Supporting Information II

for

Peloruside B, a New and Potent Antitumor Macrolide from the New Zealand Marine Sponge *Mycale hentscheli*: Isolation, Total Synthesis and Bioactivity

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Contents

Experimental for Natural Peloruside B

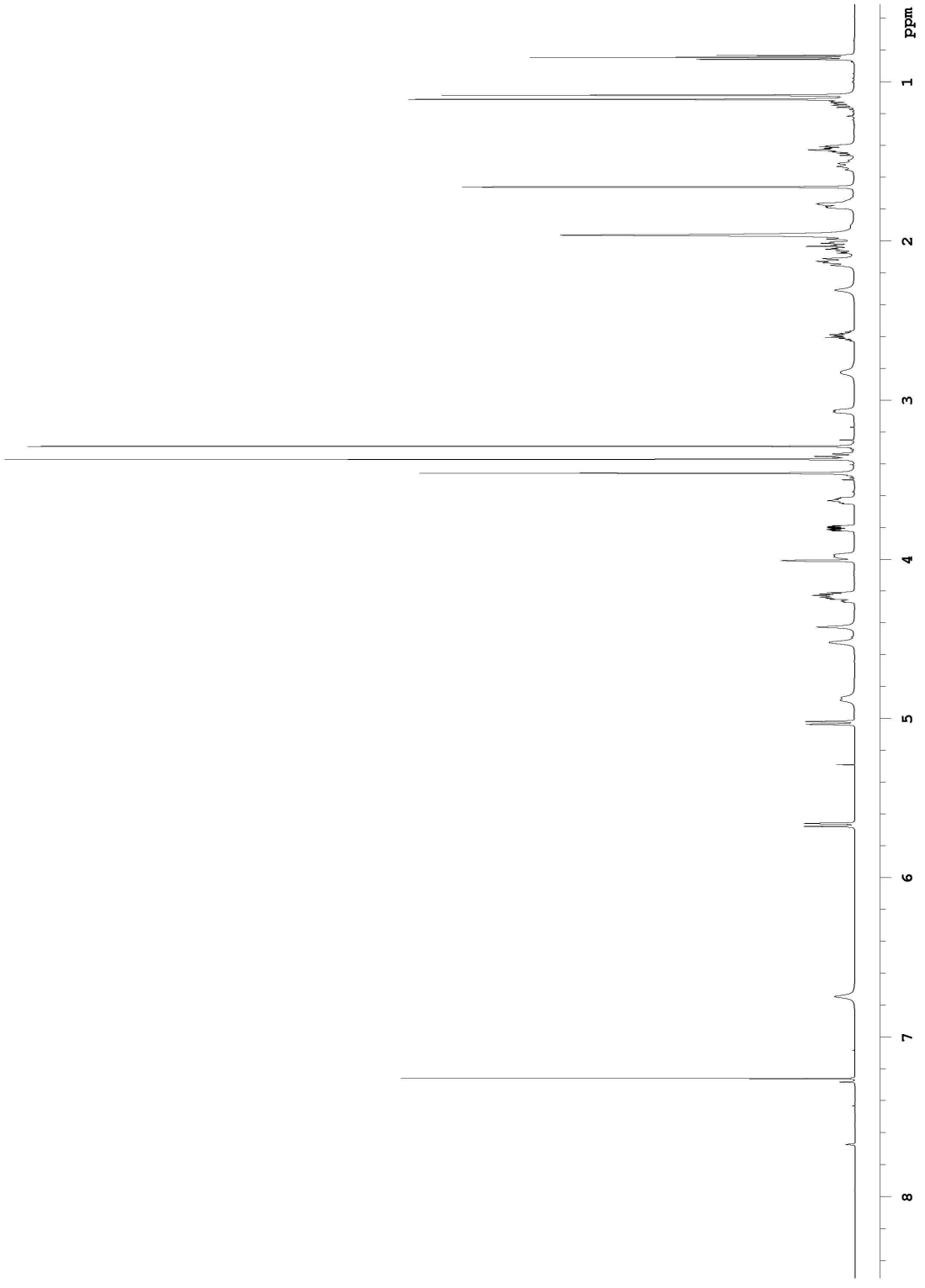
General: 600 MHz NMR spectra for both natural and synthetic peloruside B (**2**) were obtained using the same spectrometer equipped with a triple resonance HCN cryogenic probe, operating at 600 MHz or 150 MHz for ^{1}H and ^{13}C nuclei respectively. Chemical shifts δ (ppm) were referenced to the residual solvent peak (δ_H 7.26 ppm, δ_C 77.16 ppm for $CDCl_3$). Catalytic amounts (1–2 μL) of d_5-pyridine were added to each NMR sample performed in $CDCl_3$ to prevent compound degradation due to trace acidity associated with the solvent. High-resolution positive-ion mass spectra were recorded on a TOF electrospray mass spectrometer. Normal-phase column chromatography was carried out using 2,3-dihydroxypropoxypropyl-derivatized silica (DIOL). Reversed-phase column chromatography was achieved using HP20 or Amberchrom poly(styrene divinylbenzene) (PSDVB) chromatographic resin. HPLC was performed using a solvent delivery module equipped with 25 mL pump heads. Solvents used for flash normal- and reversed-phase column chromatography were of HPLC or analytical grade quality. All other solvents were purified by glass-distillation. Solvent mixtures are reported as % vol/vol unless otherwise stated. Specimens were stored at −20 °C until required

Isolation of Natural Peloruside B: *Mycale hentscheli*, (230 g, NIWA no. MNP 0026), collected at a depth of 23 m from Kapiti Island, New Zealand, was cut into small segments and extracted with MeOH (2 × 700 mL) for 24 h. The combined extracts were loaded on to HP20 PSDVB beads, washed with H_2O and eluted with i) 20% Me_2CO/H_2O, ii) 55% Me_2CO/H_2O, iii) 55% Me_2CO/0.2 M NH_4OH and iv) 55% Me_2CO/0.2 M NH_4OH adjusted to pH 4 with AcOH. Fraction ii) was concentrated to dryness to yield 82.0 mg of a viscous brown oil. The resulting oil was dissolved in MeOH, loaded onto Amberchrom PSDVB and eluted with increasing concentrations of MeOH in H_2O (10–100%). The 48–54% MeOH/H_2O fractions were concentrated to dryness to yield mycalamide D (3 mg). The 56–60% MeOH/H_2O fractions were

