Supplementary Information

Synthesis and Conformational Analysis of Peptides Embodying 2,3-Methanopipecolic Acids

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Table of contents

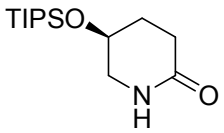
I. Chemistry General.

Chromatographic separations were performed under pressure on silica gel 60 (Merck,70–230 mesh) using flash column techniques; R_f values refer to TLC carried out on 0.25 mm silica gel plates with the same eluent indicated for column chromatography. ^{1}H NMR (400 MHz) and ^{13}C NMR (100.4 MHz) spectra were recorded on Varian Inova and Mercury spectrometers in the solvent indicated. ^{1}H NMR (500 MHz) were recorded on Bruker Avance II 500 MHz in the solvent indicated. Mass spectra were carried out by direct inlet on a LCQ Fleet^{TM} Ion Trap LC/MS system (Thermo Fisher Scientific) with an ESI interface in the positive mode. Compounds **25** and **35** were purified on a Dionex Ulltimate 3000 HPLC system equipped with a reverse-phase semi-preparative column (Alltima C18 10 μm, 250 mm × 10 mm, Alltech). Analytical HPLC analyses were performed on a Dionex Ulltimate 3000 system equipped with a reverse-phase column (Acclaim 120, C18, 5 μm, 4.6-250 mm). Anhydrous solvents were either commercial or prepared according to standard techniques. Compounds **1**[1] and **14**[2] were prepared as reported.

II. NMR Methods

NMR experiments were performed at a temperature of 298 K on Varian Inova and Varian Mercury 400 MHz NMR spectrometers and Bruker Avance II 500 MHz using diluted $CDCl_3$, CD_3OD, D_2O and D_2O/H_2O 1:9 solutions. All proton chemical shifts were assigned unambiguously for all compounds by gCOSY and/or TOCSY spectra. NOESY1D, NOESY and ROESY spectra provided the data used in the conformational analyses. 2D Experiments (gCOSY, TOCSY, NOESY, ROESY, gHSQC) and variable temperature 1D experiments were carried out at the sample concentration of 5 mM for **16**, 10 mM for **25**, and 3.9 mM for **35** in D_2O/H_2O 1:9 solutions. Water peak suppression in the spectra of D_2O/H_2O 1:9 solutions was obtained by either presaturation or excitation sculpting techniques using standard Bruker sequences. One-dimensional ^{1}H NMR spectra in D_2O/H_2O 1:9 solution for determining chemical shift temperature coefficients were obtained at 293–328 K (for **16** and **35**) or at 293-338 K (for **25**) with increments of 5 K, using sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as internal standard. Sample temperatures were controlled with the variable-temperature unit of the instrument. NOESY spectra were recorded using mixing times of 300, 500 and 800 ms for compound **16** in D_2O/H_2O 1:9 solution; using mixing time of 800 ms for compound **25** in D_2O/H_2O 1:9 solution; and using mixing times of 300 ms for compound **35** in D_2O/H_2O 1:9 solution and 600 ms in D_2O solution. For all NOESY spectra and NOESY1D experiments carried out in $CDCl_3$, mixing time was 800 ms for all compounds. ROESY spectra for compound **35** in D_2O/H_2O 1:9 solution were recorded using a spinlock of 200 ms. TOCSY spectrum of compound **35** in D_2O/H_2O 1:9 solution was recorded with a spinlock of 80 ms.

III. Synthetic procedures



(5*S*)-5-Triisopropylsilanyloxy-2-oxopiperidine [(-)-2]

To a stirred solution of **1**[1] (729 mg, 6.33 mmol) in anhydrous DMF (6.5 mL) were added imidazole (905 mg, 13.30 mmol) and TIPSCl (1.9 mL, 8.86 mmol) and the reaction was stirred, under an N_2 atmosphere, for 21 h at 35 °C (external bath); meanwhile additional imidazole (388 mg, 5.70 mmol) and TIPSCl (813 µL, 3.80 mmol) were added. After cooling to r.t., water (65 mL) was added and the solution was extracted with Et_2O (3 x 65 mL). The combined organic layers were dried with Na_2SO_4. After filtration and evaporation of the solvent, the oily residue was purified by chromatography (EtOAc/CH_3OH, 10:1; R_f = 0.32) to give **2** (1.46 g, 85%) as a pale yellow oil.

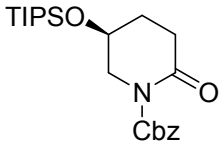
$[\alpha]_D^{19}$ -10.3 (*c* 0.95, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.05 (br s, 1 H, NH), 4.21 – 4.07 (m, 1 H, 5-H), 3.39 (ddd, *J* = 12.0, 4.0, 2.0 Hz, 1 H, 6-H), 3.21 (ddd, *J* = 12.0, 5.0, 2.6 Hz, 1 H, 6-H′), 2.56 (dt, *J* = 17.7, 7.5 Hz, 1 H, 3-H), 2.28 (dt, *J* = 17.7, 6.1 Hz, 1 H, 3-H′), 1.93-184 (m, 2 H, 4-H), 1.02 {s, 21 H, $Si[CH(CH_3)_2]_3$ + $Si[CH(CH_3)_2]_3$}.

^{13}C NMR($CDCl_3$, 100.4 MHz) δ (ppm): 172.4, 64.2, 49.5, 29.2, 27.7, 18.1, 12.2.

MS (ESI) m/z (%): 565 (100) $[2M + Na]^+$, 294 (10) $[M + Na]^+$, 272 (5) $[M + 1]^+$.

$C_{14}H_{29}NO_2Si$ (271.47): calcd. C, 61.94; H, 10.77; N, 5.16. Found: C, 61.77; H, 10.45; N, 5.18



(5*S*)-Benzyl 5-Triisopropylsilanyloxy-2-oxopiperidine-1-carboxylate [(+)-3]

n-BuLi (3.36 mL of a 1.6 M solution in hexane, 5.38 mmol) was added dropwise, under an N_2 atmosphere, to a solution of lactam **2** (1.46 g, 5.38 mmol) in anhydrous THF (54 mL) cooled to –78 °C, keeping the temperature below –70 °C during the addition. The mixture was stirred for 15 min and then benzyl chloroformate (922 µL, 6.46 mmol) was added dropwise. After 10 min the cooling bath was removed and the temperature was allowed to reach 0 °C. Keeping the reaction flask in an ice bath, a saturated solution of $NaHCO_3$ (27 mL) was added and the mixture was stirred for 10 min. Afterward, the layers were separated, the aqueous layer was extracted with CH_2Cl_2 (3 x 27

mL) and the combined organic layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was purified by flash chromatography (*n*-hexane/ EtOAc, 4:1; R_f 0.28), to afford pure **3** (1.75 g, 80%) as a colourless oil.

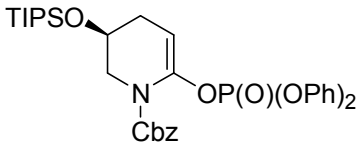
$[\alpha]_D^{20}$ +10.7 (*c* 1.00, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.42 – 7.40 (m, 2 H, Ph), 7.36 – 7.25 (m, 3 H, Ph), 5.27 (s, 2 H, C*H*$_2$Ph), 4.29 – 4.25 (m, 1 H, 5-H), 3.84 (ddd, *J* = 13.1, 4.7, 1.5 Hz, 1 H, 6-H), 3.69 (dd, *J* = 13.1, 3.3 Hz, 1 H, 6-H′), 2.77 (ddd, *J* = 17.2, 9.9, 7.1 Hz, 1 H, 3-H), 2.46 (dt, *J* = 17.2, 5.9 Hz, 1 H, 3-H′), 2.02 – 1.86 (m, 2 H, 4-H + 4-H′), 1.02 {s, 21 H, Si[C*H*$(CH_3)_2]_3$ + Si[CH(C*H*$_3)_2]_3$}.

^{13}C NMR ($CDCl_3$, 100.4 MHz) δ (ppm): 170.8, 154.0, 135.4, 128.5, 128.2, 128.0, 68.4, 64.2, 53.0, 30.9, 29.0, 17.9, 12.1.

MS (ESI) *m/z* (%): 833 (100) $[2M + Na]^+$, 406 (26) $[M + 1]^+$. MS/MS (ESI of $[M + 1]^+$) *m/z* (%): 362 (72), 272 (100), 228 (29).

$C_{22}H_{35}NO_4Si$ (405.60): calcd. C, 65.15; H, 8.70; N, 3.45. Found: C, 65.37; H, 8.62; N, 3.18.



(3*S*)-Benzyl 6-[(Diphenoxyphosphoryl)oxy]-3-triisopropylsilanyloxy-3,4-dihydropyridine-1(2*H*)-carboxylate [(–)-4]

A solution of **3** (1.75 g, 4.31 mmol) in anhydrous THF (10.4 mL) was added dropwise to a solution of KHMDS (12.94 mL of a 0.5 M solution in toluene, 6.47 mmol) in anhydrous THF (27.6 mL) cooled to –78 °C, under an N_2 atmosphere, and the resulting mixture was stirred for 1.5 h at –78 °C. Afterward, a solution of diphenyl chlorophosphate (1.34 mL, 6.47 mmol) in anhydrous THF (8.2 mL) was added dropwise and the reaction was left under stirring for 1 h at –78 °C, before allowing the temperature to reach 0 °C. Then a 5% NaOH aqueous solution (84 mL) was slowly added and the product was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with 5% NaOH (65 mL) and dried over anhydrous K_2CO_3 for 30 min. After filtration and evaporation of the solvent (without heating and leaving a small volume of solvent), the crude phosphate was chromatographed (*n*-hexane/EtOAc, 8:1 with 1% Et_3N; R_f 0.23), affording **4** (2.06 g, 75%) as a colourless oil, which was immediately used in the next step.

$[\alpha]_D^{23}$ –2.6 (*c* 0.70, $CDCl_3$).

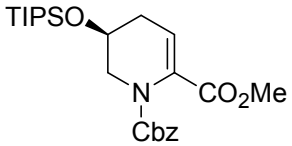
^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.35 – 7.24 (m, 10 H, Ph), 7.20 – 7.15 (m, 5 H, Ph), 5.10 – 5.02 (m, 3 H, C*H*$_2$Ph + 5-H), 4.13 – 4.11 (m, 1 H, 3-H), 3.75 (dd, *J* = 12.5, 6.4 Hz, 1 H, 2-H), 3.57

(dd, J = 12.5, 2.4 Hz, 1 H, 2-H′), 2.43 (dq, J = 18.1, 4.7 Hz, 1 H, 4-H), 2.14 (dq, J = 18.1, 4.0 Hz, 1 H, 4-H′), 1.03 {s, 21 H, $Si[CH(CH_3)_2]_3$ + $Si[CH(CH_3)_2]_3$}.

^{13}C NMR ($CDCl_3$, 100.4 MHz) δ (ppm): 154.4, 150.4, 139.5, 135.7, 129.7, 128.4, 128.1, 128.0 , 125.5, 120.1, 98.6, 67.9, 64.9, 51.6, 32.3, 17.9, 12.1.

MS (ESI) *m/z* (%): 1298 (100) $[2M + Na]^+$, 638 (8) $[M + 1]^+$.

$C_{34}H_{44}NO_7PSi$ (637.77): calcd. C, 64.03; H, 6.95; N, 2.20. Found: C, 63.88; H, 7.04; N, 2.03.



(5*S*)-1-Benzyl 2-Methyl 5-Triisopropylsilanyloxy-5,6-dihydropyridine-1,2(4*H*)-dicarboxylate [(+)-5]

$Pd(OAc)_2$ (69 mg, 0.306 mmol) and Ph_3P (176 mg, 0.673 mmol) were added to a solution of phosphate **4** (1.95 g, 3.06 mmol) in anhydrous DMF (9 mL), under an N_2 atmosphere. The flask was flushed and saturated with carbon monoxide and the mixture was vigorously stirred for 10 min at 25 °C under carbon monoxide atmosphere. Afterward, Et_3N (848 µL, 6.12 mmol) and anhydrous methanol (5.2 mL, 128.7 mmol) were added and the mixture was stirred at 60 °C (external bath) for 1.5 h under carbon monoxide atmosphere (balloon). After cooling, water (90 mL) was added, the product was extracted with Et_2O (4 × 90 mL) and the combined organic extracts were dried over Na_2SO_4. After filtration and evaporation of the solvent, the crude was purified by flash chromatography (*n*-hexane/EtOAc, 9:1; R_f 0.17), to afford pure **5** (1.02 g, 74%) as a thick colourless oil.

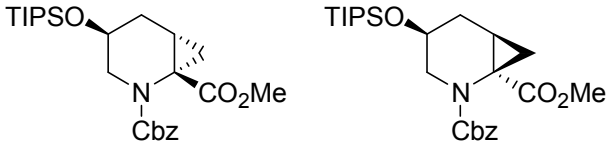
$[\alpha]_D^{22}$ +16.8 (*c* 0.96, $CHCl_3$).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.36 – 7.29 (m, 5 H, Ph), 6.04 (t, J = 3.7 Hz, 1 H, 3-H), 5.20 – 5.07 (m, 2 H, CH_2Ph), 4.13 (br s, 1 H, 5-H), 3.78 – 3.71 (m, 1 H, 6-H), 3.55 (br s, 4 H, 6-H′ + OCH_3), 2.51 (ddd, J = 19.5, 5.5, 3.9 Hz, 1 H, 4-H), 2.19 (dt, J = 19.5, 4.5 Hz, 1 H, 4- H′), 1.03 {s, 21 H, $Si[CH(CH_3)_2]_3$ + $Si[CH(CH_3)_2]_3$}.

^{13}C NMR ($CDCl_3$, 100.4 MHz) δ (ppm): 164.7, 154.4, 135.7, 131.9, 128.4, 128.3, 128.2, 121.5, 68.0, 64.7, 52.0, 49.9, 33.6, 17.9, 12.1.

MS (ESI) *m/z* (%): 917 (100) $[2M + Na]^+$, 448 (67) $[M + 1]^+$. MS/MS (ESI of $[M + 1]^+$) *m/z* (%): 404 (100).

$C_{24}H_{37}NO_5Si$ (447.64): calcd. C, 64.39; H, 8.33; N, 3.13. Found: C, 64.67; H, 8.65; N, 3.47.



2-Benzyl 1-Methyl (1*S*,4*S*,6*S*)-4-Triisopropylsilanyloxy-2-azabicyclo[4.1.0]heptane-1,2-dicarboxylate [(+)-6] and 2-Benzyl 1-Methyl (1*R*,4*S*,6*R*)-4-Triisopropylsilanyloxy-2-azabicyclo[4.1.0]heptane-1,2-dicarboxylate [(+)-7]

NaH (60% in weight in mineral oil, 97 mg, 2.42 mmol), previously washed with dry *n*-hexane (2 × 5 mL), was suspended in dry DMSO (10 mL), under an N_2 atmosphere. Trimethylsulfoxonium iodide (709 mg, 3.22 mmol) was added to the resulting suspension in three portions and the mixture was stirred at room temperature for 30 min, until complete disappearance of the precipitate. Then, a cooling bath at 15 °C was applied and a solution of **5** (722 mg, 1.61 mmol) in dry DMSO (5 mL) was added dropwise to the reaction mixture. The cooling bath was removed and the reaction mixture was stirred at room temperature for 45 min. Afterward, a cooling bath at 15 °C was applied again and water (100 mL) was added to the mixture, which was then extracted with Et_2O (5 × 75 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated, affording a colourless oil containing a 1.4:1 mixture of *trans* (**6**) and *cis* (**7**) isomers. The two isomers were separated and purified by flash chromatography (*n*-hexane/EtOAc, 9:1, R_f 0.27 and R_f 0.15), to afford pure **6** (301 mg, 40%) and pure **7** (264 mg, 35%) both as thick colourless oils.

6: $[\alpha]_D^{21}$ +11.4 (*c* 0.95, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz) (2.3:1 mixture of rotamers) δ (ppm): 7.36 – 7.27 (m, 5 H + 5H, Ph, both rotamers), 5.28 (part A of an AB system, *J* = 12.6 Hz, 1 H, C*H*$_2$Ph major rotamer), 5.16 (AB system, *J* = 12.3 Hz, 2 H, C*H*$_2$Ph minor rotamer), 5.06 (part B of an AB system, *J* = 12.6 Hz, 1 H, C*H*$_2$Ph major rotamer), 3.95 – 3.84 (m, 2 H, 4-H major rotamer + 3-H_{eq} both rotamers), 3.79 – 3.72 (m, 1 H, 4-H minor rotamer), 3.69 (s, 3 H, OCH_3 minor rotamer), 3.52 (s, 3 H, OCH_3 major rotamer), 2.93 – 2.84 (m, 1 H + 1H, 3-H_{ax} both rotamers), 2.10-2.03 (m, 1 H, 5-H major rotamer), 2.01 – 1.79 (m, 2 H, 5-H minor rotamer + 5-H′ minor rotamer + 4 H, 6-H both rotamers + 7-H both rotamers), 1.73 – 1.65 (m, 1 H, 5-H′ major rotamer), 1.03 {s, 21 H, Si[C*H*$(CH_3)_2]_3$ + Si[CH(C*H*$_3)_2]_3$ major rotamer}, 0.99 {s, 21 H, Si[C*H*$(CH_3)_2]_3$ + Si[CH(C*H*$_3)_2]_3$ minor rotamer}, 0.89 – 0.84 (m, 1 H, 7-H′ minor rotamer), 0.81 (dd, *J* = 7.4, 4.7 Hz, 1 H, 7-H′ major rotamer).

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 172.9, 156.5, 136.7, 128.4, 127.8, 127.6, 67.1, 65.8, 52.1, 49.2, 37.4, 31.2, 24.1, 23.3, 17.9, 12.1.

MS (ESI) *m/z* (%): 945 (14) $[2M + Na]^+$, 462 (100) $[M + 1]^+$. MS/MS (ESI of $[M + 1]^+$) *m/z* (%): 429 (81), 418 (58), 295 (100), 287 (63).

$C_{25}H_{39}NO_5Si$ (461.67): calcd. C, 65.04; H, 8.51; N, 3.03. Found: C, 65.33; H, 8.19; N, 3.40

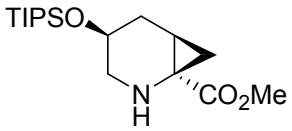
7: $[\alpha]_D^{22}$ +19.5 (*c* 1.74, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) (2.5:1 mixture of rotamers) δ (ppm): 7.37 – 7.23 (m, 5 H, Ph), 5.27 – 5.22 (m, 1 H + 1 H, C*H*$_2$Ph both rotamers), 5.05 – 5.00 (m, 1 H + 1 H, C*H*$_2$Ph both rotamers), 4.11 – 4.03 (m, 3 H, 4-H both rotamers + 3-H_{eq} major rotamer), 3.95 (dd, *J* = 13.2, 3.9 Hz, 1 H, 3-H_{eq} minor rotamer), 3.69 (s, 3 H, OCH_3 minor rotamer), 3.52 (s, 3 H, OCH_3 major rotamer), 2.92 (dd, *J* = 13.2, 1.6 Hz, 1 H, 3-H_{ax} minor rotamer), 2.88 – 2.78 (m, 1 H, 3-H_{ax} major rotamer), 2.04-1.96 (m, 2 H, 5-H both rotamers + 1 H, 5-H′ major rotamer), 1.92-1.88 (m, 2 H, 5-H′ minor rotamer + 7-H minor rotamer), 1.83 (dd, *J* = 9.9, 4.1 Hz, 1 H, 7-H major rotamer), 1.69 – 1.59 (m, 1 H + 1 H, 6-H both rotamers), 1.36 (dd, *J* = 8.0, 4.1 Hz, 1 H + 1H, 7-H′ both rotamers), 1.02 {s, 21 H, Si[C*H*$(CH_3)_2]_3$ + Si[CH(C*H*$_3)_2]_3$ major rotamer}, 0.96 {s, 21 H, Si[C*H*$(CH_3)_2]_3$ + Si[CH(C*H*$_3)_2]_3$ minor rotamer}.

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 173.4, 157.1, 137.1, 128.2, 127.7, 127.6, 67.0, 65.8, 52.2, 47.2, 38.3, 29.3, 23.7, 22.8, 18.0, 12.1.

MS (ESI) *m/z* (%): 945 (100) $[2M + Na]^+$, 462 (12) $[M + 1]^+$.

$C_{25}H_{39}NO_5Si$ (461.67): calcd. C, 65.04; H, 8.51; N, 3.03. Found: C, 65.24; H, 8.27; N, 3.37.



Methyl (1*R*,4*S*,6*R*)-4-Triisopropylsilanyloxy-2-azabicyclo[4.1.0]heptane-1-carboxylate [(+)-8]

Pd/C (66 mg, 10%) was added to a solution of **7** (260 mg, 0.56 mmol) in ethyl acetate (20 mL), under an N_2 atmosphere. The resulting suspension was stirred under a H_2 atmosphere (balloon) at room temperature for 18 h. After filtration over a celite layer and evaporation of the solvent, pure **8** (174 mg, 95%) was obtained as a colourless oil.

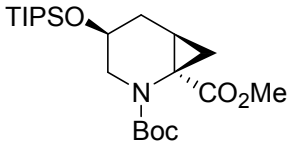
$[\alpha]_D^{21}$ +42.8 (*c* 1.12, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.82 (d, *J* = 3.5 Hz, 1 H, 4-H), 3.69 (s, 3 H, OCH_3), 2.75 (ddd, *J* = 13.6, 3.5, 1.1 Hz, 1 H, 3-H_{eq}), 2.54 (d, *J* = 13.6 Hz, 1 H, 3-H_{ax}), 2.08 – 1.98 (m, 2 H, 5-H + 5-H′), 1.92 (br s, 1 H, NH), 1.60 – 1.51 (m, 2 H, 6-H + 7-H), 1.34 – 1.28 (m, 1 H, 7-H′), 1.01 {s, 21 H, Si[C*H*$(CH_3)_2]_3$ + Si[CH(C*H*$_3)_2]_3$}.

^{13}C NMR (100.4 MHz, $CDCl_3$) δ (ppm): 176.0, 64.4, 52.3, 48.3, 38.2, 29.0, 26.6, 20.4, 18.0, 12.0.

MS (ESI) *m/z* (%): 677 (61) $[2M + Na]^+$, 328 (100) $[M + 1]^+$.

$C_{17}H_{33}NO_3Si$ (327.53): calcd. C, 62.34; H, 10.16; N, 4.28. Found: C, 62.30; H, 9.86; N, 4.07.



2-*tert*-Butyl 1-Methyl (1*R*,4*S*,6*R*)-4-Triisopropylsilanyloxy-2-azabicyclo[4.1.0]heptane-1,2-dicarboxylate [(+)-9]

Triethylamine (31 µL, 0.22 mmol) and Boc_2O (49 mg, 0.22 mmol) were added to a solution of **8** (36 mg, 0.11 mmol) in anhydrous methanol (4.5 mL), under an N_2 atmosphere, and the resulting reaction mixture was heated to reflux for 2 h. Afterward, water (7 mL) was added and the product was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic extracts were washed with a 5% solution of $KHSO_4$ (7 mL), a satd. solution of $NaHCO_3$ (7 mL), water (7 mL) and brine (7 mL) and then dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was purified by flash chromatography (*n*-hexane/EtOAc, 8:1; R_f 0.23), affording pure **9** (35 mg, 74%) as a colourless oil.

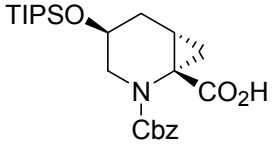
$[\alpha]_D^{23}$ +17.5 (*c* 1.15, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) (2.5:1 mixture of rotamers) δ (ppm): 4.10 – 4.06 (m, 1 H, 4-H major rotamer), 4.04 – 3.98 (m, 1 H, 3-H_{eq} major rotamer + 1H, 4-H minor rotamer), 3.87 – 3.80 (m, 1 H, 3-H_{eq} minor rotamer), 3.69 (s, 3 H, OCH_3 major rotamer), 3.68 (s, 3 H, OCH_3 minor rotamer), 2.89 (dd, *J* = 13.3, 1.8 Hz, 1 H, 3-H_{ax} minor rotamer), 2.74 (dd, *J* = 13.0, 1.5 Hz, 1 H, 3-H_{ax} major rotamer), 2.01-1.90 (m, 2 H + 2 H, 5-H + 5-H′ both rotamers), 1.87 (dd, *J* = 9.9, 4.4 Hz, 1 H, 7-H minor rotamer), 1.77 (dd, *J* = 10.0, 4.2 Hz, 1 H, 7-H major rotamer), 1.63 – 1.55 (m, 1 H + 1H, 6-H both rotamers), 1.46 [s, 9 H, $OC(CH_3)_3$ minor rotamer], 1.41 [s, 9 H, $OC(CH_3)_3$ major rotamer], 1.29 (dd, *J* = 7.9, 4.2 Hz, 1 H + 1 H, 7-H′ both rotamers), 1.03 {s, 21 H + 21 H, $Si[CH(CH_3)_2]_3$ + $Si[CH(CH_3)_2]_3$ both rotamers}.

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 173.7, 156.0, 79.5, 66.0, 52.0, 46.1, 38.4, 29.2, 28.3, 23.4, 22.9, 18.0, 12.0.

MS (ESI) *m/z* (%):877 (74) $[2M + Na]^+$, 450 (36) $[M + Na]^+$, 328 (98). MS/MS (ESI of $[M + Na]^+$) *m/z* (%): 395 (27), 351 (100).

$C_{22}H_{41}NO_5Si$ (427.65): calcd. C, 61.79; H, 9.66; N, 3.28. Found: C, 61.65; H, 9.38; N, 3.02.



(1*S*,4*S*,6*S*)-4-Triisopropylsilanyloxy-2-benzyl-2-azabicyclo[4.1.0]heptane-1-carboxylic Acid [(+)-10]

NaOH (330 µL of a 1 N solution in water, 0.33 mmol) was added to a solution of **6** (101 mg, 0.22

mmol) in methanol (1.5 mL) and the resulting mixture was vigorously stirred for 24 h at 50 °C. Afterward, the methanol was evaporated, the remaining aqueous layer was acidified to pH 3 by adding a 1 N solution of HCl. The resulting suspension was extracted with CH_2Cl_2 (3 x 8 mL) and the combined organic layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, compound **10** (87 mg, 88%) was obtained as a thick pale yellow oil.

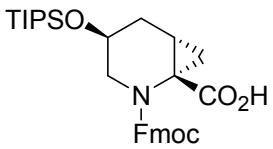
$[\alpha]_D^{25}$ +10.9 (*c* 0.65, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz) (1.7:1 mixture of rotamers) δ (ppm): 9.02 (br s, 1 H, COOH), 7.34 – 7.29 (m, 5 H + 5 H, Ph both rotamers), 5.22 – 5.03 (m, 2 H + 2 H, C*H*$_2$Ph both rotamers), 3.98 – 3.89 (m, 1 H + 1 H, 3-H_{eq} both rotamers), 3.87 – 3.82 (m, 1 H, 4-H major rotamer), 3.69 – 3.60 (m, 1 H, 4-H minor rotamer), 2.83 (dd, *J* = 12.6, 6.8 Hz, 1 H, 3-H_{ax} major rotamer), 2.79 – 2.72 (m, 1 H, 3-H_{ax}, minor rotamer), 2.07 – 1.97 (m, 1 H + 1 H, 5-H both rotamers), 1.94 – 1.87 (m, 2 H + 2 H, 6-H + 7-H, both rotamers), 1.84 – 1.78 (m, 1 H + 1 H, 5-H′ both rotamers), 1.04 {s, 21 H, Si[C*H*$(CH_3)_2]_3$ + Si[CH(C*H*$_3)_2]_3$ major rotamer}, 0.98 {s, 21 H, Si[C*H*$(CH_3)_2]_3$ + Si[CH(C*H*$_3)_2]_3$ minor rotamer}, 0.85 – 0.78 (m, 1 H + 1 H, 7-H′ both rotamers).

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 178.0, 156.4, 136.7, 128.4, 128.0, 127.4, 67.2, 65.8, 49.0, 37.6, 31.3, 24.9, 24.4, 17.9, 12.1.

MS (ESI) *m/z* (%): 933 (23) $[2M + K]^+$, 448 (100) $[M + 1]^+$.

$C_{24}H_{37}NO_5Si$ (447.64): calcd. C, 64.39; H, 8.33; N, 3.13. Found: C, 64.65; H, 8.05; N, 2.87.



(1*S*,4*S*,6*S*)-4-Triisopropylsilanyloxy-2-(9-Fluorenylmethoxycarbonyl)-2-azabicyclo[4.1.0]heptane-1-carboxylic Acid [(+)-12]

Pd/C (21 mg, 10%) was added to a solution of **10** (85 mg, 0.19 mmol) in ethyl acetate (6 mL), under an N_2 atmosphere. The resulting suspension was stirred under a H_2 atmosphere (balloon) at room temperature for 18 h. Afterward, methanol (12 mL) was added and the mixture was filtered over a celite layer to remove the catalyst. Subsequent evaporation of the solvent led to **11** (57 mg, 95%) as a pale yellow solid, which was employed for the next step without further purification.

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 4.02 – 3.96 (m, 1 H, 4-H), 2.96-2.92 (m, 1 H, 3-H), 2.91-2.86 (m, 1 H, 3-H′), 2.13-2.06 (m, 1 H, 5-H), 2.05 – 1.99 (m, 1 H, 5-H′), 1.84 – 1.74 (m, 1 H, 6-H), 1.56 (dd, *J* = 10.0, 5.8 Hz, 1 H, 7-H), 1.10 {s, 21 H, Si[C*H*$(CH_3)_2]_3$ + Si[CH(C*H*$_3)_2]_3$}, 0.93 (dd, *J* = 7.5, 5.8 Hz, 1 H, 7-H′).

MS (ESI) *m/z* (%): 649 (100) $[2M + Na]^+$, 336 (43) $[M + Na]^+$, 314 (17) $[M + 1]^+$. MS/MS (ESI of $[M + 1]^+$) *m/z* (%): 140 (100).

Amino acid **11** was suspended in THF (400 µL) and a 10% solution of Na_2CO_3 in water (500 µL) was added to this suspension. The resulting mixture was cooled to 0 °C and a solution of Fmoc-OSu (60 mg, 0.18 mmol) in THF (1.2 mL) was added. This reaction mixture was vigorously stirred at room temperature for 24 h. Afterward, the solvent was evaporated under *vacuum*, the resulting residue was taken up in EtOAc (3 mL) and a satd. solution of NH_4Cl (3 mL) was added. The mixture was extracted with EtOAc (4 x 3 mL) and the combined organic layers were dried over Na_2SO_4. After filtration and evaporation of the solvent the crude was purified by flash chromatography (*n*-hexane/EtOAc, 4:1 + 0.5% CH_3COOH; R_f 0.16) to afford compound **12** (78 mg, 81%) as a white solid.

M.p. 58.1-60.3 °C.

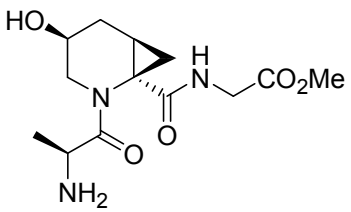
$[\alpha]_D^{24}$ +1.2 (*c* 0.82, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 400 MHz) (2:1 mixture of rotamers) δ (ppm): 9.11 (br s, 1 H, COOH), 7.73-7.64 (m, 2 H, Fmoc), 7.58-7.50 (m, 2 H, Fmoc), 7.37 – 7.28 (m, 2 H, Fmoc), 7.27-7.20 (m, 2 H, Fmoc), 4.51 – 4.28 (m, 2 H, CH_2 Fmoc), 4.20 – 4.08 (m, 1 H, CH Fmoc), 3.90 (dd, *J* = 12.7, 4.2 Hz, 1 H, 3-H_{eq} major rotamer), 3.87 – 3.81 (m, 1 H, 3-H_{eq} minor rotamer), 3.71 – 3.60 (m, 1 H + 1 H, 4-H both rotamers), 2.78 – 2.62 (m, 1 H + 1 H, 3-H_{ax} both rotamers), 1.96 – 1.72 (m, 3 H + 3 H, 5-H + 5-H′ + 6-H both rotamers), 1.67 – 1.56 (m, 1 H + 1 H, 7-H both rotamers), 1.01 {s, 21 H + 21 H, $Si[CH(CH_3)_2]_3$ + $Si[CH(CH_3)_2]_3$ both rotamers}, 0.63 – 0.51 (m, 1 H + 1 H, 7-H′ both rotamers).

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 177.7, 156.4, 143.8, 141.2, 127.5, 126.9, 125.0, 119.8, 67.4, 65.8, 48.7, 47.3, 37.8, 31.4, 29.7, 25.0, 17.9, 12.1.

MS (ESI) *m/z* (%): 1093 (100) $[2M + Na]^+$, 558 (9) $[M + Na]^+$.

$C_{31}H_{41}NO_5Si$ (535.75): calcd. C, 69.50; H, 7.71; N, 2.61. Found: C, 69.44; H, 7.95; N, 2.43.



[2-(Ala)-4-hydroxyCPA-Gly-OMe]·HCl [(+)-16]

Pd/C (15 mg, 10%) was added to a solution of **14**[2] (19 mg, 0.04 mmol) in EtOH (3 mL), under an N_2 atmosphere. The resulting suspension was stirred under a H_2 atmosphere (balloon) at room temperature for 18 h. After filtration over a celite layer and evaporation of the solvent, pure **15** (15 mg, 100%) was obtained as a colourless solid.

^{1}H NMR (400 MHz, CD_3OD) (3:1 mixture of rotamers) δ (ppm): 4.74 (dd, *J* = 6.4 Hz, 1 H, Ala H_α minor rotamer), 4.45 (q, *J* = 6.8 Hz, 1 H, Ala H_α major rotamer), 4.20 (dd, *J* = 13.2, 5.2 Hz, 1 H, 3-H_{eq} minor rotamer), 4.13 (d, *J* = 17.6 Hz, 1 H, Gly H_α major rotamer), 4.07 – 3.97 (m, 1 H + 1H, 4-H both rotamers), 3.86 (dd, *J* = 13.6, 3.6 Hz, 1 H, 3-H_{eq} major rotamer), 3.59 (d, *J* = 17.6 Hz, 1 H, Gly H'_α major rotamer), 3.37 (dd, *J* = 13.6, 1.6 Hz, 1 H, 3-H_{ax} major rotamer), 3.04 (dd, *J* = 13.2, 3.2 Hz, 1 H, 3-H_{ax} minor rotamer), 2.31 – 2.12 (m, 1 H + 1 H, 5-H both rotamers), 2.06 (dd, *J* = 9.2, 4.4 Hz, 1 H, 7-H minor rotamer), 1.89 – 1.82 (m, 1 H, 5-H′ major rotamer), 1.78 (dd, *J* = 10.0, 4.4 Hz, 1 H, 7-H major rotamer), 1.74 – 1.60 (m, 1 H, 6-H major rotamer + 1 H, 5-H′ minor rotamer), 1.60 – 1.53 (m, 1 H, 6-H minor rotamer), 1.43 [s, 9 H, $OC(CH_3)_3$, major rotamer], 1.42 [s, 9 H, $OC(CH_3)_3$, minor rotamer], 1.29 (d, *J* = 6.4 Hz, 3 H, Ala C*H*$_3$ minor rotamer), 1.28 (d, *J* = 6.8 Hz, 3 H, Ala C*H*$_3$ major rotamer and 1 H, 7-H′ minor rotamer), 1.06 (dd, *J* = 7.6, 4.4 Hz, 1 H, 7-H′ major rotamer).

15 (15 mg, 0.04 mmol) was dissolved in 1.25 M solution of HCl in CH_3OH. The resulting mixture was vigorously stirred for 3 h. Afterward, the methanol was evaporated and pure **16** (13 mg, 100%) was obtained as a HCl salt.

$[\alpha]_D^{18}$ +21.3 (*c* 0.98, CH_3OH).

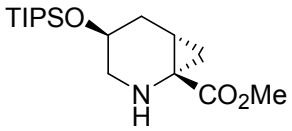
^{1}H NMR (400 MHz, D_2O) (1.3:1 mixture of rotamers) δ (ppm): 4.64 – 4.58 (m, 1 H, Ala H_α minor rotamer), 4.57 – 4.45 (m, 1 H, Ala H_α major rotamer), 4.24 (br d, *J* = 13.1 Hz, 1 H, 3-H_{eq} minor rotamer), 4.17 (br. s, 1H + 1 H, 4-H both rotamers), 4.07 – 3.86 (m, 2 H + 2 H, Gly H_α both rotamers), 3.72 (s, 3 H + 3 H, CO_2Me both rotamers), 3.67 (br d, *J* = 13.6 Hz, 1 H, 3-H_{eq} major rotamer), 3.43 (br d, *J* = 13.6 Hz, 1 H, 3-H_{ax} major rotamer), 3.02 (br d, *J* = 13.1 Hz, 1 H, 3-H_{ax} minor rotamer), 2.37 – 2.22 (m, 1 H + 1 H, 5-H both rotamers), 2.15 (dd, *J* = 10.0, 4.8 Hz, 1 H, 7-H minor rotamer), 1.98 – 1.83 (m, 2 H, 5-H′ + 7-H major rotamer), 1.83 – 1.67 (m, 1 H + 2 H, 5-H′ minor rotamer + 6-H both rotamers), 1.58–1.46 (m, 3 H + 3 H, Ala C*H*$_3$ both rotamers), 1.41 – 1.35 (m, 1 H, 7-H′ minor rotamer), 1.06 – 0.99 (m, 1 H, 7-H′ major rotamer).

^{1}H NMR (500 MHz, H_2O + D_2O 9:1, *water suppression*) (1.5:1 mixture of rotamers) *δ*(ppm): 8.72 (br t, 1 H, Gly NH minor rotamer), 8.30 (br t, 1 H, Gly NH major rotamer) 4.64 – 4.58 (m, 1 H, Ala H_α minor rotamer), 4.57 – 4.45 (m, 1 H, Ala H_α major rotamer), 4.24 (br d, *J* = 13.1 Hz, 1 H, 3-H_{eq} minor rotamer), 4.17 (br. s, 1H + 1 H, 4-H both rotamers), 4.07 – 3.86 (m, 2 H + 2 H, Gly H_α both rotamers), 3.72 (s, 3 H + 3 H, CO_2Me both rotamers), 3.67 (br d, *J* = 13.6 Hz, 1 H, 3-H_{eq} major rotamer), 3.43 (br d, *J* = 13.6 Hz, 1 H, 3-H_{ax} major rotamer), 3.02 (br d, *J* = 13.1 Hz, 1 H, 3-H_{ax} minor rotamer), 2.37 – 2.22 (m, 1 H + 1 H, 5-H both rotamers), 2.15 (dd, *J* = 10.0, 4.8 Hz, 1 H, 7-H minor rotamer), 1.98 – 1.83 (m, 2 H, 5-H′ + 7-H major rotamer), 1.83 – 1.67 (m, 1 H + 2 H, 5-H′

minor rotamer + 6-H both rotamers), 1.56 – 1.48 (m, 3 H + 3 H, Ala C*H*$_3$ both rotamers), 1.41 – 1.35 (m, 1 H, 7-H′ minor rotamer), 1.06 – 0.99 (m, 1 H, 7-H′ major rotamer).

^{13}C NMR (100.4 MHz, D_2O) δ (ppm): 173.3, 172.2, 171.7, 63.7, 52.7, 48.4, 47.3, 41.4, 39.9, 27.1, 24.0, 21.5, 15.6.

MS (ESI) m/z (%): 600 (16), 599 (46) $[2M+1]^+$, 322 (7) $[M+Na]^+$, 301 (17), 300 (100) $[M+1]^+$.



1-Methyl (1*S*,4*S*,6*S*)-4-Triisopropylsilanyloxy-2-azabicyclo[4.1.0]heptane-1-carboxylate [(-)-17]

Pd/C (15 mg, 10%) was added to a solution of **6** (66 mg, 0.14 mmol) in ethyl acetate (6 mL), under an N_2 atmosphere. The resulting suspension was stirred under a H_2 atmosphere (balloon) at room temperature for 18 h. After filtration over a Celite layer and evaporation of the solvent, pure **17** (45 mg, 96%) was obtained as a yellow oil.

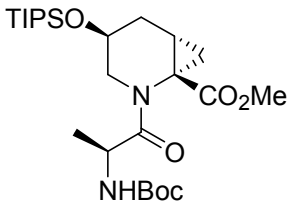
$[\alpha]_D^{20}$ -17.6 (*c* 0.5, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.70 (s, 3 H, OCH_3), 3.66 – 3.56 (m, 1 H, 4-H); 2.80 (dd, *J* = 12.1, 2.7 Hz, 1 H, 3-H_{eq}), 2.46 (dd, *J* = 12.1, 9.0 Hz, 1 H, 3-H_{ax}), 2.21 – 2.10 (m, 2 H, 5-H + 6-H), 1.88 – 1.73 (m, 2 H, 5-H′ + N-H), 1.54 (dd, *J* = 10.1, 4.5 Hz, 1 H, 7-H), 1.02 {s, 21 H, Si[C*H*$(CH_3)_2$]$_3$ + Si[CH(C*H*$_3$)$_2$]$_3$}, 0.74 (dd, *J* = 7.2, 4.4 Hz, 1 H, 7-H′).

^{13}C NMR (100.4 MHz, $CDCl_3$) δ (ppm): 175.5, 66.8, 52.5, 49.9, 39.1, 31.8, 24.9, 22.7, 18.1, 12.4.

MS (ESI) m/z (%): 328 (100) $[M+1]^+$.

$C_{17}H_{33}NO_3Si$ (327.53): calcd. C, 62.34; H, 10.16; N, 4.28. Found: C, 62.31; H, 10.21; N, 4.07.



2-(Boc-Ala)-4-triisopropylsilanyloxyCPA-OMe [(-)-18]

DEPBT (90 mg, 0.30 mmol) and DIPEA (52 µL, 0.30 mmol) were added under an N_2 atmosphere to a solution of Boc-Ala-OH (34 mg, 0.18 mmol) in anhydrous THF (2 mL), cooled to 0°C, and the resulting mixture was allowed to warm to room temperature. After 15 min this solution was slowly added to a solution of compound **17** (45 mg, 0.14 mmol) in anhydrous THF (1 mL) precooled to 0°C. The resulting reaction mixture was stirred at 35°C for 3 days.

Afterward, EtOAc (5 mL) was added and the mixture was washed with a saturated solution of NH_4Cl (2 x 4 mL), a saturated solution of $NaHCO_3$ (2 x 4 mL), and H_2O (2 x 4 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under *vacuum*. The residue was purified by flash chromatography (*n*-hexane/ EtOAc, 5:1; R_f=0.23) affording pure **18** (55 mg, 81%) as a colourless oil.

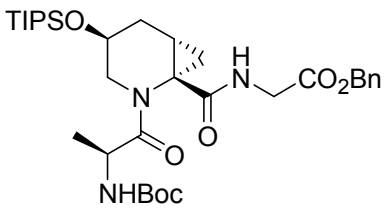
$[\alpha]_D^{23}$ +7.2 (*c* 1.02, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) (1.6:1 mixture of rotamers) δ (ppm): 5.46 (d, *J* = 7.9 Hz, 1 H, Ala NH minor rotamer), 5.23 (d, *J* = 9.2 Hz, 1 H, Ala NH major rotamer), 4.75 (dq, *J* = 9.3, 6.7 Hz, 1 H, Ala H_α, major rotamer), 4.60 – 4.44 (m, 1 H, Ala H_α, minor rotamer), 4.12 – 4.05 (m, 1 H, 4-H major rotamer), 3.99 – 3.94 (m, 1 H, 4-H, minor rotamer), 3.91 (dd, *J* = 13.5, 5.2 Hz, 1 H, 3-H_{ax}, major rotamer), 3.71 – 3.61 (m, 4 H, 3-H_{eq} minor rotamer + OC*H*$_3$ minor rotamer + 3 H, OC*H*$_3$ major rotamer), 3.13 (dd, *J* = 12.2, 6.0 Hz, 1 H, 3-H_{ax} minor rotamer), 3.01 (dd, *J* = 13.5, 2.9 Hz, 1 H, 3-H_{ax}, major rotamer), 2.33 – 2.23 (m, 1 H, 5-H major rotamer), 2.14 – 2.04 (m, 1 H, 5-H minor rotamer + 1 H, 7-H major rotamer), 2.03 – 1.93 (m, 1 H, 6-H major rotamer + 1 H, 7-H minor rotamer), 1.87 – 1.77 (m, 1 H, 6-H minor rotamer), 1.75 – 1.66 (m, 1 H, 5-H′ minor rotamer), 1.44 [s, 9 H, OC(C*H*$_3$)$_3$ minor rotamer], 1.40 [s, 9 H, OC(C*H*$_3$)$_3$ major rotamer], 1.37 (d, *J* = 6.9 Hz, 3 H, Ala C*H*$_3$ minor rotamer), 1.36 – 1.29 (m, 1 H, 5-H′ major rotamer), 1.23 (d, *J* = 6.7 Hz, 3 H, Ala C*H*$_3$ major rotamer) 1.04 {s, 21 H, Si[C*H*(CH$_3$)$_2$]$_3$+Si[CH(C*H*$_3$)$_2$]$_3$ minor rotamer}, 1.01 ppm {s, 21 H, Si[C*H*(CH$_3$)$_2$]$_3$+Si[CH(C*H*$_3$)$_2$]$_3$ major rotamer}, 0.93 (dd, J = 7.3, 4.7 Hz, 1 H, 7-H′ major rotamer), 0.77 (dd, *J* = 7.7, 5.4 Hz, 1 H, 7-H′ minor rotamer).

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 176.4, 172.3, 154.7, 79.4, 65.3, 53.1, 49.1, 46.5, 37.8, 31.3, 28.6, 23.9, 20.3, 18.2, 12.3.

MS (ESI) m/z (%): 537 (7) $[M+K]^+$, 521 (30) $[M+Na]^+$, 499 (15) $[M+1]^+$.

$C_{25}H_{46}N_2O_6Si$ (498.73): calcd. C, 60.21; H, 9.30; N, 5.62. Found: C, 60.34; H, 9.11; N, 5.24.



2-(Boc-Ala)-4-triisopropylsilanyloxyCPA-Gly-OBn (20)

A 1N solution of NaOH (165 µL) was added to a solution of **18** (55 mg, 0.110 mmol) in methanol (400 µL), and the resulting mixture was vigorously stirred for 24 h at 40°C. Afterward, the methanol was evaporated and the remaining aqueous layer was diluted with H_2O (6 mL). The resulting solution was acidified to pH 6 by adding dropwise a 1N solution of HCl, and the product

was extracted with CH_2Cl_2 (3 x 6 mL). Then the aqueous layer was further acidified to pH 3, and the product was extracted again with CH_2Cl_2 (3 x 6 mL). The combined organic layers were dried over Na_2SO_4 and, after filtration and evaporation of the solvent, compound **19** (47 mg, 88%) was obtained as a thick colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$) (1:1 mixture of rotamers) δ (ppm): 8.84 (br s, 1 H, COO*H*), 5.54 – 5.39 (m, 1 H + 1 H, Ala NH both rotamers), 4.83 – 4.64 (m, 1 H, Ala H_α cis rotamer), 4.64 – 4.43 (m, 1 H, Ala H_α trans rotamer), 4.07 (dd, *J* = 26.0, 9.6 Hz, 1 H, 3-H_{eq} cis rotamer), 4.01 – 3.83 (m, 1 H + 1 H, 4-H both rotamers), 3.70 (dd, *J* = 24.8, 8.4 Hz, 1 H, 3-H_{eq} trans rotamer), 3.06 (dd, *J* = 24.8, 14.0 Hz, 1 H, 3-H_{ax} trans rotamer), 2.86 (dd, *J* = 26.0, 10.0 Hz, 1 H, 3-H_{ax} cis rotamer), 2.28 – 1.76 (m, 4 H + 4 H, 5-H + 5-H′ + 6-H + 7-H both rotamers), 1.41 [s, 9 H, OC(C*H*$_3$)$_3$, 1.39 (s, 9 H, OC(C*H*$_3$)$_3$], 1.35 (d, *J* = 14 Hz, 3 H, Ala C*H*$_3$ trans rotamer), 1.26 (d, *J* = 13.2 Hz, 3 H, Ala C*H*$_3$ cis rotamer), 1.05 {s, 21 H, Si[C*H*(CH$_3$)$_2$]$_3$+Si[CH(C*H*$_3$)$_2$]$_3$}, 1.03 {s, 21 H, Si[C*H*(CH$_3$]$_2$)$_3$+Si[CH(C*H*$_3$)$_2$]$_3$}, 0.97 – 0.87 (m, 1 H, 7-H′ cis rotamer), 0.85 – 0.76 (m, 1 H, 7-H′ trans rotamer).

DEPBT (58 mg, 0.194 mmol) and DIPEA (68 µL, 0.388 mmol) were added under an N_2 atmosphere to a solution of intermediate **19** (47 mg, 0.097 mmol) in anhydrous THF (1 mL) cooled to 0°C, and the resulting mixture was allowed to warm to room temperature. After 15 min the reaction was cooled again to 0°C, and H-Gly-OBn·HCl (29 mg, 0.145 mmol) was added. The resulting reaction mixture was stirred at 35°C for 4 days. Afterward, EtOAc (5 mL) was added, and the mixture washed with a saturated solution of NH_4Cl (2 x 3 mL), a saturated solution of $NaHCO_3$ (2 x 3 mL), and H_2O (2 x 3 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by flash chromatography (*n*-hexane/ EtOAc, 2:1; R_f=0.35) to afford pure **20** (52 mg, 85%) as a colourless gummy solid.

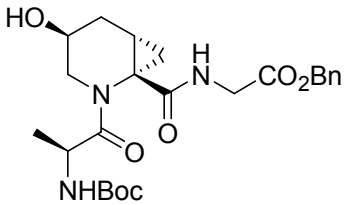
^{1}H NMR (400 MHz, $CDCl_3$) (11.5:1 mixture of rotamers) δ (ppm): 8.27 (t, *J* = 5.6 Hz, 1 H, Gly NH major rotamer), 7.44 – 7.28 (m, 5 H + 5 H, Ph both rotamers), 6.50 – 6.44 (m, 1 H, Gly NH minor rotamer), 5.35 (d, *J* = 7.2 Hz, 1 H, Ala NH minor rotamer), 5.24 – 5.01 (m, 2 H + 2 H, C*H*$_2$Ph both rotamers), 4.79 (d, *J* = 5.6 Hz, 1 H, Ala NH major rotamer), 4.58 – 4.49 (m, 1 H, Ala H_α minor rotamer), 4.49 – 4.41 (m, 1 H, Ala H_α major rotamer), 4.34 (dd, *J* = 12.8, 4.0 Hz, 1 H, 3-H_{eq} major rotamer), 4.15 (dd, *J* = 17.6, 5.6 Hz, 1 H, Gly H_α major rotamer), 4.06 – 4.03 (m, 2 H, Gly H_α minor rotamer), 3.92 (dd, *J* = 17.6, 5.6 Hz, 1 H, Gly H'_α major rotamer and 1 H, 3-H_{eq} minor rotamer), 3.72 – 3.64 (m, 1 H + 1 H, 4-H both rotamers), 3.05 (dd, *J* = 12.8, 8.4 Hz, 1 H, 3-H_{ax} minor rotamer), 2.61 (dd, *J* = 12.8, 8.0 Hz, 1 H, 3-H_{ax} major rotamer), 2.06 – 1.86 (m, 4 H + 4 H, 5-H + 5-H′ + 6-H + 7-H both rotamers), 1.44 (d, *J* = 6.4 Hz, 3 H, Ala C*H*$_3$ minor rotamer), 1.35 [s, 9 H + 9 H, OC(C*H*$_3$)$_3$ both rotamers], 1.17 (d, *J* = 6.8 Hz, 3 H, Ala C*H*$_3$ major rotamer), 1.03 {br s, 21 H +

21 H, Si[C*H*$(CH_3)_2]_3$+Si[CH(C*H*$_3)_2]_3$ both rotamers}, 0.76 (m, 1 H, 7-H′ major rotamer), 0.69 (dd, *J* = 6.9, 4.6 Hz, 1 H, 7-H′ minor rotamer);

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 175.3, 171.3, 169.5, 155.4, 135.3, 128.5, 128.3, 128.2, 110.0, 80.0, 67.0, 65.6, 48.0, 47.4, 41.9, 39.9, 31.4, 28.1, 24.1, 23.7, 17.9, 12.0.

