Supporting Information

Development of potent inhibitors for strigolactone receptor DWARF 14

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Table of Contents

1.	General	S2
2.	Synthesis of DL1 analogues	S3–S13
3.	YLG-based competition assay	S14
4.	Shoot branching assay	S15
5.	NMR spectrum	S16–S34
6.	Supplementary Figures	S35–S40

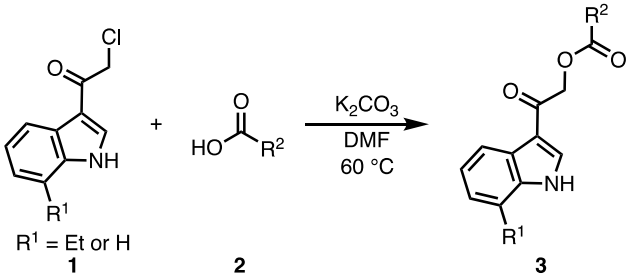
1. General

Unless otherwise noted, all reactants or reagents including dry solvents were obtained from commercial suppliers and used as received. Unless otherwise noted, all reactions were performed with dry solvents under an atmosphere of argon and N_2 in dried glassware using standard vacuum-line techniques. All work-up and purification procedures were carried out with reagent-grade solvents in air.

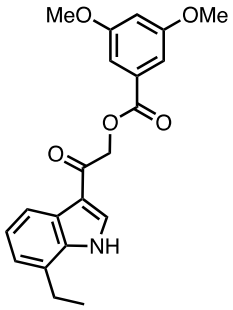
Analytical thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F_{254} precoated plates (0.25 mm). The developed chromatogram was analyzed by UV lamp (254 nm). Flash column chromatography was performed with E. Merck silica gel 60 (230–400 mesh) and Biotage Isolera® equipped with Biotage SNAP Cartridge KP-Sil columns and hexane/EtOAc as an eluent. Preparative thin-layer chromatography (PTLC) was performed using Wakogel B5-F silica coated plates (0.75 mm) prepared in our laboratory. High-resolution mass spectra was conducted on Thermo Fisher Scientific Exactive (ESI). Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-ECA-600 (^{1}H 600 MHz, ^{13}C 150 MHz) spectrometer, a JEOL JNM-ECA-400 (^{1}H 400 MHz, ^{13}C 100 MHz) and JEOL JMN-ECA-600II with Ultra COOL™ probe (^{1}H 600 MHz, ^{13}C 150 MHz) spectrometer. Chemical shifts for ^{1}H NMR are expressed in parts per million (ppm) relative to tetramethylsilane (δ 0.00 ppm). Chemical shifts for ^{13}C NMR are expressed in ppm relative to $CDCl_3$ (δ 77.0 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, t = triplet, dt = doublet of triplets, td = triplet of doublets, q = quartet, quin = quintet, m = multiplet, br = broad signal), coupling constant (Hz), and integration. Docking study was performed by using Maestro software (Schrödinger).

2. Synthesis of Inhibitors of DL1 analogues

2-1. General procedure for synthesis of DL1 analogues

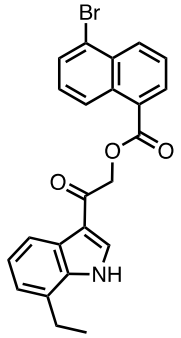


To a screw cap tube containing a magnetic stirring bar, α-chloroacetyl indole **1** (20 mg, 1.0 equiv), the carboxylic acid **2** (3.0 equiv), K_2CO_3 (2.0 equiv) and DMF (1.0 mL) were added. After stirring the mixture for several hours at 60 °C with monitoring reaction progress with TLC, the reaction was quenched with saturated $NaHCO_3$aq and extracted three times with EtOAc. The combined organic layer was dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The residue was purified by reprecipitation or silica-gel chromatography to afford the desired product **3**.



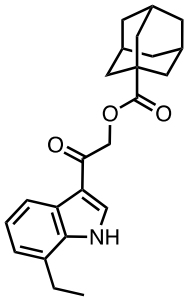
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 3,5-dimethoxybenzoate (DL1a)

Purification by reprecipitation (hexane/DCM) afforded **DL1a** as a brown solid (8.3 mg, 25% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.66 (bs, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.99 (d, J = 3.0 Hz, 1H), 7.31 (d, J = 2.4 Hz, 2H), 7.27 (t, J = 7.2 Hz, 1H), 7.15 (d, J = 7.2 Hz, 1H), 6.68 (t, J = 2.4 Hz, 1H), 5.42 (s, 2H), 3.84 (s, 6H), 2.88 (q, J = 7.8 Hz, 2H), 1.37 (t, J = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.8, 166.0, 160.7, 134.9, 131.4, 130.3, 126.8, 125.2, 123.3, 122.7, 120.0, 115.5, 107.5, 106.3, 66.9, 55.6, 23.9, 13.9; HRMS (ESI) *m/z* calcd for $C_{21}H_{21}NO_5Na^+$ $[M + Na]^+$: 390.1312 found 390.1312.



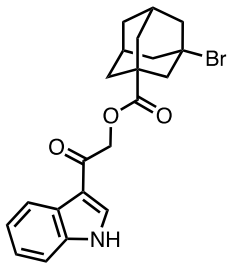
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 5-bromo-1-naphthoate (DL1b)

Purification by reprecipitation (hexane/DCM) afforded **DL1b** as a off-white solid (24.5 mg, 62% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (d, *J* = 9.2 Hz, 1H), 8.73 (bs, 1H), 8.53 (d, *J* = 8.8 Hz, 1H), 8.42 (dd, *J* = 7.2, 1.2 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 7.99 (d, *J* = 3.2 Hz, 1H), 7.85 (d, *J* = 6.8 Hz, 1H), 7.64 (dd, *J* = 8.8, 7.6 Hz, 1H), 7.45 (dd, *J* = 8.8, 7.6 Hz, 1H), 7.28 (t, *J* = 7.6 Hz, 1H), 7.15 (d, *J* = 6.8 Hz, 1H), 5.53 (s, 2H), 2.88 (q, *J* = 7.6 Hz, 2H), 1.36 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.7, 166.8, 135.0, 132.8, 132.5, 132.2, 131.3, 130.6, 130.2, 128.0, 127.3, 126.9, 126.0, 125.9, 125.1, 123.4, 123.2, 122.7, 119.9, 115.5, 66.8, 23.9, 13.9; HRMS (ESI) *m/z* calcd for $C_{23}H_{19}BrNO_3^+$ $[M + H]^+$: 434.0397 found 434.0388.



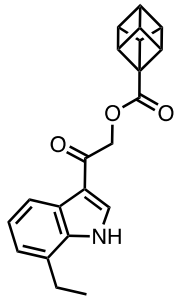
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl (3*r*,5*r*,7*r*)-adamantane-1-carboxylate (DL1c)

Purification by reprecipitation (hexane/DCM) afforded **DL1c** as a brown solid (8.6 mg, 26% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.74 (bs, 1H), 8.15 (d, *J* = 7.2 Hz, 1H), 7.89 (d, *J* = 3.0 Hz, 1H), 7.23 (t, *J* = 7.2 Hz, 1H), 7.13 (d, *J* = 7.2 Hz, 1H), 5.17 (s, 2H), 2.87 (q, *J* = 7.8 Hz, 2H), 2.07–2.01 (m, 9H), 1.75 (bs, 6H), 1.36 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.1, 177.4, 134.9, 130.1, 126.8, 125.1, 123.2, 122.6, 119.9, 115.5, 65.9, 40.8, 38.9, 36.5, 27.9, 23.8, 13.9; HRMS (ESI) *m*/*z* calcd for $C_{23}H_{27}NO_3Na^+$ $[M + Na]^+$: 388.1883 found 388.1881.



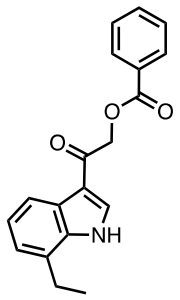
2-(1*H*-Indol-3-yl)-2-oxoethyl (1*r*,3*s*,5*R*,7*S*)-3-bromoadamantane-1-carboxylate (DL1d)

Purification by reprecipitation (hexane/DCM) afforded **DL1d** as a brown solid (15.8 mg, 42% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.67 (bs, 1H), 8.33–8.29 (m, 1H), 7.90 (d, J = 3.0 Hz, 1H), 7.46–7.41 (m, 1H), 7.33–7.29 (m, 2H), 5.21 (s, 2H), 2.62 (s, 2H), 2.36–2.33 (m, 4H), 2.27–2.22 (m, 2H), 2.08–2.00 (m, 4H), 1.75–1.72 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.5, 175.2, 136.1, 130.5, 125.2, 124.1, 123.0, 122.2, 114.9, 111.5, 65.9, 63.7, 49.5, 48.1, 45.0, 37.2, 34.5, 31.7; HRMS (ESI) *m*/*z* calcd for $C_{21}H_{22}BrNO_3Na^+$ $[M + Na]^+$: 438.0675 found 438.0675.



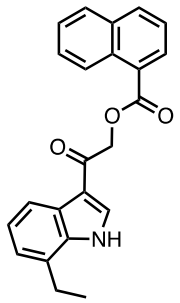
2-(7- Ethyl-1*H*-indol-3-yl)-2-oxoethyl cubane-1-carboxylate (DL1f)

Purification by reprecipitation (hexane/DCM) afforded **DL1f** as a white solid (9.2 mg, 31% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (bs, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.94 (d, J = 3.2 Hz, 1H), 7.26 (t, J = 7.6 Hz, 1H), 7.14 (d, J = 7.6 Hz, 1H), 5.23 (s, 2H), 4.40–4.35 (m, 3H), 4.08–3.98 (m, 4H), 2.89 (q, J = 7.6 Hz, 2H), 1.37 (t, J = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.1, 171.9, 135.0, 130.3, 126.9, 125.1, 123.2, 122.6, 119.9, 115.4, 65.9, 55.5, 49.6, 47.8, 45.3, 23.8, 13.9; HRMS (ESI) *m*/*z* calcd for $C_{21}H_{18}NO_3^-$ $[M - H]^-$: 332.1292 found 332.1285.



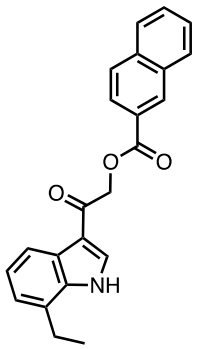
2-(7- Ethyl-1*H*-indol-3-yl)-2-oxoethyl cubane-1-carboxylate (DL1g)

Purification by silica-gel chromatography (20% EtOAc in hexane) afforded **DL1g** as a white solid (29.1 mg, 42% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (bs, 1H), 8.21-8.12 (m, 3H), 7.90 (d, J = 3.1 Hz, 1H), 7.61-7.50 (m, 1H), 7.44 (t, J = 7.6 Hz, 2H), 7.23 (t, J = 7.3 Hz, 2H), 7.11 (d, J = 7.3 Hz, 1H), 5.37 (s, 2H), 2.84 (q, J = 7.5 Hz, 2H), 1.32 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.0, 166.3, 135.0, 133.3, 130.6, 129.9, 129.5, 128.5, 127.0, 125.1, 123.3, 122.6, 119.8, 115.2, 66.6, 23.8, 13.9; HRMS (ESI) *m/z* calcd for $C_{19}H_{16}NO_3$ $[M - H]^-$: 306.1125 found 306.1127.



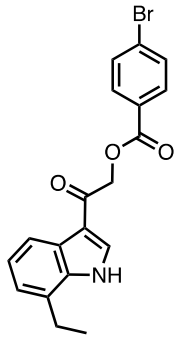
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 1-naphthoate (DL1h)

Purification by reprecipitation (hexane/DCM) afforded **DL1h** as a brown solid (6.4 mg, 20% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.00 (d, J = 8.4 Hz, 1H), 8.67 (bs, 1H), 8.40 (dd, J = 7.2, 1.2 Hz, 1H), 8.23 (d, J = 8.4 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H), 8.02 (d, J = 3.0 Hz, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.63 (td, J = 7.2, 1.2 Hz, 1H), 7.57–7.52 (m, 2H), 7.28 (t, J = 7.8 Hz, 1H), 7.16 (d, J = 7.2 Hz, 1H), 5.34 (s, 2H), 2.89 (q, J = 7.8 Hz, 2H), 1.37 (t, J = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.0, 167.1, 135.0, 133.8, 133.7, 131.5, 130.8, 130.3, 128.5, 127.9, 126.9, 126.5, 126.3, 125.2, 124.6, 123.3, 122.7, 120.0, 115.5, 66.7, 23.9, 13.9; HRMS (ESI) *m/z* calcd for $C_{23}H_{19}NO_3Na^+$ $[M + Na]^+$: 380.1257 found 380.1258.



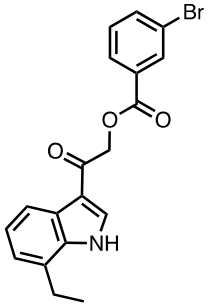
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 2-naphthoate (DL1i)

Purification by reprecipitation (hexane/DCM) afforded **DL1i** as a brown solid (8.9 mg, 28% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.64 (bs, 1H), 8.23 (d, J = 7.8 Hz, 1H), 8.16 (d, J = 7.2 Hz, 1H), 8.05–7.86 (m, 4H), 7.65–7.54 (m, 2H), 7.26–7.15 (m, 2H), 5.51 (s, 2H), 2.89 (q, J = 7.2 Hz, 2H), 1.37 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.0, 166.4, 135.7, 135.0, 132.5, 131.6, 130.2, 129.5, 128.4, 128.3, 127.8, 126.8, 126.7, 125.4, 125.2, 123.4, 122.7, 120.0, 115.6, 66.8, 23.9, 14.0; HRMS (ESI) *m/z* calcd for $C_{23}H_{19}NO_3Na^+$ $[M + Na]^+$: 380.1257 found 380.1258.



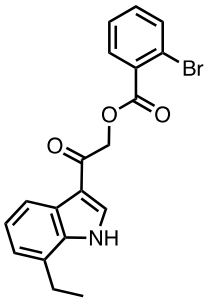
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 4-bromobenzoate (DL1j)

Purification by reprecipitation (hexane/DCM) afforded **DL1j** as a brown solid (7.0 mg, 20% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.66 (bs, 1H), 8.18 (d, J = 8.4 Hz, 1H), 8.02 (d, J = 8.4 Hz, 2H), 7.97 (d, J = 3.6 Hz, 1H), 7.61 (d, J = 8.4 Hz, 2H), 7.27 (t, J = 7.8 Hz, 1H), 7.13 (d, J = 7.8 Hz, 1H), 5.44 (s, 2H), 2.89 (q, J = 7.8 Hz, 2H), 1.37 (t, J = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.5, 165.5, 135.0, 131.8, 131.5, 130.2, 128.48, 128.46, 126.9, 125.1, 123.4, 122.7, 119.9, 115.4, 66.7, 23.9, 13.9; HRMS (ESI) *m/z* calcd for $C_{19}H_{17}BrNO_3^+$ $[M + H]^+$: 384.0241 found 384.0234.



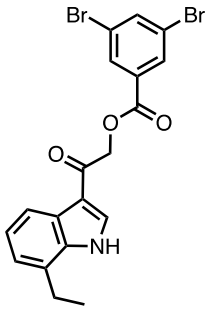
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 3-bromobenzoate (DL1k)

Purification by reprecipitation (hexane/DCM) afforded **DL1k** as a brown solid (10.4 mg, 30% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.76 (bs, 1H), 8.29 (s, 1H), 8.17 (d, *J* = 7.8 Hz, 1H), 8.07 (d, *J* = 6.6 Hz, 1H), 7.93 (d, *J* = 4.2 Hz, 1H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.26 (t, *J* = 7.8, 1H), 7.14 (d, *J* = 7.2 Hz, 1H), 5.43 (s, 2H), 2.88 (q, *J* = 7.8 Hz, 2H), 1.36 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.4, 165.0, 136.2, 135.0, 132.9, 131.5, 130.2, 130.0, 128.5, 126.9, 125.1, 123.4, 122.7, 122.5, 119.9, 115.3, 66.8, 23.8, 13.9; HRMS (ESI) *m*/*z* calcd for $C_{19}H_{16}BrNO_3Na^+$ $[M + Na]^+$: 408.0206 found 408.0206.



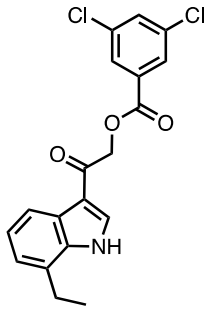
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 2-bromobenzoate (DL1l)

Purification by reprecipitation (hexane/DCM) afforded **DL1l** as a brown solid (4.8 mg, 14% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.87 (bs, 1H), 8.17 (d, *J* = 8.4 Hz, 1H), 8.05 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.94 (d, *J* = 3.6 Hz, 1H), 7.68 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.41–7.33 (m, 2H), 2.56 (t, *J* = 8.4 Hz, 1H), 7.14 (d, *J* = 7.2 Hz, 1H), 5.43 (s, 2H), 2.87 (q, *J* = 7.8 Hz, 2H), 1.35 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.4, 165.6, 135.0, 134.4, 132.9, 132.0, 131.3, 130.4, 127.3, 127.0, 125.1, 123.3, 122.7, 122.0, 119.8, 115.3, 66.8, 23.8, 13.9; HRMS (ESI) *m*/*z* calcd for $C_{19}H_{16}BrNO_3Na^+$ $[M + Na]^+$: 408.0206 found 408.0206.



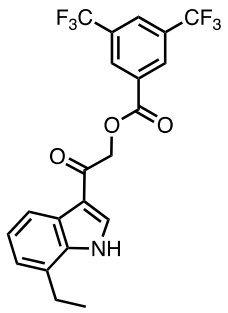
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 3,5-dibromobenzoate (DL1m)

Purification by reprecipitation (hexane/DCM) afforded **DL1m** as a brown solid (5.3 mg, 13% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.65 (bs, 1H), 8.23 (s, 2H), 8.17 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 3.0 Hz, 1H), 7.88 (s, 1H), 7.28 (t, J = 7.2 Hz, 1H), 7.17 (d, J = 7.2, 1H), 5.47 (s, 2H), 2.90 (q, J = 7.8 Hz, 2H), 1.38 (t, J = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 186.9, 163.7, 138.6, 135.0, 132.8, 131.7, 130.0, 126.9, 125.0, 123.5, 123.1, 122.8, 119.9, 115.4, 67.0, 23.9, 13.9; HRMS (ESI) *m*/*z* calcd for $C_{19}H_{16}Br_2NO_3^+$ $[M + H]^+$: 463.9325 found 463.9314.



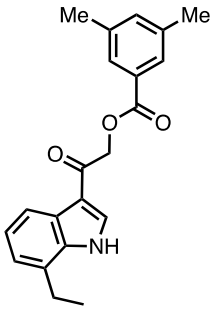
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 3,5-dichlorobenzoate (DL1n)

Purification by reprecipitation (hexane/DCM) afforded **DL1n** as a brown solid (14.2 mg, 42% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.68 (bs, 1H), 8.17 (d, J = 7.8 Hz, 1H), 8.03 (d, J = 1.2 Hz, 2H), 7.96 (d, J = 3.6 Hz, 1H), 7.58 (t, J = 1.2 Hz, 1H), 7.28 (t, J = 7.2 Hz, 1H), 7.16 (d, J = 7.2 Hz, 1H), 5.47 (s, 2H), 2.89 (q, J = 7.8 Hz, 2H), 1.38 (t, J = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 186.9, 164.0, 135.3, 135.0, 133.1, 132.3, 130.1, 128.3, 126.9, 125.0, 123.5, 122.8, 119.9, 115.3, 66.9, 23.9, 13.9; HRMS (ESI) *m/z* calcd for $C_{19}H_{14}Cl_2NO_3^-$ $[M - H]^-$: 374.0356 found 374.0349.



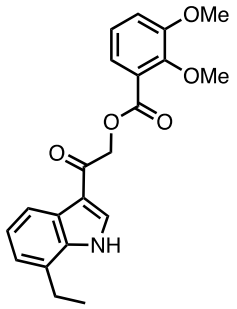
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 3,5-bis(trifluoromethyl)benzoate (DL1o)

Purification by reprecipitation (hexane/DCM) afforded **DL1o** as a brown solid (11.3 mg, 28% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (bs, 1H), 8.62 (s, 2H), 8.15 (d, J = 8.0 Hz, 1H), 8.10 (s, 1H), 7.98 (d, J = 2.8 Hz, 1H), 7.31–7.26 (m, 1H), 7.17 (d, J = 7.6 Hz, 1H), 5.56 (s, 2H), 2.90 (q, J = 7.6 Hz, 2H), 1.38 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.5, 163.7, 135.0, 132.2 (q, J = 34.4 Hz), 131.8, 130.1, 127.0, 126.7, 125.0, 123.5, 122.86, 122.84 (q, J = 270.0 Hz), 119.8, 115.2, 67.1, 23.9, 13.9; HRMS (ESI) *m/z* calcd for $C_{21}H_{14}F_6NO_3^-$ [M – H]$^-$: 442.0883 found 442.0876.



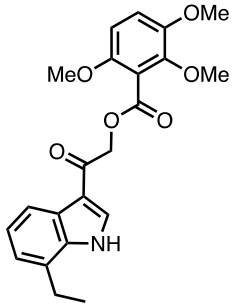
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 3,5-dimethylbenzoate (DL1p)

Purification by reprecipitation (hexane/DCM) afforded **DL1p** as a brown solid (13.3 mg, 44% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.66 (bs, 1H), 8.21 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 3.0 Hz, 1H), 7.78 (s, 2H), 7.28–7.25 (m, 1H), 7.22 (s, 1H), 7.15 (d, J = 7.2 Hz, 1H), 5.42 (s, 2H), 2.89 (q, J = 7.8 Hz, 2H), 2.37 (s, 6H), 1.37 (t, J = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.0, 166.6, 138.1, 135.0, 130.3, 129.4, 127.7, 126.8, 125.2, 123.3, 122.6, 120.0, 115.5, 66.6, 23.9, 21.1, 13.9; HRMS (ESI) *m/z* calcd for $C_{21}H_{20}NO_3^-$ [M – H]$^-$: 334.1449 found 334.1443.