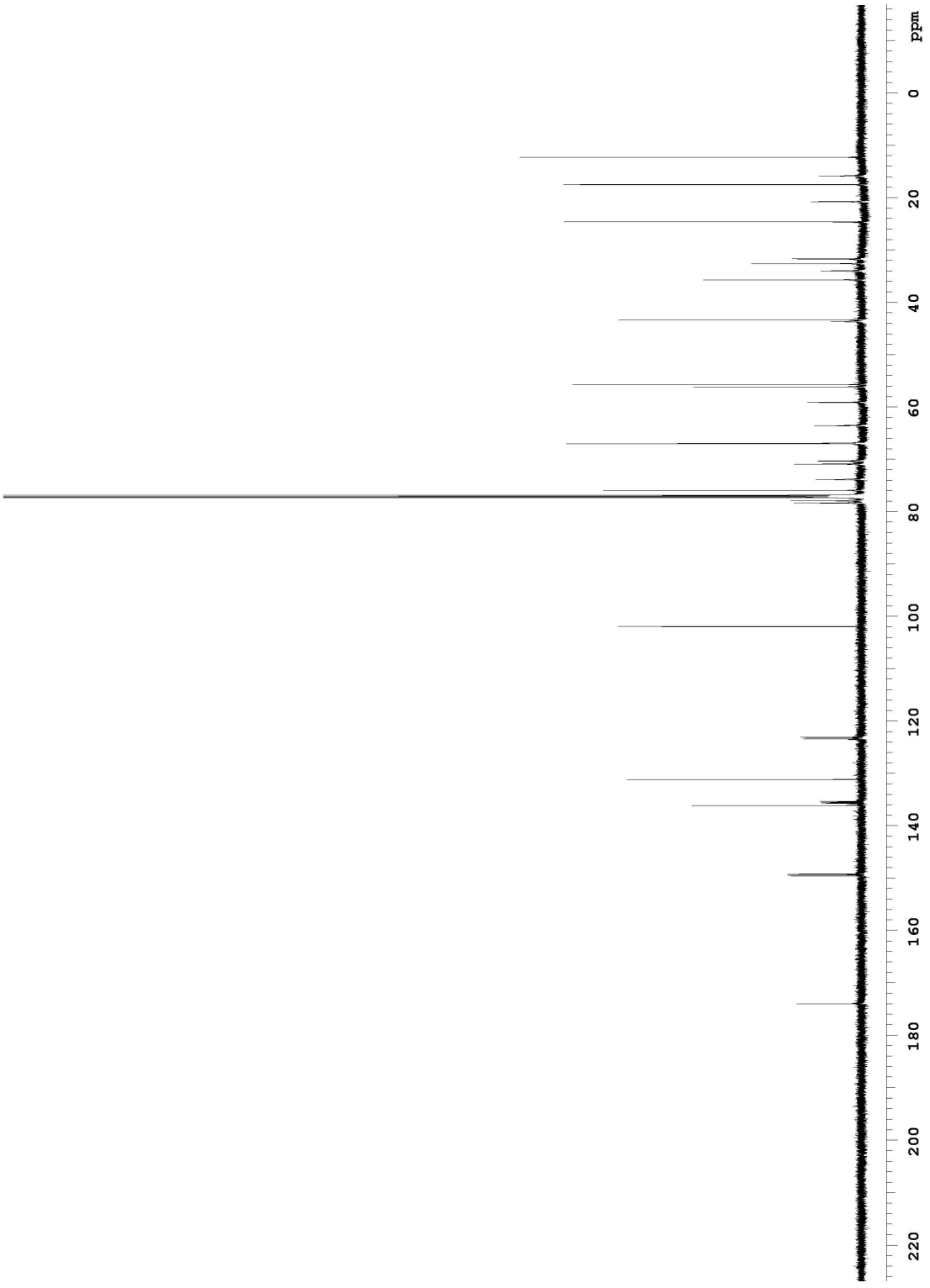
concentrated to dryness to yield peloruside A (**1**) (1.1 mg), and concentration of the 68–72% MeOH/H_2O fractions gave mycalamide A (7.0 mg). The 54–56% MeOH/H_2O fractions were concentrated to dryness to yield 5.0 mg of brown oil. A portion of this oil (3.0 mg) was recycled twice on DIOL with increasing concentrations of MeCN in CH_2Cl_2 (1–10%), and 50% MeOH/CH_2Cl_2. The 10% MeCN/CH_2Cl_2 and 50% MeOH/CH_2Cl_2 fractions were concentrated to dryness to yield a pale yellow oil (1.5 mg). This oil was then purified using HPLC (DIOL, 5 µm, 4 mm × 250 mm), with 20% i-PrOH/n-hexane as the mobile phase, collecting fractions at 1 min intervals to give 13.9 µg of mycalamide D, 9.4 µg of peloruside A (**1**) and 327 µg of peloruside B (**2**).

Peloruside A (1): Colorless film; all other data as previously published.

