Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

CAL-B Catalyzed Desymmetrization of 3-Alkylglutarate: ″Olefin Effect″ and Asymmetric Synthesis of Pregabalin

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1. General Information

Almost reactions were carried out under an atmosphere of nitrogen in oven-dried glassware with magnetic stirring. Reactions were monitored by thin layer chromatography (TLC) with 0.25 mm E. Merck pre-coated silica gel plates (60 F254). Visualization was accomplished with either UV light, or by immersion in solutions of ninhydrin, phosphomolybdic acid (PMA) followed by heating on a hot plate for 10 – 15 sec. The reaction products were purified by flash column chromatography using Kieselgel 60 Art 9385 (230 – 400 mesh). ^{1}H-NMR and ^{13}C-NMR spectra were obtained using a Varian 200 (200 MHz for ^{1}H and 50.3 MHz for ^{13}C) spectrometer and deuterated solvents ($CDCl_3$ + 0.03% TMS, CD_3OD; euriso-top®). The pH was measured with a pH meter (Thermo scientific; Orion 3 star pH portable). Optical rotation was obtained using a polarimeter (Rudolph Autopol IV digital polarimeter). The % ee value was obtained using HPLC (YL 9100 HPLC system) and chiral column (Chiralcel® OD-RH, Chiralpak® IA; Diacel). Low and high resolution mass spectrometry analyses were performed using Macromass ZQ4000 LC/MS system and AB Sciex 4800 Plus MALDI TOF/TOF™ (2,5-dihydroxybenzoic acid (DHB) matrix was used to prepare samples for MS. Data was obtained in the reflector positive mode with interrnal standards for calibration), respectively.

2. Preparation of Substrates

Dimethyl 3-isobutylpentanedioate (1a)

CAN (5.45 g, 9.94 mmol, 2.4 equiv.) was added to a solution of 3-isobutylpentanedioic acid (0.78 g, 4.14 mmol, 1.0 equiv.) in MeOH (0.1 M, 41 mL) at room temperature was added. The reaction mixture was stirred at room temperature for 12 h. This was concentrated *in vacuo*. The crude compound was purified by flash column chromatography on silica gel (9:1 Hex-EtOAc) to furnish *title compound* **1a** (0.85 g, 95%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.89 (d, *J* = 6.4 Hz , 6H), 1.19 (t, *J* = 6.8 Hz, 2H), 1.61 (m, 1H), 3.34 – 3.40 (m, 5H), 3.67 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 173.1, 51.5, 43.5, 38.6, 30.0, 25.2, 22.6; MS (*m/z*): $[M + Na]^+$ calcd for $C_{11}H_{20}O_4Na$, 239.13; found, 238.88

Diethyl 3-isobutylpentanedioate (1b)

The procedure was conducted in the same manner as **1a**. The 3-isobutylpentanedioic acid (0.82 g, 4.36 mmol) and EtOH (0.1 M, 43.6 mL) was used as starting material and solvent. The reaction time was 24 h. *Title compound* **1b** (0.99 g, 93%) as a colorless oil was given. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.89 (d, *J* = 6.6 Hz, 6H), 1.20 (t, *J* = 6.6 Hz, 2H), 1.26 (t, *J* = 7.2 Hz, 6H), 1.64 (m, 1H), 2.33 – 2.48 (m, 5H), 4.13 (q, *J* = 7.2 Hz, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.3, 60.0, 43.2, 38.6, 29.8, 25.0, 22.4, 14.0; MS (*m/z*): $[M + Na]^+$ calcd for $C_{13}H_{24}O_4Na$, 267.16; found, 266.97

Dipropyl 3-isobutylpentanedioate (1c)

The procedure was conducted in the same manner as **1a**. The 3-isobutylpentanedioic acid (0.78 g, 4.15 mmol) and *n*-PrOH (0.1 M, 41.5 mL) was used as starting material and solvent. The reaction time was 24 h. *Title compound* **1c** (0.90 g, 80%) as a colorless oil was given. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.89 (d, *J* = 6.6 Hz, 6H), 0.94 (t, *J* = 7.4 Hz, 6H), 1.21 (t, *J* = 6.6 Hz, 2H), 1.63 (m, 1H), 1.65 (6^{th}, *J* = 7.4 Hz, 4H), 2.36 (m, 5H), 4.03 (t, *J* = 7.4 Hz, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.5, 65.8, 43.3, 38.6, 29.8, 25.0, 22.4, 21.8, 10.3; MS (*m/z*): $[M + Na]^+$ calcd for $C_{15}H_{28}O_4Na$, 295.19; found, 295.03

Diallyl 3-isobutylpentanedioate (1d)

DBU (2.16 g, 14.19 mmol, 3 equiv.) was added to a solution of 3-isobutylpentanedioic acid (0.89 g, 4.73 mmol, 1 equiv.) in ACN (0.1 M, 47.3 mL) at room temperature. The solution was cooled to 0°C. The reaction mixture was stirred for 10 min and then allyl iodide (1.29 mL, 11.83 mmol, 2.5 equiv.) was added dropwise over 15 min and then stirred at 0°C for 12 h. The reaction mixture was diluted with Et_2O (20 mL), washed with H_2O (3 x 10 mL), brine (10 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography on silica gel (9:1 Hex-EtOAc) to furnish *title compound* **1d** (1.18 g, 93%) as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.90 (d, *J* = 6.6 Hz, 6H), 1.22 (t, *J* = 6.2 Hz, 2H), 1.62 (m, 1H), 2.40 (m, 5H), 4.57 (d, *J* = 5.6 Hz, 4H), 5.27 (m, 4H), 5.92 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.9, 132.0, 118.0, 64.8, 43.2, 38.4, 29.7, 25.0, 22.4; MS (*m/z*): $[M + Na]^+$ calcd for $C_{15}H_{24}O_4Na$, 291.16; found, 291.02

Diisobutyl 3-isobutylpentanedioate (1e)

The procedure was conducted in the same manner as **1d**. The 3-isobutylpentanedioic acid (0.22 g, 1.16 mmol) and *i*-BuBr (0.35 mL, 2.91 mmol) was used as starting material and reagent. The reaction time was 12 h. *Title compound* **1e** (0.33 g, 95%) as a colorless oil was given. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.91 (t, *J* = 6.2 Hz, 18H), 1.20 (t, *J* = 6.2 Hz, 2H), 1.52 (q, *J* = 6.8 Hz, 4H), 1.64 (m, 2H), 2.35 (m, 5H), 4.10 (t, *J* = 6.8 Hz, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.7, 62.9, 43.4, 38.8, 37.4, 30.1, 25.2, 25.1, 22.6, 22.5

Dipropyl 3-propylpentanedioate (2c)

The procedure was conducted in the same manner as an **1c**. Starting material was 3-propylpentanedioic acid (0.57 g, 3.27 mmol). *Title compound* **2c** (0.73 g, 87%) as a colorless oil was given. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.83 – 1.03 (m, 9H), 1.23 – 1.44 (m, 4H), 1.53 – 1.77 (m, 4H), 2.35 (m, 5H), 4.02 (t, *J* = 6.6 Hz, 4H); ^{13}C NMR (50 MHz, CD_3OD) 174.35, 67.09, 39.47, 37.36, 33.28, 23.04, 20.77, 14.43, 10.76; HRMS-MALDI (*m/z*): $[M + Na]^+$ calcd for $C_{14}H_{26}O_4Na$, 281.1723; found, 281.1713

Diallyl 3-propylpentanedioate (2d)

The procedure was conducted in the same manner as **1d**. Starting material was 3-propylpentanedioic acid (0.72 g, 4.13 mmol). *Title compound* **2d** (0.96 g, 92%) as a colorless oil was given. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.90 (m, 3H), 1.31 – 1.35 (m, 4H), 2.37 (m, 5H), 4.56 (dt, *J* = 5.6, 1.4 Hz, 4H), 5.17 – 5.36 (m, 4H), 5.93 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 171.9, 131.8, 116.5, 64.2, 37.4, 35.4, 31.3, 18.9, 12.5; HRMS-MALDI (*m/z*): $[M + Na]^+$ calcd for $C_{14}H_{22}O_4Na$, 277.1410; found, 277.1411

Dipropargyl 3-propylpentanedioate (2e)

The procedure was conducted in the same manner as **1d**. 3-propylpentanedioic acid (0.51 g, 2.92 mmol) and propargyl bromide (0.65 mL, 7.26 mmol) was used as starting material and reagent. *Title product* **2e** (0.68 g, 93%) as a colorless oil was given. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.85 – 0.95 (m, 3H), 1.25 – 1.41 (m, 4H), 2.42 (m, 5H), 2.47 (t, *J* = 2.5 Hz, 2H), 4.68 (t, *J* = 2.5 Hz, 4H); ^{13}C NMR (50 MHz, CD_3Cl): δ =171.74, 74.91, 51.92, 38.17, 36.19, 31.92, 29.01, 19.81, 14.11; HRMS-MALDI (*m/z*): $[M + Na]^+$ calcd for $C_{14}H_{18}O_4Na$, 273.1097; found, 273.1092

Dicyclopropylmethyl 3-propylpentanedioate (2f)

The procedure was conducted in the same manner as **1c**. 3-propylpentanedioic acid (0.52 g, 2.97 mmol) and cyclopropylmethyl alcohol (0.94 mL, 11.88 mmol) was used as starting material and reagent. *Title compound* **2f** (0.71 g, 85%) as a colorless oil was given. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.28 – 0.81 (m, 4H), 0.50 – 0.63 (m, 4H), 0.87 - 0.94 (m, 3H), 1.02 – 1.20 (m, 2H), 1.34 – 1.36 (m, 4H), 2.38 (m, 5H), 3.89 – 3.93 (d, *J* = 7.3 Hz, 4H); ^{13}C NMR (50 MHz, CD_3Cl) : δ = 172.86, 69.16, 38.78, 36.31, 19.85, 14.22, 9.94, 3.33; HRMS-MALDI (*m/z*): $[M + Na]^+$ calcd for $C_{16}H_{26}O_4Na$, 305.1723 found, 305.1720

Dipropyl 3-methylpentanedioate (3c)

The procedure was conducted in the same manner as **1c**. Starting material was 3-methylpentanedioic acid (0.51 g, 3.49 mmol). *Title compound* **3c** (0.76 g, 95%) as a colorless oil was given. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 0.94 (t, *J* = 7.4 Hz, 6H), 1.03 (d, *J* = 6.5 Hz, 3H), 1.70 – 1.56 (m, 4H), 2.53 – 2.17 (m, 5H), 4.04 (t, *J* = 6.7 Hz, 4H); ^{13}C NMR (50 MHz, CD_3OD): δ = 174.18, 67.09, 41.59, 28.76, 23.05, 20.01, 10.76; HRMS-MALDI (*m/z*): $[M + Na]^+$ calcd for $C_{12}H_{22}O_4Na$, 253.1410; found, 253.1415

Diallyl 3-methylpentanedioate (3d)

The procedure was conducted in the same manner as **1d**. Starting material was 3-methylpentanedioic acid (0.52 g, 3.56 mmol). *Title compound* **3d** (0.74 g, 92%) as a colorless oil was given. ^{1}H NMR (200 MHz, CD_3OD): δ = 1.01 (d, *J* = 6.1 Hz, 3H), 2.55 – 2.17 (m, 5H), 4.58 (dt, *J* = 5.6, 1.4 Hz, 4H), 5.22 (ddd, *J* = 10.4, 2.7, 1.3 Hz, 2H), 5.37 – 5.18(m, 4H), 6.04 – 5.85 (m, 2H); ^{13}C NMR (50 MHz, CD_3OD): δ = 173.61, 133.70, 118.35, 66.05, 41.41, 28.69, 20.01; HRMS-MALDI (*m/z*): $[M + Na]^+$ calcd for $C_{12}H_{18}O_4Na$, 249.1097; found, 249.1102

3. Molecular Modeling

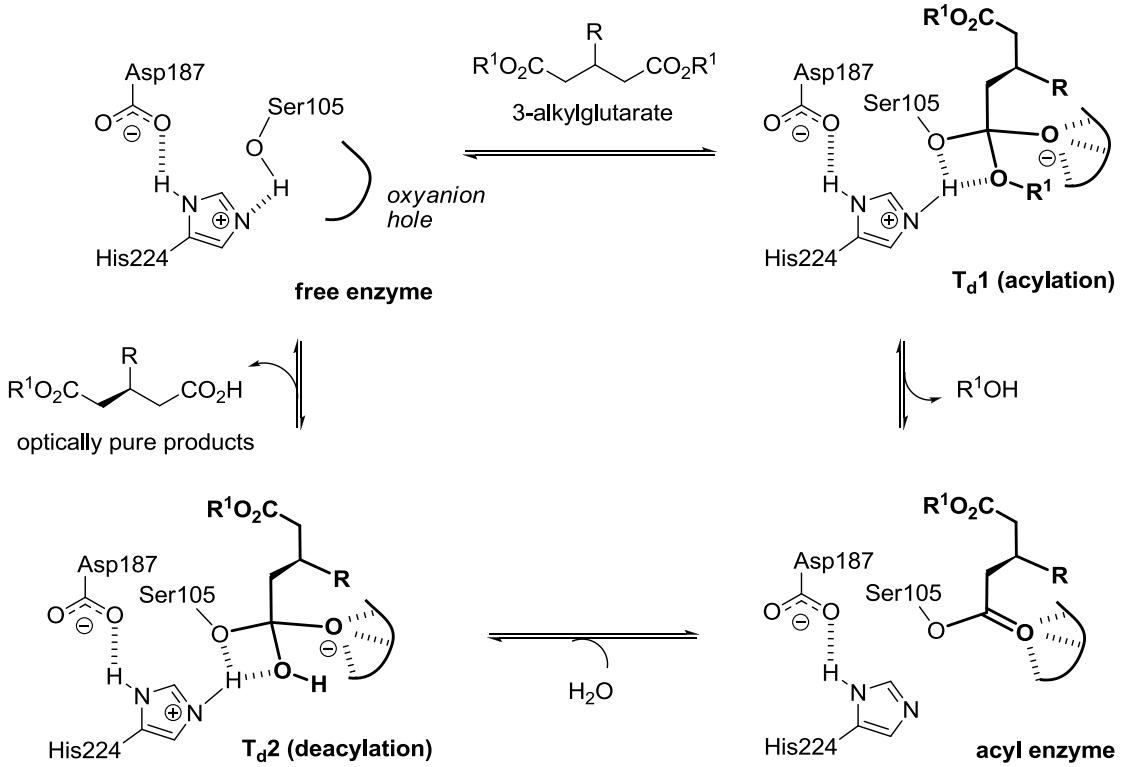


Fig. S1 Mechanism of CAL-B catalyzed hydrolysis (desymmetrization) of 3-alkylglutarate. The formation and collapse of T_d1 (acylation step) determine the reaction rate.

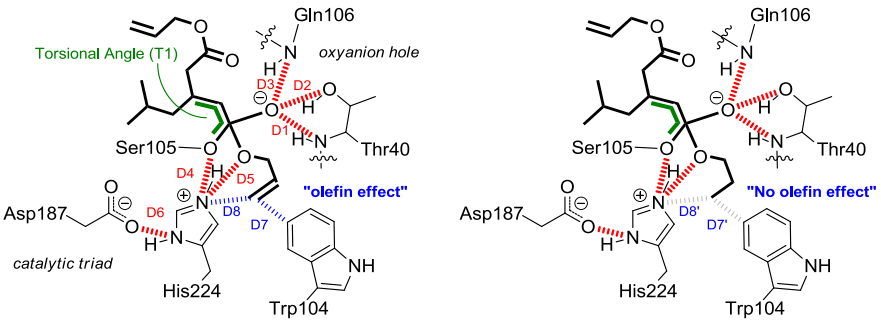


Fig. S2 Tetrahedral intermediate (T_d1) of diallyl and dipropyl 3-isobutylglutarates. Diallyl-3-isobutylglutarate would have olefin effect based on the examination of D7 or D8.

Table S1. Prediction of enantioselectivity based on the fraction of catalytically essential H-bonds maintained during molecular dynamics simulation. MD calculation was perforemd with CHARMm forcefield at 300K during 15 ns production run. The average value of distance and angle of six essential H-bonds were described. In parenthesis percentage criteria are distance of N-O or O-O < 3.1 Å and angle of N-H-O or O-H-O > 120˚. All productive H-bonds cannot properly evaluate because all percentage values are incomparable within error range.

Substrates	D1 / Å (%) A1 / ° (%)	D2 / Å (%) A2 / ° (%)	D3 / Å (%) A3 / ° (%)	D4 / Å (%) A4 / ° (%)	D5 / Å (%) A5 / ° (%)	D6 / Å (%) A6 / ° (%)	All six productive H-bonds / %
Fast-1c	2.88 (97.3) 161.7 (100)	2.67 (100) 169.3 (100)	2.92 (91.0) 157.7 (100)	2.64 (100) 140.6 (99.7)	2.74 (100) 148.8 (100)	2.87 (96.3) 152.2 (100)	85.3
Slow-1c	2.79 (99.3) 160.6 (100)	2.66 (100) 167.7 (100)	2.94 (87.3) 165.5 (100)	2.66 (100) 145.7 (100)	2.71 (100) 141.9 (100)	2.84 (99.0) 156.6 (100)	86.0
Fast-1d	2.87 (97.3) 161.8 (100)	2.67 (100) 166.7 (100)	2.94 (91.0) 166.5 (100)	2.75 (100) 152.4 (100)	2.64 (100) 136.3 (99.0)	2.86 (99.0) 158.0 (100)	87.0
Slow-1d	2.77 (99.3) 160.0 (100)	2.69 (100) 166.6 (100)	2.90 (95.0) 165.5 (100)	2.79 (98.0) 149.4 (100)	2.65 (100) 139.9 (99.0)	2.85 (98.0) 156.1 (100)	89.3

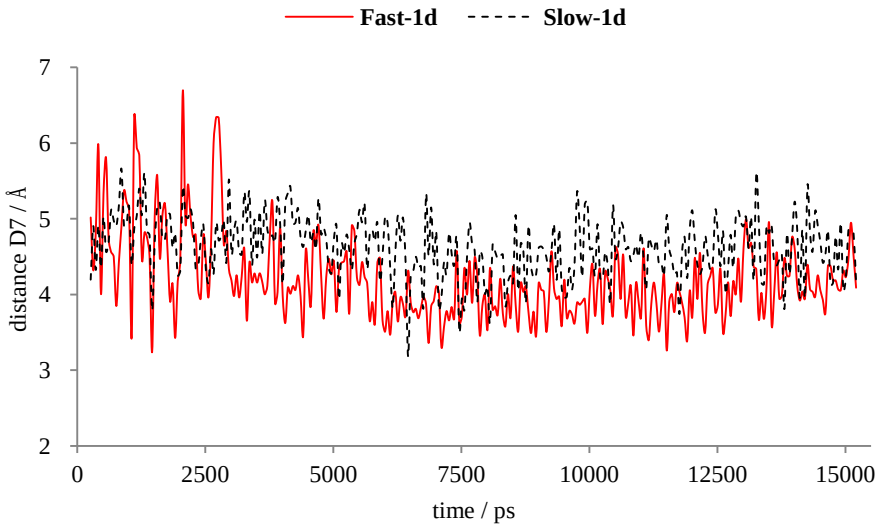


Fig. S3 Trace of distance D7 between the olefinic carbon in the substrates and the nearest arylic carbon in the side chain of Trp104. For the case of **Fast-1d** (red solid line), in the initial step below 3000 ps D7 showed slight fluctuantion but after that gradually closer to the carbon of Trp104 than **Slow-1d** (black dotted line).

