Supporting Information

Modular Strategies for Structure and Function Employed by Marine Cyanobacteria: Characterization and Synthesis of Pitinoic Acids

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General Experimental Procedures

Optical rotations were measured on a Perkin–Elmer 341 polarimeter, whereas UV was measured on a SpectraMax M5 (Molecular Devices). ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer operating at 100 MHz, and ^{1}H and 2D NMR spectra were acquired on a Bruker Avance II 600 MHz spectrometer or Varian 400 MHz spectrometer. Spectra obtained in $CDCl_3$ using residual solvent signals (δ_H 7.26, δ_C 77.16 ppm) as internal standards. HSQC and HMBC experiments were optimized for $^{1}J_{CH}$ = 145 and $^{1}J_{CH}$ = 7 Hz, respectively. HRMS data was recorded on an Agilent LC–TOF mass spectrometer equipped with an APCI/ESI multimode ion source detector in positive or negative ion mode, whereas LRMS data were obtained using an API 3200 triple quadrupole MS (Applied Biosystems). ESIMS fragmentation data were recorded on an API 3200 by direct injection with a syringe driver. Malic acid standards were purchased from Sigma.

Extraction and Isolation

A cyanobacterial sample morphologically similar to *Lyngbya* sp. was collected from a channel at the north end of Piti Bay, Guam in July 2000. The freeze-dried sample was extracted three times with EtOAc-MeOH (1:1) to afford an organic extract (24.3 g). The resulting extract was partitioned between hexanes and 80% aqueous MeOH; the methanolic phase was evaporated to dryness and the residue was further partitioned between *n*-BuOH and H_2O. After concentrating the *n*-BuOH extract in vacuo, the resulting residue (5.47 g) was subjected to flash chromatography over silica gel, eluting with CH_2Cl_2 followed by increasing gradients of *i*-PrOH in CH_2Cl_2 then MeOH in CH_2Cl_2, and finally with MeOH. ^{1}H NMR profiles of the generated silica fractions showed a major simple fatty acid (**1**) dominating most of the fractions.

The silica fraction eluting with 10% *i*-PrOH/ CH_2Cl_2 was fractionated on a semi-preparative reversed-phase HPLC column (Synergi Hydro-RP, 250 × 10 mm, 5 μm, 2 mL/min; UV detection at 220/254 nm) using a MeOH/0.05% aqueous TFA linear gradient (80% to 100% over 20 min and then 100% MeOH for 10 min) to give pitinoic acid A (**1**) as the major peak at t_R 16.3 min as a colorless oil. Cyanobacterial samples collected at Cocos Lagoon, Guam, have also yielded pitinoic acid A (**1**). The ^{1}H NMR spectrum of **1** showed typical peaks for fatty acids: two methylene groups at $\delta_H \approx 1.2$ ppm, a terminal methyl group at δ_H 0.89 ppm, an α-methylene group at δ_H 2.35 ppm, and the fatty acid carbonyl carbon appeared in the ^{13}C NMR spectrum at δ_C 180.2 ppm. Additionally, a methylene group at δ_H 4.73 ppm and δ_C 109.5 ppm showed an HMBC correlation to a quaternary carbon at δ_C 148.7 ppm, indicating the presence of an exo-double bond along the fatty acid chain. The information obtained from 1D, 2D NMR spectra and MS data (HRESIMS: *m/z* 183.1398 for $[M-H]^-$ corresponding to $C_{11}H_{19}O_2$) led to the characterization of the simple fatty acid pitinoic acid A (**1**) as the major fatty acid in this sample (Table 1).

The silica fraction eluting with 40% MeOH/ CH_2Cl_2 was fractionated on a semi-preparative reversed-phase HPLC column (YMC-Pack ODS-AQ, 250 × 10 mm, 5 μm, 2 mL/min; UV detection at 200/220 nm) using a MeOH/0.05% aqueous TFA linear gradient (60% to 100% over 30 min and then 100% MeOH for 10 min) to yield 13 fractions. The fraction

eluting at t_R 32.8 min was re-purified on a semi-preparative reversed-phase HPLC column (Luna C18, 250 × 10 mm, 5 μm, 2.0 mL/min; UV detection at 200/220 nm) using a MeOH/0.05% aqueous TFA linear gradient (75% to 100% over 20 min followed by 100% MeOH for 10 min) to yield 0.9 mg of the pure chlorinated ester pitinoic acid B (**2**) at t_R 16.2 min. The ^{1}H and ^{13}C NMR spectra for **2** included all the peaks corresponding to **1** as well as peaks for five additional carbons and attached protons: one carbonyl at δ_C 173.5 ppm, an oxygenated methine at δ_H 5.09 and δ_C 70.7 ppm, a methylene at δ_H 2.64 and δ_C 32.5 ppm and olefinic methines at δ_H 5.9; δ_C 127.3 ppm and δ_H 6.12 and δ_C 121.4 ppm (Table 1). COSY, TOCSY and HMBC data led to the assignment of the additional portion as a 2-hydroxy-pent-4-enoic acid, and a chloride attachment to the terminal olefinic methine was suggested by the remaining molecular mass and the isotopic cluster detected in the HRESIMS analysis (*m/z* 315.1384, 317.1348 (3:1) for $[M-H]^-$ corresponding to $C_{16}H_{24}{}^{35}ClO_4$ and $C_{16}H_{24}{}^{37}ClO_4$, respectively). Notably, the chemical shift of the carbonyl carbon in the decanoic acid part was shifted upfield compared to the free fatty acid (Table 1), and therefore the compound appeared to be a fatty acid ester, **2**. The geometry of the alkene in the 2-hydroxy-5-chloro-pent-4-enoic acid moiety was determined to be *E* based on the large vicinal coupling constant ($^3J_{H,H}$ 13.4 Hz) between the olefinic protons. The configuration of the stereogenic center C2' was assigned through ozonolysis followed by oxidative workup to yield the corresponding malic acid, which was then analyzed by chiral HPLC compared to malic acid standards to reveal *S* configuration.

Pitinoic acid A (1): colorless oil; ^{1}H NMR, ^{13}C NMR and HMBC data, see Table 1; HRESI/APCIMS *m/z* 183.1398 $[M - H]^-$ (calcd $C_{11}H_{19}O_2$ 183.1391).

Pitinoic acid B (2): colorless oil; $[\alpha]^{20}_D$ −12 (*c* 0.05, MeCN); ^{1}H NMR, ^{13}C NMR and HMBC data, see Table 1; HRESI/APCIMS *m/z* 315.1384/ 317.1348 (3:1) $[M-H]^-$ (calcd $C_{16}H_{24}ClO_4$ 315.1369/ 317.1369).

Enantioselective Analysis

A sample of compound **2** (100 μg) was dissolved in 3 mL CH_2Cl_2 and subjected to ozonolysis at -78°C for 10 min. The solvent was evaporated and the residue was dissolved in $HCOOH/H_2O_2$ (2:1) and heated at 75°C for 30 min. After removing the solvent, the residue was subjected to hydrolysis with 6 N HCl at 110°C for 12 h. The hydrolyzed product was dried and subjected to chiral HPLC (column: Phenomenex Chirex phase 3126 *N*,*S*-dioctyl-(D)-penicillamine, 4.6 × 250 mm, 5 μm; solvent: 0.5 mM $Cu(OAc)_2$, 0.1 M NH_4OAc in 85:15 H_2O/MeCN, pH 4.6; flow rate: 1.0 mL/min; detection: 254 nm). Retention times were as follows: L-malic acid eluted at t_R 7.0 min, D-malic acid eluted at t_R 13.8 min. Malic acid in the hydrolysate eluted after 7.0 min, indicating the presence of (*S*)-hydroxy acid in compound **2**.

Initial Synthetic Trials (Strategy 1):

Our initial retrosynthetic strategy relied on the obvious disconnection at the ester linkage to the fatty acid pitinoic acid A (**1**), which could be obtained from the cyanobacterial sample, and the chlorinated α-hydroxy acid fragment pitinoic acid C. The selective introduction of the *E*-vinyl chloride in the alcohol moiety could be achieved through Takai-Utimoto olefination

reaction from an α-hydroxy-4-oxobutanoic acid.[1] The latter could be obtained by selective reduction of L-malic acid at C4 (Scheme 1, strategy 1a). Since this selective reduction of malic acid was not successful, we tried the method by Padron et al.,[2] to selectively reduce the di-Boc protected aspartic acid to the corresponding C4 semialdehyde (Scheme 1, strategy 1b). Indeed, the selective reduction starting from aspartic acid proceeded smoothly with a 67% yield. However, Takai-Utimoto olefination for the aspartate semialdehyde proceeded with a low yield (32%) and relatively low selectivity (E:$Z \approx$ 4:1). Notably, one Boc group was also lost during this reaction as determined by analyzing the ^{1}H NMR spectrum. Trials with the acid-stable amine protecting group Fmoc did not achieve the initial selective reduction at C4 as with the di-Boc protected aspartate.

Synthetic Procedures

(*S*)-2-(2,2-dimethyl-[1,3]dioxolan-4-yl)-acetaldehyde (3): PCC (10.6 g, 49 mmol, 3.6 eq) was added slowly to a suspension of freshly activated molecular sieves (3°A, 10.6 g) in dry CH_2Cl_2 (50 mL). The commercially available alcohol (2 g, 13.6 mmol, 1 eq) was dissolved in 5 mL CH_2Cl_2 and added to the above mixture which was stirred at r.t. for 2 h. The mixture was then diluted using Et_2O, filtered through silica gel and concentrated under reduced pressure, to give 1.8 g of the pure aldehyde **3** as a colorless oil (76% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.80 (s, 1H), 4.53 (quint, J = 6.6 Hz, 1H), 4.18 (dd, J = 8.2, 6.3 Hz, 1H), 3.58 (dd, J = 8.2, 7.0 Hz, 1H), 2.84 (ddd, J = 17.1, 6.7, 1.6 Hz, 1H), 2.64 (ddd, J = 17.1, 6.2, 1.6 Hz, 1H), 1.41 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.22, 109.46, 70.84, 69.33, 48.05, 26.99, 25.84. HRESIMS: m/z 167.0679 $[M + Na]^+$ (calcd $C_7H_{12}O_3Na$ 167.0684), 311.1480 $[2M + Na]^+$ (calcd $C_{14}H_{24}O_6Na$ 311.1465).

(*S*,*E*)-4-(3-chloroallyl)-2,2-dimethyl-1,3-dioxolane (4): To a dry flask, anhydrous $CrCl_2$ (4 g, 33.3 mmol, 10 eq) was added and then heated using a heat gun under vacuum. After cooling, the vacuum was released under argon, and dry THF (30 mL) was added. The greenish suspension was stirred in an oil bath and heated to 65°C. The aldehyde **3** (500 mg, 3.4 mmol, 1 eq) was dissolved in 5 mL dry THF in another vial, and mixed with dry $CHCl_3$ (1.3 mL, 16.7 mmol, 4 eq). This solution was then added drop wise to the heated flask containing chromous chloride, which turned violet upon stirring. The reaction mixture was stirred for 3 h at 65 °C, and then cooled down to r.t. The mixture was diluted with brine, filtered and extracted three times with Et_2O. The combined organic layers were dried over $MgSO_4$, filtered and the solvent was removed. The crude green residue was then purified using silica gel column chromatography (Hex/ Et_2O, 14:1) to yield the pure yellow oily product as a mixture of isomers (226 mg, 37%, E:$Z \approx$ 8:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.05 (d, J = 13.3 Hz, 1H), 5.89 (m, 1H), 4.12 (quint, J = 6.2 Hz, 1H), 4.01 (dd, J = 8.0, 6.1 Hz, 1H), 3.55 (dd, J = 7.9, 6.9 Hz, 1H), 2.32 (m, 2H), 1.40 (s, 3H), 1.33 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 129.13, 119.81, 109.48, 74.87, 68.83,

[1] Takai, K.; Nitta, K.; Utimoto, K. *J. Am. Chem. Soc.* **1986**, 108, 7408-7410.
[2] Padrón, J. M.; Kokotos, G.; Martín, T.; Markidis, T.; Gibbons, W. A.; Martín, V. C. S. *Tetrahedron: Asymmetry* **1998**, *9*, 3381-3394.

35.26, 27.06, 25.75. The compound could not be detected by positive mode HRESI-TOF-MS and positive mode GC-EI-MS.

***(S,E)*-5-chloropent-4-ene-1,2-diol (5):** Compound **4** (122 mg) was dissolved in MeOH (1 mL). 200 mg of MeOH-washed DOWEX 50WX4 were added to the solution and stirred at 50 °C for 3 h. The mixture was filtered through celite using Et_2O, and residual MeOH and H_2O were removed using toluene via azeotropic distillation to give 90.3 mg (95%) of the diol product **4**, which was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.05 (d, J = 13.3 Hz, 1H), 5.91 (m, 1H), 3.70 (m, 1H), 3.61 (dd, J = 11.2, 2.3 Hz, 1H), 3.42 (dd, J = 11.2, 7.5 Hz, 1H), 2.19 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 129.57, 119.83, 71.41, 66.17, 34.84. The compound could not be detected by negative mode HRESI-TOF-MS and positive mode GC-EI-MS.

***(S,E)*-1-((*tert*-butyldimethylsilyl)oxy)-5-chloropent-4-en-2-ol (6):** Imidazole (32.5 mg, 0.478 mmol, 1.3 eq) and TBS-Cl (66.5 mg, 0.441 mmol, 1.2 eq) were added sequentially to a solution of diol **5** (50 mg, 0.367 mmol, 1 eq) in DMF (0.5 mL). The solution was allowed to warm up to r.t. and stirred for 4 h. The reaction mixture was then diluted with EtOAc, washed three times with saturated $NaHCO_3$, washed twice with brine, dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product (74 mg) was then purified by column chromatography (Hex/Et_2O, 12:1) to yield 43.1 mg (47%) of pure product. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.03 (d, J = 13.3 Hz, 1H), 5.9 (m, 1H), 3.67 (m, 1H), 3.61 (dd, J = 9.9, 3.7 Hz, 1H), 3.44 (dd, J = 9.9, 6.6 Hz, 1H), 2.22 (dd br, 6.7 Hz, 2H), 0.89 (s, 9H), 0.06 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 129.89, 119.29, 71.00, 66.49, 34.76, 26.07, -5.20, -5.17. HRESIMS: *m/z* 273.1051/ 275.1034 (3:1) $[M + Na]^+$ (calcd $C_{11}H_{23}ClO_2SiNa$ 273.1048/275.1022).

