SUPPLEMENTARY INFORMATION

Targeted 1,3-dipolar cycloaddition with acrolein for cancer prodrug activation

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MATERIALS AND METHODS

Computational methods (Figure 3). The 1,3-dipolar cycloaddition of acrolein with aryl azides **1a** (or **1d**) was systematically investigated using the AFIR method implemented in the GRRM program.[1] All DFT calculations were performed with the Gaussian 09 program.[2] The present AFIR calculations were carried out at the ωB97XD level in the gas phase with D95V(d) for N and O atoms and D95V for H and C atoms to reduce the computational costs.[3,4]

For azide **1d**, conformation search for azide and isopropyl groups was performed by the single-component AFIR method with the model collision energy parameter γ = 50 kJ/mol. All conformers found were reoptimized at the ωB97XD/D95V(d) level with the SMD solvation model (DMSO) method[5] to identify the lowest energy conformer.

The multi-component AFIR method was applied to explore the reaction pathways of **1a** (or **1d**) cyclization with acrolein. Based on the putative reaction mechanism, the ethylene moieties of the acrolein and azide group of the aryl compounds were specified as target atoms in the AFIR calculations with γ = 200 kJ/mol. Initial structures were automatically generated by a random distribution of each reactant. For each reaction, 10 AFIR paths were computed.

The obtained AFIR paths were refined by the locally updated plane method[6] to gain initial transition state (TS) optimization structures. After the TS optimizations, intrinsic reaction coordinate calculations were performed to confirm path connections. Harmonic vibrational frequency calculations followed the geometry optimizations to check either local minima or saddle points on potential energy surfaces. These calculations were carried out at the ωB97XD/D95V(d) level with the SMD solvation model (DMSO) throughout.

References:

1. S. Maeda, Y. Harabuchi, M. Takagi, K. Saita, K. Suzuki, T. Ichino, Y. Sumiya, K. Sugiyama and Y. Ono, *J. Comput. Chem*., 2018, **39**, 233-251.
2. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Gaussian 09, Revision D.01*; Gaussian, Inc., Wallingford CT, 2013.
3. J.-D. Chai and M. Head-Gordon, Phys. Chem. Chem. Phys., 2008, **10**, 6615-6620.
4. T. H. Dunning Jr. and P. J. Hay, in *Modern Theoretical Chemistry*, Ed. H. F. Schaefer III, Vol. 3 (Plenum, New York, 1977) 1-28.
5. V. Marenich, C. J. Cramer and D. G. Truhlar, *J. Phys Chem. B*, 2009, **113**, 6378-6396
6. Choi and R. Elber, *J. Chem. Phys.*, 1991, **94**, 751-760.

Kinetics studies (HPLC analysis, Figure S5). The 1,3-dipolar cycloaddition rate measurement of phenyl azide **1a** or 2,6-diisopropylphenyl azide **1d** in the presence of excess acrolein were performed by monitoring their HPLC reaction profile (peak area).
Acrolein (10 eq) was added to three different concentrations of phenyl azide **1a** in THF (1.5 M, 1.0 M, and 0.5 M) or 2,6-diisopropylphenyl azide **1d** in THF (1.5 M, 1.0 M, and 0.5 M). The reaction was monitored by directly injecting 5 μL of the reaction mixtures to HPLC at different time intervals (5, 45, 75, 120, 180, and 240 min).
Condition of reversed-phase HPLC for the reaction of phenyl azide **1a** with acrolein: Column, Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm; Mobile phase A, 0.1% TFA in H_2O; B, 0.1% TFA in CH_3CN; Gradient elution, 0-4 min at 50% B, 4-14 min at 50-80% B, 14-15 min at 80% B; Flow rate at 1 mL/min; UV detection at 254 nm. The production of triazoline **3a** was observed by monitoring the peak area increase at 4 minutes of retention time.
Condition of reversed-phase HPLC for the reaction of 2,6-diisopropylphenyl azide **1d** with acrolein: Column, Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm; Mobile phase A, 0.1% TFA in H_2O; B, 0.1% TFA in CH_3CN; Gradient elution, 0-4 min at 65% B, 4-15 min at 65-98% B, 15-20 min at 98% B; Flow rate at 1 mL/min; UV detection at 254 nm. The production of triazole **4d** and heterocycle **6** was observed by monitoring the peak area increase at 6 and 17 minutes of retention time.
The peak area ratios of 1,3-dipolar cycloaddition products were plotted versus time and fitted to a second-order exponential decay curve using GraphPad Prism 8, which allowed the determination of observed rate constants (Figure S5). The obtained rate constant data were then plotted versus the concentration of phenyl azide **1a** or 2,6-diisopropylphenyl azide **1d** and fitted to a straight line by linear regression method using GraphPad Prism 8 (Figure S5). The slope of the straight line indicates the second-order rate constant (k) for each product.

Kinetics studies - coumarin release (HPLC analysis, Figure S9). Acrolein (1000 eq) was added to three different concentrations (1.5 M, 1.0 M, and 0.5 M) of coumarin-ABC **7** in PBS (2% DMSO). The reaction was monitored by directly injecting 10 μL of the reaction mixtures to HPLC at different time intervals (5, 60, 115, 170, 225, and 280 min). Condition of reversed-phase HPLC for the reaction of phenyl azide **1a** with acrolein: Column, Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm; Mobile phase A, 0.1% TFA in H_2O; B, 0.1% TFA in CH_3CN; Gradient elution, 0-4 min at 25% B, 4-39 min at 25-95% B, 39-44 min at 95% B; Flow rate at 1 mL/min; UV detection at 330 nm. The reaction was observed by monitoring the peak area (coumarin-ABC **7**) decrease at 37 minutes of retention time.
The peak area ratios were plotted versus time and fitted to a pseudo-first-order exponential decay curve using GraphPad Prism 8, which allowed the determination of observed rate constants (Figure S9). The obtained rate constant data were then plotted versus the concentration of coumarin-ABC **7** and fitted to a straight line by linear regression method using GraphPad Prism 8 (Figure S9). The slope of the straight line indicates the second-order rate constant (k).

Fluorescence release (HPLC analysis, Figure 5a). A mixture of coumarin-ABC **7** (20 μM, 2 x 10^{-5} mmol) and glutathione (20 μM, 2 x 10^{-5} mmol) in 1 mL DMSO was incubated at room temperature. The reaction mixture at different time intervals (30 min, 1 h, 2 h, and 12 h) was subjected to reversed-phase HPLC analysis.
A mixture of coumarin-ABC **7** (20 μM, 2 x 10^{-5} mmol) and acrolein (20 μM, 2 x 10^{-5} mmol) in 1 mL DMSO was incubated at room temperature. The reaction mixture at different time intervals (30 min, 1 h, 2 h, and 4 h) was subjected to reversed-phase HPLC analysis.
Conditions of the HPLC: Column, Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm; mobile phase A, 0.1% TFA in H_2O; Mobile phase B, 0.1% TFA in CH_3CN; gradient elution, 0–4 min at 65% B, 4–14 min at 65–95% B, 14–20 min at 95% B; flow rate at 1 mL/min; fluorescence detection at 360/450 nm; and amount per injection 6 μL.

Fluorescence release (DMEM cell culture medium, Figure 5b). The coumarin-ABC **7** (20 μM) in 100 μL DMEM cell culture medium (2% DMSO) on a 96-well plate was incubated in the presence or absence of acrolein (20 mM). The increase of fluorescence intensity was immediately observed in a real-time manner for 30 minutes. The fluorescence intensity was measured using a spectrofluorometer (SpectraMax iD3, Molecular Devices).

Fluorescence release (cell-based assay; time-dependent, Figure 5c). Three cell lines (MCF10A, MCF7, A549, and HeLa S3) were seeded on 96 wells (2 x 10^4 cells/well) and left to attach for 24 h at 37 °C. The cells were treated with 100 μL of 2 μM or 20 μM of coumarin-ABC **7** solutions in the culture medium (1% DMSO). The increase in fluorescence intensity was immediately observed in a real-time manner for 30 min. The fluorescence intensity was measured using a spectrofluorometer (SpectraMax iD3, Molecular Devices). Fluorescence intensity was normalized for each cell line per 10,000 cells.

Fluorescence release (cell-based assay; concentration-dependent, Figure S10). Three cell lines (MCF10A, A549, and HeLa S3) were seeded on 96 wells (2 x 10^4 cells/well) and left to attach for 24 h at 37 °C. Each of the cells was then used for three different conditions as follows: (1) the cells were treated with 100 μL of coumarin-ABC **7** solutions (2 μM, 5 μM, 10 μM, 15 μM, 20 μM) in the culture medium (1% DMSO) and incubated for 60 min at room temperature; (2) the cells were treated with 100 μL of 7-amino-4-methylcoumarin solution (2 μM, 5 μM, 10 μM, 15 μM, 20 μM) in the culture medium (1% DMSO) and incubated for 60 min at room temperature; and (3) the cells were treated with 1 mM of N-acetyl cysteine in the culture medium and incubated for 2 h at 37 °C. The cells were then treated with 100 μL of coumarin-ABC **7** solutions (2 μM, 5 μM, 10 μM, 15 μM, 20 μM) in the culture medium (1% DMSO) and incubated for 60 min at room temperature. After the incubation, the fluorescence intensity was measured using a spectrofluorometer (SpectraMax iD3, Molecular Devices). Fluorescence intensity was normalized for each cell line per 10,000 cells.

Fluorescence release in cancer cells (HPLC analysis, Figure 5d). A549 cells (2.5 x 10^5 cells/mL) in 10 cm dishes were incubated with coumarin-ABC **7** (20 μM) at 37 °C. The cell culture medium at two different incubation time intervals (1 min and 30 min) was subjected to reversed-phase HPLC analysis. Condition of the HPLC: column, Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm; mobile phase A, 0.1% TFA in H_2O; mobile phase B, 0.1% TFA in CH_3CN; gradient elution, 0–4 min at 65% B, 4–14 min at 65–95% B, 14–20 min at 95% B; flow rate at 1 mL/min; fluorescence detection at 360/450 nm; and amount per injection, 20 μL.

Stability of 7 in 2 mM glutathione (Figure S8e-i). The coumarin-ABC **7** (20 μM) and glutathione (2 mM) in PBS (1 mL, 1% DMSO) were incubated at room temperature. At various time intervals (5, 60, 115, 170, 225 min), the reaction mixture was subjected to reversed-phase HPLC analysis. Conditions of the HPLC: Column, Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm; mobile phase A, 0.1% TFA in H_2O; Mobile phase B, 0.1% TFA in CH_3CN; gradient elution, 0–4 min at 25% B, 4–39 min at 25–95% B, 39–44 min at 95% B; flow rate at 1 mL/min; UV detection at 330 nm; and amount per injection 10 μL.

Stability of 7 in mouse serum (Figure S8e-ii). The DMSO solutions of samples (1 mM) were added to 20% mouse serum (48 μL) in PBS (190 μL) that had been pre-incubated at 37 ºC for 15 min. The resulting 20% mouse serum solution containing each sample (10 μM) was separated into Eppendorf tubes containing 40 μL each, and the tubes were incubated at 37 ºC for a certain period (8, 24, and 48 min). The incubation was stopped by adding MeOH (80 μL), and the mixtures were vortexed and centrifuged at 14,000 g at 4 ºC for 15 min. The supernatant was then analyzed by reversed-phase HPLC with a linear gradient of CH_3CN (20-90%, 35 min) in H_2O at a flow rate of 1.0 mL/min detected by UV scanning at 330 nm [Column: Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm]. A decay curve to determine the half-life of coumarin-ABC **7** in 20% mouse serum in PBS was generated in GraphPad Prism 8.

Stability of 7 in mouse liver microsome (Figure S8e-iii). PBS (198 μL) containing mouse liver microsome (final concentration: 0.5 mg/mL protein) and NADPH (final concentration: 1 mM) was incubated at 37 ºC for 5 min. Then, the DMSO solution of samples (2 μL, 1 mM stock) were added to the mixture and was separated into tubes containing 40 μL each, and the tubes were incubated at 37 ºC for a certain period (5, 15, 30, and 45 min). The supernatant was then analyzed by reversed-phase HPLC with a linear gradient of CH_3CN (20-90%, 35 min) in H_2O at a flow rate of 1.0 mL/min detected by UV scanning at 330 nm [Column: Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm]. A decay curve to determine the half-life of coumarin-ABC **7** in mouse liver microsome was generated in GraphPad Prism 8.

Solubility test of coumarin-ABC 7 (Figure S8c). The coumarin-ABC **7** solution (0.2 mM) in DMSO or PBS (2% DMSO) were subjected to reversed-phase HPLC analysis. Conditions of the HPLC: Column, Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm; mobile phase A, 0.1% TFA in H_2O; Mobile phase B, 0.1% TFA in CH_3CN; gradient elution, 0–4 min at 25% B, 4–39 min at 25–95% B, 39–44 min at 95% B; flow rate at 1 mL/min; UV detection at 330 nm; and amount per injection 10 μL.

Fluorescence release in the presence of HSA (DMEM cell culture medium, Figure S8d). The coumarin-ABC **7** (20 μM) and human serum albumin (40 μM) in 100 μL DMEM cell culture medium (2% DMSO) on a 96-well plate were incubated in the presence or absence of acrolein (20 mM). The increase of fluorescence intensity was immediately observed in a real-time manner for 30 minutes. The fluorescence intensity (normalized to the HSA fluorescence intensity) was measured using a spectrofluorometer (SpectraMax iD3, Molecular Devices).

Plasma protein binding. The protein binding was determined using Rapid Equilibrium Dialysis Device (Thermo Fisher Scientific) following manufactures protocol. The DMSO solution of samples (free compound or ABC-derivative) were spiked with mouse serum at the 10 μM with 1% final concentration of DMSO. The sample solution (100 μL) is placed into the sample chamber, and then 350 μL of 1% DMSO in PBS is added to the buffer chamber. The units were covered with sealing tape. After shaking for 4 h at 37 ºC, 40 μL of both the buffer and the plasma chambers are pipetted and diluted with 80 μL of MeOH to precipitate the serum proteins. The solution was vortexed and centrifuged at 14,000 g for 15 min at 4 ºC. The supernatant was then analyzed by reversed-phase HPLC with a linear gradient of CH_3CN (20-90%, 35 min) in H_2O at a flow rate of 1.0 mL/min detected by UV scanning at 330 nm [Column: Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm]. The value of protein binding is determined by the following equation: % Bound = (1 – Peak area of buffer chamber/Peak area of plasma chamber) x 100.

ATP assay (cytotoxicity assay, Table 1 and Figure S11). One normal cell line (MCF10A) and two cancer cell lines (A549, HeLa S3) were seeded on 96 wells (2 x 10^4 cells/well) and left to attach for 24 h at 37 °C. The cells were treated with 100 μL of prodrug or drug solution (0.05 μM, 0.1 μM, 0.5 μM, 1 μM, 5 μM, 10 μM, 50 μM) in the culture medium (1% DMSO) and incubated for 72 h at 37 °C, 5% CO_2. After the incubation, 100 μL of ATP lite 1 step reagent was added to the cells, and the fluorescence intensity was measured using a spectrofluorometer (SpectraMax iD3, Molecular Devices). The same procedure was also performed on the cells that were pretreated with 1 mM N-acetyl cysteine.

Quantitative analysis of the released compound (Figure S12). To 150 μM solution of coumarin-ABC **7** or MMC-ABC **8** or DOX-ABC **11** in PBS (10% DMSO) was added acrolein (1000 eq) and mixed at room temperature. After 4 hours, 10 μL of the reaction mixtures were analyzed by RP-HPLC (column, Cosmosil 5C_{18}-AR300, 4.6 x 250 mm; mobile phase A, 0.1% TFA in H_2O; mobile phase B, 0.1% TFA in CH_3CN; flow rate at 1 mL/min). The peak area of released compounds and starting materials were plotted to the standard curve.

Condition of the HPLC for:

a) Coumarin-ABC **7**: gradient elution, 0–4 min at 25% B, 4–39 min at 25–95% B, 39–44 min at 95% B; UV detection at 330 nm.
b) MMC-ABC **8**: gradient elution, 0–4 min at 1% B, 4–37 min at 5–100% B, 37–44 min at 100% B; UV detection at 350 nm.
c) DOX-ABC **11**: gradient elution, 0–4 min at 1% B, 4–37 min at 5–100% B, 37–44 min at 100% B; UV detection at 480 nm.

Animal experiments. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of RIKEN and approved by the Animal Ethics Committee of RIKEN (W2019-2-049). For all intratumoral injections and tumor measurements, the mice were anesthetized with 2% isoflurane in oxygen at a 2.5–3.0 L/min flow rate.

Cell lines and reagents: A549 cells were obtained from our stock kept in liquid nitrogen. They were cultured in Dulbecco's Modified Eagle's medium (DMEM) (Wako-Fujifilm, Japan) supplemented with 10% fetal bovine serum (FBS) (Biowest, France) and 1% penicillin-streptomycin (Gibco, Saint Aubin, France). The cells were then incubated at 37 °C in a 5% CO_2 humidified atmosphere.

A549 bearing mice xenograft models: The A549 breast cancer xenograft tumors were established in 6-week-old female nude mice BALB/cAJcl-nu/nu by subcutaneous injection of 2.5–6.0 x 10^6 cells in 100 µL of cold 50% Matrigel in unnourished DMEM into the right and left shoulder, and tumor growth was monitored. The mice were kept in a room with controlled temperature, salinity, and aeration and with sufficient food and water for 12 h day and 12 h night. After 5 weeks, tumor sizes reached 300–500 mm^3, and the A549 tumor-bearing mice were ready to be used for cancer therapy.

Acrolein assessment in the tumor tissue of mouse xenograft models (Figure S11): Groups of A549 tumor-bearing mice, with tumors ranging in size from 1000–1500 mm^3, were anesthetized and the tumors were exposed. CTS probe **1b** (20 µM in H_2O/DMSO 9:1) was sprayed carefully once to the exposed area. The fluorescent intensities were measured by calculating the region of interest (ROI) of the selected images.

In vivo drug release analysis (Figure S20): The compounds were dissolved in DMSO:EtOH:saline (1:4:5). Groups of A549 tumor-bearing mice, with tumors ranging in size from 1000–1500 mm^3, were intratumorally injected with 20 µl of mitomycin C (240 nmol, 3.6 mg/kg, n = 2) or 20 µl of MMC-phenyl azide **10** (240 nmol, 5.6 mg/kg, n = 2). The mice were sacrificed after 2 h post-drug and prodrug injections. The tumor and urine of the treated mice were collected, lysed, and extracted with methanol at 4 °C overnight. The extracts were filtered, evaporated, and partitioned to give the organs crude extracts and then analyzed by reversed-phase HPLC analysis. Condition of the HPLC: Column, Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm; mobile phase A, H_2O; mobile phase B, CH_3CN; gradient elution, 0–5 min at 0–10% B, 5–35 min at 10–100% B, 35–40 min at 100% B; flow rate at 1 mL/min; and fluorescence detection at 254 nm.

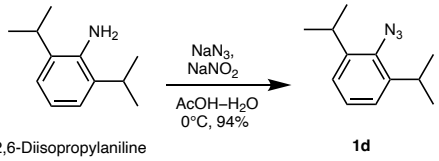
Intratumor therapy (Figure 6): The mice were randomized, divided into five groups, and treated with the prodrugs and controls. The compounds were dissolved in DMSO:EtOH:saline (1:4:5). The group 1 was intratumorally injected with 5 μL of the vehicle solution (DMSO:EtOH:saline = 1:4:5, n = 6). Group 2 was injected with 5 μL of compound **9** (120 nmol, 1.2 mg/kg, n = 6). Group 3 was injected with 5 μL of mitomycin C (120 nmol, 1.8 mg/kg, n = 6). Group 4 was injected with 5 μL of MMC-ABC **8** (120 nmol, 3.2 mg/kg, n = 6). Group 5 was injected with 5 μL of MMC- phenyl azide **10** (120 nmol, 2.8 mg/kg, n = 6). The administrations were repeated 12 times in consecutive days with the same doses. The tumor volume and body weight of the mice were recorded daily by the equation of $V = W^2L/2$, where W and L represented the minor and major length of the tumor, respectively. When the tumor reached 2000 mm^3, the mice were sacrificed, and the survival rates were calculated using the Kaplan-Meier method. After the tumors in the treated mice reached 2000 mm^3, the mice were sacrificed and transcardially perfused with 0.9% saline, followed by 4% paraformaldehyde. The selected organs (liver, kidney, spleen, heart, lung, and tumor) were then removed, dipped in 4% paraformaldehyde overnight, followed by 15% sucrose overnight and 30% sucrose overnight, and kept at -80 °C until used for hematoxylin and eosin (H&E) analysis.

Intravenous therapy (Figure 7): The compounds were dissolved in DMSO:EtOH:saline (1:4:5). The mice were randomly assigned to the following groups: Group 1 was intravenously injected with 100 μL of the vehicle solution (DMSO:EtOH:saline = 1:4:5, n = 8). Group 2 was injected with 100 μL of compound **9** (240 nmol, 2.2 mg/kg, n = 8). Group 3 was injected with 100 μL of mitomycin C (240 nmol, 3.6 mg/kg, n = 8). Group 4 was injected with 100 μL of MMC-ABC **8** (240 nmol, 6.2 mg/kg, n = 8). Group 5 was injected with 100 μL of MMC-phenyl azide **10** (240 nmol, 5.2 mg/kg, n = 8). The therapies were conducted once every three days for 4 injections in total. The tumor volume and bodyweight of the mice were recorded every single day by the equation of $V = W^2L/2$, where W and L represented the minor and major length of the tumor, respectively. When the tumor reached 2000 mm^3, the mice were sacrificed, and the survival rates were calculated by using the Kaplan-Meier method. The treated mice after its tumor reached 2000 mm^3 were sacrificed and transcardially perfused with 0.9% saline, followed by 4% paraformaldehyde. The selected organs (liver, kidney, spleen, heart, lung, and tumor) were then removed, dipped in 4% paraformaldehyde overnight and followed by 15% sucrose overnight and 30% sucrose overnight, and kept at -80 °C until used for hematoxylin and eosin (H&E) analysis.

Acrolein of the tumor was eliminated before intravenous therapy (Figure S21): The mice were treated intratumorally with 5 μL of compound **9** (120 nmol, 1.2 mg/kg, n = 8) and then after 1 h, treated (intravenous) with 100 μL of MMC-ABC **8** (240 nmol, 6.2 mg/kg, n = 8). The therapies were conducted once every three days for 4 injections in total. The tumor volume and bodyweight of the mice were recorded every single day by the equation of $V= W^2xL/2$, where W and L represented the minor and major length of the tumor, respectively. When the tumor reached 2000 mm^3, the mice were sacrificed, and the survival rates were calculated by using the Kaplan Meier method.

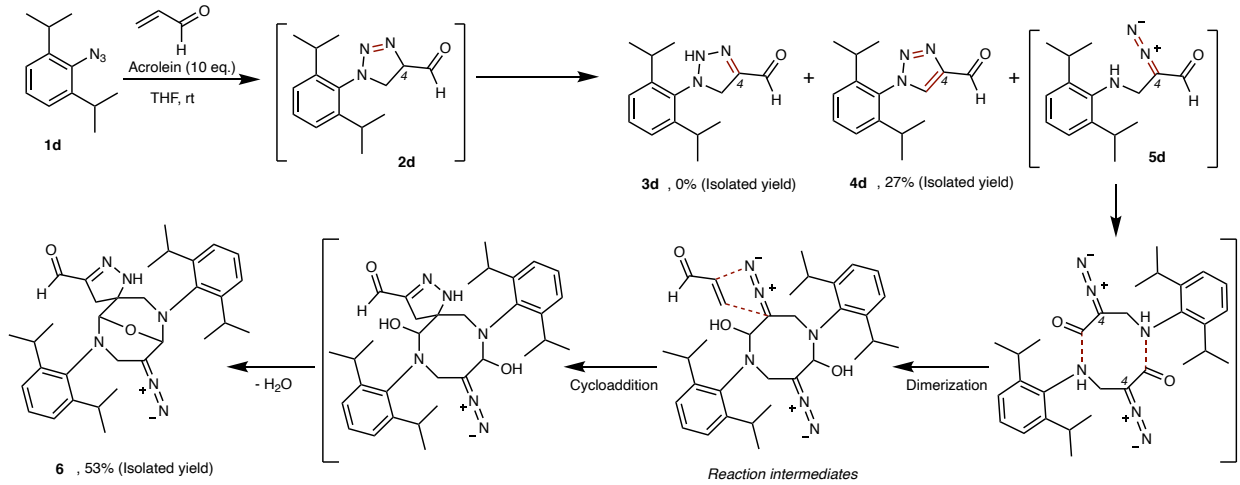
Chemical Synthesis. All commercially available reagents were used without further purification. The preparative separation was performed by column chromatography on Merck Silica gel 60 (230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on the JEOL RESONANCE AL400 NMR spectrometer. Unless otherwise mentioned, $CDCl_3$ was used as a solvent, and chemical shifts were represented as δ-values relative to the internal standard TMS. High-resolution mass spectrometry (HRMS) was recorded on micrOTOF-QIII. Note that caution is required as azide-containing compounds are presumed to be potentially explosive. Although we have never experienced such an explosion with the azide compounds used in this study, all manipulations should be carefully carried out in a hood.

Synthesis of 2,6-diisopropylphenyl azide **1d**



Sodium nitrite (814 mg, 11.5 mmol, 2.4 eq) was slowly added to a mixture of 2,6-diisopropylaniline (846 mg, 4.77 mmol, 1.0 eq) and sodium azide (791 mg, 11.9 mmol, 2.5 eq) dissolved in acetic acid and distilled water (9:1) (50 mL, [2,6-diisopropylaniline] = 0.1 M) at 0 °C. After stirring for 30 min at 0 °C, a saturated aqueous solution of $NaHCO_3$ was added until the mixture achieved a pH of 7. The mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (n-hexane only) to give the desired 2,6-diisopropylphenyl azide **1d** as a yellow oil (912 mg, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.25–7.07 (m, 3H), 3.36 (hept, J = 7.0 Hz, 2H), 1.26 (d, J = 6.8 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 143.33, 135.54, 127.01, 124.12, 28.91, 23.59.

The reaction of 2,6-diisopropylphenyl azide **1d** with acrolein

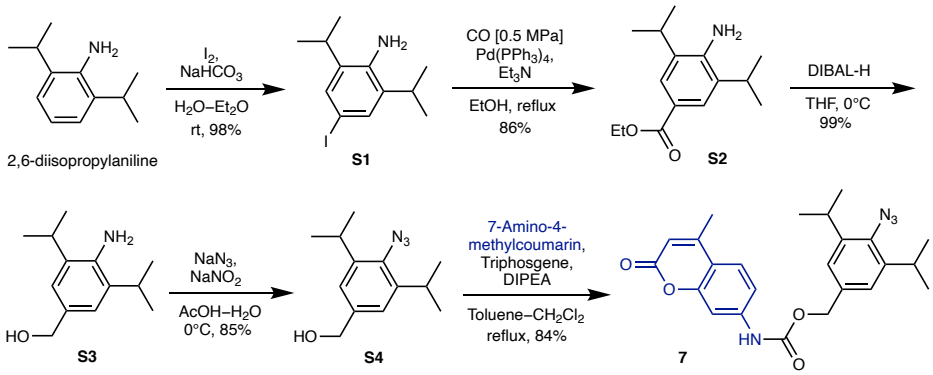


Acrolein (164 µL, 2.33 mmol, 10 eq) was added to a solution of 2,6-diisopropylphenyl azide **1d** (47.3 mg, 0.23 mmol, 1.0 eq) in THF (stabilizer free, 310 µL, [**1d**] = 0.7 M). After being stirred at ambient temperature for 24 h, the reaction mixture was concentrated to dryness under reduced pressure. The resulting crude product was purified by preparative TLC 10 x 20 cm (n-hexane/EtOAc 3:1) to afford 4-formyl-1,2,3-triazole **4d** (Rf 0.66, yellow oil, 17 mg, 27% yield) and heterocycle 6 (Rf 0.42, red oil, 35 mg, 53% yield).

4d: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 10.29 (s, 1H), 8.19 (s, 1H), 7.54 (t, J = 7.8 Hz, 1H), 7.33 (d, J = 7.8 Hz, 2H), 2.16 (hept, J = 6.9 Hz, 2H), 1.14 (dd, J = 10.4, 6.8 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 185.60, 145.97, 131.65, 128.19, 124.27, 28.63, 24.17, 24.02; ESI-HRMS m/z calcd for $C_{15}H_{19}N_3NaO$ ($[M+Na]^+$) 280.1420, found 280.1419.

6: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 9.38 (s, 1H), 7.38 (t, J = 7.7 Hz, 1H), 7.29 (t, J = 7.7 Hz, 1H), 7.22 (d, J = 7.7 Hz, 2H), 7.15 (dd, J = 7.6, 3.1 Hz, 2H), 5.85 (s, 1H), 5.54 (s, 1H), 4.20 (q, J = 26.3 Hz, 2H), 4.11 (d, J = 11.0 Hz, 1H), 3.31 (d, J = 11.0 Hz, 1H), 3.12 (s, 2H), 2.94 (p, J = 6.9 Hz, 1H), 2.83 (p, J = 7.1 Hz, 3H), 1.23 – 1.18 (m, 24H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 189.33, 147.46, 146.89, 143.09, 140.10, 134.52, 129.87, 128.57, 124.37, 124.32, 120.59, 109.18, 92.90, 84.51, 53.37, 51.37, 39.40, 28.76, 28.23, 28.17, 24.78, 24.71, 24.68, 24.48; ESI-HRMS m/z calcd for $C_{33}H_{45}N_6O_2$ ($[M+H]^+$) 557.3599, found 557.3597.

Synthesis of coumarin-ABC **7**



*4-Iodo-2,6-diisopropylaniline **S1**:* Iodine (13.7 g, 53.8 mmol, 1.2 eq) was added to a solution of 2,6-diisopropylaniline (8.8 g, 44.9 mmol, 1.0 eq) in saturated aqueous $NaHCO_3$ and Et_2O (1:1) (200 mL, [2,6-diisopropylaniline] = 0.2 M). After being stirred for 5 h at ambient temperature, $Na_2S_2O_3$•H_2O was added, and the mixture was stirred for 15 min. The resulting mixture was extracted with Et_2O. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure to give the desired 4-iodo-2,6-diisopropylaniline **S1** as red-black oil (13.4 g, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.27 (s, 2H), 3.70 (bs, 2H, NH_2), 2.81 (hept, J = 6.7 Hz, 2H), 1.21 (d, J = 6.8 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 140.27, 135.08, 131.79, 81.12, 27.82, 22.21; ESI-HRMS m/z calcd for $C_{12}H_{19}IN$ ($[M+H]^+$) 304.0557, found 304.0558.

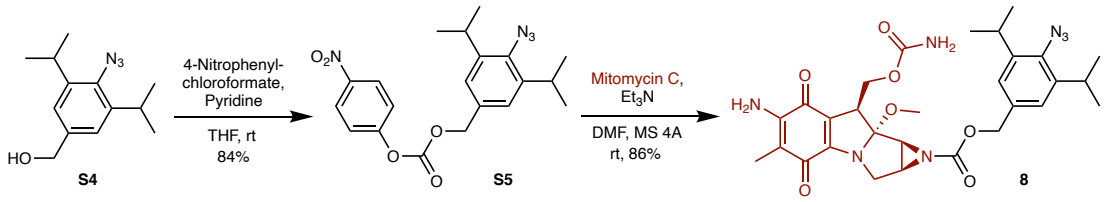
*Ethyl 4-amino-3,5-diisopropylbenzoate **S2**:* Et_3N (4.3 mL, 30.7 mmol, 1.5 eq) was added to a mixture of 4-iodo-2,6-diisopropylaniline **S1** (6.2 g, 20.5 mmol, 1.0 eq) and $Pd(PPh_3)_4$ (0.5 g, 0.41 mmol, 0.02 eq) in EtOH (17 mL, [**S1**] = 1.2 M). The resulting solution was stirred under CO pressure (0.5 MPa) at 110 °C for 8 h. The suspension was filtered through Celite and the filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of eluents [n-hexane/EtOAc (25:1 to 15:1)] to give the desired compound **S2** as yellow oil (4.4 g, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.75 (s, 2H), 4.34 (q, J = 7.1 Hz, 2H), 4.16 (bs, 2H, NH_2), 2.88 (hept, J = 6.9 Hz, 2H), 1.38 (t, J = 7.2 Hz, 3H), 1.29 (d, J = 6.8 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 167.64, 145.10, 131.37, 125.04, 119.77, 60.30, 27.97, 22.24, 14.55; ESI-HRMS m/z calcd for $C_{15}H_{24}NO_2$ ($[M+H]^+$) 250.1802, found 250.1805.

*Synthesis of 4-hydroxymethyl-2,6-diisopropyl aniline **S3**:* DIBAL-H (9 mL of a 1.0 M solution in toluene, 9.2 mmol, 3.0 eq) was added slowly to a solution of compound **S2** (764 mg, 3.1 mmol, 1.0 eq) in THF (30 mL, [**S2**] = 0.1 M) at 0 °C. The reaction was stirred under nitrogen atmosphere at 0 °C for 10 min, and then for 1 h at ambient temperature. The excess DIBAL-H was quenched carefully with MeOH (20 mL) at 0 °C, stirred for 30 min, and allowed to warm to ambient temperature. The resulting mixture was filtered through Celite (washed with MeOH), and the filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of eluents [n-hexane/EtOAc (15:1 to 7:1)] to give the desired compound **S3** as an orange oil (627 mg, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.04 (s, 2H), 4.55 (s, 2H), 3.74 (bs, 2H, NH_2), 2.92 (hept, J = 6.7 Hz, 2H), 1.27 (d, J = 6.8 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 140.06, 132.71, 130.89, 122.46, 66.09, 27.99, 22.44; ESI-HRMS m/z calcd for $C_{13}H_{22}NO_2$ ($[M+H]^+$) 208.1696, found 208.1699.

*Synthesis of (4-azido-3,5-diisopropylphenyl)-methanol **S4**:* Sodium nitrite (628 mg, 8.83 mmol, 2.4 eq) was slowly added to a mixture of compound **S3** (763 mg, 3.68 mmol, 1.0 eq) and sodium azide (610 mg, 9.20 mmol, 2.5 eq) dissolved in acetic acid and distilled water (5:2) (35 mL, [**S3**] = 0.1 M) at 0 °C. After stirring for 4 h at 0 °C, a saturated aqueous solution of $NaHCO_3$ was added until the mixture achieved a pH of 7. The mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of eluents [n-hexane/EtOAc (30:1 to 15:1)] to give the desired compound **S4** as a yellow oil (730 mg, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.13 (s, 2H), 4.64 (s, 2H), 3.36 (p, J = 6.8 Hz, 2H), 1.27 (d, J = 6.7 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 143.51, 139.31, 134.84, 122.79, 65.27, 28.90, 23.52; ESI-HRMS m/z calcd for $C_{13}H_{20}N_3O$ ($[M+H]^+$) 234.1601, found 234.1605.

Synthesis of coumarin-ABC 7: Triphosgene (140 mg, 0.46 mmol, dissolved in 4 mL toluene) was slowly added to a mixture of 7-amino-4-methylcoumarin (69 mg, 0.39 mmol, 1.0 eq) and DIPEA (250 mL, 1.45 mmol, 3.8 eq) dissolved in toluene (4 mL, [7-amino-4-methylcoumarin] = 0.1 M) at 0 °C. The reaction mixture was refluxed with stirring under nitrogen atmosphere for 4 h, and then cooled to ambient temperature. CH_2Cl_2 (3 mL) was added to the reaction mixture and stirred for 5 min until the solution turned dark brown, and compound **S4** (117 mg, 0.5 mmol, 1.3 eq, in 5 mL CH_2Cl_2) was added. The reaction mixture was stirred and heated at 55 °C for 13 h, and then cooled to ambient temperature. The resulting solution was concentrated to dryness under reduced pressure while maintaining the temperature under 30 °C. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH 25:1) to give the desired coumarin-ABC 7 as pale yellow solid (141 mg, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.53 (d, J = 8.7 Hz, 1H), 7.48 (d, J = 2.2 Hz, 1H), 7.39 (dd, J = 8.7, 2.2 Hz, 1H), 7.17 (s, 2H), 7.08 (bs, s, 1H), 6.19 (d, J = 1.2 Hz, 1H), 5.18 (s, 2H), 3.36 (hept, J = 6.7 Hz, 2H), 2.40 (d, J = 1.2 Hz, 3H), 1.28 (d, J = 6.9 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 161.21, 154.62, 152.89, 152.37, 143.75, 141.54, 135.72, 134.01, 125.52, 124.46, 115.73, 114.54, 113.34, 106.13, 67.63, 28.99, 23.59, 18.69; ESI-HRMS m/z calcd for $C_{24}H_{26}N_4NaO_4$ ([M+Na]$^+$) 457.1846, found 457.1840.

