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Supporting Information

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Synthesis, Biosynthesis and absolute Configuration of Vioxanthin

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General Remarks: All reagents were used in analytical grade. Solvents were desiccated by standard methods if necessary. Thin Layer Chromatography (TLC) was carried out on aluminum sheets precoated with silica gel $60F_{254}$ (Merck). Detection was performed by UV-light (λ = 254 nm) and fluorescence (λ = 366 nm). Preparative column chromatography was carried out on silica gel 60 (Merck) (particle size 40-63 µm). NMR spectra were recorded on an Avance DRX 400 (Bruker Physik AG, Germany). Chemical shifts δ are reported in ppm relative to $CHCl_3$ (^{1}H, δ = 7.25) and $CDCl_3$ (^{13}C, δ= 77.00) as internal standard. Optical rotations were measured on a polarimeter P-1020 (Jasco). CD-spectra were recorded on a Jasco J-720 spectralpolarimeter. Molar circular-dichroic absorptions $\Delta\varepsilon$ are reported in $cm^2 \cdot mmol^{-1}$.

Cultivation of *Penicillium citreo-viride* and isolation of natural products: Potato dextrose agar is inoculated with *Penicillium citreo-viride* ATCC 42743 (Tü 553), after 10 days incubation at 30 °C the mycelium is suspended with saline solution (9 g/L sodium chloride and 0.5 g/L Tween® 80 in water; 8 mL per agar plate). The mycelium suspensions are mixed with glycerol and stored at –20 °C. For isolation of natural product a 100 mL culture (10 g/L malt extract, 4 g/L yeast extract, 4 g/L glucose) is spiked with 150 mg Amberlite XAD-7, inoculated with 1 mL mycelium suspension and incubated at 21 °C, 120 rpm for 10-14 days, until an intensive red-brown color appears. The mycelium is filtered,[1] 3 mL 2 M hydrochloric acid are added, then it is extracted with chloroform / methanol 1:1 twice. The extract is concentrated under reduced pressure; the residue is taken up in dichloromethane and water. The aqueous phase is extracted with dichloromethane; the organic phase is dried ($MgSO_4$), and the solvent removed under reduced pressure. The crude extract was purified by column chromatography on silica gel (silica gel conditioned with 2 M HCl, eluent: dichloromethane / methanol 97:3) to obtain different fractions with secondary metabolites: R_f = 0.56, turquoise fluorescence, pale yellow solid (*semi*-vioxanthin (**2**), 39 mg / 100 mL culture); R_f = 0.20, green fluorescence, yellow solid (vioxanthin (**1**), 10 mg / 100 mL culture); R_f < 0.20, red color, red-brown solid (mixed fraction, containing vioxanthin and oxidized metabolites viomellein, xanthomegnin and rubrosulphin).

Feeding experiments: Preparation of the substrate: 15 mg ^{13}C-labeled (*R*)-, (*S*)-, or *rac*-**4** (57 µmol), or (*R*)-[O^{13}CH$_3$]-**2** are dissolved in acetone, 150 mg Amberlite® XAD-7 are added, and the solvent is removed under reduced pressure. Feeding experiments are done in 100 mL cultures under the same conditions as for isolation of natural products, the cultures are spiked with the substrate loaded Amberlite® XAD-7. After 10 days incubation at 21 °C and 120 rpm, the dark red mycelium was extracted and the metabolites isolated by column chromatography on silica gel (silica gel conditioned with 2 M HCl, eluent: dichloromethane / methanol 97:3). Analysis of the isolated metabolites was done by ^{1}H- and ^{13}C-NMR and mass spectrometry for determination of the incorporation rate.

(*P,R,R*)-9,9'-Bismethoxy vioxanthin (*P,R,R*-10): To a solution of 0.42 mL lithiumdiisopropylamide (2.8 mmol) in anhydrous THF (10 mL) at –70 °C 168 mg (0.40 mmol) dimeric orsellinate (*P*)-**9**, dissolved in 2 mL THF, are added. The dark red solution is stirred for 20 minutes at –70 °C, prior to the addition of 342 mg (2.40 mmol) Michael acceptor (*R*)-**5**, dissolved in 2 mL THF. After stirring for 40 minutes at –70 °C 1.5 mL dry ethanol are added to the orange-brown solution. The reaction mixture is allowed to warm up to room temperature within 20 minutes, then hydrolyzed with 1.5 mL acetic acid, and mixed with saturated ammonium chloride solution. The aqueous phase is extracted with ethyl acetate; the organic phase is dried ($MgSO_4$), and the solvent removed under reduced pressure. The crude product was purified by column chromatography on silica gel (1. ethyl acetate / cyclohexane 1:2; 2. chloroform / acetone 9:1). The obtained product fraction is further purified by preparative thin layer chromatography (silica gel, 1 mm on glass layer, eluent: toluene / ethyl acetate / acetic acid 50:49:1, R_f = 0.62, dark blue fluorescence) to give (*P,R,R*)-**10** (21 mg, 9 %) as a pale yellow solid.
^{1}H-NMR: (400 MHz, $CDCl_3$, 24 °C), δ = 1.54 (d, $^3J_{HH}$ = 6.3 Hz, 6 H, 2 x CH_3), 3.01 (m, 4 H, 2 x CH_2), 3.68 (s, 6 H, 2 x OCH_3), 3.79 (s, 6 H, 2 x OCH_3), 4.73 (m, 2 H, 2 x CH), 6.89 (s, 2 H, 2 x 6-H_{ar}), 6.97 (s, 2 H, 2 x 5-H_{ar}), 13.05 (s, 2 H, 2 x OH). ^{13}C-NMR: (100 MHz, $CDCl_3$, 24 °C), δ = 20.79 (3-CH_3), 35.07 (CH_2), 55.73 (7-OCH_3), 62.08 (OCH_3), 75.83 (CH), 100.85 (C_q), 101.96 (C-6), 113.71 (C_q), 115.38 (C-5), 118.29 (C_q), 133.72 (C_q), 140.76 (C_q), 157.72 (C_q), 160.13 (C_q), 163.21 (C_q), 171.28 (C=O). CD: (acetonitrile, c = $7.379 \cdot 10^{-5}$ mol/l, l = 1 cm): λ [nm] (Mol. CD) = 368 (6.59), 331 (3.18), 276 (54.35), 252 (–81.74), 237 (–31.00), 232 (–32.57), 194 (12.34). $[\alpha]_D^{24}$ = + 157.7 (1.0, $CHCl_3$).

(*M,R,R*)-9,9'-Bismethoxy vioxanthin (*M,R,R*-10): The synthesis of (*M,R,R*)-**10** is done analogously to (*P,R,R*)-**10** starting with 160 mg (0.38 mmol) dimeric orsellinate (*M*)-**9** and 326 mg (2.30 mmol) Michael acceptor (*R*)-**5**. After purification by column chromatography on silica gel (1. ethyl acetate / cyclohexane 1:2; 2. chloroform / acetone 9:1) and preparative thin layer chromatography (silica gel, 1 mm on glass layer, eluent: toluene / ethyl acetate / acetic acid 50:49:1, R_f = 0.62, dark blue fluorescence) (*M,R,R*)-**10** (17 mg, 8 %) was obtained as a pale yellow solid.
^{1}H-NMR: (400 MHz, $CDCl_3$, 25 °C), δ = 1.54 (d, $^3J_{HH}$ = 6.3 Hz, 6 H, 2 x CH_3), 3.01 (m, 4 H, 2 x CH_2), 3.67 (s, 6 H, 2 x OCH_3), 3.80 (s, 6 H, 2 x OCH_3), 4.74 (m, 2 H, 2 x CH), 6.89 (s, 2 H, 2 x 6-H_{ar}), 6.97 (s, 2 H, 2 x 5-H_{ar}), 13.04 (s, 2 H, 2 x OH). ^{13}C-NMR: (100 MHz, $CDCl_3$, 25 °C), δ = 20.80 (3-CH_3), 35.11 (CH_2), 55.74 (7-OCH_3), 62.06 (OCH_3), 75.80 (CH), 100.88 (C_q), 101.96 (C-6), 113.73 (C_q), 115.38 (C-5), 118.31 (C_q), 133.75 (C_q), 140.76 (C_q), 157.69 (C_q), 160.19 (C_q), 163.21 (C_q), 171.30 (C=O). CD: (acetonitrile, c = $1.184 \cdot 10^{-5}$ mol/l, l = 1 cm): λ [nm] (Mol. CD) = 370 (–2.81), 320 (0.19), 270 (–159.41), 254 (110.58). $[\alpha]_D^{21}$ = – 155.7 (0.8, $CHCl_3$).

(*P,R,R*)-Vioxanthin (*P,R,R*-1): To a solution of 21 mg (36.5 µmol) (*P,R,R*)-**10** in 2.2 mL dry dichloromethane at 0 °C 73 µmol BBr_3 (73 µL of a 1 M dichloromethane solution) are added. The mixture is stirred for one hour at room temperature, then 5 mL half saturated $NaHCO_3$ solution is added and the mixture is acidified to pH 2 with 2 M HCl. The aqueous phase is extracted with ethyl acetate; the organic phase is dried ($MgSO_4$), and the solvent removed under reduced pressure. The crude product was purified by column chromatography on silica gel (silica gel conditioned with 2 M HCl, eluent: dichloromethane / methanol 97:3, R_f = 0.20, green fluorescence) to give (*P,R,R*)-**1** (5 mg, 25 %) as a yellow solid.
^{1}H-NMR: (400 MHz, $CDCl_3$, 24 °C), δ = 1.55 (d, $^3J_{HH}$ = 6.2 Hz, 6 H, 2 x CH_3), 3.01 (m, 4 H, 2 x CH_2), 3.84 (s, 6 H, 2 x OCH_3), 4.76 (m, 2 H, 2 x CH), 6.70 (s, 2 H, 2 x 6-H_{ar}), 6.95 (s, 2 H, 2 x 5-H_{ar}), 9.69 (d, J_{HH} = 1.0 Hz, 2 H, 2 x 9-OH), 13.79 (d, J_{HH} = 1.0 Hz, 2 H, 2 x 10-OH). ^{13}C-NMR: (100 MHz, $CDCl_3$, 25 °C), δ = 20.7 (CH_3), 34.7 (CH_2), 56.0 (OCH_3), 76.5 (CH), 98.1 (C-6), 99.3 (C-10a), 108.1 (C-8), 108.5 (C-9a), 116.1 (C-5), 132.8 (C-4a), 140.0 (C-5a), 155.4 (C-9), 161.4 (C-7), 162.8 (C-10), 171.57 (C=O, C-1). CD: (acetonitrile, c = $3.660 \cdot 10^{-5}$ mol/l, l = 1 cm): λ [nm] (Mol. CD) = 402 (–2.16), 374 (10.94), 322 (2.26), 272 (65.74), 255 (–78.20), 228 (–11.39), 221 (–13.12), 192 (15.55). $[\alpha]_D^{25}$ = + 275.9° (0.3, $CHCl_3$).