***(S,E)*-1-((*tert*-butyldimethylsilyl)oxy)-5-chloropent-4-en-2-yl 5-methylene-decanoate (7):** Et_3N (13.6 μL, 0.098 mmol, 1 eq) was added to a solution of **1** (18 mg, 0.098 mmol, 1 eq) in dry CH_2Cl_2 (0.2 mL). EDC·HCl (19 mg, 0.098 mmol, 1 eq) and DMAP (4 mg, 0.033 mmol, 0.3 eq) were then added and the mixture was stirred at r.t. for 10 min. A solution of HOAT (14.6 mg, 0.12 mmol, 1.1 eq) in DMF (0.12 mL) was then added to the above mixture and cooled to 0°C. Compound **6** (15 mg, 0.059 mmol, 0.6 eq) was then dissolved in 0.2 mL CH_2Cl_2 and added to the cooled mixture, which was then stirred at r.t. overnight. The reaction was then quenched by adding 0.7 mL of Sorensen buffer (0.4 M, pH 7). The mixture was extracted three times with CH_2Cl_2, dried over $MgSO_4$ and the solvent was removed under reduced pressure to give 33.4 mg of crude product. Purification using preparative TLC (Hex/Et_2O, 2:1) yielded 18.3 mg of the pure ester **7** as a colorless oil (73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.01 (d, J = 13.2 Hz, 1H), 5.85 (m, 1H), 4.88 (m, 1H), 4.75 (s, 1H), 4.72 (s, 1H), 3.63 (m, 2H), 2.29 (t, J = 7.4 Hz, 2H), 2.04 (t, J = 7.3 Hz, 2H), 1.98 (t, J = 7.7 Hz, 2H), 1.75 (m, 2H), 1.41 (m, 2H), 1.32 (m, 2H), 1.28 (m, 2H), 0.88 (t, 3H), 0.88 (s, 9H), 0.04 (s, 6H) . ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.29, 149.08, 128.94, 119.86, 109.67, 72.95, 63.54, 36.02, 35.51, 34.13, 32.24, 31.84, 29.94, 27.64, 26.01, 23.18, 22.79, 14.31, -5.21, -5.19. HRESIMS: *m/z* 439.2410/ 441.2388 (3:1) $[M + Na]^+$ (calcd $C_{22}H_{41}ClO_3SiNa$ 439.2406/441.2386).

(*S*,*E*)-5-chloro-1-hydroxy-pent-4-en-2-yl 5-methylenedecanoate (8): AcOH (3.4 μL, 2.5 eq) was added to TBAF (33 μL of a 1 M solution in THF, 2 eq) to give a neutral reagent, which was added to a solution of compound **7** (6.8 mg, 0.017 mmol, 1 eq) in THF (0.2 mL) at r.t. The reaction mixture was stirred at r.t. for 3 h, and then quenched by adding 0.7 mL of saturated $NaHCO_3$ solution. The aqueous layer was extracted 3 × with EtOAc, dried over $MgSO_4$ and concentrated under reduced pressure. Purification using preparative TLC (Hex/Et_2O, 2:1) yielded 3.5 mg of the pure alcohol **8** as a colorless oil (72%, 85% BRMS). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.06 (d, *J* = 13.3 Hz, 1H), 5.87 (m, 1H), 4.93 (m, 1H), 4.75 (s, 1H), 4.72 (s, 1H), 3.69 (m, 2H), 2.40 (m, 2H), 2.35 (t, *J* = 7.4 Hz, 2H), 2.05 (t, *J* = 7.4 Hz, 2H), 2.00 (t, *J* = 7.4 Hz, 2H), 1.78 (m, 2H), 1.46-1.26 (m, 6H), 0.89 (t, *J* = 6.65 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.83, 148.98, 128.47, 120.36, 109.76, 73.69, 63.96, 35.97, 35.44, 34.03, 32.26, 31.81, 27.62, 23.11, 22.78, 14.29. HRESIMS: *m/z* 325.1541/327.1517 (3:1) $[M + Na]^+$ (calcd $C_{16}H_{27}ClO_3Na$ 325.1546/327.1517).

(*S*, *E*)-5-chloro-2-((5-methylenedecanoyl)oxy)pent-4-enoic acid (2): Freshly prepared Jones reagent (1.1 mL) was added over 5 min to a solution of alcohol **8** (30.6 mg, 0.1 mmol) in acetone (7 mL) at 0 °C. The mixture was warmed up to r.t. and stirred for 1 h. Excess reagent was quenched by the addition of *i*-PrOH (1.5 mL) followed by filtration through a pad of celite. The solvent was removed and then the residue was resuspended in water and extracted twice with EtOAc. The combined EtOAc layers were dried over $MgSO_4$, filtered and the solvent was removed. The crude product was purified using silica gel column chromatography to give 18 mg of a semi-pure fraction, which was re-purified by reversed-phase HPLC (YMC-Pack ODS-AQ, 250 × 10 mm, 5 μm, 2 mL/min; UV detection at 220/200 nm) using a MeOH/0.05% aqueous TFA linear gradient (60% to 100% over 30 min and then 100% MeOH for 10 min) to give 8 mg of the pure product **2** as a colorless oil (mixture of *Z* and *E* isomers; *Z*:*E* ≈ 1:8). $[\alpha]^{20}_D$ –3.4° (*c* 0.32, DCM). ^{1}H NMR (400 MHz, $CDCl_3$): $^\delta$ 6.12 (d, *J* = 13.3 Hz, 1H), 5.95 – 5.86 (m, 1H), 5.11(br dt, *J* = 5.1 Hz, 1H), 4.75 (s, 1H), 4.72 (s, 1H), 2.64 (m, 2H), 2.41 (td, *J* = 7.4, 3.2 Hz, 2H), 2.07 (t, *J* = 7.4 Hz, 2H), 1.99 (t, *J* = 7.6 Hz, 2H), 1.79 (m, 2H), 1.46 – 1.36 (m, 2H), 1.36 – 1.22 (m, 4H), 0.89 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.22, 148.91, 127.14, 121.51, 109.85, 35.98, 35.35, 33.62, 32.62, 31.83, 27.63, 22.93, 22.79, 14.3. LRMS: *m/z* 315.2/317.0 $[M – H]^-$, 182.9 $[M-C_5H_7ClO_2]^-$ (acid fragment), 149.0/150.8 (3:1) $[M-C_{11}H_{19}O]^-$ (alcohol fragment), 130.8/132.8 (3:1) $[M- C_{11}H_{21}O]^-$ (alcohol fragment, McLafferty rearrangement or loss of water). HRESIMS: *m/z* 315.1375/317.1357 (3:1) $[M - H]^-$ (calcd $C_{16}H_{24}ClO_4$ 315.1363/317.1334), 631.2816/ 633.2802 $[2M – H]^-$ (calcd $C_{32}H_{49}Cl_2O_8$ 631.2810/633.2775).

Pyocyanin and Elastase Quantification in *Pseudomonas aeruginosa*[1]

An overnight culture of *Pseudomonas aeruginosa* (strain PAO1) was diluted 100-fold and incubated at 37 °C for 2 h with shaking. This was followed by another 100-fold dilution and incubation for 1 h. Then, 100 μL of this culture were transferred to another culture tube containing 890 μL LB broth and 10 μL compound (1 mM and 100 μM final concentrations) or

EtOH control, and incubated at 37 °C with shaking for 6 h. No problems with compound solubility were detected at the tested concentrations. The culture was then spun down for 10 min at 9700 × *g*, and the supernatant was filtered using 0.2 μM Eppendorf filters. 100 μL of this sterile supernatant were added to 900 μL Elastin Congo Red (ECR) suspension (prepared in 1 mM $CaCl_2$, 100 mM Tris buffer, pH 7.2) and incubated at 37 °C with shaking for 18 h. After 18 h, the solid ECR was removed by centrifugation and the UV absorbance was measured at 495 nm for the quantitation of the soluble Congo red liberated by the enzyme LasB, reflecting the enzyme activity.

For Pyocyanin quantification, 500 μL of the sterile supernatant (stored overnight at -80 °C and used next day) were added to 500 μL $CHCl_3$ in an Eppendorf tube. Tube shaking allowed for the extraction of Pyocyanin in the $CHCl_3$ layer. This layer was then added to 150 μL of 0.2 N HCl in another Eppendorf tube. After shaking, the aqueous layer containing Pyocyanin turned red. 100 μL of this layer were transferred to a clear bottomed 384-well plate and the absorbance was measured at 385 nm to quantify the amount of Pyocyanin. Data was analyzed using GraphPad Prism 5 software.

RT-qPCR in *Pseudomonas aeruginosa*[1]

A culture of *Pseudomonas aeruginosa* (strain PAO1) was grown overnight and diluted as mentioned above. Then, 100 μL of this culture were transferred to another culture tube containing 890 μL LB broth and 10 μL compound or EtOH control, and incubated at 37 °C with shaking for 6 h. The culture was then spun down for 10 min at maximum speed; the supernatant was removed (used for quantitation mentioned above) and the cell pellet was resuspended in 500 μL LB broth. 1 mL of RNAprotect bacteria reagent (Qiagen) was added to the resuspended pellet, mixed and incubated at r.t. for 5 min to stabilize RNA. After centrifugation at maximum speed for 10 min, the supernatant was decanted, and RNA was extracted using the RNeasy Kit (Qiagen) according to the manufacturer's instructions (using enzymatic lysis and proteinase K digestion protocol). On column DNase digestion was done using the RNase-Free DNase set (Qiagen).

DNA contamination was quantified by qPCR of the RNA samples using a primer/probe set for *rpsL.* To further reduce DNA contamination, the TURBO DNA-free kit (Ambion) was used according the manufacturer's protocol (rigorous DNase treatment). RNA samples were then re-quantified by UV absorbance (Nanodrop 8000, Thermo) and RNA integrity was assessed by measuring RNA Integrity Number (RIN) using Agilent 2100 Bioanalyzer. Samples used for RT-qPCR showed RIN values > 9.2.

Total RNA (2 μg) was reverse-transcribed using Superscript II reverse transcriptase (Invitrogen) and Oligo $(dT)_{12\text{-}18}$ Primers (Invitrogen). For qPCR, 0.5 μL of the synthesized cDNA was added to 12.5 μL of 2 × TaqMan gene expression master mix, 1.25 μL of 20 × TaqMan gene expression assay mix and 10.75 μL sterile water, in a total of 25 μL reaction volume. TaqMan primers/probes used were custom designed by Applied Biosystems[1] for the target genes *lasB* and *phzG1* and the endogenous control *rpoD*. Real-time PCR was performed

[1] Kwan, J. C.; Meickle, T.; Ladwa, D.; Teplitski, M.; Paul, V.; Luesch, H. *Mol. Biosyst.* **2011,** 7, (4), 1205-16.

on an ABI 7300 sequence detection system with the following thermocycler program: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 sec at 95 °C and 1 min at 60 °C. Experiments were done in triplicate. Data was analyzed using GraphPad Prism 5 software.

THP-1 Cell Culture and RT-qPCR

THP-1 human acute monocytic leukemia cells were purchased from American Type Culture Collection (ATCC, TIB-202). The cells were maintained and propagated in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 0.05 mM *β*-mercaptoethanol at 37 °C humidified air and 5% CO_2. For the anti-inflammatory assays, cells were seeded (5×10^6 cells/ well) in 6-well plate in RPMI-1640 medium supplemented with 10% FBS. The monocytes were differentiated to macrophages by incubating the cells in the presence of 100 nM PMA (Sigma) for 48 h. The medium was then aspirated and the adherent macrophages were washed twice with PBS and fresh medium was added. Cells were treated with the compound (100 *μ*M in DMSO) for 1 h before they were stimulated with LPS (5 *μ*g/mL) for 4, 12 or 24 h. At the end of each time point, the medium was aspirated and the RNeasy mini kit (QIAGEN) was used for RNA extraction and purification according to the manufacturer's protocol. Total RNA was quantified using UV absorbance. Total RNA (2 *μ*g) was reverse-transcribed using Superscript II reverse transcriptase (Invitrogen) and Oligo $(dT)_{12\text{-}18}$ Primers (Invitrogen). For qPCR, 0.5 *μ*L of the synthesized cDNA was added to 12.5 μL of 2 × TaqMan gene expression master mix, 1.25 *μ*L of 20 × TaqMan gene expression assay mix and 10.75 *μ*L sterile water, in a total of 25 *μ*L reaction volume. TaqMan primers/probes sets used for this experiment are the recommended inventoried sets, designed for best coverage by Applied Biosystems for the target genes *TNF-α*, *IL-6*, *IL-1β* and *IL-8* and the endogenous control *GAPDH*. Real-time PCR was performed on an ABI 7300 sequence detection system with the following thermocycler program: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 sec at 95 °C and 1 min at 60 °C. Experiments were done in triplicate. Data was analyzed using GraphPad Prism 5 software.

Cell viability assay

THP-1 human acute monocytic leukemia cells were maintained, propagated and differentiated to macrophages as mentioned above. Differentiated cells in 96-well plate (2×10^4 cells/well) were treated with the compound or solvent control (1% DMSO) in the presence or absence of LPS (5 *μ*g/mL). After 48 hours of incubation, cell viability was measured using MTT according to the manufacturer's instructions (Promega, Madison, WI). Cell viability at 100 *μ*M was 60% without LPS and 73% in the presence of LPS.

