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A Multicomponent Macrocyclization Strategy to Natural Product-Like Cyclic Lipopeptides: Synthesis and Anticancer Evaluation of Surfactin and Mycosubtilin Analogues

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General procedure for the solid-phase peptide synthesis: Coupling reactions were carried out in an Automated Solid-Phase Peptide Synthesizer by stepwise Fmoc strategy using the amino acid-functionalized Wang resin in 0.10-0.30 mmol scale. The coupling cycle from the standard Intavis protocol was used. The intermediate linear peptides, further used in the solution-phase macrocyclization, were cleaved from the resin with the cocktail TFA/CH_2Cl_2/TIPS (49:49:2, *v*/*v*, 2 mL) and the purity was assessed by analytical RP-HPLC. Prior to solution-phase macrocyclization, the peptides were re-dissolved in 4 M HCl/dioxane, stirred at room temperature for 15 minutes and then dropped over frozen diethyl ether. The precipitate product was centrifuged, then taken up in 1:2 acetonitrile/water and lyophilized to yield the corresponding peptide hydrochloride salt. Peptido-aldehyde **6** was obtained by coupling 3,3-dimethoxypropanoic acid at the last position of the *N*-terminus. Upon resin cleavage conditions, the acetal is deprotected to render the terminal free aldehyde. The modified peptide was precipitated over frozen diethyl ether and the purity was assessed by analytical RP-HPLC.

General procedure for the solution-phase peptide synthesis. The Boc-protected amino acid (1.0 mmol, 1.0 equiv.), HOBt (168 mg, 1.1 mmol, 1.1 equiv.), EDC (210 mg, 1.1 mmol, 1.1 equiv.) and the amino acid benzyl ester hydrochloride (1.0 mmol, 1.0 equiv.) are dissolved either in dry CH_2Cl_2 (15 mL) or in a CH_2Cl_2/DMF mixture (15 mL, 3:1 *v*/*v*). Et_3N (0.15 mL, 1.1 mmol, 1.1 equiv.) is syringed in one portion and the resulting solution is stirred at room temperature overnight (~12 h). The reaction mixture is then diluted with 100 mL EtOAc, transferred to a separatory funnel and sequentially washed with 0.5 M aqueous solution of citric acid (2×50 mL), saturated aqueous suspension $NaHCO_3$ (2×50 mL) and brine (2×50 mL). The organic phase is dried over $MgSO_4$, filtered and concentrated under reduced pressure.

General Boc removal procedure: The peptide is dissolved in a 4 M HCl solution in dioxane (2 mL) and the solution is stirred at room temperature. As the material dissolved, gas evolution could be detected and the pressure that built up inside the reaction flask is regularly relieved by opening the reaction flask. After 30 min, usually no starting material is detected by thin layer chromatography and the reaction is concentrated under a stream of dry N_2. The volatiles are then fully removed by concentrating the resulting thick oily residue under reduced pressure in the rotary evaporator and then placing the flask under high vacuum for 2 h. The resulting salt was used forward assuming quantitative yield.

General procedure for the benzyl ester/ether deprotection: The peptide is dissolved in dry THF (4.0 mL) and the palladium catalyst supported on charcoal (Pd/C, 10wt %) is added. The mixture is subjected successively to vacuum and filling with hydrogen atmosphere and then stirred under hydrogen atmosphere for 12 h. The catalyst was removed by filtration over a pad of Celite and the filtrate was evaporated under reduced pressure.

General procedure for the methyl ester deprotection: The peptide (0.3 mmol) is dissolved in THF/H_2O (2:1, 6.0 mL) and the mixture is cooled down to $^{\circ}0$ C in an ice bath. Then, LiOH is added (3 equiv. per methyl ester) and the resulting mixture is stirred for 2-4 h as the conversion is checked by thin layer chromatography. The solution is brought to pH 3 by careful drop-wise addition of $NaHSO_4$ (1 M) and the product is extracted with EtOAc (3×20 mL) and the combined organic phases are transferred to a separatory funnel and washed with brine (2×20 mL). The organic phase is dried over $MgSO_4$, filtered and concentrated under reduced pressure.

HCl·H-β-Ala-Asn-D-Tyr(Bzl)-D-Asn-Gln-Pro-D-Ser-Asn-OH (3): The linear peptide **3** was produced (93 mg, 0.09 mmol) in 91% purity according to the general protocol for solid-phase peptide synthesis using the Automated Solid-Phase Peptide Synthesizer starting from 0.10 mmol

of Fmoc-Asn(Trt)-Wang resin. An analytical sample was purified by RP-HPLC to 97% purity (254 nm) for ESI-MS analysis. R_t = 8.11 min. HR-MS (ESI) *m/z*: 997.4382 $[M+H]^+$, calcd. for $[C_{44}H_{61}O_{15}N_{12}]^+$ 997.4374.

OCCH$_2$CO-Glu(OBzl)-Leu-D-Leu-Val-Asp(OBzl)-D-Leu-Leu-OH (6): The linear peptide **6** was produced in 78% purity according to the general protocol for solid-phase peptide synthesis using the Automated Solid-Phase Peptide Synthesizer starting from 0.30 mmol of Fmoc-Leu-Wang resin. Preparative RP-HPLC purification afforded peptide **6** (192 mg, 98% purity). R_t = 13.55 min. HRMS (ESI) *m/z*: 1064.5906 $[M+H]^+$, calcd. for $[C_{55}H_{82}O_{14}N_7]^+$ 1064.5914.

Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl: *N*-Boc-D-Leu-OH (694 mg, 3.0 mmol) was coupled to HCl·Leu-OBzl (773 mg, 3.0 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal. The same protocol was employed for the sequential coupling of *N*-Boc-Asp(OMe)-OH (742 mg, 3.0 mmol) and *N*-Boc-Val-OH (652 mg, 3.0 mmol). Flash column chromatography purification (*n*-hexane/AcOEt 1:1) furnished the title tetrapeptide (1.41 g, 71%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37 – 7.30 (m, 5H), 7.10 – 6.96 (m, 2H), 5.19 – 5.08 (m, 3H), 4.83 (dt, *J* = 8.4, 5.3 Hz, 1H), 4.62 (td, *J* = 8.4, 4.9 Hz, 1H), 4.51 (ddd, *J* = 10.2, 7.8, 4.4 Hz, 1H), 4.01 – 3.95 (m, 1H), 3.69 (s, 3H), 3.13 (dd, *J* = 17.3, 4.3 Hz, 1H), 2.75 (dd, *J* = 17.4, 5.6 Hz, 1H), 2.18 – 2.11 (m, 1H), 1.76 – 1.69 (m, 1H), 1.67 – 1.55 (m, 5H), 1.43 (s, 9H), 0.99 – 0.94 (m, 3H), 0.93 – 0.85 (m, 15H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.8, 19.4, 21.5, 21.9, 22.9, 23.3 (CH_3), 24.7, 24.8 (CH), 28.4 (CH_3), 30.8 (CH), 36.0, 40.2, 41.1 (CH_2), 49.4 (CH), 50.9 (CH_3), 52.1, 52.2, 60.6 (CH), 66.8 (CH_2), 80.7 (C), 128.1, 128.3, 128.6 (CH), 135.7 (C), 156.5, 170.6, 171.8, 171.9, 172.5, 172.7 (CO). HRMS (ESI) *m/z*: 663.3974 $[M+H]^+$, calcd. for $[C_{34}H_{55}O_9N_4]^+$ 663.3969.

H-Val-Asp(OMe)-D-Leu-Leu-OH: Tetrapeptide Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl (220 mg, 0.33 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the Boc removal procedure to furnish the title peptide (162 mg, 96%) in 94% purity (210 nm). R_t = 8.99 min. HRMS (ESI) *m/z*: 473.2965 $[M+H]^+$, calcd. for $[C_{22}H_{41}O_7N_4]^+$ 473.2970.

Boc-Glu(OMe)-Leu-D-Leu-OBzl: *N*-Boc-Leu-OH (462 mg, 2.0 mmol) was coupled to HCl·D-Leu-OBzl (516 mg, 2.0 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal. The same protocol was employed for the sequential coupling of *N*-Boc-Glu(OMe)-OH (523 mg, 2.0 mmol). Flash column chromatography purification (*n*-hexane/AcOEt 2:1) furnished the title tripeptide (1.02 g, 88%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31 – 7.20 (m, 5H), 7.00 – 6.92 (m, 1H), 6.78 – 6.68 (m, 1H), 5.54 – 5.46 (m, 1H), 5.12 – 4.97 (m, 2H), 4.51 (q, *J* = 7.4, 1H), 4.46 – 4.38 (m, 1H), 4.04 (q, *J* = 6.8 Hz, 1H), 3.59 (s, 3H), 2.44 – 2.28 (m, 2H), 2.07 – 1.97 (m, 1H), 1.92 – 1.81 (m, 1H), 1.69 – 1.45 (m, 6H), 1.35 (s, 9H), 0.91 – 0.73 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.7, 21.8, 22.9, 23.1 (CH_3), 24.8, 24.9 (CH), 27.2 (CH_2), 28.3 (CH_3), 30.4, 40.6, 40.8 (CH_2), 51.0 (CH_3), 51.8, 52.0, 54.4 (CH), 66.9 (CH_2), 80.5 (C), 128.2, 128.3, 128.6 (CH), 135.6 (C), 156.1, 171.8, 171.9, 172.7, 174.0 (CO). HRMS (ESI) m/z: 578.3444 $[M+H]^+$, calcd. for $[C_{30}H_{48}O_8N_3]^+$ 578.3441.

Boc-β-Ala-Glu(OMe)-Leu-D-Leu-OBzl: Tripeptide Boc-Glu(OMe)-Leu-D-Leu-OBzl (242 mg, 0.42 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure, followed by the coupling of *N*-Boc-β-Ala-OH (79 mg, 0.42 mmol). Flash column chromatography purification (CH_2Cl_2/AcOEt 3:2) furnished the title tetrapeptide (223 mg, 82%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31 – 7.21 (m, 5H), 7.11 – 6.85

(m, 2H), 5.05 (q, *J* = 12.5 Hz, 2H), 4.61 – 4.47 (m, 2H), 4.45 – 4.35 (m, 1H), 4.18 – 4.06 (m, 1H), 3.59 (s, 3H), 3.37 – 3.21 (m, 2H), 2.48 – 2.29 (m, 4H), 2.08 – 1.87 (m, 2H), 1.71 – 1.63 (m, 1H), 1.62 – 1.47 (m, 5H), 1.36 (s, 9H), 1.25 – 1.15 (m, 1H), 0.90 – 0.76 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.7, 21.9, 22.9, 23.0 (CH_3), 24.9, 25.0 (CH), 27.2, 27.4 (CH_2), 28.5 (CH_3), 30.4, 36.3, 37.2, 40.8 (CH_2), 51.1 (CH_3), 51.7, 52.1, 53.5 (CH), 67.0 (CH_2), 79.5 (C), 128.2, 128.4, 128.6 (CH), 135.6 (C), 156.4, 171.2, 171.9, 172.7, 173.0, 174.2 (CO). HRMS (ESI) m/z: 649.3810 $[M+H]^+$, calcd. for $[C_{33}H_{53}O_9N_4]^+$ 649.3813.

Boc-β-Ala-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl (9a): Tetrapeptide Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl (99 mg, 0.30 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure to furnish HCl·Val-Asp(OMe)-D-Leu-Leu-OBzl. In parallel, tetrapeptide Boc-β-Ala-Glu(OMe)-Leu-D-Leu-OBzl (194 mg, 0.30 mmol) was submitted to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially coupled to tetrapeptide HCl·Val-Asp(OMe)-D-Leu-Leu-OBzl. Purification by recrystallization (*n*-hexane/CH_2Cl_2/MeOH, 6:3:1) furnished the pure peptide **9a** (232 mg, 70%; 51% overall yield from HCl·Leu-OBzl) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.31 – 8.23 (m, 2H), 8.12 (d, *J* = 7.9 Hz, 1H), 8.05 (d, *J* = 7.7 Hz, 1H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.88 – 7.82 (m, 2H), 7.41 – 7.29 (m, 5H), 6.71 – 6.64 (m, 1H), 5.11 (s, 2H), 4.66 – 4.59 (m, 1H), 4.37 – 4.30 (m, 3H), 4.30 – 4.22 (m, 2H), 4.12 (dd, *J* = 8.5, 6.7 Hz, 1H), 3.57 (s, 6H), 3.16 – 3.07 (m, 2H), 2.75 – 2.66 (m, 2H), 2.35 – 2.25 (m, 4H), 2.06 – 1.96 (m, 2H), 1.95 – 1.84 (m, 2H), 1.80 – 1.68 (m, 2H), 1.59 – 1.51 (m, 4H), 1.49 – 1.42 (m, 4H), 1.37 (s, 9H), 1.21 – 1.10 (m, 1H), 0.90 – 0.76 (m, 30H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 13.9, 17.9, 19.2, 21.3, 21.3, 21.4, 21.7, 22.7, 22.9, 23.0 (CH_3), 23.1, 24.1, 24.2, 24.3 (CH), 27.1 (CH_2), 28.3 (CH_3), 29.9 (CH_2), 30.2 (CH), 35.6, 36.1, 36.7, 40.7, 40.8, 41.3 (CH_2), 49.7, 50.4 (CH), 51.0, 51.2 (CH_3), 51.3, 51.5,

51.6, 51.8, 57.8 (CH), 65.9 (CH_2), 77.6 (C), 127.8, 128.1, 128.5 (CH), 135.9 (C), 155.5, 169.8, 17057, 170.8, 171.0, 171.1, 171.9, 172.1, 172.2, 172.3, 172.8 (CO). HRMS (ESI) m/z: 1103.6607 $[M+H]^+$, calcd. for $[C_{55}H_{91}O_{15}N_8]^+$ 1103.6604.

H-β-Ala-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH (9b): Peptide **9a** (200 mg, 0.18 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the Boc removal procedure to furnish peptide **9b** (165 mg, 96%) in 97% purity (210 nm). R_t = 10.89 min. HRMS (ESI) *m/z*: 913.5596 $[M+H]^+$, calcd. for $[C_{43}H_{77}O_{13}N_8]^+$ 913.5605.

Boc-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl: Tetrapeptide Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl (220 mg, 0.33 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure, followed by the coupling of *N*-Boc-D-Leu-OH (76 mg, 0.33 mmol). Flash column chromatography purification (CH_2Cl_2/AcOEt 1:2) furnished the title pentapeptide (213 mg, 83%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.35 – 7.32 (m, 5H), 7.12 – 6.96 (m, 2H), 5.17 – 5.10 (m, 2H), 5.08 – 5.02 (m, 1H) 4.80 (dt, *J* = 8.2, 5.2 Hz, 1H), 4.67 (td, *J* = 8.1, 4.4 Hz, 1H), 4.58 – 4.52 (m, 2H), 4.22 – 4.16 (m, 1H), 3.71 (s, 3H), 3.13 – 3.05 (m, 3H), 2.73 – 2.68 (m, 1H), 2.17 – 2.12 (m, 1H), 1.78 – 1.70 (m, 1H), 1.67 – 1.55 (m, 6H), 1.44 (s, 9H), 0.97 – 0.92 (m, 6H), 0.93 – 0.85 (m, 18H). ^{13}C NMR (100 MHz, CD_3OD): δ = 18.6, 19.2, 21.5, 21.9, 22.2, 22.7, 22.8, 23.1 (CH_3), 23.9, 24.6, 24.8 (CH), 28.3 (CH_3), 30.8 (CH), 36.1, 40.2, 40.5, 41.0 (CH_2), 49.9, 50.5 (CH), 51.9 (CH_3), 52.3, 52.9, 59.6 (CH), 66.6 (CH_2), 80.3 (C), 128.2, 128.3, 128.4 (CH), 135.6 (C), 156.4, 170.8, 171.6, 171.9, 172.2, 172.8, 173.6 (CO). HRMS (ESI) m/z: 776.4807 $[M+H]^+$, calcd. for $[C_{40}H_{66}O_{10}N_5]^+$ 776.4810.

H-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH: Pentapeptide Boc-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl (182 mg, 0.23 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the Boc removal procedure to furnish the title peptide (140 mg, 98%) in 97% purity (210 nm). R_t = 9.59 min. HRMS (ESI) *m/z*: 586.3807 $[M+H]^+$, calcd. for $[C_{28}H_{52}O_8N_5]^+$ 586.3810.

Boc-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBz: Tetrapeptide Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl (220 mg, 0.33 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure, followed by sequential coupling of *N*-Boc-D-Leu-OH (76 mg, 0.33 mmol) and *N*-Boc-Leu-OH (76 mg, 0.33 mmol). Flash column chromatography purification (CH_2Cl_2/AcOEt 1:1) furnished the title hexapeptide (232 mg, 79%) as a white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.39 – 7.27 (m, 5H), 5.48 (s, 1H), 5.22 – 5.10 (m, 2H), 4.61 (dd, *J* = 8.2, 5.3 Hz, 1H), 4.52 (dd, *J* = 10.0, 4.4 Hz, 1H), 4.46 – 4.36 (m, 2H), 4.04 (t, *J* = 7.5 Hz, 1H), 3.98 (d, *J* = 6.5 Hz, 1H), 3.66 (s, 3H), 3.00 – 2.93 (m, 1H), 2.91 – 2.81 (m, 1H), 2.18 (h, *J* = 6.8 Hz, 1H), 1.81 – 1.69 (m, 3H), 1.68 – 1.56 (m, 7H), 1.55 – 1.49 (m, 2H), 1.44 (s, 9H), 1.00 – 0.85 (m, 30H). ^{13}C NMR (100 MHz, CD_3OD): δ = 18.9, 19.6, 21.5, 21.9, 22.0, 22.1, 23.3, 23.4, 23.5, 23.7 (CH_3), 25.8, 25.91, 25.98 (CH), 28.8 (CH_3), 30.8 (CH), 36.3, 41.2, 41.5, 41.6, 41.8 (CH_2), 52.0 (CH_3), 52.3, 52.5, 53.1, 53.4, 54.8, 61.4 (CH), 67.9 (CH_2), 80.7 (C), 129.1, 129.2, 129.3, 129.6 (CH), 137.2 (C), 157.9, 172.5, 172.7, 173.8, 173.9, 174.8, 175.3, 176.1 (CO). HRMS (ESI) m/z: 889.5652 $[M+H]^+$, calcd. for $[C_{46}H_{77}O_{11}N_6]^+$ 889.5650.

H-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH: Hexapeptide Boc-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl (200 mg, 0.22 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the Boc removal

procedure to furnish the title peptide (159 mg, 98%) in 95% purity (210 nm). R_t = 10.83 min. HRMS (ESI) *m/z*: 699.4642 $[M+H]^+$, calcd. for $[C_{34}H_{63}O_9N_6]^+$ 699.4651.

Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-OBzl: *N*-Boc-Val-OH (91 mg, 0.42 mmol) was coupled to HCl·Asp(OMe)-OBzl (115 mg, 0.42 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal to furnish HCl·Val-Asp(OMe)-OBzl. In parallel, tripeptide Boc-Glu(OMe)-Leu-D-Leu-OBzl (242 mg, 0.42 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially coupled to dipeptide HCl·Val-Asp(OMe)-OBzl. Flash column chromatography purification (CH_2Cl_2/AcOEt 2:1) furnished the title pentapeptide (261 mg, 77%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39 – 7.29 (m, 5H), 7.18 (d, *J* = 7.6 Hz, 1H), 7.14 – 7.00 (m, 3H), 5.62 (d, *J* = 6.7 Hz, 1H), 5.25 – 5.13 (m, 2H), 4.88 – 4.80 (m, 1H), 4.50 – 4.41 (m, 2H), 4.25 (dd, *J* = 8.5, 5.7 Hz, 1H), 4.17 – 4.10 (m, 1H), 3.63 (s, 3H), 3.61 (s, 3H), 2.99 – 2.94 (m, 2H), 2.46 (t, *J* = 6.8 Hz, 2H), 2.22 – 2.13 (m, 1H), 2.11 – 2.01 (m, 1H), 2.00 – 1.91 (m, 2H), 1.80 – 1.71 (m, 2H), 1.67 – 1.53 (m, 4H), 1.40 (s, 9H), 0.97 – 0.84 (m, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.8, 19.4, 21.9, 22.0, 23.0, 23.1 (CH_3), 24.8, 25.0 (CH), 27.3 (CH_2), 28.4 (CH_3), 30.3 (CH), 36.2, 39.3, 40.0 (CH_2), 49.1 (CH), 51.8, 52.0 (CH_3), 52.1, 52.2, 54.5, 59.1 (CH), 67.9 (CH_2), 80.6 (C), 128.4, 128.6, 128.7 (CH), 135.1 (C), 156.2, 171.0, 171.3, 171.4, 172.0, 172.2, 173.1, 174.4 (CO). HRMS (ESI) m/z: 806.4555 $[M+H]^+$, calcd. for $[C_{40}H_{64}O_{12}N_5]^+$ 806.4551.

H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-OH: Pentapeptide Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-OBzl (210 mg, 0.26 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the Boc removal procedure to furnish the title peptide (160 mg, 94%) in 96% purity (210 nm). R_t = 9.05 min. HRMS (ESI) *m/z*: 616.3547 $[M+H]^+$, calcd. for $[C_{28}H_{50}O_{10}N_5]^+$ 616.3552.

Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-OBzl: *N*-Boc-Asp(OMe)-OH (104 mg, 0.42 mmol) was coupled to HCl·D-Leu-OBzl (108 mg, 0.42 mmol) according to the peptide coupling procedure, following by deprotection of the *N*-terminus by Boc removal. The same protocol was employed for the sequential coupling of *N*-Boc-Val-OH (91 mg, 0.42 mmol), following by deprotection of the *N*-terminus by Boc removal to furnish HCl·Val-Asp(OMe)-D-Leu-OBzl. In parallel, tripeptide Boc-Glu(OMe)-Leu-D-Leu-OBzl (242 mg, 0.42 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially coupled to tripeptide HCl·Val-Asp(OMe)-D-Leu-OBzl. Flash column chromatography purification (CH_2Cl_2/AcOEt 1:1) furnished the title hexapeptide (266 mg, 69%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41 – 7.32 (m, 5H), 7.17 (d, *J* = 7.4 Hz, 1H), 7.10 – 7.03 (m, 3H), 5.60 – 5.66 (m, 2H), 5.25 – 5.13 (m, 2H), 4.87 – 4.82 (m, 2H), 4.49 – 4.40 (m, 2H), 4.20 (dd, *J* = 8.3, 5.5 Hz, 1H), 4.16 – 4.11 (m, 1H), 3.65 (s, 3H), 3.59 (s, 3H), 3.01 – 2.97 (m, 2H), 2.47 – 2.40 (m, 2H), 2.21 – 2.16 (m, 1H), 2.15 – 2.00 (m, 1H), 1.97 – 1.90 (m, 2H), 1.80 – 1.78 (m, 4H), 1.65 – 1.50 (m, 4H), 1.42 (s, 9H), 0.95 – 0.85 (m, 24H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.8, 19.4, 21.5, 21.9, 22.1, 22.6, 23.2, 23.4 (CH_3), 24.4, 24.7, 25.1 (CH), 26.8 (CH_2), 28.5 (CH_3), 30.1 (CH_2), 30.3 (CH), 36.7, 38.8, 39.5, 40.5 (CH_2), 49.7, 50.1 (CH), 51.6, 51.9 (CH_3), 52.1, 52.0, 54.8, 59.0 (CH), 67.3 (CH_2), 80.4 (C), 127.8, 128.1, 128.3 (CH), 135.9 (C), 156.7, 171.0, 171.2, 171.3, 171.5, 172.1, 172.3, 173.4, 174.0 (CO). HRMS (ESI) m/z: 919.5389 $[M+H]^+$, calcd. for $[C_{46}H_{75}O_{13}N_6]^+$ 919.5392.

