

Supporting Information

Total synthesis of (29*S*,37*S*)-isomer of malevamide E, a potent ion-channel inhibitor

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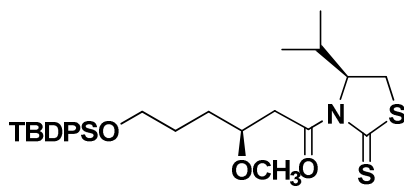
General Experimental Procedures. All reactions were carried out in oven or flame-dried glassware with magnetic stirring under nitrogen atmosphere using dry, freshly distilled solvents, unless otherwise noted. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm silica gel plates with UV light, I₂, 7% ethanolic phosphomolybdic acid-heat and 2.5% ethanolic anisaldehyde (with 1% AcOH and 3.3% conc. H₂SO₄)-heat as developing agents. Silica gel finer than 200 mesh was used for flash column chromatography. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated. Melting points are uncorrected.

Optical rotations were measured using Autopol III manufactured by Rudolph using sodium (589, D line) lamp and are reported as follows: $[\alpha]_D^{25}$ ($c = \text{g}/100 \text{ ml}$, solvent). MALDI-TOF was recorded on ABSciex ToF spectrometer. Analytical HPLC analyses were performed on a Merck Hitachi HPLC system equipped with a 5 μ Thermo C-8 column (250 \times 4.6 mm) and a UV/vis detector setting of $\lambda = 254 \text{ nm}$.

IR spectra were recorded as neat liquids or KBr pellets. Mass spectra were obtained under electron impact ionisation (EI), liquid secondary ion mass spectrometric (LSIMS) technique, electron spray ionisation (ESI) and MALDI techniques. Optical rotations were measured with a digital polarimeter.

NMR spectra were recorded on 600, 400, 300 and 200 MHz spectrometers at 30 °C with 2-10 mM solutions in appropriate solvents using TMS as internal standard or the solvent signals as secondary standards and the chemical shifts are shown in δ scales. Multiplicities of NMR signals are designated as s (singlet), d (doublet), t (triplet), q (quartet), br (broad), m (multiplet, for unresolved lines), etc. ¹³C NMR spectra were recorded on 100, 75 and 50 MHz spectrometers with complete proton decoupling.

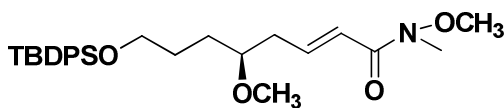
Compound 10:



10

A solution of thione **9** (2.18 gm, 10.74 mmol) in 100 mL CH₂Cl₂ was cooled to 0 °C. Neat TiCl₄ (1.25 mL, 11.81 mmol) was added dropwise and the resulting slurry was stirred for 5 min. The reaction was then cooled to -78 °C and *i*-Pr₂NEt (2.06 mL, 11.81 mmol) in 2 mL of CH₂Cl₂ was added dropwise. The resulting dark red solution was warmed to -40 °C and stirred for 2 h. After 2 h, the reaction was cooled to -78 °C and freshly distilled, neat SnCl₄ (0.63 mL, 5.37 mmol) was added dropwise, followed by the addition of acetal **8** (2.0 gm, 5.37 mmol) in 3 mL CH₂Cl₂. After addition was complete, the reaction was allowed to stir at -78 °C for an additional 15 min, and then transferred into a -20 °C bath where it was allowed to stir for 2 h. The reaction was then quenched by the addition of a saturated aqueous NH₄Cl solution. The layers were separated and the aqueous layer was then extracted into CH₂Cl₂ (2 x 50 mL). The organic extracts were dried (Na₂SO₄) and evaporated to give a crude yellow oil which was purified by flash chromatography (8% EtOAc/hexanes to 10% EtOAc /hexanes) to afford the product **10** (1.57 gm, 54%) as a yellow oil along with the other isomer. *R_f* = 0.3 (SiO₂, 15% EtOAc in petroleum ether); specific rotation [*α*]_D²⁵ = 94.1 (*c* 1.2, CHCl₃); IR (neat): *v*_{max} 3749, 3180, 2932, 2862, 2361, 1704, 1466, 1217, 1103, 768, 705 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 7.78-7.61 (m, 4H), 7.50-7.35 (m, 6H), 5.14 (t, *J* = 7.2 Hz, 1H), 3.85 (m, 1H), 3.76-3.66 (m, 2H), 3.60-3.41 (m, 2H), 3.35 (m, 1H), 3.34 (s, 3H), 3.02 (m, 1H), 2.40 (m, 1H), 1.75-1.56 (m, 4H), 1.15-0.93 (m, 15H); ¹³C NMR (50 MHz, CDCl₃): δ 201.80, 131.75, 135.48, 133.85, 129.49, 127.56, 71.64, 69.93, 63.69, 56.87, 43.10, 35.92, 31.98, 30.78, 30.58, 29.42, 27.91, 26.79, 19.12, 19.03, 18.74, 18.19, 17.70; MS (ESIMS): *m/z* (%): 544 (90) [M + H]⁺; HRMS (ESIMS): calcd for C₂₉H₄₂NO₃S₂Si [M + H]⁺: 544.2375, found: 544.2372.

Compound 12:

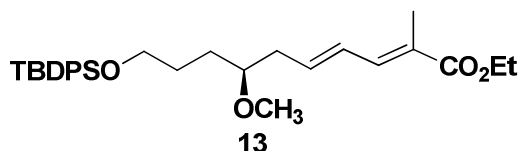


12

Compound **10** (1.10 g, 2.0 mmol) was taken in dry CH₂Cl₂ (6 mL), cooled to -78 °C and DIBAL-H (4.0 mL, 1 M in toluene, 4.0 mmol) was added portion-wise and stirred at that temperature for 15 min. The reaction was then quenched by slow addition of dry methanol and brought to room temperature. Saturated aqueous potassium-sodium tartrate solution was added to the reaction mixture and stirred (~ 2 h) until two clear layers got separated. Solvent was evaporated under reduced pressure and the aqueous layer was extracted with EtOAc. The organic layer was washed with H₂O, brine, dried (Na₂SO₄) and concentrated in *vacuo*. The aldehyde thus obtained by flash chromatography (745 mg, 96%) was directly used in the next step without further characterization.

To a solution of aldehyde (745 mg, 1.94 mmol) in dry THF (3 mL) at $-78\text{ }^{\circ}\text{C}$ was added NaH (79 mg, 1.98 mmol) in portion wise followed by *N*-methyl diethylphosphoacetamide (**11**) (0.41 mL, 1.98 mmol), and slowly the reaction mixture was brought to $-40\text{ }^{\circ}\text{C}$ and allowed to stir at the same temperature for 1 h. The reaction was then quenched by slow addition of NH_4Cl solution (5 mL). THF was removed under reduced pressure and the product was extracted from aqueous layer with EtOAc (2 x 50 mL). The combined organic layer was washed with brine (10 mL), dried (Na_2SO_4), filtered and concentrated in *vacuo*. Purification by column chromatography (SiO_2 , 30% EtOAc in petroleum ether eluant) afforded the Wenreb amide **12** (702 mg, 78%) as colorless oil. $R_f = 0.3$ (SiO_2 , 40% EtOAc in petroleum ether); specific rotation $[\alpha]_{\text{D}}^{25} = 13.4$ (*c* 0.24, CHCl_3); IR (neat): ν_{max} 3415, 2355, 1634, 1381, 1218, 1103, 769, 703 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 7.75-7.60 (m, 4H), 7.49-7.33 (m, 4H), 6.98 (m, 1H), 6.47 (d, $J = 15.56$ Hz, 1H), 3.76-3.62 (m, 5H), 3.69 (s, 3H), 3.33 (s, 3H), 3.29 (m, 1H), 3.25 (s, 3H), 2.51-2.31 (m, 2H) 1.70-1.52 (m, 4H), 1.07 (s, 9H); ^{13}C NMR (50 MHz, CDCl_3): δ 166.68, 143.88, 135.56, 133.99, 129.54, 127.61, 120.76, 79.70, 63.85, 61.66, 56.73, 36.91, 32.35, 29.90, 28.14, 26.87, 19.20; MS (ESIMS): m/z (%): 470 (100) $[\text{M} + \text{H}]^+$; HRMS (ESIMS): calcd for $\text{C}_{27}\text{H}_{40}\text{NO}_4\text{Si}$ $[\text{M} + \text{H}]^+$: 470.2724, found: 470.2715.

Compound 13:

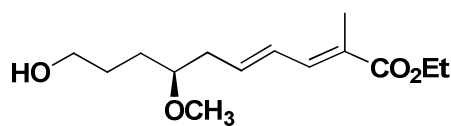


Compound **12** (650 mg, 1.38 mmol) was taken in dry THF (4 mL), cooled to $-78\text{ }^{\circ}\text{C}$ and DIBAL-H (4.15 mL, 1.0 M in toluene, 4.15 mmol) was added portion-wise and stirred at that temperature for 15 min. The reaction was then quenched by slow addition of dry methanol and brought to room temperature. Saturated aqueous potassium-sodium tartrate solution was added to the reaction mixture and stirred (~ 2 h) until two clear layers got separated. Solvent was evaporated under reduced pressure and the aqueous layer was extracted with EtOAc. The organic layer was washed with H_2O , brine, dried (Na_2SO_4) and concentrated *vacuo*. The aldehyde thus obtained by flash chromatography (550 mg, 97%) was directly used in the next step without further characterization.

To a solution of aldehyde obtained above (550 mg, 1.34 mmol) in dry benzene (4 mL) was added (1-ethoxycarbonylethylidene)-triphenylphosphorane (971 mg, 2.68 mmol) at room temperature. The mixture was stirred for 4 h at reflux conditions. It was then concentrated in *vacuo*. Purification by column chromatography (SiO_2 , 10% EtOAc/hexanes to 15% EtOAc /hexanes eluant) furnished **13** (530 mg, 80%) as a colorless liquid. $R_f = 0.3$ (SiO_2 , 20% EtOAc in petroleum ether); specific rotation $[\alpha]_{\text{D}}^{25} = 24.97$ (*c* 0.38, CHCl_3); IR (neat): ν_{max} 3441, 2933, 2359, 1700, 1360, 1219, 1104, 769, 704 cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 7.77-7.61 (m, 4H), 7.50-7.33 (m, 6H), 7.20 (m, 1H), 6.41 (dd, $J = 15.34$ Hz, 11.16 Hz, 1H), 6.08 (m, 1H), 4.23 (q, $J = 7.01$ Hz, 2H), 3.77-3.63 (m, 2H), 3.34 (s, 3H), 3.28 (m, 1H), 2.40 (t, $J = 6.83$ Hz, 2H), 1.95 (s, 3H), 1.73-1.49 (m, 4H), 1.32 (t, $J = 7.01$ Hz, 1H), 1.07 (s, 9H); ^{13}C NMR (50 MHz, CDCl_3): δ 168.61, 138.57, 138.24, 135.57, 133.97, 129.57, 128.08, 127.62, 125.63, 80.12, 63.82, 60.49, 56.67, 37.34, 29.75, 28.18, 26.87, 19.22, 14.35, 12.63; MS

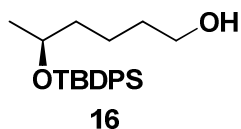
(ESIMS): m/z (%): 517 (100) [M + Na HRMS (ESIMS): calcd for $C_{30}H_{42}O_4SiNa$ [M + Na]⁺: 517.2750, found: 517.2798.

Compound 14:



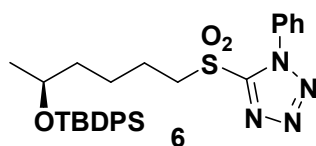
To a solution of **13** (500 mg, 1.01 mmol) in dry THF (3 mL), TBAF (1M in THF, 2.02 mL, 2.02 mmol) was added at 0 °C, the reaction mixture was warmed to room temperature and stirred for 1 h. It was quenched with saturated aqueous NH_4Cl solution, extracted with EtOAc, washed with brine, dried (Na_2SO_4) and concentrated in *vacuo*. Purification by column chromatography (SiO_2 , 25 to 30% EtOAc in petroleum ether eluant) afforded compound **14** (220 mg, 85%) as a clear oil. R_f = 0.3 (SiO_2 , 30% EtOAc in petroleum ether); specific rotation $[\alpha]_D^{25} = 48.3$ (c 0.12, $CHCl_3$); IR (neat): ν_{max} 3422, 2931, 2359, 1707, 1373, 1221, 1095, 770, 676 cm^{-1} ; 1H NMR (300 MHz, $CDCl_3$): δ 7.18 (m, 1H), 6.42 (dd, $J = 15.27, 11.81$ Hz, 1H), 6.07 (m, 1H), 4.22 (q, $J = 7.11$ Hz, 2H), 3.71-3.60 (m, 2H), 3.39 (s, 3H), 3.33 (m, 1H), 2.53-2.34 (m, 2H) 1.95 (s, 3H), 1.76-1.51 (m, 5H), 1.31 (t, $J = 7.11$ Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.57, 138.04, 128.25, 125.86, 80.38, 62.85, 60.50, 56.72, 37.08, 30.32, 28.60, 14.30, 12.59; MS (ESIMS): m/z (%): 279 (80) [M + Na]⁺; HRMS (ESIMS): calcd for $C_{14}H_{24}O_4Na$ [M + Na]⁺: 279.1572, found: 279.1586.

Compound 16:



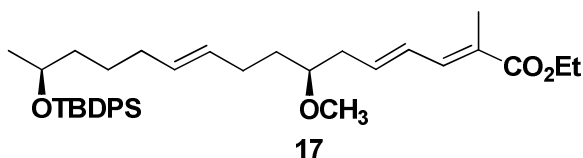
BMS (0.561 mL, 5.91 mmol) was added to olefin **15** (1.0 gm, 2.95 mmol) in anhydrous THF (15 mL). The resulting solution was stirred at room temperature for 6 h. Basic workup: To the reaction solution were added 12 mL of EtOH/THF (1:1) and then 12 mL of H_2O_2 (30% aq), and the resulting mixture was stirred at room temperature for 4h and extracted by ethyl acetate (EtOAc). The combined organic layers were washed with water, brine, dried over Na_2SO_4 , filtered, and concentrated in *vacuo*. Purification on silica gel (15 to 20% EtOAc in petroleum ether eluant) gave 842 mg of **16** in 80% yield. R_f = 0.3 (SiO_2 , 20% EtOAc in petroleum ether); specific rotation $[\alpha]_D^{25} = -6.8$ (c 2.52, $CHCl_3$); IR (neat): ν_{max} 3477, 3402, 2934, 2861, 1725, 1590, 1466, 1218, 1109, 764, 704 cm^{-1} ; 1H NMR (300 MHz, $CDCl_3$): δ 7.81-7.59 (m, 4H), 7.48-7.26 (m, 6H), 3.85 (m, 1H), 3.50 (t, $J = 6.82$ Hz, 2H), 1.60-1.22 (m, 6H), 1.18-0.91 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 135.93, 134.89, 129.55, 127.55, 69.50, 62.82, 39.16, 32.16, 32.68, 27.12, 23.25, 21.39, 19.32; MS (ESIMS): m/z (%): 357 (100) [M + H]⁺; HRMS (ESIMS): calcd for $C_{22}H_{24}O_2Si$ [M + H]⁺: 357.2250, found: 357.2268.

Compound 6:



Triphenylphosphine (884 mg, 3.37 mmol), 1-phenyl-1*H*-tetrazole-5-thiol (PT-SH, 600 mg, 3.37 mmol) and **16** (800 mg, 2.24 mmol) were dissolved in anhydrous THF (22 mL), to which was added DIAD (0.66 g, 3.37 mmol) at room temperature. After being stirred for 0.5 h, the reaction mixture was diluted with EtOH (25 mL) and cooled to 0 °C. In a separate flask were mixed 30% aqueous H₂O₂ (5.09 mL, 44.80 mmol) and ammonium molybdate (554 mg, 0.45 mmol), producing a bright yellow solution that was added to the reaction via pipette. After being stirred overnight at room temperature, the reaction mixture was diluted by the addition of water and CH₂Cl₂. The layers were separated and the aqueous layer was extracted three times with CH₂Cl₂. The combined organic layers were washed with water and brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification on silica gel (15 to 20% EtOAc in petroleum ether eluant) provided 1.04 g (85% yield) of **6** as colorless oil. *R*_f = 0.3 (SiO₂, 10% EtOAc in petroleum ether); specific rotation [α]_D²⁵ = -14.4 (*c* 3.04, CHCl₃); IR (neat): ν_{max} 3848, 2936, 2860, 1724, 1594, 1344, 1149, 1107, 760, 702 cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 7.85-7.58 (m, 8H), 7.54-7.34 (m, 7H), 3.92 (m, 1H), 3.73-3.56 (m, 2H), 1.97-1.79 (m, 2H), 1.65-1.40 (m, 4H), 1.22-0.96 (m, 12H); ¹³C NMR (50 MHz, CDCl₃): δ 153.30, 135.69, 134.64, 134.38, 134.14, 132.89, 131.25, 129.51, 129.41, 127.46, 127.33, 124.91, 68.70, 55.72, 38.24, 26.89, 23.57, 23.03, 21.79, 19.09; MS (ESIMS): *m/z* (%): 549 (100) [M + H]⁺; HRMS (ESIMS): calcd for C₂₉H₃₇N₄O₃SSi [M + H]⁺: 549.2356, found: 549.2348.

Compound 17:

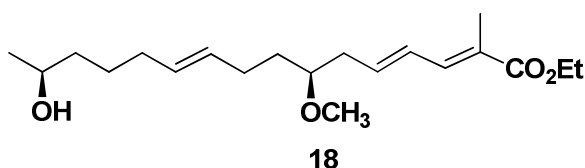


To a solution of compound **14** (200 mg, 0.78 mmol) in dry CH₂Cl₂ (2.0 mL) was added, with stirring, DMSO (1.6 mL), Et₃N (0.54 mL, 3.90 mmol) and SO₃-Py complex (622 mg, 3.90 mmol) portion wise at 0°C under nitrogen atmosphere. After 1h of stirring at 0°C, the reaction was quenched with saturated aqueous NH₄Cl (5 mL) and extracted with EtOAc (2 x 50 mL). The combined organic extracts were washed with saturated aqueous NH₄Cl (10 mL), water (10 mL), brine (10 mL), dried (Na₂SO₄), and concentrated in *vacuo*. The aldehyde **7** (*R*_f = 0.55, 15% EtOAc in petroleum ether) thus obtained by flash chromatography (190 mg, 96%) was directly used in the next reaction.

To a solution of sulfone **6** (615 mg, 1.12 mmol) in 4 mL of THF/HMPA (4:1 v/v) was added LiHMDS (1 M in THF, 0.75 mL, 0.75 mmol) at -78 °C. After the mixture was stirred for 15 min, a solution of the aldehyde **7** (190 mg, 0.75 mmol) in 1 mL of THF/HMPA (4:1 v/v) obtained above was added dropwise. The reaction was stirred at -78 °C for 3 h. Saturated NH₄Cl (aq) was added. The mixture was then extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (4 to 6%

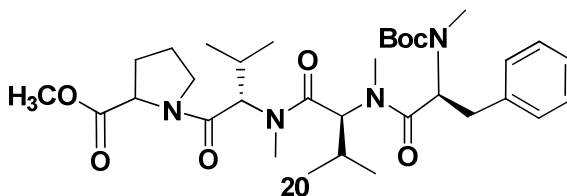
EtOAc in petroleum ether eluant) to afford a mixture of two geometrical isomers **17** (310 mg, 72% yield, *E/Z* 13 based on ^1H NMR): $R_f = 0.3$ (SiO₂, 10% EtOAc in petroleum ether). Specific rotation $[\alpha]_D^{25} = -9.4$ (*c* 2.46, CHCl₃); IR (neat): ν_{max} 3620, 3404, 2929, 2360, 1704, 1365, 1219, 1105, 769, 702 cm⁻¹; ^1H NMR (300 MHz, CDCl₃): δ 7.76-7.57 (m, 4H), 7.46-7.31 (m, 6H), 7.19 (m, 1H), 6.41 (dd, *J* = 15.33, 11.38 Hz, 1H), 6.08 (m, 1H), 5.42-5.25 (m, 2H), 4.21 (q, *J* = 7.15 Hz, 2H), 3.93-3.78 (m, 2H), 3.61 (m, 1H), 3.35 (s, 3H) 3.26 (m, 1H), 2.40 (t, *J* = 6.55 Hz, 2H), 2.12-1.77 (m, 8H), 1.94 (s, 3H), 1.58-1.21 (m, 9H), 1.06 (s, 9H); ^{13}C NMR (75 MHz, CDCl₃): δ 168.59, 138.56, 135.88, 134.81, 131.44, 129.71, 129.37, 127.60, 127.45, 127.38, 125.08, 79.80, 69.50, 68.86, 60.48, 56.77, 55.93, 38.95, 38.95, 38.42, 37.31, 33.65, 32.52, 29.70, 28.37, 27.05, 25.15, 23.76, 23.18, 19.28, 14.33, 12.60; MS (ESIMS): *m/z* (%): 578 (100) [M + 2H]⁺; HRMS (ESIMS): calcd for C₃₆H₅₄O₄Si [M + 2H]⁺: 578.3791, found: 578.3767.

