Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Replication of Biosynthetic Reactions Enables Efficient Synthesis of A-factor, a γ-Butyrolactone Autoinducer from *Streptomyces griseus*

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GENERAL PROCEDURES

All reactions were carried out in oven-dried glassware under a nitrogen atmosphere and with dry solvents unless otherwise stated. All chemicals were purchased from Aldrich and used as received unless noted otherwise. Meldrum's acid was recrystallized in acetone prior to use. Flash chromatography was performed using silica gel 60, 230 x 400 mesh (Whatman). Analytical thin layer chromatography was performed using silica gel 60 F_{254} TLC plates, 200 µm (EMD Chemicals) and *p*-anisaldehyde staining. All NMR spectra were recorded in $CDCl_3$, D_2O using a Bruker 400 or 300 MHz DRX Avance Spectrometer. Mass spectra were acquired using a JEOL JMS-600H double focusing magnetic sector mass spectrometer using FAB+ or EI+ ionization or a Thermo LCQ Deca XP MAX high sensitivity MSn ion trap mass spectrometer. IR Spectra were recorded using a Jasco FT/IR 4100 using NaCl plates.

PREPARATION OF STARTING MATERIALS

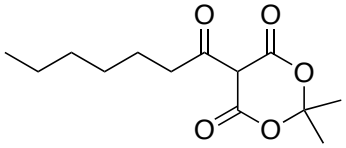
6-methylheptanoic acid[1] was prepared as in Weber, W.; Schoenmakers, R.; Spielmann, M.; Daoud El-Baba, M.; Folcher, M.; Keller, M.; Weber, C. C.; Link, N.; van de Wetering, P.; Heinzen, C.; Jolivet, B.; Sequin, E.; Aubel, D.; Thompson, C. J.; Fussenegger, M. *Nucleic Acids Res.* **2003**, *31*, e71.

acyl Meldrum's acids

Acid Chloride Preparation: 1 eq of 6-methylheptanoic acid was placed in a round-bottomed flask. $SOCl_2$ (2 eq) and catalytic DMF were added slowly and the reaction was stirred overnight at room temperature. Excess $SOCl_2$/SO was removed under vacuum with a NaOH trap. The resulting acid chloride was used without further purification. (Heptanoyl chloride is commercially available and did not need to be prepared.)

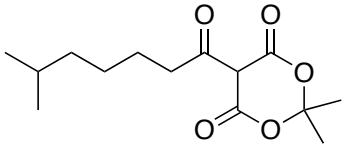
Acylation of Meldrum's acid: These compounds were prepared as reported by Oikawa and co-workers.[2] The acylated Meldrum's acid products were purified using flash column chromatography (8:1 hexanes:ethyl acetate). Meldrum's acids exist in an equilibrium between the enol and keto tautomers so NMR spectra contain a mixture of both. This causes inaccurate integration.

heptanoyl Meldrum's acid



^{1}H NMR: δ 0.9 (m, 7H) 1.2-1.3 (m, 10H), 1.43 (m, 2H), 1.63-1.73 (m, 3 H), 1.75 (s, 6H), 3.08 (t, *J*= 7.7 Hz, 2H). ^{13}C NMR: δ 14.4, 22.9, 25.0, 26.5, 27.2, 29.4, 31.8, 34.5, 36.2, 91.6, 105.1, 160.6, 171.0, 198.8. MS (FAB+, m/z): Calcd. for $C_{13}H_{20}O_5Na$: 279.1, found $[M+Na]^+$: 279.3.

11, 6-methylheptanoyl Meldrum's acid



^{1}H NMR: δ 0.88 (d, *J*= 6.6 Hz, 11H) 1.2-1.72 (m, 13H), 1.75 (s, 6H), 3.09 (t, *J*= 7.6 Hz, 2H). ^{13}C NMR: δ 22.9, 24.9, 26.4, 26.8, 27.2, 34.0, 35.8, 38.5, 91.2, 104.8, 160.2, 170.6, 198.3. MS (FAB+, m/z): Calcd. for $C_{14}H_{22}O_2Na$: 293.1, found $[M+Na]^+$: 293.3.

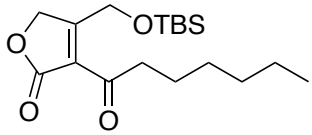
10, monosilyl DHA (1-((*tert*-butyldimethylsilyl)oxy)-3-hydroxypropan-2-one)[3] was prepared as in Schroer, J.; Welzel, P. *Tetrahedron*, **1994**, *50*, 6839-6858.

EXPERIMENTAL PROCEDURES

A. BUTENOLIDE FORMATION

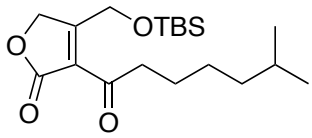
To a two-necked flask equipped with a septum and a condenser in a oil bath already heated to 110°C was added 1 equivalent of monosilyl DHA and 1.2 equivalents of an acylated Meldrum's acid along with 2 mL of toluene. The reaction was refluxed with stirring for five hours at which point was added 0.5 more equivalents of acylated Meldrum's acid. The reaction was allowed to stand at -20°C for at least one night before purification (without removal of toluene) on a column using a column with 4 times the typical amount of silica and about 4 times the diameter and 60:1 hexanes:acetone. Butenolides were isolated as bright yellow oils in 60-75% yield.

heptanoyl butenolide



71% yield, ^{1}H NMR: δ 0.09 (s, 6H), 0.81-0.93 (m, 12H), 1.13 (m, 10H), 1.59 (t, *J*= 7.3 Hz, 2H), 4.98 (s, 2H), 5.06 (s, 2H). ^{13}C NMR: δ -5.7, 14.0, 18.1, 22.5, 23.1, 25.4, 28.8, 31.6, 41.6, 61.9, 70.2, 122.4, 181.2, 197.1. LRMS (FAB+, m/z): 363.2. HRMS (FAB+, m/z): Calcd. for $C_{18}H_{32}O_4SiNa$: 363.1968, found $[M+Na]^+$: 363.1956.

13, 6-methylheptanoyl butenolide



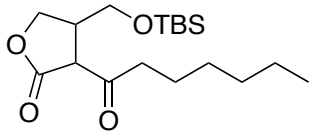
51% yield, ^{1}H δ NMR: 0.013 (s, 6H), 0.83-1.00 (m, 15H), 1.16-1.35 (m, 10H), 3.00 (t, *J*= 7.3 Hz, 2H), 5.01 (s, 2H), 5.10 (s, 2H). LRMS (FAB+, m/z): 377.2. HRMS (FAB+, m/z): Calcd. for $C_{19}H_{34}O_4SiNa$: 377.2124, found $[M+Na]^+$: 377.2118.

B. BUTENOLIDE REDUCTION

Procedure for reduction of butenolide with $NaBH_3CN$

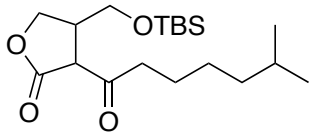
1 equivalent of butenolide was dissolved in ethanol (0.25 M) at room temperature. To this was added 1.1 equivalent of $NaBH_3CN$. The reaction was stirred until complete by TLC (approximately 30 minutes). *The reaction must be closely monitored as once conjugate reduction is complete, the ketone can be reduced, leading to an over-reduced product.* The reaction was quenched with water (2.5 times the volume of ethanol) and allowed to stir for 15 minutes before extraction with ethyl acetate. The ethyl acetate extracts were combined, dried over sodium sulfate, and filtered. The solvent was removed by rotary evaporation and the crude product was either used directly in the next reaction or purified by flash column chromatography using 70:1 hexanes:ethyl acetate.

protected heptanoyl γ-butyrolactone



65-70% yield for reactions with either $NaBH_3CN$ or Pd/C. ^1H NMR: δ 0.06 (s, 6H), 0.89 (m, 15H), 1.28 (m, 10H), 1.61 (m, 2H), 2.63 (m, *J*=7.2, 17.6 Hz, 1H), 2.95 (m, *J*=7.6, 17.6 Hz, 1H), 3.19, (m, 1H), 3.65 (m, 3H), 4.14 (t, *J*=7.3 Hz, 1H), 4.40 (t, *J*=8.3 Hz, 1H). ^{13}C NMR: δ -5.6, 14.0, 14.1, 18.1, 22.5, 23.3, 25.7, 28.7, 31.6, 39.3, 42.6, 54.7, 61.9, 69.2, 172.5, 202.8. LRMS (FAB+, m/z): 365.2. HRMS (FAB+, m/z): Calcd. for $C_{18}H_{34}O_4SiNa$: 365.2124, found $[M+Na]^+$: 365.2130.

