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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 flaks were used. The preculture medium had the following composition: 2.5 g L^{-1} yeast extract, 3.0 g L^{-1} NH_4Cl, 0.7 g L^{-1} KH_2PO_4, 0.35 g L^{-1} $MgSO_4 \times 7\ H_2O$, 3.5 mg L^{-1} $FeSO_4 \times 7\ H_2O$, 5 g L^{-1} $CaCO_3$ and 5 mL L^{-1} trace elements with the following composition: 4.0 g L^{-1} $CuSO_4 \times 5\ H_2O$, 4.0 g L^{-1} $MnSO_4 \times 5\ H_2O$, 2.1 g L^{-1} $ZnCl_2$, 0.5 g L^{-1} $CoSO_4 \times 7\ H_2O$ and 5.7 g L^{-1} H_3BO_3.[1] The concentration of refined sunflower oil was 15 g·L^{-1}. 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^{-1} $(NH_4)_2SO_4$, 0.7 g L^{-1} KH_2PO_4, 0.35 g L^{-1} $MgSO_4 \times 7\ H_2O$, 3.5 mg L^{-1} $FeSO_4 \times 7\ H_2O$, 30 mg L^{-1} $CaCl_2$; b) trace elements: 20 mg L^{-1} $CuSO_4 \times 5\ H_2O$, 20 mg L^{-1} $MnSO_4 \times 5\ H_2O$, 10 mg L^{-1} $ZnCl_2$, 2.5 mg L^{-1} $CoSO_4 \times 7\ H_2O$ and 28.5 mg L^{-1} H_3BO_3; c) vitamin: 1 mg L^{-1} 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^{-1}. 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_2 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^{-1}; 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 µm pore size. The filter cake was dried at 105 °C until stability of weight (MA 40, Sartorius AG, Göttingen, Germany).

1.3 Results of cultivation and further downstream processing

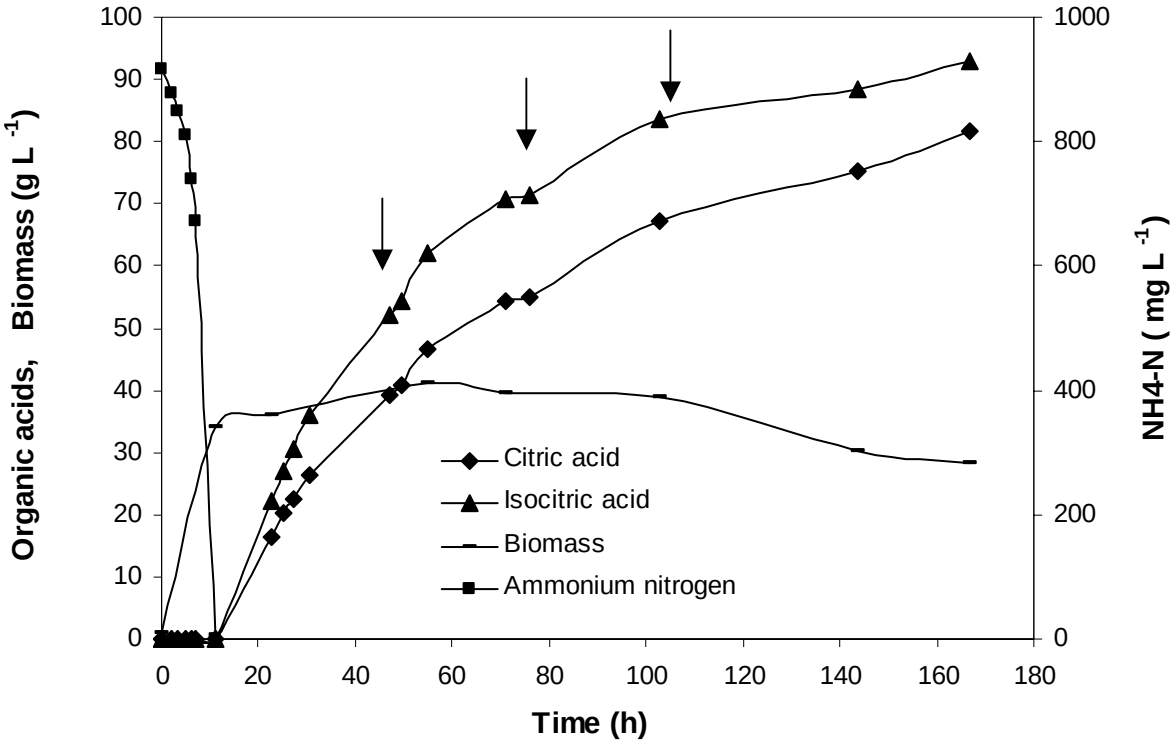


Figure 1: Fed-batch cultivation of the *Yarrowia lipolytica* EH59 on sunflower oil. Concentration of sunflower oil at cultivation start was 85 g L^{-1}; first (25 g L^{-1}), second (20 g L^{-1}) and third (15 g L^{-1}) oil dosage. Arrows indicate additional sunflower oil feedings.

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^{-1} and citric acid concentration of 82.8 g L^{-1}, 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^{-1}. At the end of cultivation a total organic acid concentration of 175.8 g L^{-1} was detected.

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

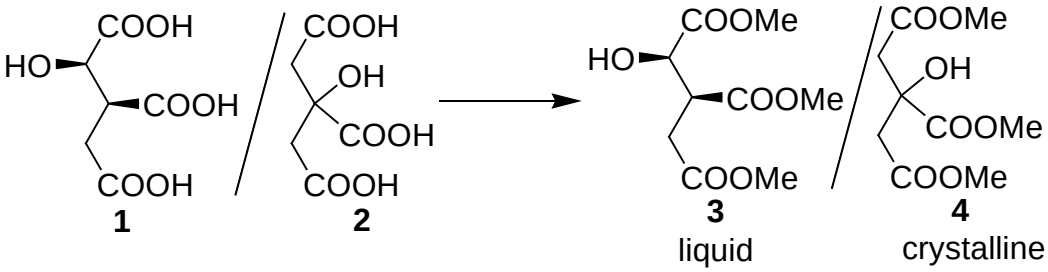
The transformation of the (2*R*,3*S*)-isocitric/citric acid sodium salts from the cell-free culture solution to their corresponding acids and sodium hydroxide was performed by laboratory electrodialysis equipment (Deukum Co., Frickenhausen, Germany) with bipolar membranes (Neosepta BP-1, Tokuyama Co.,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

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}H and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 and Varian Gemini-200BB (200 MHz for ^{1}H; 50 MHz for ^{13}C), Varian Gemini-300BB and Varian Mercury-300BB (300 MHz for ^{1}H; 75 MHz for ^{13}C), Varian Mercury-400BB (400 MHz for ^{1}H; 100 MHz for ^{13}C) and Bruker DRX 600 (600 MHz for ^{1}H; 150 MHz for ^{13}C). The chemical shifts are reported relative to the residual solvent peak, which was used as an internal reference (δ 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_{254}, 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 $CHCl_3$ (700 mL) and washed once with saturated aqueous $NaHCO_3$ solution (300 mL) and two times with water (100 mL). After drying with Na_2SO_4 the solvent was

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].[2]

$[\alpha]_D^{22}$ ±0.00 (*c* 1.00; MeOH).

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

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.75 (d, ^2J = 15.3 Hz, 2 H, C**H**$_2$), 2.90 (d, ^2J = 15.6 Hz, 2 H, C**H**$_2$), 3.59 (s, 6 H, 2 x COOC**H**$_3$), 3.68 (s, 3H, COOC**H**$_3$), 5.78 (s, 1 H, O**H**).

^{13}C-NMR (75 MHz, DMSO-d_6): δ = 43.1 (2 x **C**H$_2$), 51.5 (2 x COO**C**H$_3$), 52.2 (COO**C**H$_3$), 73.1 (**C**H), 169.7 (2 x **C**OOCH$_3$), 173.1 (**C**OOCH$_3$).

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 ^{1}H-NMR.

B.p.: 105 °C (0.02 mbar).

n_D^{22} = 1.4523.

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

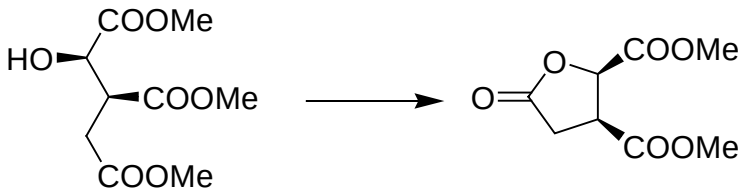
IR (film): $\tilde{\nu}$ = 3486, 3005, 2957, 1799, 1738, 1439 cm^{-1}.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 2.60-2.72 (m, 2H, C**H$_2$**), 3.28-3.32 (m, 1H, C**H**), 3.59, 3.66, 3.69 (s, 3 x 3 H, 3 x COOC**H$_3$**), 4.34-4.37 (m, 1 H, C**H**), 5.91 (d, ^3J = 5.1 Hz, 1 H, O**H**).

^{13}C-NMR (75 MHz, DMSO-d_6): δ = 31.8 (**C**H$_2$), 45.0 (**C**H), 51.6, 51.8, 51.9 (3 x **C**H$_3$), 70.3 (**C**H), 171.1, 171.9, 172.4 (3 x **C**O).

HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_9H_{14}O_7Na$: 257.06317; found 257.06321, $[2M+Na]^+$ calcd for $C_{18}H_{28}O_{14}Na$: 491.13731; found 491.13745.

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



3 **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 $CHCl_3$ (25 mL) and washed once with saturated aqueous $NaHCO_3$ solution (25 mL), then water (25 mL) and dried with Na_2SO_4. 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*-toluene-sulfonic 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].[3]

$[\alpha]_D^{24}$ –66.8 (*c* 8.56, acetone) [Lit: $[\alpha]_D^{26}$ –66.3 (*c* 8.47, acetone)].[3]

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

^{1}H-NMR (600 MHz, DMSO-d_6): δ = 2.74 (dd, ^2J = 17.5 Hz, ^3J = 6.9 Hz, 1 H, C**H**$_2$), 2.89 (dd, ^2J = 17.5 Hz, ^3J = 9.2 Hz, 1 H, C**H**$_2$), 3.63, 3.69 (s, 2 x 3 H, COOC**H**$_3$), 3.89 (ddd, ^3J = 6.9 Hz, 8.1 Hz, 9.2 Hz, 1 H, C**H**COOCH$_3$), 5.32 (d, ^3J = 8.1 Hz, 1 H, C**H**(O)COOCH$_3$).

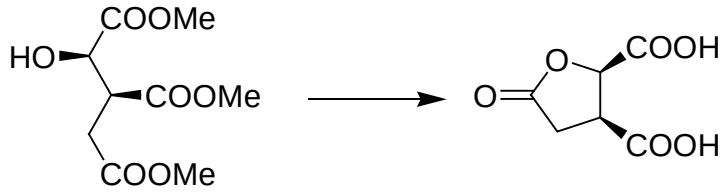
^{13}C-NMR (150 MHz, DMSO-d_6): δ = 30.6 (**C**H$_2$), 42.7 (CH$_2$**C**H), 52.4, 52.5 (2 x COO**C**H$_3$), 76.0 (**C**H(O)), 168.3, 170.3 (2 x **C**OOCH$_3$), 174.3 (**C**OOCH).

HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_8H_{10}O_6Na$: 225.03696; found 225.03694.

Elemental analysis: calcd for $C_8H_{10}O_6$: C 47.53, H 4.99; found: C 47.60, H 4.90.

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

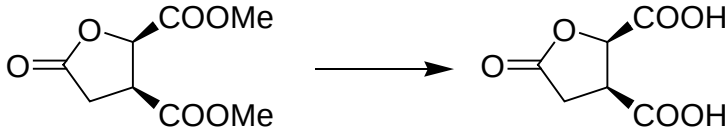
From (2*R*,3*S*)-Isocitric acid trimethylester **3**:



3 **6**

A solution of (2*R*,3*S*)-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 (2*R*,3*S*)-isocitric acid lactone-2,3-dicarboxylic acid **6** (1.74 g, 10.0 mmol, quantitative) as a colourless solid.

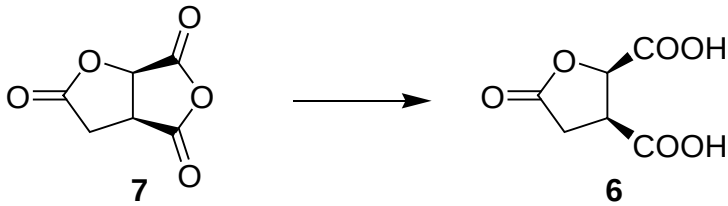
From (2*R*,3*S*)-isocitric acid lactone-2,3-dicarboxylic acid dimethylester **5**:[4]



5 **6**

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



7 **6**

A solution of (2*R*,3*S*)-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].[5]

$[\alpha]_D^{22}$ –69.9 (*c* 1.06, H_2O) [Lit: $[\alpha]_D^{22}$ –60.3 (*c* 1.03, H_2O)].[5]

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

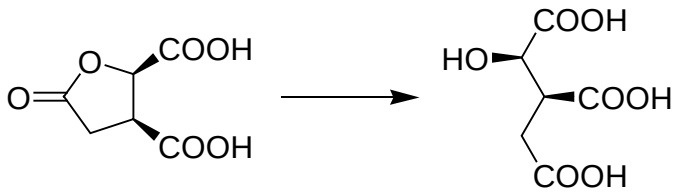
^{1}H-NMR (400 MHz, DMSO-d_6): δ = 2.67 (dd, ^2J = 17.2 Hz, ^3J = 6.8 Hz, 2 H, C**H**$_2$), 2.81 (dd, ^2J = 17.6 Hz, ^3J = 8.8 Hz, 2 H, C**H**$_2$), 3.67 (ddd, ^3J = 6.8, 7.6, 8.8 Hz, 1 H, C**H**COOH), 5.09 (d, ^3J = 8.0 Hz, 1 H, C**H**(O)COOH).

^{13}C-NMR (100 MHz, DMSO-d_6): δ = 31.3 (**C**H_2), 43.2 (CH_2**C**HCOOH), 76.8 (**C**H(O)COOH), 169.5 (CH(O)**C**OOH), 171.7 (**C**O), 175.2 (CH_2CH**C**OOH).

HRMS-ESI: *m/z* $[2M-H]^-$ calcd for $C_{12}H_{11}O_{12}$: 347.02560; found 347.02402.

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

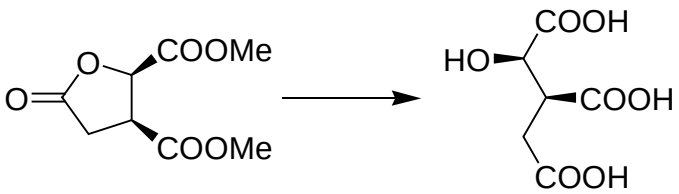
From (2*R*,3*S*)-isocitric acid lactone-2,3-dicarboxylic acid **6**:[6a]



6 **1**

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^3) 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 ^{1}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**:[6b]

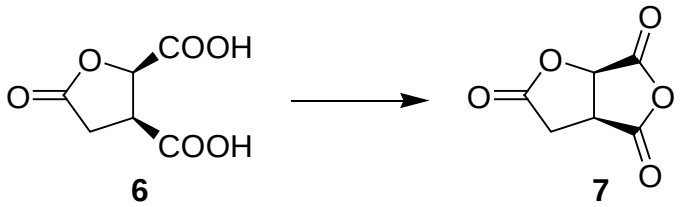


5 **1**

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 ^{1}H-NMR to be a 1 : 9 mixture of starting material **5** and the desired (2*R*,3*S*)-isocitric acid **1**.

$[\alpha]_D^{22}$ +15.8 (*c* 4.67, H_2O) [Lit: $[\alpha]_D^{22}$ +30.6 (H_2O),[6a] $[\alpha]_D^{22}$ +9.0[6c]].
IR (Film): $\tilde{\nu}$ = 3407, 1732, 1238 cm^{-1}.
^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.40-2.50 (m, 2 H, C**H$_2$**), 3.03-3.12 (m, 1 H, C**H**COOH), 4.15 (d, ^3J = 3.6 Hz, 1 H, C**H**OH**)**.
^{13}C-NMR (50 MHz, DMSO-d_6): δ = 32.2 (**C**H$_2$), 45.1, 70.3 (2 x **C**H), 172.6, 173.3, 173.8 (3 x **C**O).
HRMS-ESI: *m/z* $[M-H]^-$ calcd for $C_6H_7O_7$: 191.01973; found 191.01946.

2.5 (2*R*,3*S*)-Isocitric acid lactone-2,3-dicarboxylic acid anhydride 7[7]

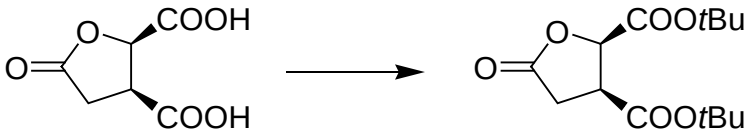


6 **7**

(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].[7]
$[\alpha]_D^{22}$ +11.4 (*c* 5.01, DMF).
IR (KBr): $\tilde{\nu}$ = 3039, 3005, 2962, 1850, 1798, 1775, 1412, 1160 cm^{-1}.
^{1}H-NMR (200 MHz, DMSO-d_6): δ = 2.80-3.08 (m, 2 H, C**H$_2$**), 4.02 (ddd, ^3J = 6.9, 8.7, 9.6 Hz, 1 H, C**H**C(O)O), 5.40 (d, ^3J = 8.7 Hz, 1 H, C**H**(O)COCH$_2$).
^{13}C-NMR (50 MHz, DMSO-d_6): δ = 29.9 (**C**H$_2$), 40.4, 76.3 (2 x **C**H), 168.0, 171.1, 174.1 (3 x **C**O).
HRMS-EI: *m/z* $[M+H]^+$ calcd for $C_6H_5O_5$: 157.01370; found 157.00927.

