Supporting Information

"Fluorogenic cyclopropenones for multi-component, real-time imaging"

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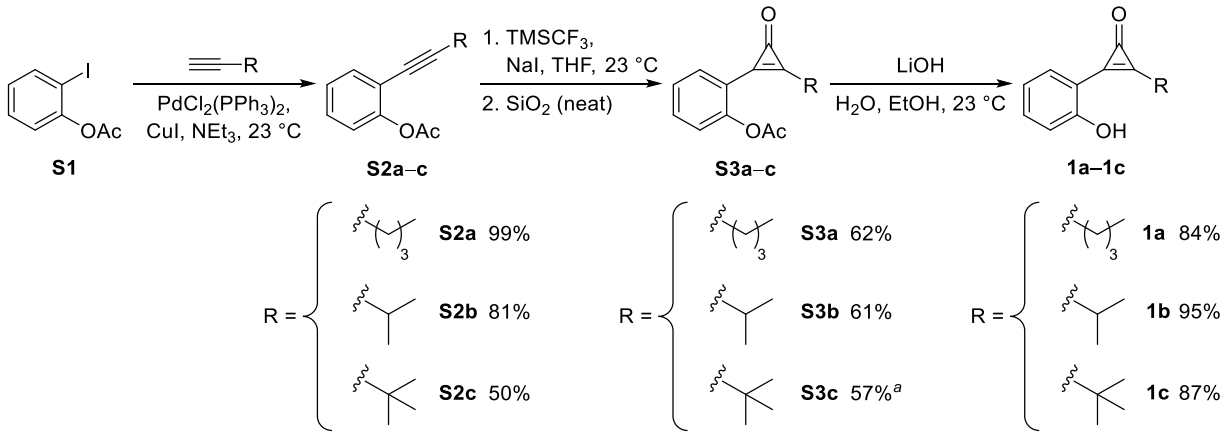
Supplementary Figures

Scheme S1. Synthesis of CpO probes **1a–1c**. [a]Insertion was performed at 110 °C for 2 d



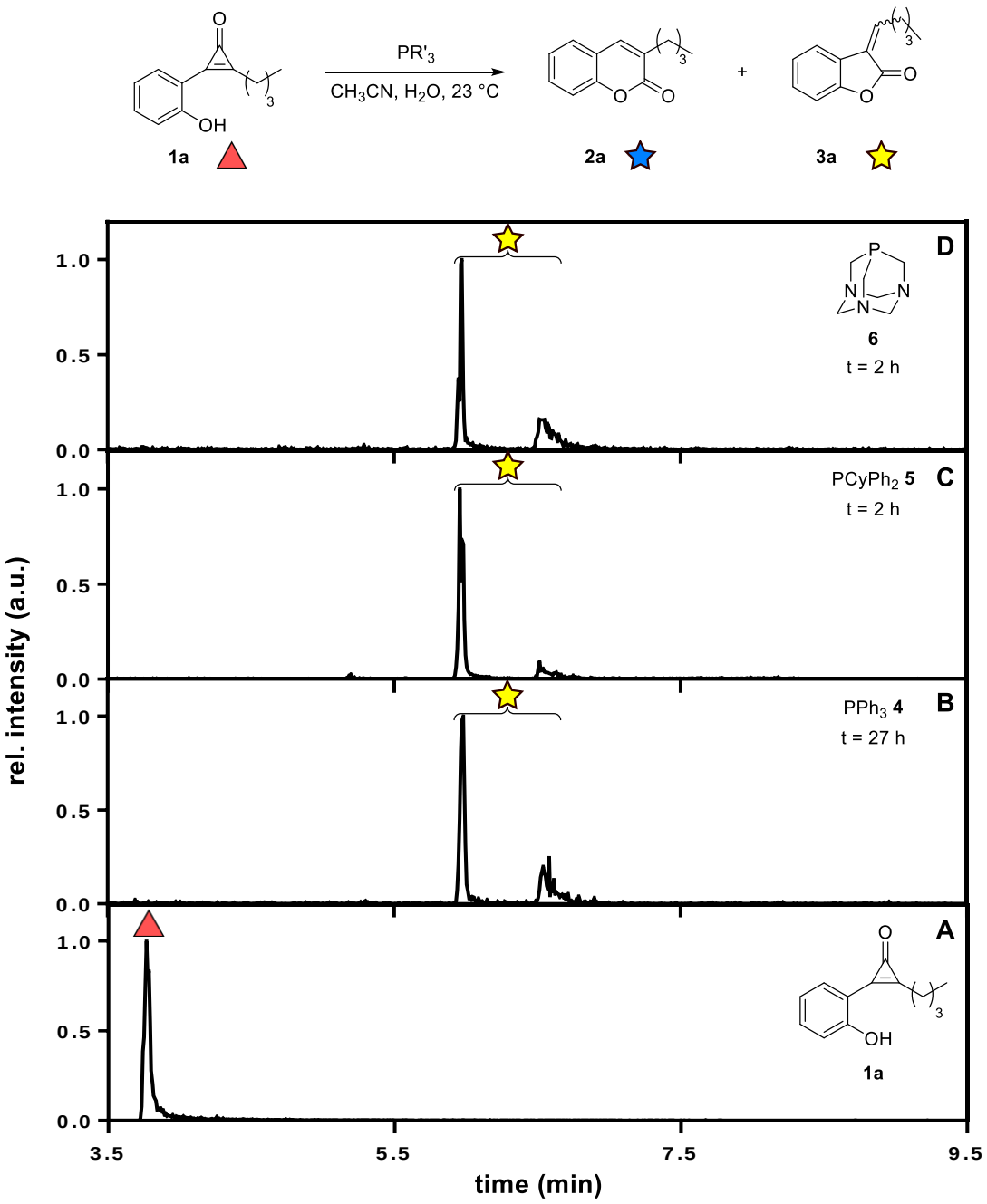


Figure S1. CpO **1a** reacts with phosphines **4**–**6** to afford the undesired product **3a**. Reaction progress was monitored using LC-MS (EIC, *m/z* 203). (A) CpO **1a** standard. (B)-(D) Reaction between CpO **1a** and (B) PPh_3 (**4**), (C) $PCyPh_2$ (**5**) or (D) phosphine **6.**