MS (ESI) m/z (%): 1286 (92), 1285 (100) $[2M+Na]^+$, 670 (3) $[M+K]^+$, 655 (26), 654 (67) $[M+Na]^+$, 632 (7) $[M+1]^+$.

$C_{33}H_{53}N_3O_7Si$ (631.88): calcd. C, 62.73; H, 8.45; N, 6.65. Found: C, 62.99; H, 8.09; N, 6.21.



2-(Boc-Ala)-4-hydroxyCPA-Gly-OBn [(-)-21]

TBAF (31 µL of a 1 M solution in THF, 0.031 mmol) was slowly added to a solution of **20** (17 mg, 0.027 mmol) in anhydrous THF (1 mL), under an N_2 atmosphere. After 2 h the solvent was evaporated under vacuum and the residue was taken up in Et_2O (1 mL), then the solvent was evaporated again. After purification by flash chromatography (EtOAc; R_f 0.26), pure **21** (8.3 mg, 64%) was obtained as a colourless gummy solid.

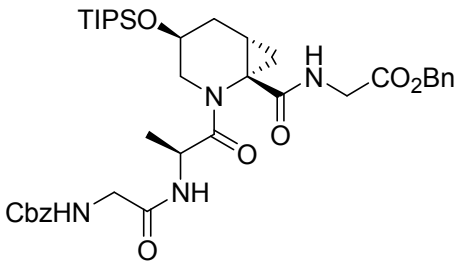
$[\alpha]_D^{21}$ -25.0 (*c* 0.25, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) (4:1 mixture of rotamers) δ (ppm): 8.37 – 8.23 (m, 1 H, Gly NH major rotamer), 7.42 – 7.28 (m, 5 H + 5 H, Ph both rotamers), 6.65 – 6.55 (m, 1 H, Gly NH minor rotamer), 5.32 (d, *J* = 7.2 Hz, 1 H, Ala NH minor rotamer), 5.20 – 5.04 (m, 2 H + 2 H, C*H*$_2$Ph both rotamers), 4.81 (d, *J* = 4.7 Hz, 1 H, Ala NH major rotamer), 4.65 – 4.55 (m, 1 H, Ala H_α minor rotamer), 4.50 – 4.42 (m, 1 H, Ala H_α major rotamer), 4.39 (dd, *J* = 12.8, 4.0 Hz, 1 H, 3-H_{eq} major rotamer), 4.12 (dd, *J* = 17.7, 6.0 Hz, 1 H, Gly H_α major rotamer), 4.06 (dd, *J* = 9.2, 4.8 Hz, 2 H, Gly H_α minor rotamer), 3.99 (dd, *J* = 17.7, 5.6 Hz, 1 H, Gly H'_α major rotamer), 3.86 – 3.79 (m, 1 H, 3-H_{eq} minor rotamer), 3.74 – 3.60 (m, 1 H + 1 H, 4-H both rotamers), 2.96 (dd, *J* = 12.8, 9.2 Hz, 1 H, 3-H_{ax} minor rotamer), 2.59 (dd, *J* = 12.8, 8.8 Hz, 1 H, 3-H_{ax} major rotamer), 2.22 – 1.74 (m, 4 H + 4 H, 5-H + 5-H′ + 6-H + 7-H both rotamers), 1.41 (s, 12 H, OC(C*H*$_3)_3$ + Ala C*H*$_3$ minor rotamer), 1.36 (s, 9 H, , OC(C*H*$_3)_3$ major rotamer), 1.18 (d, *J* = 6.8 Hz, 3 H, Ala C*H*$_3$ major rotamer), 0.82 – 0.78 (m, 1 H, 7-H′ major rotamer), 0.75 – 0.70 (m, 1 H, 7-H′ minor rotamer).

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 175.6, 171.3, 169.7, 155.5, 135.3, 128.6, 128.4, 128.3, 80.1, 67.0, 65.0, 47.5, 42.0, 40.2, 29.9, 28.3, 28.2, 23.9, 23.7, 17.4.

MS (ESI) m/z (%): 973 (15) $[2M+Na]^+$, 499 (27), 498 (100) $[M+Na]^+$.

$C_{24}H_{33}N_3O_7$ (475.53): calcd. C, 60.62; H, 6.99; N, 8.84. Found: C, 60.37; H, 7.17; N, 8.45.



2-(Z-Gly-Ala)-4-triisopropylsilanyloxyCPA-Gly-OBn [(-)-23]

$Sn(OTf)_2$ (33 mg, 0.30 mmol) was added under an N_2 atmosphere to a solution of **20** (42 mg, 0.066 mmol) in anhydrous CH_2Cl_2 (1 mL), cooled to 0°C, and the resulting mixture was stirred at room temperature for 22 h; meanwhile additional $Sn(OTf)_2$ (22 mg, 0.053 mmol) was added. Afterward, the mixture was neutralized with a saturated solution of $NaHCO_3$ and further basificated to pH 9 by adding solid Na_2CO_3. The product was extracted with AcOEt (4 x 10 mL). After filtration the organic layer was evaporated under vacuum and the residue was taken up in $CHCl_3$ (20 mL) and washed with H_2O (10 mL). The organic layer were dried over Na_2SO_4 and, after filtration and evaporation of the solvent, compound **22** (35 mg, 100%) was obtained as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) (7.3:1 mixture of rotamers) δ (ppm): 7.41 – 7.29 (m, 5H + 5H, Ph both rotamers), 7.03 (br dd, J = 7.3, 4.1 Hz, 1 H, Gly NH major rotamer), 6.46 – 6.41 (m, 1 H, Gly NH minor rotamer), 5.24 – 5.09 (m, 4 H + 4 H, CH_2Ph both rotamers), 4.42 (dd, J = 18.1, 7.6 Hz, 1 H, Gly H_α major rotamer), 4.17 (dd, J = 12.9, 4.1 Hz, 1 H, 3-H_{eq} major rotamer), 4.07 – 4.03 (m, 1 H, Gly H_α minor rotamer), 3.90 (q, J = 6.5 Hz, 1 H, Ala H_α major rotamer), 3.81 (m, 1 H, 4-H major rotamer), 3.76 – 3.68 (m, 1H, Gly H'_α major rotamer + 2 H, 4-H + Ala H_α minor rotamer), 3.68 – 3.60 (m, 1 H, 3-H_{eq} minor rotamer), 3.03 – 2.91 (m, 1 H, 3-H_{ax} minor rotamer), 2.74 (dd, J = 12.9, 6.7 Hz, 1 H, 3-H_{ax} major rotamer), 2.14 – 1.83 (m, 3 H, 5-H + 7-H + 6-H major rotamer + 2 H, 5-H minor rotamer), 1.83 – 1.74 (m, 1H, 5-H′ major rotamer + 1 H, 6-H minor rotamer), 1.70 – 1.61 (m, 1 H, 7-H minor rotamer), 1.41 (d, J = 6.8 Hz, 3 H, Ala CH_3 minor rotamer), 1.19 (d, J = 6.5 Hz, 3 H, Ala CH_3 major rotamer), 1.03 (br s, 21H + 21H, $Si[CH(CH_3)_2]_3$ + $Si[CH(CH_3)_2]_3$ both rotamers), 0.75 (dd, J = 7.6, 4.6 Hz, 1 H, 7-H′ major rotamer), 0.67 – 0.62 (m, 1 H, 7-H′ minor rotamer).

DEPBT (43 mg, 0.145 mmol) and DIPEA (25 µL, 0.145 mmol) were added under an N_2 atmosphere to a solution of Z-Gly-OH (18 mg, 0.086 mmol) in anhydrous THF (1 mL) cooled to 0°C, and the resulting mixture was allowed to warm to room temperature. After 15 min the reaction was cooled again to 0°C, and a solution of intermediate **22** (35 mg, 0.066 mmol) in anhydrous THF (500 µL) was added. The resulting reaction mixture was stirred at 35°C for 4 days. Afterward, EtOAc (5 mL) was added, and the mixture washed with a saturated solution of NH_4Cl (2 x 3 mL), a

saturated solution of $NaHCO_3$ (2 x 3 mL), and H_2O (2 x 3 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by flash chromatography (CH_2Cl_2/ CH_3OH, 45:1; R_f = 0.14) to afford pure **23** (32 mg, 67%) as a colourless gummy solid.

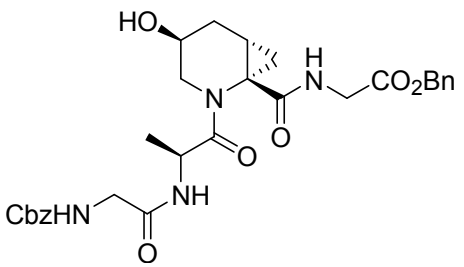
$[\alpha]_D^{19}$ -30.7 (*c* 0.5, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) (6.1:1 mixture of rotamers) δ (ppm): 7.91 (t, *J* = 5.2 Hz, 1 H, Gly4 NH, major rotamer), 7.41 – 7.26 (m, 5H + 5H, 2 x Ph both rotamers), 6.99 (d, *J* = 7.2 Hz, 1 H, NH Ala, minor rotamer), 6.41 (d, *J* = 5.2 Hz, 1 H, Ala NH, major rotamer and 1 H, Gly4 NH minor rotamer), 5.47 – 5.41 (m, 1 H, Gly1 NH minor rotamer), 5.38 – 5.29 (m, 1 H, Gly1 NH major rotamer), 5.20 – 4.98 (m, 4 H + 4 H, 2 x C*H*$_2$Ph both rotamers), 4.85 – 4.76 (m, 1 H, Ala H_α minor rotamer), 4.76 – 4.68 (m, 1 H, Ala H_α major rotamer), 4.26 (dd, *J* = 12.8, 4.0 Hz, 1 H, 3-H_{eq} major rotamer), 4.21 (dd, *J* = 18.0, 6.6 Hz, 1 H, Gly4 H_α major rotamer), 4.11 – 3.99 (m, 2 H, Gly4 H_α, minor rotamer), 3.95 – 3.88 (m, 2 H,2 x Gly1 H_α minor rotamer), 3.87 – 3.77 (m, 3 H, 3-H_{eq} minor rotamer + Gly1 H_α major rotamer + Gly4 H'_α major rotamer), 3.76 – 3.70 (m, 2 H, 4-H both rotamers), 3.67 (dd, *J* = 17.0, 5.5 Hz, 1 H, Gly1 H'_α major rotamer), 3.14 – 2.99 (m, 1 H, 3-H_{ax} minor rotamer), 2.66 (dd, *J* = 12.8, 7.7 Hz, 1 H, 3-H_{ax} major rotamer), 2.31 – 2.23 (m, 1 H, 6-H minor rotamer), 2.11 – 1.93 (m, 3 H, 5-H + 6-H + 7-H major rotamer + 2 H, 5-H minor rotamer), 1.93 – 1.85 (m, 1 H, 5-H′ major rotamer), 1.48 (d, *J* = 6.8 Hz, 3 H, Ala C*H*$_3$ minor rotamer), 1.29 – 1.25 (m, 1 H, 7-H minor rotamer), 1.23 (d, *J* = 6.7 Hz, 3 H, Ala C*H*$_3$ major rotamer), 1.03 (br s, 21 H + 21 H, Si[C*H*$(CH_3)_2]_3$ + Si[CH(C*H*$_3)_2]_3$ both rotamers).

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 174.8, 171.0, 169.8, 168.8, 156.4, 136.1, 135.4, 128.5, 128.5, 128.4, 128.2, 128.2, 128.1, 67.2, 67.0, 65.5, 48.1, 47.1, 44.3, 41.9, 39.9, 31.3, 24.2, 23.7, 17.9, 17.4, 12.1.

MS (ESI) m/z (%): 1468 (52), 1467 (100) $[2M+Na]^+$, 761 (7) $[M+K]^+$, 746 (30), 745 (65) $[M+Na]^+$, 723 (4) $[M+1]^+$.

$C_{38}H_{54}N_4O_8Si$ (722.94): calcd. C, 63.13; H, 7.53; N, 7.75. Found: C, 63.42; H, 7.18; N, 7.60.



2-(Z-Gly-Ala)-4-hydroxyCPA-Gly-OBn [(-)-24]

TBAF (33 µL of a 1 M solution in THF, 0.033 mmol) was slowly added to a solution of **23** (21 mg, 0.029 mmol) in anhydrous THF (1 mL), under an N_2 atmosphere. After 2 h the solvent was

evaporated under vacuum and the residue was taken up in Et_2O (1 mL), then the solvent was evaporated again. After purification by flash chromatography (CH_2Cl_2/CH_3OH 20:1; R_f 0.26), pure **24** (10.7 mg, 65%) was obtained as a colourless gummy solid.

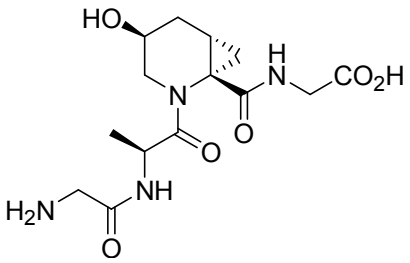
$[\alpha]_D^{17}$ -22.4 (*c* 0.9, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) (3.8:1 mixture of rotamers) δ (ppm): 7.68 – 7.59 (m, 1 H, Gly4 NH major rotamer), 7.39 – 7.25 (m, 5 H + 5 H, 2 x Ph both rotamers and 1 H, Ala NH minor rotamer), 7.17 (d, *J* = 5.6 Hz, 1 H, Ala NH major rotamer), 6.59 – 6.53 (m, 1 H, Gly4 NH minor rotamer), 5.69 – 5.55 (m, 1 H + 1 H, Gly1 NH both rotamers), 5.18 – 5.00 (m, 4 H + 4 H, C*H*$_2$Ph both rotamers), 4.98 – 4.85 (m, 1 H, Ala CH_α minor rotamer), 4.85 – 4.76 (m, 1 H, Ala CH_α major rotamer), 4.15 (dd, *J* = 18.0, 6.0 Hz, 1 H, Gly4 H_α major rotamer), 4.08 – 3.95 (m, 1 H, Gly4 H'_α major rotamer + 2 H, Gly4 H_α minor rotamer and 1 H, Gly1 H_α minor rotamer), 3.95 – 3.85 (m, 2 H, Gly1 H_α, major rotamer + 1 H Gly1 H'_α minor rotamer), 3.85 – 3.74 (m, 1 H, 3-H_{eq} minor rotamer), 3.62 – 3.45 (m, 1 H + 1 H, 4-H both rotamers), 2.93 (dd, *J* = 12.8, 9.6 Hz, 1 H, 3-H_{ax} minor rotamer), 2.54 (dd, *J* = 12.0, 9.2 Hz, 1 H, 3-H_{ax} major rotamer), 2.23 – 2.12 (m, 2 H, 5-H both rotamers), 2.09 (dd, *J* = 10.4, 4.4 Hz, 1 H, 7-H major rotamer), 2.06 – 1.97 (m, 1 H, 6-H major rotamer), 1.97 – 1.79 (m, 2 H, 5-H′ both rotamers + 2 H, 6-H and 7-H minor rotamer), 1.44 (d, *J* = 6.8 Hz, 3 H, Ala C*H*$_3$ minor rotamer), 1.24 (d, *J* = 8.4 Hz, 3 H, Ala C*H*$_3$ major rotamer), 0.79 (dd, *J* = 7.6, 4.4 Hz, 1 H, 7-H′ major rotamer), 0.71 – 0.64 (m, 1 H, 7-H′ minor rotamer).

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 175.4, 170.5, 169.8, 168.4, 156.4, 136.0, 135.2, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 67.2, 67.1, 64.7, 47.6, 46.7, 44.2, 42.0, 40.5, 29.7, 24.0, 23.5, 17.9.

MS (ESI) m/z (%): 1156 (12), 1155 (24) $[2M+Na]^+$, 590 (34), 589 (100) $[M+Na]^+$.

$C_{29}H_{34}N_4O_8$ (566.60): calcd. C, 61.47; H, 6.05; N, 9.89. Found: C, 61.26; H, 6.01; N, 9.67.



[2-(Gly-Ala)-4-hydroxyCPA-Gly-OH]·TFA [(-)-25]

Pd/C (4 mg, 10%) was added to a solution of **24** (9 mg, 0.016 mmol) in CH_3OH (2.5 mL), under an N_2 atmosphere. The resulting suspension was stirred under a H_2 atmosphere (balloon) at room temperature for 23 h. After filtration over a celite layer, TFA (2.5 µL, 0.032 mmol) was added and the solvent was evaporated under *vacuum*, affording the deprotected

tetrapeptide as a trifluoroacetate salt. This crude was purified by semi-preparative HPLC (C18 column, 10 µm, 250 mm × 10 mm i.d.) using acetonitrile (0.1% TFA) in H_2O (0.1% TFA), 5-60% linear gradient over 27 min at room temperature with a flow rate of 5 mL/min (λ = 223 nm; R_t = 7.0 min). The HPLC sample was concentrated under *vacuum*, affording pure **25** (7 mg, 100%) as a colourless glassy solid. Purity was checked by HPLC analysis (C18 column, 5 µm, 250 mm x 4.6-mm i.d.), using acetonitrile (0.1% TFA) in water (0.1% TFA) as eluant, 5-60% linear gradient over 27 min at room temperature.

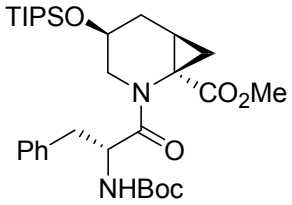
$[\alpha]_D^{22}$ -50.4 (*c* 0.5, CH_3OH).

^{1}H NMR (400 MHz, D_2O) (1.4:1 mixture of rotamers) δ (ppm): 4.90 – 4.83 (m, 1 H + 1 H, Ala H_α both rotamers), 4.18 (dd, *J* = 12.8, 4.0 Hz, 1 H, 3-H_{eq} minor rotamer), 4.04 – 3.94 (m, 4 H + 4 H, Gly1 H_α both rotamers + Gly4 H_α both rotamers), 3.91 (dd, *J* = 12.9, 3.6 Hz, 1 H, , 3-H_{eq} major rotamer), 3.86 – 3.72 (m, 1 H + 1 H, 4-H both rotamers), 3.15 (dd, *J* = 12.9, 9.6 Hz, 1 H, 3-H_{ax} major rotamer), 2.81 (dd, *J* = 12.8, 3.7 Hz, 1 H, 3-H_{ax} minor rotamer), 2.29 (dd, *J* = 13.2, 4.8 Hz, 1 H, 5-H major rotamer), 2.13 – 2.04 (m, 2 H, 6-H + 7-H minor rotamer), 2.01 – 1.83 (m, 3 H + 3 H, 5-H′ + 6-H + 7-H major rotamer + 5-H + 5-H′ + 7-H minor rotamer), 1.44 (d, *J* = 6.8 Hz, 3 H, Ala CH_3 major rotamer), 1.33 (d, *J* = 6.7 Hz, 3 H, Ala CH_3 minor rotamer), 1.22 (dd, *J* = 7.6, 5.2 Hz, 1 H, 7-H′ minor rotamer), 0.80 (dd, *J* = 5.2, 3.6 Hz, 1 H, 7-H′ major rotamer).

^{1}H NMR (500 MHz, H_2O + D_2O 9:1, *water suppression*) (1.4:1 mixture of rotamers) *δ* (ppm): 8.66 (d, *J* = 6.0 Hz, 1 H, Ala NH major rotamer), 8.48 (d, *J* = 7.0 Hz, 1 H, Ala NH minor rotamer), 8.37 (t, *J* = 5.5 Hz, 1 H, Gly NH minor rotamer), 7.86 (t, *J* = 5.5 Hz, 1 H, Gly NH major rotamer), 4.89 – 4.84 (m, 1 H + 1 H, Ala H_α both rotamers), 4.16 (dd, *J* = 12.8, 4.0 Hz, 1 H, 3-H_{eq} minor rotamer), 3.98 – 3.84 (m, 5 H + 4 H, Gly1 H_α both rotamers + Gly4 H_α both rotamers + 3-H_{eq} major rotamer), 3.13 (dd, *J* = 13.0, 9.5 Hz, 1 H, 3-H_{ax} major rotamer), 2.79 (dd, *J* = 12.8, 8.5 Hz, 1 H, 3-H_{ax} minor rotamer), 2.27 (dd, *J* = 12.7, 5.2 Hz, 1 H, 5-H major rotamer), 2.11 – 1.99 (m, 2 H, 6-H + 7-H minor rotamer), 1.99 – 1.81 (m, 3 H + 3 H, 5-H′ + 6-H + 7-H major rotamer + 5-H + 5-H′ + 7-H minor rotamer), 1.43 (d, *J* = 7.0 Hz, 3 H, Ala CH_3 major rotamer), 1.31 (d, *J* = 6.5 Hz, 3 H, Ala CH_3 minor rotamer), 1.18 (dd, *J* = 8.0, 5.5 Hz, 1 H, 7-H′ minor rotamer), 0.78 (dd, *J* = 5.1, 2.5 Hz, 1 H, 7-H′ major rotamer).

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 175.8, 173.2, 166.3, 163.1, 64.5, 49.3, 46.4, 41.3, 40.4, 40.1, 28.9, 23.7, 20.1, 15.7.

MS (ESI) m/z (%): 382 (23) $[M + K]^+$, 381 (100) $[M + K]^+$.



Methyl (1*R*,4*S*,6*R*)-4-Triisopropylsilanyloxy-2-(Boc-D-Phe)-2-azabicyclo[4.1.0]heptane-1-carboxylate [(+)-29]

DEPBT (176 mg, 0.59 mmol) and DIPEA (103 µL, 0.59 mmol) were added under an N_2 atmosphere to a solution of Boc-D-Phe-OH (113 mg, 0.43 mmol) in anhydrous THF (4 mL), cooled to 0 °C, and the resulting mixture was allowed to warm to room temperature. After 15 min this solution was slowly added to a solution of compound **8** (100 mg, 0.3 mmol) in anhydrous THF (1.6 mL) precooled to 0 °C. The resulting reaction mixture was stirred at 35°C for 4 days. Afterward, EtOAc (10 mL) was added and the mixture was washed with a saturated solution of NH_4Cl (2 x 5 mL), a saturated solution of $NaHCO_3$ (2 x 5 mL), and H_2O (2 x 5 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under *vacuum*. The residue was purified by flash chromatography (*n*-hexane/ EtOAc, 4:1; R_f=0.13) affording pure **29** (132 mg, 75%) as a yellow oil.

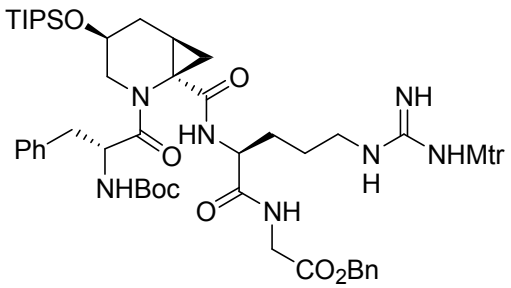
$[\alpha]_D^{23}$ +8.0 (*c* 0.95, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) (1.2:1 mixture of rotamers) δ(ppm): 7.39 – 7.09 (m, 5 H + 5 H, D-Phe CH_{arom} both rotamers), 5.65 (d, J = 7.2 Hz, 1 H, D-Phe NH minor rotamer), 5.21 (d, J = 9.6 Hz, 1 H, D-Phe NH major rotamer), 4.88 – 4.79 (m, 1 H, D-Phe H_α major rotamer), 4.76 – 4.69 (m, 1 H, D-Phe H_α minor rotamer), 4.09 – 4.01 (m, 1 H, 4-H major rotamer), 3.95 – 3.89 (m, 1 H, 4-H minor rotamer), 3.74 (s, 3 H, CO_2Me minor rotamer), 3.64 (s, 3 H, CO_2Me major rotamer), 3.26 (dd, J = 13.2, 4.0 Hz, 1 H, 3-H minor rotamer), 3.05 – 2.97 (m, 3 H, D-Phe H_β + 3-H + 3-H′ major rotamer + 2 H, D-Phe H_β minor rotamer), 2.76 (dd, J = 13.2, 8.4 Hz, 1 H, D-Phe H'_β major rotamer), 2.52 (d, J = 13.2, 1 H, 3-H′ minor rotamer), 2.16 – 2.07 (m, 1 H, 5-H major rotamer), 1.95-1.89 (m, 1 H + 1 H, 7-H both rotamers + 2 H, 5-H minor rotamer), 1.71 – 1.66 (m, 1 H + 1 H, 6-H both rotamers), 1.38 (s, 9 H, + C(CH_3)$_3$ minor rotamer + 1 H, 5-H′ major rotamer), 1.30 (s, 9 H, C(CH_3)$_3$ major rotamer), 1.18 (dd, J = 8.0, 4.8 Hz, 1 H, 7-H′ minor rotamer), 1.04 (s, 21 H, Si[CH(CH_3)$_2$]$_3$ major rotamer), 1.00 (s, 21 H, Si[CH(CH_3)$_2$]$_3$ minor rotamer), 0.74 (dd, J = 7.2, 4.0 Hz, 1 H, 7-H′ major rotamer).

^{13}C NMR (100.4 MHz, CD_3Cl_3) (major rotamer) δ (ppm): 175.2, 172.1, 154.3, 136.9, 129.4, 128.2, 120.0, 79.7, 65.7, 54.3, 52.1, 48.7, 46.8, 40.7, 38.5, 30.7, 28.2, 23.7, 17.9, 12.0.

MS (ESI) m/z (%): 1172 (44), 1171 (60) $[2M + Na]^+$, 613 (19) $[M + K]^+$, 598 (38), 597 (100) $[M + Na]^+$, 576 (19), 575 (53) $[M + 1]^+$.

$C_{31}H_{50}N_2O_6Si$ (574.82): calcd. C, 64.77; H, 8.77; N, 4.87. Found: C, 64.54; H, 8.40; N, 4.68.



4-Triisopropylsilanyloxy-2-(Boc-D-Phe)CPA-Arg(Mtr)-Gly-OBn [(-)-31]

A 1 N solution of NaOH (345 µL) was added to a solution of **29** (130 mg, 0.23 mmol) in methanol (493 µL), and the resulting mixture was vigorously stirred for 72 h at room temperature. Afterward, the methanol was evaporated and the remaining aqueous layer was diluted with H_2O (6 mL). The resulting solution was acidified to pH 6 by adding dropwise a 1 N solution of HCl, and the product was extracted with $CHCl_3$ (3 x 6 mL). Then the aqueous layer was further acidified to pH 3, and the product was extracted again with $CHCl_3$ (3 x 6 mL). The combined organic layers were dried over Na_2SO_4 and, after filtration and evaporation of the solvent, compound **30** (100 mg, 78%) was obtained as a white solid.

^{1}H NMR (200 MHz, $CDCl_3$) (mixture of rotamers) δ(ppm): 7.36 – 7.09 (m, 5 H + 5 H, D-Phe CH_{arom} both rotamers), 5.66 (br s, 1 H, D-Phe NH minor rotamer), 5.37 (br s, 1 H, D-Phe NH major rotamer), 4.87 – 4.78 (m, 1 H, D-Phe H_α major rotamer), 4.72 – 4.64 (m, 1 H, D-Phe H_α minor rotamer), 4.06 (br s, 1 H, 4-H major rotamer), 3.90 (br s, 1 H, 4-H minor rotamer), 3.29 – 3.21 (m, 1 H, 3-H minor rotamer), 3.12 (br d, J = 7.0 Hz, 2 H, D-Phe H_β minor rotamer), 3.05 – 2.96 (m, 1 H, 3-H major rotamer), 2.94 – 2.83 (m, 1 H, 3-H′ major rotamer), 2.77 (br d, J = 6.2 Hz, 2 H, D-Phe H_β major rotamer), 2.63 – 2.52 (m, 1 H, 3-H′ minor rotamer), 2.06 – 1.48 (m, 2 H + 4 H, 5-H + 5-H′ major rotamer + 5-H + 5-H′ + 6-H + 7-H minor rotamer), 1.34 (s, 9 H, C(CH_3)$_3$ minor rotamer), 1.18 (s, 10 H, 6-H + C(CH_3)$_3$ major rotamer), 1.02 (s, 22 H + 1 H, Si[CH(CH_3)$_2$]$_3$ and 7-H major rotamer + 7-H′ minor rotamer), 0.98 (s, 21 H, Si[CH(CH_3)$_2$]$_3$ minor rotamer), 0.90 – 0.81 (m, 1 H, 7-H′ major rotamer).

MS (ESI) m/z (%): 584 (37), 583 (100) $[M + Na]^+$, 561 (10) $[M + 1]^+$.

MS (ESI) m/z (%) (negative mode): 1120 (27), 1119 (39) $[2M – 1]^–$, 560 (34), 559 (100) $[M – 1]^–$.

DEPBT (107 mg, 0.36 mmol) and DIPEA (62 µL, 0.36 mmol) were added under an N_2 atmosphere to a solution of intermediate **30** (100 mg, 0.18 mmol) in anhydrous THF (1.5 mL) cooled to 0°C, and the resulting mixture was allowed to warm to room temperature. After 15 min the reaction was cooled again to 0 °C, and a solution of H-Arg(Mtr)-Gly-OBn (143 mg, 0.27 mmol) in anhydrous THF (1.5 mL) was added. The resulting reaction mixture was stirred at 35°C for 4 days. Afterward,

EtOAc (20 mL) was added, and the mixture washed with a saturated solution of NH_4Cl (2 x 5 mL), a saturated solution of $NaHCO_3$ (2 x 5 mL), and H_2O (2 x 5 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by flash chromatography (*n*-hexane/EtOAc, 1:2; R_f=0.14) to afford pure **31** (137 mg, 77%) as a yellow oil.

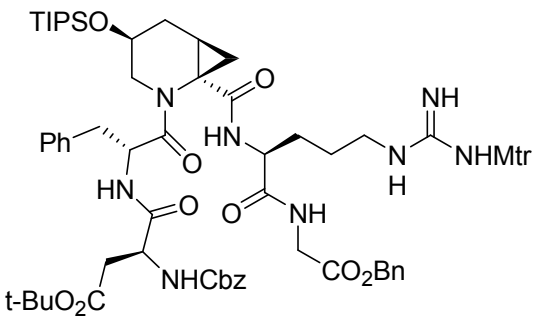
$[\alpha]_D^{23}$ -1.1 (*c* 1.28, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) (1.4:1 mixture of rotamers) δ(ppm): 7.76 – 7.62 (m, 1 H, Gly NH minor rotamer), 7.59 – 7.45 (m, 1 H, Gly NH major rotamer) 7.42 – 7.12 (m, 10 H + 10 H, D-Phe CH_{arom} + Ph CH_{arom} both rotamers), 6.86 (d, J = 8.4 Hz, 1 H, Arg NH major rotamer and Arg NH minor rotamer), 6.51 (s, 1 H + 1 H, Mtr CH_{arom} both rotamers), 6.25 – 5.92 (m, 3 H + 3 H, Arg NH guanidinium both rotamers), 5.57 (d, J = 6.8 Hz, 1 H, D-Phe NH major rotamer), 5.29 (d, J = 8.0 Hz, 1 H, D-Phe NH minor rotamer), 5.13 (s, 2 H + 2H, OCH_2Ph both rotamers), 4.79 – 4.72 (m, 1 H, D-Phe H_α major rotamer), 4.67 – 4.50 (m, 1 H + 1 H, Arg H_α both rotamers + 1 H, D-Phe H_α minor rotamer), 4.16 – 3.91 (m, 3 H + 3 H, Gly H_α + 4-H both rotamers), 3.81 (s, 3 H + 3 H, Mtr OMe both rotamers), 3.40 (br d, J = 14.0 Hz, 1 H, 3-H major rotamer), 3.33 – 3.18 (m, 2 H + 2 H, Arg H_δ both rotamers), 3.08 – 3.00 (m, 2 H, D-Phe H_β major rotamer), 2.93 – 2.83 (m, 1 H, 3-H minor rotamer), 2.79 – 2.72 (m, 1 H, 3-H′ minor rotamer), 2.69 (s, 3 H + 3 H, Mtr CH_3 both rotamer and 1 H, 3-H′ major rotamer), 2.62 (s, 3 H + 3 H, Mtr CH_3 both rotamers), 2.59 – 2.53 (m, 2 H, D-Phe H_β minor rotamer), 2.21 – 2.14 (m, 2 H, 5-H minor rotamer), 2.12 (s, 3 H + 3 H, Mtr CH_3 both rotamers), 2.02 – 1.87 (m, 2 H, 5-H major rotamer), 1.87 – 1.79 (m, 1 H, 7-H major rotamer), 1.78 – 1.65 (m, 4 H + 4 H, Arg H_β + Arg H_γ both rotamers), 1.65 – 1.52 (m, 1 H, 6-H major rotamer), 1.38 (s, 9 H, C(CH_3)$_3$ major rotamer), 1.28 – 1.21 (m, 1 H, 6-H minor rotamer), 1.19 (s, C(CH_3)$_3$ minor rotamer), 1.15 – 1.07 (m, 1 H, 7-H′ major rotamer), 1.04 (s, 21 H, Si[CH(CH_3)$_2$]$_3$ major rotamer and 1 H, 7-H minor rotamer), 1.01 (s, 21 H, Si[CH(CH_3)$_2$]$_3$ minor rotamer), 0.91 – 0.82 (m, 1 H, 7-H′ minor rotamer).

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 174.9, 172.1, 171.3, 170.6, 169.7, 158.3, 156.6, 154.3, 138.5, 136.4, 135.3, 133.6, 129.6, 128.6, 128.2, 126.9, 124.7, 116.6, 79.1, 67.0, 65.6, 55.4, 52.5, 48.9, 41.2, 40.6, 40.3, 39.7, 28.3, 28.2, 24.8, 24.1, 18.3, 17.9, 12.0, 11.9.

MS (ESI) m/z (%): 1114 (19) $[M + K]^+$, 1099 (25), 1098 (40) $[M + Na]^+$, 1077 (20), 1076 (32) $[M + 1]^+$.

$C_{55}H_{81}N_7O_{11}SSi$ (1076.42): calcd. C, 61.37; H, 7.58; N, 9.11. Found: C, 61.52; H, 7.38; N, 9.03.



4-Triisopropylsilanyloxy-2-[Z-Asp(OtBu)-D-Phe]CPA-Arg(Mtr)-Gly-OBn (33)

$Sn(OTf)_2$ (62 mg, 0.15 mmol) was added under an N_2 atmosphere to a solution of **31** (134 mg, 0.12 mmol) in anhydrous CH_2Cl_2 (1.4 mL), cooled to 0°C, and the resulting mixture was stirred at room temperature for 24 h; meanwhile additional $Sn(OTf)_2$ (41 mg, 0.10 mmol) was added. Afterward, the mixture was neutralized with a saturated solution of $NaHCO_3$ and further basificated to pH 9 by adding solid Na_2CO_3. The product was extracted with AcOEt (4 x 20 mL). After filtration the organic layer was evaporated under vacuum and the residue was taken up in $CHCl_3$ (20 mL) and washed with H_2O (10 mL). The organic layer were dried over Na_2SO_4 and, after filtration and evaporation of the solvent, compound **32** (116 mg, 96%) was obtained as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers) δ (ppm): 7.60 – 7.51 (m, 1 H + 1 H, Gly NH both rotamers), 7.36 – 7.04 (m, 10 H + 10 H, D-Phe CH_{arom} + Bn CH_{arom} both rotamers), 6.76 – 6.66 (d, J = 8.0 Hz, 1 H + 1 H, Arg N-H both rotamers), 6.49 (s, 1 H + 1 H, Mtr CH_{arom}, both rotamers), 6.38 – 6.04 (m, 3 H + 3 H, Arg N-H guanidinium both rotamers), 5.10 (s, 2 H, Gly-CH_2Ph, minor rotamer), 5.07 (s, 2 H, Gly-CH_2Ph major rotamer), 4.61 – 4.54 (m, 1 H + 1 H, Arg H_α both rotamers), 5.52 – 4.39 (m, 1 H + 1 H, D-Phe H_α both rotamers), 4.15 – 3.85 (m, 3 H+ 3 H, 4-H + Gly H_α both rotamers), 3.79 (s, 3 H + 3 H, Mtr OMe both rotamers), 3.28 – 2.88 (m, 5 H + 2 H, D-Phe H_β + D-Phe H'_β + 3-H major rotamer, Arg H_δ both rotamers), 2.79 (br d, J = 12.8 Hz, 1 H, 3-H minor rotamer), 2.71 – 2.49 (m, 4-H + 6-H, D-Phe H_β + D-Phe H'_β minor rotamer, 3-H′ + 2 x Mtr CH_3 both rotamers), 2.10 (s, 3 H + 3 H, Mtr CH_3 both rotamers), 2.06 – 1.98 (m, 1 H, 7-H major rotamer), 1.97 – 1.74 (m, 2 H, 5-H + 5-H′ major rotamer), 1.72 – 1.33 (m, 5 H + 6 H, 6-H major rotamer, Arg H_β + Arg H_γ both rotamers, 5-H + 5-H′ minor rotamer), 1.30 – 1.14 (m, 1 H + 1 H, 7-H′ major rotamer + 6-H minor rotamer), 1.04 (s, 21 H, $Si[CH(CH_3)_2]_3$ major rotamer and 1 H, 7-H minor rotamer), 1.02 (s, 21 H, $Si[CH(CH_3)_2]_3$ minor rotamer), 0.91 – 0.82 (m, 1 H, 7-H′ minor rotamer).

MS (ESI) m/z (%): 1975 (20), 1974 (27), 1973 (24) $[2M + Na]^+$, 1954 (34), 1953 (56), 1952 (100) $[2M + 1]^+$, 998 (12) $[M + Na]^+$, 978 (16), 977 (36), 976 (60) $[M + 1]^+$.

DEPBT (22 mg, 0.074 mmol) and DIPEA (13 µL, 0.074 mmol) were added under an N_2 atmosphere to a solution of Z-Asp(O*t*Bu)-OH·H_2O (13 mg, 0.037 mmol) in anhydrous THF (1.0

mL) cooled to 0°C, and the resulting mixture was allowed to warm to room temperature. After 15 min the reaction was cooled again to 0°C, and a solution of intermediate **32** (33 mg, 0.034 mmol) in anhydrous THF (300 µL) was added. The resulting reaction mixture was stirred at 35°C for 4 days. Afterward, EtOAc (10 mL) was added, and the mixture washed with a saturated solution of NH_4Cl (2 x 5 mL), a saturated solution of $NaHCO_3$ (2 x 5 mL), and H_2O (2 x 5 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under vacuum. The residue was purified by flash chromatography (*n*-hexane/EtOAc, 1:2; R_f=0.28) to afford pure **33** (32 mg, 75%) as a white solid.

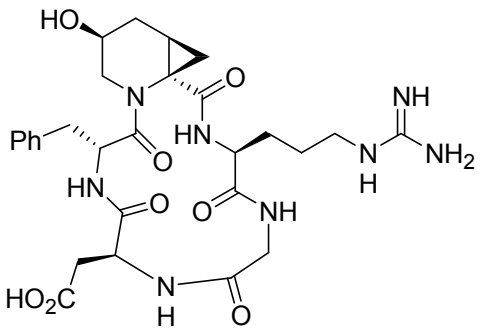
^{1}H NMR (400 MHz, $CDCl_3$) (1.4:1 mixture of rotamers) δ (ppm): 7.75 – 7.55 (m, 1 H, Gly NH, minor rotamer), 7.54 – 7.41 (m, 2 H, Gly NH + D-Phe NH, major rotamer), 7.41-7.21 (m, 15 H + 15 H, D-Phe CH_{arom} + Bn CH_{arom} both rotamers), 7.19 – 7.07 (m, 1 H, D-Phe NH minor rotamer), 6.87 (d, J = 6.8 Hz, 1 H + 1 H, Arg N-H, both rotamers), 6.49 (s, 1 H + 1 H, Mtr CH_{arom} both rotamers), 6.41 – 6.01 (m, 3 H + 3 H, Arg N-H guanidinium both rotamers), 6.09 (d, J = 7.6 Hz, 1 H, Asp NH major rotamer), 6.00 – 5.91 (m, 1 H, Asp NH minor rotamer), 5.12 (s, 2 H + 2 H, Gly-CH_2Ph, both rotamers), 5.10 (s, 2 H + 2 H, Asp-CH_2Ph both rotamers), 5.09 – 5.03 (m, 1 H, D-Phe H_α major rotamer), 5.02 – 4.90 (m, 1 H, D-Phe H_α minor rotamer), 4.67-4.59 (m, 1 H + 1 H, Arg H_α both rotamers), 4.54 – 4.45 (m, 1 H, Asp H_α minor rotamer), 4.41 – 4.31 (m, 1 H, Asp H_α, major rotamer), 4.06 – 3.89 (m, 3 H + 3 H, 4-H + Gly H_α both rotamers), 3.78 (s, 3 H + 3 H, Mtr OMe both rotamers), 3.37 (br d, J = 11.6 Hz, 1 H, 3-H major rotamer), 3.25-3.12 (m, 2 H + 2 H, Arg H_δ, both rotamers), 3.12 – 2.98 (m, 2 H, D-Phe H_β major rotamer), 2.80 (d, J = 13.0 Hz, 1 H, 3-H, minor rotamer), 2.75 – 2.70 (m, 1 H + 1 H, 3-H′ minor rotamer + 3-H′ major rotamer), 2.68 (s, 3 H, Mtr CH_3 major rotamer), 2.67 (s, 3 H, Mtr CH_3 minor rotamer), 2.61 (s, 3 H, Mtr CH_3 major rotamer and 3 H, D-Phe H_β + D-Phe H'_β + Asp H_β minor rotamer), 2.59 (s, 3 H, Mtr CH_3 minor rotamer), 2.53 (dd, J = 16.8, 6.4 Hz, 1 H, Asp H'_β minor rotamer), 2.48 – 2.39 (m, 2 H, Asp H_β major rotamer), 2.31 – 2.16 (m, 2 H, 5-H minor rotamer), 2.10 (s, 3 H + 3 H, Mtr CH_3 both rotamers), 1.91 – 1.80 (m, 2 H, 5-H + 7-H major rotamer), 1.76 – 1.64 (m, 2 H, Arg H_β both rotamers), 1.64 – 1.49 (m, 2 H, Arg H_γ, both rotamers and 1 H, 6-H major rotamer), 1.37 (s, 9 H + 9 H, C(CH_3)$_3$, both rotamers), 1.29 – 1.20 (m, 1 H, 6-H minor rotamer), 1.03 (s, 21 H, Si[CH(CH_3)$_2$]$_3$ major rotamer and 1 H, 7-H′ major rotamer and 1 H, 7-H minor rotamer), 0.97 (s, 21 H, Si[CH(CH_3)$_2$]$_3$ minor rotamer), 0.90 – 0.84 (m, 1 H, 7-H′ minor rotamer).

^{13}C NMR (100.4 MHz, $CDCl_3$) (major rotamer) δ (ppm): 174.1, 173.8, 172.1, 171.9, 171.2, 170.53, 170.4, 170.2, 169.9, 169.7, 169.3, 158.2, 156.5, 156.3, 155.9, 138.5, 136.5, 136.3, 136.1, 136.0, 135.2, 133.7, 129.4, 129.2, 129.2, 128.9, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.2, 127.1, 124.6, 124.6, 111.6, 82.0, 81.6, 67.0, 65.6, 65.3, 55.3, 53.7, 52.4, 52.2, 51.3, 51.1, 49.0, 45.9, 41.2, 40.5, 40.3, 39.9, 38.9, 37.6, 31.9, 30.4, 29.6, 29.2, 28.3, 28.0, 27.3,

25.4, 24.8, 24.1, 24.0, 23.2, 22.7, 21.7, 21.2, 18.3, 18.3, 18.0, 18.0, 17.9, 17.9, 14.1, 12.0, 11.9, 11.8.

MS (ESI) m/z (%): 1305 (36), 1304 (60), 1303 (92) $[M + Na]^+$, 1283 (41), 1282 (74), 1281 (100) $[M + 1]^+$.

$C_{66}H_{92}N_8O_{14}SSi$ (1281.63): calcd. C, 61.85; H, 7.24; N, 8.74. Found: C, 61.57; H, 7.22; N, 8.64.



Cyclo[Arg-Gly-Asp-D-Phe-4-OHCPA]·TFA (35)

Pd/C (10 mg, 10%) was added to a solution of **33** (32 mg, 0.025 mmol) in ethanol (1.5 mL), under an N_2 atmosphere. The resulting suspension was stirred under a H_2 atmosphere (balloon) at room temperature for 18 h. After filtration over a celite layer and evaporation of the solvent, compound **34** (25 mg, 95%) was obtained as a white solid. This crude was suspended in THF (7 mL) under an N_2 atmosphere. The suspension was cooled to 0 °C and DEPBT (22 mg, 0.072 mmol) and DIPEA (13 µL, 0.072 mmol) were added. The resulting reaction mixture was stirred at 35 °C for 4 days. Afterward, EtOAc (10 mL) was added and the mixture washed with a satd. solution of NH_4Cl (2 x 5 mL), a satd. solution of $NaHCO_3$ (2 x 5 mL) and H_2O (2 x 5 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated under *vacuum*. The residue was eluted through a short pad of silica gel (CH_2Cl_2/MeOH, 25:1) and used directly in the next step.

MS (ESI) m/z (%): 1078 (23), 1077 (28) $[M + K]^+$, 1061 (19) $[M + Na]^+$.

TBAF (28 µL of a 1 M solution in THF, 0.086 mmol) was slowly added to a solution of protected tetrapeptide in anhydrous THF (1.5 mL), under an N_2 atmosphere. After 2 h the solvent was evaporated under vacuum and the residue was taken up in Et_2O (2 mL), then the solvent was evaporated again. The crude cyclic tetrapeptide was obtained as an orange solid. This crude was dissolved in a 95:2.5:2.5 trifluoroacetic acid/triisopropylsilane/H_2O mixture (5 mL) and the resulting solution was stirred at room temperature for 18 h. Afterward, the mixture was evaporated under *vacuum* and the residue was taken up in H_2O (10 mL) and washed with Et_2O (4 x 12 mL). The aqueous layer was then concentrated under *vacuum*, affording the deprotected cyclic tetrapeptide as a trifluoroacetate salt. This crude was purified by semi-preparative HPLC (C18

column, 10 μm, 250 mm × 10 mm i.d.) using acetonitrile (0.1% TFA) in H_2O (0.1% TFA), 5-60% linear gradient over 27 min at room temperature with a flow rate of 5 mL/min (λ = 223 nm; R_t = 13.56 min). The HPLC sample was concentrated under *vacuum* and lyophilized, affording pure **35** (2.1 mg, 12% over 3 steps) as a colourless glassy solid. Purity was checked by HPLC analysis (C18 column, 5 μm , 250 mm x 4.6-mm i.d.), using acetonitrile (0.1% TFA) in water (0.1% TFA) as eluant, 5-60% linear gradient over 35 min at room temperature.

^{1}H NMR (400 MHz, D_2O) δ(ppm): 7.48 – 7.16 (m, 5 H, D-Phe H_{arom}), 4.91 (dd, J = 9.6, 5.2 Hz, 1 H, D-Phe H_α), 4.73 (dd, J = 8.0, 6.4 Hz, 1 H, Asp H_α), 4.21 (d, J = 14.0 Hz, 1 H, Gly H_α), 4.00 – 3.91 (m, 1 H, 4-H), 3.88 (t, J = 7.5 Hz, 1 H, Arg H_α), 3.69 (dd, J = 13.2, 5.6 Hz, 1 H, 3-H), 3.39 (d, J = 14.0 Hz, 1 H, Gly H'_α), 3.27 – 3.13 (m, 3 H, D-Phe H_β + Arg H_δ + Arg H'_δ), 3.08 (dd, J = 13.2, 4.8 Hz, 1 H, 3-H′), 3.06 (dd, J = 12.8, 5.2 Hz, 1 H, D-Phe H'_β), 2.88 (dd, J = 16.8, 8.0 Hz, 1 H, Asp H_β), 2.75 (dd, J = 16.8, 6.4 Hz, 1 H, Asp H'_β), 2.22 – 2.13 (m, 1 H, 5-H), 1.85 (dd, J = 9.6, 6.4 Hz, 1 H, 7-H), 1.79 – 1.47 (m, 5 H, Arg H_β + Arg H_γ + H-6), 0.96 – 0.86 (m, 1 H, 5-H′), 0.11 (t, J = 6.4 Hz, 1 H, 7-H′).

^{1}H NMR (500 MHz, H_2O + D_2O 9:1, *water suppression*) δ (ppm): 8.76 (pseudo t, J = 6.5 Hz, 1 H, Gly NH), 8.25 (d, J = 7.0 Hz, 1 H, D-Phe NH), 7.96 (d, J = 9.5 Hz, 1 H, Asp NH), 7.42 – 7.18 (m, 6 H, D-Phe H_{arom} + Arg NH), 7.15 (t, J = 6.0 Hz, 1 H Arg NH guanidinium), 6.85 – 6.42 (m, 2 H, Arg NH guanidinium), 4.19 (dd, J = 14.5, 7.5 Hz, 1 H, Gly H_α), 3.97 – 3.89 (m, 1 H, 4-H_{eq}), 3.86 (td, J = 8.0, 5.0 Hz, 1 H, Arg H_α), 3.66 (dd, J = 13.5, 5.5 Hz, 1 H, 3-H), 3.38 (dd, J = 14.5, 5.5 Hz, 1 H, Gly H'_α), 3.22 – 3.12 (m, 3 H, Arg H_δ e H'_δ + D-Phe H_β), 3.08 (dd, J = 13.5, 5.0 Hz, 1 H, 3-H′), 3.04 (dd, J = 12.5, 5.0 Hz, 1 H, D-Phe H'_β), 2.82 (dd, J = 16.5, 7.5 Hz, 1 H, Asp H_β), 2.69 (dd, J = 16.5, 6.5 Hz, 1 H, Asp H'_β), 2.16 (ddd, J = 12.5, 7.0, 5.0 Hz, 1 H, 5-H), 1.83 (dd, J = 9.5, 6.5 Hz, 1 H, 7-H), 1.70 – 1.44 (m, 4 H, Arg H_β + Arg H'_β + Arg H_γ + Arg H'_γ), 1.62 – 1.52 (m, 1 H, 6-H), 0.86 (ddd, J = 12.5, 8.0, 6.0, 1 H, 5-H′), 0.06 (pseudo t, J = 7.0 Hz, 1 H, 7-H′).

