Supporting Information

Red-shifted backbone N–H photocaging agents

Alicia E. Mangubat-Medina,[†] Hallie O. Trial,[†] Reyner D. Vargas,[†] Mekedlawit T. Setegne,[†] Taysir Bader,[‡] Mark D. Distefano,[‡] Zachary T. Ball*[†]

[†]Department of Chemistry, Rice University, Houston, Texas 77005

[‡]Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

Contents

General information

Generally, chemicals were purchased from commercial companies and used without purification. Specifically, the 10% Pd/C was purchased from Strem chemicals and THF was purified through a PPt solvent system. Peptides pep2 and pep3 were purchased from Sigma and Alfa Aesar, respectively. Peptide pep3 was synthesized by SPPS. Photocleavage reactions were conducted with a commercial blue LED flashlight (nominal 455 nm) or a Penn PhD Photoreactor M2 with a blue LED light lamp (nominal 450 nm).

Instrumentation

NMR spectra were obtained on Bruker AVANCE 600 or AVANCE 500 spectrometers.

ESI-MS was performed on a Bruker Daltonics MicroTOF spectrometer.

MALDI-MS was performed on a Bruker Daltonics Autoflex Speed MALDI-TOF/TOF spectrometer. Sinapinic acid sat. aq matrix solns were made by dissolving 1 mg of matrix in 50 μL (20 mg/mL) of a 1:1 soln of water/MeCN with 0.1% TFA.

General procedure for MALDI analysis: Sample aliquots (1 μL) of the crude reaction mixture were diluted 1:5 with 5 μL of water and 1 μL of this soln was spotted on the MALDI target. The sat. aq matrix soln (1 μL) was spotted and mixed with sample on the MALDI target for analysis. All MS/MS experiments were performed on the MALDI spectrometer.

HPLC

Reverse-phase HPLC was performed on Shimadzu CBM-20A instrument with Phenomenex Jupiter 4μ Proteo 90A (250 x 4.6 mm analytical) and Phenomenex Jupiter 4μ Proteo 90A (250 × 15 mm preparative) columns. The columns were eluted with a gradient of MeCN in water (20–70%) with 0.1% v/v TFA using a flow rate of 1 mL/min and 8 mL/min for analytical and preparative columns, respectively. Purification was monitored using 220 nm and 330 nm UV for peptide detection.

General procedure for HPLC analysis: Sample aliquots (20 μL) of crude reaction mixture were diluted with MeCN (1:1 ratio, 20 μL) before injection onto the HPLC.

Buffers

NMM buffer: To water was added *N*-methylmorpholine (11 μL, 0.1 mM), and the solution was adjusted to pH 7.0 or pH 6.0 using 2 M HCl before bringing the final volume to 10 mL.

Photocleavage buffer: To water was added *N*-methylmorpholine (110 μL, 1.0 mmol), isoamylamine (116 μL, 1.0 mmol), and NaCl (584 mg, 10.0 mmol). The buffer was adjusted to pH 6.8 using 2 M HCl before bringing the final volume to 100 mL.

Peptide synthesis

Collagen mimetic peptide (**pep1**, sequence: Ac–$(POG)_4HOG(POG)_3$–NH_2) was synthesized according to the reported procedure using standard solid phase peptide synthesis methods with rink amide AM resin (P3Biosystems, #52001) and Fmoc-amino acids purchased from NovaBiochem. Peptide was purified by reverse phase HPLC and characterized by MALDI-MS and circular dichroism.[1]

Overview of peptide sequences

Table S1. Summary of peptide sequences

name	peptide sequence	MALDI-MS spectra
pep1	Ac–$(POG)_3$POGHOG$(POG)_3$–NH_2	Figure 2
pep2	pE–HWSYGLRPG–NH_2	Figure 3
pep3	pE–HWSY–^{D}Leu–LRP–NHEt	Figure 4, 5

Overview of experimental procedures for peptide modification

Modification of **pep1**

A collagen mimetic peptide (**pep1**, 200 μM) was incubated with boronate **1b** (2 mM) and $Cu(NO_3)_2$ (1 mM) in NMM buffer (pH 7.0) with 10 v/v% DMSO at rt overnight. Specifically, **pep1** (160 nmol, 64 μL of 2.5 mM aq soln), DMSO (80 μL), boronate **1b** (1.6 μmol, 32 μL of 50 mM DMSO soln), and $Cu(NO_3)_2$ (0.8 μmol, 16 μL of 50 mM aq soln) were added to a soln of NMM buffer (pH 7.0, 608 μL) and heated to 45 °C for 2 d. The crude reaction mixture was analyzed according to the general procedure for HPLC analysis, which indicated 70% conversion to the modified product.

Modification of **pep2** with **1b**

Pep2 (200 μM) was incubated with boronic acid **1b** (1 mM) and $Cu(NO_3)_2$ (3 mM) in NMM buffer (pH 7) with 14% v/v DMSO at 37 °C for 96 h. Specifically, **pep2** (40 nmol, 16 μL of 2.5 mM aq soln), boronate **1c** (250 nmol, 10 μL of 50 mM DMSO soln), and $Cu(NO_3)_2$ (1500 nmol, 30 μL of 50 mM aq soln) were added to a soln of NMM buffer (pH 7.0, 370 μL) and heated at 37 °C for 1 h. At this point an additional 250 nmol of boronate **1b** were added to the soln for a total boronate **1b** concentration of 1 mM, and the reaction was heated at 37 °C for 1h. The crude reaction mixture was analyzed according to the

general procedure for HPLC and MALDI analysis, which indicated complete conversion to the modified product.

Modification of **pep2** with **1c**

Pep2 (100 μM) was incubated with boronic acid **1c** (2 mM) and $Cu(NO_3)_2$ (1 mM) in NMM buffer (pH 6) with 40% v/v DMSO at 37 °C for 4 days. Specifically, **pep2** (40 nmol, 16 μL of 5 mM aq soln), boronate **1c** (800 nmol, 160 μL of 5 mM DMSO soln), and $Cu(NO_3)_2$ (800 nmol, 16 μL of 50 mM aq soln) were added to a soln of NMM buffer (pH 6.0, 448 μL) and heated at 37 °C for 24 h. At this point additional boronate **1b** (800 nmol, 160 μL of 5 mM DMSO soln) was added to the soln for a total boronate **1b** concentration of 2 mM, and the reaction was heated at 37 °C for 72 h. The crude reaction mixture was analyzed according to the general procedure for HPLC and MALDI analysis, which indicated complete conversion to the modified product.

Modification of **pep3** with **1a**

Leuprolide (**pep3**, 100 μM) was incubated with boronate **1a** (1 mM) and $Cu(NO_3)_2$ (1 mM) in NMM buffer (pH 7.0) at 37 °C for 2 h. Specifically, leuprolide (80 nmol, 32 μL of 2.5 mM aq soln), boronate **1a** (400 nmol, 48 μL of 8.3 mM DMSO soln), and $Cu(NO_3)_2$ (800 nmol, 16 μL of 50 mM aq soln) were added to a soln of NMM buffer (pH 7.0, 604 μL) and heated at 37 °C for 2 h. The crude reaction mixture was analyzed according to the general procedure for HPLC and MALDI analysis, which indicated complete conversion to the modified product.

Modification of **pep3** with **1b**

P**ep3** (200 μM) was incubated with boronate **1b** (2 mM) and $Cu(NO_3)_2$ (3 mM) in NMM buffer (pH 7.0) with 16% v/v DMSO at 37 °C for 2 h. Specifically, **pep3** (160 nmol, 64 μL of 2.5 mM aq soln), boronate **1b** (800 nmol, 96 μL of 8.3 mM DMSO soln), and $Cu(NO_3)_2$ (2.4 μmol, 48 μL of 50 mM aq soln) were added to a soln of NMM buffer (pH 7.0, 524 μL) and heated at 37 °C for 1 h. At this point additional boronate **1b** (800 nmol, 96 μL of 8.3 mM DMSO soln) was added to the soln for a total boronate **1b** concentration of 2 mM, and the reaction was heated at 37 °C for 1 h. The crude reaction mixture was analyzed according to the general procedure for HPLC and MALDI analysis, which indicated complete conversion to the modified product.

Modification of **pep3** with **1c**

P**ep3** (100 μM) was incubated with boronate **1c** (4 mM) and $Cu(NO_3)_2$ (1 mM) in NMM buffer (pH 6.0) with 20% v/v DMSO at 37 °C for 1 h. Specifically, **pep3** (80 nmol, 32 μL of 2.5 mM aq soln), boronate **1c** (1600 nmol, 80 μL of 20 mM DMSO soln), and $Cu(NO_3)_2$ (800 nmol, 16 μL of 50 mM aq soln) were added to a soln of NMM buffer (pH 6.0, 572 μL) and heated at 37 °C for 24 h. At this point additional

boronate **1c** (1600 nmol, 80 μL of 20 mM DMSO soln) was added to the soln for a total boronate **1c** concentration of 4 mM, and the reaction was heated at 37 °C for 5 days. The crude reaction mixture was analyzed according to the general procedure for HPLC and MALDI analysis, which indicated quantitative conversion to the modified product.

One-photon uncaging experiments

Modified peptide (**pep3a**, **pep3b**, or **pep3c**) in photocleavage buffer was sealed in an 8-mL screwcap vial. The vial was then placed in the Penn PhD photoreactor with a blue LED lamp (nominal 450 nm) for 2–4 h. Aliquots (150 μL) were removed at time points 2 h and 4 h for HPLC analysis. Modified peptides **pep2b**, **pep2c**, **pep3b**, and **pep3c** in photocleavage buffer with 10 mM DTT were sealed in 8 mL screwcap vials. The vials were placed in the photoreactor with a blue LED lamp (nominal 450 nm) for 2-4 h. From the samples, 150 μL were removed at time points 2 h and 4 h for HPLC analysis.

Experimental overview for irradiation experiments

One-photon kinetics experiments

Pep3 (100 μM, 700 μL total rxn volume) was modified with boronic acids **1a**, **1b**, and **1c** as described above. The crude reaction was diluted with 400 μL of MeCN before being purified using size exclusion chromatography in photocleavage buffer. For each timepoint, 80 μL of modified peptide (**pep3a**, **pep3b**, or **pep3c**) in photocleavage buffer were placed in 4-mL borosilicate glass vials and irradiated using a Penn PhD Photoreactor M2 with a 450-nm LED light source set to 1% intensity. The intensity of this light source was determined to be $5.776*10^{-9}$ Einstein. sec^{-1}. A cm^{-2} by actinometry using a potassium ferrioxalate standard.[2] A 45-μL aliquot from each irradiated sample was injected onto the HPLC, and percent conversion to uncaged peptide was determined by comparison of the caged peptide peak area to that of an unirradiated sample. Irradiation timepoints were selected for each modified peptide such that >80% conversion to uncaged peptide was achieved by the last timepoint. The chosen timepoints were 0, 30, 60, 180, and 300 s for pep3a; 0, 5, 10, 15, 20, and 30 s for pep3b; and 0, 1, 5, 10, 20, and 30 m for pep3c. Irradiation experiments were performed in triplicate.

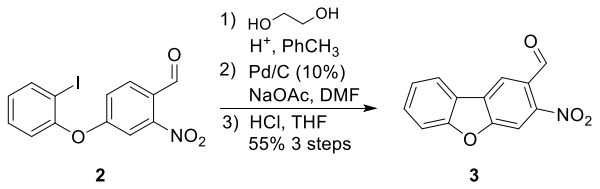
Two-photon uncaging experiments

Two-photon experiments were performed according to reported procedures described in detail elsewhere.[3] A home-built regeneratively amplified Ti:Sapphire laser operating at 1 kHz with the pulse power maintained at 70 mW centered around 800 nm was used. Each pulse had a Gaussian profile with a full width at half maximum of 80 fs. The beam was sent through a 35 cm focusing lens and then through the

sample. Samples (30 μL) were irradiated in a quartz microcuvette (Starna 16.10-Q-10/Z15, 1mm x 1 mm sample window, 10 mm path length) 15 cm after the focal plane of the lens. Samples were irradiated in Photocleavage buffer with DTT (15 mM) for 20 and 40 min before being analyzed using LCMS.

Synthetic procedures

Preparation of aldehyde **3**.



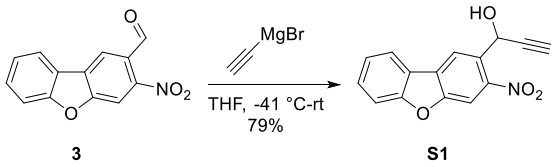
Step 1: acetalization of the carboxaldehyde. Ether **2** was synthesized from previously reported methods.[4] To a 25-mL round bottom containing ether **2** (1.0 g, 2.72 mmol) and p-toluenesulfonic acid (152.4 mg, 0.81 mmol) was added toluene (12 mL) and ethylenglycol (1.0 mL, 17.9 mmol). The reaction was stirred under reflux in a Dean Stark apparatus for 18 h. After completion, the reaction was diluted with EtOAc and washed with sat. aq $NaHCO_3$, water, and brine before being dried over Na_2SO_4. The solvent was concentrated under reduce pressure and the crude product was dry loaded on SiO_2 and purified by flash chromatography (10% Et_2O/hexanes) to yield the protected aldehyde (1.31 g, 83%). Partial characterization: ^{1}H NMR (600 MHz, chloroform-*d*) δ 7.91 (dd, 1H, *J* = 7.9, 1.3 Hz), 7.75 (d, 1H, *J* = 8.6 Hz), 7.41 (d, 1H, *J* = 2.6 Hz), 7.39 (td, 1H, *J* = 7.9, 1.5 Hz), 7.15 (dd, 1H, *J* = 8.6, 2.4 Hz), 7.03 (dd, 1H, *J* = 8.1, 1.3 Hz), 6.99 (td, 1H, *J* = 7.5, 1.3 Hz), 6.40 (s, 1H), 4.05 (d, 4H, *J* = 1.9 Hz).

Step 2: palladium-catalyzed cyclization. A 25-mL vial was charged with product from the previous step (120 mg, 0.30 mmol). To the vial was added sodium acetate (75 mg, 0.915 mmol), and 10% Pd/C (15 mg, 0.125 mmol) and the vial was purged with nitrogen before adding dry DMF (6 mL). The reaction was stirred at 140 °C for 18 h. The reaction was diluted with EtOAc and passed through a pad of silica to remove Pd/C particles. The resulting soln was washed with water and brine before being dried over Na_2SO_4 and then was concentrated under reduced pressure. The resulting crude product was transferred to a 20-ml vial.