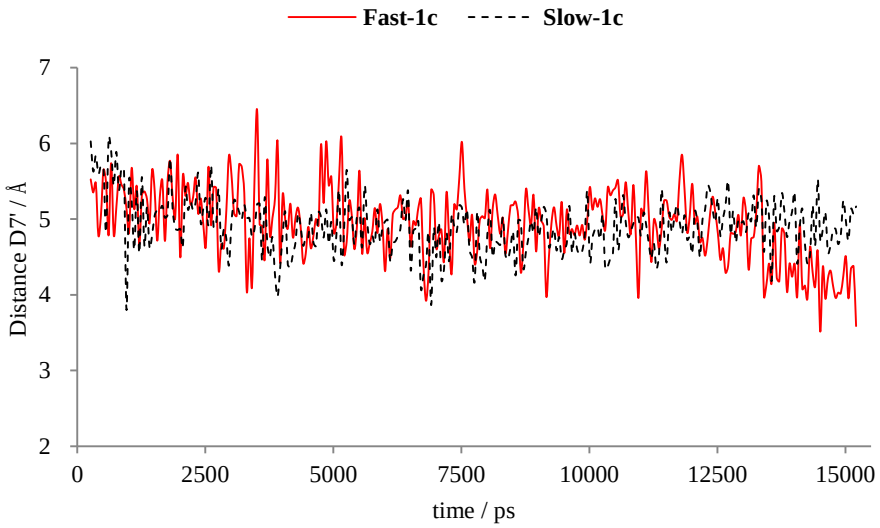


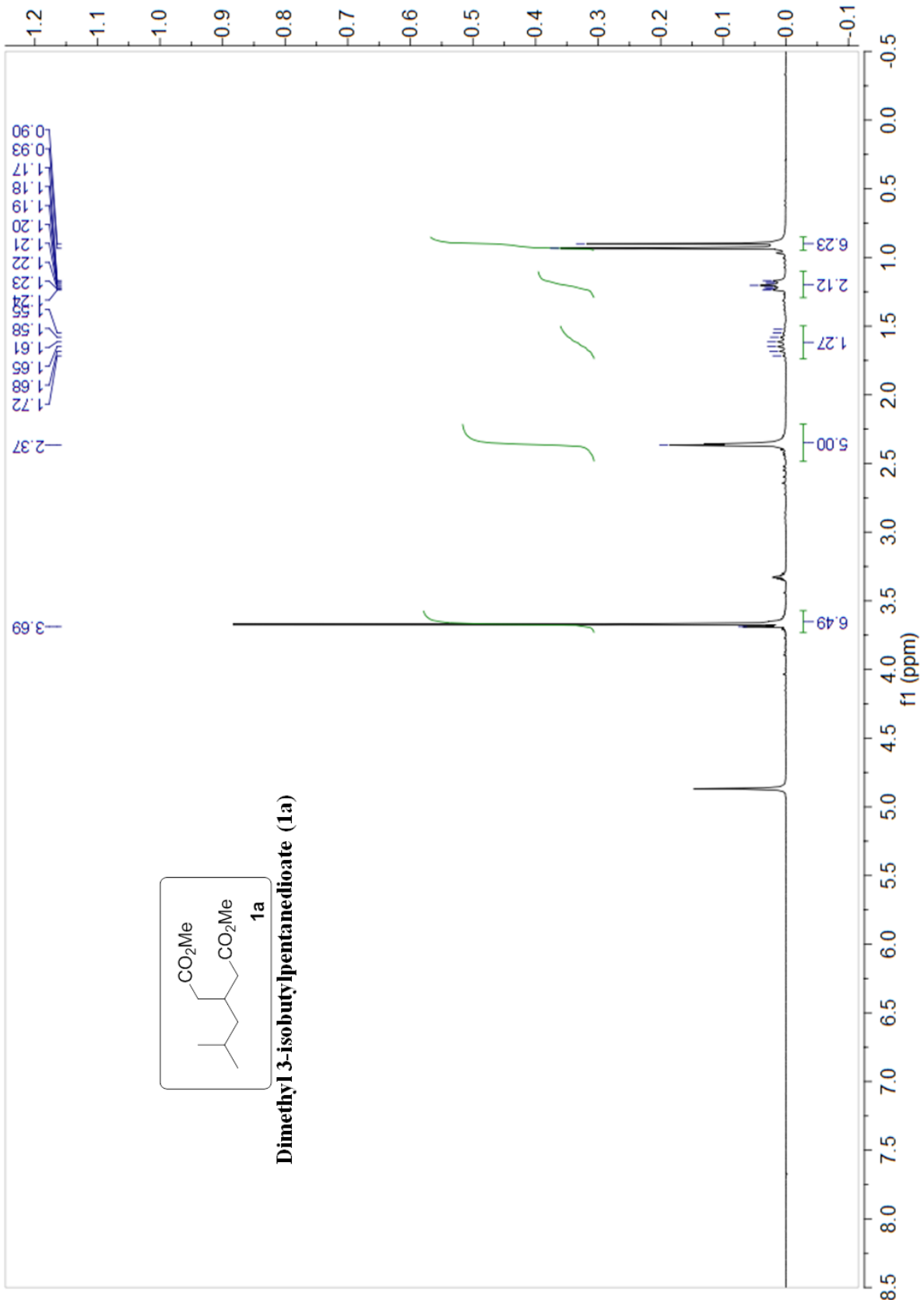
Fig. S4 Trace of distance D7´between the terminal carbon in propyl group of the substrate and the nearest arylic carbon in the side chain of Trp104. No discrimination on D7´between **Fast-1c** (red solid line) and **Slow-1c** (black dotted line) means that propyl which has no olefin element of the ester group in the reactive site would not be the recognition factor for kinetic resolution.

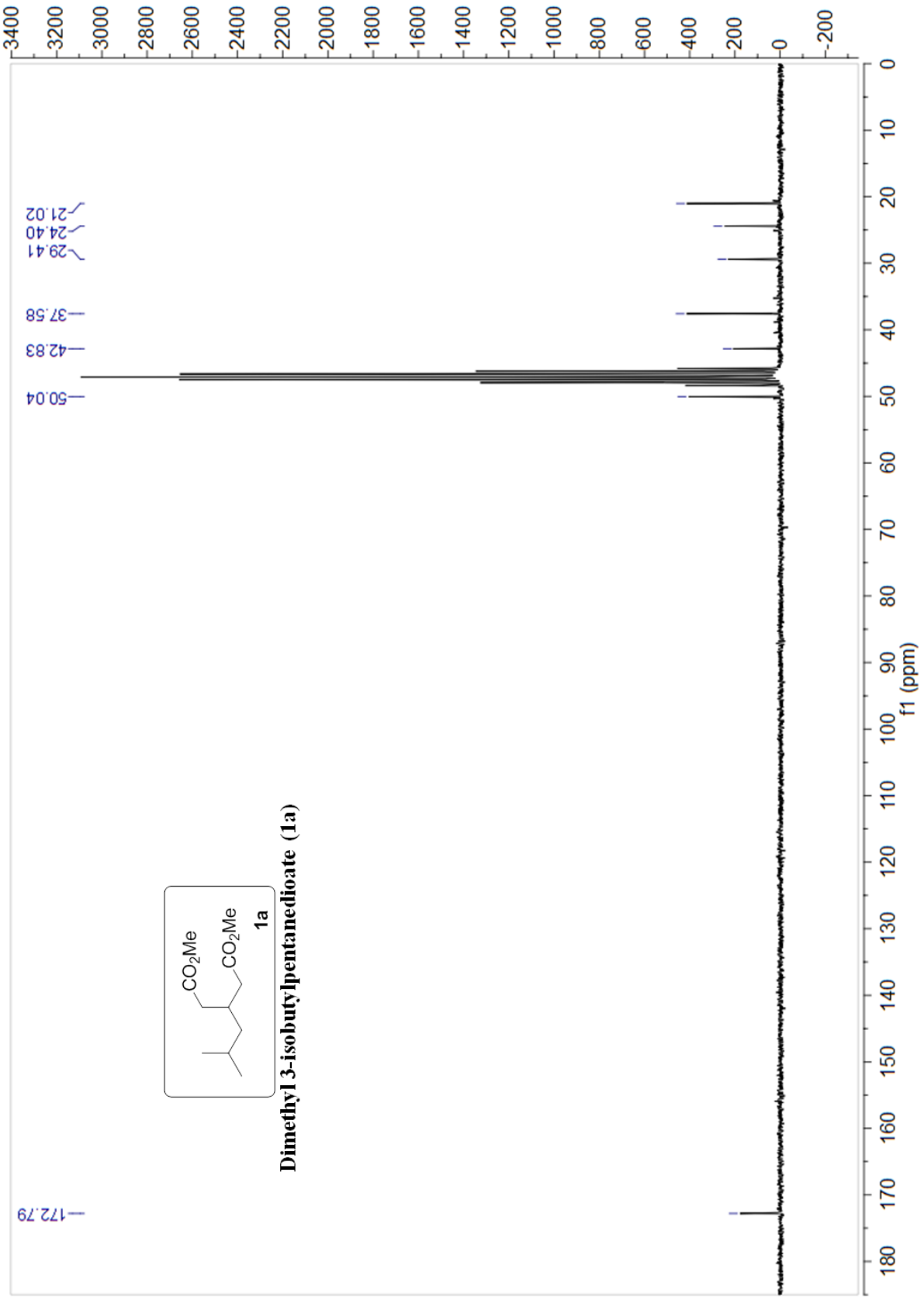
Table S2. Average of distances and percentage within distance criteria for **Fast-** and **Slow-1c** (D7´) which are not possessed any olefin effect in the unreactive ester group during MD. Only few snapshots were effective during the simulation, therefore, explanation of difference of reaction rate between **Fast-1d** vs. **Slow-1d** was not able to.

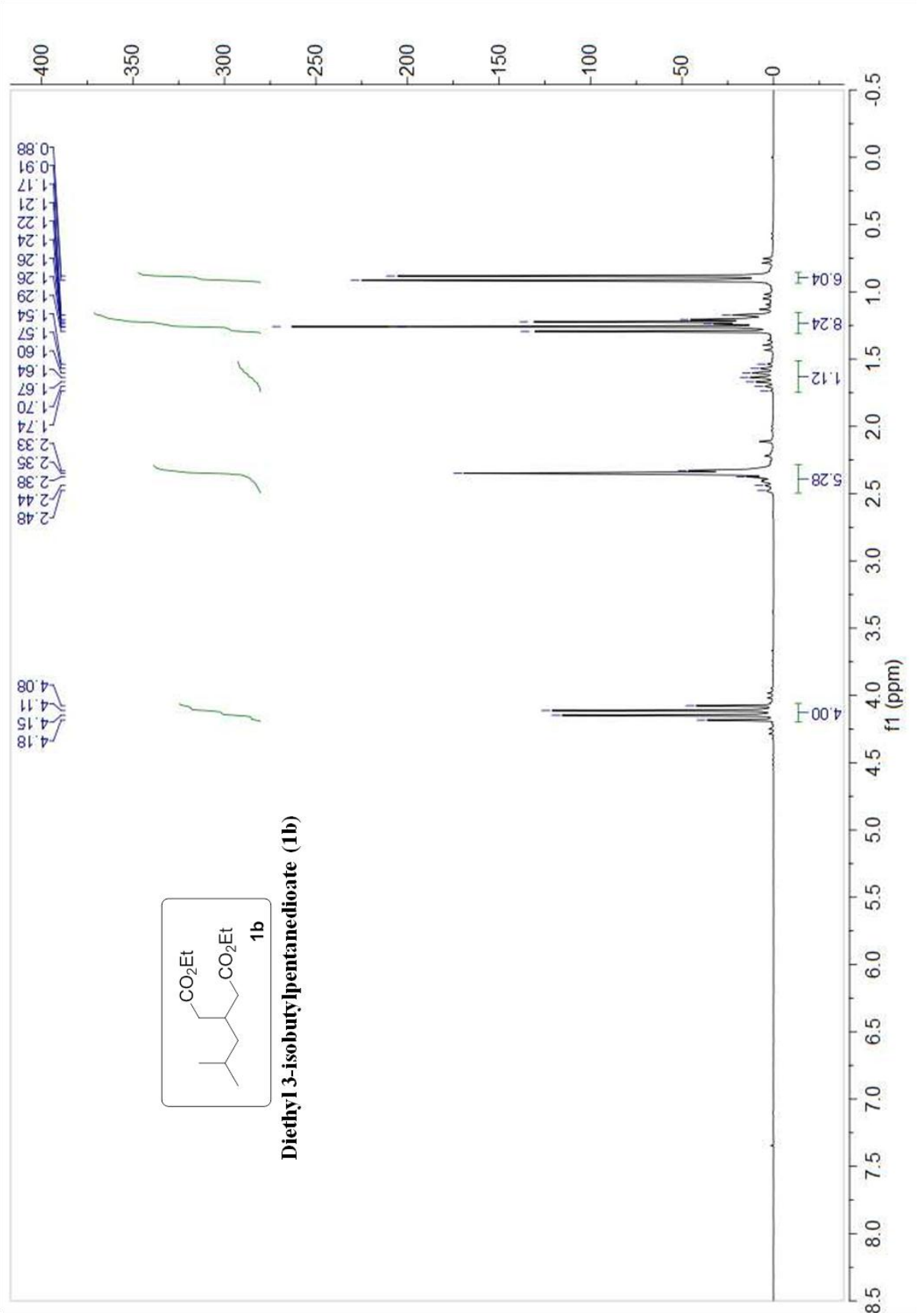
	Fast-1c (D7´)	**Slow-1c** (D7´)
Distance Average (Å)	4.95	4.90
Total data (15 ns)	300	300
Within criteria[a]	11	3
%[a]	4	1

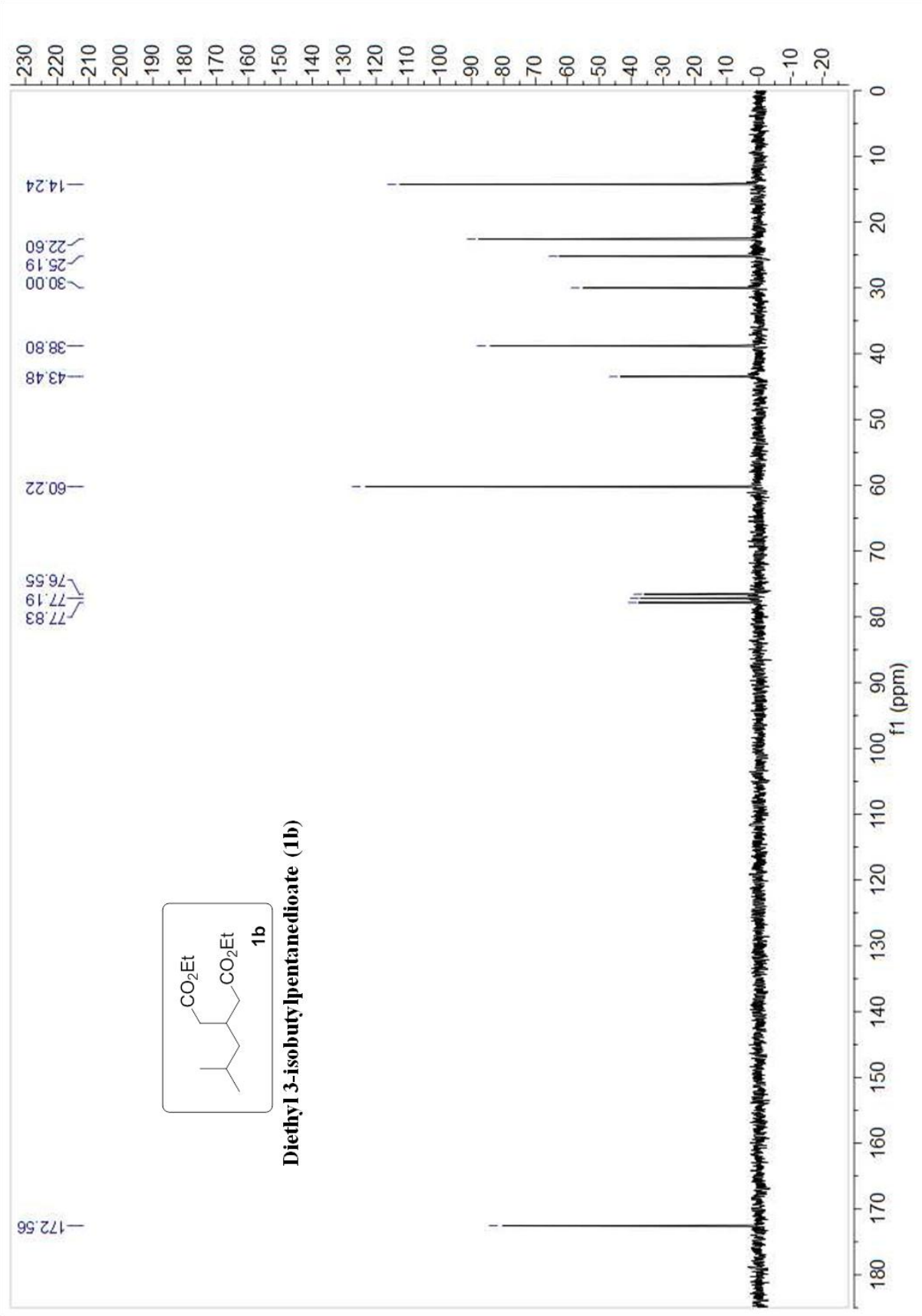
[a] Distance criteria as less than 4.0 Å between terminal carbon in propyl group (D7´) in the substrates and the nearest arylric carbon in the side chain of Trp104.