MS (ESI) m/z (%): 617 (8), 616 (34), 615 (100) $[M + 1]^+$.

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IV. Table S1

Table S1. ^{1}H NMR chemical shifts for compounds **13-16** and **18-25**

Entry	Compd	***trans***				***cis***			
		3-H_{ax}	3-H_{eq}	NH (Gly)[a]	α-CH (Ala)	3-H_{ax}	3-H_{eq}	NH (Gly)[a]	α-CH (Ala)
1	**13**[b]	3.35	3.69	-	4.63	2.76	4.36	-	4.80
2	**14**[b]	3.29	3.77	7.42	4.44	2.72	4.56	6.77	4.75
3	**15**[c]	3.37	3.86	n.d.[e]	4.45	3.04	4.20	n.d.[e]	4.74
4	**16**[d]	3.43	3.67	8.30	4.53	3.02	4.24	8.72	4.59
5	**18**[b]	3.13	3.69	-	4.53	3.00	3.91	-	4.75
6	**19**[b]	3.06	3.70	-	4.53	2.85	4.08	-	4.73
7	**20**[b]	3.05	3.71	6.47	4.53	2.61	4.34	8.27	4.45
8	**21**[b]	2.93	3.83	6.56	4.61	2.55	4.41	8.37	4.45
9	**22**[b]	2.97	3.64	6.44	3.69	2.74	4.17	7.03	3.90
10	**23**[b]	3.03	3.84	6.41	4.81	2.66	4.26	7.91	4.72
11	**24**[b]	2.93	3.77	6.56	4.92	2.54	4.39	7.63	4.81
12	**25**[d]	3.15	3.91	7.71	4.87	2.81	4.18	8.20	4.87

[a]Gly (i+2) or (i+3) in the tri- and tetrapeptides, respectively; [b]Recorded in $CDCl_3$; [c]Recorded in CD_3OD; [d]Recorded in D_2O/H_2O 1:9; [e]Not detected due to exchange with the deuterated solvent.

V. Figure S1

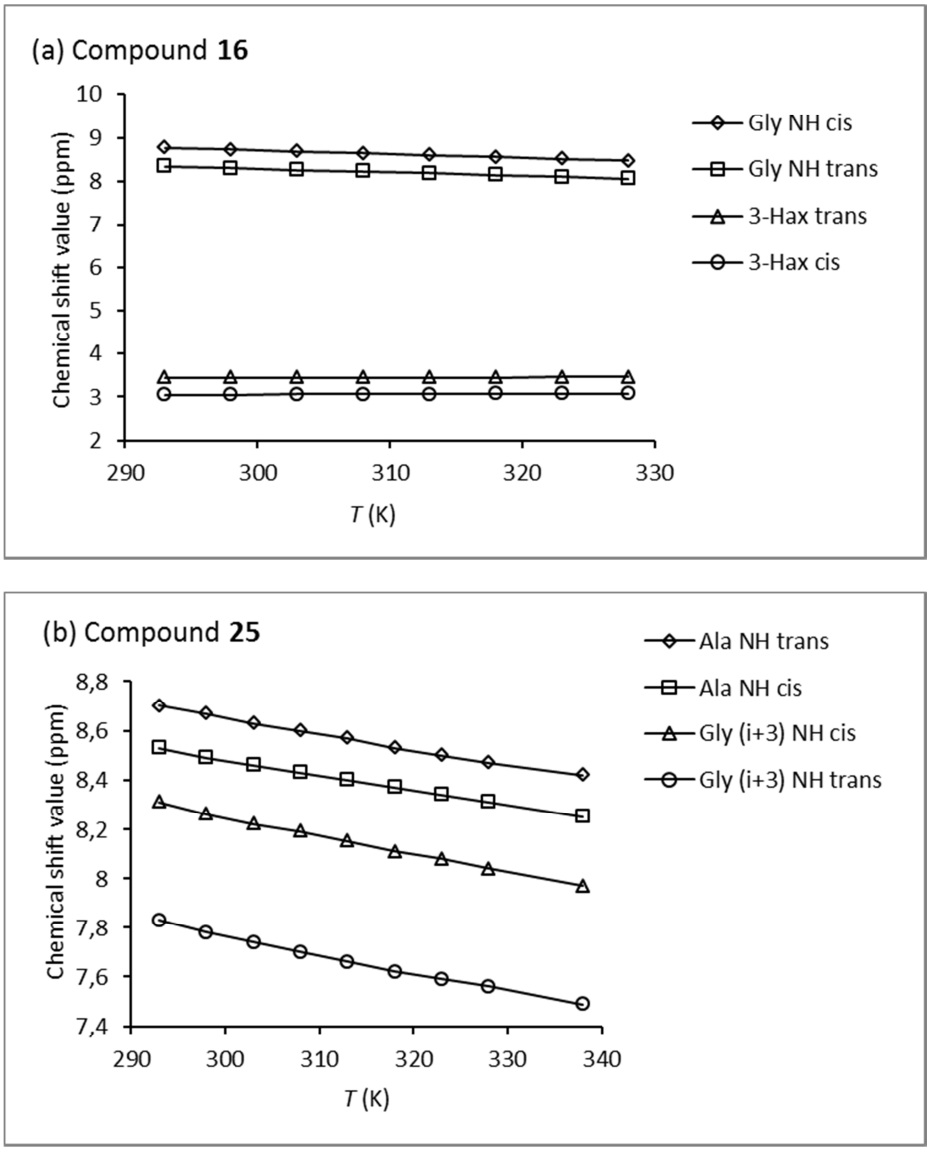

Figure S1. Variable temperature ^{1}H NMR experiments in D_2O/H_2O 1:9 on compounds **16** (a) and **25** (b).

VI. Computational Calculations

Umbrella Sampling (US) and Potential of Mean Force (PMF) calculations. The starting geometries were obtained from REMD simulations,[1] followed by cluster analysis of the 300K trajectory, as described below. A modified version of the ff14SB force field,[2] where parameters for the peptide bond rotation were modified as suggested by Doshi and Hemelberg,[3] was used. The system was solvated by a TIP3P water box and neutralized by adding Cl^- ions. The geometry was minimized and the solvent box was equilibrated by a constant volume (NVT) simulation (100 ps) were restraints (force constant = 50 kcal · mol^{-1} · $Å^2$) were applied on the solute. A constant pressure (NPT) equilibration was then performed for 100 ps, where the restraint force constant was lowered to 25 kcal · mol^{-1} · $Å^2$. The system was then minimized and then gently heated up to 300 K during 30 ns of simulation. In the meanwhile, backbone restraints were relaxed from 10 to 5 kcal · mol^{-1} · $Å^2$. The final steps consisted in 200 ps of NVT equilibrations (backbone restraints = 5 kcal · mol^{-1} · $Å^2$) followed by 1 ns of NPT simulations with backbone restraints relaxed from 5 to 1 kcal · mol^{-1} · $Å^2$ and 1 ns od unrestrained NPT simulation. The final coordinate set was then used for US calculations that were performed accordingly to the procedure described in the Amber documentation.[4] The amide bond linking Ala1 to CPA was rotated from 0 to 180 degrees, with a step of 3 degrees per rotation. Each step was composed by a minimization (500 steps by using the steepest descent algorithm followed by 1500 steps of conjugated gradient), followed by NPT equilibration (500 ps, final temperature = 300 K) and production (1000 ps, 300 K). Restraints for the rotating dihedral were imposed using a force constant of 200 kcal · mol^{-1} · rad^2. The suitability of this value was assessed by evaluating the dihedral distribution over the whole production run. In all the MD simulations, the temperature was controlled by the Langevin dynamics with a collision frequency $\gamma = 2$. Covalent bonds involving hydrogens were restrained using the SHAKE algorithm and the Particle Mesh Ewald method was used for long-range electrostatics. Using this setup, the overlap of dihedral distributions among each window was excellent (Figure S2), even in the transition state region that, for peptide **16**, was observed for dihedral values of about 100 deg (see Figure 5, main text).

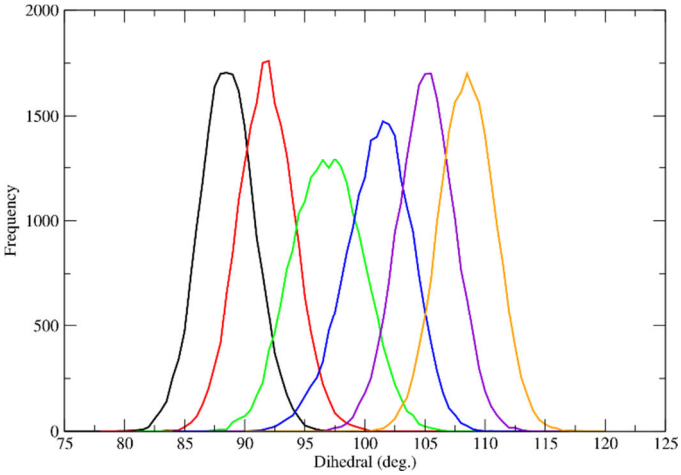

Figure S2. Dihedral distribution frequency (windows from 90 to 105 deg.) from the Umbrella Sampling simulation on peptide **16**.

PMF of the -1 to 181 degree rotation was evaluated using the Weighted Histogram Analysis Method,[5] at a temperature of 300 K, using 61 bins with a tolerance of 0.01 kcal · mol^{-1} and using the WHAM code developed by Grossfield.[6]

Conformational search. A "Low mode" conformational search was performed on peptide **24** by using the MOE software package.[7] The Amber10EHT force field and the R-Field solvation model, implemented in MOE, were used and the exterior dielectric constant was set to 4.8 to simulate chloroform as the solvent. The termination criteria of the conformational search were increased by a factor of 50 compared to default settings (rejection limit and iteration limit = 5000 and 500000, respectively) and the enforcement of chair conformation for hexacyclic rings was removed; all the other setting were left as the software defaults. The conformational search was performed by using dihedral and distance restraints derived from the NOE experiments on peptide **24**, as shown in Table S2 and Figure S3.

Table S2. Restraints derived from NOE measurements. A weight of 2.0 kcal/mol was used for all restraints.

Type	Range[a]	Atoms[b]
dihedral	120 – 130 deg.	ALA2.(HA CA N H)
dihedral	160 – 180 deg.	CPA3.(OD CD CE N)
distance	2.5 – 4.0 Å	ALA2.(H CB)
distance	2.5 – 4.0 Å	CPA3.HP2 ALA2.CB
distance	1.8 – 3.5 Å	GLY4.H ALA2.HA
distance	1.8 – 3.5 Å	GLY4.(HA3 H)

[a]The restraint operates when the geometrical parameter moves outside the specified range. [b]See Figure S3 for atom labels.

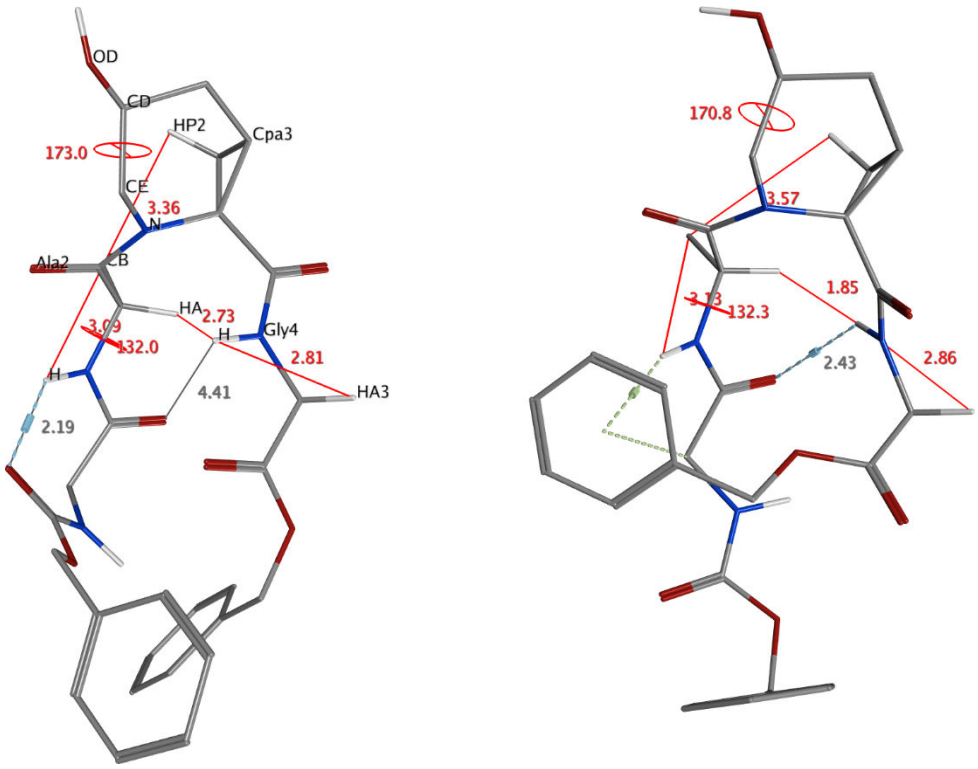

Figure S3. Lowest energy conformation (left) and lowest energy conformation with Gly1-C=O···HN-Gly4 H-bond (ΔE = 1.6 kcal/mol compared to the lowest energy conformation) obtained from a restrained conformational search on peptide **24**. Final distances of restrained atoms (see Table S2) are shown in red. H-bond distances are shown in dark grey.

Replica Exchange Molecular Dynamics (REMD).

REMD simulations for peptides **16**, **25** and **35** were carried out using the *pmemd.MPI* software of the Amber14 package.[8] Simulations were started from the results of a preliminary conformational

search, performed with MOE as described above for peptide **24**, but using the external dielectric of water and no experimentally derived restraints. The ff96 force field coupled with the GB-OBC(II) solvent model,[9] a combination that was shown to provide a good description of the conformational preferences of peptides that fold into beta secondary structures,[10] was adopted for all REMD simulations. Geometries were minimized by 500 steps of steepest-descendant followed by 500 steps of conjugated-gradient minimization. Twelve replicas were then run at the following temperatures 300.00, 330.69, 364.36, 401.31, 441.89, 486.42, 535.31, 588.95, 647.82, 712.41, 783.26, and 860.93 K. A short equilibration run (200 ps) was performed on each replica prior to the actual production run. REMD simulations were conducted on each peptide using Langevin dynamics, at constant temperature, for 400 ns and with different seeds for every simulation.[11] A time step of 0.002 ps and an infinite cut-off for electrostatic were used, while the SHAKE algorithm was adopted to constrain all bonds involving hydrogens. Exchanges were attempted every 2 ps and were accepted with an average probability above 50% probability. To evaluate convergence, the 300 K trajectory was subjected to a cluster analysis every 50 ns. The simulation was considered converged when the population of the three principal clusters differed by less than 15% between consecutive 50 ns batches. All simulations were considered converged after 300 ns. Moreover, two simulations were performed for each system by starting from different conformations, and the results were compared. After 300 ns of simulation, no significant differences were observed in the population of the three main cluster as well as in their representative geometries. Cluster analyses were performed with *cpptraj* (AmberTools15),[8] sampling one frame every 4 ps, using the average-linkage algorithm, the pairwise mass-weighted RMSD on backbone heavy atoms as a metric and requesting 10 clusters. For every simulation, the first four clusters accounted at least the 95% of the total population.

Parameterization of non standard residues. Charge parameterization for CPA (*R* or *S* configuration at the α carbon), and for pipecolic acid (*S* configuration at the α carbon) was performed using the R.E.D. procedure.[12] The amino acid structures were capped by a acetyl and a NHMe group at the N and C termini, respectively, and subjected to a conformational search (low mode method, MMFF94x force field as implemented in MOE). The two conformations corresponding to the *trans* and *cis* configurations at the peptide bond linking the acetyl cap to the residue were used for charge parameterization. Moreover, for each conformation, two different orientations were used to derive conformation and orientation independent RESP charges. The Gaussian09 software was used for quantum mechanical calculations,[13] performed at the HF/6-31G* level accordingly to the force field specifications.

Additional Figures

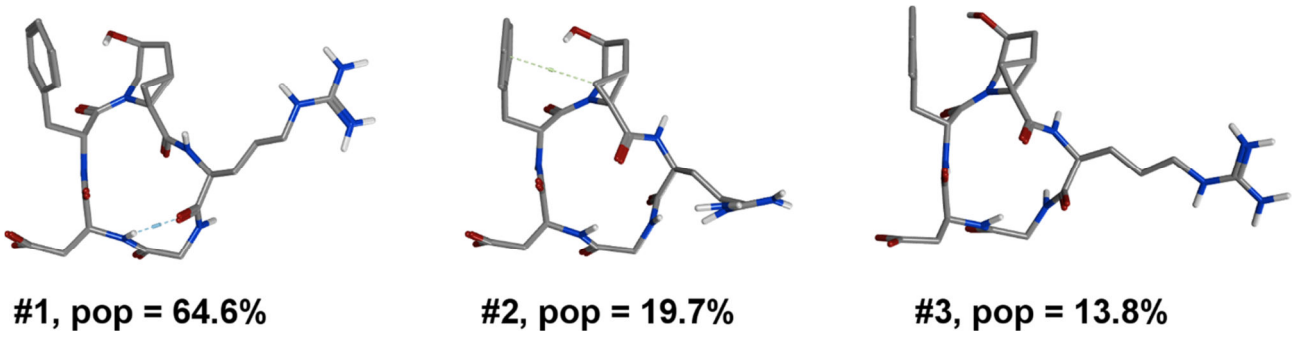

Figure S4. Representative conformation of the three principal clusters obtained from the analysis of the 300 K trajectory obtained by REMD simulations on cyclopeptide **35**. Populations are referred to the cluster analysis of the last 50 ns of a 400 ns REMD trajectory.

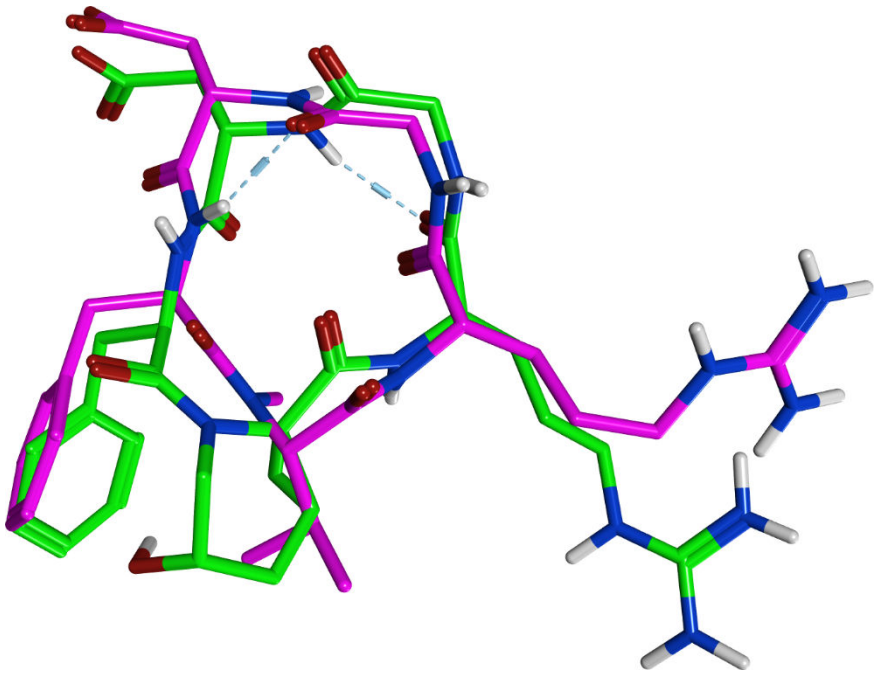

Figure S5. Superposition of the representative geometry of the most populated cluster obtained by the analysis of the 300K REMD trajectory of **35** (green carbons) and the crystal structure of Cilengitide (purple carbons) bound to the extracellular segment of Integrin $\alpha_V\beta_3$.[14] The RMSD evaluated between the backbone atoms of the two molecules is 1.07 Å.

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VII. Table S3. Chemical shift values for cyclic peptidomimetic **35**, temperature coefficients and NOE correlations

Proton	δ (ppm)	$\Delta\delta/\Delta T$ (ppb/K)	Notes
3-H	3.66 (dd, *J* = 13.5, 5.5 Hz)		
3-H'	3.08 (dd, *J* = 13.5, 5.0 Hz)		
4-H (eq)	3.97-3.89 (m)		
5-H	2.16 (ddd, *J* = 12.0, 7.0, 4.5 Hz)		
5-H'	0.860 (ddd, *J* = 12.0, 8.0, 6.0 Hz)		
6-H	1.62-1.52 (m)		
7-H	1.83 (dd, *J* = 9.5, 6.0 Hz)		NOE with Arg N-H
7-H'	0.064 (dd, *J* = 7.0, 6.0 Hz)		
D-Phe CHα	4.94-4.83 (m)		NOE with *endo* 7-H
D-Phe CHβ	3.22-3.12 (*J* = n.d.)		
D-Phe CHβ'	3.04 (dd, *J* = 12.5, 5.0 Hz)		
D-Phe N-H	8.27 (d, *J* = 7.5 Hz)	-6.0	
Asp CHα	4.78-4.68 (m)		
Asp CHβ	2.84 (dd, *J* = 16.5, 8.0 Hz)		
Asp CHβ'	2.71 (dd, *J* = 16.5, 6.5 Hz)		
Asp N-H	7.97 (d, *J* = 9.0 Hz)	-4.28	NOE with Gly CHα
Gly CHα	4.19 (dd, *J* = 14.0, 7.5 Hz)		NOE with Asp N-H
Gly CHα'	3.37 (dd, *J* = 14.0, 5.5 Hz)		
Gly N-H	8.77 (dd, *J* = 7.5,5.5 Hz)	-7.1	NOE with Arg CHα
Arg CHα	3.88-3.82 (m)		NOE with Gly N-H
Arg CHβ and CHβ'	1.70-1.44 (m)		
Arg CHγ and CHγ'	1.70-1.44 (m)		
Arg CHδ and CHδ'	3.22-3.12 (m)		
Arg N-H	7.21 (*J* = n.d.)	-5.4	NOE with *exo* 7-H
Arg N-H (guanidinium)	7.15 (t, *J* = 6.0 Hz)		

VIII. Figure S6

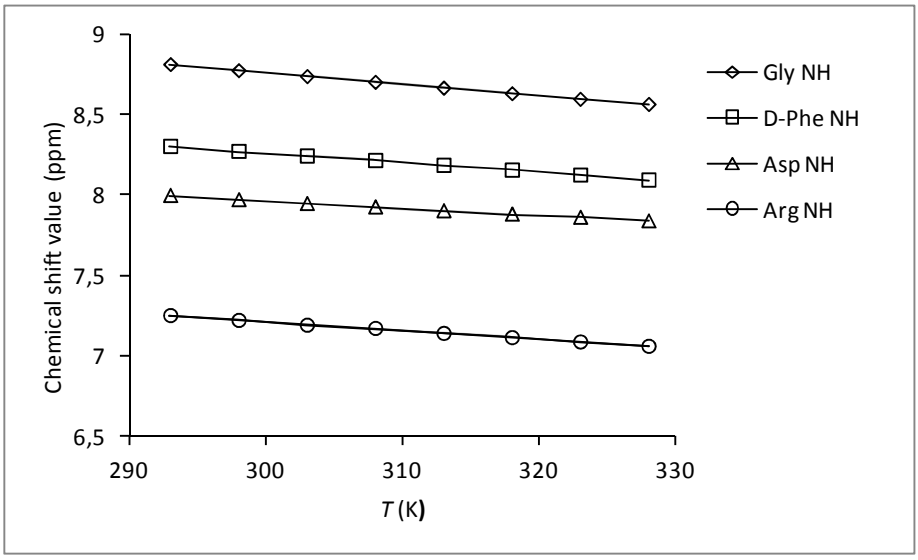
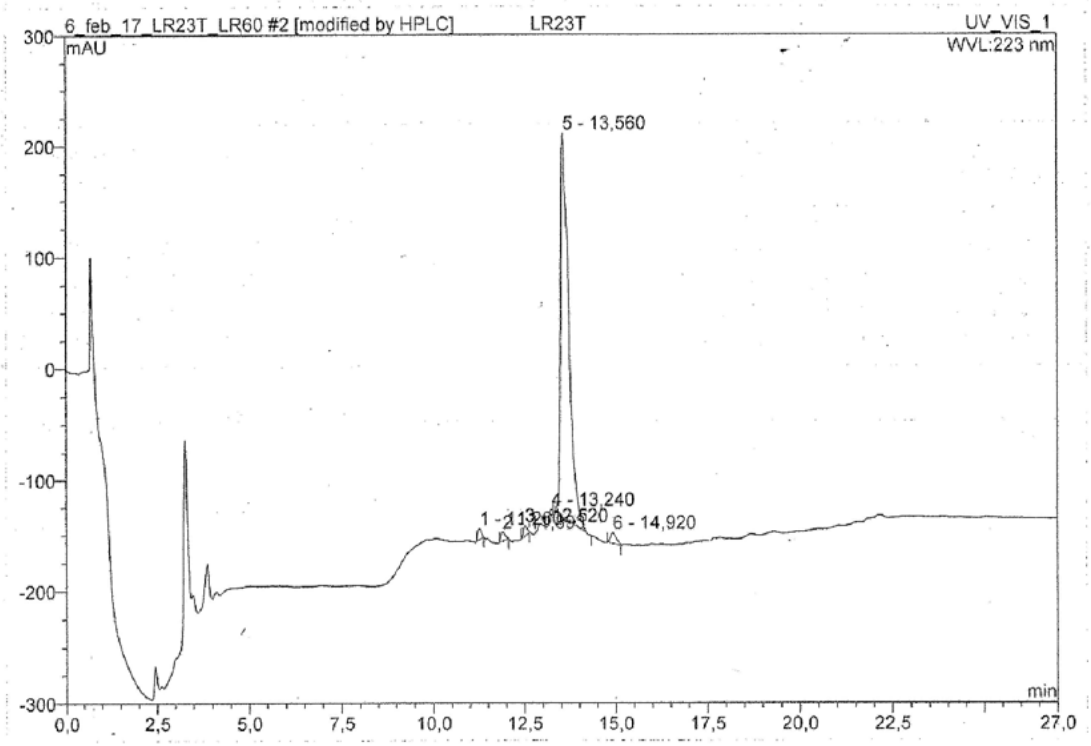


Figure S6. Variable temperature ^{1}H NMR experiment in D_2O/H_2O 1:9 on compound **35**.

IX. Figure S7. HPLC analysis of purified cyclopeptide **35**.

2 LR23T

Sample Name:	**LR23T**	*Injection Volume:*	**5,0**
Vial Number:	**BB2**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**223**
Control Program:	**Rampa_5_60**	*Bandwidth:*	**n.a.**
Quantif. Method:	**INSTRUMENTS_IQ_Iso17D**	*Dilution Factor:*	**1,0000**
Recording Time:	**6/2/2017 10.59**	*Sample Weight:*	**1,0000**
Run Time (min):	**27,00**	*Sample Amount:*	**1,0000**

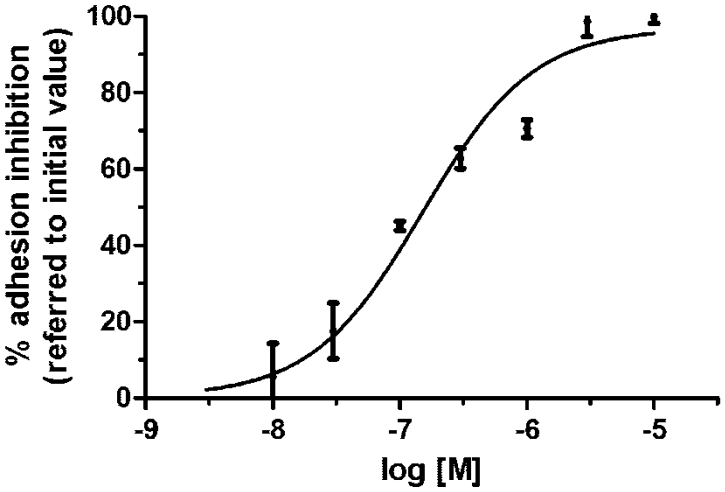


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11,26	n.a.	9,832	0,998	1,05	n.a.	BMB*
2	11,89	n.a.	7,786	1,010	1,06	n.a.	BMB*
3	12,52	n.a.	9,347	1,005	1,06	n.a.	BMB*
4	13,24	n.a.	5,678	0,546	0,57	n.a.	BMb*
5	13,56	n.a.	344,948	89,866	94,42	n.a.	bMB*
6	14,92	n.a.	9,706	1,748	1,84	n.a.	BMB*
Total:			387,297	95,173	100,00	0,000	

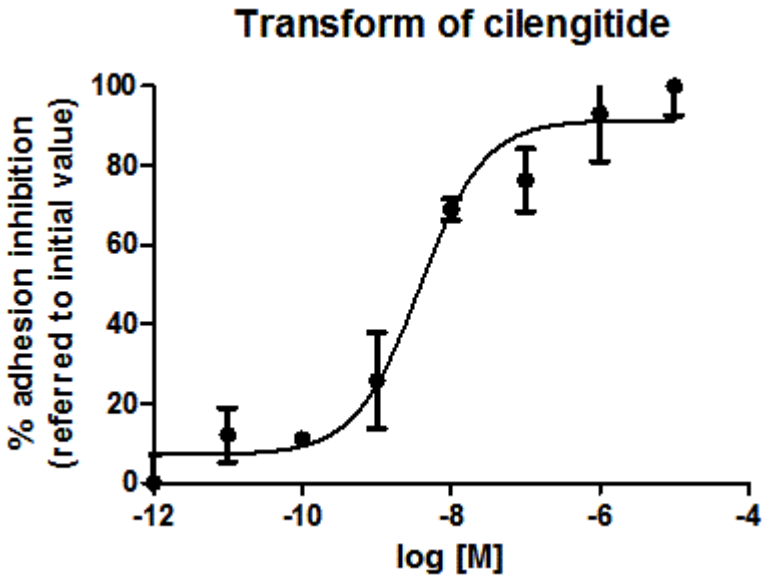
X. Biological assay

Cell lines and culture conditions. The M21 human melanoma cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD). Melanoma cells were grown in Dulbecco's modified Eagle medium, containing 4500 mg/L glucose (DMEM 4500, GIBCO) supplemented with 10% foetal calf serum (FCS) at 37 °C in a humidified incubator containing 10% CO_2. 5.0×105 melanoma cells were seeded in 100 mm Sarstedt dishes and propagated every 3 days by incubation with a trypsin−EDTA solution. Cultures were periodically monitored for mycoplasma contamination. For the use in the experiments, cells (passages 2-4) were grown to confluence in plates coated with 1% bovine gelatin (Sigma, St. Louis). *Citofluorimetric assay*. Cells were detached by gentle treatment with Accutase (Lonza), a 0.5 mM EDTA solution, washed, and incubated for 1 h at 4°C in the presence of anti-$\alpha_v\beta_3$ monoclonal antibody (1 μg/50 μL, anti-integrin $\alpha_v\beta_3$, clone LM609, Millipore) and anti-$\alpha_v\beta_5$ monoclonal antibody (1 μg/50 μL, anti-integrin $\alpha_v\beta_5$, Santa Cruz 13588). Cells were then washed and incubated for 1 h at 4 °C with a specific secondary antibody, 5 μg/mL goat antimouse IgG conjugated with FITC (Santa Cruz Biotecnology, Inc., Santa Cruz, CA). Integrin-Positive cells were analyzed at 488 nm on the flow cytometer FACScan system (BD-FACS Canto). *Cell adhesion assay*. Plates (96 wells) were coated with vitronectin (10 μg/mL) by overnight incubation at 4 °C. Plates were washed with PBS and then incubated at 37 °C for 1 h with PBS−1% BSA. After being washed tumor cells were counted and resuspended in serum free medium, and exposed to the compound (final concentration was 0.1, 1, and 10 pM, 0.1, 1, and 10 nM, 0.1, 1, and 10 μM) at 37 °C for 30 min to allow the ligand-receptor equilibrium to be reached. Assays were performed in the presence of 2 mmol/L $MnCl_2$. Cells were then plated (4−5×104 cells/well) and incubated at 37 °C for 1 h. All the wells were washed with PBS to remove the non adherent cells, and 0.5% crystal violet solution in 20% methanol was added. After 2 h of incubation at 4 °C, plates were examined at 540 nm in a counter ELX800 (Bio TEK Instruments). Experiments were conducted in triplicate and were repeated at least three times. The values are expressed as % inhibition ± SEM of cell adhesion relative to untreated cells. *Data Analysis*. The IC50 values were determined by fitting binding inhibition data by non-linear regression using GraphPad Prism 4.0 Software Package (GraphPad Prism, San Diego, CA).

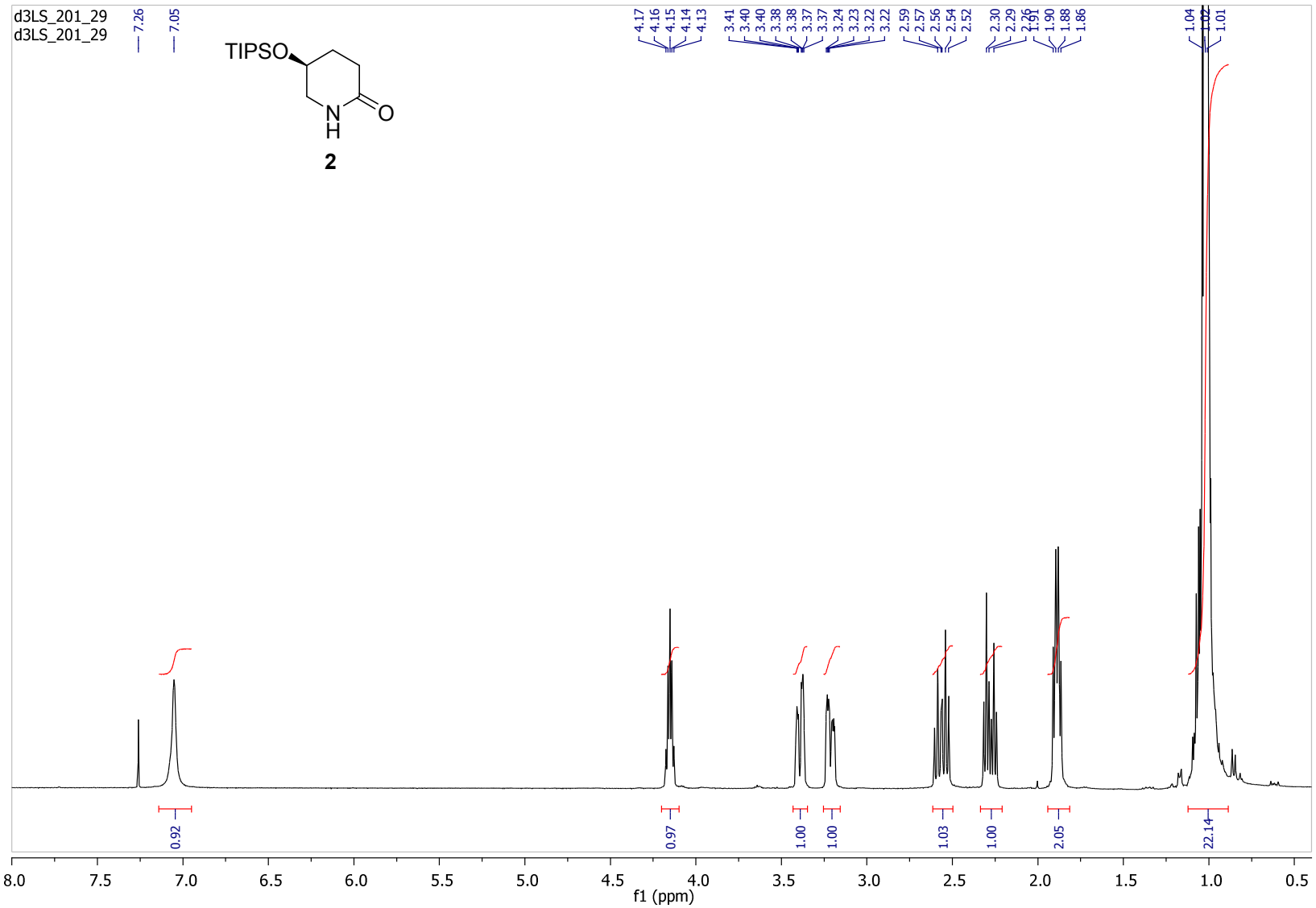
Figure S8.



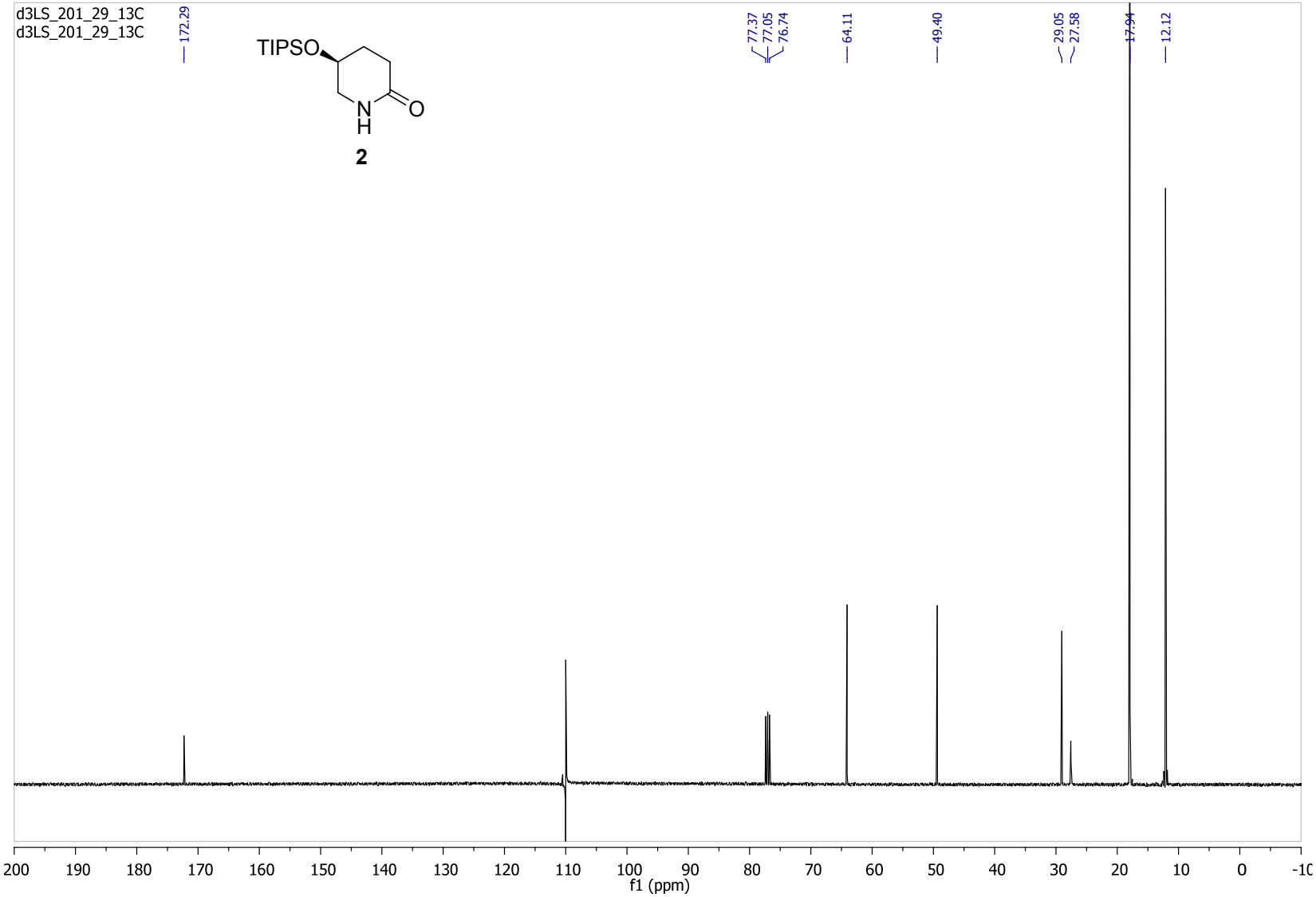
a) Percentage of inhibition of integrin-mediated M21 cell adhesion to vitronectin by compound **35**. Experiments were conducted in triplicate and repeated at least three times. Data are presented as means ±SEM from three independent experiments.



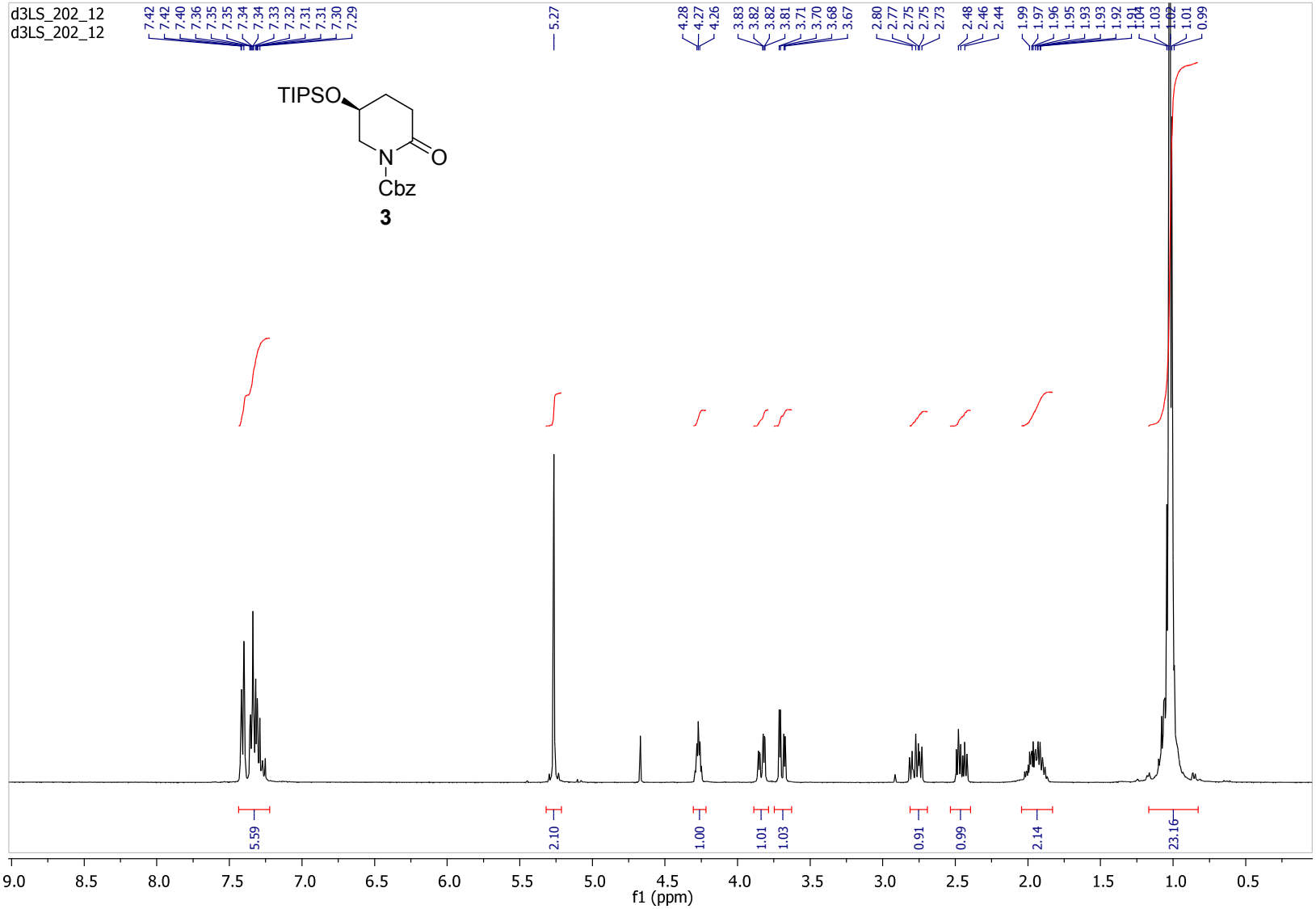
b) Percentage of inhibition of integrin-mediated M21 cell adhesion to vitronectin by cilengitide. Experiments were conducted in triplicate and repeated at least three times. Data are presented as means ±SEM from three independent experiments.



