Supporting Information

Total Synthesis of Microcystin-LF and Derivatives Thereof

Ivan Zemskov,[†] Stefan Altaner,[‡] Daniel R. Dietrich,[‡] and Valentin Wittmann[†,*]

[†]*Department of Chemistry and Konstanz Research School Chemical Biology (KoRS-CB), University of Konstanz, 78457 Konstanz (Germany)*
[‡]*Department of Biology and Graduate School Biological Sciences (GBS), University of Konstanz, 78457 Konstanz (Germany)*

E-mail: mail@valentin-wittmann.de

Table of Contents

Colorimetric Protein Phosphatase Inhibition Assay

Materials

Protein phosphatase 1 (PP1) (New England Biolabs, #P0754S)

para-Nitrophenylphosphate (pNPP) (Fisher Scientific, #10655562)

Beckman DU-640 spectrophotometer

The assay was performed following published procedures[1-2] with slight modifications. The assay was performed 3-5 times, each in technical duplicates or triplicates. The concentration of natural and synthetic MCs was determined UV spectroscopically at λ = 238 nm by using the published extinction coefficient of MC-LR.[3] Solutions of MC derivatives in dH_2O (20 µL) with ten different concentrations obtained by serial dilutions were mixed with a solution of PP1 in enzyme diluent buffer (20 µL, 1.5 U mL^{-1})[1] in a 96-well plate. The mixture was incubated for 5-10 min at 37°C and substrate solution (200µL, 60 mM pNPP)[1] was added. Absorption (λ = 405 nm) was directly measured after the addition of the substrate (t = 0h) and after an incubation time of 3h at 37°C. For every assay a 100% control (20 µL dH_2O, no toxin) and a 0% control (20 µL enzyme diluent buffer,[1] no PP1) was performed. The protein phosphatase activity was calculated by subtracting the absorption value at t = 0h from the value determined after 3h. Afterwards the mean of the 0% control (blank) was subtracted from the means of the samples (blank correction). Individual enzyme activity was normalized to the 100% control. The IC_{50} values were calculated using GraphPad Prism 5.0 software by performing a 5-PL non-linear regression.

References

(1) Heresztyn, T.; Nicholson, B. C. *Water Res.* **2001**, *35*, 3049-3056.

(2) Fischer, A.; Hoeger, S. J.; Stemmer, K.; Feurstein, D. J.; Knobeloch, D.; Nussler, A.; Dietrich, D. R. *Toxicol. Appl. Pharmacol.* **2010**, *245*, 9-20.

(3) Harada, K.-i.; Matsuura, K.; Suzuki, M.; Watanabe, M. F.; Oishi, S.; Dahlem, A. M.; Beasley, V. R.; Carmichael, W. W. *Toxicon* **1990**, *28*, 55-64.

LC-MS Analyses of Microcystin Derivatives

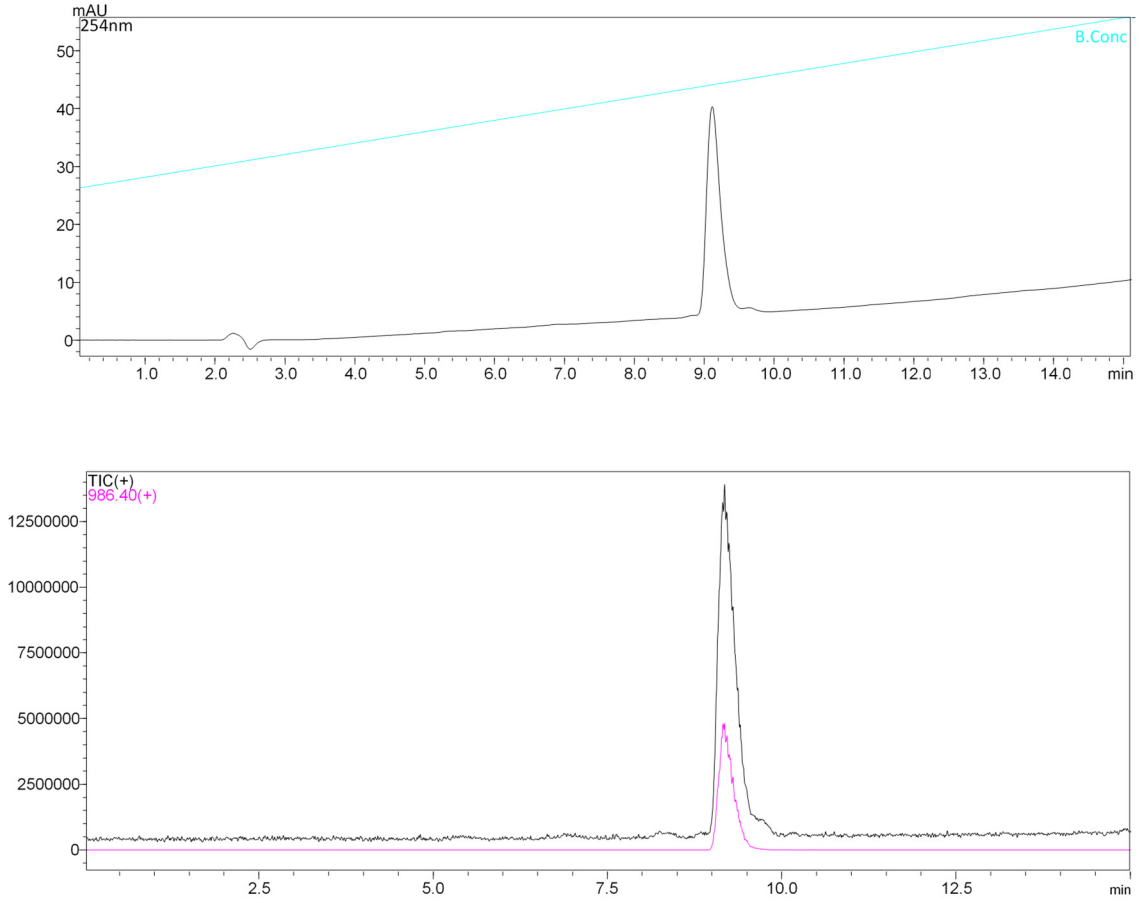


Figure S1. LC-MS analysis of natural MC-LF (Gradient: 50-100% B in 15 min, t_R = 9.1 min).

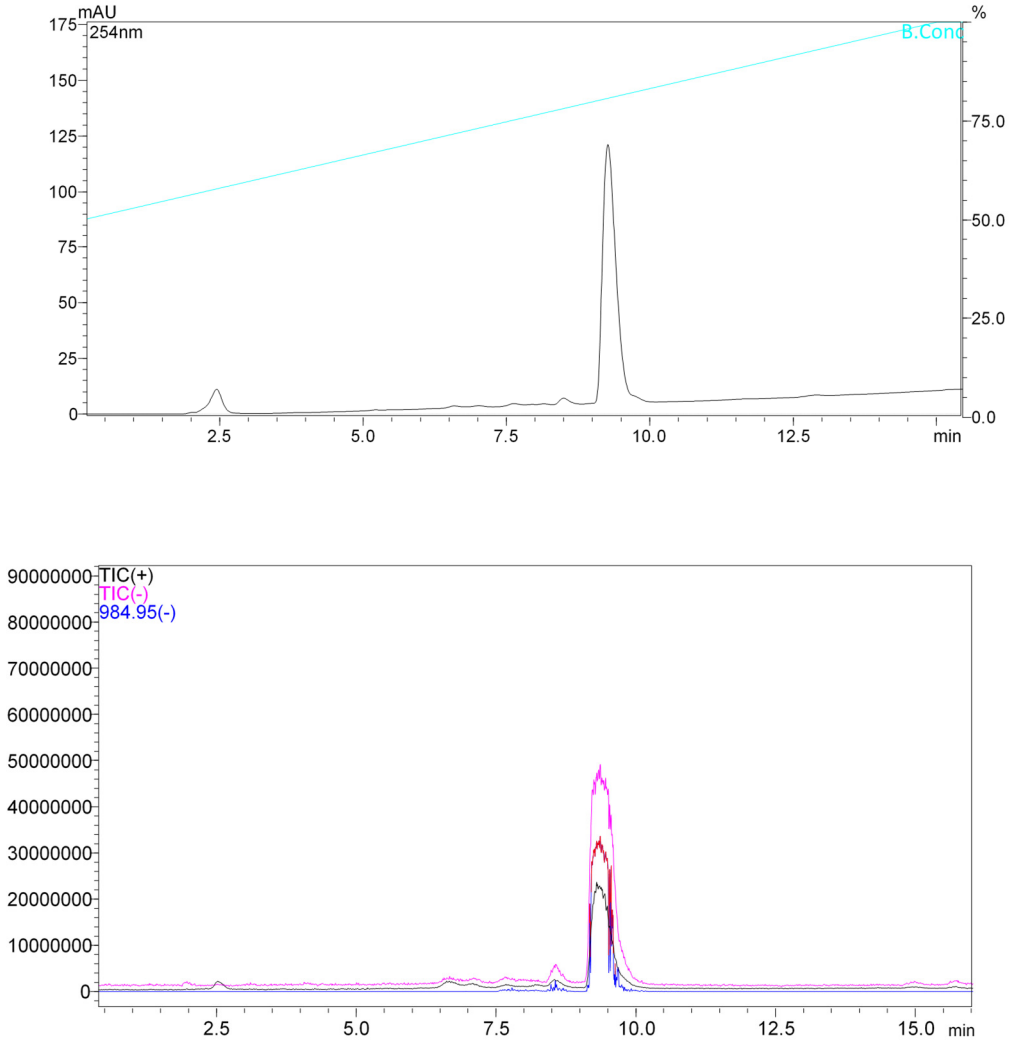


Figure S2. LC-MS analysis of synthetic MC-LF **1a** (gradient: 50-100% B in 15 min, t_R = 9.1 min).

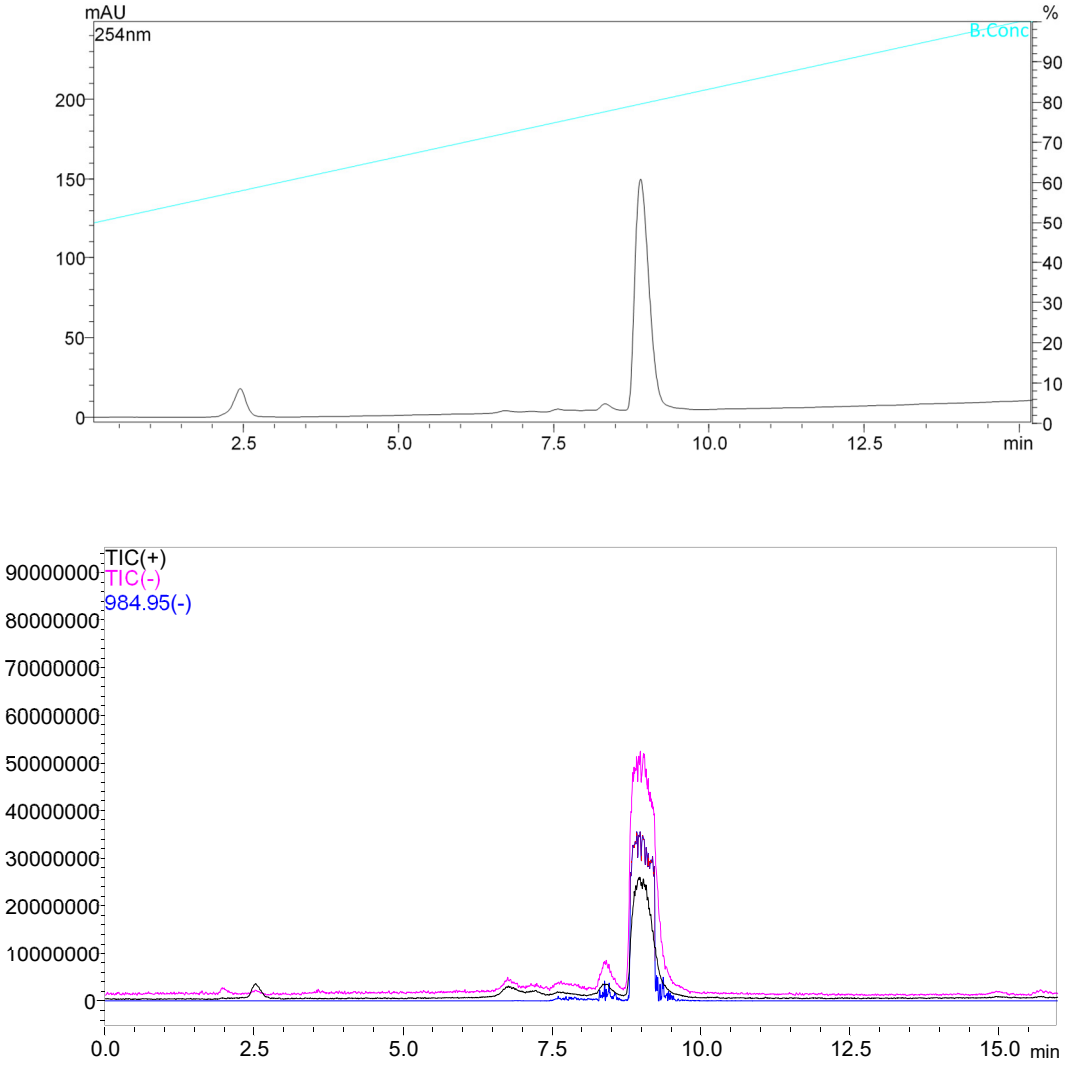


Figure S3. LC-MS analysis of a coinjection of natural and synthetic MC-LF **1a** (gradient: 50-100% B in 15 min, t_R = 9.1 min)

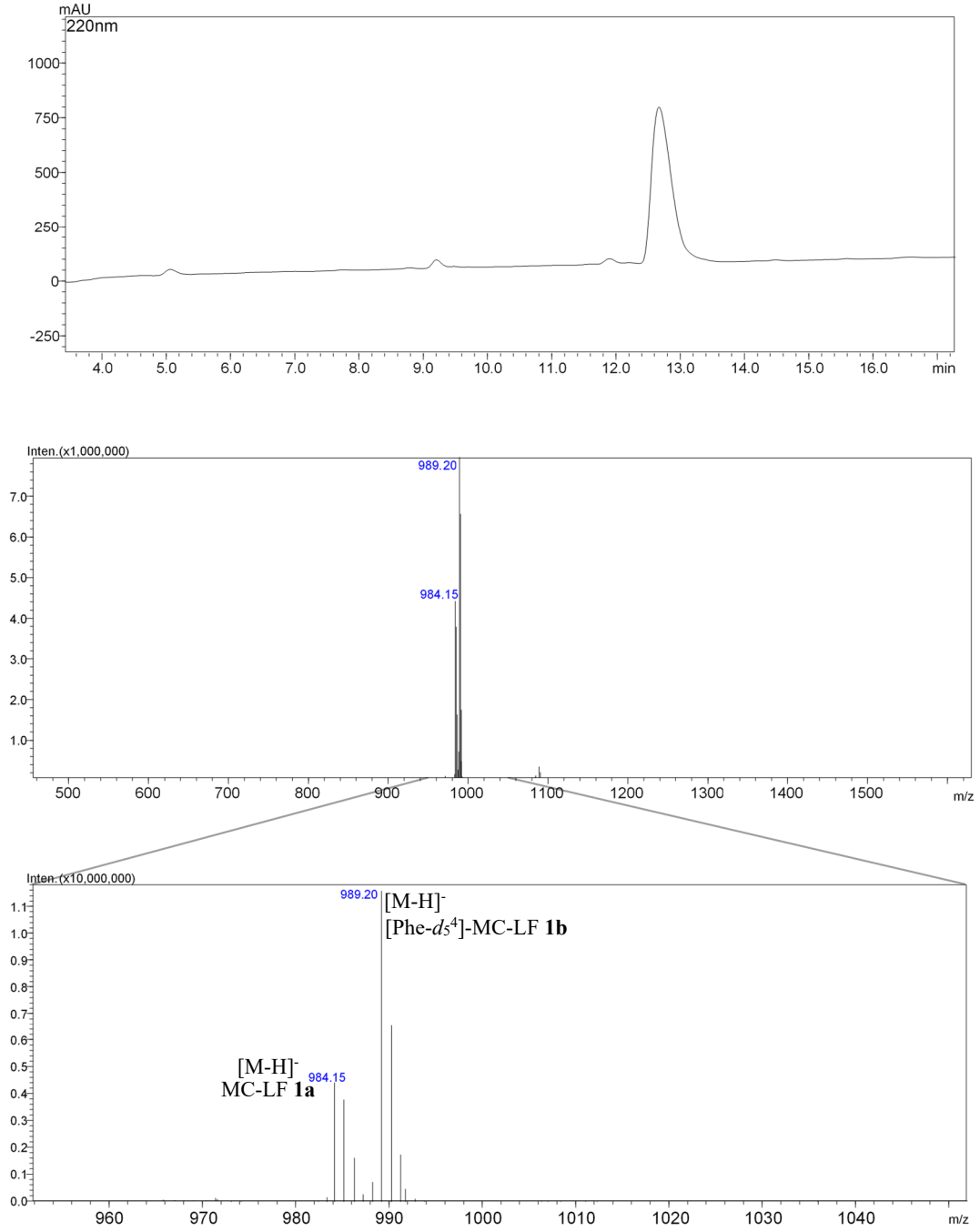


Figure S4. LC-MS analysis of a coinjection of synthetic MC-LF **1a** and [Phe-d_5^4]-MC-LF **1b** (gradient: 40-90% B in 15 min, t_R = 12.4 min). The ESI-MS (-) spectrum corresponds to the peak with t_R = 12.4 min.

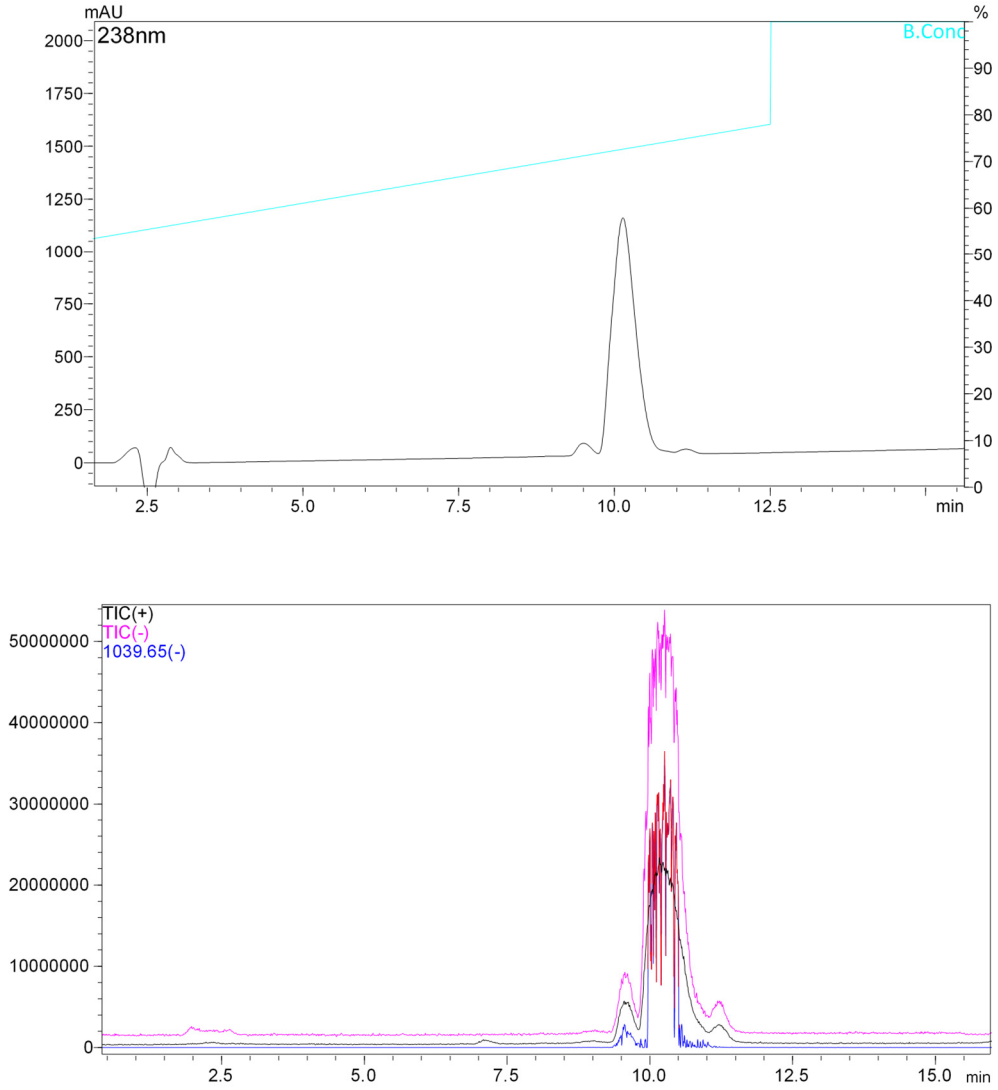


Figure S5. LC-MS analysis of MC-LY(Prg) **1c** (gradient: 50-78% B in 12.5 min, t_R = 10.1 min).