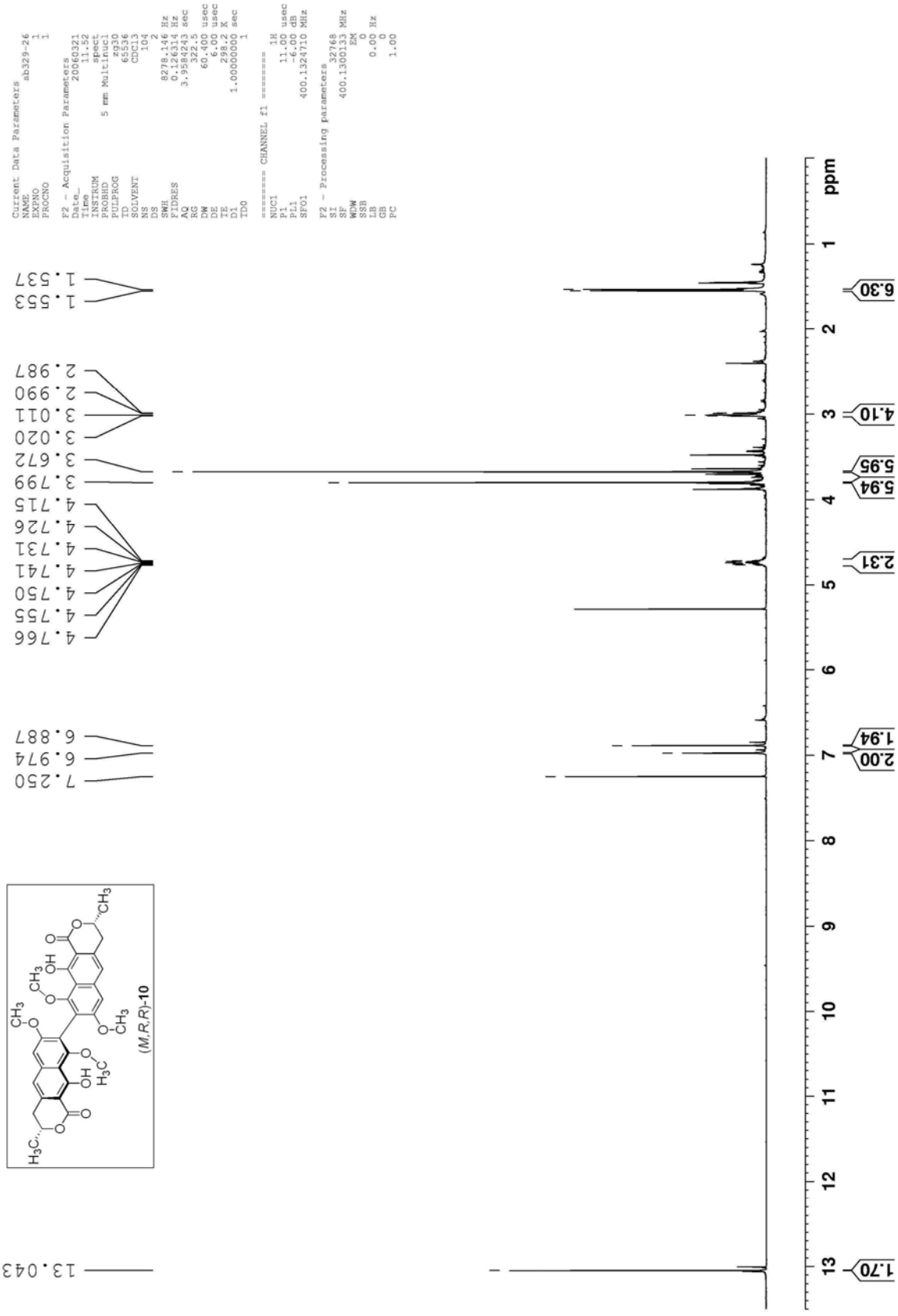
(*M,R,R*)-Vioxanthin (*M,R,R*-1): The synthesis of (*M,R,R*)-**1** is done analogously to (*P,R,R*)-**1** starting with 17 mg (29.6 µmol) (*M,R,R*)-**10** and 60 µmol BBr_3 (60 µL of a 1 M dichloromethane solution). After

[1] Extraction of the filtrate yields *semi*-vioxanthin (**2**): after acidification with 2 M hydrochloric acid the filtrate is extracted with ethyl acetate; the organic phase is dried ($MgSO_4$), and the solvent removed under reduced pressure. The crude extract is purified by column chromatography (silica gel conditioned with 2 M HCl, eluent: dichloromethane / methanol 97:3).

purification by column chromatography on silica gel (silica gel conditioned with 2 M HCl, eluent: dichloromethane / methanol 97:3, R_f = 0.20, green fluorescence) (*M*)-**1** (2 mg, 12 %) was obtained as yellow solid.

^{1}H-NMR: (400 MHz, $CDCl_3$, 25 °C), δ = 1.55 (d, $^3J_{HH}$ = 6.2 Hz, 6 H, 2 x CH_3), 3.01 (m, 4 H, 2 x CH_2), 3.84 (s, 6 H, 2 x OCH_3), 4.76 (m, 2 H, 2 x CH), 6.70 (s, 2 H, 2 x 6-H_{ar}), 6.95 (s, 2 H, 2 x 5-H_{ar}), 9.69 (s, 2 H, 2 x 9-OH), 13.79 (s, 2 H, 2 x 10-OH). ^{13}C-NMR: (100 MHz, $CDCl_3$, 26 °C), δ = 20.7 (CH_3), 34.7 (CH_2), 56.0 (OCH_3), 76.5 (CH), 98.1 (C-6), 99.3 (C-10a), 108.1 (C-8), 108.5 (C-9a), 116.1 (C-5), 132.8 (C-4a), 140.0 (C-5a), 155.4 (C-9), 161.4 (C-7), 162.8 (C-10), 171.61 (C=O, C-1). CD: (acetonitrile, c = $2.928 \cdot 10^{-5}$ mol/l, l = 1 cm): λ [nm] (Mol. CD) = 400 (3.48), 363 (–12.09), 320 (0.85), 270 (–61.01), 253 (53.30).

^{1}H-NMR of (*M*,*R*,*R*)-**10** (400 MHz, $CDCl_3$, 25 °C)



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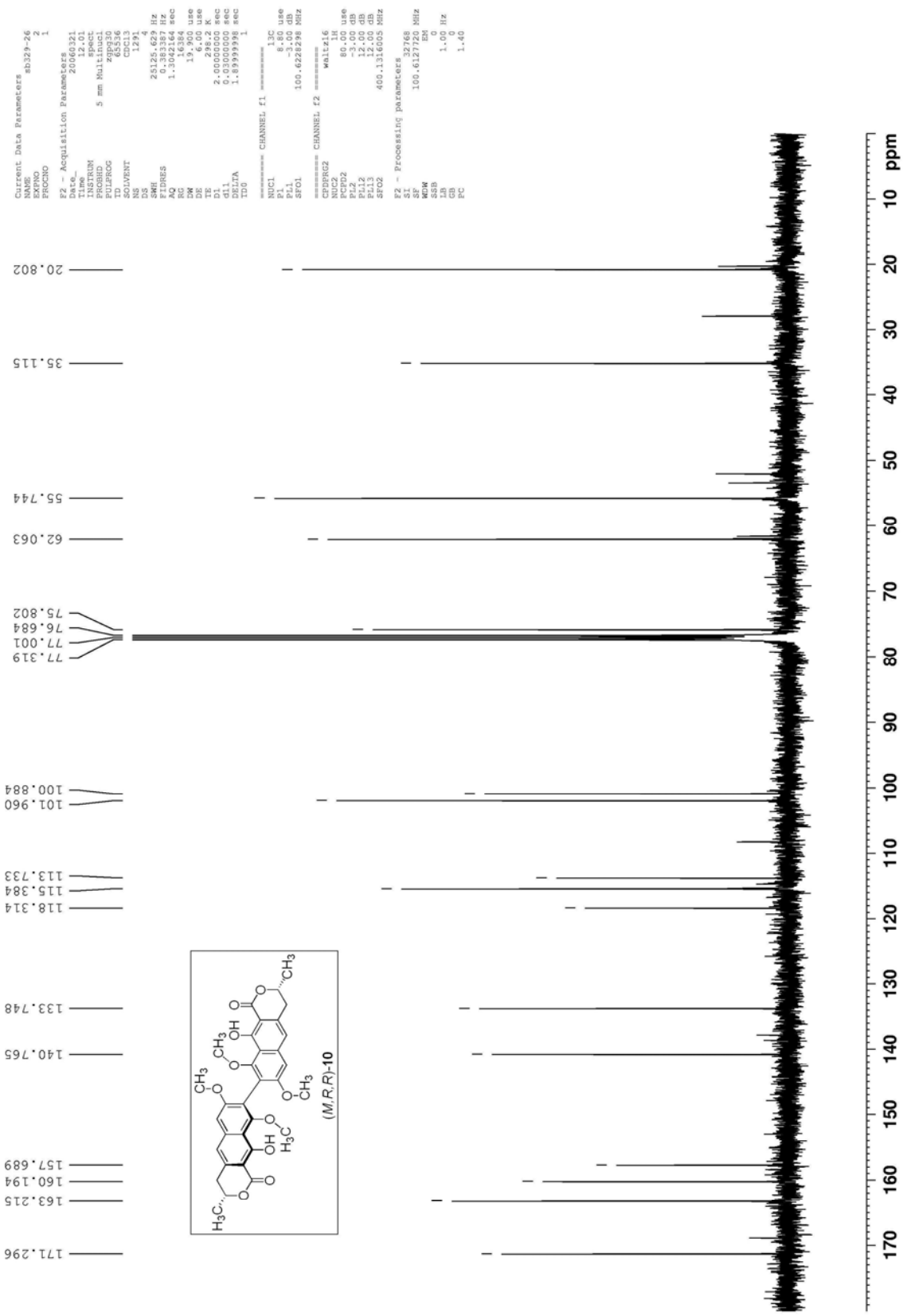
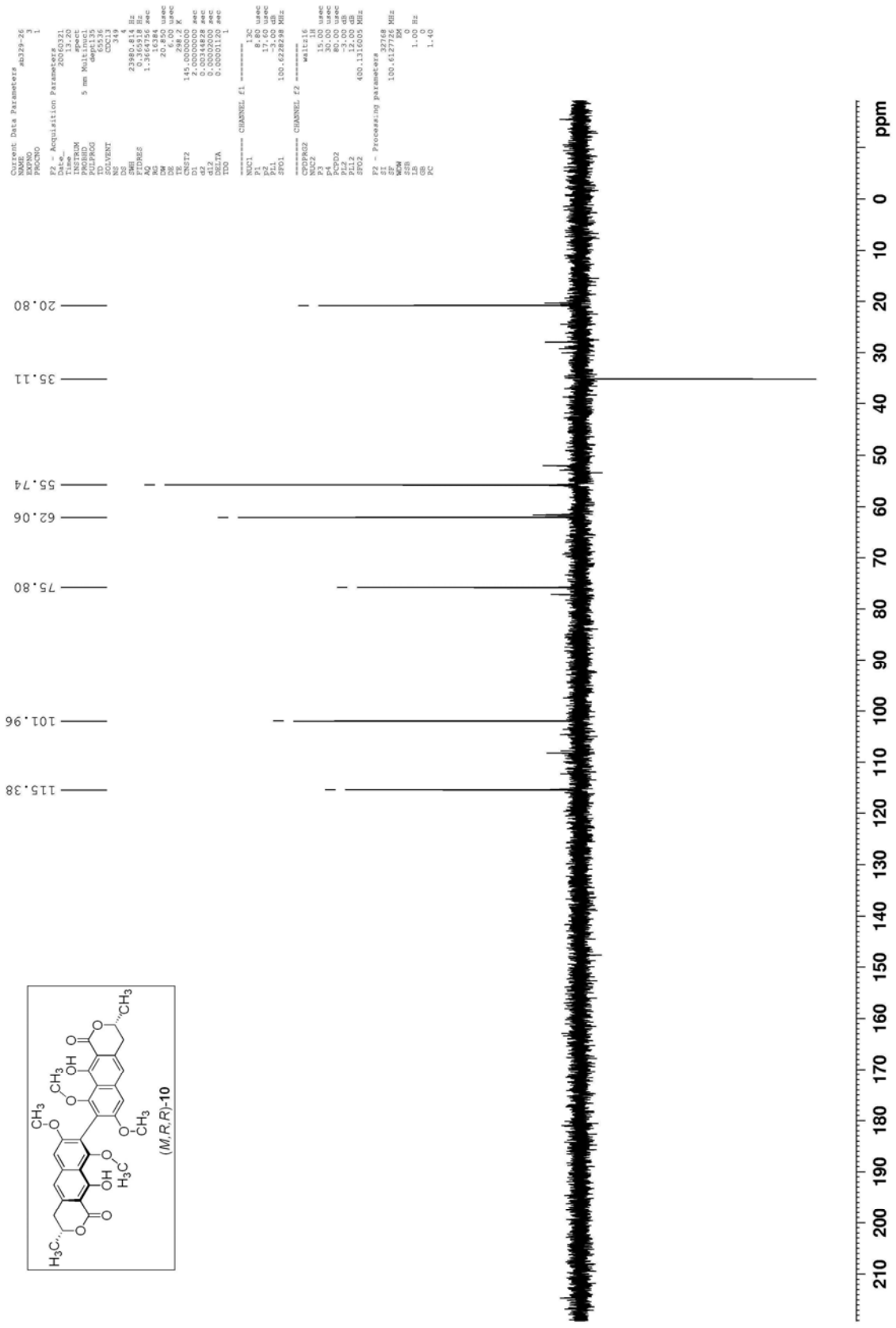


