Supporting Information

A prochelator with a modular masking group featuring hydrogen peroxide activation with concurrent fluorescent reporting

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Methods and Materials

General Notes

Dry dimethylformamide (DMF) was purchased from Sigma-Aldrich Corp. Dry CH_2Cl_2 was obtained by degassing with argon and passing the solvent through an activated alumina column. The HBTU coupling agent was purchased from Chem-Impex International, Inc., 8-hydroxyquinoline was purchased from Acros Organics, and 4-(bromomethyl)benzeneboronic acid pinacol ester was purchased from Ark Pharm, Inc. Potassium *tert*-butoxide was from Sigma-Aldrich Corp. as a 1M solution in dry THF. All other solvents and reagents were obtained as reagent grade from Sigma-Aldrich Corp. and were used without further purification. The starting material 7-allyloxycoumarin (**4**) was synthesized as described in the literature.[1]

Solutions for absorbance and fluorescence spectroscopy were prepared using pH 7.4 phosphate-buffered saline solution (PBS) from Lonza Corp. (catalog no. 17-516F), which contained 144 mg/L KH_2PO_4, 9000 mg/L NaCl, and 795 mg/L Na_2HPO_4. The direct hydrogen peroxide sources used in these experiments were either 100 mM or 500 mM working solutions freshly prepared daily by dilution from a refrigerated 50 wt. % stock.

Nuclear magnetic resonance spectroscopy was performed on Varian 400 MHz or 500 MHz spectrometers. Exact mass measurements were acquired on an Agilent Model 6224 TOF-LC/MS system with an Ascentis C18 column.

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[1] Mizukami, S., Watanabe, S. and Kikuchi, K. *ChemBioChem* **2009**, *10,* 1465–1468.

Synthesis

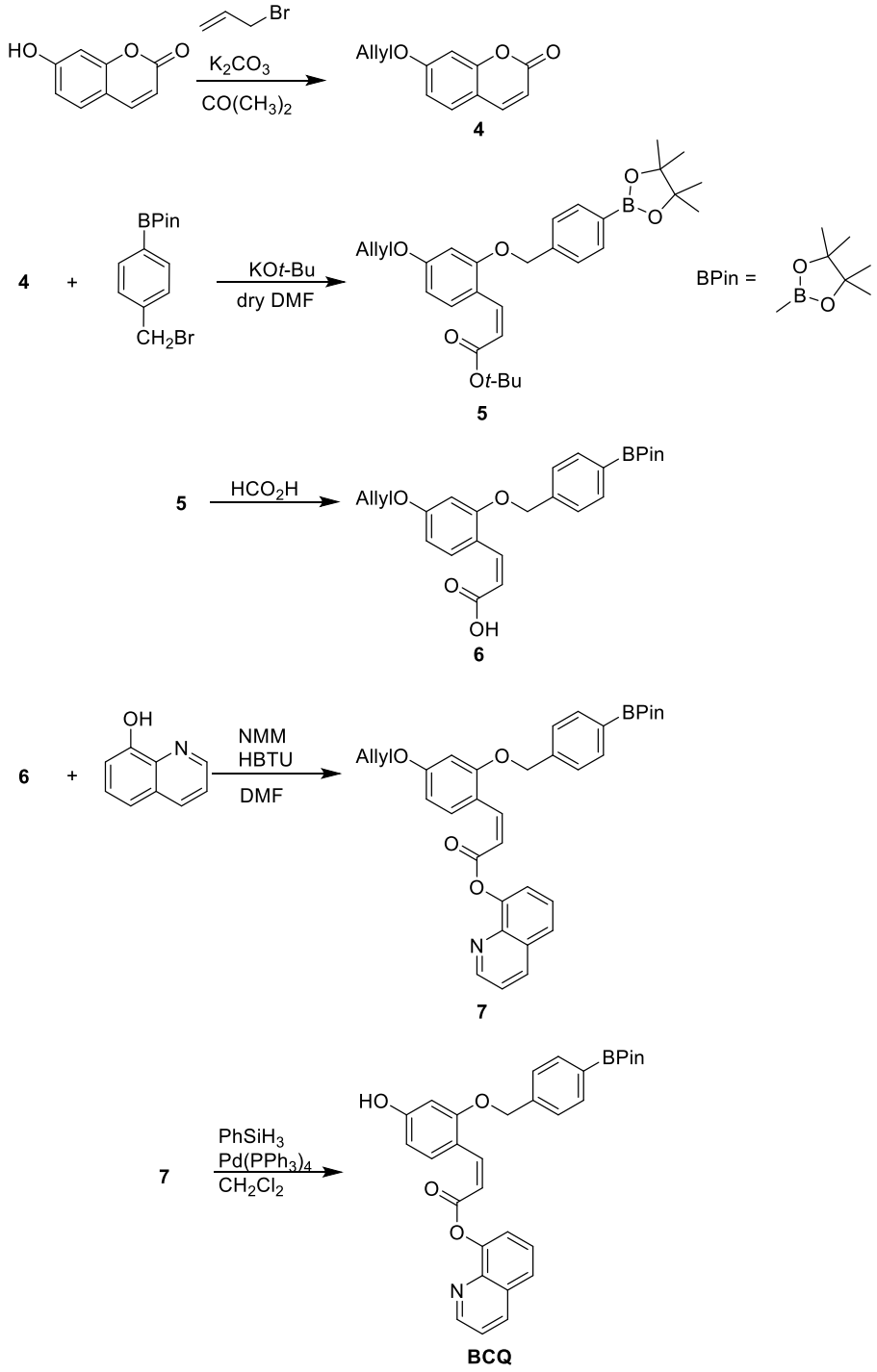


Figure S1: Synthetic scheme for BCQ

tert-butyl(*Z*)-3-(4-(allyloxy)-2-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)phenyl)acrylate (5)

A portion of 7-allyloxycoumarin **4** (285 mg, 1.4 mmol, 1 equiv) was dissolved in dry DMF (5 mL) then cooled to 0 °C in an ice bath under Ar atmosphere. To the stirring solution, potassium *t*-butoxide (1.0 M in THF, 1.73 mL) was added dropwise, which turned the reaction mixture orange. Once the dropwise addition was complete, 4-(bromomethyl)benzeneboronic acid pinacol ester (544 mg, 1.83 mmol, 1.3 equiv) was added rapidly as a solid. After stirring for 15 min, the ice bath was removed and the reaction mixture was allowed to reach room temperature. After 45 min of stirring, the solvent was removed and the residue was taken up in ethyl acetate (25 mL) and washed with water (10 mL) then brine (10 mL). The organic phase was then dried over $MgSO_4$, concentrated and purified by silica column chromatography (7:3 ethyl acetate:hexanes). Product was a colorless oil (421 mg, 61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.89 (d, J = 7.8 Hz, 2H), 7.77 (d, J = 8.4 Hz, 1H), 7.45 (d, J = 7.8 Hz, 2H), 7.16 (d, J = 12.6 Hz, 1H), 6.55-6.50 (m, 2H), 6.05 (tdd, J = 17.1, 10.5, 5.3 Hz, 1H), 5.85 (d, J = 12.6 Hz, 1H), 5.45-5.38 (m, 1H), 5.33-5.26 (m, 1H), 5.08 (s, 2H), 4.54-4.51 (m, 2H), 1.49 (s, 9H), 1.38 (s, 12H); ^{13}C (500 MHz, $CDCl_3$) δ ppm 166.0, 160.6, 157.7, 139.9, 13.3, 135.1, 133.1, 132.0, 126.5, 119.8, 117.7, 117.5, 105.4, 99.9, 83.8, 80.0, 70.2, 68.8, 28.2, 24.9 HR-ESIMS (*m*/*z*): calcd for $[M + Na]^+$ $C_{29}H_{37}BO_6Na$ (^{11}B isotope) is 515.2575, found 515.2580