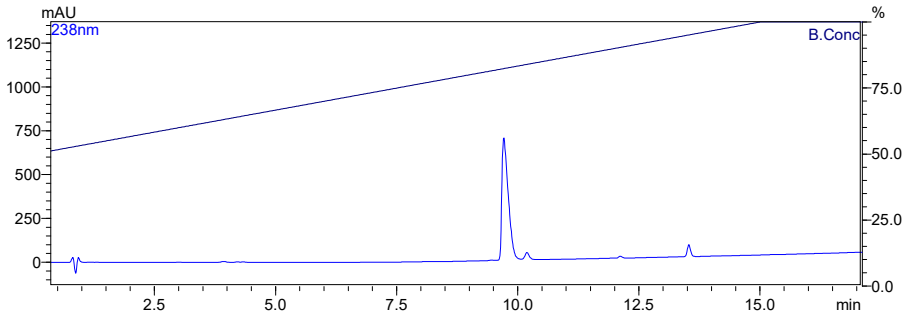
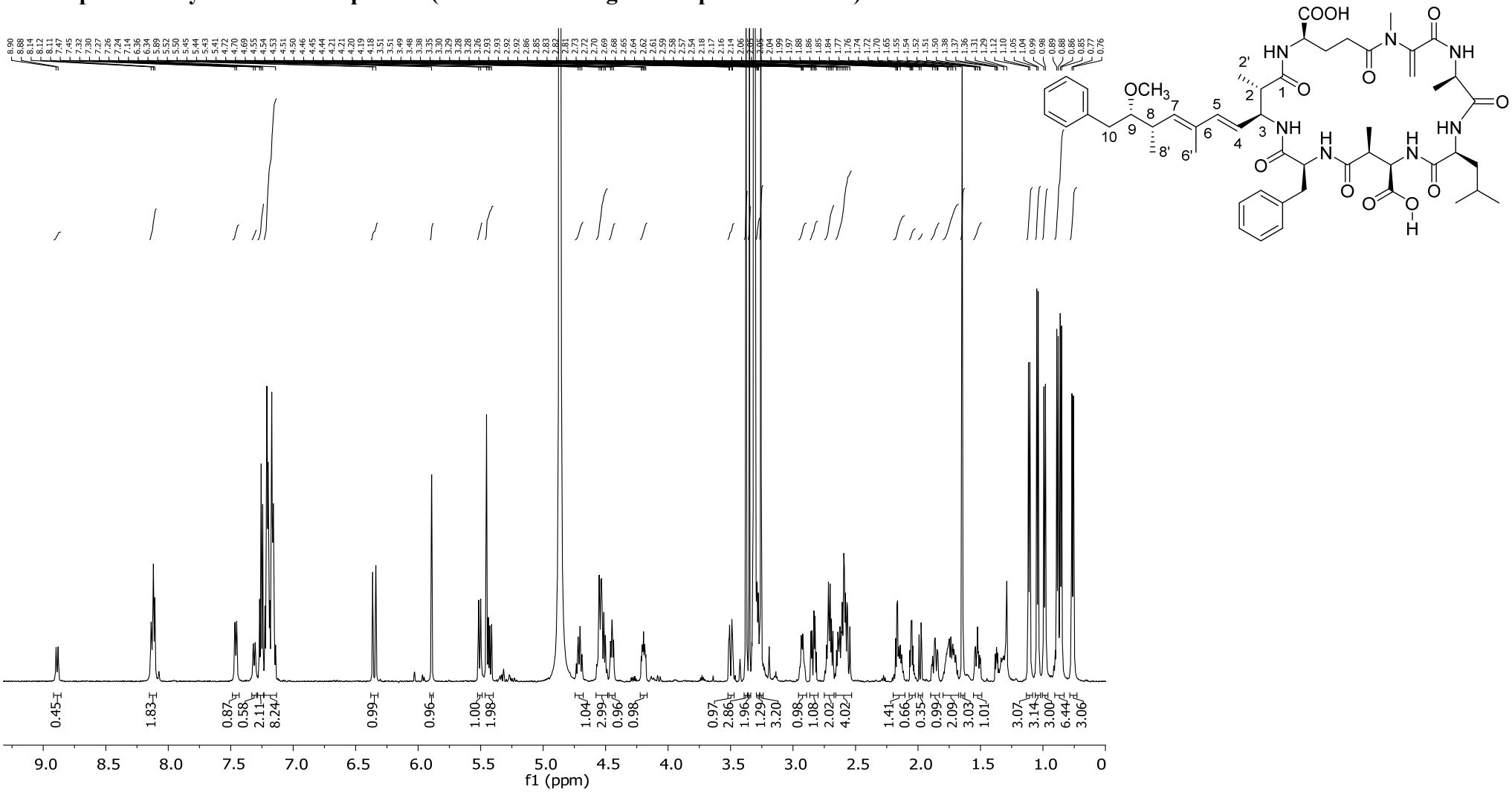
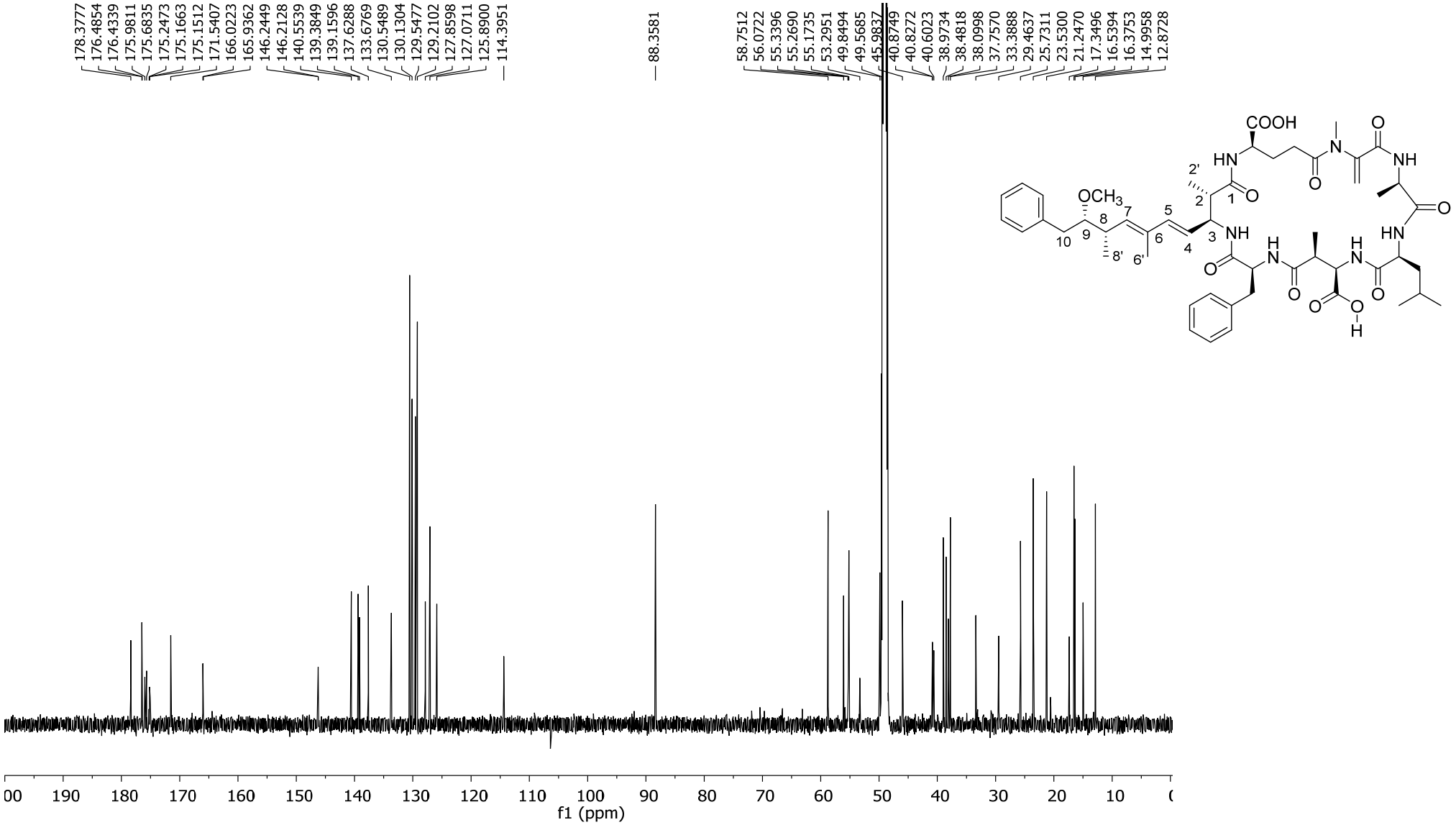


Figure S6. Analytical RP-HPLC analysis of **23c** (gradient: 50-100% B in 15 min, t_R = 9.6 min). Column: Kinetex 2.6 µm C18 100A, 100 x 3.0 mm (*Phenomenex*), flow rate: 0.6 mL min^{-1}.

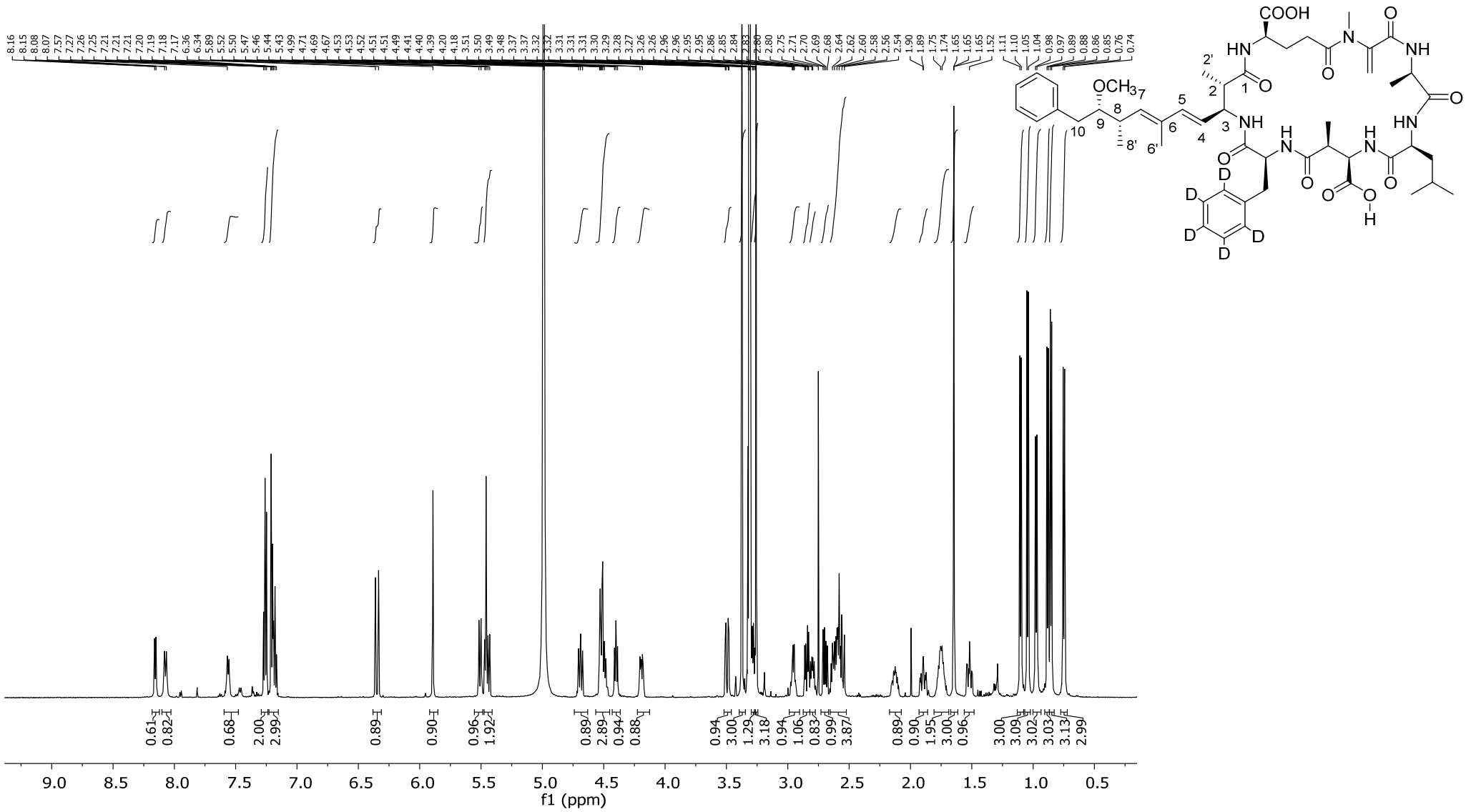
NMR Spectra of Synthesized Compounds (Sorted According to Compound Number)



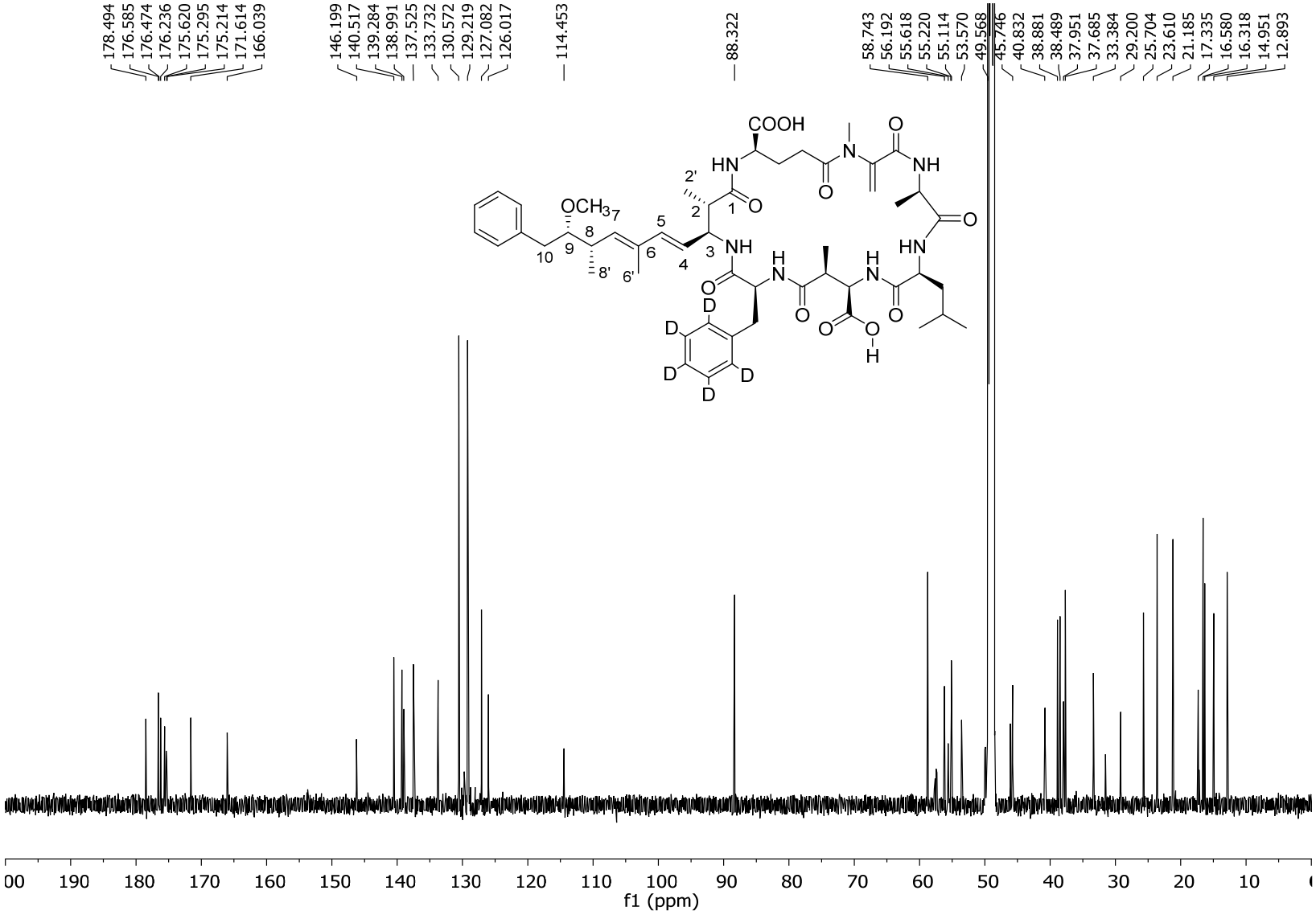
^{1}H NMR spectrum (600 MHz, 300 K, CD_3OD) of **1a.** Different H/D-exchange rates can be estimated from the integrals of remaining incompletely exchanged NH signals.



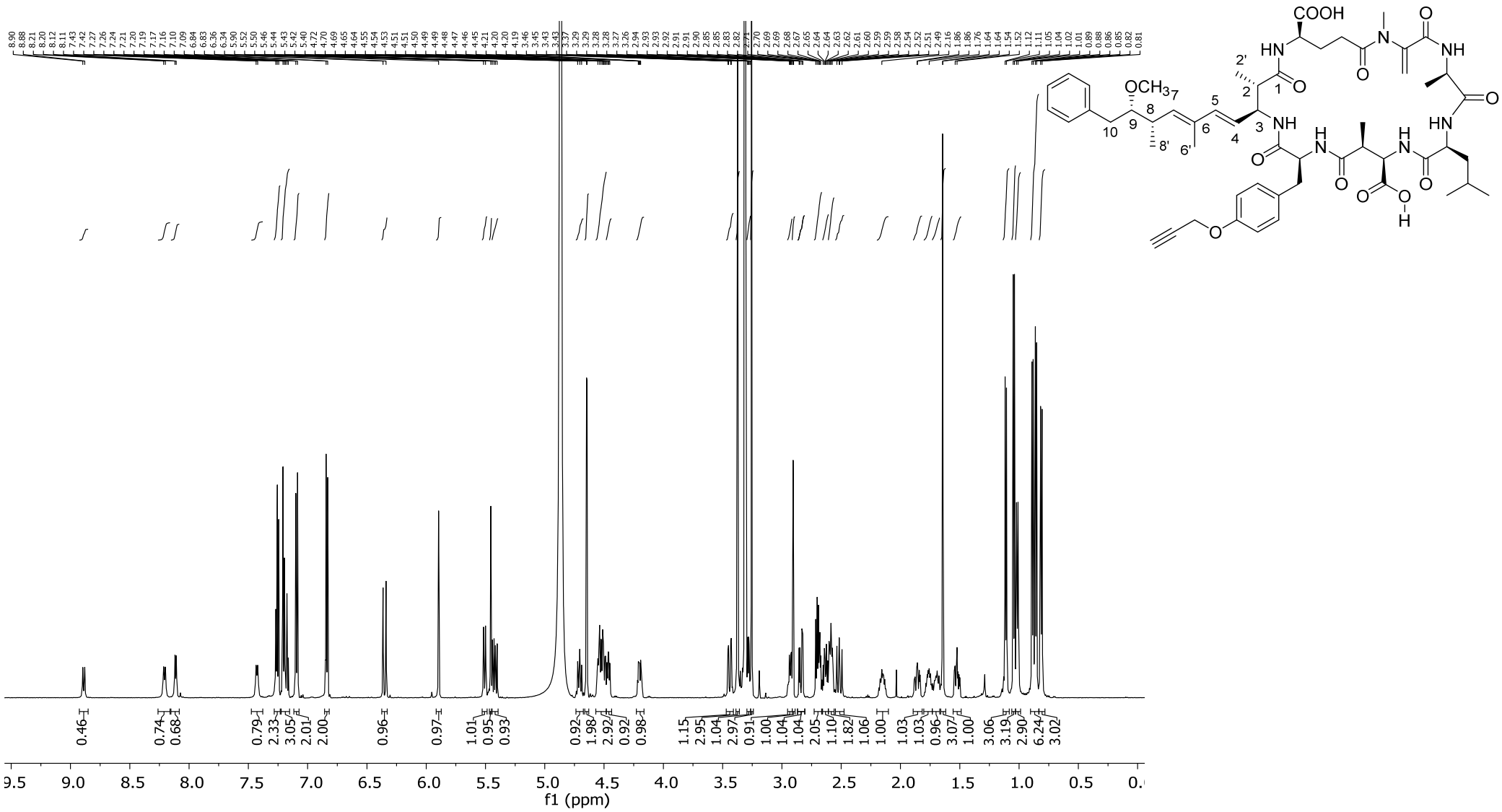
^{13}C NMR spectrum (151 MHz, 300 K, CD_3OD) of **1a.**



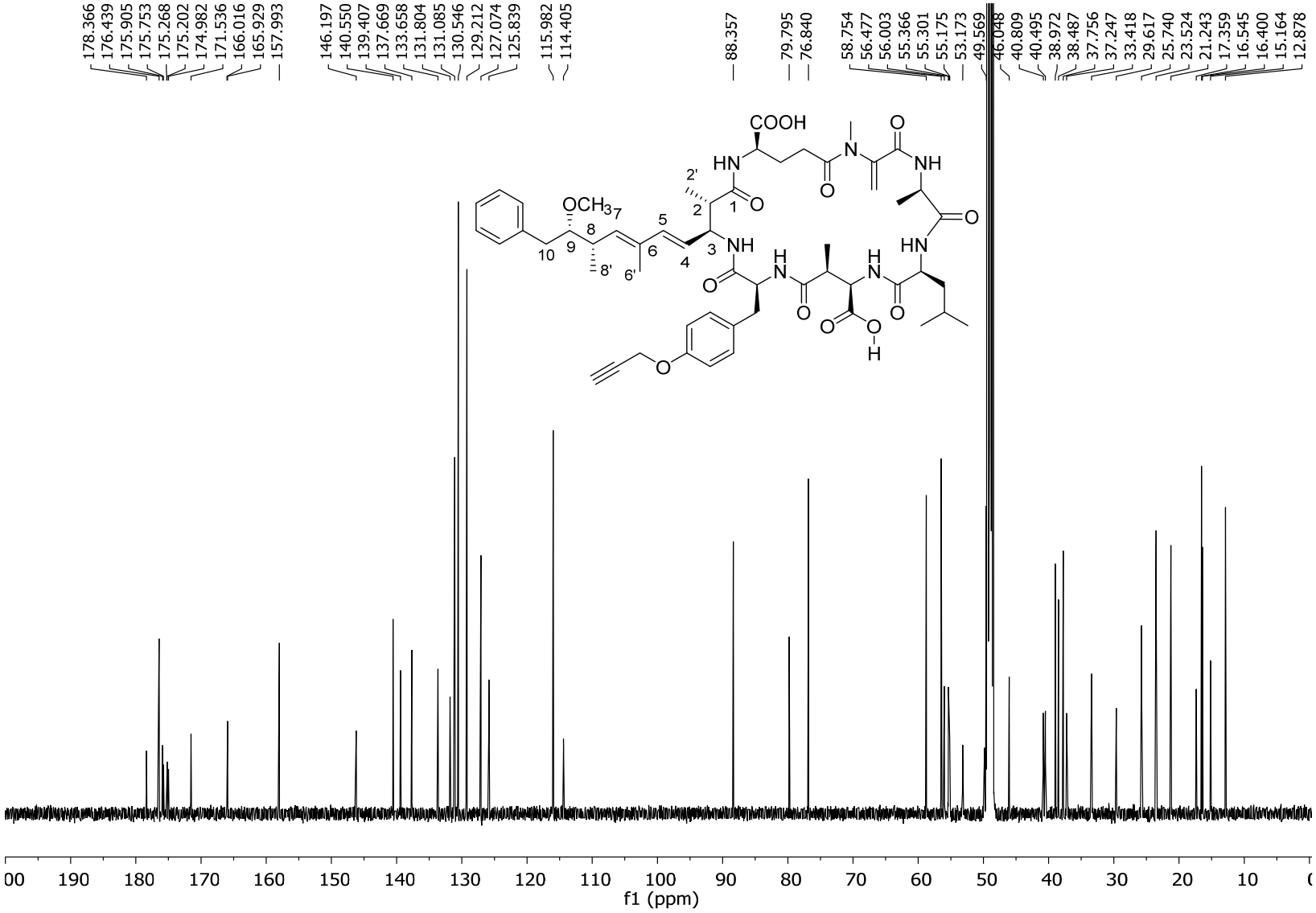
^{1}H NMR spectrum (600 MHz, 284 K, CD_3OD) of **1b.** Different H/D-exchange rates can be estimated from the integrals of remaining incompletely exchanged NH signals.