Compound 18:



To a solution of **17** (250 mg, 0.43 mmol) in dry DMF (3.0 mL), TBAF (1M in THF, 2.17 mL, 2.17 mmol) was added at room temperature, followed by AcOH (0.12 mL, 2.17 mmol) and H₂O (0.19 mL, 10.75 mmol). The reaction mixture was warmed to 50 °C and stirred for 12 h. It was quenched with saturated aqueous NH₄Cl solution, extracted with EtOAc, washed with brine, dried (Na₂SO₄) and concentrated in *vacuo*. Purification by column chromatography (SiO₂, 15 to 20% EtOAc in petroleum ether eluant) afforded compound **18** (125 mg, 85%) as a clear oil. $R_f = 0.3$ (SiO₂, 20% EtOAc in petroleum ether); specific rotation $[\alpha]_D^{25} = -2.0$ (*c* 0.15, CHCl₃); IR (neat): ν_{max} 3416, 2364, 1635, 1426, 1220, 771 cm⁻¹; ^1H NMR (300 MHz, CDCl₃): δ 7.18 (m, 1H), 6.41 (dd, *J* = 15.10, 11.33 Hz, 1H), 6.08 (m, 1H), 5.41 (m, 2H), 4.21 (q, *J* = 7.06 Hz, 2H), 3.81 (m, 1H), 3.36 (s, 3H), 3.27 (m, 1H) 2.47-2.32 (m, 2H), 2.15-1.98 (m, 5H), 1.94 (s, 3H), 1.62-1.14 (m, 11H); ^{13}C NMR (75 MHz, CDCl₃): δ 168.63, 138.48, 134.79, 130.51, 129.97, 128.08, 125.66, 79.81, 68.05, 60.49, 56.75, 38.77, 37.27, 33.62, 32.46, 28.35, 27.09, 23.50, 14.30, 12.58; MS (ESIMS): *m/z* (%): 339 (100) [M + H]⁺; HRMS (ESIMS): calcd for C₂₀H₃₅O₄ [M + H]⁺: 339.2535, found: 339.2523.

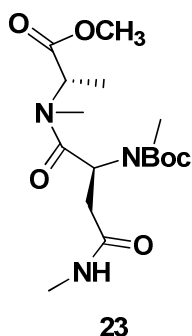
Compound 20:



Silver oxide (1.20 g, 5.22 mmol) was added portion wise to a stirred solution of compound **19** (500 mg, 0.87 mmol) in DMF (3 mL) at 0 °C under nitrogen atmosphere. After the addition was completed, the reaction mixture was stirred at 0 °C for 5 min. Then MeI (0.33 mL, 5.22 mmol) was added slowly to the stirred reaction mixture. The reaction mixture was warmed to room temperature and stirred for 12 h. It was then diluted with EtOAc, extracted with EtOAc, washed with brine, dried (Na₂SO₄) and concentrated in *vacuo*. Purification by

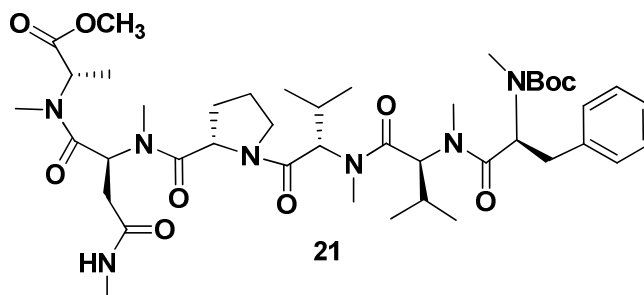
column chromatography (SiO₂, 50% to 60% EtOAc in petroleum ether eluant) afforded compound **20** (443 mg, 72%) as colorless oil. $R_f = 0.3$ (SiO₂, 60% EtOAc in petroleum ether); specific rotation $[\alpha]_D^{25} = -3.9$ (c 0.10, CHCl₃); IR (neat): ν_{\max} 3507, 3247, 3022, 2698, 1638, 1461, 1217, 769, 670 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) for chemical shifts of four isomers see Tables 1a-1d; ¹³C NMR (50 MHz, CDCl₃, a mixture of rotamers): δ 172.47, 170.20, 169.67, 168.38, 155.46, 137.95, 129.40, 128.29, 126.40, 58.89, 58.37, 57.47, 55.02, 52.08, 46.99, 35.53, 31.88, 30.02, 29.65, 29.27, 28.13, 27.90, 26.92, 25.98, 24.71, 19.85, 19.39, 18.97, 18.03; MS (ESIMS): m/z (%): 639 (100) [M + Na]⁺; HRMS (ESIMS): calcd for C₃₇H₅₂N₄O₇Na [M + Na]⁺: 639.3734, found: 639.3737.

Compound 23:

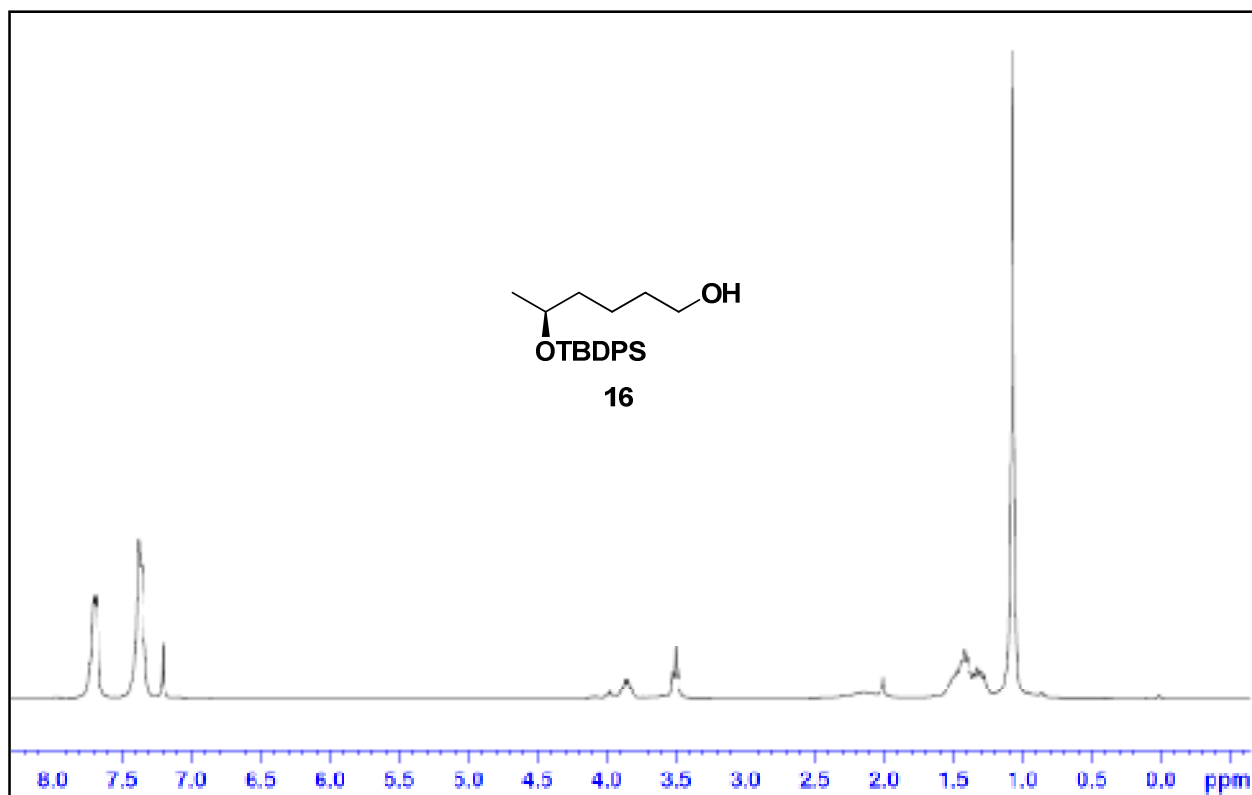


Silver oxide (811 mg, 3.50 mmol) was added portion wise to a stirred solution of compound **21** (300 mg, 0.95 mmol) in DMF (3 mL) at 0 °C under nitrogen atmosphere. After the addition was completed, the reaction mixture was stirred at 0 °C for 5 min. Then MeI (0.22 mL, 3.50 mmol) was added slowly to the stirred reaction mixture. The reaction mixture was warmed to room temperature and stirred for 12 h. The reaction mixture was diluted with EtOAc, extracted with EtOAc, washed with brine, dried (Na₂SO₄) and concentrated in *vacuo*. Purification by column chromatography (SiO₂, 80% to 90% EtOAc in petroleum ether eluant) afforded compound **23** (230 mg, 68%) as colorless oil. $R_f = 0.3$ (SiO₂, EtOAc); specific rotation $[\alpha]_D^{25} = -28.6$ (c 0.05, CHCl₃); IR (neat): ν_{\max} 3449, 3375, 2863, 1668, 1540, 1158, 1056, 758 cm⁻¹; ¹H NMR (300 MHz, CDCl₃, a mixture of rotamers): δ 6.15 (m, 1H), 5.51-5.20 (m, 1H), 4.84 (m, 1H), 3.70 (s, 3H), 3.13-2.55 (m, 10H), 2.38 (m, 1H), 1.64-1.33 (m, 12H); ¹³C NMR (50 MHz, CDCl₃, a mixture of rotamers): δ 172.48, 171.67, 170.48, 155.30, 54.32, 52.25, 35.79, 32.28, 29.65, 28.26, 26.28, 15.98; MS (ESIMS): m/z (%): 382 (100) [M + H]⁺; HRMS (ESIMS): calcd for C₁₆H₂₉N₃O₆Na [M + Na]⁺: 382.1954, found: 382.1960.

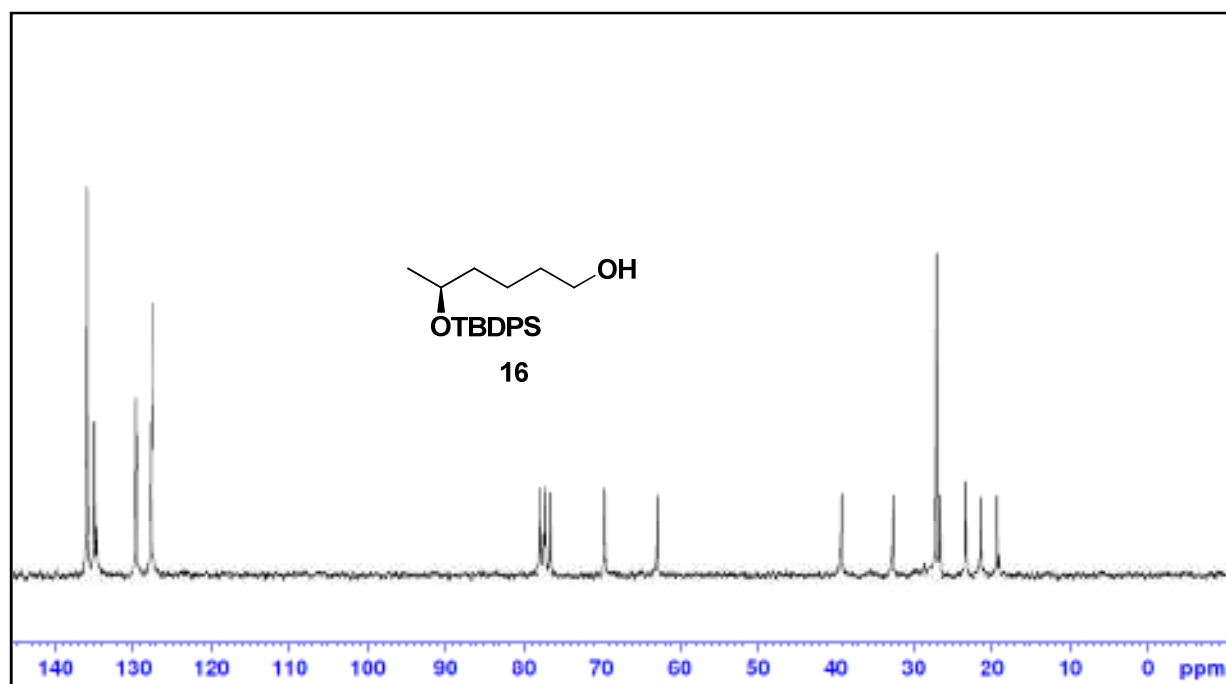
Compound 21:



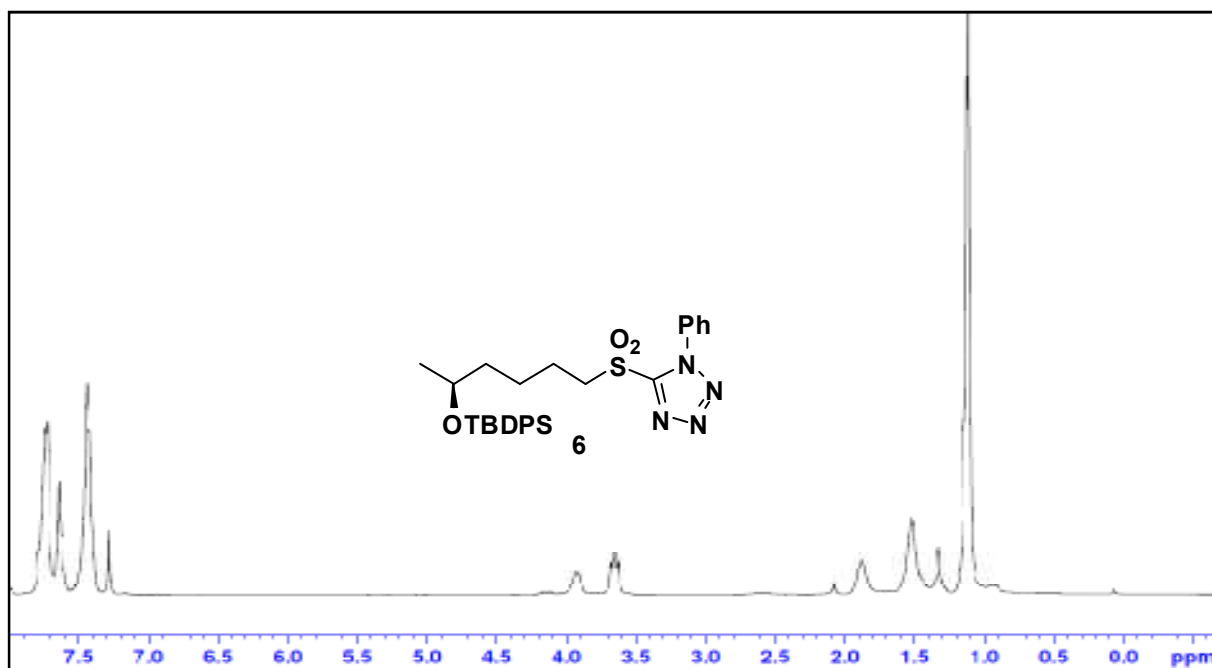
The Boc-D-Val-OH (52 mg, 0.23 mmol) was dissolved in CH₂Cl₂ (3 mL) and cooled to 0 °C. Then to it was added BOP-Cl (86 mg, 0.34 mmol). After 10 minute, Boc-protected TFA salt of **21** prepared above, and dissolved in CH₂Cl₂ (1 mL), was added to the reaction mixture followed by the addition of DIPEA (0.2 mL, 1.13 mmol). After stirring for 12 h at room temperature, the reaction mixture was diluted with CHCl₃, washed with saturated NH₄Cl solution, 1N HCl, saturated NaHCO₃ solution, water, brine, dried (Na₂SO₄), filtered and concentrated in *vacuo*. Purification by column chromatography (silica gel, 2% to 3% MeOH in CHCl₃ as eluant) afforded the **24** (167 mg, 79%) as white solid. $R_f = 0.3$ (SiO₂, 5% MeOH in CHCl₃, a mixture of rotamers); specific rotation $[\alpha]_D^{25} = -39.8$ (c 0.23, CHCl₃); IR (neat): ν_{\max} 3434, 2967, 2374, 1636, 1450, 1220, 1017, 771, 670 cm⁻¹; ¹H NMR (100 MHz, CDCl₃): For major conformation chemical shifts, see Table 3; ¹³C NMR (100 MHz, CDCl₃, a mixture of rotamers): δ 173.17, 172.24, 171.61, 170.15, 170.09, 169.85, 169.71, 168.28, 155.96, 137.55, 129.45, 129.13, 128.99, 128.48, 126.95, 126.60, 79.81, 60.24, 60.05, 59.01, 58.78, 58.16, 56.74, 55.56, 55.21, 53.55, 53.27, 52.22, 51.13, 47.34, 35.76, 35.60, 32.74, 31.91, 30.75, 30.61, 30.11, 29.78, 29.58, 29.42, 27.04, 26.44, 26.01, 24.93, 22.76, 19.99, 19.89, 19.31, 18.44, 18.02, 16.95, 14.19, 14.10; MS (ESIMS): m/z (%): 965 (100) [M + Na]⁺; HRMS (ESIMS): calcd for C₄₈H₇₉N₈O₁₁ [M + H]⁺: 943.5868, found: 943.5876.



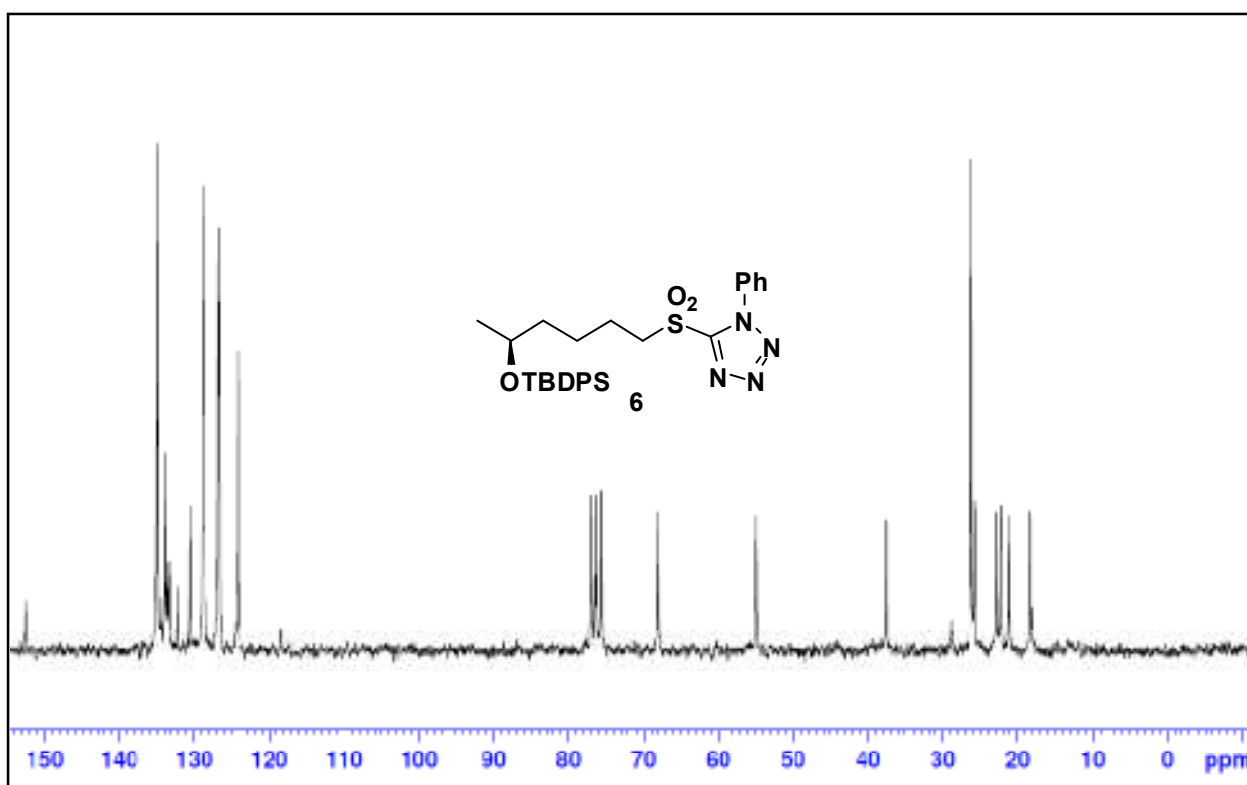
¹H Spectrum of compound **16** in CDCl₃ (300 MHz)



