Supporting Information for: Kinetics-Based Measurement of Hypoxia in Living Cells and Animals Using an Acetoxymethyl Ester Chemiluminescent Probe

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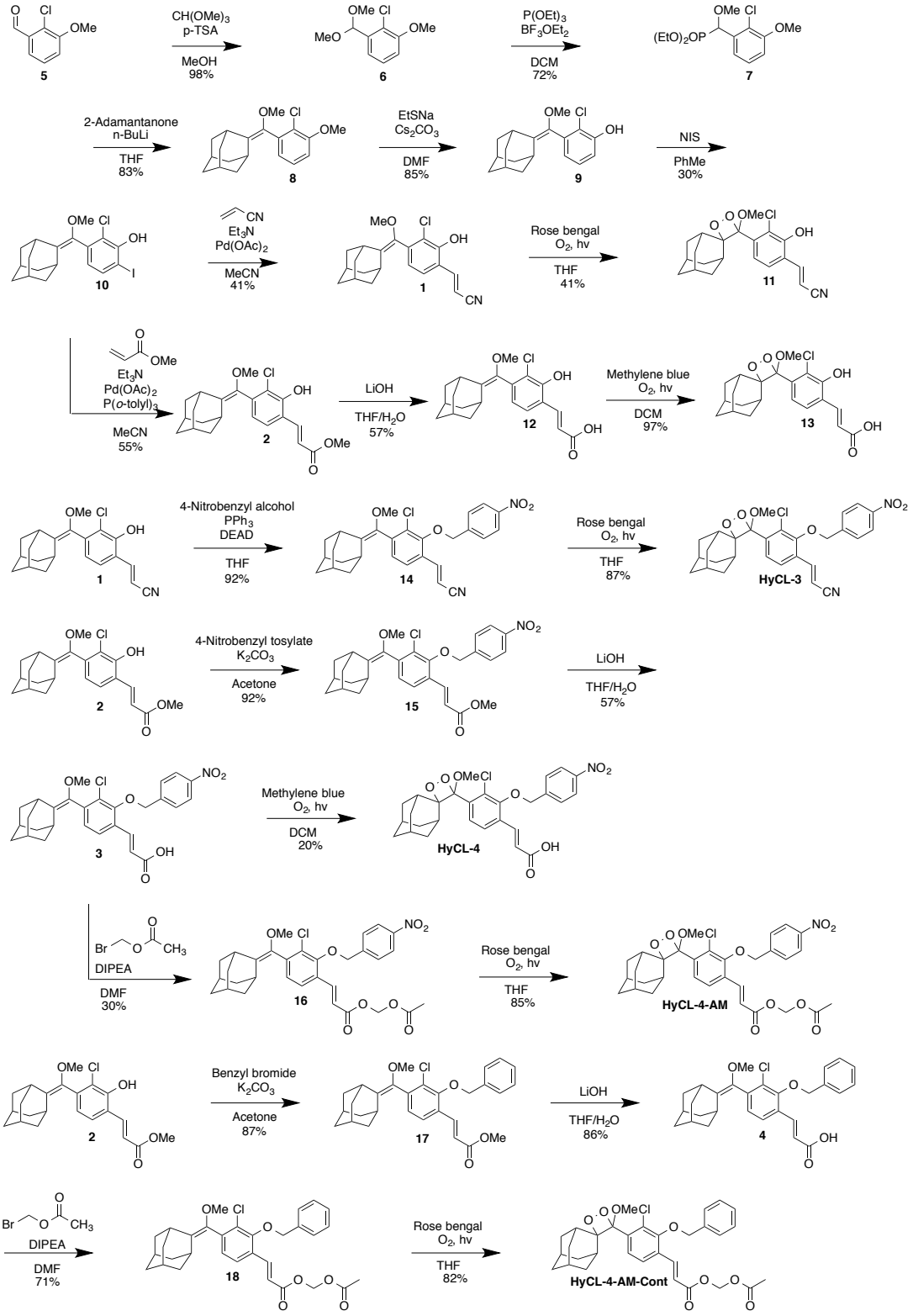
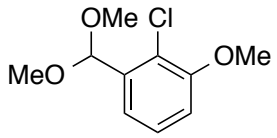
Supporting Information:

1. General synthetic methods All reactions were performed in dried glassware under an atmosphere of dry N_2. Silica gel P60 (SiliCycle) was used for column chromatography and SiliCycle 60 F254 silica gel (precoated sheets, 0.25 mm thick) was used for analytical thin layer chromatography. Plates were visualized by fluorescence quenching under UV light or by staining with iodine. Other reagents were purchased from Sigma-Aldrich (St. Louis, MO), Alfa Aesar (Ward Hill, MA), EMD Millipore (Billerica, MA), Oakwood Chemical (West Columbia, SC), and Cayman Chemical (Ann Arbor, MI) and used without further purification. ^{1}H NMR for compounds **13**, **14**, **15**, **3**, **16**, **HyCL-4-AM**, **17**, **4**, **18**, **HyCL-4** and **HyCL-4-AM-Cont** and ^{13}C NMR for compounds **13**, **15**, **3**, **16**, **4**, and **8** were collected on a Bruker 400 MHz spectrometer in the Department of Chemistry at Southern Methodist University. **^{1}**H NMR for compound **HyCL-3** and ^{13}C NMR for compounds **14**, **HyCL-3**, **HyCL-4-AM**, **17**, **HyCL-4** and **HyCL-4-AM-Cont** were measured on a JEOL 500 MHz spectrometer in the Department of Chemistry at Southern Methodist University. All ^{1}H and ^{13}C NMR spectra for characterization of new compounds and monitoring reactions were collected in $CDCl_3$ (Cambridge Isotope Laboratories, Cambridge, MA). All chemical shifts are reported in the standard notation of parts per million using the peak of residual proton signals of the deuterated solvent as an internal reference. Coupling constant units are in Hertz (Hz) Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets. High resolution mass spectroscopy was performed on a Shimadzu IT-TOF (ESI source) at the Shimadzu Center for Advanced Analytical Chemistry at the University of Texas, Arlington, and low resolution mass spectrometry was performed on an Advion Expression^L CMS (ESI source) at Southern Methodist University.

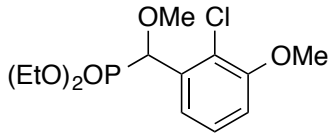


Scheme S1. Full synthesis of **HyCL-3**, **HyCL-4-AM**, **HyCL-4**, **HyCL-4-AM-Cont**, and dioxetane phenols.



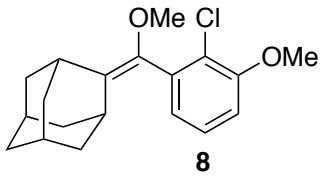
6

4-nitrobenzyl 4-methylbenzenesulfonate (6). 2-chloro-3-methoxybenzaldehyde **5** (10 g, 59 mmol, 1.0 equiv) was dissolved in 100 mL anhydrous methanol in a round bottom flask under a nitrogen atmosphere at room temperature. *p*-Toluenesulfonic acid (1115 mg, 5.86 mmol, 0.1 equiv) and trimethyl orthoformate (6.41 mL, 58.6 mmol, 1.0 equiv) were then added. The reaction was stirred at room temperature for 24 hr. Upon completion, the crude mixture was poured into a separatory funnel and washed with saturated $NaHCO_3$ and brine mixture. The organic layer was eluted with 3 x 50 mL EtOAc. The combined organic layers were collected and dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give **6** as a colorless oil without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (m, 2H), 6.93 (m, 1H), 5.65 (s, 1H), 3.90 (s, 3H), 3.38 (s, 6H).



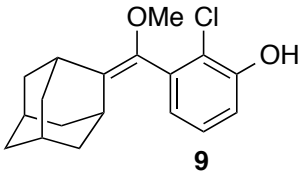
7

Diethyl ((2-chloro-3-methoxyphenyl)(methoxy)methyl)phosphonate (7). Acetal **6** (19 g, 88 mmol, 1.0 equiv) was dissolved in 100 mL anhydrous DCM in a dry round bottom flask under N_2 atmosphere and cooled to 0 ºC. Boron trifluoride diethyl etherate (11.4 mL, 90.9 mmol, 1.0 equiv) was added dropwise and allowed to stir for 10 min. Then, triethyl phosphite (15.6 mL, 90.9 mmol, 1.03) equiv was also added dropwise and stirred for 10 min. The reaction was then heated to 45 ºC and refluxed for 1.5 hr and monitored by TLC. Upon completion, the reaction was transferred into a separatory funnel, washed with brine, eluted with 3 x 50 mL DCM. The combined organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (50% EtOAc/ hexanes) yielded **7** as a beige oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 2H), 6.94 (m, 1H), 5.23 (d, 1H, J = 16.0 Hz), 4.17–4.21 (m, 4H), 4.16 (s, 3H), 3.37 (s, 1H), 1.31–1.35 (t, 3H, J = 6.8 Hz), 1.20–1.24 (t, 3H, J = 6.8 Hz).



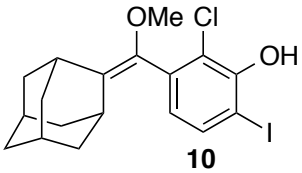
8

(1*r*,3*r*,5*R*,7*S*)-2-((2-chloro-3-methoxyphenyl)(methoxy)methylene)adamantane (8). Phosphonate **7** (4.33 g, 13.4 mmol, 1.0 equiv) was dissolved in 50 mL anhydrous THF in an oven-dried round bottom flask under N_2 atmosphere at –78 ºC. 2.6 M n-BuLi (6.2 mL 16.12 mmol, 1.2 equiv), was added dropwise over 10 min and allowed to stir for 20 min. Then, 2-adamantanone (2420 mg, 16.12 mmol, 1.2 equiv) dissolved in 15 mL anhydrous THF was added dropwise and allowed to stir for 5 min. Then, the reaction was taken out of the –78 ºC bath and allowed to stir for 2 hr. The temperature was raised to 90 ºC and the reaction was refluxed for 1 hr. Upon completion as determined by TLC, the reaction was transferred to a separatory funnel and quenched with sat. NH_4Cl. The organic layer was eluted with 3 x 30 mL EtOAc, dried with Na_2SO_4, and concentrated under reduced pressure. Column chromatography (5% EtOAc/ hexanes) yielded **8** (3.56 g, 11.2 mmol, 83%) as an amber oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (t, 1H, *J* = 7.6 Hz), 6.89–6.94 (m, 2H), 3.95 (s, 3H), 3.36 (s, 3H), 3.30 (s, 1H), 1.34–2.20 (m, 12H).



9

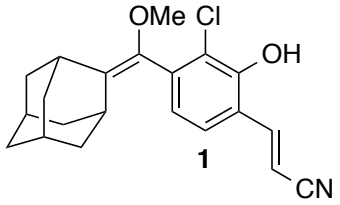
3-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-2-chlorophenol (9).[1] Ether **8** (4.5 g, 14 mmol, 1.0 equiv) was dissolved in 50 mL anhydrous DMF in an oven-dried round bottom flask under N_2 atmosphere. Sodium ethanethiolate (1430 mg, 17.00 mmol, 1.2 equiv) and Cs_2CO_3 (5540 mg, 17.00 mmol, 1.2 equiv) were added to the solution. The reaction was heated to 90 ºC and stirred for 12 hr. Upon completion as determined by TLC, the crude reaction was washed with sat. NH_4Cl and brine, and eluted with 3 x 30 mL EtOAc. The organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography (10% EtOAc/ hexanes) yielded compound **9** (3.68 g, 12.06 mmol, 85%) as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (t, 1H, *J* = 7.5 Hz), 6.92 (d, 1H, *J* = 6.0 Hz), 6.73 (d, 1H, *J* = 6.0 Hz), 3.22 (s, 3H), 3.19 (s, 1H), 1.72–2.20 (m, 12H).



10

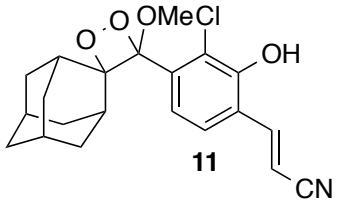
3-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-2-chloro-6-iodophenol (10).[1] Compound **9**[1] (3677 mg, 12.06 mmol, 1.0 equiv) was dissolved in 50 mL anhydrous toluene under N_2 atmosphere at 0 ºC. *N*-Iodosuccinimide (2714 mg, 12.06 mmol, 1.0 equiv) was then added in 1

portion. The reaction was stirred for 1 hr. Upon completion of the reaction as determined by TLC, the reaction was transferred into a separatory funnel and washed with brine. A few crystals of sodium thiosulfate pentahydrate were added and the separatory funnel was shaken vigorously to quench any remaining iodine. Upon quenching, the organic layer changed from pink to colorless. The organic layer was then eluted with 3 x 30 mL EtOAc, dried with Na_2SO_4, and concentrated under reduced pressure. Washing the crude residue with hexanes yielded compound **10** (1.68 mg, 3.9 mmol, 30%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, 1H, J = 8.0 Hz), 6.65 (d, 1H, J = 8.0 Hz), 6.16 (s, 1H), 3.33 (s, 3H), 3.28 (s, 1H), 1.63–2.11 (m, 12H).



1

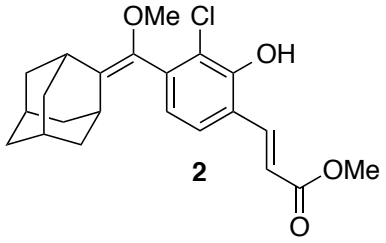
(*E*)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-3-chloro-2-hydroxyphenyl)acrylonitrile (1).[1] Iodophenol **10**[1] (500 mg, 1.16 mmol, 1.0 equiv) was dissolved in 3 mL anhydrous MeCN under N_2 atmosphere in a 10 mL microwave flask. Acrylonitrile (0.22 mL, 3.48 mmol, 3.0 equiv), anhydrous Et_3N (0.24 mL, 1.74 mmol, 1.5 equiv), and $Pd(OAc)_2$ were added to the solution. The reaction was capped and microwaved at 120 ºC for 70 min. Upon completion, the reaction was transferred to a separatory funnel and washed with NH_4Cl and brine mixture. The organic layer was eluted with 3 x 30 mL EtOAc, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Column chromatography (100 % DCM) yielded compound **1** (201 mg, 0.56 mmol, 48%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, 1H, J = 16.8 Hz), 7.29 (d, 1H, J = 8.0 Hz), 6.90 (d, 1H, J = 8.0 Hz), 6.21 (d, 1H, J = 16.8 Hz), 3.33 (s, 3H), 3.28 (s, 1H), 1.63–2.18 (m, 12H).



11

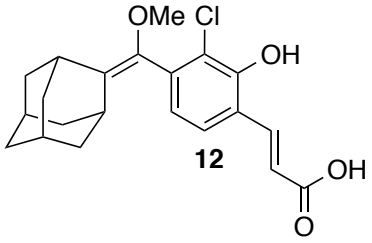
Compound (11).[1] Compound **1**[1] (112 mg, 0.341 mmol, 1 equiv) and Rose bengal (18.1 mg, 0.0186 mmol, 0.0545 equiv) added to a 2-neck round bottom flask and dissolved in 5 mL THF. Oxygen was bubbled through the reaction mixture, while irradiating with a 120 W light bulb (Home Depot, Dallas, TX) at 0 °C. After 2 hr of reaction, TLC showed no starting material left and the mixture was then concentrated under vacuum at 0 ºC. The residue was purified by column chromatography

(5% EtOAc/ hexanes) to deliver compound **10** (74.8 mg, 0.129 mmol, 41%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, 1H, *J* = 8.0 Hz), 7.60 (d, 1H, *J* = 16.8 Hz), 6.44 (d, 1H, *J* = 8.0 Hz), 6.23 (d, 1H, *J* = 16.8 Hz), 3.24 (s, 3H), 3.04 (s, 1H), 1.22–2.26 (m, 12H).



2

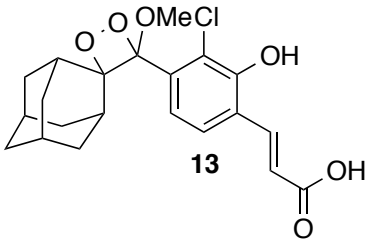
(*E*)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-3-chloro-2-hydroxyphenyl)acrylic acid (2).[1] In an oven-dried round bottom flask, iodophenol **10**[1] (431 mg, 1.00 mmol, 1.0 equiv) was dissolved in 5 mL anhydrous MeCN under N_2 atmosphere. Methyl acrylate (0.26 mL, 3.0 mmol, 3.0 equiv), Et_3N (0.20 mL, 1.5 mmol, 1.5 equiv), $Pd(OAc)_2$ (11 mg 0.05 mmol, 0.05 equiv), and tri(*o*-tolyl) phosphine (3 mg, 0.01 mmol, 0.01 equiv) were added into the solution. The reaction was heated to 120 ºC and refluxed for 2 hr. Upon completion of the reaction as determined by TLC, the crude mixture was transferred to a separatory funnel and washed with NH_4Cl. The organic layer was eluted with 3 x 30 mL EtOAc, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (15% EtOAc/ hexanes) yielded compound **2** (215 mg, 0.55 mmol, 55%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, 1H, *J* = 16.0 Hz), 7.41 (d, 1H, *J* = 8.0 Hz), 6.90 (d, 1H, *J* = 8.0 Hz), 6.67 (d, 1H, *J* = 16.0 Hz), 6.22 (s, 1H), 3.84 (s, 3H), 3.33 (s, 3H), 3.29 (s, 1H), 1.63–2.20 (m, 12H).



12

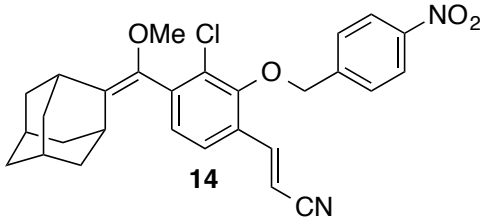
(*E*)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-3-chloro-2-hydroxyphenyl)acrylic acid (12).[1] Acrylic methyl ester phenol **2**[1] (0.668 mmol, 260 mg 1 equiv) was dissolved in 5.0 mL THF in a dry round bottom flask flushed with nitrogen. 5 mL of 1.0 M LiOH in H_2O was added to the solution. The reaction was then heated to 80 ºC and stirred under reflux for 4 hr. Upon completion of the reaction as determined by TLC, the crude was washed with 1 M HCl and eluted with 3 x 30 mL EtOAc. The organic layer was collected, dried with Na_2SO_4, and evaporated under reduced pressure, yielding compound **12** (257 mg, 0.668 mmol, quantitative)

without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, 2H, *J* = 16.1 Hz), 7.49 (d, 2H, *J* = 8.0 Hz), 6.83 (d, 2H, *J* = 8.0 Hz), 6.59 (d, 2H, *J* = 16.1 Hz), 3.30 (s, 3H), 3.24 (s, 1H), 1.40–2.22 (m, 12H).



13

(*E*)-3-(3-chloro-2-hydroxy-4-((1*r*,3*r*,5*r*,7*r*)-4'-methoxyspiro[adamantane-2,3'-[1,2]dioxetan]-4'-yl)phenyl)acrylic acid (13). Enol ether **12**[1] (20 mg, 0.053 mmol, 1 equiv) and methylene blue (5.0 mg, 0.015 equiv, 0.028 equiv) were added into a dry flask and dissolved in 5 mL THF. Oxygen was bubbled through the reaction mixture, while irradiating with a 120 W light bulb (Home Depot, Dallas, TX) at 0 °C. After 1.5 hr of reaction, TLC showed no starting material left and the mixture was then concentrated under vacuum at 0 ºC. The residue was purified by column chromatography (50% EtOAc/ hexanes) to deliver **13** (19.5 mg, 97%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, 1H, *J* = 16.4 Hz), 7.72 (d, 1H, *J* = 8.0 Hz), 7.58 (d, 1H, *J* = 8.0 Hz), 6.70 (d, 1H, *J* = 16.4 Hz), 6.65 (br s, 1H), 3.26 (s, 3H), 3.04 (s, 1H), 1.27-2.26 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) 171.80, 151.04, 140.73, 134.25, 127.16, 124.76, 123.49, 120.37, 96.32, 49.72, 39.28, 36.51, 34.07, 33.45, 32.82, 32.16, 31.56, 26.12, 25.78; LRMS data for $C_{21}H_{23}ClO_6$ $[M-H]^-$ found 405.3.