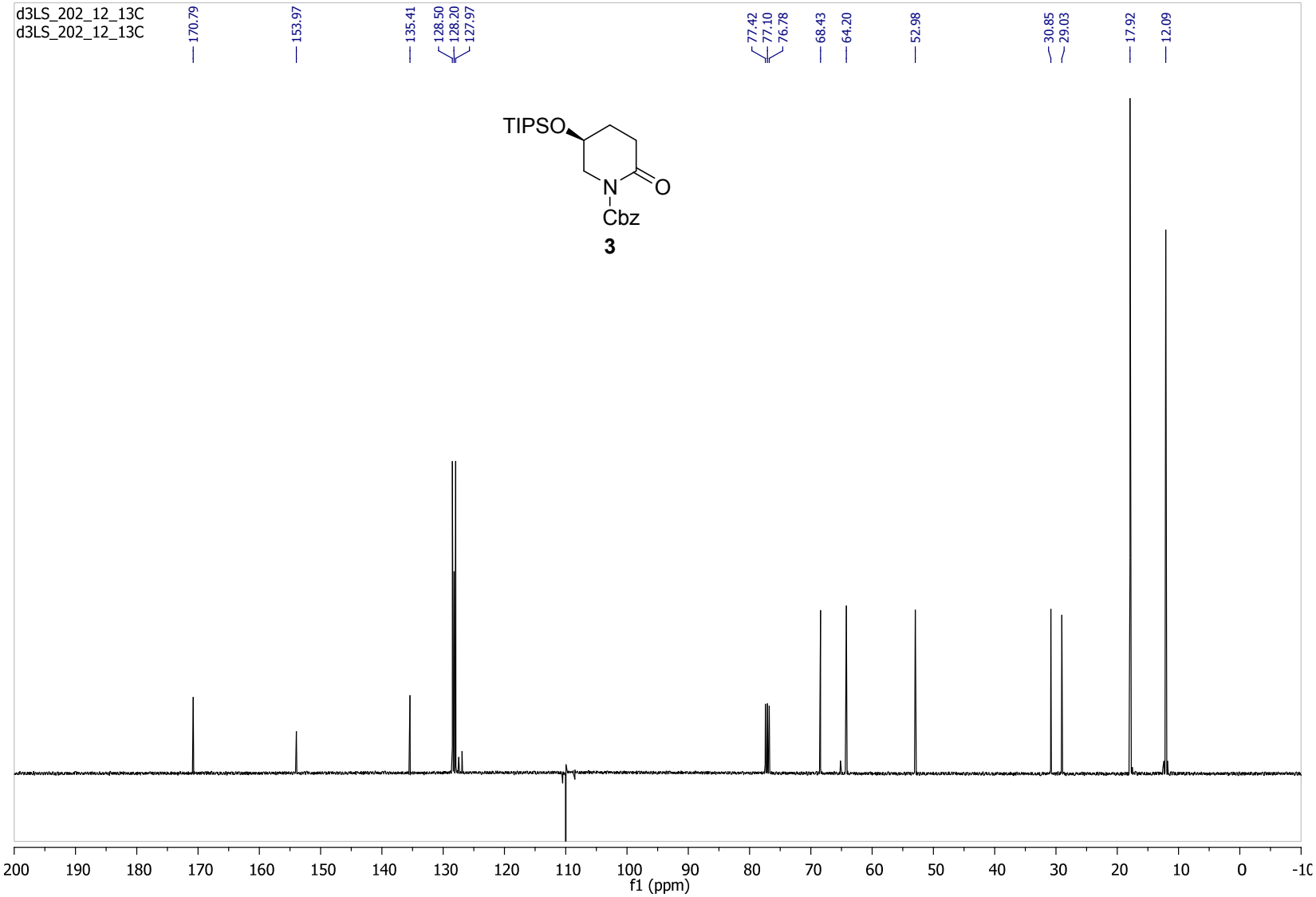
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **2**



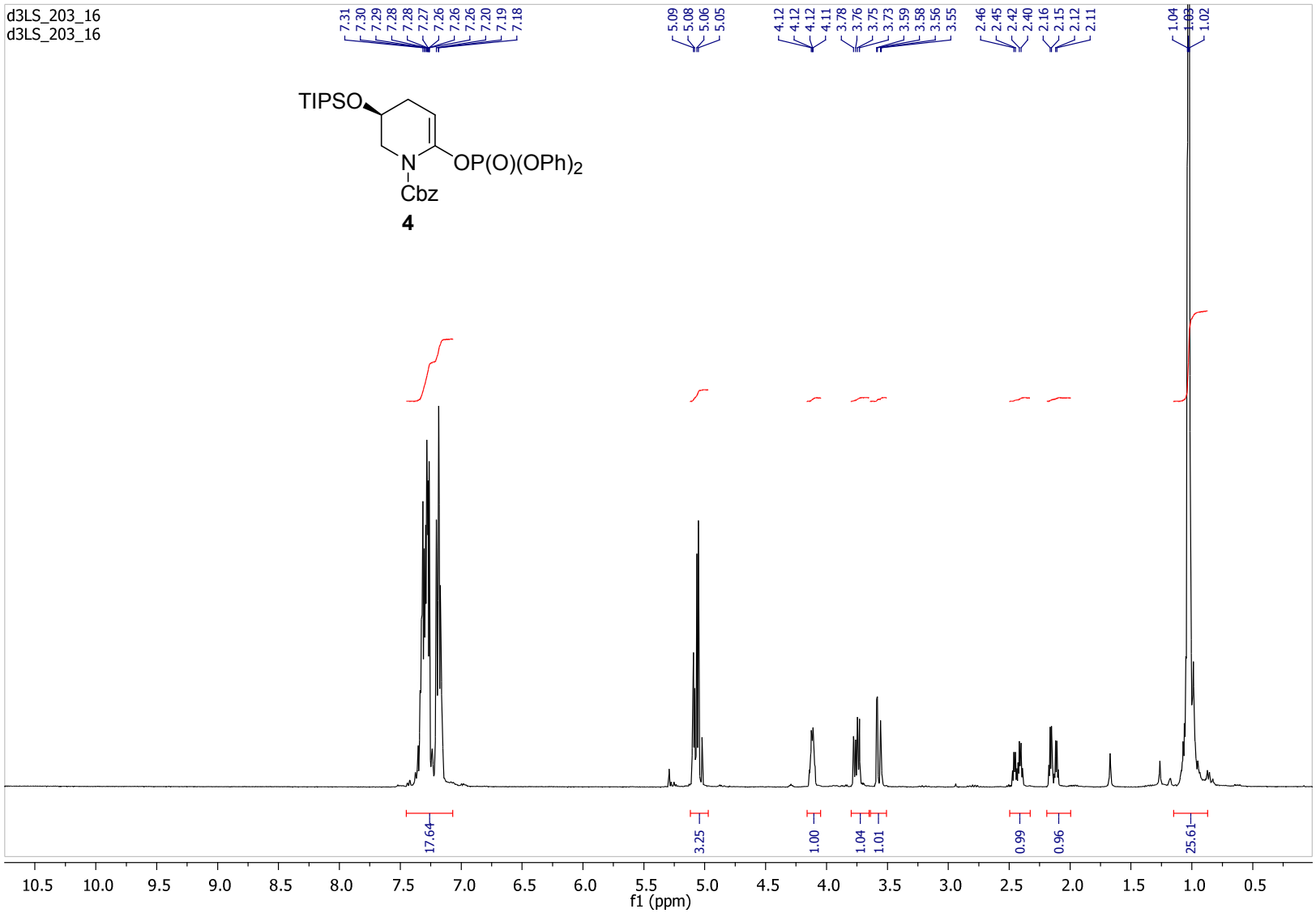
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **2**



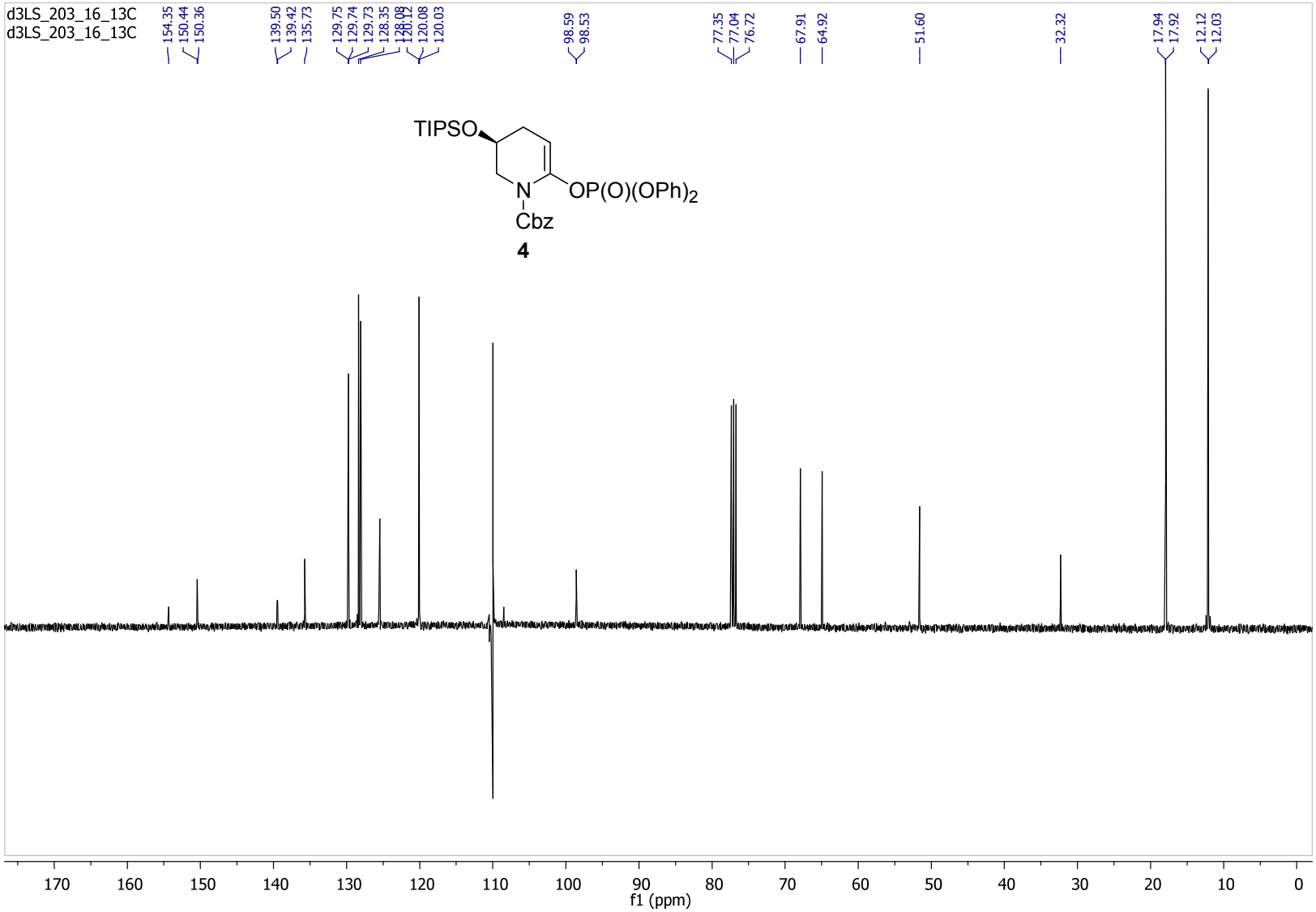
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **3**



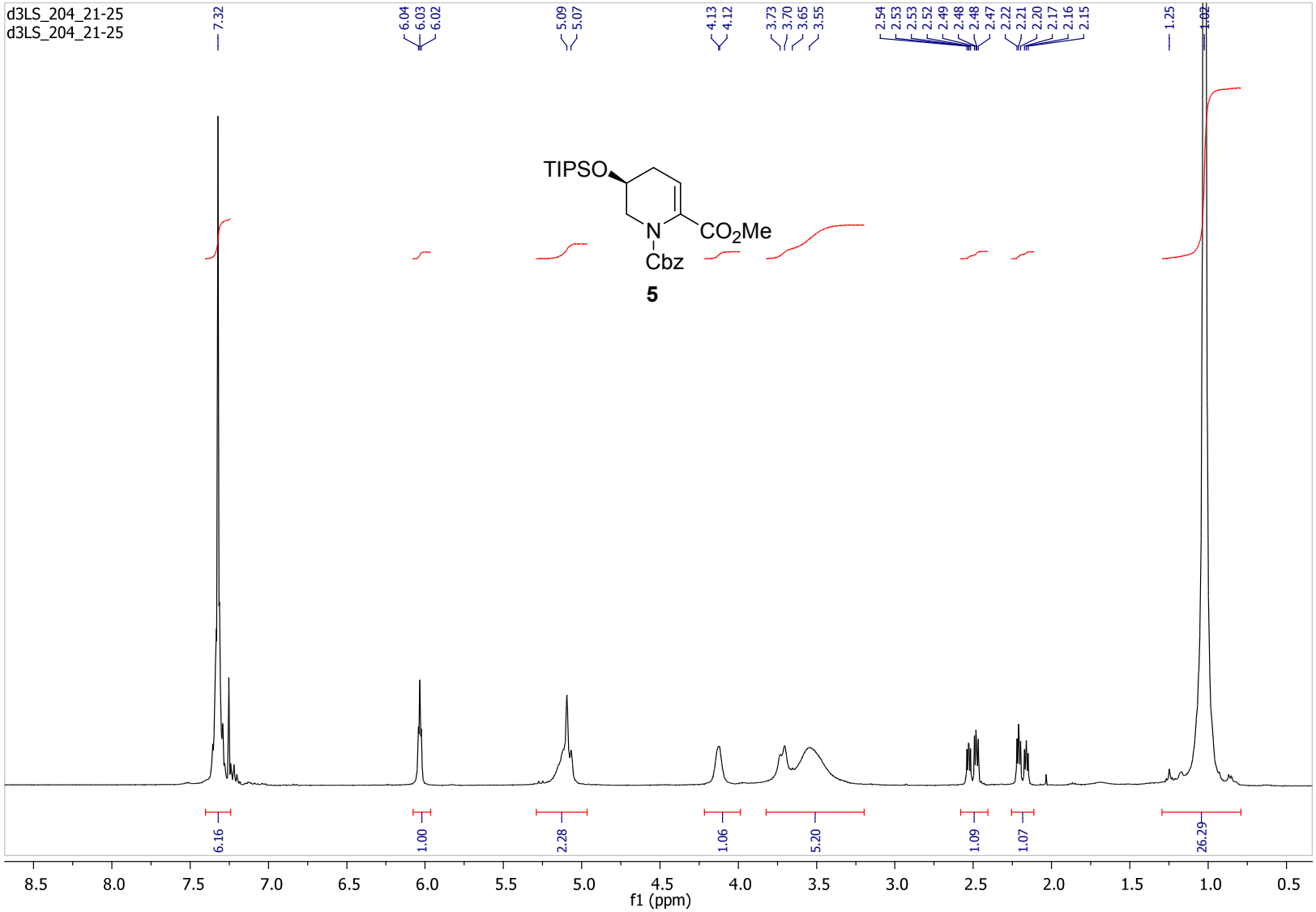
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **3**



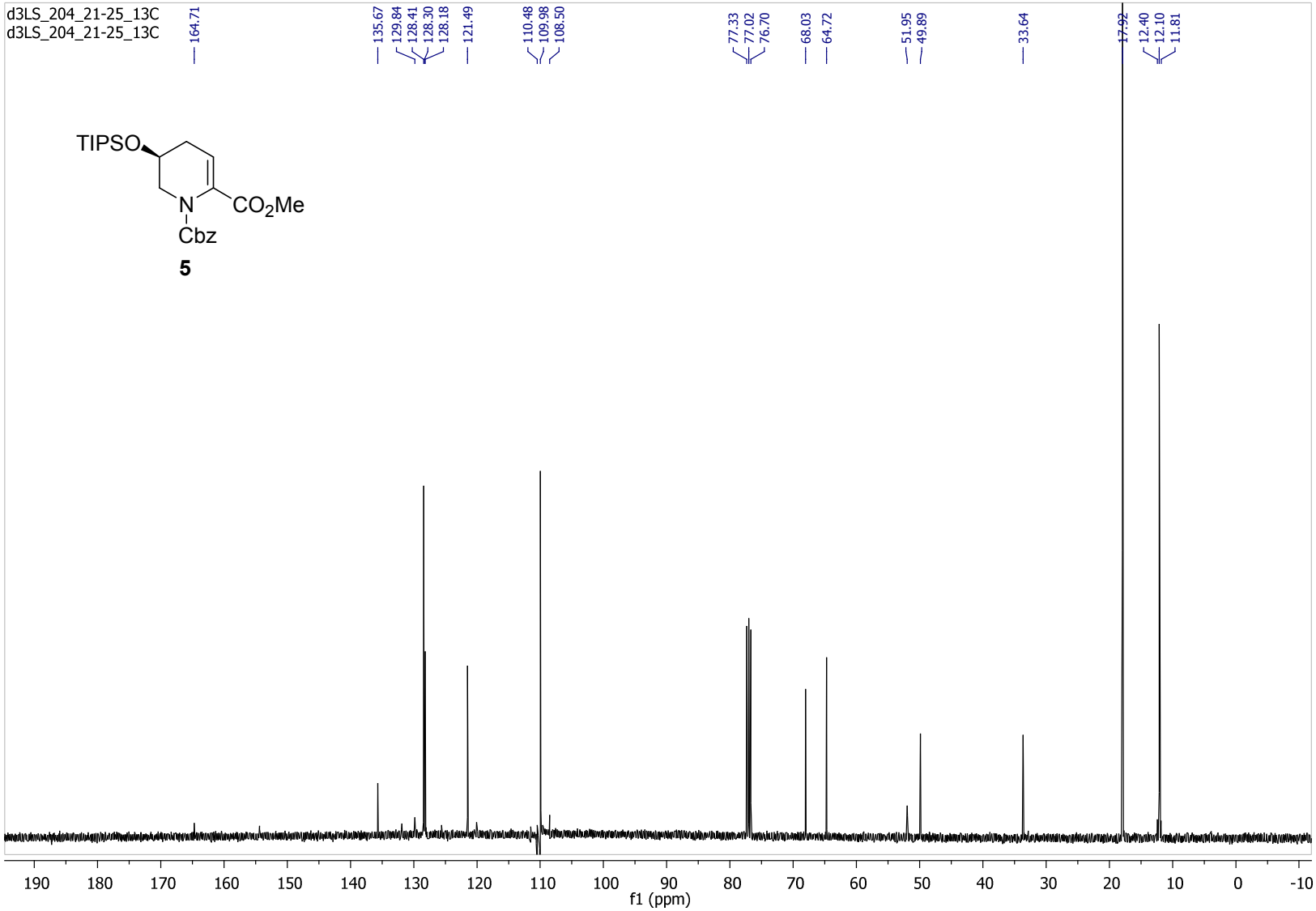
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **4**



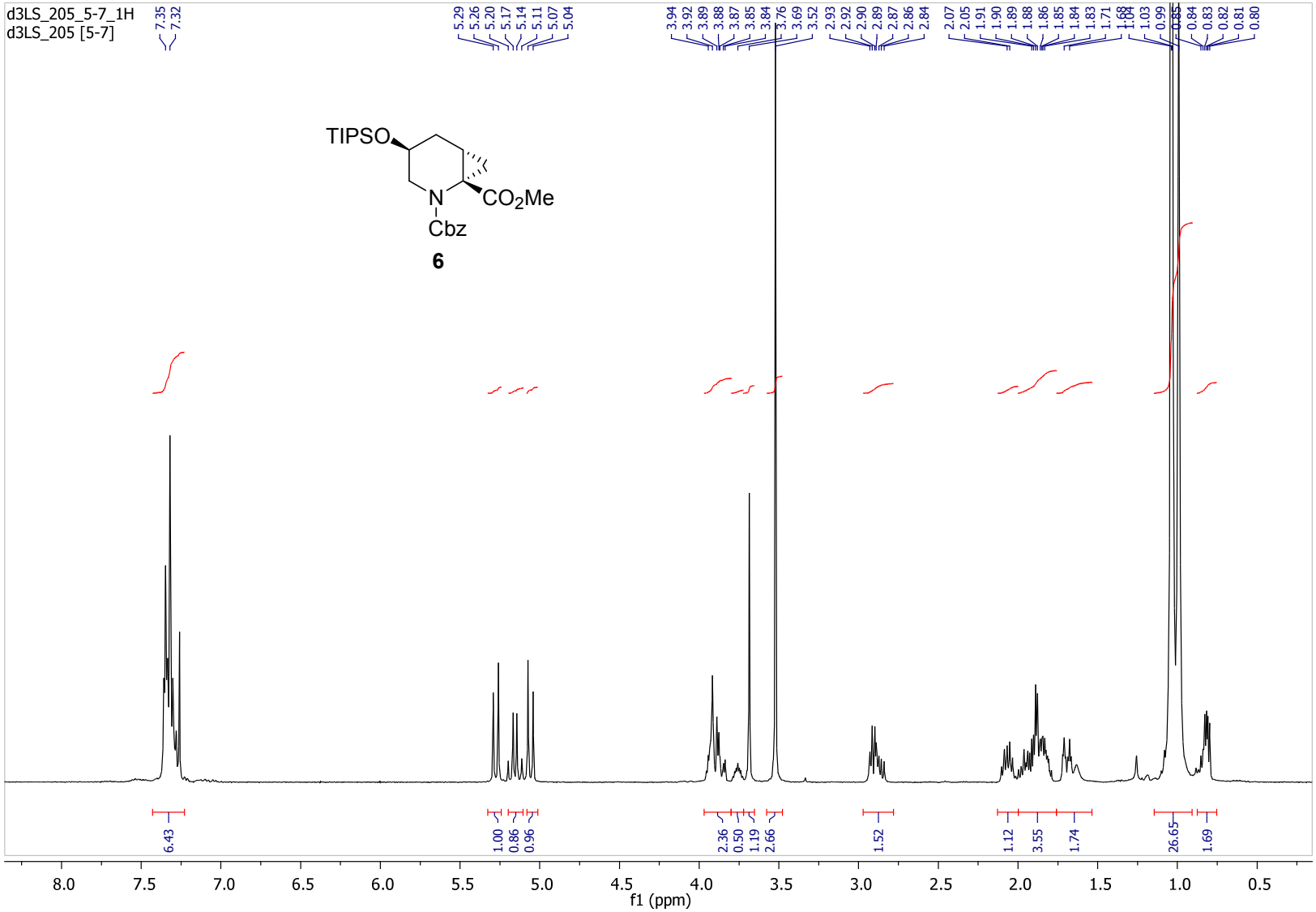
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **4**



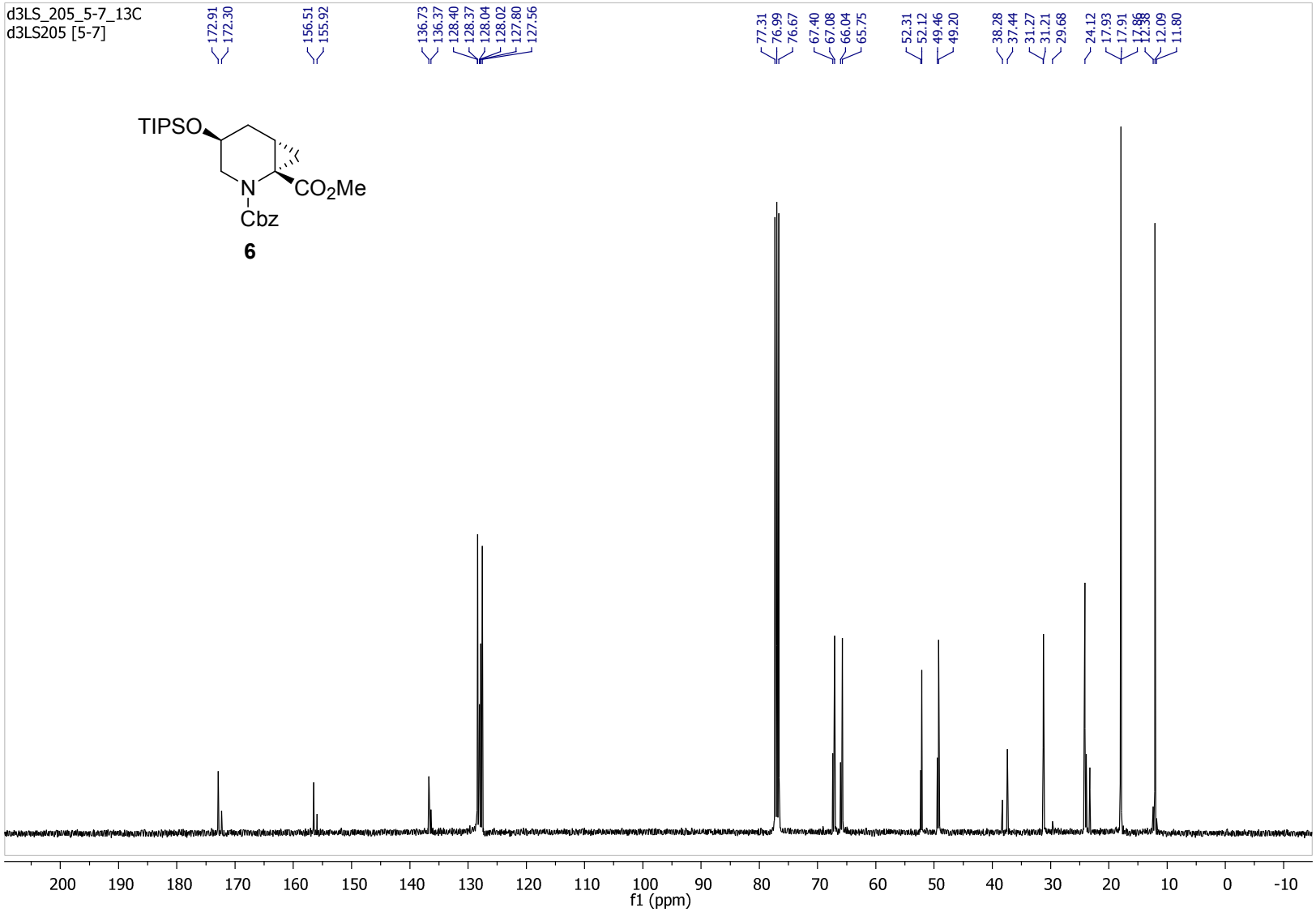
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **5**



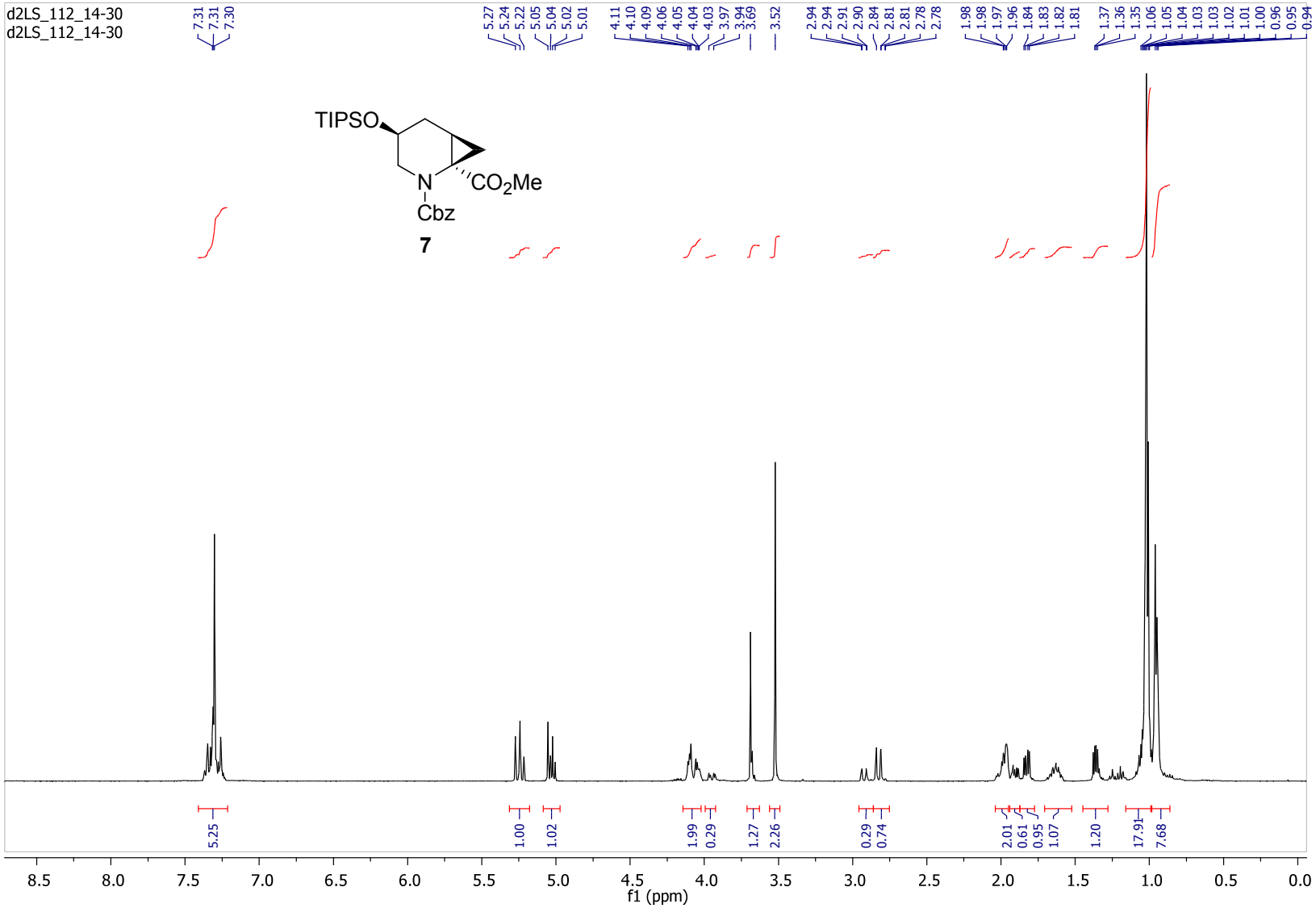
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **5**



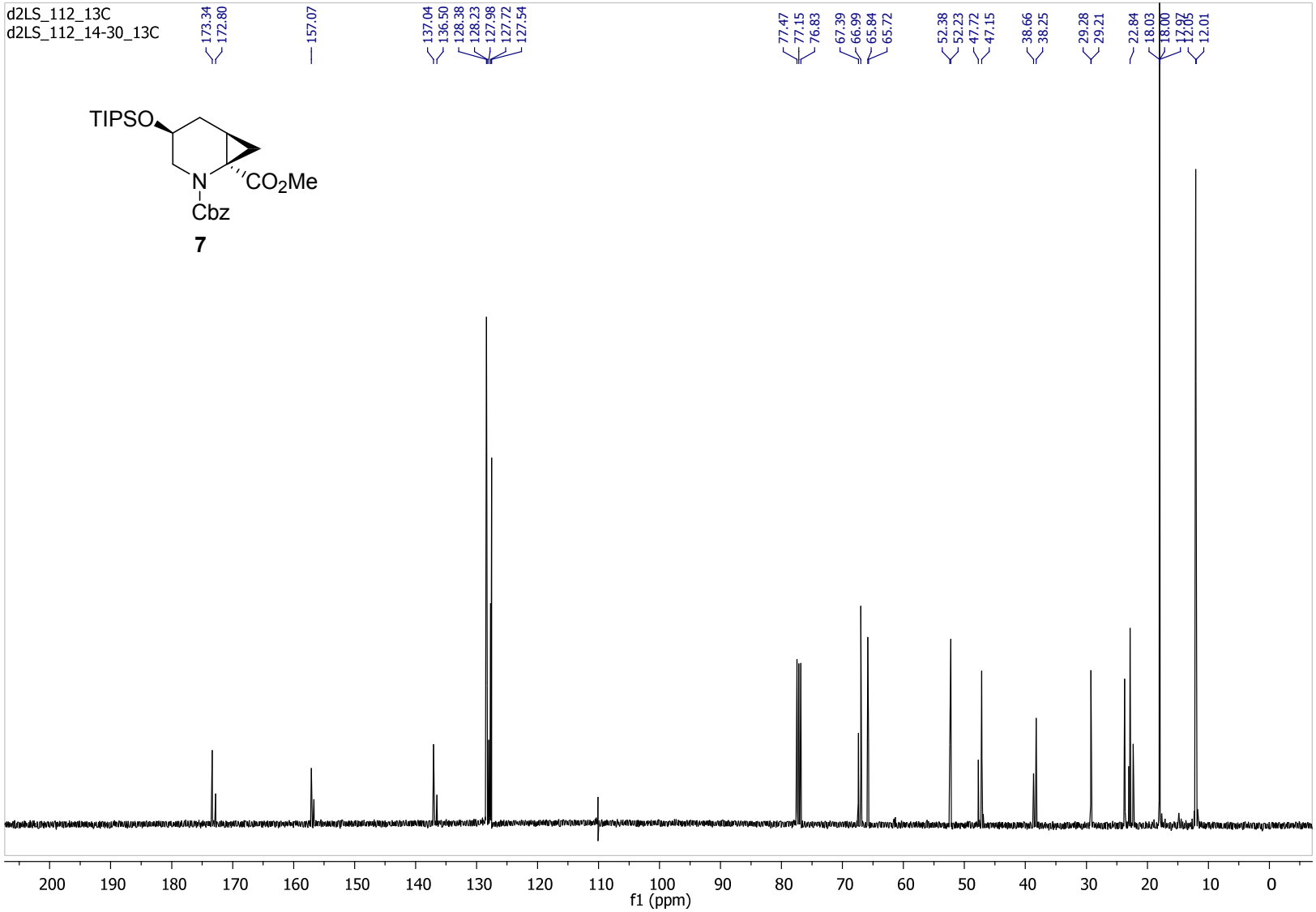
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **6**



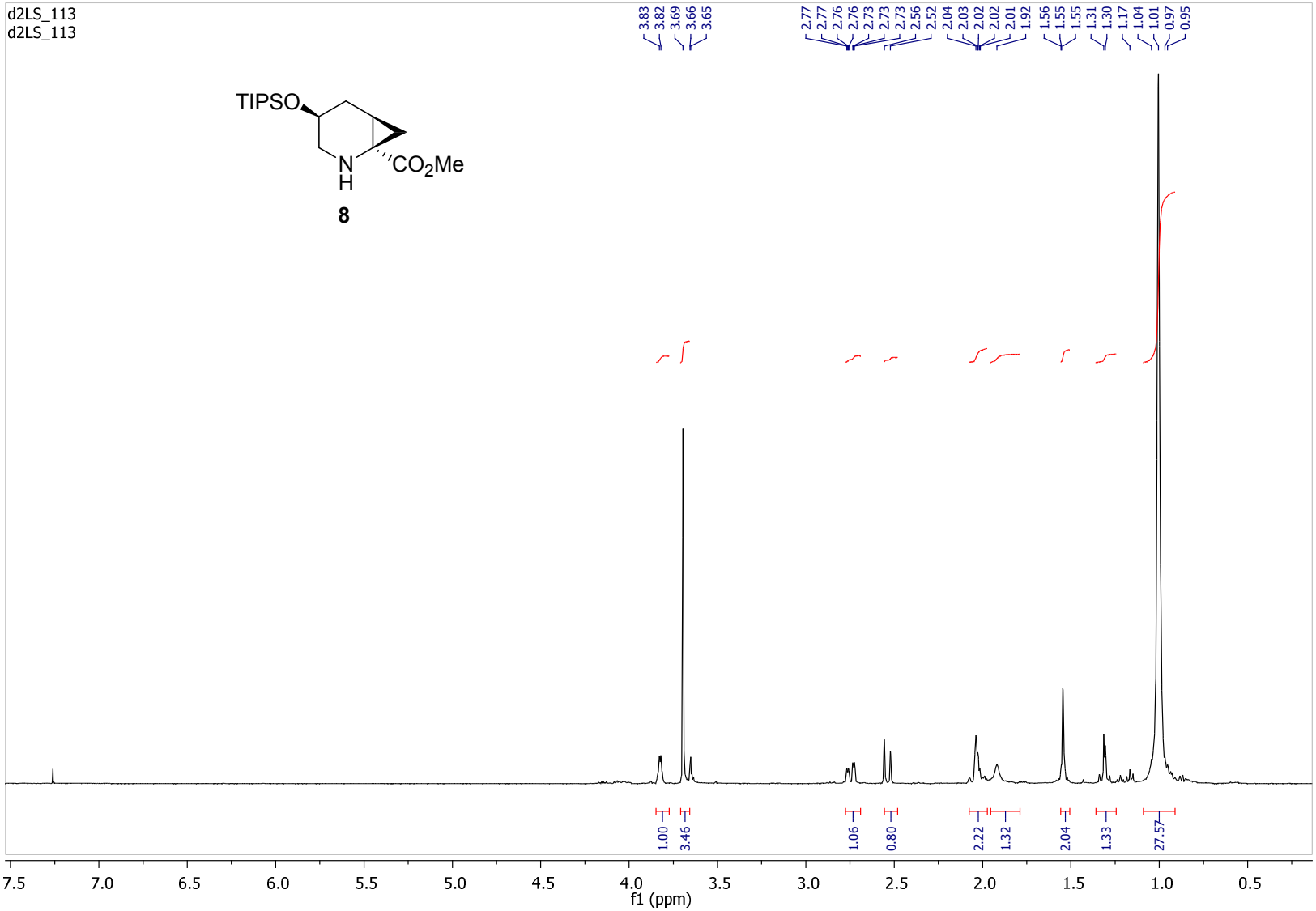
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **6**



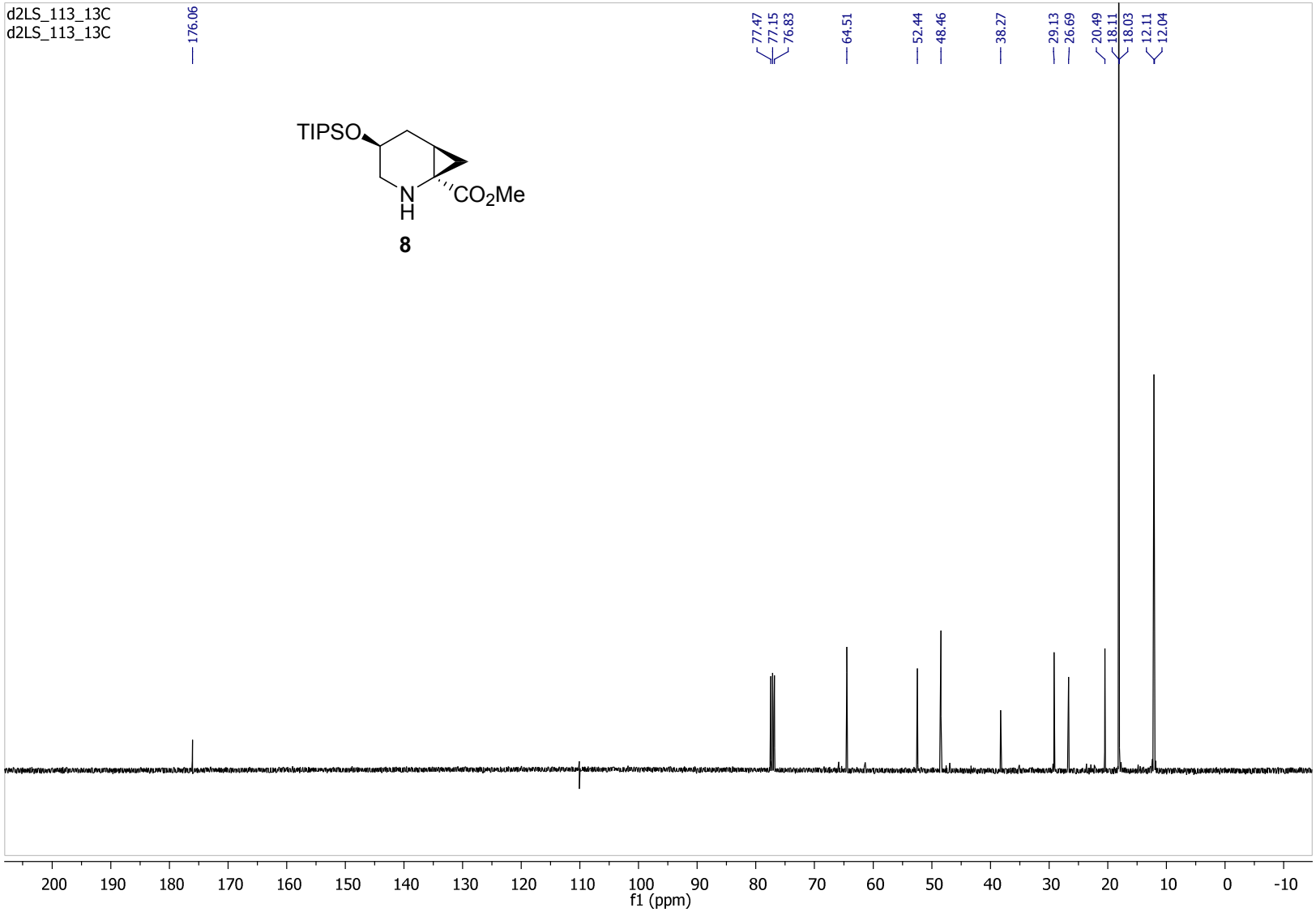
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **7**



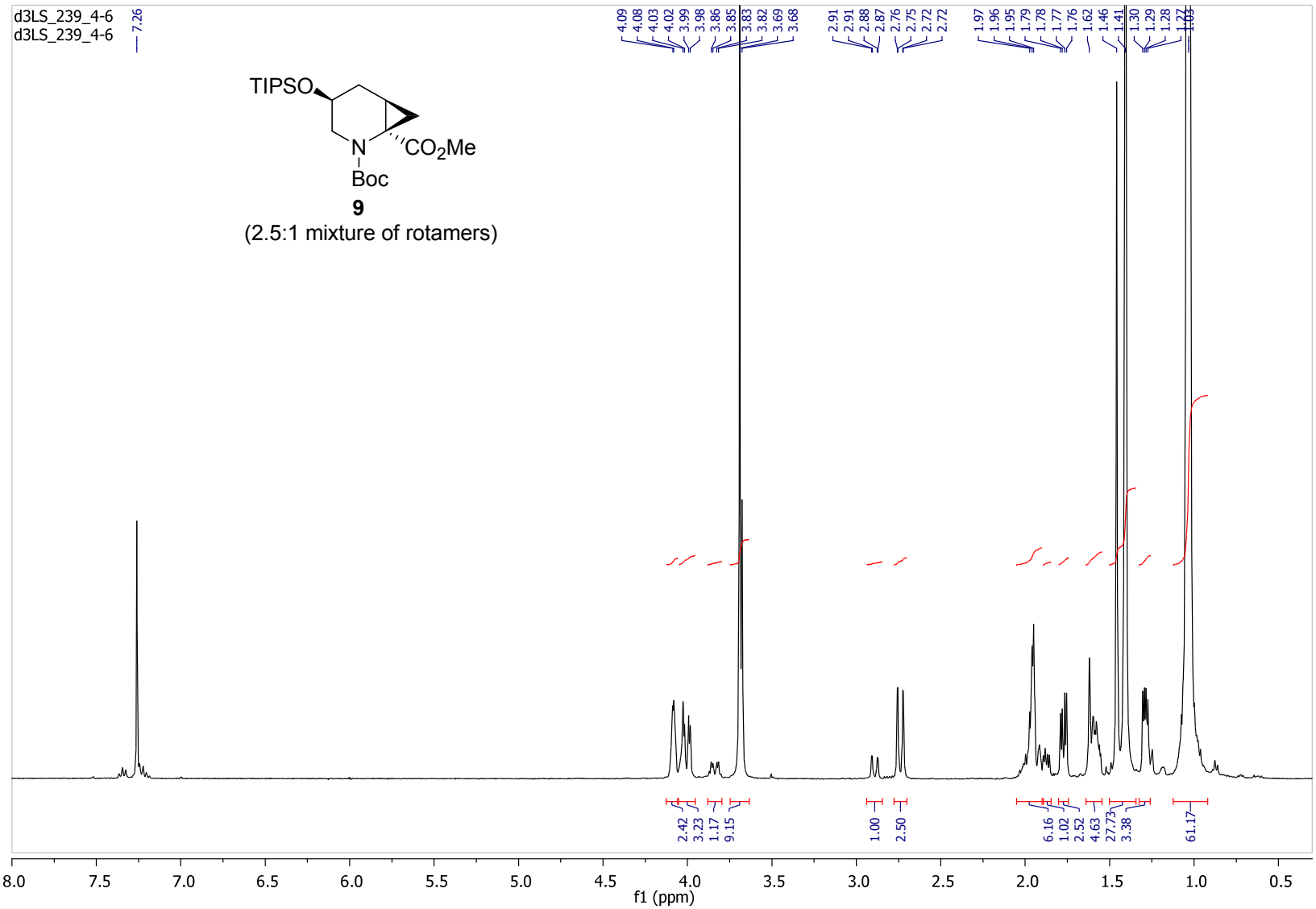
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **7**



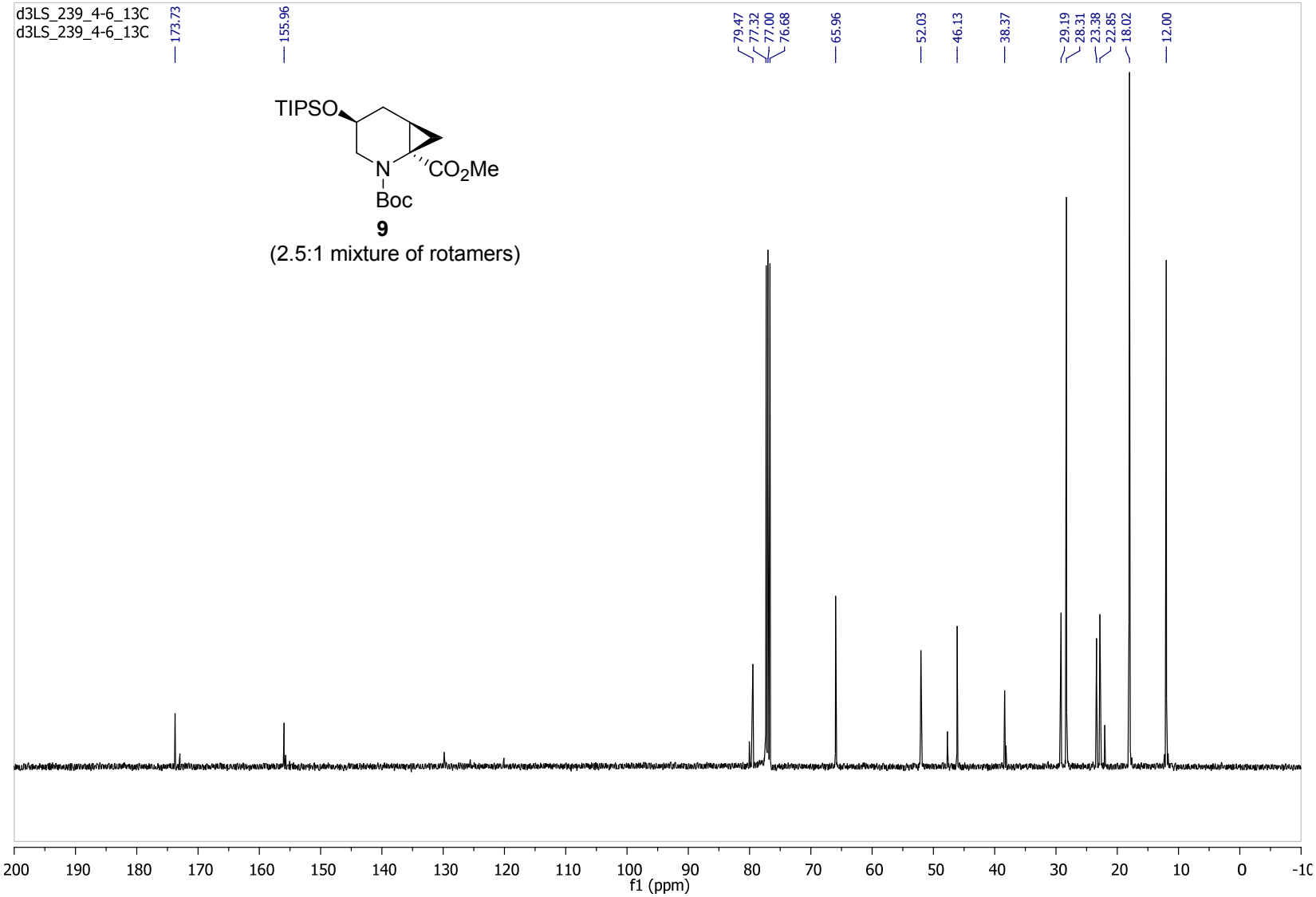
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **8**



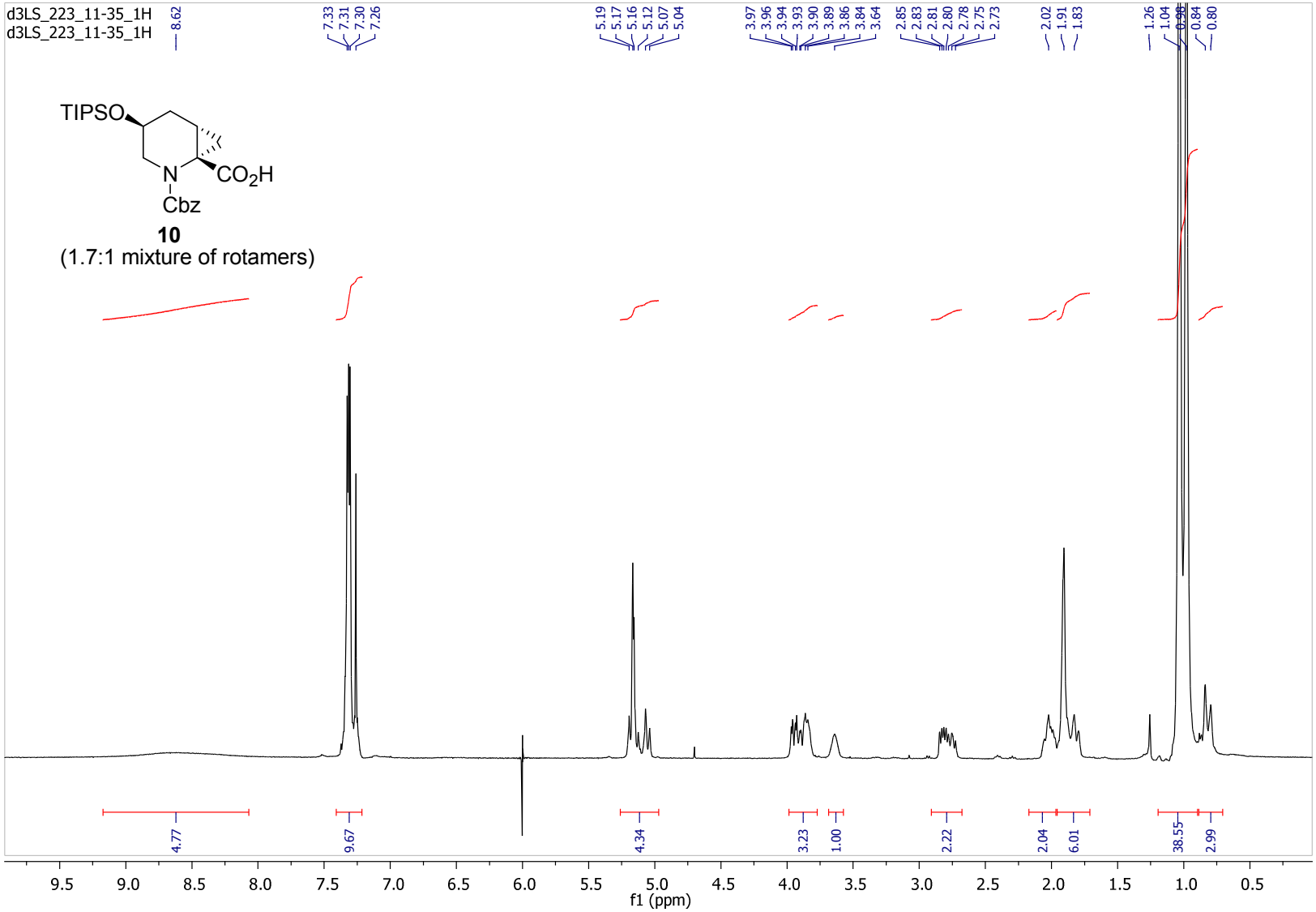
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **8**



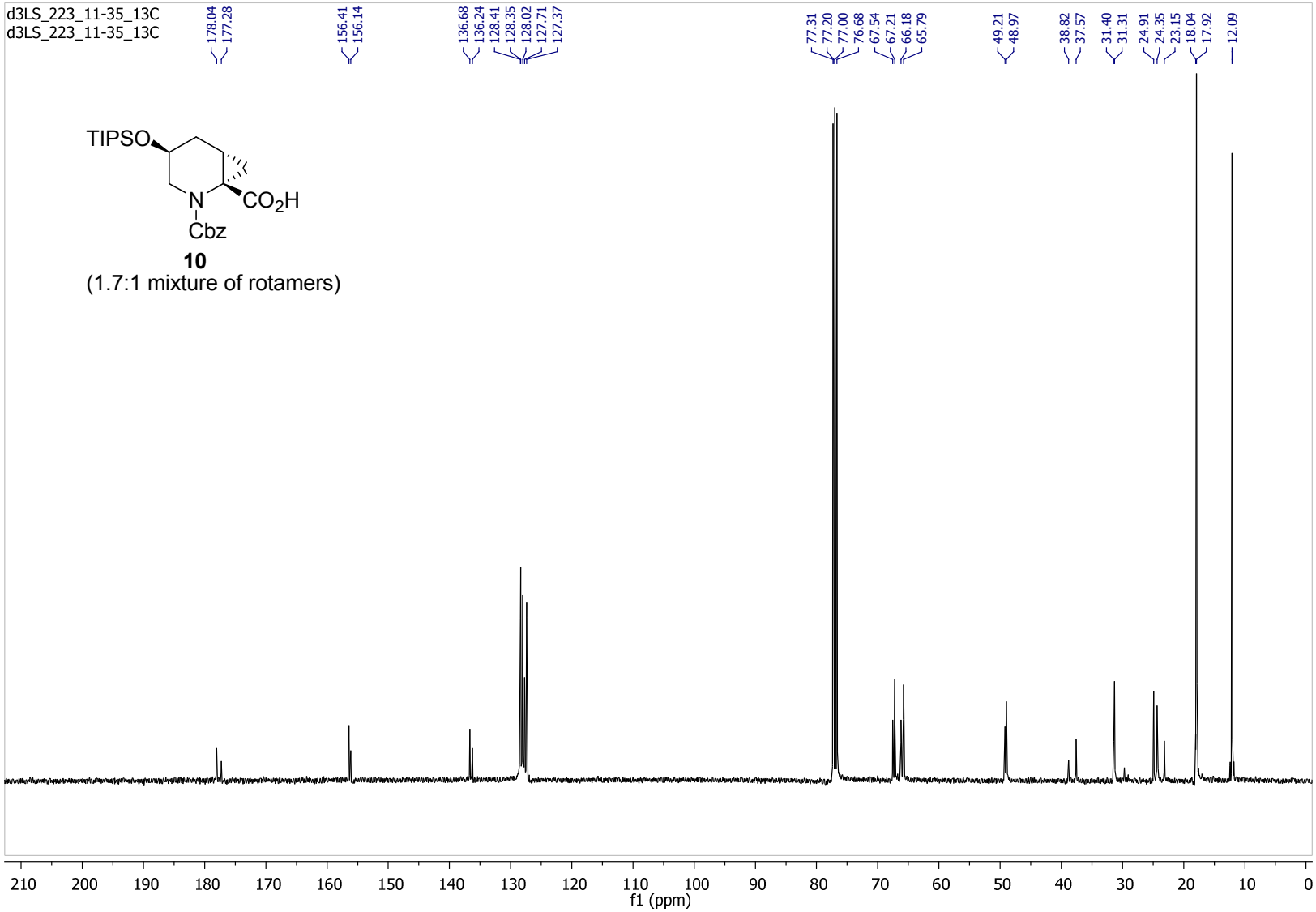
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **9**



