Self-assembling Releasable Thiocolchicine-Diphenylbutenylaniline Conjugates

Gaia Fumagalli,[a] Laura Polito,[b] Eleonora Colombo,[a] Francesca Foschi,[a] Michael S. Christodoulou,[a] Francesco Galeotti,[b] Dario Perdicchia,[a] Ivan Bassanini,[c] Sergio Riva,[c] Pierfausto Seneci,[a] Aída García-Argáez,[d,e] Lisa Dalla Via,[d] Daniele Passarella*[,a]

Chemistry

General

All reactions were carried out in oven-dried glassware and dry solvents under nitrogen atmosphere.

Unless otherwise stated, all solvents were purchased from Sigma Aldrich and used without further purification.

Substrates and reagents were purchased from Sigma Aldrich and used as received.

Thin layer chromatography (TLC) was performed on Merck precoated $60F_{254}$ plates.

Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by charring with 1% permanganate solution.

Flash chromatography was performed using silica gel (240-400 mesh, Merck).

^{1}H-NMR spectra were recorded on Bruker DRX-400 And Bruker DRX-300 instruments and are reported relative to residual $CDCl_3$ and CD_3OD.

^{13}C-NMR spectra were recorded on the same instruments (100 and 75 MHz) and are reported relative to $CDCl_3$ and CD_3OD.

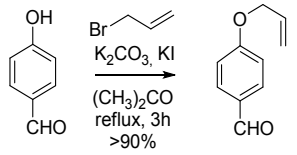
Chemical Shifts (δ) for proton and carbon resonances are quoted in parts per million (ppm) relative to tetramethylsilane (TMS), which was used as an internal standard.

MS spectra were recorded using Electrospray ionization (ESI) technique on a Waters Micromass Q-Tof micro mass spectrometer and HR-ESI mass spectra were recorded on FT-ICR $APEX_{II}$ (Bruker Daltonics), EI mass spectra were recorded at an ionizing voltage of 6 kEv on a VG 70-70 EQ.

Specific rotations were measured with a P-1030-Jasco polarimeter with 10 cm optical path cells and 1 ml capacity (Na lamp, λ = 589 nm). Microwave assisted reactions were performed with Emrys Creator single-mode (power range 0-400 W from magnetron at 2.45 GHz).

IR spectra were recorded on a Jasco FT-IR 4100 Spectrometer using CH_2Cl_2 in NaCl rectangular windows.

Synthesis of 4



3 **4**

To a solution of 4-hydroxybenzaldehyde **3** (2.005 g, 16.377 mmol) in acetone (100 mL), allybromide (3.950 g, 32.653 mmol), K_2CO_3 (4.502 g, 32.574 mmol) and KI (0.273 g, 1.644 mmol) are added and the reaction mixture is stirred at room temperature for 3h. Brine (70 mL) is added and extracted with AcOEt (5x15 mL). The organic layers are dried over Na_2SO_4 and the solvent is removed under reduced pressure to obtain **4** as a yellow oil (2.660 g, Yield: > 90%) without any further purification. [1]

^{1}H-NMR ($CDCl_3$, 300 MHz): δ(ppm) = 9.91 (s, 1H), 7.86 (d, *J*=8.8 Hz, 2H), 7.04 (d, *J*=8.8 Hz, 2H), 6.13-6.03 (m, 1H), 5.48-5.35 (m, 2H), 4.66 (m, 2H).

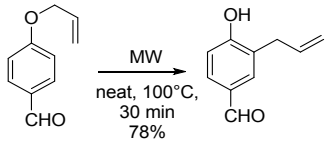
^{13}C-NMR ($CDCl_3$, 100 MHz): δ (ppm) = 190.6, 163.6, 132.3, 131.9, 130.1 (2 CH_{Ar}), 118.2, 115.0 (2 CH_{Ar}), 69.0.

ESI-HRMS : (*m*/*z*) calcd for $C_{10}H_{10}O_2Na$: 185.0578; found : 185.0580.

IR (neat): 3076, 2925, 2837, 1686, 1596, 1575, 1507 cm^{-1}.

Anal. Calcd for $C_{10}H_{10}O_2$: C, 74.06; H, 6.21. **Found**: C, 73.95; H, 6.32.

Synthesis of 5



4 **5**

Compound **4** (3.492 g, 21.011 mmol) is exposed to microwave radiation in a microwave oven at 190°C for 30 minutes. The crude is purified by flash chromatography (Hex/AcOEt 8:2) to obtain **5** (2.657 g, Yield: 78%).[2]

^{1}H-NMR ($CDCl_3$, 300 MHz): δ(ppm): 9.81 (s, 1H), 7.68-7.66 (m, 2H), 6.95 (d, *J*=8.5 Hz, 1H), 6.77 (bs, 1H), 6.07-5.93 (m, 1H), 5.18-5.13 (m, 2H), 3.46-3.45 (m, 2H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ (ppm): 191.9, 160.5, 135.4, 132.6, 130.9, 129.5, 126.9, 117.1, 116.1, 34.4.

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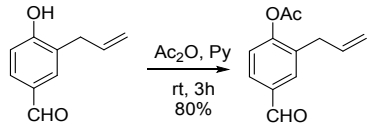
[1] The characterization of product **4** is consistent with that reported in the literature: Z. Liu, L. Tang et al*., European Journal of Medicinal Chemistry*, **2014**, *74*, 671-682.

[2] The characterization of product **5** is consistent with that reported in the literature: W.A.L. van Otterlo, G.L. Morgans et al., *Tetrahedron*, **2005**, *61*, 7746

Synthesis of 6



5 **6**

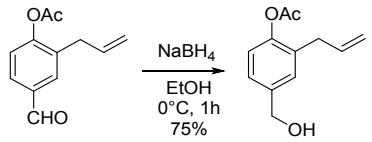
To a mixture of Ac_2O/Pyridine 1:1 (40 mL) **5** (2.003 g, 12.301 mmol) is added and the reaction mixture is stirred at room temperature for 2 h. The reaction mixture is then cooled at 0°C and solid $NaHCO_3$ is added. The reaction mixture is extracted with AcOEt (3x20 mL) and organic layers are washed with H_2O. The organic layers are dried over Na_2SO_4 and the solvent is removed under reduced pressure. The crude is purified by flash chromatography (Hex/AcOEt 85:15) to obtain **6** (2.302 g, Yield: 80%).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ(ppm): 9.99 (s, 1H), 7.81-7.79 (m, 2H), 7.26 (d, *J*=11.1 Hz, 1H), 5.97-5.87 (m, 1H), 5.17-5.09 (m, 2H), 3.40-3.39 (m, 2H), 2.35 (s, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ(ppm): 191.9, 170.5, 157.6, 135.4, 132.7, 130.9, 129.5, 126.9, 117.1, 116.1, 34.4, 20.4.

ESI-HRMS : (*m*/*z*) calcd for $C_{12}H_{12}O_3Na$: 227.0684; found : 227.0688.

Synthesis of 7



6 **7**

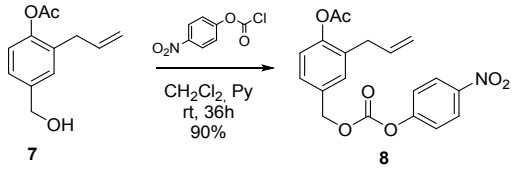
To a solution of **6** (0.203 g, 0.984 mmol) in EtOH (10 mL) at 0°C, $NaBH_4$ (0.074 g, 1.965 mmol) is added and the reaction mixture is stirred at 0°C for 30 minutes. The solvent is then removed under reduced pressure, H_2O (20 mL) is added and extracted with CH_2Cl_2 (5x10 mL). The organic layers are dried over Na_2SO_4 and the solvent is removed under reduced pressure. The crude is purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to obtain **7** (0.151 g, Yield: 75%).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ(ppm): 7.07 (s, 1H), 7.01 (d, *J*=8.5 Hz, 1H), 6.70 (d, *J*=8.5 Hz, 1H), 6.05-5.92 (m, 1H), 5.11-5.07 (m, 2H), 4.52 (s, 2H), 3.37-3.35 (m, 3H), 2.19 (s, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz): δ (ppm): 170.9, 135.8, 136.6, 132.2, 129.4, 127.04, 126.4, 116.1, 115.7, 64.9, 34.4, 20.2.

ESI-HRMS : (*m*/*z*) calcd for $C_{12}H_{14}O_3Na$: 229.0841; found : 229.0845.

Synthesis of 8



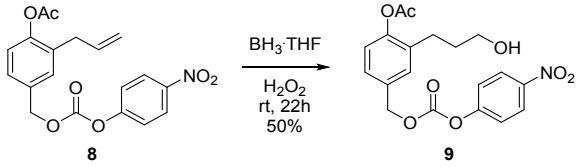
To a solution of **7** (1.194 g, 5.791 mmol) in dry CH_2Cl_2 (80 mL), 4-nitrophenyl chloroformate (2.343 g, 11.594 mmol) and dry pyridine (0.917 g, 11.594 mmol) are added and the reaction mixture is stirred at room temperature for 36 h. The solvent is then removed under reduced pressure. The crude is purified by flash chromatography (Hex/AcOEt 8:2) to obtain **8** (2.101 g, Yield: 90 %).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ(ppm): 8.28 (d, *J*=9.4 Hz, 2H), 7.38 (d, *J*=9.4 Hz, 2H), 7.33-7.30 (m, 2H), 7.10 (d, *J*=8.8, 1H), 6.07-5.96 (m, 1H), 5.27 (s, 2H), 5.17-5.11 (m, 2H), 3.41-3.39 (m, 2H), 2.35 (s, 3H).

^{13}C-NMR: ($CDCl_3$, 100 MHz): δ (ppm): 169.6, 155.5, 152.5, 149.5, 145.4, 136.4, 135.0, 132.8, 130.7, 130.0 (2 CH_{Ar}), 129.6 (2 CH_{Ar}), 129.4, 127.6, 116.4, 70.5, 34.8, 20.9.

ESI-HRMS : (*m*/*z*) calcd for $C_{19}H_{17}NO_7Na$: 394.0902; found : 394.0909.

Synthesis of 9



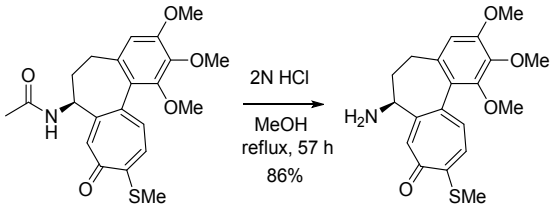
To a solution of **8** (1.106 g, 5.654 mmol) in dry THF (50 mL), BH_3·THF (7.771 mL, 3.885 mmol) is added and the reaction mixture is stirred at rt for 22 h. At 0°C H_2O_2 (0.166 mL, 7.079 mmol) is added and the reaction mixture is stirred at rt for 30 minutes. Solid NaCl and H_2O (50 mL) are added and extracted with Et_2O (3x40 mL). The organic layers are washed with brine, dried over Na_2SO_4 and the solvent is removed under reduced pressure. The crude is purified by flash chromatography (Hex/AcOEt 1:1) to obtain **9** (1.221 g, Yield: 50 %).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ(ppm): 8.26 (d, *J*=9.4 Hz, 2H), 7.40-7.30 (m, 4H), 7.08 (d, *J*=8.8, 1H), 5.26 (s, 2H), 3.65 (t, *J*=6.1, 2H), 2.66 (t, *J*=7.1, 2H), 2.34 (s, 3H), 1.90-1.80 (m, 2H), OH signal not detected.

^{13}C-NMR ($CDCl_3$, 100 MHz): δ (ppm): 169.6, 155.5, 152.4, 149.5, 145.4, 134.3, 132.1, 129.1, 125.3, 122.5 (2 CH_{Ar}), 121.8, 121.1 (2 CH_{Ar}), 70.4, 61.8, 32.6, 26.2, 20.9.

ESI-HRMS : (*m*/*z*) calcd for $C_{19}H_{19}NO_8Na$: 412.1008; found : 412.1012.

Synthesis of 11a



10 **11a**

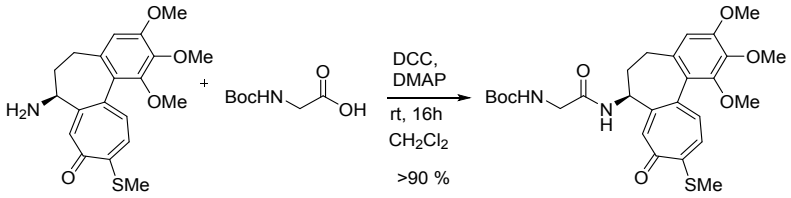
To a solution of (-)-thiocolchicine **10** (500 mg, 1.205 mmol) in MeOH (20 mL), HCl 2N (9.650 mL, 19.312 mmol) is added and the reaction mixture is stirred at reflux at 90 °C for 57 h. The solvent is removed under reduced pressure, H_2O (20 mL) is added and extracted with CH_2Cl_2 (3x15 mL). The aqueous layer is neutralized with NaOH and extracted with CH_2Cl_2. The organic layers are washed with brine and dried over Na_2SO_4. The solvent is removed under reduced pressure. The crude is purified by flash chromatography (CH_2Cl_2/MeOH 9:1) to obtain **11a** (0.387 g, Yield: 86 %).[3]

^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm): 7.61 (s, 1H), 7.22 (d, *J*=10.5 Hz, 1H), 7.05 (d, *J*=10.5 Hz, 1H), 6.56 (s, 1H), 3.93 (s, 6H), 3.83-3.79 (m, 1H), 3.69 (s, 3H), 2.54-2.50 (m, 1H), 2.47-2.38 (m, 5H), 1.79-1.93 (m, 1H), NH_2 signal not detected.

Anal. calcd for ($C_{20}H_{23}NO_4S$): C, 64.32, H, 6.21, N, 3.75; **Found**: C, 64.27, H, 6.35, N, 3.68.

$[\alpha]_D^{20}$**:** -160.6 ° (c 0.35; MeOH).

Synthesis of 11b



11a **11b**

To a solution of **11a** (0.303 g, 0.812 mmol) in CH_2Cl_2 (30 mL), N-Boc-glycine (0.312 g, 0.59 mmol), DCC (1.009 g, 4.862 mmol) and DMAP (0.198 g, 1.624 mmol) are added. The reaction mixture is stirred at rt for 16 h, then filtered through Celite. The solvent is removed under reduced pressure. The crude is purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to afford **11b** (0.420 g, > 90%).[4]

^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm): 8.20 (bs, 1H), 7.54-7.50 (m, 1H), 7.44-7.41 (m, 1H), 7.25 (s, 1H), 6.57 (s, 1H), 5.80 (bs, 1H), 4.78-4.58 (m, 1H), 4.05-3.63 (m, 11H), 2.58-2.52 (m, 4H), 2.46-2.32 (m, 2H), 2.13-2.03 (m, 1H) 1.42 (s, 9H).

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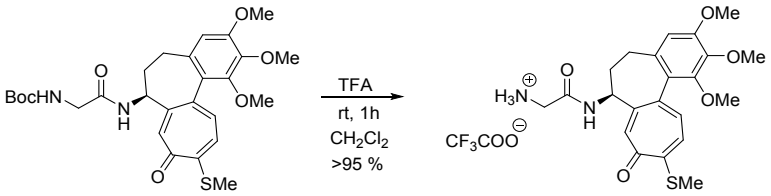
[3] The characterization of product **11a** is consistent with that reported in the literature: A. Muzaffar, A. Brossi, *Synthetic Communications*, **1990**, *20*, 713-717

[4] The characterization of product **11b** is consistent with that reported in the literature: G. Cappelletti, D. Cartelli, B. Peretto *et al*., *Tetrahedron*, **2011**, *67*, 7354-7357

^{13}C-NMR ($CDCl_3$, 100 MHz, detected signals): δ (ppm): 182.3, 169.6, 158.2, 156.3, 154.5, 151.0, 141.8, 139.1, 135.5, 131.4, 128.1, 107.2, 80.6, 61.5, 61.3, 56.1, 52.1, 45.0, 36.4, 29.9, 28.3, 15.3.

Anal. Calcd for $C_{27}H_{34}N_2O_7S$: C, 61.11; H, 6.46; N, 5.28. **Found**: C, 61.14; H, 6.41; N, 5.30.

Synthesis of 11



11b **11**

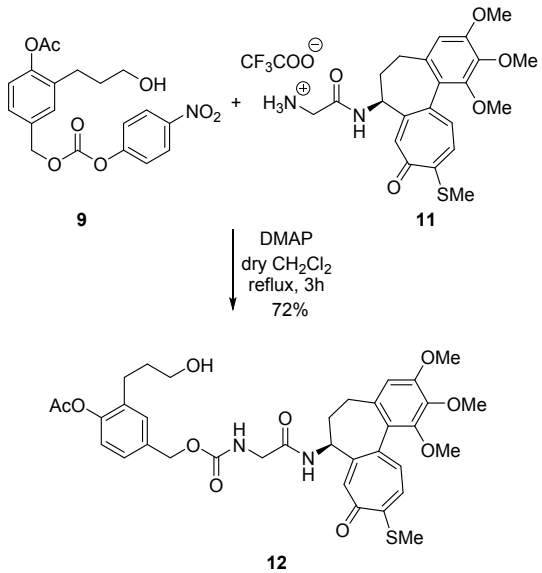
To a solution of **11b** (0.420 g, 0.812 mmol) in dry CH_2Cl_2 (20 mL) at 0°C TFA (2.418 g, 21.085 mmol) is added and the reaction mixture is stirred at rt for 1 h. The solvent is removed under reduced pressure to obtain **11** (0.430 g, Yield: > 95%) without any further purification.[5]

characterization of the free amine: **^{1}H-NMR** ($CDCl_3$, 300 MHz): δ (ppm): 8.89 (bs, 2H), 8.03-8.00 (m, 1H), 7.77-7.73 (m, 1H), 7.54 (s, 1H), 6.60 (s, 1H), 4.52 (bs, 1H), 4.41-4.36 (m, 1H), 3.91 (s, 6H), 3.64 (s, 3H), 2.60-2.54 (m, 4H), 2.52-2.07 (m, 3H), 1.25-1.15 (m, 2H).

^{13}C-NMR ($CDCl_3$, 100 MHz, detected signals): δ (ppm): 182.3, 158.2, 156.3, 154.5, 151.0, 141.8, 139.1, 135.5, 131.4, 128.1, 107.2, 61.5, 61.3, 56.1, 52.1, 45.0, 36.4, 29.9, 15.3.

Anal. Calcd for $C_{22}H_{26}N_2O_5S$: C, 61.38; H, 6.09; N, 6.51. **Found**: C, 61.42; H, 6.08; N, 6.48.

