# Precursor-directed fungal generation of novel halogenated chaetoglobosins with more preferable immunosuppressive action

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# 1 Experimental section

## 1.1 General Experimental Procedures.

$^{1}H$ and $^{13}C$ NMR spectra were accomplished on Bruker DPX-300 and DRX-500 NMR spectrometers using TMS as internal standard. HRESIMS spectra were recorded on an Angilent 6210 mass spectrometer. Silica gel (200-300 mesh) for column chromatography and silica $GF_{254}$ (10-20 m) for TLC were products of Qingdao Marine Chemical Factory, China. Semipreparative HPLC was carried out on a Hitachi L-7110 pump equipped with an Hypersil OBS 5 $\mu$m column (250 x 10 mm) from Thermo Fisher Scientific, Inc.

## 1.2 Fungal Material.

The strain of *Chaetomium globosum* was isolated from the the gut of an ocean fish *Epinephelus drummondhayi* collected in the Yellow sea, China. The working strain was preserved on potato dextrose agar slants containing 10% NaCl and stored at 4 °C.

## 1.3 General procedure for the feeding experiments.

All fermentations were performed in 1L Erlenmeyer flasks containing 400 ml Czapek's medium (3% sucrose, 0.1% yeast extract, 0.3% $NaNO_3$, 0.05% $MgSO_4 \cdot 7H_2O$, 0.001% $FeSO_4 \cdot 7H_2O$, 0.1% $K_2HPO_4$, 0.05%KCl) made in deionized water on a rotary shaker at 160 rpm and 28℃. Main cultures were inoculated with 5% starter cultures that have been cultivated for 3 days. Filter-sterilized aqueous solutions of the halo-tryptophan was added pulsely to the main culture to a final concentration of 1 mM 2 days after inoculation. Incubation was continued for another 10 days.

## 1.4 Extraction, Isolation and Purification.

The culture was harvested by lyophilization followed by extraction with EtOAc. Evaporation of the solvent under reduced pressure yielded a brown residue. The EtOAc extract was separated on a silica gel column eluted with $CHCl_3$-MeOH mixtures ($CHCl_3$:MeOH=1:0; 100:1; 100:2; 100:4; 0:1) to yield 5 fractions. The subfractions Fr.2 and Fr.3 were combined, ant then further separated into 9 fractions using Sephadex LH-20 by elution of MeOH. Fr.2-2 to Fr.2-5 was pooled as determined by LC-MS, and subjected to semipreparative HPLC to give chaetoglobosins.

## 1.5 Measurement of cell proliferation.

Lymph node cells isolated from Balb/c mice were seeded in 96 well-plate at a density of $3\times10^5$ cells per well and activated by Con A (5 g/ml) in the presence or absence of various concentrations of compounds for 72 hr. 20 μl of MTT (Sigma, MO; 4 mg/ml in PBS) were added per well 4 hrs before the end of the incubation. MTT formazan production was dissolved by dimethyl sulfoxide replacing the medium. The optical density at 540nm ($OD_{540}$) was measured by a microplate reader. The $IC_{50}$ value was calculated from the correlation curve between the compound concentration and the $OD_{540}$.

## 1.6 Measurement of CD25 and CD69 expression.

Lymph node cells isolated from Balb/c mice were activated by Con A (5 g/ml) in the presence or absence of various concentrations of compounds for 24 hr. After washing, cells were stained with anti-CD25 or anti-CD69 antibodies (BD pharmingen, CA) for 30 min at 4°C in the dark, and then analyzed on FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA).

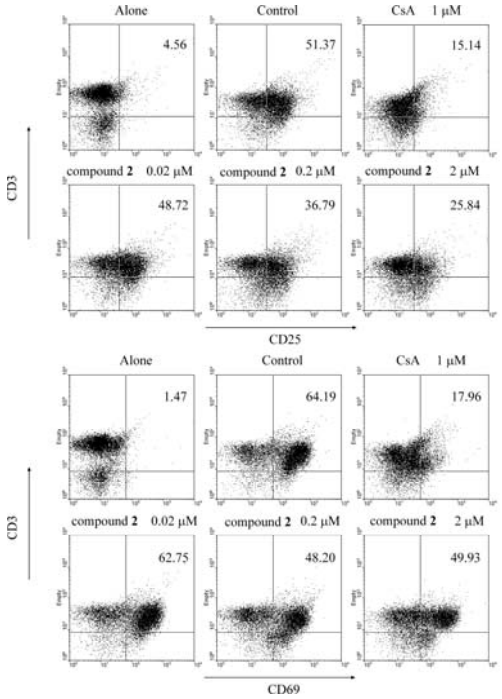



Figure S1. Compound **2** inhibited T cell activation.
freshly isolated lymph node cells ($3\times10^5$) were stimulated with 5 μg/ml Con A for 24 hr, then they were collected and analyzed by flow cytometry.

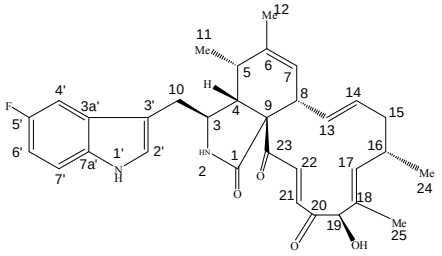
### 1.7 Measurement of apoptotic activated T cells.

Lymph node cells isolated from Balb/c mice were activated by Con A (5 μg/ml) in the presence or absence of various concentrations of compounds for 24 hr, and then fixed with 75% ethanol at 4°C overnight. After washing, the cells were stained with 50 μg/ml of propidium iodide (PI) in the dark at room temperature for 30 min. Samples were analyzed by flow cytrometry on a FACScan.

## NMR data of halogenated chaetoglobosins

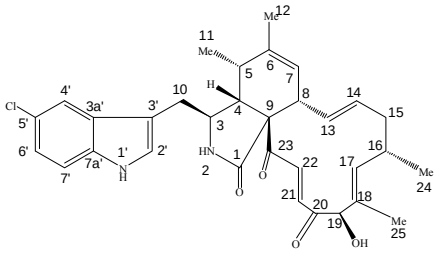
Chaetoglobosin J ( **1** )

**$^1$H NMR** (acetone-$d_6$, 500 MHz): δ 8.04 (d, J=16.8 Hz, 1H, H-22), 7.53 (d, J=7.5 Hz, 1H, H-4'), 7.34 (d, J=7.5 Hz, 1H, H-7'), 7.10 (br s, 1H, H-2'), 7.05 (t, J=7.5 Hz, 1H, H-5'), 7.00 (t, J=7.5 Hz, 1H, H-6'), 6.21 (d, J=16.8 Hz, 1H, H-21), 6.01 (dd, J=11.4, 14.8 Hz, 1H, H-13), 5.61 (d, J=9.3 Hz, 1H, H-17), 5.30 (br s, 1H, H-7), 5.15 (m, 1H, H-14 ), 5.06 (s, 1H, H-19), 3.44 (m, 1H, H-3), 3.04 (t, J=4.4 Hz, 1H, H-4), 2.99 (dd, J=6.4, 14.5 Hz, 1H, H-10a), 2.78 (dd, J=3.7, 14.5 Hz, 1H, H-10b), 2.50 (m, 2H, H-16 and H-5), 2.36 (m, 1H, H-8), 2.26 (m, 1H, H-15a), 1.99 (m, 1H, H-15b), 1.80 (s, 3H, $CH_3$-12), 1.31 (s, 3H, $CH_3$-25), 1.27 (d, J=7.3 Hz, 3H, $CH_3$-11), 0.99 (d, J=6.7 Hz, 3H, $CH_3$-24); **$^{13}$C NMR** ($CDCl_3$, 500 MHz): δ 201.6 (C-20), 198.1 (C-23), 172.8 (C-1), 141.0 (C-6), 140.6 (C-17), 138.0 (C-21), 136.5 (C-7a'), 132.4 (C-18), 132.1 (C-22), 131.2 (C-14), 130.8 (C-13), 126.9 (C-3a'), 125.7 (C-7), 122.6 (C-5'), 122.5 (C-2'), 119.9 (C-6'), 118.5 (C-4'), 111.8 (C-3'), 111.5 (C-7'), 81.4 (C-19), 66.1 (C-9), 54.0 (C-3), 49.7 (C-8), 46.9 (C-4), 41.8 (C-15), 34.8 (C-5), 34.6 (C-10), 31.0 (C-16), 20.9 (C-24), 20.3 (C-12), 14.9 (C-11), 10.6 (C-25); HRESI-MS *m/z*: 535.2569 $[M + Na]^+$ (calcd for $C_{32}H_{36}N_2O_4Na$ 535.2567).



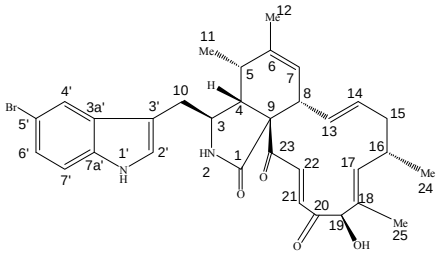

5'-F-chaetoglobosin J ( **1a** )

**$^1$H NMR** (acetone-$d_6$, 500 MHz): δ 7.99 (d, J=16.8 Hz, 1H, H-22), 7.22 (dd, J=2.4, 10.1 Hz, 1H, H-7'), 7.32 (dd, J=4.5, 8.8 Hz, 1H, H-4'), 7.17 (br s, 1H, H-2'), 6.84 (dt, J=9.0, 2.4 Hz, 1H, H-6'), 6.14 (d, J=16.8 Hz, 1H, H-21), 6.01 (dd, J=10.4, 15.2 Hz, 1H, H-13), 5.60 (d, J=9.0 Hz, 1H, H-17), 5.30 (br s, 1H, H-7), 5.13 (m, 1H, H-14), 5.04 (s, 1H, OH-19), 3.45 (m, 1H, H-3), 3.01 (t, J=4.4 Hz, 1H, H-4), 2.96 (dd, J=4.0, 14.7 Hz, 1H, H-10a), 2.78 (dd, J=5.7, 14.7 Hz, 1H, H-10b), 2.51 (m, 1H, H-16), 2.50 (m, 1H, H-5), 2.36 (m, 1H, H-8), 2.26 (m, 1H, H-15a), 1.99 (m, 1H, H-15b), 1.81 (s, 3H, $CH_3$-12), 1.30 (s, 3H, $CH_3$-25), 1.27 (d, J=7.3 Hz, 3H, $CH_3$-11), 0.98 (d, J=6.8 Hz, 3H, $CH_3$-24); **$^{13}$C NMR** ($CDCl_3$, 300 MHz): δ 201.5 (C-20), 197.9 (C-23), 172.8 (C-1), 157.8 (C-5'), 141.0 (C-6), 140.6 (C-17), 137.9 (C-21), 132.9 (C-7a'), 132.4 (C-18), 132.1 (C-22), 131.1 (C-14), 130.7 (C-13), 127.3 (C-3a'), 125.7 (C-7), 124.4 (C-2'), 112.2 (C-7'), 111.3 (C-3'), 111.0 (C-6'), 103.4 (C-4'), 81.4 (C-19), 66.0 (C-9), 53.7 (C-3), 49.6 (C-8), 46.9 (C-4), 41.8 (C-15), 34.6 (C-10 and C-5), 32.3 (C-16), 20.9 (C-24), 20.4 (C-12), 14.8 (C-11), 10.6 (C-25); HRESI-MS *m/z*: 553.2468 $[M + Na]^+$ (calcd for $C_{32}H_{35}FN_2O_4Na$ 553.2473).



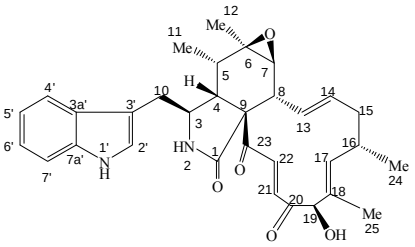
5’-Cl-chaetoglobosin J ( **1b** )

**$^{1}$H NMR** (acetone-$d_6$, 500 MHz): δ 7.99 (d, J=16.8 Hz, 1H, H-22), 7.52 (s, 1H, H-4’), 7.34 (d, J=8.6 Hz, 1H, H-7’), 7.17 (br s, H-2’), 7.02 (d, J=8.6 Hz, H-6’), 6.14 (d, J=16.8 Hz, H-21), 6.01 (dd, J=10.4, 15.2 Hz, 1H, H-13), 5.60 (d, J=9.0 Hz, 1H, H-17), 5.30 (br s, 1H, H-7), 5.13 (m, 1H, H-14), 5.04 (s, 1H, H-19), 3.45 (m, 1H, H-3), 3.01 (t, J=4.4 Hz, H-4), 2.96 (dd, J=4.0, 14.7Hz, 1H, H-10a), 2.80 (dd, J=5.7, 14.7Hz, 1H, H-10b), 2.50 (m, 2H, H-5 and H-16), 2.36 (m, 1H, H-8), 2.26 (m, 1H, H-15a), 1.99 (m, 1H, H-15b), 1.81 (s, 3H, $CH_3$-12), 1.30 (s, 3H, $CH_3$-25), 1.27 (d, J=7.3 Hz, 3H, $CH_3$-11), 0.98 (d, J=6.8Hz, 3H, $CH_3$-24); **$^{13}$C NMR** (acetone-$d_6$, 500 MHz): δ 202.1 (C-20), 199.0 (C-23), 172.5 (C-1), 141.8 (C-6), 140.5 (C-17), 138.5 (C-21), 137.5 (C-7a’), 133.9 (C-18), 132.3 (C-22), 131.8 (C-14), 130.9 (C-13), 128.6 (C-3a’), 127.0 (C-5’), 126.9 (C-7), 126.5 (C-6’), 122.2 (C-2’), 118.9 (C-4’), 113.6 (C-7’), 110.7 (C-3’), 82.0 (C-19), 66.0 (C-9), 54.4 (C-3), 49.2 (C-8), 48.1 (C-4), 42.7 (C-15), 35.6 (C-5), 33.6 (C-10), 32.8 (C-16), 21.3 (C-24), 20.3 (C-12), 14.5 (C-11), 10.8 (C-25); HRESI-MS *m/z*: 569.2183 $[M + Na]^+$ (calcd for $C_{32}H_{35}{}^{35}ClN_2O_4Na$ 569.2178).



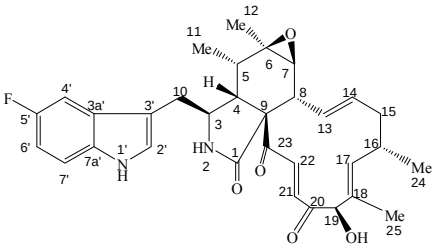
5’-Br-chaetoglobosin J ( **1c** )

**$^{1}$H NMR** (acetone-$d_6$, 500 MHz): δ 8.01 (d, J=16.8 Hz, 1H, H-22), 7.67 (d, J=1.8 Hz, 1H, H-4’), 7.29 (d, J=8.6 Hz, 1H, H-7’), 7.17 (br s, 1H, H-2’), 7.13 (dd, J=8.6, 1.8 Hz, 1H, H-6’), 6.12 (d, J=16.8 Hz, 1H, H-21), 6.02 (ddd, J=1.4,10.4, 15.1 Hz, 1H, H-13), 5.60 (d, J=9.2 Hz, 1H, H-17), 5.30 (br s, 1H, H-7), 5.14 (m, 1H, H-14), 5.06 (s, 1H, H-19), 3.46 (m, 1H, H-3), 3.00 (t, J=4.4 Hz, 1H, H-4), 2.98 (dd, J=3.6, 14.7 Hz, 1H, H-10a), 2.79 (dd, J=5.9, 14.7 Hz, 1H, H-10b), 2.51 (m, 1H, H-16), 2.50 (m, 1H, H-5), 2.36 (m, 1H, H-8), 2.26 (m, 1H, H-15a), 1.99 (m, 1H, H-15b), 1.81 (s, 3H, $CH_3$-12), 1.30 (s, 3H, $CH_3$-25), 1.27 (d, J=7.3 Hz, $CH_3$-11), 0.98 (d, J=6.8 Hz, $CH_3$-24); **$^{13}$C NMR** ($CDCl_3$, 300 MHz): δ 202.0 (C-20), 199.0 (C-23), 172.5 (C-1), 141.8 (C-6), 140.4 (C-17), 138.5 (C-21), 136.6 (C-7a’), 133.3 (C-18), 132.3 (C-22), 131.7 (C-14), 130.8 (C-13), 128.9 (C-3a’), 126.7 (C-6’), 126.5 (C-7), 124.9 (C-7’), 122.1 (C-2’), 114.2 (C-5’), 114.0 (C-4’), 112.3 (C-3’), 82.0 (C-19), 67.0 (C-9), 54.3 (C-3), 49.2 (C-8), 47.2 (C-4), 42.7 (C-15), 36.1 (C-5), 33.6 (C-10), 32.8 (C-16), 21.3 (C-24), 20.2 (C-12), 14.5 (C-11), 10.7 (C-25); HRESI-MS *m/z*: 613.1674 $[M + Na]^+$ (calcd for $C_{32}H_{35}{}^{79}BrN_2O_4Na$ 613.1672).



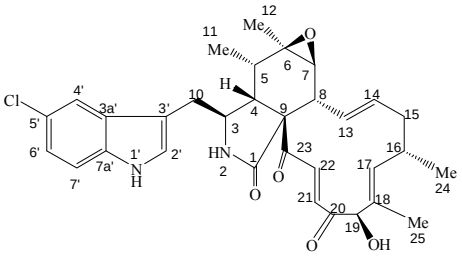
Chaetoglobosin A ( **2** )

**$^1$H NMR** (acetone-$d_6$, 500 MHz): δ 10.1 (br s,1H, NH-1'), 7.53 (d, J=7.1 Hz, 1H, H-21), 7.52 (d, J=16.5 Hz, 1H, H-4') 7.43 (br s, 1H, NH-2), 7.33 (d, J=7.1 Hz, 1H, H-7'), 7.12 (s, 1H, H-2'), 7.05 (t, J=7.1 Hz, 1H, H-6'), 7.00 (t, J=7.1 Hz, 1H, H-5'), 6.41 (d, J=16.5 Hz, 1H, H-22), 6.16 (dd, J=10.1, 15.3 Hz, 1H, H-13), 5.52 (d, J=9.3 Hz, 1H, H-17), 5.18 (dt, J=3.3, 10.3 Hz, 1H, H-14), 4.97 (d, J=4.2 Hz, 1H, H-19), 4.21 (d, J=4.2 Hz, 1H, OH-19), 3.92 (br t, 1H, H-3), 2.85 (dd, J=5.3, 14.2 Hz, 1H, H-10a), 2.79 (dd, J=6.2, 14.2 Hz, 1H, H-10b), 2.72 (d, J=5.1 Hz, 1H, H-7), 2.52 (m, 1H, H-16), 2.27 (m, 1H, H-15a), 2.13 (m, 1H, H-8), 1.99 (m, 1H, H-15b), 1.75 (m, 1H, H-5), 1.23 (s, 3H, $CH_3$-12), 1.33 (s, 3H, $CH_3$-25), 0.99 (d, J=6.4 Hz, 3H, $CH_3$-11), 0.98 (d, J=7.1 Hz, 3H, $CH_3$-24); **$^{13}$C NMR** (acetone-$d_6$, 300 MHz): δ 201.9 (C-20), 198.8 (C-23), 173.7 (C-1), 139.8 (C-17), 137.6 (C-7a'), 136.5 (C-21), 133.7 (C-22), 133.5 (C-18), 133.2 (C-14), 129.8 (C-13), 128.7 (C-3a'), 125.2 (C-5'), 122.2 (C-2'), 119.8 (C-16'), 119.4 (C-4'), 112.4 (C-7'), 110.6 (C-3'), 82.7 (C-19), 64.1 (C-9), 62.9 (C-7), 58.2 (C-6), 53.4 (C-3), 49.2 (C-8), 47.6 (C-4), 42.2 (C-15), 37.3 (C-5), 34.1 (C-10), 32.9 (C-16), 21.4 (C-24), 19.9 (C-12), 13.3 (C-11), 10.8 (C-25); HRESI-MS *m/z*: 551.2525 $[M + Na]^+$ (calcd for $C_{32}H_{36}N_2O_5Na$ 551.2516).