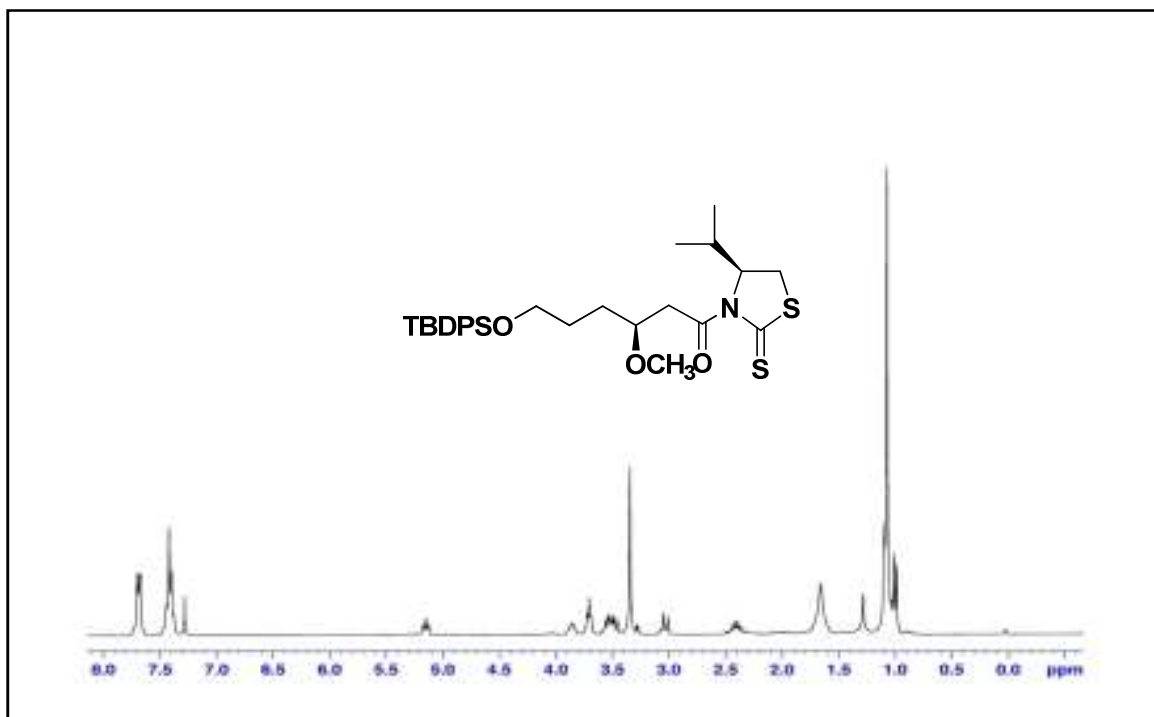
¹³C Spectrum of compound **16** in CDCl₃ (50 MHz)



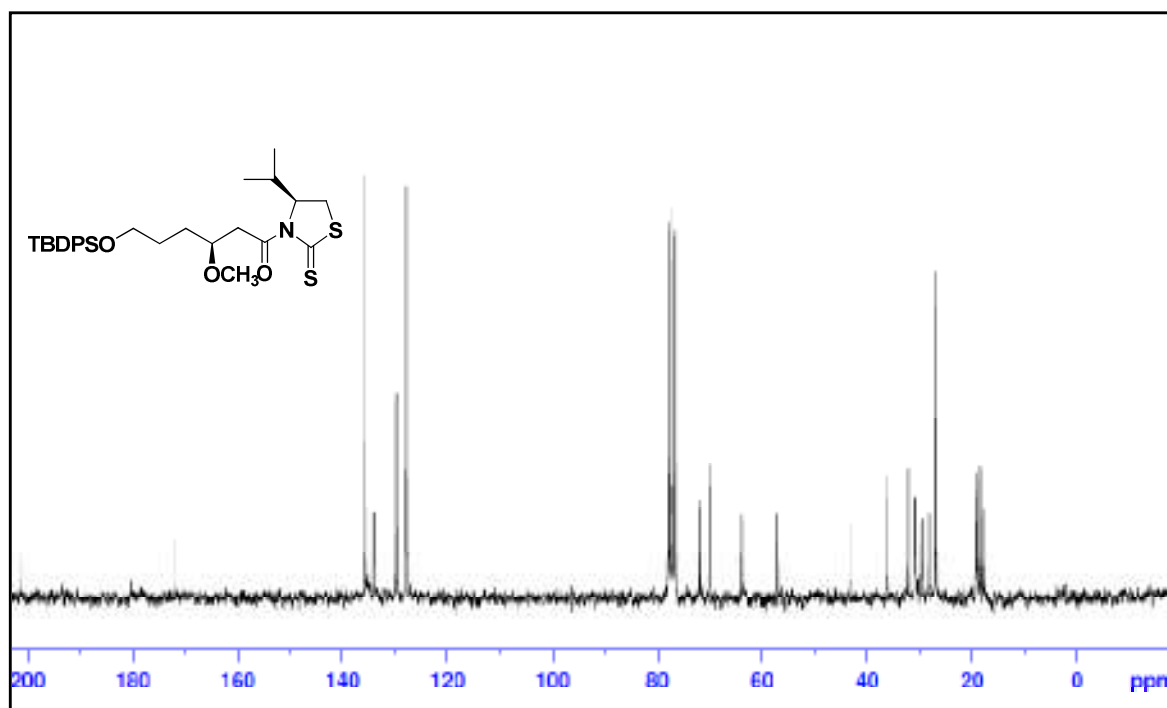
¹H Spectrum of compound **6** in CDCl₃ (300 MHz)



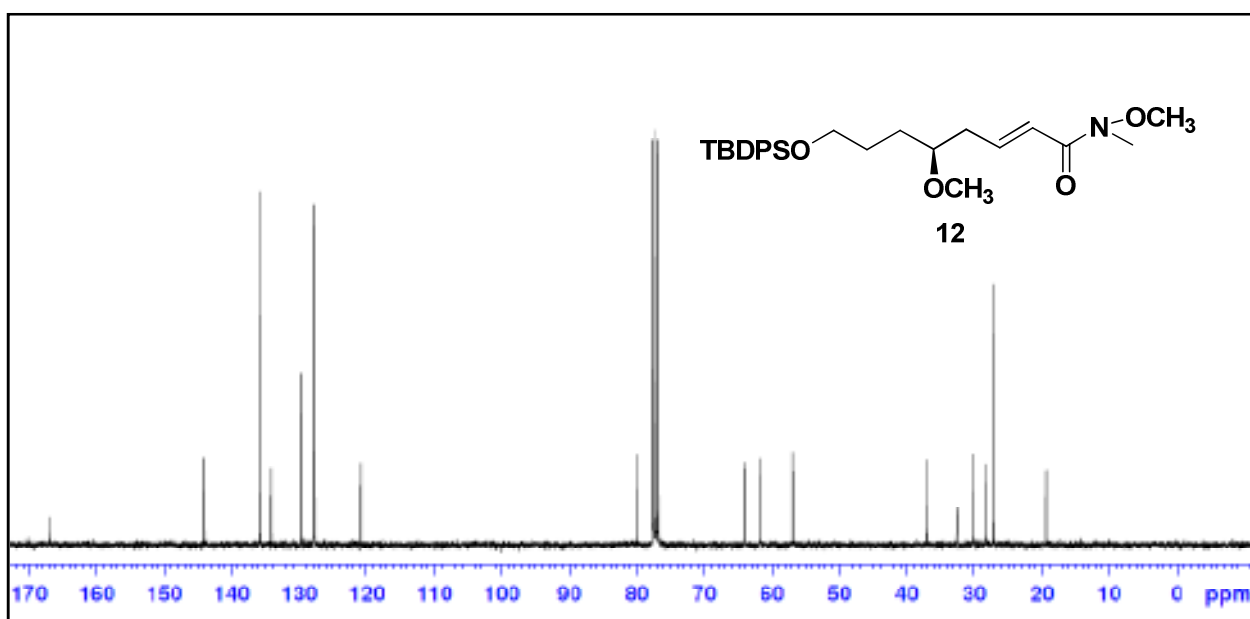
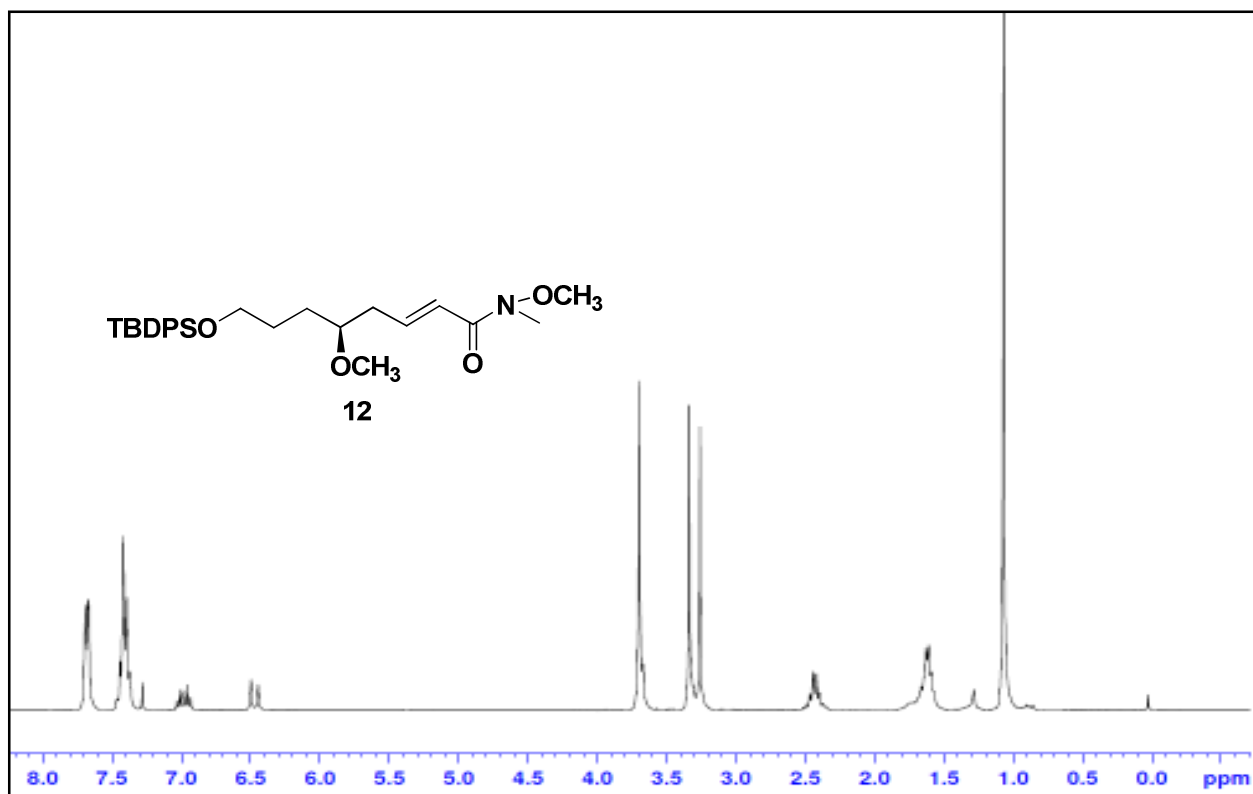
¹³C Spectrum of compound **6** in CDCl₃ (50 MHz)

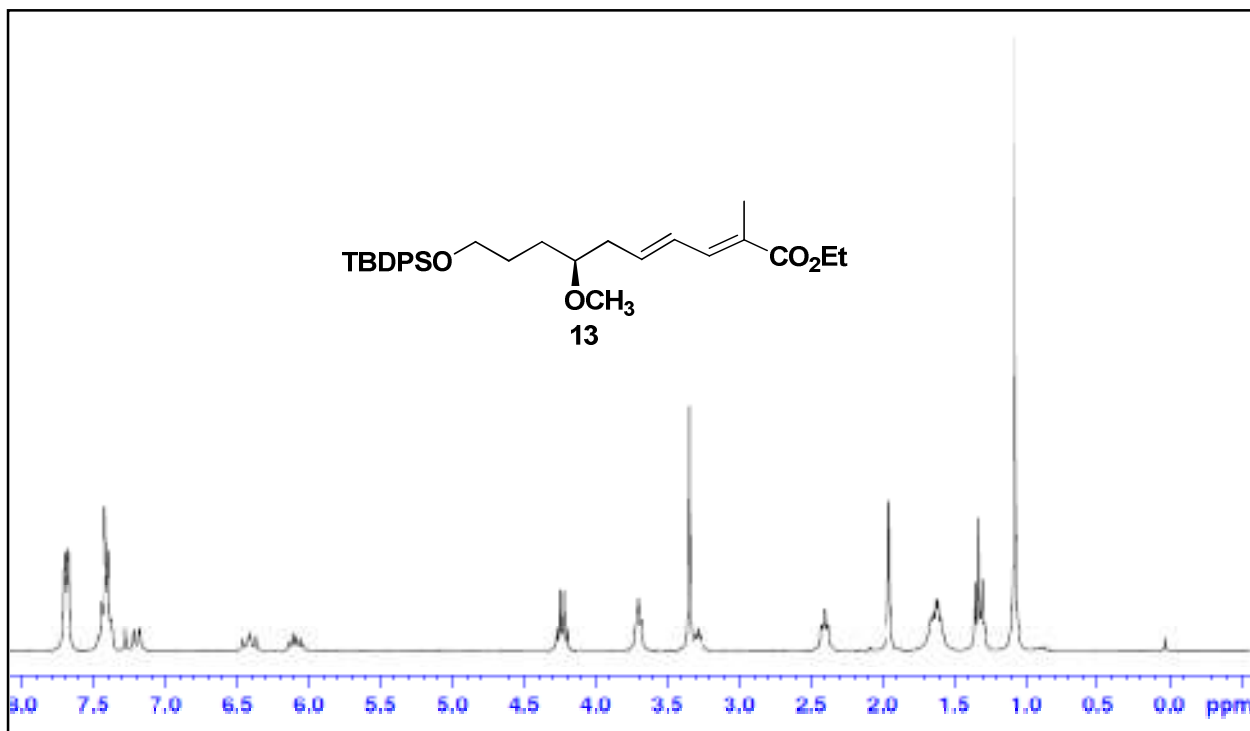


^1H Spectrum of compound **10** in CDCl_3 (300 MHz)

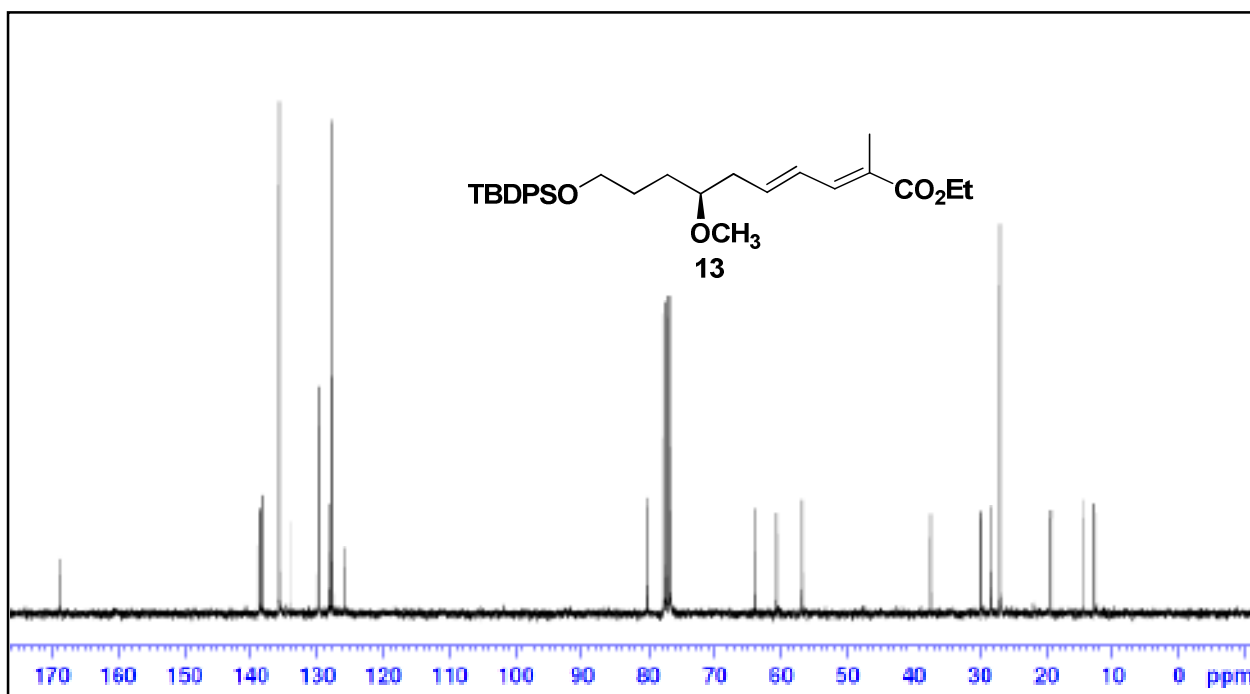


^{13}C Spectrum of compound **10** in CDCl_3 (50 MHz)

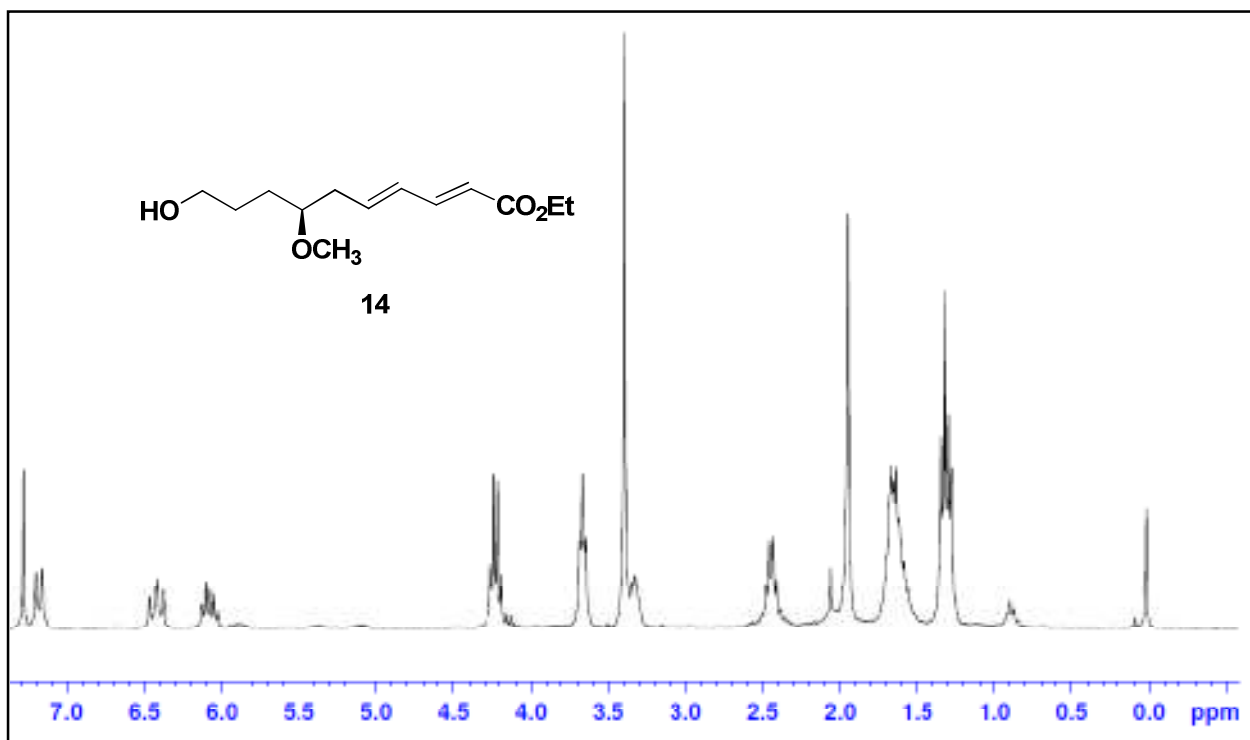




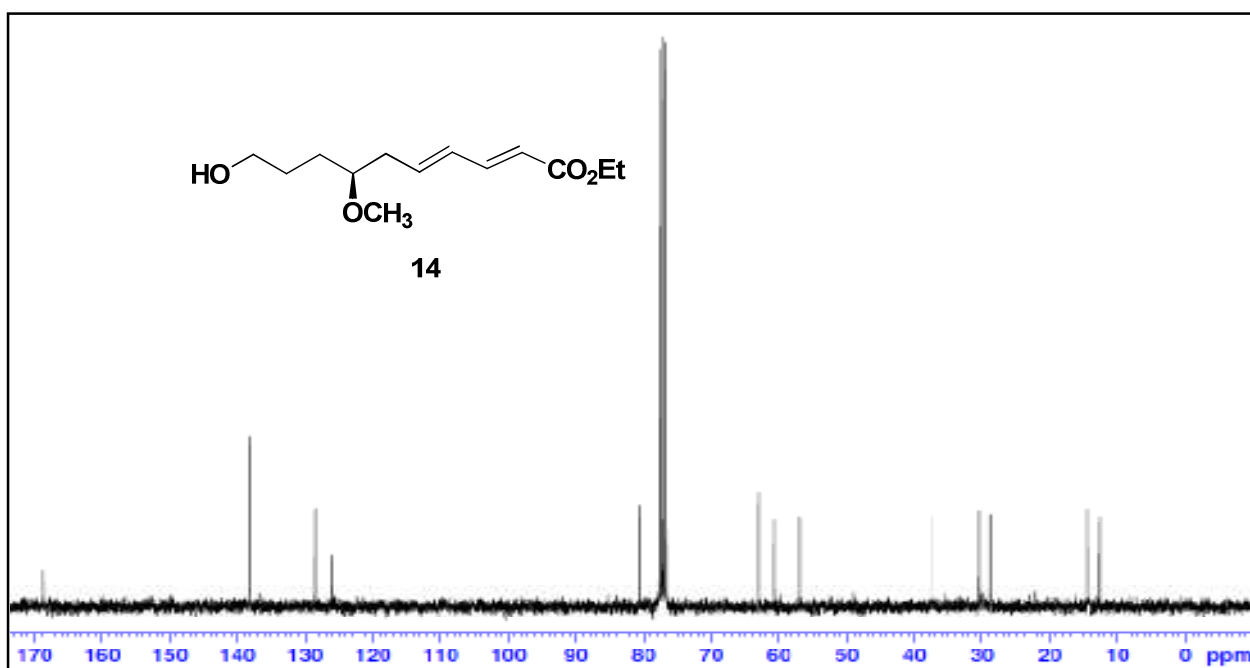
¹H Spectrum of compound **13** in CDCl₃ (300 MHz)