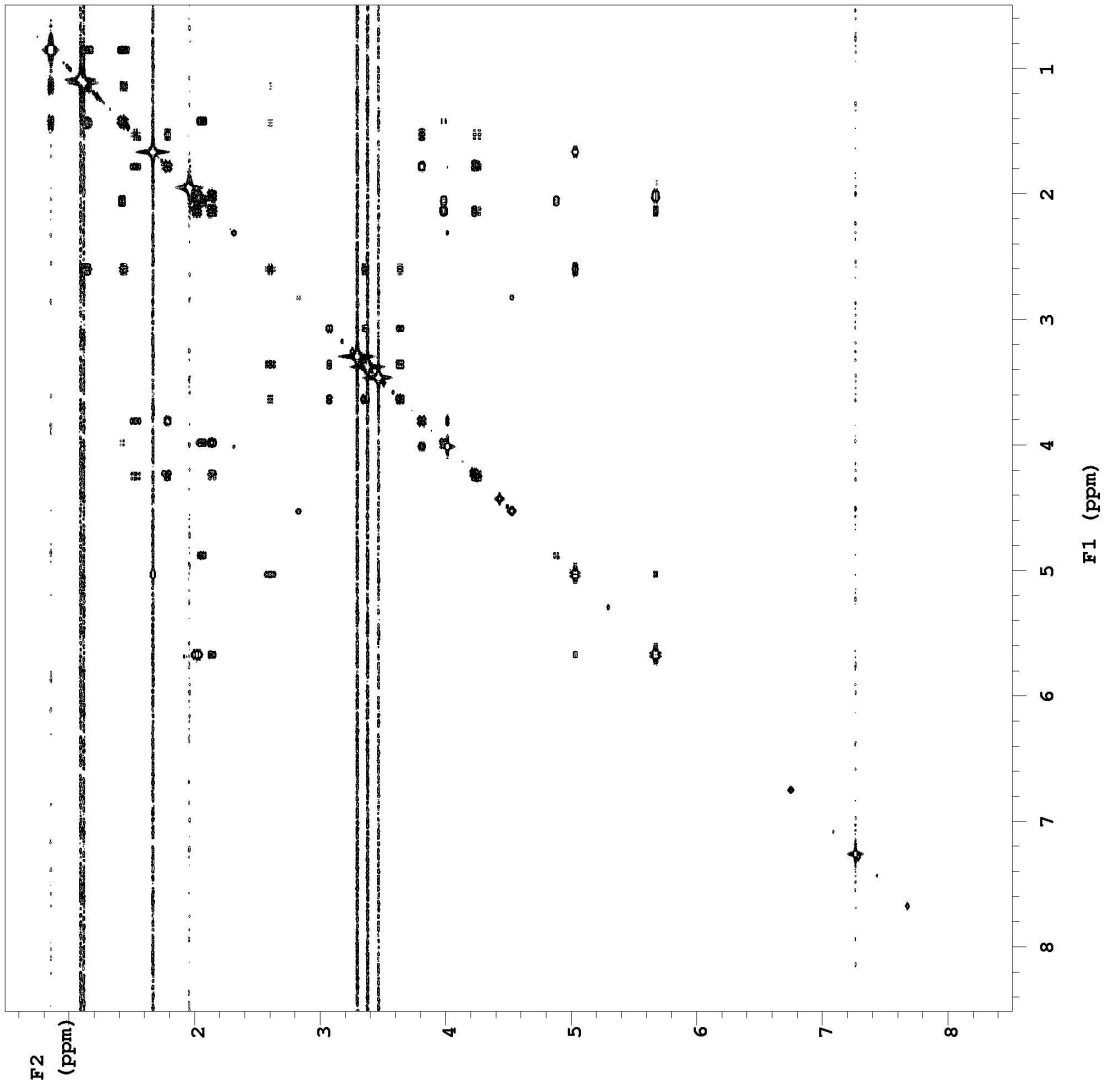
Peloruside B (2): Colorless film; $[\alpha]_D^{25}$ could not be determined accurately because of very small quantity of natural sample and magnitude of rotation was very small; NMR data see Table 1; HRESIMS, $[M + Na]^+$, observed m/z 557.29356, calculated 557.29323 for $C_{26}H_{46}O_{11}Na$, Δ = 0.58 ppm.



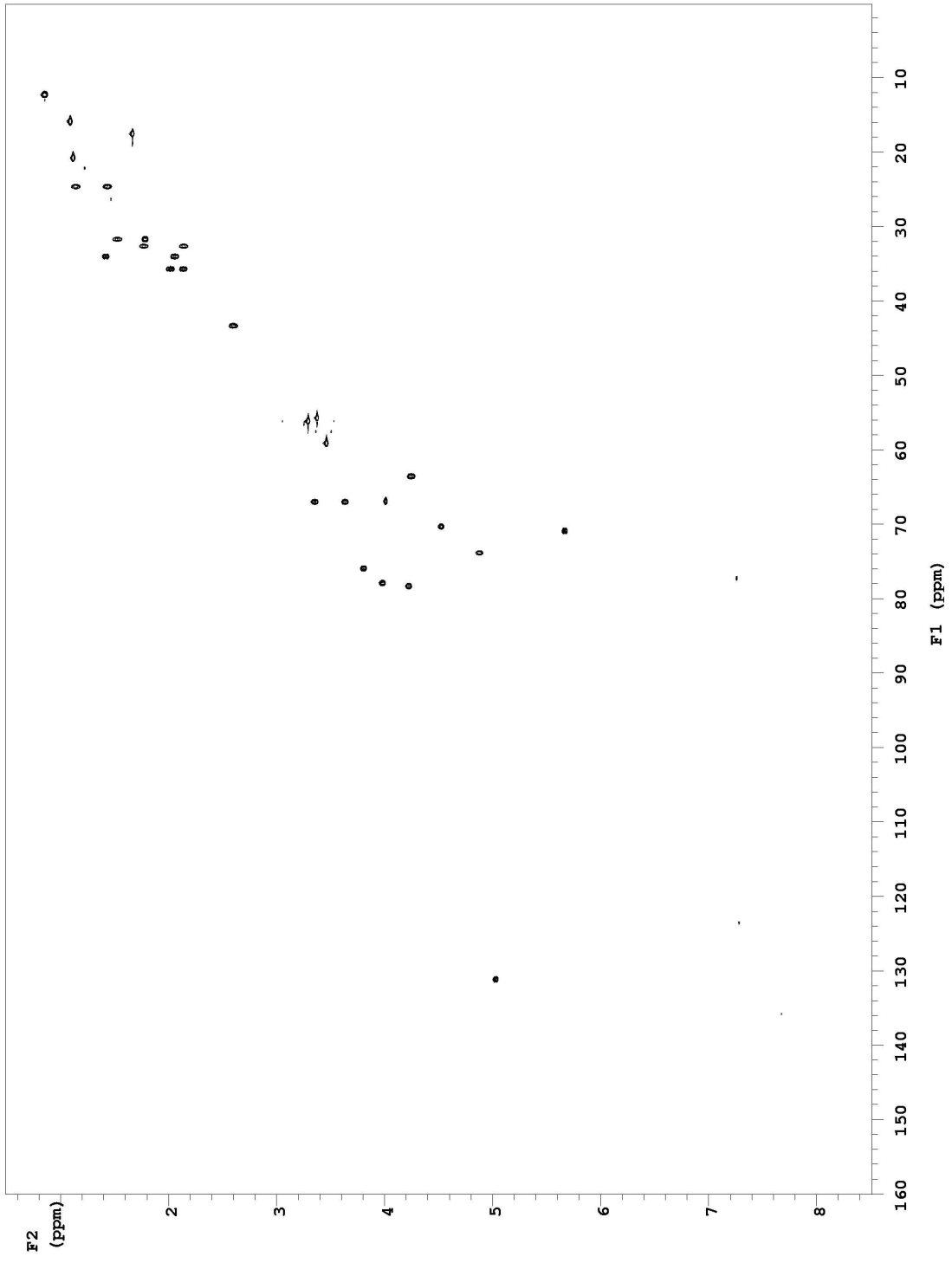
^{1}H NMR spectrum of peloruside A (**1**) in $CDCl_3$ (600 MHz).



