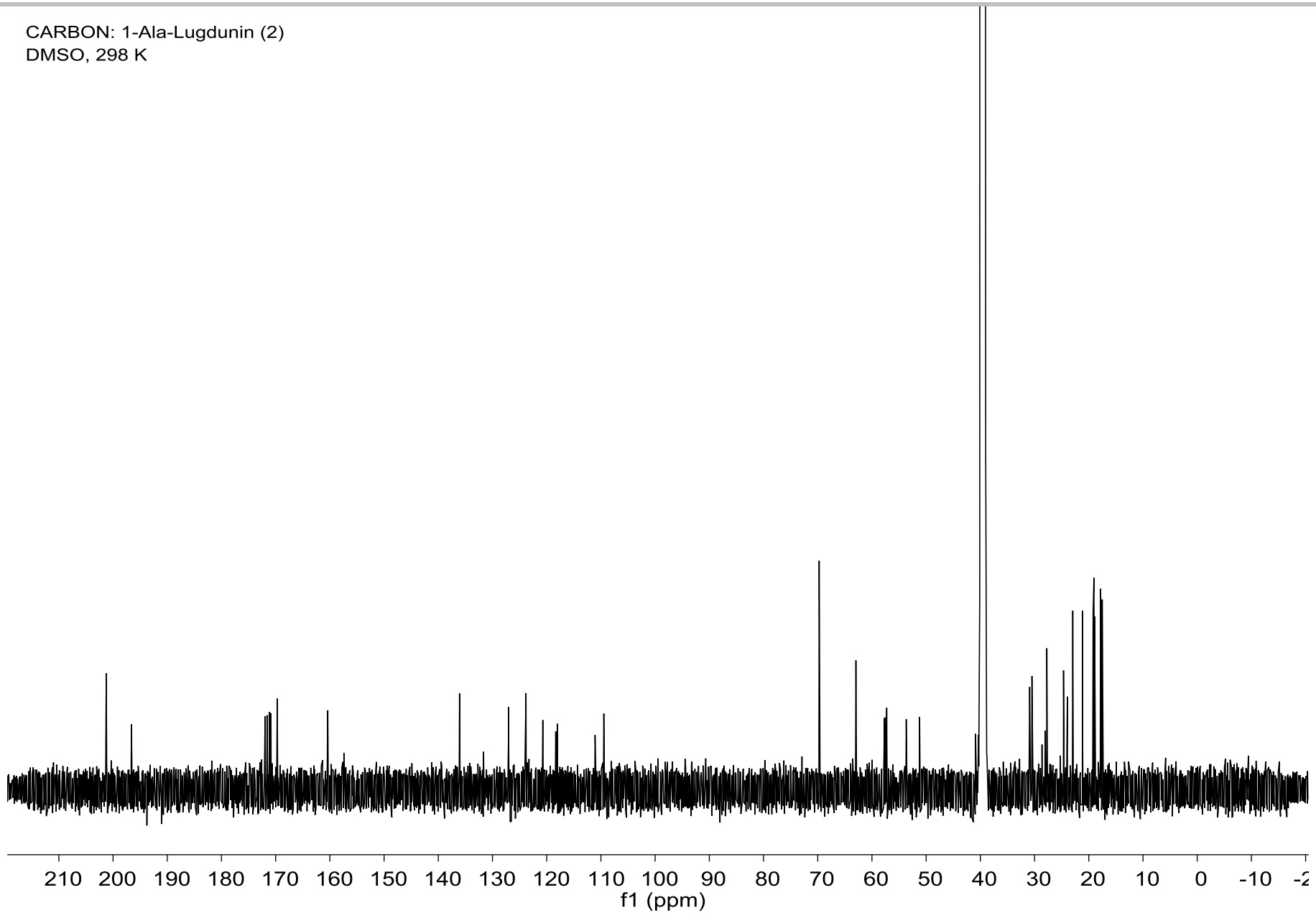
PROTON: alpha protons: Lugdunin natural product
DMSO, 2 mg, 298 K

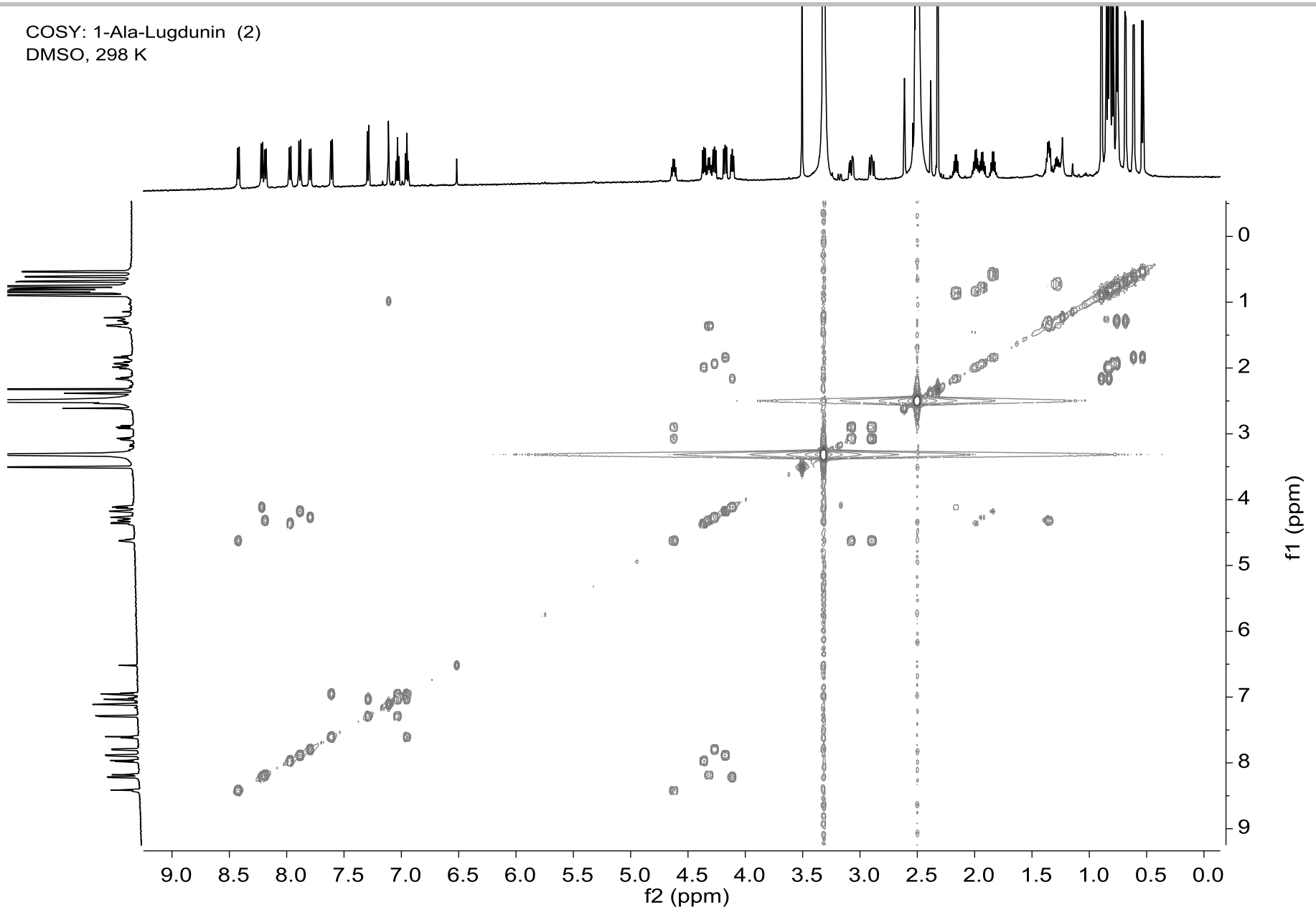
PROTON: alpha protons: Lugdunin synthetic product
DMSO, 2 mg, 298 K

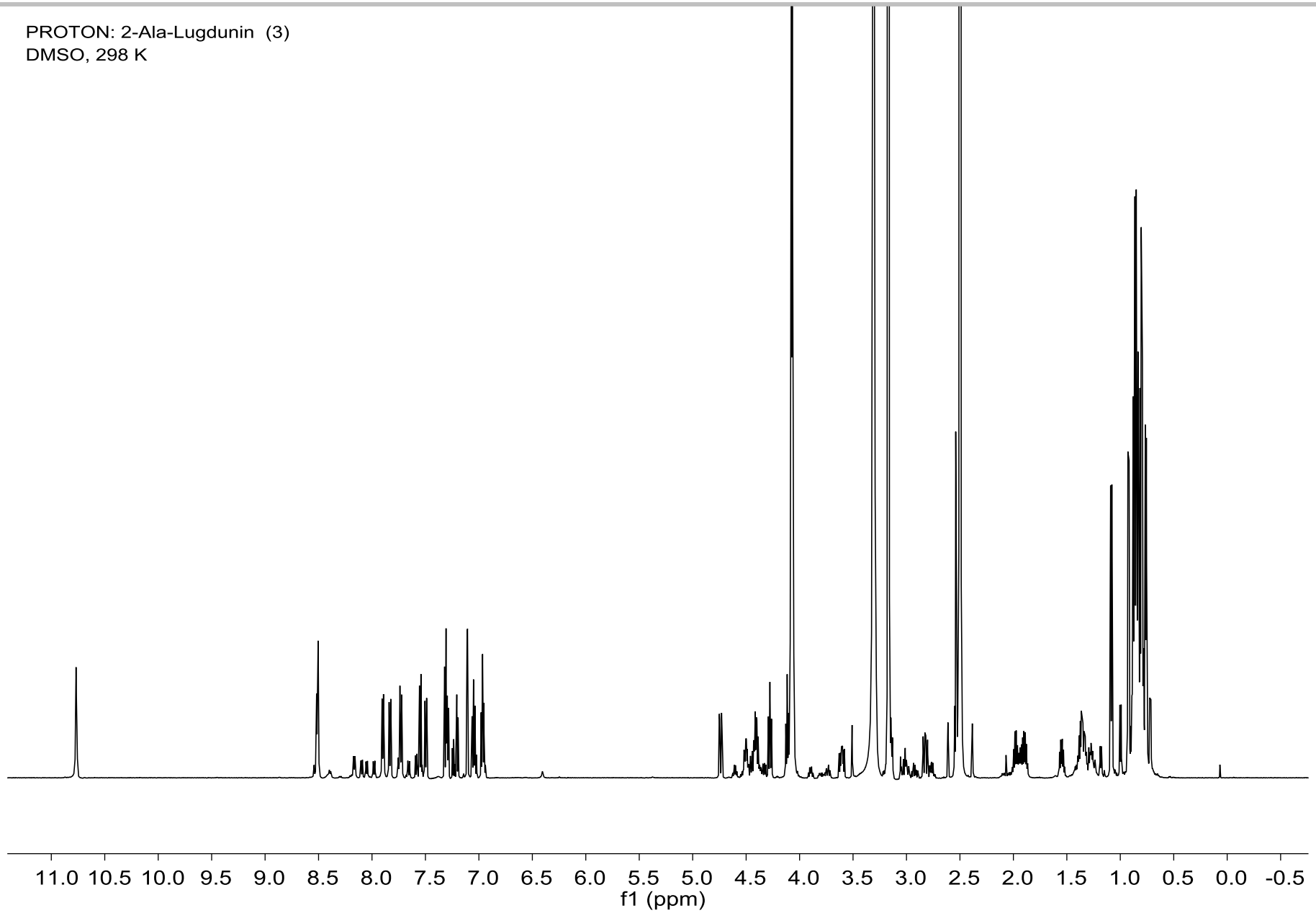


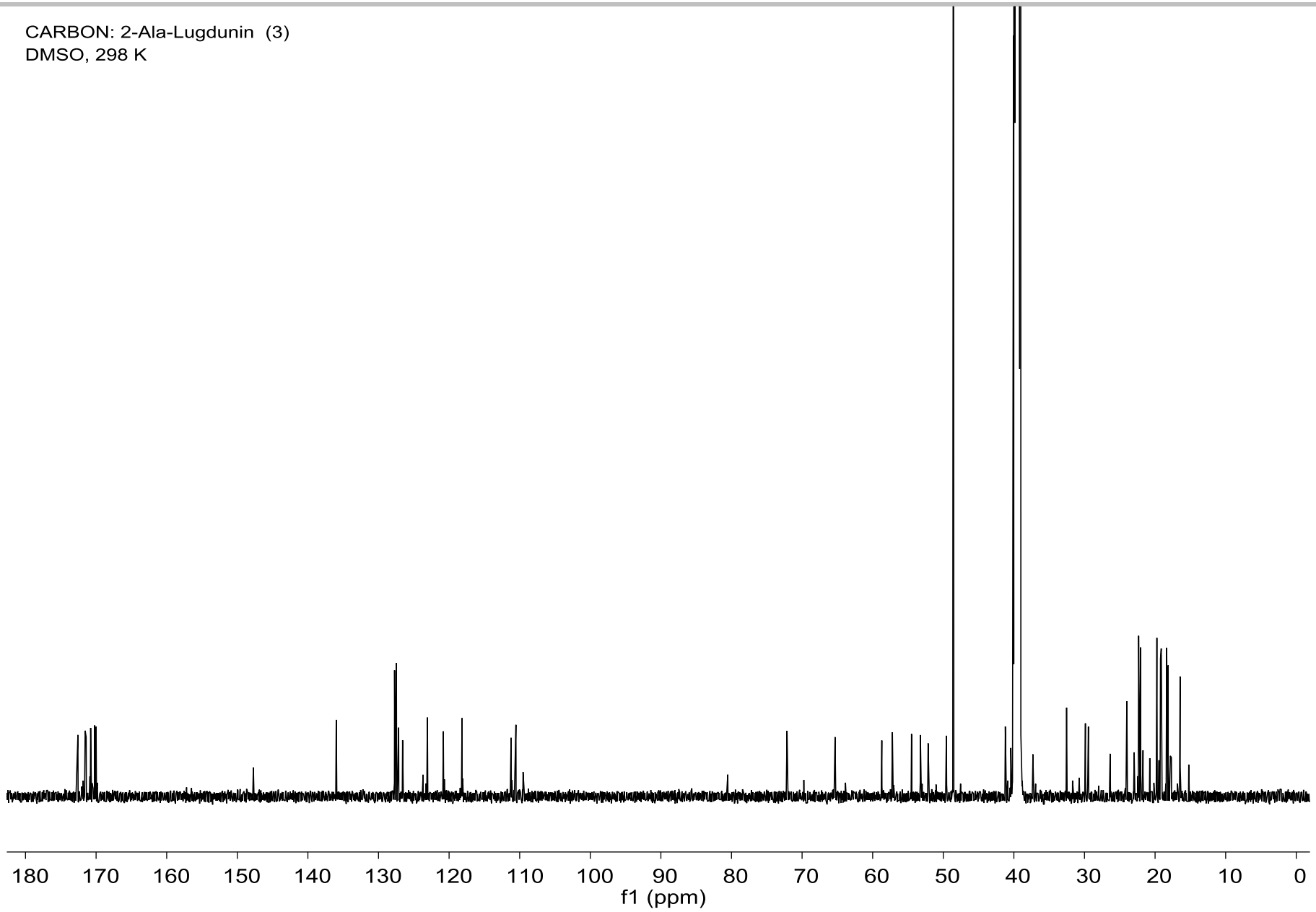

PROTON: aliphatic: Lugdunin natural product
DMSO, 2 mg, 298 K
PROTON:aliphatic: Lugdunin synthetic product
DMSO, 2 mg, 298 K
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
f1 (ppm)

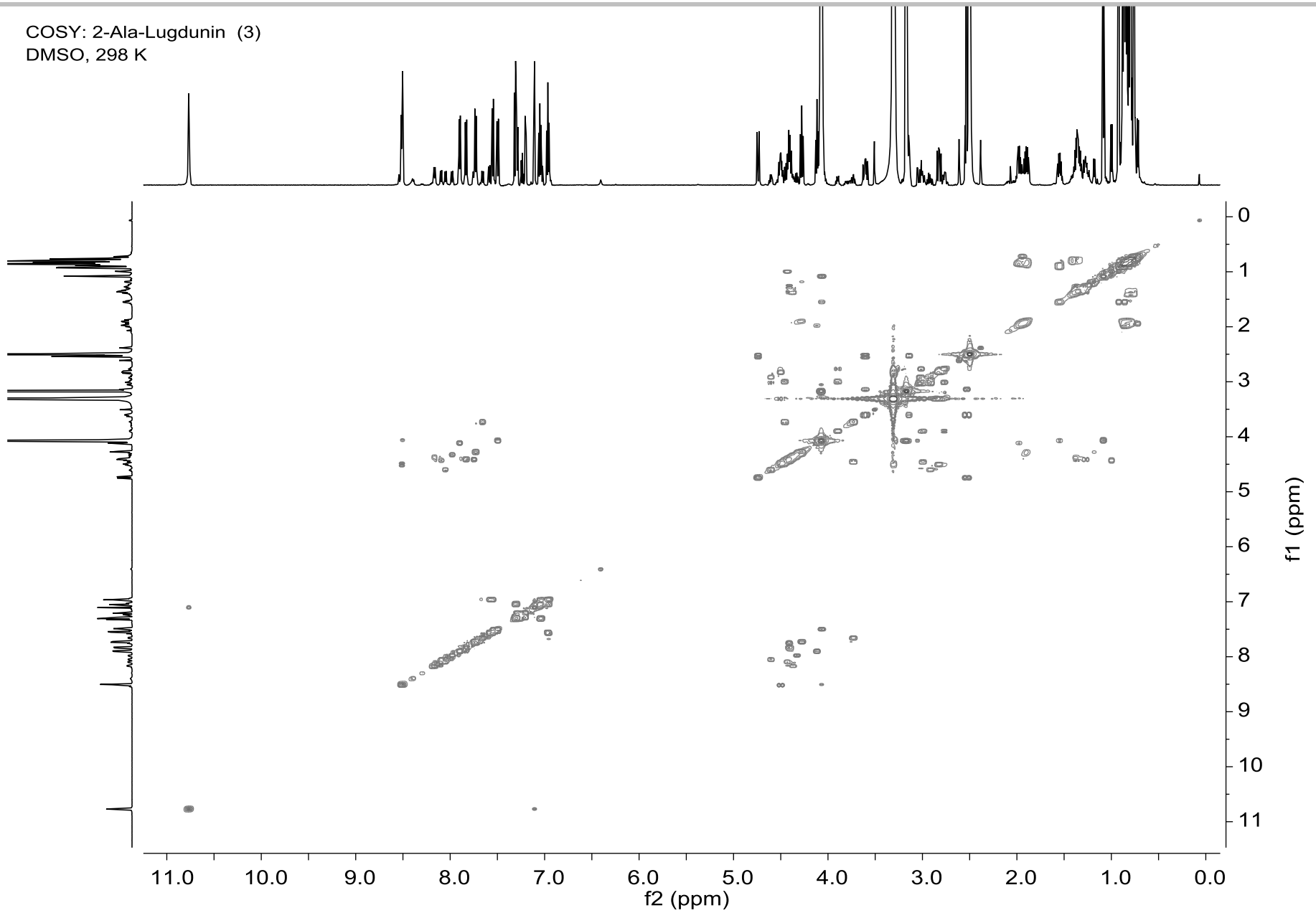

PROTON: 1-Ala-Lugdunin (2)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

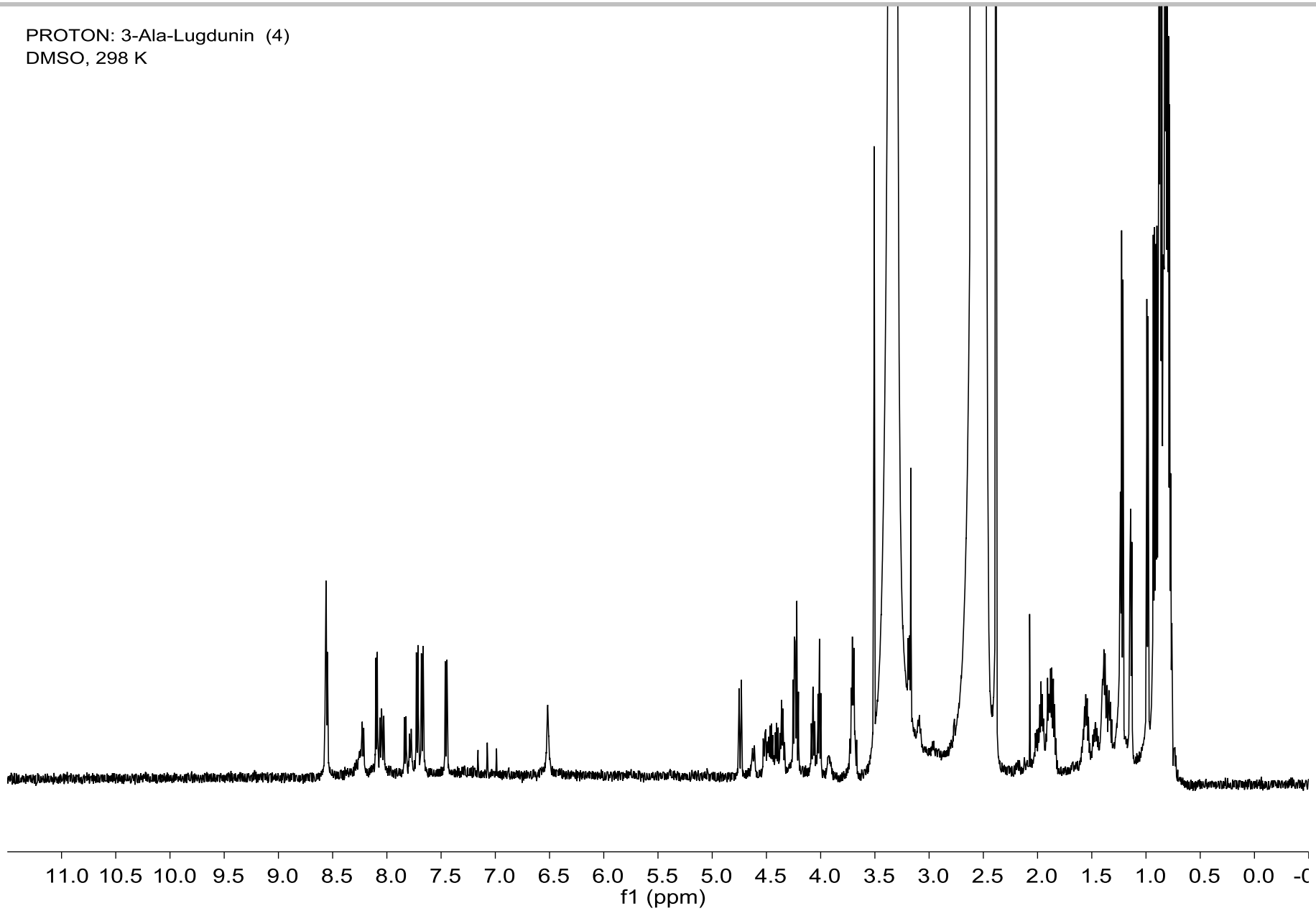

CARBON: 1-Ala-Lugdunin (2)
DMSO, 298 K
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

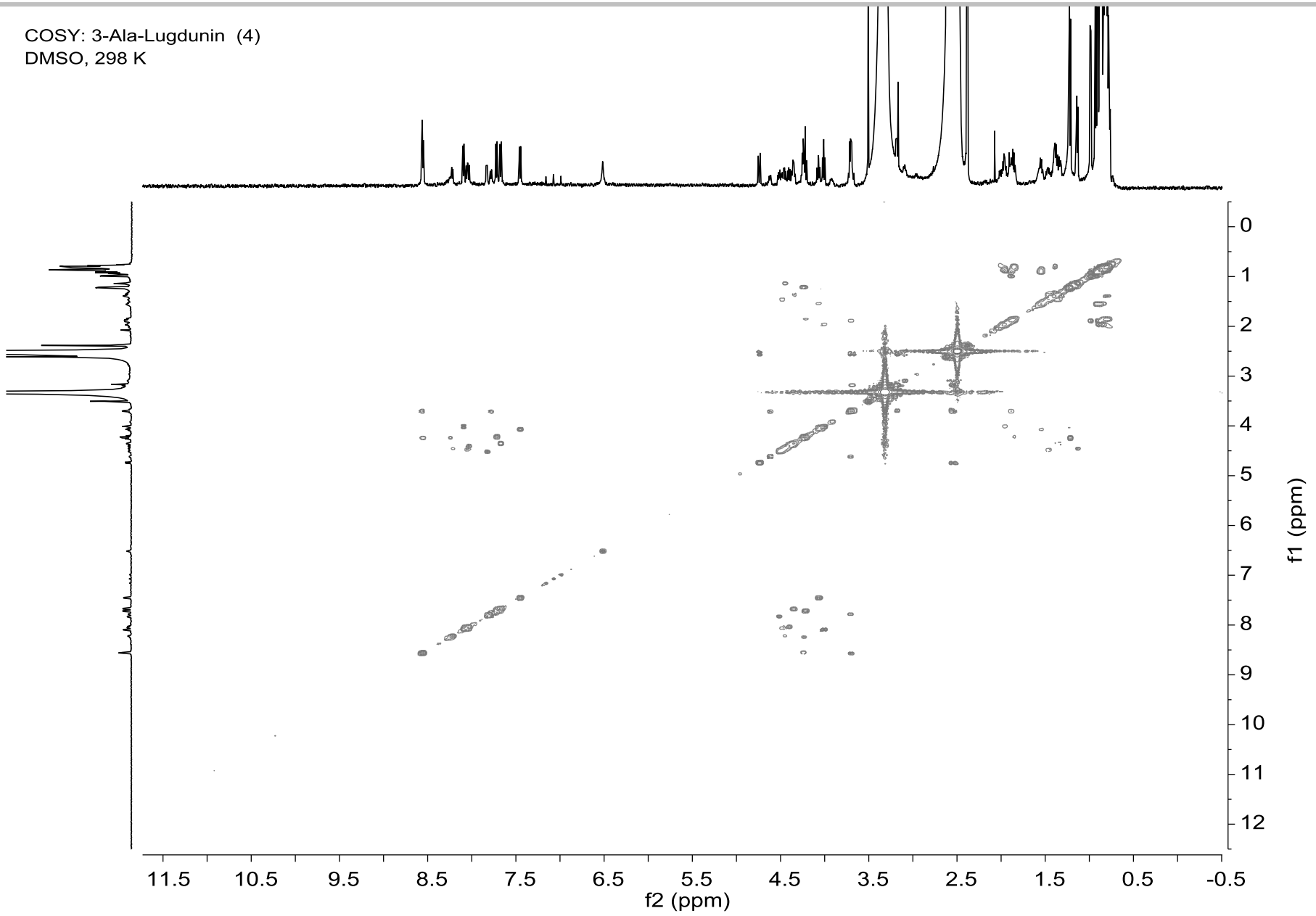

COSY: 1-Ala-Lugdunin (2)
DMSO, 298 K
f2 (ppm)
f1 (ppm)

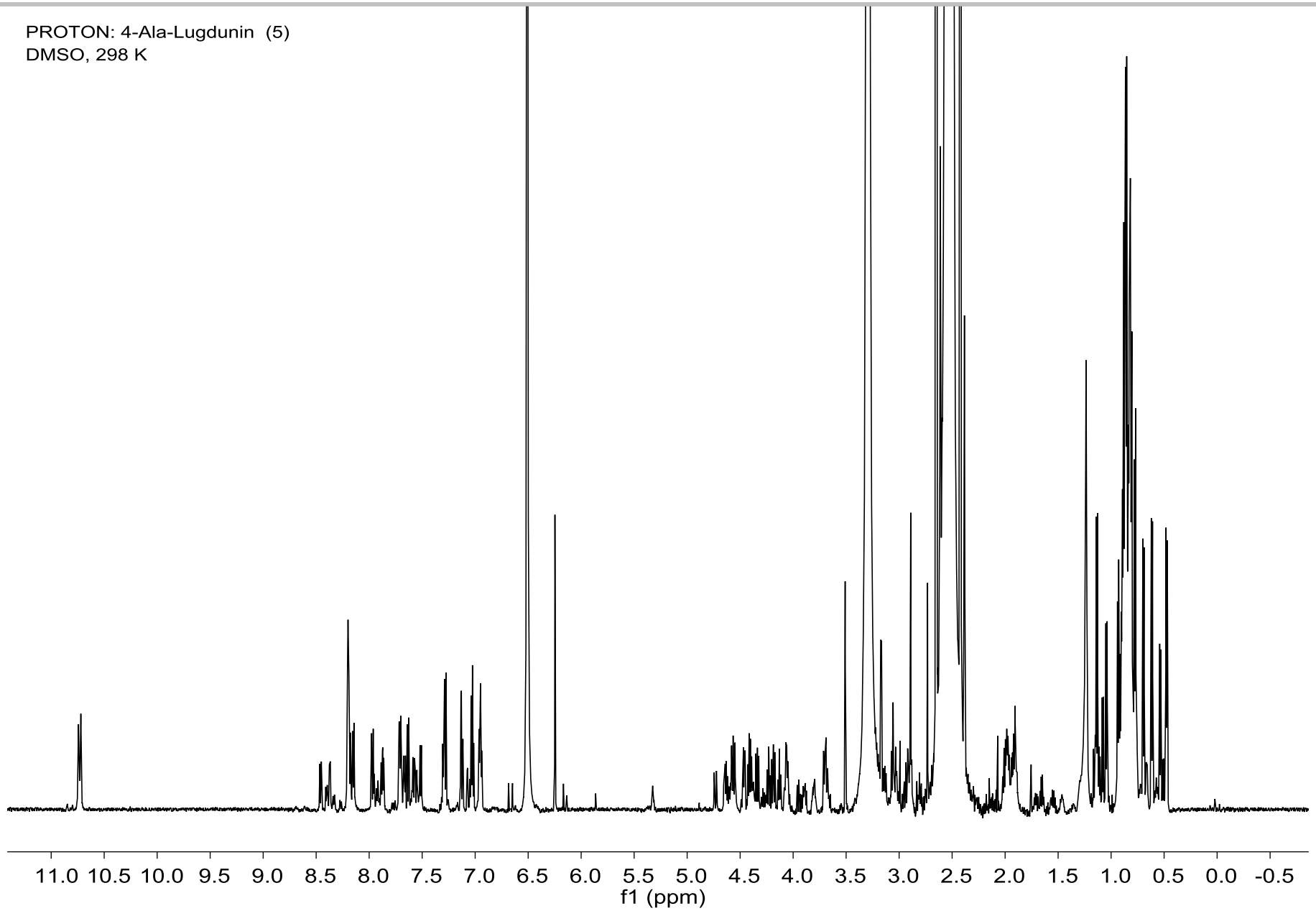

PROTON: 2-Ala-Lugdunin (3)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

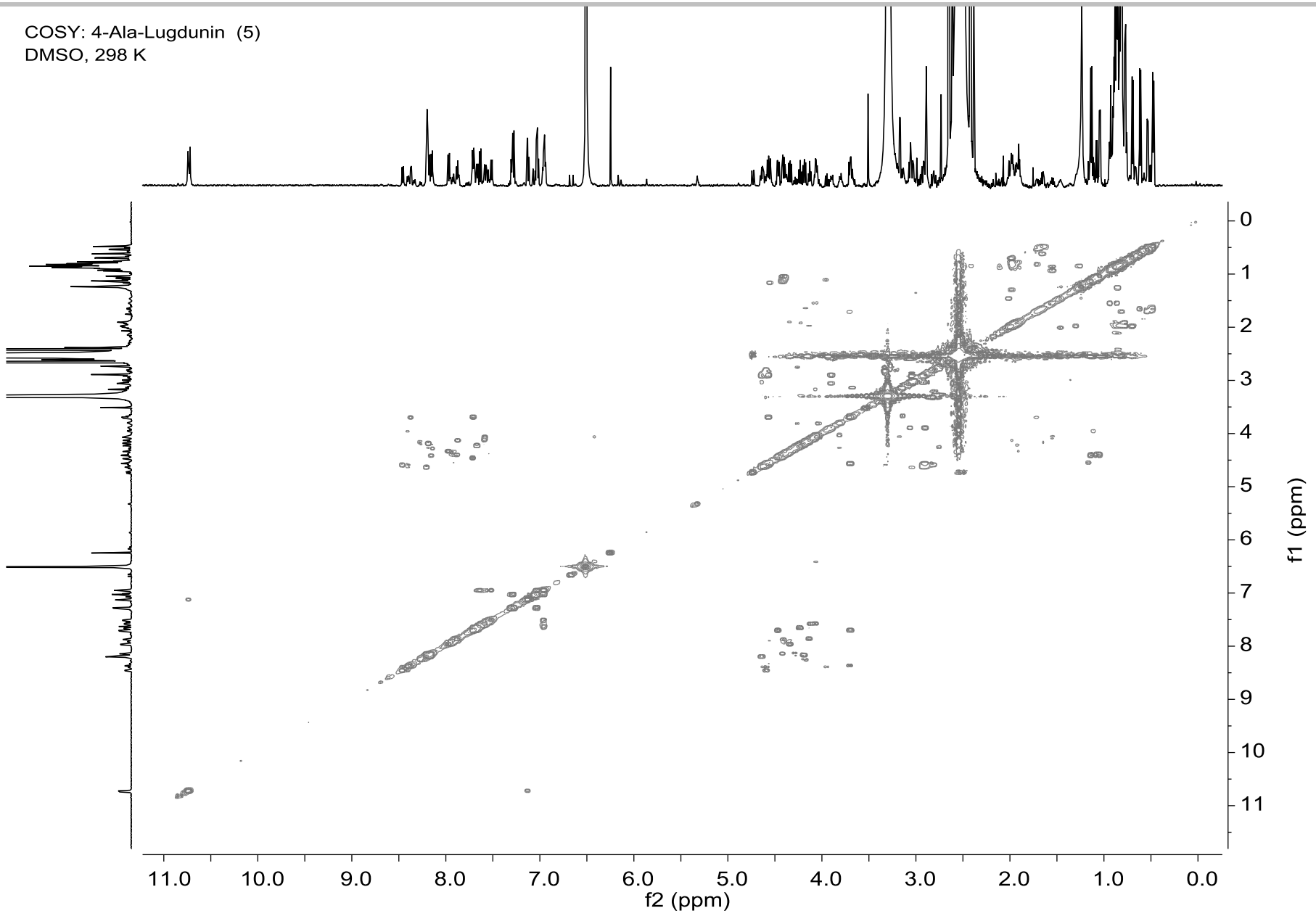

CARBON: 2-Ala-Lugdunin (3)
DMSO, 298 K
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