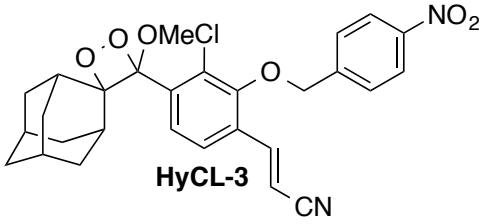


14

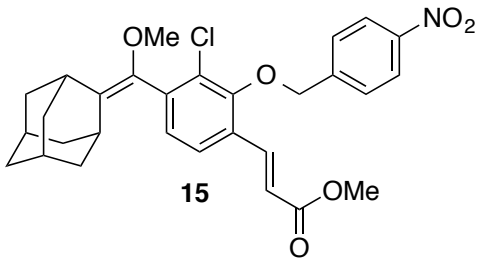
(*E*)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-3-chloro-2-((4-nitrobenzyl)oxy)phenyl)acrylonitrile (14). Acrylonitrile phenol **1**[1] (85 mg, 0.22 mmol, 1.0 equiv), triphenyl phosphite (69 mg, 0.26 mmol, 1.2 equiv) and 4-nitrobenzyl alcohol (33 mg, 0.22 mmol, 1.0 equiv) were added to a dry round bottom flask flushed with nitrogen. The reaction contents were dissolved in 3.0 mL THF, and the reaction was cooled to 0 °C. Diethyl

(1) Green, O.; Eilon, T.; Hananya, N.; Gutkin, S.; Bauer, CR.; Shabat, D. Opening a gateway for chemiluminescence cell imaging: distinctive methodology for design of bright chemiluminescent dioxetane probes. *ACS Central Sci*. **2017**, *4*, 349–358.

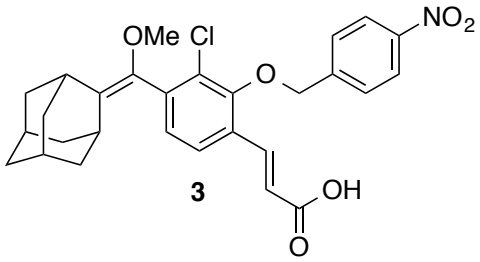
azodicarboxylate (41 µL, 0.26 mmol, 1.2 equiv) was added dropwise to the solution, and stirred for 1 hr. The crude mixture was combined with 30 mL brine solution, and mixture was washed with 2 x 20 mL EtOAc. The combined organic layers were collected and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (10% EtOAc/Hexanes) yielded **14** (99 mg, 92%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, 2H, *J* = 8.8 Hz), 7.69 (d, 2H, *J* = 8.8 Hz), 7.60 (d, 1H, *J* = 16.8 Hz), 7.41 (d, 1H, *J* = 8.0 Hz), 7.16 (d, 1H, *J* = 8.0 Hz), 6.98 (d, 1H *J* =16.8 Hz), 5.13 (d, 2H, *J* = 6.8 Hz), 3.32 (s, 3H), 3.30 (s, 1H), 1.61–2.08 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.07, 148.03, 144.38, 143.05, 139.69, 139.07, 133.62, 129.87, 128.53, 128.38, 124.01, 117.91, 99.12, 77.86, 76.86, 57.69, 38.74, 37.02, 33.10, 29.84, 28.36, 28.19; HRMS calcd for $C_{28}H_{27}ClN_2O_4$ $[M-H]^-$ 489.1587, found 489.1592.



(*E*)-3-(3-chloro-4-((1*r*,3*r*,5*r*,7*r*)-4'-methoxyspiro[adamantane-2,3'-[1,2]dioxetan]-4'-yl)-2-((4-nitrobenzyl)oxy)phenyl)acrylonitrile (HyCL-3). Enol ether **14** (40 mg, 0.081 mmol, 1.0 equiv) and Rose bengal (8.5 mg, 0.0087 mmol, 0.11 equiv) were added into a dry flask and dissolved in 5 mL THF. Oxygen was bubbled through the reaction mixture, while irradiating with a 120 W light bulb (Home Depot, Dallas, TX) at 0 °C. After 3.5 h of reaction, TLC showed no starting material left. The mixture was then concentrated under vacuum at 0 ºC and the residue was purified by column chromatography (10% EtOAc/ hexanes) to deliver **HyCL-3** (56.4 mg, 87%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (d, 2H, *J* = 6.25 Hz), 7.99 (d, 1H, *J* = 7.45 Hz), 7.67 (d, 2H, *J* = 6.25 Hz), 7.57 (d, 1H, *J* = 17.2 Hz), 7.25 (d, 1H, *J* = 7.45 Hz), 6.02 (d, 1H, *J* =17.2 Hz), 5.02 (s, 2H), 3.22 (s, 3H), 3.02 (s,1H) 1.71–2.28 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.53, 148.11, 143.86, 142.69, 136.79, 130.33, 129.77, 128.36, 127.93, 124.91, 124.07, 117.55, 111.59, 100.59, 96.44, 72.14, 49.87, 36.58, 33.73, 32.23, 31.61, 26.16, 25.86; LRMS data for $C_{28}H_{27}ClN_2O_6$ $[M+CH_3CN+H]^+$ found 564.8.

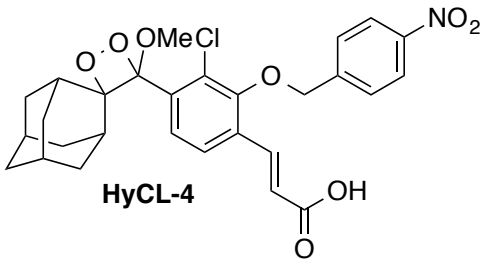


Methyl (*E*)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-3-chloro-2-((4-nitrobenzyl)oxy)phenyl)acrylate (15). Methyl acrylate phenol **2**[1] (215 mg, 0.55 mmol, 1.0 equiv) was dissolved in 5.0 mL anhydrous acetone in a dry round bottom flask flushed with nitrogen. *p*-Nitrobenzyl tosylate (253 mg, 0.825 mmol, 1.5 equiv) and K_2CO_3 were added in 2 separate portions. The reaction was stirred at room temperature for 12 hr, at which time TLC inspection showed complete consumption of starting material **2**. The crude mixture was combined with 30 mL brine solution, and mixture was washed with 2 x 20 mL EtOAc. The combined organic layers were collected and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (10% EtOAc/ hexanes) yielded **15** (228 mg, 92%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, 2H, *J* = 8.8 Hz), 7.90 (d, 1H, *J* = 16.0 Hz), 7.70 (d, 2H, *J* = 8.8 Hz), 7.48 (d, 1H, *J* = 8.0 Hz), 7.13 (d, 1H, *J* = 8.0 Hz), 6.47 (d, 1H, *J* = 16.0 Hz), 5.11 (d, 2H, *J* = 5.2 Hz), 3.81 (s, 3H), 3.35 (s, 3H), 3.30 (s, 1H), 1.77–2.10 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.95, 153.32, 147.79, 143.40, 139.28, 128.26, 132.87, 129.42, 128.63, 128.28, 125.26, 123.76, 123.38, 74.40, 57.36, 51.88, 39.17, 39.02, 38.67, 36.99, 32.95, 29.71, 28.31, 28.16; HRMS calcd for $C_{29}H_{30}ClNO_6$ $[M+Na]^+$ 546.1654, found 546.1642.

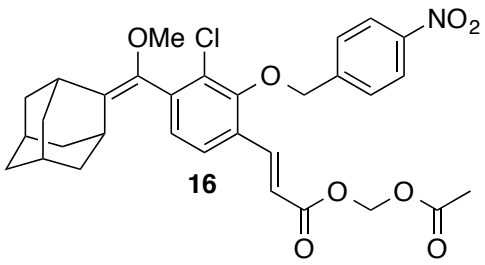


(*E*)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-3-chloro-2-((4-nitrobenzyl)oxy)phenyl)acrylic acid (3). Acrylate phenol **15** (228 mg, 0.49 mmol, 1.0 equiv) was dissolved in 5 mL THF in a 20 mL round bottom flask. 5 mL of 1 M LiOH was then added to the mixture. The reaction was placed under an inert atmosphere, heated to 80 ºC, and refluxed for 2 hr. Upon completion, the reaction was transferred into a separatory funnel. The organic layer was washed with 1 M HCl and eluted with 2 x 20 mL EtOAc. The combined organic layers were

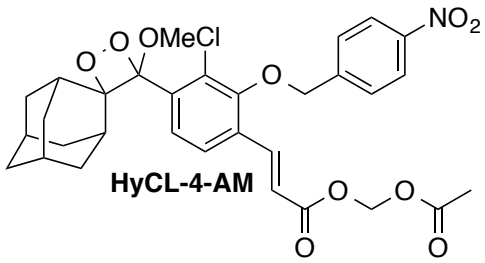
collected and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (50% EtOAc/ hexanes) yielded **3** (142 mg, 57%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, 2H, *J* = 8.4 Hz), 7.99 (d, 1H, *J* = 16.0 Hz), 7.70 (d, 2H, *J* = 8.4 Hz), 7.52 (d, 1H, J = 8.0 Hz), 7.15 (d, 1H, *J* = 8.0 Hz*)*, 6.47 (d, 1H, *J* = 16.0 Hz), 5.14 (d, 2H, *J* = 5.2 Hz), 3.36 (s, 3H), 3.30 (s, 1H), 1.73–2.19 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.65, 153.60, 147.89, 143.26, 140.44, 139.20, 138.88, 133.16, 129.63, 129.11, 128.72, 128.33, 125.43, 123.83, 119.85, 74.61, 57.41, 39.20, 39.05, 38.67, 38.58, 37.00 33.01, 29.76, 28.32, 28.17; HRMS calcd for $C_{28}H_{28}ClNO_6$ $[M-H]^-$ 508.1532, found 508.1524.



(*E*)-3-(3-chloro-4-((1*r*,3*r*,5*r*,7*r*)-4'-methoxyspiro[adamantane-2,3'-[1,2]dioxetan]-4'-yl)-2-((4-nitrobenzyl)oxy)phenyl)acrylic acid (HyCL-4). Compound **3** (13 mg, 0.048 mmol, 1.0 equiv) and methylene blue (5.0 mg, 0.015 mmol, 0.31 equiv) were added into a dry flask and dissolved in 5 mL THF. Oxygen was bubbled through the reaction mixture, while irradiating with a 120 W light bulb (Home Depot, Dallas, TX) at 0 °C. After 2 h of reaction, TLC showed no starting material left and the mixture was then concentrated under vacuum. The residue was purified by column chromatography (15% EtOAc/hexanes) to deliver **HyCL-4** as a white solid (2.6 mg, 20 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, 2H, *J* = 8.8 Hz), 8.00 (d, 1H, *J* = 8.0 Hz), 7.96 (d, 1H, *J* = 16.4 Hz), 7.66 (d, 2H, *J* = 8.0 Hz), 5.07 (s, 2H), 3.25 (s, 3H), 3.05 (s, 1H), 1.67–2.11 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.42, 154.10, 148.06, 142.96, 139.92, 136.04, 131.08, 129.62, 128.82, 125.71, 124.01, 121.02, 111.70, 96.45, 74.04, 49.86, 36.62, 33.70, 32.27, 31.64, 26.20, 25.90.

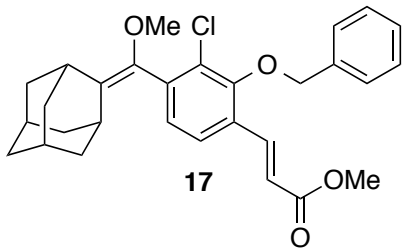


Acetoxymethyl (*E*)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-3-chloro-2-((4-nitrobenzyl)oxy)phenyl)acrylate (16). Acrylate **3** (98 mg, 0.19 mmol, 1.0 equiv) was dissolved in 1 mL anhydrous DMF and quantitatively transferred into a dry 10 mL round bottom flask flushed with nitrogen. Anhydrous DIPEA (0.12 mL, 0.69 mmol, 3.6 equiv) was added and allowed to mix for 2 min. Bromomethyl acetate (0.08 mL, 0.79 mmol, 4.1 equiv) was then added dropwise. The reaction was stirred for 24 hr at RT, upon which the starting material was fully consumed as determined by TLC. The crude reaction was then diluted with 4 mL EtOAc and transferred into a separatory funnel. The organic layer was washed with 1 M HCl and eluted with 2 x 20 mL EtOAc. The combined organic layers were collected and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (10–30% EtOAc/ hexanes gradient) yielded **16** (33 mg, 30%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, 2H, *J* = 8.8 Hz), 7.98 (d, 1H, *J* = 16.0 Hz), 7.69 (d, 2H, *J* = 8.8 Hz), 7.49 (d, 1H, *J* = 8.0 Hz), 7.14 (d, 1H, *J* = 8.0 Hz), 6.47 (d, 1H, *J* = 16.0 Hz), 5.87 (s, 2H), 5.12 (d, 2H, *J* = 5.6 Hz), 3.36 (s, 3H), 3.30 (s, 1H), 2.16 (s, 3H), 1.62–2.14 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.73, 165.05, 153.53, 147.94, 143.13, 140.13, 139.21, 138.99, 133.16, 129.65, 129.03, 128.79, 128.33, 125.26, 123.86, 119.16, 57.44, 39.20, 39.20, 39.05, 38.69, 38.59, 37.00, 33.00, 31.59, 29.76, 28.32, 28.16, 22.66, 20.75, 14.13; HRMS calcd for $C_{31}H_{32}ClNO_8$ $[M-H]^-$ 580.1744, found 580.1742.



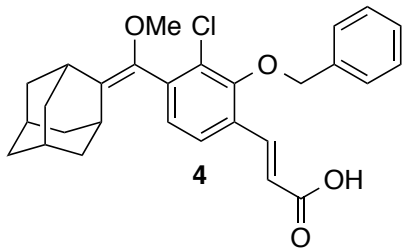
Acetoxymethyl (*E*)-3-(3-chloro-4-((1*r*,3*r*,5*r*,7*r*)-4'-methoxyspiro[adamantane-2,3'-[1,2]dioxetan]-4'-yl)-2-((4-nitrobenzyl)oxy)phenyl)acrylate (HyCL-4-AM). Enol ether **16** (22 mg, 0.038 mmol, 1.0 equiv) and Rose bengal (9.8 mg, 0.0087 mmol, 0.107 equiv) were added into a dry flask and dissolved in 5 mL THF. Oxygen was bubbled through the reaction mixture, while irradiating with a 120 W light bulb (Home Depot, Dallas, TX) at 0 °C. After 3.5 h of reaction, TLC showed no starting material left and the mixture was then concentrated under vacuum. The residue was purified by column chromatography (10% EtOAc/ hexanes) to deliver **HyCL-4-AM** as a pale yellow solid (20 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (d, 2H, 8.8 Hz), 7.99 (d, 1H, *J* = 8.4 Hz), 7.97 (d, 1H, *J* = 16.0 Hz), 7.71 (d, 1H, *J* = 8.4 Hz), 7.69, (d, 2H, *J* = 8.8 Hz) 6.53 (d, 1H,

J = 16.0 Hz), 5.87 (s, 2H), 5.05 (s, 2H), 3.25 (s, 3H), 3.05 (s, 1H), 2.16 (s, 3H), 1.60–2.14 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.76, 164.84, 148.11, 142.82, 139.60, 129.60, 128.92, 125.55, 124.01, 120.57, 111.68, 96.42, 49.84, 36.62, 33.71, 32.26, 31.64, 26.20, 25.90, 20.80; LRMS for $C_{31}H_{32}ClNO_{10}$ $[M-H]^-$ found 613.0.



17

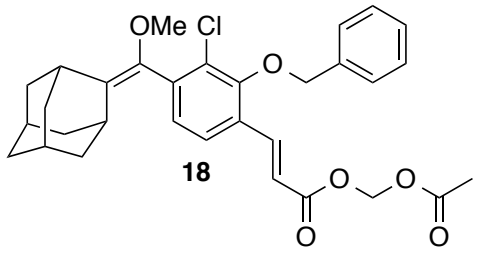
Methyl (*E*)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-2-(benzyloxy)-3-chlorophenyl)acrylate (17). Acrylate phenol **2** (90 mg, 0.231 mmol 1.0 equiv) was dissolved in anhydrous acetone and transferred into a dry round bottom flask flushed with nitrogen. K_2CO_3 (64 mg, 0.46 mmol, 2.0 equiv) was added in one portion and stirred for 2 min. Then, benzyl bromide (0.04 mL, 0.34 mmol, 1.2 equiv) was added in one portion and the reaction was monitored for 3 hr. Additional benzyl bromide (0.03 mL, 0.25 mmol, 1.0 equiv) was added to drive the reaction to completion. The reaction was stirred for 16 hr, then transferred into a separatory funnel. The reaction was then washed with brine and eluted with 3 x 15 mL EtOAc. The combined organic layers were collected and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (10–30% EtOAc/ hexanes) yielded **6** (97 mg, 88%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, 1H, *J* = 16.0 Hz), 7.53 (m, 2H), 7.45 (d, 2H, *J* = 8.0 Hz), 7.39–7.44 (m, 3H), 6.45 (d, 1H, *J* = 16.0 Hz), 5.03 (d, 2H, *J* = 5.6 Hz), 3.83 (s, 3H), 3.35 (s, 3H), 3.30 (s,1H), 1.72–2.10 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.08, 153.66, 139.50, 138.94, 132.51, 129.76, 128.89, 128.63, 127.86, 125.11, 119.96, 57.35, 51.88, 38.73, 37.14, 33.01, 29.79, 28.44, 28.28; LRMS for $C_{28}H_{29}ClO_4$ $[M+H]^+$ found 479.4.



4

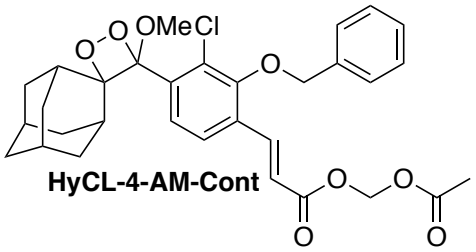
(*E*)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-3-chloro-2-((4-nitrobenzyl)oxy)phenyl)acrylic acid (4). Methyl acrylate **17** (90 mg, 0.19 mmol, 1.0 equiv) was dissolved in 5 mL THF in a 20 mL round bottom flask. 5 mL of 1 M LiOH was then added to the

mixture. The reaction was placed under an inert atmosphere, heated to 80 ºC and refluxed for 6 hr. Upon completion, the reaction was transferred into a separatory funnel. The organic layer was washed with 1 M HCl and eluted with 2 x 20 mL EtOAc. The combined organic layers were collected and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (50% EtOAc/ hexanes) yielded **4** (77 mg, 86%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, 1H, *J* = 16.0 Hz), 7.51 (m, 3H) 7.38–7.49 (m, 3H), 5.06 (d, 2H, *J* = 5.6 Hz), 3.37 (s, 3H), 3.32 (s, 1H), 1.73–2.21 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.92, 153.92, 140.98, 139.36, 138.62, 135.83, 132.59, 129.96, 129.43, 128.93, 128.64, 128.62, 127.85, 125.23, 119.32,57.29, 39.22, 39.06, 38.65, 37.07, 32.97, 30.94, 29.74, 28.37, 28.21; HRMS calcd for $C_{28}H_{29}ClO_4$ $[M-H]^-$ 463.1682, found 463.1675.