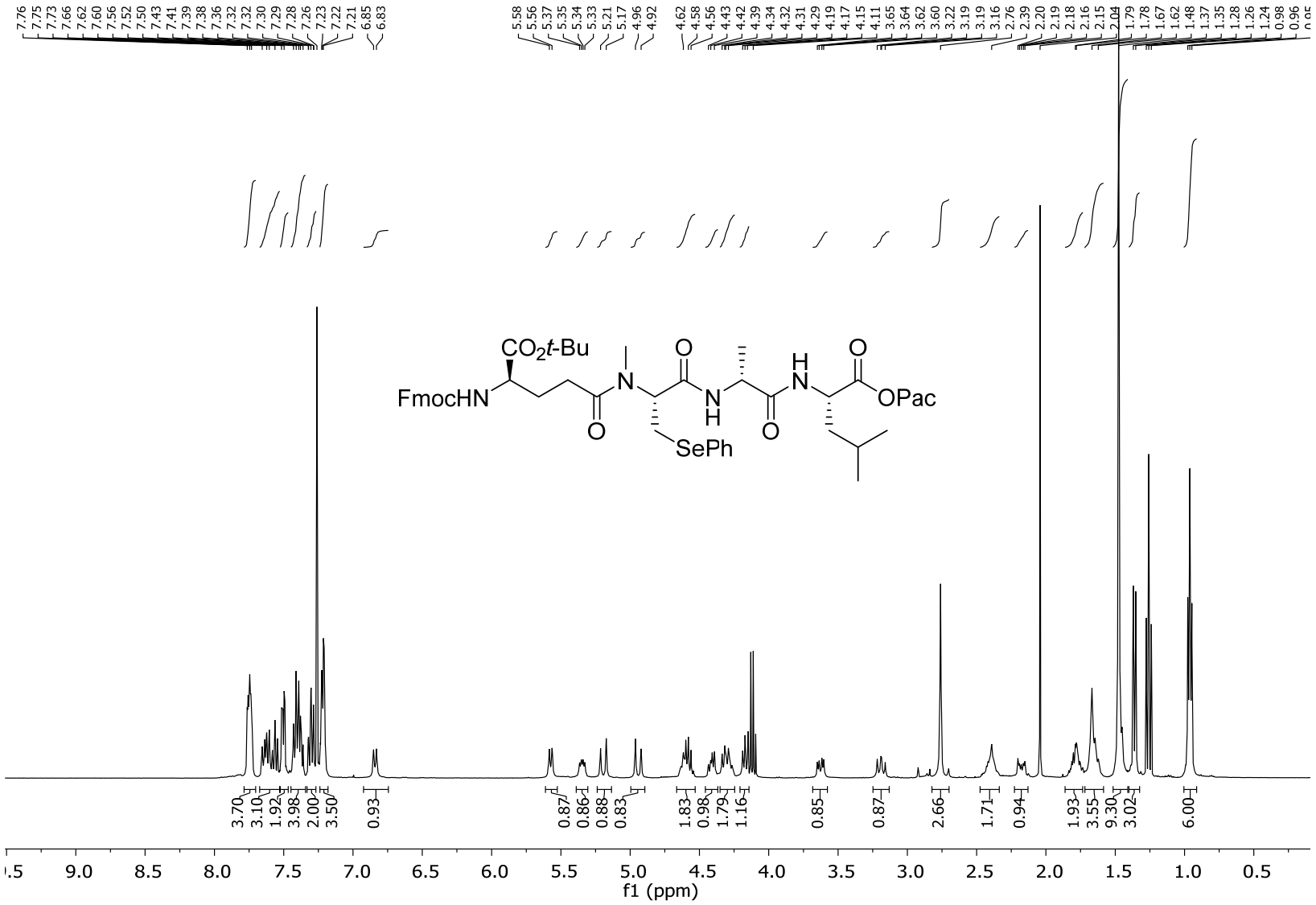
^{13}C NMR spectrum (151 MHz, 284 K, CD_3OD) of **1b**.



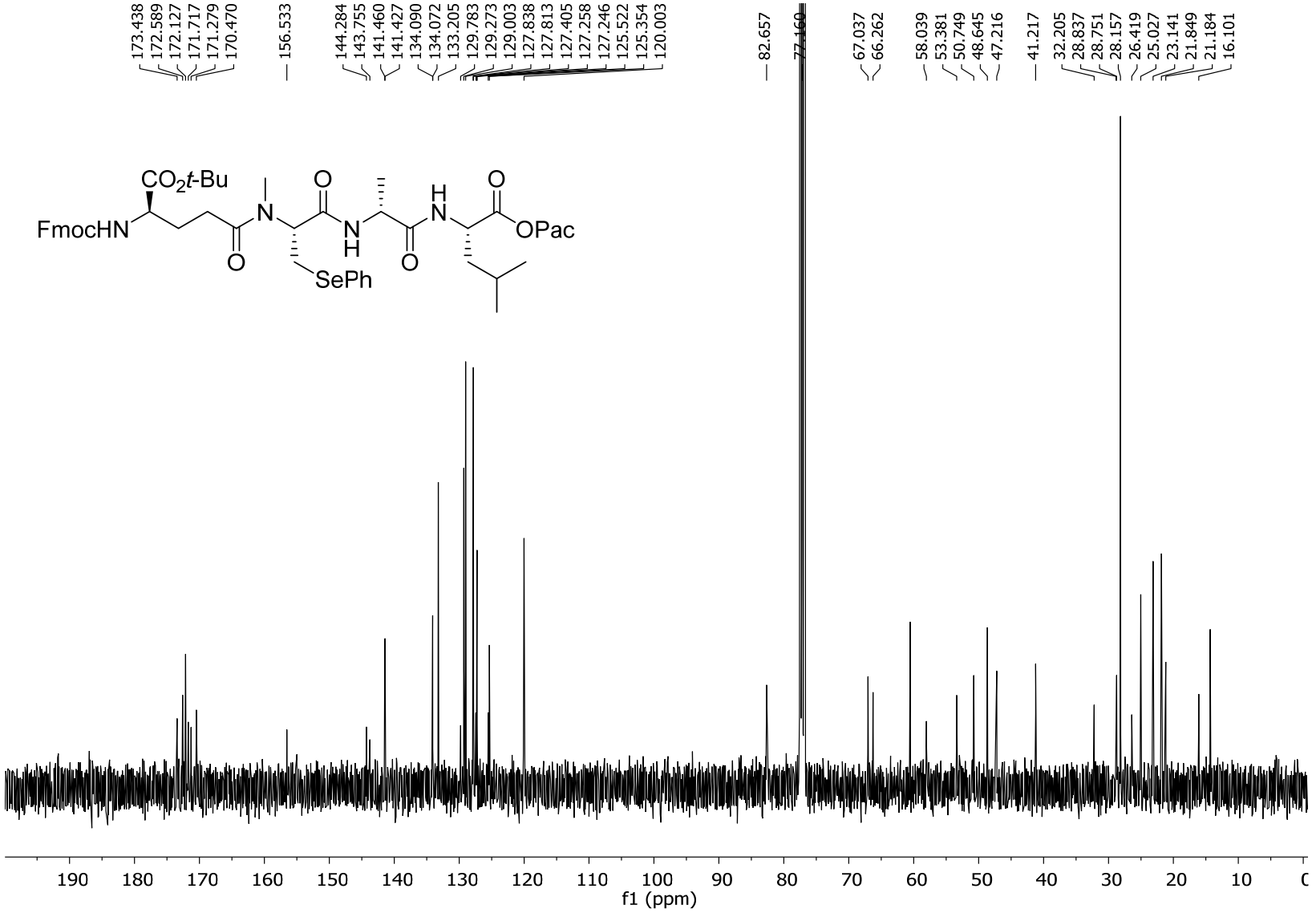
^{1}H NMR spectrum (600 MHz, 300 K, CD_3OD) of **1c**. Different H/D-exchange rates can be estimated from the integrals of remaining incompletely exchanged NH signals.



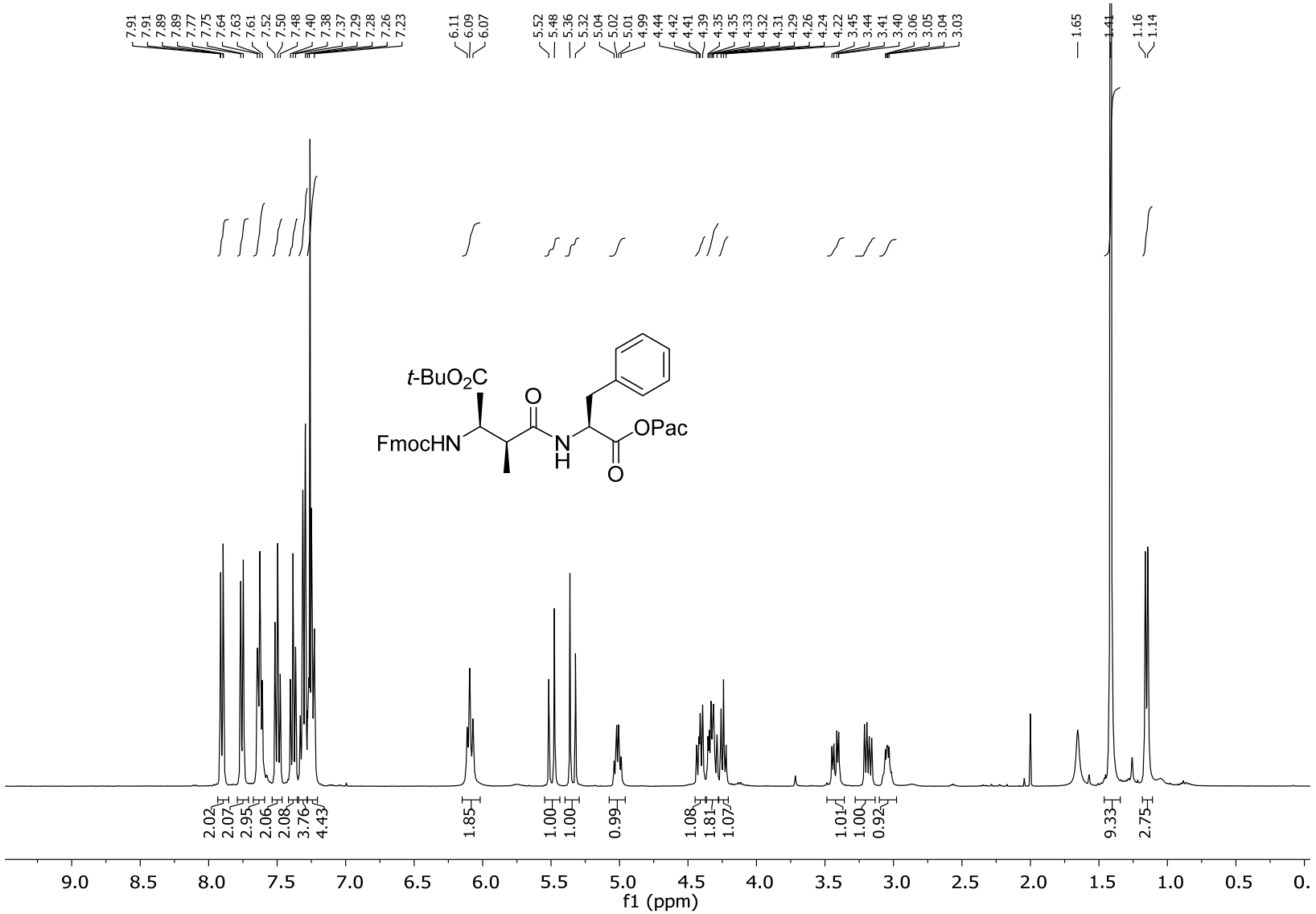
^{13}C NMR spectrum (151 MHz, 300 K, CD_3OD) of **1c**.



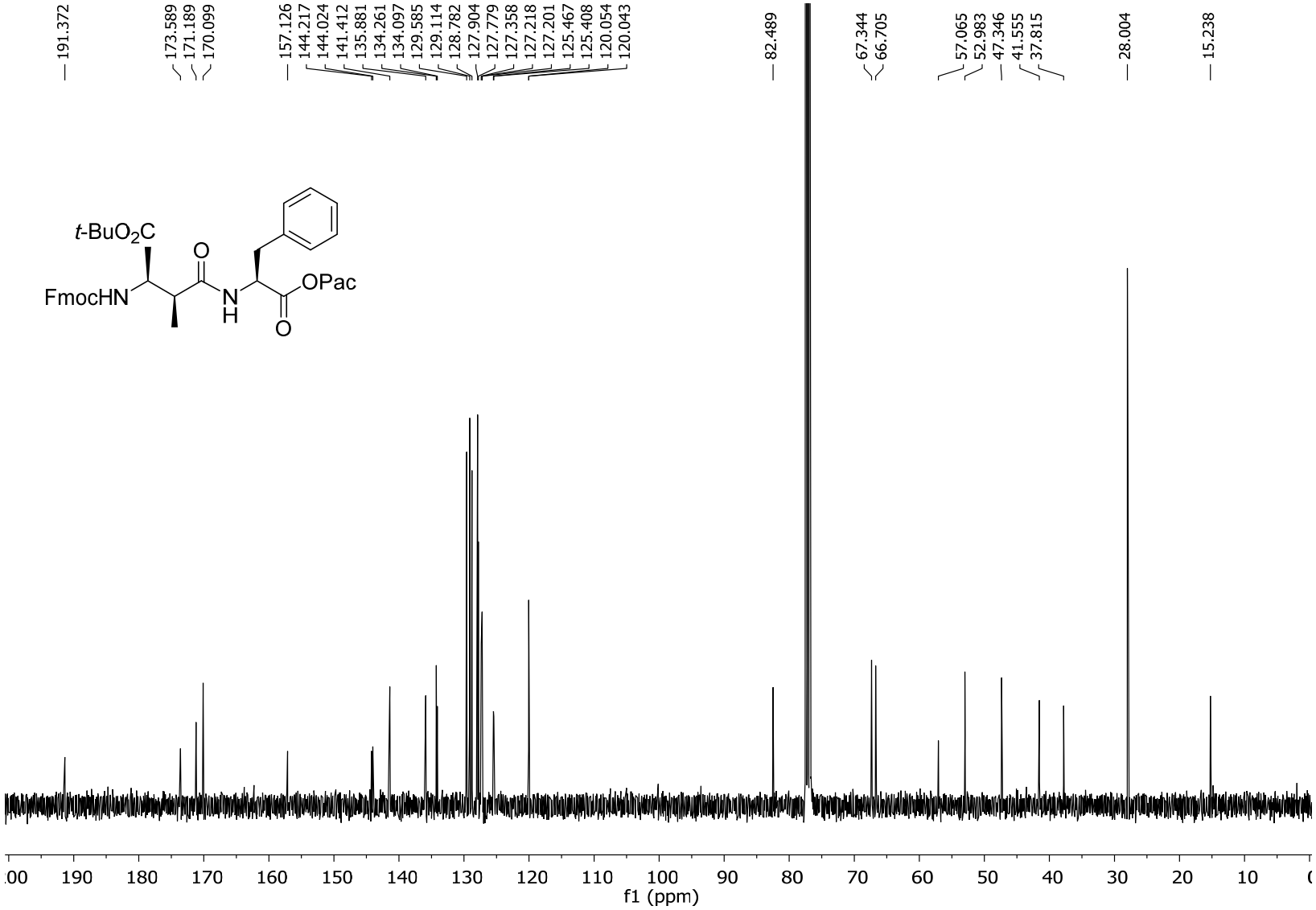
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of **4.**



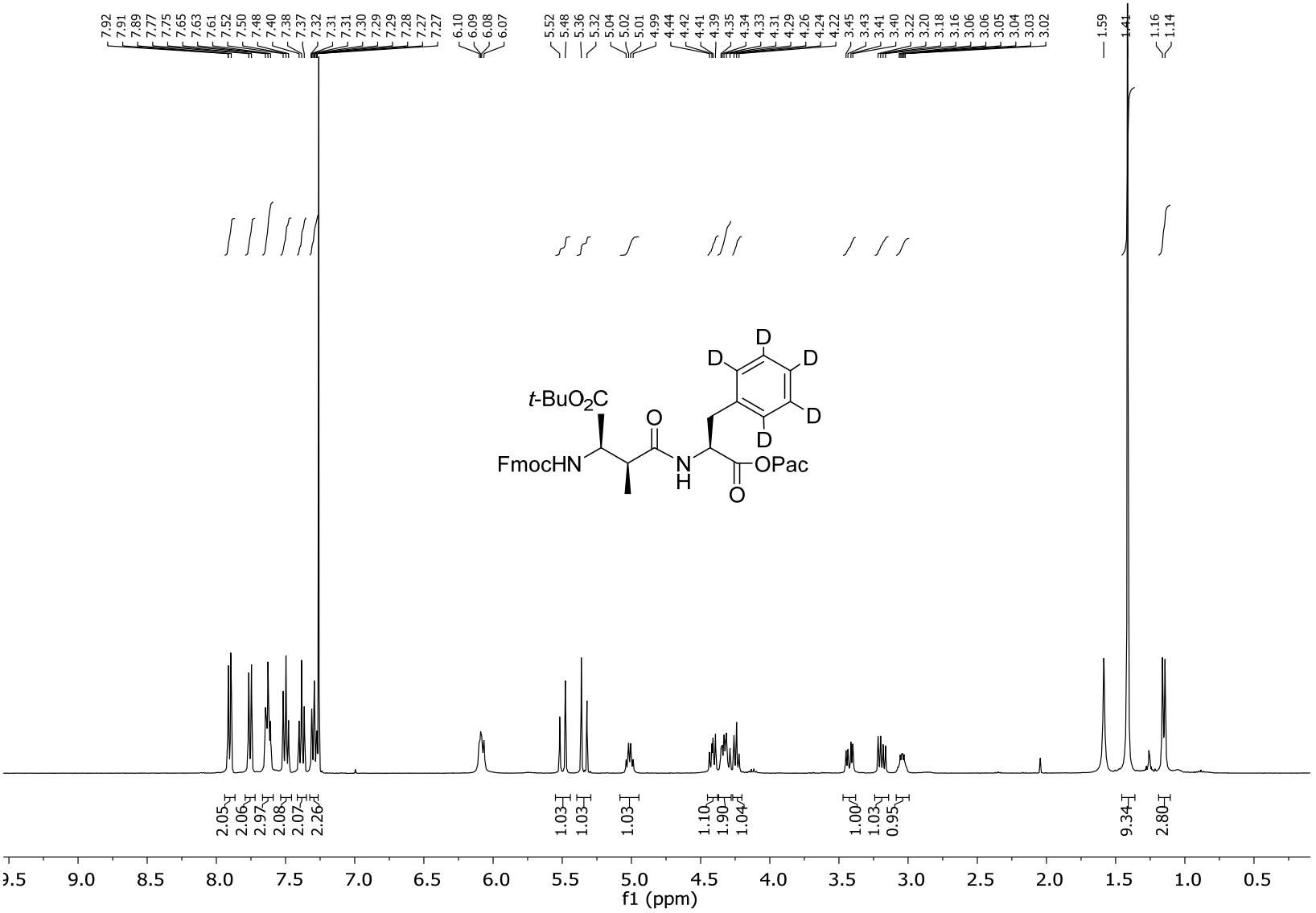
^{13}C NMR spectrum (101 MHz, 300 K, $CDCl_3$) of **4**.



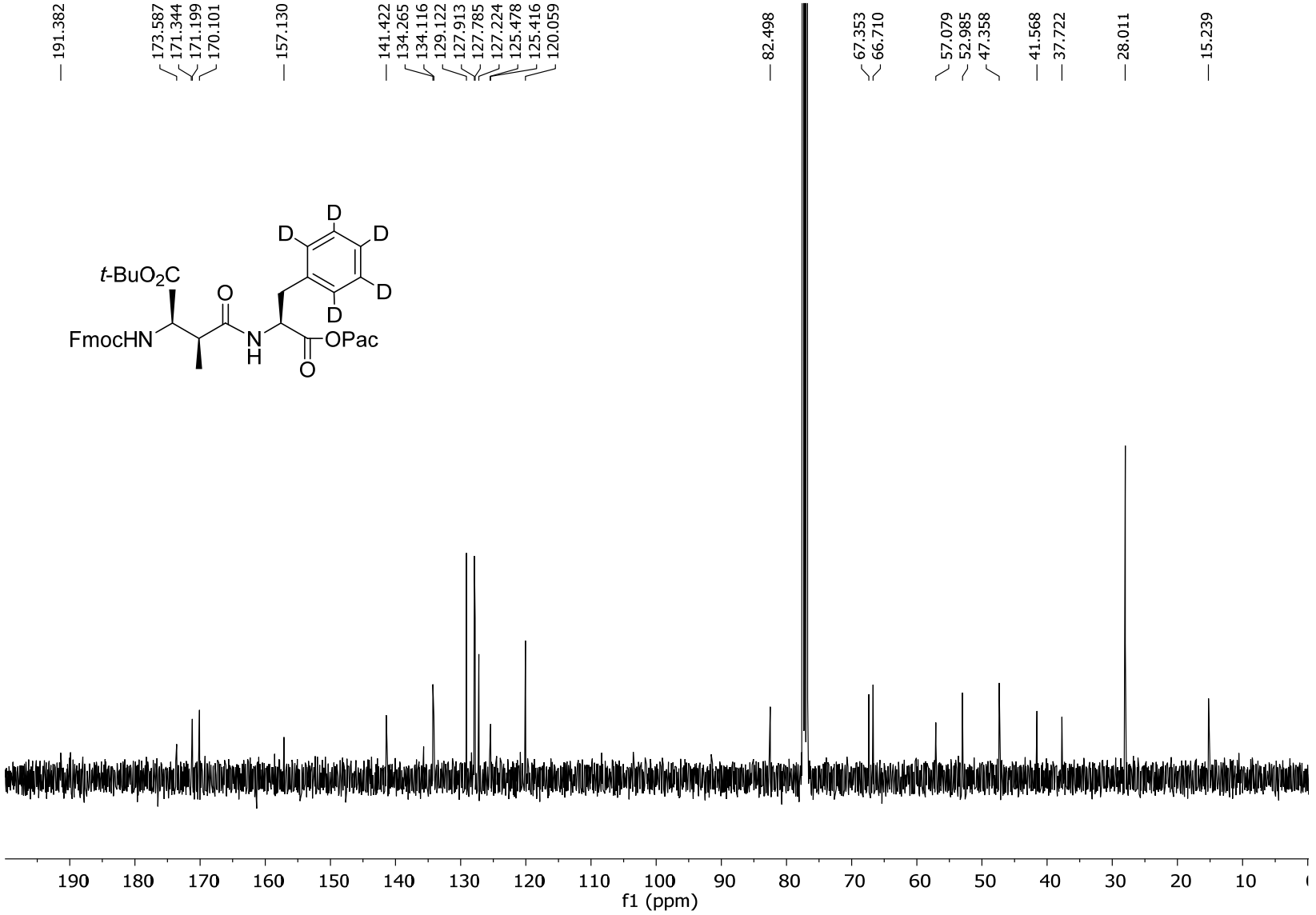
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of **5a**.