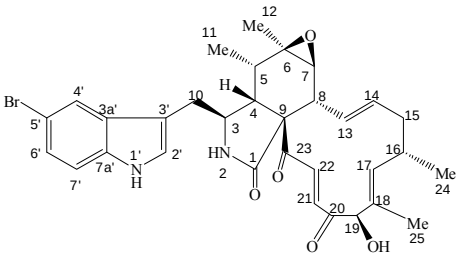
5'-F-chaetoglobosin A ( **2a** )

**$^1$H NMR** (acetone-$d_6$, 500 MHz): δ 10.21 (br s, 1H, NH-1'), 7.51 (d, J=16.5 Hz, 1H, H-21), 7.43 (br s, 1H, NH-2), 7.30 (dd, J=4.6, 7.8 Hz, 1H, H-7'), 7.25 (dd, J=10.1, 2.3 Hz, 1H, H-4'), 7.19 (s, 1H, H-2'), 6.82 (dt, J=9.2, 2.3 Hz, 1H, H-6'), 6.32 (d, J=16.5 Hz, 1H, H-22), 6.15 (dd, J=15.1, 9.8 Hz, 1H, H-13), 5.52 (d, J=9.2 Hz, 1H, H-17), 5.17 (dt, J=3.7, 15.1 Hz, 1H, H-14), 4.95 (d, J=4.6 Hz, 1H, H-19), 4.18 (d, J=4.6 Hz, OH-19), 3.93 (br t, 1H, H-3), 2.82 (d, J=4.9 Hz, 2H, H-10), 2.90 (m, 1H, H-4), 2.71 (d, J=5.2 Hz, 1H, H-7), 2.51 (m, 1H, H-16), 2.27 (m, 1H, H-15a), 2.10 (m, 1H, H-8), 1.99 (m, 1H, H-15b), 1.75 (m, 1H, H-5), 1.32 (s, 3H, $CH_3$-25), 1.24 (3H, s 3H, $CH_3$-12), 1.03 (3H, d, J=7.3 Hz, 3H, $CH_3$-11), 0.99 (3H, d, J=6.7 Hz, 3H, $CH_3$-24); **$^{13}$C NMR** (acetone-$d_6$, 300 MHz): δ 201.9 (C-20), 198.7 (C-23), 173.7 (C-1), 158.6 (C-5'), 139.8 (C-17), 136.5 (C-21), 134.2 (C-18), 133.6 (C-7a'), 133.5 (C-14), 132.8 (C-22), 129.9 (C-13), 129.2 (C-3a'), 127.5 (C-2'), 113.2 (C-7'), 110.5 (C-3'), 110.3 (C-6'), 104.2 (C-4'), 82.7 (C-19), 64.1 (C-9), 62.9 (C-7), 53.2 (C-6), 53.2 (C-3), 49.4 (C-8), 47.5 (C-4), 42.3 (C-15), 37.3 (C-5), 33.8 (C-10), 30.4 (C-16), 21.4 (C-24), 19.9 (C-12), 13.3 (C-11), 10.8 (C-25); HRESI-MS *m/z*: 569.2427 $[M + Na]^+$ (calcd for $C_{32}H_{35}FN_2O_5Na$ 569.2422).



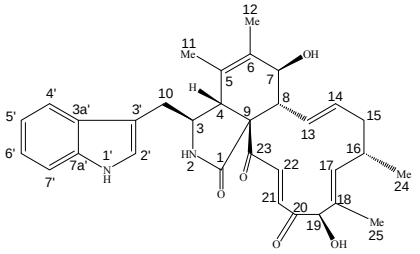

5’-Cl-chaetoglobosin A ( **2b** )

**$^{1}$H NMR** (acetone-$d_6$, 500 MHz): δ 7.52 (d, J=16.6 Hz, 1H, H-21), 7.55 (s, 1H, H-4’), 7.34 (d, J=8.6 Hz, 1H, H-7’), 7.19 (s, 1H, H-2’), 7.03 (d, J=8.6 Hz, 1H, H-6’), 6.31 (d, J=16.6 Hz, 1H, H-22), 6.15 (dd, J=15.4, 10.0 Hz, 1H, H-13), 5.56 (d, J=9.3 Hz, H-17), 5.18 (dt, J=3.6, 15.4 Hz, 1H, H-14), 5.02 (s, 1H, H-19), 3.94 (br t, 1H, H-3), 2.87 (m, 1H, H-4), 2.86 (dd, J=4.7, 14.4 Hz, H-10a), 2.80 (dd, J=6.0, 14.4 Hz, H-10b), 2.74 (d, J=5.1 Hz, 1H, H-7), 2.51 (m, 1H, H-16), 2.28 (m, 1H, H-15a), 2.10 (m, 1H, H-8), 2.00 (m, 1H, H-15b), 1.75 (m,1H, H-5), 1.33 (s, 3H, $CH_3$-25), 1.25 (s, 3H, $CH_3$-12), 1.01 (d, J=7.4 Hz, 3H, $CH_3$-11), 0.99 (d, J=6.8 Hz, 3H, $CH_3$-24); **$^{13}$C NMR** (acetone-$d_6$, 500 MHz): δ 202.0 (C-20), 198.6 (C-23), 173.9 (C-1), 140.0 (C-17), 136.5 (C-21), 135.9 (C-7a’), 133.6 (C-22), 133.5 (C-18), 132.9 (C-14), 129.9 (C-3a’), 129.8 (C-13), 127.1 (C-6’), 125.2 (C-5’), 122.3 (C-2’), 118.9 (C-4’), 113.7 (C-7’), 110.2 (C-3’), 82.6 (C-19), 64.1 (C-9), 62.9 (C-7), 58.3 (C-6), 53.2 (C-3), 49.4 (C-8), 47.4 (C-4), 42.4 (C-15), 37.3 (C-5), 33.6 (C-10), 32.8 (C-16), 21.4 (C-24), 19.8 (C-12), 13.3 (C-11), 10.8 (C-25); HRESI-MS *m/z*: 585.2135 $[M + Na]^+$ (calcd for $C_{32}H_{35}{}^{35}ClN_2O_5Na$ 585.2127).



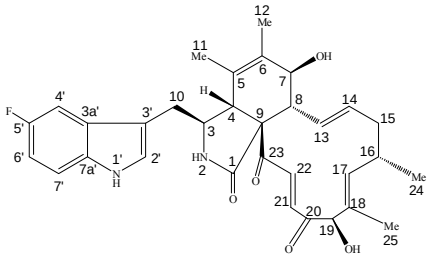

5’-Br-chaetoglobosin A ( **2c** )

**$^{1}$H NMR** (acetone-$d_6$, 500 MHz): δ 7.70(s, 1H, H-4’), 7.53 (d, J=16.6 Hz, 1H, H-21), 7.30 (d, J=8.5 Hz, 1H, H-7’), 7.17 (s, 1H, H-2’), 7.14 (d, J=8.5 Hz, 1H, H-6’), 6.30 (d, J=16.6 Hz, 1H, H-22), 6.15 (dd, J=14.9, 9.9 Hz, 1H, H-13), 5.56 (d, J=9.2 Hz, 1H, H-17), 5.18 (dt, J=3.1, 10.2 Hz, 1H, H-14), 5.03 (s, 1H, H-19), 3.94 (br t, 1H, H-3), 2.83 (dd, J=4.7, 14.2 Hz, 1H, H-10a), 2.80 (dd, J=5.9, 14.2 Hz, 1H, H-10b), 2.74 (d, J=5.1 Hz, 1H, H-7), 2.51 (m, 1H, H-16), 2.28 (m, 1H, H-15a), 2.19 (m, 1H, H-8), 1.99 (m, 1H, H-15b), 1.75 (m, 1H, H-5), 1.33 (s, 3H, $CH_3$-25), 1.25 (s, 3H, $CH_3$-12), 1.01 (d, J=7.7 Hz, 3H, $CH_3$-11), 0.99 (d, J=6.8 Hz, 3H, $CH_3$-24); **$^{13}$C NMR** (acetone-$d_6$, 500 MHz): δ 140.0, 136.5, 132.9, 129.8, 128.6, 124.9, 122.0, 117.0, 114.2, 82.6, 63.0, 53.2, 49.4, 53.2, 49.4, 37.3, 35.5, 33.6, 32.8, 21.3, 19.8, 13.3, 10.8; HRESI-MS *m/z*: 629.1615 $[M + Na]^+$ (calcd for $C_{32}H_{35}{}^{79}BrN_2O_5Na$ 629.1622).



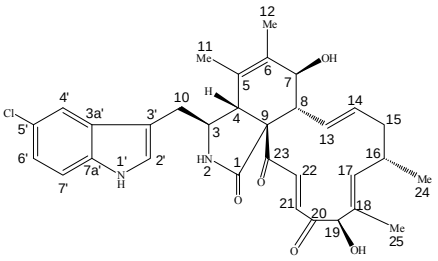
Chaetoglobosin B ( **3** )

**$^1$H NMR** (acetone-$d_6$, 500 MHz): δ 10.15 (br s, 1H, NH-1'), 7.66 (d, J=16.5 Hz, 1H, H-21), 7.53 (d, J=7.0 Hz, 1H, H-4'), 7.38 (d, J=7.0 Hz, 1H, H-7'), 7.28 ( br s, 1H, NH-2), 7.16 (s, 1H, H-2'), 7.09 (t, J=7.0 Hz, 1H, H-5'), 7.01 (t, J=7.0 Hz, 1H, H-6'), 6.76 (d, J=16.5 Hz, 1H, H-22), 6.18 (dd, J=10.0, 15.5 Hz, 1H, H-13), 5.58 (d, J=9.5 Hz, H-17), 5.18 (m, 1H, H-14), 5.09 (d, J=5.0 Hz, H-19), 4.24 (d, J=5.0 Hz, OH-19), 3.91 (m, 1H, H-7), 3.60 (br t, J=7.4 Hz, 1H, H-3), 3.53 (d, J=5.5 Hz, 1H, OH-7), 3.31 (br s, 1H, H-4), 2.85 (dd, J=2.5, 14.1 Hz, 1H, 10a), 2.64 (dd, J=7.4, 14.1 Hz, 1H, 10b), 2.56 (m, 1H, H-16), 2.29 (brd, J=14.1 Hz, 1H, H-15a), 2.08 (d, J=7.4 Hz, 1H, H-8), 2.01 (m, 1H, H-15b), 1.65 (s, 3H, $CH_3$-11), 1.40 (s, 3H, $CH_3$-25), 1.33 (s, 3H, $CH_3$-12), 1.00 (d, J=6.7 Hz, 3H, $CH_3$-24); **$^{13}$C NMR** (acetone-$d_6$, 500 MHz): δ 202.0 (C-20), 200.0 (C-23), 173.7 (C-1), 140.2 (C-17), 137.9 (C-7a'), 137.0 (C-21), 135.6 (C-14), 134.4 (C-22), 134.0 (C-6), 133.6 (C-18), 129.7 (C-13), 128.7 (C-3a'), 127.0 (C-5), 124.7 (C-2'), 122.4 (C-5'), 119.9 (C-6'), 119.4 (C-4'), 112.5 (C-7'), 111.7 (C-3'), 83.2 (C-19), 69.8 (C-7), 62.5 (C-9), 59.1 (C-3), 53.2 (C-8), 48.7 (C-4), 42.5 (C-15), 33.5 (C-10), 33.1 (C-16), 21.5 (C-24), 17.8 (C-12), 14.5 (C-11), 11.0 (C-25); HRESI-MS *m/z*: 551.2519 $[M + Na]^+$ (calcd for $C_{32}H_{36}N_2O_5Na$ 551.2516).



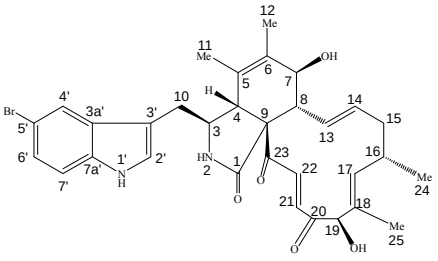
5'-F-chaetoglobosin B ( **3a** )

**$^1$H NMR** (acetone-$d_6$, 500 MHz): δ 10.26 (br s, 1H, NH-1'), 7.66 (d, J=16.5 Hz, 1H, H-21), 7.38 (dd, J=4.5, 9.0 Hz, 1H, H-7'), 7.27 (br s, 1H, NH-2), 7.25 (dd, J=2.5, 9.0 Hz, 1H, H-4'), 6.89 (dt, J=2.5, 9.3 Hz, 1H, H-6'), 6.72 (d, J=16.5 Hz, 1H, H-22), 6.18 (dd, J=10.0, 15.5 Hz, 1H, H-13), 5.58 (d, J=9.4 Hz, 1H, H-17), 5.18 (m, 1H, H-14), 5.08 (d, J=4.3 Hz, 1H, H-19), 4.24 (d, J=4.3 Hz, 1H, OH-19), 3.91 (m, 1H, H-7), 3.58 (br t, J=6.7 Hz, 1H, H-3), 3.54 (d, J=5.5 Hz, 1H, OH-7), 3.31 (br s, 1H, H-4), 2.85 (m, 1H, H-10a), 2.63 (dd, J=8.5, 14.5 Hz, 1H, H-10b), 2.56 (m, 1H, H-16), 2.29 (brd, J=13.5 Hz, 1H, H-15a), 2.06 (d, J=7.4 Hz, 1H, H-8), 2.01 (m, 1H, H-15b), 1.65 (s, 3H, $CH_3$-11), 1.39 (s, 3H, $CH_3$-25), 1.37 (s, 3H, $CH_3$-12), 1.00 (d, J=6.7 Hz, 3H, $CH_3$-24); **$^{13}$C NMR** (acetone-$d_6$, 500 MHz): δ 202.0 (C-20), 199.9 (C-23), 173.8 (C-1), 158.1 (C-5'), 140.1 (C-17), 136.9 (C-21), 135.6 (C-14), 134.5 (C-6), 134.3 (C-22), 133.6 (C-7a'), 133.4 (C-18), 129.8 (C-13), 129.6 (C-3a'), 127.0 (C-5), 127.0 (C-2'), 113.4 (C-7'), 110.7 (C-3'), 110.5 (C-6'), 104.1 (C-4'), 83.1 (C-19), 69.8 (C-7), 62.5 (C-9), 59.0 (C-3), 53.2 (C-8), 48.7 (C-4), 42.6 (C-15), 33.4 (C-10), 33.1 (C-16), 21.6 (C-24), 17.8 (C-12), 14.5 (C-11), 11.0 (C-25); HRESI-MS *m/z*: 569.2427 $[M + Na]^+$ (calcd for $C_{32}H_{35}FN_2O_5Na$ 569.2422).



5’-Cl-chaetoglobosin B ( **3b** )

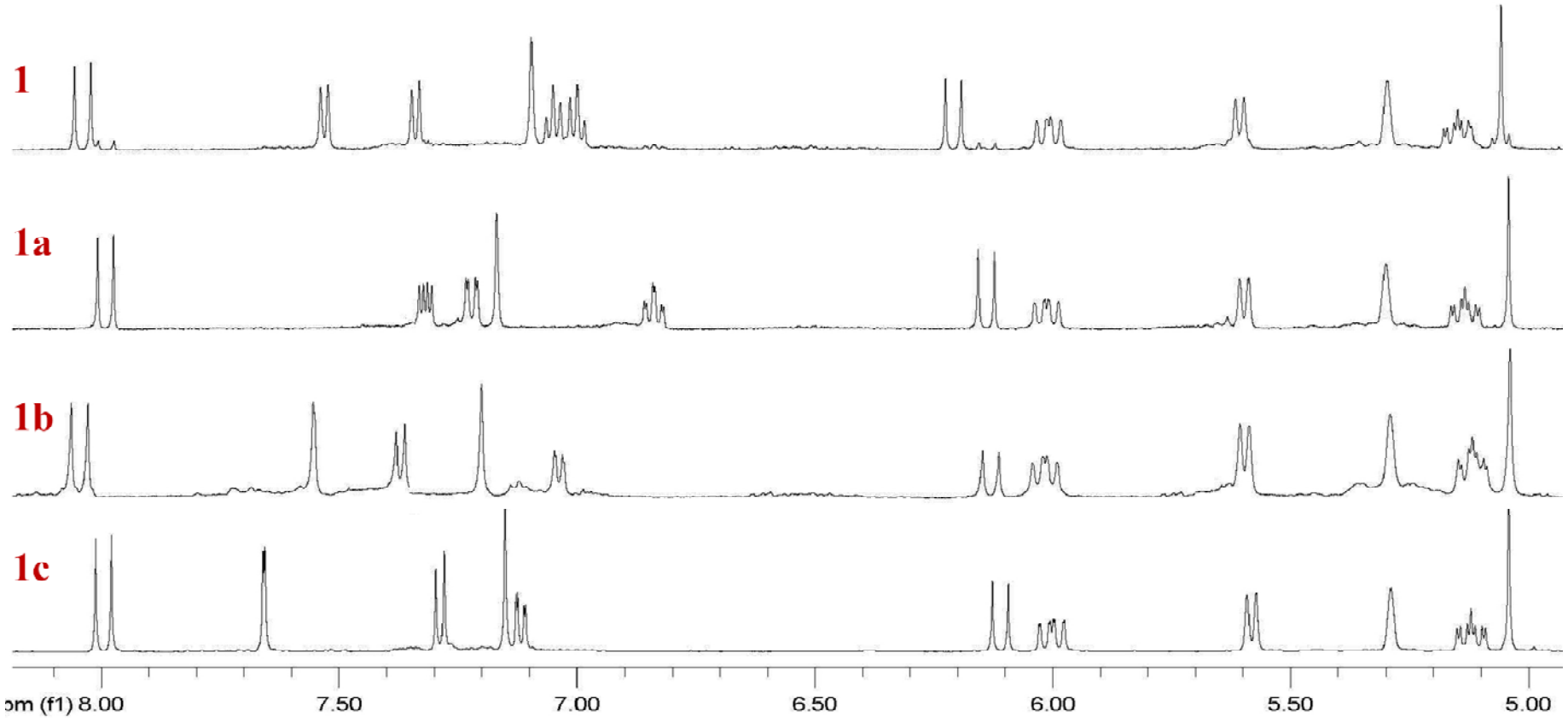
**$^1$H NMR** (acetone-$d_6$, 500 MHz): δ 7.67 (d, J=16.4 Hz, 1H, H-21), 7.57 (d, J=1.6 Hz, 1H, H-4’), 7.41 (d, J=8.6 Hz, 1H, H-7’), 7.25 (s, 1H, H-2’), 7.08 (dd, J=1.6, 8.6 Hz, 1H, H-6’), 6.71 (d, J=16.4 Hz, 1H, H-22), 6.17 (dd, J=10.2, 14.8 Hz, 1H, H-13), 5.62 (d, 9.4 Hz, 1H, H-17), 5.19 (m, 1H, H-14), 5.13 (s, 1H, H-19), 3.90 (m, 1H, H-7), 3.58 (br t, J=7.4 Hz, 1H, H-3), 3.31 (br s, 1H, H-4), 2.89 (dd, J=5.8, 14.3 Hz, 1H, H-10a), 2.61 (dd, J=8.5, 14.3 Hz, 1H, H-10b), 2.56 (m, 1H, H-16), 2.29 (brd, J=13.9 Hz, 1H, H-15a), 2.06 (d, J=7.4 Hz, 1H, H-8), 2.01 (m, 1H, H-15b), 1.66 (s, 3H, $CH_3$-11), 1.40 (s, 3H, $CH_3$-25), 1.30(s, 3H, $CH_3$-12), 1.00 (d, J=6.7 Hz, 3H, $CH_3$-24); **$^{13}$C NMR** (acetone-$d_6$, 500 MHz): δ 140.2, 135.5, 135.4, 134.5, 129.3, 126.7, 126.4, 122.2, 118.6, 113.7, 82.9, 69.5, 58.8, 52.8, 48.2, 42.3, 32.9, 21.4, 14.6, 10.9; HRESI-MS *m/z*: 585.2130 $[M + Na]^+$ (calcd for $C_{32}H_{35}{}^{35}ClN_2O_5Na$ 585.2127).