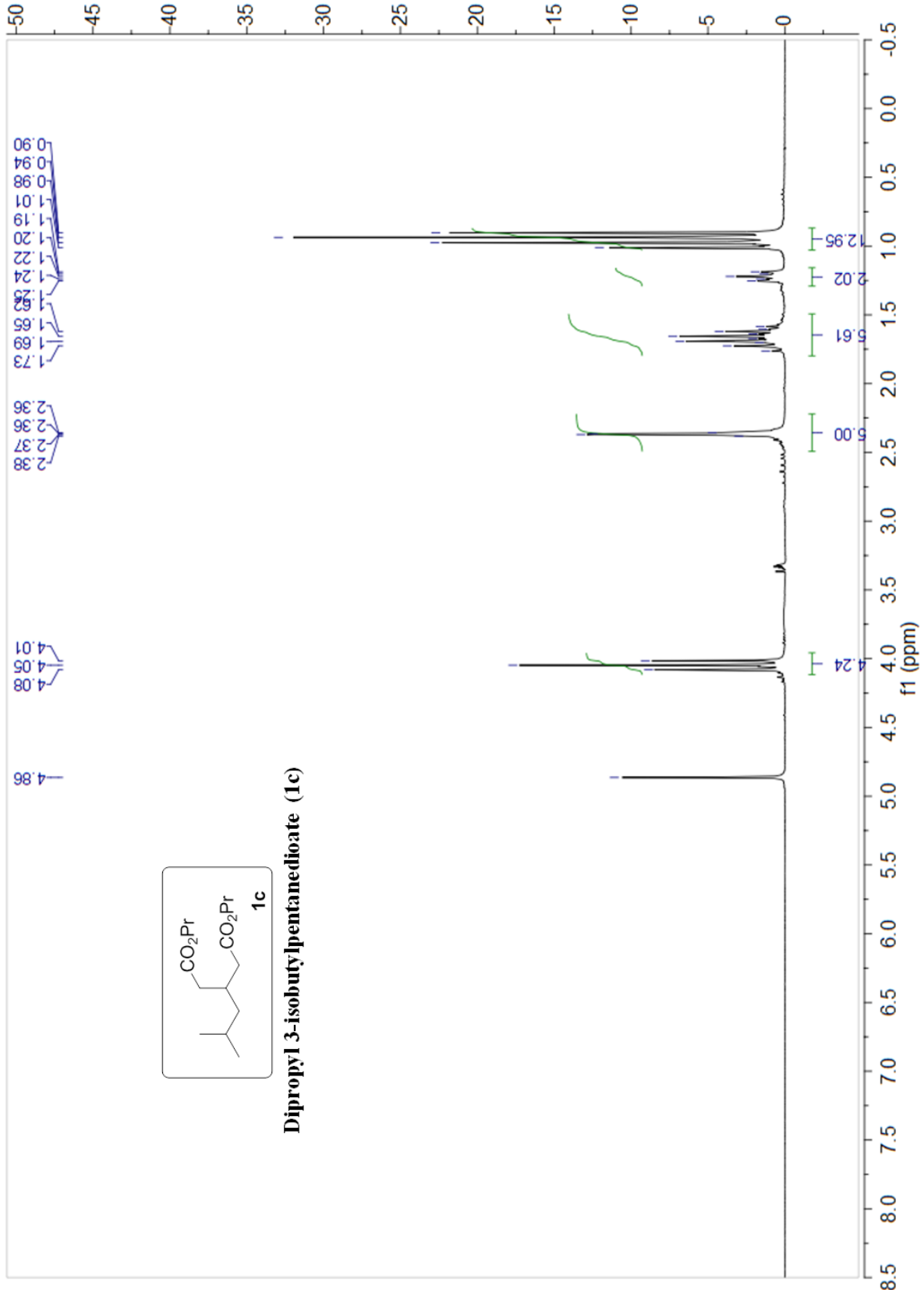
4. NMR (^{1}H and ^{13}C spectral data)