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



6 **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_2Cl_2 (4 mL) and cooled to –25 °C. Catalytic amounts of conc. H_2SO_4 (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 $NaHCO_3$ solution (20 mL). Et_2O (50 mL) was added, the layers were separated and the organic layer was washed with water (20 mL). After drying with Na_2SO_4 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.

$[\alpha]_D^{22}$ –47.3 (*c* 1.02, toluene).

R_f = 0.87 (*n*-hexane-EtOAc 10:1 v/v).

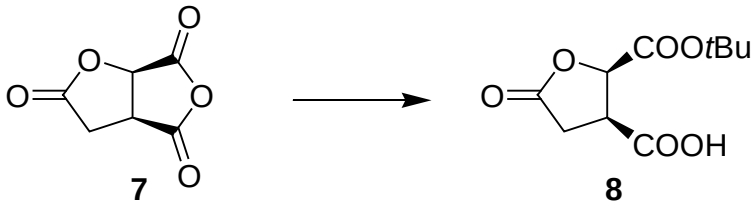
IR (KBr): $\tilde{\nu}$ = 2981, 2937, 1792, 1739, 1730, 1370, 1153 cm^{-1}.

^{1}H-NMR (600 MHz, DMSO-d_6): δ = 1.40 (s, 9 H, 3 x C**H$_3$**), 1.42 (s, 9 H, 3 x C**H$_3$**), 2.68 (dd, ^2J = 17.5 Hz, ^3J = 7.2 Hz, 1 H, C**H**$_2$), 2.83 (dd, ^2J = 17.5 Hz, ^3J = 9.3 Hz, 1 H, C**H**$_2$), 3.68 (ddd, ^3J = 7.2, 7.9, 9.3 Hz, 1 H, CH_2C**H**), 5.05 (d, ^3J = 7.9 Hz, 1 H, C**H**(O)).

^{13}C-NMR (150 MHz, DMSO-d_6): δ = 27.5 (3 x **C**H_3), 27.6 (3 x **C**H_3), 31.0 (**C**H_2COO), 43.4 (CH_2**C**H), 76.7 (**C**H(O)), 81.7 (**C(**$CH_3)_3$), 82.6 (**C(**$CH_3)_3$), 166.6 (CH(O)**C**), 168.9 (CH_2**C**O(O)), 174.4 (CH**C**OOC$(CH_3)_3$).

HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{14}H_{22}O_6Na$: 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).

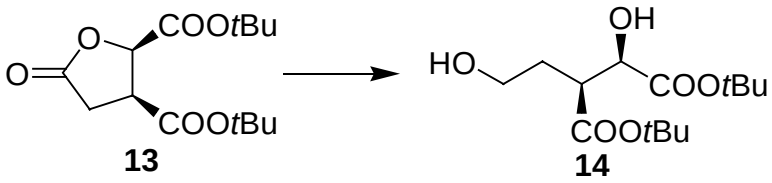
IR (KBr): $\tilde{\nu}$ = 3268, 3012, 2996, 2946, 1778, 1746, 1709, 1373, 1152 cm^{-1}.

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

^{13}C-NMR (150 MHz, DMSO-d_6): δ = 27.4 (3 x **C**H$_3$), 30.6 (**C**H$_2$), 42.9 (**C**HCOOH), 76.5 (**C**H(O)), 82.5 (COO**C**(CH$_3$)$_3$), 166.7 (**C**OOC(CH$_3$)$_3$), 171.1 (**C**O(O)), 174.6 (**C**OOH).

HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{10}H_{14}O_6Na$: 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_2O (2 x 50 mL). After drying the organic layer with Na_2SO_4 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).

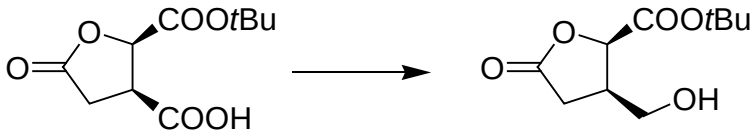
IR (film): $\tilde{\nu}$ = 3446, 2978, 2934, 1732, 1369, 1152 cm^{-1}.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.37 (s, 9 H, 3 x C**H$_3$**), 1.40 (s, 9 H, 3 x C**H$_3$**), 1.47-1.55 (m, 1 H, C**H**$_2$), 1.64-1.73 (m, 1 H, C**H**$_2$), 2.64 (td, ^3J = 9.8, 5.1 Hz, 1 H, C**H**), 3.28-3.36 (m, 1 H, C**H**$_2$), 3.37-3.44 (m, 1 H, C**H**$_2$), 3.99 (t, ^3J = 6.0 Hz, 1 H, CH_2O**H**), 4.46 (t, ^3J = 4.8 Hz, 1 H, C**H**(OH)), 5.32 (d, ^3J = 5.6 Hz, 1 H, CHO**H**).

^{13}C-NMR (75 MHz, DMSO-d_6): δ = 27.7 (6 x **C**H_3), 30.7 (**C**H_2), 46.5 (**C**H), 57.8 (**C**H_2OH), 71.7 (**C**HOH), 79.8, 80.5 (2 x **C**$(CH_3)_3$), 171.2, 171.5 (2 x **C**O).

HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{14}H_{26}O_6Na$: 313.16216; found 313.16199.

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



8 **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 ($CHCl_3$-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 ($CHCl_3$-MeOH 10:1 v/v).

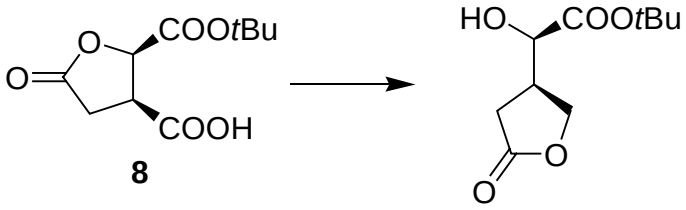
IR (film): $\tilde{\nu}$ = 3520, 3004, 2934, 2885, 1791, 1724, 1342, 1155 cm^{-1}.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.42 (s, 9 H, 3 x C**H**$_3$), 2.36 (dd, ^2J = 17.4 Hz, ^3J = 8.5 Hz, 1 H, C**H**$_2$), 2.59 (dd, ^2J = 17.6 Hz, ^3J = 8.7 Hz, 1 H, C**H**$_2$), 2.82-2.95 (m, 1 H, C**H**), 3.33-3.40 (m, 1 H, C**H**), 3.42-3.51 (m, 1 H, C**H**$_2$)), 4.83 (t, ^3J = 4.8 Hz, 1 H, O**H**), 4.90 (d, ^3J = 7.8 Hz, 1 H, C**H**COO).

^{13}C-NMR (75 MHz, $CDCl_3$): δ = 27.5 (**C**H) 27.6 (3 x **C**H_3), 30.5, 59.2 (2 x **C**H_2), 77.4 (**C**H), 82.2 (**C**$(CH_3)_3$), 167.5, 175.9 (2 x **C**O).

HRMS-ESI: *m/z* $[2M+Na]^+$ calcd for $C_{20}H_{32}O_{10}Na$: 455.18877; found 455.18863.

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



8 **11**

This reaction was carried out analogously to **2.9**, workup included dilution with EtOAc and washing the organic layer with saturated aqueous NH_4Cl solution. After drying the organic layer with Na_2SO_4 the solvent was removed under reduced pressure to give a colourless solid, which was purified by column chromatography ($CHCl_3$-MeOH 30:1 v/v) to yield pure **11** (78%) as colourless crystals.

M.p.: 81-83 °C.

$[\alpha]_D^{22}$ +9.8 (*c* 1.02, MeOH).

R_f = 0.60 ($CHCl_3$-MeOH 10:1 v/v).

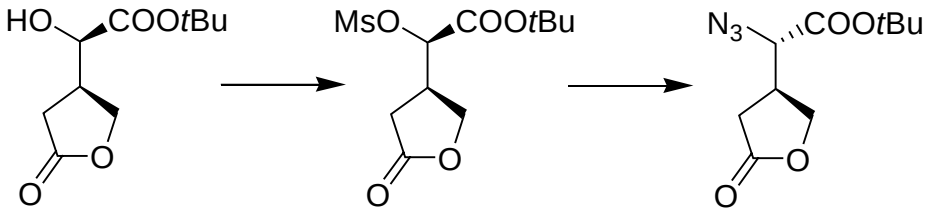
IR (KBr): $\tilde{\nu}$ = 3423, 1773, 1716, 1122 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.49 (s, 9 H, 3 x C**H**$_3$), 2.61 (d, 2 H, ^3J = 8.4 Hz, C**H**$_2$), 2.85-2.95 (m, 1 H, C**H**CH), 4.07 (d, 1 H, ^3J = 5.6 Hz, C**H**OH), 4.19 (dd, 1 H, ^2J = 9.2 Hz, ^3J = 6.8 Hz, C**H**$_2$(O)), 4.32 (dd, 1 H, ^2J = 9.2 Hz, ^3J = 8.4 Hz, C**H**$_2$(O)).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.1 (3 x **C**H_3), 31.0 (CH_2**C**HCH_2), 38.9 (**C**H_2CH), 68.8 (CH**C**H_2), 70.8 (**C**HOH), 84.2 (**C**$(CH_3)_3$), 172.3 (**C**O), 176.5 (CH_2**C**O).

HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{10}H_{16}O_5Na$: 239.08899; found 239.08905, $[2M+Na]^+$ calcd for $C_{20}H_{32}O_{10}Na$: 455.18877; found 455.18863.

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



11 **11a** **11b**

tert-Butyl (2*R*)-hydroxy[(3*S*)-5-oxotetrahydrofuran-3-yl)acetate **11** (726 mg, 3.36 mmol) was dissolved in anhydrous CH_2Cl_2 (25 mL). At 0 °C pyridine (268 mg, 3.39 mmol) was added followed by methane sulfonyl chloride (387 mg, 3.38 mmol). After stirring for 16 h at RT the organic layer was washed with saturated aqueous $NaHCO_3$ solution (30 mL) and saturated aqueous NaCl solution (30 mL). After drying the organic layer with Na_2SO_4 the solvent was removed under reduced pressure. The resulting oil was dissolved in anhydrous DMF (50 mL), NaN_3 (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_2SO_4 the solvent was removed under reduced pressure. The resulting oil was purified by column chromatography ($CHCl_3$-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, $CHCl_3$).

R_f = 0.77 ($CHCl_3$-MeOH 10:1 v/v).

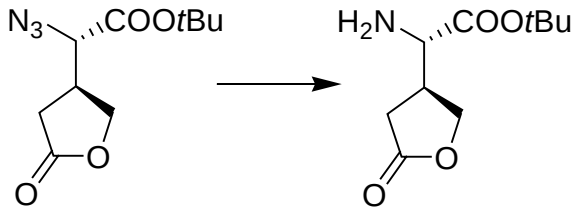
IR (KBr): $\tilde{\nu}$ = 2980, 2934, 2114, 1782, 1735, 1370, 1258, 1226, 1151, 1086, 1027, 626 cm^{-1}.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.51 (s, 9 H, 3 x C**H**$_3$), 2.50 (dd, 1 H, ^2J = 18.0 Hz, ^3J = 7.2 Hz, C**H**$_2$), 2.60 (dd, 1 H, ^2J = 18.0 Hz, ^3J = 8.8 Hz, C**H**$_2$), 2.90-3.05 (m, 1 H, C**H**), 3.98 (d, 1 H, ^3J = 6.0 Hz, C**H**N_3), 4.16 (dd, 1 H, ^2J = 9.2 Hz, ^3J = 6.4 Hz, C**H**$_2$), 4.42 (dd, 1 H, ^2J = 9.2 Hz, ^3J = 7.8 Hz, C**H**$_2$).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.1 (3 x **C**H_3), 30.3 (**C**H_2) 36.8 (**C**H), 63.5 (**C**H), 69.7 (**C**H_2), 84.6 (**C**$(CH_3)_3$), 167.4, 175.7 (2 x **C**O).

HRMS-ESI: *m/z* $[M+Na]^+$ calcd for $C_{10}H_{15}N_3O_4Na$: 264.09548; found 264.09541, $[2M+Na]^+$ calcd for $C_{20}H_{30}N_6O_8Na$: 505.20173; found 505.20127.

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



11b **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, $CHCl_3$).

R_f = 0.52 ($CHCl_3$-MeOH 10:1 v/v).

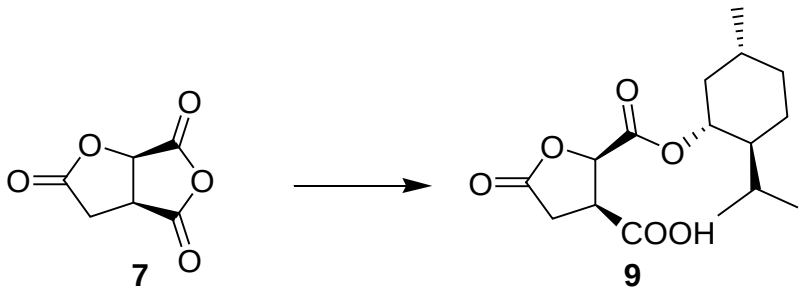
IR (KBr): $\tilde{\nu}$ = 3435, 2980, 2934, 1770, 1727, 1650, 1394, 1370, 1251, 1154, 626 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.46 (s, 9 H, 3 x C**H**$_3$), 1.81 (br, 2 H, N**H**$_2$), 2.51 (d, 1 H, ^3J = 1.2 Hz, C**H**$_2$), 2.53 (d, 1 H, ^3J = 1.2 Hz, C**H**$_2$), 2.80-2.89 (m, 1 H, C**H**), 3.36 (d, 1 H, ^3J = 6.0 Hz, C**H**NH$_2$), 4.21 (dd, 1 H, ^2J = 9.6 Hz, ^3J = 6.4 Hz, C**H**$_2$), 4.40 (dd, 1 H, ^2J = 9.6 Hz, ^3J = 7.2 Hz, C**H**$_2$).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.0 (3 x **C**H$_3$), 30.5 (**C**H$_2$) 39.4 (**C**H), 56.0 (**C**HNH$_2$), 70.6 (**C**H$_2$), 82.6 (**C**(CH$_3$)$_3$), 173.0, 176.7 (2 x **C**O).

HRMS-ESI: *m/z* $[2M+H]^+$ calcd for $C_{20}H_{35}N_2O_8$: 431.23879; found 431.23900, $[3M+H]^+$ calcd for $C_{30}H_{52}N_3O_{12}$: 646.35455; found 646.35510, $[4M+H]^+$ calcd for $C_{40}H_{69}N_4O_{16}$: 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



7 **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.

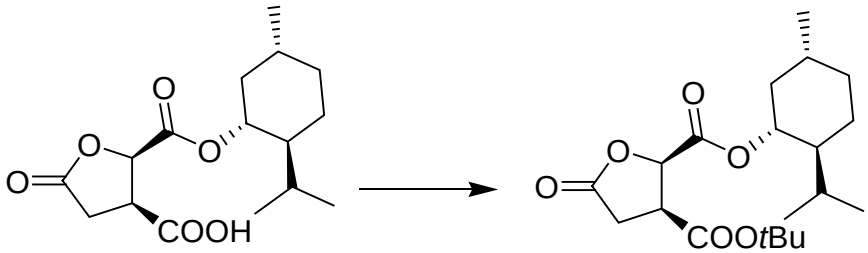
IR (KBr): $\tilde{\nu}$ = 3212, 2954, 2937, 2860, 1777, 1754, 1184, 1056 cm^{-1}.

^{1}H-NMR (400 MHz, CD_3OD): δ = 0.76 (d, 3 H, ^3J = 6.8 Hz, C**H$_3$**), 0.90 (d, 3 H, ^3J = 7.6 Hz, C**H$_3$**), 0.92 (d, 3 H, ^3J = 7.6 Hz, C**H$_3$**), 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, C**H**$(CH_3)_2$), 2.00-2.08 (m, 1 H), 2.81 (dd, 1 H, ^2J = 17.6 Hz, ^3J = 9.6 Hz, (O)CC**H$_2$**), 2.92 (dd, 1 H, ^2J = 17.6 Hz, ^3J = 8.0 Hz, (O)CC**H$_2$**), 3.79-3.86 (m, 1 H, C**H**COOH), 4.92 (dt, 1 H, ^3J = 11.2, 4.4 Hz, OC**H**CH_2), 5.11 (d, 1 H, ^3J = 8.4 Hz, C**H**COO).

^{13}C-NMR (100 MHz, CD_3OD): δ = 16.4, 21.1, 22.4 (3 x **C**H_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 **C**O).

HRMS-ESI: *m/z* $[M-H]^-$ calcd for $C_{16}H_{23}O_6$: 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 CH_2Cl_2 (3 mL) and cooled to –20 °C. Catalytic amounts of conc. H_2SO_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 CH_2Cl_2 (10 mL) and washed with saturated aqueous $NaHCO_3$ solution (5 mL). After drying with $MgSO_4$ 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, $CHCl_3$).

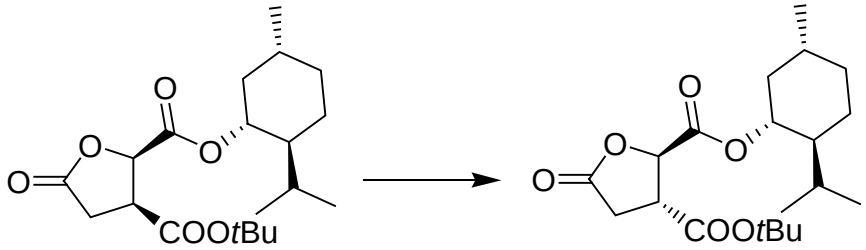
IR (KBr): $\tilde{\nu}$ = 2962, 2931, 2859, 1800, 1745, 1718, 1144, 1036 cm^{-1}.

