



Supporting Information

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Syntheses with a chiral building block from the citric acid cycle:  
(2*R*,3*S*)-Isocitric acid by fermentation of sunflower oil

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## 1. Cultivation

### 1.1 Strains, media and culture conditions

The wild-type strain *Yarrowia lipolytica* EH59 obtained from the strain collection of the UFZ was used for the production of (2*R*,3*S*)-isocitric acid.

For the preculture 500 mL shaking flasks were used. The preculture medium had the following composition: 2.5 g L<sup>-1</sup> yeast extract, 3.0 g L<sup>-1</sup> NH<sub>4</sub>Cl, 0.7 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 0.35 g L<sup>-1</sup> MgSO<sub>4</sub> × 7 H<sub>2</sub>O, 3.5 mg L<sup>-1</sup> FeSO<sub>4</sub> × 7 H<sub>2</sub>O, 5 g L<sup>-1</sup> CaCO<sub>3</sub> and 5 mL L<sup>-1</sup> trace elements with the following composition: 4.0 g L<sup>-1</sup> CuSO<sub>4</sub> × 5 H<sub>2</sub>O, 4.0 g L<sup>-1</sup> MnSO<sub>4</sub> × 5 H<sub>2</sub>O, 2.1 g L<sup>-1</sup> ZnCl<sub>2</sub>, 0.5 g L<sup>-1</sup> CoSO<sub>4</sub> × 7 H<sub>2</sub>O and 5.7 g L<sup>-1</sup> H<sub>3</sub>BO<sub>3</sub>.<sup>[1]</sup> The concentration of refined sunflower oil was 15 g·L<sup>-1</sup>. The yeast *Y. lipolytica* EH59 was cultivated on a rotary shaker (Bühler, Göttingen, Germany) at 30 °C and 130-150 rpm for 24 h.

The main cultivation was carried out in a stirred tank bioreactor (ISF215, Infors, Botmingen, Switzerland) with a working volume of 15 L. The production medium had the following composition a) mineral salts: 3.0 g L<sup>-1</sup> (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.7 g L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 0.35 g L<sup>-1</sup> MgSO<sub>4</sub> × 7 H<sub>2</sub>O, 3.5 mg L<sup>-1</sup> FeSO<sub>4</sub> × 7 H<sub>2</sub>O, 30 mg L<sup>-1</sup> CaCl<sub>2</sub>; b) trace elements: 20 mg L<sup>-1</sup> CuSO<sub>4</sub> × 5 H<sub>2</sub>O, 20 mg L<sup>-1</sup> MnSO<sub>4</sub> × 5 H<sub>2</sub>O, 10 mg L<sup>-1</sup> ZnCl<sub>2</sub>, 2.5 mg L<sup>-1</sup> CoSO<sub>4</sub> × 7 H<sub>2</sub>O and 28.5 mg L<sup>-1</sup> H<sub>3</sub>BO<sub>3</sub>; c) vitamin: 1 mg L<sup>-1</sup> thiamine hydrochloride. All media and instruments were sterilized at 121 °C for 20 min. The production media was inoculated with 10% (v/v) preculture broth. The total concentration of sunflower oil was 145 g L<sup>-1</sup>. To obtain a high concentration of citrates the cultivation was realized in a fedbatch-mode. During cultivation, temperature was maintained at 30 °C, the pH was adjusted to 6.0 with 10 N NaOH and the value of dissolved oxygen pO<sub>2</sub> was maintained at 60% saturation.

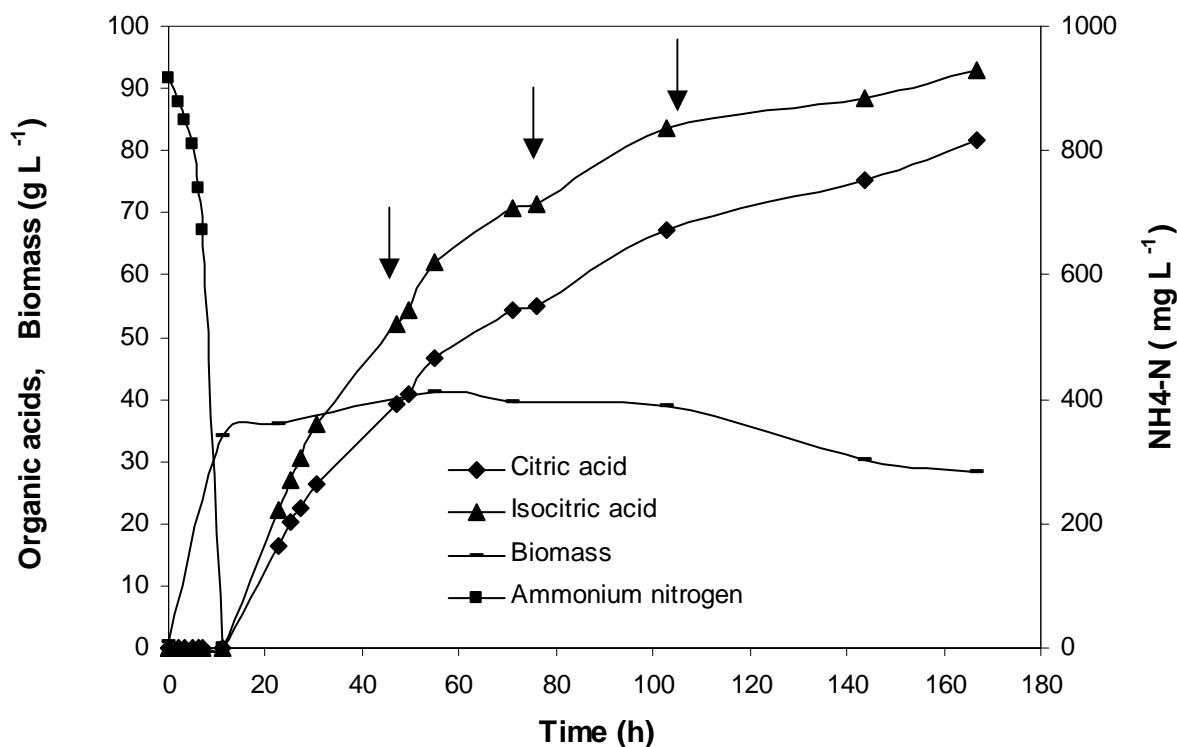
### 1.2 Analytical methods

The determination of organic acids (citric and (2*R*,3*S*)-isocitric acid) was carried out by using an ion chromatography (IC) system DX 600, equipped with an EG 40 KOH eluent generator, quaternary gradient pump GP 50-2 and conductivity detector CD25a (Dionex, Sunnyvale, CA, USA). For anion separation an IonPac AS 11 (4 mm) analytical column with an IonPac AG 11 (4 mm) guard column (both Dionex) was used. The ion separation was carried out under the following conditions: sample injection volume: 10 µL; eluent flow rate: 1.5 mL min<sup>-1</sup>; KOH eluent gradient: isocratic at 0.5 mM hold for 2 min, linear to 5 mM from 2-6 min, linear to 40 mM from 6-22 min, isocratic to 0.5 mM hold for 22-25 min. The organic acids were quantified with the software Chromeleon 6.5 (Dionex) using calibration curves.

The ammonium nitrogen concentration was determined by using test kits LCK 302 and 303 (Dr. Lange GmbH, Düsseldorf, Germany).

For determination of biomass 20 mL samples were filtrated via a vacuum filtration apparatus (Sartorius AG, Göttingen, Germany) using a membrane filter with 0.45  $\mu\text{m}$  pore size. The filter cake was dried at 105  $^{\circ}\text{C}$  until stability of weight (MA 40, Sartorius AG, Göttingen, Germany).

### 1.3 Results of cultivation and further downstream processing



**Figure 1:** Fed-batch cultivation of the *Yarrowia lipolytica* EH59 on sunflower oil. Concentration of sunflower oil at cultivation start was 85 g L<sup>-1</sup>; first (25 g L<sup>-1</sup>), second (20 g L<sup>-1</sup>) and third (15 g L<sup>-1</sup>) oil dosage. Arrows indicate additional sunflower oil feedings.

In *figure 1* the time course of (2*R*,3*S*)-isocitric/citric acid-cultivation by *Yarrowia lipolytica* EH59 is shown. After exhaustion of ammonium nitrogen at 10 h, the production of biomass stopped and the secretion of the citric and (2*R*,3*S*)-isocitric acid started. Sunflower oil was added again after 45, 75 and 105 h. The cultivation was stopped after 167 h at a (2*R*,3*S*)-isocitric acid concentration of 93 g L<sup>-1</sup> and citric acid concentration of 82.8 g L<sup>-1</sup>, which is equivalent to a 1.14 : 1 ratio of (2*R*,3*S*)-isocitric/citric acid and a substrate related yield of (2*R*,3*S*)-isocitric acid at 0.65 g g<sup>-1</sup>. At the end of cultivation a total organic acid concentration of 175.8 g L<sup>-1</sup> was detected.

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The yeast cells and residual sunflower oil were separated from the culture solution by cross-flow microfiltration (Sartoflow<sup>®</sup> Alpha, Sartorius, Göttingen, Germany) with Hydrosart<sup>®</sup> type membranes (Sartorius) with 0.2 µm pore sizes.

The transformation of the (2*R*,3*S*)-isocitric/citric acid sodium salts from the cell-free culture solution to their corresponding acids and sodium hydroxide was performed by laboratory electro dialysis equipment (Deukum Co., Frickenhausen, Germany) with bipolar membranes (Neosepta BP-1, Tokuyama Co., Japan).

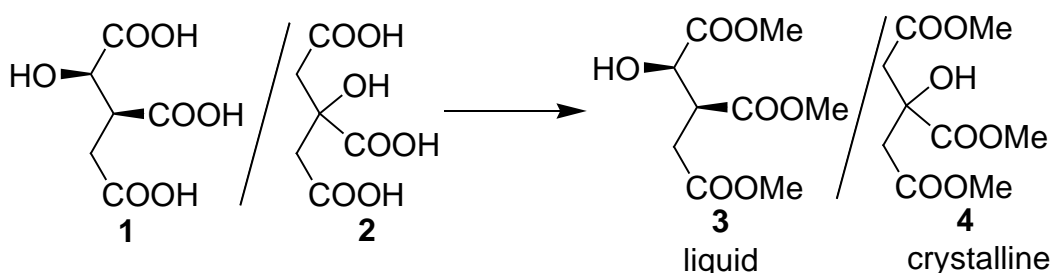
For the following chemical experiments the water was removed under reduced pressure to get a brown concentrate of (2*R*,3*S*)-isocitric acid and citric acid.

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## 2. Experimental Section

All reagents were obtained commercially from Acros, Alfa Aesar, Sigma-Aldrich or Merck and used without further purification. Melting points were measured with a BOETIUS-micro-hot-stage and are uncorrected. The rotation angles were determined with a half-automatic polarimeter Polartronic D (eloptron) from Schmidt + Haensch. IR spectra were recorded on an FT-IR-spectrometer GENESIS SERIES from ATI/MATTSON.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Varian Gemini 2000 and Varian Gemini-200BB (200 MHz for  $^1\text{H}$ ; 50 MHz for  $^{13}\text{C}$ ), Varian Gemini-300BB and Varian Mercury-300BB (300 MHz for  $^1\text{H}$ ; 75 MHz for  $^{13}\text{C}$ ), Varian Mercury-400BB (400 MHz for  $^1\text{H}$ ; 100 MHz for  $^{13}\text{C}$ ) and Bruker DRX 600 (600 MHz for  $^1\text{H}$ ; 150 MHz for  $^{13}\text{C}$ ). The chemical shifts are reported relative to the residual solvent peak, which was used as an internal reference ( $\delta$  values in ppm,  $J$  in Hz). HRMS were obtained on a Bruker Daltonics APEX II for ESI and on an FG Masslab Manchester VG-12-250 with an ionisation energy of 70 eV and an ion source temperature of 250 °C for EI; elemental analysis was performed on a CHN-O-Rapid (HERAEUS). Reactions involving moisture-sensitive reactants were performed in flame dried glassware under an atmosphere of argon, reactants being added via syringe. Flash column chromatography was performed on silica gel (Merck 60, 0.040-0.63 mm) and analytical TLC on pre-coated silica gel plates (Merck 60 F<sub>254</sub>, 0.25 mm).

### 2.1 (2*R*,3*S*)-Isocitric acid trimethylester **3**



The fermentation concentrate of (2*R*,3*S*)-isocitric acid **1** and citric acid **2** (306.5 g, 1.60 mol) was dissolved in MeOH (1.0 L) and 2,2-dimethoxypropane (1.1 L) was added. Under vigorous stirring TMSCl (75 mL, 0.59 mol) was carefully introduced. The flask was equipped with a drying tube and the mixture was stirred for three days at RT, afterwards the solvents were removed under reduced pressure. The residue was dissolved in  $\text{CHCl}_3$  (700 mL) and washed once with saturated aqueous  $\text{NaHCO}_3$  solution (300 mL) and two times with water (100 mL). After drying with  $\text{Na}_2\text{SO}_4$  the solvent was



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removed under reduced pressure (bath temperature not higher than 40 °C) and the residue was kept in the refrigerator for three days. The precipitated citric acid trimethylester **4** was filtered off from an oil containing (2*R*,3*S*)-isocitric acid trimethylester **3** to give **4** (153.4 g, 0.66 mol, 88%) as colourless crystals.

M.p.: 75-75.5 °C [Lit: 75.0-76.5 °C].<sup>[2]</sup>

$[\alpha]_{\text{D}}^{22} \pm 0.00$  (*c* 1.00; MeOH).

IR (film):  $\tilde{\nu} = 3482, 3032, 2962, 1742, 1721, 1440 \text{ cm}^{-1}$ .

<sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>):  $\delta = 2.75$  (d, <sup>2</sup>*J* = 15.3 Hz, 2 H, **CH**<sub>2</sub>), 2.90 (d, <sup>2</sup>*J* = 15.6 Hz, 2 H, **CH**<sub>2</sub>), 3.59 (s, 6 H, 2 x **COOCH**<sub>3</sub>), 3.68 (s, 3H, **COOCH**<sub>3</sub>), 5.78 (s, 1 H, **OH**).

<sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>):  $\delta = 43.1$  (2 x **CH**<sub>2</sub>), 51.5 (2 x **COOCH**<sub>3</sub>), 52.2 (**COOCH**<sub>3</sub>), 73.1 (**CH**), 169.7 (2 x **COOCH**<sub>3</sub>), 173.1 (**COOCH**<sub>3</sub>).

The oily brown filtrate was distilled under reduced pressure to give (2*R*,3*S*)-isocitric acid trimethylester **3** (158.3 g, 0.68 mol, 80%) as a yellow oil of a purity > 92% judged by <sup>1</sup>H-NMR.

B.p.: 105 °C (0.02 mbar).

$n_{\text{D}}^{22} = 1.4523$ .

$[\alpha]_{\text{D}}^{22} + 11.4$  (*c* 1.00, MeOH).

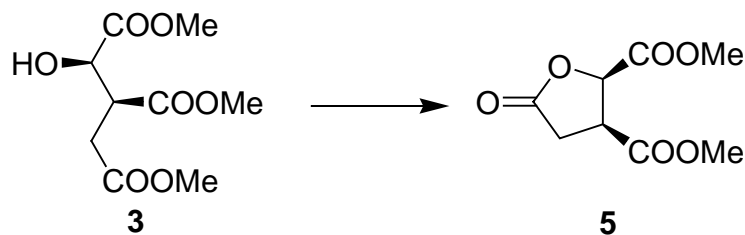
IR (film):  $\tilde{\nu} = 3486, 3005, 2957, 1799, 1738, 1439 \text{ cm}^{-1}$ .

<sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>):  $\delta = 2.60$ -2.72 (m, 2H, **CH**<sub>2</sub>), 3.28-3.32 (m, 1H, **CH**), 3.59, 3.66, 3.69 (s, 3 x 3 H, 3 x **COOCH**<sub>3</sub>), 4.34-4.37 (m, 1 H, **CH**), 5.91 (d, <sup>3</sup>*J* = 5.1 Hz, 1 H, **OH**).

<sup>13</sup>C-NMR (75 MHz, DMSO-*d*<sub>6</sub>):  $\delta = 31.8$  (**CH**<sub>2</sub>), 45.0 (**CH**), 51.6, 51.8, 51.9 (3 x **CH**<sub>3</sub>), 70.3 (**CH**), 171.1, 171.9, 172.4 (3 x **CO**).

HRMS-ESI: *m/z* [M+Na]<sup>+</sup> calcd for C<sub>9</sub>H<sub>14</sub>O<sub>7</sub>Na: 257.06317; found 257.06321, [2M+Na]<sup>+</sup> calcd for C<sub>18</sub>H<sub>28</sub>O<sub>14</sub>Na: 491.13731; found 491.13745.

## 2.2 (2*R*,3*S*)-Isocitric acid lactone-2,3-dicarboxylic acid dimethylester **5**



From distilled (2*R*,3*S*)-isocitric acid trimethylester **3**:

A solution of (2*R*,3*S*)-isocitric acid trimethylester **3** (3.99 g, 17.0 mmol) and *para*-toluenesulfonic acid (0.35 g, 1.80 mmol) in toluene (130 mL) was heated at reflux for 6 h using a Dean-Stark trap. After the solvent had been removed under reduced pressure, the yellow solid was resolved in CHCl<sub>3</sub> (25 mL) and washed once with saturated aqueous NaHCO<sub>3</sub> solution (25 mL), then water (25 mL) and dried with Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed under reduced pressure and the remaining solid was recrystallised from methanol to give **5** (2.70 g, 13.4 mmol, 80%) as colourless needles.

From crude (2*R*,3*S*)-isocitric acid trimethylester **3**:

The reaction with crude (2*R*,3*S*)-isocitric acid trimethylester **3** (60.90 g, 0.26 mol) and *para*-toluenesulfonic acid (4.95 g, 0.03 mol) in toluene (500 mL) was performed analogously to obtain a solid, which could be recrystallised from methanol to give pure **5** (30.05 g, 0.15 mol, 58%) as colourless needles.

M.p.: 104-104.5 °C [Lit: 104-106 °C].<sup>[3]</sup>

$[\alpha]_{\text{D}}^{24}$  -66.8 (*c* 8.56, acetone) [Lit:  $[\alpha]_{\text{D}}^{26}$  -66.3 (*c* 8.47, acetone)].<sup>[3]</sup>

IR (KBr):  $\tilde{\nu}$  = 3010, 2966, 1782, 1753, 1740, 1366, 1070 cm<sup>-1</sup>.

<sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 2.74 (dd, <sup>2</sup>*J* = 17.5 Hz, <sup>3</sup>*J* = 6.9 Hz, 1 H, CH<sub>2</sub>), 2.89 (dd, <sup>2</sup>*J* = 17.5 Hz, <sup>3</sup>*J* = 9.2 Hz, 1 H, CH<sub>2</sub>), 3.63, 3.69 (s, 2 x 3 H, COOCH<sub>3</sub>), 3.89 (ddd, <sup>3</sup>*J* = 6.9 Hz, 8.1 Hz, 9.2 Hz, 1 H, CH(O)COOCH<sub>3</sub>), 5.32 (d, <sup>3</sup>*J* = 8.1 Hz, 1 H, CH(O)COOCH<sub>3</sub>).

<sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 30.6 (CH<sub>2</sub>), 42.7 (CH<sub>2</sub>CH), 52.4, 52.5 (2 x COOCH<sub>3</sub>), 76.0 (CH(O)), 168.3, 170.3 (2 x COOCH<sub>3</sub>), 174.3 (COOCH).

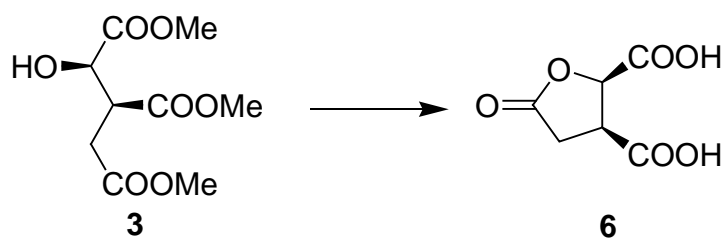
HRMS-ESI: *m/z* [M+Na]<sup>+</sup> calcd for C<sub>8</sub>H<sub>10</sub>O<sub>6</sub>Na: 225.03696; found 225.03694.

Elemental analysis: calcd for C<sub>8</sub>H<sub>10</sub>O<sub>6</sub>: C 47.53, H 4.99; found: C 47.60, H 4.90.

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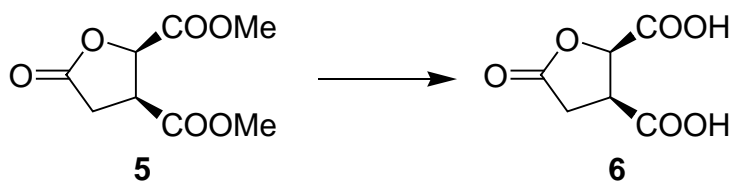
### 2.3 (2R,3S)-Isocitric acid lactone-2,3-dicarboxylic acid **6**

From (2R,3S)-Isocitric acid trimethylester **3**:



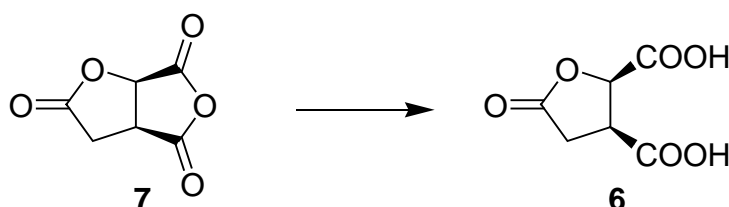
A solution of (2R,3S)-isocitric acid trimethylester **3** (2.36 g, 10.0 mmol) in 4.0 M hydrochloric acid (8 mL) was heated at reflux for 8 h. After solvent evaporation the residue was dried to give (2R,3S)-isocitric acid lactone-2,3-dicarboxylic acid **6** (1.74 g, 10.0 mmol, quantitative) as a colourless solid.

From (2R,3S)-isocitric acid lactone-2,3-dicarboxylic acid dimethylester **5**:<sup>[4]</sup>



A solution of (2R,3S)-isocitric acid lactone-2,3-dicarboxylic acid dimethylester **5** (36.47 g, 0.18 mol) in 1.0 M hydrochloric acid (500 mL) was heated at reflux for 4 h. After solvent evaporation the residue was dried to give **6** (31.32 g, 0.18 mol, quantitative) as a colourless solid.

From (2R,3S)-isocitric acid lactone-2,3-dicarboxylic acid anhydride **7**:



A solution of (2R,3S)-isocitric acid lactone-2,3-dicarboxylic acid anhydride **7** (20.0 g, 0.13 mol) in 1.0 M hydrochloric acid (150 mL) was heated at reflux for 1 h. After solvent evaporation the residue was dried to give **6** (22.6 g, 0.13 mol, quantitative) as a colourless solid.

M.p.: 148-52 °C [Lit: 150-152 °C].<sup>[5]</sup>

$[\alpha]_D^{22} -69.9$  (*c* 1.06, H<sub>2</sub>O) [Lit:  $[\alpha]_D^{22} -60.3$  (*c* 1.03, H<sub>2</sub>O)].<sup>[5]</sup>

IR (KBr):  $\tilde{\nu} = 3083, 1797, 1732, 1246 \text{ cm}^{-1}$ .

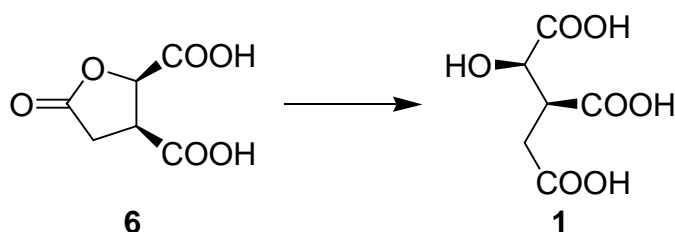
<sup>1</sup>H-NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta = 2.67$  (dd, <sup>2</sup>*J* = 17.2 Hz, <sup>3</sup>*J* = 6.8 Hz, 2 H, CH<sub>2</sub>), 2.81 (dd, <sup>2</sup>*J* = 17.6 Hz, <sup>3</sup>*J* = 8.8 Hz, 2 H, CH<sub>2</sub>), 3.67 (ddd, <sup>3</sup>*J* = 6.8, 7.6, 8.8 Hz, 1 H, CHCOOH), 5.09 (d, <sup>3</sup>*J* = 8.0 Hz, 1 H, CH(O)COOH).

<sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta = 31.3$  (CH<sub>2</sub>), 43.2 (CH<sub>2</sub>CHCOOH), 76.8 (CH(O)COOH), 169.5 (CH(O)COOH), 171.7 (CO), 175.2 (CH<sub>2</sub>CHCOOH).

HRMS-ESI: *m/z* [2M-H]<sup>-</sup> calcd for C<sub>12</sub>H<sub>11</sub>O<sub>12</sub>: 347.02560; found 347.02402.

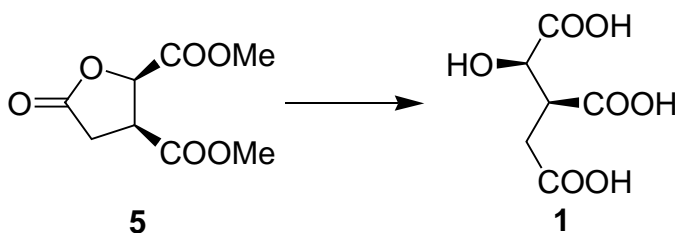
## 2.4 (2*R*,3*S*)-Isocitric acid 1

From (2*R*,3*S*)-isocitric acid lactone-2,3-dicarboxylic acid **6**.<sup>[6a]</sup>



To a stirred solution of (2*R*,3*S*)-isocitric acid lactone-2,3-dicarboxylic acid **6** (4.36 g, 25.0 mmol) in water (50 mL) was added 1.00 M NaOH (75.0 mL, 75.0 mmol) drop wise at 60 °C. After completion of addition stirring and heating was continued for additional 15 min. Subsequently the solution was allowed to cool to RT and then Amberlite IR-120 (20 cm<sup>3</sup>) was introduced. After filtration and solvent evaporation under reduced pressure, the residue was dried in vacuo to give a colourless oil (4.81 g, 25.04 mmol, quantitative), which was judged by <sup>1</sup>H-NMR to be a 1 : 9 mixture of starting material **6** and the desired (2*R*,3*S*)-isocitric acid **1**.

From (2*R*,3*S*)-isocitric acid lactone-2,3-dicarboxylic acid dimethylester **5**.<sup>[6b]</sup>



Analogously (2*R*,3*S*)-isocitric acid lactone-2,3-dicarboxylic acid dimethylester **5** (1.01 g, 5.0 mmol) in water (20 mL) was titrated with 1.00 M NaOH (15.0 mL, 15.0 mmol) to give a colourless oil (0.96 g, 5.0 mmol, quantitative), which was judged by <sup>1</sup>H-NMR to be a 1 : 9 mixture of starting material **5** and the desired (2*R*,3*S*)-isocitric acid **1**.

$[\alpha]_{\text{D}}^{22} +15.8$  (*c* 4.67, H<sub>2</sub>O) [Lit:  $[\alpha]_{\text{D}}^{22} +30.6$  (H<sub>2</sub>O),<sup>[6a]</sup>  $[\alpha]_{\text{D}}^{22} +9.0$ <sup>[6c]</sup>].

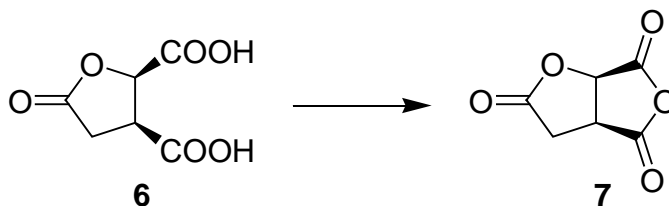
IR (Film):  $\tilde{\nu} = 3407, 1732, 1238 \text{ cm}^{-1}$ .

<sup>1</sup>H-NMR (200 MHz, DMSO-*d*<sub>6</sub>):  $\delta = 2.40\text{-}2.50$  (m, 2 H, **CH**<sub>2</sub>), 3.03-3.12 (m, 1 H, **CHCOOH**), 4.15 (d, <sup>3</sup>*J* = 3.6 Hz, 1 H, **CHOH**).

<sup>13</sup>C-NMR (50 MHz, DMSO-*d*<sub>6</sub>):  $\delta = 32.2$  (**CH**<sub>2</sub>), 45.1, 70.3 (2 x **CH**), 172.6, 173.3, 173.8 (3 x **CO**).