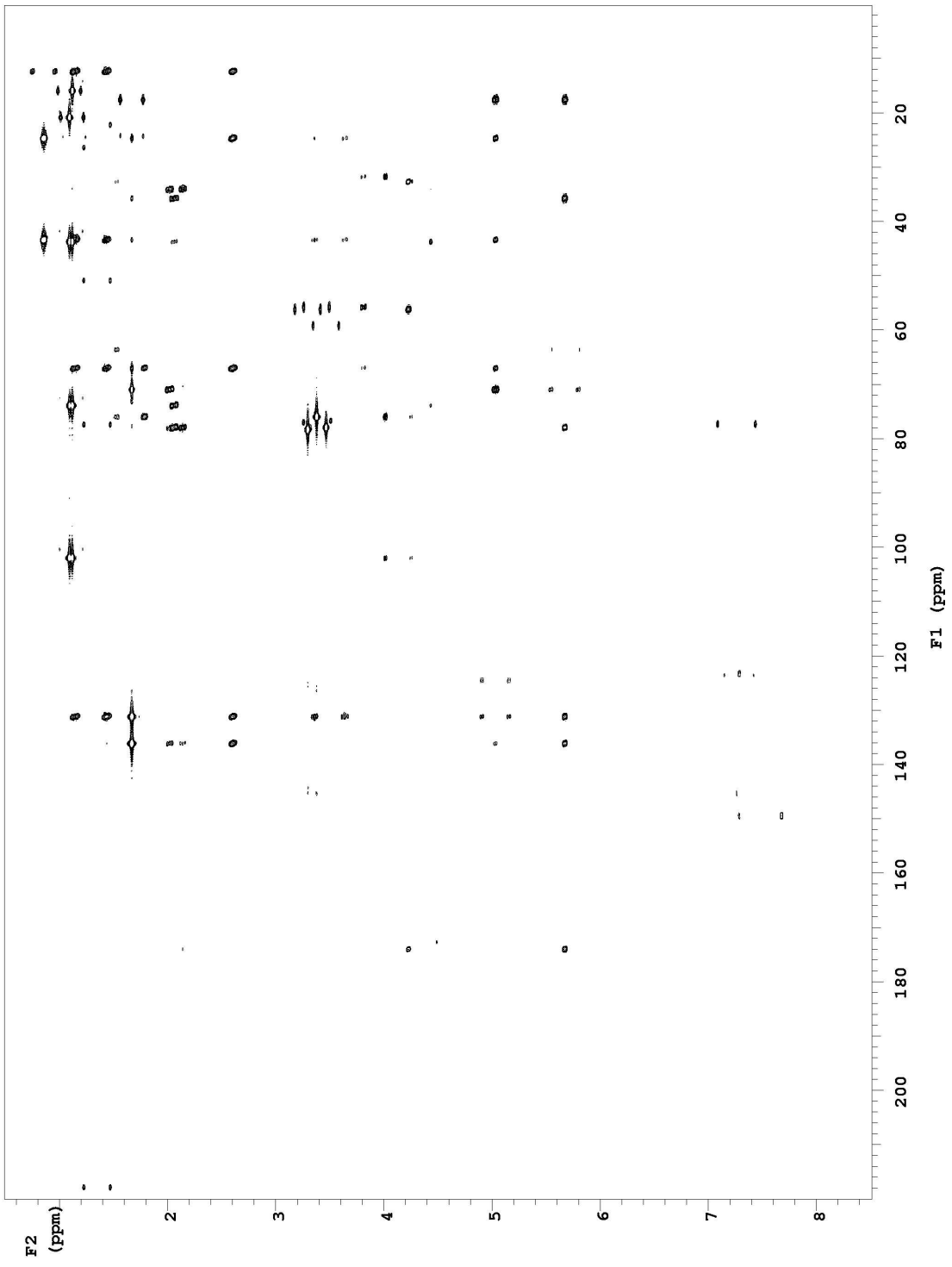
^{13}C NMR spectrum of peloruside A (**1**) in $CDCl_3$ (600 MHz).



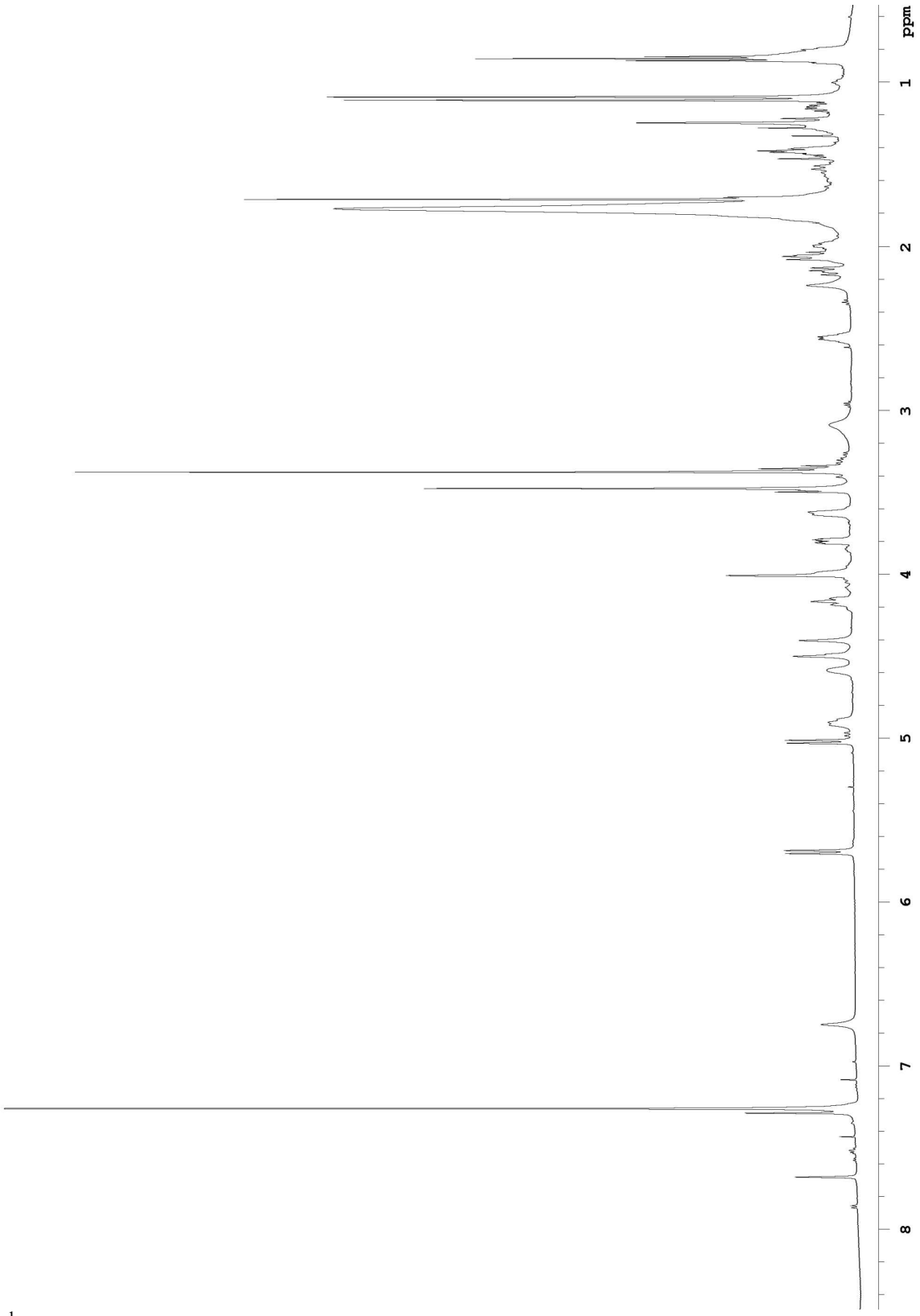
COSY NMR spectrum of peloruside A (**1**) in $CDCl_3$ (600 MHz).



