Supplementary Material

Continuous flow synthesis of L-menthyl glyoxylate monohydrate: an important intermediate in the manufacture of antiretrovirals

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1. General

Reagents were sourced from Sigma Aldrich and were used as supplied. Nuclear magnetic resonance (NMR) spectra were recorded at room temperature as solutions in deuterated chloroform ($CDCl_3$). A Bruker Avance-400 spectrometer (400 MHz) was used to record the spectra and the chemical shifts are reported in parts per million (ppm) with coupling constants in Hertz (Hz). Infra-red spectra were recorded from 4000 to 500 cm^{-1} using a Bruker spectrometer and peaks (ν_{max}) reported in wavenumbers (cm^{-1}). Continuous flow reactions were performed on various systems were monitored by Agilent 1200 HPLC fitted with a diode array and Agilent 7890A Gas Chromatography (GC). HPLC analysis was performed on Agilent Zorbax C18-column (250 mm x 4.6 mm i.d, 5µm) at ambient temperature using an isocratic system. The mobile phase consisted of 70 % acetonitrile and 30 % water. Sample injection volume was 5 µl, eluted at a flow rate of 1 ml/min and detected at 194 nm with a run time of 16 min. GC analysis was performed on a ZB-5 MS capillary column of length 30 m and an internal diameter of 0.25 mm.

2. Data analysis

To determine the validity of the data the experimental conditions for each entry or run for LMGH selectivity and glyoxylic acid conversion were repeated, in triplicates and the average used for reporting.

2.1 Determination of reaction conversion

The conversion of starting material was calculated using the equation below (Equation 1).

$$\boldsymbol{Reaction\ Conversion}\ (\%) = \frac{C_{Ai} - C_{Af}}{C_{Ai}}\ \mathbf{x\ Dilution\ factor}\ \boldsymbol{x}\ \mathbf{100}\ \% \qquad \textbf{Equation 1}$$

Where C_{Ai} is defined as the initial concentration of glyoxylic acid and C_{Af} is the final concentration of glyoxylic acid, determined after the reaction.

To calculate the initial and final concentrations, the total volume of sample (TVS) was determined using the formula given below, in which the total flow rate of the reactant solutions (TFR) was multiplied by the total sample collection time (T).

$$\boldsymbol{TVS = TFR\ x\ T} \qquad \textbf{Equation 2}$$

2.2 Determination of product selectivity

The selectivity of L-menthyl glyoxylate hydrate (LMGH) was calculated by dividing the moles of LMGH with the moles of products formed from the reaction.

$$\boldsymbol{LMGH\ selectivity} = \frac{\boldsymbol{Amount\ of\ LMGH\ (mol)}}{\boldsymbol{Amount\ of\ products\ (mol)}}\ \boldsymbol{x}\ \mathbf{100}\% \qquad \textbf{Equation 3}$$

The moles of the MGH formed were calculated by multiplying its final concentration by the sample volume collected. The final sample concentration used was determined by using a calibration curve approach as elaborated above.

3. LMGH synthesis using continuous flow systems

3.1 Continuous flow glyoxylic acid esterification in Chemtrix Labtrix flow system. A Chemtrix Labtrix Start system fitted with a 19.5 µl glass reactor was used to perform all solution phase esterification investigations (Figure 1 and 2). Two syringe pumps were used to pump reagents from two 10 ml SGE Luer lock gas tight glass syringes into the thermally controlled micro-reactor

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system, which was fitted with a 10-bar back pressure regulator. Glyoxylic acid **3** (0.1 M) and catalyst were both dissolved in acetonitrile and L-menthol **2** dissolved in acetonitrile prepared according to the mole equivalents under investigation were pumped into the flow system separately (Figure 2). The reagents were prepared at various mole ratios with glyoxylic acid as the limiting reagent and *p*-TSA (0.5 % v/v) as the catalyst. Samples were collected and analysed using HPLC. LMGH **4** and the diester **5** were observed at the following retention times; 2.310 and 3.565 mins, respectively.

Figure 1. Chemtrix Labtrix® Start continuous flow system.