Step 3: acetal deprotection. To the 20-mL vial was added THF (1.5 mL) and 4 M HCl (1.5 ml, 6 mmol), and stirred at rt for 1h. The crude reaction was extracted using EtOAc, and the organic layer was washed with water and brine before being dried over Na_2SO_4. The solvent was concentrated under reduced pressure and the remaining crude product was purified by recrystallization in MeCN to yield aldehyde **3** as a yellow crystalline solid (45.8 mg, 66% over 2 steps). ^{1}H NMR (600 MHz, chloroform-*d*) δ 10.51 (s, 1H), 8.58 (s,

1H), 8.34 (s, 1H), 8.09 (d, 1H, *J* = 7.9 Hz), 7.70 (d, 1H, *J* = 8.5 Hz), 7.60 (td, 1H, *J* = 7.3, 1.1 Hz), 7.5 (t, 1H, *J* = 7.3 Hz). ^{13}C NMR (150 MHz, chloroform-*d*) δ 187.7, 158.6, 157.1, 148.7, 130.3, 129.4, 127.2, 124.6, 122.2, 122.12, 122.08, 112.5, 108.9. HRMS–APCI (m/z): $[M+H]^+$ calcd for $C_{13}H_7NO_4$: 242.0448, found: 242.0447. mp: 173-180 °C.

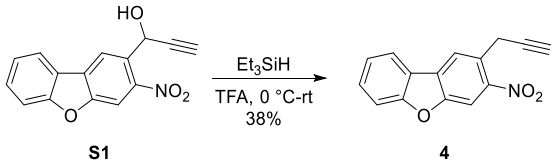
Preparation of propargyl alcohol **S1**.



3 **S1**

A 50-mL round bottom flask was charged with aldehyde **3** (137 mg, 0.567 mmol) and the flask was purged with nitrogen before adding dry THF (8 mL). Aldehyde **3** was completely dissolved before the flask was cooled to –46 °C in a dry ice-MeCN bath. To the soln, a 0.5 M ethynylmagnesium bromide soln in THF (1.48 mL, 0.738 mmol) was added dropwise. The reaction was allowed to warm to rt and was stirred for 18 h. The reaction was quenched with sat. aq NH_4Cl (3 mL) and aq HCl (1 mL, 4 M soln). The resulting soln was diluted with EtOAc, washed with water and brine, and dried over Na_2SO_4. The solvent was concentrated under reduce pressure and the crude product was dry loaded on SiO_2 and purified by flash chromatography (20% Et_2O/hexanes) to afford propargyl alcohol **S1** (120 mg, 79%). ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.54 (s, 1H), 8.25 (s, 1H), 8.06 (d, 1H, *J* = 7.7 Hz), 7.65 (d, 1H, *J* = 8.4 Hz), 7.61 (t, 1H, *J* = 7.4 Hz), 7.45 (t, 1H, *J* = 7.4 Hz), 6.20 (d, 1H, *J* = 2.2 Hz), 2.71 (d, 1H, *J* = 2.2 Hz). ^{1}H NMR (600 MHz, chloroform-*d*) δ 158.4, 154.4, 146.3, 130.5, 129.6, 129.1, 123.9, 122.5, 121.8, 120.9, 112.3, 109.3, 82.2, 74.7, 70.0. HRMS–ESI (m/z): $[M+H]^+$ calcd for $C_{15}H_9NO_4$: 268.0604, found: 268.0594. mp: 141-143 °C.

Preparation of alkyne **4**.

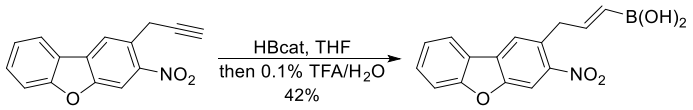


S1 **4**

A 25-ml vial was charged with propargyl alcohol **S1** (115 mg, 0.431 mmol) and purged with nitrogen before adding TFA (7.9 mL). The vial was cooled in an ice bath before adding triethylsilane (0.690 mL, 4.32 mmol) dropwise. The reaction was allowed to warm to rt and was stirred for 18 h. The reaction was then diluted with toluene (10 mL) and all solvents were removed under reduced pressure. The resulting residue was dry loaded on SiO_2 and purified by flash chromatography (100% hexanes) to afford the pure alkyne **3**

(35.2 mg, 38%). ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.38 (s, 1H), 8.30 (s, 1H), 8.06 (d, 1H, *J* = 7.6 Hz), 7.65 (d, 1H, *J* = 8.3 Hz), 7.60 (t, 1H, *J* = 7.9 Hz), 7.44 (t, 1H, *J* = 7.3 Hz), 4.16 (d, 2H, J=2.6), 2.37 (t, 1H, J=2.6). ^{13}C NMR (150 MHz, chloroform-*d*) δ 158.4, 153.9, 146.6, 129.5, 129.1, 127.9, 126.3, 123.8, 122.4, 122.2, 121.8, 112.3, 109.2, 80.2, 72.5, 23.5. HRMS–ESI (m/z): $[M+H]^+$ calcd for $C_{15}H_9NO_3$: 252.0655, found: 252.0646. mp: 110-112 °C.

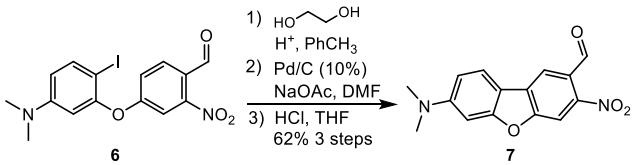
Preparation of vinyl boronic acid **1b**.



4 **1b**

A 4-ml vial was charged with alkyne **4** (30 mg, 0.12 mmol) and purged with nitrogen before adding THF (210 µL). A soln of catechol borane (0.359 mL, 0.359 mmol, 1 M in THF) was added, and the reaction was stirred for 24 h at 75 °C. To the completed reaction was added aq TFA (0.5 mL., 0.1% soln) and the soln was stirred for 4 h at 75 °C. The resulting soln was diluted with THF and passed through a syringe filter before purification by reverse-phase HPLC (50-65% MeCN in H_2O, 0.1% TFA) to afford boronic acid **1b** as a white solid (14.8 mg, 42%). ^{1}H NMR (600 MHz, acetone-d6) δ 8.29 (s, 1H), 8.25 (d, 1H, J = 7.75 Hz), 8.21 (d, 1H, J = Hz), 7.74 (d, 1H, J = 8.45 Hz), 7.67 (ddd, 1H, J = 8.85, 7.2, 1.3 Hz), 7.50 (td, 1H, J = 7.5, 0.8 Hz), 6.87 (s, 2H), 6.76 (dt, 1H, J = 17.8, 6 Hz), 5.44 (dt, 1H, J = 17.8, 1.5 Hz), 3.92 (dd, 2H, J = 6, 1.5 Hz). ^{13}C NMR (150 MHz, acetone-d6) δ 159.95, 155.36, 149.91, 148.75, 131.32, 131.25, 130.3, 125.8, 125.71, 124.24, 123.93, 113.81, 110.25, 71.98, 40.3. HRMS–ESI (m/z): $[M+H]^+$ calcd for $C_{15}H_{12}BNO_5$: 298.0881, found: 298.0873. mp: 121-122 °C (dec).

Preparation of aldehyde **6** (3 steps).



6 **7**

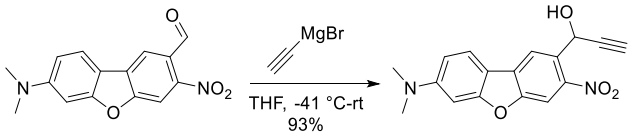
Step 1: acetalization of the carboxaldehyde. Ether **6** was synthesized by previously reported methods.[5] To a 25-mL round bottom containing ether **6** (175 mg, 0.426 mmol) and p-toluenesulfonic acid (28 mg, 0.110 mmol) was added toluene (4 mL) and ethylenglycol (0.157 mL, 2.81 mmol). The reaction was stirred under reflux in a Dean Stark apparatus for 40 min, darkening to a mulberry color as the reaction progressed. After completion, the reaction was diluted with EtOAc and washed with sat. aq $NaHCO_3$, water, and brine before being dried over Na_2SO_4. The solvent was concentrated under reduce pressure and the crude product was

dry loaded on SiO_2 and purified by flash chromatography (5-15% EtOAc/hexanes) to yield the protected aldehyde (152 mg, 78%).

Step 2: palladium-catalyzed cyclization. A 25-mL vial was charged with the product from the previous step (73.6 mg, 0.16 mmol), sodium acetate (39.6 mg, 0.483 mmol), and 10% Pd/C (6.9 mg, 0.0064 mmol) and purged with nitrogen before adding dry DMF (3.6 mL). The reaction stirred at 140 °C for 48 h. The reaction was diluted with EtOAc and passed through a pad of silica to remove Pd/C particles. The resulting soln was washed with water and brine before being dried over Na_2SO_4 and then was concentrated under reduced pressure. The resulting crude was transferred to a 20-ml vial.

Step 3: acetal deprotection. To the 20-mL vial was added THF (2 mL) and 4 M HCl (2 mL, 8 mmol), and the reaction was stirred at rt for 1h. The crude reaction was diluted with EtOAc and washed with $NaHCO_3$, DI water, and brine before being dried over Na_2SO_4. The solvent was concentrated under reduced pressure and the remaining crude was purified by recrystallization in MeCN to yield aldehyde **7** as a red/brown crystalline solid (36.7 mg, 80% over 2 steps). ^{1}H NMR (600 MHz, chloroform-*d*) δ 10.51 (s, 1H), 8.27 (s, 1H), 8.22 (s, 1H), 7.82 (d, 1H, *J* = 8.7 Hz), 6.84 (dd, 1H, *J* = 8.7, 1.9 Hz), 6.81 (d, 1H, *J* = 1.9 Hz), 3.13 (s, 6H). ^{13}C NMR (150 MHz, chloroform-*d*) δ 188.7, 161.6, 156.6, 152.7, 146.1, 130.8, 127.9, 122.5, 119.3, 110.7, 110.3, 108.0, 93.7, 40.7. HRMS–ESI (m/z): $[M+H]^+$ calcd for $C_{15}H_{12}N_2O_4$: 285.0870; found: 285.0868. mp: 232-234 °C (dec).

Preparation of propargyl alcohol **S2**.

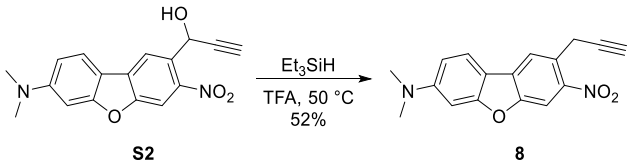


7 **S2**

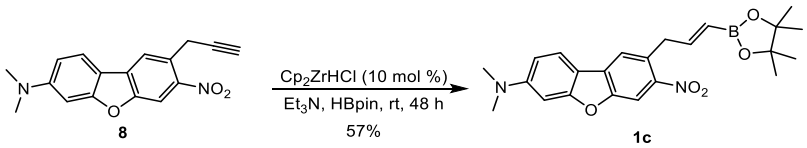
A 25-mL round bottom flask was charged with aldehyde **7** (210 mg, 0.739 mmol) and the flask was purged with nitrogen before adding dry THF (2 mL). Aldehyde **7** was completely dissolved before being cooled to –46 °C in a dry ice-MeCN bath. To the soln, a 1.0 M ethynylmagnesium bromide soln in THF (1.12 mL, 1.12 mmol) was added dropwise. The reaction was allowed to warm to rt and was stirred for 2 h. The reaction was quenched with sat. aq NH_4Cl (3 mL) and 4 M HCl (1 mL). The resulting soln was extracted with EtOAc, and the combined organic layers were washed with water and brine before being dried over Na_2SO_4. The solvent was concentrated under reduced pressure, and the crude product was dry loaded on SiO_2 and purified by flash chromatography (10-30% EtOAc/hexanes) to afford propargyl alcohol **S2** (212 mg, 93%). ^{1}H NMR (600 MHz, acetone-*d6*) δ 8.45 (s, 1H), 8.17 (s, 1H), 8.01 (d, 1H, *J* = 8.6 Hz), 6.92 (dd, 1H, *J* = 8.6, 2.2 Hz), 6.91 (d, 1H, *J* = 2.2 Hz), 6.29 (ddd, 1H, *J* = 5.8, 2.2, 0.6 Hz), 5.52 (d, 1H, *J* = 5.8 Hz),

3.13 (s, 6H), 3.09 (d, 1H, *J* = 2.2 Hz). ^{13}C NMR (150 MHz, acetone-d6) δ 162.2, 154.6, 153.8, 145.0, 133.4, 131.0, 123.4, 118.7, 112.0, 111.0, 108.9, 94.5, 84.7, 74.9, 60.84, 60.75, 40.9. HRMS–APCI (m/z): $[M+H]^+$ calcd for $C_{17}H_{14}N_2O_4$: 311.1026; found: 311.1022. mp: 175-180 °C (dec).

Preparation of alkyne **8**.



A 25-ml vial was charged with propargyl alcohol **S2** (20.2 mg, 0.064 mmol) and purged with nitrogen and TFA (1.5 mL) was added, turning the rxn colorless. The vial was cooled in an ice bath and triethylsilane was added (0.690 mL, 4.32 mmol) dropwise. The reaction was heated to 50 °C and stirred for 3 h. The reaction was then diluted with toluene (10 mL) and all solvents were removed under reduced pressure. The resulting residue was dry loaded on SiO_2 and purified by flash chromatography (5-10% EtOAc/hexanes) to afford the pure alkyne **8** (9.9 mg, 51%).^{1}H NMR (600 MHz, chloroform-*d*) δ 8.20 (s, 1H), 8.09 (s, 1H), 7.77 (d, 1H, *J* = 9.3 Hz), 6.78 (m, 2H), 4.13 (d, 2H, *J* = 2.3 Hz), 3.10 (s, 6H), 2.35 (t, 2H, *J* = 2.3 Hz). ^{13}C NMR (600 MHz, chloroform-*d*) δ 161.2, 153.5, 152.3, 143.9, 130.6, 126.9, 122.2, 112.0, 111.2, 109.6, 108.5, 93.8, 80.7, 72.2, 40.7, 29.7, 23.9. HRMS–ESI (m/z): $[M+H]^+$ calcd for $C_{17}H_{14}N_2O_3$: 295.1077; found: 295.1071. mp: 133-136 °C (dec).