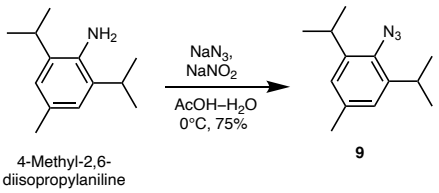
Synthesis of prodrug MMC-ABC **8**



*Synthesis of 4-azido-3,5-diisopropylbenzyl-(4-nitrophenyl)-carbonate **S5**:* Pyridine (206 mL, 2.55 mmol, 2.0 eq) was added to a mixture of compound **S4** (297 mg, 1.27 mmol, 1.0 eq) and 4-nitrophenyl chloroformate (321 mg, 1.53 mmol, 1.2 eq) dissolved in THF (13 mL, [**S4**] = 0.1 M) at 0 °C. The reaction mixture was stirred under a nitrogen atmosphere at ambient temperature for 24 h. The THF solvent was removed by rotary evaporation, and the resulting crude was partitioned with EtOAc-H_2O. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (n-hexane/EtOAc 25:1) to give the desired compound **S5** as a yellow oil (424 mg, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 8.27 (d, J = 9.1 Hz, 2H), 7.20 (s, 2H), 5.26 (s, 2H), 3.38 (p, J = 6.9 Hz, 2H), 1.29 (d, J = 6.9 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 155.65, 152.50, 145.52, 143.82, 136.11, 132.62, 125.41, 124.63, 121.86, 71.07, 28.94, 23.53.

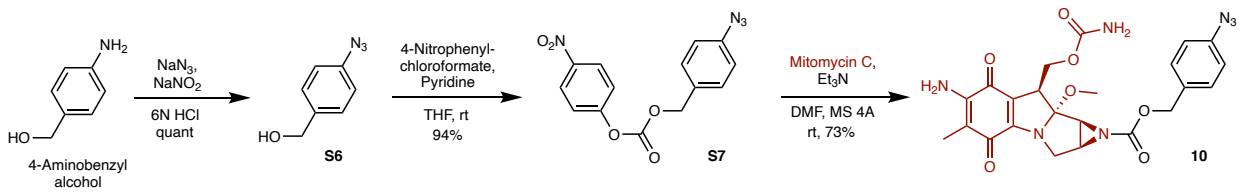
*Synthesis of prodrug MMC-ABC **8**:* Et_3N (130 μL, 0.93 mmol, 1.8 eq) was added to a mixture of compound **S5** (307 mg, 0.77 mmol, 1.0 eq), mitomycin C (170 mg, 0.51 mmol, 1.5 eq), and activated powder molecular sieves (MS 4A) dissolve in DMF (8 mL, [**S5**] = 0.1 M). The reaction mixture was stirred under nitrogen atmosphere at ambient temperature for 16 h. The resulting mixture was filtered through Celite (washed with EtOAc) and the filtrate was concentrated to dryness under reduced pressure. The resulting crude was partitioned with EtOAc-H_2O. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH 30:1) to give the desired prodrug MMC-ABC **8** as a purple oil (260 mg, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.12 (s, 2H), 5.25 (bs, 2H, NH_2), 5.04 (s, 2H), 4.89 (dd, J = 10.8, 4.7 Hz, 1H), 4.73 (bs, 2H, NH_2), 4.44 (d, J = 13.3 Hz, 1H), 4.32 (t, J = 11.0 Hz, 1H), 3.70 (dd, J = 11.0, 4.8 Hz, 1H), 3.48 (dd, J = 13.3, 1.9 Hz, 1H), 3.45 (d, J = 4.6 Hz, 1H), 3.37 – 3.27 (m, 3H), 3.19 (s, 3H), 1.76 (s, 3H), 1.25 (dd, J = 6.9, 4.1 Hz, 12H).; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 178.53, 176.03, 160.82, 156.46, 154.47, 147.15, 143.52, 135.60, 133.83, 124.67, 110.67, 105.58, 105.31, 68.80, 62.29, 49.90, 48.81, 43.52, 42.01, 40.21, 28.93, 23.55, 8.01; ESI-HRMS m/z calcd for $C_{29}H_{36}N_7O_7$ ($[M+H]^+$) 594.2671, found 594.2673.

Synthesis of 2-azido-1,3-diisopropyl-5-methylbenzene **9**



To a mixture of 4-methyl-2,6-diisopropyl aniline (0.8 g, 4.18 mmol, 1.0 eq) and sodium azide (0.7 g, 10.5 mmol, 2.5 eq) dissolved in acetic acid and distilled water (5:2) (21 mL, [4-methyl-2,6-diisopropyl aniline] = 0.2 M) was slowly added sodium nitrite (0.7 mg, 10.0 mmol, 2.4 eq) at 0°C. After stirring for 3 hours at 0°C, a saturated aqueous solution of $NaHCO_3$ was added until the mixture became pH 7. The mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane only) to give the desired compound **18** as a colorless oil (0.7 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 6.92 (s, 2H), 3.32 (hept, J = 7.1 Hz, 2H), 2.31 (s, 3H), 1.25 (d, J = 7.2 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 142.98, 136.42, 124.75, 124.07, 28.88, 23.66, 21.47.

Synthesis of MMC-phenyl azide **10**



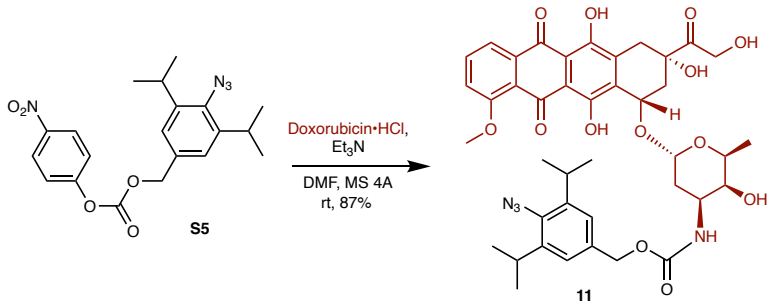
*Compound **S6***: To a mixture of 4-aminobenzyl alcohol (2.0 g, 16 mmol, 1.0 eq) and sodium azide (3.2 g, 48 mmol, 3.0 eq) dissolved in 6N HCl (16 mL, [4-aminobenzyl alcohol] = 1.0 M) was slowly added sodium nitrite (1.7 g, 24 mmol, 1.5 eq). After stirring for 2 hours at ambient temperature, the resulting mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel using a gradient of eluents [*n*-hexane/EtOAc (30:1 to 15:1)] to give the desired compound **S6** as a yellow oil (2.4 g, quant). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.21 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 8.5 Hz, 2H), 4.48 (s, 2H), 3.78 (bs, 1H, O*H*); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 139.06, 137.57, 128.37, 118.90, 63.99.

*Compound **S7***: To a mixture of compound **S6** (500 mg, 3.35 mmol, 1.0 eq) and 4-nitrophenyl chloroformate (845 mg, 4.02 mmol, 1.2 eq) dissolved in THF (34 mL, [**S6**] = 0.1 M) at 0 °C was added pyridine (543 μL, 6.71 mmol, 2.0 eq). The reaction mixture was stirred under a nitrogen atmosphere at ambient temperature for 24 hours. The THF solvent was removed by rotary evaporation, and the resulting crude was partitioned with EtOAc–H_2O. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane/EtOAc 25:1) to give the desired compound **S7** as a colorless oil (990 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 8.27 (d, J = 9.2 Hz, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.38 (d, J = 9.1 Hz, 2H), 7.07 (d, J = 8.3 Hz, 2H), 5.26 (s, 2H).; ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 155.66, 152.62, 145.62, 141.18, 130.97, 130.66, 125.49, 121.92, 119.52, 70.47.

*MMC-phenyl azide **10***: To a mixture of compound **S7** (245 mg, 0.78 mmol, 1.5 eq), mitomycin C (170 mg, 0.51 mmol, 1.0 eq), and activated powder molecular sieves (MS 4A) dissolve in DMF (8 mL, [**S7**] = 0.1 M) was added Et_3N (129 μL, 0.92 mmol, 1.8 eq). The reaction mixture was stirred under a nitrogen atmosphere at ambient temperature for 16 hours. The resulting mixture was filtered through Celite (washed with EtOAc), and the filtrate was concentrated to dryness under reduced pressure. The resulting crude was partitioned with EtOAc–H_2O. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH 30:1) to give the desired

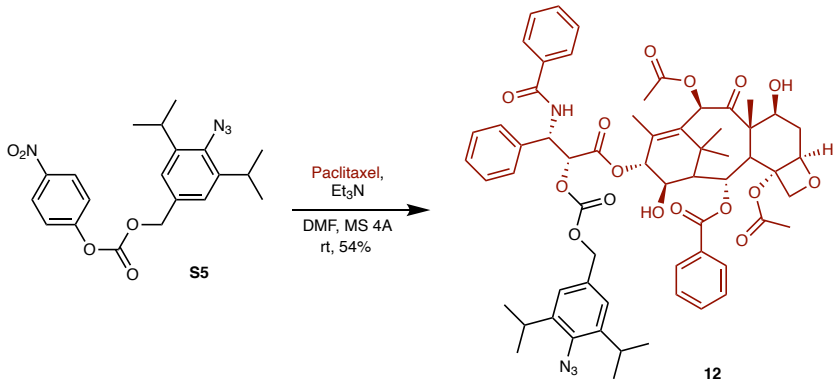
MMC-phenyl azide **10** as a purple oil (189 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.33 (d, J = 8.6 Hz, 2H), 6.97 (d, J = 8.5 Hz, 2H), 5.41 (bs, 2H, N*H*$_2$), 5.05 (dd, J = 20.0, 12.0 Hz, 2H), 4.98 (bs, 2H, N*H*$_2$), 4.89 (dd, J = 10.8, 4.7 Hz, 1H), 4.43 (d, J = 13.4 Hz, 1H), 4.30 (t, J = 10.9 Hz, 1H), 3.67 (dd, J = 11.0, 4.7 Hz, 1H), 3.50 (dd, J = 13.4, 2.0 Hz, 1H), 3.45 (d, J = 4.6 Hz, 1H), 3.32 (dd, J = 4.7, 1.8 Hz, 1H), 3.19 (s, 3H), 1.76 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 178.31, 175.97, 160.94, 157.16, 154.74, 147.64, 141.06, 131.91, 130.48, 126.29, 110.27, 105.49, 105.31, 68.32, 62.41, 49.93, 48.83, 43.49, 42.16, 40.28, 8.02; ESI-HRMS *m/z* calcd for $C_{23}H_{24}N_7O_7$ ($[M+H]^+$) 510.1732, found 510.1738.

Synthesis of DOX-ABC **11**



*DOX-ABC **11***: To a mixture of compound **S5** (80.2 mg, 0.20 mmol, 1.0 eq), doxorubicin·HCl (105 mg, 0.17 mmol, 0.9 eq), and activated powder molecular sieves (MS 4A) dissolve in DMF (2 mL, [**S7**] = 0.1 M) was added Et_3N (57 μL, 0.40 mmol, 2.0 eq). The reaction mixture was stirred under a nitrogen atmosphere at ambient temperature for 20 hours. The resulting mixture was filtered through Celite (washed with EtOAc), and the filtrate was concentrated to dryness under reduced pressure. The resulting crude was partitioned with EtOAc–H_2O. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH 25:1) to give the desired DOX-ABC **11** as a red solid (120 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 7.95 (d, J = 7.7 Hz, 1H), 7.74 (t, J = 8.0 Hz, 1H), 7.36 (d, J = 8.7 Hz, 1H), 7.05 (s, 2H), 5.49 (bs, 1H, N*H*), 5.34 – 5.18 (m, 2H), 4.97 (s, 2H), 4.76 (d, J = 4.6 Hz, 2H), 4.54 (s, 1H), 4.14 (dd, J = 14.7, 7.2 Hz, 1H), 4.05 (s, 3H), 3.93 – 3.82 (m, 1H), 3.68 (d, J = 7.8 Hz, 1H), 3.30 (p, J = 6.9 Hz, 2H), 3.18 (d, J = 18.5 Hz, 1H), 3.12 (t, J = 6.0 Hz, 1H), 2.85 (d, J = 18.8 Hz, 1H), 2.33 (d, J = 14.6 Hz, 2H), 2.15 (dd, J = 14.8, 4.1 Hz, 1H), 1.84 (ddd, J = 27.1, 13.1, 4.5 Hz, 2H), 1.30 (d, J = 6.5 Hz, 3H), 1.22 (d, J = 6.9 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 213.96, 186.91, 186.51, 161.05, 156.22, 155.62, 155.55, 143.42, 135.84, 135.38, 135.25, 134.70, 133.64, 124.01, 120.71, 119.88, 118.57, 111.52, 111.36, 100.86, 76.66, 69.81, 69.67, 67.41, 66.85, 65.62, 56.69, 47.15, 35.67, 33.95, 30.26, 28.88, 23.49, 16.92; ESI-HRMS *m/z* calcd for $C_{41}H_{46}N_4NaO_{13}$ ($[M+Na]^+$) 825.2954, found 825.2954.

Synthesis of PCX-ABC **12**



*PCX-ABC **12***: To a mixture of compound **S5** (11.7 mg, 0.03 mmol, 1.0 eq), paclitaxel (25.6 mg, 0.03 mmol, 1.0 eq), and activated powder molecular sieves (MS 4A) dissolve in DMF (1 mL, [**S7**] = 30 μM) was added Et_3N (5 μL, 0.04 mmol, 1.2 eq). The reaction mixture was stirred under a nitrogen atmosphere at ambient temperature for 23 hours. The resulting mixture was filtered through Celite (washed with EtOAc), and the filtrate was concentrated to dryness under reduced pressure. The resulting crude was partitioned with EtOAc–H_2O. The combined organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH 50:1) to give the desired PCX-ABC **12** as a white solid (18 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ 8.14 (d, J = 7.6 Hz, 2H), 7.72 (d, J = 7.7 Hz, 2H), 7.61 (t, J = 7.3 Hz, 1H), 7.50 (q, J = 7.6 Hz, 3H), 7.42 – 7.35 (m, 7H), 7.10 (s, 2H), 6.91 (d, J = 9.3 Hz, 1H), 6.30 (s, 2H), 5.99 (dd, J = 9.1, 2.6 Hz, 1H), 5.70 (d, J = 7.2 Hz, 1H), 5.44 (d, J = 2.7 Hz, 1H), 5.13 (q, J = 11.9 Hz, 2H), 4.98 (dd, J = 9.7, 2.2 Hz, 1H), 4.45 (dd, J = 11.0, 6.5 Hz, 1H), 4.32 (d, J = 8.6 Hz, 1H), 4.21 (d, J = 8.5 Hz, 1H), 3.82 (d, J = 7.0 Hz, 1H), 3.35 (q, J = 6.8 Hz, 2H), 2.56 (ddd, J = 15.7, 9.6, 6.5 Hz, 1H), 2.46 (s, 3H), 2.41 (dd, J = 15.8, 9.7 Hz, 2H), 2.24 (s, 4H), 1.94 (s, 3H), 1.69 (s, 3H), 1.30 – 1.20 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ 203.96, 171.42, 170.01, 168.03, 167.26, 154.28, 143.78, 142.83, 136.89, 135.99, 133.84, 133.66, 133.02, 132.82, 132.19, 130.38, 129.32, 129.25, 128.91, 128.84, 128.66, 127.29, 126.71, 124.33, 84.59, 81.25, 79.36, 75.73, 75.26, 72.30, 72.20, 70.83, 58.69, 52.80, 45.70, 43.35, 35.72, 35.66, 28.95, 26.98, 23.54, 22.87, 22.33, 20.96, 14.96, 9.75; ESI-HRMS *m/z* calcd for $C_{61}H_{69}N_4O_{16}$ ($[M+H]^+$) 1113.4703, found 1113.4705.

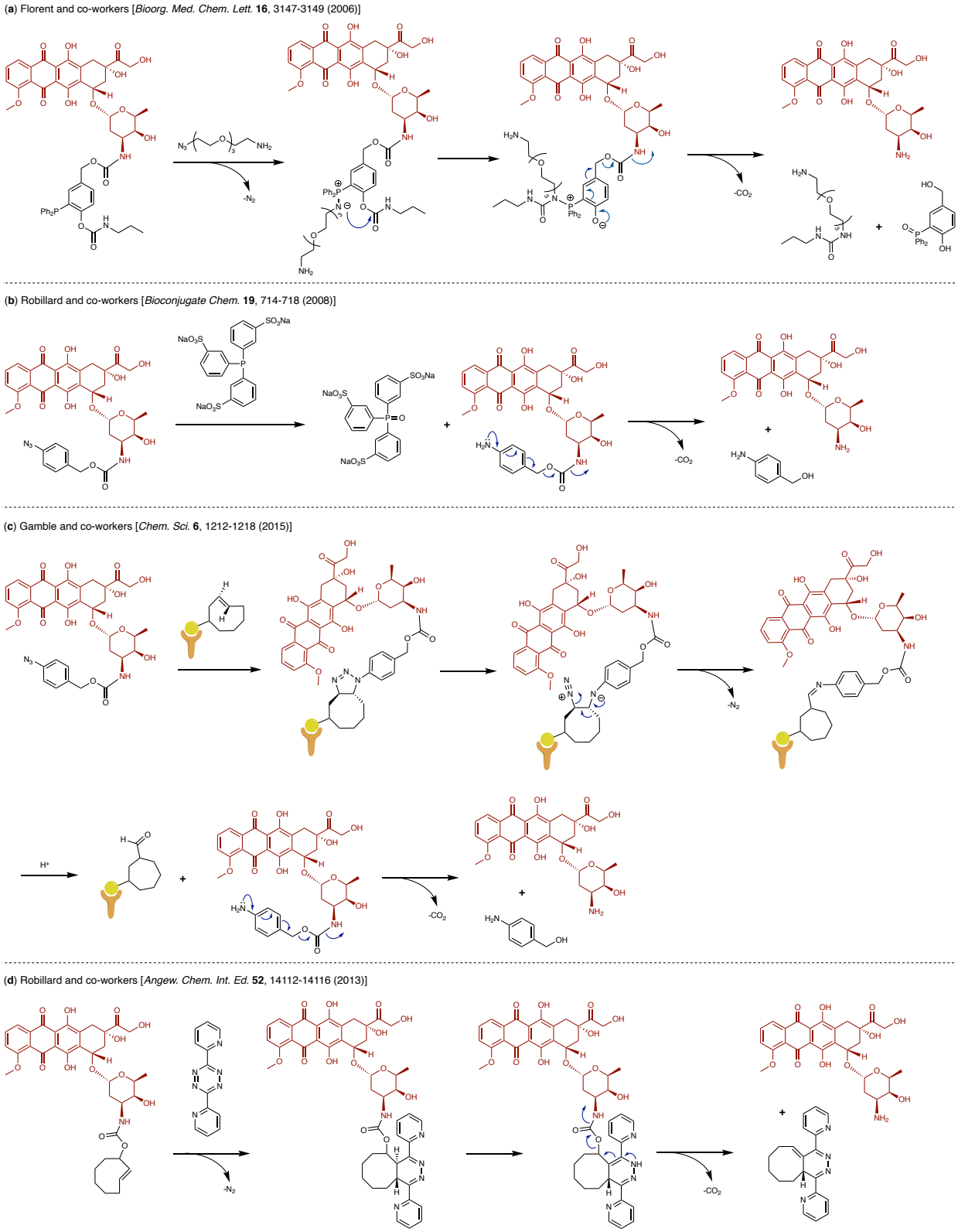


Figure S1. (**a**) Prodrug activation via Staudinger ligation. (**b**) Prodrug activation via the Staudinger reaction. (**c**) Prodrug activation via 1,3-dipolar cycloaddition between an azido group and *trans*-cyclooctene. (**d**) A click-to-release strategy via an inverse-electron-demand Diels-Alder reaction between *trans*-cyclooctene and tetrazine.

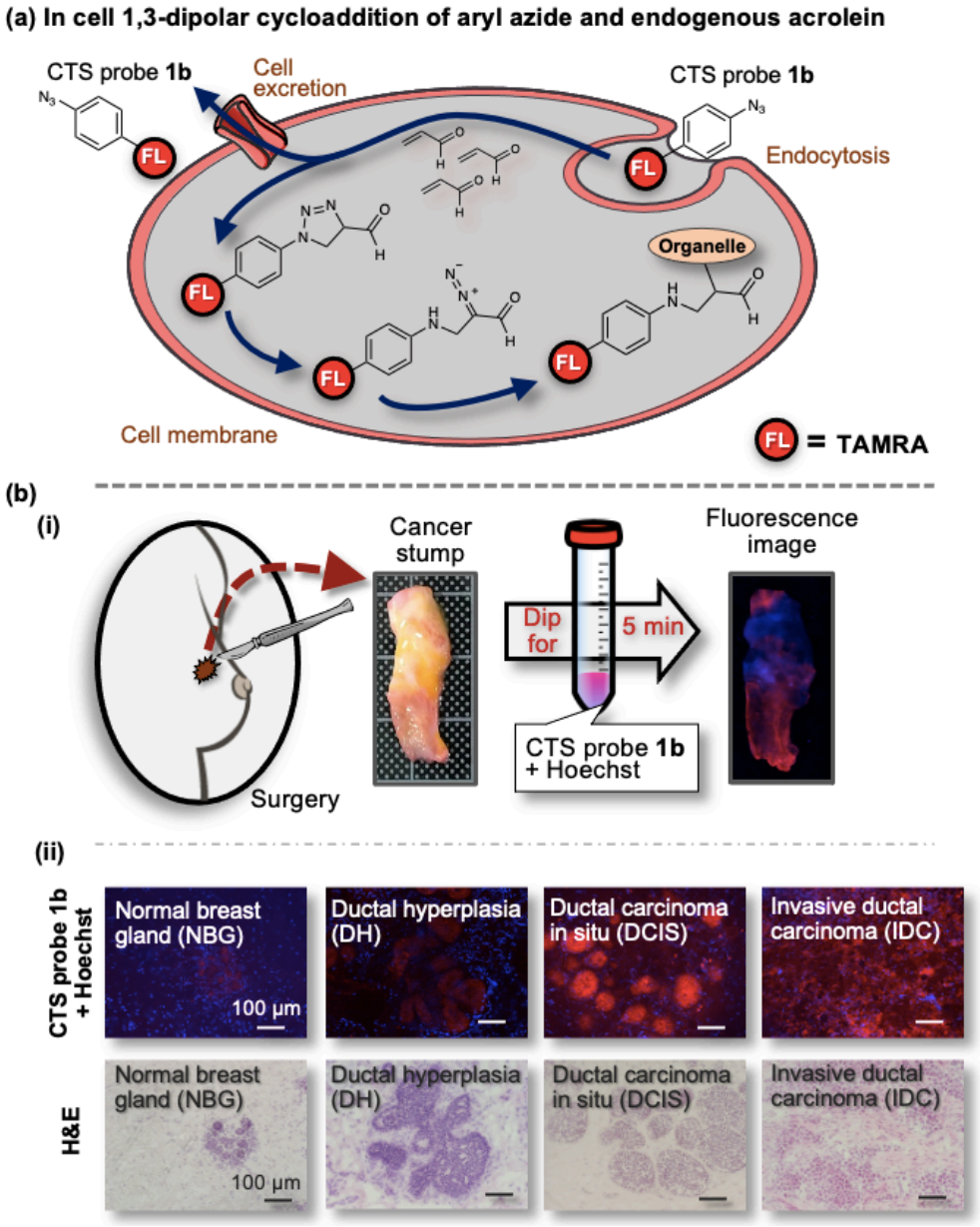


Figure S2. **(a)** The click-to-sense (CTS) probe **1b** and acrolein reaction at the cellular level underlying mechanism of intracellular acrolein detection. TAMRA = Tetramethylrhodamine. **(b-i)** CTS probe **1b** distinguishes cancer and normal tissue from breast cancer patients with high selectivity and sensitivity. **(b-ii)** Morphology of normal breast gland (NBG), (DH), carcinoma in (DCIS), and invasive carcinoma (IDC) labeled with 20 μM of CTS probe **1b** at 200x magnification. The cancer morphology detected was consistent with the morphology in images with and (H&E) staining in frozen sections. The scale bar indicates 100 μM.

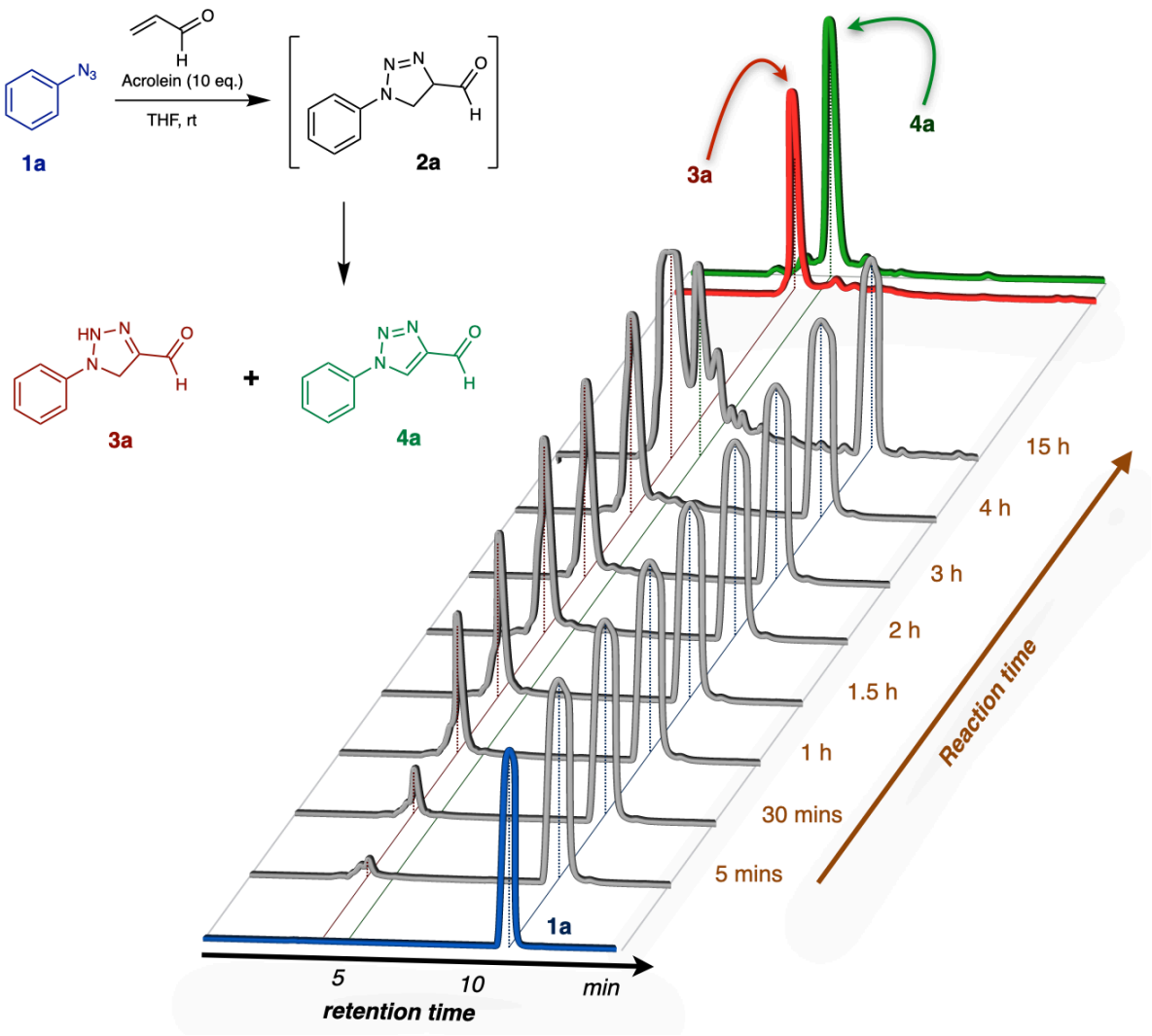


Figure S3. The reaction between phenyl azide **1a** (1.0 eq) and acrolein (10 eq) in THF ([**1a**] = 1.5 mM) was monitored by directly injecting the 5 µL of the reaction mixtures to HPLC at different time intervals (5, 30, 60, 90, 120, 180, 240, 900 min). Condition of reversed-phase HPLC: Column, Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm; Mobile phase A, 0.1% TFA in H_2O; B, 0.1% TFA in CH_3CN; Gradient elution, 0-4 min at 50% B, 4-14 min at 50-80% B, 14-15 min at 80% B; Flow rate at 1 mL/min; UV detection at 254 nm.

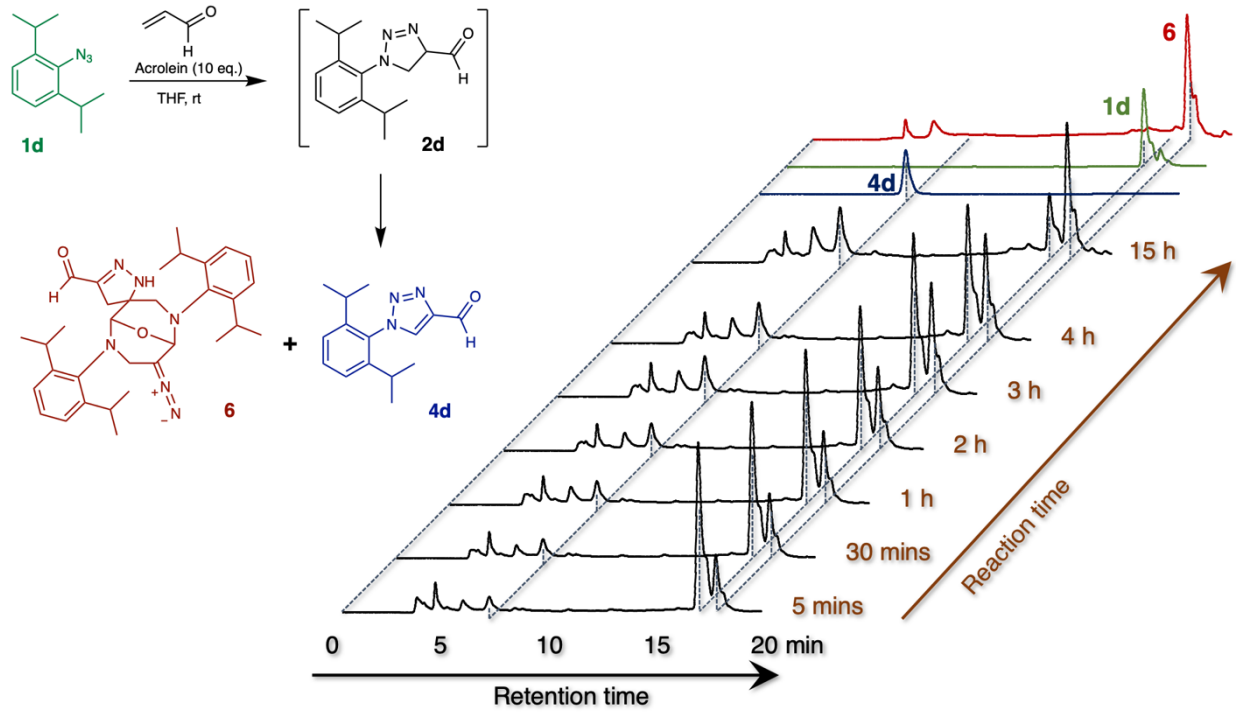


Figure S4. The reaction between 2,6-diisopropylphenyl azide **1d** (1.0 eq) and acrolein (10 eq) in THF ([**1**] = 1.5 mM) was monitored by directly injecting 5 µL of the reaction mixtures to HPLC at different time intervals (5, 30, 60, 120, 180, 240, 900 min). Condition of reversed-phase HPLC: Column, Cosmosil 5C_{18}-AR300 (Nacalai Tesque, Inc.) 4.6 x 250 mm; Mobile phase A, 0.1% TFA in H_2O; B, 0.1% TFA in CH_3CN; Gradient elution, 0-4 min at 65% B, 4-15 min at 65-98% B, 15-20 min at 98% B; Flow rate at 1 mL/min; UV detection at 254 nm.

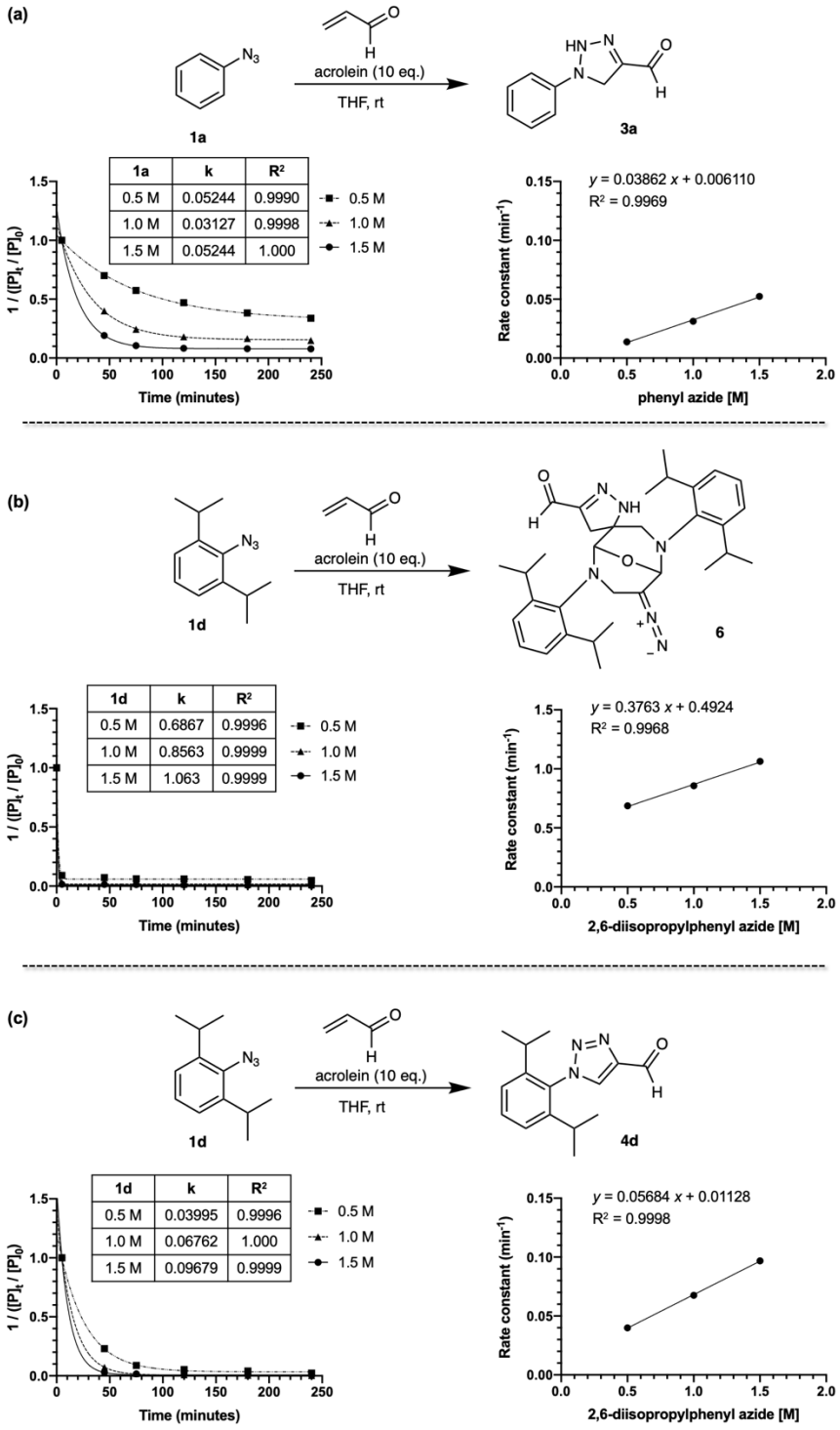


Figure S5. Kinetics of the reaction between: **(a)** phenyl azide **1a** and acrolein to give triazoline **3a** ($k = 3.9\times10^{-2}$ M^{-1} min^{-1}); **(b)** 2,6-diisopropylphenyl azide **1d** and acrolein to give heterocycle **6** ($k = 3.8\times10^{-1}$ M^{-1} min^{-1}); **(c)** 2,6-diisopropylphenyl azide **1d** and acrolein to give triazole **4d** ($k = 5.7\times10^{-2}$ M^{-1} min^{-1}). *Left graph*: The HPLC chromatogram peak area ratios were plotted versus reaction time. *Right graph*: The rate constants data were plotted versus the concentration of starting material to determine the second-order rate constant (k). $[P]_t$=HPLC chromatogram peak area at t min. $[P]_0$=HPLC chromatogram peak area at 0 min.