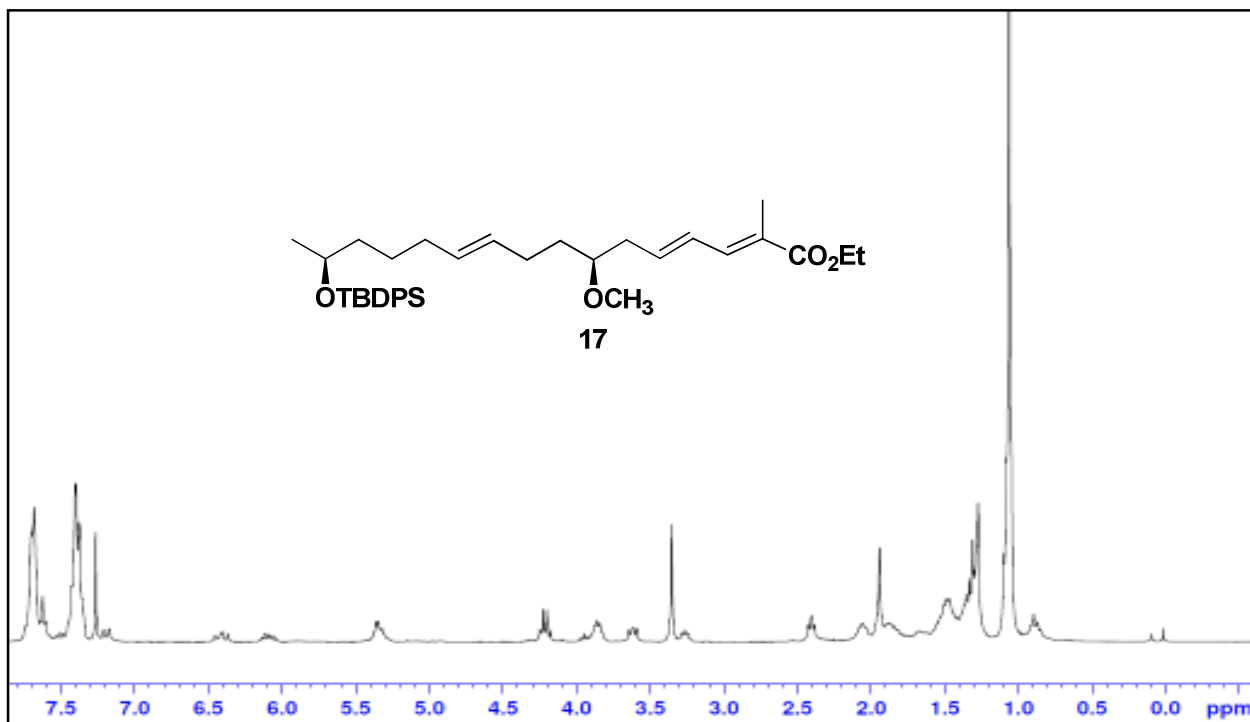
¹³C Spectrum of compound **13** in CDCl₃ (50 MHz)



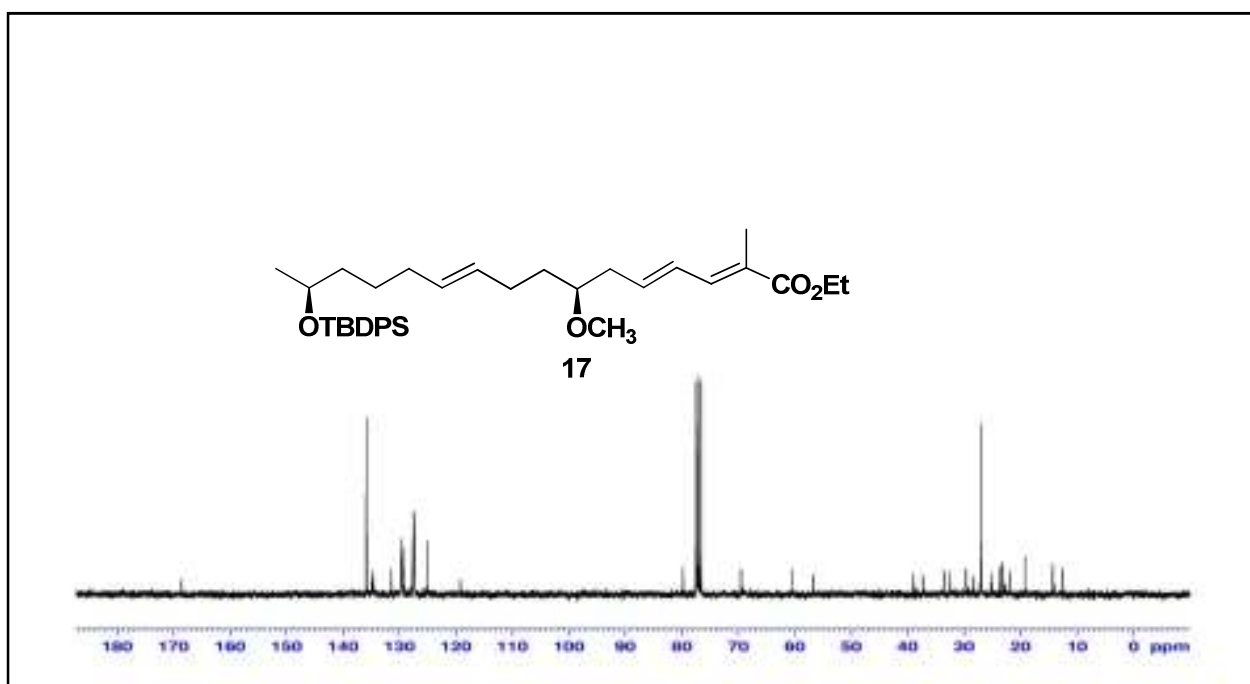
¹H Spectrum of compound **14** in CDCl₃ (300 MHz)



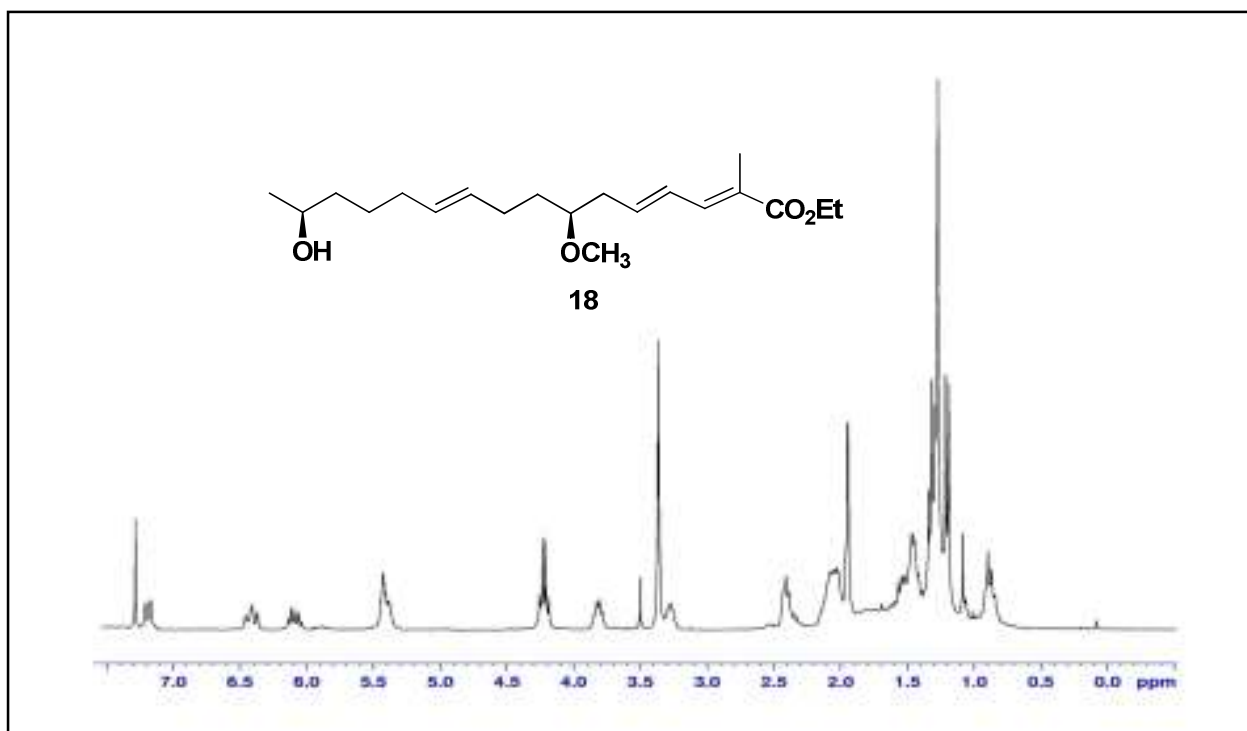
¹³C Spectrum of compound **14** in CDCl₃ (50 MHz)



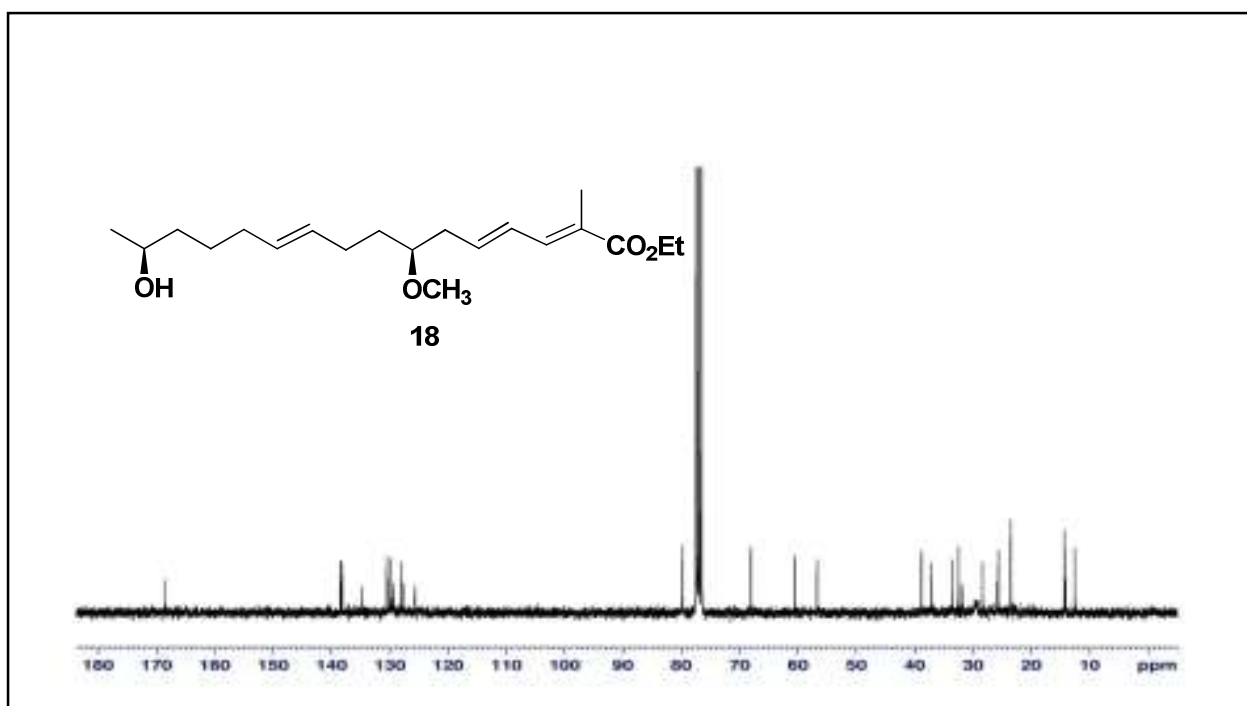
¹H Spectrum of compound 17 in CDCl₃ (300 MHz)



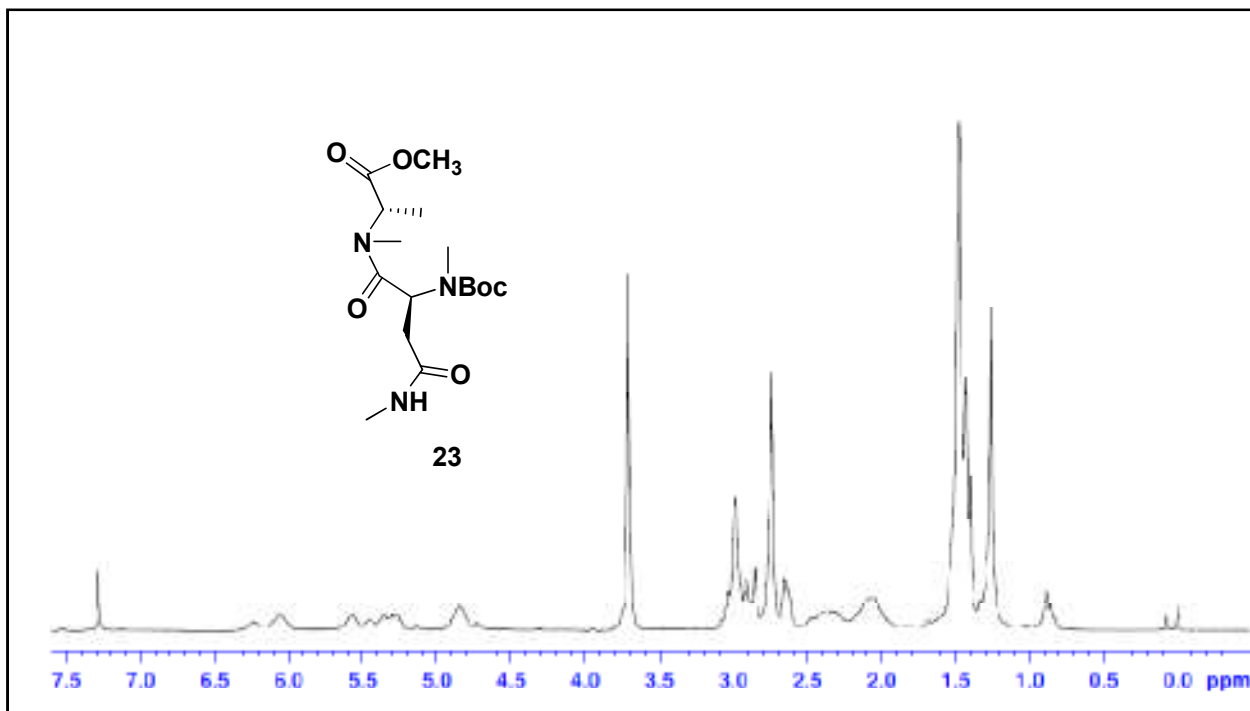
¹³C Spectrum of compound 17 in CDCl₃ (75 MHz)



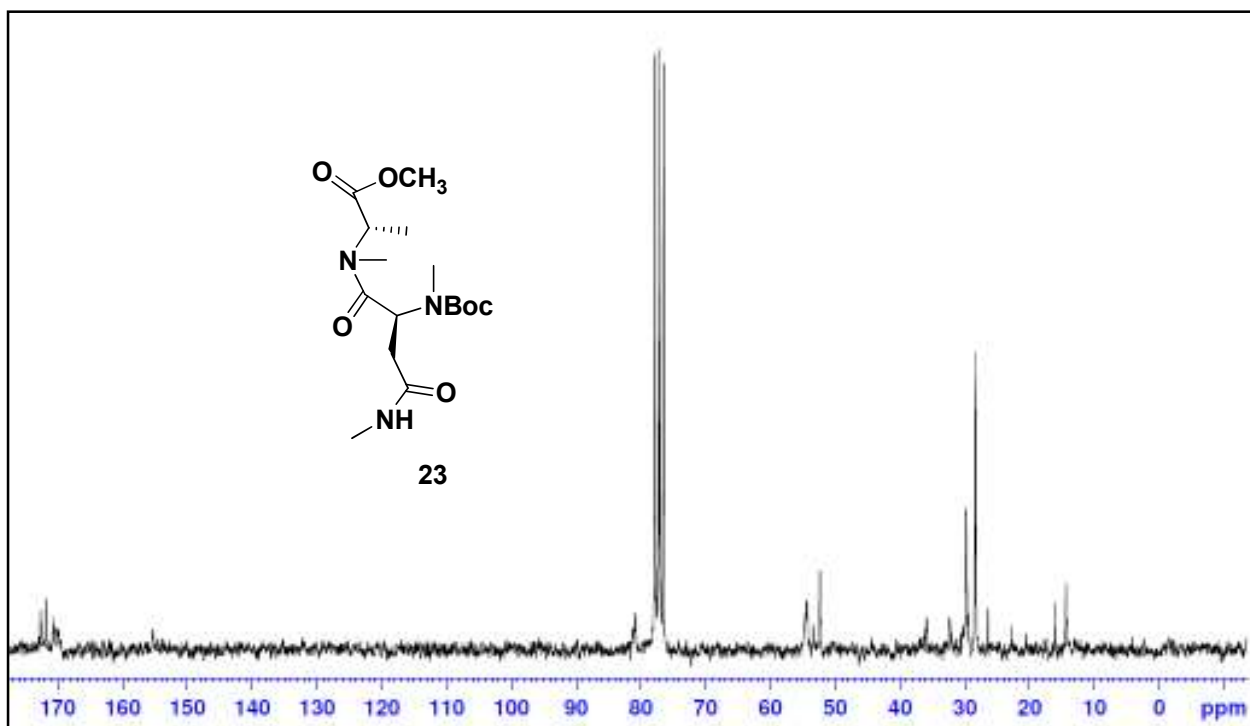
¹H Spectrum of compound **18** in CDCl₃ (300 MHz)