HRMS-ESI: *m/z* [M-H]<sup>-</sup> calcd for C<sub>6</sub>H<sub>7</sub>O<sub>7</sub>: 191.01973; found 191.01946.

## 2.5 (2*R*,3*S*)-Isocitric acid lactone-2,3-dicarboxylic acid anhydride **7**<sup>[7]</sup>



(2*R*,3*S*)-Isocitric acid lactone-2,3-dicarboxylic acid **6** (50.0 g, 0.29 mol) was dissolved in acetic anhydride (80 mL) and heated to 160 °C for 15 min. After cooling (2*R*,3*S*)-isocitric acid lactone-2,3-dicarboxylic acid anhydride **7** precipitated and was filtered off under inert conditions to give pure **7** (38.1 g, 0.24 mol, 85%) as colourless crystals.

M.p.: 190-193 °C [Lit: 190-195 °C].<sup>[7]</sup>

$[\alpha]_{\text{D}}^{22} +11.4$  (*c* 5.01, DMF).

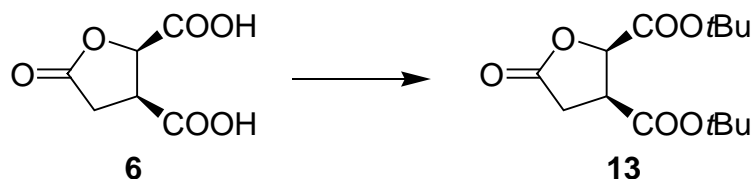
IR (KBr):  $\tilde{\nu} = 3039, 3005, 2962, 1850, 1798, 1775, 1412, 1160 \text{ cm}^{-1}$ .

<sup>1</sup>H-NMR (200 MHz, DMSO-*d*<sub>6</sub>):  $\delta = 2.80\text{-}3.08$  (m, 2 H, **CH**<sub>2</sub>), 4.02 (ddd, <sup>3</sup>*J* = 6.9, 8.7, 9.6 Hz, 1 H, **CHC(O)O**), 5.40 (d, <sup>3</sup>*J* = 8.7 Hz, 1 H, **CH(O)COCH**<sub>2</sub>).

<sup>13</sup>C-NMR (50 MHz, DMSO-*d*<sub>6</sub>):  $\delta = 29.9$  (**CH**<sub>2</sub>), 40.4, 76.3 (2 x **CH**), 168.0, 171.1, 174.1 (3 x **CO**).

HRMS-EI: *m/z* [M+H]<sup>+</sup> calcd for C<sub>6</sub>H<sub>5</sub>O<sub>5</sub>: 157.01370; found 157.00927.

## 2.6 (2*R*,3*S*)-Isocitric acid lactone-2,3-dicarboxylic acid di-*tert*-butylester **13**



Ten pyrex vessels (volume 7.5 mL) were each filled with (2*R*,3*S*)-isocitric acid lactone-2,3-dicarboxylic acid **6** (0.35 g, 20 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (4 mL) and cooled to -25 °C. Catalytic amounts of conc. H<sub>2</sub>SO<sub>4</sub> (3 drops) and liquid 2-methylpropene (2 mL) were added and the vessels were sealed and shaken at RT for 5 d. The vessels were cooled and reopened carefully. Their content was combined and stirred with saturated aqueous NaHCO<sub>3</sub> solution (20 mL). Et<sub>2</sub>O (50 mL) was added, the layers were separated and the organic layer was washed with water (20 mL). After drying with Na<sub>2</sub>SO<sub>4</sub> the solvents were removed under reduced pressure to give a solid which was purified by column chromatography (*n*-hexane-EtOAc 10:1 v/v) to yield **13** (5.04 g, 17.6 mmol, 88%) as a colourless solid.

M.p.: 68-70 °C.

[α]<sub>D</sub><sup>22</sup> -47.3 (*c* 1.02, toluene).

R<sub>f</sub> = 0.87 (*n*-hexane-EtOAc 10:1 v/v).

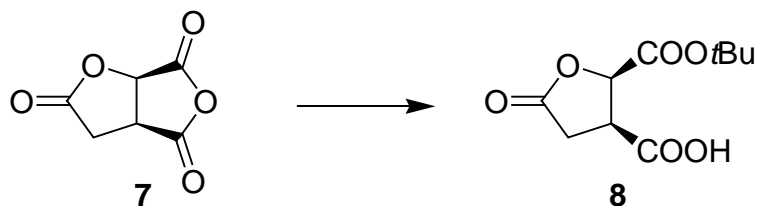
IR (KBr):  $\tilde{\nu}$  = 2981, 2937, 1792, 1739, 1730, 1370, 1153 cm<sup>-1</sup>.

<sup>1</sup>H-NMR (600 MHz, DMSO-*d*<sub>6</sub>): δ = 1.40 (s, 9 H, 3 x CH<sub>3</sub>), 1.42 (s, 9 H, 3 x CH<sub>3</sub>), 2.68 (dd, <sup>2</sup>*J* = 17.5 Hz, <sup>3</sup>*J* = 7.2 Hz, 1 H, CH<sub>2</sub>), 2.83 (dd, <sup>2</sup>*J* = 17.5 Hz, <sup>3</sup>*J* = 9.3 Hz, 1 H, CH<sub>2</sub>), 3.68 (ddd, <sup>3</sup>*J* = 7.2, 7.9, 9.3 Hz, 1 H, CH<sub>2</sub>CH), 5.05 (d, <sup>3</sup>*J* = 7.9 Hz, 1 H, CH(O)).

<sup>13</sup>C-NMR (150 MHz, DMSO-*d*<sub>6</sub>): δ = 27.5 (3 x CH<sub>3</sub>), 27.6 (3 x CH<sub>3</sub>), 31.0 (CH<sub>2</sub>COO), 43.4 (CH<sub>2</sub>CH), 76.7 (CH(O)), 81.7 (C(CH<sub>3</sub>)<sub>3</sub>), 82.6 (C(CH<sub>3</sub>)<sub>3</sub>), 166.6 (CH(O)C), 168.9 (CH<sub>2</sub>CO(O)), 174.4 (CHCOOC(CH<sub>3</sub>)<sub>3</sub>).

HRMS-ESI: *m/z* [M+Na]<sup>+</sup> calcd for C<sub>14</sub>H<sub>22</sub>O<sub>6</sub>Na: 309.13086; found 309.13076.

## 2.7 (2*R*,3*S*)-Isocitric acid lactone-2-carboxylic acid-*tert*-butylester-3-carboxylic acid **8**



(2*R*,3*S*)-Isocitric acid lactone-2,3-dicarboxylic acid anhydride **7** (10.2 g, 65.3 mmol) in anhydrous *t*BuOH (80 mL) was heated under reflux for 15 h. After removing the solvent under reduced pressure and then high vacuum (0.01 mbar) the oily product solidified to yield pure **8** (15.0 g, 65.3 mmol, quantitative) as a beige solid.

M.p.: 88-90 °C.

$[\alpha]_D^{22}$  -51.3 (*c* 1.17, MeOH).

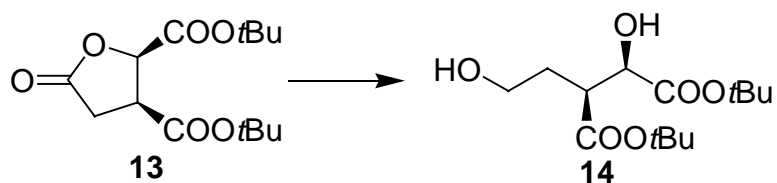
IR (KBr):  $\tilde{\nu}$  = 3268, 3012, 2996, 2946, 1778, 1746, 1709, 1373, 1152  $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  (600 MHz, DMSO- $d_6$ ):  $\delta$  = 1.41 (s, 9 H, 3 x  $\text{CH}_3$ ), 2.70 (dd,  $^2J$  = 17.4 Hz,  $^3J$  = 7.7 Hz, 1 H,  $\text{CH}_2$ ), 2.80 (dd,  $^2J$  = 17.4 Hz,  $^3J$  = 9.2 Hz, 1 H,  $\text{CH}_2$ ), 3.68-3.73 (m, 1 H,  $\text{CHCOOH}$ ), 5.08 (d,  $^3J$  = 9.2 Hz, 1 H,  $\text{CH(O)COOC}(\text{CH}_3)_3$ ) 13.00-13.10 (br, 1 H,  $\text{COOH}$ ).

$^{13}\text{C-NMR}$  (150 MHz, DMSO- $d_6$ ):  $\delta$  = 27.4 (3 x  $\text{CH}_3$ ), 30.6 ( $\text{CH}_2$ ), 42.9 ( $\text{CHCOOH}$ ), 76.5 ( $\text{CH(O)}$ ), 82.5 ( $\text{COOC}(\text{CH}_3)_3$ ), 166.7 ( $\text{COOC}(\text{CH}_3)_3$ ), 171.1 ( $\text{CO(O)}$ ), 174.6 ( $\text{COOH}$ ).

HRMS-ESI:  $m/z$   $[\text{M}+\text{Na}]^+$  calcd for  $\text{C}_{10}\text{H}_{14}\text{O}_6\text{Na}$ : 253.06826; found 253.06834.

## 2.8 (2*R*,3*S*)-2-Hydroxy-3-(2-hydroxy-ethyl)-succinic acid di-*tert*-butylester **14**



(2*R*,3*S*)-Isocitric acid lactone-2,3-dicarboxylic acid di-*tert*-butylester **13** (1.43 g, 5.0 mmol) was dissolved in a mixture of anhydrous THF (40 mL) and anhydrous *i*PrOH (20 mL) and a solution of calcium borohydride bis(THF) complex (536 mg, 5.0 mmol) in anhydrous THF (20 mL) was added over a period of 5 min at -10 °C. This solution was stirred for an additional 15 min (TLC-control).

Afterwards 1 m% aqueous citric acid solution (50 mL) was added to the mixture and the aqueous phase was extracted twice with Et<sub>2</sub>O (2 x 50 mL). After drying the organic layer with Na<sub>2</sub>SO<sub>4</sub> the solvents were removed under reduced pressure. The resulting oil was purified by column chromatography (*n*-hexane-EtOAc 1:1 v/v) to yield **14** (1.22 g, 4.2 mmol, 84%) as a colourless oil.

$[\alpha]_D^{22} -7.4$  (*c* 1.89, MeOH).

$R_f = 0.20$  (*n*-hexane-EtOAc 1:1 v/v).

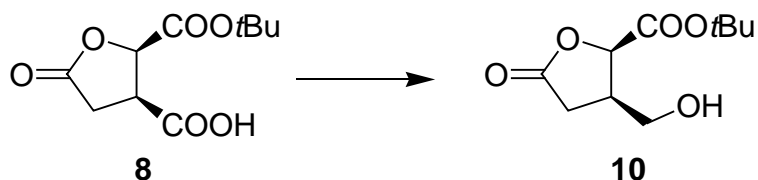
IR (film):  $\tilde{\nu} = 3446, 2978, 2934, 1732, 1369, 1152$  cm<sup>-1</sup>.

<sup>1</sup>H-NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta = 1.37$  (s, 9 H, 3 x CH<sub>3</sub>), 1.40 (s, 9 H, 3 x CH<sub>3</sub>), 1.47-1.55 (m, 1 H, CH<sub>2</sub>), 1.64-1.73 (m, 1 H, CH<sub>2</sub>), 2.64 (td, <sup>3</sup>*J* = 9.8, 5.1 Hz, 1 H, CH), 3.28-3.36 (m, 1 H, CH<sub>2</sub>), 3.37-3.44 (m, 1 H, CH<sub>2</sub>), 3.99 (t, <sup>3</sup>*J* = 6.0 Hz, 1 H, CH<sub>2</sub>OH), 4.46 (t, <sup>3</sup>*J* = 4.8 Hz, 1 H, CH(OH)), 5.32 (d, <sup>3</sup>*J* = 5.6 Hz, 1 H, CHOH).

<sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>):  $\delta = 27.7$  (6 x CH<sub>3</sub>), 30.7 (CH<sub>2</sub>), 46.5 (CH), 57.8 (CH<sub>2</sub>OH), 71.7 (CHOH), 79.8, 80.5 (2 x C(CH<sub>3</sub>)<sub>3</sub>), 171.2, 171.5 (2 x CO).

HRMS-ESI: *m/z* [M+Na]<sup>+</sup> calcd for C<sub>14</sub>H<sub>26</sub>O<sub>6</sub>Na: 313.16216; found 313.16199.

## 2.9 (2*R*,3*R*)-Tetrahydro-3-(hydroxymethyl)-5-oxofuran-2-carboxylic acid-*tert*-butylester **10**



A borane THF complex solution (1 M in THF, 58 mL, 58.0 mmol) was added slowly to (2*R*,3*S*)-isocitric acid lactone-2-carboxylic acid-*tert*-butylester-3-carboxylic acid **8** (7.20 g, 31.3 mmol) in anhydrous THF (150 mL) at 0 °C. After stirring for 5 h at RT the solution was cooled to 0 °C, treated with anhydrous MeOH (100 mL) and the solvents were removed under reduced pressure. The residue was diluted with MeOH (100 mL) and the solvent was again removed under reduced pressure to give a colourless solid, which was purified by column chromatography (CHCl<sub>3</sub>-MeOH 30:1 v/v) to yield pure **10** (5.94 g, 27.5 mmol, 88%) as colourless crystals.

M.p.: 81-83 °C.

$[\alpha]_D^{22} -48.5$  (*c* 0.45, MeOH).

$R_f = 0.49$  (CHCl<sub>3</sub>-MeOH 10:1 v/v).



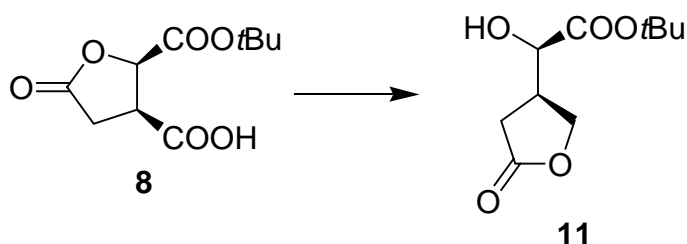
IR (film):  $\tilde{\nu}$  = 3520, 3004, 2934, 2885, 1791, 1724, 1342, 1155  $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.42 (s, 9 H, 3 x  $\text{CH}_3$ ), 2.36 (dd,  $^2J$  = 17.4 Hz,  $^3J$  = 8.5 Hz, 1 H,  $\text{CH}_2$ ), 2.59 (dd,  $^2J$  = 17.6 Hz,  $^3J$  = 8.7 Hz, 1 H,  $\text{CH}_2$ ), 2.82-2.95 (m, 1 H,  $\text{CH}$ ), 3.33-3.40 (m, 1 H,  $\text{CH}$ ), 3.42-3.51 (m, 1 H,  $\text{CH}_2$ ), 4.83 (t,  $^3J$  = 4.8 Hz, 1 H,  $\text{OH}$ ), 4.90 (d,  $^3J$  = 7.8 Hz, 1 H,  $\text{CHCOO}$ ).

$^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 27.5 ( $\text{CH}$ ) 27.6 (3 x  $\text{CH}_3$ ), 30.5, 59.2 (2 x  $\text{CH}_2$ ), 77.4 ( $\text{CH}$ ), 82.2 ( $\text{C}(\text{CH}_3)_3$ ), 167.5, 175.9 (2 x  $\text{CO}$ ).

HRMS-ESI:  $m/z$  [ $2\text{M}+\text{Na}$ ] $^+$  calcd for  $\text{C}_{20}\text{H}_{32}\text{O}_{10}\text{Na}$ : 455.18877; found 455.18863.

## 2.10 *tert*-Butyl (2*R*)-hydroxy[(3*S*)-5-oxotetrahydrofuran-3-yl]acetate **11**



This reaction was carried out analogously to **2.9**, workup included dilution with EtOAc and washing the organic layer with saturated aqueous  $\text{NH}_4\text{Cl}$  solution. After drying the organic layer with  $\text{Na}_2\text{SO}_4$  the solvent was removed under reduced pressure to give a colourless solid, which was purified by column chromatography ( $\text{CHCl}_3$ -MeOH 30:1 v/v) to yield pure **11** (78%) as colourless crystals.

M.p.: 81-83  $^\circ\text{C}$ .

$[\alpha]_{\text{D}}^{22} +9.8$  ( $c$  1.02, MeOH).

$R_f = 0.60$  ( $\text{CHCl}_3$ -MeOH 10:1 v/v).

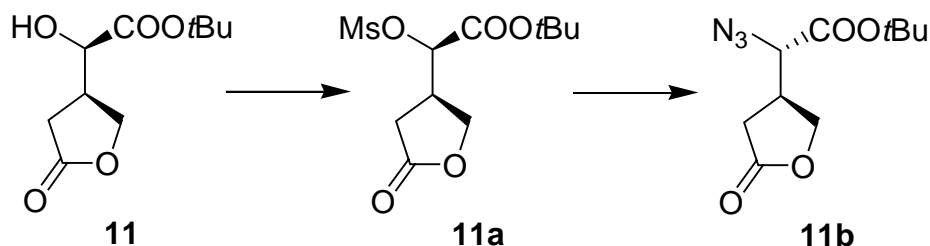
IR (KBr):  $\tilde{\nu}$  = 3423, 1773, 1716, 1122  $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.49 (s, 9 H, 3 x  $\text{CH}_3$ ), 2.61 (d, 2 H,  $^3J$  = 8.4 Hz,  $\text{CH}_2$ ), 2.85-2.95 (m, 1 H,  $\text{CHCH}$ ), 4.07 (d, 1 H,  $^3J$  = 5.6 Hz,  $\text{CHOH}$ ), 4.19 (dd, 1 H,  $^2J$  = 9.2 Hz,  $^3J$  = 6.8 Hz,  $\text{CH}_2(\text{O})$ ), 4.32 (dd, 1 H,  $^2J$  = 9.2 Hz,  $^3J$  = 8.4 Hz,  $\text{CH}_2(\text{O})$ ).

$^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 28.1 (3 x  $\text{CH}_3$ ), 31.0 ( $\text{CH}_2\text{CHCH}_2$ ), 38.9 ( $\text{CH}_2\text{CH}$ ), 68.8 ( $\text{CHCH}_2$ ), 70.8 ( $\text{CHOH}$ ), 84.2 ( $\text{C}(\text{CH}_3)_3$ ), 172.3 ( $\text{CO}$ ), 176.5 ( $\text{CH}_2\text{CO}$ ).

HRMS-ESI:  $m/z$  [ $\text{M}+\text{Na}$ ] $^+$  calcd for  $\text{C}_{10}\text{H}_{16}\text{O}_5\text{Na}$ : 239.08899; found 239.08905, [ $2\text{M}+\text{Na}$ ] $^+$  calcd for  $\text{C}_{20}\text{H}_{32}\text{O}_{10}\text{Na}$ : 455.18877; found 455.18863.

## 2.11 *tert*-Butyl (2*S*)-azido[(3*S*)-5-oxotetrahydrofuran-3-yl]acetate **11b**



*tert*-Butyl (2*R*)-hydroxy[(3*S*)-5-oxotetrahydrofuran-3-yl]acetate **11** (726 mg, 3.36 mmol) was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (25 mL). At 0 °C pyridine (268 mg, 3.39 mmol) was added followed by methanesulfonyl chloride (387 mg, 3.38 mmol). After stirring for 16 h at RT the organic layer was washed with saturated aqueous NaHCO<sub>3</sub> solution (30 mL) and saturated aqueous NaCl solution (30 mL). After drying the organic layer with Na<sub>2</sub>SO<sub>4</sub> the solvent was removed under reduced pressure. The resulting oil was dissolved in anhydrous DMF (50 mL), NaN<sub>3</sub> (225 mg, 3.46 mmol) was added and this mixture was stirred for 16 h at 55 °C. Afterwards EtOAc (50 mL) and water (50 mL) were added and the aqueous layer was extracted three times with EtOAc (3 x 50 mL). After drying the organic layer with Na<sub>2</sub>SO<sub>4</sub> the solvent was removed under reduced pressure. The resulting oil was purified by column chromatography (CHCl<sub>3</sub>-MeOH 30:1 v/v) to yield **11b** (379 mg, 1.57 mmol, 47% after two steps) as a pale yellow oil.

$[\alpha]_D^{22} -102.9$  (*c* 1.30, CHCl<sub>3</sub>).

$R_f = 0.77$  (CHCl<sub>3</sub>-MeOH 10:1 v/v).

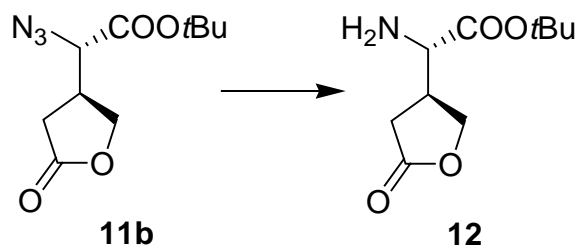
IR (KBr):  $\tilde{\nu} = 2980, 2934, 2114, 1782, 1735, 1370, 1258, 1226, 1151, 1086, 1027, 626$  cm<sup>-1</sup>.

<sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>):  $\delta = 1.51$  (s, 9 H, 3 x CH<sub>3</sub>), 2.50 (dd, 1 H, <sup>2</sup>*J* = 18.0 Hz, <sup>3</sup>*J* = 7.2 Hz, CH<sub>2</sub>), 2.60 (dd, 1 H, <sup>2</sup>*J* = 18.0 Hz, <sup>3</sup>*J* = 8.8 Hz, CH<sub>2</sub>), 2.90-3.05 (m, 1 H, CH), 3.98 (d, 1 H, <sup>3</sup>*J* = 6.0 Hz, CHN<sub>3</sub>), 4.16 (dd, 1 H, <sup>2</sup>*J* = 9.2 Hz, <sup>3</sup>*J* = 6.4 Hz, CH<sub>2</sub>), 4.42 (dd, 1 H, <sup>2</sup>*J* = 9.2 Hz, <sup>3</sup>*J* = 7.8 Hz, CH<sub>2</sub>).

<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):  $\delta = 28.1$  (3 x CH<sub>3</sub>), 30.3 (CH<sub>2</sub>), 36.8 (CH), 63.5 (CH), 69.7 (CH<sub>2</sub>), 84.6 (C(CH<sub>3</sub>)<sub>3</sub>), 167.4, 175.7 (2 x CO).

HRMS-ESI: *m/z* [M+Na]<sup>+</sup> calcd for C<sub>10</sub>H<sub>15</sub>N<sub>3</sub>O<sub>4</sub>Na: 264.09548; found 264.09541, [2M+Na]<sup>+</sup> calcd for C<sub>20</sub>H<sub>30</sub>N<sub>6</sub>O<sub>8</sub>Na: 505.20173; found 505.20127.

## 2.12 *tert*-Butyl (2*S*)-amino[(3*S*)-5-oxotetrahydrofuran-3-yl]acetate **12**



*tert*-Butyl (2*S*)-azido[(3*S*)-5-oxotetrahydrofuran-3-yl]acetate **11b** (94 mg, 0.38 mmol) in EtOAc (20 mL) was hydrogenated with catalytic amounts of Pd/C at RT for 8 h. After filtration the solvent was removed under reduced pressure to yield **12** (73 mg, 0.34 mmol, 90%) as a pale yellow oil.

$[\alpha]_D^{22} +17.1$  (*c* 0.47, CHCl<sub>3</sub>).

$R_f = 0.52$  (CHCl<sub>3</sub>-MeOH 10:1 v/v).

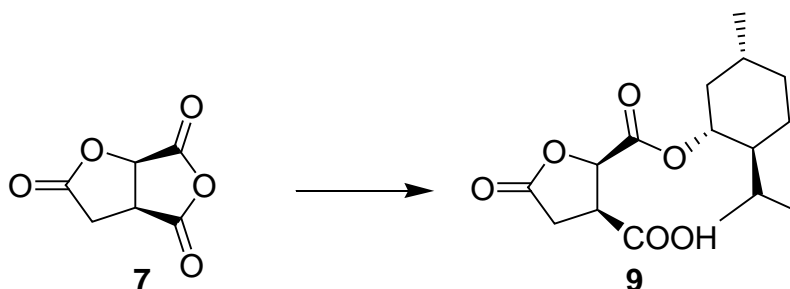
IR (KBr):  $\tilde{\nu} = 3435, 2980, 2934, 1770, 1727, 1650, 1394, 1370, 1251, 1154, 626$  cm<sup>-1</sup>.

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta = 1.46$  (s, 9 H, 3 x CH<sub>3</sub>), 1.81 (br, 2 H, NH<sub>2</sub>), 2.51 (d, 1 H, <sup>3</sup>*J* = 1.2 Hz, CH<sub>2</sub>), 2.53 (d, 1 H, <sup>3</sup>*J* = 1.2 Hz, CH<sub>2</sub>), 2.80-2.89 (m, 1 H, CH), 3.36 (d, 1 H, <sup>3</sup>*J* = 6.0 Hz, CHNH<sub>2</sub>), 4.21 (dd, 1 H, <sup>2</sup>*J* = 9.6 Hz, <sup>3</sup>*J* = 6.4 Hz, CH<sub>2</sub>), 4.40 (dd, 1 H, <sup>2</sup>*J* = 9.6 Hz, <sup>3</sup>*J* = 7.2 Hz, CH<sub>2</sub>).

<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):  $\delta = 28.0$  (3 x CH<sub>3</sub>), 30.5 (CH<sub>2</sub>) 39.4 (CH), 56.0 (CHNH<sub>2</sub>), 70.6 (CH<sub>2</sub>), 82.6 (C(CH<sub>3</sub>)<sub>3</sub>), 173.0, 176.7 (2 x CO).

HRMS-ESI:  $m/z$  [2M+H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>35</sub>N<sub>2</sub>O<sub>8</sub>: 431.23879; found 431.23900, [3M+H]<sup>+</sup> calcd for C<sub>30</sub>H<sub>52</sub>N<sub>3</sub>O<sub>12</sub>: 646.35455; found 646.35510, [4M+H]<sup>+</sup> calcd for C<sub>40</sub>H<sub>69</sub>N<sub>4</sub>O<sub>16</sub>: 861.47031; found 861.47060.

## 2.13 (2*R*,3*S*)-Isocitric acid lactone-2-carboxylic acid-[(1'*R*,2'*S*,5'*R*)-(-)-menthylester]-3-carboxylic acid **9**



(1*R*,2*S*,5*R*)-(-)-Menthol (4.45 g, 28.5 mmol) was molten at 50 °C and (2*R*,3*S*)-isocitric acid lactone anhydride **7** (1.06 g, 6.8 mmol) was added. The resulting suspension was heated to 100 °C for 36 h and then to 140 °C until a clear solution was obtained (12 h). Excess (-)-menthol was distilled off (6 mbar, 200 °C) and the crude product was recrystallised from hot EtOAc (15 ml) to yield pure **9** (575 mg, 1.8 mmol, 27%) as colourless needles.

M.p.: 194-197 °C.

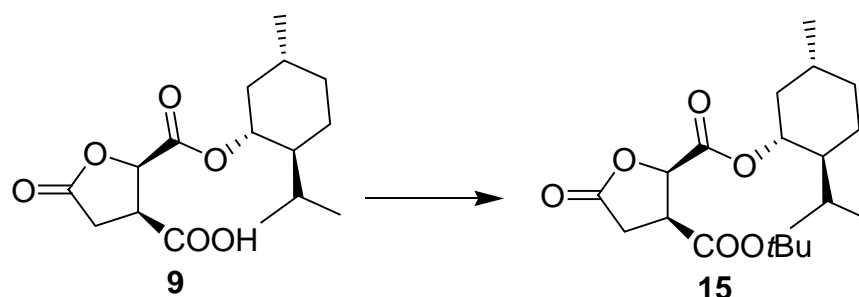
IR (KBr):  $\tilde{\nu}$  = 3212, 2954, 2937, 2860, 1777, 1754, 1184, 1056  $\text{cm}^{-1}$ .