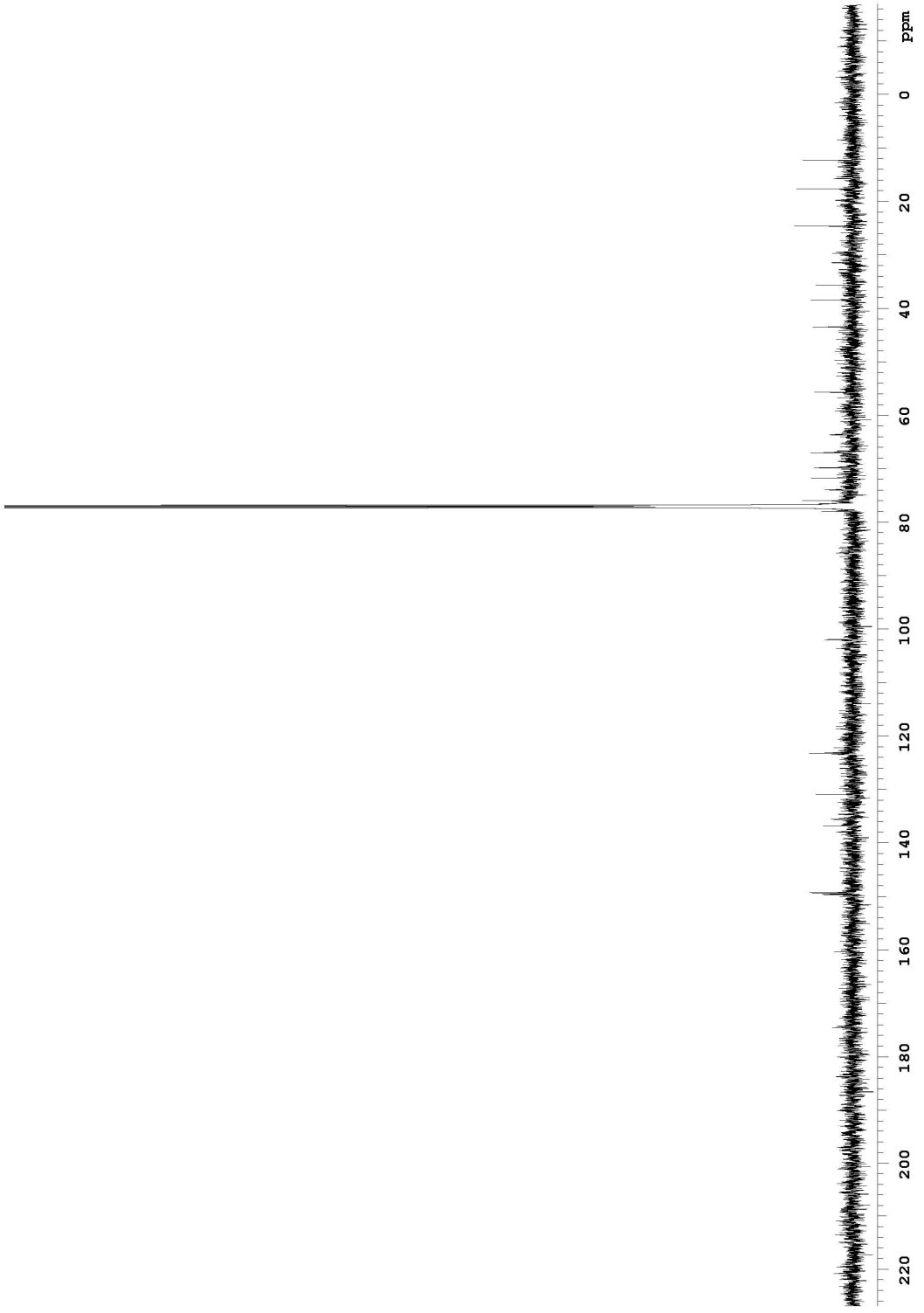
$HSQC_{ad}$ NMR spectrum of peloruside A (**1**) in $CDCl_3$ (600 MHz).