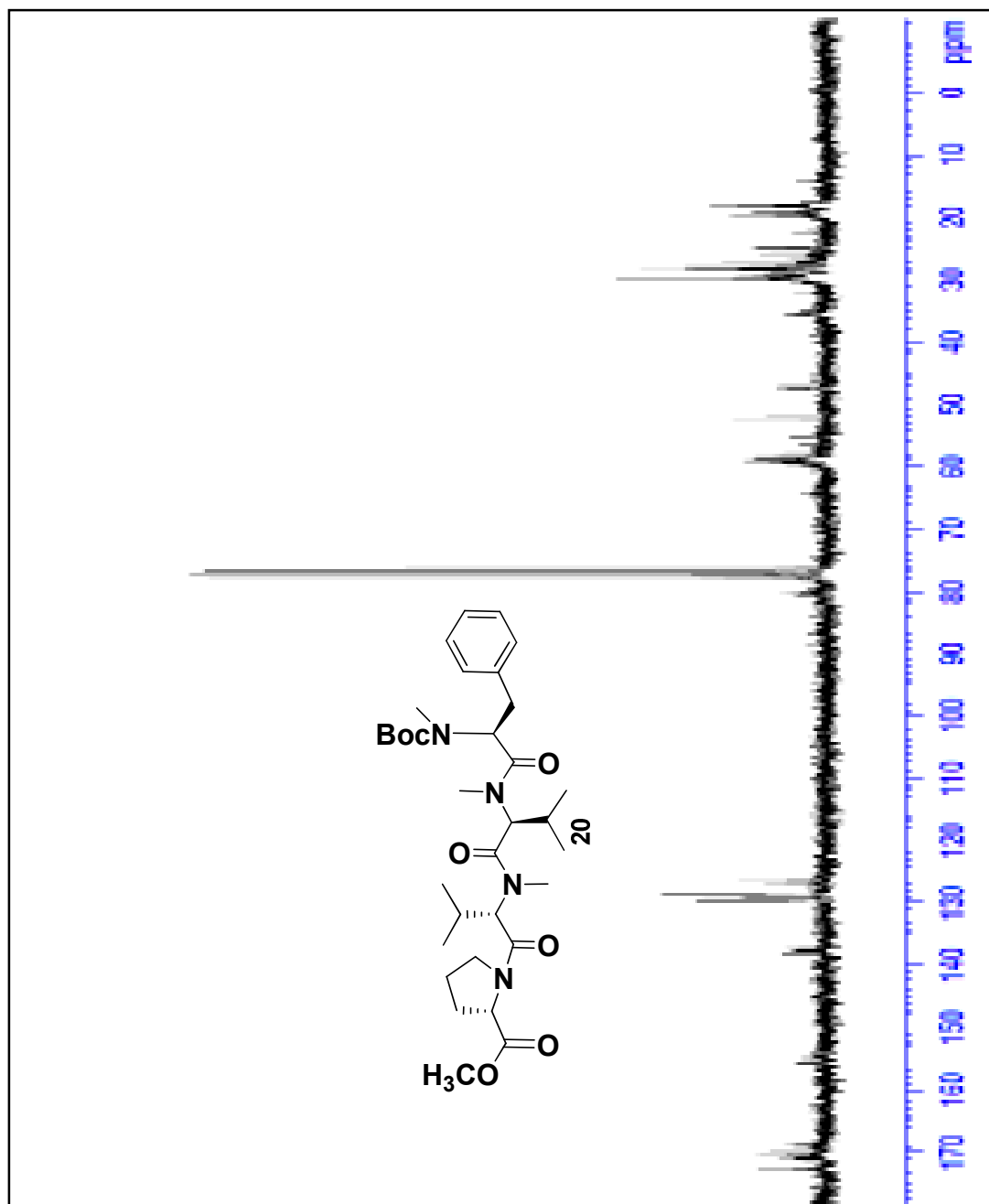
¹³C Spectrum of compound **18** in CDCl₃ (75 MHz)



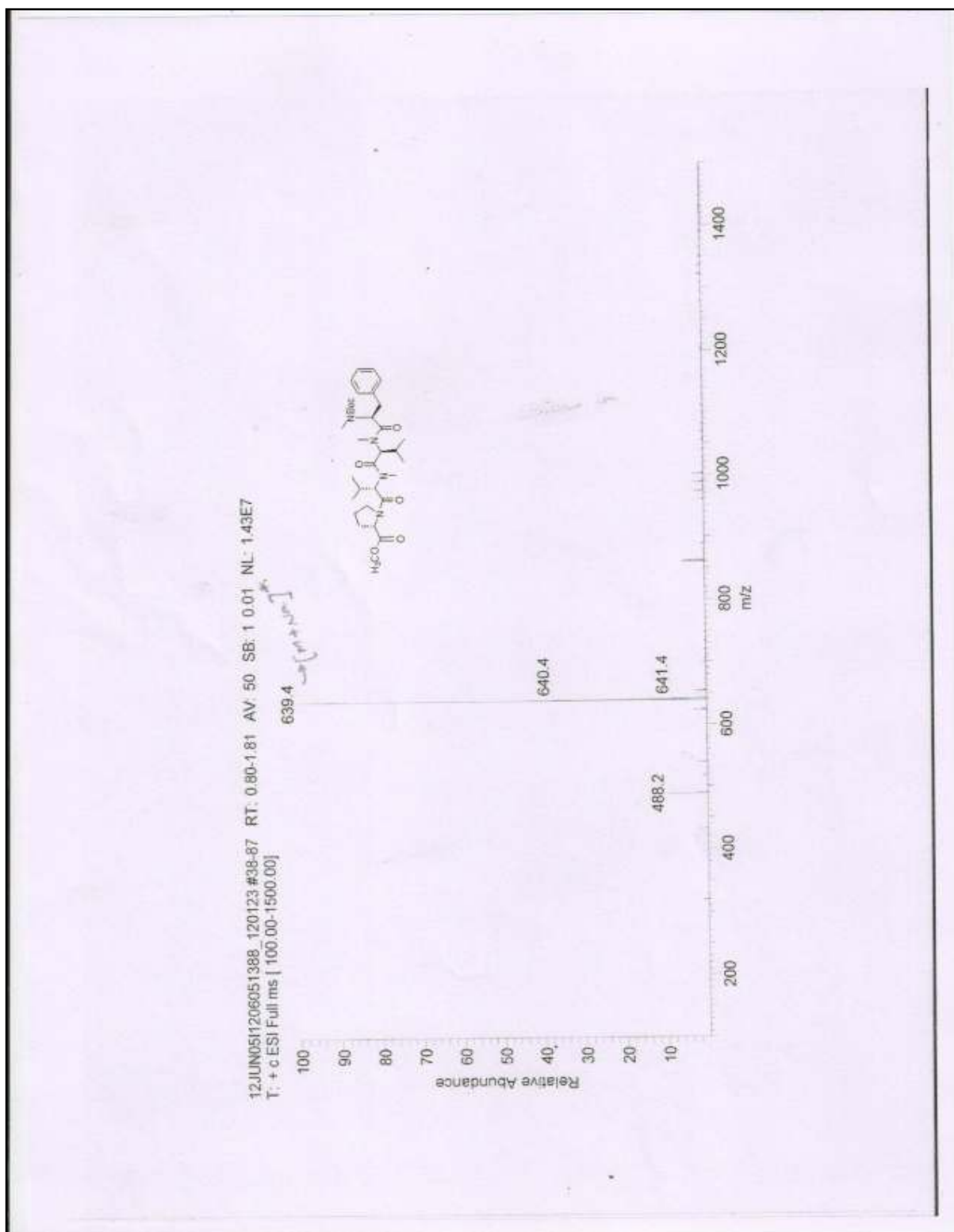
^1H Spectrum of compound **23** in CDCl_3 (300 MHz)



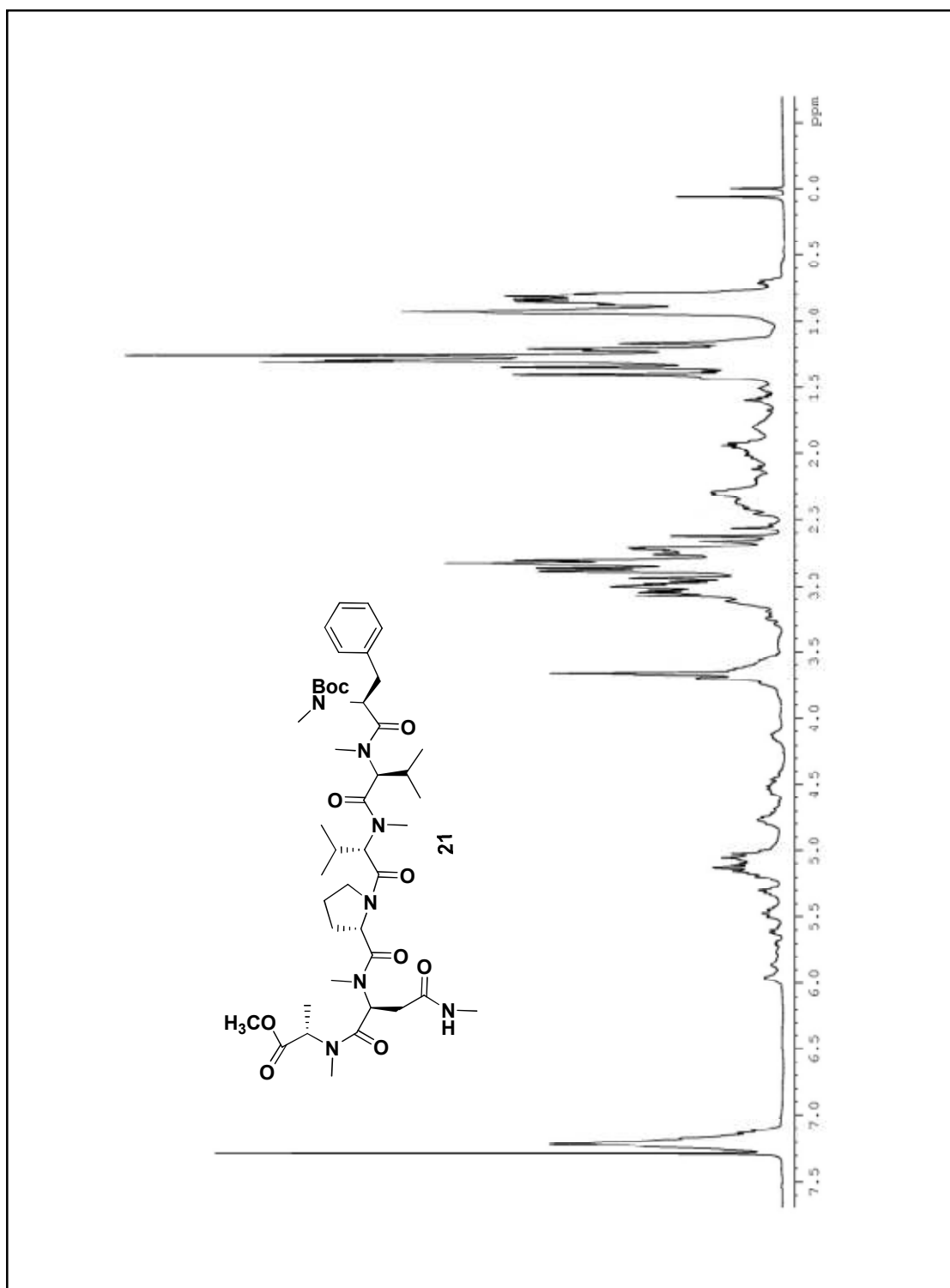
^{13}C Spectrum of compound **23** in CDCl_3 (50 MHz)



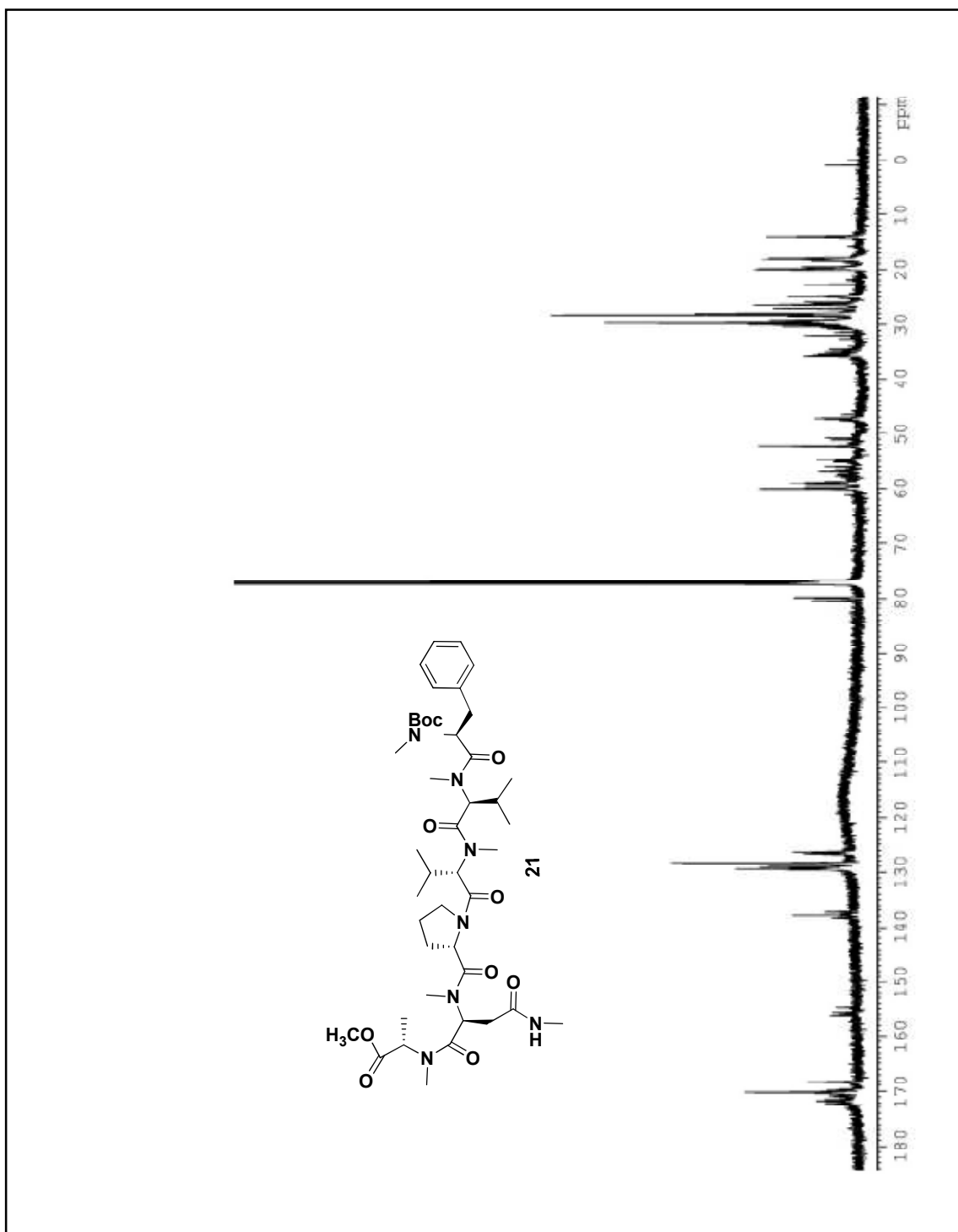
^{13}C Spectrum of compound **20** in CDCl_3 (50 MHz)



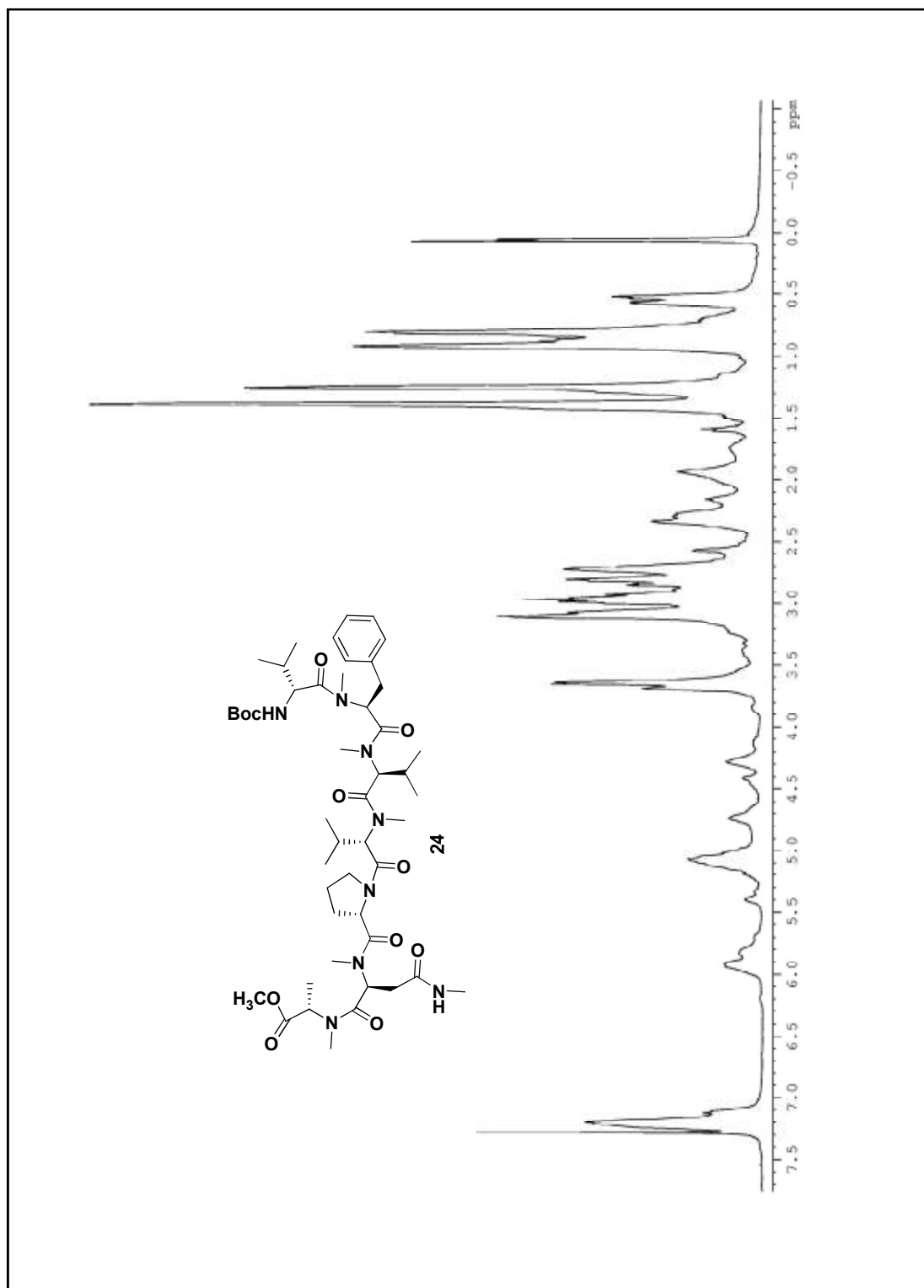
ESI-MS Spectrum of compound **20**



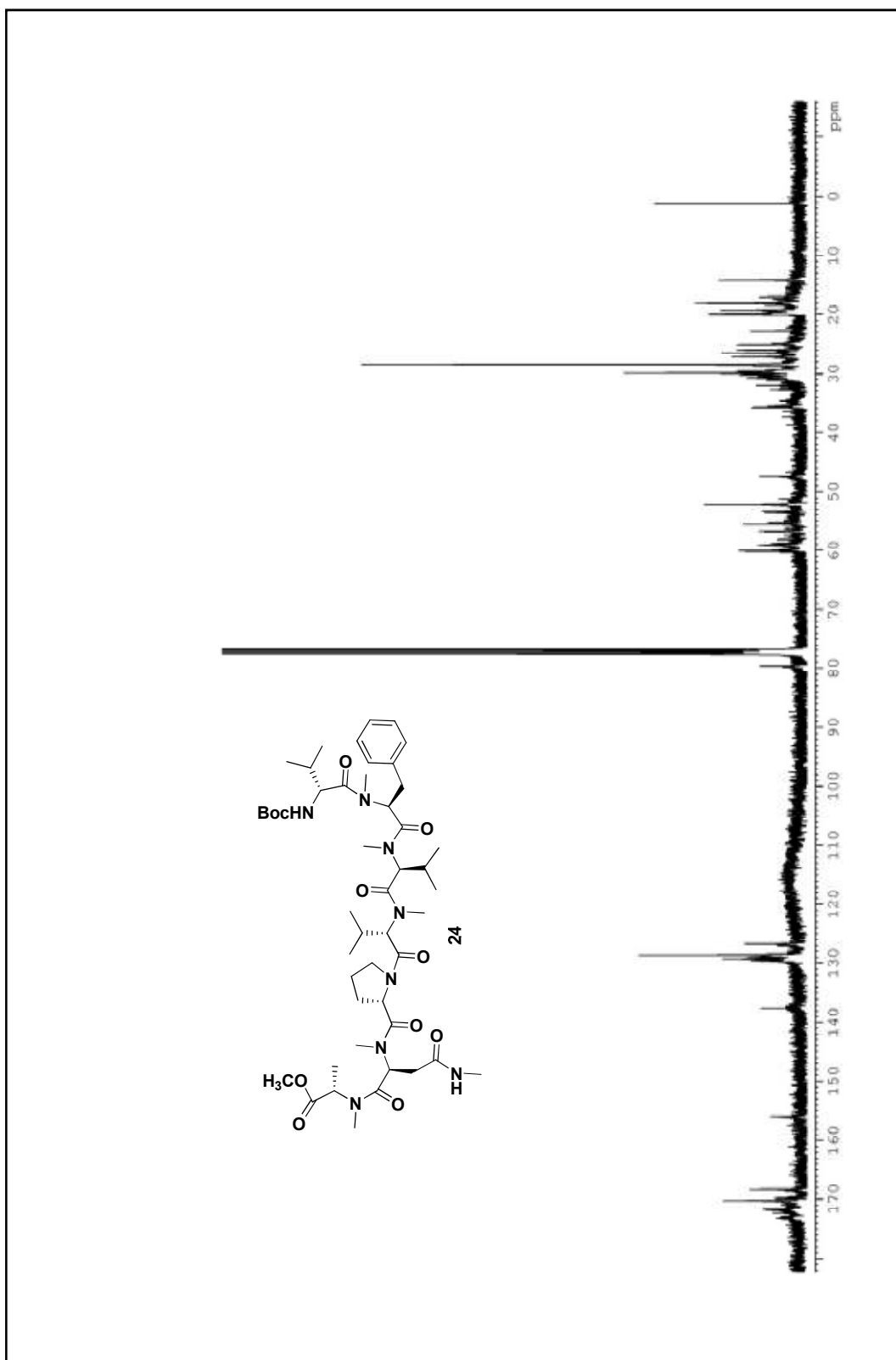
^1H Spectrum of compound **21** in CDCl_3 (400 MHz)