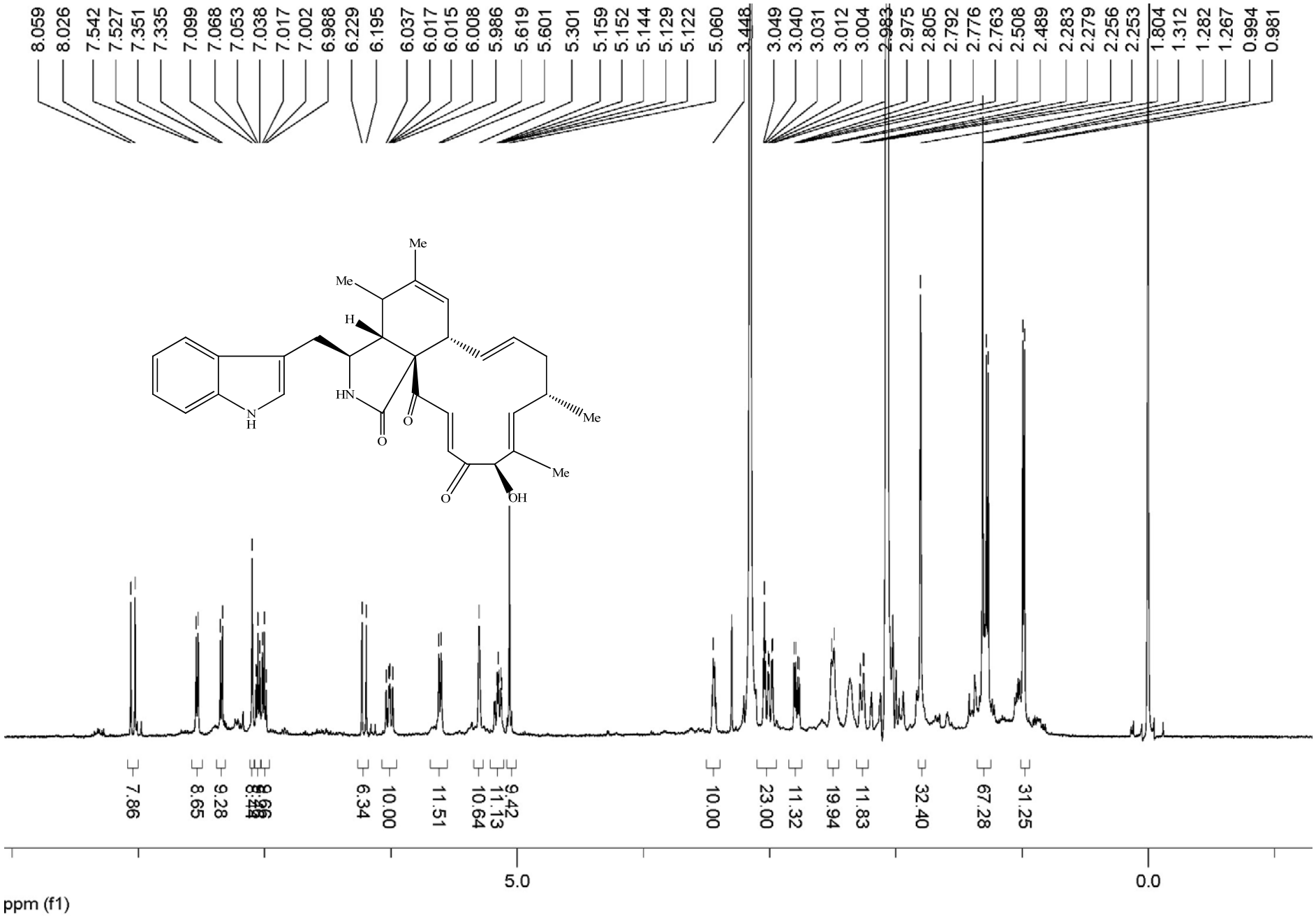
5’-Br-chaetoglobosin B ( **3c** )

**$^1$H NMR** (acetone-$d_6$, 500 MHz): δ 7.72 (s, 1H, H-4’), 7.68 (d, J=16.4 Hz, 1H, H-21), 7.37 (d, J=8.5 Hz, 1H, H-7’), 7.23 (s, 1H, H-2’), 7.20 (d, J=8.5 Hz, 1H, H-6’), 6.71 (d, J=16.4 Hz, 1H, H-22), 6.17 (dd, 10.5, 13.1 Hz, 1H, H-13), 5.62 (d, J=9.1 Hz, 1H, H-17), 5.18 (m, 1H, H-14), 5.08 (d, J=4.3 Hz, 1H, H-19), 3.91 (m, 1H, H-7), 3.58 (br t, J=6.7 Hz, 1H, H-3), 3.31 (br s, 1H, H-4), 2.88 (dd, 5.6, 14.1 Hz, 1H, H-10a), 2.61 (dd, 8.4, 14.1 Hz, 1H, H-10b), 2.56 (m, 1H, H-16), 2.29 (brd, J=12.4 Hz, 1H, H-15a), 2.06 (d, J=7.4 Hz, 1H, H-8), 2.01 (m, 1H, H-15b), 1.66 (s, 3H, $CH_3$-11), 1.40 (s, 3H, $CH_3$-25), 1.34 (s, 3H, $CH_3$-12), 1.00 (d, J=6.5 Hz, 3H, $CH_3$-24); **$^{13}$C NMR** (acetone-$d_6$, 500 MHz): δ 140.2, 136.7, 135.4, 134.4, 129.4, 124.9, 121.8, 114.3, 114.2, 82.9, 69.6, 58.8, 52.9, 48.3, 42.4, 32.9, 21.4, 17.7, 14.6, 10.9; HRESI-MS *m/z*: 629.1617 $[M + Na]^+$ (calcd for $C_{32}H_{35}{}^{79}BrN_2O_5Na$ 629.1622).

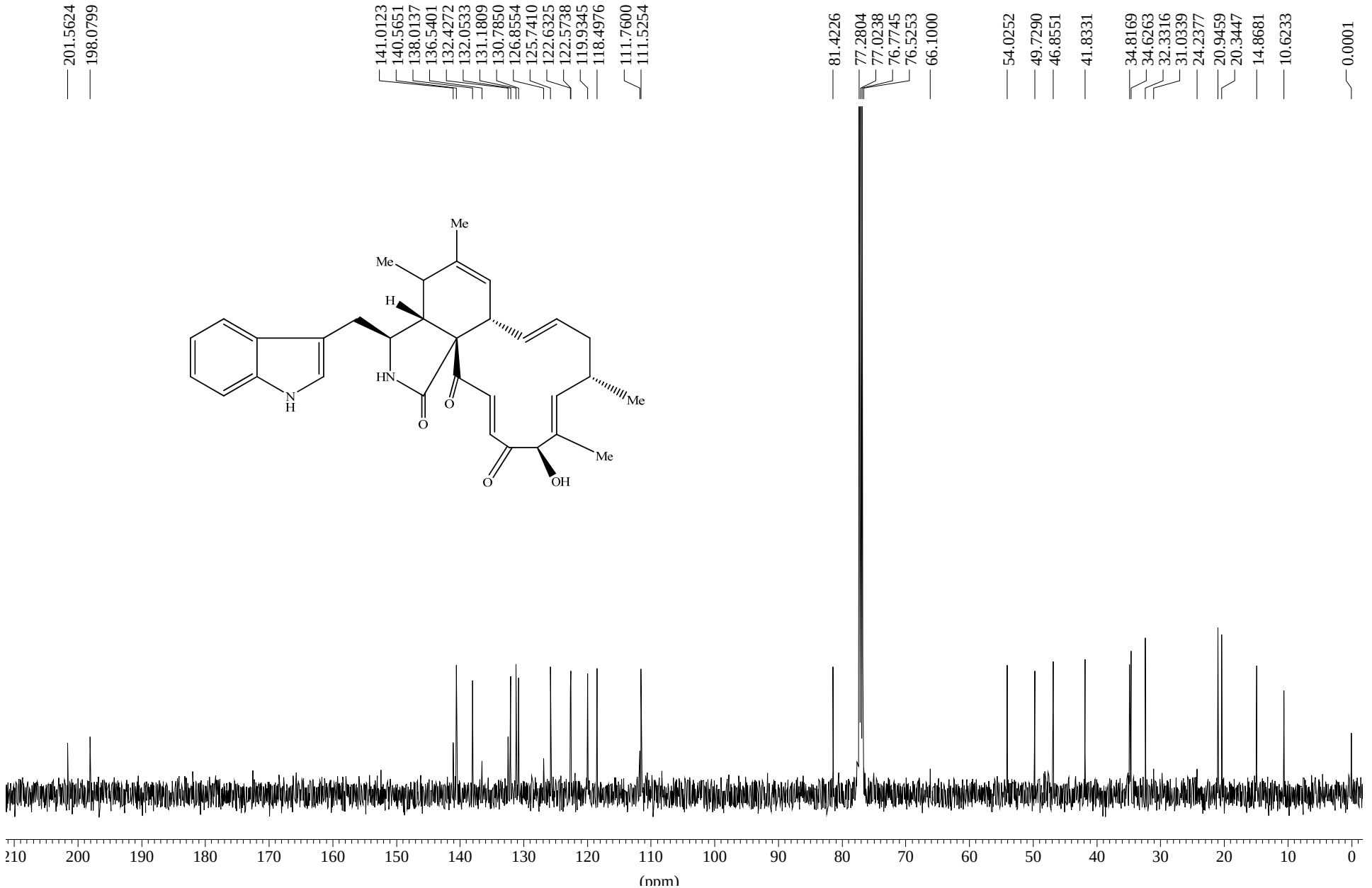
# **S11** Comparison $^{1}$H NMR spectra of compounds **1** and **1a-1c**



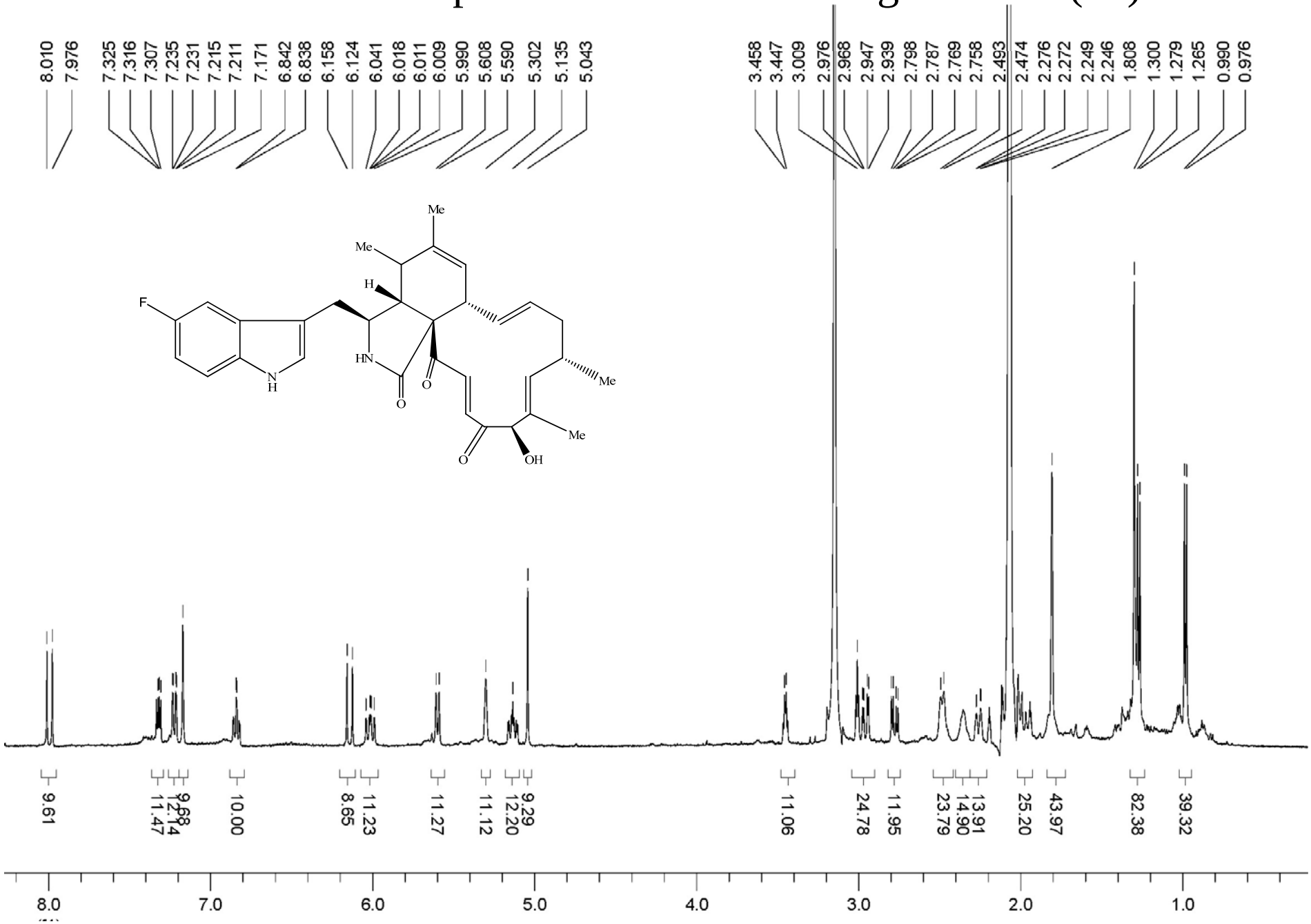
# **S12** $^{1}$H NMR spectrum for chaetoglobosin J (**1**)



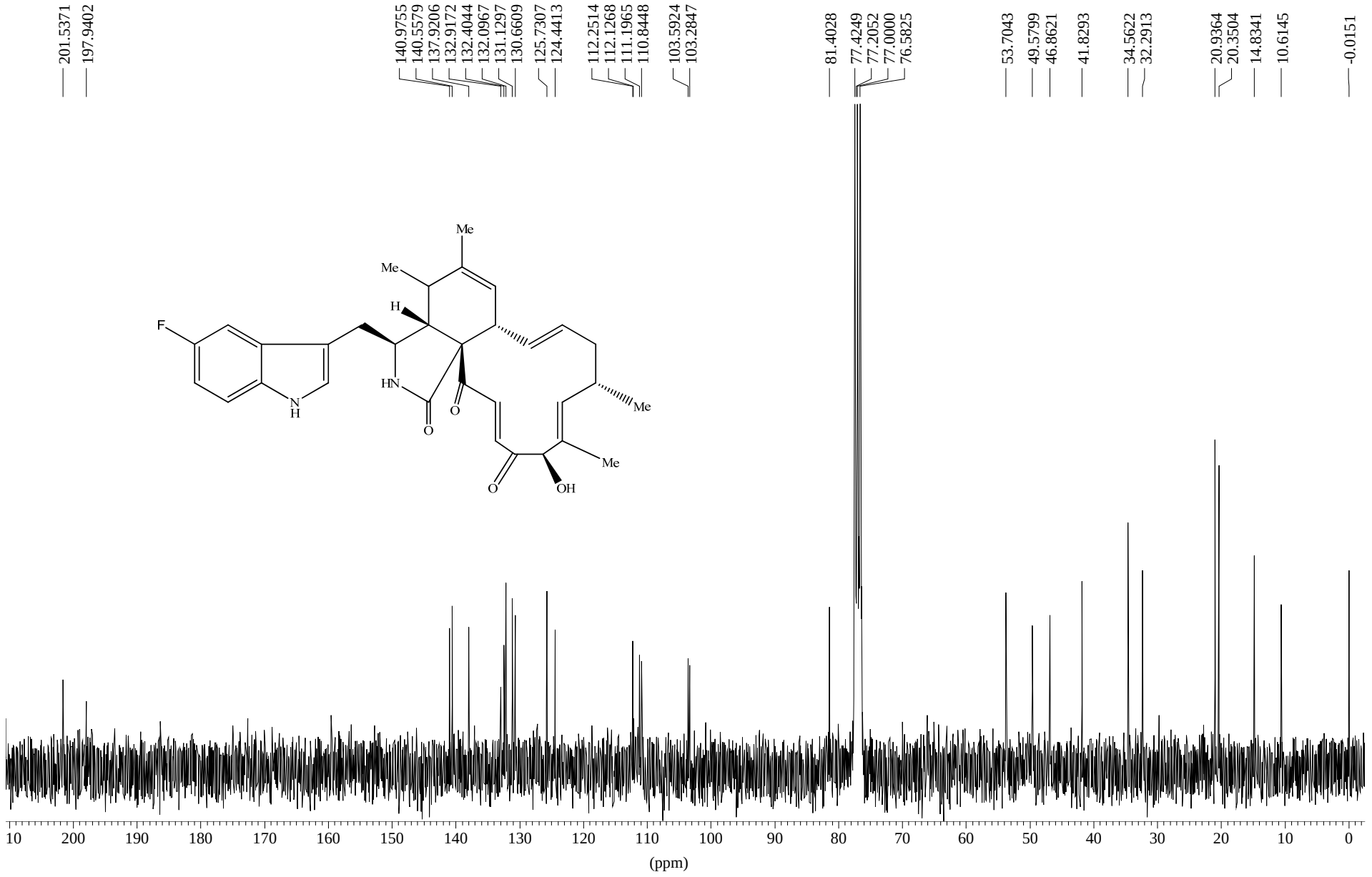
# **S13** $^{13}$C NMR spectrum for chaetoglobosin J (**1**)



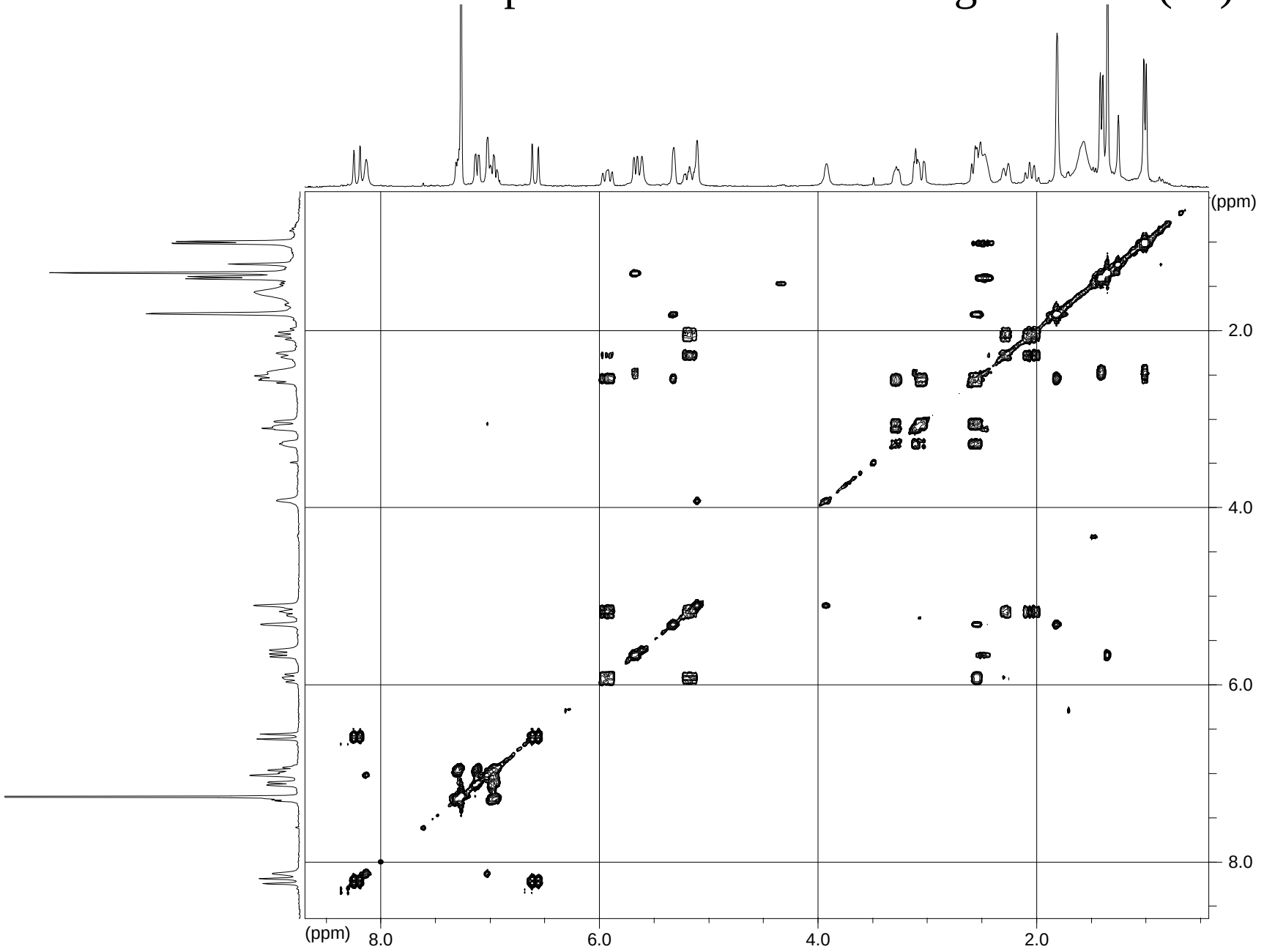
# **S14** $^{1}$H NMR spectrum for 5'-F-chaetoglobosin J (**1a**)