In a glovebox, alkyne **8** (24.0 mg, 0.082 mmol) in a 2-mL vial was treated sequentially with Schwartz's reagent (2 mg, 0.008 mmol), pinacolborane (300 µL, 2.06 mmol), and triethylamine (1.13 µL, 0.0082 mmol). The resulting mixture was stirred at room temperature for 18 h. As the reaction progressed, it changed from a heterogeneous red mixture to an opaque pale orange slurry. The crude reaction was removed from the glovebox and quenched with MeOH (1 mL). The resulting crude product was dry loaded onto silica and purified by flash chromatography (3-10% EtOAc/hexanes) to afford boronate **1c** as a bright orange solid (20.3 mg, 59%). ^{1}H NMR (600 MHz, chloroform-*d*) δ 8.15 (s, 1H), 7.74 (d, 1H, *J* = 8.3 Hz), 7.61 (s, 1H), 6.85 (dt, 1H, *J* = 17.9, 6 Hz), 6.78 (m, 2H), 5.46 (d, 1H, *J* = 17.9 Hz), 3.95 (dd, 2H, *J* = 6, 1.1 Hz), 3.10 (s, 6H), 1.25 (s, 12 H). ^{13}C NMR (150 MHz, chloroform-*d*) δ 161.1, 153.4, 152.3, 150.9, 144.9, 130.3, 129.8, 122.0, 121.5, 111.2, 109.6, 108.3, 93.9, 83.2, 83.1, 40.7, 39.9, 24.8. MS–ESI (m/z) was

performed after hydrolysis to the boronic acid in TFA/THF/H_2O: $[M + H]^+$ calcd for $C_{17}H_{18}BN_2O_5$: 341.1, found: 341.1. mp: 137-140 °C (dec).

HPLC and additional MALDI-MS

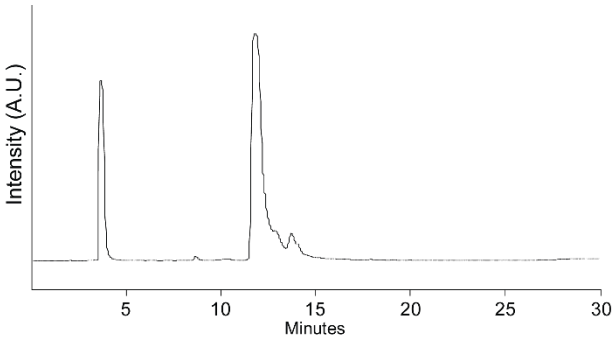


Figure S1. HPLC of purified **1b**. Ramp: 20-70% MeCN/H_2O, UV detection at 220 nm.

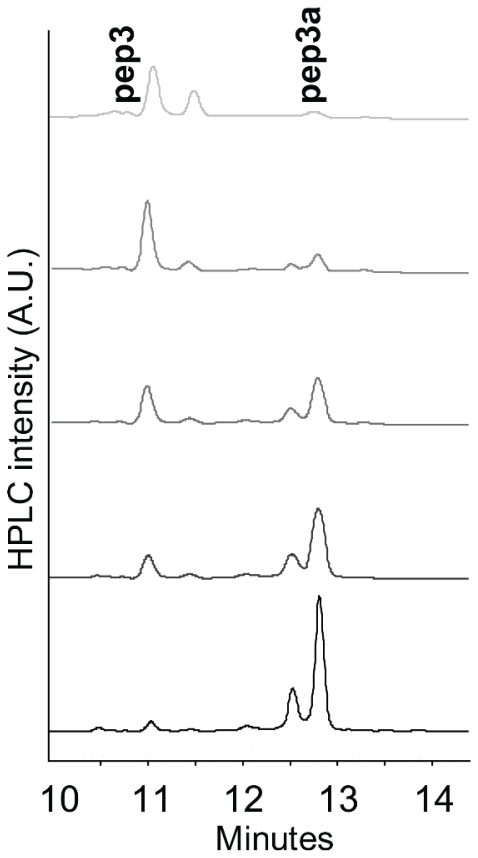


Figure S2. HPLC kinetics of **pep3** modification with **1a** photocleavage with blue LED light (nominal 450 nm). Samples (80 µL) were irradiated for 0, 30, 60, 180, and 300 s. From each irradiated sample, 45 µL were injected for analysis. Ramp: 20-70% MeCN/H_2O, UV detection at 220 nm.

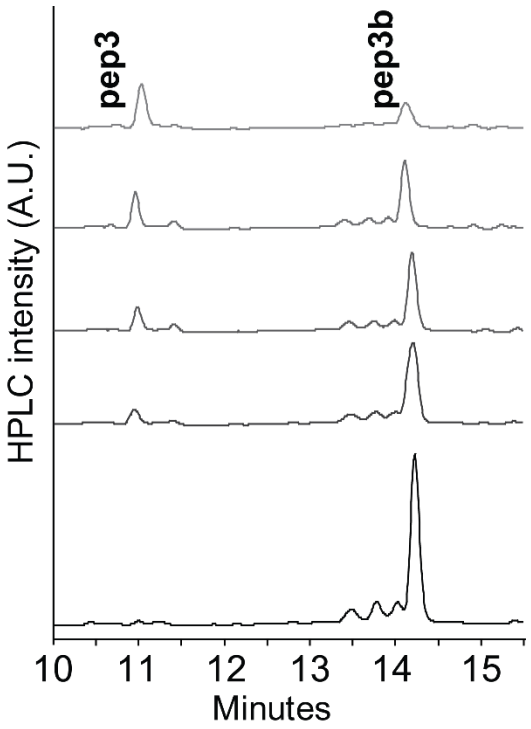


Figure S3. HPLC kinetics of **pep3** modified with **1b** photocleavage with blue LED light (nominal 450 nm). Samples (80 µL) were irradiated for 0, 1, 5, 10, 20, and 30 m. From each irradiated sample, 45 µL were injected for analysis. Ramp: 20-70% MeCN/H_2O, UV detection at 220 nm.

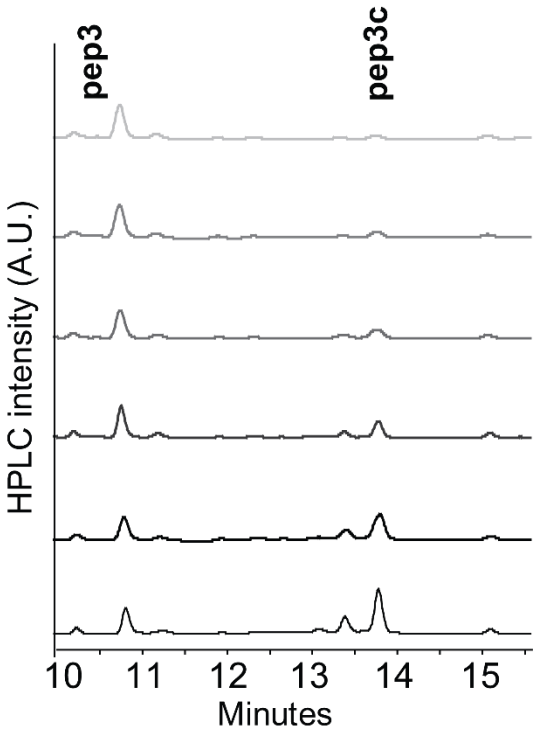


Figure S4. HPLC kinetics of **pep3** modified with **1c** photocleavage with blue LED light (nominal 450 nm). Samples (80 µL) were irradiated for 0, 5, 10, 15, 20, and 30 s. From each irradiated sample, 45 µL were injected for analysis. Ramp: 20-70% MeCN/H_2O, UV detection at 220 nm.

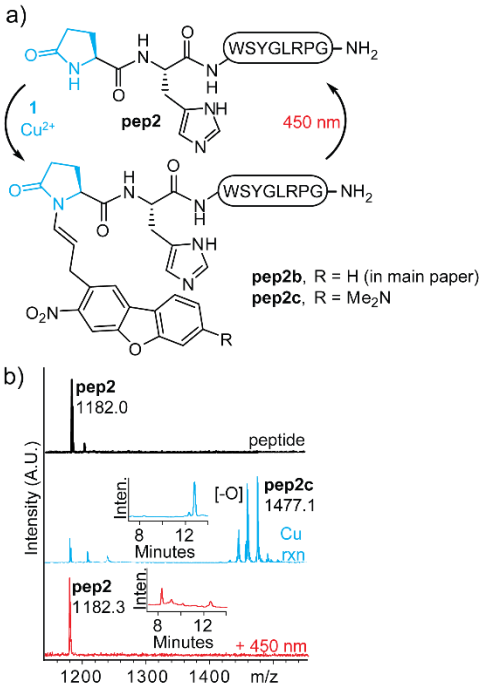


Figure S5. (a) Schematic depiction of modification and photorelease of hormone releasing peptide (**pep2**) with boronic acid **1b** and **1c**. (c) MALDI–TOF MS of **pep2** $[M+H]^+$ before (black) and after (cyan) copper-mediated N–H photocaging with **1c**. Irradiation with a blue LED (nominal 450 nm) (red) causes photocleavage. (insets) HPLC analysis before (cyan) and after (red) irradiation. Condns: peptide (100 μM), **1c** (2 mM), $Cu(NO_3)_2$ (1 mM), NMM buffer + 40% v/v DMSO (pH 6.0), 37 °C.

LC/MS analysis of 2-photon uncaging

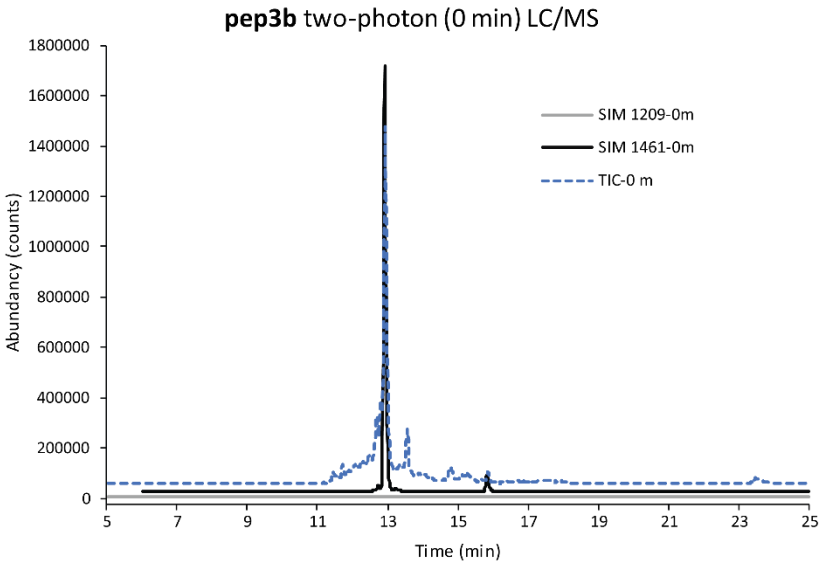


Figure S6. LC/MS analysis of **pep3b** before irradiation with 800 nm light.

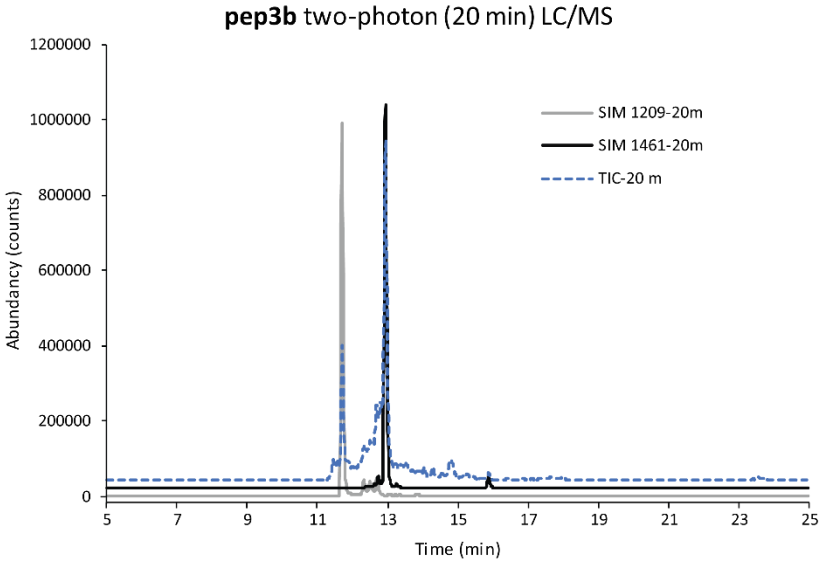


Figure S7. LC/MS analysis of **pep3b** after 20 min irradiation with 800 nm light.

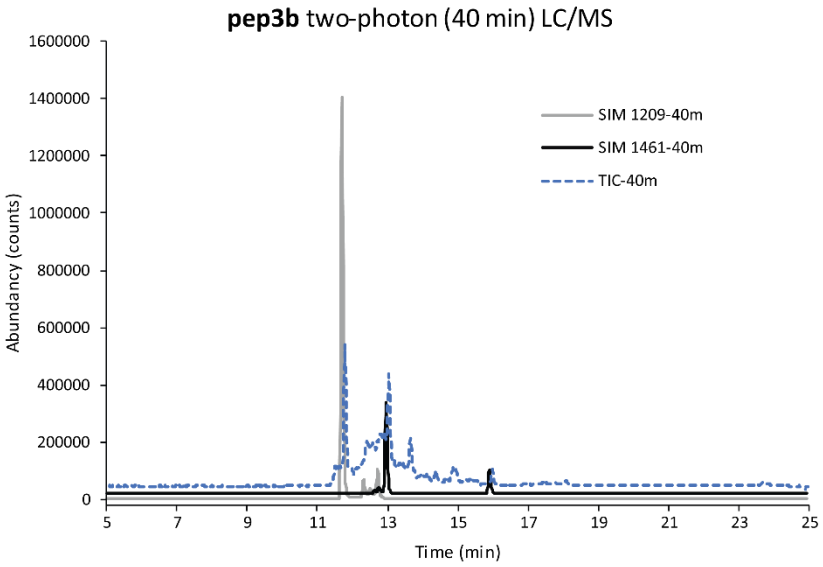


Figure S8. LC/MS analysis of **pep3b** after 40 min irradiation with 800 nm light.