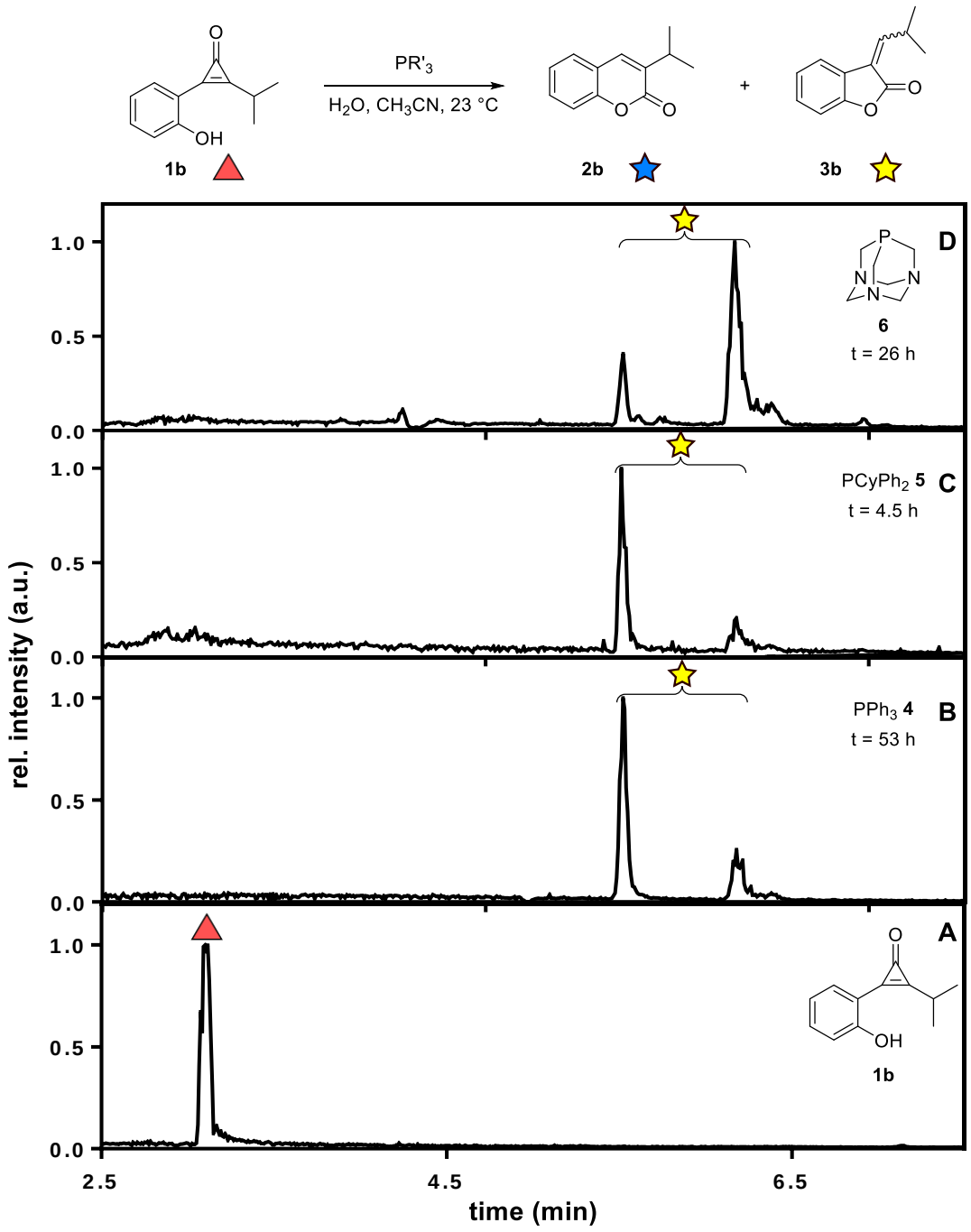


Figure S2. CpO **1b** reacts with phosphines **4**–**6** to afford the undesired product **3b**. Reaction progress was monitored using LC-MS (EIC, *m/z* 203). (A) CpO **1b** standard. (B)-(D) Reaction between CpO **1b** and (B) PPh_3 (**4**), (C) $PCyPh_2$ (**5**) or (D) phosphine **6.**

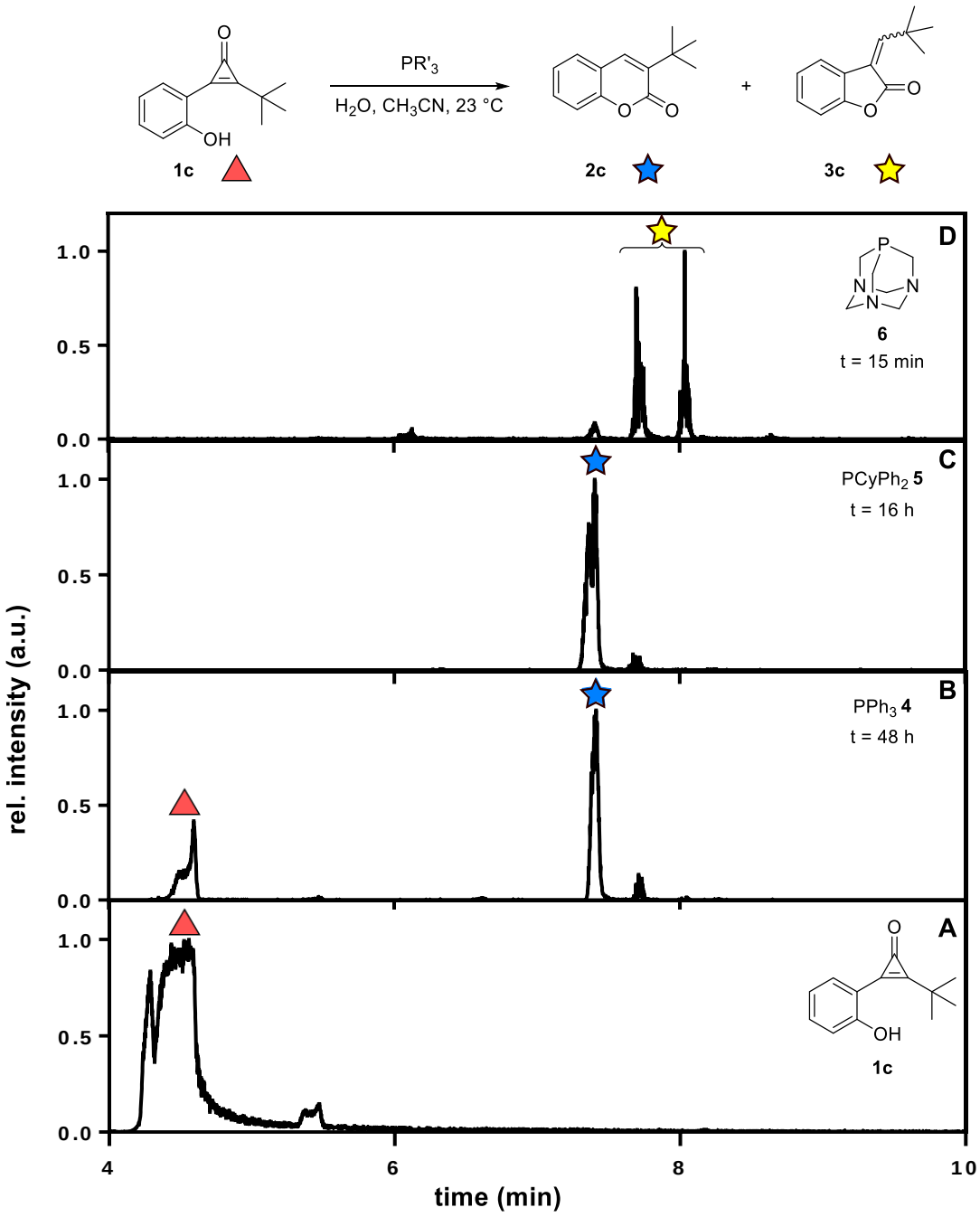
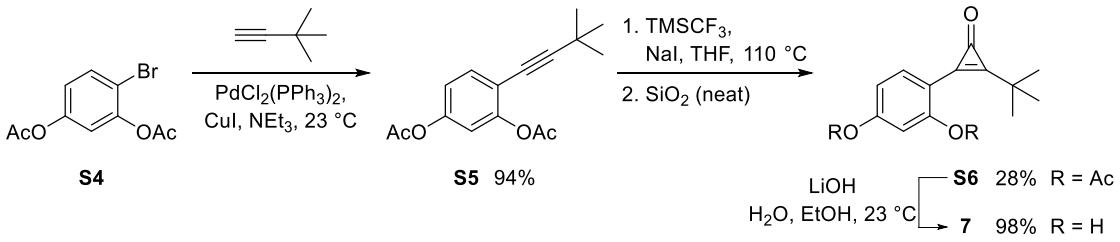


Figure S3. CpO **1c** reacts with phosphines **4**–**6** to afford the undesired product **3c**. Reaction progress was monitored using LC-MS (EIC, *m/z* 203). (A) CpO **1c** standard. (B)-(D) Reaction between CpO **1c** and (B) PPh_3 (**4**), (C) $PCyPh_2$ (**5**) or (D) phosphine **6.**

Scheme S2. Synthesis of fluorogenic CpO probe **7**.