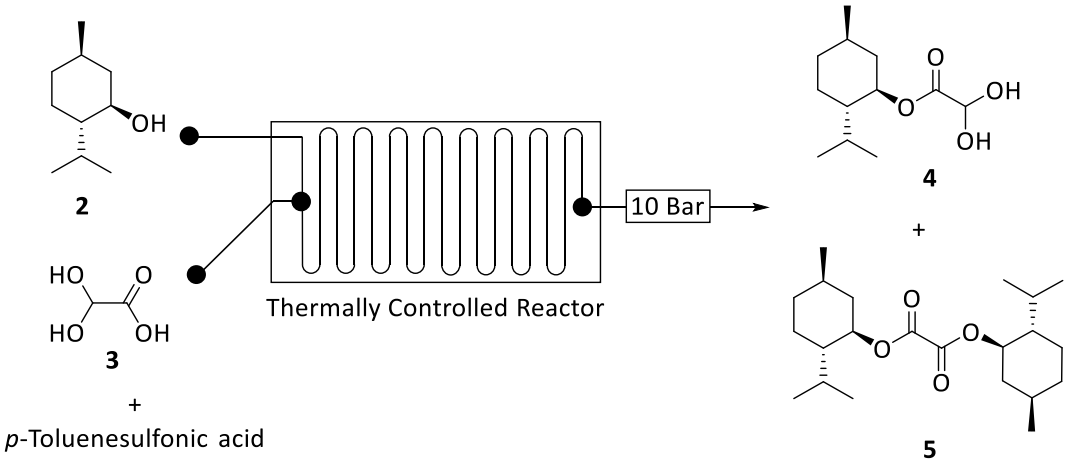

Figure 2. Schematic manifold used for LMGH synthesis and optimisation studies.

3.2. Microwave-assisted continuous flow glyoxylic acid esterification. The microwave-assisted continuous-flow reactor set-up was used in the study of the reaction fitted with a 10-bar back pressure regulator (Figure 3). Glyoxylic acid **3** (0.1 M) and L-menthol **2** (0.6 M) in acetonitrile were premixed and pumped into the reactor column packed with a mixture 2.0 g of Amberlyst-15 catalyst using an HPLC pump. The reactor temperatures were controlled by use of pre-installed WaveCraft software. Samples were collected and analysed by HPLC. The reactor was allowed to equilibrate between sample runs.

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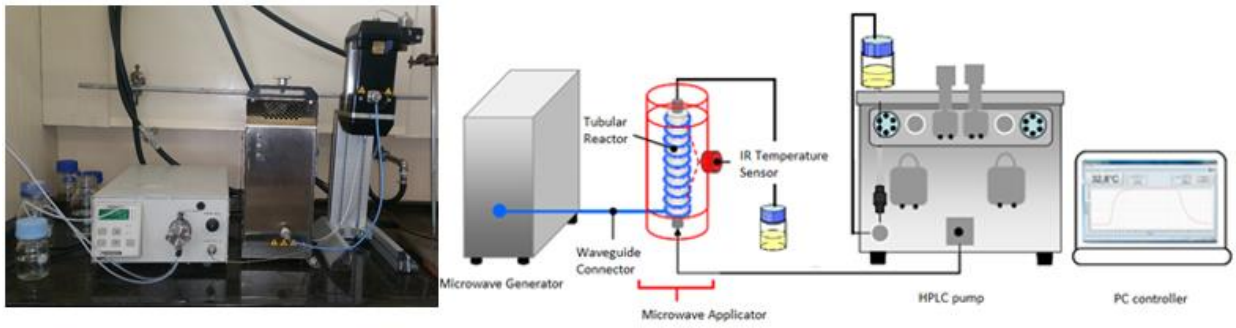

Figure 3. Microwave-assisted continuous-flow reactor/furnace setup for LMGH.

3.3 Continuous flow glyoxylic acid esterification in a packed column. A Uniqsis glass column reactor (1 cm ID x 10 cm) packed with Amberlyst-15 (4 g) and activated molecular sieves in the ratio 2:1 (5 cm bed height, 3,9 cm^3 reactor volume) was used. The column reactor was heated using the Uniqsis heating frame and the system was pressurised using a 10-bar back pressure regulator. Glyoxylic acid (0.1 M) and L-menthol **2** were both dissolved in acetonitrile were pumped into the column reactor using a peristaltic HPLC pump (Figure 4). The reagents were prepared at various mole ratios with glyoxylic acid as the limiting reagent. Samples were collected and analysed using HPLC.

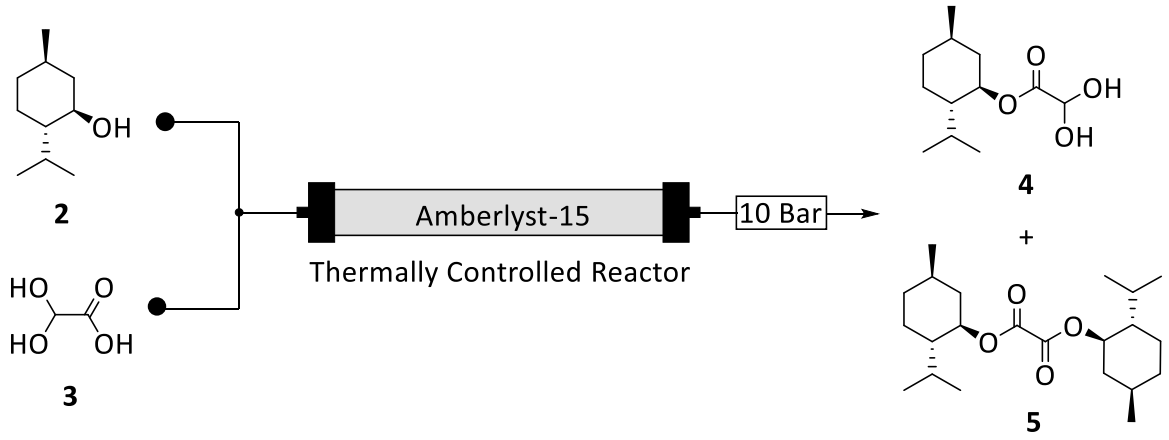

Figure 4. Amberlyst-15 packed column reactor for LMGH flow synthesis and optimisation studies.