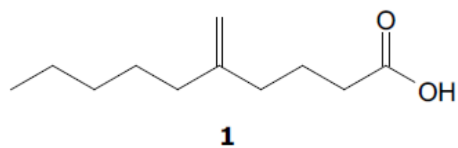
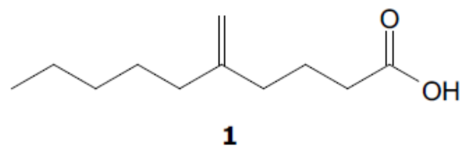


Fig. S1: ^{1}H NMR Spectrum of Pitinoic acid A (**1**) in $CDCl_3$ (600 MHz)



180.24
148.69
109.53
35.78
33.54
31.59
27.39
22.54
14.03

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

Fig. S2: ^{13}C NMR Spectrum of Pitinoic acid A (**1**) in $CDCl_3$ (150 MHz)

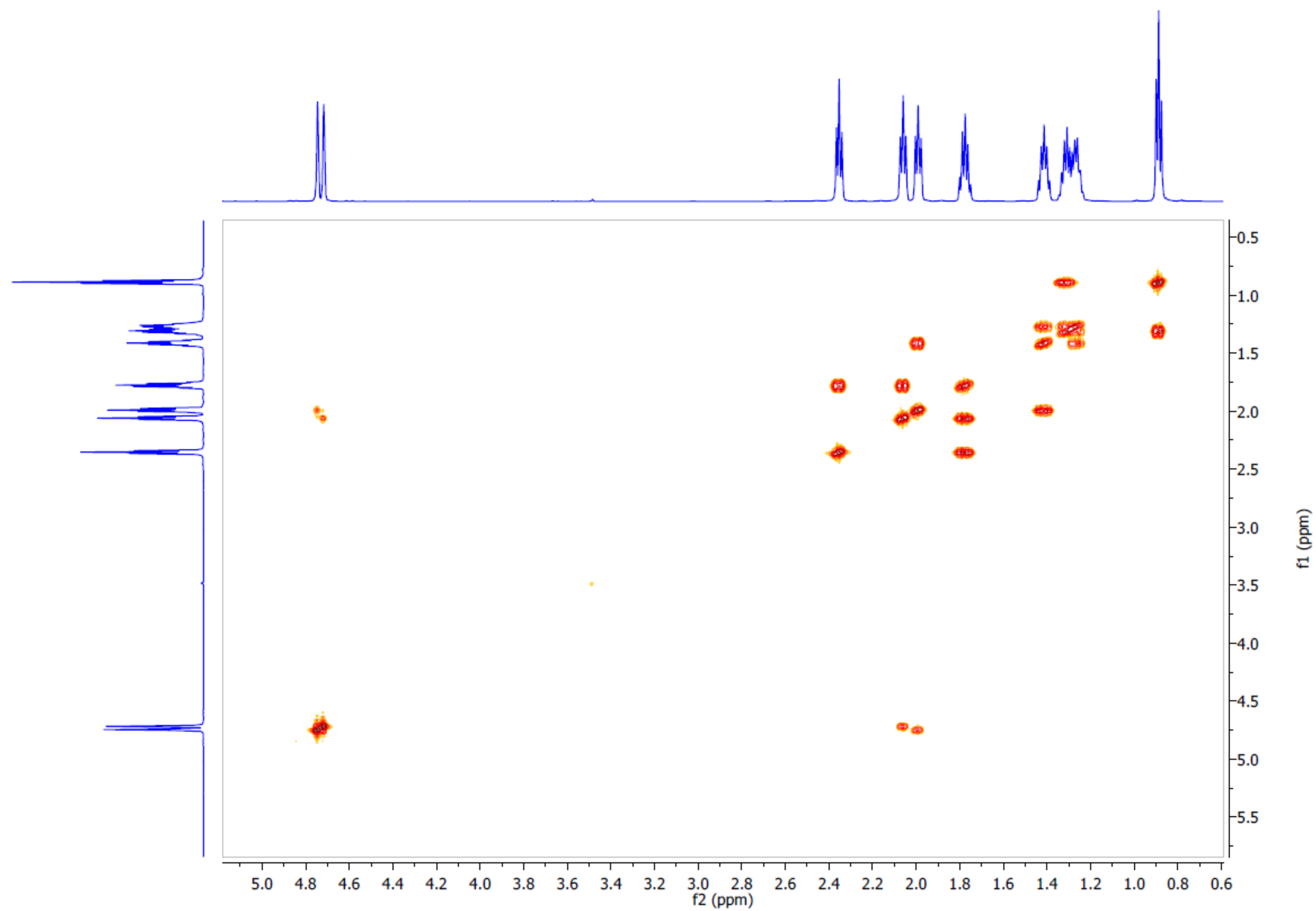


Fig. S3: COSY Spectrum of Pitinoic acid A (**1**) in $CDCl_3$ (600 MHz)

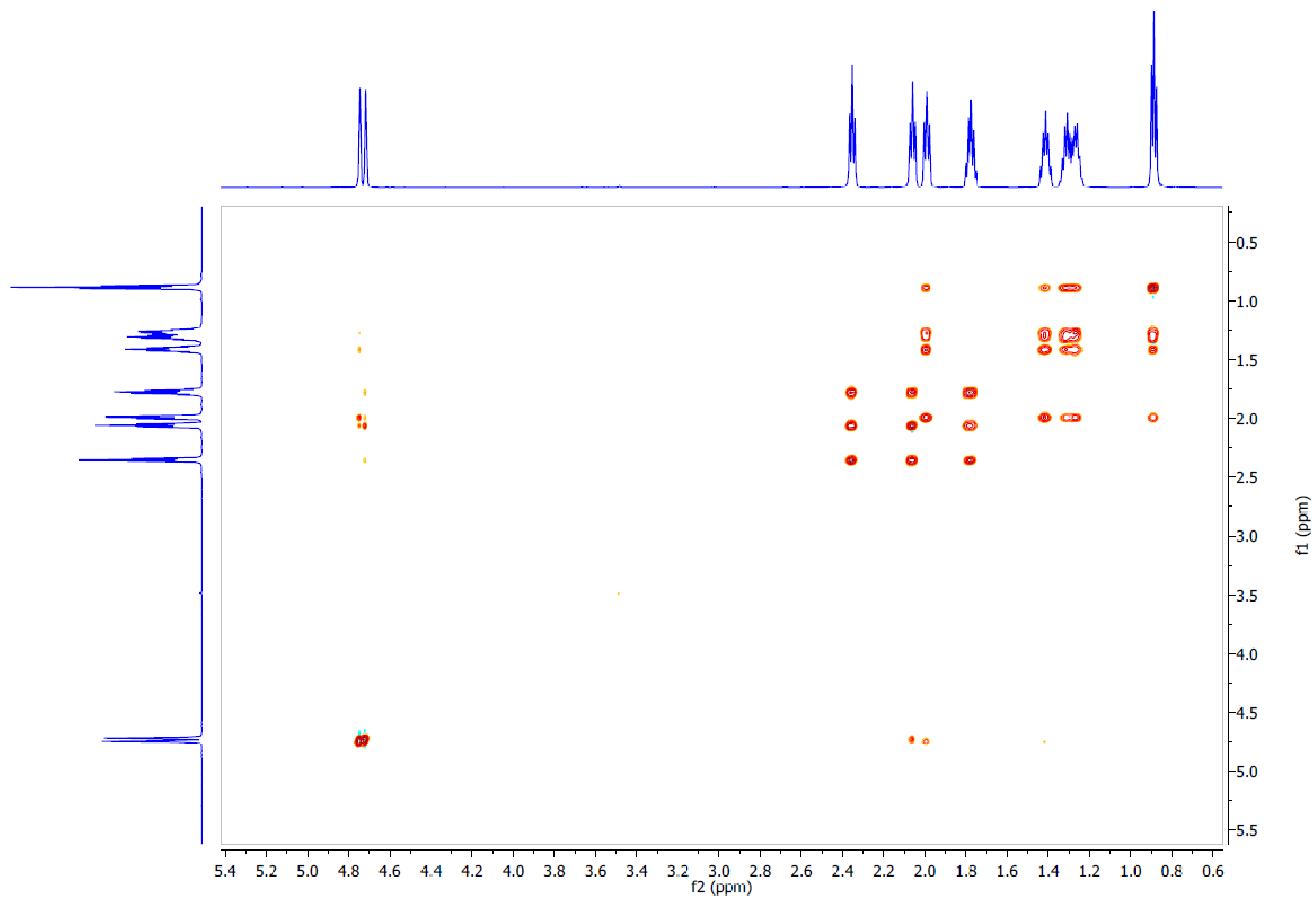


Fig. S4: TOCSY Spectrum of Pitinoic acid A (**1**) in $CDCl_3$ (600 MHz)

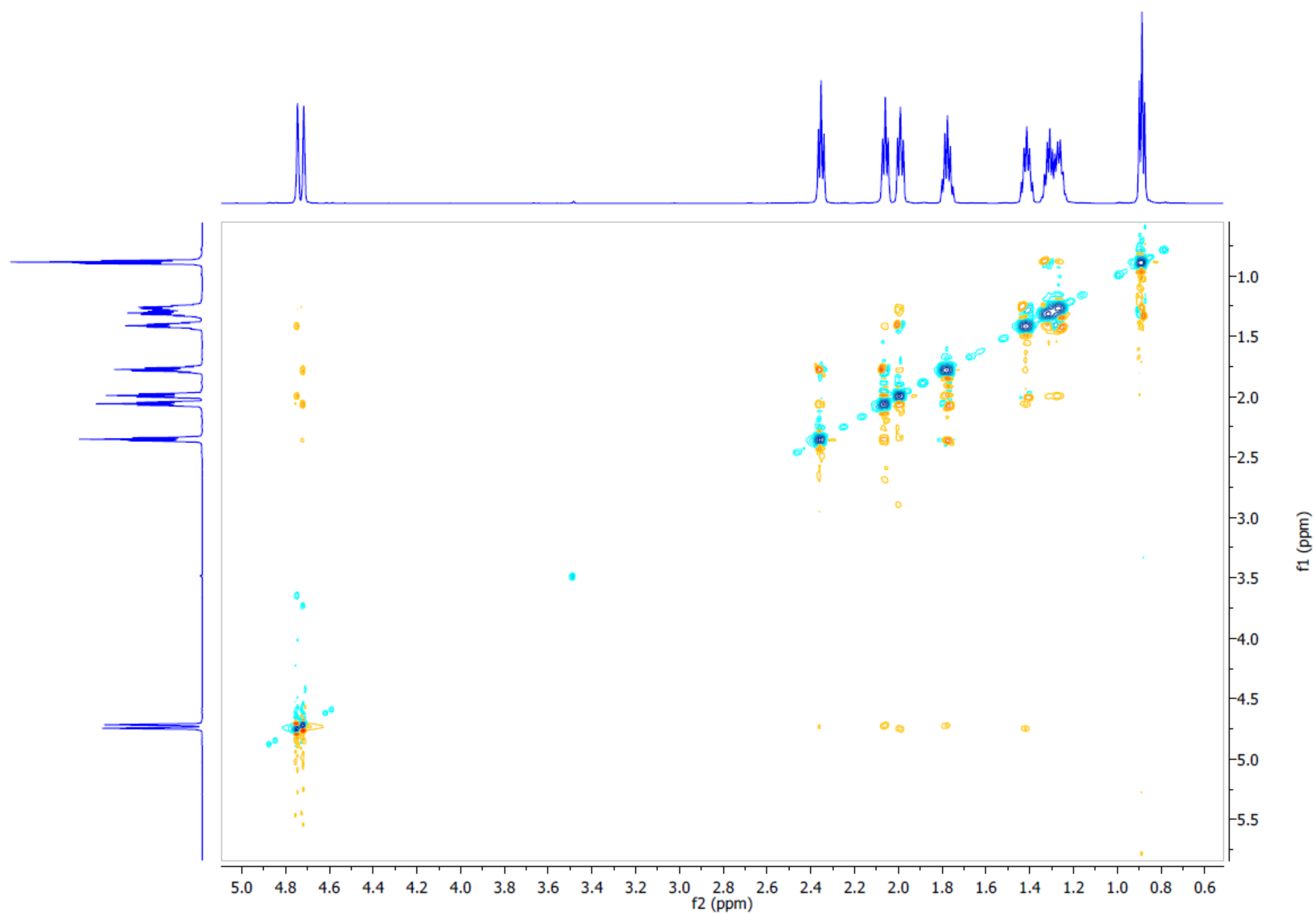


Fig. S5: NOESY Spectrum of Pitinoic acid A (**1**) in $CDCl_3$ (600 MHz)

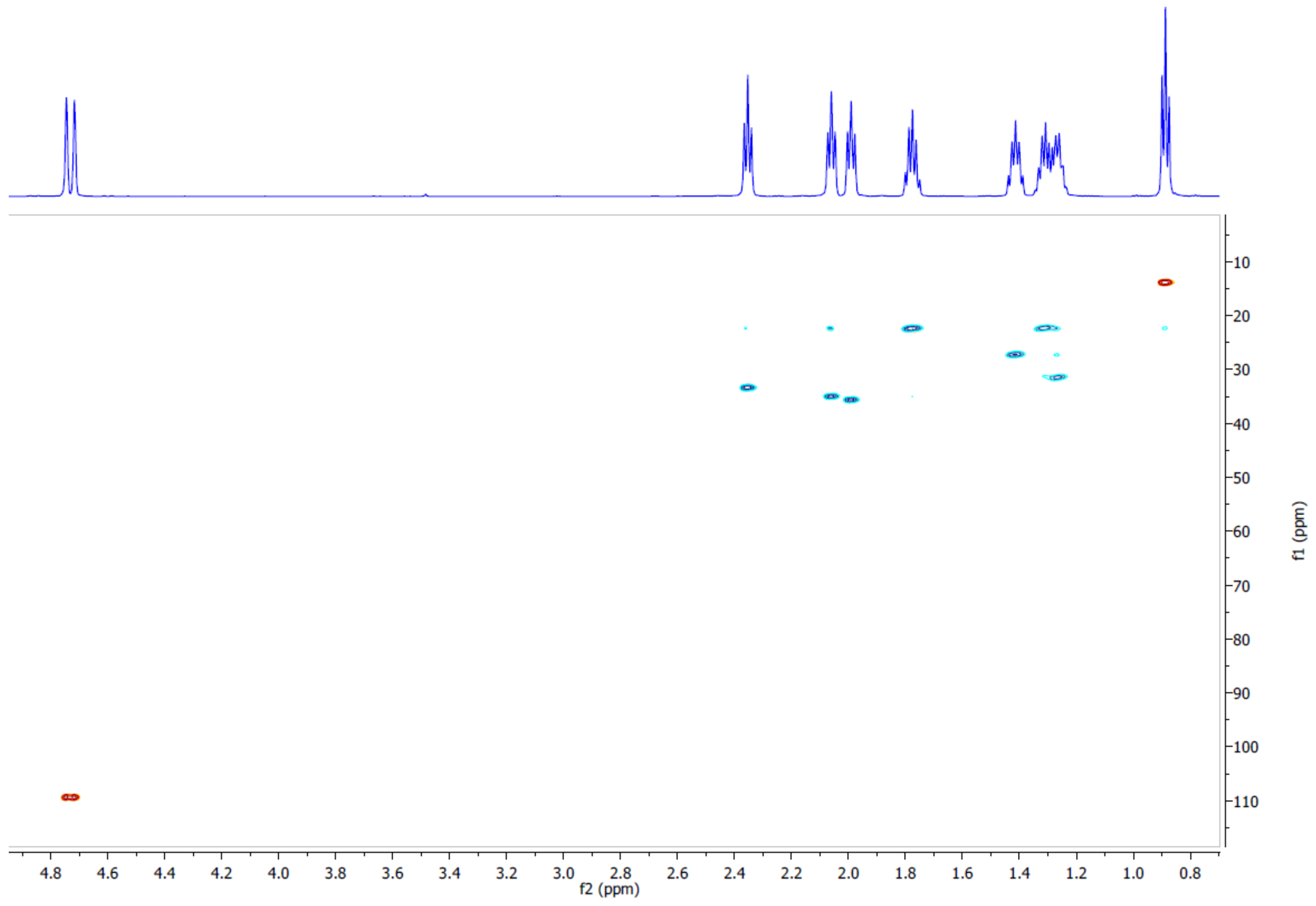


Fig. S6: HSQC Spectrum of Pitinoic acid A (**1**) in $CDCl_3$ (600 MHz)