(*Z*)-3-(4-(allyloxy)-2-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)phenyl)acrylic acid (6)

To a flask containing **5** (370 mg, 0.75 mmol) was added 5 mL of formic acid. The heterogeneous mixture was stirred vigorously for 30 min, during which time the oil slowly dissolved. The acid was removed in vacuo to yield the crude product as a colorless oil, which was taken on without further purification (323 mg, 98% yield). ^{1}H NMR (400 MHz): 7.83 (d, J = 8.1 Hz, 2H), 7.77 (d, J = 9.2 Hz, 1H), 7.40 (d, J = 8.1 Hz, 2H), 7.31 (d, J = 12.5 Hz, 1H), 6.54-6.44 (m, 2H), 6.02 (tdd, J = 17.1, 10.5, 5.3 Hz, 1H), 5.84 (d, J = 12.6 Hz, 1H), 5.39 (dtd, J = 17.3, 1.5, 1.5 Hz, 1H), 5.31-5.25 (m, 1H), 5.07 (s, 2H), 4.51 (ddd, J = 5.4, 1.5, 1.5 Hz, 2H), 1.34 (s, 12H); ^{13}C (500 MHz): 171.78, 161.19, 158.11, 141.33, 139.60, 135.09, 132.87, 132.52, 126.57, 118.07, 116.72, 116.28, 105.43, 99.79, 83.90, 70.26, 68.92, 24.86; HR-ESIMS (*m*/*z*): calcd for $[M + H]^+$ $C_{25}H_{30}BO_6$ (^{11}B isotope) is 437.2130, found 431.2142

quinolin-8-yl (Z)-3-(4-(allyloxy)-2-((4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)phenyl)acrylate (7)

In a dry flask under inert atmosphere, a portion of **6** (102 mg, 0.234 mmol, 1 equiv) was dissolved in 3 mL dry DMF. N-methylmorpholine (77 µL, 0.702 mmol, 3 equiv) then HBTU (106 mg, 0.281 mmol, 1.2 equiv) were added to the reaction mixture. The solution was stirred at room temperature for 48 h, quenched with water, then extracted with ethyl acetate (3 × 25 mL).

The combined organic phases were dried over sodium sulfate and evaporated to yield a yellow oil that was purified by flash chromatography using silica gel and an eluent gradient from 10% to 30% ethyl acetate in hexanes. Compound **7** was isolated as a yellow oil (46 mg, 35% yield).

^{1}H (400 MHz): 8.94 (dd, J = 4.3, 1.7 Hz, 1H), 8.18 (dd, J = 8.5, 1.7 Hz, 1H), 8.14 (d, J = 8.7 Hz, 1H), 7.85 (d, J = 8.1 Hz, 2H), 7.71 (d, J = 8.8 Hz, 1H), 7.55-.49 (m, 2H), 7.47-7.41 (m, 4H), 6.48 (d, J = 2.3 Hz, 1H), 6.42 (dd, J = 8.8, 2.4 Hz, 1H), 6.33 (d, J = 12.7 Hz, 1H), 5.99 (tdd, J = 17.1, 10.5, 5.3 Hz, 1H), 5.36 (dd, J = 17.3, 1.6 Hz, 1H), 5.28-5.22 (m, 1H) 5.10 (s, 2H), 4.47 (d, J = 5.2 Hz, 2H), 1.36 (s, 12H); ^{13}C: 165.1, 161.3, 158.3, 150.4, 147.3, 141.7, 141.3, 139.8, 136.2, 135.1, 133.0, 132.9, 129.6, 126.6, 126.3, 125.7, 121.9, 121.7, 117.9, 116.9, 115.9, 115.6, 105.5, 99.7, 83.9, 70.3, 68.9, 24.9; HR-ESIMS (*m*/*z*): calcd for $[M + H]^+$ $C_{31}H_{30}BNO_6$ (^{11}B isotope) is 564.2552, found 564.2564

BCQ

A portion of **7** (76 mg, 0.135 mmol) was combined with a portion of tetrakis(triphenylphosphine)palladium(0) (3 mg, 0.003 mmol, 0.02 equiv) under inert atmosphere. Dichloromethane (250 μL) was added to the reaction before addition of phenylsilane (33.5 μL, 0.271 mmol, 2 equiv). The reaction mixture was left to stir for several hours, until reaction was complete as determined by thin-layer chromatography. The solvent was reduced by flowing N_2 over the reaction mixture, then the residue was taken up in ethyl acetate (5 mL) and washed sequentially with water and brine. The organic phase was separated, dried over Na_2SO_4 and the solvent was removed to produce a crude yellow oil. The final purification was performed by semi-preparative HPLC using a Waters XBridge Prep column (C18 10μ, 19 × 250 mm) with unbuffered acetonitrile/H_2O eluent. After removal of solvent, product was obtained as a white solid (20 mg, 29% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.86 (dd, J = 4.3, 1.6 Hz, 1H), 8.23 (dd, J = 8.3, 1.6 Hz, 1H), 8.10 (d, J = 8.7 Hz, 1H), 7.80 (d, J = 7.9 Hz, 2H), 7.74 (dd, J = 8.2, 1.3 Hz, 2H), 7.57 (dd, J = 7.8, 7.7 Hz, 1H), 7.50-7.40 (m, 3H), 7.33 (d, J = 7.9 Hz, 2H), 6.31 (d, J = 2.3 Hz, 1H), 6.25 (dd, J = 8.7, 2.3 Hz, 1H), 6.20 (d, J = 12.9 Hz, 1H), 4.94 (s, 2H), 1.36 (s, 12H); ^{13}C (500 MHz): 165.3, 160.6, 158.7, 149.9, 146.83, 141.6, 140.8, 140.0, 137.1, 135.0, 133.2, 129.6, 1 26.7, 126.5, 126.3, 125.6, 122.7, 121.7, 115.4, 113.3, 107.5, 99.8, 83.8, 69.94, 24.9; HR-ESIMS (*m*/*z*): calcd for $[M + H]^+$ $C_{31}H_{30}BNO_6$ is 524.2244, found 524.2265; UV (1:1 H_2O:CH_3OH) λ_{max} (ε): 302 (7800), 315 (8500), 332 (7900)

LC-MS

Liquid chromatography was performed on an Agilent 1100 Series system using a Supelco Ascentis (C18 3μ, 50 × 1 mm) column. Compound standard solutions and reaction mixtures were separated using a linear gradient of 95%A / 5%B to 20%A/ 80%B over 15 min followed by 4 min of isocratic flow at 20%A / 80%B, where A is 98:2 H_2O:CH_3CN and B is 98:2 CH_3CN:H_2O. A photodiode array detector was used to measure UV absorbance of the eluting species. In-line mass spectrometry was performed using an Agilent 1100 Series LC/MSD ion trap mass spectrometer. Samples were typically analyzed by injecting 4 μL aliquots of 50 μM solutions in

1:1 MeOH:PBS. Stocks for these solutions were in DMSO, though the DMSO content was kept below 2% of the total volume.