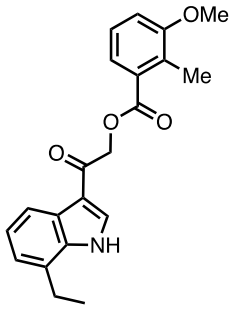
2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 2,3-dimethoxybenzoate (DL1q)

Purification by reprecipitation (hexane/DCM) afforded **1** as a brown solid (13.7 mg, 41% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.72 (bs, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 8.07 (d, *J* = 3.0 Hz, 1H), 7.54 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.26 (t, *J* = 7.8 Hz, 1H), 7.15–7.09 (m, 3H), 5.40 (s, 2H), 3.94 (s, 3H), 3.91 (s, 3H), 2.88 (q, *J* = 7.8 Hz, 2H), 1.36 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.0, 165.7, 153.5, 149.4, 134.9, 130.8, 126.8, 125.34, 125.26, 124.0, 123.3, 122.9, 122.6, 120.0, 116.2, 115.5, 67.0, 61.7, 56.1, 23.9, 13.9; HRMS (ESI) *m/z* calcd for $C_{21}H_{21}NO_5Na^+$ [M + Na]$^+$: 390.1312 found 390.1312.



2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 2,3,6-trimethoxybenzoate (DL1r)

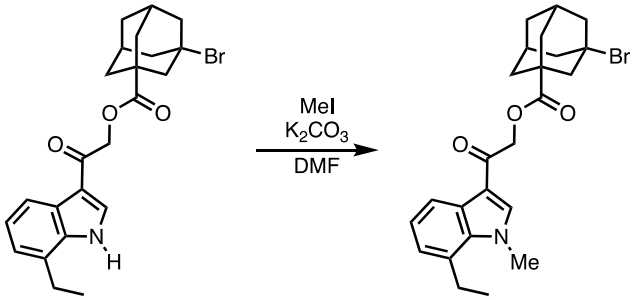
Purification by reprecipitation (hexane/DCM) afforded **DL1r** as a brown solid (23.8 mg, 66% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.99 (bs, 1H), 8.22 (d, *J* = 7.8 Hz, 1H), 8.04 (d, *J* = 3.6 Hz, 1H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.12 (d, *J* = 7.2 Hz, 1H), 6.91 (d, *J* = 9.0 Hz, 1H), 6.59 (d, *J* = 9.0 Hz, 1H), 5.36 (s, 2H), 3.90 (s, 3H), 3.83 (s, 3H), 3.75 (s, 3H), 2.87 (q, *J* = 7.8 Hz, 2H), 1.34 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.0, 165.5, 150.8, 147.2, 146.9, 134.9, 131.6, 126.9, 125.4, 123.1, 122.5, 120.0, 118.6, 115.4, 114.6, 106.4, 67.7, 61.7, 56.6, 56.4, 23.8, 14.0; HRMS (ESI) *m/z* calcd for $C_{22}H_{23}NO_6Na^+$ [M + Na]$^+$: 420.1418 found 420.1416.



2-(7-Ethyl-1*H*-indol-3-yl)-2-oxoethyl 3-methoxy-2-methylbenzoate (DL1s)

Purification by reprecipitation (hexane/DCM) afforded **DL1s** as a brown solid (19.6 mg, 62% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.72 (bs, 1H), 8.20 (d, *J* = 8.4 Hz, 1H), 7.96 (d, *J* = 3.0 Hz, 1H), 7.61 (d, *J* = 7.2 Hz, 1H), 7.28–7.21 (m, 2H), 7.14 (d, *J* = 7.2 Hz, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 5.40 (s, 2H), 3.86 (s, 3H), 2.88 (q, *J* = 7.2 Hz, 2H), 2.49 (s, 3H), 1.34 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 188.0, 167.6, 158.1, 134.9, 131.0, 130.3, 129.0, 126.8, 126.1, 125.2, 123.3, 122.6, 122.4, 120.0, 115.5, 113.7, 66.6, 55.9, 23.9, 13.9, 12.8; HRMS (ESI) *m/z* calcd for $C_{21}H_{21}NO_4Na^+$ $[M + Na]^+$: 374.1363 found 474.1363.

2-1. Synthesis of DL1e



DL1 **DL1e**

2-(7-Ethyl-1-methyl-1*H*-indol-3-yl)-2-oxoethyl (1*r*,3*s*,5*R*,7*S*)-3-bromoadamantane-1-carboxylate (DL1e)

To a screw cap tube containing a magnetic stirring bar, **DL1** (10 mg, 22.5 μmol, 1.0 equiv), K_2CO_3 (9.33 mg, 67.5 μmol, 3.0 equiv), DMF (0.10 mL) and MeI (7.0 μL, 112.5 μmol, 5.0 equiv) were added. After stirring the mixture for several hours at 60 °C with monitoring reaction progress with TLC, the reaction was quenched with saturated $NaHCO_3$aq and extracted three times with EtOAc. The combined organic layer was dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The residue was purified by PTLC (hexane/EtOAc = 2:1) to afford **DL1e** as a white solid (10.2 mg, 99% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.21 (d, *J* = 7.2 Hz, 1H), 7.67 (s, 1H), 7.21 (t, *J* = 7.2 Hz, 1H), 7.09 (d, *J* = 7.2 Hz, 1H), 5.16 (s, 2H), 4.09 (s, 3H), 3.10 (q, *J* = 7.8 Hz, 2H), 2.61 (s, 2H), 2.36–2.33 (m, 4H), 2.27–2.22 (m, 2H), 2.08–

2.00 (m, 4H), 1.75–1.72 (m, 2H), 1.35 (t, J = 7.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 186.8, 175.0, 136.6, 135.2, 128.2, 127.6, 124.7, 123.2, 120.4, 112.9, 65.8, 63.8, 49.6, 48.1, 45.0, 37.9, 37.2, 34.5, 31.8, 25.2, 16.6; HRMS (ESI) *m/z* calcd for $C_{24}H_{28}BrNO_3Na^+$ $[M + Na]^+$: 480.1145 found 480.1145.

3. YLG-based competition assay

Preparation of recombinant proteins

1 L of Luria broth (LB) medium, inoculated into 10 mL of overnight culture, was incubated at 37 °C until optical density at 600 nm (OD_{600}) reached around 0.6. Protein expression was induced by addition of 100 μM isopropyl-β-D-1-thiogalactopyranoside (IPTG). After the incubation for protein expression overnight at 20 °C, the *Escherichia coli* culture was lysed using a homogenizer (UD-211, TOMY) in 20 ml of the extraction buffer (100 mM HEPES, 150 mM NaCl, 10% glycerol [pH 7.0]). His-tagged AtD14 was trapped by the nickel resin at 4 °C. After the resin was washed with extraction buffer and 30 mM imidazole buffer, the His-tagged AtD14 was eluted with 150 mM imidazole buffer. Further purification was conducted by gel filtration chromatography using AKTA system equipped with Superdex 200 increase (GE healthcare) with running buffer (10 mM HEPES, 150 mM NaCl, pH 7.0). Aliquots of purified recombinant AtD14 were kept at –80 °C until use.

YLG-based competition assays

For YLG hydrolysis assays, 1 μg of recombinant AtD14 was incubated with 1 μM YLG and various concentrations of DL1 analogues in 100 μL of the reaction buffer (100 mM HEPES, 150 mM NaCl, pH 7.0) with 0.2% DMSO on a 96-well black plate (Greiner). After the incubation at room temperature for 1 hour, the fluorescence intensity was measured by spectraMax i3 (Molecular Devices) at excitation by 480 nm and detection by 520 nm. IC_{50} values were calculated by a curve-fitting program in Kaleidagraph software.

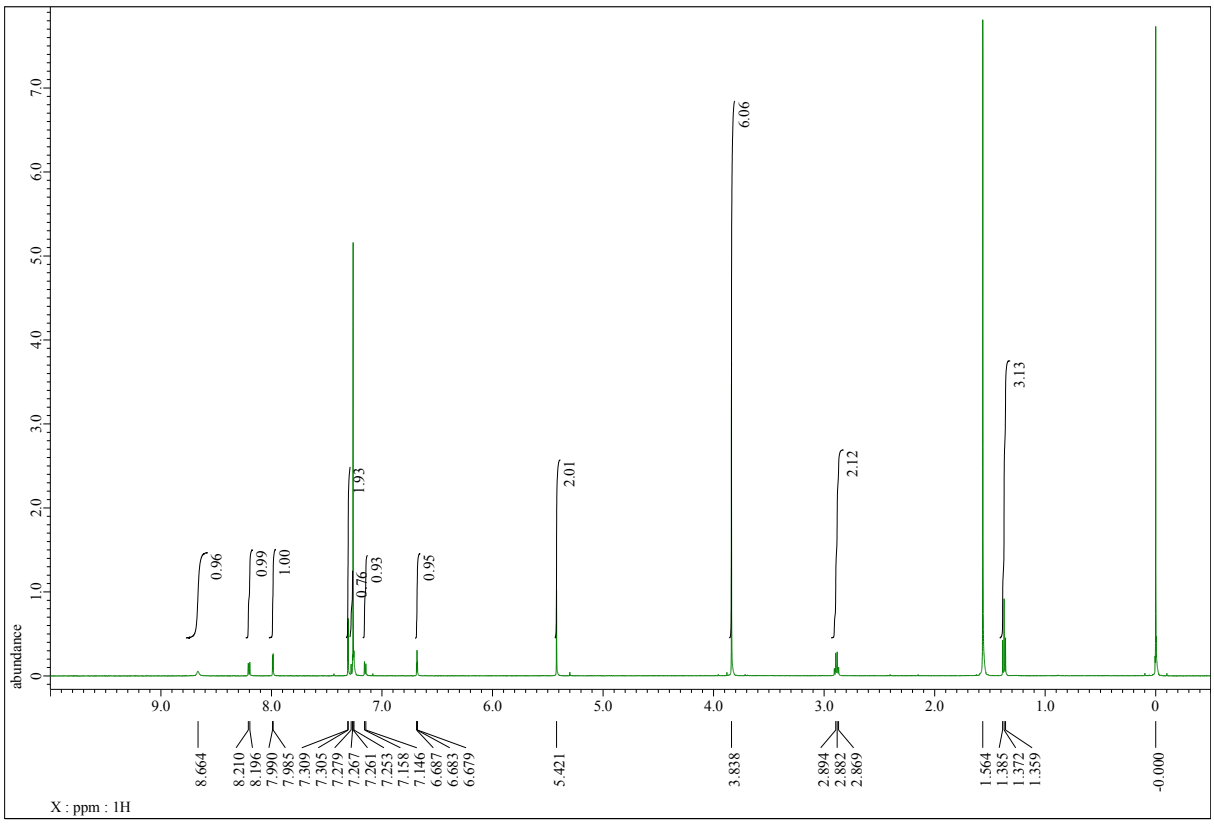
4. Shoot branching assay

Plant culture and treatment

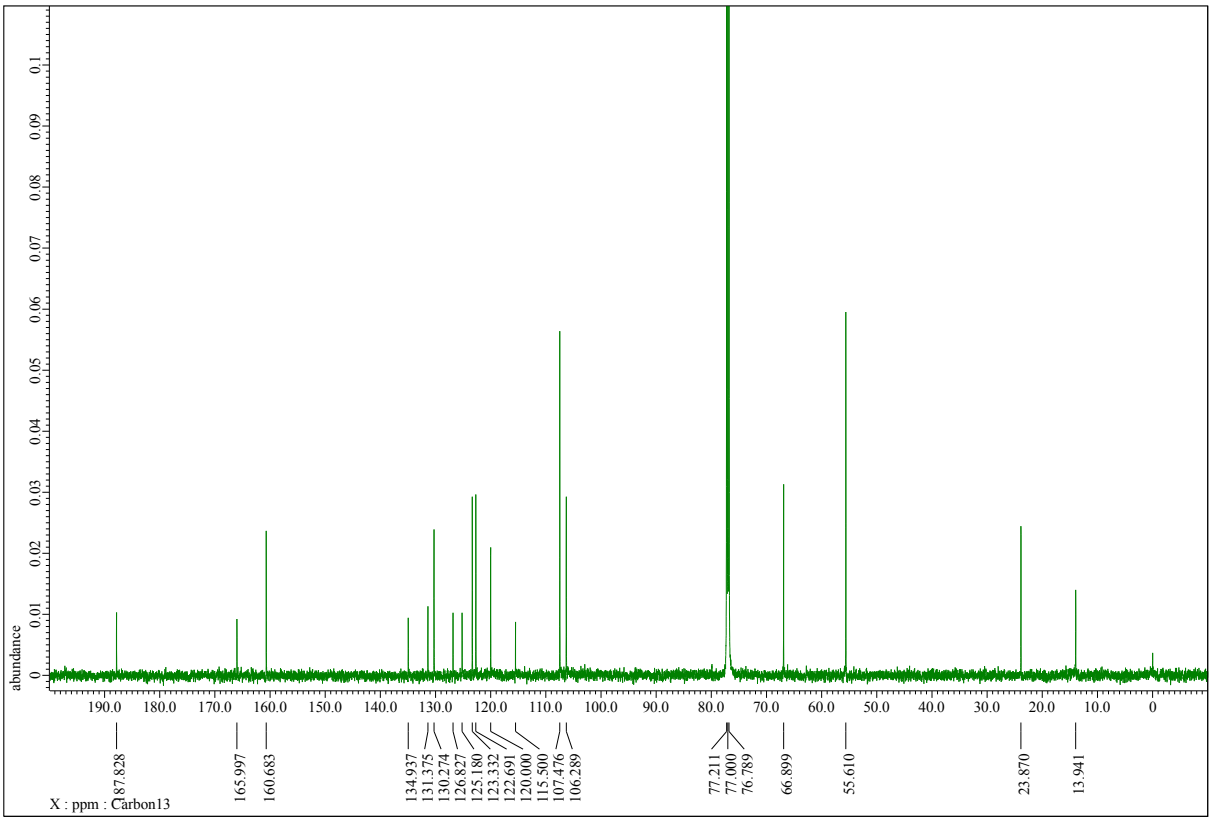
For shoot branching assays, the *Arabidopsis thaliana* Columbia (Col) accession was used. The seeds of *Arabidopsis* were sterilized and stored in the dark at 4 °C for a few days before use. The seeds were transferred to 1/2 Murashige and Skoog (MS) culture medium (0.5% [w/v] MES, 1% [w/v] sucrose, pH 5.7 with KOH and 0.8% [w/v] agar, 0.1% DMSO) including 1 μM of DL1 analogues and grown under a 16 h/ 8 h light/dark cycle at 22 °C for 30 days. After the incubation, the number of primary rosette branches at least 0.5 cm long was counted.