$^1\text{H-NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  = 0.76 (d, 3 H,  $^3J$  = 6.8 Hz,  $\text{CH}_3$ ), 0.90 (d, 3 H,  $^3J$  = 7.6 Hz,  $\text{CH}_3$ ), 0.92 (d, 3 H,  $^3J$  = 7.6 Hz,  $\text{CH}_3$ ), 1.01 (q, 2 H,  $^3J$  = 12.0 Hz), 1.09 (qd, 1 H,  $^3J$  = 13.2, 3.6 Hz), 1.38-1.54 (m, 2 H), 1.67-1.76 (m, 2 H), 1.93 (sepd, 1 H,  $^3J$  = 7.6, 3.6 Hz,  $\text{CH}(\text{CH}_3)_2$ ), 2.00-2.08 (m, 1 H), 2.81 (dd, 1 H,  $^2J$  = 17.6 Hz,  $^3J$  = 9.6 Hz,  $(\text{O})\text{CCH}_2$ ), 2.92 (dd, 1 H,  $^2J$  = 17.6 Hz,  $^3J$  = 8.0 Hz,  $(\text{O})\text{CCH}_2$ ), 3.79-3.86 (m, 1 H,  $\text{CHCOOH}$ ), 4.92 (dt, 1 H,  $^3J$  = 11.2, 4.4 Hz,  $\text{OCHCH}_2$ ), 5.11 (d, 1 H,  $^3J$  = 8.4 Hz,  $\text{CHCOO}$ ).

$^{13}\text{C-NMR}$  (100 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  = 16.4, 21.1, 22.4 (3 x  $\text{CH}_3$ ), 24.2, 26.9, 31.7, 32.7, 35.3, 41.4, 44.3, 48.3, 77.6, 78.9, 169.2, 172.4, 176.7 (3 x CO).

HRMS-ESI:  $m/z$   $[\text{M-H}]^-$  calcd for  $\text{C}_{16}\text{H}_{23}\text{O}_6$ : 311.15001, found: 311.15007.

#### 2.14 (2*R*,3*S*)-Isocitric acid lactone-2-carboxylic acid-[(1'*R*,2'*S*,5'*R*)-(-)-menthylester]-3-carboxylic acid-*tert*-butylester **15**



A pyrex vessel (volume 7.5 mL) was filled with (2*R*,3*S*)-Isocitric acid lactone-2-carboxylic acid-[(1'*R*,2'*S*,5'*R*)-(-)-menthylester]-3-carboxylic acid **9** (200 mg, 0.64 mmol) in anhydrous  $\text{CH}_2\text{Cl}_2$  (3 mL) and cooled to  $-20$  °C. Catalytic amounts of conc.  $\text{H}_2\text{SO}_4$  (3 drops) and liquid 2-methylpropene (2 mL) were added. The vessel was sealed and shaken at RT for 48 h. The cooled vessel was reopened carefully, the reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (10 mL) and washed with saturated aqueous  $\text{NaHCO}_3$  solution (5 mL). After drying with  $\text{MgSO}_4$  the solvent was removed under reduced pressure to yield **15** (236 mg, 0.64 mmol, quantitative) as colourless fused needles.

M.p.: 151-155 °C.

$[\alpha]_D^{24} -80.8$  (*c* 1.040, CHCl<sub>3</sub>).

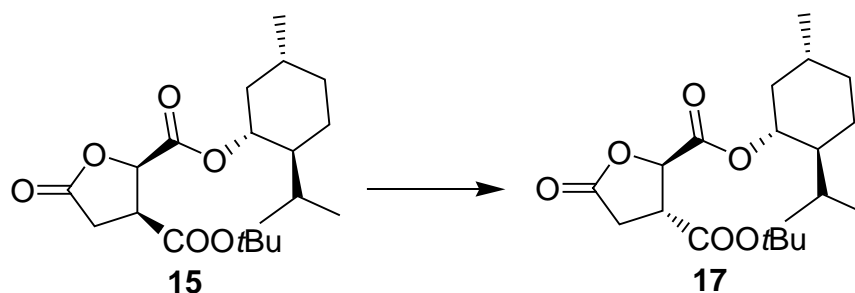
IR (KBr):  $\tilde{\nu} = 2962, 2931, 2859, 1800, 1745, 1718, 1144, 1036$  cm<sup>-1</sup>.

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>):  $\delta = 0.75$  (d, <sup>3</sup>*J* = 6.8 Hz, 3 H, CH<sub>3</sub>), 0.89 (d, <sup>3</sup>*J* = 7.2 Hz, 3 H, CH<sub>3</sub>), 0.91 (d, <sup>3</sup>*J* = 7.2 Hz, 3 H, CH<sub>3</sub>), 0.94-1.10 (m, 3 H), 1.44 (s, 9 H, 3 x CH<sub>3</sub>), 1.38-1.54 (m, 2 H), 1.63-1.74 (m, 2 H), 1.91 (septd, <sup>3</sup>*J* = 7.6, 2.8 Hz, 1 H, CH(CH<sub>3</sub>)<sub>2</sub>), 1.98-2.07 (m, 1 H), 2.65 (dd, <sup>2</sup>*J* = 17.6 Hz, <sup>3</sup>*J* = 9.2 Hz, 1 H, (O)CCH<sub>2</sub>), 3.05 (dd, <sup>2</sup>*J* = 17.6 Hz, <sup>3</sup>*J* = 8.0 Hz, 1 H, (O)CCH<sub>2</sub>), 3.59 (m, 1 H, CH<sub>2</sub>CHCOO), 4.73 (dt, <sup>3</sup>*J* = 11.2, 4.8 Hz, 1 H, OCHCH<sub>2</sub>), 5.00 (d, <sup>3</sup>*J* = 8.0 Hz, 1 H, OCHCOO).

<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):  $\delta = 16.3, 20.9, 22.1$  (3 x CH<sub>3</sub>), 23.3, 26.0, 28.0 (3 x CH<sub>3</sub>), 30.3, 31.5, 34.2, 40.6, 44.4, 47.0, 77.0, 77.3, 83.2 (C(CH<sub>3</sub>)<sub>3</sub>), 167.4, 167.8, 174.5 (3 x CO).

HRMS-ESI: *m/z*: [M+Na]<sup>+</sup> calcd for C<sub>20</sub>H<sub>32</sub>O<sub>6</sub>Na: 391.20911, found: 391.20936.

## 2.15 (2*R*,3*R*)-Isocitric acid lactone-2-carboxylic acid-[(1'*R*,2'*S*,5'*R*)-(-)-menthylester]-3-carboxylic acid-*tert*-butylester) **17**



In a pyrex vessel (2*R*,3*S*)-Isocitric acid lactone-2-carboxylic acid-[(1'*R*,2'*S*,5'*R*)-(-)-menthylester]-3-carboxylic acid-*tert*-butylester **15** (80 mg, 0.22 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (4 mL) and DBU (0.15 mL) was heated to 50 °C for 1 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and washed with saturated aqueous NH<sub>4</sub>Cl solution. After drying with MgSO<sub>4</sub> the solvent was removed under reduced pressure to give an oil which was purified by column chromatography (*n*-hexane-EtOAc 10:1 v/v) to yield **17** (51 mg, 0.14 mmol, 64%) as colourless fused needles and recovered starting material **15** (20 mg, 0.06 mmol, 25%).

M.p.: 74-76 °C.

*R<sub>f</sub>* = 0.20 (*n*-hexane/EtOAc, 10:1 v/v)

$[\alpha]_D^{24} -83.0$  (*c* 2.17; CHCl<sub>3</sub>).

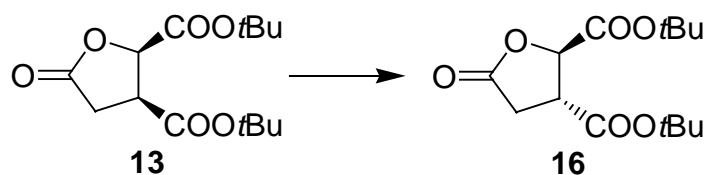
IR (KBr):  $\tilde{\nu} = 2957, 2942, 2871, 1809, 1741, 1723, 1152, 1036$  cm<sup>-1</sup>.

<sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>): δ = 0.76 (d, <sup>3</sup>J = 6.3 Hz, 3 H, CH<sub>3</sub>), 0.89 (d, <sup>3</sup>J = 6.3 Hz, 3 H, CH<sub>3</sub>), 0.91 (d, <sup>3</sup>J = 6.3 Hz, 3 H, CH<sub>3</sub>), 0.91-1.07 (m, 2 H), 1.48 (s, 9 H, 3 x CH<sub>3</sub>), 1.40-1.54 (m, 3 H), 1.67-1.72 (m, 2 H), 1.84 (dtd, <sup>3</sup>J = 13.9, 7.0, 2.8 Hz, 1 H, CHCH(CH<sub>3</sub>)<sub>2</sub>), 1.97-2.03 (m, 1 H), 2.81 (d, <sup>3</sup>J = 8.6 Hz, 1 H, (O)CCH<sub>2</sub>), 2.82 (d, <sup>3</sup>J = 7.0 Hz, 1 H, (O)CCH<sub>2</sub>), 3.31 (ddd, <sup>3</sup>J = 8.6, 7.0, 4.7 Hz, 1 H, CH<sub>2</sub>CHCOO), 4.78 (dt, <sup>3</sup>J = 10.9, 3.9 Hz, 1 H, OCHCH<sub>2</sub>), 5.04 (d, <sup>3</sup>J = 4.7 Hz, 1 H, OCHCOO).

<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>): δ = 16.3, 20.9, 22.0 (3 x CH<sub>3</sub>), 23.4, 26.4, 28.0 (3 x CH<sub>3</sub>), 30.8, 31.5, 34.2, 40.7, 44.6, 46.9, 76.9, 77.8, 83.3 (C(CH<sub>3</sub>)<sub>3</sub>), 168.3, 169.4, 173.9 (3 x CO).

HRMS-ESI (*m/z*): [M+Na]<sup>+</sup> calcd for C<sub>20</sub>H<sub>32</sub>O<sub>6</sub>Na: 391.20911, found: 391.20912.

## 2.16 (2*R*,3*R*)-Isocitric acid lactone-2,3-carboxylic acid di-*tert*-butylester **16**



In a Pyrex vessel (2*R*,3*S*)-isocitric acid lactone-2,3-carboxylic acid di-*tert*-butylester **13** (286 mg, 1.0 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (4 mL) and DBU (0.75 mL) were heated to 50 °C for 30 min. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (10 mL) and washed with saturated aqueous NH<sub>4</sub>Cl solution (5 mL). After drying with MgSO<sub>4</sub> the solvent was removed under reduced pressure to give an oil which was purified by column chromatography (*n*-hexane-EtOAc 10:1 v/v) to yield **16** (185 mg, 0.65 mmol, 65%) as a colourless oil and recovered starting material **13** (42 mg, 0.15 mmol, 15%).

*R<sub>f</sub>* = 0.32 (*n*-hexane/EtOAc 5:1 v/v).

[α]<sub>D</sub><sup>24</sup> -44.7 (*c* 1.21, CHCl<sub>3</sub>).

IR (KBr):  $\tilde{\nu}$  = 2981, 2936, 1799, 1732, 1370, 1145, 841 cm<sup>-1</sup>.

<sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>): δ = 1.48 (s, 9 H, 3 x CH<sub>3</sub>), 1.50 (s, 9 H, 3 x CH<sub>3</sub>), 2.80 (d, <sup>3</sup>J = 7.9 Hz, 1 H, CH<sub>2</sub>), 2.81 (d, <sup>3</sup>J = 7.5 Hz, 1 H, CH<sub>2</sub>), 3.30 (ddd, <sup>3</sup>J = 7.9, 7.5, 4.9 Hz, 1 H, CH<sub>2</sub>CH), 4.94 (d, <sup>3</sup>J = 4.9 Hz, 1 H, CH(O)).

<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>): δ = 28.0 (3 x CH<sub>3</sub>), 28.1 (3 x CH<sub>3</sub>), 30.8 (CH<sub>2</sub>COO), 44.6 (CH<sub>2</sub>CH), 78.2 (CH(O)), 83.2 (C(CH<sub>3</sub>)<sub>3</sub>), 83.8 (C(CH<sub>3</sub>)<sub>3</sub>), 167.7, 169.4, 174.1 (3 x CO).

HRMS-ESI (*m/z*): [M+Na]<sup>+</sup> calcd for C<sub>20</sub>H<sub>32</sub>O<sub>6</sub>Na: 309.13086, found: 309.13103.

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### 3. References

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- [2] W. E. Donaldson, R. F. McCleary, E. F. Degering, *J. Am. Chem. Soc.* **1934**, *56*(2), 459-460.
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### 4. NMR-Spectra

P.Heretsch; PH8-2B2

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Solvent: DMSO  
Ambient temperature  
GEMINI-300BB "jutta"

PULSE SEQUENCE

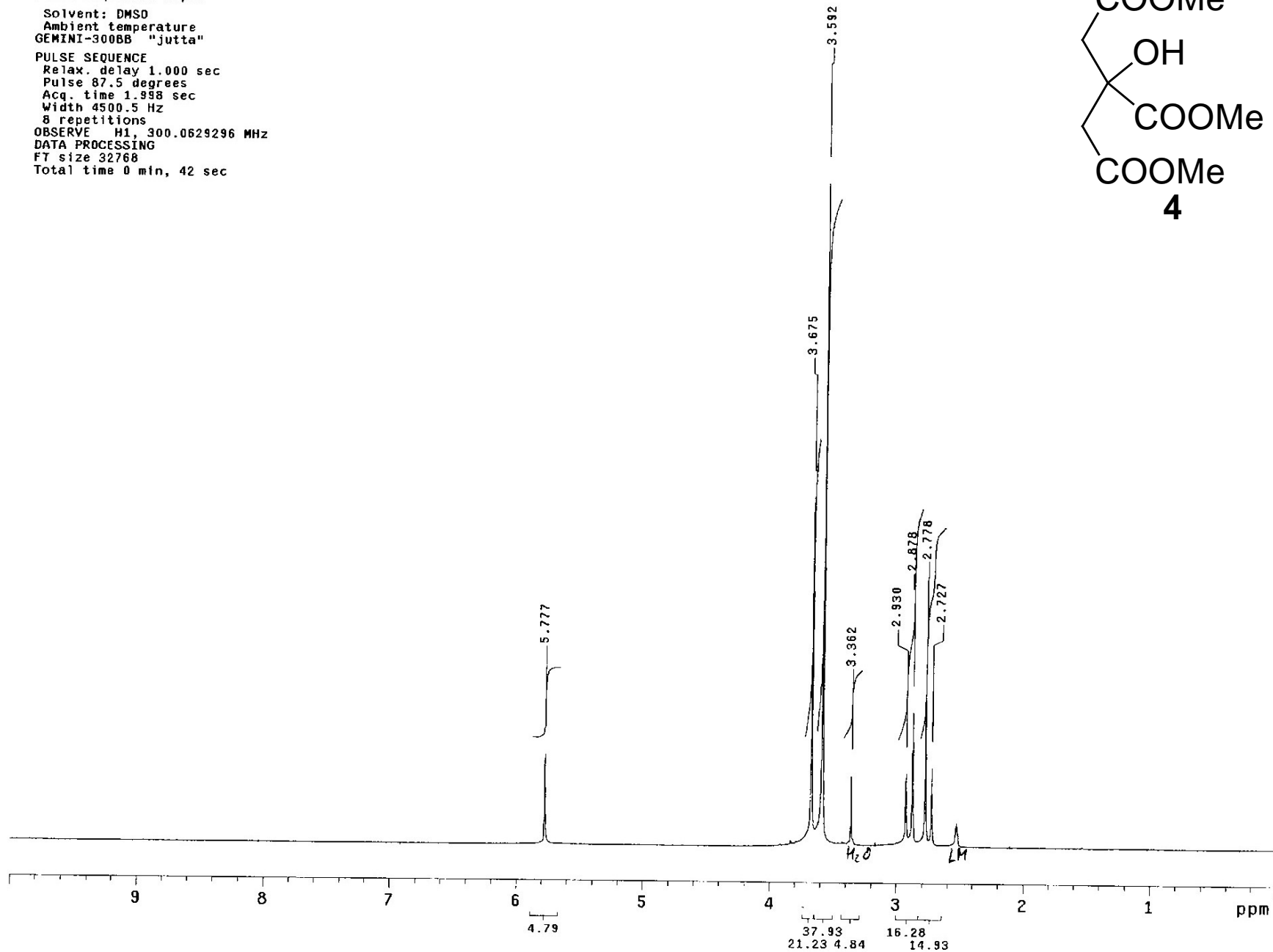
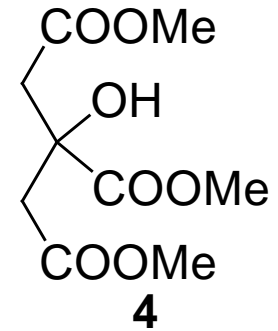
Relax. delay 1.000 sec  
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Acq. time 1.998 sec  
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8 repetitions

OBSERVE H1, 300.0629296 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 42 sec





P.Heretsch; PH 8-2B3

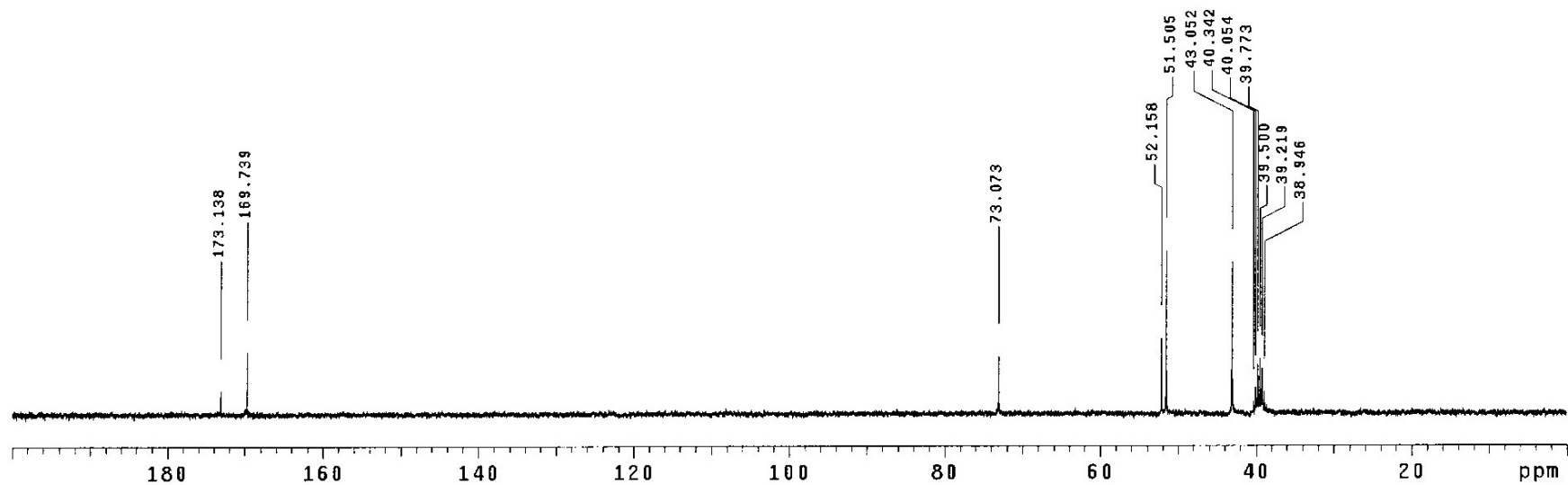
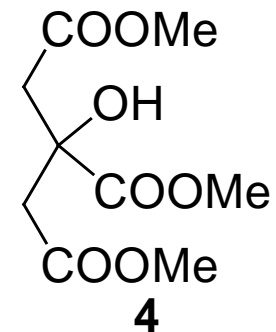
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Ambient temperature  
GEMINI-300BB "Jutta"

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Acq. time 1.500 sec  
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48 repetitions

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DECOUPLE H1, 300.0644431 MHz  
Power 36 dB  
continuously on  
WALTZ-16 modulated

DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 65536  
Total time 1 hr, 3 min, 2 sec



P. Heretsch; PH8-6

Pulse Sequence: s2pu1

Solvent: DMSO

Ambient temperature

GEMINI-300BB "jutta"

PULSE SEQUENCE

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Pulse 87.5 degrees

Acq. time 1.998 sec

Width 7000.0 Hz

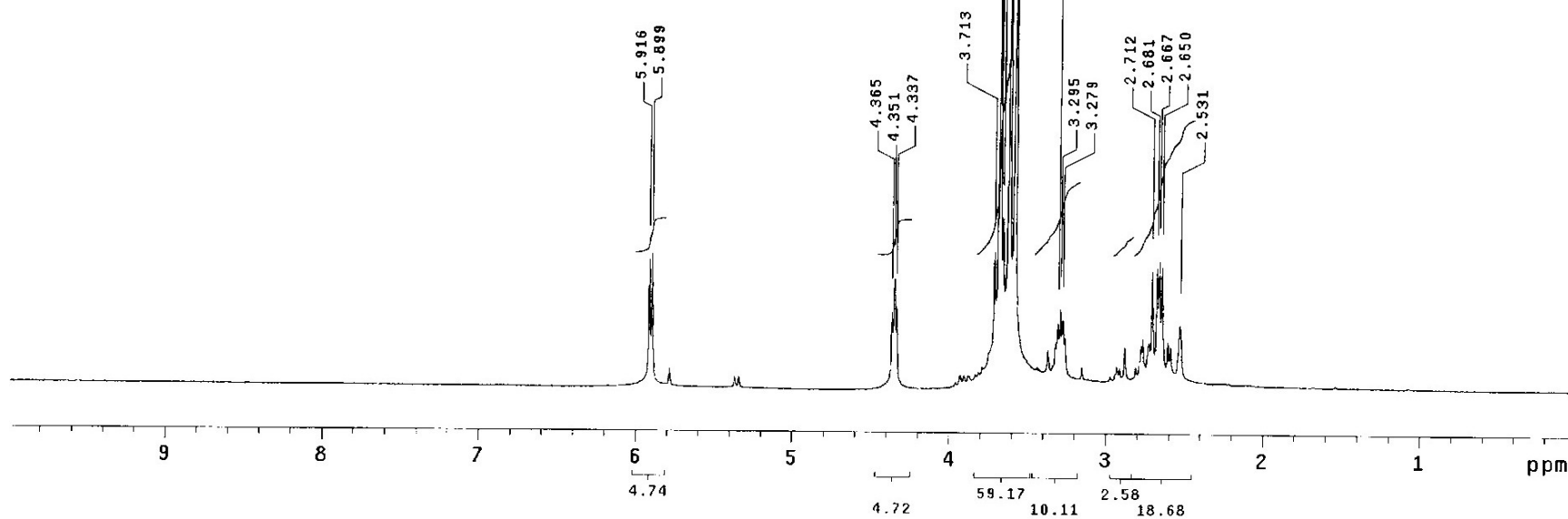
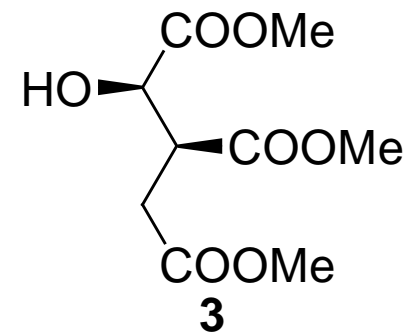
8 repetitions

OBSERVE H1, 300.0629296 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 45 sec



P.Heretsch; PH8-6

Pulse Sequence: s2pul

Solvent: DMSO  
Ambient temperature  
GEMINI-300BB "jutta"

PULSE SEQUENCE

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Acq. time 1.500 sec  
Width 18761.7 Hz  
96 repetitions

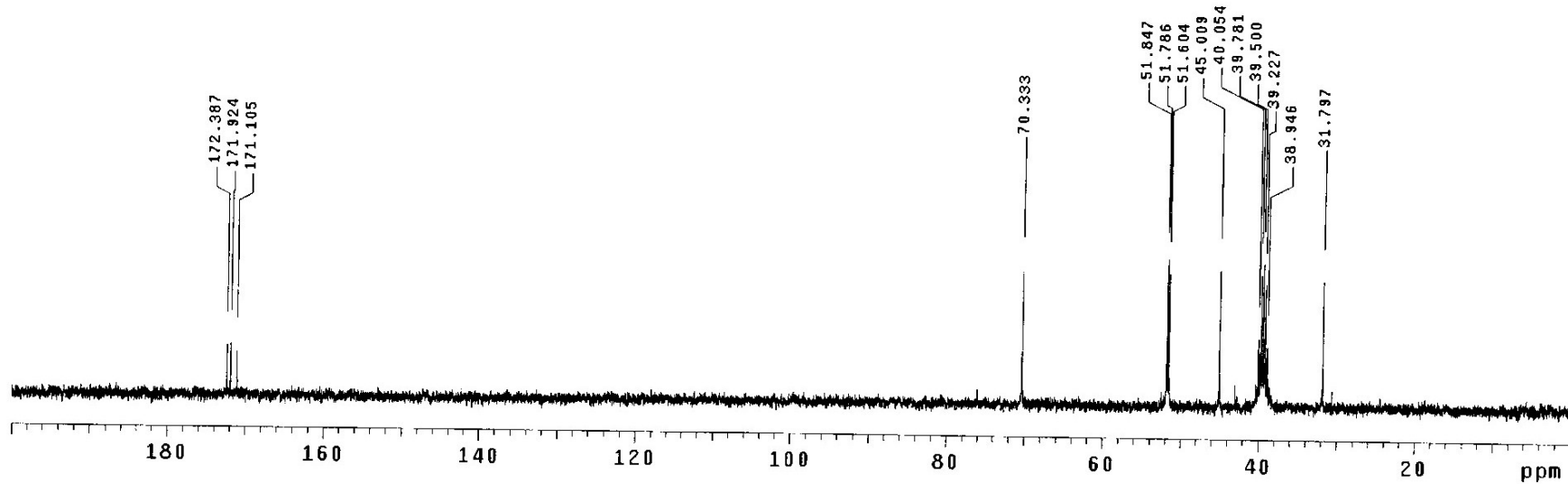
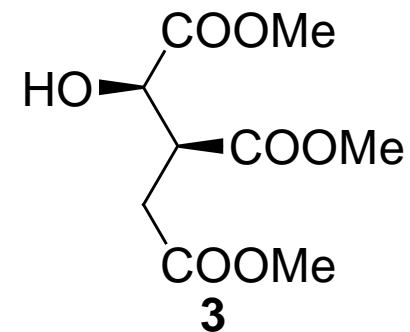
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Power 36 dB

continuously on  
WALTZ-16 modulated

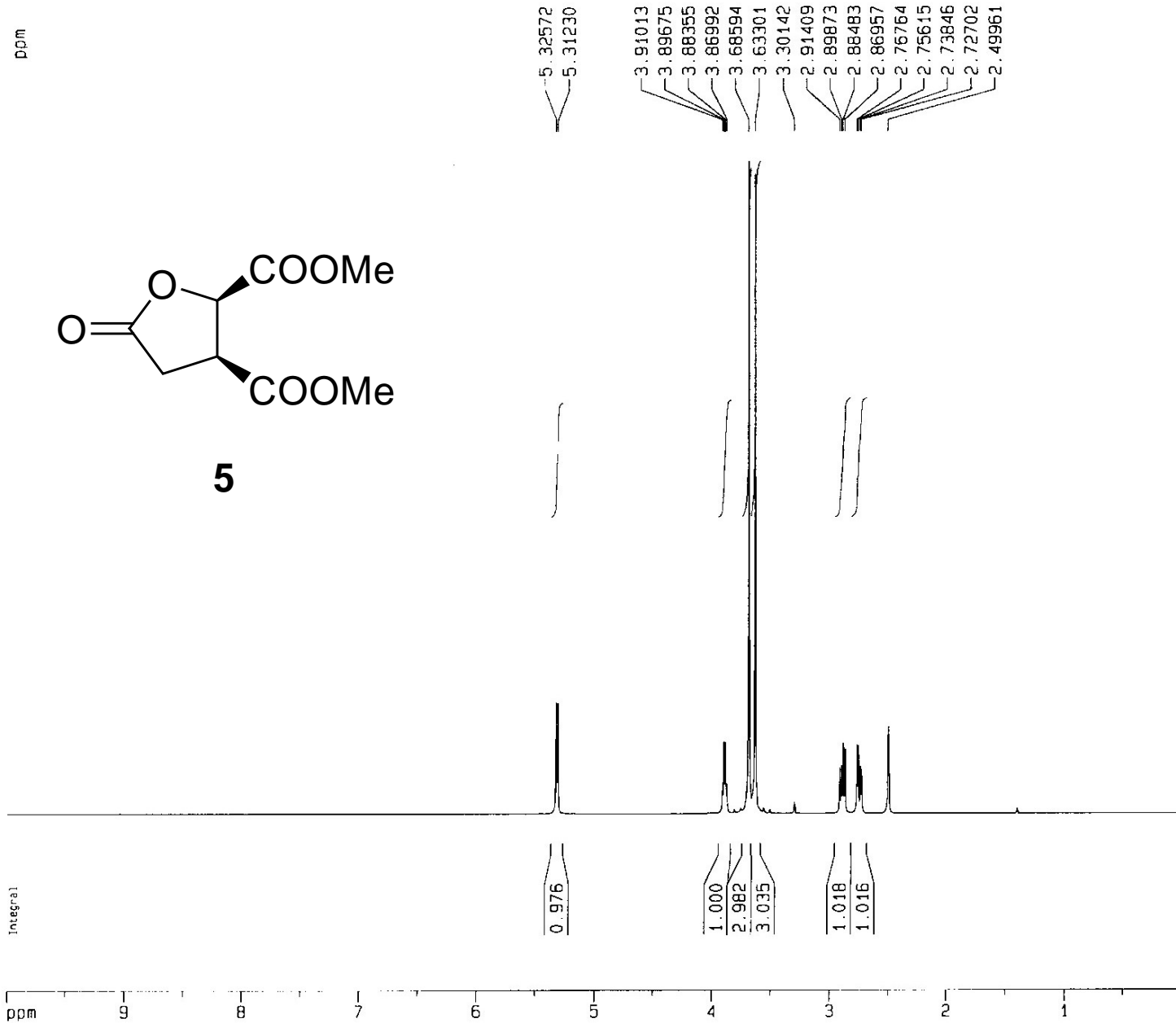
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Ph. Heretsch; PH8-26; 1H