4. Batch synthesis of L-menthyl glyoxylate monohydrate

A solution of L-menthol (7.83 g, 3M) and glyoxylic acid monohydrate (1.53 g, 1.0 M) was prepared by dissolving the reagents in acetonitrile (15 mL) in a 100 mL two neck, round-bottomed flask. The flask was connected to a Dean Stark apparatus (Figure 5). *p*-Toluenesulfonic acid (*p*-TSA) (0.5 % v/v) was added to the reaction mixture before commencing the reaction. A magnetic stirrer

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bar was inserted into the reactor flask for agitation. The mixture was heated to boiling point with concomitant removal of water by azeotropic entrainment.

Figure 5. Batch-scale reactor set-up.

The progress of the reaction was monitored at half hour intervals, sampling the reaction mixture and analysing it on a HPLC and Thin Layer Chromatography (TLC). TLC visualisation was possible by spotting the reaction mixture on a TLC silica gel plate. The plate was allowed to develop in an aqueous solution of potassium permanganate stain which was prepared by dissolving $KMnO_4$ (3.0 g), K_2CO_3 (20 g), and 10% NaOH (2.50 mL) in water (400 mL). The spots where visualised by using hot air gun to dry the stain.

Upon completion, the reaction mixture was cooled down to ambient temperature and the organic layer washed with 10 % $NaHCO_3$ (5 mL). The undesired aqueous layer removed using a separating funnel. The organic layer was dried with anhydrous Na_2SO_4. The sodium hydrogen carbonate serves to react with any excess acid in the resultant reaction mixture. Final purification was done by recrystallisation, in which it was dissolved in solvent and heated before filtration. The filtrate was then cooled in ice and agitated to induce crystallisation.

LMGH **4** was isolated as a white powder with a melting point of 79 °C (Lit. value mp 77.0 – 79.0 °C). FT-IR (cm^{-1}) υ: 3420, 3345, 2959, 2873, 1738, 1455, 1219, 1095, 1030, 9556, 796. ^{1}H-NMR (400 MHz, DMSO) δ ppm: 0.72-0.70 (d, 3H), 0.89-0.84 (m; 7H), 1.06-0.94 (m; 2H), 1.47-1.35 (m; 2H), 1.65-1.62 (m, 2H), 1.87-1.84 (m, 2H), 4.64-4.57 (m, 1H), 4.97 (s; 1H), 6.59 (bs; 2H). ^{13}C-NMR (400 MHz, DMSO): δ ppm 170.6, 87.4, 73.9, 46.9, 34.1, 31.3, 25.9, 23.4, 22.3, 20.9, 16.2.

Diester **5**

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The continuous flow run with the highest selectivity towards the diester by HPLC was collected and washed with aqueous NaOH and the product was extracted into EtOAc and concentrated in *vacuo* to afford a diester **5**. FT-IR (cm^{-1}) υ: 2957, 2928, 2872, 1795, 1750, 1456, 1260, 979, 965, 796. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 0.72-0.70 (d, 6H), 0.85-0.83 (m, 14H), 1.07-1.00 (m, 4H), 1.46 (t, 4H), 1.65-1.62 (d, 4H), 1.82 (m, 2H), 1.98 (m, 2H), 4.76-4.74 (m, 2H). ^{13}C-NMR (400 MHz, $CDCl_3$): δ ppm 158.2, 76.7, 53.4, 46.8, 46.7, 40.3, 40.2, 34.0, 31.4, 26.3, 26.1, 23.6, 23.3, 21.8, 20.6, 20.5, 16.4, 16.2, 16.1