5. NMR Spectrum

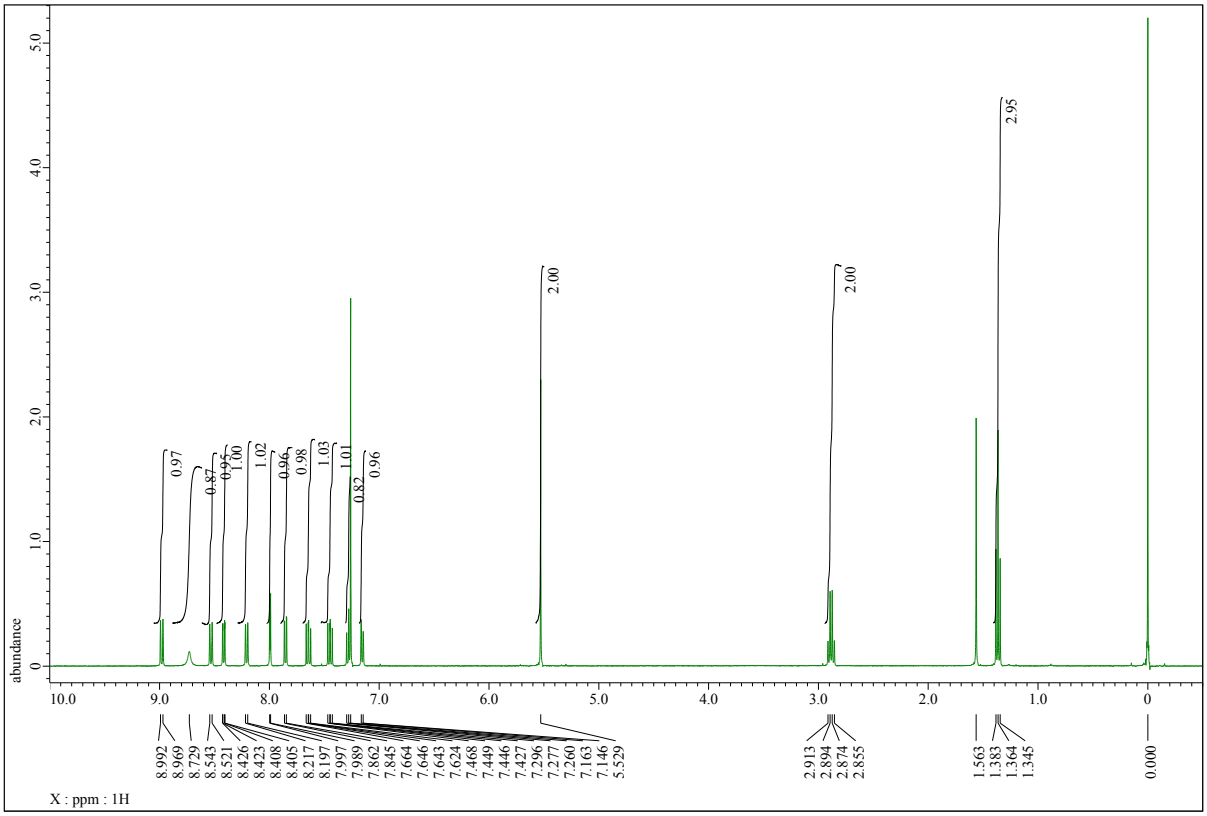
DL1a; ^{1}H NMR



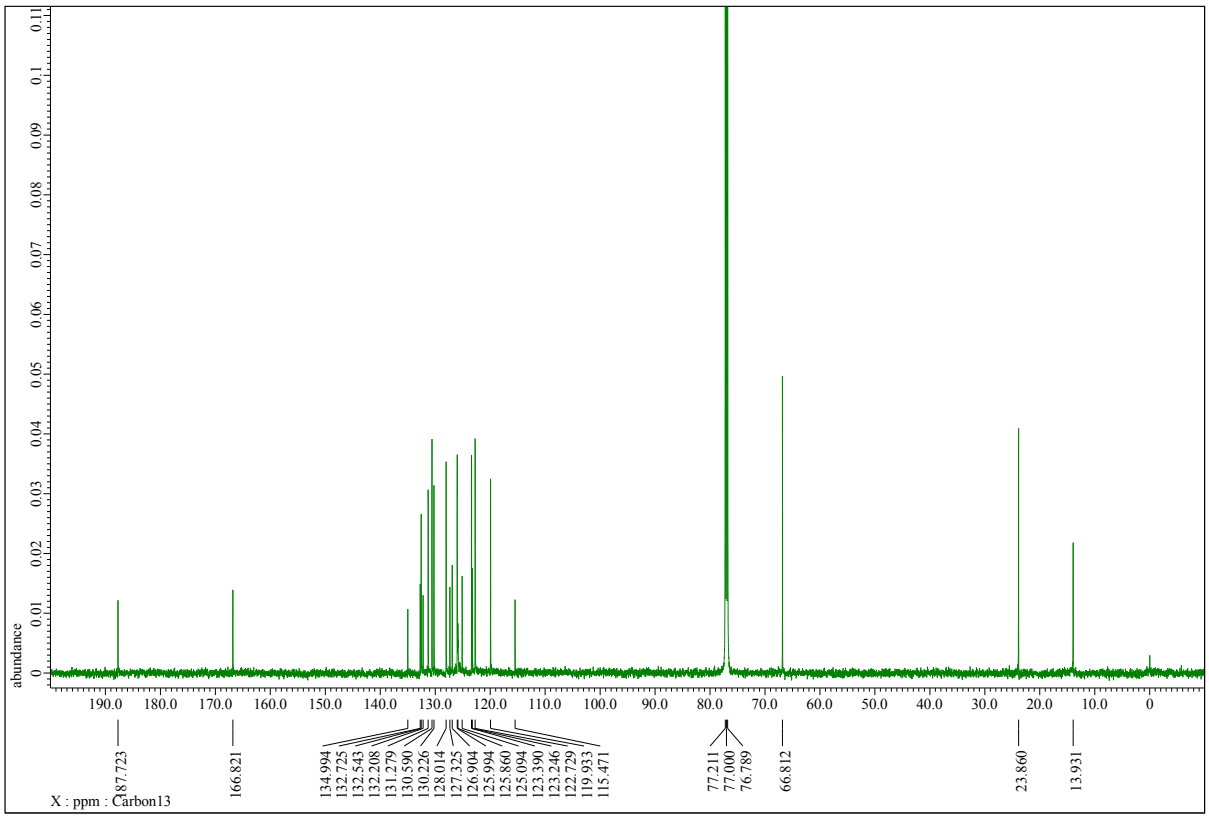
DL1a; ^{13}C NMR



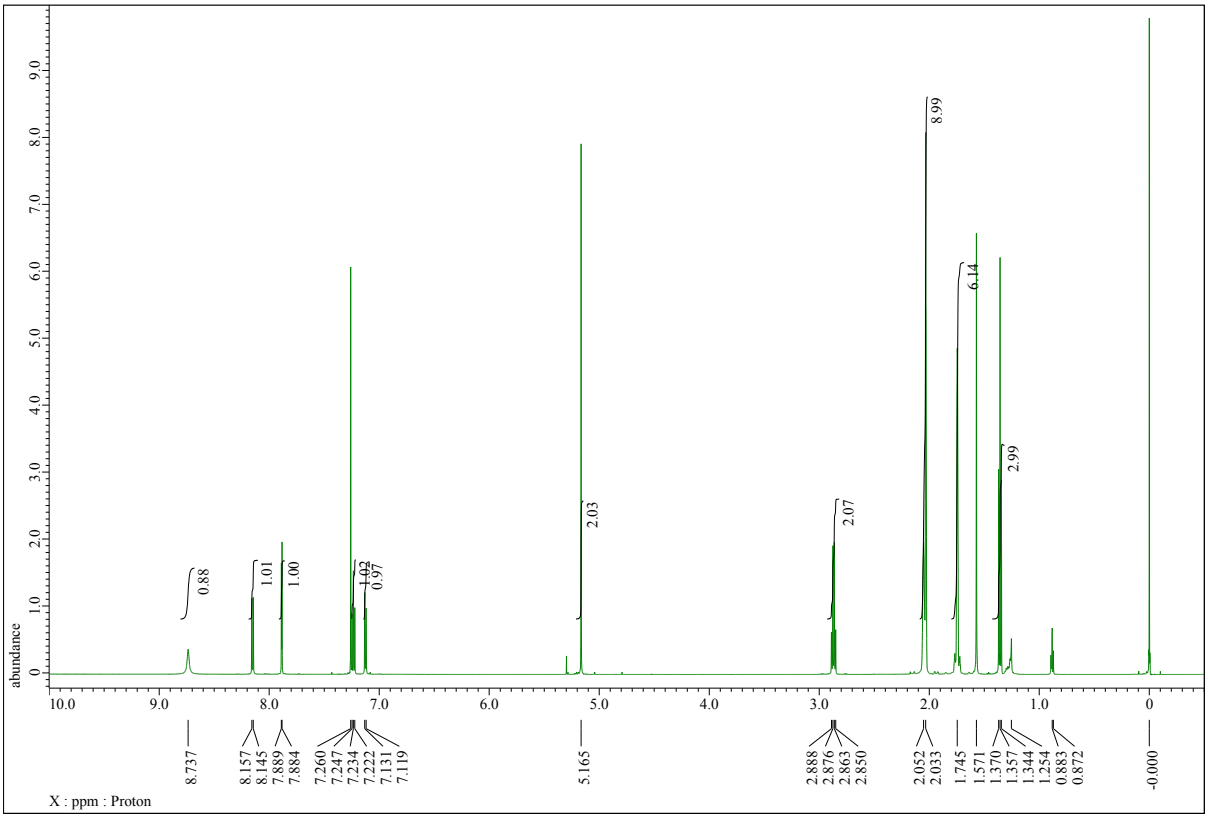
DL1b; ^{1}H NMR



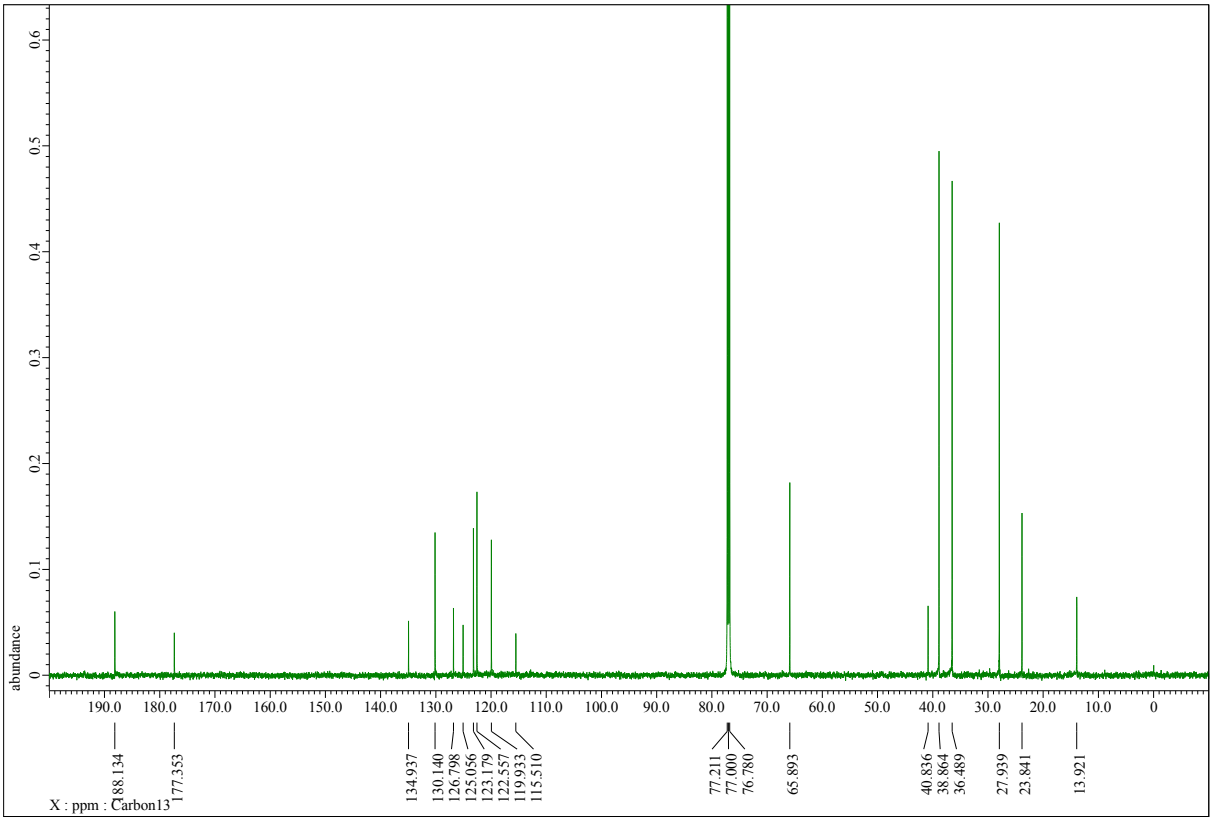
DL1b; ^{13}C NMR



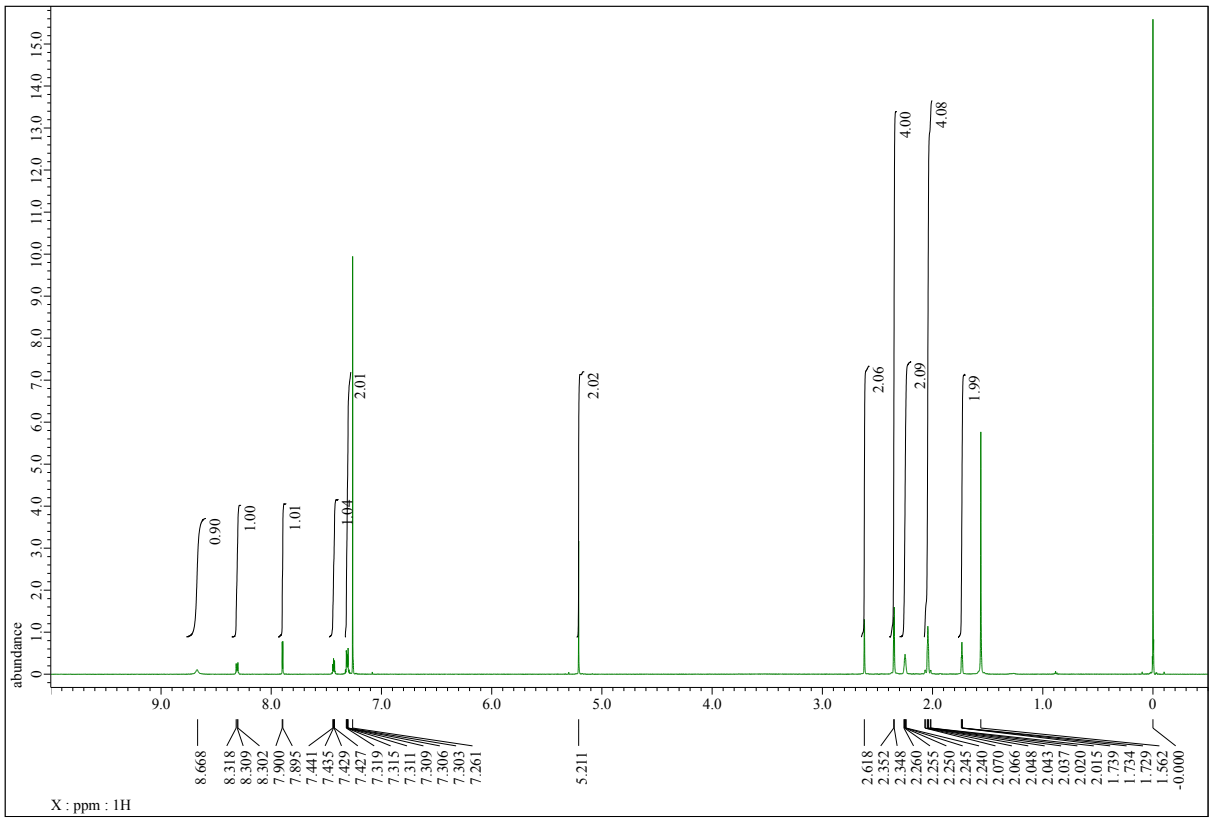
DL1c; ^{1}H NMR



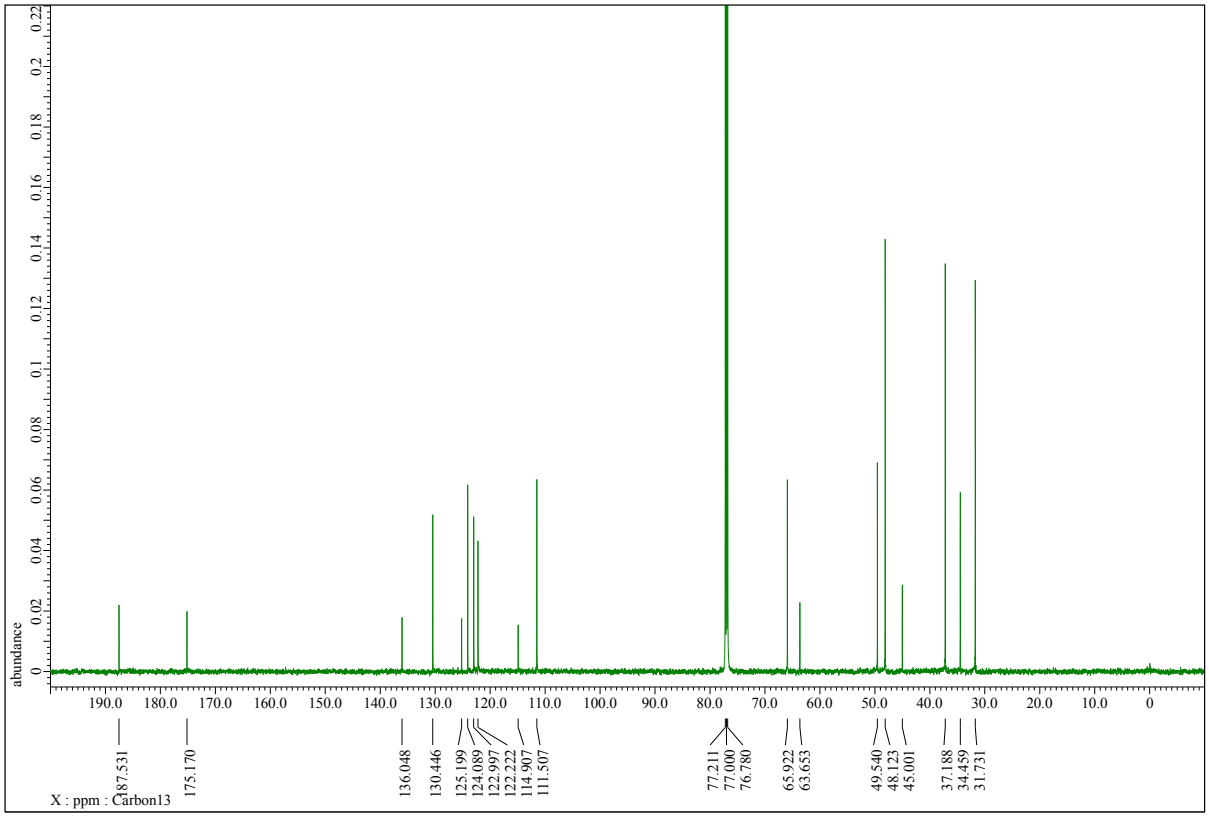
DL1c; ^{13}C NMR



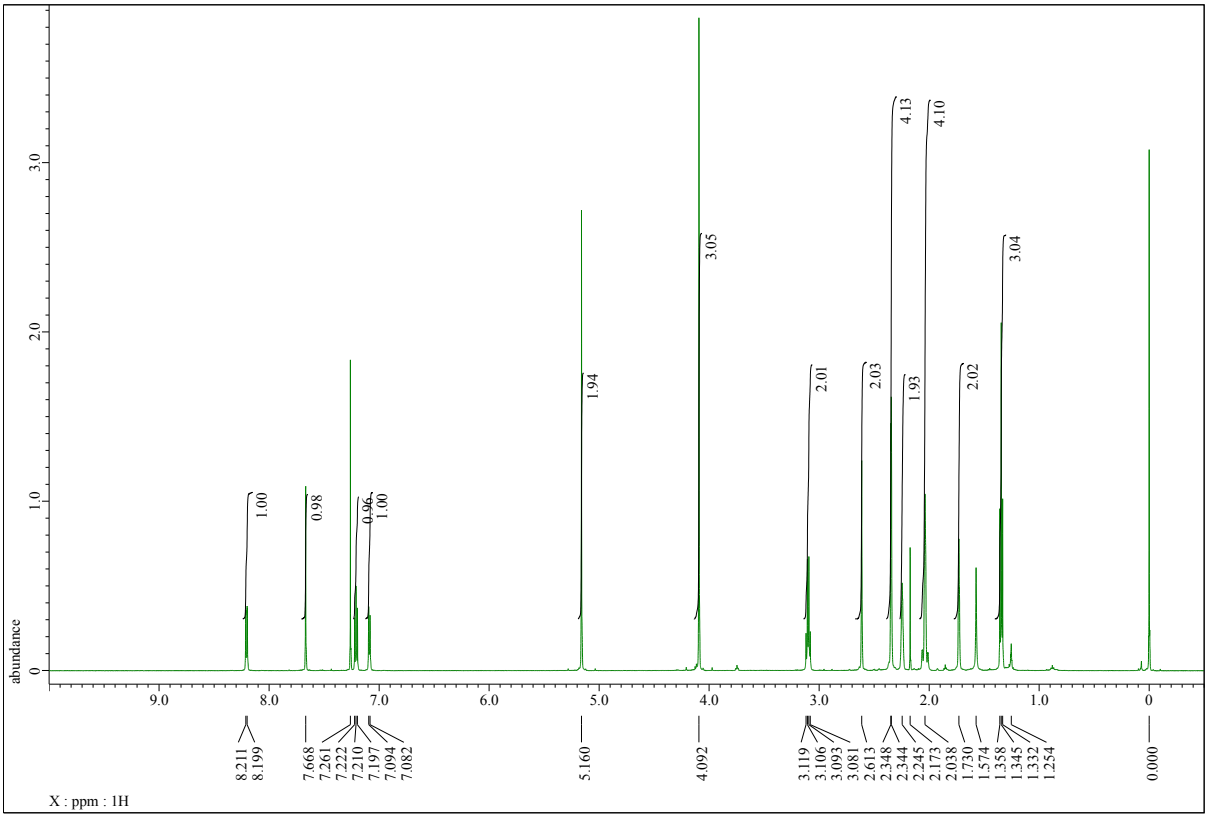
DL1d; ^{1}H NMR



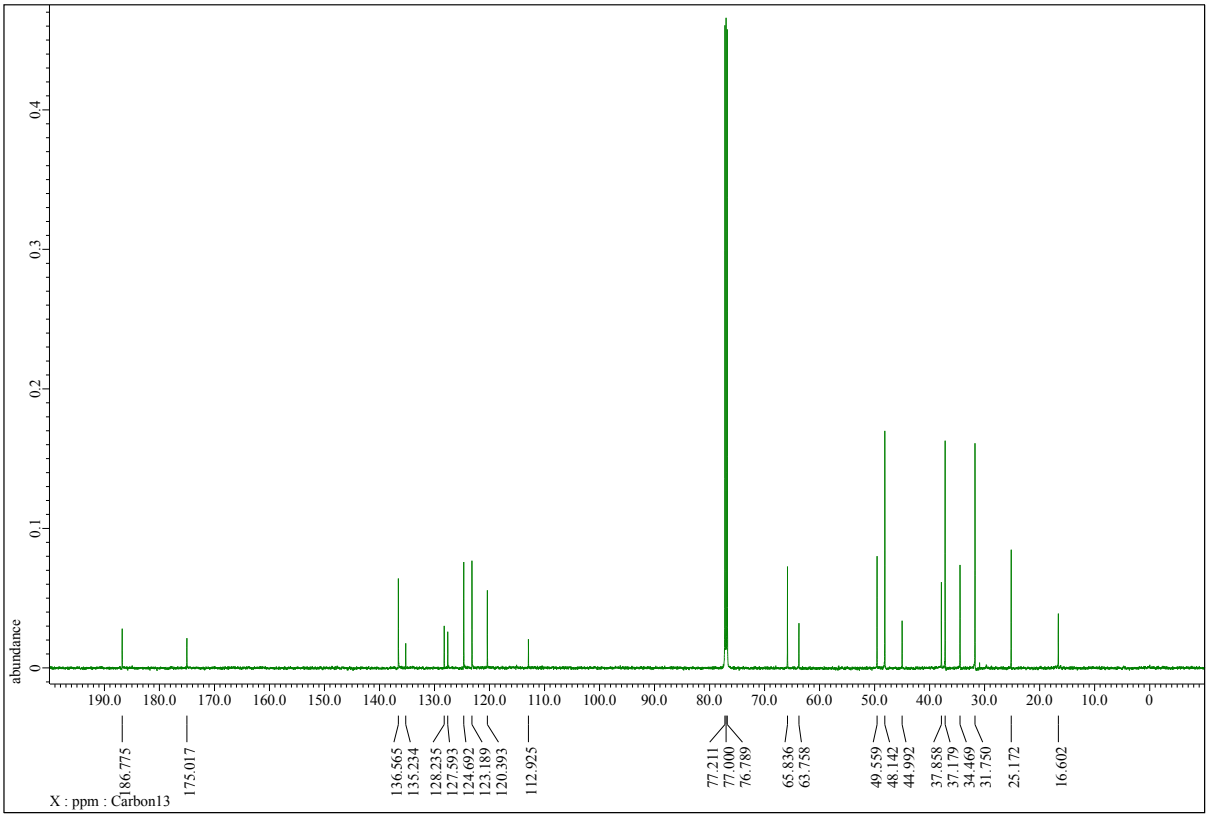
DL1d; ^{13}C NMR



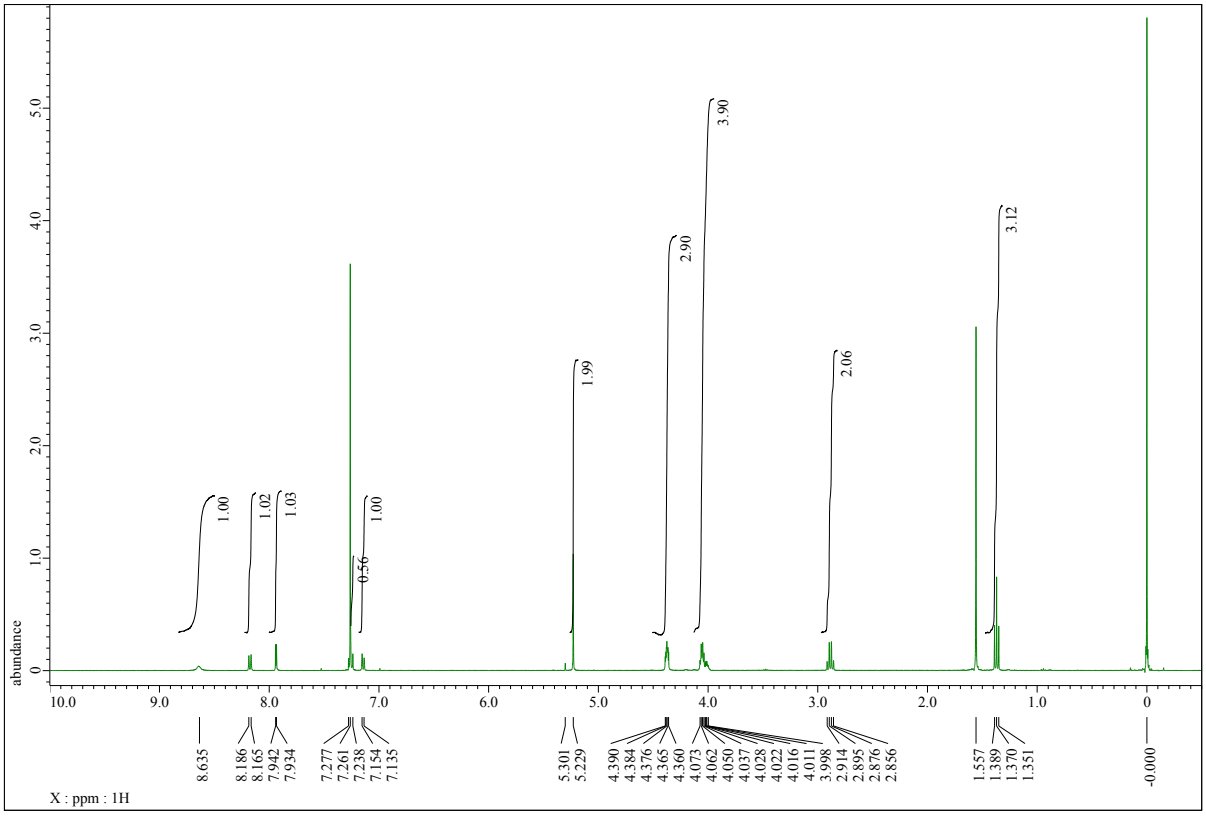
DL1e; ^{1}H NMR



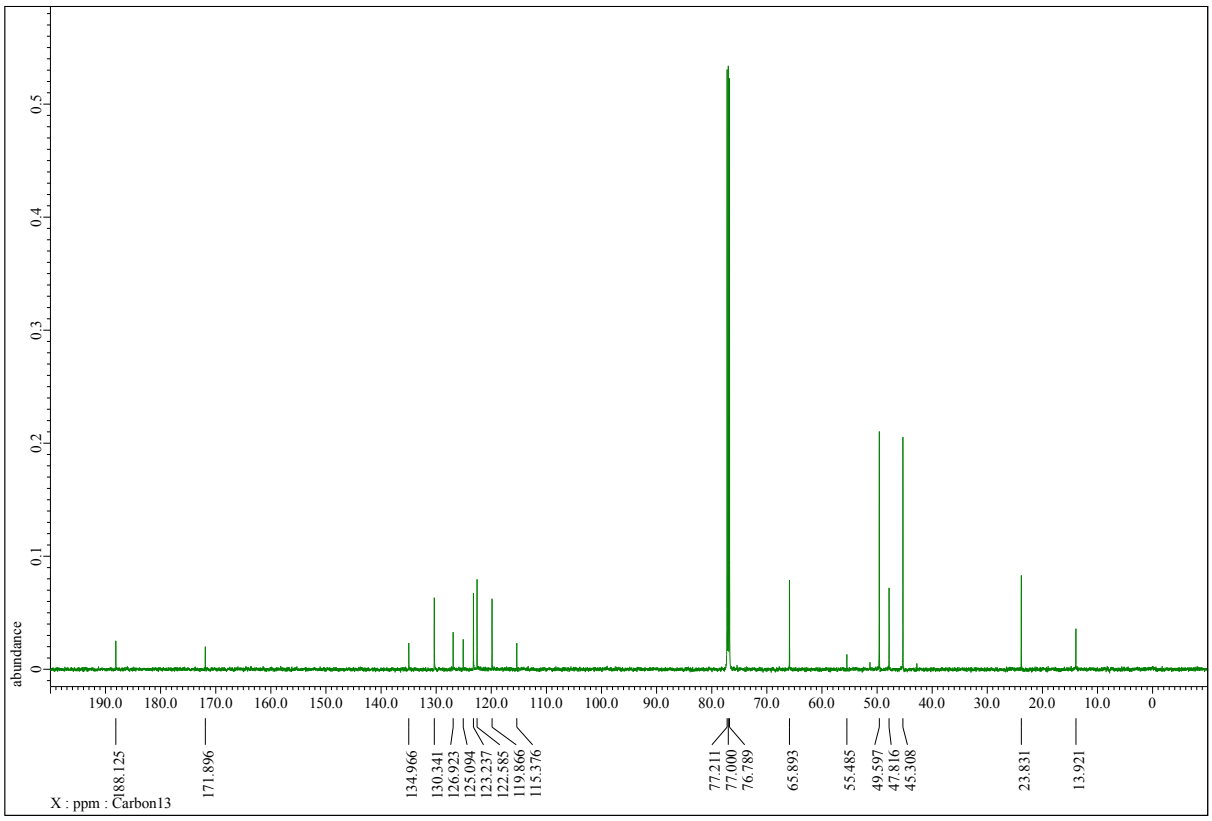
DL1e; ^{13}C NMR