Synthesis of 12



9 **11** **12**

[5] The characterization the free amine **11** is consistent with that reported in the literature: G. Cappelletti, D. Cartelli, B. Peretto *et al*., *Tetrahedron*, **2011**, *67*, 7354-7357

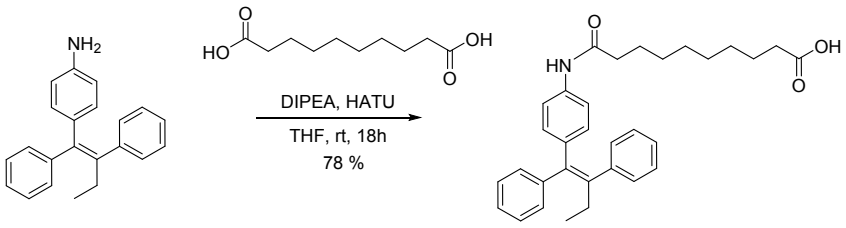
To a solution of **11** (0.438 g, 0.812 mmol) in dry CH_2Cl_2 (10 mL) DMAP (0.247 g, 2.015 mmol) and a solution of **9** (0.315 g, 0.812 mmol) are added and the reaction mixture is stirred at reflux at 40°C for 3 h. The solvent is then removed under reduced pressure. The crude is purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to afford **12** (0.399 g, 72%).

^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm): 8.37 (bs, 1H), 7.40 (s, 1H), 7.30 (s, 1H), 7.16 (s, 1H), 7.08-7.04 (m, 2H), 6.88 (d, J=8.2 Hz, 1H), 6.52 (s, 1H), 6.09 (bs, 1H), 4.89 (s, 2H), 4.68-4.60 (m, 1H), 3.90-3.87 (m, 8H), 3.60 (s, 3H), 3.53-3.48 (m, 2H), 2.53-2.48 (m, 3H), 2.41 (s, 3H), 2.29-2.11 (m, 4H), 2.01-1.96 (m, 2H), 1.76-1.67 (m, 2H), OH signal not detected.

^{13}C-NMR ($CDCl_3$, 100 MHz): δ (ppm): 182.2, 169.7, 169.4, 158.3, 156.8, 153.7, 151.7, 151.0, 148.6, 141.5, 138.7, 134.9, 134.5, 134.2, 133.8, 129.9, 128.6, 127.0, 126.6, 125.6, 122.3, 107.5, 66.3, 61.6, 61.4, 61.2, 56.2, 53.50, 52.3, 36.2, 32.4, 29.6, 26.1, 20.9, 15.0.

ESI-HRMS : (*m*/*z*) calcd for $C_{35}H_{40}N_2O_{10}SNa$: 703.2301; found : 703.2307.

Synthesis of 13a



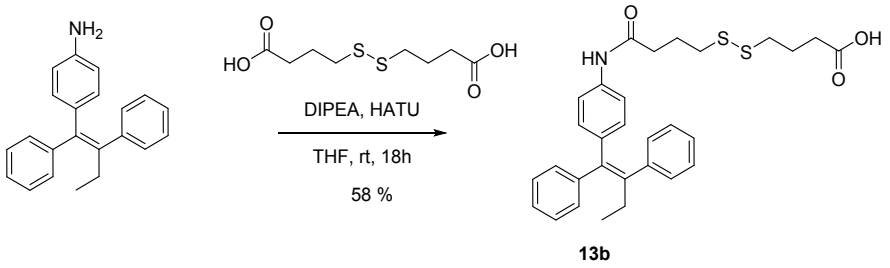
To a solution of sebacic acid (0.255 g, 1.263 mmol) in dry THF (20 mL) HATU (0.527 g, 1.386 mmol) and DIPEA (0.326 g, 2.523 mmol) are added and the reaction mixture is stirred for 30 minutes. Then 4-(1,2-diphenylbut-1-en-1-yl)aniline (0.378 g, 1.265 mmol) is added and the reaction mixture is stirred at rt overnight. The solvent is removed under reduced pressure, AcOEt is added and it is washed with water and brine. The organic layer is then dried over Na_2SO_4 and concentrated under reduced pressure. The crude is purified by flash chromatography (AcOEt/Hex 4:6) to obtain pure **13a** (0.476 g, Yield: 78%).[6]

^{1}H-NMR ($CDCl_3$, 300 MHz): δ(ppm): 7.95 (bs, 1H), 7.33-6.78 (m, 14H), 2.51-2.41 (m, 2H), 2.36-2.27 (m, 4H), 1.87-1.61 (m, 4H), 1.35-1.24 (m, 8H), 0.94-0.88 (m, 3H), OH signal not detected.

^{13}C-NMR ($CDCl_3$, 75 MHz, detected signals): δ(ppm): 175.3, 174.6, 145.1, 143.2, 143.1, 141.3, 139.7, 135.4, 131.9, 129.6, 129.4, 128.2, 127.7, 127.3, 126.3, 116.1, 37.6, 35.0, 31.8, 30.8, 27.0, 26.2, 13.6.

[6] The characterization of product **13a** is consistent with that reported in the literature: G. Fumagalli, M.S. Christodoulou, B. Riva, *Organic & Biomolecular Chemistry*, **2017**, *15*, 1106-1109

Synthesis of 13b



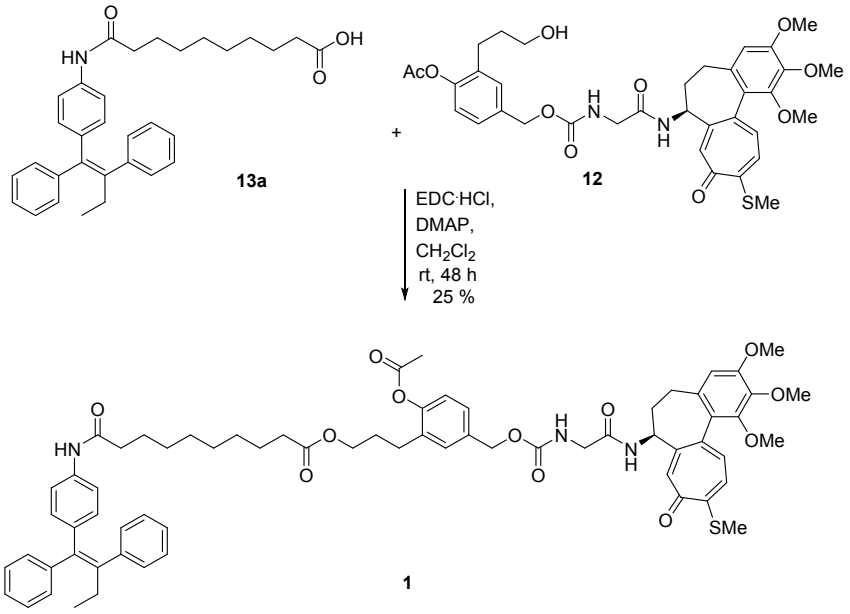
To a solution of 4,4'-dithiobutyric acid (0.228 g, 0.958 mmol) in dry THF (15 mL) HATU (0.401 g, 1.054 mmol) and DIPEA (0.248 g, 1.921 mmol) are added and the reaction mixture is stirred for 30 minutes. Then 4-(1,2-diphenylbut-1-en-1-yl)aniline (0.827 g, 0.958 mmol) is added and the reaction mixture is stirred at rt overnight. The solvent is then removed under reduced pressure, H_2O (20 mL) is added, acidified with HCl and extracted with CH_2Cl_2 (3x15 mL). The combined organic layers are dried over Na_2SO_4 and evaporated under reduced pressure. The crude is purified by flash chromatography (Hex/EtOAc 6:4 + 1% of glacial acetic acid) to provide **13b** (0.289 g, Yield: 58%).

^{1}H-NMR ($CDCl_3$, 300 MHz) δ(ppm): 7.38-7.13 (m, 12H), 6.84 (d, *J*=8.5 Hz, 2H), 2.77-2.71 (m, 4H), 2.50 (m, 6H), 2.08-2.01 (m, 4H), 0.96 (t, *J*=7.7 Hz, 3H), NH and OH signals not detected.

^{13}C-NMR ($CDCl_3$, 100 MHz): δ(ppm): 178.3, 170.6, 143.4, 142.2 (2 C_{Ar}), 139.2, 138.1, 135.4, 131.4 (2 CH_{Ar}), 129.7 (2 CH_{Ar}), 129.5 (2 CH_{Ar}), 128.2 (2 CH_{Ar}), 127.9 (2 CH_{Ar}), 126.7, 126. 2, 118.8 (2 CH_{Ar}), 37.8, 37.5, 35.4, 32.3, 29.0, 24.5, 24.0, 13.5.

ESI-HRMS : (*m*/*z*) calcd for $C_{30}H_{33}NO_3S_2Na$: 542.1800; found : 542.1803.

Synthesis of 1



To a suspension of **13a** (0.106 g, 0.221 mmol) in dry CH_2Cl_2 (5 mL) EDC·HCl (0.050 g, 0.262 mmol), DMAP (0.019 g, 0.154 mmol) are added and the reaction mixture is stirred for 30 minutes. Then **12** (0.150 g, 0.221

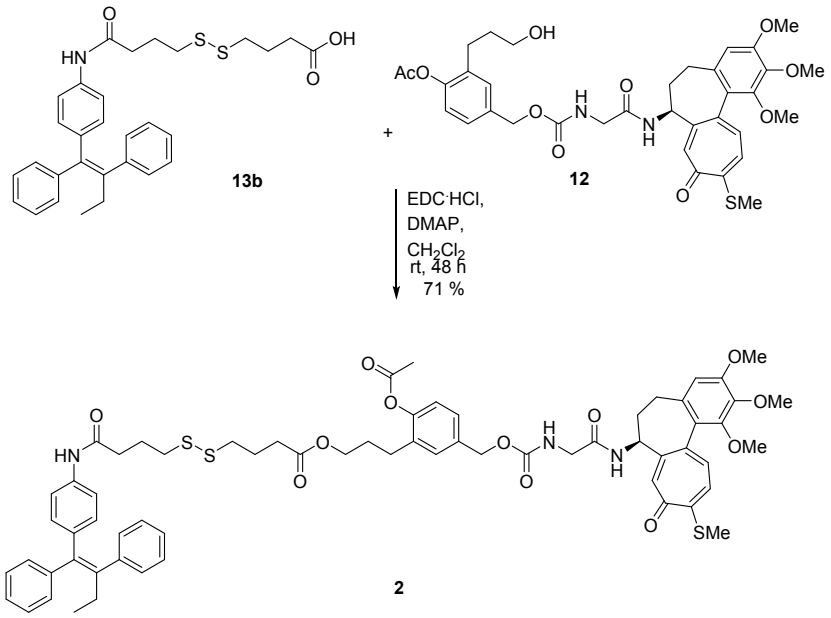
mmol) is added and the reaction mixture is stirred at rt for 48 h. The solvent is removed under reduced pressure. The crude is purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to afford **1** (0.063 g, 25%).

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.56 (bs, 1H), 7.53 (bs, 1H), 7.38 – 7.31 (m, 3H), 7.27 – 7.07 (m, 12H), 7.01 – 6.96 (m, 2H), 6.88 – 6.87 (m, 1H), 6.81 (d, *J* = 8.4 Hz, 2H), 6.56 (s, 1H), 5.78 (bs, 1H), 5.05 – 5.01 (m, 2H), 4.70 – 4.66 (m, 1H), 4.10 – 4.06 (m, 2H), 3.96 (s, 3H), 3.96 – 3.90 (m, 5H), 3.65 (s, 3H), 2.59 – 2.39 (m, 10H), 2.37 – 2.28 (m, 6H), 2.26 – 2.22 (m, 2H), 2.06 (q, *J* = 7.1 Hz, 2H), 1.99 – 1.88 (m, 6H), 1.74 – 1.60 (m, 6H), 0.99 – 0.91 (m, 3H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 182.8, 174.6, 172.0, 170.1, 169.5, 159.1, 157.3, 154.4, 151.8, 149.4, 144.1, 142.8, 143.0, 142.3, 139.4, 139.1, 138.8, 136.8, 135.6, 135.0, 134.9, 133.8, 131.8 ($2CH_{ar}$), 131.4, 130.7, 130.3 ($2CH_{ar}$), 130.1 ($2CH_{ar}$), 129.1, 128.8 ($2CH_{ar}$), 128.6 ($2CH_{ar}$), 128.0, 127.2, 126.8, 126.3, 123.1, 119.2 ($2CH_{ar}$), 114.7, 108.1, 67.2, 64.2, 62.2, 62.1, 56.8, 52.7, 45.2, 38.3, 37.2, 35.0, 30.5, 30.4, 30.2, 30.0, 29.6, 29.4, 27.4, 26.2, 26.2, 25.6, 21.6, 15.8,14.2.

ESI-HRMS : (*m*/*z*) calcd for $C_{67}H_{75}N_3O_{12}SNa$: 1168.4969; found : 1168.4976.

Synthesis of 2



To a suspension of **13b** (0.114 g, 0.221 mmol) in dry CH_2Cl_2 (5 mL) EDC·HCl (0.050 g, 0.262 mmol), DMAP (0.019 g, 0.154 mmol) are added and the reaction mixture is stirred for 30 minutes. Then **12** (0.150 g, 0.221 mmol) is added and the reaction mixture is stirred at rt for 48 h. The solvent is removed under reduced pressure. The crude is purified by flash chromatography (CH_2Cl_2/MeOH 95:5) to afford **2** (0.184 g, 71%).

^{1}H-NMR (400 MHz, $CDCl_3$,) δ 7.89 (bs, 1H), 7.82 (bs, 1H), 7.46 – 7.03 (m, 17H), 6.95 (d, *J* = 8.1 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 2H), 6.56 (s, 1H), 5.82 (bs, 1H), 4.98 (q, *J* = 13.0, 12.4 Hz, 2H), 4.77 – 4.59 (m, 1H), 4.07 (t, *J* = 6.2 Hz, 2H), 3.95 (s, 3H), 3.92 – 3.89 (m, 5H), 3.63 (s, 3H), 2.71 – 2.69 (m, *J* = 7.1 Hz, 4H), 2.60 – 2.35 (m, 13H), 2.32 (s, 3H), 2.10 – 1.82 (m, 8H), 0.94 (t, *J* = 7.4 Hz, 3H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ (ppm): 182.91, 173.76, 171.08, 170.09, 169.61, 159.07, 157.34, 154.36, 151.84, 149.35, 144.13, 142.84, 142.73, 142.32, 139.50, 139.09, 138.83, 136.51, 135.52, 135.06, 134.93, 133.68, 131.94 ($2CH_{ar}$), 131.45, 130.67, 130.32 ($2CH_{ar}$), 130.12 ($2CH_{ar}$), 129.19, 128.79 ($2CH_{ar}$), 128.56 ($2CH_{ar}$), 127.83, 127.38, 127.28, 126.85, 126.29, 123.12, 119.25 ($2CH_{ar}$), 108.14, 67.12, 64.52, 62.22, 62.05, 56.80, 52.90, 45.12, 38.65, 38.35, 37.09, 36.07, 33.26, 30.58, 29.73, 29.29, 27.38, 25.14, 24.97, 21.59, 15.80, 14.20.

ESI-MS: m/z 1204.7 $[M+Na]^+$.

ESI-HRMS : (*m*/*z*) calcd for $C_{65}H_{71}N_3O_{12}S_3Na$: 1204.4098; found : 1204.4010.

NP Characterization: Dynamic light scattering and ζ-potential measurements were carried out by a 90 plus particle size analyzer (Brookhaven Instrument Corporation) equipped with a solid state He−Ne laser (wavelength = 661 nm). Experiments were carried out at 298 K and the scattering angle was setted at 90°. Each sample was sonicated allowed to equilibrate for 3 min before the experiment. Ten independent measurements of 60 s duration were performed for each sample. The hydrodynamic diameters were calculated using Mie theory, considering absolute viscosity and refractive index values of the medium to be 0.890 cP and 1.33, respectively. The ζ-potential was calculated from electrophoretic mobility of nanoparticles, by using the Smoluchowski theory.

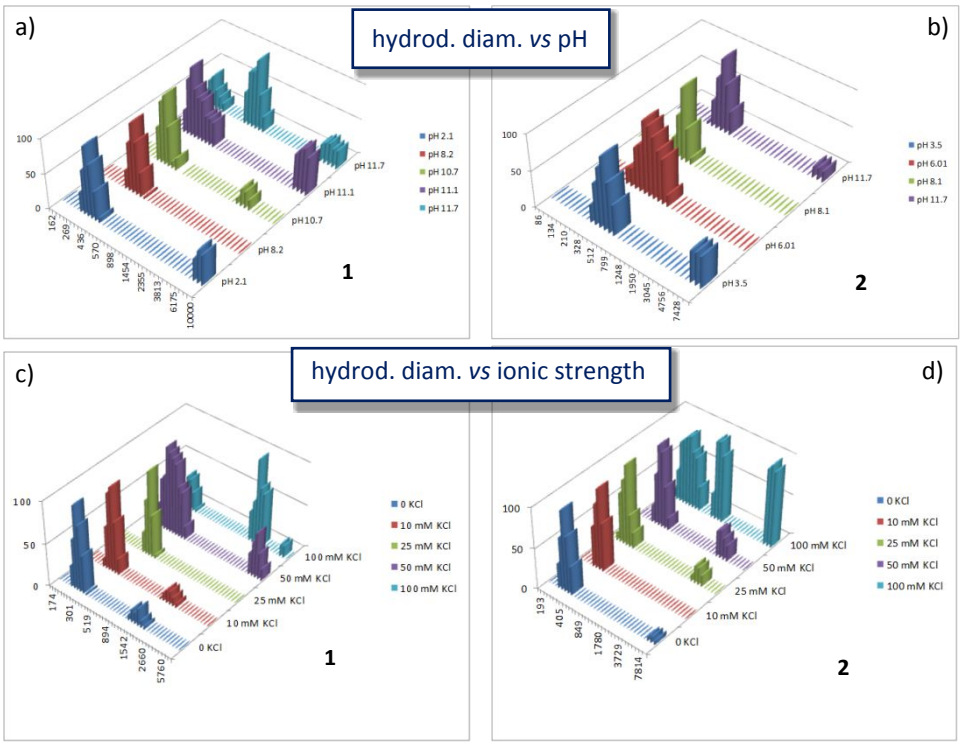


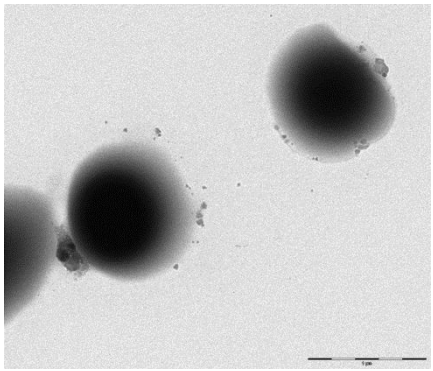
Figure 1-Supp.Info: a) and b) hydrodynamic diameter in function of pH; c) and d) hydrodynamic diameter in function of ionic strength.