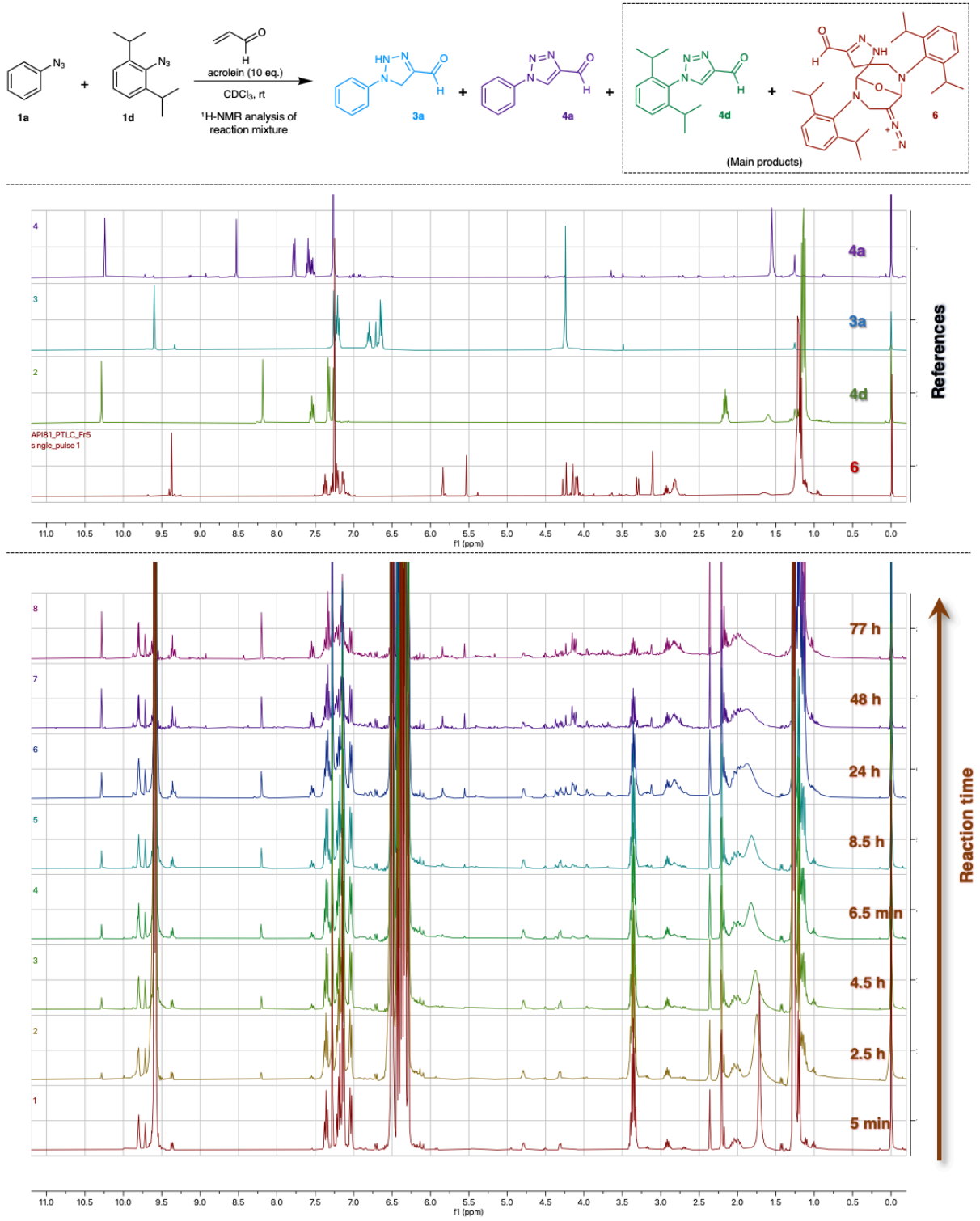
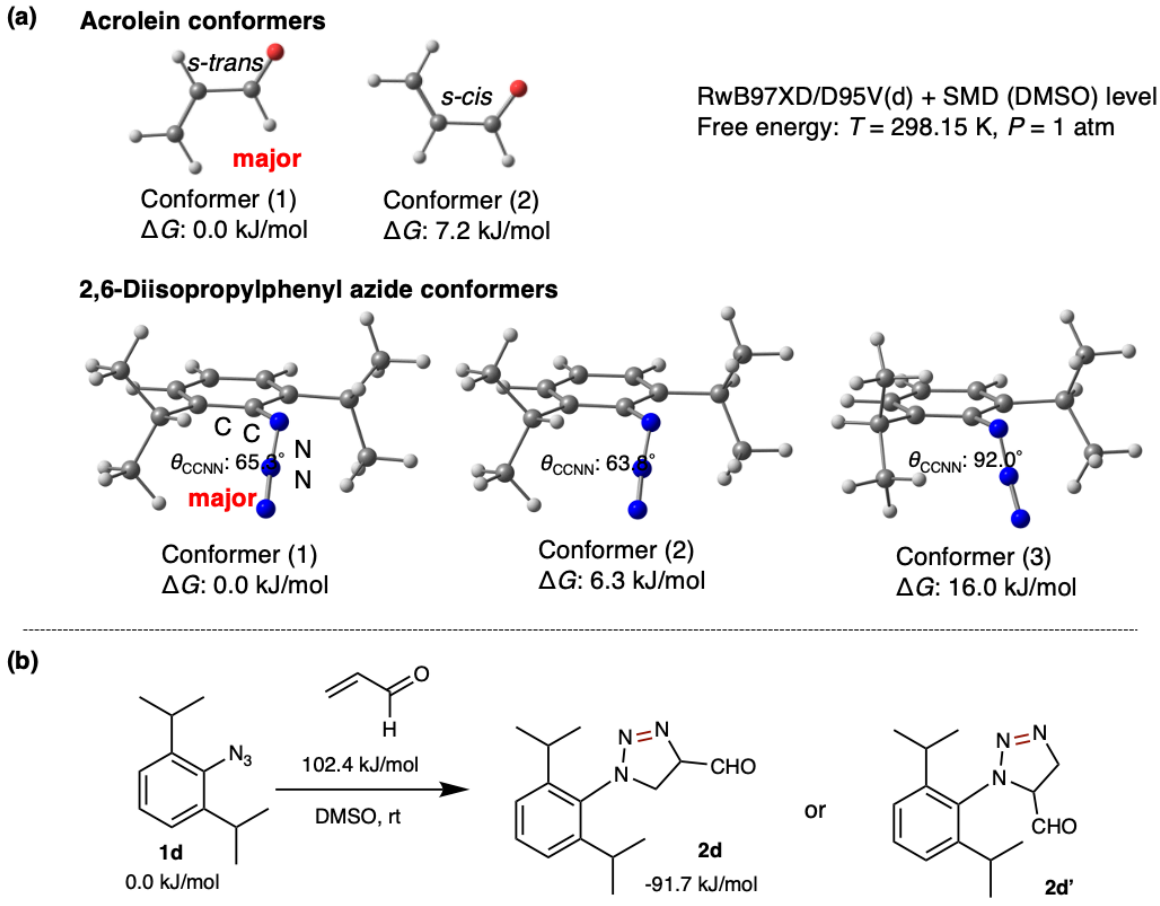


Figure S6. The reaction of a 1:1 mixture of **1a** and **1d** with an excess amount of acrolein in $CDCl_3$ was monitored by ^{1}H-NMR spectroscopy. The result shows that 2,6-diisopropylphenyl azide **1d** is more reactive toward acrolein than phenyl azide **1a**. The reaction mixture was monitored at 5 minutes, 2.5 hours, 4.5 hours, 6.5 hours, 8.5 hours, 24 hours, 48 hours, and 77 hours. We observed that cycloaddition products from **1d** (i.e., triazoline **4d** and heterocycle **6**) were produced exclusively, whereas cycloaddition products from **1a** were hardly observed.



Entry	Acrolein	2,6-Diispropylphenyl azide **1d**	Product	ΔΔG‡
1	*s-trans*	Conformer (1)	**2d**	102.4
2	*s-trans*	Conformer (1)	**2d'**	109.1
3	*s-trans*	Conformer (2)	**2d**	110.1
4	*s-trans*	Conformer (2)	**2d'**	116.6
5	*s-trans*	Conformer (3)	**2d**	132.1
6	*s-trans*	Conformer (3)	**2d'**	141.6
7	*s-cis*	Conformer (1)	**2d**	104.0
8	*s-cis*	Conformer (1)	**2d'**	114.3
9	*s-cis*	Conformer (2)	**2d**	104.0
10	*s-cis*	Conformer (2)	**2d'**	122.7
11	*s-cis*	Conformer (3)	**2d**	133.9
12	*s-cis*	Conformer (3)	**2d'**	147.6

Figure S7. **(a)** The conformation of isopropyl groups controls the relative conformation of azide to the benzene ring. (**b**) The path from entry 1 path is preferred to give **2d** as the primary product. On the other hand, because of the kinetic disadvantages, the path from entry 2 is not preferred.

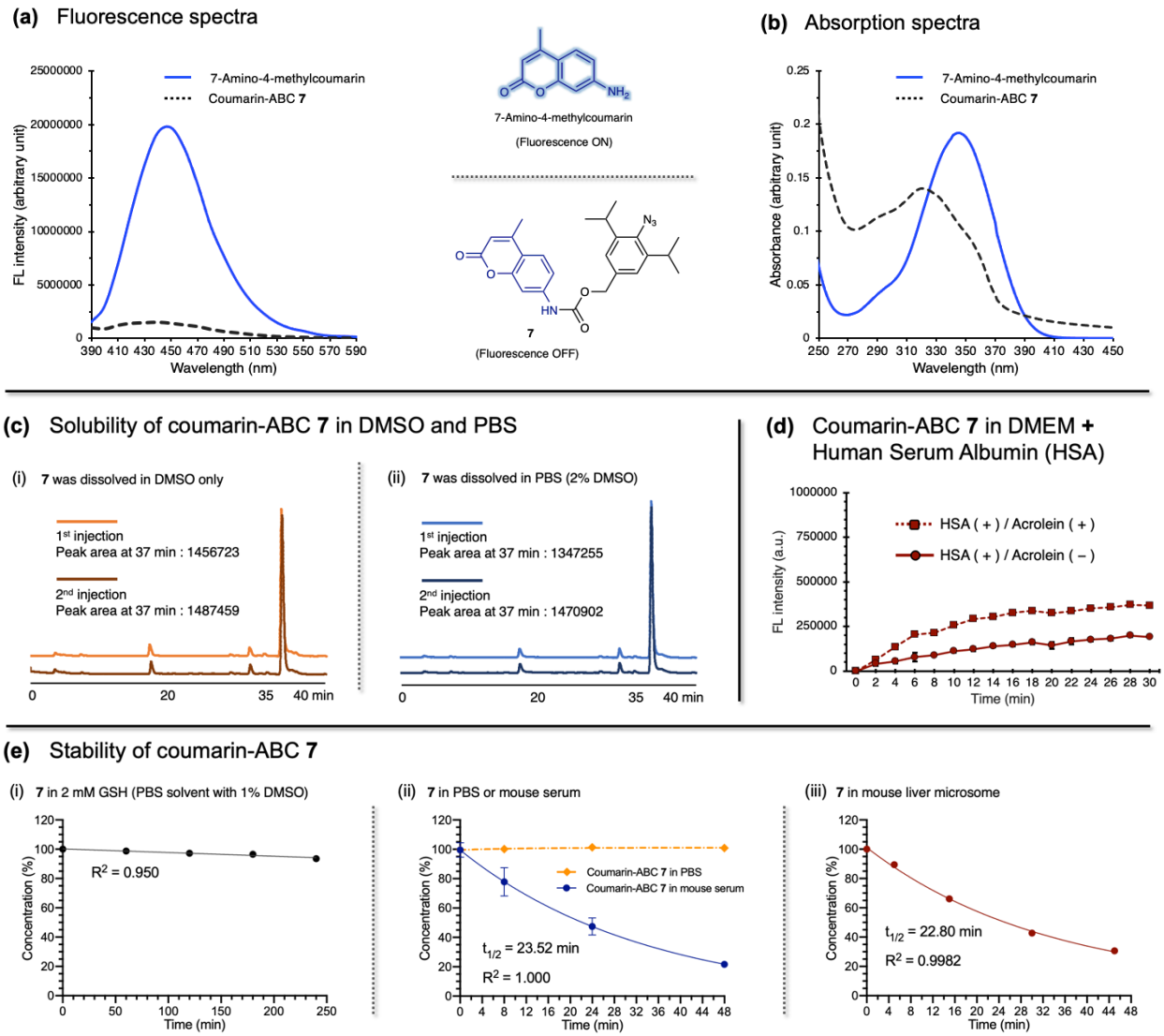


Figure S8. **(a)** The fluorescence spectra; and **(b)** the absorption spectra of coumarin-ABC **7** (2 μM, dashed black line) and 7-amino-4-methylcoumarin (2 μM, solid blue line) in water (0.2% DMSO). **(c)** The solubility of coumarin-ABC **7** in PBS (2% DMSO) was examined by comparing the chromatogram peak area to that when dissolved only in DMSO. The average peak area of compound **7** dissolved in both PBS (2% DMSO) or DMSO were almost similar (P_{PBS}/P_{DMSO} = 0.96), suggesting good solubility in both solvent systems. Gradient elution, 0–4 min at 25% B, 4–39 min at 25–95% B, 39–44 min at 95% B; flow rate at 1 mL/min; UV detection at 330 nm. **(d)** The coumarin-ABC **7** (20 µM) and human serum albumin (40 µM) were incubated in DMEM solution (2% DMSO) with the presence (dashed line) or absence (solid line) of acrolein at room temperature. Normalized to the HSA fluorescence intensity. **(e)** The coumarin-ABC **7** was incubated at room temperature in (i) 2 mM GSH in PBS (1% DMSO); (ii) PBS or mouse serum; and (iii) mouse liver microsome. The samples were analyzed by RP-HPLC at specific time intervals, and the chromatogram peak areas were measured to observe the compound degradation. We found that coumarin-ABC **7** was almost not degraded even when incubated with 2 mM GSH or PBS only. Nevertheless, the half-lives of coumarin-ABC **7** in mouse serum and mouse liver microsome are 23.5 min and 22.8 min, respectively.

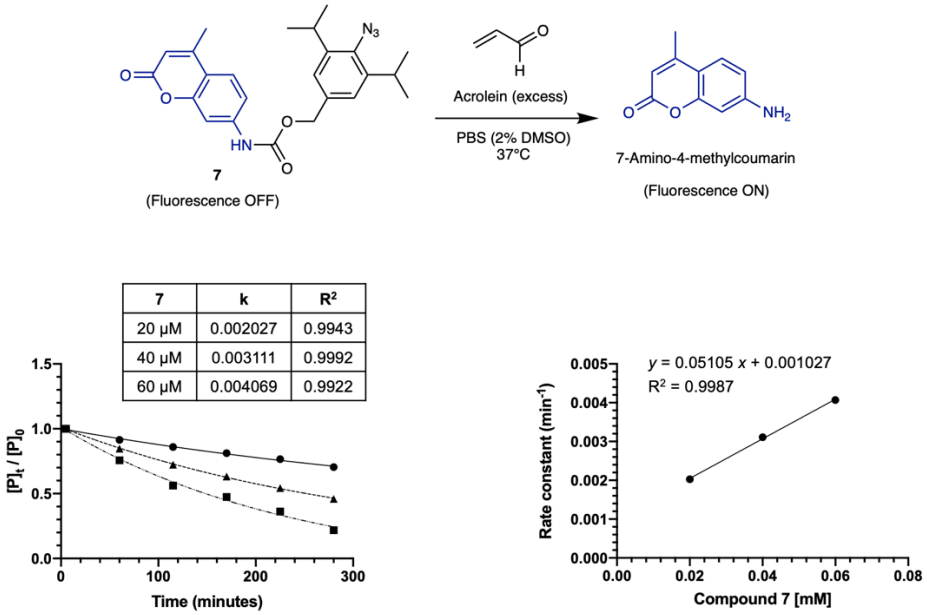


Figure S9. Kinetics of the coumarin release reaction. *Left graph*: The HPLC chromatogram peak area ratios were plotted versus reaction time. *Right graph*: The rate constants data were plotted versus the concentration of starting material to determine the second-order rate constant (k = 5.1×10^{-2} mM^{-1} min^{-1}). $[P]_t$ = HPLC chromatogram peak area at t minute. $[P]_0$ = HPLC chromatogram peak area at 0 minute. The reaction was observed by monitoring the peak area (coumarin-ABC **7**) decrease.

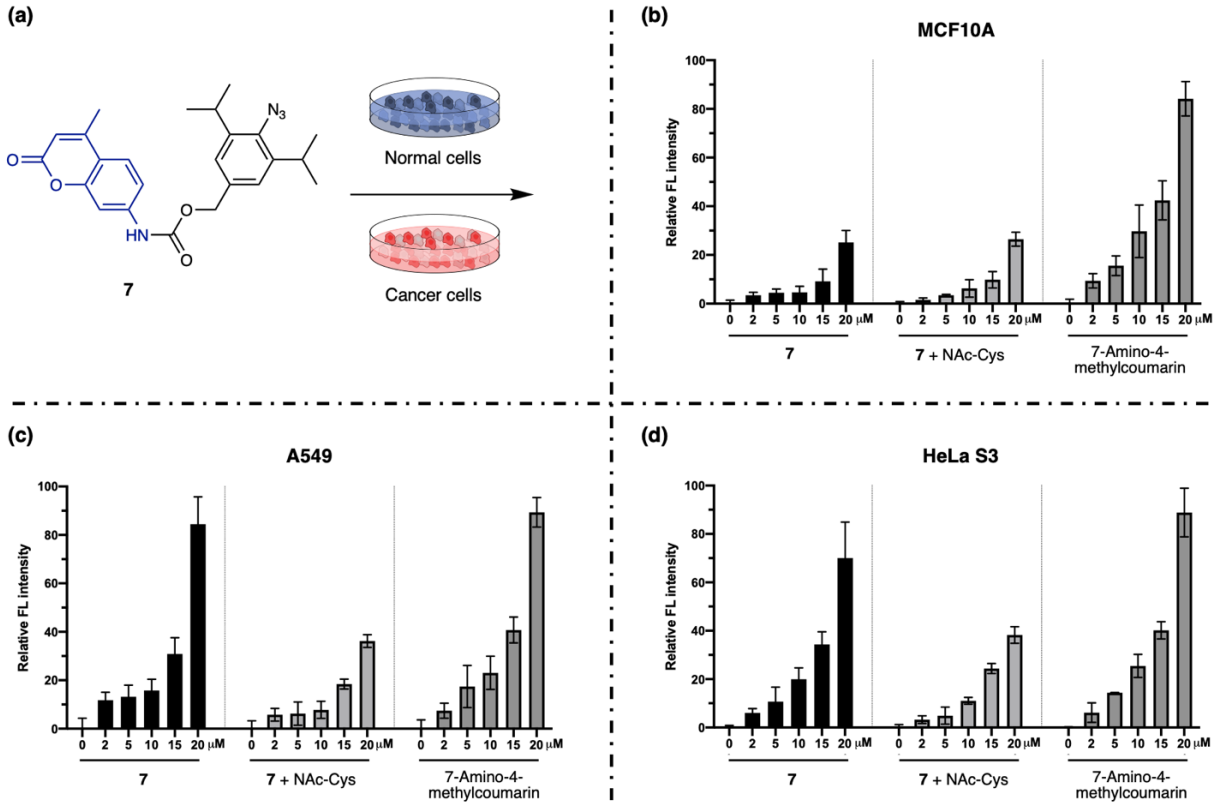


Figure S10. (**a**) The reaction between coumarin-ABC **7** and endogenous acrolein generated by cells could trigger the 7-amino-4-methylcoumarin release, which could easily observe by a fluorescence detector. One normal cell and two cancer cells were treated with various concentrations of (i) coumarin-ABC **7**; (ii) coumarin-ABC **7** with the cells were pretreated by 1 mM of *N*-acetyl cysteine (NAc-Cys) for 2 hours, and; (iii) 7-amino-4-methylcoumarin. Comparison of fluorescence intensity from (**b**) normal human mammary MCF10A cells, (**c**) human lung cancer A549 cells, (**d**) human cervical cancer HeLa S3 cells. Fluorescence intensity was normalized for each cell line by the number of 10,000 cells.

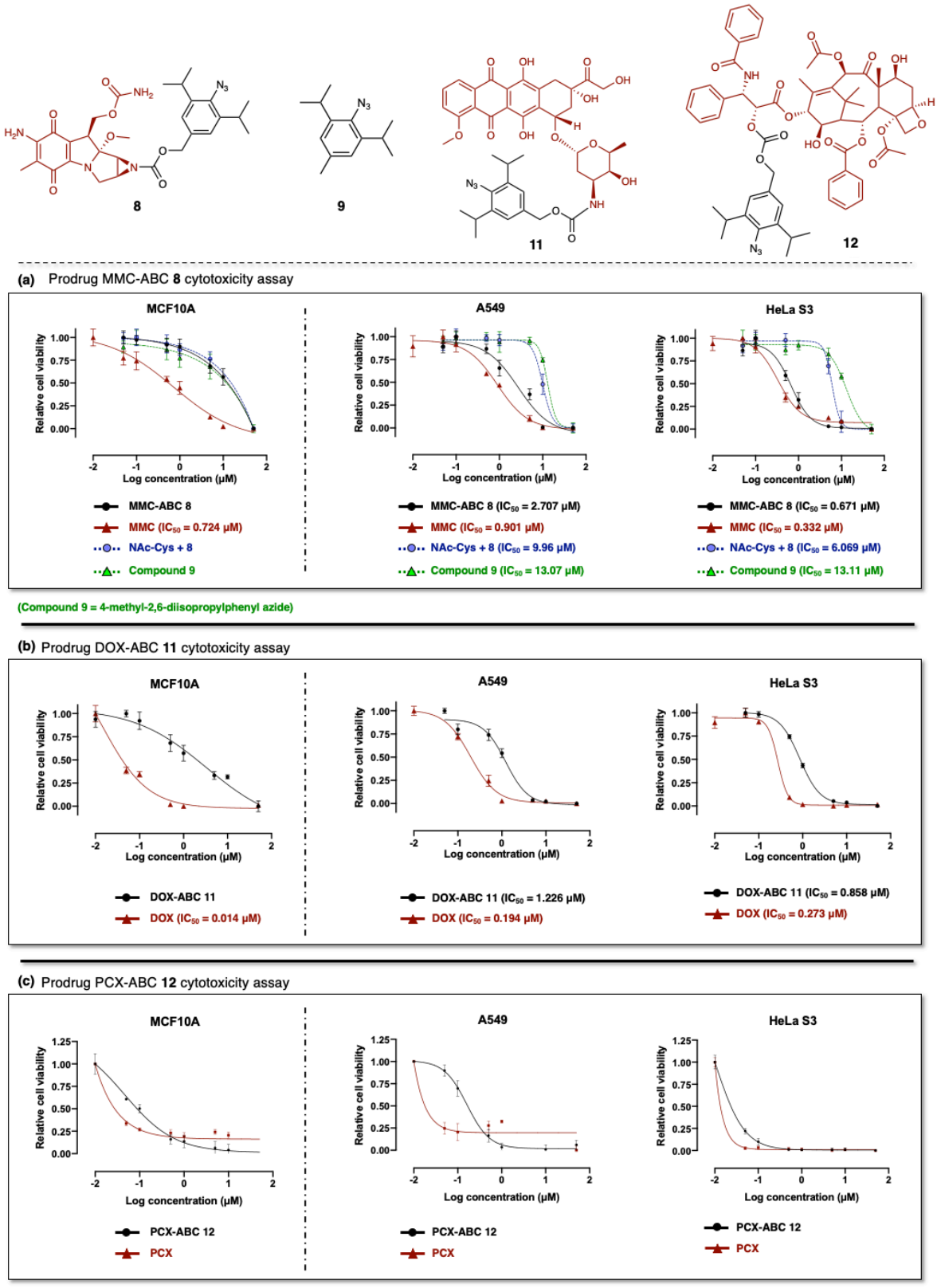


Figure S11. ATP assay of the (**a**) MMC-ABC **8**; (**b**) DOX-ABC **11**; (**c**) PCX-ABC **12** towards MCF10A, A549, and Hela S3 cells. Compound 9 = 4-methyl-2,6-diisopropylphenyl azide.

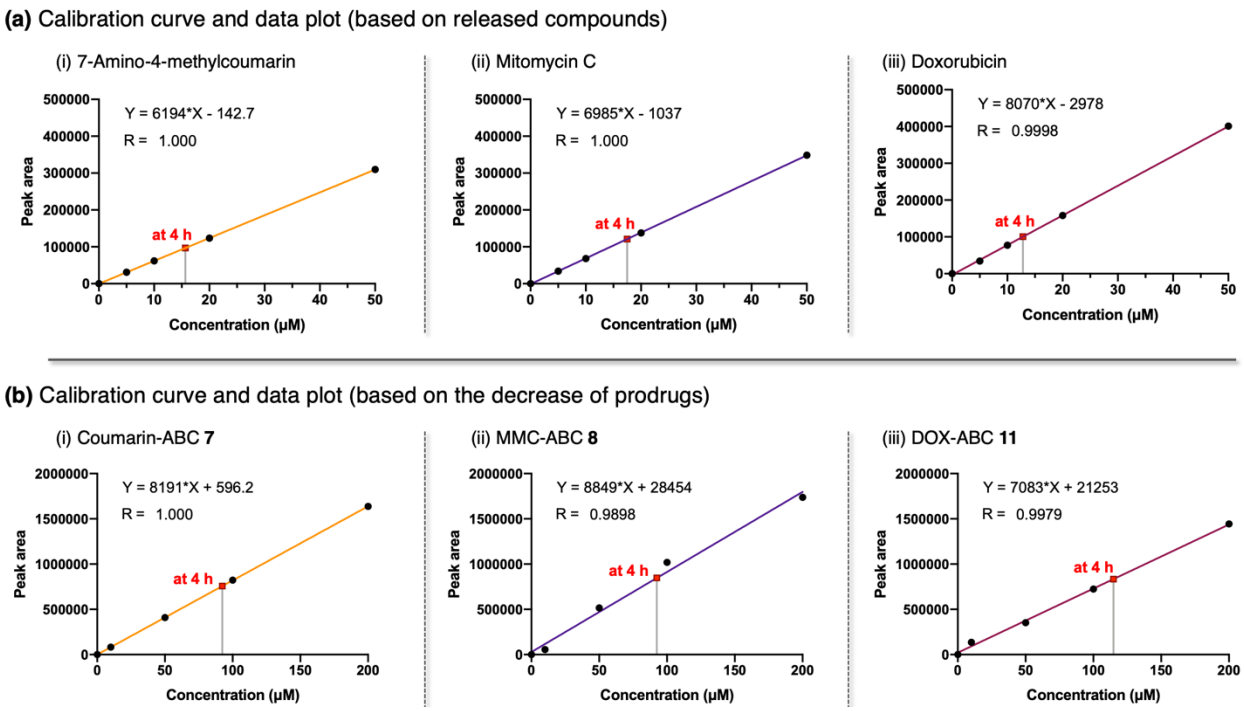


Figure S12. Quantitative analysis by RP-HPLC to estimate the amount of released compound. Incubation of 150 μM of coumarin-ABC **7** with acrolein approximately release 16 μM of coumarin (peak area = 96812). Incubation of 150 μM of MMC-ABC **8** with acrolein approximately release 18 μM of MMC (peak area = 121015). Incubation of 150 μM of DOX-ABC **11** with acrolein approximately release 13 μM of DOX (peak area = 100551). After 4 h of incubation the remaining starting materials, i.e., approximately 92 μM of coumarin-ABC **7** (peak area = 757601), 93 μM of MMC-ABC **8** (peak area = 847230), 115 μM of DOX-ABC **11** (peak area = 834659), were detected in the reaction mixtures.

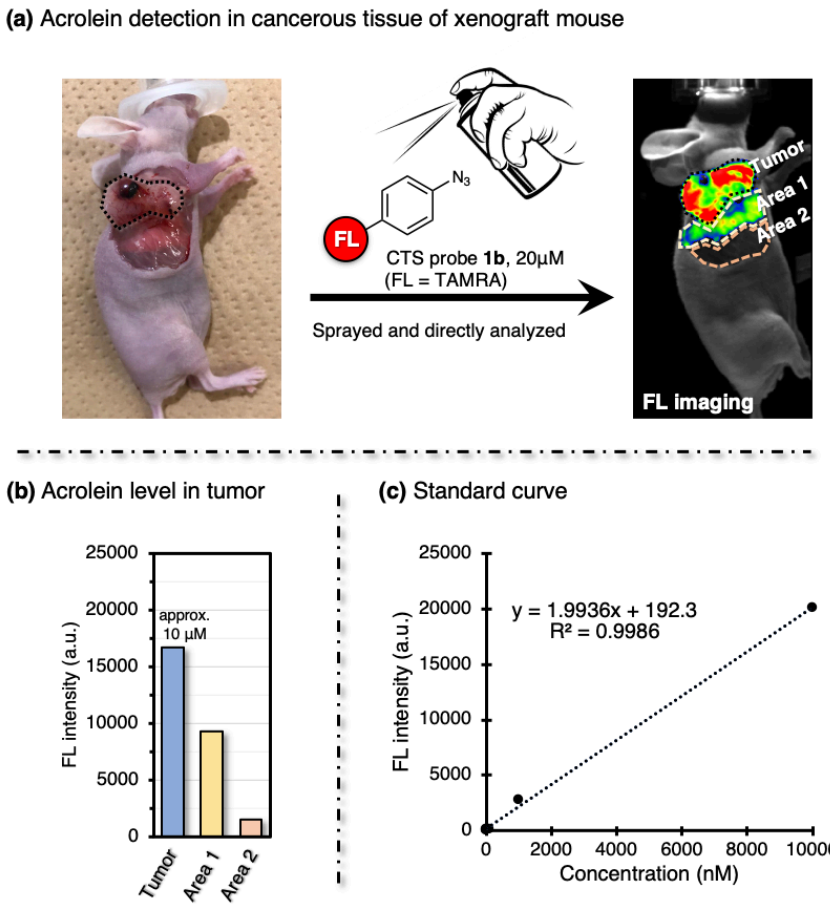


Figure S13. **(a)** The acrolein level on A549 cell xenograft-bearing nude mice could be determined by utilizing 20 μM of CTS probe **1b**. The tumor area shows higher fluorescence intensity than the border of cancer-healthy tissue (area 1) and healthy tissue (area 2). **(b)** Comparison of fluorescence intensities on the tumor, area 1, and area 2. **(c)** Based on the standard fluorescence, approximately 10 μM of acrolein were generated on the xenograft tumor mouse.

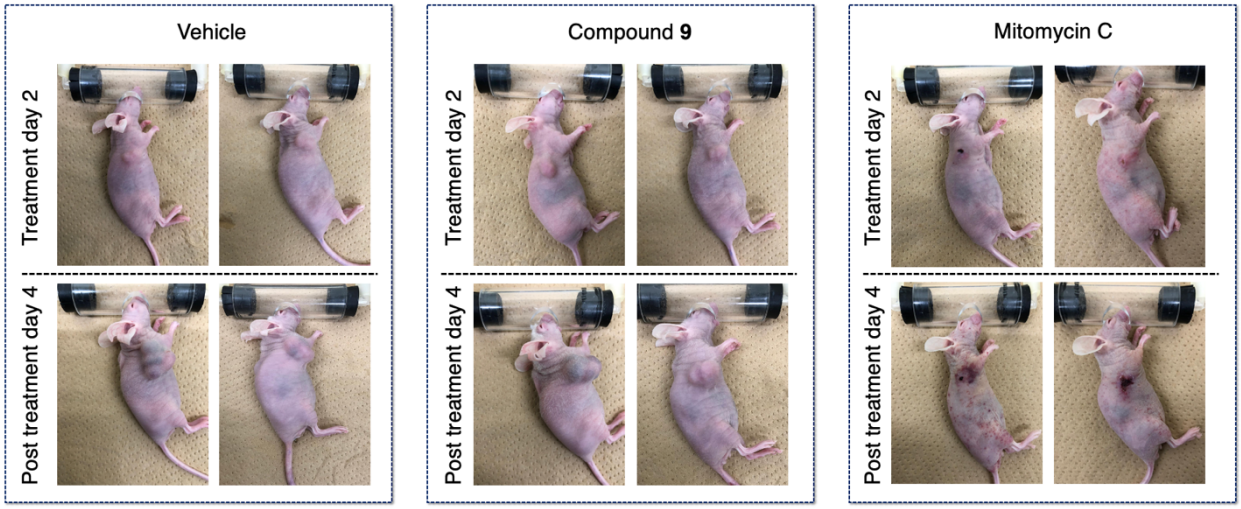


Figure S14. The A549 cell xenograft-bearing nude mice were treated with vehicle, compound **9**, and free MMC. The compounds were administered intratumorally every day for 12 days. The photos were taken during the second day of treatment and the fourth day after treatment.

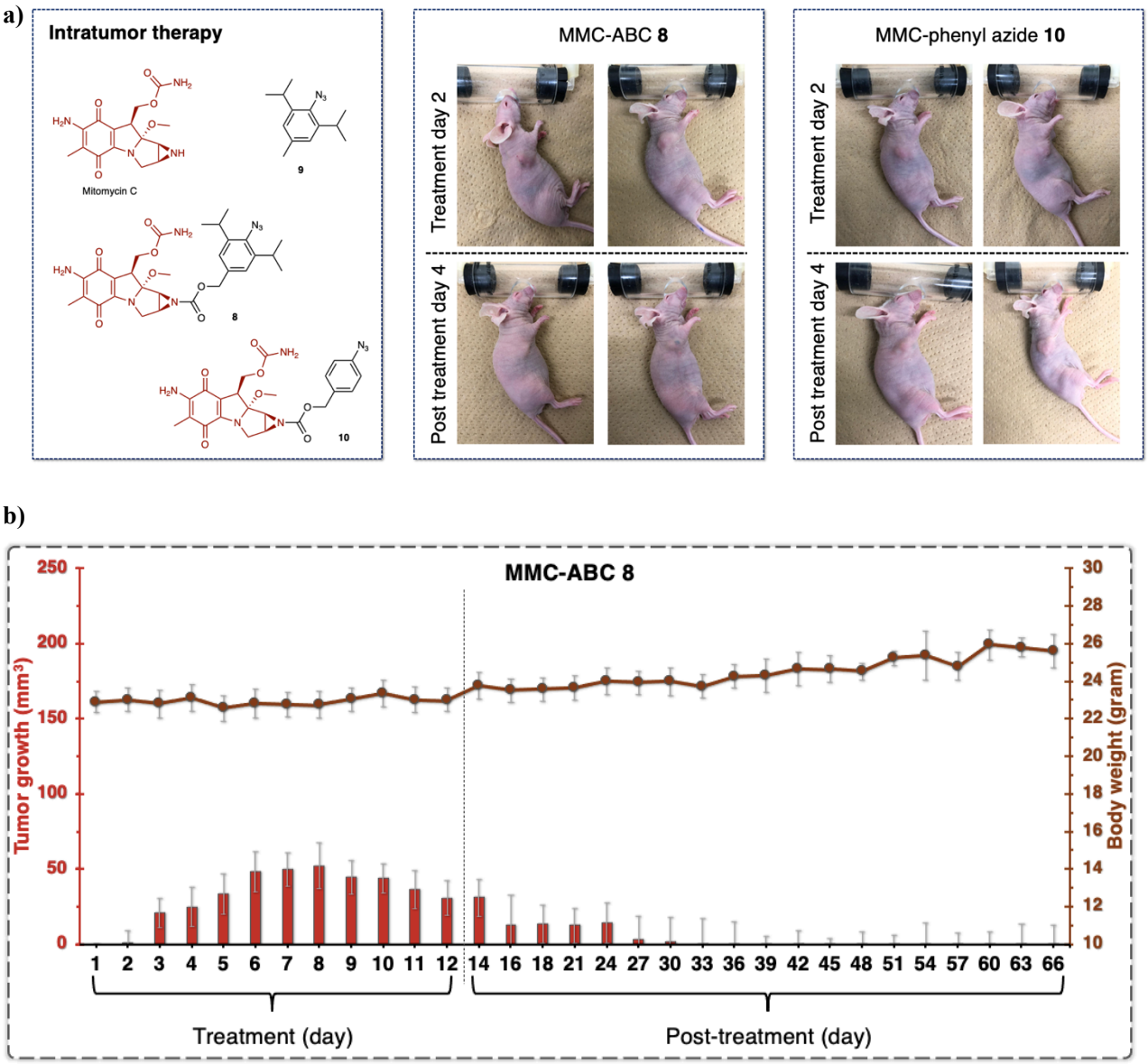


Figure S15. **(a)** The A549 cell xenograft-bearing nude mice were treated with MMC-ABC **8** and MMC-phenyl azide **10**. The compounds were administered intratumorally every day for 12 days. The photos were taken during the second day of treatment and the fourth day after treatment. **(b)** Tumor growth level (red bar) and body weight change (dark brown line) of mice treated with MMC-ABC **8** were observed (63 days).