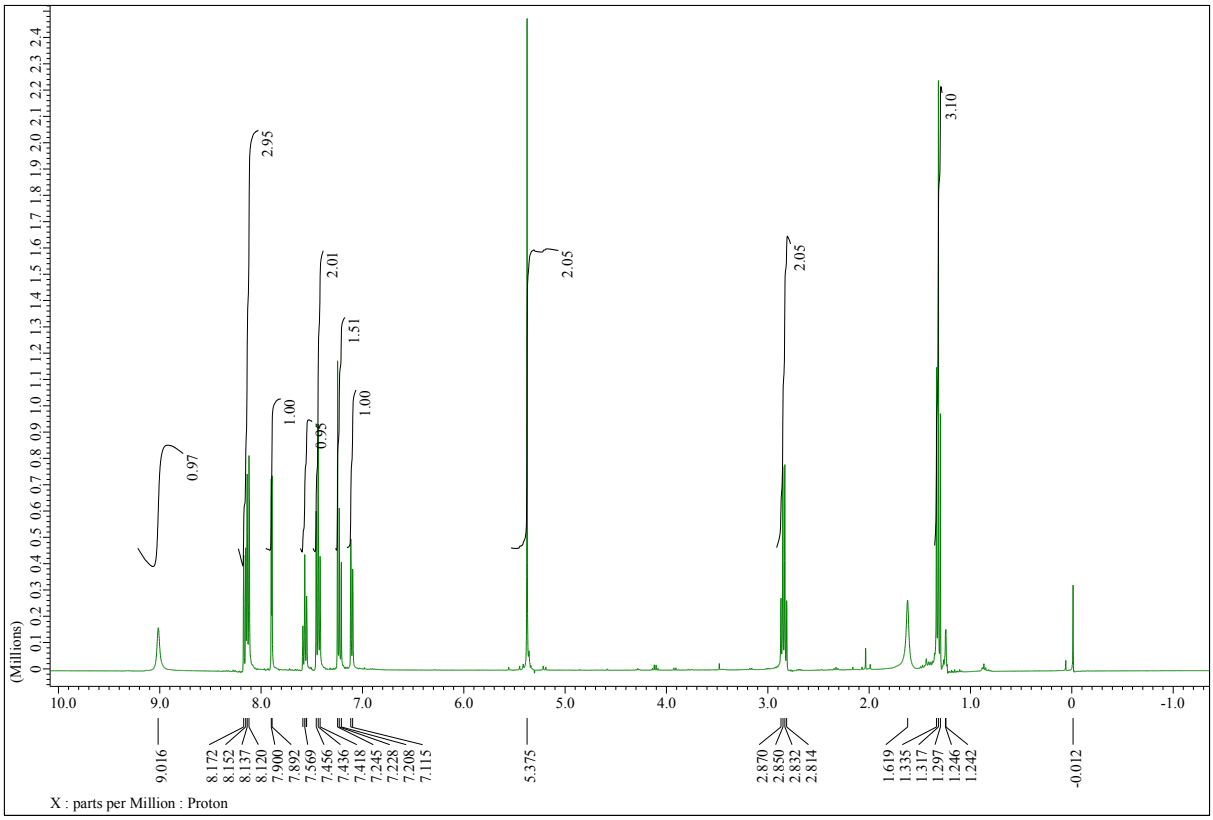
DL1f; ^{1}H NMR



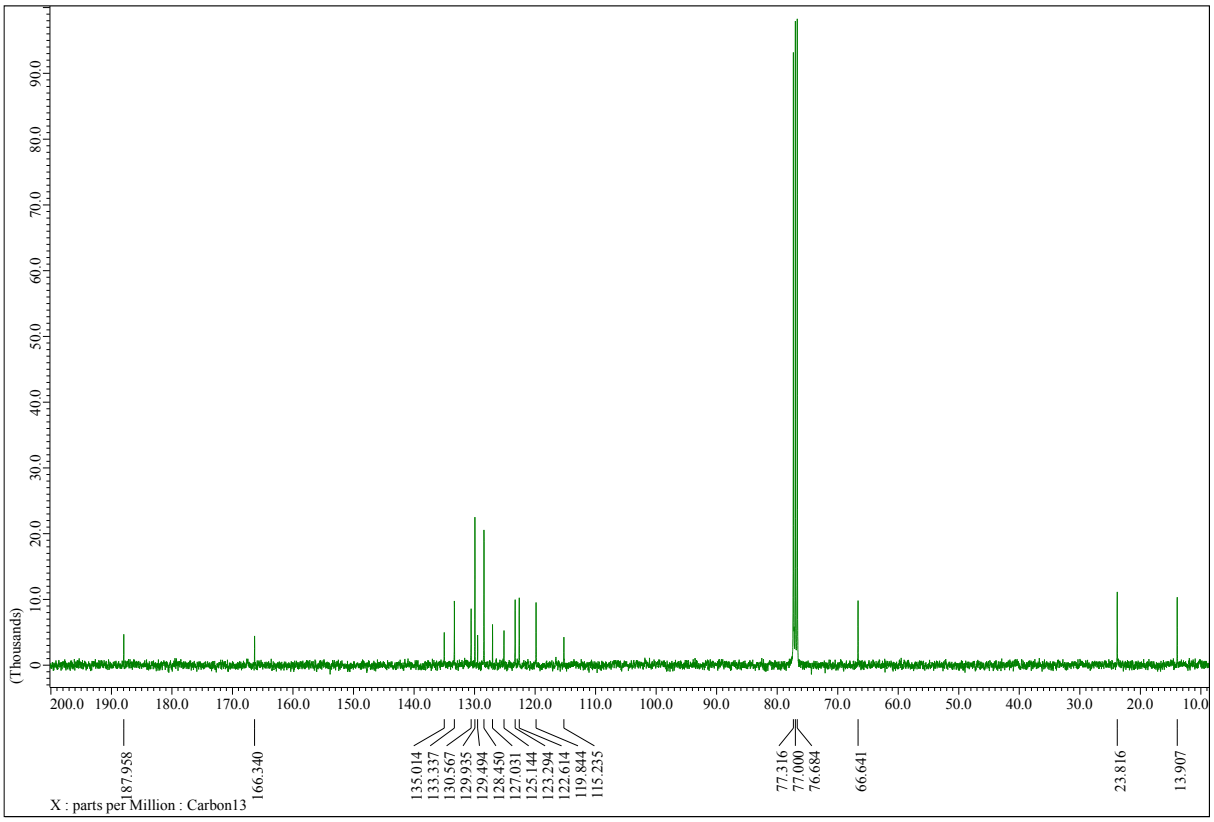
DL1f; ^{13}C NMR



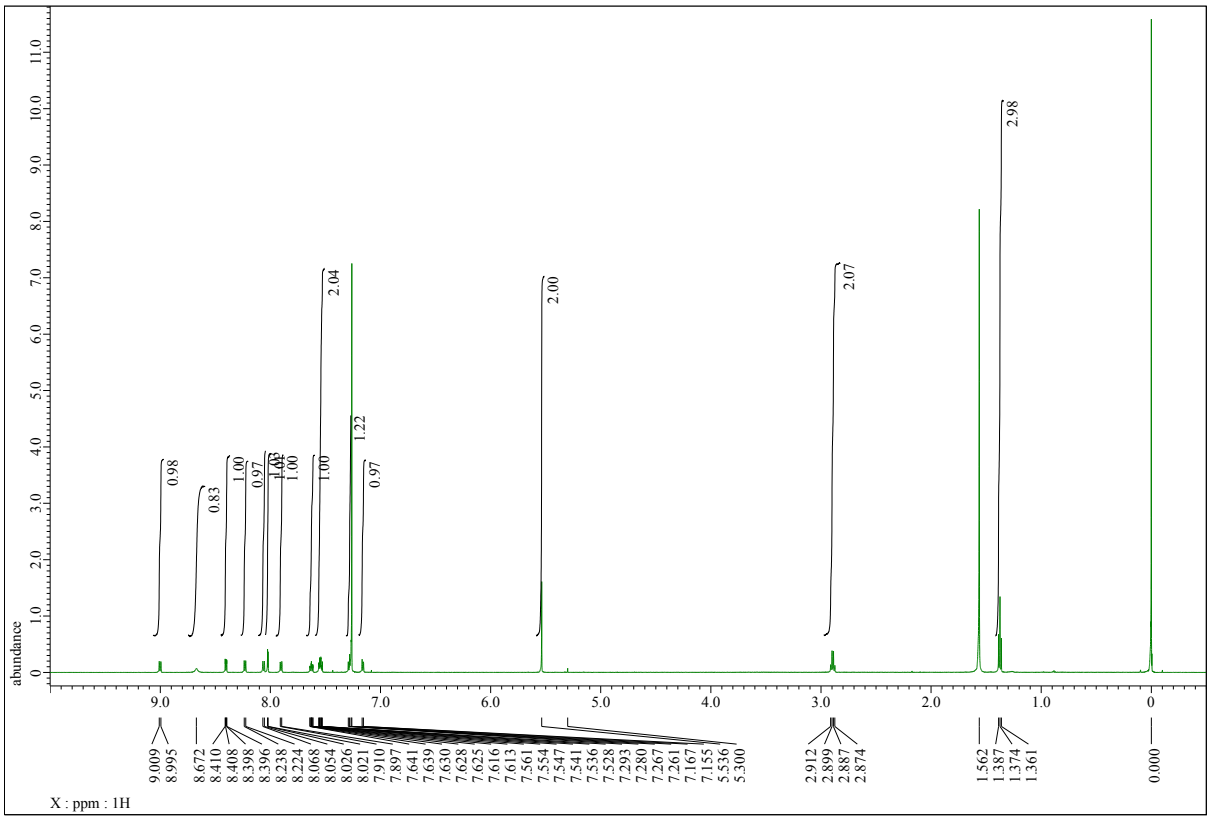
DL1g; ^{1}H NMR



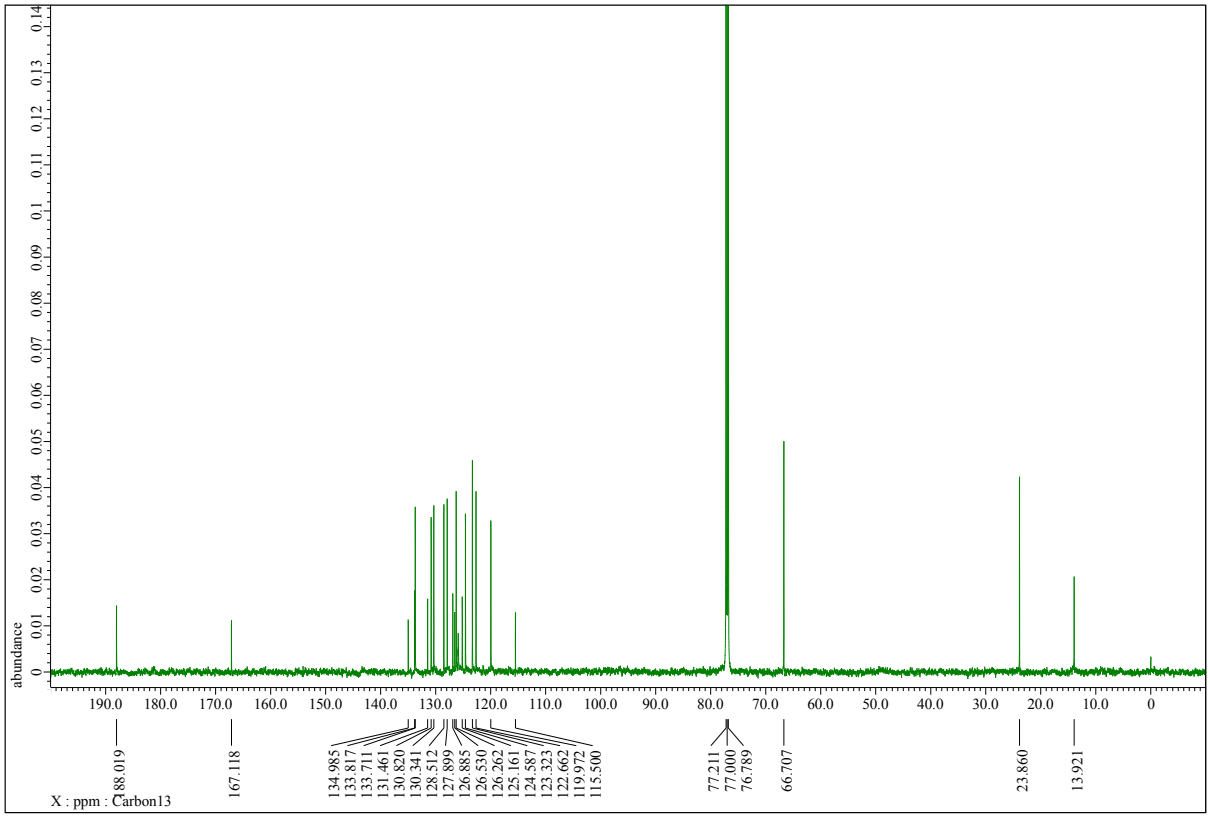
DL1g; ^{13}C NMR



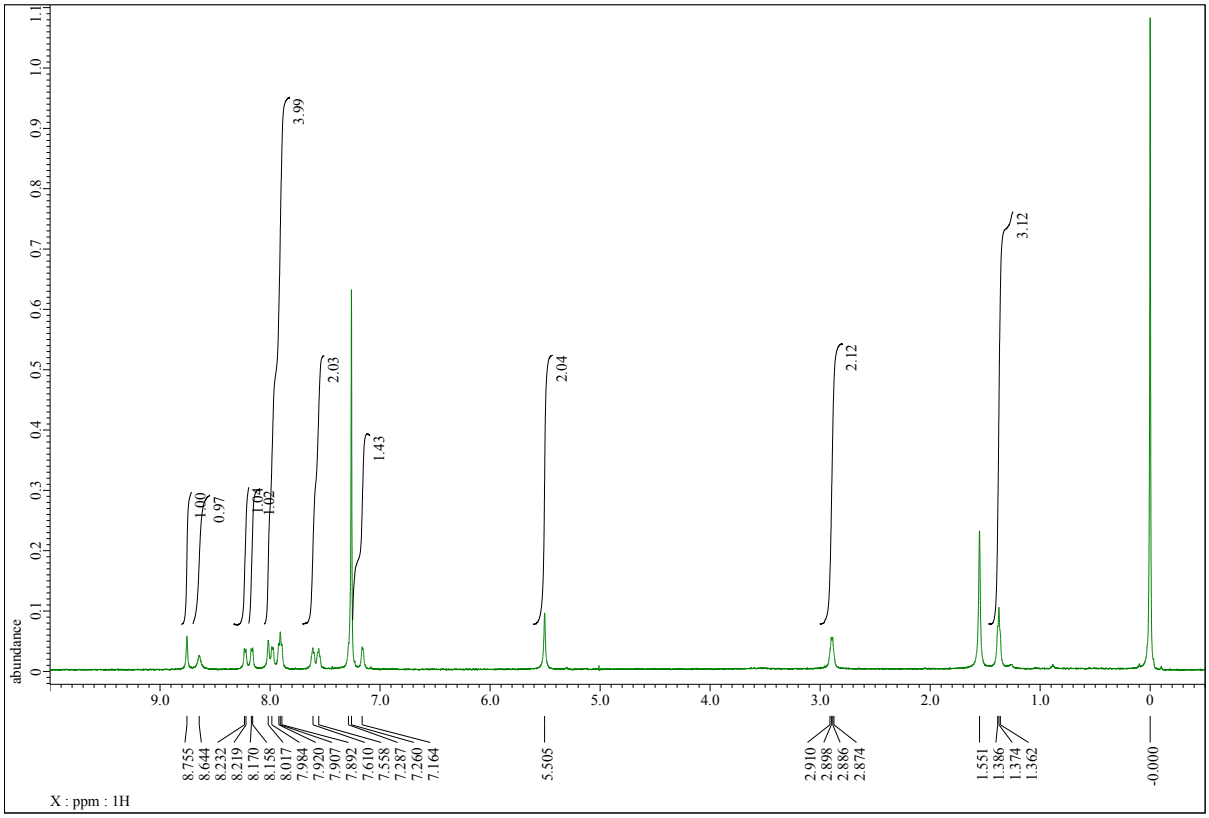
DL1h; ^{1}H NMR



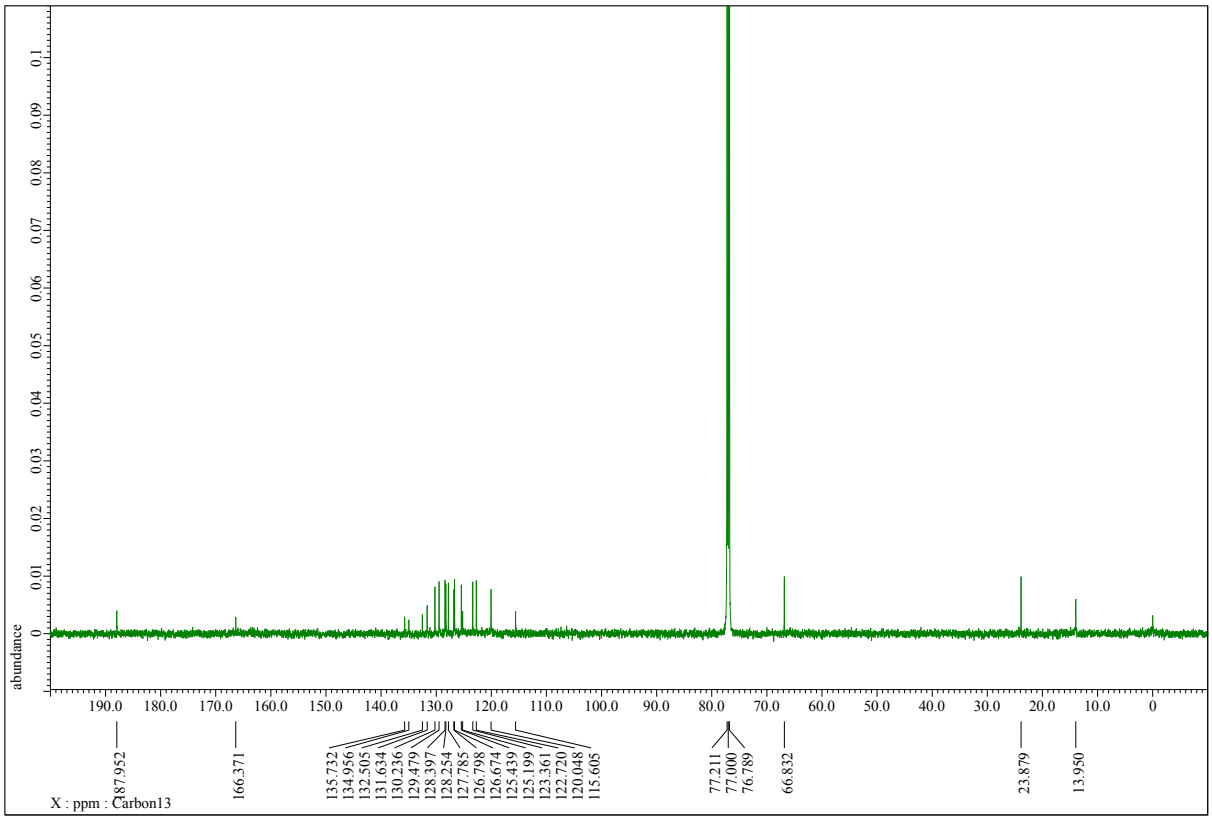
DL1h; ^{13}C NMR



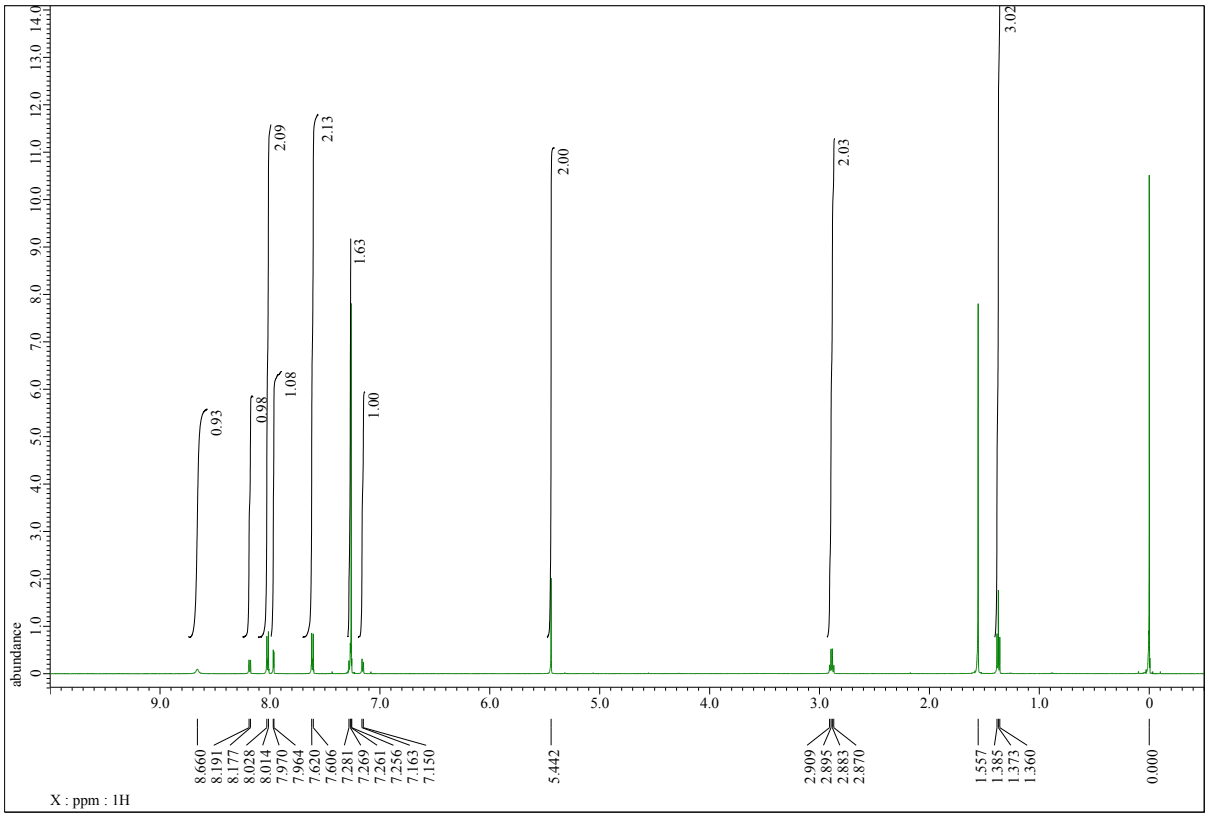
DL1i; ^{1}H NMR



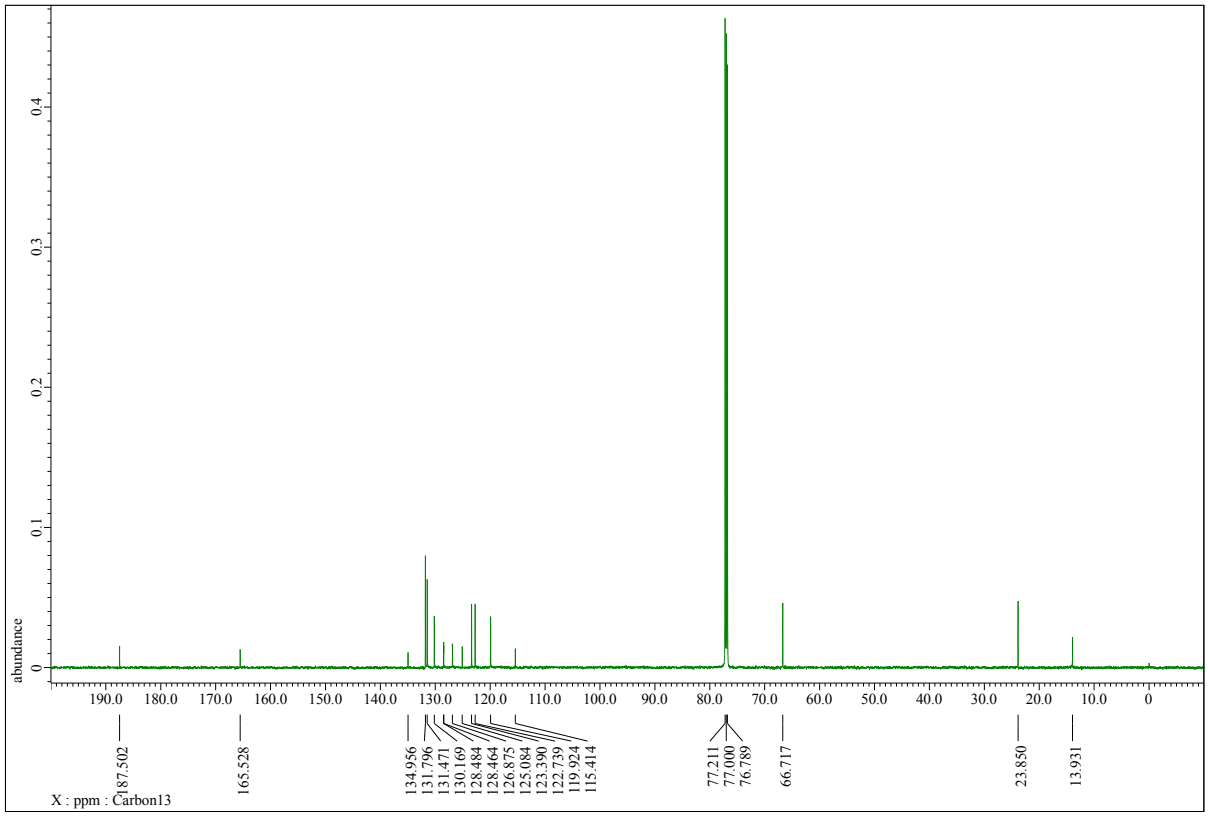
DL1i; ^{13}C NMR



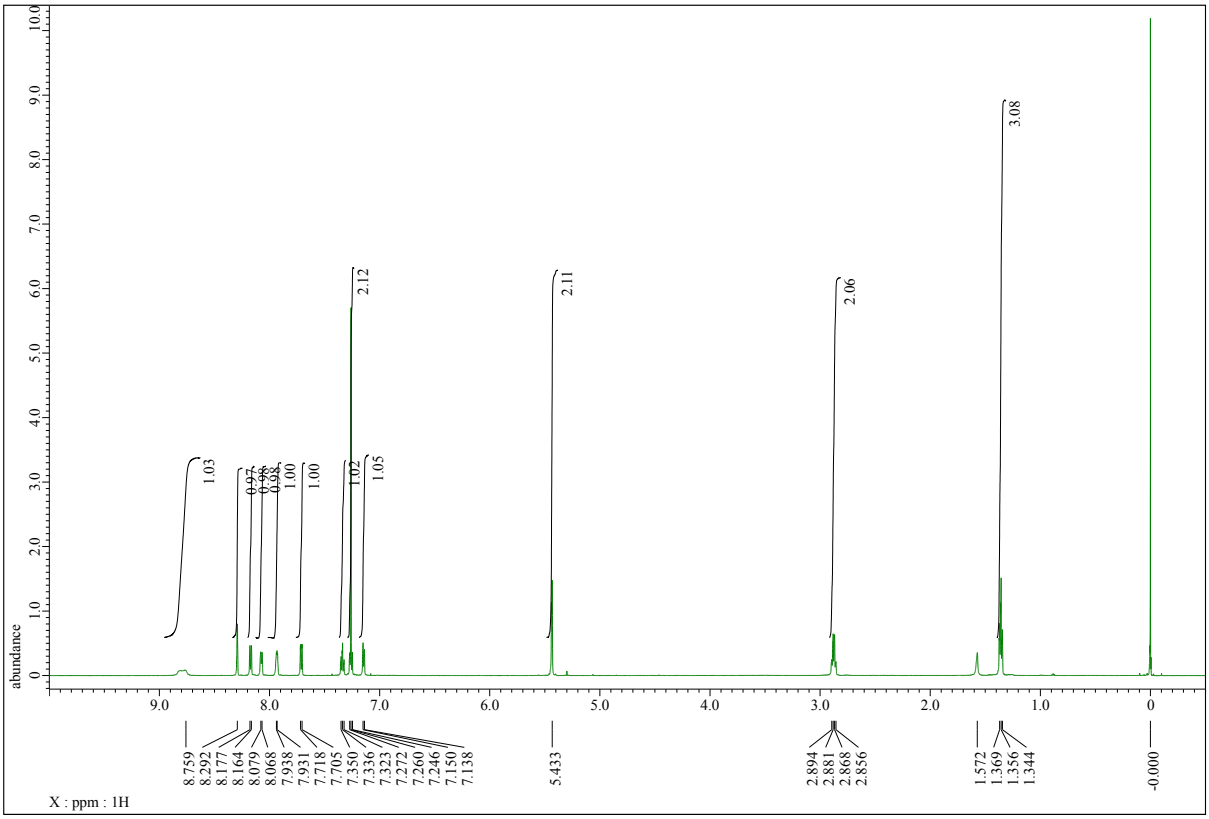
DL1j; ^{1}H NMR



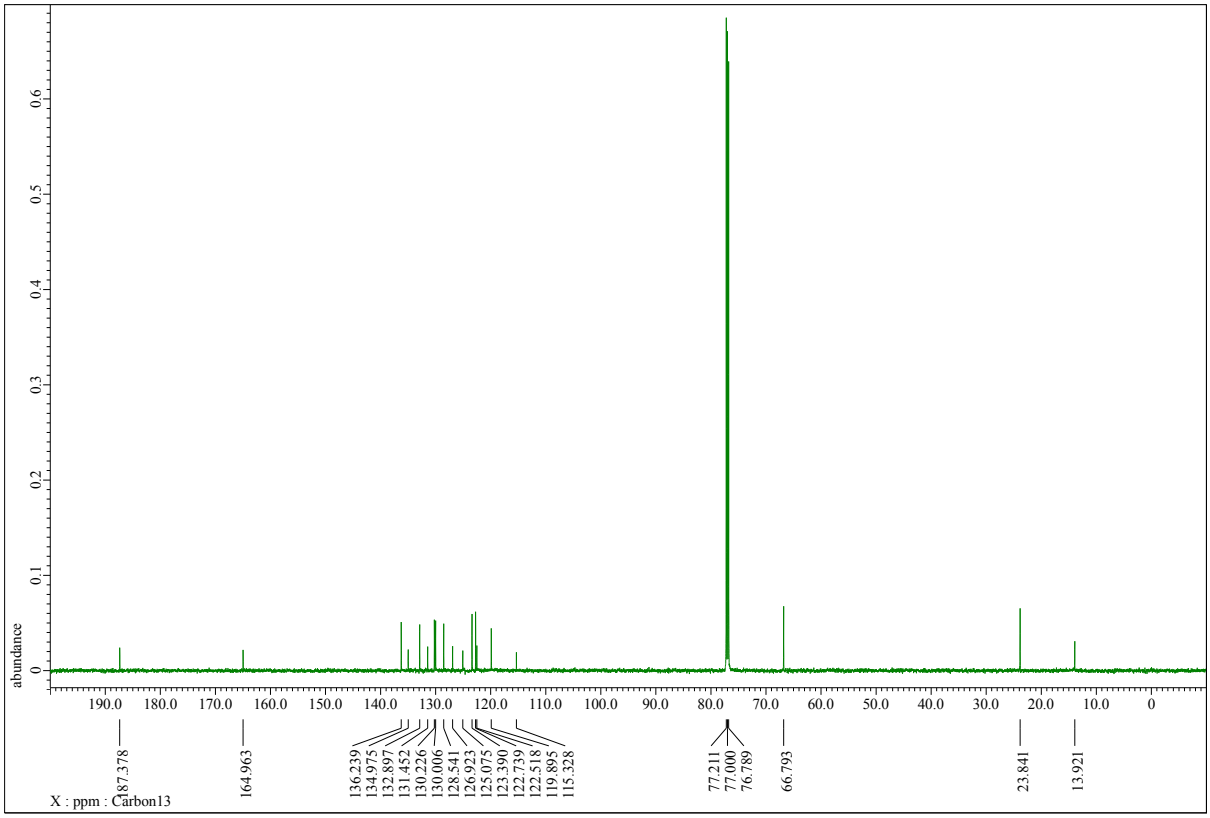
DL1j; ^{13}C NMR