^{13}C-NMR dept-135 of (*M*,*R*,*R*)-**10** (100 MHz, $CDCl_3$, 25 °C)



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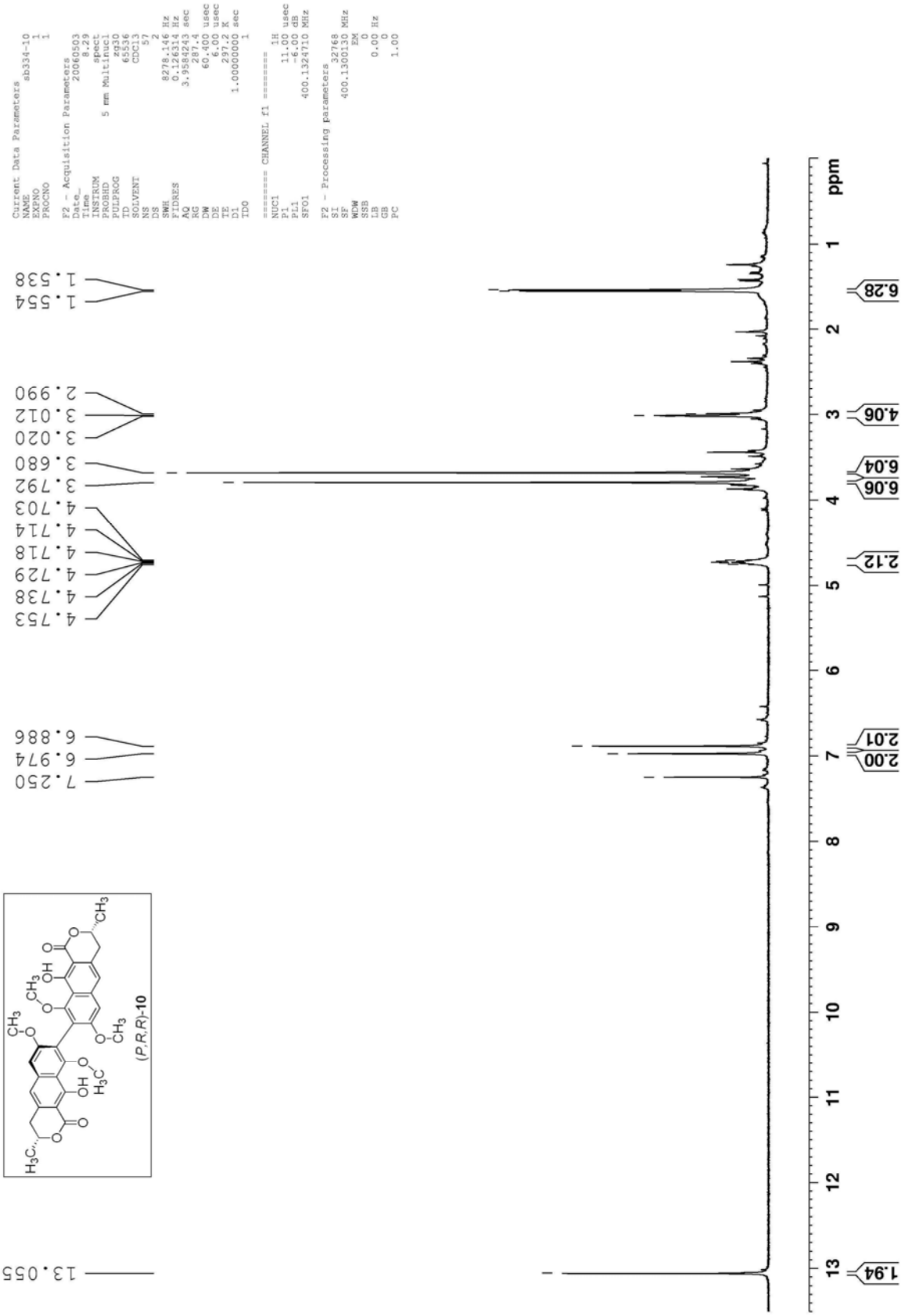