^{1}H-NMR (400 MHz, $CDCl_3$): δ= 0.75 (d, ^3J = 6.8 Hz, 3 H, C**H$_3$**), 0.89 (d, ^3J = 7.2 Hz, 3 H, C**H$_3$**), 0.91 (d, ^3J = 7.2 Hz, 3 H, C**H$_3$**), 0.94-1.10 (m, 3 H), 1.44 (s, 9 H, 3 x C**H$_3$**), 1.38-1.54 (m, 2 H), 1.63-1.74 (m, 2 H), 1.91 (septd, ^3J = 7.6, 2.8 Hz, 1 H, C**H**$(CH_3)_2$), 1.98-2.07 (m, 1 H), 2.65 (dd, ^2J = 17.6 Hz, ^3J = 9.2 Hz, 1 H, (O)CC**H$_2$**), 3.05 (dd, ^2J = 17.6 Hz, ^3J = 8.0 Hz, 1 H, (O)CC**H$_2$**), 3.59 (m, 1 H, CH_2C**H**COO), 4.73 (dt, ^3J = 11.2, 4.8 Hz, 1 H, OC**H**CH_2), 5.00 (d, ^3J = 8.0 Hz, 1 H, OC**H**COO).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 16.3, 20.9, 22.1 (3 x **C**H_3), 23.3, 26.0, 28.0 (3 x **C**H_3), 30.3, 31.5, 34.2, 40.6, 44.4, 47.0, 77.0, 77.3, 83.2 (**C**$(CH_3)_3$), 167.4, 167.8, 174.5 (3 x **C**O).

HRMS-ESI: *m/z*: $[M+Na]^+$ calcd for $C_{20}H_{32}O_6Na$: 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_2Cl_2 (4 mL) and DBU (0.15 mL) was heated to 50 °C for 1 h. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with saturated aqueous NH_4Cl solution. After drying with $MgSO_4$ 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_f = 0.20 (*n*-hexane/EtOAc, 10:1 v/v)

$[\alpha]_D^{24}$ –83.0 (c 2.17; $CHCl_3$).

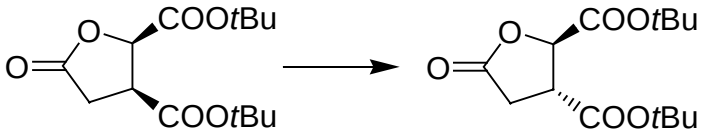
IR (KBr): $\tilde{\nu}$ = 2957, 2942, 2871, 1809, 1741, 1723, 1152, 1036 cm^{-1}.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 0.76 (d, ^3J = 6.3 Hz, 3 H, C**H$_3$**), 0.89 (d, ^3J = 6.3 Hz, 3 H, C**H$_3$**), 0.91 (d, ^3J = 6.3 Hz, 3 H, C**H$_3$**), 0.91-1.07 (m, 2 H), 1.48 (s, 9 H, 3 x C**H$_3$**), 1.40-1.54 (m, 3 H), 1.67-1.72 (m, 2 H), 1.84 (dtd, ^3J = 13.9, 7.0, 2.8 Hz, 1 H, C**H**CH$(CH_3)_2$), 1.97-2.03 (m, 1 H), 2.81 (d, ^3J = 8.6 Hz, 1 H, (O)CC**H$_2$**), 2.82 (d, ^3J = 7.0 Hz, 1 H, (O)CC**H$_2$**), 3.31 (ddd, ^3J = 8.6, 7.0, 4.7 Hz, 1 H, CH_2C**H**COO), 4.78 (dt, ^3J = 10.9, 3.9 Hz, 1 H, OC**H**CH_2), 5.04 (d, ^3J = 4.7 Hz, 1 H, OC**H**COO).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 16.3, 20.9, 22.0 (3 x **C**H_3), 23.4, 26.4, 28.0 (3 x **C**H_3), 30.8, 31.5, 34.2, 40.7, 44.6, 46.9, 76.9, 77.8, 83.3 (**C**$(CH_3)_3$), 168.3, 169.4, 173.9 (3 x **C**O).

HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{20}H_{32}O_6Na$: 391.20911, found: 391.20912.

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



13 **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_2Cl_2 (4 mL) and DBU (0.75 mL) were heated to 50 °C for 30 min. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with saturated aqueous NH_4Cl solution (5 mL). After drying with $MgSO_4$ 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_f = 0.32 (*n*-hexane/EtOAc 5:1 v/v).

$[\alpha]_D^{24}$ –44.7 (*c* 1.21, $CHCl_3$).

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

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.48 (s, 9 H, 3 x C**H$_3$**), 1.50 (s, 9 H, 3 x C**H$_3$**), 2.80 (d, ^3J = 7.9 Hz, 1 H, C**H$_2$**), 2.81 (d, ^3J = 7.5 Hz, 1 H, C**H$_2$**), 3.30 (ddd, ^3J = 7.9, 7.5, 4.9 Hz, 1 H, CH_2C**H**), 4.94 (d, ^3J = 4.9 Hz, 1 H, C**H**(O)).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 28.0 (3 x **C**H_3), 28.1 (3 x **C**H_3), 30.8 (**C**H_2COO), 44.6 (CH_2**C**H), 78.2 (**C**H(O)), 83.2 (**C**$(CH_3)_3$), 83.8 (**C**$(CH_3)_3$), 167.7, 169.4, 174.1 (3 x **C**O).

HRMS-ESI (*m/z*): $[M+Na]^+$ calcd for $C_{20}H_{32}O_6Na$: 309.13086, found: 309.13103.

3. References

[1] U. Behrens, E. Weißbrodt, W. Lehmann, *Z. Allg. Mikrobiol.* **1978**, *18*, 549-558.

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

[3] W. F. Bruce, *J. Am. Chem. Soc.* **1935**, *57*, 1725-1729.

[5] C. Schmitz, A.-C. Rouanet-Dreyfuss, M. Tueni, J.-F. Biellmann, *J. Org. Chem.* **1996**, *61*, 1817-1821.

[6] a) J. P. Greenstein, N. Izumiya, M. Winitz, S. M. Birnbaum, *J. Am. Chem. Soc.* **1955**, *77(3)*, 707-716; b) G. W. Pucher, M. D. Abraham, H. Bradford Vickery, *J. Biol. Chem.* **1948**, *172*, 579-588; c) S. B. Singh, D. L. Zink, G. A. Doss, J. D. Polishook, C. Ruby, E. Register, T. M. Kelly, C. Bonfiglio, J. M. Williamson, R. Kelly, *Org. Lett.* **2004**, *6(3)*, 337-340.

[7] R. Carrington, G. Halek, *Ger. Offen.* **1973**, DE226473.

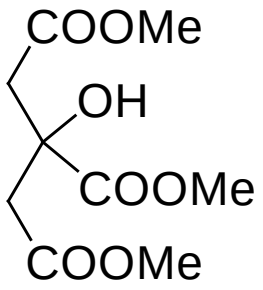
4. NMR-Spectra

P.Heretsch; PH8-2B2

Pulse Sequence: s2pul

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

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 87.5 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
8 repetitions
OBSERVE H1, 300.0629296 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 42 sec



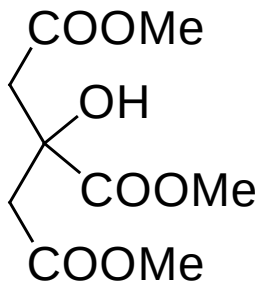
4

P.Heretsch; PH 8-2B3

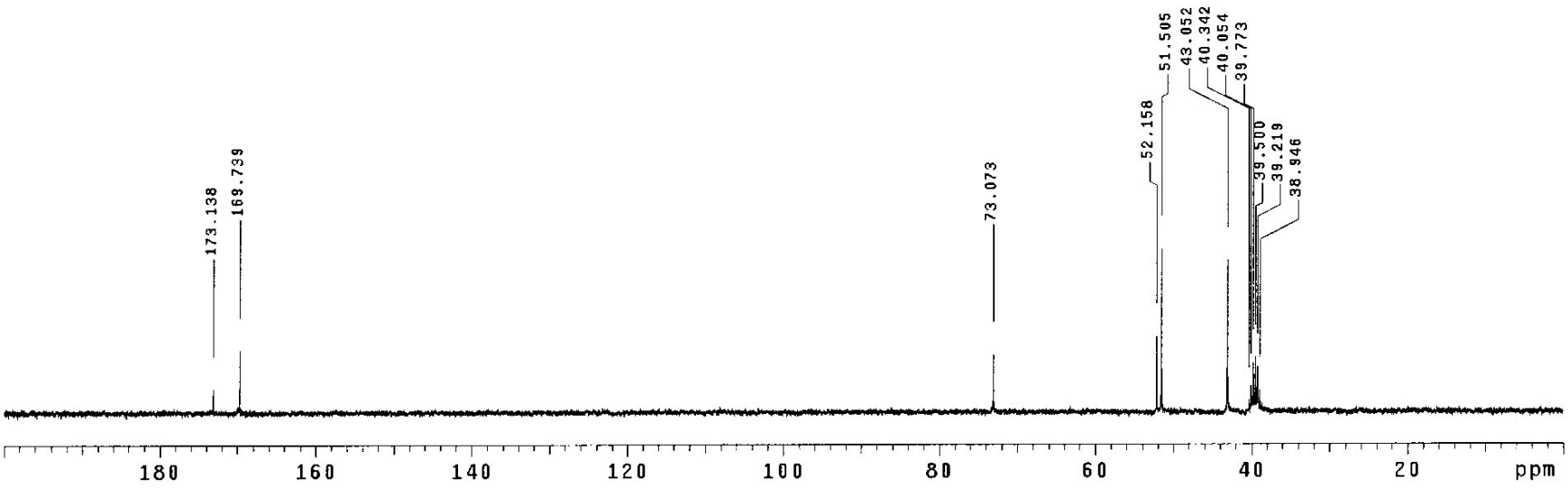
Pulse Sequence: s2pul

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

PULSE SEQUENCE
Relax. delay 2.000 sec
Pulse 68.4 degrees
Acq. time 1.500 sec
Width 18761.7 Hz
48 repetitions
OBSERVE C13, 75.4509172 MHz
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



4

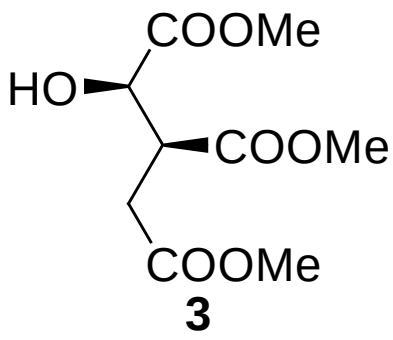


P.Neretsch; PH8-6

Pulse Sequence: s2pul

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

PULSE SEQUENCE
Relax. delay 1.000 sec
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



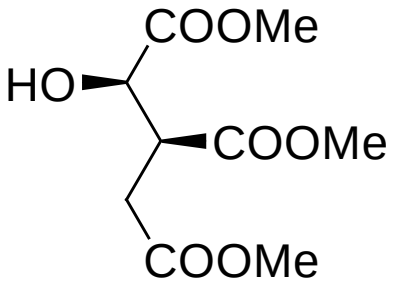
3

P.Heretsch; PH8-6

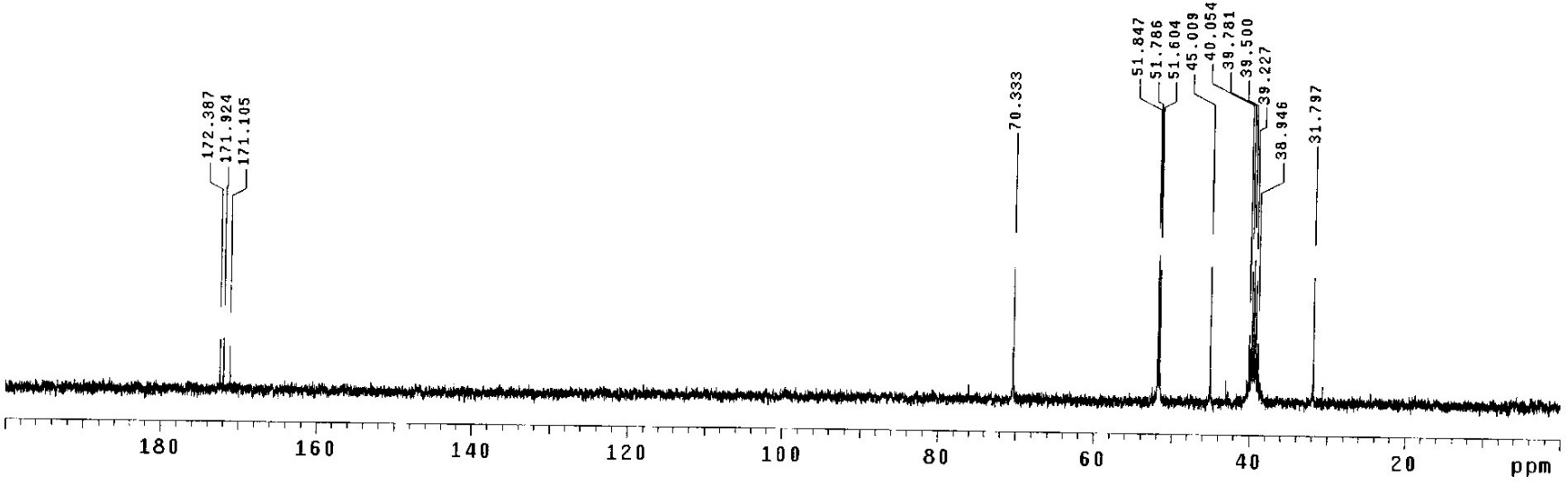
Pulse Sequence: s2pul

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

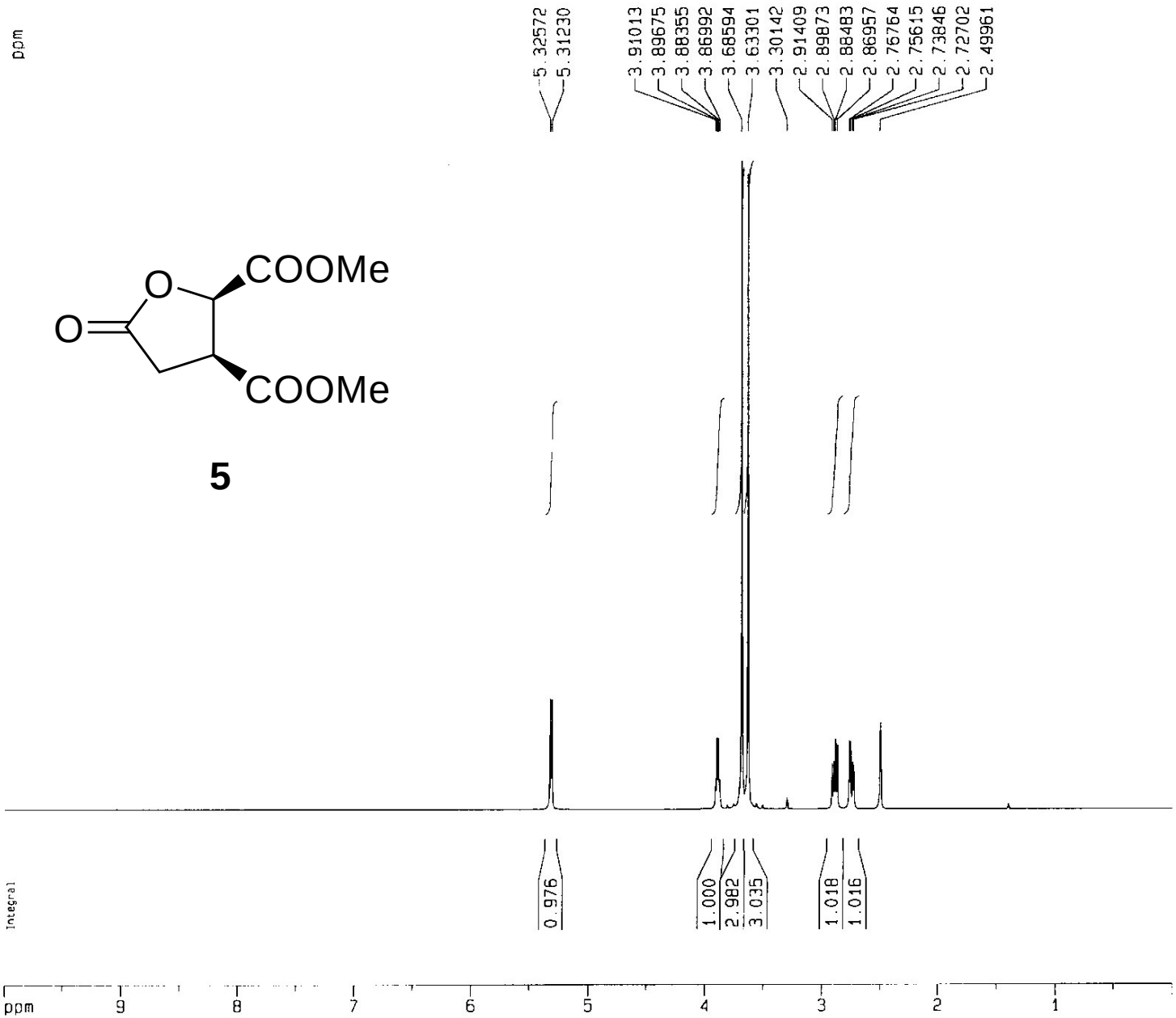
PULSE SEQUENCE
Relax. delay 2.000 sec
Pulse 68.4 degrees
Acq. time 1.500 sec
Width 18761.7 Hz
96 repetitions
OBSERVE C13, 75.4509184 MHz
DECOUPLE H1, 300.0644431 MHz
Power 36 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 1 hr, 3 min, 2 sec