H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-OH: Hexapeptide Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-OBzl (200 mg, 0.22 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus by the

Boc removal procedure to furnish the title peptide (163 mg, 97%) in 94% purity (210 nm). R_t = 9.90 min. HRMS (ESI) *m/z*: 729.4387 $[M+H]^+$, calcd. for $[C_{34}H_{61}O_{11}N_6]^+$ 729.4393.

Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl: Tetrapeptide Boc-Val-Asp(OMe)-D-Leu-Leu-OBzl (220 mg, 0.33 mmol) was subjected to deprotection of the *N*-terminus by the Boc removal procedure to furnish HCl·Val-Asp(OMe)-D-Leu-Leu-OBzl. In parallel, tripeptide Boc-Glu(OMe)-Leu-D-Leu-OBzl (191 mg, 0.33 mmol) was submitted to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially coupled to tetrapeptide HCl·Val-Asp(OMe)-D-Leu-Leu-OBzl. Flash column chromatography purification (CH_2Cl_2/MeOH 10:1) furnished the title heptapeptide (238 mg, 70%) as a white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.21 – 8.15 (m, 2H), 8.10 – 8.07 (m, 1H), 8.06 – 8.01 (m, 1H), 7.88 – 7.82 (m, 2H), 7.41 – 7.29 (m, 5H), 6.65 – 6.59 (m, 1H), 5.11 (s, 2H), 4.68 – 4.65 (m, 1H), 4.39 – 4.35 (m, 2H), 4.34 – 4.16 (m, 3H), 4.08 (dd, *J* = 8.5, 6.7 Hz, 1H), 3.61 (m, 6H), 3.15 – 3.08 (m, 2H), 2.75 – 2.66 (m, 2H), 2.45 – 2.35 (m, 2H), 2.00 – 1.95 (m, 2H), 1.94 – 1.83 (m, 2H), 1.77 – 1.64 (m, 2H), 1.54 – 1.45 (m, 2H), 1.46 – 1.42 (m, 4H), 1.39 (s, 9H), 1.21 – 1.10 (m, 1H), 0.92 – 0.74 (m, 30H). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 14.1, 17.7, 19.5, 21.1, 21.3, 21.4, 21.7, 22.8, 22.9, 23.2 (CH_3), 23.5, 24.2, 24.3, 24.5 (CH), 27.3 (CH_2), 28.5 (CH_3), 29.9 (CH_2), 30.6 (CH), 35.5, 36.5, 36.7, 40.9 (CH_2), 49.9, 50.7 (CH), 51.1, 51.2 (CH_3), 51.4, 51.5, 51.7, 51.9, 57.6 (CH), 65.8 (CH_2), 79.5 (C), 127.8, 128.1, 128.3 (CH), 136.1(C), 155.9, 169.1, 170.7, 170.8, 171.3, 171.7, 172.0, 172.6, 172.8, 172.9 (CO). HRMS (ESI) m/z: 1032.6230 $[M+H]^+$, calcd. for $[C_{52}H_{86}O_{14}N_7]^+$ 1032.6233.

H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH: Heptapeptide Boc-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OBzl (200 mg, 0.19 mmol) was subjected to deprotection of the *C*-terminus by the Bzl removal procedure and sequentially to deprotection of the *N*-terminus

by the Boc removal procedure to furnish the title peptide (162 mg, 97%) in 96% purity (215 nm). R_t = 10.97 min. HRMS (ESI) *m/z*: 842.5228 $[M+H]^+$, calcd. for $[C_{40}H_{72}O_{12}N_7]^+$ 842.5233.

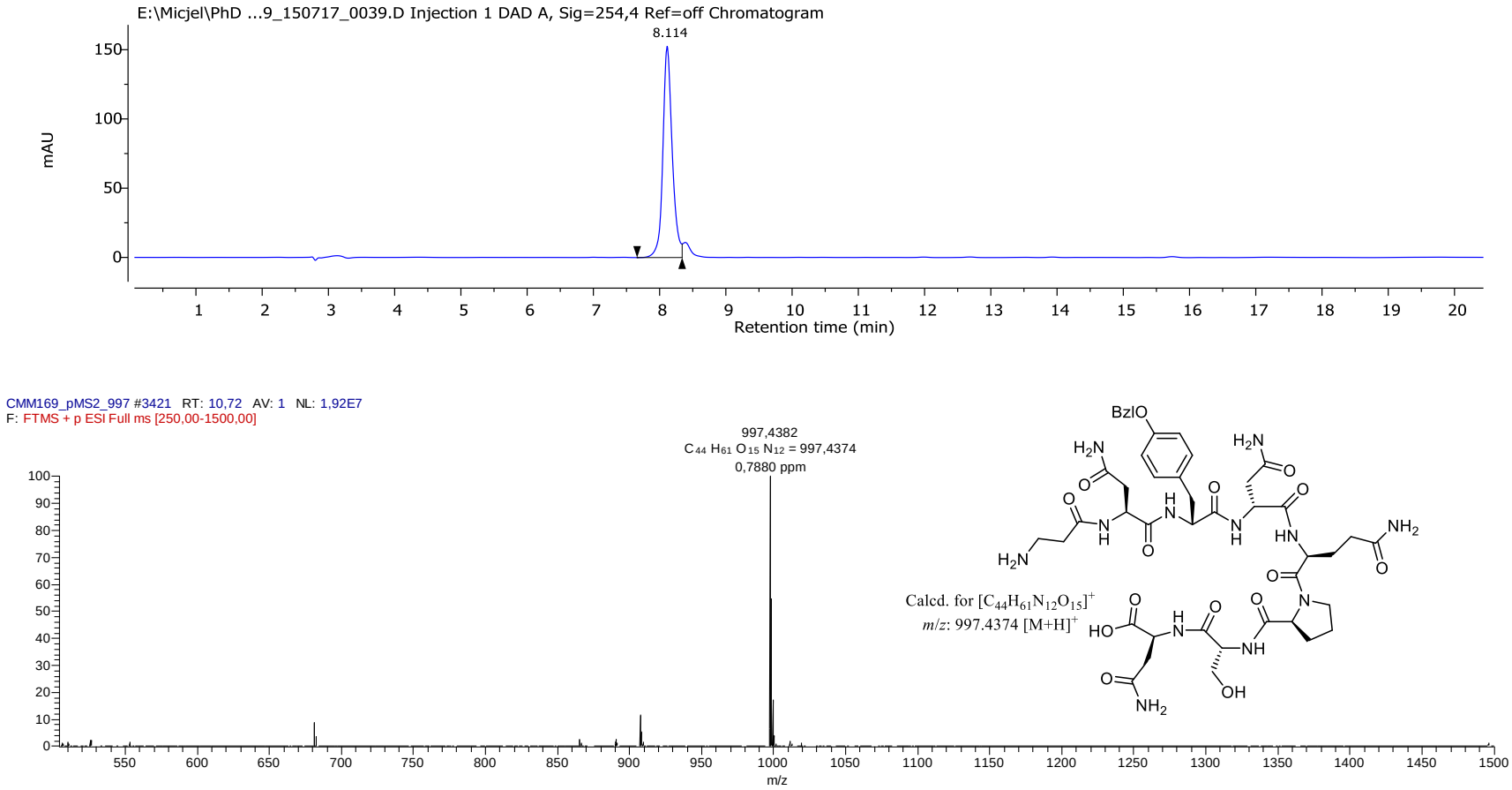

Figure 1. RP-HPLC chromatogram and ESI-HRMS of pure peptide **3**.

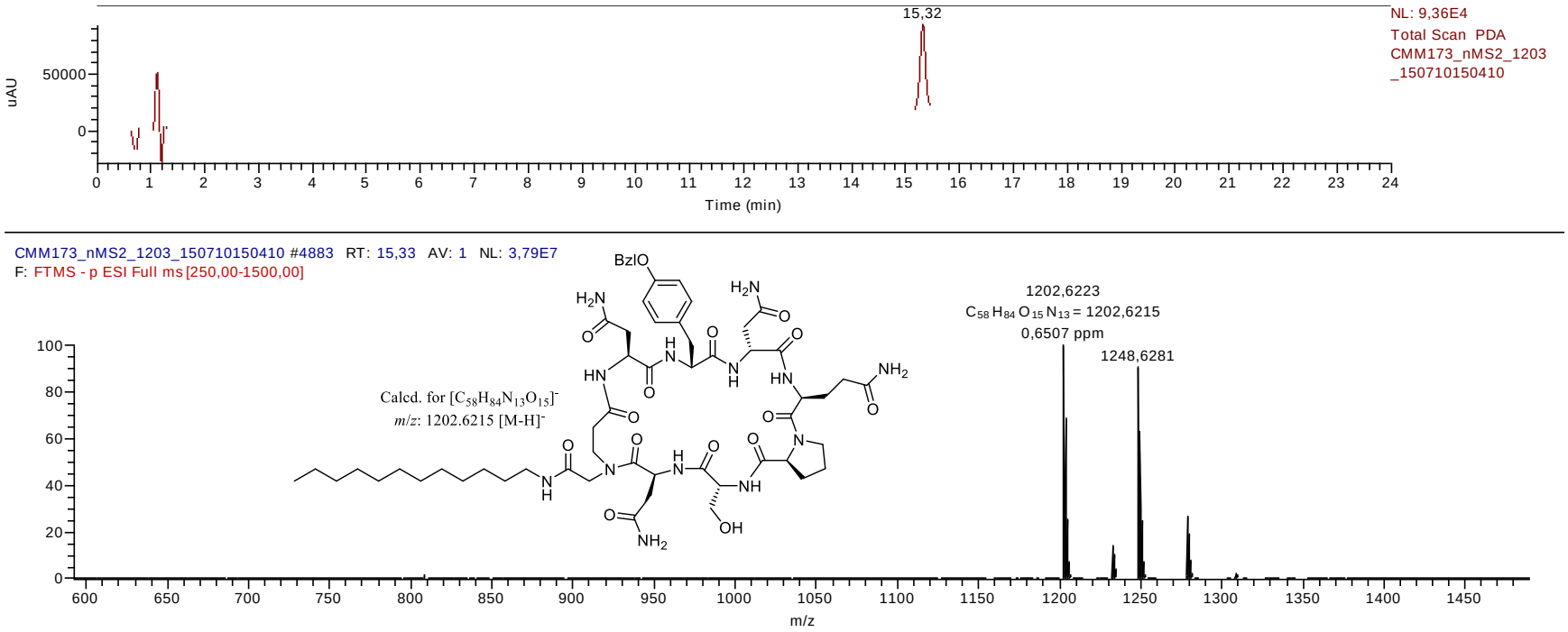

Figure 2. RP-UHPLC chromatogram and ESI-HRMS of pure cyclic lipopeptide **4**.

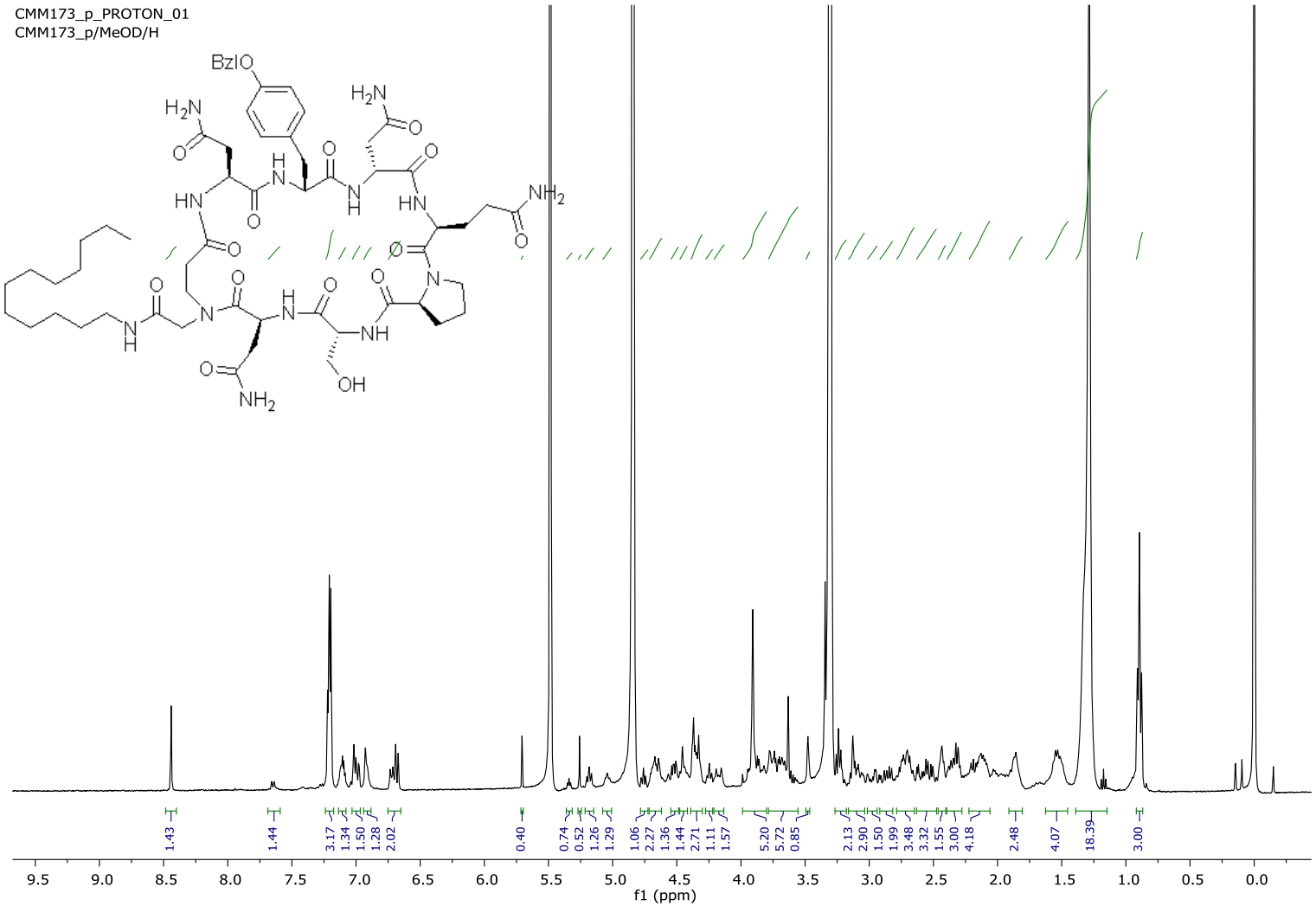

Figure 3. 400 MHz ^{1}H NMR spectrum of cyclic lipopeptide **4** in CD_3OD.

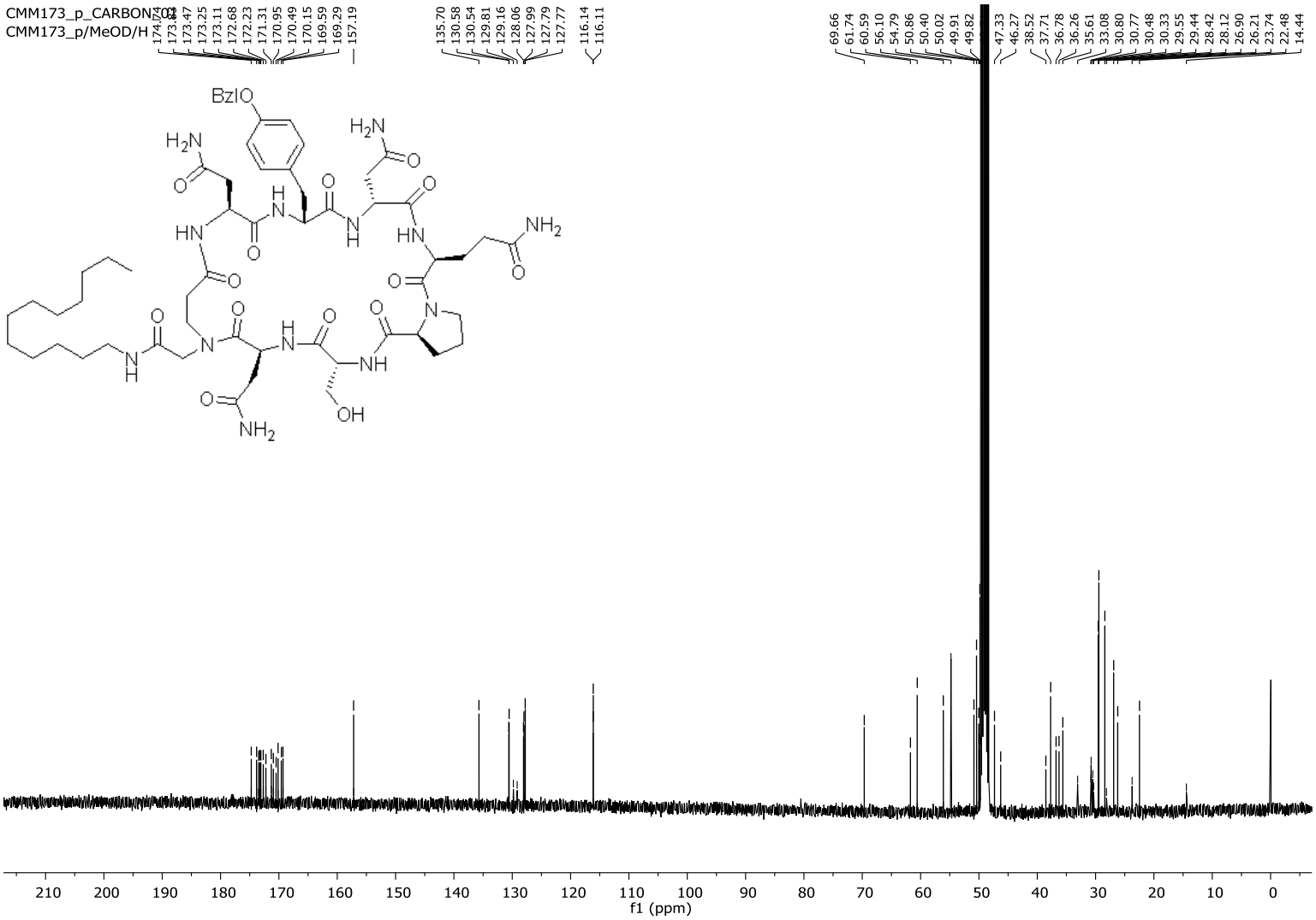

Figure 4. 100 MHz ^{13}C NMR spectrum of cyclic lipopeptide **4** in CD_3OD.

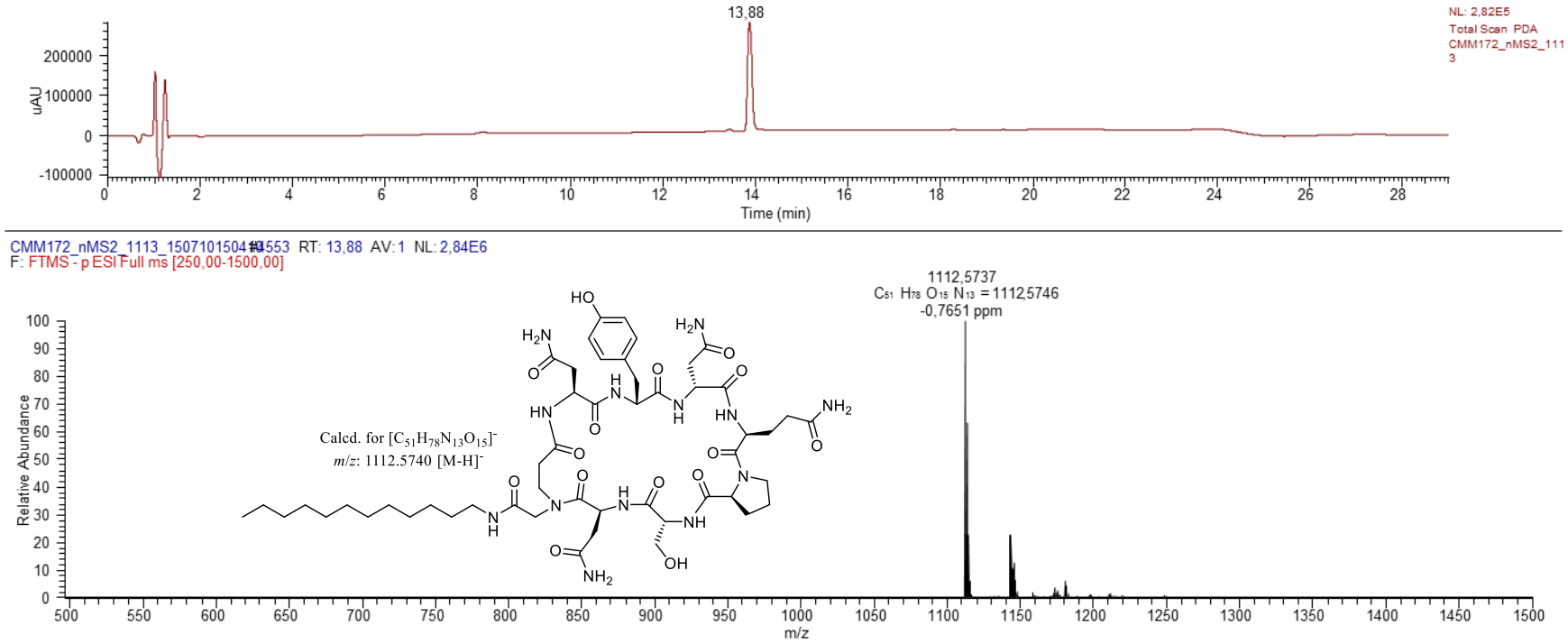

Figure 5. RP-UHPLC chromatogram and ESI-HRMS of pure cyclic lipopeptide **5**.