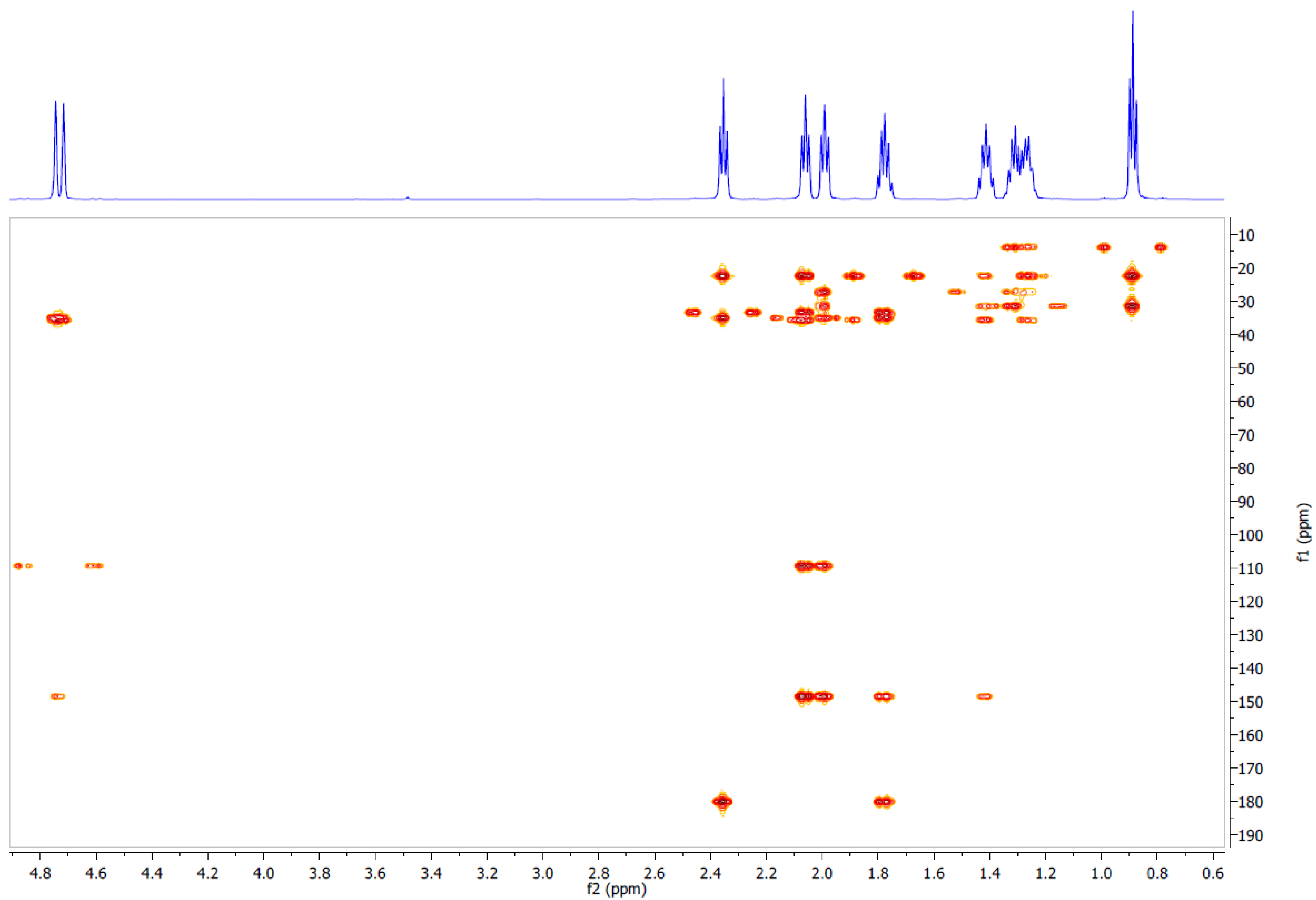


Fig. S7: HMBC Spectrum of Pitinoic acid A (**1**) in $CDCl_3$ (600 MHz)

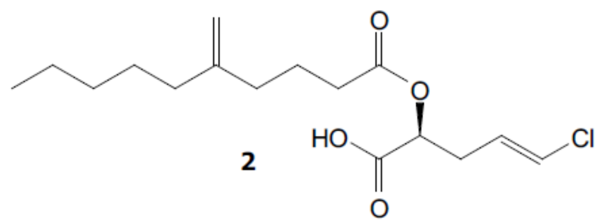


Fig. S8: ^{1}H NMR Spectrum of Pitinoic acid B (**2**) in $CDCl_3$ (600 MHz)

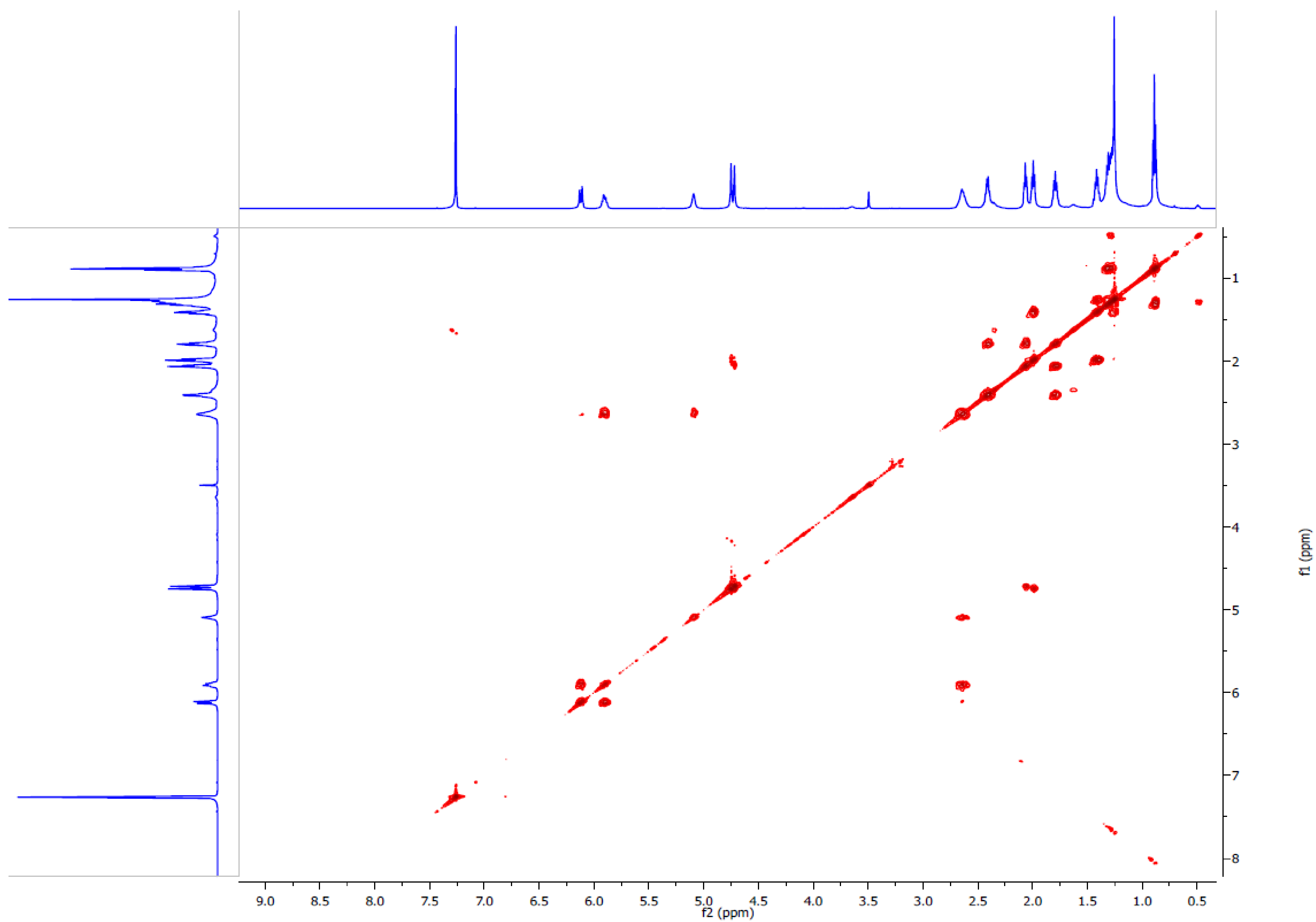


Fig. S9: COSY Spectrum of Pitinoic acid B (**2**) in $CDCl_3$ (600 MHz)

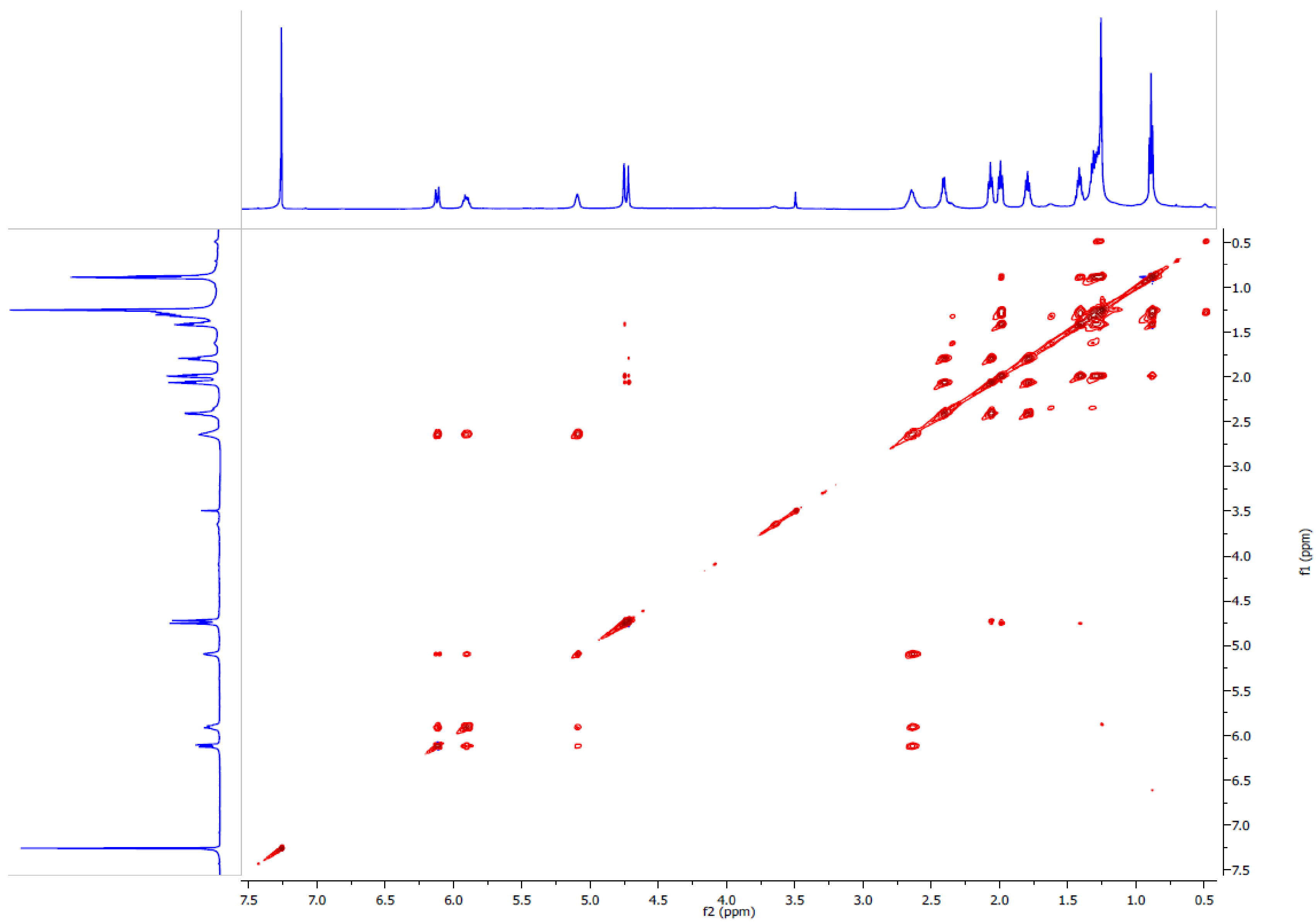


Fig. S10: TOCSY Spectrum of Pitinoic acid B (**2**) in $CDCl_3$ (600 MHz)