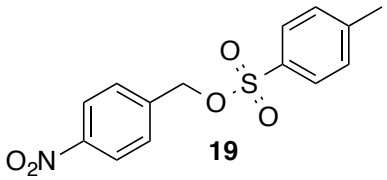


18

Acetoxymethyl (*E*)-3-(4-(((1*r*,3*r*,5*R*,7*S*)-adamantan-2-ylidene)(methoxy)methyl)-2-(benzyloxy)-3-chlorophenyl)acrylate (18). Acrylic acid **4** (72 mg, 0.15 mmol, 1.0 equiv) was dissolved in 1 mL anhydrous DMF and quantitatively transferred into a dry 10 mL round bottom flask flushed with nitrogen. Anhydrous DIPEA (0.096 mL, 0.55 mmol, 3.6 equiv) was added and allowed to mix for 2 min. Bromomethyl acetate (0.08 mL, 0.79 mmol, 4.1 equiv) was then added dropwise. The reaction was stirred for 24 hr at RT, upon which the starting material was consumed as determined by TLC. The crude reaction was then diluted with 4 mL EtOAc and transferred into a separatory funnel. The organic layer was washed with saturated NH_4Cl and eluted with 2 x 20 mL EtOAc. The combined organic layers were collected and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by column chromatography (10–30% EtOAc/ hexanes gradient) yielded **6** (33 mg, 71%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, 1H, *J* = 16.4 Hz), 7.37–7.51 (m, 6H), 7.10 (d, 1H, *J* = 8.0 Hz), 6.44 (d, 1H, *J* = 16.4 Hz), 5.90 (s, 2H), 5.03 (d, 2H, *J* = 5.6 Hz), 3.53 (s, 3H), 3.31 (s, 1H), 1.68–2.19 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.72, 165.18, 153.92, 140.76, 139.37, 138.71, 135.79, 132.57, 129.96, 129.35, 128.94, 128.63, 128.61, 127.84, 125.08, 118.59, 57.30, 39.21, 39.05, 38.64, 37.06, 32.96, 30.94, 29.73, 28.36, 28.21, 20.80; HRMS calcd for $C_{31}H_{33}ClO_6$ $[M+Na]^+$ 559.1858, found 559.1844.



Acetoxymethyl (*E*)-3-(3-chloro-4-((1*r*,3*r*,5*r*,7*r*)-4'-methoxyspiro[adamantane-2,3'-[1,2]dioxetan]-4'-yl)-2-((4-nitrobenzyl)oxy)phenyl)acrylate (HyCL-4-AM-Control). Enol ether **18** (29 mg, 0.081 mmol, 1.0 equiv) and Rose bengal (7.9 mg, 0.0081 mmol, 0.1 equiv) were added into a dry flask and dissolved in 5 mL THF. Oxygen was bubbled through the reaction mixture, while irradiating with a 120 W light bulb (Home Depot, Dallas, TX) at 0 °C. After 3.5 h of reaction, TLC showed no starting material left and the mixture was then concentrated under vacuum. The residue was purified by column chromatography (20% EtOAc/ hexanes) to deliver **HyCL-4-AM-Cont** as a pale yellow solid (25 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.99 (d, 1H, *J* = 16.0 Hz), 7.94 (d, 1H, *J* = 8.0 Hz), 7.58 (d, 1H, *J* = 8.0 Hz), 7.48 (m, 2H), 7.38–7.44 (m, 3H), 6.48 (d, 1H, *J* = 16.0 Hz), 5.90 (s, 2H), 4.97 (s, 2H), 3.25 (s, 3H), 3.05 (s, 1H), 1.75–2.36 (m, 15H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.77, 164.98, 154.42, 140.22, 135.77, 135.53, 131.33, 129.08, 128.88, 128.79, 125.34, 119.94, 111.81, 86.46, 49.84, 33.68, 32.30, 33.68, 32.30, 31.65, 29.80, 26.24, 25.89, 20.87, 14.22; HRMS calcd for $C_{31}H_{33}ClO_8$ $[M+Na]^+$ 591.1756, found 591.1702.



***p*- Nitrobenzyl tosylate (19)**.[2] In a 100 mL round bottom flask under nitrogen atmosphere, *p*-nitrobenzyl alcohol (2002 mg, 13.07 mmol, 1.1 equiv) and *p*-toluenesulfonyl chloride (2265 mg 11.88 mmol, 1.0 equiv) were dissolved in 25 mL THF. In a separate 50 mL roundbottom flask, NaOH (713 mg, 17.82 mmol, 1.5 equiv was dissolved in 25 mL H_2O and poured into the flask. The reaction was stirred at rt for 3 hr. Upon completion as determined by TLC, the reaction was transferred into a separatory funnel and washed with sat. NH_4Cl, brine and EtOAc. The organic layer was then eluted with 3 x 50 mL EtOAc. The combined organic layer was dried with Na_2SO_4,

(2) Yang, Y.; Voak, A.; Wilkinson, S. R.; Hu, L. Design, synthesis, and evaluation of potential prodrugs of DFMO for reductive activation. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6583–6586.

filtered, and concentrated under reduced pressure. Product was recrystallized in hot EtOH to give *p*-nitrobenzyl tosylate (3655 mg, 91%) as a burnt orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89–6.94 (m, 2H), 3.95 (s, 3H), 3.34 (s, 3H), 3.30 (s, 1H), 1.34–2.20 (m, 12H).

2. Dose dependent response of HyCL-3 to NTR and NADH. Dose dependent responses to NTR and NADH for **HyCL-3** were acquired using a Hitachi F-7000 Fluorescence Spectrophotometer (Hitachi, Tokyo, Japan) using the luminescence detection module and setting luminescence emission at 525 nm. 10 µM **HyCL-3** was treated with 400 µM NADH and 0, 2.5, 5, 7.5, 10, and 12.5 µg mL^{-1} NTR. Scans were started immediately after addition of all agents, and luminescence was measured over 30 min. The integrated emission intensity was measured on a Cytation 5 BioTek plate reader via luminescence detection mode at 37 ºC using a gain set to 135. Measurements were taken every 20 minutes over 4 hours. Reported values are the average of 3 technical replicates.

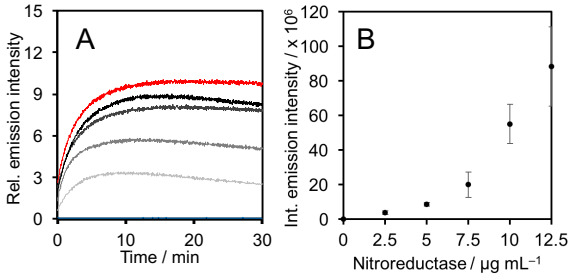


Figure S1. Response of HyCL-3. (A) Time-course and (B) integrated emission intensity of the chemiluminescence emission of 10 µM **HyCL-3** alone (blue trace) or with 2.5, 5, 7.5, 10, and (red trace) 12.5 mg mL^{-1} NTR, and 0.4 mM NADH. All experiments were performed in 20 mM PBS (pH 7.4) containing ≤ 1% DMSO. Values are the average of 3 technical replicates. Error bars are ± S.D.

3. GC/MS determination of HyCL-3 and HyCL-4-AM products. In a 20 mL vial, 1.5 mg of **HyCL-3** or **HyCL-4-AM** was dissolved in 10 mL acetone. Then, 9.9 mL of 100 µM NADPH were added to the vial and 100 µL of 20 mg mL^{-1} RLM were added for a final concentration of 122 µM **HyCL-4-AM** or 144 µM **HyCL-3**, 50 µM NADPH and 200 µg mL^{-1} RLM. The reaction was capped and allowed to stir for 12 hr. Upon completion, the reaction was transferred to a separatory funnel and washed with sat. NH_4Cl. The organic layer was eluted with 3 x 10 mL EtOAc. The organic layer was then dried with Na_2SO_4, filtered, and concentrated under reduced pressure, reconstituted with 1.5 mL DCM, and transferred into a 2 mL GC/MS vial. GC/MS was conducted immediately using a 6850 Series GC/MS (Agilent Technologies, Santa Clara, CA). Mass spectra were averaged across the major peaks in the chromatogram and molecular ions for m/z = 150.1 (Figure S1) and m/z = 138.8 (Figure S2) were found. The spectrum of 2-adamantanone

was matched against the NIST standard, which can be found via web at: https://webbook.nist.gov/cgi/cbook.cgi?ID=C700583&Units=CAL&Mask=200#MassSpec, accessed September 26, 2018.

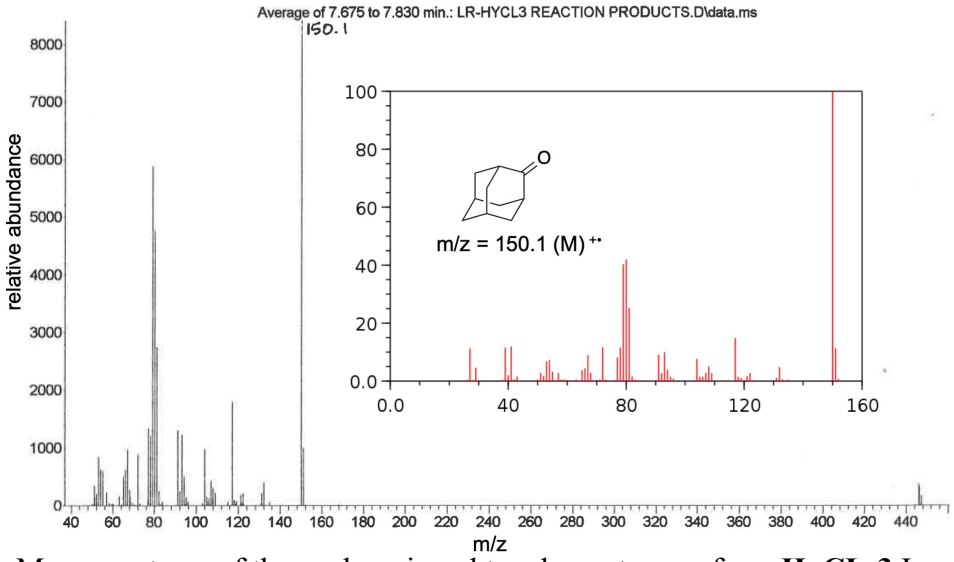


Figure S2. Mass spectrum of the peak assigned to adamantanone from **HyCL-3** Inset is the NIST standard.

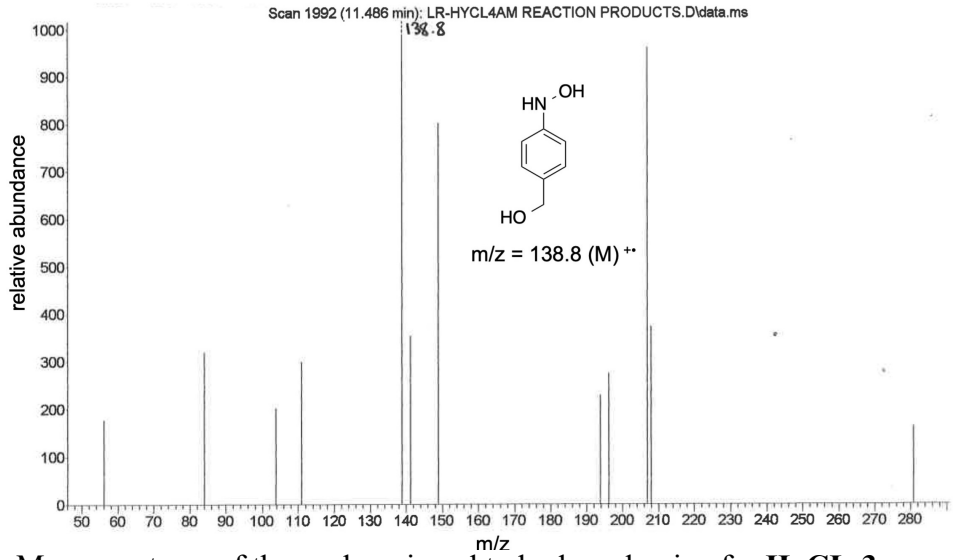


Figure S3. Mass spectrum of the peak assigned to hydroxylamine for **HyCL-3.**

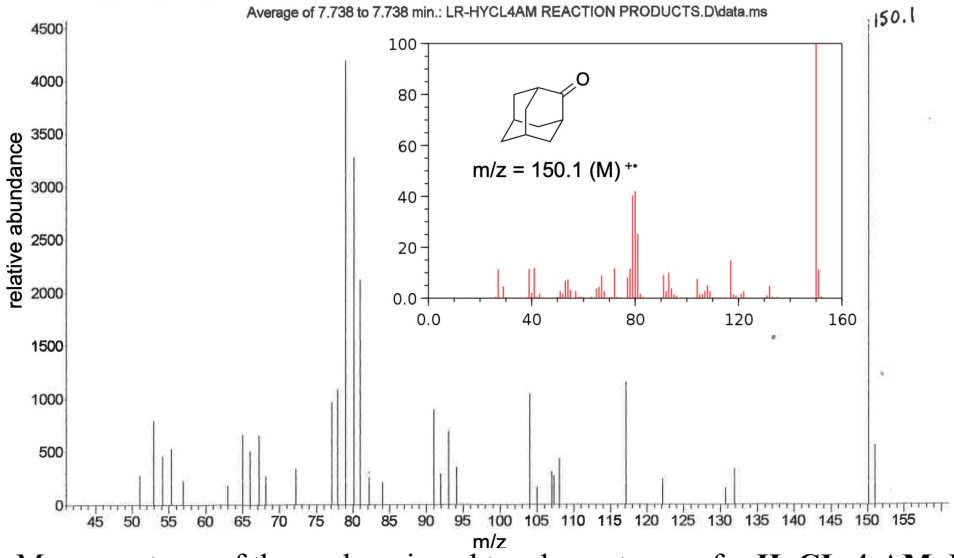


Figure S4. Mass spectrum of the peak assigned to adamantanone for **HyCL-4-AM**. Inset is the NIST standard.

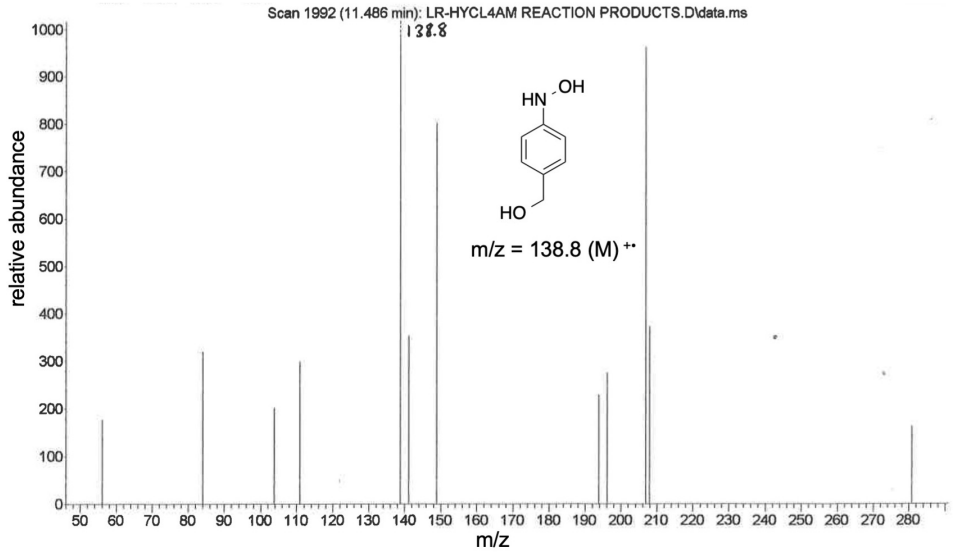


Figure S5. Mass spectrum of the peak assigned to the hydroxylamine for **HyCL-4-AM**.

4. Selectivity studies. Selectivity for **HyCL-3** was measured by monitoring the time-dependent full-spectrum chemiluminescence emission using a Cytation 5 BioTek plate reader (Winooski, VT) by using the luminescence detection method, endpoint read type, and setting gain to 135 and temperature to 37 ºC. All assays were performed in 20 mM PBS buffered to pH 7.4. All analytes were tested with a final concentration of 200 µM for all metal species, 5 mM for GSH, 1 mM for cysteine, and 200 µM for Na_2S. Values are the average of 3 technical replicates.

Cu^{2+} (200 μM): 1 μL of 50 mM $Cu(OTf)_2$ in DI H_2O was added to a solution of 245 μL 20 mM PBS (pH 7.43) buffer and 1 μL of 5 mM **HyCL-3** or **HyCL-4-AM** (20 μM final concentration) in DMSO.

Fe^{2+} (200 μM): 1 μL of 50 mM $Fe(OTf)_2$ in DI H_2O was added to a solution of 245 μL 20 mM PBS (pH 7.43) buffer and 1 μL of 5 mM **HyCL-3** or **HyCL-4-AM** (20 μM final concentration) in DMSO.

Fe^{3+} (200 μM): 1 μL of 50 mM $Fe(OTf)_3$ in DI H_2O was added to a solution of 245 μL 20 mM PBS (pH 7.43) buffer and 1 μL of 5 mM **HyCL-3** or **HyCL-4-AM** (20 μM final concentration) in DMSO.

Mn^{2+} (200 μM): 1 μL of 50 mM $Mn(OTf)_2$ in DI H_2O was added to a solution of 245 μL 20 mM PBS (pH 7.43) buffer and 1 μL of 5 mM **HyCL-3** or **HyCL-4-AM** (20 μM final concentration) in DMSO.

Co^{2+} (200 μM): 1 μL of 50 mM $Co(OAc)_2$ in DI H_2O was added to a solution of 245 μL 20 mM PBS (pH 7.43) buffer and 1 μL of 5 mM **HyCL-3** or **HyCL-4-AM** (20 μM final concentration) in DMSO.

Ni^{2+} (200 μM): 1 μL of 50 mM $Ni(OTf)_2$ in DI H_2O was added to a solution of 245 μL 20 mM PBS (pH 7.43) buffer and 1 μL of 5 mM **HyCL-3** or **HyCL-4-AM** (20 μM final concentration) in DMSO.

Zn^{2+} (200 μM): 1 μL of 50 mM $Zn(OAc)_2$ in DI H_2O was added to a solution of 245 μL 20 mM PBS (pH 7.43) buffer and 1 μL of 5 mM **HyCL-3** or **HyCL-4-AM** (20 μM final concentration) in DMSO.

GSH (5 mM): 10 μL of 125 mM GSH in 0.01 M NaOH was added to a solution of 236 μL 20 mM PBS (pH 7.43) buffer and 1 μL of 5 mM **HyCL-3** or **HyCL-4-AM** (20 μM final concentration) in DMSO.

Cysteine (1 mM): 1 μL of 250 mM cysteine in 0.01 M NaOH was added to a solution of 245 μL 20 mM PBS (pH 7.43) buffer and 1 μL of 5 mM **HyCL-3** or **HyCL-4-AM** (20 μM final concentration) in DMSO.

Na_2S (200 μM): 1 uL of 50 mM Na_2S in 0.01 M NaOH was added to a solution of 245 μL 20 mM PBS (pH 7.43) buffer and 1 μL of 5 mM **HyCL-3** or **HyCL-4-AM** (20 μM final concentration) in DMSO.

5. Cell culture and biological studies. Human lung adenocarcinoma epithelial cells (A549) cells were purchased from ATCC and cultured in Ham's F-12K (Kaighn's) Medium supplemented with 10% Fetal Bovine Serum (FBS) and 1% antibiotics (penicillin/streptomycin, 100 U/mL). Cells were maintained in a humidified incubator at 37 °C with 5% CO_2. One or two days before the experiment, cells were passaged and plated on Costar® 12-well plates by adding 150K–200K of cells per well, filling each well up to 1 mL of media. Chemiluminescence responses were measured using a Cytation 5 BioTek plate reader (Winooski, VT).