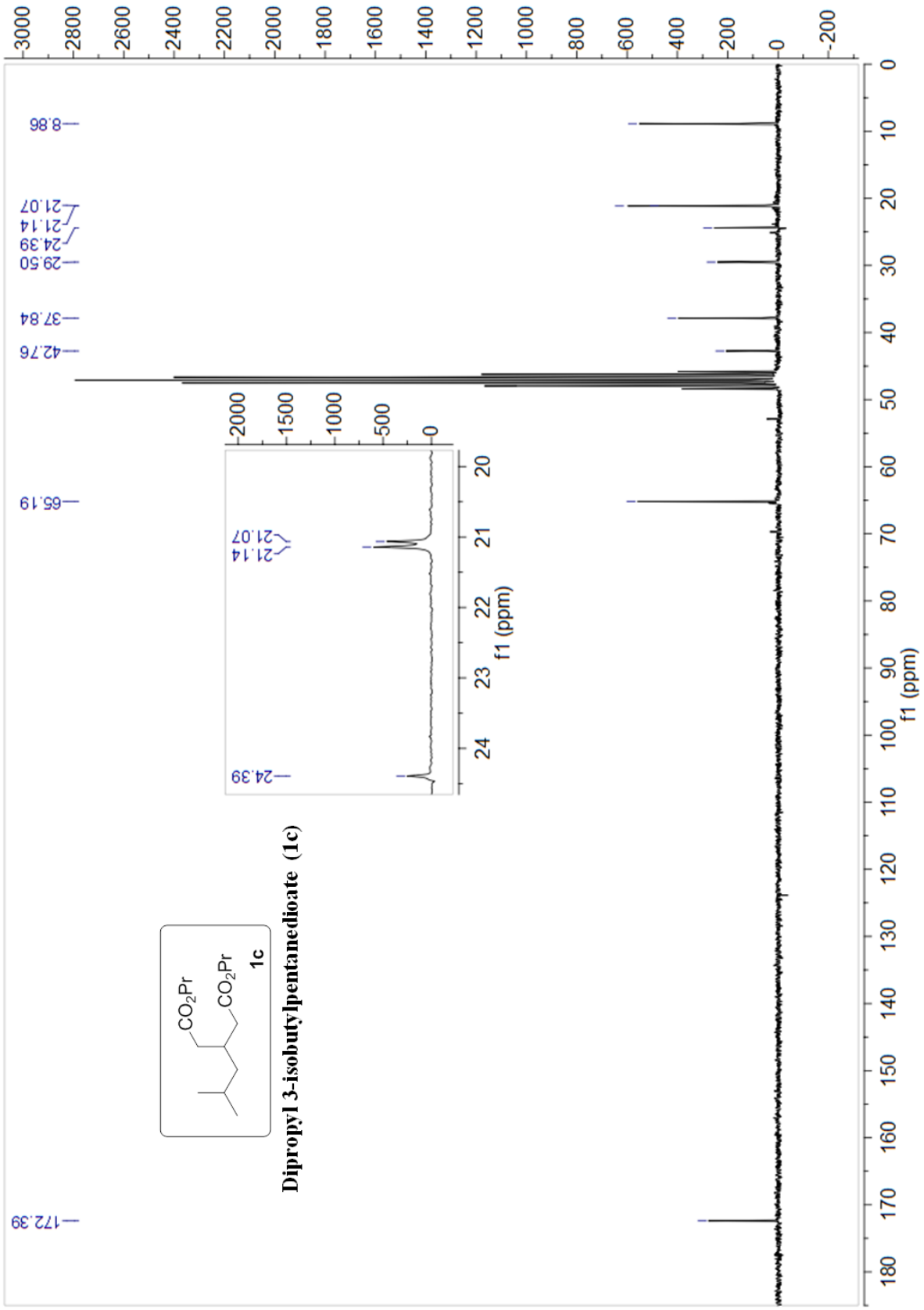


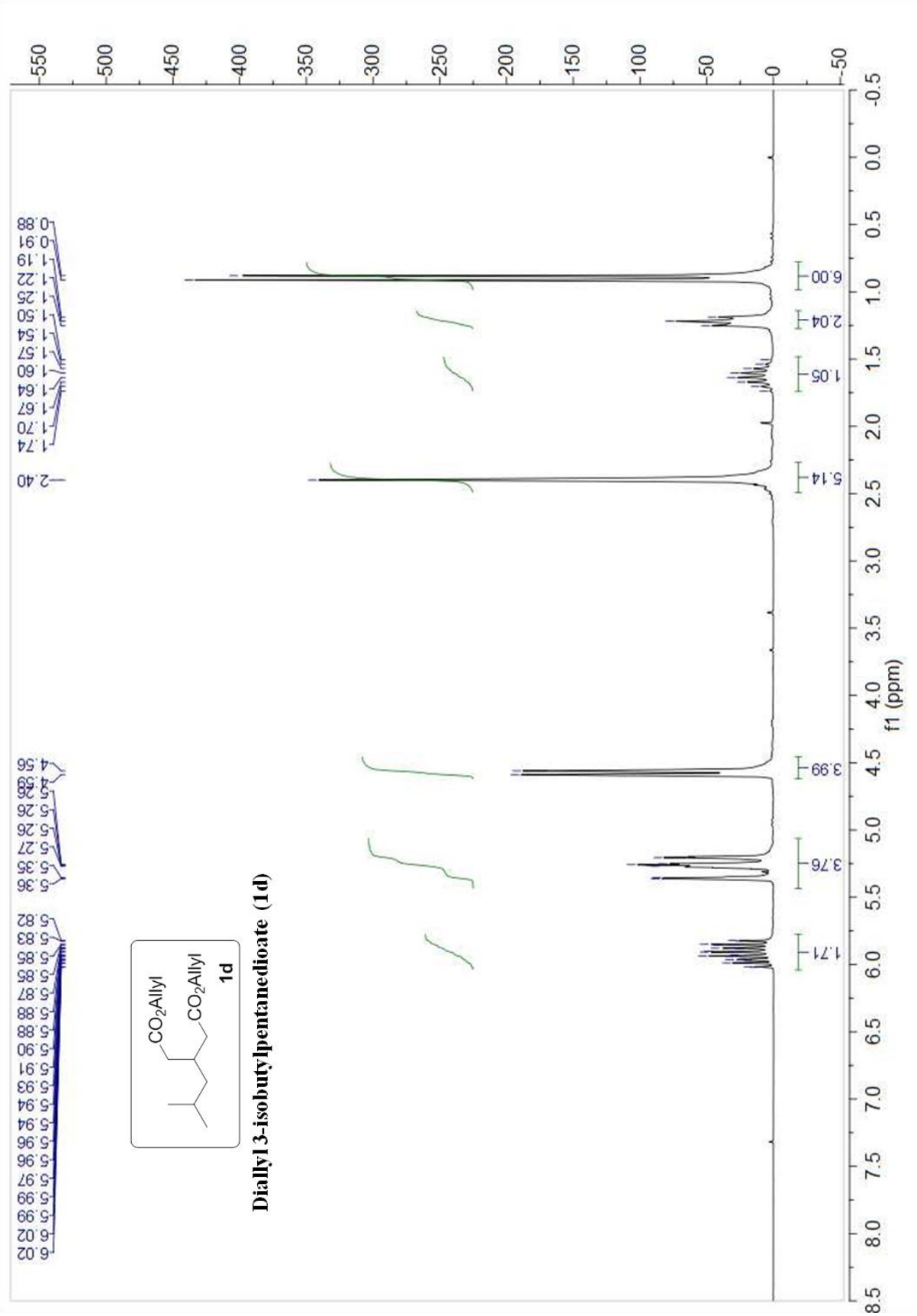


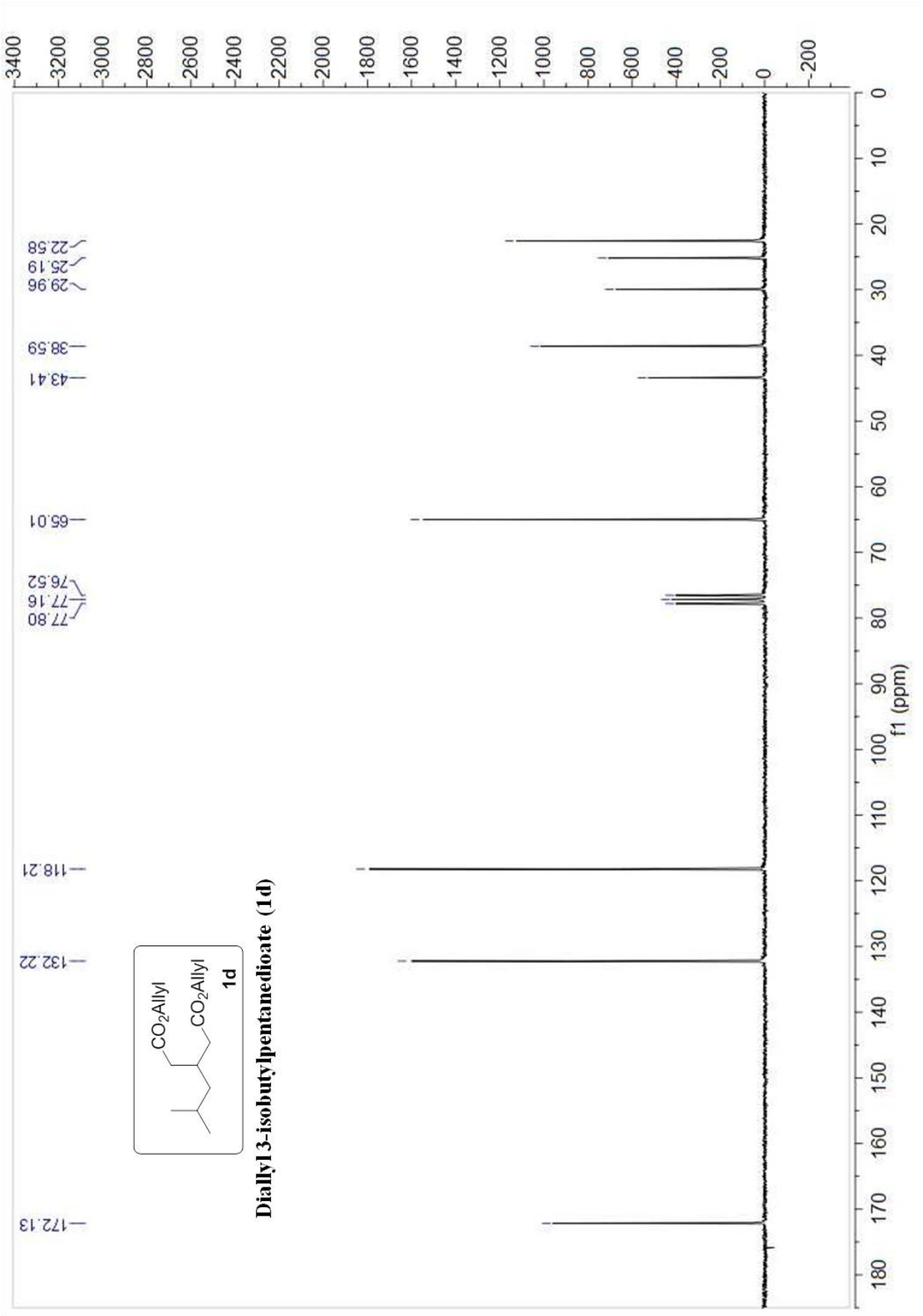


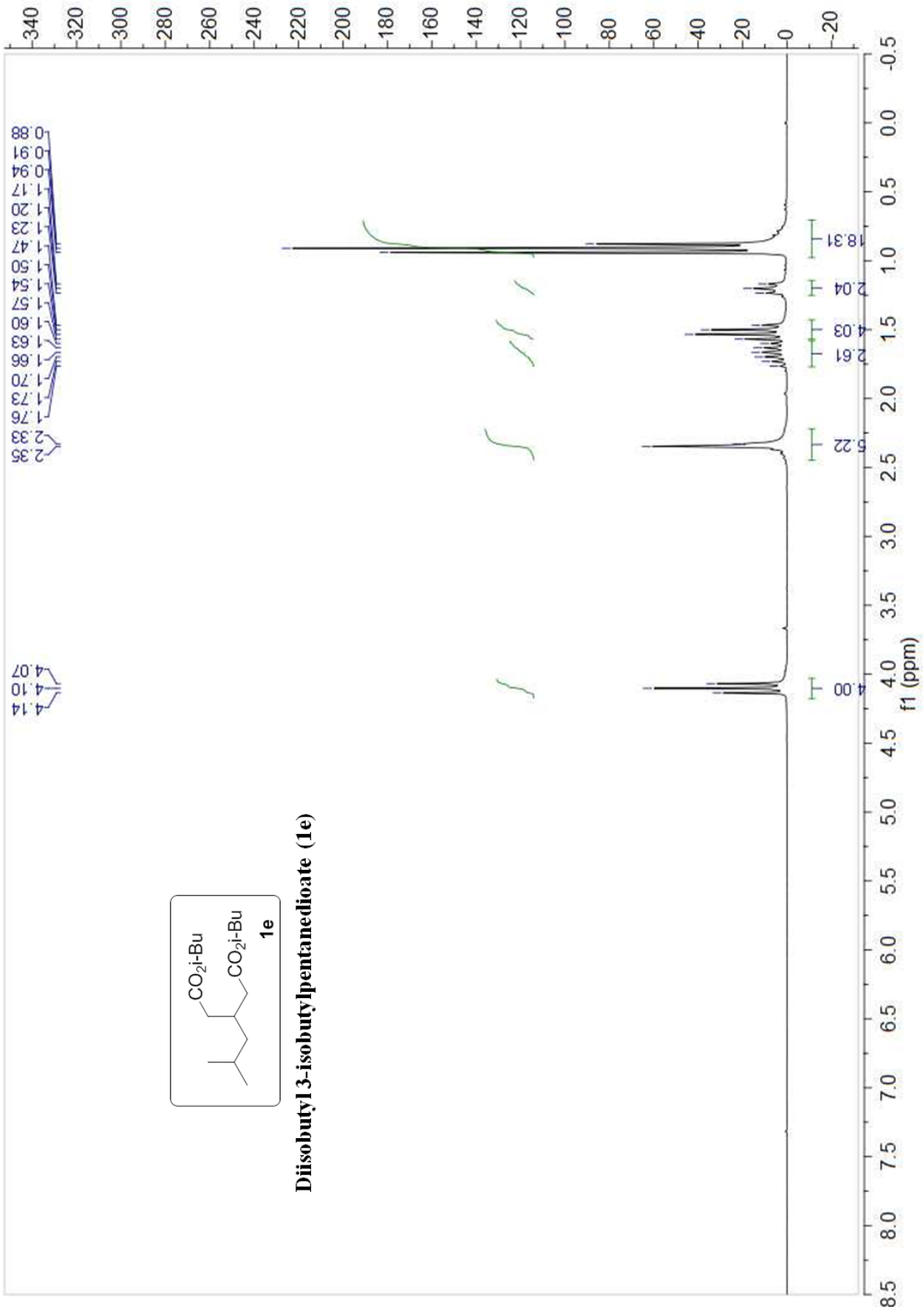


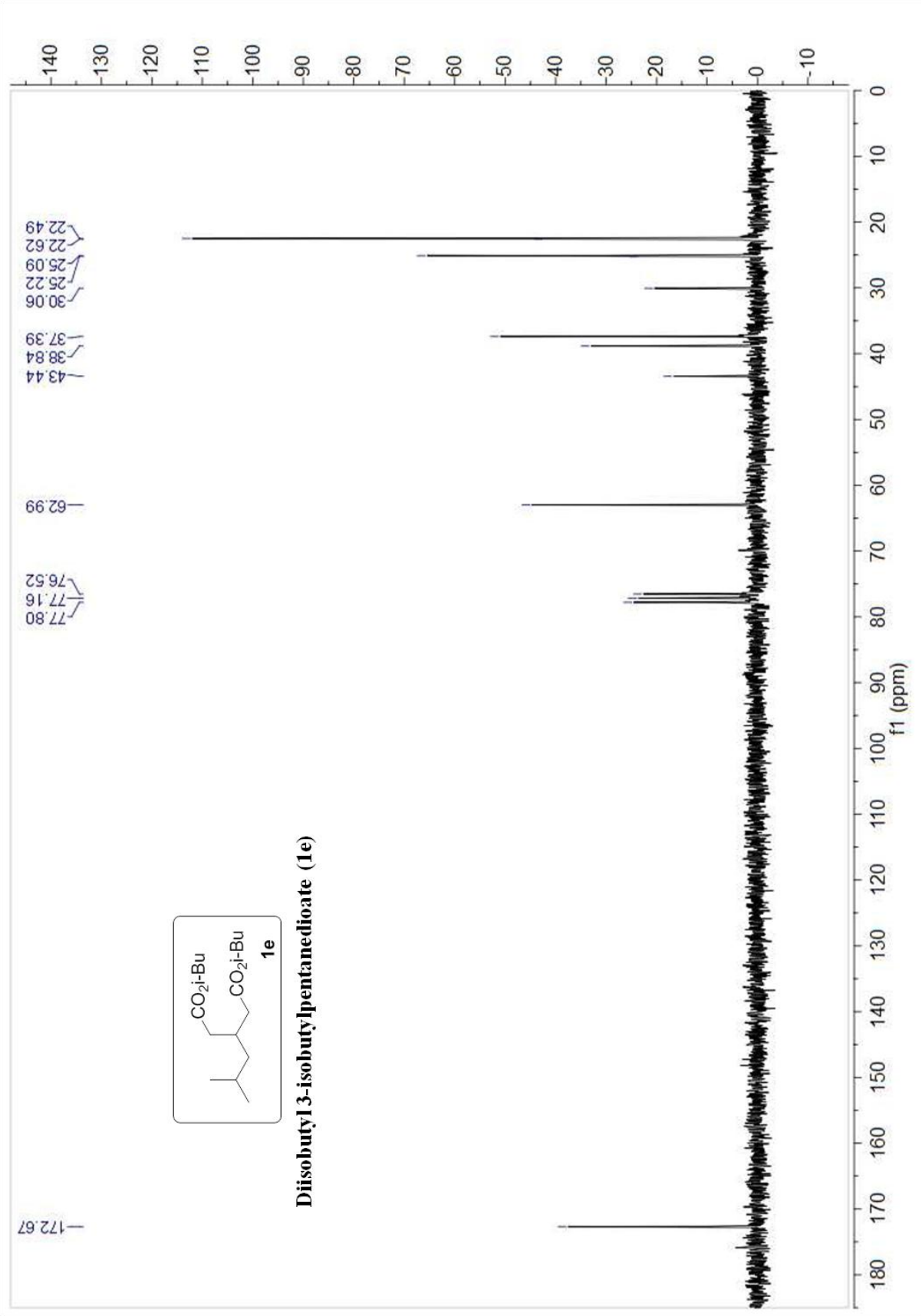


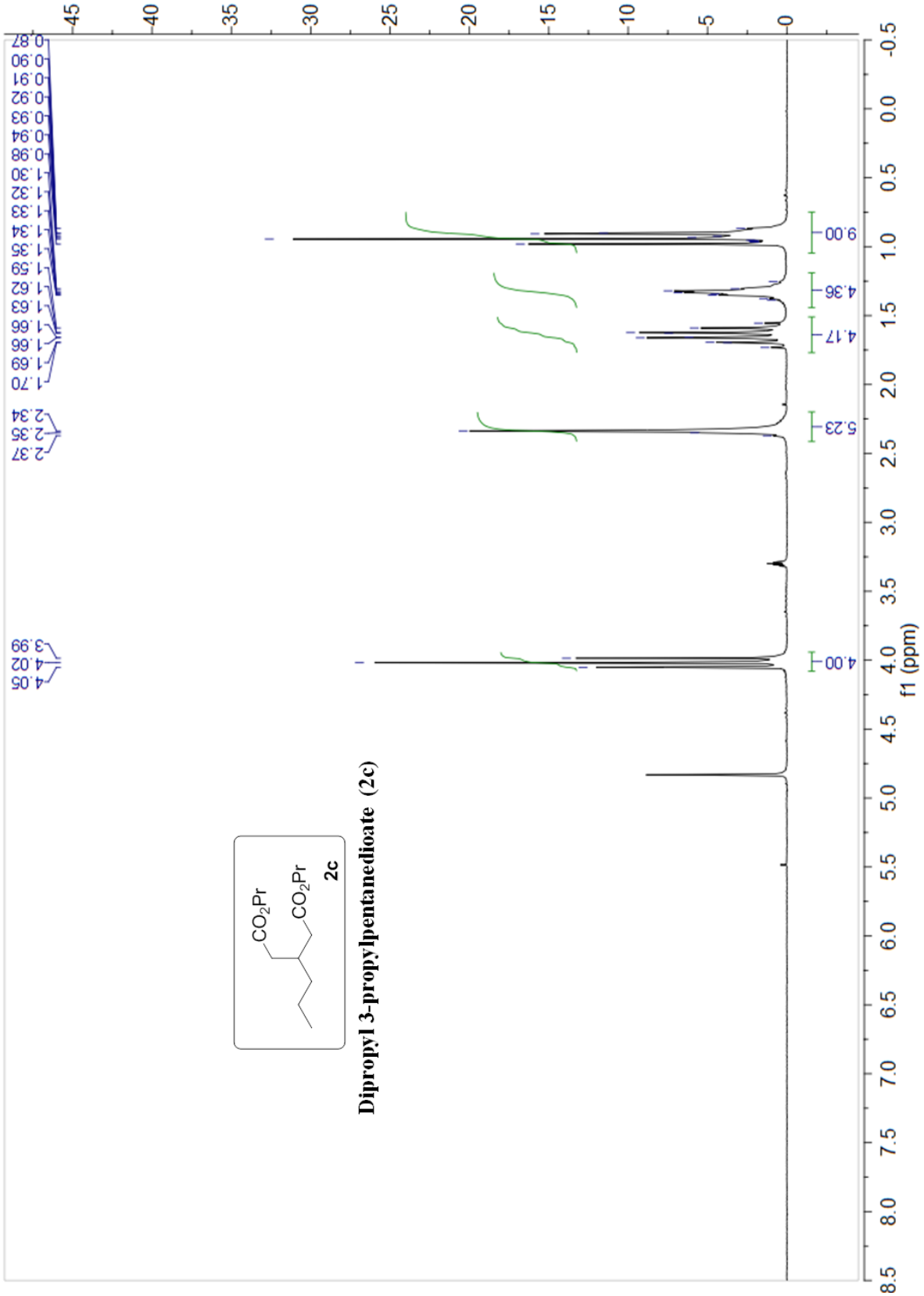


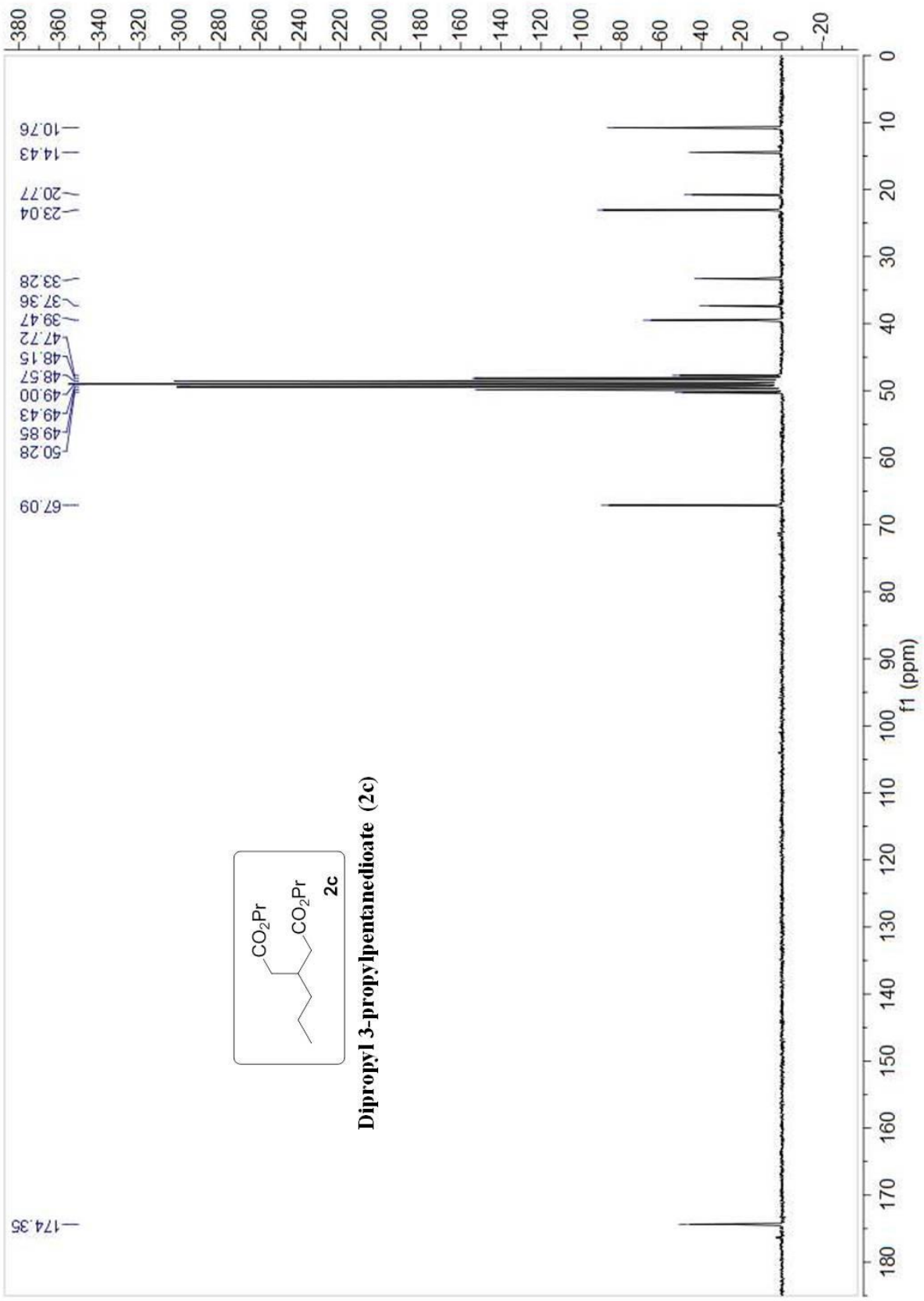

Diallyl 3-isobutylpentanedioate (1d)
CO2Allyl
CO2Allyl
1d
f1 (ppm)