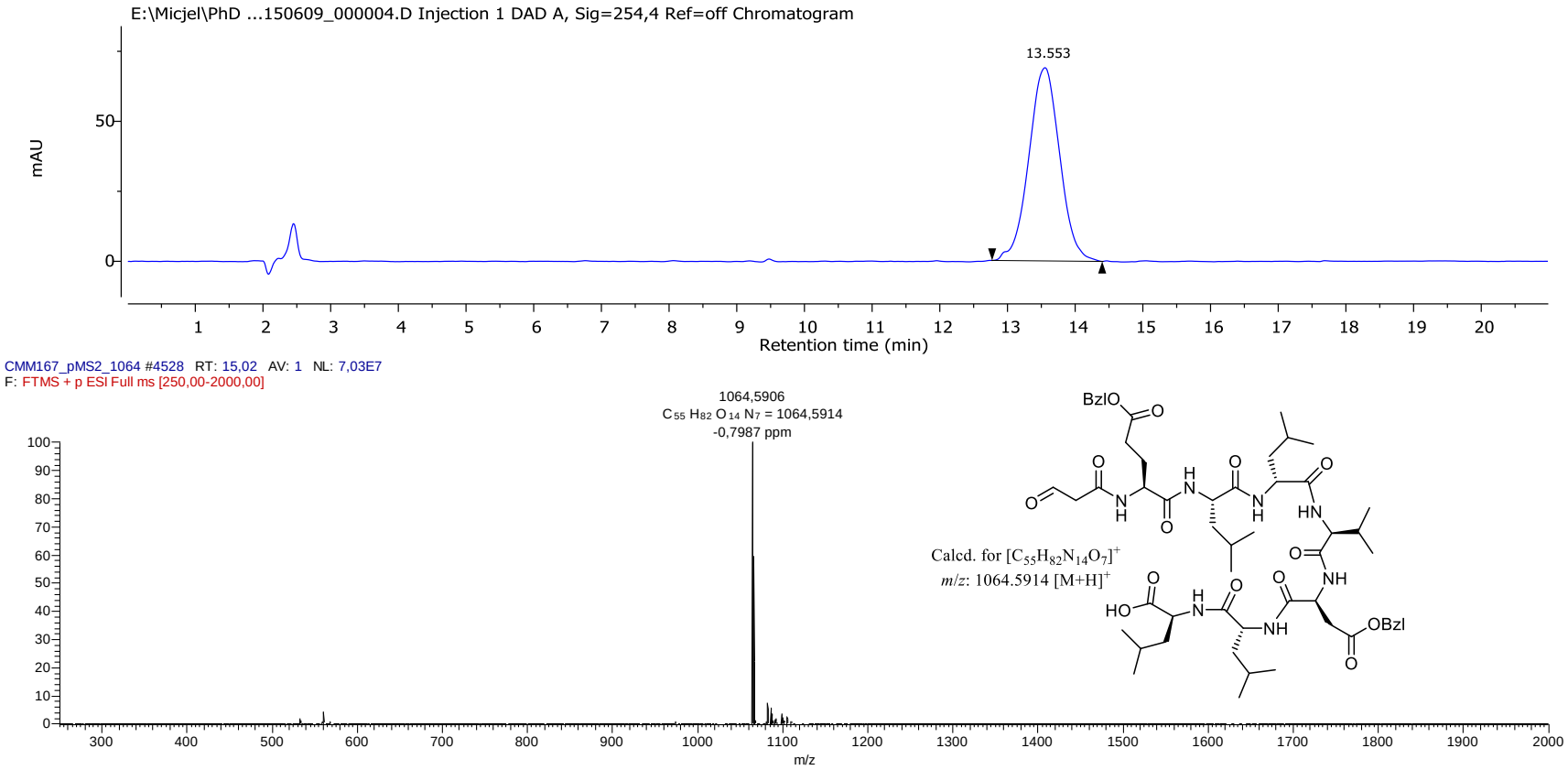

Figure 6. RP-HPLC chromatogram and ESI-HRMS of pure peptide **6**.

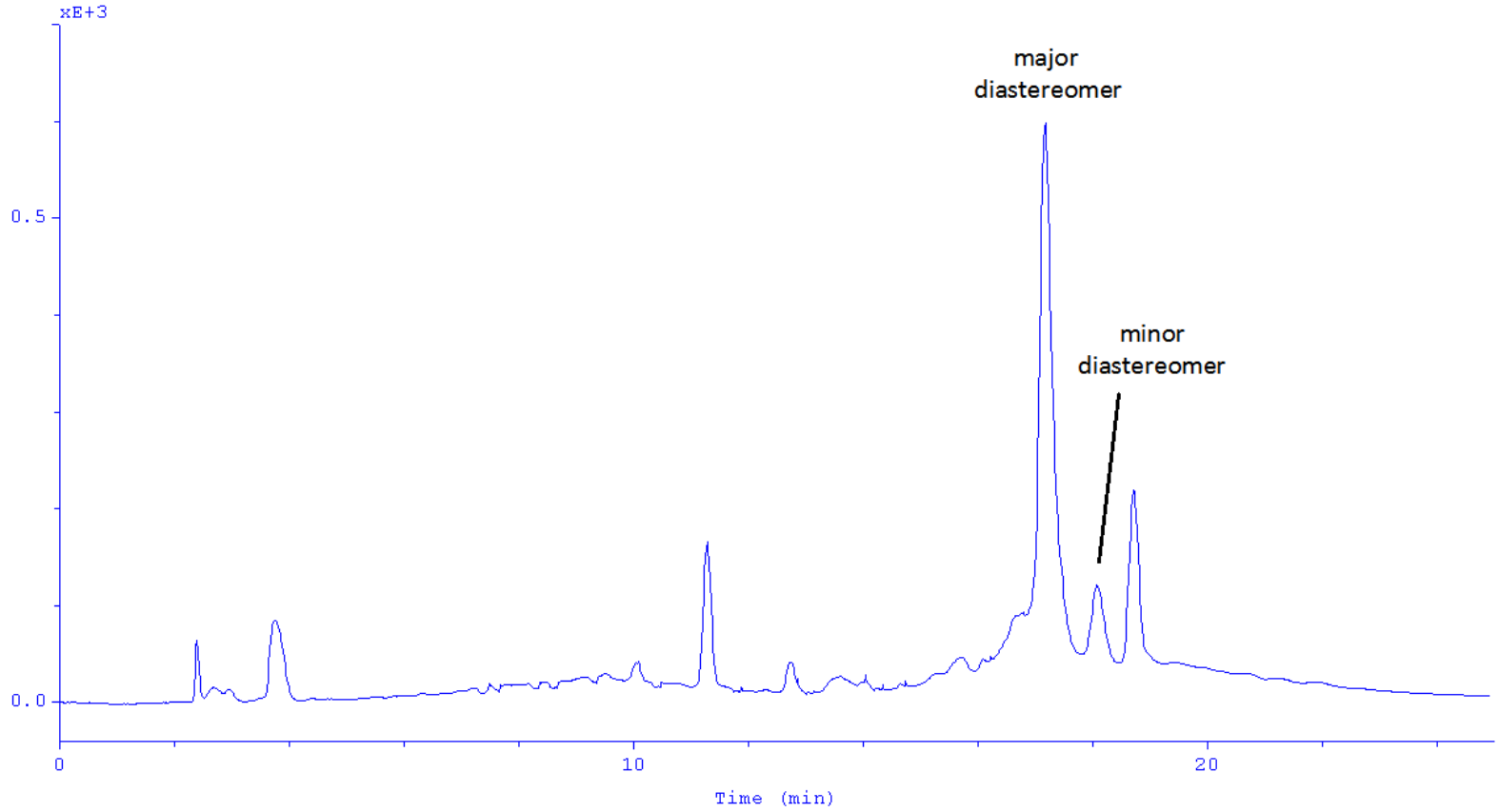

Figure 7. RP-HPLC chromatogram of the crude cyclic lipopeptide **7**.

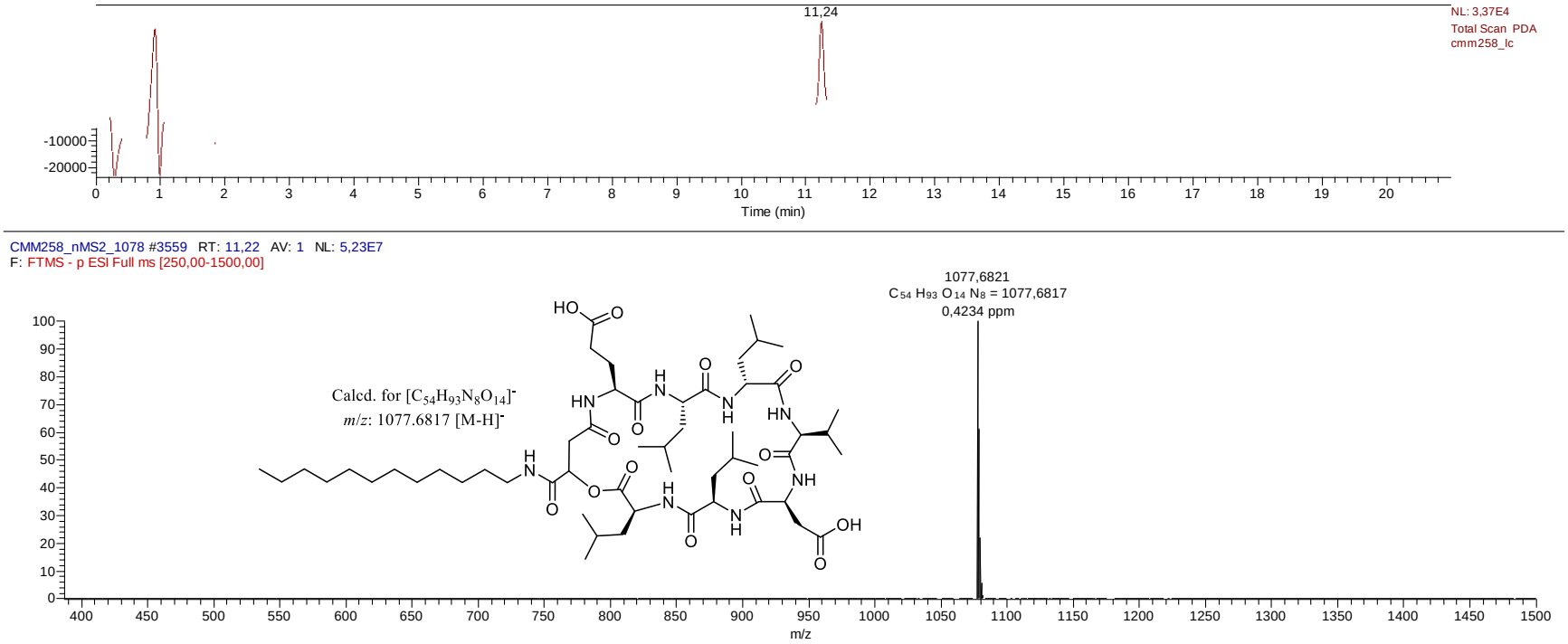

Figure 8. RP-UHPLC chromatogram and ESI-HRMS of pure cyclic lipopeptide **7** (major diastereomer).

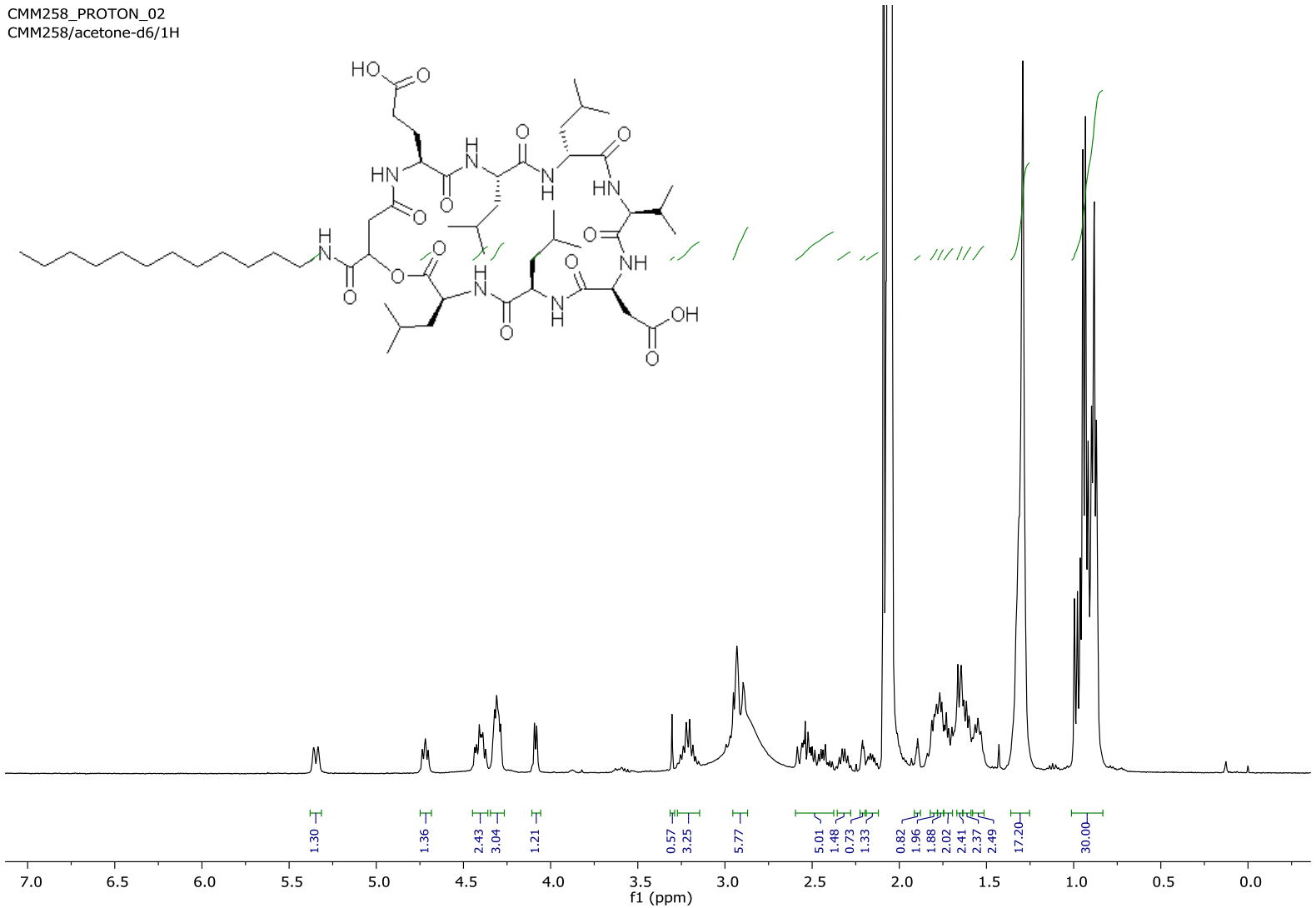

Figure 9. 400 MHz ^{1}H NMR spectrum of cyclic lipopeptide **7** (major diastereomer) in Acetone-d_6.

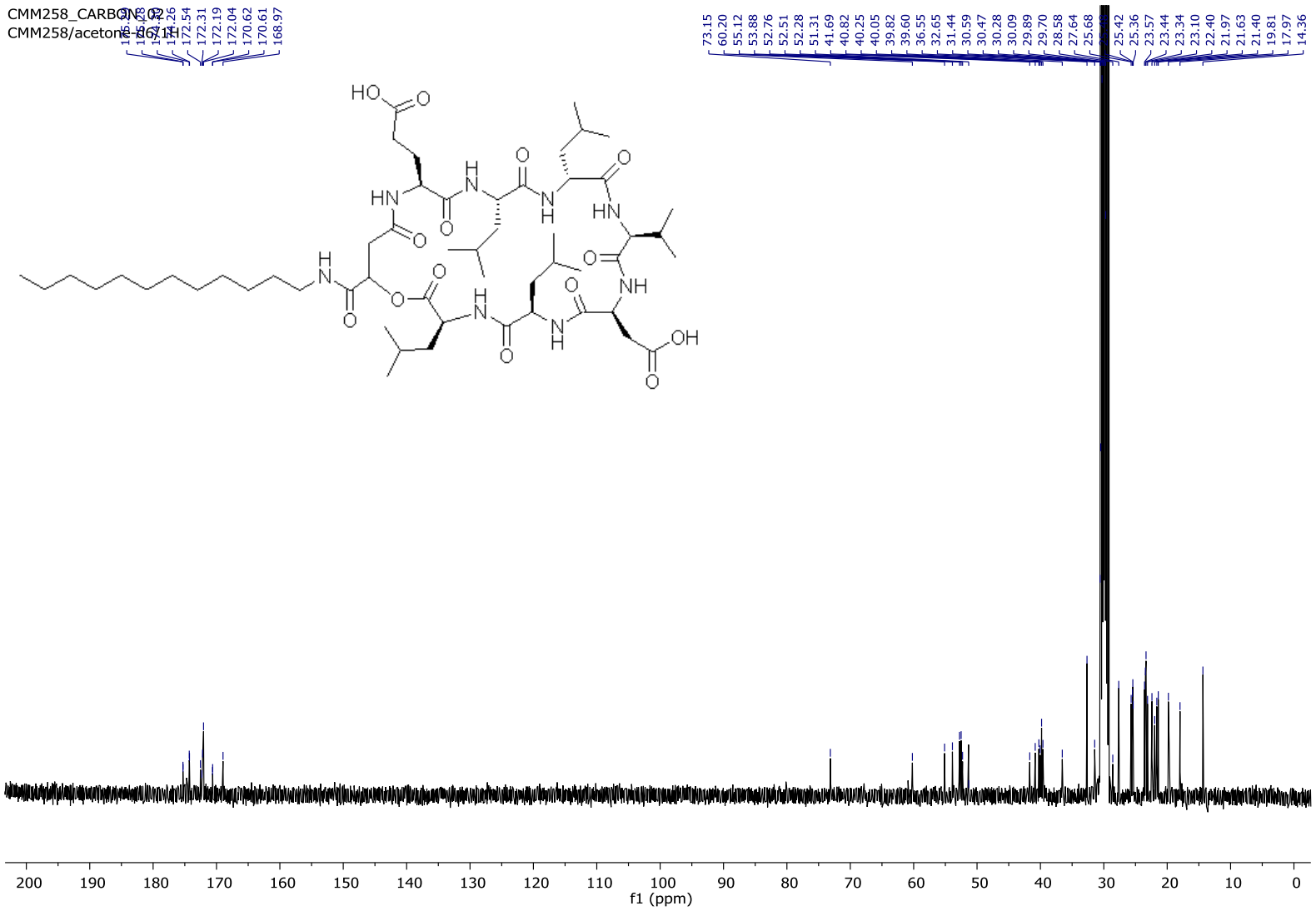

Figure 10. 100 MHz ^{13}C NMR spectrum of cyclic lipopeptide **7** (major diastereomer) in Acetone-d_6.

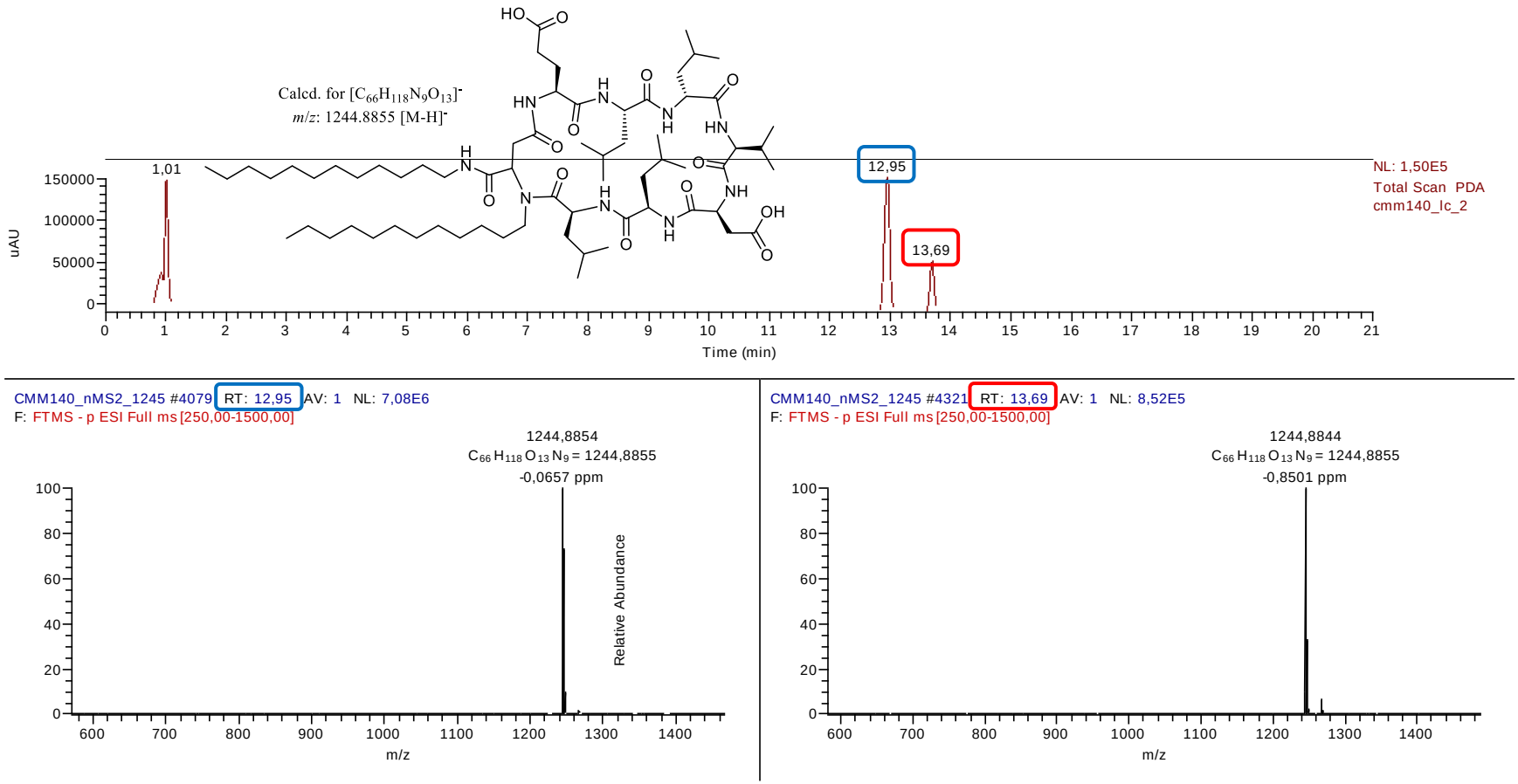

Figure 11. RP-UHPLC chromatogram and ESI-HRMS of the pure diastereomeric mixture of cyclic lipopeptide **8**.[1]

[1] The two diastereomers could not be separated by preparative RP-HPLC, despite of appearing as separate chromatographic bands in analytical RP-UHPLC.