Hydrodynamic diameters of Compound **1** and **2** after one week of observation:

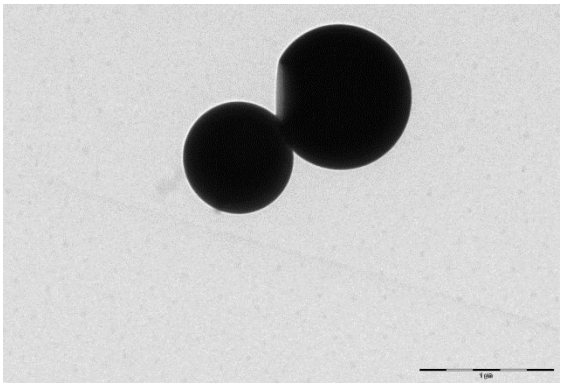
	Hydrodynamic Diameter (nm) Over 1 week of observation	Polydispersity Index
1	417.0 ±8.2	0.190±0.072
2	388.3 ±8.5	0.153±0.045

AFM investigations were performed using a NT-MDT NTEGRA apparatus in tapping mode under ambient conditions. Fluorescence microscopy images were taken with a Nikon Eclipse TE2000-U inverted microscope.

TEM investigations on samples were performed using at an EFTEM Leo 912 ab (Zeiss, Germany) operating at 100 KV and digital images were acquired by a CCD camera 1kx1k (Proscan, Germany) and iTEM software (Olympus, Germany).



Compound **1**



Compound **2**

Lipase-incubation: general procedure.

The selected lipase (5 mg/ml) and n-BuOH (10 μL/ml) were added to solution of drug-drug conjugate (2 mg/ml) prepared in a 15 : 85 mixture of DMSO and phosphate buffer (20 mM, pH 7) which was subsequently incubated in a thermoshaker (45 °C, 180 rpm) overnight. After that, the reacting media was extracted with AcOEt. The combined organic layers were dried over Na_2SO_4, evaporated in vacuo and dissolved again in a 2:1 mixture of CH_3CN and MeOH to be analyzed by means of reversed-phase HPLC.

Compound 1: The incubation of **1** (4 mg, 0,0034 mmol) with porcine pancreas lipase type II (PPL, 10 mg) and n-BuOH (0.020 mL, 0.22 mmol) in the experimental conditions described above, gave the 88% of enzymatic-promoted hydrolysis of **1** calculated on the bases of the total products area.

*Compound **2***: The incubation of 2 (4 mg, 0,0035 mmol) with Celite®-supported lipase PS (10 mg) and n-BuOH (0.020 ml, 0.22 mmol) in the experimental conditions described above, gave the 85% of enzymatic-promoted hydrolysis of **2** calculated on the bases of the total products area.

HPLC analysis: *general information and methods*. All the samples were analysed using a Kinetex 5µ EVO C18 100Å column working with a gradient of CH_3CN (A) and milliQ water + 0.001 % of TFA (B) (0 min: A = 90 %, 30 min: A = 20 %; 35 min: A = 0 %; 60 min: A = 0 % ; 75 min: A = 90 %) at a flow rate of 0.5 mL min-1 and collecting the chromatograms at 256 nm.

compound	Retention time (min.)
1	43.4
2	43.2
11	20.4
13a	39.9
13b	39.4
14	4.5
15	27.1

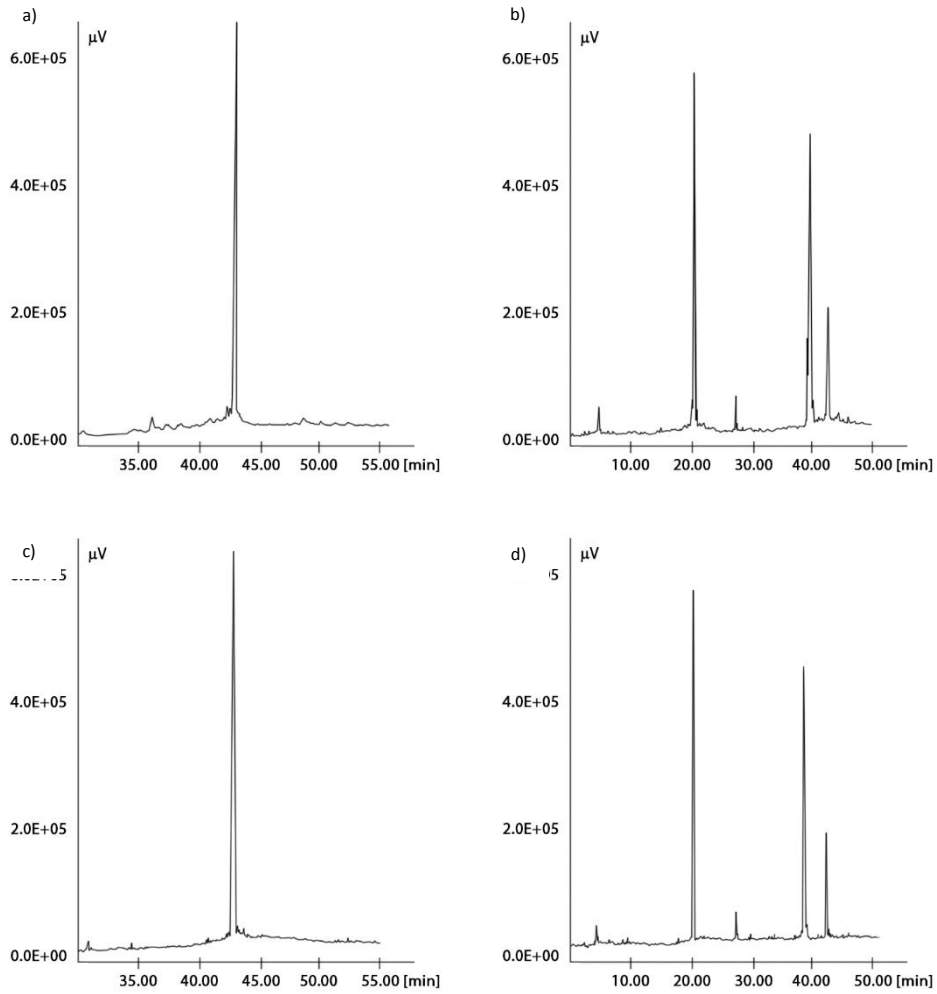


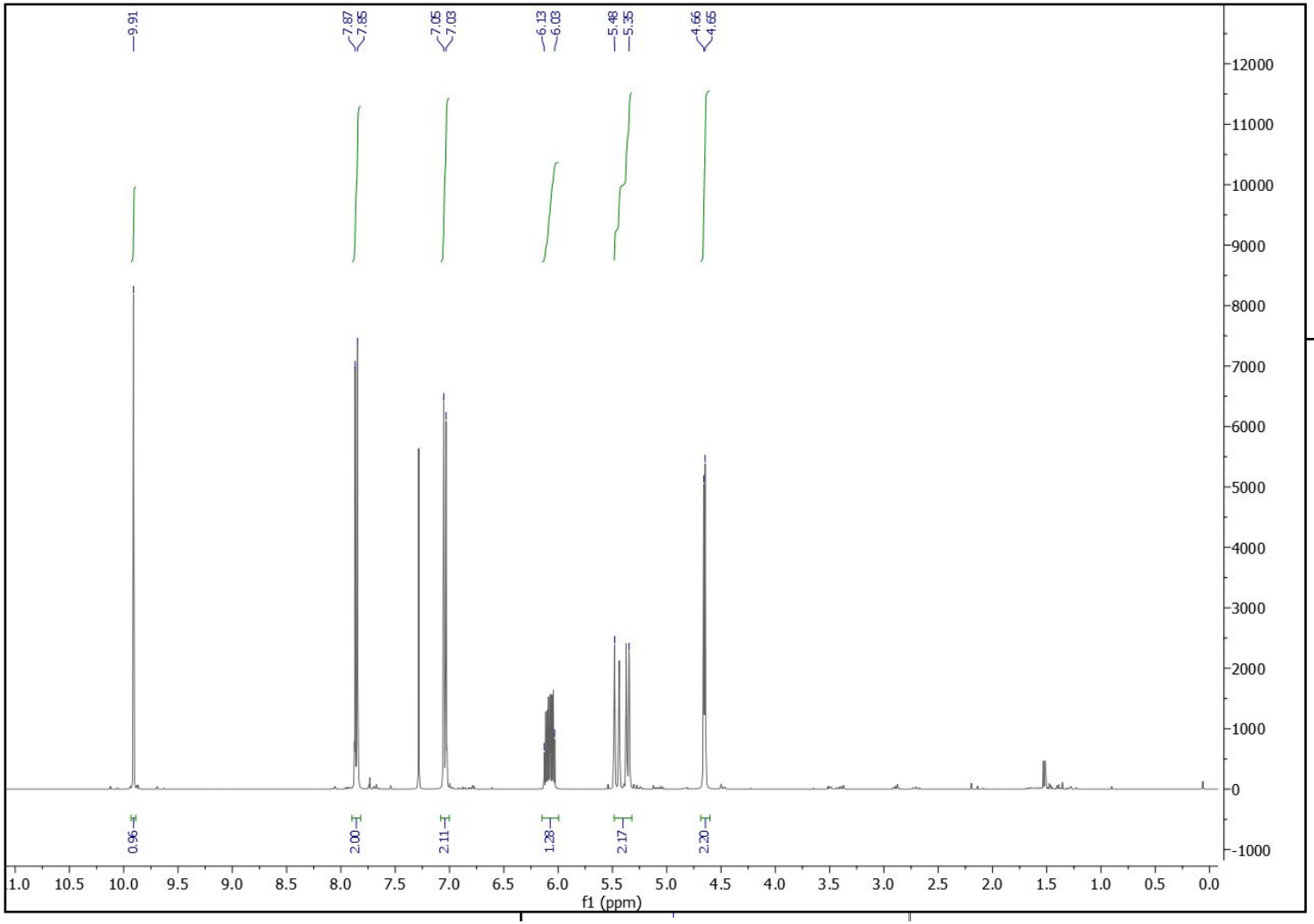
Figure 2-Supp.Info: HPLC chromatograms of a) compound **1** at t=0; b) compound **1** after overnight incubation with lipase; c) compound **2** at t=0; d) compound **2** after overnight incubation with lipase.

ESI-MS of the enzymatic reaction mixture of **1** after overnight incubation with lipase:

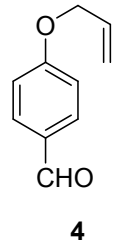
Compound	**ESI-MS (*m/z*) calcd**	**found**
1 ($C_{67}H_{75}N_3O_{12}SNa$)	1168.4969	1168.4978
11 ($C_{22}H_{26}N_2NaO_5S$)	453.1460	453.1465
13a ($C_{32}H_{37}NNaO_3$)	506.2671	506.2678
14 ($C_{20}H_{23}NNaO_4S$)	396.1245	396.1249
15 ($C_{22}H_{21}NNa$)	322.1572	322.1575

ESI-MS of the enzymatic reaction mixture of **2** after overnight incubation with lipase:

Compound	**ESI-HRMS (*m/z*) calcd**	**found**
2 ($C_{65}H_{71}N_3O_{12}S_3Na$)	1204.4098	1204.4101
11 ($C_{22}H_{26}N_2NaO_5S$)	453.1460	453.1468
13b ($C_{30}H_{33}NNaO_3S_2$)	542.1799	542.1804
14 ($C_{20}H_{23}NNaO_4S$)	396.1245	396.1241
15 ($C_{22}H_{21}NNa$)	322.1572	322.1575



*[1]H-NMR of compound **4** in $CDCl_3$*



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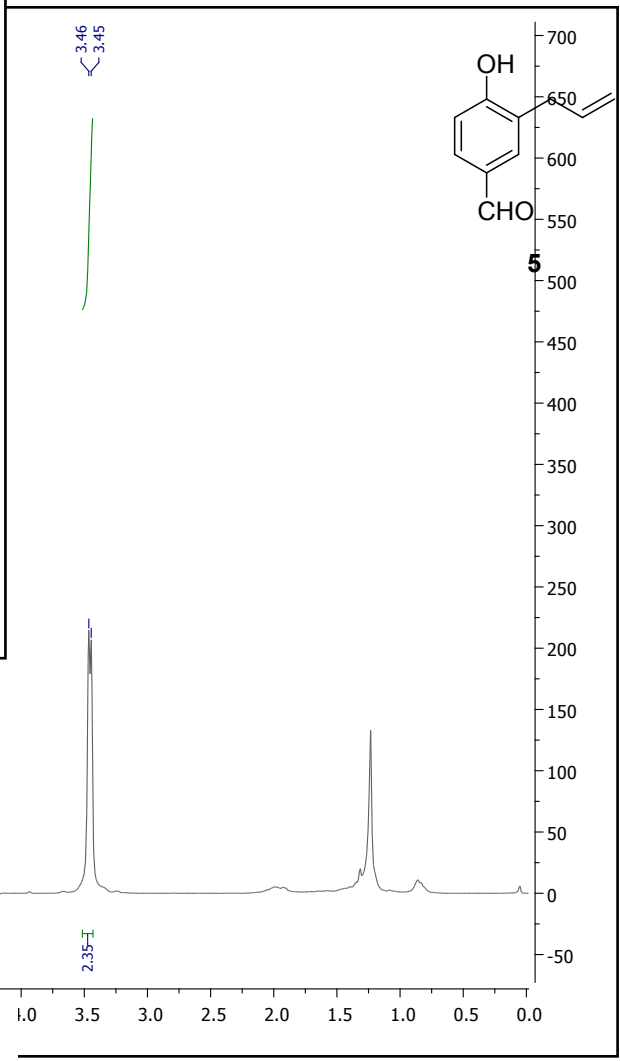
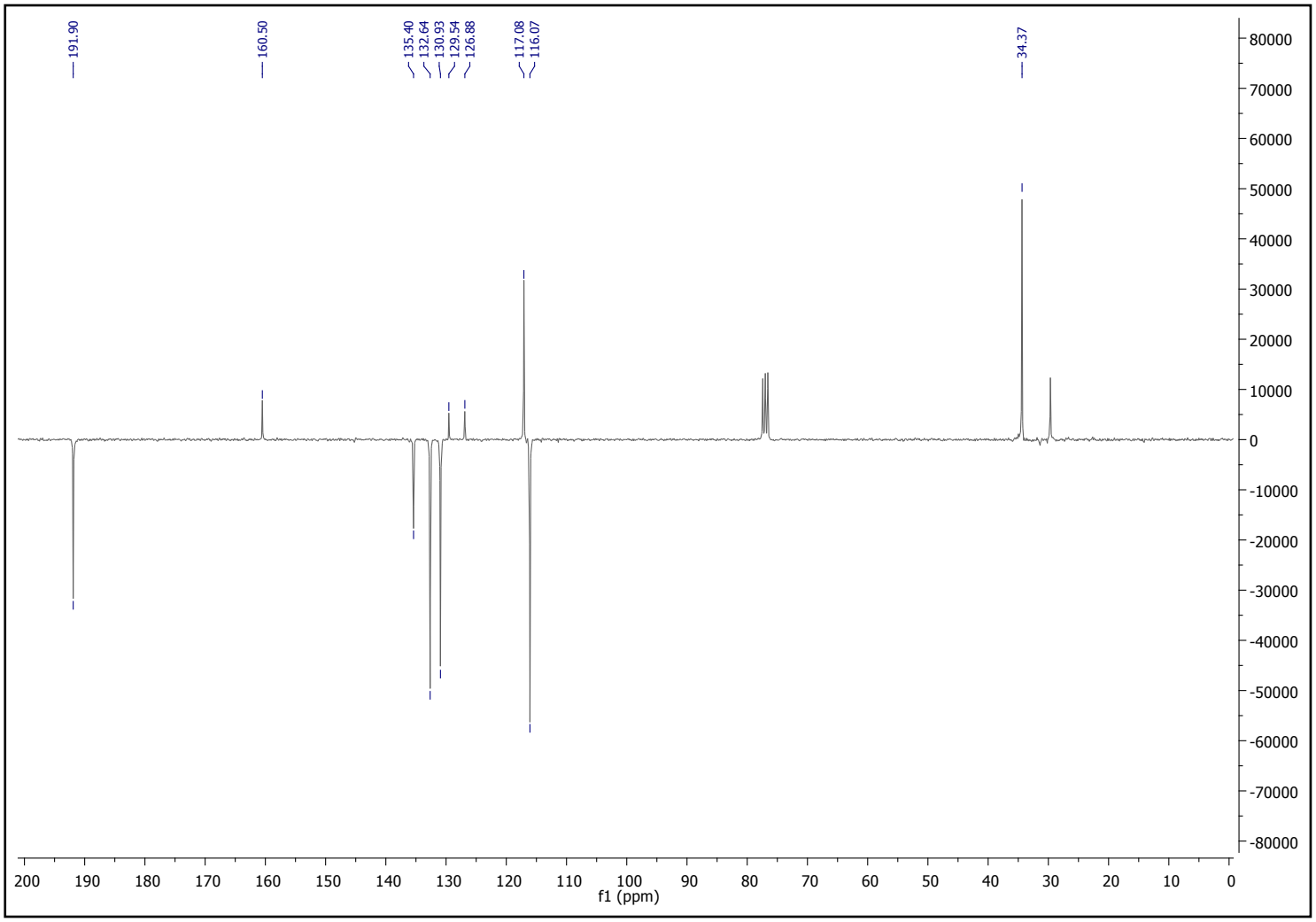
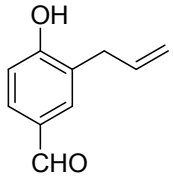