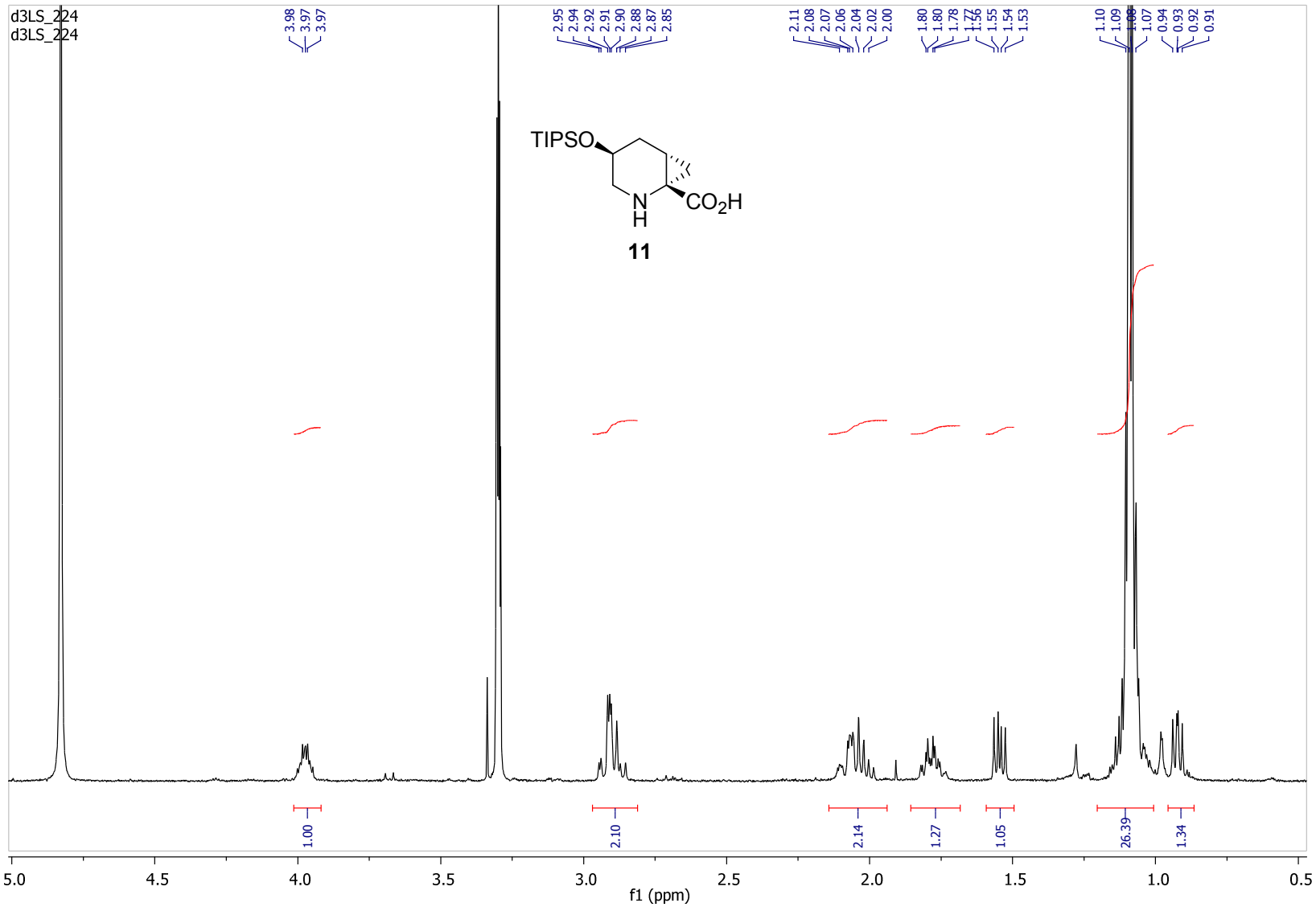
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **9**



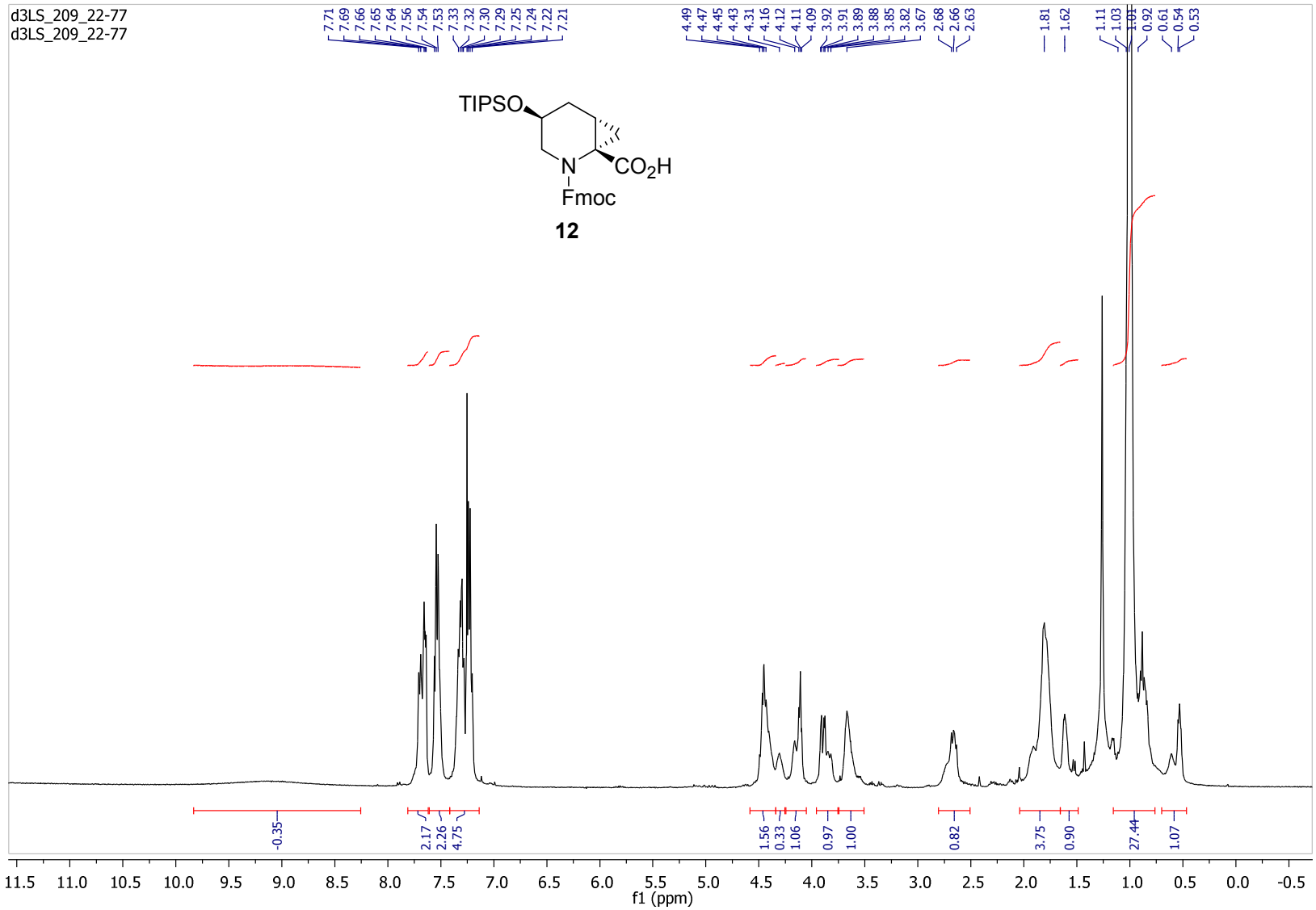
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **10**



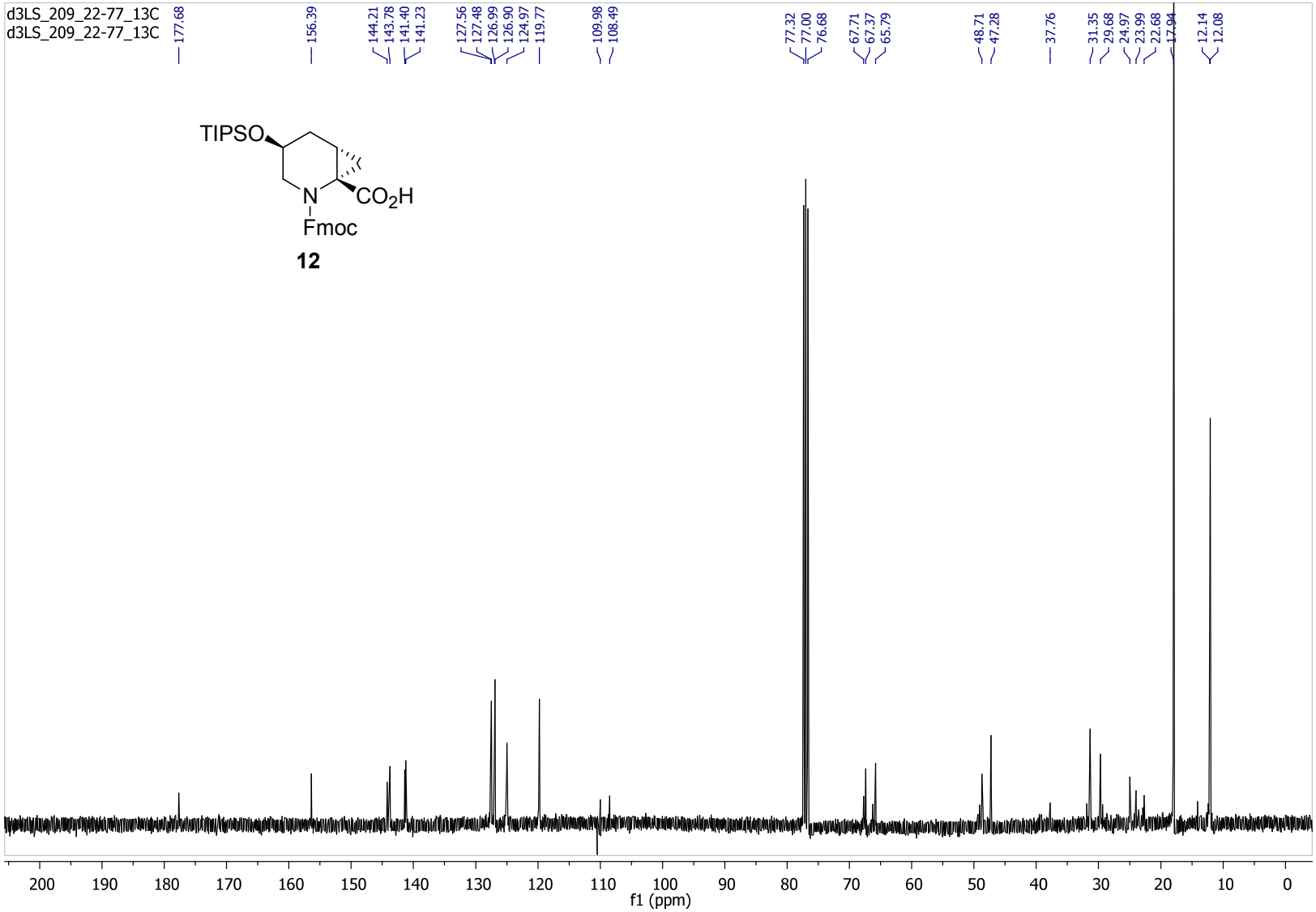
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **10**



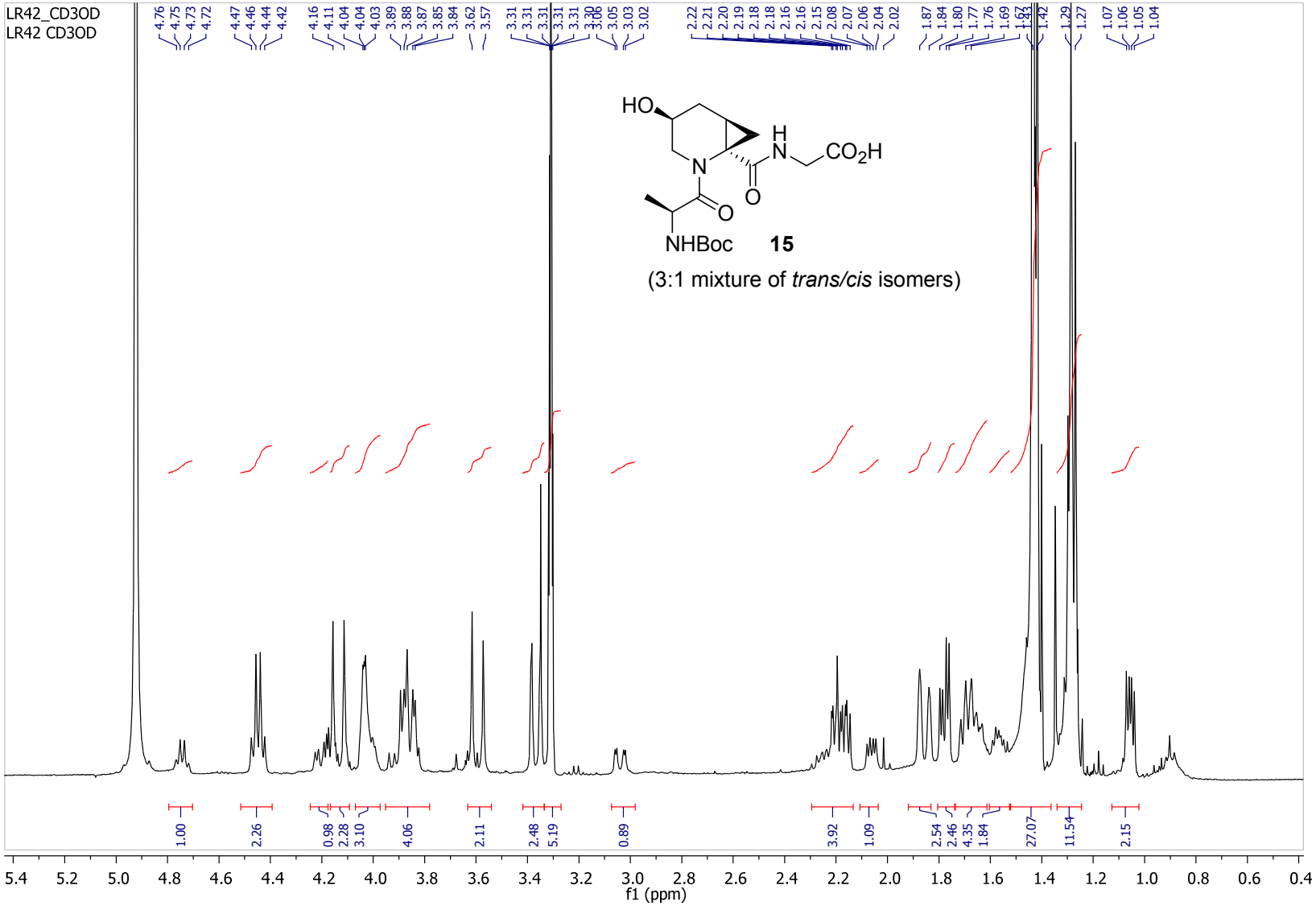
^{1}H NMR (CD_3OD, 400 MHz) of compound **11**



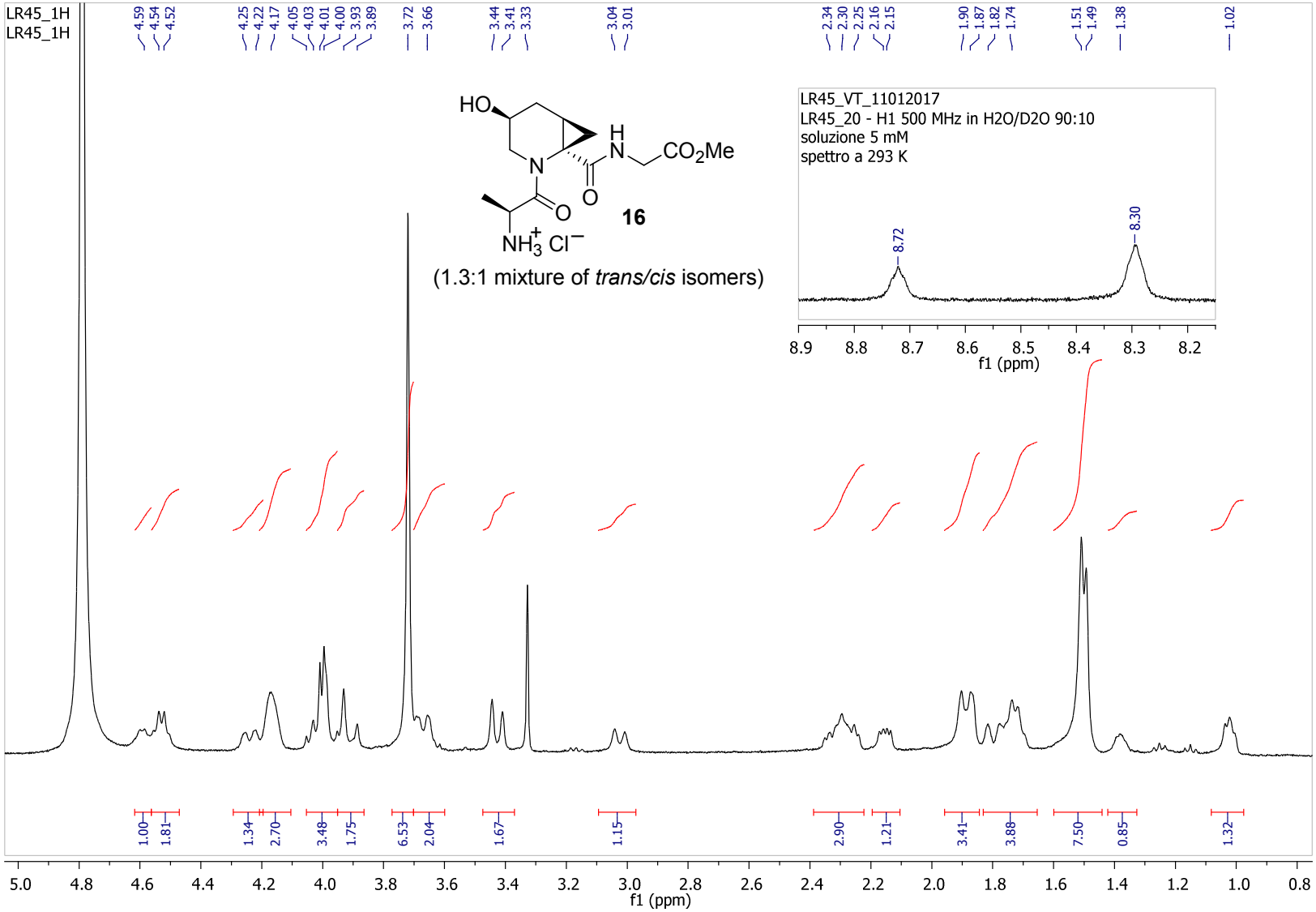
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **12**



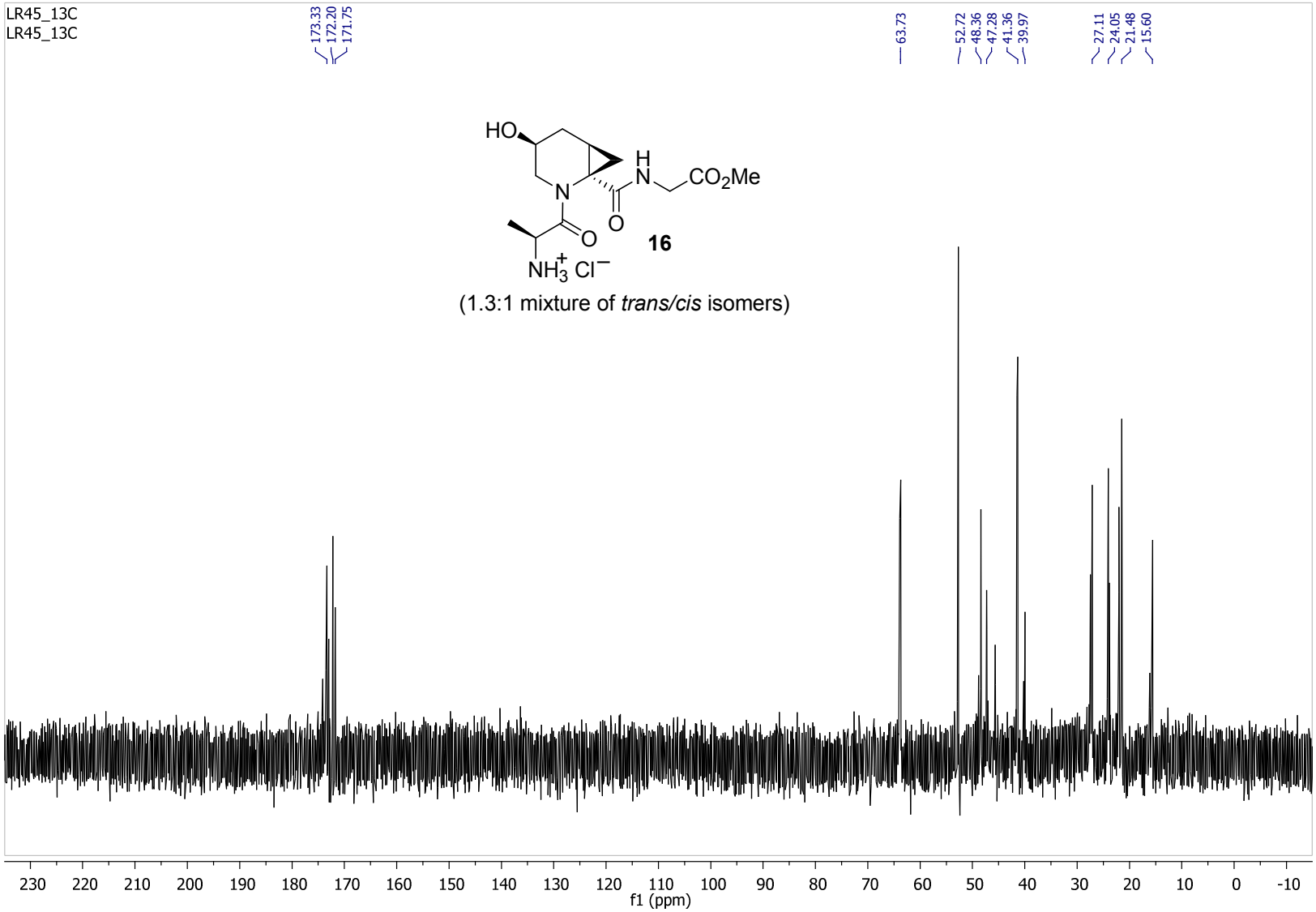
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **12**



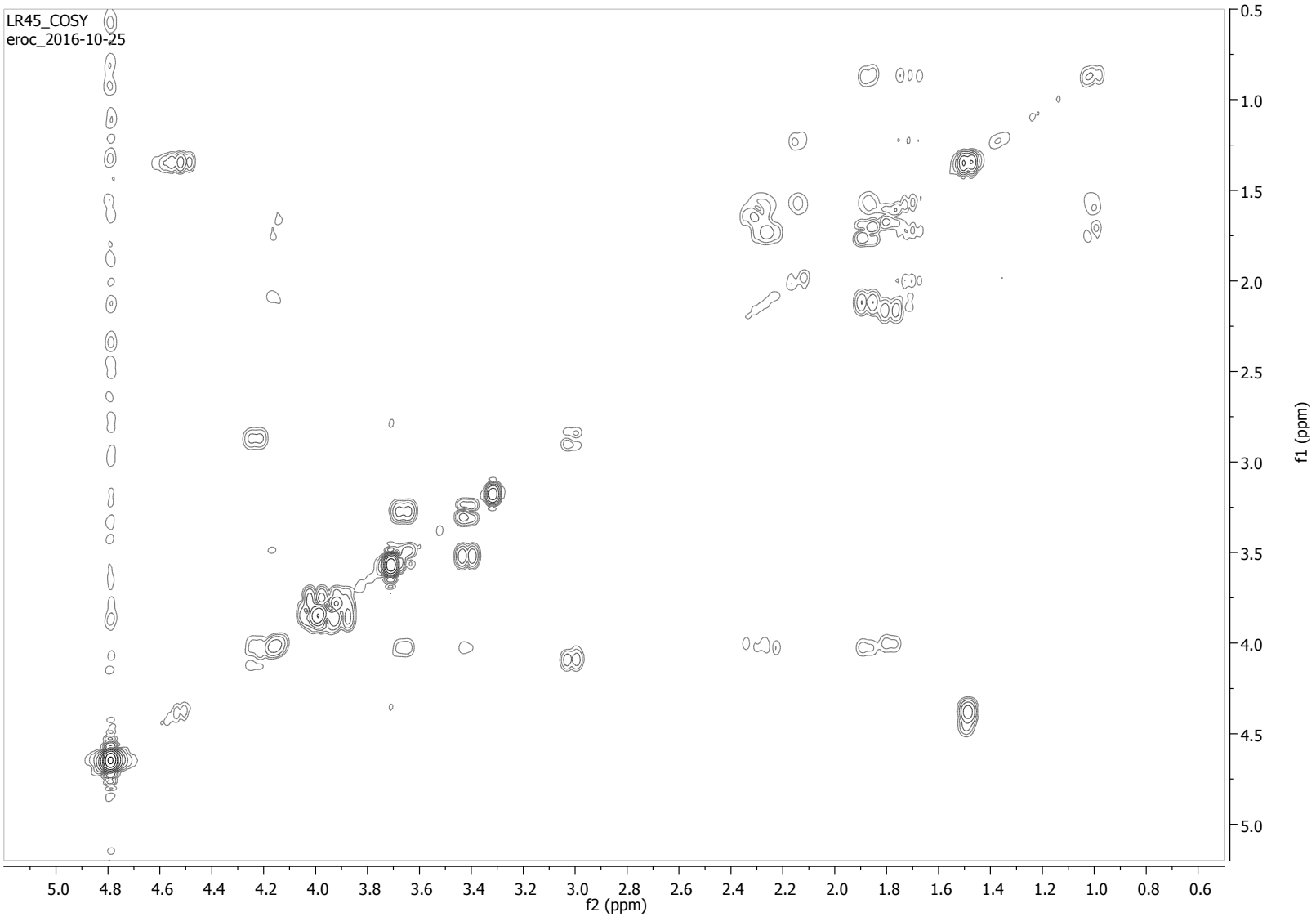
^{1}H NMR (CD_3OD, 400 MHz) of compound **15**



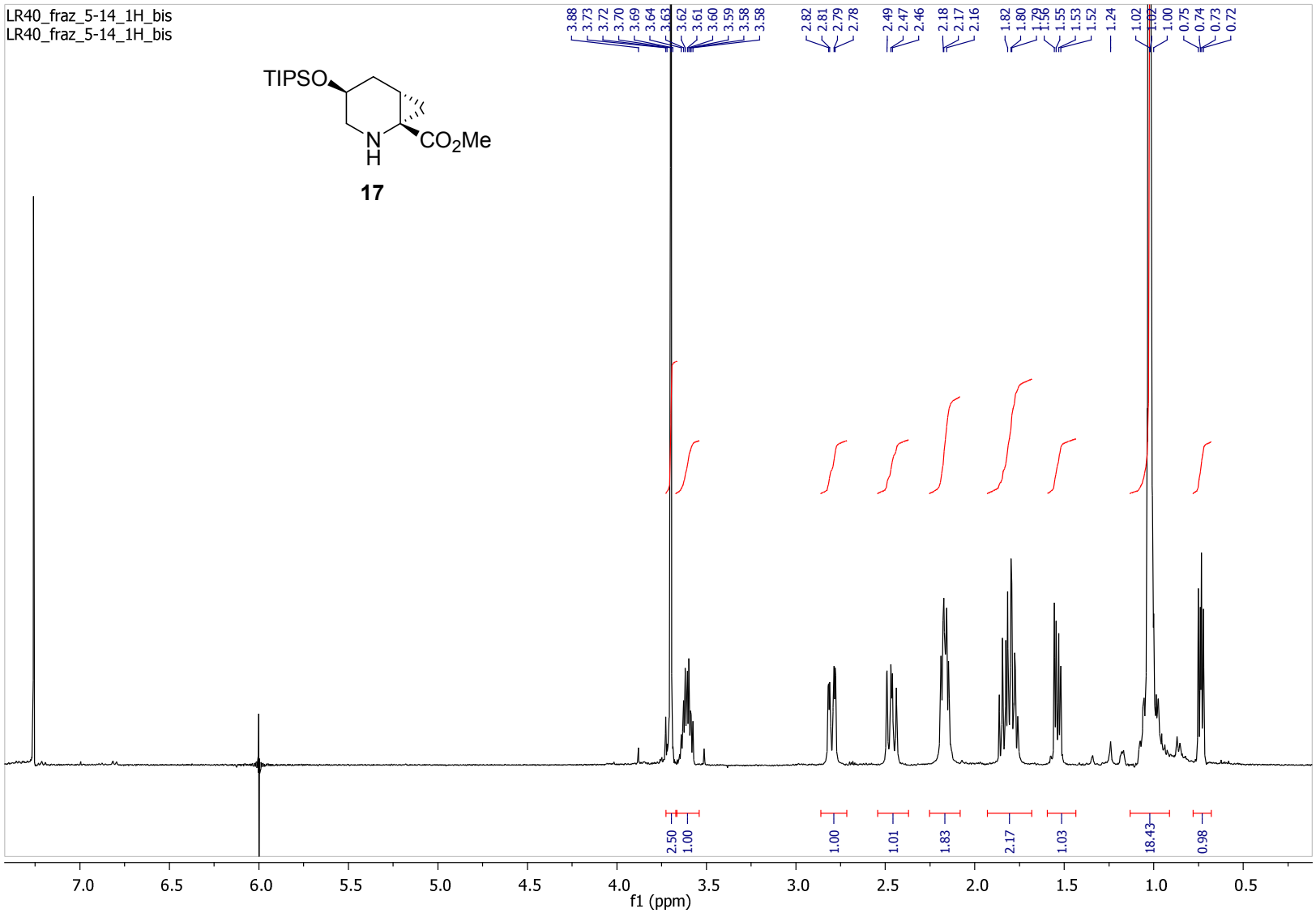
^{1}H NMR (400 MHz) of compound **16** in D_2O and in H_2O/D_2O 9:1 (500 MHz, inset)



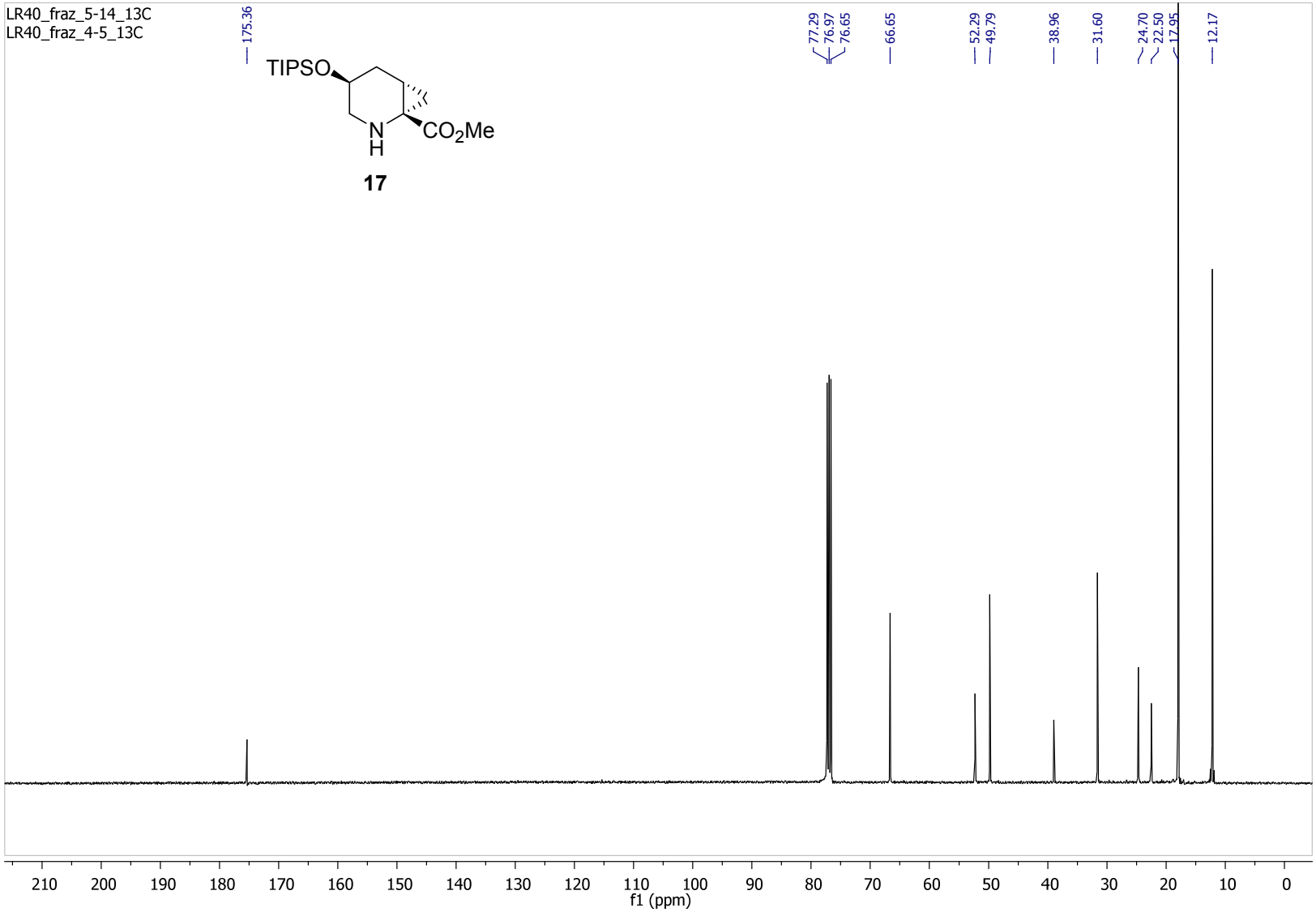
^{13}C NMR (D_2O, 100.4 MHz) of compound **16**



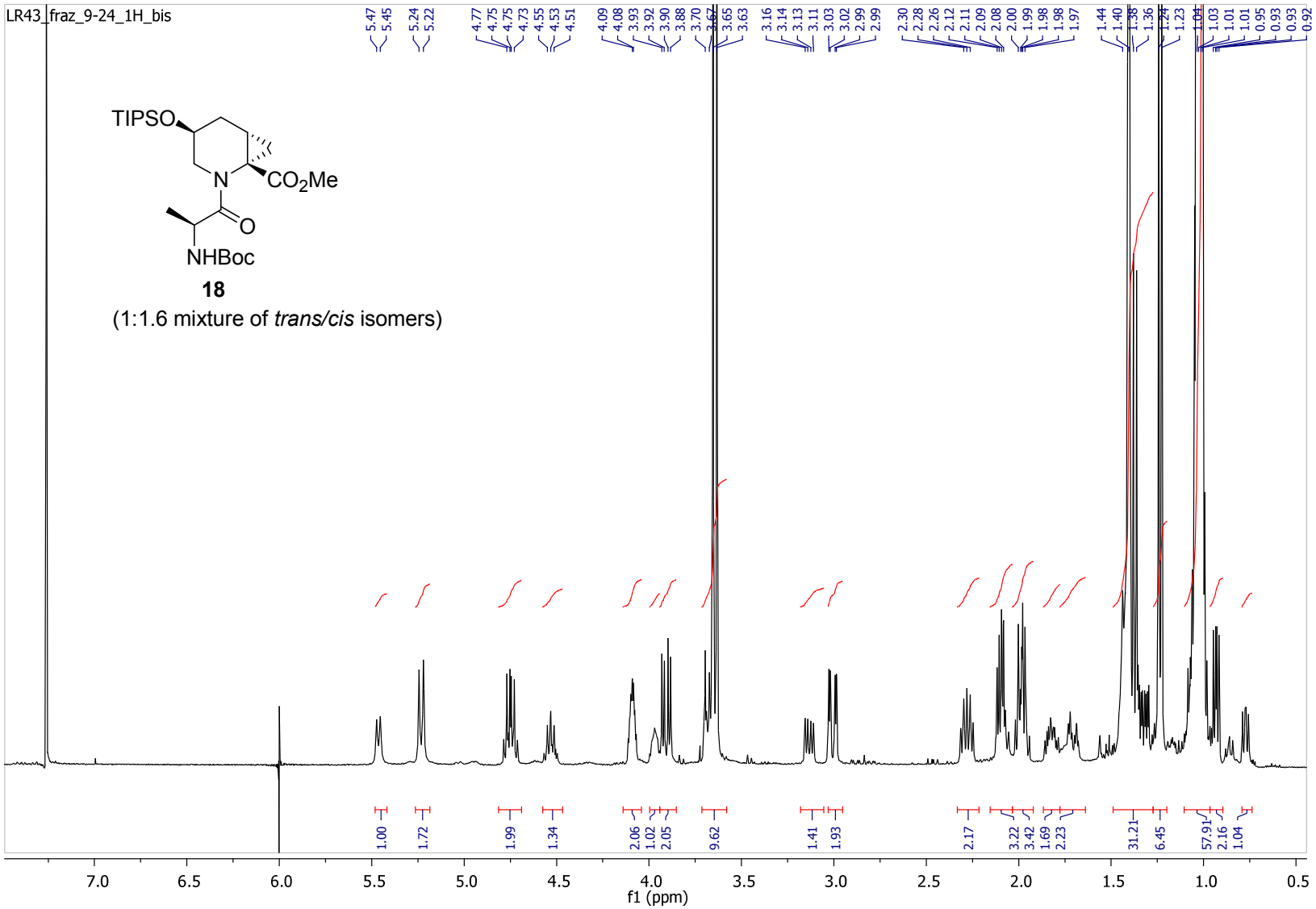
gCOSY (D_2O, 400 MHz) of compound **16**



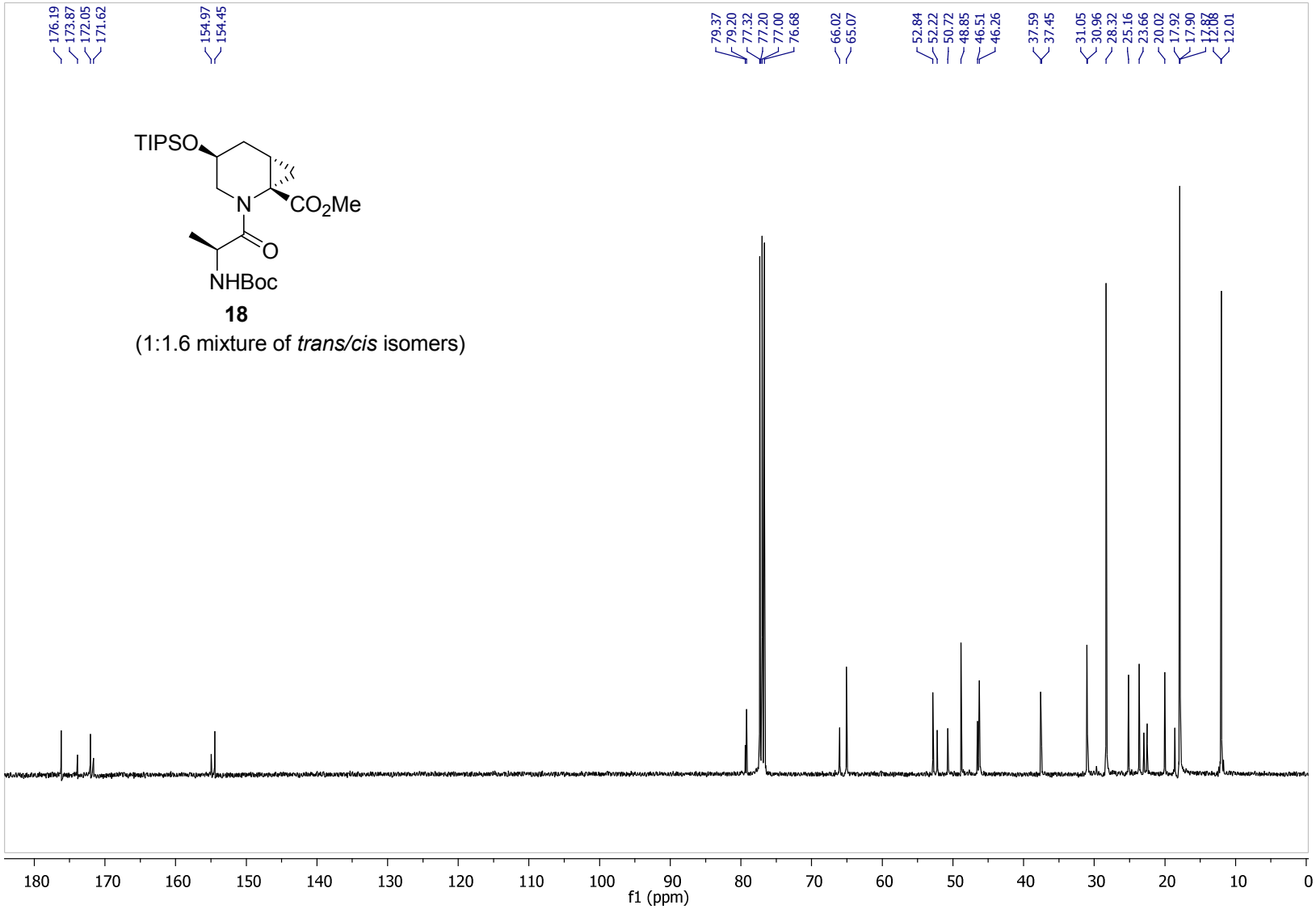
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **17**



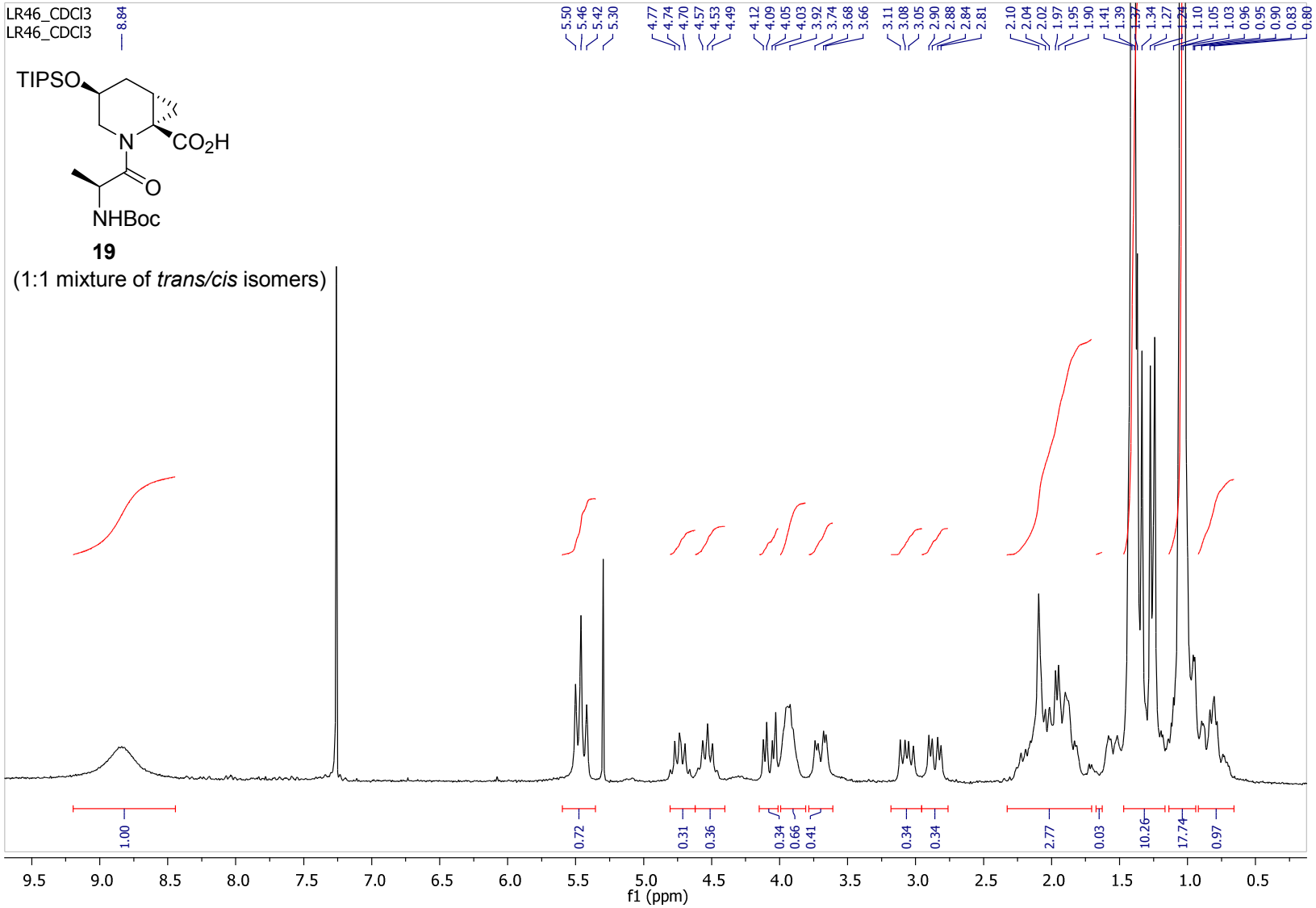
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **17**



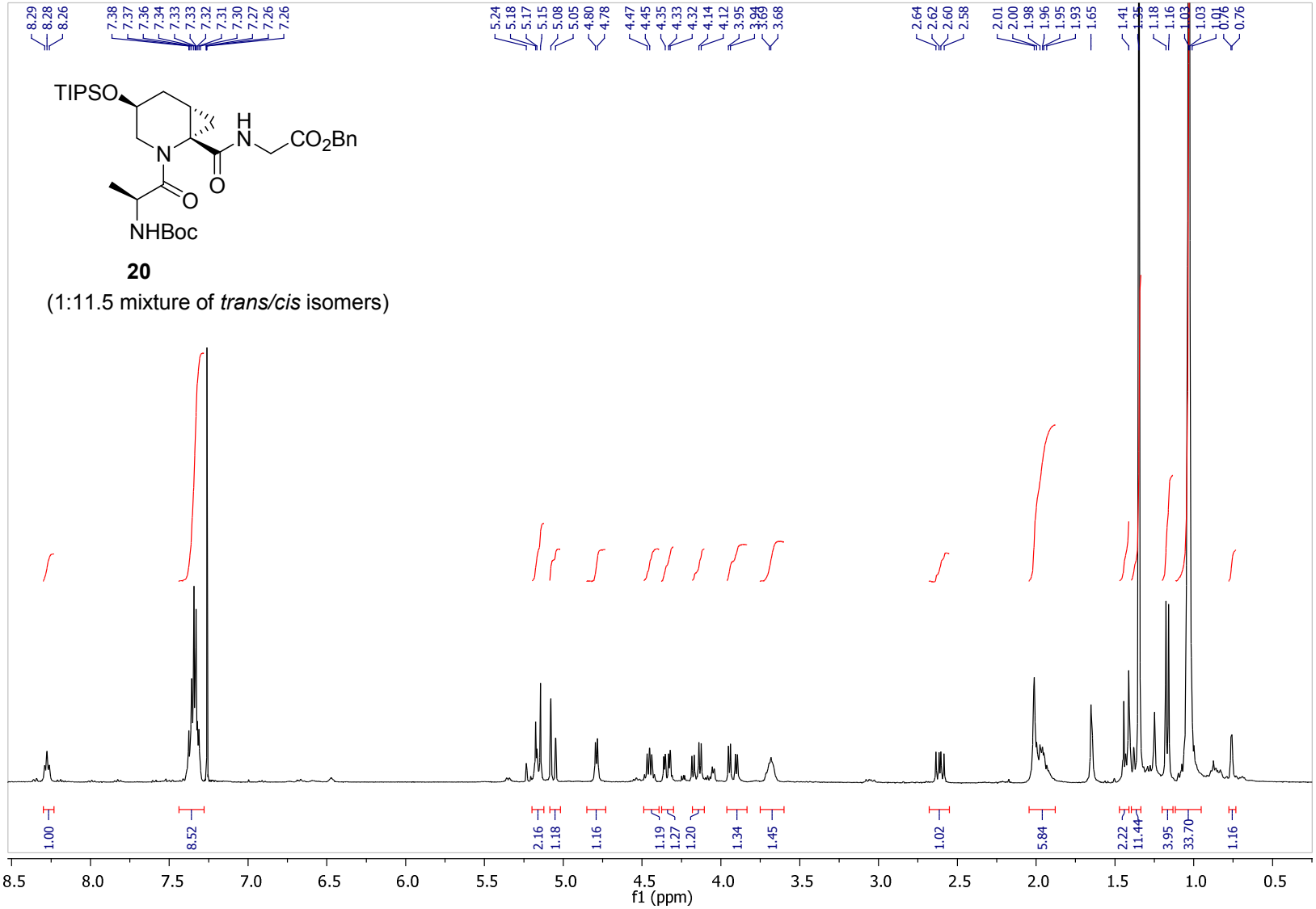
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **18**



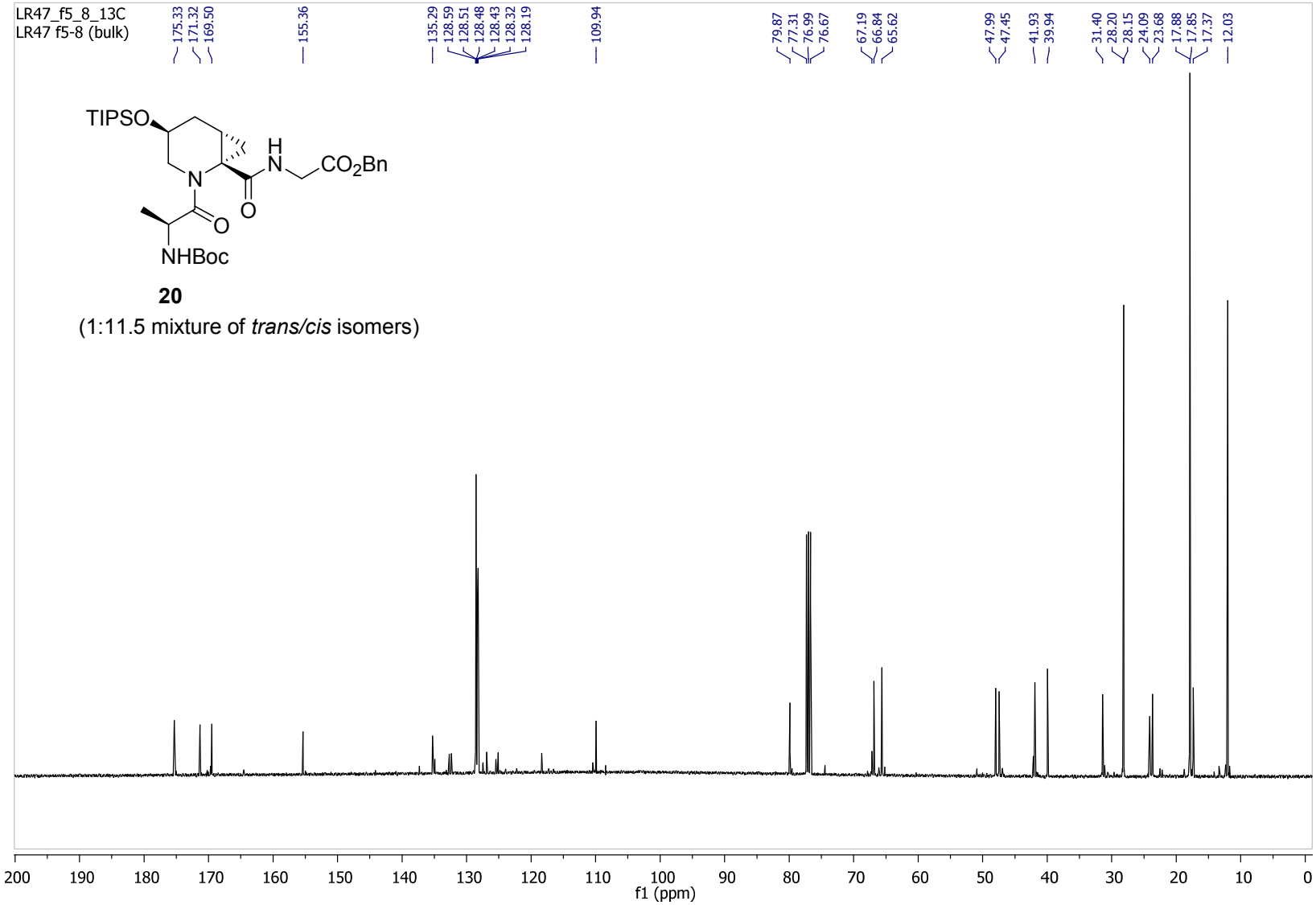
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **18**