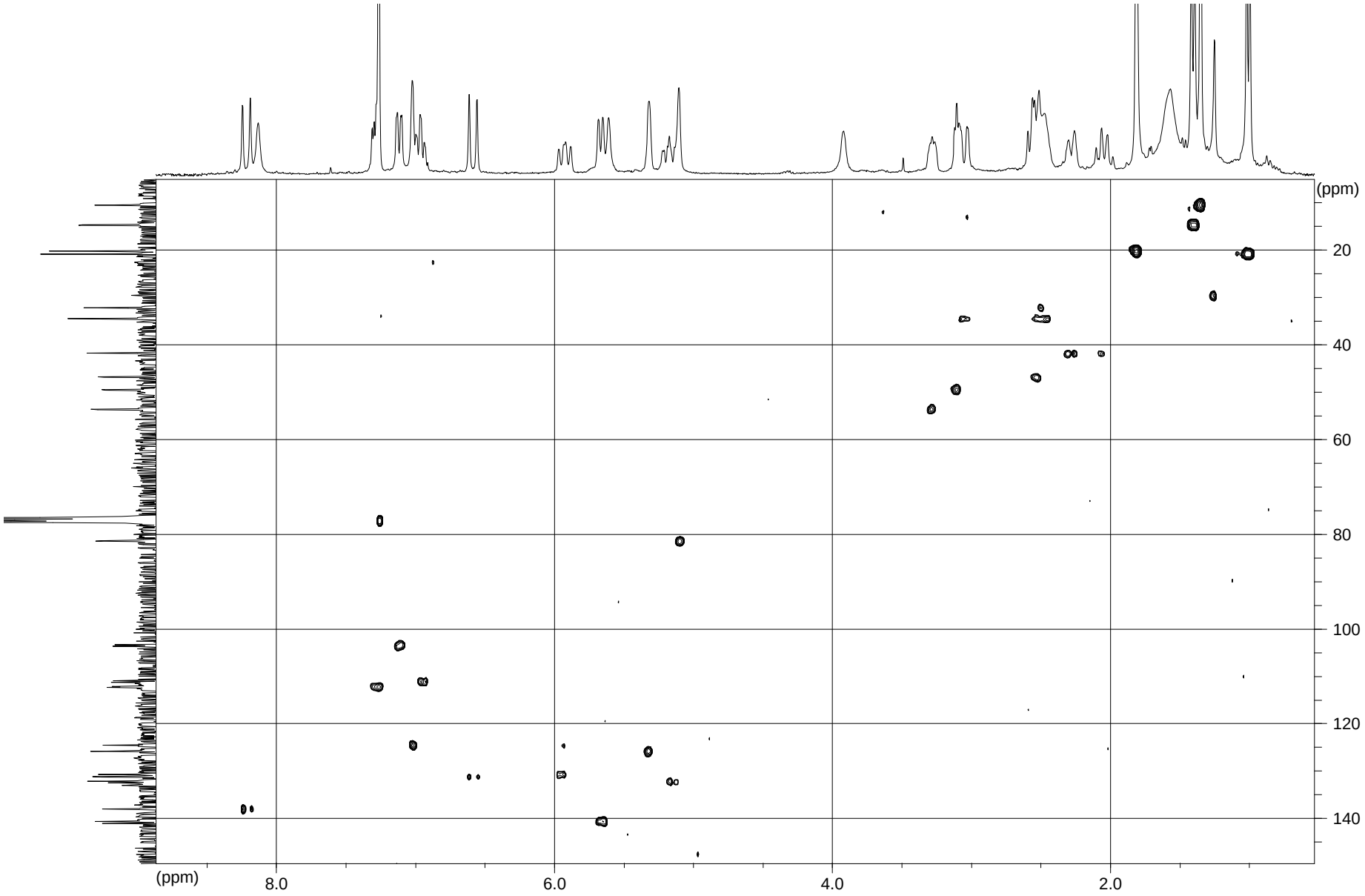
# **S15** $^{13}$C NMR spectrum for 5'-F-chaetoglobosin J (**1a**)



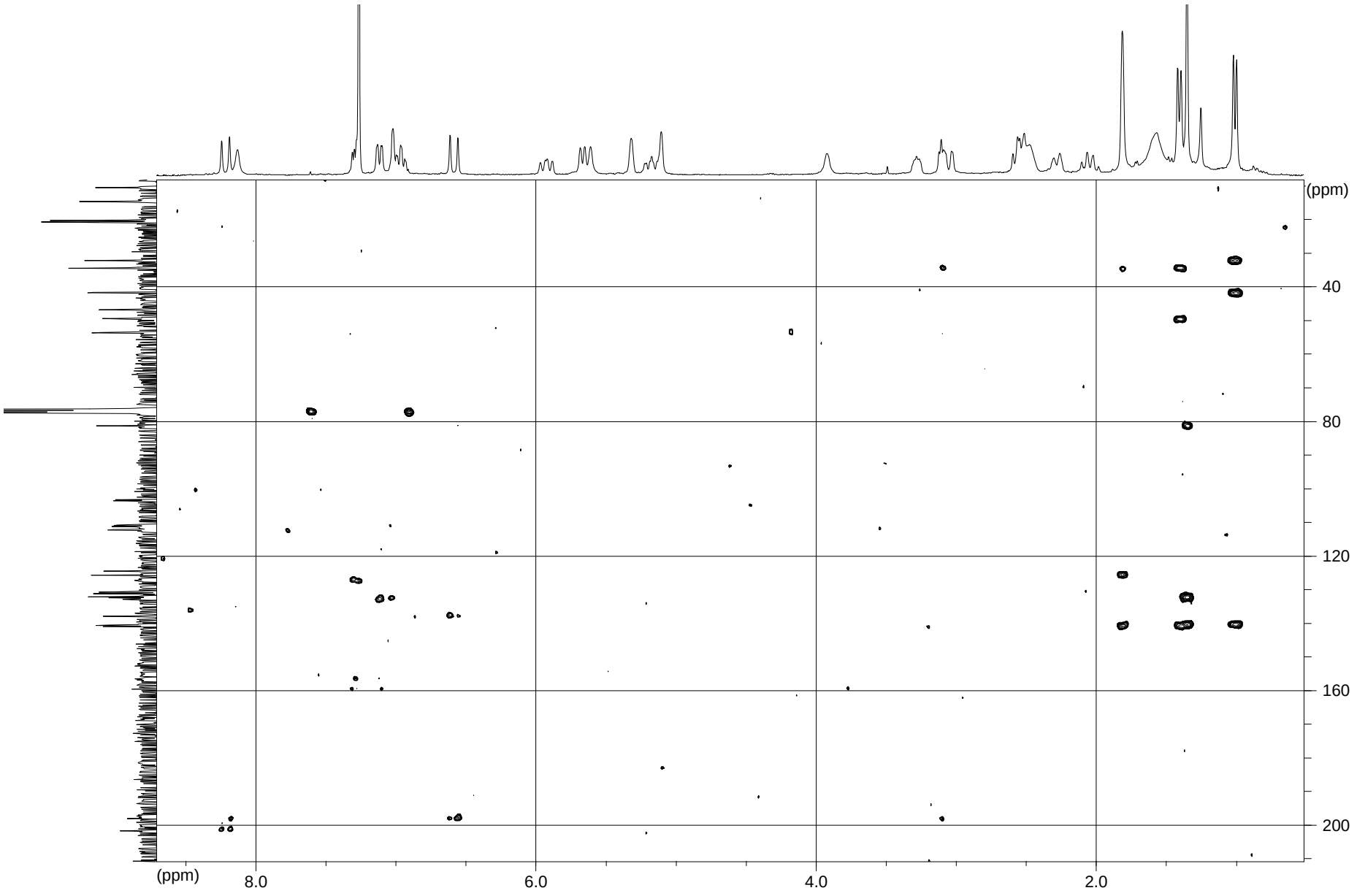
# **S16** $^{1}$H-$^{1}$HCOSY spectrum for 5'-F-chaetoglobosin J (**1a**)



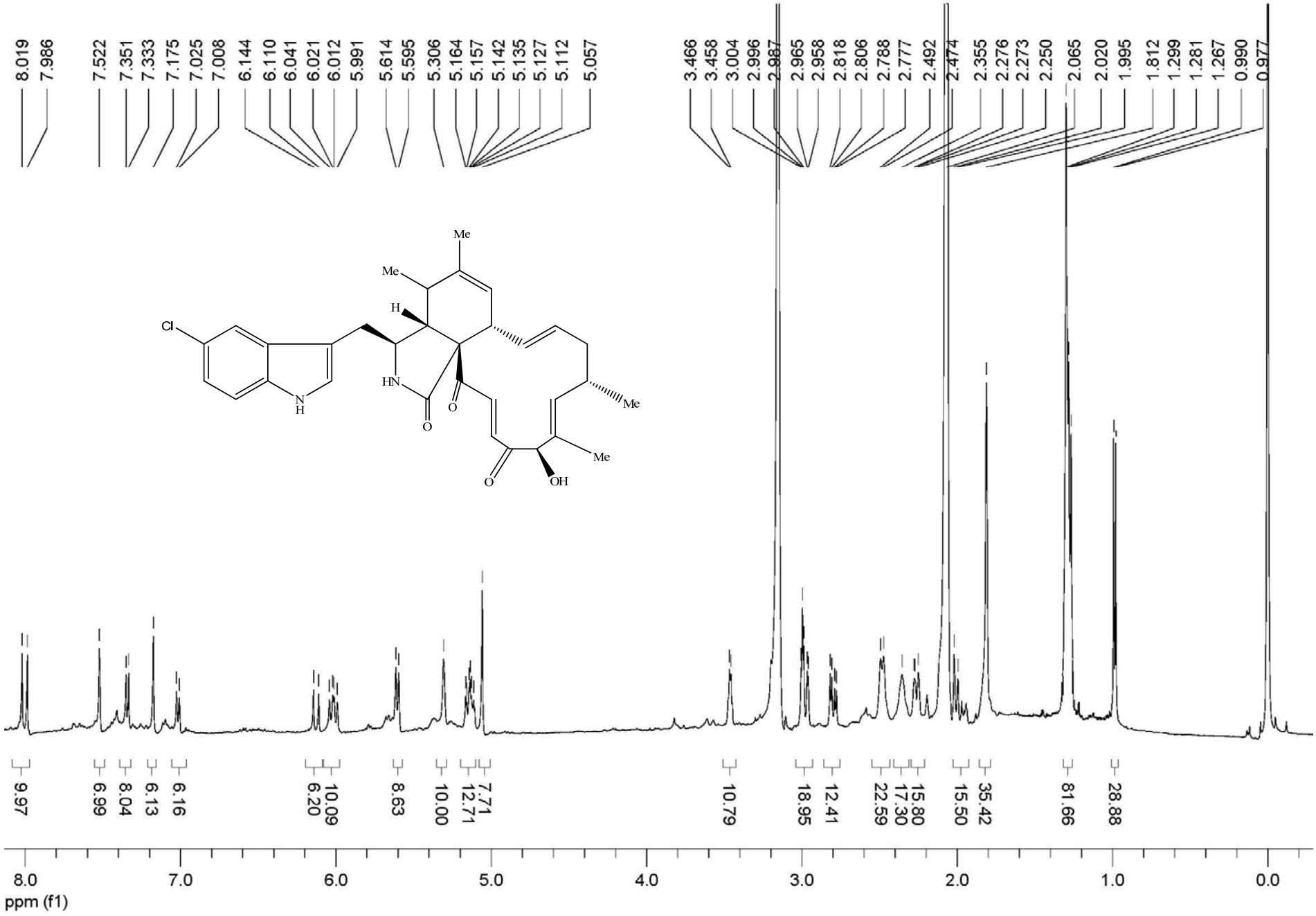
# **S17** HSQC spectrum for 5'-F-chaetoglobosin J (**1a**)



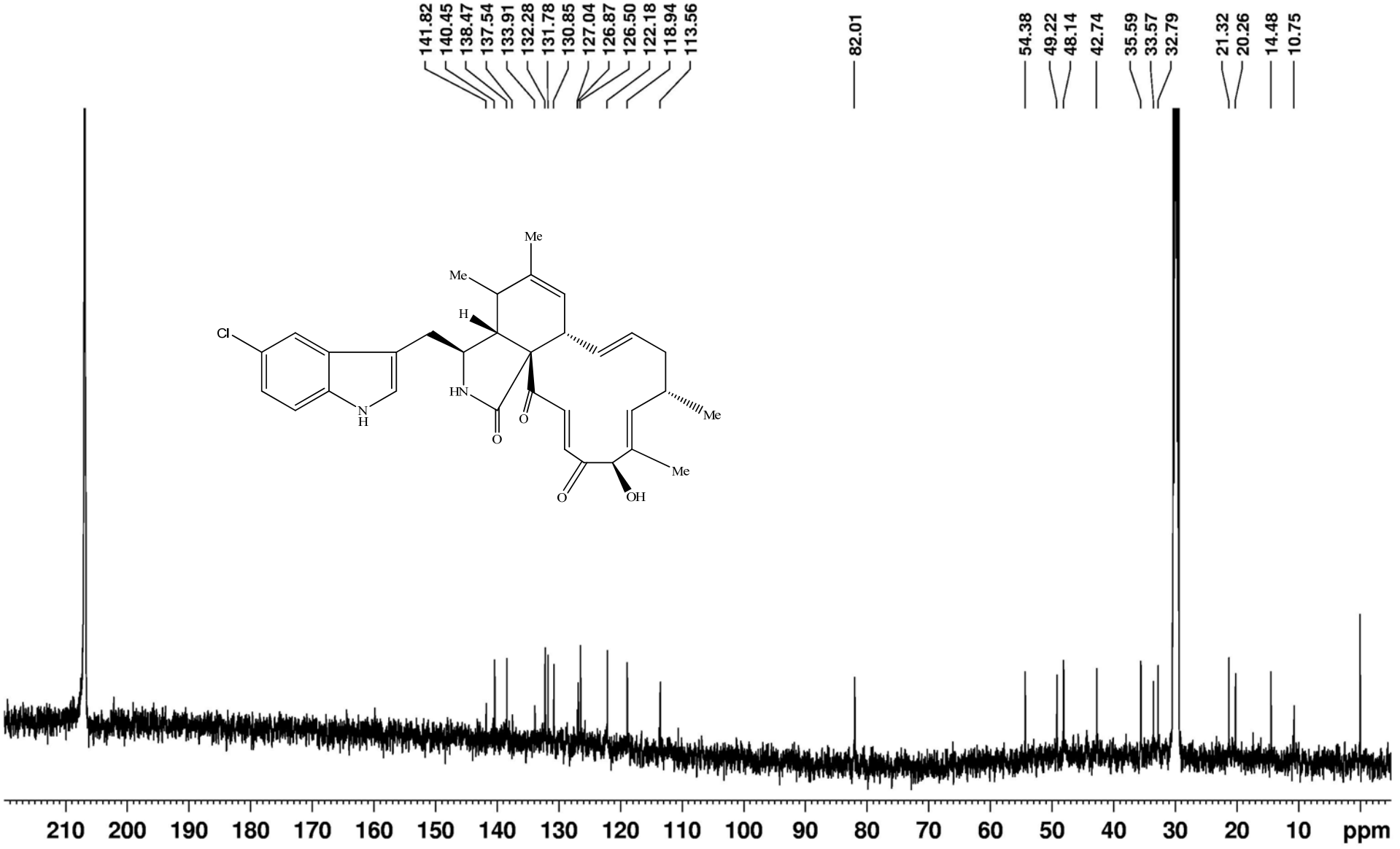
# **S18** HMBC spectrum for 5'-F-chaetoglobosin J (**1a**)



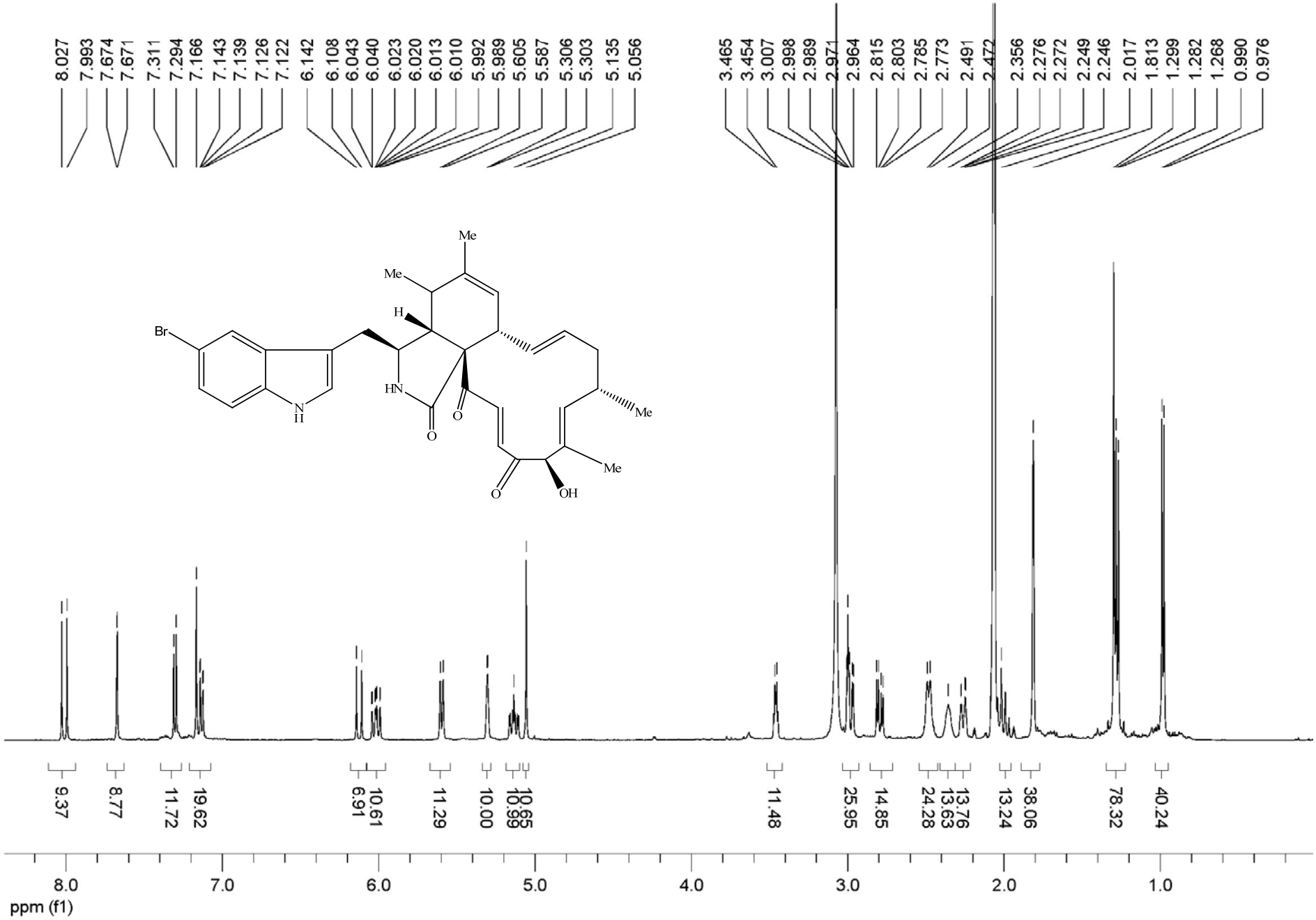
# **S19** $^{1}$H NMR spectrum for 5'-Cl-chaetoglobosin J (**1b**)



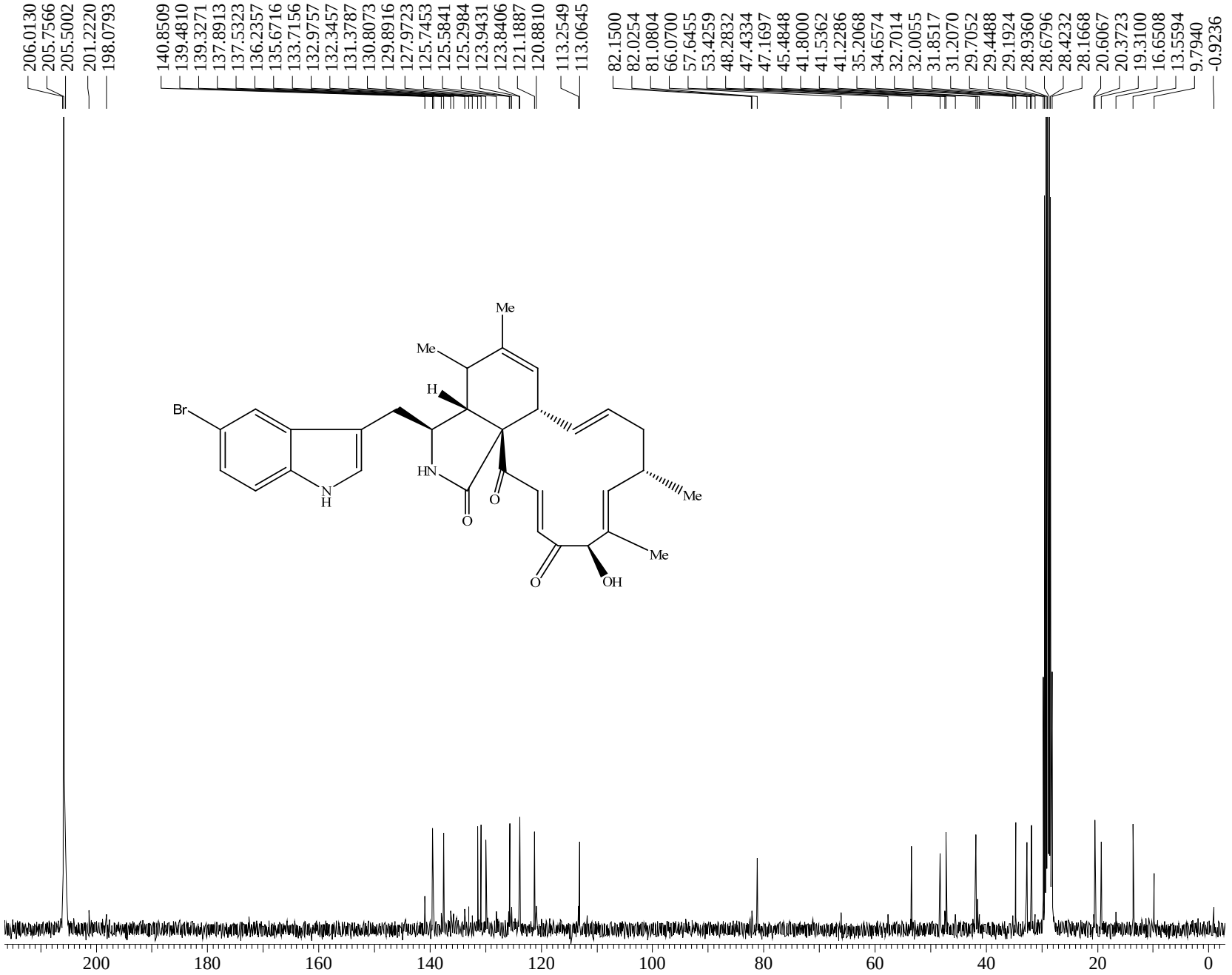
# **S20** $^{13}$C NMR spectrum for 5'-Cl-chaetoglobosin J (**1b**)



