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Supporting Information

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Supplementary Information

Structure and Total Synthesis of Lysobactin (Katanosin B)**

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[•] J. B.-B.: X-ray crystallography. L. M.: NMR solution structure.

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Experimental Section

General Methods: *Reactions* were carried out under argon atmosphere, if not indicated otherwise. Reaction control was performed with HPLC monitoring. Crude products were immediately purified with preparative HPLC methodology or gel chromatography. The fractions obtained were concentrated in vacuo to remove organic solvents. All products were freeze-dried, yielding colorless lyophilisates. *Chemicals* were obtained in analytical grade from Bachem (Swiss), Merck KGaA (Germany) or Sigma-Aldrich (Germany). *Gel chromatography* was carried out with Sephadex LH-20 (Pharmacia). *Structural representation and nomenclature:* Lysobactins are salts. Counterions were omitted in the figures and schemes. Peptide atoms were numbered according to IUPAC recommendations.[1]

Mass-spectrometry: The FT-ICR instrument (*HR-FT-ICR-MS*) was a APEX II mass spectrometer (Bruker Daltonics, Billerica, MA, USA) equipped with a 160 mm room temperature 7 tesla actively shielded magnet, electrostatic ion transfer optics, octupole ion storage device and external off-axis electrospray ion source. *HPLC-FT-ICR-MS* was performed on a HP 1100 HPLC-system synchronized to the FT-ICR spectrometer via contact closure. The whole effluent (250 µL/min) was directed to the external electrospray source. A heated nitrogen flow of 8 l/min each with a temperature of 250°C was used as nebulizing and drying gas. *TOF-HR-ESI-MS* spectra were obtained on a Micromass-LCT mass spectrometer (capillary 3.2 KV, cone 42 V, source 120°C). Samples were injected with a syringe pump (Harvard Apparatus). Leucine-enkephaline was used as standard.

NMR Spectroscopy: Spectra were recorded on a Bruker Avance 400 or a Bruker DRX 500 instrument with a TXI CryoProbe. ^{1}H and ^{13}C chemical shifts are given with respect to TMS or the solvent as internal standard ([D_6]DMSO: d H 2.49, d C 39.5; $CDCl_3$ d H 7.25, d C 77.0; [D_5]pyridine H 7.19, d C 123.5). If no certain assignment was possible # and † indicate interchangeable assignment of NMR signals. Peaks that were under the solvent signal are also indicated ‡. Several NMR

spectra were visualized with NPNMR.[2] Two-dimensional NMR experiments were carried out on a Bruker DMX-600 spectrometer equipped with a triple gradient inverse triple resonance probe. Solvent suppressed one-dimensional ^{1}H spectra were recorded with either a WATERGATE scheme utilizing a binomial pulse sandwich[3] or with a noesypresat[4] sequence. Homonuclear correlation (COSY)[5] experiments were collected using gradients for coherence selection[6] and in the absolute value mode. Phase sensitive nuclear Overhauser spectroscopy (NOESY)[7] was carried out with a WATERGATE sequence for water suppression with mixing times of 100 msec, 200 msec, 300 msec and 500 msec and with time proportional phase incrementation[8] (TPPI). No spin diffusion effects were observed up to 300 msec. Rotating frame Overhauser enhancement spectroscopy (ROESY)[9] was acquired with the pulse sequence of Hwang and Shaka,[10] with the same mixing times as the NOESY spectra and also with TPPI.[8] Total correlation spectroscopy (TOCSY)[11] was carried out with a MLEV17[12] spin-lock of 120 msec duration in the echo-antiecho mode,[13] applying WATERGATE solvent suppression. One-bond ^{1}H–^{13}C heteronuclear multiple quantum coherence (HMQC) spectroscopy[14] was run in a phase sensitive mode with TPPI, using bilinear rotation decoupling (BIRD)[15] for suppressing protons coupled to ^{12}C carbons. Heteronuclear multiple bond (HMBC) spectroscopy[16] was acquired with a gradient enhanced pulse sequence[17] in absolute value mode, using a constant time evolution delay.[18] Phase sensitive ^{1}H–^{15}N heteronuclear single quantum coherence (HSQC)[19] spectroscopy was carried out with gradient selection[20] in echo-antiecho mode with sensitivity enhancement.[21] Molecular visualization and simple modeling was performed using the software package SYBYL on a Silicon Graphics Indy workstation. Modeling was based on molecular mechanics[22] using the Tripos force-field[23] and the Gasteiger-Hückel[24] method for calculation of charges as implemented in version 7.1 of the software.

NMR solution structure:[25] Oligopeptides, particularly linear ones, exist usually in solution in a dynamic exchange of multiple conformations, often in an exchange regime that precludes the establishment of a meaningful 3D structure. Cyclic peptides on the

other hand may be constrained enough to yield at least partially stable conformations with relevance to their binding interactions.[26] The NMR studies were carried out with a trifluoroacetate salt of the natural product **1**. The distinct ^{1}H-NMR signals in deuterated water, DMSO, and pyridine clearly hinted at the absence of rotamers. Although, **1** showed signs of concentration-dependent aggregation, still complete ^{1}H, ^{13}C and ^{15}N assignments were possible. The general strategy was to identify the spin systems of the individual amino acids from COSY, TOCSY and, if needed, HMBC data sets, followed by sequential assignments from HMBC and ROESY or NOESY experiments. The latter two were also the source of through-space ^{1}H-^{1}H distance information, which, together with torsion angle information from coupling constant data, formed the basis of the 3D structure analysis. ^{1}H–^{15}N HSQC data provided the ^{15}N assignments. Chemical shift assignments are presented (Table 1). Variable temperature studies were completed to assess the potential hydrogen bond forming and solvent accessibility of the amide hydrogens. It was also noted that the positive charges were localized on the amino group of the *N*-terminal D-Leu^{1} and on the guanidine group of D-Arg^{6}. In aqueous environment, ^{1}H assignments were achieved in two solvent systems: in a 4:1 H_2O/D_2O system with 2.5% [D_6]DMSO present, and in a 5:1 H_2O:CD_3CO_2D system. The ester bond of **1** was sensitive to hydrolysis at basic or neutral pH. In the first aqueous solvent system about 50% of the cyclic compound hydrolyzed to the linear peptide in ~10 days. In the water/acetic acid mixture no signs of ring-opening hydrolysis were observed over a month. In addition, the latter system yielded significantly better spectral line shape. Only minor chemical shift differences were observed in the two solvent systems, mostly among the amide resonances, as can be seen from presented data (Table 2 and Table 3). All detected NOE correlations and coupling constants were consistent with a single well-defined backbone conformation, essentially identical to that of the crystal structure. The side-chains, e.g. the D-Arg^{6} folding back and the guanidine stacking up with the phenyl group of *Hy*Phe^{3} phenyl moiety was unequivocally defined by NOE constraints. By the N-terminal D-Leu^{1} the NMR structure was not defined due to conformational freedom. No relevant NOEs were observed here.

X-ray Crystallography: Compound **1** crystallizes from a saturated methanol/water 50:50 solution at 35°C in form of twinned crystals. After different trials, a full data set with a resolution of 0.88 Å (2Θ = 119.9° Cu_K) could be collected using a crystal with the dimensions 0.20 × 0.20 × 0.03 mm^3. Measurements were carried out on a Bruker-Nonius diffractometer equipped with a Proteum CCD area detector, a FR591 rotating anode with $Cu_{K\alpha}$ radiation, Montel mirrors as monochromator and a Kryoflex low temperature device. Software: Data collection Proteum V. 1.37 (Bruker-Nonius 2002), data reduction Saint Plus Version 6.22 (Bruker-Nonius 2002), absorption correction SADABS V. 2.03 (2002) and structure solution and refinement SHELXTL Version 6.12 (Sheldrick, 2000)[27]. *Crystal data and structure refinement:* Empirical formula: $C_{62}H_{109}F_6N_{15}O_{27.5}$ (corresponds to one diprotonated molecule of lysobactin, two bistrifluoroacetate anions and 6 ½ molecules of water); formula weight: 1618.64 g/mol; temperature: 90(2) K; wavelength: 1.54178 Å; crystal system: monoclinic; space group: $P2_1$; unit cell dimensions: a = 13.2471(4) Å; b = 12.8927(4) Å; c = 25.0255(8) Å; ß = 103.709(2)°; volume: 4152.4(2) $Å^3$; Z = 2; density calcd: 1.295 mg/m^3; absorption coefficient: 0.943 mm^{-1}; theta range for data collection: 3.43 to 59.95; reflections collected: 33386; independent reflections: 10745 [R(int) = 0.0497; goodness-of-fit on F^2: 1.061; final R indices [I>2sigma(I)]: R1 = 0.0790, wR2 = 0.2115; R indices (all data): R1 = 0.1021, wR2 = 0.2320; absolute structure parameter: -0.21(27) (twin and Basf: 0.0(3)), taking in account that the described molecule has several chiral centers and is a pure natural product, the assigned stereochemistry is in spite of the relative high standard deviation of the Flack-Parameter probably right; largest diff. peak and hole: 0.551 and -0.313 e.$Å^{-3}$. The orthoscopic views were produced with PyMol.[28]

Analytical HPLC & LC-MS Methods: *Method 1:* Instrument: HP1100 with DAD (G1315A), autosampler (G1329A), autosampler therme (G1330A, 5°C), degasser (G1322A)and binary pump (G1312A); stationary phase: precolumn: Waters Symmetry C_{18} 3.5 µm, 10 × 2.1 mm, column: Waters Symmetry C_{18} 3.5 µm, 50 × 2.1 mm; column thermostat: 45°C; UV detection

at 210 and 254 nm; eluent A: 0.05% TFA in water; eluent B: 0.05% TFA in acetonitrile; flow: 0.4 ml/min; gradient: ramp 0-100% B in 9 min, 9-12 min 100% B, regeneration. *Method 2:* Agilent 1100 with DAD (G1315B), binary pump (G1312A), autosampler (G1313A), degasser (G1379A) and column thermostat (G1316A); column: Agilent Zorbax Eclipse XDB-C_8 5 µm, 150 × 4.6 mm; column thermostat: 30°C; UV detection at 210 and 254 nm; eluent A: 0.05% 70% perchloric acid in water; eluent B: acetonitrile; flow rate: 2.00 ml/min; gradient: 0–1 min 10% B, ramp, 4–5 min 90% B, regeneration. *Method 3 (LC-MS):* Agilent 1100 with DAD (G1315B) and Micromass Platform LCZ; column: Thermo Hypersil GOLD 3 µm, 20 × 4 mm; column thermostat: 50°C; UV-detection at 210 nm; flow: 0.8 ml/min; eluent A: 0.05% 50% formic acid in water, eluent B: 0.05% 50% formic acid in acetonitrile; gradient: 0.0–0.2 min 0% B, ramp, 2.9 min 70% B, ramp, 3.1-5.5 min 90% B, regeneration. *Method 4 (LC-MS):* Agilent 1100 with DAD (G1315B) and Micromass ZQ; column thermostat: 50°C; UV-detection at 210 nm; column: Phenomenex Gemini 3 µm, 30 × 3 mm; eluent A: 0.05% 50% formic acid in water, eluent B: 0.05% 50% formic acid in acetonitrile; flow: 0.0 min 1 ml/min, ramp, 2.5-4.5 min 2 ml/min; gradient: 0.0 min 10% B, ramp, 2.5 min 70% B, ramp, 3.0–4.5 min 95% B, regeneration. *Method 5*: HP 1050 Series with UV DAD; column: Agilent Zorbax 300 mSB-C_{18} 3.5 µm, 150 × 4.6 mm; column thermostat: 40°C; UV-detection at 210 nm; flow: 1 ml/min; eluent A: 0.1% TFA in water, eluent B: 0.1% TFA in water-acetonitrile 6:4; gradient: 0.0 min 100% A, ramp, 1.3 min 10% B, ramp, 18.0-20 min 80% B, ramp, 21.0–25 min 100% B, regeneration. *Method 6 (LC-MS):* Waters Alliance 2795 with Micromass ZQ; column: Merck Chromolith SpeedROD RP_{18e} 100 × 4.6 mm; UV-detection at 210 nm; column thermostat 35°C; flow: 0.0 min 1.0 ml/min, ramp, 7.0–9.0 min 2.0 ml/min; eluent A: 0.05% 50%ige formic acid in water; eluent B: 0.05% 50% formic acid in acetonitrile; gradient: 0.0 min 10% B, ramp, 7.0–9.0 min 95% B, regeneration. *Method 7*: HP 1050 Series with UV DAD; column: Agilent Zorbax 300 mSB-C_{18} 3.5 µm, 150 × 4.6 mm; column thermostat: 40°C; UV-detection at 210 nm; flow: 0.7 ml/min; eluent A: 0.1% TFA in water, eluent B: 0.1% TFA in water-acetonitrile 6:4; gradient: 0.0 min 100% A, ramp, 2.0 min 10% B, ramp, 50.0 min 80% B,