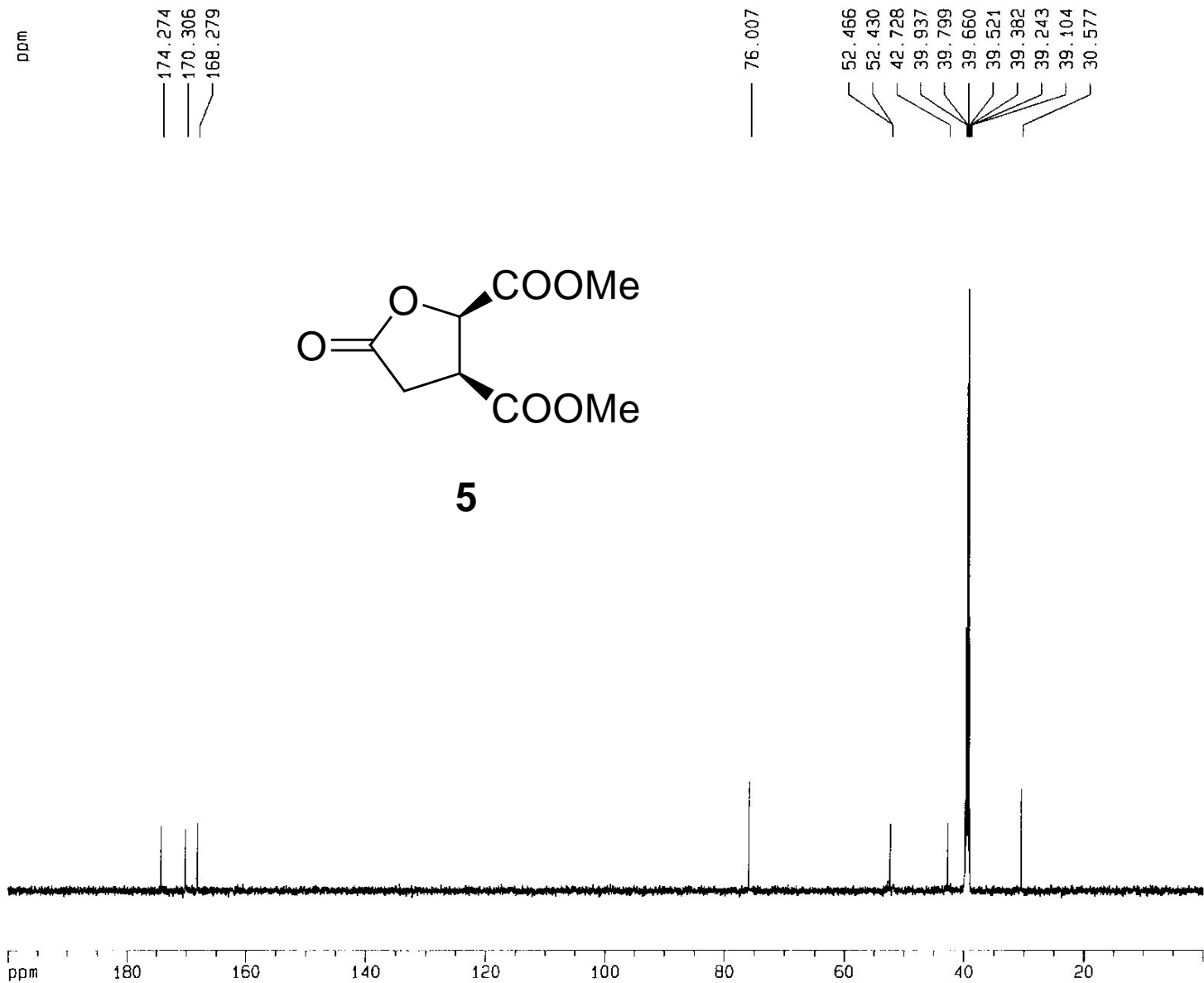
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TD 32768  
SOLVENT DMSO  
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DS 4  
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AQ 1.9137596 sec  
RG 574.7  
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DE 6.00 usec  
TE 300.0 K  
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MCREST 0.00000000 sec  
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GB 0  
PC 1.00

1D NMR plot parameters  
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F2P 0.000 ppm  
F2 0.00 Hz  
PPMCM 0.50000 ppm/cm  
HZCM 300.06500 Hz/cm



Current Data Parameters  
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 EXPNO 2  
 PROCNO 1

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 TE 300.0 K  
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 MCREST 0.0000000 sec  
 MCWPK 0.0150000 sec

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 PL1 0.00 dB  
 SF01 150.9178393 MHz

===== CHANNEL f2 =====  
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 NUC2 1H  
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 PL12 20.00 dB  
 SF02 600.1324005 MHz

F2 - Processing parameters  
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 SSB 0  
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 GB 0  
 PC 1.40

1D NMR plot parameters  
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 CY 10.00 cm  
 F1P 200.000 ppm  
 F1 30180.56 Hz  
 F2P 0.000 ppm  
 F2 0.00 Hz  
 PPMCM 10.00000 ppm/cm  
 HZCM 1509.02881 Hz/cm

nomas

Sample: FT-IC 40

Pulse Sequence: s2pul

Date: Sep 10 2007

Solvent: dmsc

Temp. 26.0 C / 299.1 K

Operator: walkup

Mercury-400BB "felix"

Relax. delay 1.000 sec

Pulse 30.0 degrees

Acq. time 1.996 sec

Width 6402.0 Hz

16 repetitions

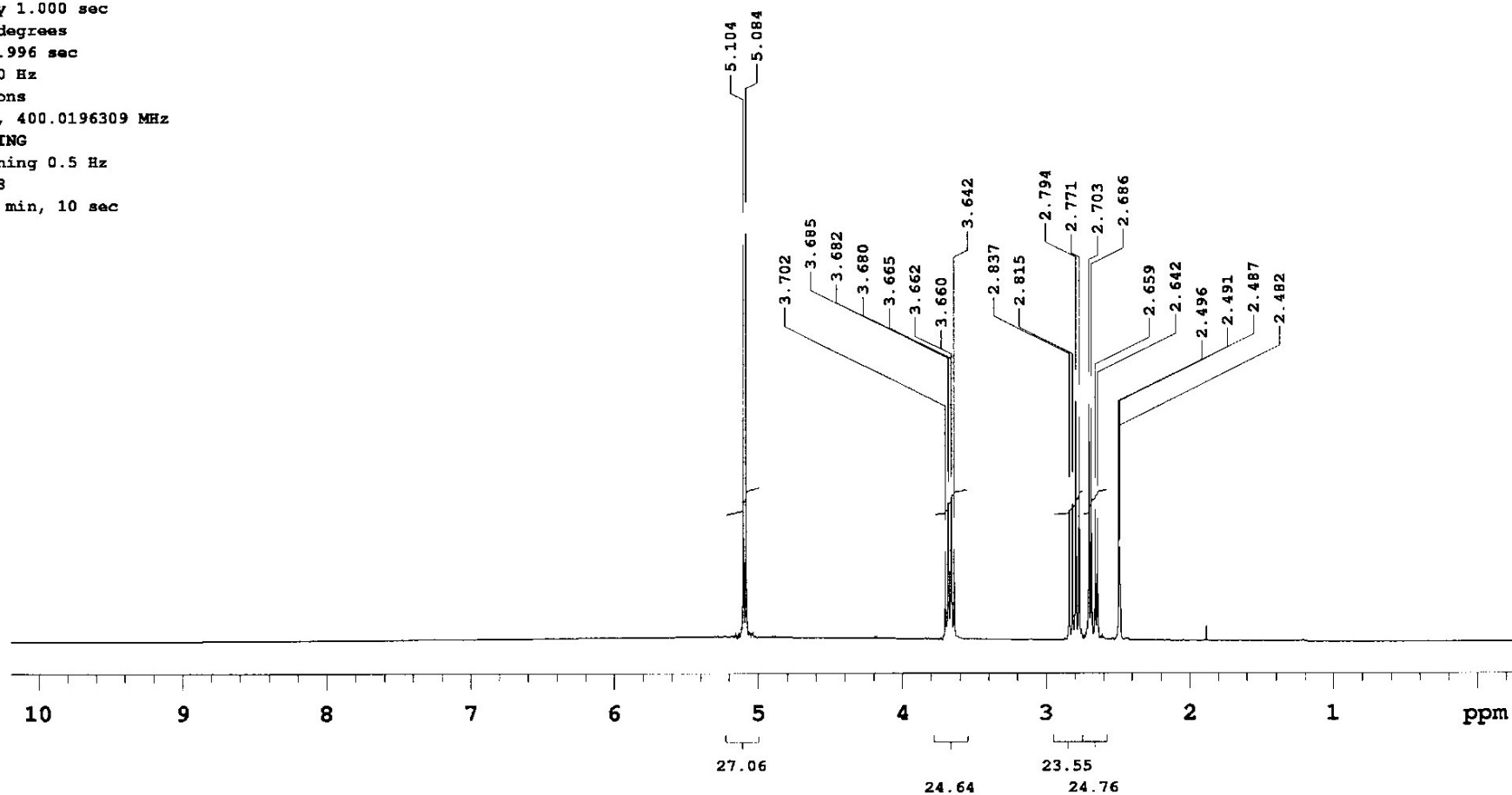
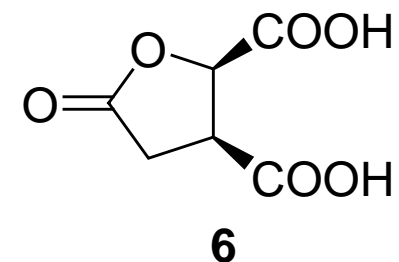
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DATA PROCESSING

Line broadening 0.5 Hz

FT size 32768

Total time 1 min, 10 sec



F. Thomas

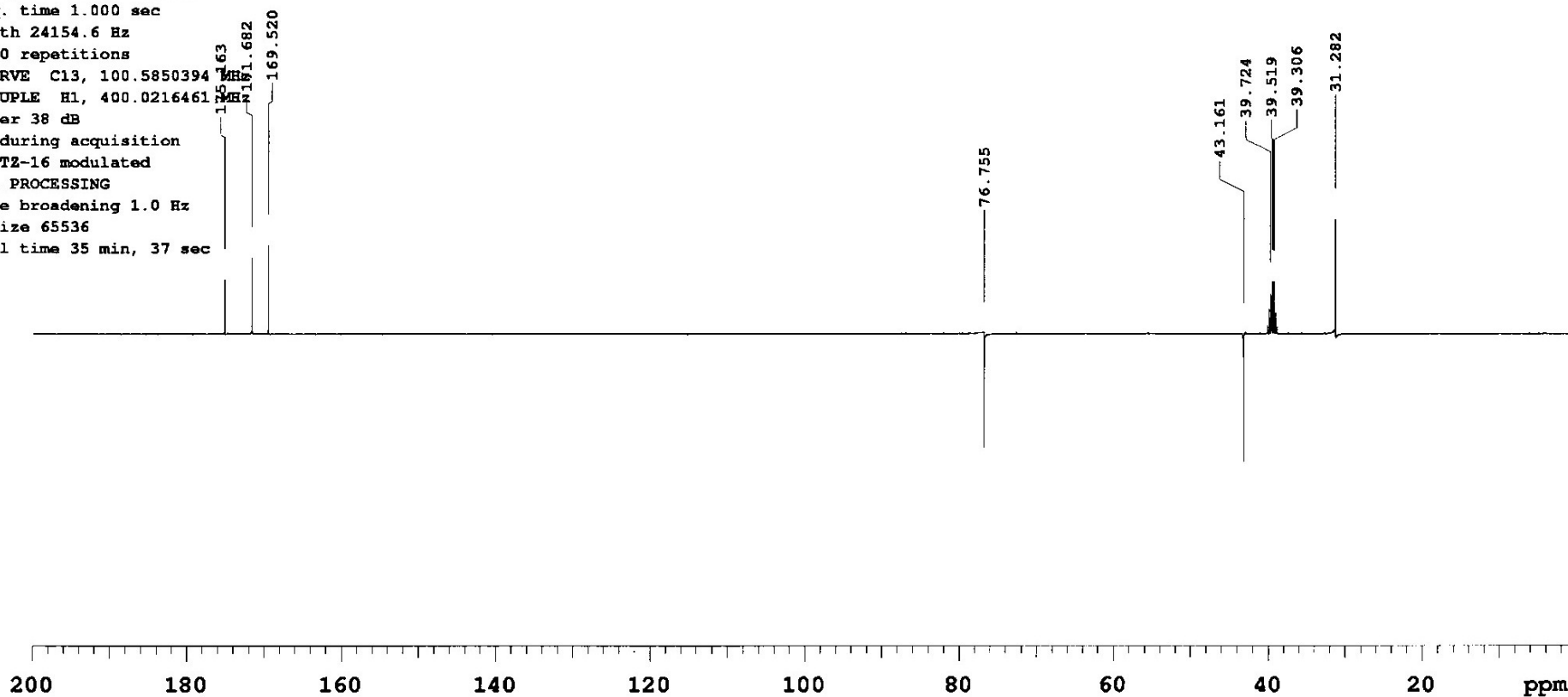
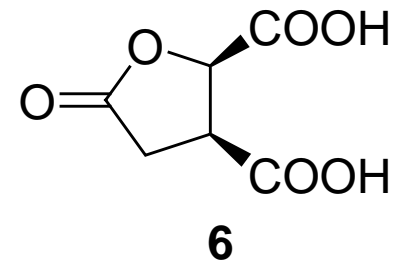
Sample: FT-IC 40

Pulse Sequence: APT

Date: Sep 11 2007

Solvent: dmsc  
Temp. 26.0 C / 299.1 K  
Operator: walkup  
Mercury-400BB "felix"

Relax. delay 1.000 sec  
1st pulse 90.0 degrees  
2nd pulse 135.0 degrees  
Acq. time 1.000 sec  
Width 24154.6 Hz  
1000 repetitions  
OBSERVE C13, 100.5850394  
DECOUPLE H1, 400.0216461  
Power 38 dB  
on during acquisition  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 65536  
Total time 35 min, 37 sec

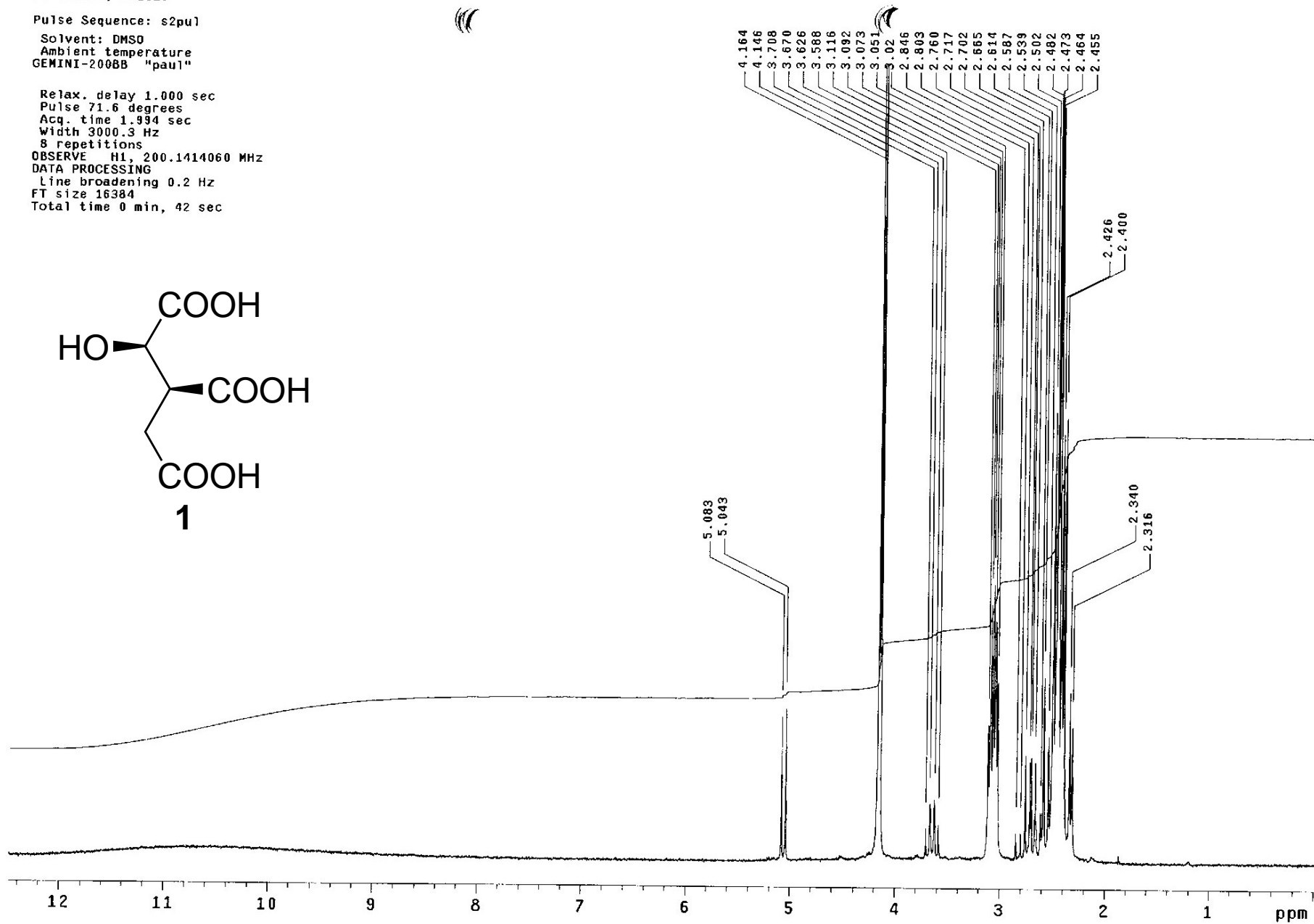
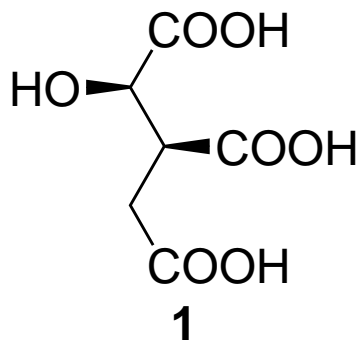


F. Thomas; FTIC25

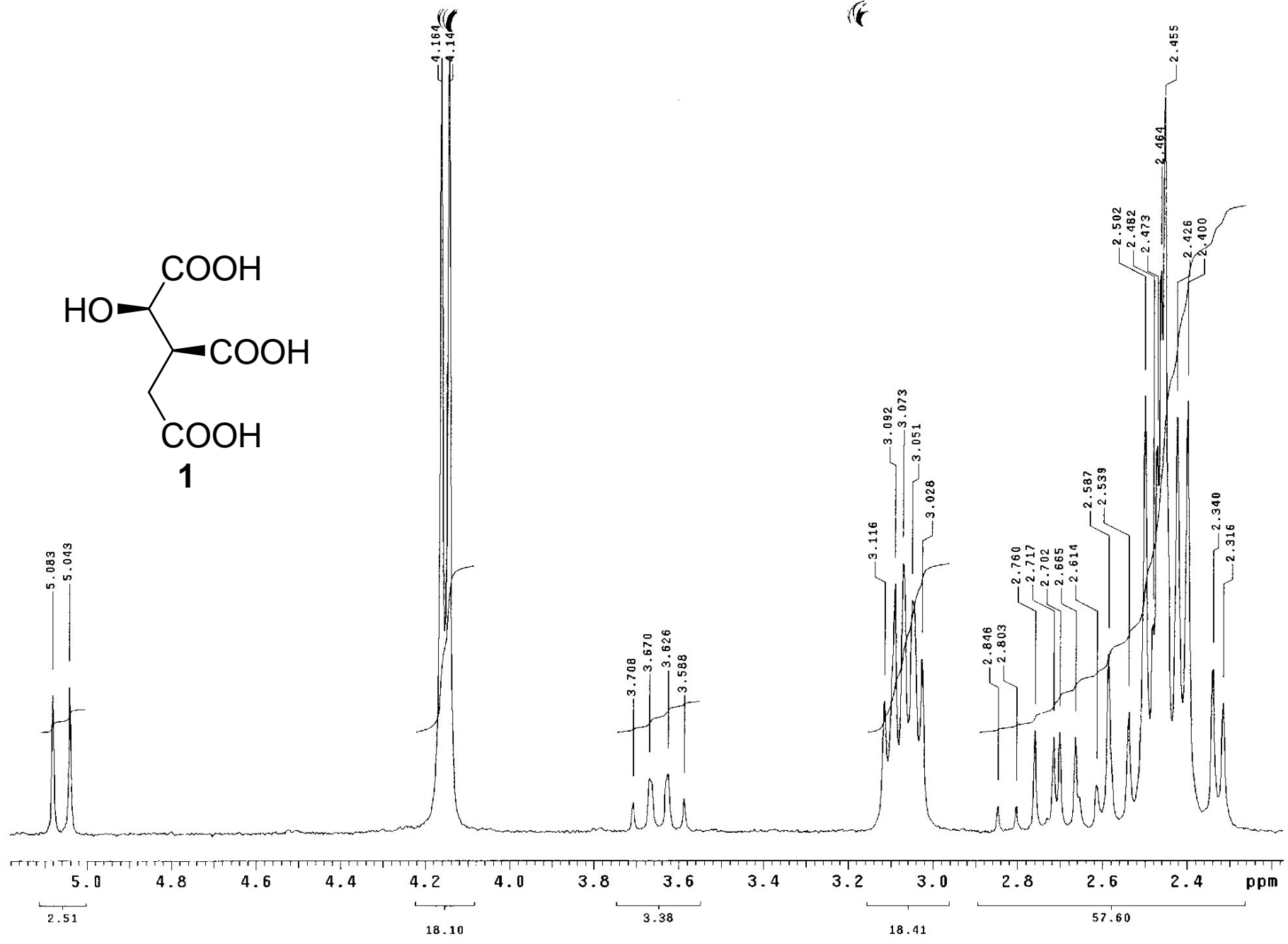
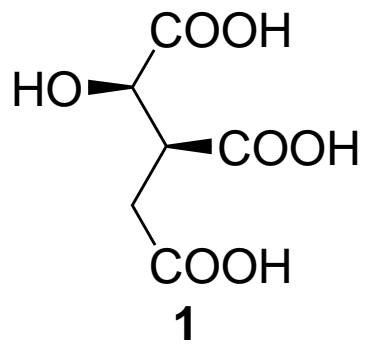
Pulse Sequence: s2pu1

Solvent: DMSO  
Ambient temperature  
GEMINI-200BB "pau1"

Relax. delay 1.000 sec  
Pulse 71.6 degrees  
Acq. time 1.994 sec  
Width 3000.3 Hz  
8 repetitions  
OBSERVE H1, 200.1414060 MHz  
DATA PROCESSING  
Line broadening 0.2 Hz  
FT size 16384  
Total time 0 min, 42 sec







F. Thomas; FTIC25

Pulse Sequence: s2pu1

Solvent: DMSO

Ambient temperature

GEMINI-200BB "paul"

Relax. delay 1.000 sec

Pulse 65.0 degrees

Acq. time 1.498 sec

Width 12500.0 Hz

13936 repetitions

OBSERVE C13, 50.3256143 MHz

DECOUPLE H1, 200.1424037 MHz

Power 36 dB

continuously on

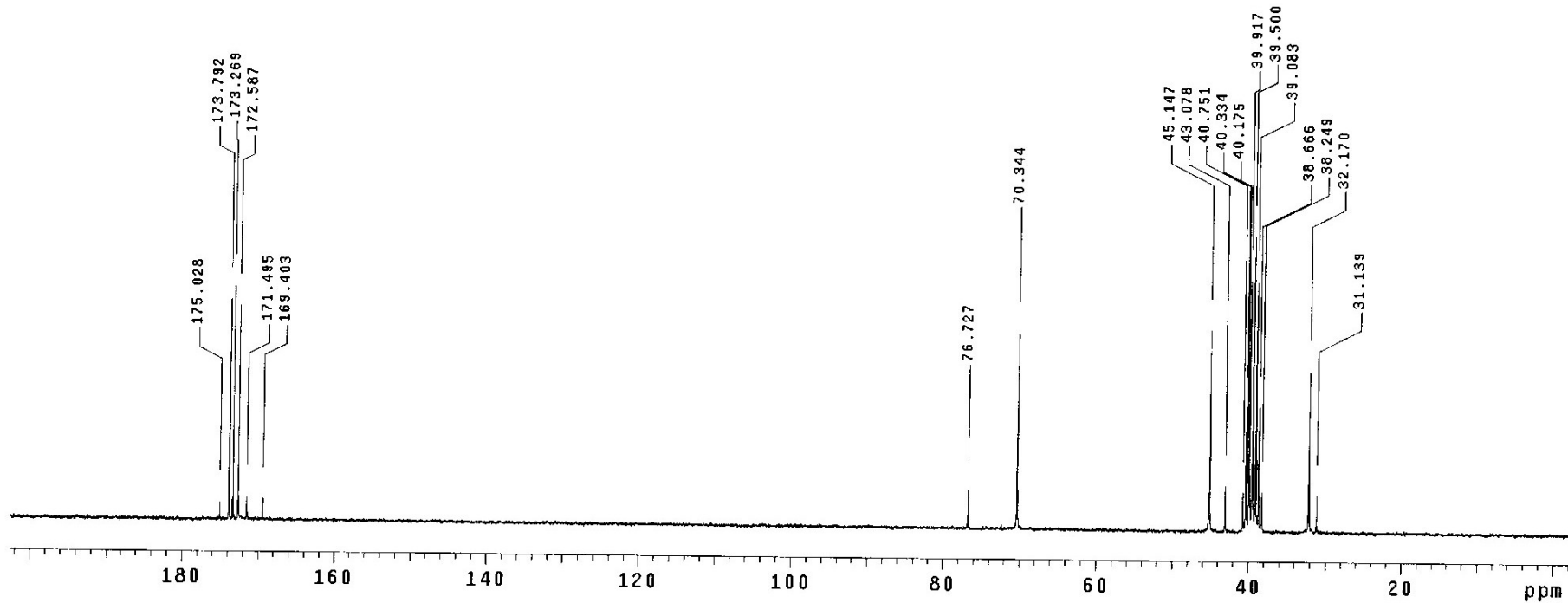
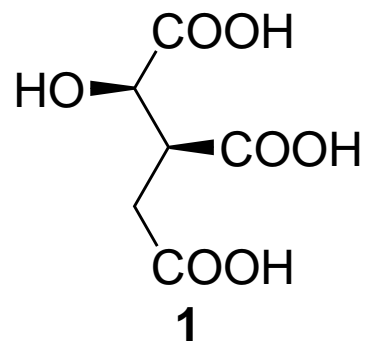
WALTZ-16 modulated