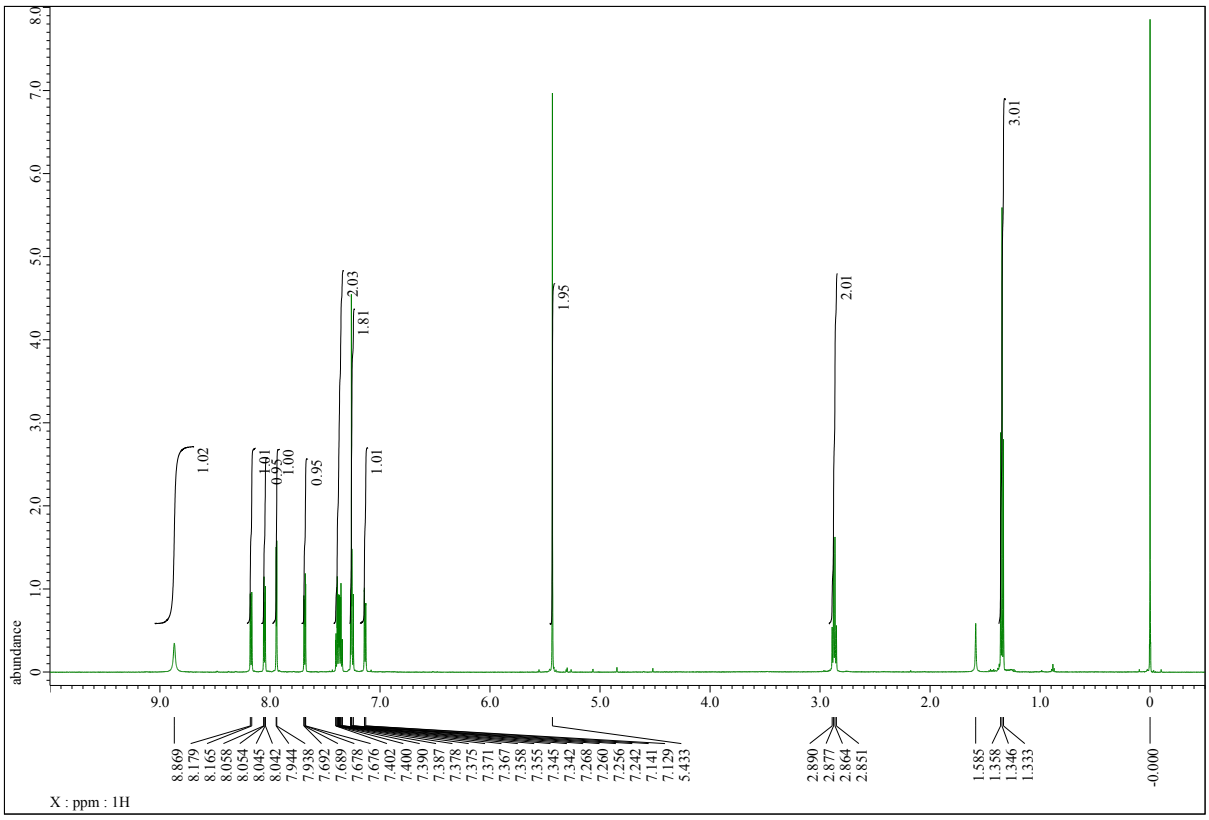
DL1k; ^{1}H NMR



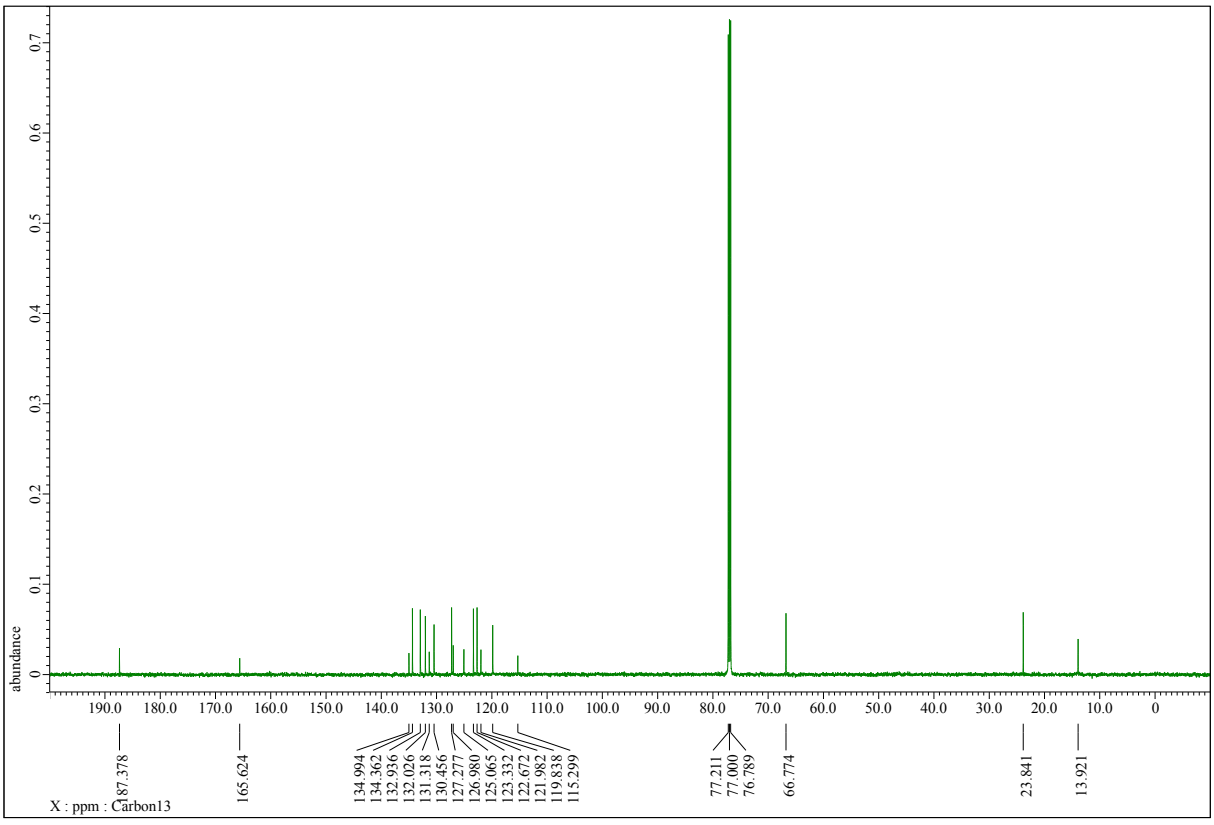
DL1k; ^{13}C NMR



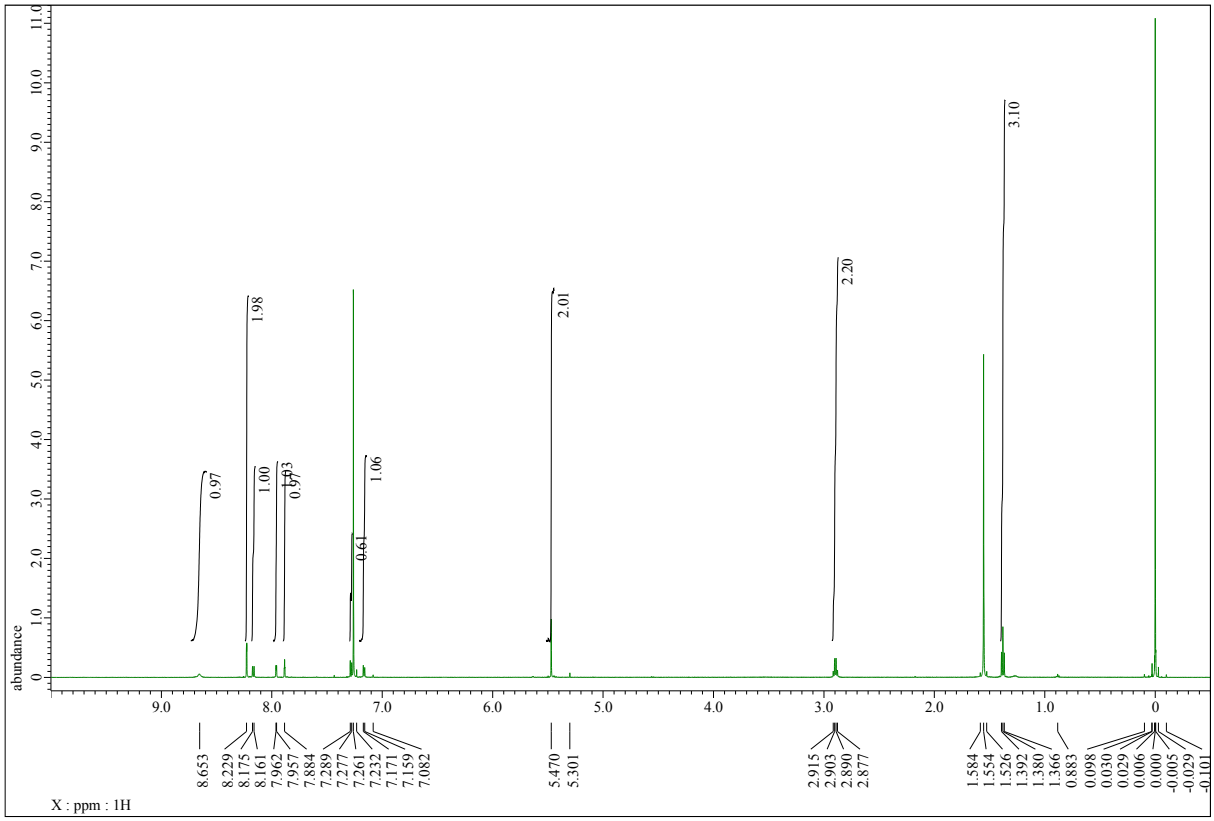
DL1l; ^{1}H NMR



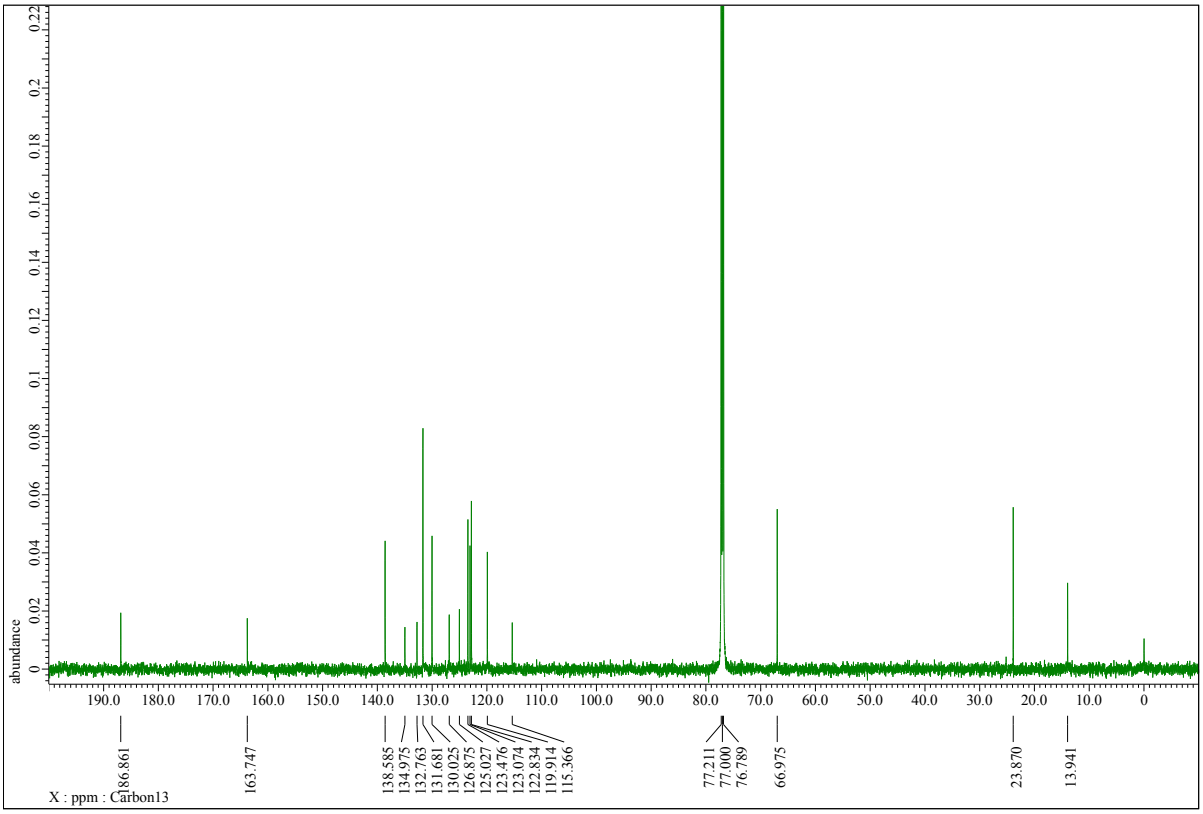
DL1l; ^{13}C NMR



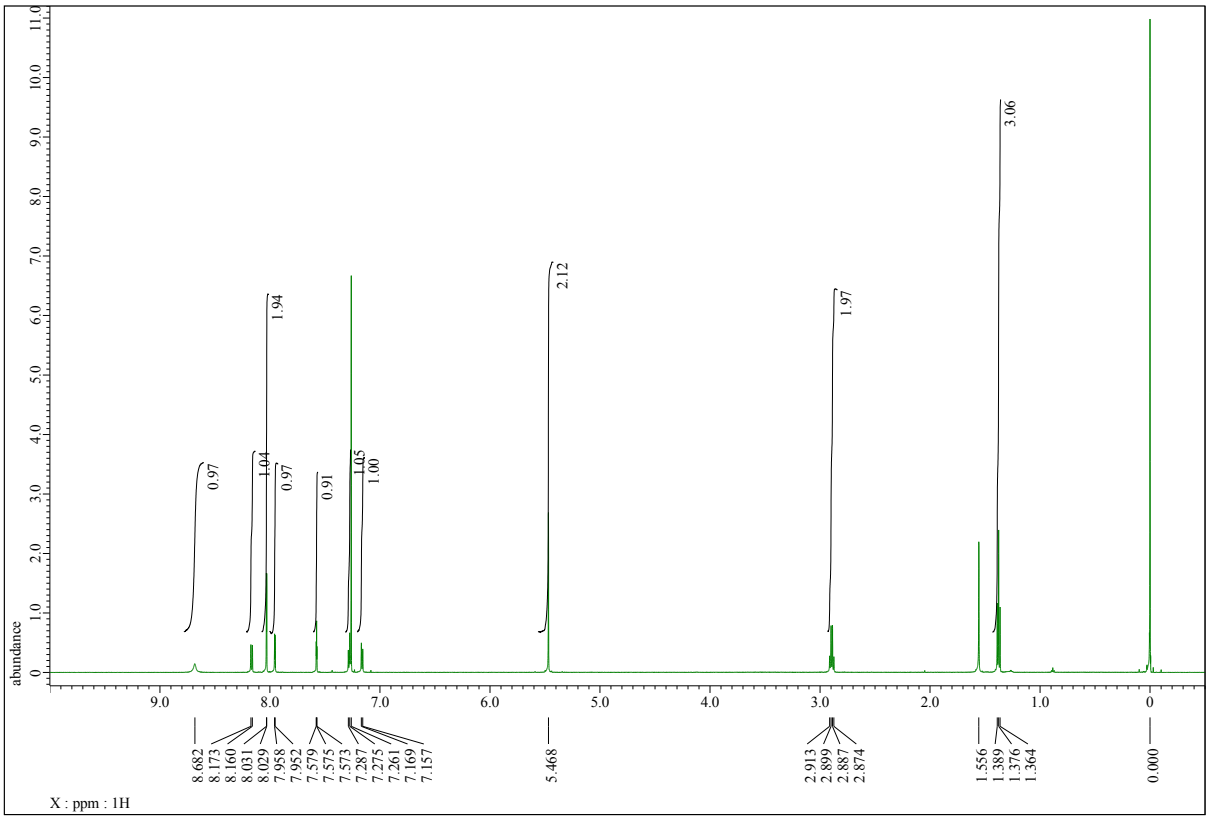
DL1m; ^{1}H NMR



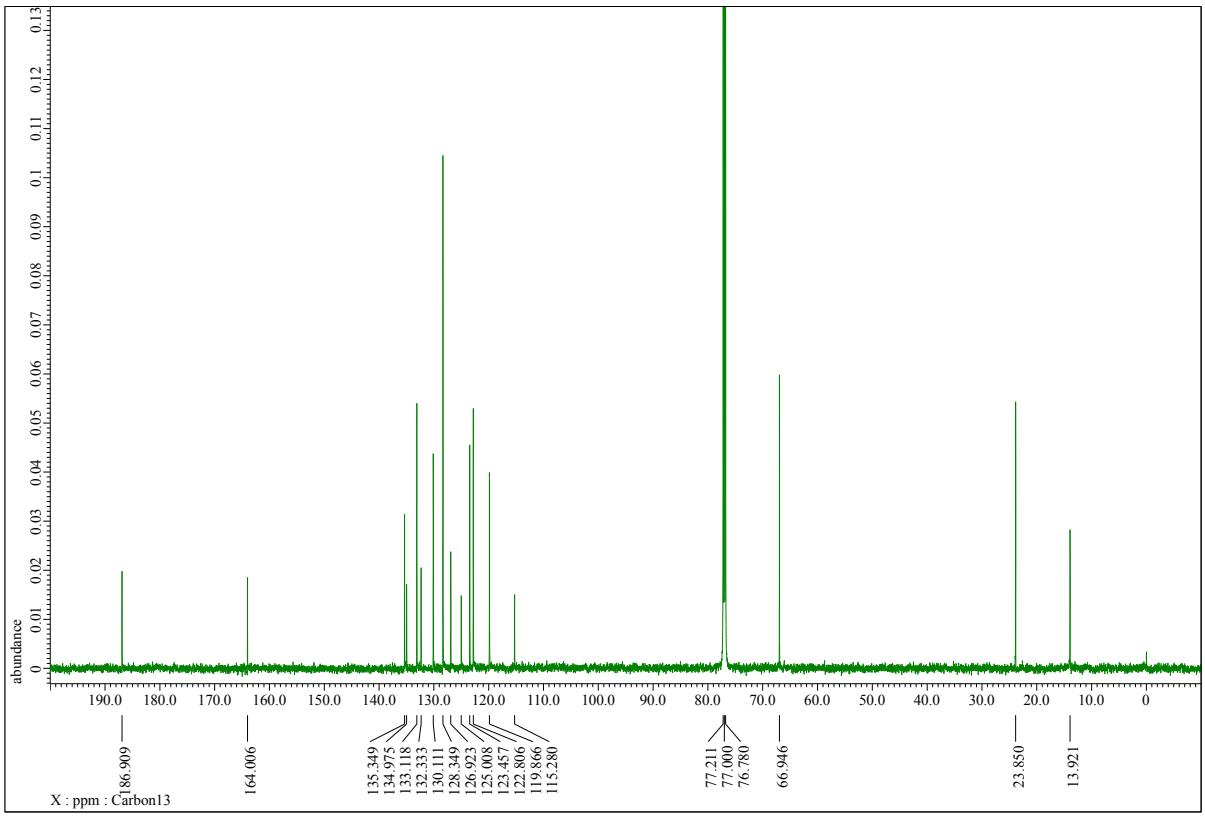
DL1m; ^{13}C NMR



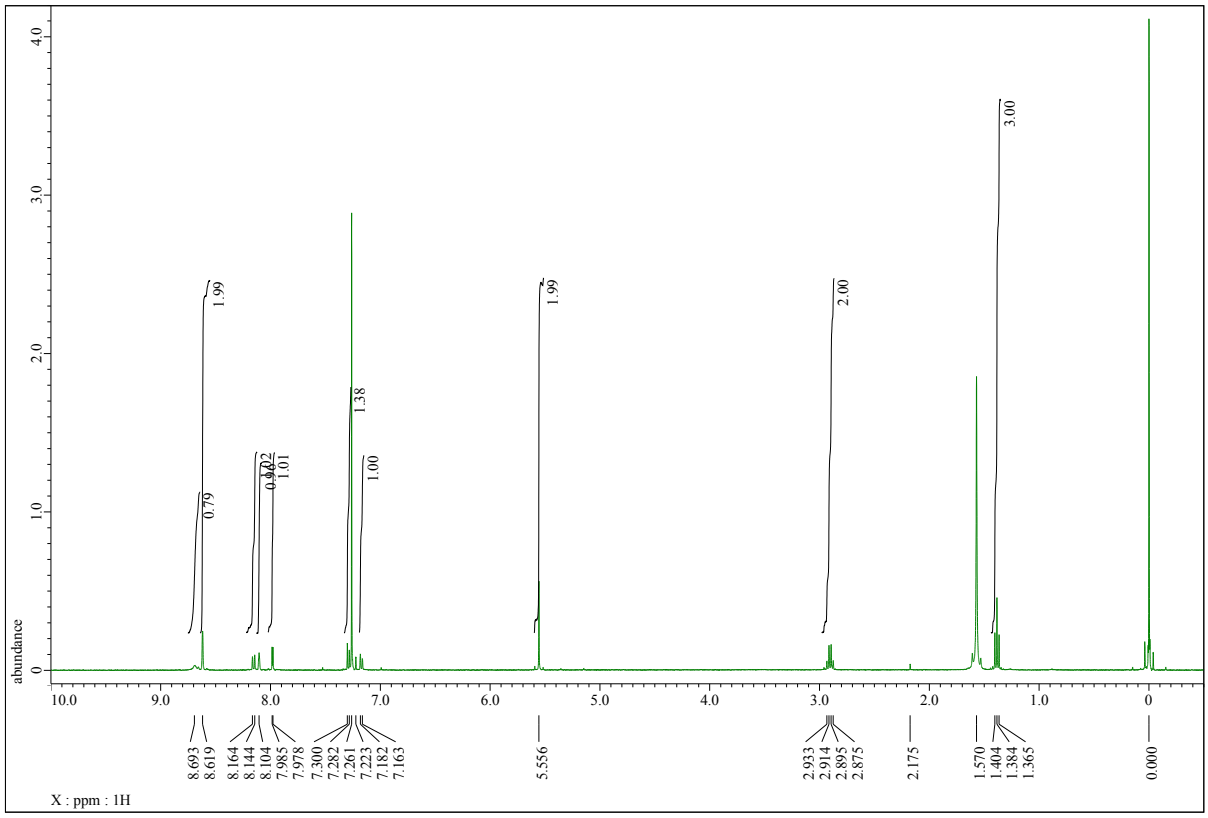
DL1n; ^{1}H NMR



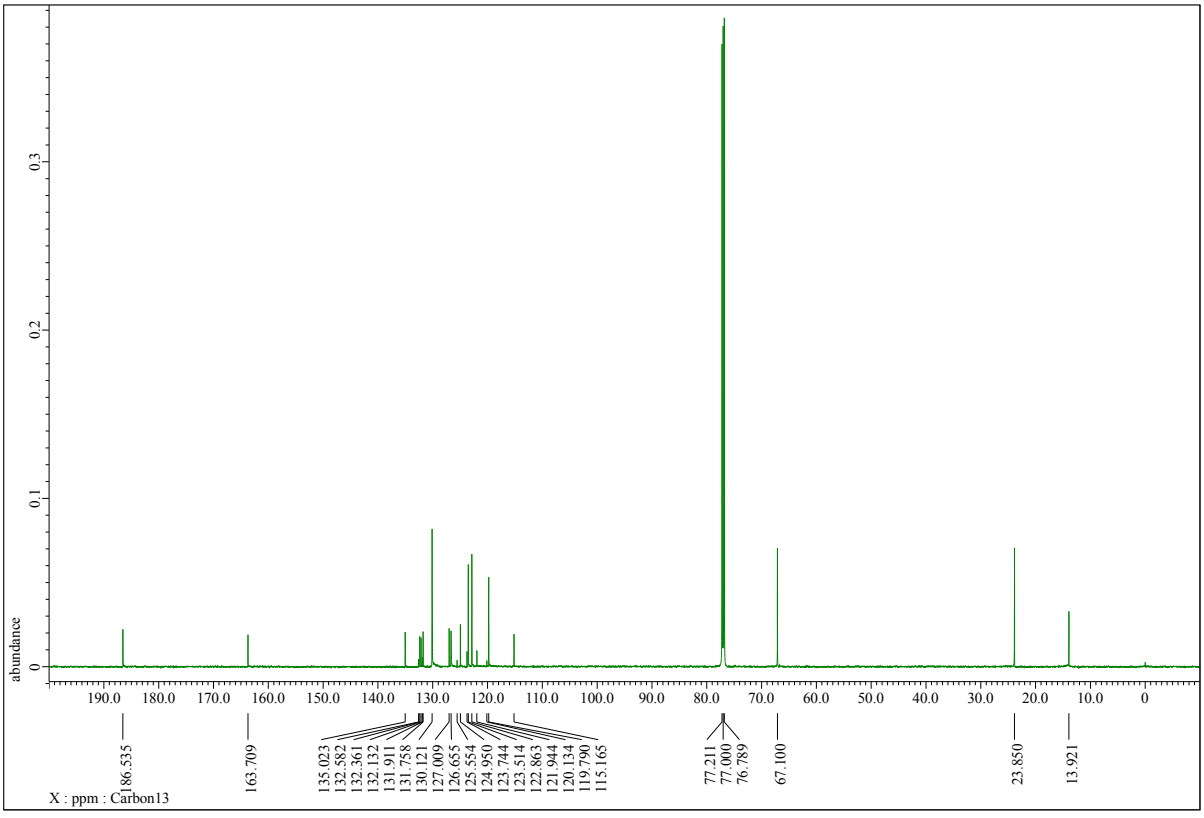
DL1n; ^{13}C NMR



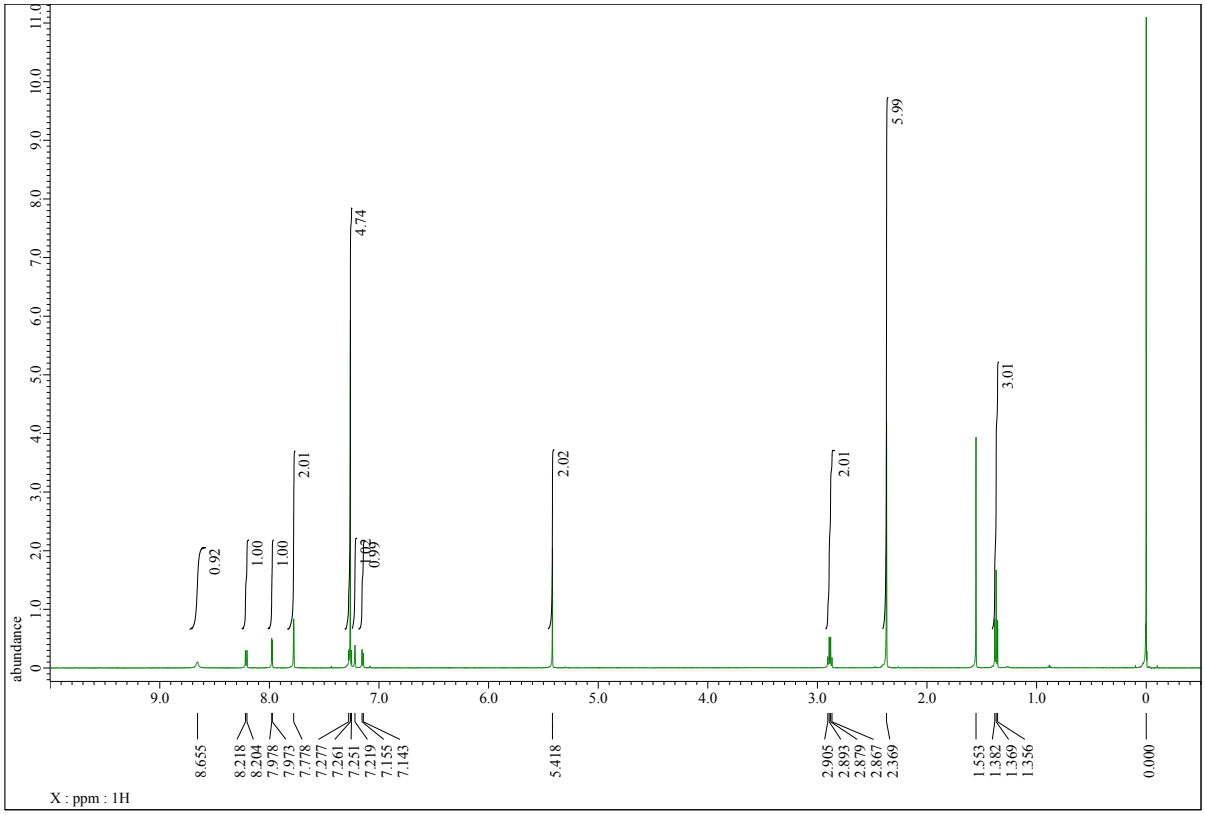
DL1o; ^{1}H NMR



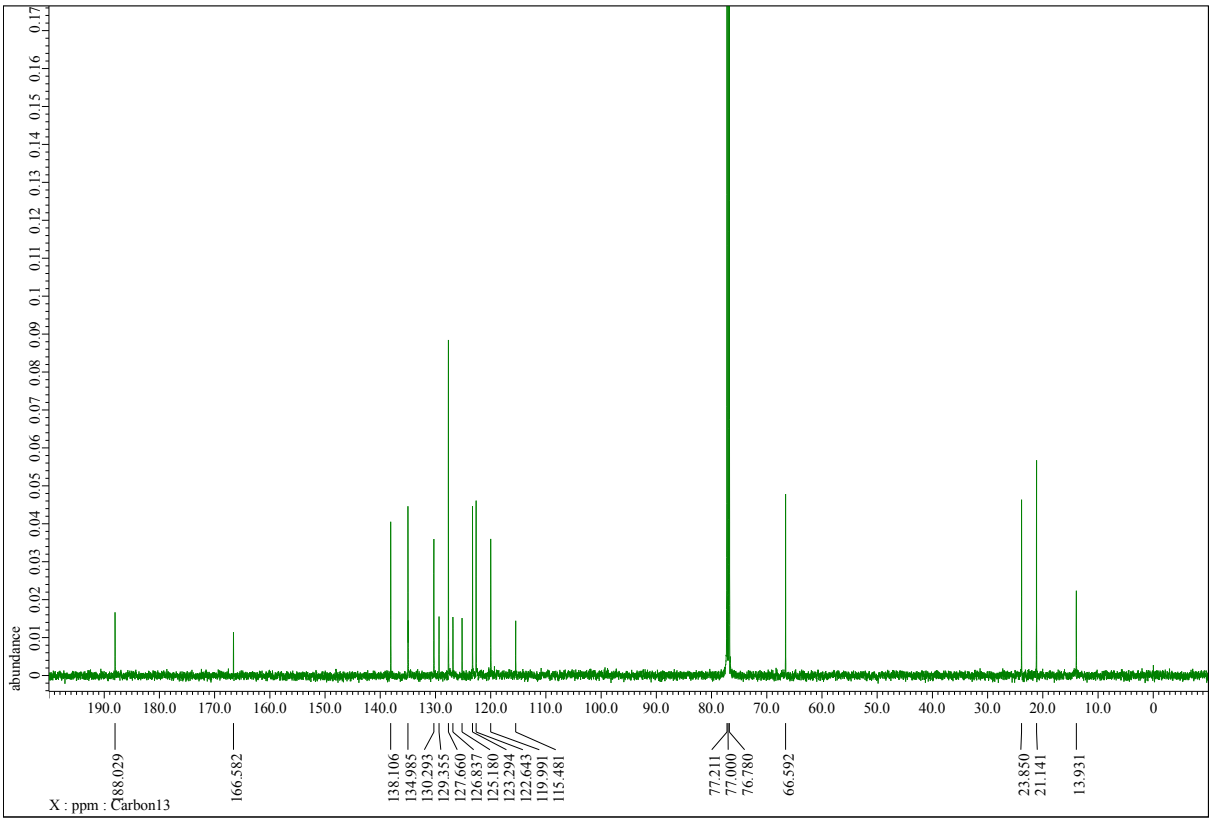
DL1o; ^{13}C NMR



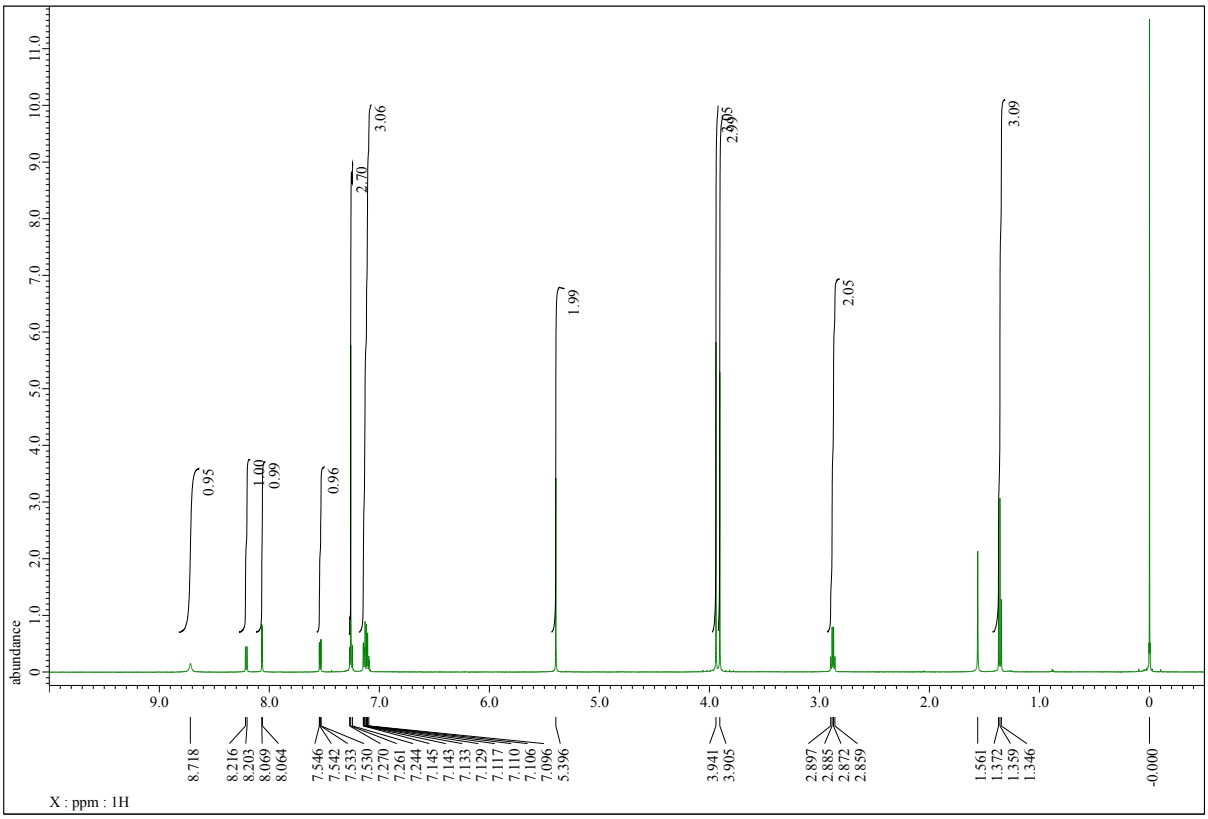