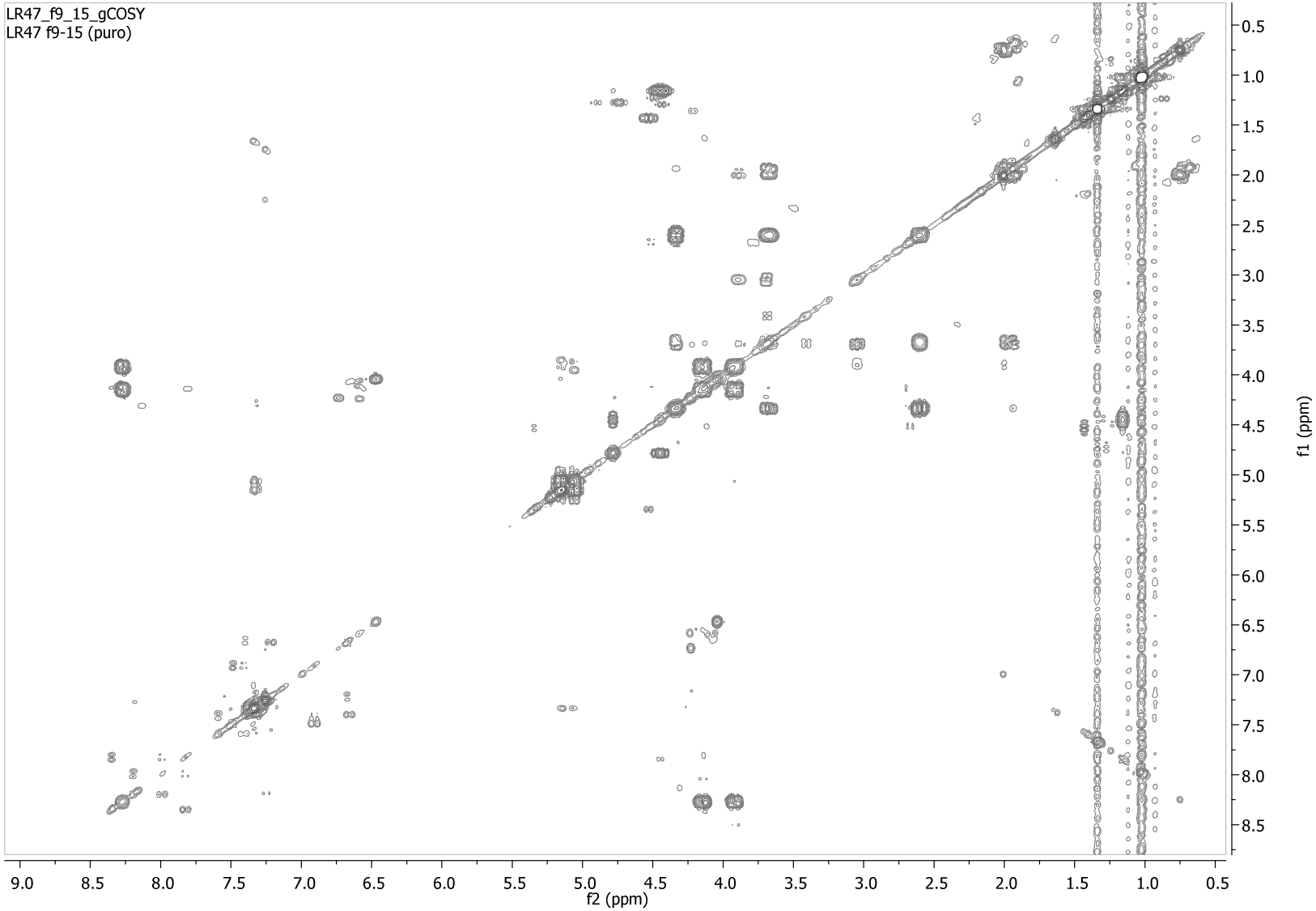
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **19**



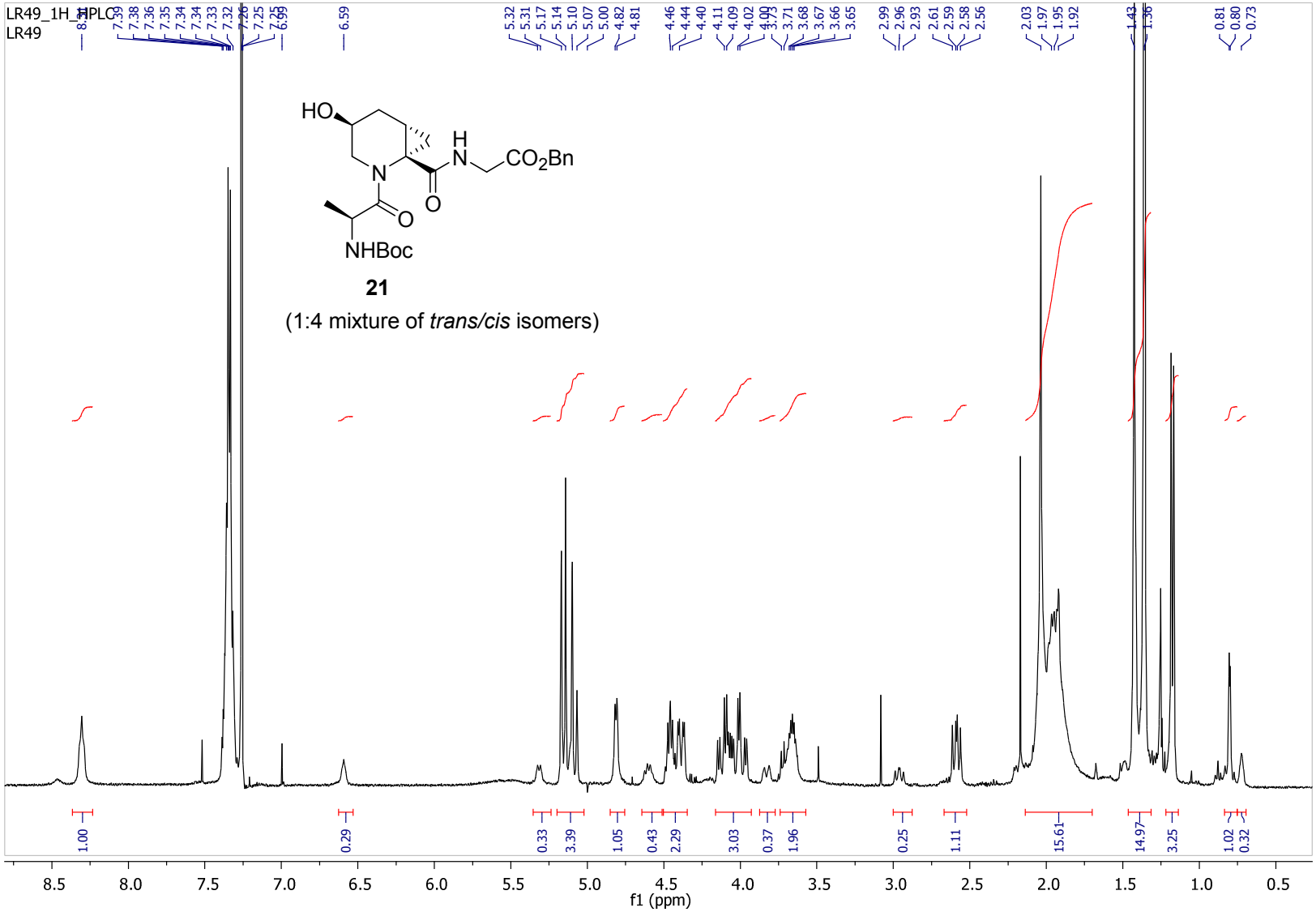
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **20**



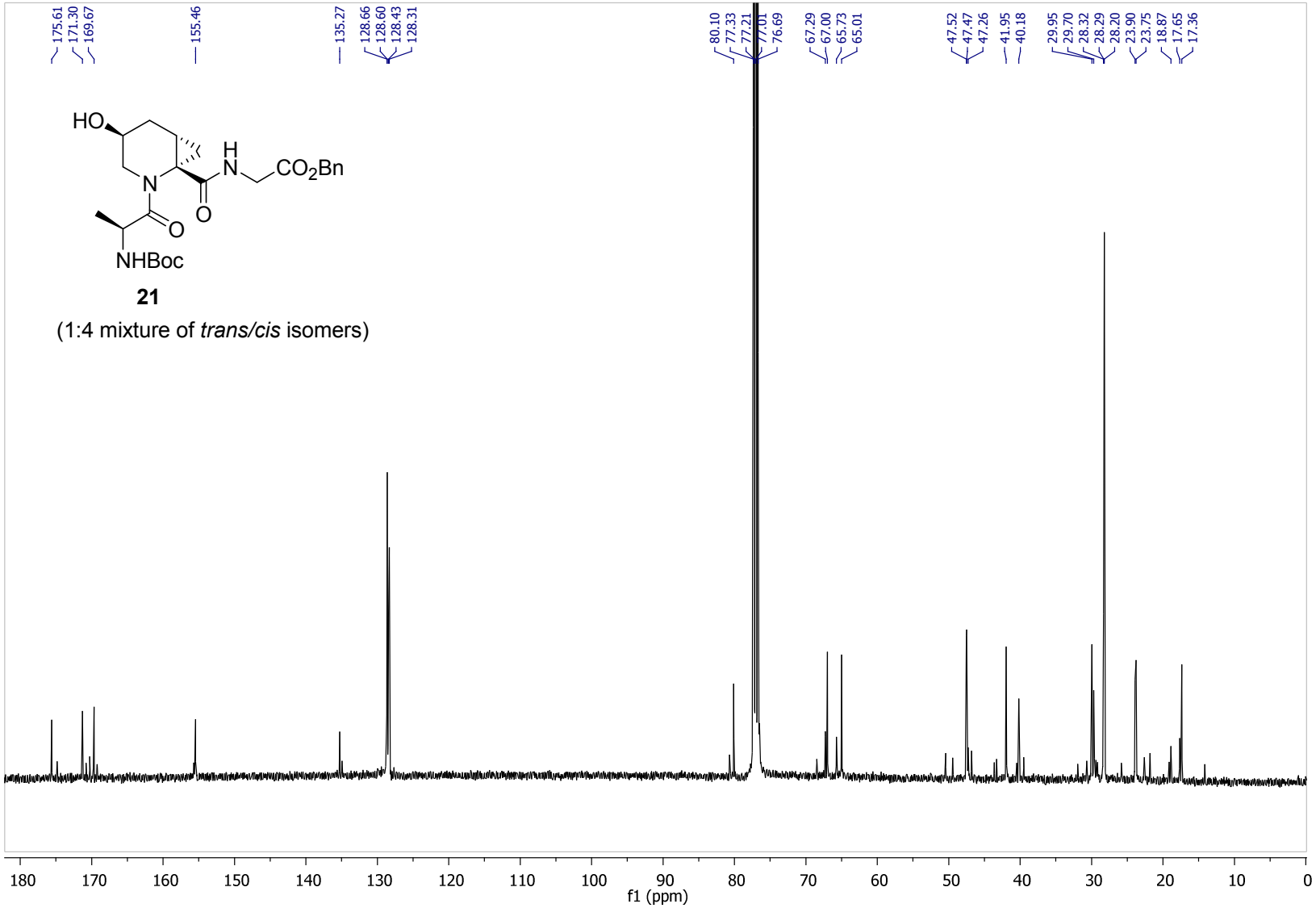
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **20**



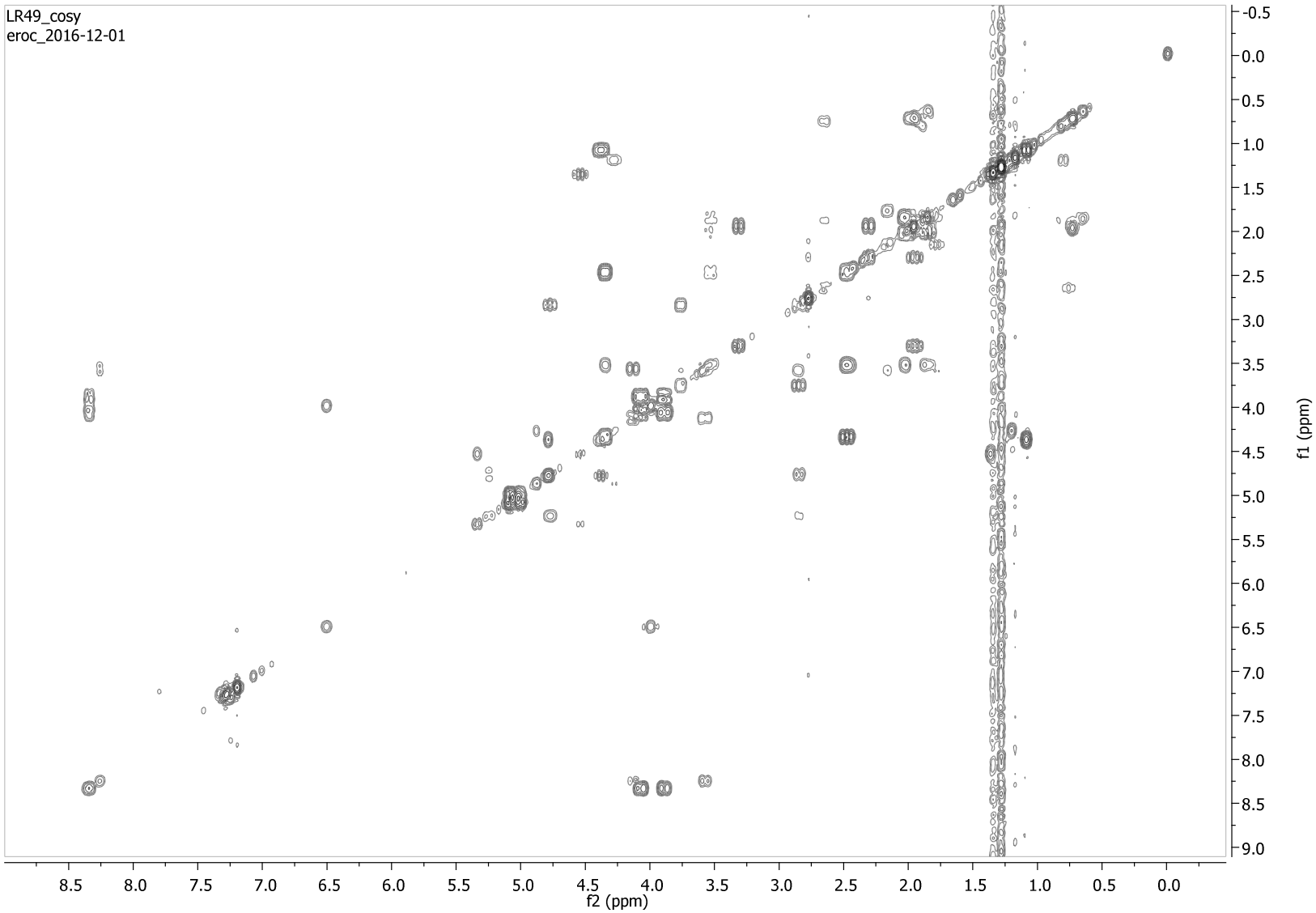
gCOSY ($CDCl_3$, 400 MHz) of compound **20**



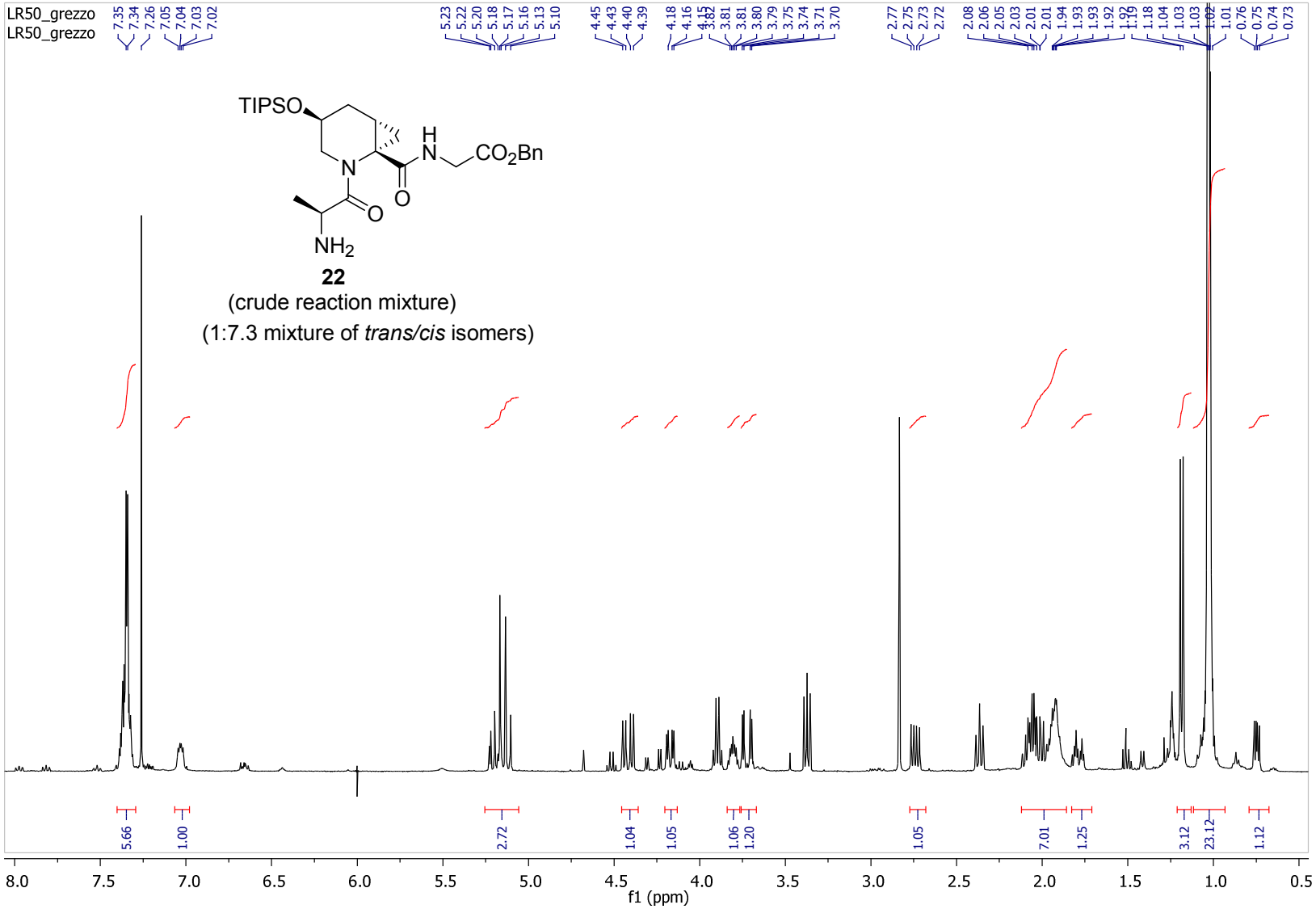
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **21**



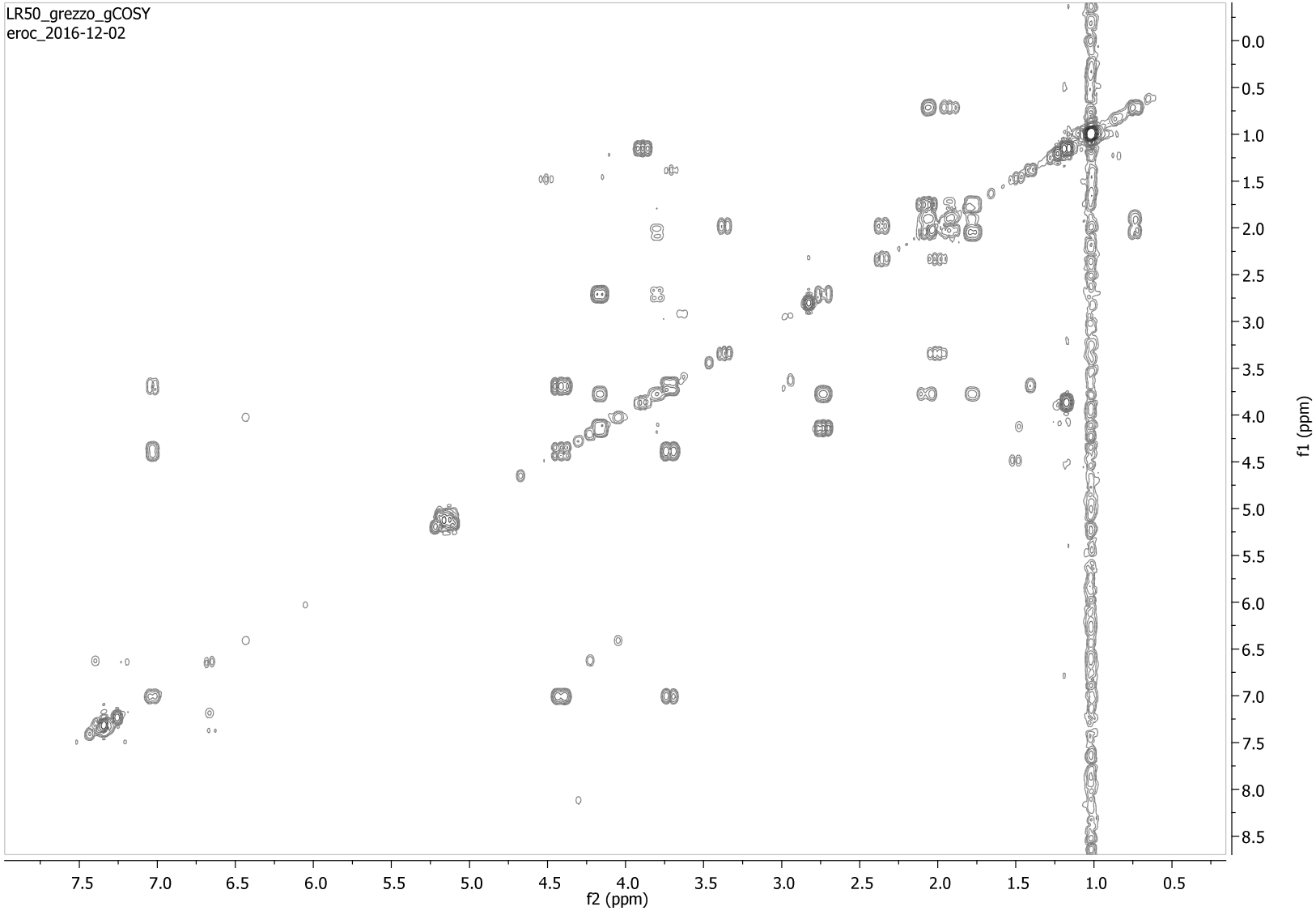
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **21**



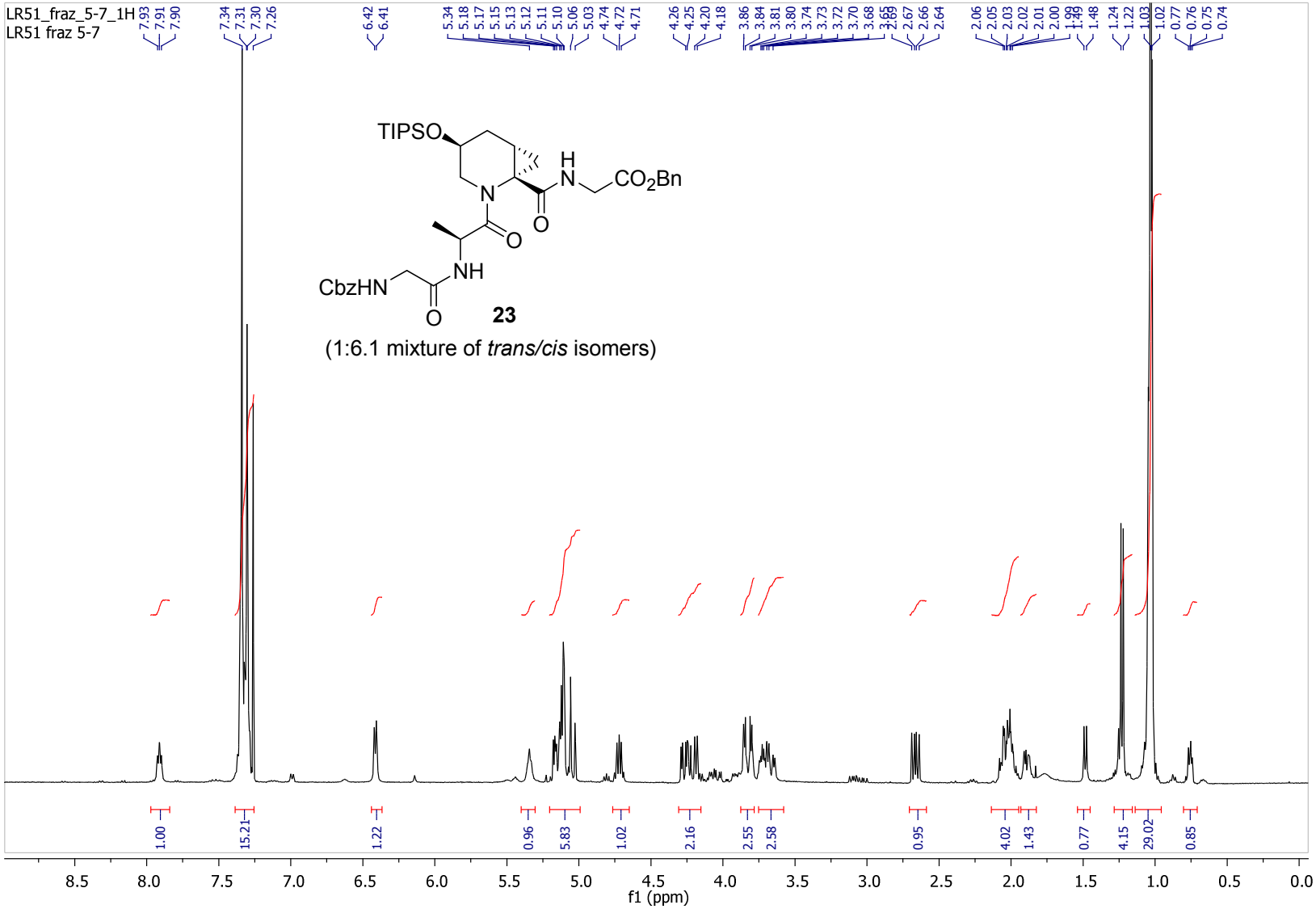
gCOSY ($CDCl_3$, 400 MHz) of compound **21**



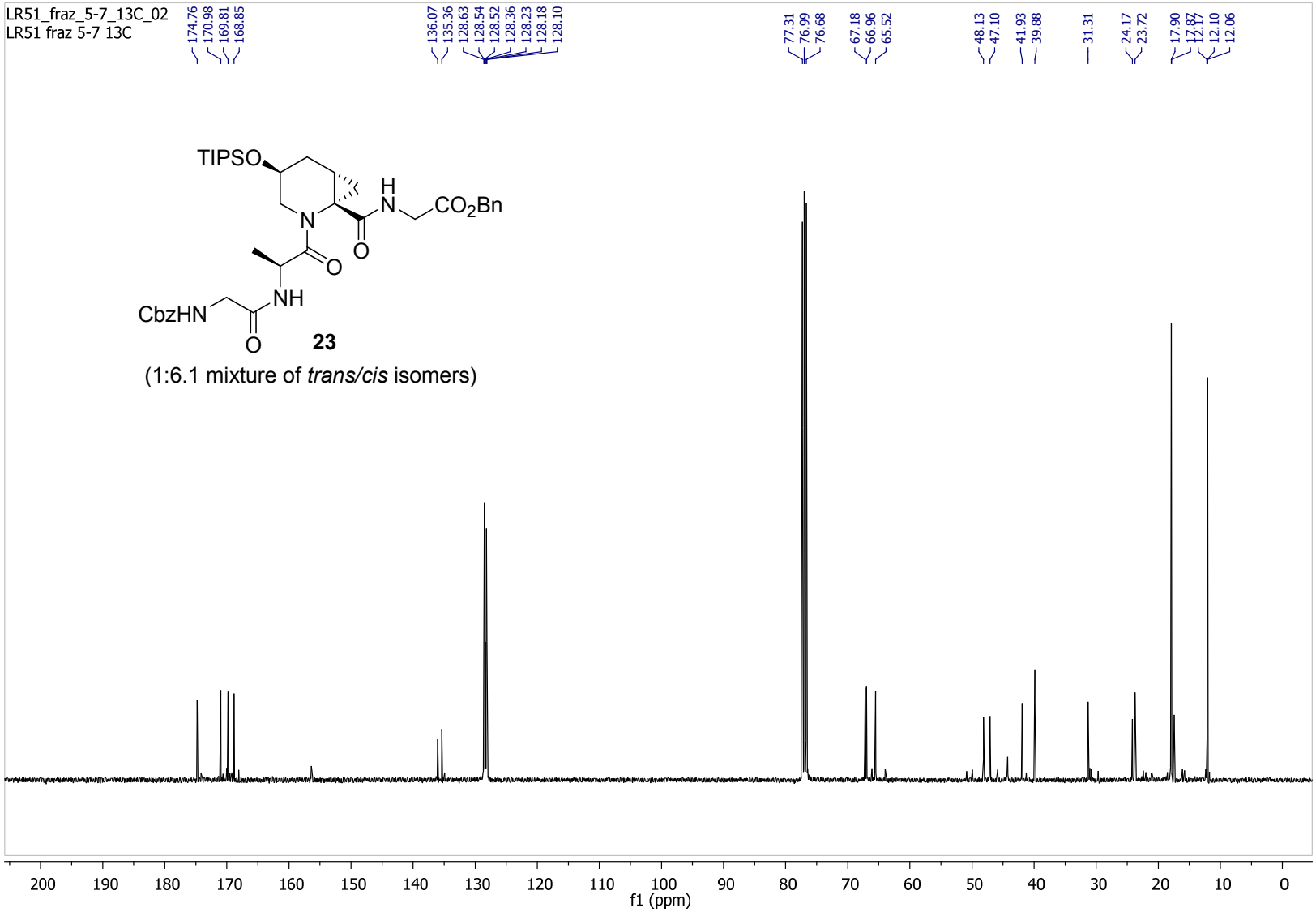
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **22** (crude reaction mixture)



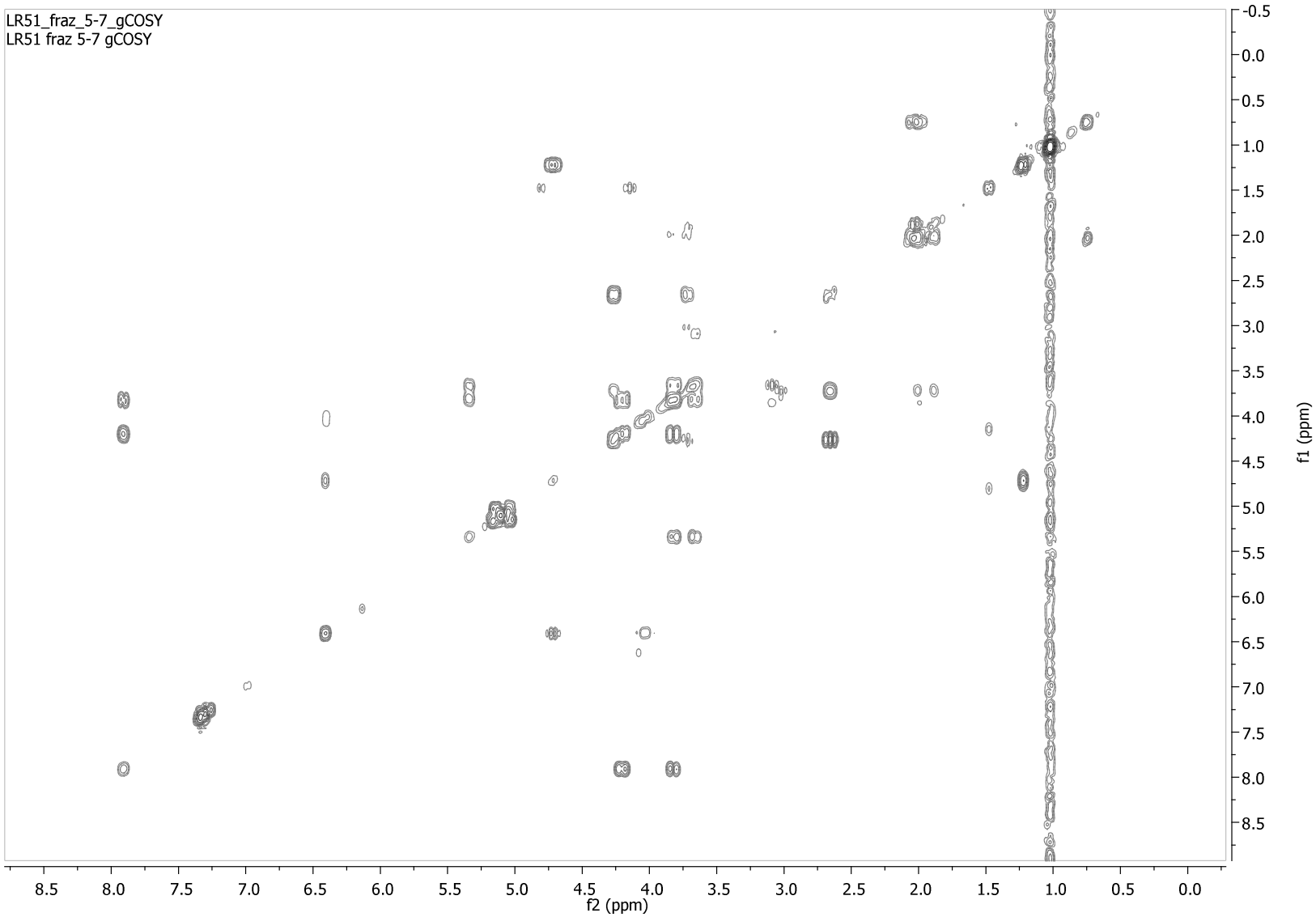
gCOSY ($CDCl_3$, 400 MHz) of compound **22** (crude reaction mixture)



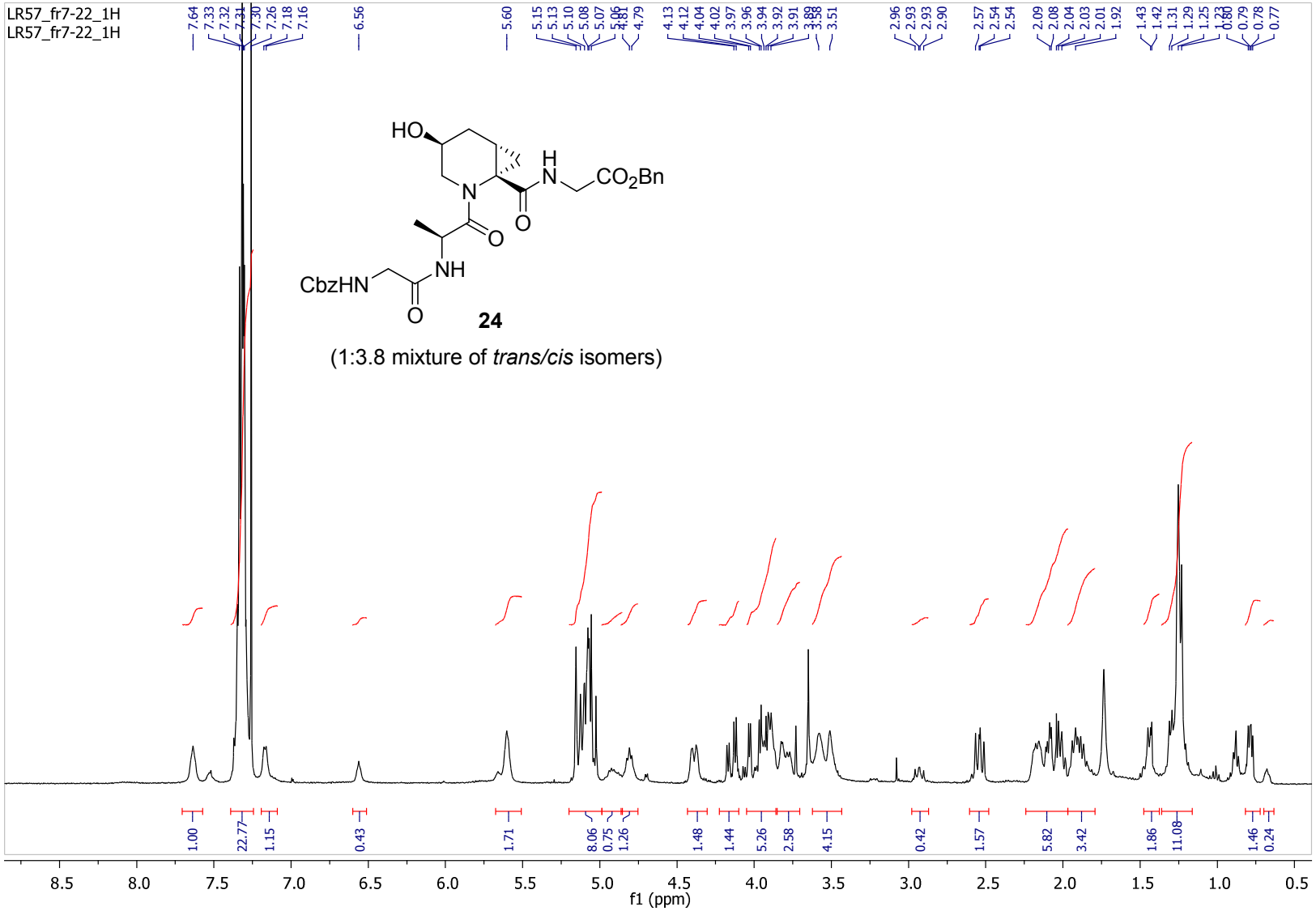
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **23**



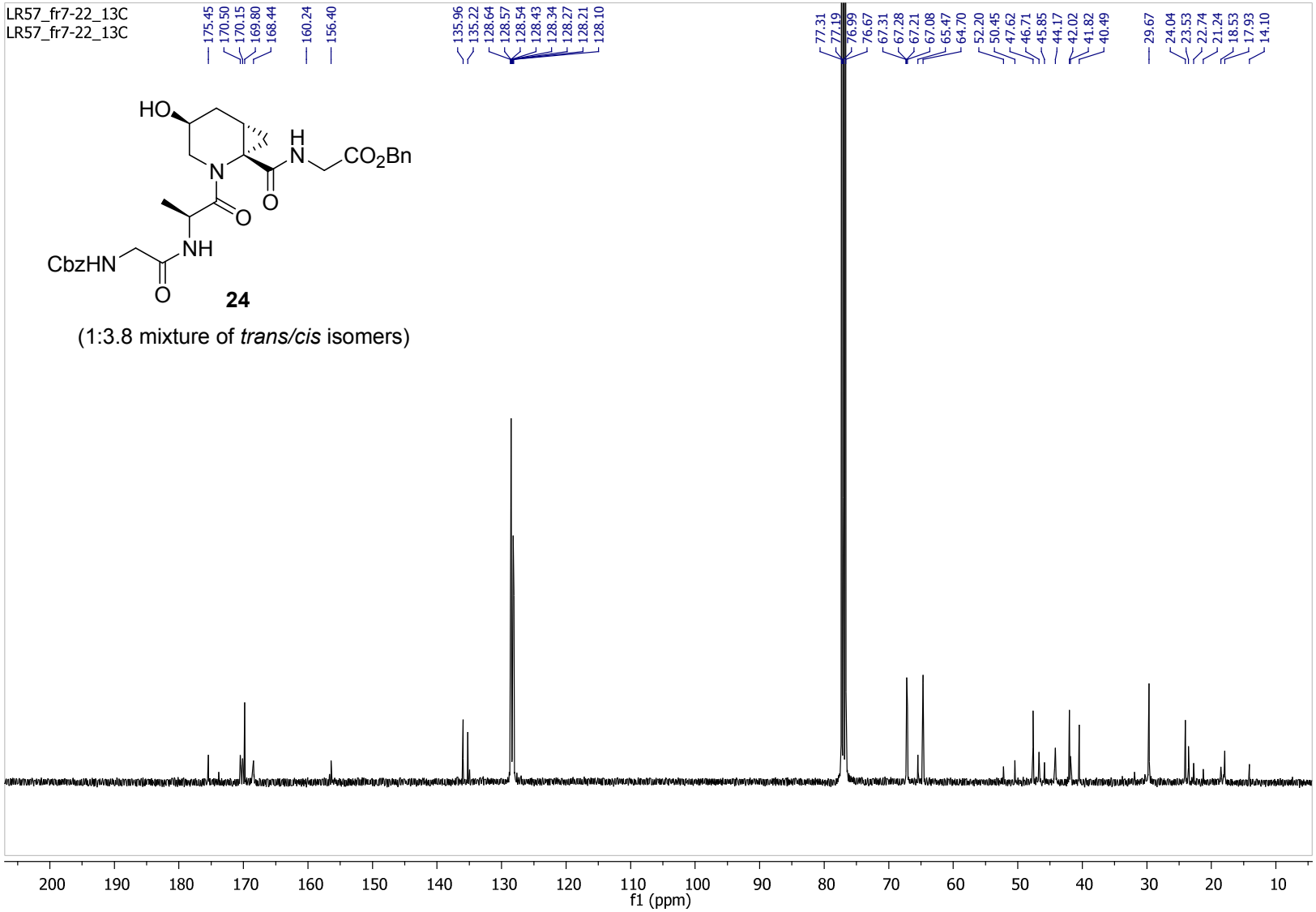
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **23**



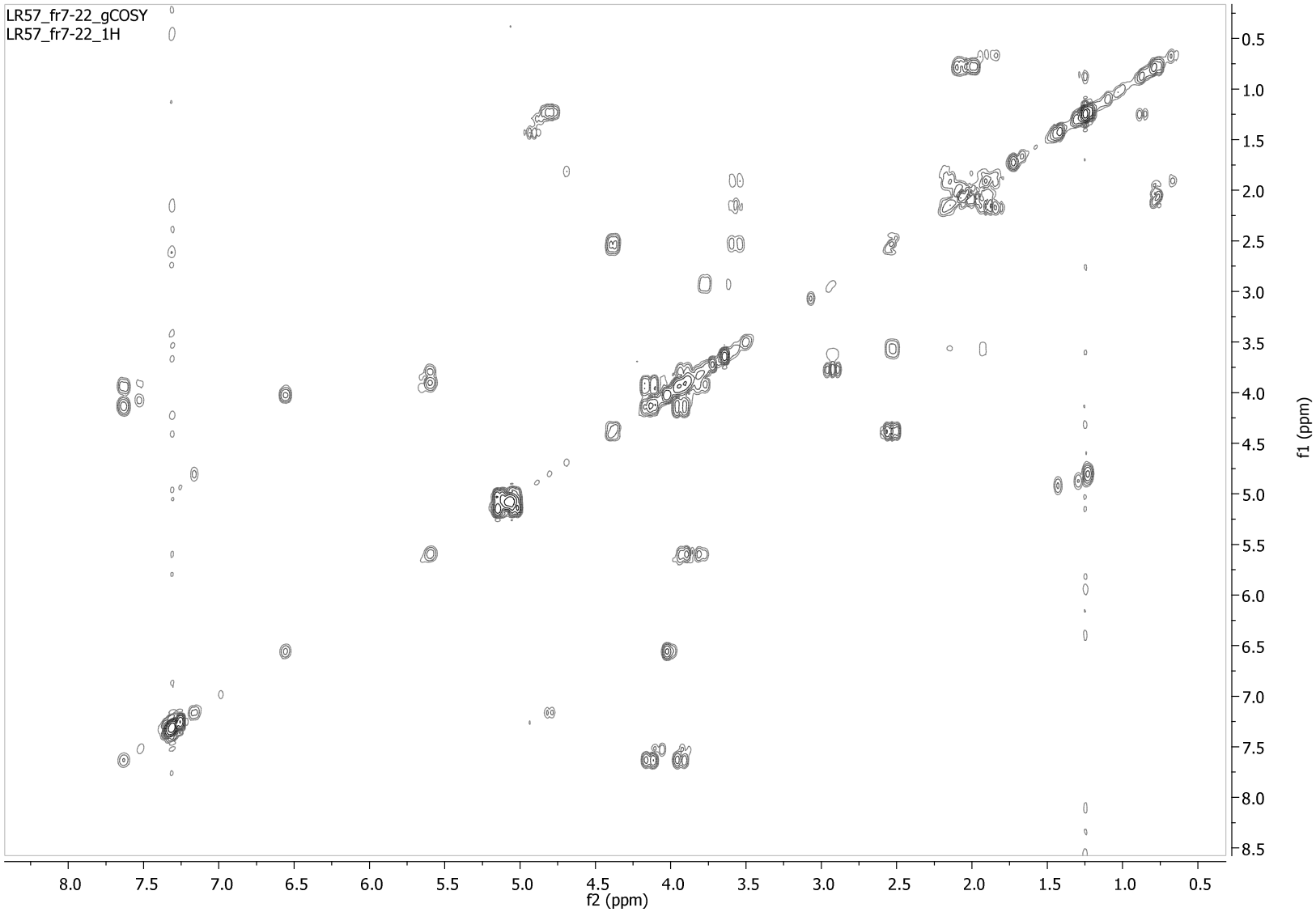
gCOSY ($CDCl_3$, 400 MHz) of compound **23**



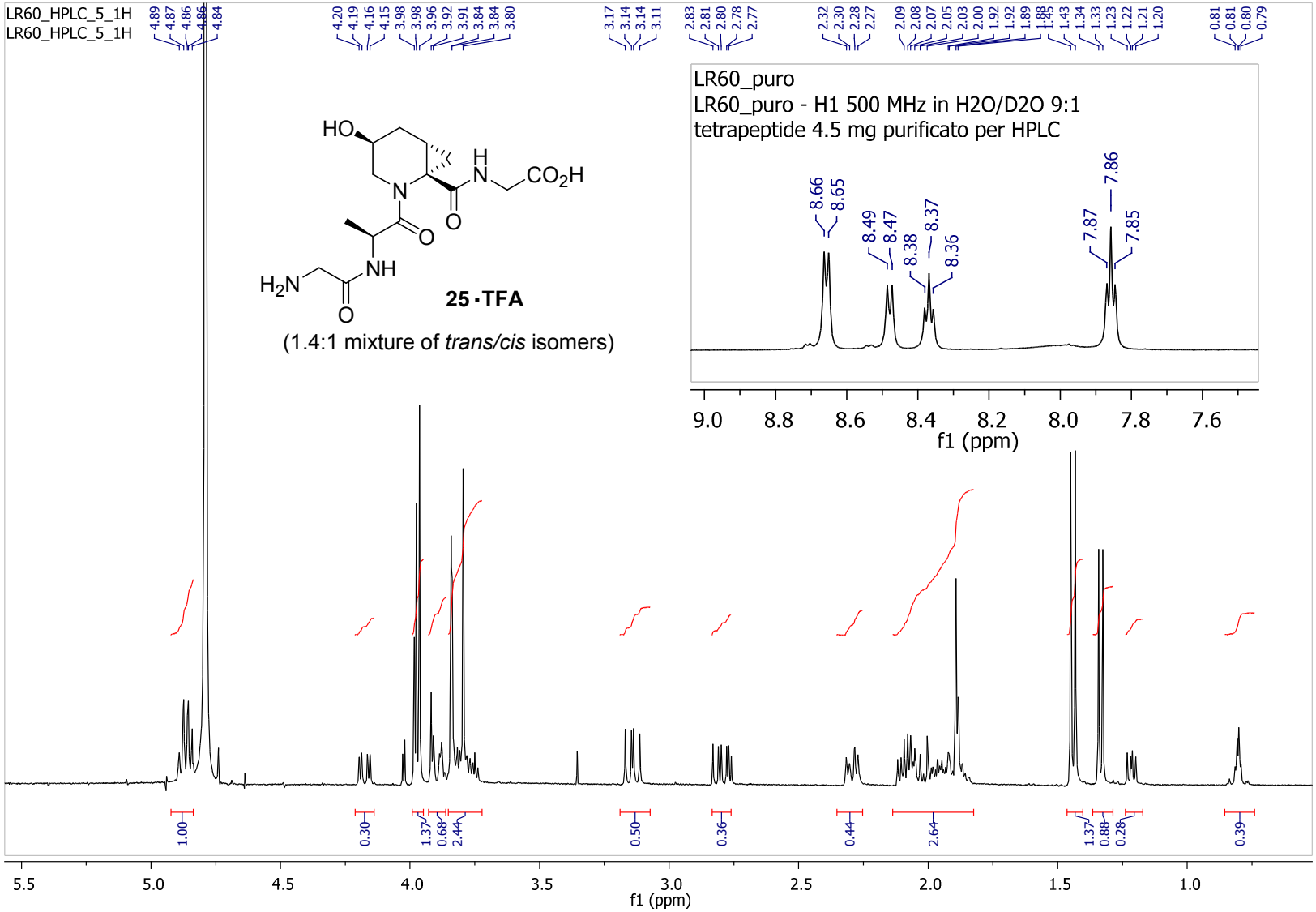
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **24**