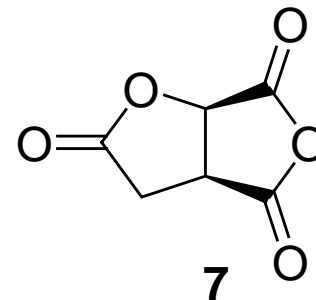
DATA PROCESSING

Line broadening 1.0 Hz

FT size 65536

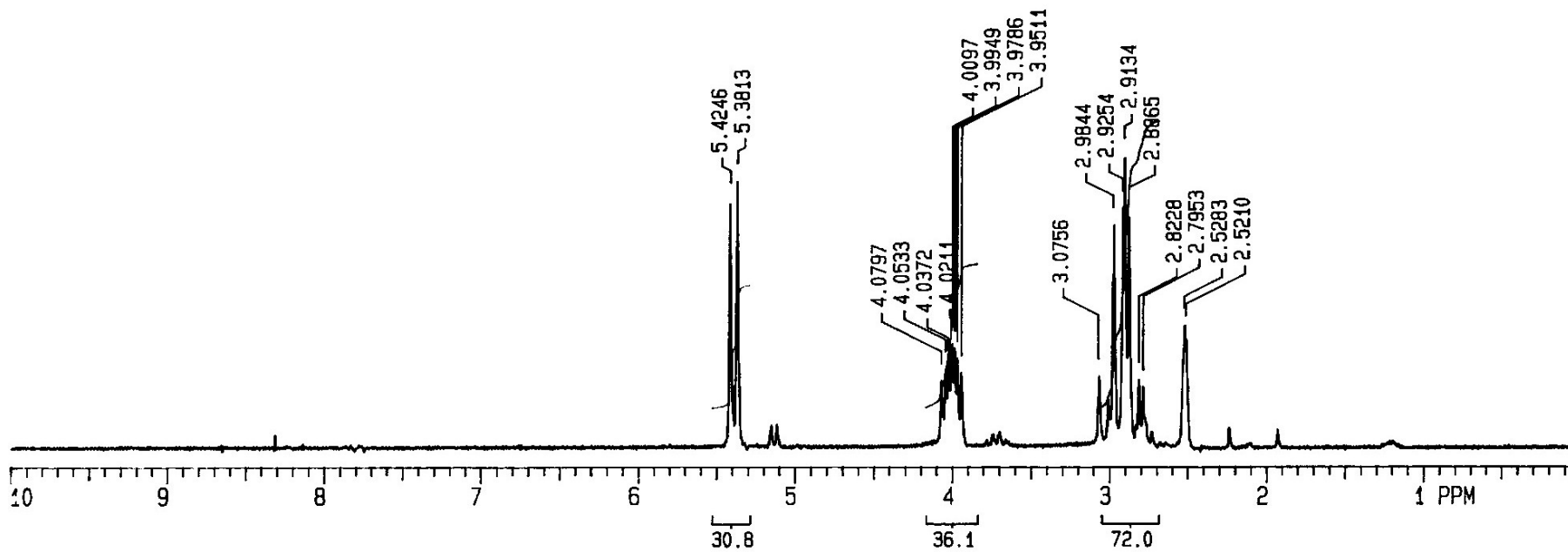
Total time 15 hr, 46 min, 2 sec

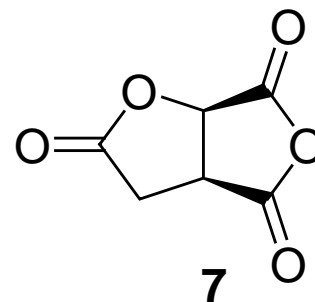




HERETSCH  
PH8-5E

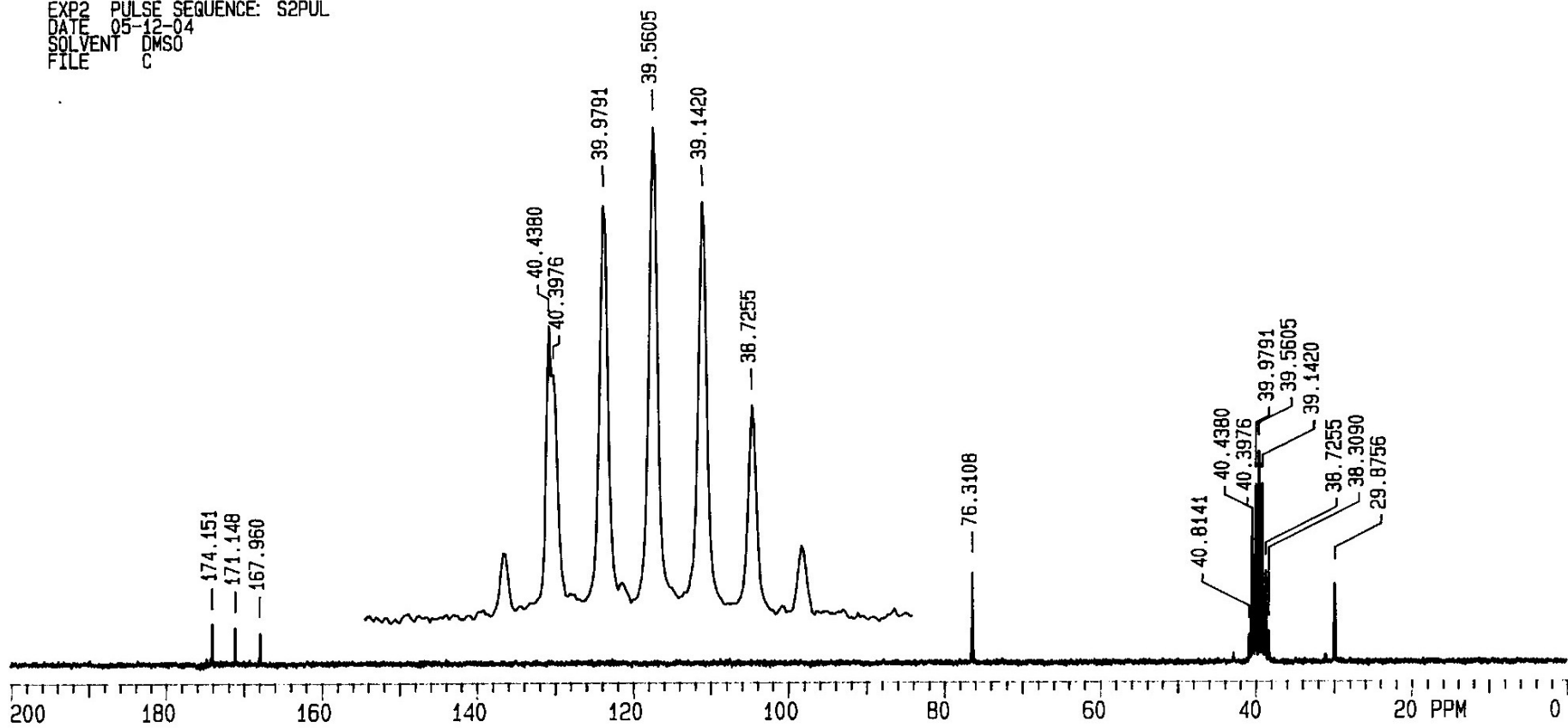
EXP1 PULSE SEQUENCE: S2PUL  
DATE 05-12-04  
SOLVENT DMSO  
FILE H



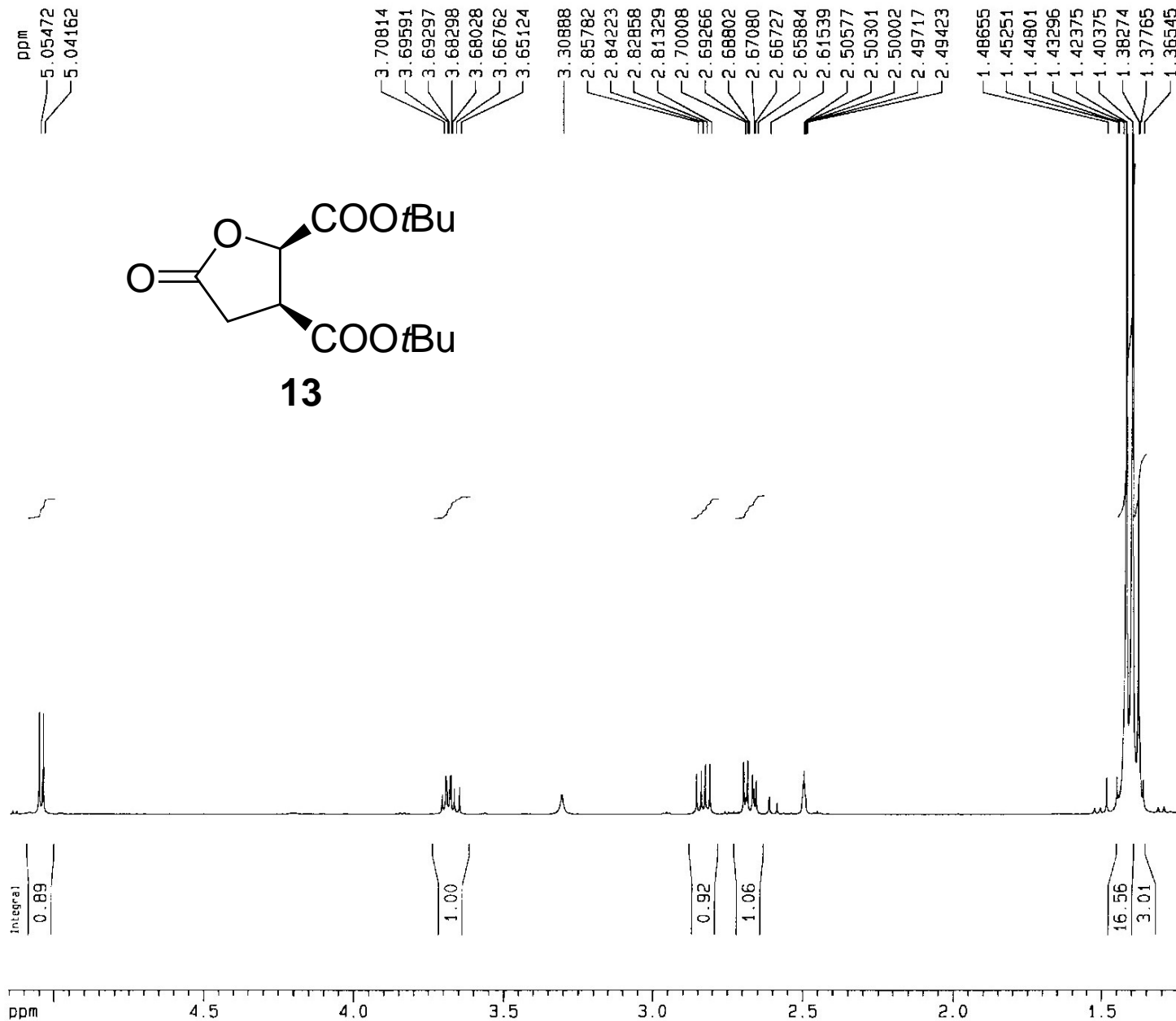


HERETSCH  
PH8-5E

EXP2 PULSE SEQUENCE: S2PUL  
DATE 05-12-04  
SOLVENT DMSO  
FILE C



Ph. Heretsch; PH 9C; 1H



Current Data Parameters  
NAME org351  
EXPNO 1  
PROCNO 1

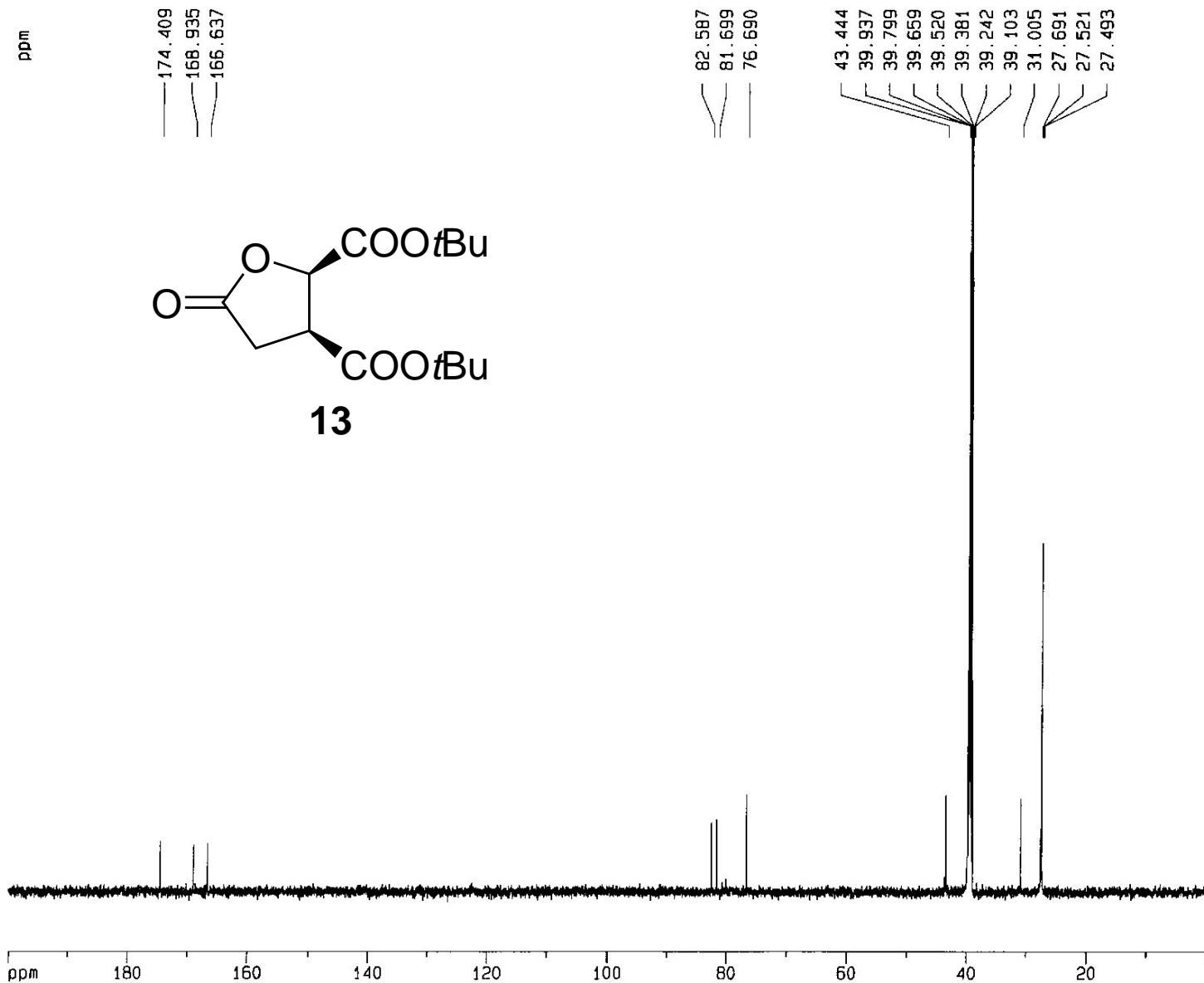
F2 - Acquisition Parameters  
Date\_ 20051011  
Time 9.49  
INSTRUM spect  
PROBHD 5 mm TBI 1H/13  
PULPROG zg30  
TD 32768  
SOLVENT DMSO  
NS 8  
DS 4  
SWH 8561.644 Hz  
FIDRES 0.261281 Hz  
AQ 1.9137596 sec  
RG 161.3  
DW 58.400 usec  
DE 6.00 usec  
TE 300.0 K  
D1 2.0000000 sec  
MCREST 0.0000000 sec  
MCWRK 0.0150000 sec

===== CHANNEL f1 =====  
NUC1 1H  
P1 7.30 usec  
PL1 5.00 dB  
SF01 600.1339172 MHz

F2 - Processing parameters  
SI 16384  
SF 600.1300069 MHz  
WDW EM  
SSB 0  
LB 0.10 Hz  
GB 0  
PC 1.00

1D NMR plot parameters  
CX 20.00 cm  
CY 25.00 cm  
F1P 5.153 ppm  
F1 3092.27 Hz  
F2P 1.240 ppm  
F2 744.44 Hz  
PPMCM 0.19561 ppm/cm  
HZCM 117.39184 Hz/cm

Ph. Heretsch; PH 9C



Current Data Parameters

NAME org351  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters

Date\_ 20051011  
Time 10.06  
INSTRUM spect  
PROBHD 5 mm TBI 1H/13  
PULPROG zgdc  
TO 65536  
SOLVENT DMSO  
NS 256  
DS 4  
SWH 37593.984 Hz  
FIDRES 0.573639 Hz  
AQ 0.8716921 sec  
RG 3649.1  
DW 13.300 usec  
DE 6.00 usec  
TE 300.0 K  
D1 3.0000000 sec  
d11 0.0300000 sec  
MCREST 0.0000000 sec  
MCWAK 0.0150000 sec

----- CHANNEL f1 -----

NUC1 13C  
P1 4.00 usec  
PL1 0.00 dB  
SF01 150.9178393 MHz

----- CHANNEL f2 -----

CPDPRG2 waltz16  
NUC2 1H  
PCPD2 80.00 usec  
PL2 20.00 dB  
PL12 20.00 dB  
SF02 600.1324005 MHz

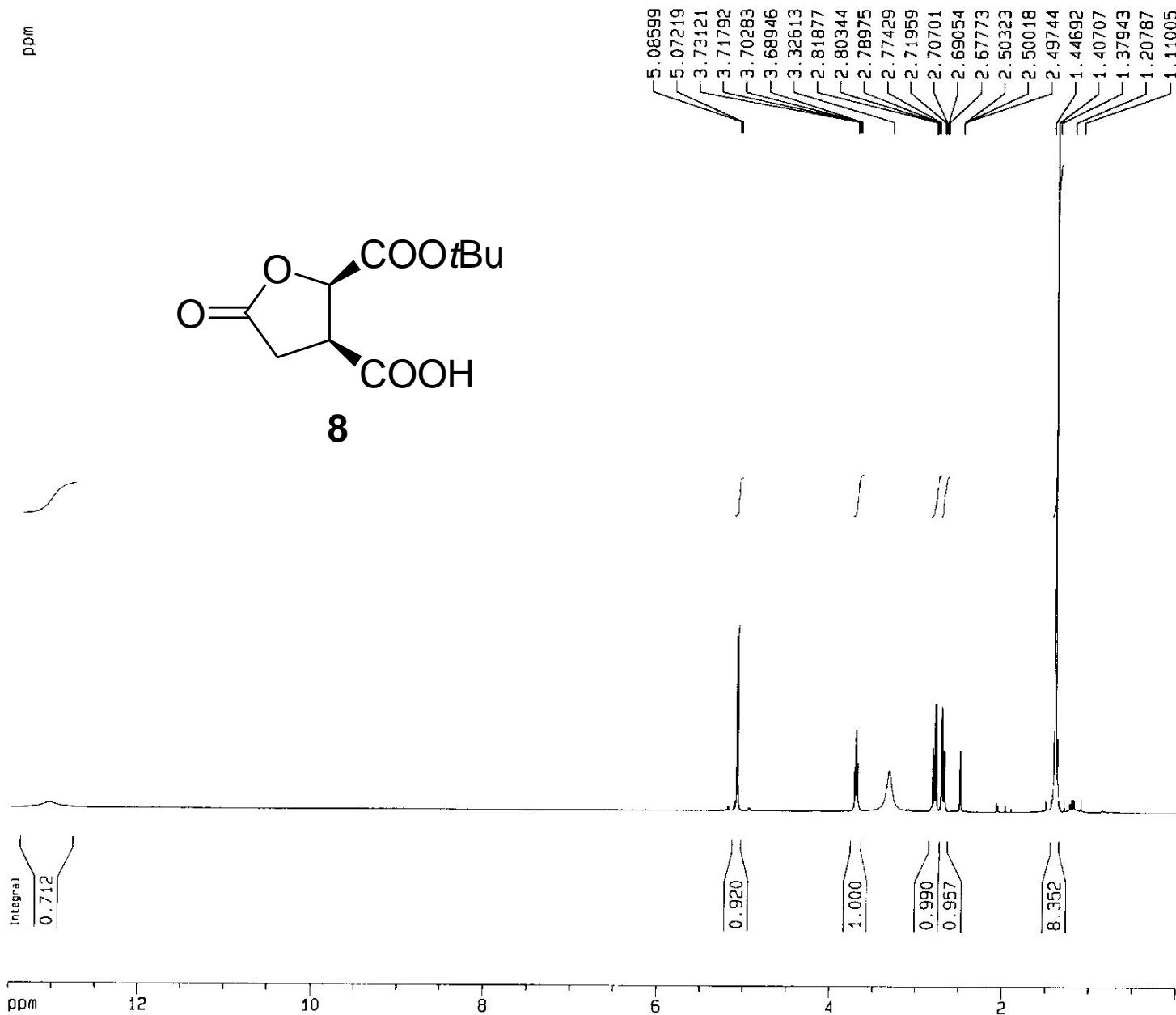
F2 - Processing parameters

SI 32768  
SF 150.9028878 MHz  
WDW EM  
SSB 0  
LB 2.00 Hz  
GB 0  
PC 1.40

1D NMR plot parameters

CX 20.00 cm  
CY 25.00 cm  
F1P 200.000 ppm  
F1 30180.58 Hz  
F2P 0.000 ppm  
F2 0.00 Hz  
PPMCH 10.00000 ppm/cm  
HZCM 1509.02893 Hz/cm

Ph. Her ch; PH 8C; 1H



Current Data Parameters

NAME org350  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters

Date\_ 20051010  
Time 17.23  
INSTRUM spect  
PROBHD 5 mm TBI 1H/13  
PULPROG zg30  
TD 32768  
SOLVENT DMSO  
NS 8  
DS 4  
SWH 8561.644 Hz  
FIDRES 0.261281 Hz  
AQ 1.9137536 sec  
RG 161.3  
DW 58.400 usec  
DE 6.00 usec  
TE 300.0 K  
D1 2.00000000 sec  
MCREST 0.00000000 sec  
MCWAK 0.01500000 sec

==== CHANNEL f1 =====

NUC1 1H  
P1 7.30 usec  
PL1 5.00 dB  
SF01 600.1339172 MHz

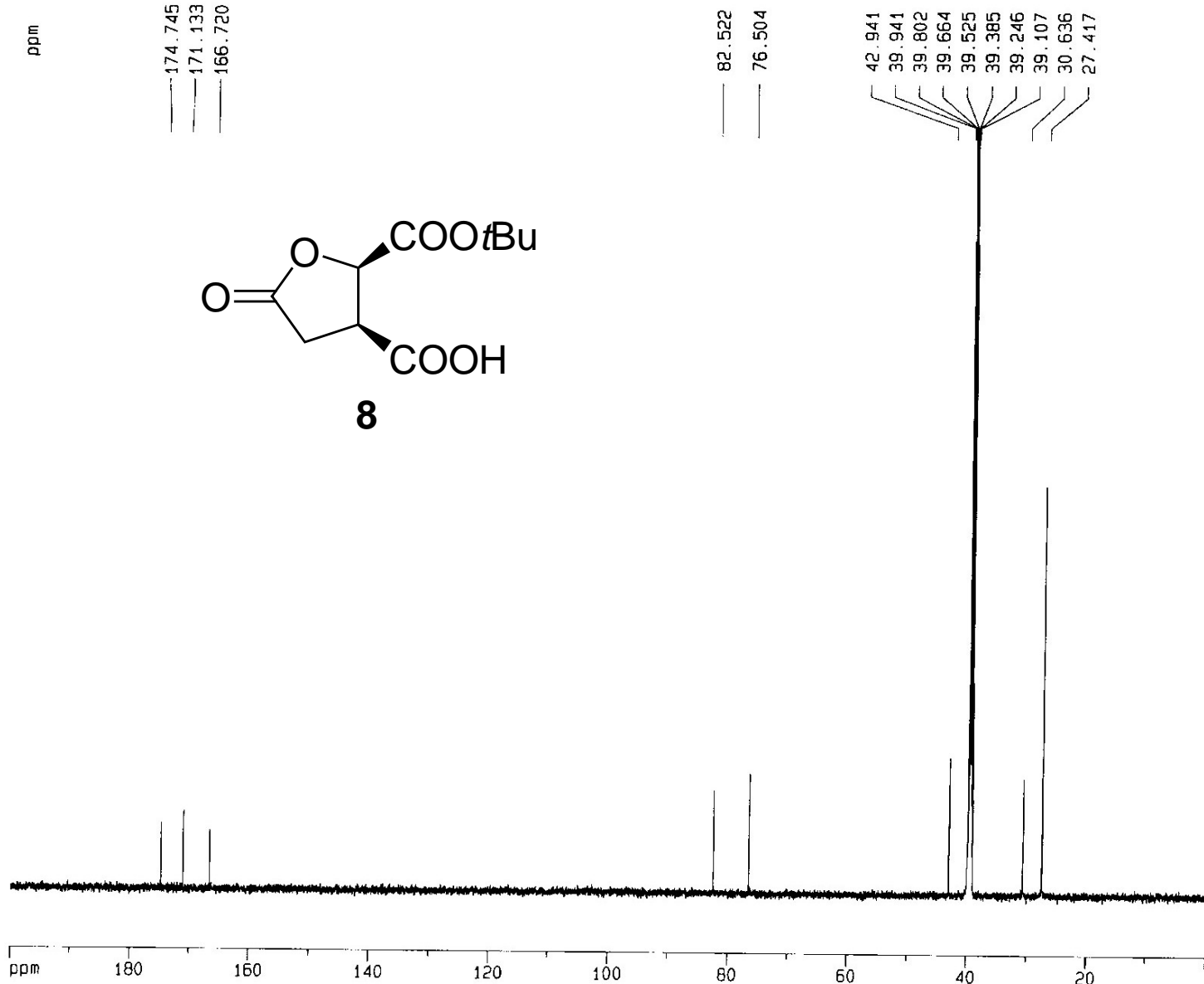
F2 - Processing parameters

SI 16384  
SF 600.1300069 MHz  
WDW EM  
SSB 0  
LB 0.10 Hz  
GB 0  
PC 1.00

1D NMR plot parameters

CX 20.00 cm  
CY 40.00 cm  
F1P 13 500 ppm  
F1 8101.75 Hz  
F2P 0.000 ppm  
F2 0.00 Hz  
PPMCM 0.67500 ppm/cm  
HZCM 405.08777 Hz/cm

Ph. Heretsch; PH 8C; 13C



Current Data Parameters  
NAME org350  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20051010  
Time 17.31  
INSTRUM spect  
PROBHD 5 mm TBI 1H/13  
PULPROG zgdc  
TD 65536  
SOLVENT DMSO  
NS 512  
DS 4  
SWH 37593.984 Hz  
FIDRES 0.573639 Hz  
AQ 0.8716921 sec  
RG 2298.8  
DW 13.300 usec  
DE 6.00 usec  
TE 300.0 K  
D1 3.0000000 sec  
d11 0.0300000 sec  
MCREST 0.0000000 sec  
MCWAK 0.0150000 sec

===== CHANNEL f1 =====  
NUC1 13C  
P1 4.00 usec  
PL1 0.00 dB  
SFO1 150.9178393 MHz

===== CHANNEL f2 =====  
CPDPRG2 waltz16  
NUC2 1H  
PCPD2 80.00 usec  
PL2 20.00 dB  
PL12 20.00 dB  
SFO2 500.1324005 MHz

F2 - Processing parameters  
SI 32768  
SF 150.9028832 MHz  
WDW EM  
SSB 0  
LB 2.00 Hz  
GB 0  
PC 1.40

1D NMR plot parameters  
CX 20.00 cm  
CY 25.00 cm  
F1P 200.000 ppm  
F1 30180.58 Hz  
F2P 0.000 ppm  
F2 0.00 Hz  
PPMCM 10.00000 ppm/cm  
HZCM 1509.02881 Hz/cm



Std Proton parameters

Automation directory:

Sample : Thomas; FT-IC-4B

Pulse Sequence: s2pul

Solvent: dmsc

Temp. 26.0 C / 299.1 K

Operator: vnmr1

Mercury-400BB "localhost"