14, protected 6-methylheptanoyl γ-butyrolactone



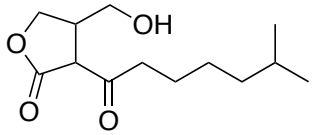
60% yield with $NaBH_3CN$. ^1H NMR: δ 0.06 (s, 6H), 0.89 (m, 26H), 1.30 (m, 6H), 1.62 (m, 4H), 2.64 (m, *J*=**7.2, 17.6** Hz, 1H), 2.97 (m, ***J*=7.6, 17.6 Hz**, 1H), 3.20 (m, 1H), 3.66 (m, 3H), 4.14 (t, ***J*=7.3 Hz**, 1H), 4.41 (t, ***J*=8.3** Hz, 1H). LRMS (FAB+, m/z): 379.2. HRMS (FAB+, m/z): Calcd. for $C_{19}H_{36}O_4SiNa$: 365.2124, found $[M+Na]^+$: 379.2276.

C. SILYL DEPROTECTION

Procedure for silyl deprotection:

TBS-protected butenolides were dissolved in 6:3:1 THF:HCOOH:H_2O (0.1 M) and stirred for 18 hours at room temperature. Reactions were brought up to ~pH=4 with saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. Ethyl acetate extracts were combined and dried over sodium sulfate and filtered before removal of the solvent by rotary evaporation. The products were purified by flash column chromatography using 7:1 hexanes:ethyl acetate.

Racemic A-factor[4]



72% yield. ^{1}H NMR: δ 0.87 (d, 6H), 1.20 (m, 2H), 1.34 (m, 2H), 1.50-1.62 (m, 3H), 2.65 (m, 1H), 2.98 (m, 1H), 3.70 (m, 2H), 4.18 (m, 1H), 4.45 (m, 1H) ^{13}C NMR: δ 23.0, 23.9, 27.2, 28.2, 39.1, 39.6, 42.9, 55.4, 62.2, 69.5 Calcd. for $C_{12}H_{22}O_4Na$: 251.2, found, $[M+Na]^+$ 251.2.

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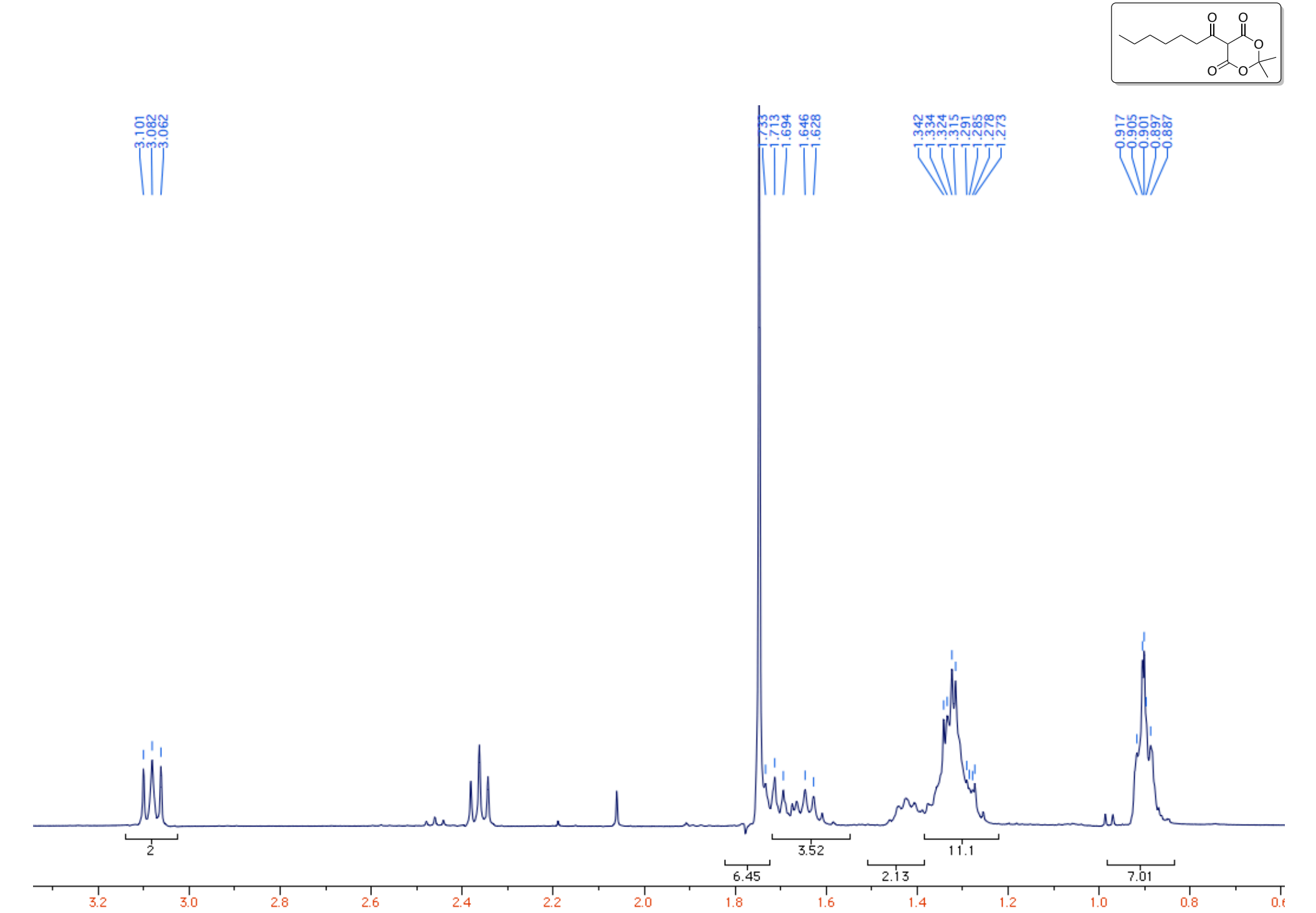
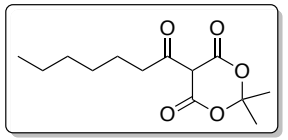
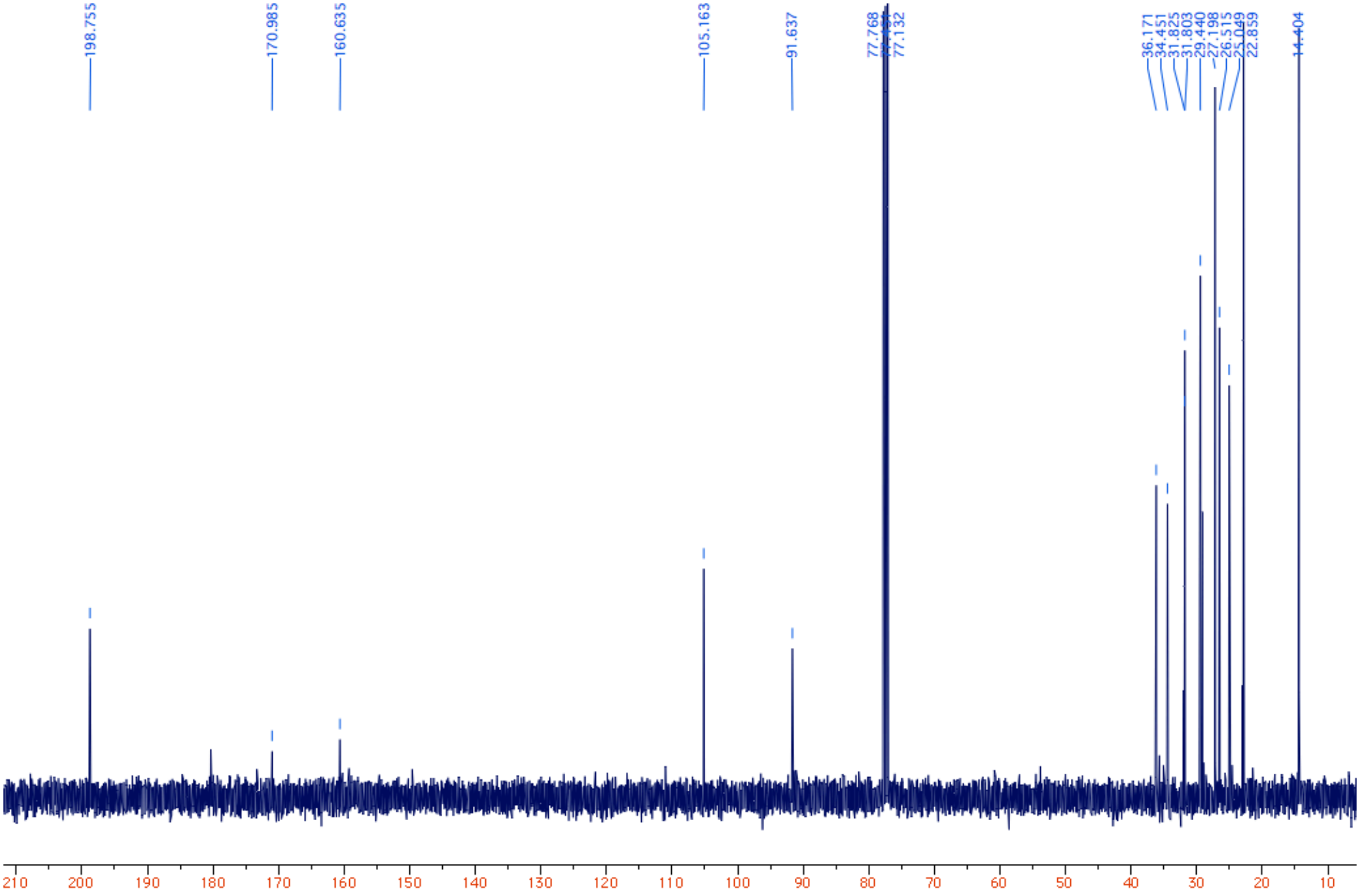
(2) Oikawa, Y.; Yoshioka, T.; Sugano, K.; Yonemitsu, O. *Org. Synth.* **1990**, *CV 7*, 359–360.