ramp, 52.0-55.0 min 100% B, regeneration. *Method 8*: HP 1050 Series with UV DAD; column: Agilent Zorbax 300 mSB-C_{18} 3.5 µm, 150 × 4.6 mm; column thermostat: 40°C; UV-detection at 210 nm; flow: 1 ml/min; eluent A: 0.05% formic acid in water, eluent B: acetonitrile; gradient: 0.0 min 100% A, ramp, 1.5 min 95% B, ramp, 15.0-17 min 100% B, ramp, regeneration. *Method 9:* Agilent 1100 with DAD column thermostat: 40°C; UV-detection at 210 nm; column: Phenomenex, : Phenomenex Gemini 5 µ C_{18}, 50 × 2 mm, 20 × 4 mm; eluent A: 0.05% aqueous formic acid, eluent B: acetonitrile; flow: 2 ml/min, 0.0-1 min 0% B, ramp, 1-5 min 100% B, ramp, 5.5 min 100% B, regeneration. Method 10 (LC-MS): Agilent 1100 with DAD (G1315B) and Micromass ZQ; column thermostat: 50°C; UV-detection at 210 nm; column: Phenomenex Synergi 2 µ, Hydro-RP Mercury 20 × 4 mm; eluent A: 0.05% 50% formic acid in water, eluent B: 0.05% 50% formic acid in aceto-nitrile; flow: 0.0 min 1 ml/min, ramp, 2.5-4.5 min 2 ml/min; gradient: 0.0 min 10% B, ramp, 2.5 min 70% B, ramp, 3.0–4.5 min 95% B, regeneration.

Selected Procedures and Physical Data:

Lysobactin (1, bistrifluoroacetate, synthetic product): At room temperature aqueous TFA (0.1%, 3 ml) and palladium (10wt% on activated carbon, 10 mg) were added to a suspension of compound **13** (6.2 mg, 4.07 µmol) in dioxane (2 ml). The reaction mixture was hydrogenated under strictly controlled conditions at room temperature and atmospheric pressure for 15 minutes. After complete Cbz deprotection, the catalyst was removed immediately by filtration (PTFE syringe filter), the reaction mixture was concurrently concentrated in vacuo and the residual solution was then purified by preparative HPLC using a Macherey-Nagel column NUCLEODUR C_{18} Gravity, 5 µ, 250 × 21 mm (eluent: acetonitrile + 0.1% aqueous TFA). Yield: 5.4 mg, 88%. Analytical HPLC-coinjection with authentic lysobactin yielded a single, symmetric peak (methods 5 and 7). ^{1}H and ^{13}C NMR (500 MHz and 126 MHz, [D_5]pyridine, 35 mM) spectra were identical for synthetic and for natural lysobactin bistrifluoroacetate. HPLC (method 5): R_t = 16.31

min HPLC (method 7) R_t = 38.10 min. $[\alpha]^{20}_{Na}$ = -69° (*c* = 0.07, methanol). LC-MS (method 6): R_t = 2.54 min, MS (ESIpos): *m/z* (%) = 639.9 (100) $[M+2H]^{2+}$, 1276.8 (5) $[M+H]^{+}$; MS (ESIneg): *m/z* (%) = 637.0 (100) $[M-2H]^{2-}$, 1274.7 (40) $[M-H]^{-}$. HR-TOF-MS: $C_{58}H_{98}N_{15}O_{17}$ calcd. 1276.7260, found. 1276.7264 $[M+H]^{+}$. HR-FT-ICR-MS: calcd for $C_{58}H_{99}N_{15}O_{17}$ $[M + 2H]^{2+}$: 638.866620, found 638.867184. ^{1}H-NMR (500 MHz, $[D_5]$pyridine, 35 mM): δ (ppm) = 0.68 (d, *J* = 6.6 Hz, 3H), 0.76 (d, *J* = 6.5 Hz, 3H), 0.86 (d, *J* = 5.8 Hz, 3H), 0.99 (d, *J* = 5.8 Hz, 3H), 1.00 (d, *J* = 8.1 Hz, 3H), 1.03 (d, *J* = 7.7 Hz, 3H), 1.06 (d, *J* = 6.3 Hz, 3H), 1.12 (d, *J* = 6.5 Hz, 3H), 1.24 (d, *J* = 6.6 Hz, 3H), 1.28 (m, 1H), 1.38 (s, br, 1H), 1.57 (d, *J* = 6.0 Hz, 3H), 1.82 („t“, *J* = 12.5 Hz, 1H), 2.03 (m, 3H), 2.09–2.41 (m, 10H), 2.46 (s, br, 1H), 3.17 (s, br, 1H), 3.34 (s, br, 1H), 3.95 („d”, *J* = 8.8 Hz, 1H), 4.00 (dd, *J* = 7.7, 5.4 Hz, 1H), 4.16 (m, 2H), 4.34 (m, 2H), 4.50 (“d”, *J* = 13.1 Hz, 1H), 4.60 (dd, *J* = 11.0, 6.2 Hz, 1H), 4.68 (m, 2H), 4.76 (s, br, 1H), 5.09 (m, 1H), 5.20‡ (m, 1H), 5.29 (“t”, *J* = 9.6 Hz, 1H), 5.37 (dd, *J* = 15.0, 5.7 Hz, 1H), 5.46 (s, 1H), 6.09 (d, *J* = 10.0 Hz, 1H), 6.38 (“t”, *J* = 9.3 Hz, 2H), 6.76 (s, br, 1H), 7.26–7.31 (m, 2H), 7.57‡ (m, 1H), 7.69 (d, *J* = 5.2 Hz, 1H), 7.74 (d, *J* = 9.3 Hz, 1H), 7.94–7.98 (m, 3H), 8.02‡ (s, br, 1H), 8.15–8.19 (m, 3H), 8.31 (d, *J* = 8.8 Hz, 1H), 8.35 (s, 1H), 8.66 (d, *J* = 10.6 Hz, 1H), 8.91 (m, 1H), 9.26 (s, br, 1H), 9.83 (s, br, 1H), 10.94 (s, br, 1H), 11.07 (t, *J* = 5.8 Hz, 1H); 10 protons were not observed due to overlap and/or H/D exchange. ^{13}C NMR (126 MHz, $[D_6]$DMSO) d (ppm) = 11.09, 16.33, 19.08, 19.89, 20.48, 20.83, 21.31, 21.74, 22.80, 23.35, 24.26, 24.80, 25.06, 25.15, 26.74, 27.15, 28.99, 31.01, 36.47, 40.07, 41.29, 41.98 (2C), 44.62, 52.59, 53.33, 56.17, 56.27, 56.89, 58.47, 60.82, 60.91, 62.32, 62.89, 70.35, 72.20, 75.29, 75.41, 118.16 (q, *J* = 295 Hz, *C*F_3CO_2H), 128.50 (2C), 129.09, 129.14 (2C), 137.23, 158.05, 162.46 (q, *J* = 33.5 Hz, CF_3*C*O_2H), 168.49, 169.49, 170.30, 172.53, 173.28, 173.53, 173.69, 173.99, 174.12, 174.20, 175.19, 176.73; 1 carbon was not observed due to overlap.

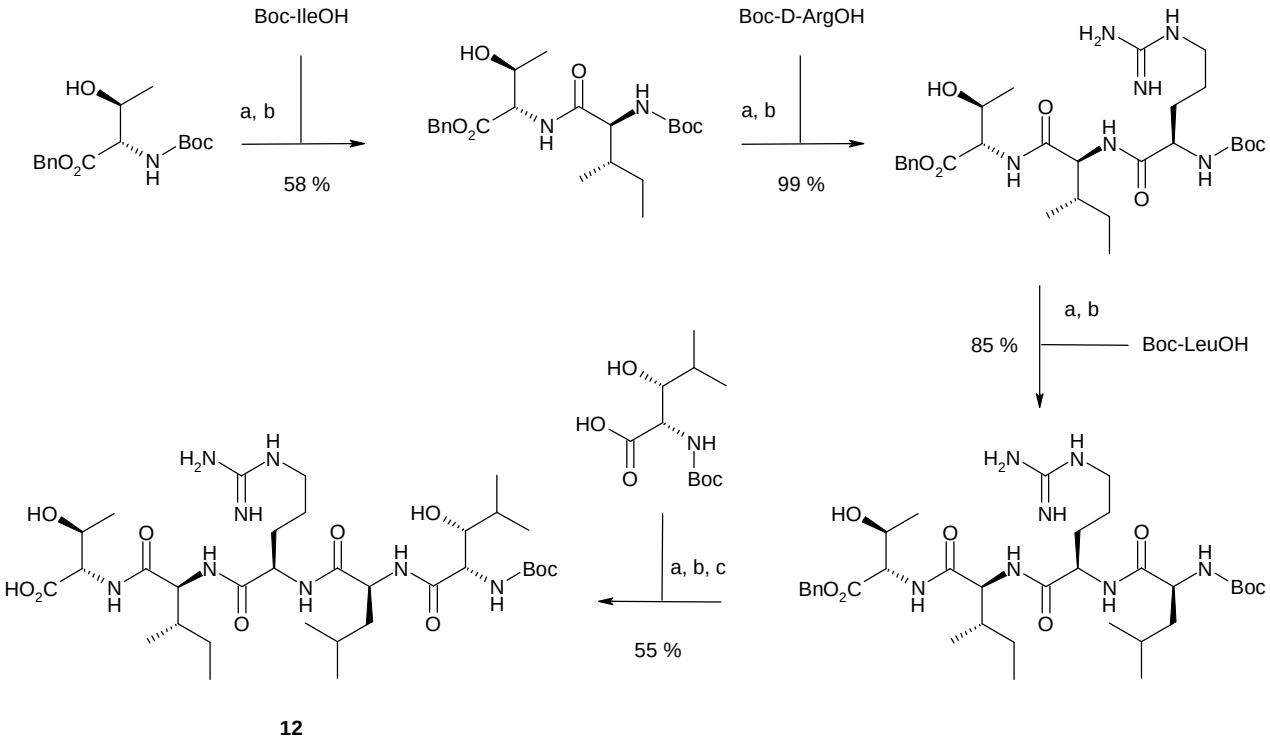
Lysobactin (1, bistrifluoroacetate, natural product): Natural lysobactin was obtained by fermentation.[29] $[\alpha]^{20}_{Na}$ = -66° (*c* = 0.245, methanol). HPLC (method 1): R_t = 6.19 min. HPLC (method 5): R_t =

16.04 min. LC-MS (method 6): R_t = 2.56 min, MS (ESIpos): *m/z* (%) = 639.2 (100) $[M+2H]^{2+}$, 1277.1 (5) $[M+H]^+$; MS (ESIneg): *m/z* (%) = 637.3 (100) $[M-2H]^{2-}$.