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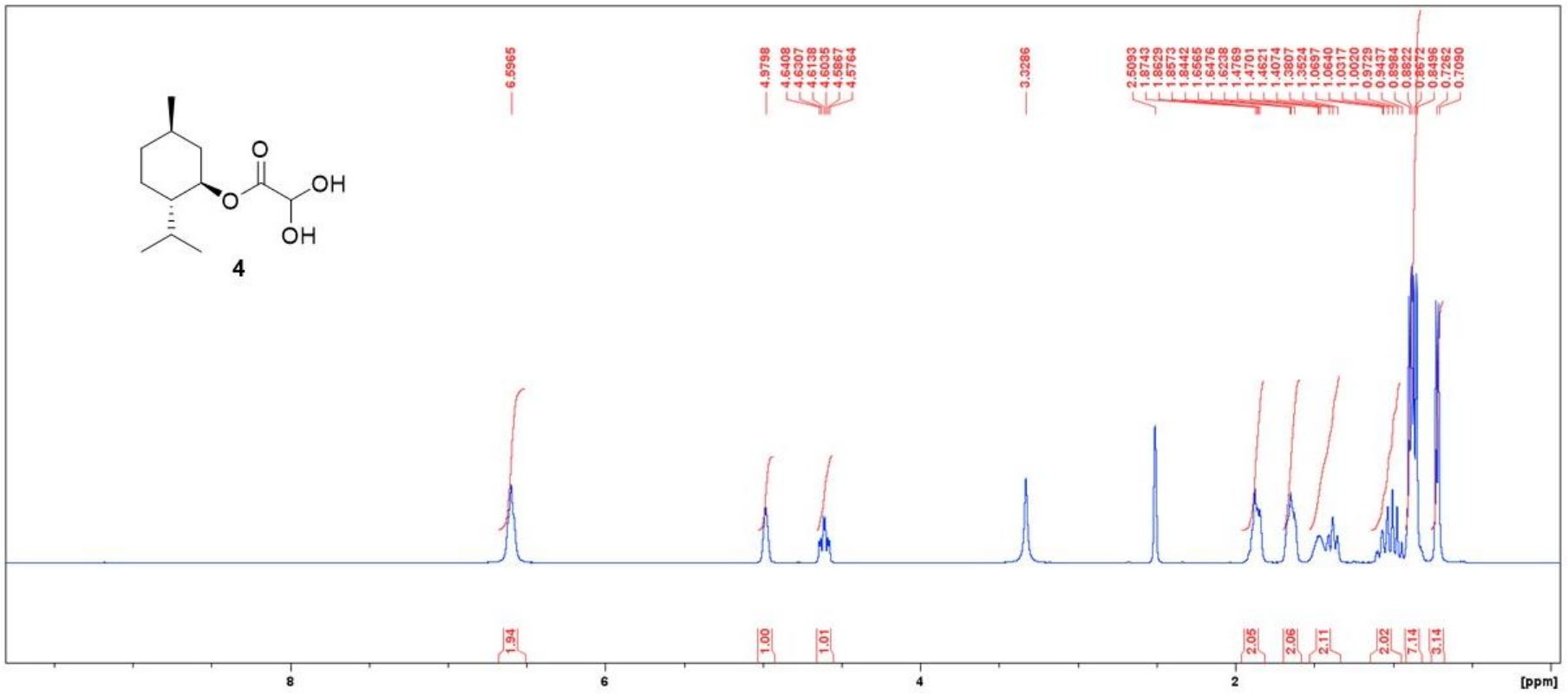

Figure 6. ^{1}H-NMR of L-menthyl glyoxylate 4.

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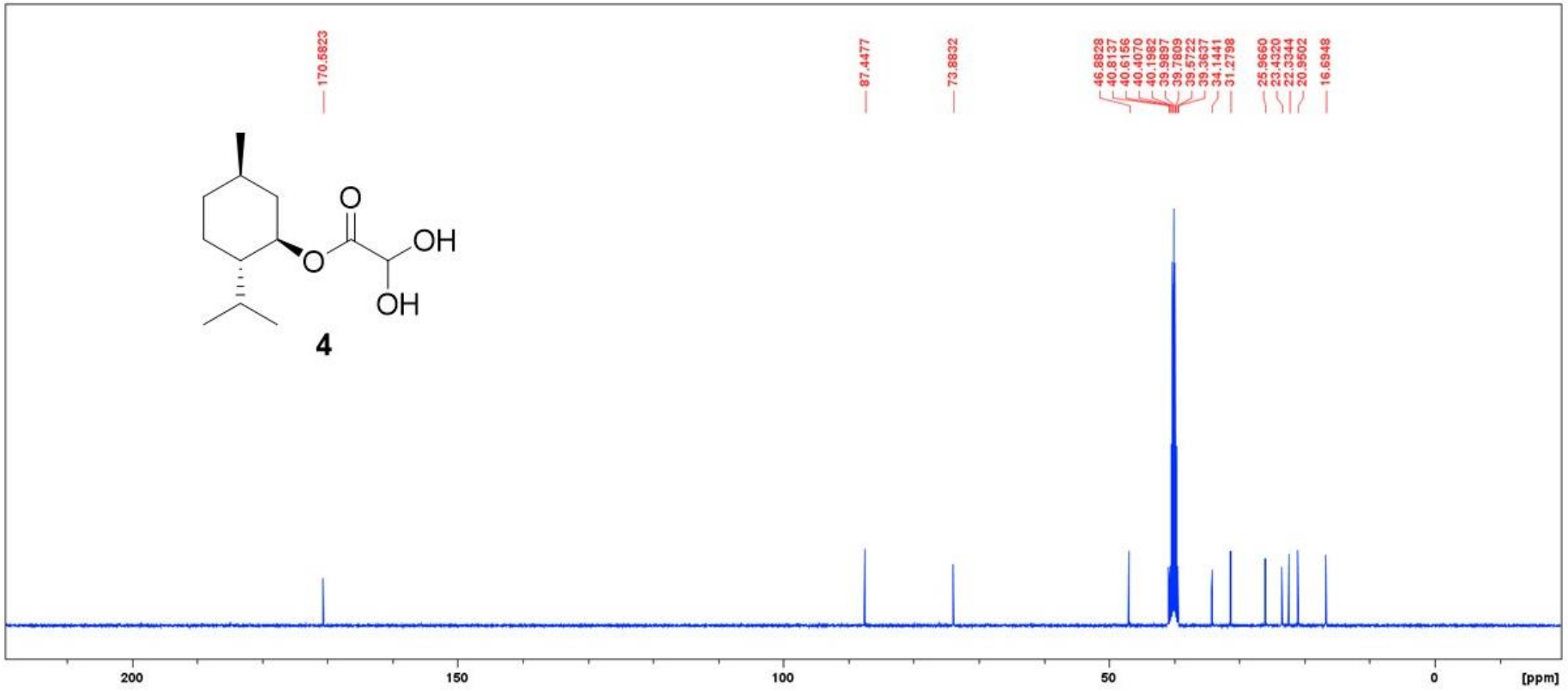