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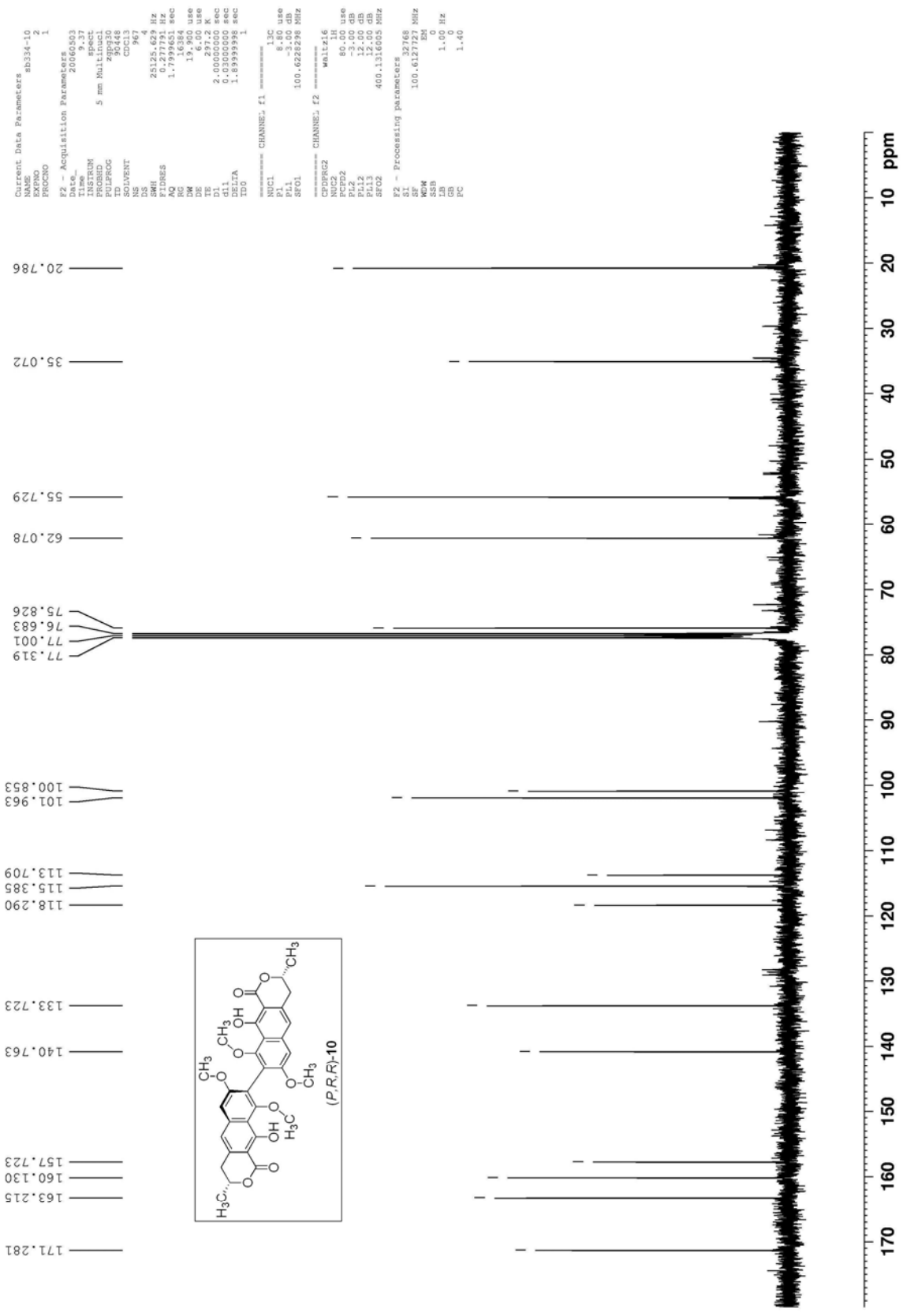
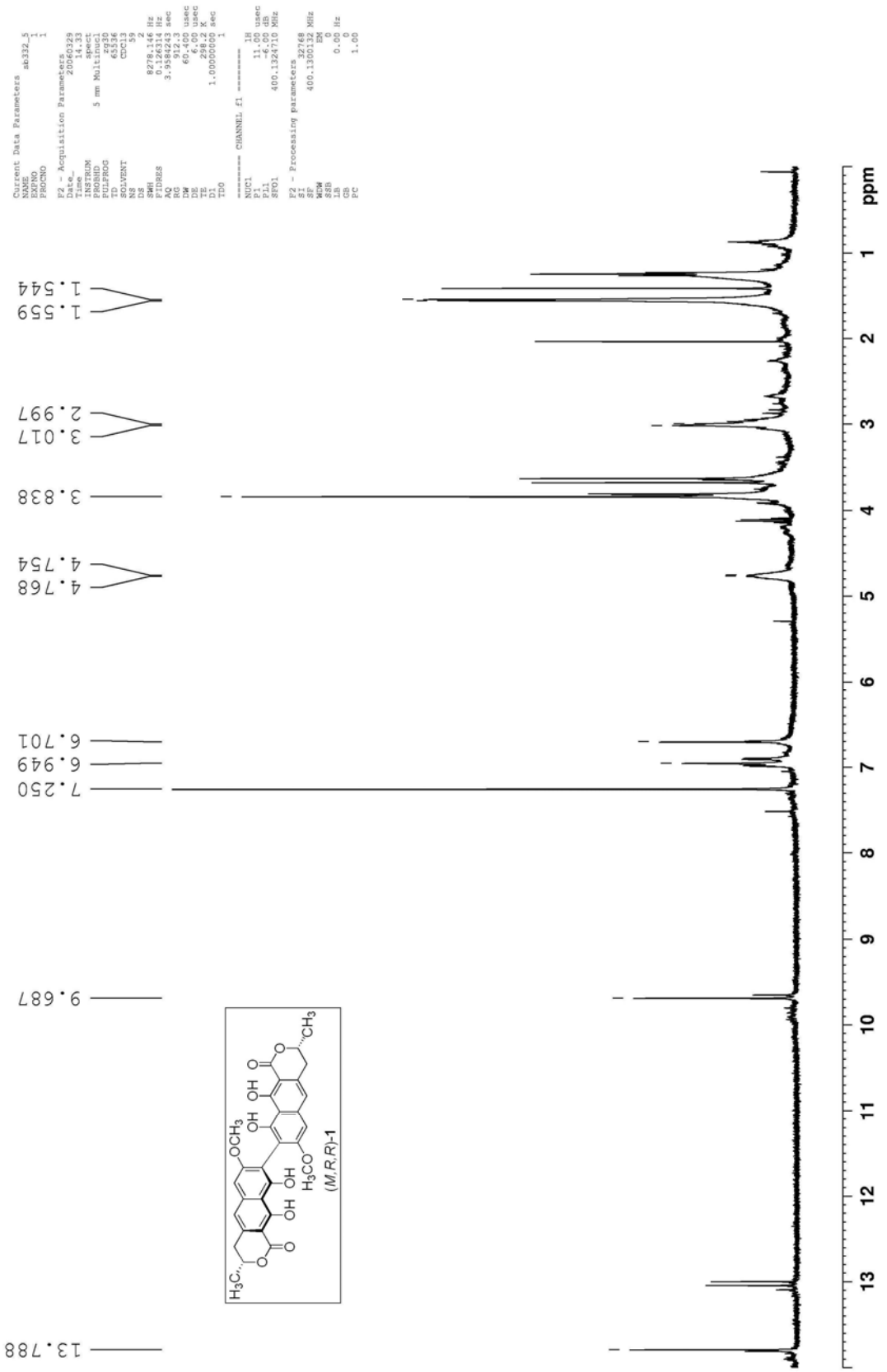
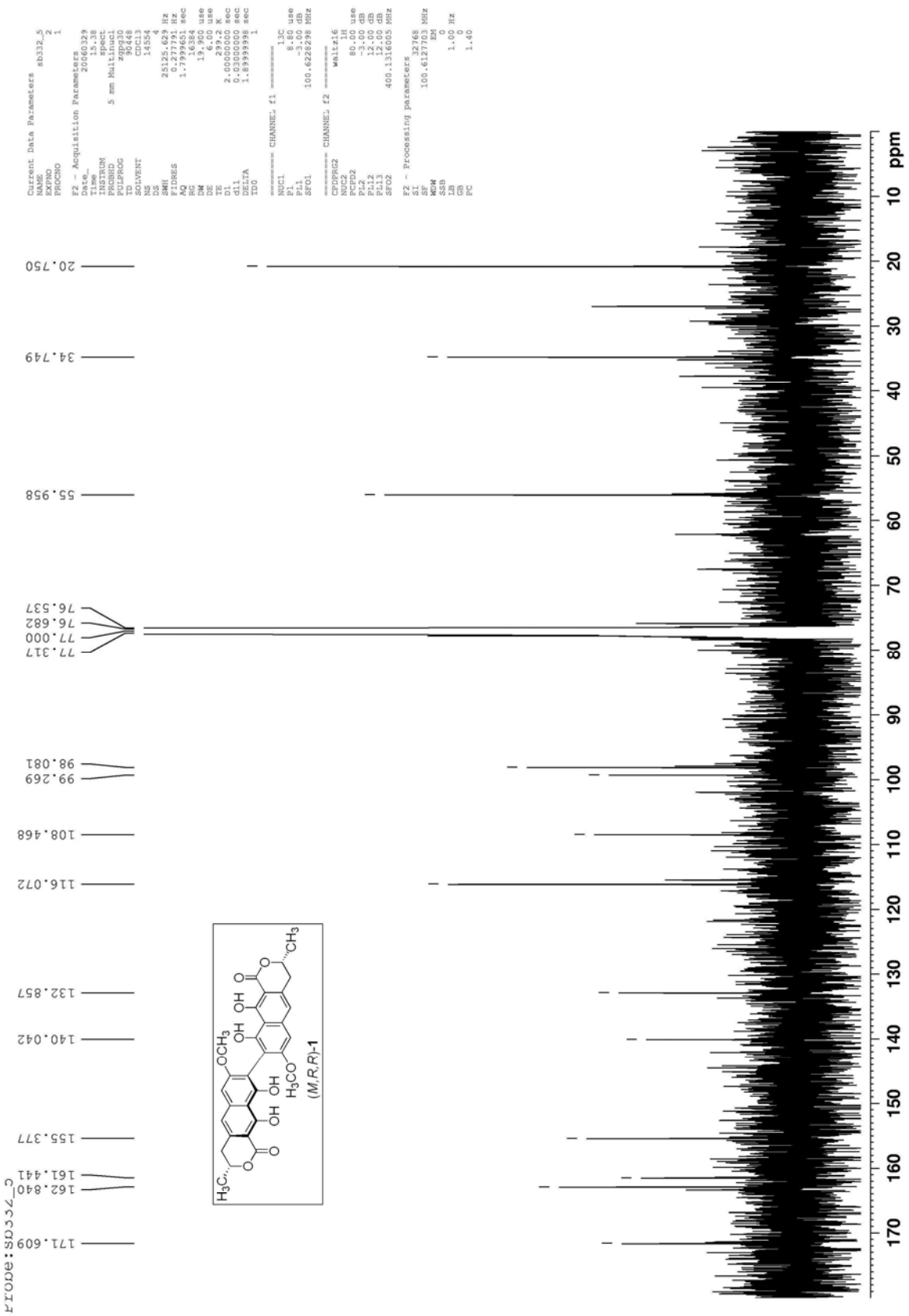


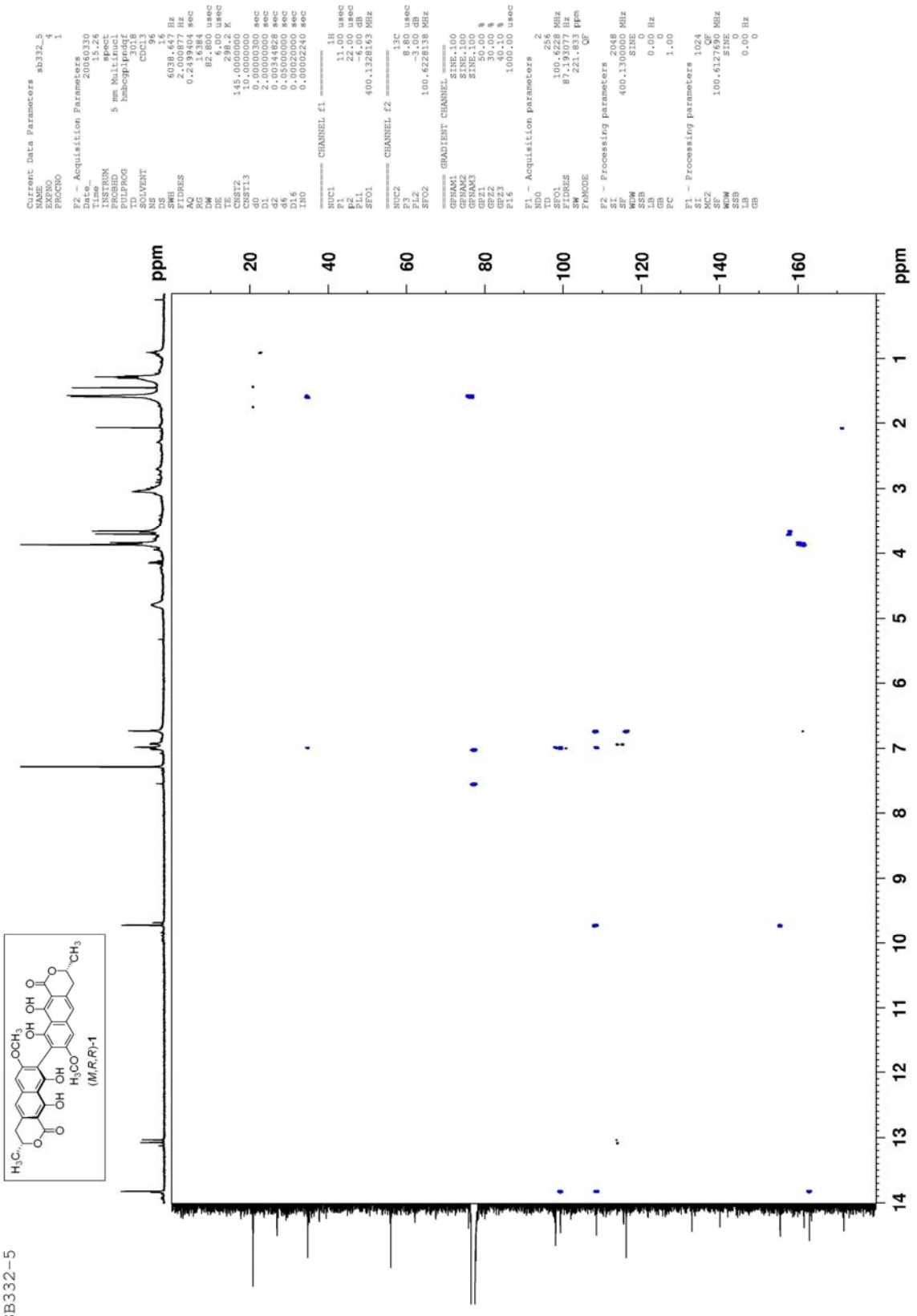
^{1}H-NMR of (*M*,*R*,*R*)-vioxanthin (*M*,*R*,*R*-**1**) (400 MHz, $CDCl_3$, 25 °C)



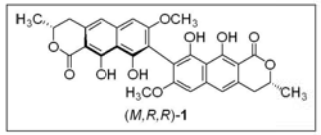
^{13}C-NMR of (*M*,*R*,*R*)-vioxanthin (*M*,*R*,*R*-**1**) (100 MHz, $CDCl_3$, 26 °C)



HMBC of (*M*,*R*,*R*)-vioxanthin (*M*,*R*,*R*-**1**) ($CDCl_3$, 25 °C, ^{1}H: 400 MHz, ^{13}C: 100 MHz)



SB332-5



(*M*,*R*,*R*)-**1**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CNST13                  10.0000000
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D1                      2.00000000 sec
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PL1                          -6.00 dB
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========== CHANNEL f2 ==========
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SFO2                  100.6228138 MHz