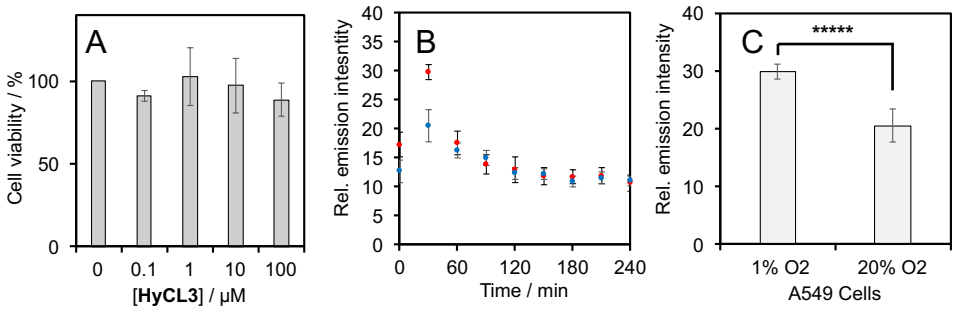


Figure S6. HyCL-3 Cellular Data. (A) A549 cells were incubated with 0–100 µM **HyCL-3** for 16 hours and then viability was evaluated using the MTT assay. Error bars are ± S.D. from n = 3 replicates. (B) Time-course of the chemiluminescence emission of A549 cells incubated with 40 µM **HyCL-3** at (red trace) 1% O_2 or (blue trace) 20% O_2. (C) Luminescence intensity of 40 µM **HyCL-3** at t = 30 min in 1% O_2 and 20% O_2 in A549 cells. Error bars are ± S.D. from n = 9 wells across 3 biological replicates. Statistical significance was assessed using a Student's two-tailed *t*-test. ***** $p<5x10^{-6}$.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Human lung adenocarcinoma epithelial cells (A549) were purchased from ATCC and were seeded in a 96-well plate to a total volume of 120 µL/ well. The plate was maintained at 37 °C with 5% CO_2 for 12 h. Then the medium was removed upon reaching 70%–80% confluency and the cells were washed with PBS. The cells were then incubated for 18 h after adding **HyCL-3** or **HyCL-4-AM** at 0, 0.1, 1, 10, and 100 µM respectively in 125 µL completed F12K media. 10 µL of the MTT reagent (Cayman Chemical, Ann Arbor, MI) was then added to each well, and mixed gently. After 4 h incubation, 100 µL of crystal dissolving solution was added to each well to dissolve the formazan crystals. Absorbance was measured at 570 nm in a Cytation 5 BioTek plate reader and cell viability was expressed as a percent of the control.

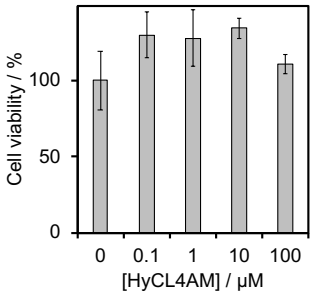


Figure S7. HyCL-4-AM MTT assay. A549 cells were incubated with 0–100 µM **HyCL-3** for 16 hours and then viability was evaluated using the MTT assay. Error bars are ± S.D. from n = 3 technical replicates.

Hypoxia detection in A549 cells. Human lung adenocarcinoma epithelial cells (A549) were seeded in a 12-well plate to a total volume of 1 mL per well. Before imaging, the media was removed upon 70%–80% confluency and the cells were washed with 1 mL PBS. Each well was filled with 996 µL F-12K media. Then, 4 µL of 10 mM **HyCL-3** in DMSO (40 µM final concentration) was added to each well and immediately placed into a Cytation 5 BioTek plate reader. Then, the luminescence was recorded using the luminescence detection mode, end point read type. The temperature was set at 37 °C, O_2 level was set to 1% or 20%, and CO_2 was set at 5%. Each experiment consisted of three technical replicates for each condition, and each experiment was repeated with three biological replicates on three separate days. The reported integrated chemiluminescence intensity values are the average of a total of nine wells across three biological replicates. Control experiments with **HyCL-4-AM** and **HyCL-4** were performed in F12K media in the absence of cells and reported values are the average of three technical replicates.

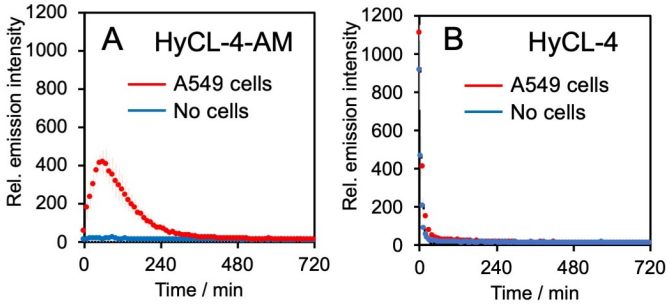


Figure S8. Comparison of the cellular response of **HyCL-4-AM** and **HyCL-4**. (A) Time-course of the chemiluminescent emission of 40 µM **HyCL-4-AM** in the (red trace) presence and (blue trace) absence of A549 cells at 1% O_2 and 37 ºC. (B) Time-course of the chemiluminescent emission of 40 µM **HyCL-4** in the (red trace) presence and (blue trace) absence of A549 cells at 1% O_2 and 37 ºC. Values are the average of n = 3 technical replicates, except for the red trace in Figure S8A, which are the average of n = 9 wells across three biological replicates.

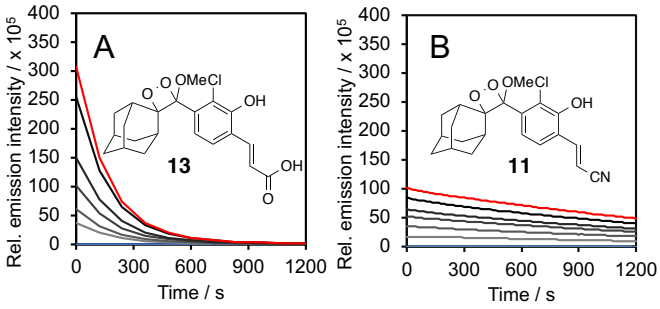


Figure S9. (A) Time-course of the chemiluminescent emission of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and (red trace) 3.0 μM acrylic acid phenol dioxetane in 20 mM PBS (pH = 7.4) and ≤ 1% DMSO. (B) Time-course of the chemiluminescent emission of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and (red trace) 3.0 μM acrylonitrile phenol dioxetane in 20 mM PBS (pH = 7.4) and ≤ 1% DMSO.

6. Kinetic modeling.

Measurement of chemiluminescent decomposition of acrylonitrile and acrylic acid dioxetane phenols. The chemiluminescence decomposition rate of the acrylonitrile and acrylic acid dioxetane phenols were obtained using a Cytation 5 BioTek plate reader (Winooski, VT) by using the luminescence detection method, endpoint read type, and setting gain to 135 and temperature to 25–28 ºC. 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 μM **11** or **13** were dissolved in 20 mM PBS buffered to pH 7.4 and placed immediately into the plate reader for measurement. The procedure was timed from the initial addition of probe into solution until the first read to account for time differences for kinetic modeling.

Kinetic fits for the cellular response of HyCL-4-AM. A549 cells were incubated with 40 μM **HyCL-4-AM** and the chemiluminescence response was immediately measure in a Cytation 5 BioTek plate reader using the luminescence detection mode at 37 ºC, gain set to 135, and either 0.5–1% O_2 or 20% O_2. The average of 3 technical replicates in a single plate were fit to the rate equation given in Figure S9B, based on the reactions given in Figure S9A using Mathematica 11.0.0.0 for Mac OS X. The values reported in the manuscript, Figure E are the average values of fits from three or four biological replicates. Example fits are given in Figure S9C–D.

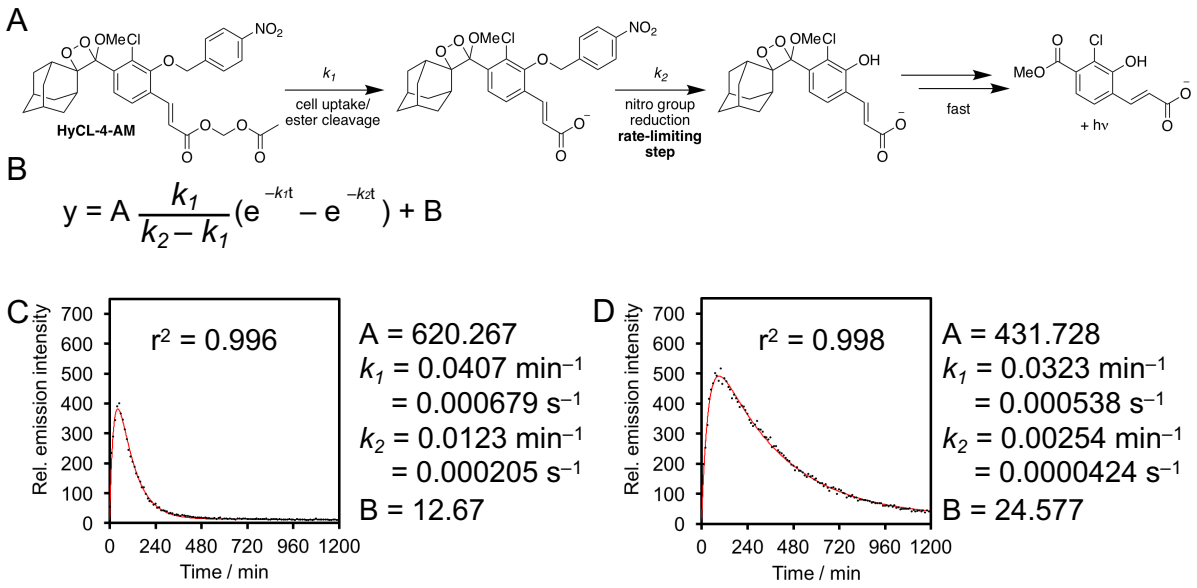


Figure S10. Kinetic model. (A) Reactions used to model the chemiluminescence emission. (B) Rate equation for the chemiluminescence emission. (C) Example fit of the chemiluminescence response of 40 µM **HyCL-4-AM** in A549 cells at 1% O_2. (D) Example fit of the chemiluminescence response of 40 µM **HyCL-4-AM** in A549 cells at 20% O_2.

7. Supplementary references.

(1) Green, O.; Eilon, T.; Hananya, N.; Gutkin, S.; Bauer, C. R.; Shabat, D. Opening a gateway for chemiluminescence cell imaging: distinctive methodology for design of bright chemiluminescent dioxetane probes. *ACS Cent. Sci.* **2017**, *3*, 349–358.

(2) Yang, Y.; Voak, A.; Wilkinson, S. R.; Hu, L. Design, synthesis, and evaluation of potential prodrugs of DFMO for reductive activation. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6583–6586.

8. Scanned spectra.

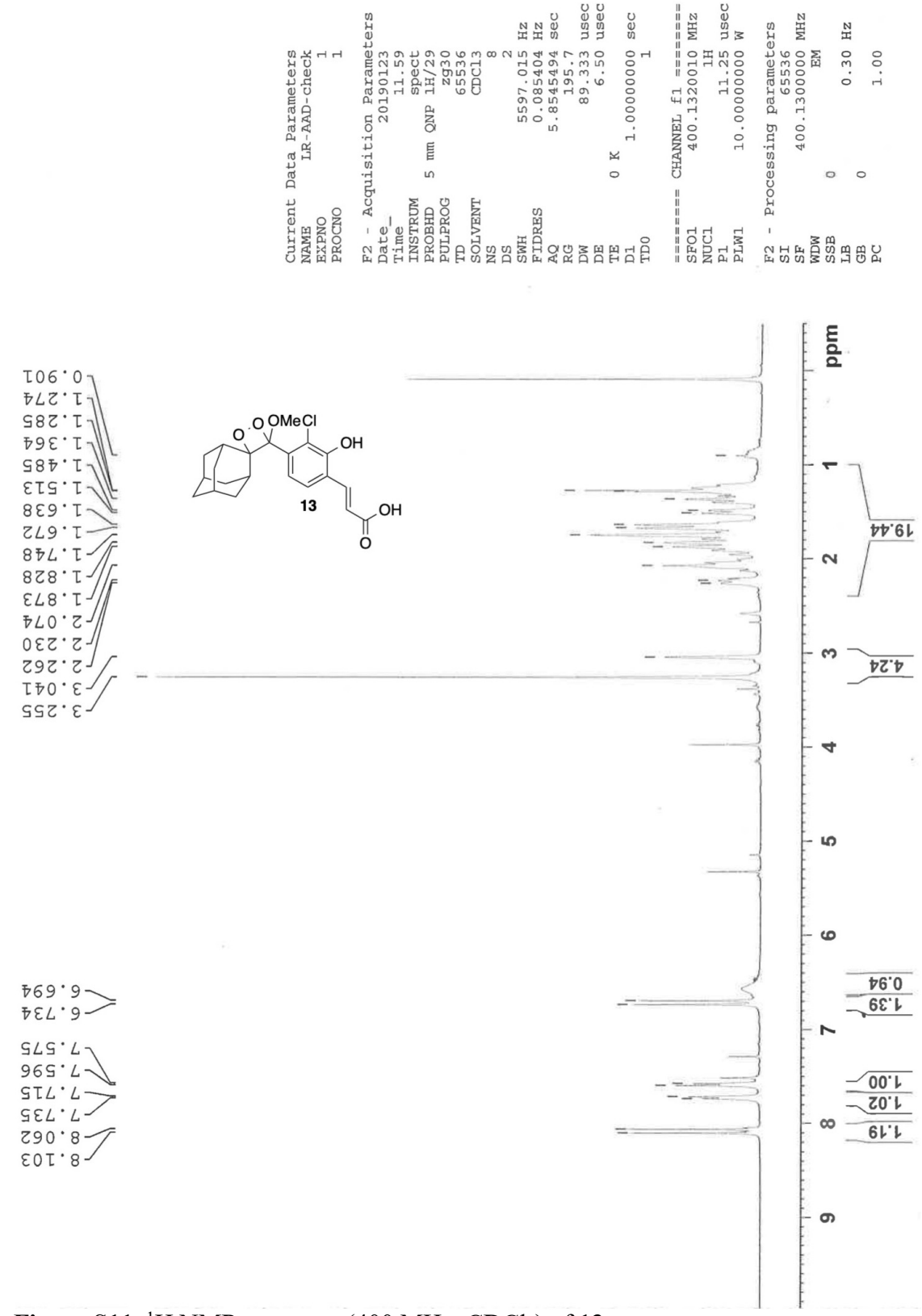


Figure S11. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **13**

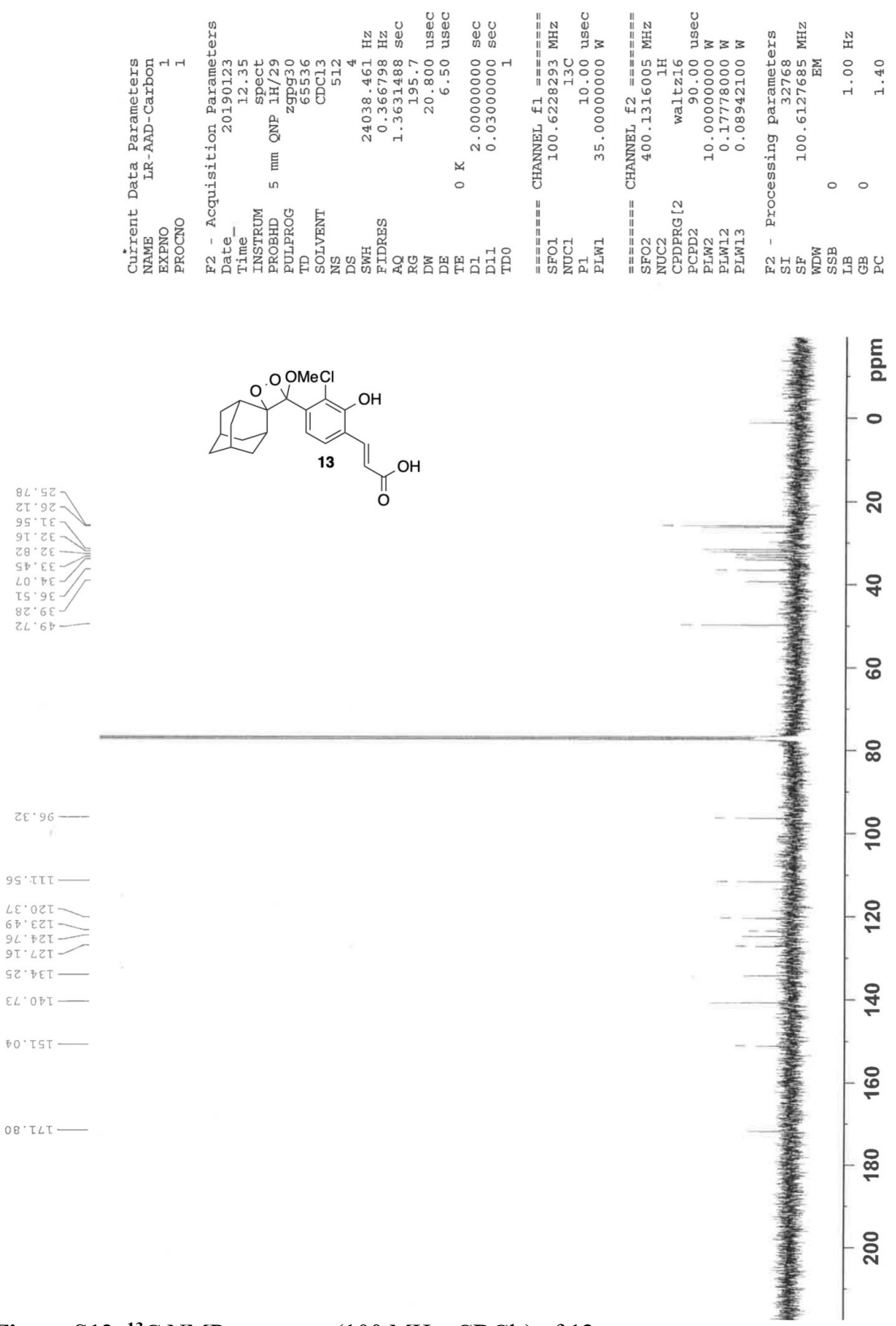


Figure S12. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **13**.

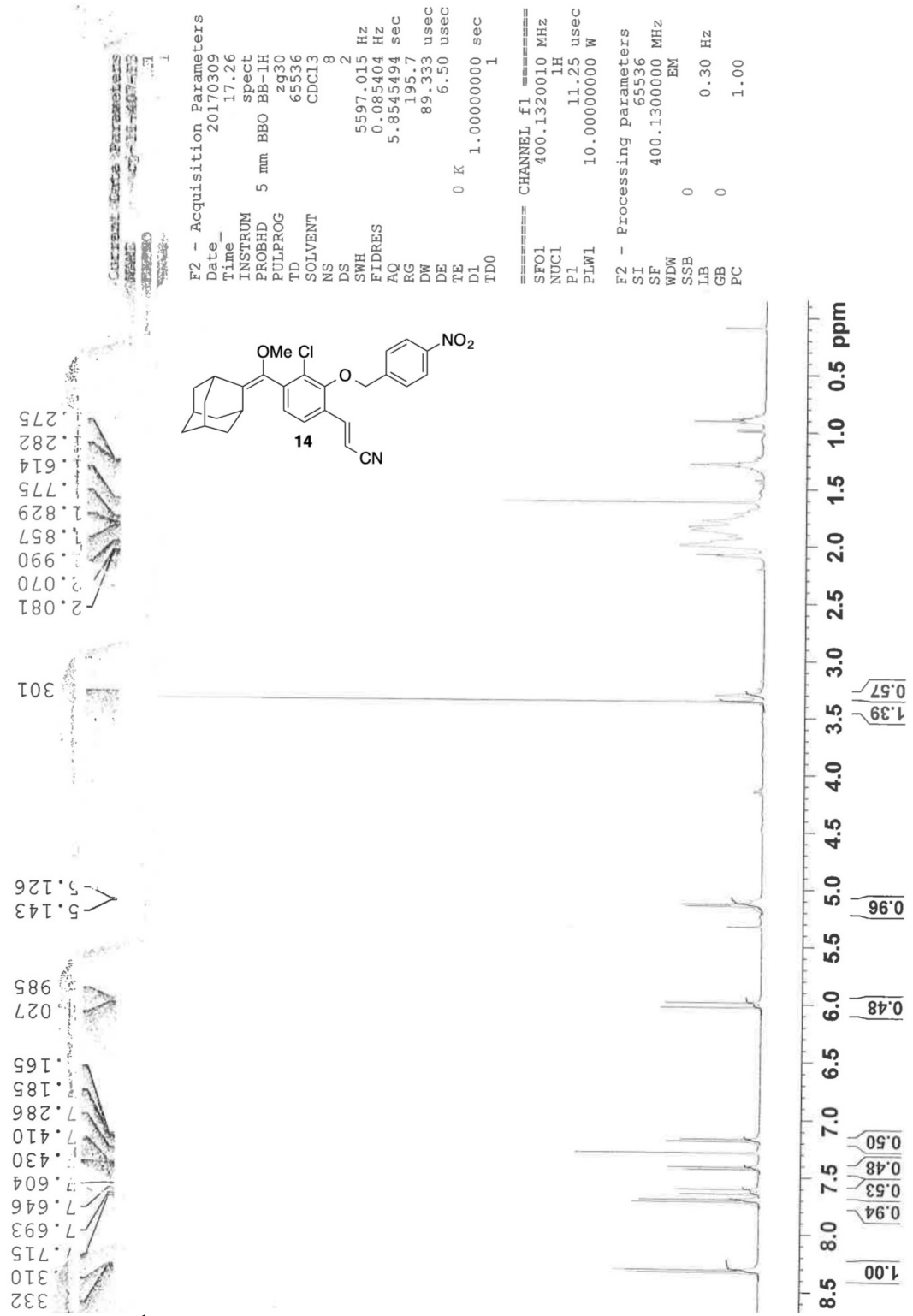


Figure S13. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **14**.