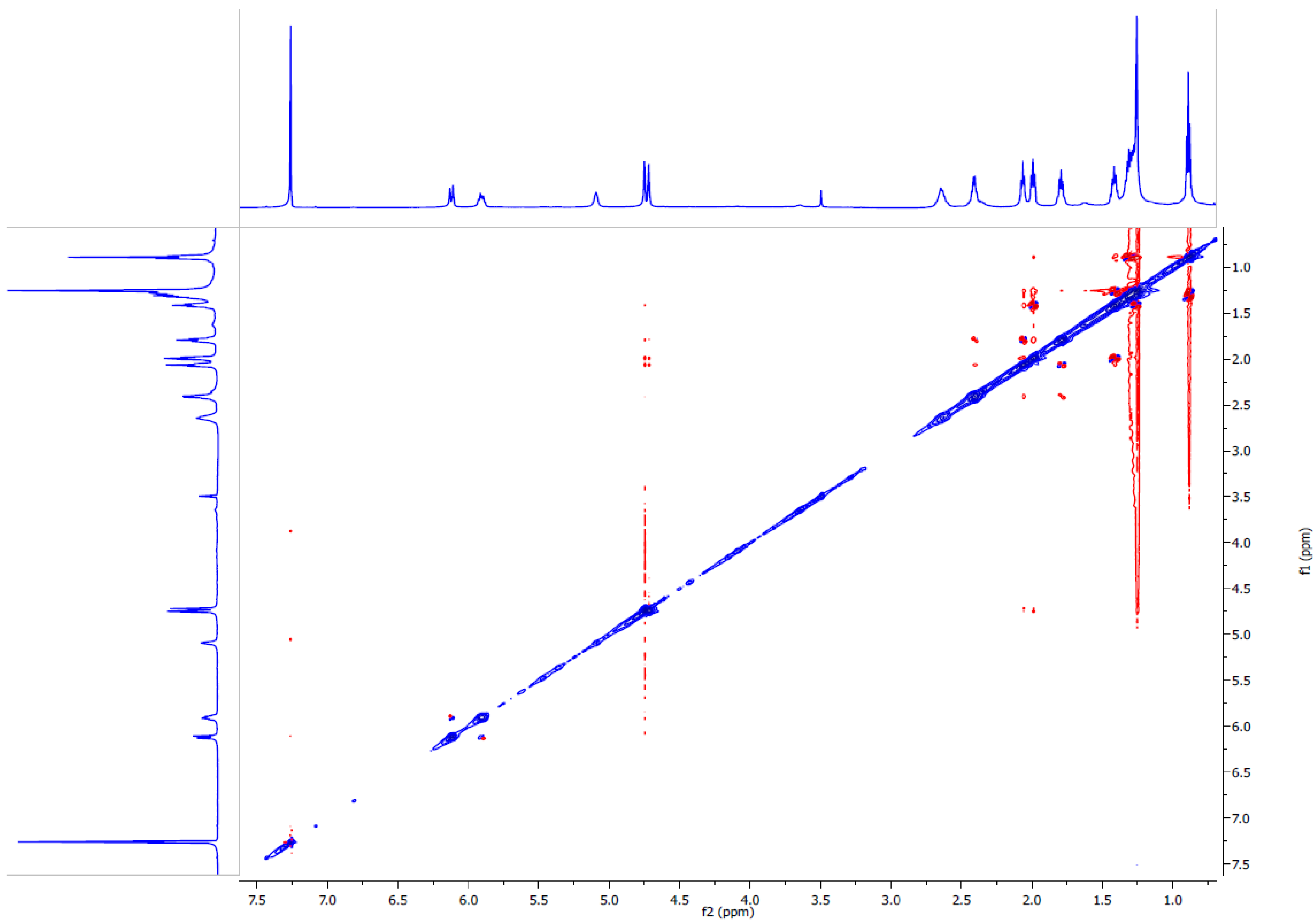


Fig. S11: NOESY Spectrum of Pitinoic acid B (**2**) in $CDCl_3$ (600 MHz)

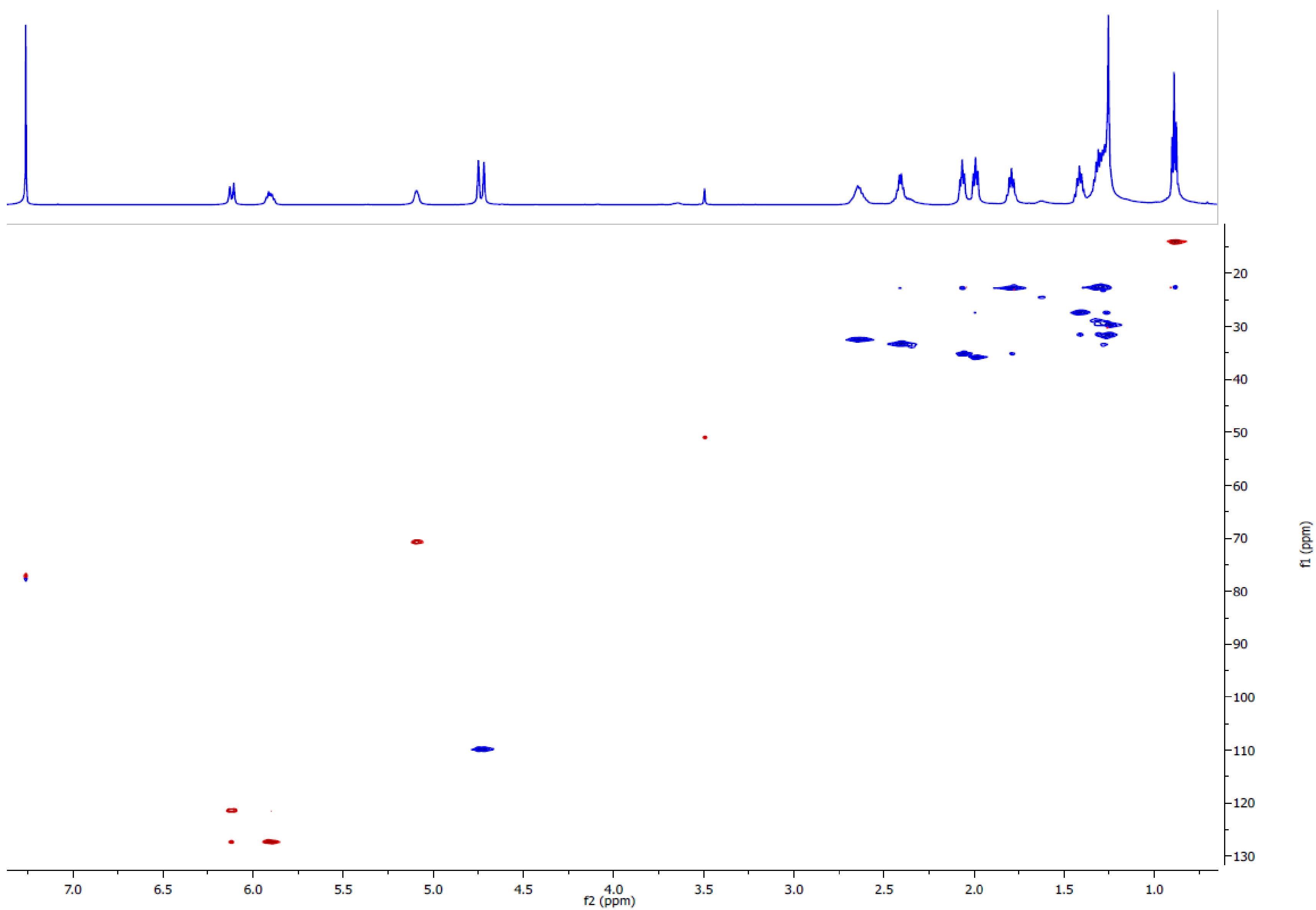


Fig. S12: HSQC Spectrum of Pitinoic acid B (**2**) in $CDCl_3$ (600 MHz)

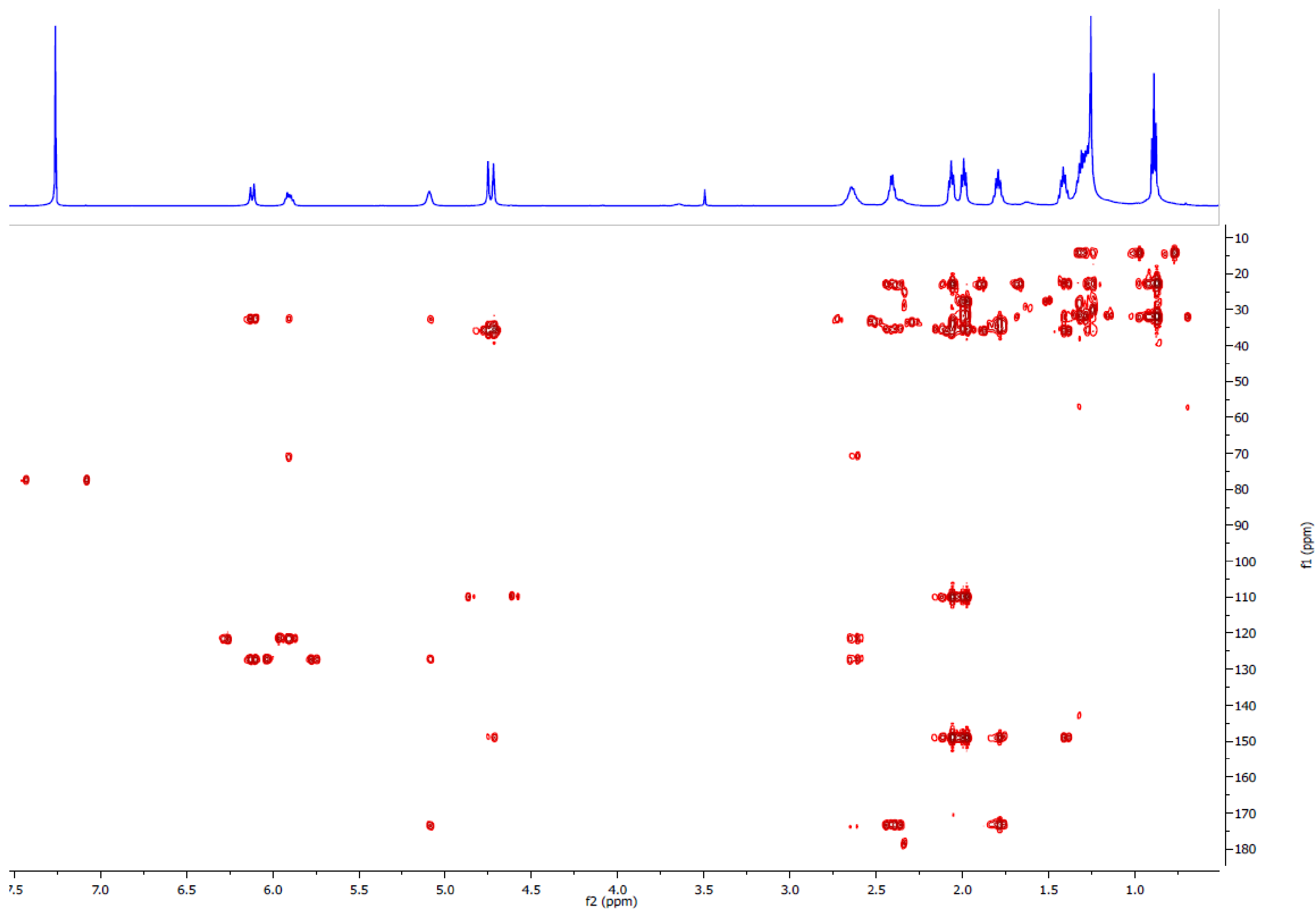


Fig. S13: HMBC Spectrum of Pitinoic acid B (**2**) in $CDCl_3$ (600 MHz)

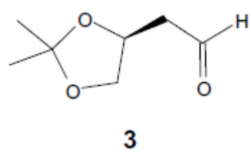
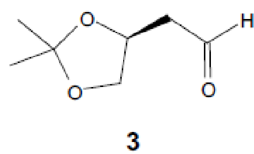


Fig. S14: ^{1}H NMR Spectrum of Intermediate **3** in $CDCl_3$ (400 MHz). Residual Solvent Peak (DCM) is Denoted with Asterisks.



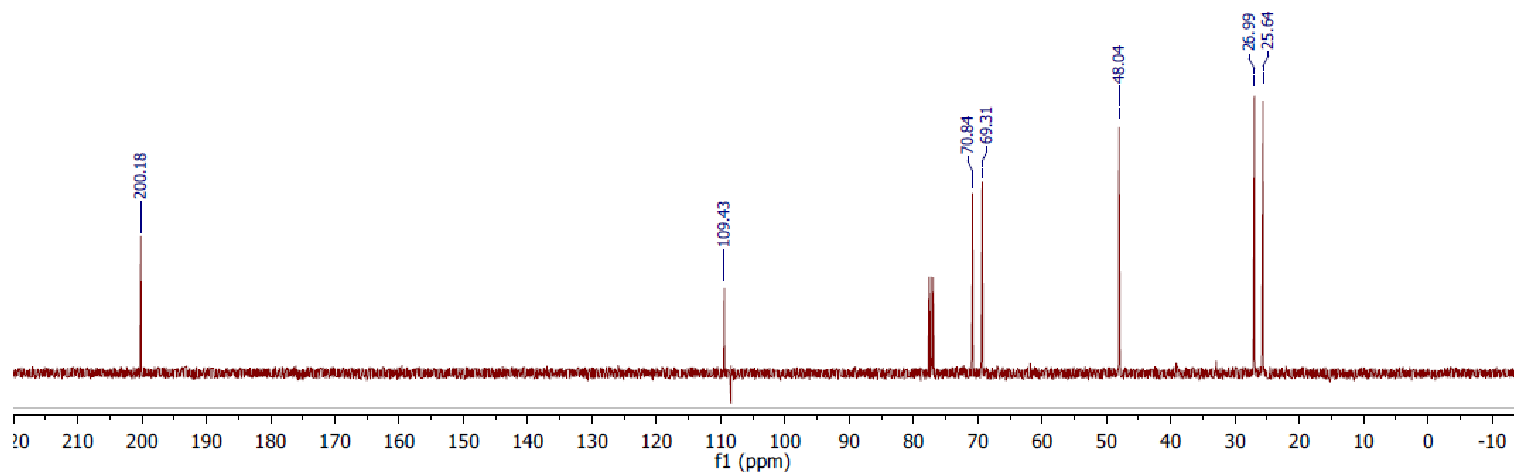
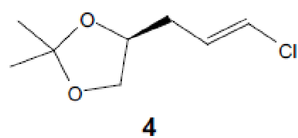


Fig. S15:^{13}C NMR Spectrum of Intermediate **3** in $CDCl_3$ (100 MHz)