3



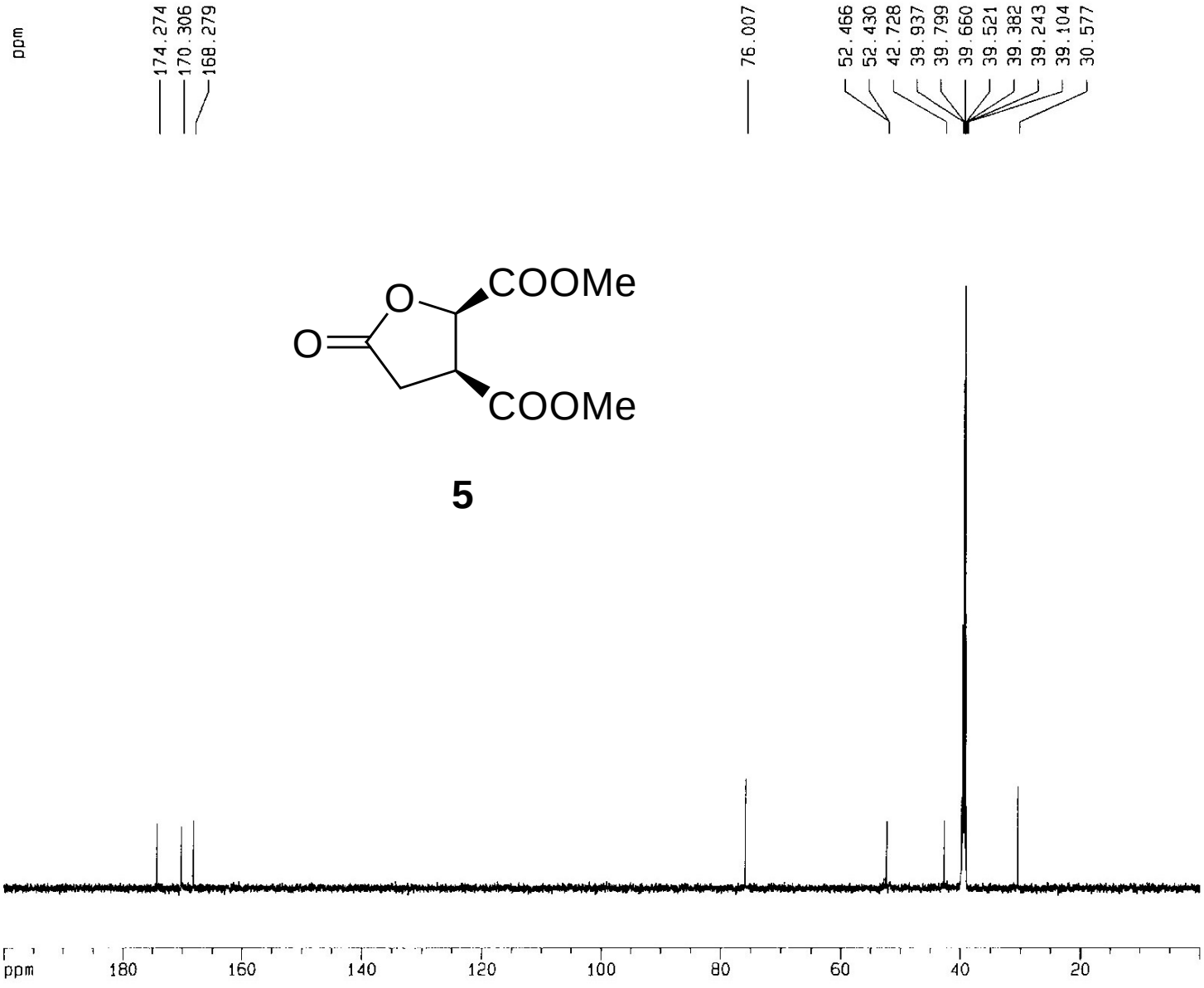
Ph. Heretsch; PH8-26; 1H



5

```
Current Data Parameters
NAME                 org299
EXPNO                     1
PROCNO                    1

F2 - Acquisition Parameters
Date_             20050517
Time                  9.45
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                   574.7
DW                  58.400 usec
DE                   6.00 usec
TE                  300.0 K
D1              2.00000000 sec
MCREST          0.00000000 sec
MCWRK           0.01500000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   7.30 usec
PL1                  5.00 dB
SFO1          600.1339172 MHz

F2 - Processing parameters
SI                   16384
SF            600.1300063 MHz
WDW                     EM
SSB                      0
LB                    0.10 Hz
GB                       0
PC                    1.00

1D NMR plot parameters
CX                   20.00 cm
CY                   11.00 cm
F1P                 10.000 ppm
F1                6001.30 Hz
F2P                  0.000 ppm
F2                    0.00 Hz
PPMCM               0.50000 ppm/cm
HZCM              300.06500 Hz/cm
```

Ph. Heretsch; PH8-2G; 13C




```
Current Data Parameters
NAME                 org299
EXPNO                     2
PROCNO                    1

F2 - Acquisition Parameters
Date_             20050517
Time                  9.53
INSTRUM               spect
PROBHD   5 mm TBI 1H/13
PULPROG               zgdc
TD                    65536
SOLVENT               DMSO
NS                      109
DS                        4
SWH              37593.984 Hz
FIDRES           0.573639 Hz
AQ               0.8716921 sec
RG                  2580.3
DW                 13.300 usec
DE                  6.00 usec
TE                  300.0 K
D1               3.00000000 sec
d11              0.03000000 sec
MCREST           0.00000000 sec
MCWRK            0.01500000 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          600.1324005 MHz

F2 - Processing parameters
SI                    32768
SF            150.9028821 MHz
WDW                   EM
SSB                   0
LB                  2.00 Hz
GB                    0
PC                  1.40

1D NMR plot parameters
CX                 20.00 cm
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
```

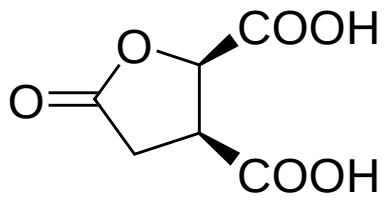
nomas

Sample: FT-IC 40

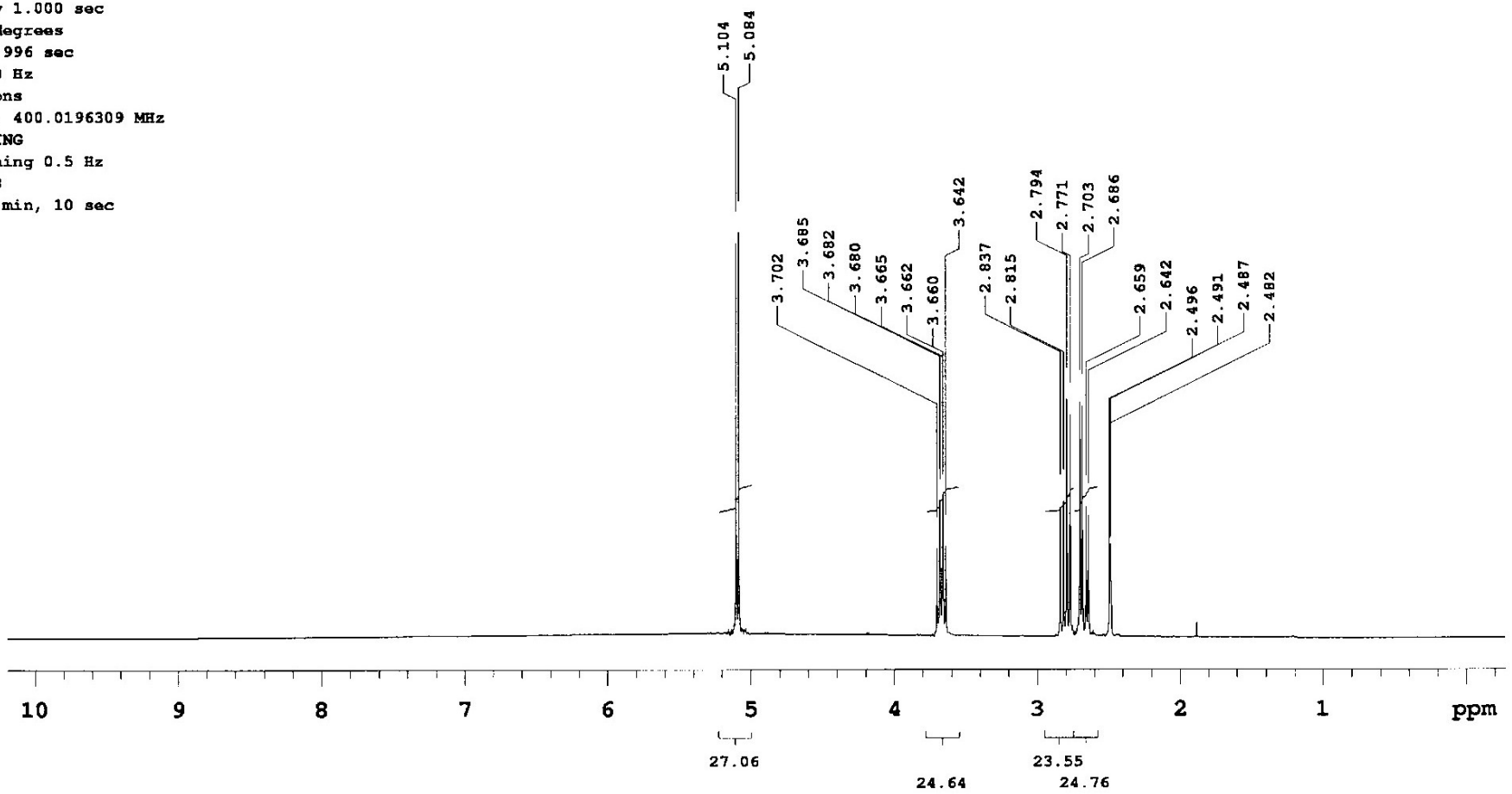
Pulse Sequence: s2pul
Date: Sep 10 2007

Solvent: dmso
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, 400.0196309 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 32768
Total time 1 min, 10 sec



6



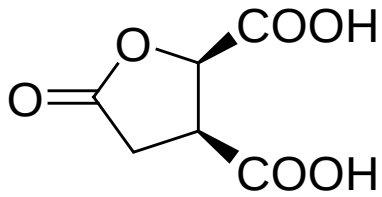
F. Thomas

Sample: FT-IC 40

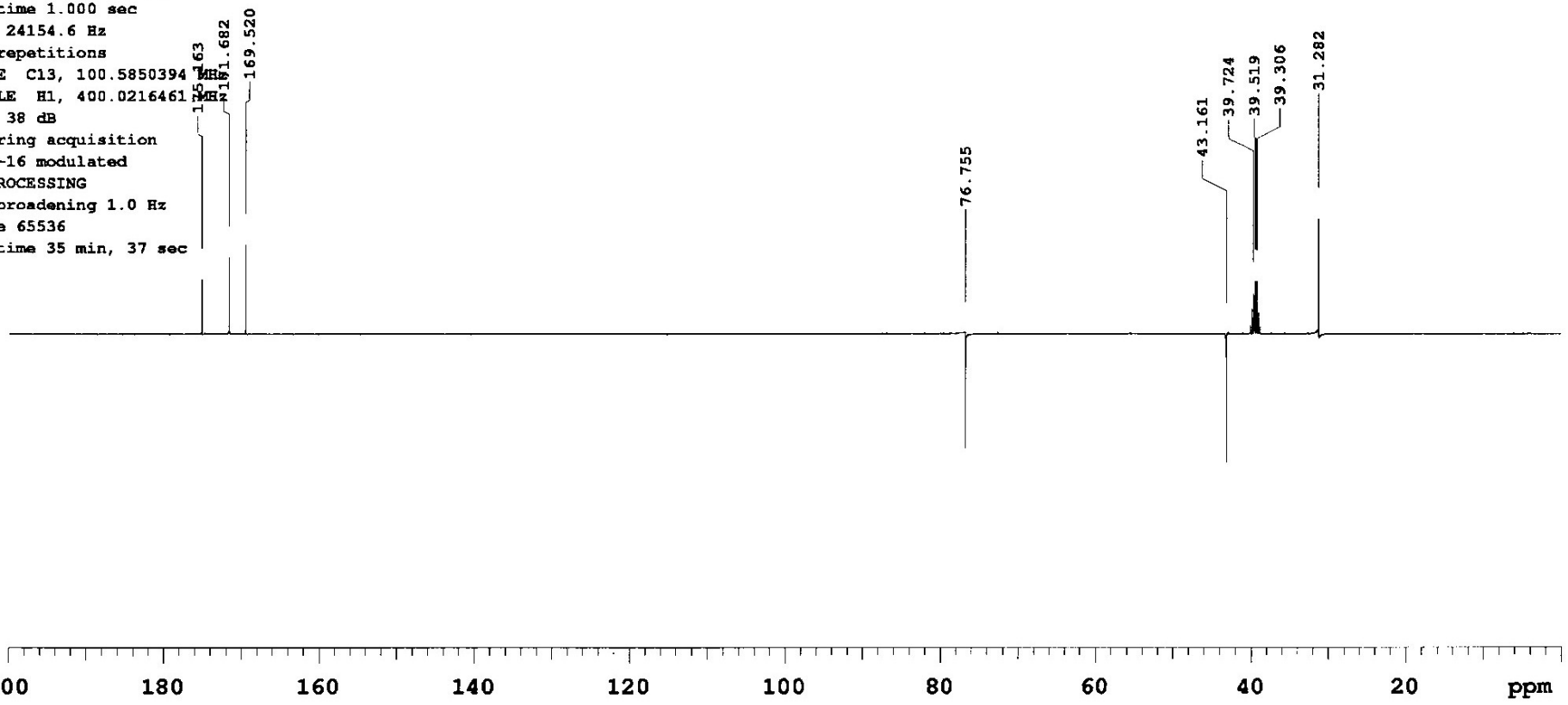
Pulse Sequence: APT
Date: Sep 11 2007

Solvent: dmso
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 MHz
DECOUPLE H1, 400.0216461 MHz
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



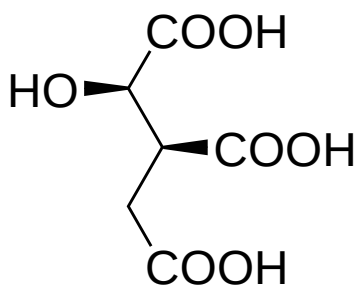
6



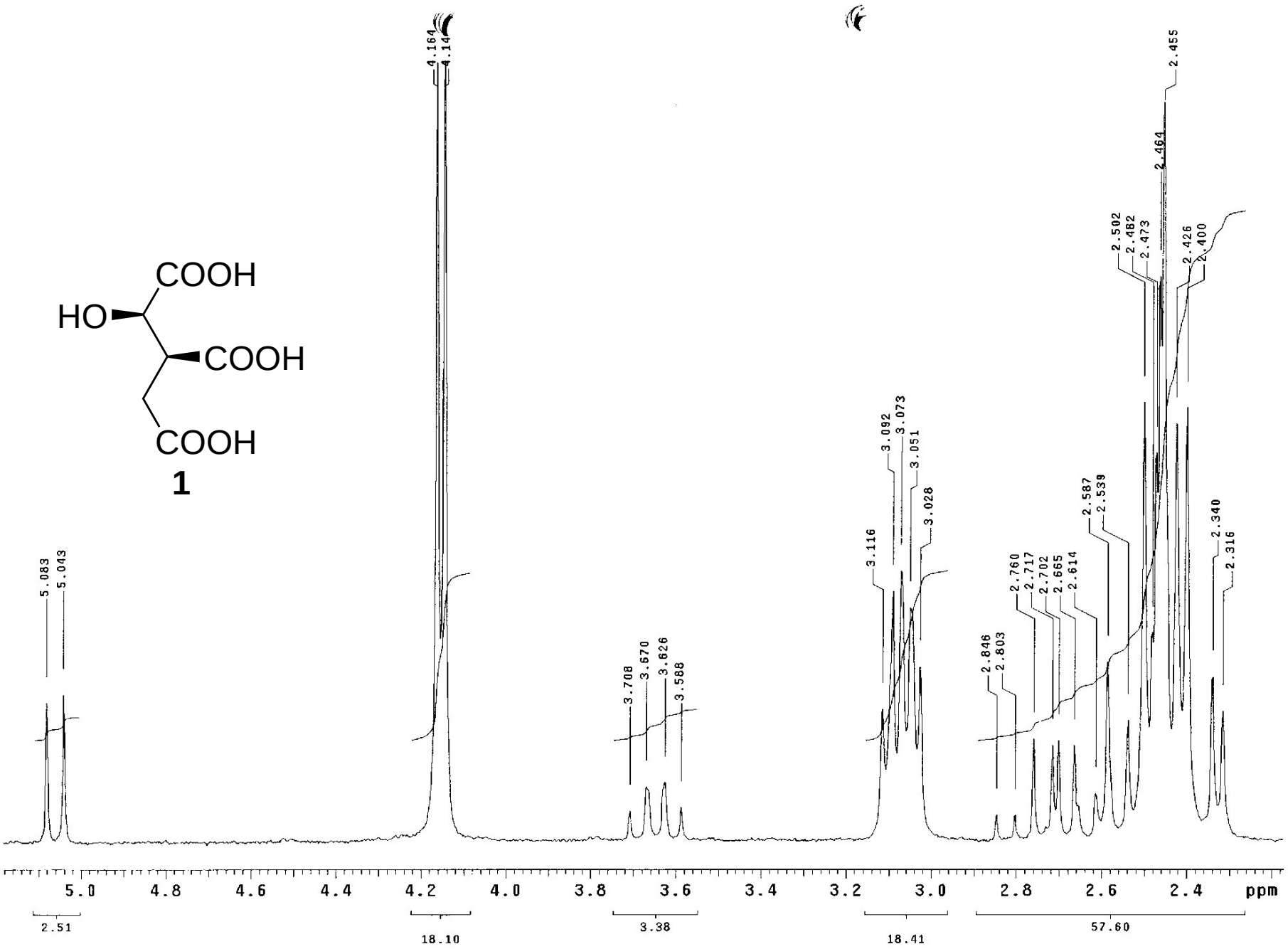
F. Thomas; FTIC25

Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
GEMINI-2000B "paul"