***N*-(*tert*-Butoxycarbonyl)glycyl-(3*S*)-3-hydroxy-L-asparagine (7):** *N*-(*tert*-Butoxycarbonyl)glycyl-(3*S*)-3-hydroxy-O^4-methoxy-L-aspartate (353 mg, 1.10 mmol) was dissolved in 25% aqueous ammonia (1.67 ml). The reaction was stirred at ambient temperature for 2 h, then the solvent was evaporated *in vacuo*. The residue was purified by HPLC (Kromasil-100 Å C_{18}, 5 µm; 250 × 30 mm; eluent A: 0.05% TFA in water, eluent B: acetonitrile; gradient: 0-3 min 5% B, 3-35 min ramp, 35-45 min 90% B; flow: 15 ml/min; UV detection at 210 nm. HPLC (method 2): R_t = 2.70 min. $[\alpha]^{20}_{Na}$ = -7° (*c* = 0.275, methanol). LC-MS (method 3): R_t = 2.21 min, MS (ESIpos): *m/z* (%) = 306 (70) $[M+H]^+$; MS (ESIneg): *m/z* (%) = 304 (100) $[M-H]^-$. ^{1}H NMR (500 MHz, [D_6]DMSO) d (ppm) = 3.55 (dd, *J* = 6.3, 17.3 Hz, 1H, 2`-$H_A$), 3.61 (dd, *J* = 6.5, 17.3 Hz, 1H, 2`-H_B), 4.33 (s, 1H, 3-H), 4.67 (d, *J* = 9.0 Hz, 1H, 2-H), 6.04 (br s, 1H, O*H*), 6.99 (m, 1H, N*H*), 7.15 (s, 1H, N*H*), 7.34 (s, 1H, N*H*), 7.58 (d, *J* = 9.0 Hz, 1H, N*H*), 12.89 (br s, 1H, COO*H*). ^{13}C NMR (126 MHz, [D_6]DMSO) d (ppm) = 28.1 (CH_3, OC(*C*H_3)$_3$), 43.0 (CH_2, C-2´), 54.5 (CH, C-2), 71.1 (CH, C-3), 78.0 (quat. O*C*(CH_3)$_3$), 155.7 (quat., N*C*OOC(CH_3)$_3$), 169.6 (quat, *C*=O), 171.6 (quat. *C*=O), 172.9 (quat., *C*=O). HR-TOF-MS: $C_{11}H_{19}N_3O_7$ calcd. 306.1296, found. 306.1300 $[M+H]^+$.

2-(Trimethylsilyl)ethyl-N^2-[(benzyloxy)carbonyl]-D-leucyl-L-leucyl-(3*R*)-3-{[glycyl-(3*S*)-3-hydroxy-L-asparaginyl-O^3-(*tert*-butyl)seryl]oxy}-L-phenylalaninat-trifluoroacetate (11): The $N^{2.1}$-Boc precursor (90 mg, 84 µmol) was dissolved in dichloromethane (3 ml). 15% TFA in dichlormethane (20 ml) was added, after 10 minutes the volatile phase was evaporated in vacuo at room temperature. The residue was suspended in dichloromethane and the volatiles were again removed in vacuo (2×). Purification by HPLC followed. Yield: 73 mg, 80%. HPLC (method 9): R_t = 2.65 min. LC-MS (method 10): R_t = 2.13 min, MS (ESIpos): *m/z* (%) = 972.6 (100) $[M+H]^+$; MS (ESIneg): *m/z* (%) = 970.7 (100) $[M-H]^-$. HR-TOF-MS: $C_{47}H_{74}N_7O_{13}Si$ calc. 972.5109, found 972.5103 $[M+H]^+$. ^{1}H NMR

(500 MHz, [D_5]pyridine) δ (ppm) = 1.10 (s, 9H), 2.13-1.93 (m, 27H), 3.17-3.00 (m, 5H), 4.79 (m, 1H), 4.94 (m, 1H), 5.45-5.38 (dd, *J* = 5.8, 8.0 Hz, 3H), 5.97 (m, 1 H), 6.46 (m, 1 H), 6.62 (m, 2H), 6.9 (dd, *J* = 4,2, 7.2 Hz, 1H), 7.18 (m, 1 H), 7.90 (m, 1 H), 8.53-8.43 (m, 6H), 8.62 (d, *J* = 5.8 Hz, 2H), 8.91-8.80 (m, 3H), 9.47 (s, br, 1H), 9.53 (s, br, 1H), 9.77 (m, 1H), 10.29 (m 1H), 10.69-10.79 (m, 1H), 11.05-11.15 (m, 1H); 3 protons were not identified due to overlap with the water peak and/or H/D exchange. ^{13}C NMR (126 MHz, [D_5]pyridine) δ (ppm) = 0.0 (3C), 19.06 (2C), 23.33 (2C), 23.48 (2C), 24.86 (4C), 26.75, 26.83, 28.90 (3C), 43.38, 43.77, 53.78, 55.90, 56.38, 58.77, 58.92, 63.91, 65.85, 68.49, 73.90, 75.07, 78.31, 129.41, 129.81, 129.89, 130.35, 130.40, 130.47, 138.98, 139.45, 155.08, 159.20, 171.61, 171.73, 172.68, 175.21, 175.36, 176.83.



Scheme 1. Liquid phase synthesis of the southern fragment **12** with a Boc protection strategy. Reagents and conditions: a) 30% TFA, DCM, 30 min, room temp., b) *N*-Boc amino acid as indicated (1.2 equiv.), HATU (1.3 equiv.), NMM (6 eqiv.) DMF (3-10 mL / mmol of *N*-Boc amino acid), -20 °C – room temp, 16 h, c) H_2, 10% Pd/C, AcOH, room temp. 2 h. DCM = dichloromethane; HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate; NMM = *N*-methylmorpholine.

[(*3R*)-N^2-(*tert*-Butoxycarbonyl)-3-hydroxy-L-leucyl]-L-leucyl-D-arginyl-L-isoleucyl-L-allothreonine-acetate (12): The precursor peptide benzyl [(*3R*)-N^2-(*tert*-butoxycarbonyl)-3-hydroxy-L-leucyl]-L-leucyl-D-arginyl-L-isoleucyl-L-allothreoninate-trifluoracetate was synthesized by a liquid phase peptide coupling strategy using Boc protection, HATU as the coupling agent and a benzyl ester group for C-terminal protection. The last step of this sequence was hydrogenolytic *C*-terminal deprotection: (100 mg, 0.11 mmol) of crude [(*3R*)-N^2-(*tert*-butoxycarbonyl)-3-hydroxy-L-leucyl]-L-leucyl-D-arginyl-L-isoleucyl-L-allothreoninate-trifluor-acetate was dissolved in glacial acetic acid (4.3 ml), 10% palladium on charcoal (22 mg) was added and the mixture was hydrogenated at room temperature and ambient pressure over 2 h. The catalyst was removed by filtration, the solvent was distilled and the residue was purified by HPLC (UV-Detector 210 nm; column: Kromasil RP_{18} 5 µm, 100 Å, 250 × 20 mm; eluent A: water + 0.05% TFA, eluent B: acetonitrile + 0.05% TFA: flow: 10 ml/min; 0-3 min 10% B, ramp, 30-38 min 90% B, 38-45 min 10% B. 58 mg (60 µmol, 55%) of the pure title compound were isolated. HPLC (method 2): R_t = 3.75 min. $[\alpha]^{20}_{Na}$ = -14.4 (*c* = 1.0, methanol). LC-MS (method 4): R_t = 1.80 min, MS (ESIpos): *m/z* (%) = 731.8 (100) $[M+H]^+$. ^{1}H NMR (500 MHz, [D_6]DMSO) d (ppm) = 0.77–0.91 (m, 22 H), 1.08–1.09 (m, 4 H), 1.34–1.75 (m, 22 H), 3.06–3.07 (m, 2 H), 3.47 (m, 1 H), 3.89 (m, 1 H), 4.08–4.12 (m, 2 H), 4.34 (m, 3 H), 4.56 (d, *J* = 6.9 Hz, 1 H), 6.46 (d, *J* = 9.0 Hz, 1 H), 7.83 (d, *J* = 7.3 Hz, 1 H), 7.87 (d, *J* = 7.4 Hz, 1 H), 7.99 (d, *J* = 7.7 Hz, 1 H). ^{13}C NMR (126 MHz, [D_6]DMSO) d (ppm) = 10.8 (CH_3), 15.1 (CH_3), 18.2 (CH_3), 19.1 (CH_3), 21.5 (CH_3), 23.0 (CH_3), 22.9 (CH_3), 23.9 (CH_3), 24.0 (CH_2), 24.8 (CH_2), 28.0 (C(*C*H_3)$_3$, 3C), 29.5 (CH_2), 30.4 (CH_3), 36.7 (CH_3), 40.0 (CH_2), 40.2 (CH_2), 40.8 (CH_2), 51.3 (CH), 51.9 (CH), 56.1 (CH_2), 57.1 (CH), 58.4, (CH), 66.5 (CH), 68.3 (CH), 75.7 (quat.), 78.2 (CH), 155.3 (quat.), 156.5 (quat.), 170.9 (quat.), 171.0 (quat.), 171.9 (quat.). HR-TOF-MS: $C_{33}H_{63}N_8O_{10}$ calcd. 731.4662, found 731.4677 $[M+H]^+$.

***N*²-[(Benzyloxy)carbonyl]-D-leucyl-L-leucyl-(3*R*)-(3*R*)-3-hydroxy-L-leucyl-L-leucyl-D-arginyl-L-isoleucyl-L-allothreonyl-glycyl-(3*S*)-3-hydroxy-L-asparaginyl-seryl]oxy}-L-phenylalanine-bistrifluoracetate (13):** A mixture consisting of triisopropylsilane (86 µl), TFA (3.4 ml) and water (19 µl) was added to the *N*$^{2.1}$-Boc-*N*$^{2.8}$-*tert*-butyl precursor (17.2 mg, 10.1 µmol). After stirring for 1 h at room temperature the solvent was evaporated in vacuo. The residue was suspended in dichloromethane and the volatiles were again removed in vacuo (2×). Purification by preparative HPLC followed immediately. Yield: 16.2 mg, 96%. HPLC (method 9): R_t = 1.97 min. LC-MS (method 4): R_t = 1.65 min, MS (ESIpos): *m/z* (%) = 715.3 (100) $[M+2H]^{2+}$; MS (ESIneg) *m/z* (%) = 1427.7 (100) $[M-H]^-$. HR-TOF-MS: $C_{66}H_{106}N_{15}O_{20}$ calcd. 1428.7734, found 1428.7700 $[M+H]^+$. The NMR spectra showed conformers.