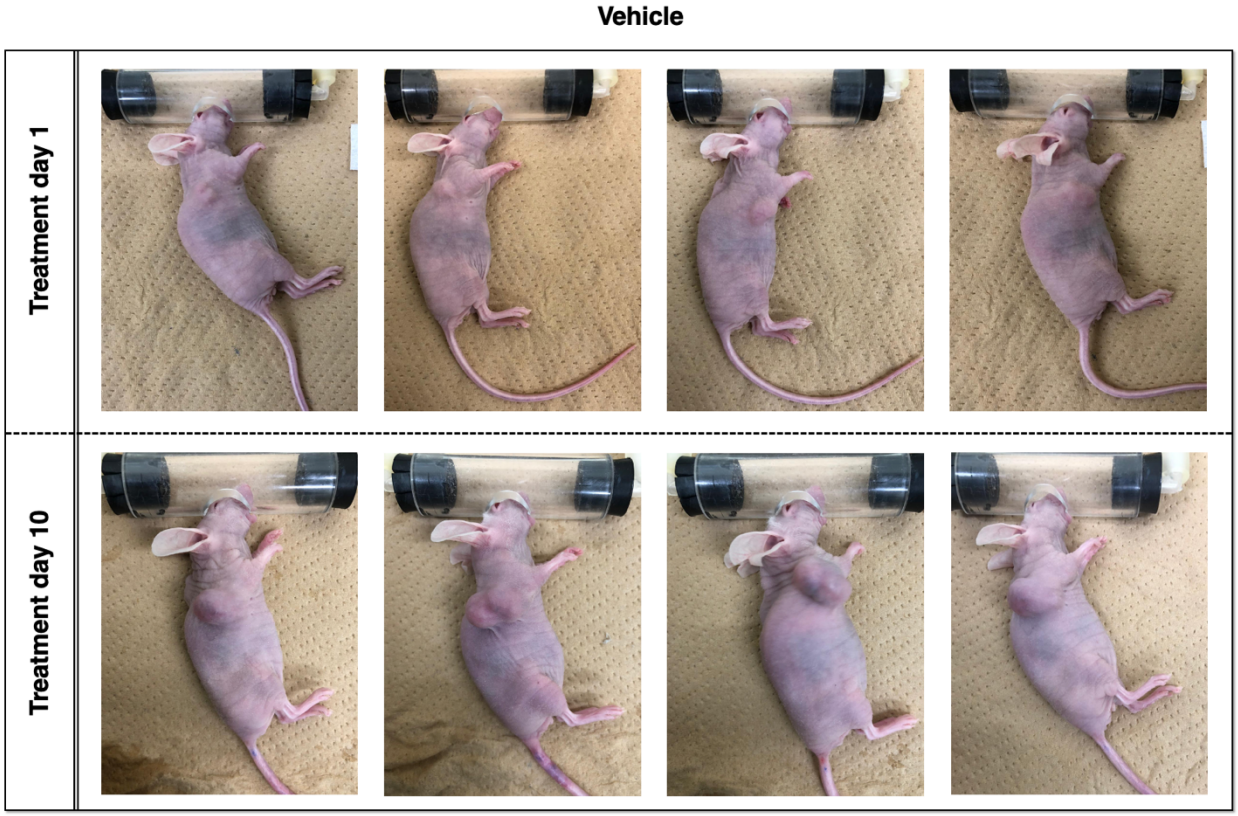


Figure S16. The A549 cell xenograft-bearing nude mice were treated with a vehicle. The solution was administered intravenously every three days for 10 days. The photos were taken during the first day and tenth day of treatment.

Figure S17. The A549 cell xenograft-bearing nude mice were treated with compound **9**. The compound was administered intravenously every three days for 10 days. The photos were taken during the first day and tenth day of treatment.

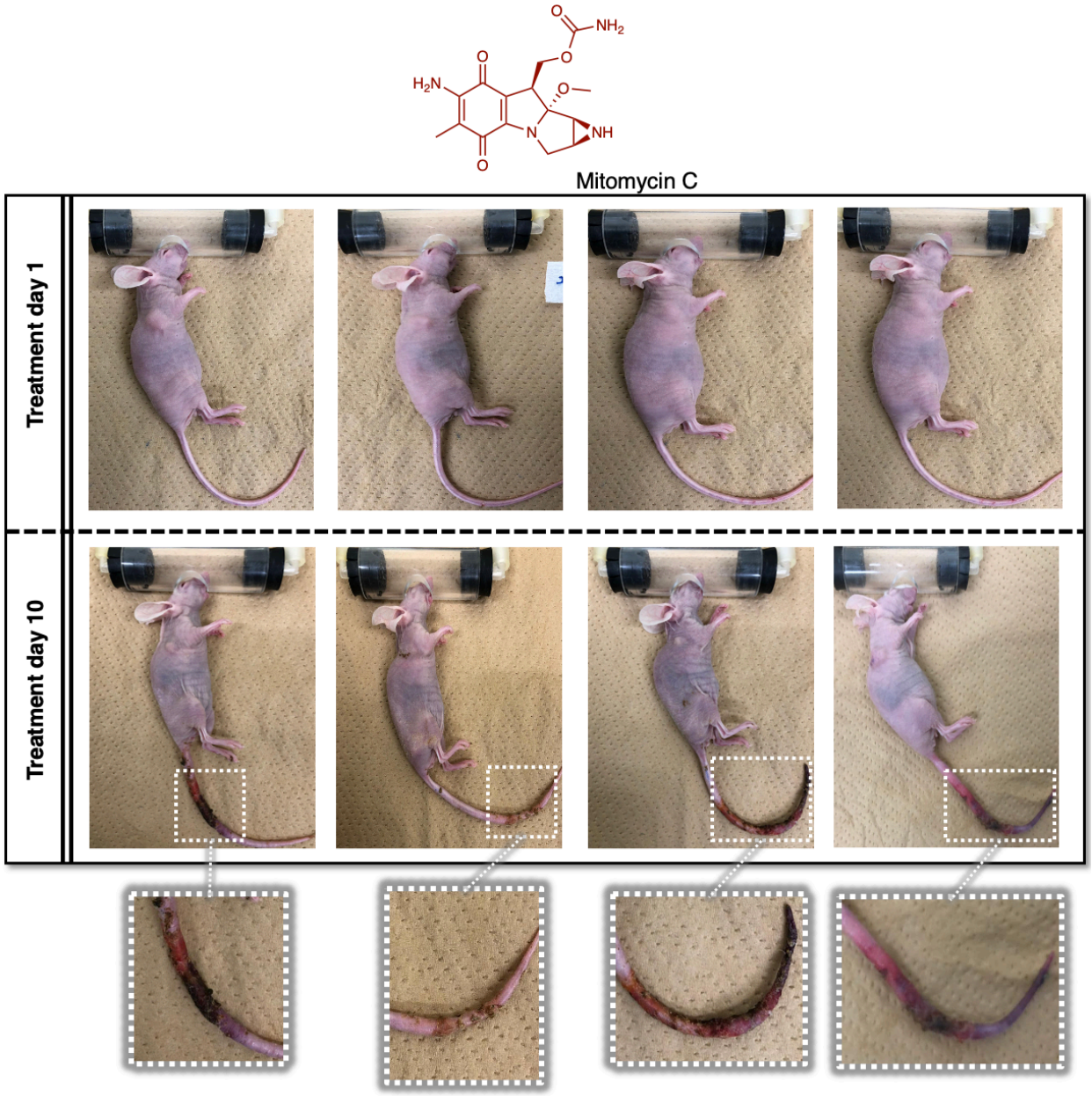


Figure S18. The A549 cell xenograft-bearing nude mice were treated with mitomycin C. The compound was administered intravenously every three days for 10 days. The photos were taken during the first day and tenth day of treatment.

Figure S19. The A549 cell xenograft-bearing nude mice were treated with MMC-ABC **8**. The compound was administered intravenously every three days for 10 days. The photos were taken during the first day and tenth day of treatment.

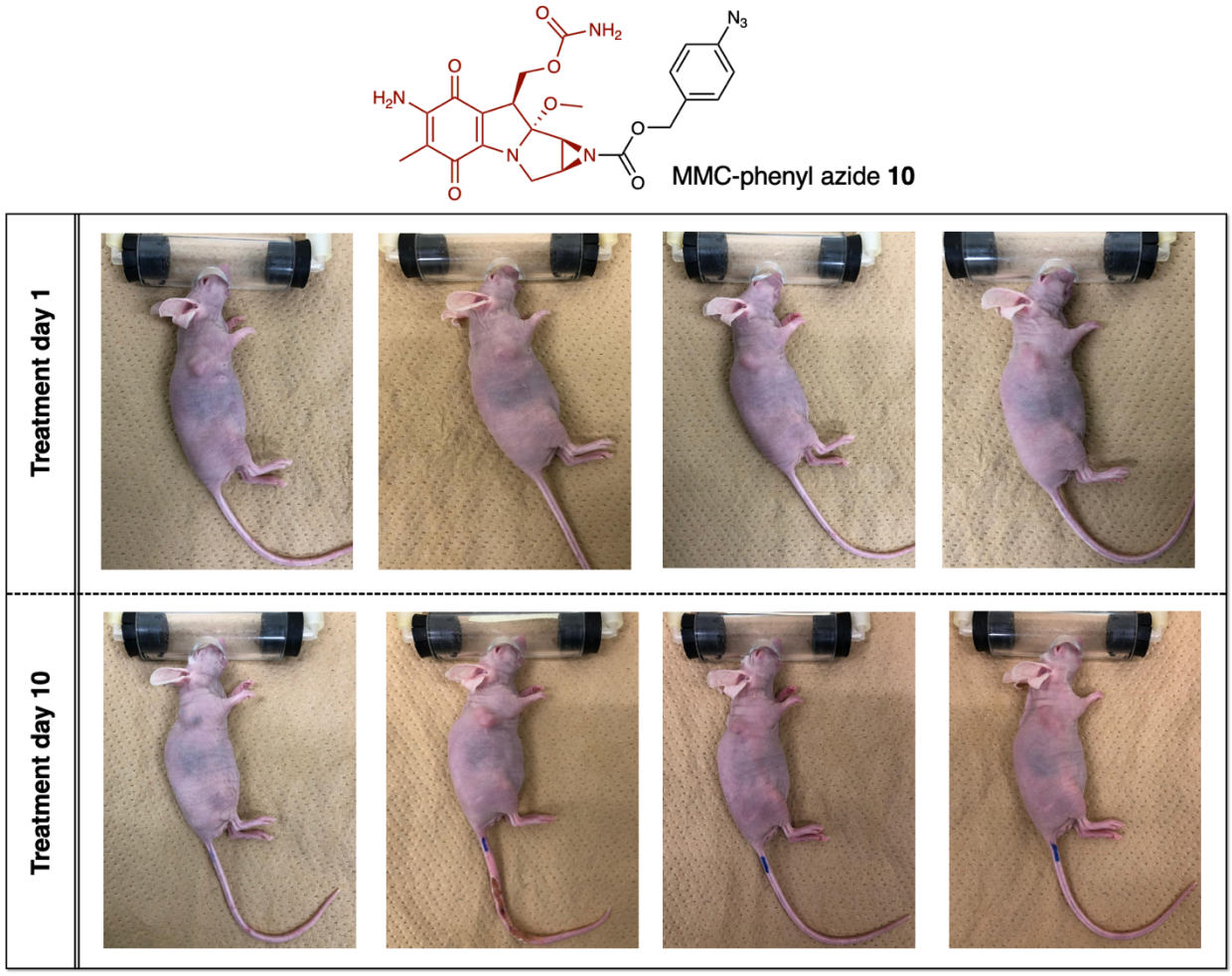


Figure S20. The A549 cell xenograft-bearing nude mice were treated with MMC-phenyl azide **10**. The compound was administered intravenously every three days for 10 days. The photos were taken during the first day and tenth day of treatment.

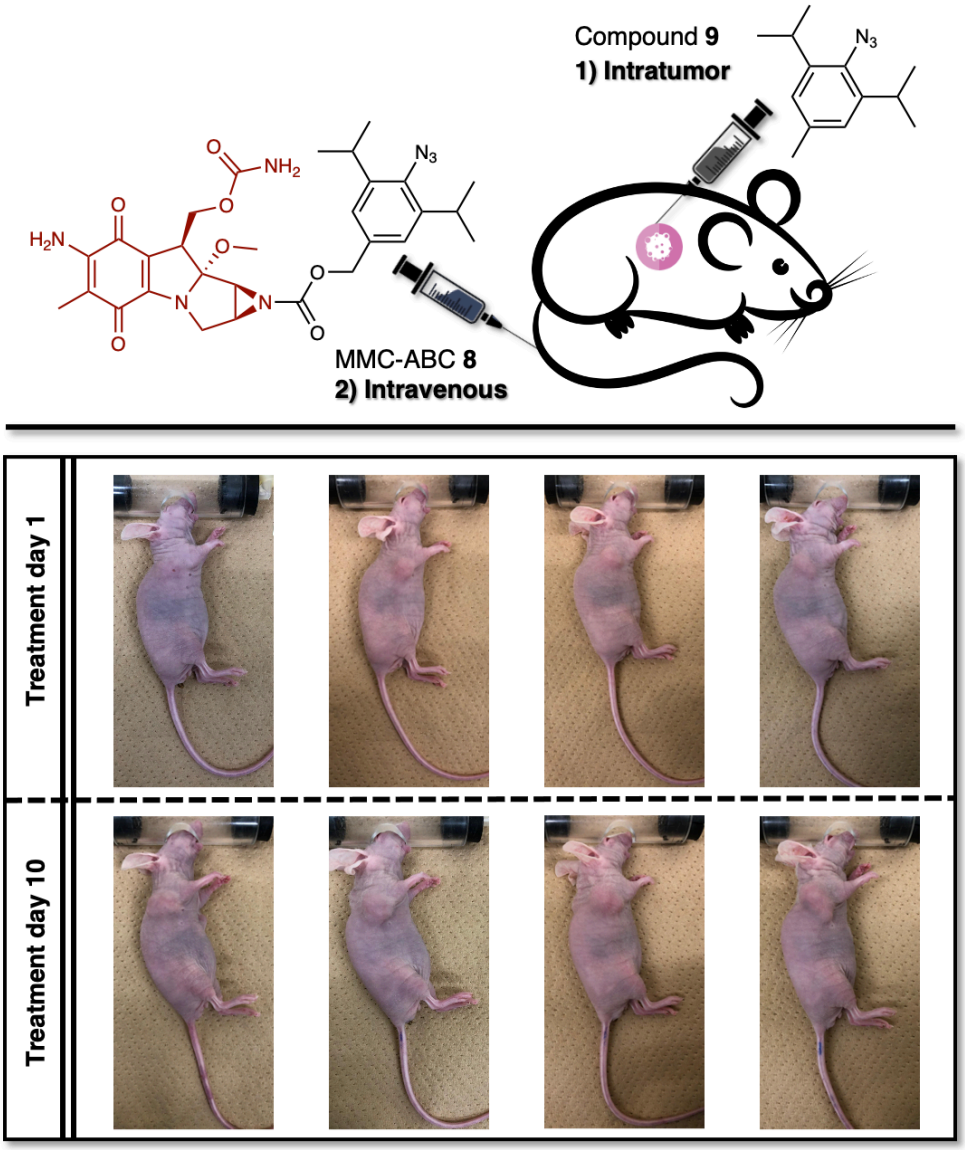


Figure S21. The A549 cell xenograft-bearing nude mice were treated with compound **9** (administered intratumorally) and then 1 hour later treated with MMC-phenyl azide **10** (administered intravenously). The compounds were administered every three days for 10 days. The photos were taken during the first day and tenth day of treatment.

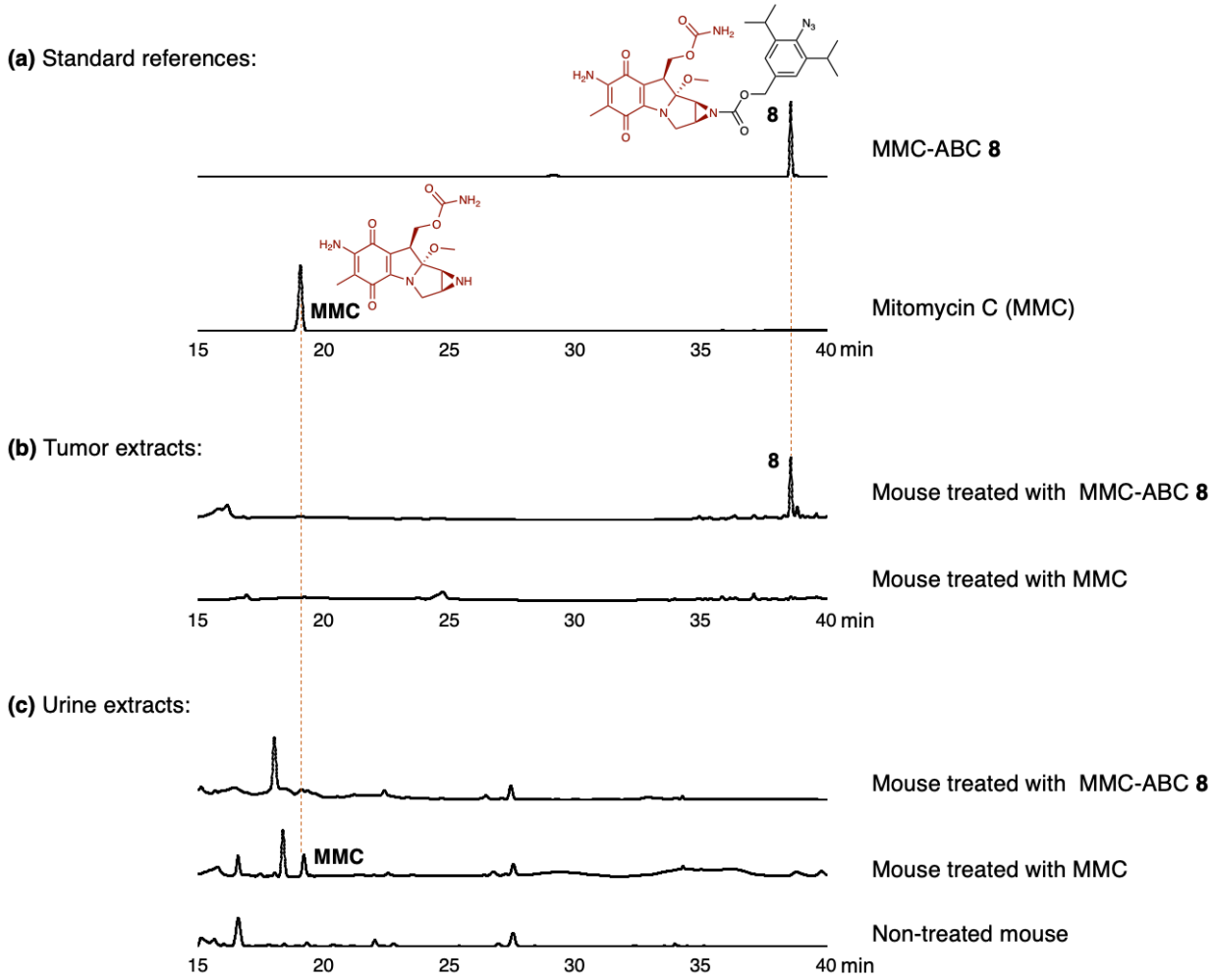


Figure S22. **(a)** HPLC chromatogram of mitomycin C (MMC) and MMC-ABC **8**. **(b)** HPLC analysis of tumor extracts from the A549 cell xenograft-bearing nude mice treated with MMC and MMC-ABC **8**. **(c)** HPLC analysis of urine extracts from xenograft mouse after treated with MMC and MMC-ABC **8**. UV detection at 254 nm.

Optimized DFT structure

Refer to **Figure S6**.

DFT level: ωB97XD/D95V(d) + SMD(DMSO)

Aclorein (*s-trans*)

E_{tot} [hartree] = -191.879084847306

C	-1.132554533	-0.589066871	0.286307270
C	0.002759684	0.024295754	0.654519016
H	-2.104747081	-0.181747852	0.557663364
H	-1.113480733	-1.511613877	-0.289484216
H	0.976168404	-0.383609238	0.389188838
C	0.003726519	1.283500209	1.444062847
O	-0.998098663	1.891053962	1.786579660
H	1.007206100	1.662568233	1.716870395

Aclorein (*s-cis*)

E_{tot} [hartree] = -191.879118462813

C	0.415730422	0.024028349	-0.505983255
C	-0.408739004	0.157630719	0.544189261
H	1.357357665	0.567900372	-0.554016207
H	0.167327459	-0.632220265	-1.336996237
H	-1.350038947	-0.385915793	0.596493364
C	-0.093300345	1.048447369	1.691253450
O	0.906893748	1.742879254	1.777367533
H	-0.848967313	1.051365882	2.500018090

Phenyl azide 1a

E_{tot} [hartree] = -395.744280626524

C	-0.968567594	0.560279417	-1.716379862
C	-2.299846468	0.994341909	-1.782051326
C	-0.266230932	0.601767951	-0.505192503
H	-2.852628059	0.966199367	-2.718842610
H	0.765446816	0.260604572	-0.473082478
C	-2.921204390	1.469584305	-0.624102515
C	-0.899780750	1.078608071	0.642699893
H	-3.953484949	1.806770670	-0.675562412
H	-0.352758702	1.109404081	1.581613322
C	-2.228773946	1.514609689	0.589673950
H	-2.718887204	1.886790620	1.485465547
N	-0.240151574	0.058245199	-2.834977587
N	-0.848953969	-0.005024682	-3.914266435
N	-1.292773050	-0.112490052	-4.956207665

2,6-diisopropylphenyl azide 1d (conformer 1)

E_{tot} [hartree] = -631.593238391333

C	-2.357514300	1.806695401	-1.526712641
C	-1.068676998	2.184124222	-1.111001685
C	-3.112342376	0.816873882	-0.866515938
C	-0.510474059	1.489634345	-0.028296492
C	-2.519580779	0.166394716	0.220268797
H	0.493464780	1.738670693	0.307907805
H	-3.066381776	-0.608873208	0.750486859
C	-1.224087446	0.490752670	0.630216115
H	-0.774547965	-0.032391336	1.470904078
N	-3.007209387	2.488424140	-2.624947744
N	-2.482901829	2.364677271	-3.738883950
N	-2.100667244	2.335218608	-4.811776006
C	-4.495001764	0.435888783	-1.373871279
H	-4.946161619	1.335518819	-1.805620089
C	-5.433898512	-0.074902886	-0.276520888
C	-4.367249517	-0.604562446	-2.500649994
H	-3.734856229	-0.239169653	-3.318108261
H	-3.924241944	-1.532995047	-2.119357844
H	-5.353538768	-0.841850126	-2.916957998
H	-5.114964456	-1.046640220	0.118047355
H	-6.441035882	-0.205186105	-0.689135814
H	-5.499248815	0.631408441	0.559330871
C	-0.276291331	3.291913153	-1.790521837
H	-0.937052960	3.828261465	-2.478167662
C	0.885018930	2.705187002	-2.609238406
C	0.235989418	4.328387803	-0.779845948
H	-0.588155741	4.747411638	-0.191149221
H	0.966656694	3.896826014	-0.086211380
H	0.728717868	5.152487632	-1.309114871
H	1.602277596	2.194786752	-1.954937613
H	1.418189433	3.502890702	-3.139870653
H	0.527838064	1.980678798	-3.350687031

2,6-diisopropylphenyl azide 1d (conformer 2)

E_{tot} [hartree] = -631.591650566678

C	-1.809574537	1.556253717	-1.483032067
C	-0.945913164	2.394089328	-0.748462977
C	-2.645595752	0.605698897	-0.868168730
C	-0.971063538	2.281115596	0.646084021
C	-2.645882940	0.546671399	0.532741988
H	-0.320476627	2.919558406	1.240339373
H	-3.297767271	-0.162221447	1.037960058
C	-1.819735843	1.375767215	1.286794942
H	-1.830109333	1.314726882	2.372397884
N	-1.752420572	1.651197924	-2.924135403
N	-2.794365489	1.985180550	-3.500952100
N	-3.676679813	2.283000769	-4.157583469

C	-0.007117891	3.413374524	-1.382018349
H	0.516875357	3.890297763	-0.546170241
C	-0.751335656	4.532688349	-2.128204979
C	1.068227328	2.764224259	-2.266898398
H	1.629131470	2.003407186	-1.711358237
H	0.631890550	2.289256694	-3.151490971
H	1.779686660	3.526558342	-2.606964275
H	-0.047413387	5.330402265	-2.394124356
H	-1.536326559	4.971715539	-1.501495531
H	-1.211602288	4.174203547	-3.054781543
C	-3.542095904	-0.334908307	-1.661745031
H	-3.260011425	-0.285140158	-2.717665750
C	-5.015227367	0.088969956	-1.542560530
C	-3.361193387	-1.798135606	-1.232590661
H	-3.953471310	-2.451093934	-1.884298882
H	-2.311970471	-2.106687603	-1.303964635
H	-3.697991934	-1.965453686	-0.203182135
H	-5.361476268	0.001554745	-0.505390177
H	-5.648051130	-0.553286912	-2.166439861
H	-5.163626538	1.127314392	-1.861485177

2,6-diisopropylphenyl azide 1d (conformer 3)

E_{tot} [hartree] = -631.588072685951

C	-1.946744794	1.449613200	-1.292622591
C	-0.844717304	2.180268167	-0.806569924
C	-2.382008114	0.249215299	-0.697849297
C	-0.212625305	1.703988779	0.350377350
C	-1.712787022	-0.180676778	0.456452379
H	0.635835594	2.253408419	0.753152807
H	-2.033019038	-1.100749084	0.941095960
C	-0.644009941	0.540740110	0.985986864
H	-0.140285596	0.190244615	1.883624638
N	-2.602455641	1.903061952	-2.503835139
N	-3.557808358	2.671627807	-2.342750431
N	-4.450550881	3.379486775	-2.334445626
C	-0.264790927	3.422213684	-1.475302540
H	0.566310850	3.730328383	-0.832541481
C	-1.222619716	4.623460468	-1.552893996
C	0.334286910	3.102417615	-2.853915045
H	-0.439361079	2.791498654	-3.564395794
H	0.829595092	3.992220389	-3.261128694
H	1.079924064	2.301828982	-2.785271764
H	-0.643008927	5.541104201	-1.706577888
H	-1.800082734	4.742144998	-0.628857134
H	-1.920808387	4.544950604	-2.392435651
C	-3.484727385	-0.643347890	-1.257365594
H	-3.600946615	-1.449578568	-0.525548269
C	-3.051355622	-1.295717888	-2.579882842
C	-4.865835750	0.018518675	-1.397836660

H	-5.636831281	-0.759418341	-1.440859101
H	-5.095136131	0.669736137	-0.546470982
H	-4.952850465	0.602715448	-2.319609247
H	-2.903286997	-0.544682487	-3.364250037
H	-3.821409773	-1.996951155	-2.923706419
H	-2.115378951	-1.852986424	-2.457142863

Product 2a

E_{tot} [hartree] = -587.687757097854

C	-0.664197348	1.892135813	-3.378749935
O	-0.723905225	1.540055257	-4.536672271
H	-1.377435028	2.630319472	-2.960293692
C	0.192193183	2.001965712	1.230904596
C	-0.654578192	1.081038937	1.866214891
C	0.841141099	2.993925047	1.987404613
H	-1.161620044	0.308791444	1.294657560
H	1.497348271	3.708634700	1.501380243
C	-0.849356011	1.157081578	3.248066288
C	0.634586649	3.053962196	3.364612969
H	-1.508946339	0.438567864	3.728466043
H	1.139892088	3.825709928	3.940403642
C	-0.209597131	2.139798857	4.006860126
H	-0.365924289	2.195991467	5.080877264
N	1.248424845	2.545937871	-2.037775774
N	1.185057671	2.767286102	-0.803692402
C	0.377419713	1.388519422	-2.390825049
C	-0.242872686	0.947615140	-1.057005249
N	0.376235502	1.917503171	-0.154430662
H	-1.334039896	1.046401514	-1.034957375
H	0.031673339	-0.074563189	-0.784749215
H	0.987603695	0.619349771	-2.868308456

Product 2d

E_{tot} [hartree] = -823.537514350939

C	-0.857610603	-4.280883177	-2.440111198
O	-1.168552386	-5.447288935	-2.326786806
H	-1.079344500	-3.711217725	-3.365178064
C	-0.009045614	0.196346089	-1.116076185
C	-0.367396591	0.850895924	-2.307665712
C	0.150113330	0.881850788	0.105038969
C	-0.544786635	2.241047323	-2.260771776
C	-0.044133508	2.266361169	0.108294513
H	-0.812432970	2.780192163	-3.167024361
H	0.074256425	2.829685229	1.030040300
C	-0.385932157	2.940686244	-1.066582172
H	-0.531019483	4.018495800	-1.047749266
C	-0.553386368	0.112689907	-3.624820783
H	-0.411467961	-0.958079938	-3.456952016

C	0.503993346	0.553581933	-4.647419615
C	-1.974331707	0.300111106	-4.175993926
H	-2.726283566	-0.035567196	-3.452235864
H	-2.177120308	1.350524915	-4.416001263
H	-2.100541191	-0.284193301	-5.095098854
H	0.403085966	1.618527122	-4.888755274
H	0.393066824	-0.014557420	-5.578488526
H	1.516368173	0.385602825	-4.261696745
C	0.578432388	0.133967638	1.358221441
H	0.184786456	-0.885204008	1.289580726
C	0.041593307	0.752130968	2.653035528
C	2.112680012	0.032991909	1.402194868
H	2.505850309	-0.464871179	0.508501661
H	2.564404373	1.031033275	1.463025798
H	2.433278539	-0.539538225	2.280919066
H	0.504421934	1.722841289	2.865730352
H	0.267982697	0.090928040	3.497303476
H	-1.044974455	0.892453446	2.613669780
N	1.152245455	-2.994392796	-1.908732388
N	1.244565488	-1.752321187	-1.732522297
C	-0.150711757	-3.478389972	-1.363232453
C	-0.909071952	-2.192599329	-1.002628997
N	0.181025037	-1.220936263	-1.112228182
H	-1.708717008	-1.950939053	-1.715918710
H	-1.321301893	-2.211145791	0.007870184
H	0.046652710	-4.118121099	-0.498862667

Transition state TS1 (reaction 1)

E_{tot} [hartree] = -587.606134625657

C	-0.763528845	0.195687788	-3.658692365
O	-0.657435654	0.506071227	-4.837741970
H	-1.503438753	0.705545240	-3.006185985
C	0.449180701	0.069706282	0.899619044
C	-0.155828801	-0.934373014	1.666082284
C	0.642758178	1.352863110	1.430749596
H	-0.295482794	-1.925699287	1.242250546
H	1.115050622	2.129124261	0.833259925
C	-0.573199008	-0.648712278	2.966608274
C	0.230403267	1.619814844	2.737718275
H	-1.046181961	-1.428559293	3.557985311
H	0.384930208	2.613111662	3.151846344
C	-0.381058203	0.626191420	3.509828966
H	-0.701929754	0.843120750	4.525336196
N	1.531562887	0.769604492	-2.309505392
N	1.394075669	0.544462037	-1.175756087
C	0.026199111	-0.838664827	-2.982895103
C	-0.375597062	-1.305460302	-1.735715229
N	0.822601683	-0.290396643	-0.417660381
H	-1.300896927	-0.936554052	-1.296134196

H	-0.037458463	-2.278738988	-1.394136488
H	0.757483268	-1.388144430	-3.571544459

Transition state TS2 (reaction 2)

E_{tot} [hartree] = -823.459348044308

C	-0.944682695	-3.835710634	-1.442527290
O	-0.725110223	-4.921707712	-1.964988851
H	-1.421805493	-3.020108478	-2.027688057
C	0.162779771	0.434178007	0.231028680
C	-0.262513213	0.971950113	-0.997688398
C	0.318598174	1.216930560	1.392697377
C	-0.473976222	2.355376300	-1.060226370
C	0.080111988	2.589969488	1.286191148
H	-0.783251050	2.809782922	-1.998670513
H	0.195190137	3.230210699	2.156274162
C	-0.303636744	3.156155133	0.067941622
H	-0.478607838	4.227436828	0.001406389
C	-0.470998846	0.113716053	-2.234999143
H	-0.413385313	-0.938917548	-1.948880976
C	0.638718597	0.372674294	-3.265349534
C	-1.861581872	0.321322181	-2.849329251
H	-2.651653674	0.139744355	-2.111238110
H	-1.986647939	1.337221116	-3.241571688
H	-2.007746385	-0.377004349	-3.681746533
H	0.604850463	1.407674470	-3.626788764
H	0.517776741	-0.293227972	-4.127812130
H	1.630258770	0.195193453	-2.832388999
C	0.791850984	0.581296840	2.690752565
H	0.371173943	-0.428914542	2.735785506
C	0.335773144	1.332657830	3.945239256
C	2.324066460	0.444187679	2.671735858
H	2.664879694	-0.146859515	1.813855324
H	2.799062069	1.431391601	2.613940054
H	2.674415917	-0.051049142	3.585110533
H	0.828062342	2.307495679	4.041680068
H	0.593583474	0.747944210	4.835740455
H	-0.748529860	1.494278975	3.949243549
N	1.458715837	-2.661816838	-0.710560883
N	1.225658991	-1.601008380	-0.292853147
C	-0.643524914	-3.485451660	-0.054333154
C	-1.160474987	-2.313577289	0.487125417
N	0.358919303	-0.976254860	0.377174374
H	-1.840919413	-1.706332080	-0.108318001
H	-1.251378149	-2.209539309	1.564260099
H	-0.210595092	-4.251308478	0.585211266

Transition state TS in entry 2

E_{tot} [hartree] = -823.456896075143

C	-2.399696114	-0.488208643	2.365061072
C	-1.203769788	-1.115420275	2.047555084
H	-2.446216842	0.164515315	3.235206207
H	-3.337620170	-0.923253510	2.033411924
H	-1.184396288	-1.991590762	1.402571798
C	0.007854011	-0.853621894	2.847098675
O	0.990646137	-1.578153618	2.865080539
H	-0.033290515	0.067723446	3.462884663
C	0.313091061	-0.042824278	-0.635917598
C	1.669928649	0.343108629	-0.628946082
C	-0.248159872	-0.824637087	-1.666201018
C	2.461913463	-0.039941446	-1.715531523
C	0.579568696	-1.168288478	-2.741466511
H	3.510129039	0.244041380	-1.751501203
H	0.177478398	-1.750435348	-3.566839082
C	1.919595286	-0.781830933	-2.766685354
H	2.547572786	-1.064974716	-3.608330328
N	-0.452062144	0.310328018	0.512766033
N	-1.512159635	0.987507760	0.449271297
N	-2.521872059	1.101626902	1.040521065
C	2.216688477	1.218546342	0.487681396
H	1.668437508	0.972893432	1.402105014
C	1.930689847	2.695320647	0.165742587
C	3.705826098	0.997848242	0.768002453
H	3.926731985	-0.061007707	0.946206292
H	4.338553142	1.347335988	-0.056120949
H	3.997269738	1.558486343	1.663659132
H	2.455423060	3.000891047	-0.748053173
H	2.269299816	3.339568754	0.986003339
H	0.858389632	2.869616011	0.017065857
C	-1.708248164	-1.250005121	-1.651842730
H	-2.085043602	-1.167075651	-0.629076932
C	-1.904978117	-2.712344422	-2.071794963
C	-2.541578951	-0.307871208	-2.536272156
H	-2.435677655	0.737598750	-2.223295425
H	-2.223762615	-0.380709052	-3.583678158
H	-3.604044209	-0.573184856	-2.481962301
H	-1.661314518	-2.874582265	-3.127757429
H	-2.954024934	-2.996297011	-1.929101370
H	-1.285830425	-3.385776373	-1.467970119

Transition state TS in entry 3

E_{tot} [hartree] = -823.456814240279

C	0.350040491	0.682457498	-2.114340528
C	-0.384070927	1.823415387	-2.423985489
H	1.435943818	0.735991546	-2.107855595
H	-0.073104340	-0.299878305	-2.308446814
H	-1.420442077	1.750147801	-2.744347149
C	0.309142681	3.096501396	-2.621870960

O	-0.188012266	4.107545974	-3.102609383
H	1.371458650	3.094534323	-2.296657317
C	0.137550890	-0.749242836	0.585564832
C	1.272168421	-1.176961578	1.304573430
C	-1.016293135	-1.547231812	0.447172950
C	1.216555200	-2.440228951	1.904164652
C	-1.027064076	-2.796838782	1.079267282
H	2.074551893	-2.796869793	2.470083576
H	-1.908753507	-3.428829931	1.009257474
C	0.079926017	-3.243478996	1.797778297
H	0.058255465	-4.218856943	2.277910930
N	0.213380405	0.507602269	-0.098362659
N	-0.542043136	1.472628332	0.203982368
N	-1.021036727	2.426342148	-0.259665034
C	2.524100815	-0.334031958	1.512347182
H	3.215368961	-0.973160638	2.072216373
C	2.246371872	0.896007956	2.391258787
C	3.239424557	0.064917602	0.213433540
H	3.349178929	-0.790002204	-0.463818345
H	2.701834990	0.858530587	-0.313859703
H	4.242267473	0.441284243	0.447799604
H	3.188291189	1.406113306	2.626029865
H	1.772164172	0.608845839	3.336764959
H	1.594229633	1.615959919	1.885251184
C	-2.248988793	-1.067013466	-0.304516884
H	-1.972156369	-0.219587491	-0.936524403
C	-3.307842796	-0.573436913	0.694981624
C	-2.829679591	-2.134952352	-1.240073305
H	-3.645614752	-1.700650713	-1.829116073
H	-2.070928226	-2.510899541	-1.936167433
H	-3.240931478	-2.987221599	-0.687611145
H	-3.644642604	-1.394312098	1.339875283
H	-4.180409122	-0.177724345	0.162061449
H	-2.912127074	0.221072047	1.338737260