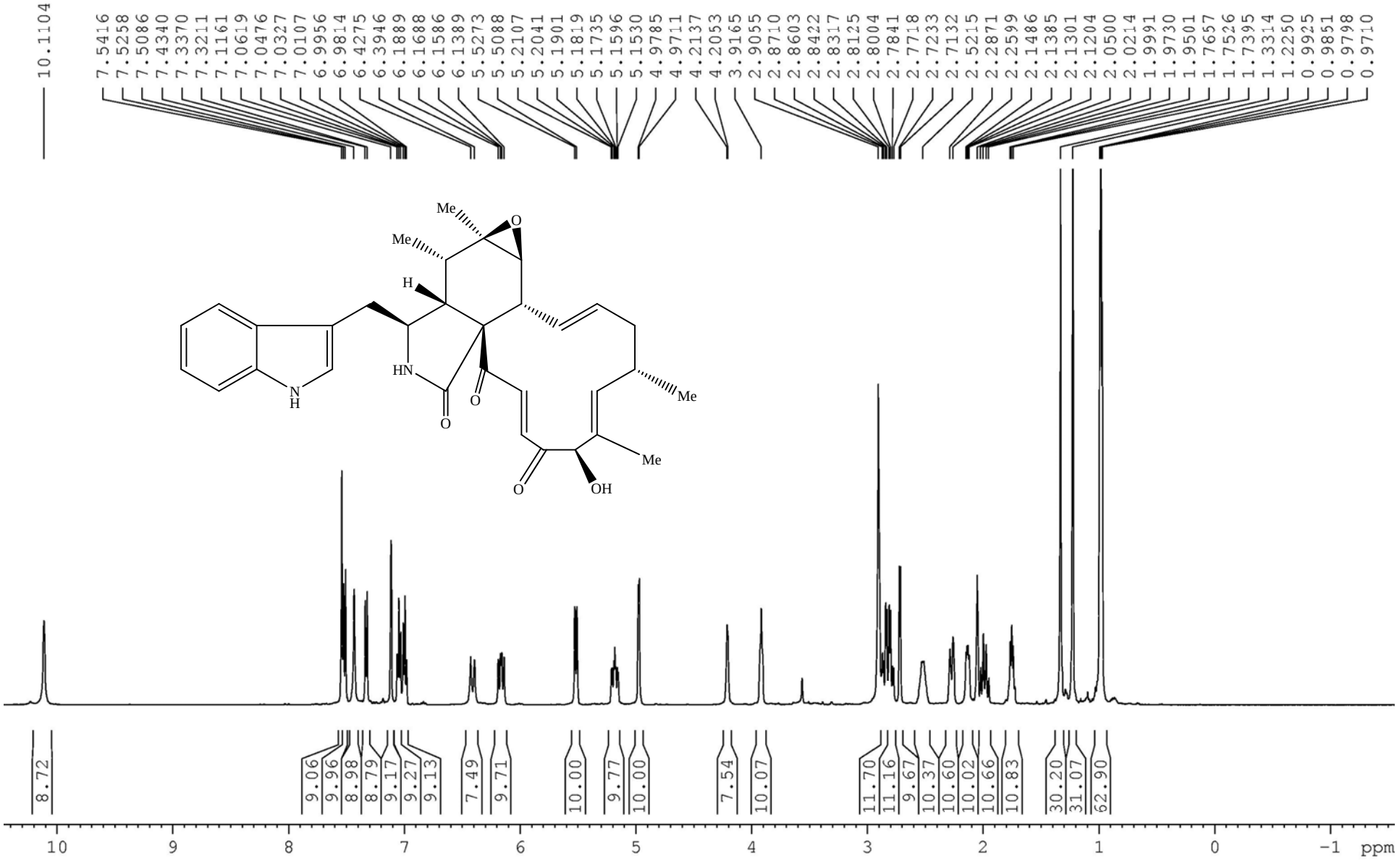
# **S21** $^{1}$H NMR spectrum for 5'-Br-chaetoglobosin J (**1c**)



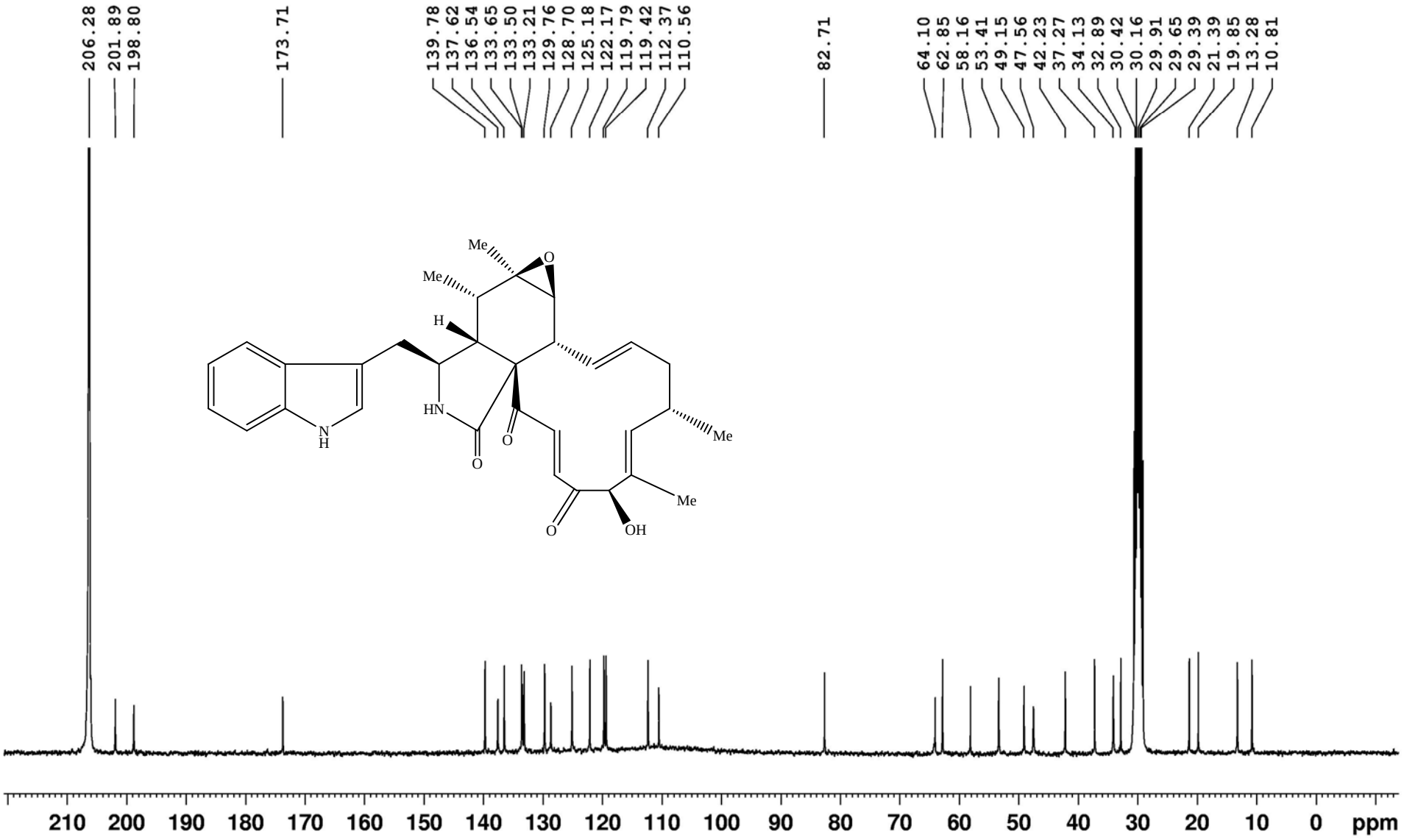
# **S22** $^{13}$C NMR spectrum for 5'-Br-chaetoglobosin J (**1c**)



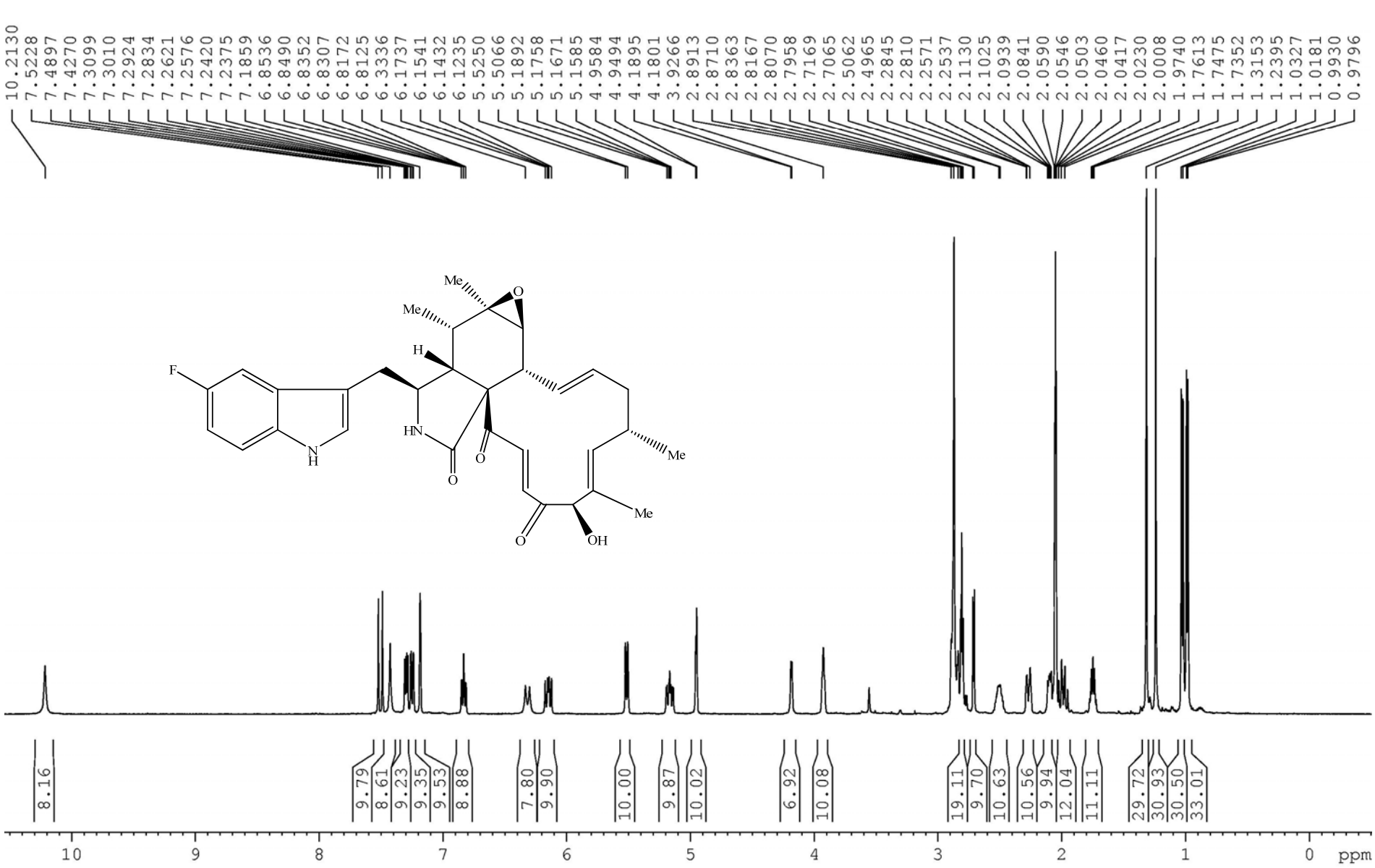
# **S23** $^{1}$H NMR spectrum for chaetoglobosin A (**2**)



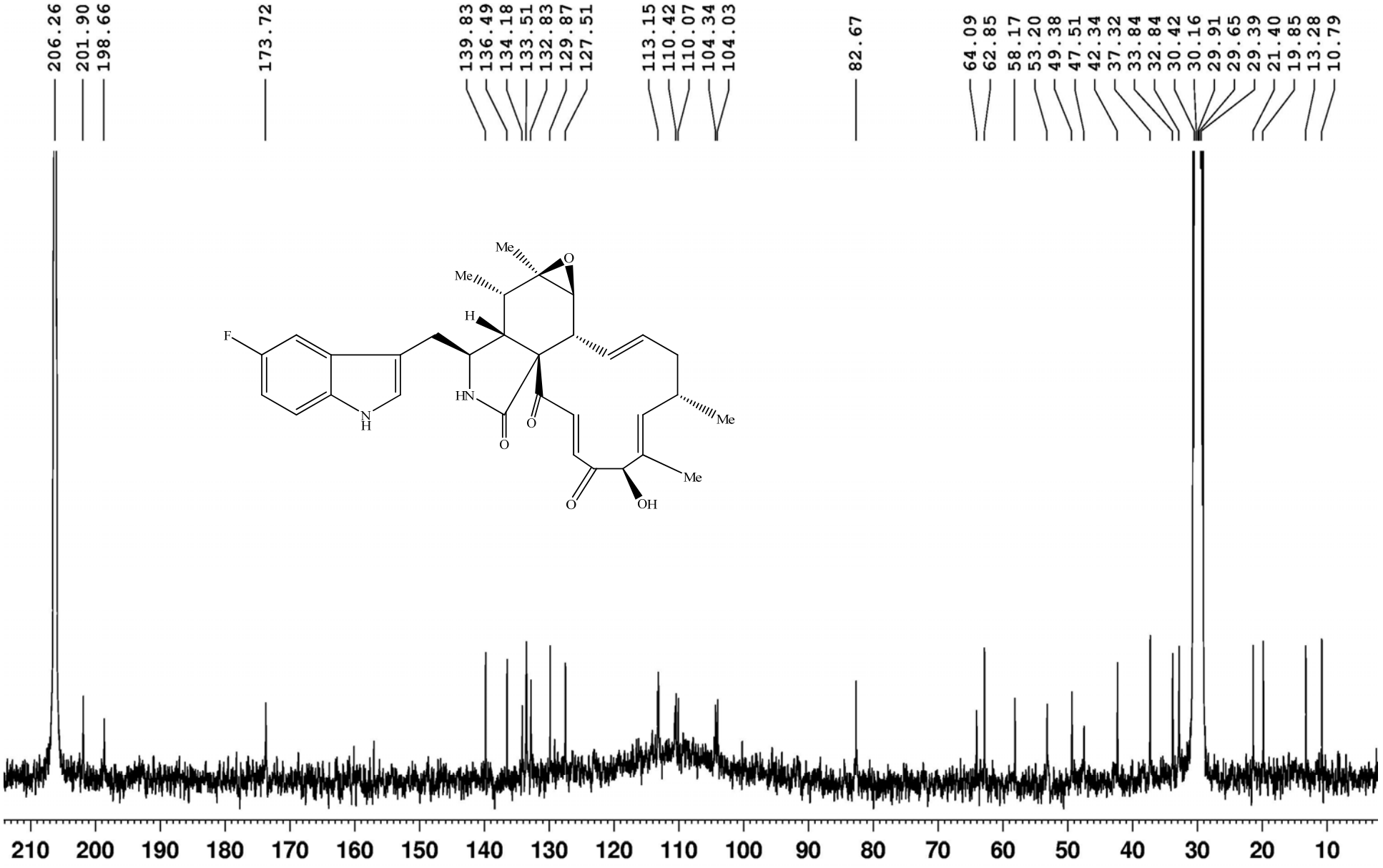
# **S24** $^{13}$C NMR spectrum for chaetoglobosin A (**2**)



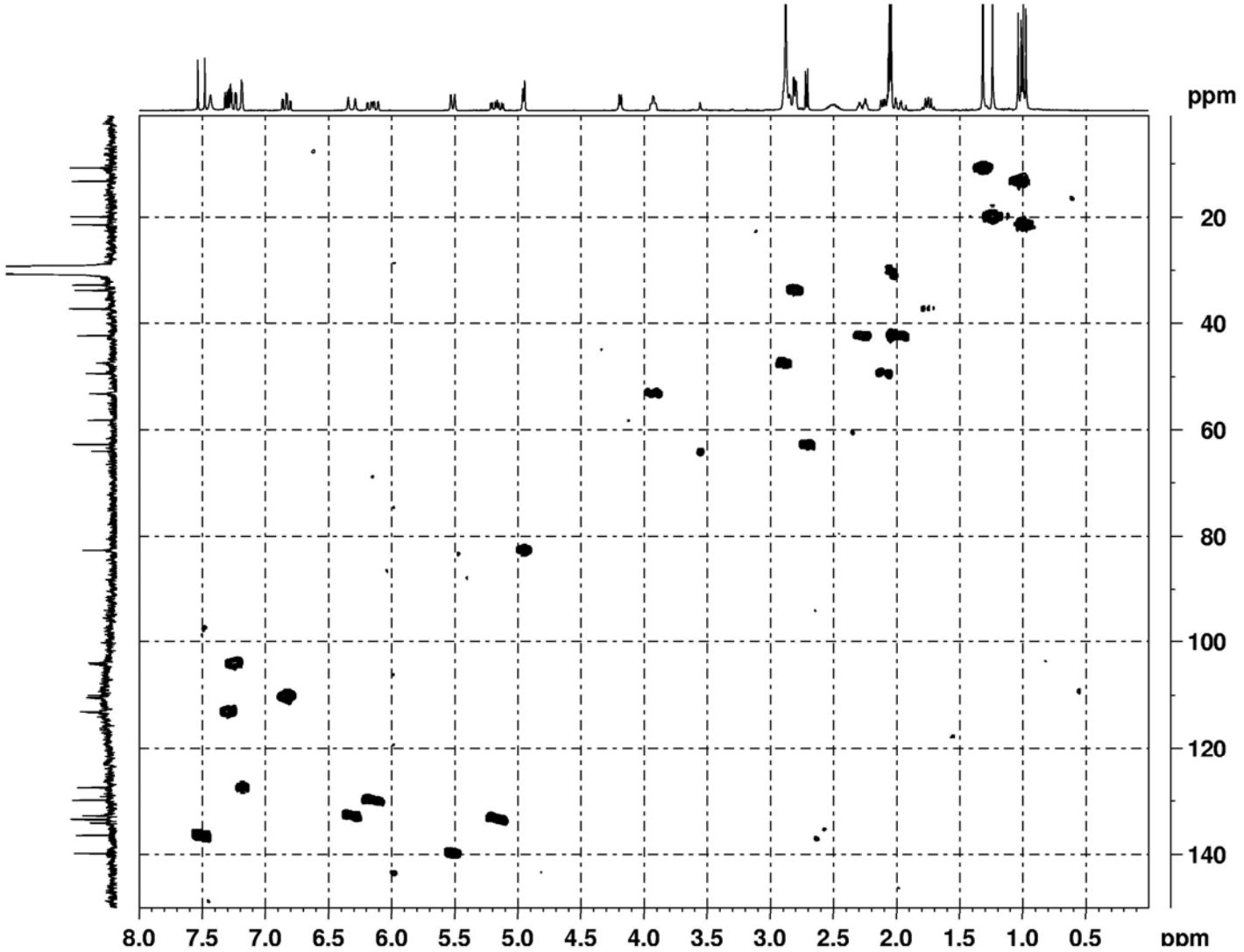
# **S25** $^{1}$H NMR spectrum for 5'-F-chaetoglobosin A (**2a**)