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GPNAM2                    SINE.100
GPNAM3                    SINE.100
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GPZ2                         30.00 %
GPZ3                         40.10 %
P16                        1000.00 usec

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LB                            0.00 Hz
GB                               0
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F1 - Processing parameters
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WDW                           SINE
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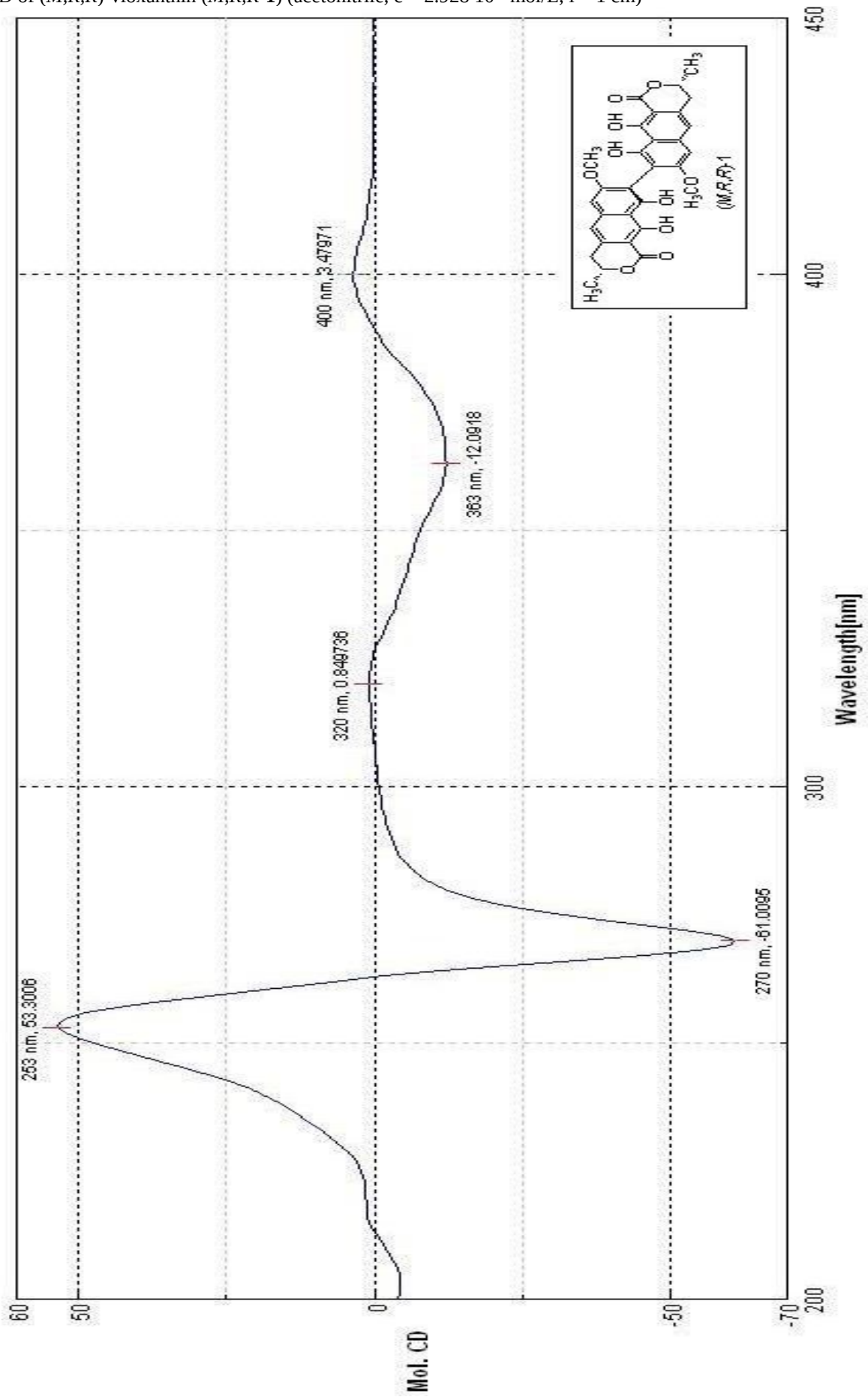
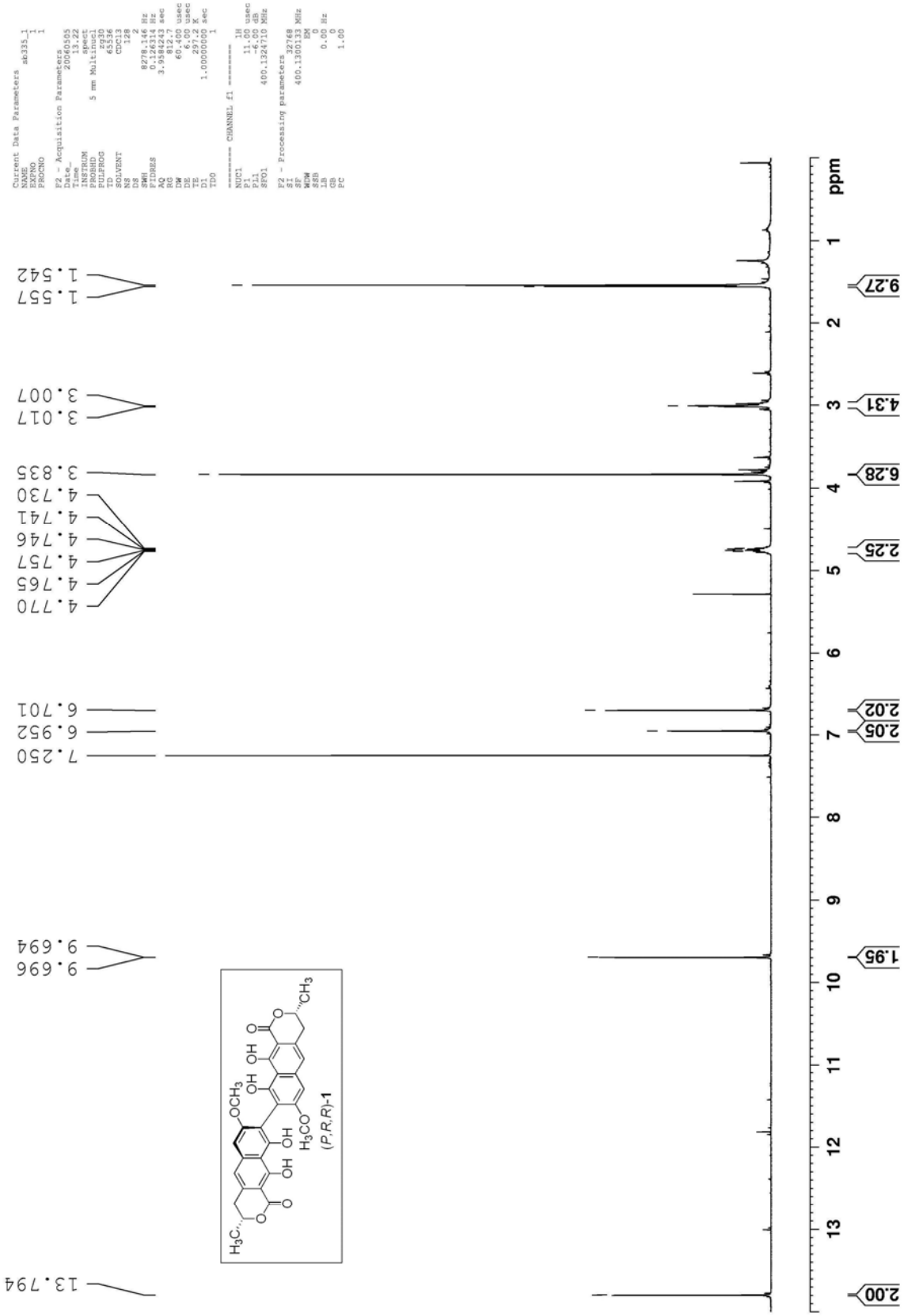
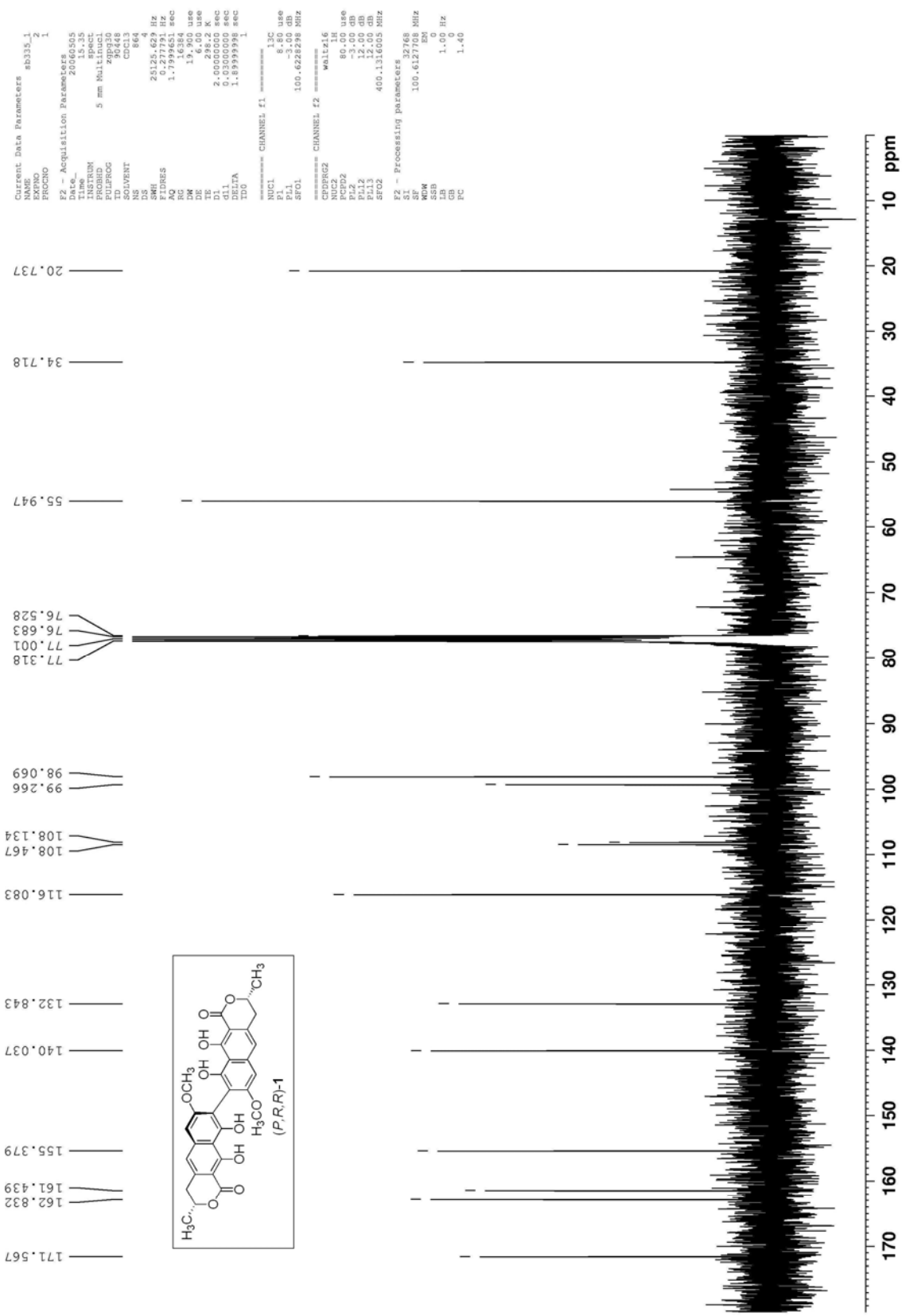
CD of (*M*,*R*,*R*)-vioxanthin (*M*,*R*,*R*-**1**) (acetonitrile, c = $2.928 \cdot 10^{-5}$ mol/L, l = 1 cm)