Mass spectrometry data were viewed and processed using Agilent DataAnalysis software; UV chromatograms were analyzed using Agilent ChemStation software.

Fluorometry

Fluorescence emission spectra were collected on a Horiba Jobin Yvon Fluorolog-3 spectrofluorometer fitted with a xenon arc lamp. Solutions were measured in 3-mL quartz cuvettes with caps to prevent evaporation. Samples were excited with monochromatic light (λ_{ex} = 350 nm) and emission was observed between 360 and 600 nm. A solution of BCQ (2 μM in PBS) was prepared from 1 mM stock in DMSO. A fluorescence emission spectrum was collected then an aliquot of 100 mM H_2O_2 was added to the cuvette so that $[H_2O_2]_{final}$ = 200 μM. The solution was mixed then spectra were collected over 14 h.

Plate reader assay

Experiments were performed at 25 °C using black-walled 96-well plates with clear bottoms in a Wallac Victor 1420 plate reader (PerkinElmer Inc.) equipped with 355 nm (40 nm bandwidth) excitation and 420 nm (15 nm bandwidth) emission filters. A working solution of BCQ was prepared at 2 μM in PBS from 1 mM DMSO stock. Wells in two plates were filled with 100 μL aliquots of BCQ solution. All conditions were tested on each plate, and each condition was tested in triplicate wells. A series of H_2O_2 working solutions were prepared at 50× concentrations (63 μM – 10 mM). The respective H_2O_2 working solutions were added as 2-μL aliquots to the wells. Umbelliferone emission was measured immediately after mixing and then monitored over at least 4 h. Between readings, one plate was kept in the dark at 25 °C while the other was stored in a 37 °C incubator. The average emission observed in wells containing only buffer (no fluorescent compounds) was subtracted from all reported values for BCQ-containing wells.

UV-visible spectroscopy

BCQ solutions used for UV-visible spectroscopy were prepared in 2-mL volumes at a final concentration of 20 μM in 1:1 PBS:methanol from 1 mM BCQ stocks in DMSO. Absorbance measurements were collected in a Cary 50 spectrophotometer. For kinetic studies, either the entire spectrum or absorbance at 379 nm was measured once per min for at least 90 min (and at least three half-lives of pseudo-first order BCQ consumption). For determination of k_{obs}, absorbance values at 379 nm were converted to BCQ concentration at time t using the following equation:

$$[BCQ]/[BCQ]_0 = (A_t - A_{inf}) / (A_0 - A_{inf})$$

where $[BCQ]_0$ is the initial concentration of BCQ prior to reaction with H_2O_2. A_t, A_0 and A_{inf} are the absorbances measured respectively at reaction time *t*, *t* = 0, and *t* = infinity (reaction completion). Concentration values were then entered into Origin 8.5 and tested for mathematical fits. The model that provided the best fit for the data was an exponential decay model described by the equation $[BCQ] = [BCQ]_0 * e^{-kt}$ where *k* is the observed rate constant k_{obs}.

Spectra

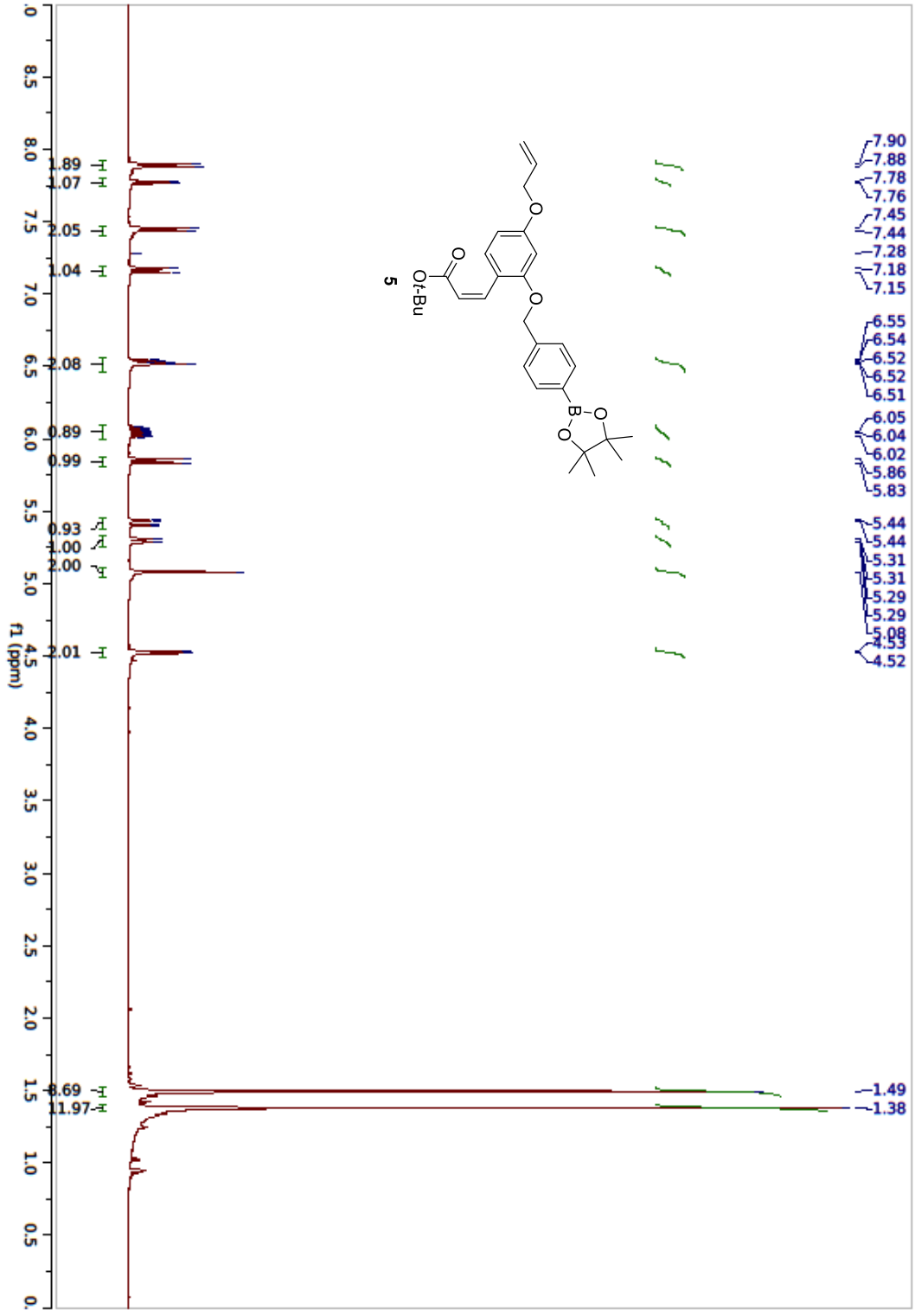


Figure S2. Compound **5;** ^{1}H NMR (400 MHz in $CDCl_3$)