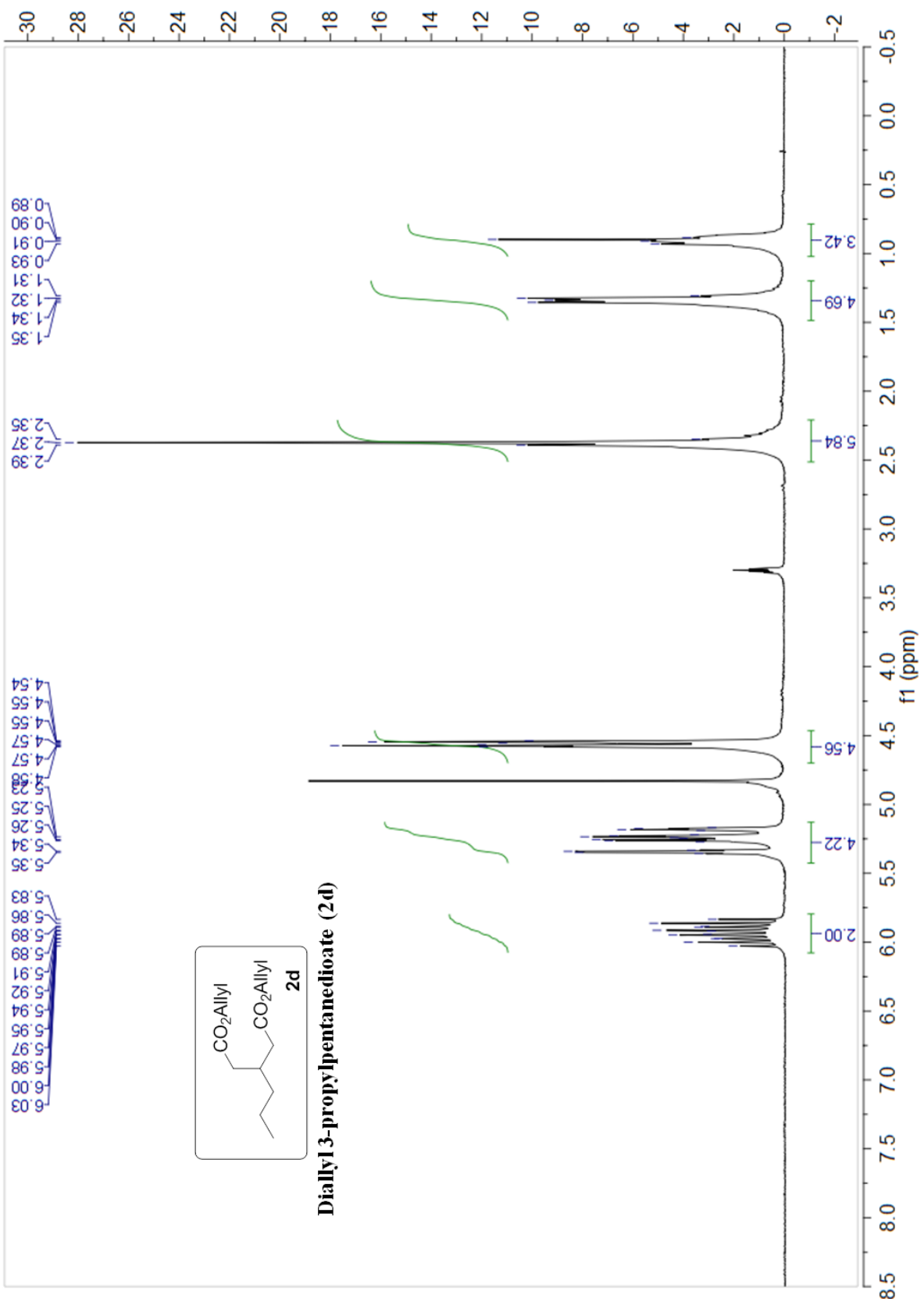


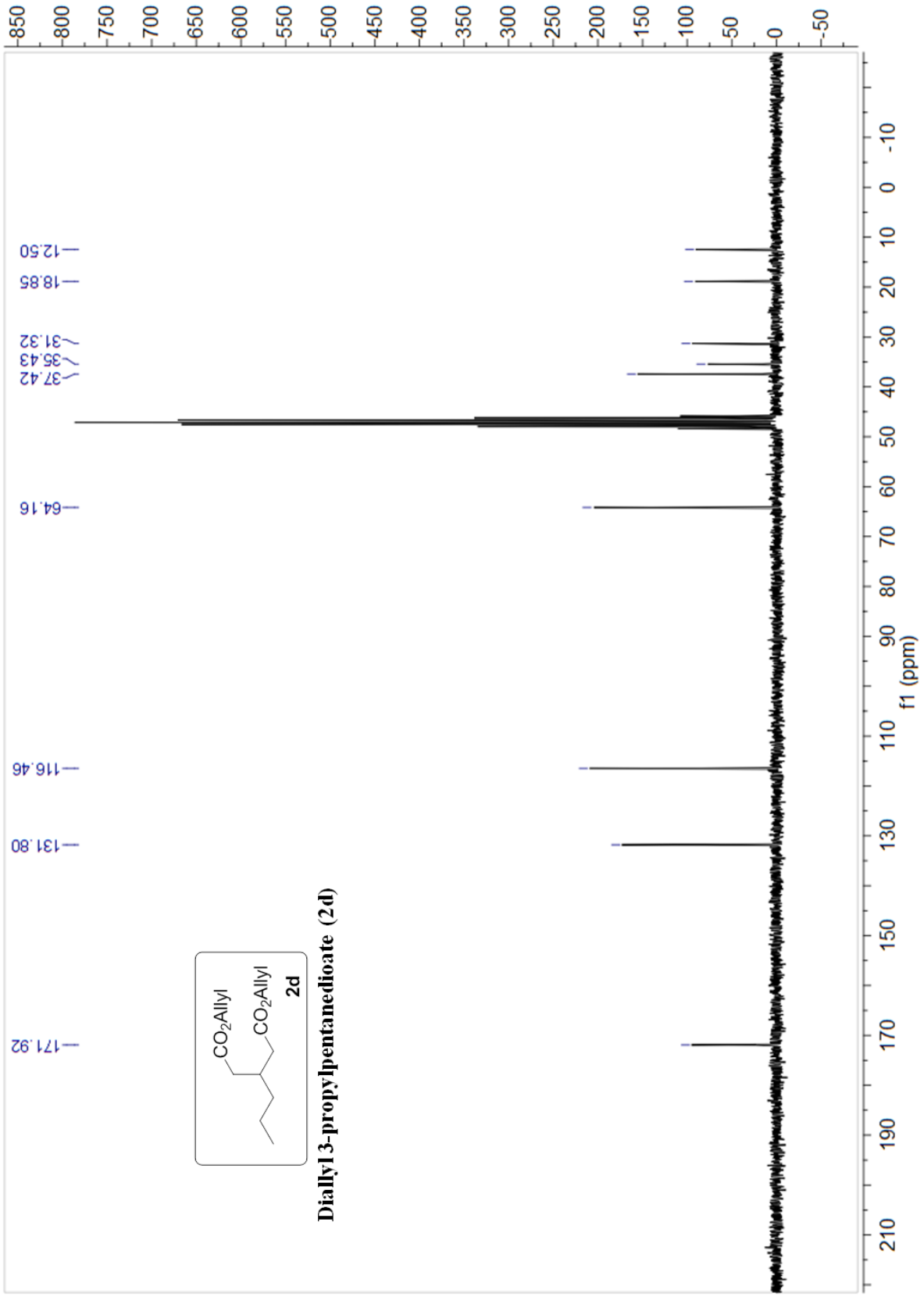


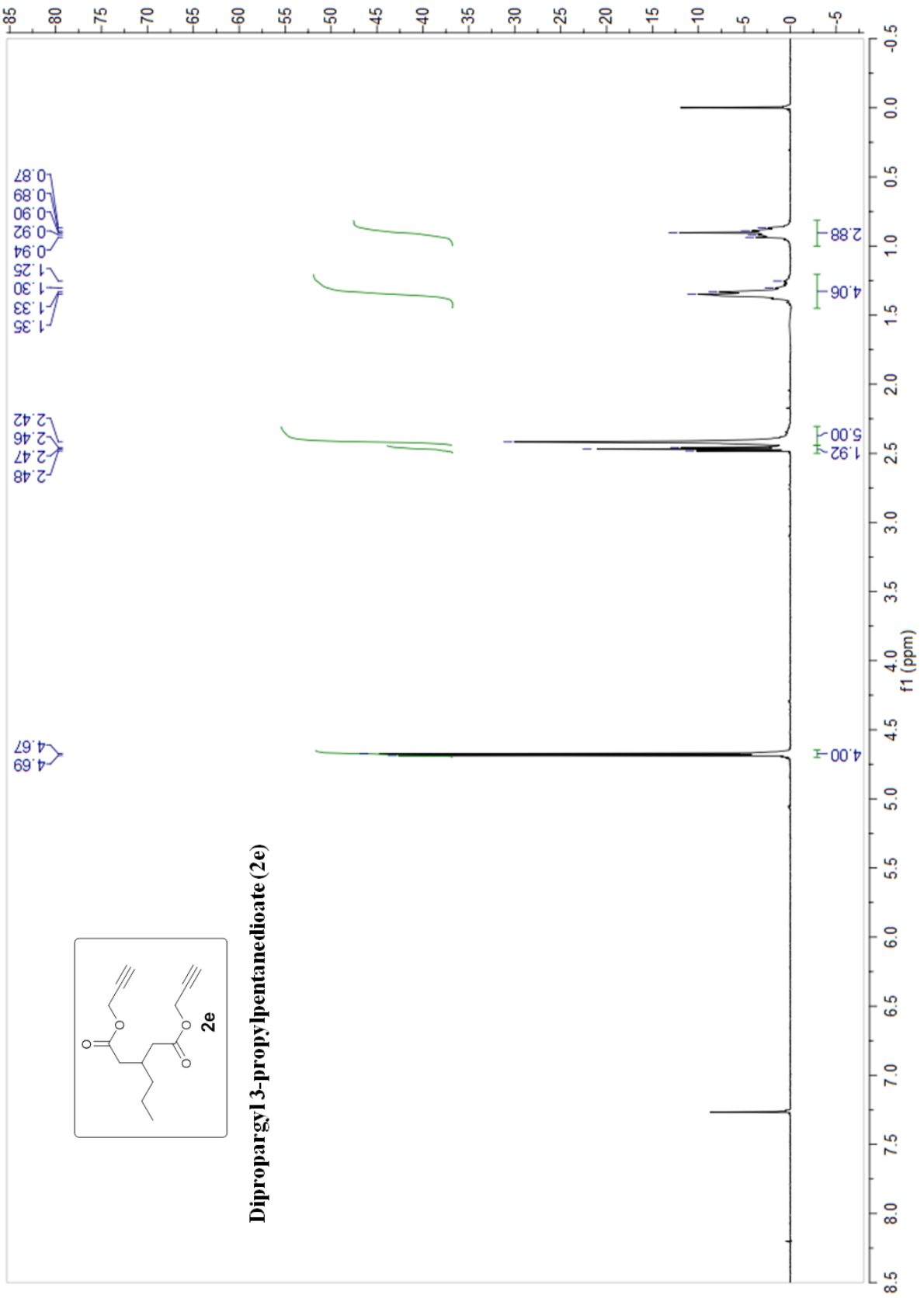


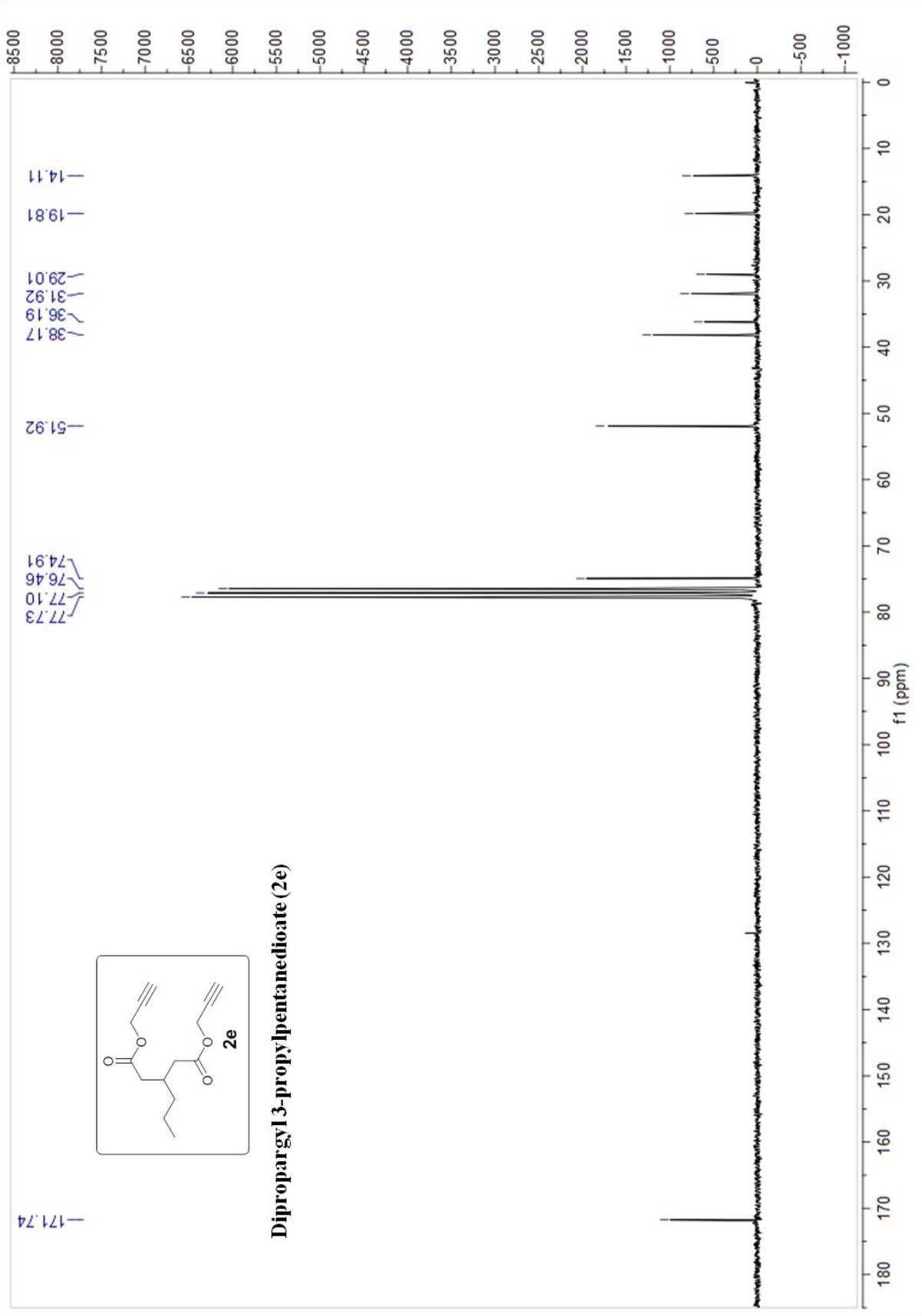

Dipropyl 3-propylpentanedioate (2c)
CO2Pr
CO2Pr
2c
f1 (ppm)
9.00
4.36
4.17
5.23
4.00