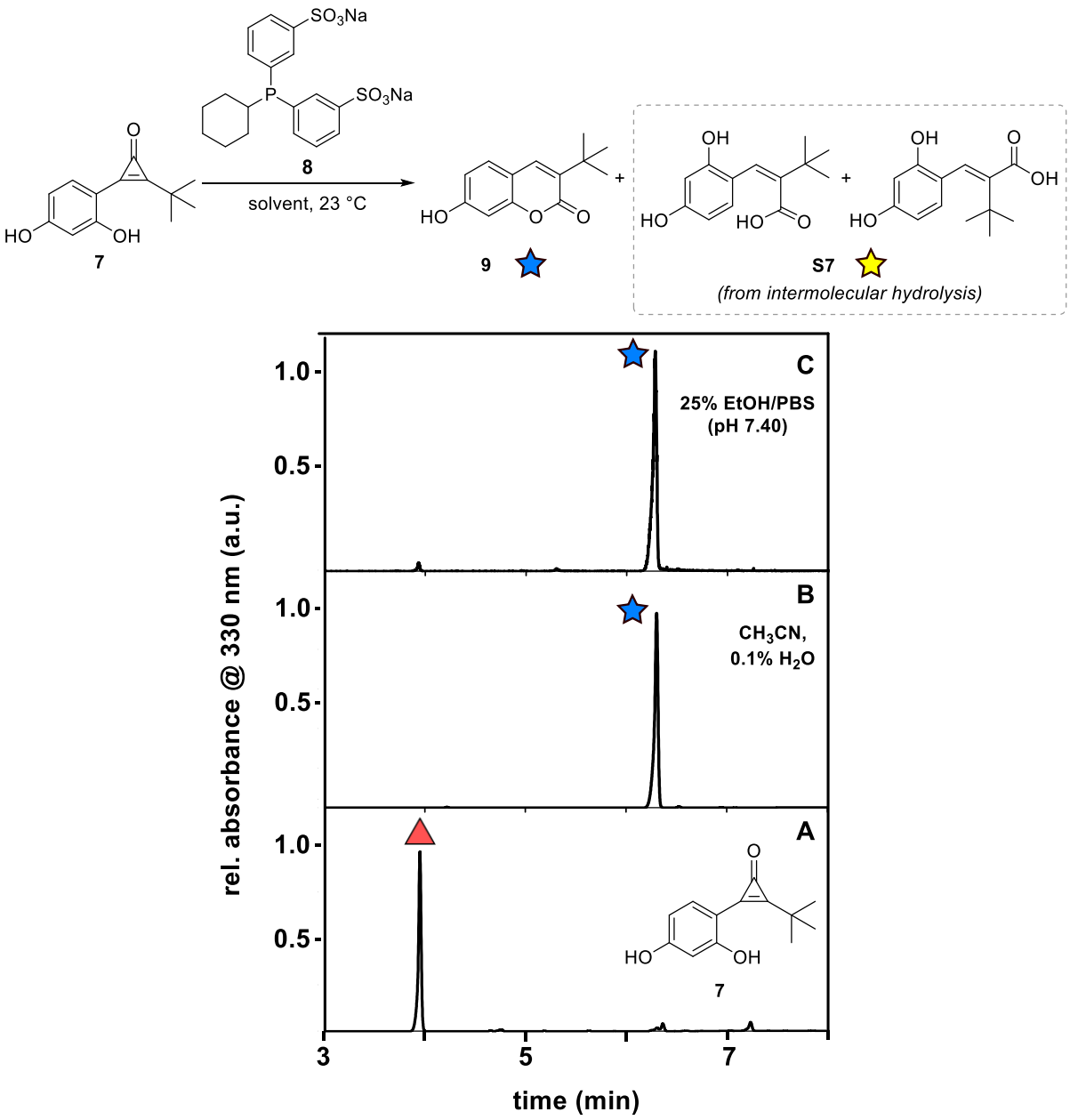


Figure S4. CpO **7** reacts with phosphine **8** to yield coumarin **9** in aqueous buffer. Reaction progress was tracked using UPLC (PDA λ_{abs} = 330 nm). (A) CpO **7** standard. (B-C) Reaction between CpO **1a** and phosphine **8** (5 mM, 15 mM, respectively) in (B) organic or (C) aqueous buffer.

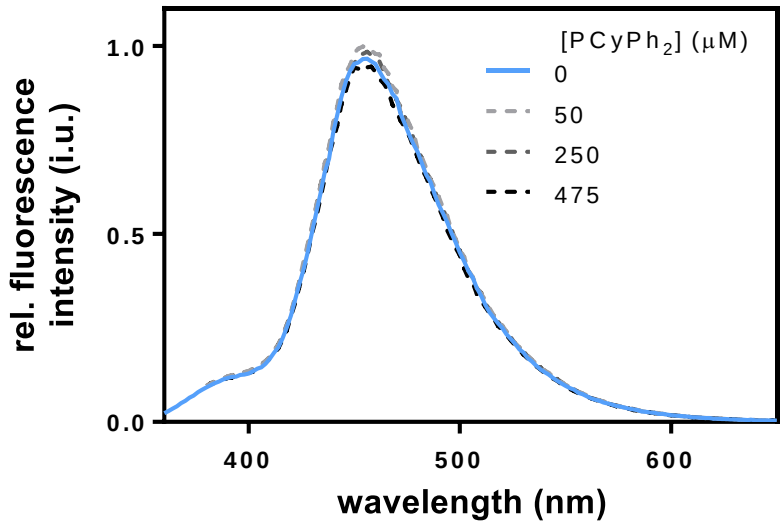
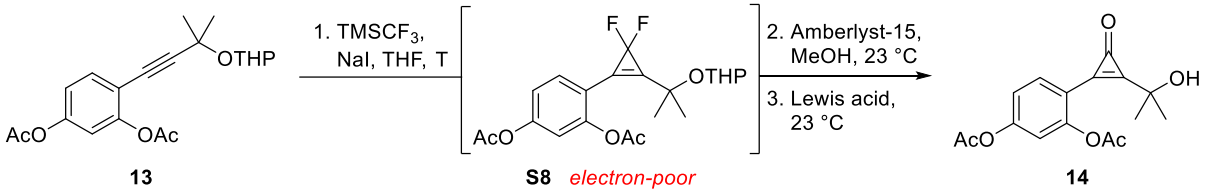


Figure S5. $PCyPh_2$ (**5**) does not quench the fluorescence of coumarin **9**. Coumarin **9** (50 μM) was incubated with $PCyPh_2$ (**5**) over a range of concentrations (0–475 μM, EtOH), and fluorescence spectra were recorded.



13 **S8** *electron-poor* **14**

A

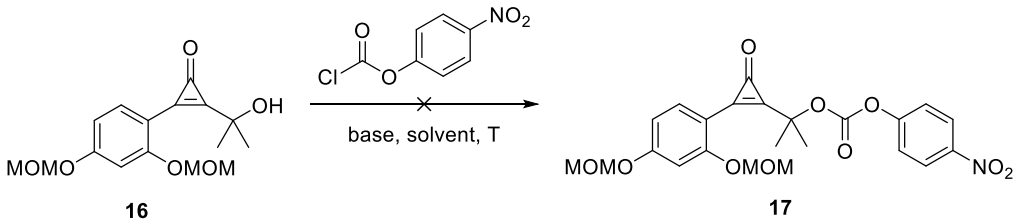
T (°C)	**13** : **S8**
23	>95 : <5
70	>95 : <5
110	75 : 25
110	86 : 14

B

Lewis acid	time	conditions	**S8** : **14**
SiO_2	2 d	neat	25 : 75
SiO_2	2 d	CH_2Cl_2 (+ H_2O)	50 : 50
BF_3•OEt_2	2 d	THF (+ H_2O)	25 : 75

Figure S6. Electron-deficient aryl alkyne **13** is not readily converted to CpO **14**. (A) Minimal carbene insertion was observed across a range of temperatures. Difluorocarbene reactants oligomerize at high temperatures, which may decrease insertion yields.[1] (B) Difluorocarbene hydrolysis was similarly slow. Incubation with silica or BF_3•OEt_2 required long periods of time and resulted in incomplete conversion.[2–4]

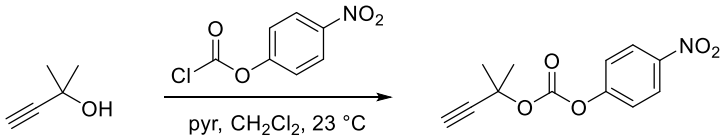
Table S1. Carbonate **17** was not accessible from CpO **16**. (A) Conditions explored for carbonate formation. (B) More polar solvents led to reagent degradation. (C) Conditions explored for deprotonation of CpO **16**. Strong bases degraded CpO **16**. (D) Control probe 2-methylbut-3-yn-2-ol (**S9**) is readily converted to the corresponding carbonate.[5]



16 **17**

	solvent	base (equiv)	T (°C)	time (h)	result
A	CH_2Cl_2	pyr (2.0)	23	26	CpO decomp., chloroformate recov.
	CH_2Cl_2	pyr (6.0)	55	18	CpO decomp., chloroformate recov.
	CH_2Cl_2	DMAP (0.50)	23	4	CpO & chloroformate recov.
	CH_2Cl_2	DMAP (0.50)	50	15	CpO & chloroformate recov.
B	pyr	pyr (neat)	23	18	CpO decomp., chloroformate recov.
	DMF	DMAP (10)	80	16	CpO & chloroformate decomp.
	DMSO	pyr (6.0)	60	20	CpO recov., chloroformate decomp.
C	THF	NaH (5.0)	23	0.5	CpO decomp.
	THF	KHMDS (1.0)	-78	0.5	CpO decomp.
	THF	KHMDS (1.0)	-78	0.5	CpO decomp.
	THF	LiHMDS (1.0)	-78	0.5	CpO decomp.