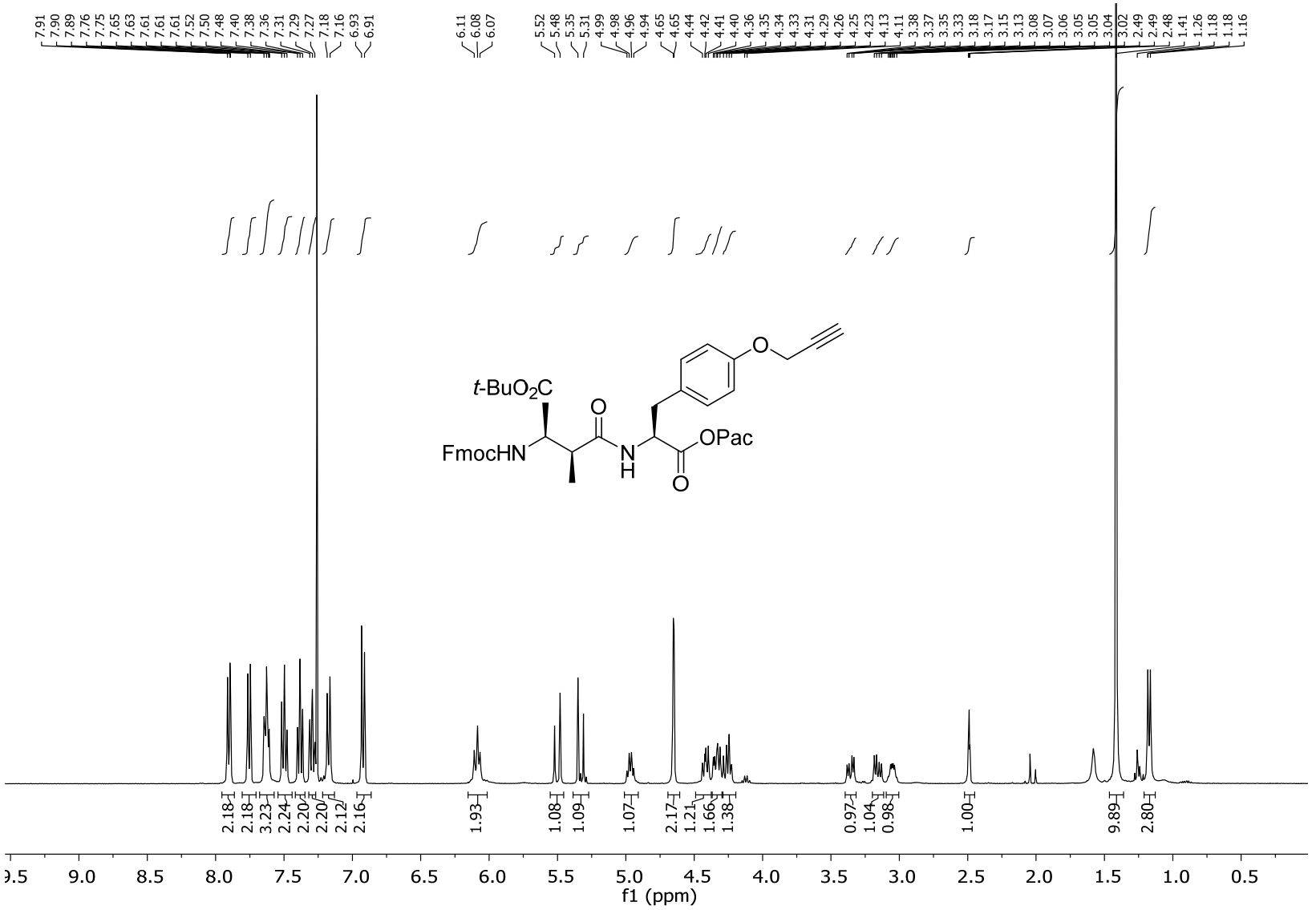
^{13}C NMR spectrum (101 MHz, 300 K, $CDCl_3$) of **5a**.



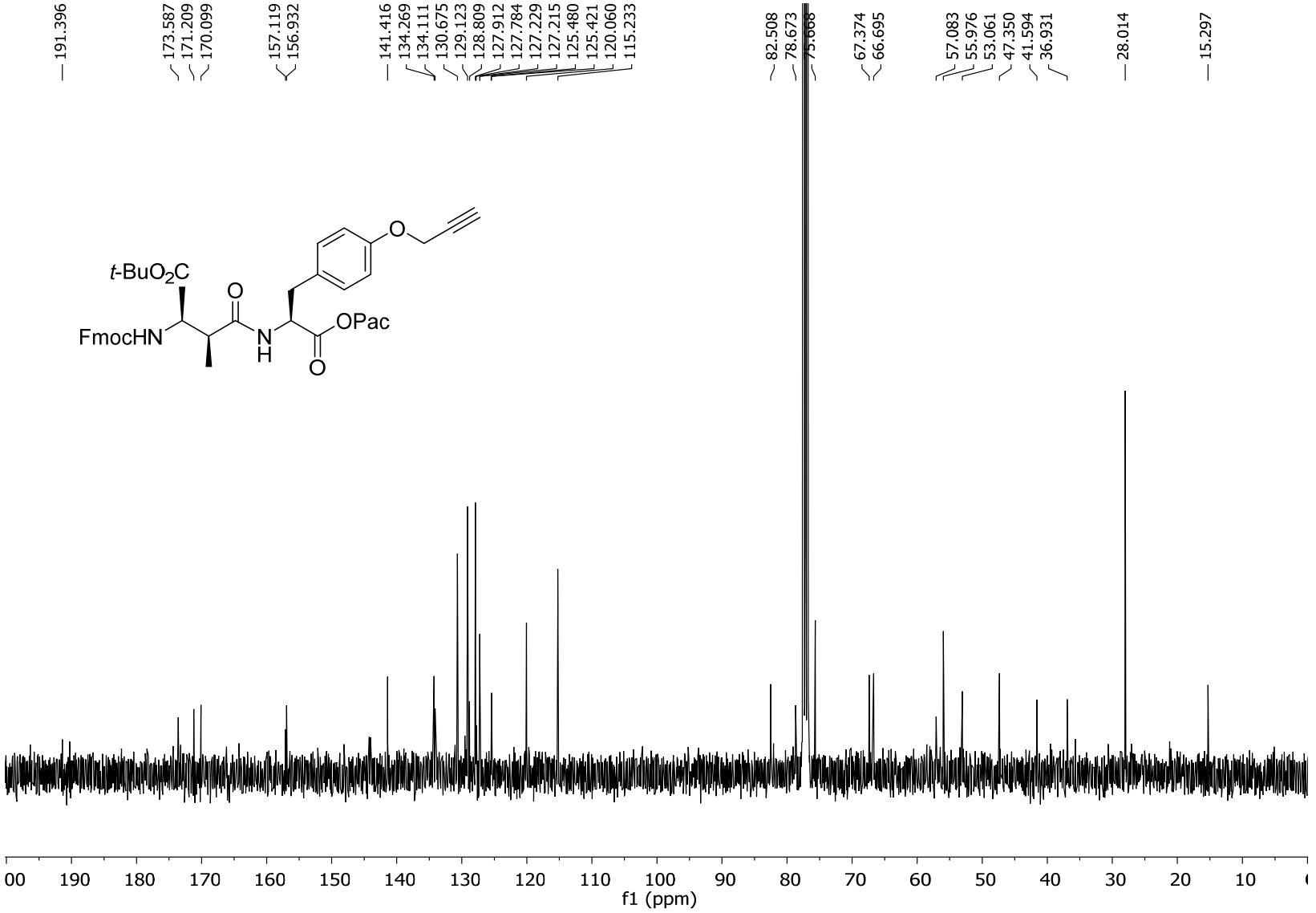
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of **5b.**



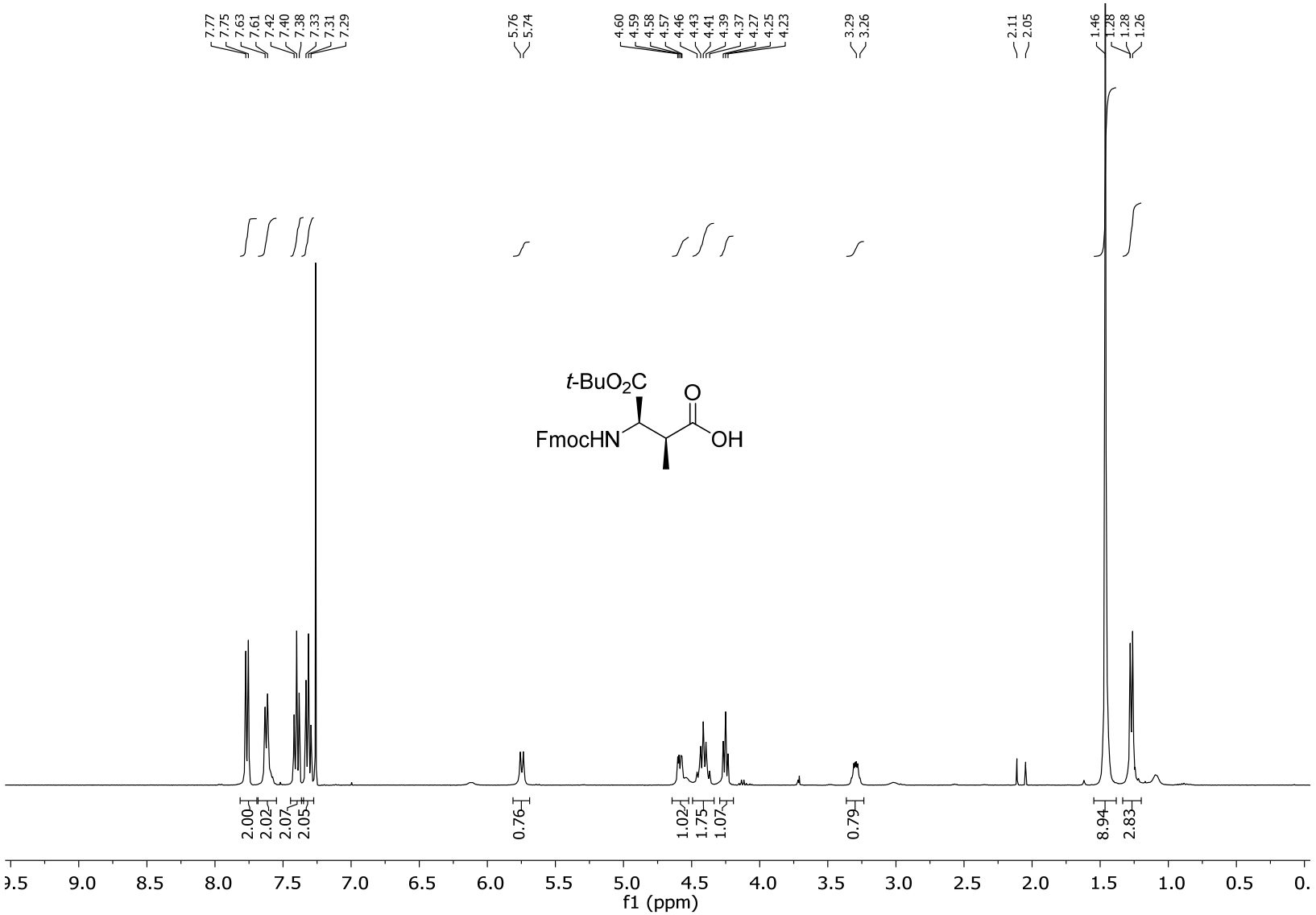
^{13}C NMR spectrum (101 MHz, 300 K, $CDCl_3$) of **5b.**



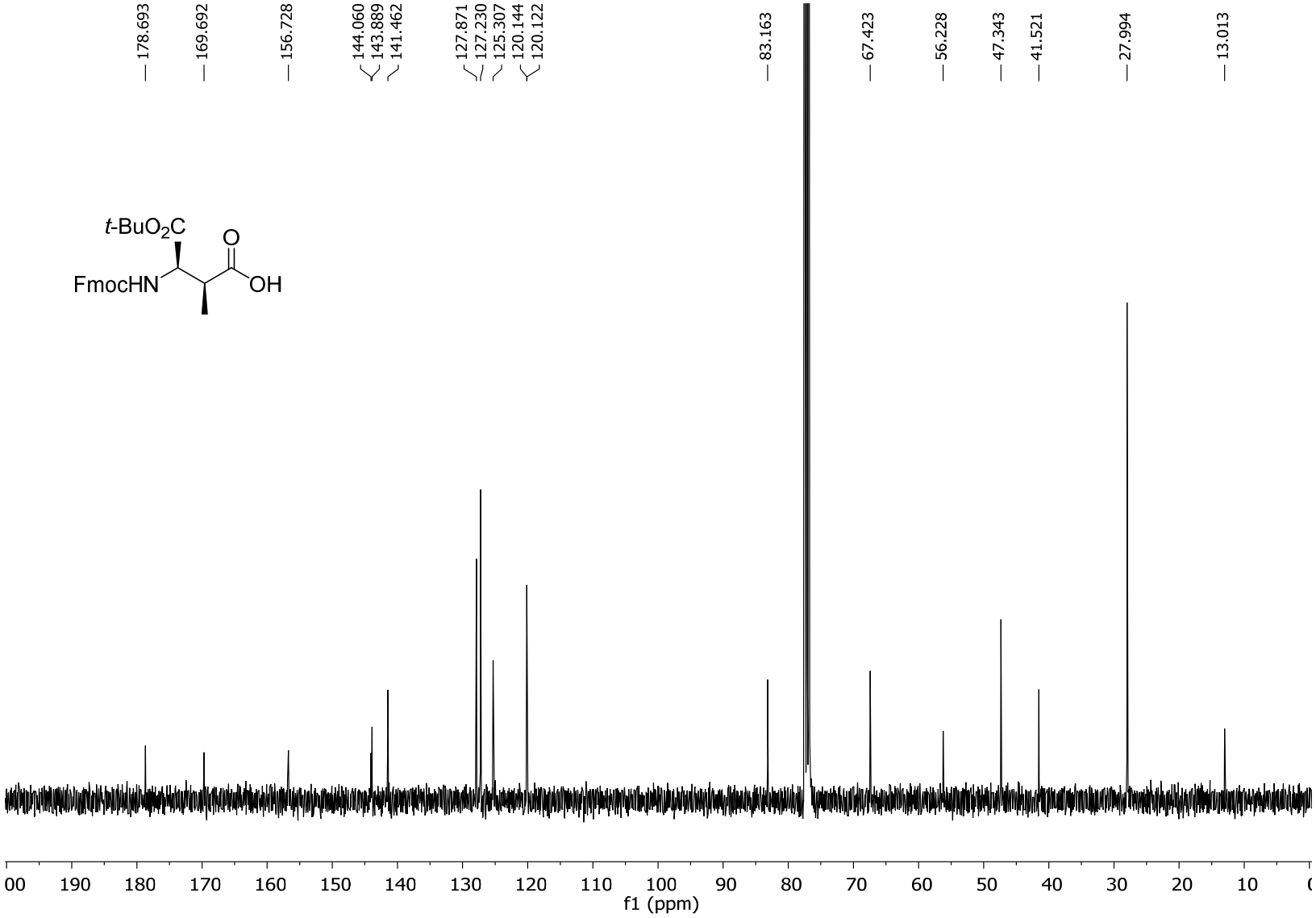
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of **5c**.



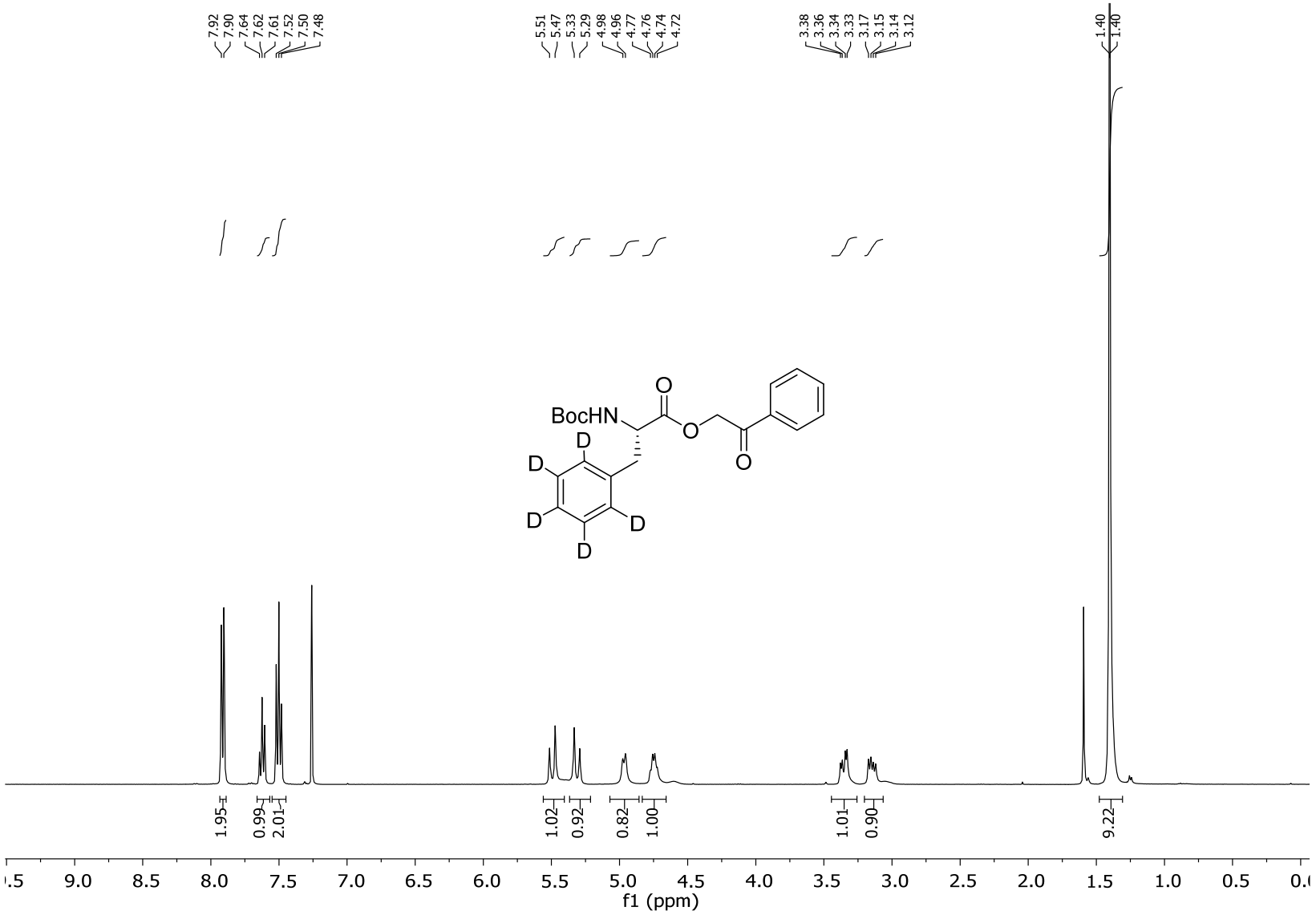
^{13}C NMR spectrum (101 MHz, 300 K, $CDCl_3$) of **5c.**



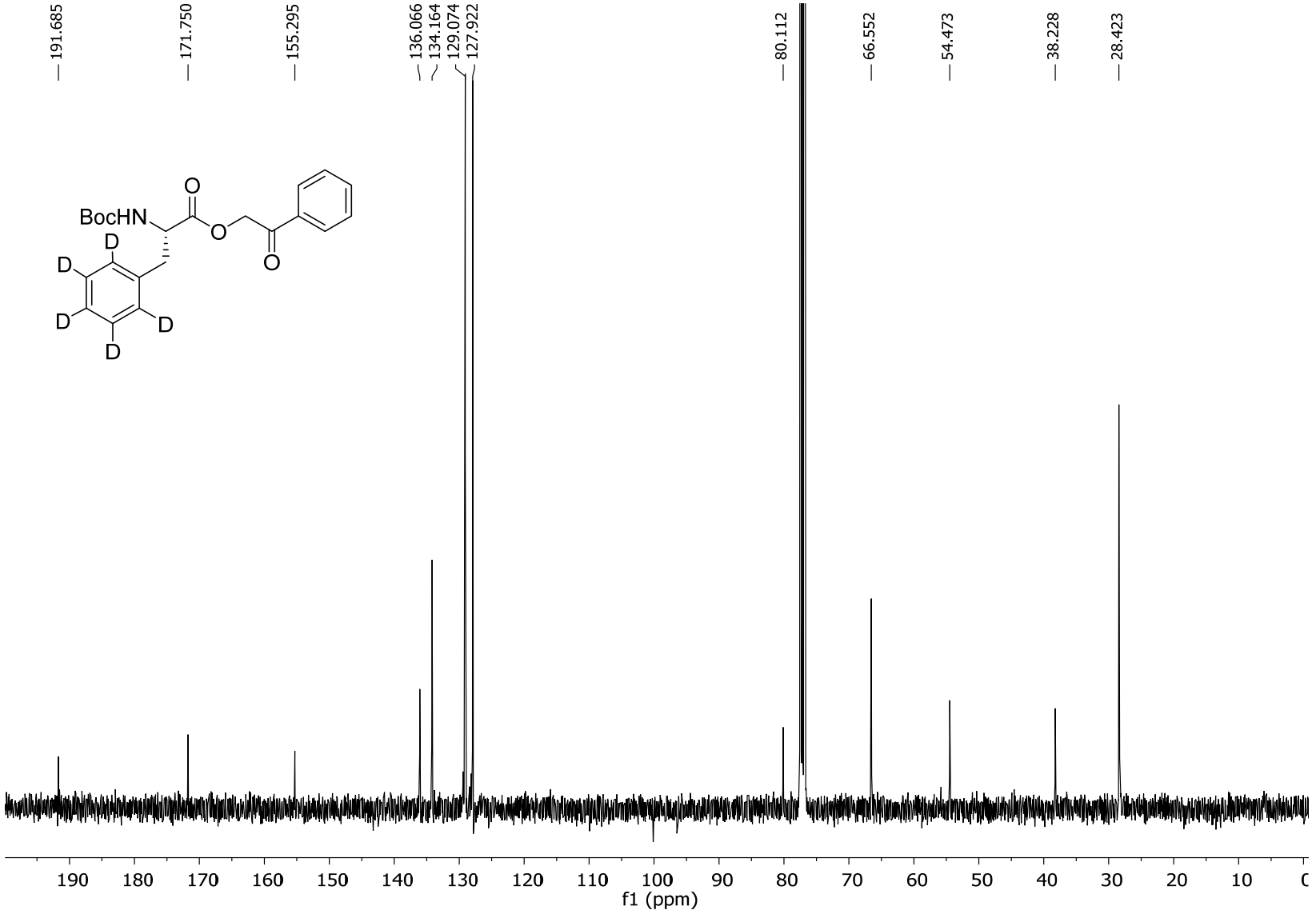
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of **7**.