Transition state TS in entry 4

E_{tot} [hartree] = -823.455146042590

C	1.925194046	2.177776147	-1.391624936
C	1.455027130	2.132364781	-0.084846673
H	2.804118487	1.596410365	-1.663414821
H	1.727643393	3.060350632	-1.993908749
H	0.792758303	2.906890187	0.290297740
C	2.132132744	1.294219572	0.920175733
O	1.954122496	1.381722866	2.125594392
H	2.865758487	0.571498297	0.509664574
C	-0.722403623	-0.392750768	0.394645277
C	-1.884584940	-0.184685618	1.169655547
C	0.015978535	-1.595261863	0.458063090
C	-2.302106267	-1.225865233	2.005494867

C	-0.454821392	-2.611398228	1.298260437
H	-3.198775200	-1.094455575	2.607303522
H	0.082056008	-3.554967463	1.355105272
C	-1.600106268	-2.430200329	2.070318259
H	-1.947433352	-3.226680521	2.724175134
N	-0.263079742	0.698232084	-0.402028789
N	-0.057970777	0.580277332	-1.640158761
N	0.629902463	1.061134697	-2.466723649
C	-2.737723316	1.076815289	1.117949420
H	-3.557442105	0.901790685	1.823312880
C	-3.379106721	1.293109671	-0.262172255
C	-2.005123167	2.337487670	1.597828672
H	-1.513472674	2.171228894	2.563352414
H	-1.249278259	2.660078169	0.876931523
H	-2.723976201	3.156436574	1.721137997
H	-4.103885763	2.114579936	-0.211240652
H	-3.911402198	0.394614268	-0.595676948
H	-2.632545812	1.550239827	-1.020650717
C	1.259127125	-1.833735939	-0.385540545
H	1.639020816	-0.872361513	-0.738519767
C	0.902717276	-2.670080963	-1.625896568
C	2.397721109	-2.486766067	0.408778687
H	3.309457282	-2.504634666	-0.199645217
H	2.613792411	-1.930611940	1.328383418
H	2.165424491	-3.522217903	0.682291758
H	0.539918825	-3.662933455	-1.333106326
H	1.786089403	-2.802339579	-2.261656749
H	0.121497921	-2.187465294	-2.224687232

Transition state TS in entry 5

E_{tot} [hartree] = -823.450355904578

C	0.214101858	2.051906942	-0.750964734
C	-0.988996381	2.695178462	-1.022094202
H	0.777160800	1.606712736	-1.566410415
H	0.790734271	2.339122822	0.121918998
H	-1.404770965	3.415140665	-0.321402492
C	-1.524952036	2.670511111	-2.384969884
O	-2.433716017	3.378025446	-2.800241388
H	-1.031307538	1.937595436	-3.058306649
C	0.314587822	-0.867468266	0.603734772
C	0.688413677	-0.814038736	1.963863368
C	0.648961451	-1.962932576	-0.225098045
C	1.391992256	-1.908232446	2.487810160
C	1.352787854	-3.026255474	0.354098891
H	1.682990730	-1.893990184	3.536335589
H	1.614950662	-3.885012651	-0.260686028
C	1.724394438	-3.004592884	1.697447410
H	2.267110598	-3.843028356	2.127376903
N	-0.367051115	0.250883514	0.026053745

N	-1.618415260	0.335332462	-0.007697063
N	-2.455687745	1.074869062	-0.347218091
C	0.452925490	0.348843826	2.926558425
H	0.457508457	-0.108239883	3.922007106
C	-0.870797605	1.116472423	2.815148604
C	1.650552648	1.313725851	2.882037893
H	1.746069600	1.786322646	1.898311099
H	1.526163336	2.107061869	3.629005029
H	2.589141777	0.789933229	3.096568986
H	-1.024189587	1.684708208	3.739989812
H	-1.726999599	0.442695722	2.695172292
H	-0.870761484	1.835264783	1.989761516
C	0.349698475	-2.075588834	-1.718483579
H	0.486181321	-3.136964109	-1.950774477
C	1.395587248	-1.308678185	-2.542919497
C	-1.072753871	-1.715204482	-2.173846501
H	-1.262931801	-2.185050793	-3.145866310
H	-1.832702442	-2.080479613	-1.473354464
H	-1.208681243	-0.637345734	-2.308736142
H	1.325087803	-0.230050324	-2.368886737
H	1.234722504	-1.487433408	-3.612910153
H	2.413359562	-1.629201279	-2.292492141

Transition state TS in entry 6

E_{tot} [hartree] = -823.446635764857

C	-2.691793341	-1.066512383	1.636736455
C	-1.319256576	-1.245932629	1.759617993
H	-3.268132659	-1.787167940	1.059285240
H	-3.223124714	-0.550514365	2.430908777
H	-0.772304801	-0.747464710	2.554806616
C	-0.645195078	-2.364804711	1.080672640
O	0.465159623	-2.778931597	1.376567667
H	-1.239012065	-2.843883371	0.276464093
C	0.453403512	0.668815667	-0.587674492
C	0.788707527	0.191397469	-1.875387231
C	1.252148138	1.621744194	0.085528365
C	1.925893981	0.733061716	-2.492851249
C	2.381057682	2.118788159	-0.576861023
H	2.195530976	0.391161837	-3.490329967
H	3.004702317	2.857804934	-0.077792945
C	2.717614409	1.685942453	-1.857858169
H	3.594749473	2.089256661	-2.358242563
N	-0.692942289	0.144936846	0.087831844
N	-1.844723323	0.610880372	-0.084495899
N	-2.929585190	0.413305996	0.341765332
C	0.079261525	-0.916055992	-2.654805667
H	0.310102993	-0.712996690	-3.706475810
C	-1.449141305	-1.005629934	-2.567036404
C	0.721782712	-2.273102966	-2.318102817

H	0.587219169	-2.523012154	-1.260878429
H	0.263209410	-3.068181654	-2.918523896
H	1.797717933	-2.264462408	-2.527448723
H	-1.807840440	-1.643849428	-3.383342103
H	-1.926532548	-0.025760882	-2.680332137
H	-1.789470866	-1.456771613	-1.629854394
C	1.026136136	2.115314838	1.511069922
H	1.613090632	3.036475001	1.589592558
C	1.635729237	1.115588532	2.508620989
C	-0.412171260	2.488734492	1.901505623
H	-0.381673172	3.162187062	2.766195190
H	-0.931896852	3.013082837	1.090822738
H	-1.008475750	1.618378510	2.190240011
H	1.183045706	0.124331612	2.402365526
H	1.477948995	1.458222352	3.538400455
H	2.714312187	1.007577972	2.345300461

Transition state TS in entry 7

E_{tot} [hartree] = -823.458410608683

C	-0.089513382	0.789372133	2.748584907
C	-0.555219700	1.346643577	1.542556027
C	0.087583545	1.546434324	3.923377508
C	-0.785431837	2.727856440	1.518555019
C	-0.172482638	2.918477414	3.855147613
H	-1.126599262	3.201395050	0.601568167
H	-0.043721677	3.540079835	4.736792467
C	-0.595541481	3.505371014	2.660665732
H	-0.786679349	4.575329933	2.622843112
C	-0.762904024	0.498719351	0.298611118
H	-0.885655178	-0.544623192	0.604391232
C	0.472129247	0.581135176	-0.614946452
C	-2.033540007	0.875214491	-0.473223609
H	-2.916123788	0.855593985	0.176710367
H	-1.961276127	1.870583139	-0.926179217
H	-2.195440913	0.156098075	-1.284325902
H	0.603966736	1.600974532	-0.997394361
H	0.354199327	-0.092864011	-1.471694577
H	1.389798370	0.298944174	-0.085464228
C	0.607624714	0.889319078	5.192569075
H	0.212873132	-0.131678672	5.221997235
C	0.164564870	1.596053041	6.477173012
C	2.141847210	0.789953908	5.132303566
H	2.475182637	0.234920394	4.247720675
H	2.592346799	1.789525008	5.095270541
H	2.525901672	0.274727086	6.020721627
H	0.636402339	2.579282005	6.589474070
H	0.456810373	0.993930418	7.345169950
H	-0.922736877	1.731712589	6.510828399
N	1.316079924	-2.276841254	1.808139494

N	1.011813893	-1.223125475	2.192874068
C	-0.769184037	-3.168609730	2.572726444
C	-1.317053386	-2.004102598	3.096582954
N	0.130314977	-0.620186399	2.856991557
H	-2.048805796	-1.453602261	2.508573148
H	-1.365932909	-1.869790409	4.173194179
H	-0.264575349	-3.881614340	3.219643473
C	-1.010774377	-3.574961734	1.187853489
O	-1.633761867	-2.927658316	0.349999346
H	-0.572500597	-4.555277412	0.914695937

Transition state TS in entry 8

E_{tot} [hartree] = -823.454726714148

C	-2.453432940	-0.534977532	2.321684667
C	-1.210444778	-1.101445853	2.087642192
H	-2.588618494	0.099064028	3.194988107
H	-3.343470146	-1.007307338	1.916646067
H	-1.103028129	-1.964481783	1.435110639
C	-0.028712055	-0.747597132	2.906864737
O	-0.029424585	0.059991999	3.823108718
H	0.898987872	-1.286738470	2.632425237
C	0.324608672	-0.048065031	-0.639284882
C	1.681600679	0.337570057	-0.641662258
C	-0.245094094	-0.822392758	-1.670542986
C	2.462849083	-0.033187932	-1.740102080
C	0.572406219	-1.154697905	-2.757468018
H	3.510360984	0.252293528	-1.783640086
H	0.163008165	-1.730449226	-3.583683328
C	1.910966472	-0.764874259	-2.793546944
H	2.530468451	-1.038320664	-3.644618699
N	-0.431286606	0.299600051	0.519290152
N	-1.498918343	0.965601228	0.454189815
N	-2.518626400	1.062337105	1.033142363
C	2.241083158	1.196050765	0.481700525
H	1.704814468	0.935218983	1.398999615
C	1.949665274	2.677535213	0.187285020
C	3.734380430	0.974871086	0.739321567
H	3.961127048	-0.085741817	0.899112814
H	4.354958776	1.338843703	-0.087754783
H	4.035411832	1.523485722	1.639213010
H	2.463705811	2.997763127	-0.727562432
H	2.297644547	3.308408583	1.013924530
H	0.875646381	2.853480161	0.054277254
C	-1.702806924	-1.255595663	-1.643349196
H	-2.064471595	-1.191863633	-0.613846921
C	-1.898290932	-2.712362910	-2.082711741
C	-2.553868566	-0.304715217	-2.501300286
H	-2.448465528	0.736656342	-2.174871357
H	-2.251706277	-0.361067001	-3.554320903

H	-3.614083965	-0.576064583	-2.434569806
H	-1.665964405	-2.859213876	-3.143412683
H	-2.944598200	-3.002415588	-1.932391282
H	-1.270392896	-3.391819128	-1.494853551

Transition state TS in entry 9

E_{tot} [hartree] = -823.455569048449

C	0.331415986	0.644055076	-2.139836112
C	-0.322364424	1.832798182	-2.441721754
H	1.417510837	0.630499349	-2.127584412
H	-0.156765100	-0.305612415	-2.344436407
H	-1.357787608	1.829433839	-2.771078253
C	0.409425009	3.098873051	-2.536116977
O	1.594450492	3.257435114	-2.258580376
H	-0.198764806	3.958506242	-2.881562587
C	0.126324770	-0.753033939	0.571884225
C	1.265541810	-1.165416650	1.292261942
C	-1.024555860	-1.556791065	0.447456465
C	1.218732513	-2.422529076	1.905360939
C	-1.026161861	-2.800066779	1.092039992
H	2.080205256	-2.768235886	2.472784166
H	-1.904868567	-3.437361051	1.032297741
C	0.086291403	-3.233273124	1.810478917
H	0.071935841	-4.203988166	2.300243012
N	0.191673383	0.495542675	-0.127785147
N	-0.546691664	1.469362071	0.180757696
N	-1.021594094	2.435254130	-0.257435877
C	2.511258720	-0.310878665	1.490162843
H	3.208801388	-0.940448191	2.053071953
C	2.224687671	0.922705213	2.361393942
C	3.220623211	0.086817592	0.187677012
H	3.336533990	-0.771011718	-0.484823158
H	2.676289024	0.873639793	-0.342629084
H	4.220767447	0.472453804	0.418593738
H	3.163270252	1.439635051	2.594555673
H	1.750321155	0.638787955	3.307815192
H	1.570041149	1.636426407	1.849715497
C	-2.263054858	-1.087122752	-0.301263402
H	-1.994105010	-0.239143942	-0.936240555
C	-3.320828237	-0.596262652	0.700698258
C	-2.839340513	-2.162686513	-1.230724641
H	-3.662214469	-1.737809969	-1.816980729
H	-2.080812502	-2.534247231	-1.929454197
H	-3.239800575	-3.016890757	-0.673239903
H	-3.647514231	-1.415069906	1.353371919
H	-4.199540994	-0.211607779	0.169725713
H	-2.927603386	0.206390776	1.335936224

Transition state TS in entry 10

E_{tot} [hartree] = -823.451227444665

C	-2.624881384	-1.613758398	1.208050095
C	-1.397655959	-2.071719864	0.747161816
H	-3.523778163	-1.889180588	0.660715118
H	-2.750042120	-1.389453199	2.263376803
H	-0.533552654	-2.119139458	1.404725464
C	-1.280612426	-2.798846734	-0.533366989
O	-2.204738554	-3.004519995	-1.306182727
H	-0.263842208	-3.173587240	-0.758464036
C	0.662087828	0.394208348	-0.145464918
C	1.396527318	0.569204984	-1.339355555
C	1.216388843	0.670574359	1.122975465
C	2.715675385	1.022729896	-1.227524730
C	2.537194664	1.133426988	1.177504701
H	3.301934728	1.171680336	-2.131817280
H	2.986168149	1.366771536	2.139688515
C	3.286372964	1.299741057	0.015449315
H	4.312845038	1.653771436	0.075375984
N	-0.655298858	-0.159624806	-0.244598281
N	-1.664848616	0.498637419	0.130402390
N	-2.716623793	0.306386948	0.622814281
C	0.832484907	0.364334978	-2.740257520
H	1.655660351	0.608486111	-3.420471517
C	-0.306726290	1.344807117	-3.059750572
C	0.417902089	-1.080915880	-3.047835828
H	1.194702822	-1.794635931	-2.749848651
H	-0.512211000	-1.346271008	-2.539913191
H	0.252042203	-1.193697178	-4.126028920
H	-0.568737302	1.272818505	-4.122254388
H	-0.010450556	2.380102945	-2.854319903
H	-1.208008683	1.124507618	-2.478289524
C	0.418991414	0.541657487	2.412757660
H	-0.486353840	-0.033934398	2.216616791
C	-0.021619612	1.932931864	2.897190993
C	1.183292191	-0.209585739	3.510930351
H	0.524861598	-0.371151235	4.372242553
H	1.527710116	-1.188587393	3.157795199
H	2.055576948	0.352311101	3.863593700
H	0.848133769	2.557375539	3.135533811
H	-0.634391677	1.843403123	3.801991521
H	-0.613307075	2.451837929	2.133789444

Transition state TS in entry 11

E_{tot} [hartree] = -823.449097801334

C	0.236650120	2.046414471	-0.735193554
C	-0.954595346	2.688468410	-1.052590597
H	0.818425349	1.589861769	-1.529860207
H	0.786115851	2.352398358	0.148890948

H	-1.387028955	3.416191518	-0.371308448
C	-1.534276733	2.598679579	-2.395453612
O	-1.120203799	1.886811665	-3.305609311
H	-2.424154490	3.239231191	-2.555483652
C	0.308648066	-0.856835190	0.603497365
C	0.685844050	-0.808789580	1.963029206
C	0.638057519	-1.950553941	-0.229600279
C	1.388793881	-1.905899024	2.481504480
C	1.341303837	-3.016964456	0.344909170
H	1.682555768	-1.895530870	3.529282452
H	1.600115798	-3.874078330	-0.273584701
C	1.717101540	-3.000364617	1.686880655
H	2.259714946	-3.841090268	2.112426002
N	-0.372454391	0.267326721	0.033294686
N	-1.623021519	0.352441673	-0.000037288
N	-2.476402974	1.075848899	-0.326434437
C	0.454253801	0.349907984	2.931665468
H	0.458321887	-0.112433064	3.924664466
C	-0.867757438	1.121083303	2.825573111
C	1.654680096	1.311487845	2.891605511
H	1.751497809	1.788701173	1.910314916
H	1.533058121	2.101292273	3.642751701
H	2.591696407	0.783658278	3.103090571
H	-1.017796783	1.686508483	3.752671637
H	-1.725696631	0.449457669	2.705557139
H	-0.867915411	1.842536726	2.002486920
C	0.338801753	-2.059852815	-1.722976940
H	0.456468945	-3.123880016	-1.953808260
C	1.404302105	-1.314001100	-2.542099092
C	-1.074589242	-1.671843682	-2.182696551
H	-1.267515277	-2.134455946	-3.157852749
H	-1.843537046	-2.029544130	-1.488002654
H	-1.187540938	-0.591439294	-2.315238824
H	1.366887223	-0.235952645	-2.355654187
H	1.236120641	-1.476096163	-3.613630446
H	2.412224917	-1.668206580	-2.297072325

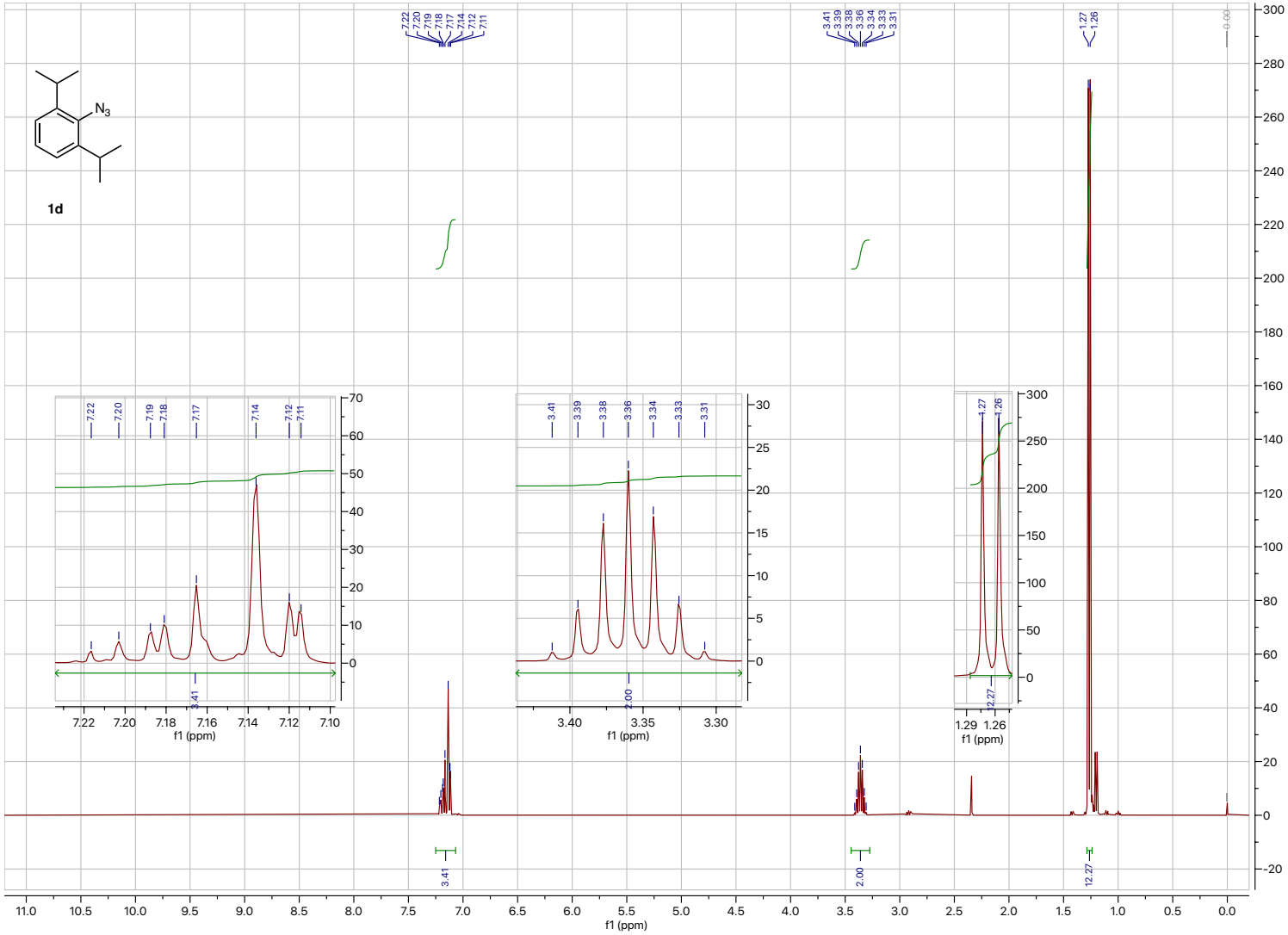
Transition state TS in entry 12

E_{tot} [hartree] = -823.444260897137

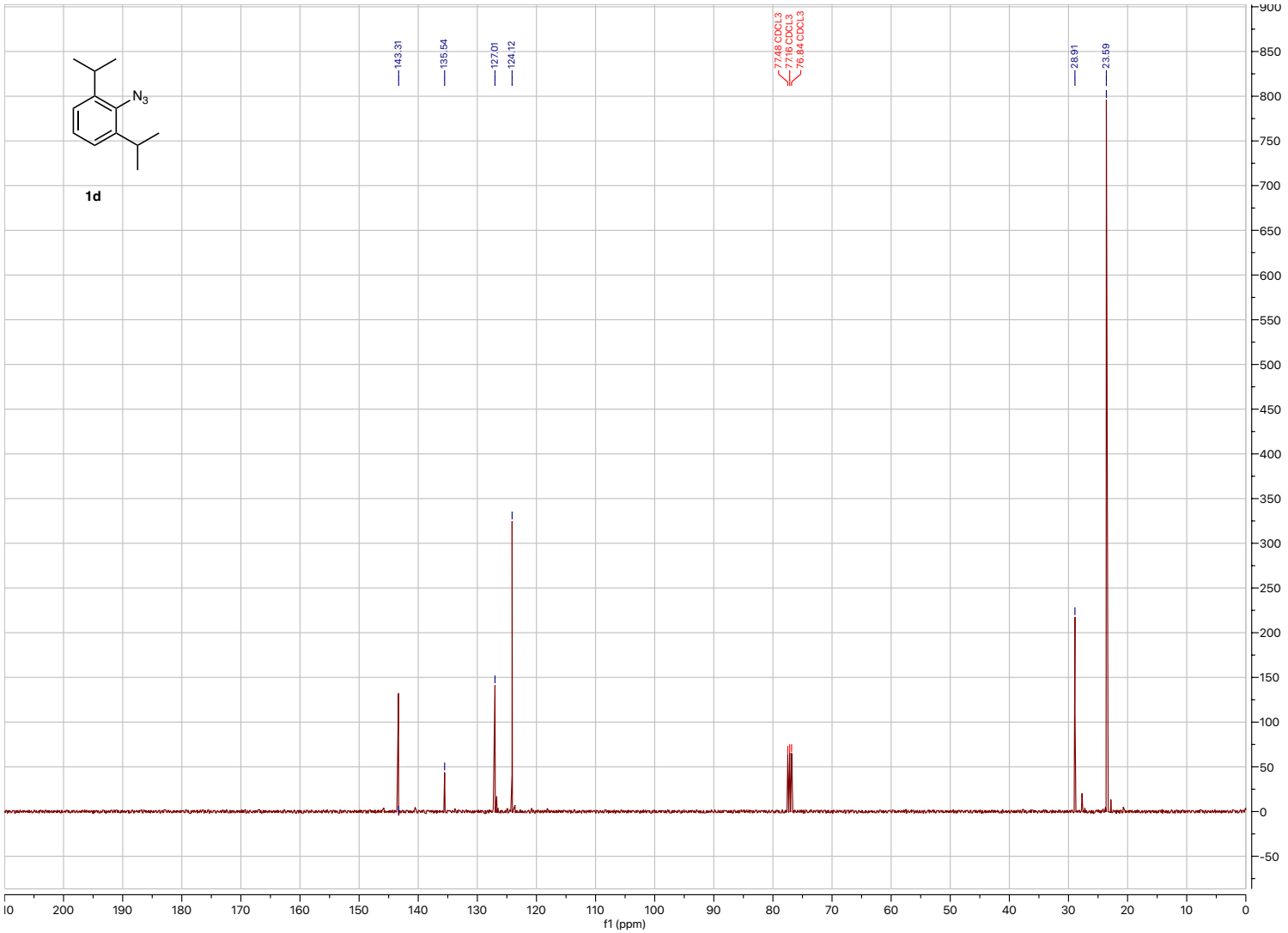
C	-2.670094992	-0.962721828	1.778244791
C	-1.314983421	-1.264689134	1.783650503
H	-3.360024074	-1.675843890	1.333153215
H	-3.068428374	-0.326409793	2.563092568
H	-0.644145382	-0.771764778	2.479283501
C	-0.773708510	-2.439200865	1.067067292
O	-1.447926221	-3.238534769	0.435314062
H	0.322049713	-2.568527211	1.156307916
C	0.468456285	0.651784885	-0.606379157
C	0.814320396	0.199350395	-1.899989188

C	1.239208752	1.621656167	0.074167623
C	1.932548975	0.779385118	-2.516089027
C	2.351616977	2.158127289	-0.586981807
H	2.208479549	0.456062842	-3.518033255
H	2.954237774	2.910571539	-0.082137548
C	2.697685386	1.749049349	-1.872964266
H	3.561269257	2.183525521	-2.370903938
N	-0.665496068	0.081959650	0.058609891
N	-1.821227333	0.544419827	-0.102584106
N	-2.897649274	0.371849194	0.356258718
C	0.129628678	-0.922001690	-2.679685470
H	0.394530654	-0.740410144	-3.727120271
C	-1.402095664	-1.000367534	-2.636762100
C	0.756657545	-2.271937484	-2.291018825
H	0.581051013	-2.496809404	-1.234296676
H	0.315704108	-3.080753247	-2.886290237
H	1.839007988	-2.269323180	-2.465163698
H	-1.742921619	-1.637719461	-3.461481769
H	-1.866819861	-0.016086936	-2.765292389
H	-1.772662796	-1.446462320	-1.708961743
C	1.004711487	2.097002861	1.504632486
H	1.573187762	3.028873425	1.591464298
C	1.634768703	1.103144888	2.495203723
C	-0.440528908	2.439969573	1.896481625
H	-0.424506207	3.102206947	2.770155440
H	-0.967599449	2.964941350	1.091055555
H	-1.021383175	1.555727733	2.173136143
H	1.201314430	0.103486504	2.385052197
H	1.471692185	1.436154416	3.527307880
H	2.715106732	1.017547040	2.330073399

^{1}H-NMR in $CDCl_3$



^{13}C-NMR in $CDCl_3$



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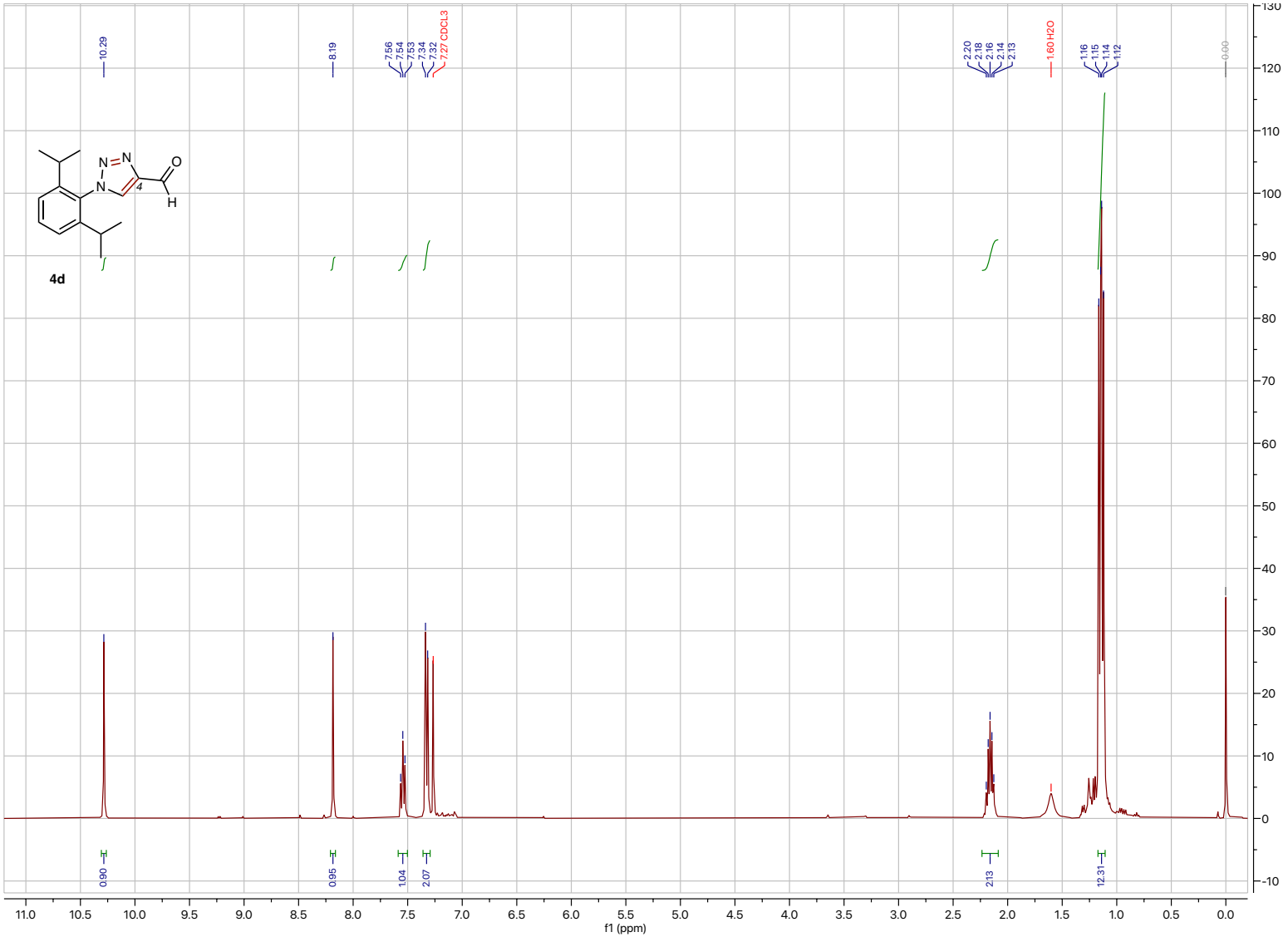

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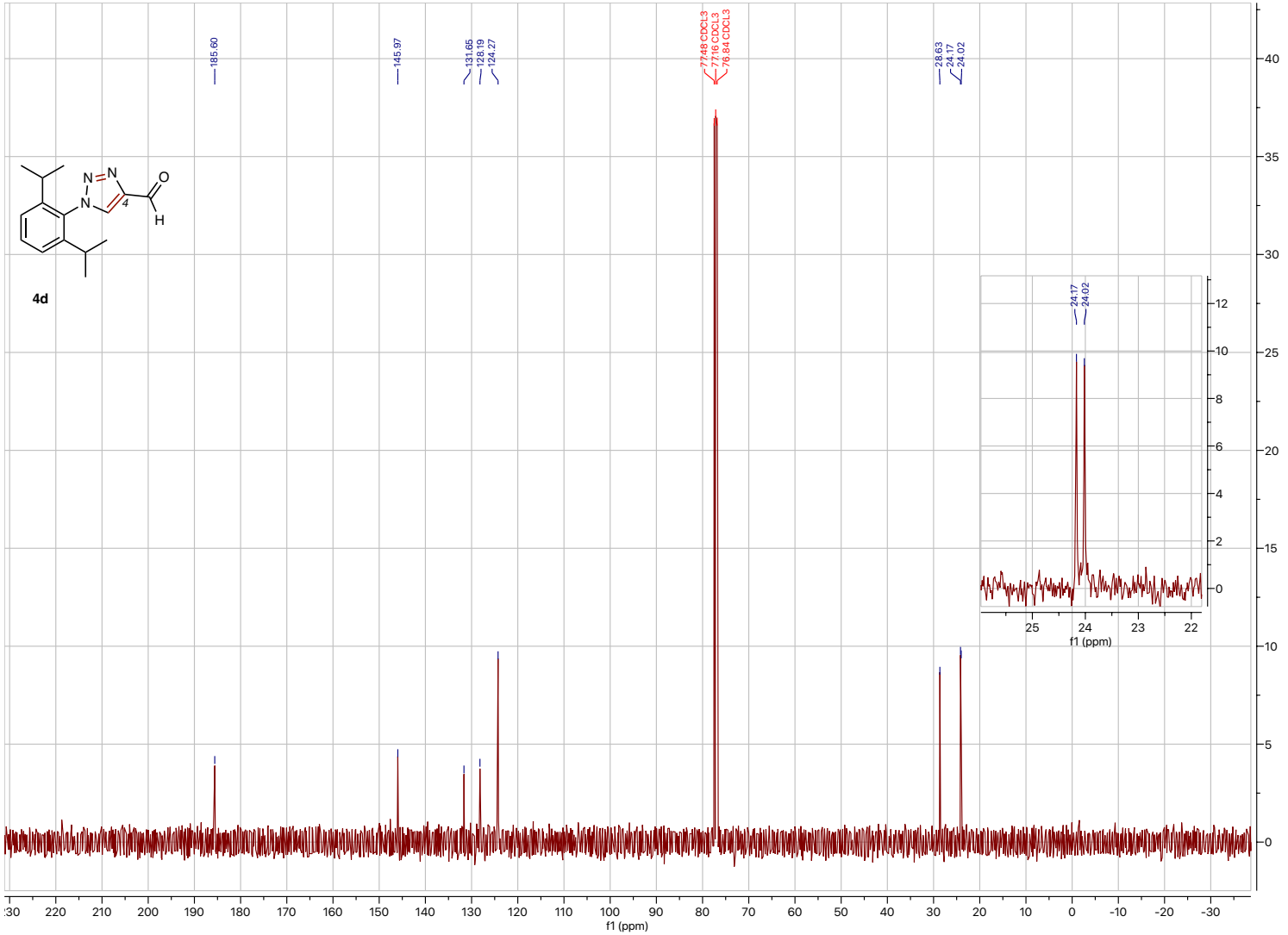