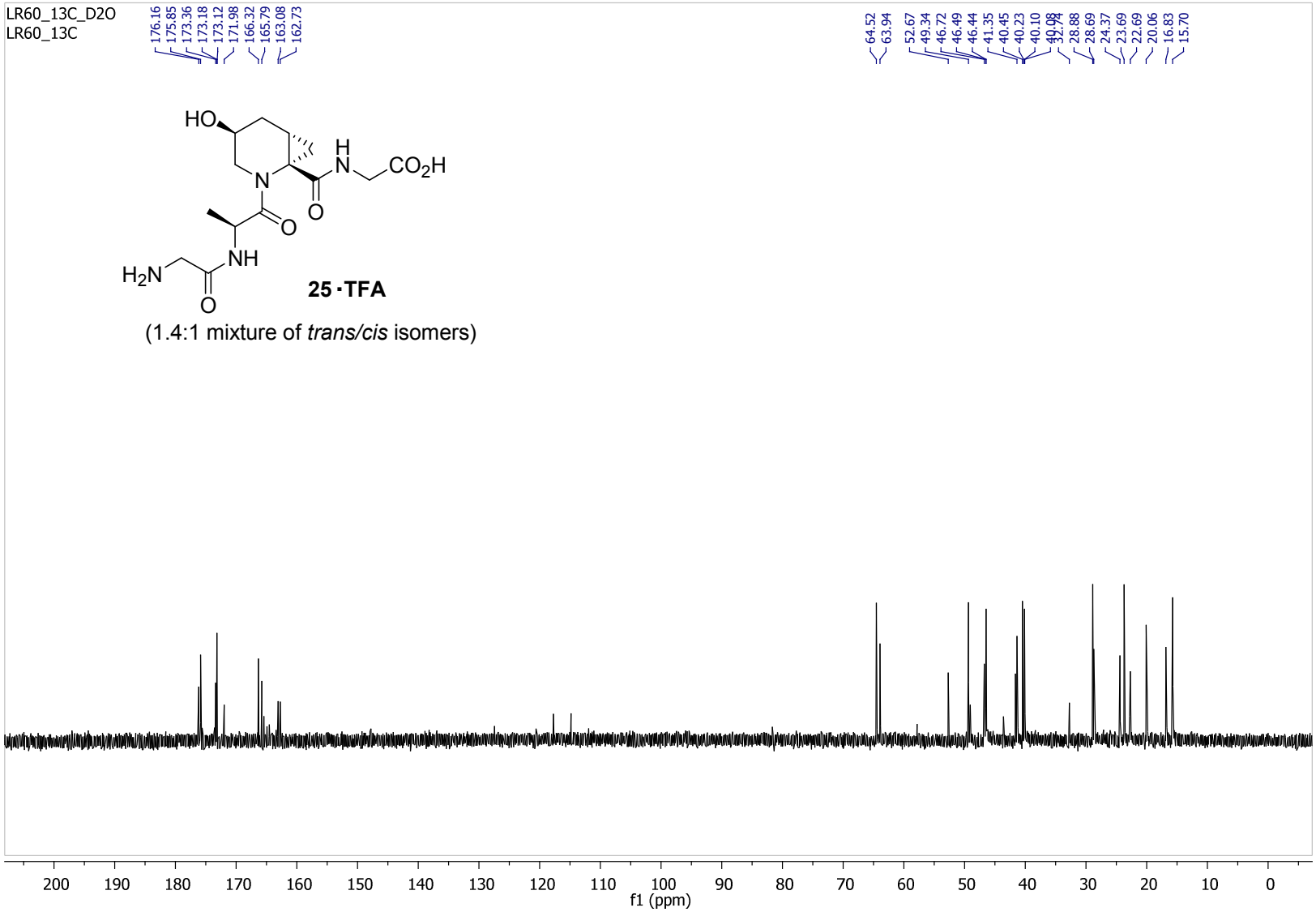
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **24**



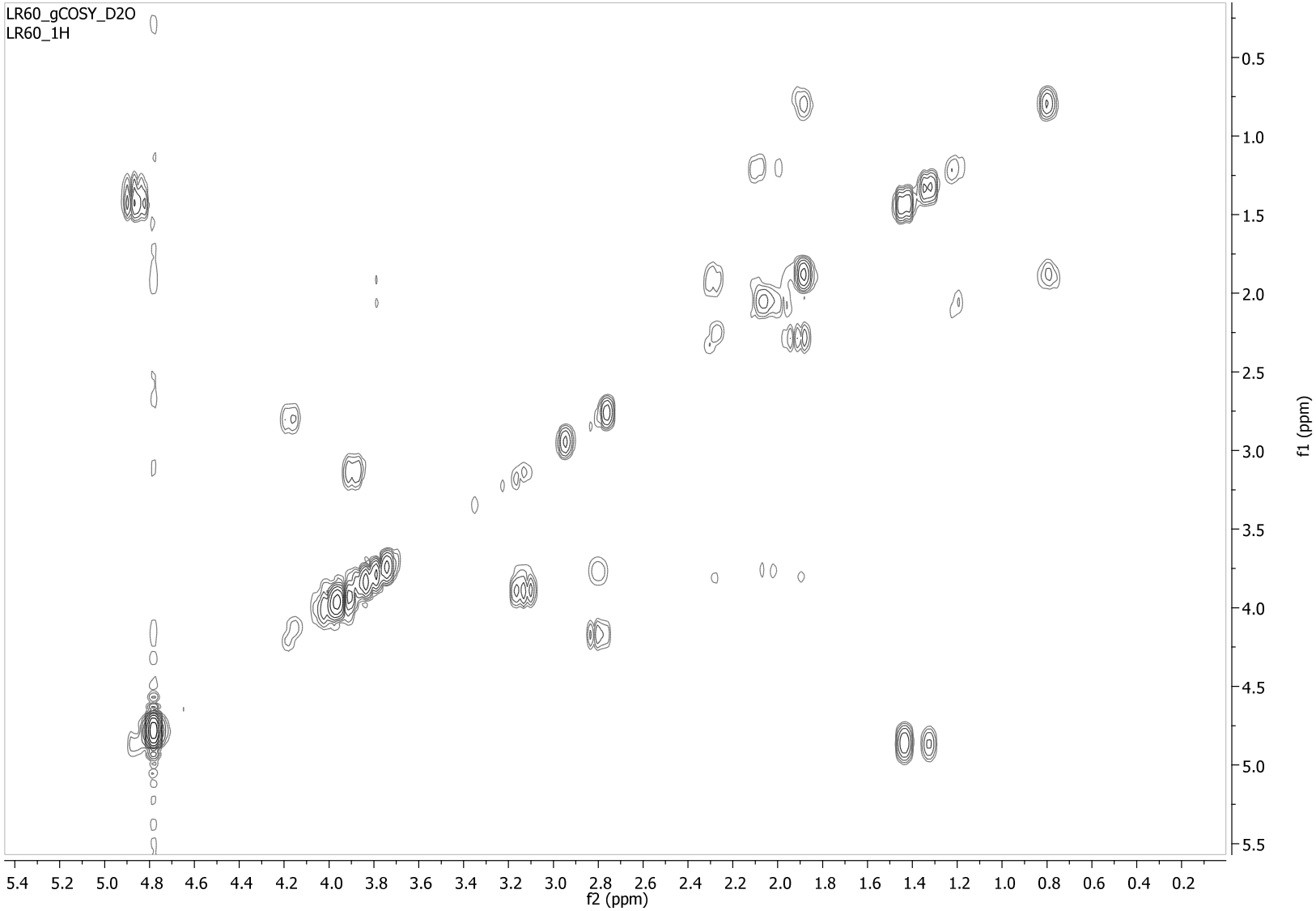
gCOSY ($CDCl_3$, 400 MHz) of compound **24**



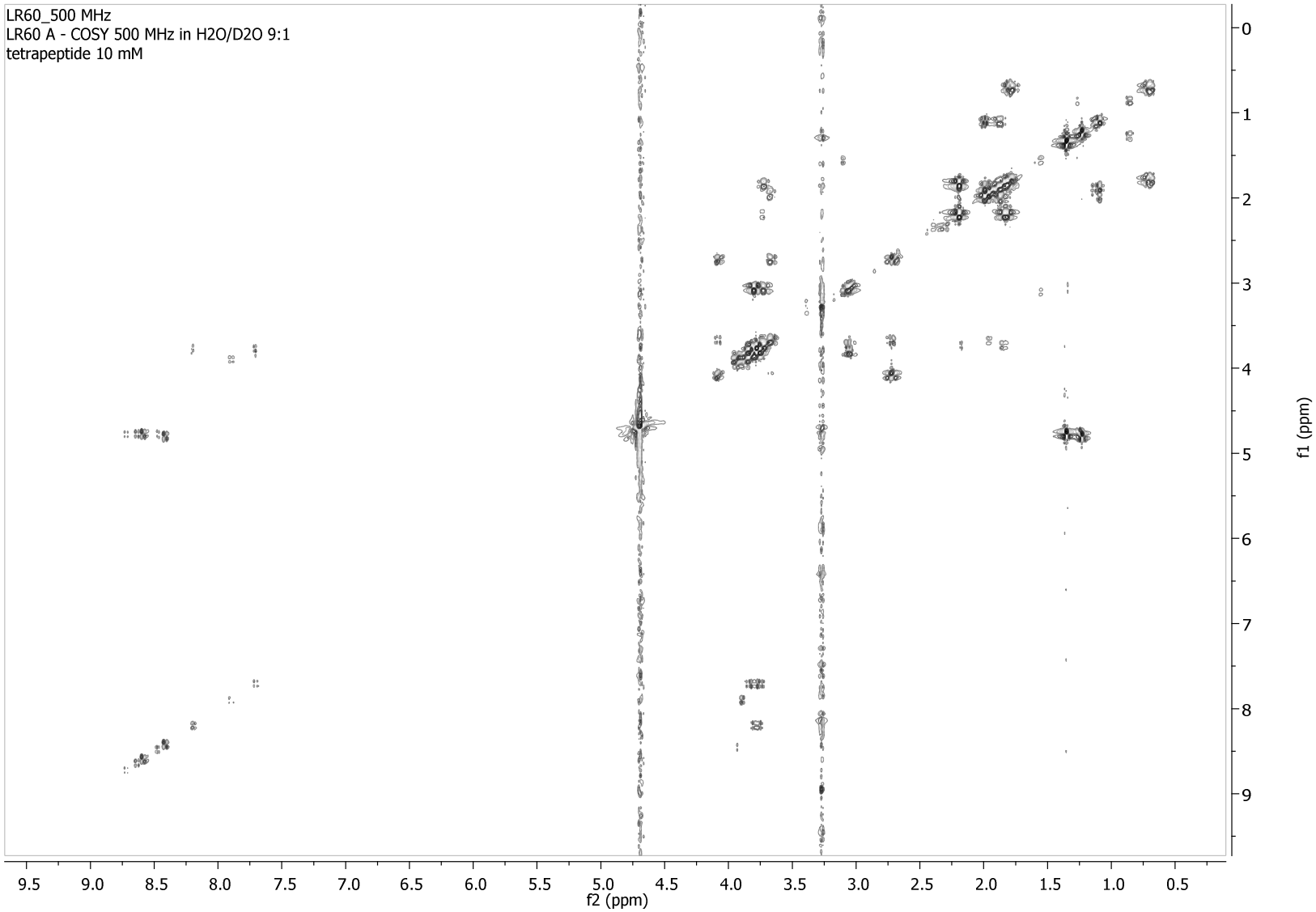
^{1}H NMR (400 MHz) of compound **25** in D_2O and in H_2O/D_2O 9:1 (500 MHz, inset)



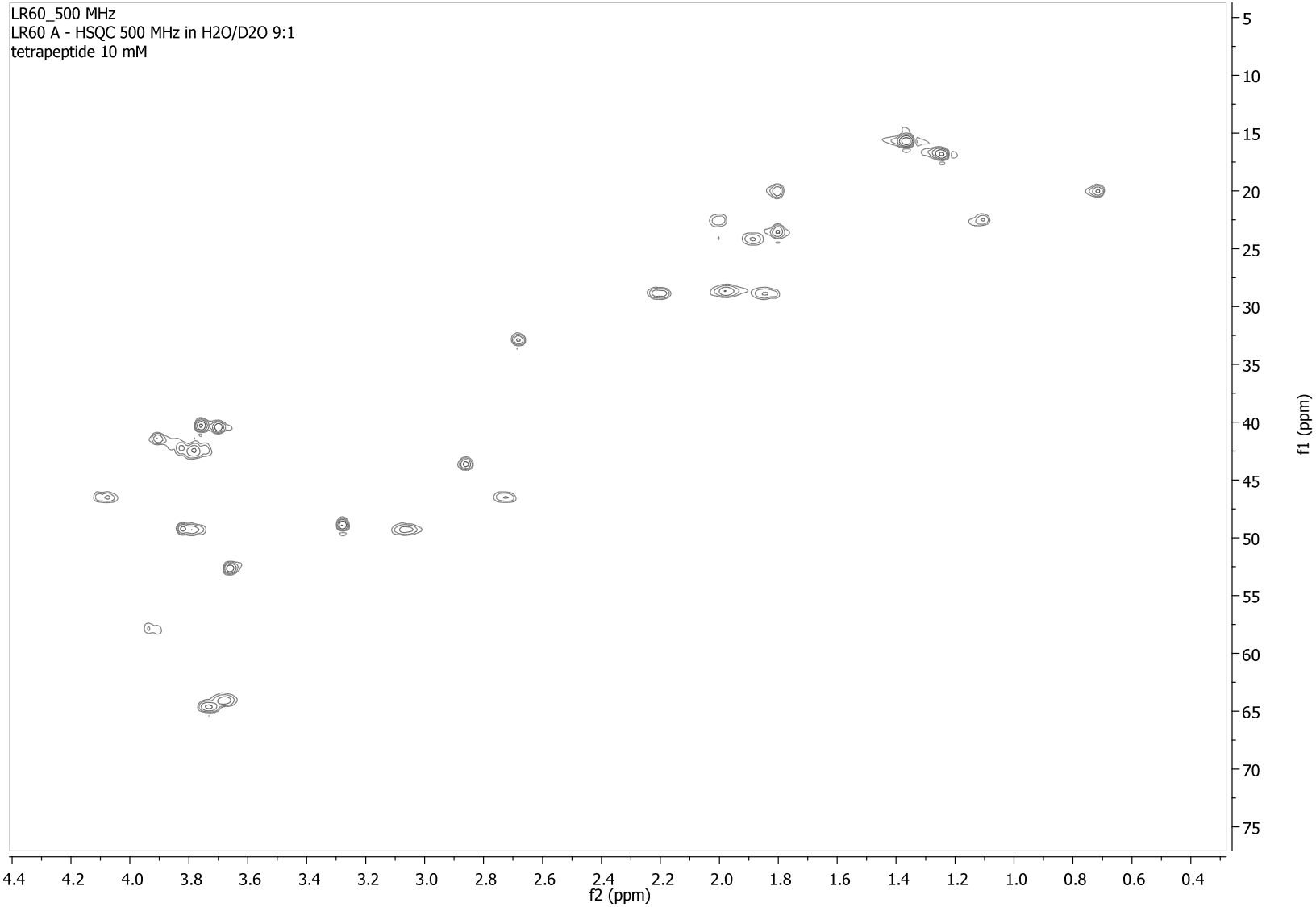
^{13}C NMR (100.4 MHz) of compound **25** in D_2O



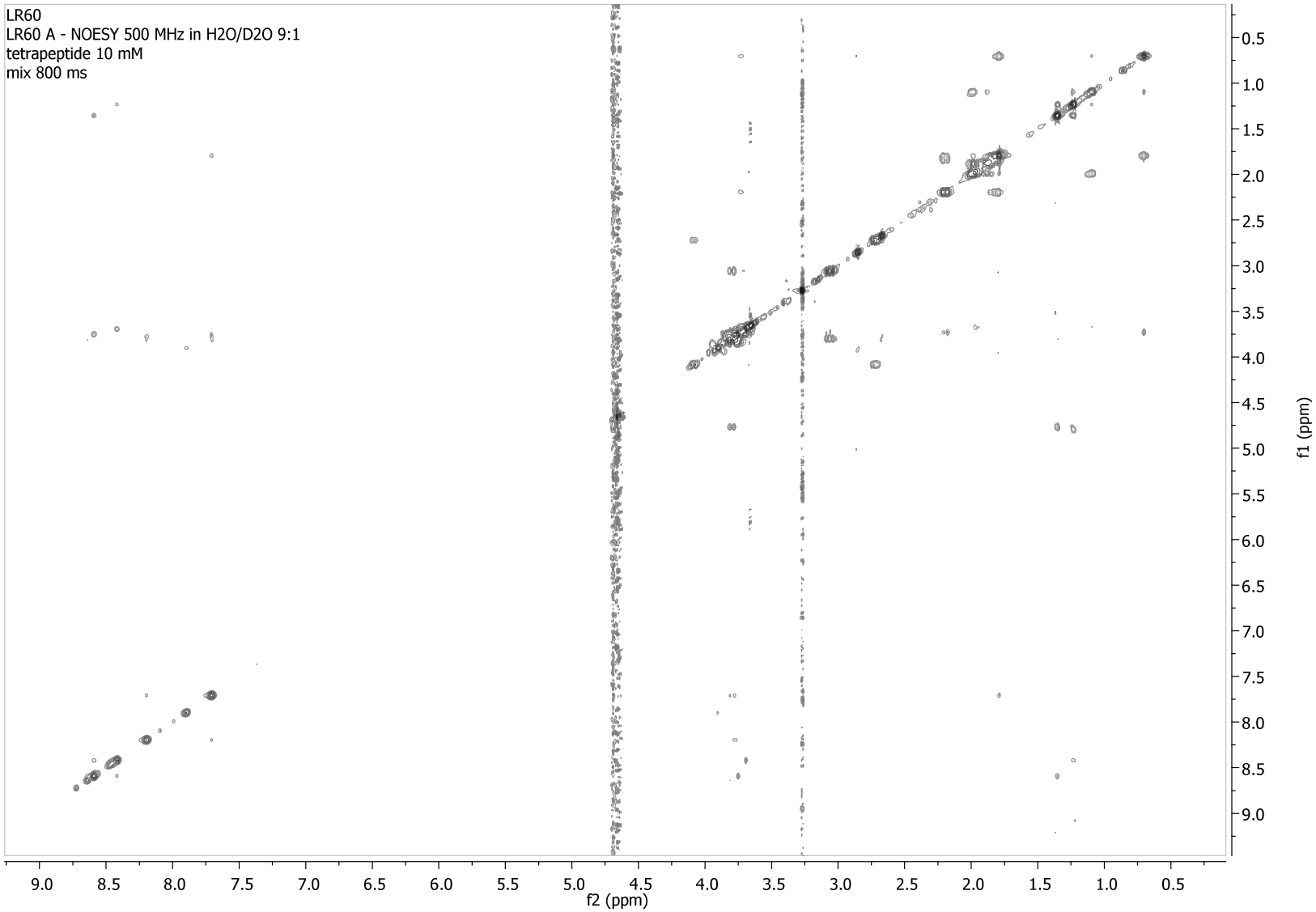
gCOSY (400 MHz) of compound **25** in D_2O



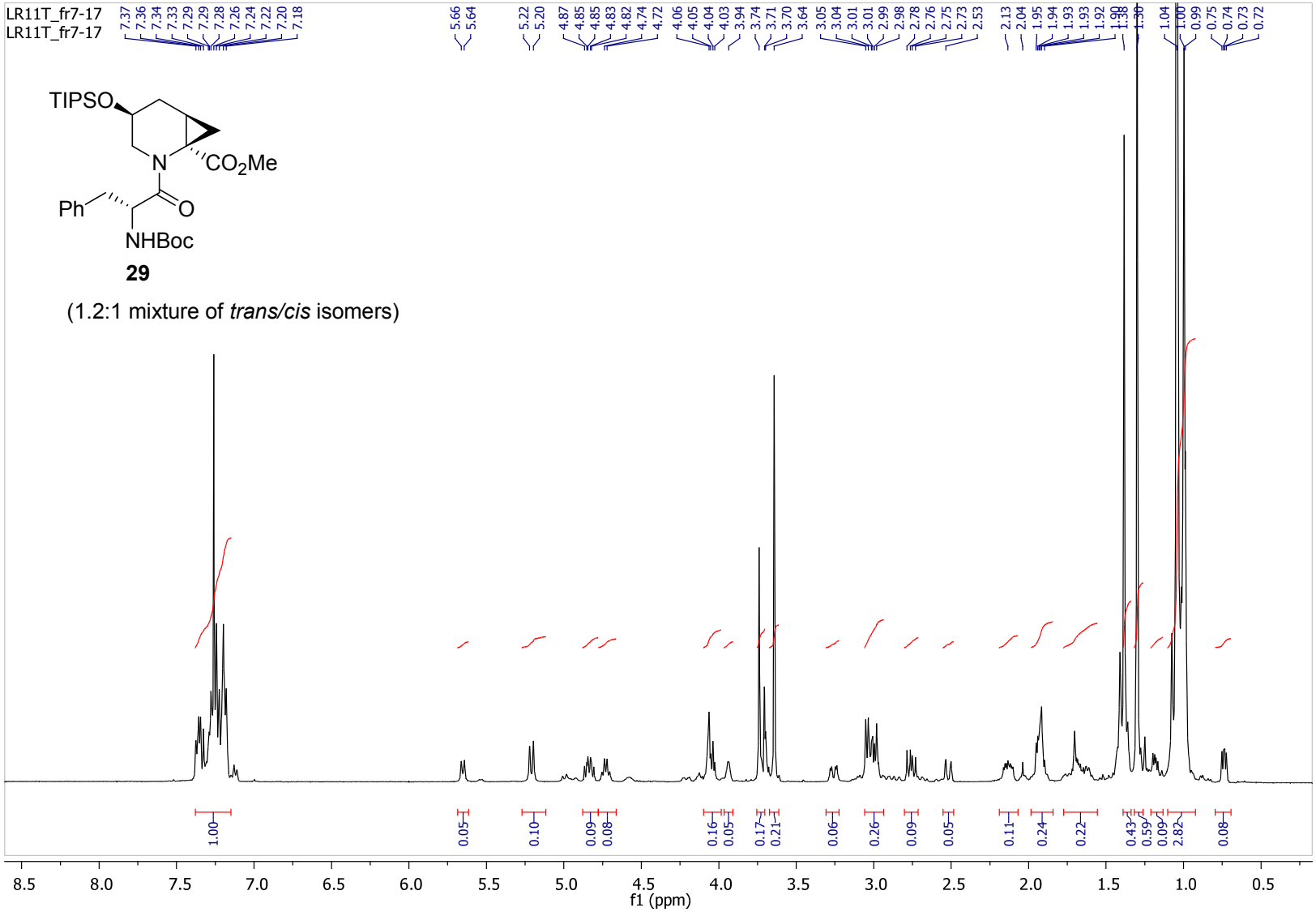
gCOSY (500 MHz) of compound **25** in H_2O/D_2O 9:1



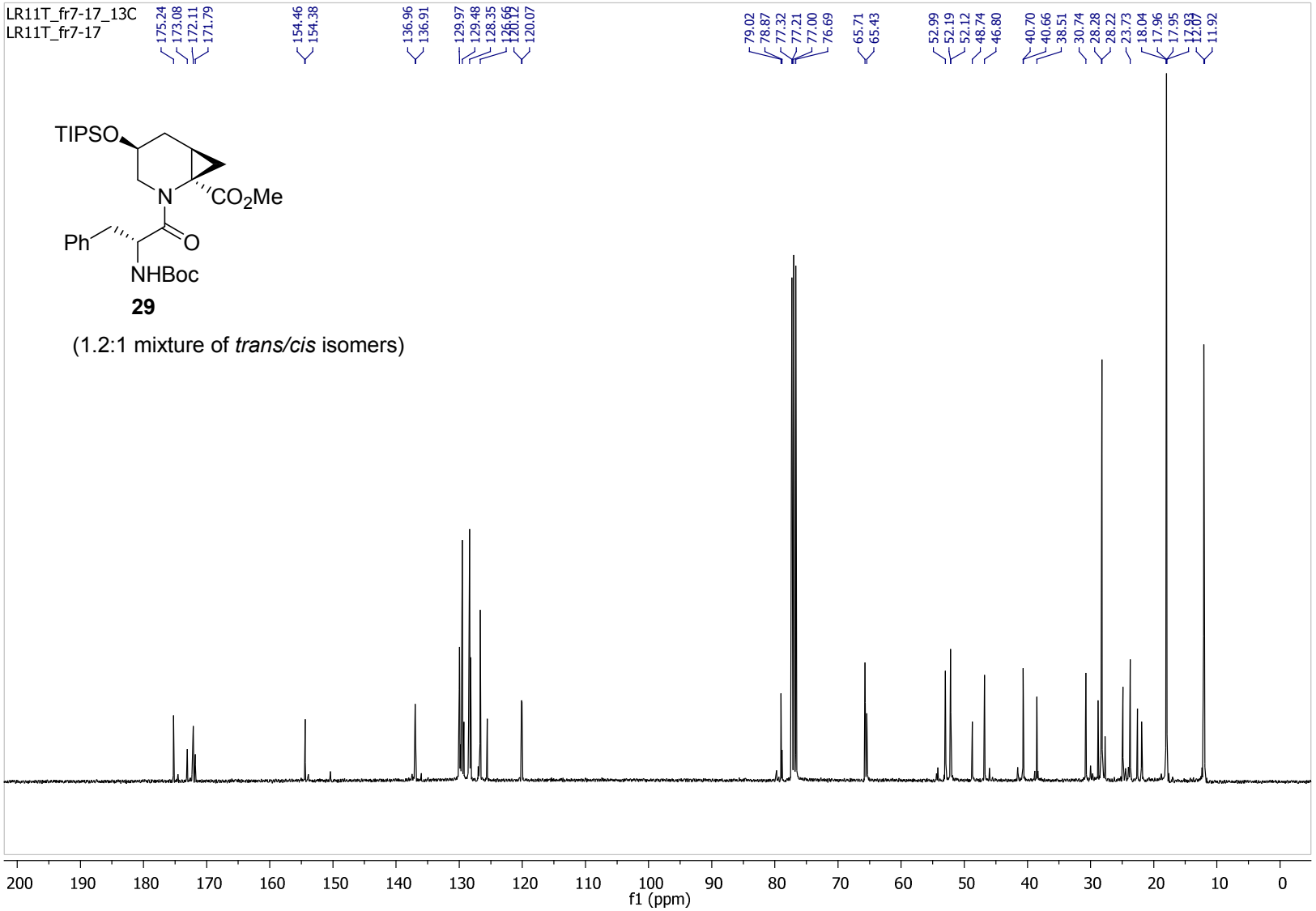
gHSQC (500 MHz) of compound **25** in H_2O/D_2O 9:1



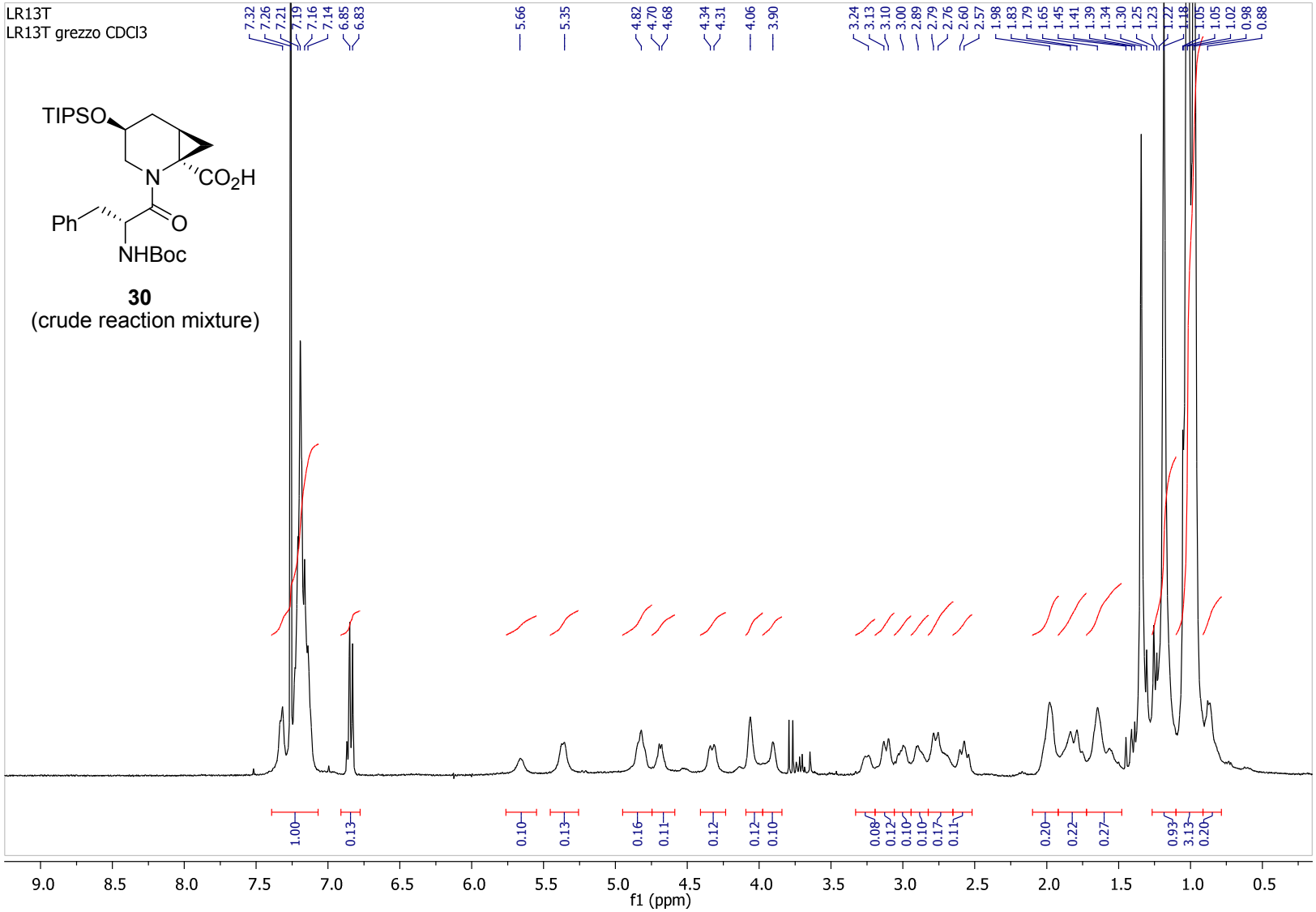
NOESY (500 MHz) of compound **25** in H_2O/D_2O 9:1



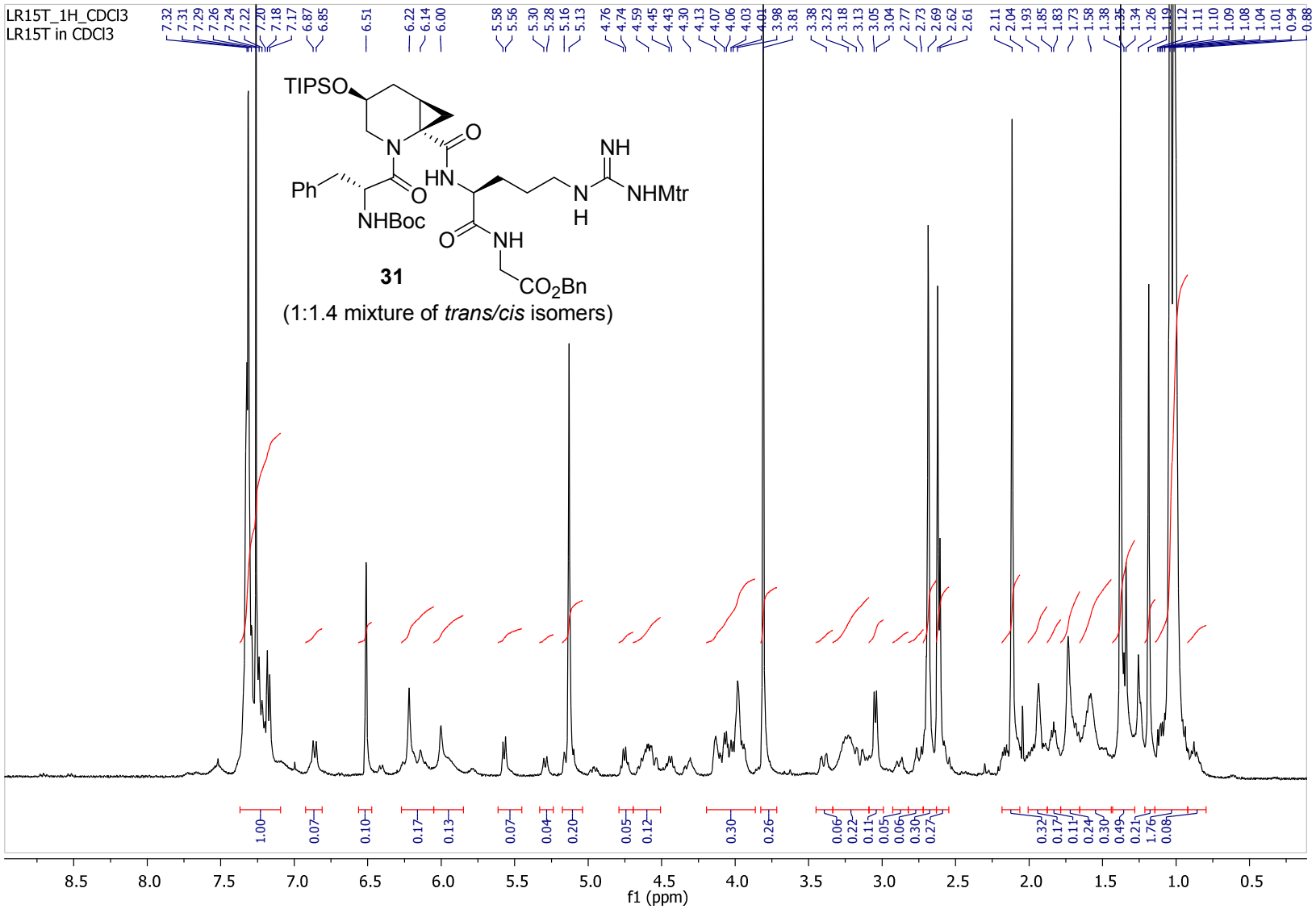
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **29**



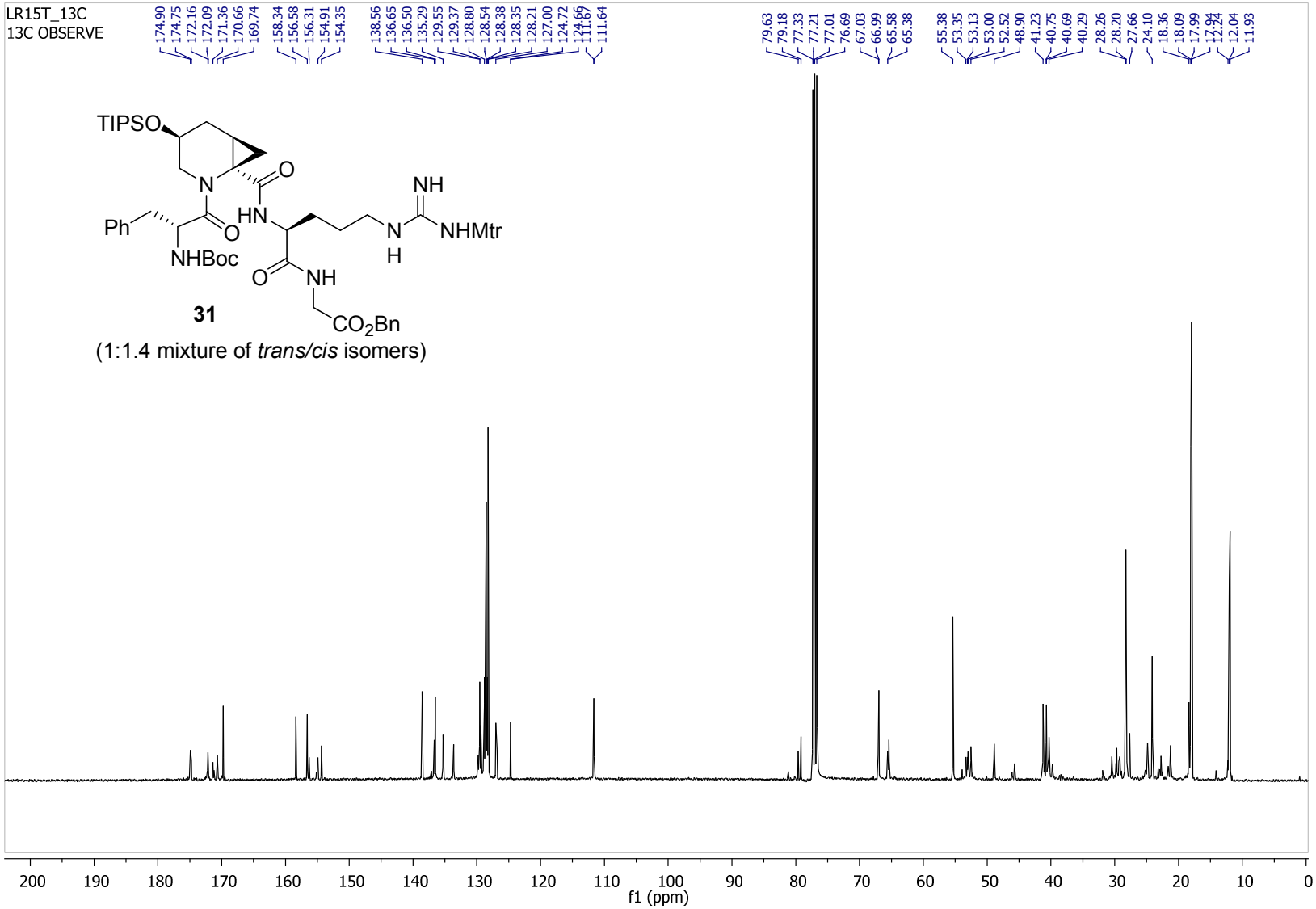
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **29**



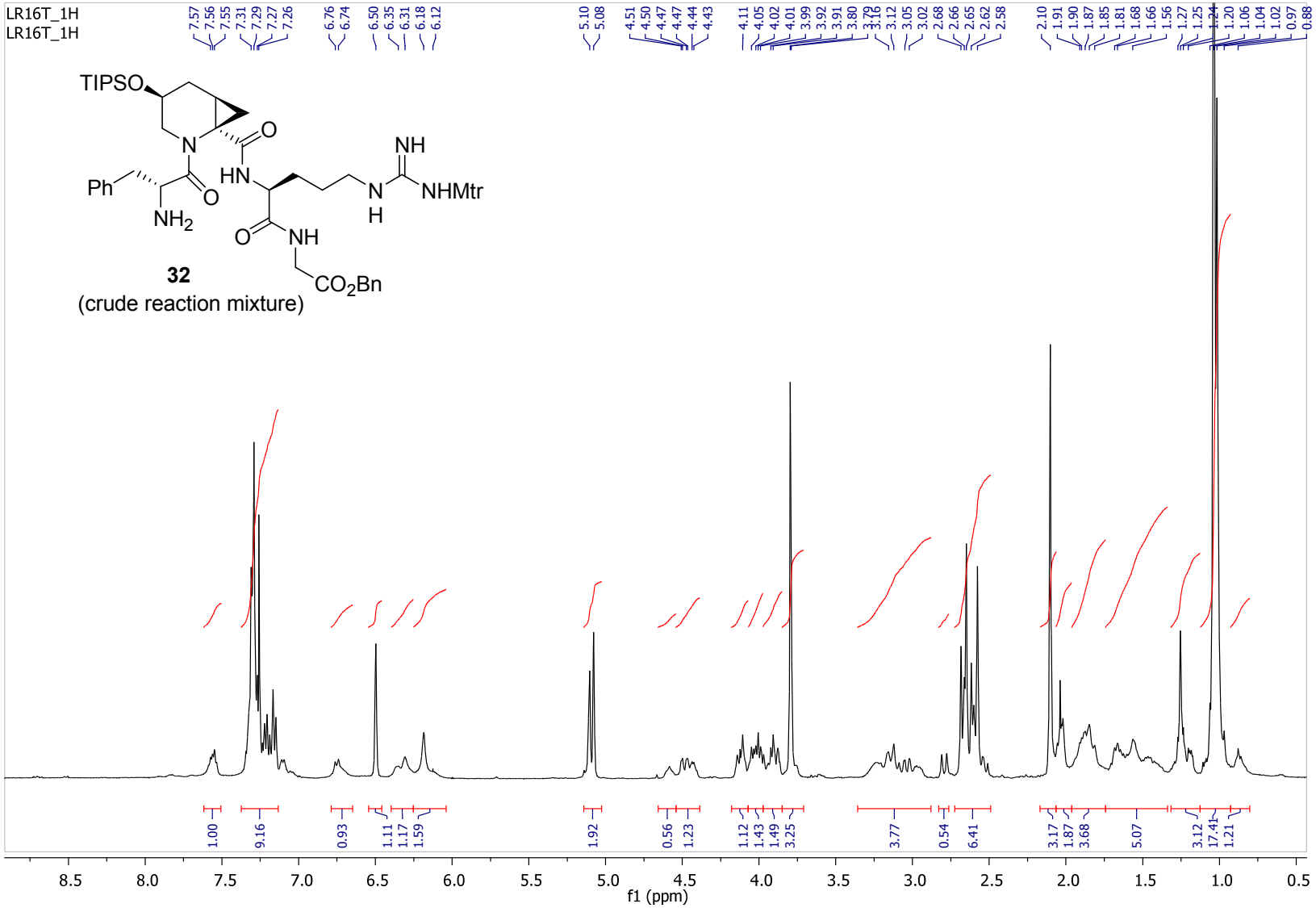
^{1}H NMR ($CDCl_3$, 200 MHz) of compound **30** (crude reaction mixture)



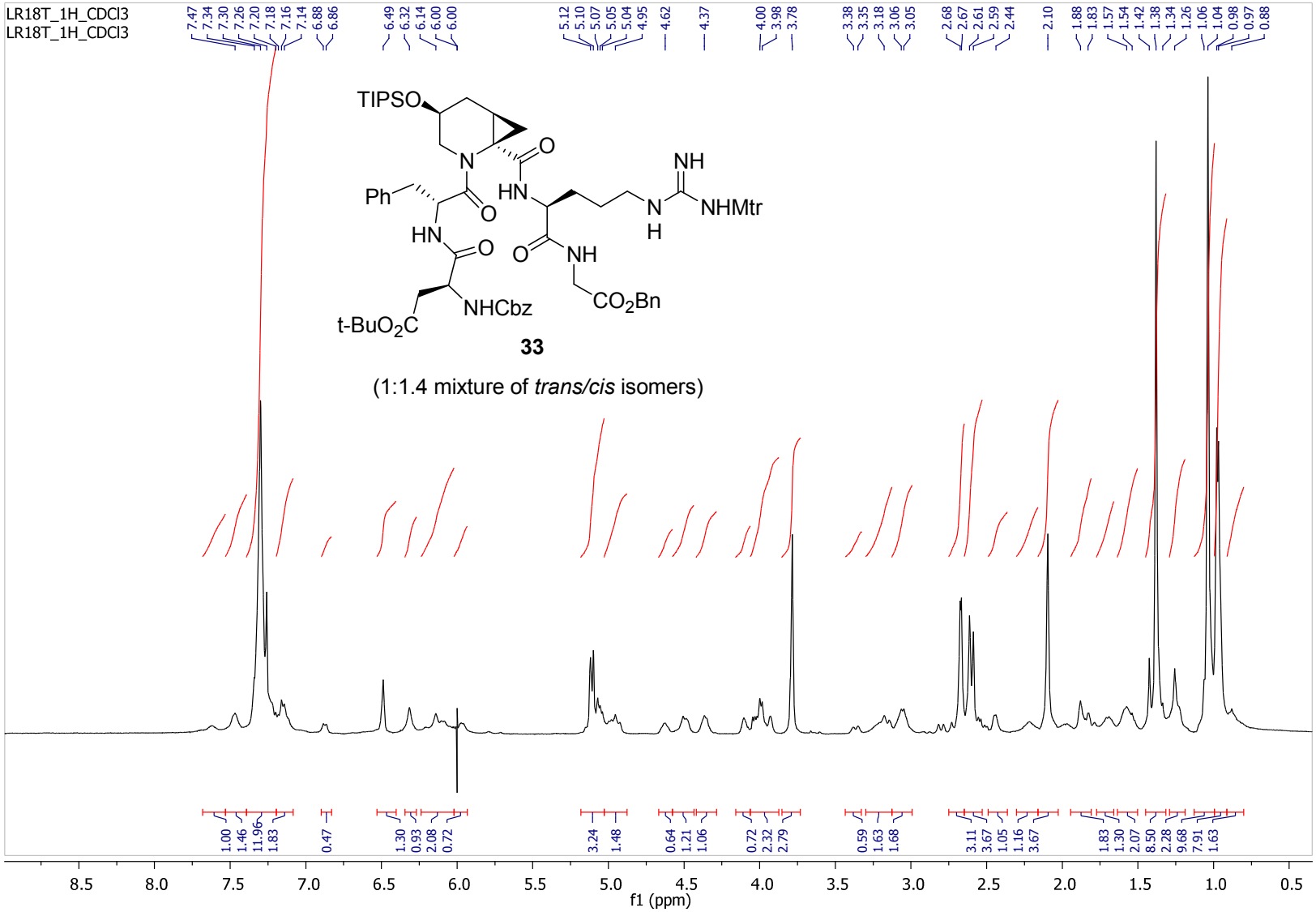
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **31**



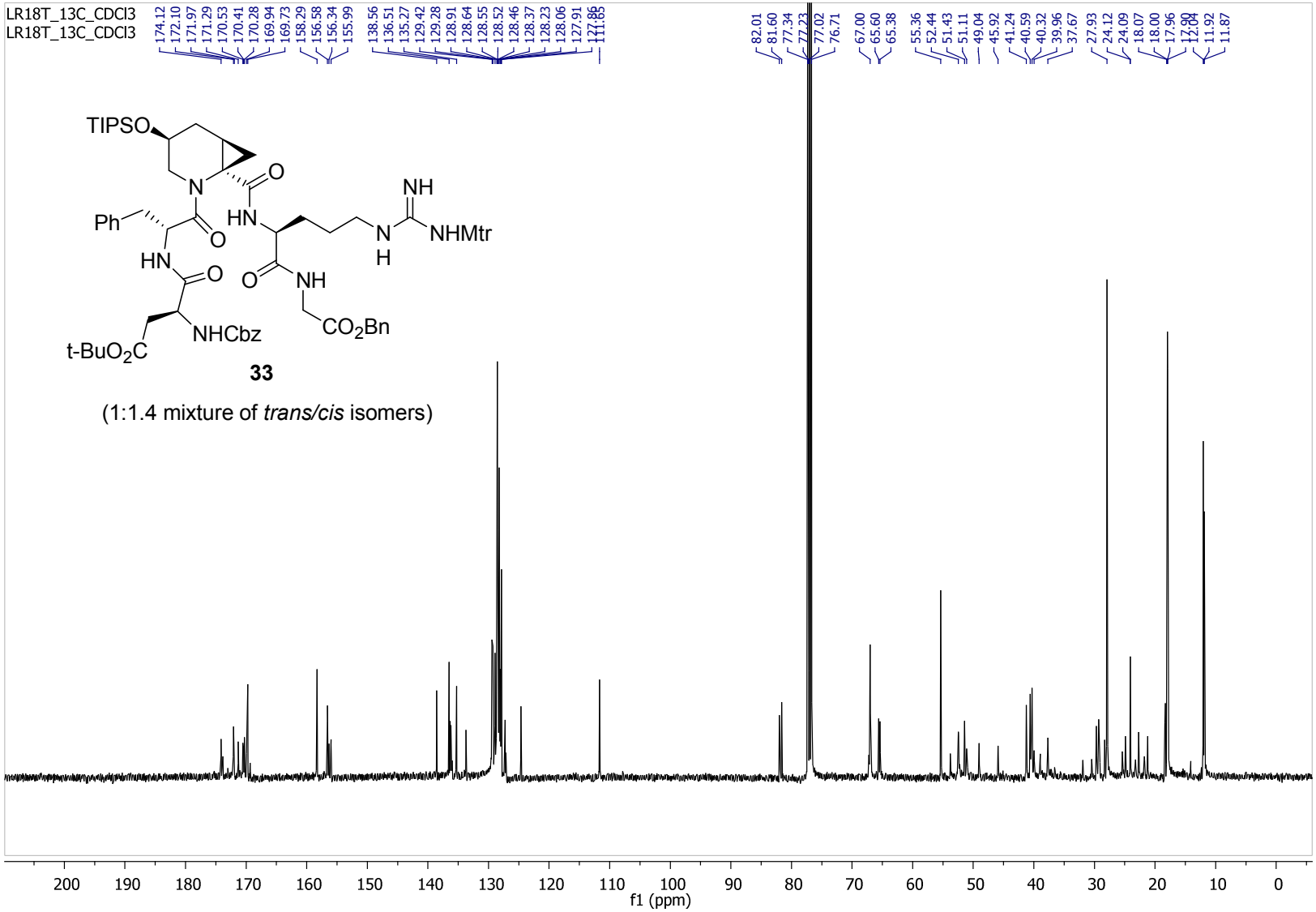
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **31**



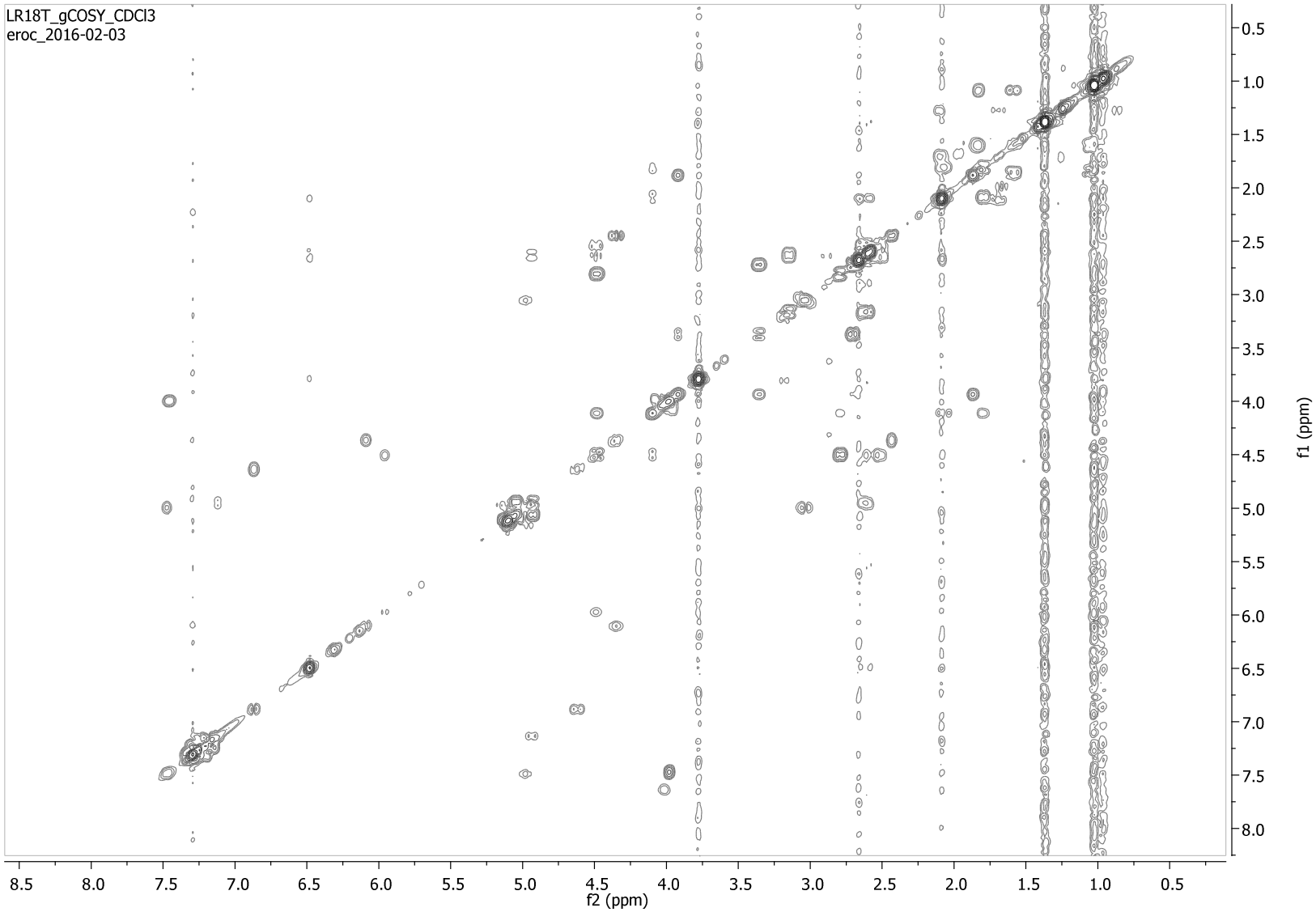
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **32** (crude reaction mixture)