5

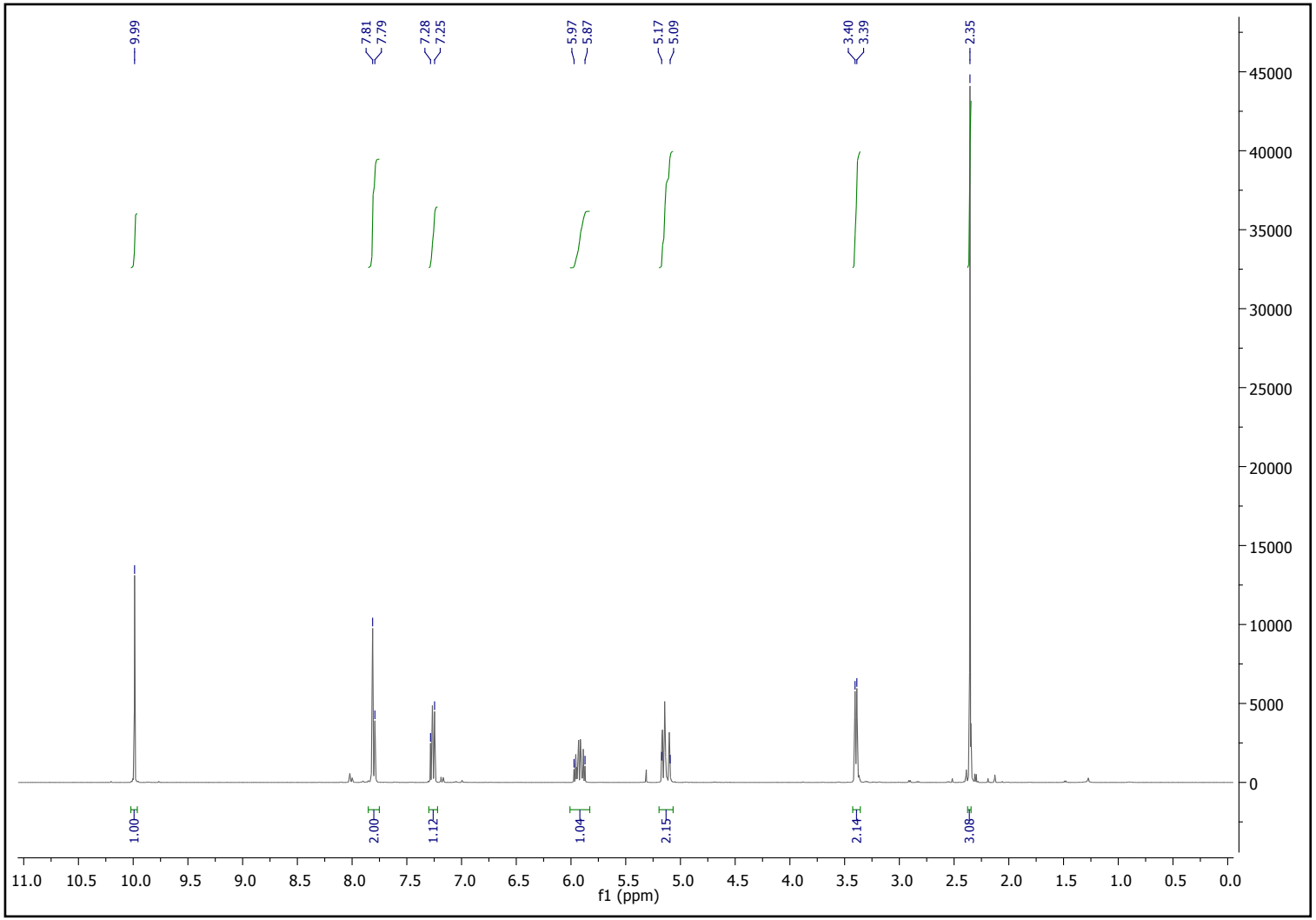
*[1]H-NMR of compound **5** in $CDCl_3$*

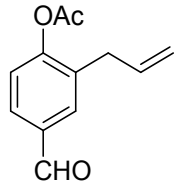




5

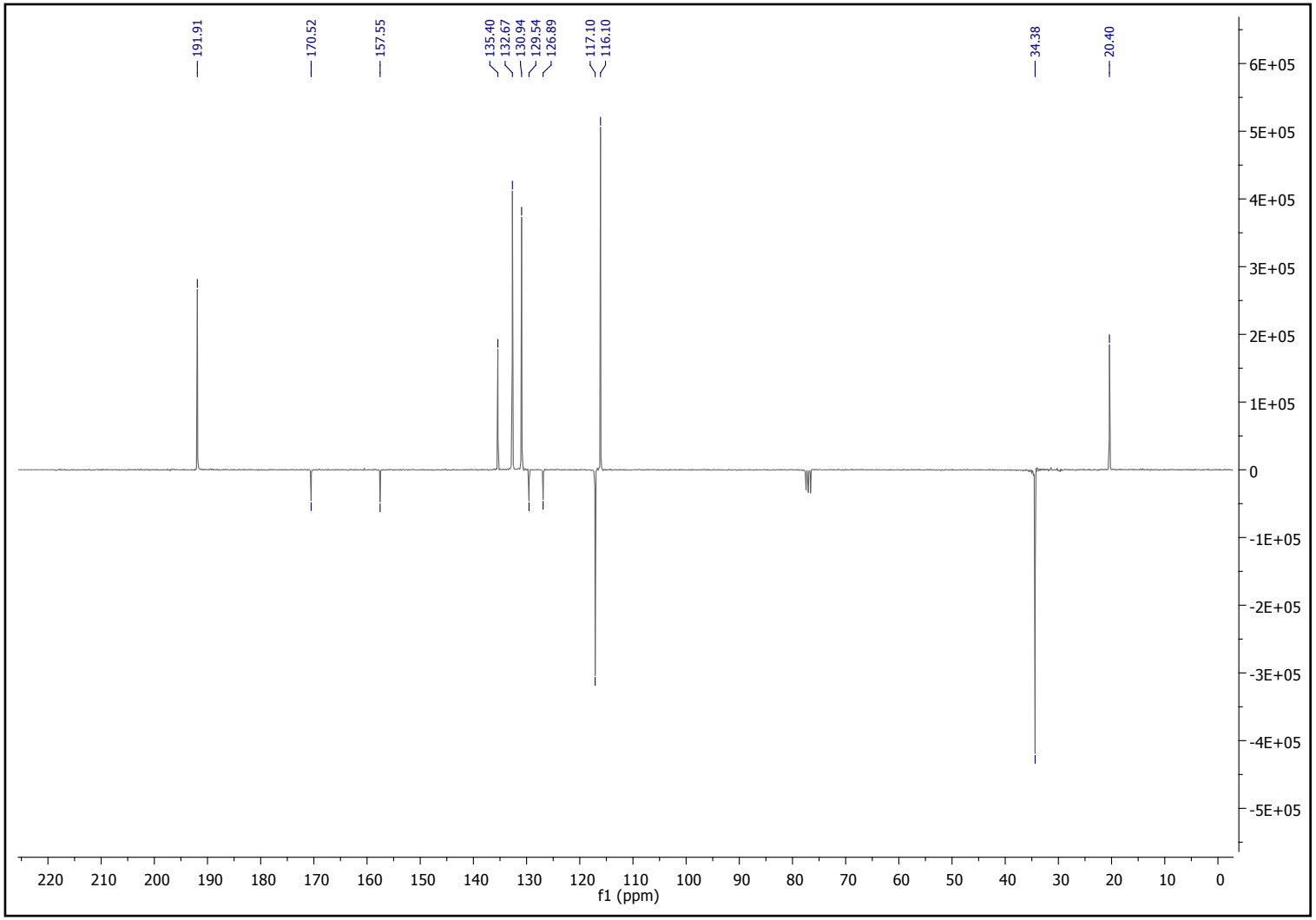
*^{13}C-NMR of compound **5** in $CDCl_3$*

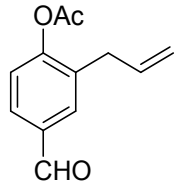




6

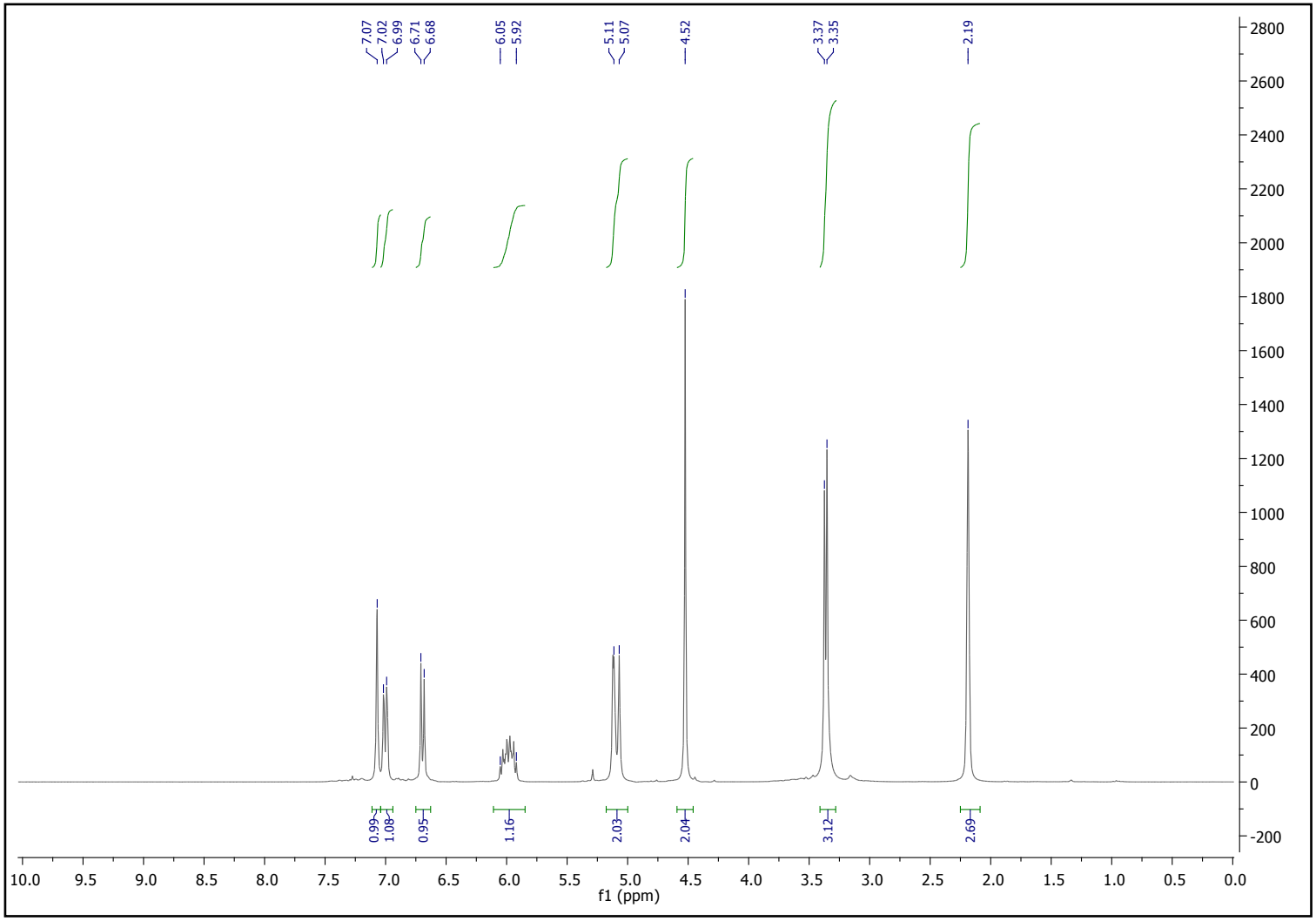
*^{1}H-NMR of compound **6** in $CDCl_3$*

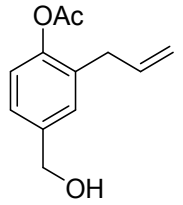




6

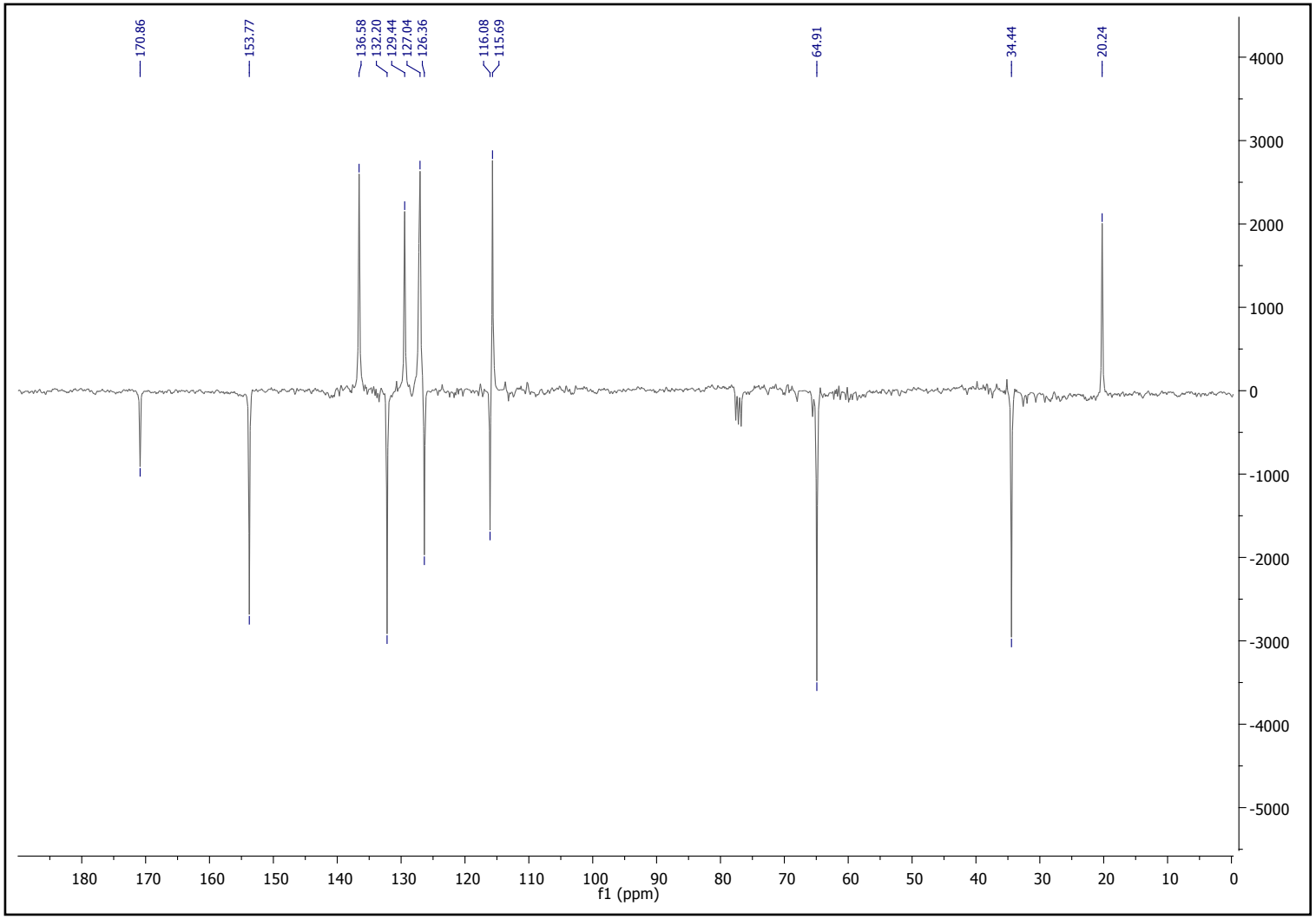
*^{13}C-NMR of compound **6** in $CDCl_3$*

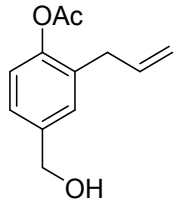




7

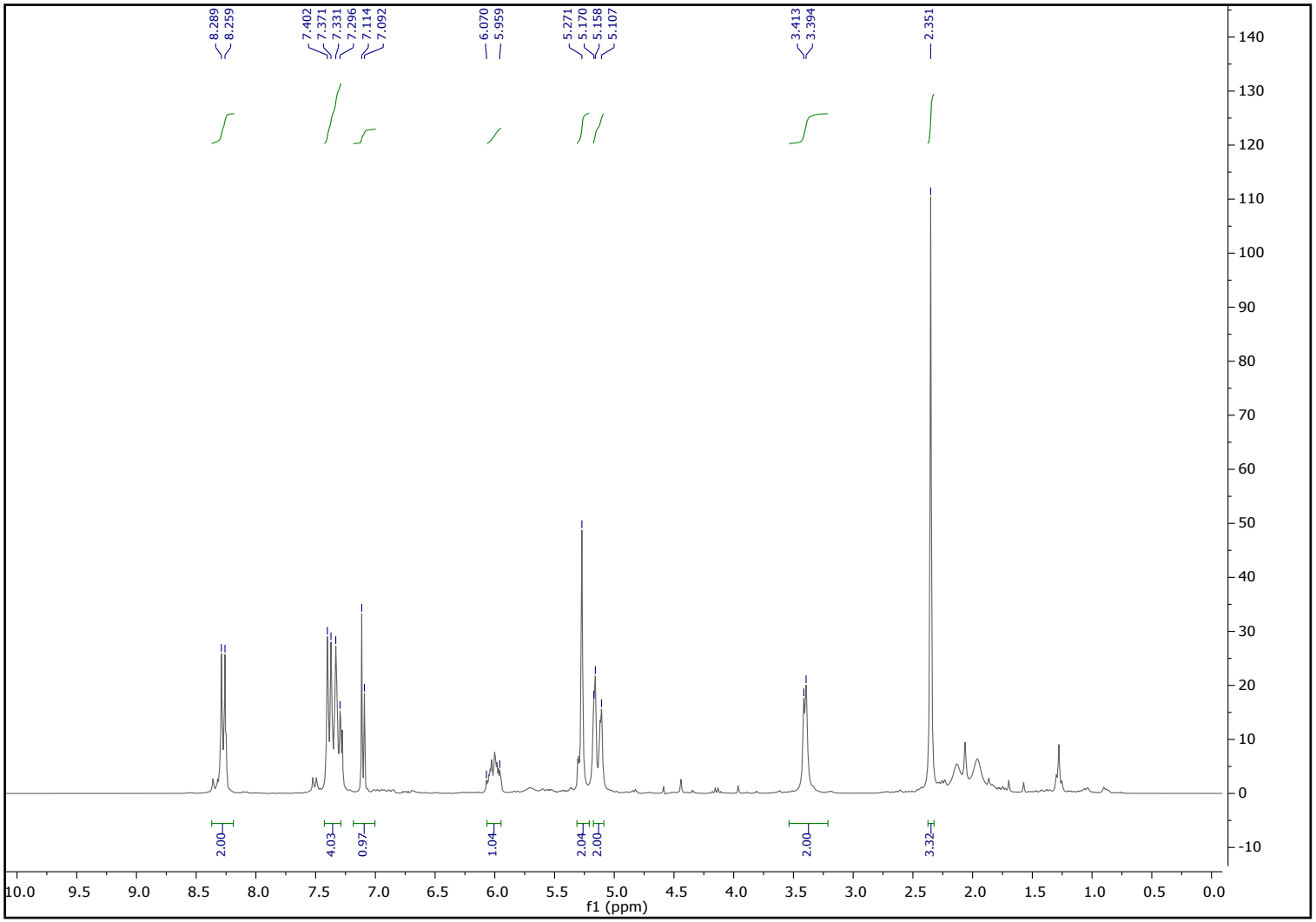
*^{1}H-NMR of compound **7** in $CDCl_3$*