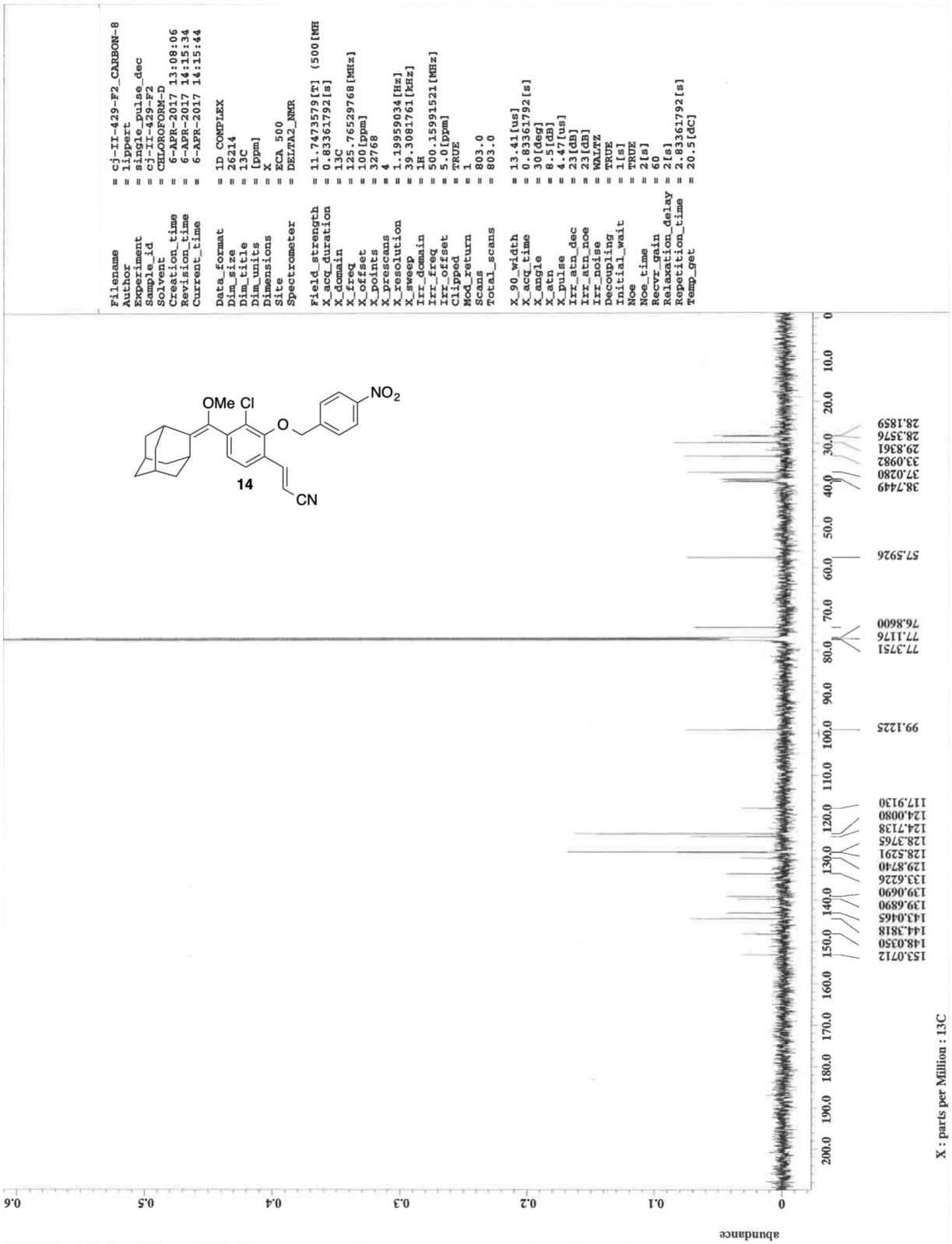


Figure S14. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of **14**.

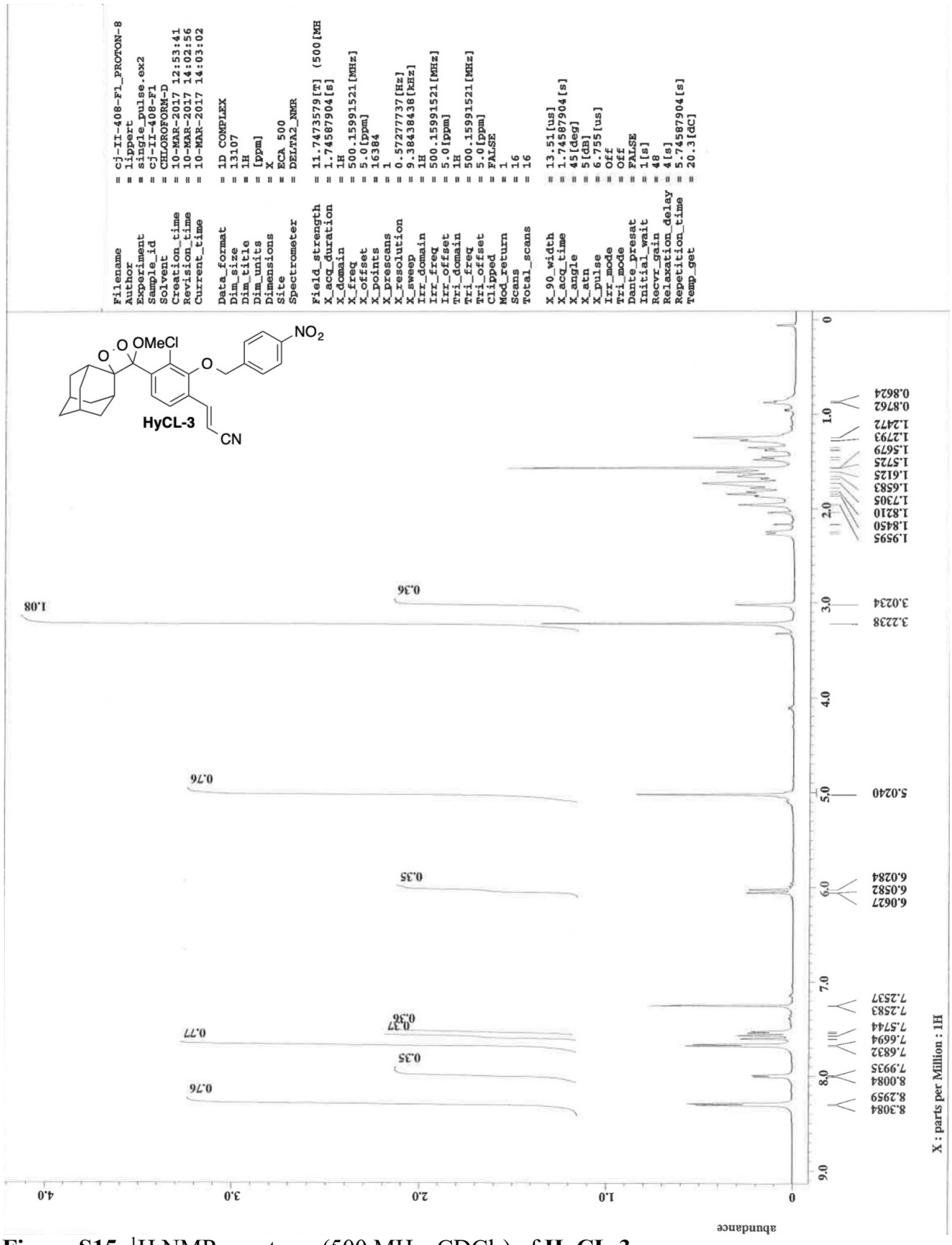


Figure S15. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **HyCL-3**.

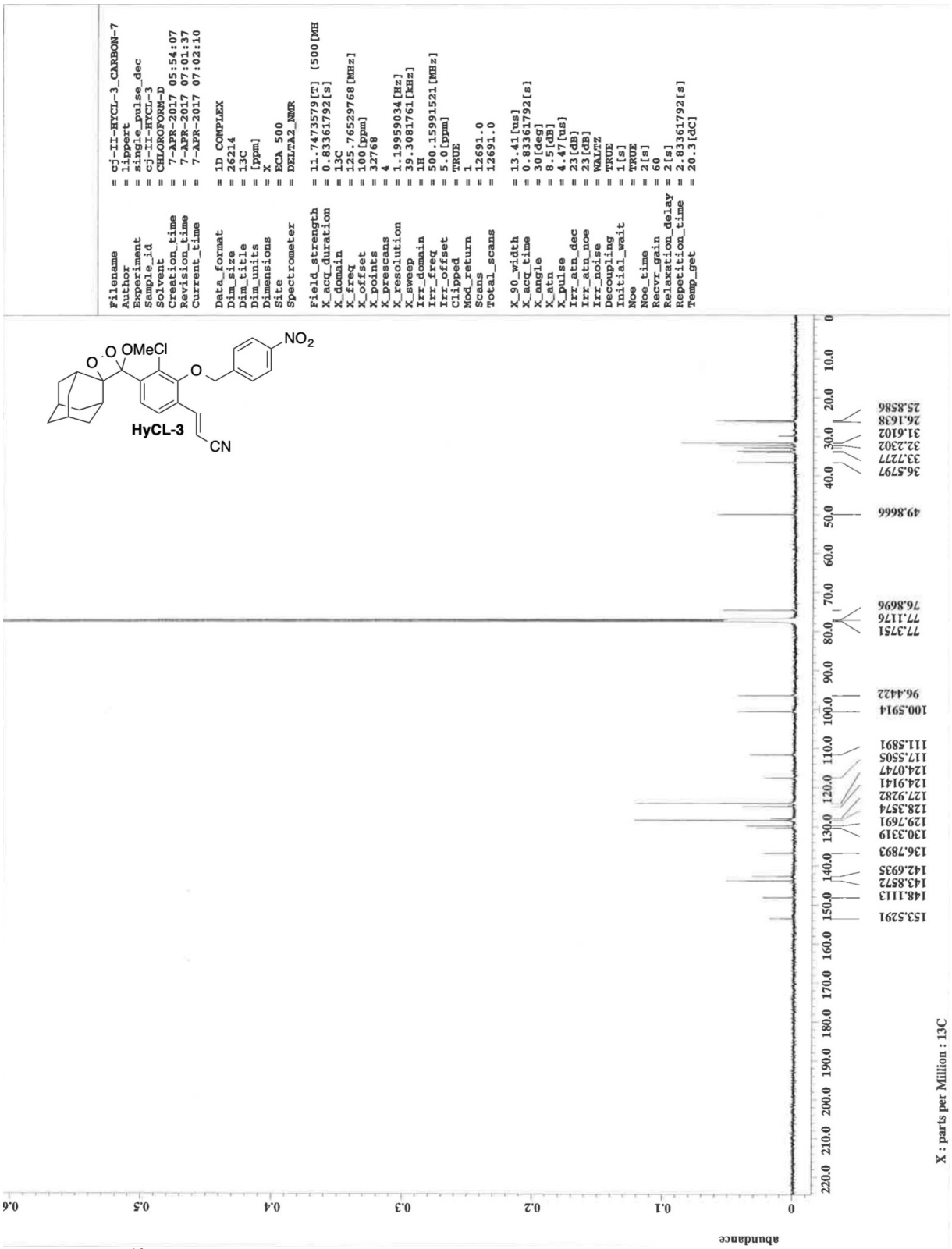


Figure S16. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of **HyCL-3**.

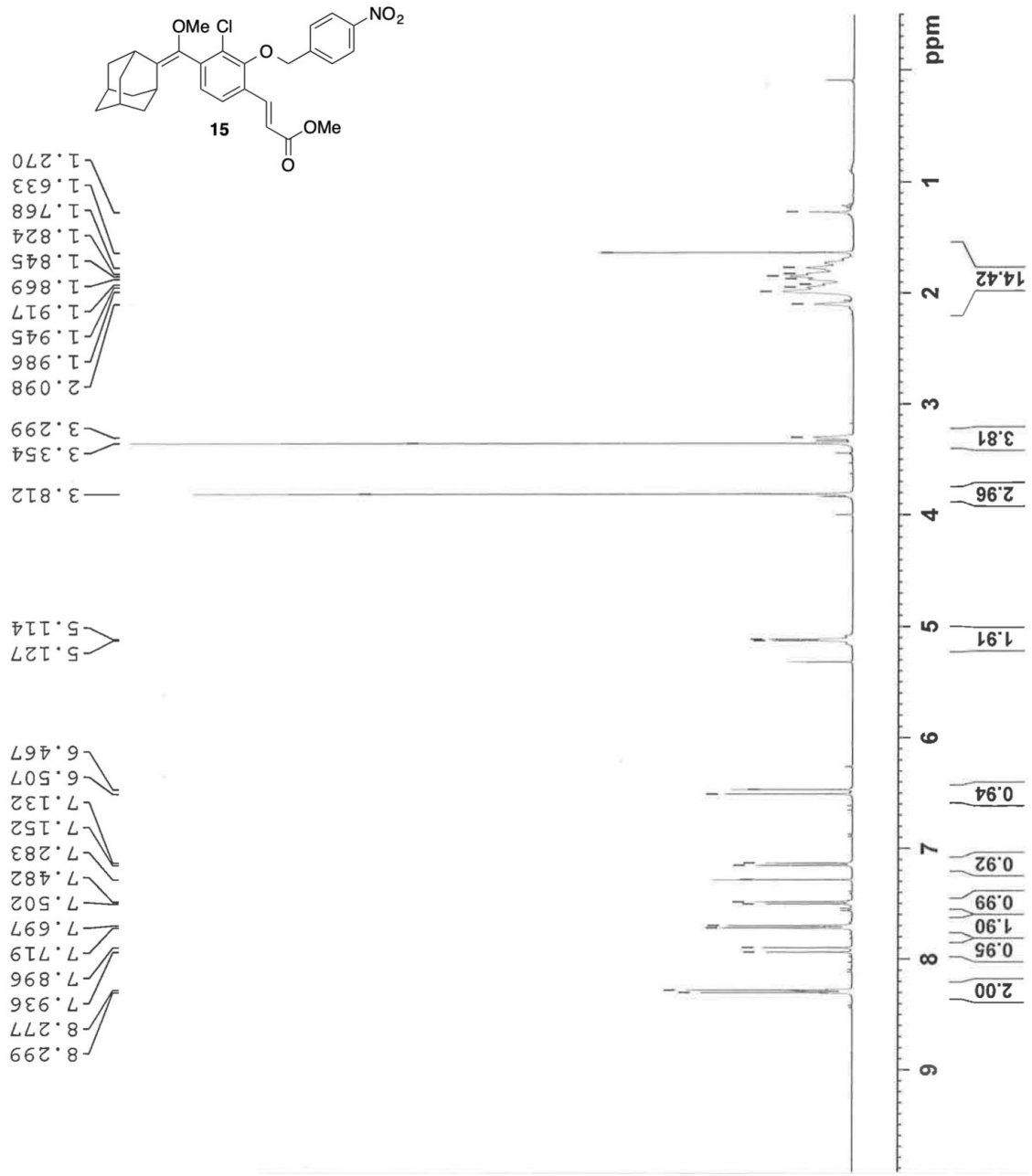


Figure S17. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **15**

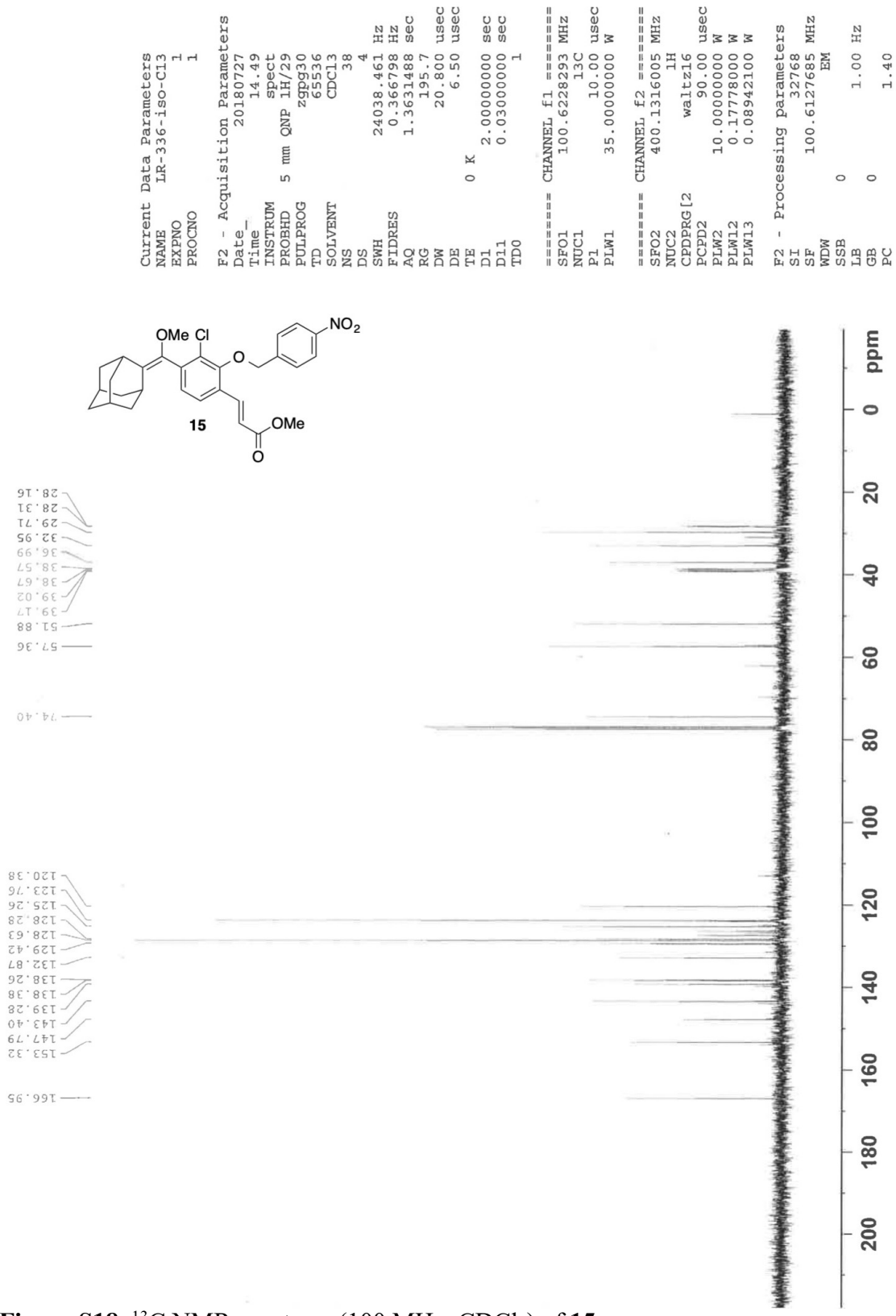


Figure S18. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **15**.