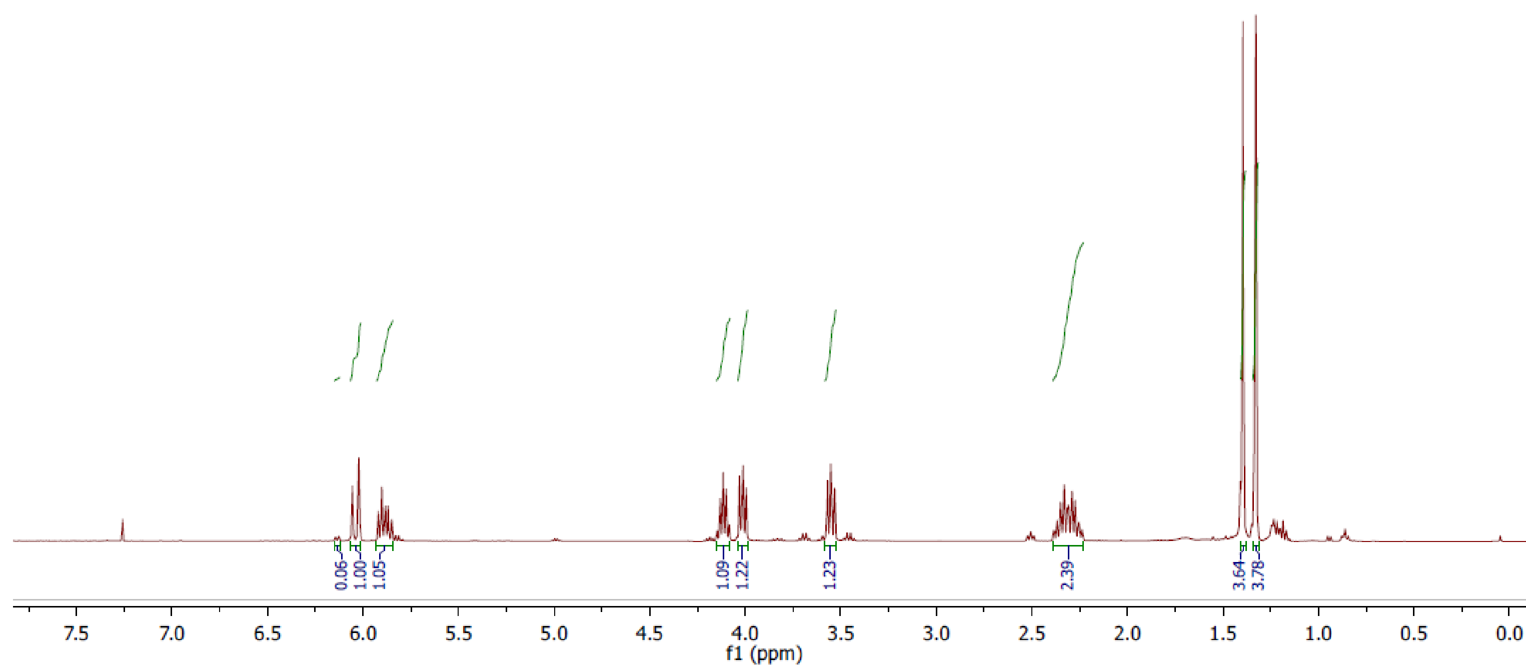
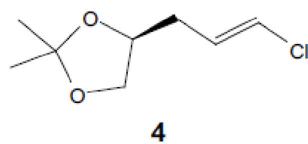


Fig. S16: ^{1}H NMR Spectrum of Intermediate **4** in $CDCl_3$ (400 MHz)



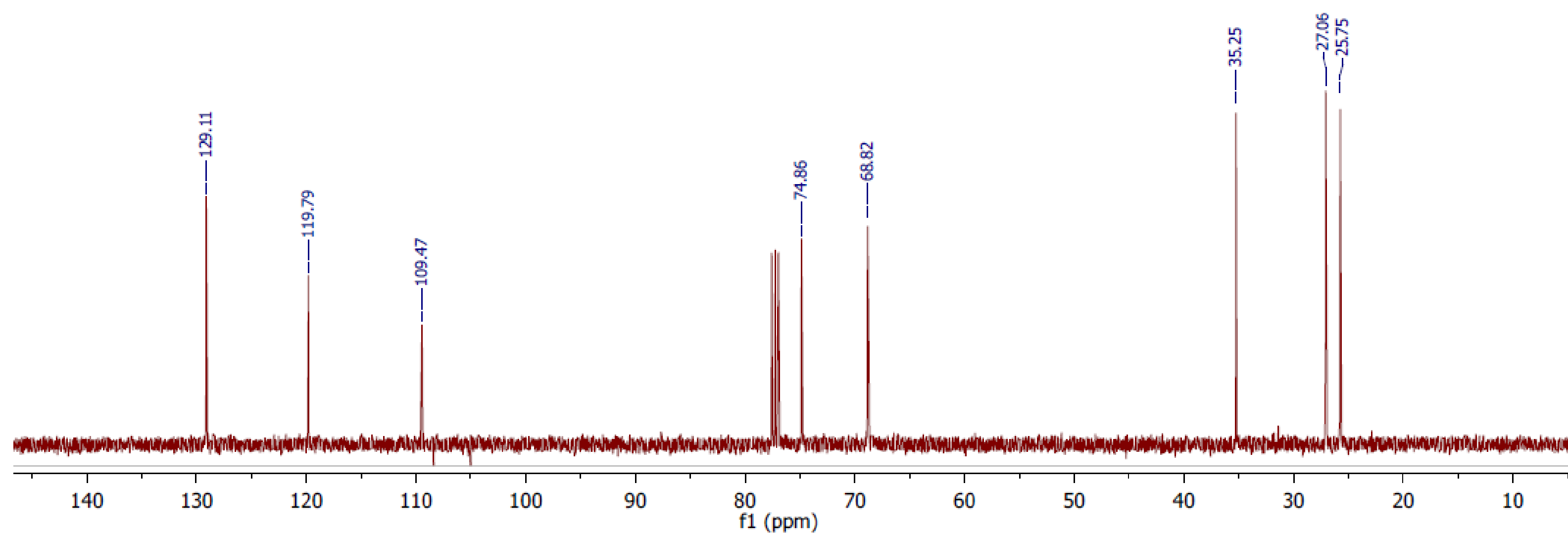
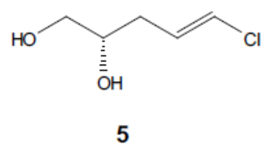


Fig. S17: ^{13}C NMR Spectrum of Intermediate **4** in $CDCl_3$ (100 MHz)



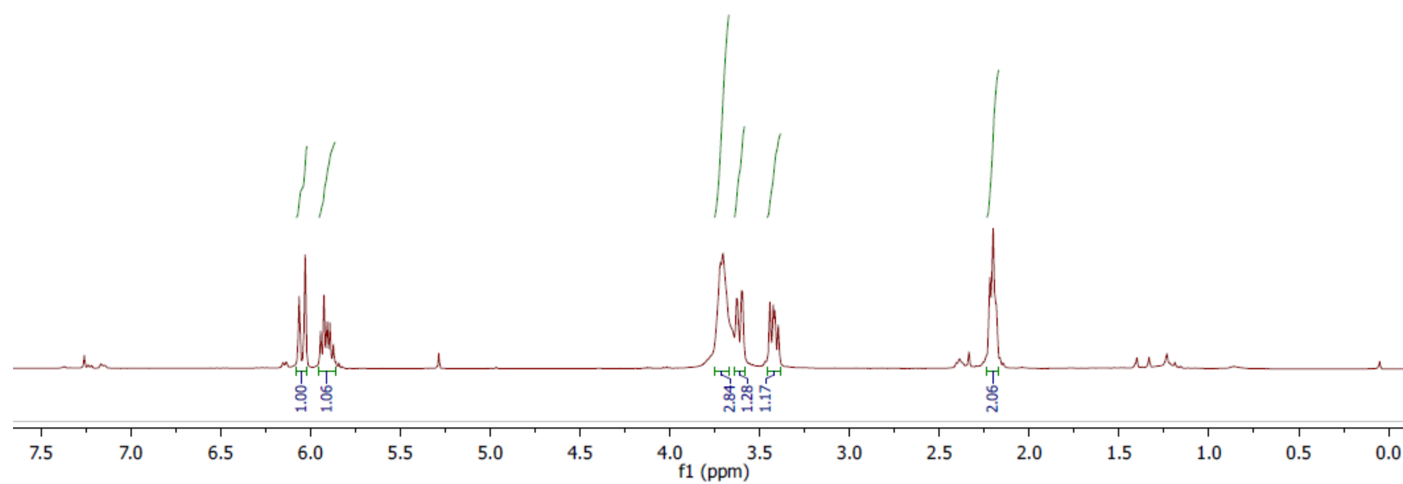
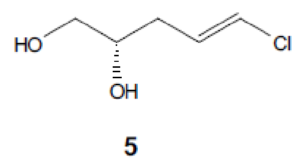


Fig. S18: ^{1}H NMR Spectrum of Intermediate **5** in $CDCl_3$ (400 MHz)



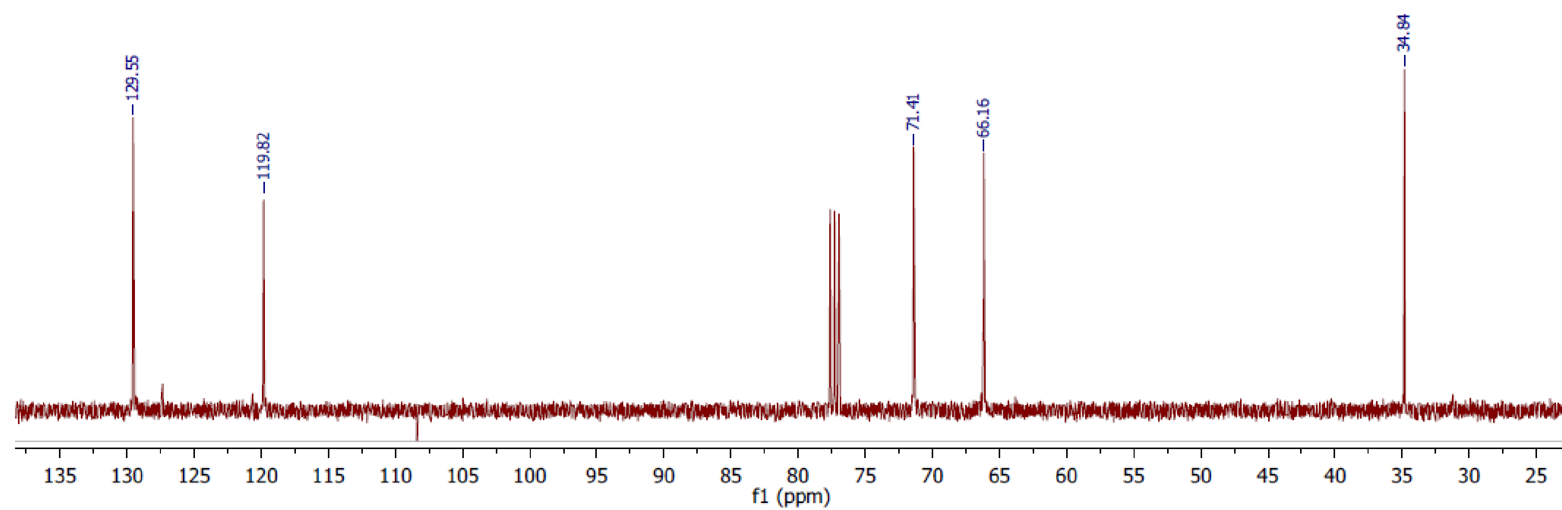


Fig. S19: ^{13}C NMR Spectrum of Intermediate **5** in $CDCl_3$ (100 MHz)

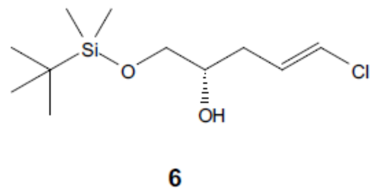


Fig. S20: ^{1}H NMR Spectrum of Intermediate **6** in $CDCl_3$ (400 MHz)

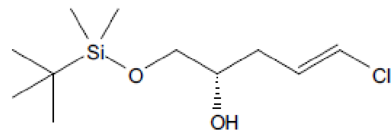


Fig. S21: ^{13}C NMR Spectrum of Intermediate **6** in $CDCl_3$ (100 MHz)

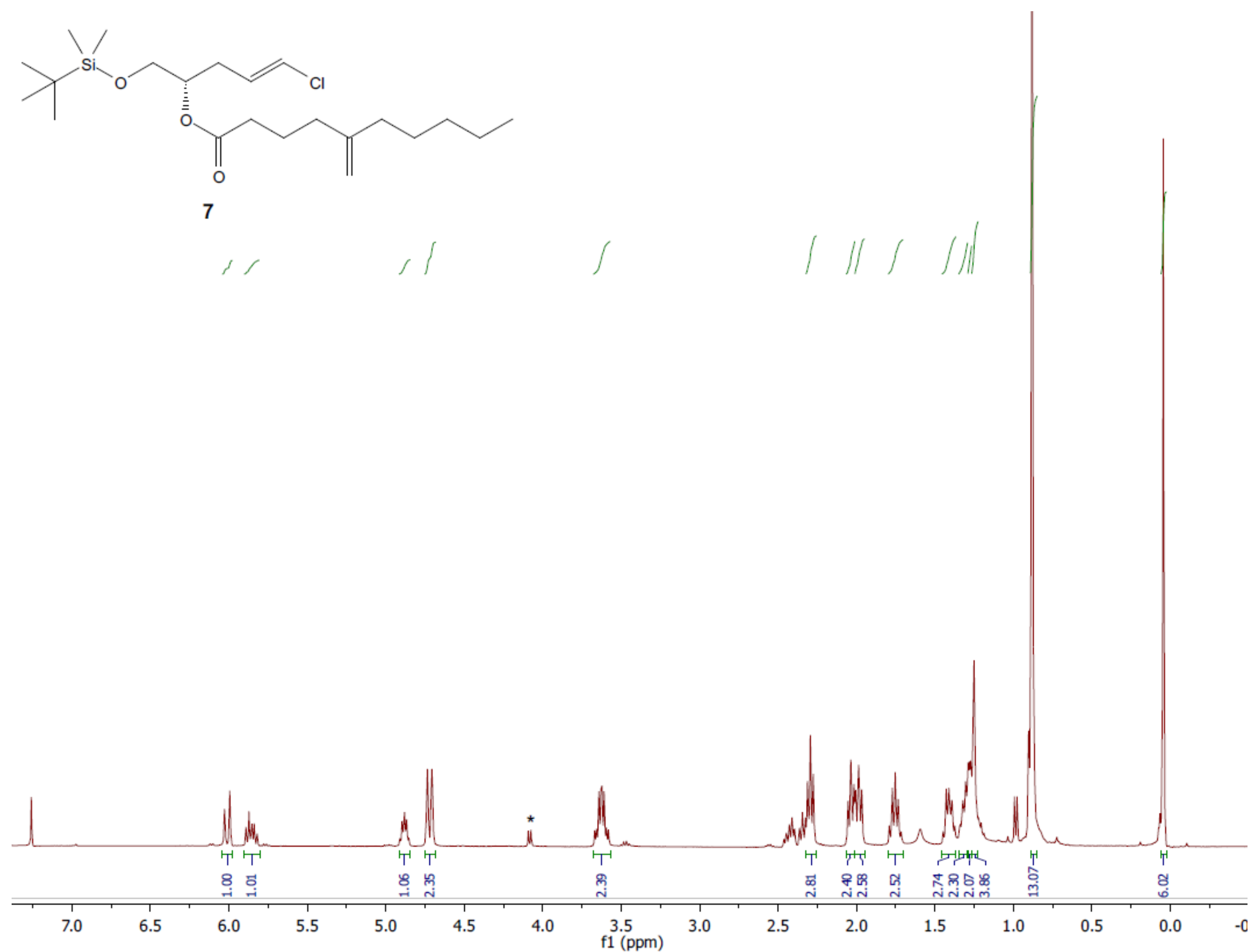
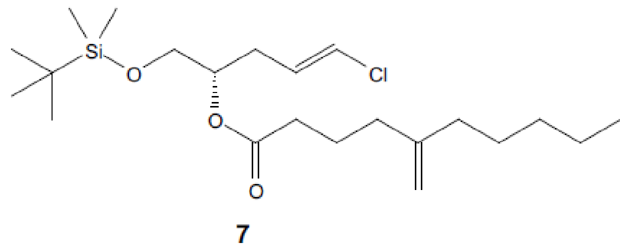


Fig. S22: ^{1}H NMR Spectrum of Intermediate **7** in $CDCl_3$ (400 MHz). Residual Solvent (EtOAc) Peaks are Denoted with Asterisks.