***N*^{1,3}-Benzyloxycarbonyl-lysobactin (3, trifluoroacetate):** Compound **13** (1.8 mg, 1.1 µmol) was dissolved in DMF (1 ml), the solution was cooled to –15°C, HATU (1.25 mg, 3.3 µmol, 3 equivalents) and NMM (0.95 µl, 8 equivalents) were added and the mixture was stirred for 3 h, HPLC control indicated completion of the reaction. The reaction was quenched with potassium dihydrogen phosphate, the solvent was distilled off and the product was purified by HPLC (acetonitrile water system + 0.1% TFA, RP_{18} phase). Yield: 1.2 mg, 72%. HPLC (method 4): R_t = 2.17 min. HPLC (method 8): R_t = 11.18 min. HR-FT-ICR-MS: calcd for $C_{66}H_{105}N_{15}O_{19}$ $[M + 2H]^{2+}$: 705.885010, found 705.885167. ^{1}H NMR (500 MHz, [D_5]pyridine) δ (ppm) = 0.89-0.92 (m, 6H), 0.92-0.95 (s,3H), 0.95-0.98 (m, 4H), 0.98-1.02 (m, 3H), 1.02-1.08 (m, 9H), 1.17-1.21 (d, *J* = 5.7 Hz 3H), 1.22-1.26 (d, *J* = 5.1 Hz, 3H), 1.32 (m, 1H), 1.42 (m, 1H), 1.54-1.58 (d, *J* = 4.9 Hz, 3H), 1.82 (m, 2H), 1.95 (m, 2H), 2.06-2.35 (m, 11H), 2.44 (t, *J* = 10.5 Hz, 3.16 (m, 1H), 3.27 (m, 1H), 3.90-4.00 (m, 2H), 4.15 (m, 1H), 4.21 (dd, *J* = 3.5, 8.1 Hz, 1H), 4.33 (m, 1H), 4.51 (m, 2H), 4.60 (m, 1H), 4.63-4.75 (m, 3H), 5.30 (m, 2H), 5.43 (s, 1H), 5.64 (d, *J* = 8.2 Hz, 1H), 5.91 (s, br, 1H), 5.95 (s, br, 1H), 6.09 (d, J = 8.4, 1H), 6.54 (m, 1H), 6.83 (m, 1H), 7.06 (d, J = 8 Hz, 1H), 7.29 (m, 2H), 7.38 (m, 2H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.54-7.57 (m, 5H), 7.58-7.66 (m, 5H), 7.79 (m, 1H), 8.05-8.16 (m, 6H), 8.35 (s,

br, 2H), 8.64 (d, *J* = 8.0 Hz, 1H), 9.12 (s, br, 1H), 9.29 (d, *J* = 5.2 Hz, 1H), 11.04 (s, br, 1H), 11.15 (s, br, 1H); one OH-signal was not identified due to overlap with the water peak. ^{13}C NMR (126 MHz, [D_5]pyridine) δ (ppm) = 11.44, 16.56, 18.45, 20.57, 20.67, 21.08, 22.40, 22.74, 23.67, 24.09, 24.33, 25.30, 25.65, 25.67, 27.32, 27.49, 29.55, 31.88, 37.46, 40.14, 41.62, 41.90, 42.18, 44.88, 53.73, 54.89, 56.28, 56.66, 56.83, 57.41, 58.73, 60.69, 62.05, 62.26, 63.21, 68.42, 70.59, 72.89, 76.11, 76.68, 128.90, 129.06, 129.33 (2C), 129.36 (2C), 129.79, 129.84, 137.03, 137.66, 158.58, 159.12, 169.73, 170.16, 170.18, 170.74, 172.48, 172.53, 173.45, 173.61, 173.96, 174.30, 176.25, 175.46, 178.09; no distinct signal was identified for D-Arg^6 C$^?$.

Table 1. ^{1}H, ^{13}C, and ^{15}N NMR chemical shift assignments of natural lysobactin (**1**, bistrifluoroacetate, 600 MHz, [D_6]DMSO).

	NH	CO	a	ß	?	? Me	d	dMe	Other
D-Leu[1]	7.99		4.11	1.62, 1.50	1.66	---	---	0.92, 0.92	---
	N.O.	172.1	51.0	40.3	23.8			22.8, 21.4	
Leu[2]	9.62		3.73	1.91, 1.24	1.89	---	---	0.91, 0.76	---
	125.1	176.6	55.4	38.7	23.7			23.1, 19.6	
*Hy*Phe[3]	8.19		5.58	6.04	---	---	---	---	7.42 (o), 7.24 (m), 7.20 (p)
	120.0	172.9	60.0	74.6					127.3 (o), 128.2 (m), 128.6 (p)
*Hy*Leu[4]	8.09		3.64	3.40	1.81	---	---	0.98, 0.80	6.42 (OH)
	121.3	174.4	59.0	74.0	23.5			19.4, 18.9	
Leu[5]	7.12		4.04	1.51, 1.38	1.83	---	---	0.74, 0.68	---
	128.8	175.1	51.8	40.8	30.5			22.6, 23.6	
D-Arg[6]	6.90		3.67	1.51, 1.48	1.44, 1.19	---	2.76, 2.71	---	7.29 (eNH)
	120.0	174.2	54.7	27.6	25.9		39.7		156.5 (guanidine), 90.2 (eNH)
Ile[7]	7.49		3.70	1.71	1.43, 0.92	0.85	---	0.72	---
	118.4	173.6	59.4	35.3	25.7	15.5		10.6	
*a*Thr[8]	6.95		4.34	3.51	---	1.16	---	---	5.36 (OH)
	113.6	172.3	57.0	69.5		20.5			
Gly[9]	9.23		4.00, 3.54	---	---	---	---	---	---
	119.0	171.2	43.1						
*Hy*Asn[10]	7.58		4.75	4.30	---	---	---	---	7.36, 7.18 ($CONH_2$), 5.65 (OH)
	107.8	170.3	55.3	70.7					176.1, 109.5 ($CONH_2$)
Ser[11]	6.94		4.53	3.65, 3.54	---	---	---	---	5.23 (OH)
	116.3	169.3	54.9	61.6					

Table 2. ^{1}H NMR chemical shift assignments of natural lysobactin (**1**, bis-trifluoroacetate, 600 MHz, H_2O/D_2O 4:1).

Residue	NH	a	ß	?	? Me	d	d**Me**	Other
D-**Leu**[1]	---	4.20	1.72, 1.72	1.62	---	---	0.97, 0.97	---
Leu[2]	9.02	3.97	1.85, 1.32	1.87	---	---	0.98, 0.87	---
***Hy*Phe**[3]	8.39	5.76	6.15	---	---	---	---	7.46 (o), 7.38 (m), 7.37 (p)
***Hy*Leu**[4]	8.21	3.82	3.57	1.81	---	---	0.99, 0.86	---
Leu[5]	7.58	4.29	1.66, 1.62	1.82	---	---	0.86, 0.84	---
D-**Arg**[6]	7.19	4.02	1.65, 1.65	1.54, 1.46	---	3.01, 2.79	---	7.10 (eNH)
Ile[7]	7.92	3.90	1.77	1.48, 1.12	0.99	---	0.84	---
***a*Thr**[8]	7.06	4.40	3.61	---	1.31	---	---	---
Gly[9]	8.94	4.18, 3.89	---	---	---	---	---	---
***Hy*Asn**[10]	8.05	4.95	4.57	---	---	---	---	---
Ser[11]	7.55	4.66	3.92, 3.79	---	---	---	---	---

Table 3. ^{1}H NMR chemical shift assignments of natural lysobactin (**1**, bis-trifluoroacetate, 600 MHz, H_2O/CD_3CO_2D 5:1).

Residue	NH	a	ß	?	? Me	d	d**Me**	a
D-**Leu**[1]	---	4.29	1.75, 1.71	1.61	---	---	0.97, 0.95	---
Leu[2]	9.22	3.95	1.89, 1.31	1.89	---	---	0.97, 0.85	---
***Hy*Phe**[3]	8.42	5.78	6.22	---	---	---	---	7.46 (o), 7.36 (m), 7.36 (p)
***Hy*Leu**[4]	8.23	3.84	3.57	1.87	---	---	1.01, 0.85	---
Leu[5]	7.54	4.33	1.62, 1.61	1.84	---	---	0.85, 0.81	---
D-**Arg**[6]	7.17	4.01	1.66, 1.65	1.57, 1.46	---	3.00, 2.80	---	7.08 (eNH)
Ile[7]	7.89	3.91	1.75	1.47, 1.10	0.97	---	0.83	---
***a*Thr**[8]	7.08	4.44	3.61	---	1.31	---	---	---
Gly[9]	8.89	4.21, 3.90	---	---	---	---	---	---
***Hy*Asn**[10]	8.07	4.99	4.63	---	---	---	---	7.86, 7.33 ($CONH_2$)
Ser[11]	7.46	4.71	3.92, 3.80	---	---	---	---	---

Table 4. Observed intraresidue NOE cross-peaks of natural lysobactin (**1**, bis-trifluoroacetate, 600 MHz, [D_6]DMSO). Strong cross-peaks observed for geminal protons are not included. Abbreviations: s = strong, m = medium, w = weak, vw = very weak, o = overlap (ambiguous). Others: *Hy*Phe NH – *Hy*Phe *ortho*-ArH (vw), *Hy*Phe α – *Hy*Phe *ortho*-ArH (m), *Hy*Pheß? – *Hy*Phe *ortho*-ArH (m), *Hy*Phe *ortho*-ArH – *Hy*Phe *meta*-ArH (m), *Hy*Phe *meta*-ArH – *Hy*Phe *para*-ArH (m).

	aNH	**a-ß**	**a-?**	**a-**d	**ßNH**	**ß-?**	**ß-**d	**?-NH**	**?-**d	d**-NH**
D-Leu[1]	---	m w	w	m	---	m w	o o o o	---	o o	---
Leu[2]	w	m w	w	m	m w	m w	m m m m	m	m m	w w
***Hy*Phe**[3]	w	w	---	---	m	---	---	---	---	---
***Hy*Leu**[4]	w	m	w	m	w	w	m m	m	m m	w w
Leu[5]	w	m w	w	m	m w	m w	m m m m	m	m m	w w
D-Arg[6]	w	m w	m w	vw vw	s w	m w m w	m w m m	w w	m m m w	w vw
Ile[7]	w	w	m w	vw	m	m w m (?-Me)	m	m w	m m	vw
***a*Thr**[8]	w	w	m	---	m	m	---	m	---	---
Gly[9]	m w	---	---	---	---	---	---	---	---	---
***Hy*Asn**[10]	w	m	---	---	w	---	---	---	---	---
Ser[11]	w	m w	---	---	m m	---	---	---	---	---

Table 5. Observed Interresidue NOE Cross-Peaks of natural lysobactin (1, bis-trifluoroacetate, 600 MHz, [D_6]DMSO). Abbreviations: s = strong, m = medium, w = weak, vw = very weak, o = overlap (ambiguous). Cross-Peaks Observed for Long-Range Interactions: Leu^2 α – Leu^5 ? (w), Leu^2 α – Leu^5 δMe_b (w), Leu^2 α – Ile δMe (m), Leu^2 α – Ile ß (m), Leu^2 α – Ile ? (w), *Hy*Phe NH – Ser $ß_a$ (w), *Hy*Phe α - Thr α (w), *Hy*Phe α – Arg $ß_a$ (w), *Hy*Phe α – Arg $ß_b$ (vw), *Hy*Phe α – Arg δ_a (w), *Hy*Phe α – Arg δ_b (vw), *Hy*Phe α – Gly α_a (vw), *Hy*Phe α – Thr NH (w), *Hy*Phe ß – *Hy*Leu ? (w), *Hy*Phe ß – Ser $ß_a$ (vw), *Hy*Phe ß – *Hy*Leu δMe_a (vw), *Hy*Phe pArH – Arg eNH (w), *Hy*Phe mArH – Arg eNH (w), *Hy*Phe pArH – Arg δ_a (w), *Hy*Phe mArH – Arg δ (m, vw), *Hy*Phe oArH – Arg δ (m, w), *Hy*Phe oArH – Arg ß (w, vw), *Hy*Phe mArH – Arg $ß_a$ (vw), *Hy*Phe oArH – Gly α (m, vw), *Hy*Phe mArH – Gly α (m, vw), *Hy*Phe oArH – *Hy*Leu ? (w), *Hy*Phe oArH – *Hy*Leu δMe_a (m), *Hy*Phe mArH – *Hy*Leu δMe_a (w), *Hy*Phe oArH – *Hy*Leu α(w), Leu^5 $ß_a$ – Ile ?Me (w), Leu^5 δMe_a – Ile ?Me (w), Leu^5 δMe_a – Ile δMe (w), Ile ß – Leu^5 $ß_a$ (m), Ile ß – Leu^5 δMe_a (w), Thr ß – Ile ß (w), Thr ß – Leu^2 ß (w, w), Thr ß – Leu^2 δMe_a (vw), Thr Me – Leu^2 δMe_b (w), Thr Me – Ile ß (w), Thr Me – Ile ?Me (m), Ser ß – Thr ß (m, w), Ser $ß_a$ – Thr Me (vw).