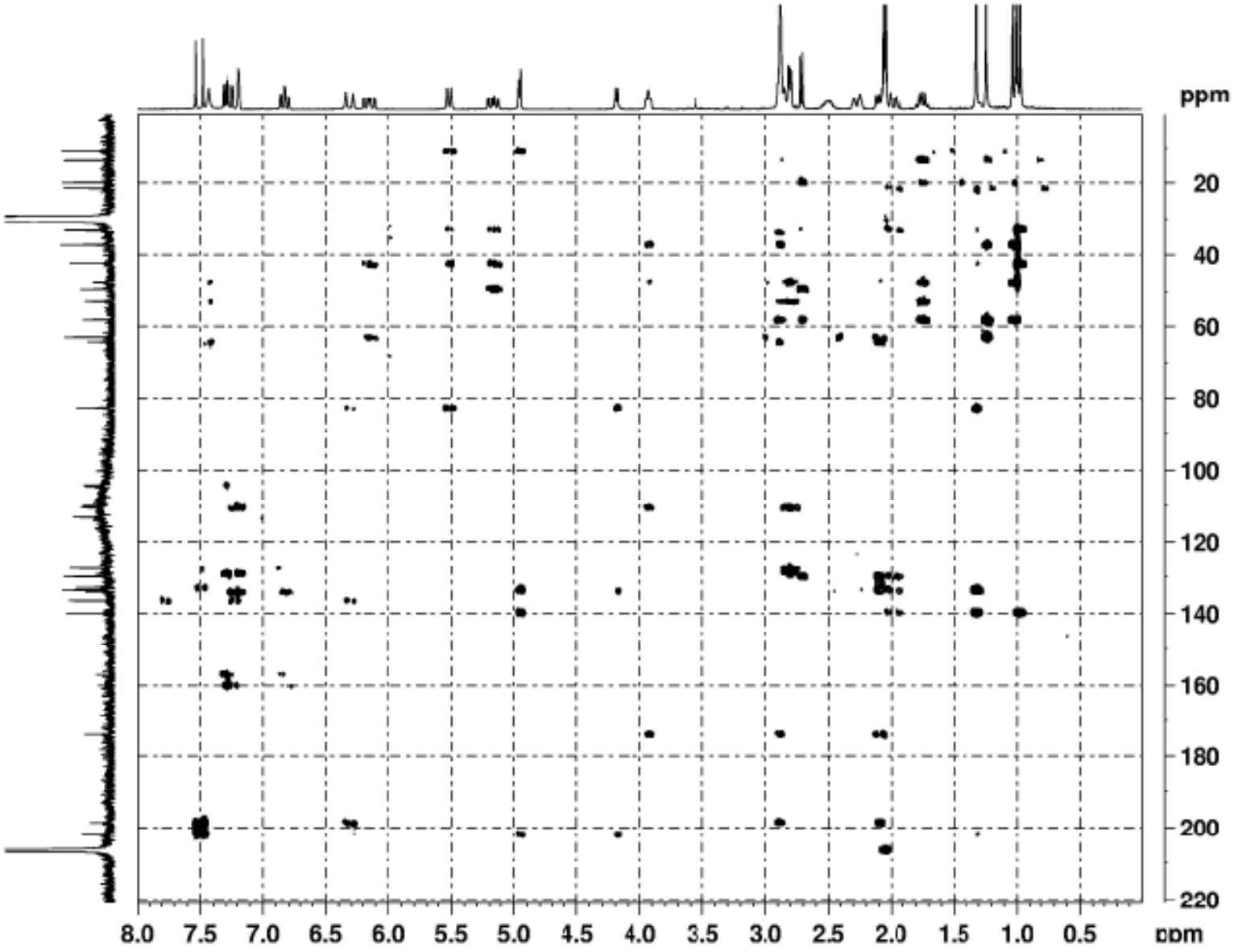
# **S26** $^{13}$C NMR spectrum for 5'-F-chaetoglobosin A (**2a**)



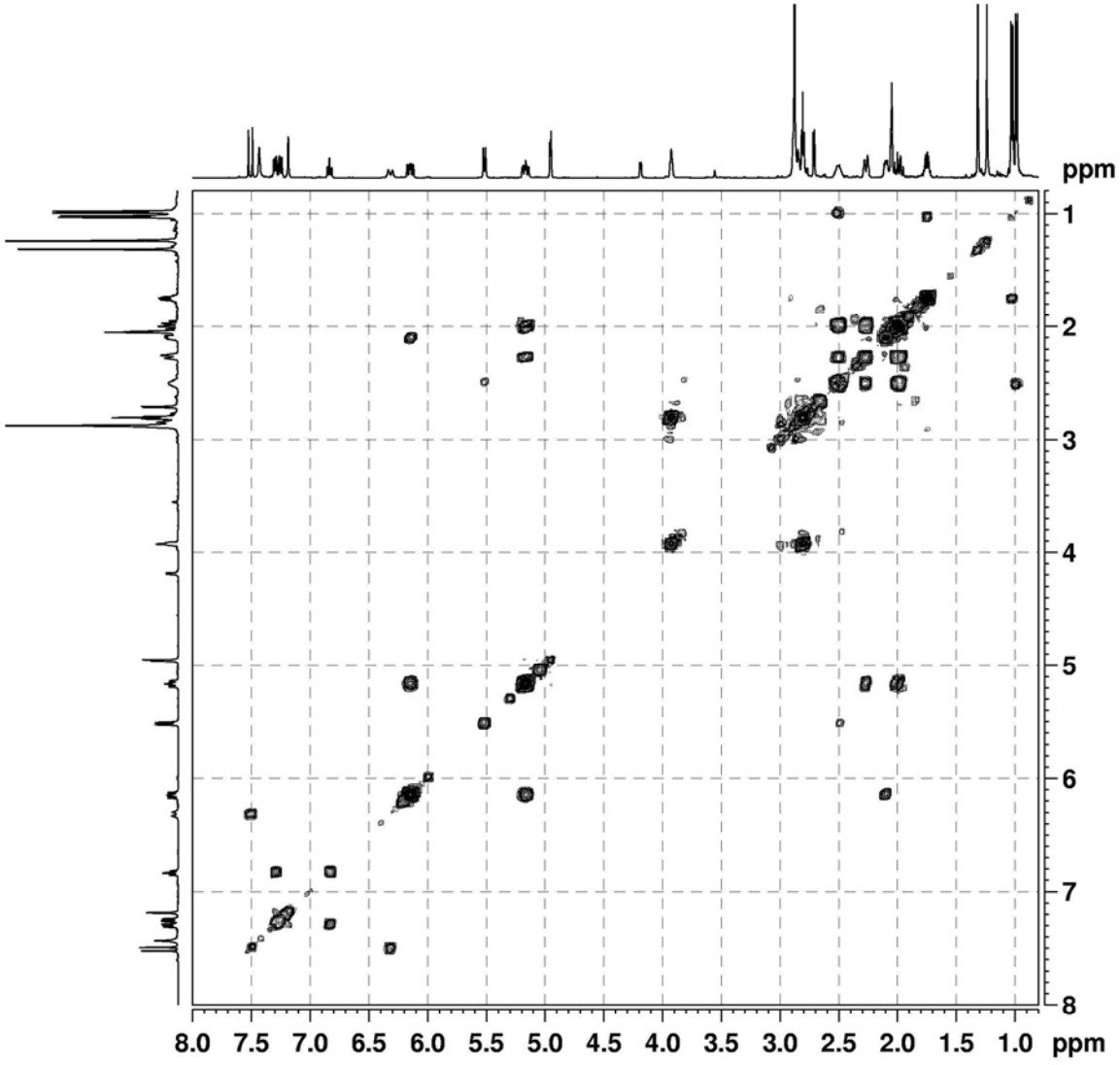
# **S27** HMQC spectrum for 5'-F-chaetoglobosin A (**2a**)



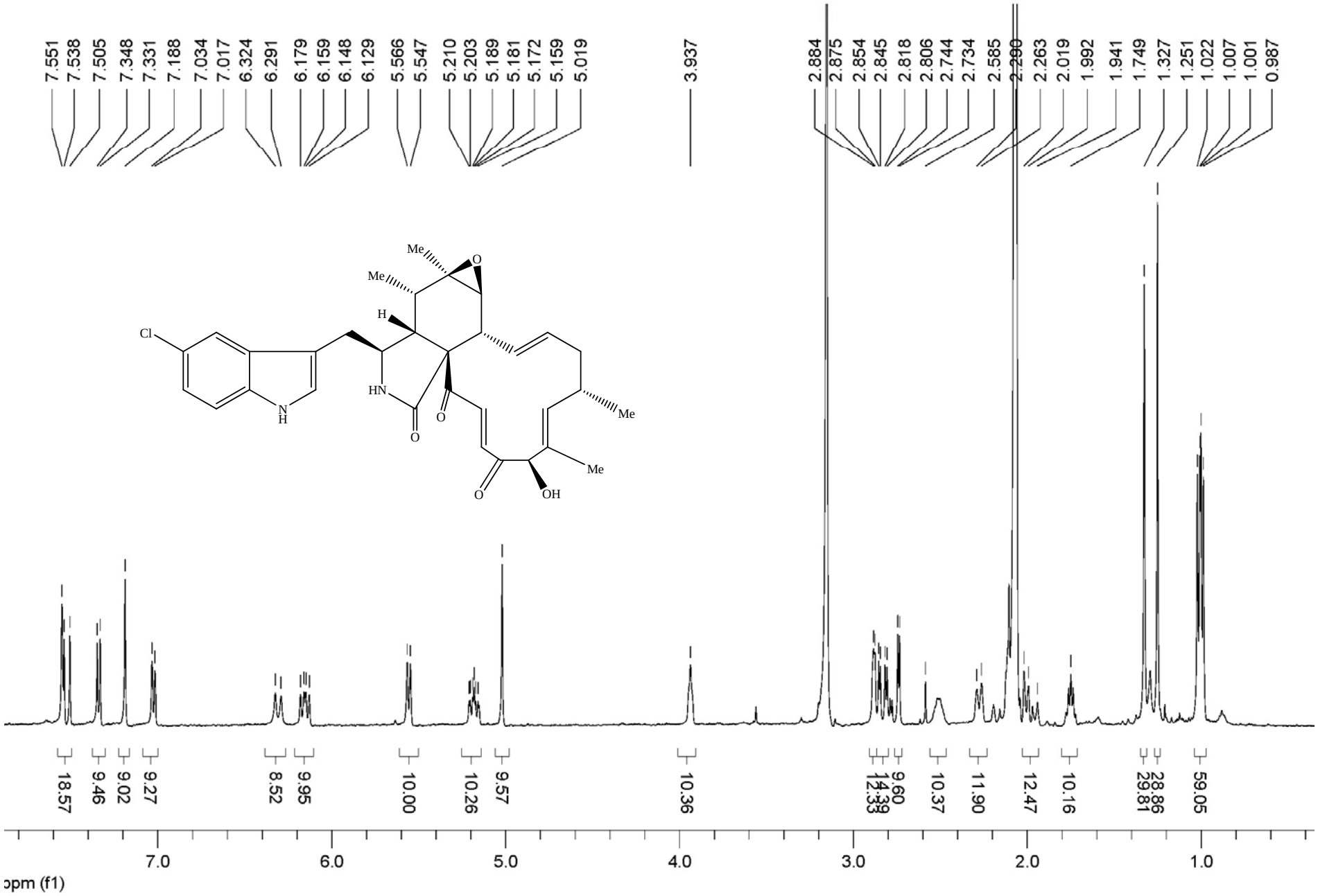
# **S28** HMBC spectrum for 5'-F-chaetoglobosin A (**2a**)



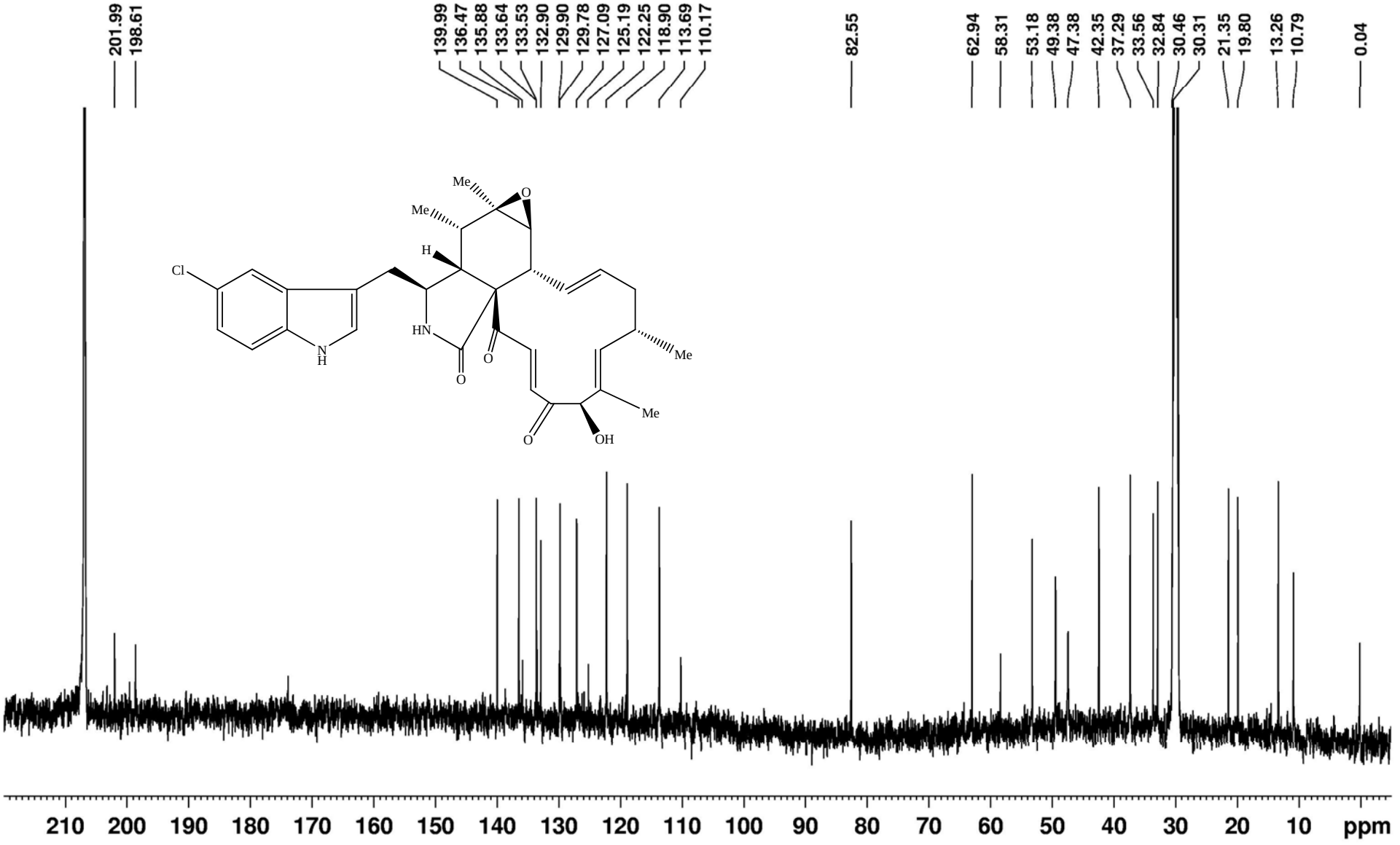
# **S29** $^{1}$H-$^{1}$HCOSY spectrum for 5'-F-chaetoglobosin A (**2a**)



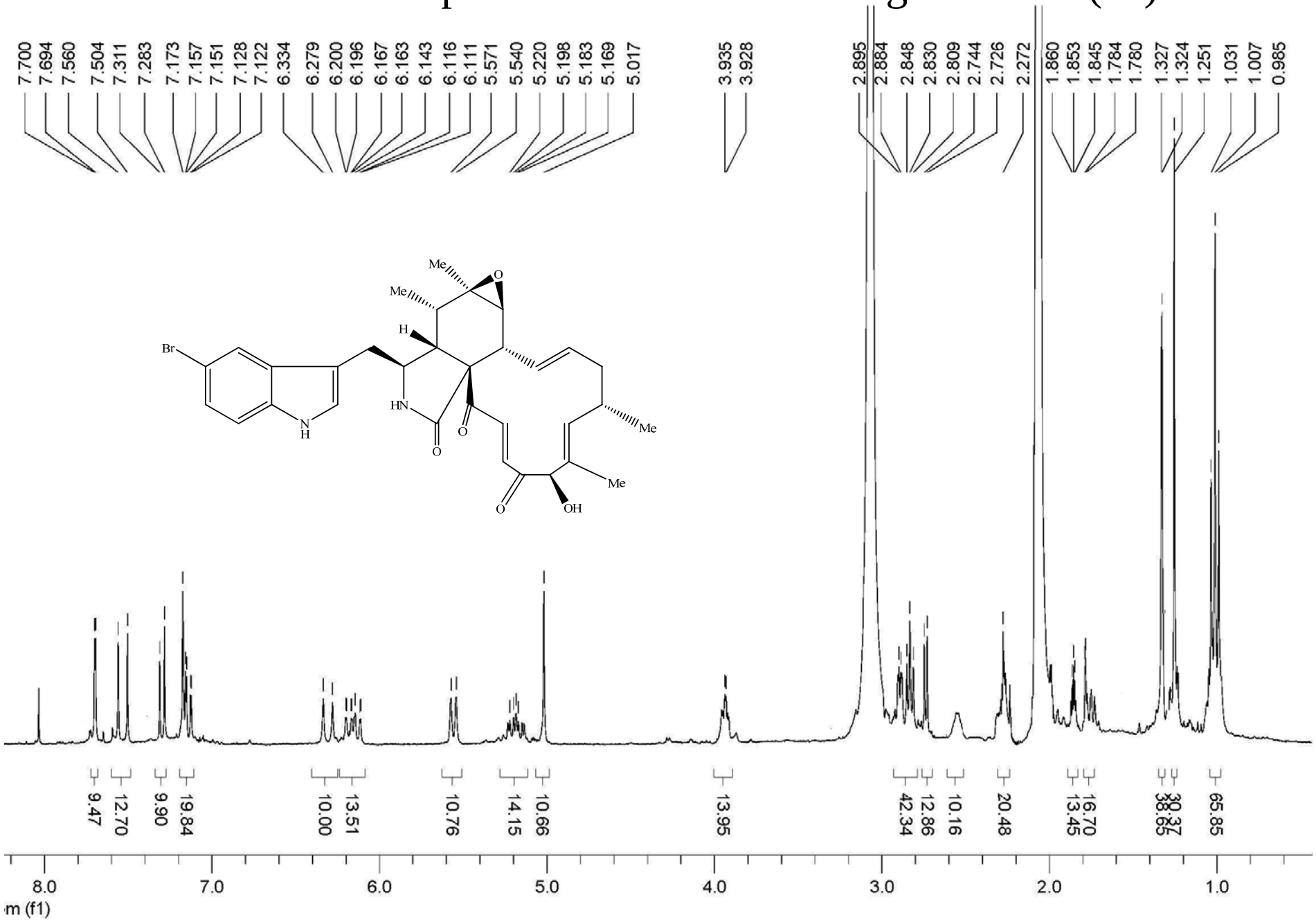
# **S30** $^{1}$H NMR spectrum for 5'-Cl-chaetoglobosin A (**2b**)