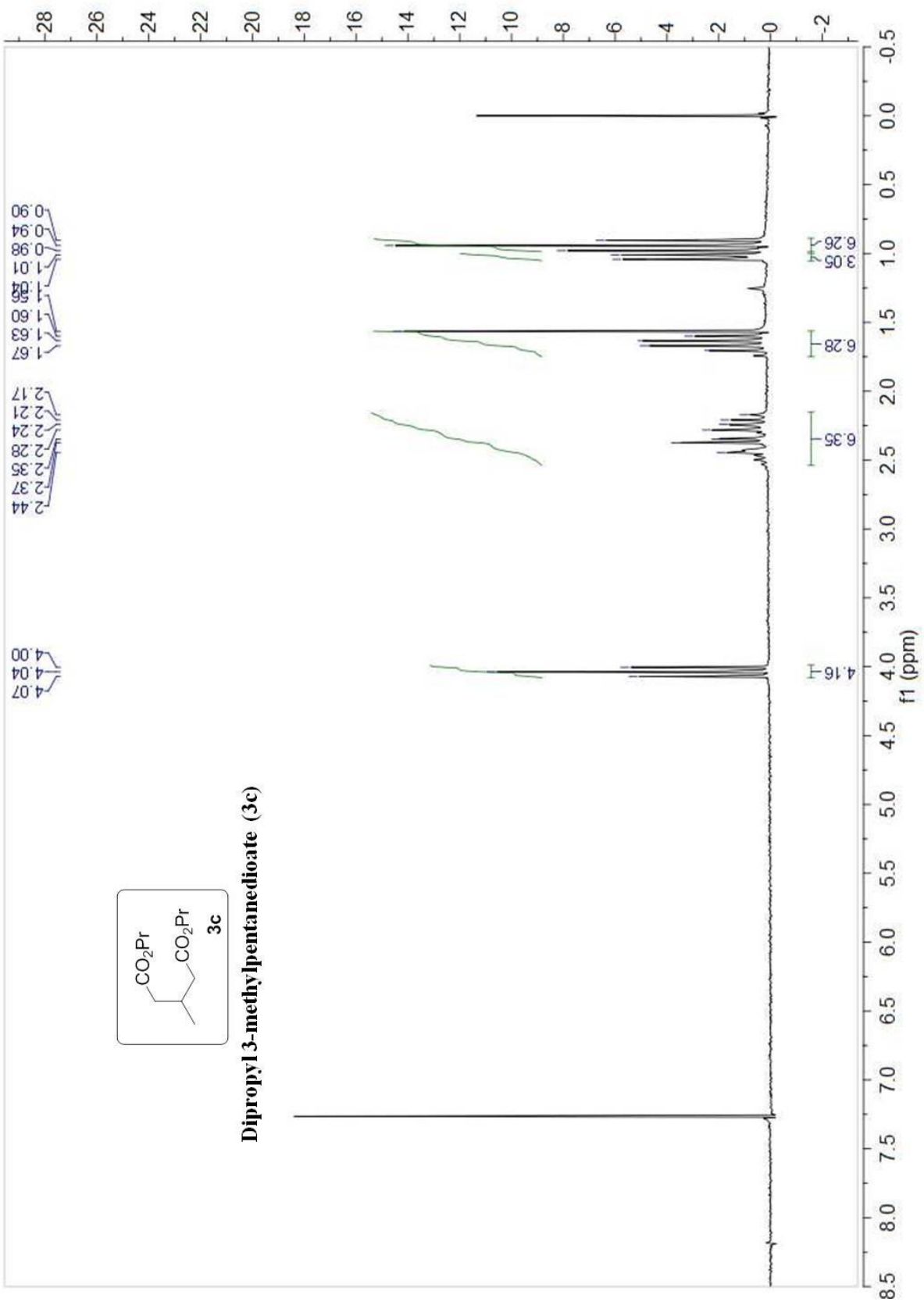


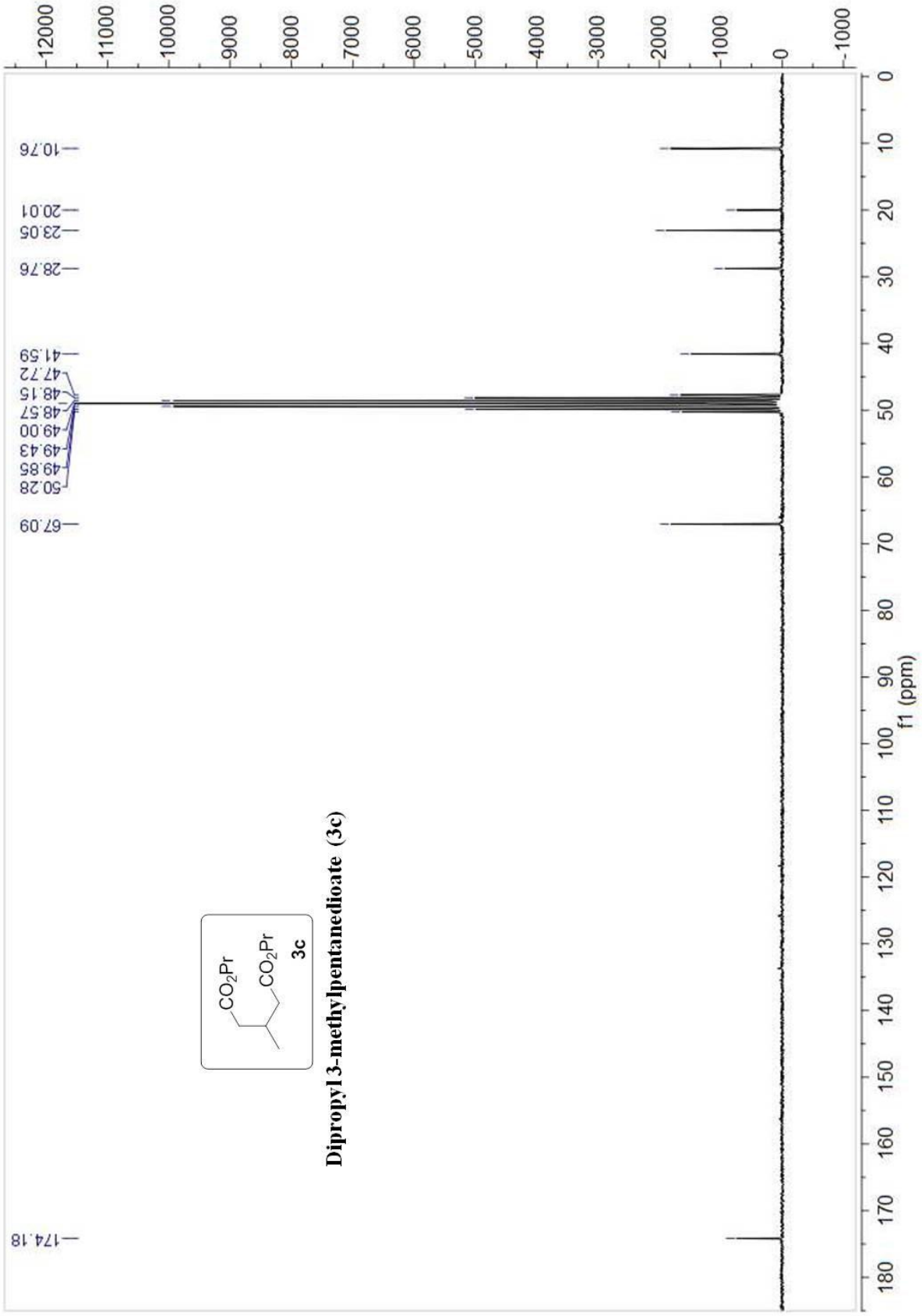


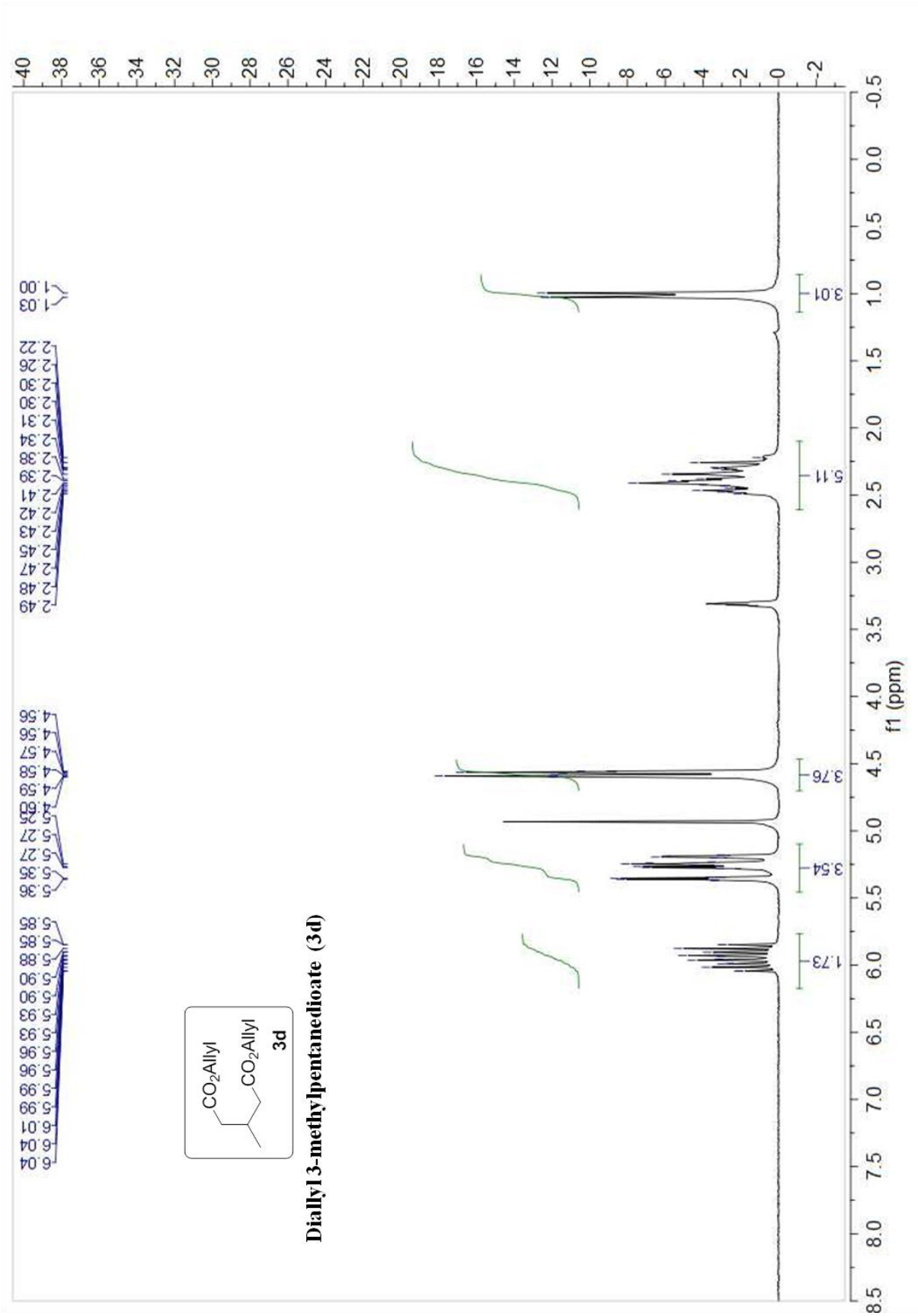


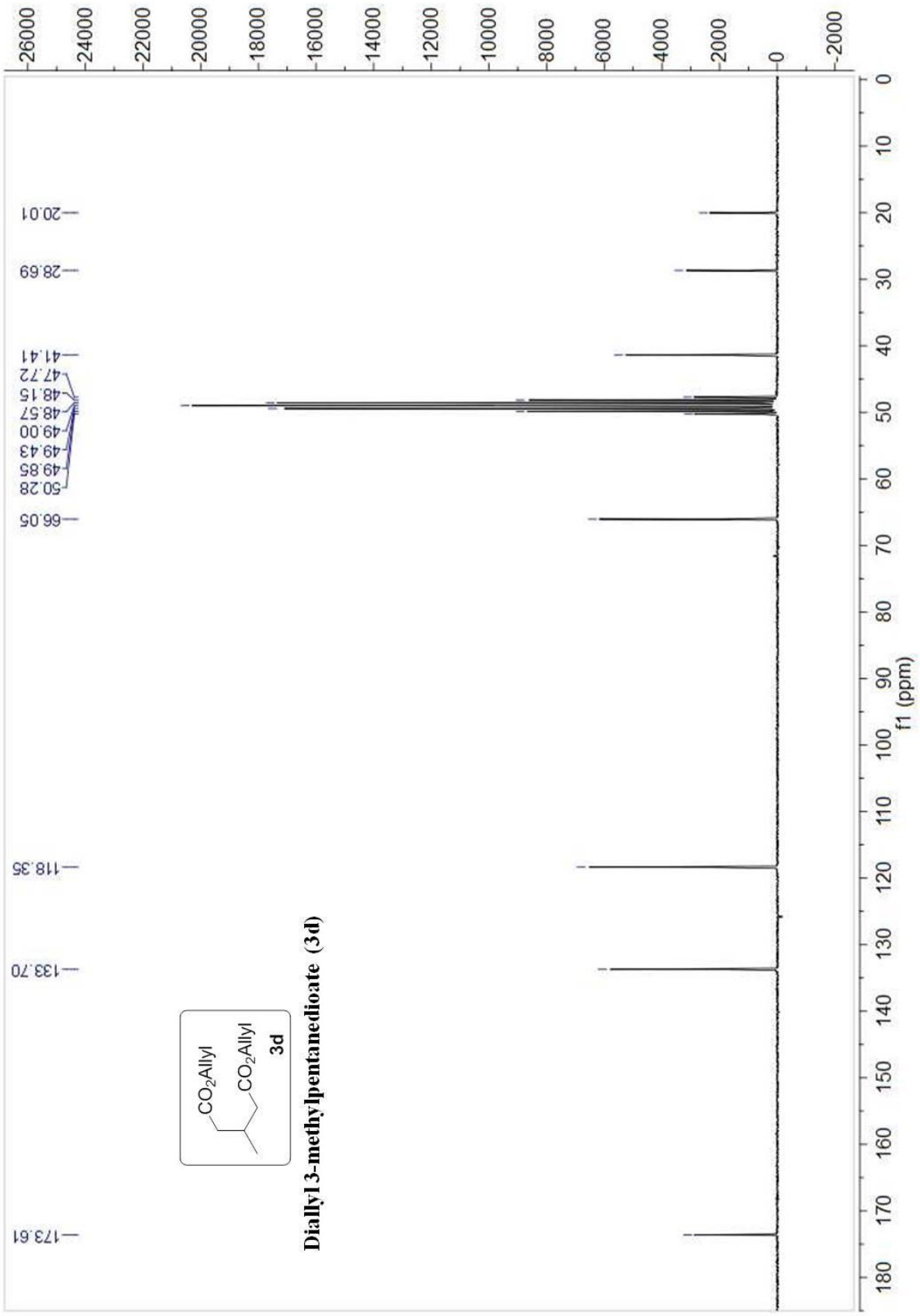


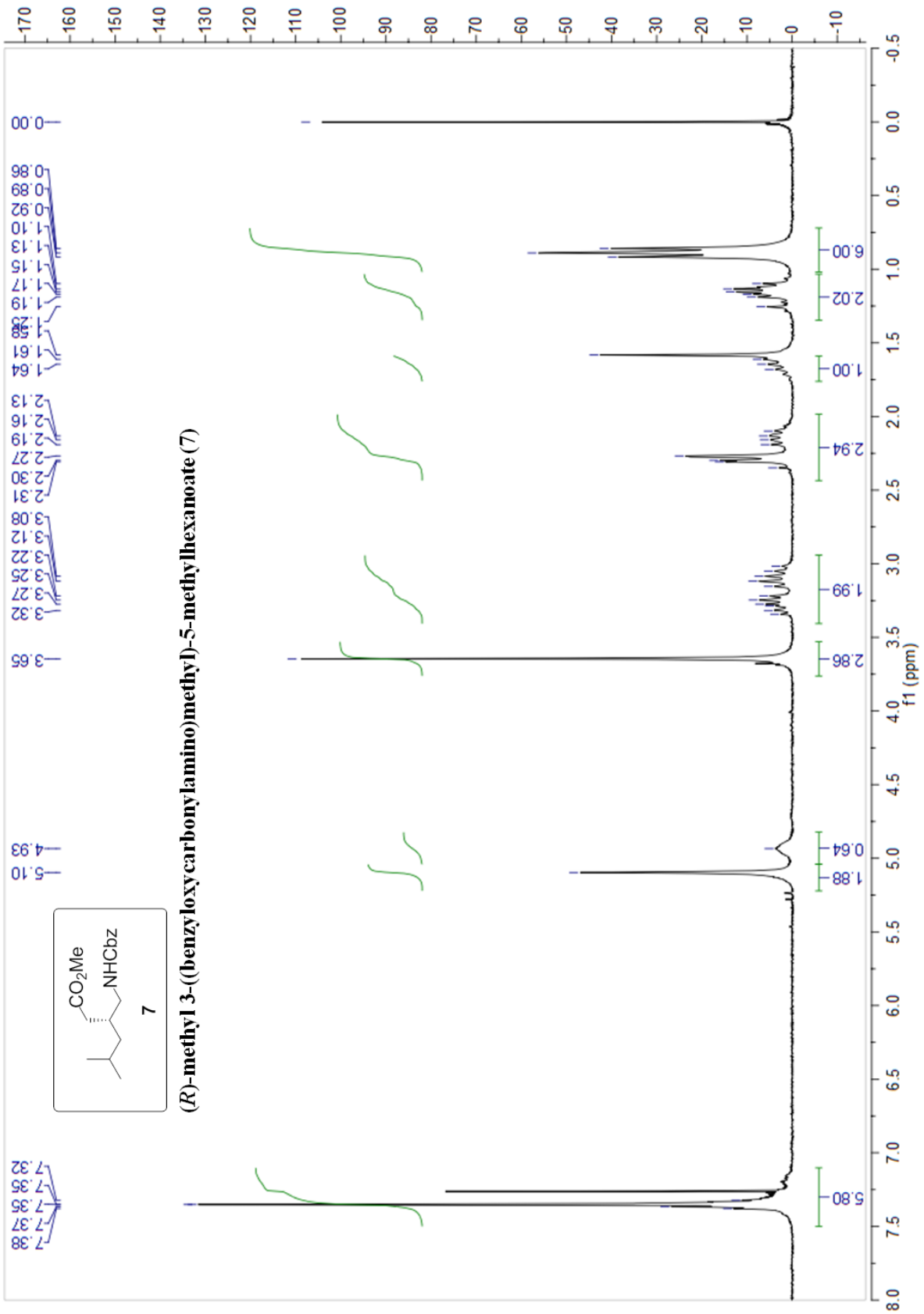

14.11
19.81
29.01
31.92
36.19
38.17
51.92
74.91
76.46
77.10
77.73
171.74
2e
Dipropargyl 3-propylpentanedioate (2e)
f1 (ppm)