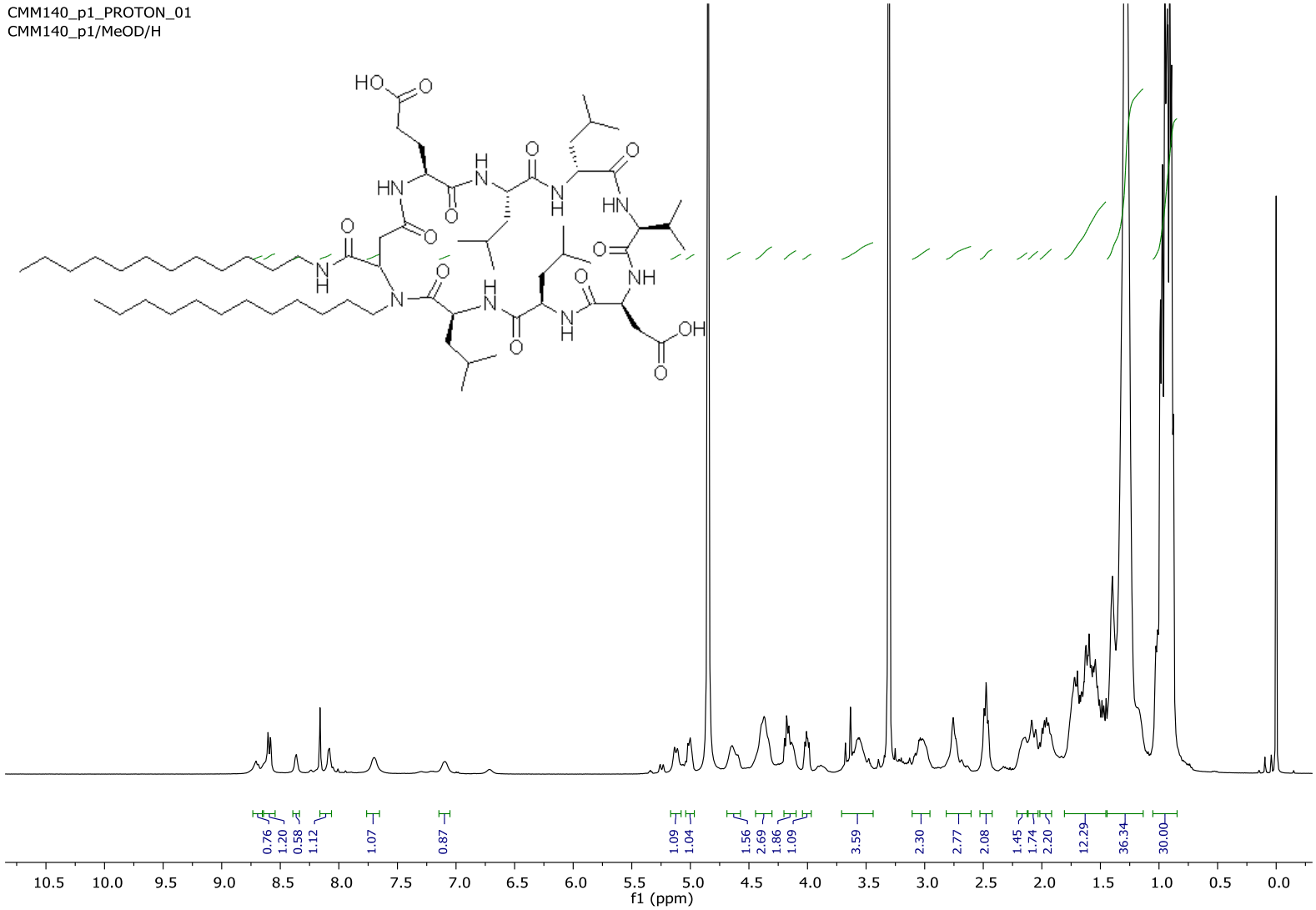

Figure 12. 400 MHz ^{1}H NMR spectrum of the pure diastereomeric mixture of cyclic lipopeptide **8** in CD_3OD.

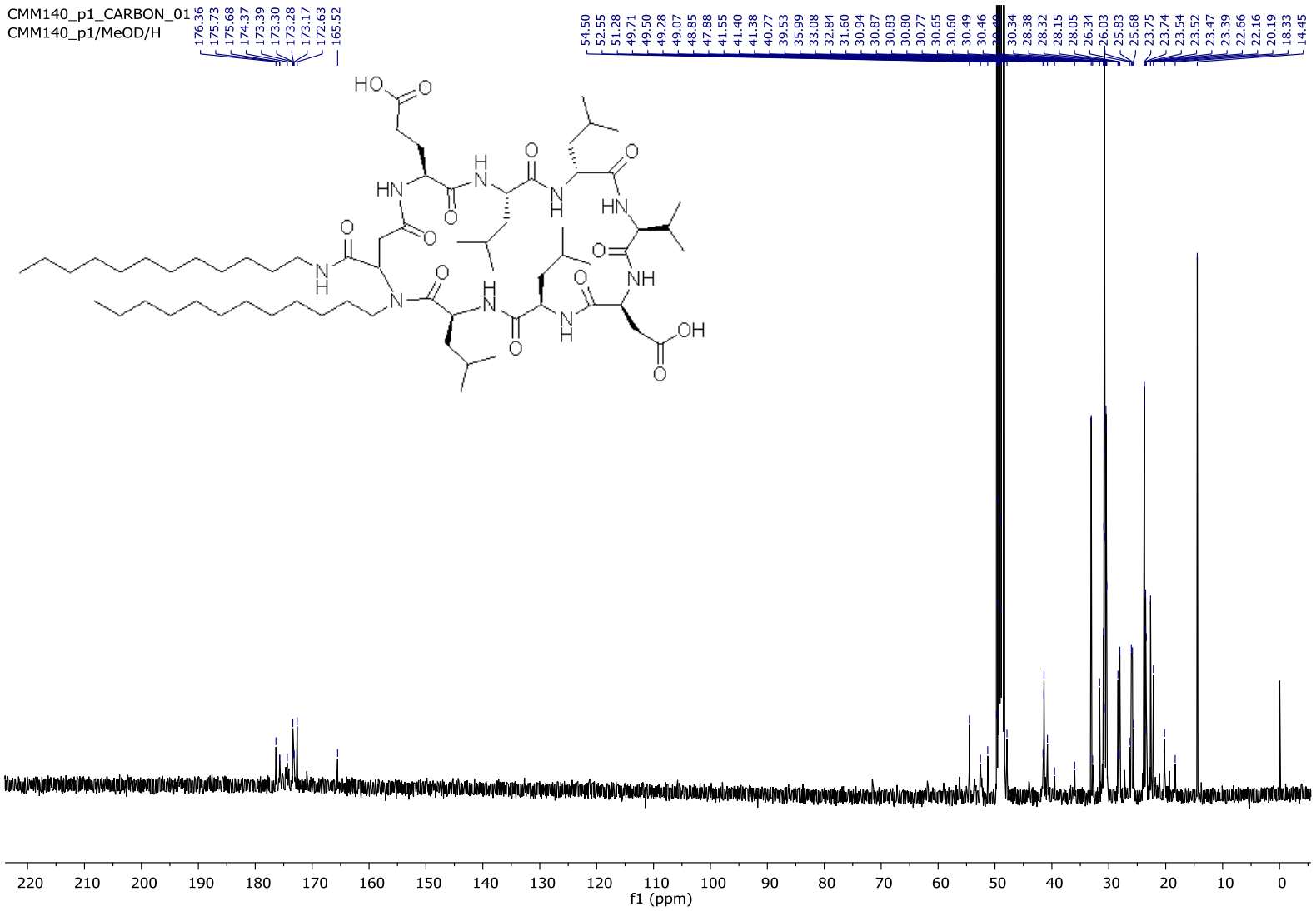

Figure 13. 100 MHz ^{13}C NMR spectrum of the pure diastereomeric mixture of cyclic lipopeptide **8** in CD_3OD.

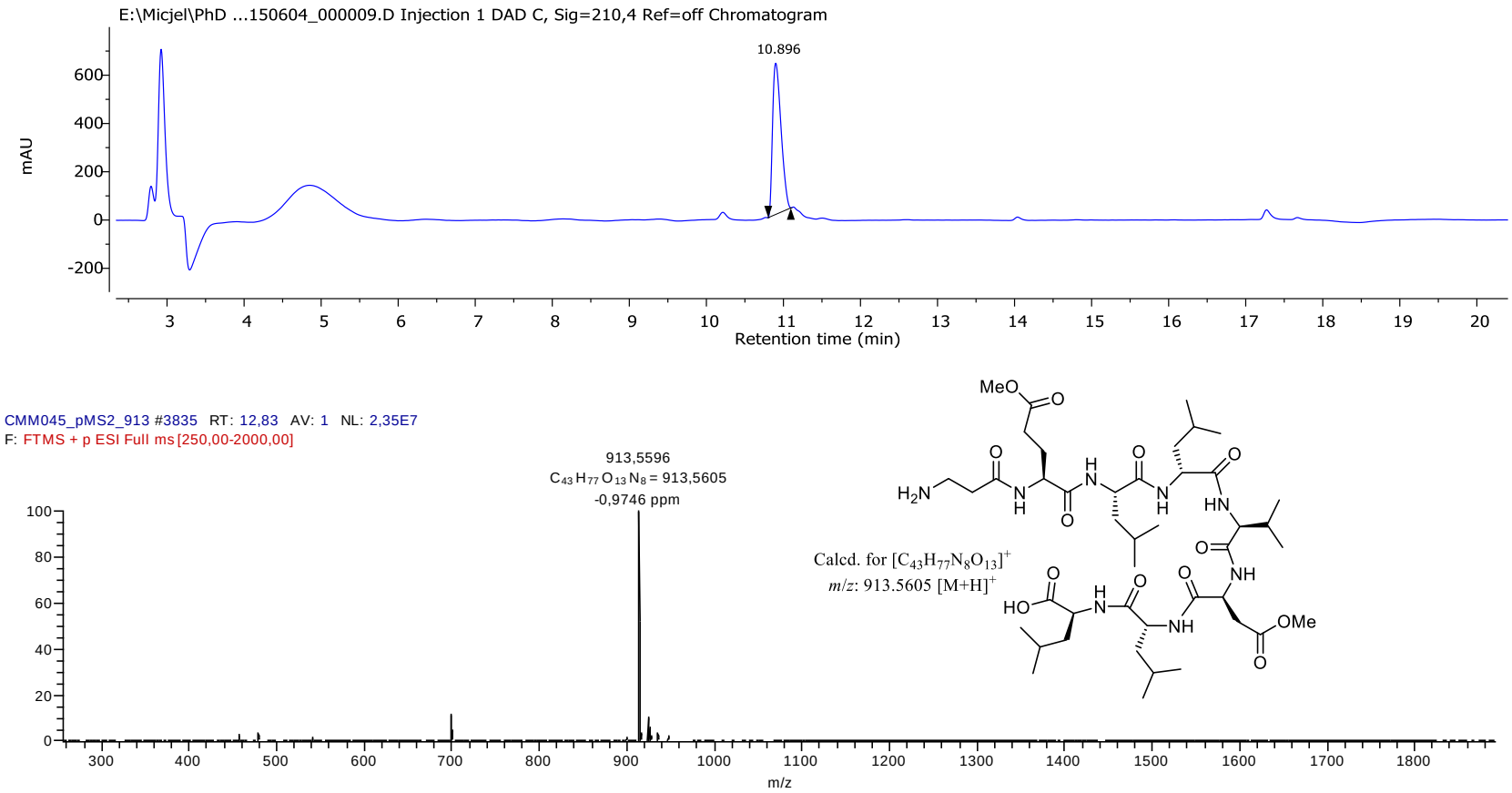

Figure 14. RP-HPLC chromatogram and ESI-HRMS of pure peptide **9b**.

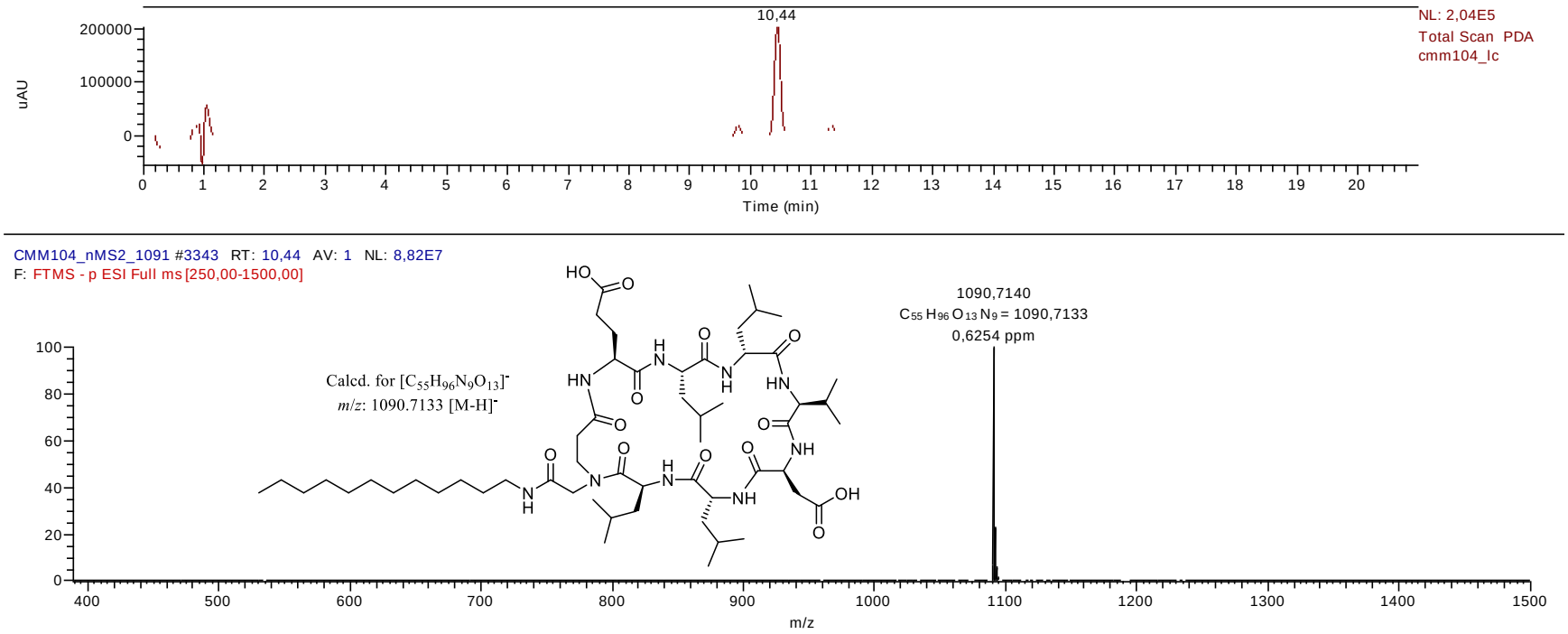

Figure 15. RP-UHPLC chromatogram and ESI-HRMS of pure cyclic lipopeptide **10**.

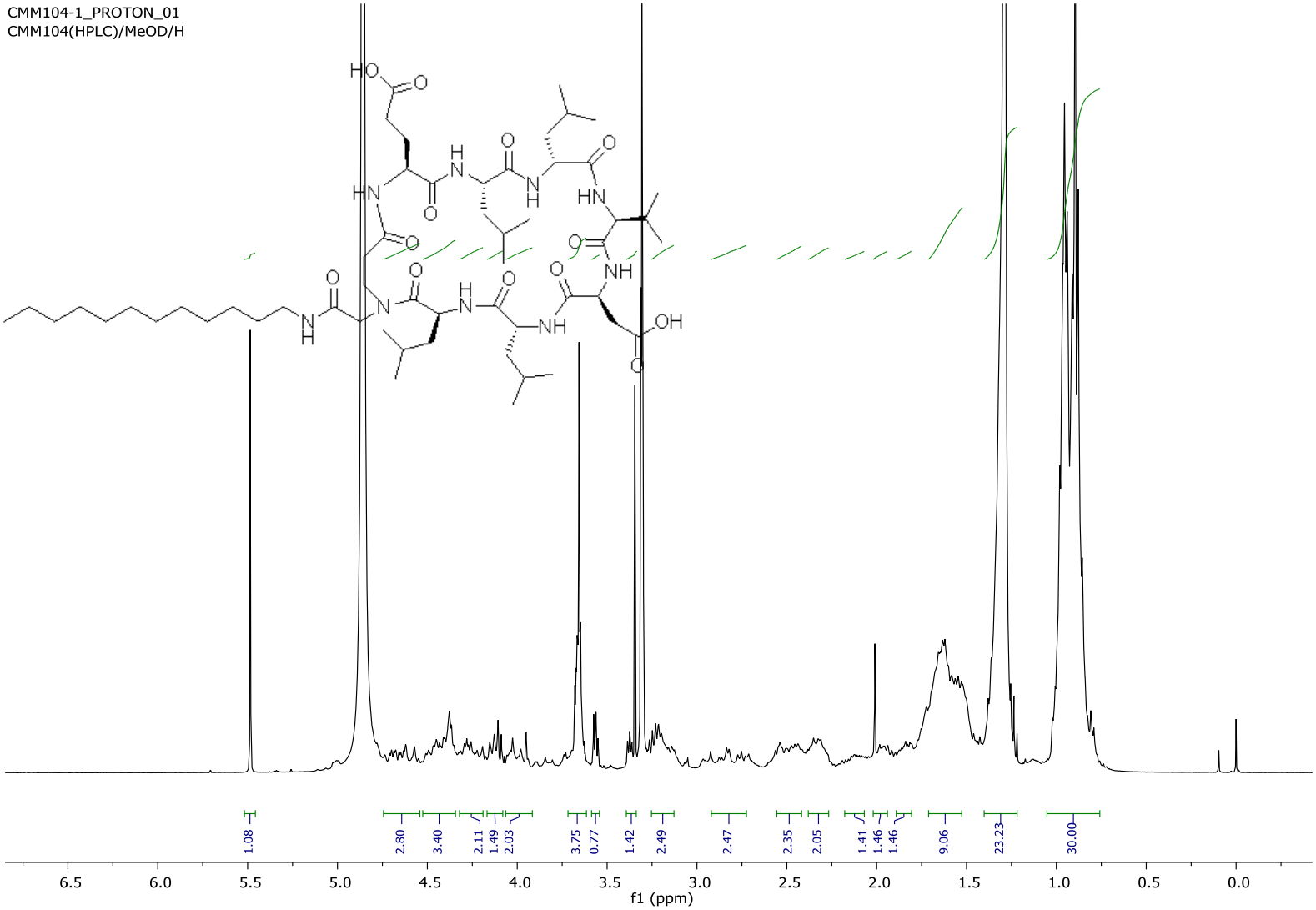

Figure 16. 400 MHz ^{1}H NMR spectrum of cyclic lipopeptide **10** in CD_3OD.

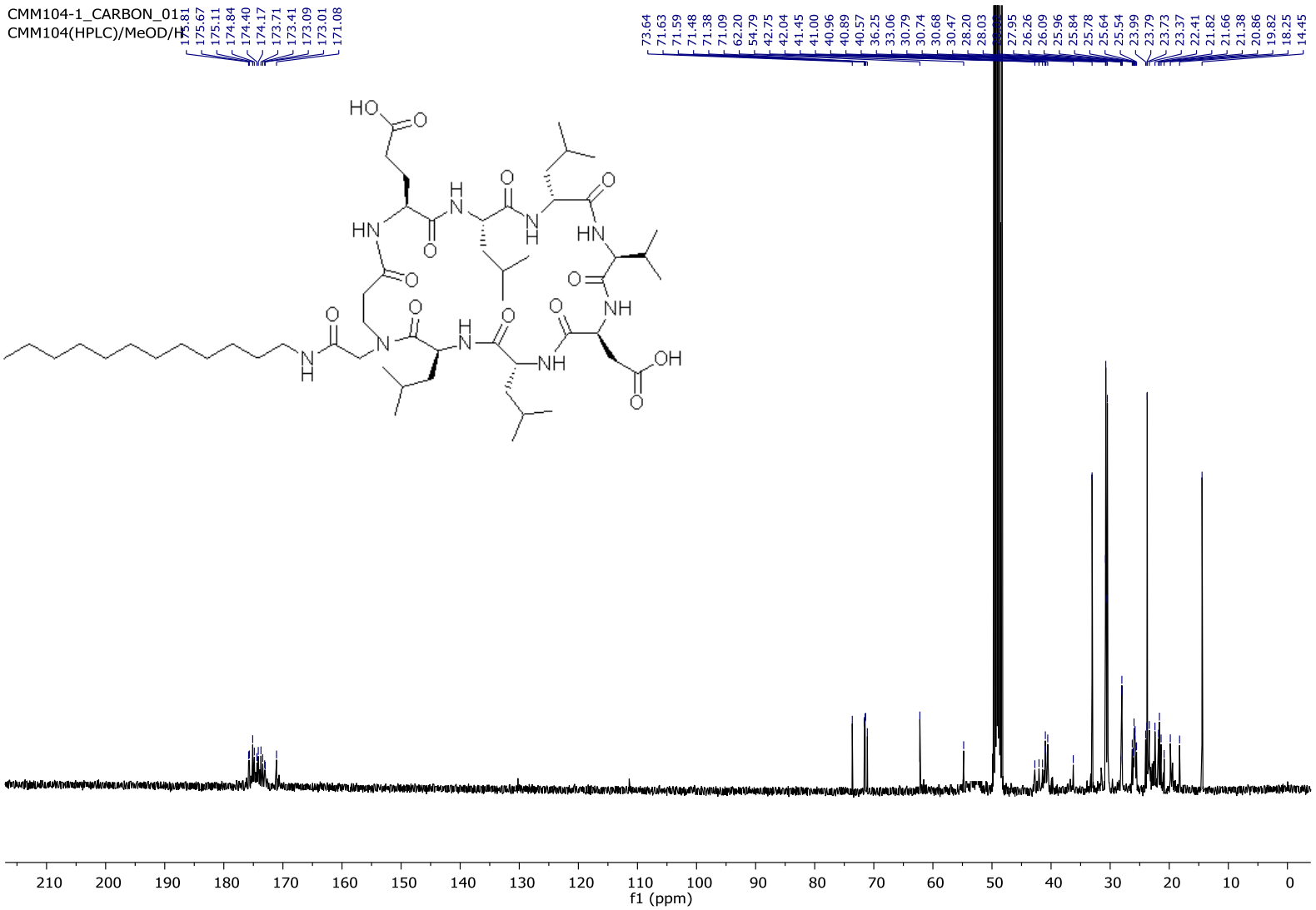

Figure 17. 100 MHz ^{13}C NMR spectrum of cyclic lipopeptide **10** in CD_3OD.