Figure 7. ^{13}C-NMR of L-menthyl glyoxylate 4.

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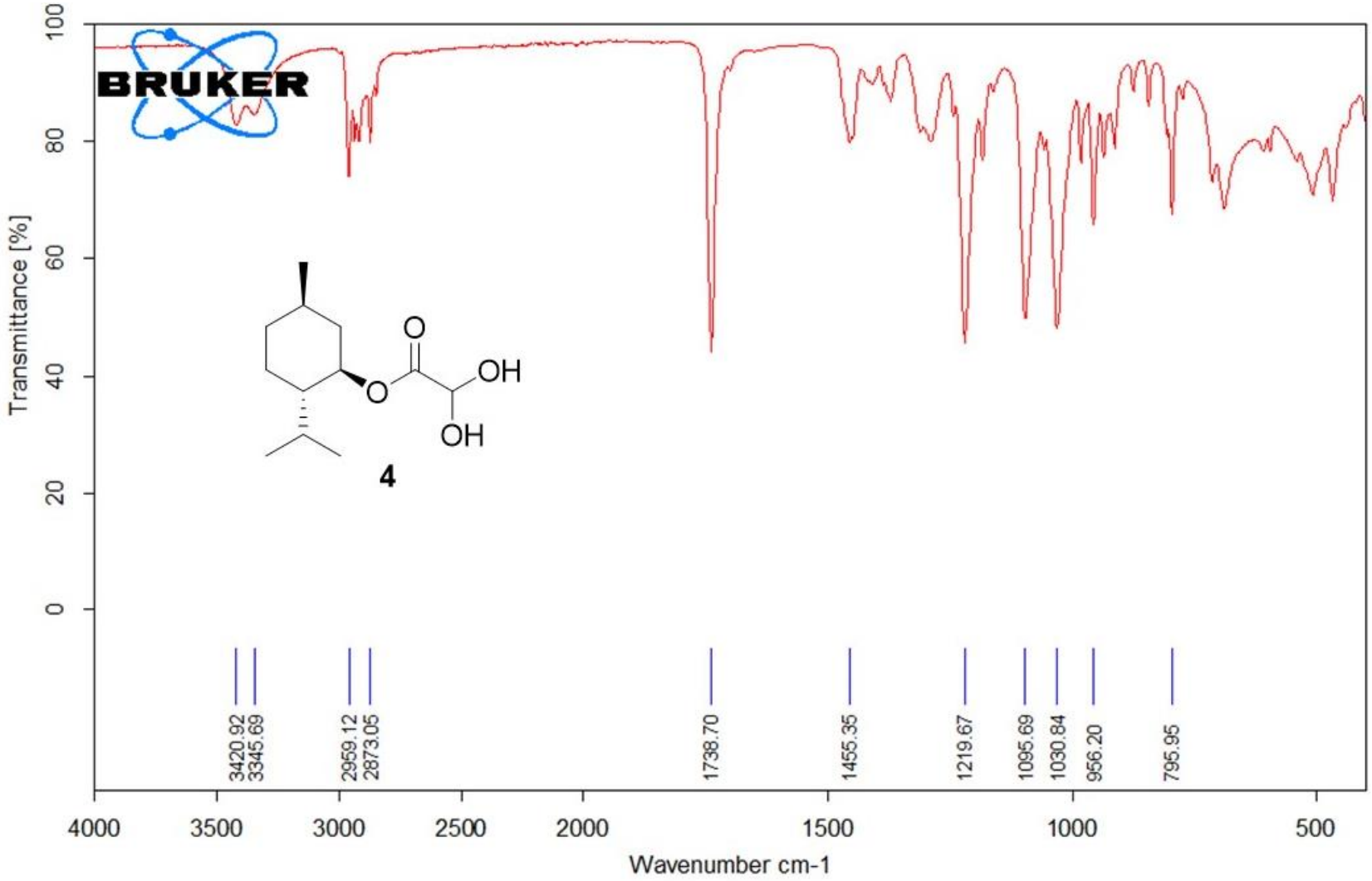

Figure 8. FT-IR of L-menthyl glyoxylate 4.