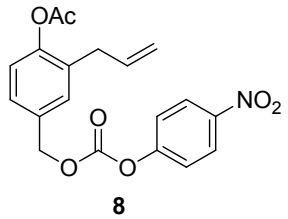




7

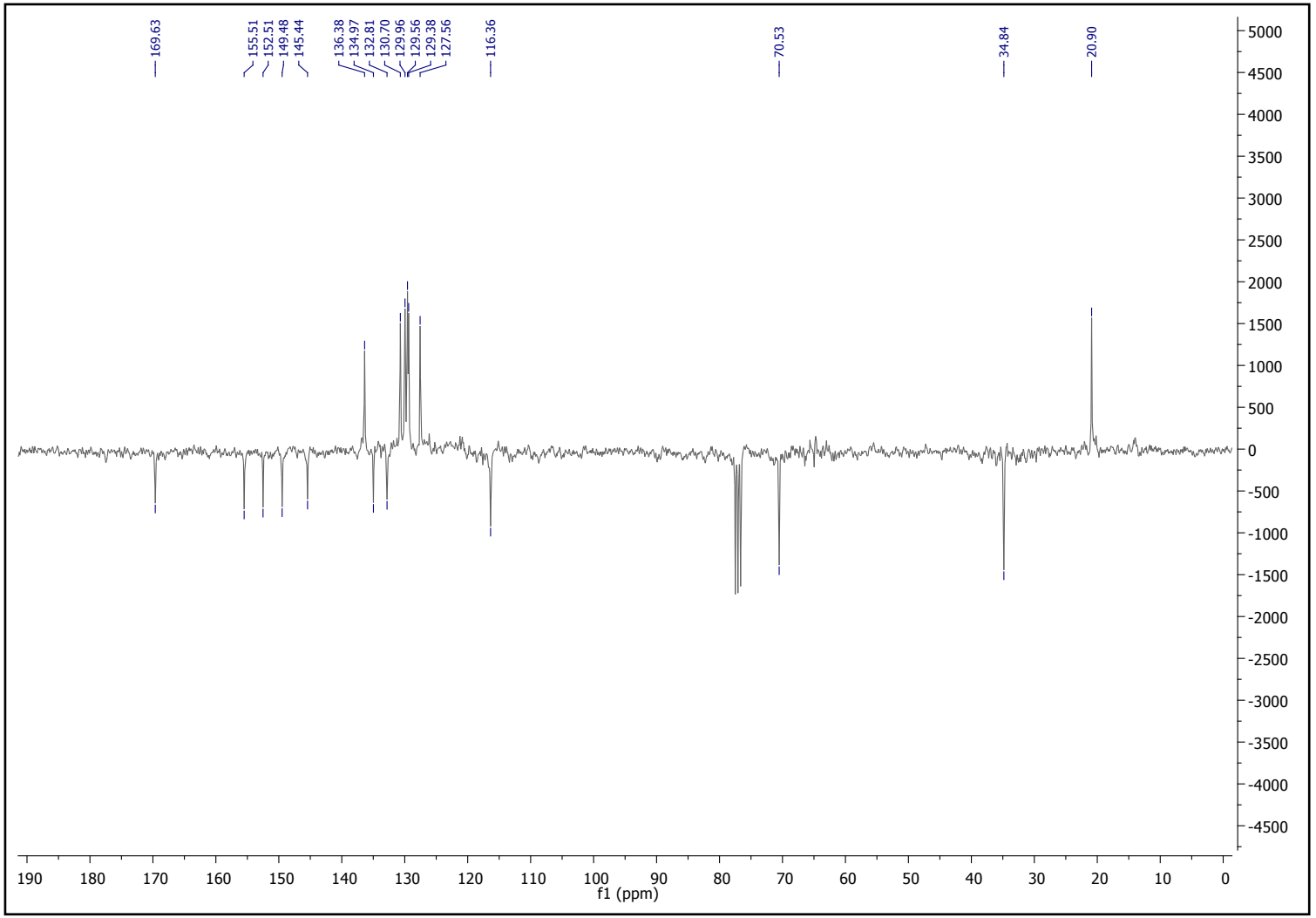
*^{13}C-NMR of compound **7** in $CDCl_3$*

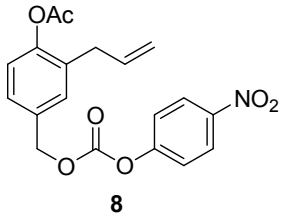




8

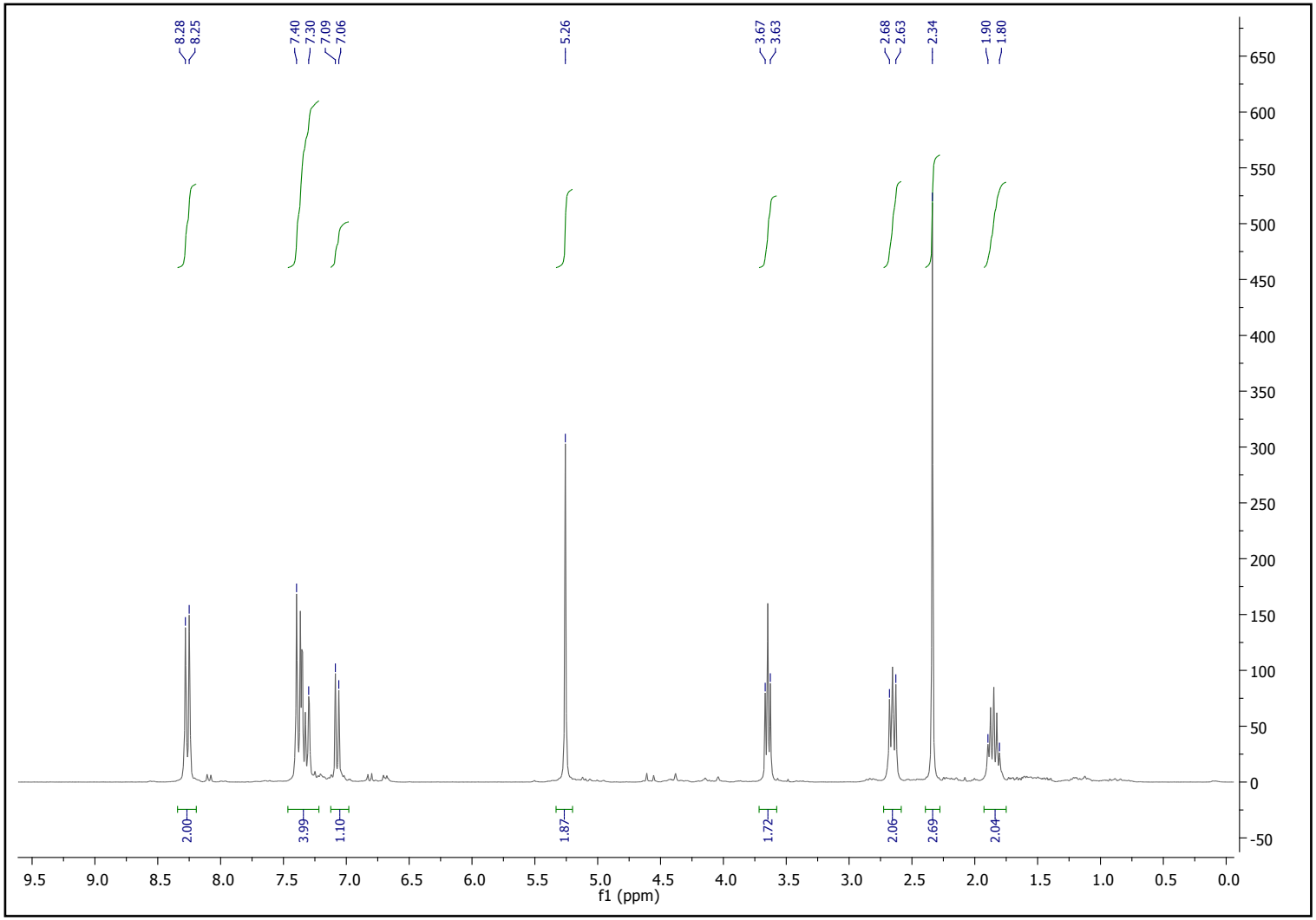
*^{1}H-NMR of compound **8** in $CDCl_3$*

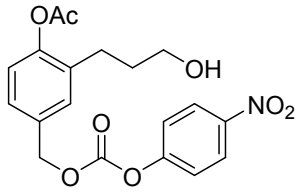




8

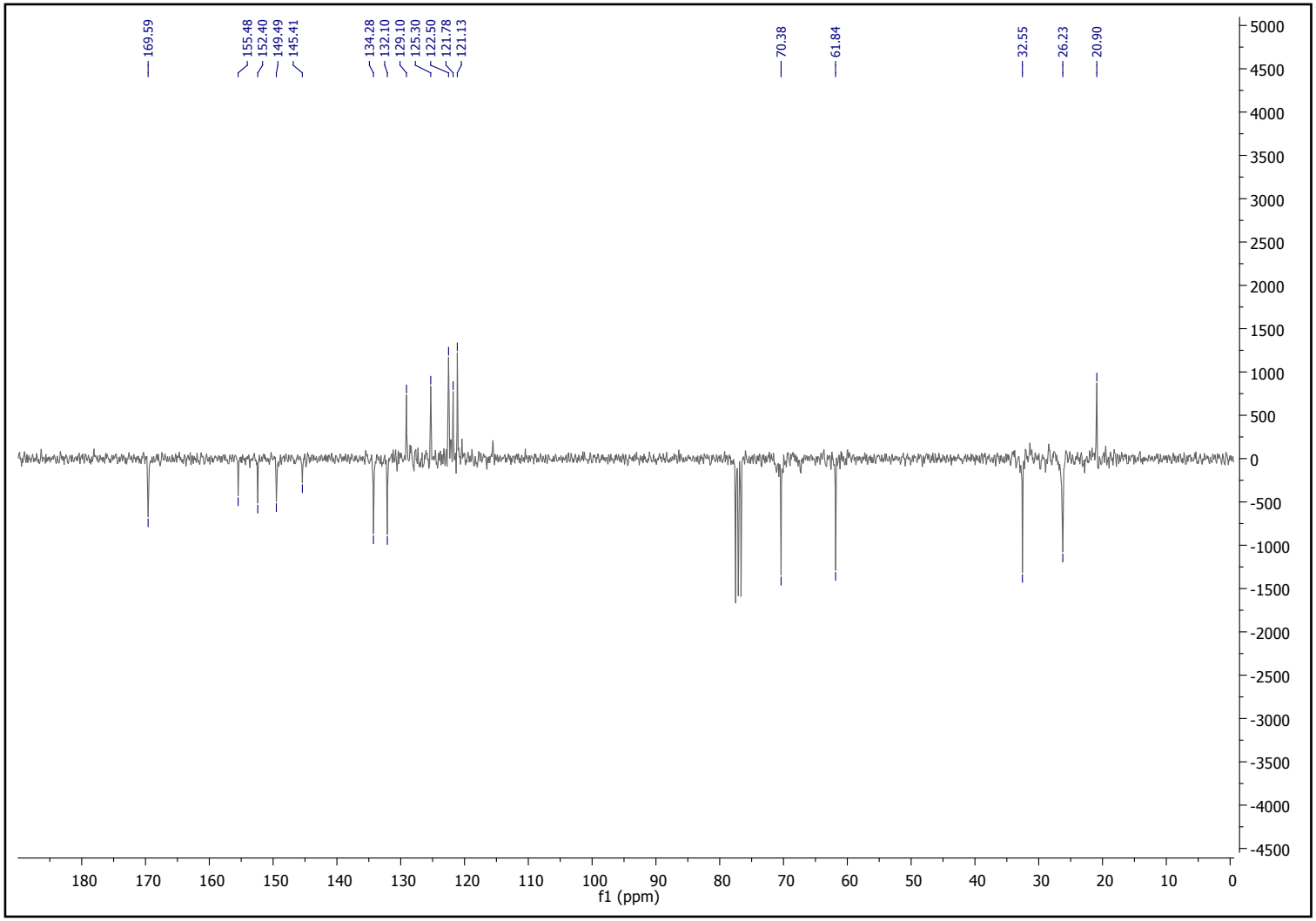
*^{13}C-NMR of compound **8** in $CDCl_3$*

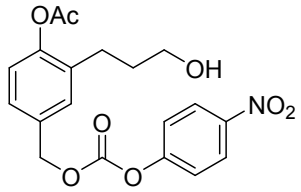




9

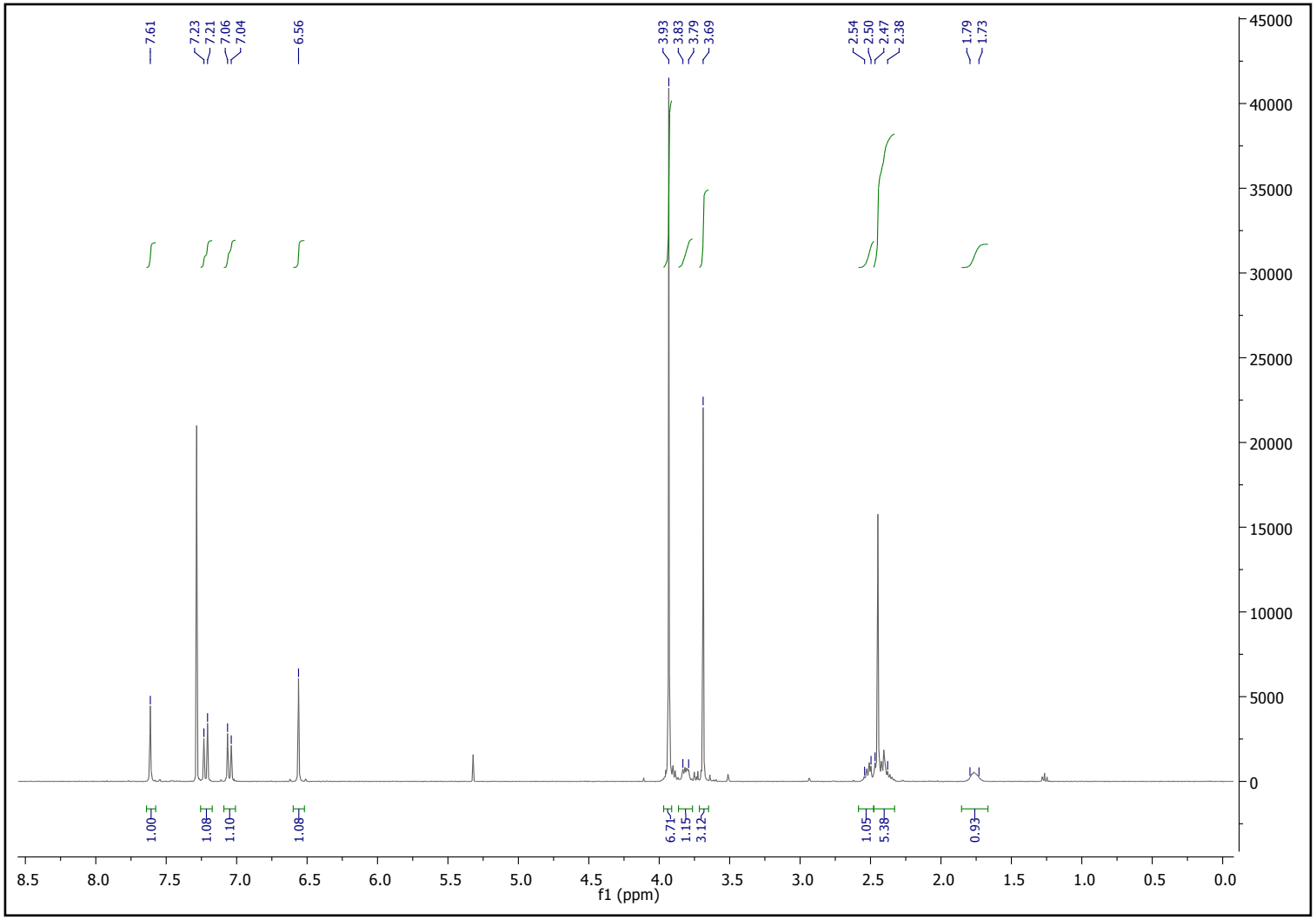
*^{1}H-NMR of compound **9** in $CDCl_3$*

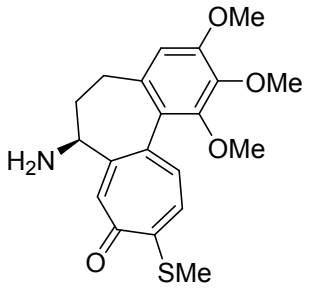




9

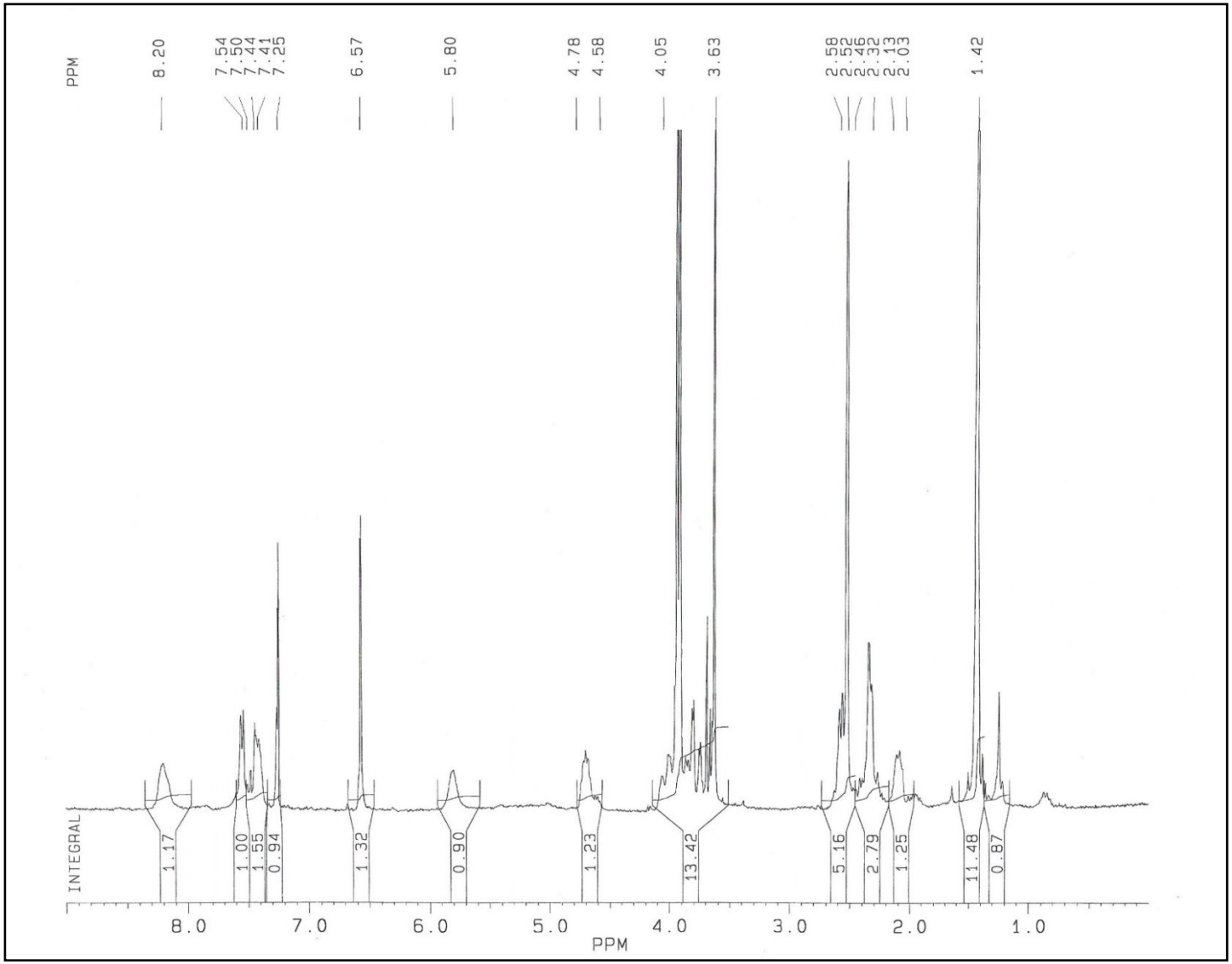
*^{13}C-NMR of compound **9** in $CDCl_3$*