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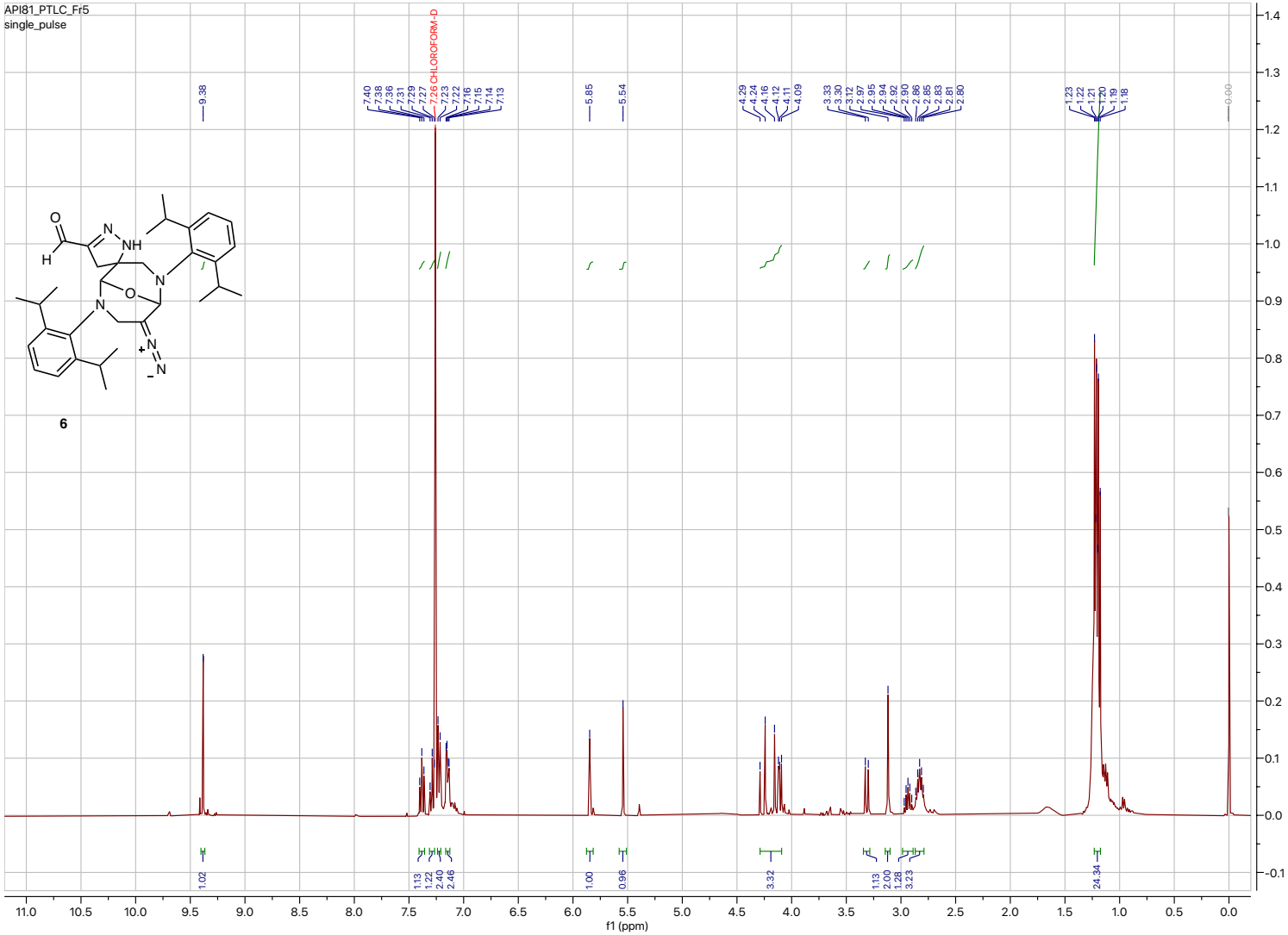
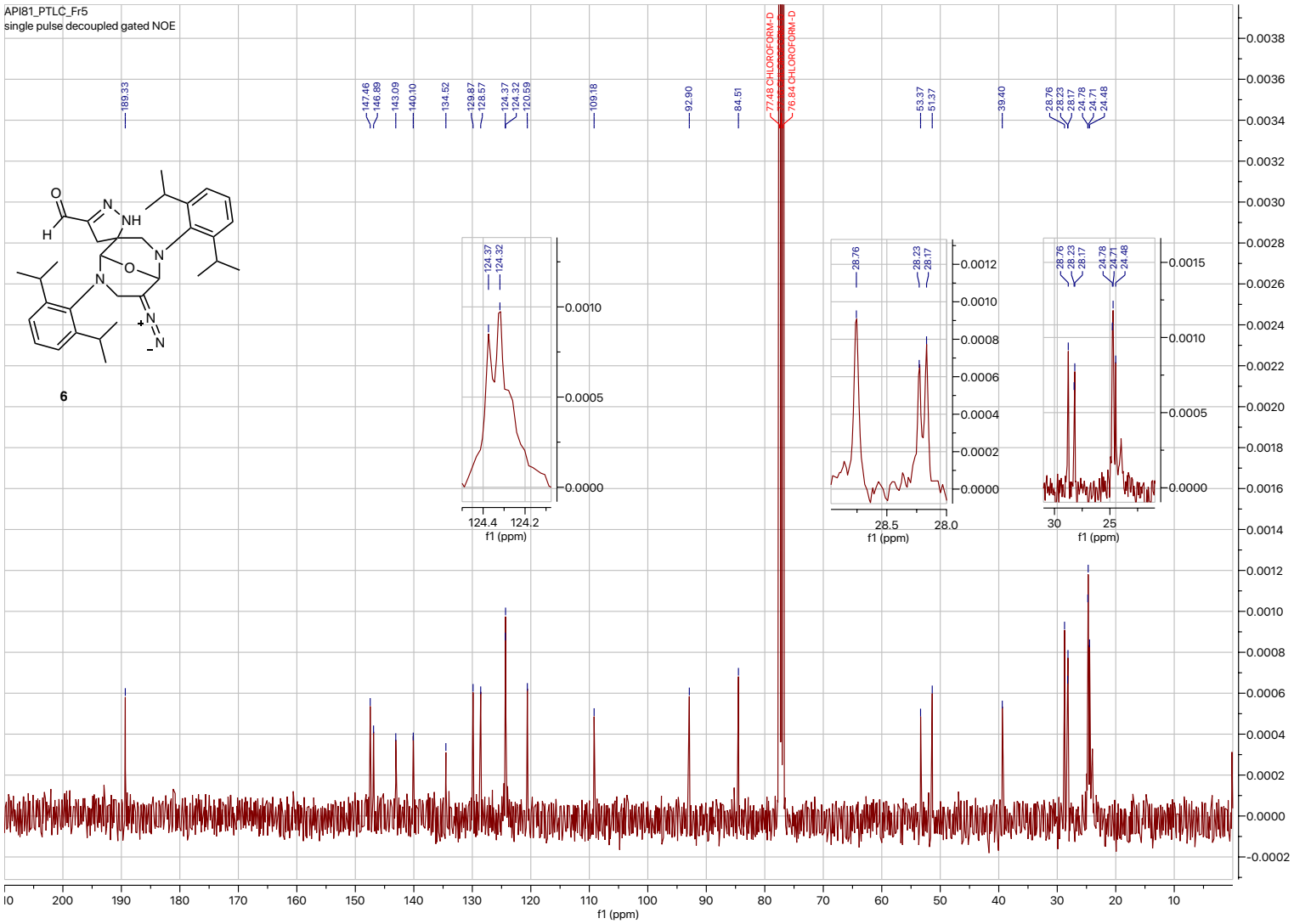
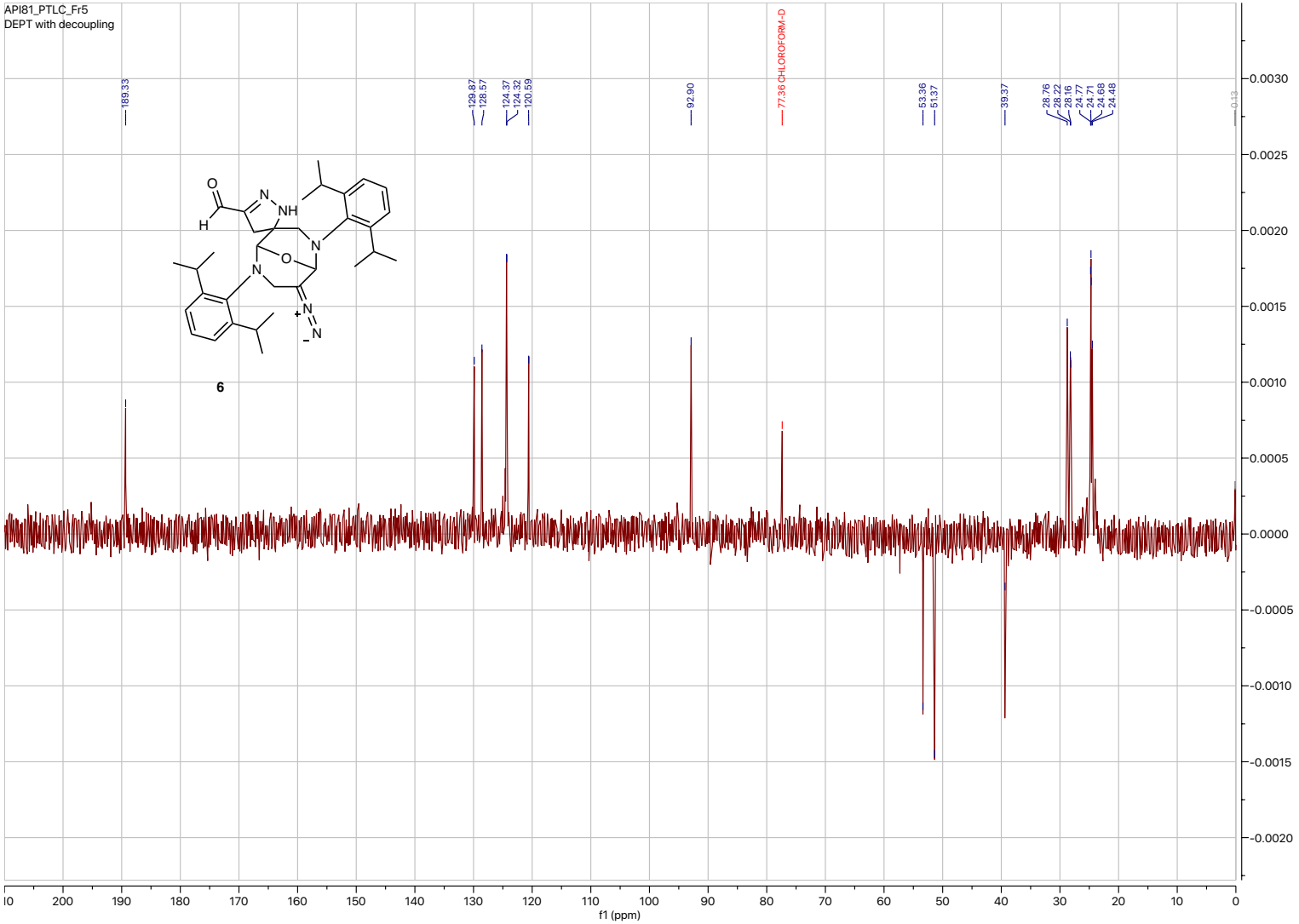


6

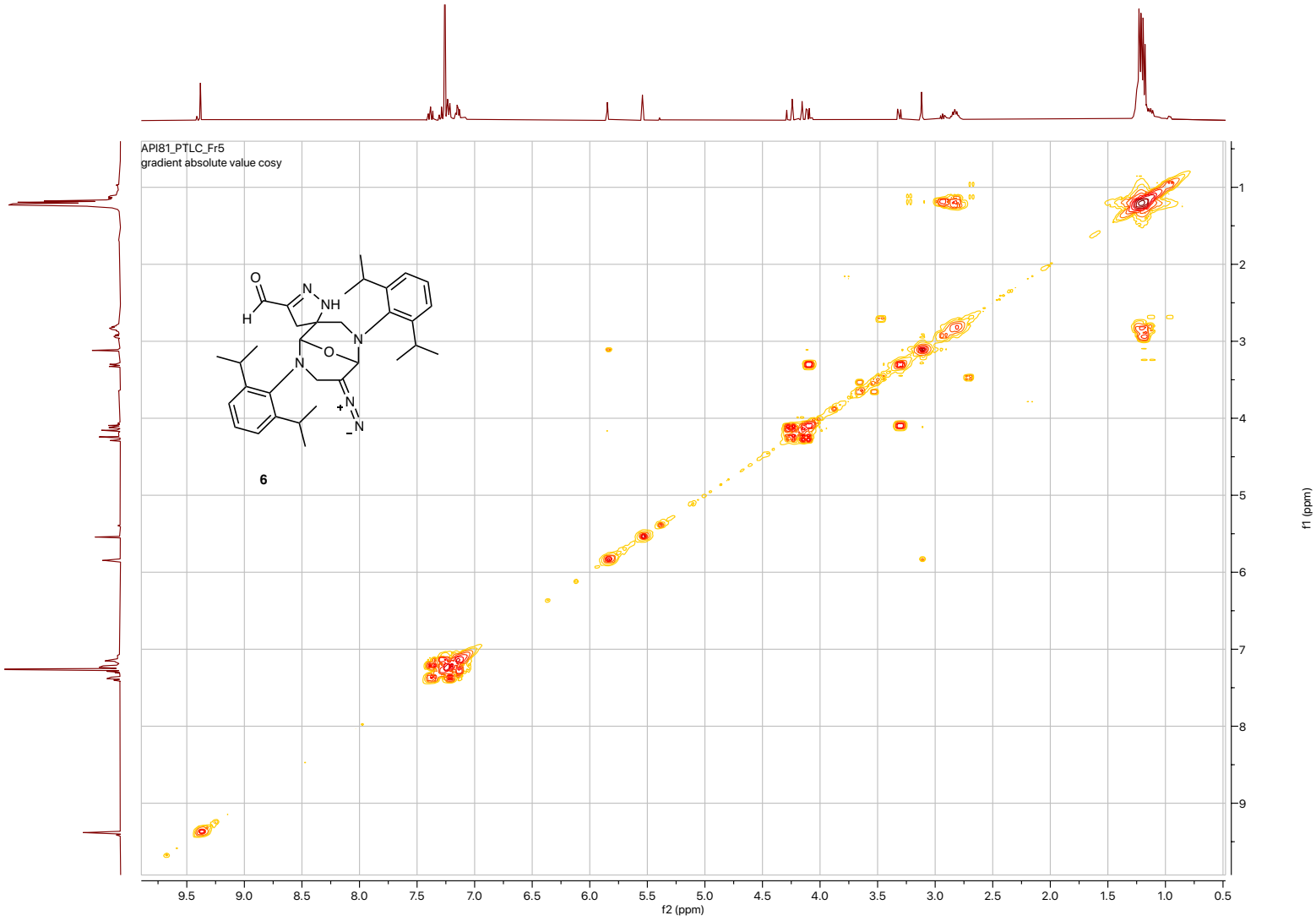
^{13}C-NMR in $CDCl_3$



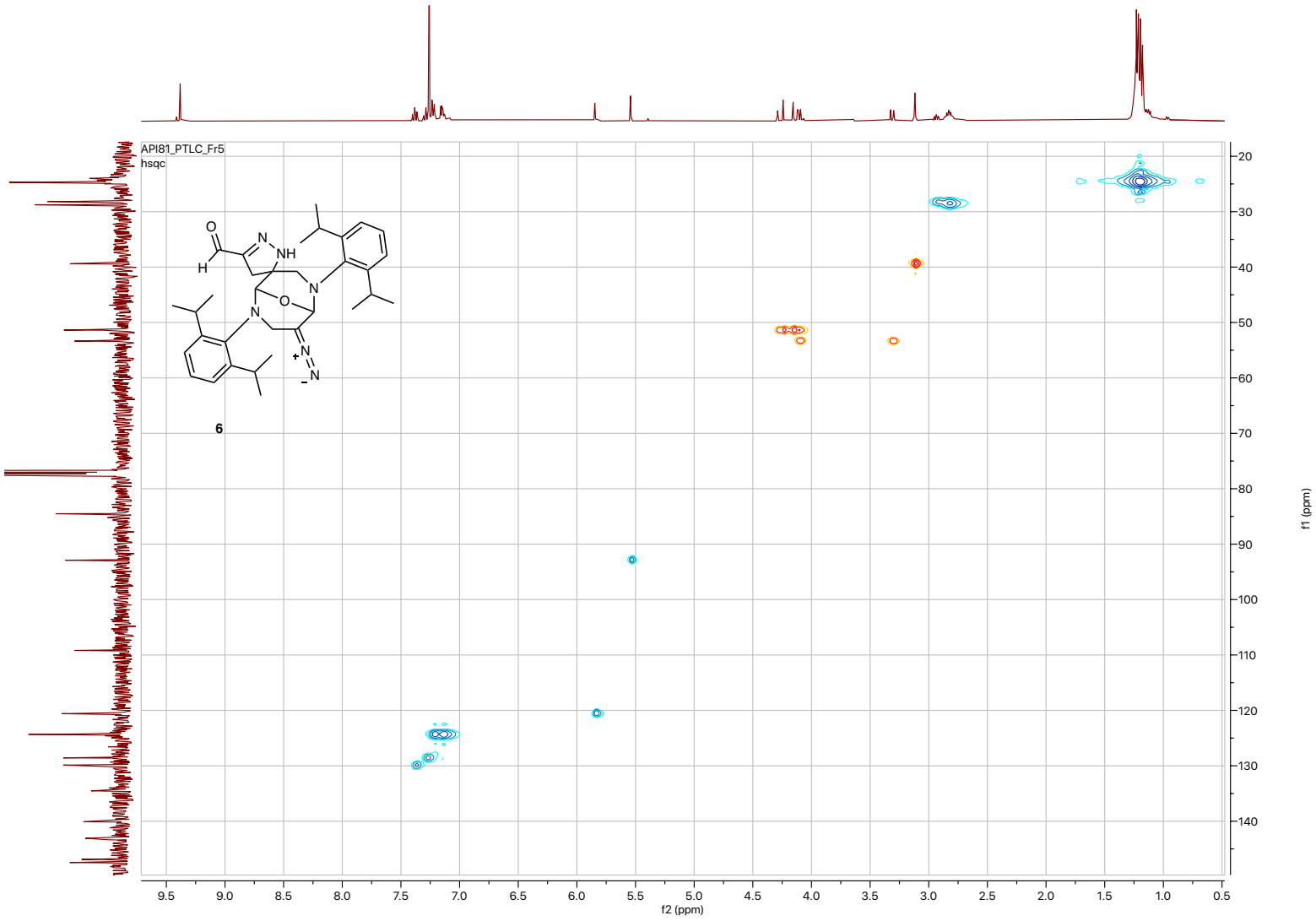
DEPT 135 spectrum



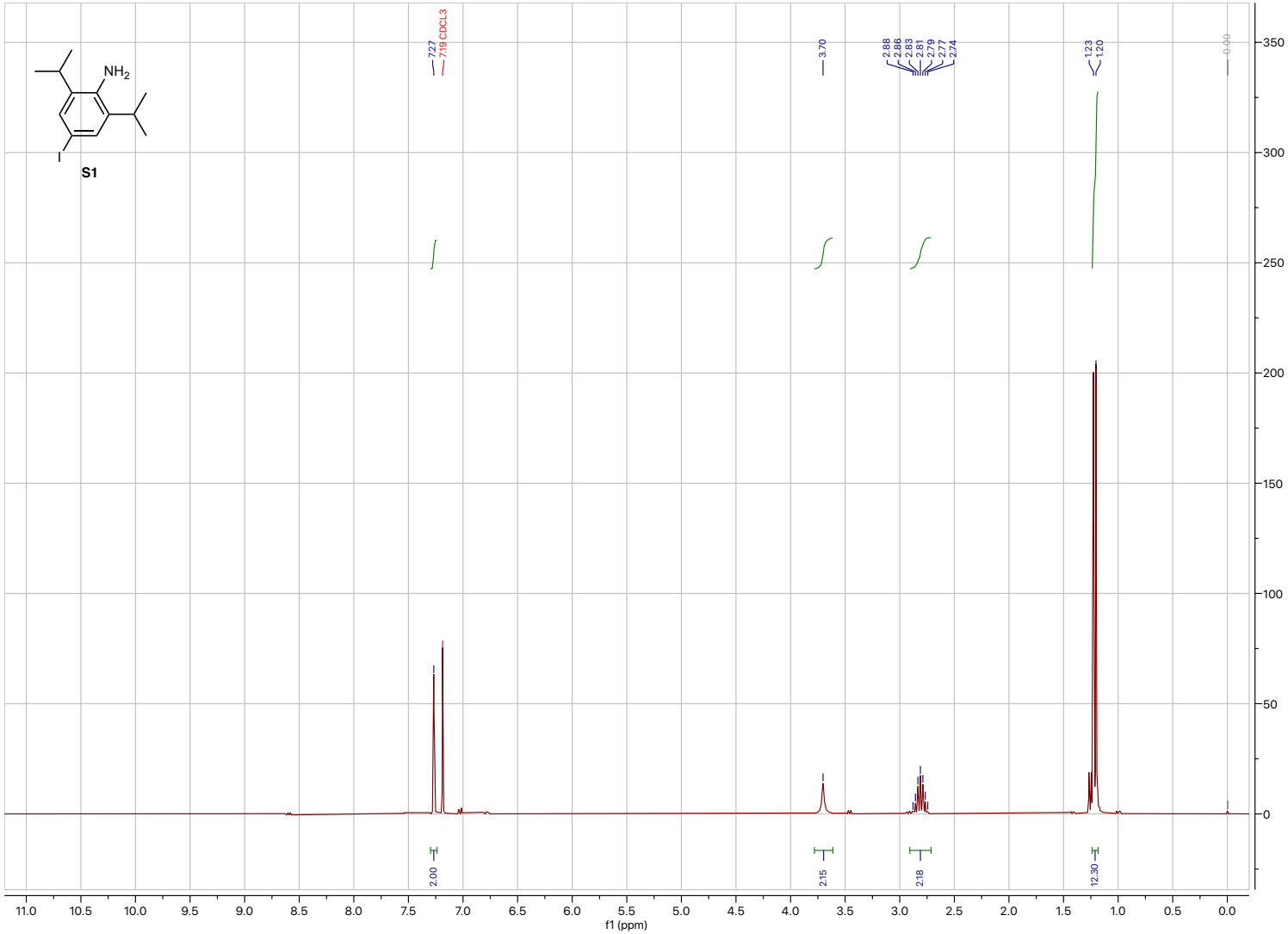
COSY spectrum



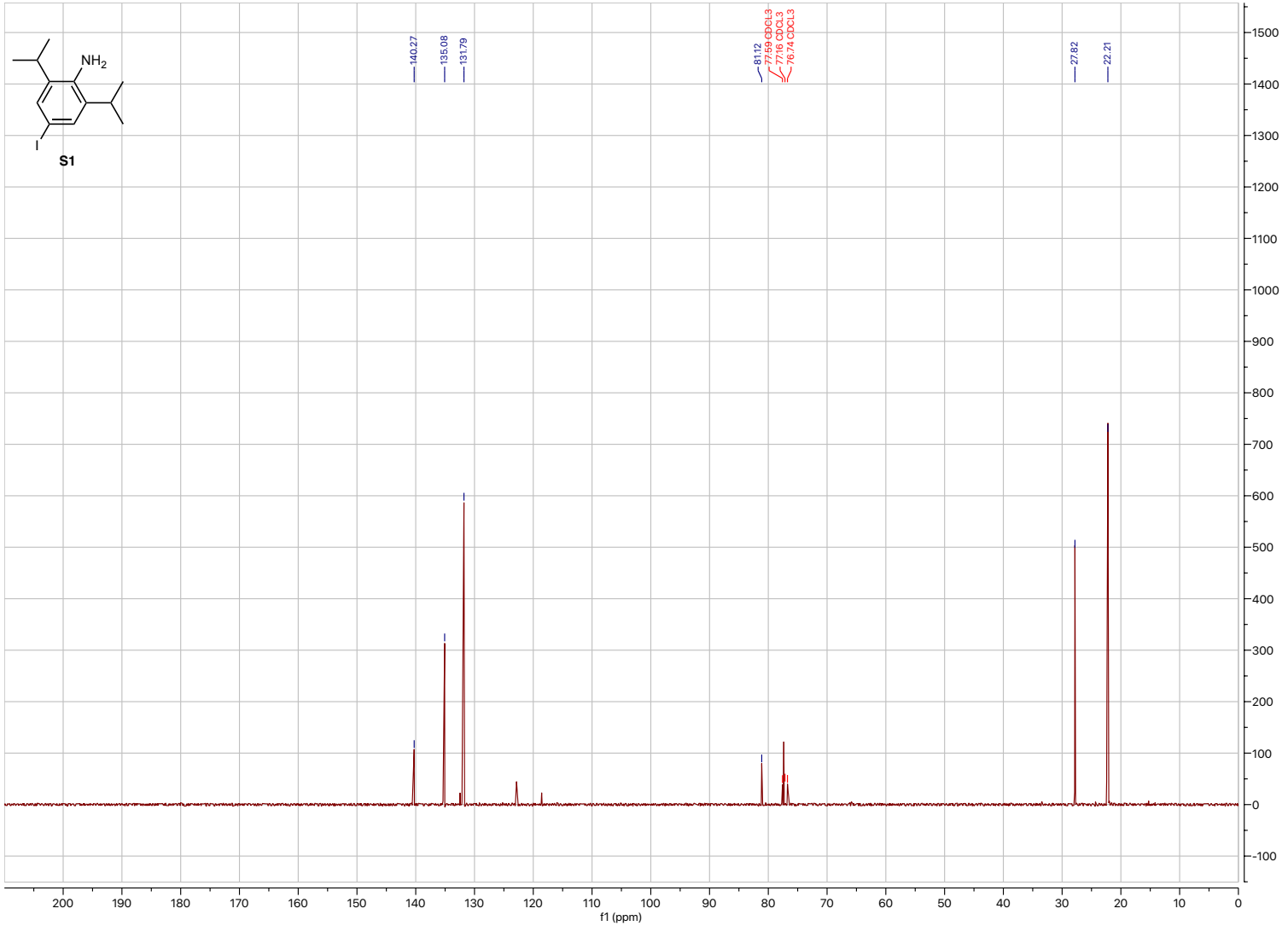
HSQC spectrum



^{1}H-NMR in $CDCl_3$



^{13}C-NMR in $CDCl_3$



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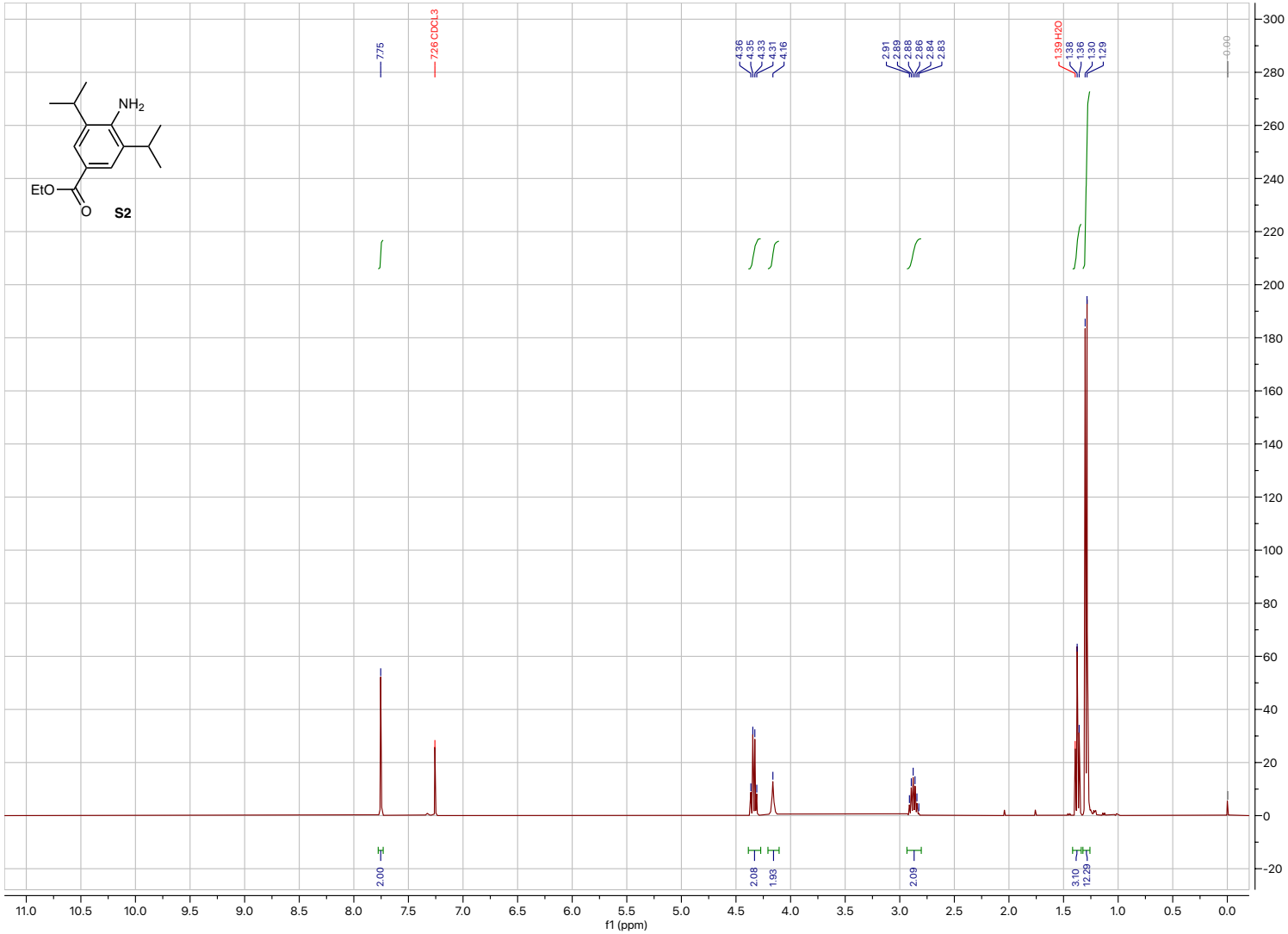

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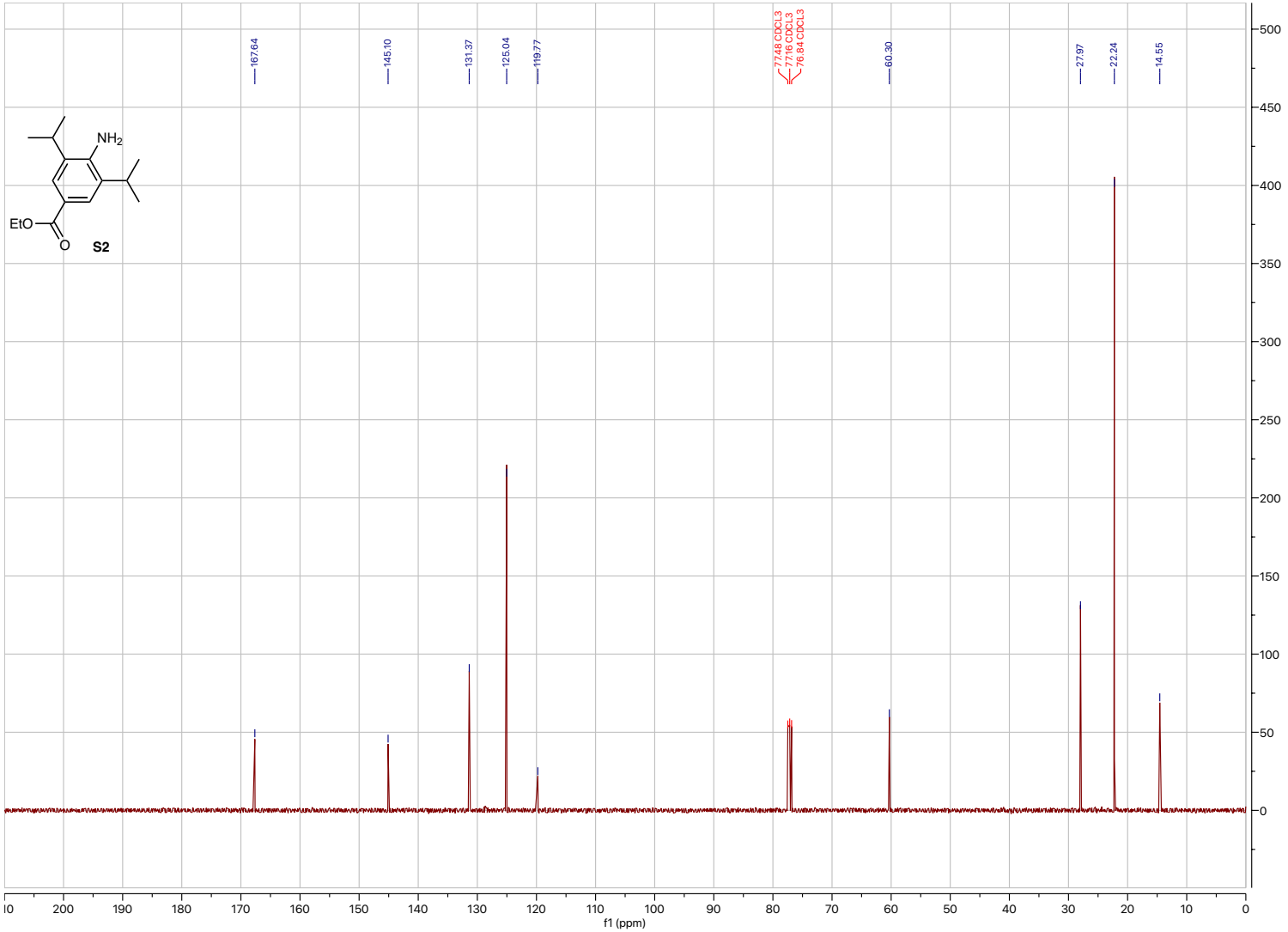

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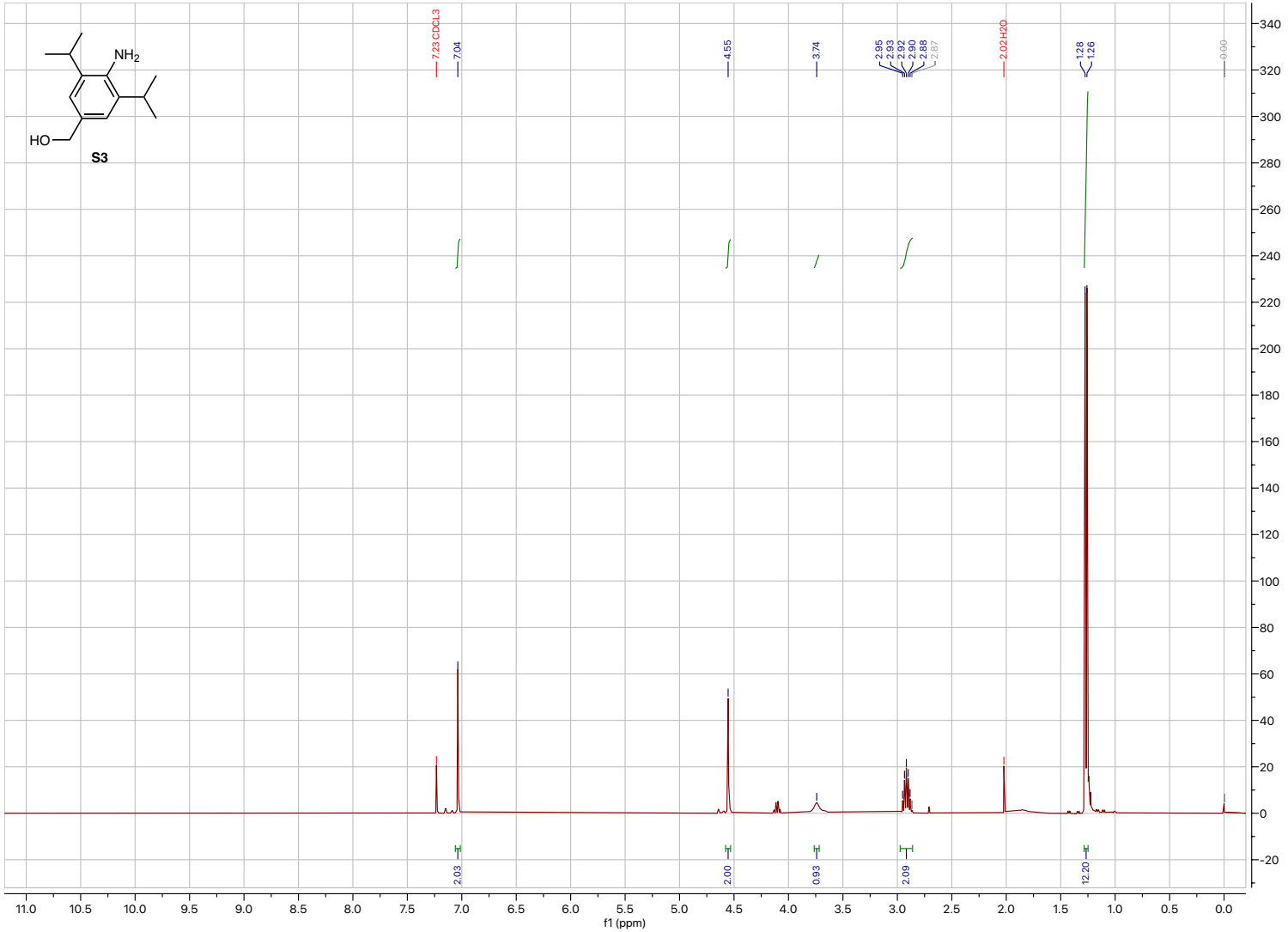