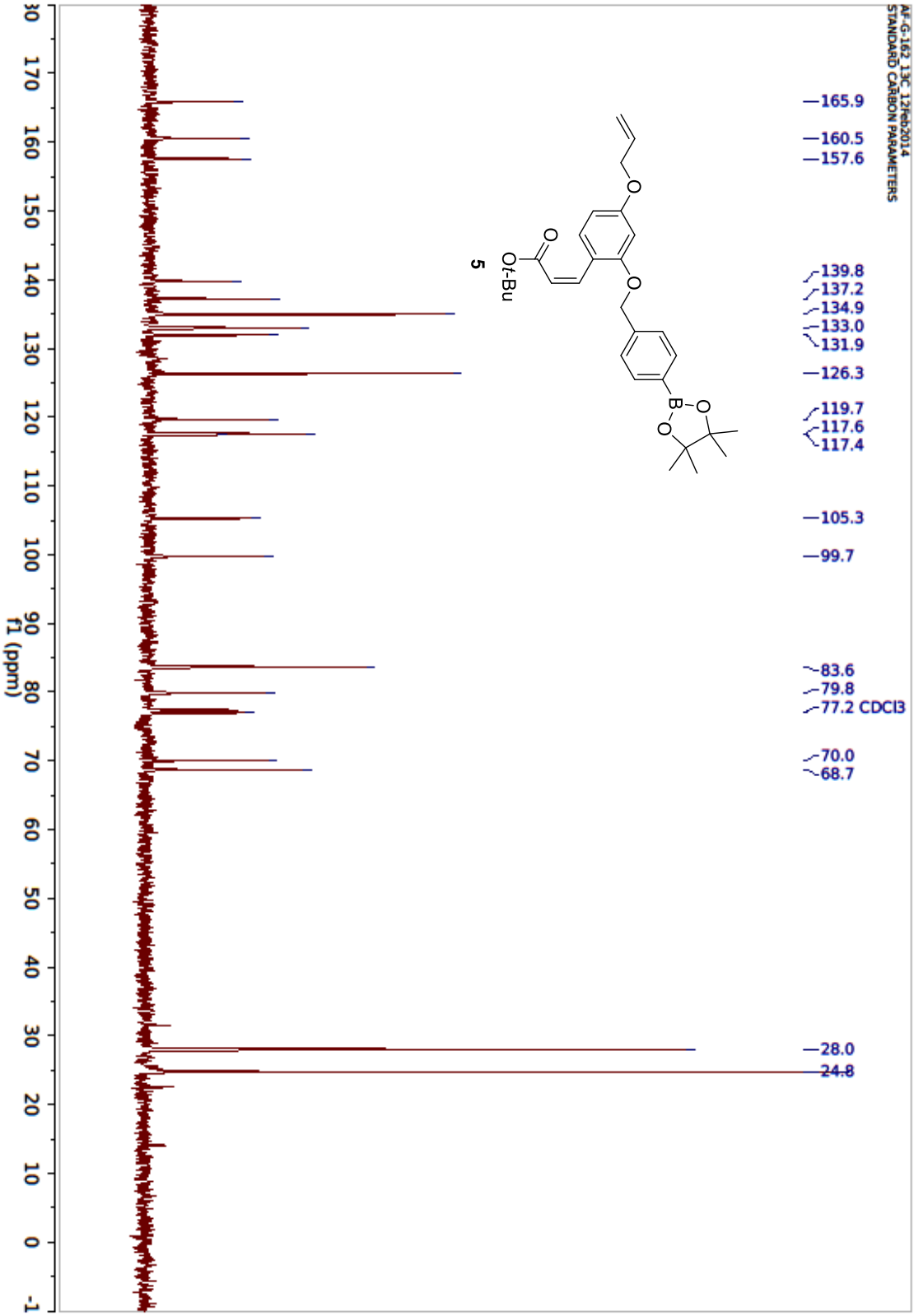


Figure S3. Compound **5;** ^{13}C NMR (500 MHz in $CDCl_3$)

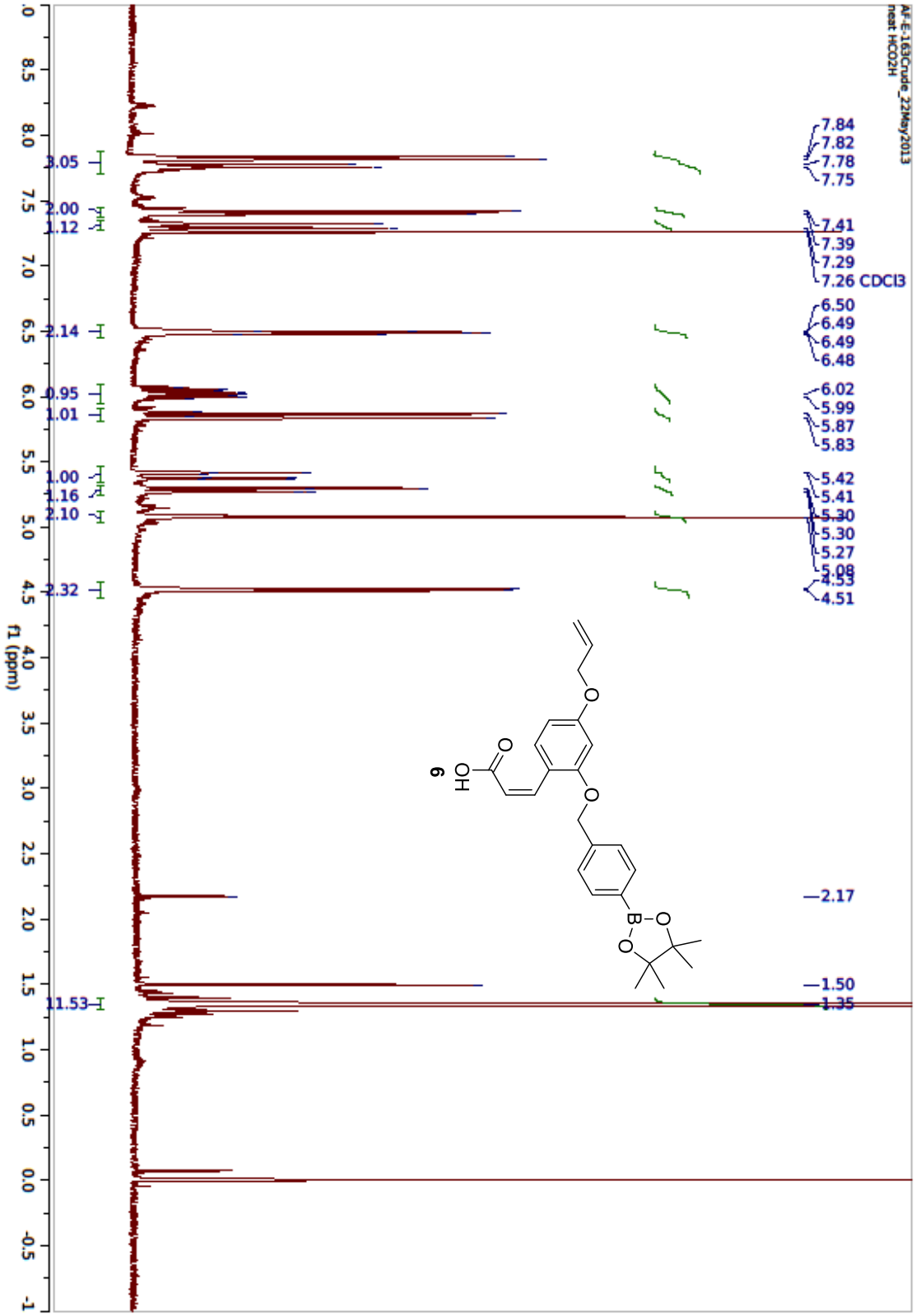


Figure S4. Crude compound **6;** ^{1}H NMR **(**400 MHz in $CDCl_3$)