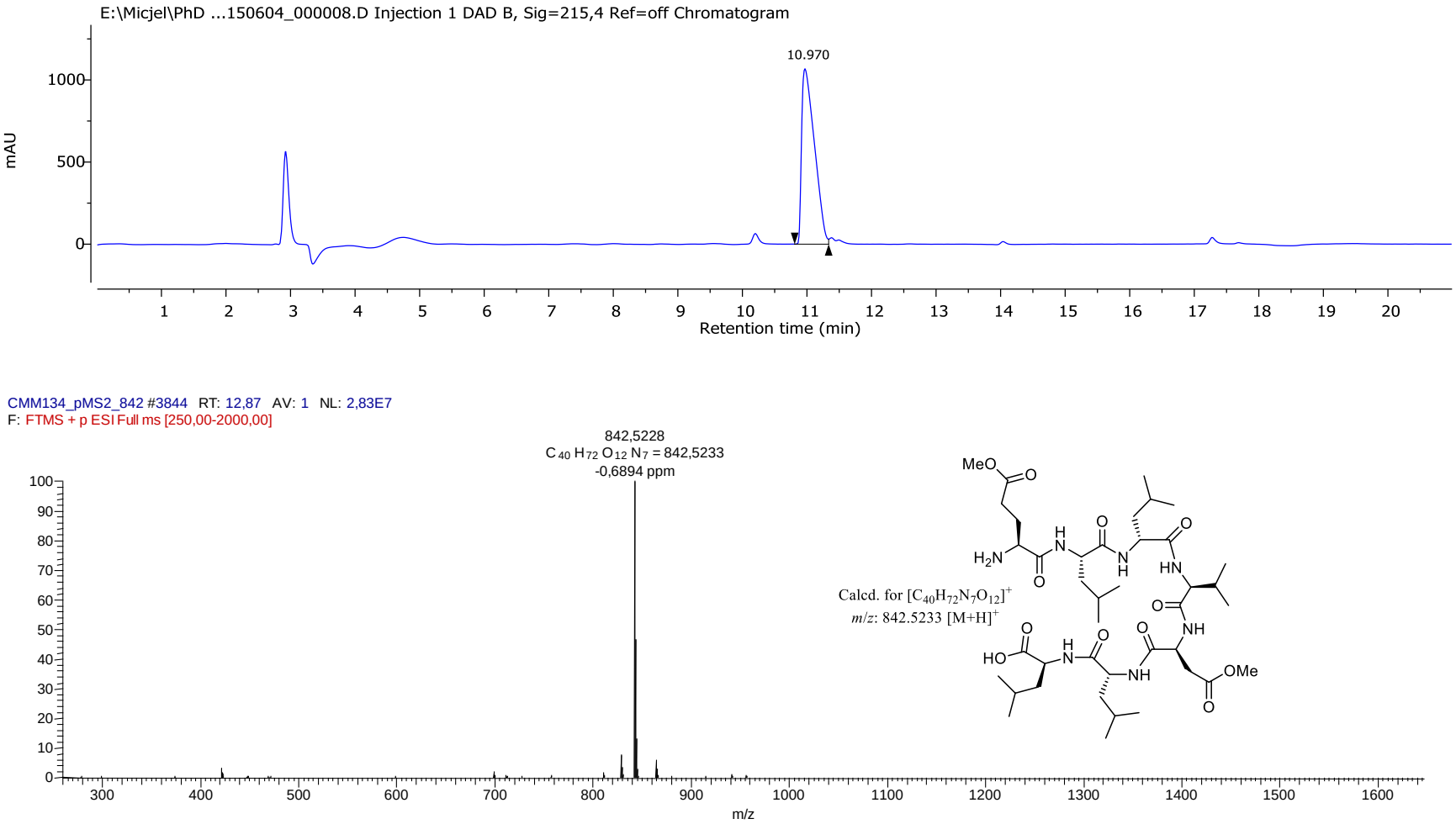

Figure 18. RP-HPLC chromatogram and ESI-HRMS of peptide H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH.

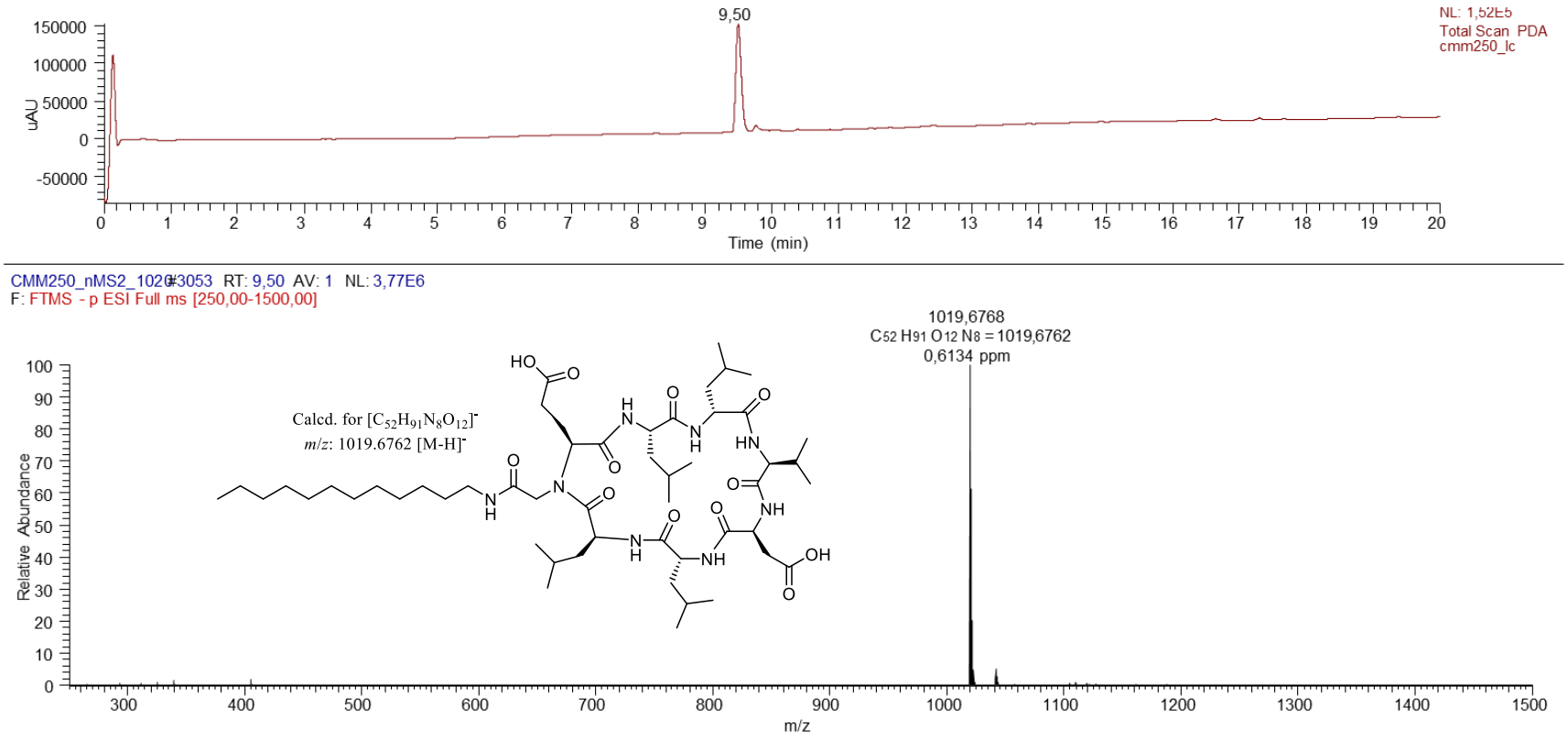

Figure 19. RP-UHPLC chromatogram and ESI-HRMS of pure cyclic lipopeptide **11**.

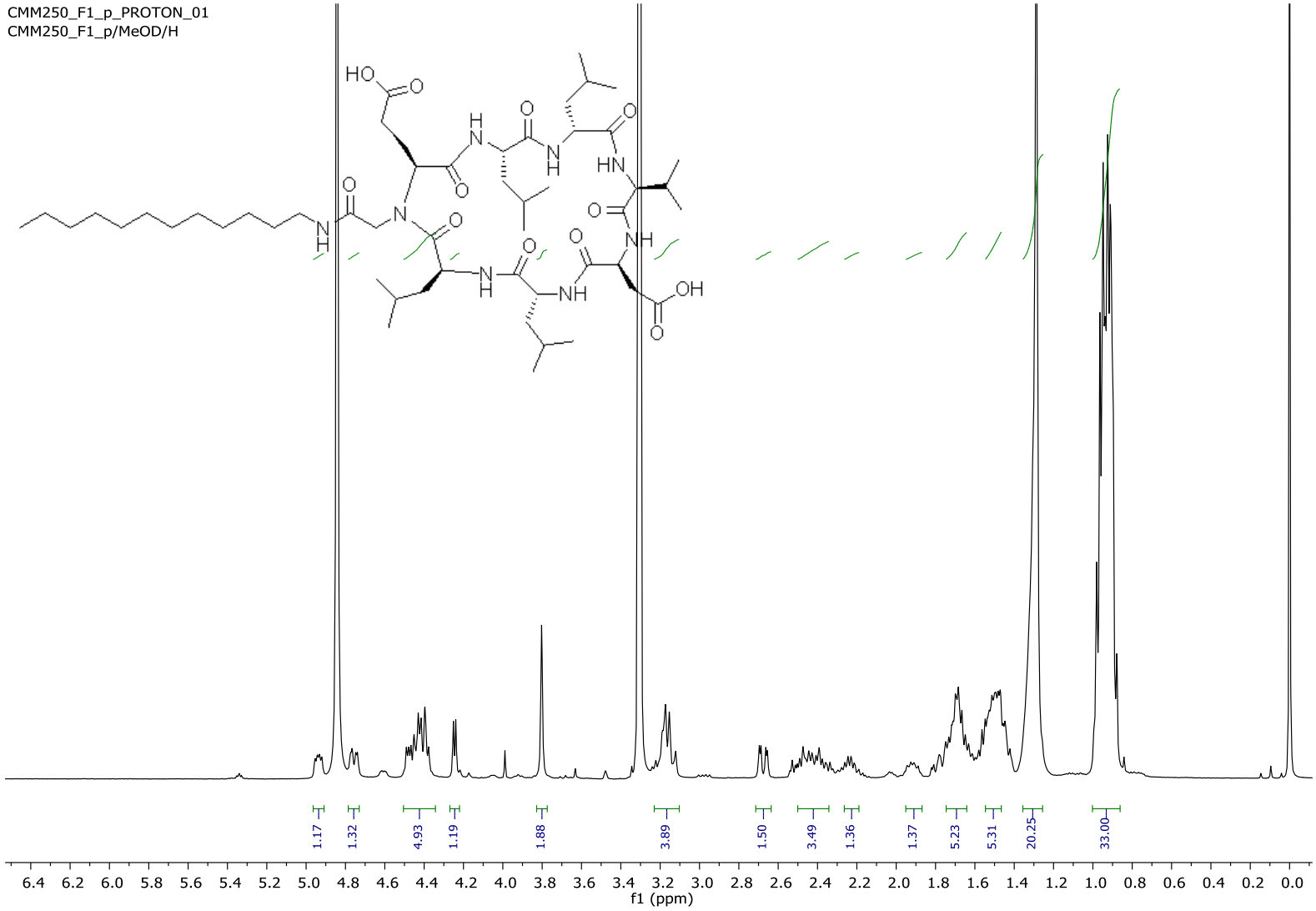

Figure 20. 400 MHz ^{1}H NMR spectrum of cyclic lipopeptide **11** in CD_3OD.

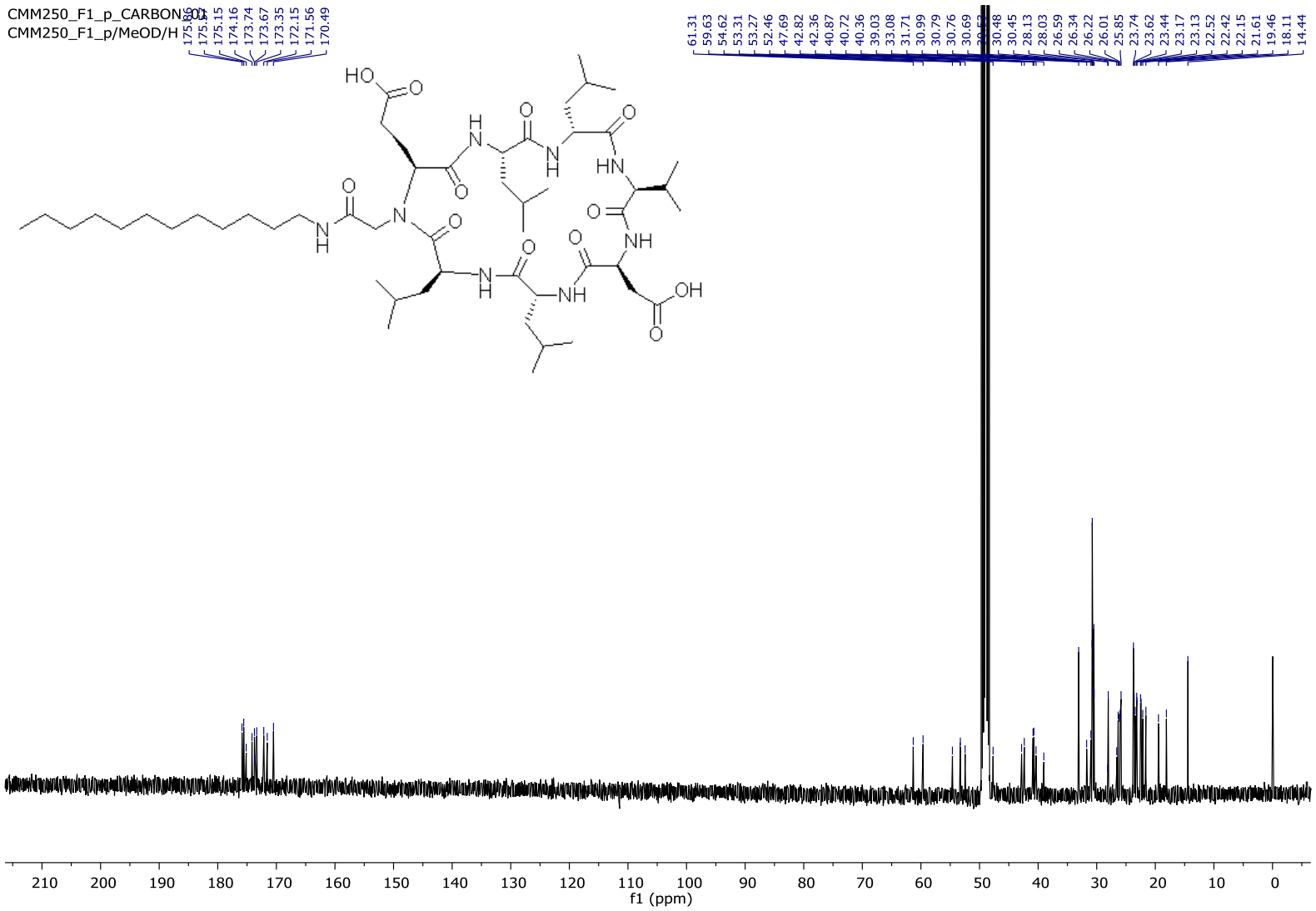

Figure 21. 100 MHz ^{13}C NMR spectrum of cyclic lipopeptide **11** in CD_3OD.

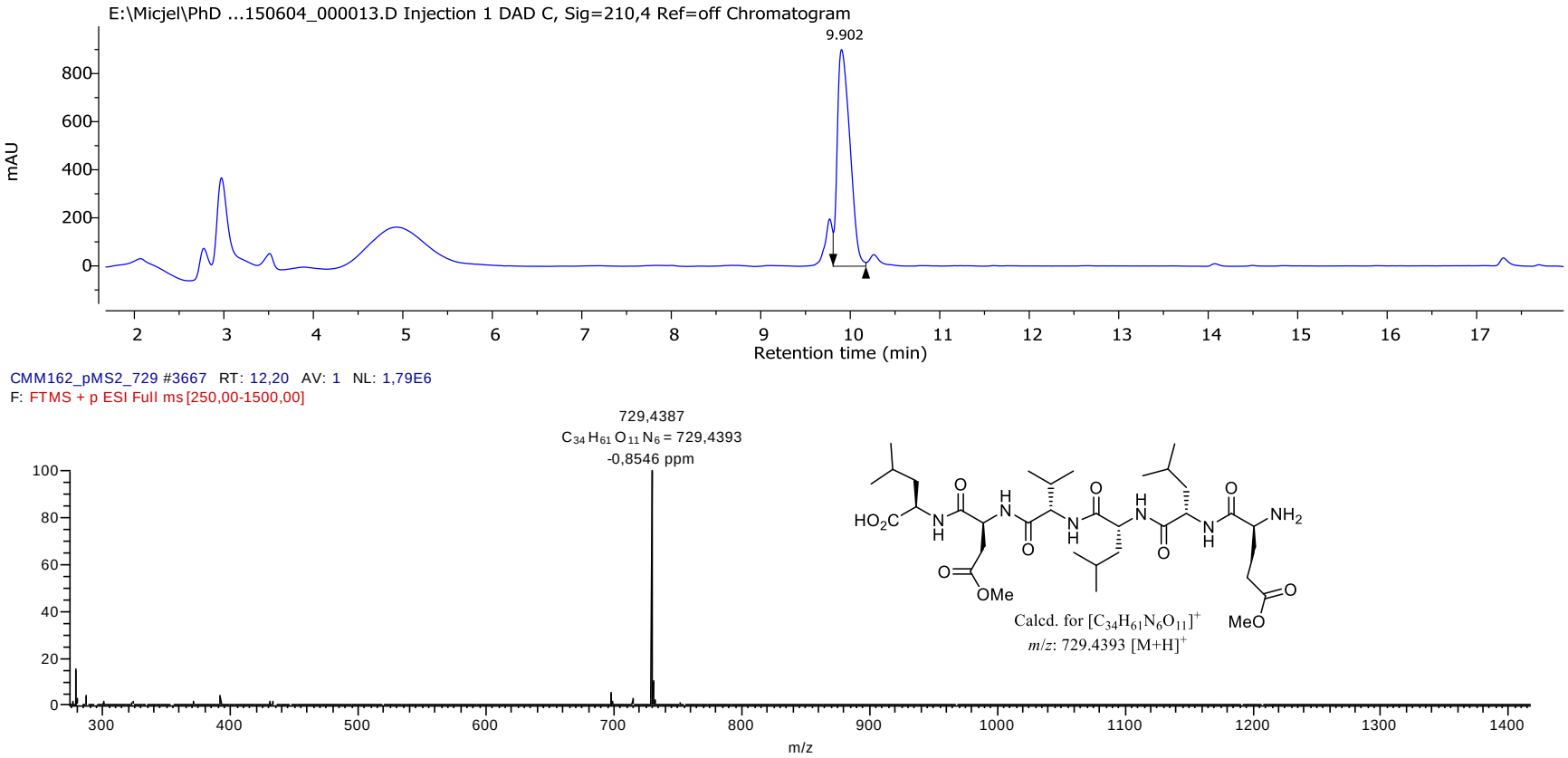

Figure 22. RP-HPLC chromatogram and ESI-HRMS of peptide H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-D-Leu-OH.

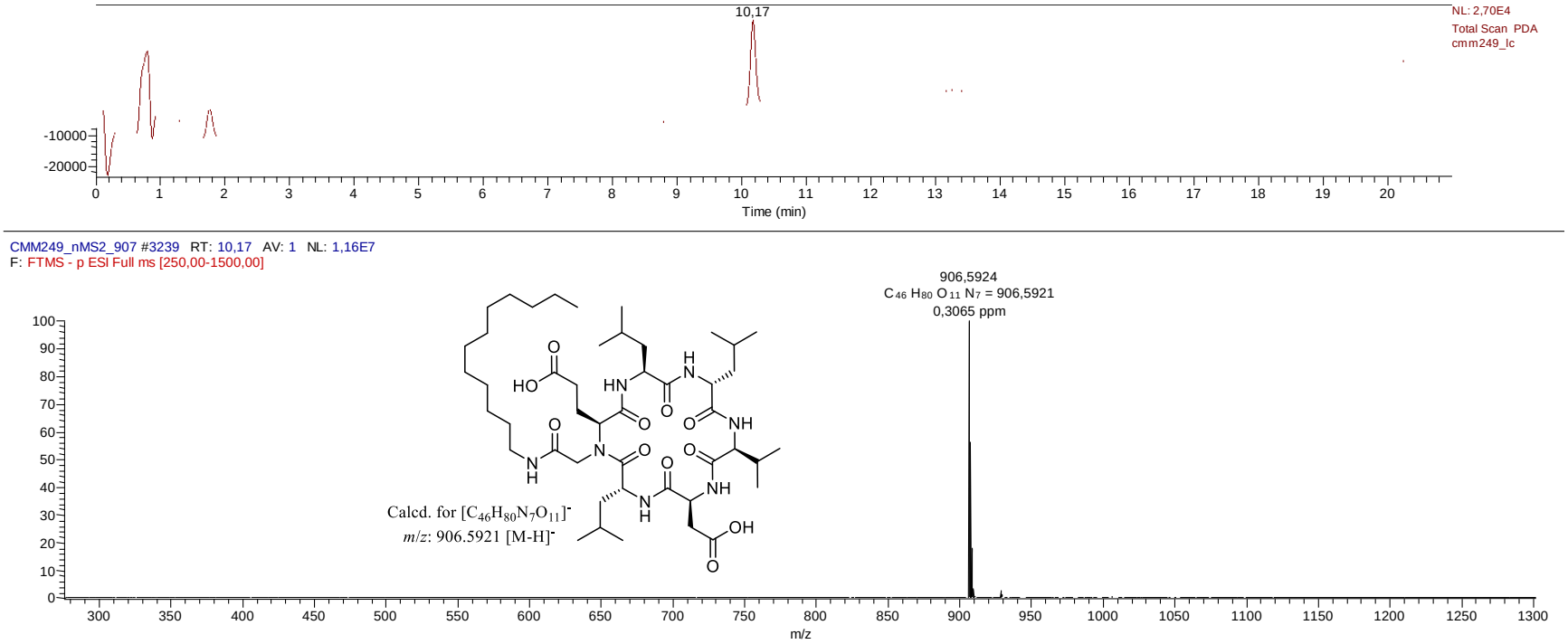

Figure 23. RP-UHPLC chromatogram and ESI-HRMS of pure cyclic lipopeptide **12**.