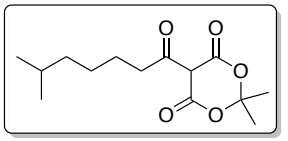
(3) Schröer, J.; Welzel, P. *Tetrahedron* **1994**, *50*, 6839–6858.

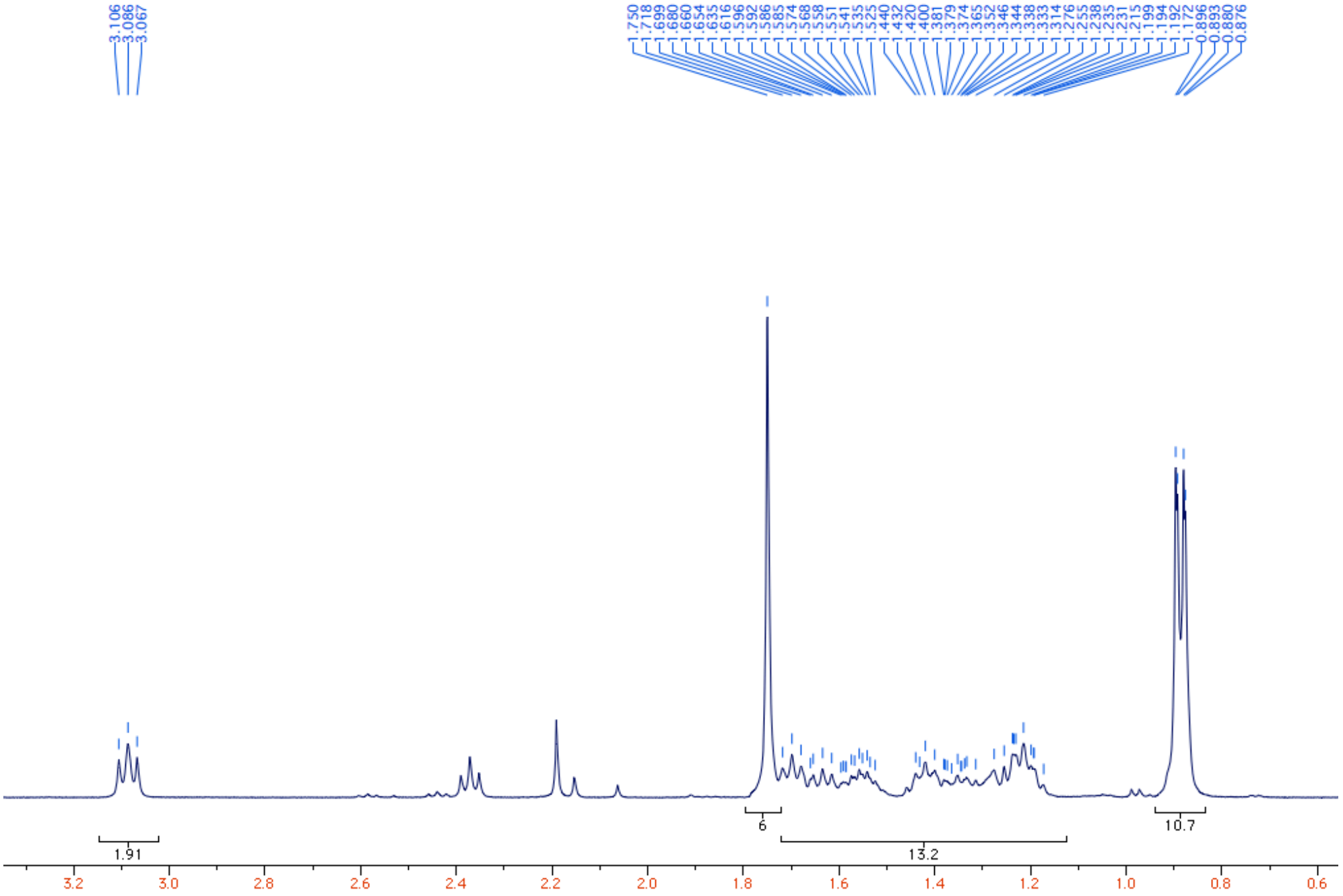
(4) E. Kleiner, S. A. Pliner, V. S. Soifer, V. V. Onoprienko, T. A. Balashova, B. V. Rozynov and A. S. Khokhlov, *Bioorg Khim.* 1976, **2**, 1142-1147.