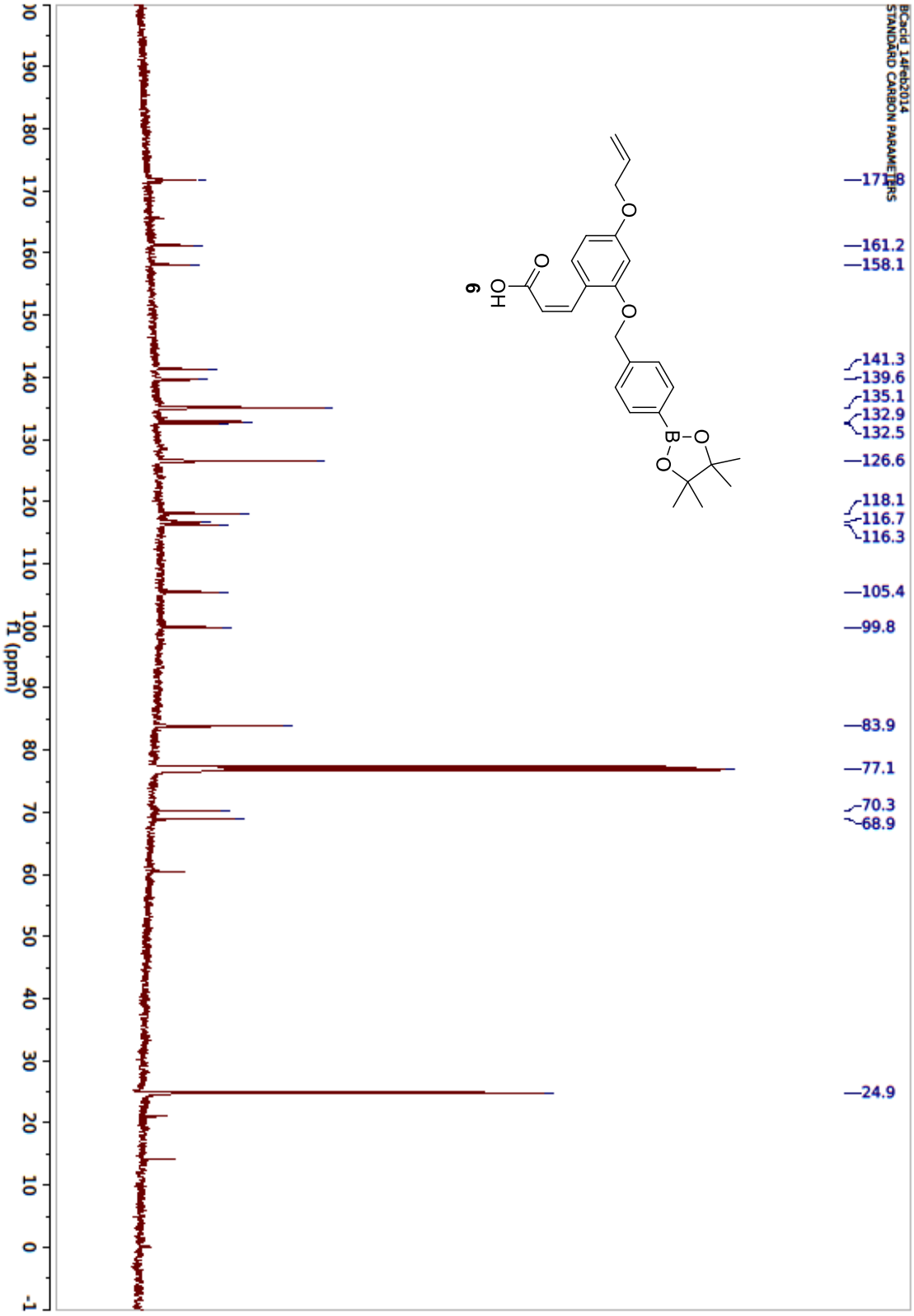
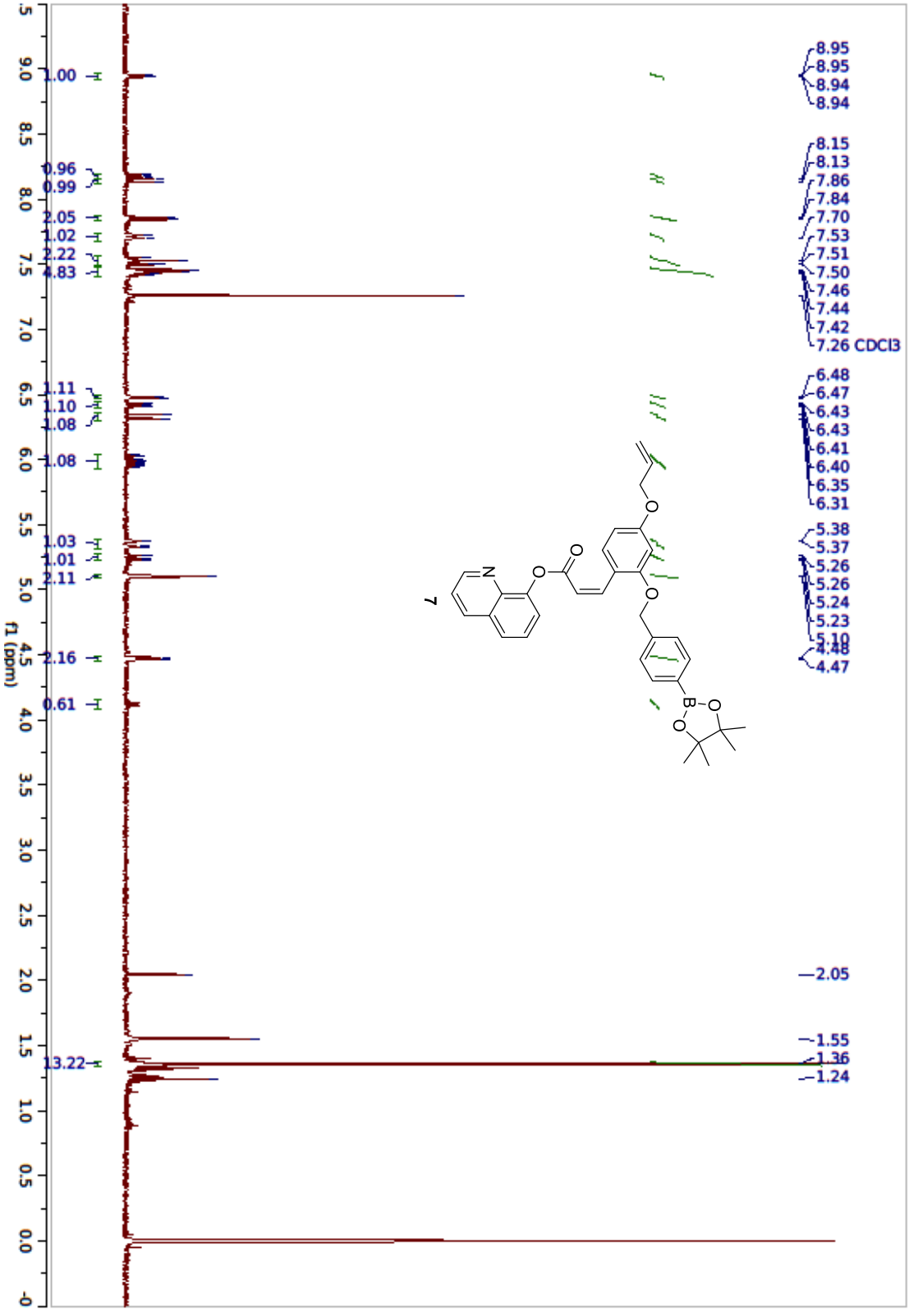