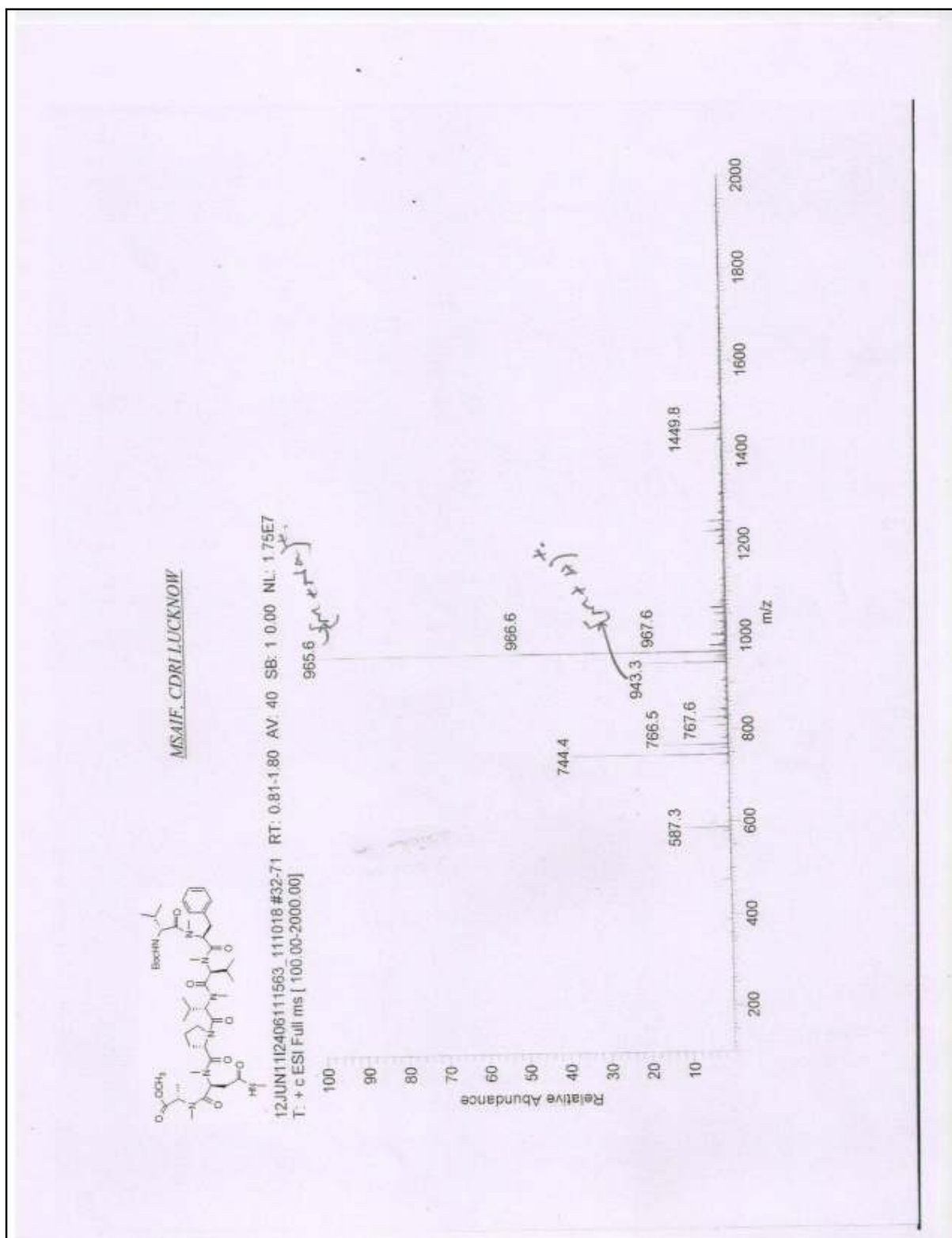
^{13}C Spectrum of compound **21** in CDCl_3 (100 MHz)



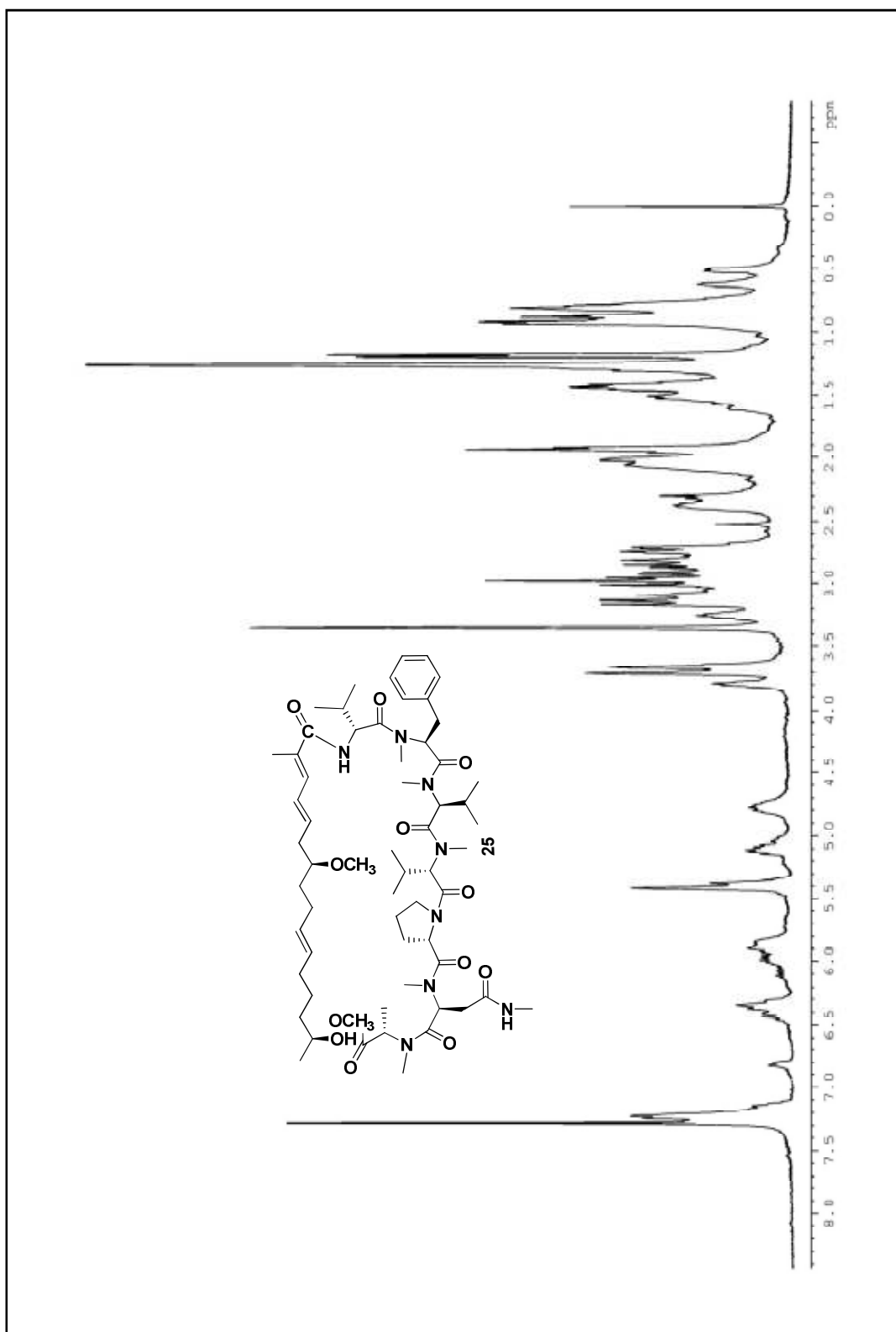
¹H Spectrum of compound **24** in CDCl₃ (400 MHz)



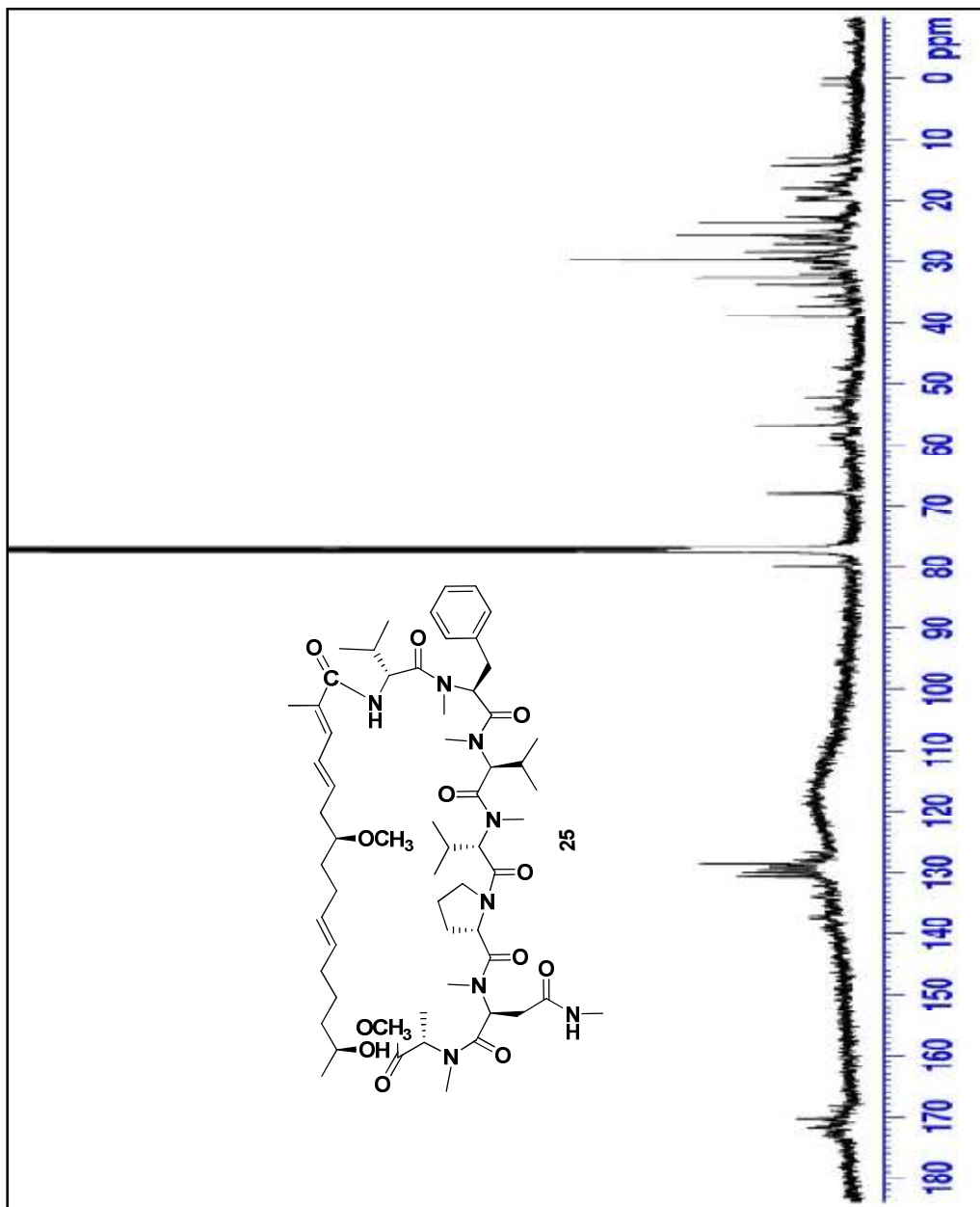
^{13}C Spectrum of compound **24** in CDCl_3 (100 MHz)



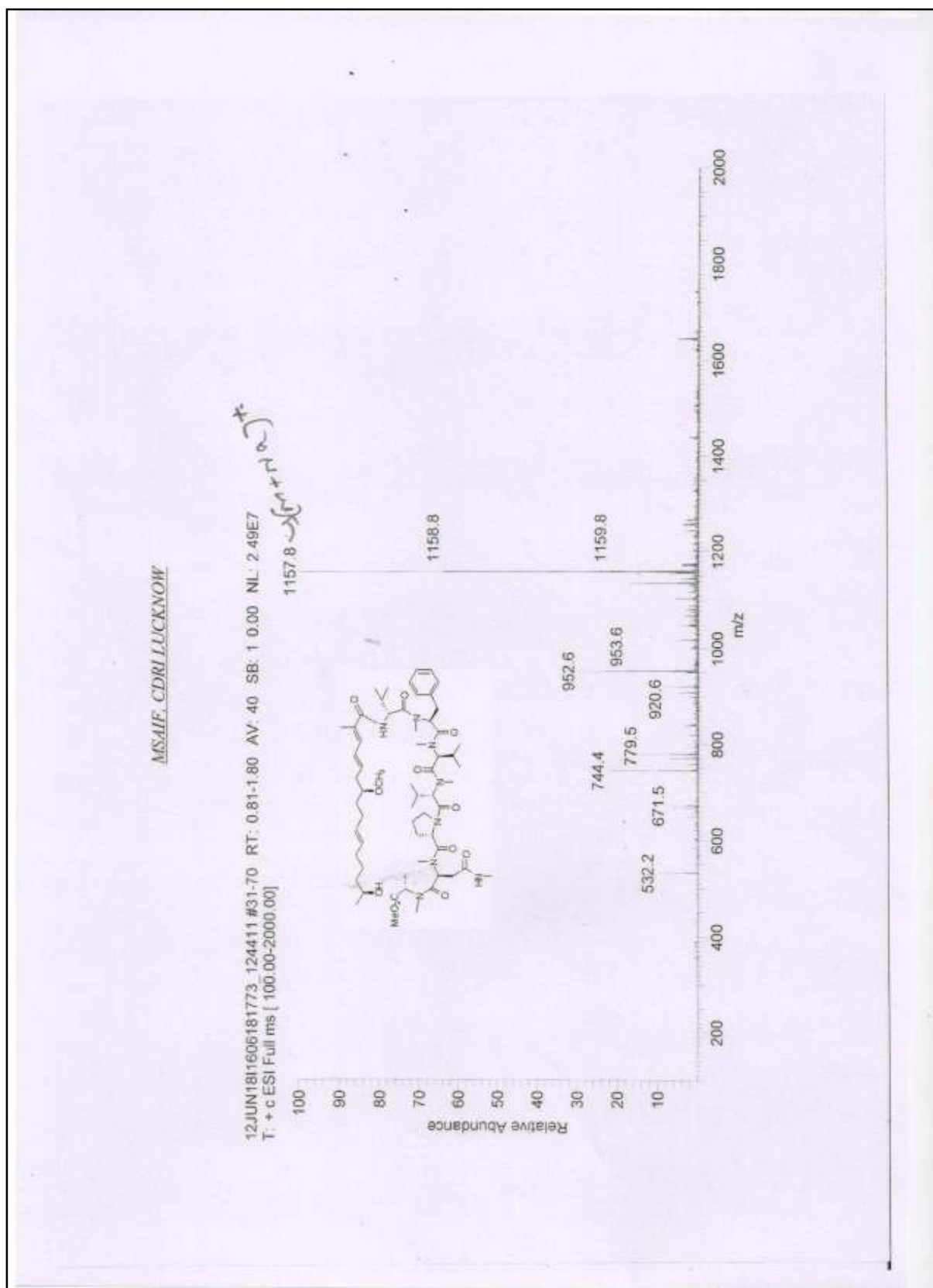
ESI-MS Spectrum of compound 24



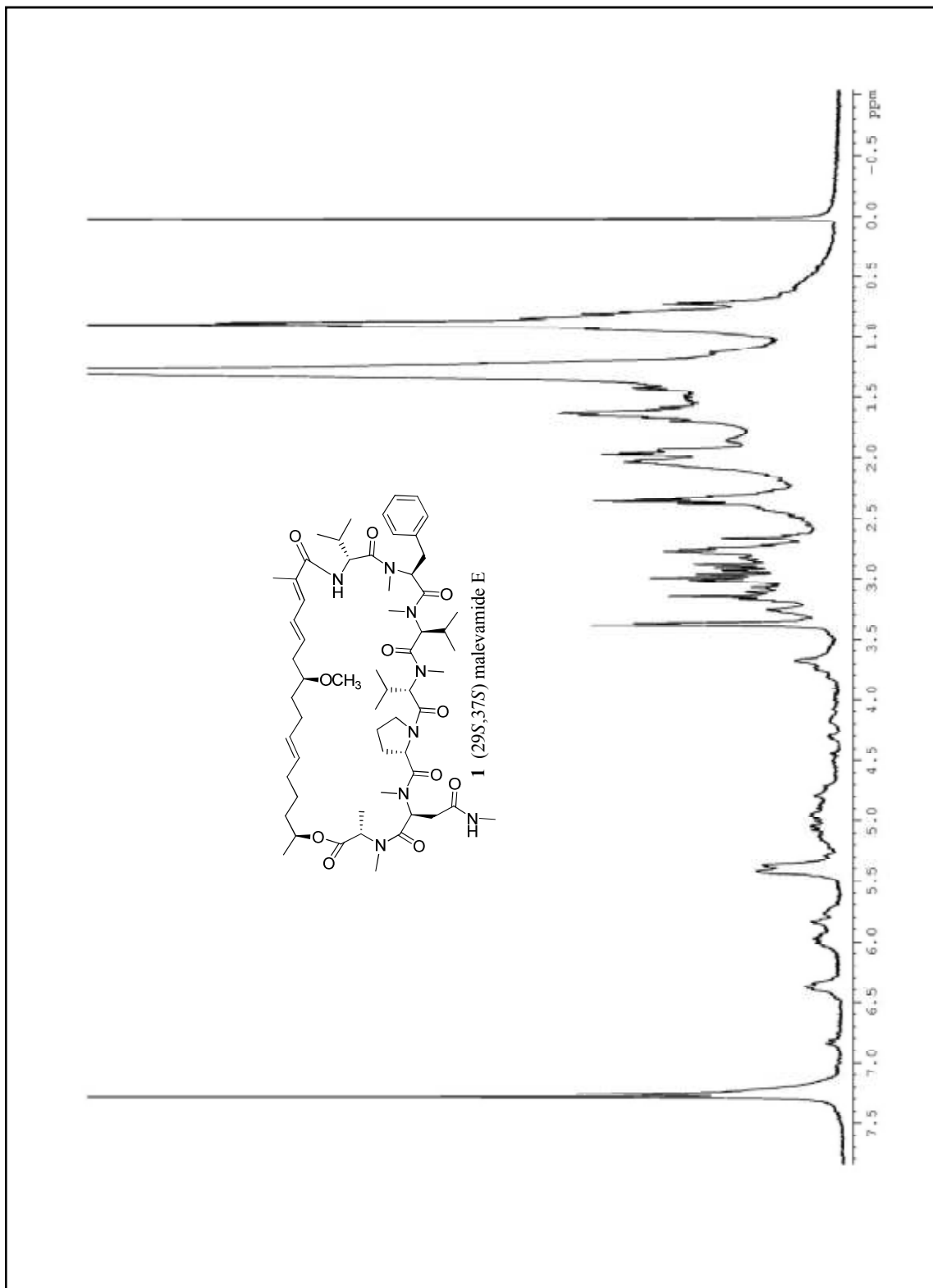
¹H Spectrum of compound **25** in CDCl₃ (400 MHz)