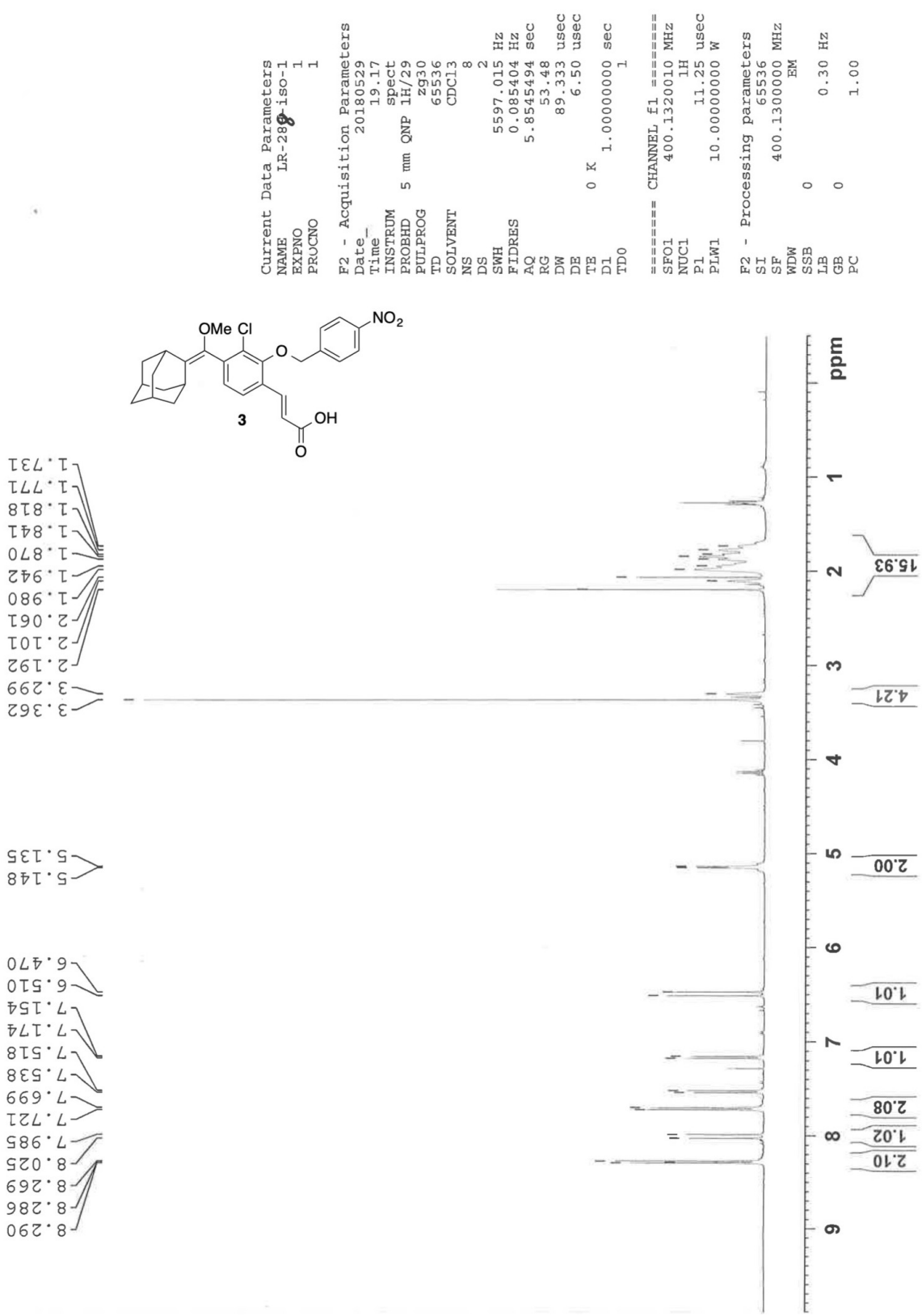


Figure S19. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **3**.

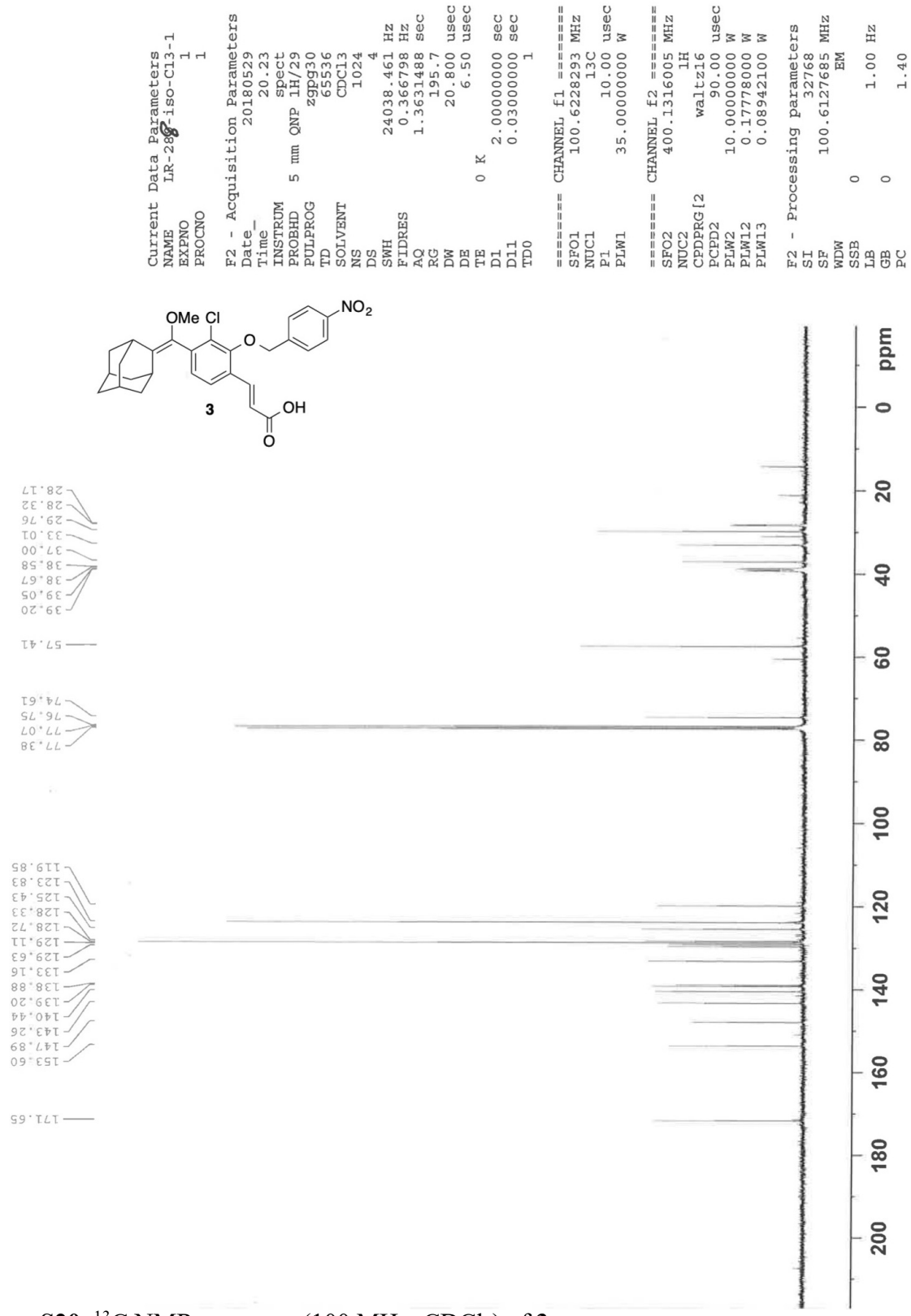


Figure S20. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **3**.

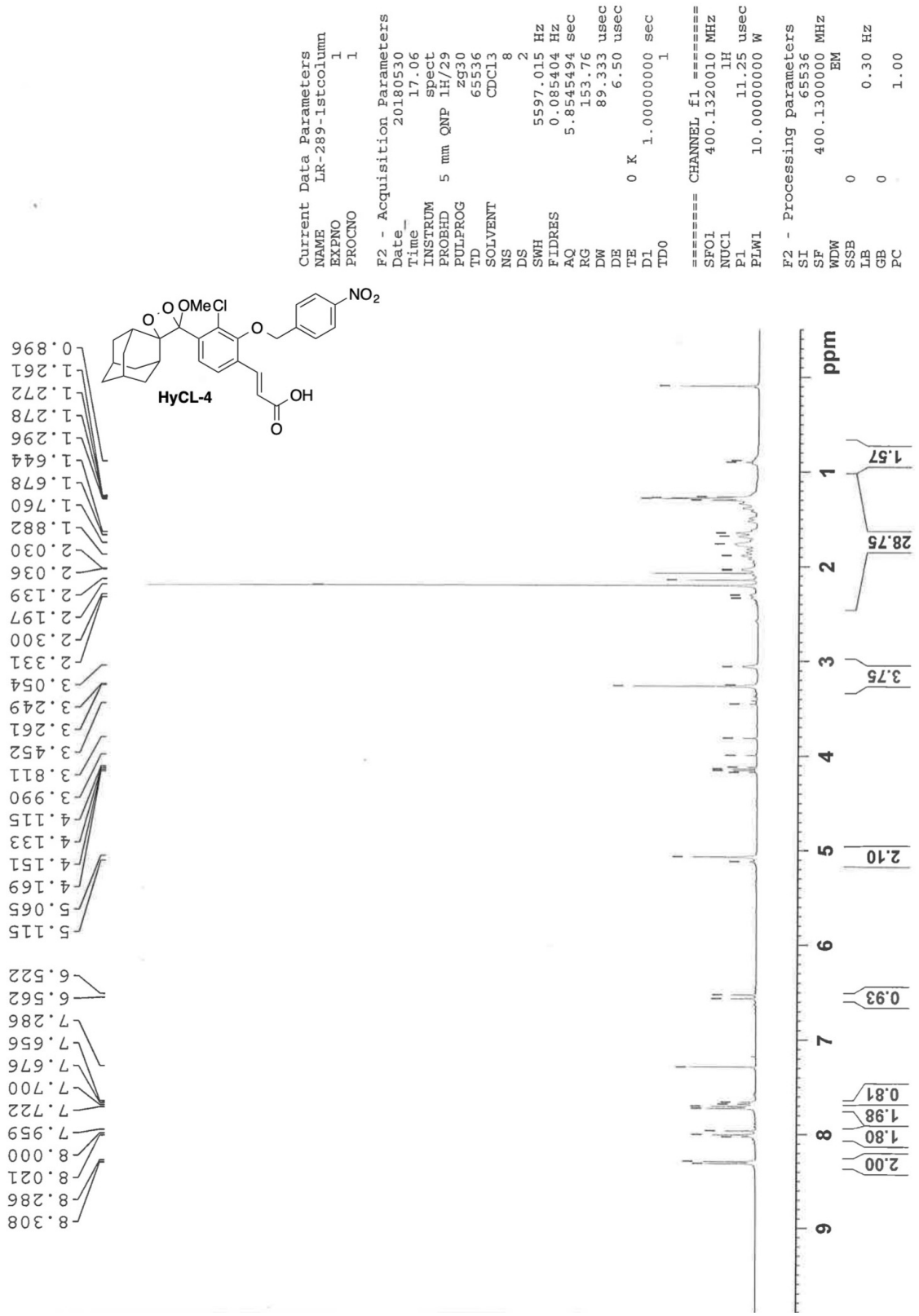


Figure S21. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **HyCL-4**.

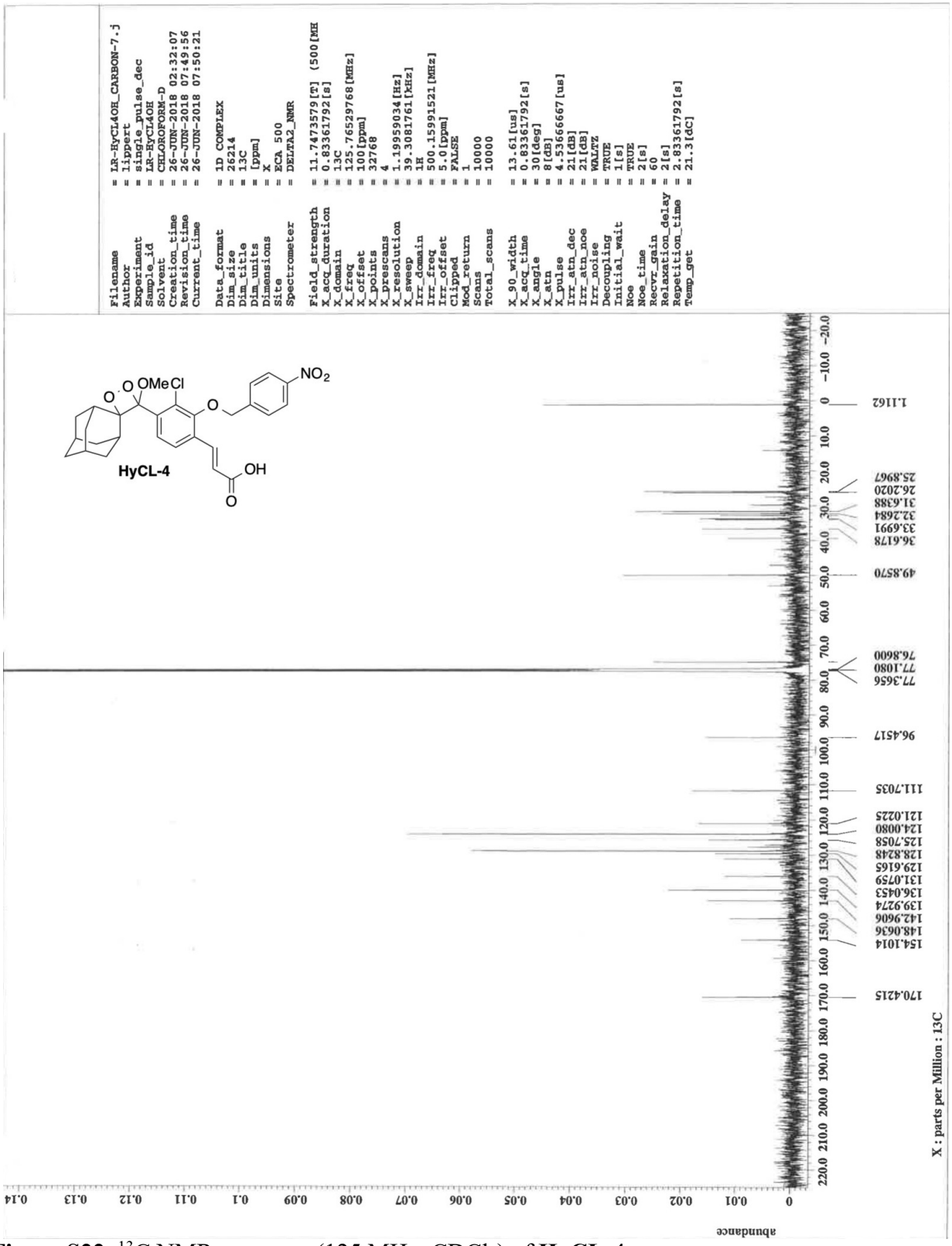


Figure S22. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of **HyCL-4**.

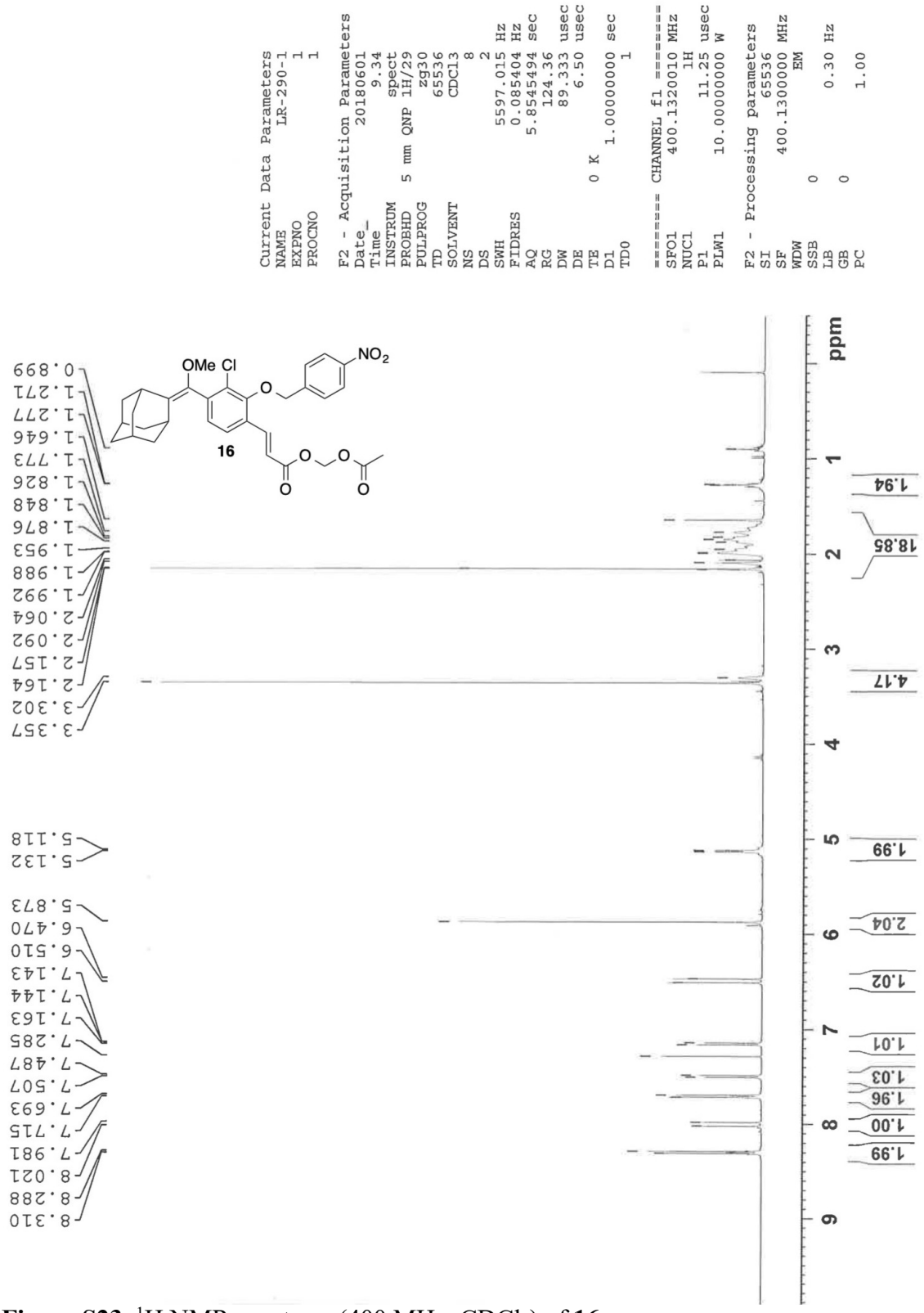


Figure S23. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **16**.