Figure S5. Crude compound **6;** ^{13}C NMR **(**500 MHz in $CDCl_3$)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7.44
7.42
7.26 CDCl3
6.48
6.47
6.43
6.43
6.41
6.40
6.35
6.31
5.38
5.37
5.26
5.26
5.24
5.23
5.10
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4.47
2.05
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2.22
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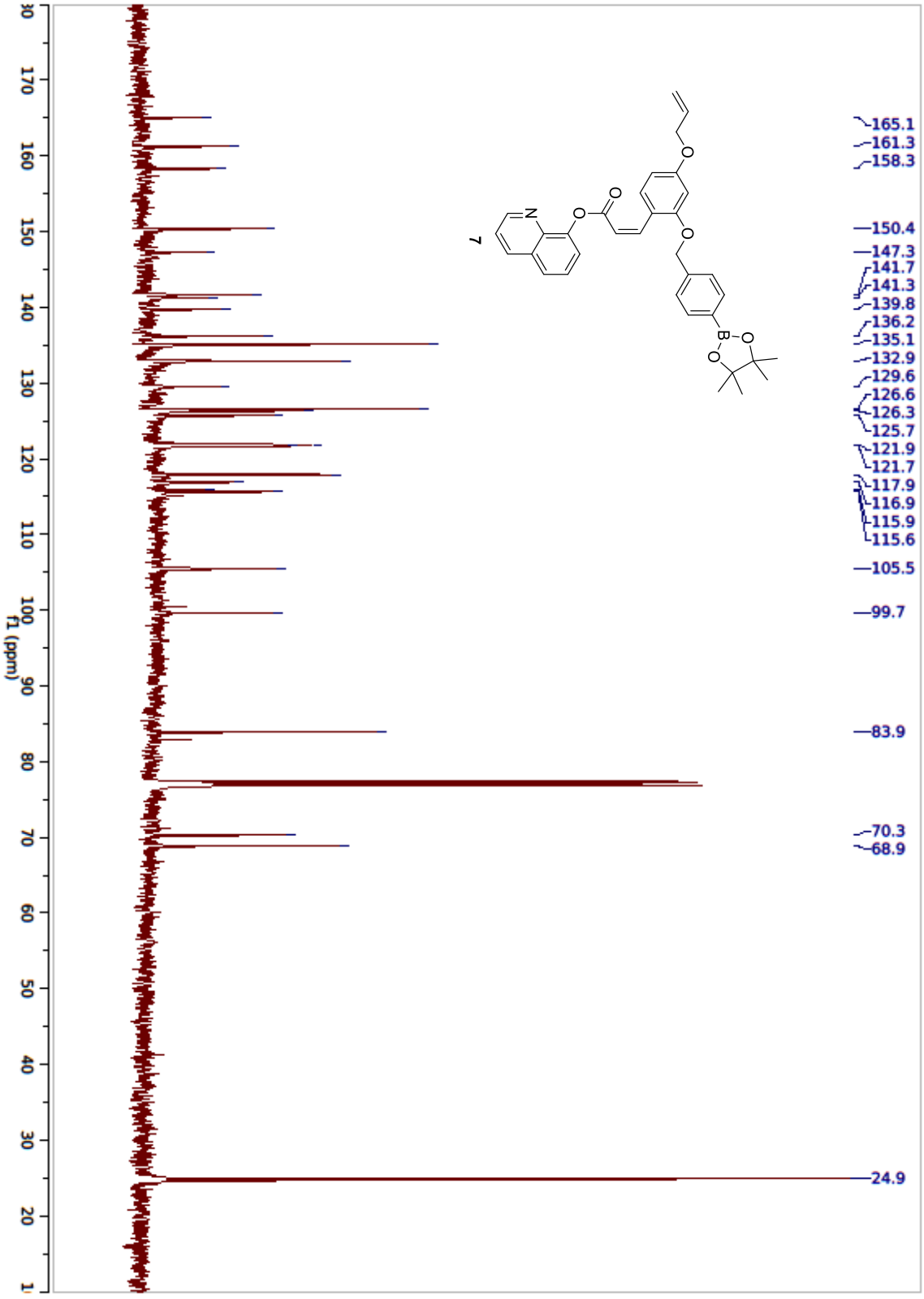
f1 (ppm)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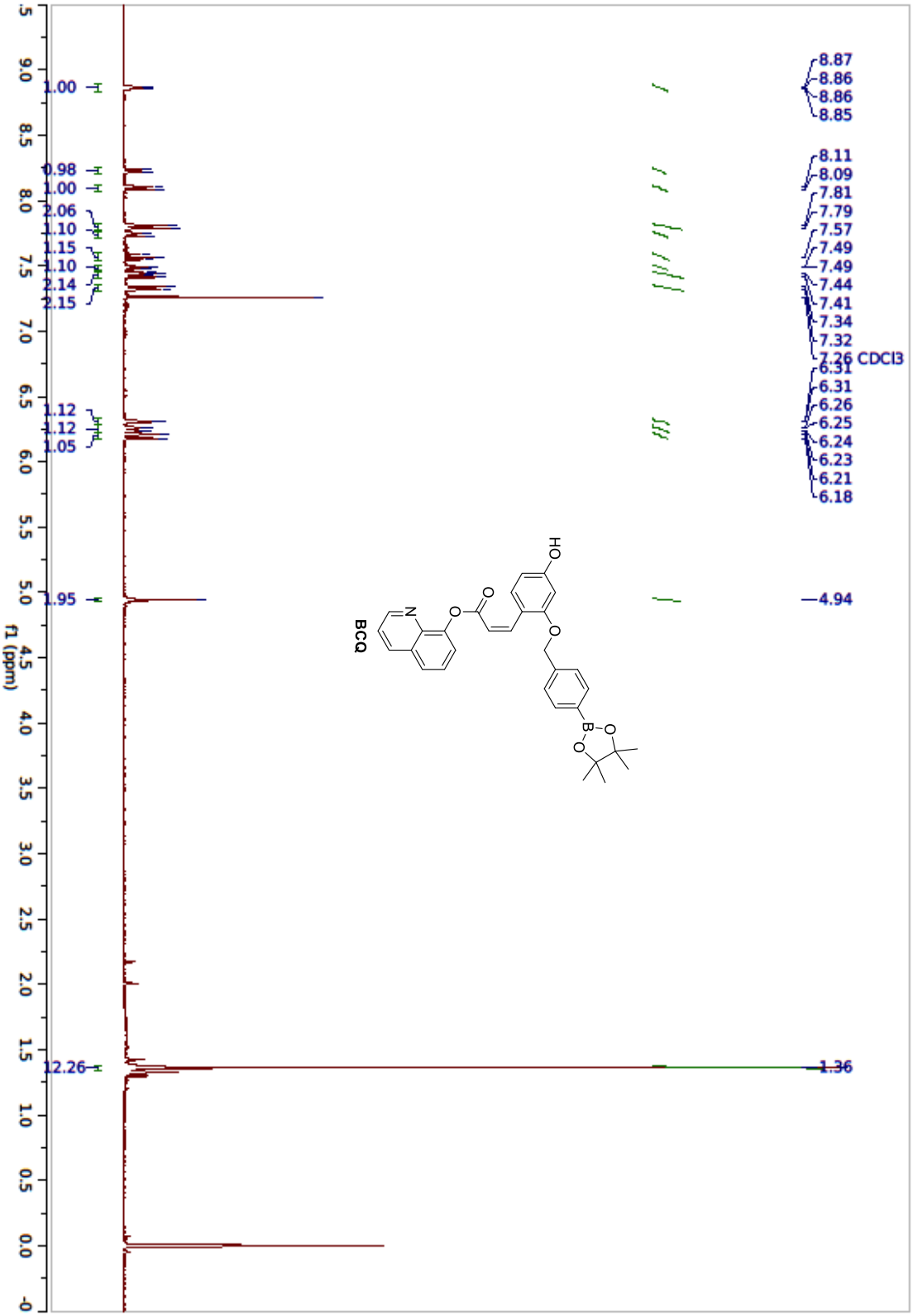
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B
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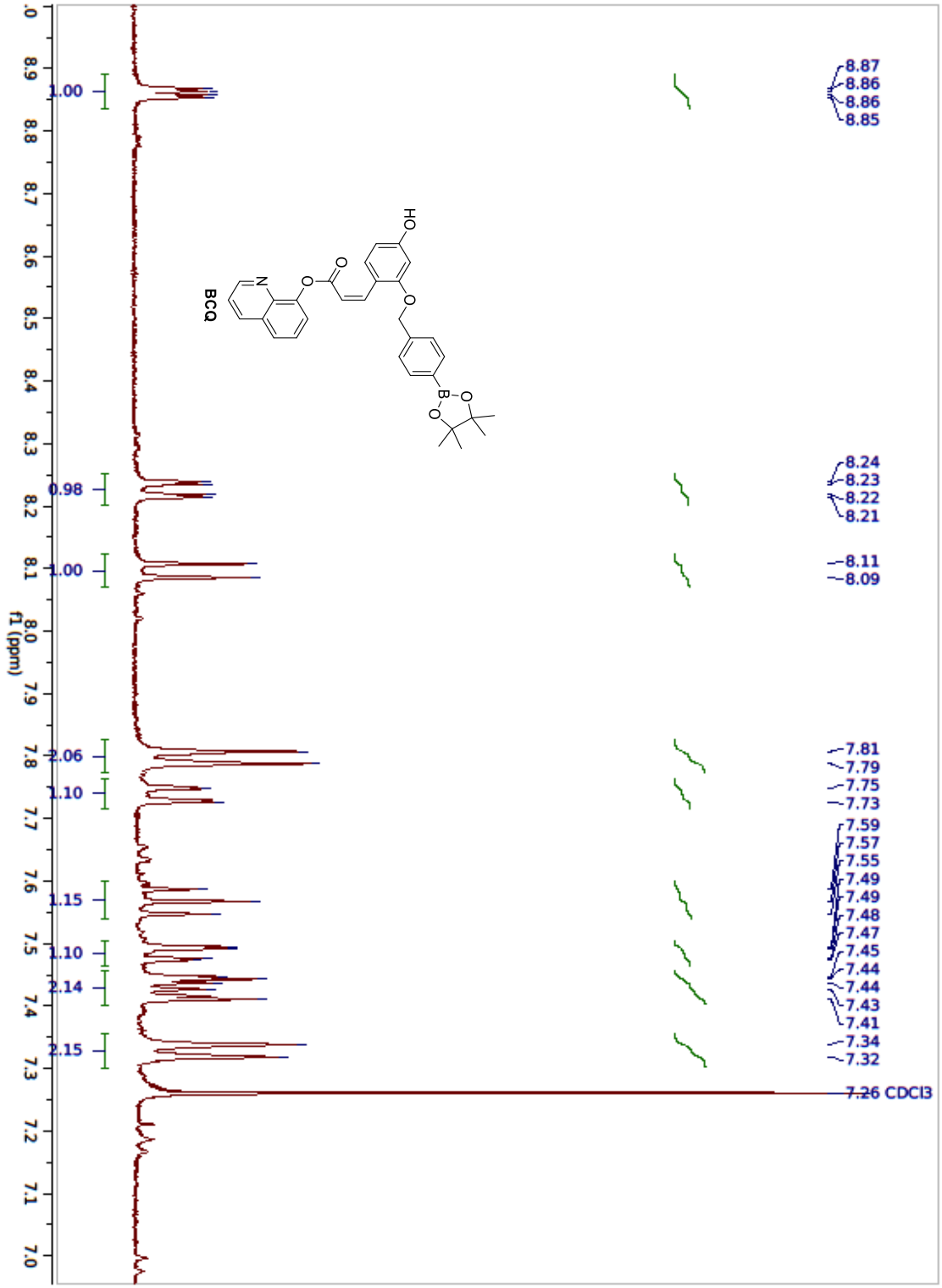
BCQ
f1 (ppm)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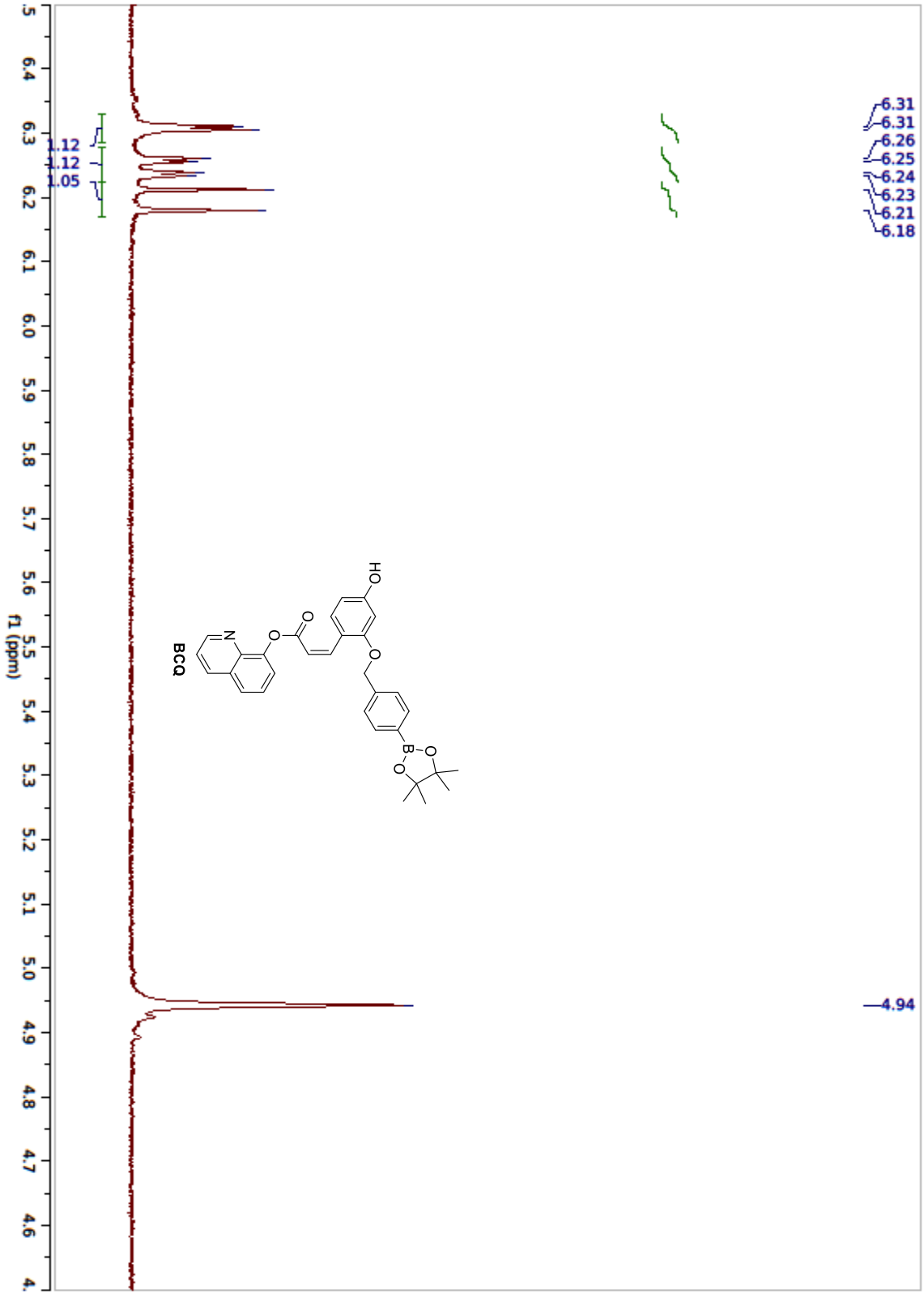