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



1

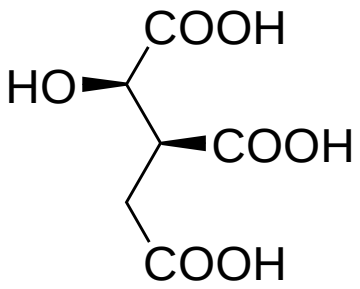

COOH
HO
COOH
COOH
1
5.083
5.043
4.164
4.144
3.708
3.670
3.626
3.588
3.116
3.092
3.073
3.051
3.028
2.846
2.803
2.760
2.717
2.702
2.665
2.614
2.587
2.539
2.502
2.482
2.473
2.464
2.455
2.426
2.400
2.340
2.316
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
ppm
2.51
18.10
3.38
18.41
57.60

F. Thomas; FTIC25

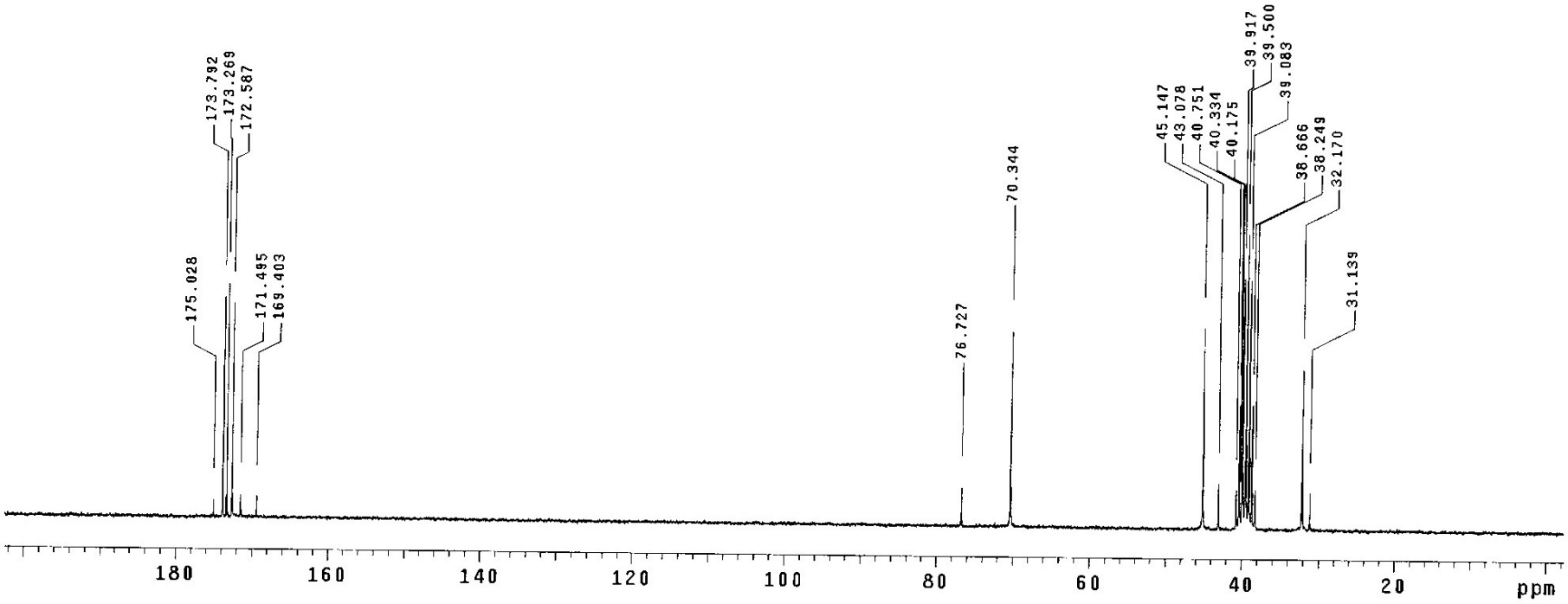
Pulse Sequence: s2pul

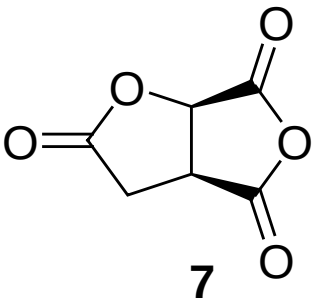
Solvent: DMSO
Ambient temperature
GEMINI-200BB "paul"

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



1

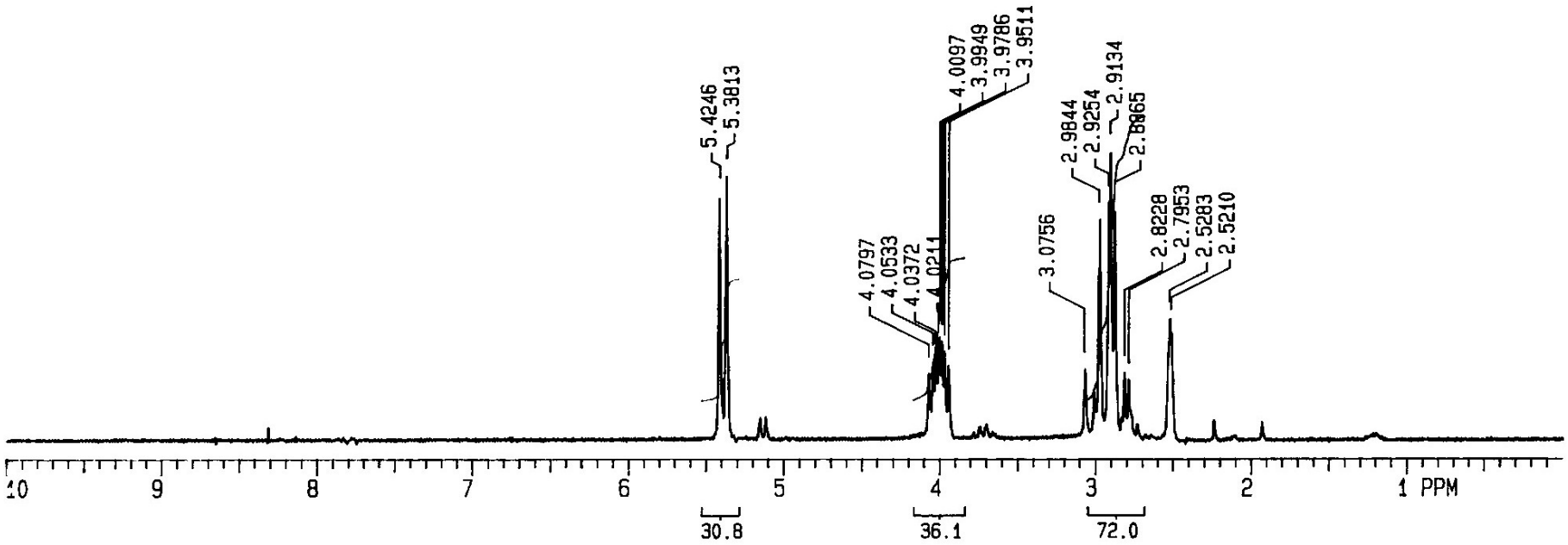


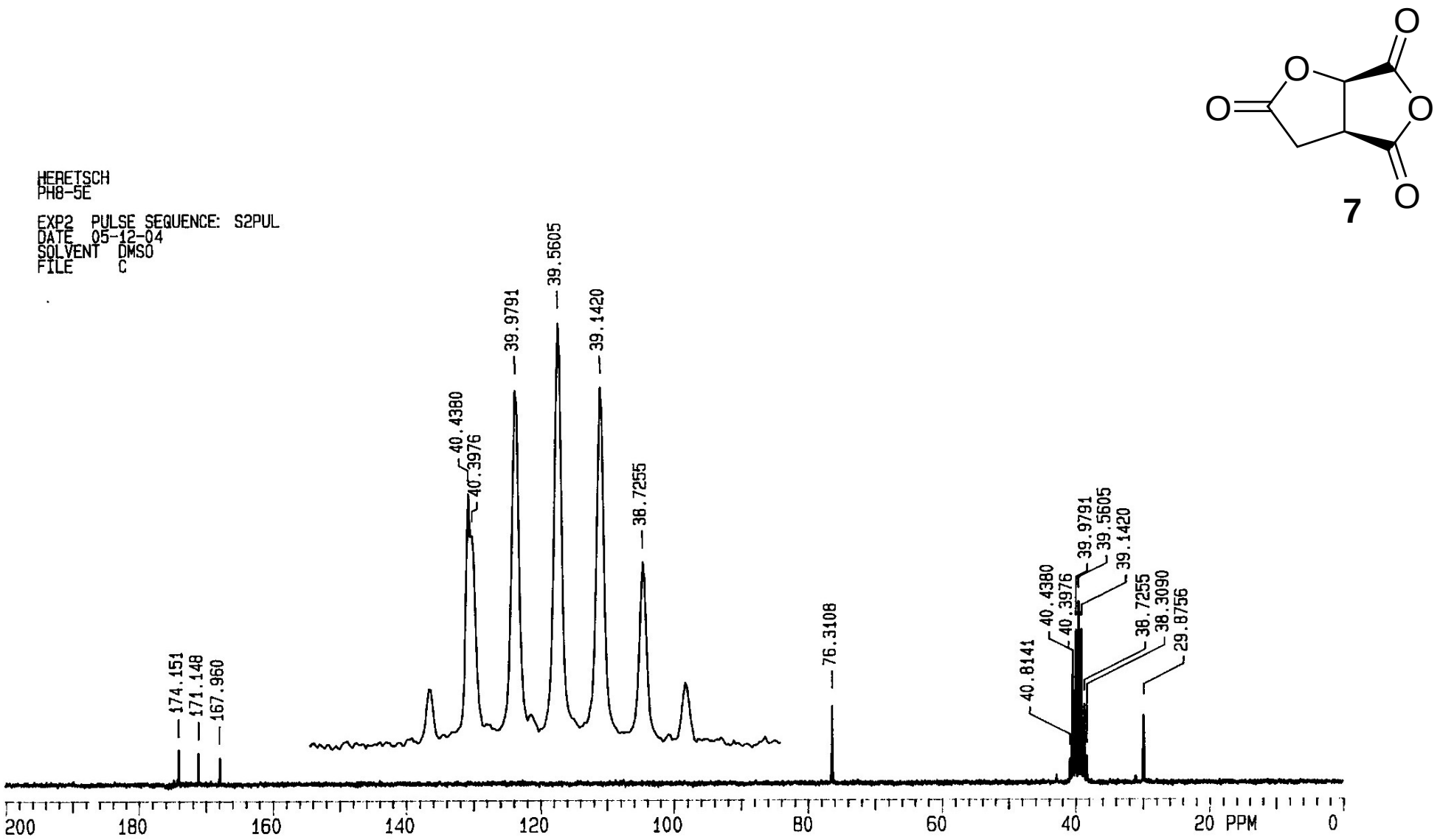


7

HERETSCH
PH8-5E

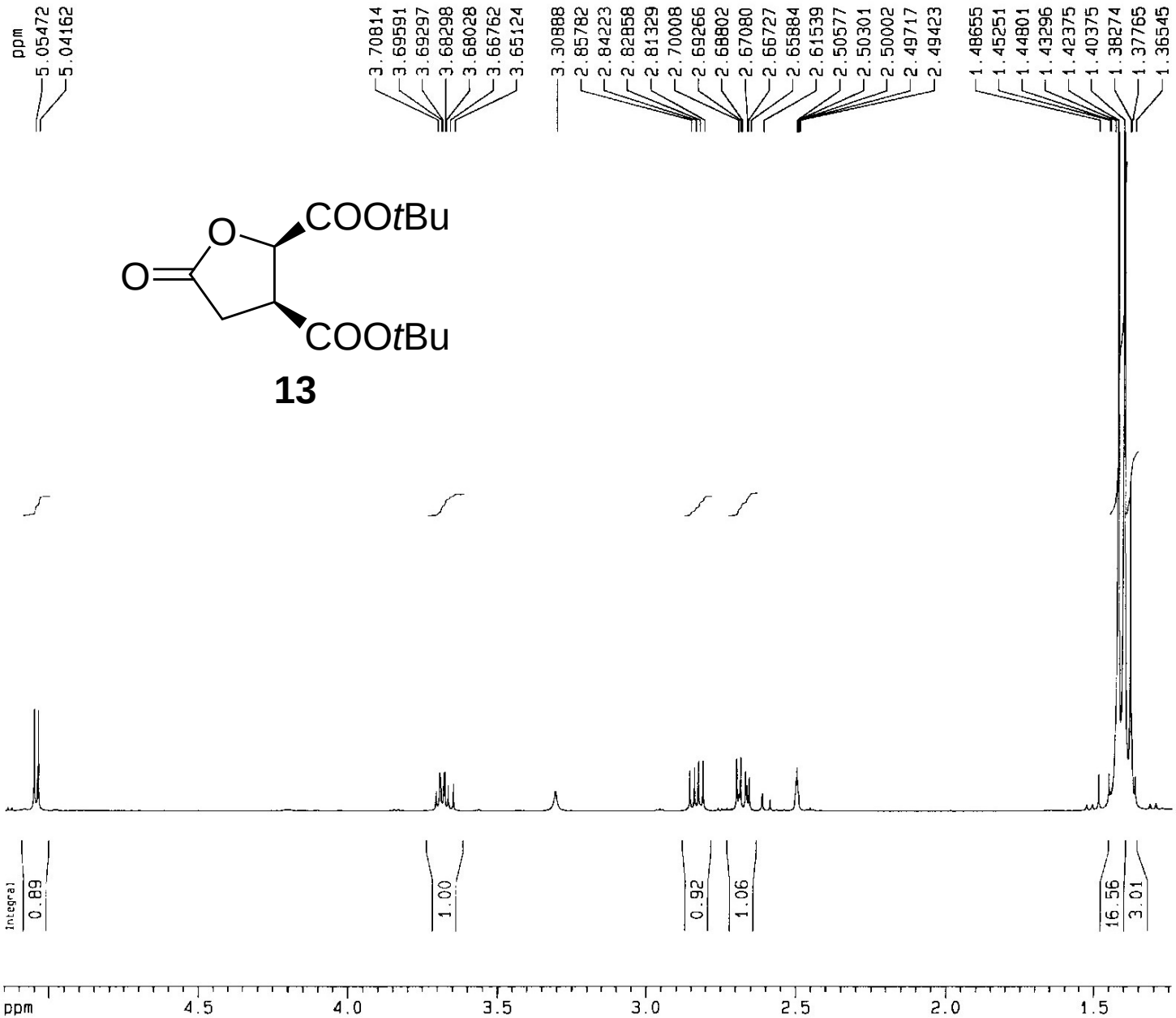
EXP1 PULSE SEQUENCE: S2PUL
DATE 05-12-04
SOLVENT DMSO
FILE H




HERETSCH
PH8-5E
EXP2 PULSE SEQUENCE: S2PUL
DATE 05-12-04
SOLVENT DMSO
FILE C
174.151
171.148
167.960
40.4380
40.3976
39.9791
39.5605
39.1420
38.7255
76.3108
40.8141
40.4380
40.3976
39.9791
39.5605
39.1420
38.7255
38.3090
29.8756
200
180
160
140
120
100
80
60
40
20 PPM
0

7

Ph. Heretsch; PH 9C; 1H



13

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	2.00000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec

======== CHANNEL f1 ========

NUC1	1H
P1	7.30 usec
PL1	5.00 dB
SFO1	600.1339172 MHz

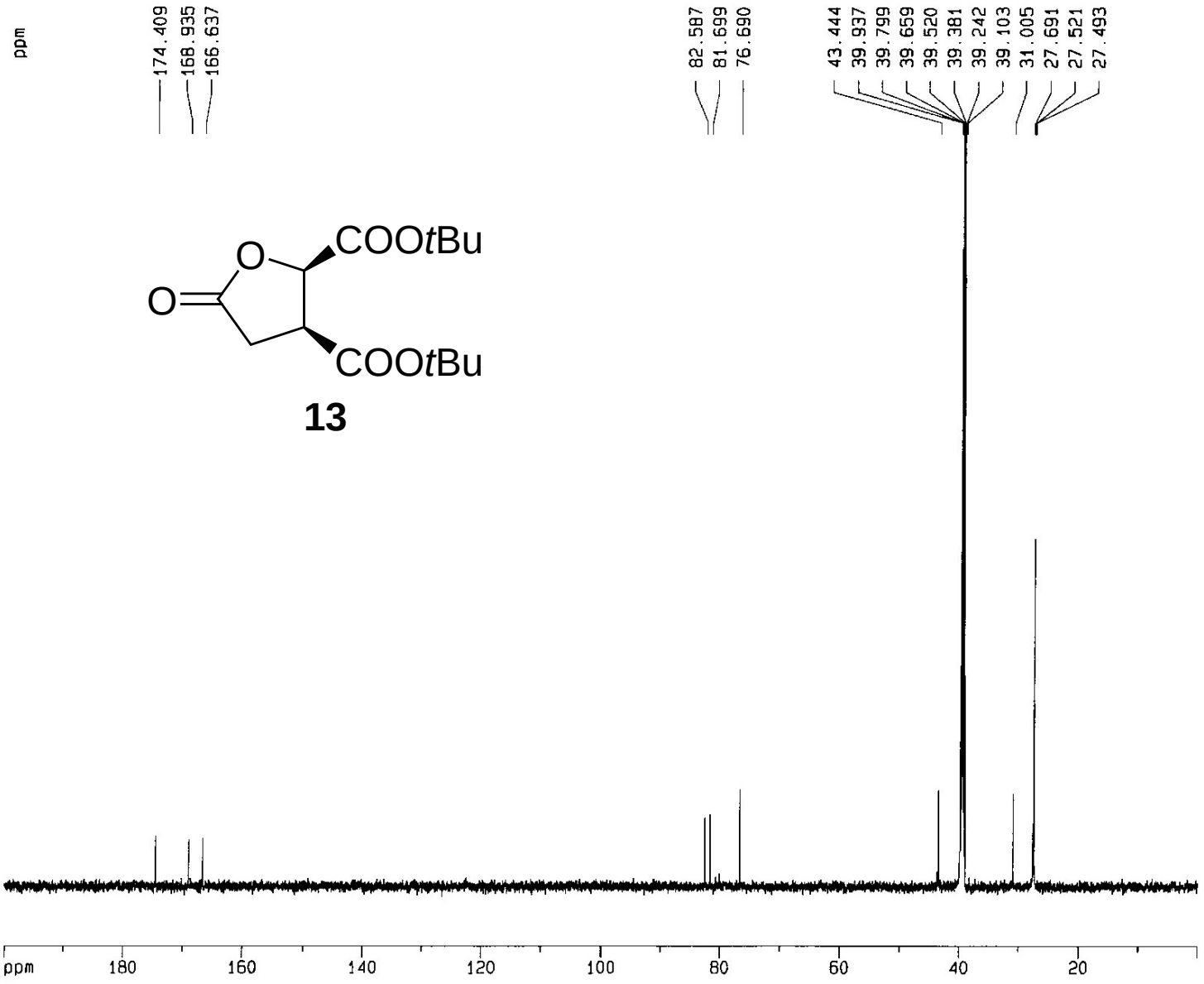
F2 - Processing parameters

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
TD	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	3.00000000 sec
d11	0.03000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 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	600.1324005 MHz