DL1p; ^{1}H NMR



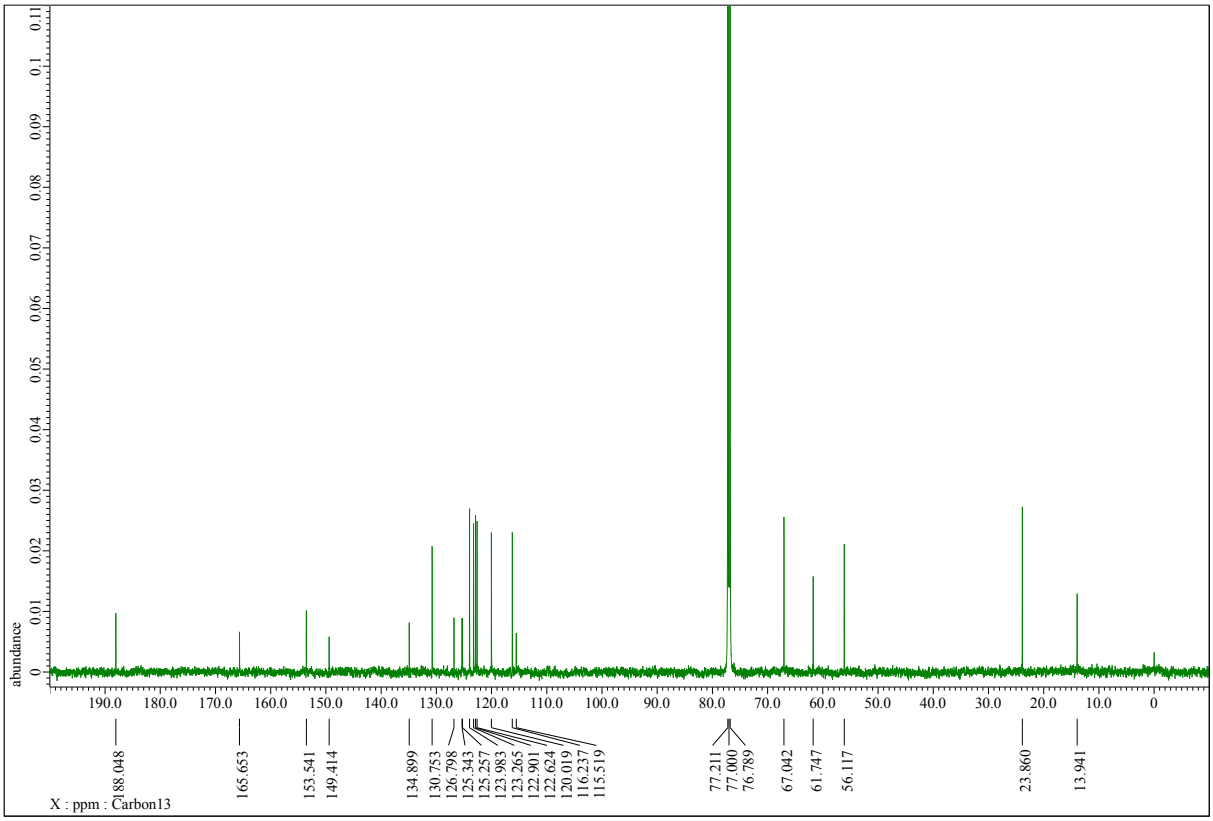
DL1p; ^{13}C NMR



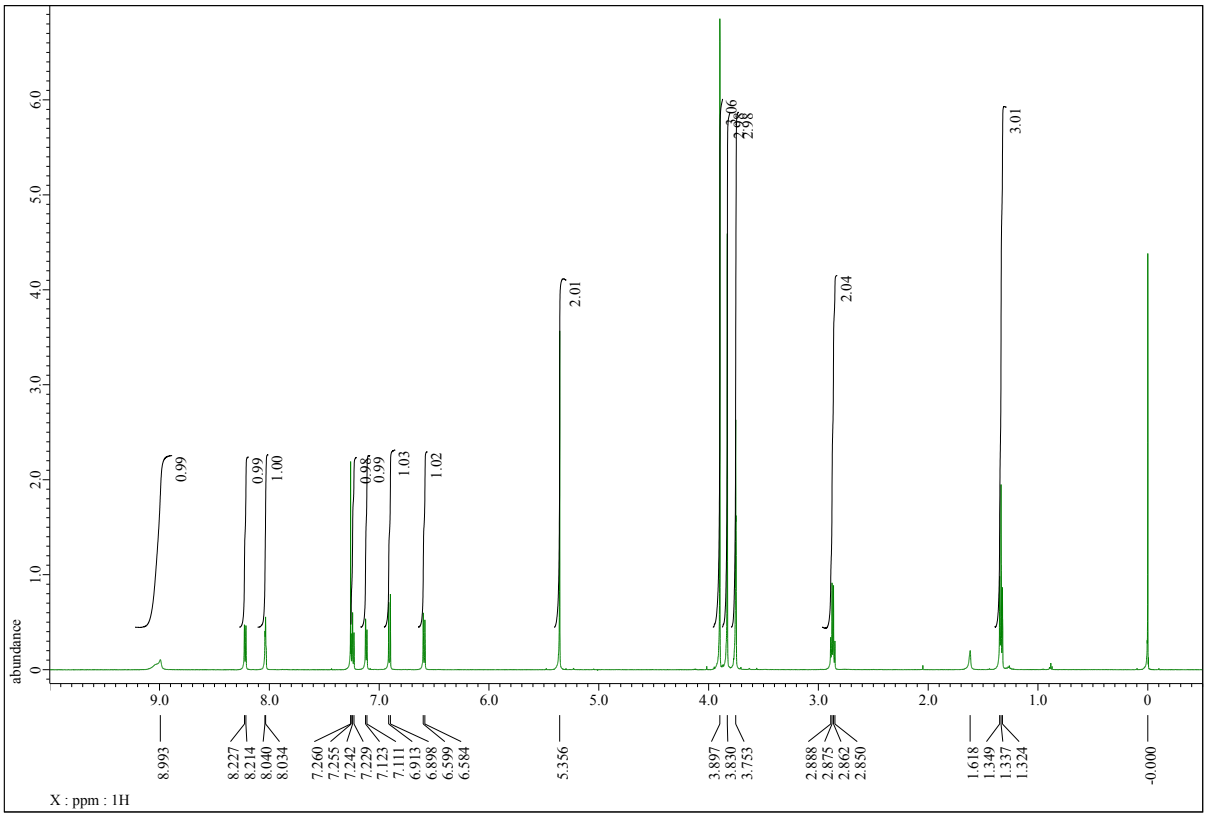
DL1q; ^{1}H NMR



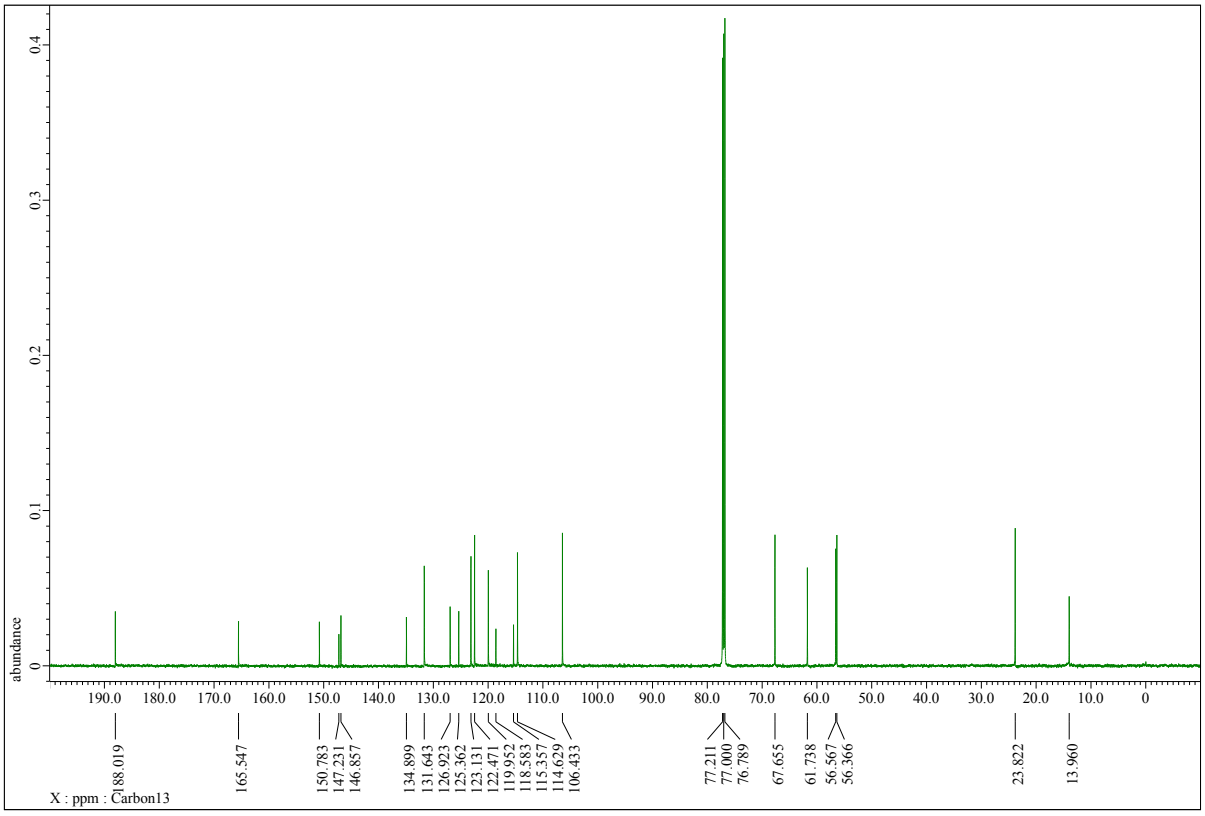
DL1q; ^{13}C NMR



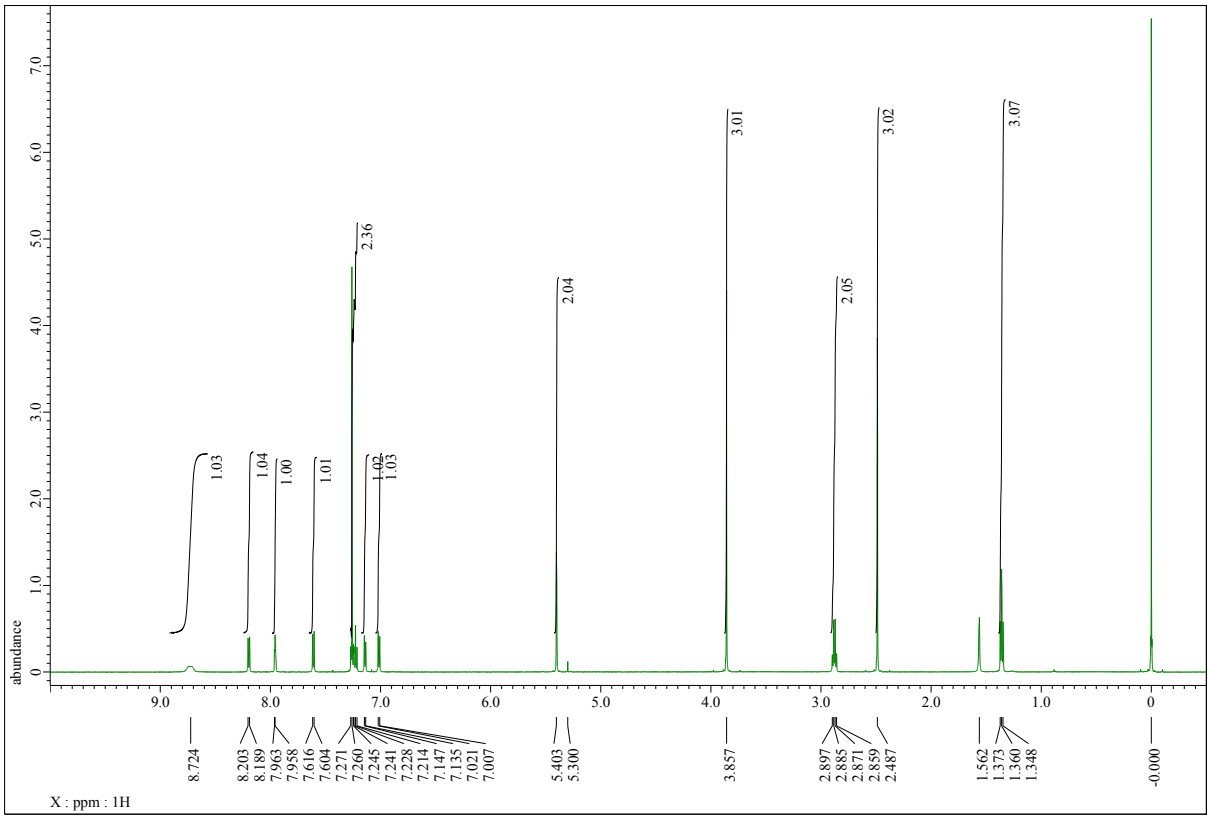
DL1r; ^{1}H NMR



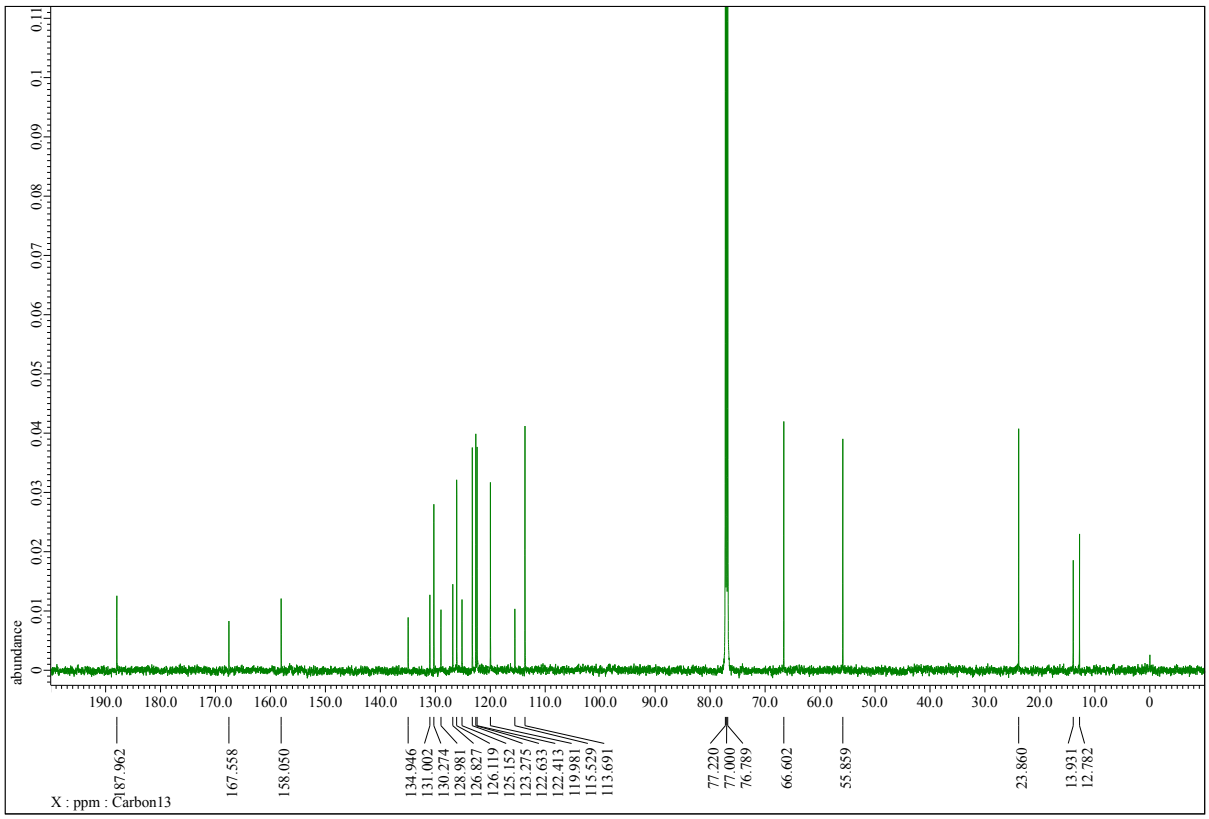
DL1r; ^{13}C NMR



DL1s; ^{1}H NMR



DL1s; ^{13}C NMR



6. Supplementary Figures

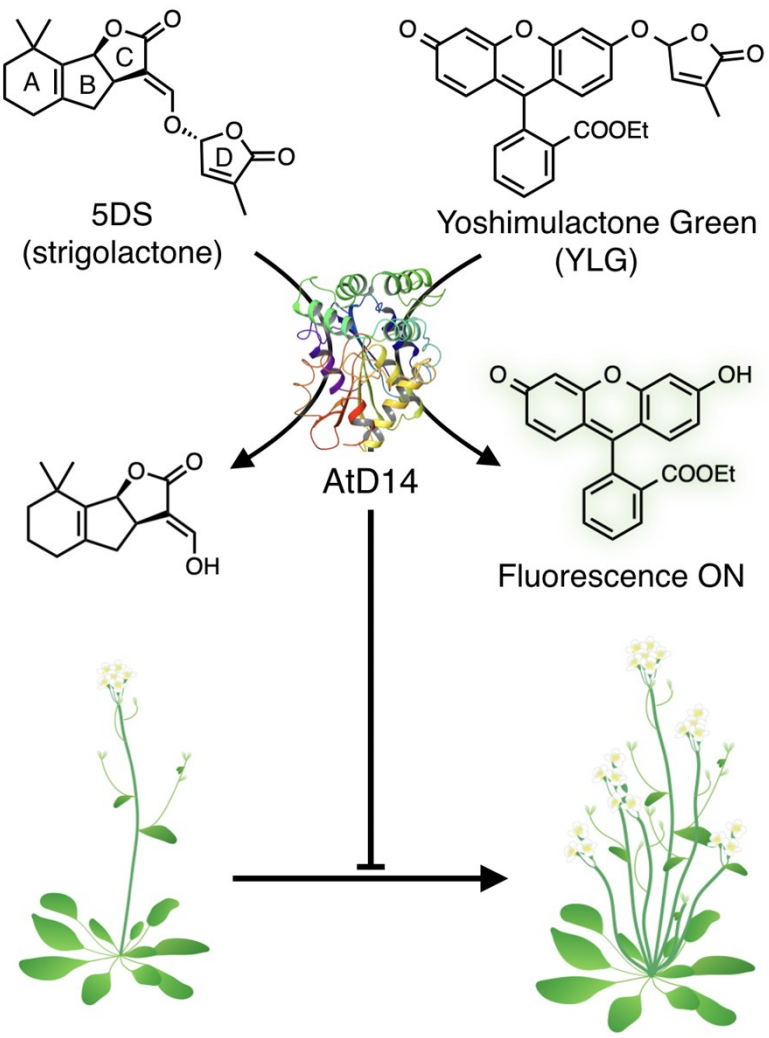


Figure S1. Strigolactone signaling that suppresses shoot branching. Strigolactones are hydrolyzed upon the perception by D14. The D14-mediated hydrolysis of Yoshimulactone Green (YLG) releases the active fluorophore and triggers downstream strigolactone signaling.