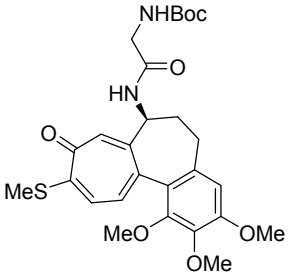




11a

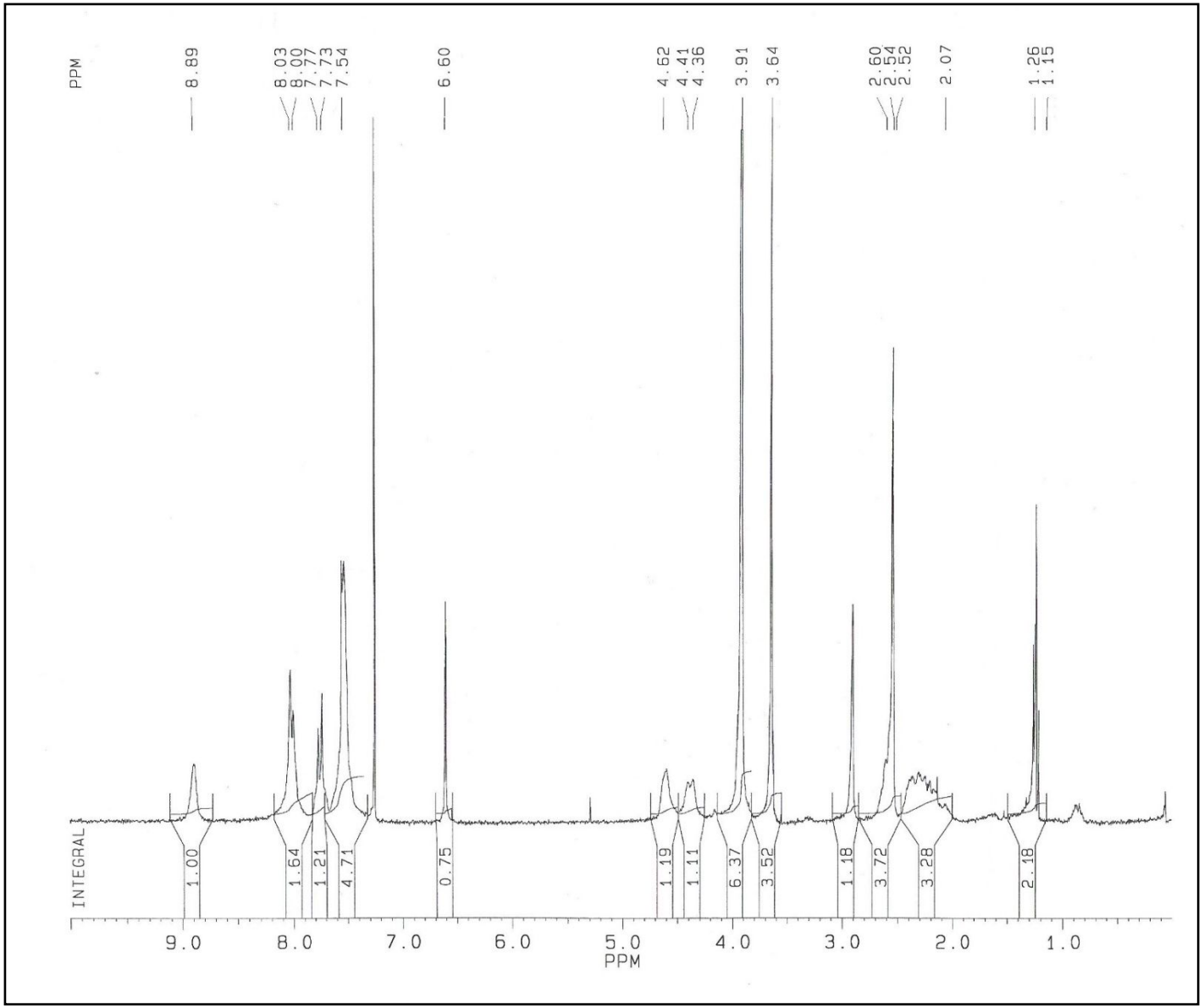
*[1]H-NMR of compound **11a** in $CDCl_3$*

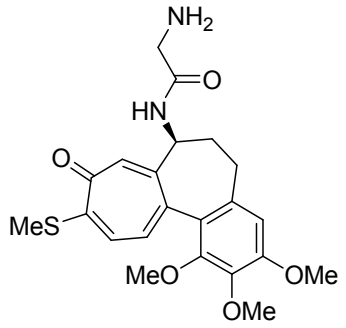




11b

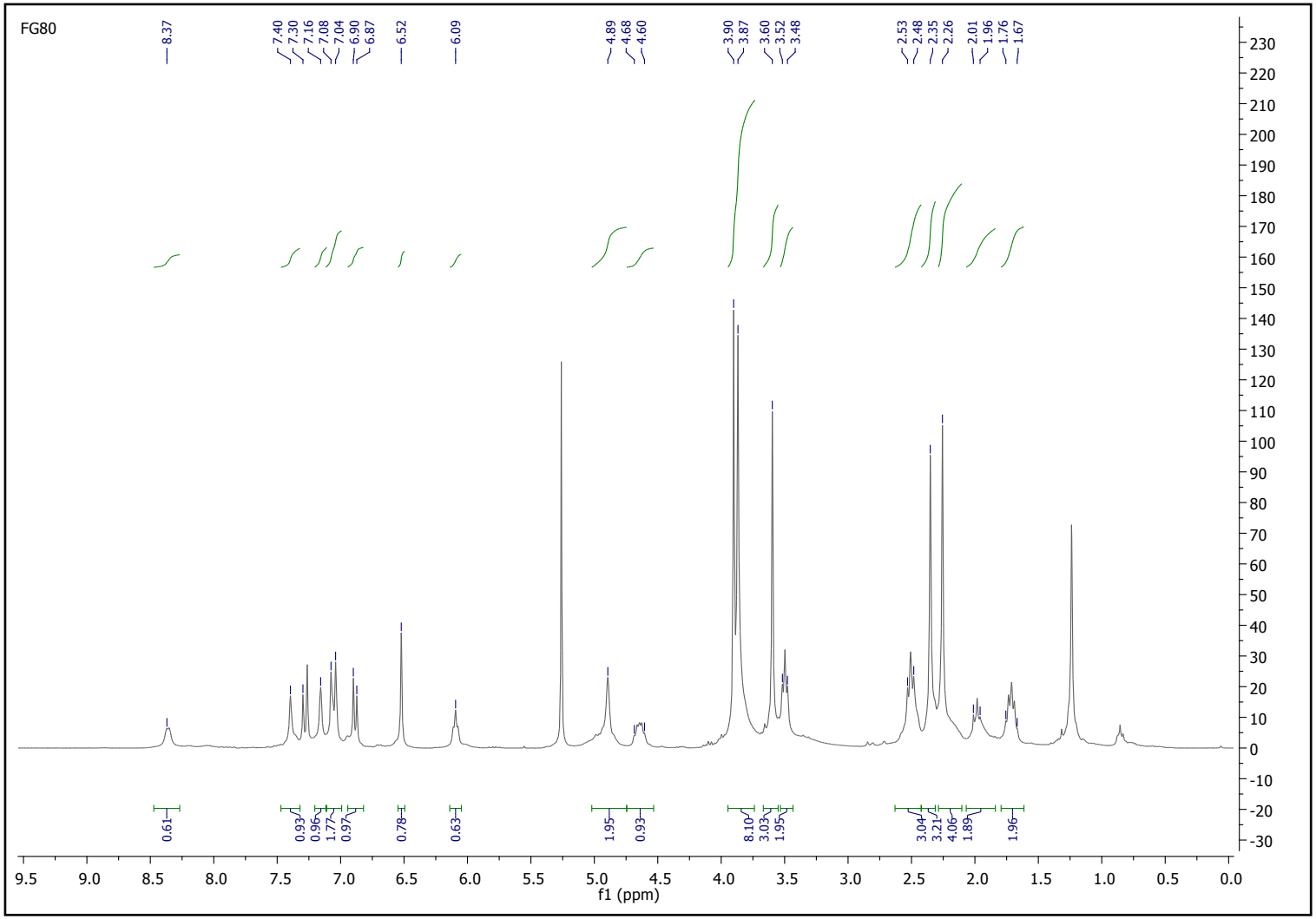
*^{1}H-NMR of compound **11b** in $CDCl_3$*

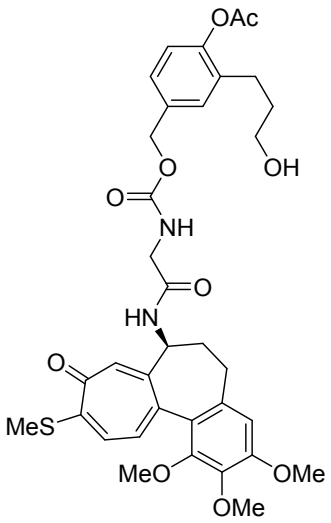




11

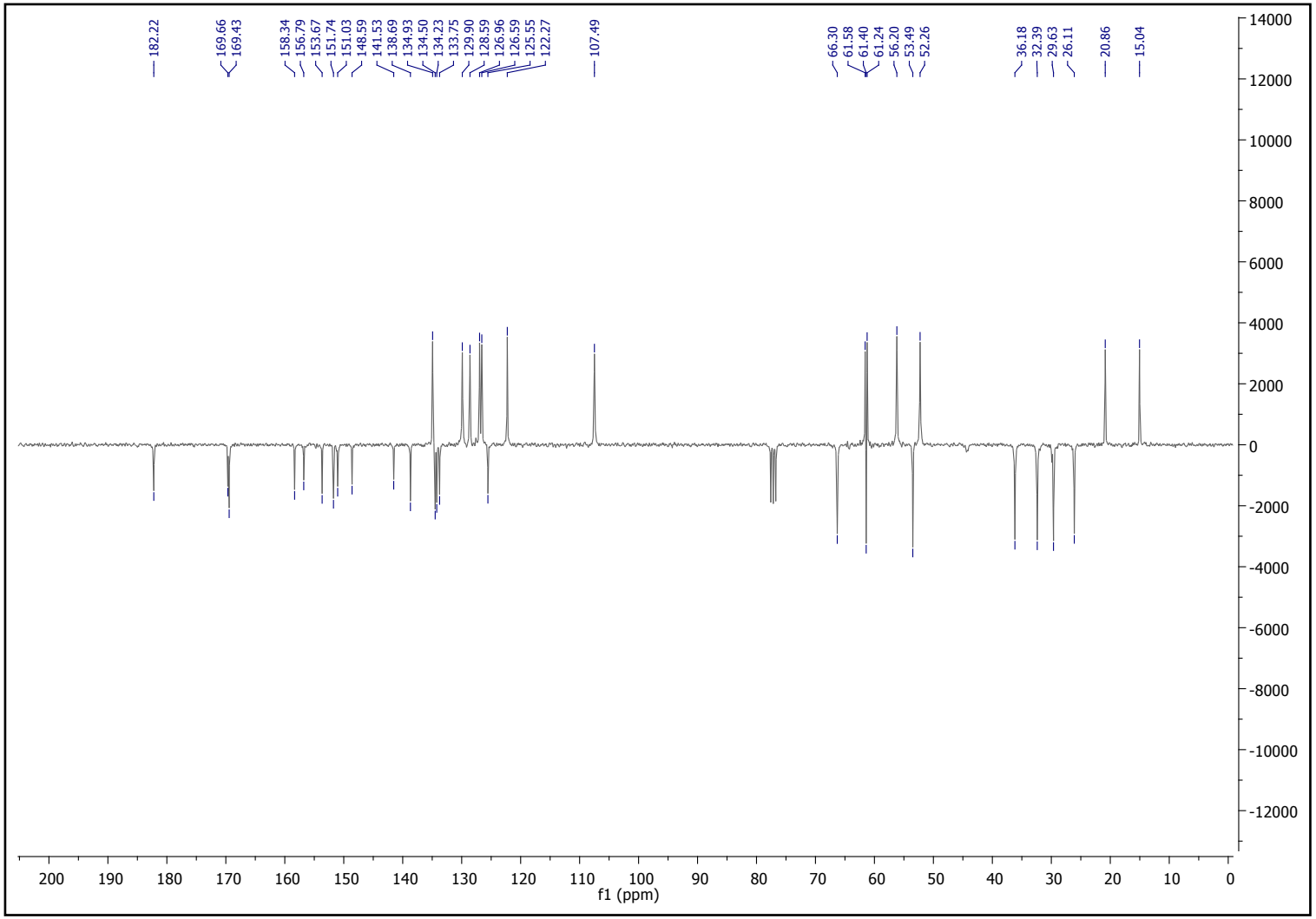
*^{1}H-NMR of compound **11** in $CDCl_3$*

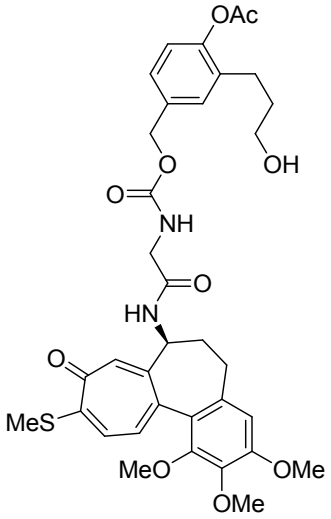




12

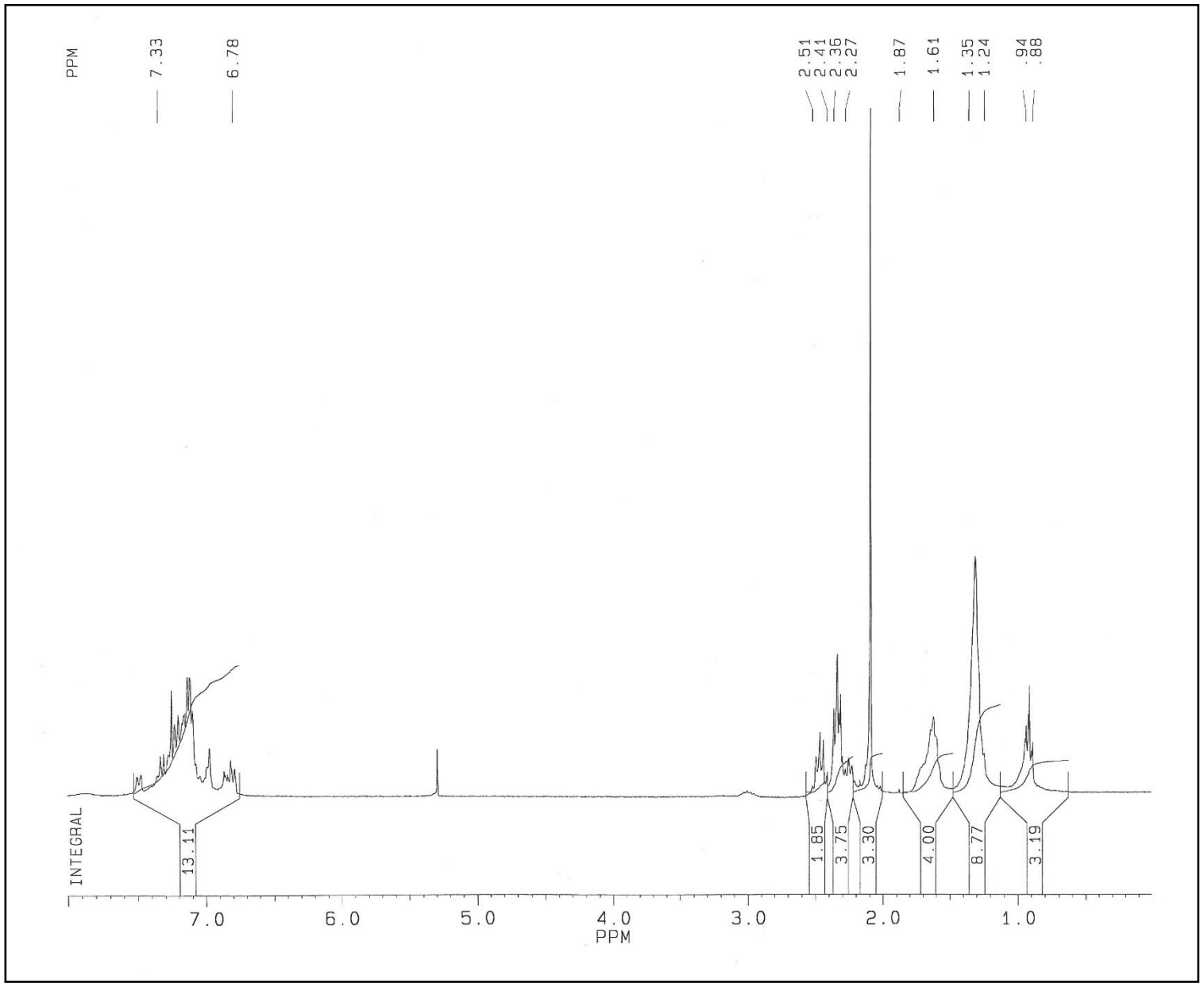
*^{1}H-NMR of compound **12** in $CDCl_3$*