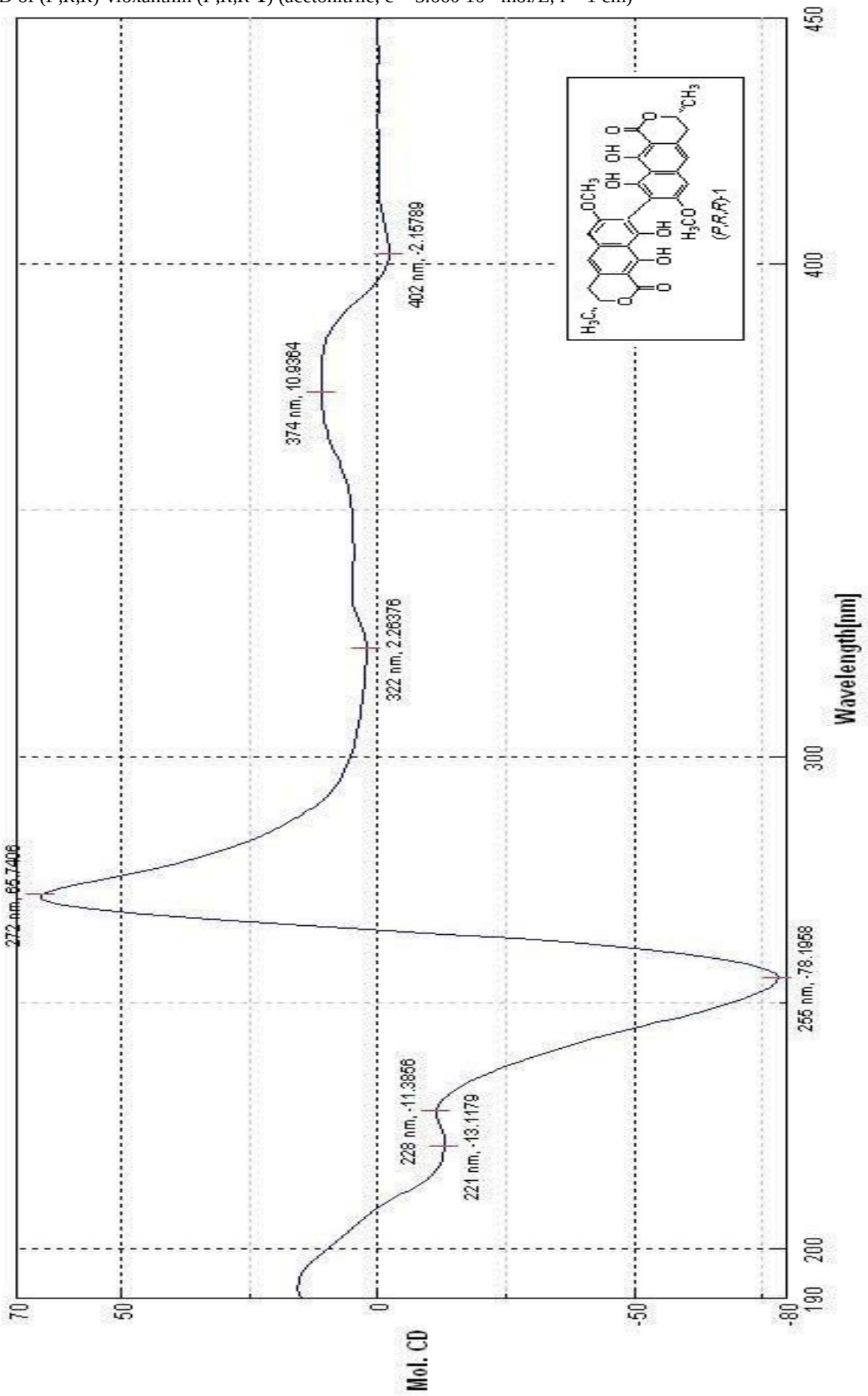
^{1}H-NMR of (*P*,*R*,*R*)-vioxanthin (*P*,*R*,*R*-**1**) (400 MHz, $CDCl_3$, 25 °C)



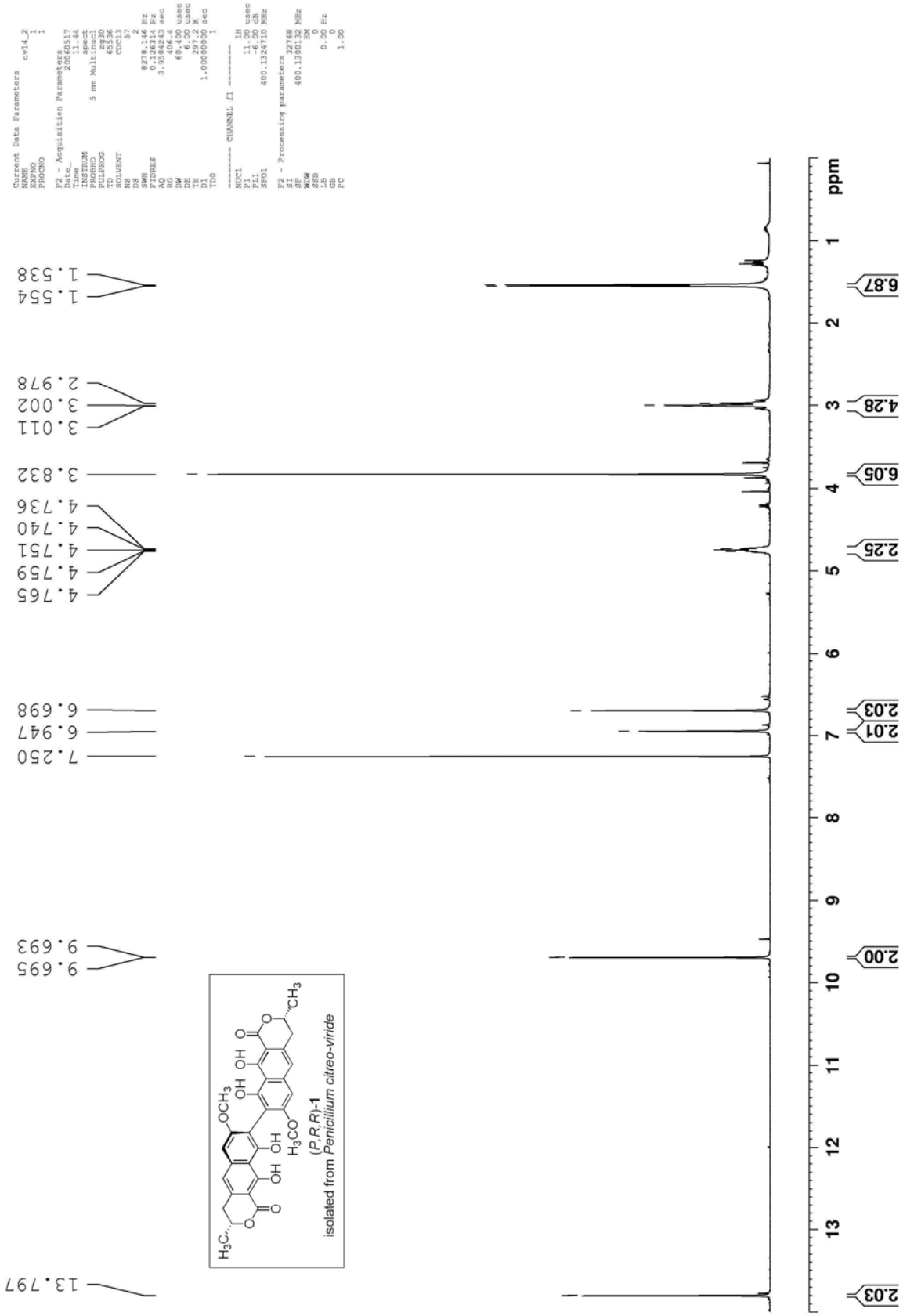
^{13}C-NMR of (*P*,*R*,*R*)-vioxanthin (*P*,*R*,*R*-**1**) (100 MHz, $CDCl_3$, 26 °C)



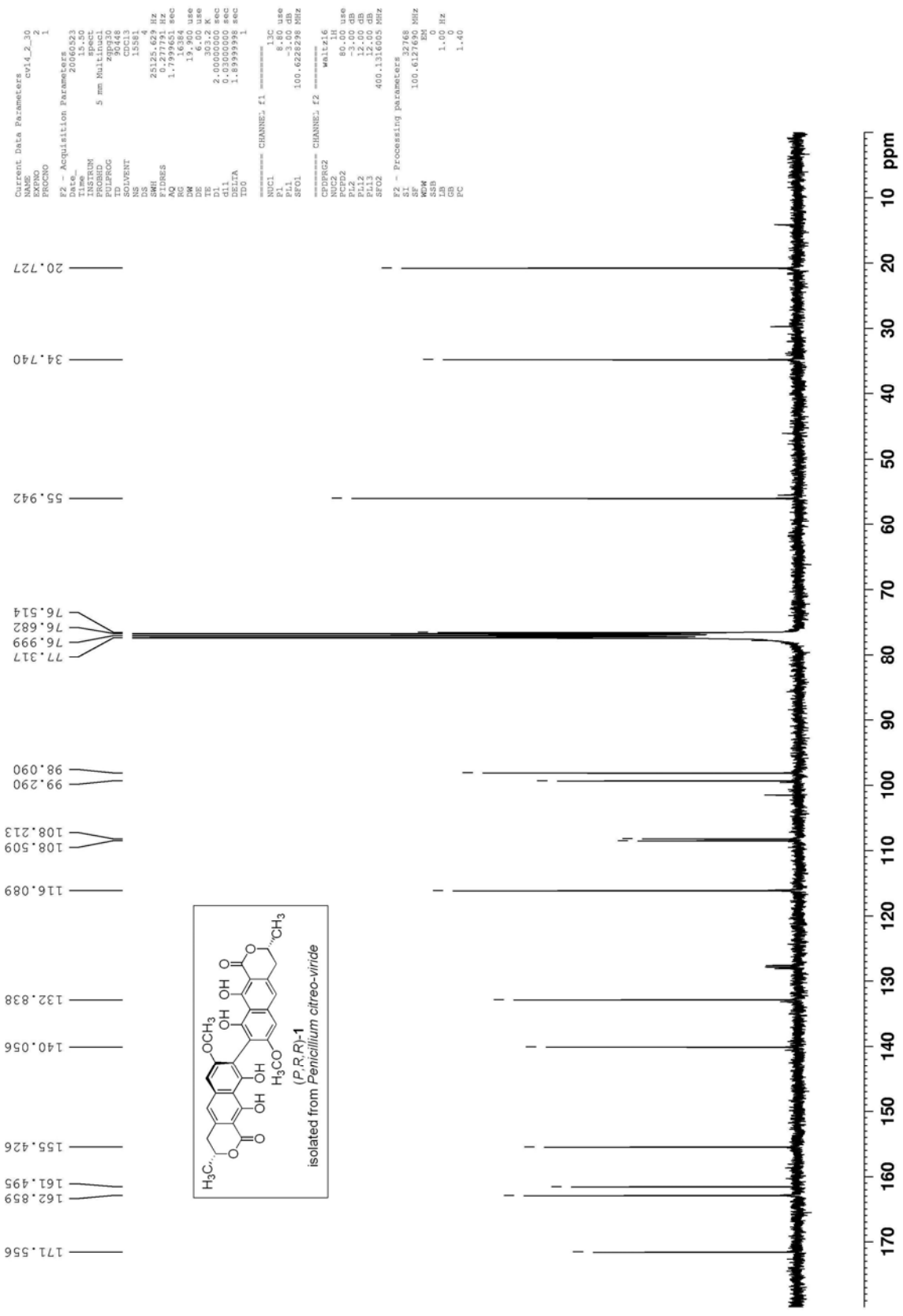
CD of (*P*,*R*,*R*)-vioxanthin (*P*,*R*,*R*-**1**) (acetonitrile, c = $3.660 \cdot 10^{-5}$ mol/L, l = 1 cm)