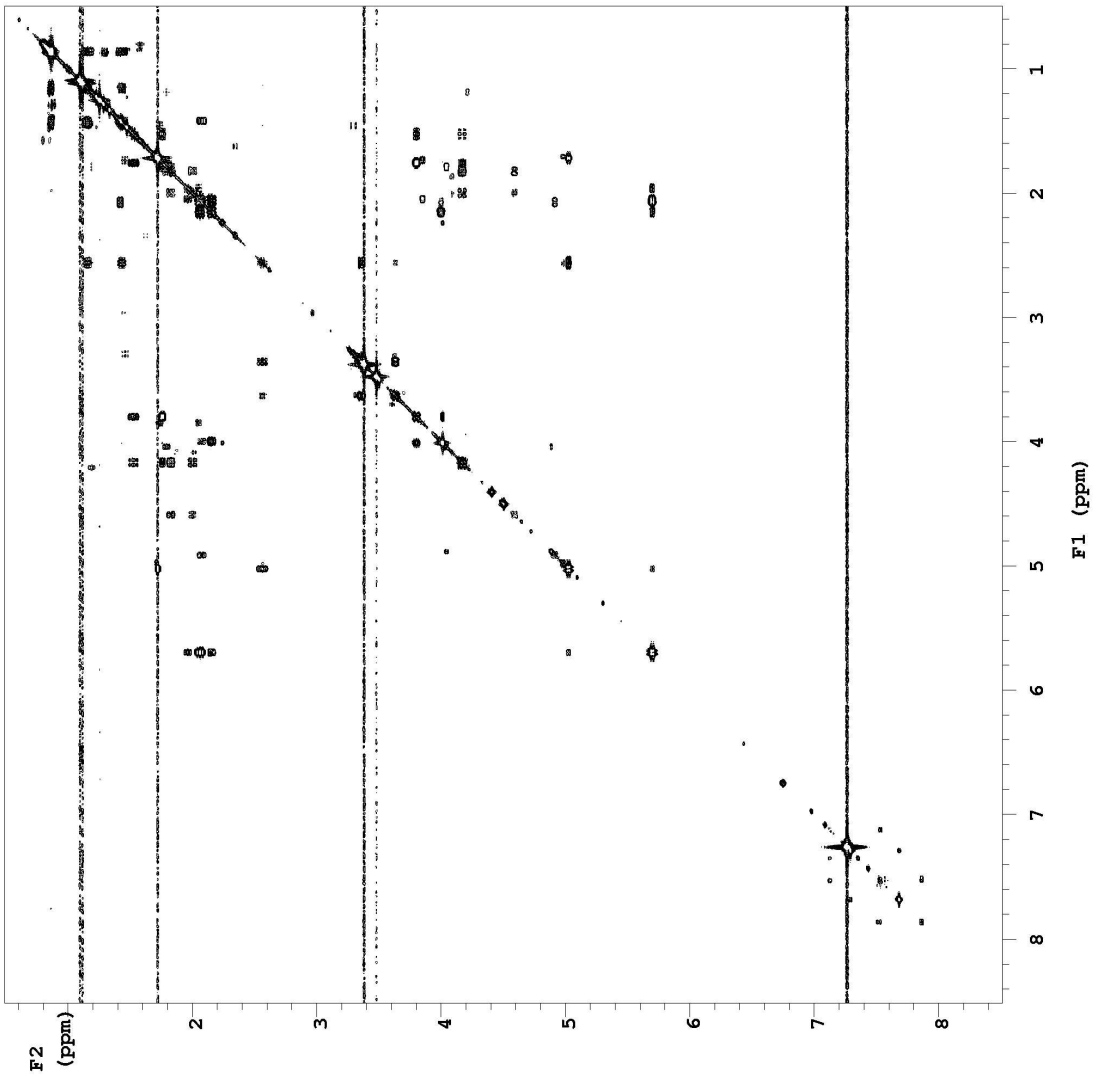
HMBC NMR spectrum of peloruside A (**1**) in $CDCl_3$ (600 MHz).



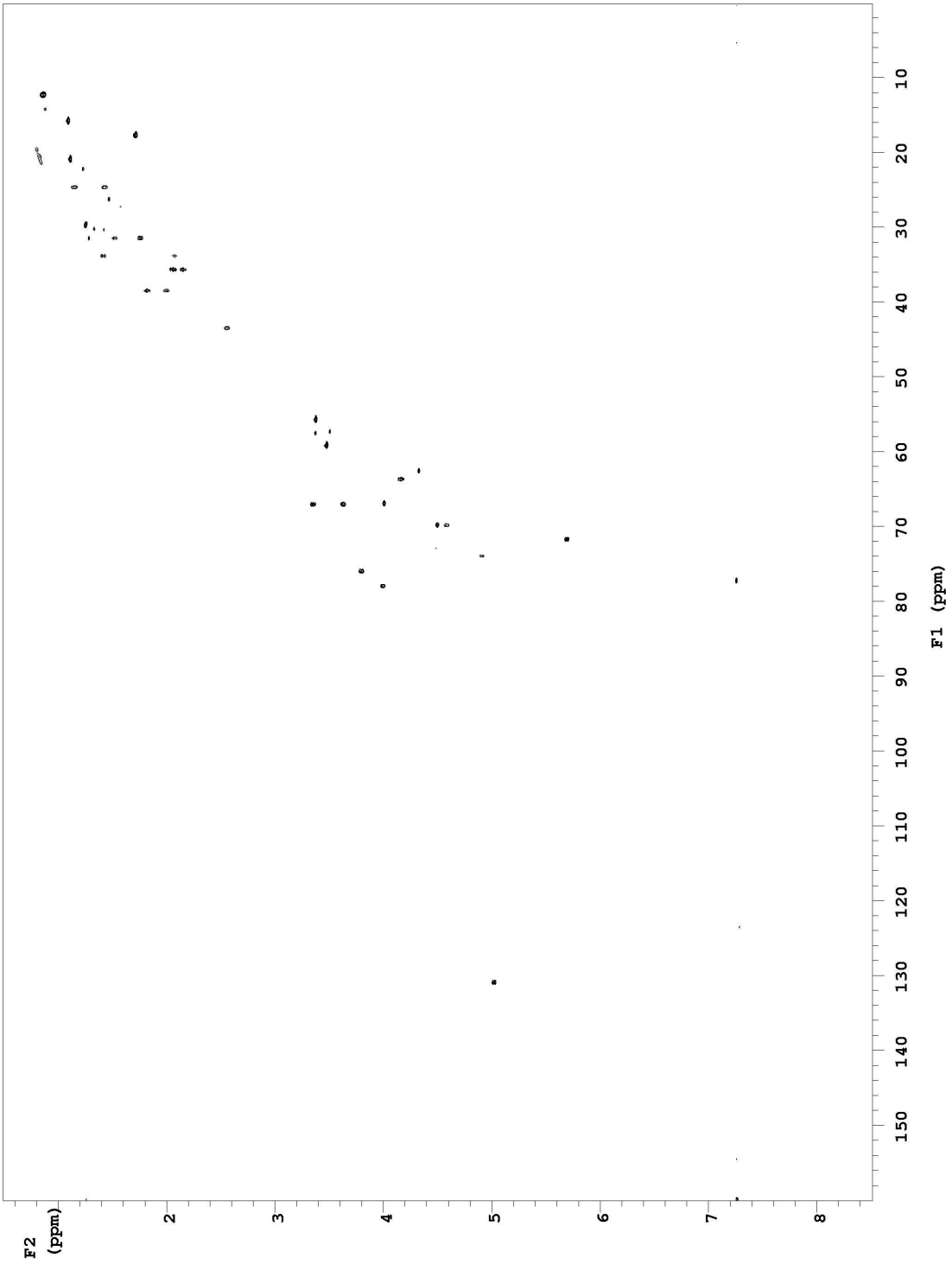
^{1}H NMR spectrum of *natural* peloruside B (**2**) in $CDCl_3$ (600 MHz).