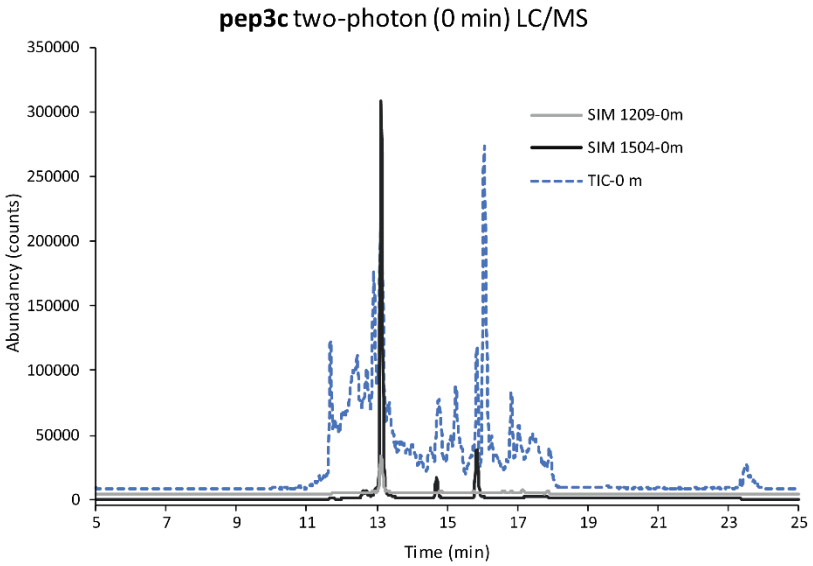


Figure S9. LC/MS analysis of **pep3c** before irradiation with 800 nm light.

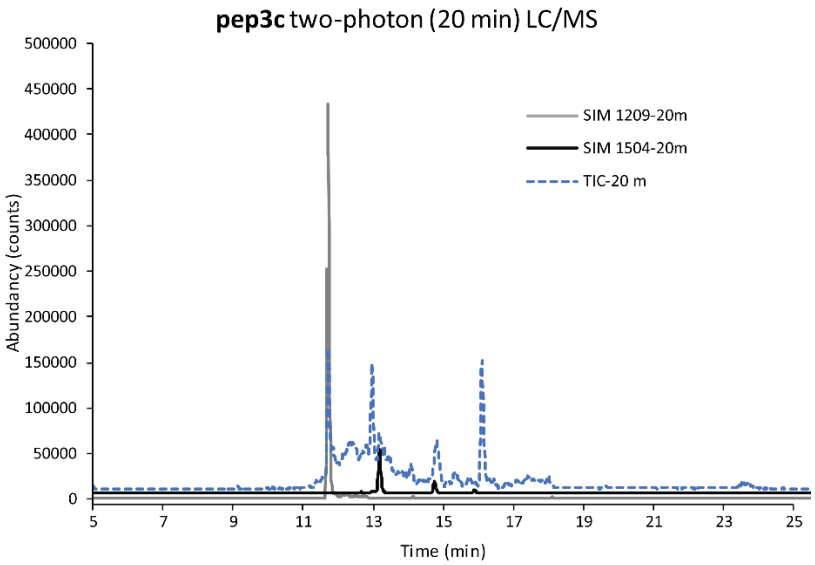


Figure S10. LC/MS analysis of **pep3c** after 20 min irradiation with 800 nm light.

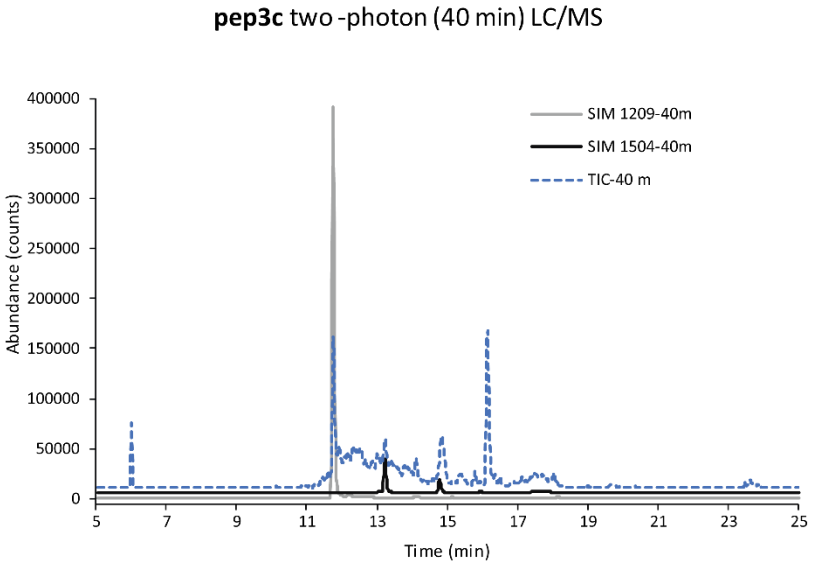


Figure S11. LC/MS analysis of **pep3c** after 40 min irradiation with 800 nm light.

UV-vis determined extinction coefficient

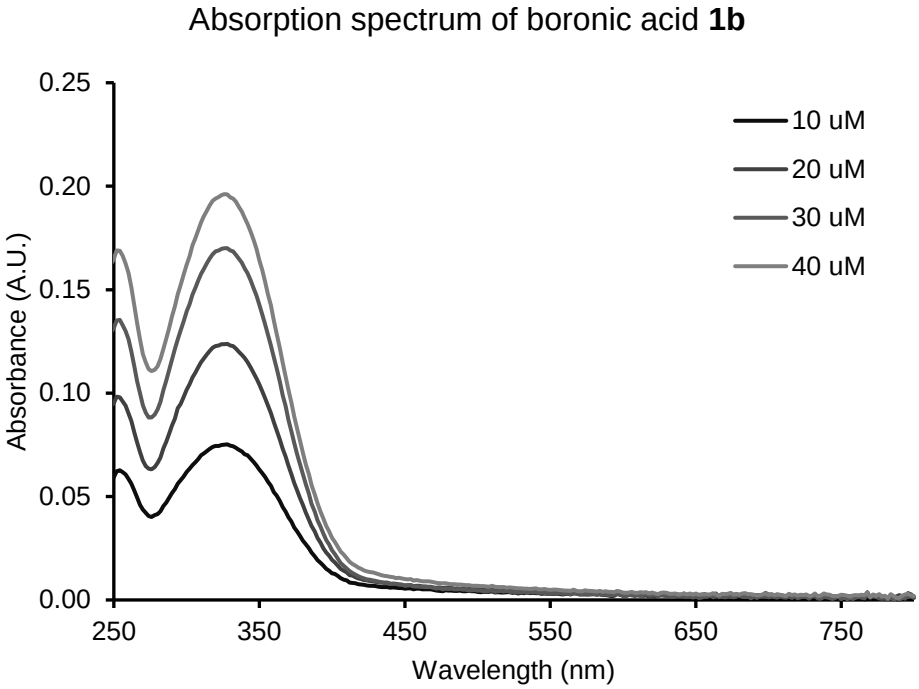


Figure S12. Absorption spectrum of boronic acid **1b** at various concentrations to calculate maximum extinction coefficient as $\varepsilon = 5200\ M^{-1}cm^{-1}$ at 324 nm. Concentrations were calculated by weighing **1b** into a solution of DMSO at a concentration of 1 mM and diluting this solution into water/DMSO solution for each sample. Extinction coefficient was calculated using Beer-Lambert law $A = \varepsilon cl$, where A = absorption, ε = extinction coefficient, c = concentration, and l = pathlength (1 cm).

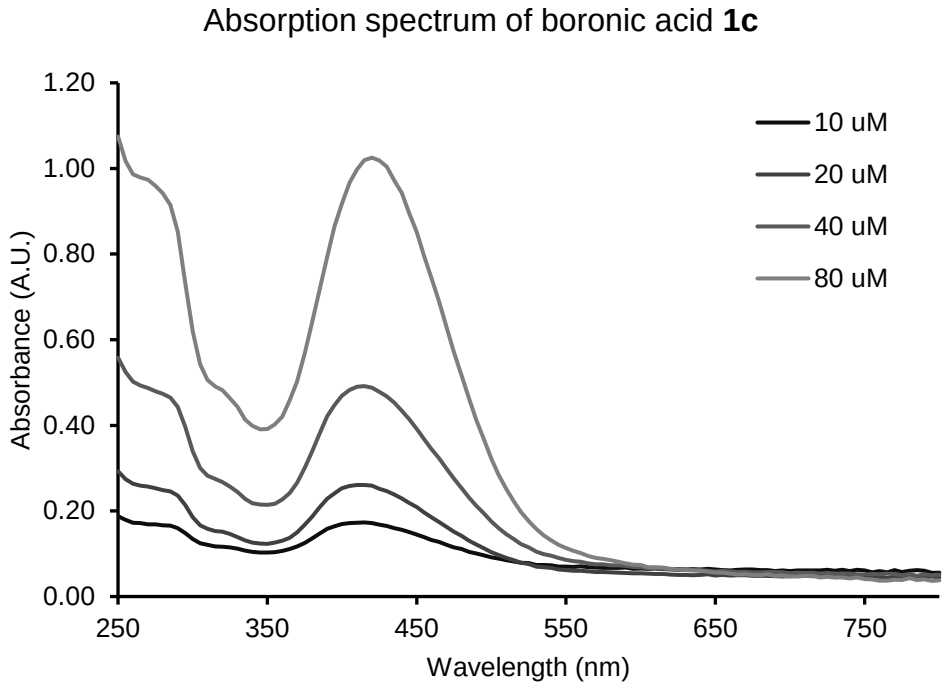
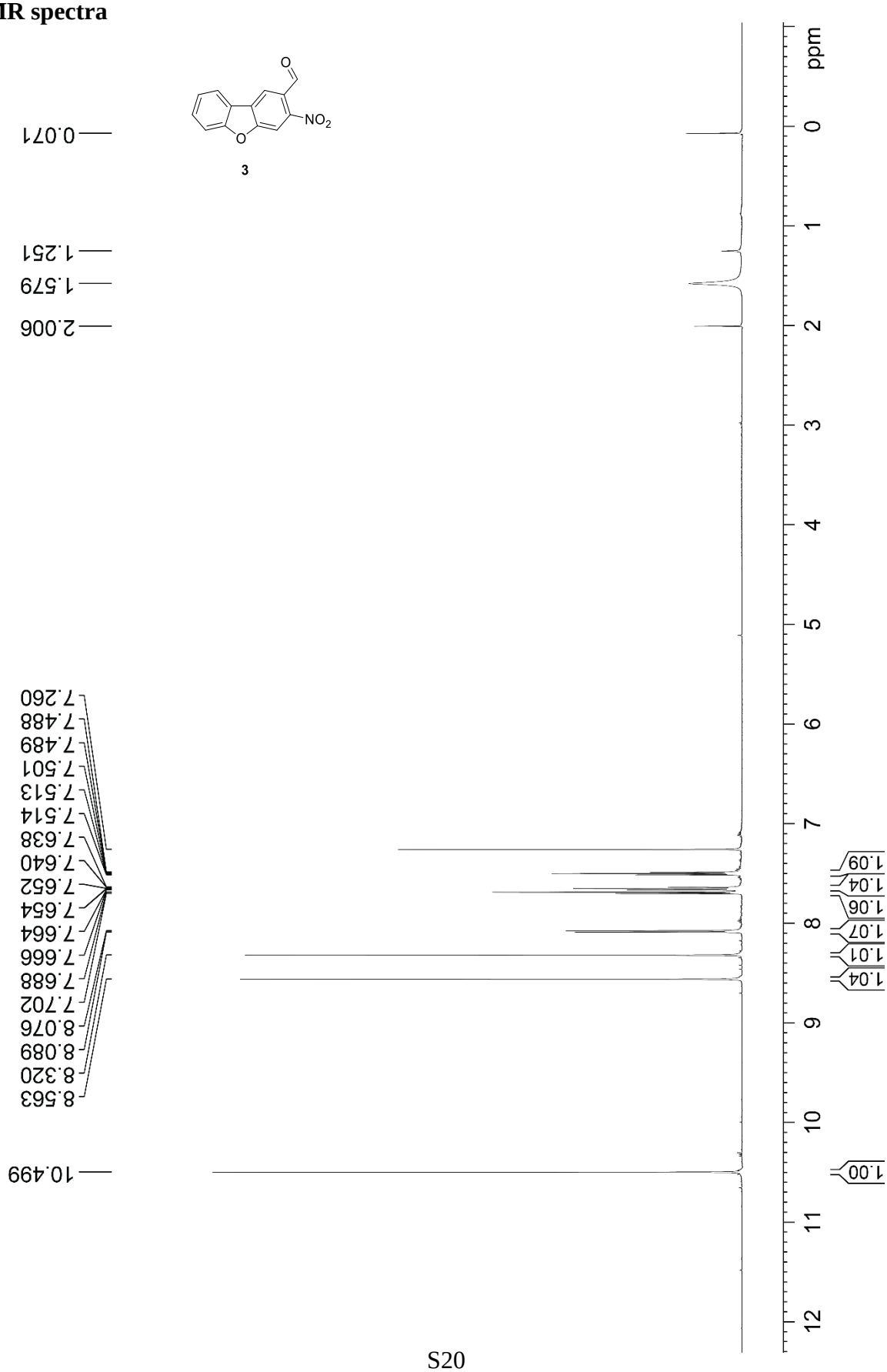
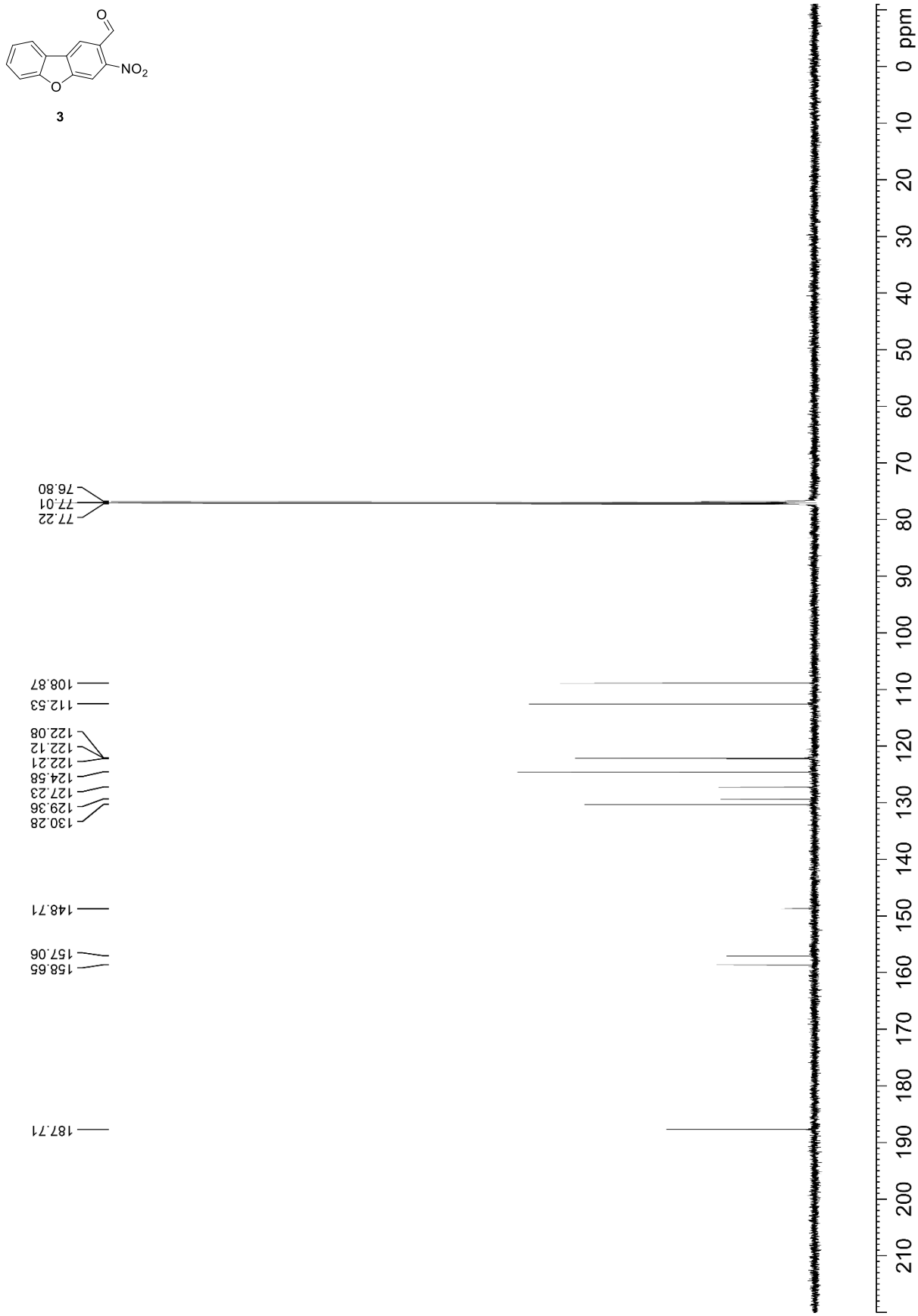
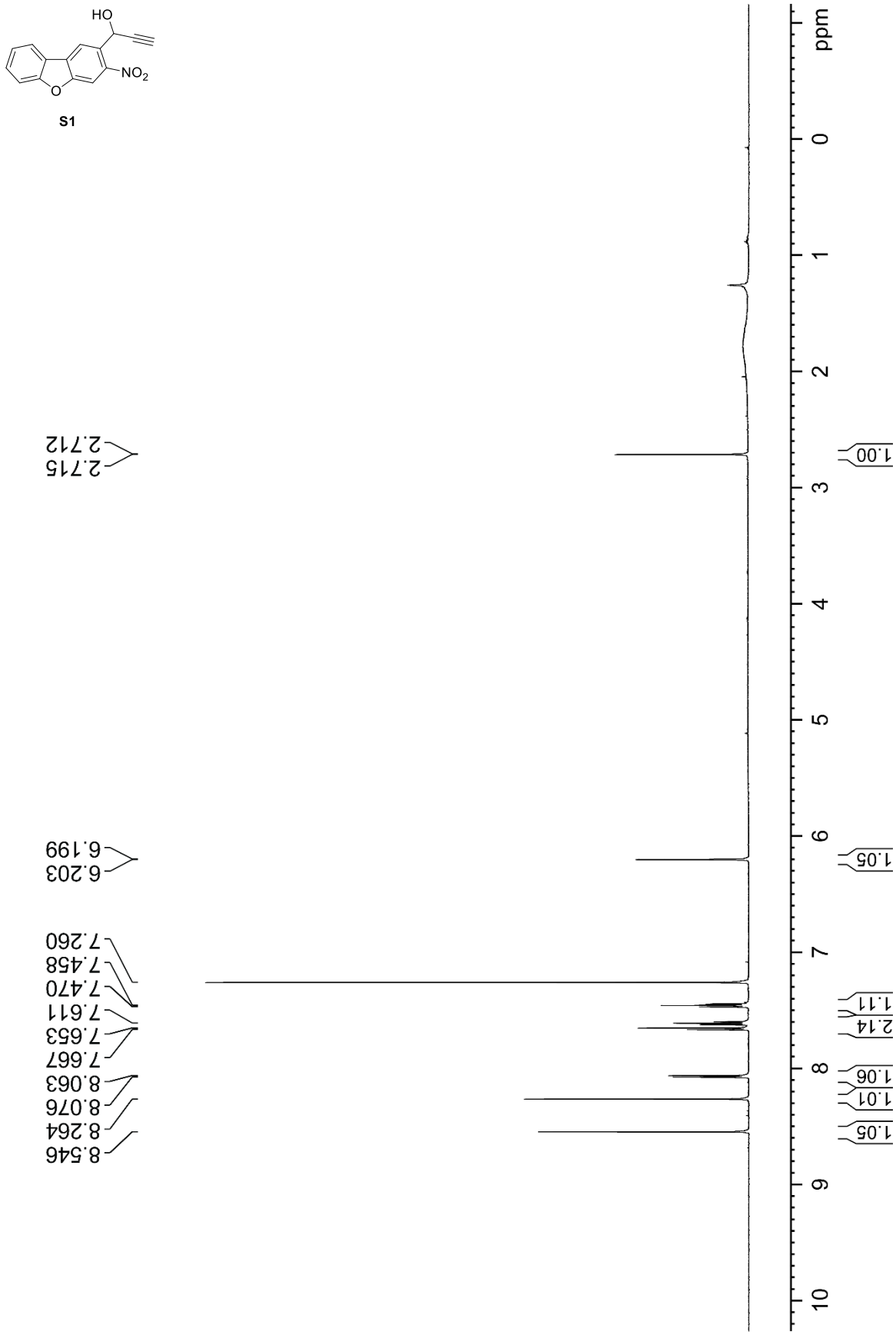