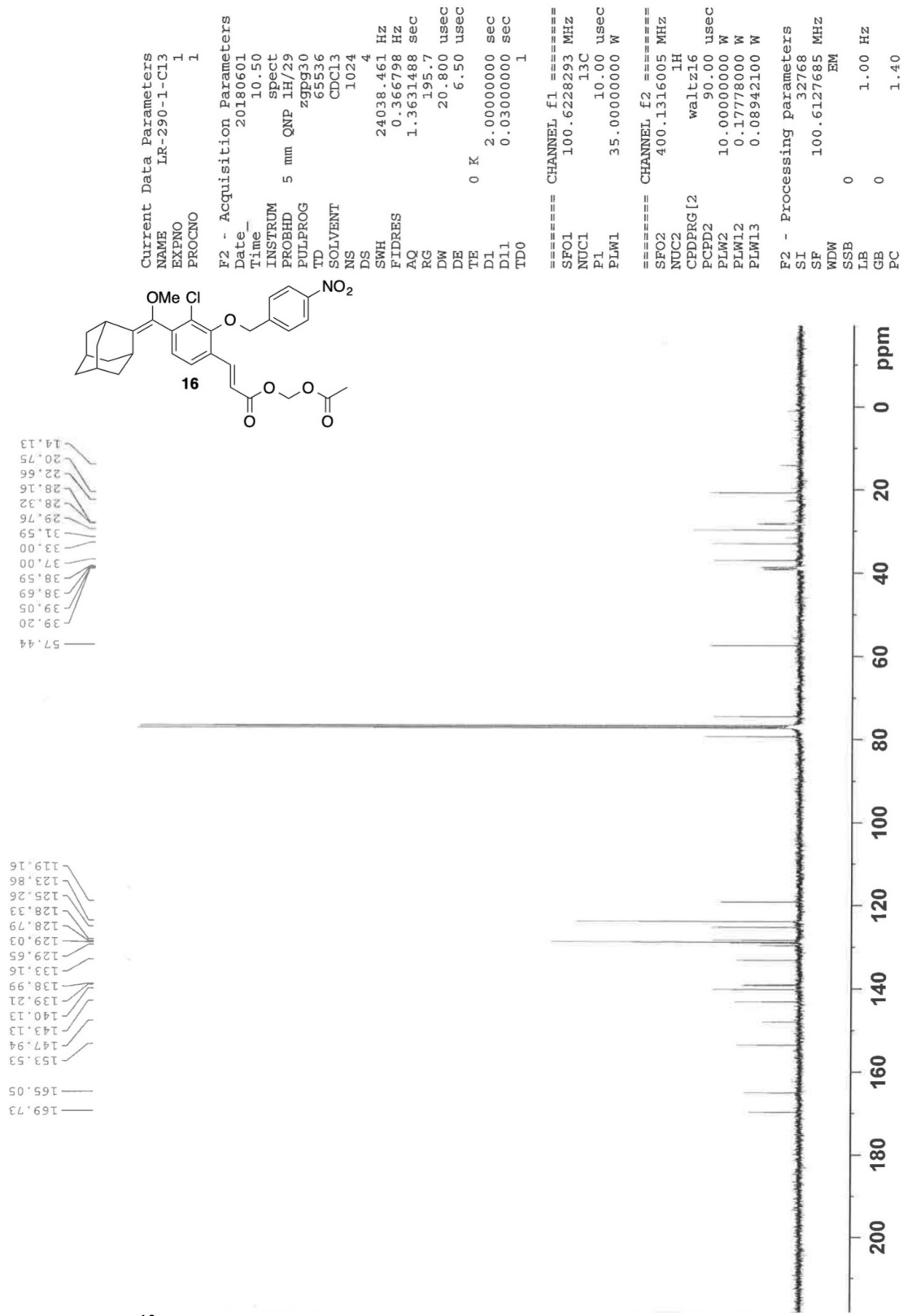


Figure S24. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **16**.

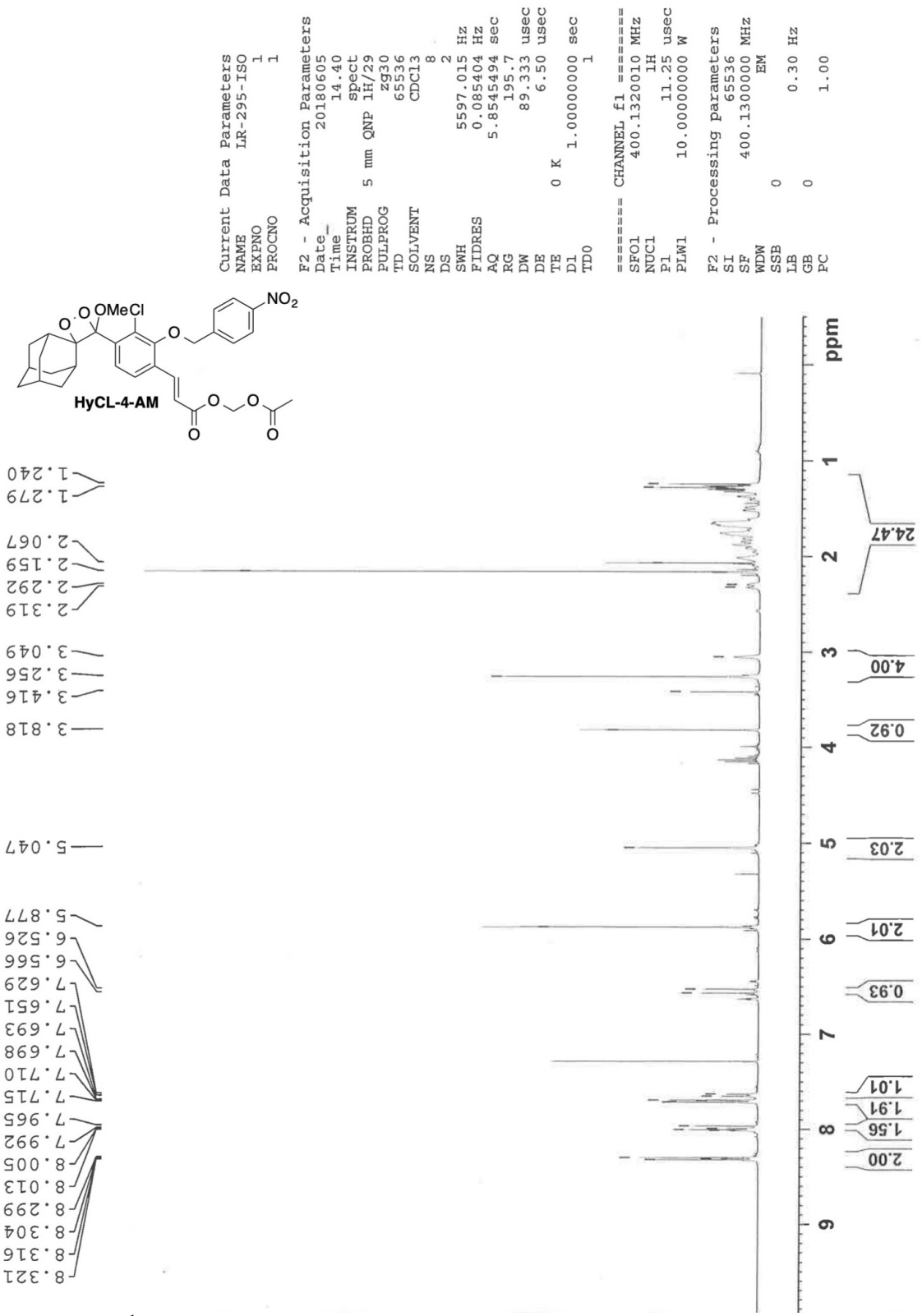


Figure S25. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **HyCL-4-AM**.

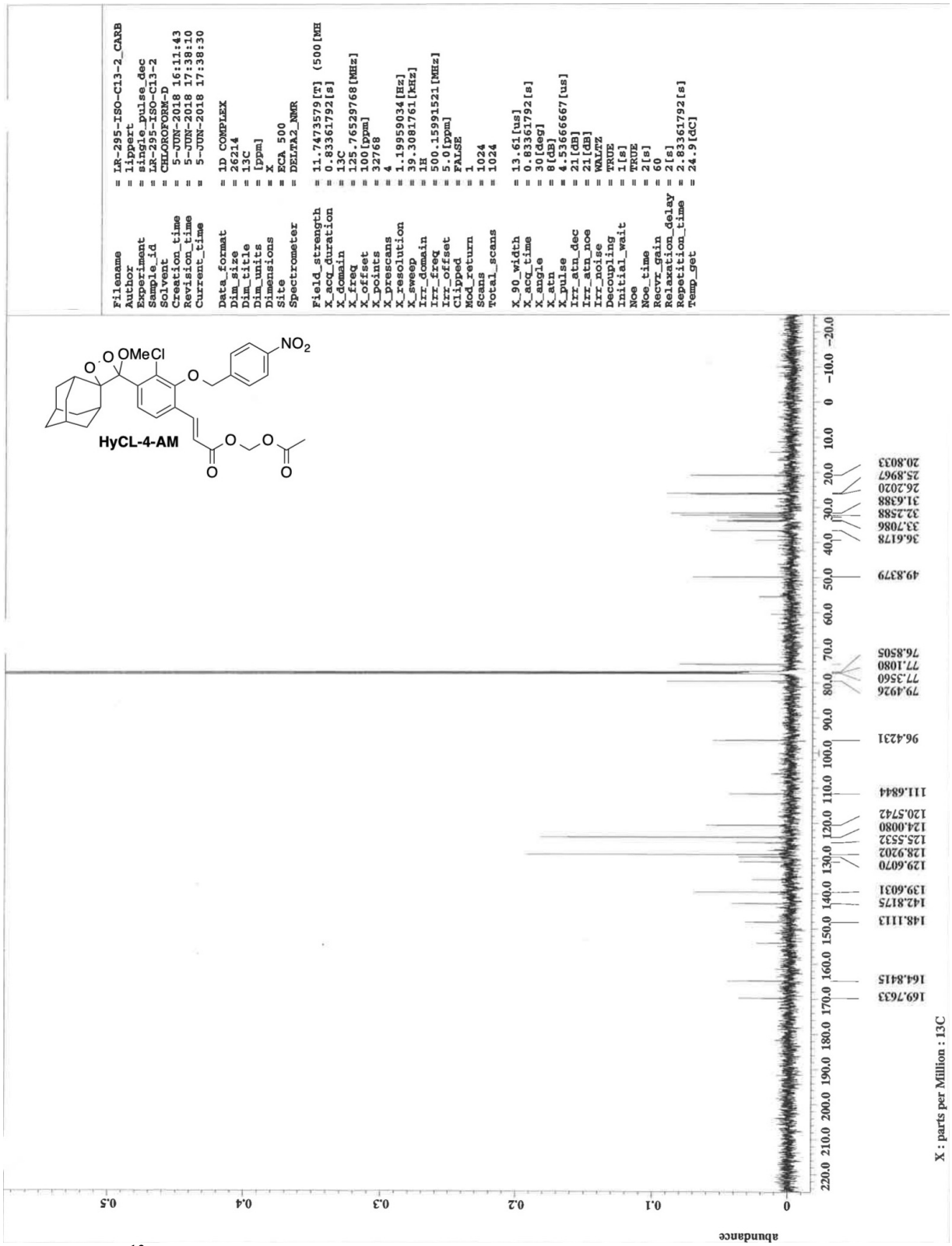


Figure S26. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of **HyCL-4-AM**.

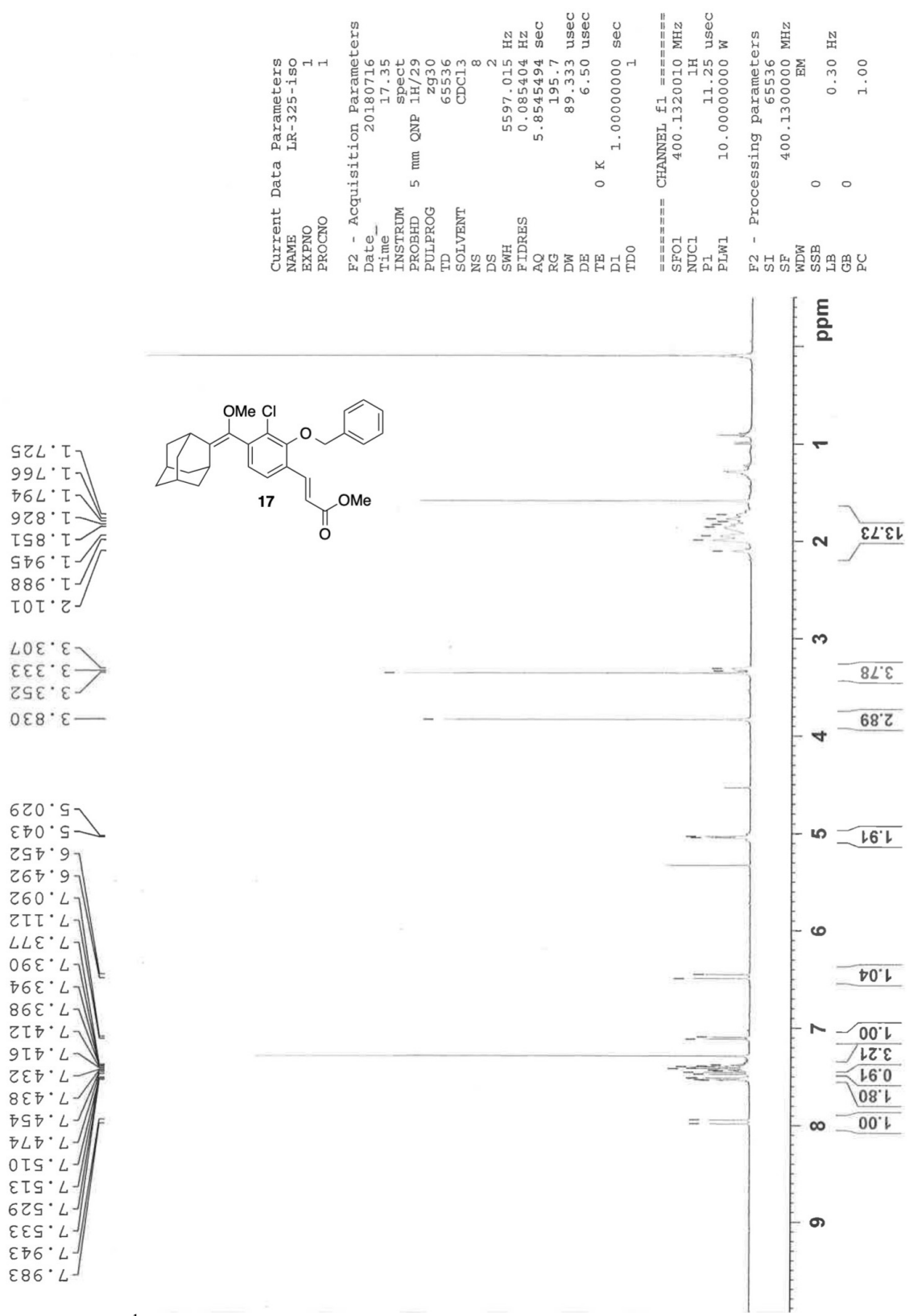


Figure S27. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **17**.

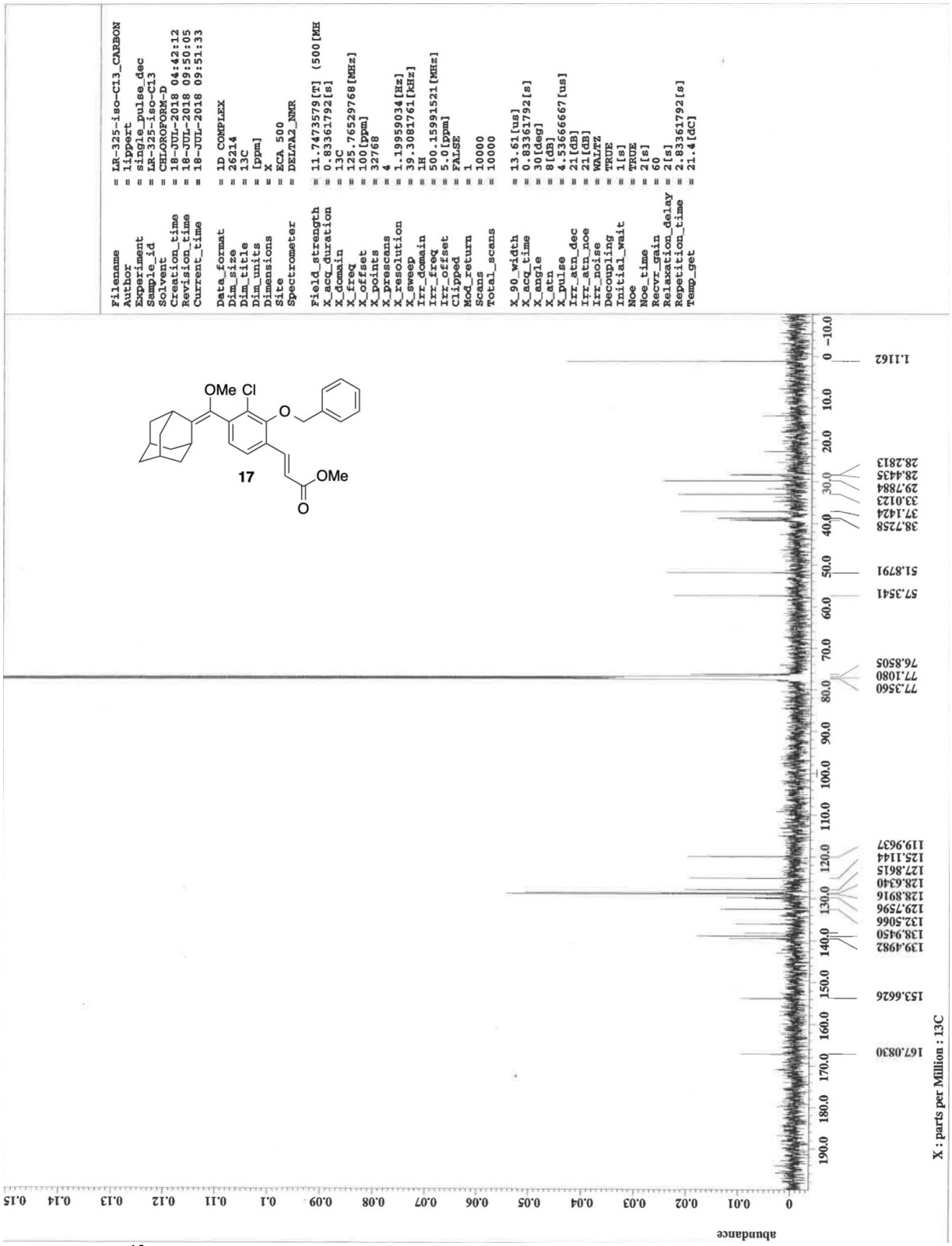


Figure S28. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of **17**.