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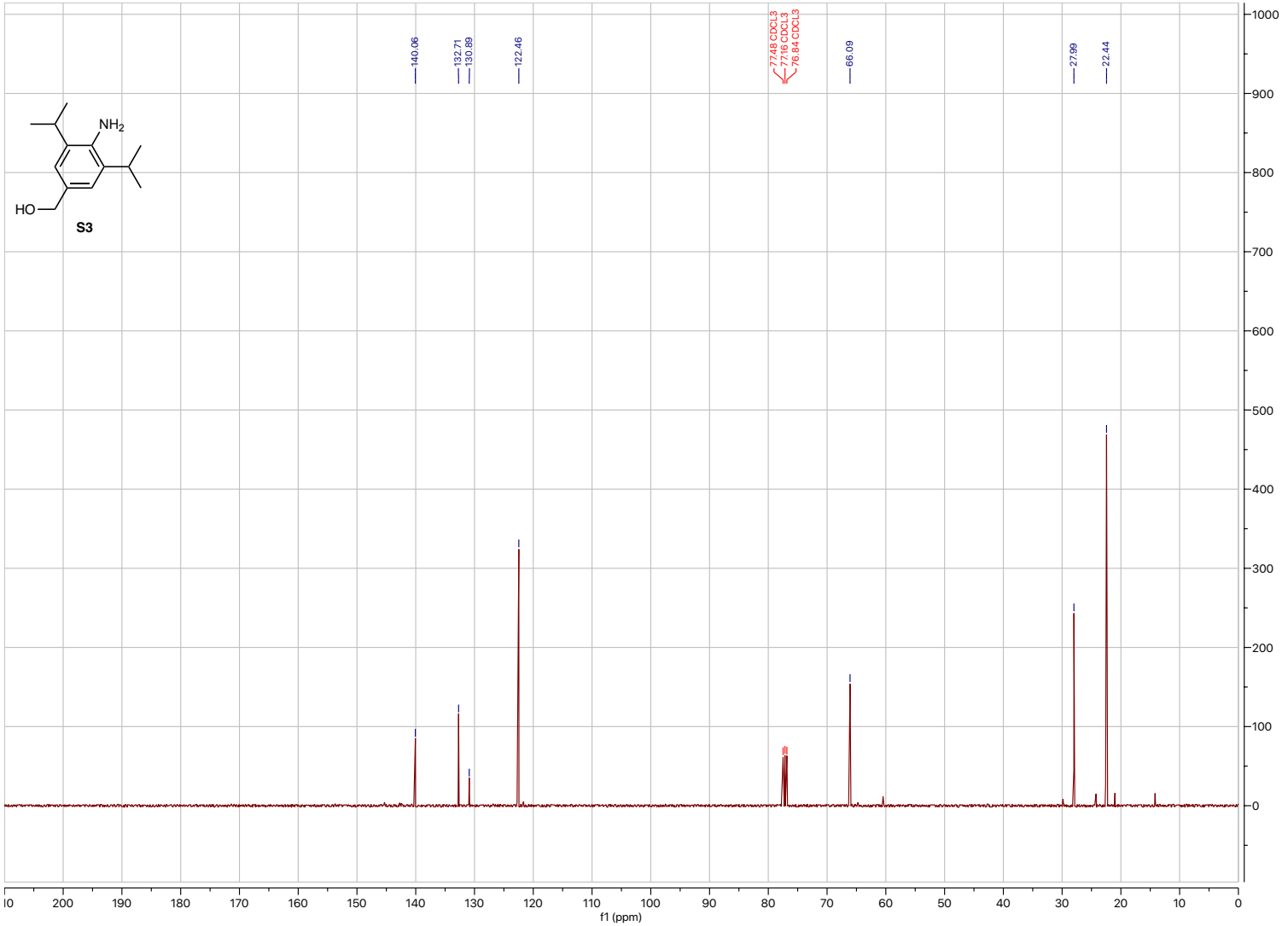

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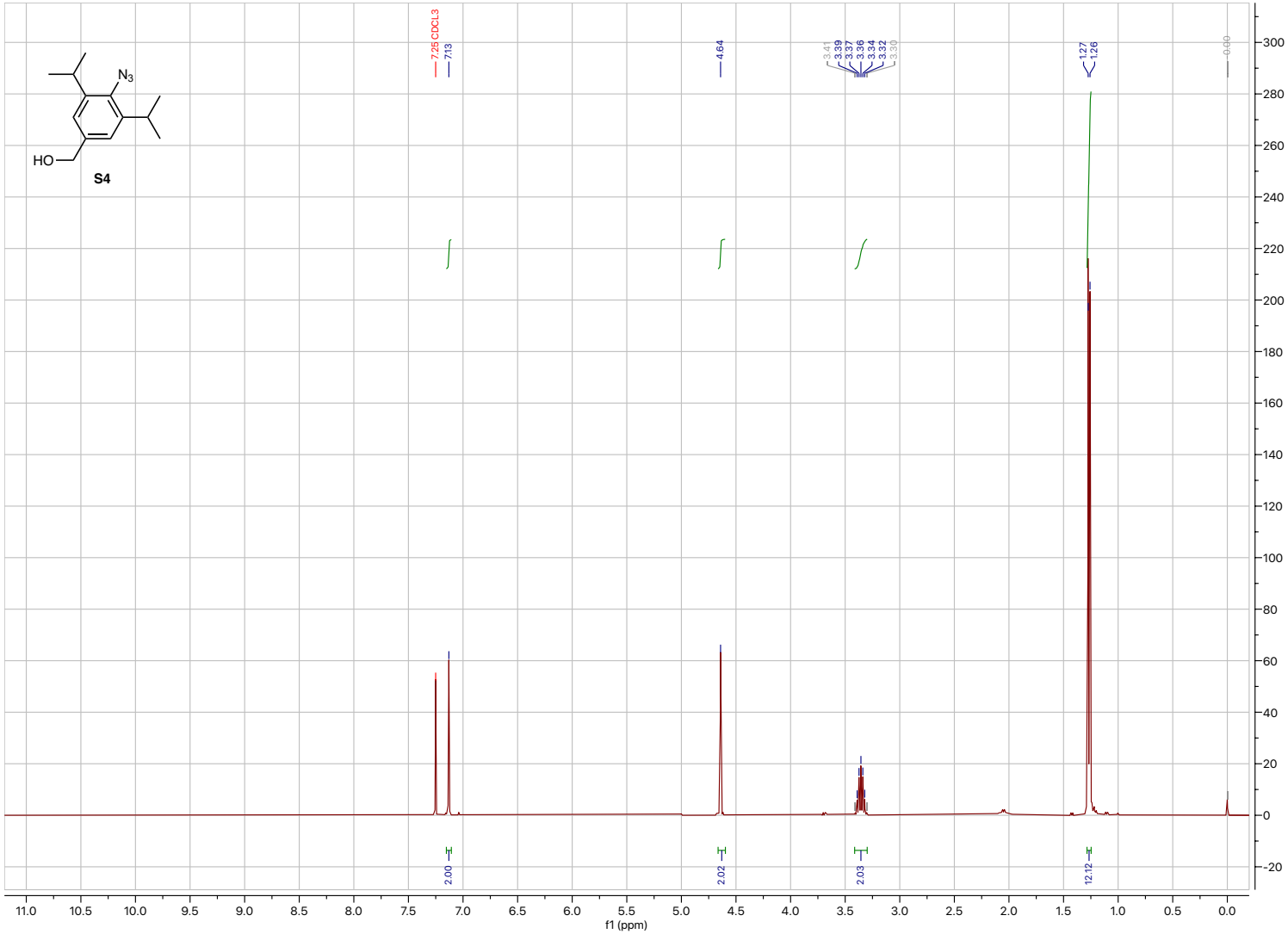

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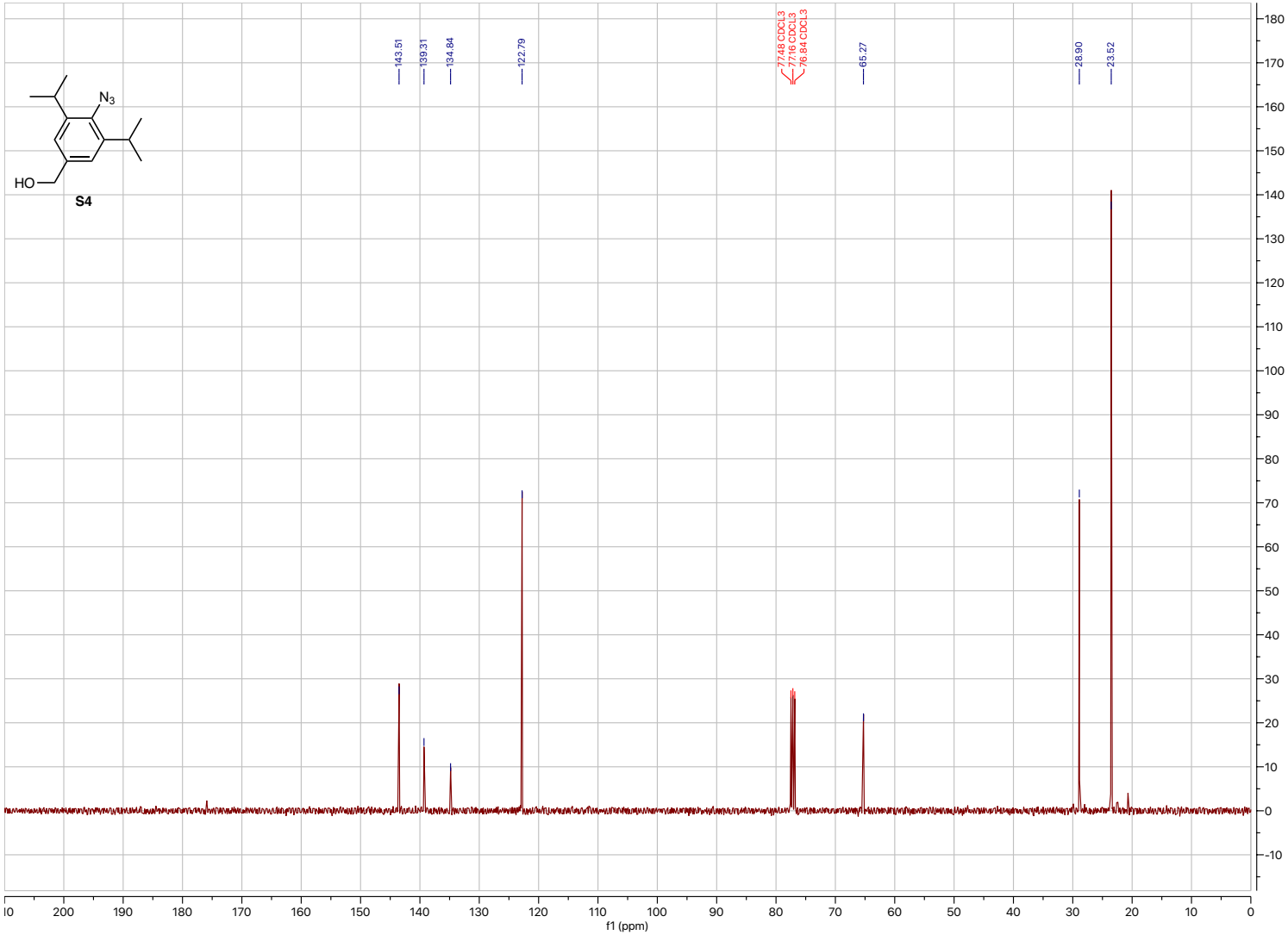

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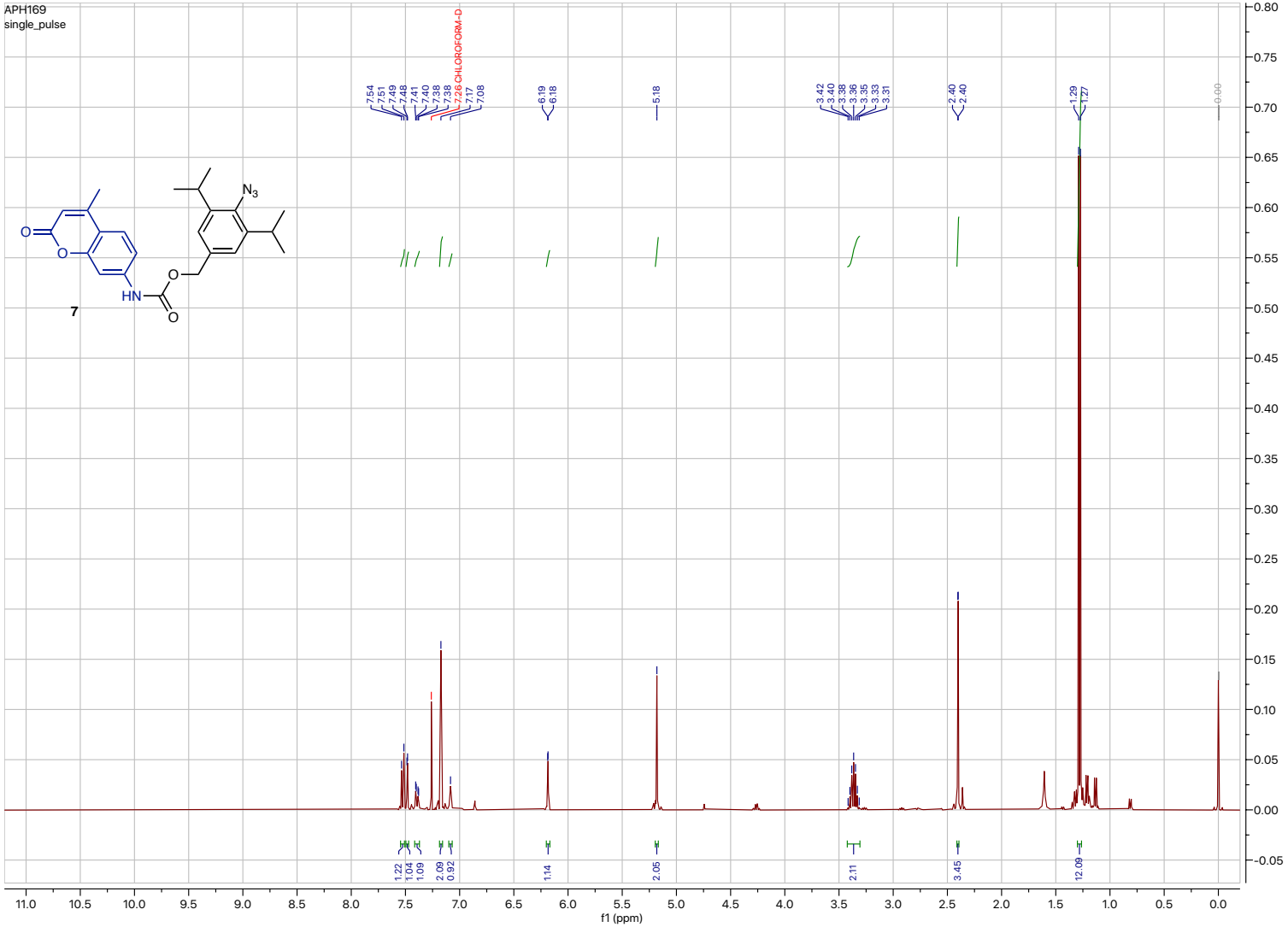

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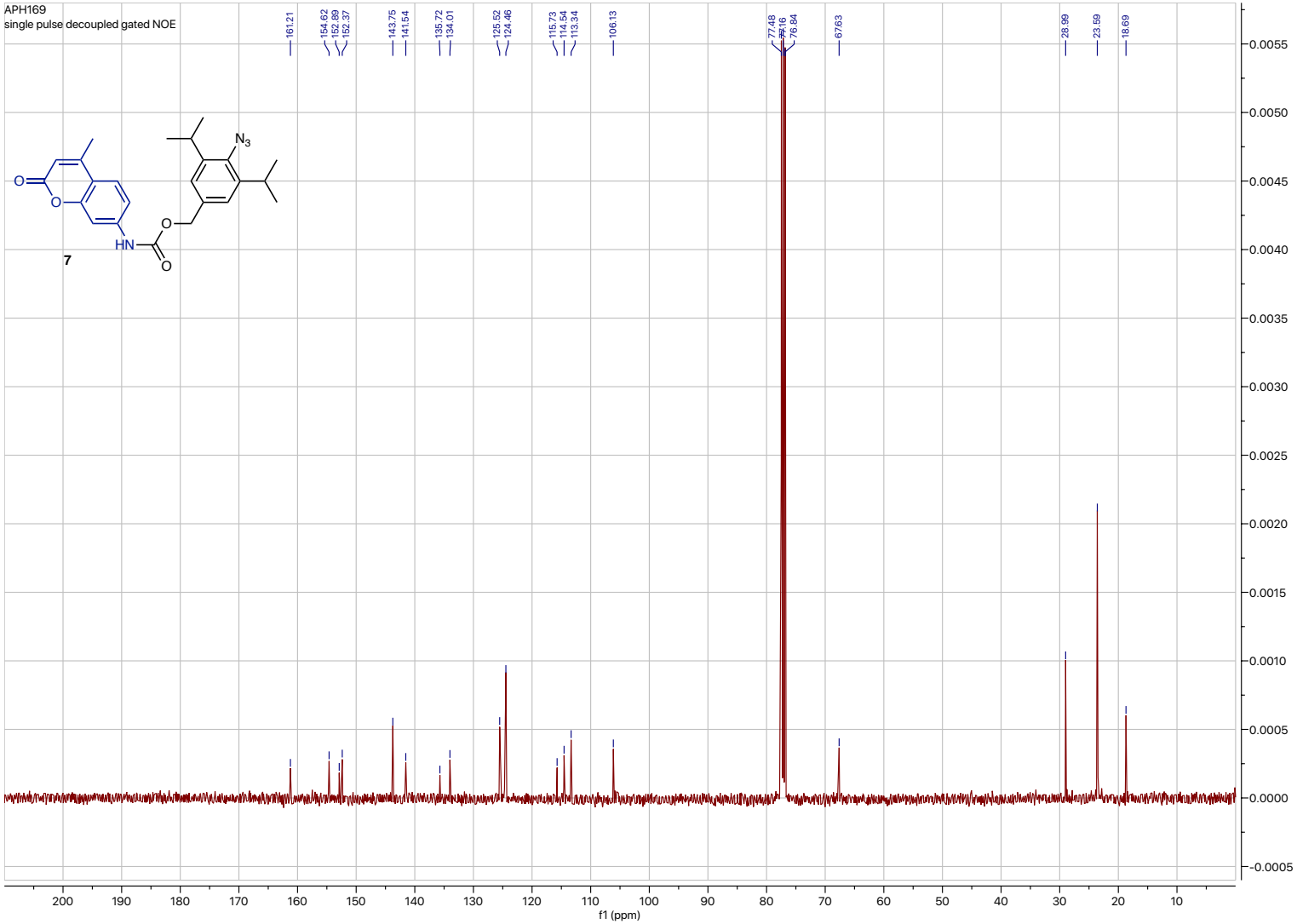

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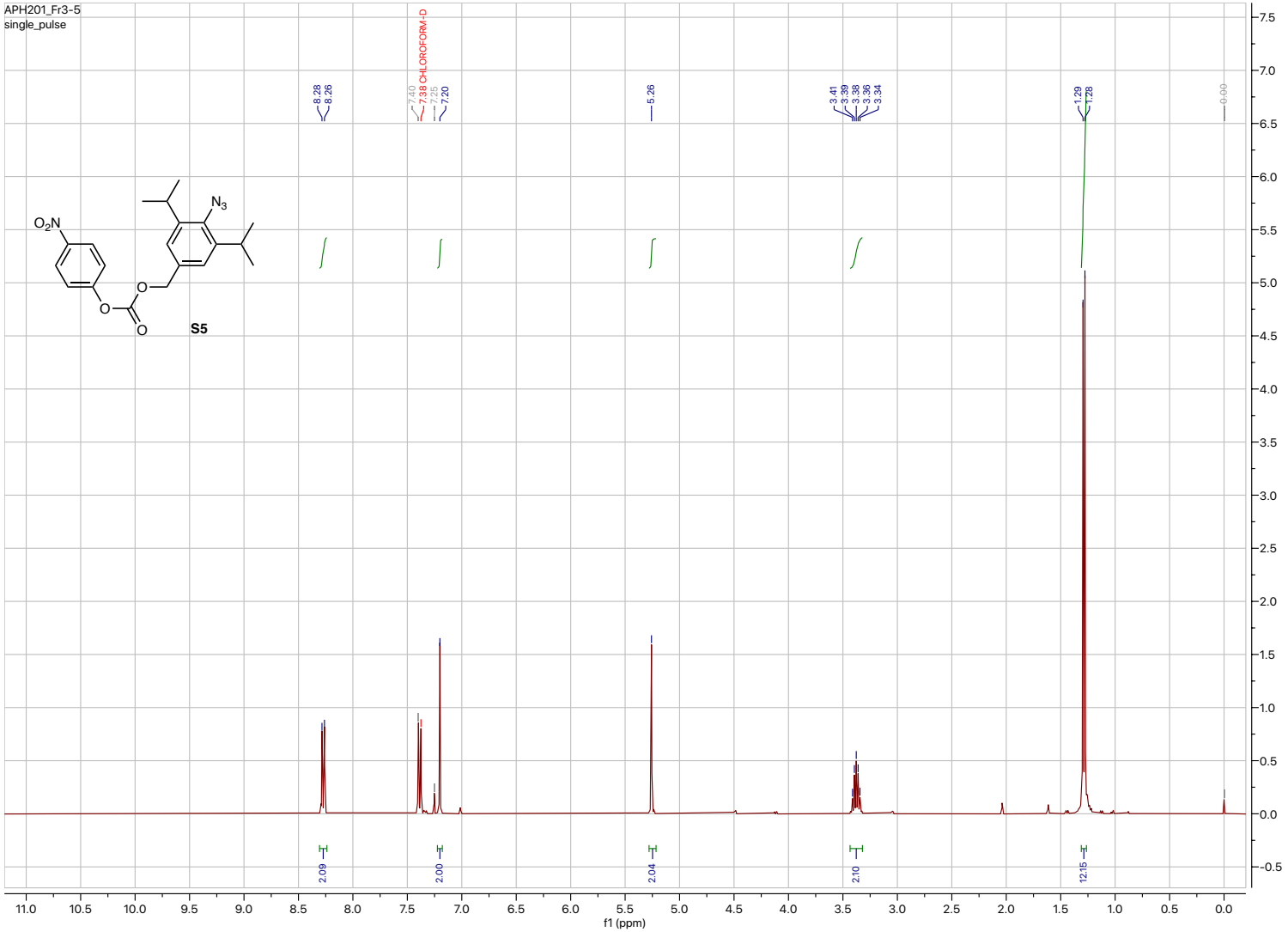

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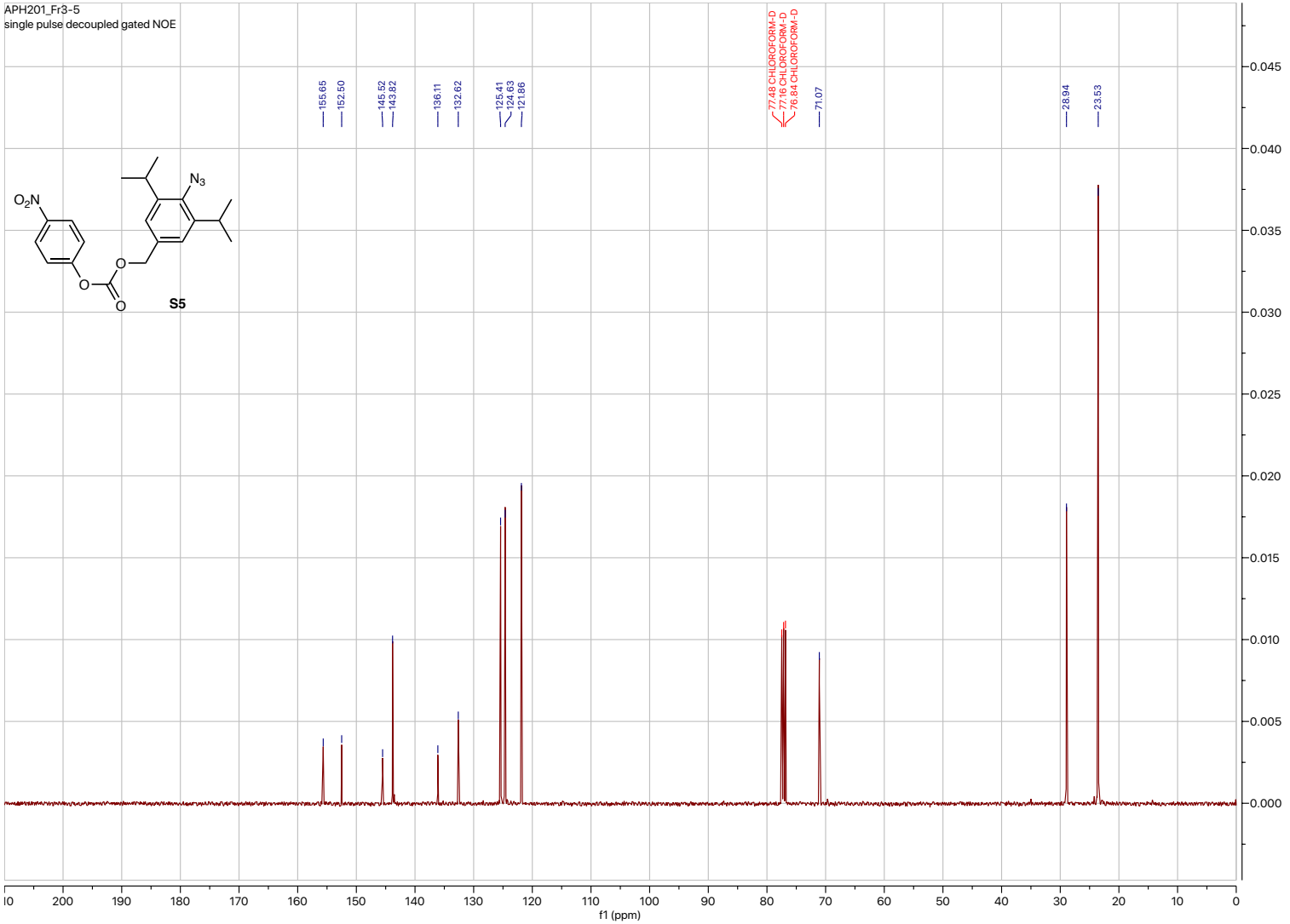

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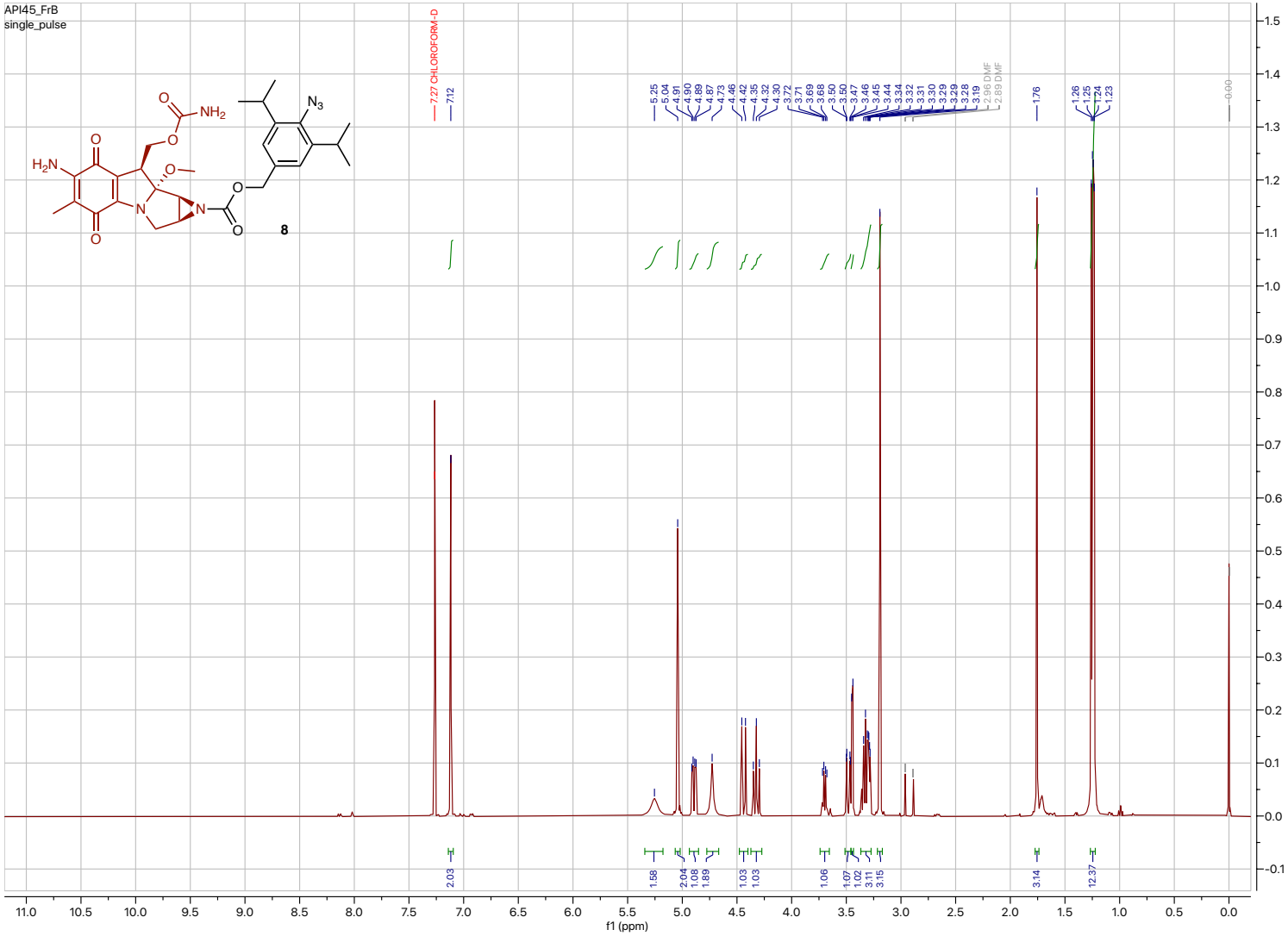

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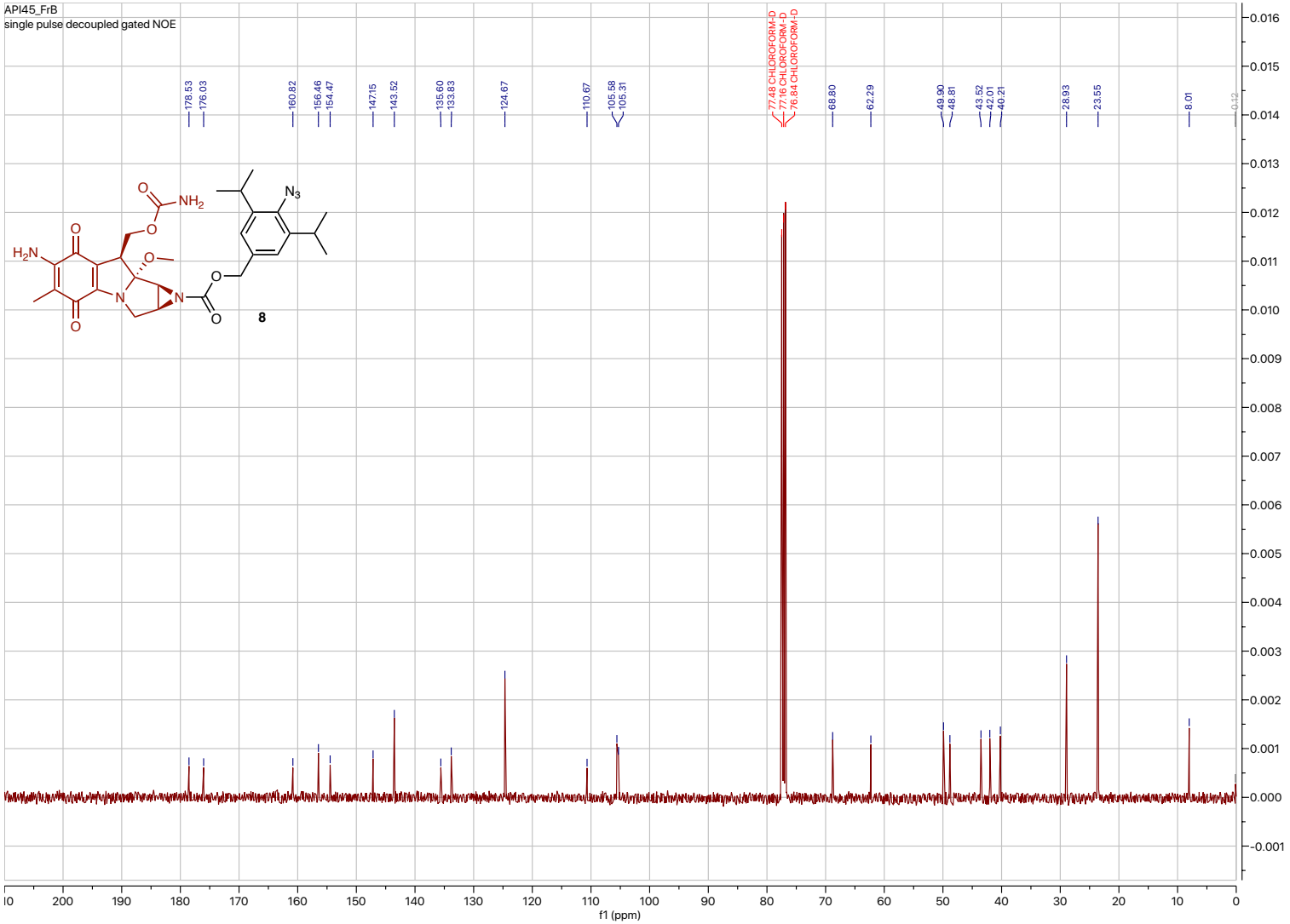

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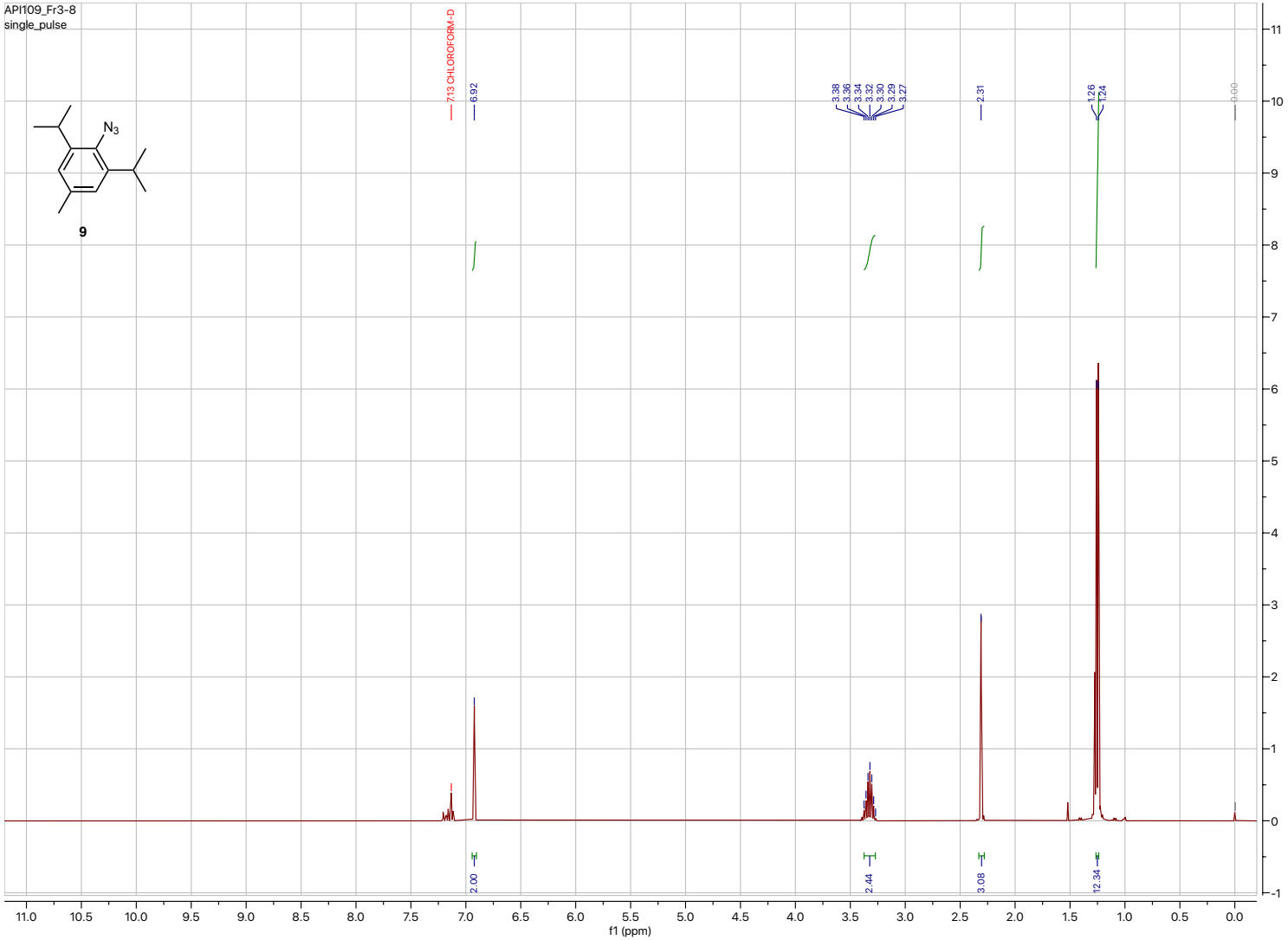