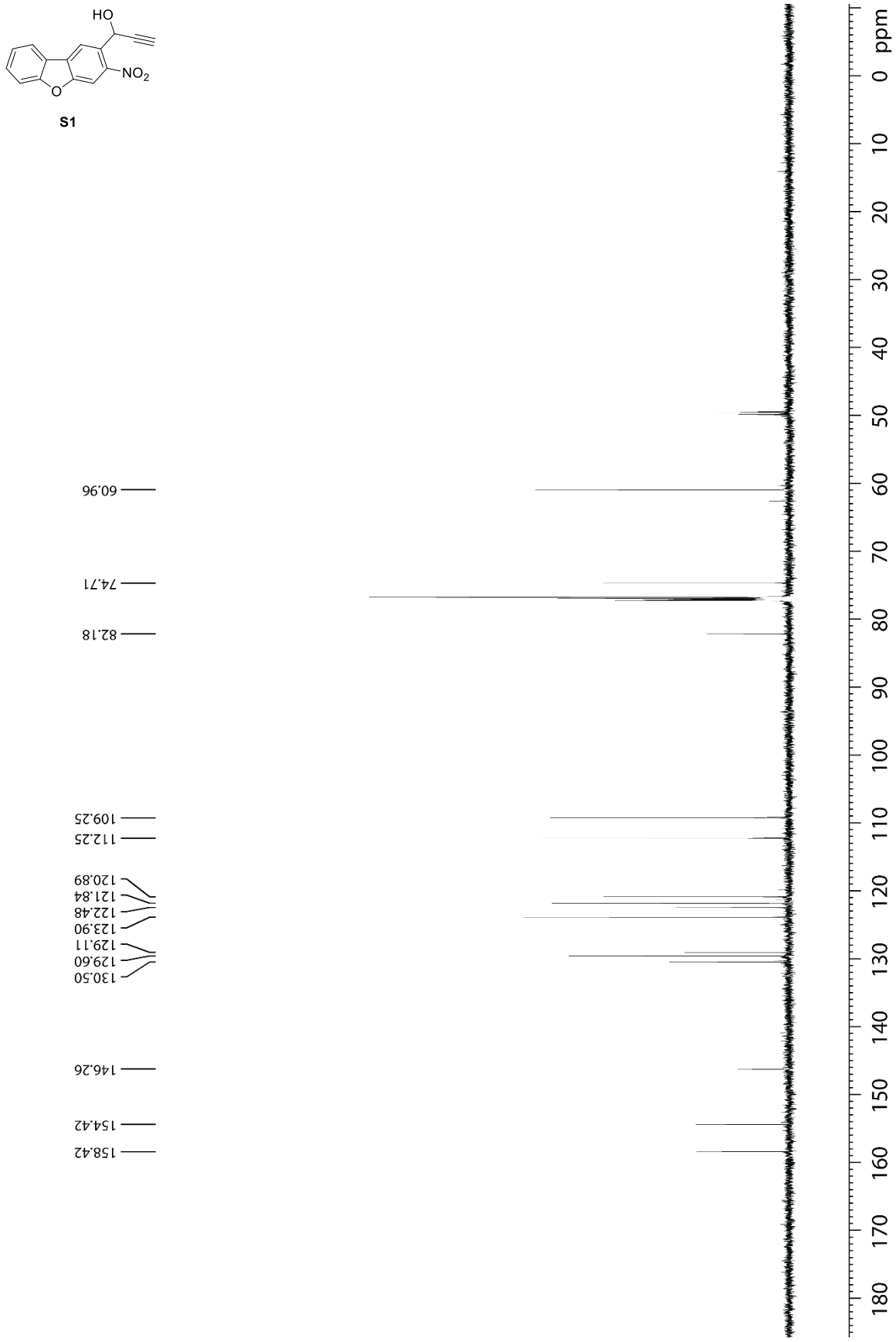
Figure S13. Absorption spectrum of boronic acid **1c** at various concentrations to calculate maximum extinction coefficient as ε = 12690 $M^{-1}cm^{-1}$ at 425 nm. Concentrations were calculated by weighing **1b** into a solution of DMSO at a concentration of 1 mM and diluting this solution into water/DMSO solution for each sample. Extinction coefficient was calculated using Beer-Lambert law A = εcl, where A = absorption, ε = extinction coefficient, c = concentration, and l = pathlength (1 cm).

NMR spectra

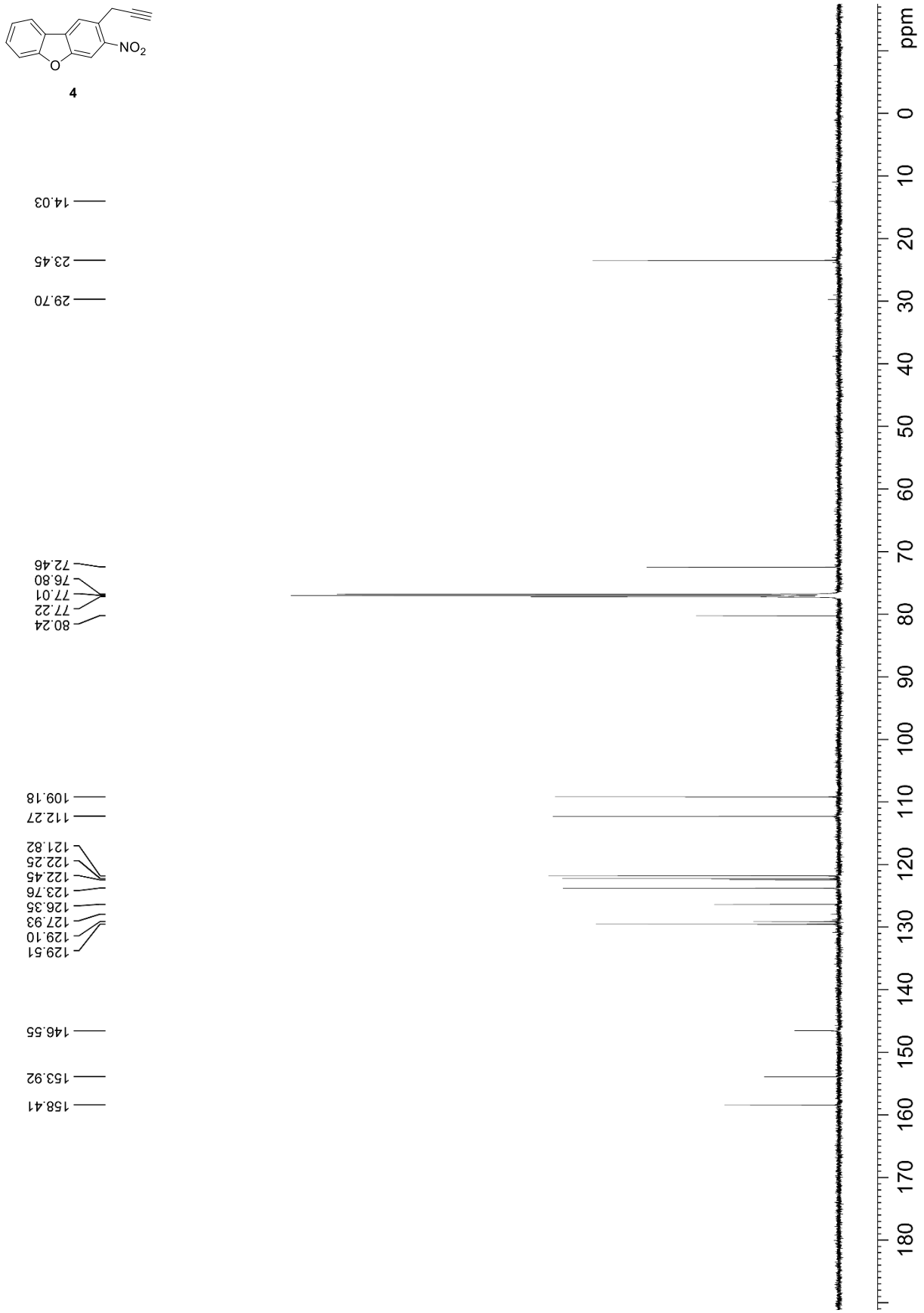

O
NO2
O
3
0.071
1.251
1.579
2.006
7.260
7.488
7.489
7.501
7.513
7.514
7.638
7.640
7.652
7.654
7.664
7.666
7.688
7.702
8.076
8.089
8.320
8.563
10.499
1.00
1.04
1.01
1.07
1.06
1.04
1.09
ppm
0
1
2
3
4
5
6
7
8
9
10
11
12