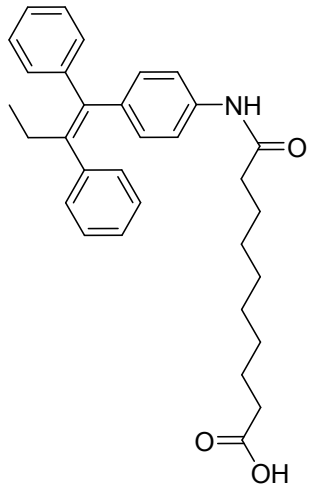




12

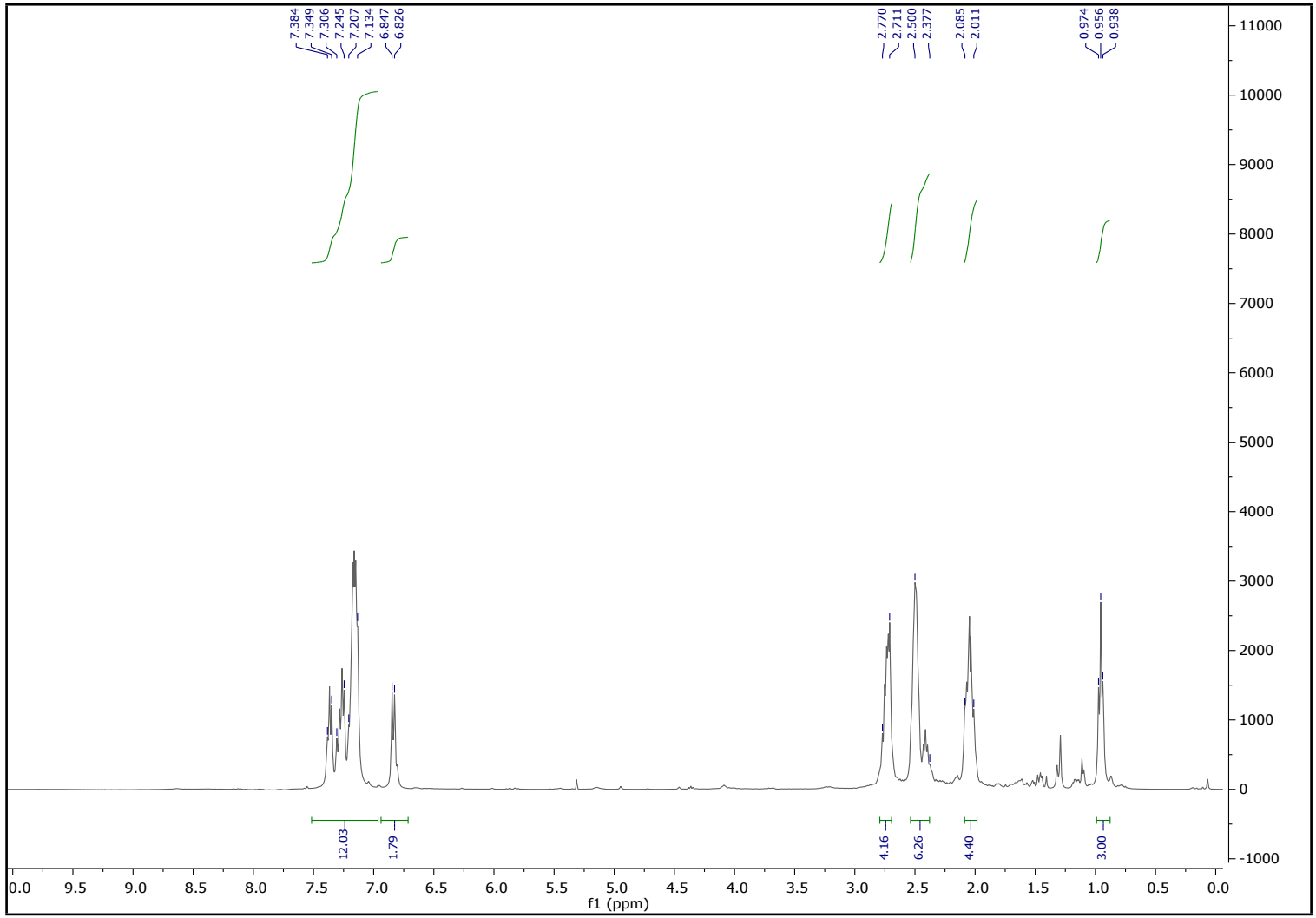
*^{13}C-NMR of compound **12** in $CDCl_3$*

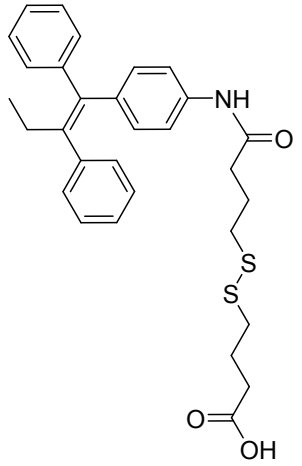




13a

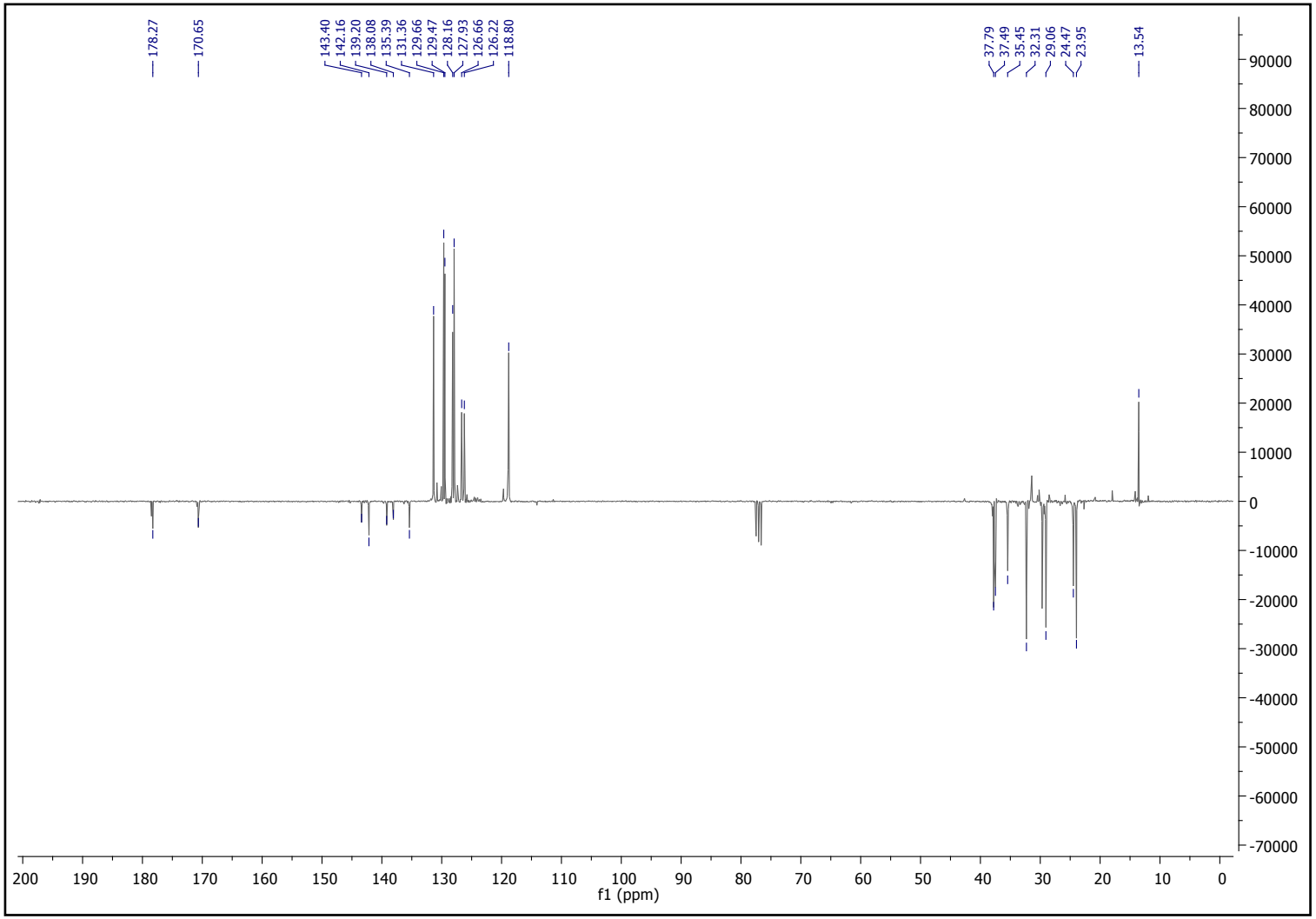
*^{1}H-NMR of compound **13a** in $CDCl_3$*

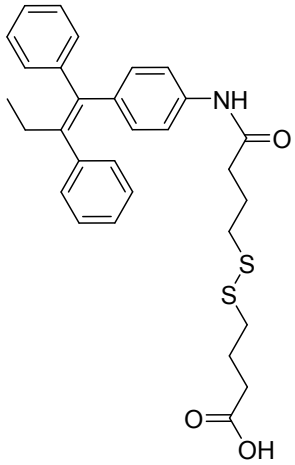




13b

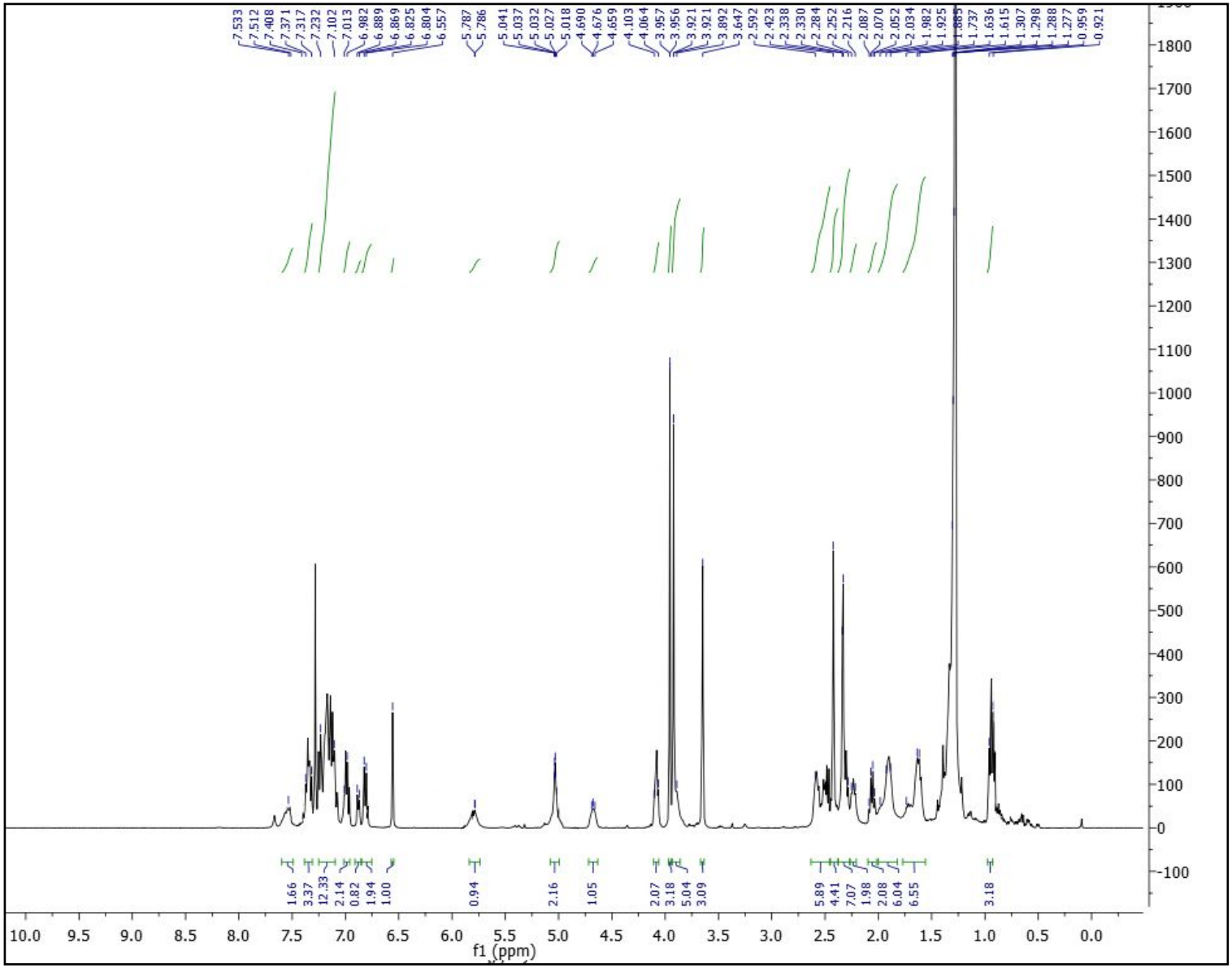
*^{1}H-NMR of compound **13b** in $CDCl_3$*