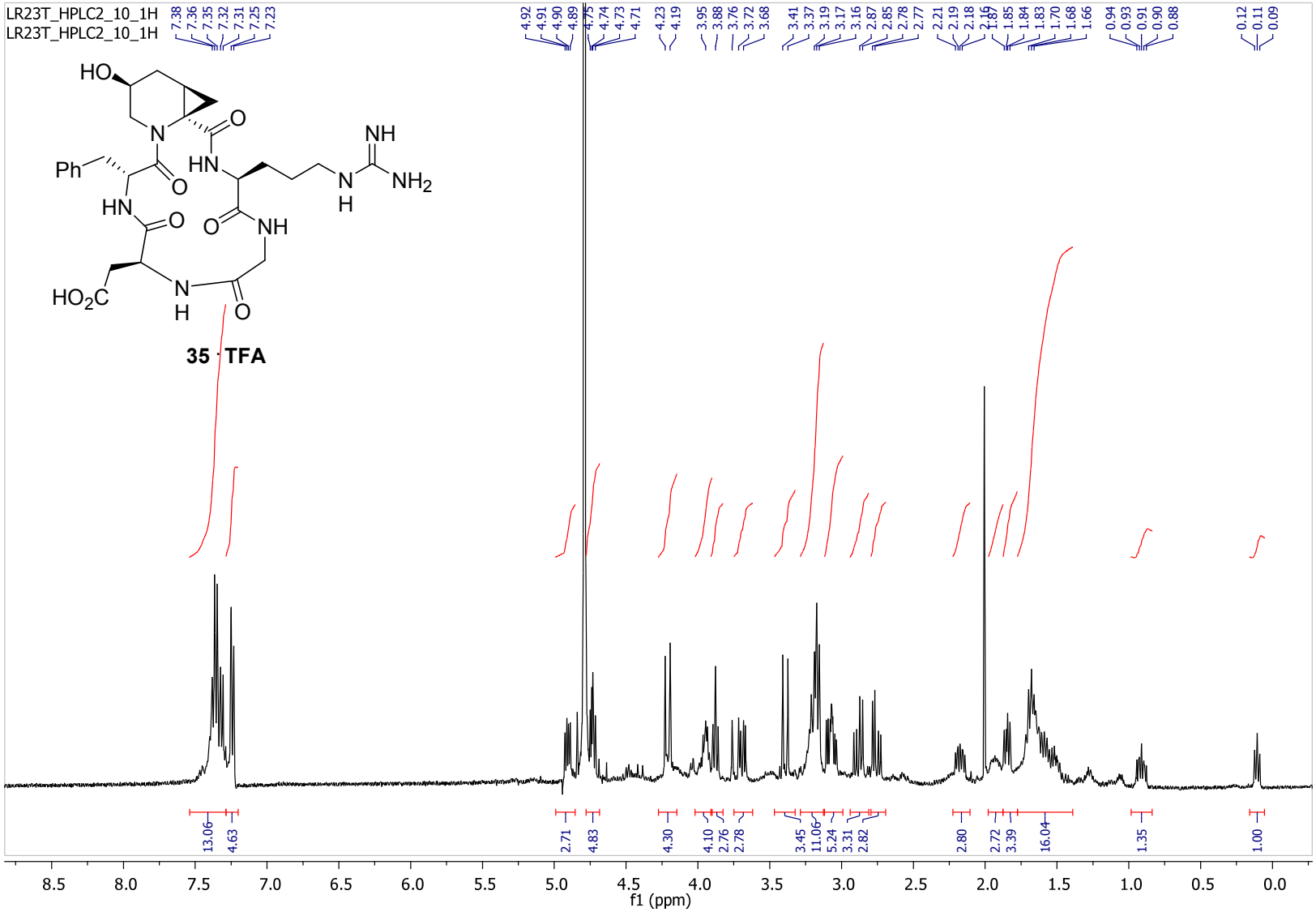
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **33**



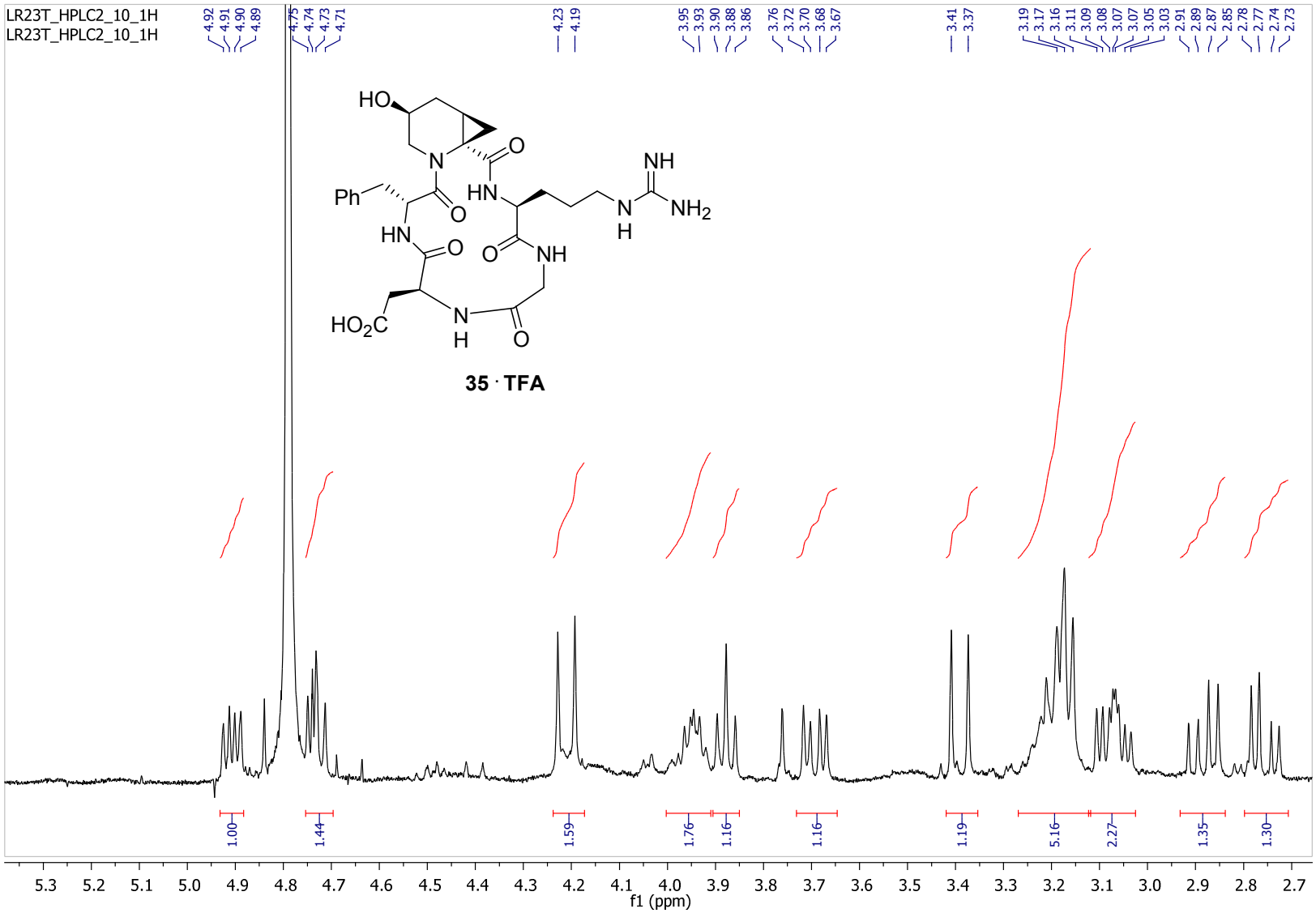
^{13}C NMR ($CDCl_3$, 100.4 MHz) of compound **33**



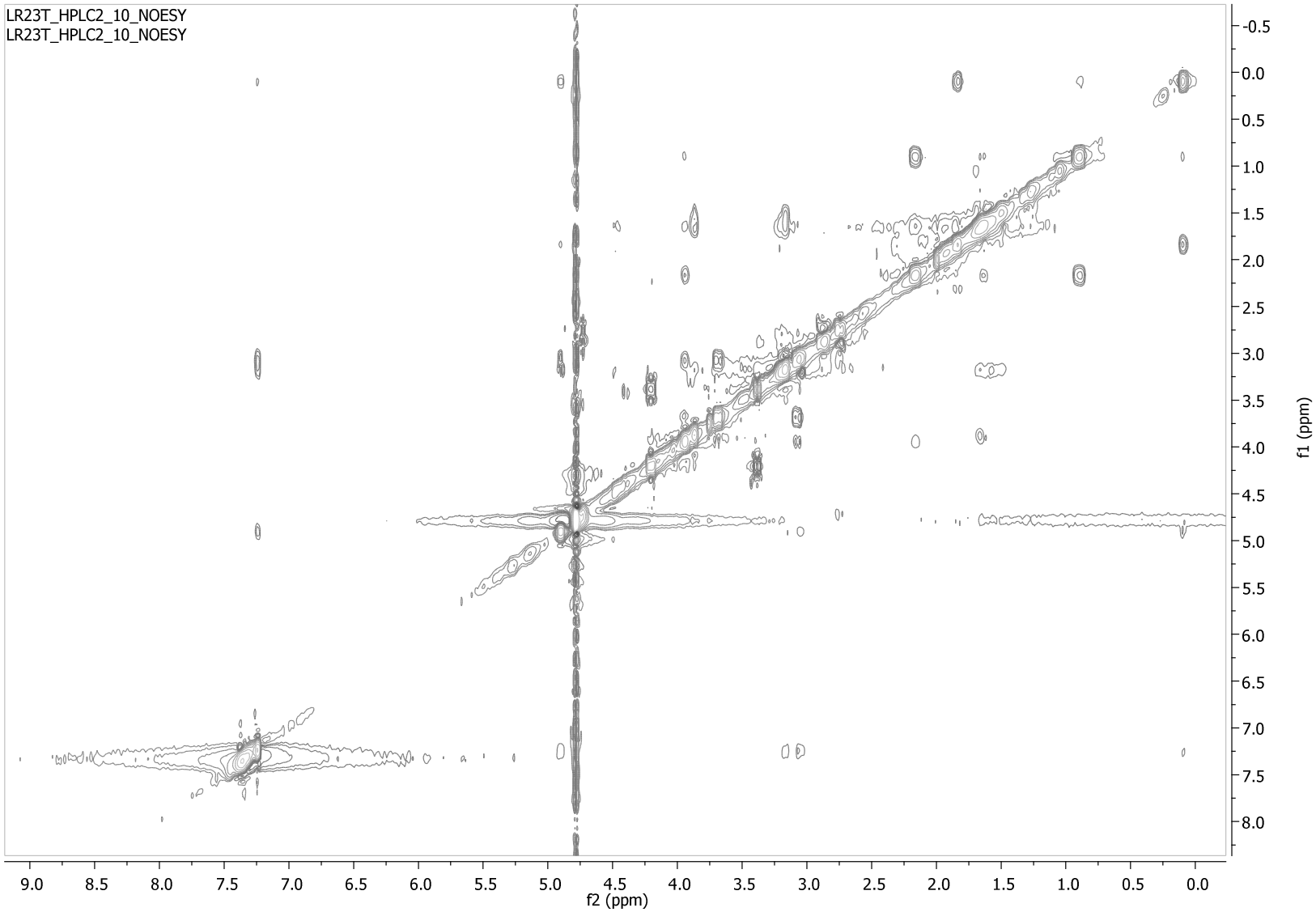
gCOSY ($CDCl_3$, 400 MHz) of compound **33**



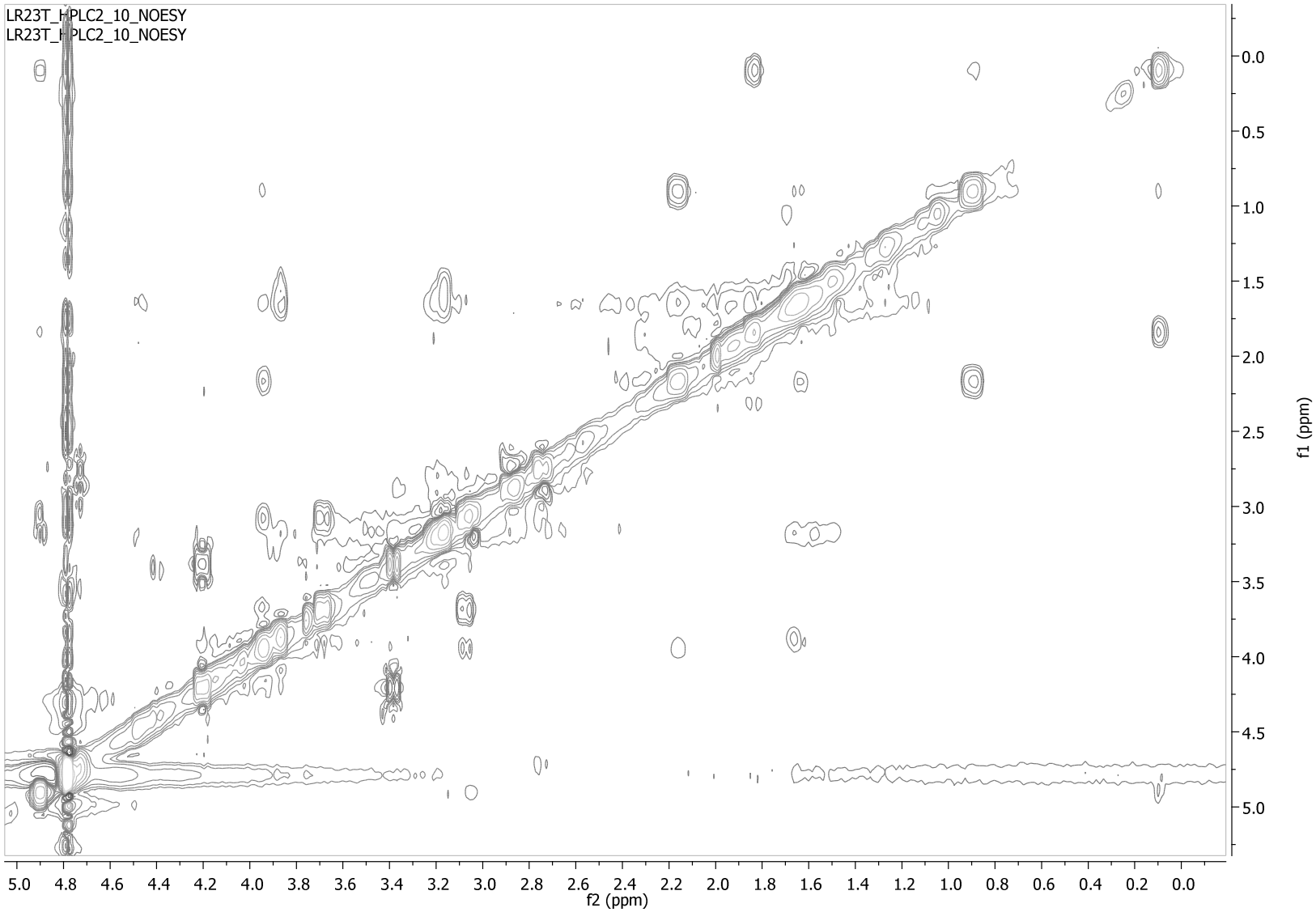
^{1}H NMR (D_2O, 400 MHz) of compound **35**



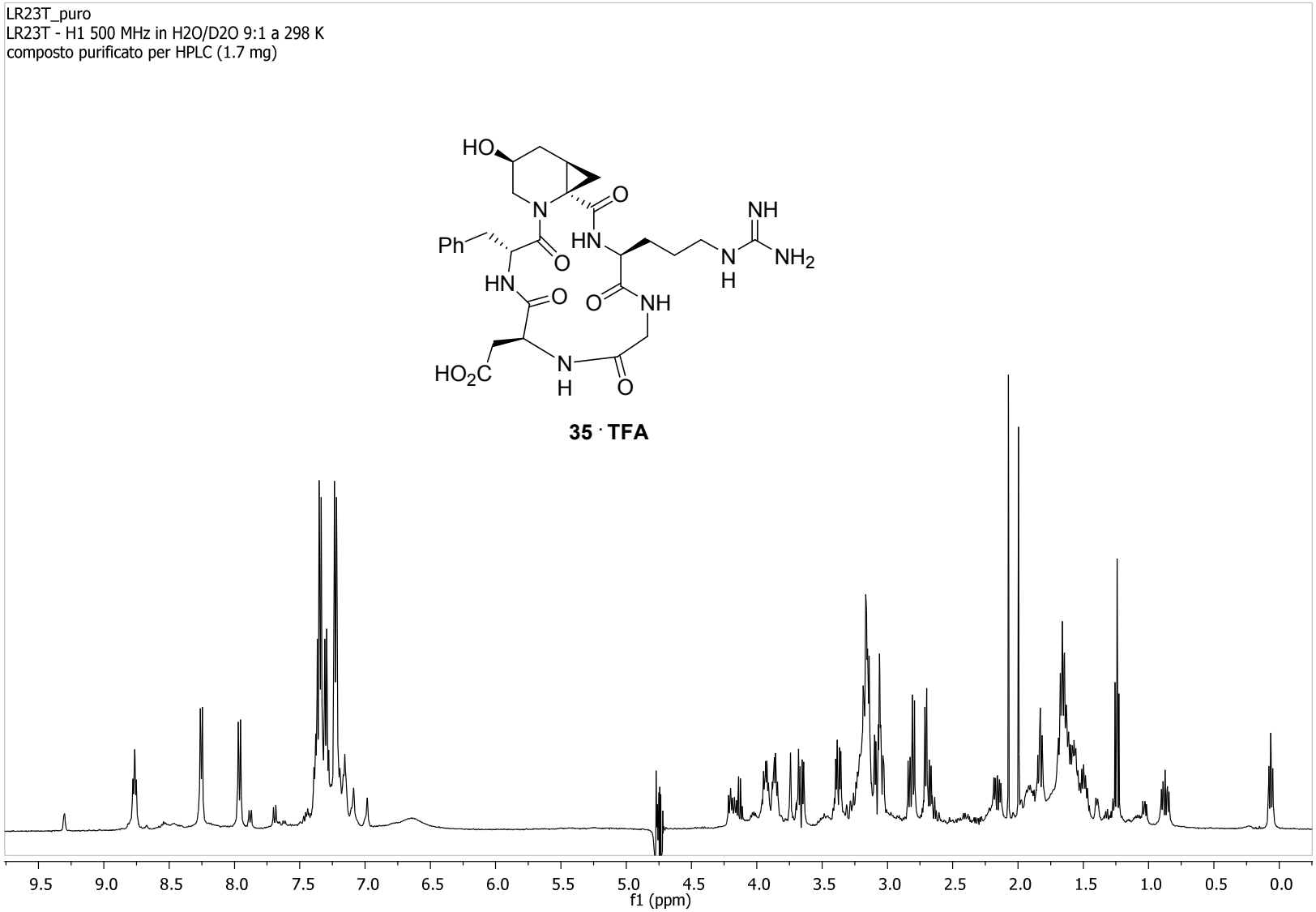
^{1}H NMR (D_2O, 400 MHz) of compound **35** (expanded 5.4-2.6 ppm area)



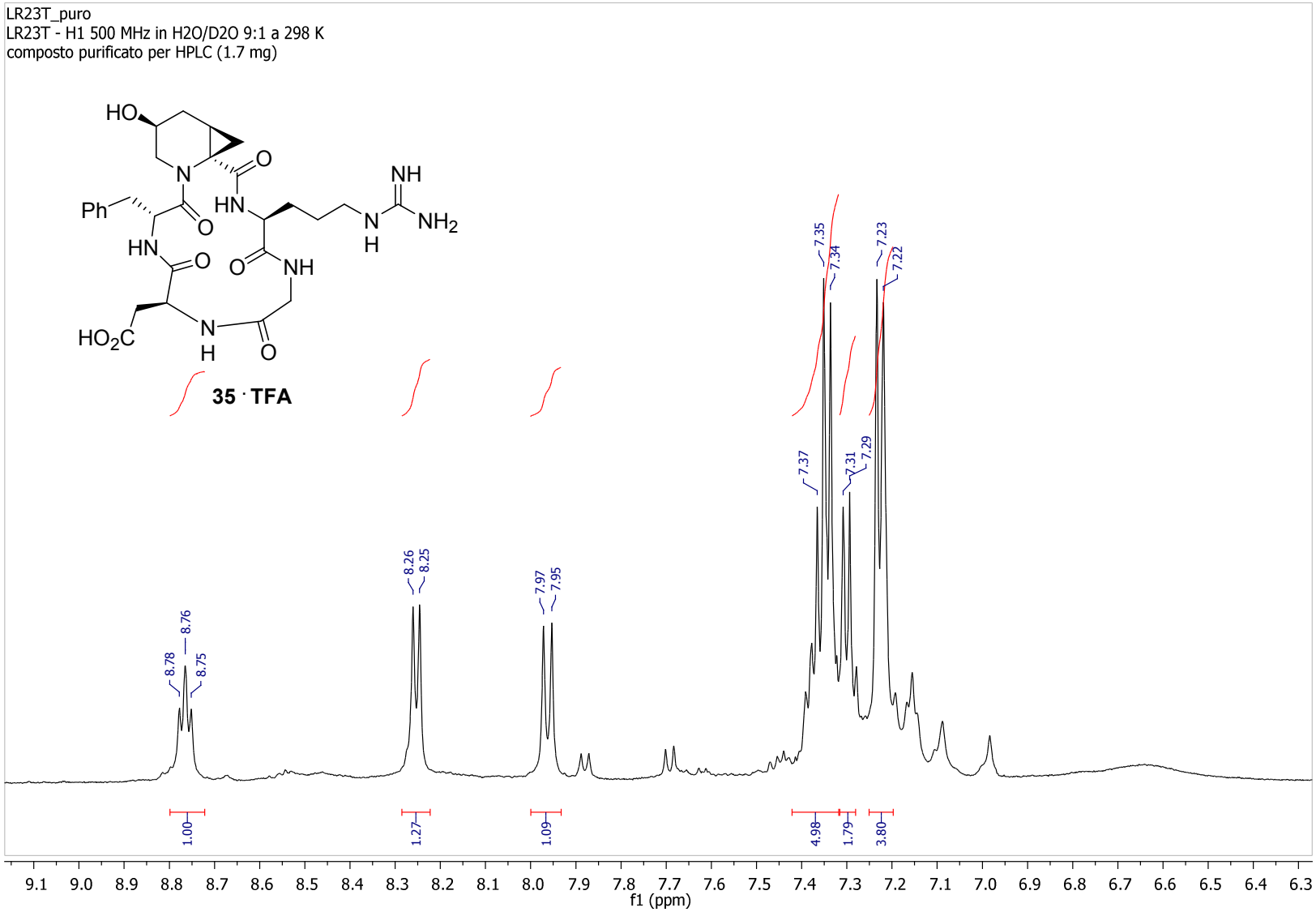
NOESY (D_2O, 400 MHz) of compound **35**



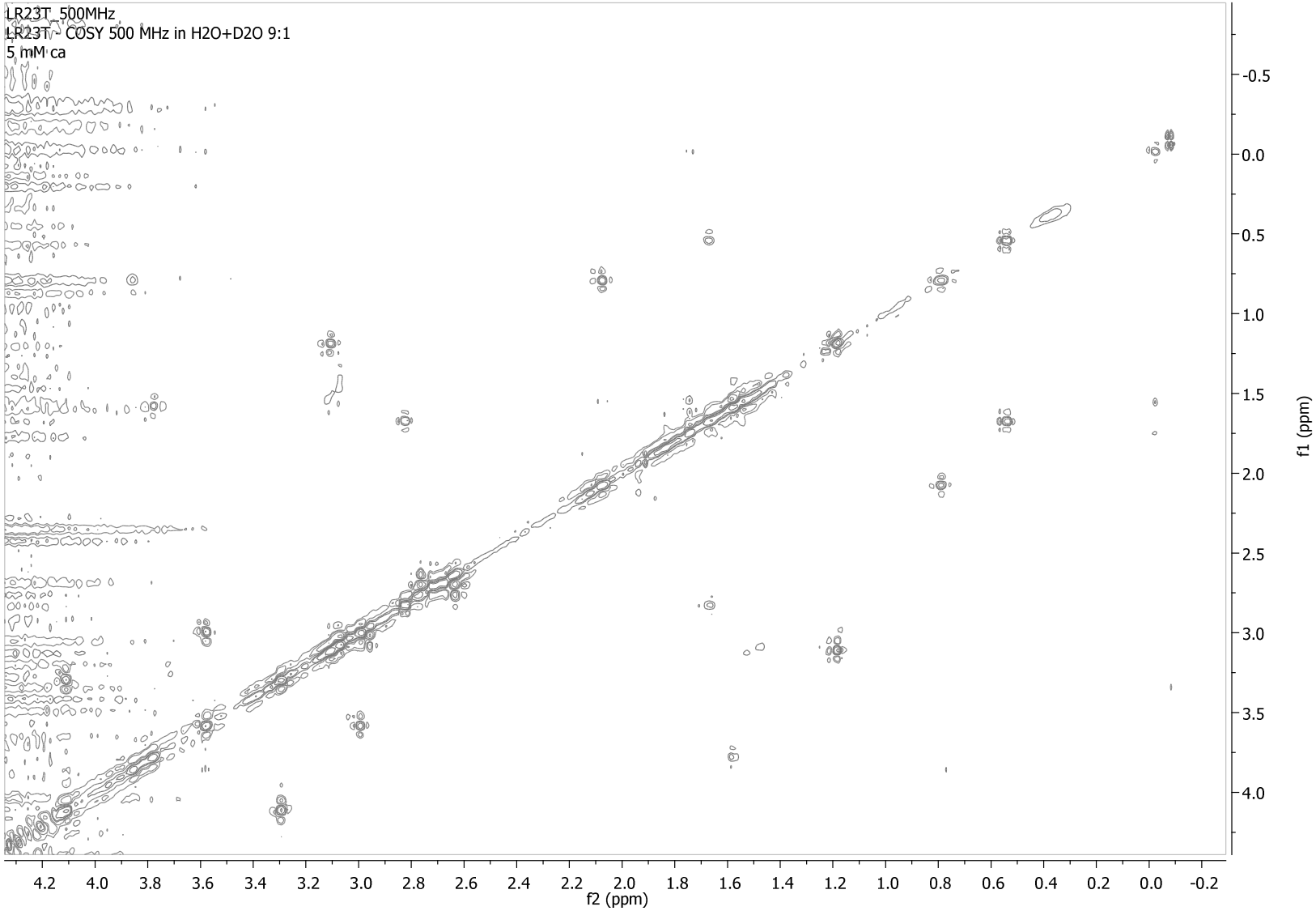
NOESY (D_2O, 400 MHz) of compound **35** (expanded area)



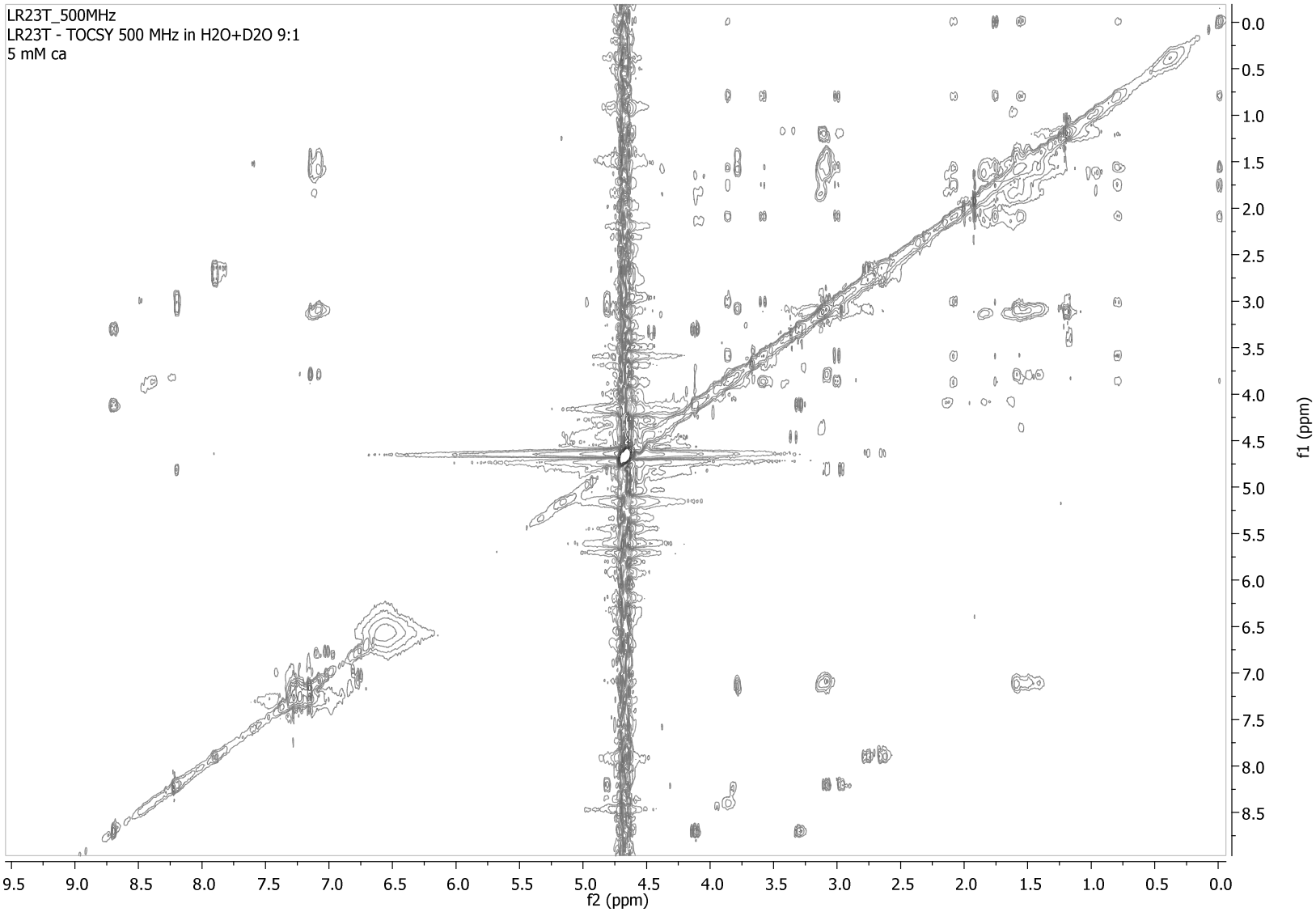
^{1}H NMR (500 MHz) of compound **35** in H_2O/D_2O 9:1



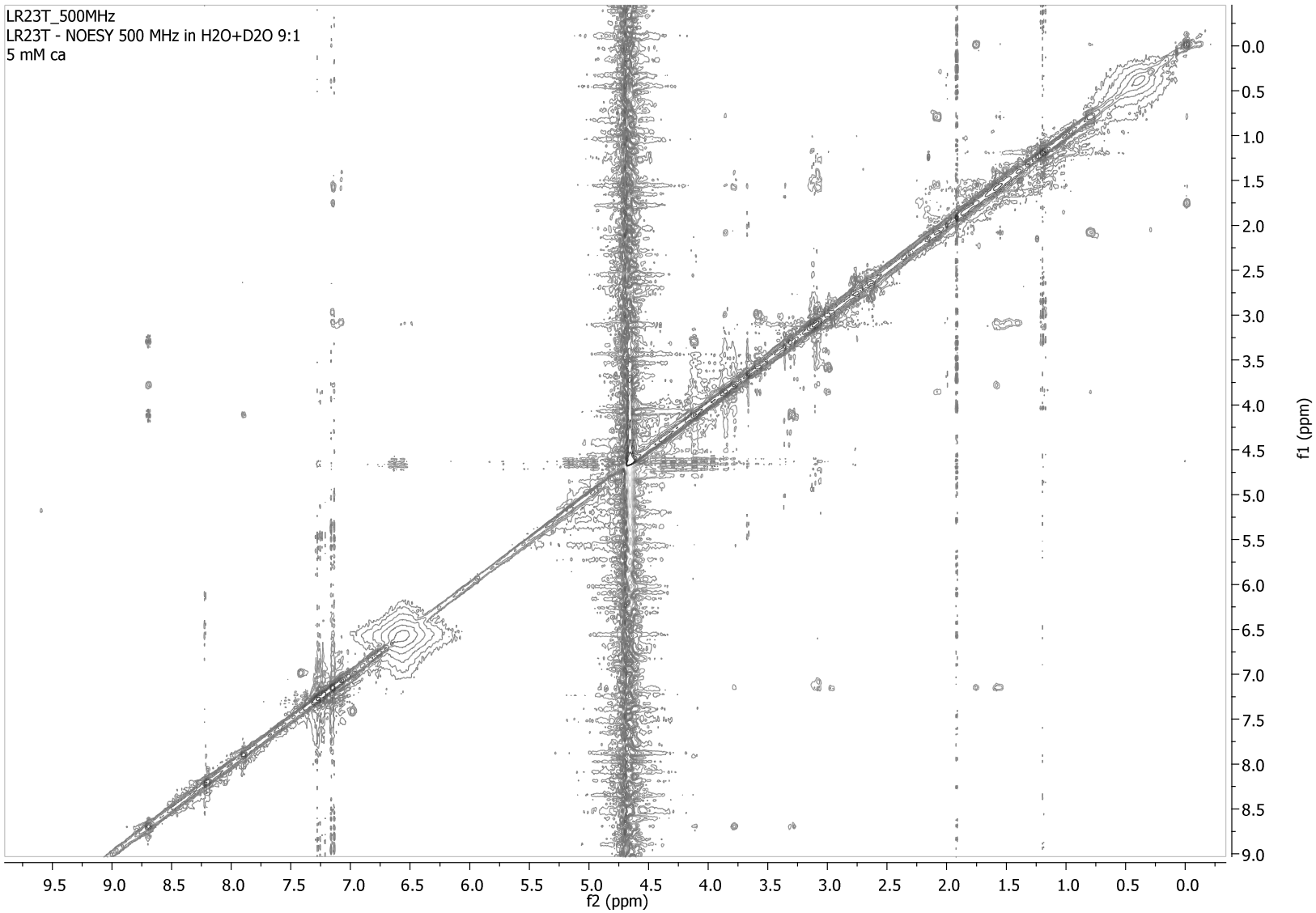
^{1}H NMR (500 MHz) of compound **35** in H_2O/D_2O 9:1 (expanded 9.1-6.3 ppm area)



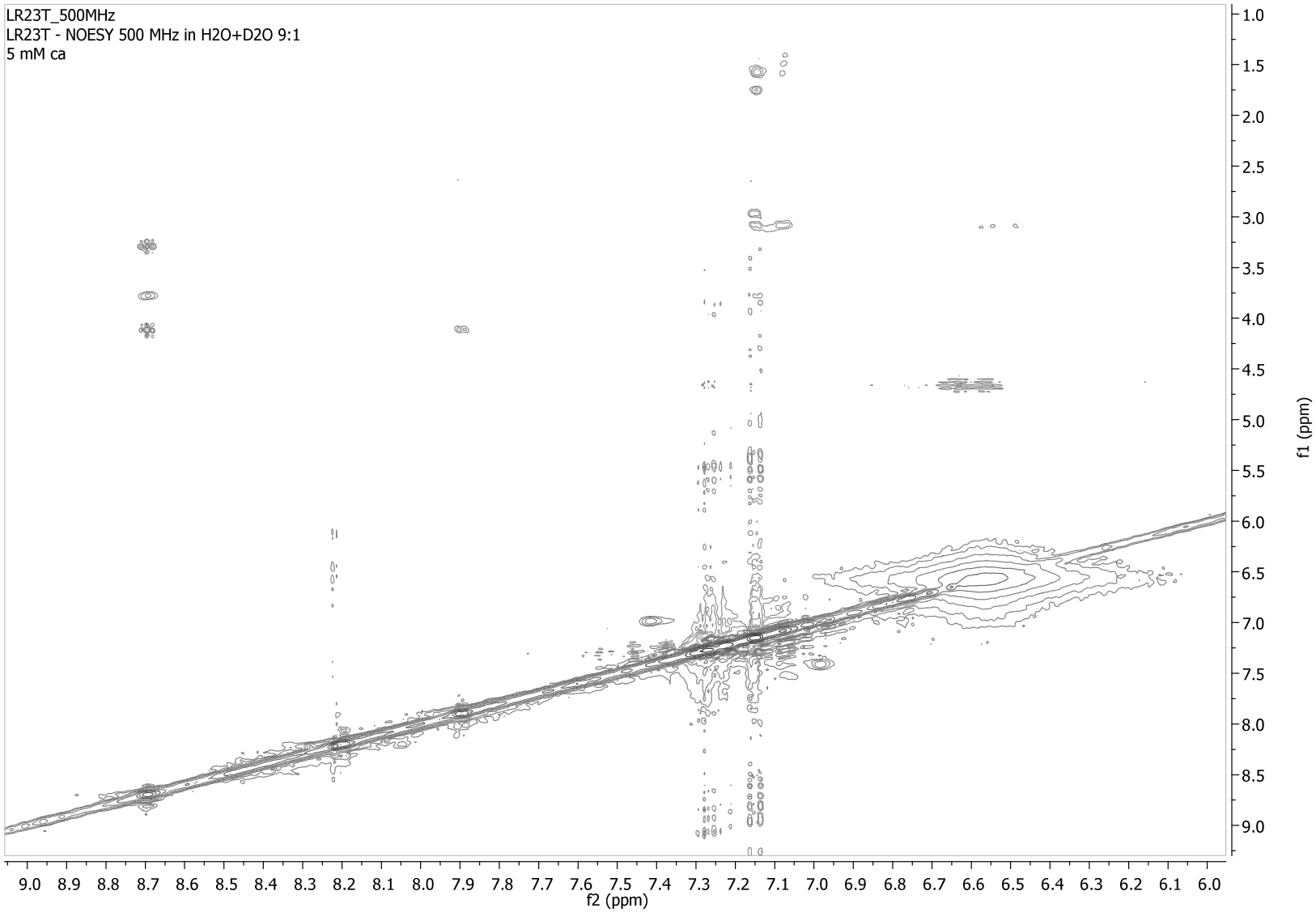
gCOSY (500 MHz) of compound **35** in H_2O/D_2O 9:1 (0.0-4.2 ppm expanded area)



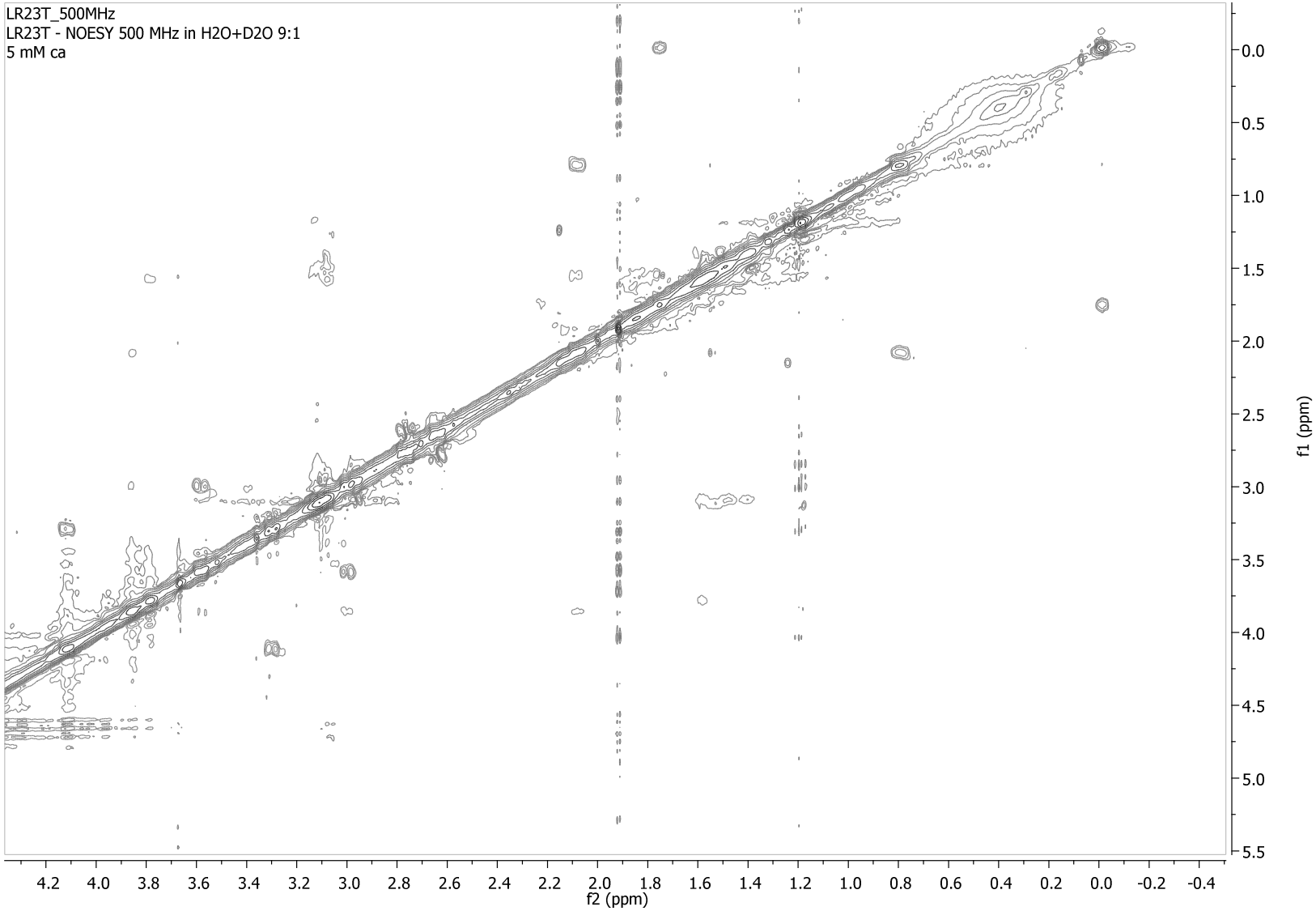
TOCSY (500 MHz) of compound **35** in H_2O/D_2O 9:1



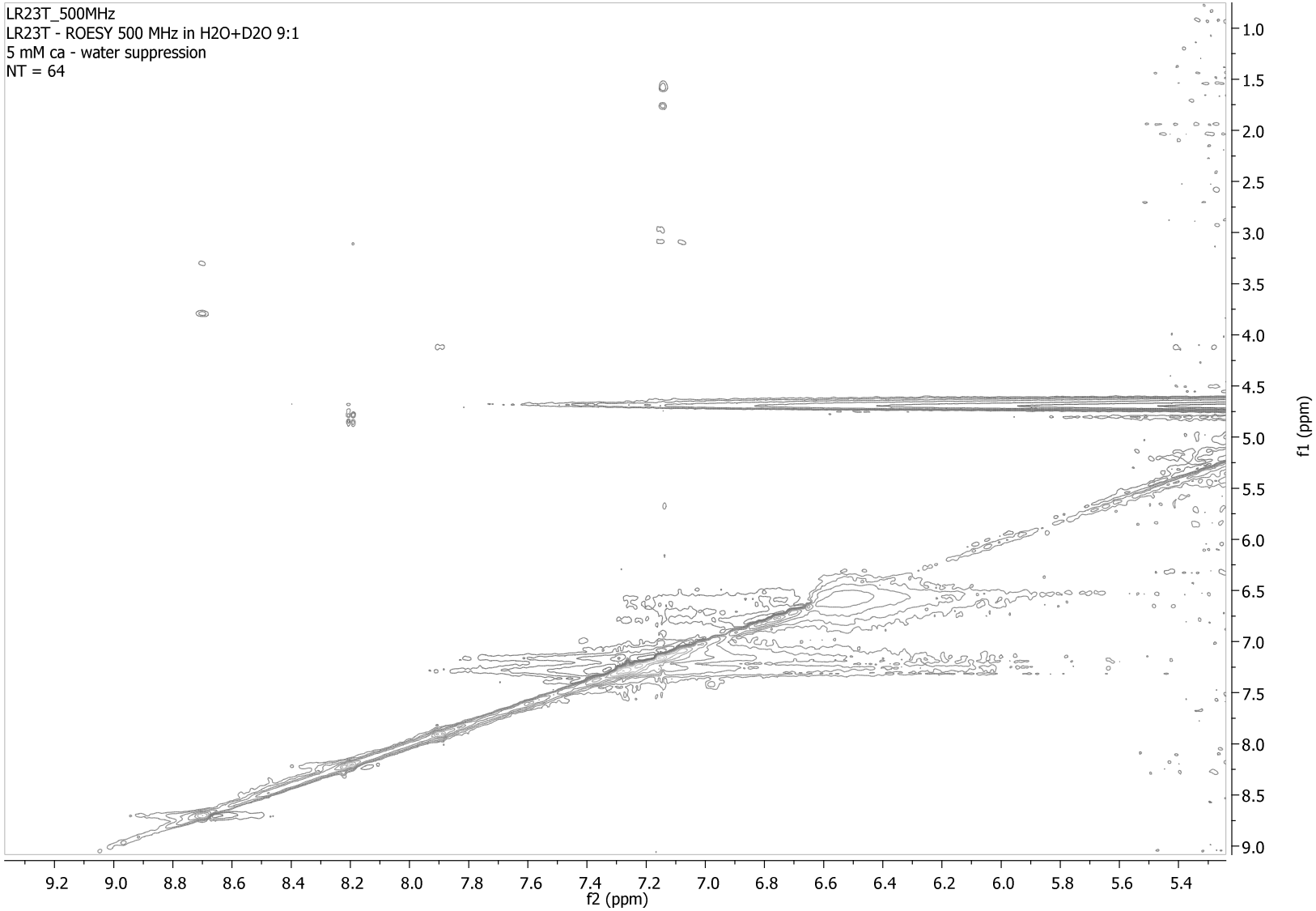
NOESY (500 MHz) of compound **35** in H_2O/D_2O 9:1



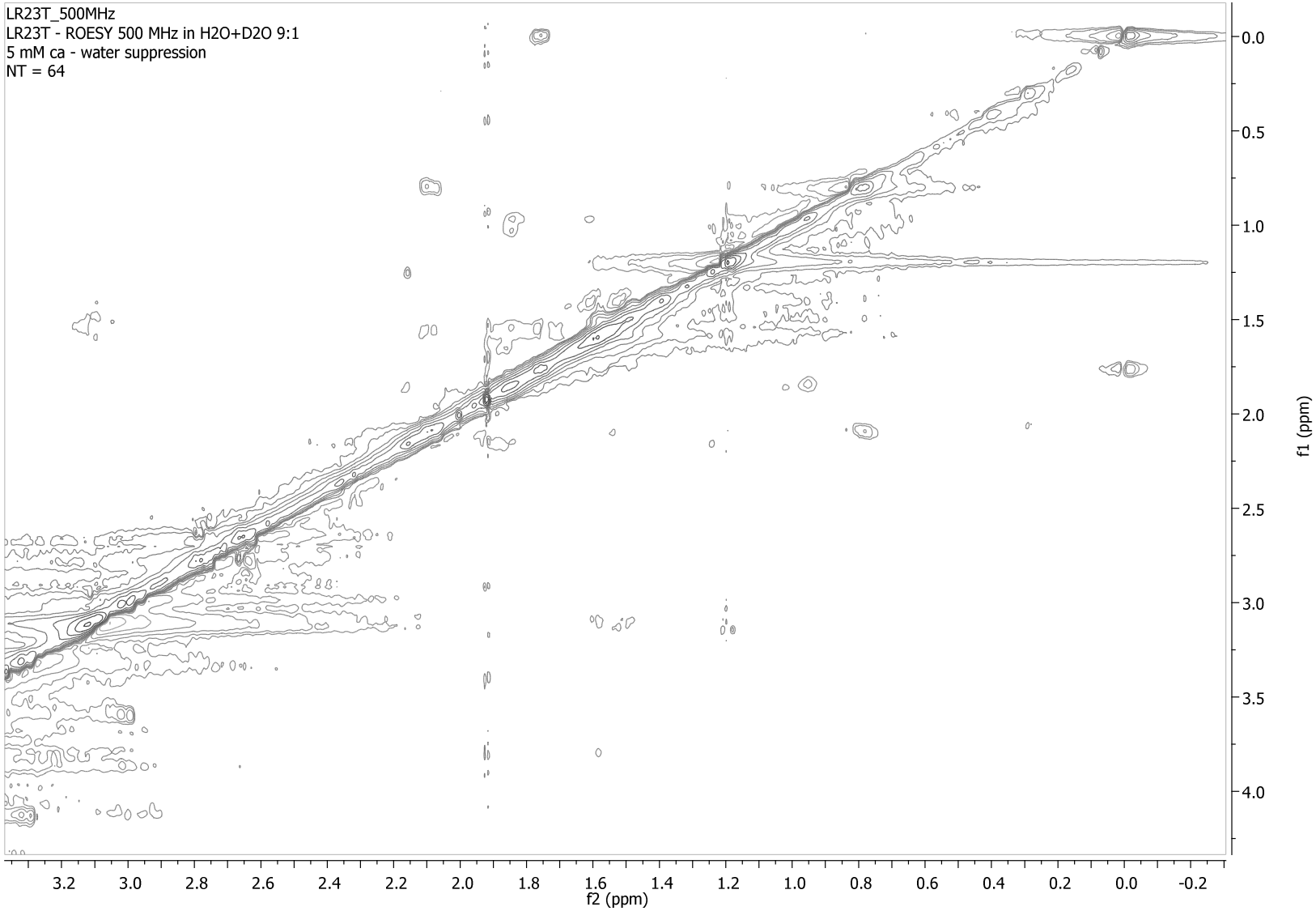
NOESY (500 MHz) of compound **35** in H_2O/D_2O 9:1 (expanded area)



NOESY (500 MHz) of compound **35** in H_2O/D_2O 9:1 (expanded area)



ROESY (500 MHz) of compound **35** in H_2O/D_2O 9:1 (expanded area)



ROESY (500 MHz) of compound **35** in H_2O/D_2O 9:1 (expanded area)