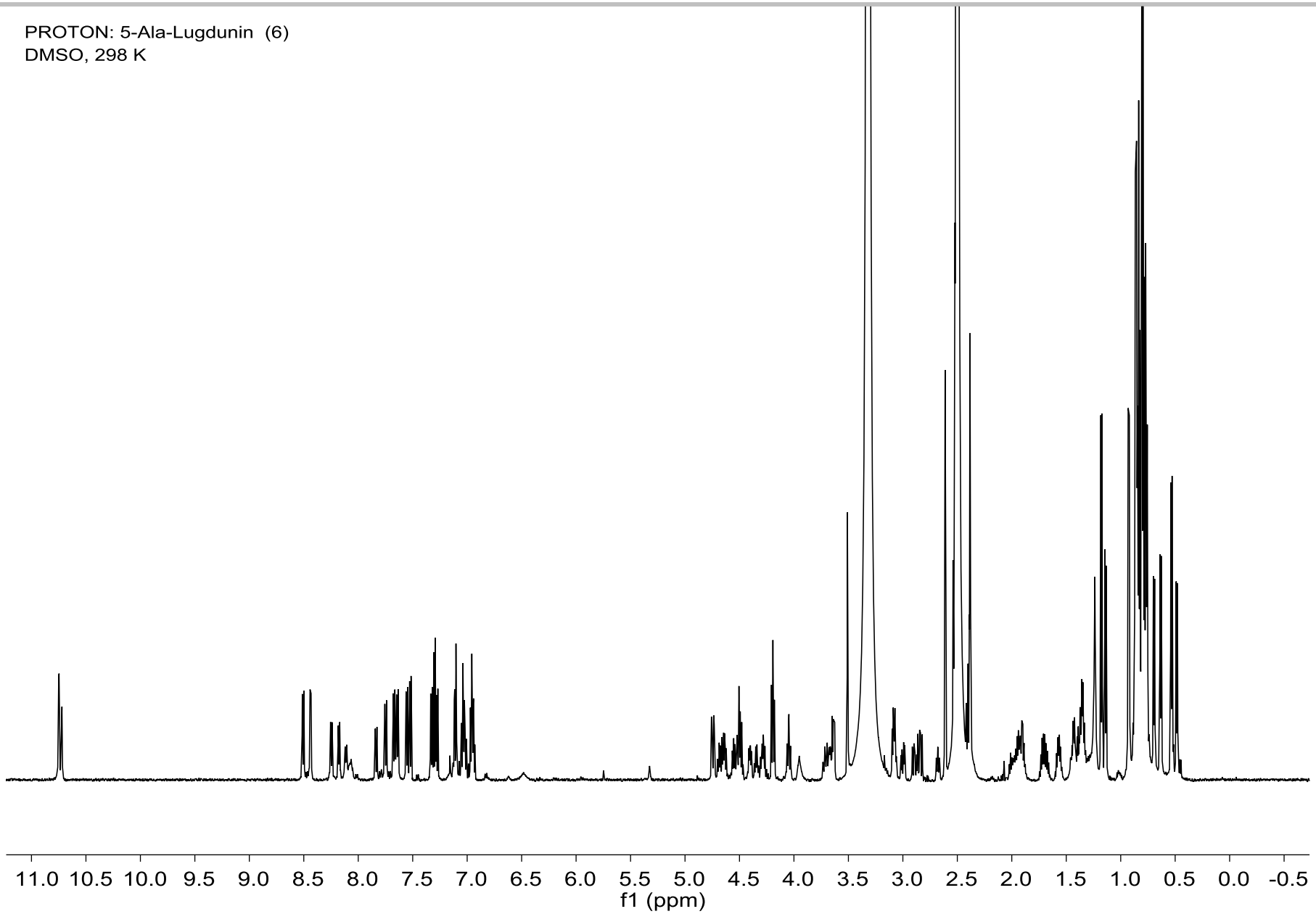

COSY: 2-Ala-Lugdunin (3)
DMSO, 298 K
f2 (ppm)
f1 (ppm)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
1
2
3
4
5
6
7
8
9
10
11

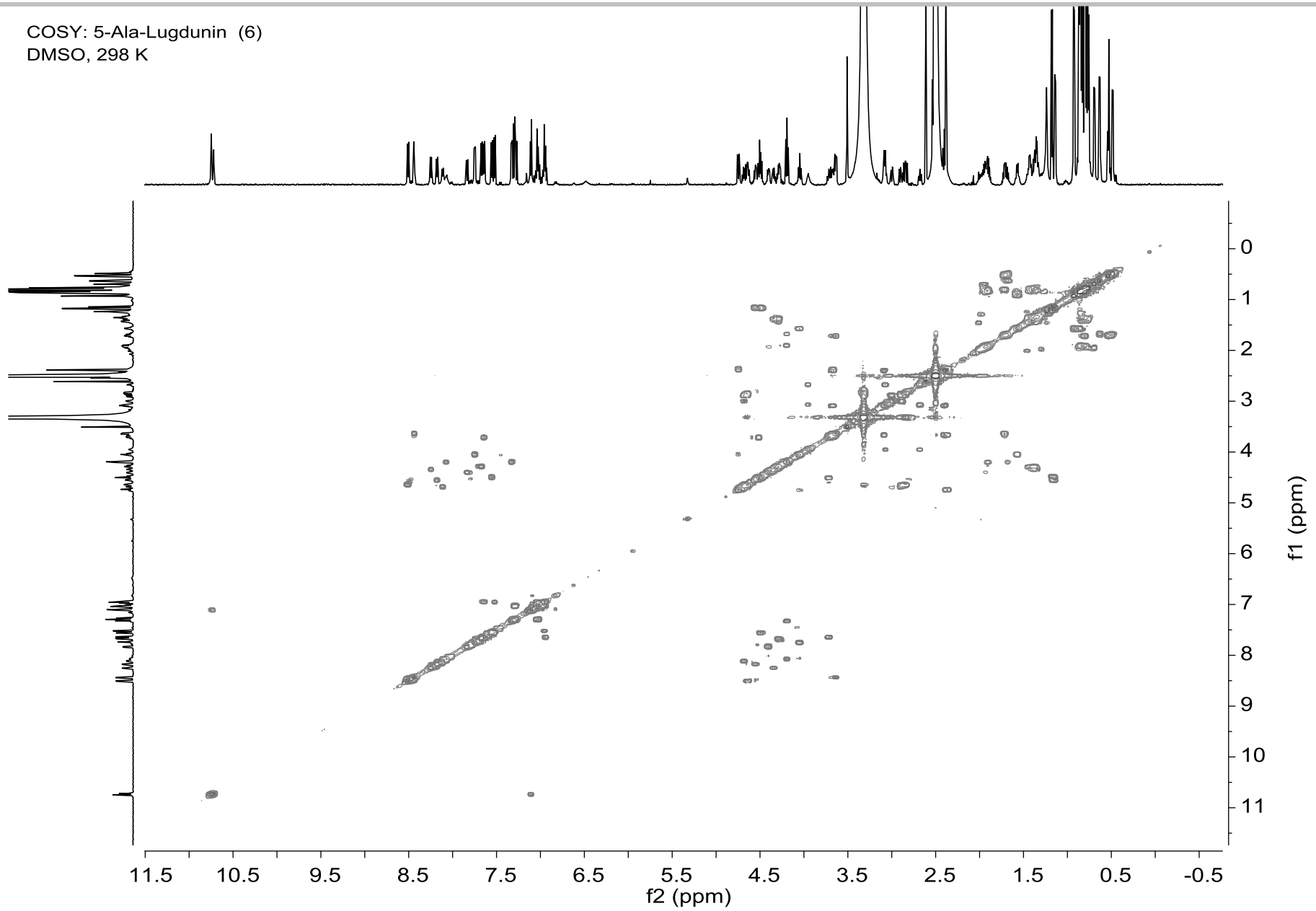

PROTON: 3-Ala-Lugdunin (4)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

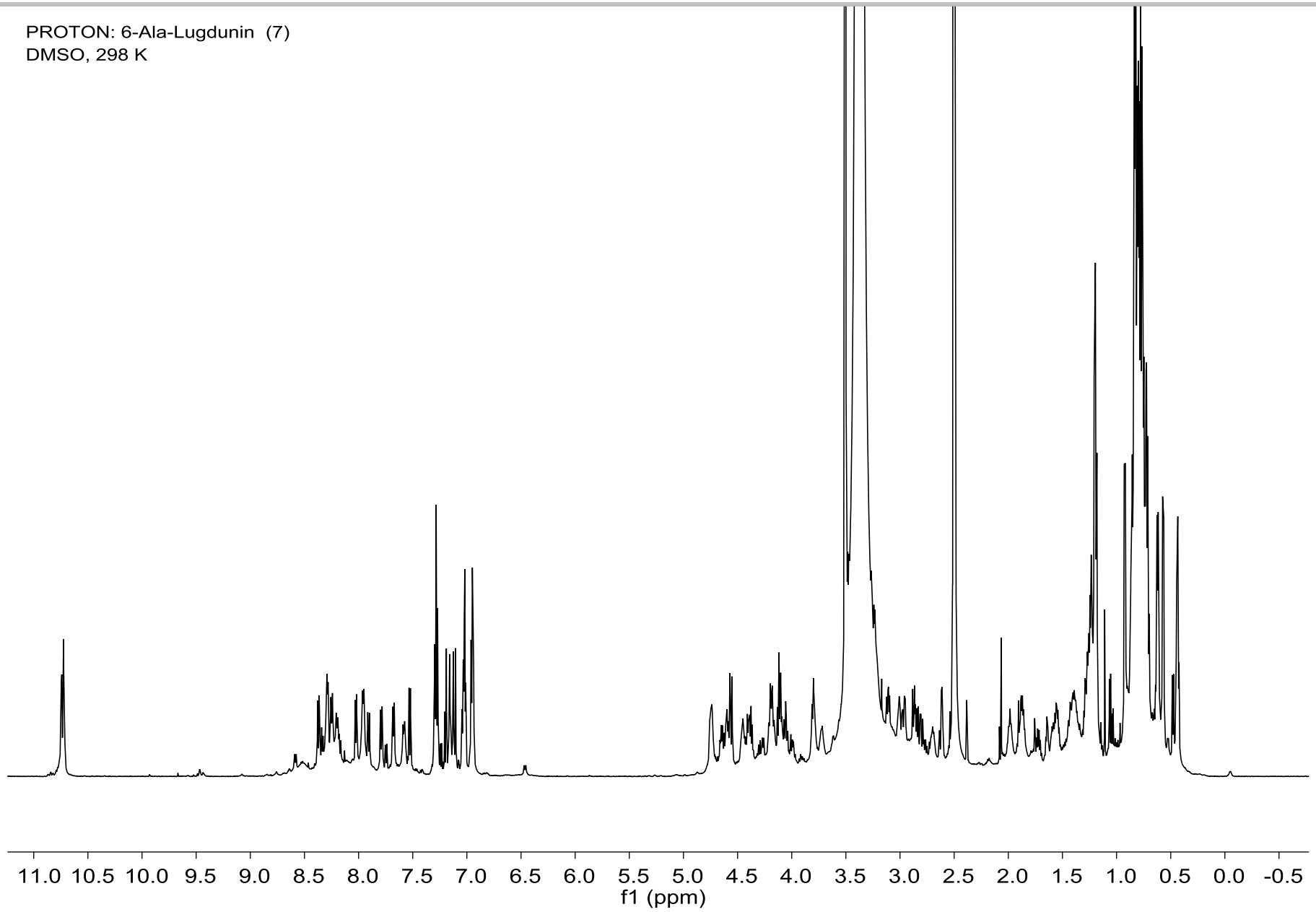

COSY: 3-Ala-Lugdunin (4)
DMSO, 298 K
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
12
f1 (ppm)

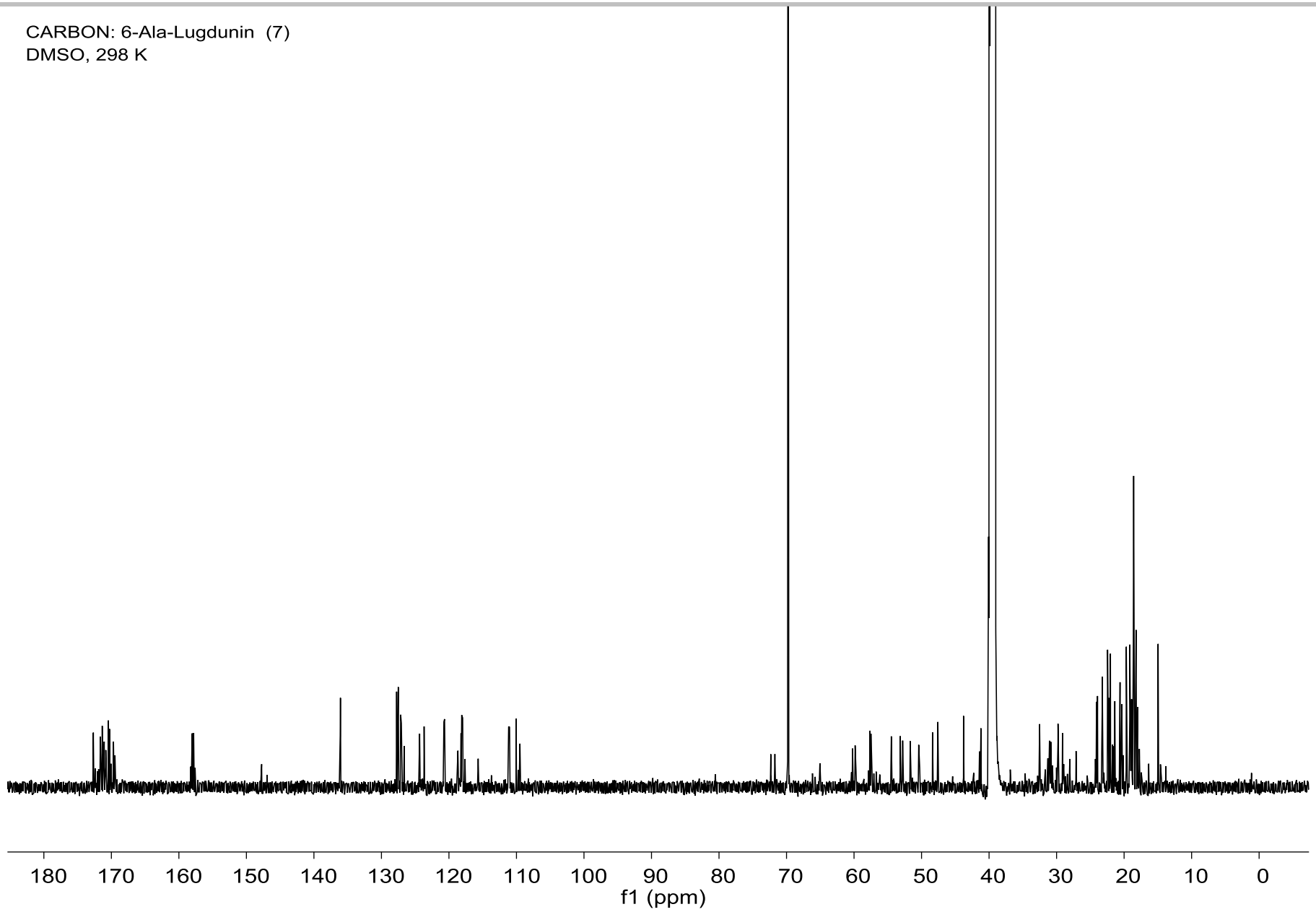

PROTON: 4-Ala-Lugdunin (5)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

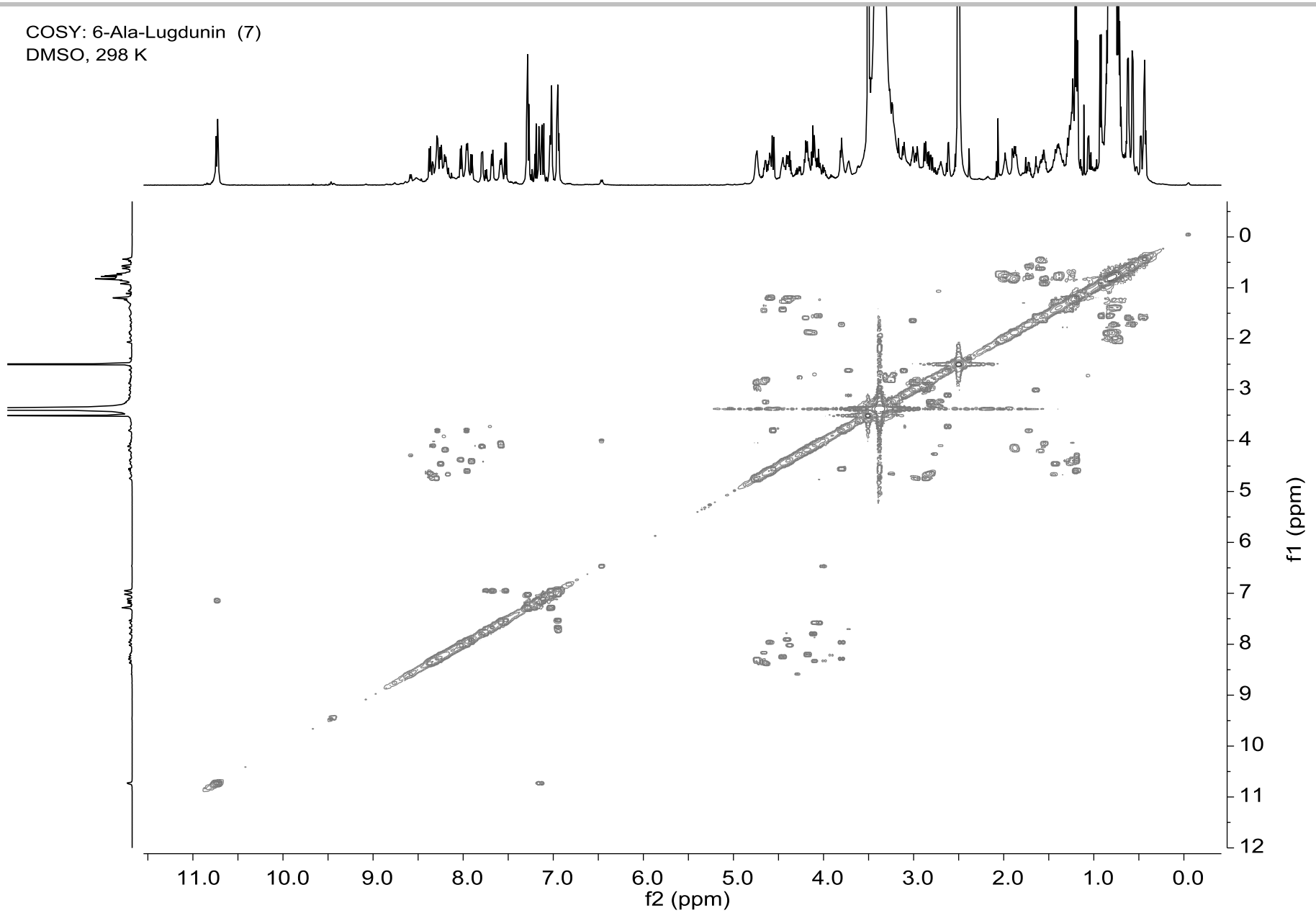

COSY: 4-Ala-Lugdunin (5)
DMSO, 298 K
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
f1 (ppm)

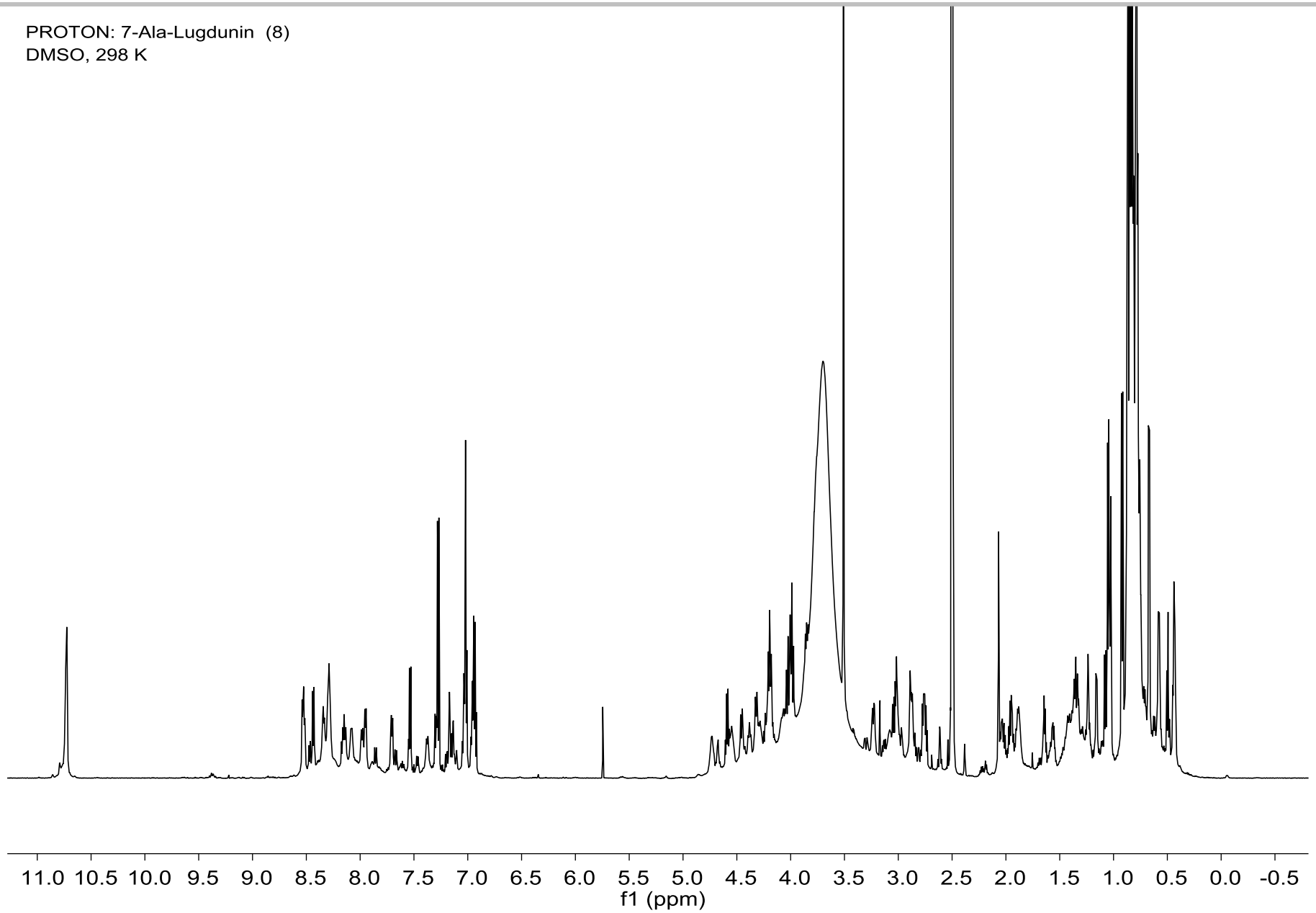

PROTON: 5-Ala-Lugdunin (6)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

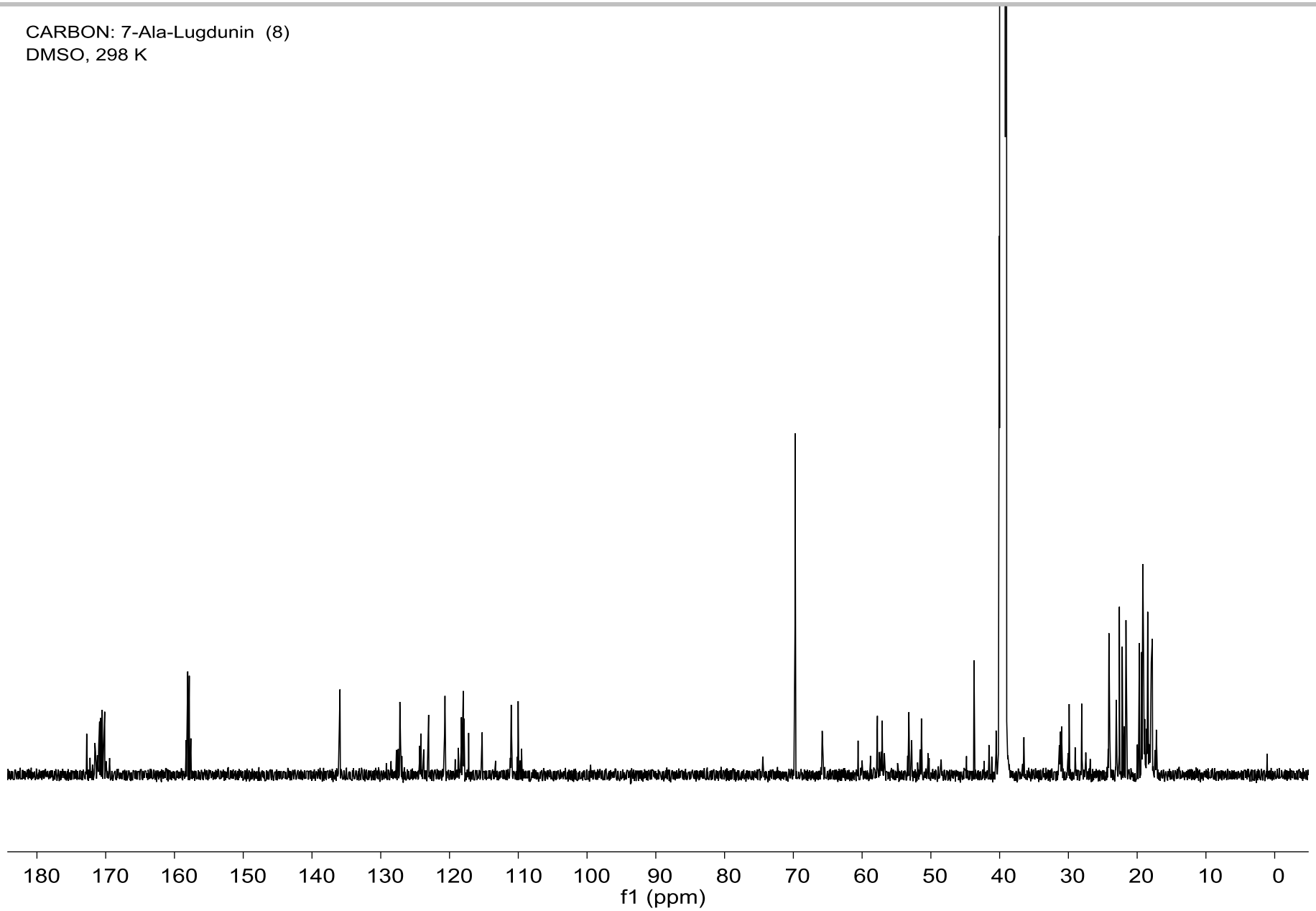

COSY: 5-Ala-Lugdunin (6)
DMSO, 298 K
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
f1 (ppm)

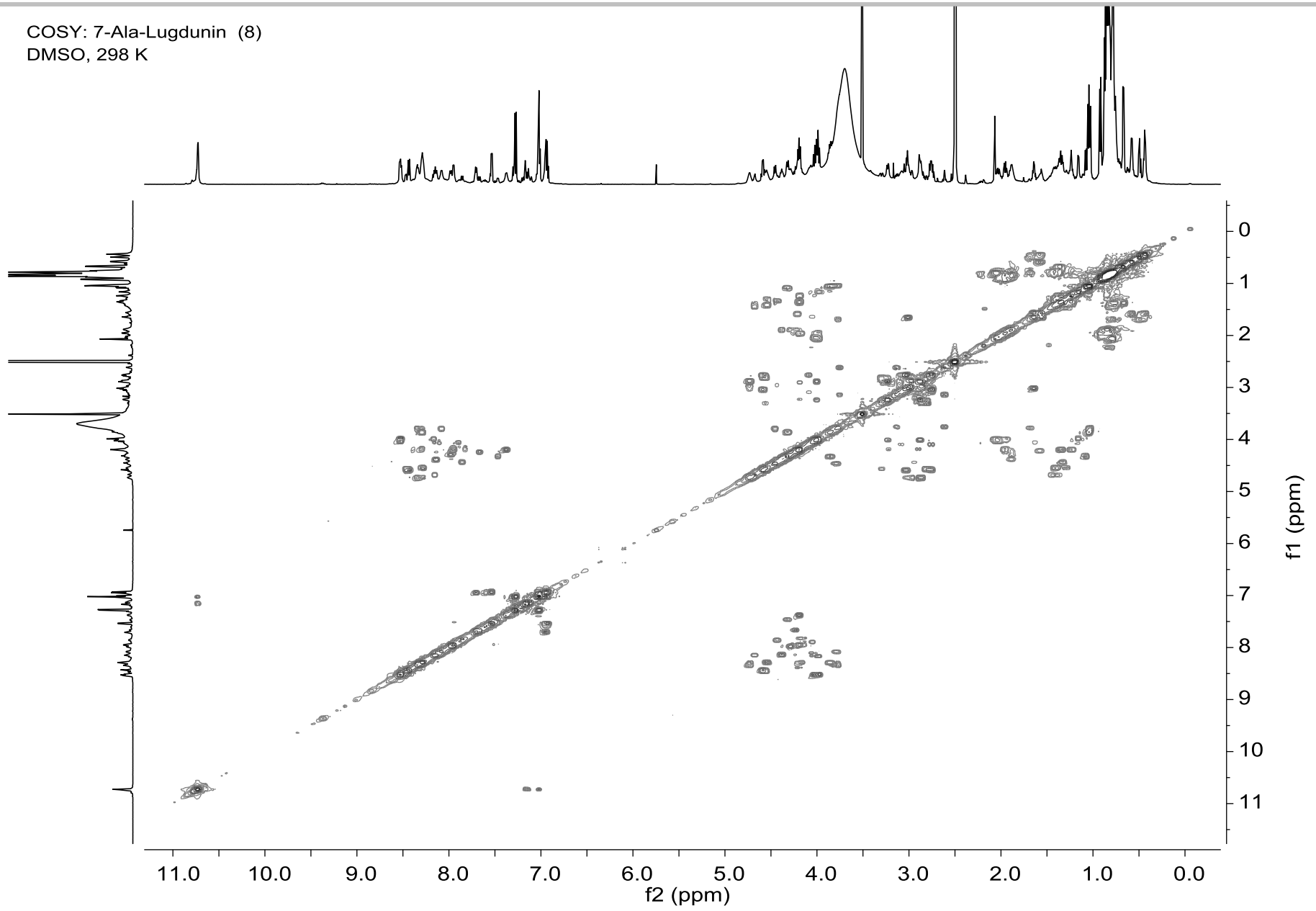

PROTON: 6-Ala-Lugdunin (7)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

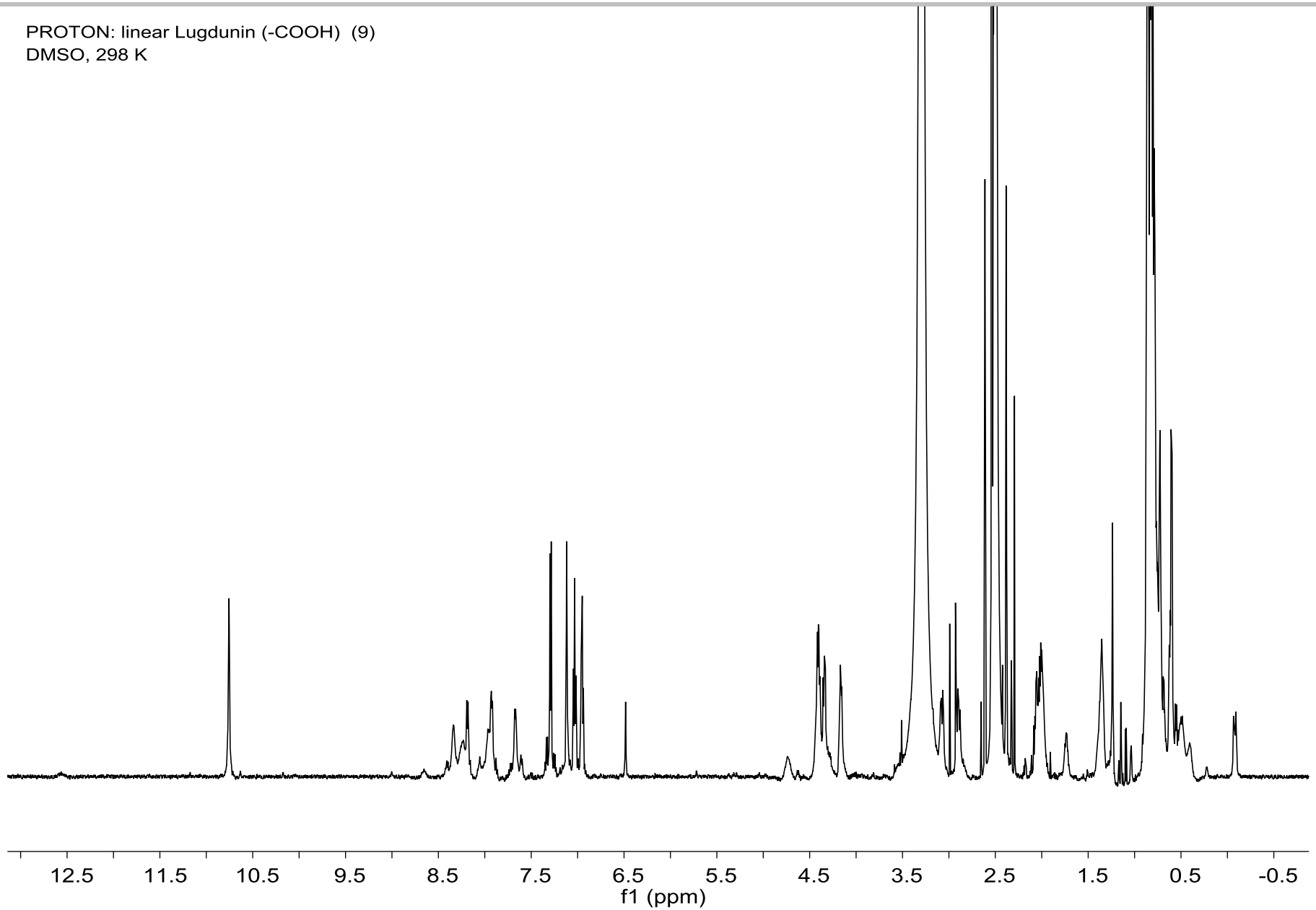

CARBON: 6-Ala-Lugdunin (7)
DMSO, 298 K
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

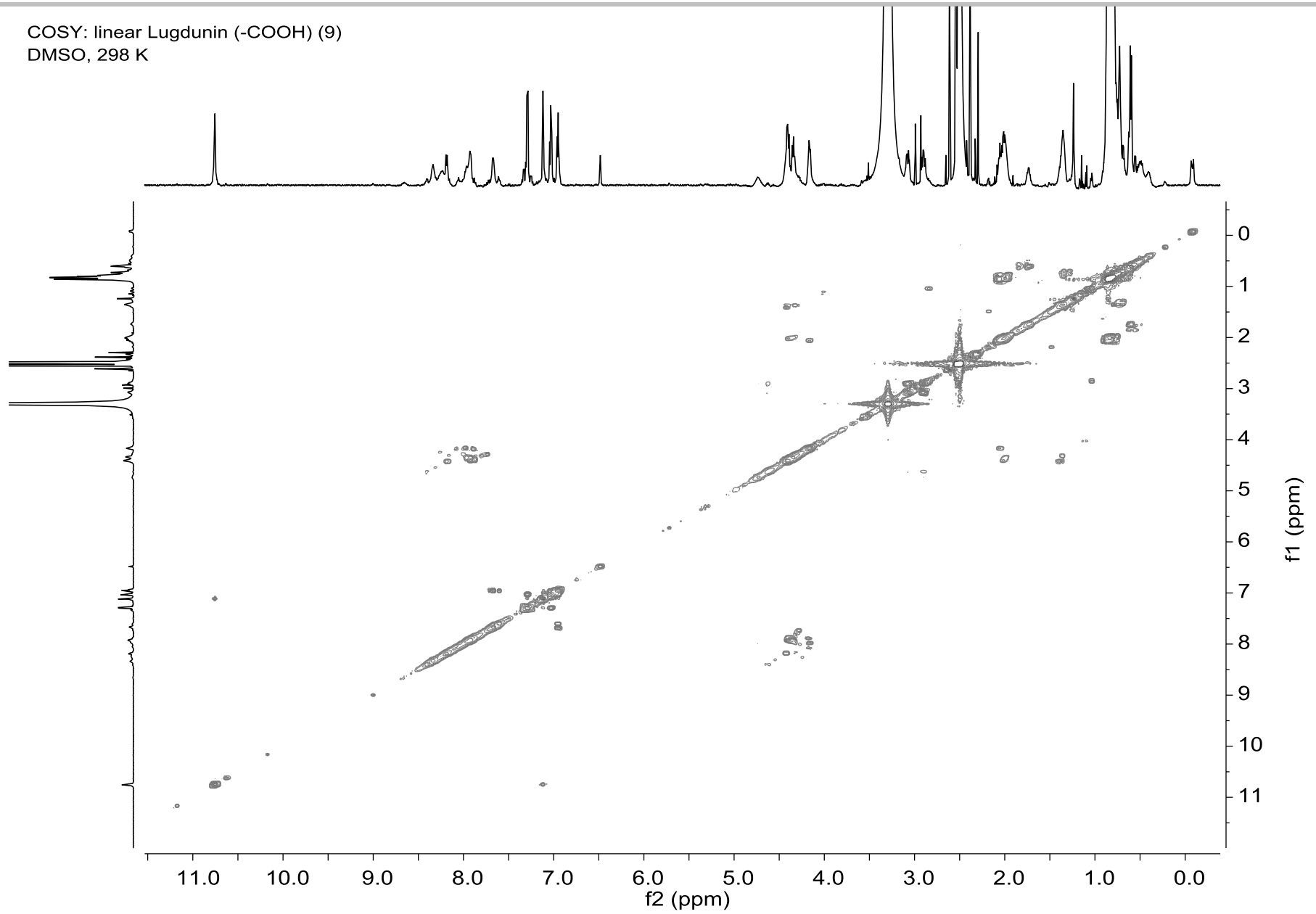

COSY: 6-Ala-Lugdunin (7)
DMSO, 298 K
f2 (ppm)
f1 (ppm)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
1
2
3
4
5
6
7
8
9
10
11
12