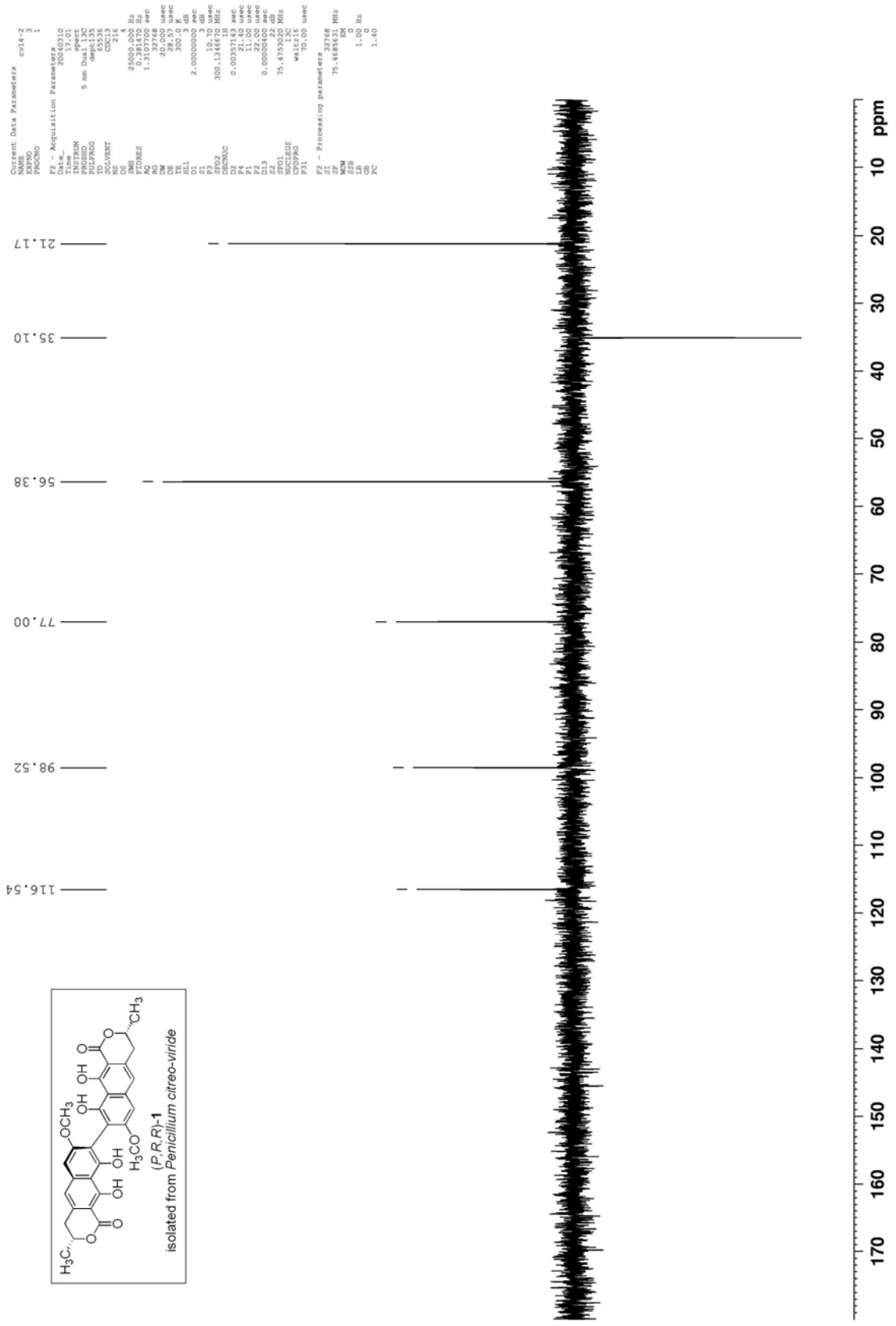
[1]H-NMR of (*P*,*R*,*R*)-vioxanthin (*P*,*R*,*R*-**1**) isolated from *Penicillium citreo-viride* (400 MHz, $CDCl_3$, 24 °C)



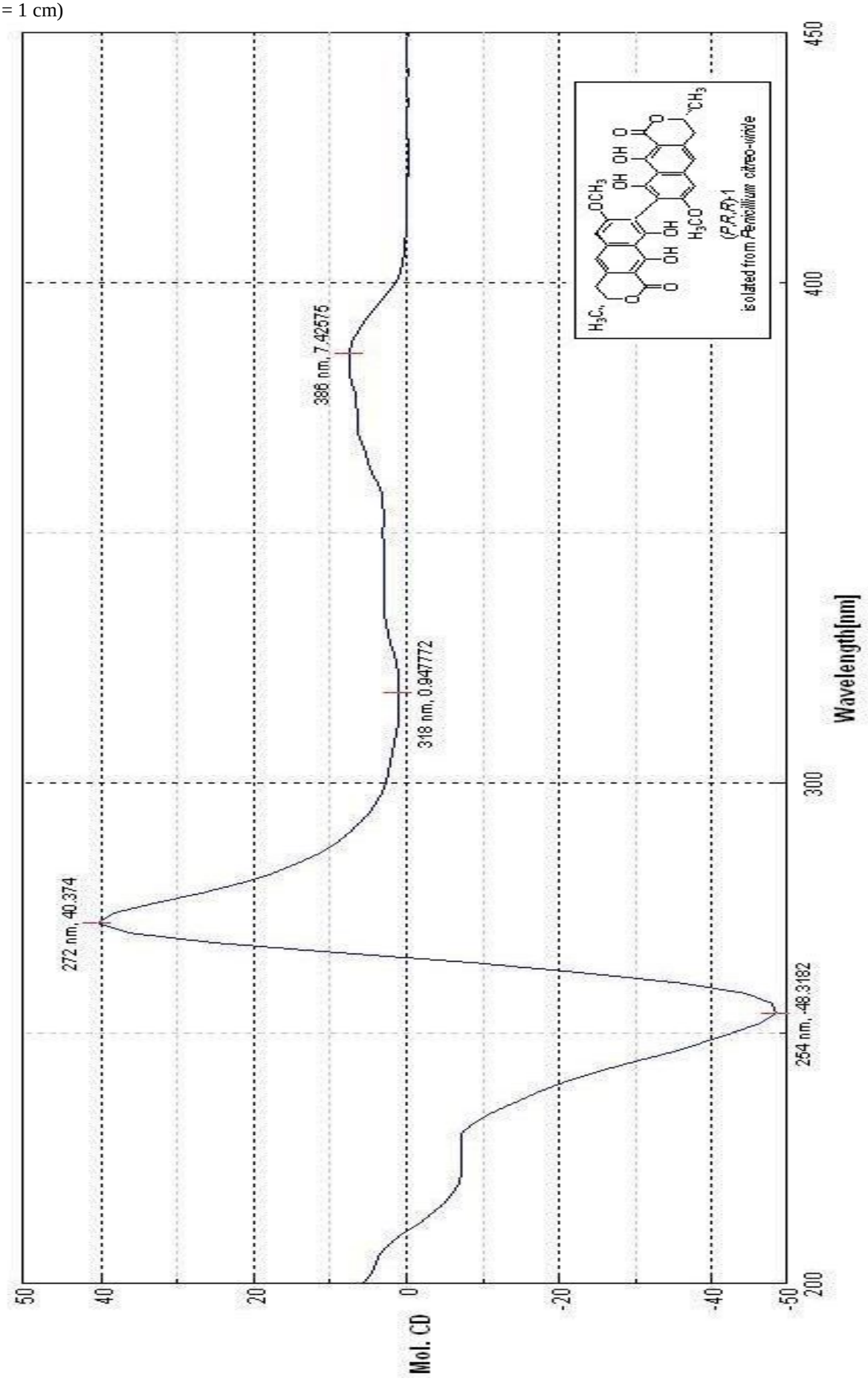
^{13}C-NMR of (*P*,*R*,*R*)-vioxanthin (*P*,*R*,*R*-**1**) isolated from *Penicillium citreo-viride* (100 MHz, $CDCl_3$, 30 °C)