D



S9 76%

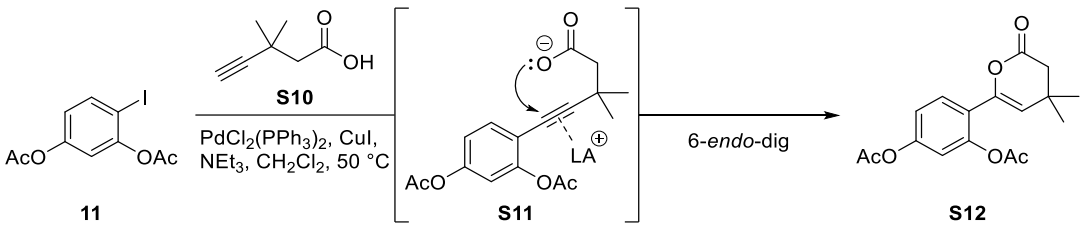


Figure S7. A lactonization reaction prevented synthesis of the desired aryl alkyne **S12**. Aryl iodide **11** and alkyne **S10** were ligated via Sonogashira coupling to form **S11**. Alkyne **S11** underwent a subsequent intramolecular lactonization *in situ* to yield lactone **S12**. Baldwin's rules permit 6-*endo*-dig cyclization reactions.[6,7]

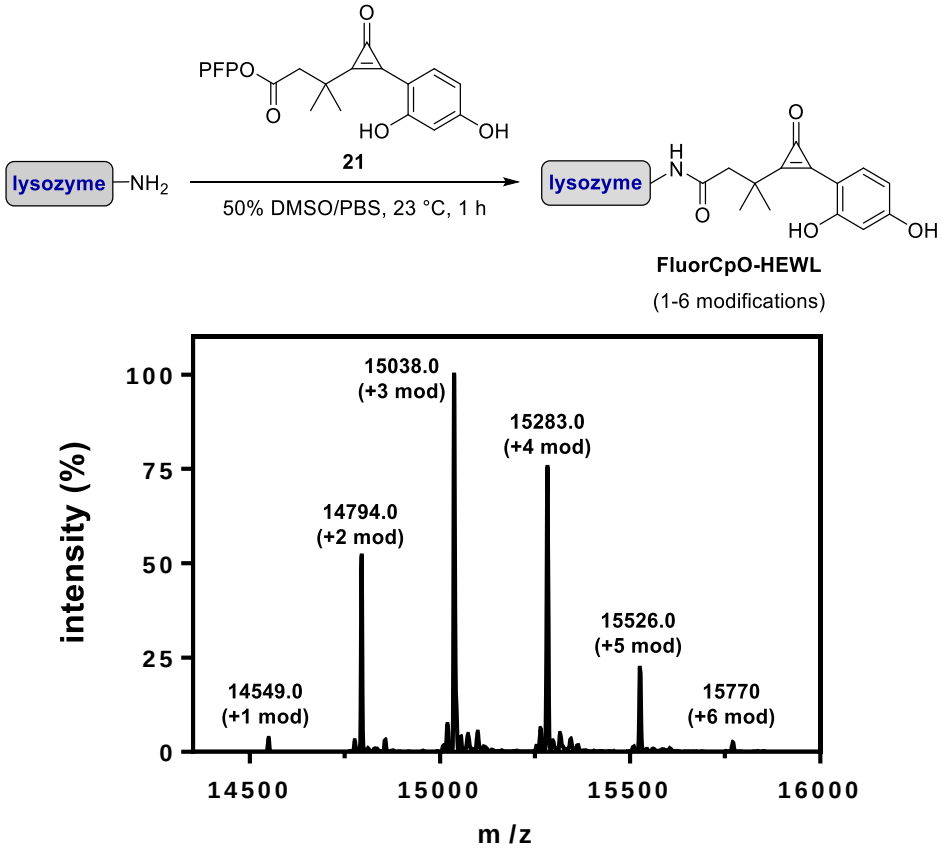


Figure S8. Mass spectrometry analysis of **FluorCpO-HEWL**. Lysozyme functionalization with CpO **21** was confirmed using mass spectrometry (ESI). The protein conjugate comprised 1–6 modifications.

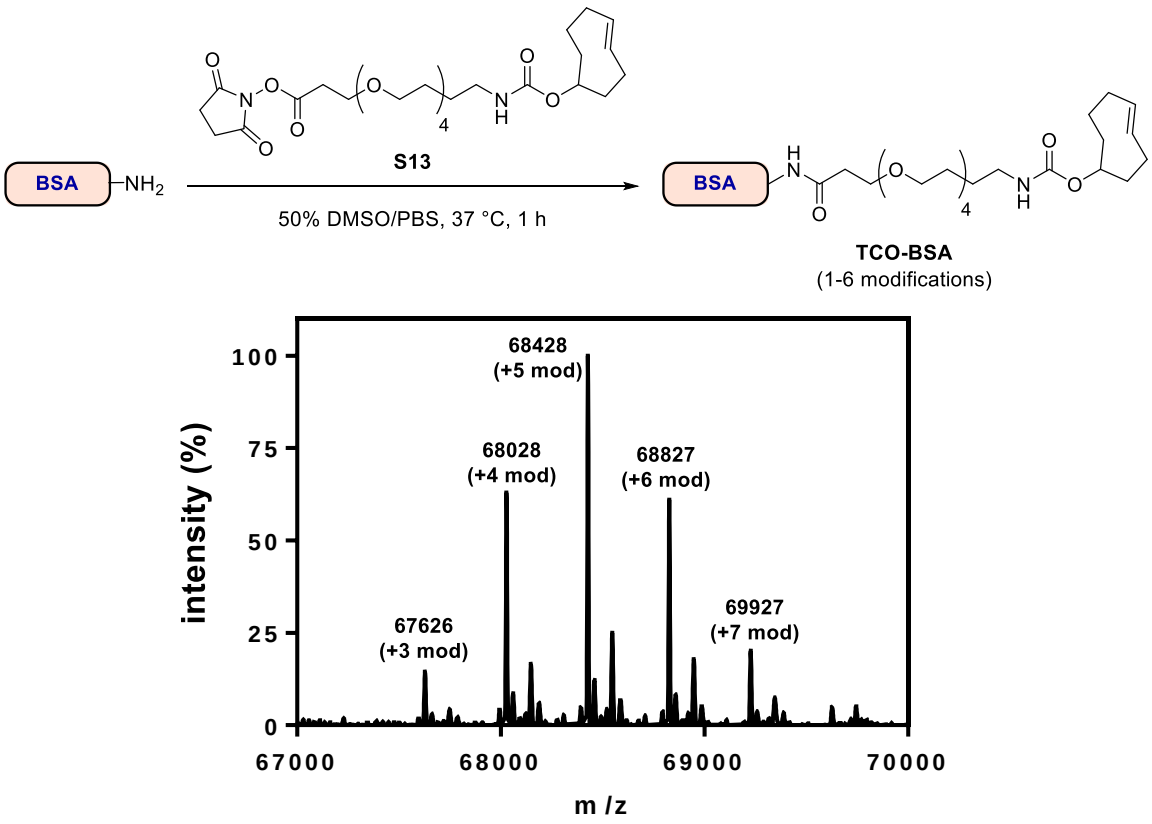


Figure S9. Mass spectrometry analysis of **BSA-TCO**. BSA functionalization with TCO-NHS ester **S13** was confirmed using mass spectrometry (ESI). The mixture comprised two proteins, and each protein was modified with 3–7 TCO modifications.