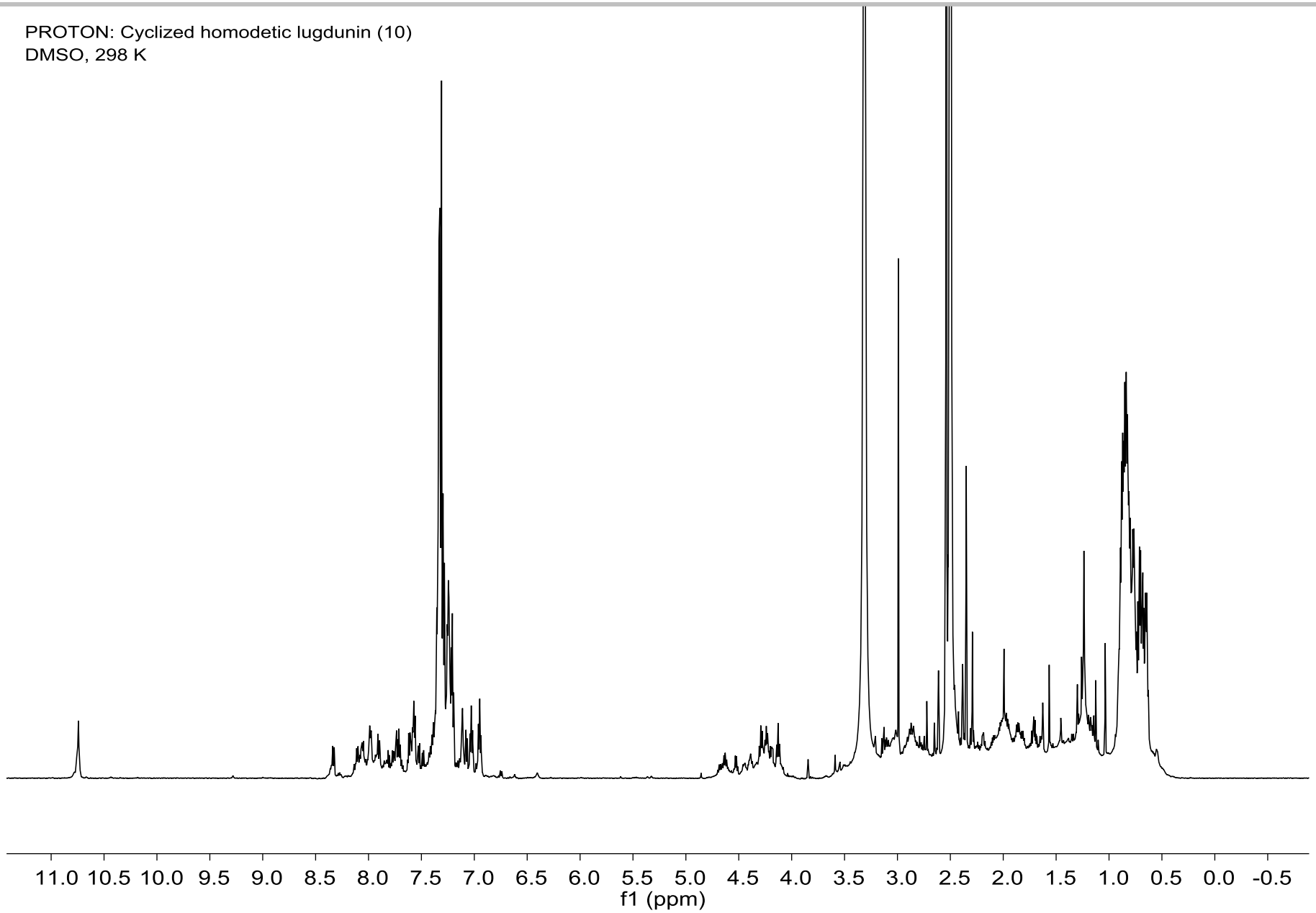

PROTON: 7-Ala-Lugdunin (8)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

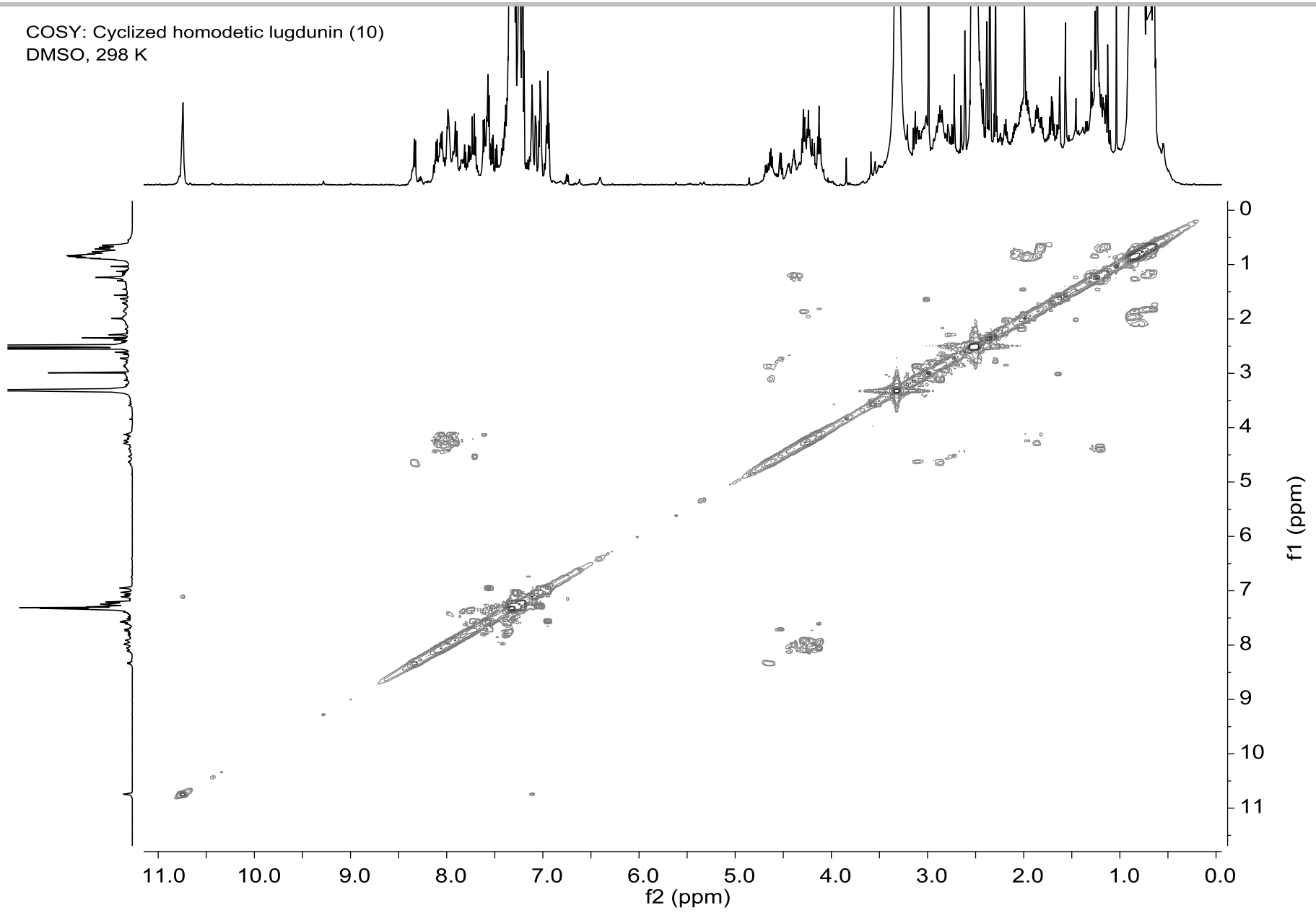

CARBON: 7-Ala-Lugdunin (8)
DMSO, 298 K
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

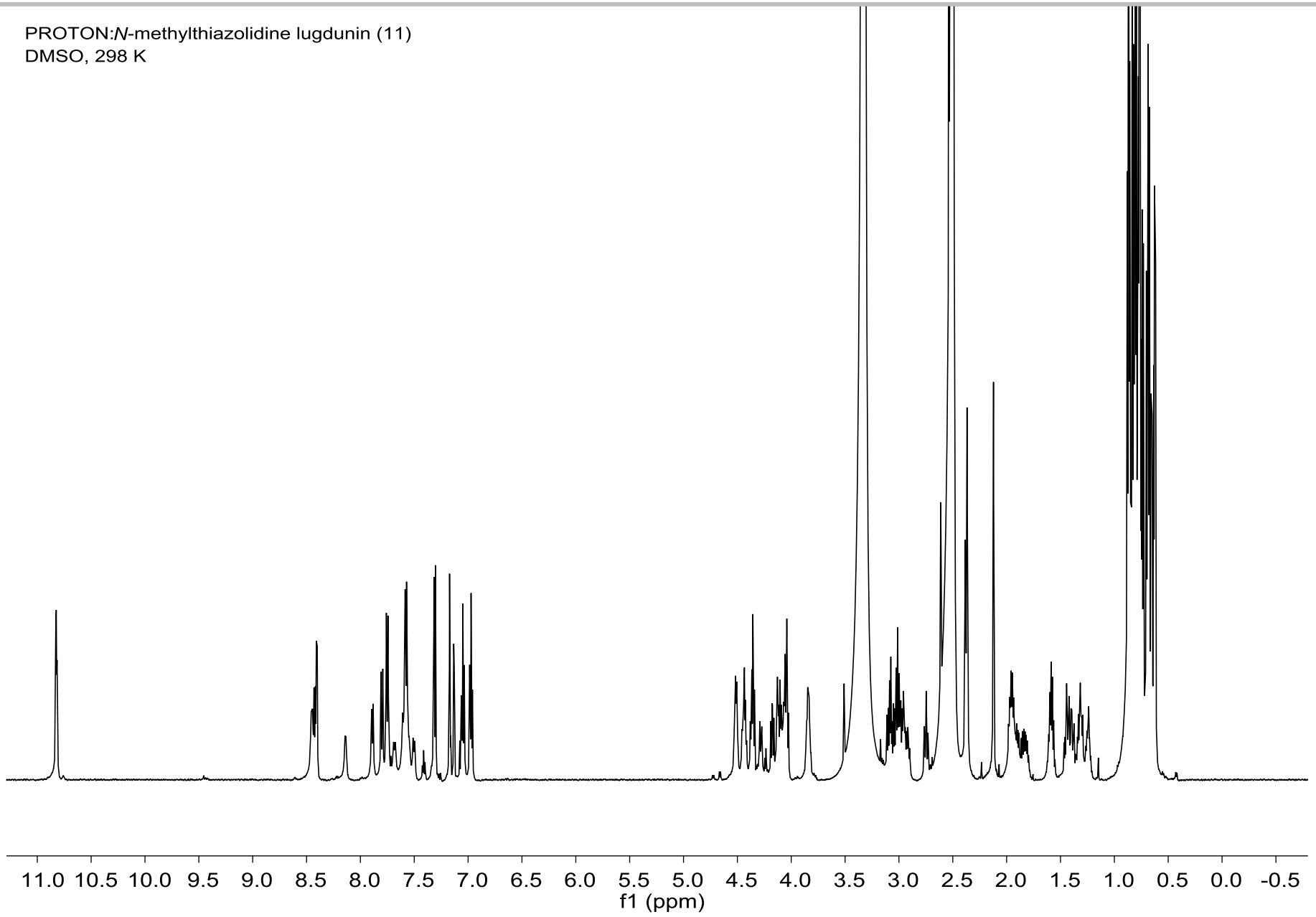

COSY: 7-Ala-Lugdunin (8)
DMSO, 298 K
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
f1 (ppm)

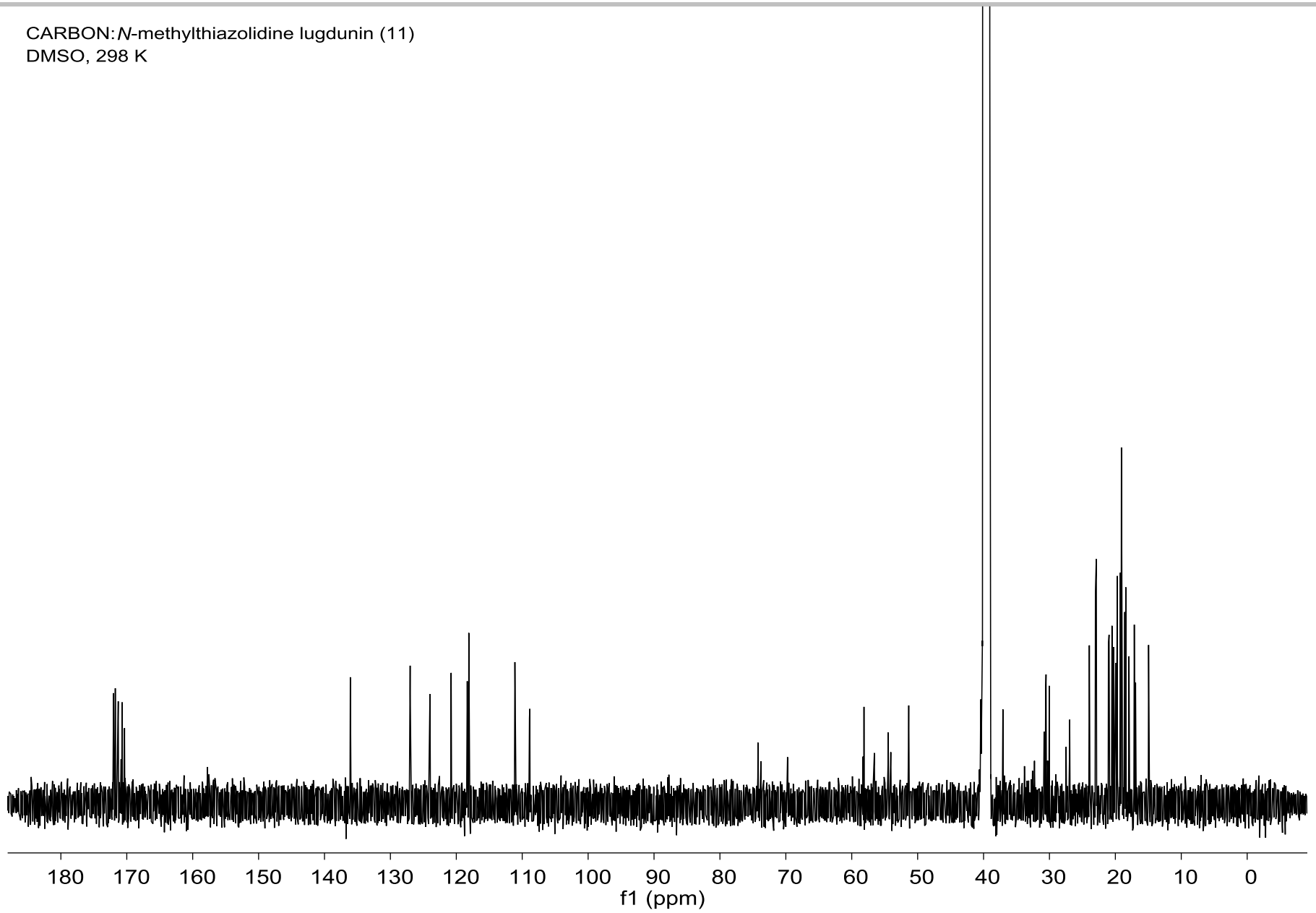

PROTON: linear Lugdunin (-COOH) (9)
DMSO, 298 K
12.5
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
f1 (ppm)

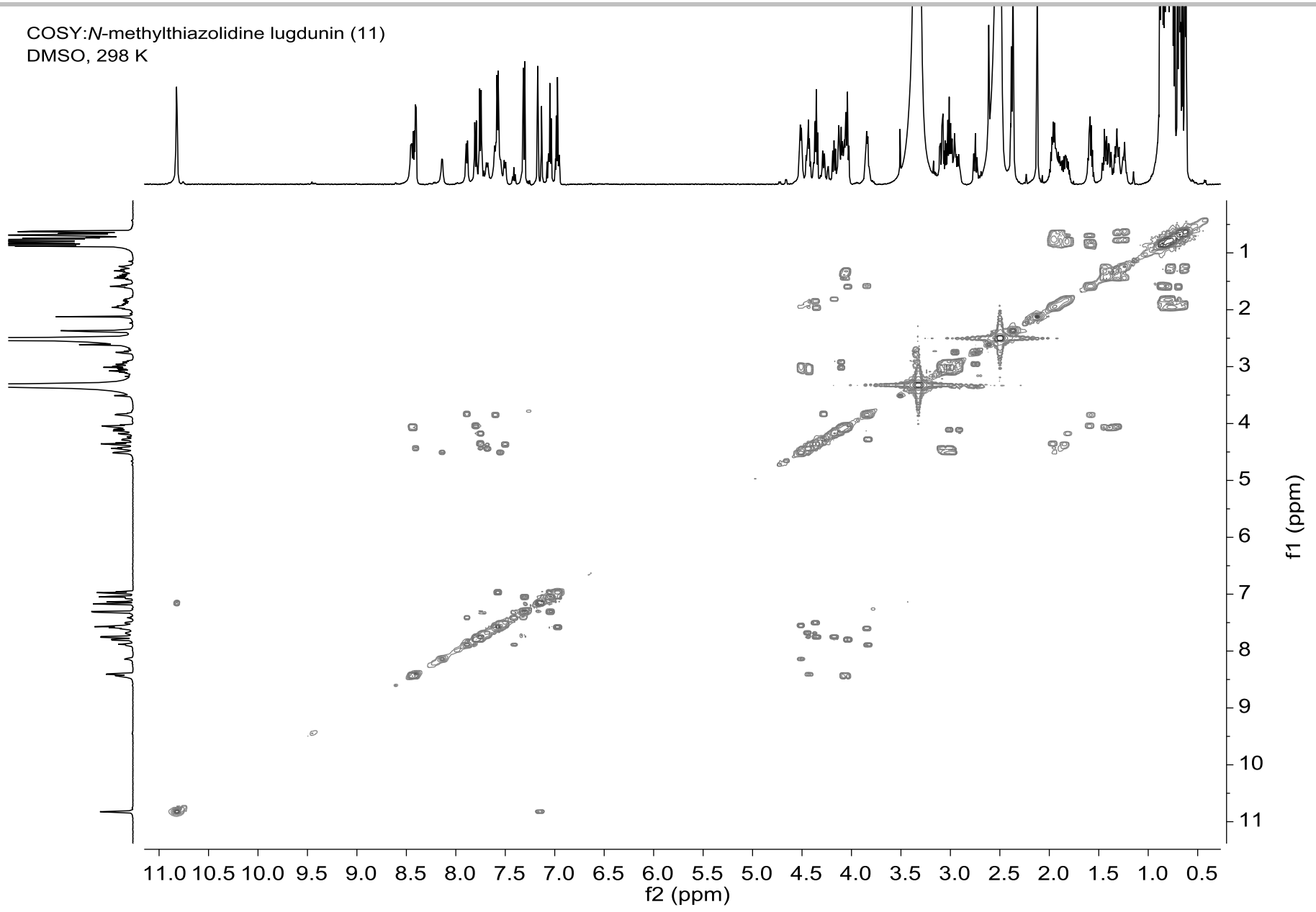

COSY: linear Lugdunin (-COOH) (9)
DMSO, 298 K
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
f1 (ppm)

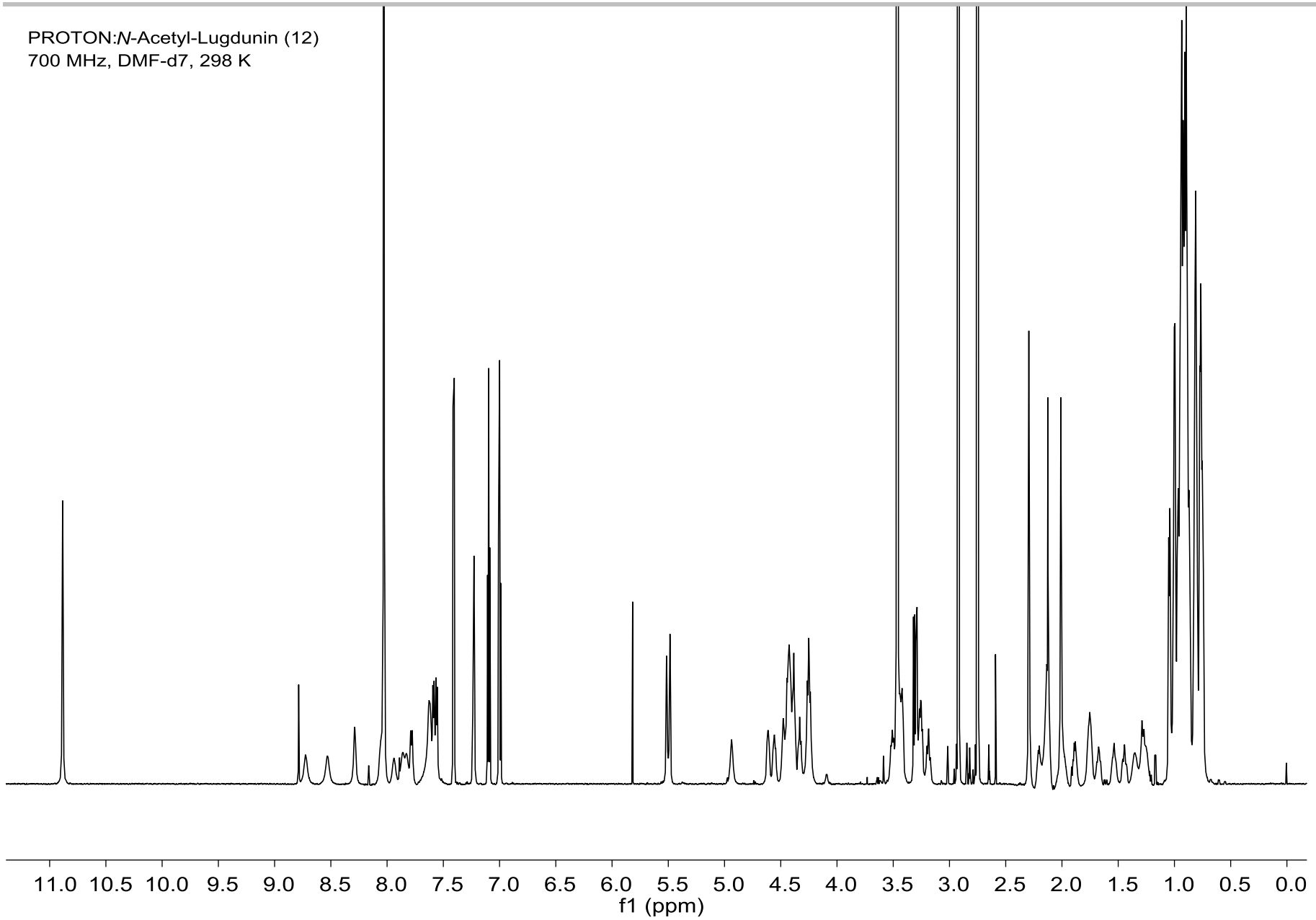

PROTON: Cyclized homodetic lugdunin (10)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

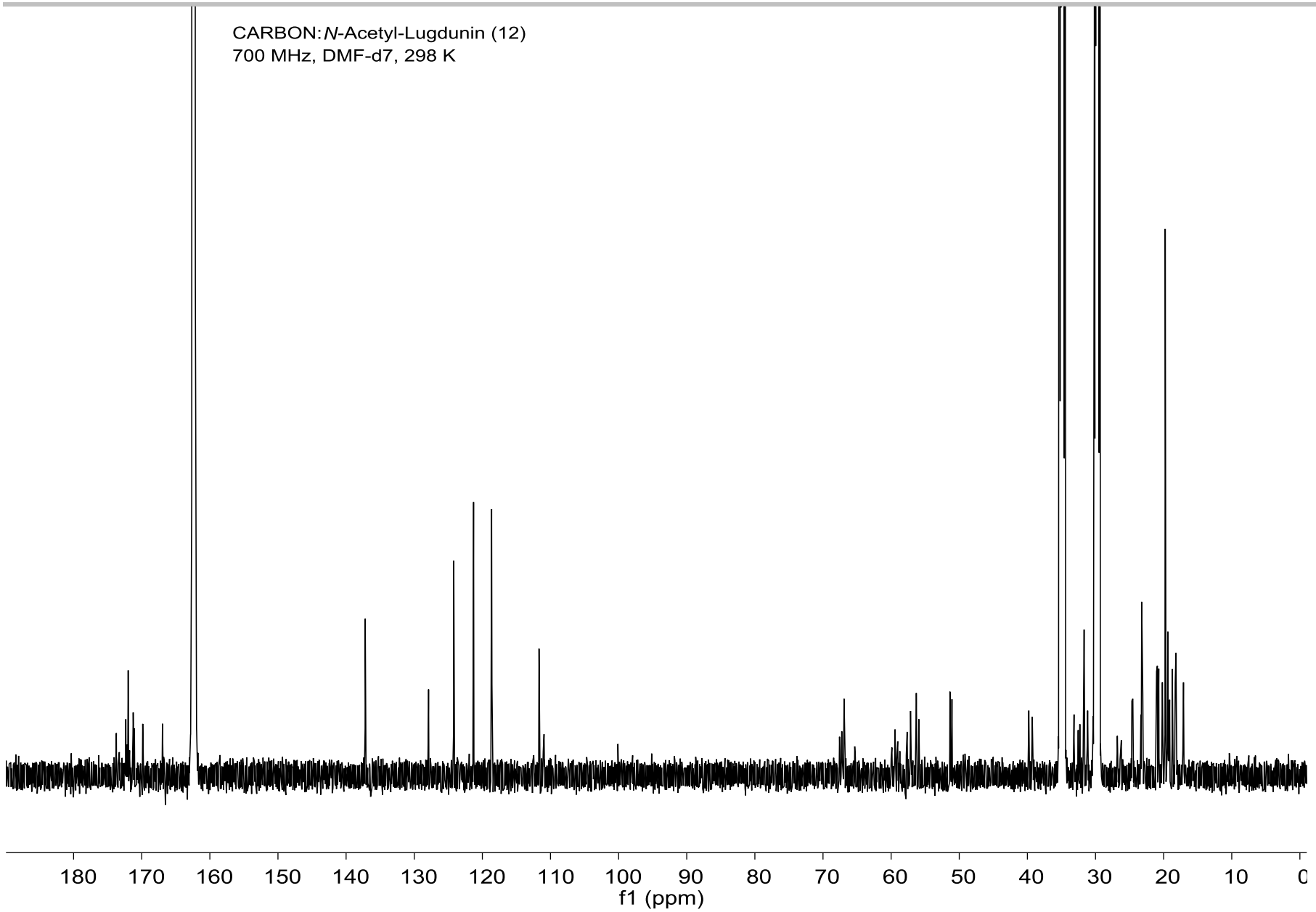

COSY: Cyclized homodetic lugdunin (10)
DMSO, 298 K
f2 (ppm)
f1 (ppm)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
1
2
3
4
5
6
7
8
9
10
11

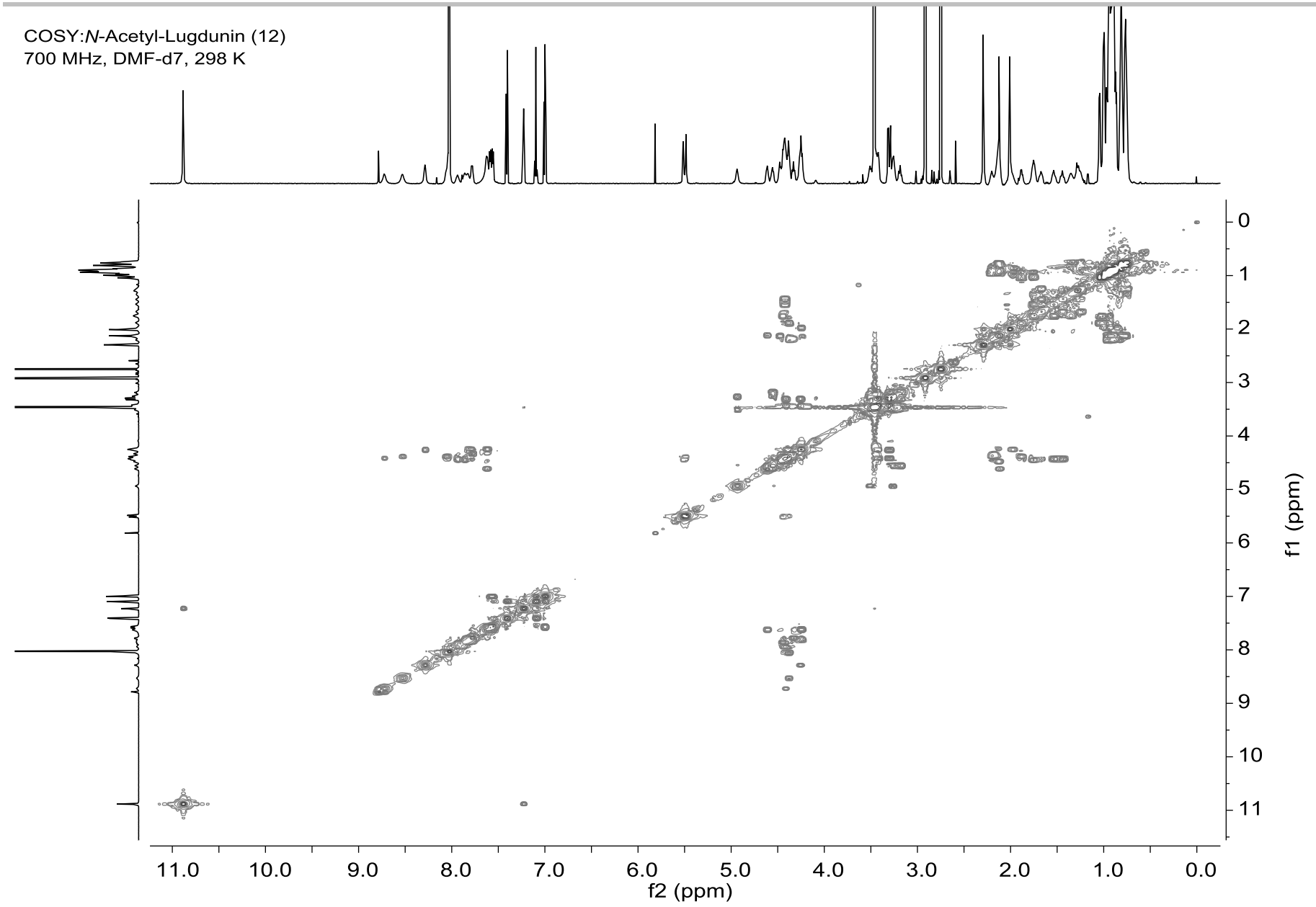

PROTON:*N*-methylthiazolidine lugdunin (11)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

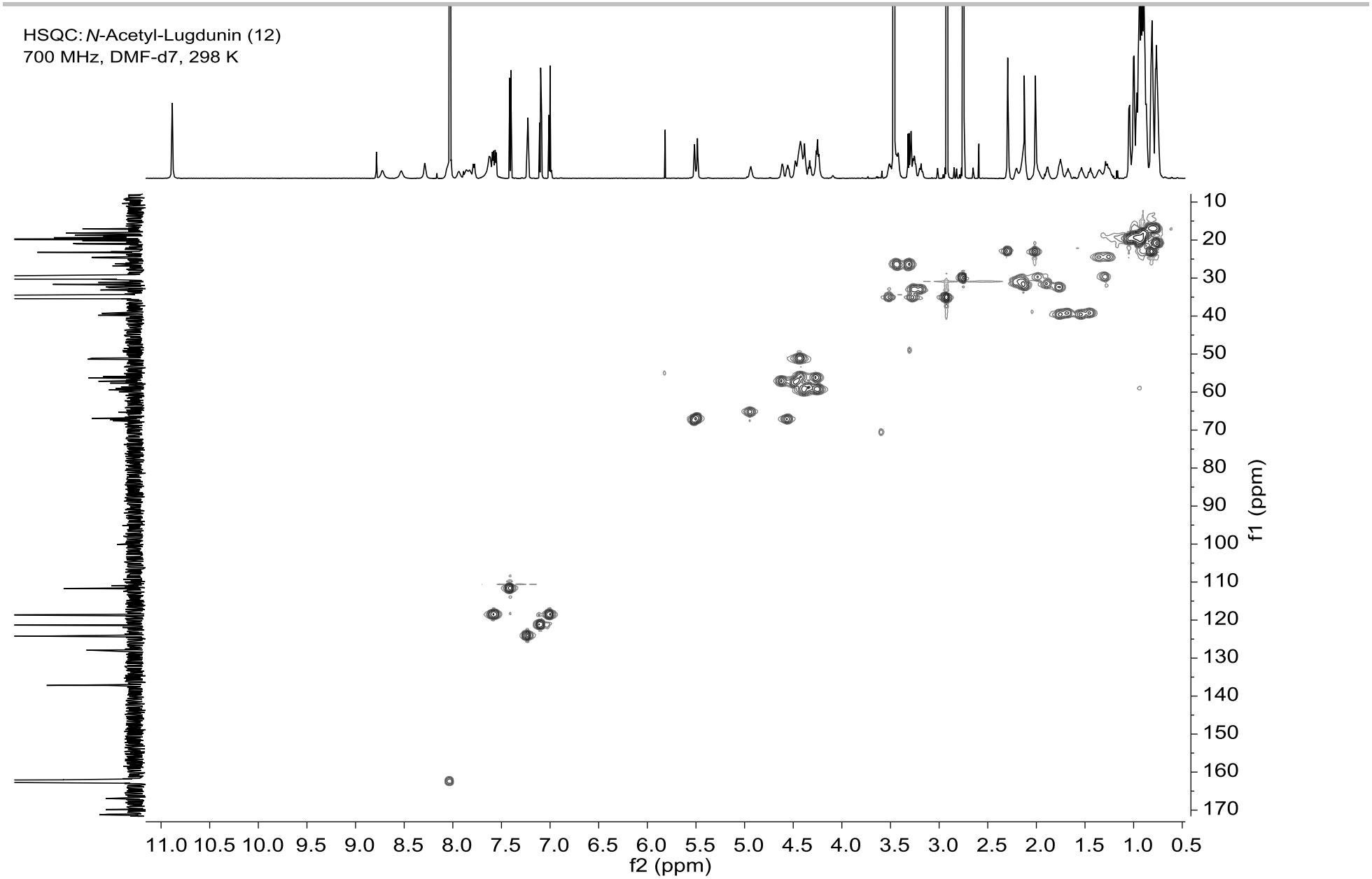

CARBON: N-methylthiazolidine lugdunin (11)
DMSO, 298 K
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