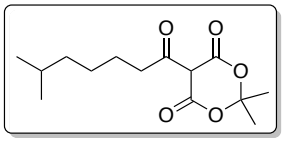


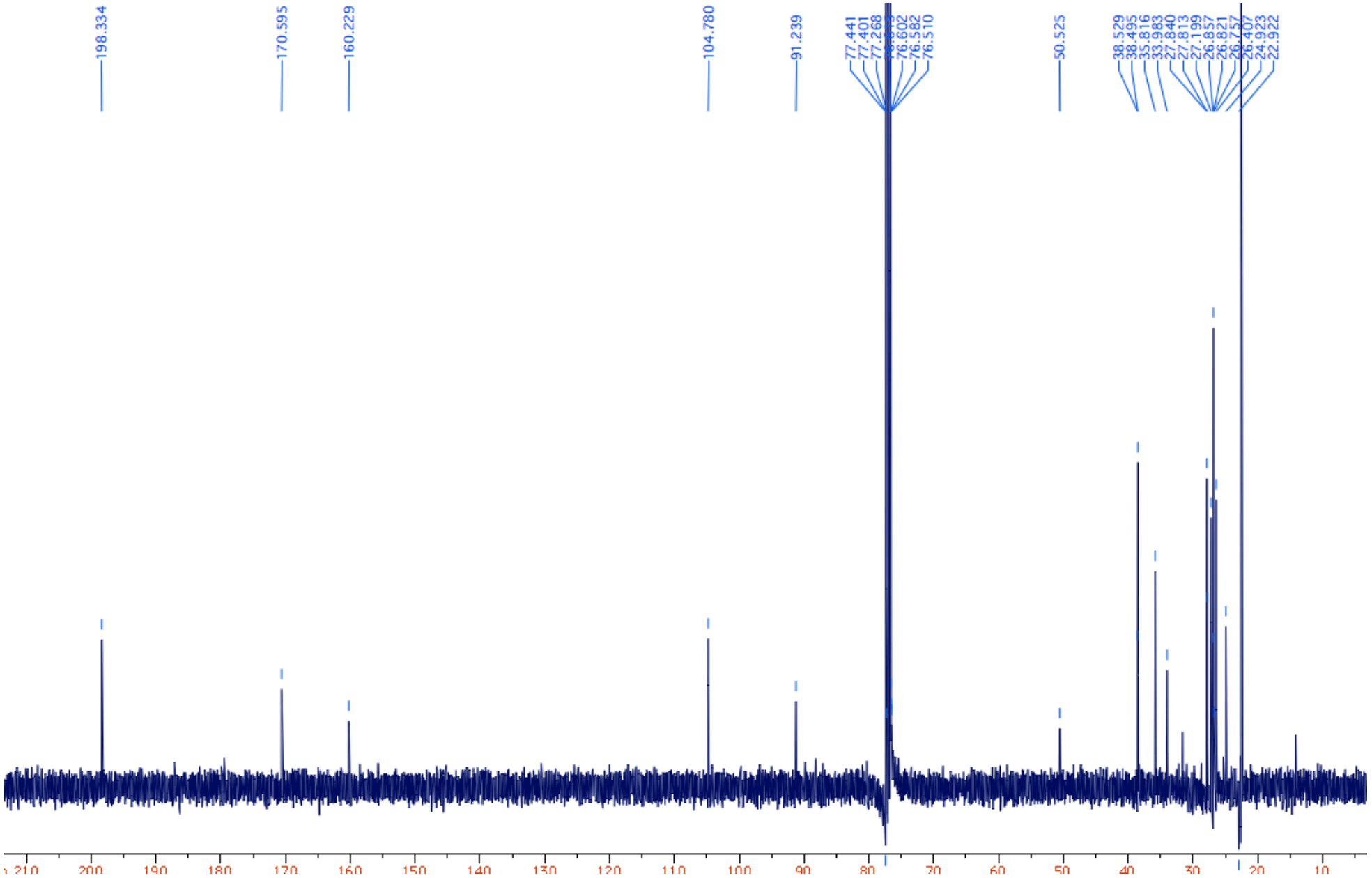


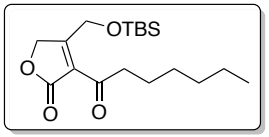


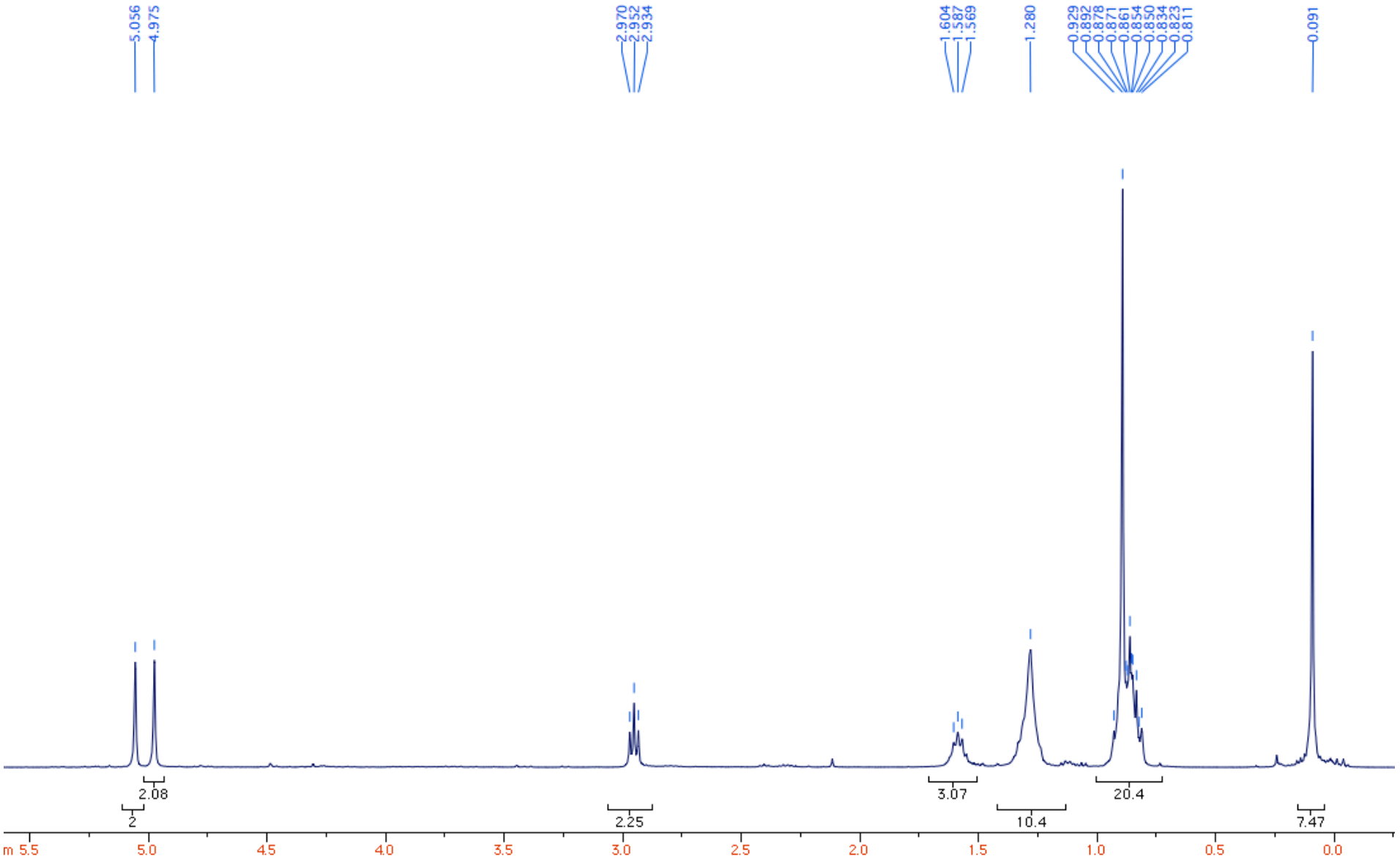


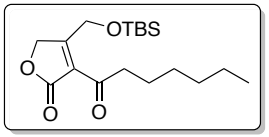


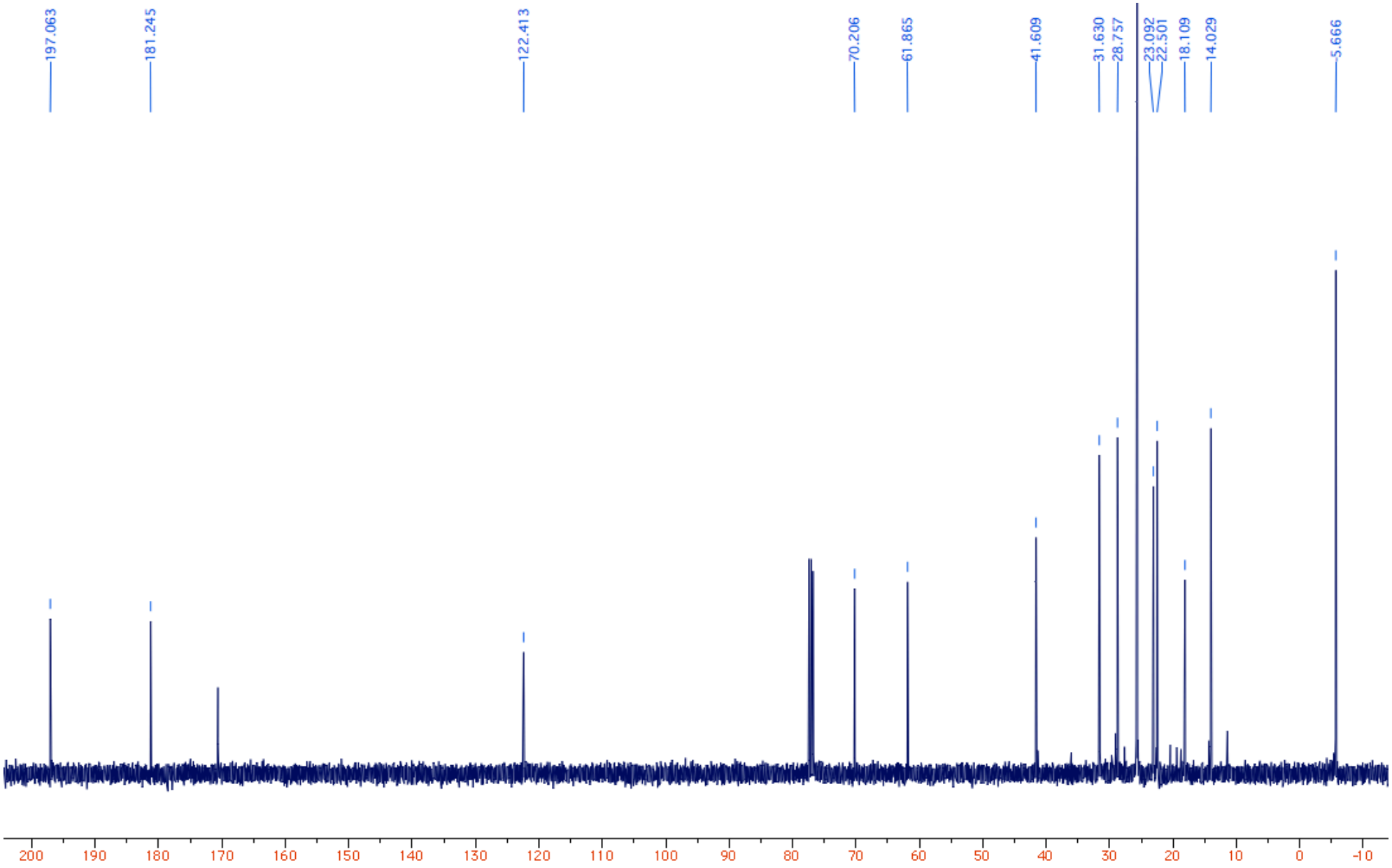