Relax. delay 1.331

Pulse 30.0 degrees

Acq. time 1.998 sec

Width 6402.0 Hz

8 repetitions

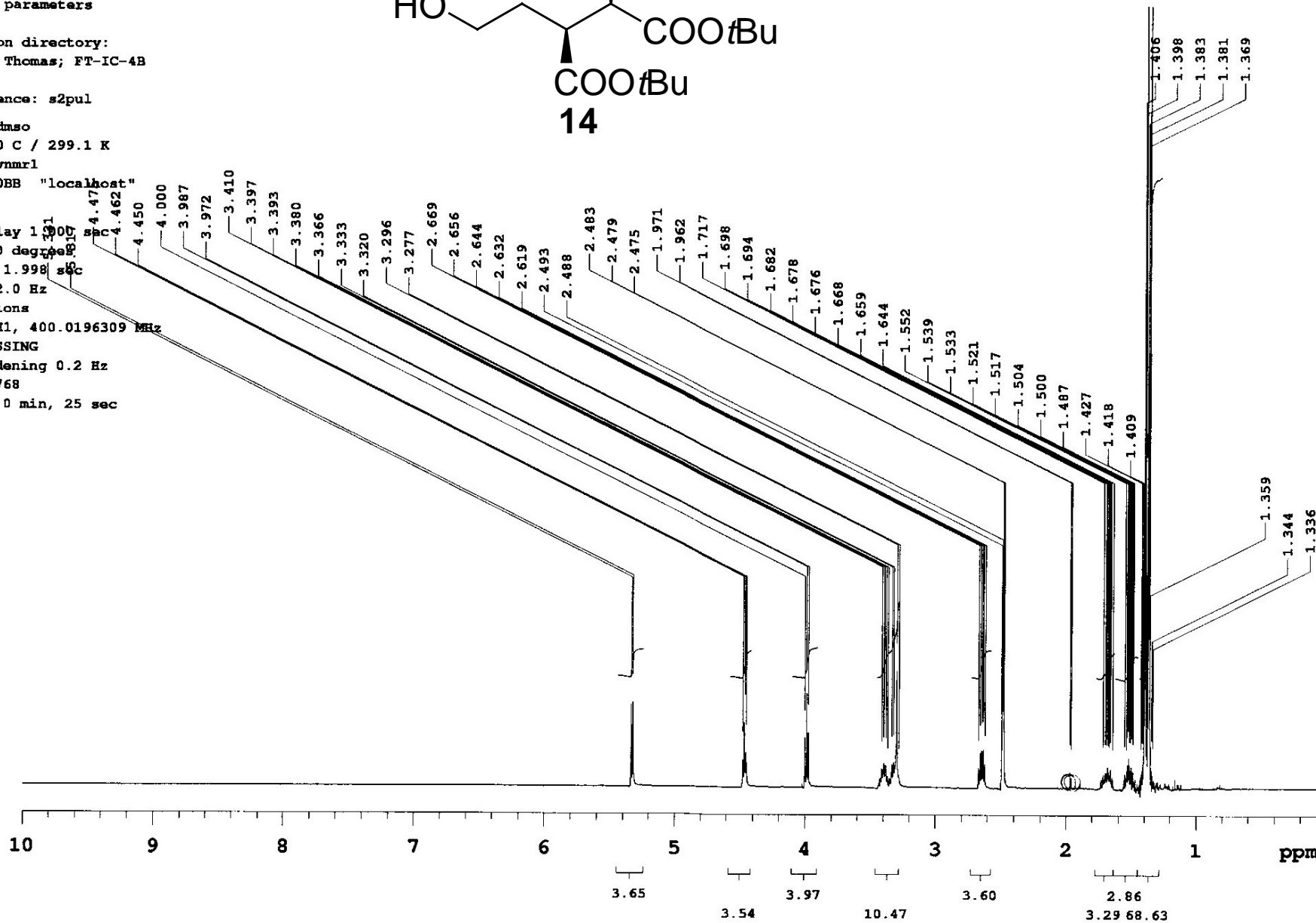
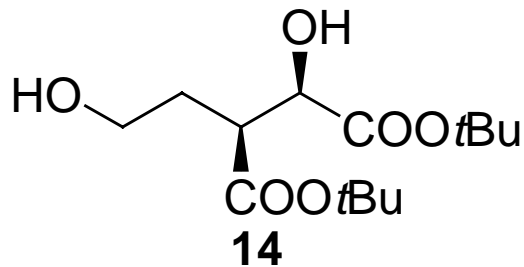
OBSERVE H1, 400.0196309 MHz

DATA PROCESSING

Line broadening 0.2 Hz

FT size 32768

Total time 0 min, 25 sec



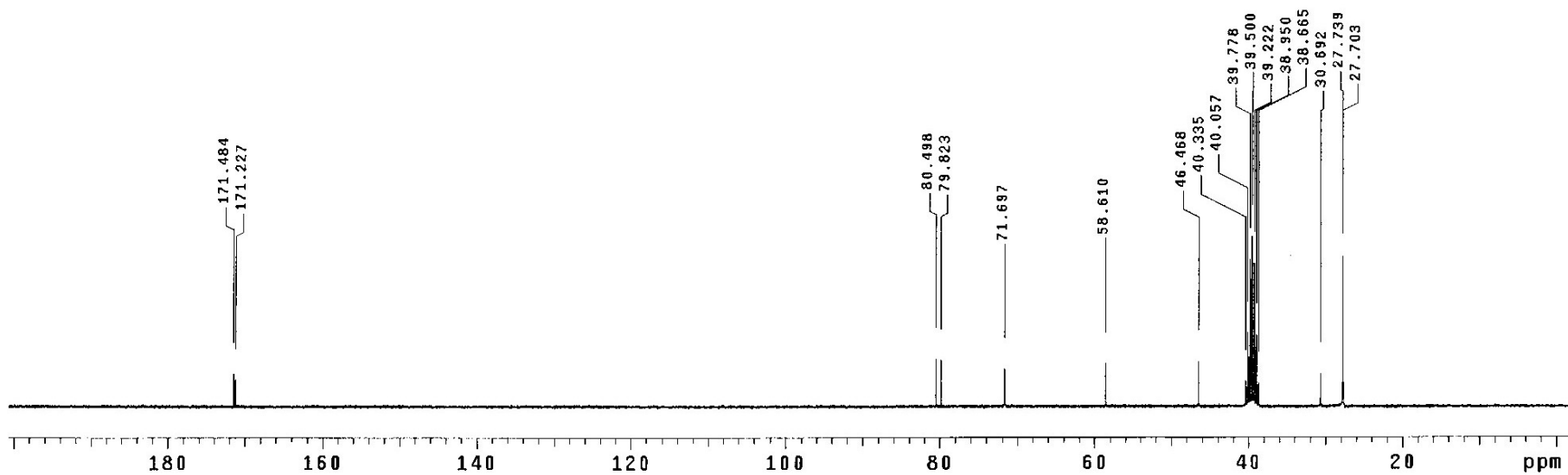
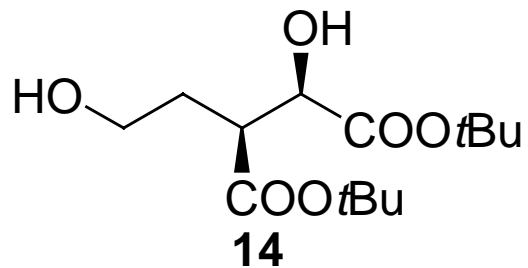
Std Carbon experiment

Automation directory:  
Sample : F.Thomas FT-IC4B

Pulse Sequence: s2pul

Solvent: dmsd  
Temp. 26.0 C / 299.1 K  
Operator: vnmr1  
Mercury-300BB "localhost.localdomain"

Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 1.301 sec  
Width 18115.9 Hz  
1488 repetitions  
OBSERVE C13, 75.4489541 MHz  
DECOUPLE H1, 300.0566153 MHz  
Power 36 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 0.5 Hz  
FT size 65536  
Total time 1 hr, 24 min, 22 sec



J. Thomas

Sample: FT IC 17

Pulse Sequence: s2pul

Date: Mar 30 2006

Solvent: dmsd

Temp. 26.0 C / 299.1 K

Operator: walkup

Mercury-300BB "jutta"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.998 sec

Width 4800.8 Hz

16 repetitions

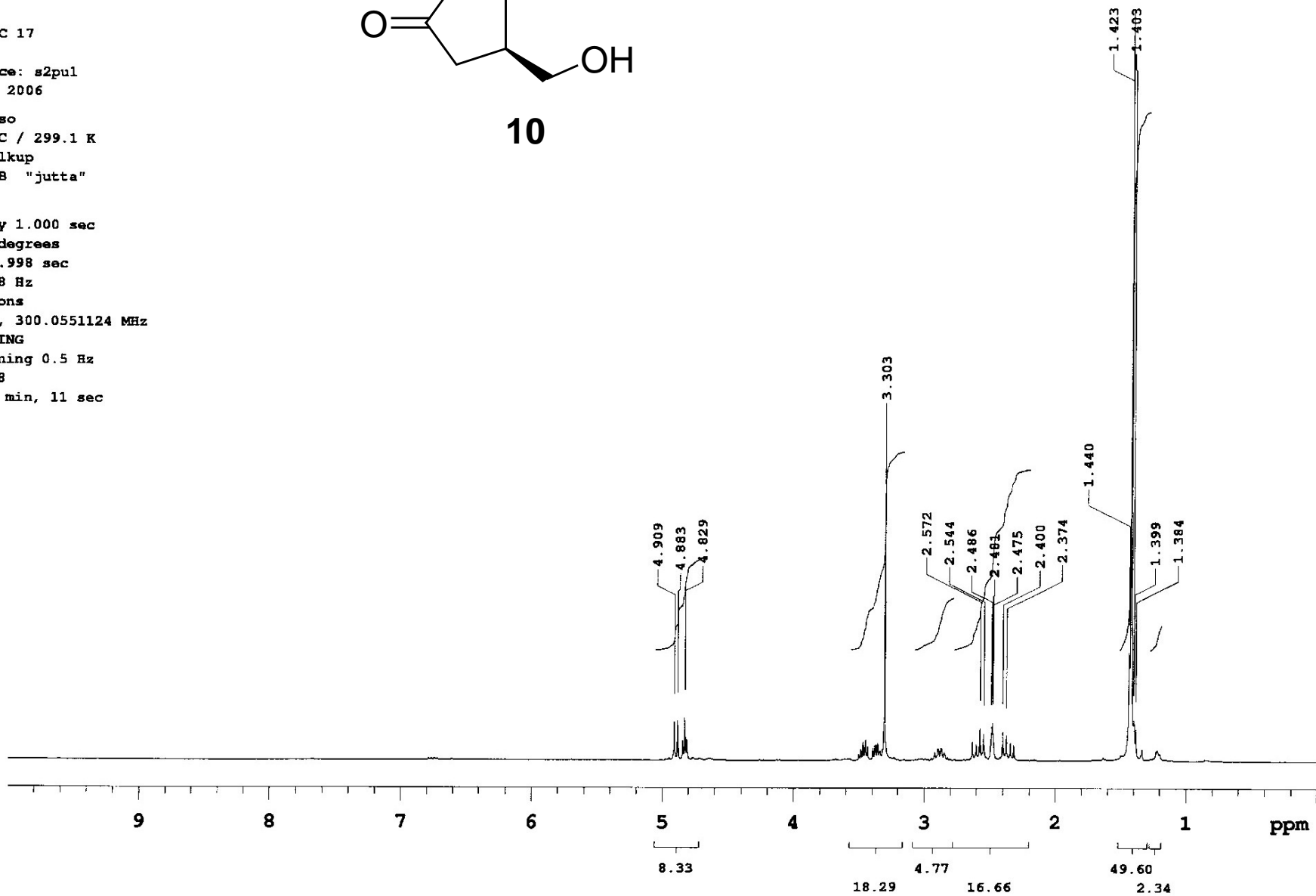
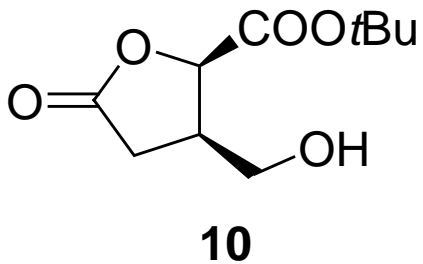
OBSERVE H1, 300.0551124 MHz

DATA PROCESSING

Line broadening 0.5 Hz

FT size 32768

Total time 1 min, 11 sec



F.Thomas

Sample: FT IC 17

Pulse Sequence: APT

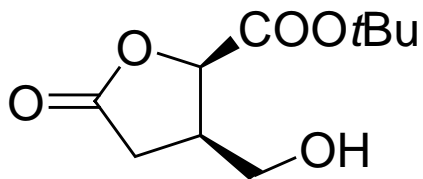
Date: Mar 30 2006

Solvent: dmsc

Temp. 26.0 C / 299.1 K

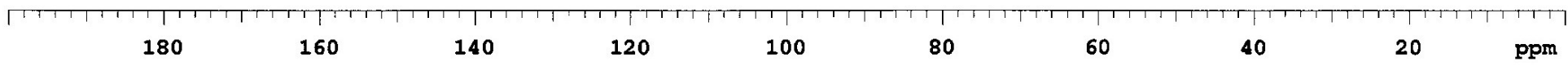
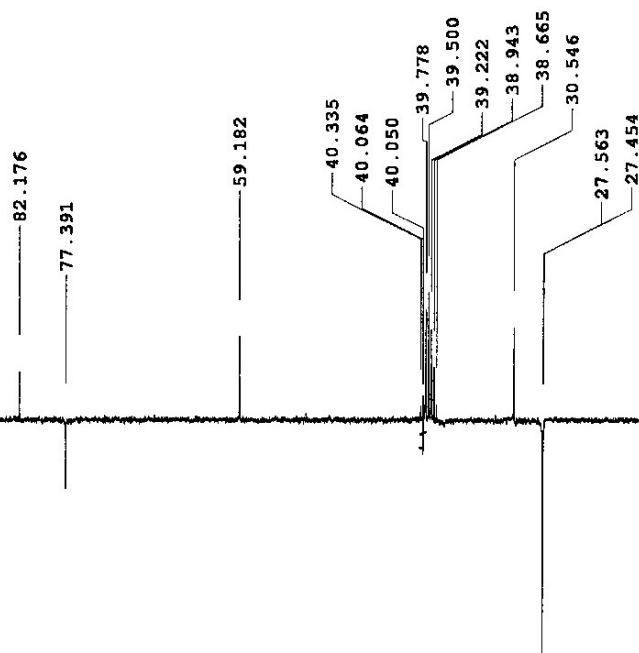
Operator: walkup

Mercury-300BB "jutta"



10

Relax. delay 1.000 sec  
1st pulse 90.0 degrees  
2nd pulse 135.0 degrees  
Acq. time 1.000 sec  
Width 18115.9 Hz  
576 repetitions  
OBSERVE C13, 75.4489536 MHz  
DECOUPLE H1, 300.0566153 MHz  
Power 36 dB  
on during acquisition  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 65536  
Total time 1 hr, 18 min, 40 sec



P. Thomas

Sample: FT-IC45B

Pulse Sequence: s2pul

Date: Apr 4 2007

Solvent: cdcl3

Temp. 26.0 C / 299.1 K

Operator: walkup

Mercury-400BB "felix"

Relax. delay 1.000 sec

Pulse 30.0 degrees

Acq. time 1.996 sec

Width 6402.0 Hz

16 repetitions

OBSERVE H1, 400.0177308 MHz

DATA PROCESSING

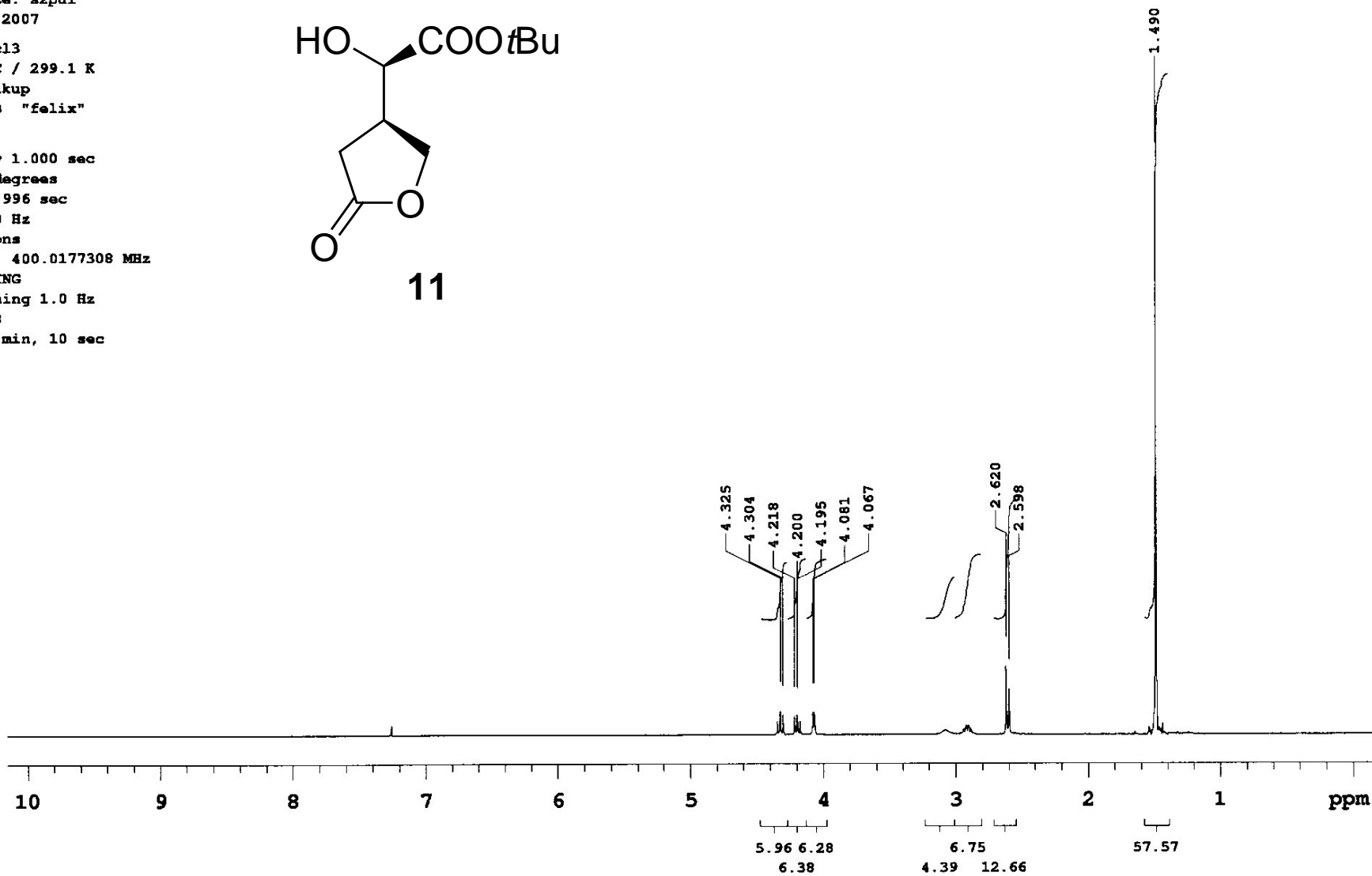
Line broadening 1.0 Hz

FT size 32768

Total time 1 min, 10 sec



11



F. Thomas

Sample: FT-IC45B

Pulse Sequence: APT

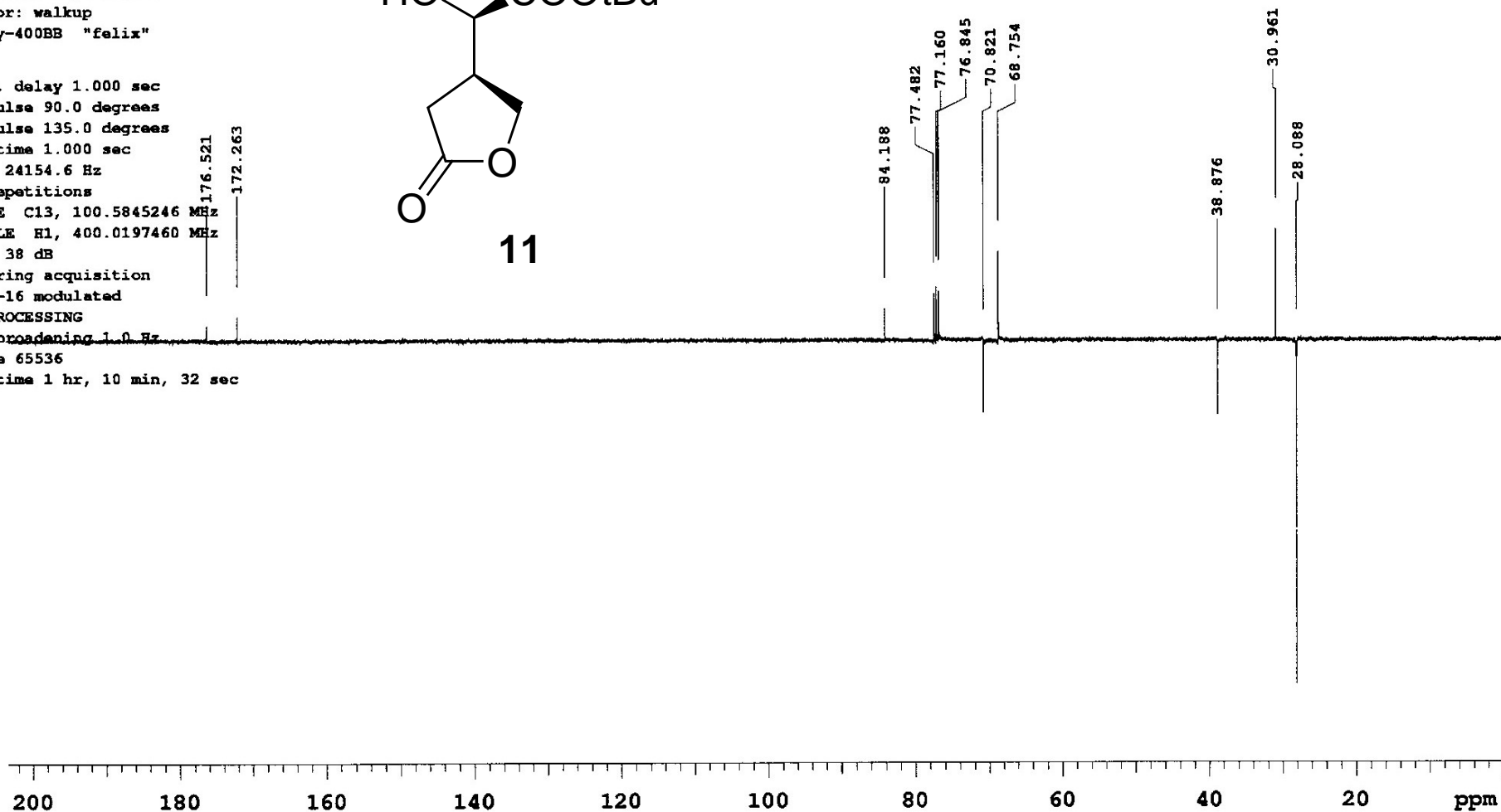
Date: Apr 4 2007

Solvent: cdcl3  
Temp. 26.0 C / 299.1 K  
Operator: walkup  
Mercury-400BB "felix"

Relax. delay 1.000 sec  
1st pulse 90.0 degrees  
2nd pulse 135.0 degrees  
Acq. time 1.000 sec  
Width 24154.6 Hz  
192 repetitions  
OBSERVE C13, 100.5845246 MHz  
DECOUPLE H1, 400.0197460 MHz  
Power 38 dB  
on during acquisition  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 65536  
Total time 1 hr, 10 min, 32 sec



11



F.Thomas

Sample: FT-IC44

Pulse Sequence: s2pul

Date: Mar 21 2007

Solvent: cdcl3

Temp. 26.0 C / 299.1 K

Operator: walkup

Mercury-300BB "jutta"

Relax. delay 1.000 sec

Pulse 30.0 degrees

Acq. time 1.998 sec

Width 4800.8 Hz

16 repetitions

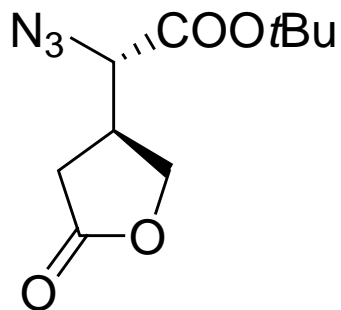
OBSERVE H1, 300.0536871 MHz

DATA PROCESSING

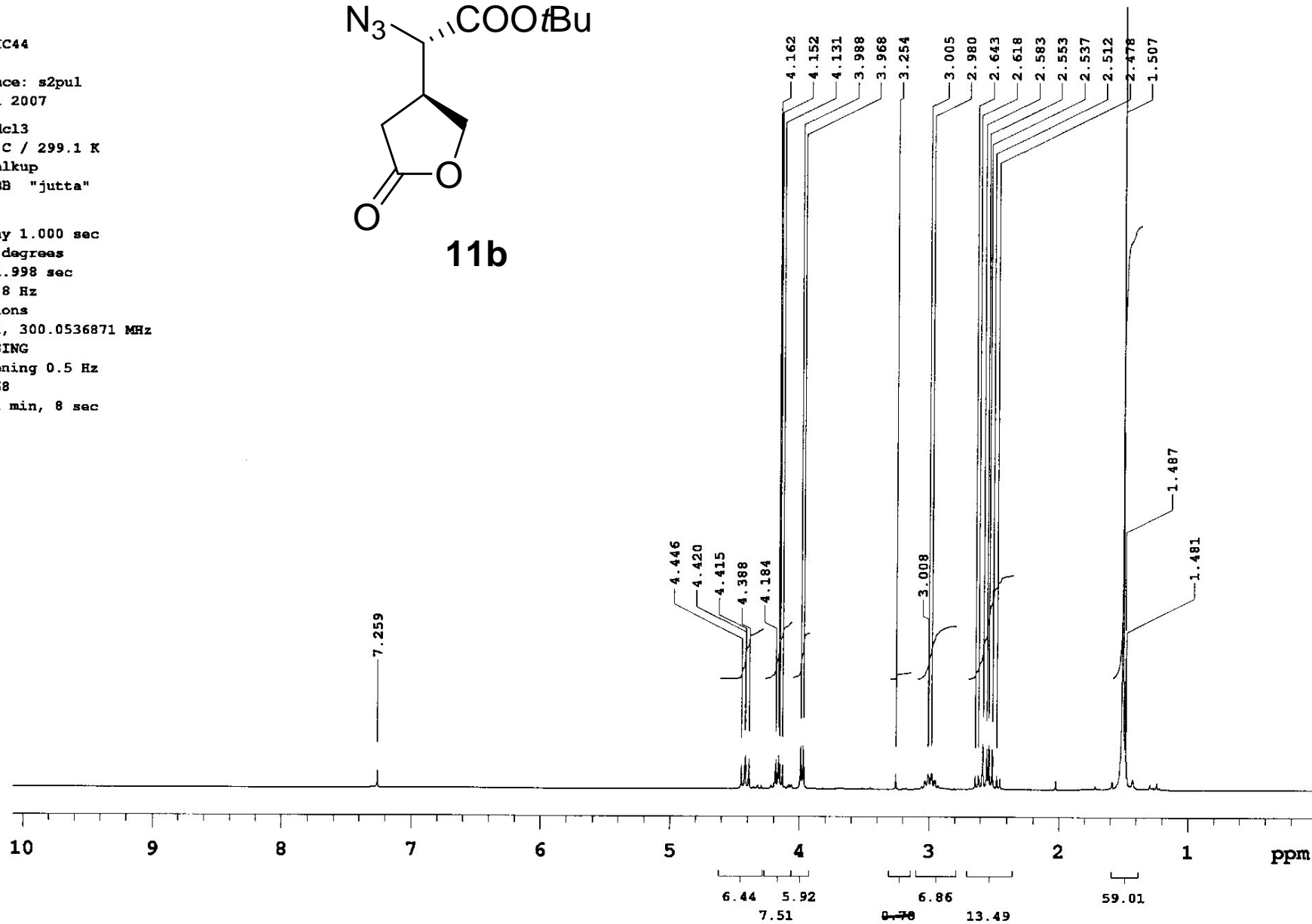
Line broadening 0.5 Hz

FT size 32768

Total time 1 min, 8 sec



11b



r. Thomas

Sample: FT-IC 44

Pulse Sequence: APT

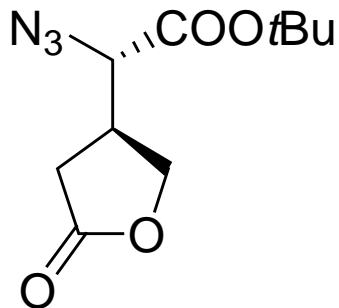
Date: Mar 21 2007

Solvent: cdcl3

Temp. 26.0 C / 299.1 K

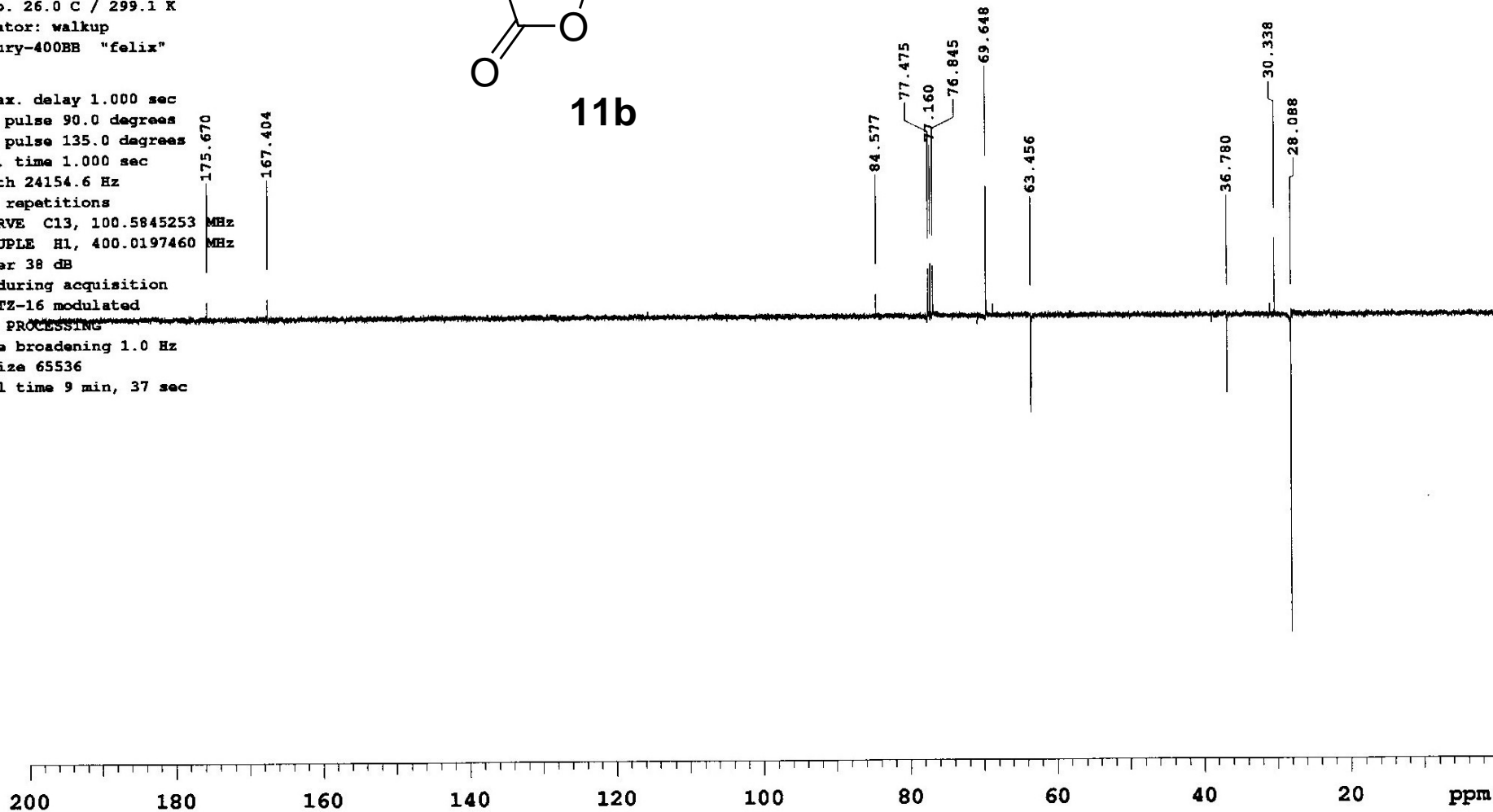
Operator: walkup

Mercury-400BB "felix"