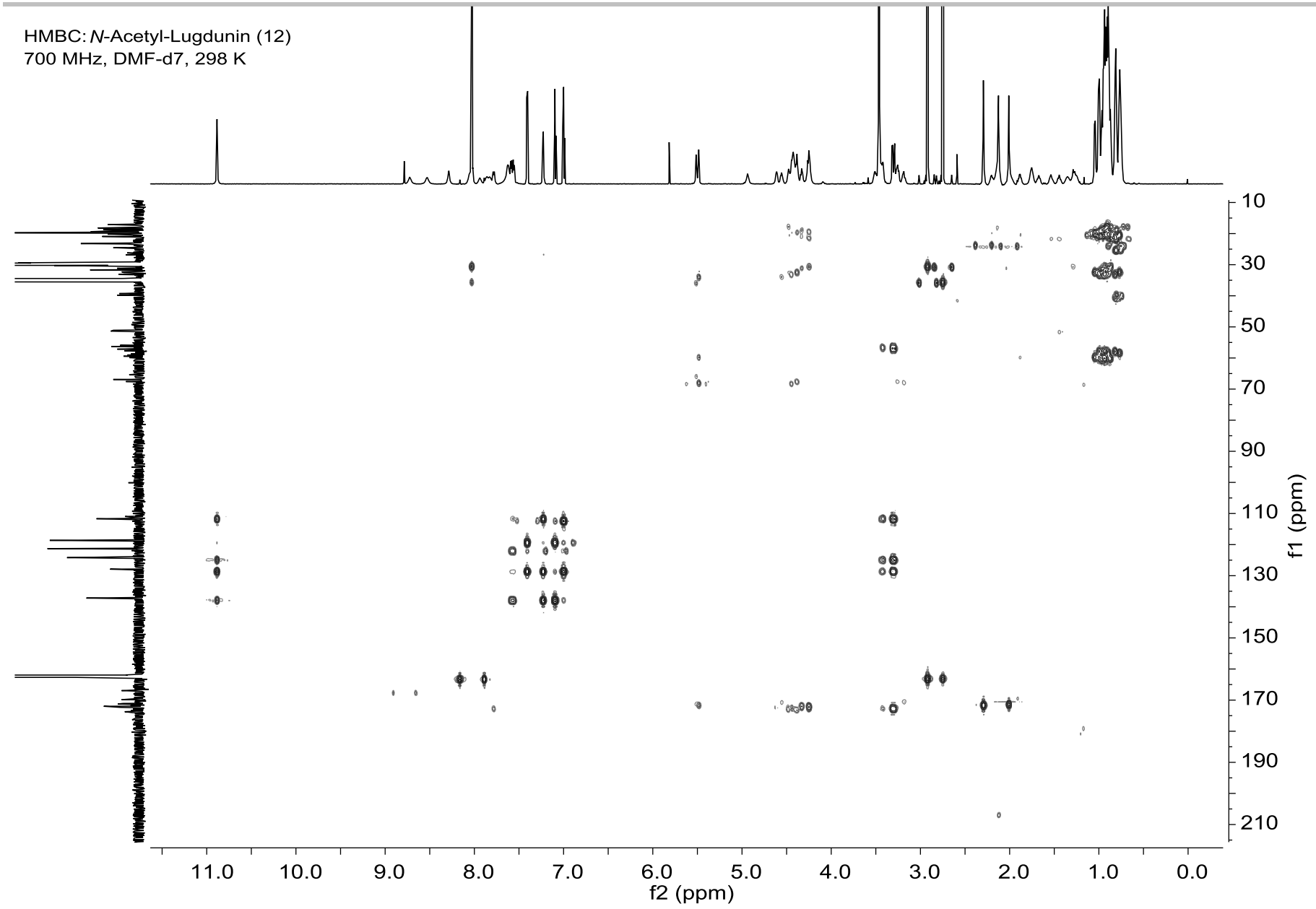

COSY:*N*-methylthiazolidine lugdunin (11)
DMSO, 298 K
f2 (ppm)
f1 (ppm)

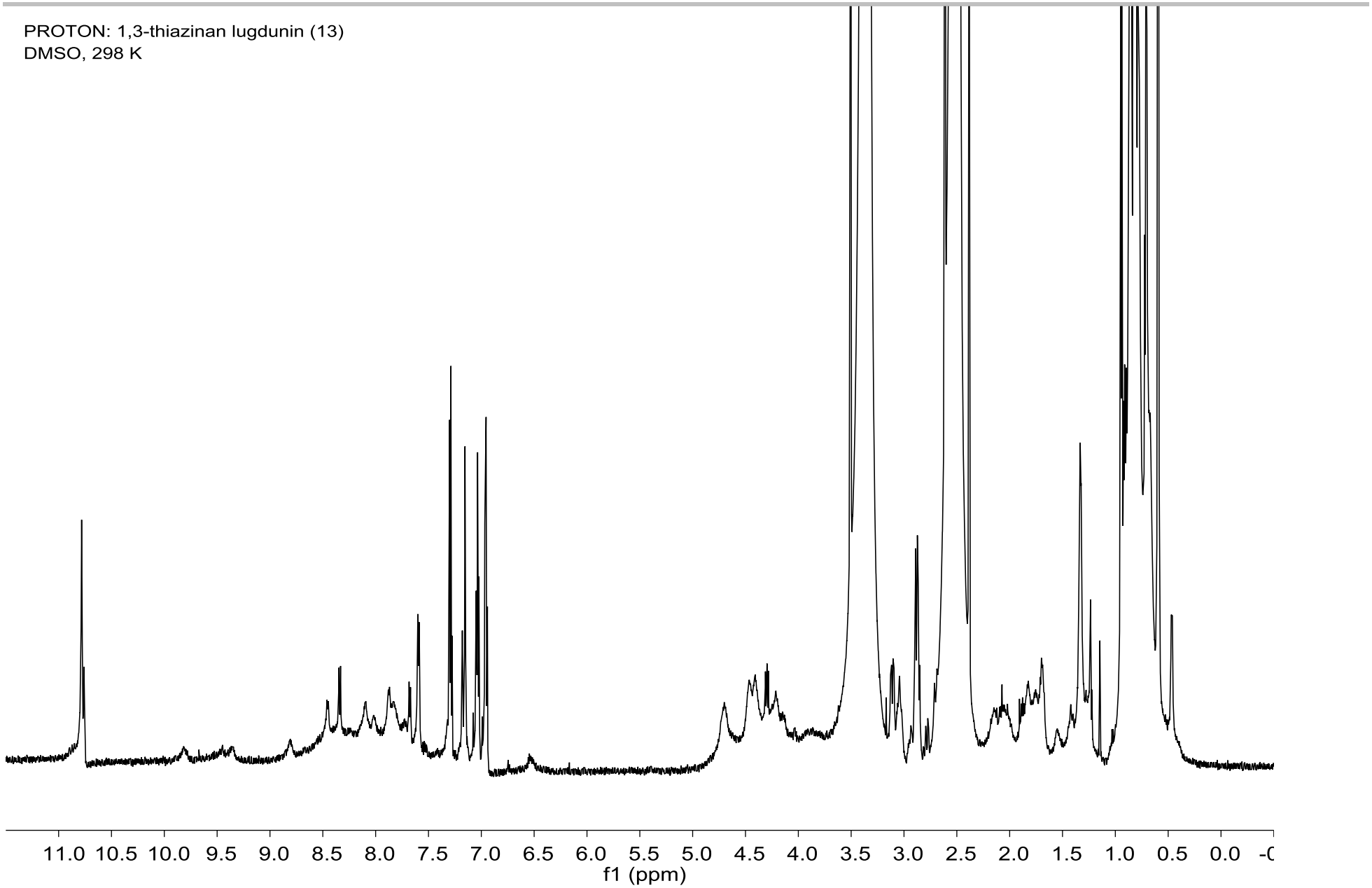

PROTON:*N*-Acetyl-Lugdunin (12)
700 MHz, DMF-d7, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

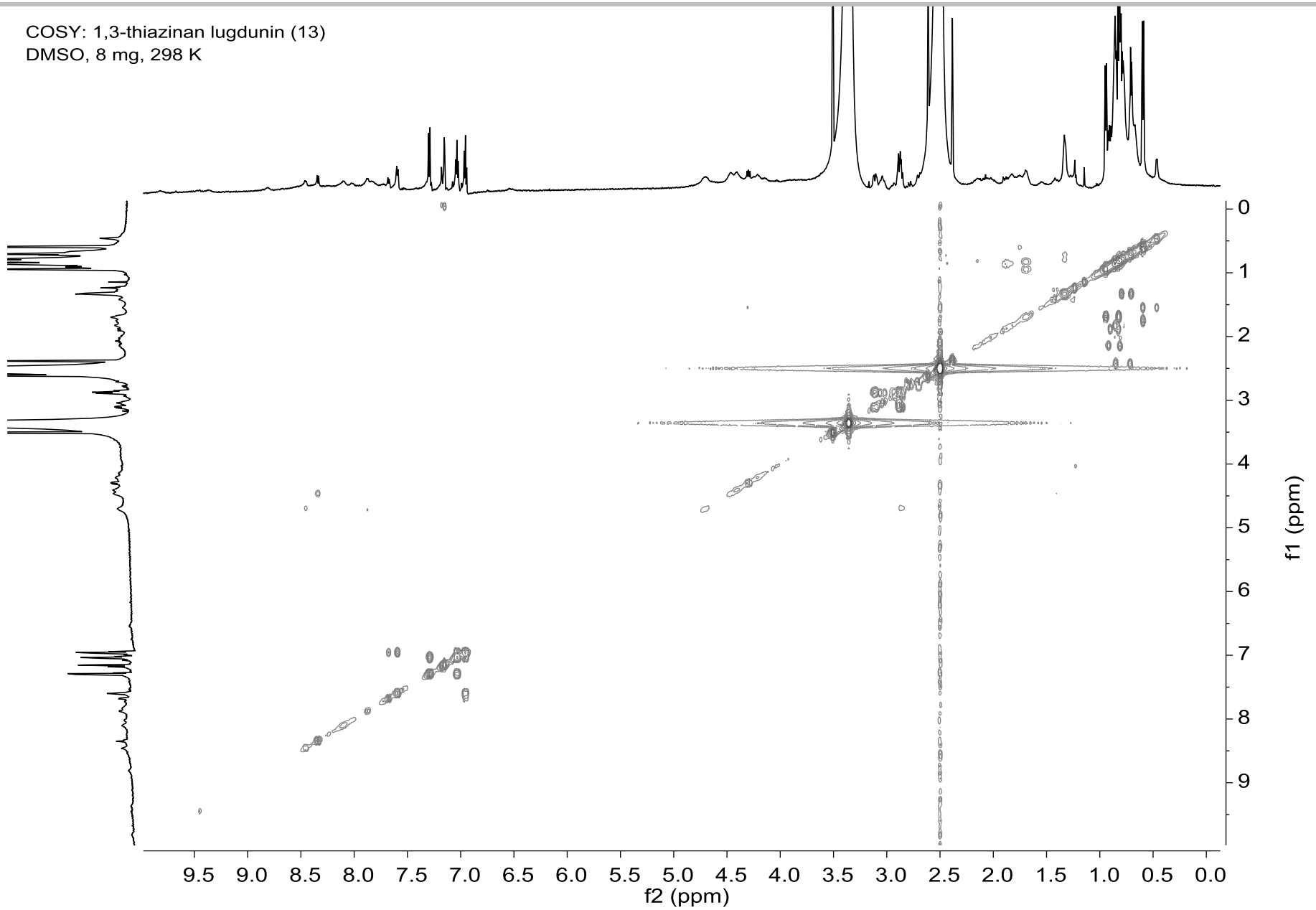

CARBON:*N*-Acetyl-Lugdunin (12)
700 MHz, DMF-d7, 298 K
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

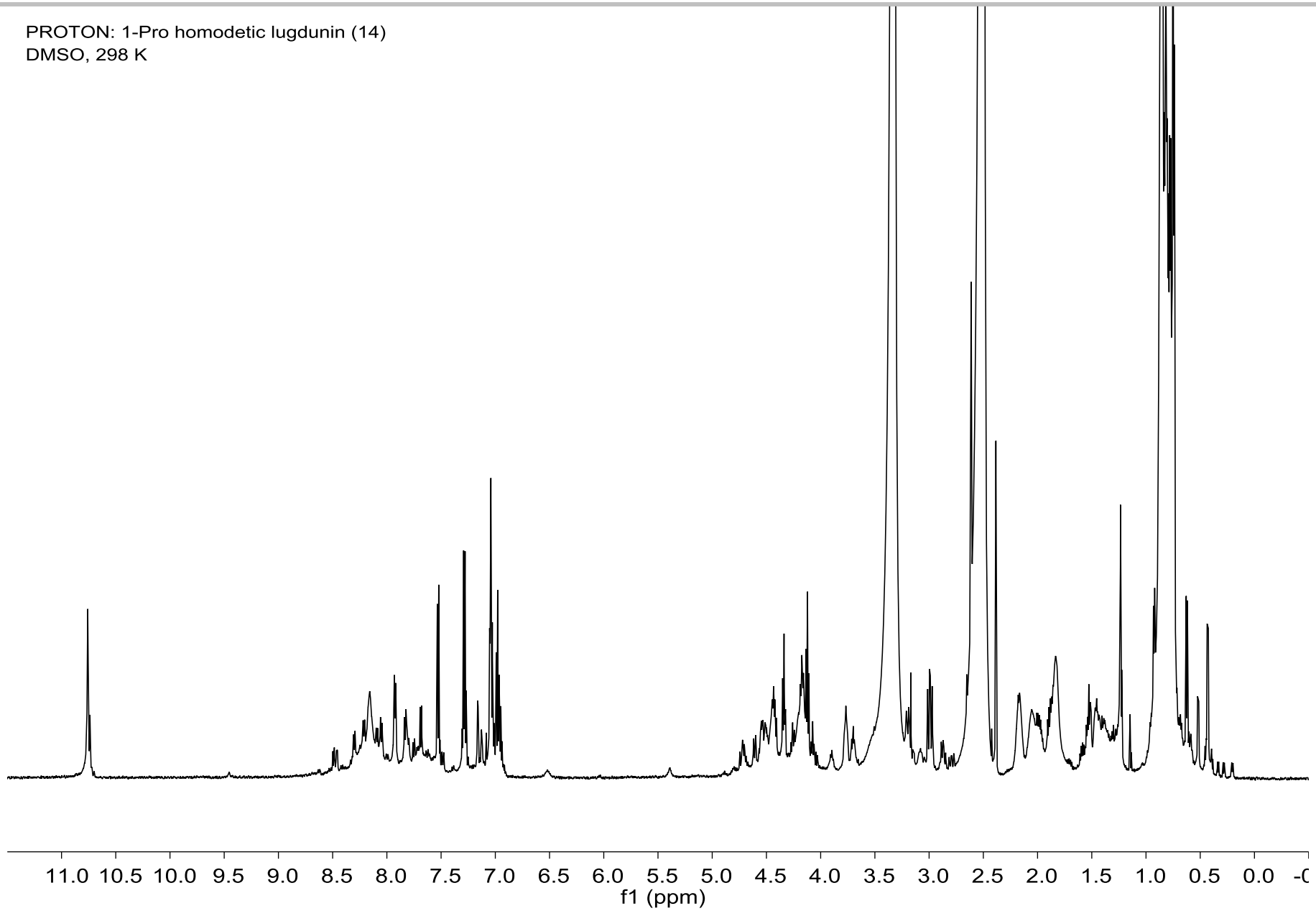

COSY:*N*-Acetyl-Lugdunin (12)
700 MHz, DMF-d7, 298 K
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
f1 (ppm)

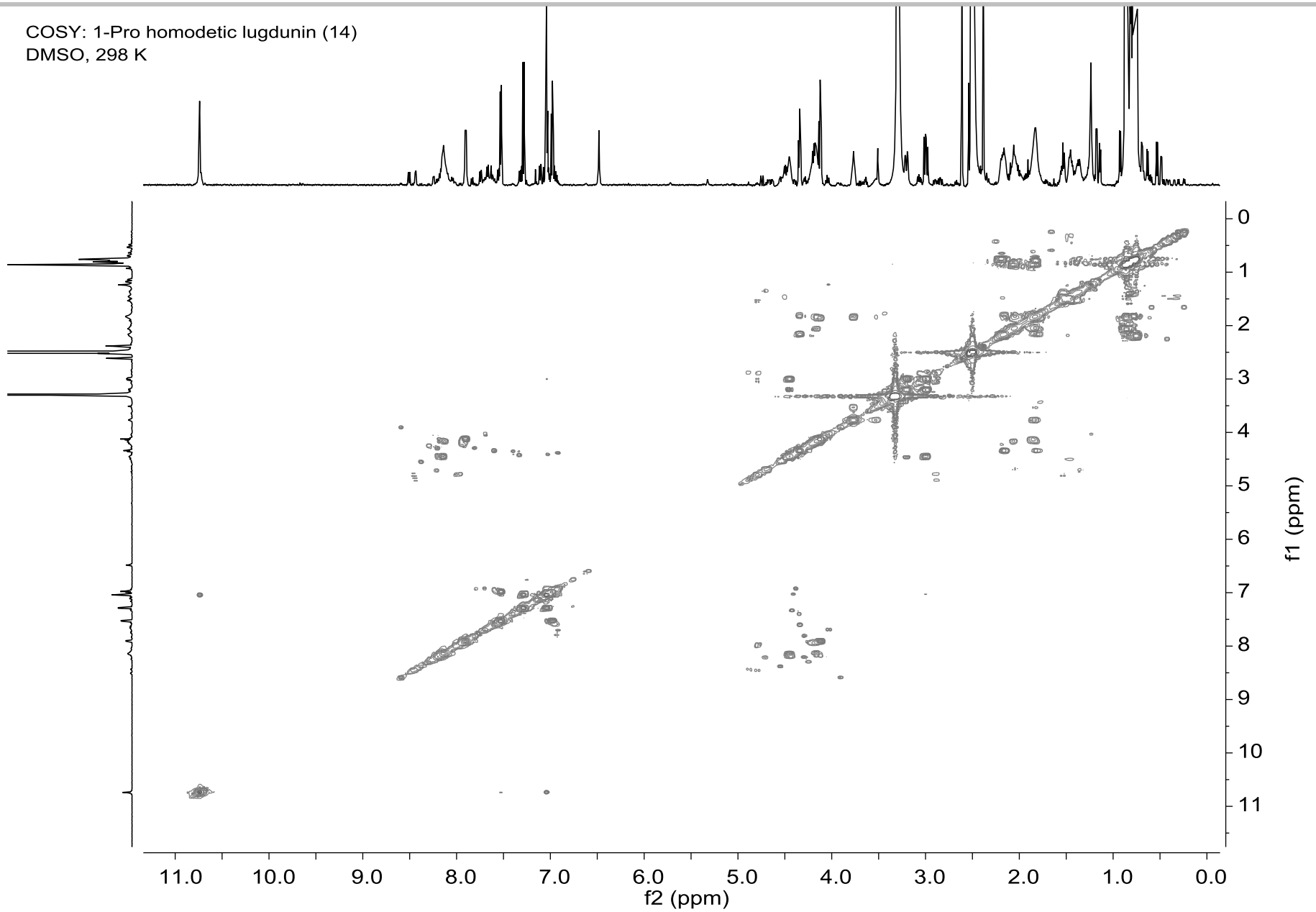

HSQC: *N*-Acetyl-Lugdunin (12)
700 MHz, DMF-d7, 298 K
f2 (ppm)
f1 (ppm)

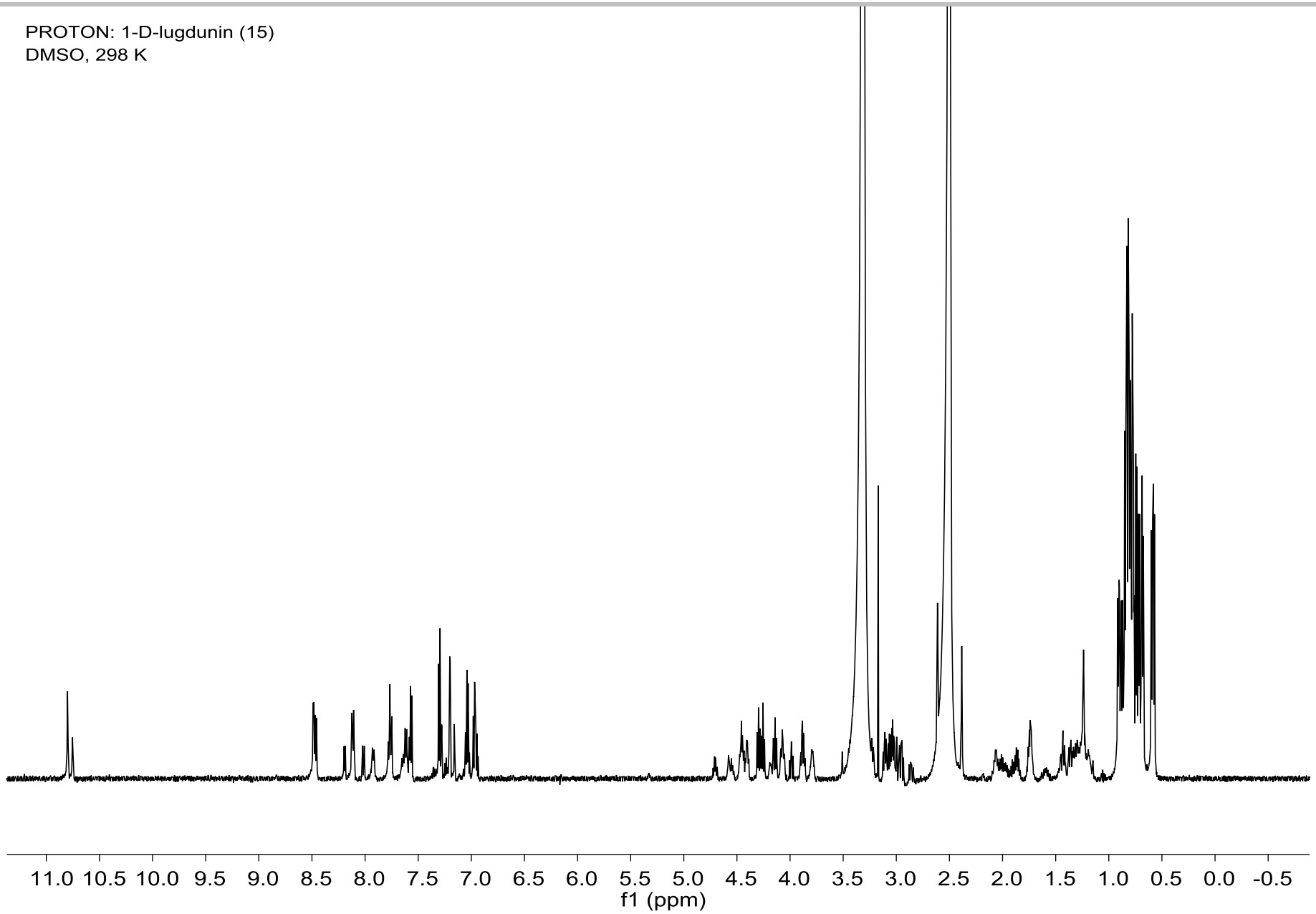

HMBC: *N*-Acetyl-Lugdunin (12)
700 MHz, DMF-d7, 298 K
f2 (ppm)
f1 (ppm)

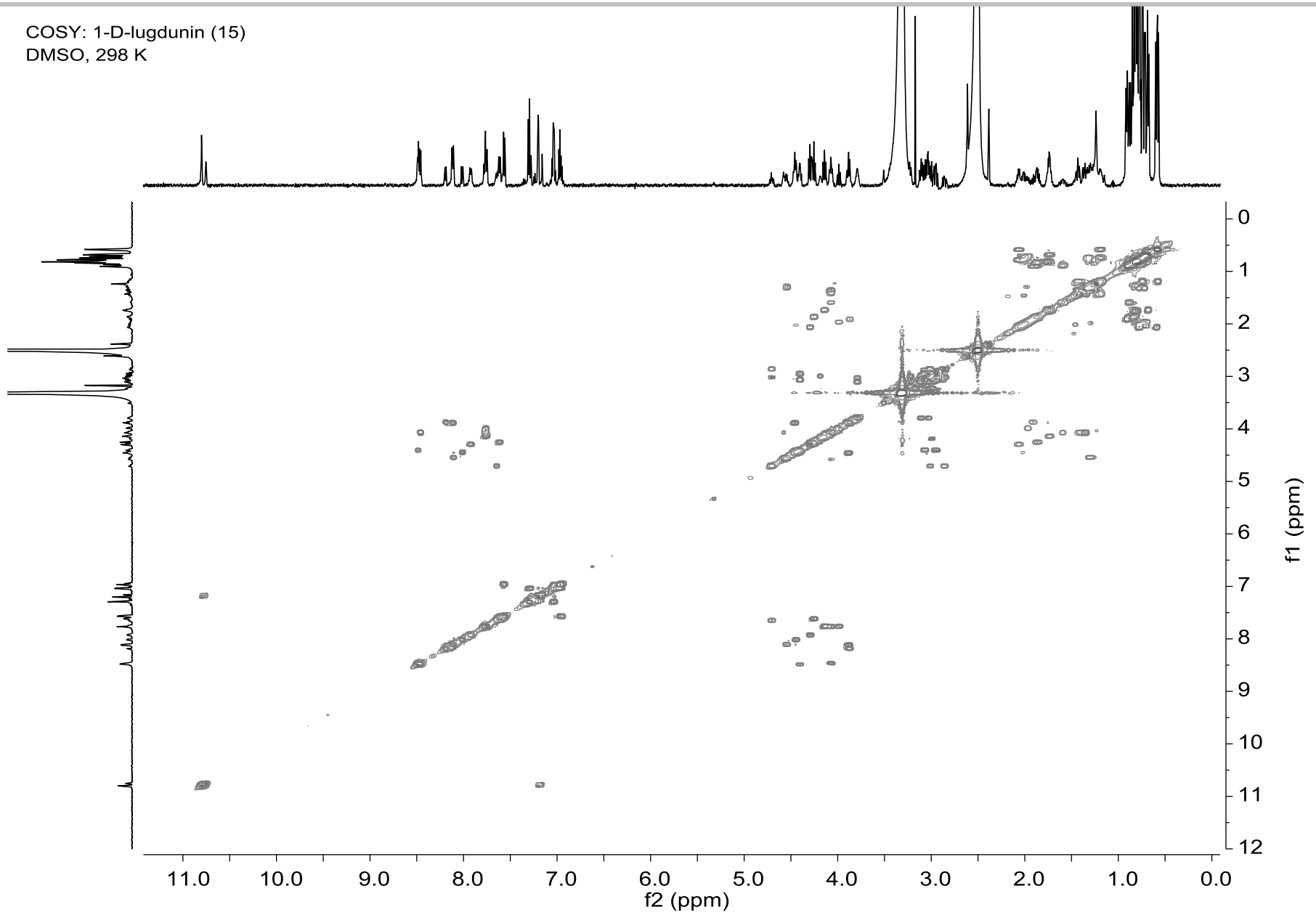

PROTON: 1,3-thiazinan lugdunin (13)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

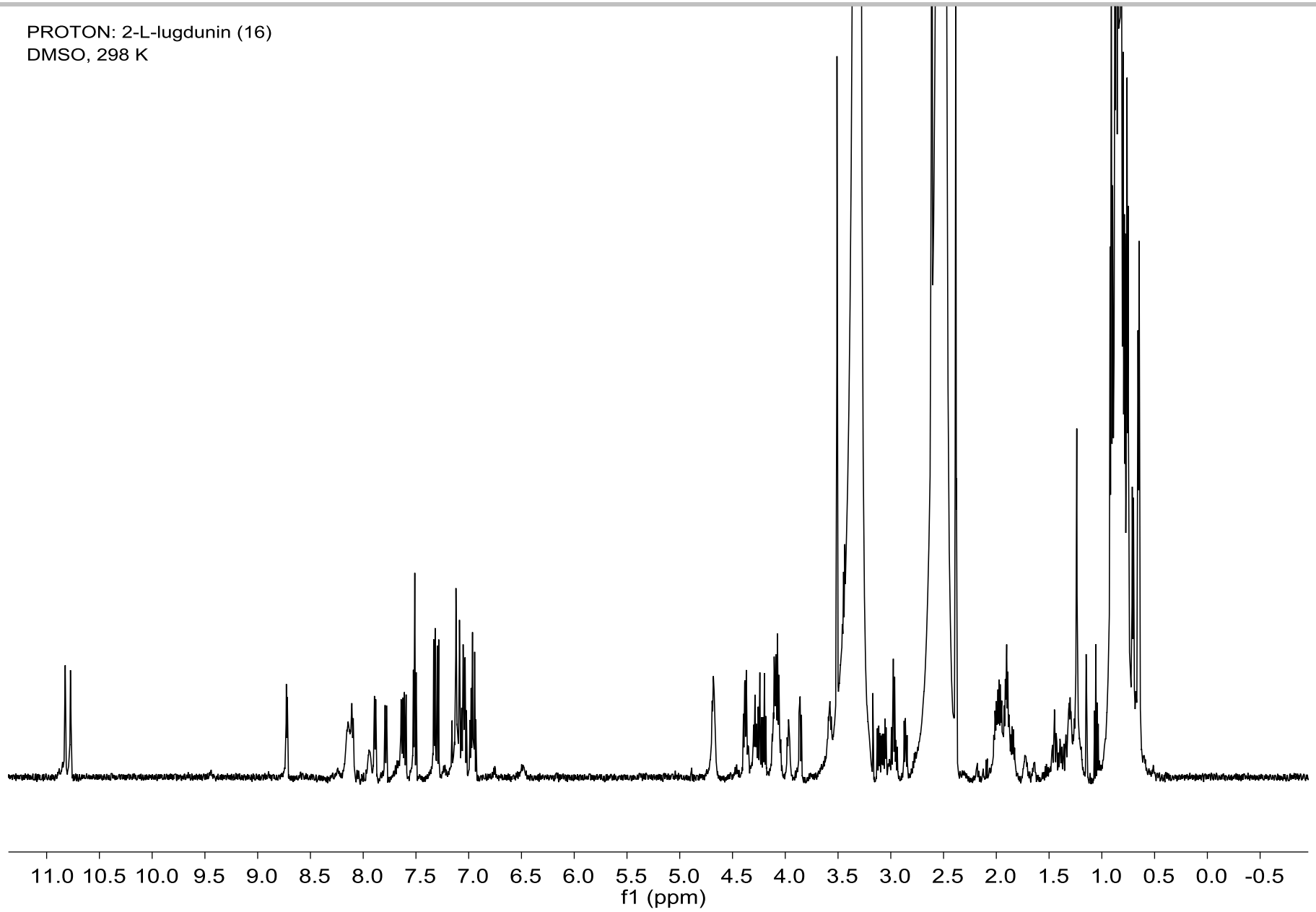

COSY: 1,3-thiazinan lugdunin (13)
DMSO, 8 mg, 298 K
f2 (ppm)
f1 (ppm)

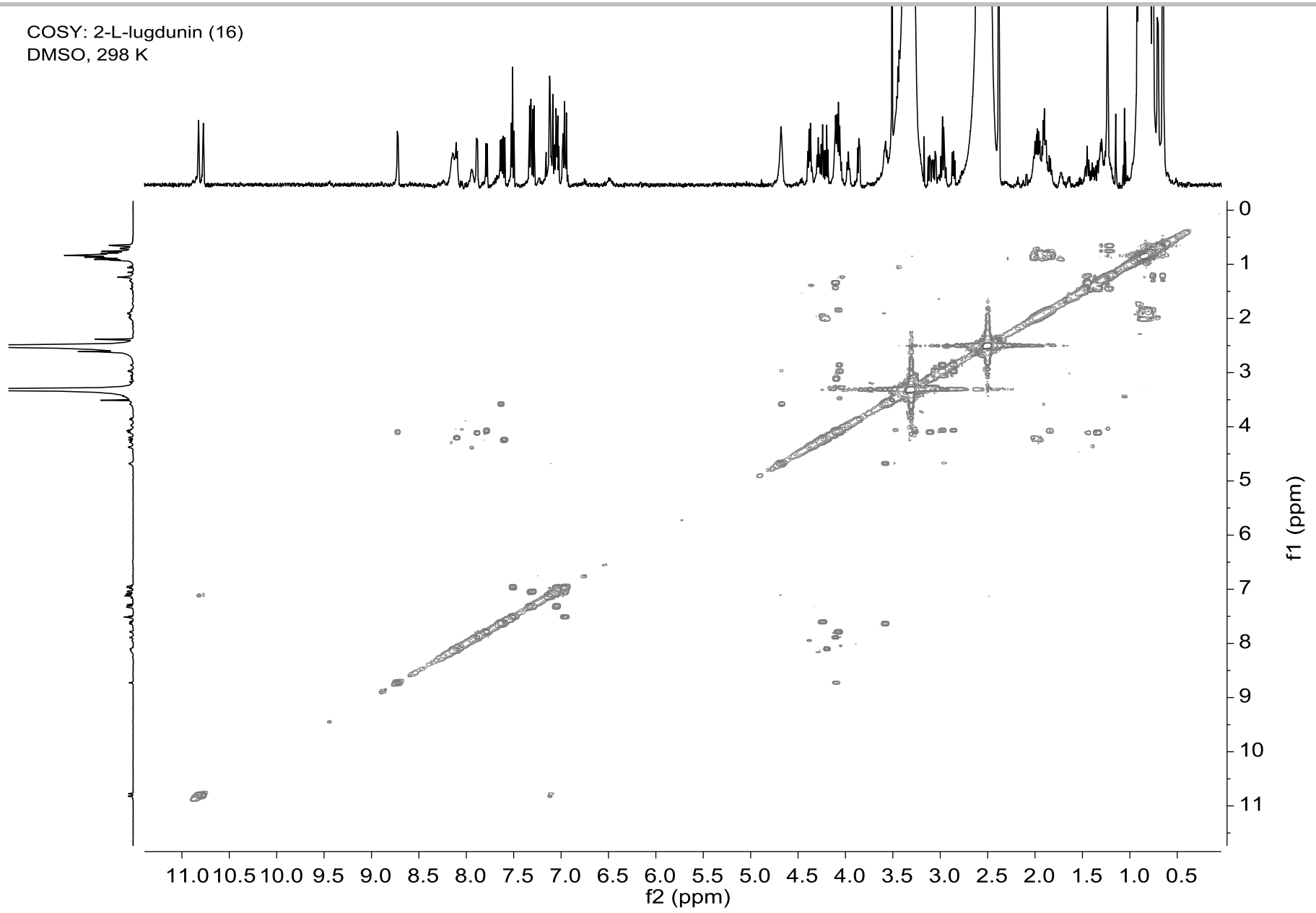

PROTON: 1-Pro homodetic lugdunin (14)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