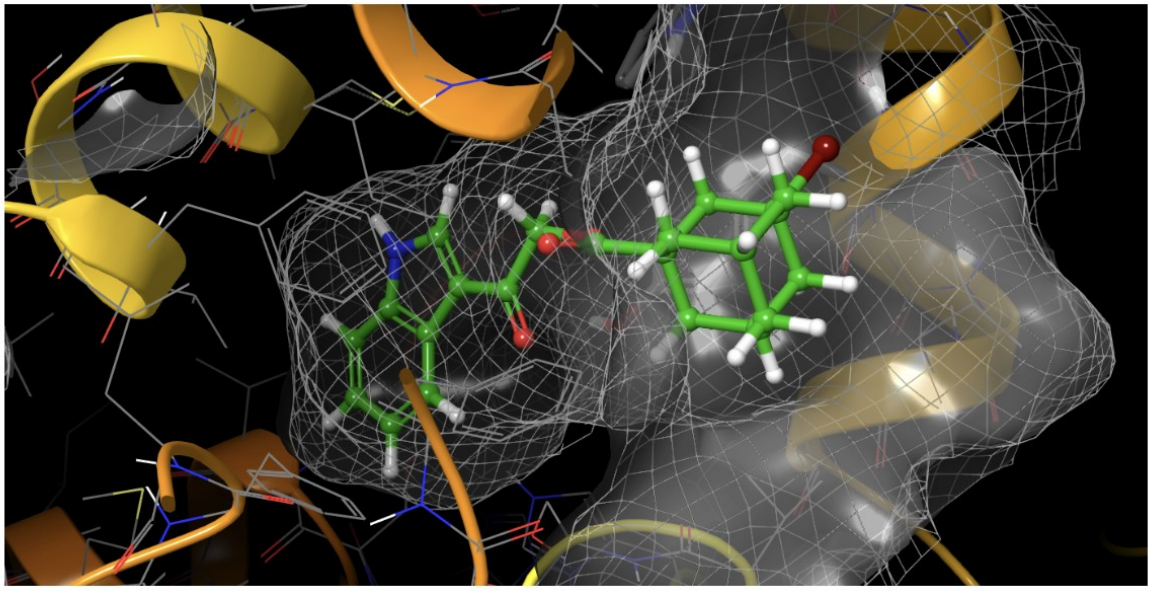


Figure S2. Docking model of DL1d bound to AtD14.

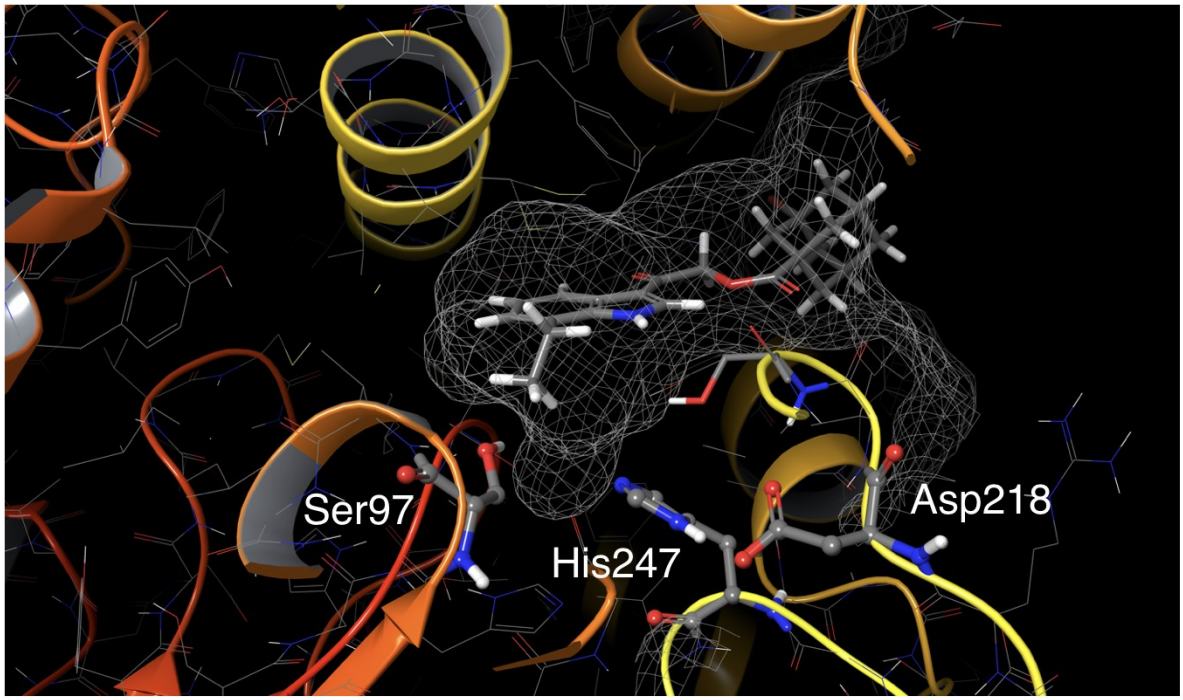


Figure S3. Docking model of DL1 bound to AtD14. The N7 proton, which is substituted to methyl group in DL1e, is located closely to residues constituting the catalytic site.

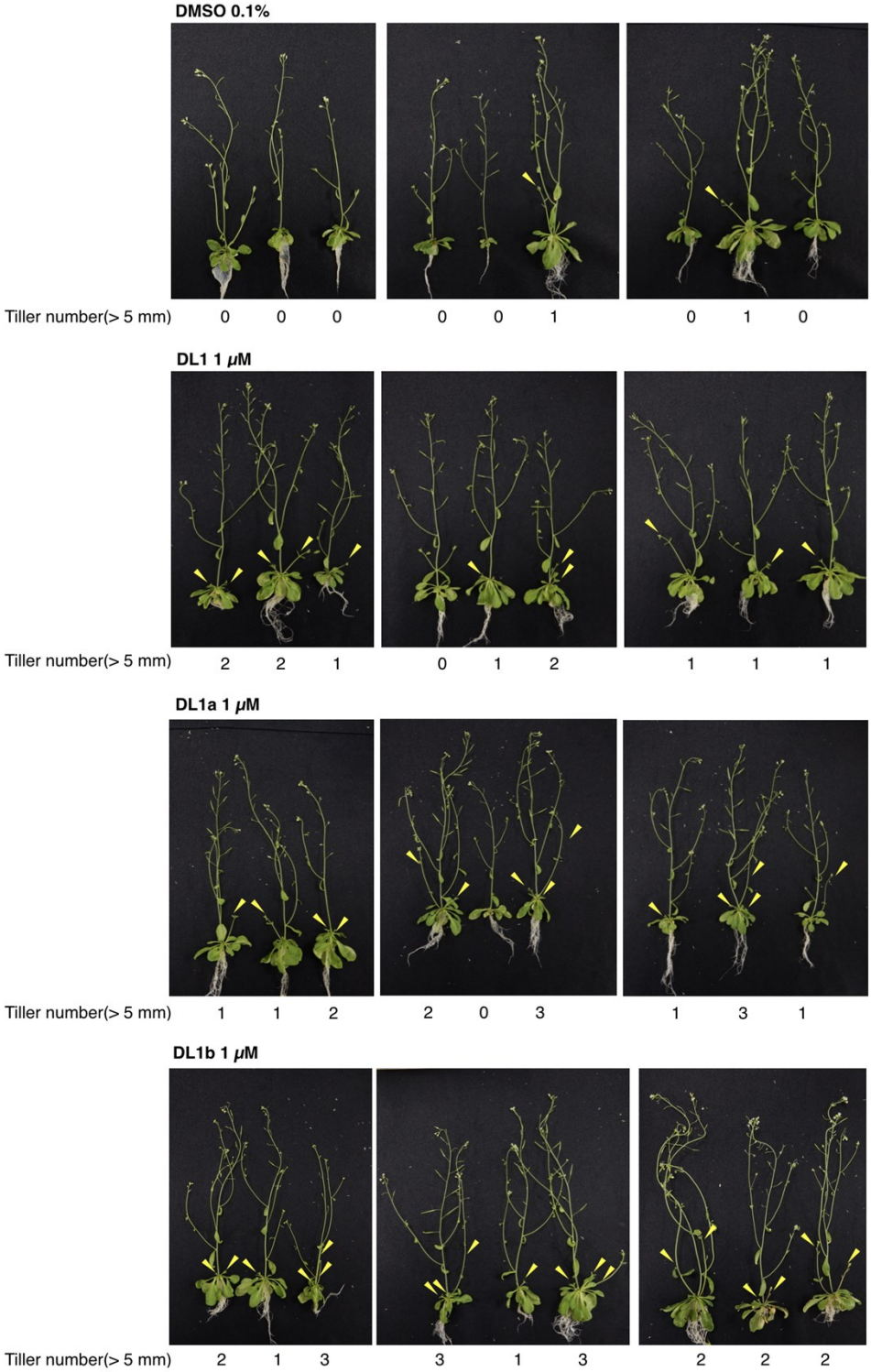


Figure S4. Enhancement of shoot branching in *Arabidopsis* by DL1 analogues. The wild-type *Arabidopsis* seedlings were treated with 1 μM DL1 analogues for 30 days. The yellow arrowheads indicate axillary buds. Numerals indicate the number of primary rosette branches.