11b

Relax. delay 1.000 sec  
1st pulse 90.0 degrees  
2nd pulse 135.0 degrees  
Acq. time 1.000 sec  
Width 24154.6 Hz  
128 repetitions  
OBSERVE C13, 100.5845253 MHz  
DECOUPLE H1, 400.0197460 MHz  
Power 38 dB  
on during acquisition  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 65536  
Total time 9 min, 37 sec





F. Thomas

Sample: FT-IC46B

Pulse Sequence: s2pul

Date: May 31 2007

Solvent: cdcl3

Temp. 26.0 C / 299.1 K

Operator: walkup

Mercury-400BB "felix"

Relax. delay 1.000 sec

Pulse 30.0 degrees

Acq. time 1.996 sec

Width 6402.0 Hz

32 repetitions

OBSERVE H1, 400.0177308 MHz

DATA PROCESSING

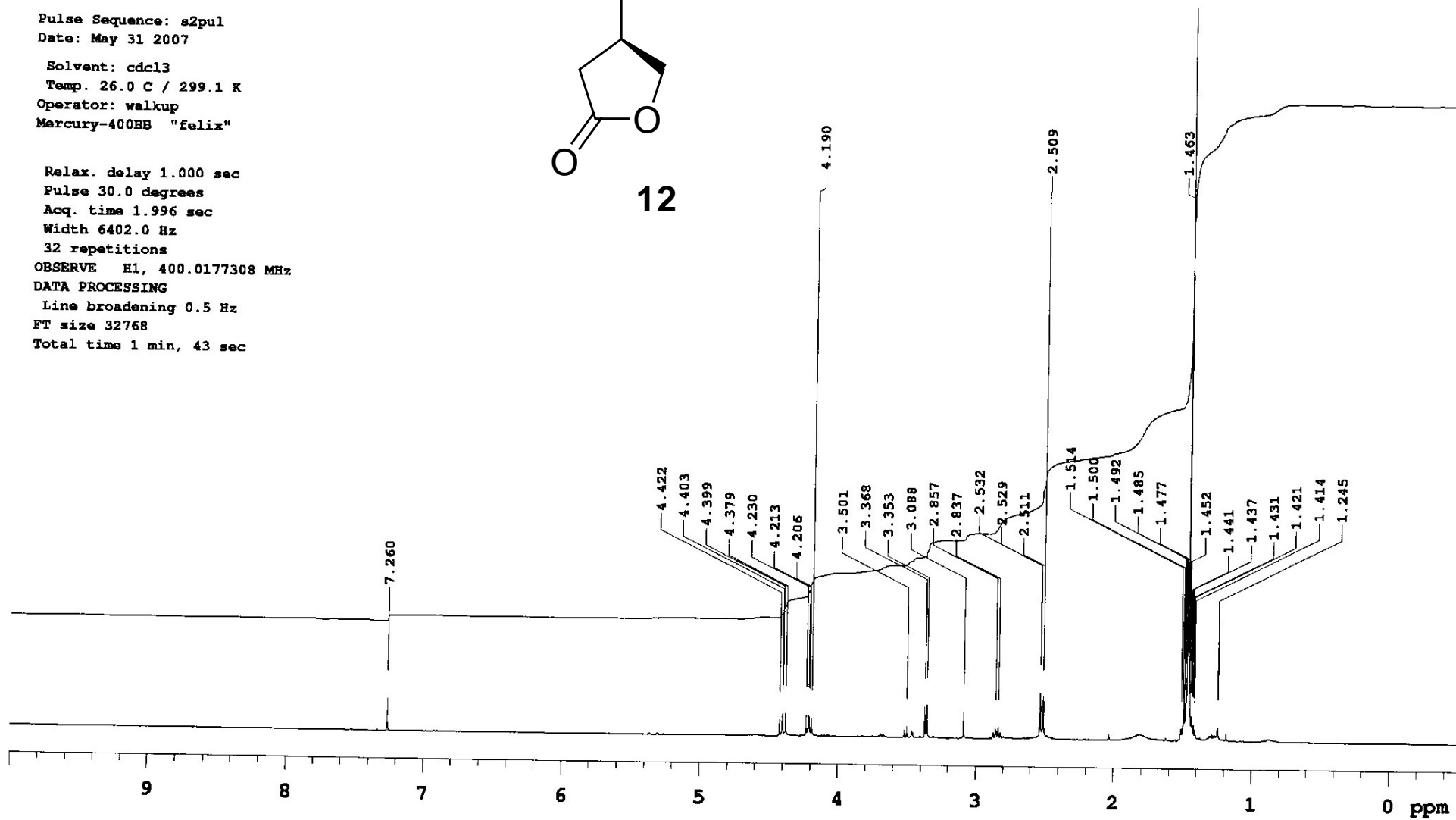
Line broadening 0.5 Hz

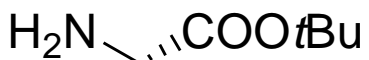
FT size 32768

Total time 1 min, 43 sec

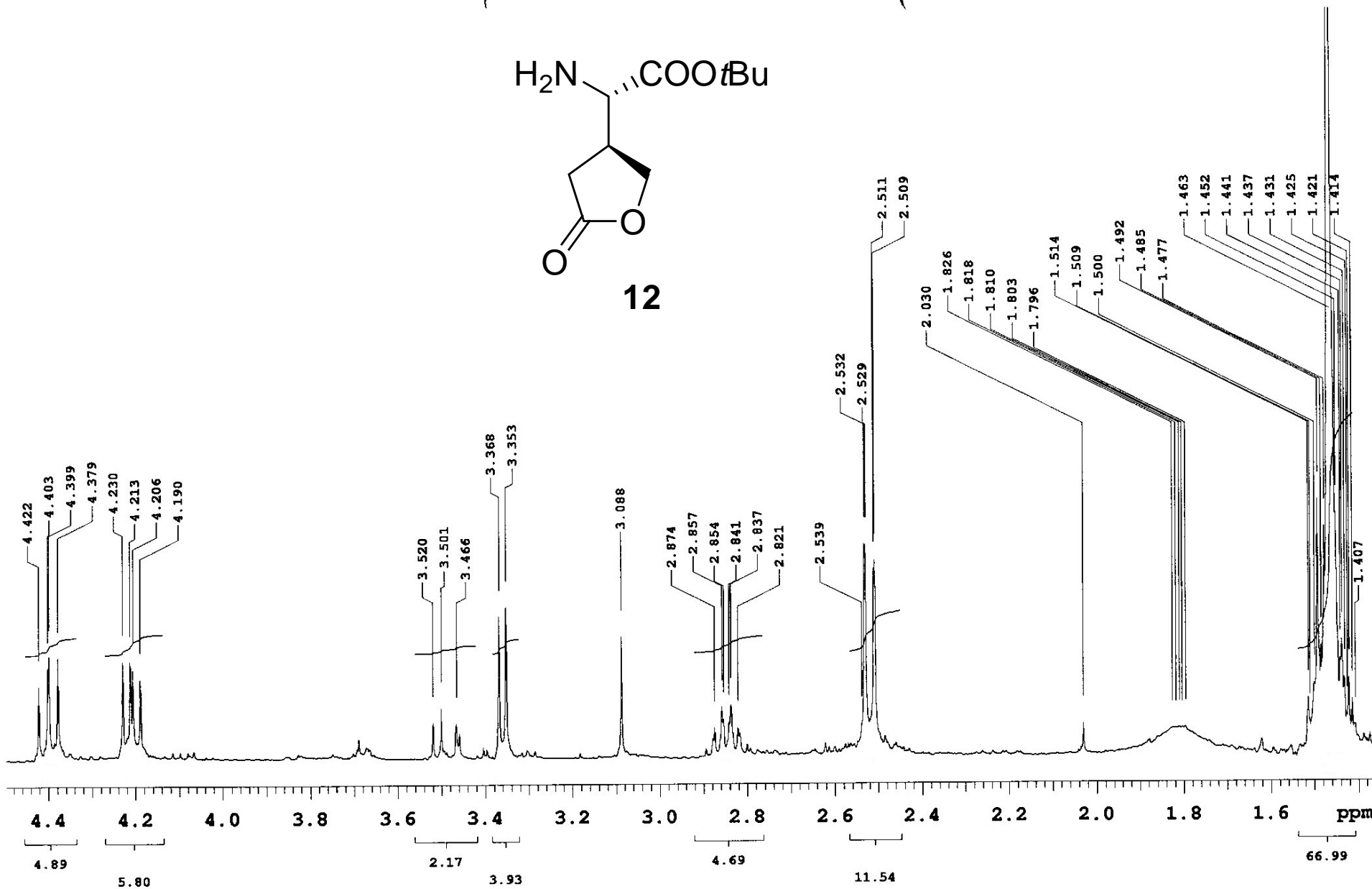


12





12



F. Thomas

Sample: FT-IC46B

Pulse Sequence: APT

Date: May 31 2007

Solvent: cdc13

Temp. 26.0 C / 299.1 K

Operator: walkup

Mercury-400BB "felix"

Relax. delay 1.000 sec

1st pulse 90.0 degrees

2nd pulse 135.0 degrees

Acq. time 1.000 sec

Width 24154.6 Hz

1088 repetitions

OBSERVE C13, 100.5845239 MHz

DECOUPLE H1, 400.0197460 MHz

Power 38 dB

on during acquisition

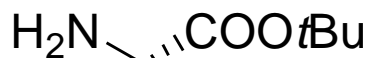
WALTZ-16 modulated

DATA PROCESSING

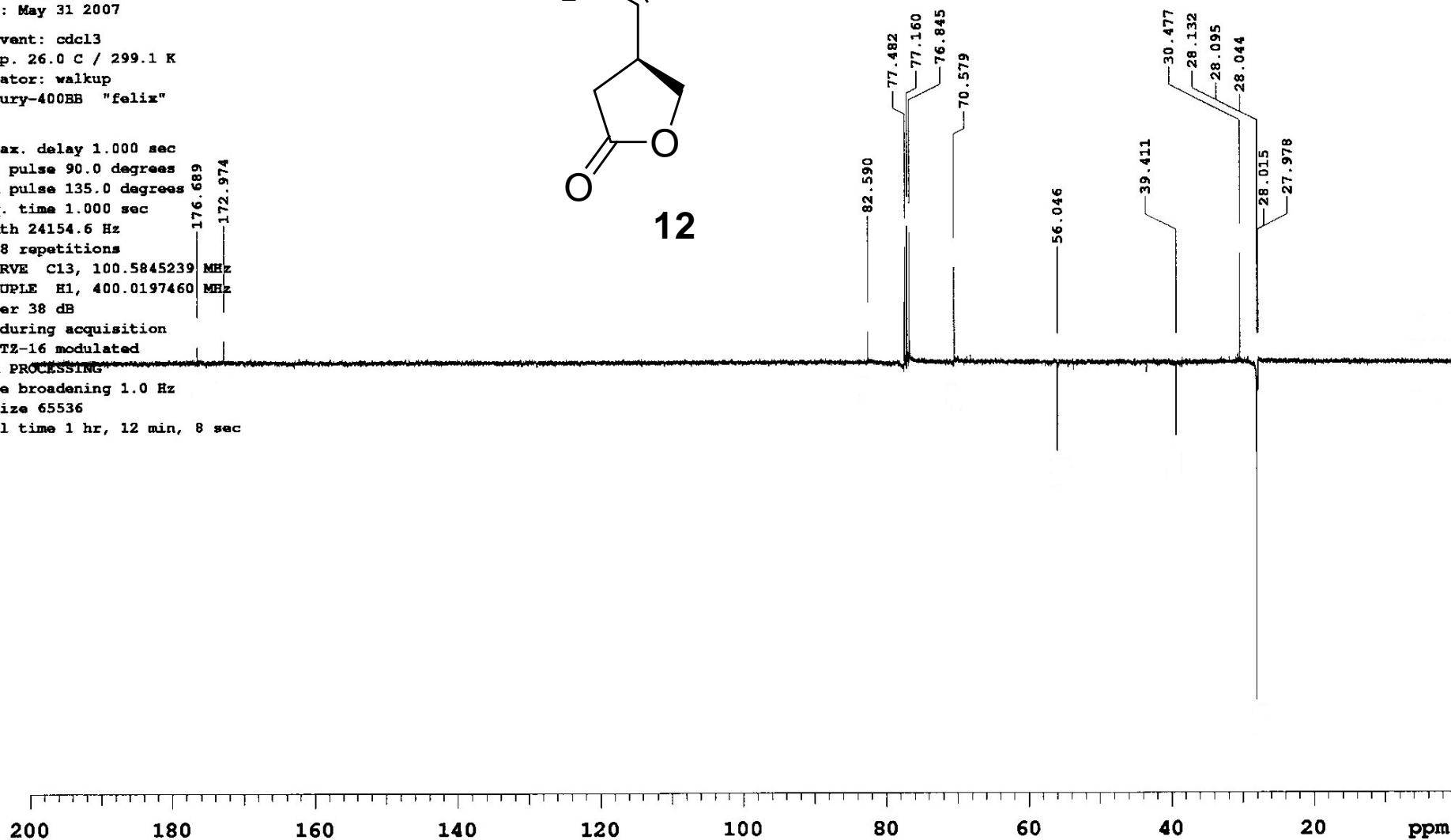
Line broadening 1.0 Hz

FT size 65536

Total time 1 hr, 12 min, 8 sec



12



P. Heretsch

Sample: PHC2

Pulse Sequence: s2pul

Date: May 18 2006

Solvent: cd3od

Temp. 25.0 C

Operator: walkup

Mercury-400BB "felix"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.996 sec

Width 6402.0 Hz

16 repetitions

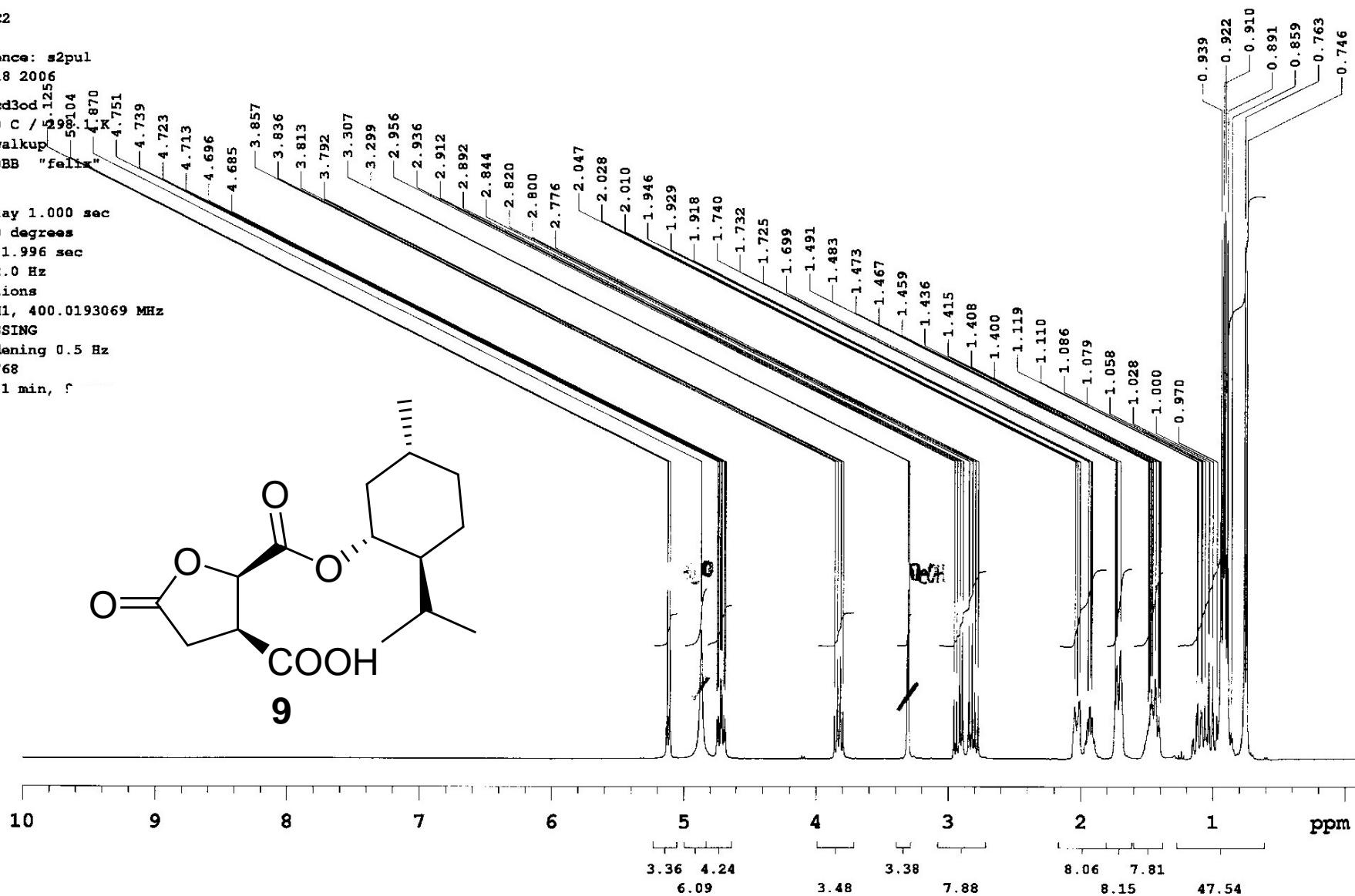
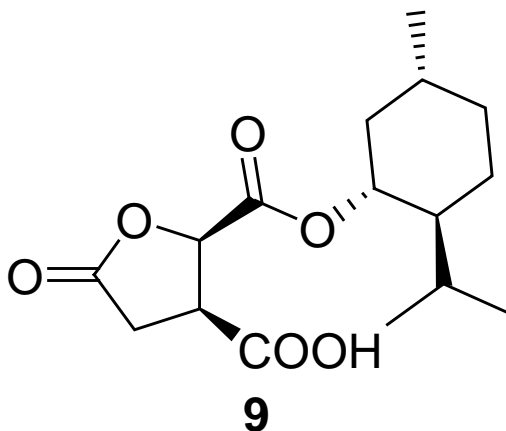
OBSERVE H1, 400.0193069 MHz

DATA PROCESSING

Line broadening 0.5 Hz

FT size 32768

Total time 1 min, 0



P. Heretsch

Sample: PHC2

Pulse Sequence: s2pul

Date: May 18 2006

Solvent: cd3od

Temp. 25.0 C / 298.1 K

Operator: walkup

Mercury-400BB "felix"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.300 sec

Width 24154.6 Hz

2000 repetitions

OBSERVE C13, 100.5847923 MHz

DECOUPLE H1, 400.0213220 MHz

Power 38 dB

continuously on

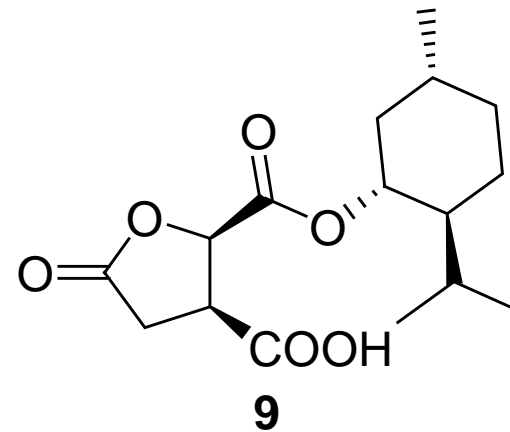
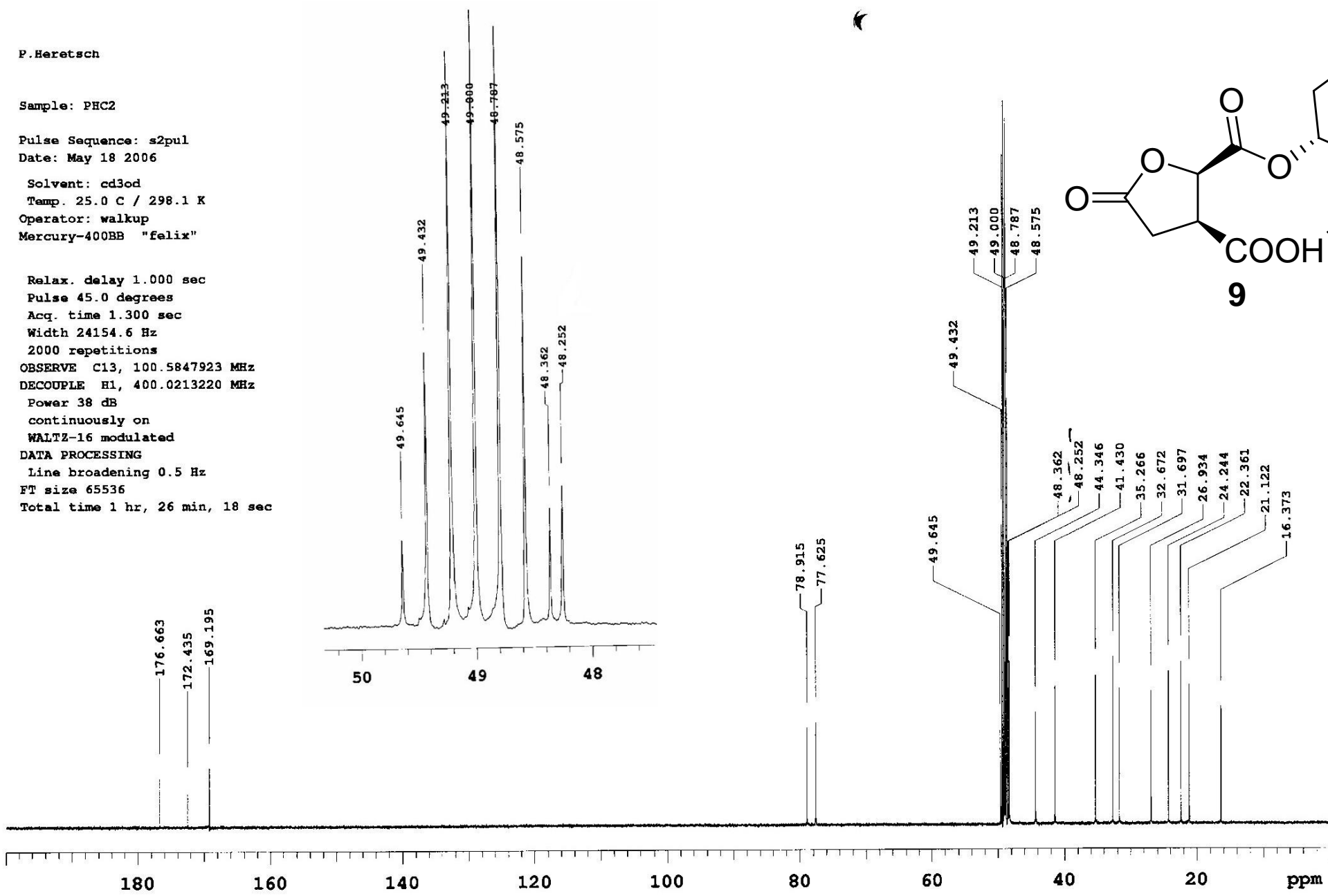
WALTZ-16 modulated

DATA PROCESSING

Line broadening 0.5 Hz

FT size 65536

Total time 1 hr, 26 min, 18 sec



Ph. Heretsch

Sample: PH IC M2

Pulse Sequence: s2pul

Date: Aug 31 2007

Solvent: cdcl3

Temp. 26.0 C / 299.1 K

Operator: walkup

Mercury-400BB "felix"