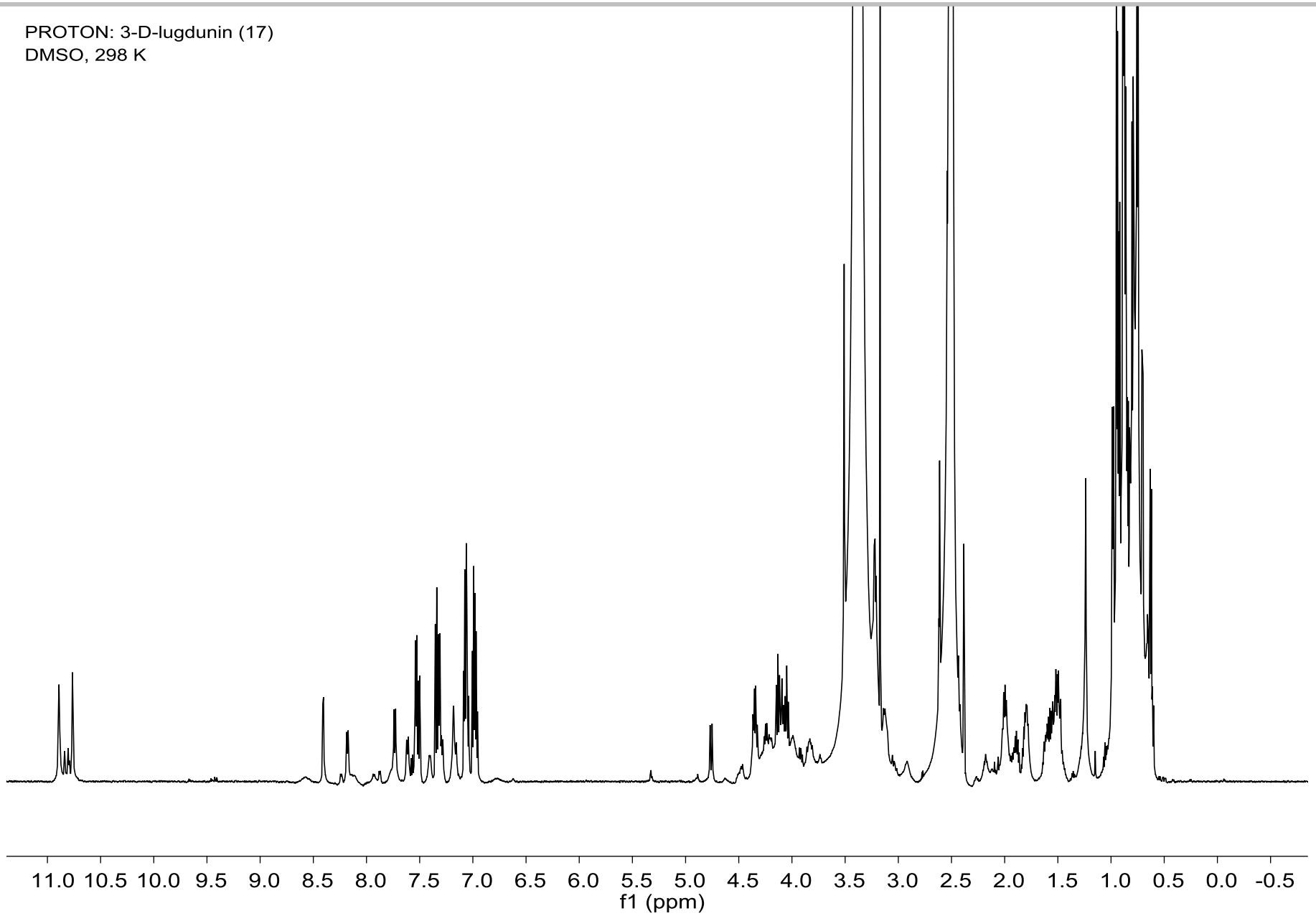

COSY: 1-Pro homodetic lugdunin (14)
DMSO, 298 K
f2 (ppm)
f1 (ppm)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
1
2
3
4
5
6
7
8
9
10
11

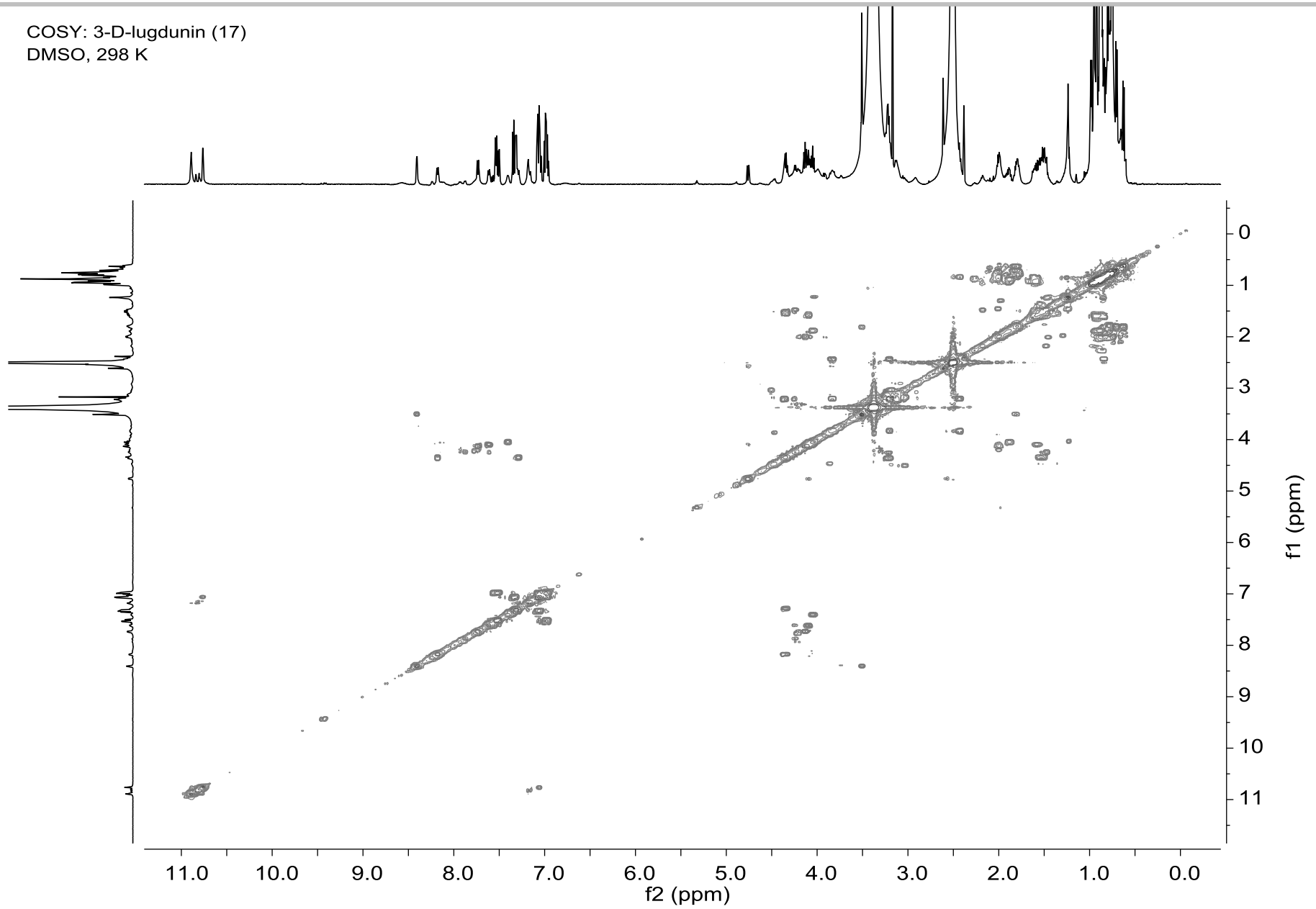

PROTON: 1-D-lugdunin (15)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

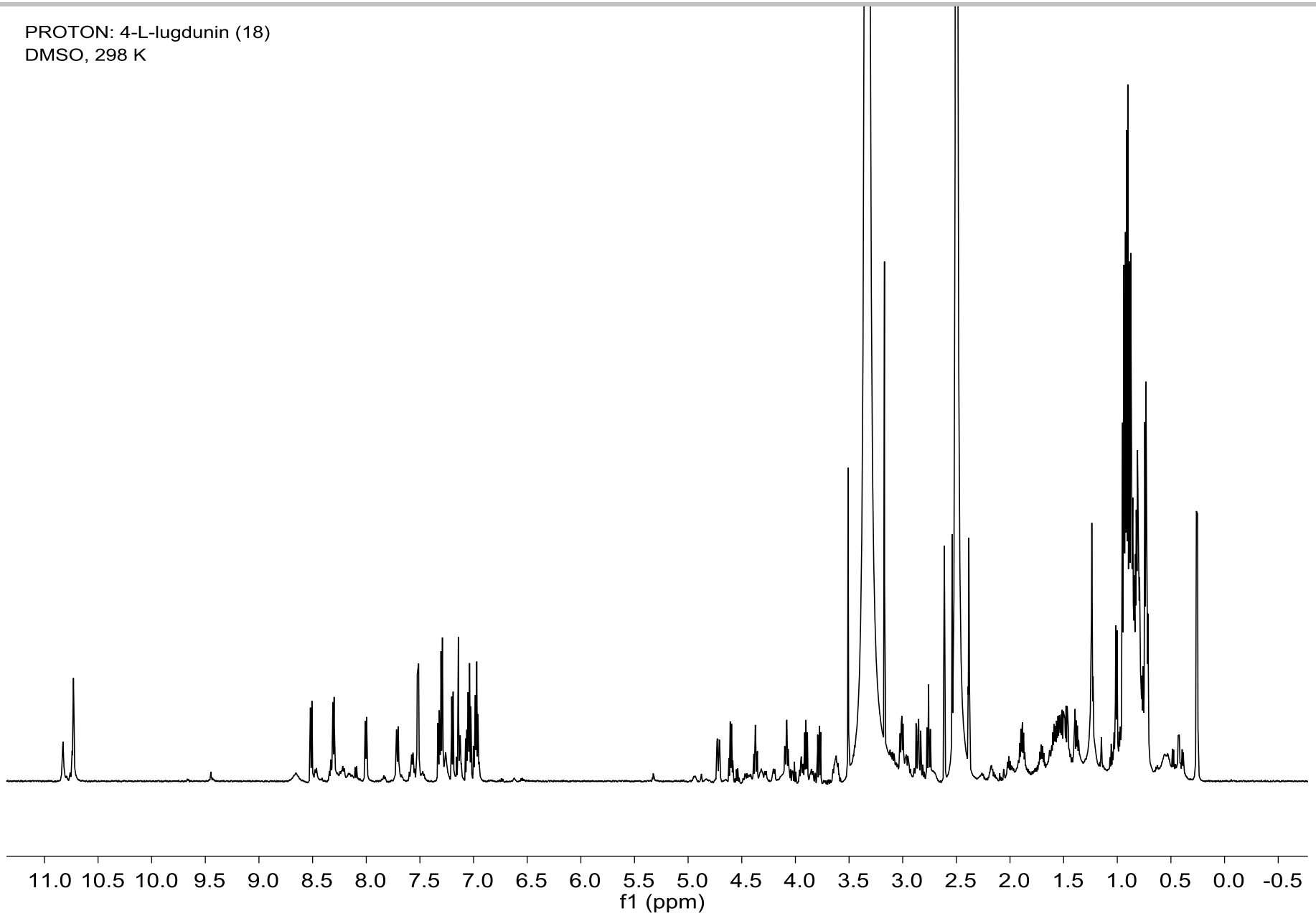

COSY: 1-D-lugdunin (15)
DMSO, 298 K
f2 (ppm)
f1 (ppm)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
1
2
3
4
5
6
7
8
9
10
11
12

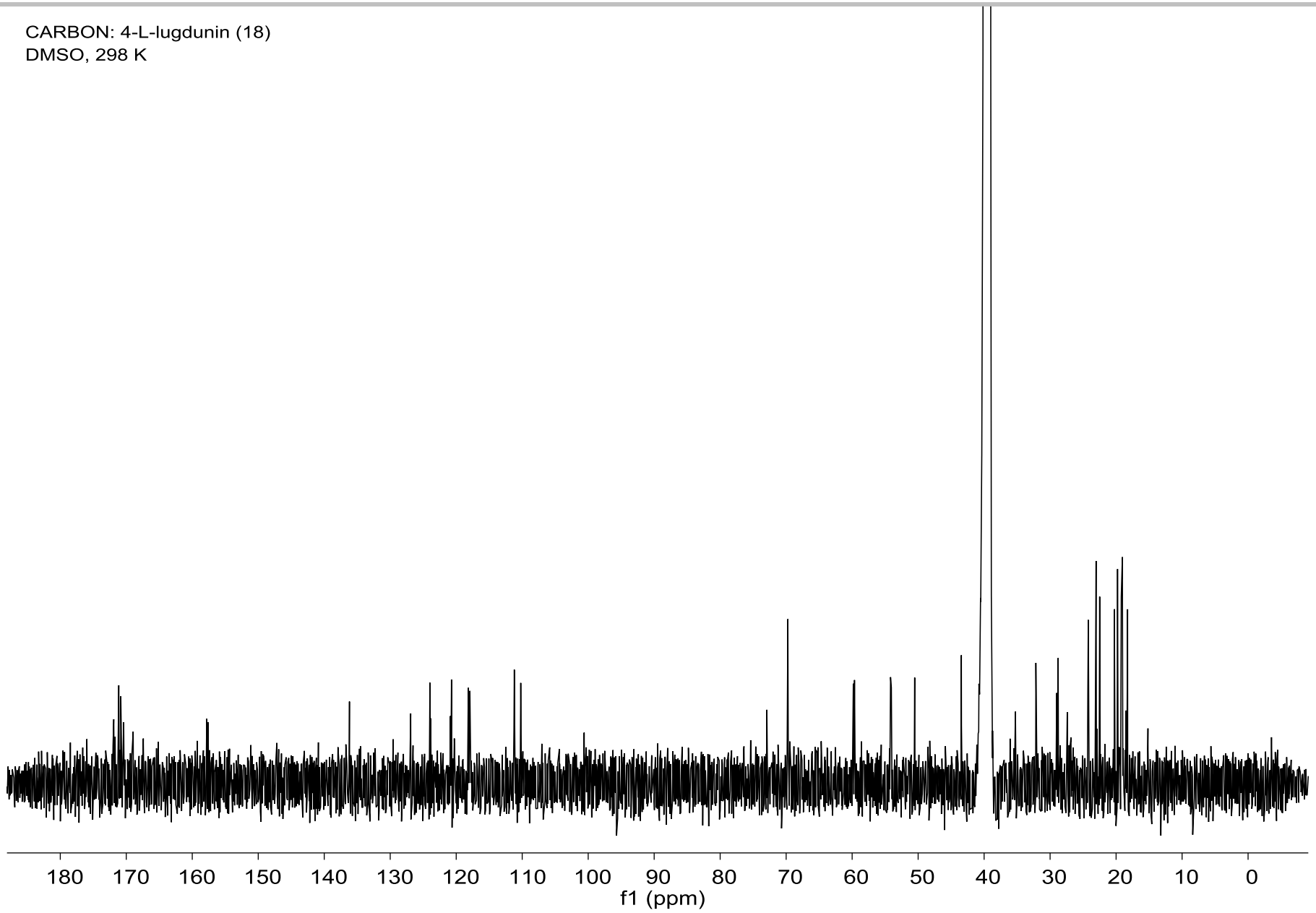

PROTON: 2-L-lugdunin (16)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

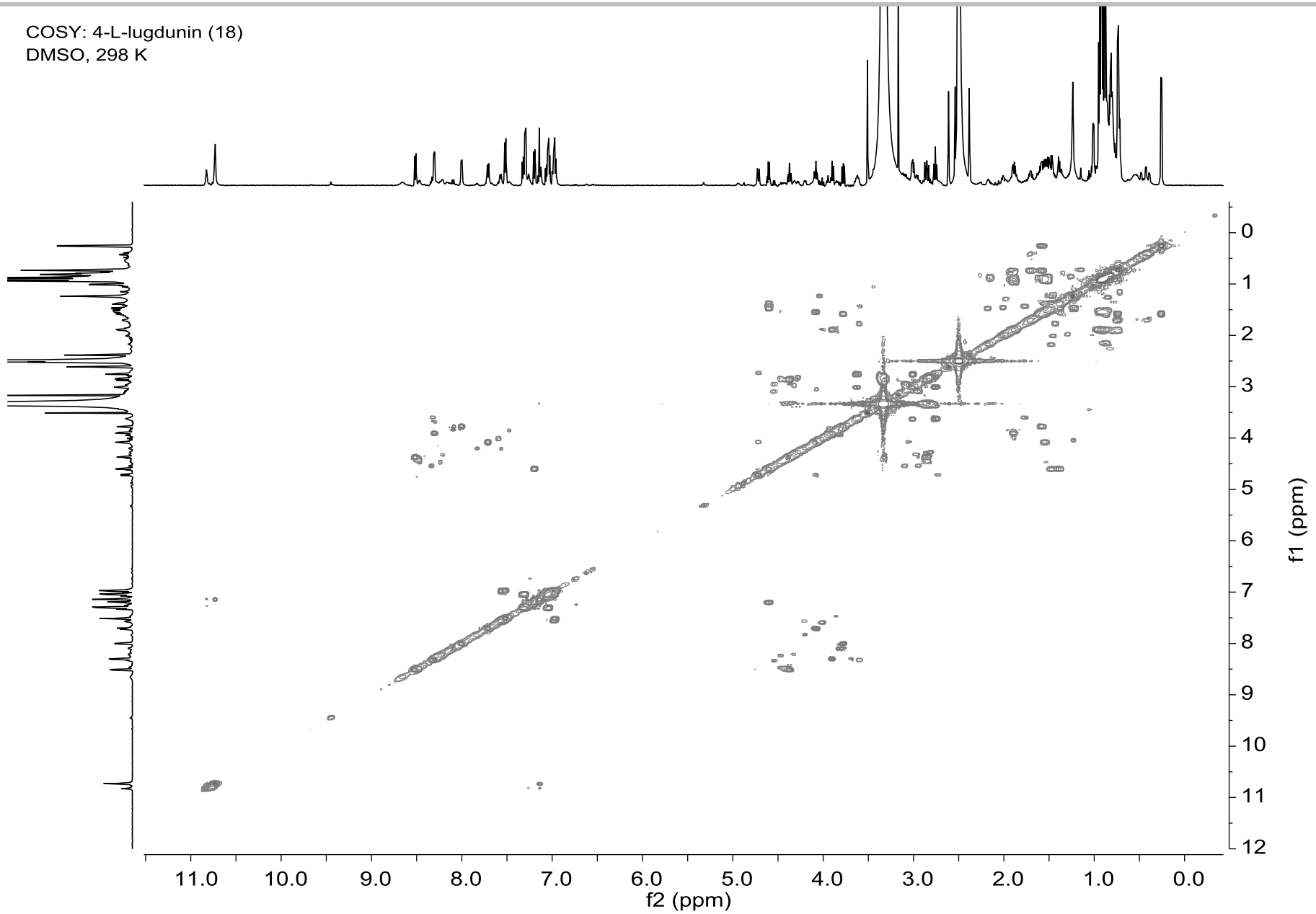

COSY: 2-L-lugdunin (16)
DMSO, 298 K
f1 (ppm)
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

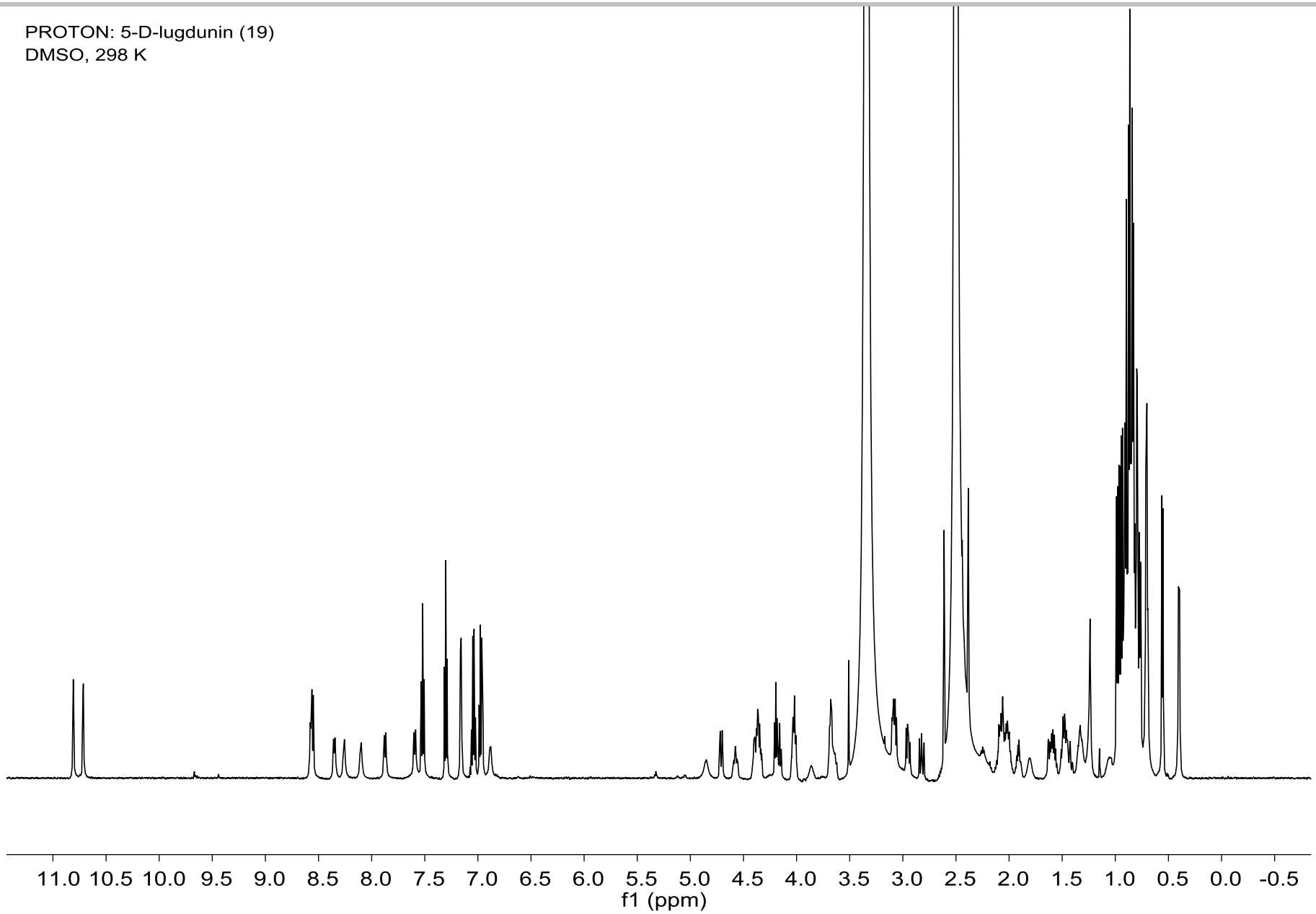

PROTON: 3-D-lugdunin (17)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

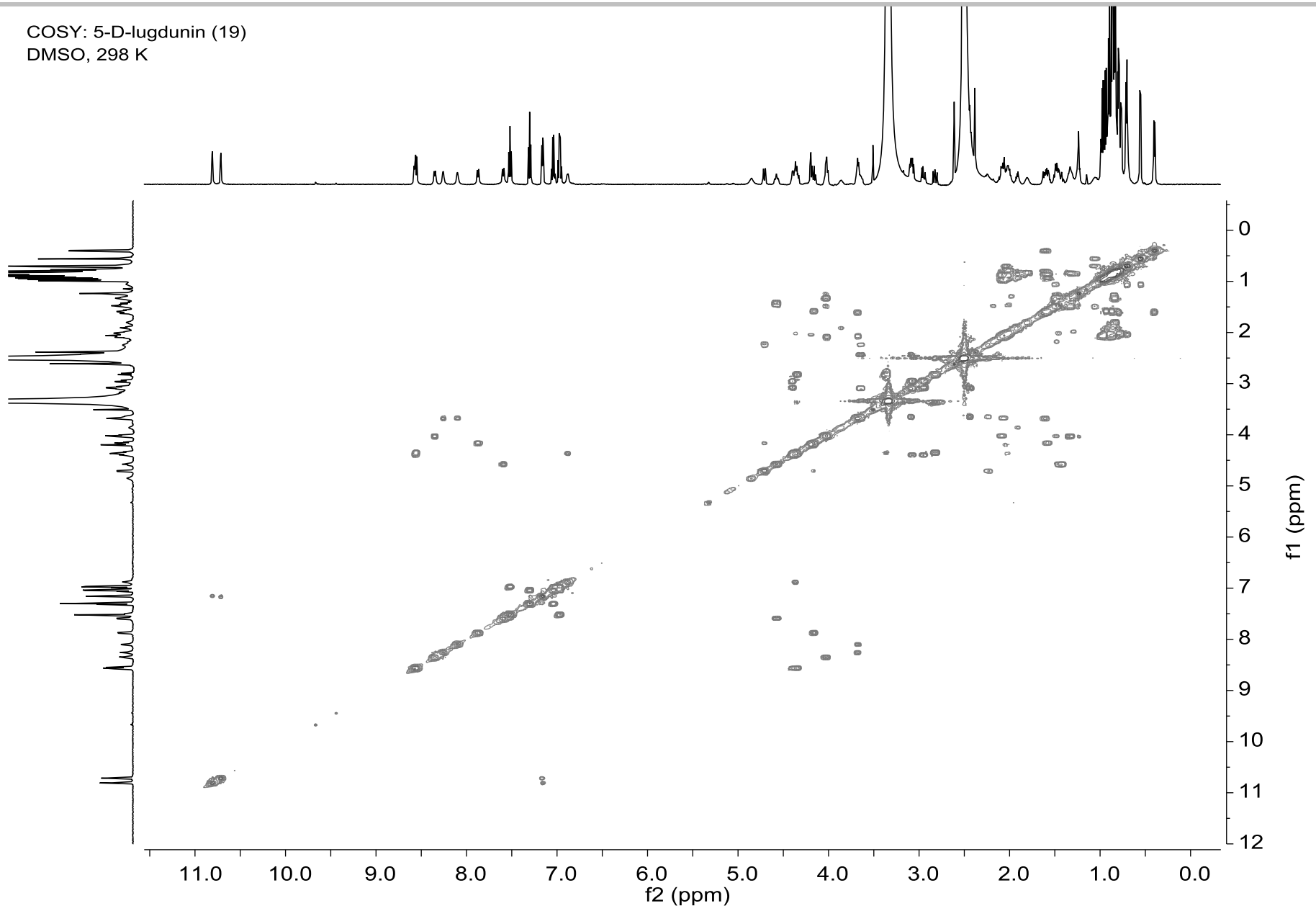

COSY: 3-D-lugdunin (17)
DMSO, 298 K
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
f1 (ppm)

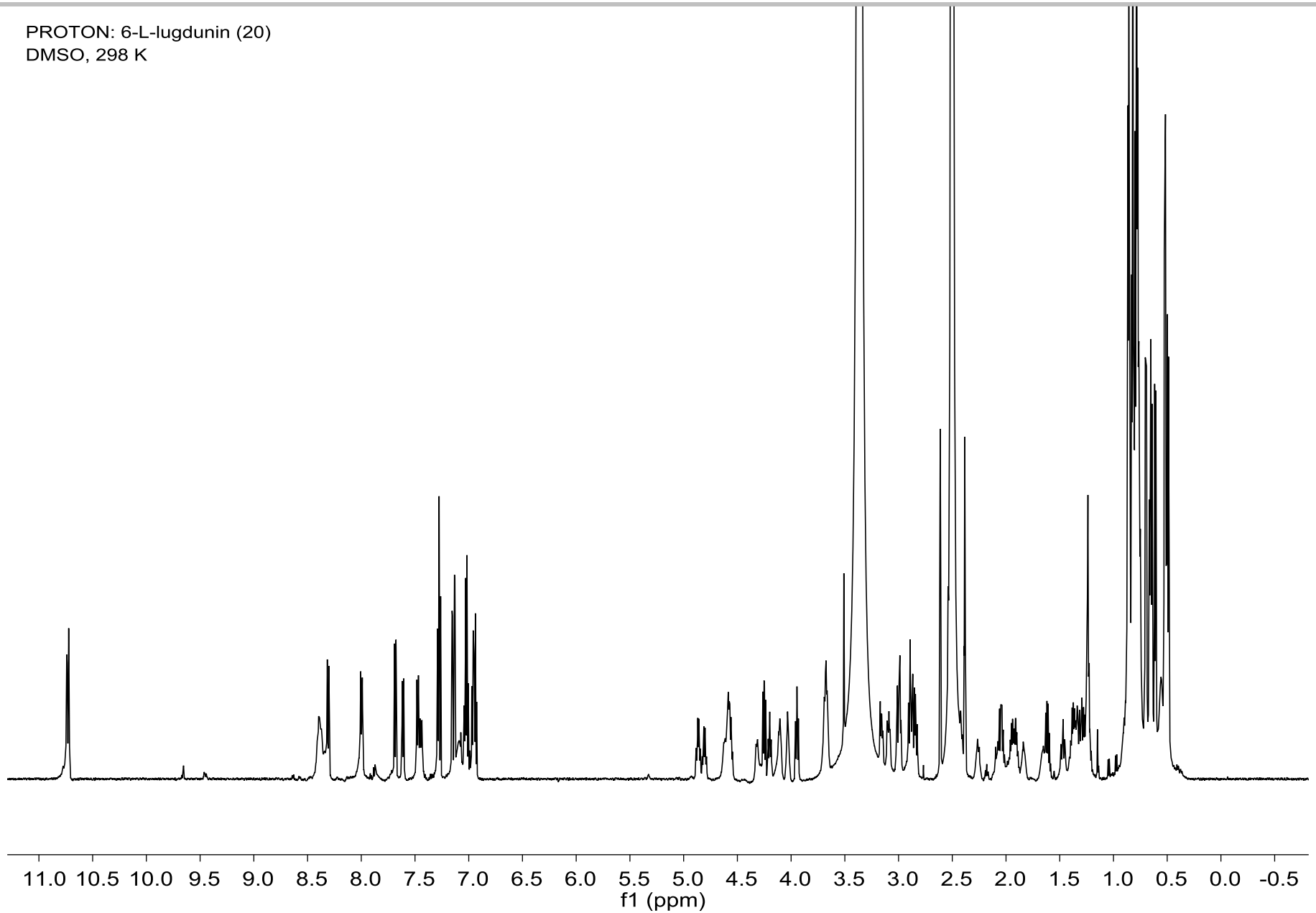

PROTON: 4-L-lugdunin (18)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

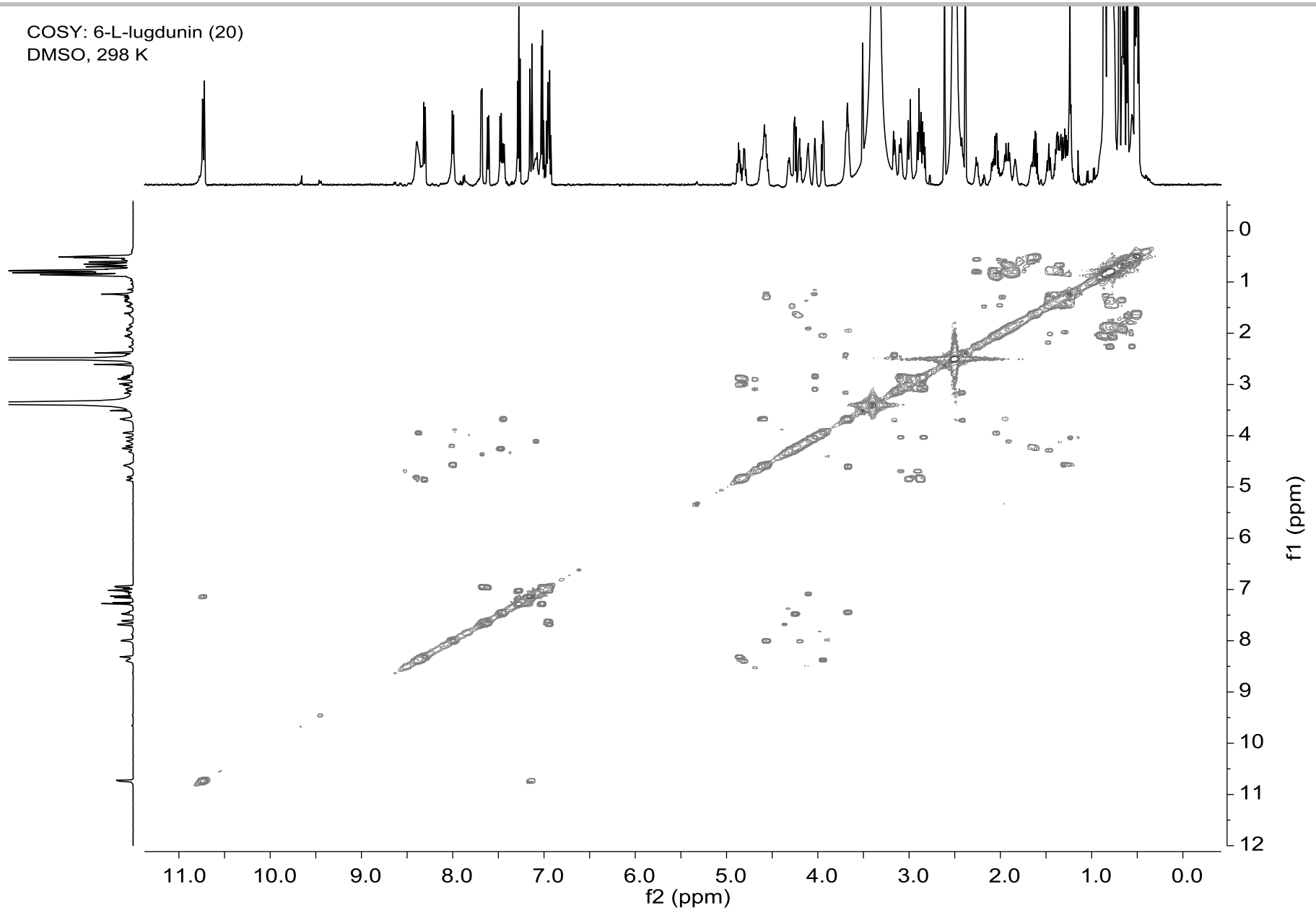

CARBON: 4-L-lugdunin (18)
DMSO, 298 K
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

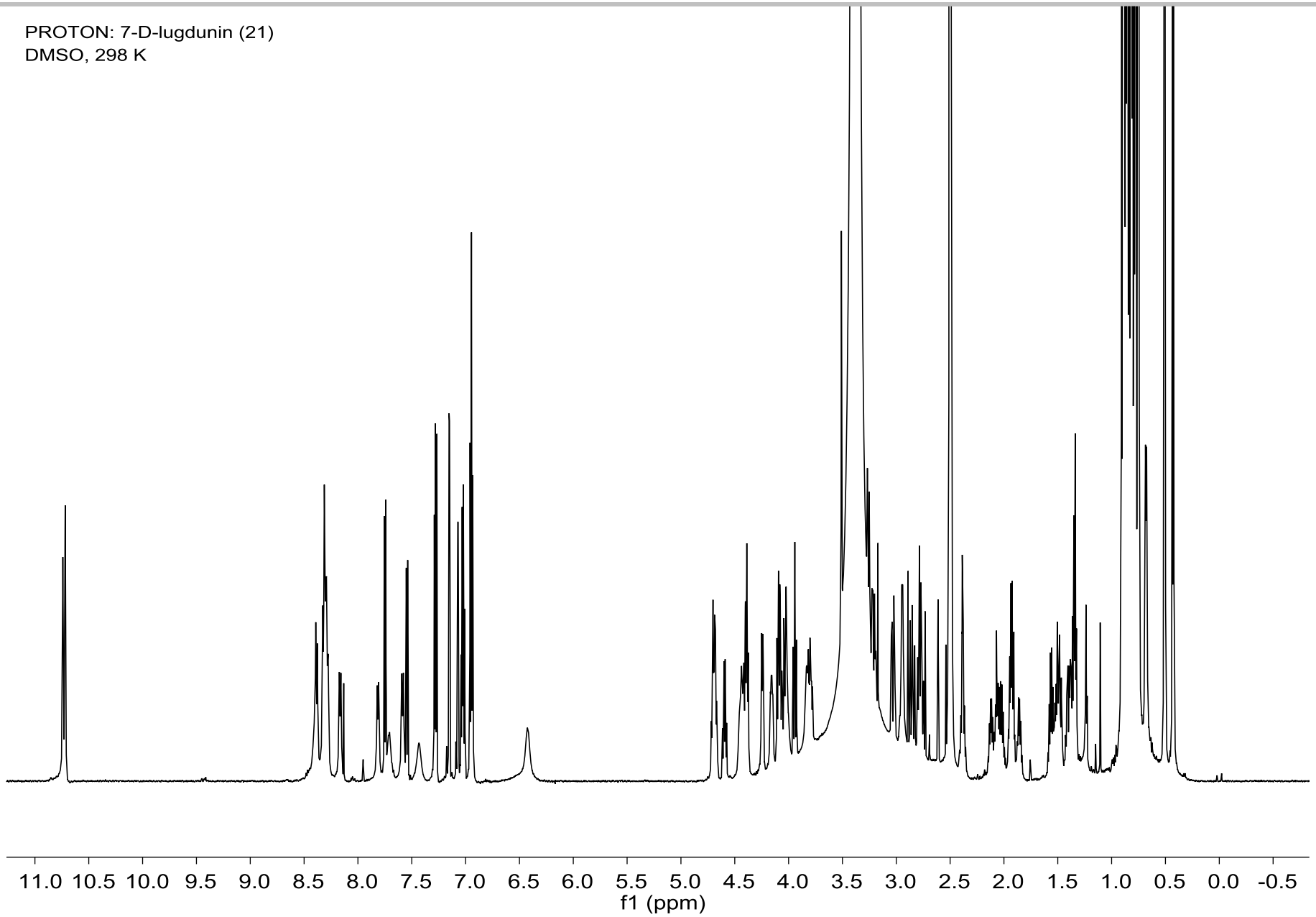

COSY: 4-L-lugdunin (18)
DMSO, 298 K
f2 (ppm)
f1 (ppm)