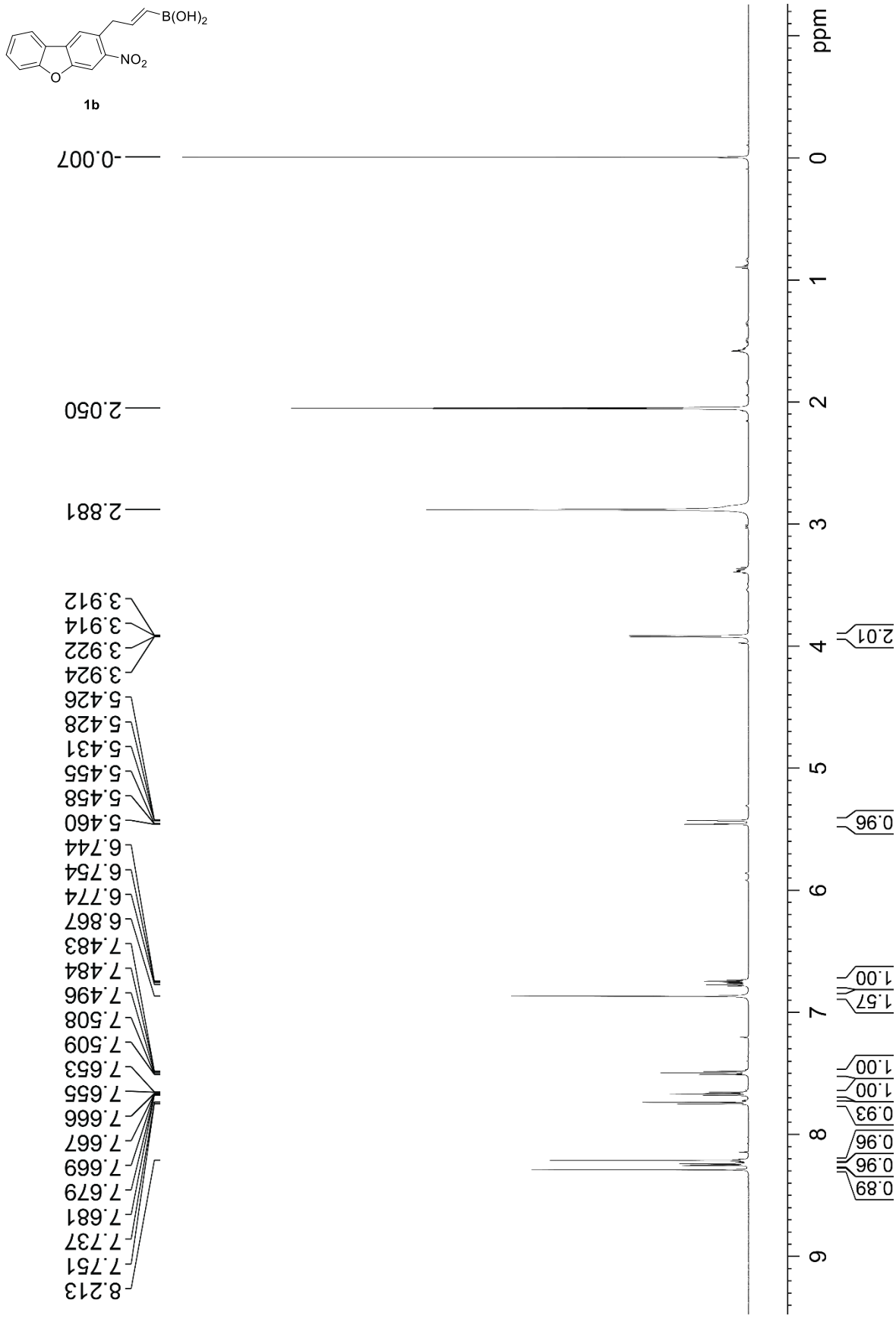

O
NO2
O
3
187.71
158.65
157.06
148.71
130.28
129.36
127.23
124.58
122.21
122.12
122.08
112.53
108.87
77.22
77.01
76.80
0 ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210

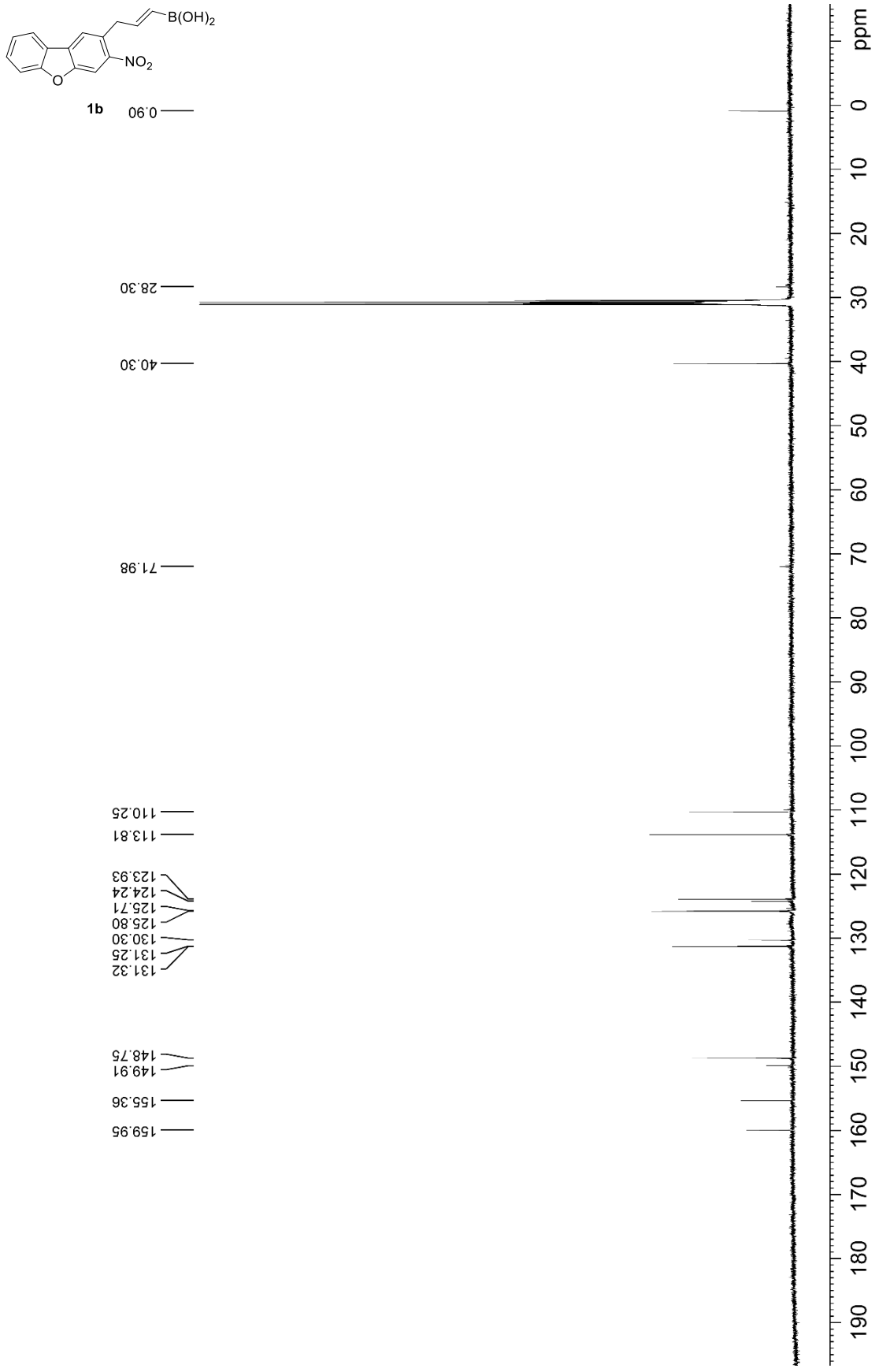

HO
NO2
O
S1
8.546
8.264
8.076
8.063
7.667
7.653
7.611
7.470
7.458
7.260
6.203
6.199
2.715
2.712
1.05
1.01
1.06
2.14
1.11
1.05
1.00
ppm

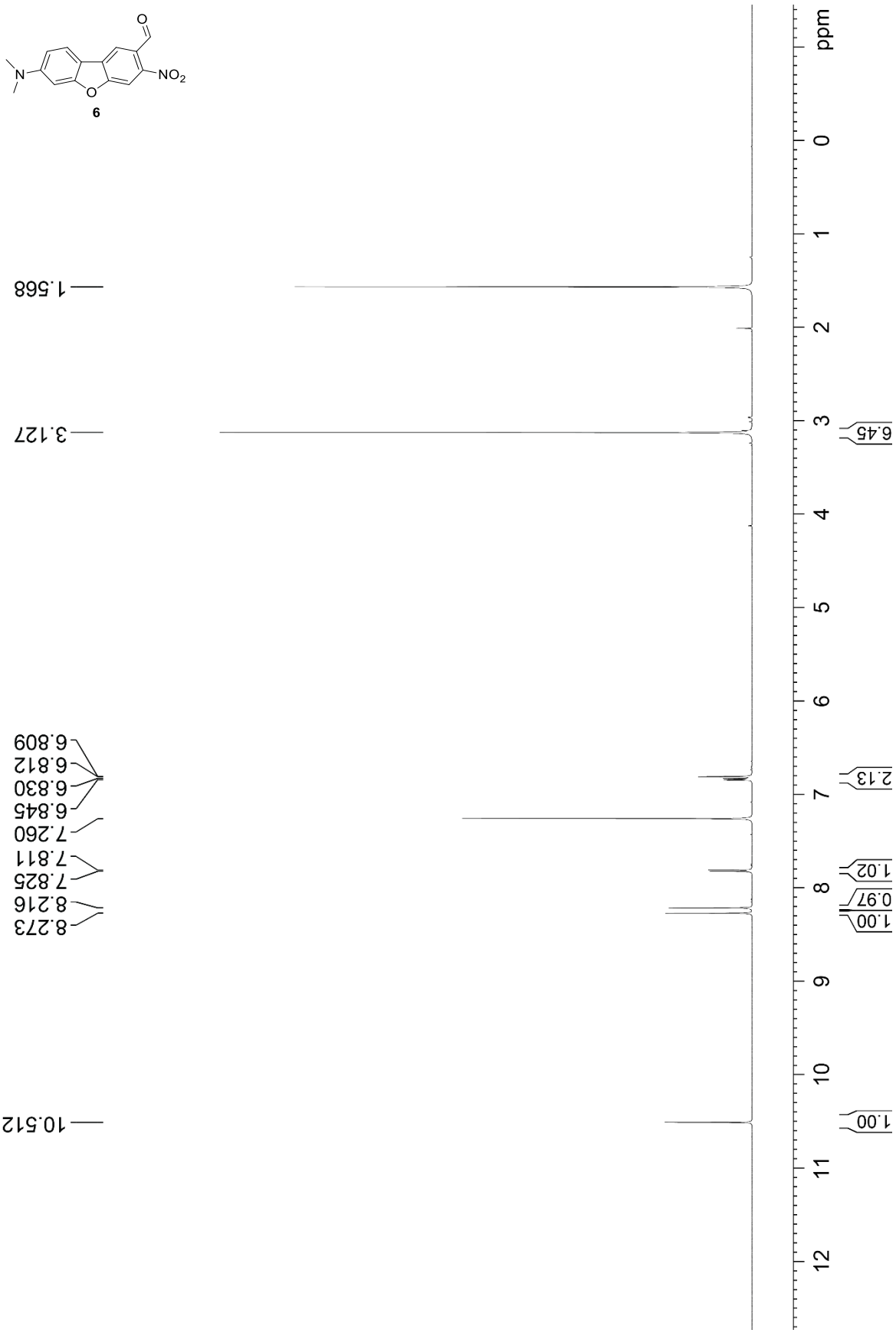

HO
NO2
O
S1
60.96
74.71
82.18
109.25
112.25
120.89
121.84
122.48
123.90
129.11
129.60
130.50
146.26
154.42
158.42
0 ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180

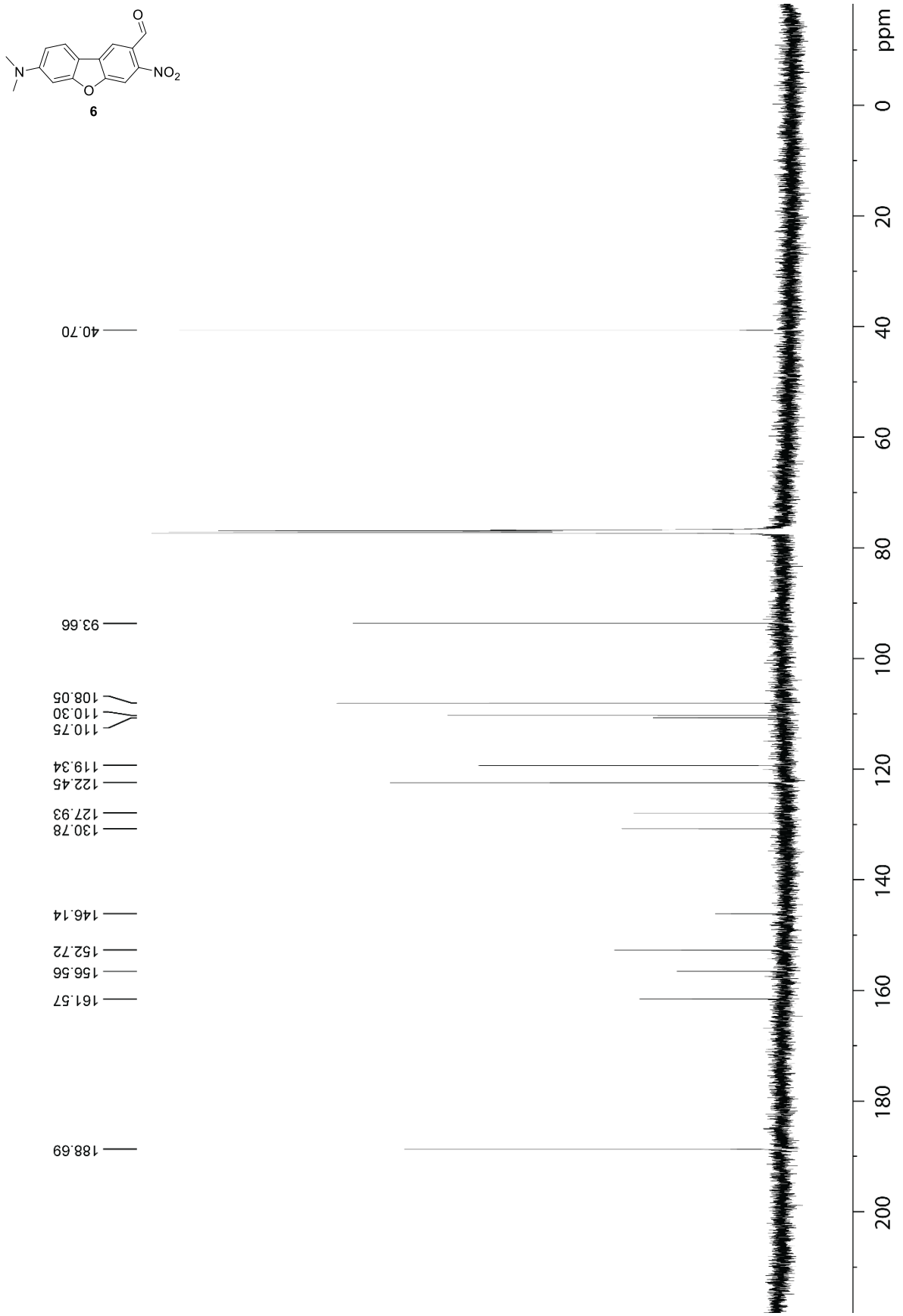
4
0.071
1.532
2.362
2.366
2.371
4.156
4.160
7.260
7.429
7.430
7.442
7.454
7.455
7.582
7.584
7.596
7.597
7.608
7.610
7.641
7.654
8.056
8.068
8.303
8.379
1.00
1.02
1.09
1.10
1.16
1.15
2.30
1.03
ppm