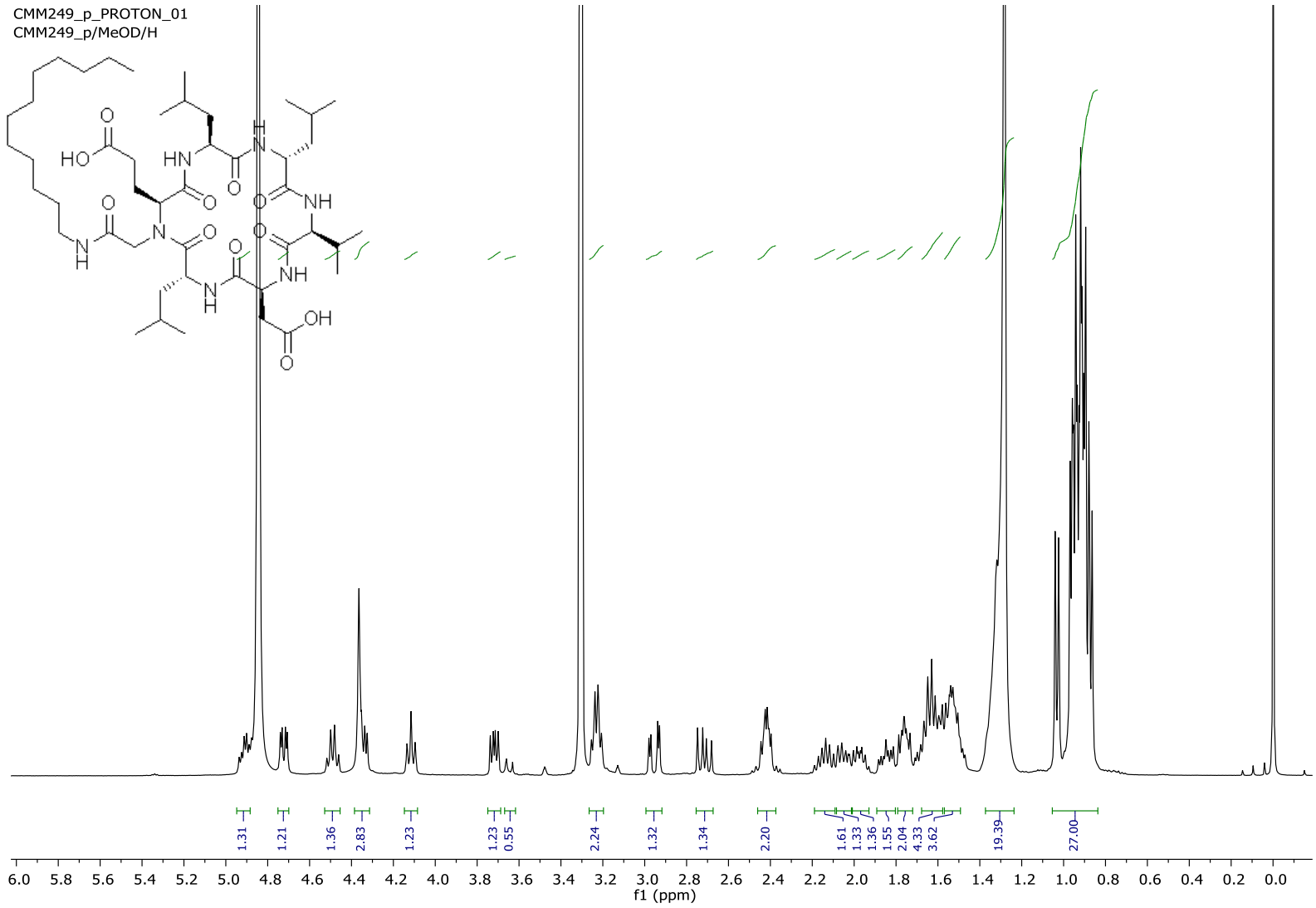

Figure 24. 400 MHz ^{1}H NMR spectrum of cyclic lipopeptide **12** in CD_3OD.

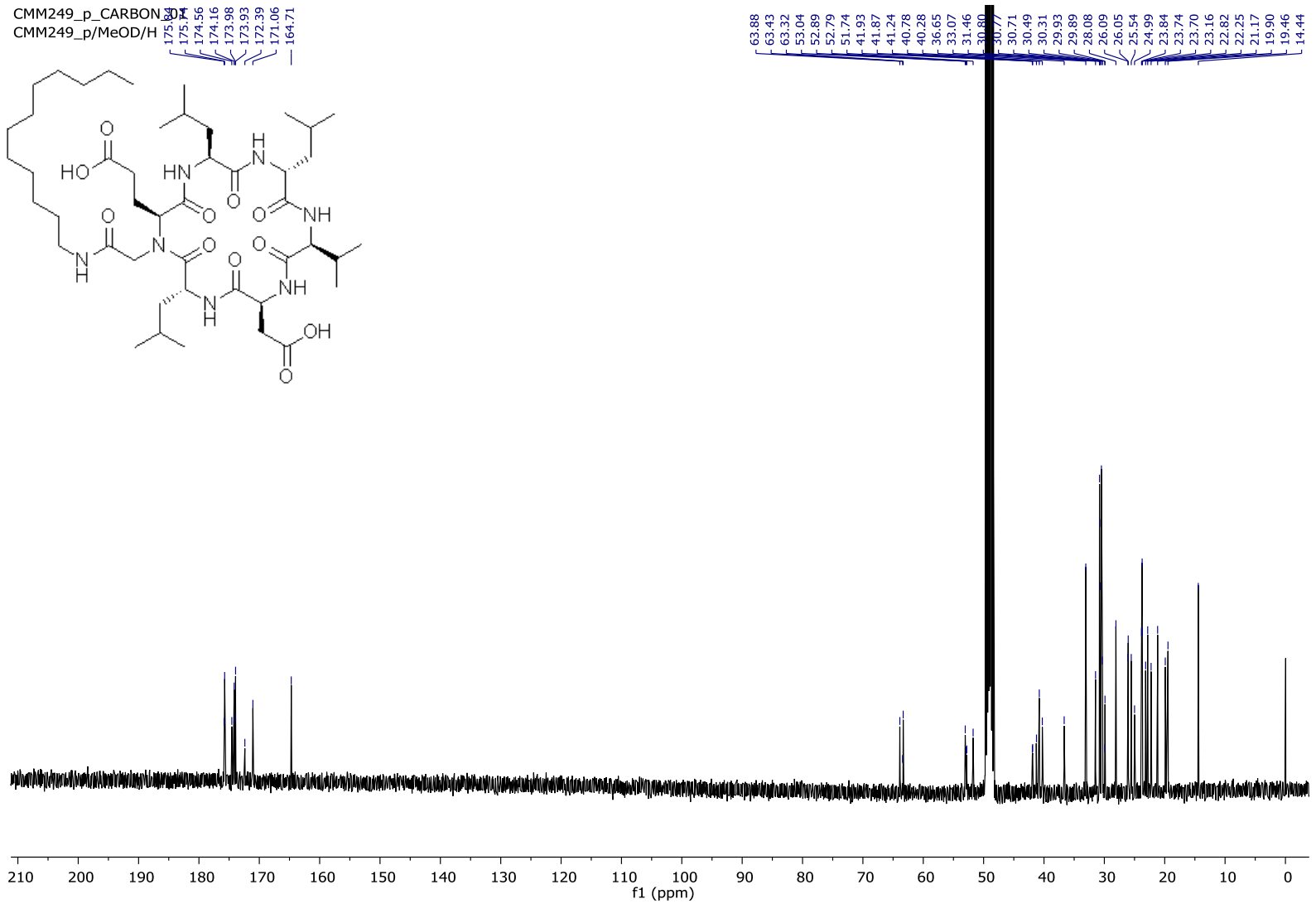

Figure 25. 100 MHz ^{13}C NMR spectrum of cyclic lipopeptide **12** in CD_3OD.

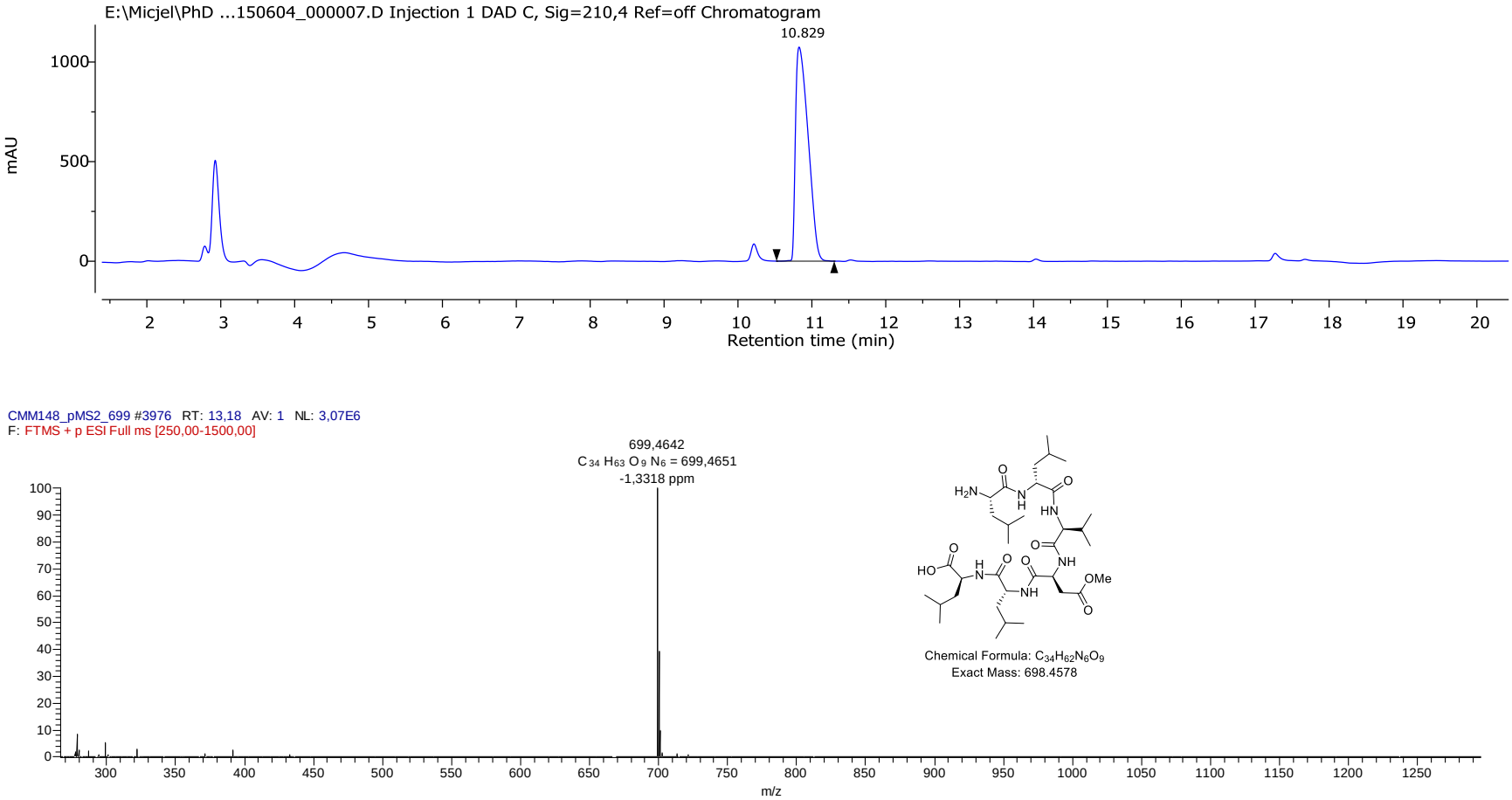

Figure 26. RP-HPLC chromatogram and ESI-HRMS of peptide H-Leu-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH.

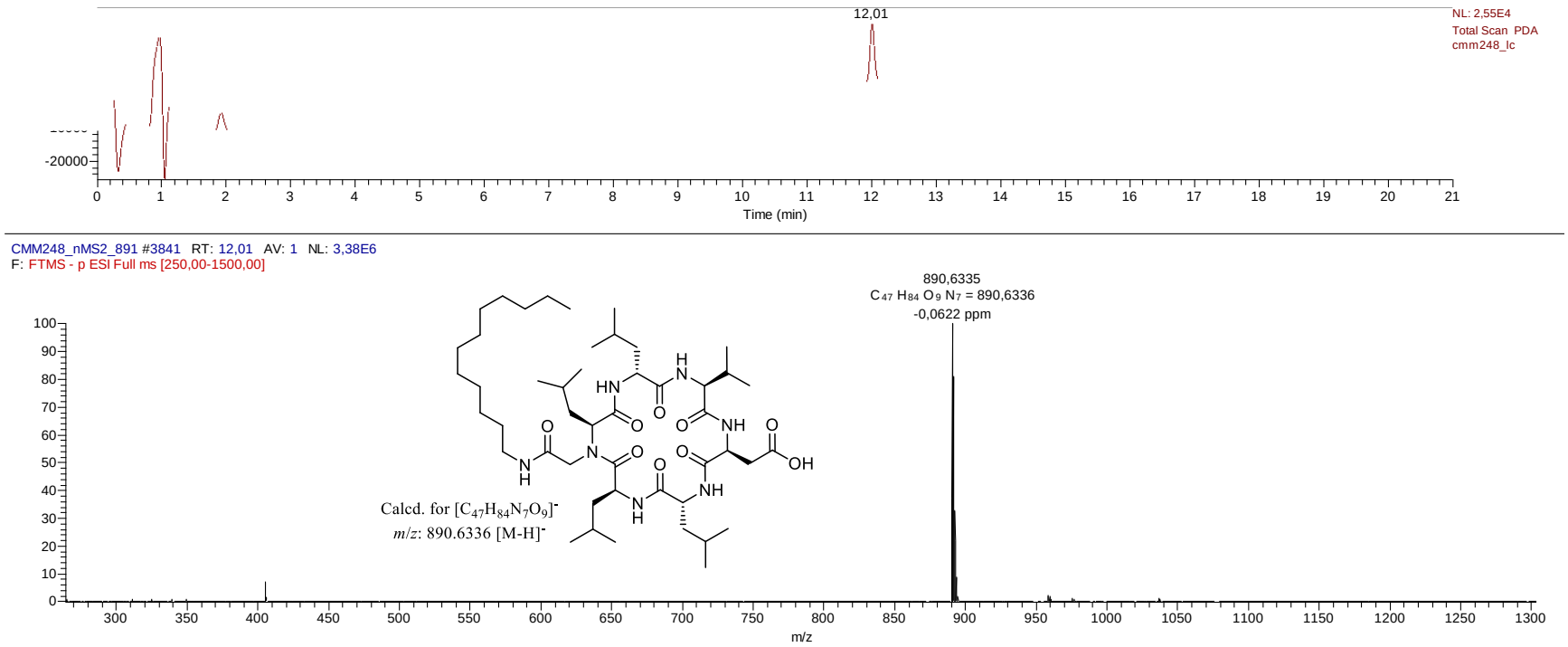

Figure 27. RP-UHPLC chromatogram and ESI-HRMS of pure cyclic lipopeptide **13**.

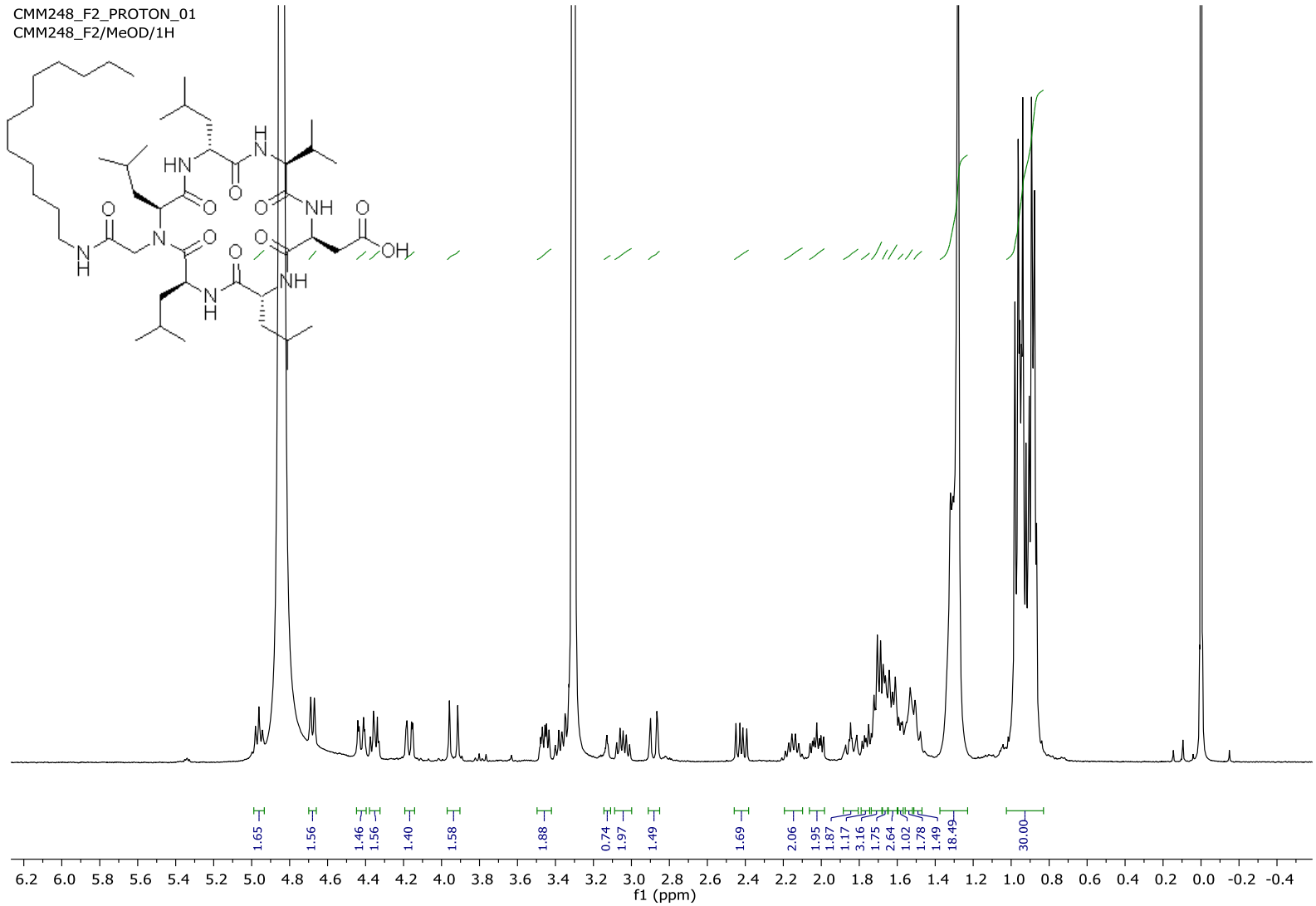

Figure 28. 400 MHz ^{1}H NMR spectrum of cyclic lipopeptide **13** in CD_3OD.

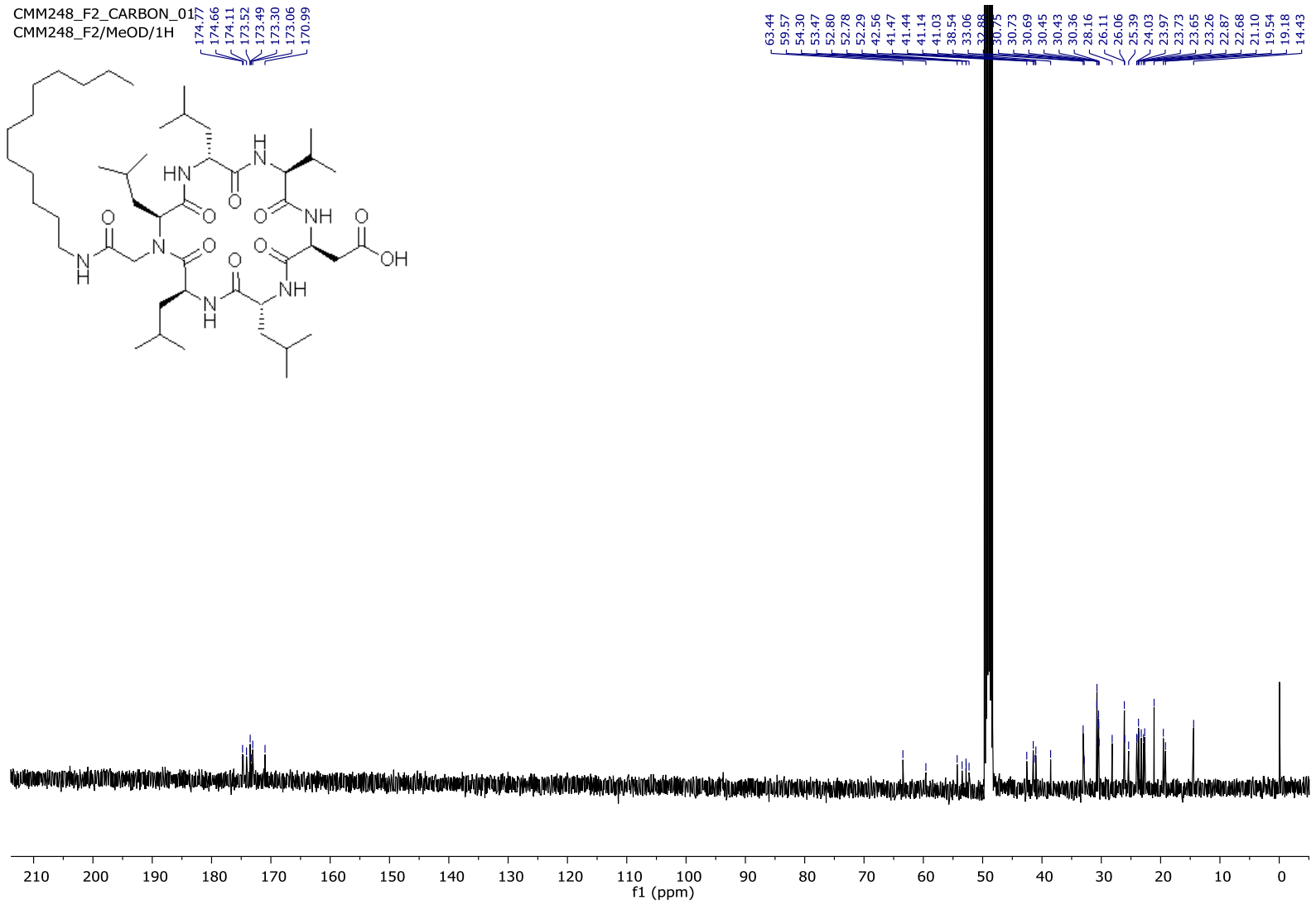

Figure 29. 100 MHz ^{13}C NMR spectrum of cyclic lipopeptide **13** in CD_3OD.