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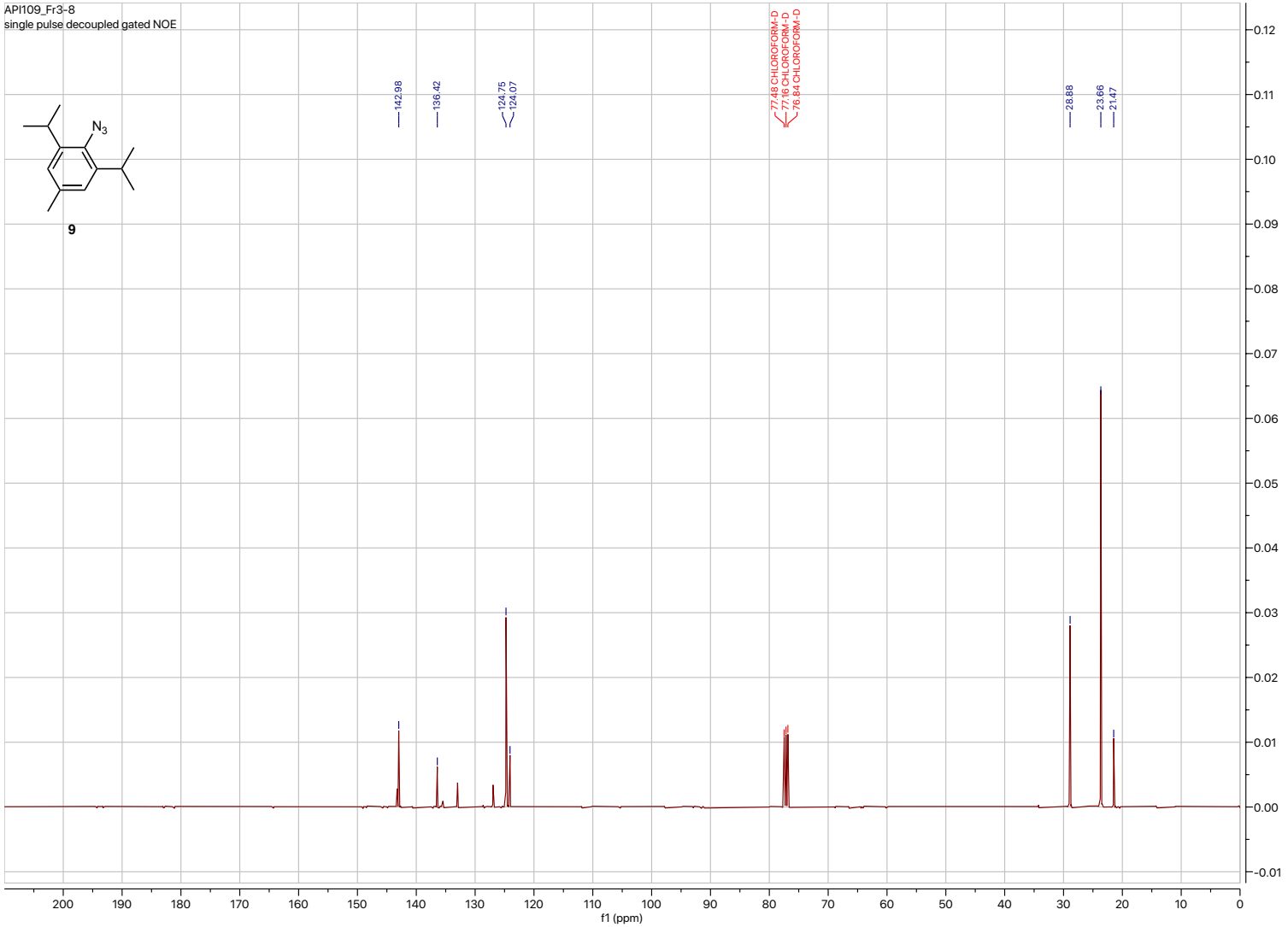

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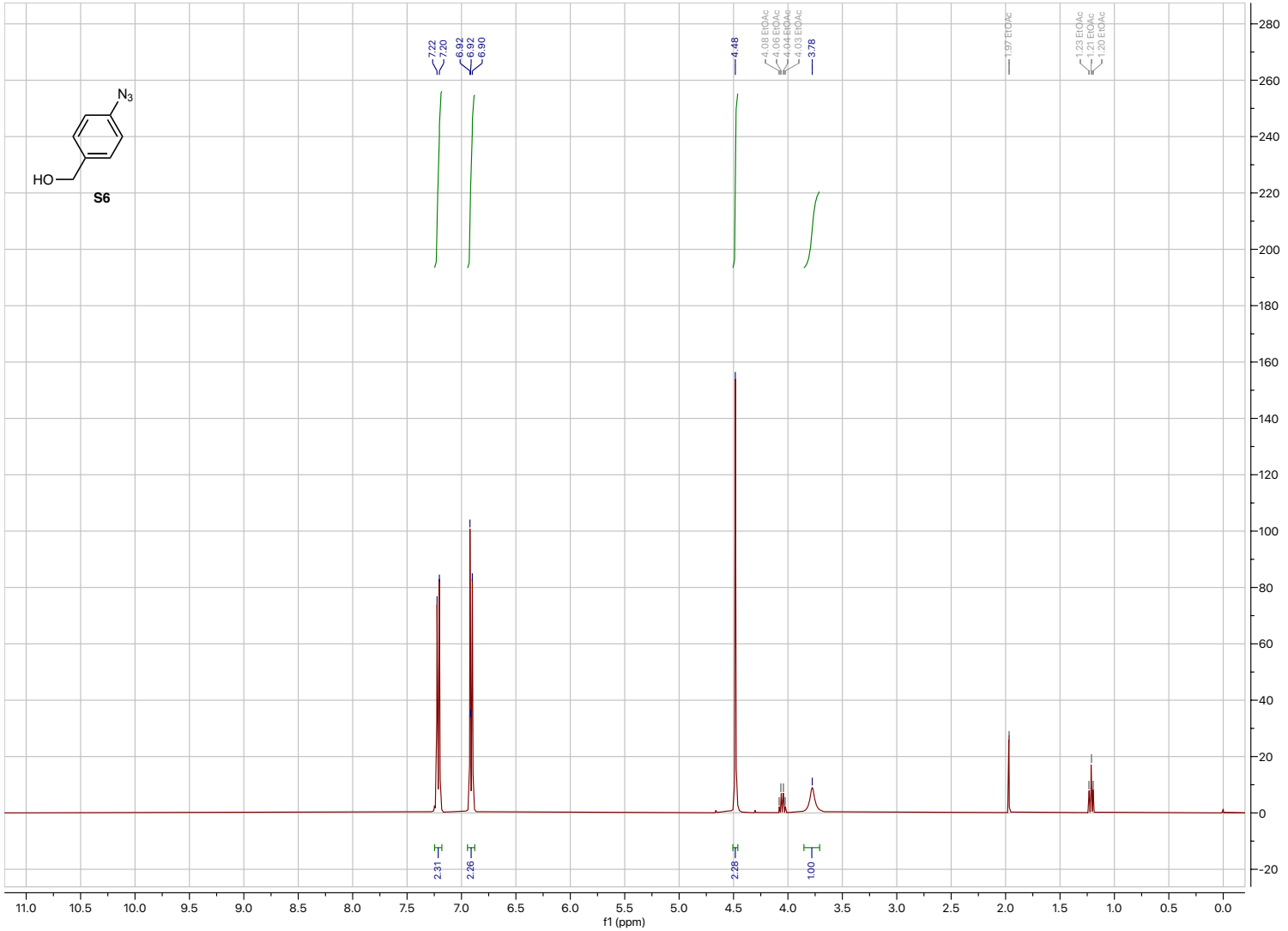

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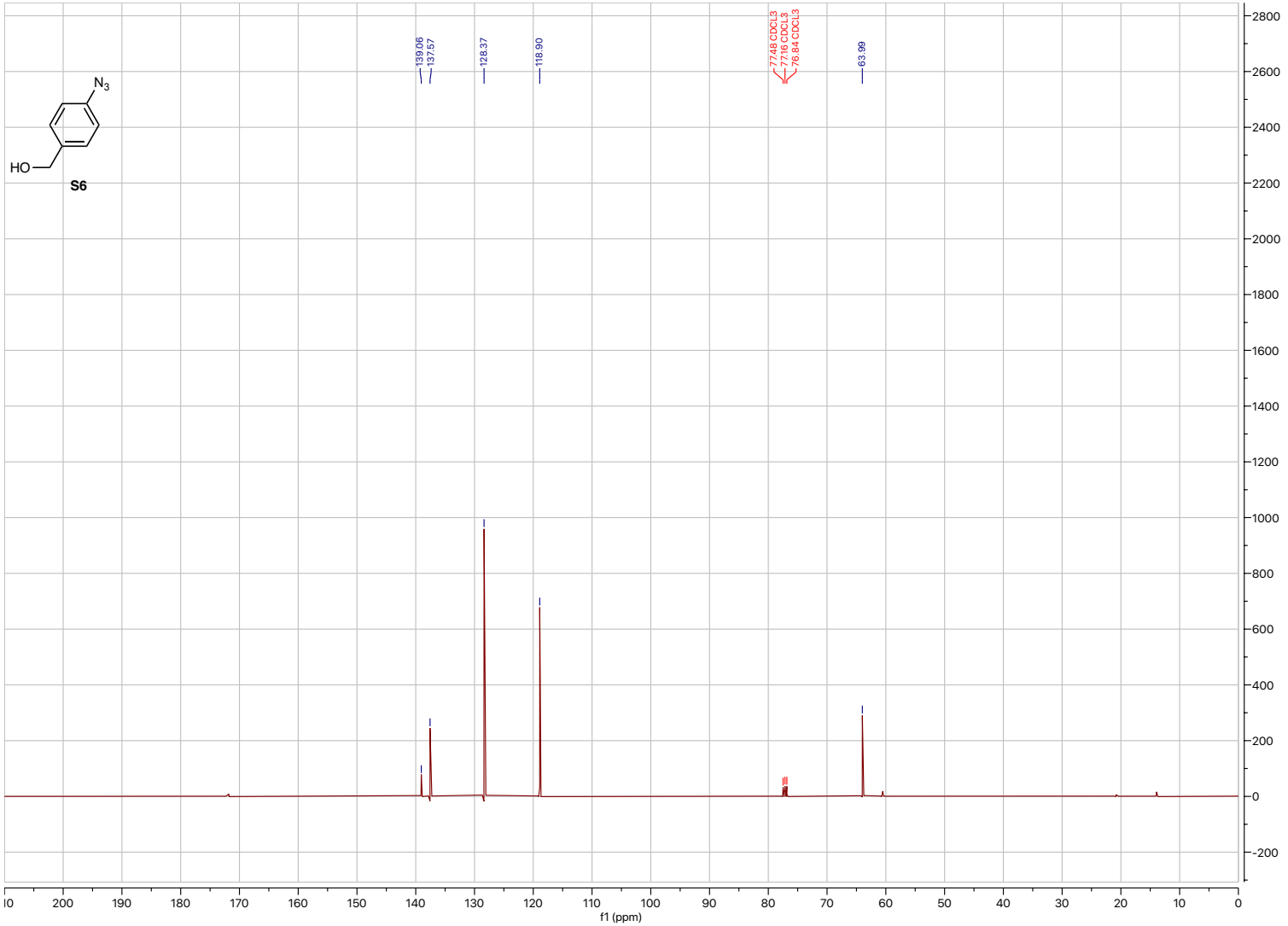

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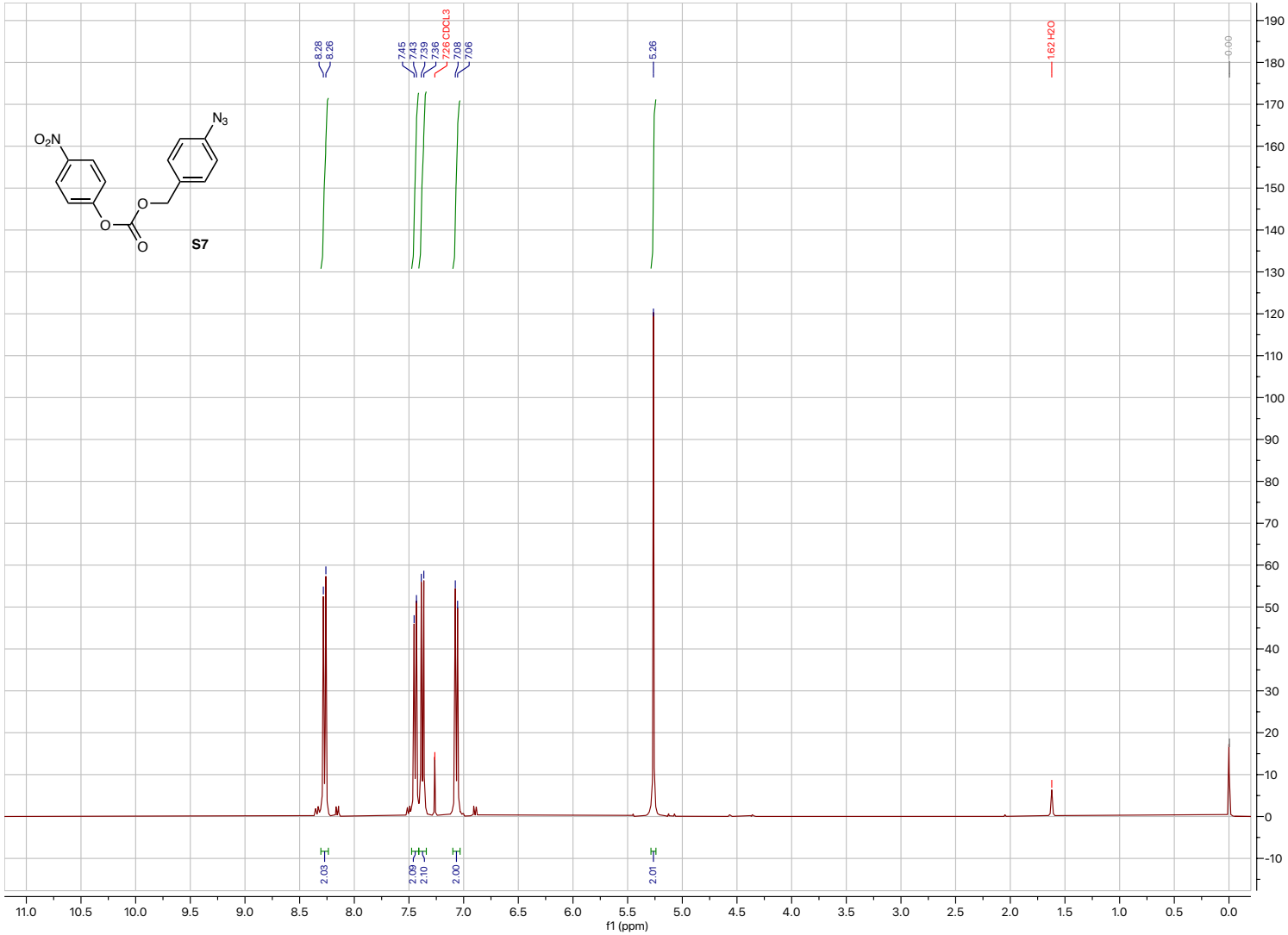

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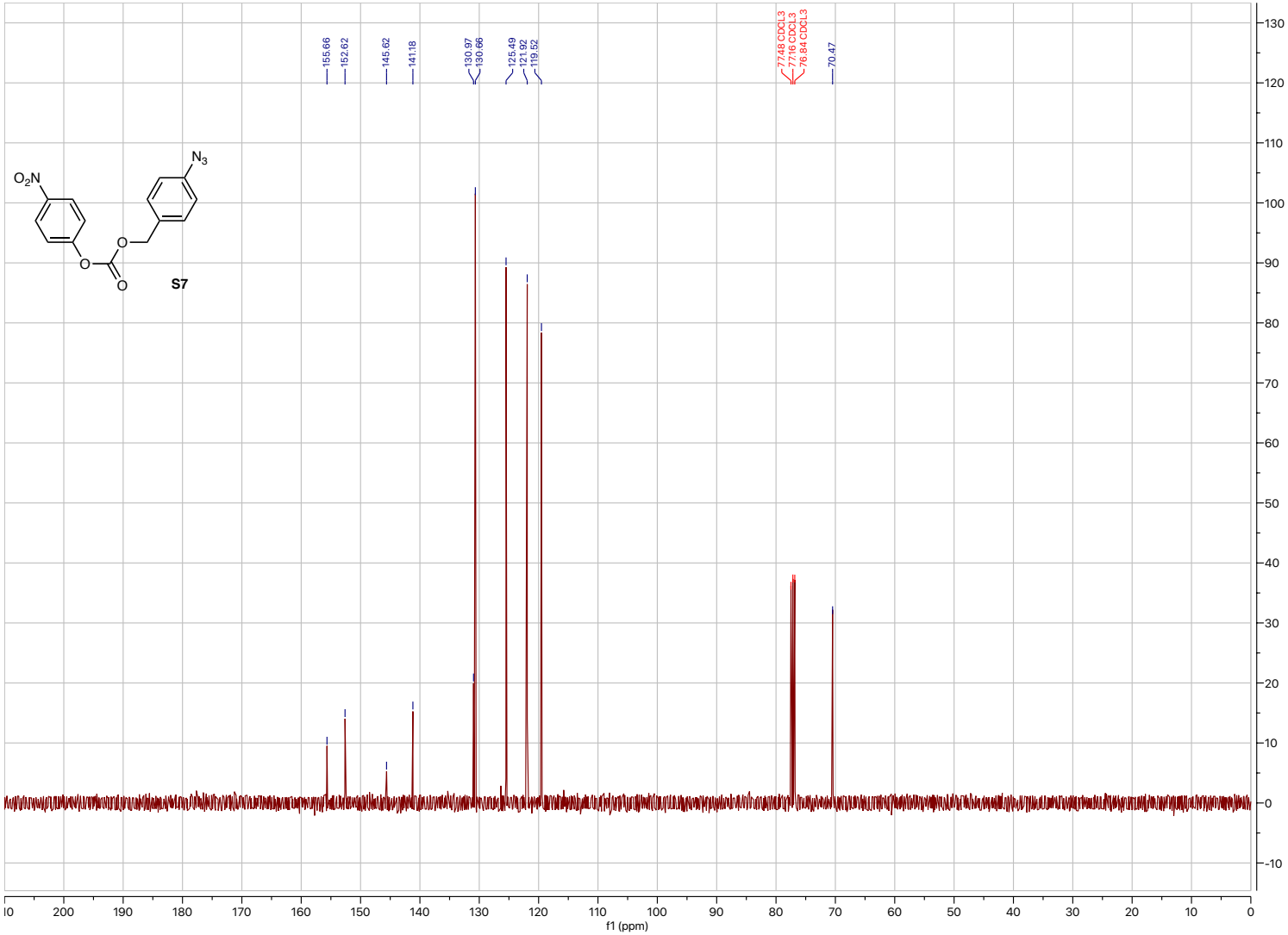

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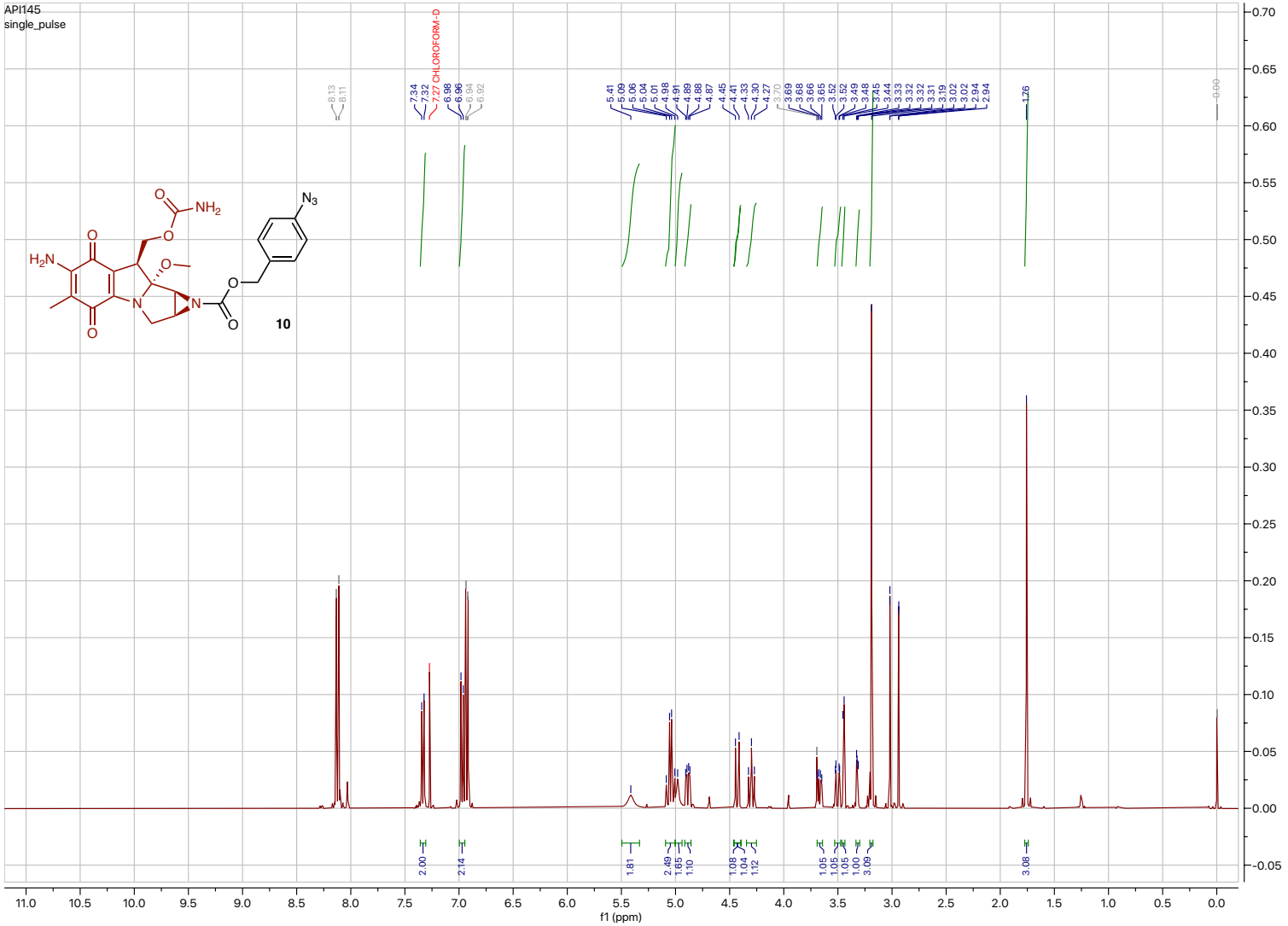

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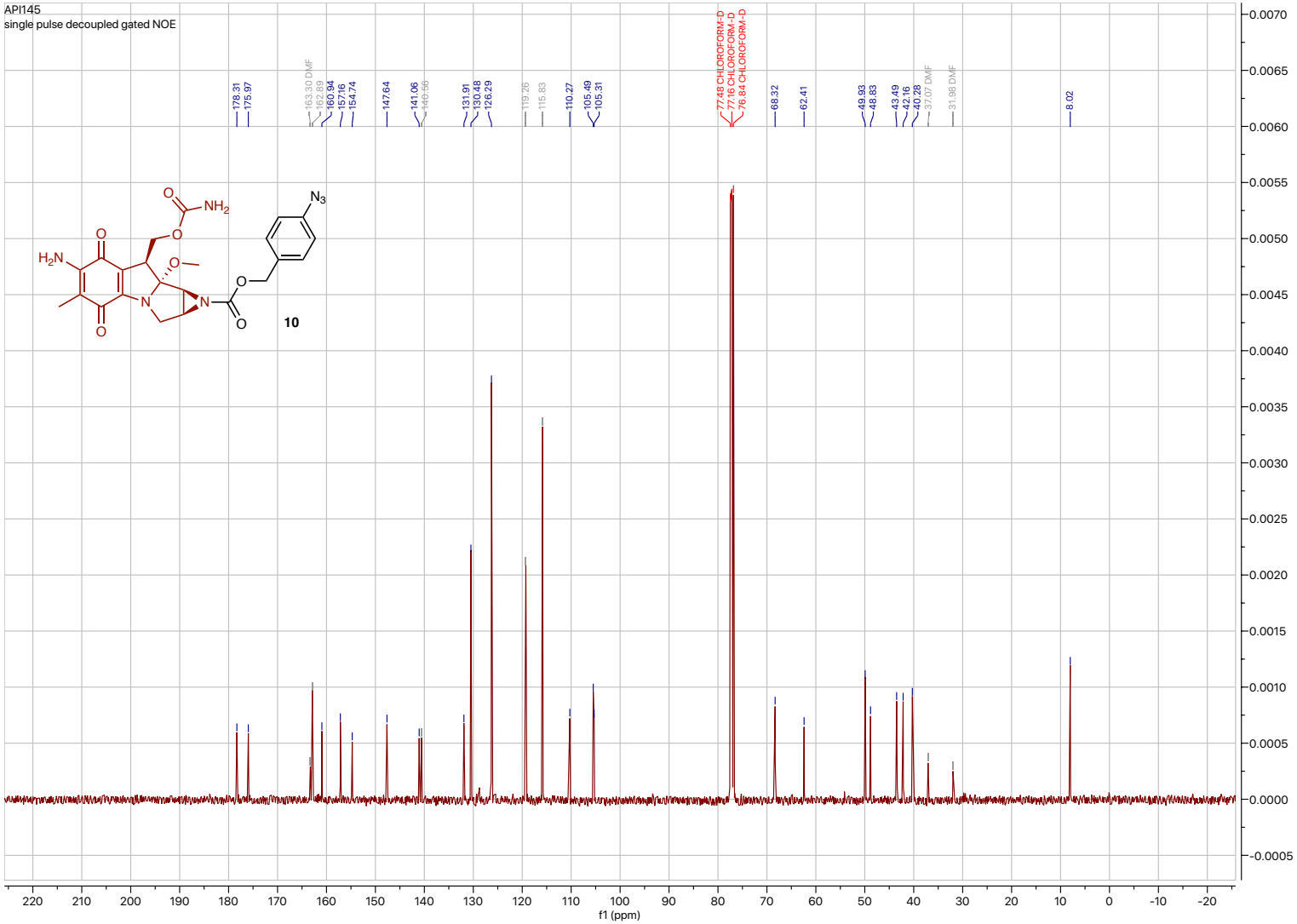

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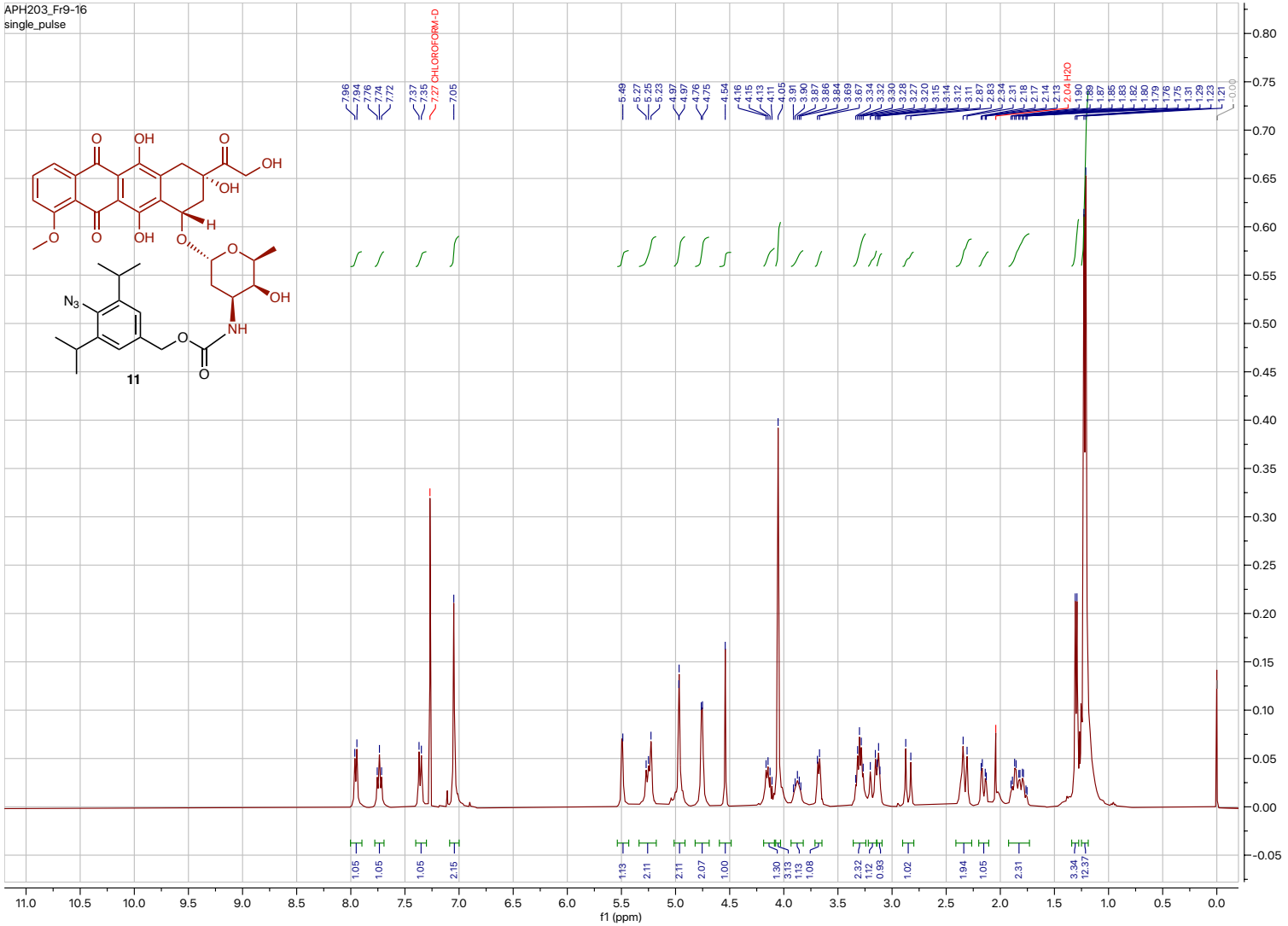

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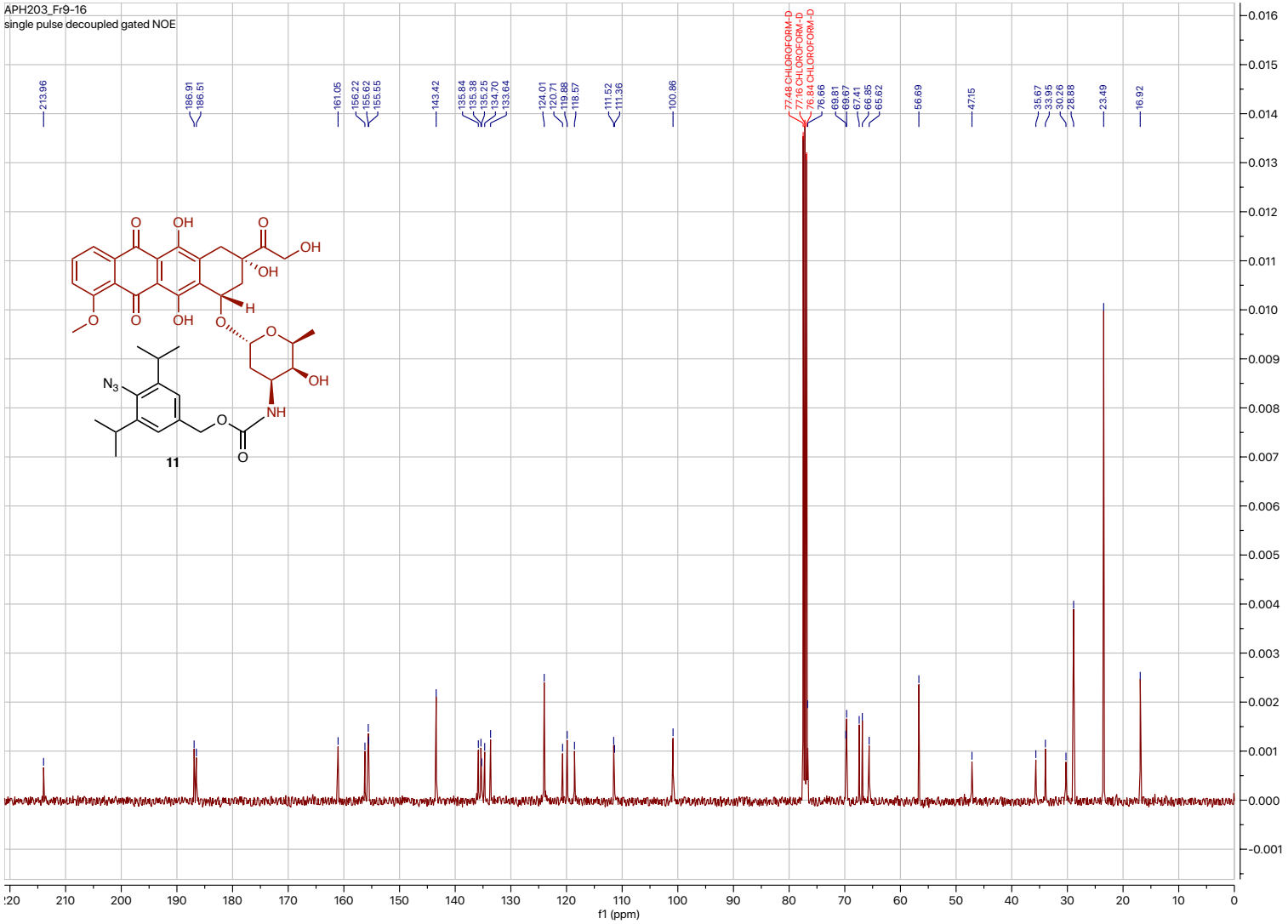

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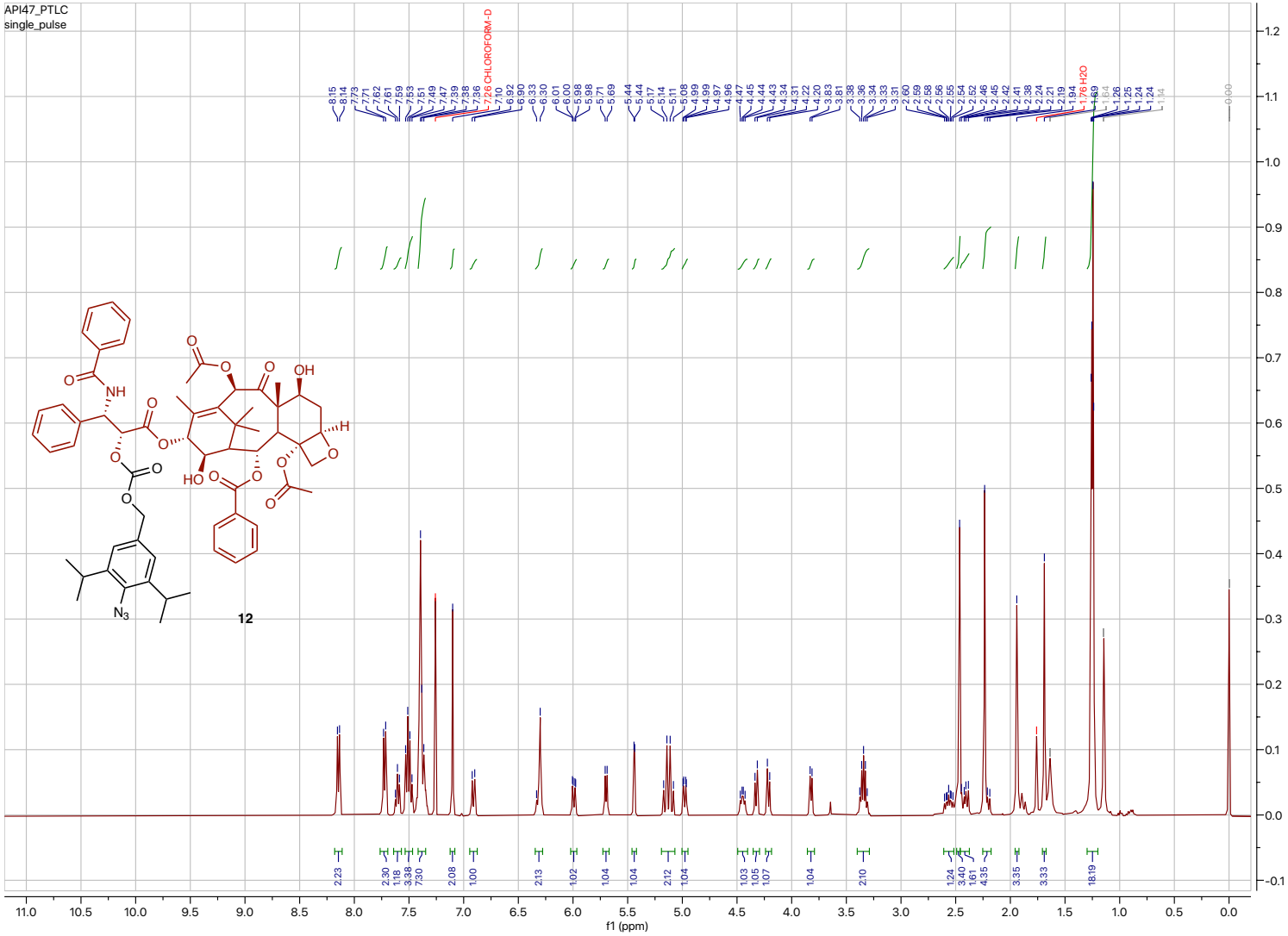

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