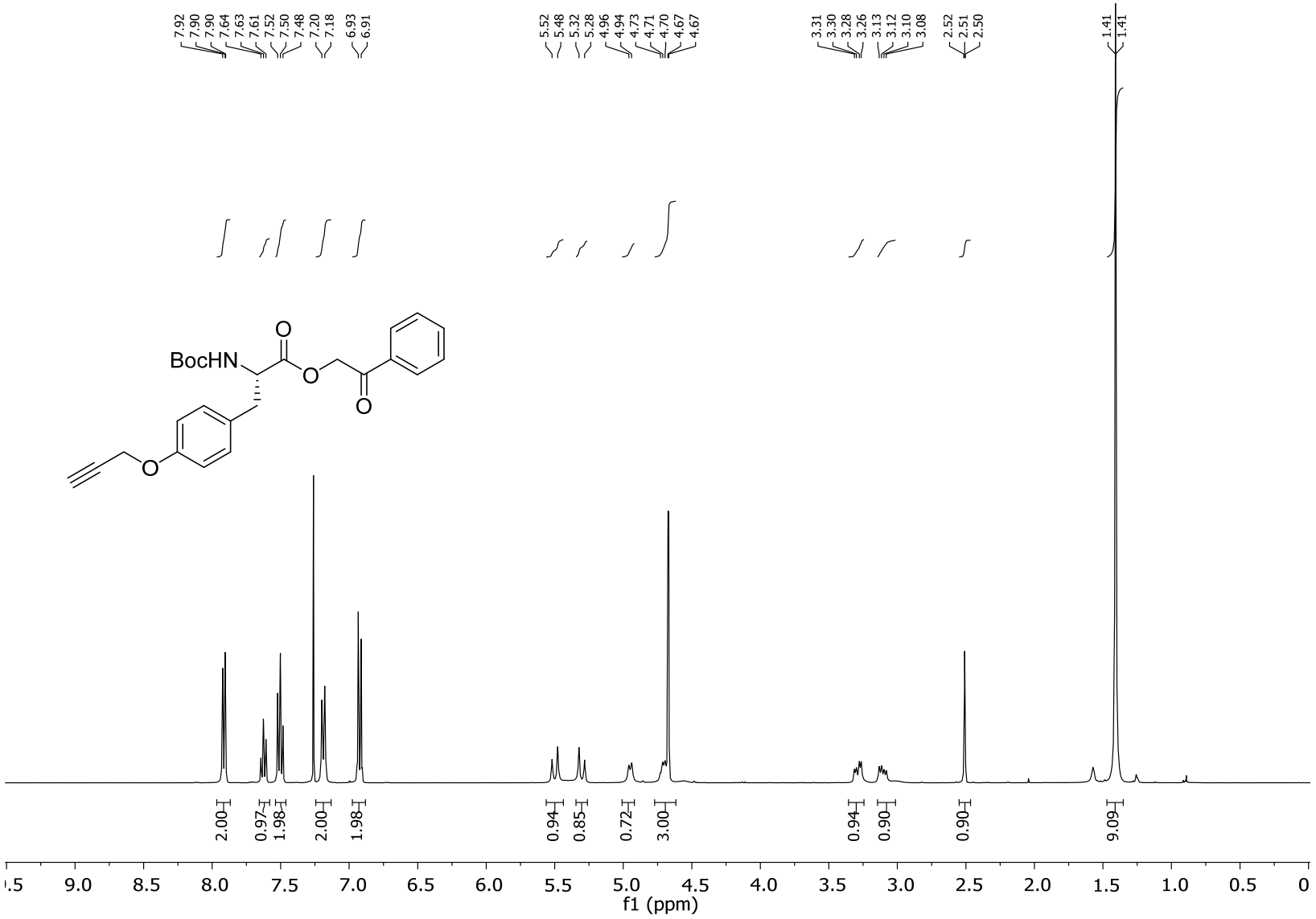
^{13}C NMR spectrum (101 MHz, 300 K, $CDCl_3$) of **7**.



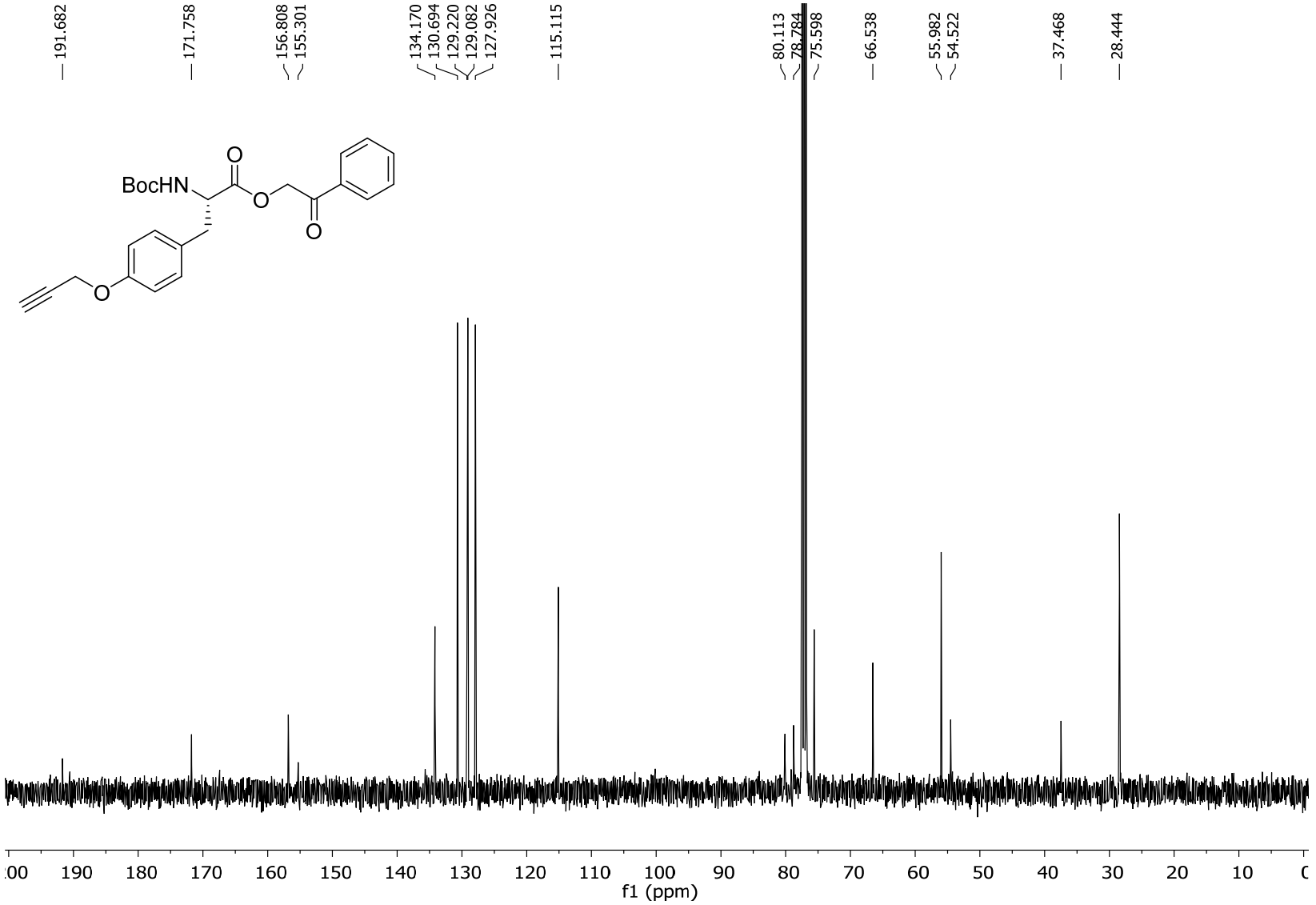
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of **8b.**



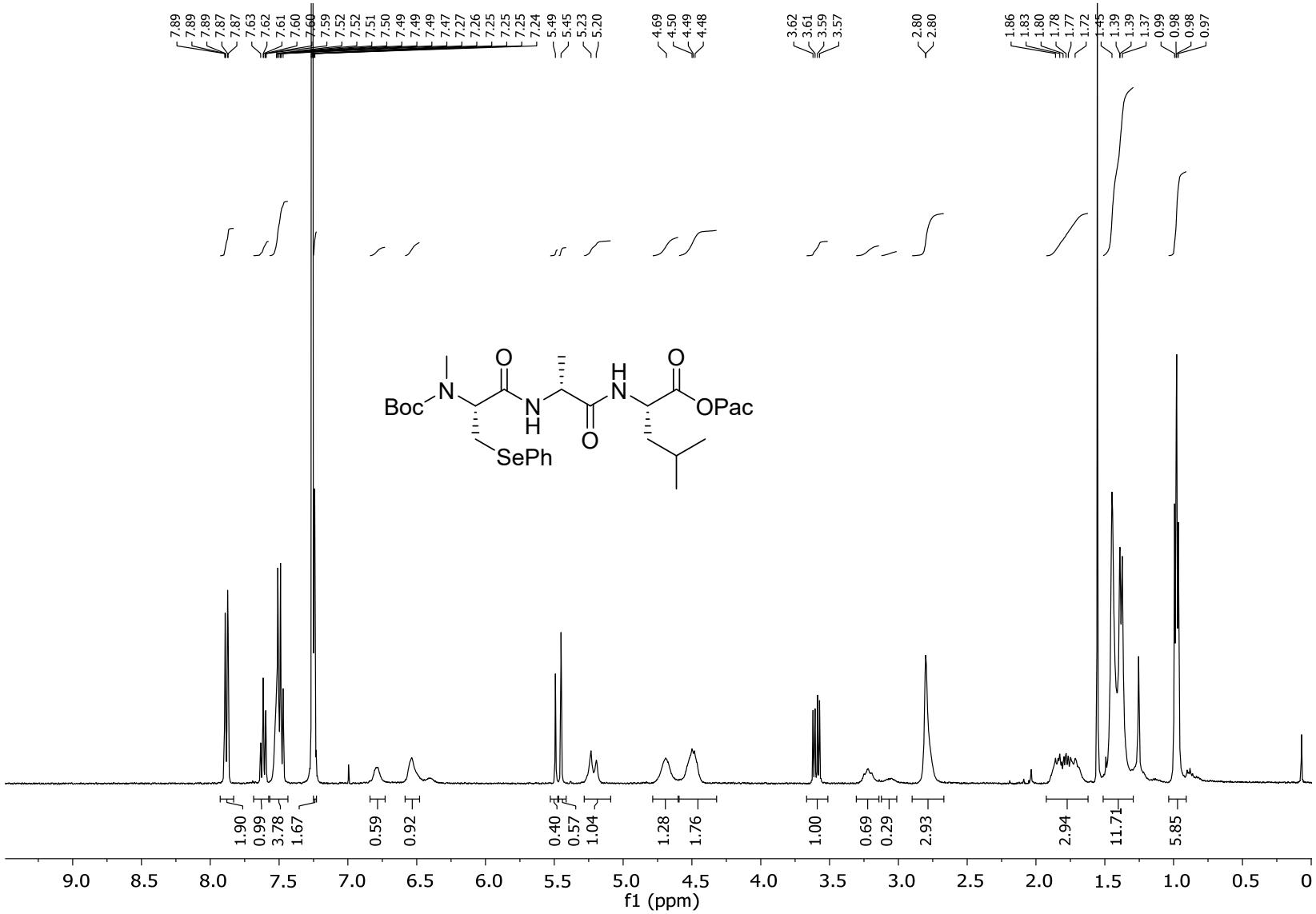
^{13}C NMR spectrum (101 MHz, 300 K, $CDCl_3$) of **8b.**



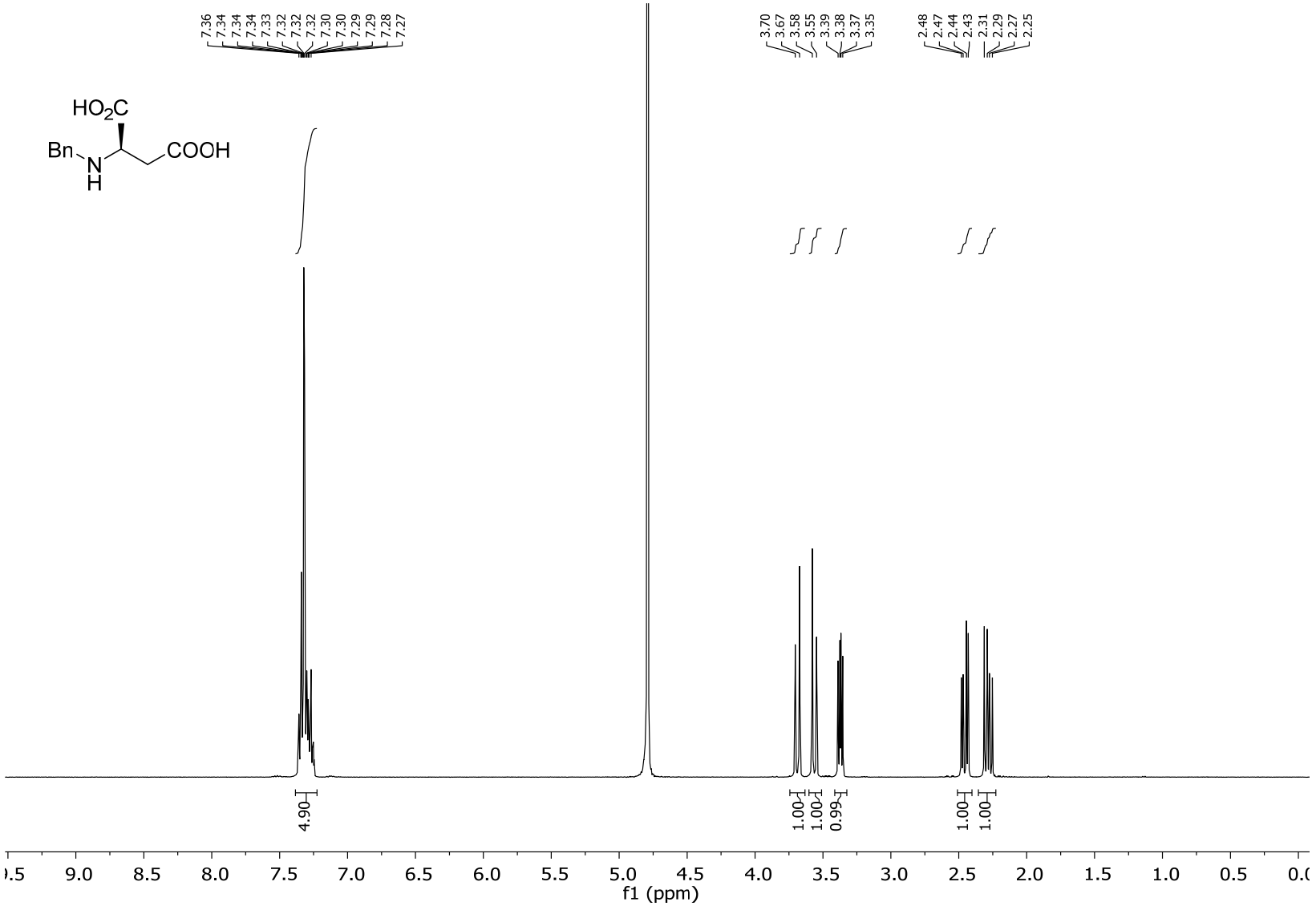
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of **8c**.



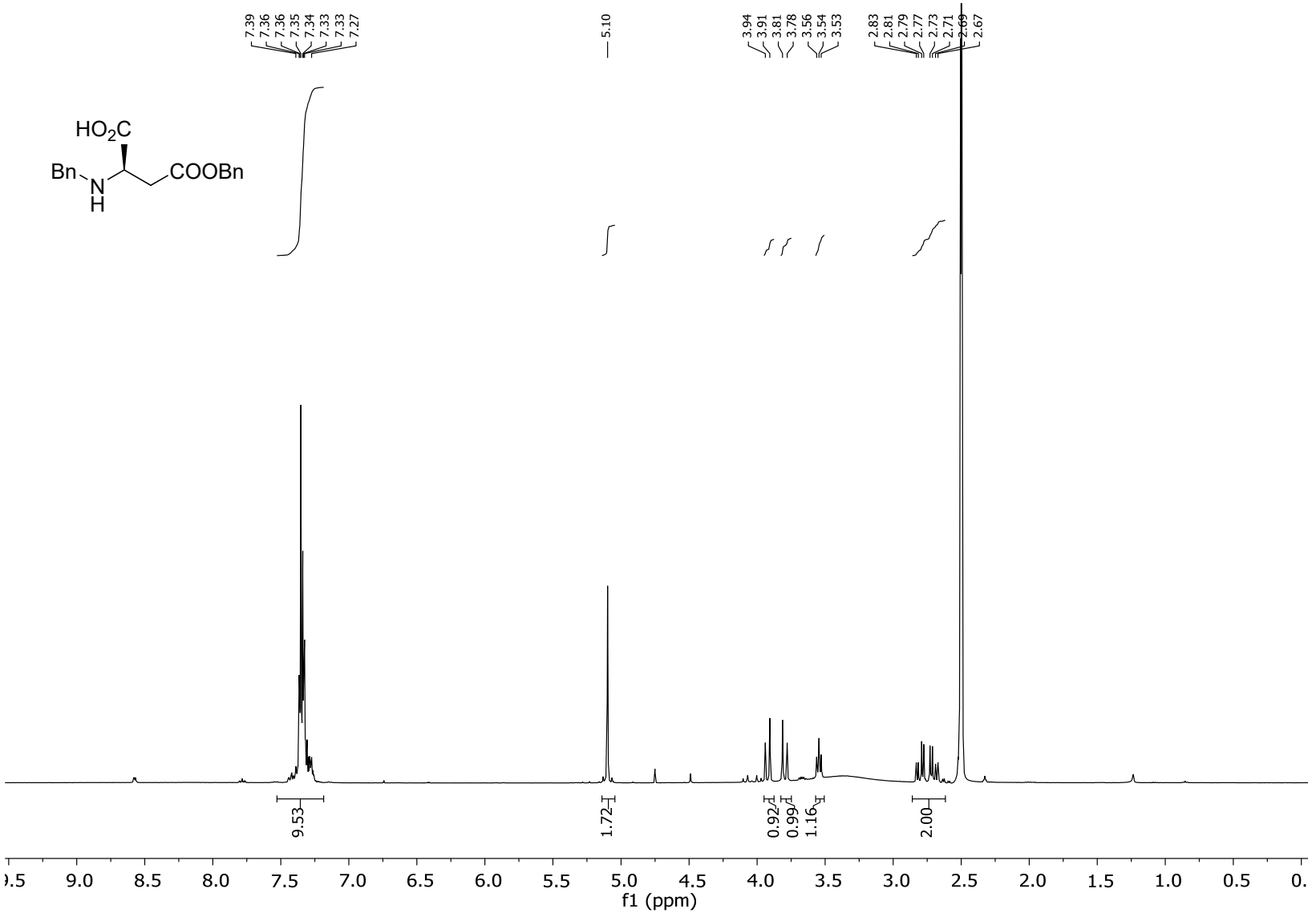
^{13}C NMR spectrum (101 MHz, 300 K, $CDCl_3$) of **8c.**



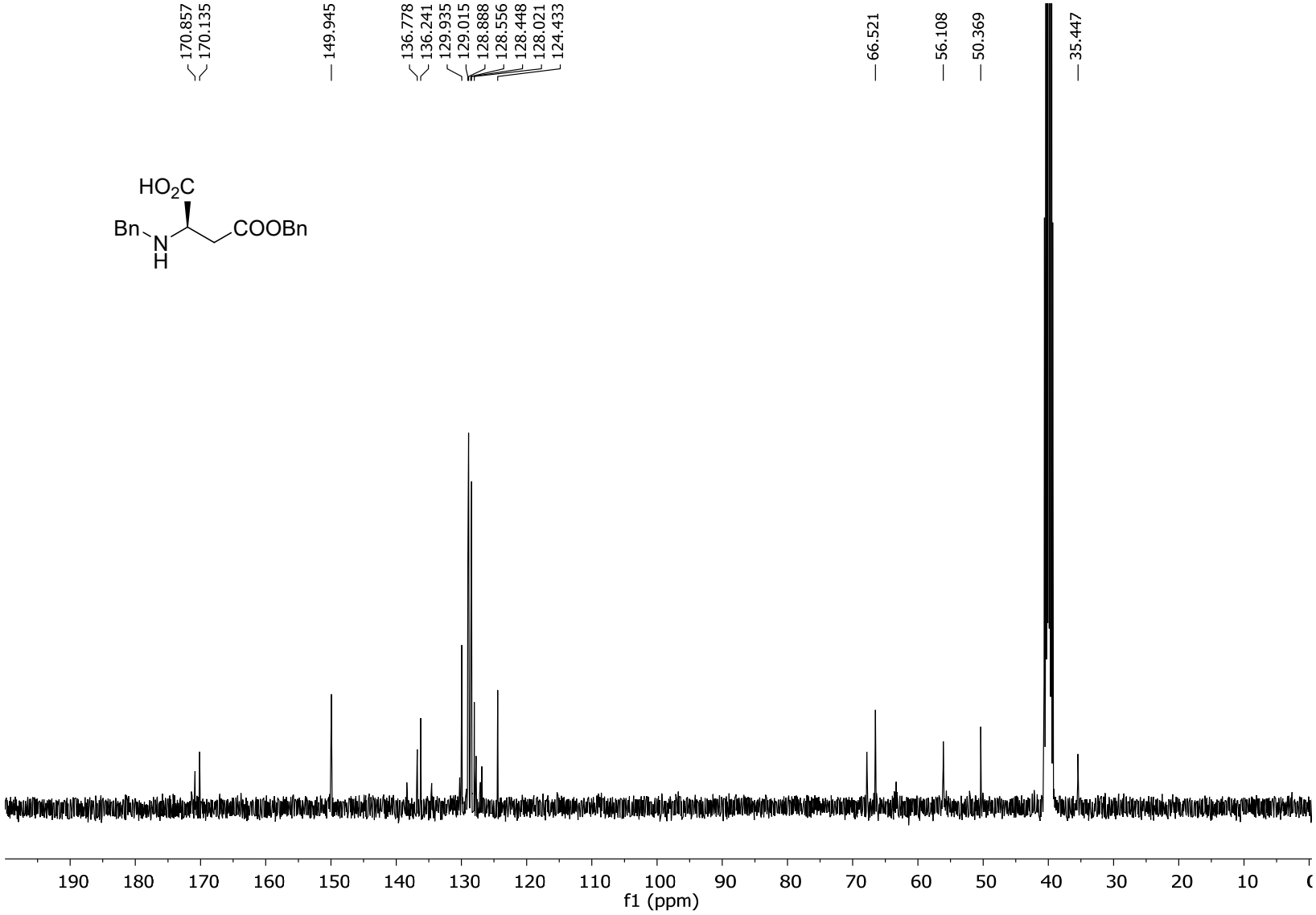
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of **12**.