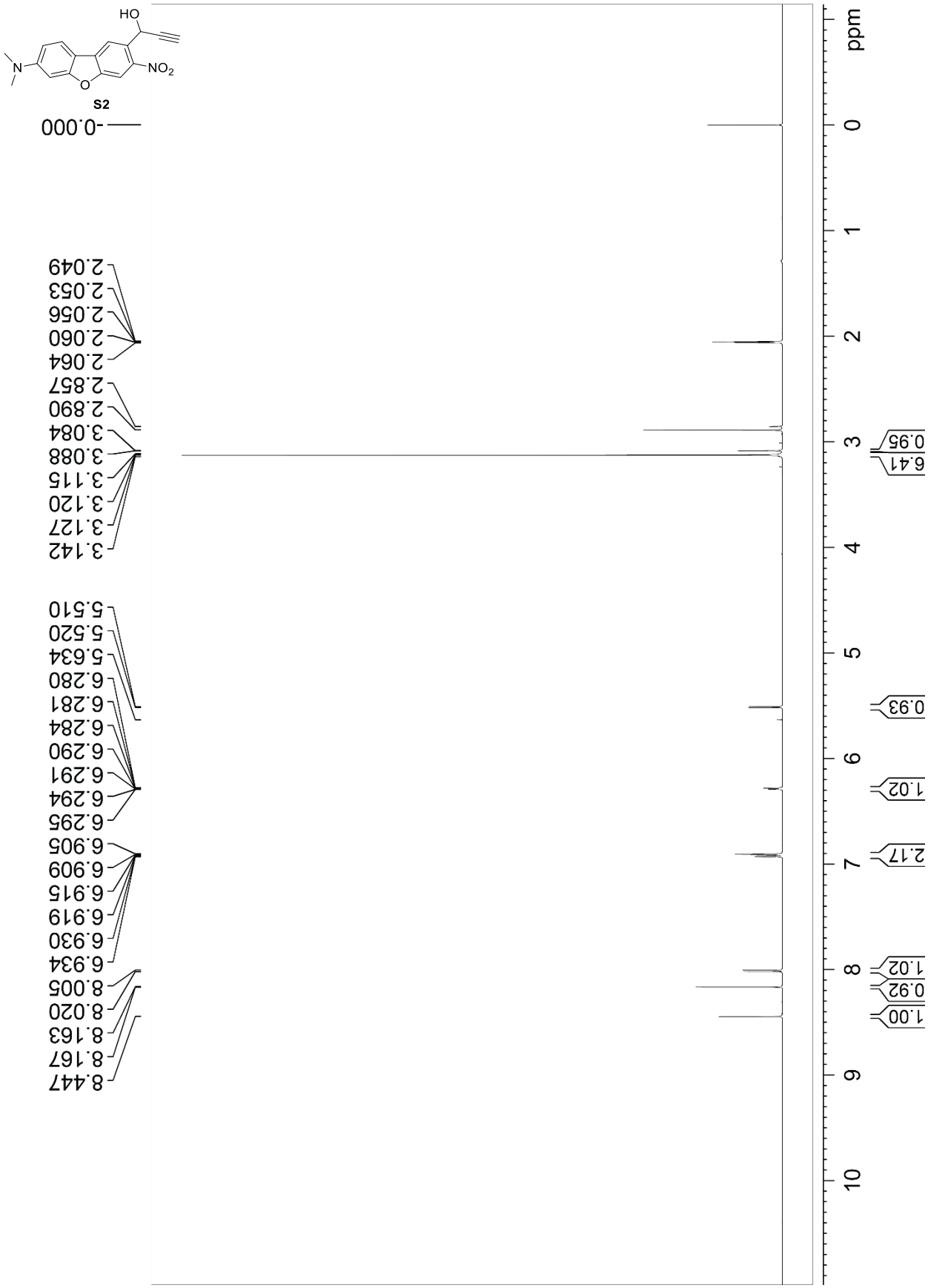

NO2
O
4
14.03
23.45
29.70
72.46
76.80
77.01
77.22
80.24
109.18
112.27
121.82
122.25
122.45
123.76
126.35
127.93
129.10
129.51
146.55
153.92
158.41
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180

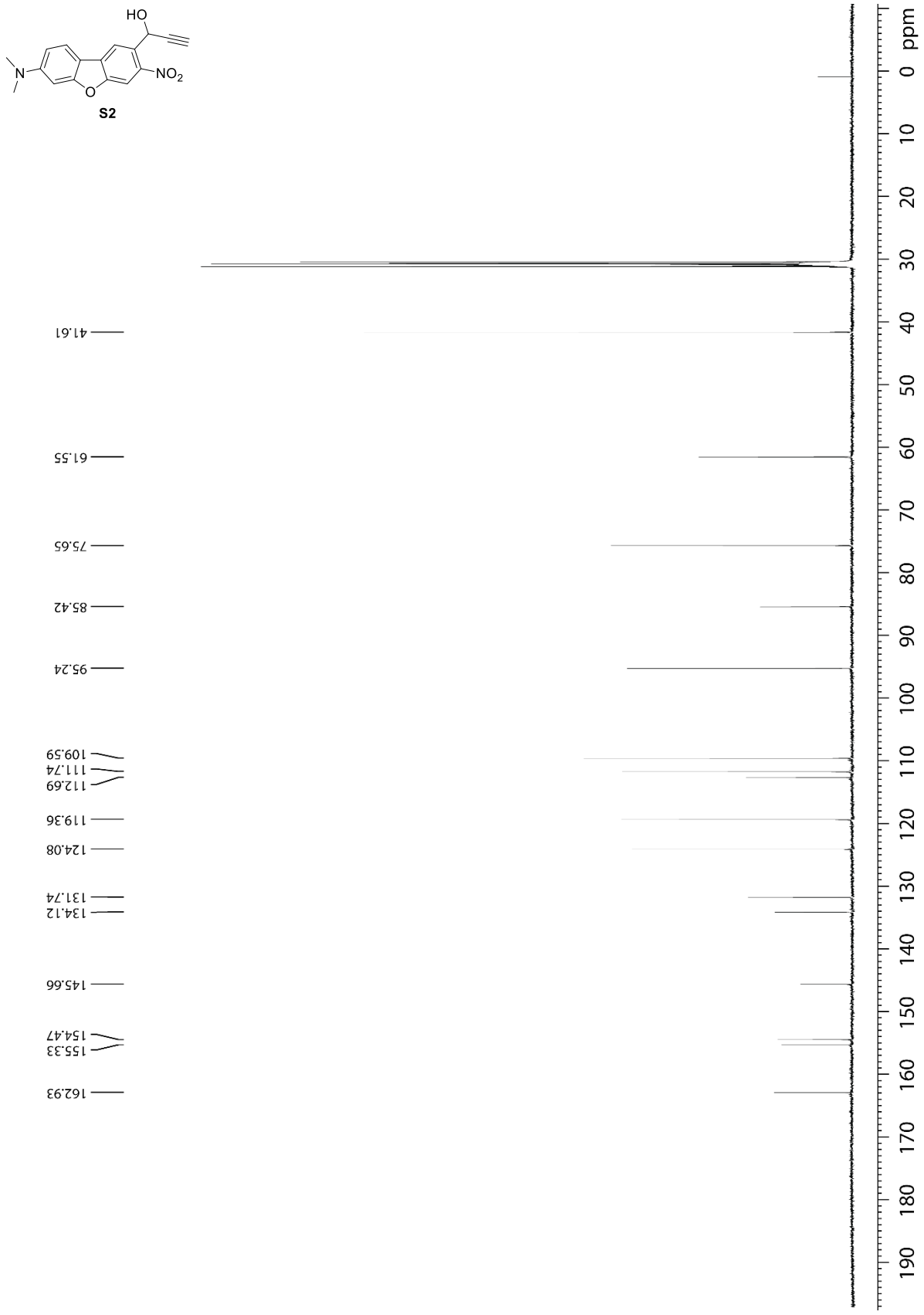

B(OH)2
NO2
O
1b
-0.007
2.050
2.881
3.912
3.914
3.922
3.924
5.426
5.428
5.431
5.455
5.458
5.460
6.744
6.754
6.774
6.867
7.483
7.484
7.496
7.508
7.509
7.653
7.655
7.666
7.667
7.669
7.679
7.681
7.737
7.751
8.213
0.89
0.96
0.96
0.93
1.00
1.00
1.57
1.00
0.96
2.01
ppm

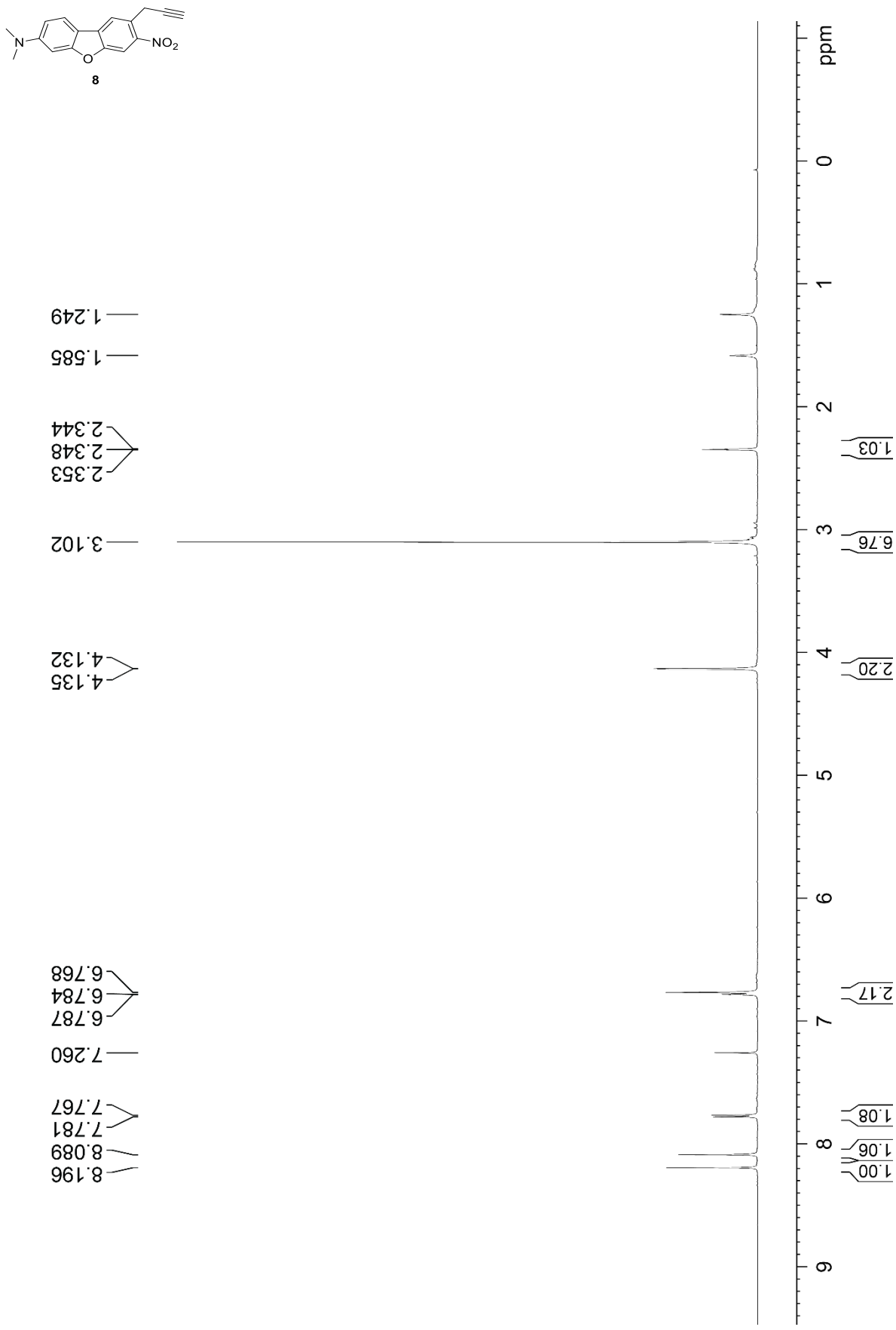

B(OH)2
NO2
O
1b
0.90
28.30
40.30
71.98
110.25
113.81
123.93
124.24
125.71
125.80
130.30
131.25
131.32
148.75
149.91
155.36
159.95
ppm