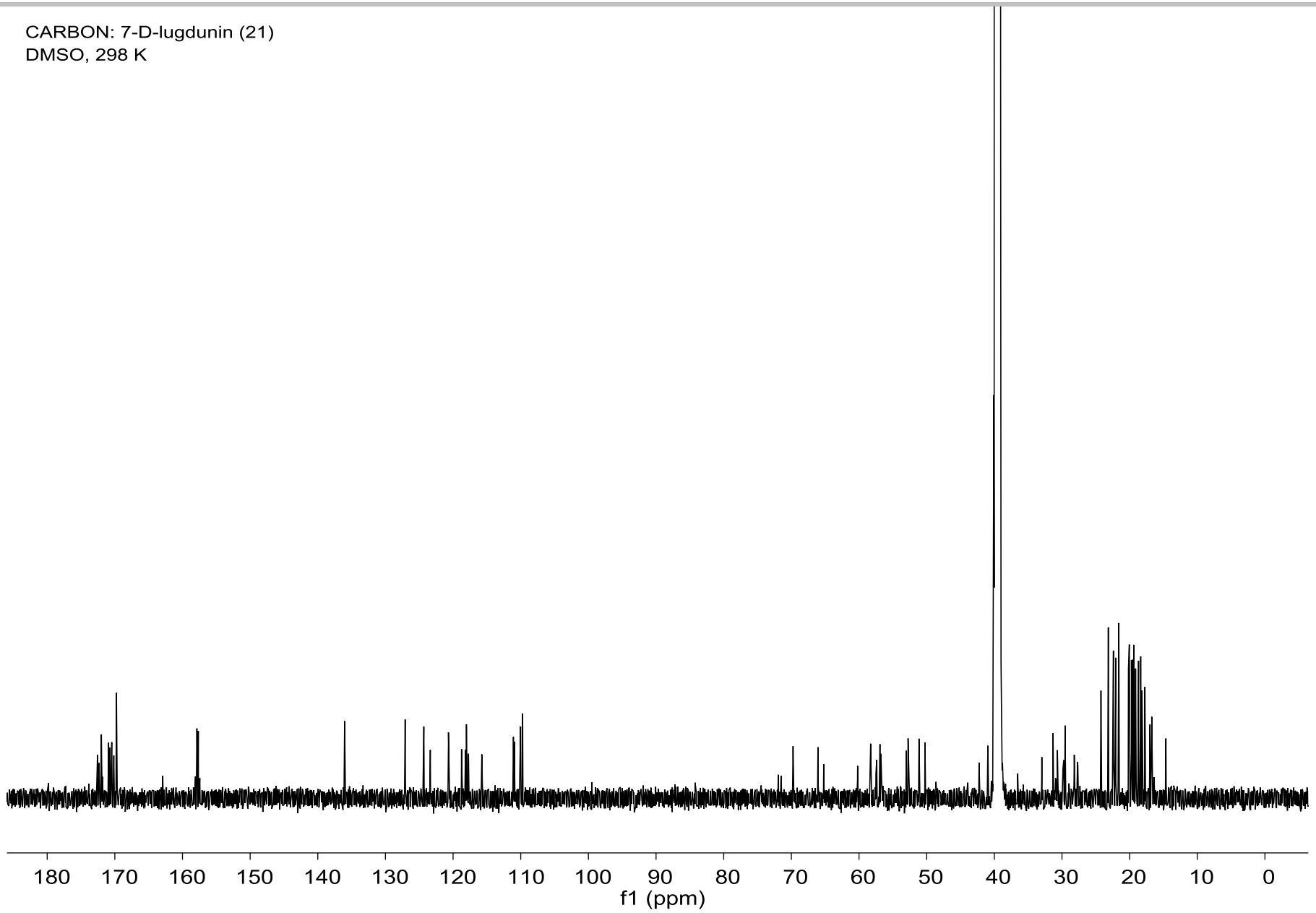

PROTON: 5-D-lugdunin (19)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

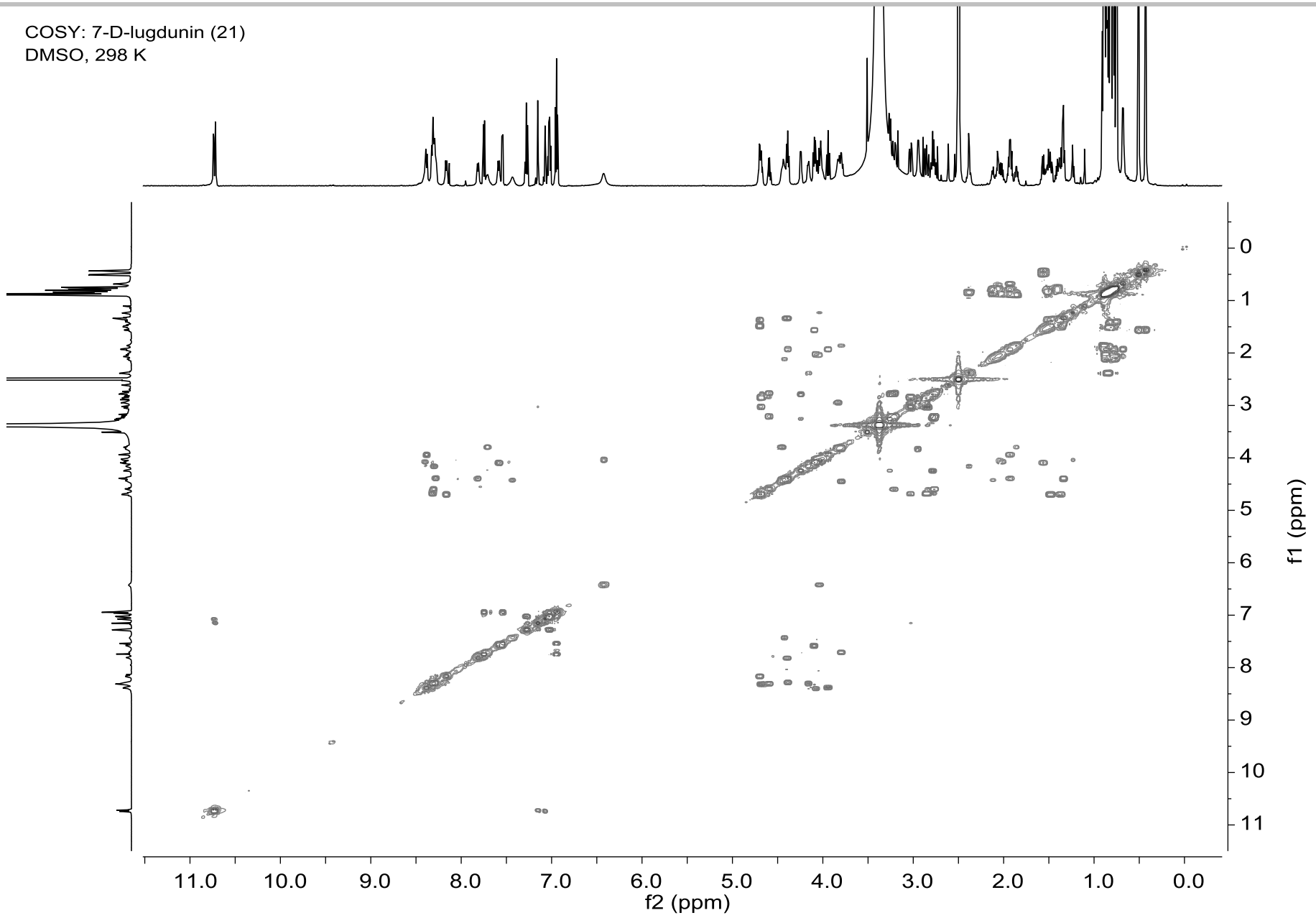

COSY: 5-D-lugdunin (19)
DMSO, 298 K
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
12
f1 (ppm)

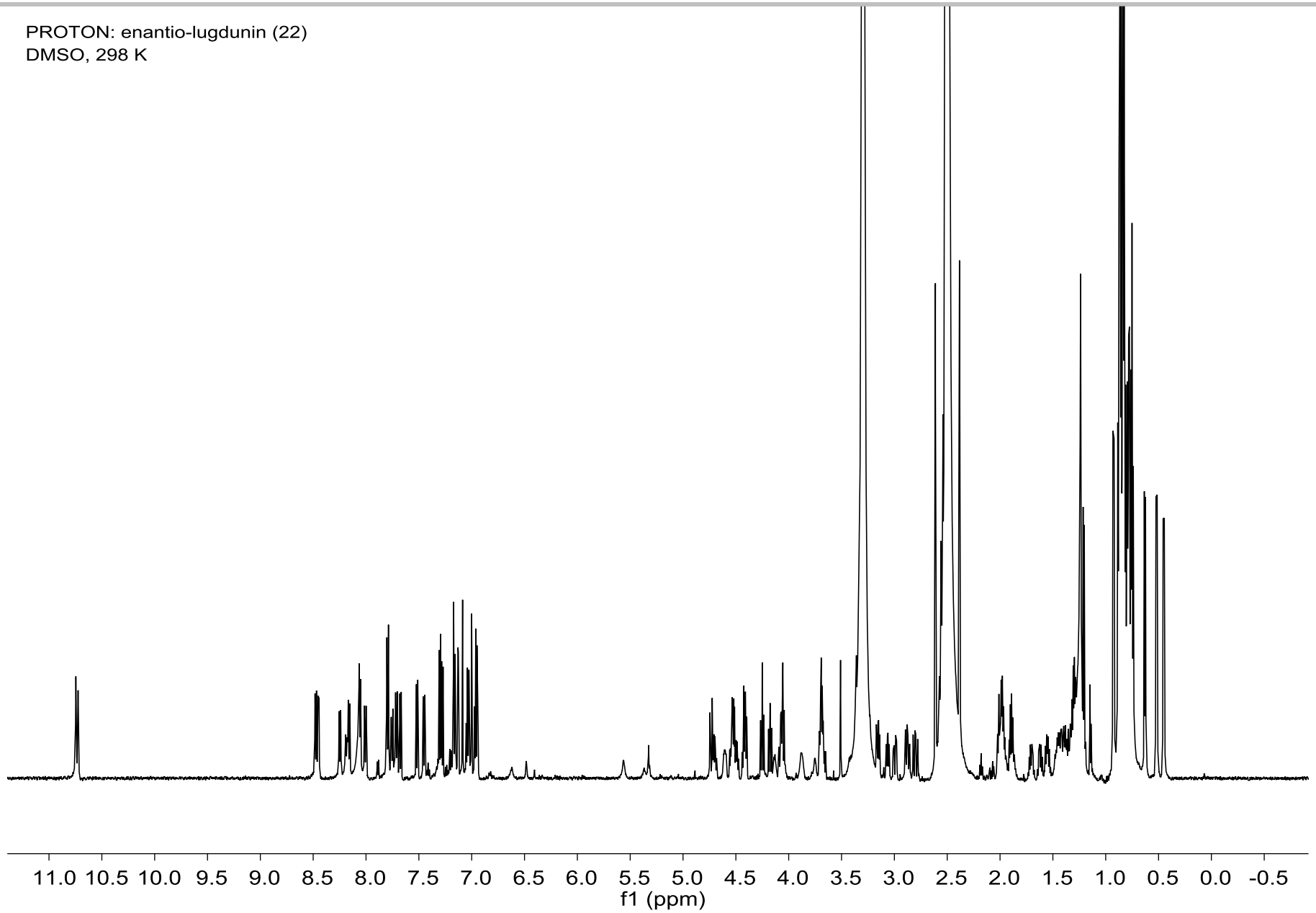

PROTON: 6-L-lugdunin (20)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

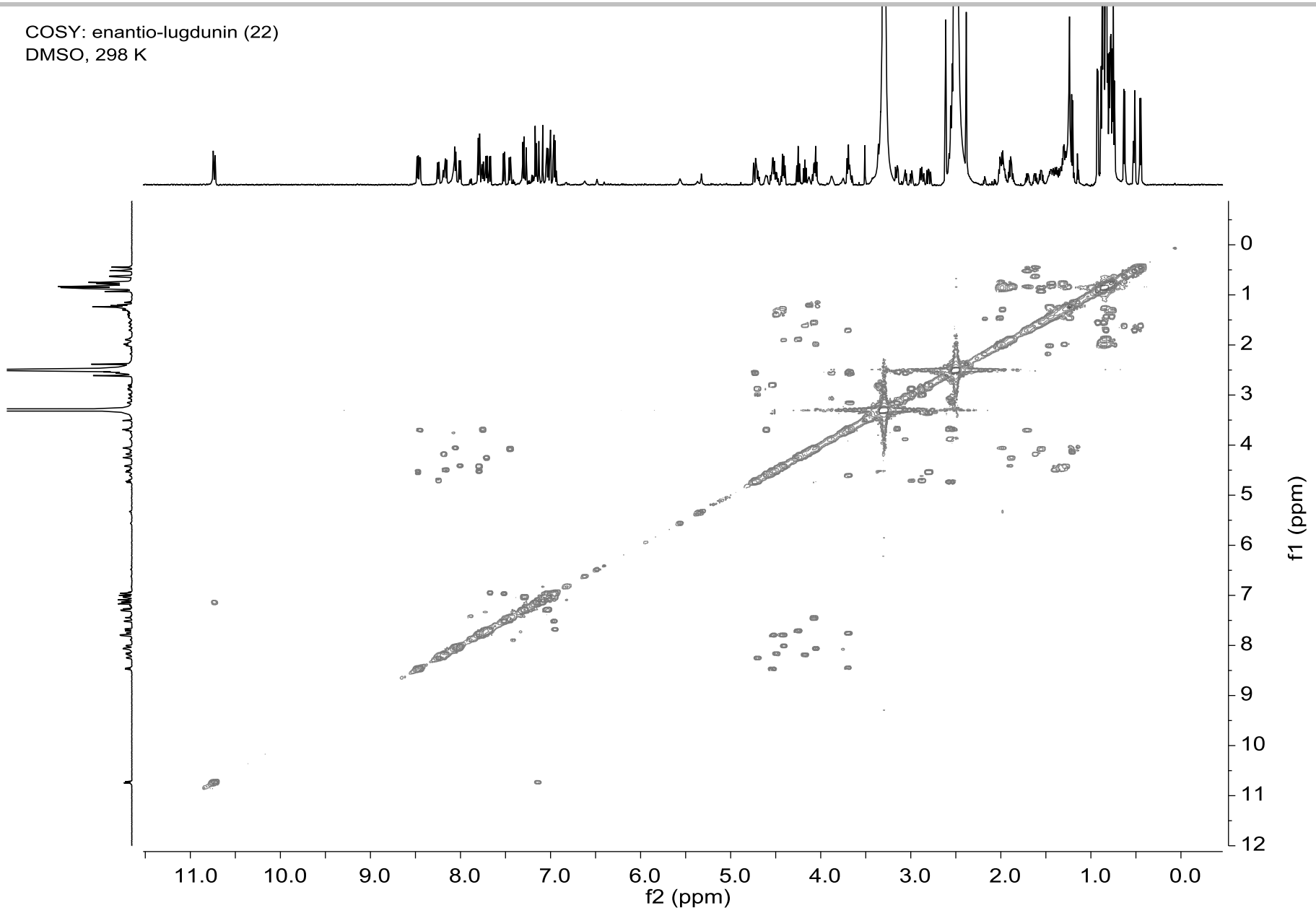

COSY: 6-L-lugdunin (20)
DMSO, 298 K
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
12
f1 (ppm)

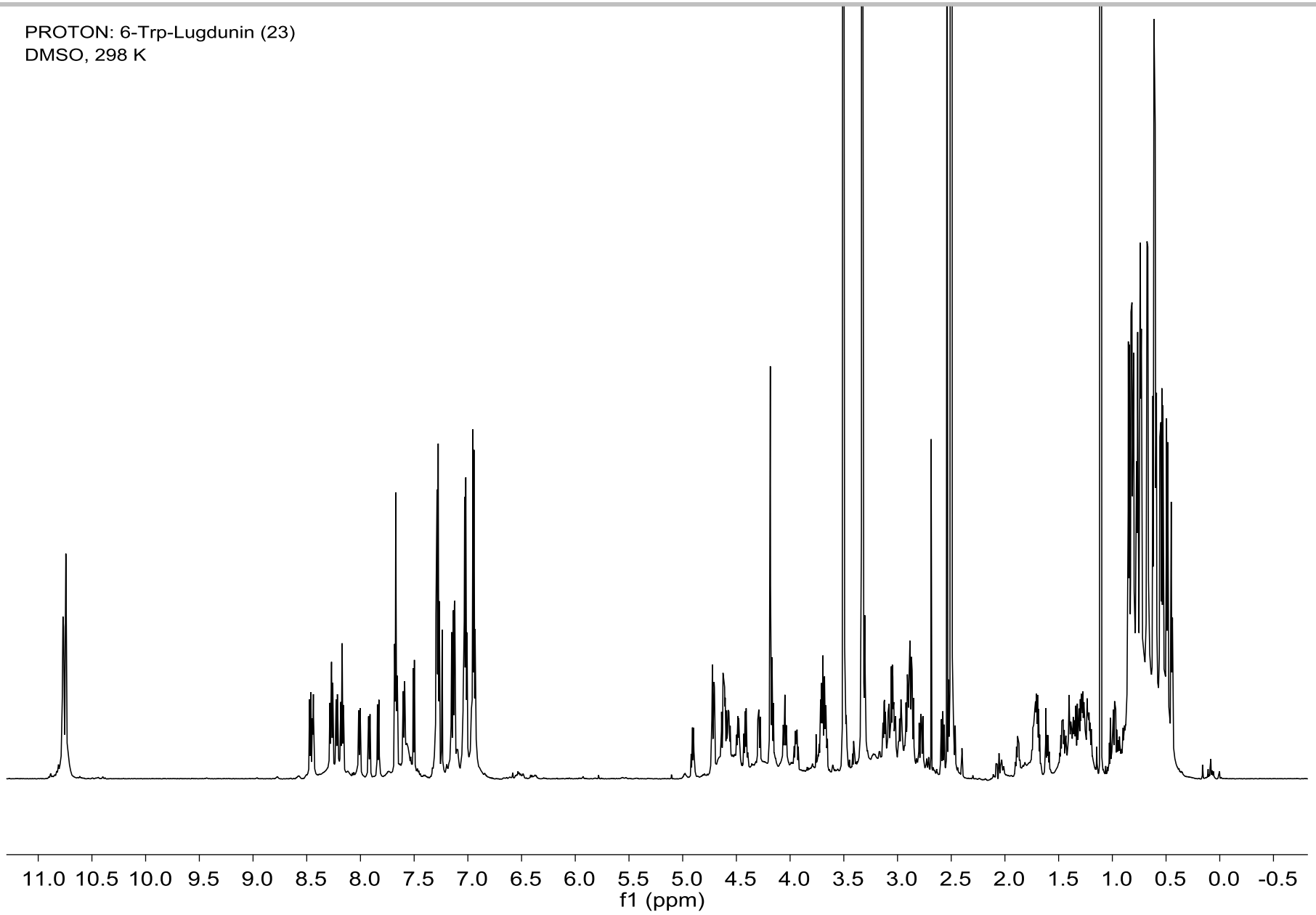

PROTON: 7-D-lugdunin (21)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

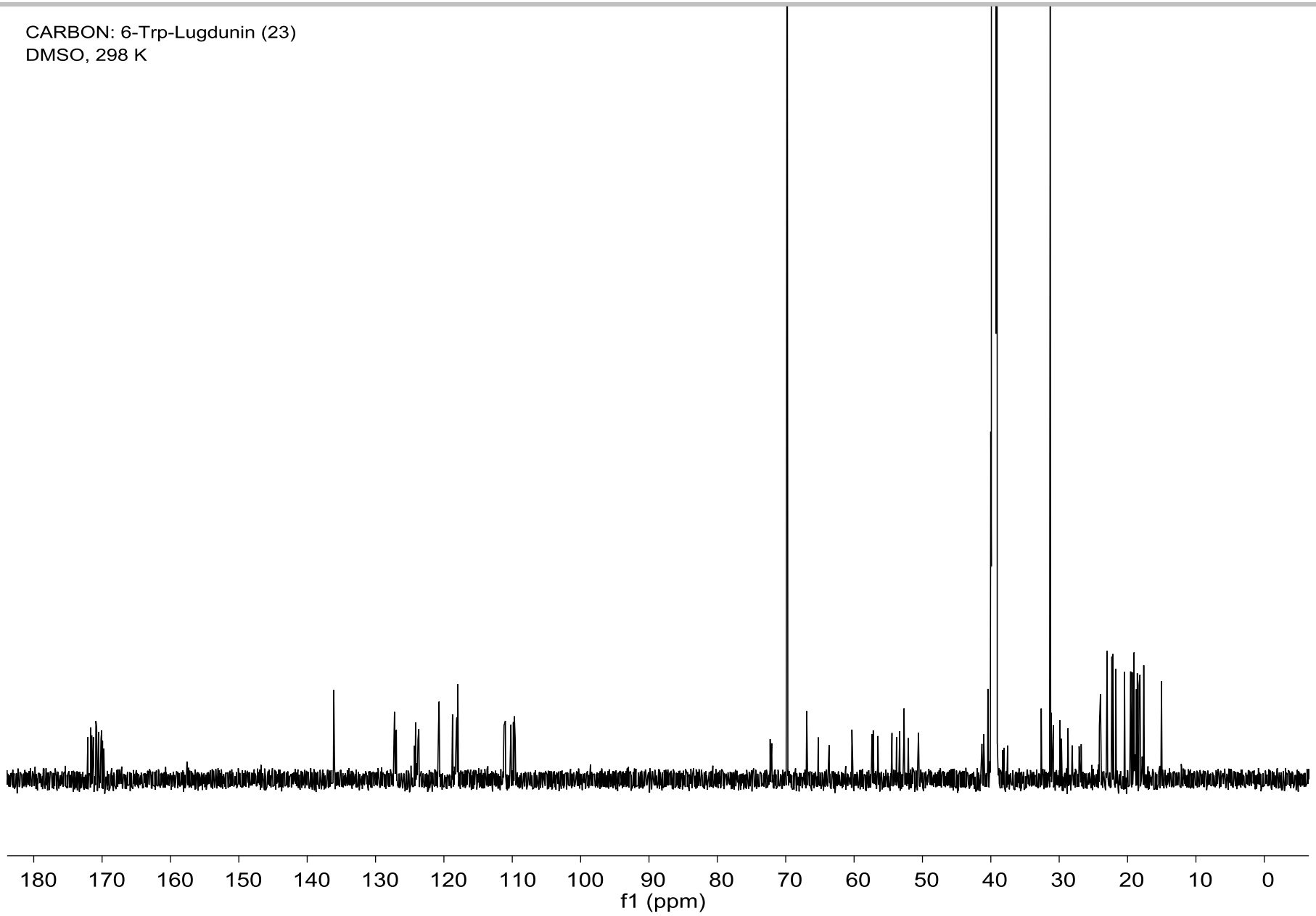

CARBON: 7-D-lugdunin (21)
DMSO, 298 K
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


COSY: 7-D-lugdunin (21)
DMSO, 298 K
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
0
1
2
3
4
5
6
7
8
9
10
11
f1 (ppm)

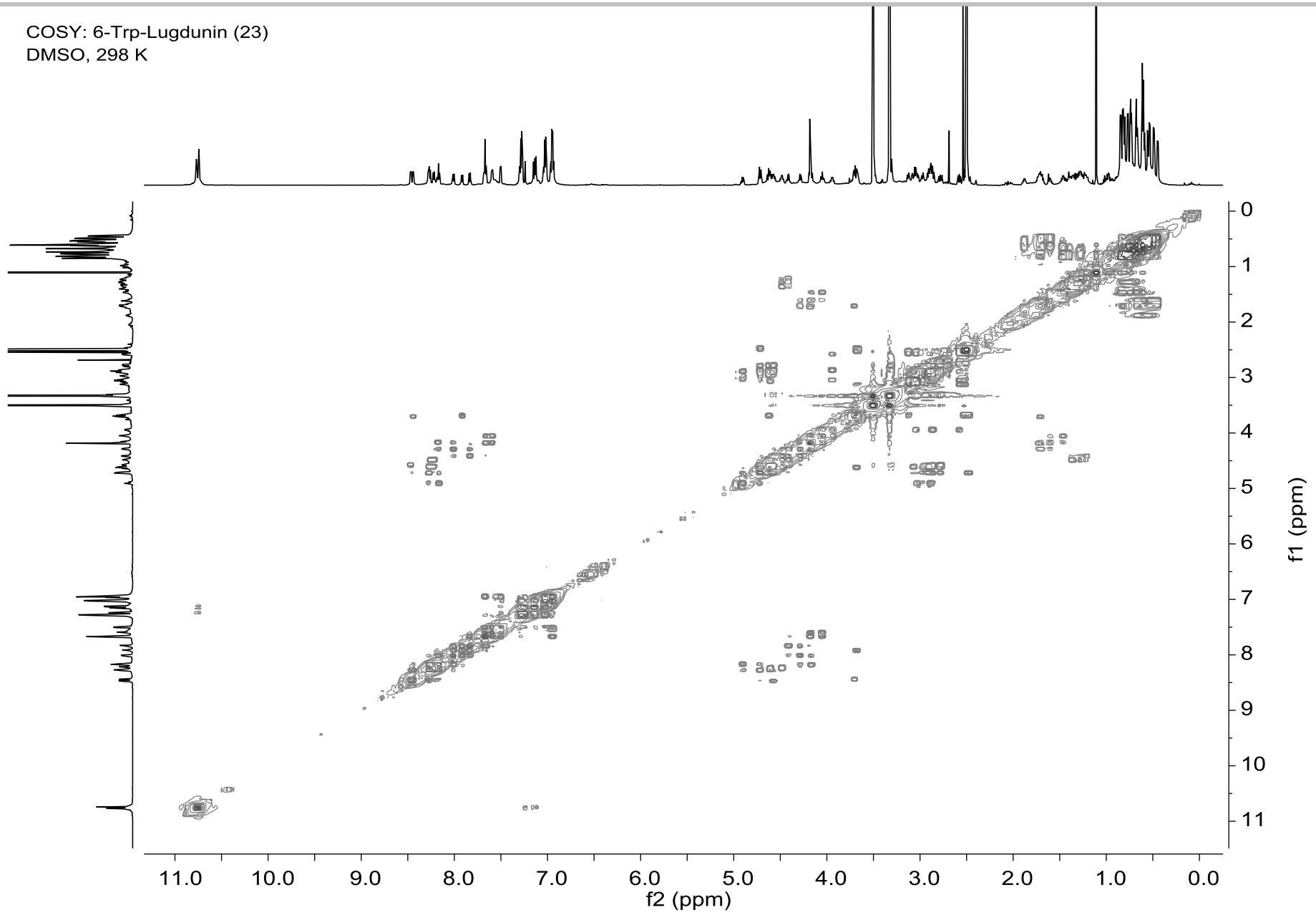

PROTON: enantio-lugdunin (22)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

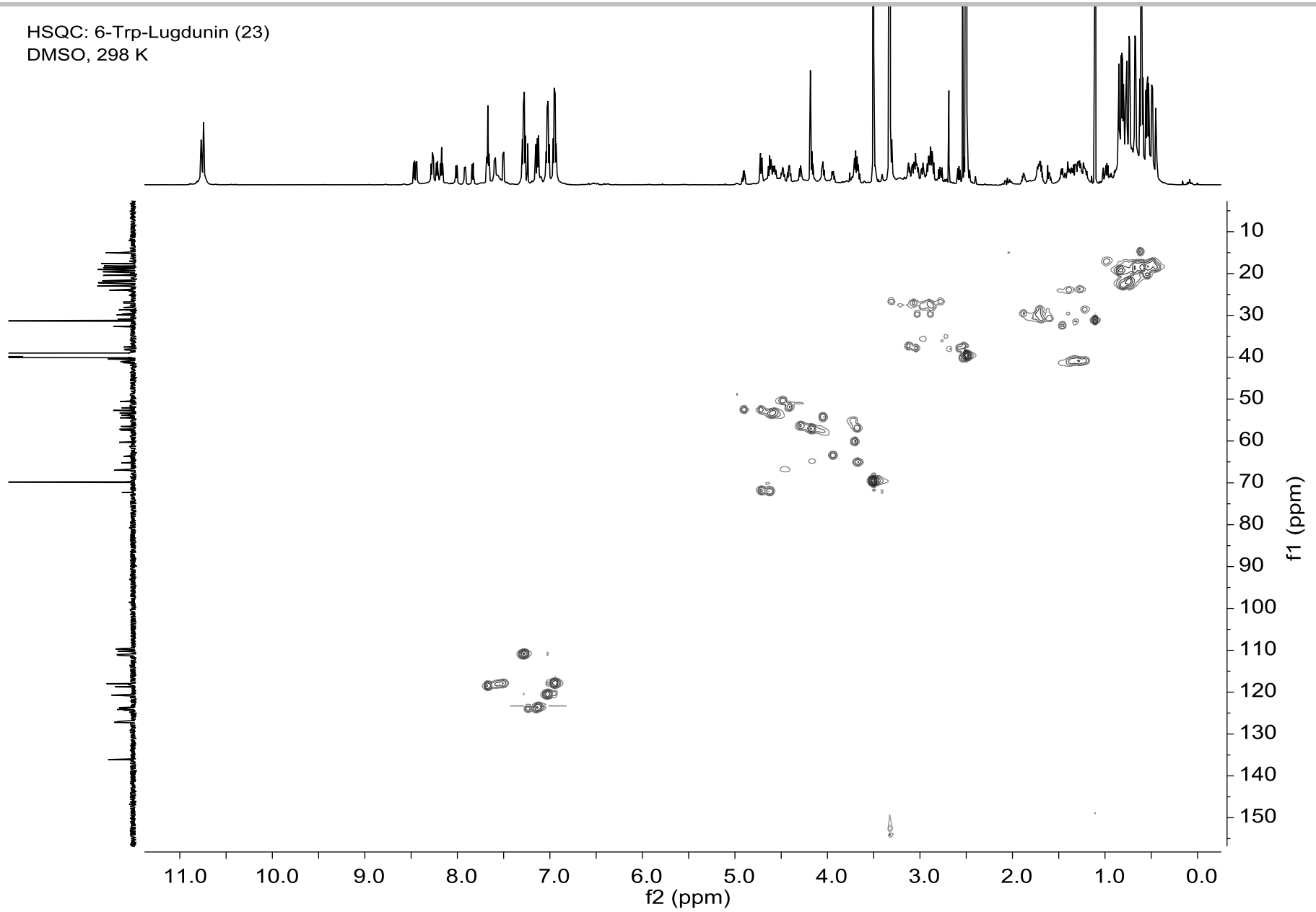

COSY: enantio-lugdunin (22)
DMSO, 298 K
f2 (ppm)
f1 (ppm)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
1
2
3
4
5
6
7
8
9
10
11
12

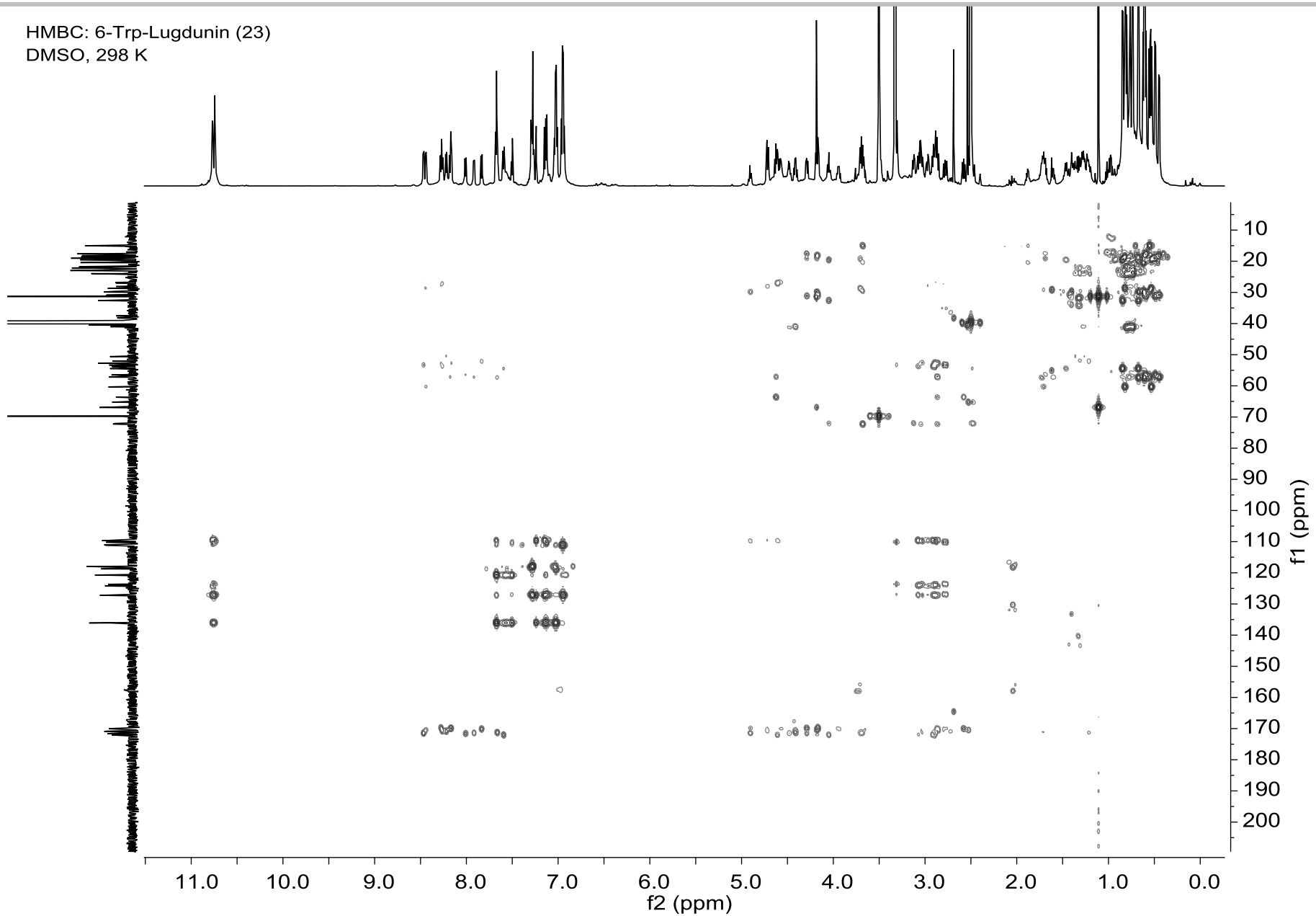

PROTON: 6-Trp-Lugdunin (23)
DMSO, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