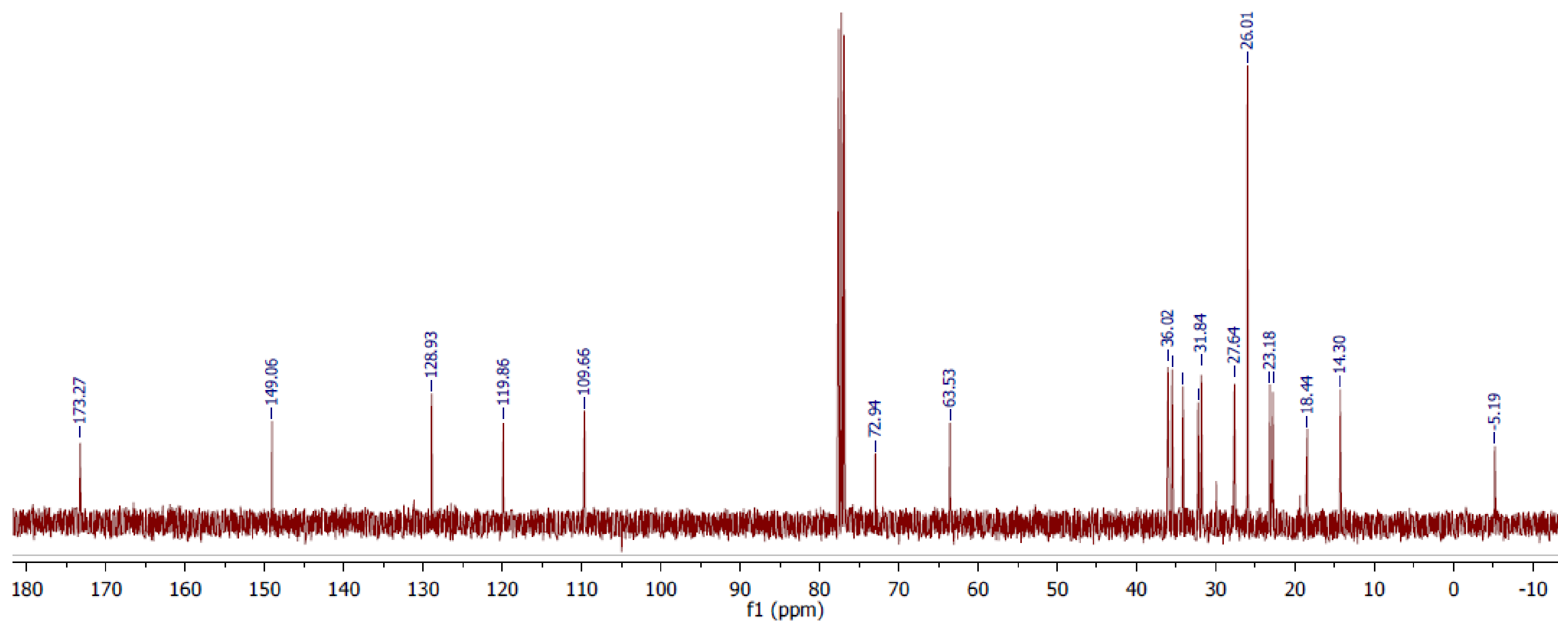
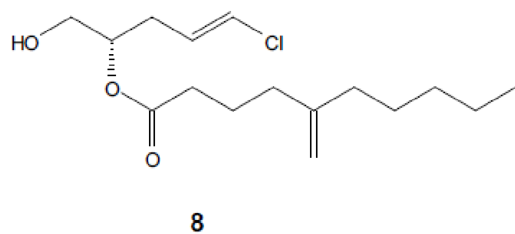


Fig. S23: ^{13}C NMR Spectrum of Intermediate **7** in $CDCl_3$ (100 MHz)



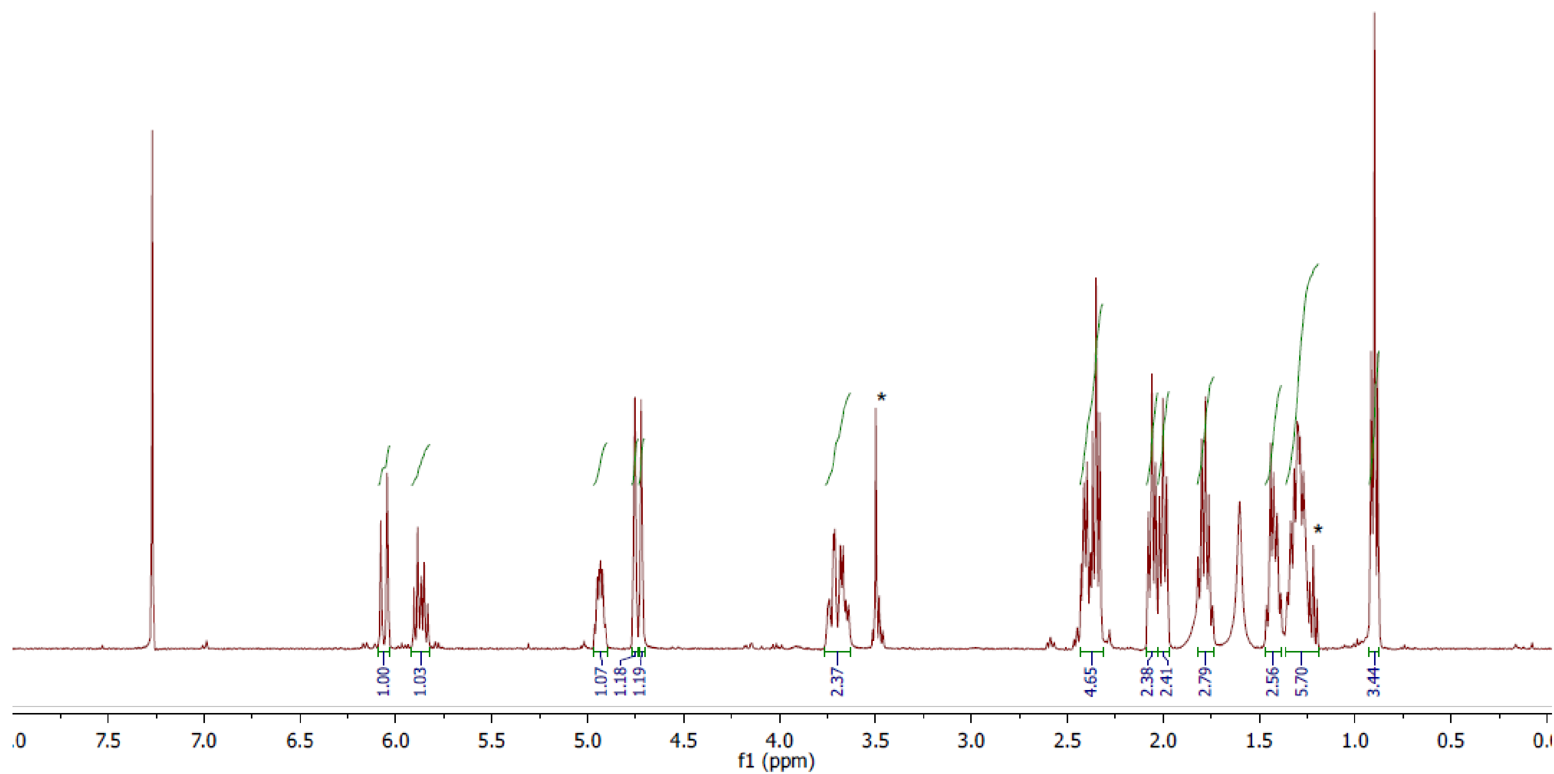
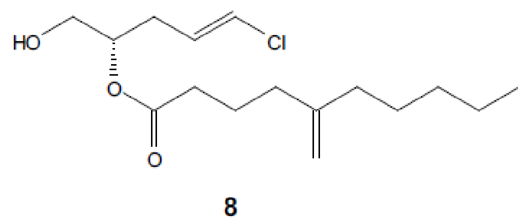


Fig.S24:^{1}H NMR Spectrum of Intermediate **8** in $CDCl_3$ (400 MHz). Residual Solvent (Et_2O) Peaks are Denoted with Asterisks.



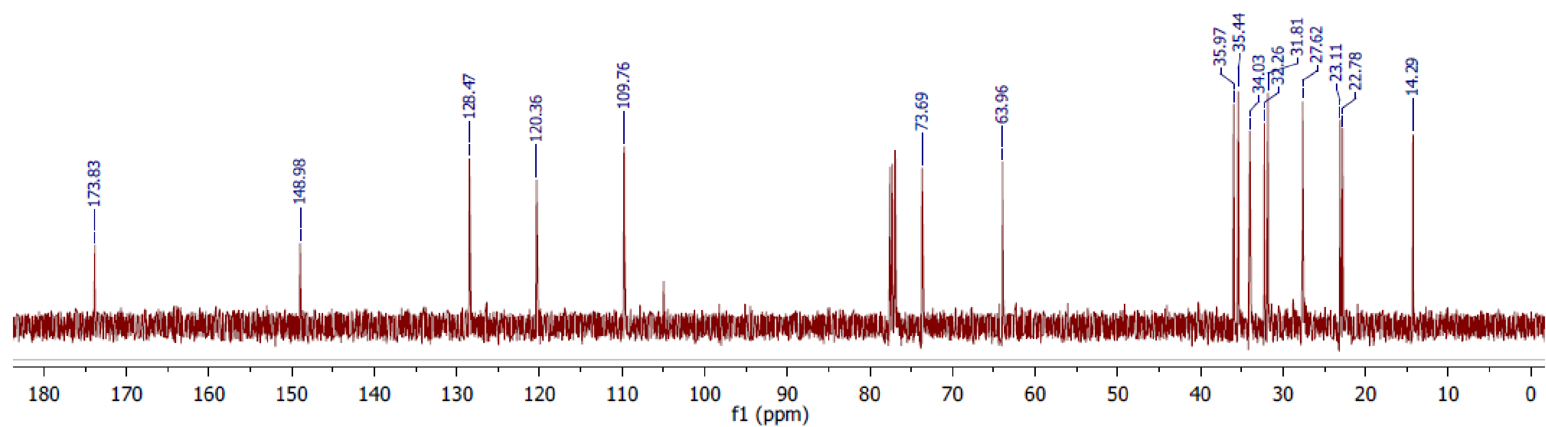


Fig. S25: ^{13}C NMR Spectrum of Intermediate **8** in $CDCl_3$ (100 MHz)

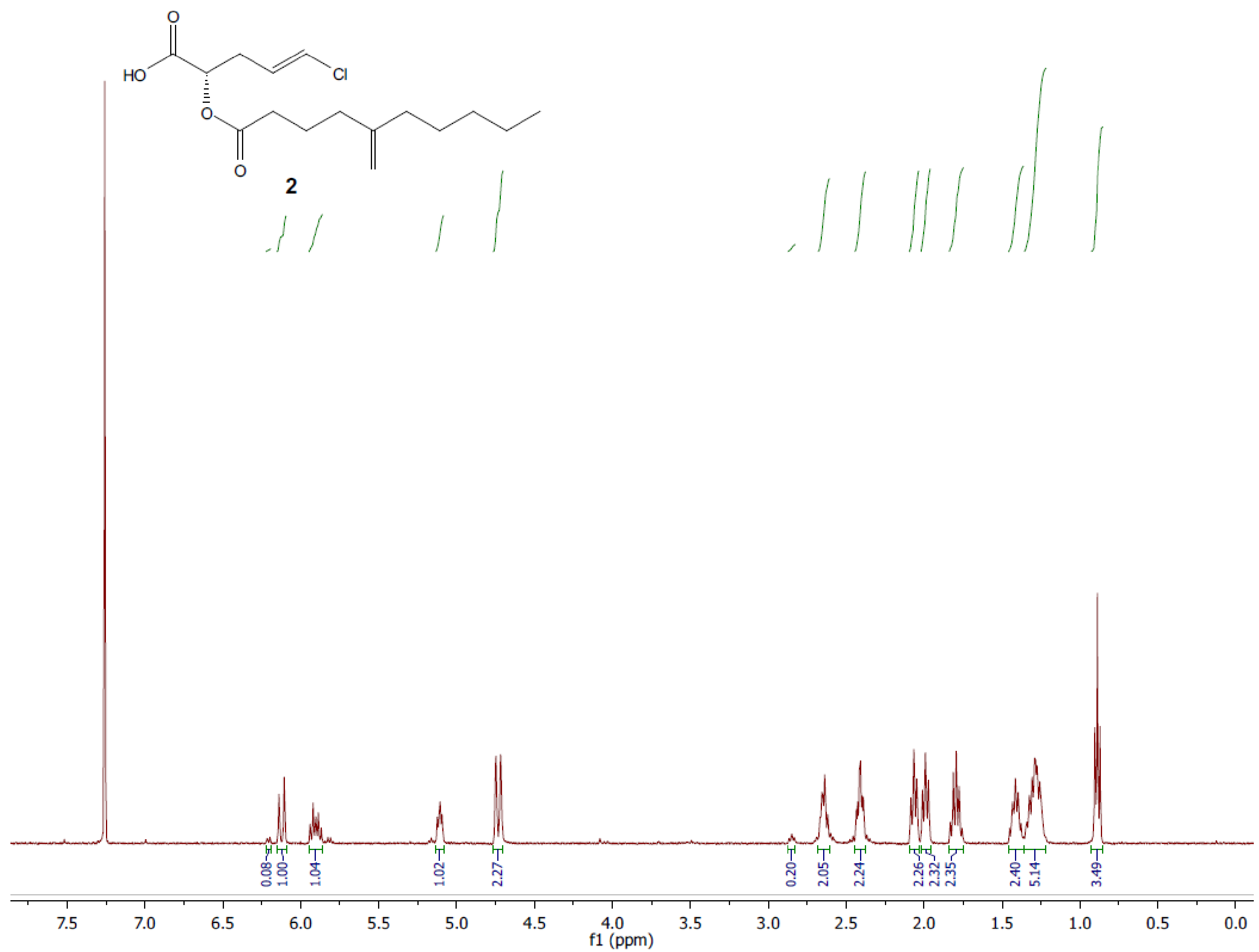


Fig.S26: ^{1}H NMR Spectrum of the Synthetic Ester **2** in $CDCl_3$ (400 MHz).