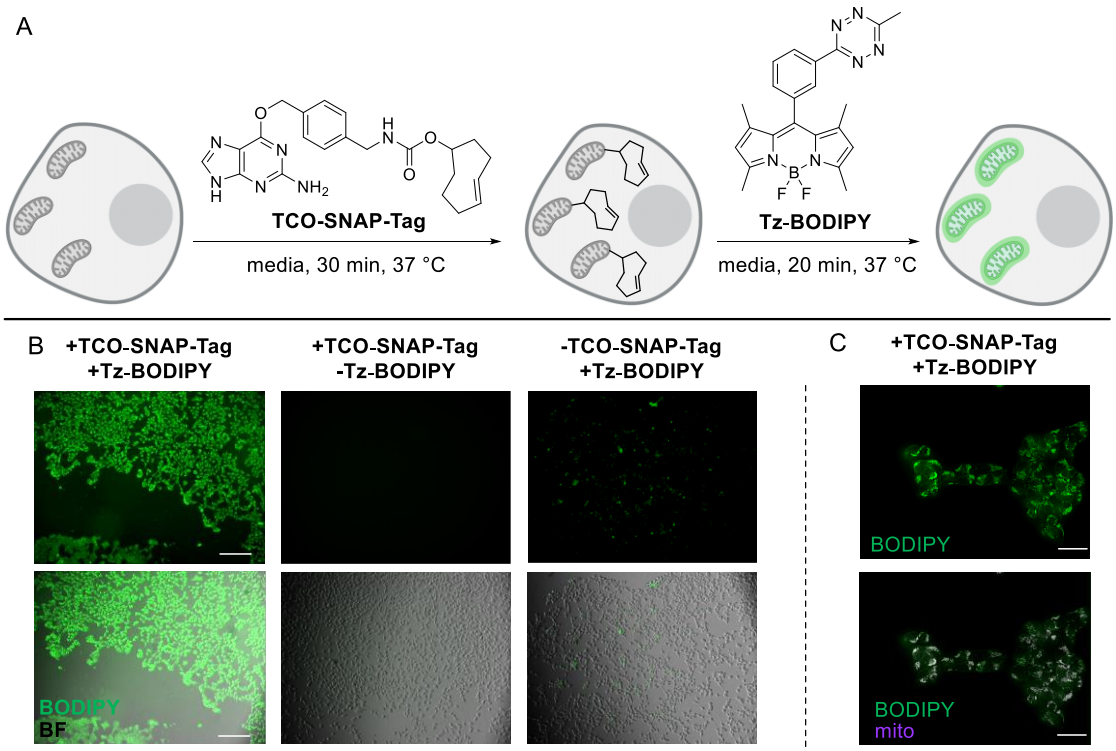


Figure S10. TCO-SNAP-Tag labels mitochondrial proteins in MitoSNAP cells. (A) **TCO**-tagged mitochondria were visualized via fluorogenic reaction with **Tz-BODIPY**. (B) Robust fluorescence was only observed in the presence of both compounds. (Scale bar: 200 µm.) (C) BODIPY fluorescence colocalized with a red mitochondrial stain (MitoView 633). Data are representative of three replicate experiments. (Scale bar: 30 µm.) Figure partially created with BioRender.com.

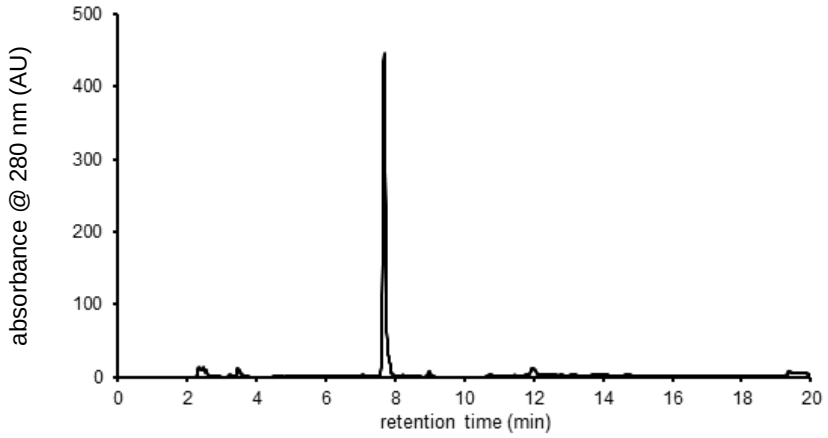


Figure S11. HPLC trace of **DCA-FluorCpO**. HPLC analysis was performed on an Agilent Technologies 1260 Infinity II equipped with a multiple wavelength detector, using an Agilent Eclipse XDB-C18 column (9.4 x 250 mm, 5 µm) and a 3 mL/min flow rate (eluting with 40–100% MeCN + 0.1% FA in H_2O + 0.1% FA over 20 min).

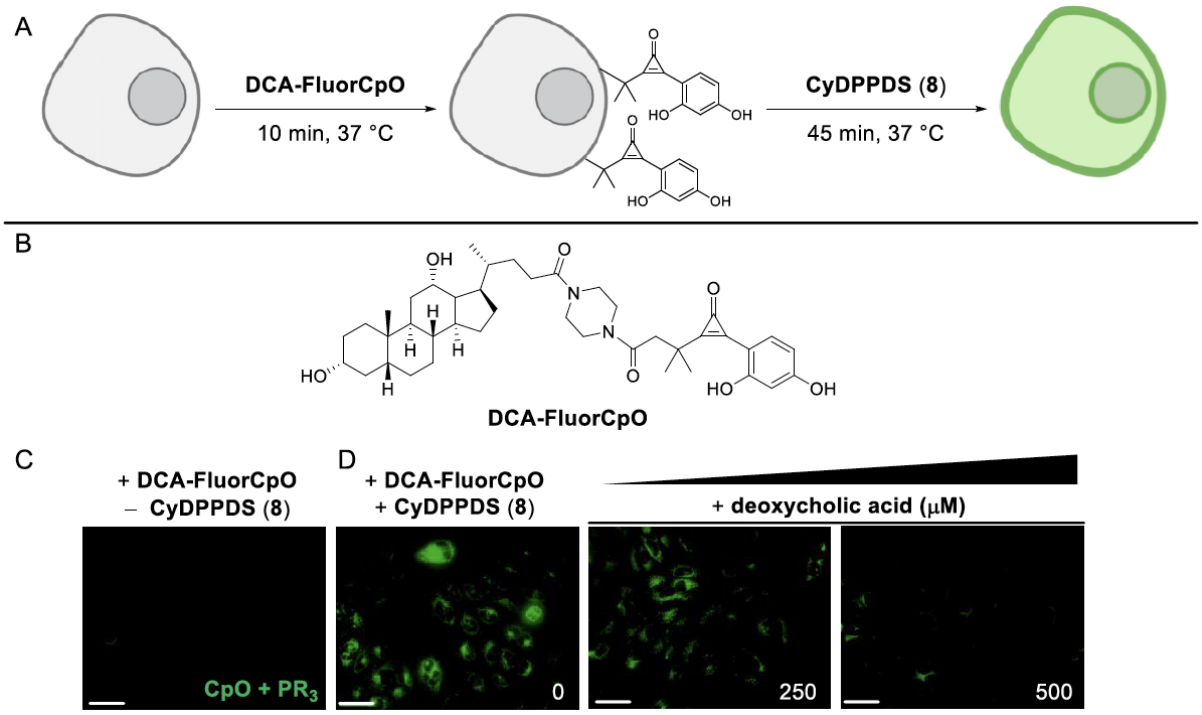


Figure S12. DCA-FluorCpO labels HeLa cells. (A) Scheme for visualizing **FluorCpO**-modified sterol probe with **CyDPPDS** (**8**). (B) Structure of **DCA-FluorCpO**. (C) No signal was observed from **DCA-FluorCpO**-treated cells in the absence of phosphine trigger **8**. (Scale bar: 50 μm.) (D) Fluorescent signal was observed from **DCA-FluorCpO**-treated cells upon treatment with phosphine **8**. Signal was diminished when **DCA-FluorCpO** was incubated with unlabeled deoxycholic acid prior to bioorthogonal reaction. Data are representative of three biological replicates. (Scale bars: 50 μm.) Figure partially created with BioRender.com.