	NH_i - NH_{i+1}	NH_i - NH_{i+2}	a_i-NH_{i+1}	a_i-NH_{i+2}	a_i-NH_{i+3}	a_i-NH_{i+4}	a_i-a_{i+1}	a_i-b_{i+3}	b_i-NH_{i+1}
D-Leu[1]	---	---	m	w	vw	---	vw	---	vw vw
Leu[2]	m	vw	w	vw	w	---	vw	w vw	w vw
***Hy*Phe[3]**	m	w	w	vw	w	w	vw	w vw	m
***Hy*Leu[4]**	m	vw	w	w	---	---	vw	---	w
Leu[5]	m	w	w	vw	---	---	vw	---	w vw
D-Arg[6]	m	vw	w	---	---	---	vw	---	w w
Ile[7]	m	---	w	---	---	---	vw	---	m
***a*Thr[8]**	vw	vw	m	w	vw	---	vw vw	---	vw
Gly[9]	m	vw	w w	vw ---	---	---	vw vw	---	---
***Hy*Asn[10]**	s	---	w	---	---	---	vw	---	vw
Ser[11]	---	---	---	---	---	---	---	---	---

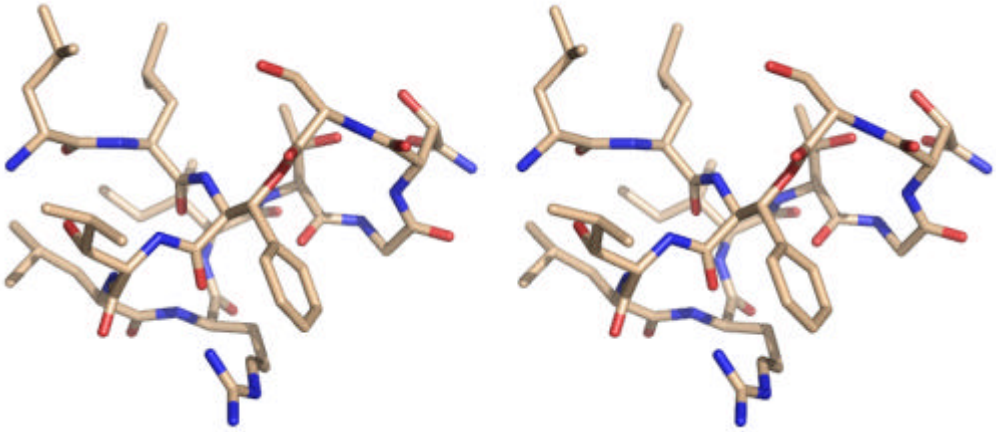


Figure 1. NMR solution structure of **1** (trifluoroacetate), orthoscopic view.

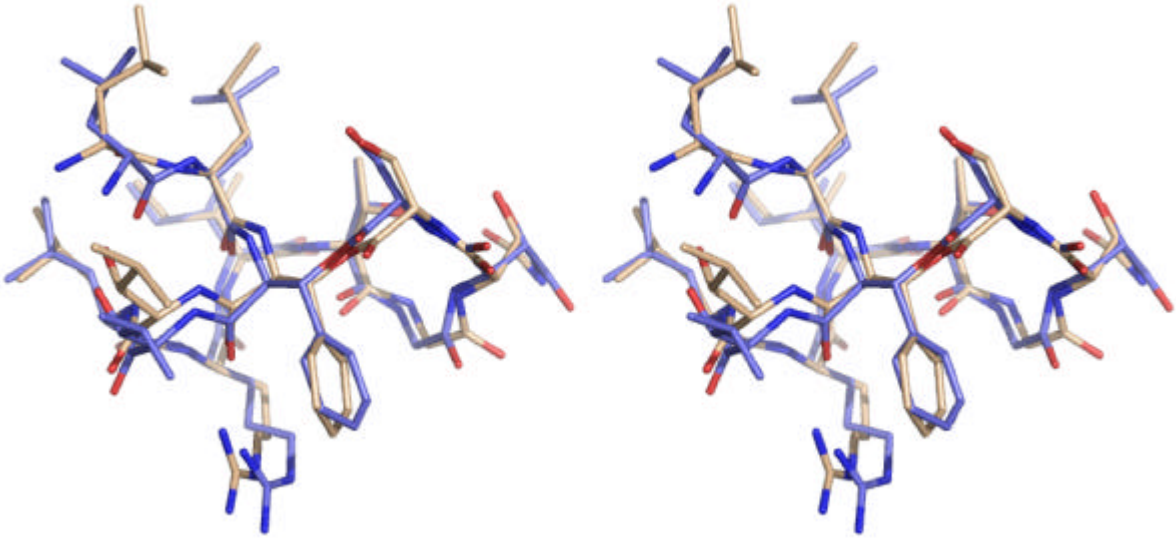


Figure 2. Alignment of the NMR solution structure (beige carbons) and the crystal structure (purple carbons) of **1** (bistrifluoroacetate), orthoscopic view.

Selected NMR spectra:

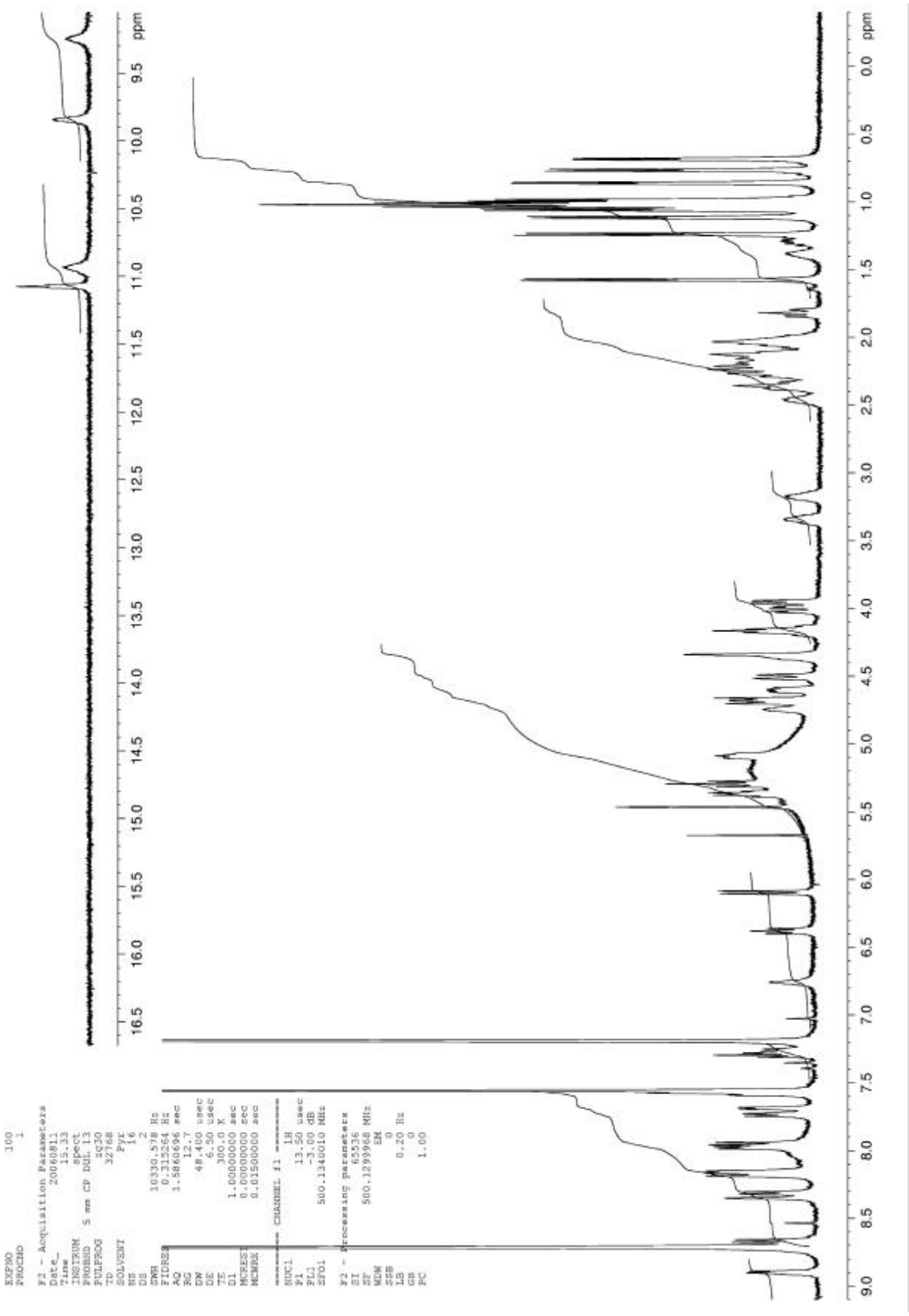


Figure 3. ^{1}H-NMR of natural lysobactin (**1**, bistrifluoroacetate, 500 MHz, [D_5]pyridine, 35 mM, reference spectrum). CH_2Cl_2 impurity at 5.65 ppm.

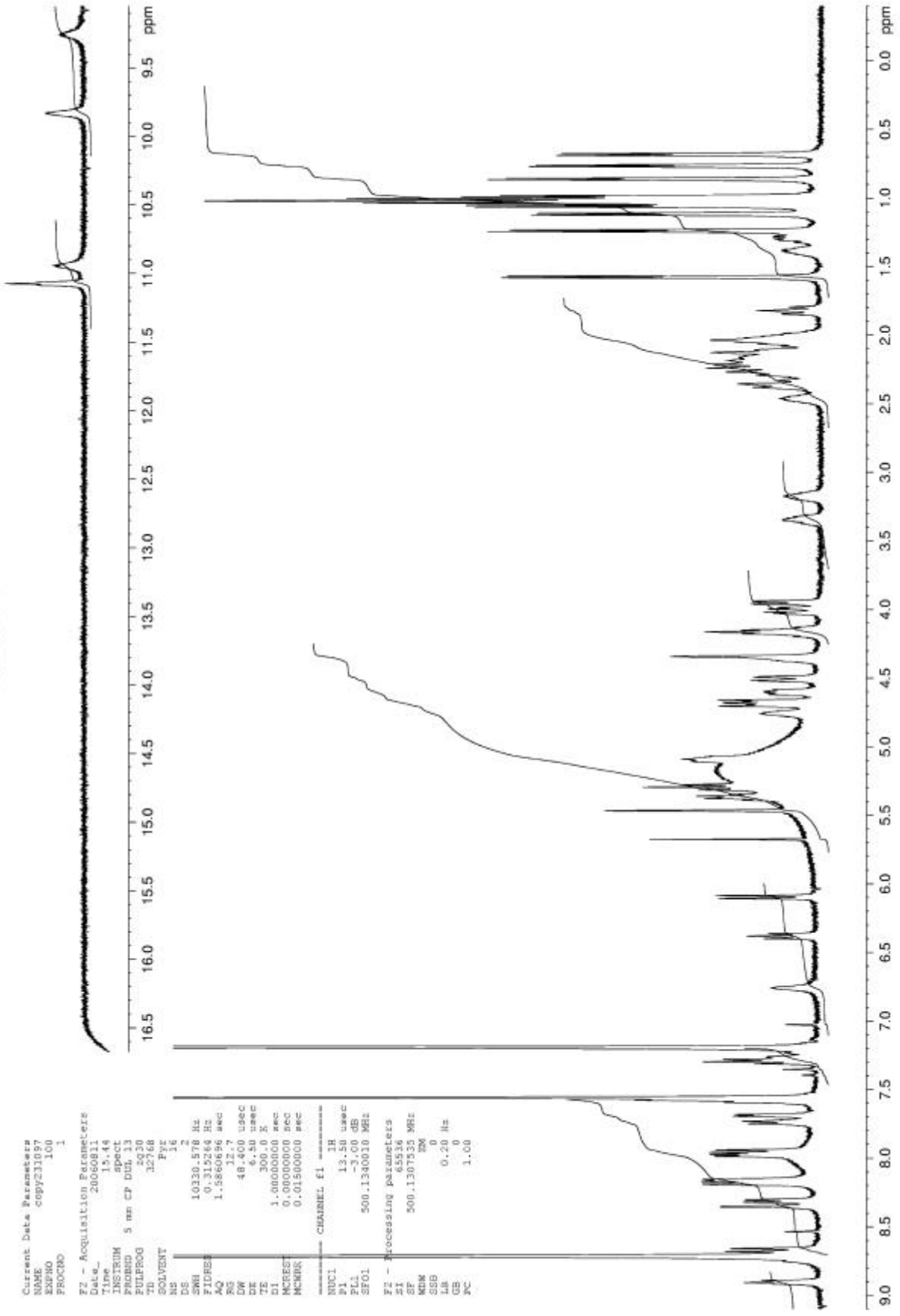


Figure 4. ^{1}H-NMR of synthetic lysobactin (**1**, bistrifluoroacetate, 500 MHz, [D_5]pyridine, 35 mM, reference spectrum). CH_2Cl_2 impurity at 5.65 ppm.