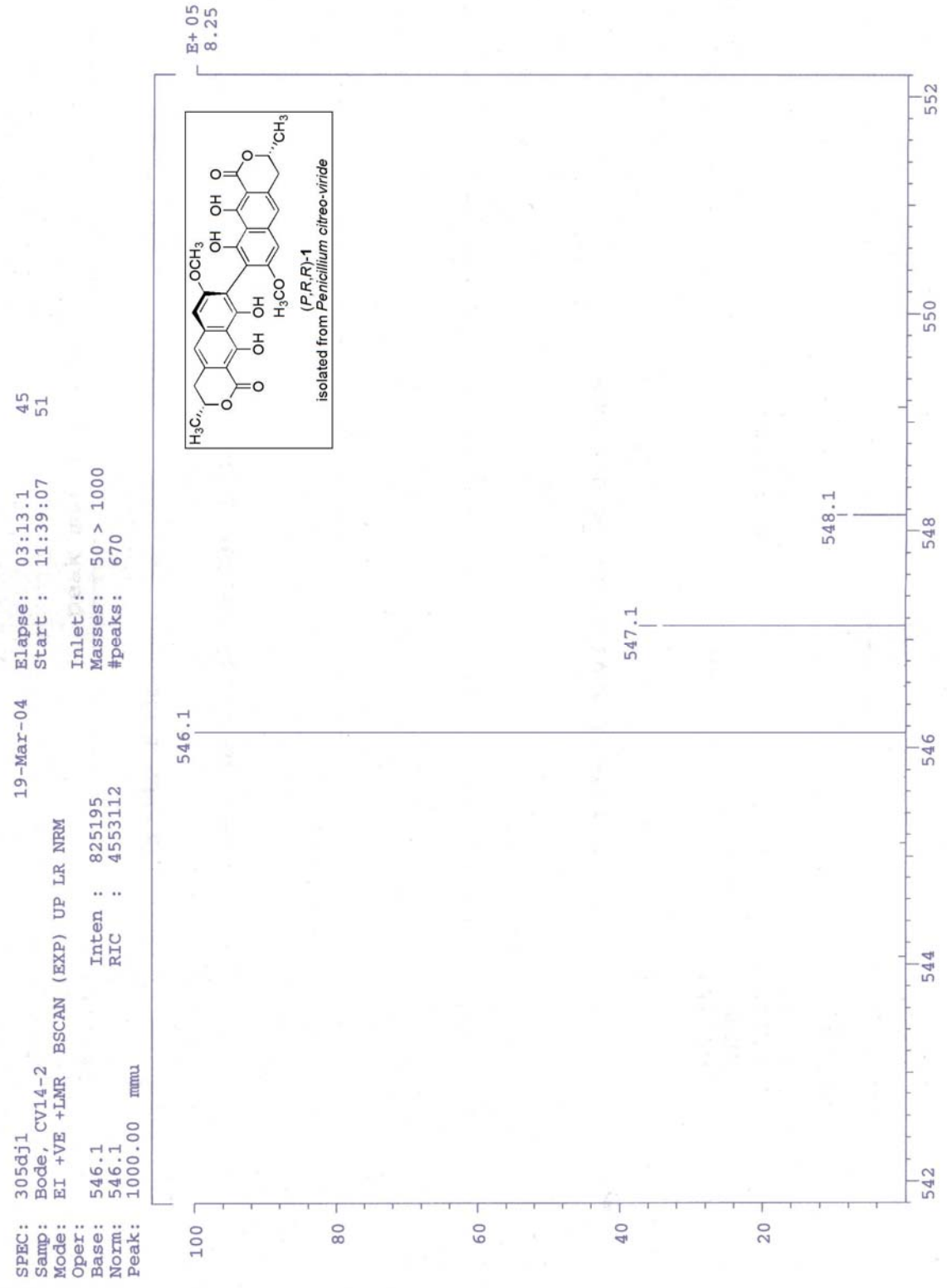
^{13}C-NMR dept-135 of (*P*,*R*,*R*)-vioxanthin (*P*,*R*,*R*-**1**) isolated from *Penicillium citreo-viride* (100 MHz, $CDCl_3$, 30 °C)



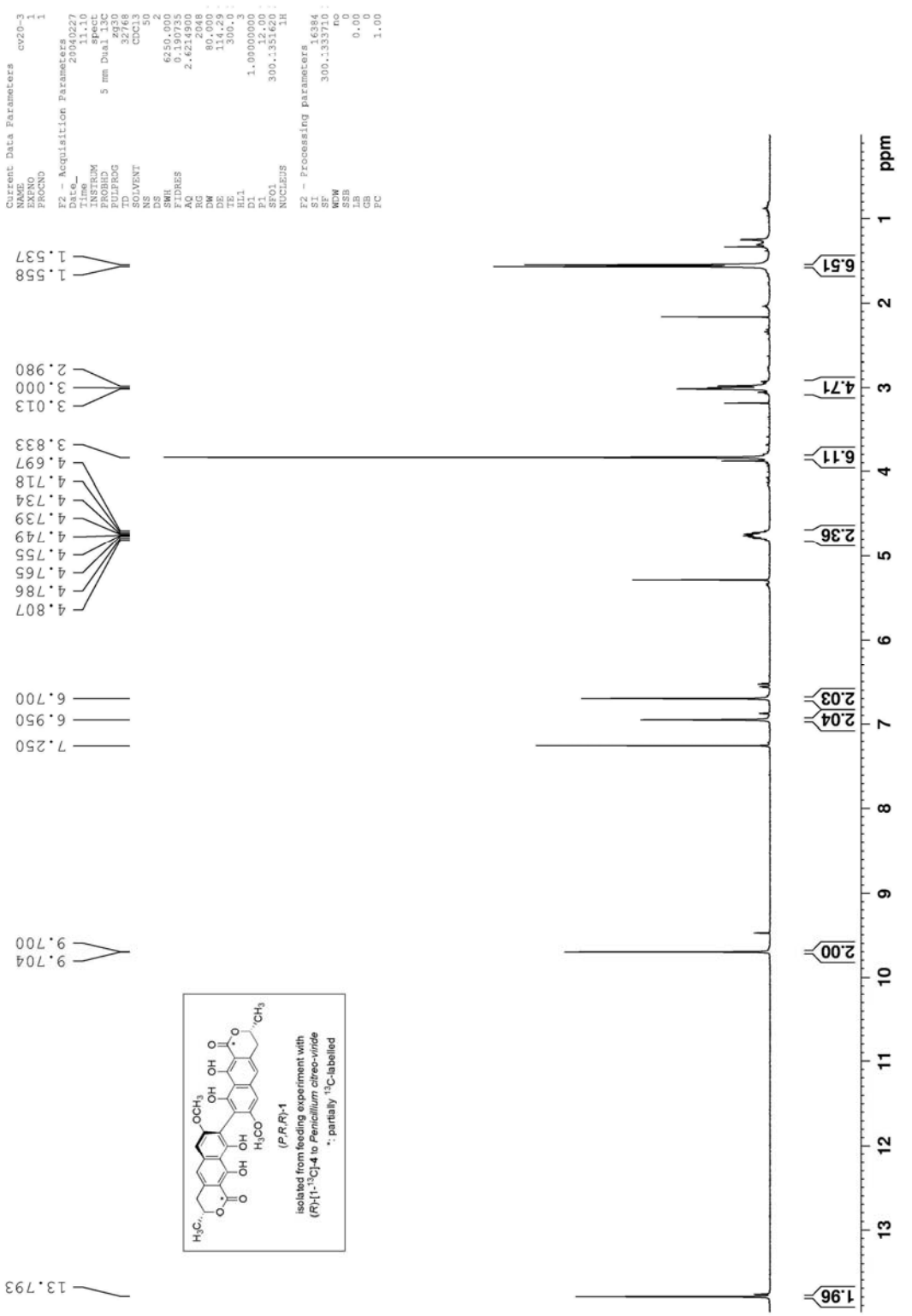
CD of (*P*,*R*,*R*)-vioxanthin (*P*,*R*,*R*-**1**) isolated from *Penicillium citreo-viride* (acetonitrile, c = $1.866 \cdot 10^{-5}$ mol/L, l = 1 cm)



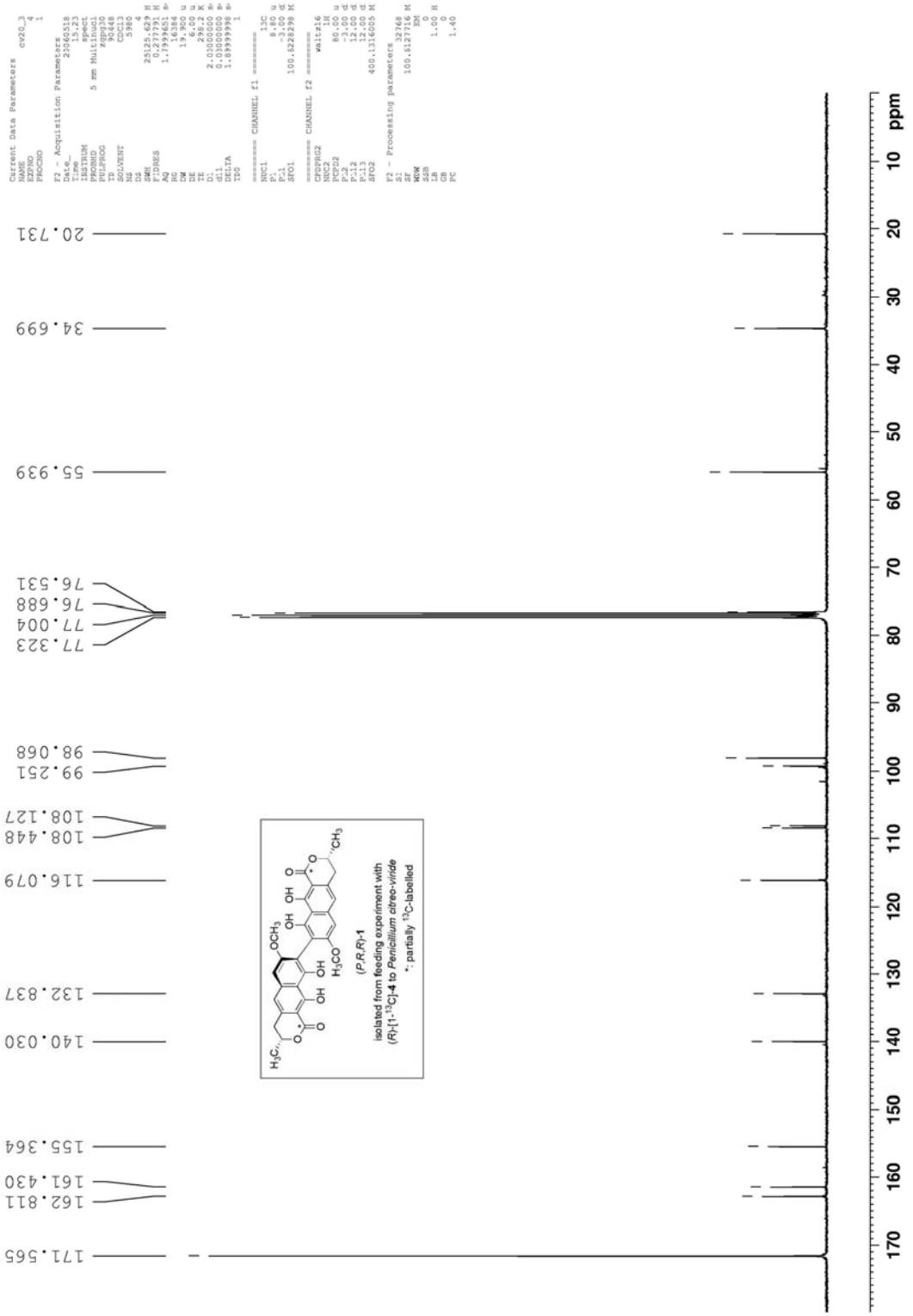
MS of (*P*,*R*,*R*)-vioxanthin (*P*,*R*,*R*-**1**) isolated from *Penicillium citreo-viride* (EI, 240 °C)



^{1}H-NMR of (*P*,*R*,*R*)-vioxanthin (*P*,*R*,*R*-**1**) isolated from feeding experiment with (*R*)-[1-^{13}C]-**4** to *Penicillium citreo-viride* (400 MHz, $CDCl_3$, 27 °C)



^{13}C-NMR of (*P*,*R*,*R*)-vioxanthin (*P*,*R*,*R*-**1**) isolated from feeding experiment with (*R*)-[1-^{13}C]-**4** to *Penicillium citreo-viride* (100 MHz, $CDCl_3$, 25 °C)



MS of (*P*,*R*,*R*)-vioxanthin (*P*,*R*,*R*-**1**) isolated from feeding experiment with (*R*)-[1-^{13}C]-**4** to *Penicillium citreo-viride* (EI, 240 °C)