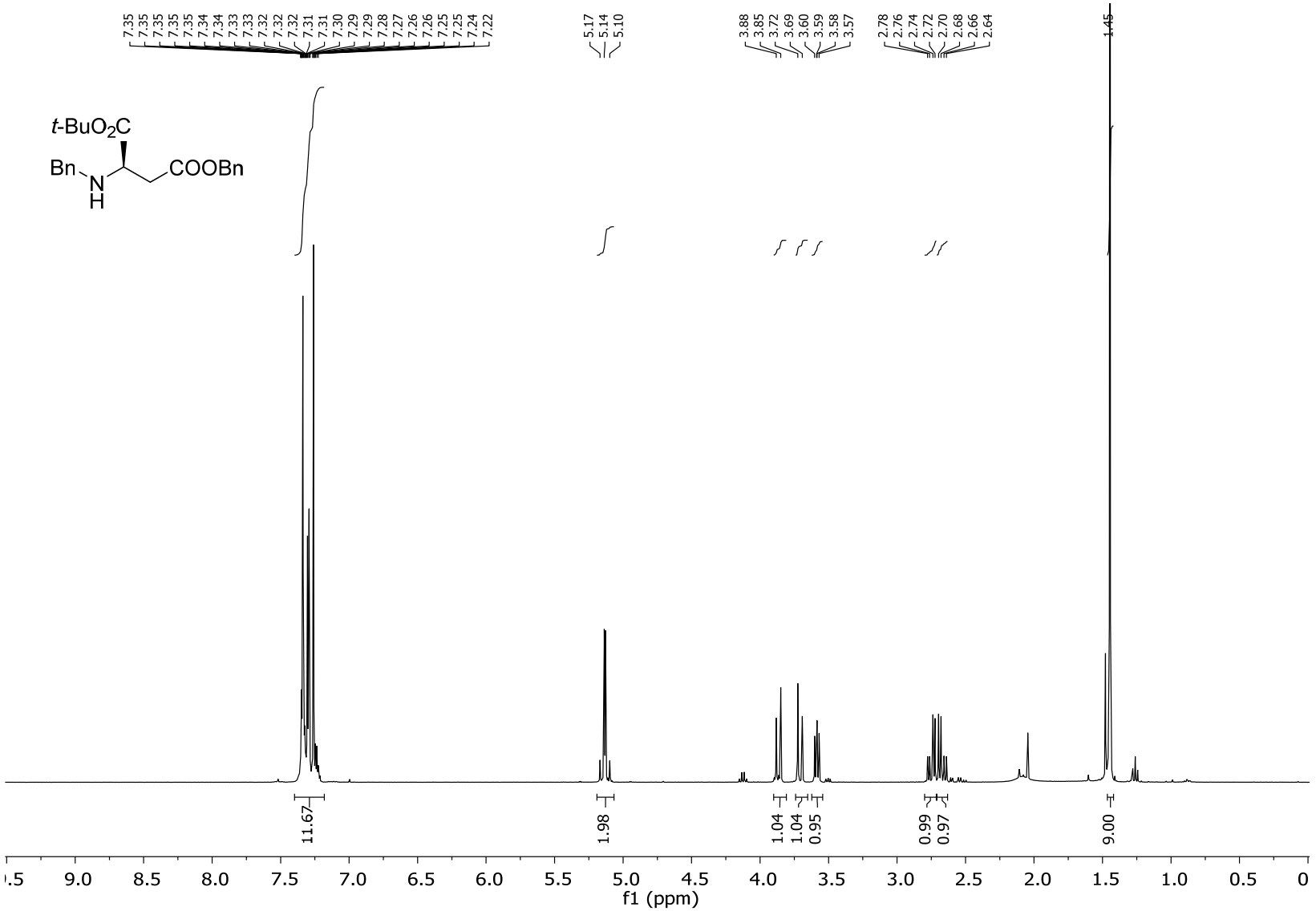
^{1}H NMR spectrum (400 MHz, 300 K, D_2O) of **Bn-D-Asp-OH**.



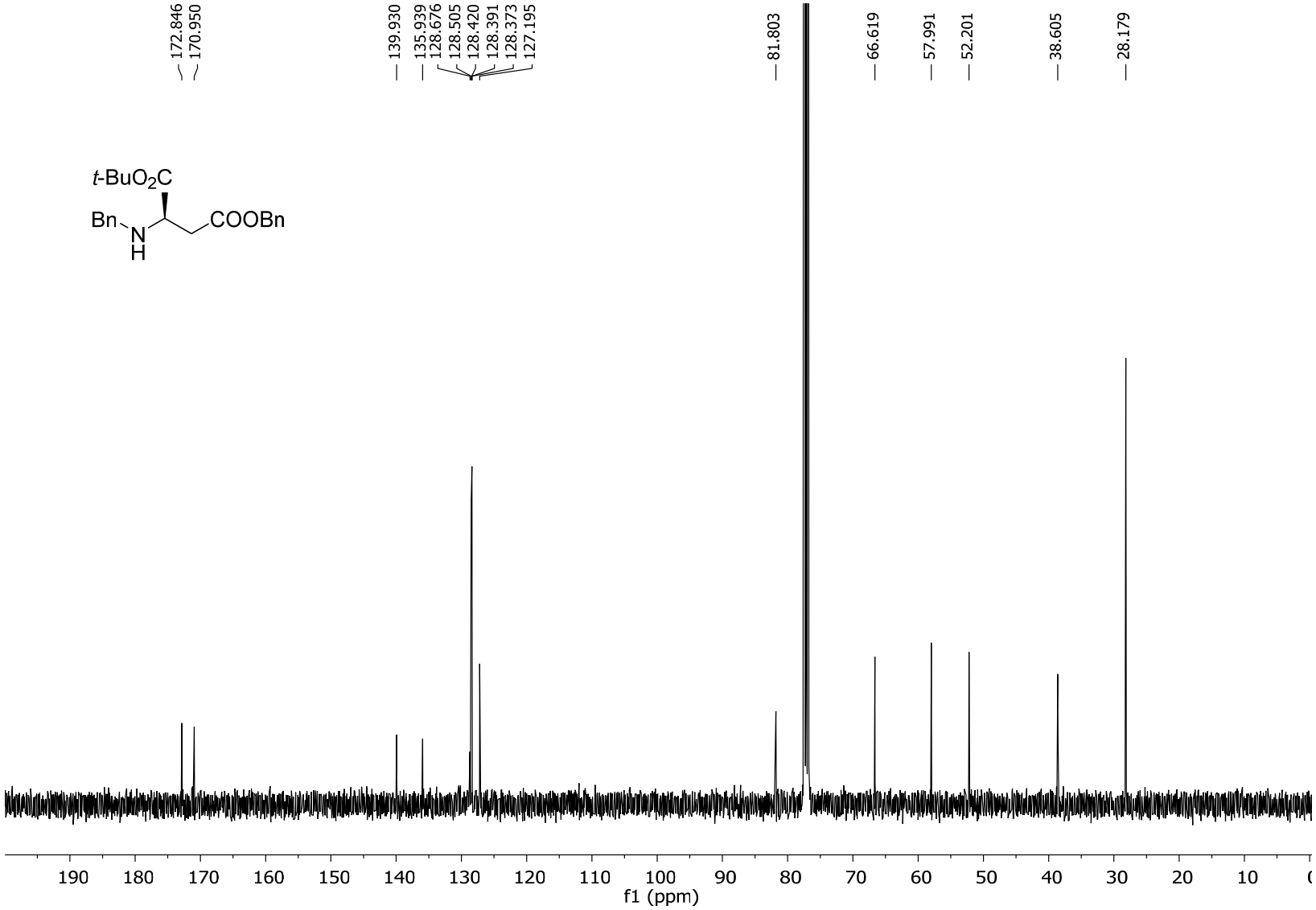
^{1}H NMR spectrum (400 MHz, 300 K, DMSO-d_6) of **14**.



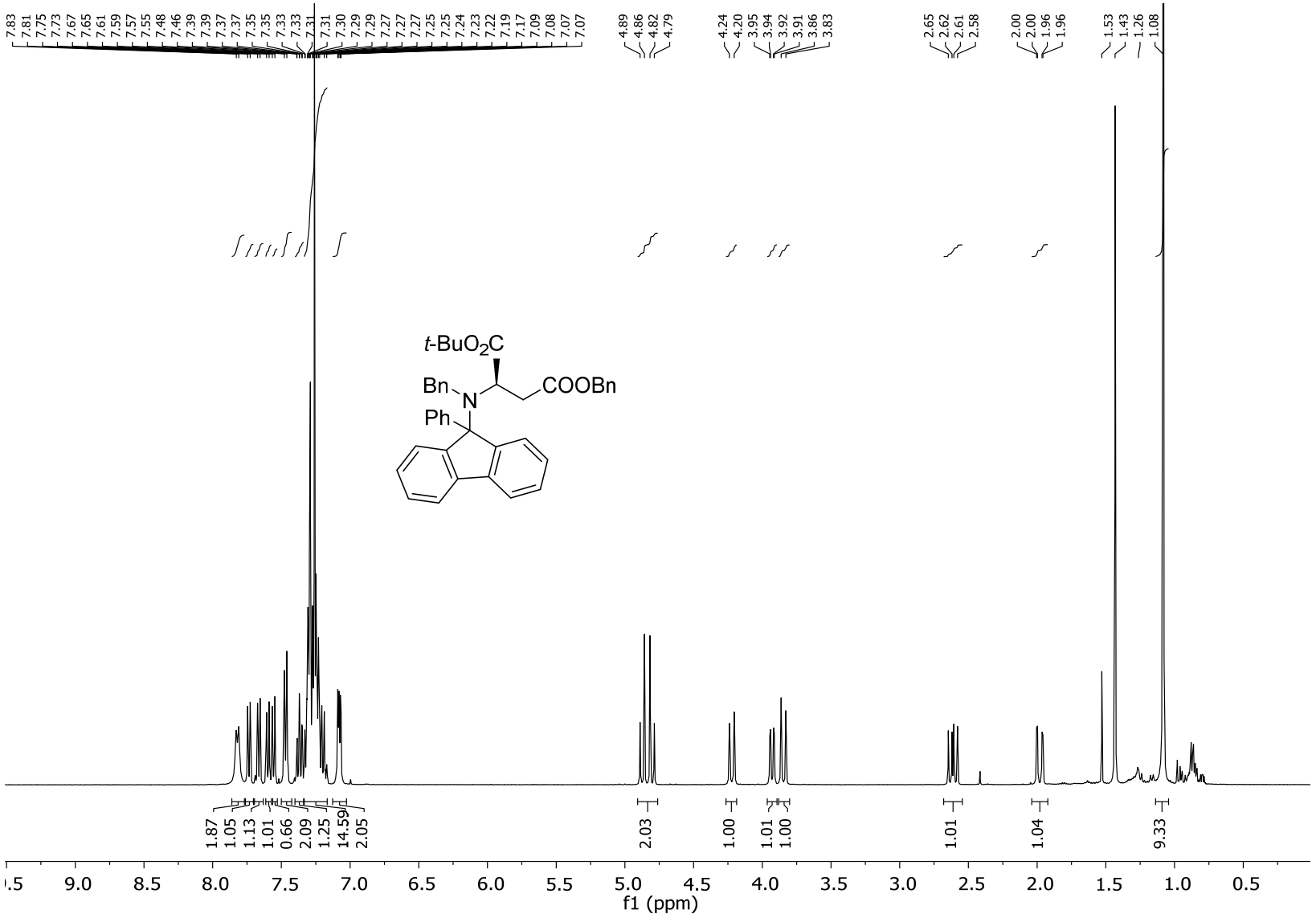
^{13}C NMR spectrum (101 MHz, 300 K, DMSO-*d*$_6$) of **14**.



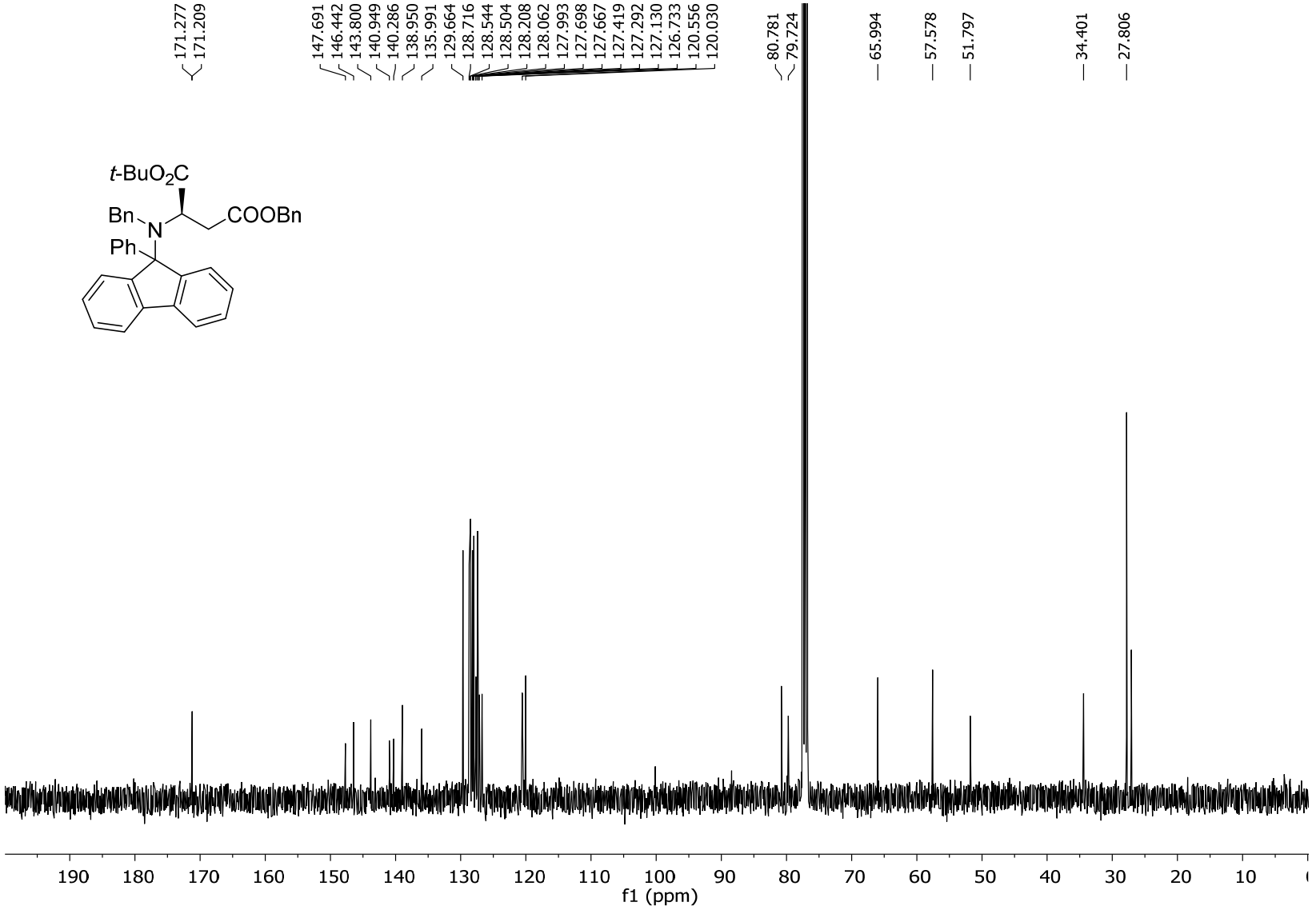
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of **Bn-D-Asp(OBn)-O*t*-Bu**.



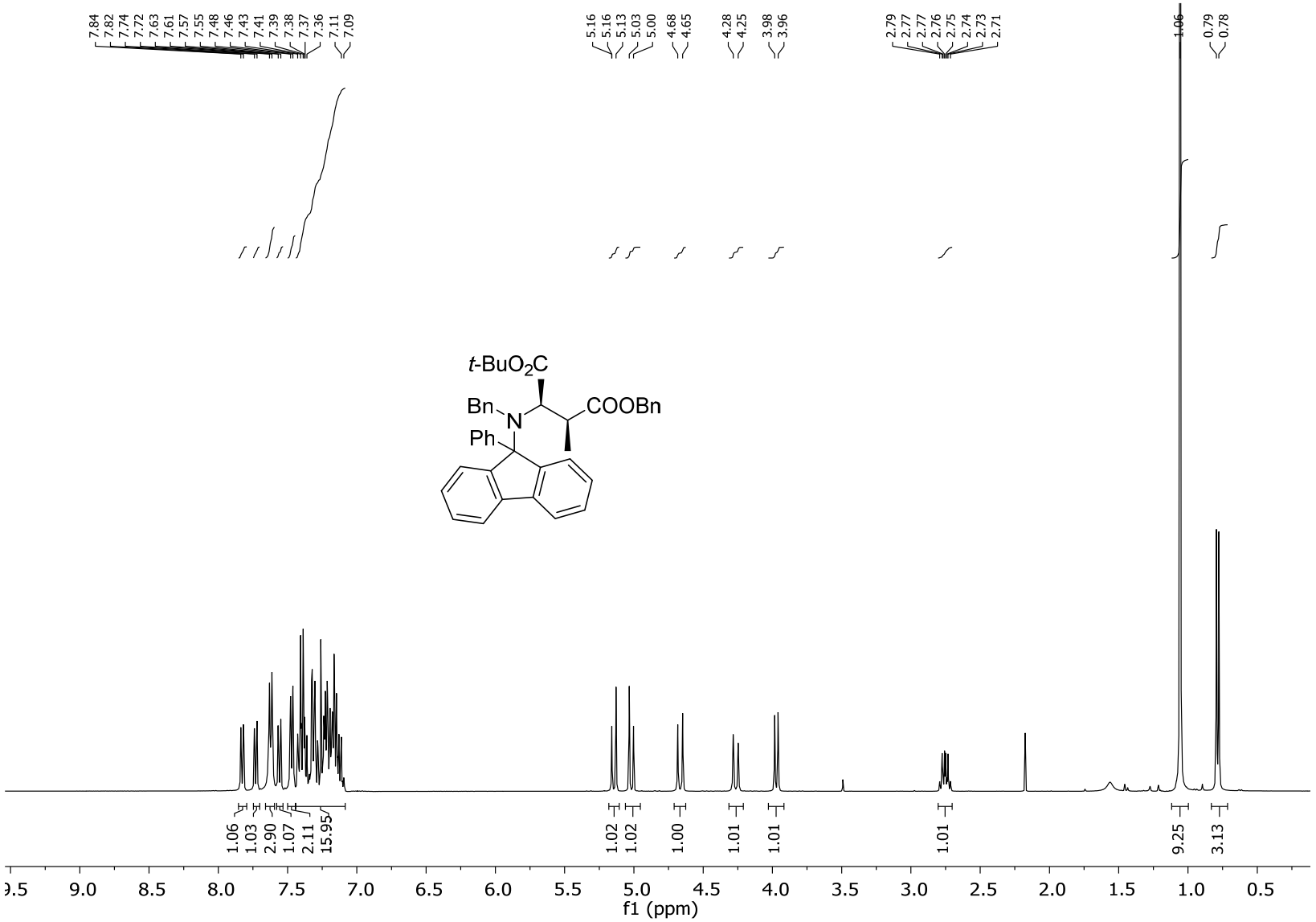
^{13}C NMR spectrum (101 MHz, 300 K, $CDCl_3$) of **Bn-D-Asp(OBn)-O*t*-Bu**.



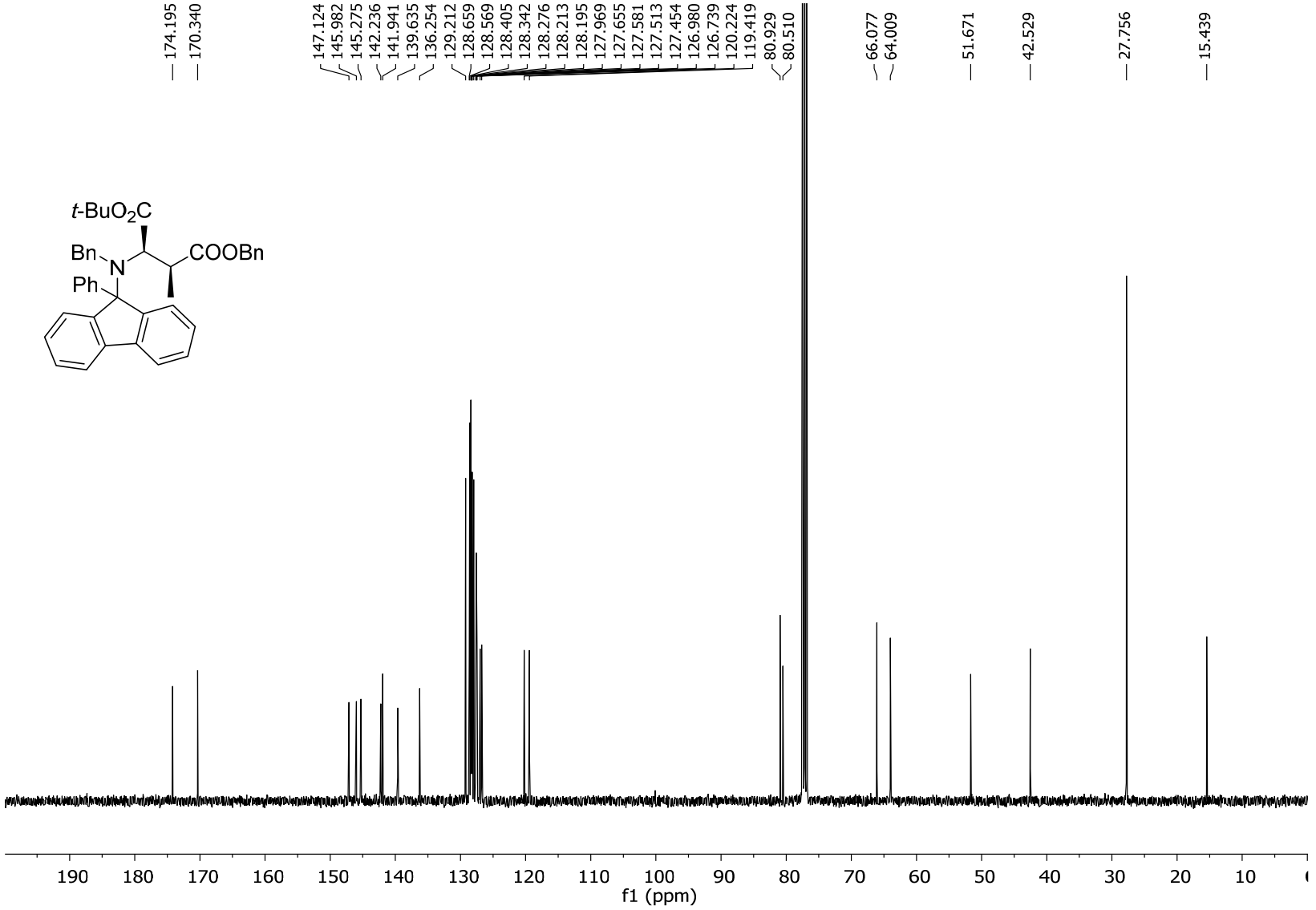
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of **15**.