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HO
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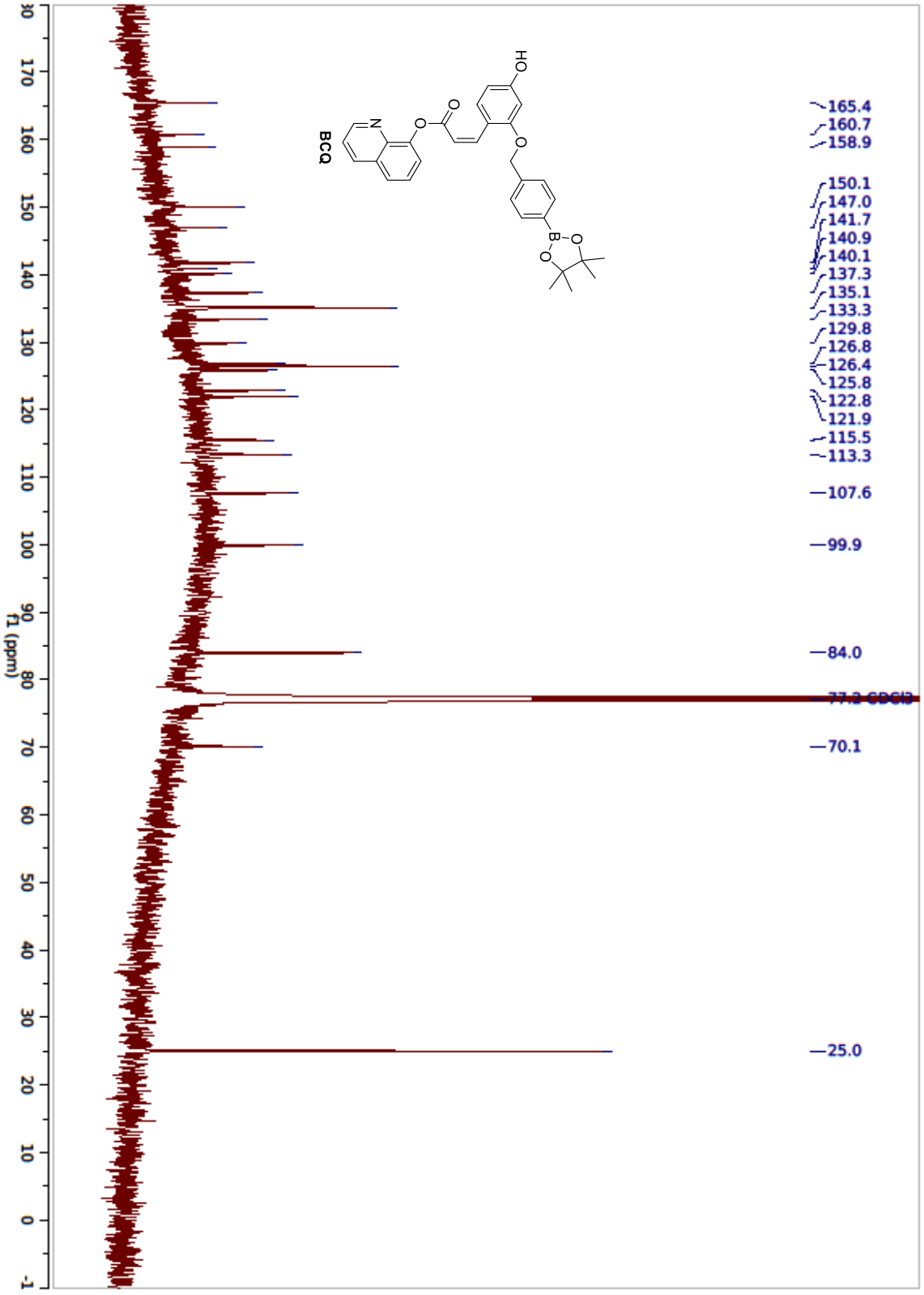
N
B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133.3
129.8
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125.8
122.8
121.9
115.5
113.3
107.6
99.9
84.0
77.2 CDCl3
70.1
25.0
f1 (ppm)