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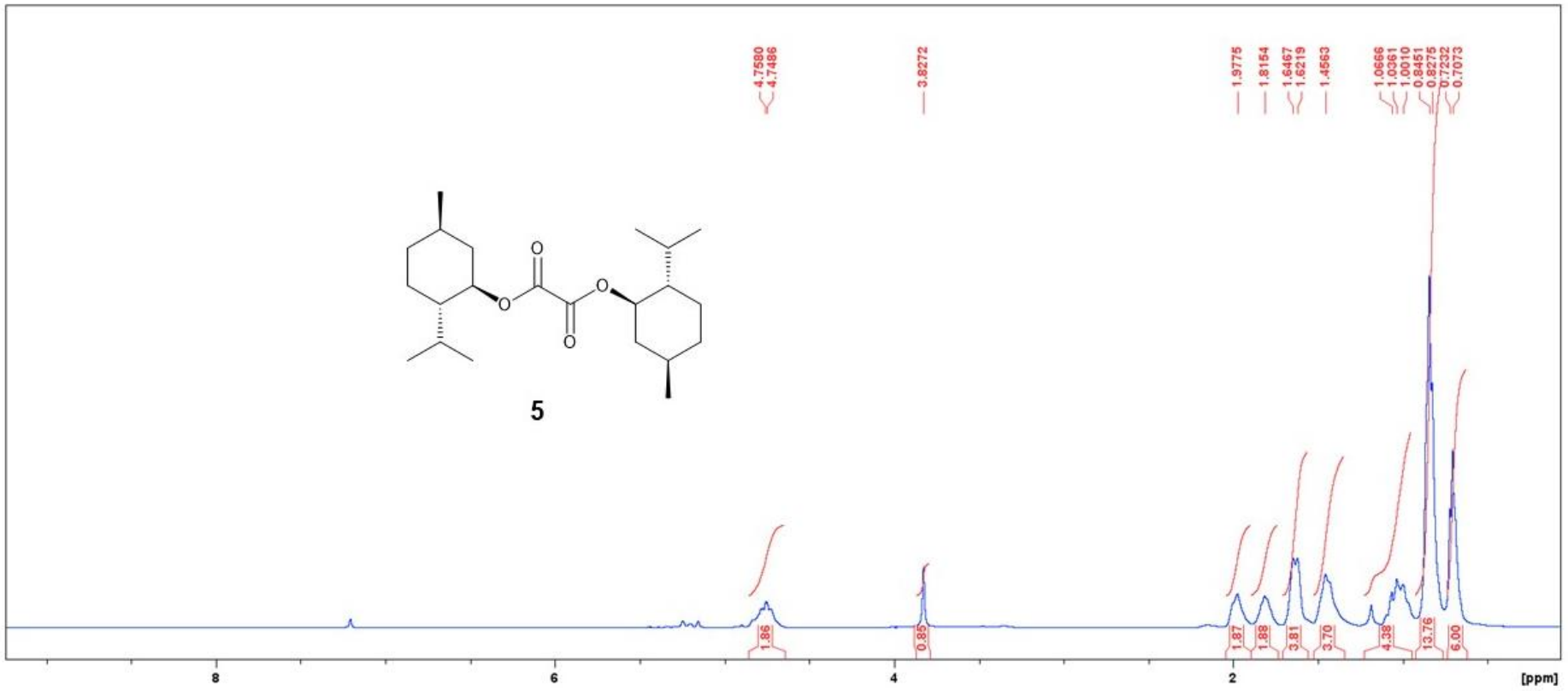

Figure 9. ^{1}H-NMR of diester 5.