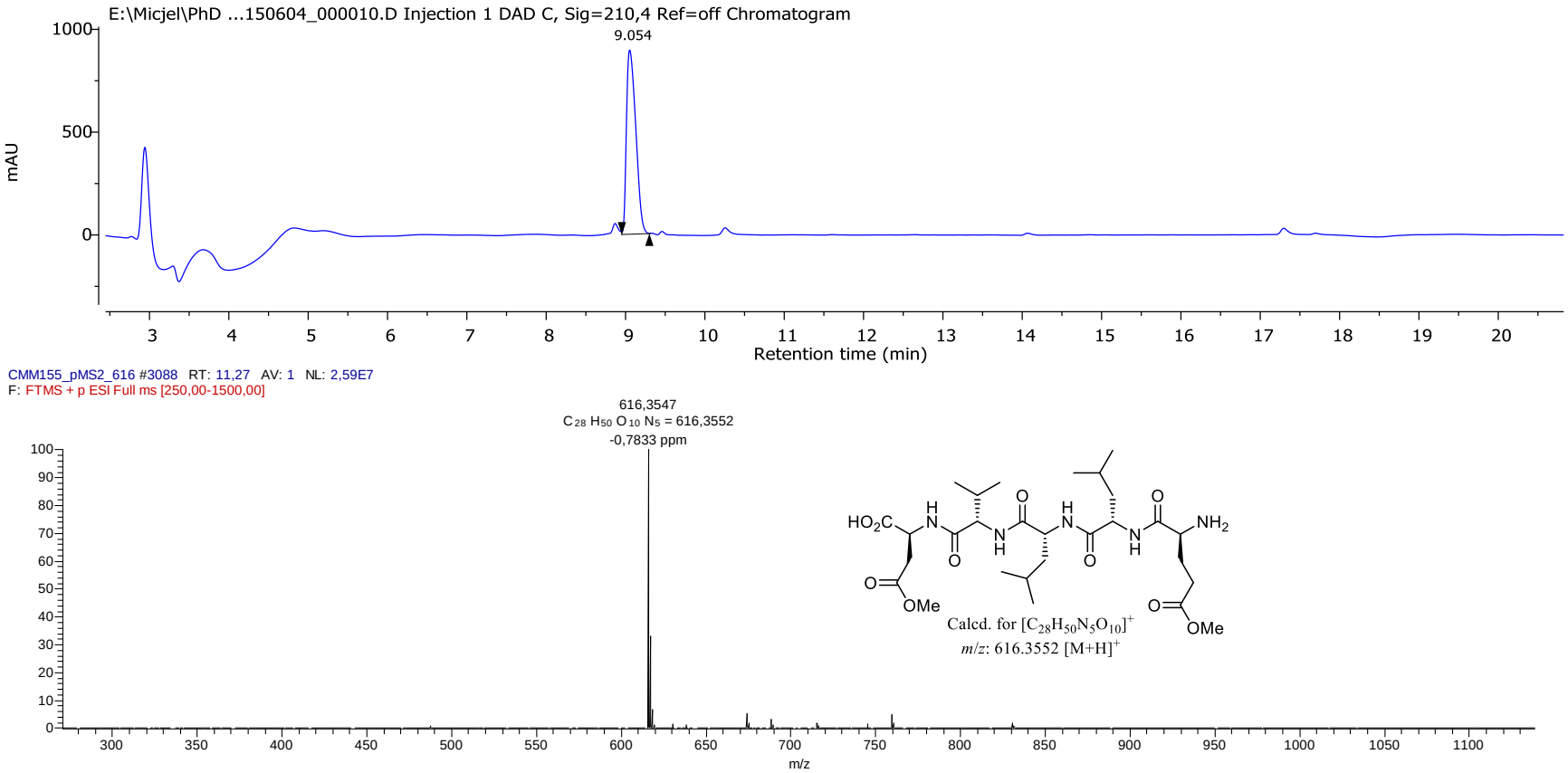

Figure 30. RP-HPLC chromatogram and ESI-HRMS of pure peptide H-Glu(OMe)-Leu-D-Leu-Val-Asp(OMe)-OH.

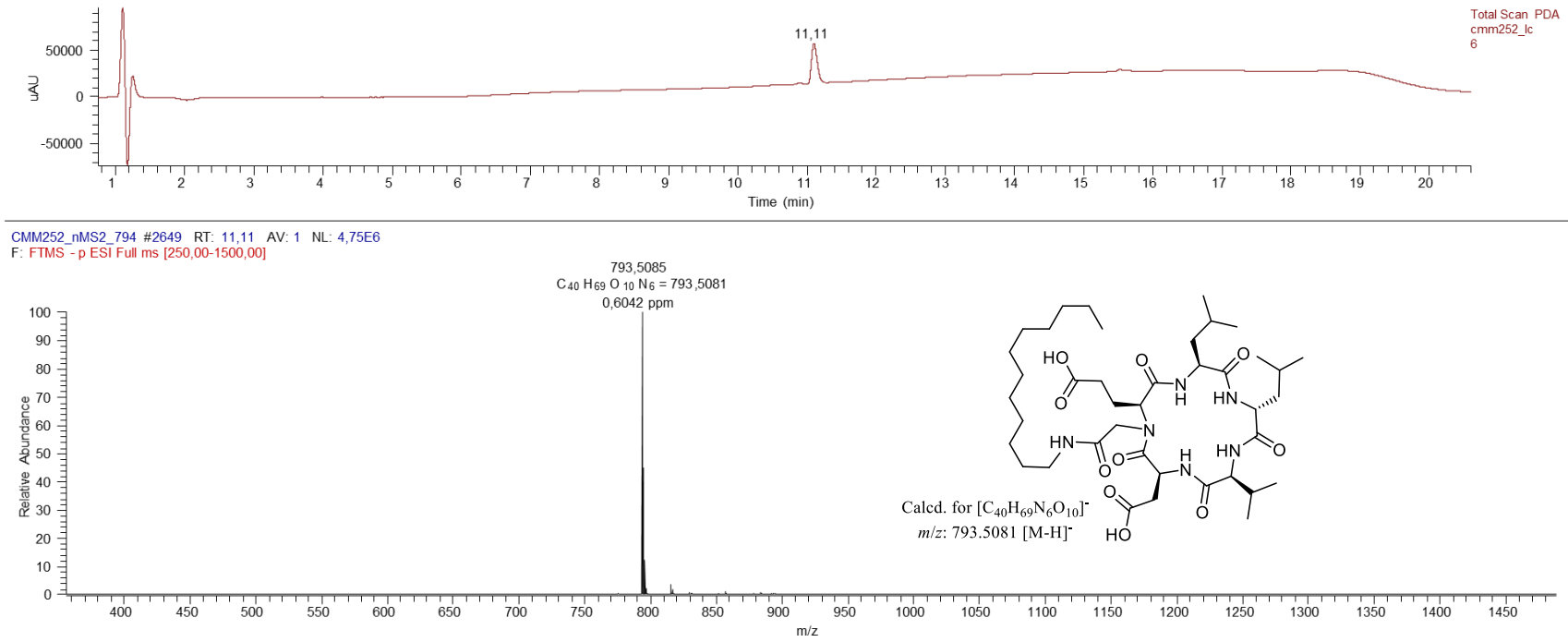

Figure 31. RP-UHPLC chromatogram and ESI-HRMS of pure cyclic lipopeptide **14**.

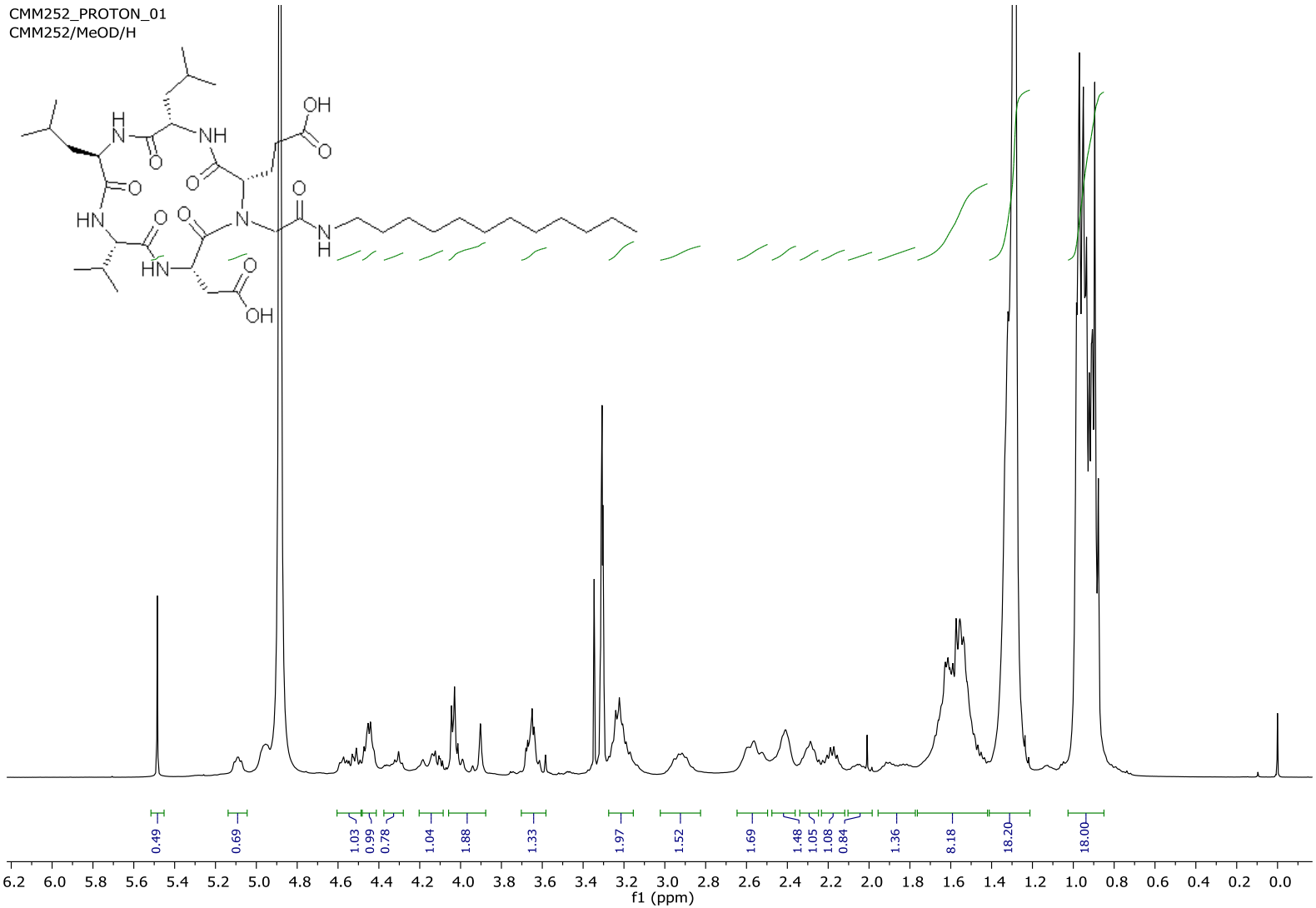

Figure 32. 400 MHz ^{1}H NMR spectrum of cyclic lipopeptide **14** in CD_3OD.

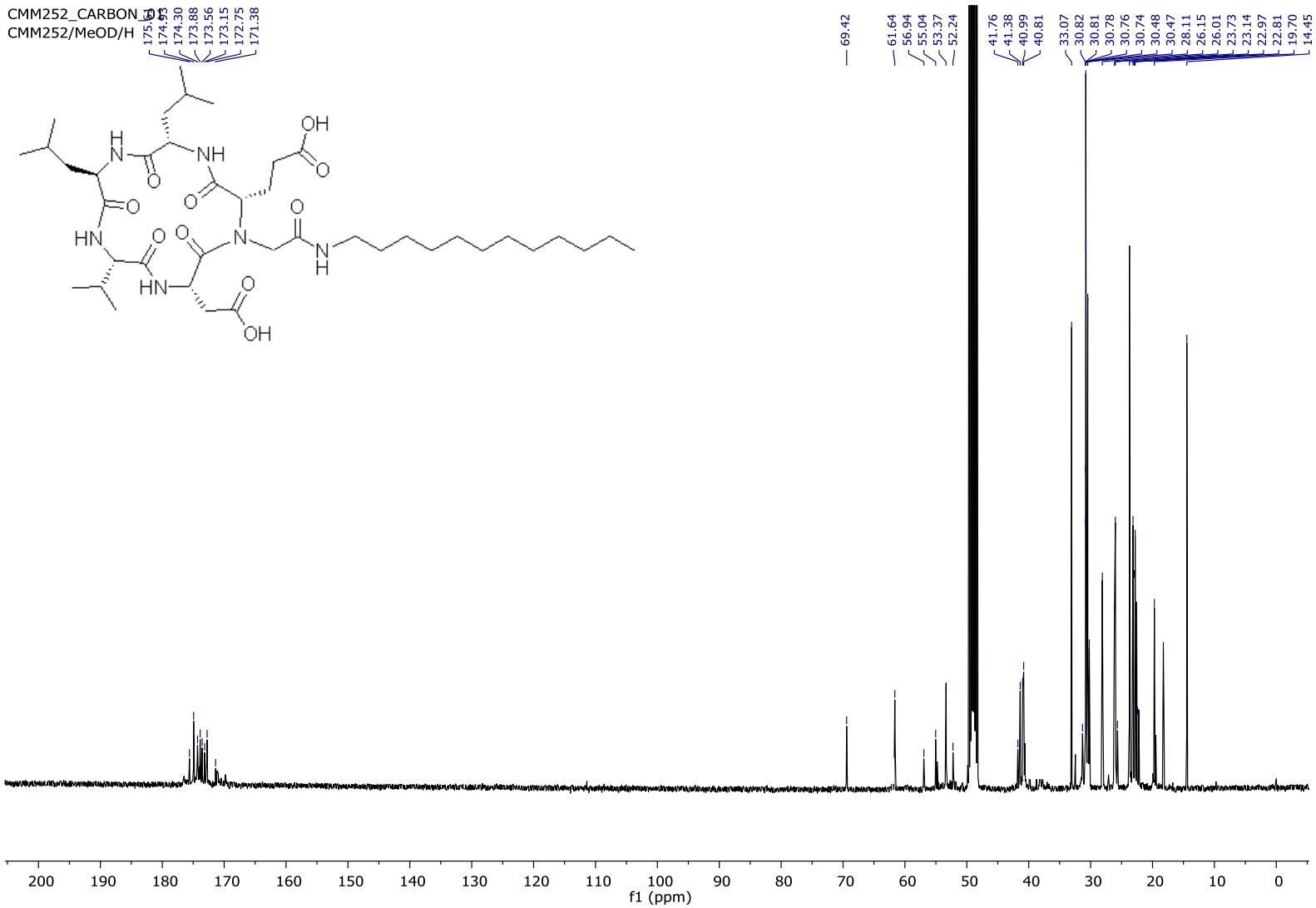

Figure 33. 100 MHz ^{13}C NMR spectrum of cyclic lipopeptide **14** in CD_3OD.

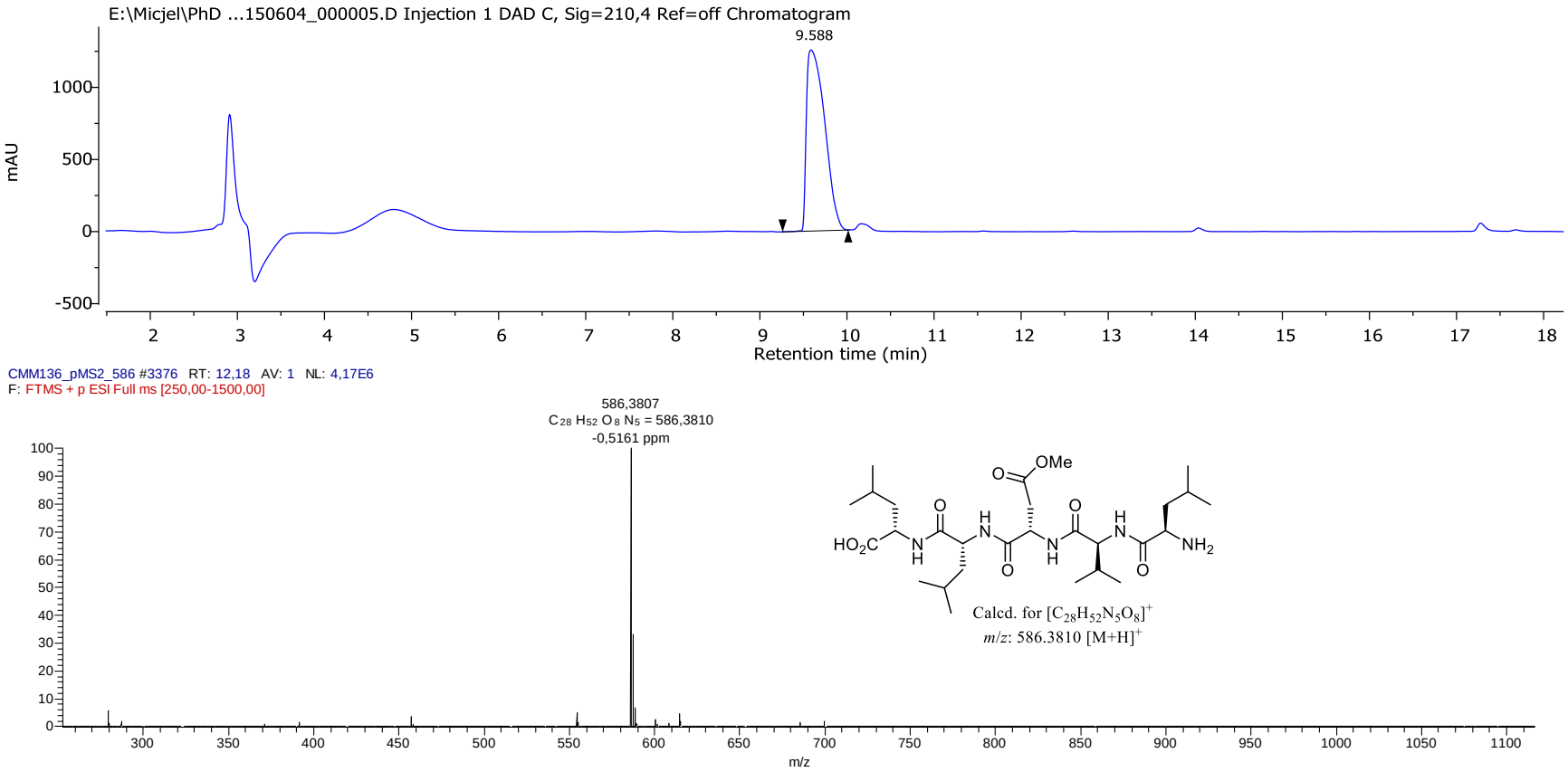

Figure 34. RP-HPLC chromatogram and ESI-HRMS of pure peptide H-D-Leu-Val-Asp(OMe)-D-Leu-Leu-OH.

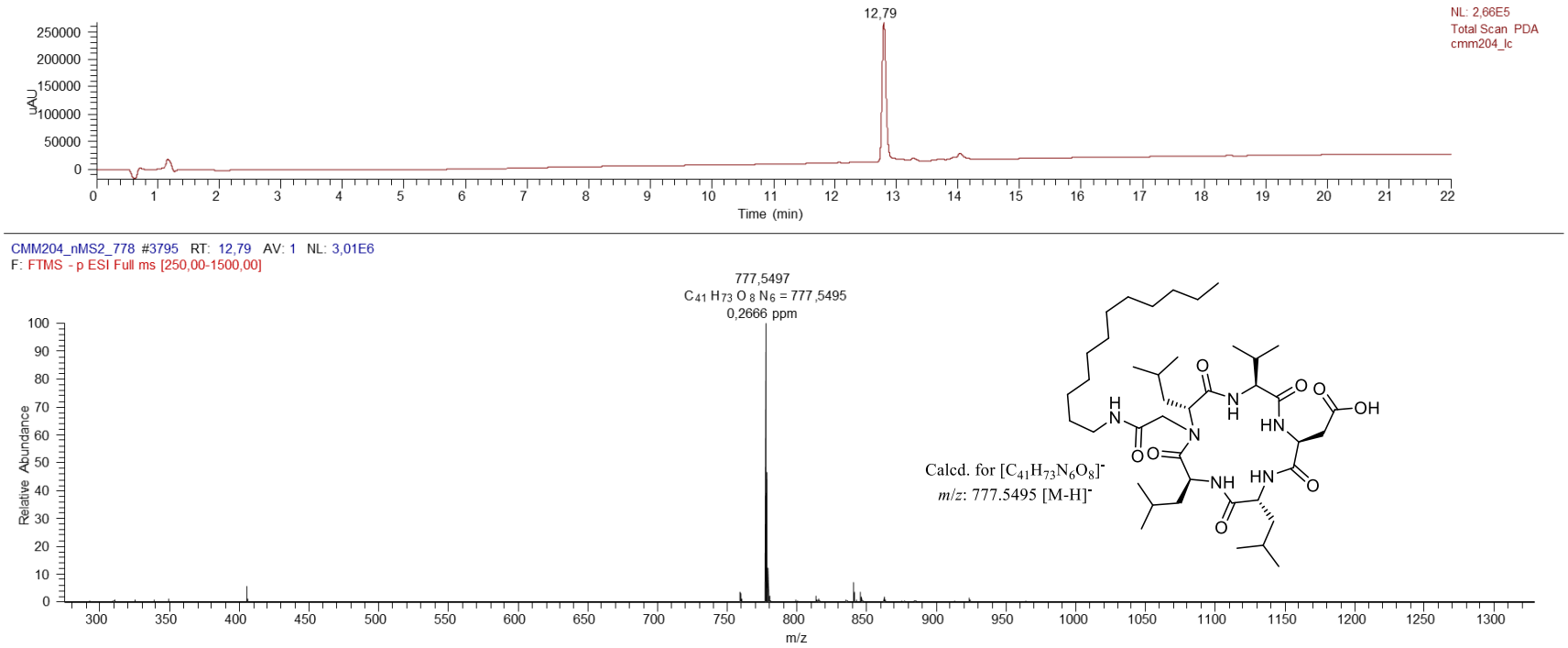

Figure 35. RP-UHPLC chromatogram and ESI-HRMS of pure cyclic lipopeptide **15**.