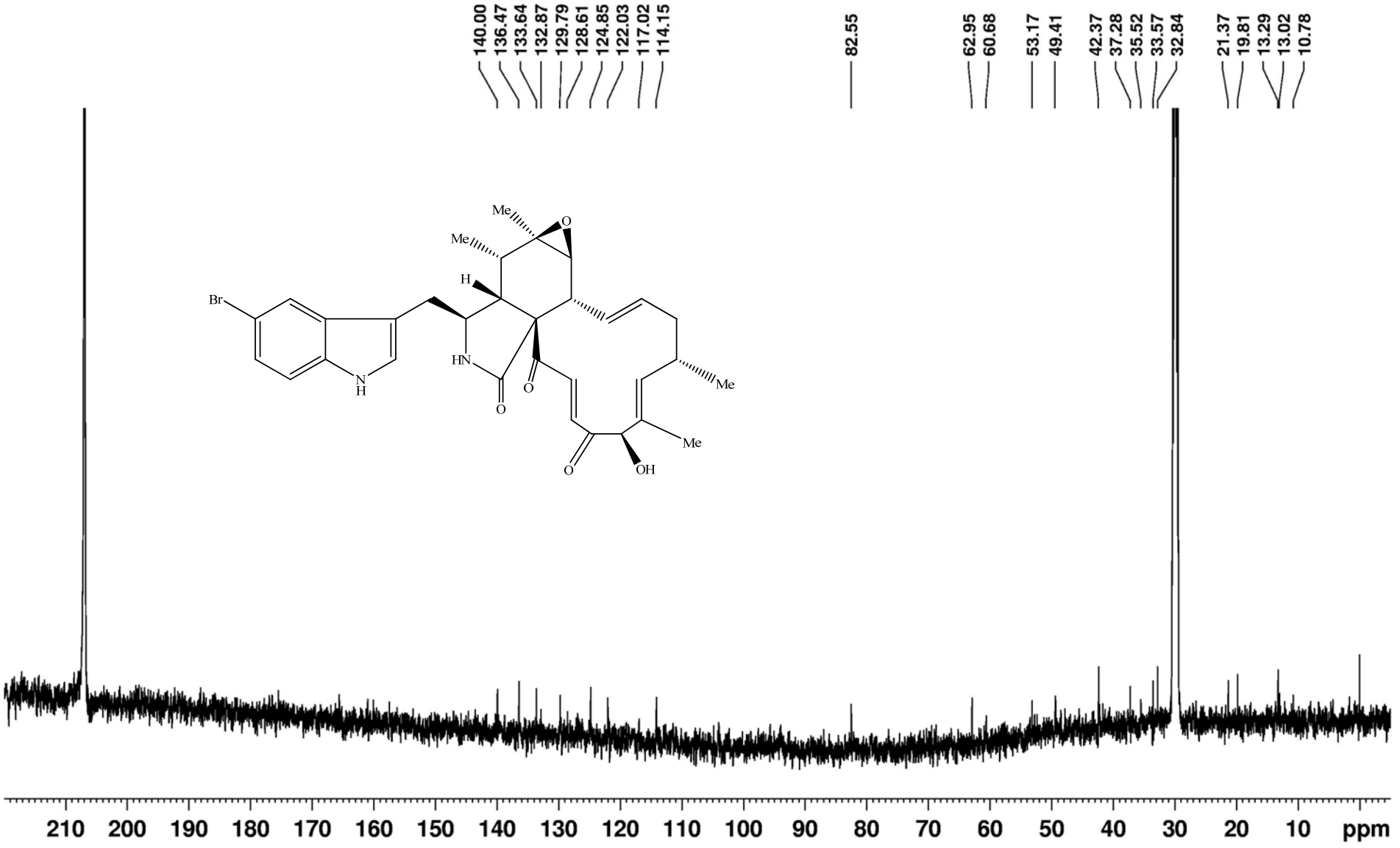
# **S31** $^{13}$C NMR spectrum for 5'-Cl-chaetoglobosin A (**2b**)



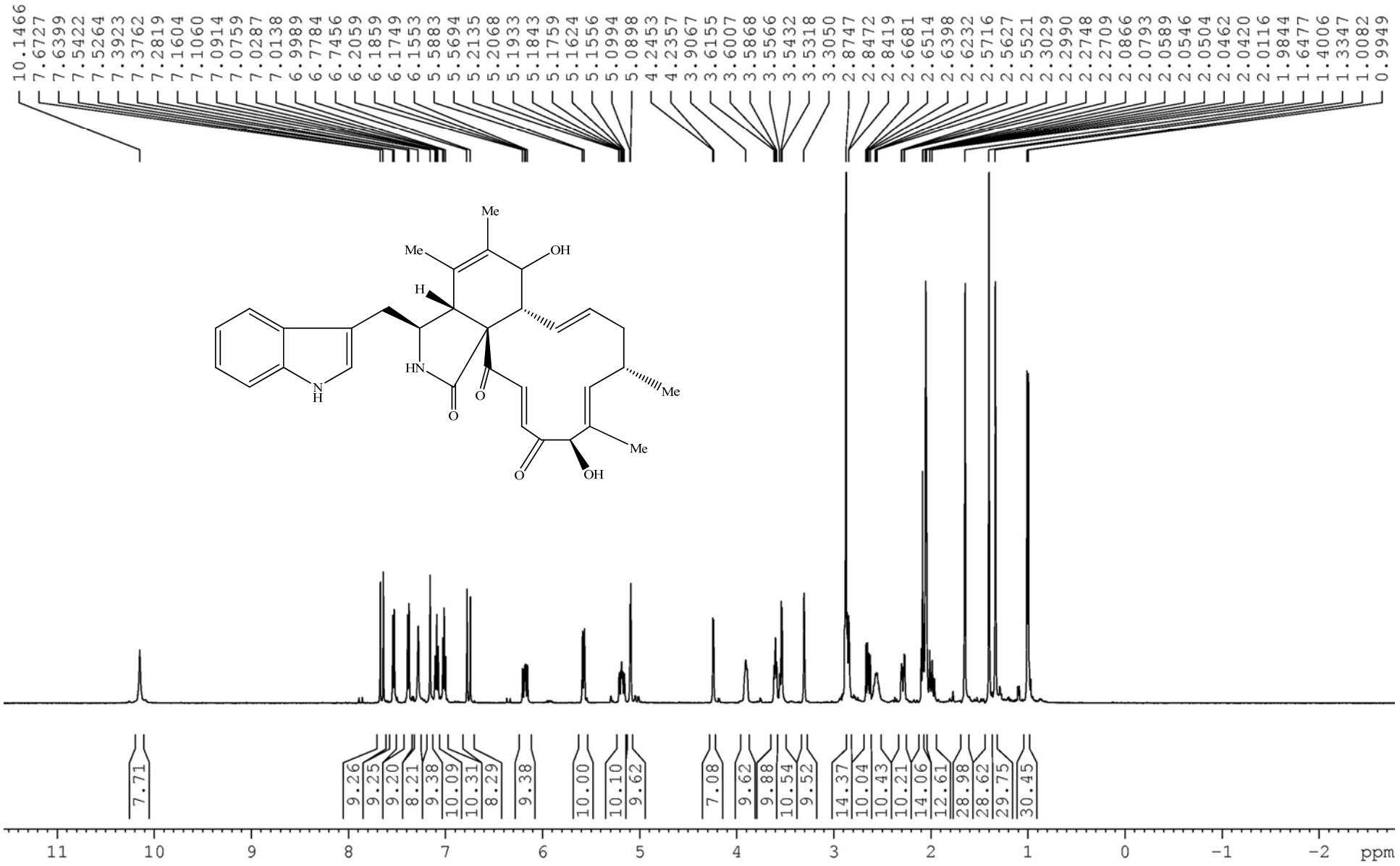
# **S32** $^{1}$H NMR spectrum for 5'-Br-chaetoglobosin A (**2c**)



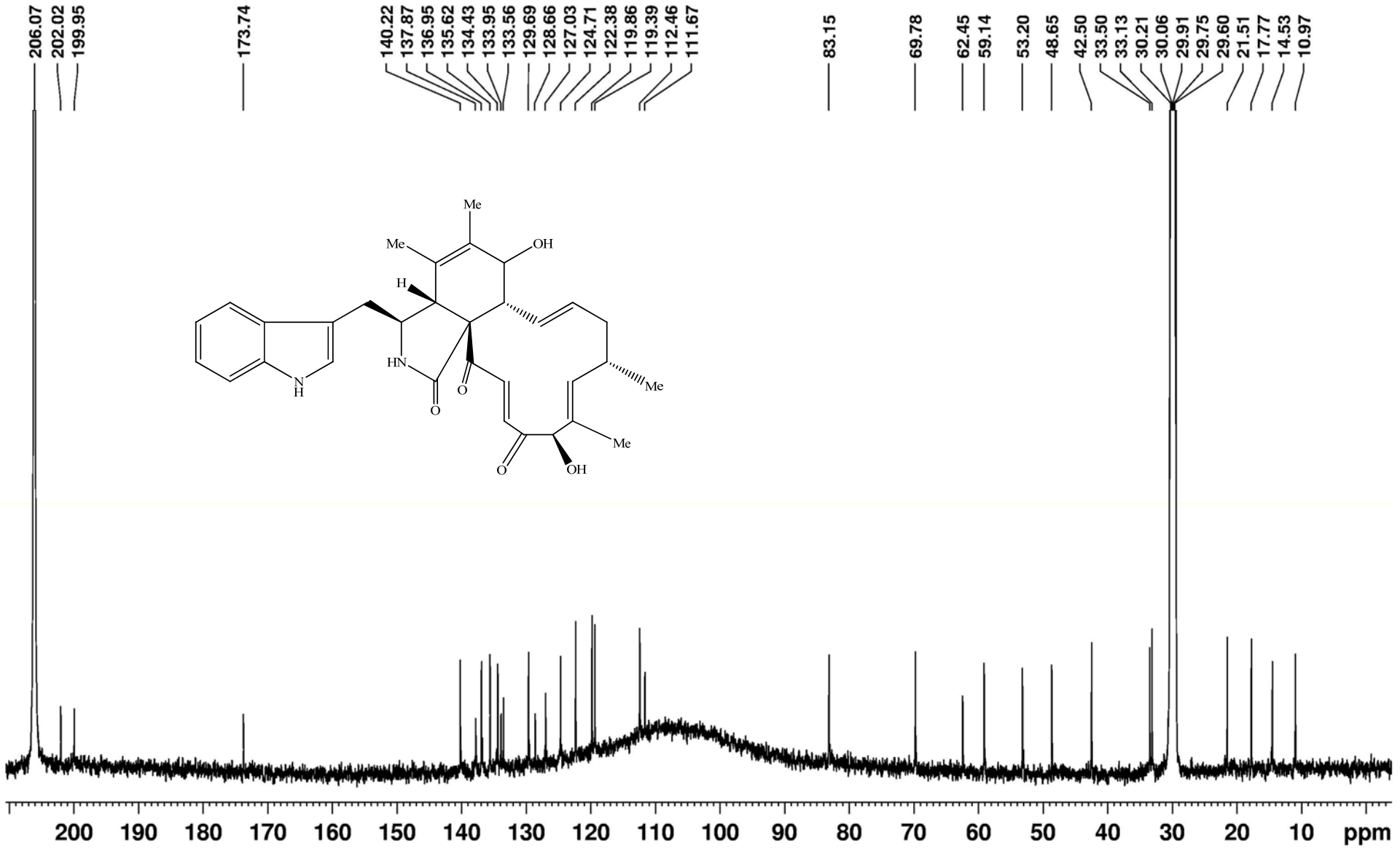
# **S33** $^{13}$C NMR spectrum for 5'-Br-chaetoglobosin A (**2c**)



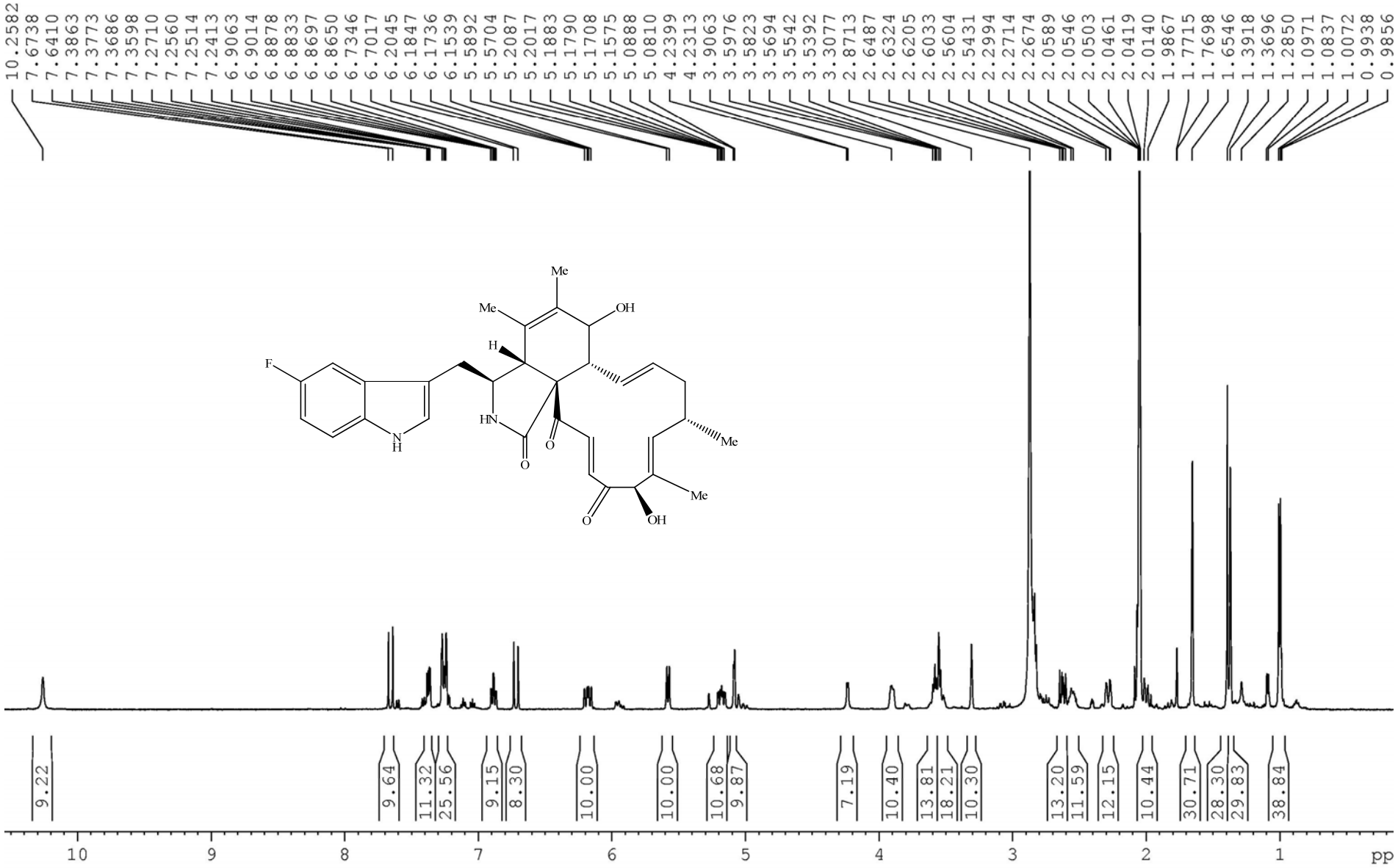
# **S34** $^{1}$H NMR spectrum for chaetoglobosin B (**3**)



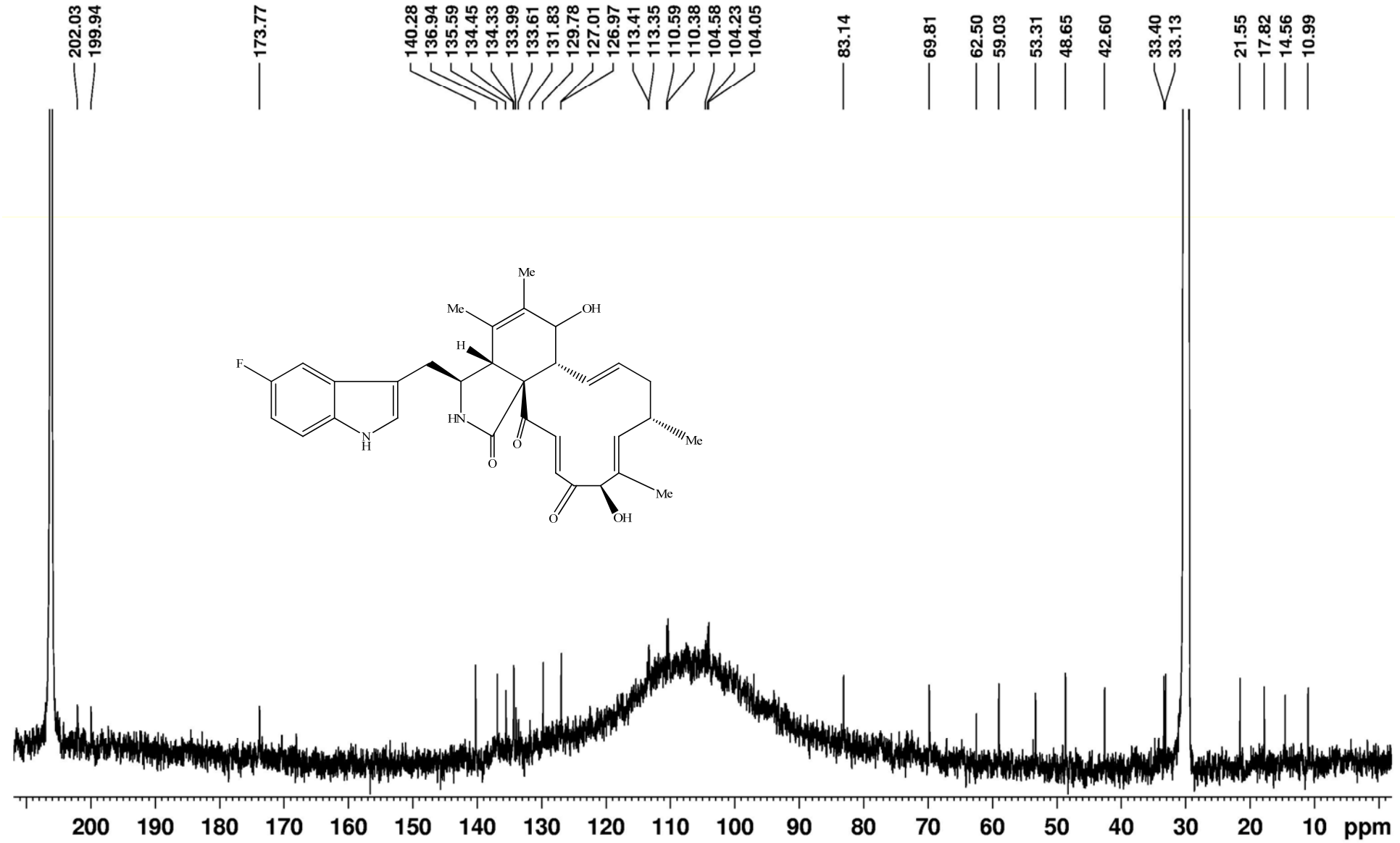
# **S35** $^{13}$C NMR spectrum for chaetoglobosin B (**3**)



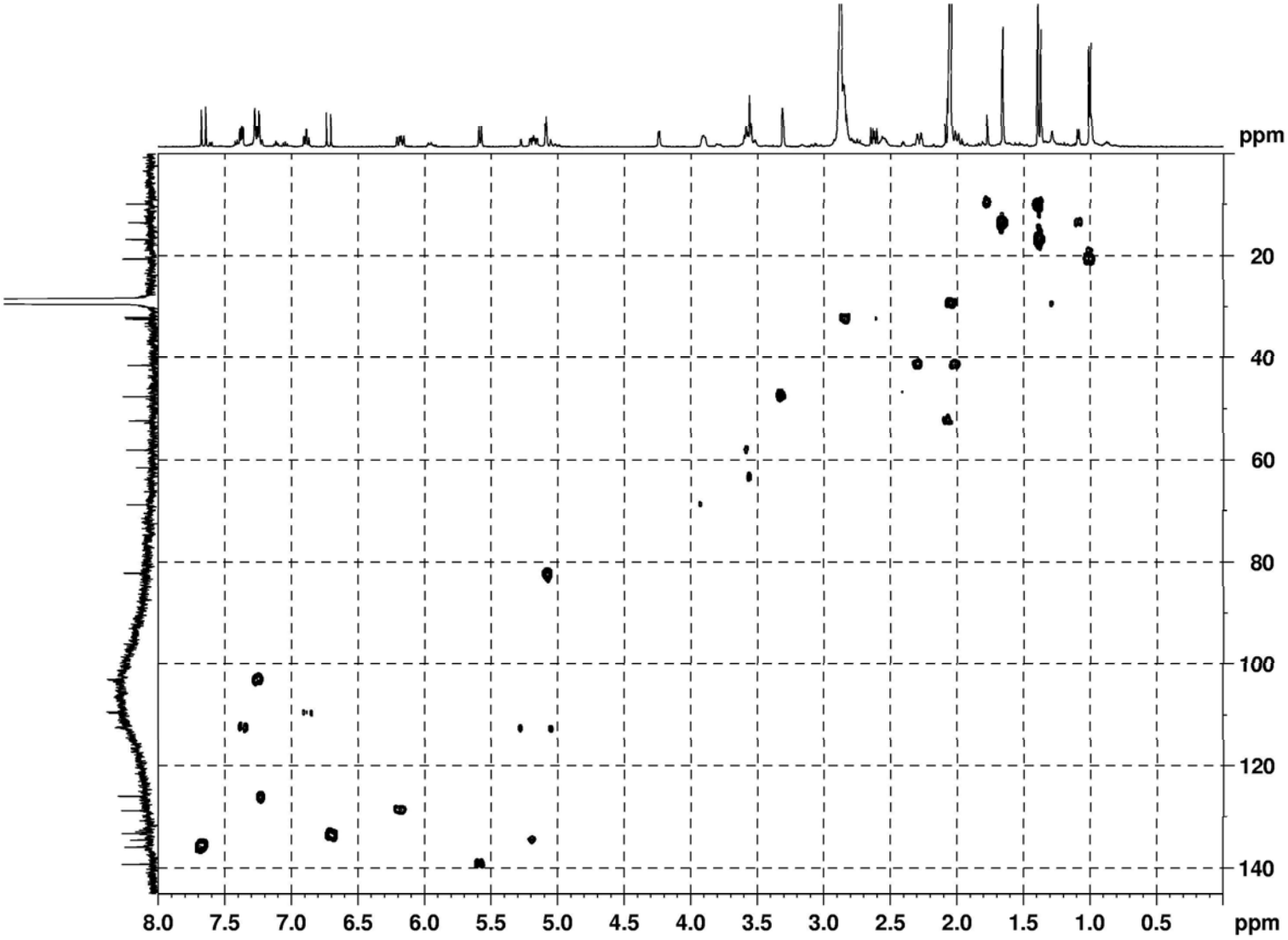
# **S36** $^{1}$H NMR spectrum for 5'-F-chaetoglobosin B (**3a**)