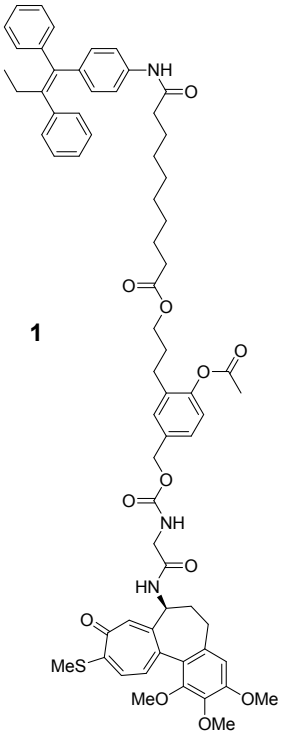




13b

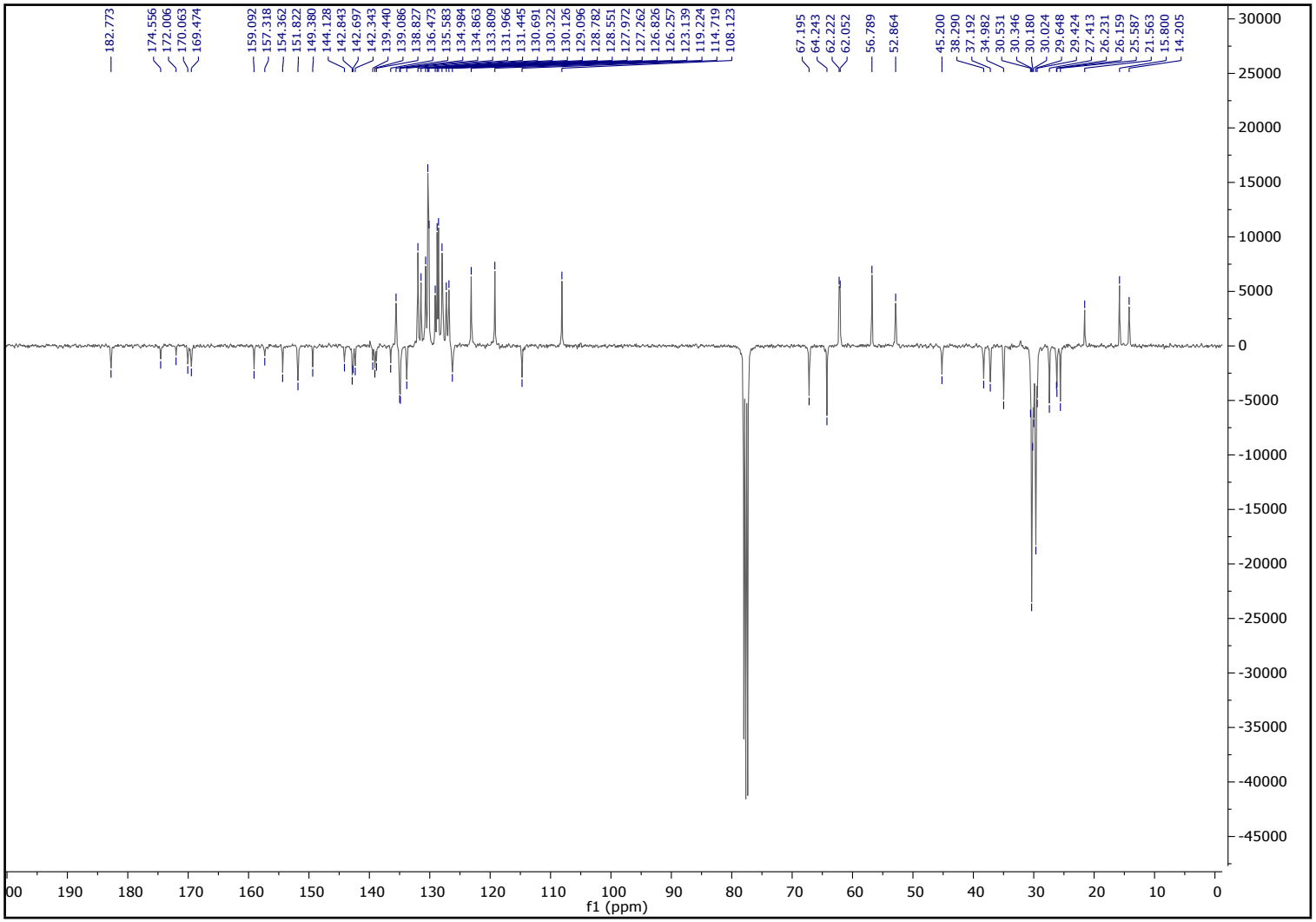
*^{13}C-NMR of **13b** in $CDCl_3$*

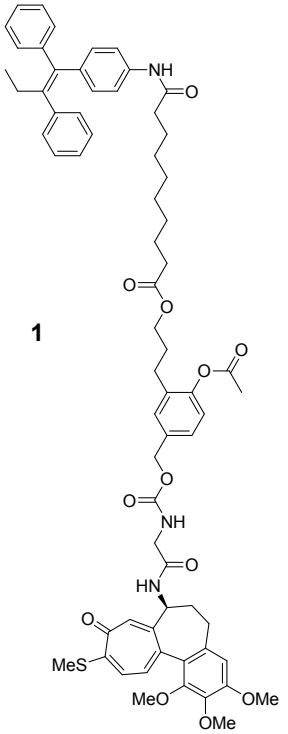




1

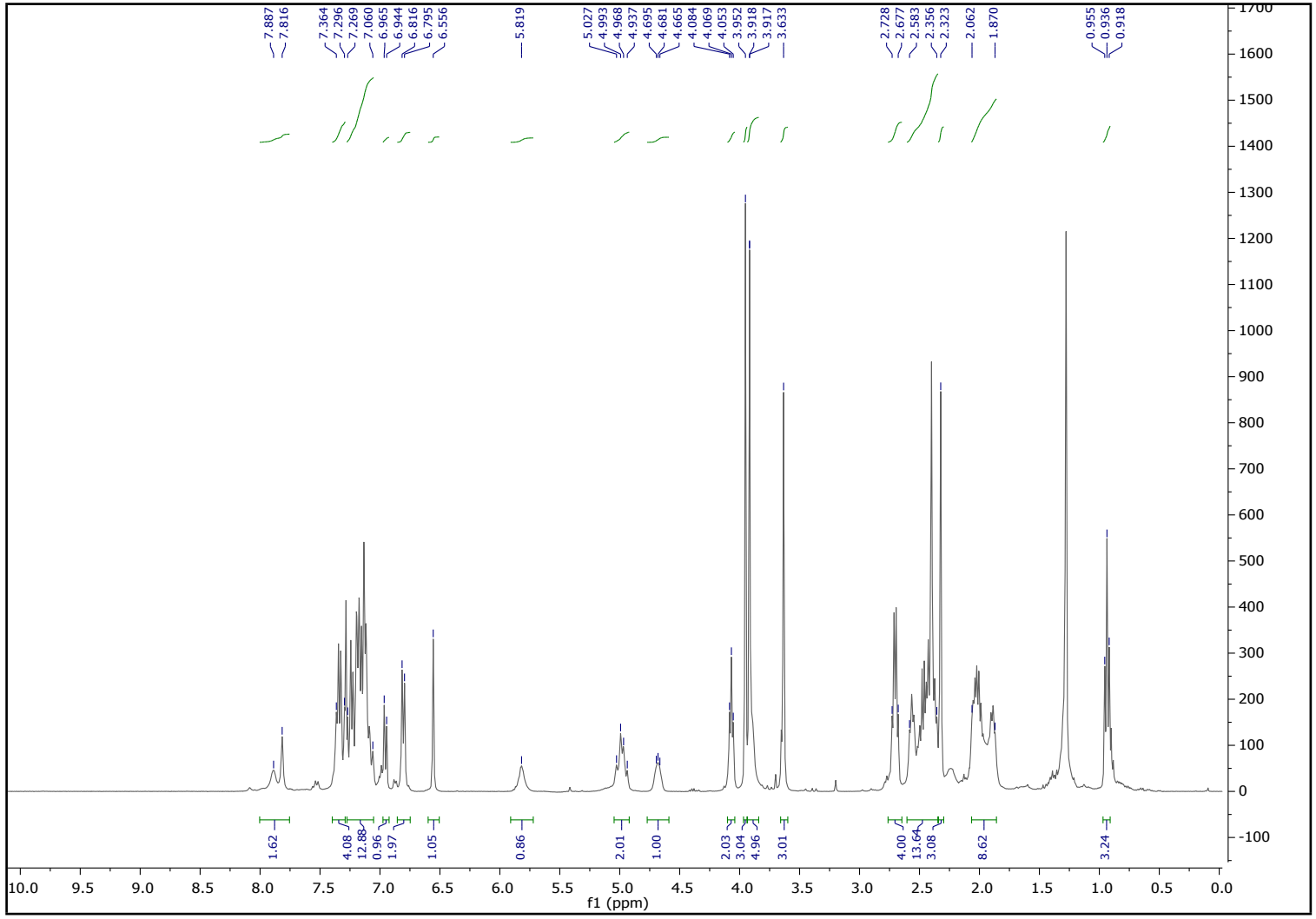
*[1]H-NMR of compound **1** in $CDCl_3$*

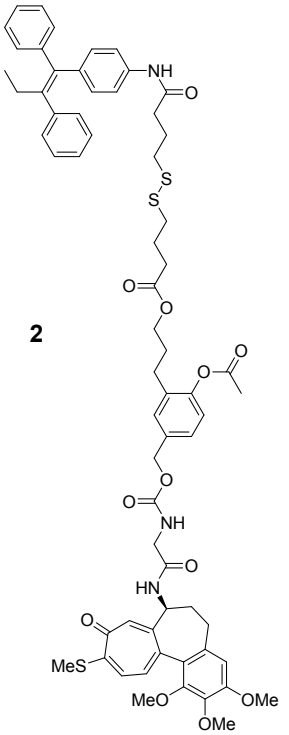




1

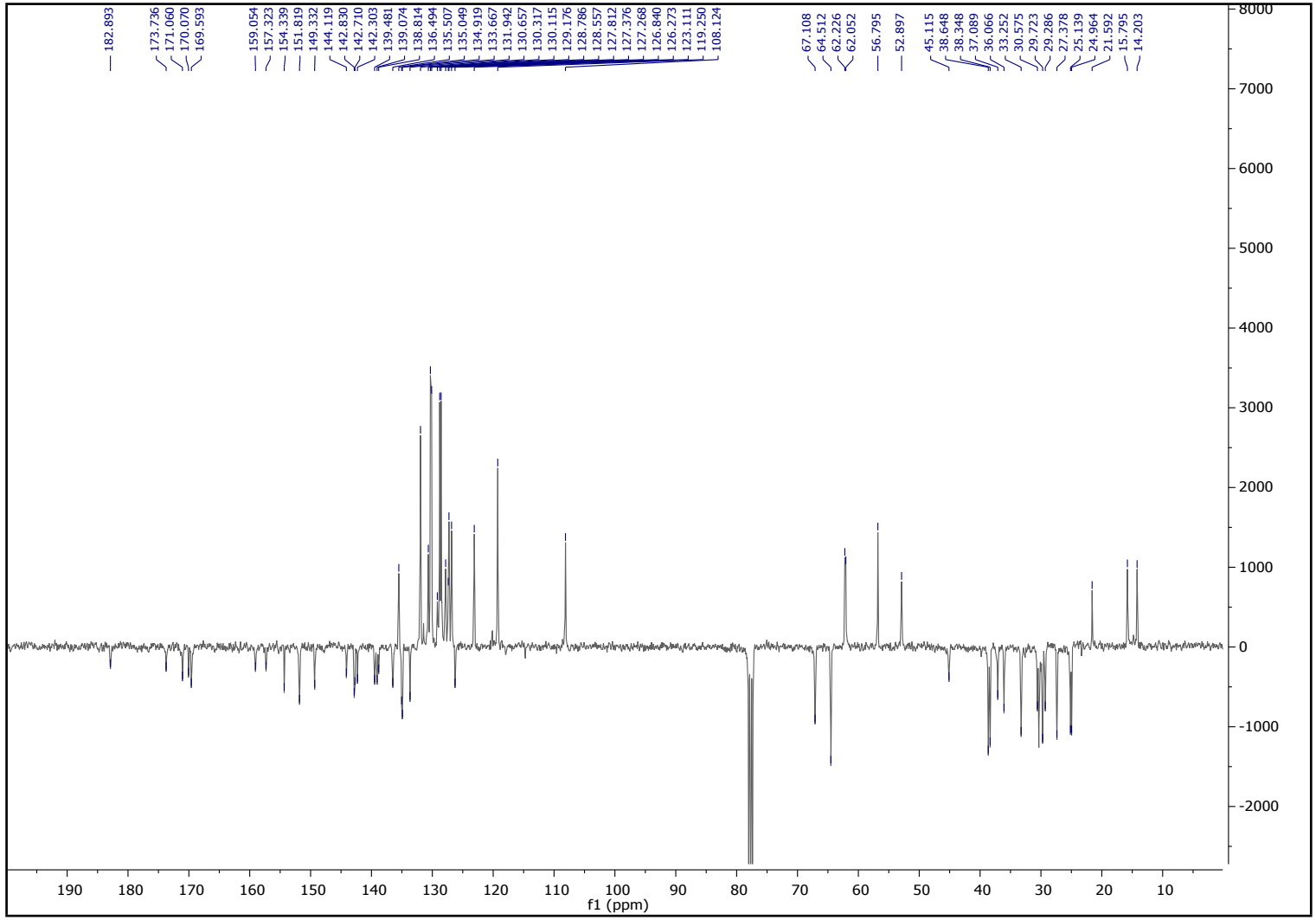
*^{13}C-NMR of compound **1** in $CDCl_3$*

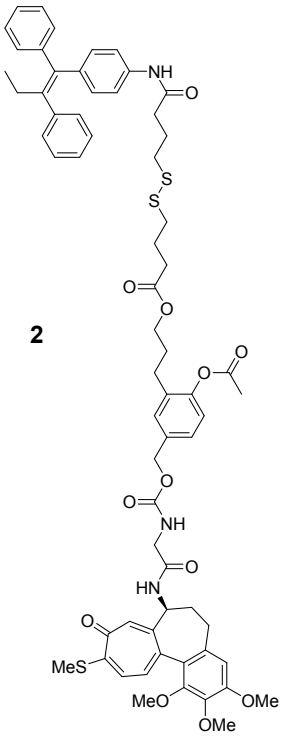




2

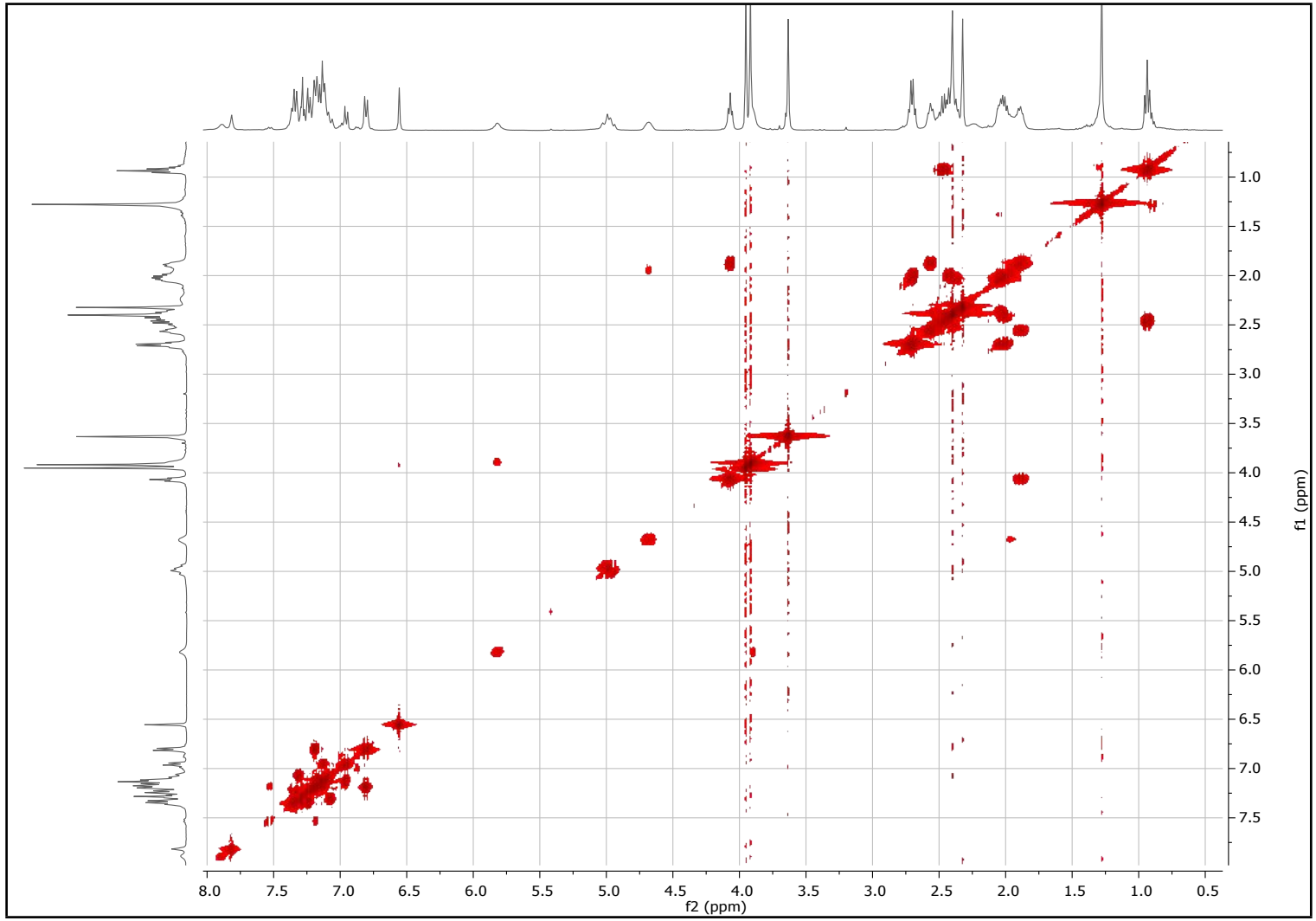
*^{1}H-NMR of compound **2** in $CDCl_3$*

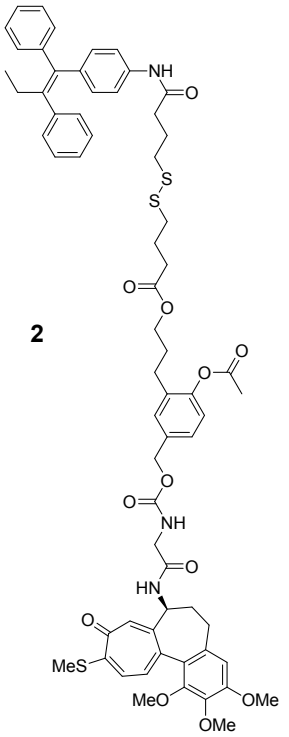




2

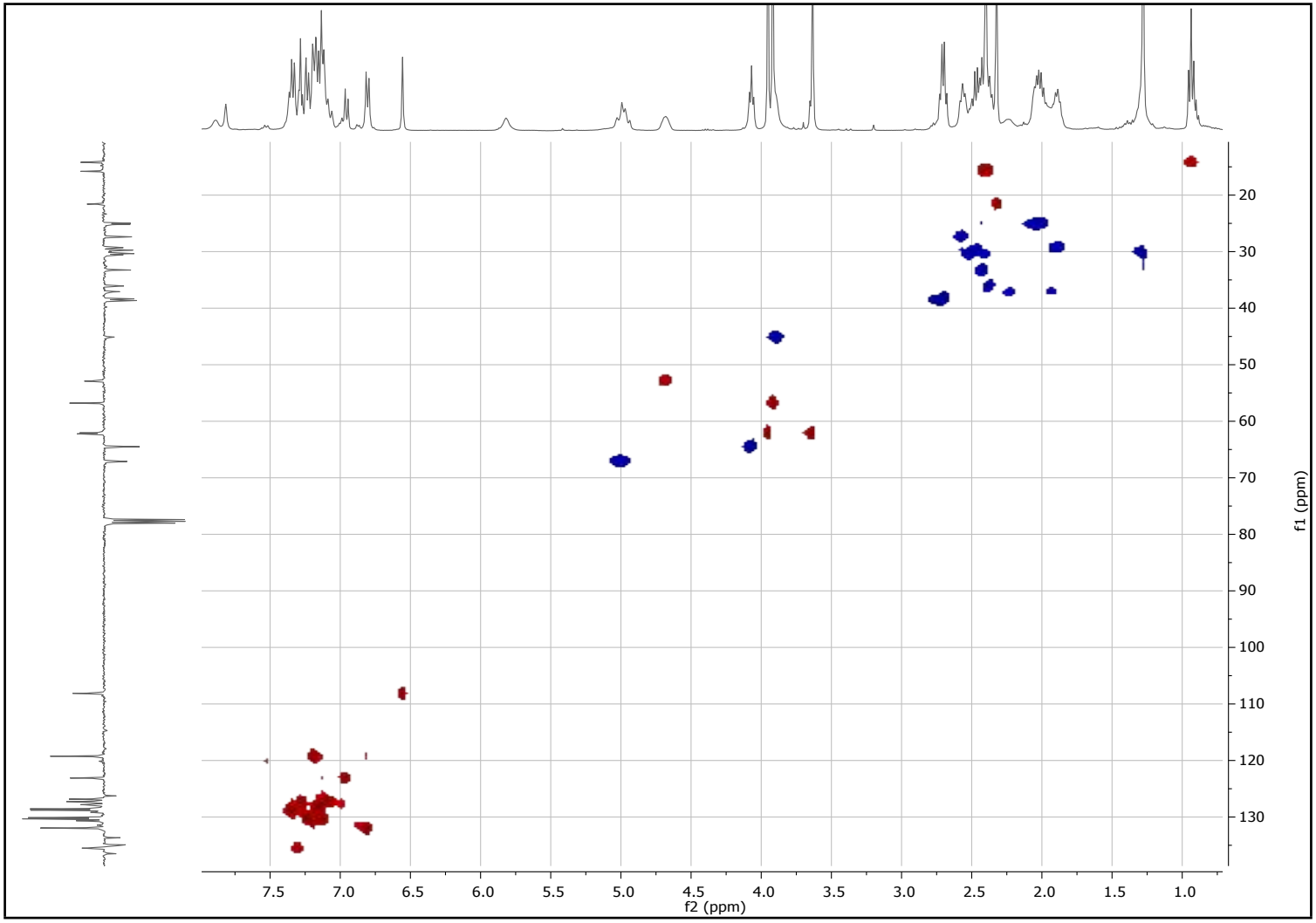
*^{13}C-NMR of compound **2** in $CDCl_3$*

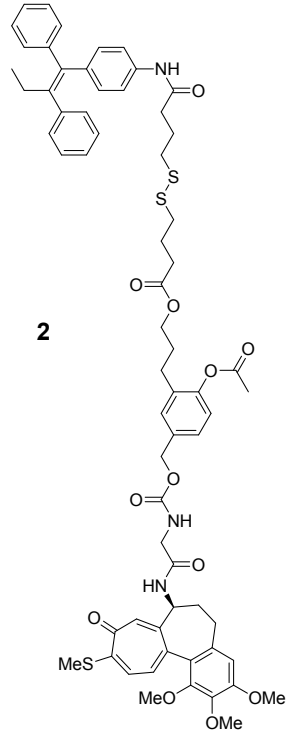




2

*2D COSY of compound **2** in $CDCl_3$*





2

*2D HSQC of compound **2** in $CDCl_3$*