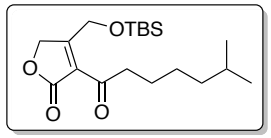


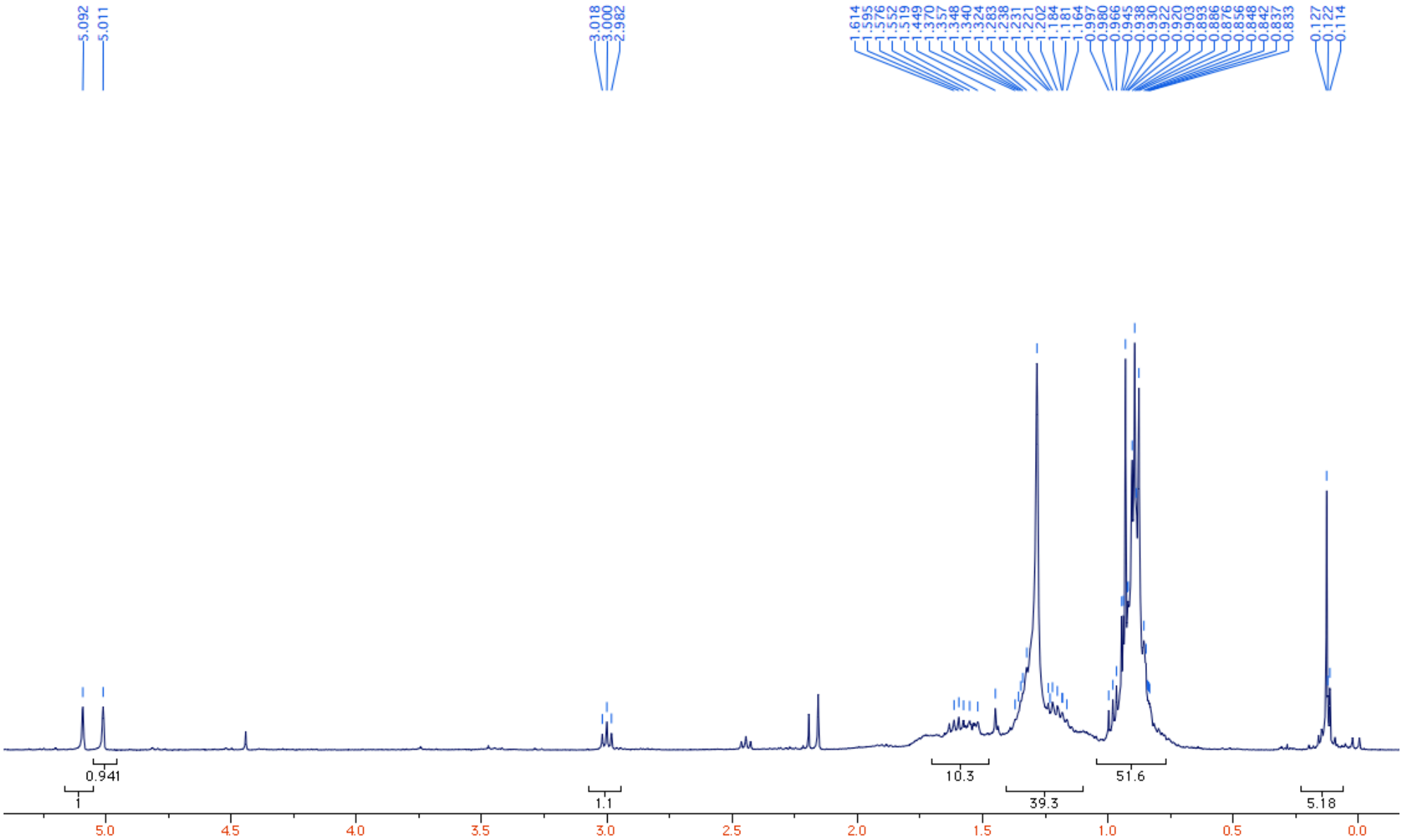


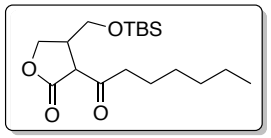


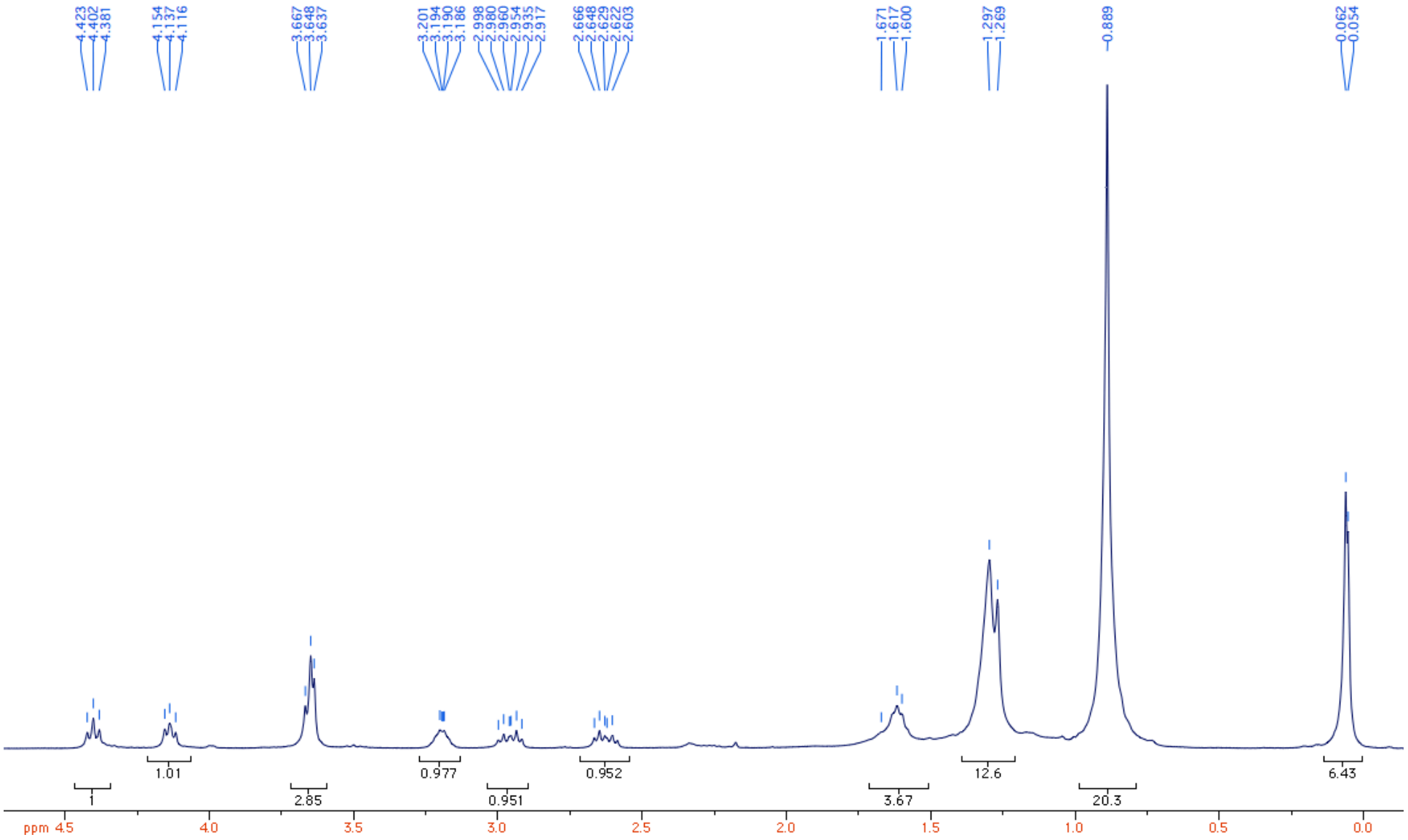


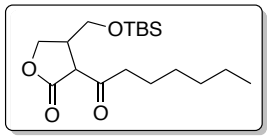


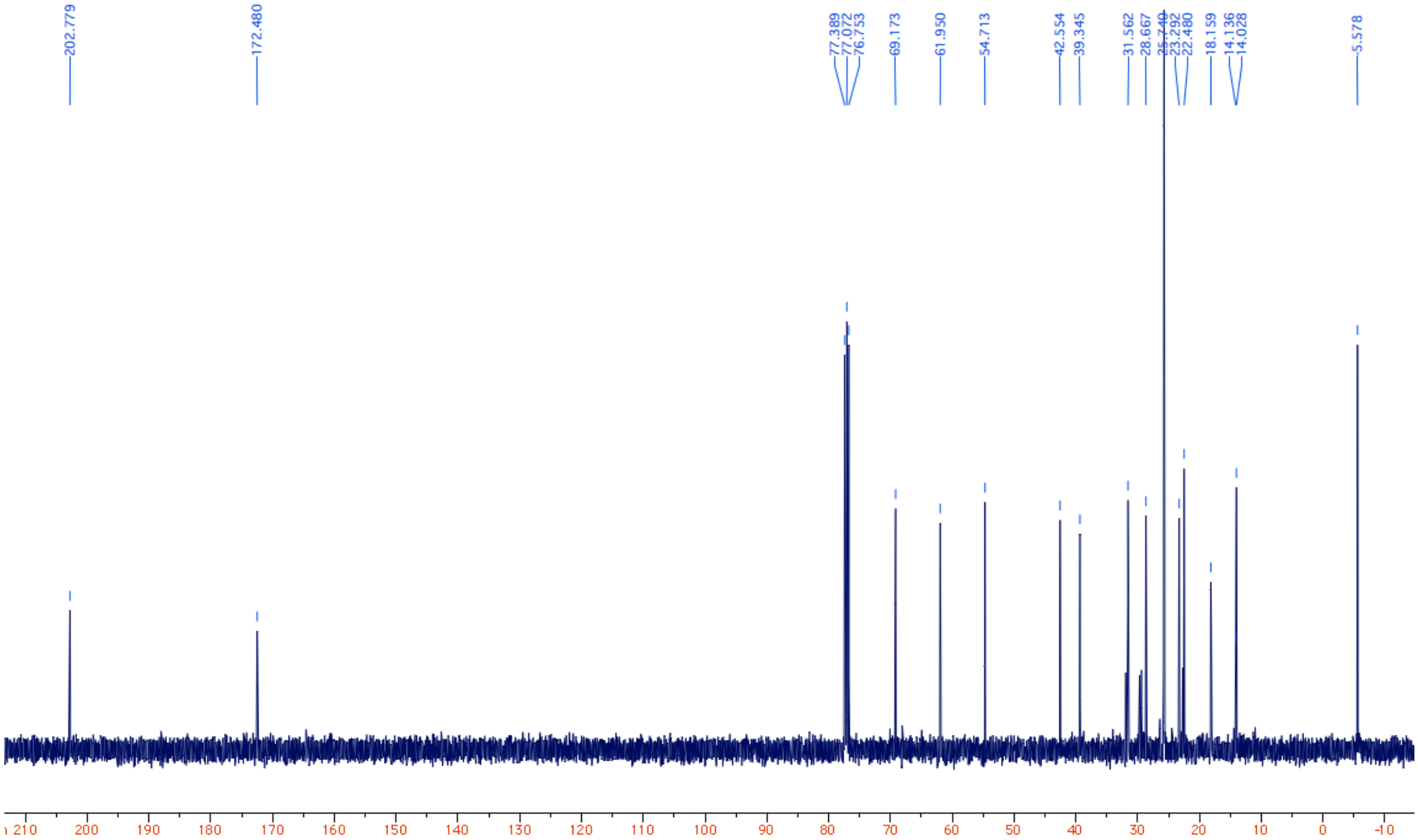