Relax. delay 1.000 sec

Pulse 30.0 degrees

Acq. time 1.996 sec

Width 6402.0 Hz

16 repetitions

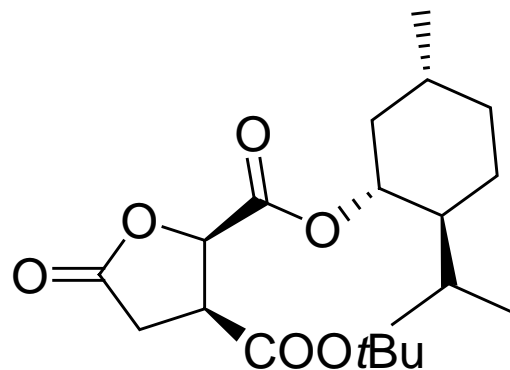
OBSERVE H1, 400.0177308 MHz

DATA PROCESSING

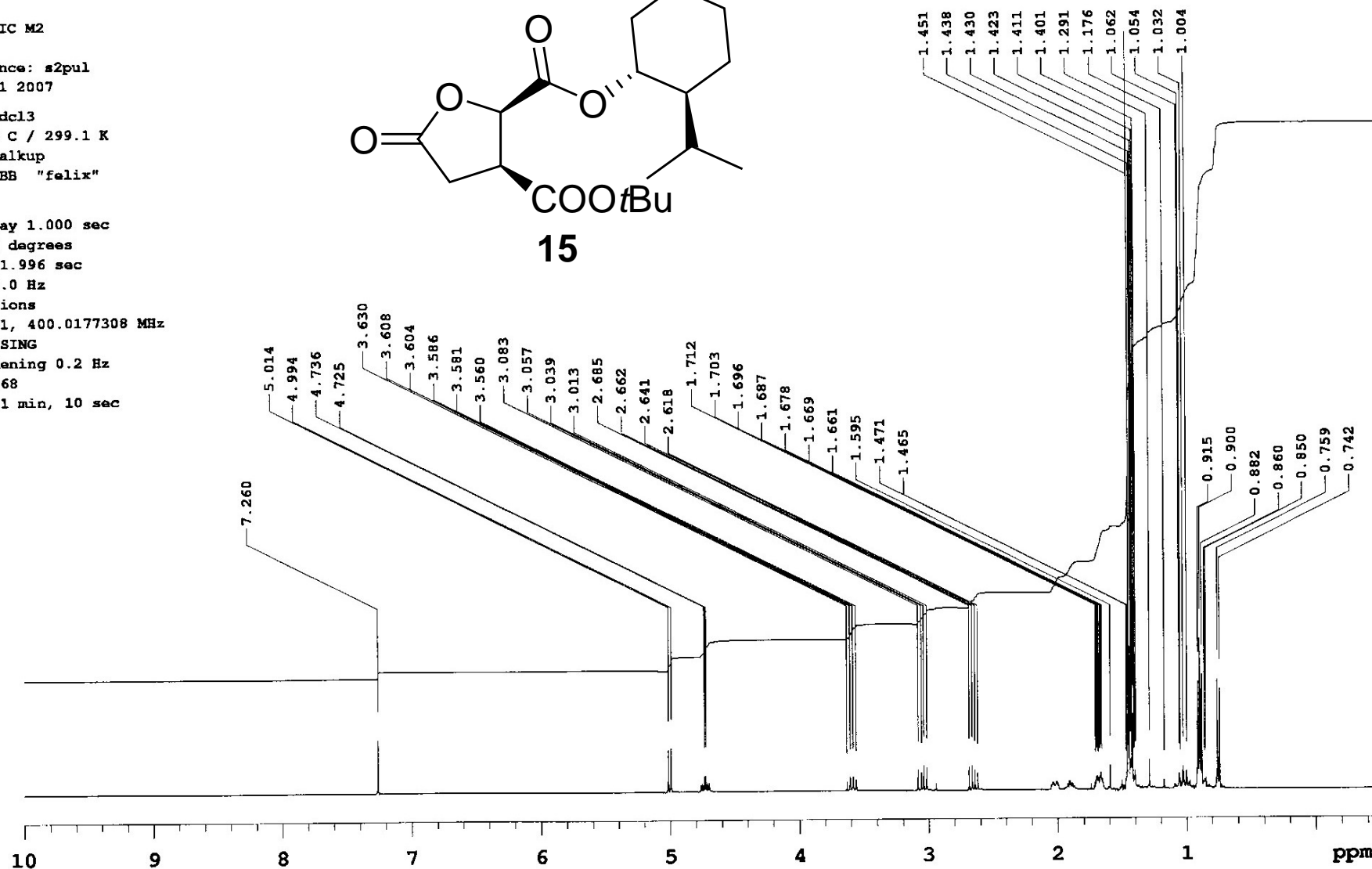
Line broadening 0.2 Hz

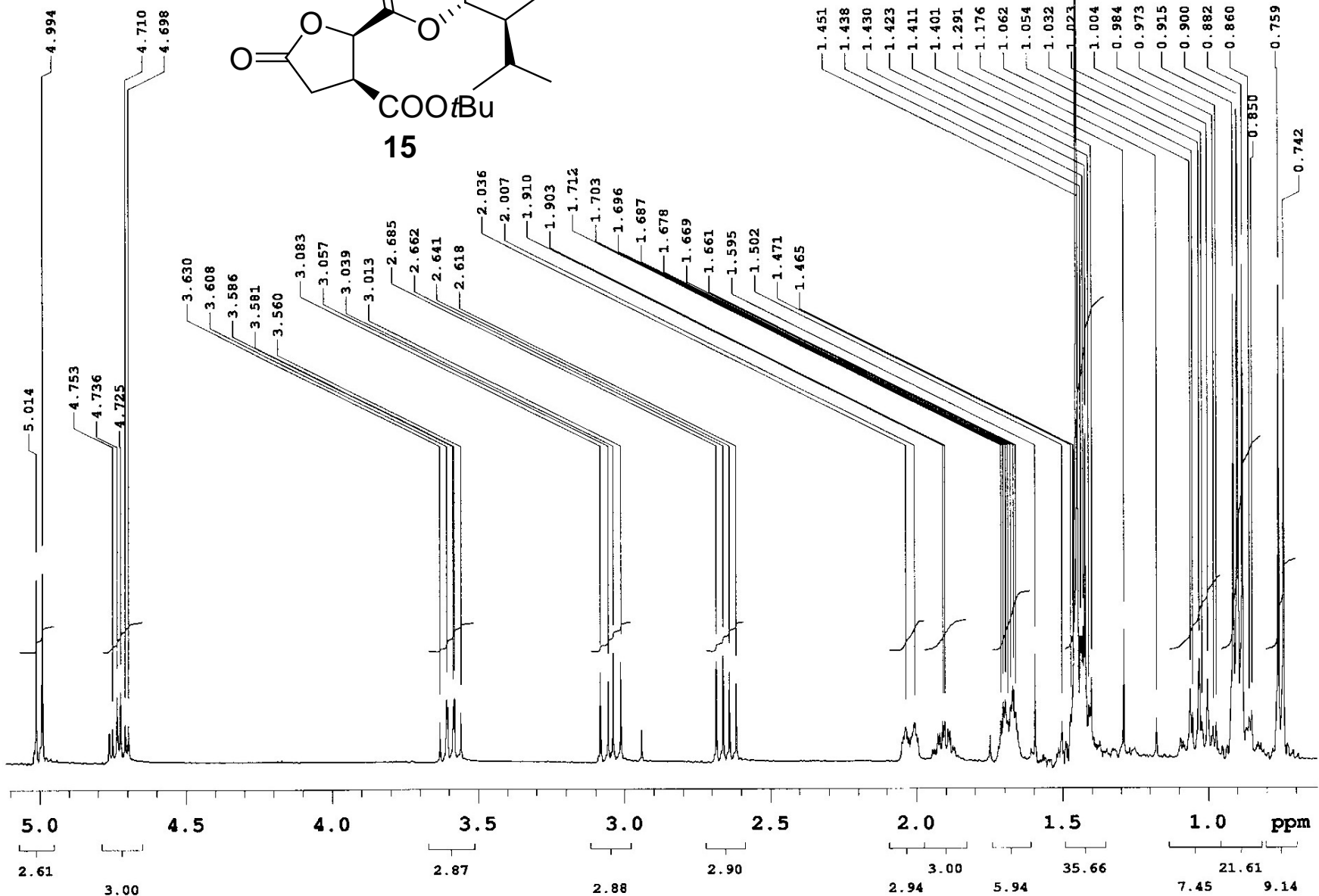
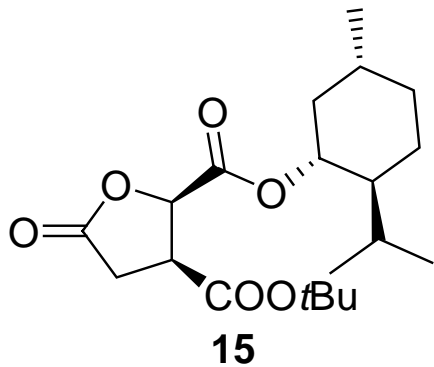
FT size 32768

Total time 1 min, 10 sec



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Ph. Heretsch

Sample: PH IC M2

Pulse Sequence: APT

Date: Aug 31 2007

Solvent: cdcl3

Temp. 26.0 C / 299.1 K

Operator: walkup

Mercury-400BB "felix"

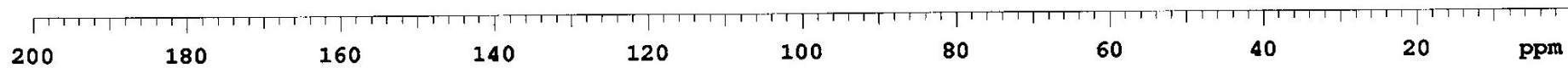
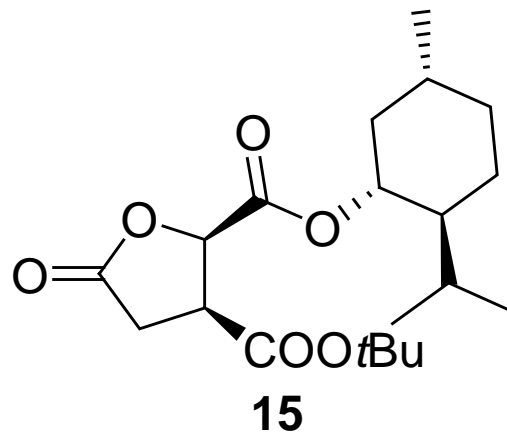
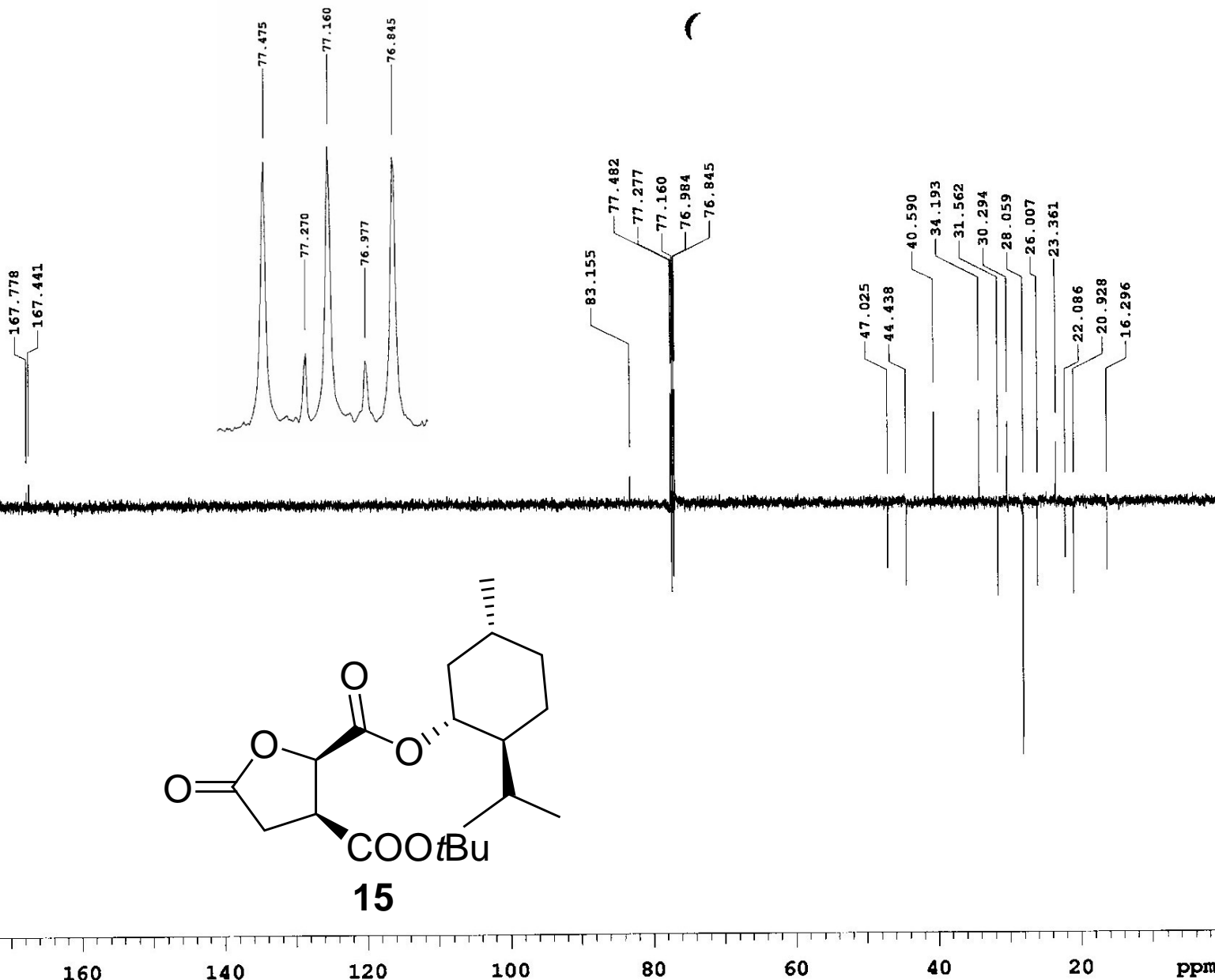
Relax. delay 1.000 sec  
1st pulse 90.0 degrees  
2nd pulse 135.0 degrees  
Acq. time 1.000 sec  
Width 24154.6 Hz

256 repetitions  
OBSERVE C13, 100.5845216 MHz  
DECOUPLE H1, 400.0197460 MHz  
Power 38 dB

on during acquisition  
WALTZ-16 modulated

DATA PROCESSING

Line broadening 1.0 Hz  
FT size 65536  
Total time 9 min, 49 sec





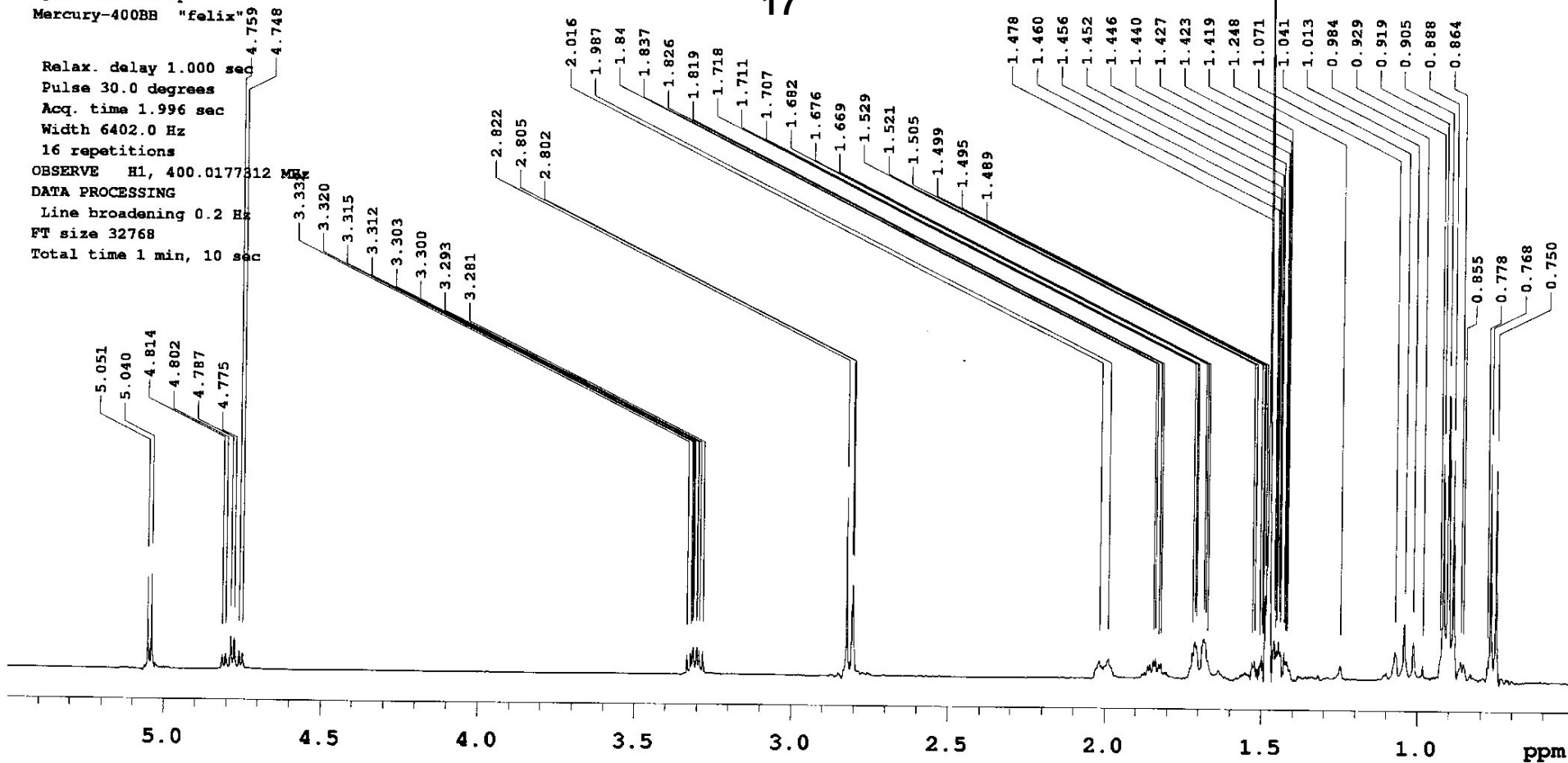
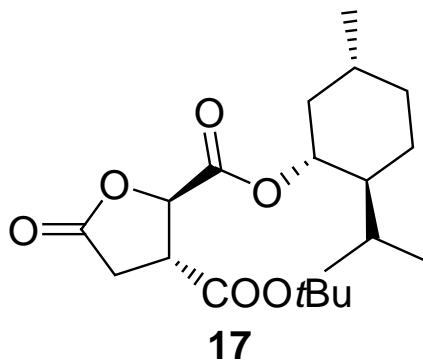
etsch

Sample: PH ICM3

Pulse Sequence: s2pul  
Date: Sep 4 2007

Solvent: cdcl3  
Temp. 26.0 C / 299.1 K  
Operator: walkup  
Mercury-400BB "felix"

Relax. delay 1.000 sec  
Pulse 30.0 degrees  
Acq. time 1.996 sec  
Width 6402.0 Hz  
16 repetitions  
OBSERVE H1, 400.0177812 MHz  
DATA PROCESSING  
Line broadening 0.2 Hz  
FT size 32768  
Total time 1 min, 10 sec



etsch

sample: PHICM3

Pulse Sequence: APT

Date: Sep 5 2007

Solvent: cdcl3

Temp. 26.0 C / 299.1 K

Operator: walkup

Mercury-400BB "felix"

Relax. delay 1.000 sec

1st pulse 90.0 degrees

2nd pulse 135.0 degrees

Acq. time 1.000 sec

Width 24154.6 Hz

1000 repetitions

OBSERVE C13, 100.5845224 MHz

DECOUPLE H1, 400.0197460 MHz

Power 38 dB

on during acquisition

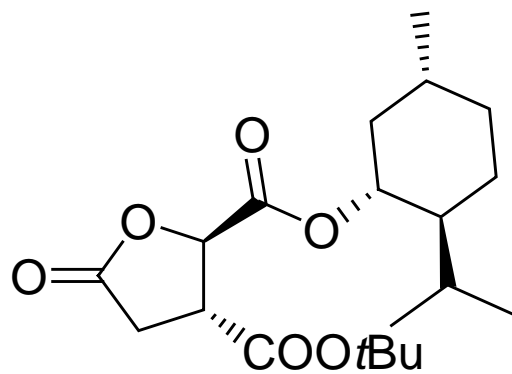
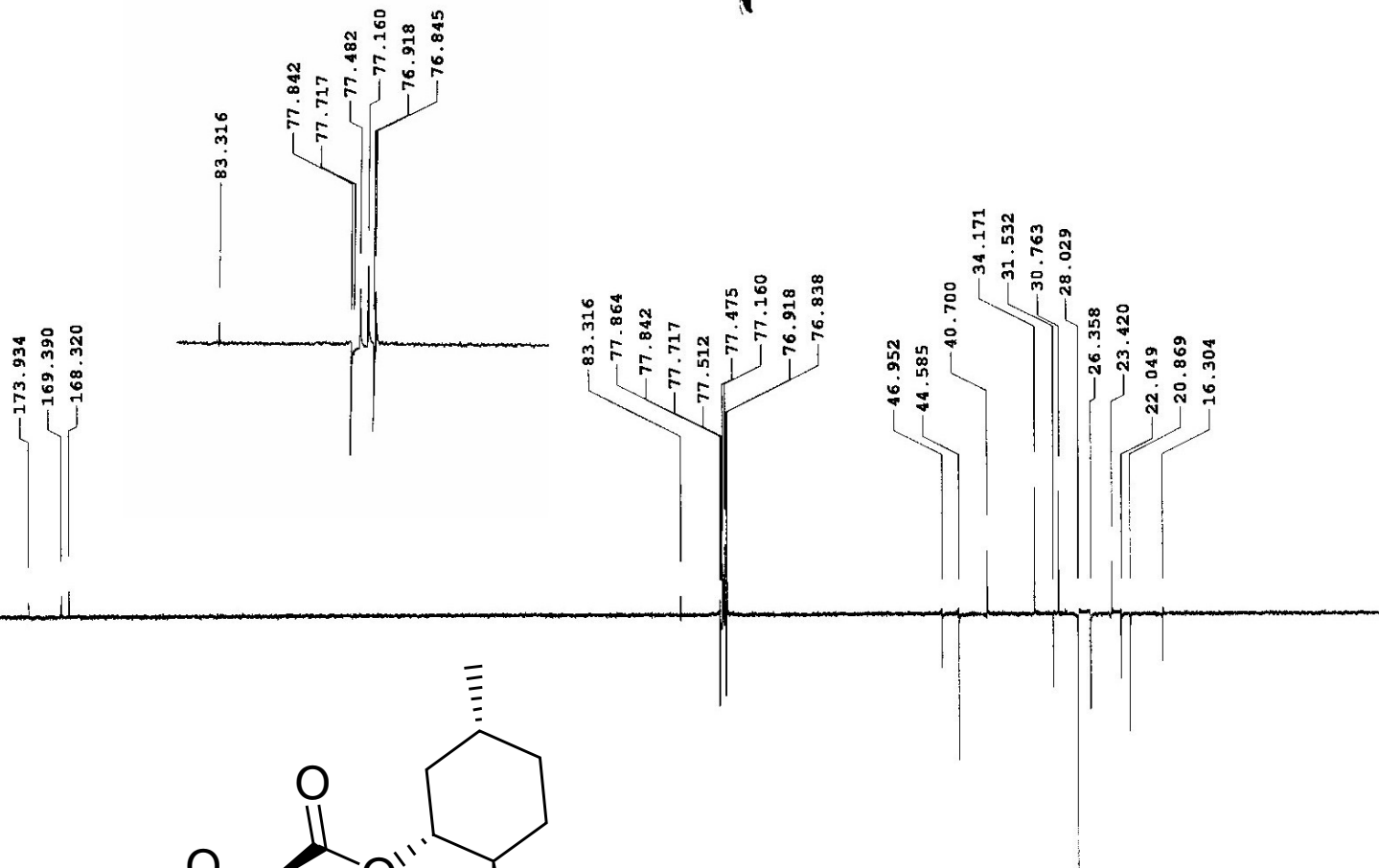
WALTZ-16 modulated

DATA PROCESSING

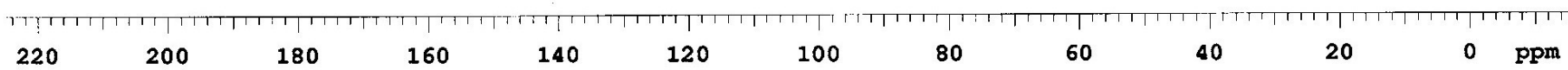
Line broadening 1.0 Hz

FT size 65536

Total time 38 min, 2 sec



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P. Heretsch

Sample: PHIC4

Pulse Sequence: s2pul

Date: Sep 7 2007

Solvent: cdcl3

Temp. 26.0 C / 299.1 K

Operator: walkup

Mercury-400BB "felix"

Relax. delay 1.000 sec

Pulse 30.0 degrees

Acq. time 1.996 sec

Width 6402.0 Hz

16 repetitions

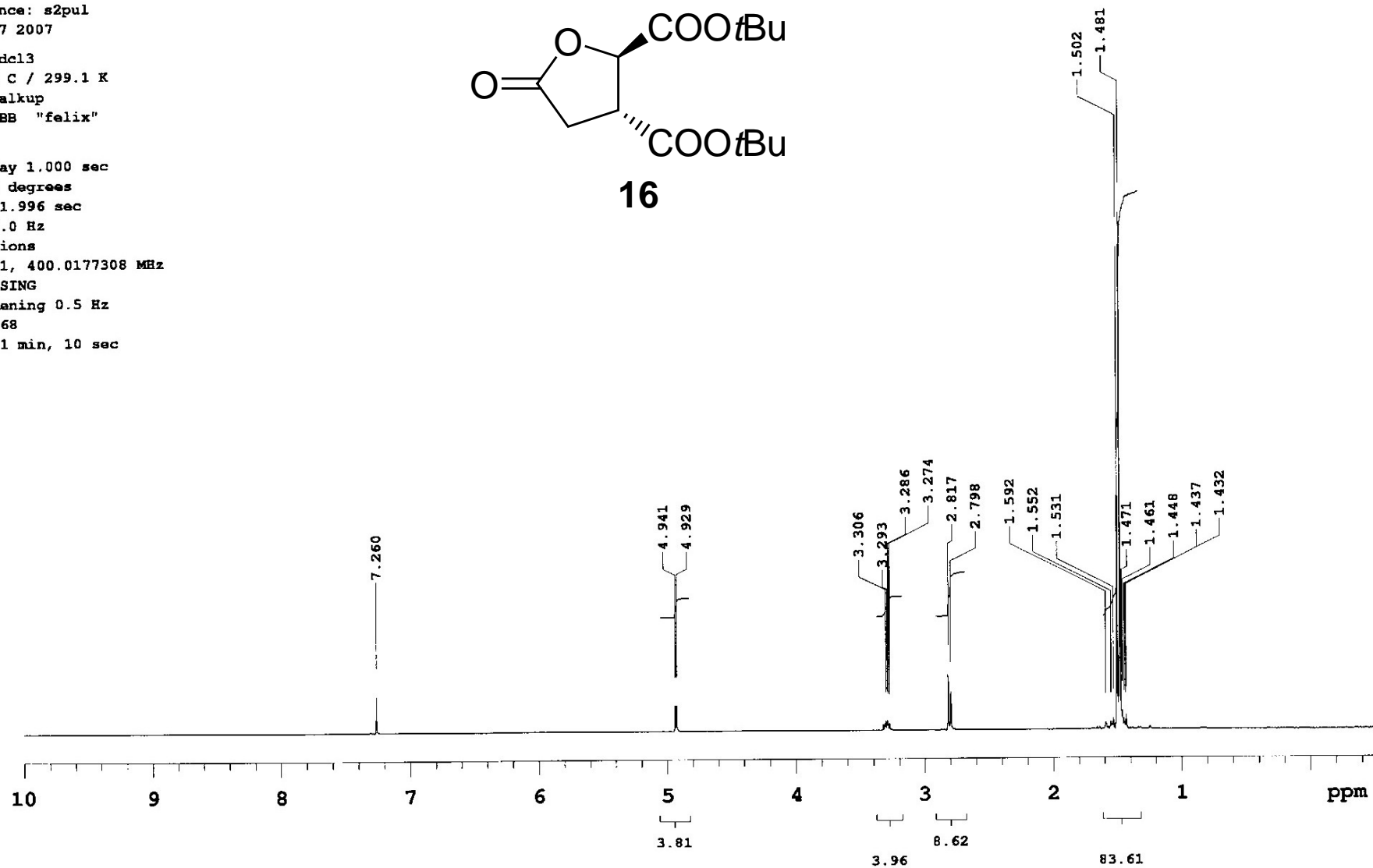
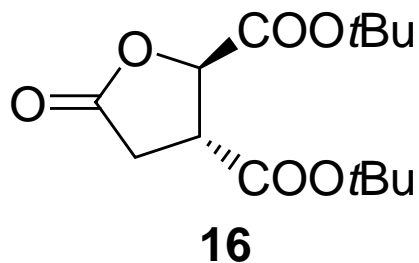
OBSERVE H1, 400.0177308 MHz

DATA PROCESSING

Line broadening 0.5 Hz

FT size 32768

Total time 1 min, 10 sec



F. Heretsch

Sample: PHIC4

Pulse Sequence: s2pul

Date: Sep 7 2007

Solvent: cdcl3

Temp. 26.0 C / 299.1 K

Operator: walkup

Mercury-400BB "felix"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.300 sec

Width 24154.6 Hz

752 repetitions

OBSERVE C13, 100.5845239 MHz

DECOUPLE H1, 400.0197460 MHz

Power 38 dB

continuously on

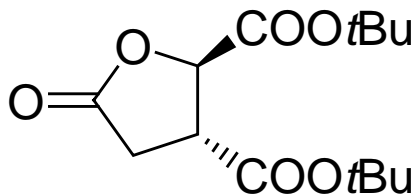
WALTZ-16 modulated

DATA PROCESSING

Line broadening 0.5 Hz

FT size 65536

Total time 1 hr, 26 min, 18 sec



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