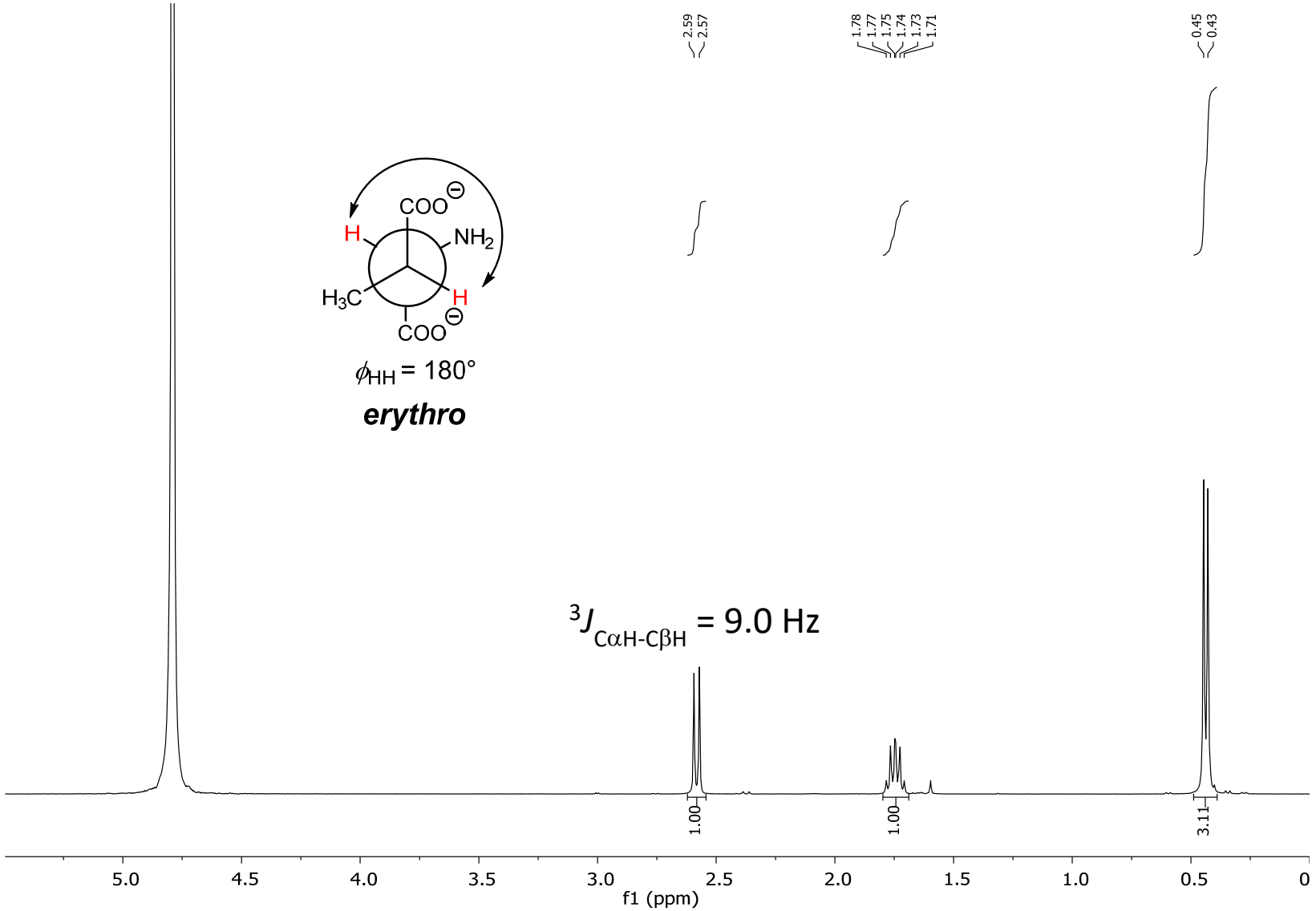
^{13}C NMR spectrum (101 MHz, 300 K, $CDCl_3$) of **15**.



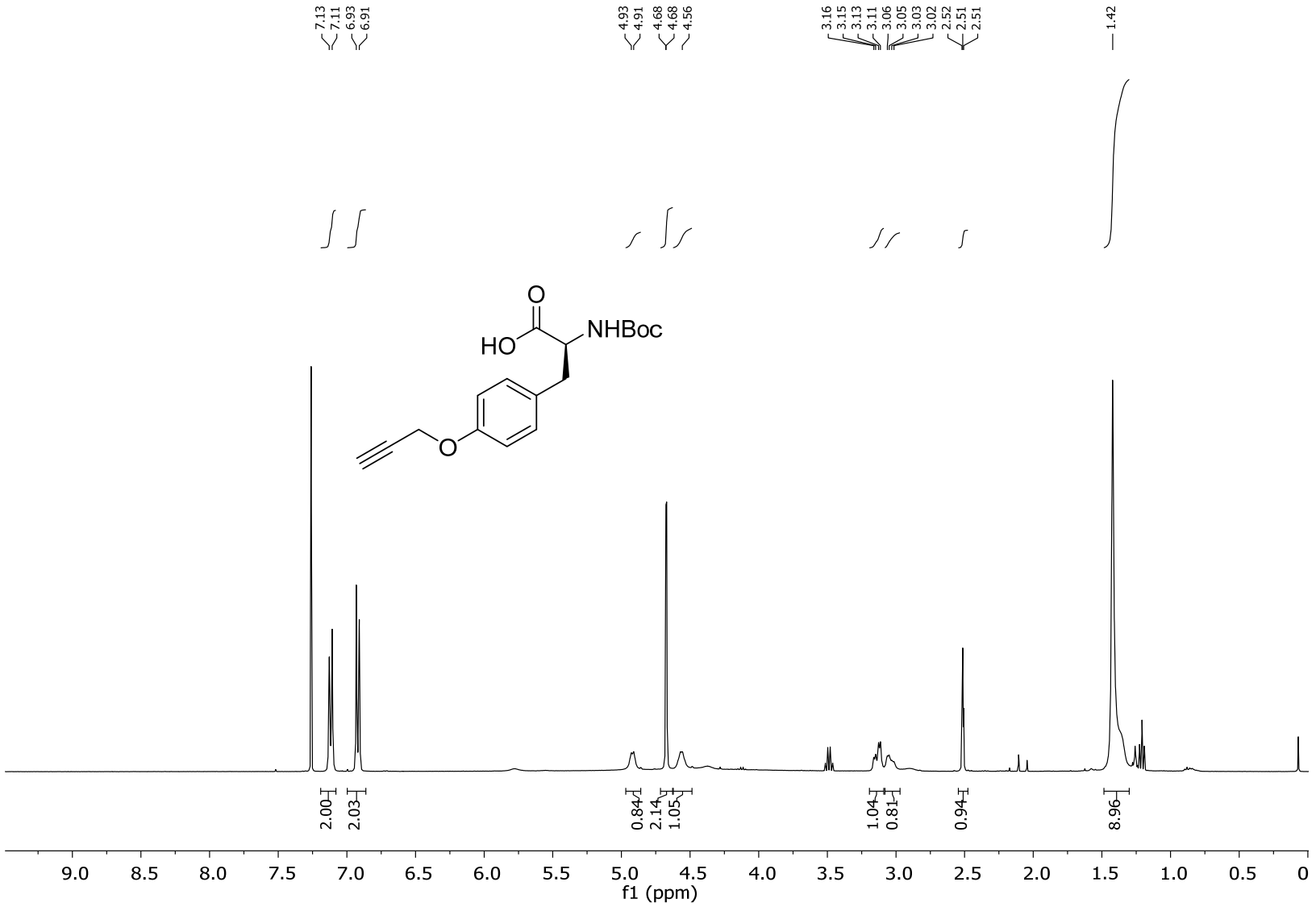
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of *erythro*-**16.**



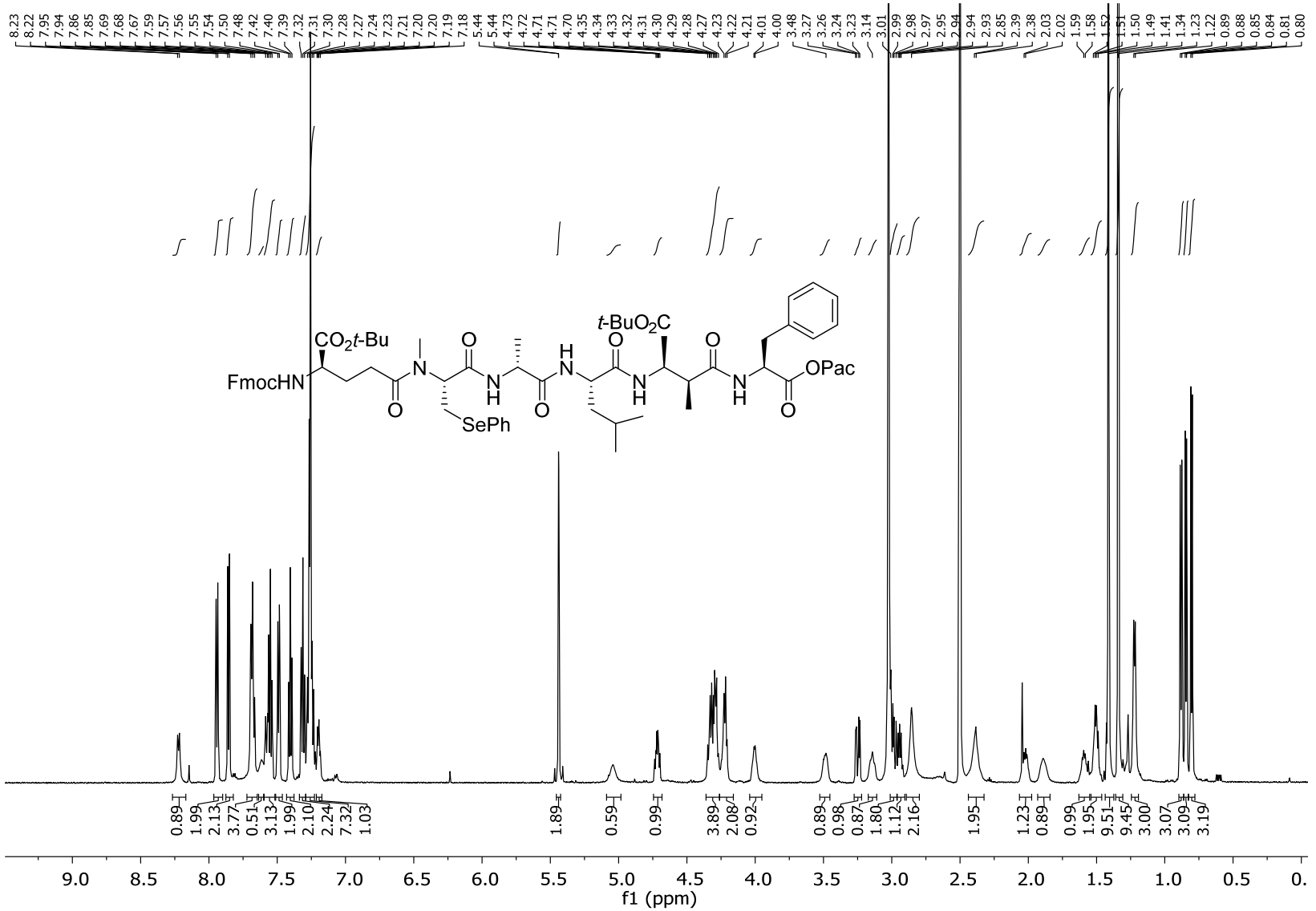
^{13}C NMR spectrum (101 MHz, 300 K, $CDCl_3$) of *erythro*-**16**.



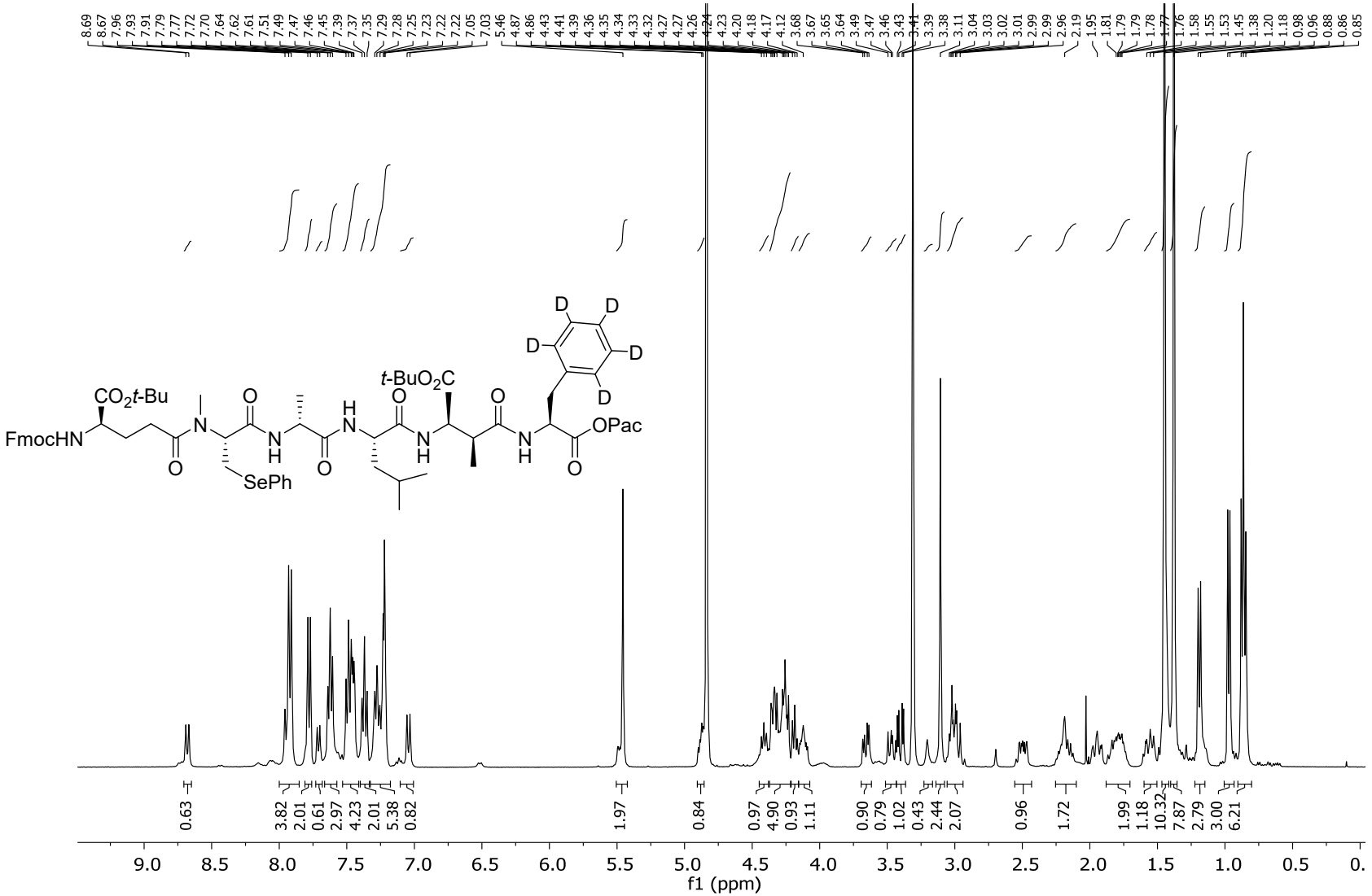
^{1}H NMR spectrum (400 MHz, D_2O, 300 K, pD > 14) of **H-MeAsp-OH**.



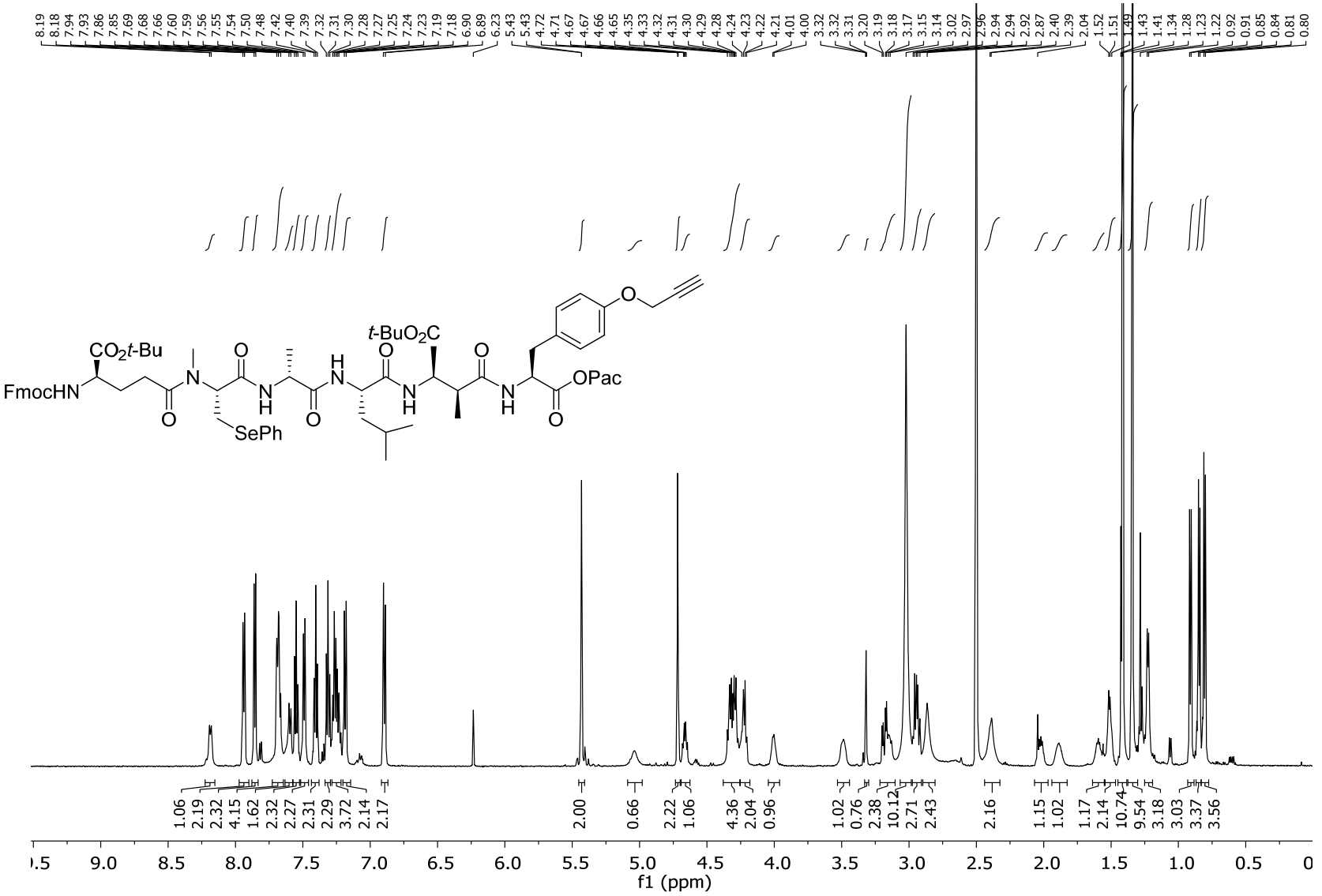
^{1}H NMR spectrum (400 MHz, 300 K, $CDCl_3$) of **18.**



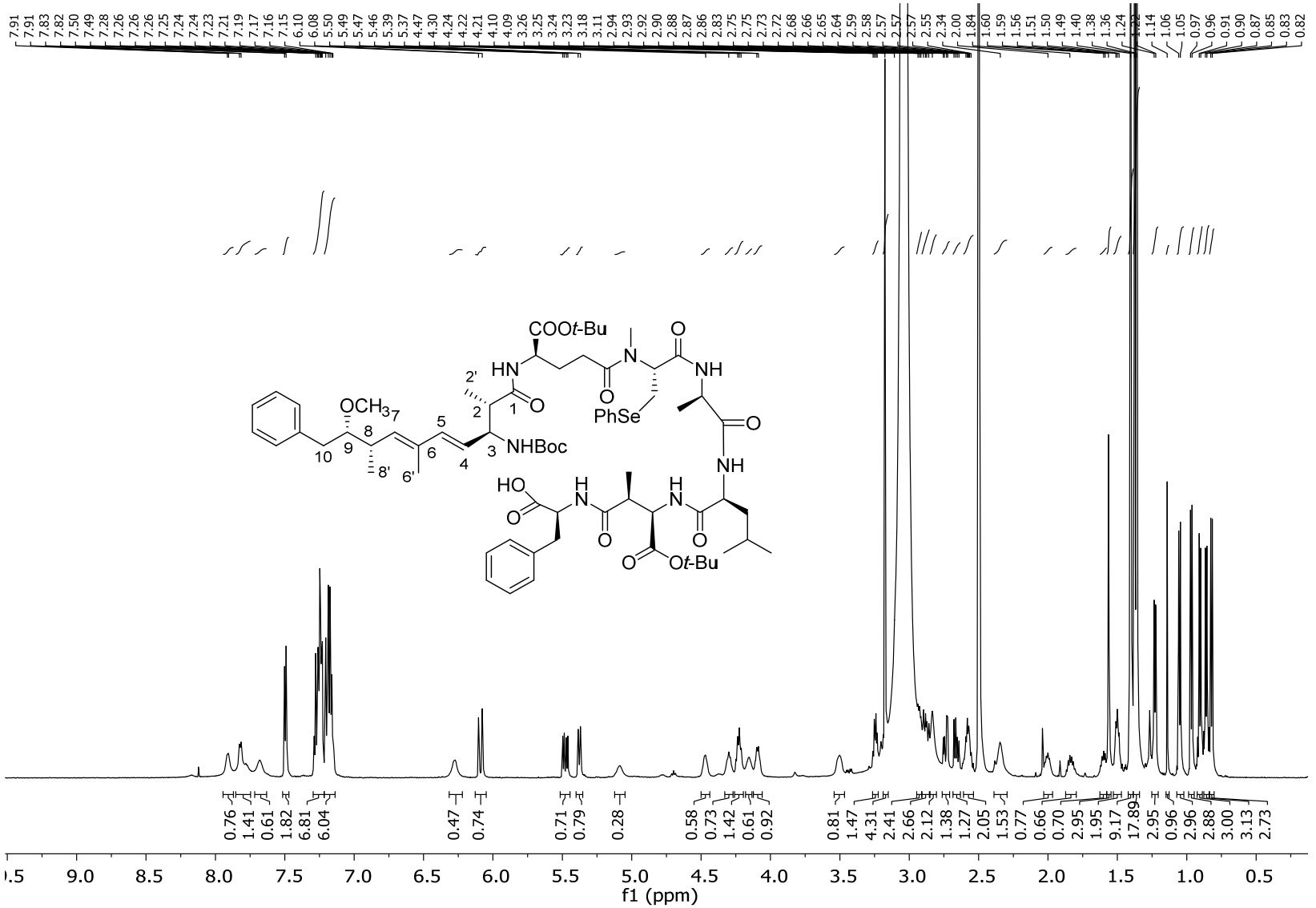
^{1}H NMR spectrum (600 MHz, 360 K, DMSO-d_6) of **21a**.