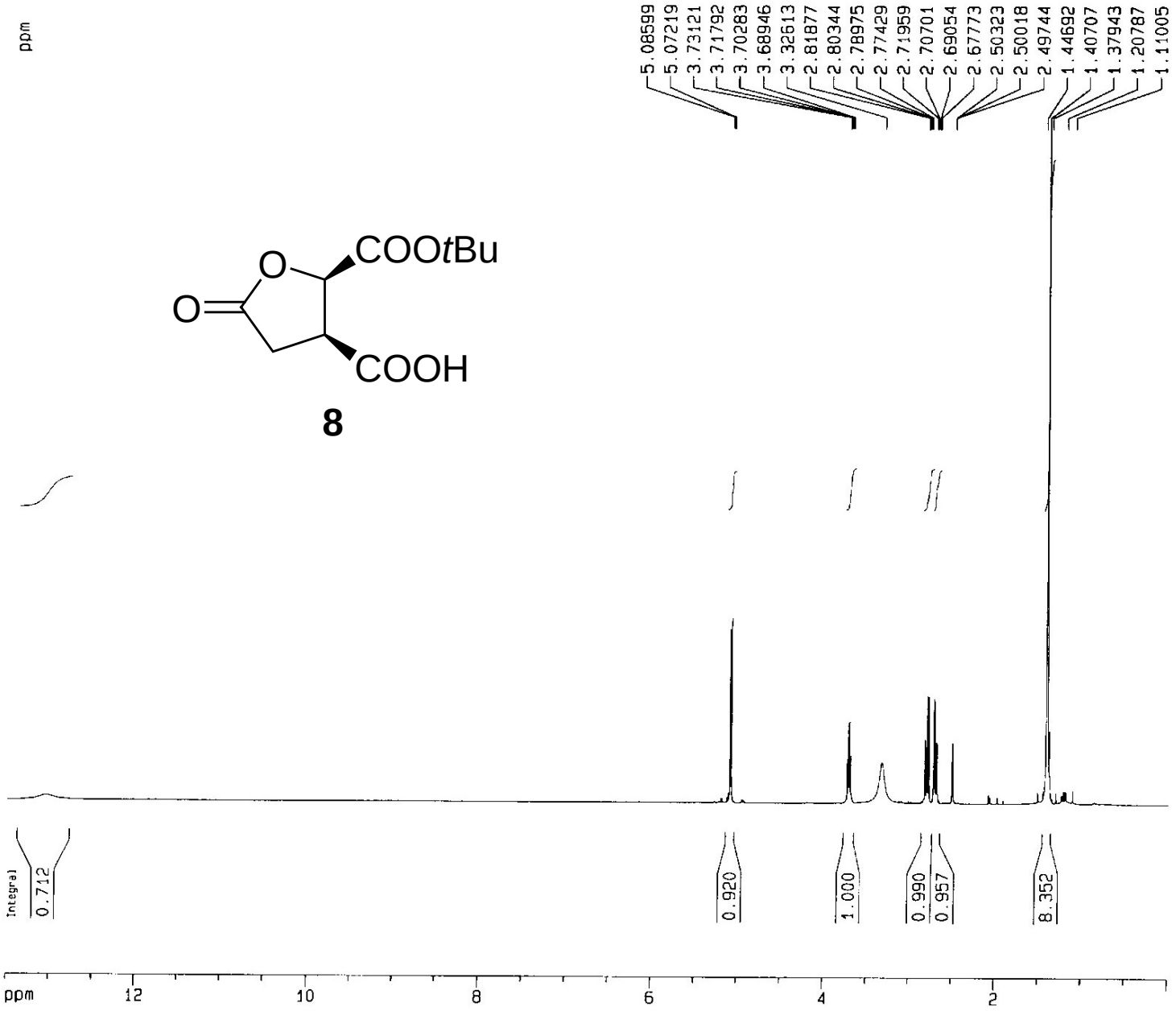
F2 - Processing parameters

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
PPMCM	10.00000 ppm/cm
HZCM	1509.02893 Hz/cm

Ph. Her ch; PH 8C; 1H



8

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
MCWRK	0.01500000 sec

======== CHANNEL f1 ========

NUC1	1H
P1	7.30 usec
PL1	5.00 dB
SFO1	600.1339172 MHz

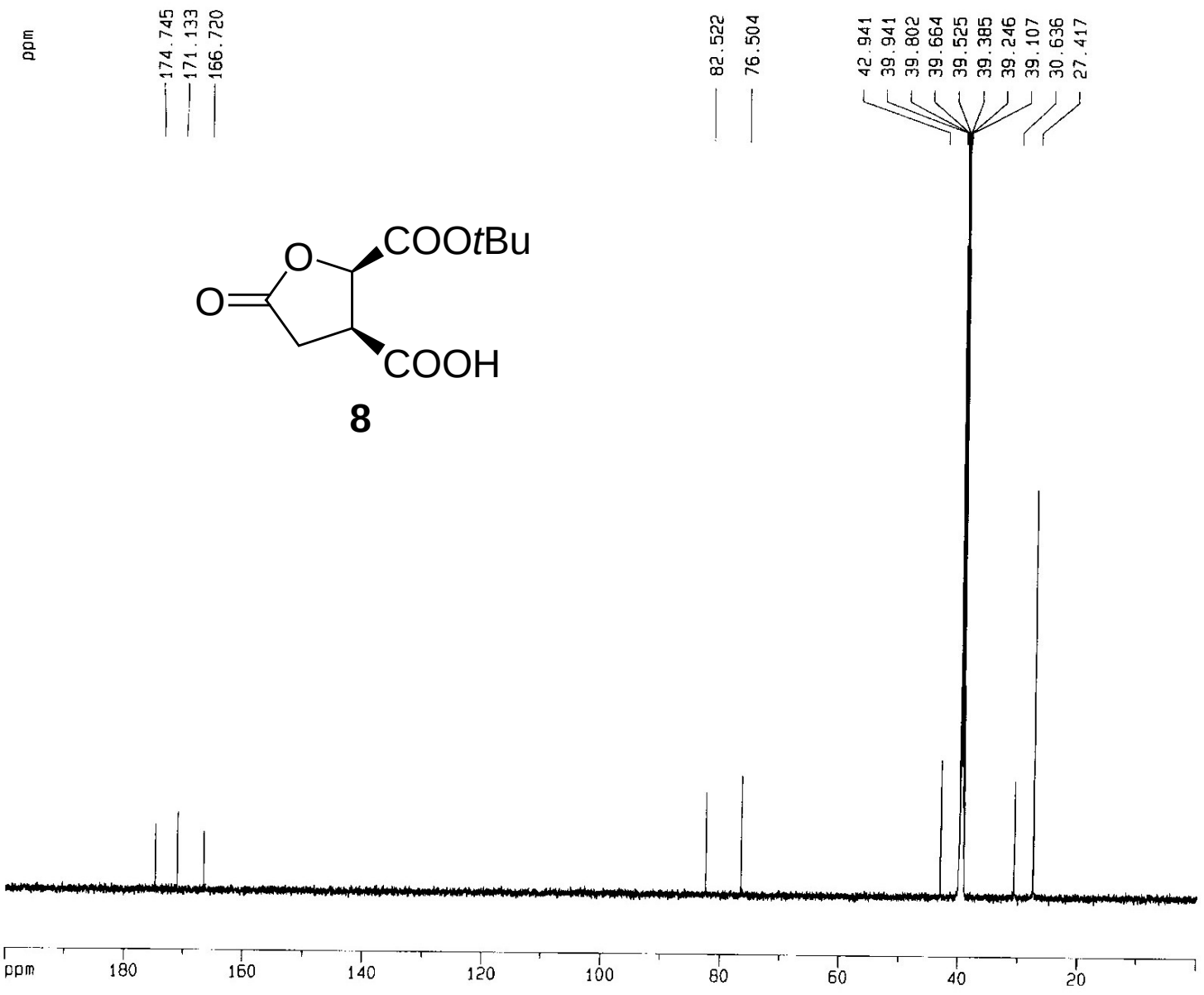
F2 - Processing parameters

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	3.00000000 sec
d11	0.03000000 sec
MCREST	0.00000000 sec
MCWRK	0.01500000 sec

======== CHANNEL f1 ========

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

======== CHANNEL f2 ========

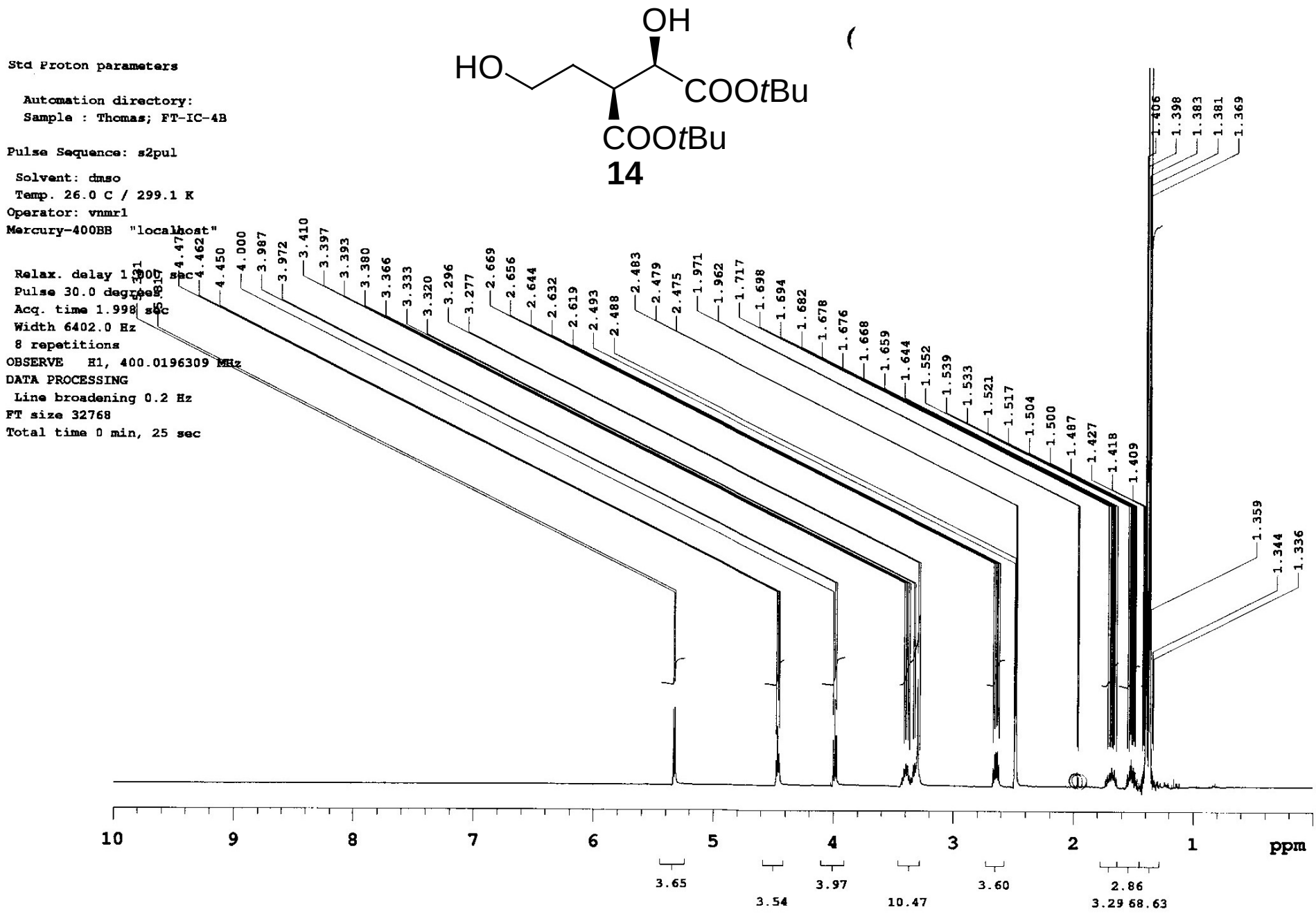
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	20.00 dB
PL12	20.00 dB
SFO2	600.1324005 MHz

F2 - Processing parameters

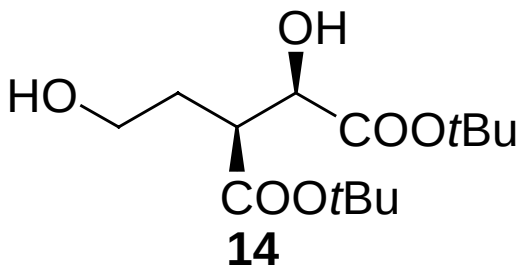
SI	32768
SF	150.9028832 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.40

1D NMR plot parameters

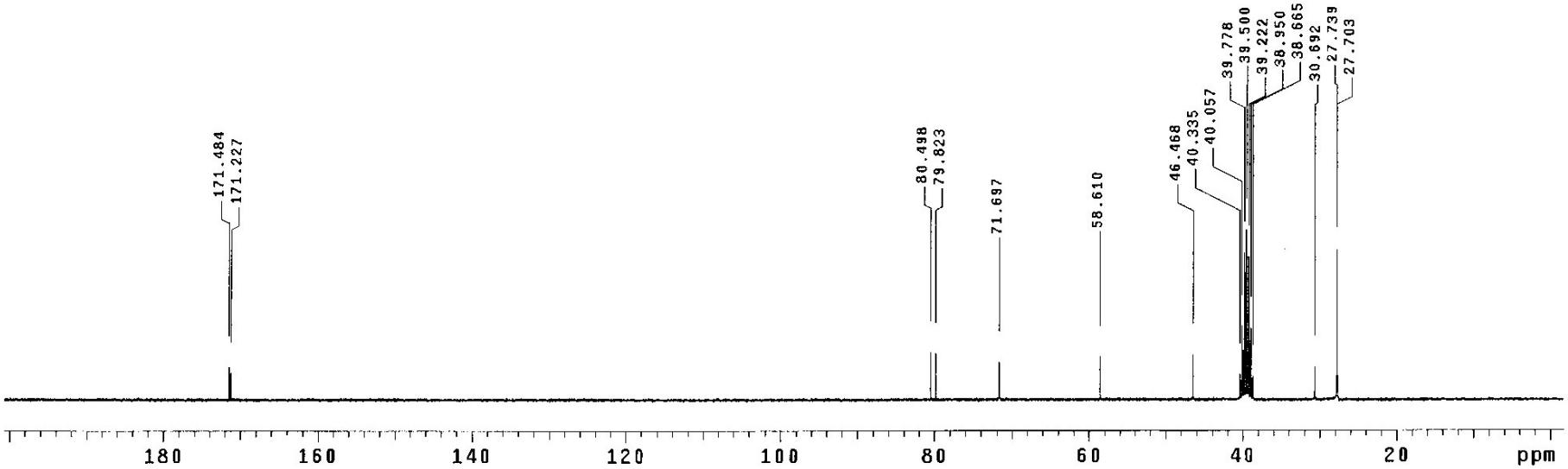
CX	20.00 cm
CY	25.00 cm
F1P	200.000 ppm
F1	30180.58 Hz
F2P	0.000 ppm
F2	0.00 Hz
PPMCM	10.00000 ppm/cm
HZCM	1509.02881 Hz/cm


Std Proton parameters
Automation directory:
Sample : Thomas; FT-IC-4B
Pulse Sequence: s2pul
Solvent: dmso
Temp. 26.0 C / 299.1 K
Operator: vnmr1
Mercury-400BB "localhost"
Relax. delay 1.000 sec
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
OH
HO
COOtBu
COOtBu
14
10
9
8
7
6
5
4
3
2
1
ppm
3.65
3.54
3.97
10.47
3.60
2.86
3.29
68.63

```
Std Carbon experiment

  Automation directory:
  Sample : F.Thomas FT-IC4B

Pulse Sequence: s2pul

 Solvent: dmso
 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
```