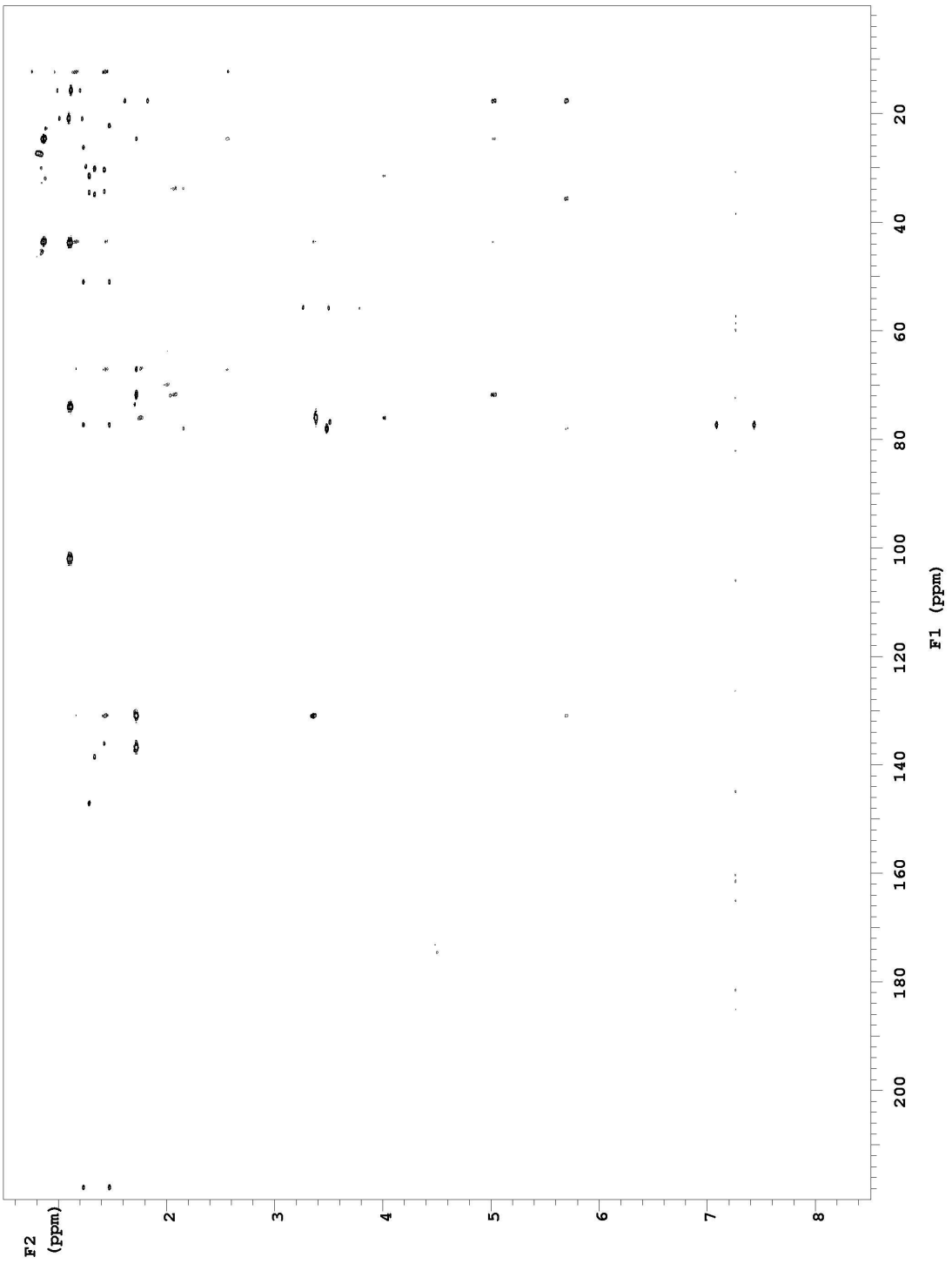
^{13}C NMR spectrum of *natural* peloruside B (**2**) in $CDCl_3$ (150 MHz).