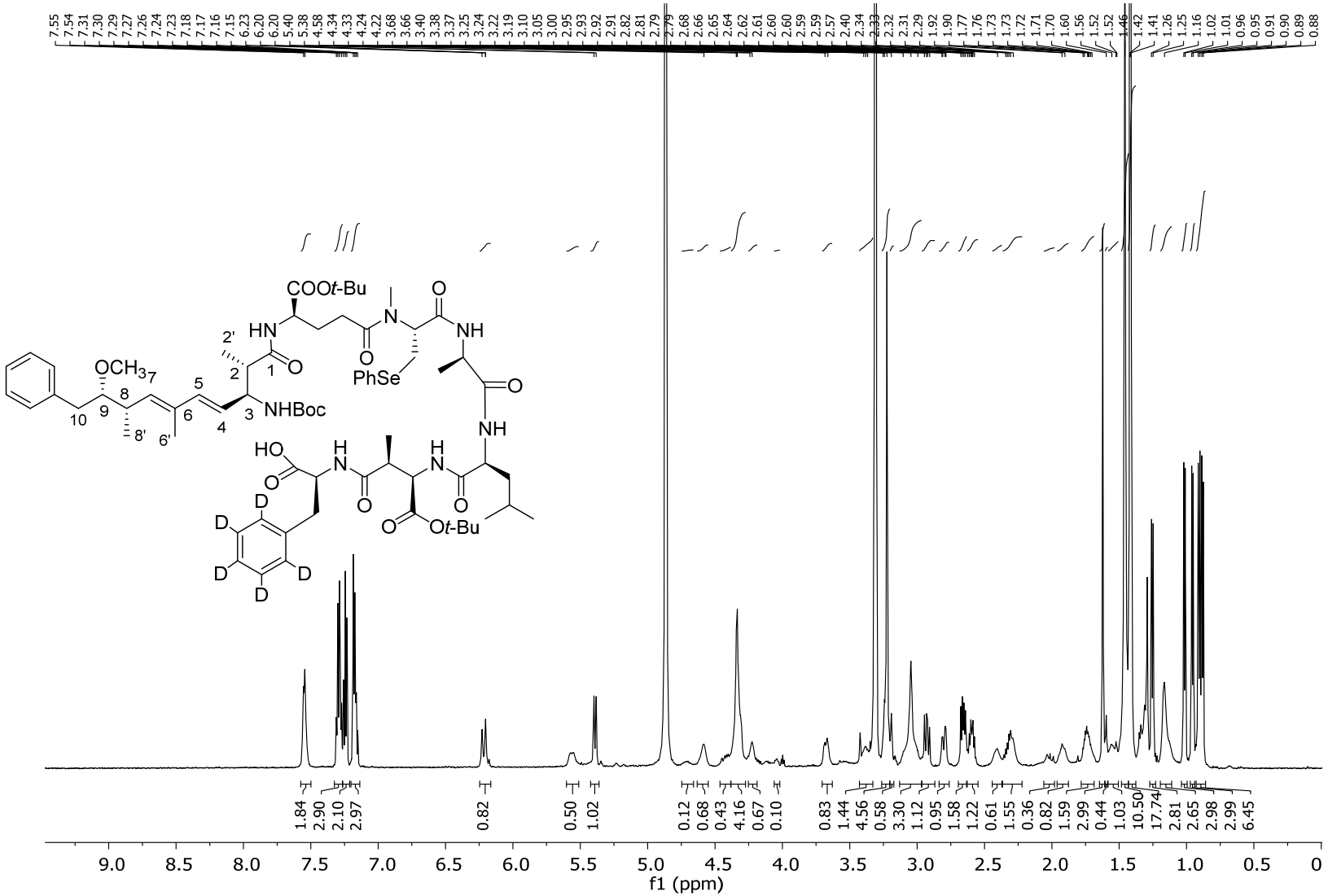
^{1}H NMR spectrum (400 MHz, 300 K, CD_3OD) of **21b.** Different H/D-exchange rates can be estimated from the integrals of remaining incompletely exchanged NH signals.



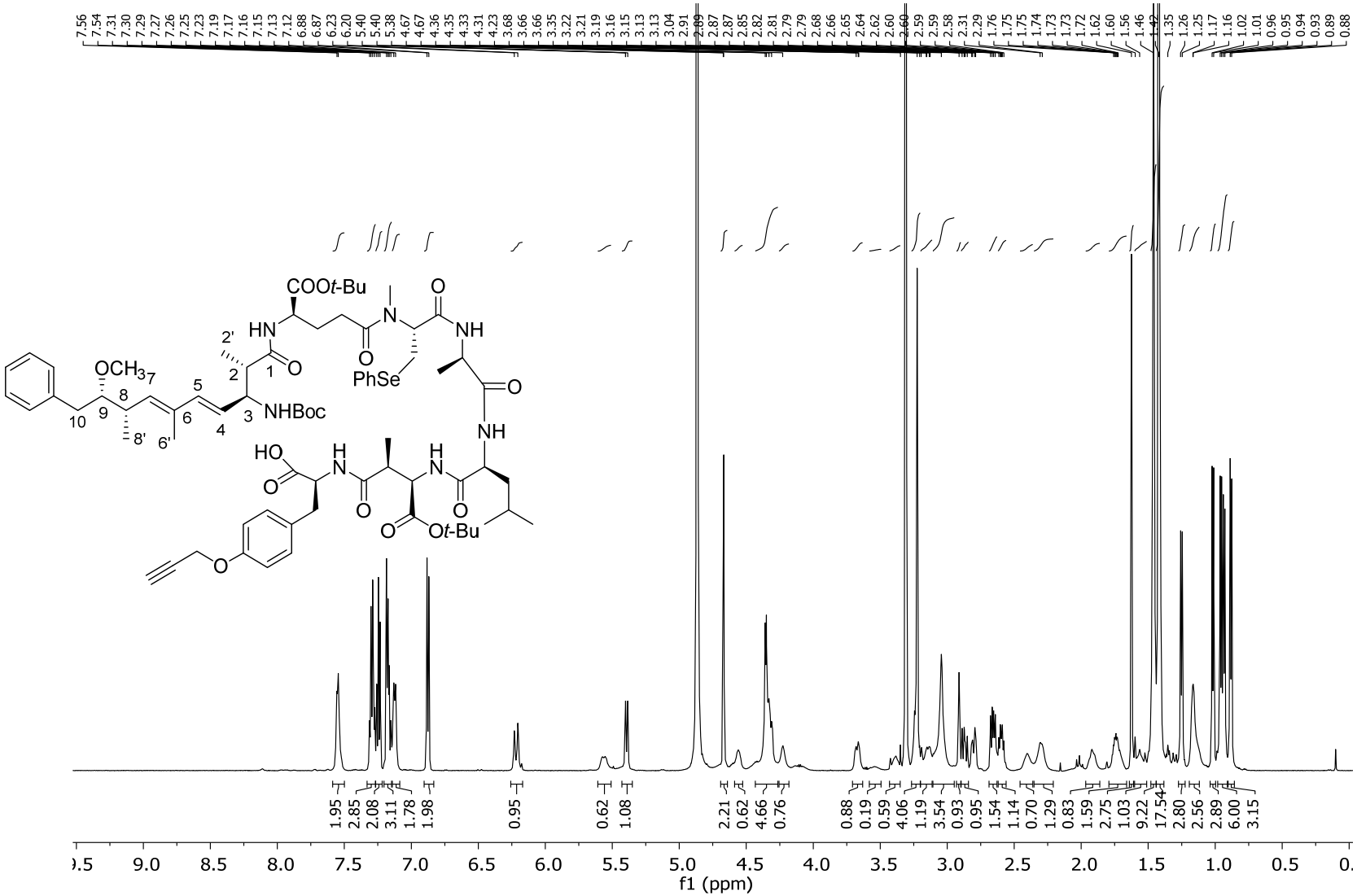
^{1}H NMR spectrum (600 MHz, 360 K, DMSO-d_6) of **21c.**



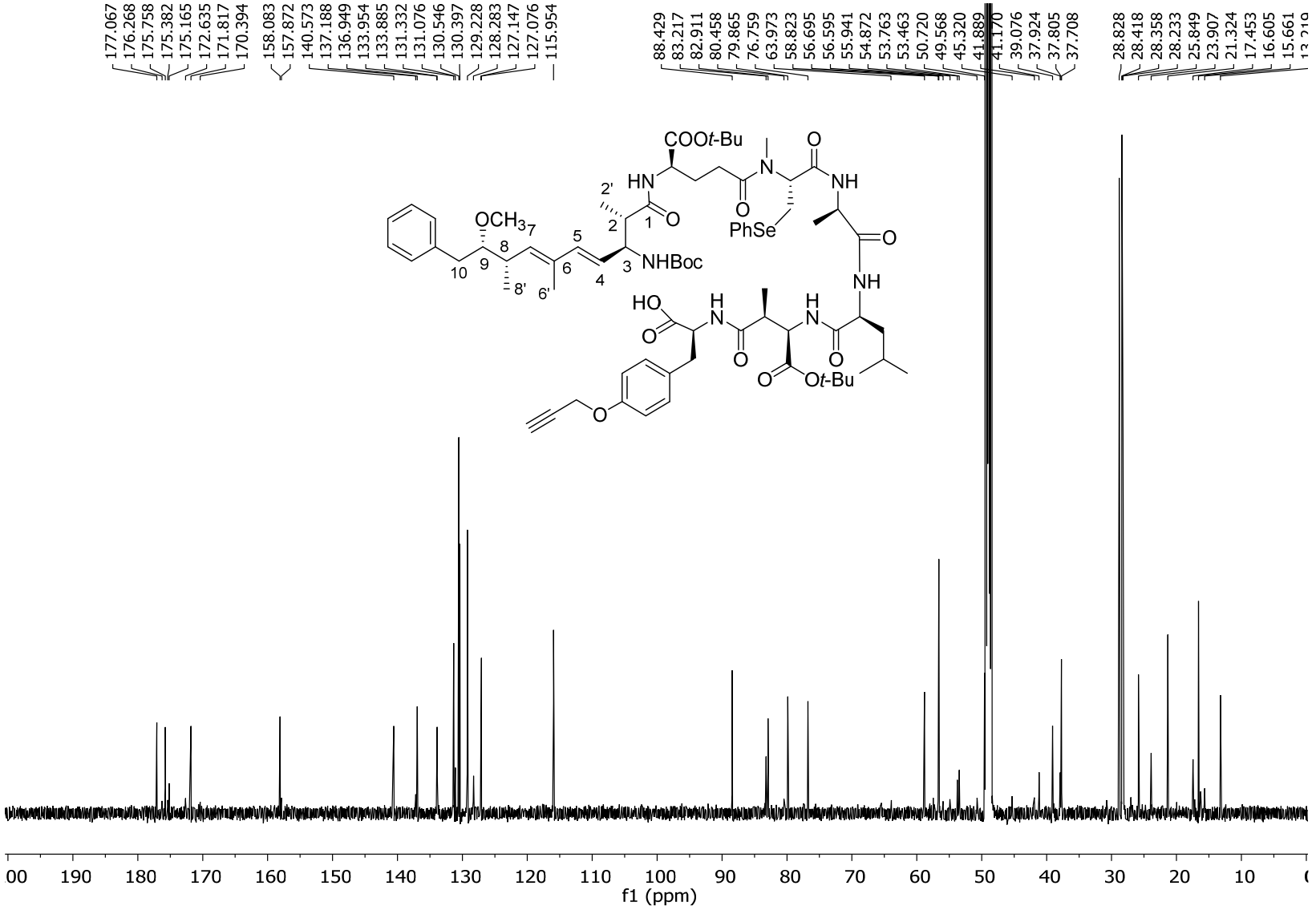
^{1}H NMR spectrum (600 MHz, 360 K, DMSO-d_6) of **22a**.



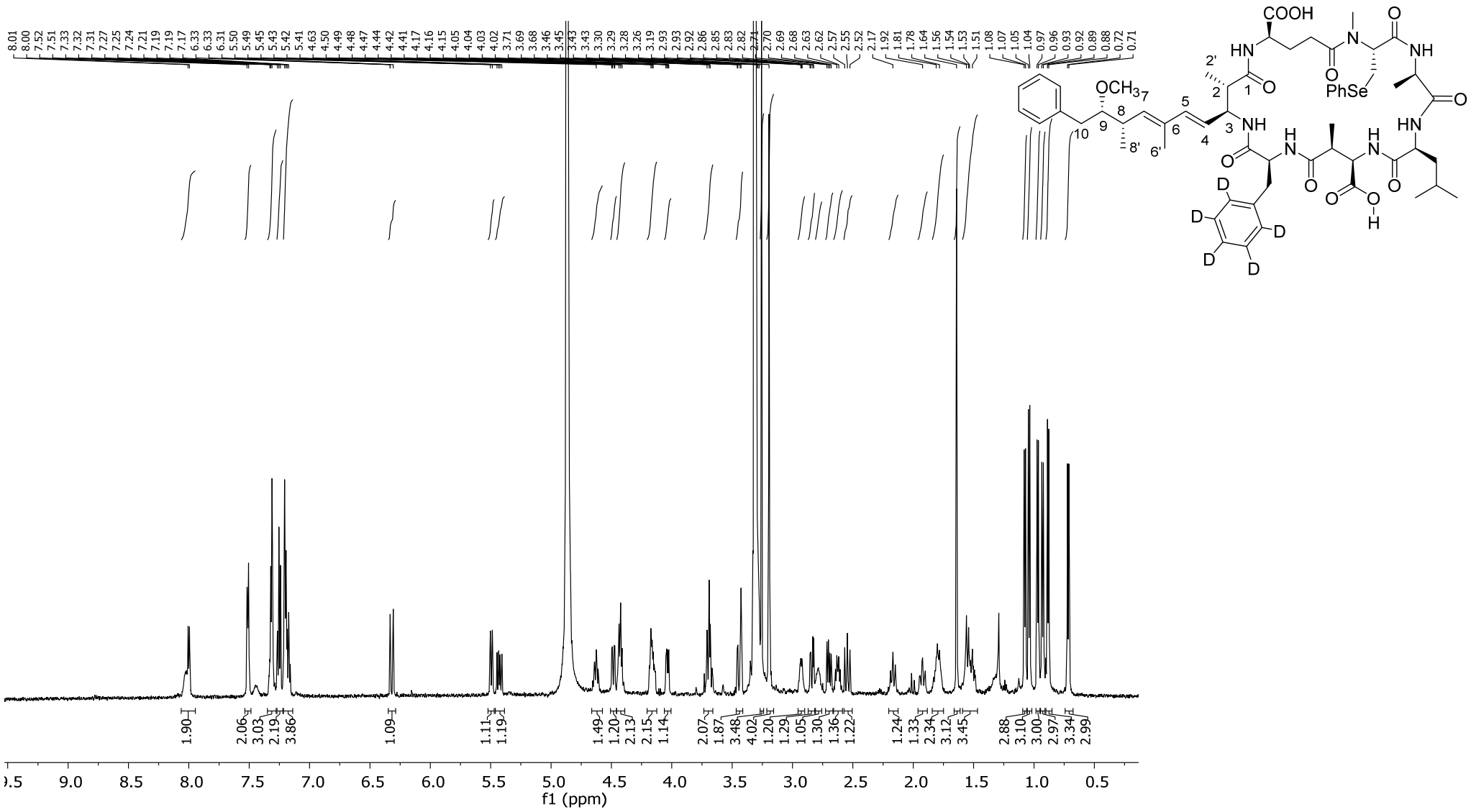
^{1}H NMR spectrum (600 MHz, 300 K, CD_3OD) of **22b**. Different H/D-exchange rates can be estimated from the integrals of remaining incompletely exchanged NH signals.



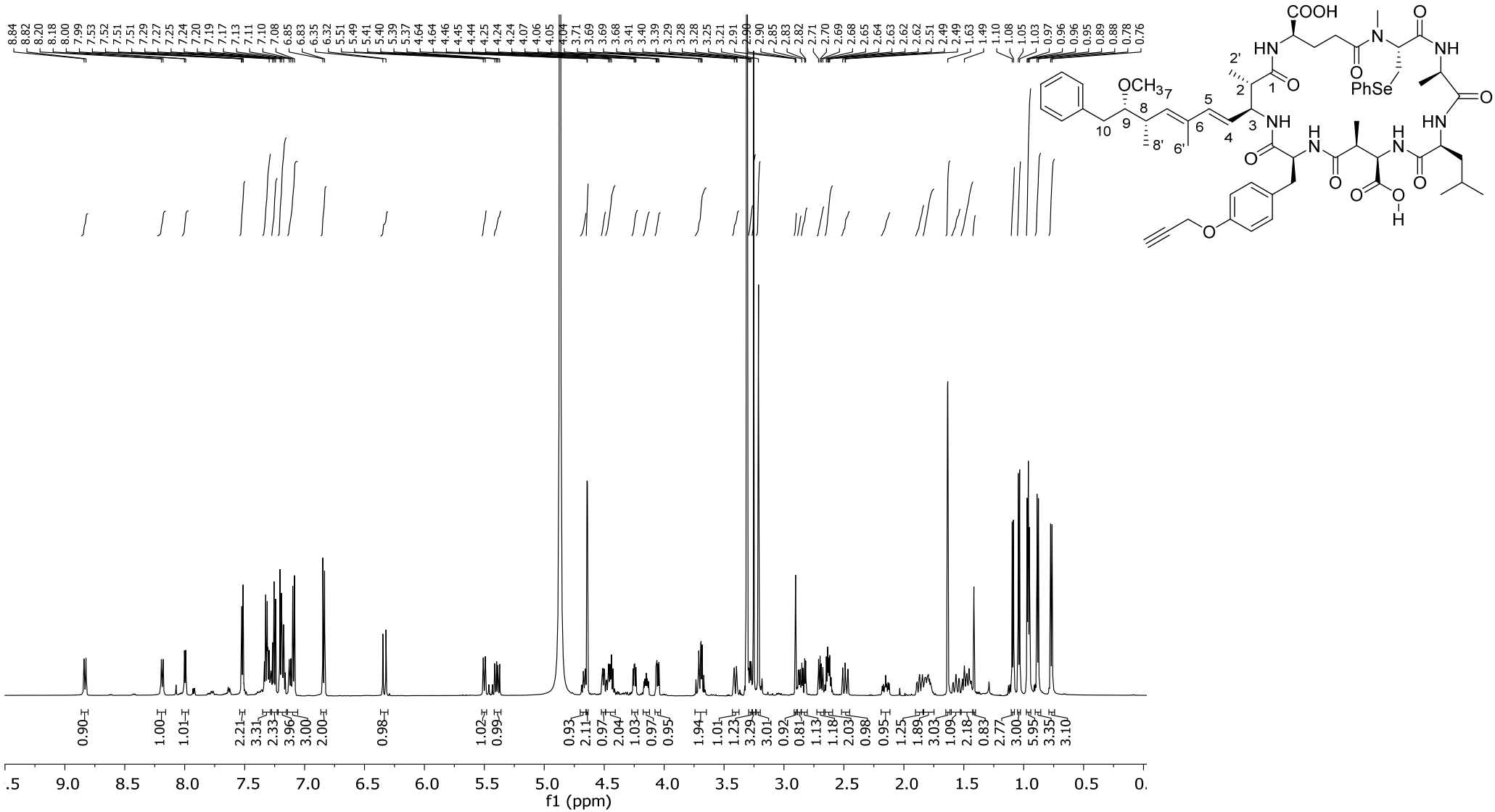
^{1}H NMR spectrum (600 MHz, 300 K, CD_3OD) of **22c**. Different H/D-exchange rates can be estimated from the integrals of remaining incompletely exchanged NH signals.



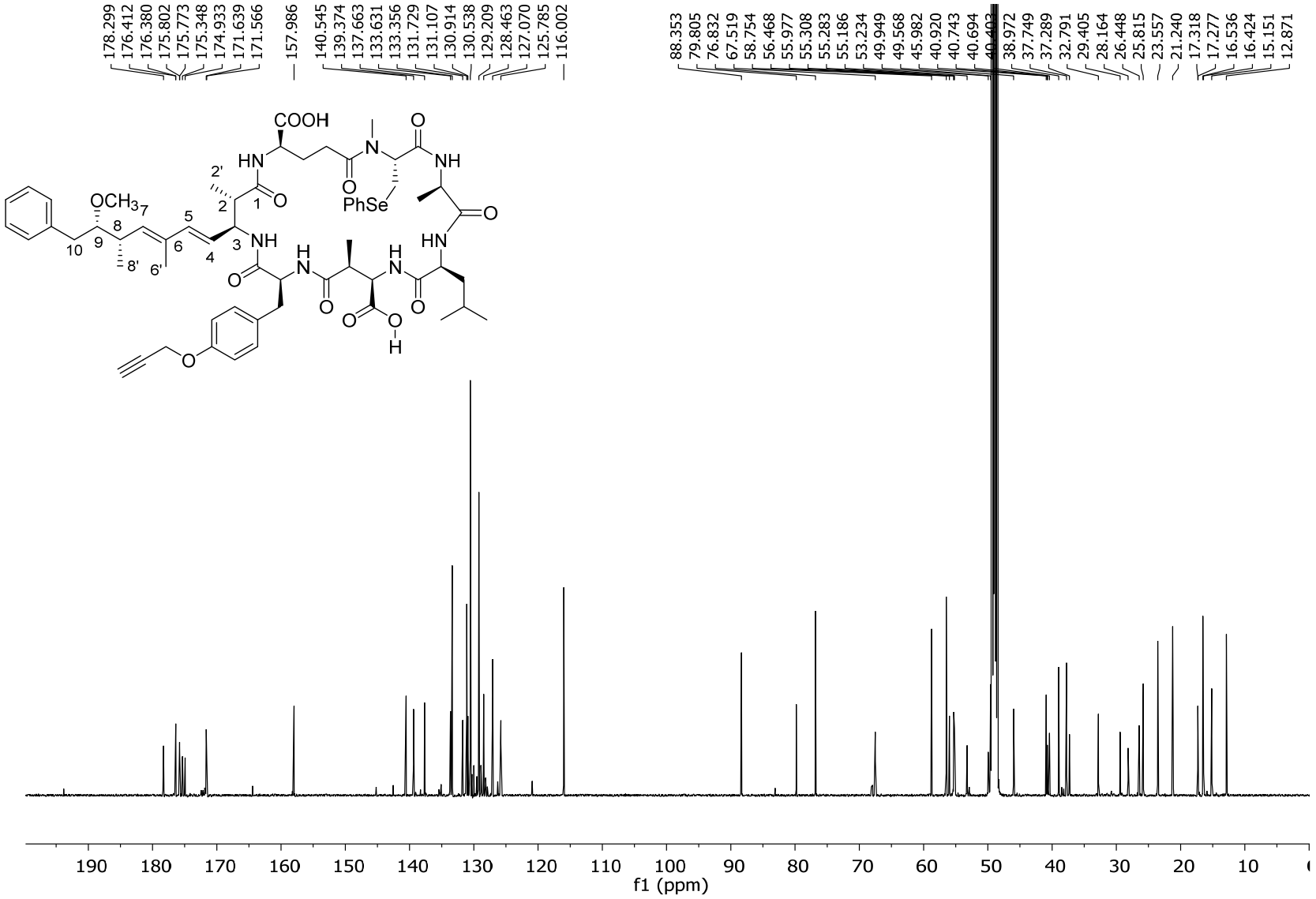
^{13}C NMR spectrum (151 MHz, 300K, CD_3OD) of **22c**.



^{1}H NMR spectrum (600 MHz, 300 K, CD_3OD) of **23b.** Different H/D-exchange rates can be estimated from the integrals of remaining incompletely exchanged NH signals.



^{1}H NMR spectrum (600 MHz, 300 K, CD_3OD) of **23c**. Different H/D-exchange rates can be estimated from the integrals of remaining incompletely exchanged NH signals.



^{13}C NMR spectrum (151 MHz, 300 K, CD_3OD) of **23c**.