14



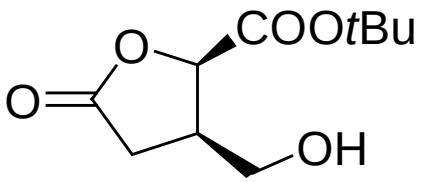
..Thomas

Sample: FT IC 17

Pulse Sequence: s2pul
Date: Mar 30 2006

Solvent: dmso
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



10

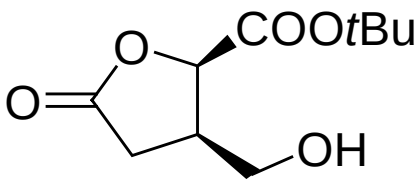
F.Thomas

Sample: FT IC 17

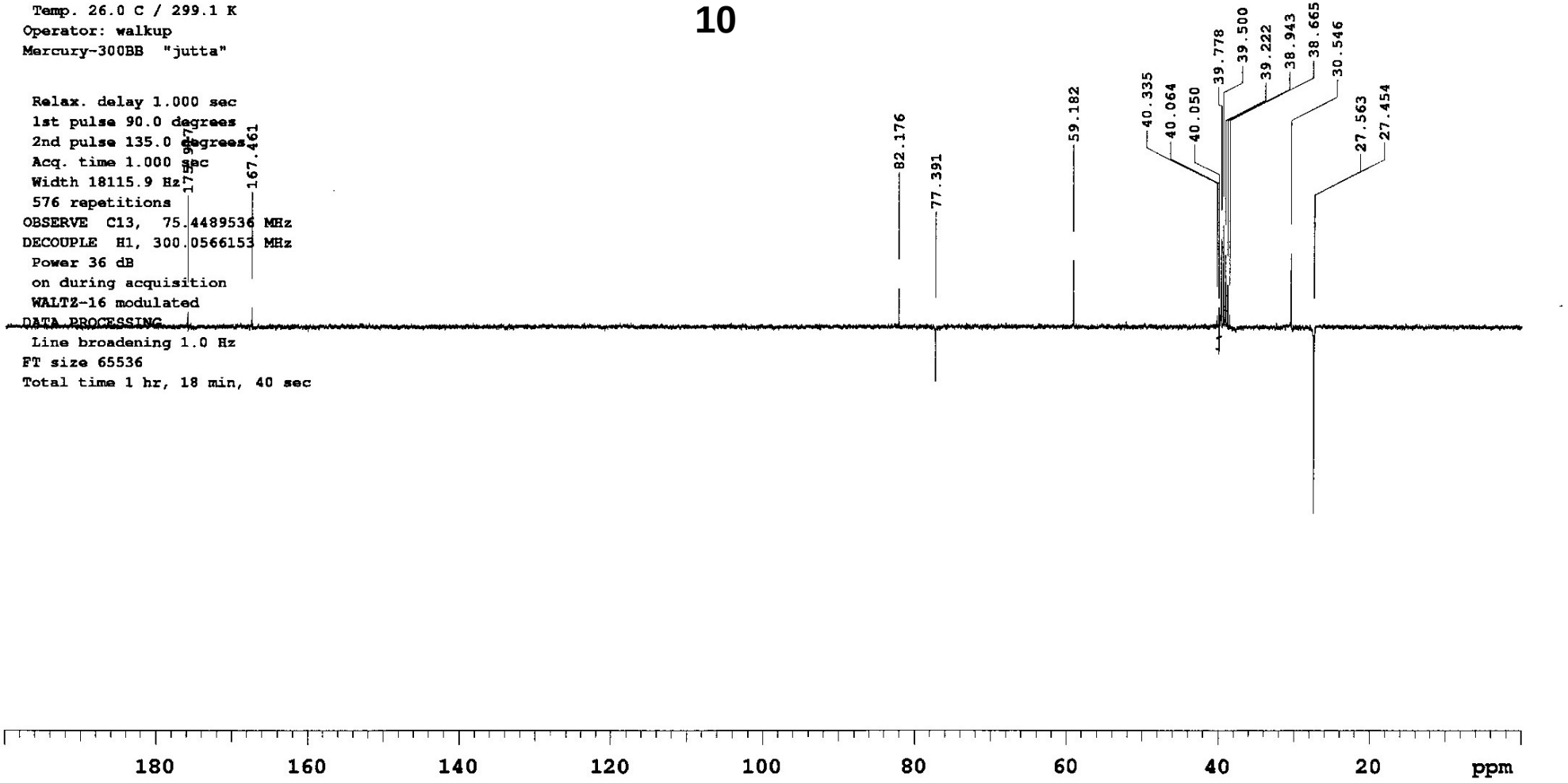
Pulse Sequence: APT
Date: Mar 30 2006

Solvent: dmso
Temp. 26.0 C / 299.1 K
Operator: walkup
Mercury-300BB "jutta"

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



10

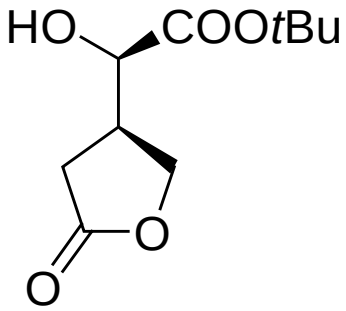


.Thomas

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

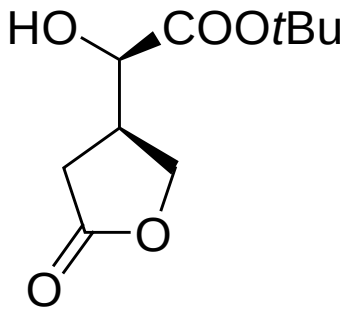
F.Thomas

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

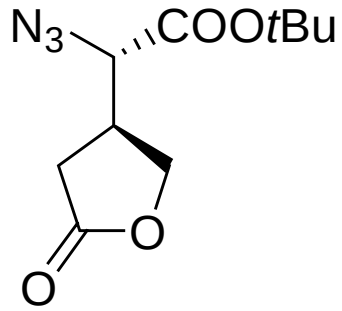
F.Thomas

Sample: FT-IC44

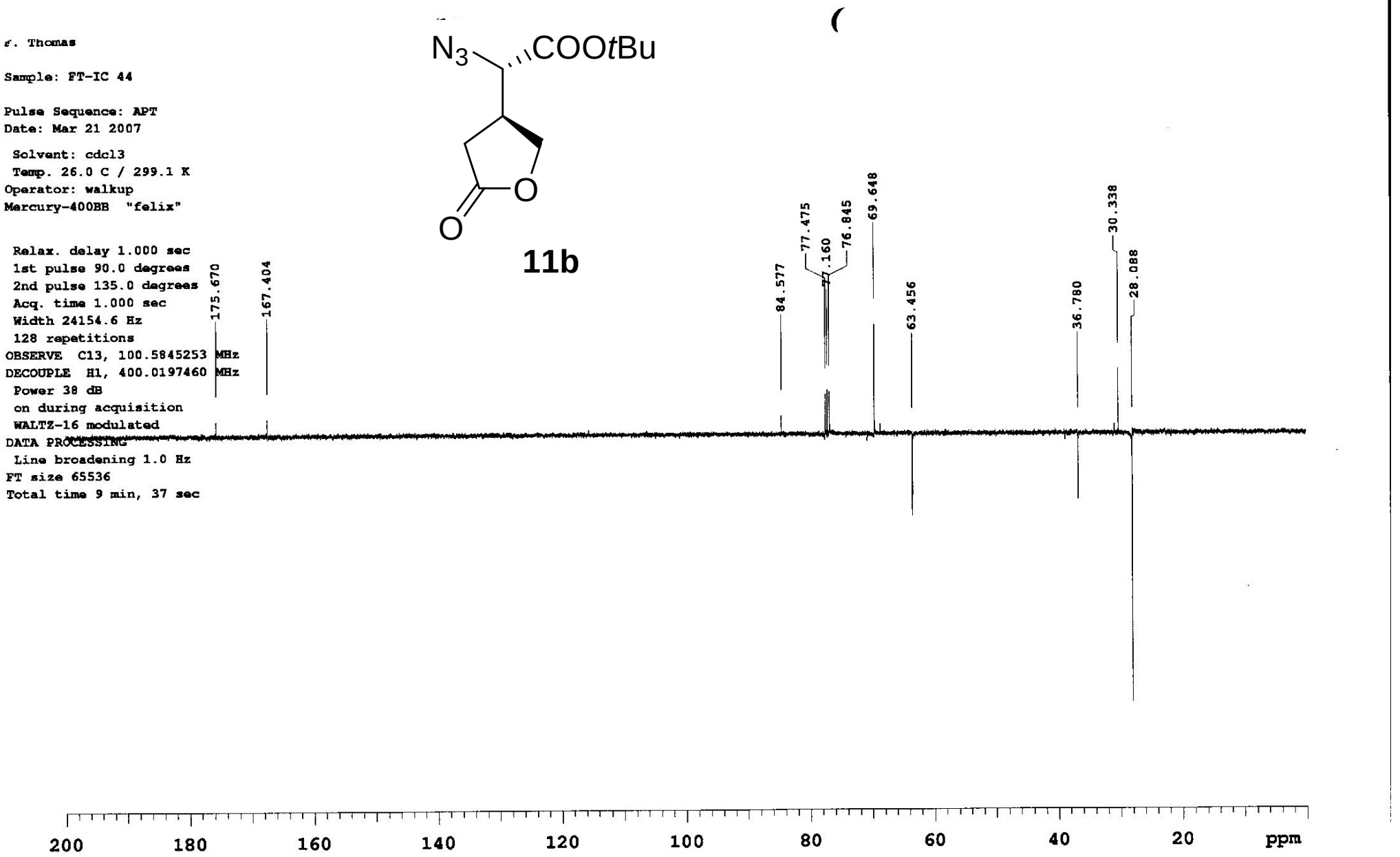
Pulse Sequence: s2pul
Date: Mar 21 2007

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


f. Thomas
Sample: FT-IC 44
Pulse Sequence: APT
Date: Mar 21 2007
Solvent: cdcl3
Temp. 26.0 C / 299.1 K
Operator: walkup
Marcury-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
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
N3
COOtBu
O
O
11b
175.670
167.404
84.577
77.475
77.160
76.845
69.648
63.456
36.780
30.338
28.088
200
180
160
140
120
100
80
60
40
20
ppm

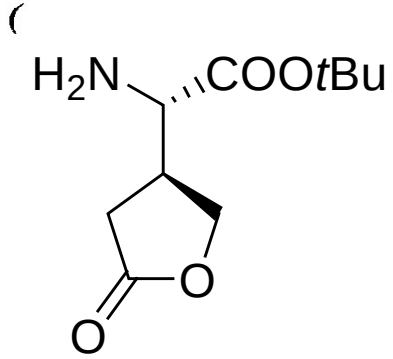
F. Thomas

Sample: FT-IC46B

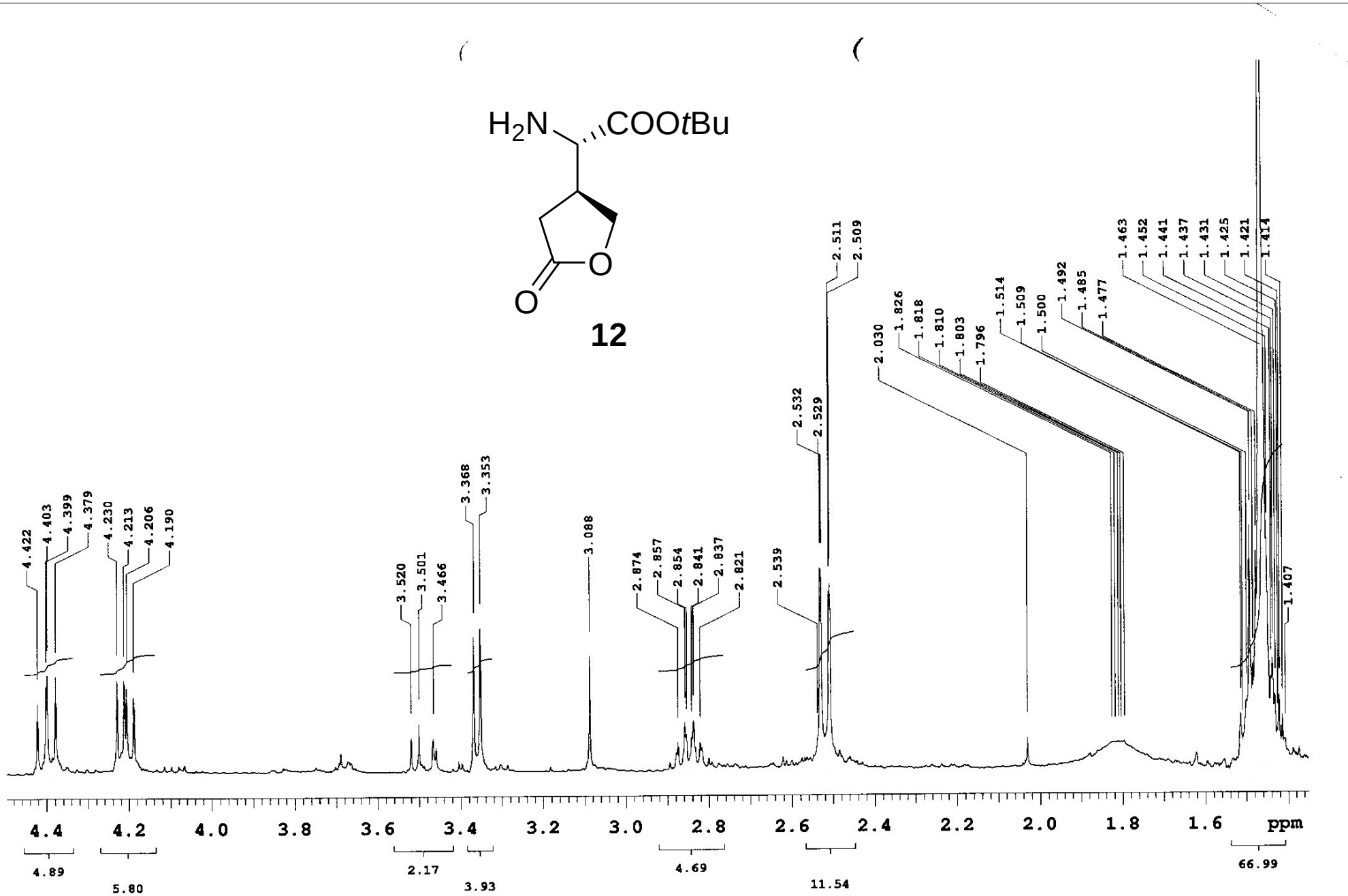
Pulse Sequence: s2pul
Date: May 31 2007

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


H2N
COOtBu
O
O
12
4.422
4.403
4.399
4.379
4.230
4.213
4.206
4.190
3.520
3.501
3.466
3.368
3.353
3.088
2.874
2.857
2.854
2.841
2.837
2.821
2.539
2.532
2.529
2.511
2.509
2.030
1.826
1.818
1.810
1.803
1.796
1.514
1.509
1.500
1.492
1.485
1.477
1.463
1.452
1.441
1.437
1.431
1.425
1.421
1.414
1.407
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
ppm
4.89
5.80
2.17
3.93
4.69
11.54
66.99

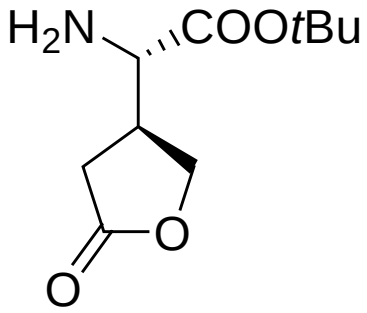
F. Thomas

Sample: FT-IC46B

Pulse Sequence: APT
Date: May 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
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



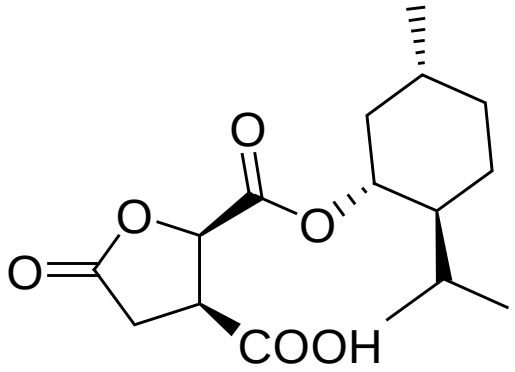
12

P.Heretsch

Sample: PHC2

Pulse Sequence: s2pul
Date: May 18 2006
Solvent: cd3od
Temp. 25.0 C / 298.1 K
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, [illegible]