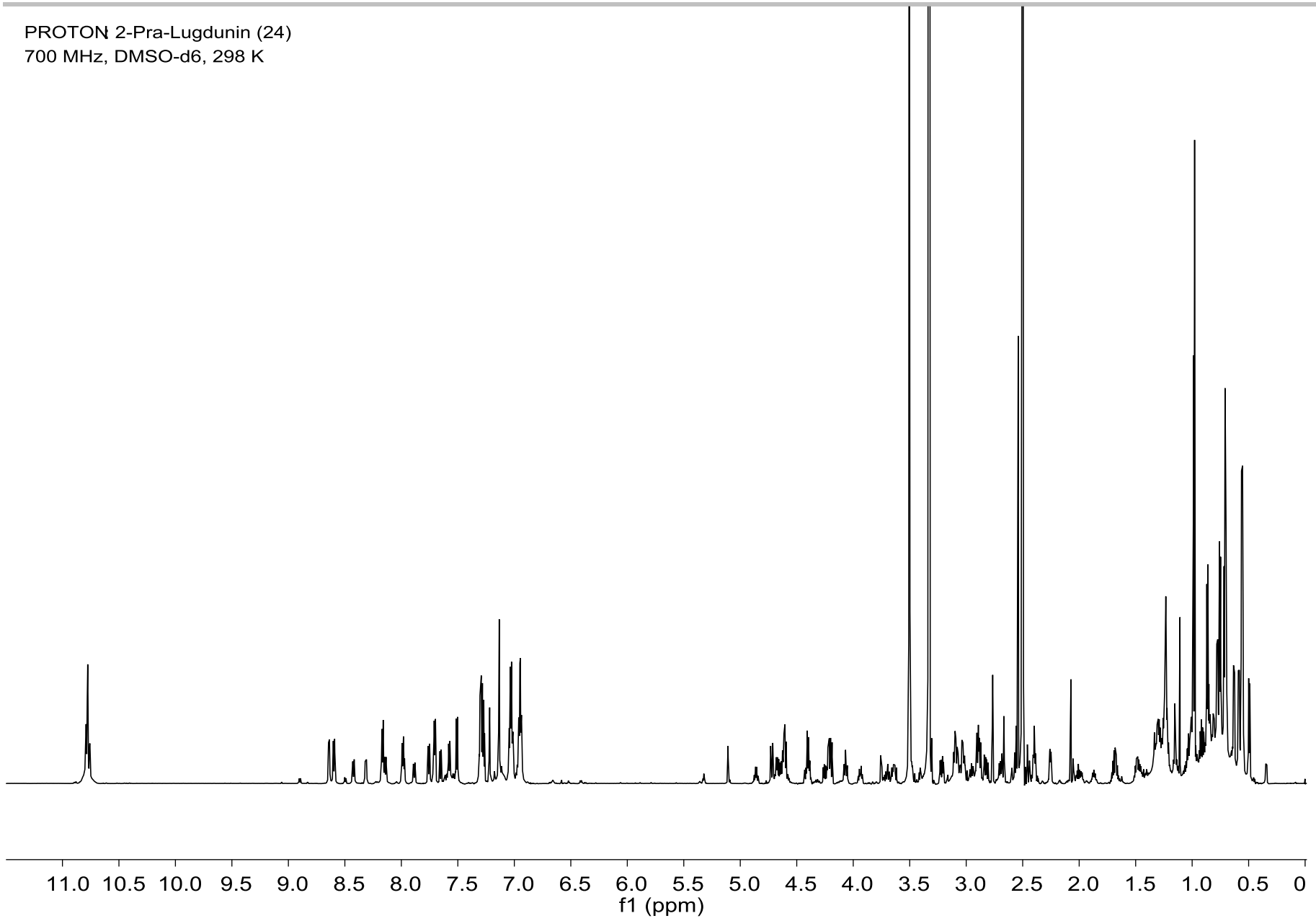

CARBON: 6-Trp-Lugdunin (23)
DMSO, 298 K
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

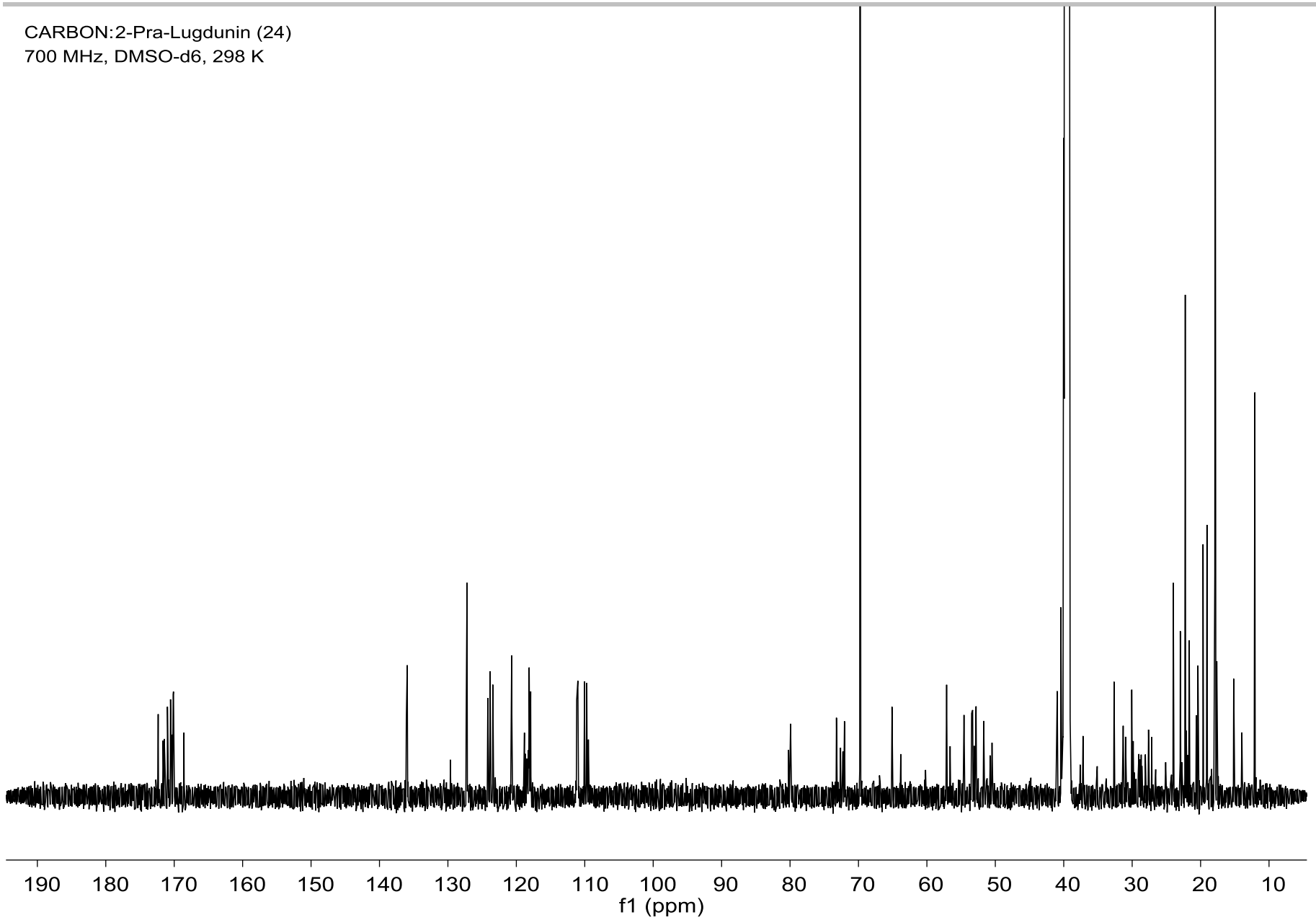
COSY: 6-Trp-Lugdunin (23)
DMSO, 298 K

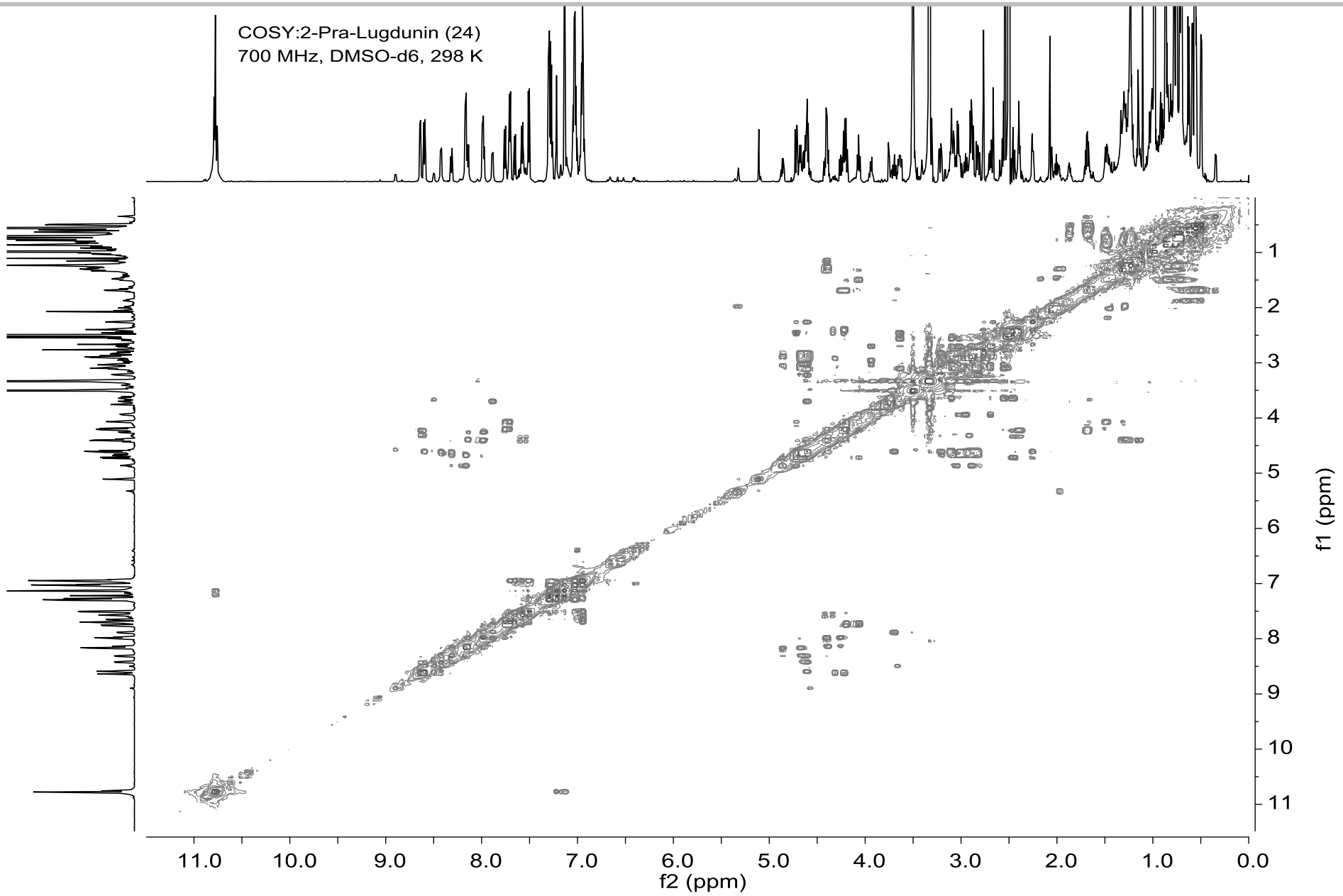


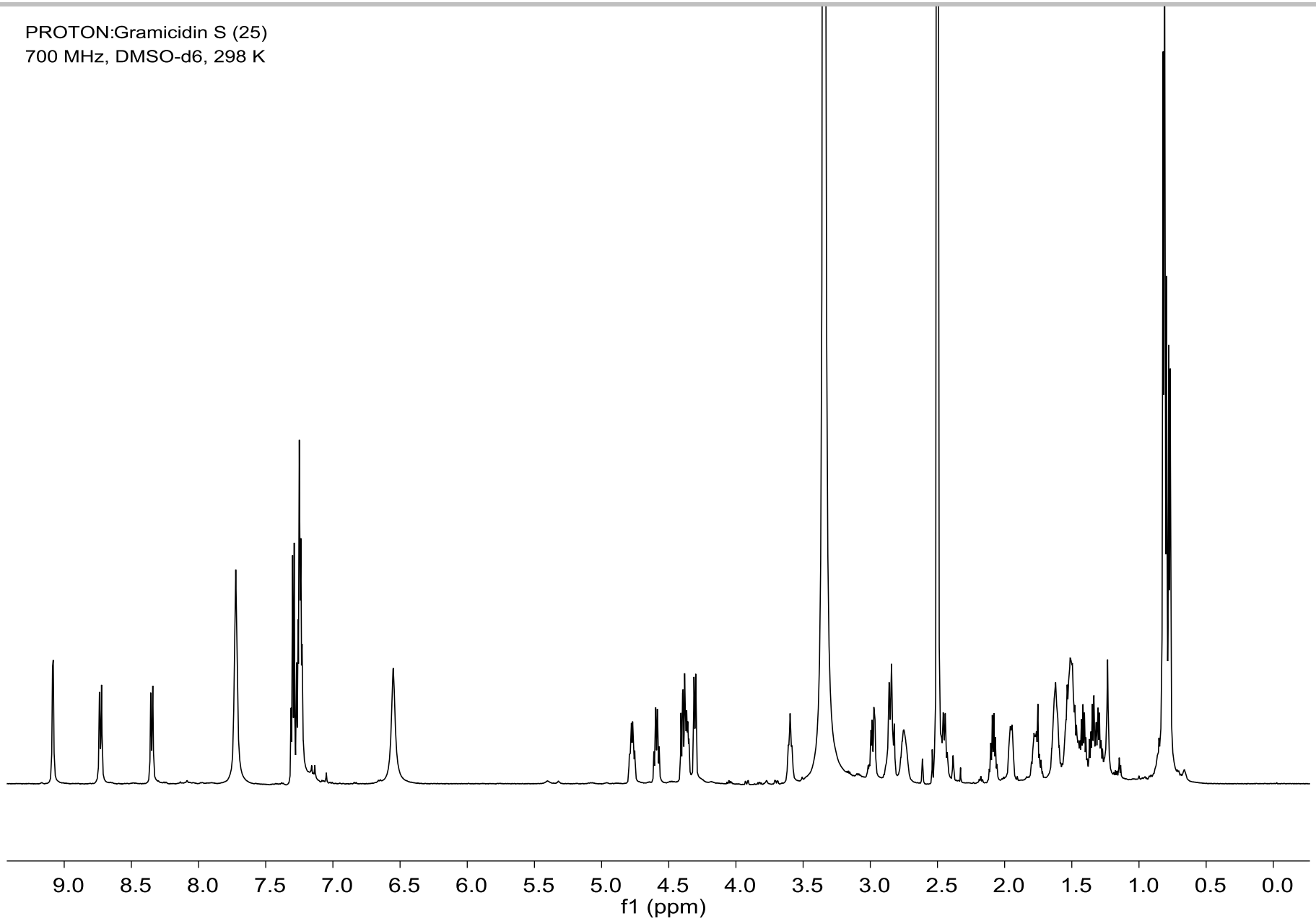

HSQC: 6-Trp-Lugdunin (23)
DMSO, 298 K
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)

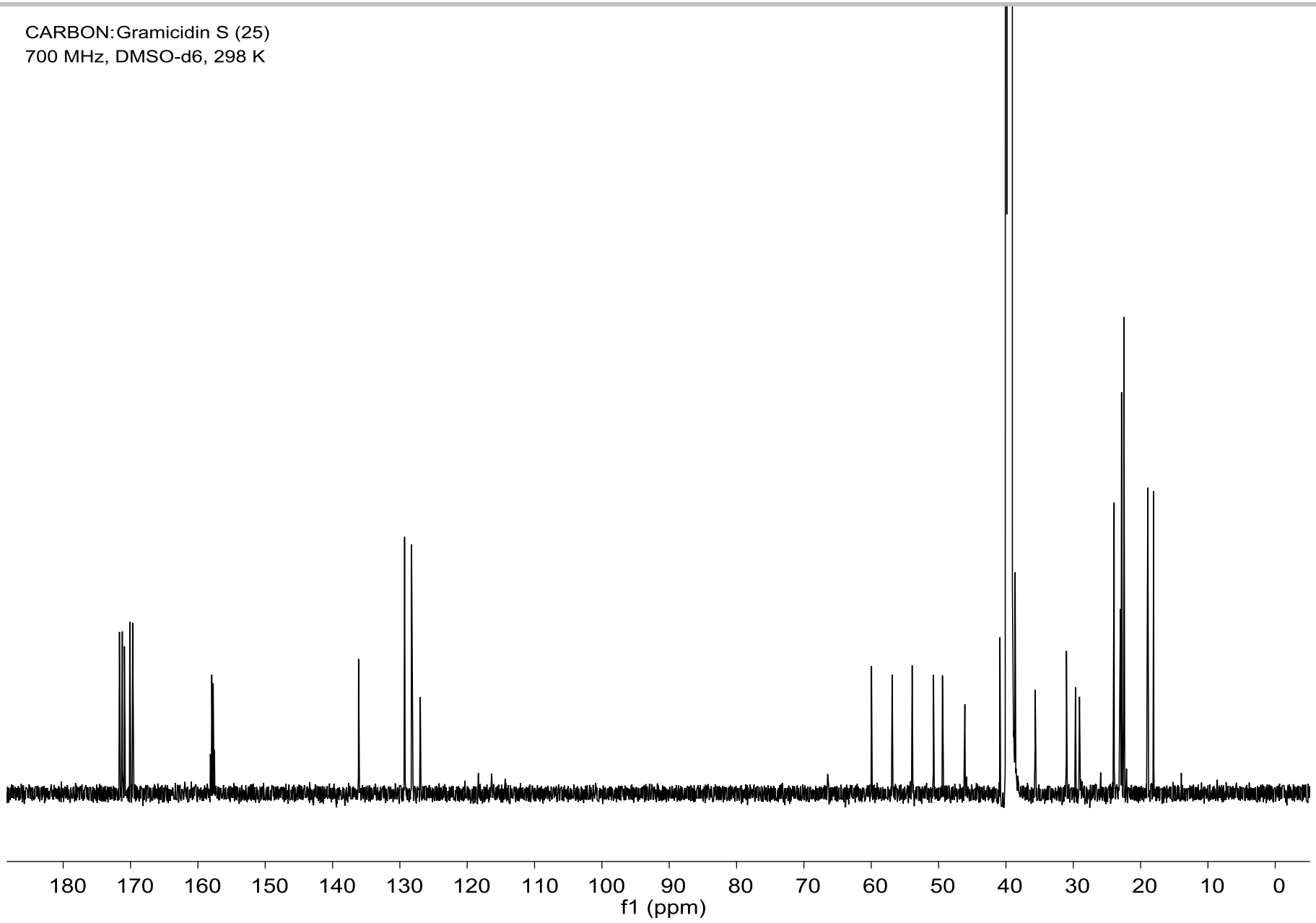

HMBC: 6-Trp-Lugdunin (23)
DMSO, 298 K
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
f1 (ppm)

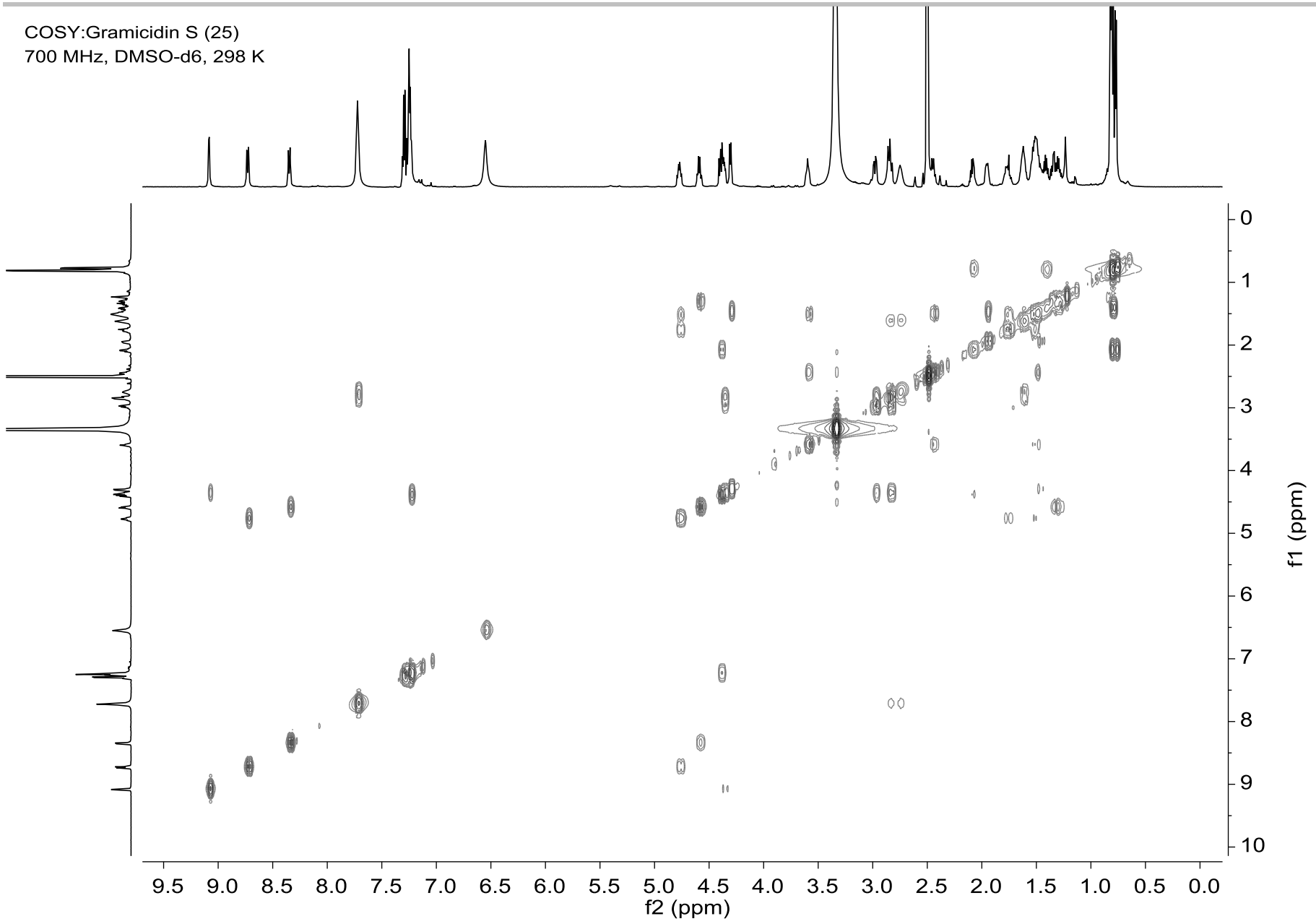

PROTON 2-Pra-Lugdunin (24)
700 MHz, DMSO-d6, 298 K
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)

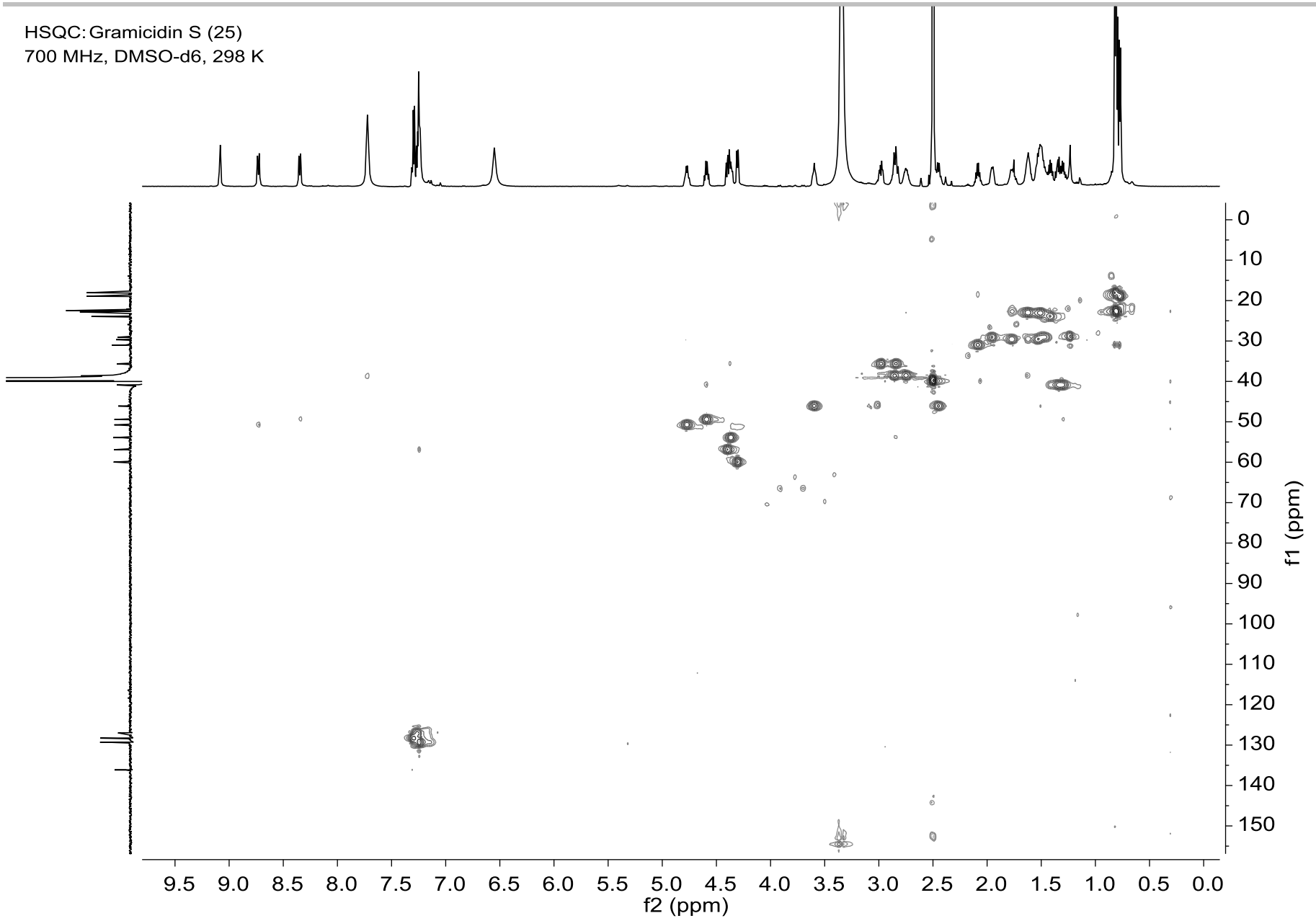

CARBON:2-Pra-Lugdunin (24)
700 MHz, DMSO-d6, 298 K
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

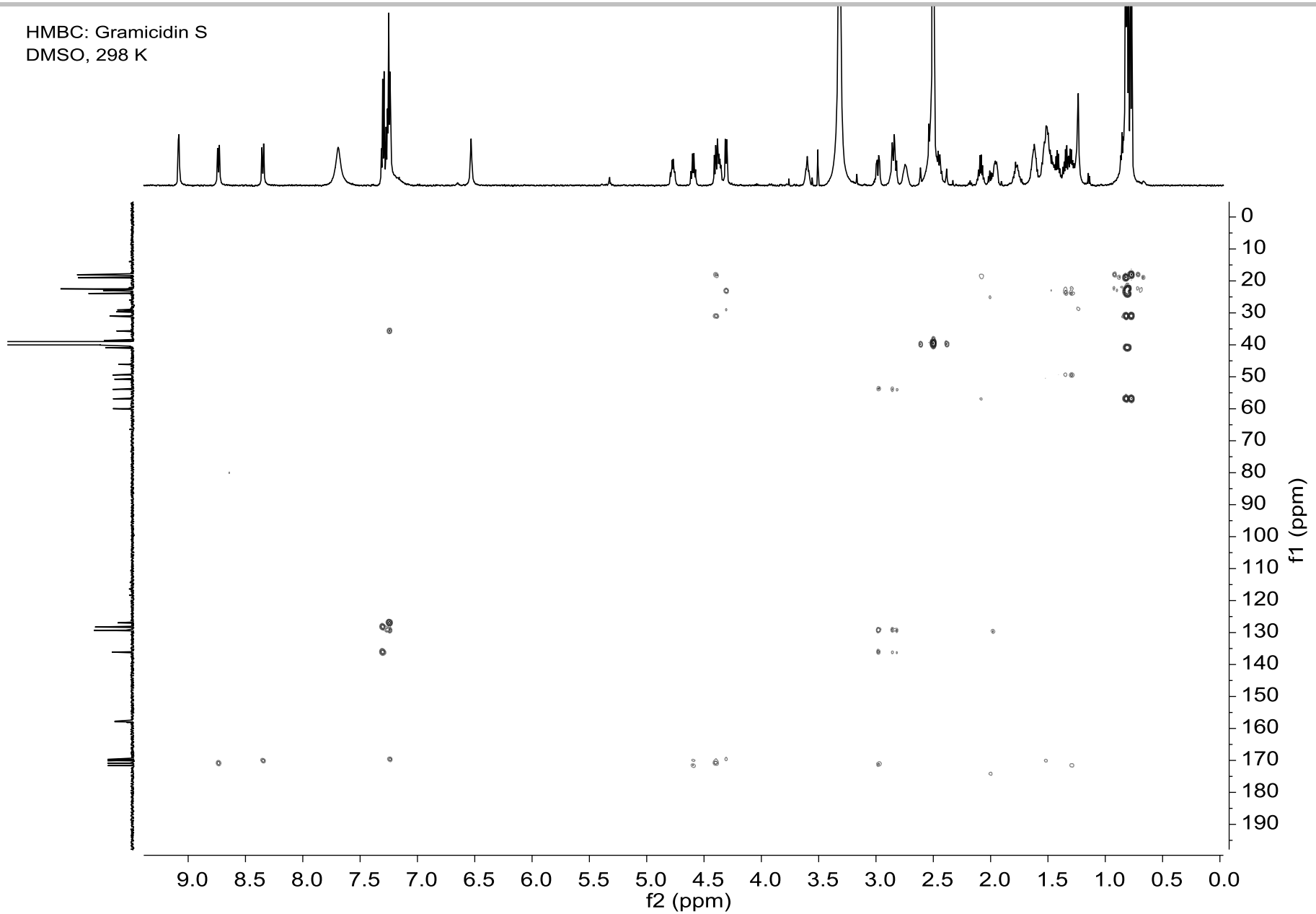

COSY:2-Pra-Lugdunin (24)
700 MHz, DMSO-d6, 298 K
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f2 (ppm)
1
2
3
4
5
6
7
8
9
10
11
f1 (ppm)

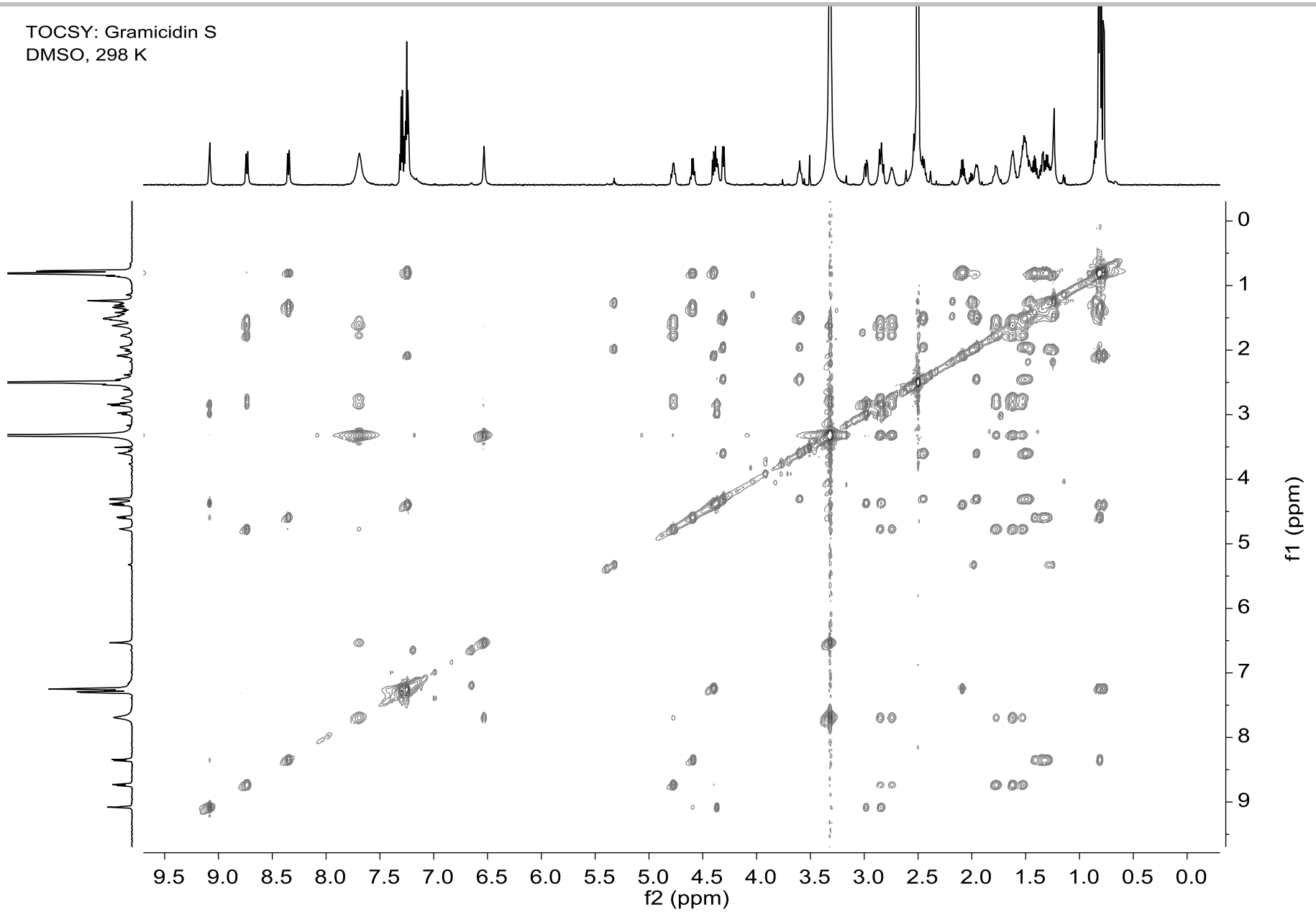

PROTON:Gramicidin S (25)
700 MHz, DMSO-d6, 298 K
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


CARBON:Gramicidin S (25)
700 MHz, DMSO-d6, 298 K
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


COSY:Gramicidin S (25)
700 MHz, DMSO-d6, 298 K
f2 (ppm)
f1 (ppm)


HSQC: Gramicidin S (25)
700 MHz, DMSO-d6, 298 K
f2 (ppm)
f1 (ppm)


HMBC: Gramicidin S
DMSO, 298 K
f2 (ppm)
f1 (ppm)


TOCSY: Gramicidin S
DMSO, 298 K
f2 (ppm)
f1 (ppm)

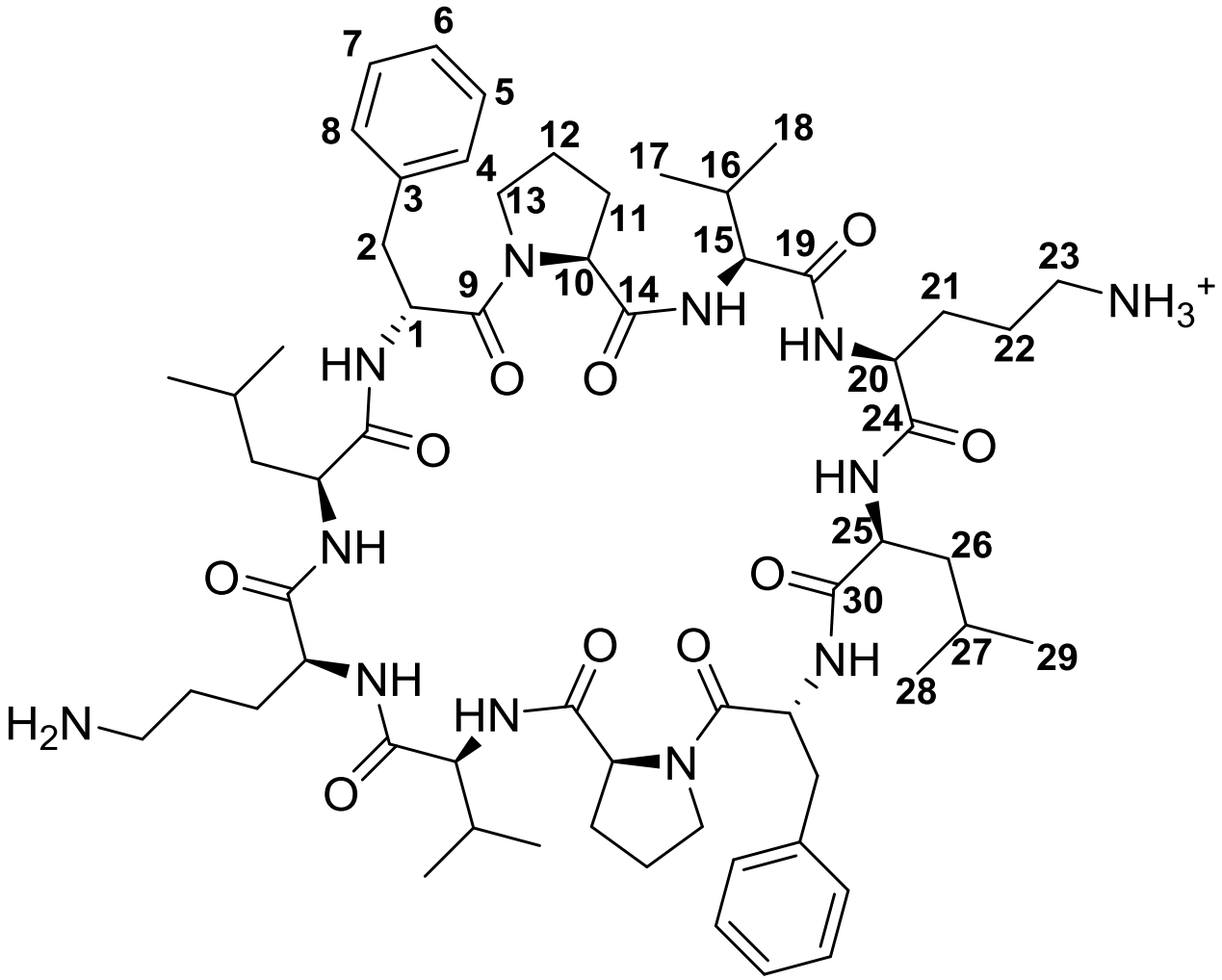


Figure S13: Structure of gramicidin S (**25**). Atom numbering is only given for one flank of the symmetric molecule.