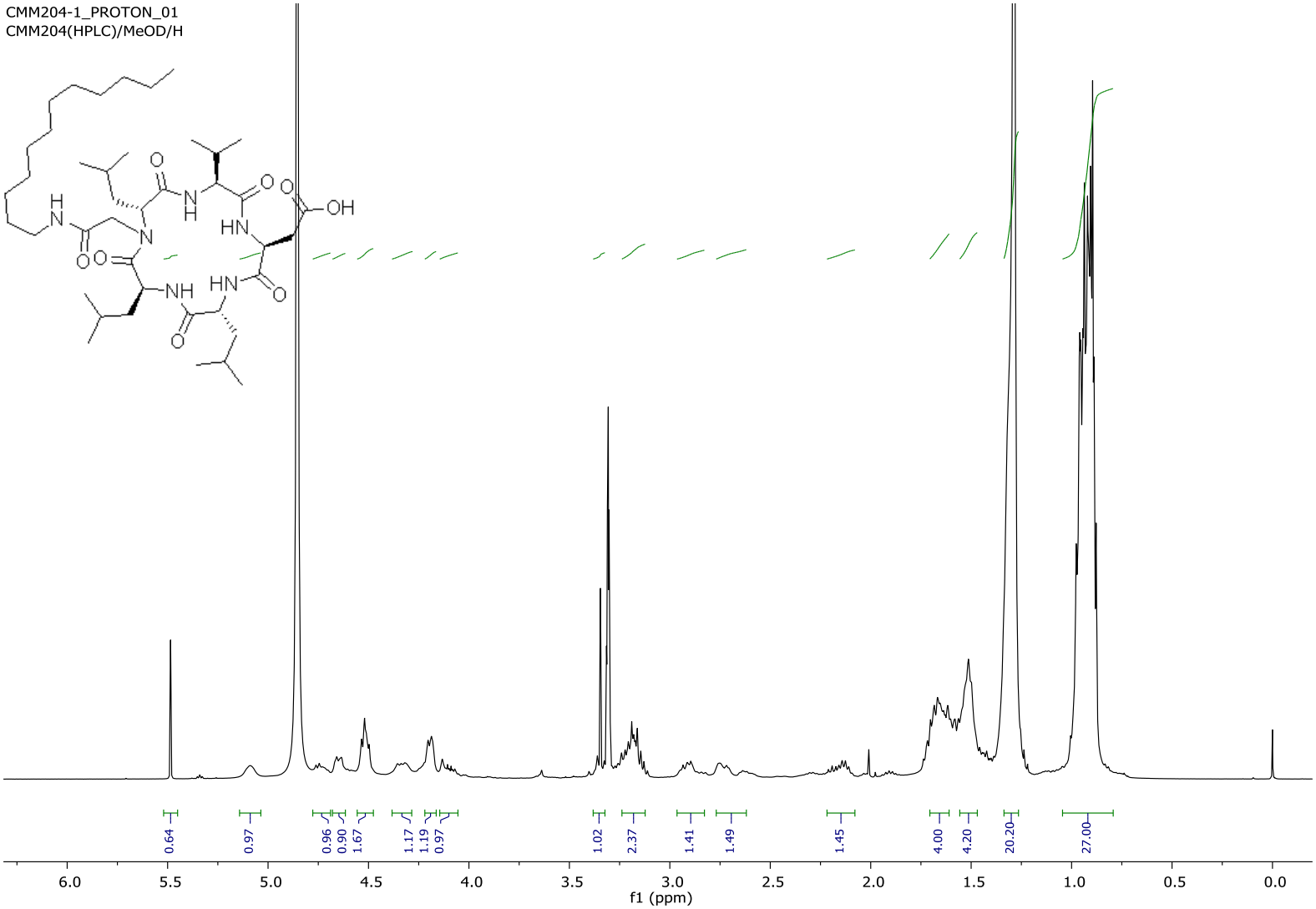

Figure 36. 400 MHz ^{1}H NMR spectrum of cyclic lipopeptide **15** in CD_3OD.

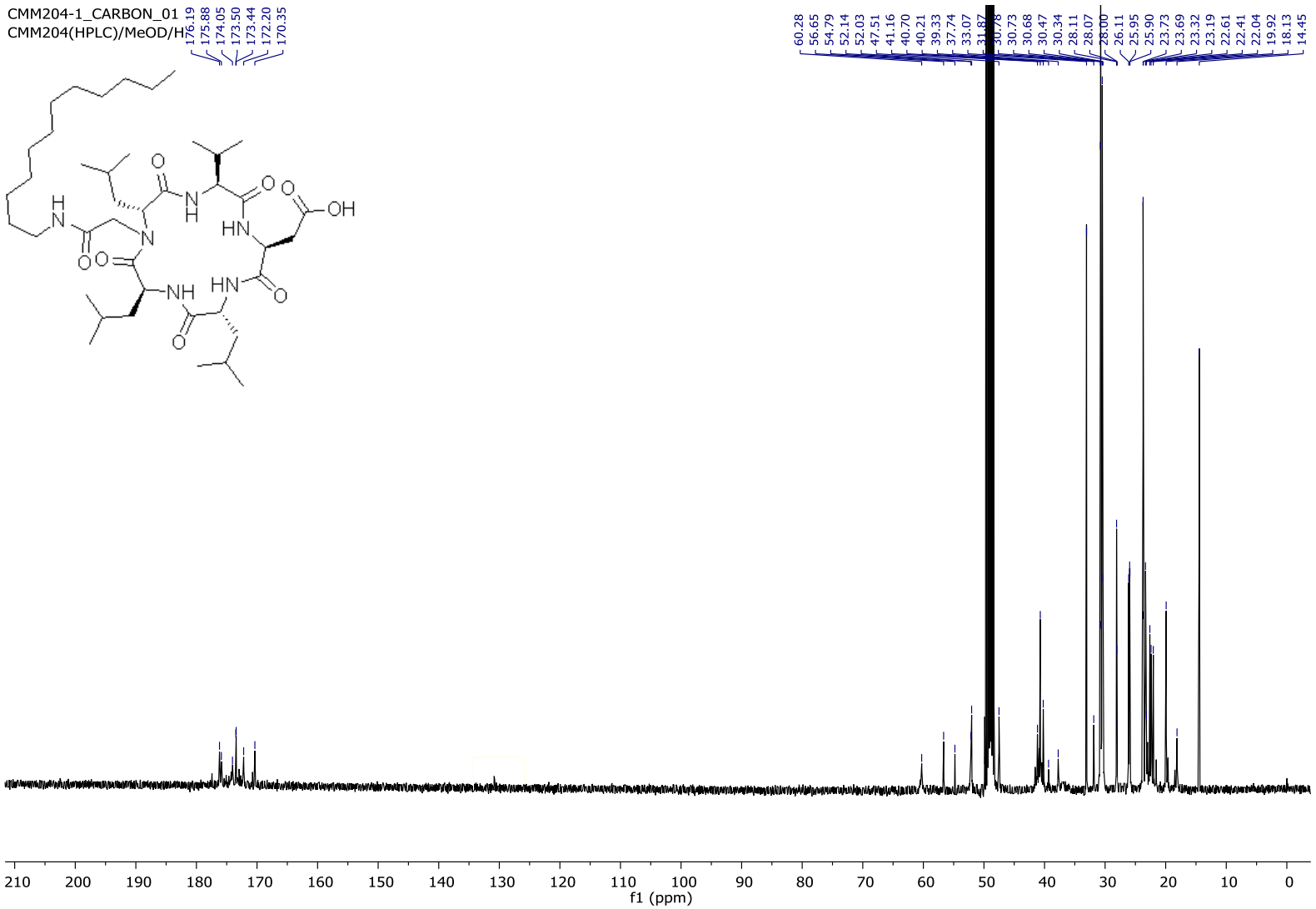

Figure 37. 100 MHz ^{13}C NMR spectrum of cyclic lipopeptide **15** in CD_3OD.

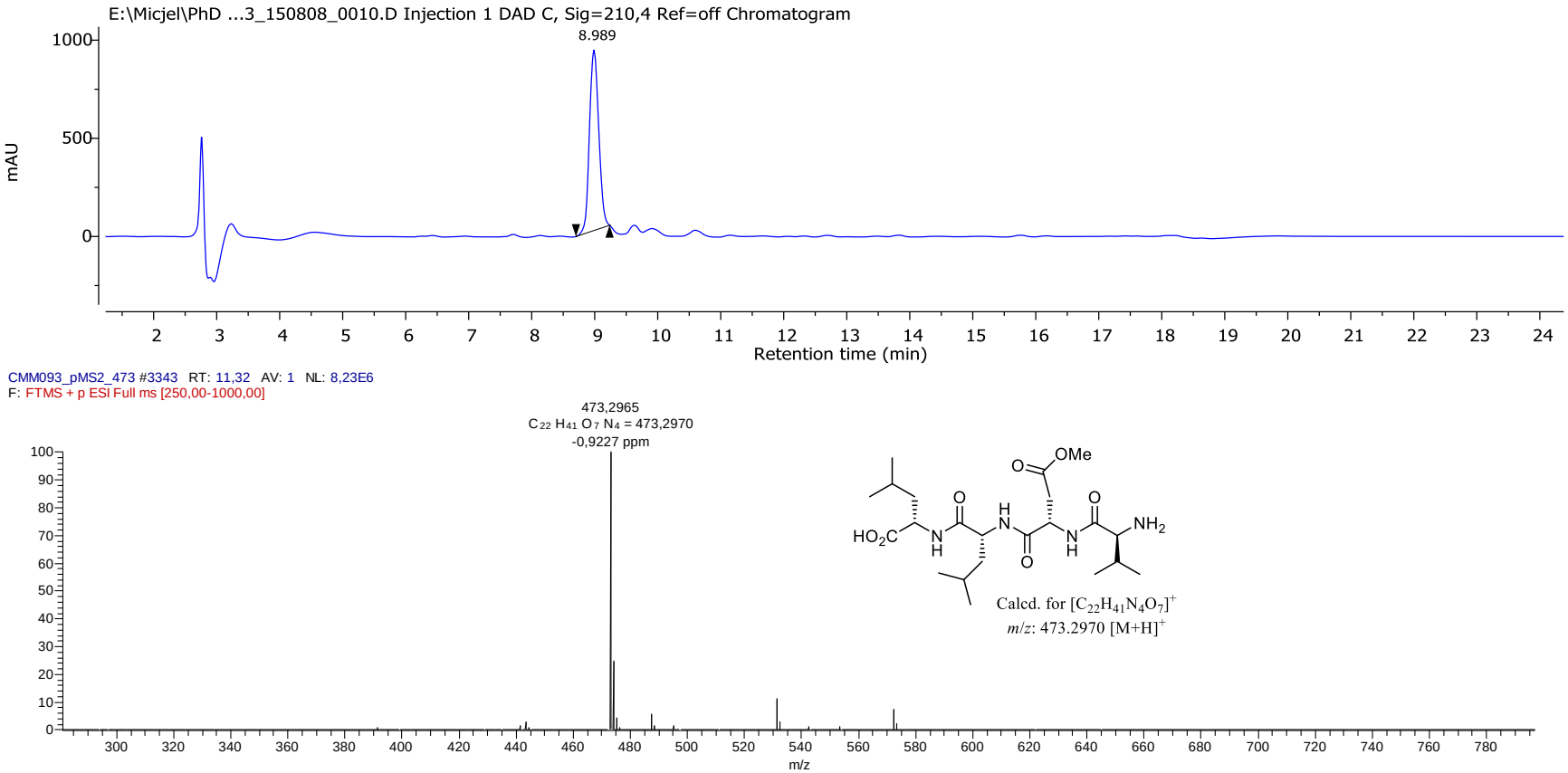

Figure 38. RP-HPLC chromatogram and ESI-HRMS of peptide H-Val-Asp(OMe)-D-Leu-Leu-OH.

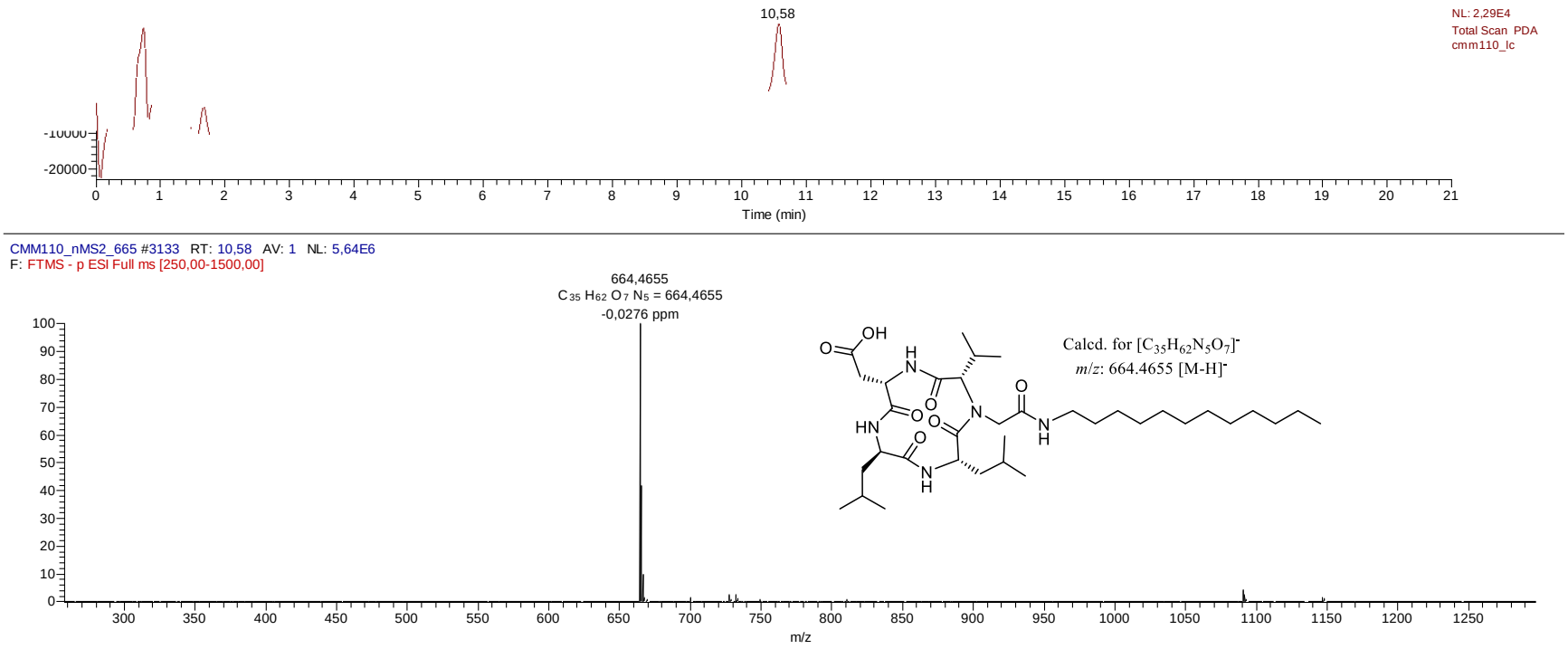

Figure 39. RP-UHPLC chromatogram and ESI-HRMS of pure cyclic lipopeptide **16**.

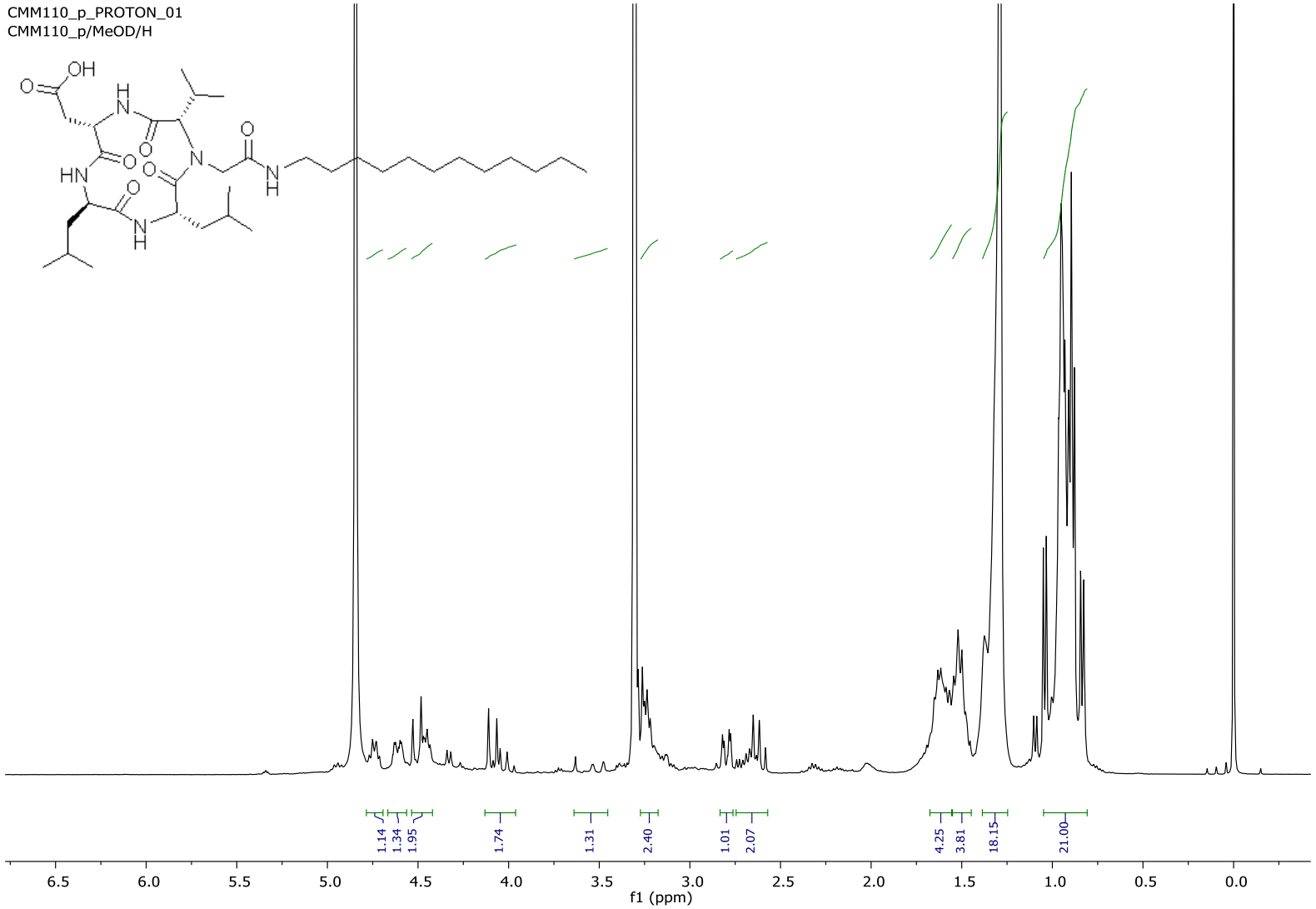

Figure 40. 400 MHz ^{1}H NMR spectrum of cyclic lipopeptide **16** in CD_3OD.

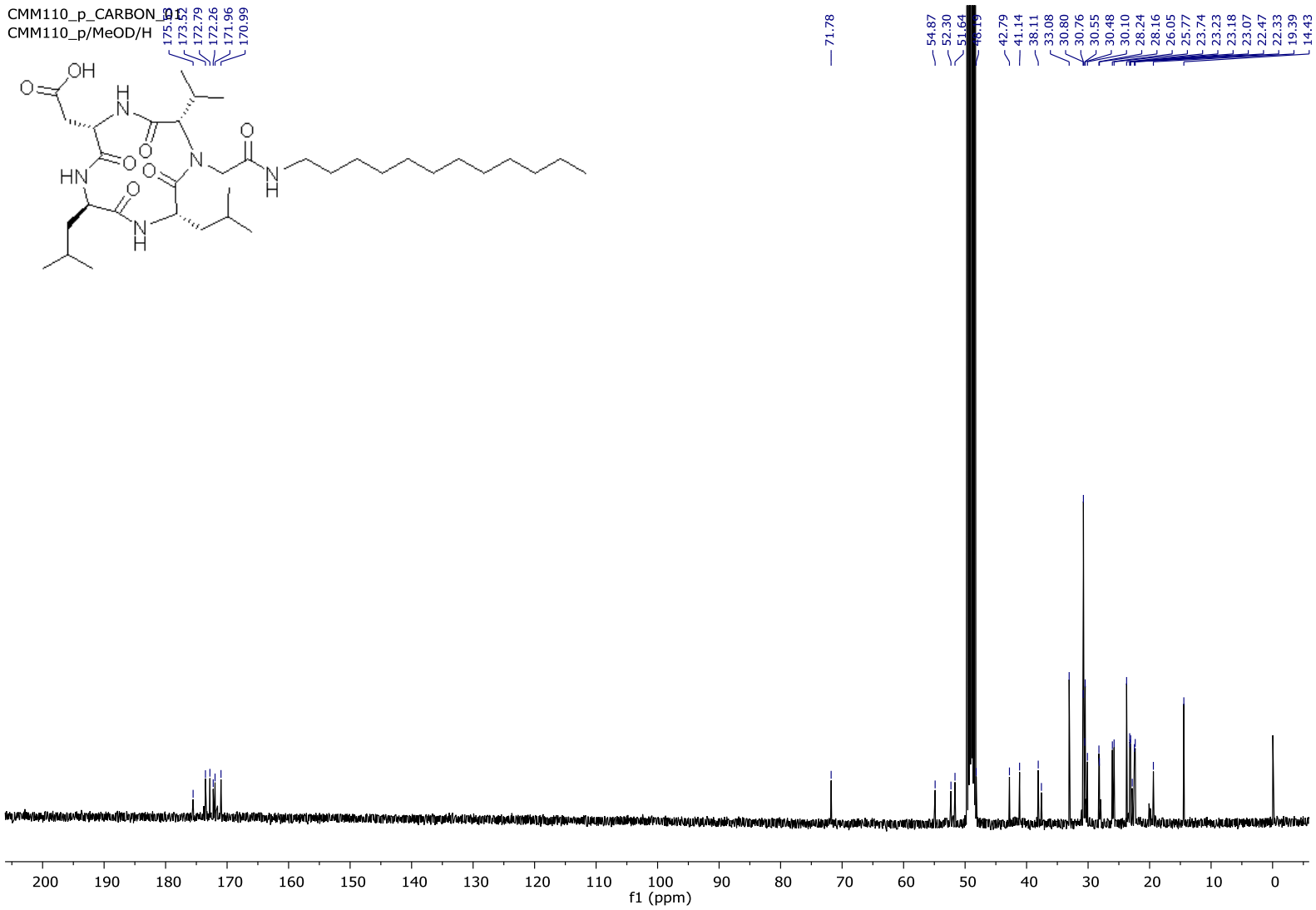

Figure 41. 100 MHz ^{13}C NMR spectrum of cyclic lipopeptide **16** in CD_3OD.