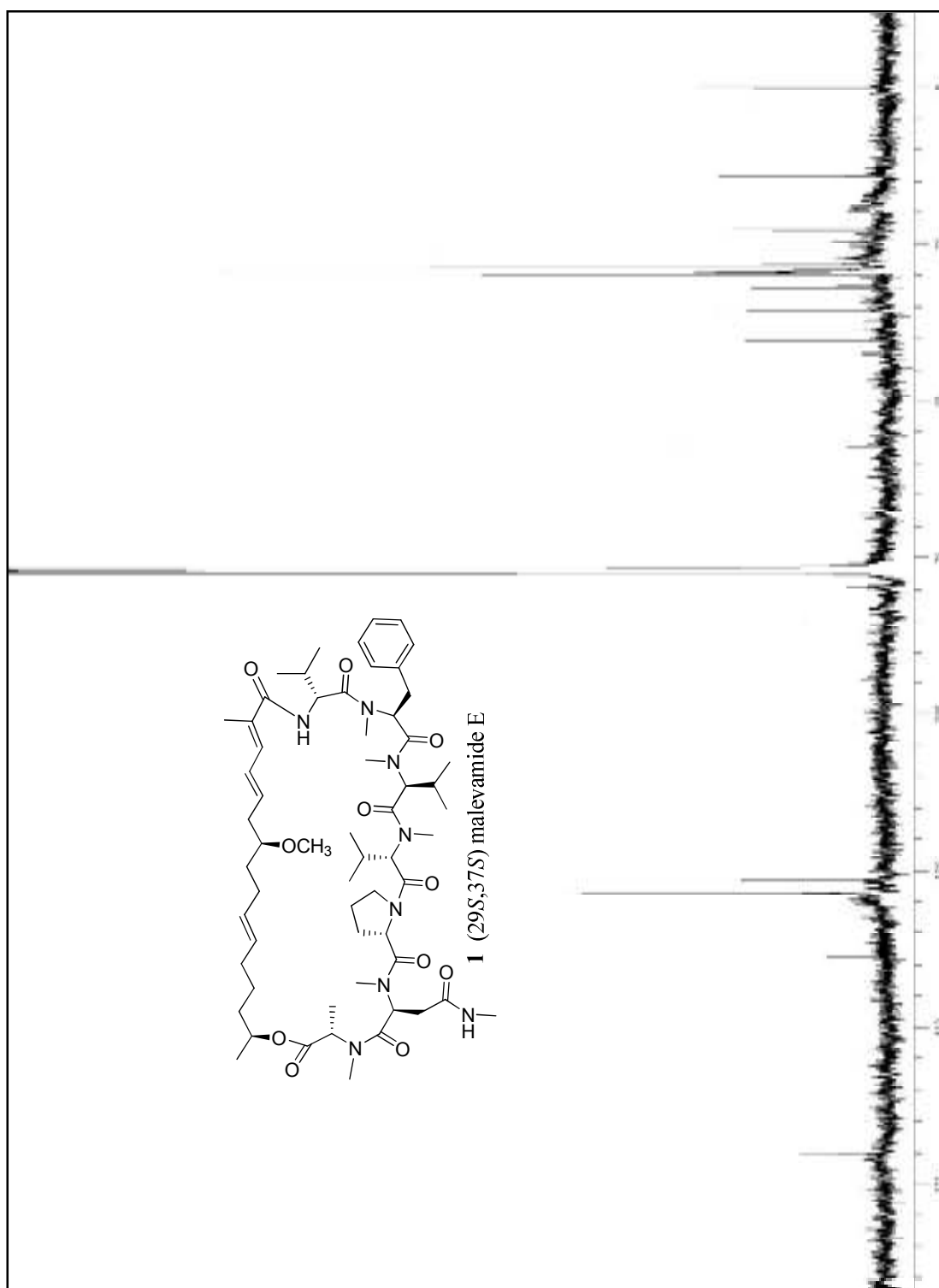
^{13}C Spectrum of compound **25** in CDCl_3 (100 MHz)



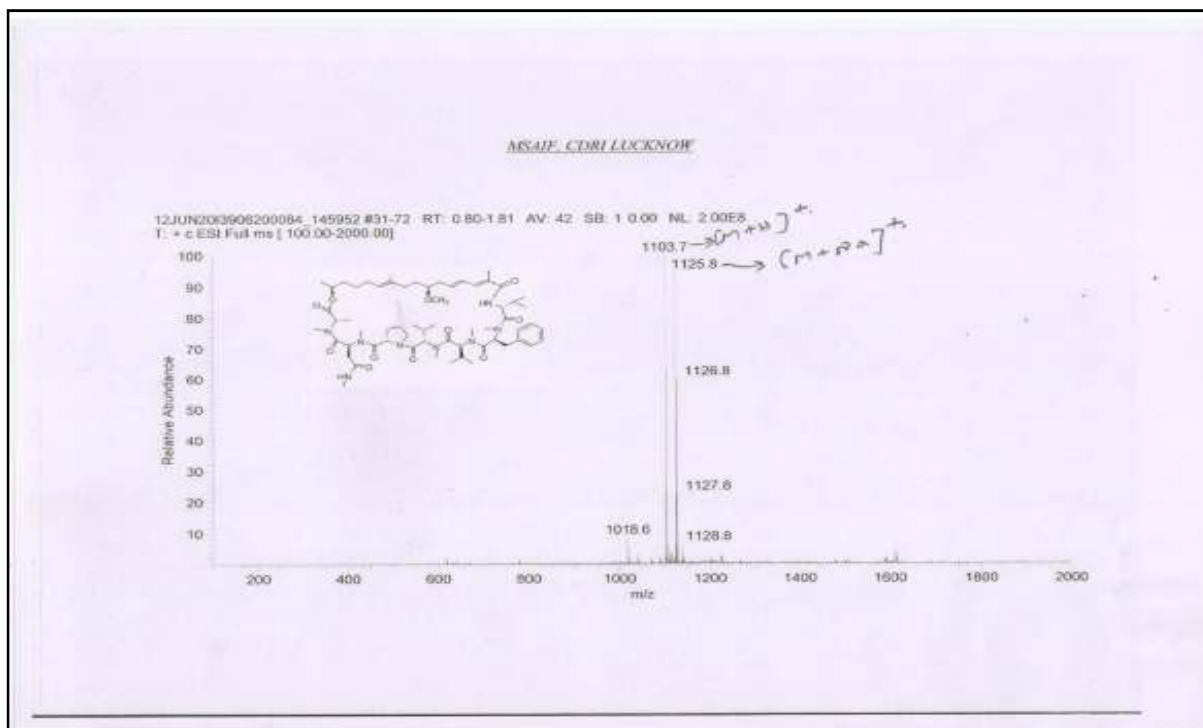
ESI-MS Spectrum of compound 25



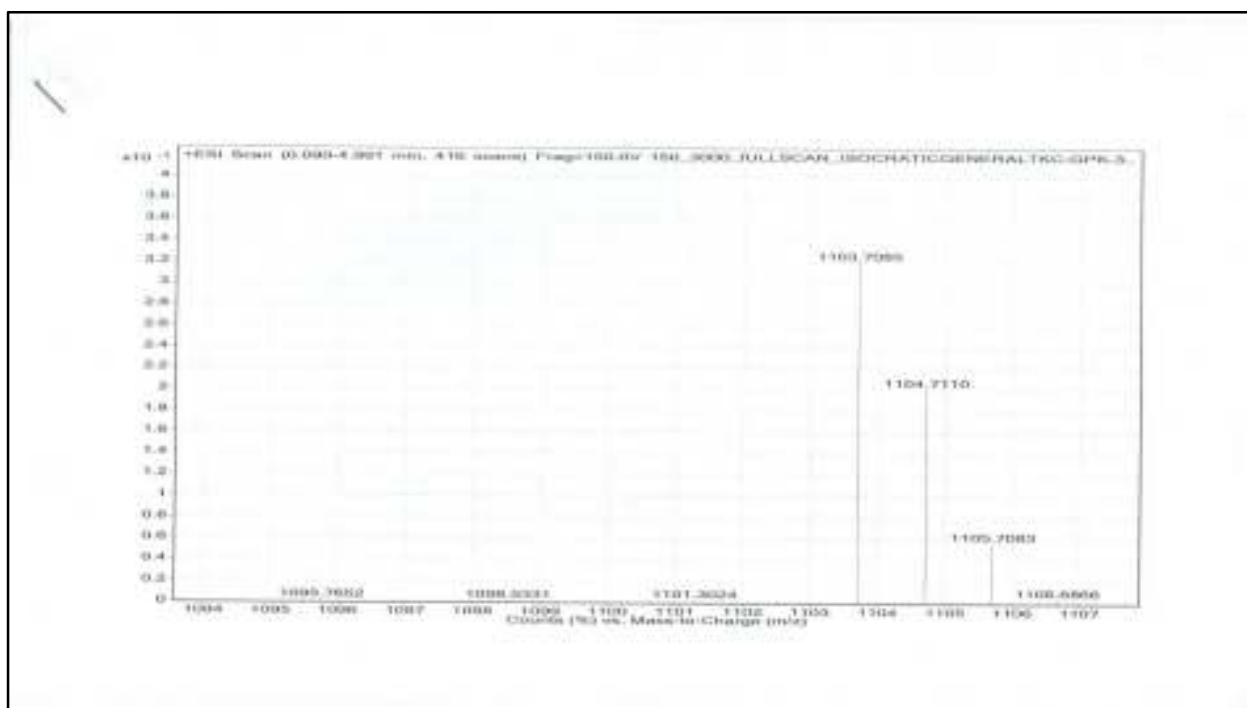
¹H Spectrum of compound **1** in CDCl₃ (400 MHz)



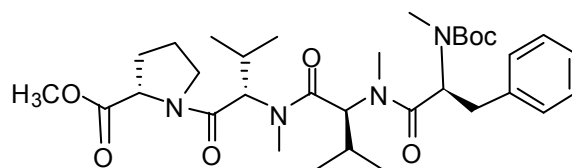
¹³C Spectrum of compound **1** in CDCl₃ (150 MHz)



ESI-MS Spectrum of compound 1



HRMS Spectrum of compound 1



Tetramer-20

Table 1a: ¹H NMR chemical shift assignments of Tetramer **20** Conformer-1A

| Proton/AA | Phe1 | Val2 | Val3 | Pro4 |
|-------------------|------|-----------------|---------------|---------------|
| C α H | 5.15 | 5.13 | 5.08 | 4.48 |
| C β H | 3.05 | 2.37 | 2.31 | 1.92, 2.17 |
| C γ H | --- | 0.848, 0.943 | 0.81, 0.92 | 2.05 |
| C δ H | --- | --- | --- | 3.57, 3.74 |
| N-CH ₃ | 2.78 | 2.81 | 2.97 | ---- |

Others: Boc=1.18, -OCH₃=3.67, PheArH=7.17-7.22

Table 1b: ¹H NMR chemical shift assignments of Tetramer **20** Conformer-1B

| Proton/AA | Phe1 | Val2 | Val3 | Pro4 |
|-------------------|------|----------------|----------------|-----------------|
| C α H | 5.47 | 5.103 | 5.105 | 4.51 |
| C β H | 3.05 | 2.35 | 2.35 | 1.933, 2.185 |
| C γ H | --- | 0.828, 0.93 | 0.828, 0.93 | 2.05 |
| C δ H | --- | --- | --- | 3.57, 3.85 |
| N-CH ₃ | 2.78 | 2.87 | 3.01 | ---- |

Others: Boc=1.31, -OCH₃=3.58, PheArH=7.17-7.22

Table 1c: ¹H NMR chemical shift assignments of Tetramer **20** Conformer-2A

| Proton/AA | Phe1 | Val2 | Val3 | Pro4 |
|-------------------|-----------------|-----------------|-----------------|---------------|
| C α H | 5.007 | 5.05 | 4.75 | 4.76 |
| C β H | 2.904, 3.114 | 2.66 | 2.336 | 1.76, 2.15 |
| C γ H | --- | 0.805, 0.899 | 0.751, 0.927 | 2.05 |
| C δ H | --- | --- | --- | 3.40, 3.57 |
| N-CH ₃ | 2.78 | 3.01 | 2.84 | ---- |

Others: Boc=1.26, -OCH₃=3.58, PheArH=7.17-7.22

Table 1d: ^1H NMR chemical shift assignments of Tetramer **20** Conformer-2B

| Proton/AA | Phe1 | Val2 | Val3 | Pro4 |
|--------------|----------------|-----------------|-----------------|-----------------|
| C α H | 5.33 | 5.20 | 4.74 | 4.837 |
| C β H | 2.841, 3.24 | 2.83 | 2.336 | 1.786, 2.176 |
| C γ H | --- | 0.864, 0.947 | 0.805, 0.928 | 2.05 |
| C δ H | --- | --- | --- | 3.43, 3.59 |
| N-CH $_3$ | 2.78 | 2.84 | 2.82 | ---- |

Others: Boc=1.40, -OCH $_3$ =3.67, PheArH=7.17-7.22

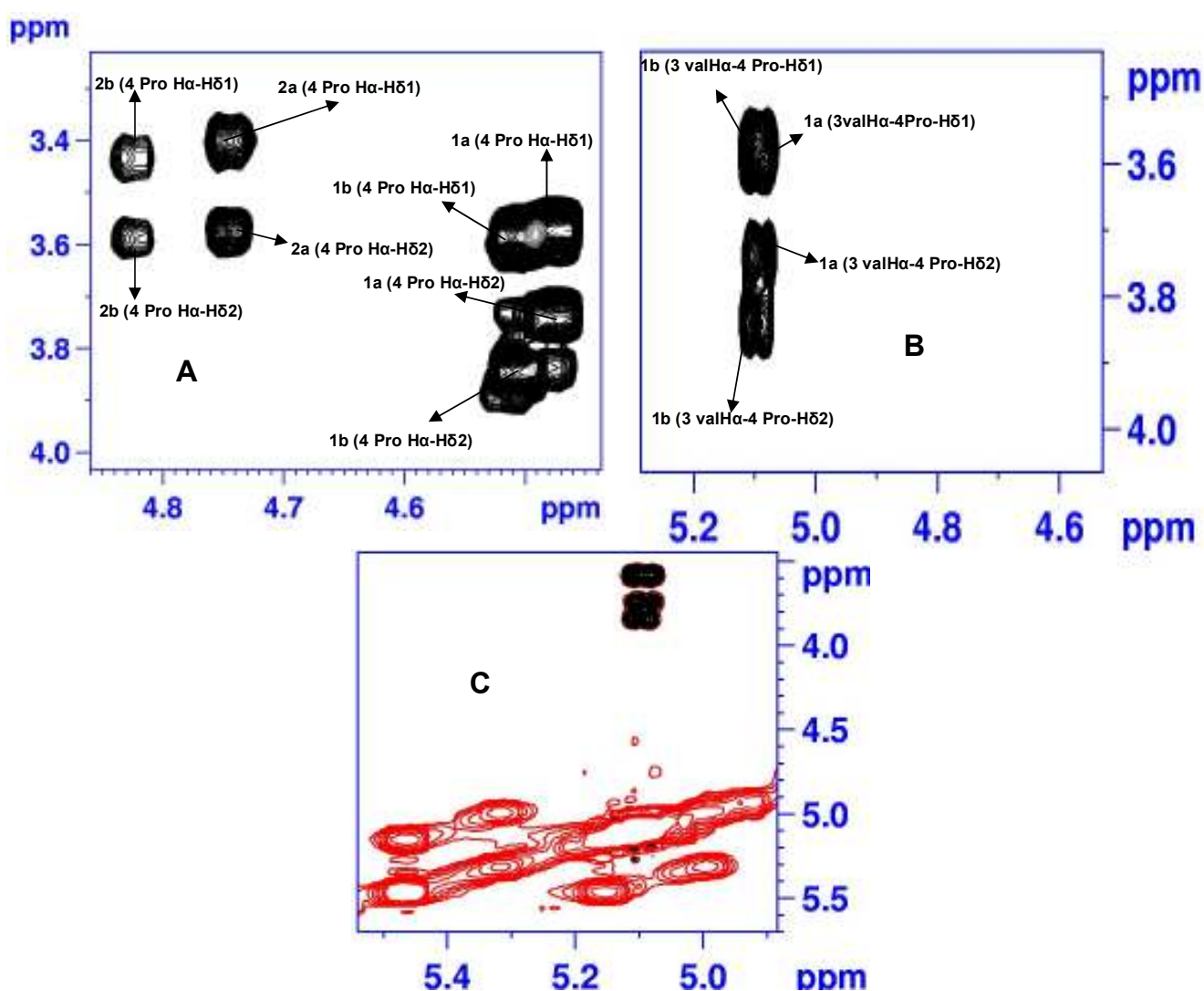


Figure 1: Multiple conformations of compound **20**. A) Four conformations in proline C α H region were found. B) Characteristic nOes in ROESY Experiment shows that proline exists predominantly in *trans* conformation for 1a, 1b and absence of such nOes for 2a, and 2b suggests *cis* configuration for 2a, 2b. C) Exchange peaks in Valine C α H and Phe C α H region.

Table 2: ¹H NMR chemical shift assignments of compound **21** (Major conformer)

| Proton/AA | Phe1 | Val2 | Val3 | Pro4 | Asn5 | Ala6 |
|-------------------|---------------|-----------------|------------------|----------------|----------------|------|
| C α H | 5.88 | 5.14 | 5.003 | 4.68 | 5.38 | 4.46 |
| C β H | 3.08, 2.65 | 2.301 | 2.22 | 2.12, 1.89 | 2.357, 2.76 | 1.35 |
| C γ H | --- | 0.783, 0.877 | 0.7971, 0.861 | 2.05 | ---- | --- |
| C δ H | --- | --- | --- | 3.51, 3.575 | --- | --- |
| N-CH ₃ | 2.81 | 2.83 | 2.78 | ---- | 2.764, | 2.75 |

Others: Boc=1.30, -OCH₃=3.66, PheArH= 7.10-7.24, NH= 5.82, AsnNH-CH₃= 2.81

Table 3: ¹H NMR chemical shift assignments of compound **24** (Major conformer)

| Proton/AA | Val1 | Phe2 | Val3 | Val4 | Pro5 | Asn6 | Ala7 |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|------|
| NH | 5.082 | --- | --- | --- | --- | --- | --- |
| C α H | 4.28 | 5.93 | 5.13 | 5.095 | 4.743 | 5.42 | 4.63 |
| C β H | 2.269 | 2.710, 3.161 | 2.347 | 2.320 | 1.97, 2.175 | 2.368, 2.859 | 1.43 |
| C γ H | 0.808, 0.920 | --- | 0.820, 0.919 | 0.825, 0.927 | 2.05 | --- | --- |
| C δ H | --- | --- | --- | --- | 3.657, 3.705 | --- | --- |
| N-CH ₃ | 2.83 | 2.81 | 2.84 | 2.80 | ---- | 2.98 | 2.97 |

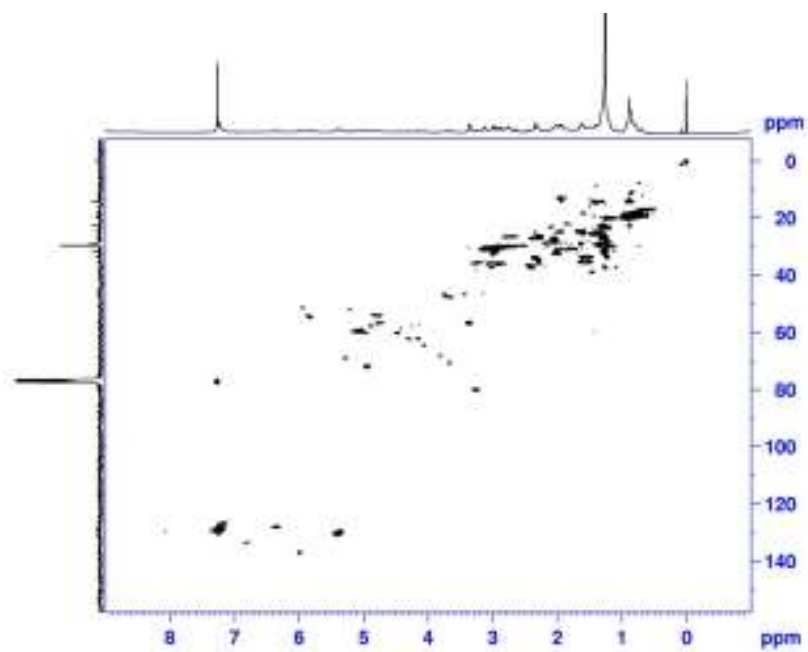
Others: Boc=1.31, -OCH₃=3.59, PheArH= 7.14-7.24, NH= 5.81, AsnNH-CH₃= 2.81

Table 6: ¹H NMR chemical shift assignments of compound **25** (Major conformer)