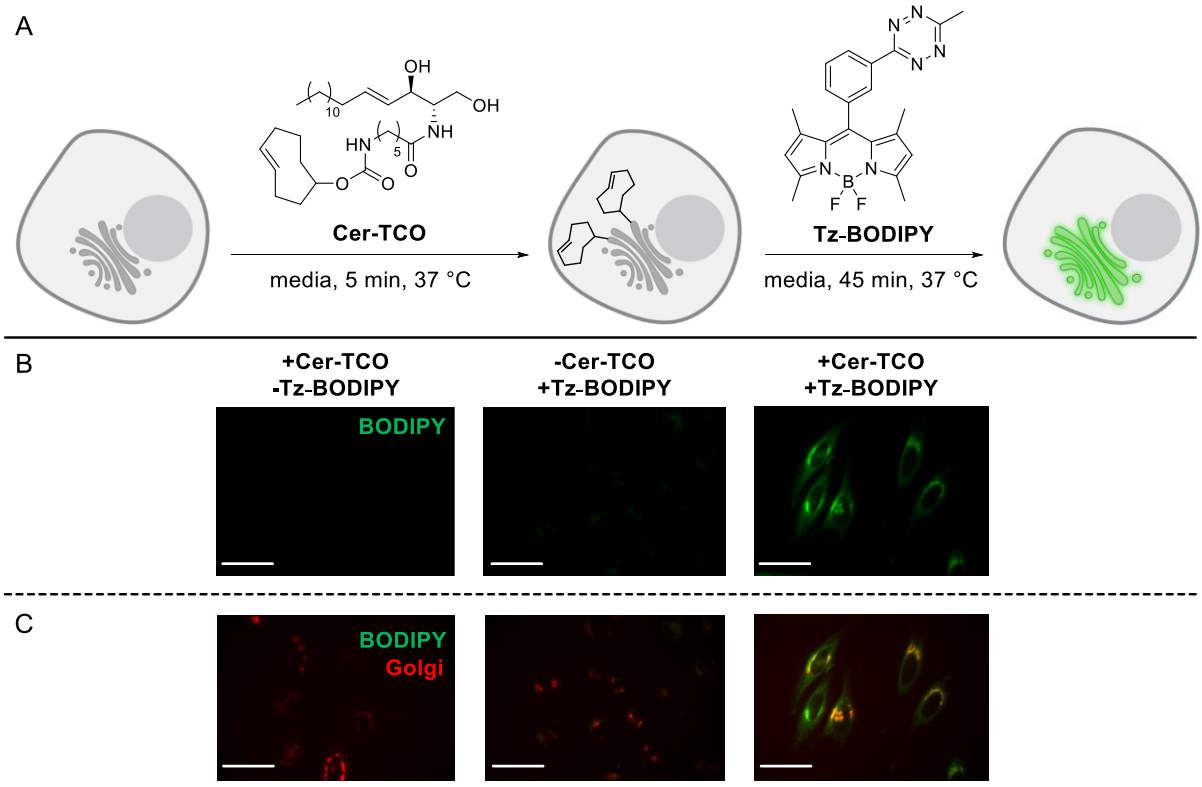


Figure S13. Cer-TCO labels the Golgi apparatus in live HeLa cells. (A) Scheme for visualizing **TCO**-tagged Golgi organelles via fluorogenic reaction with **Tz-BODIPY**. (B) HeLa cells were incubated with **Cer-TCO** (2 μM), washed, and visualized with **Tz-BODIPY** (100 nM). Robust BODIPY fluorescence was only observed in the presence of both compounds. (Scale bar: 50 μm.) (C) BODIPY fluorescence colocalized with an organellar Golgi stain, confirming Golgi localization of **Cer-TCO**. Data are representative of three replicate experiments. (Scale bar: 50 μm.) Figure partially created with BioRender.com.

General information

Reactions were run at ambient temperature under a nitrogen atmosphere, unless otherwise stated. All reagents and solvents were used as received, unless otherwise specified. Tetrahydrofuran (THF), diethyl ether (Et_2O), dichloromethane (CH_2Cl_2), dimethylformamide (DMF), and acetonitrile (CH_3CN) were prepared by degassing with argon and passing through two 4 in. × 36 in. columns of anhydrous neutral A-2 (8 × 14 mesh; LaRoche Chemicals; activated at 350 °C for 12 h under a flow of argon). Column chromatography was carried out using Silicycle 60 Å (240−400 mesh) silica gel, commercially available from Sorbent Technologies. Thin-layer chromatography (TLC) was carried out with Silica Gel 60 F_{254}-coated glass plates (0.25 mm thickness) 250 mm silica gel F-254 plates. Plates were visualized using UV light or $KMnO_4$ stain. Organic solutions were concentrated under reduced pressure using a Büchi rotary evaporator.

^{1}H, ^{13}C, ^{19}F, and ^{31}P NMR spectra were obtained using Bruker instruments: DRX400, DRX500 equipped with a cryoprobe, or AVANCE600 equipped with a cryoprobe. ^{1}H NMR spectra were acquired at 400, 500, or 600 MHz, ^{13}C spectra were acquired at 125 or 151 MHz, and ^{31}P spectra were acquired at 162 or 243 MHz. Spectra were internally referenced to residual solvent signals (7.27 ppm for ^{1}H and 77.16

ppm for ^{13}C for $CDCl_3$, 3.31 ppm for ^{1}H and 49.0 ppm for ^{13}C for CD_3OD, 1.94 ppm for ^{1}H and 1.32 ppm for ^{13}C for CD_3CN, and 4.79 ppm for ^{1}H for D_2O). ^{19}F and ^{31}P NMR spectra were referenced by an indirect absolute chemical shift to residual protio solvent signals. All spectra were acquired at 298 K unless stated otherwise. Chemical shifts are reported in ppm, and coupling constants (*J*) are reported in hertz (Hz). Spectrophotometer and fluorimeter measurements were performed in the Laser Spectroscopy Labs at the University of California, Irvine. High resolution mass spectrometry (HRMS) was performed by the University of California, Irvine Mass Spectrometry Facility.

CpO regioselective reactivity with phosphines

Solutions of CpO **1a**–**c** in CH_3CN (4–50% H_2O, final concentration = 0.01–0.1 M) were added to scintillation vials containing stir bars. Phosphines **4**–**6** (1–2 equiv.) were added, and the reactions were stirred at room temperature. In some cases, methanol and benzene were necessary to solubilize the phosphine reagents. Reaction progress was monitored using TLC and liquid chromatography mass spectrometry (LC–MS). When starting material was fully consumed, the reaction mixtures were concentrated *in vacuo*. The resulting crude solids were resuspended in $CDCl_3$ and analyzed using ^{1}H NMR spectroscopy. If necessary, the cyclized products were purified using flash chromatography. The acquired spectra were then compared to known spectra of 6-membered ring products **2a**–**c** to tabulate product distribution.[8-9]

Photophysical measurements

Samples for absorption and emission measurements were prepared from 10 mM stock solutions in 50% EtOH/PBS (pH 7.40). The dyes were diluted to various concentrations in 50% EtOH/PBS (pH 7.40). Absorbance measurements were made on a Cary-60 UV-Vis Spectrophotometer, and fluorescence spectra were measured on a Cary Eclipse Fluorescence Spectrometer.

Fluorescence quantum yields (Φ) for **7**, **9**–**10** were determined using the method of Lemieux *et al*.[11] Quinine bisulfate (λ_{abs}= 317 nm, λ_{ex} = 346 nm, λ_{em} = 448 nm, Φ = 0.546 in 1 N H_2SO_4) was used as the standard. The reported Φ values represent the mean of three independent measurements ± one standard deviation.

Kinetic experiments

Kinetic measurements were performed on a Biotek Synergy H1 instrument. CpO **7** (10 mM stock in EtOH, 100 μM final concentration) was added to a Greiner 96 well black flat bottom Fluotrac plate containing PBS (pH 7.40). CyDPPDS **8** (10 mM stock solution in H_2O, final concentration = 1–5 mM) was added to the appropriate wells (final volume 100 μL). Fluorescence measurements (λ_{ex} = 360 nm, λ_{em} = 455 nm) were collected every 70 seconds for 4 h.

The rate of fluorophore formation (k_2) was determined by tracking product formation via fluorescence and subsequent reference to a standard curve. The reported rate constant represents the mean of three independent measurements ± one standard deviation.

Protein functionalization and analysis

FluorCpO-HEWL was prepared by treating lysozyme with cyclopropenone **21**.[12-14] Hen egg-white lysozyme (Sigma Aldrich, 500 μL of a 10 mg/mL solution in PBS) was treated with CpO **21** (320 μL of a 10 mM stock in DMSO) and DMSO (180 μL, 50% DMSO/PBS final concentration). The solution was incubated at 37 °C for 0.5–2 h. Labeling was monitored using mass spectrometry. The functionalized proteins were isolated using P10 Biogel (BioRad), eluting with PBS (12 mM, pH 7.4). Fractions containing protein were identified by absorbance measurements at 280 nm (A_{280}). The fractions containing protein were pooled and concentrated to a volume of ~100 μL using a spin filter (3 kDa molecular weight cutoff). PBS (12 mM, pH 7.4) was then added to bring the total volume to 300 μL, and the sample was concentrated again. This process was repeated three times total to remove excess small molecules. Protein concentrations were measured using a Pierce® bicinchoninic acid (BCA) assay (ThermoFisher).

BSA-TCO was prepared by treating BSA (Fraction V, ThermoFisher) with TCO-NHS ester **S13** (Click Chemistry Tools) in a similar manner.

In-gel fluorescence analysis of lysozyme conjugates

FluorCpO-HEWL (2 μL of a 200 μM solution in PBS, final concentration = 20 μM) and CyDPPDS (from 1–10 mM solution in PBS, final concentration = 100 μM–1 mM) were mixed with PBS (12 mM, pH 7.40) to a total volume of 10 uL. The reactions were incubated at 37 °C for 5–120 min. For time course experiments, reactions were quenched with H_2O_2 (0.1% final concentration).