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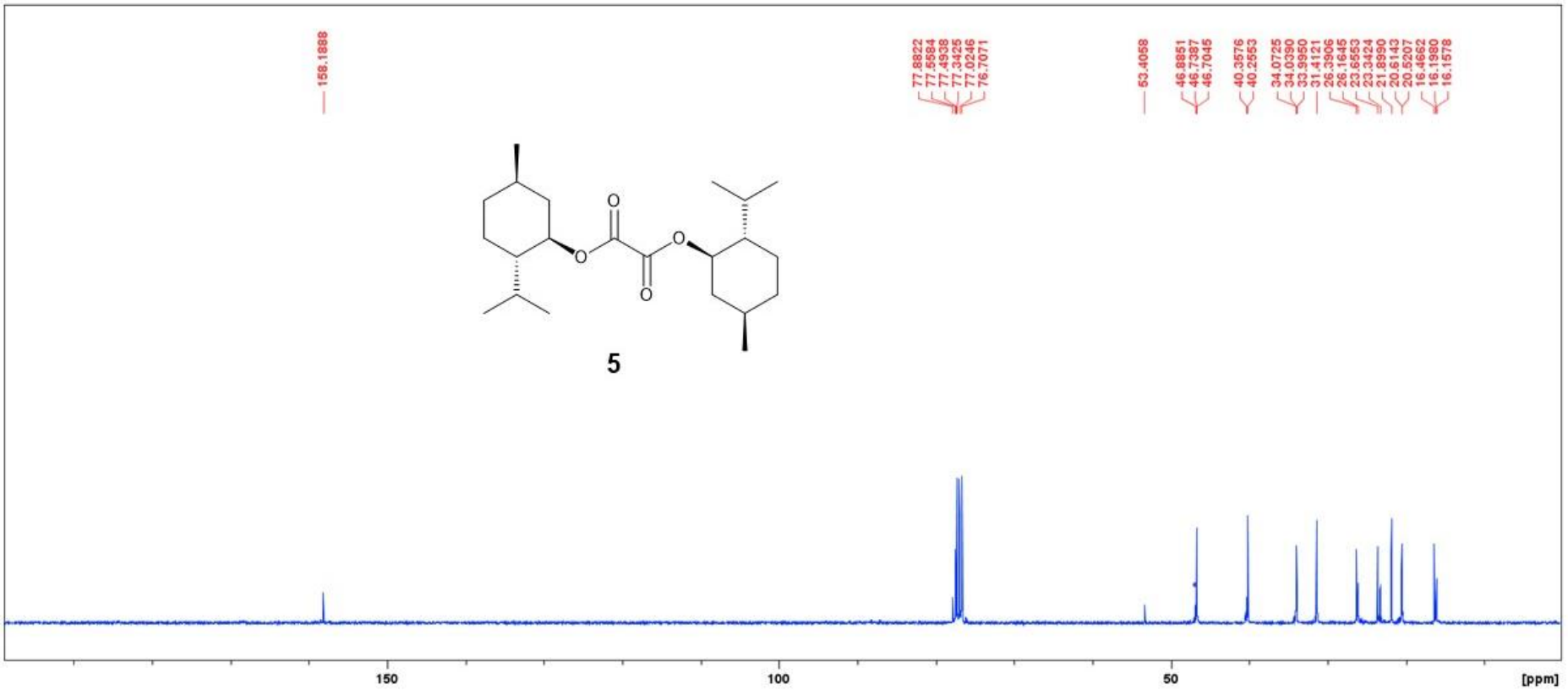

Figure 10. ^{13}C-NMR of diester 5.

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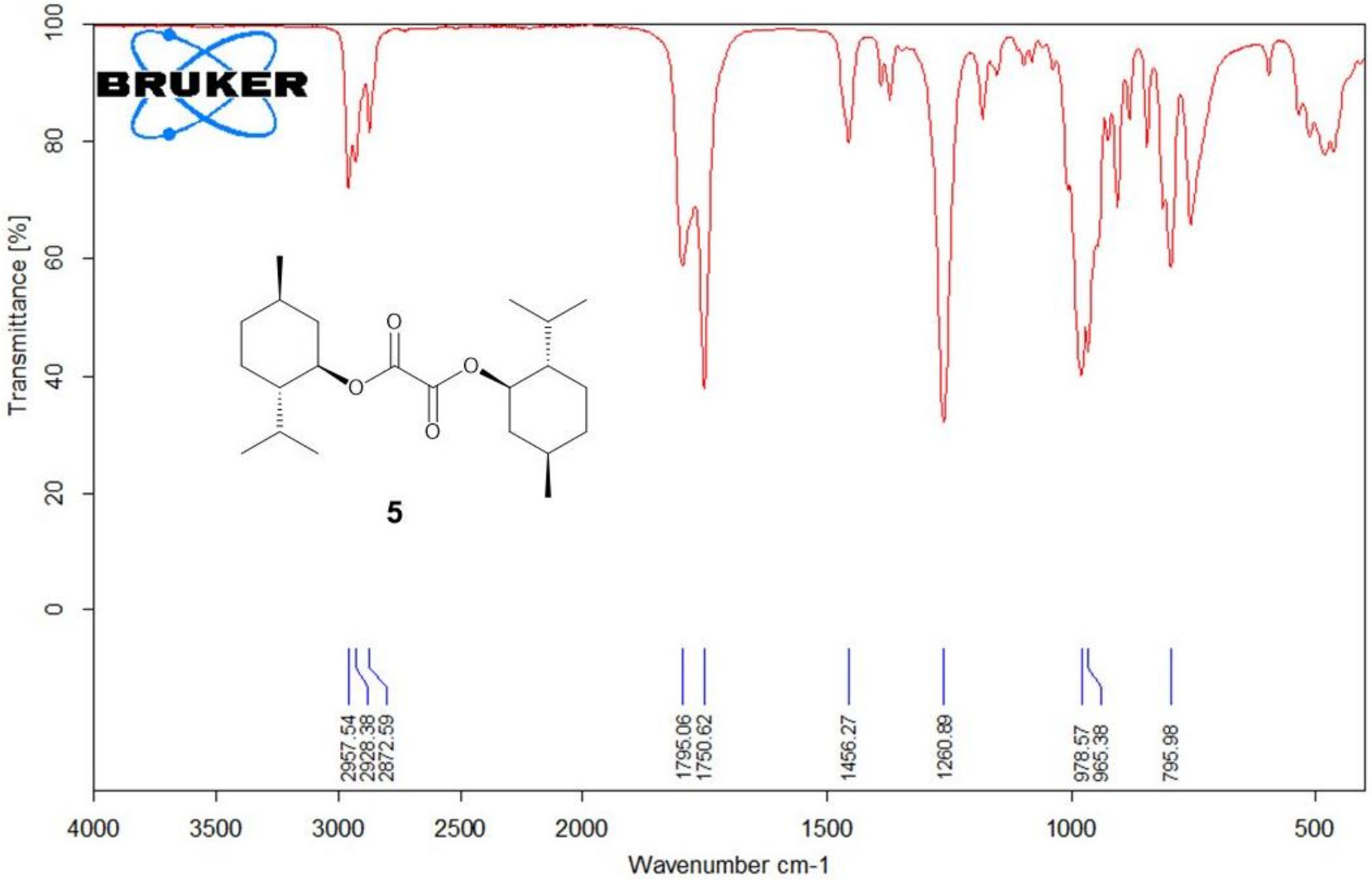