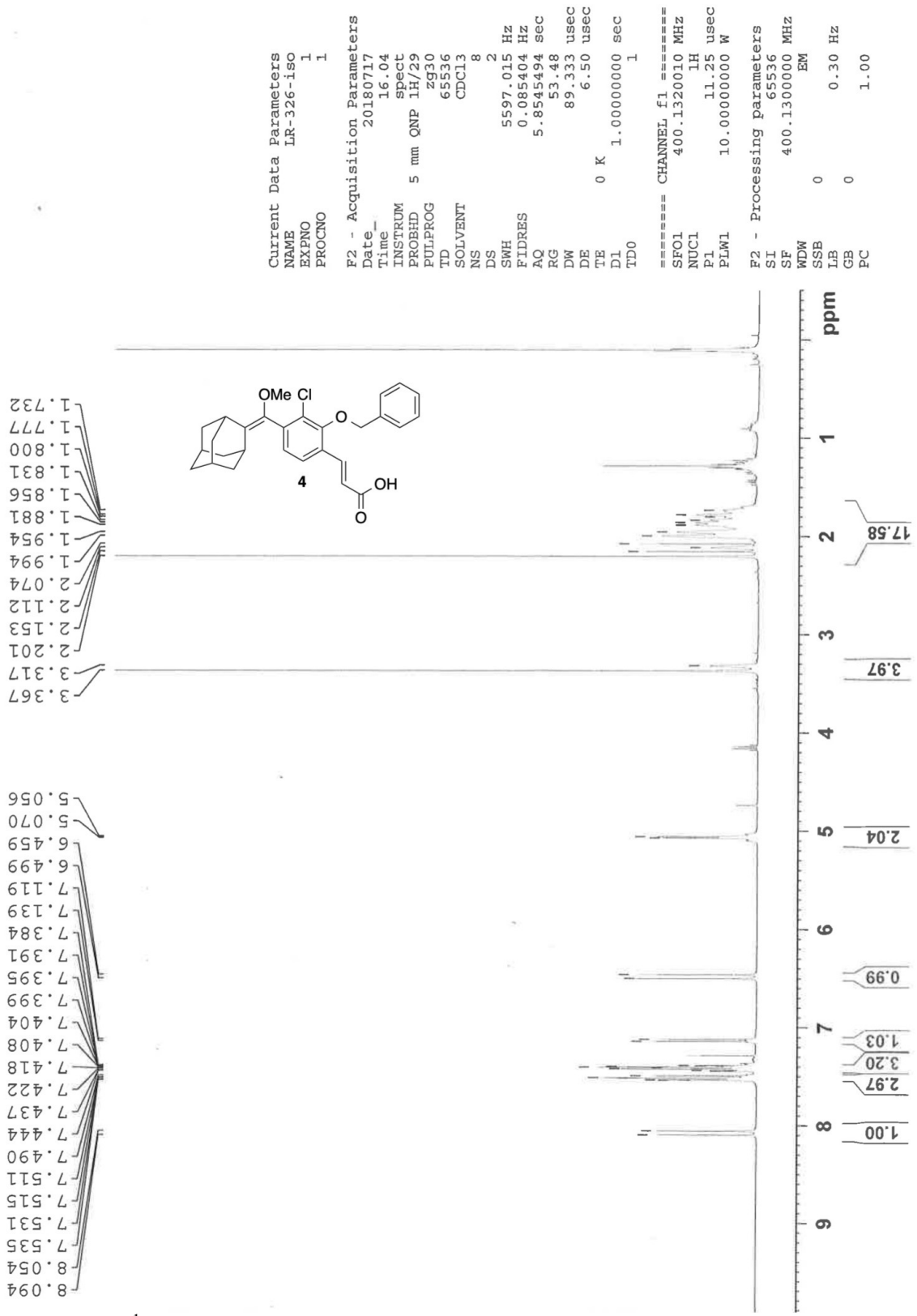


Figure S29. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **4**

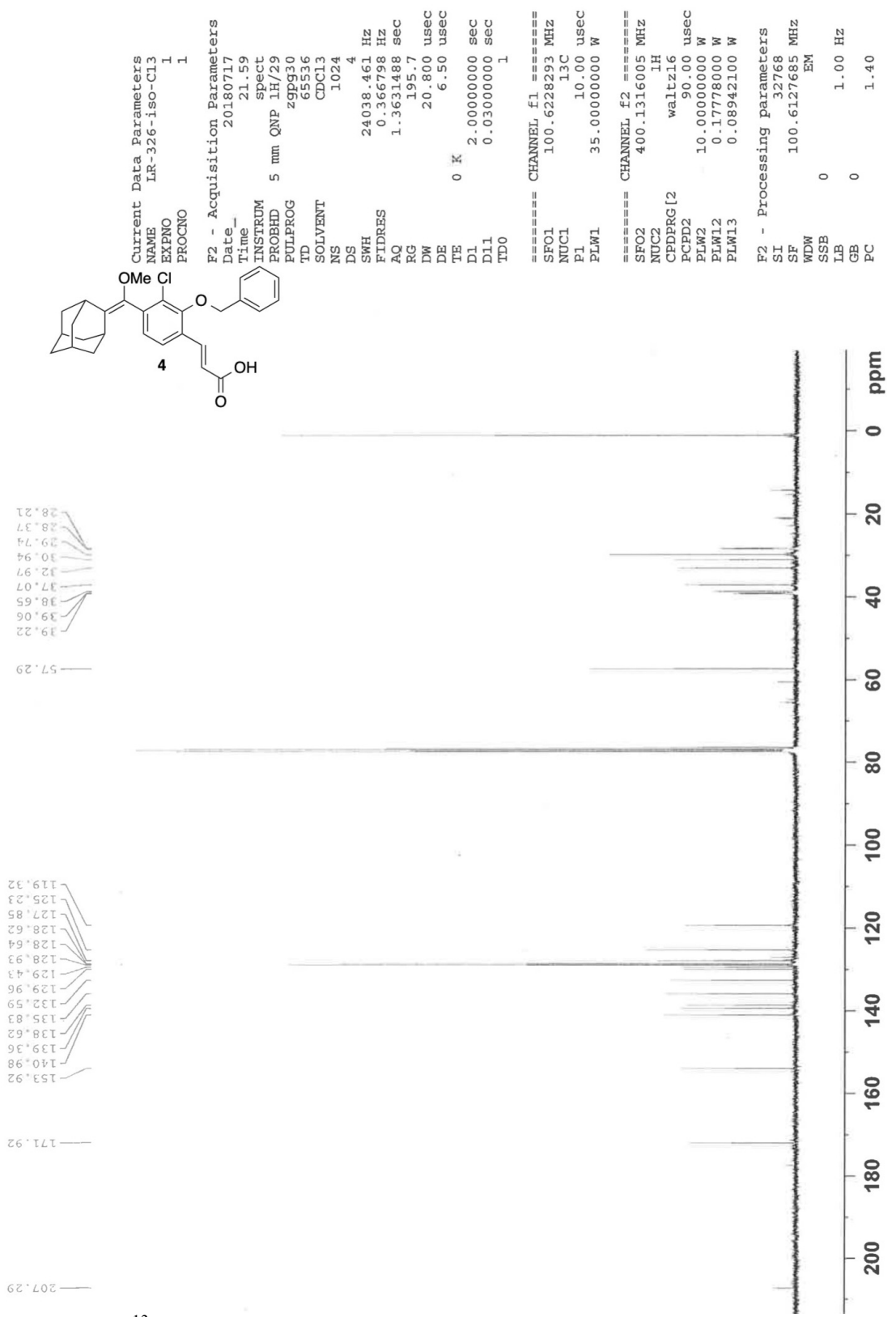


Figure S30. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **4**.

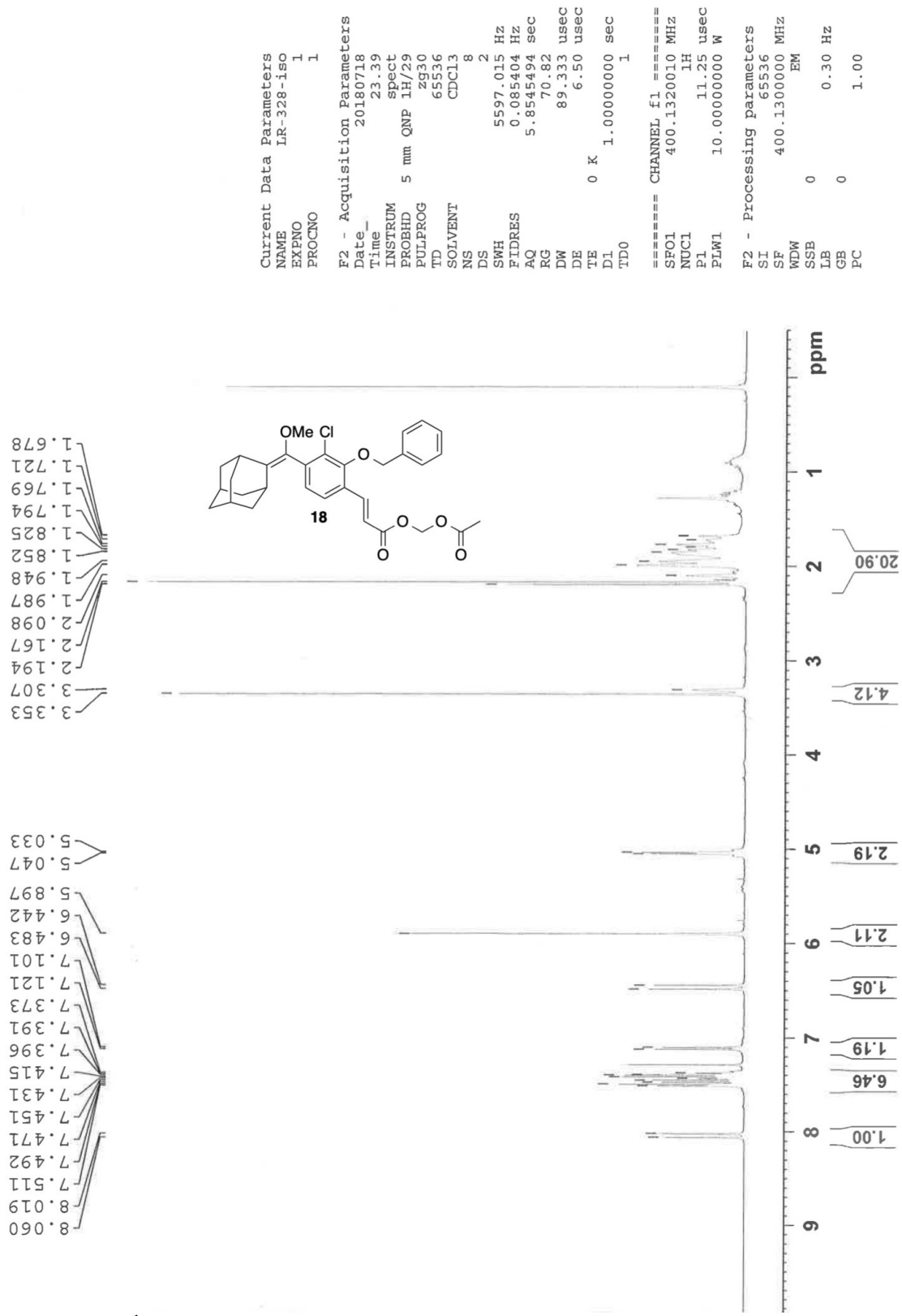


Figure S31. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **18**.

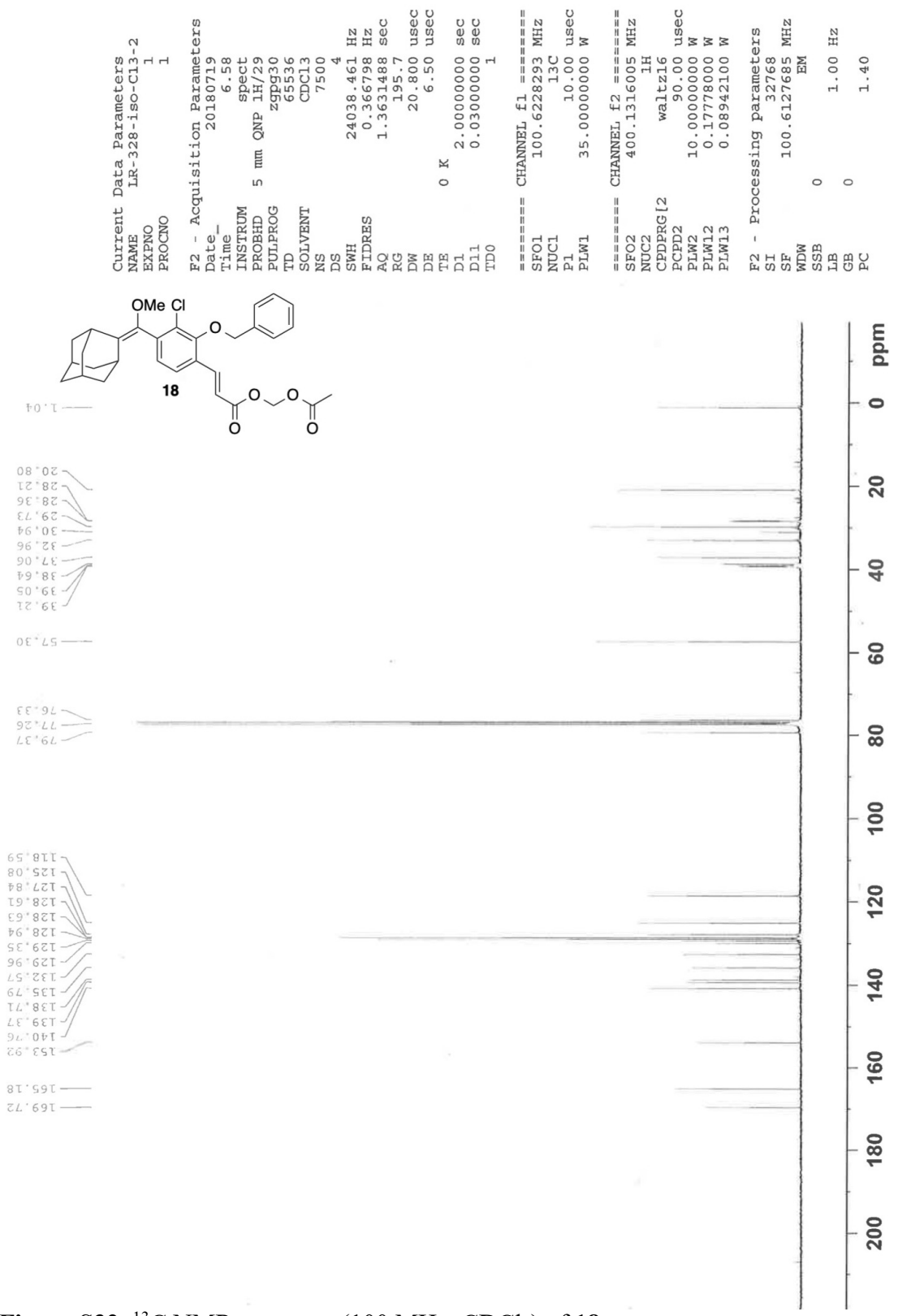


Figure S32. ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of **18**.

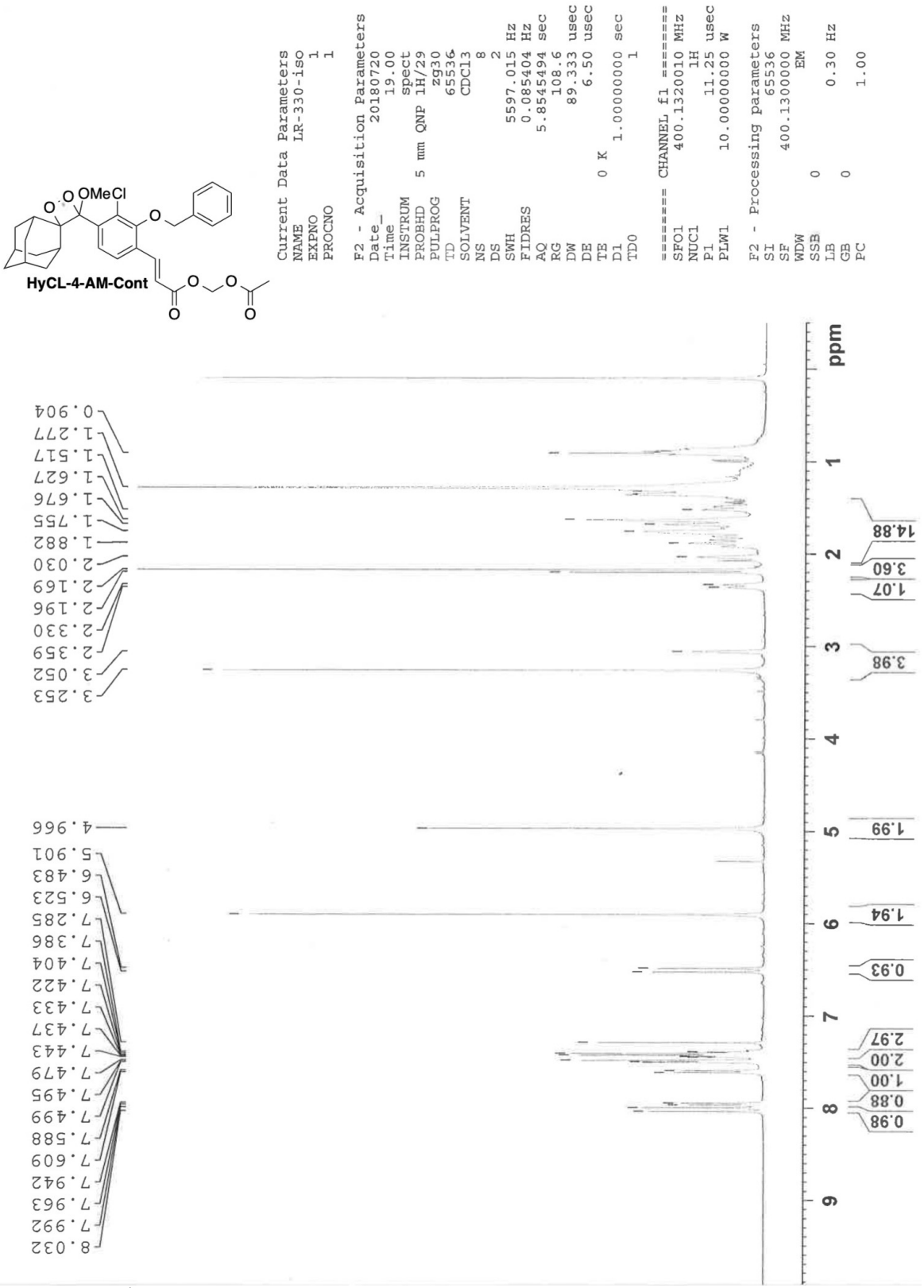


Figure S33. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **HyCL-4-AM-Cont**.

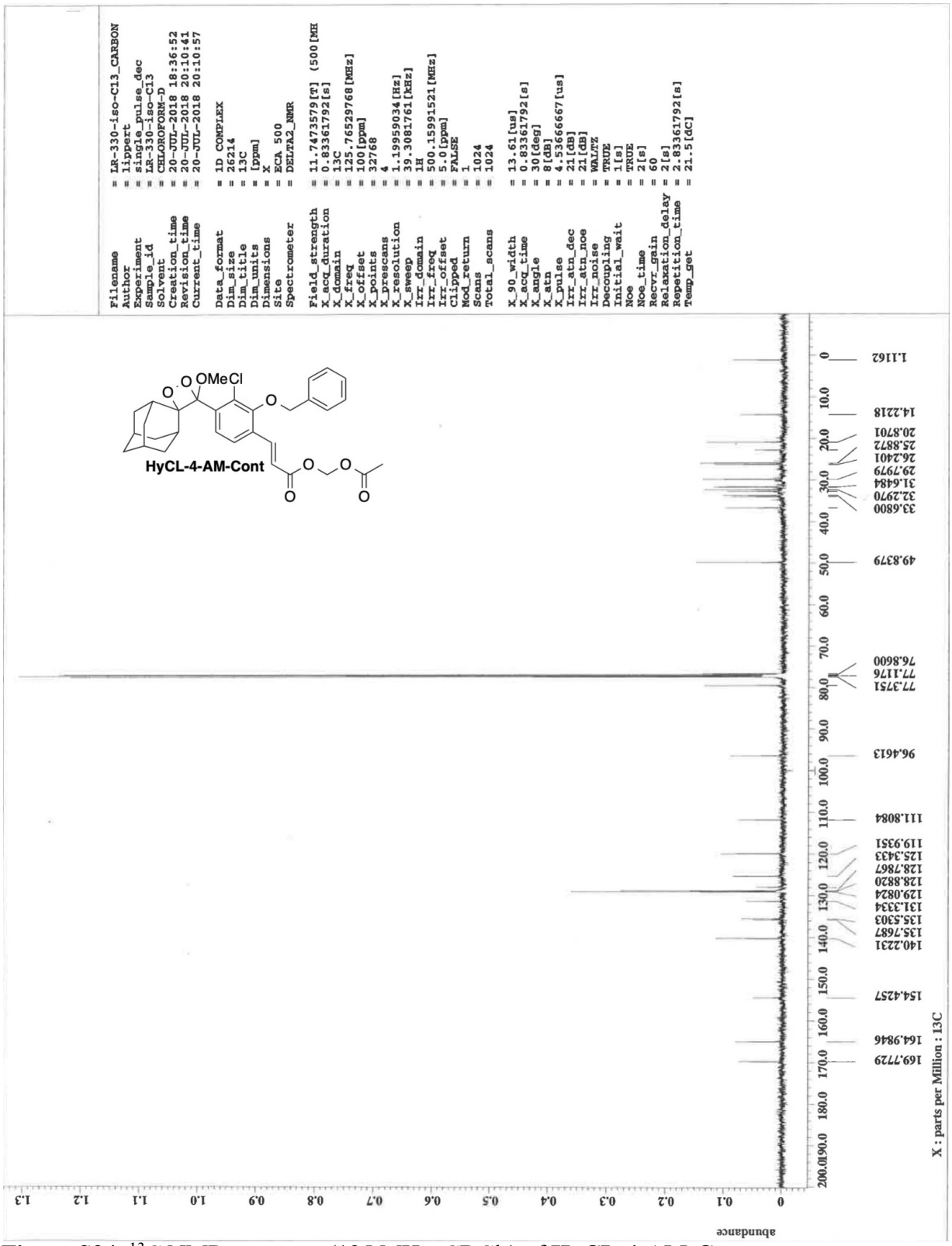


Figure S34. ^{13}C NMR spectrum (125 MHz, $CDCl_3$) of **HyCL-4-AM-Cont**.