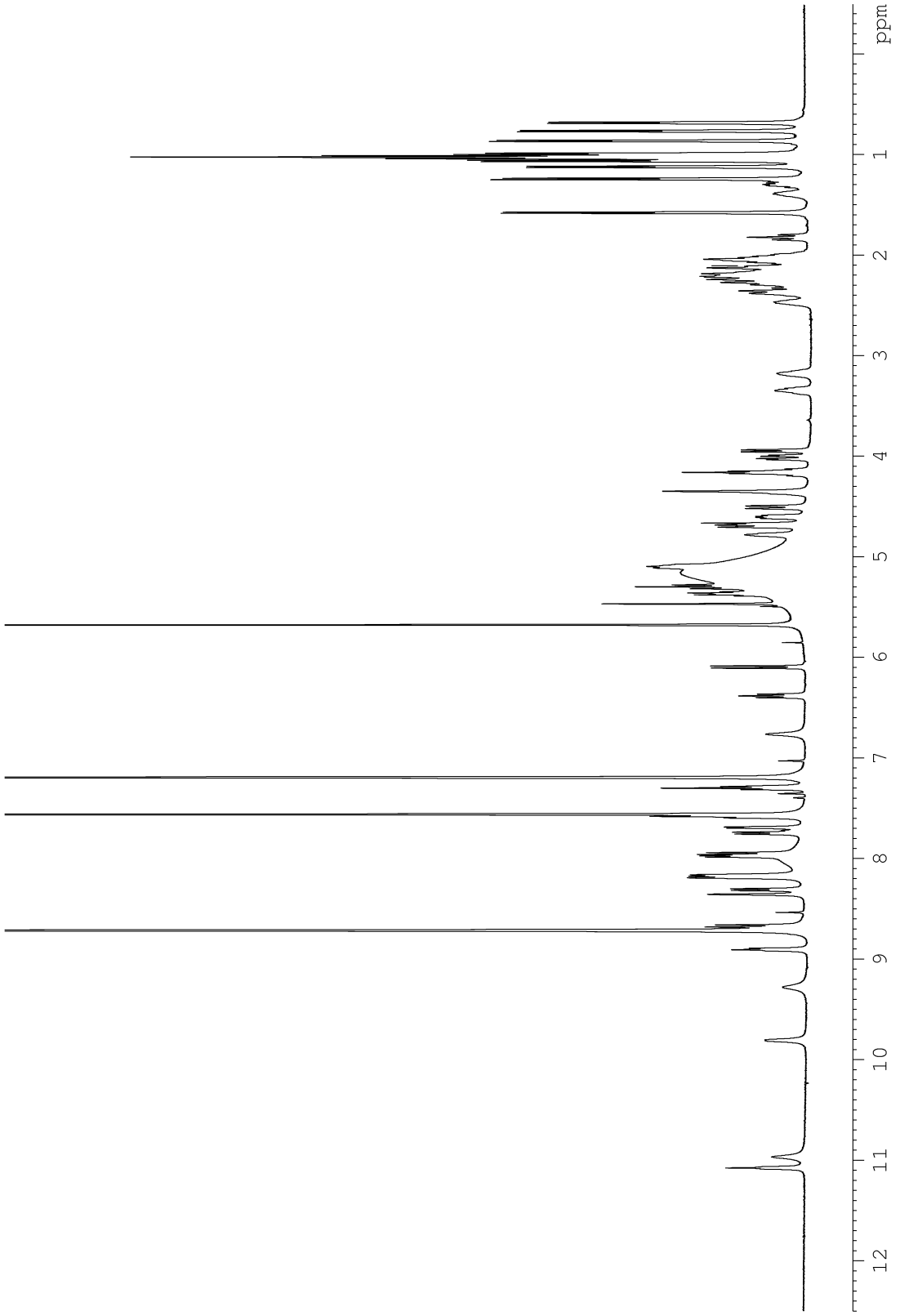


Figure 5. ^{1}H-NMR of synthetic lysobactin (**1**, bistrifluoroacetate, 500 MHz, [D_5]pyridine, 35 mM, long-term experiment). CH_2Cl_2 impurity at 5.65 ppm.

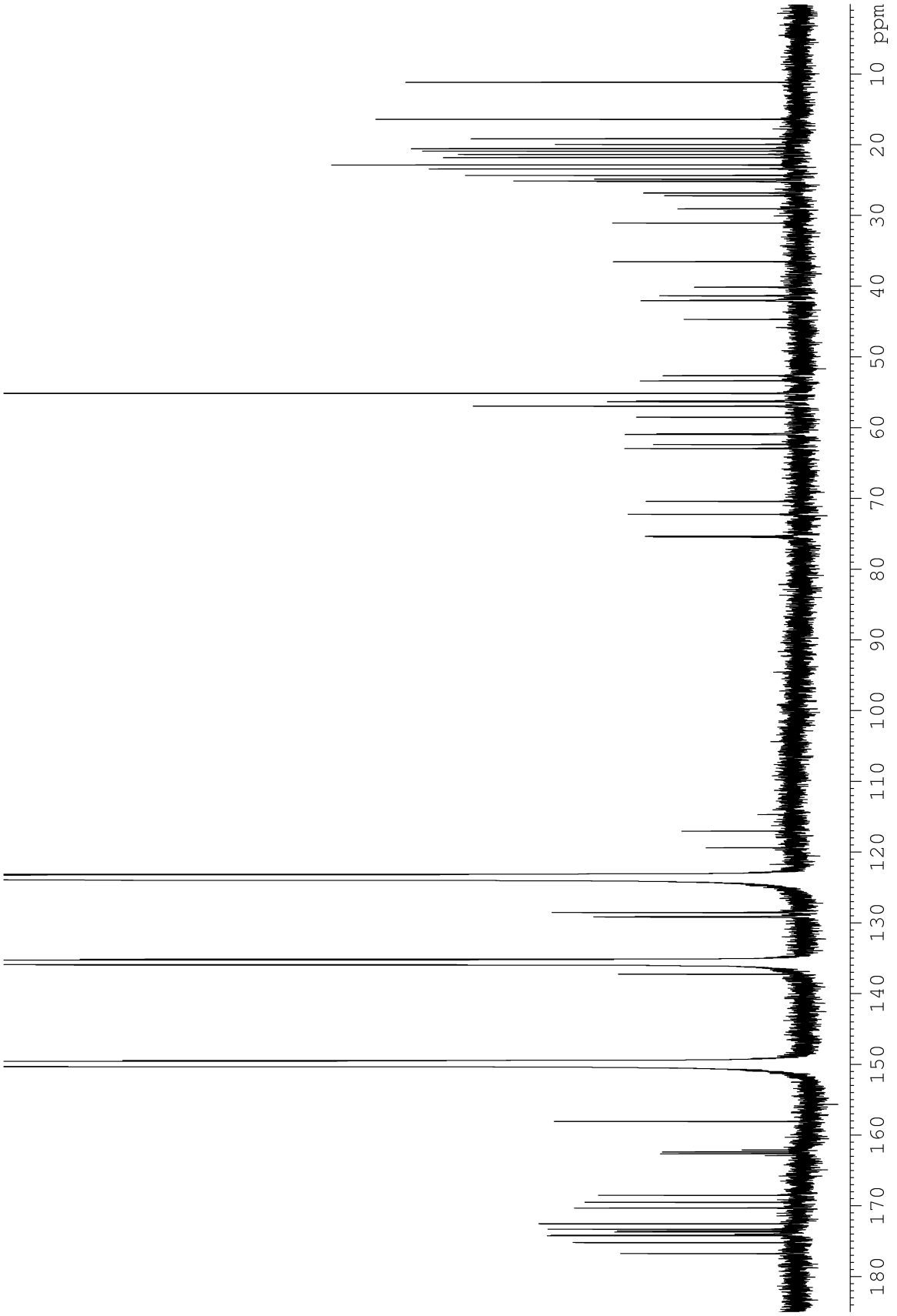


Figure 6. ^{13}C-NMR of synthetic lysobactin (**1**, bistrifluoroacetate, 126 MHz, [D_5]pyridine, 35 mM).

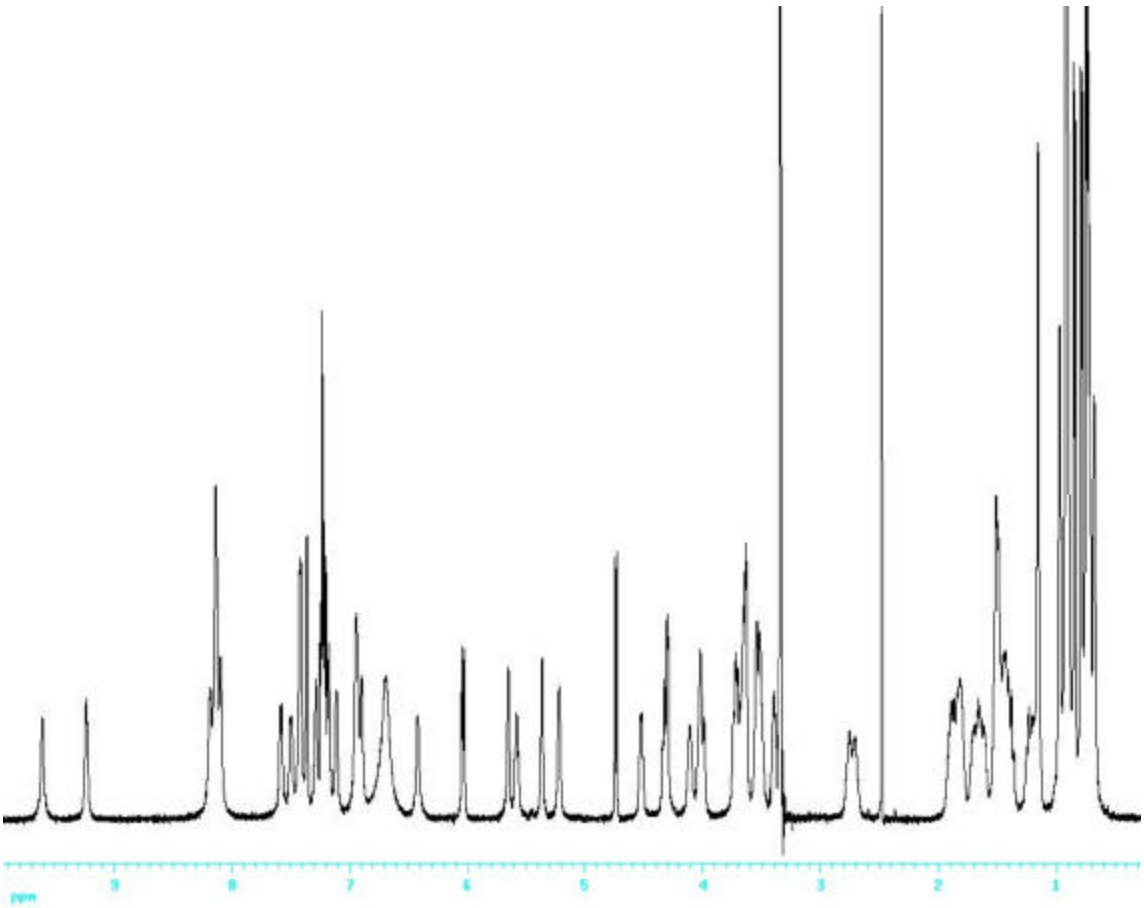


Figure 7. ^{1}H-NMR of natural lysobactin (**1**, bistrifluoroacetate, 600 MHz, [D_6]DMSO).