	Gramicidin S (25)	
Position	**δ_H [ppm]** (Integral, Type, J in Hz)[2)]	**δ_C [ppm]**
1-NH	9.08 (1H, d, 3.7)	-
1	4.36 (1H, dd, 6.6, 5.0)	53.9
2	2.98 (1H, dd, 5.41), 2.84 (1H, m)	35.7
3	-	136.1
4	7.31 (1H, d, 6.9)	128.3
5	7.26 – 7.21 (2H, m)	129.3
6	7.28 – 7.25 (1H, m)	127.0
7	7.26 – 7.21 (2H, m)	129.3
8	7.29 (1H, d, 6.9)	128.3
09	-	171.2
10	4.30 (1H, dd, 7.72, 1.26)	59.9
11	1.95 (1H, m), 1.49 (2H, m)	23.9
12	1.95 (2H, m)	29.0
13	3.59 (1H, dd, 8.35, 7.1), 2.45 (1H, dd, 8.70, 8.38)	46.1
14	-	169.7
15-NH	7.23 (1H, d, 5.22)	-
15	4.40 (1H, dd, 8.80, 7.43)	56.9
16	2.09 (1H, dqq, 6.8)	31.0
17, 18	0.82 (3H, d, 6.7), 0.77 (3H, d, 6.88)	22.5, 18.9
19	-	170.9
20-NH	8.73 (1H, d, 9.3)	-
20	4.77 (1H, dt, 9.5, 5.3)	50.7
21	1.76 (1H, m), 1.53 (1H, m)	29.7
22	1.62 (2H, m)	23.0
23	2.86 (1H, m), 2.75 (2H, m)	38.6
23-NH_3^+, -NH_2	7.72 (3H, bs), 6.55 (2H, bs)	-
24	-	171.6
25-NH	8.35(1H, d, 9.1)	-
25	4.58 (1H, dt, 8.23, 7.56)	49.4
26	1.32 (2H, m)	40.9
27	1.41 (1H, m)	23.9
28, 29	0.82 (3H, d, 7.0), 0.80 (3H, d, 6.9)	22.8, 18.0
30	-	170.1

Table S4: ^{1}H and ^{13}C NMR data and assignments of gramicidin S (**25**)
^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR data of gramicidin (**25**) in d_6-dmso at resonance frequency 600 resp. 150.8 MHz or (298K). The chemical shifts are represented by Δ ppm.

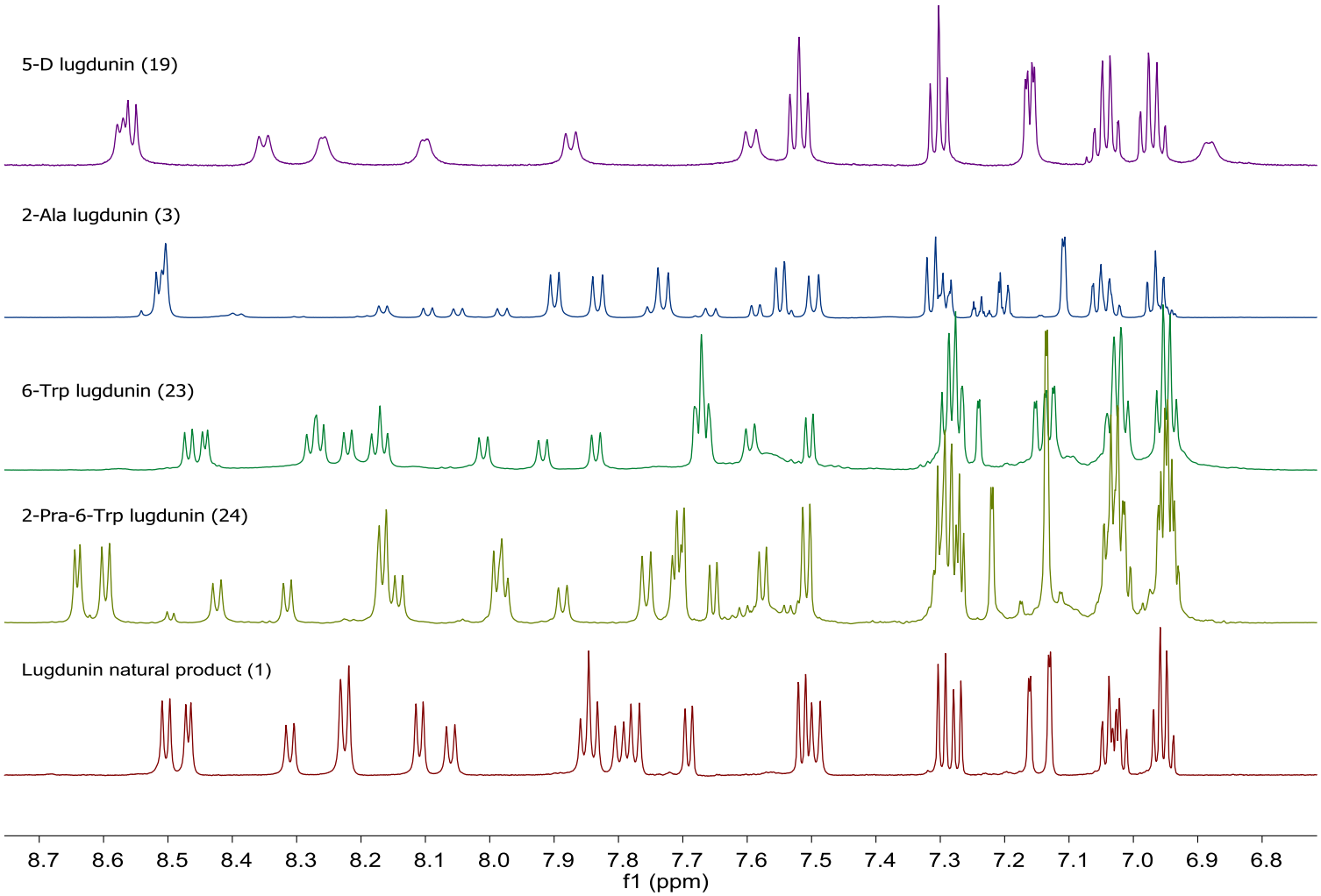


Figure S14. Expansion of ^{1}H NMR spectra (600 MHz, dmso-d6, 308 K) of lugdunin analogues **1, 3, 19, 23** and **24.** Amide protons are shown exemplarily to underline very strong chemical shifts in the lugdunin series of analogues.

SUPPORTING INFORMATION

List of ^{1}H and ^{13}C NMR data of active fibupeptides **3**, **6**, **7**, **8**, **22**, **23**, **24**. The chemical shifts are represented by Δ ppm.

All purified lugdunin analogues in indicated solvents occur as epimeric mixtures and different conformers. Therefore they show more than one set of NMR-signals in the analyzed NMR-spectra (^{1}H, ^{13}C). According to this finding, proton integrals can differ from the actual proton number of lugdunin analogues due to overlaying signals. For both epimers of lugdunin (**1**) a full proton assignment is given in the original publication.[2]

2-Ala-lugdunin (3)

^{1}H NMR (600 MHz, DMSO-d6) δ = 10.77 (d, *J*=2.5, 1H), 8.53 – 8.48 (m, 1H), 8.17 (d, *J*=7.8, H), 8.10 (d, *J*=8.4, 1H), 8.05 (d, *J*=8.3, 1H), 7.98 (d, *J*=9.0, 1H), 7.90 (d, *J*=7.9, 1H), 7.83 (d, *J*=9.2, 1H), 7.73 (d, *J*=9.5, 1H), 7.66 (d, *J*=9.5, 1H), 7.59 (d, *J*=7.9, 1H), 7.55 (dd, *J*=8.0, 1.1, 1H), 7.50 (d, *J*=9.4, 1H), 7.32 (t, *J*=0.9, 1H), 7.31 – 7.27 (m, 1H), 7.26 – 7.22 (m, 1H), 7.21 – 7.19 (m, 1H), 7.11 (d, *J*=2.3, 1H), 7.04 (dddd, *J*=9.8, 8.1, 6.9, 1.2, 1H), 6.99 – 6.93 (m, 1H), 4.74 (dd, *J*=13.0, 2.2, 1H), 4.61 (td, *J*=8.3, 5.8, 1H), 4.53 – 4.47 (m, 1H), 4.47 – 4.31 (m, 2H), 4.28 (dd, *J*=9.3, 8.2, 1H), 4.12 (t, *J*=7.8, 1H), 3.90 (dt, *J*=10.9, 7.1, 1H), 3.83 – 3.70 (m, 1H), 3.61 (ddd, *J*=13.0, 10.2, 5.9, 1H), 3.15 – 3.12 (m, 1H), 3.07 – 2.98 (m, 1H), 2.98 – 2.88 (m, 1H), 2.82 (dd, *J*=14.9, 10.0, 1H), 2.79 – 2.72 (m, 1H), 2.56 – 2.52 (m, 2H), 2.13 – 2.02 (m, 1H), 2.01 – 1.85 (m, 2H), 1.55 (dp, *J*=8.8, 6.6, 1H), 1.45 – 1.30 (m, 2H), 1.30 – 1.21 (m, 1H), 1.18 (d, *J*=7.1, 1H), 1.08 (d, *J*=6.9, 2H), 1.00 (d, *J*=6.9, 1H), 0.92 (d, *J*=6.7, 2H), 0.91 – 0.74 (m, 22H), 0.73 (s, 1H) ppm.

^{13}C NMR (151 MHz, DMSO-d_6) δ = 173.08 , 172.33 , 172.05 , 171.91 , 171.38 , 171.35 , 171.27 , 171.09 , 170.78 , 170.70 , 170.51 , 170.34 , 148.23 , 136.49 , 136.43 , 128.23 , 127.97 , 127.80 , 127.65 , 127.09 , 124.19 , 123.75 , 123.58 , 121.32 , 121.18 , 118.71 , 118.67 , 118.58 , 111.72 , 111.60 , 111.04 , 110.02 , 81.03 , 72.64 , 70.26 , 65.83 , 64.36 , 59.21 , 57.71 , 55.00 , 53.75 , 53.49 , 52.65 , 51.52 , 50.07 , 48.05 , 41.71 , 41.38 , 40.93 , 37.81 , 37.42 , 33.04 , 32.19 , 31.25 , 30.37 , 30.04 , 29.95 , 26.87 , 24.55 , 24.50 , 23.46 , 22.94 , 22.83 , 22.57 , 22.53 , 22.21 , 21.26 , 20.67 , 20.29 , 20.25 , 19.95 , 19.73 , 19.69 , 19.63 , 18.97 , 18.86 , 18.65 , 18.34 , 18.30 , 18.19 , 17.33 , 16.96 , 15.73 ppm.

5-Ala-lugdunin (6)

^{1}H NMR (600 MHz, DMSO-d6) δ = 10.75 (d, *J*=2.5, 1H), 10.72 (d, *J*=2.5, 1H), 8.51 (d, *J*=8.5, 1H), 8.44 (d, *J*=5.4, 1H), 8.25 (d, *J*=8.0, 1H), 8.18 (d, *J*=8.1, 1H), 8.11 (d, *J*=8.4, 1H), 8.06 (d, *J*=9.6, 1H), 7.84 (d, *J*=9.2, 1H), 7.75 (d, *J*=9.4, 1H), 7.66 (dd, *J*=18.1, 8.4, 2H), 7.54 (dd, *J*=19.4, 8.2, 2H), 7.35 – 7.25 (m, 3H), 7.11 (dd, *J*=9.0, 2.3, 2H), 7.03 (dtd, *J*=11.2, 8.1, 6.9, 1.2, 2H), 6.95 (dddd, *J*=9.5, 7.9, 6.8, 1.0, 2H), 4.74 (dd, *J*=12.7, 2.2, 1H), 4.66 (dtd, *J*=23.7, 8.8, 5.3, 2H), 4.58 – 4.46 (m, 2H), 4.40 (dd, *J*=9.2, 5.4, 1H), 4.35 (q, *J*=7.8, 1H), 4.31 – 4.26 (m, 1H), 4.19 (t, *J*=8.1, 2H), 4.08 – 4.01 (m, 1H), 3.95 (s, 1H), 3.75 – 3.61 (m, 3H), 3.09 (dd, *J*=9.5, 5.9, 2H), 3.00 (dd, *J*=14.3, 5.4, 1H), 2.89 (dd, *J*=14.3, 9.3, 1H), 2.84 (dd, *J*=14.7, 9.8, 1H), 2.68 (t, *J*=8.8, 1H), 2.03 – 1.86 (m, 3H), 1.70 (ddt, *J*=21.4, 14.3, 6.8, 2H), 1.57 (dq, *J*=9.2, 6.5, 1H), 1.48 – 1.40 (m, 1H), 1.40 – 1.32 (m, 2H), 1.24 (s, 3H), 1.18 (d, *J*=7.0, 3H), 1.14 (d, *J*=6.9, 2H), 0.93 (d, *J*=6.7, 3H), 0.89 – 0.74 (m, 35H), 0.69 (d, *J*=6.9, 2H), 0.63 (d, *J*=6.8, 2H), 0.53 (d, *J*=6.8, 3H), 0.48 (d, *J*=6.7, 2H), 4.26 – 4.26 (m, 1H) ppm.

6-Ala-lugdunin (7)

^{1}H NMR (600 MHz, DMSO-d_6) δ 10.74 (d, *J*=2.4, 1H), 10.73 (d, *J*=2.3, 1H), 8.37 (d, *J*=8.4, 1H), 8.35 (d, *J*=8.5, 1H), 8.29 (d, *J*=5.9, 1H), 8.25 (d, *J*=8.8, 1H), 8.20 (d, *J*=9.3, 1H), 8.17 (d, *J*=9.2, 1H), 8.02 (d, *J*=7.2, 1H), 7.96 (d, *J*=8.3, 1H), 7.91 (d, *J*=9.3, 1H), 7.79 (d, *J*=9.1, 1H), 7.75 (d, *J*=8.0, 1H), 7.68 (d, *J*=7.9, 1H), 7.61 – 7.56 (m, 1H), 7.53 (d, *J*=7.8, 1H), 7.28 (t, *J*=7.9, 2H), 7.20 (d, *J*=8.6, 1H), 7.11 (d, *J*=12.5, 1H), 7.02 (dt, *J*=7.6, 3.7, 1H), 6.95 (t, *J*=7.4, 1H), 4.75 (t, *J*=6.5, 1H), 4.64 (td, *J*=9.2, 5.5, 1H), 4.60 (t, *J*=7.3, 1H), 4.56 (d, *J*=11.1, 1H), 4.45 (tt, *J*=9.7, 5.0, 1H), 4.42 – 4.34 (m, 1H), 4.33 – 4.23 (m, 1H), 4.24 – 4.14 (m, 1H), 4.11 (q, *J*=9.3, 1H), 4.05 (td, *J*=9.4, 2.4, 1H), 4.02 – 3.95 (m, 1H), 3.80 (dd, *J*=8.5, 6.0, 1H), 3.72 (s, 1H), 3.27 (s, 1H), 3.11 (dd, *J*=9.6, 6.1, 1H), 3.01 (dd, *J*=6.7, 3.9, 1H), 2.99 – 2.93 (m, 1H), 2.91 – 2.85 (m, 1H), 2.84 (d, *J*=7.1, 1H), 2.69 (d, *J*=6.2, 1H), 2.61 (td, *J*=3.9, 2.0, 1H), 2.03 – 1.95 (m, 1H), 1.88 (ddd, *J*=12.5, 8.8, 6.2, 1H), 1.75 – 1.68 (m, 1H), 1.64 (q, *J*=6.0, 1H), 1.62 – 1.52 (m, 1H), 1.50 – 1.33 (m, 1H), 1.32 – 1.22 (m, 1H), 1.19 (dd, *J*=9.0, 6.8, 3H), 0.92 (d, *J*=6.6, 3H), 0.90 – 0.68 (m, 20H), 0.62 (d, *J*=6.8, 3H), 0.57 (d, *J*=6.7, 3H), 0.48 (d, *J*=6.8, 3H), 0.44 (d, *J*=6.6, 3H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 172.67, 172.40, 172.05, 171.89, 171.63, 171.35, 171.06, 170.99, 170.92, 170.82, 170.45, 170.27, 170.05, 169.69, 169.47, 158.25, 158.03, 157.82, 157.60, 147.75, 146.93, 136.10, 136.07, 127.75, 127.51, 127.15, 127.05, 126.63, 124.34, 123.68, 120.75, 120.65, 118.67, 118.21, 118.11, 117.97, 117.63, 115.67, 111.16, 111.02, 110.04, 109.74, 109.49, 80.56, 72.32, 71.72, 66.16, 65.75, 65.05, 60.37, 60.22, 59.84, 57.86, 57.63, 57.50, 57.41, 57.06, 56.72, 54.48, 53.16, 52.80, 52.70, 51.68, 50.42, 50.27, 48.38, 47.58, 43.78, 42.28, 41.42, 41.20, 40.06, 36.85, 32.51, 31.68, 31.29, 31.05, 30.85, 30.60, 30.01, 29.79, 29.12, 28.94, 28.04, 27.11, 24.25, 24.09, 23.94, 23.23, 23.09, 22.44, 22.21, 22.03, 21.71, 21.60, 21.37, 20.60, 20.34, 20.13, 19.68, 19.15, 19.10, 19.06, 18.97, 18.89, 18.70, 18.66, 18.64, 18.58, 18.23, 18.01, 17.74, 17.46, 16.35, 14.96, 14.61, 13.83.

7-Ala-lugdunin (8)

^{1}H NMR (600 MHz, DMSO-d_6) δ 10.77 – 10.69 (m, 1H), 8.53 (d, *J*=5.0, 1H), 8.52 (d, *J*=5.3, 1H), 8.47 (d, *J*=8.2, 1H), 8.44 (d, *J*=7.8, 1H), 8.36 – 8.32 (m, 1H), 8.31 – 8.27 (m, 1H), 8.18 – 8.16 (m, 1H), 8.14 (d, *J*=9.2, 1H), 8.08 (d, *J*=8.0, 1H), 7.98 (d, *J*=8.7, 1H), 7.95 (dd, *J*=8.1, 3.7, 1H), 7.86 (d, *J*=9.5, 1H), 7.71 (dd, *J*=8.0, 2.8, 1H), 7.67 (d, *J*=9.3, 1H), 7.54 (d, *J*=7.9, 1H), 7.47 (d, *J*=8.6, 1H), 7.38 (d, *J*=8.5, 1H), 7.30 (d, *J*=8.2, 1H), 7.27 (d, *J*=8.0, 1H), 7.17 (s, 1H), 7.14 (dd, *J*=9.0, 2.4, 1H), 7.05 – 7.00 (m, 1H), 6.94 (q, *J*=7.5, 1H), 4.77 – 4.71 (m, 1H), 4.68 (d, *J*=4.5, 1H), 4.59 (q, *J*=7.4, 1H), 4.46 (d, *J*=9.4, 1H), 4.38 (t, *J*=8.3, 1H), 4.32 (d, *J*=8.9, 1H), 4.19 (dd, *J*=8.9, 6.4, 1H), 4.03 (d, *J*=9.0, 1H), 4.02 – 3.95 (m, 1H), 3.85 (dd, *J*=9.0, 6.6, 1H), 3.30 (dd, *J*=14.6, 4.2, 1H), 3.23 (dd, *J*=10.5, 3.8, 1H), 3.13 (dd, *J*=9.6, 6.0, 1H), 3.05 (d, *J*=7.8, 1H), 3.01 (td, *J*=9.3, 8.5, 4.4, 1H), 2.91 – 2.85 (m, 1H), 2.76 (dd, *J*=14.1, 6.8, 1H), 2.26 – 2.15 (m, 1H), 2.06 – 2.00 (m, 1H), 1.99 – 1.92 (m, 1H), 1.89 (ddt, *J*=12.6, 6.5, 2.8, 1H), 1.72 – 1.66 (m, 1H), 1.64 (q, *J*=5.9, 1H), 1.61 – 1.52 (m, 1H), 1.52 – 1.27 (m, 1H), 1.23 (td, *J*=6.4, 5.7, 2.9, 1H), 1.16 (d, *J*=6.4, 3H), 1.08 (d, *J*=6.7, 3H), 1.05 (d, *J*=6.7, 3H), 1.03 (d, *J*=6.5, 3H), 0.92 (d, *J*=6.8, 3H), 0.87 (d, *J*=6.4, 3H), 0.84 (dd, *J*=8.5, 1.7, 3H), 0.82 (s, 3H), 0.78 (s, 3H), 0.67 (d, *J*=6.3, 3H), 0.58 (d, *J*=6.5, 3H), 0.49 (t, *J*=7.0, 3H), 0.43 (d, *J*=6.4, 3H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 172.76, 172.28, 171.57, 171.48, 171.20, 171.02, 170.93, 170.88, 170.75, 170.68, 170.51, 170.30, 170.24, 170.14, 169.94, 169.43, 158.30, 158.07, 157.84, 157.62, 136.11, 136.03, 135.93, 127.72, 127.46, 127.22, 127.12, 126.93, 124.35, 124.21, 124.16, 123.75, 123.04, 120.73, 120.66, 120.59, 119.17, 118.72, 118.27, 118.09, 118.00, 117.89, 117.22, 115.26, 111.03, 110.97, 110.14, 110.01, 109.73, 109.52, 74.45, 74.32,

69.75, 65.80, 60.58, 60.01, 58.79, 57.48, 57.31, 57.22, 57.09, 57.04, 56.76, 53.37, 53.20, 52.79, 51.93, 51.57, 51.37, 50.42, 50.26, 44.82, 43.72, 42.27, 41.56, 41.15, 40.51, 40.06, 36.47, 31.31, 31.22, 31.16, 30.98, 29.89, 29.02, 28.06, 27.47, 26.83, 24.11, 23.04, 23.03, 22.59, 22.46, 22.17, 21.89, 21.77, 21.63, 21.57, 19.98, 19.79, 19.67, 19.36, 19.18, 19.11, 19.06, 18.90, 18.88, 18.61, 18.59, 18.51, 18.47, 18.37, 18.02, 17.95, 17.80, 17.40, 17.21.

Enantio-lugdunin (22)

^{1}H NMR (600 MHz, DMSO-d_6) δ 10.74 (d, *J* = 12.8 Hz, 1H), 8.48 (d, *J* = 8.4 Hz, 1H), 8.45 (d, *J* = 5.5 Hz, 1H), 8.25 (d, *J* = 8.5 Hz, 1H), 8.19 (d, *J* = 9.8 Hz, 1H), 8.16 (d, *J* = 8.5 Hz, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 8.01 (d, *J* = 9.1 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 1H), 7.80 (d, *J* = 9.4 Hz, 2H), 7.75 (d, *J* = 9.3 Hz, 1H), 7.71 (d, *J* = 9.3 Hz, 1H), 7.67 (d, *J* = 7.9 Hz, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.45 (d, *J* = 9.3 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 1H), 7.14 (d, *J* = 13.8 Hz, 1H), 7.11 (d, *J* = 24.1 Hz, 1H), 7.06 – 6.99 (m, 2H), 6.98 – 6.93 (m, 2H), 4.74 (d, *J* = 2.2 Hz, 1H), 4.72 (d, *J* = 1.9 Hz, 1H), 4.70 (d, *J* = 5.5 Hz, 1H), 4.56 – 4.47 (m, 3H), 4.44 – 4.38 (m, 2H), 4.25 (t, *J* = 9.2 Hz, 1H), 4.18 (t, *J* = 8.9 Hz, 1H), 4.10 – 4.03 (m, 2H), 3.72 – 3.65 (m, 3H), 3.51 (s, 1H), 2.61 (q, *J* = 1.9 Hz, 2H), 2.38 (s, 1H), 1.98 (tq, *J* = 19.4, 6.8, 6.4 Hz, 2H), 1.92 – 1.85 (m, 2H), 1.71 (dt, *J* = 8.4, 6.4 Hz, 1H), 1.63 – 1.58 (m, 1H), 1.57 – 1.52 (m, 1H), 1.49 – 1.34 (m, 3H), 1.33 – 1.23 (m, 2H), 1.21 (s, 1H), 0.93 (d, *J* = 6.7 Hz, 3H), 0.90 – 0.72 (m, 18H), 0.63 (d, *J* = 6.7 Hz, 3H), 0.52 (d, *J* = 6.7 Hz, 3H), 0.45 (d, *J* = 6.7 Hz, 3H).

6-Trp-lugdunin (23)

^{1}H NMR (700 MHz, DMSO-d_6) δ 10.76 (d, 1H), 8.47 (d, J = 8.3 Hz, 1H), 8.44 (d, J = 5.7 Hz, 1H), 8.27 (dd, J = 10.5, 8.0 Hz, 1H), 8.22 (d, J = 8.5 Hz, 2H), 8.17 (t, J = 8.7 Hz, 1H), 8.01 (d, J = 9.3 Hz, 1H), 7.92 (d, J = 9.3 Hz, 1H), 7.84 (d, J = 9.3 Hz, 1H), 7.67 (td, J = 7.6, 2.4 Hz, 1H), 7.60 (d, J = 9.5 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.32 – 7.26 (m, 2H), 7.24 (d, J = 2.3 Hz, 1H), 7.17 – 7.11 (m, 2H), 7.07 – 6.99 (m, 2H), 6.98 – 6.90 (m, 2H), 4.90 (dd, J = 8.7, 6.1 Hz, 1H), 4.76 – 4.69 (m, 1H), 4.65 – 4.55 (m, 1H), 4.48 (dd, J = 9.1, 5.5 Hz, 1H), 4.41 (td, J = 9.2, 8.5, 6.0 Hz, 1H), 4.29 (dd, J = 9.4, 6.8 Hz, 1H), 4.22 – 4.14 (m, 1H), 4.05 (dd, J = 9.2, 2.4 Hz, 2H), 3.94 (dt, J = 11.6, 7.4 Hz, 1H), 3.77 – 3.64 (m, 1H), 3.12 (dd, J = 9.4, 6.0 Hz, 1H), 3.08 (d, J = 6.1 Hz, 1H), 3.04 (ddd, J = 14.4, 12.4, 6.1 Hz, 1H), 2.98 (d, J = 5.6 Hz, 1H), 2.96 (s, 1H), 2.94 – 2.84 (m, 2H), 2.82 – 2.75 (m, 2H), 2.69 (s, 1H), 2.62 – 2.55 (m, 2H), 1.88 (dd, J = 13.8, 6.9, 2.9 Hz, 2H), 1.76 – 1.66 (m, 1H), 1.64 – 1.56 (m, 2H), 1.51 – 1.41 (m, 2H), 1.40 (s, 1H), 0.85 (d, J = 6.7 Hz, 3H), 0.81 (dd, J = 11.3, 6.5 Hz, 6H), 0.77 (d, J = 6.2 Hz, 3H), 0.74 (dd, J = 6.4, 2.6 Hz, 6H), 0.67 (dd, J = 6.7, 2.1 Hz, 6H), 0.61 (dt, J = 9.9, 6.7 Hz, 9H), 0.54 (dd, J = 13.3, 6.8 Hz, 6H), 0.49 (d, J = 6.8 Hz, 3H), 0.45 (d, J = 6.7 Hz, 3H).

^{13}C NMR (176 MHz, DMSO-d_6) δ 172.7, 171.6, 171.5, 171.4, 171.2, 170.8, 170.7, 170.5, 170.1, 170.0, 169.9, 169.7, 136.9, 136.4, 136.0, 127.2, 127.1, 126.9, 124.2, 124.1, 123.7, 123.6, 120.7, 120.6, 120.3, 118.7, 117.9, 111.2, 111.0, 110.1, 109.8, 109.6, 109.5, 72.2, 72.0, 66.9, 65.2, 60.3, 57.3, 57.2, 57.1, 56.5, 54.4, 53.7, 53.3, 52.7, 52.0, 50.5, 41.3, 41.0, 40.4, 39.8, 39.7, 39.6, 39.5, 39.4, 39.3, 39.1, 32.6, 31.3, 31.2, 30.9, 29.9, 29.7, 28.7, 28.1, 27.1, 26.8, 24.1, 24.0, 23.0, 22.3, 22.1, 21.7, 20.5, 19.6, 19.3, 19.1, 18.7, 18.6, 18.3, 18.2, 17.6, 15.0.

2-Pra-6-Trp-lugdunin (24)

^{1}H NMR (700 MHz, DMSO-d_6) δ 10.79 (d, *J* = 10.1 Hz, 1H), 8.64 (d, *J* = 5.7 Hz, 1H), 8.60 (d, *J* = 8.3 Hz, 1H), 8.42 (d, *J* = 8.9 Hz, 1H), 8.31 (d, *J* = 8.4 Hz, 1H), 8.17 (d, *J* = 7.2 Hz, 1H), 8.14 (d, *J* = 8.3 Hz, 1H), 7.98 (dd, *J* = 9.1, 6.4 Hz, 2H), 7.89 (d, *J* = 9.4 Hz, 1H), 7.76 (d, *J* = 9.4 Hz, 1H), 7.73 – 7.69 (m, 2H), 7.65 (d, *J* = 8.6 Hz, 1H), 7.58 – 7.56 (m, 2H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.34 – 7.25 (m, 2H), 7.22 (d, *J* = 2.4 Hz, 1H), 7.13 (d, *J* = 2.3 Hz, 1H), 7.06 – 7.00 (m, 2H), 6.99 – 6.92 (m, 2H), 4.86 (d, *J* = 6.0 Hz, 1H), 4.73 (d, *J* = 2.6 Hz,1H), 4.71 (d, *J* = 2.5 Hz, 1H), 4.69 – 4.55 (m, 1H), 4.44 – 4.38 (m, 1H), 4.27 – 4.18 (m, 1H), 4.07 (td, *J* = 9.3, 2.5 Hz, 1H), 3.94 (dt, *J* = 10.8, 7.0 Hz, 1H), 3.76 – 3.67 (m, 2H), 3.67 – 3.61 (m, 1H), 3.41 (t, *J* = 5.2 Hz, 1H), 3.23 (d, *J* = 6.0 Hz, 1H), 3.21 (d, *J* = 5.9 Hz, 1H), 3.13 – 3.07 (m, 2H), 3.03 (dd, *J* = 10.6, 6.6 Hz, 2H), 2.89 (dd, *J* = 14.4, 8.9 Hz, 1H), 2.84 – 2.79 (m, 2H), 2.77 (t, *J* = 2.6 Hz, 1H), 2.67 (t, *J* = 2.6 Hz, 1H), 2.57 (s, 1H), 2.56 (s, 1H), 2.54 (s, 1H), 2.46 (s, 1H), 2.40 (ddd, *J* = 9.7, 6.6, 2.6 Hz, 1H), 2.25 (dd, *J* = 7.8, 2.7 Hz, 2H), 2.03 – 1.96 (m, 2H), 1.69 (dq, *J* = 14.3, 7.0 Hz, 1H), 1.48 (dtt, *J* = 19.3, 7.8, 4.2 Hz, 2H), 1.35 – 1.27 (m, 1H), 1.23 (td, *J* = 8.4, 6.5, 4.4 Hz, 2H), 1.17 – 1.12 (m, 1H), 1.11 (s, 1H), 0.98 (d, *J* = 7.2 Hz, 3H), 0.87 (d, *J* = 6.7 Hz, 3H), 0.78 (d, *J* = 6.2 Hz, 3H), 0.75 (d, *J* = 6.5 Hz, 3H), 0.72 – 0.69 (m, 3H), 0.63 (d, *J* = 6.9 Hz, 3H), 0.59 (d, *J* = 6.8 Hz, 3H), 0.57 – 0.54 (m, 9H), 0.49 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (176 MHz, DMSO-d_6) δ 172.3, 171.6, 171.4, 170.9, 170.5, 170.4, 170.3, 170.3, 170.2, 170.1, 170.0, 168.5, 136.1, 136.0, 135.9, 135.8, 129.6, 127.3, 127.2, 127.2, 124.1, 123.8, 123.4, 120.7, 120.6, 118.8, 118.6, 118.4, 118.2, 118.1, 118.0, 117.9, 117.8, 111.2, 111.1, 111.0, 110.0, 109.8, 109.7, 109.4, 80.2, 79.9, 72.6, 72.3, 72.2, 72.0, 69.7, 65.1, 63.8, 57.1, 56.6, 54.5, 53.4, 53.3, 53.0, 52.8, 52.7, 51.7, 50.7, 50.4, 41.0, 40.9, 40.4, 39.1, 35.1, 32.6, 31.3, 30.9, 29.8, 28.0, 27.6, 27.1, 23.9, 22.9, 22.4, 22.1, 22.0, 21.8, 21.6, 20.6, 20.3, 19.6, 19.6, 19.0, 17.9, 17.8, 17.6, 15.1, 13.9, 12.0.

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Author Contributions

N.A.S. designed and coordinated the study, optimized and performed synthesis, conducted chromatography and wrote the manuscript. A.B. performed membrane potential and pore formation assays. J.S. performed vesicle assays. J.S.S. designed, synthesized and analysed **24**, M.C.K. designed and synthesized **12** and performed NMR analyses. S.N.W. synthesized peptides, performed NMR analyses and edited the manuscript. J.M.B.-B. conducted chromatography. A.Z. and B.K. performed MIC-assays. A.P., H.K., H.B.-Ö., C.S. and S.G. acquired funding and coordinated the study. All authors contributed in analysing and discussing data and commenting on the manuscript.