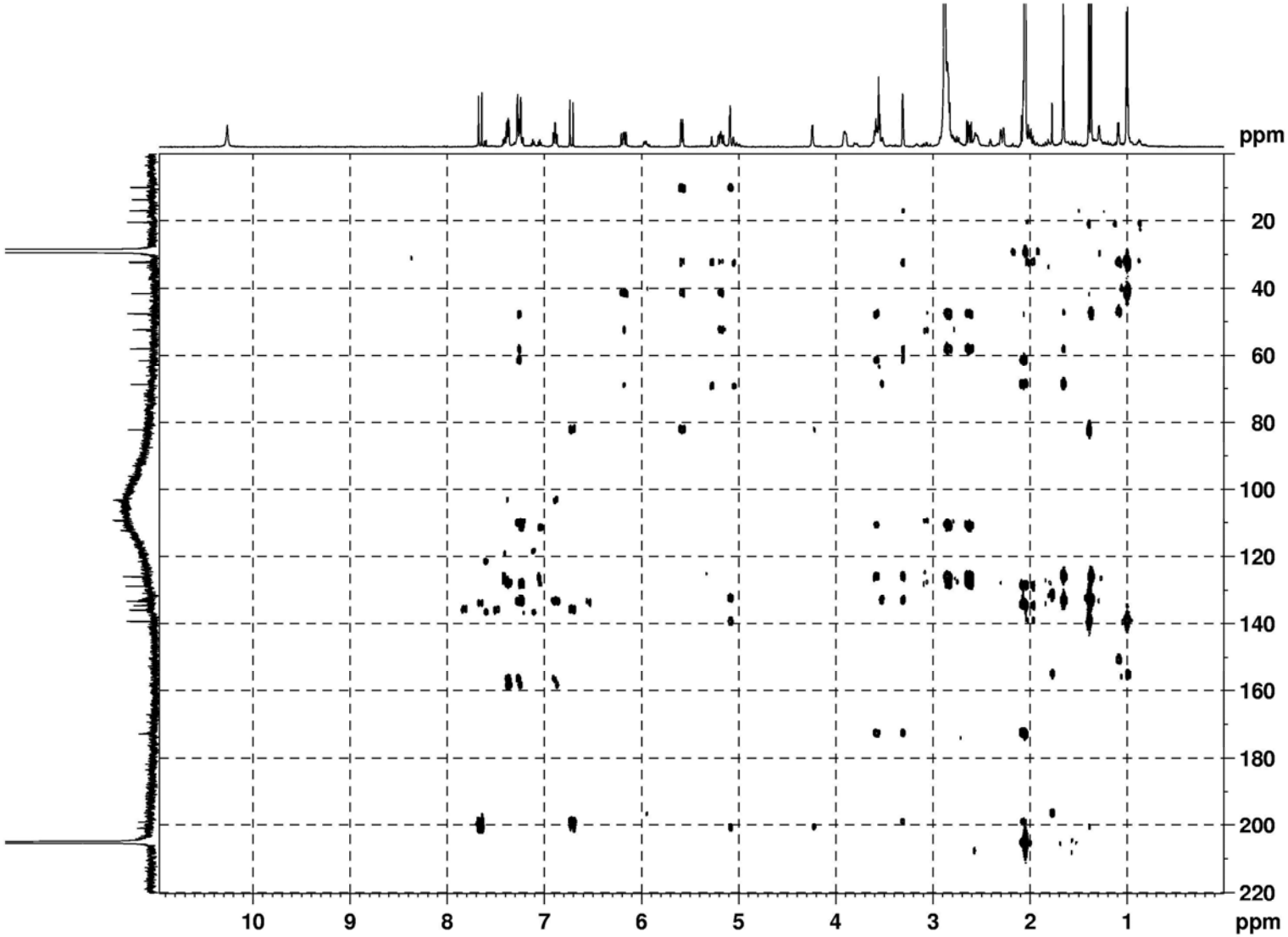
# **S37** $^{1}$H NMR spectrum for 5'-F-chaetoglobosin B (**3a**)



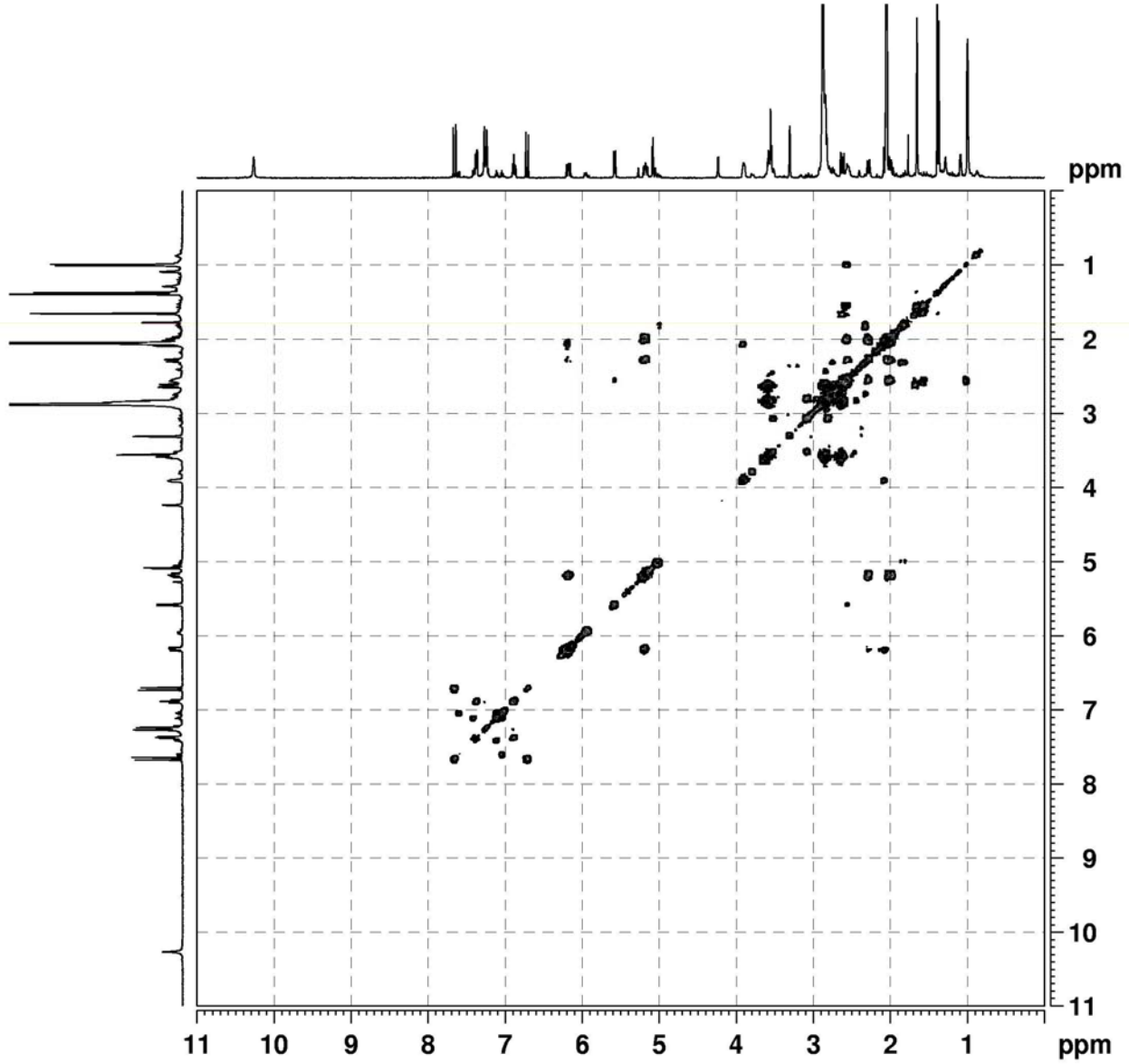
# **S38** HMQC spectrum for 5'-F-chaetoglobosin B (**3a**)



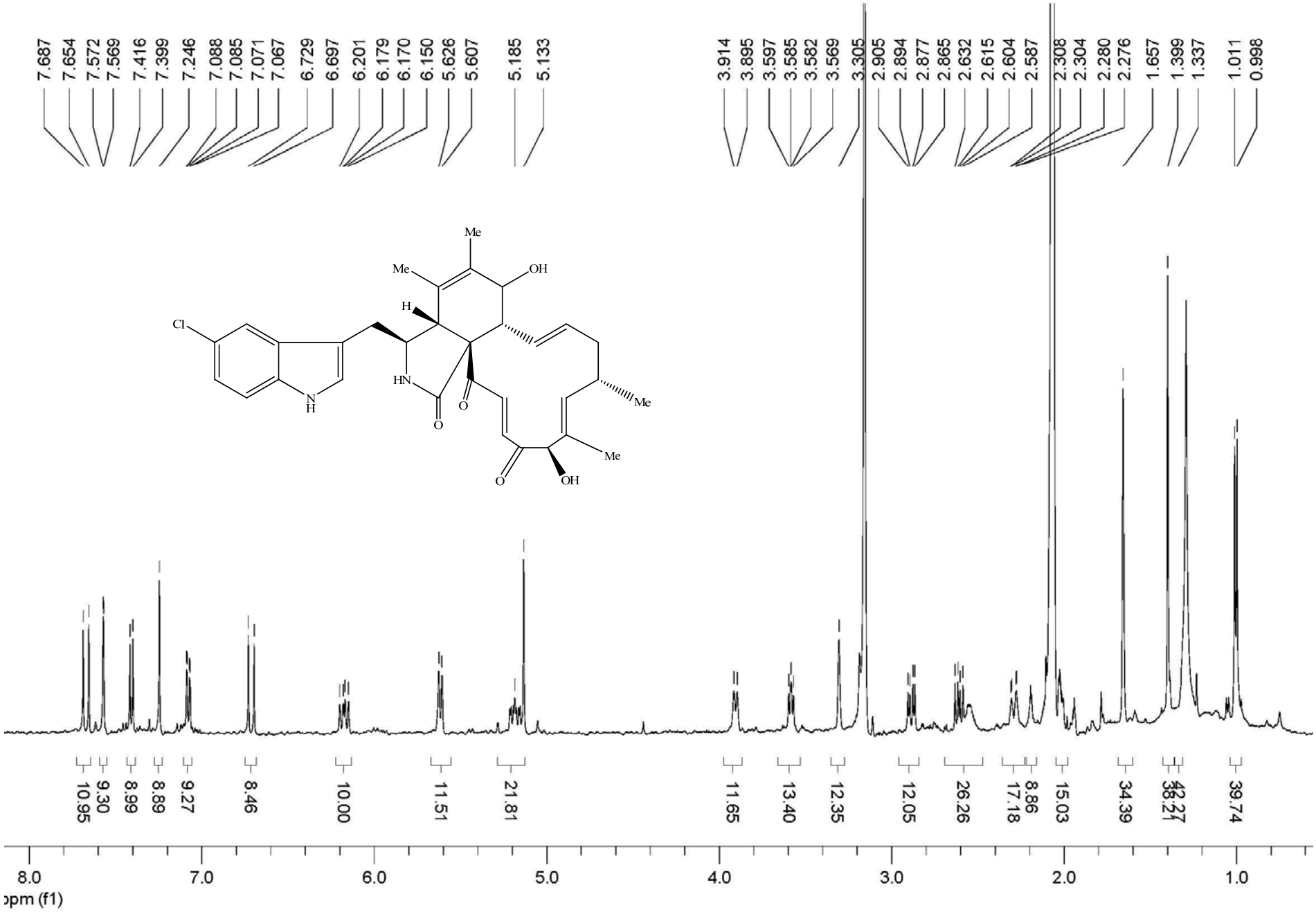
# **S39** HMBC spectrum for 5'-F-chaetoglobosin B (**3a**)



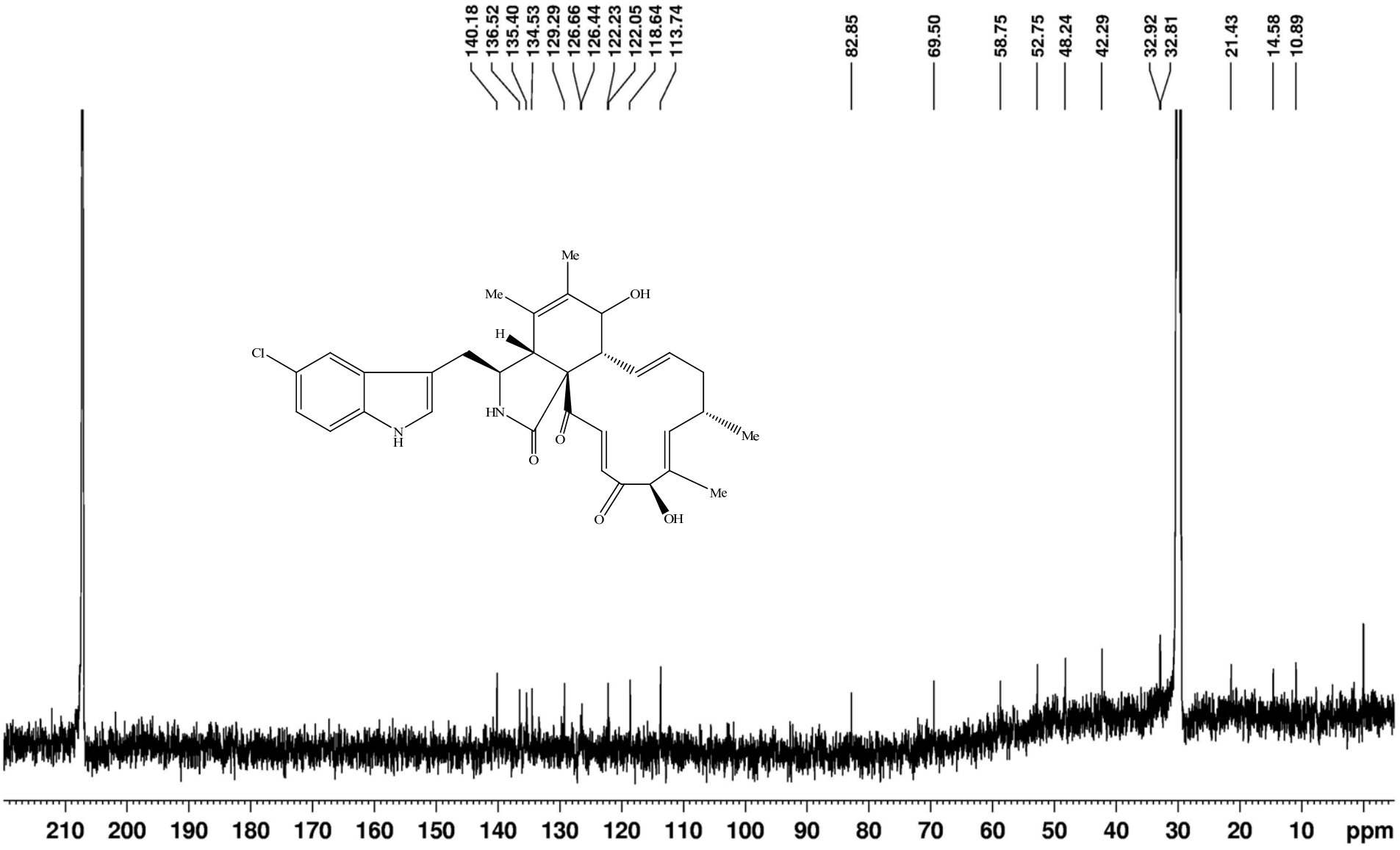
# **S40** $^{1}$H-$^{1}$HCOSY spectrum for 5'-F-chaetoglobosin B (**3a**)



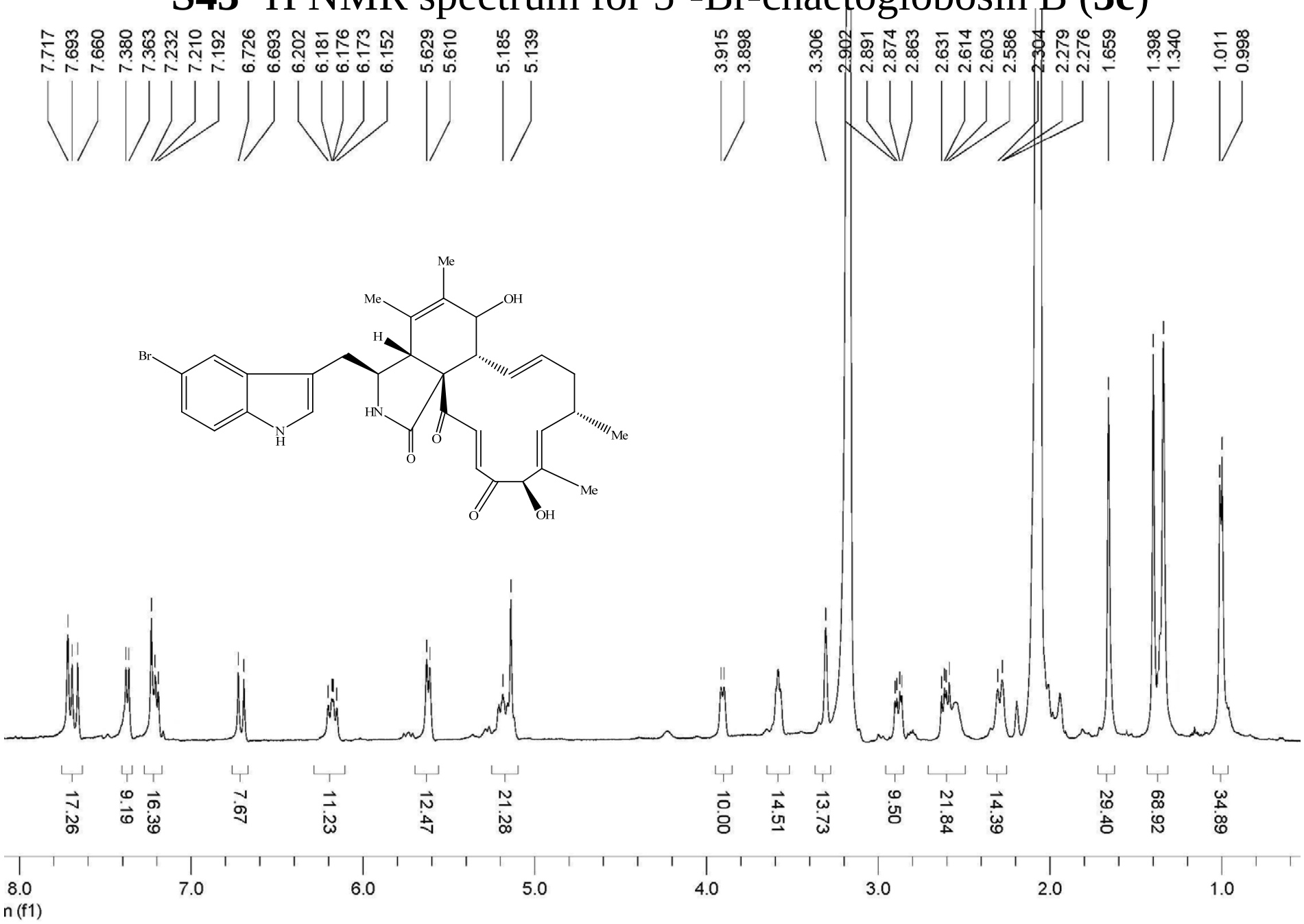
# **S41** $^{1}$H NMR spectrum for 5'-Cl-chaetoglobosin B (**3b**)



# **S42** $^{13}$C NMR spectrum for 5'-Cl-chaetoglobosin B (**3b**)



# **S43** $^{1}$H NMR spectrum for 5'-Br-chaetoglobosin B (**3c**)



# **S44** $^{13}$C NMR spectrum for 5'-Br-chaetoglobosin B (**3c**)