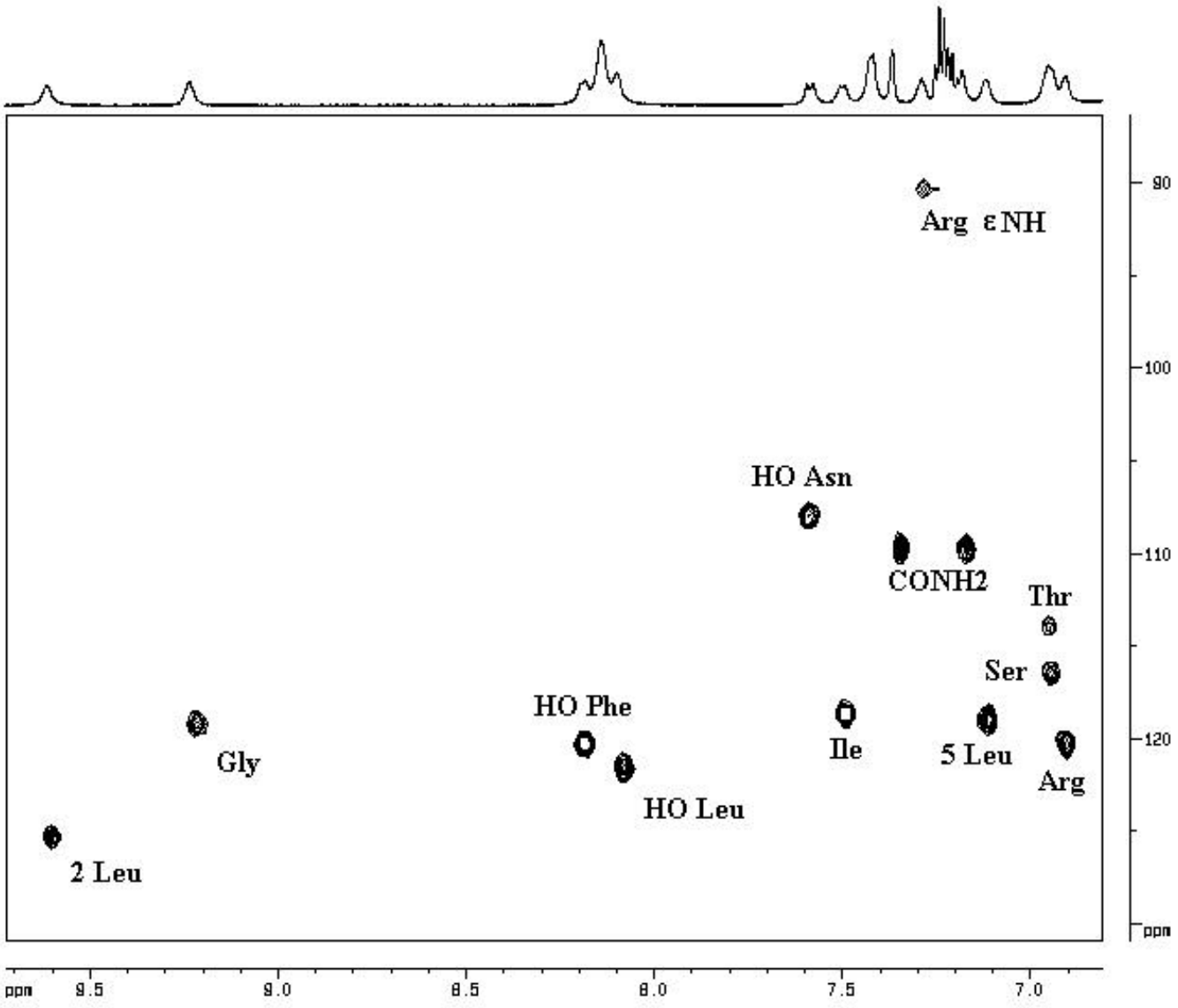


Figure 8. Natural abundance ^{1}H-^{15}N HSQC spectrum of natural lysobactin (**1**, bistrifluoroacetate, 600 MHz, [D_6]DMSO, 25 mM).

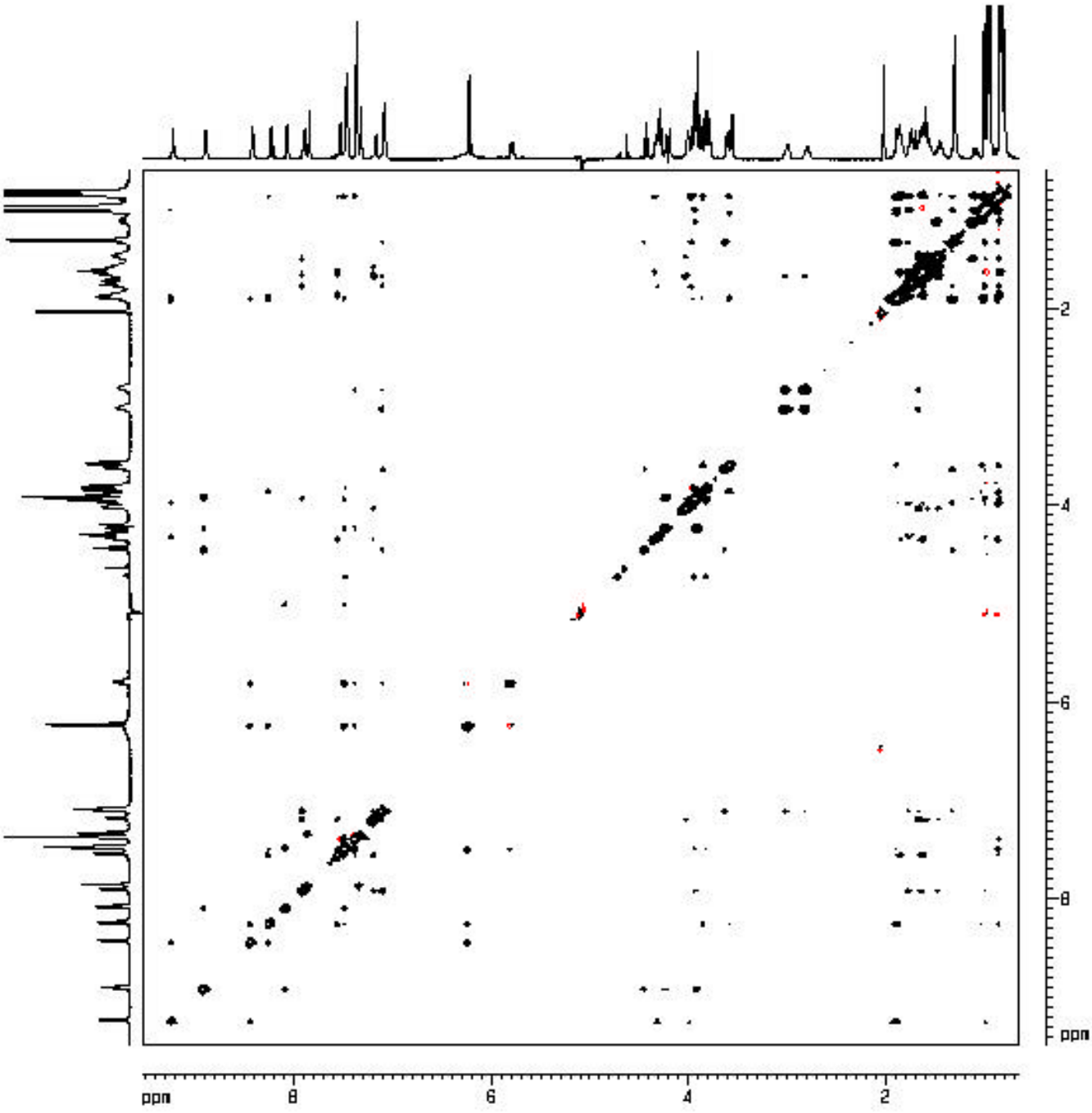


Figure 9. NOESY spectrum of natural lysobactin (**1**, bistrifluoroacetate, 600 MHz, H_2O/D_2O 4:1).

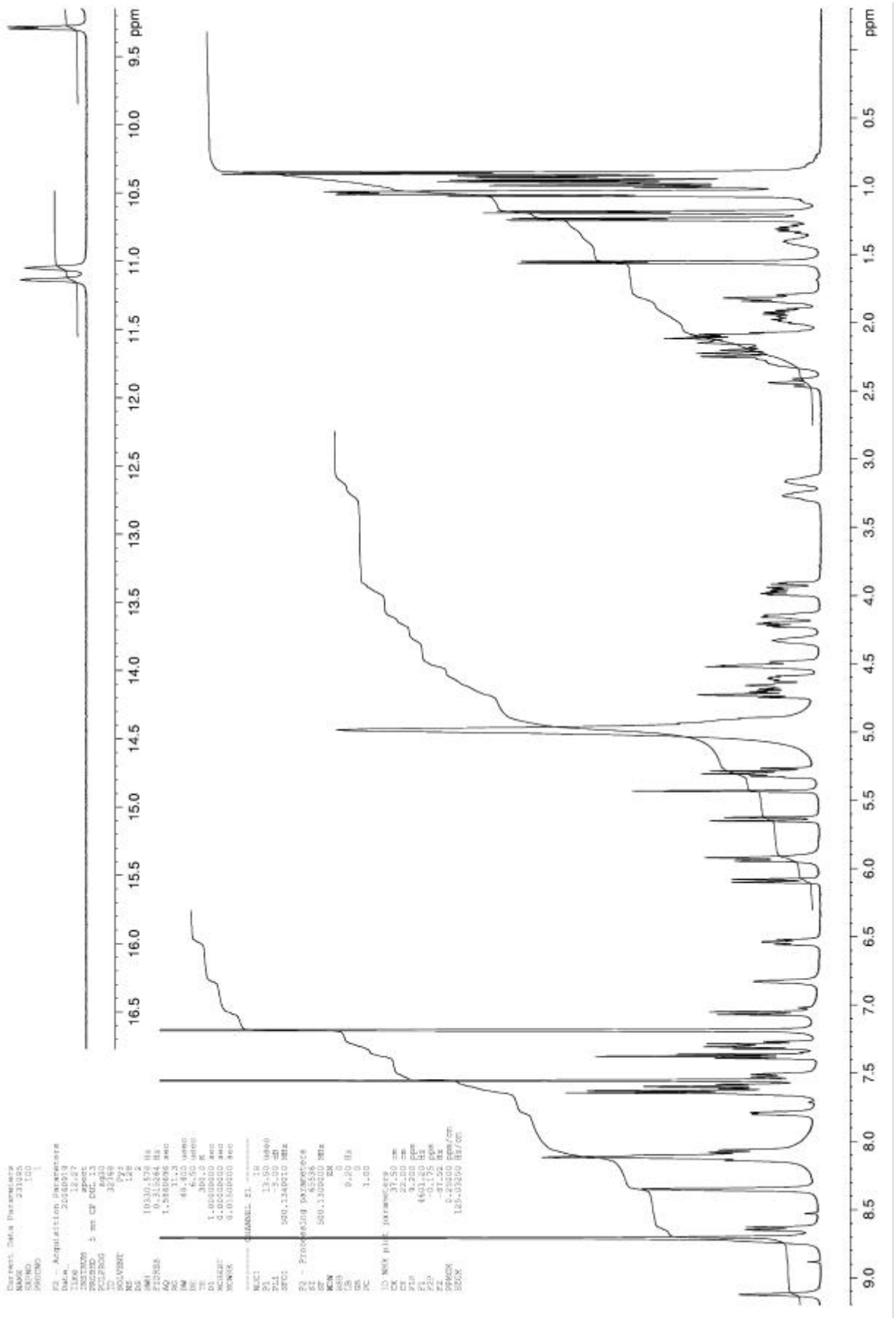


Figure 10. ^{1}H-NMR of synthetic $N^{2,1}$-Cbz-protected lysobactin (**3**) (500 MHz, [D_5]pyridine).