9

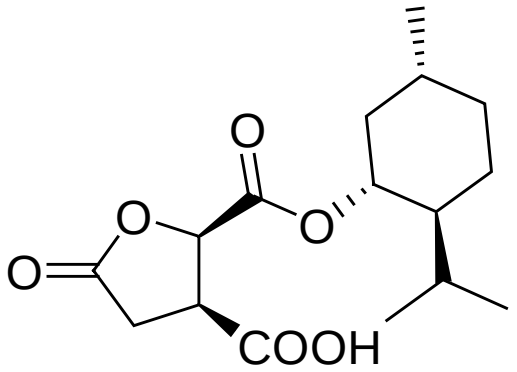
P.Heretsch

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



9

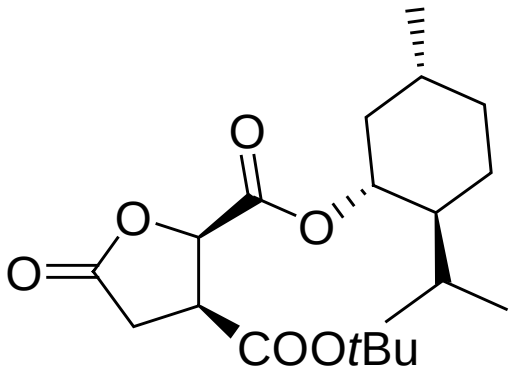
Ph. Heretsch

Sample: PH IC M2

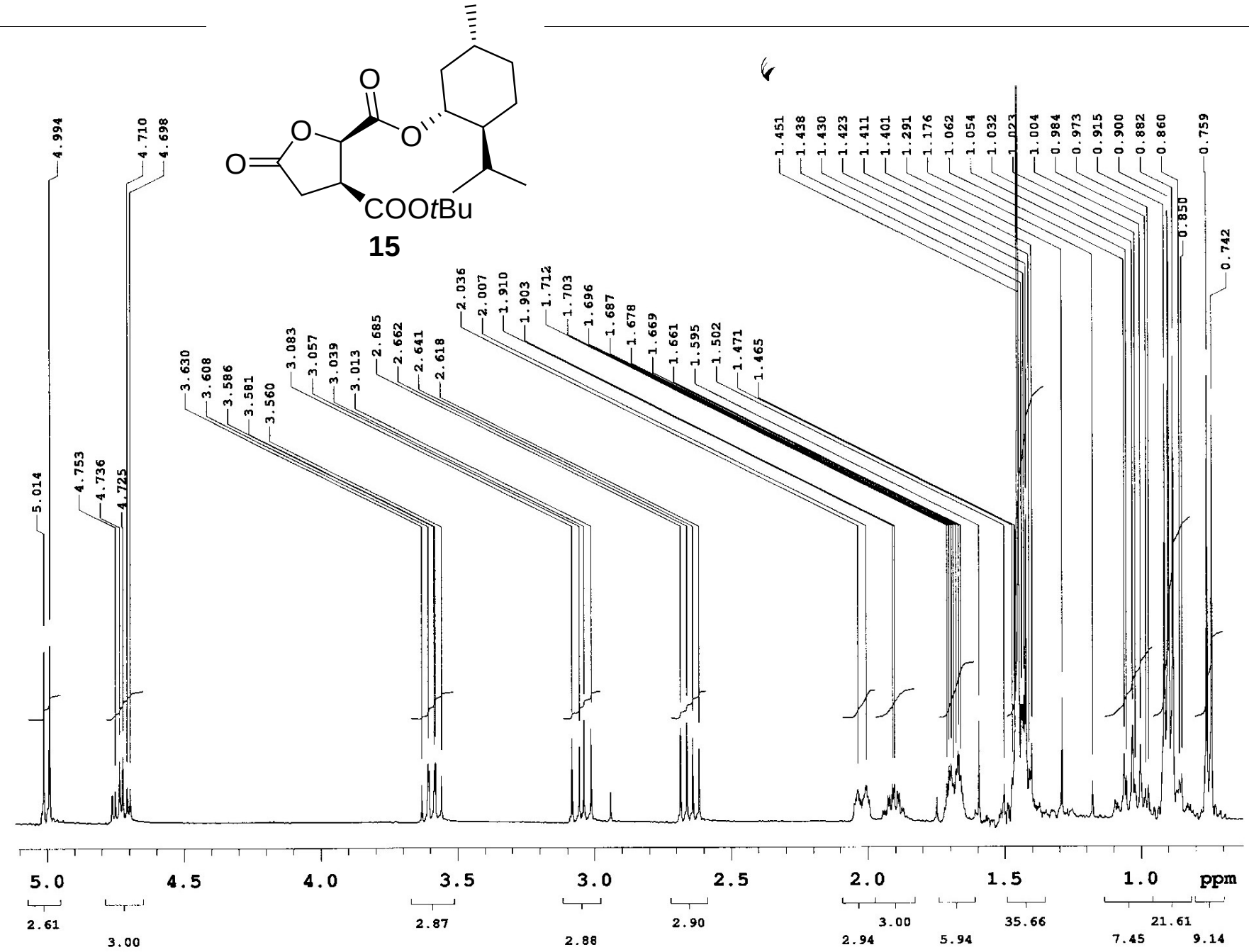
Pulse Sequence: s2pul
Date: Aug 31 2007

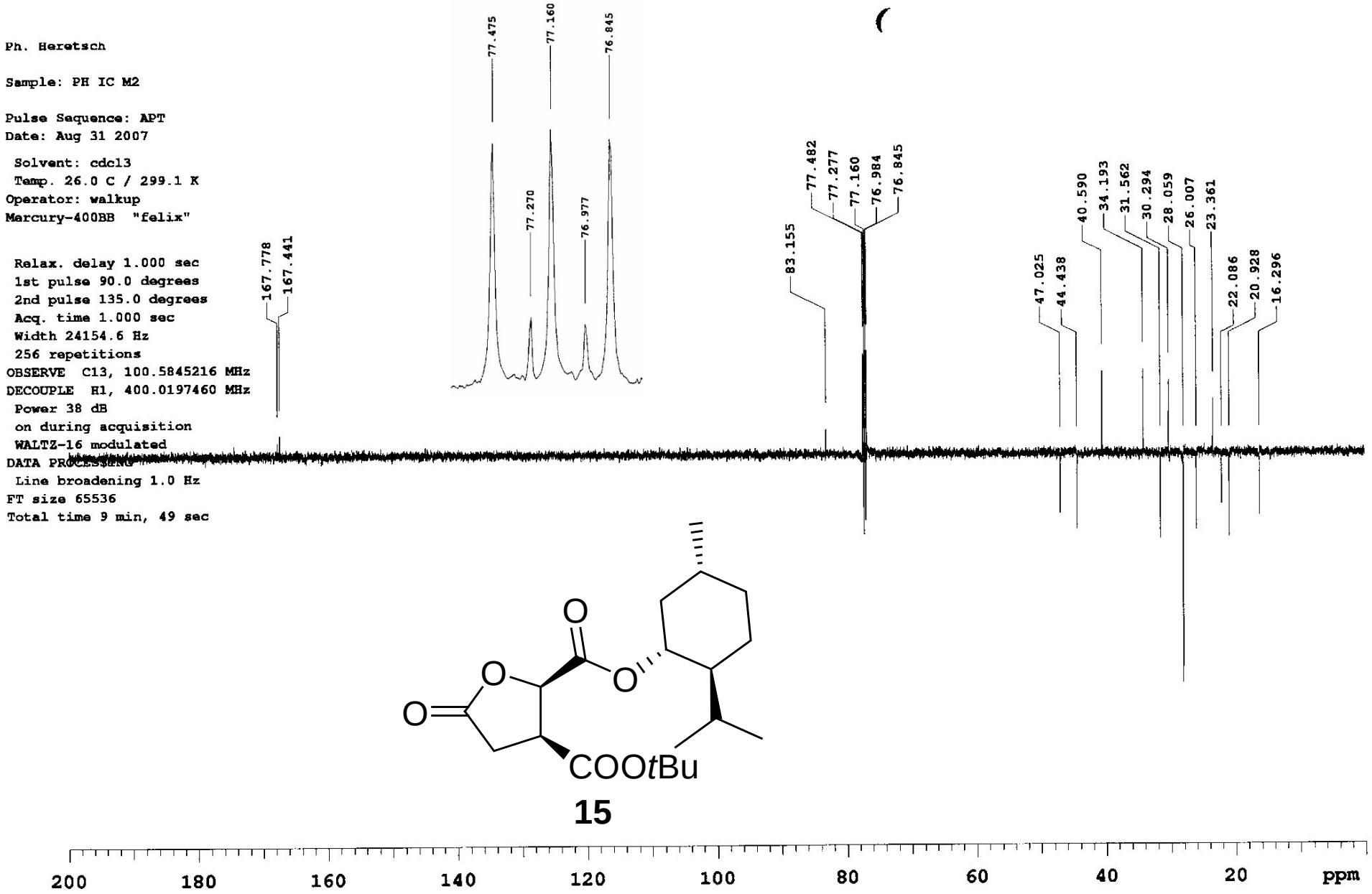
Solvent: cdcl3
Temp. 26.0 C / 299.1 K
Operator: walkup
Mercury-400BB "felix"

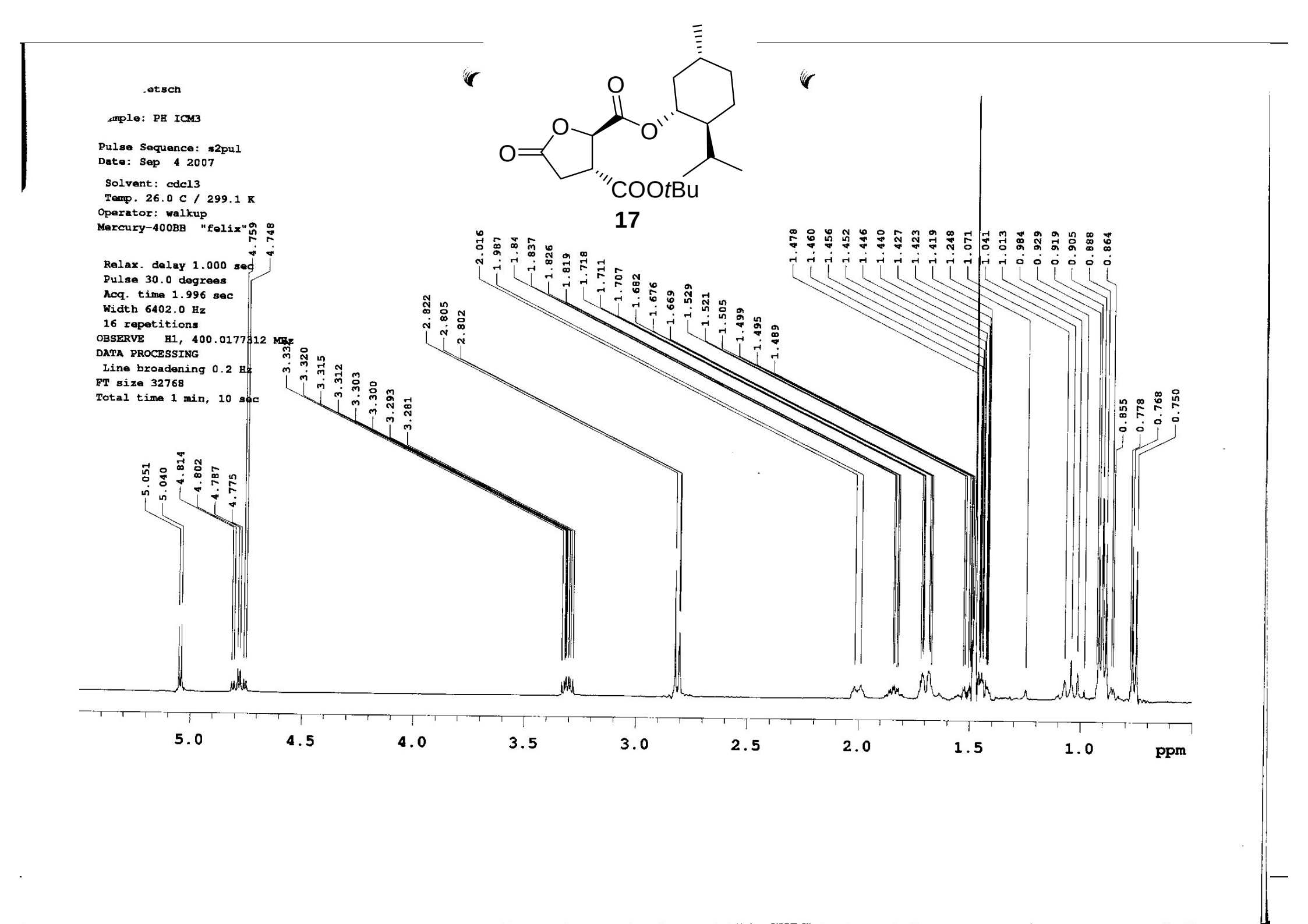
Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 1.996 sec
Width 6402.0 Hz
16 repetitions
OBSERVE H1, 400.0177308 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 32768
Total time 1 min, 10 sec

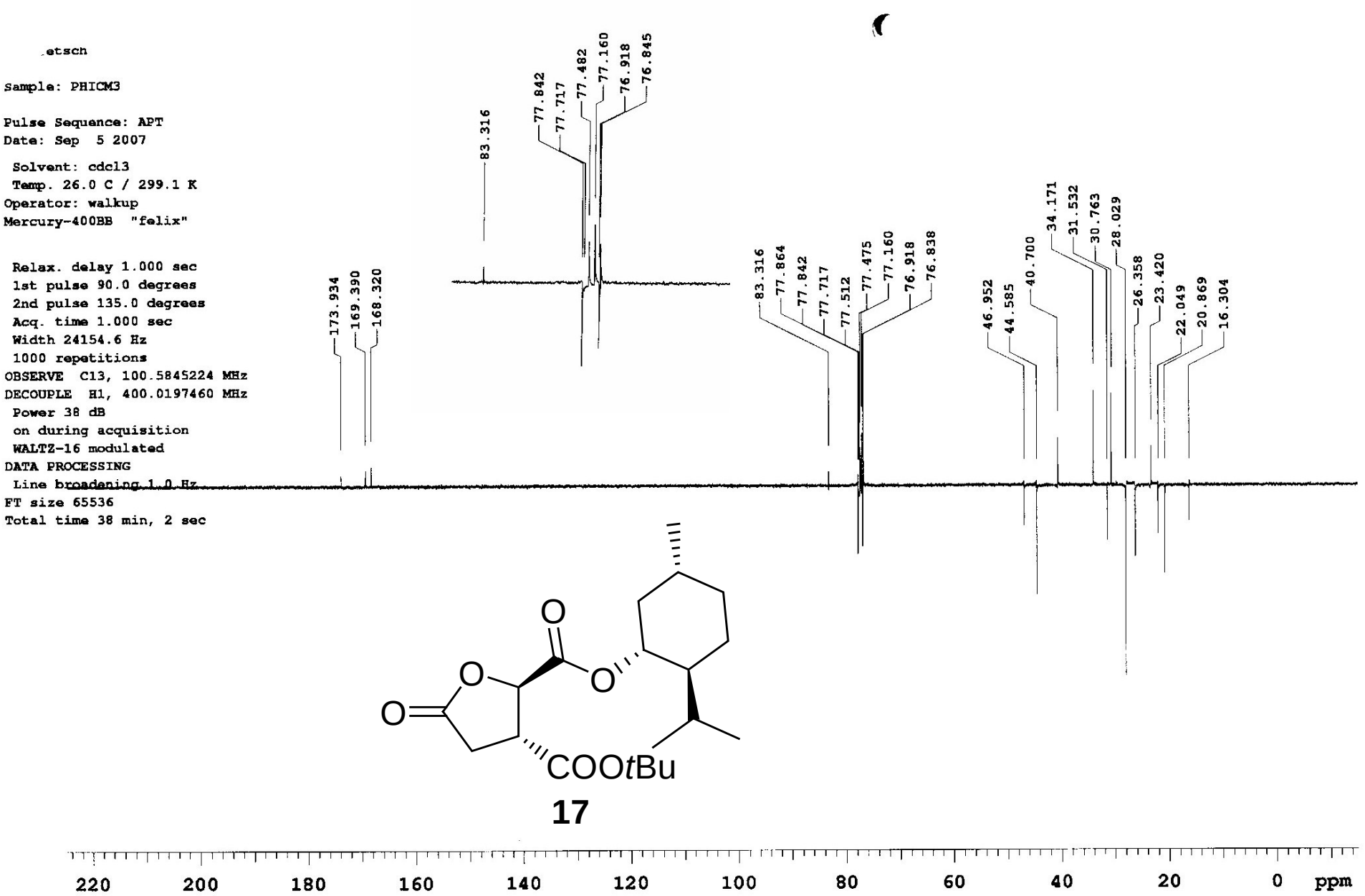


15


COO*t*Bu
15
4.994
4.753
4.736
4.725
4.710
4.698
5.014
3.630
3.608
3.586
3.581
3.560
3.083
3.057
3.039
3.013
2.685
2.662
2.641
2.618
2.036
2.007
1.910
1.903
1.714
1.703
1.696
1.687
1.678
1.669
1.661
1.595
1.502
1.471
1.465
1.451
1.438
1.430
1.423
1.411
1.401
1.291
1.176
1.062
1.054
1.032
1.023
1.004
0.984
0.973
0.915
0.900
0.882
0.860
0.850
0.759
0.742
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
2.61
3.00
2.87
2.88
2.90
3.00
2.94
5.94
35.66
21.61
7.45
9.14


Ph. Heretsch
Sample: PH IC M2
Pulse Sequence: APT
Date: Aug 31 2007
Solvent: cdcl3
Temp. 26.0 C / 299.1 K
Operator: walkup
Marcury-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
77.475
77.270
77.160
76.977
76.845
167.778
167.441
83.155
77.482
77.277
77.160
76.984
76.845
47.025
44.438
40.590
34.193
31.562
30.294
28.059
26.007
23.361
22.086
20.928
16.296
O
O
O
O
COOtBu
15
200
180
160
140
120
100
80
60
40
20
ppm


.etsch
.mple: 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, 400.0177312 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 32768
Total time 1 min, 10 sec
COOtBu
17
5.051
5.040
4.814
4.802
4.787
4.775
4.759
4.748
3.336
3.320
3.315
3.312
3.303
3.300
3.293
3.281
2.822
2.805
2.802
2.016
1.987
1.84
1.837
1.826
1.819
1.718
1.711
1.707
1.682
1.676
1.669
1.529
1.521
1.505
1.499
1.495
1.489
1.478
1.460
1.456
1.452
1.446
1.440
1.427
1.423
1.419
1.248
1.071
1.041
1.013
0.984
0.929
0.919
0.905
0.888
0.864
0.855
0.778
0.768
0.750
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


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
83.316
77.842
77.717
77.482
77.160
76.918
76.845
173.934
169.390
168.320
83.316
77.864
77.842
77.717
77.512
77.475
77.160
76.918
76.838
46.952
44.585
40.700
34.171
31.532
30.763
28.029
26.358
23.420
22.049
20.869
16.304
O
O
O
O
COOtBu
17
220
200
180
160
140
120
100
80
60
40
20
0
ppm

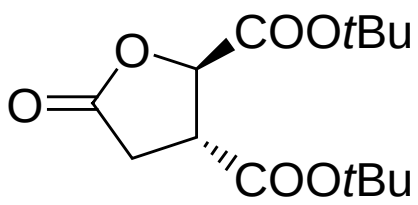
P.Heretsch

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



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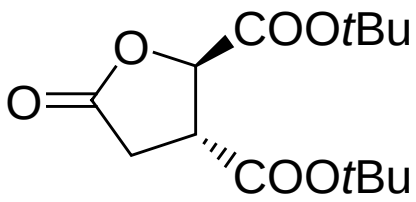
P.Heretsch

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 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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