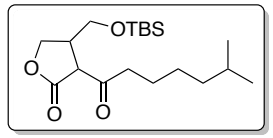


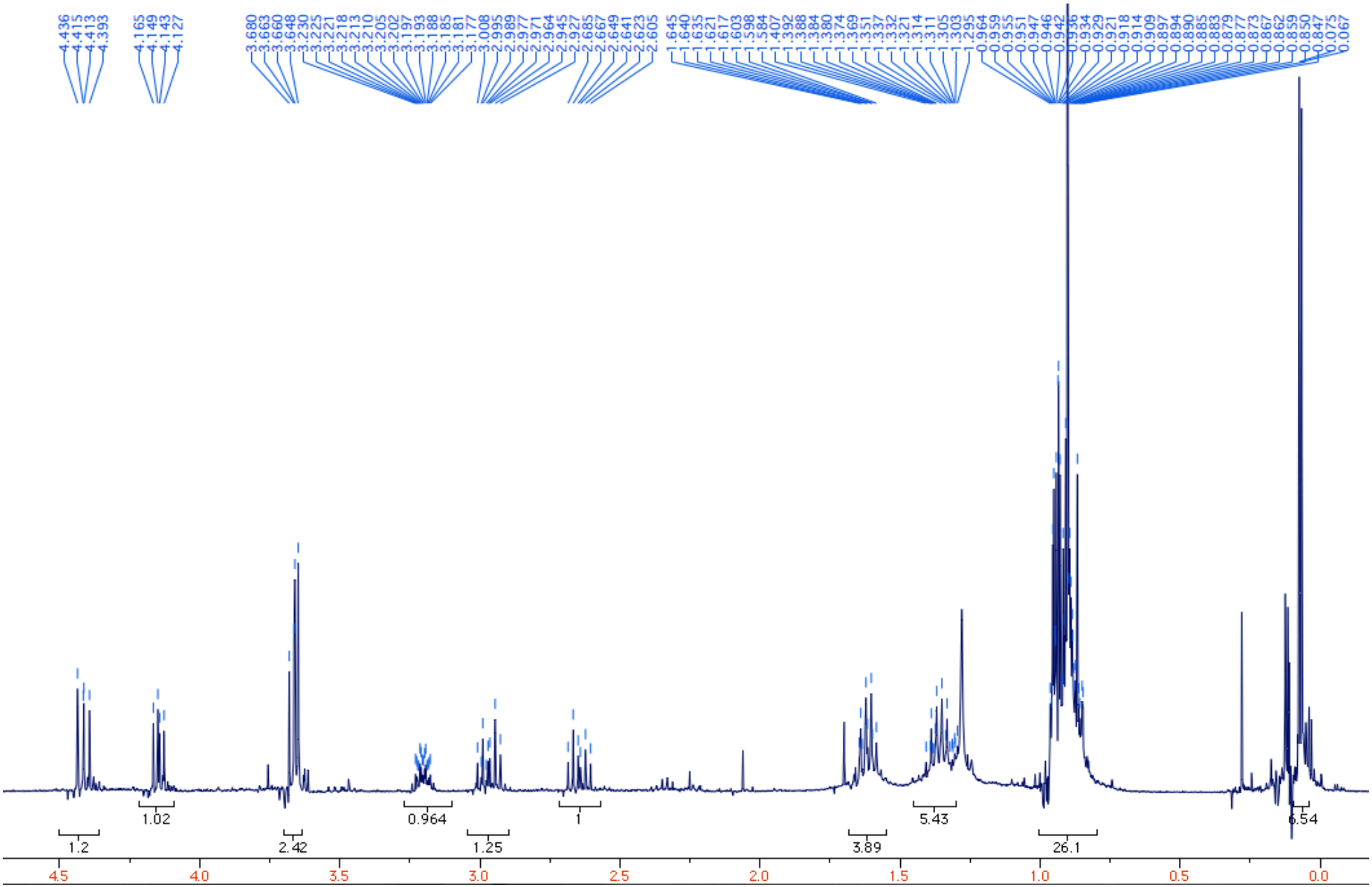




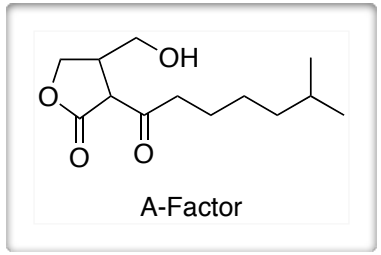




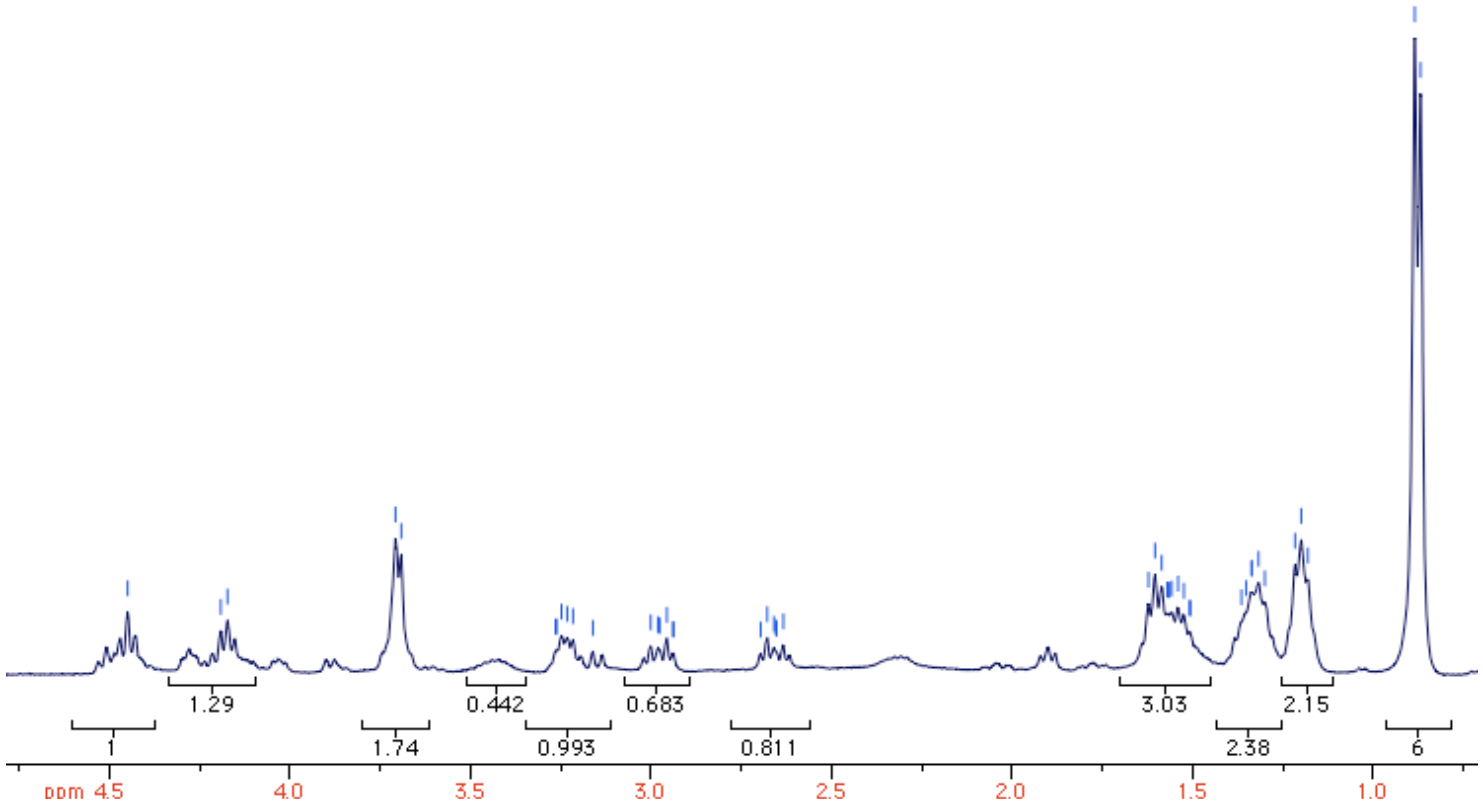


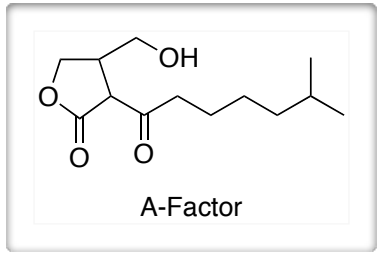


Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry



A-Factor





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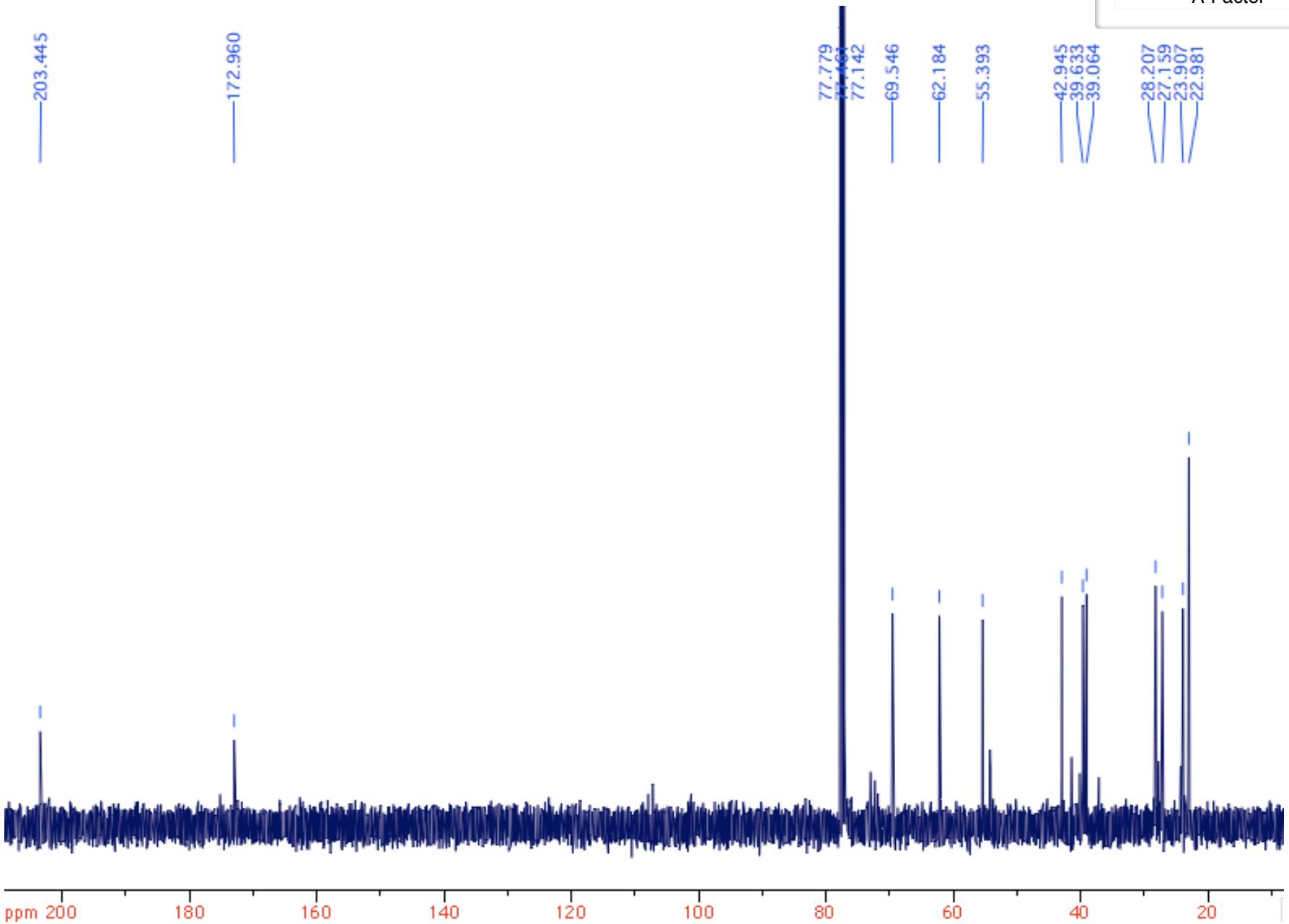
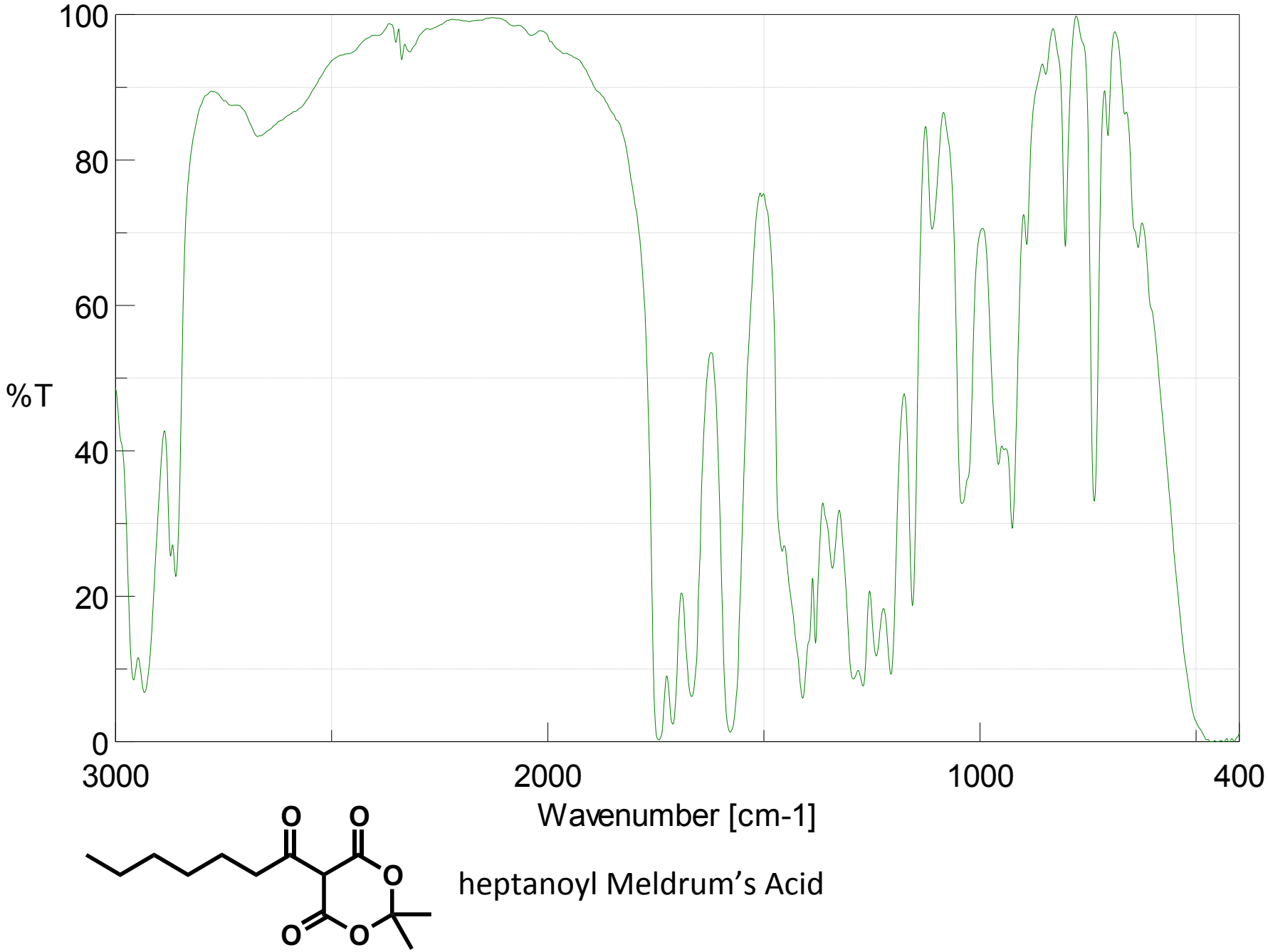
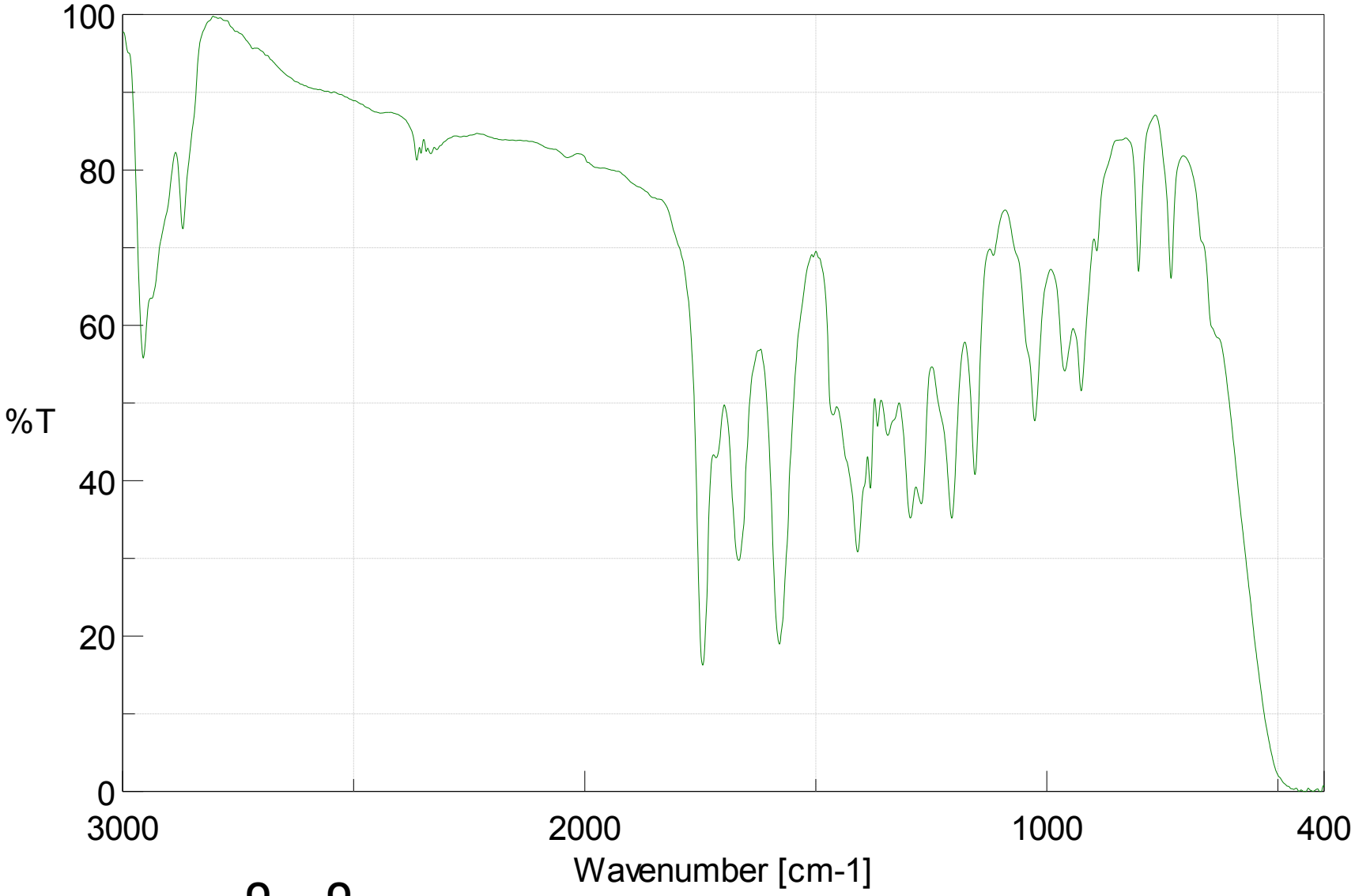
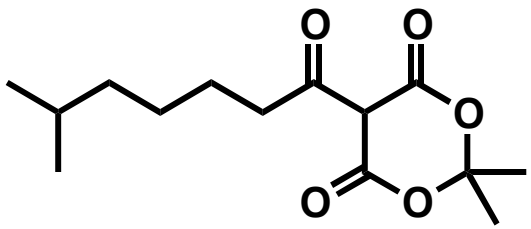