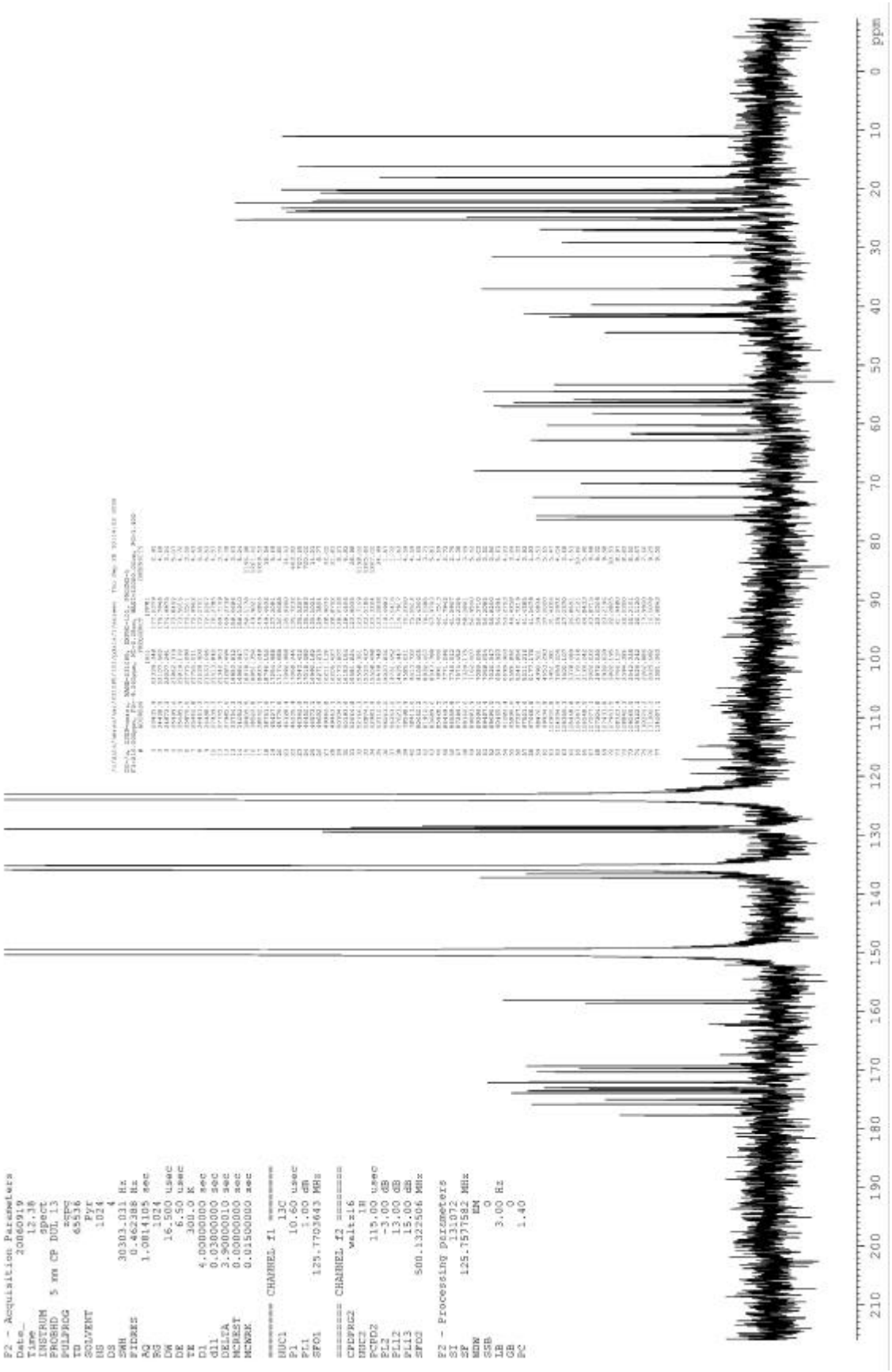


Figure 11. ^{13}C-NMR of synthetic $N^{2.1}$-Cbz protected lysobactin (**3**) (126 MHz, [D_5]pyridine).

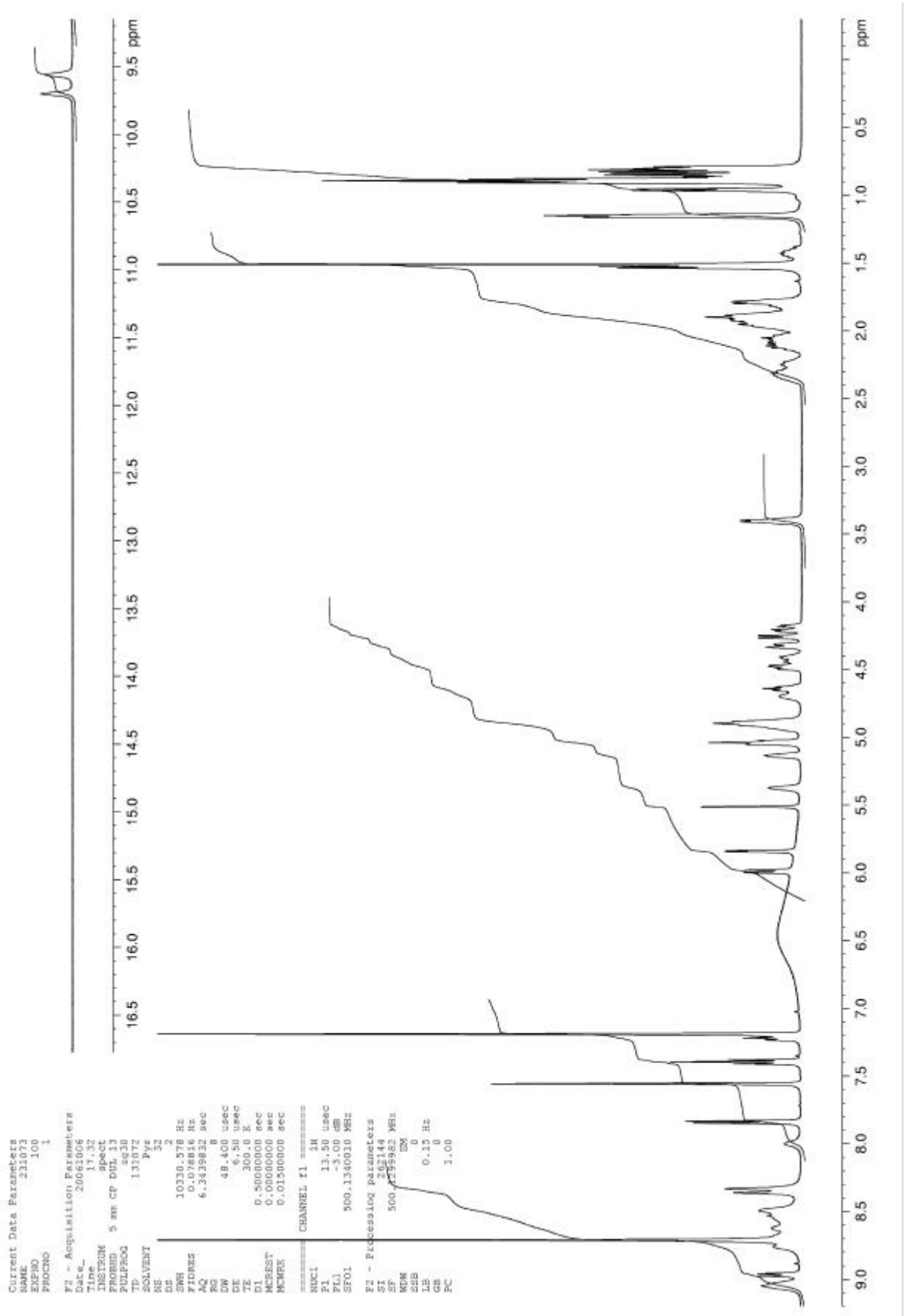


Figure 12. ^{1}H-NMR of semisynthetic $N^{2.1}$-Boc protected (ring-opened) lysobactin (**5**) (500 MHz, [D_5]pyridine).

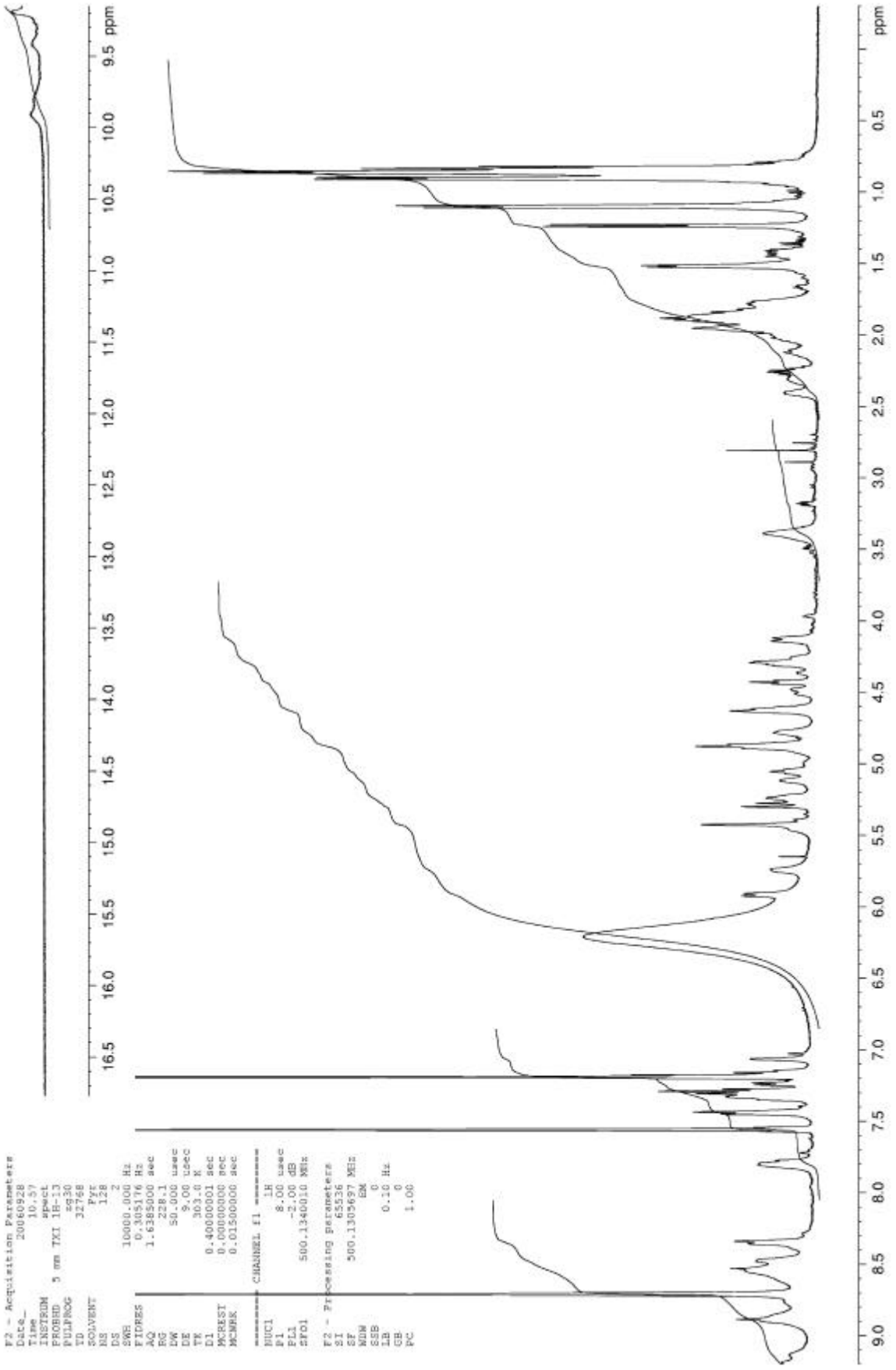


Figure 13. ^{1}H-NMR of the Cbz peptide **13** (500 MHz, [D_5]pyridine). The spectrum shows conformers.

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