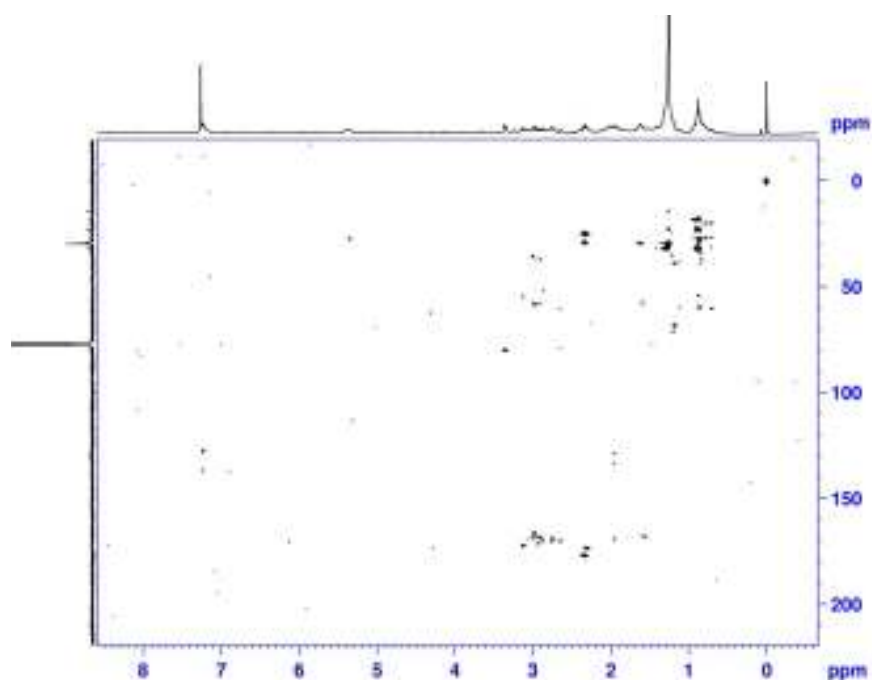
| Proton/AA | Val1 | Phe1 | Val2 | Val3 | Pro4 | Asn5 | Ala6 |
|-------------------|--|---------------|---------------|---------------|---------------|---------------|------|
| C α H | 4.78 | 5.98 | 5.09 | 5.11 | 4.74 | 5.83 | 4.75 |
| C β H | 2.36 | 3.24, 2.71 | 2.34 | 2.31 | 1.95, 2.17 | 2.37, 2.75 | 1.40 |
| C γ H | 0.81, 0.72 | --- | 0.82, 0.93 | 0.81, 0.92 | 2.04 | ---- | --- |
| C δ H | ---- | --- | --- | --- | 3.65, 3.71 | --- | --- |
| N-CH ₃ | ---- | 3.01 | 3.01 | 2.96 | ---- | 2.93, 2.73 | 2.91 |
| NH | 6.34 | ---- | ---- | ---- | ---- | 5.68 | ---- |
| DTA/others | C ₂₄ H= 1.93, C ₂₅ H=6.81, C ₂₆ H=6.33, C ₂₇ H=6.09, C ₃₂ H=5.40, C ₃₃ H=5.36, C ₂₉ H=3.25, C ₂₈ H=2.37,2.32, C ₃₁ H=2.02, C ₃₄ H=1.93, C ₃₆ H=1.51,1.42, C ₃₀ H=1.60,1.58, C ₃₅ H=1.25, C ₃₇ H=4.77,1.26, C ₂₉ -OCH ₃ =3.36, PheArH=7.13-7.26. | | | | | | |

Table 8. Comparison of NMR Assignments of Maleamide E with synthetic compound **1** (Major conformer) in CDCl₃

| Position | ¹ H | Compound | | Compound |
|----------|----------------|-------------|-----------------|---------------------------------|
| | (natural) | (synthetic) | ¹³ C | (1) ¹³ C |
| | | | (natural) | (synthetic) |
| 2 | | | 170.7 | 169.1 |
| 3 | 4.87 | 4.90 | 55.6 | 57.6 |
| 3a | 1.48 | 1.54 | 16.0 | 14.4 |
| 4a | 2.78 | 2.94 | 29.8 | 30.4 |
| 5 | | | 169.3 | 168.5 |
| 6 | 5.87 | 5.93 | 49.1 | 51.5 |
| 6a | 2.98,2.39 | 3.03,2.27 | 35.6 | 36.1 |
| 6b | | | 170.2 | 169.4 |
| 6c | 5.61 | 5.45 | | |
| 6d | 2.75 | 2.76 | 26.3 | 26.6 |
| 7a | 2.95 | 2.84 | 30.2 | 30.4 |
| 8 | | | 171.0 | 169.5 |
| 9 | 4.72 | 4.77 | 57.1 | 56.7 |
| 9a | 2.01,1.71 | 2.19,1.86 | 28.3 | 28.5 |
| 9b | 1.75,1.85 | 2.06 | 24.6 | 24.8 |
| 9c | 3.58,3.77 | 3.67 | 47.3 | 47.6 |
| 11 | | | 168.6 | 168.2 |
| 12 | 4.89 | 4.94 | 59.8 | 59.1 |
| 12a | 2.09 | 2.37 | 27.0 | 26.3 |
| 12b | 0.50 | 0.83 | 18.0 | 16.8 |
| 12c | 0.93 | 0.93 | 19.7 | 18.1 |
| 13a | 2.66 | 2.81 | 30.1 | 29.7 |
| 14 | | | 170.3 | 169.8 |
| 15 | 5.01 | 5.01 | 58.5 | 59.4 |
| 15a | 2.17 | 2.27 | 27.5 | 27.1 |
| 15b | 0.77 | 0.78 | 18.3 | 18.8 |
| 15c | 0.78 | 0.87 | 19.5 | 19.8 |
| 16a | 2.68 | 2.79 | 29.6 | 29.9 |
| 17 | | | 169.2 | 168.9 |
| 18 | 5.76 | 5.80 | 54.9 | 54.5 |
| 18a | 2.83,3.28 | 2.83,3.28 | 35.5 | 35.8 |
| 18b | | | 136.9 | 136.4 |
| 18c,e' | 7.25 | 7.24 | 129.6 | 129.5 |
| 18d,d' | 7.20 | 7.23 | 128.3 | 128.3 |
| 18e | 7.14 | 7.20 | 126.6 | 126.9 |
| 19a | 3.13 | 2.71 | 30.8 | 30.4 |
| 20 | | | 172.7 | 168.3 |
| 21 | 4.68 | 4.82 | 54.8 | 53.9 |
| 21a | 1.94 | 1.82 | 30.5 | 30.9 |
| 21b | 0.68 | 0.69 | 18.9 | 18.4 |
| 21c | 0.90 | 0.87 | 18.5 | 18.8 |
| 22 | 6.02 | 6.37 | | |
| 23 | | | 169.2 | 169.1 |
| 24 | | | 127.8 | 127.5 |
| 24a | 1.88 | 1.94 | 12.7 | 13.1 |
| 25 | 6.73 | 6.79 | 134.6 | 133.6 |
| 26 | 6.30 | 6.35 | 127.5 | 128.2 |
| 27 | 5.96 | 5.97 | 137.5 | 137.3 |
| 28 | 2.48,2.31 | 2.41,2.36 | 36.8 | 36.6 |
| 29 | 3.25 | 3.26 | 79.6 | 79.8 |
| 29a | 3.35 | 3.36 | 56.8 | 56.7 |
| 30 | 1.47,1.53 | 1.47,1.53 | 34.1 | 33.6 |
| 31 | 2.05 | 2.02 | 29.3 | 30.6 |
| 32 | 5.39 | 5.39 | 130.7 | 13.3 |
| 33 | 5.37 | 5.35 | 130.1 | 129.9 |
| 34 | 1.95 | 1.98 | 32.8 | 32.4 |
| 35 | 1.27 | 1.32 | 26.2 | 25.9 |
| 36 | 1.44,1.52 | 1.37,1.49 | 35.3 | 35.4 |
| 37 | 4.78 | 4.79 | 72.7 | 72.8 |
| 37a' | 1.19 | 1.20 | 19.7 | 19.9 |



2D-HSQC spectra of Compound 1



2D-HMBC spectra of Compound 1

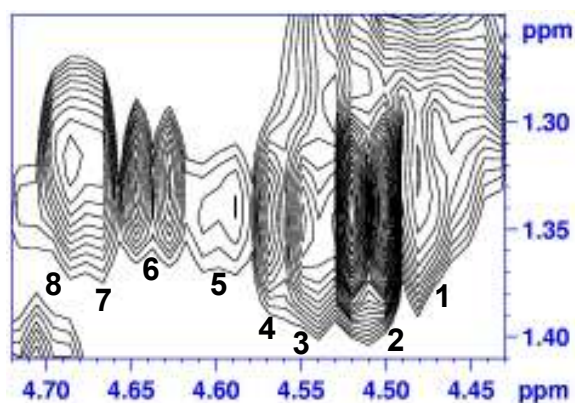


Figure 2: TOCSY expansion of **21**, Ala-C α H-C β H region, suggests at least 8 conformations.

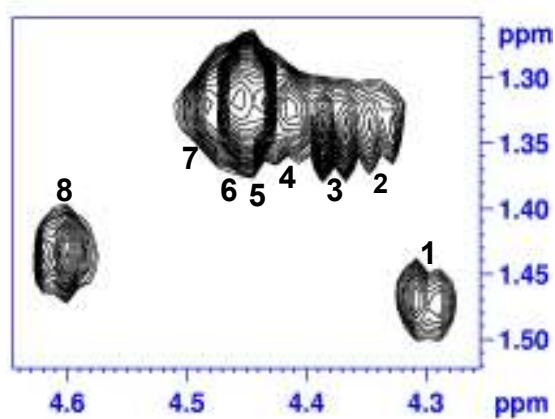


Figure 3: TOCSY expansion of **24**, Ala-C α H-C β H region.

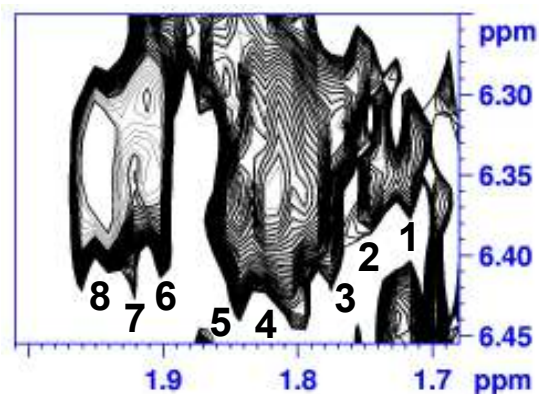


Figure 4: TOCSY expansion of compound **1**, Val NH-C β H region.

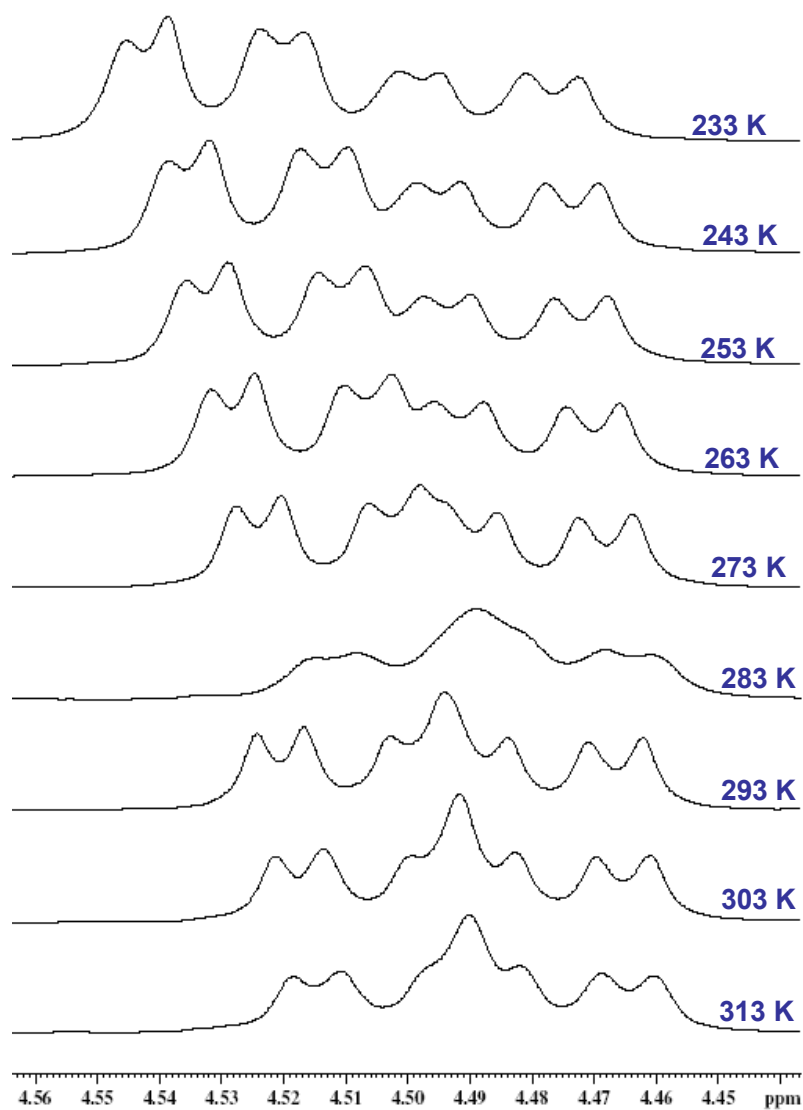


Figure 5: VT study of tetramer **20**

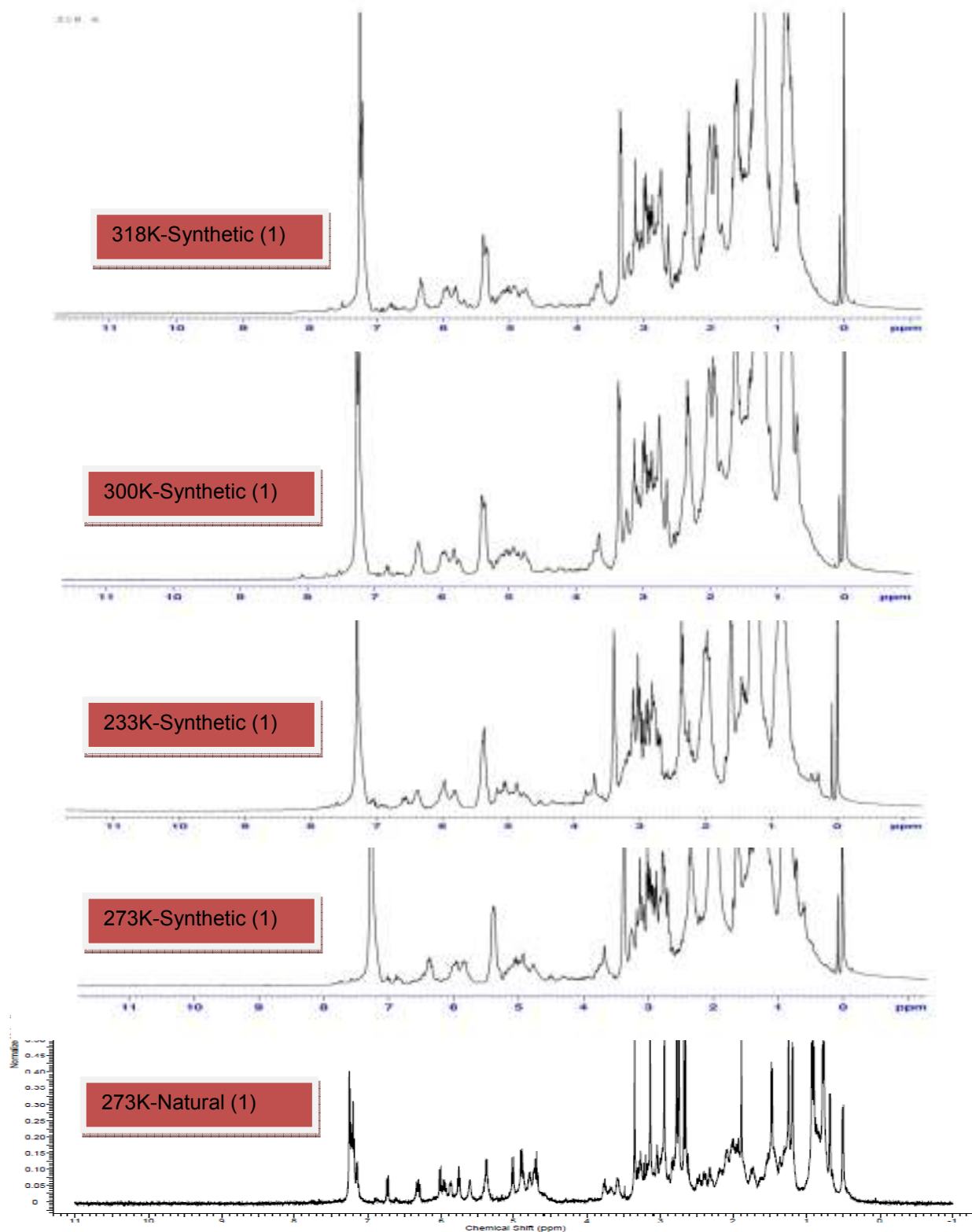
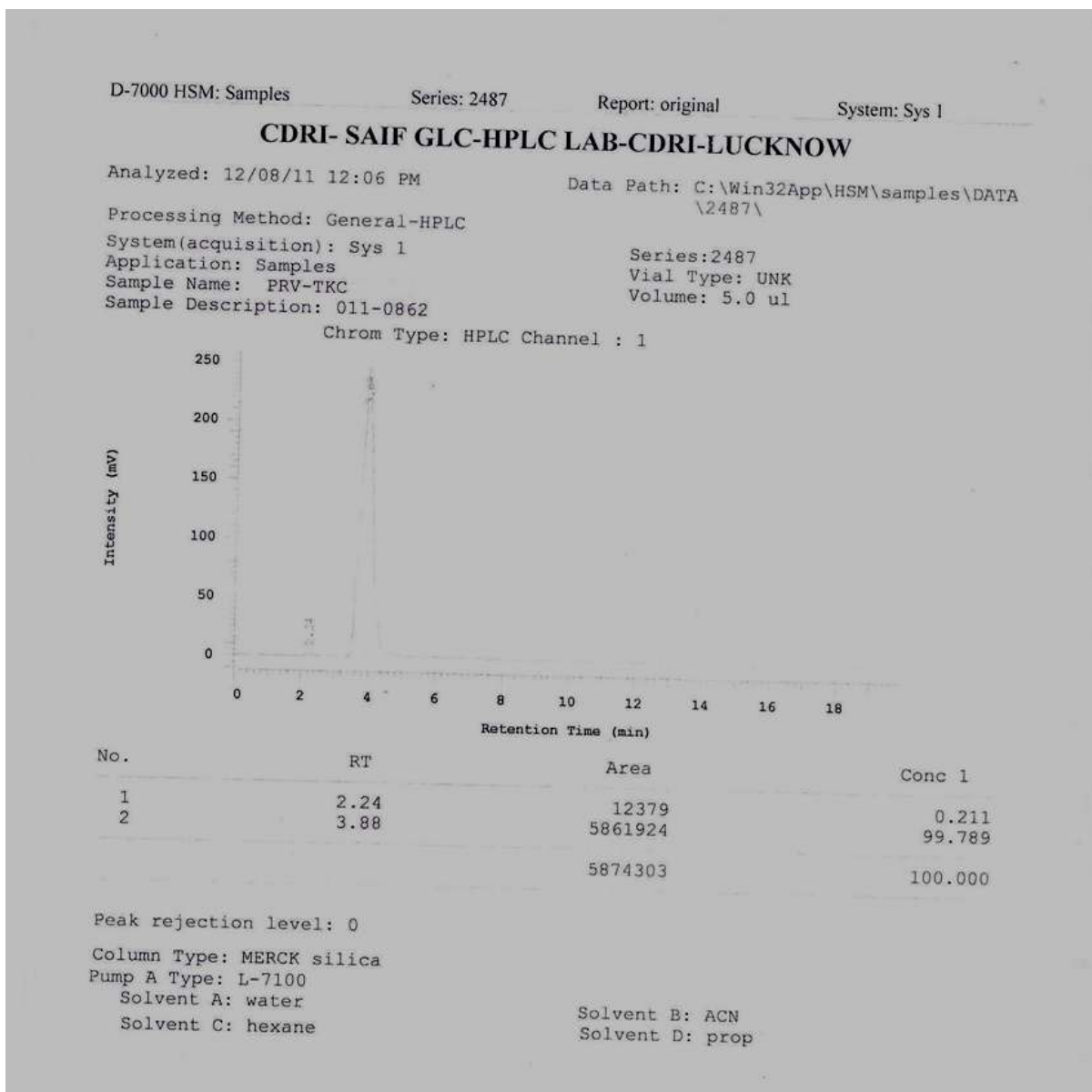


Figure 6: Comparison of compound of 1 at variable temperature with natural compound in CDCl₃



HPLC Spectrum of compound 1