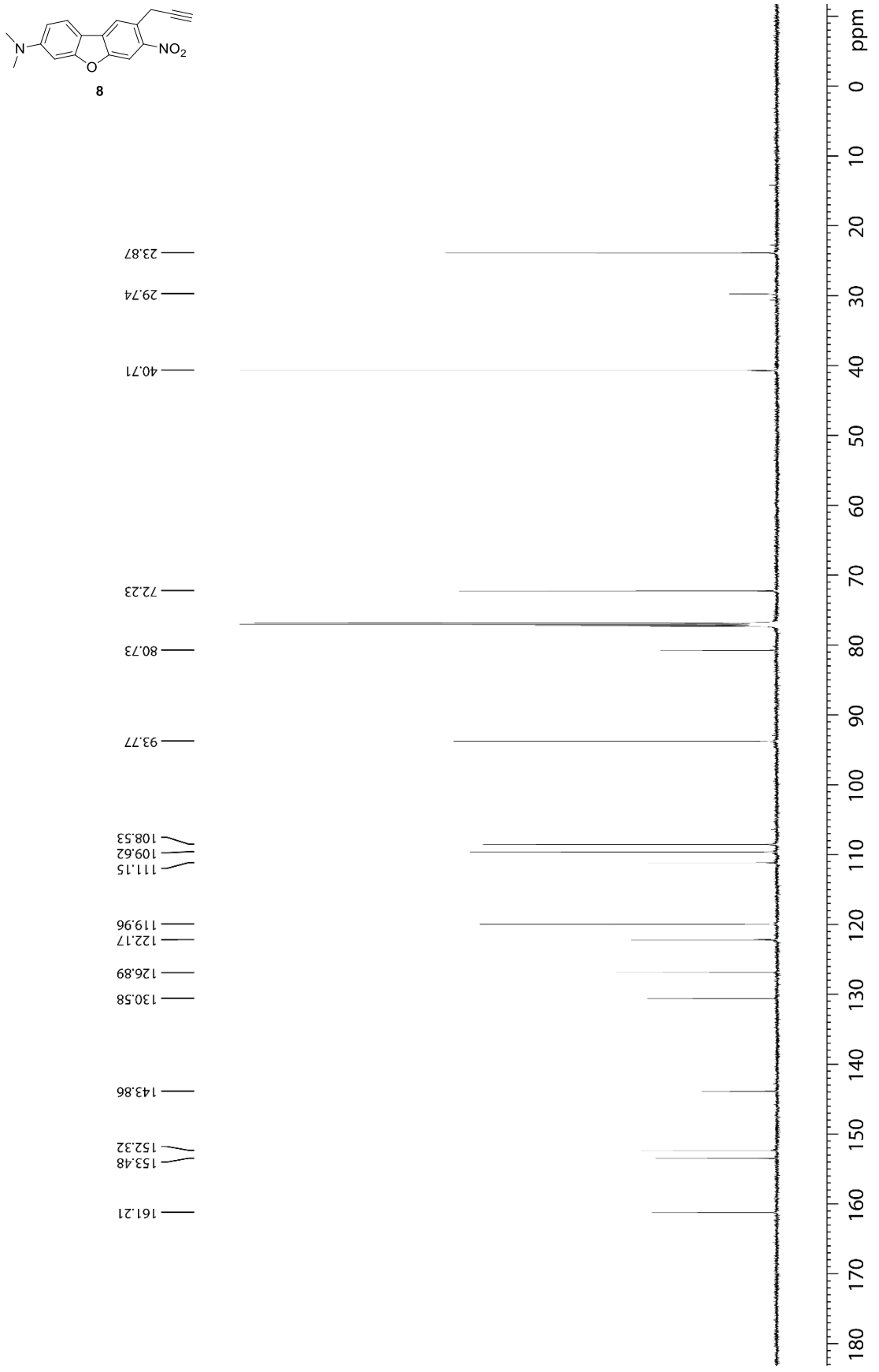

O
N
NO2
O
6
10.512
8.273
8.216
7.825
7.811
7.260
6.845
6.830
6.812
6.809
3.127
1.568
1.00
2.13
1.02
0.97
1.00
6.45
ppm
0
1
2
3
4
5
6
7
8
9
10
11
12

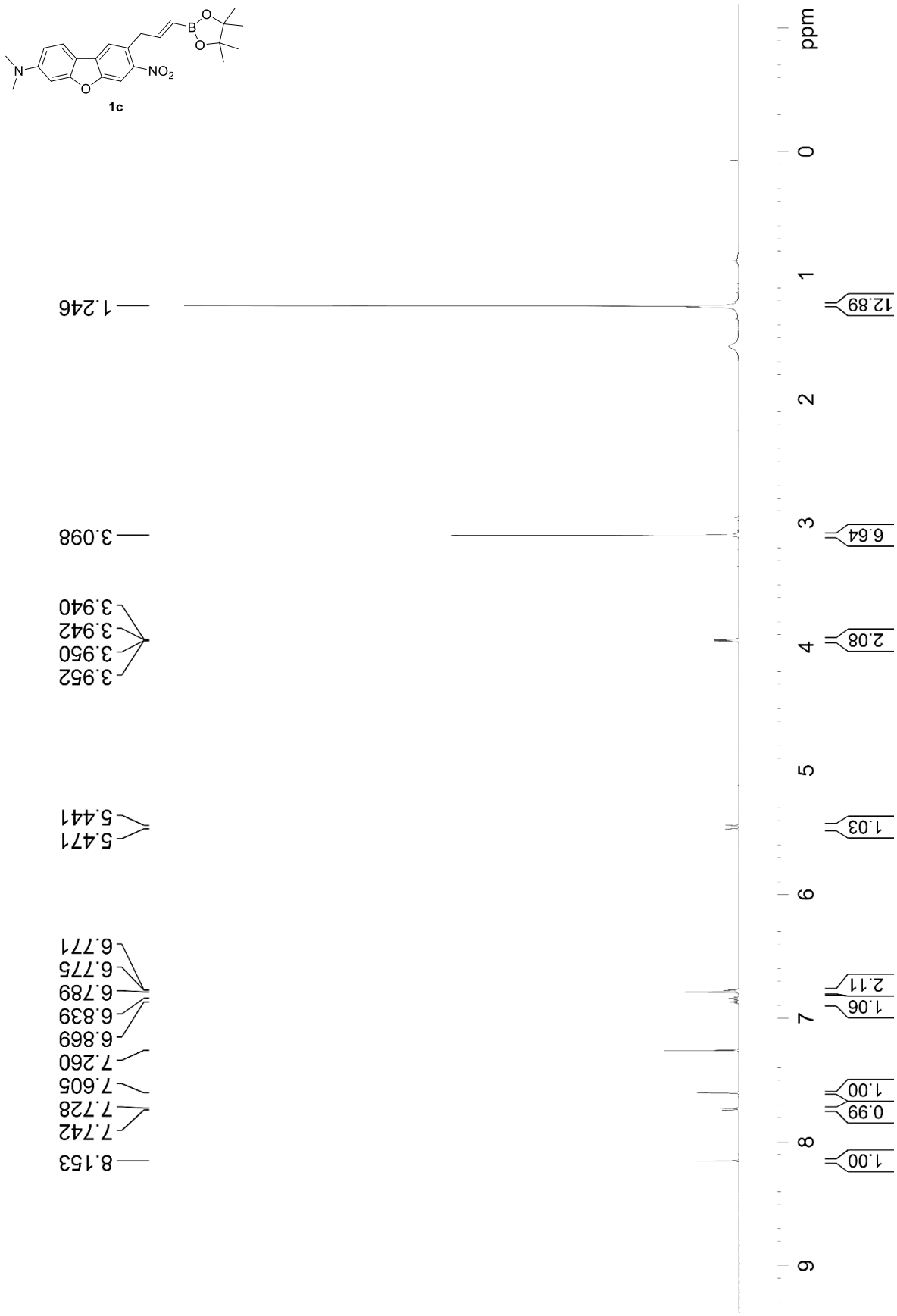

O
N
NO2
O
6
40.70
93.66
108.05
110.30
110.75
119.34
122.45
127.93
130.78
146.14
152.72
156.56
161.57
188.69
ppm
0
20
40
60
80
100
120
140
160
180
200

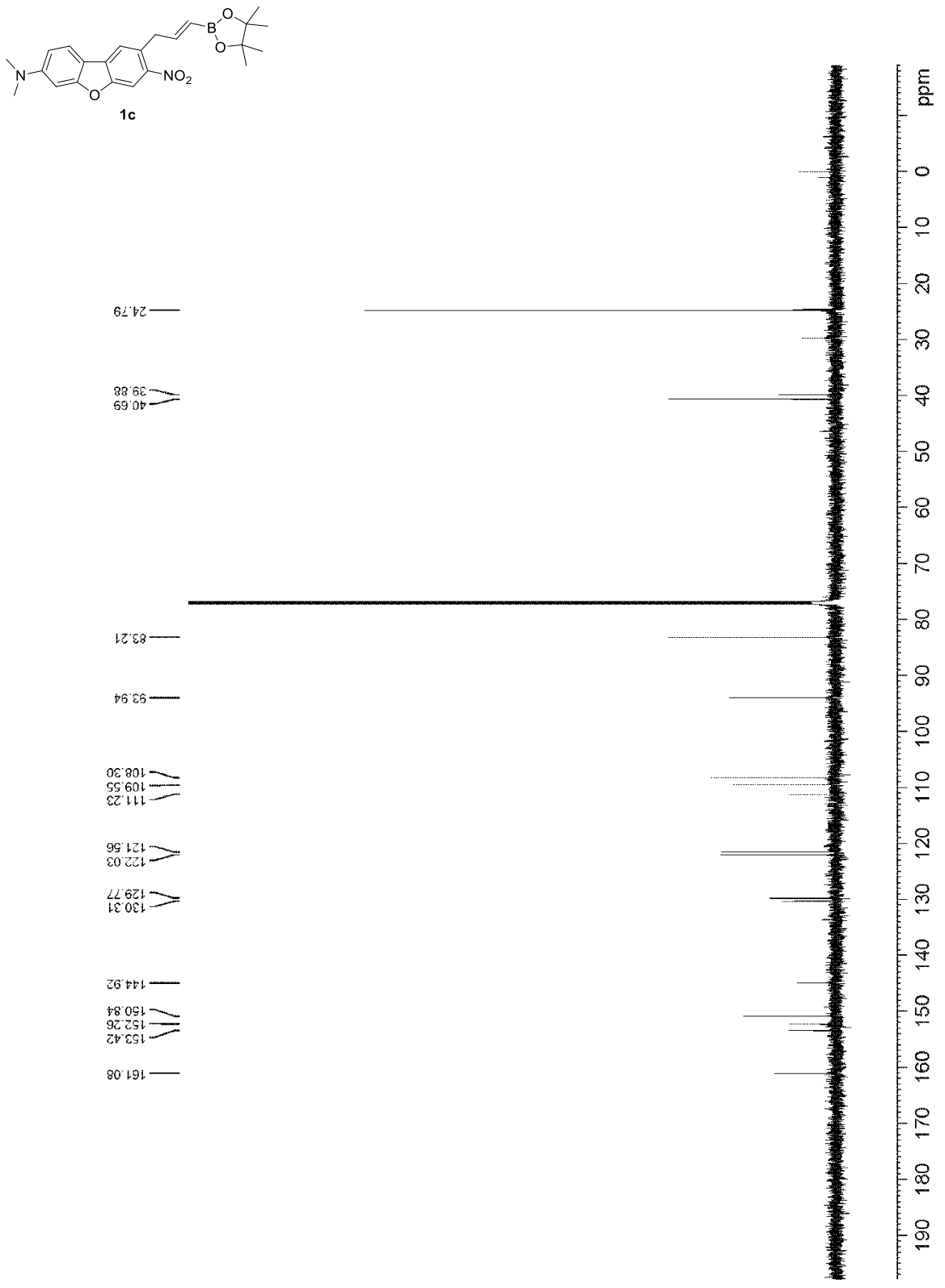

HO
N
O
NO2
S2
-0.000
2.049
2.053
2.056
2.060
2.064
2.857
2.890
3.084
3.088
3.115
3.120
3.127
3.142
5.510
5.520
5.634
6.280
6.281
6.284
6.290
6.291
6.294
6.295
6.905
6.909
6.915
6.919
6.930
6.934
8.005
8.020
8.163
8.167
8.447
ppm
0
1
2
3
4
5
6
7
8
9
10
1.00
0.92
1.02
2.17
1.02
0.93
6.41
0.95


HO
N
NO2
O
S2
41.61
61.55
75.65
85.42
95.24
109.59
111.74
112.69
119.36
124.08
131.74
134.12
145.66
154.47
155.33
162.93
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190


8
8.196
8.089
7.781
7.767
7.260
6.787
6.784
6.768
4.135
4.132
3.102
2.353
2.348
2.344
1.585
1.249
1.00
1.06
1.08
2.17
2.20
6.76
1.03
ppm


8
23.87
29.74
40.71
72.23
80.73
93.77
108.53
109.62
111.15
119.96
122.17
126.89
130.58
143.86
152.32
153.48
161.21
ppm


1c
ppm
0
1
2
3
4
5
6
7
8
9
12.89
6.64
2.08
1.03
2.11
1.06
1.00
0.99
1.00
1.246
3.098
3.940
3.942
3.950
3.952
5.441
5.471
6.771
6.775
6.789
6.839
6.869
7.260
7.605
7.728
7.742
8.153


1c
24.79
39.88
40.69
83.21
93.94
108.30
109.55
111.23
121.56
122.03
129.77
130.31
144.92
150.84
152.26
153.42
161.08
ppm
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190

References

(1) Mangubat-Medina, A. E.; Martin, S. C.; Hanaya, K.; Ball, Z. T. *J. Am. Chem. Soc.* **2018**.
(2) Hatchard, C. G.; Parker, C. A.; Bowen, E. J. *Proceedings of the Royal Society of London. Series A. Mathematical and Physical Sciences* **1956**, *235*, 518–536.
(3) Mahmoodi, M. M.; Abate-Pella, D.; Pundsack, T. J.; Palsuledesai, C. C.; Goff, P. C.; Blank, D. A.; Distefano, M. D. *J. Am. Chem. Soc.* **2016**, *138*, 5848–5859.
(4) Lusic, H.; Uprety, R.; Deiters, A. *Org. Lett.* **2010**, *12*, 916–919.
(5) Becker, Y.; Unger, E.; Fichte, M. A. H.; Gacek, D. A.; Dreuw, A.; Wachtveitl, J.; Walla, P. J.; Heckel, A. *Chem. Sci.* **2018**, *9*, 2797–2802.