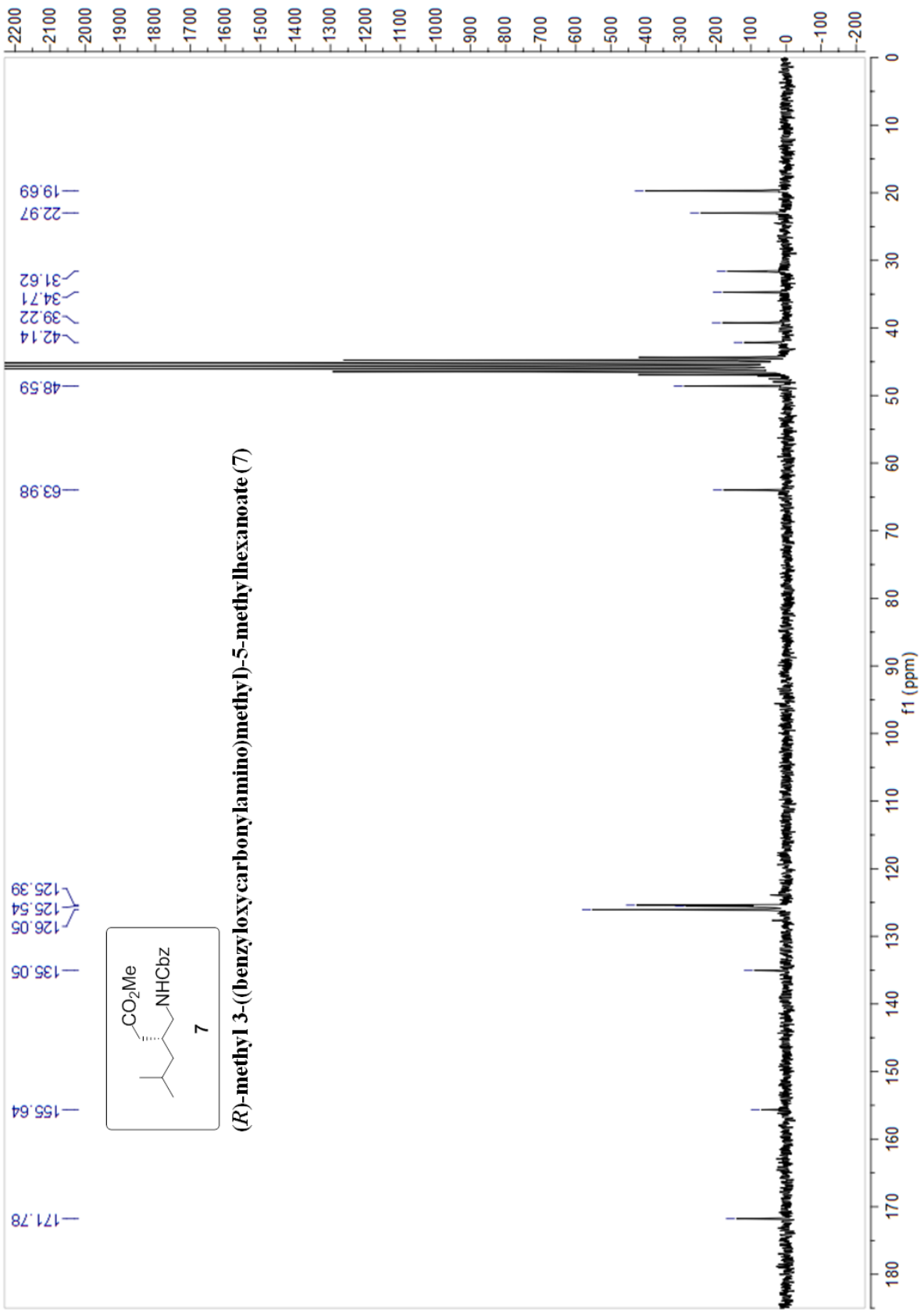


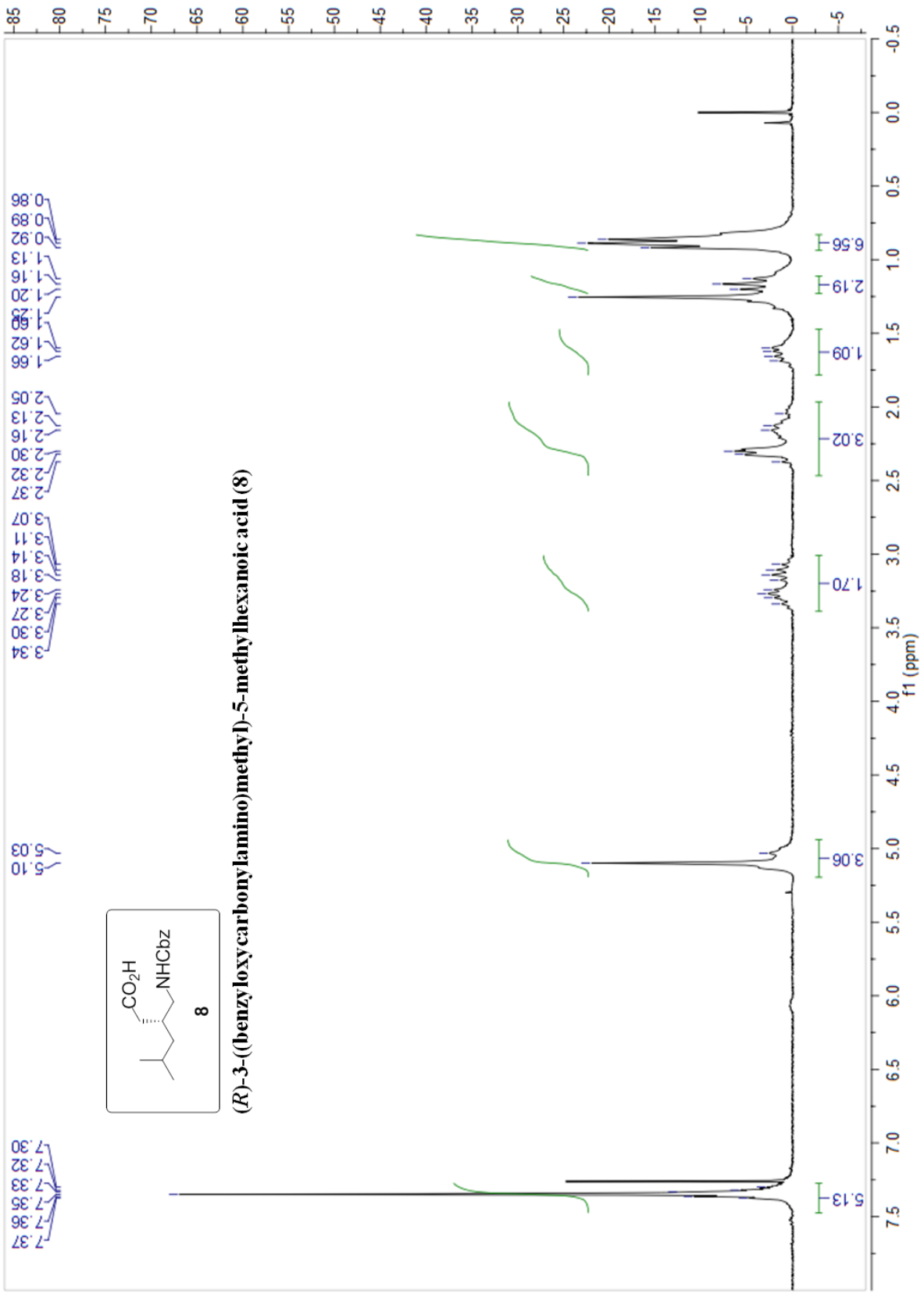


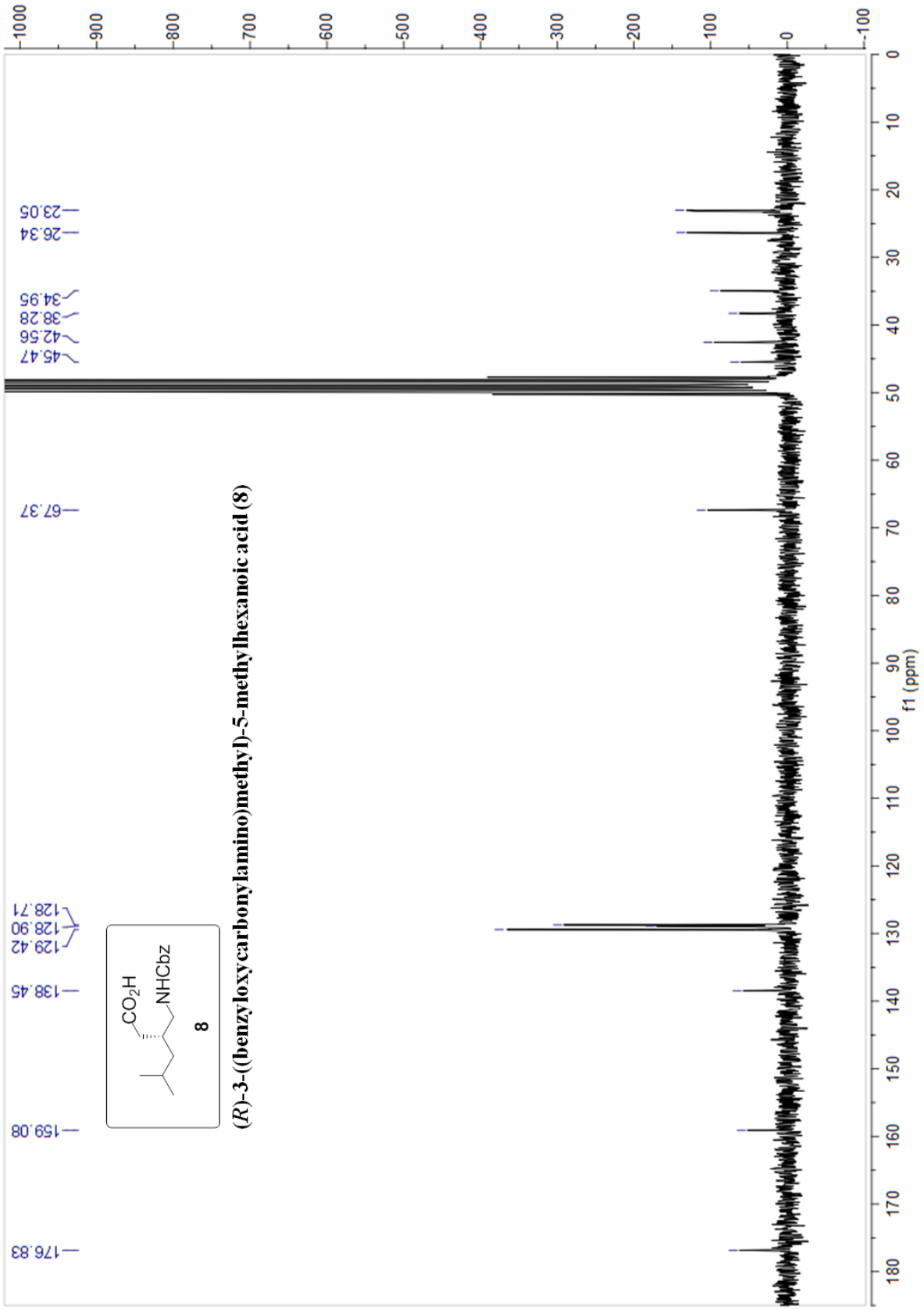


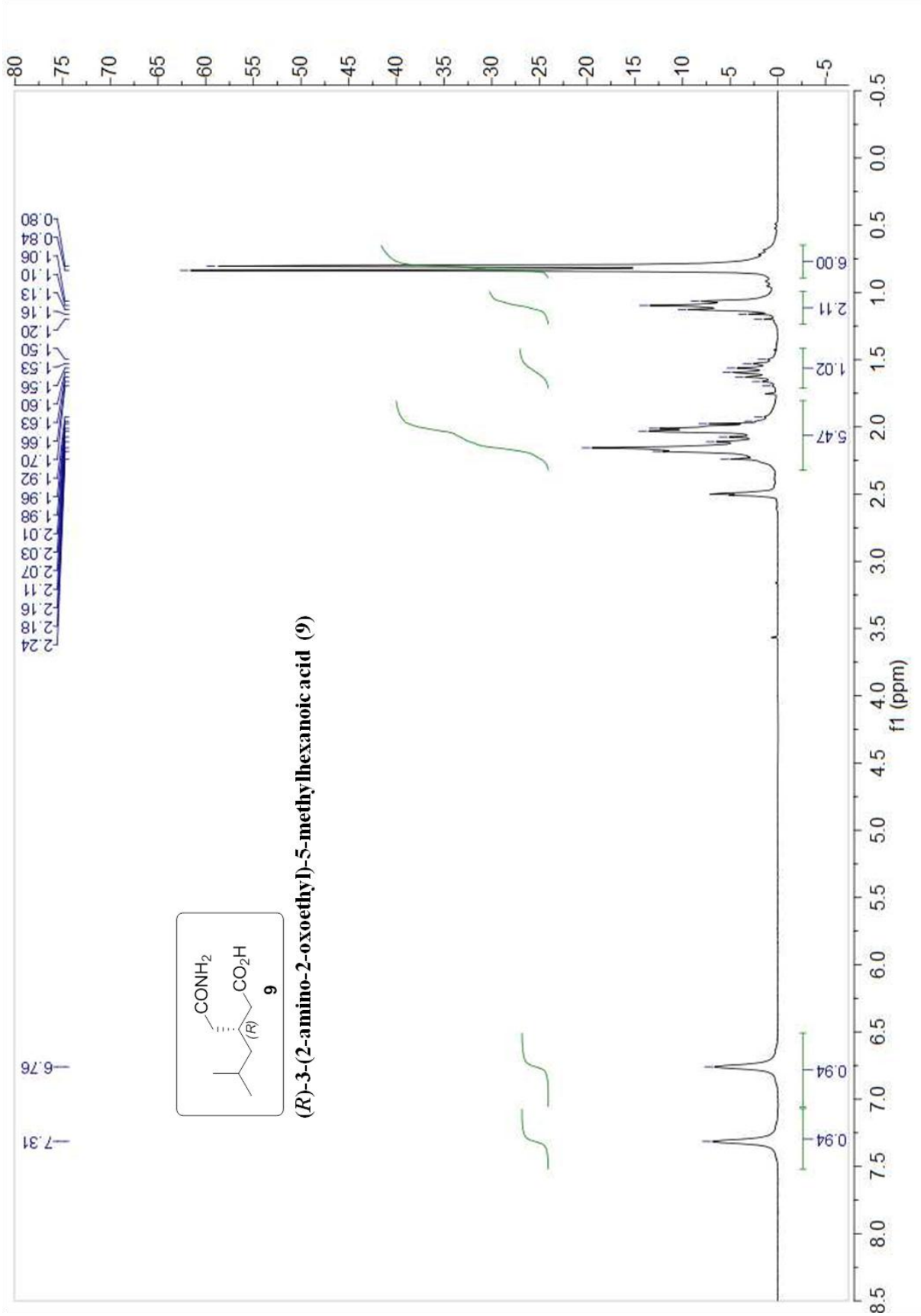

Diallyl 3-methylpentanedioate (3d)
CO2Allyl
CO2Allyl
3d
20.01
28.69
41.41
47.72
48.15
48.57
49.00
49.43
49.85
50.28
66.05
118.35
133.70
173.61
f1 (ppm)

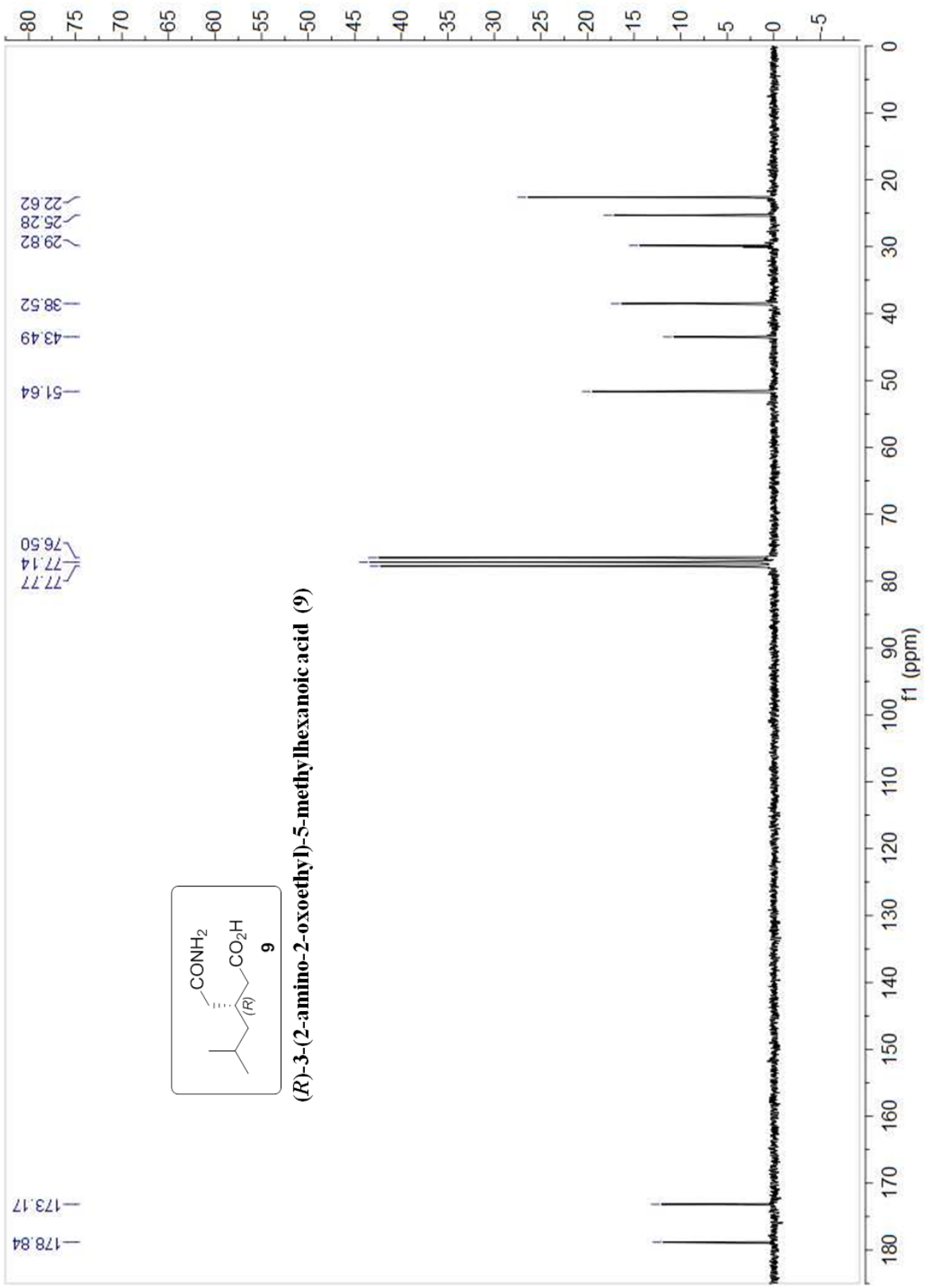






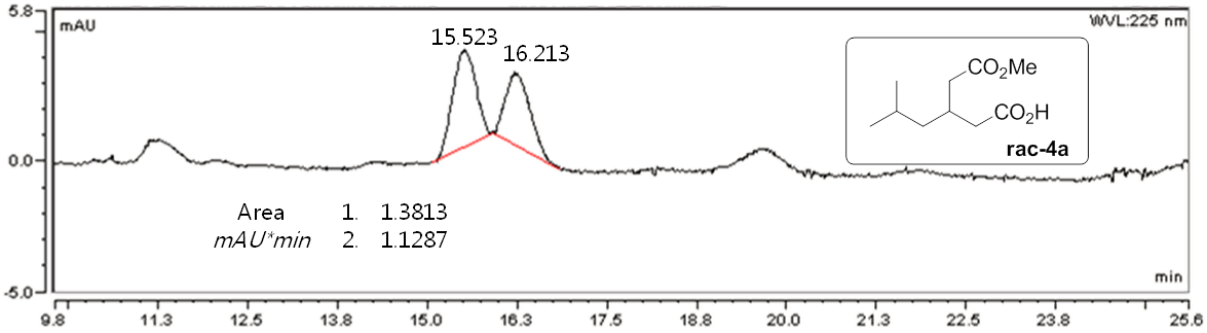



(R)-3-(2-amino-2-oxoethyl)-5-methylhexanoic acid (9)
CONH2
CO2H
(R)
9
f1 (ppm)



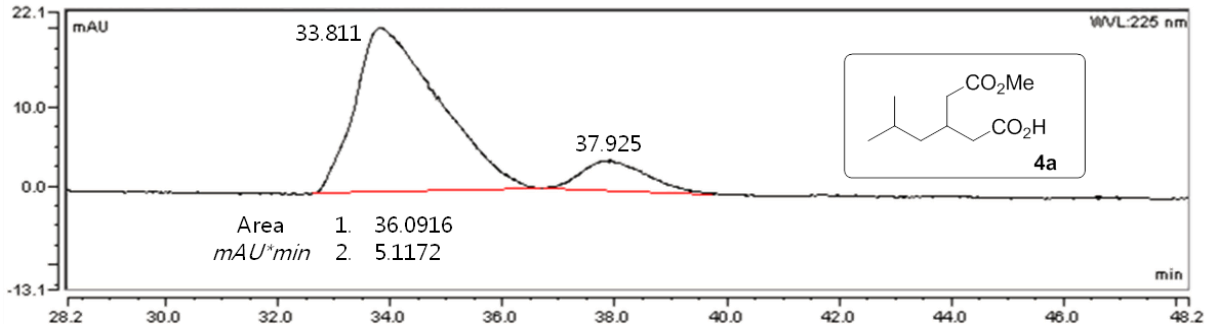
5. HPLC data

3-(2-methoxy-2-oxoethyl)-5-methylhexanoic acid (rac-4a)



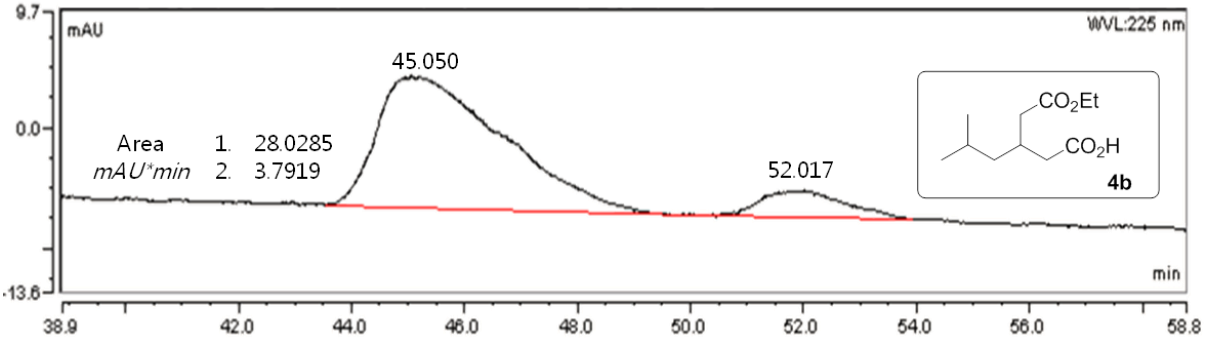
Chiralcel® OD-RH, ACN : H_2O = 20 : 80, 0.6 mL/min

3-(2-methoxy-2-oxoethyl)-5-methylhexanoic acid (4a)