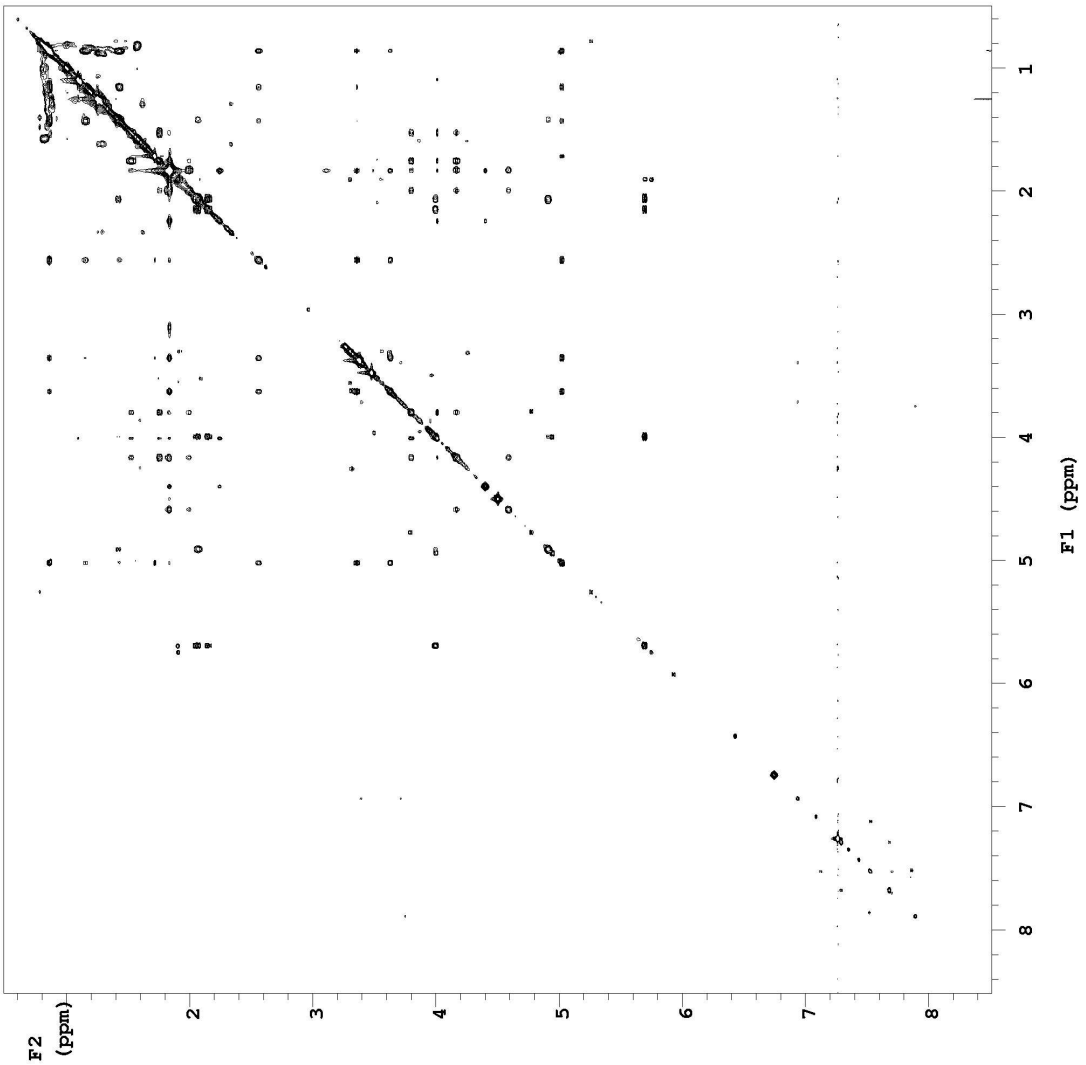
COSY NMR spectrum of *natural* peloruside B (**2**) in $CDCl_3$ (600 MHz).



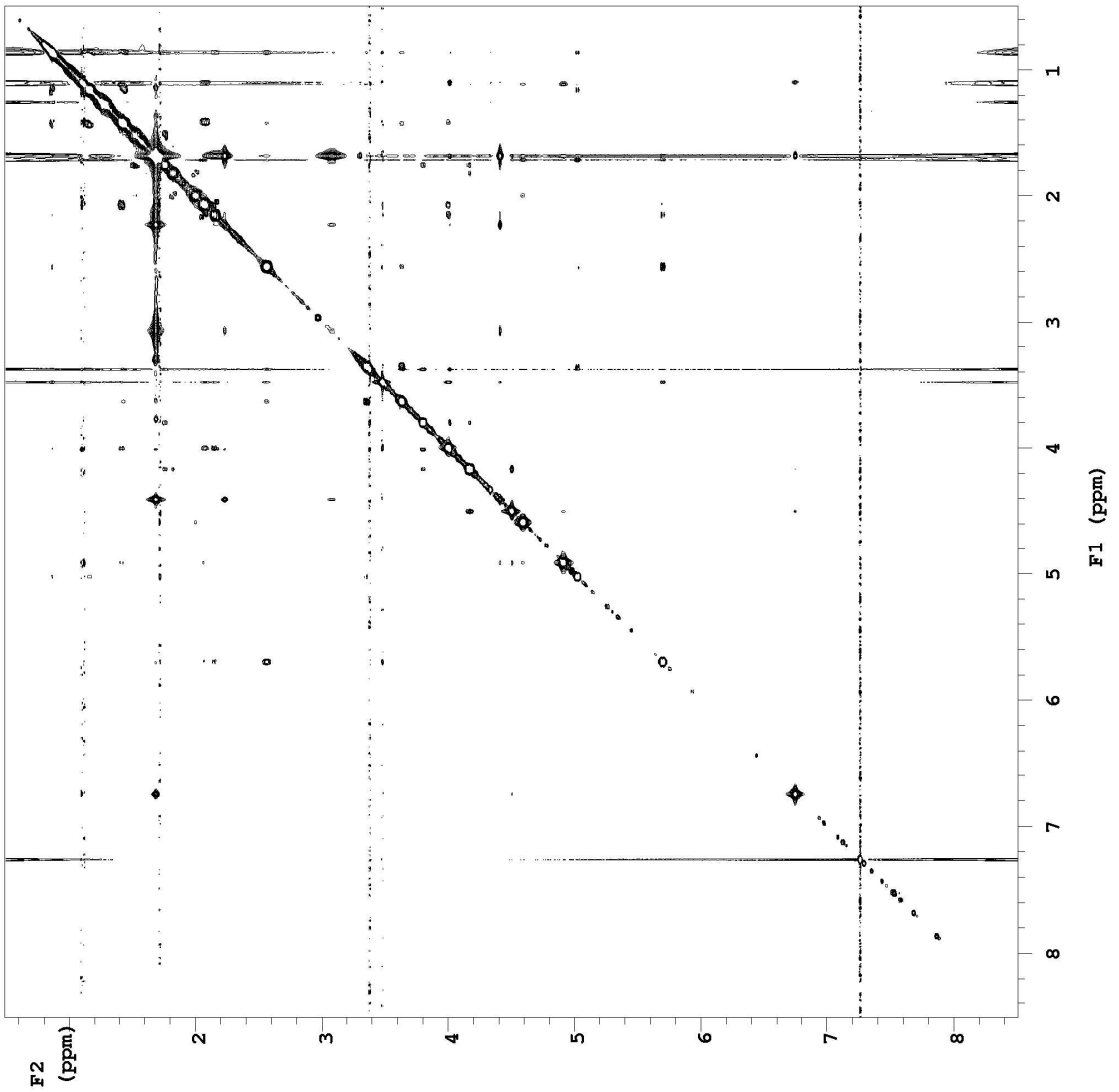
$HSQC_{ad}$ NMR spectrum of *natural* peloruside B (**2**) in $CDCl_3$ (600 MHz).



HMBC NMR spectrum of *natural* peloruside B (**2**) in $CDCl_3$ (600 MHz).



2D TOCSY NMR spectrum of *natural* peloruside B (**2**) in $CDCl_3$ (600 MHz).



NOESY$_{zq}$ NMR spectrum of *natural* peloruside B (**2**) in $CDCl_3$ (600 MHz).