To prepare samples for SDS-PAGE, 5 μL of a 4x SDS-PAGE loading buffer (containing 8% BME) and 5 μL of urea were added to each reaction mixture. The resultant mixture (20 μL) was analyzed by gel electrophoresis using 20% polyacrylamide gel (140 V for 30 min). Coumarin formation was observed by in-gel fluorescence using a Bio-Rad ChemiDoc XRS+ Imaging system, exciting with the white light filter setting. Gel staining was accomplished using Coomassie Blue.

To perform fluorogenic orthogonal bioorthogonal transformations, **FluorCpO-HEWL** (3.48 μL of a 460 μM solution in PBS, final concentration = 40 μM), **TCO-BSA** (0.5 μL of a 160 μM solution in PBS, final concentration = 2 μM), **BODIPY-Tz** (4 μL of a 1 mM solution in DMSO, final concentration = 100 μM),[15] and **CyDPPDS** (4 μL of a 100 mM solution in PBS, final concentration = 10 mM) were mixed with bacterial lysate from TOP10 *E. coli* cells (300 μg) to a total volume of 40 μL. The reactions were incubated at 37 °C for 90 min. Samples were prepared for SDS-PAGE and in-gel fluorescence analysis in a similar manner as other protein labeling experiments.

Molecular cloning and plasmid construction methods

Polymerase chain reaction (PCR) was used to amplify the Mito-SNAP fusion protein from Addgene plasmid #136623 (pCNDA3.0_mitoSNAP was a gift from Kai Johnsson).[17] The following primers were used:

Fwd primer: 5'- GGCTAGCGCTACCGGTCGCCACCTCTAGAGCCGCACCATGGTGGGC -3'

Rev primer: 5'- GCCTCTGCCCTCGCCGCTGCCCTCGAGGCCCAGGCCTGGTTTACCCAG -3'

Successfully amplified insert was ligated into a CRISPR pcDNA3.0 destination vector (gift from Drs. Theresa Loveless and Chang Liu, UCI) via Gibson assembly.[16] Linearized vector was generated via restriction enzyme digestion with *Xba*I and *Xho*I (New England Biolabs). Primer melting temperatures (T_m) were determined using the NEB T_m calculator. All PCR reactions were performed in a BioRad C3000 Thermocycler using the following conditions: 1) 95 °C for 3 min, 2) 95 °C for 30 s, 3) T_m of primers for 30 s, 4) 72 °C for 3 min, repeat steps 2-4 twenty times, then 72 °C for 5 min, and hold at 12 °C until retrieval from the thermocycler. Gibson assembly conditions were 50 ºC for 60 min and held at 12 ºC until retrieval from the thermocycler. Ligated plasmid were transformed into the TOP10 strain of *E. coli* using the heat shock method. After incubation at 37 °C for 18-24 h, colonies were picked and subjected to colony PCR to check for successful gene insertion. Select colonies were expanded overnight in 3 mL LB broth supplemented with ampicillin (100 μg/mL). DNA was extracted from colonies using a Zymo Research Plasmid Mini-prep Kit.

Cell culture and imaging

A549 cells (ATCC), HEK cells (ATCC), and HeLa cells (ATCC) were cultured in complete media (DMEM supplemented with 10% (vol/vol) fetal bovine serum (FBS, Life Technologies), penicillin (100 U/mL), and streptomycin (100 μg/mL). Cell lines stably expressing Mito-SNAP (MitoSNAP) were generated via transfection of HEK293 with Lipofectamine3000 and the following three plasmids: AAVS1 donor plasmid containing the Mito-SNAP gene, Cas9 (Addgene #41815), and AAVS1 sgRNA (Addgene #53370). Transfected cells were further cultured in complete media with puromycin (20 μg/mL) to preserve gene incorporation. Cells were incubated at 37 °C in a 5% CO_2 humidified chamber. Cells were serially passaged using trypsin (0.25% in HBSS, Gibco).

For live cell **FluorCpO** labeling experiments, A549 cells (5.0 x 10^4 cells/well) were added to glass microscope chamber slide (Ibidi, μ-Slide 8 well) and incubated overnight. Cells were then treated with CpO **21** (4 μL of a 50 mM solution in DMSO, final concentration = 1 mM) and incubated for 15 min at 37 °C. The cells were washed with complete media (2 x 200 μL), resuspended in fresh media (200 μL), and incubated at 15 min at 37 °C. A portion of the media (20 μL) was replaced with **CyDPPDS** (**8**) (20 μL of a 10 mM solution in PBS, final concentration = 1 mM), and the cells were incubated for 30 min at 37 °C. The cells were then fixed by replacing a portion of the media with 2% formaldehyde in PBS (70 μL) and incubating for 15 min at 37 °C. The cells were then completely fixed with 2% formaldehyde in PBS (200 μL) for 15 min at 37 °C. Cells were washed with PBS (2 x 200 μL). Cells were stained with a wheat-germ agglutinin fluorescein conjugate (WGA-fluorescein, Fisher Scientific, final concentration of 5 μg/mL in Hank's buffered saline solution, HBBS) for 10 min at 37 °C. The cells were washed with HBBS (2 x 200 μL) before imaging.

After washing, images were acquired on a Zeiss LSM780 confocal microscope equipped with a 40X 1.3 NA Plan Apochromat objective lens; 405-, 488-, 561-, 640-, and two-photon 710 nm lasers; and a GAsP PMT detector. Images were acquired and processed using the Zeiss Zen Black software package. To visualize the fluorogenic CpO-phosphine reaction, fluorescence images were collected by two-photon excitation with 710 nm (2% power, 800 gain). Membranes were detected using the 488 nm laser (4% power, 800 gain). Brightfield images were also collected using a 40X objective (244 gain). Images were analyzed and merged using ImageJ Fiji.

For live cell SNAP-Tag labeling, MitoSNAP cells (5.0 x 10^4 cells/well) were added to glass microscope chamber slide (Ibidi, μ-Slide 8 well) pretreated with human plasma containing fibronectin (0.1%, Sigma Aldrich). Cells were incubated overnight. The following day, media was removed and replaced with media containing **TCO-SNAP-tag** (final concentration = 20 μM). Cells were incubated for

30 min at 37 °C, then washed with complete media (3 x 200 μL) and incubated for an additional 30 min at 37 °C. During this time, cellular mitochondria were stained with MitoView 633 (200 nM, Biotium), according to the manufacturer's protocol. The media was then removed and replaced with fresh media (150 μL) containing **Tz-BODIPY** (final concentration = 1 μM). After 20 min, images were acquired using a Keyence BZ-X810 epifluorescent microscope using both the BODIPY (λ_{ex}= 450–490 nm, λ_{em}= 500–550 nm) and Cy5 (λ_{ex}= 560–680 nm, λ_{em}= 625–775 nm) cubes. Images were collected using a 10X 0.45 NA Plan Apochromat objective lens and 60X 1.42 NA Plan Apochromat oil immersion objective lens. Images were analyzed and merged using ImageJ Fiji.

For simultaneous orthogonal fluorogenic reactions, MitoSNAP cells (5.0 x 10^4 cells/well) were added to glass microscope chamber slide (Ibidi, μ-Slide 8 well) pretreated with human plasma containing fibronectin (0.1%, Sigma Aldrich). Cells were incubated overnight. The following day, media was removed and replaced with media containing **TCO-SNAP-tag** (final concentration = 20 μM). Cells were incubated for 30 min at 37 °C, then washed with complete media (3 x 200 μL) and incubated for an additional 30 min at 37 °C. Then, the cells were resuspended in fresh media (150 μL) and treated with CpO **21** (6 μL of a 25 mM solution in DMSO, final concentration = 1 mM). The cells were incubated for 15 min at 37 °C, and then washed with complete media (2 x 200 μL).

Prior to imaging, the cells were resuspended in a solution of PBS containing **Tz-BODIPY** and **CyDPPDS** (**8**) (final concentrations = 0.25 μM and 1 mM, respectively). Images were acquired using a Keyence BZ-X810 epifluorescent microscope, with both the DAPI/coumarin (λ_{ex}= 300–400 nm, λ_{em}= 410–510 nm) and eGFP/BODIPY (λ_{ex}= 450–490 nm, λ_{em}= 500–550 nm) filter cubes. Images were collected from multiple fields of view every minute for 90 min using a 10X 0.45 NA Plan Apochromat objective lens. It should be noted that the source power was decreased to 40% to minimize photobleaching. Images were analyzed and combined into movies using ImageJ Fiji (1 min/frame, movie speed 10 frames/second).