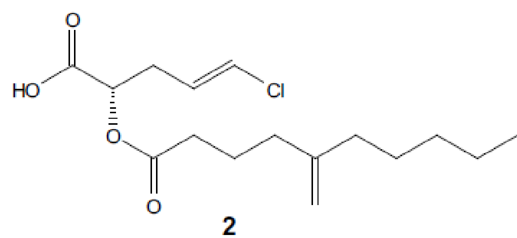


Fig. S27: ^{13}C NMR Spectrum of the Synthetic Ester **2** in $CDCl_3$ (100 MHz)

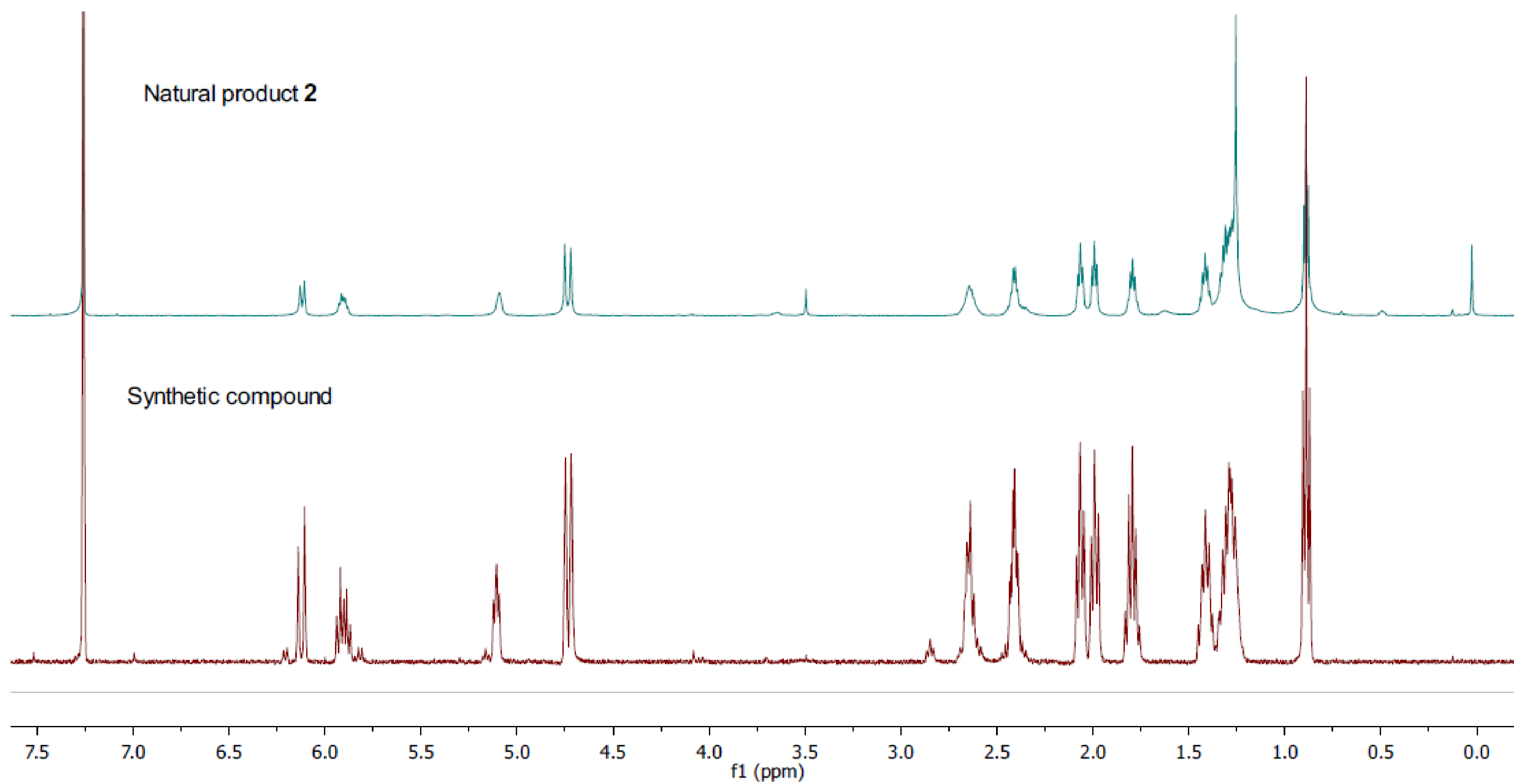


Fig. S28: Comparison of ^{1}H NMR Spectra of the Natural Product Pitinoic Acid B (**2**) and the Synthetic Compound

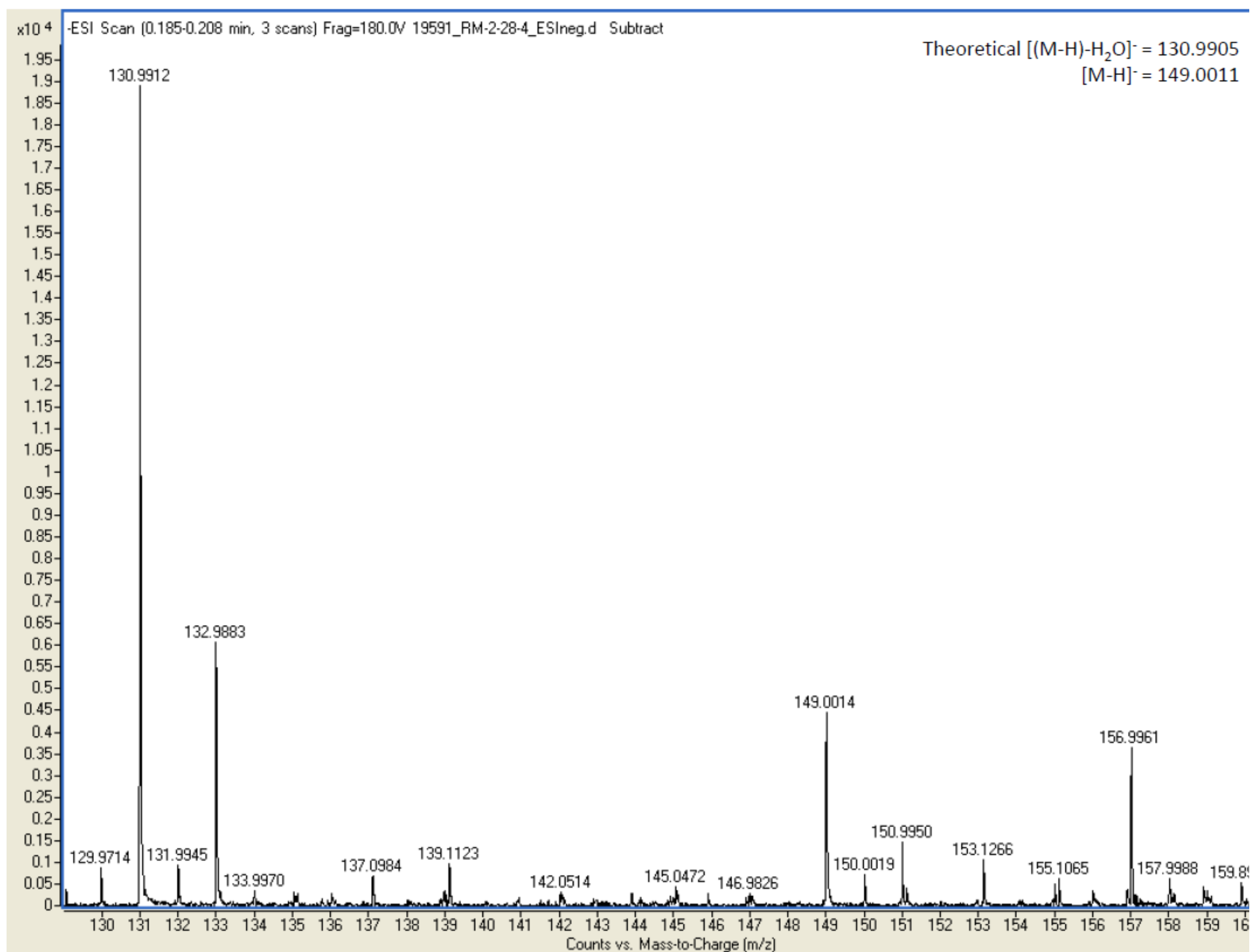


Fig. S29: HRESIMS Spectrum for the HPLC Fraction Containing the α-Hydroxy Acid Moiety Pitinoic Acid C. Peaks at *m/z* 149.0014/150.9950 [M-H]$^-$ and *m/z* 130.9912/132.9883 [(M-H)-H_2O]$^-$ confirm the presence of this fragment in this fraction.

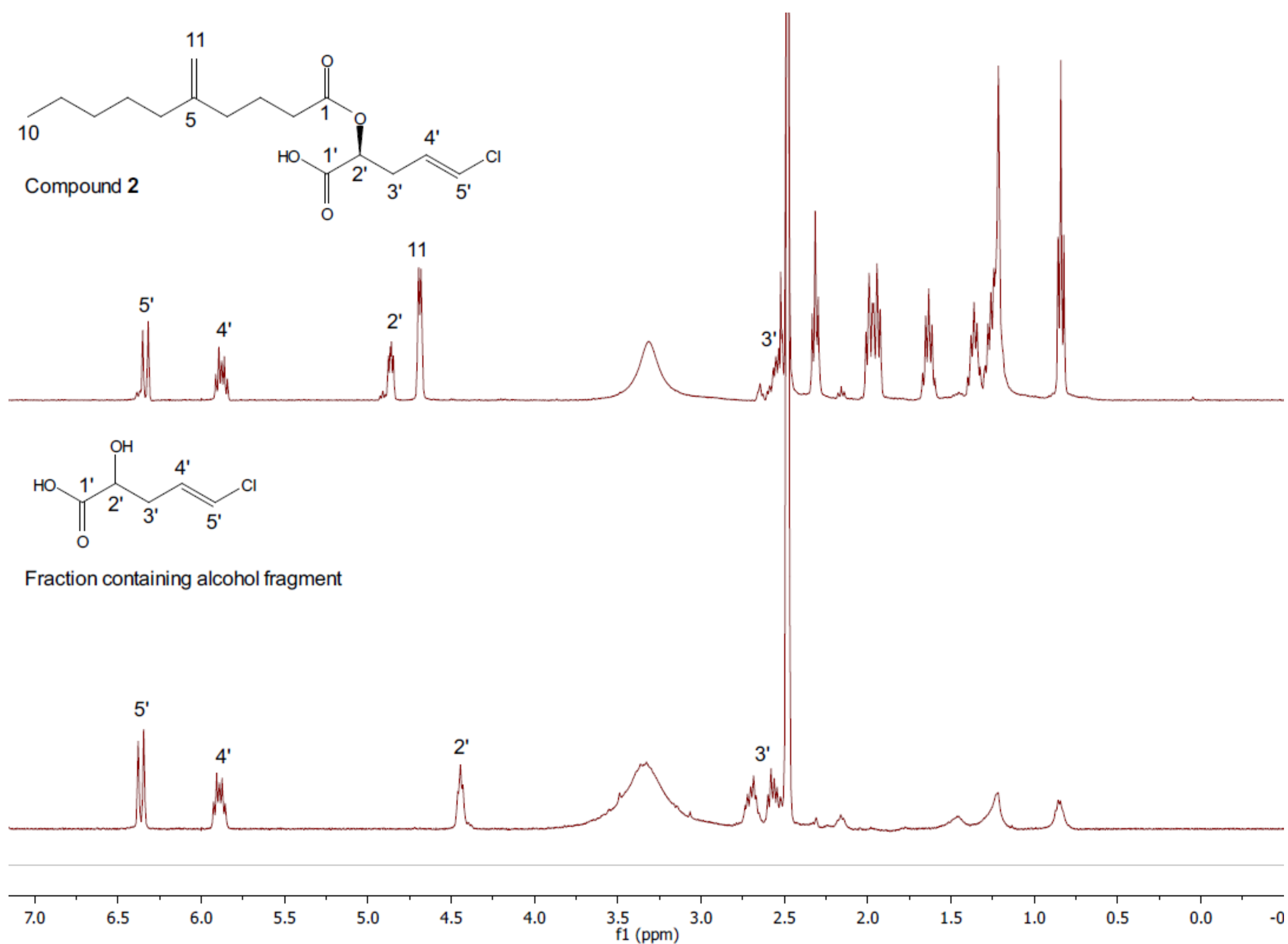


Fig. S30: Comparison of the ^{1}H NMR Spectra of Pitinoic Acid B (**2**) (Upper Spectrum) and the Fraction Containing the α-Hydroxy Acid Part, Pitinoic Acid C (Lower Spectrum), in DMSO-d_6. Pitinoic acid C appears to be the major compound in this fraction.

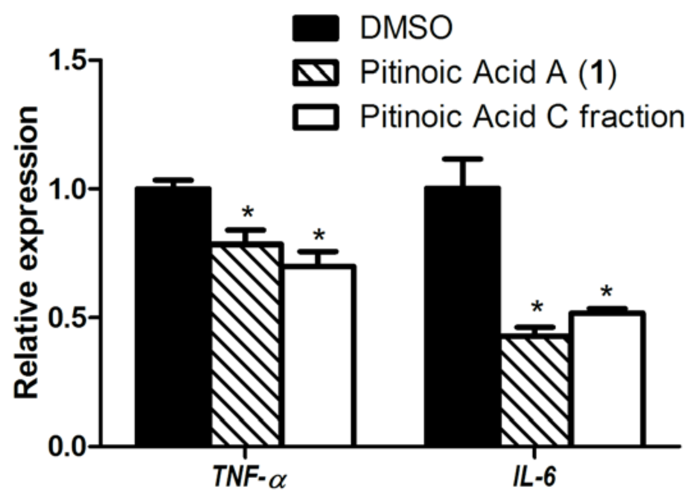


Fig. S31: Effect of Pitinoic Acid A (**1**) (100 μM) and the HPLC fraction containing the α-Hydroxy Acid Pitinoic Acid C (15 μg/mL) on Transcript Levels of Pro-inflammatory Cytokines in Differentiated THP-1 Cells after 4 h. **P*-value < 0.05, n = 3. Results are calculated relative to the endogenous control *GAPDH*. Data are presented as mean ± SD.