Figure 11. FT-IR spectrum of diester 5.

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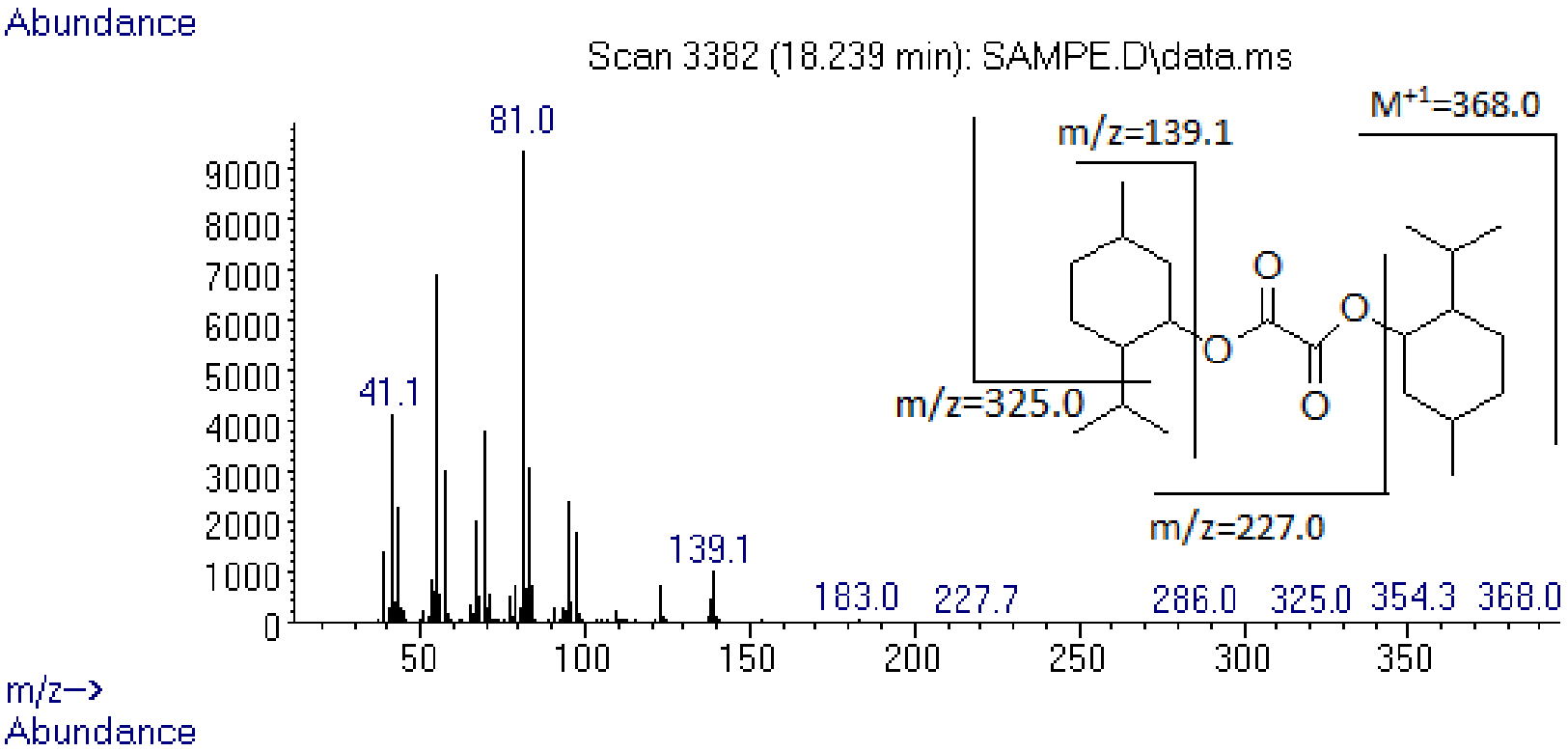

Figure 12. Di-ester 5 mass fragmentation pattern.

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