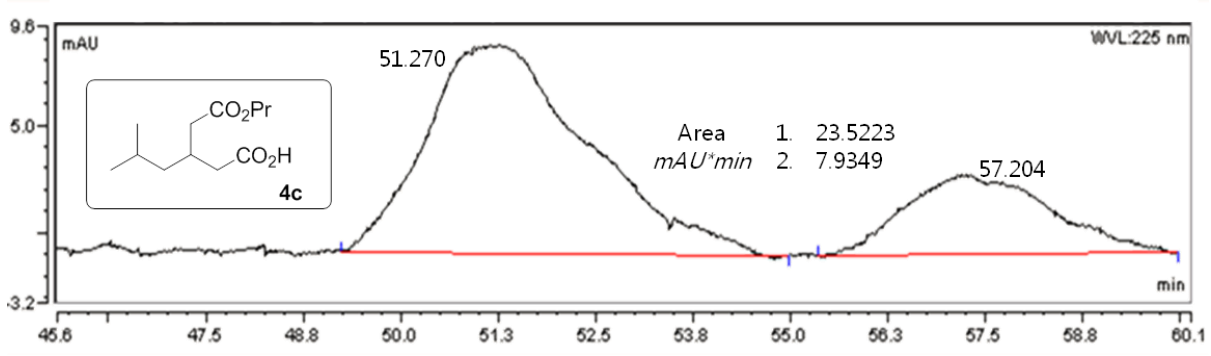
Chiralcel® OD-RH, ACN : H_2O = 20 : 80, 0.3 mL/min

3-(2-ethoxy-2-oxoethyl)-5-methylhexanoic acid (4b)



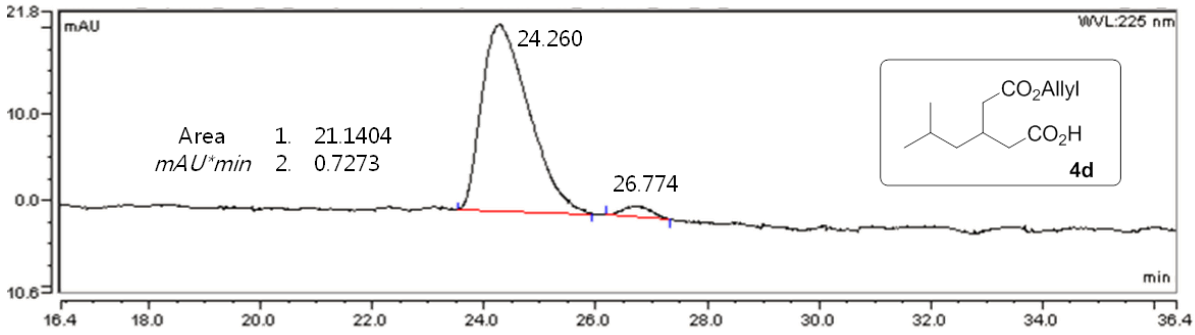
Chiralcel® OD-RH, ACN : H_2O = 20 : 80, 0.3 mL/min

5-methyl-3-(2-oxo-2-propoxyethyl)hexanoic acid (4c)



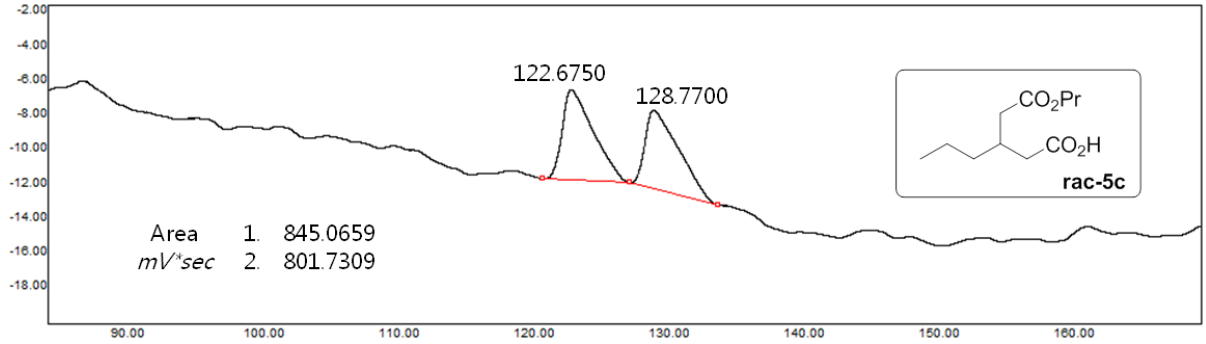
Chiralcel® OD-RH, ACN : H_2O = 20 : 80, 0.3 mL/min

3-(2-(allyloxy)-2-oxoethyl)-5-methylhexanoic acid (4d)



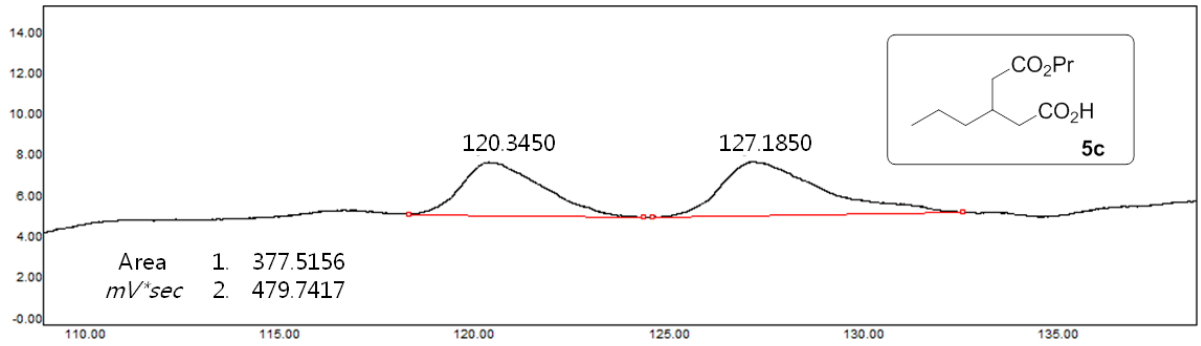
Chiralcel® OD-RH, ACN : H_2O = 20 : 80, 0.5 mL/min

3-(2-oxo-2-propoxyethyl)hexanoic acid (rac-5c)



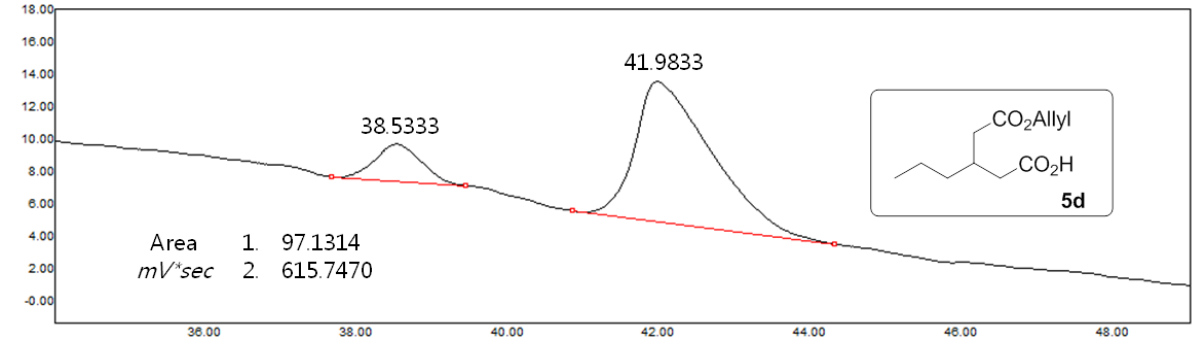
ChiralPak® IA, IPA : Hex : TFA = 1 : 99 : 0.1, 0.3 mL/min

3-(2-oxo-2-propoxyethyl)hexanoic acid (5c)



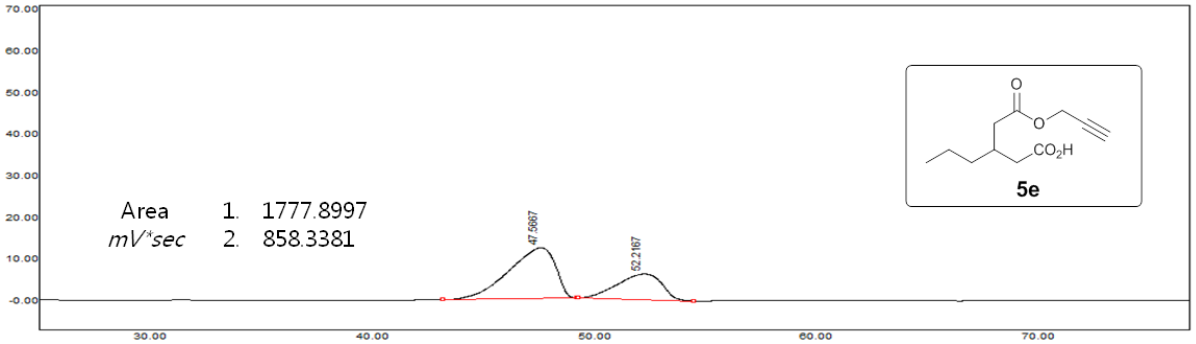
ChiralPak® IA, IPA : Hex : TFA = 1 : 99 : 0.1, 0.3 mL/min

3-(2-(allyloxy)-2-oxoethyl)hexanoic acid (5d)



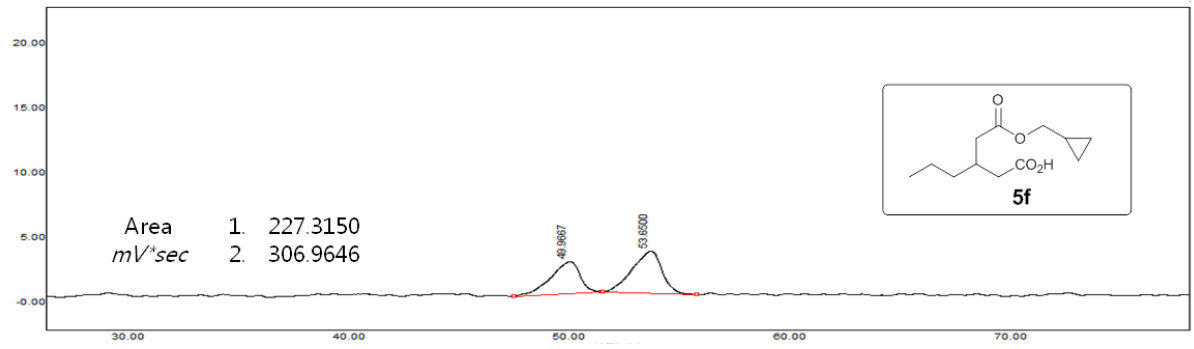
ChiralPak® IA, IPA : Hex : TFA = 1 : 99 : 0.1, 0.7 mL/min

3-(2-oxo-2-(prop-2-ynyloxy)ethyl)hexanoic acid (5e)



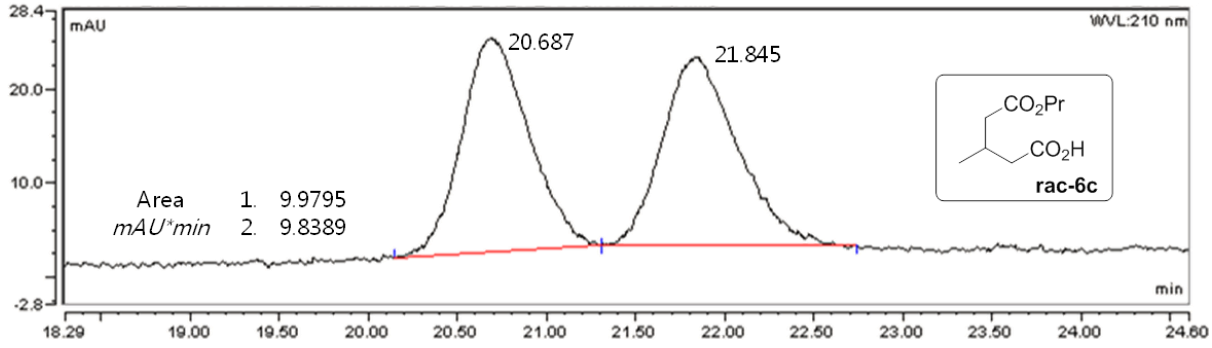
Chiralcel® OD-RH, ACN : H_2O = 20 : 80, 0.4 mL/min

3-(2-(cyclopropylmethoxy)-2-oxoethyl)hexanoic acid (5f)



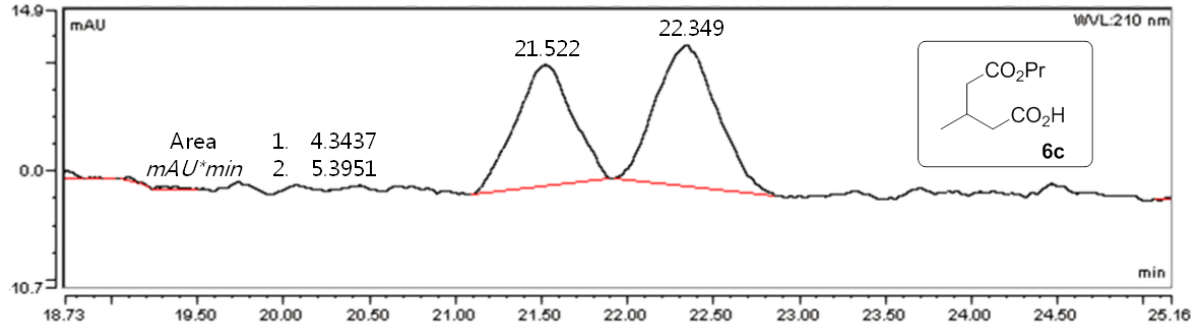
Chiralcel® OD-RH, ACN : H_2O = 20 : 80, 0.8 mL/min

3-methyl-5-oxo-5-propoxypentanoic acid (rac-6c)



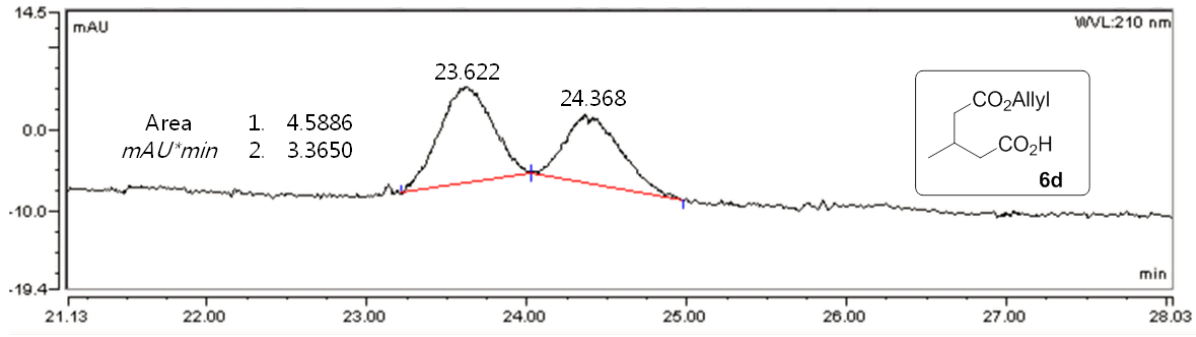
ChiralPak® IA, IPA : Hex = 2 : 98, 0.6 mL/min

3-methyl-5-oxo-5-propoxypentanoic acid (6c)



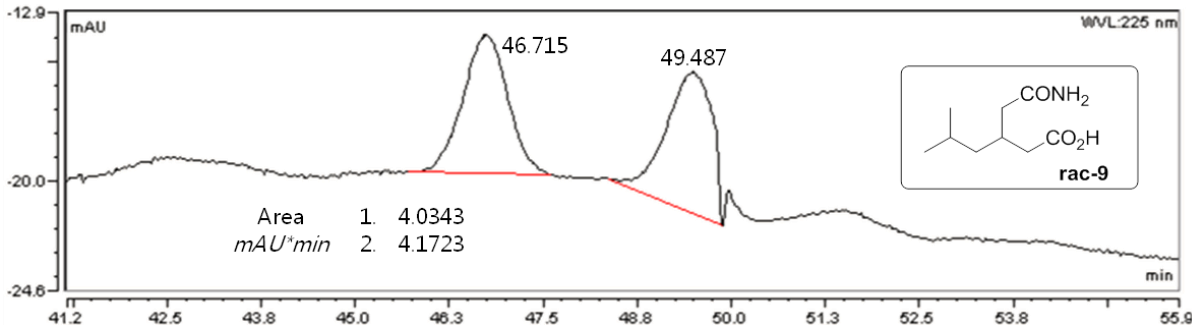
ChiralPak® IA, IPA : Hex = 2 : 98, 0.6 mL/min

5-(allyloxy)-3-methyl-5-oxopentanoic acid (6d)



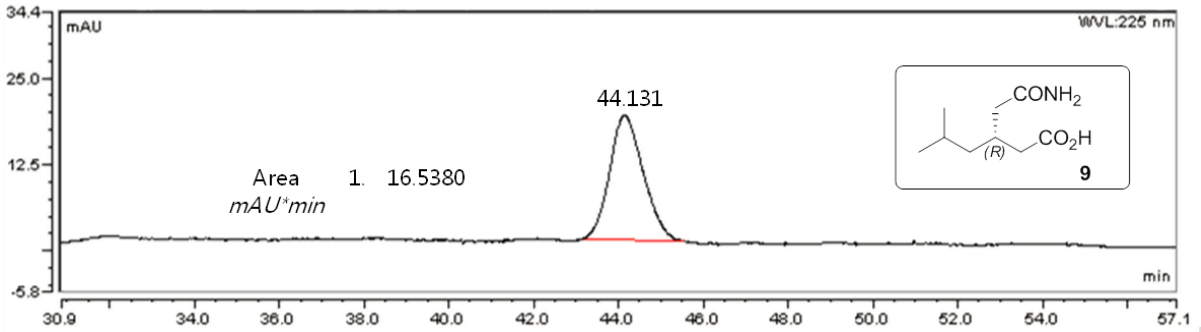
ChiralPak® IA, IPA : Hex = 2 : 98, 0.6 mL/min

3-(2-amino-2-oxoethyl)-5-methylhexanoic acid (rac-9)



ChiralPak® IA, TFA : IPA : Hex = 1 : 4 : 95, 0.4 mL/min

(*R*)-3-(2-amino-2-oxoethyl)-5-methylhexanoic acid (9)



ChiralPak® IA, TFA : IPA : Hex = 1 : 4 : 95, 0.4 mL/min