heptanoyl Meldrum's Acid





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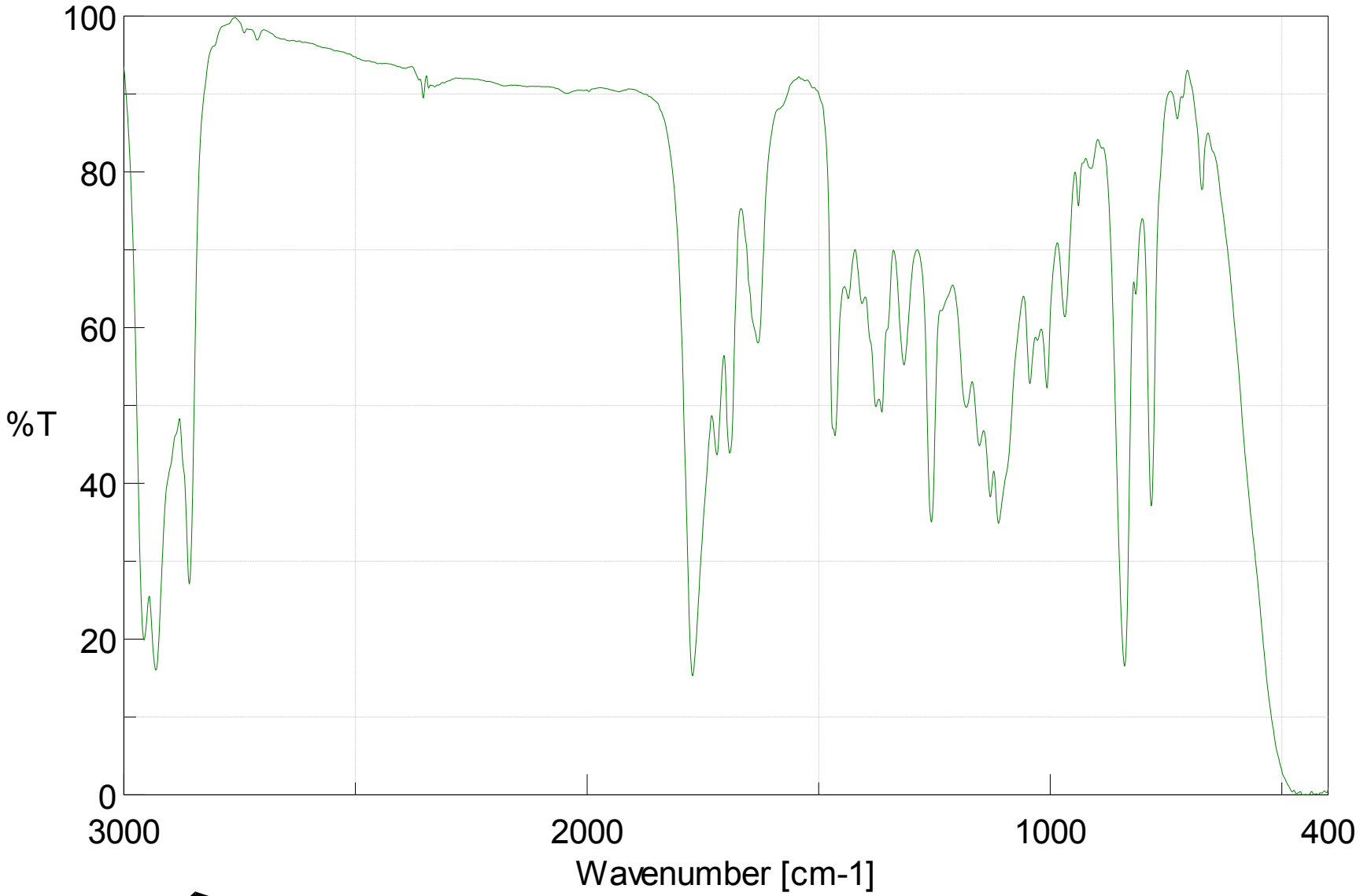

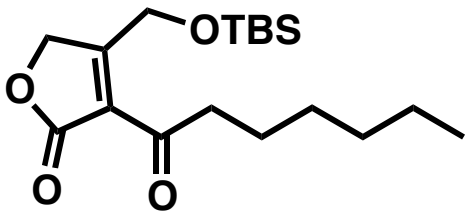

heptanoyl butenolide