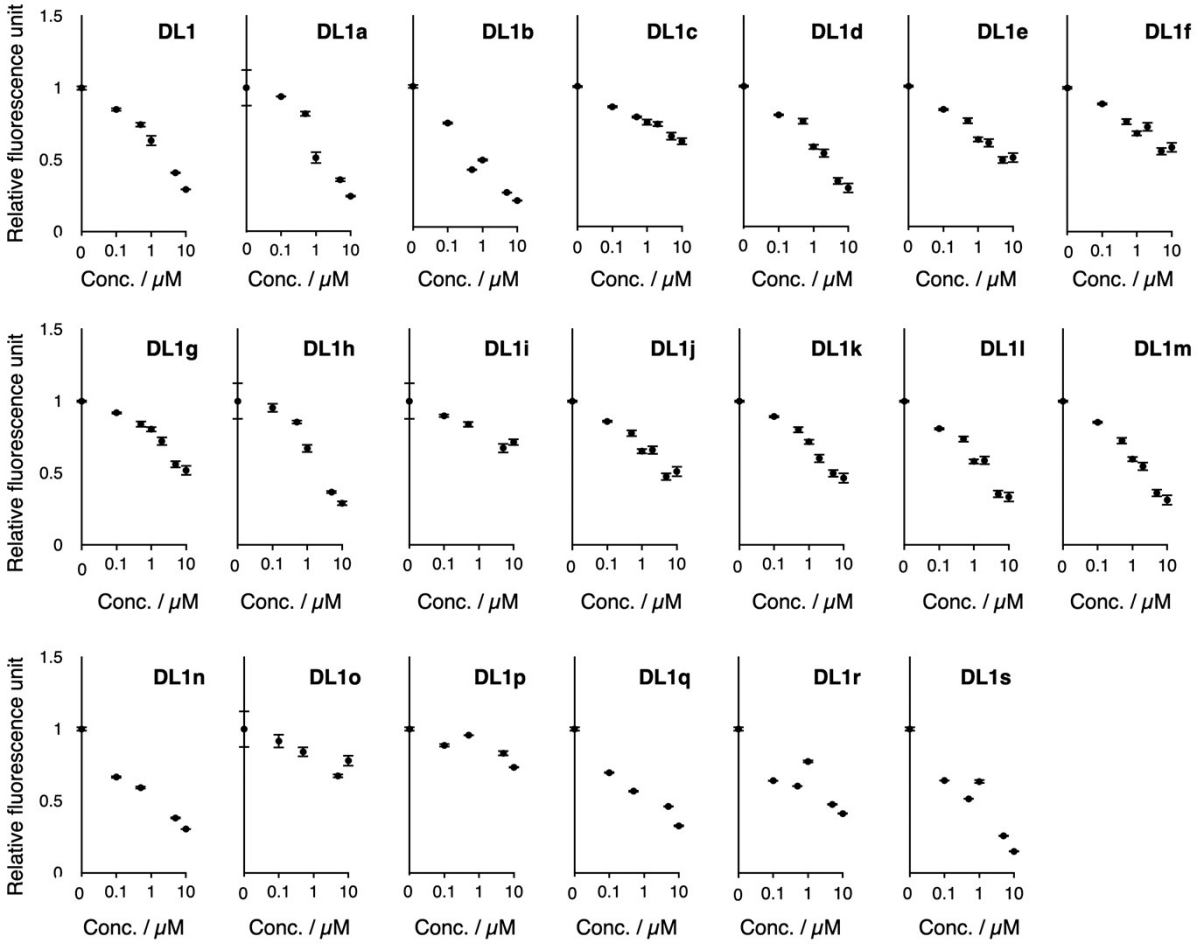
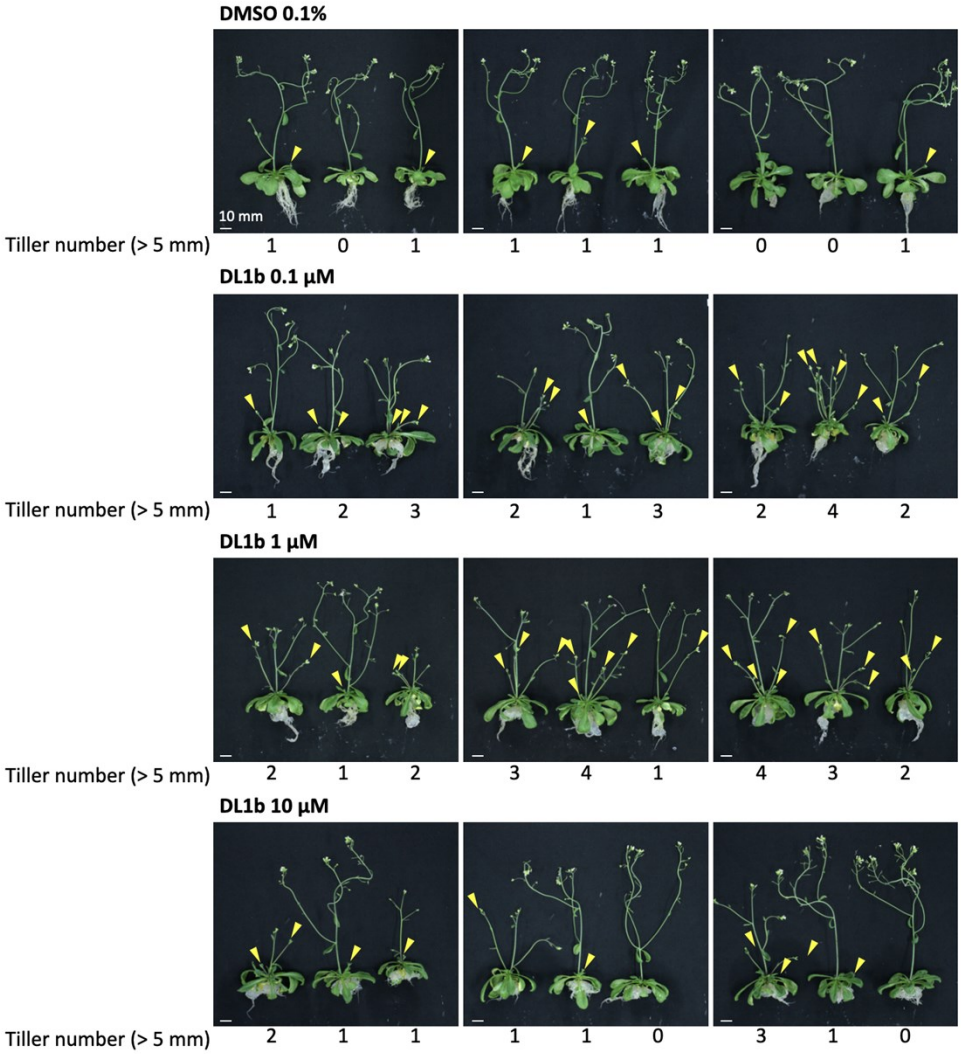


Figure S5. Inhibitory activity of DL1 analogues for AtD14-mediated YLG hydrolysis. Error bar indicates SE (n = 3 biological replicates).



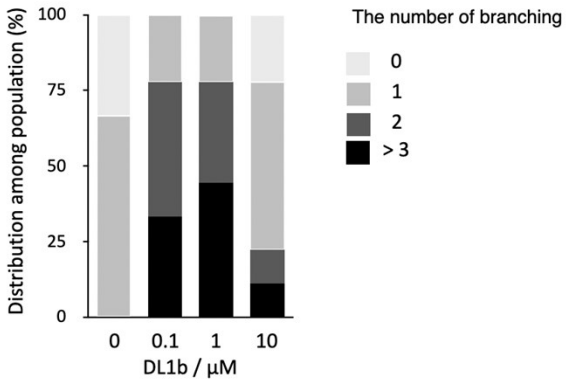


Figure S6. Enhancement of shoot branching in *Arabidopsis* by DL1b. The wild-type *Arabidopsis* seedlings were treated with 0.1, 1, 10 µM DL1 analogues for 30 days. The yellow arrowheads indicate axillary buds. Numerals indicate the number of primary rosette branches.