For live cell deoxycholate imaging, HeLa cells (3.0 x 10^4 cells/well) were added to glass microscope chamber slides (Ibidi, μ-Slide 8 well) pretreated with human plasma containing fibronectin (0.1%, Sigma Aldrich). Cells were incubated overnight. The following day, cells were washed with FluoroBrite™ DMEM (Gibco) (2 x 200 μL). Media was removed and replaced with FluoroBrite™ DMEM (Gibco) containing deoxycholate modified with a FluorCpO handle (**DCA-FluorCpO**) final concentration 500 μM) and deoxycholate (0–500 μM) and incubated for 10 min at 37 °C. Prior to imaging, the media was replaced with FluoroBrite™ DMEM (Gibco) containing CyDPPDS (**8**) (final concentration 1 mM). Images were acquired using a Keyence BZ-X810 epifluorescent microscope with both the DAPI/coumarin (λ_{ex} = 300–400 nm, λ_{em} = 410–510 nm) filter cube. Images were collected after 45 min using a 40X 1.3 NA Plan Apochromat objective lens. Images were analyzed using ImageJ Fiji.

For live cell Golgi organelle labeling, HeLa cells (3.0 x 10^4 cells/well) were added to glass microscope chamber slides (Ibidi, μ-Slide 8 well) pretreated with human plasma containing fibronectin (0.1%, Sigma Aldrich). After 8 h, Golgi organelles were labeled via treatment with CellLite™ Golgi-RFP, BacMam 2.0, according to the manufacturer's protocol (Invitrogen). Cells were incubated overnight. Labeled cells were then incubated with **Cer-TCO** (2 μM final concentration) for 5 min at 37 °C. Samples were then washed with FluoroBrite™ DMEM (Gibco) (3 x 200 μL) and incubated at 25 °C for 1 h. **Cer-TCO** was then visualized via reaction with **Tz-BODIPY** (100 nM final concentration) for 45 min. Images were acquired using a Keyence BZ-X810 epifluorescent microscope with both the eGFP/BODIPY (λ_{ex} = 450–490 nm, λ_{em} = 500–550 nm) and Texas Red/RFP (λ_{ex} = 520–600 nm, λ_{em} = 555–705 nm) filter cubes. Images were collected using a 60X 1.42 NA Plan Apochromat oil immersion objective lens. Images were analyzed and merged using ImageJ Fiji.

Dual metabolite labeling was performed similarly as above. HeLa cells (3.0 x 10^4 cells/well) were added to glass microscope chamber slides (Ibidi, μ-Slide 8 well) pretreated with human plasma containing fibronectin (0.1%, Sigma Aldrich). Cells were incubated overnight. The following day, cells were incubated

with **Cer-TCO** (2 μM final concentration) for 5 min at 37 °C. The cells were then washed with FluoroBrite™ DMEM (Gibco) (3 x 200 μL) and incubated at 25 °C for 1 h. Media was removed from each sample and FluoroBrite™ DMEM (Gibco), containing **DCA-FluorCpO** (500 μM final concentration) was added. Cells were then incubated for 10 min at 37 °C. Prior to imaging, the cells were resuspended in FluoroBrite™ DMEM (Gibco) containing Tz-BODIPY and CyDPPDS (**8**) (final concentrations 100 nM and 1 mM, respectively). Images were acquired using a Keyence BZ-X810 epifluorescent microscope with both the DAPI/coumarin (λ_{ex} = 300–400 nm, λ_{em} = 410–510 nm) and eGFP/BODIPY (λ_{ex} = 450–490 nm, λ_{em} = 500–550 nm) filter cubes. Images were collected after 45 min using a 40X 1.3 NA Plan Apochromat objective lens. Images were analyzed and merged using ImageJ Fiji.

Synthetic procedures

Compounds **8**[18], **11**[19]**, 12**[20], **18**,[19] **S1**[21], **S2a**[22], **S4**[23]**, S8**[24], **S10**[25]**, Tz-BODIPY**[15], **TCO-SNAP-tag**[26], and **Cer-TCO**[27] were synthesized as previously described.

General procedure A for Sonogashira coupling

2-Iodophenyl acetate (**S1**) was prepared as previously described.[21] To an oven-dried round-bottom flask with a stir bar was added **S1** (1.0 equiv.), $Pd(PPh_3)Cl_2$ (10 mol%), CuI (10 mol%), and triethylamine (NEt_3, 0.048–0.77 M aryl iodide), and alkyne (1.2 equiv.). The mixture was stirred at ambient temperature overnight. The resulting slurry was then dissolved in EtOAc (20 mL) and washed with sat. NH_4Cl (1 x 40 mL). The organic layers were combined, dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (eluting with EtOAc in hexanes) to afford pure product.

General procedure B for cyclopropenone synthesis

To an oven-dried Schlenk tube with a stir bar was added sodium iodide (NaI, 2.2 equiv.). The tube was gently flame-dried under vacuum, then resuspended in a solution of alkyne (1 equiv.) in anhydrous THF. Trifluoromethyltrimethylsilane ($TMSCF_3$, 2 equiv.) was added, and the vessel was sealed and stirred vigorously at ambient temperature or while heating (23–110 °C) for 2 d. The slurry was diluted with water (30 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were combined, dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting crude difluorocyclopropene was incubated on silica (3 g/mmol). The crude material was purified by flash column chromatography (eluting with acetone in CH_2Cl_2) to afford pure cyclopropenone product.

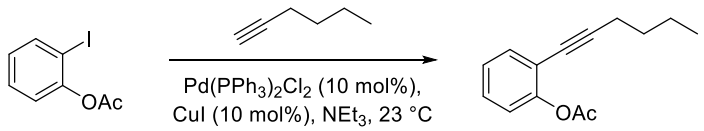
General procedure C for cyclopropenone synthesis

To an oven-dried Schlenk tube with a stir bar was added sodium iodide (NaI, 2.2 equiv.). The tube was gently flame-dried under vacuum, then resuspended in a solution of alkyne (1 equiv.) in anhydrous THF. Trifluoromethyltrimethylsilane ($TMSCF_3$, 2 equiv.) was added, and the vessel was sealed and stirred vigorously at ambient temperature or while heating (60–110 °C) for 2 d. The slurry was diluted with water (30 mL) and extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were combined, dried with $MgSO_4$, filtered, and concentrated *in vacuo*.

To an oven-dried round-bottom flask with a stir bar was added a solution of crude difluorocyclopropene in anhydrous MeOH, followed by Amberlyst-15 resin (120 mg/mmol alkyne). The mixture was stirred until starting material was fully consumed. The mixture was then filtered to remove the Amberlyst-15 resin, then concentrated *in vacuo*. The crude residue was purified by flash chromatography to afford pure product.

General procedure D for acetyl protecting group removal

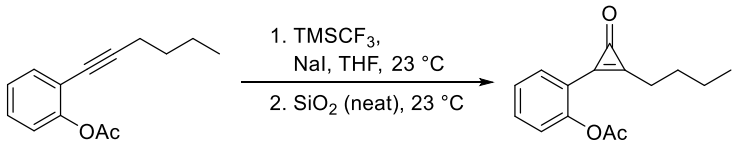
To a scintillation vial with a stir bar was added cyclopropenone (1 equiv.) and ethanol (EtOH). A solution of LiOH in water (0.4–0.5 M, 1.1–2.2 equiv.) was added dropwise, and the mixture was stirred at ambient temperatures. The reaction was acidified with 1 N HCl to pH 7 and ethanol was removed by concentration *in vacuo*. The mixture was diluted with water (10 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The organic layers were combined, washed with brine (1 x 30 mL), dried with Na_2SO_4, filtered, and concentrated *in vacuo*. If necessary, the crude residue was purified by flash column chromatography to afford pure product.



S1 **S2a**

2-(Hex-1-yn-1-yl)phenyl acetate (**S2a**)

2-Iodophenyl acetate (**S1**) was prepared as previously described.[21] General procedure A was used with the following reagents: **S1** (0.600 g, 2.30 mmol, 1.00 equiv.), $Pd(PPh_3)_2Cl_2$ (0.161 g, 0.230 mmol, 10.0 mol%), CuI, (0.044 g, 0.23 mmol, 10 mol%), 1-hexyne (0.31 mL, 2.8 mmol, 1.2 eq), and anhydrous Et_3N (30 mL, 0.08 M aryl iodide). The crude residue was purified with flash chromatography (eluting with 3% EtOAc in hexanes) to give compound **S2a** as an orange oil (0.19 g, 81%). Spectra matched those previously reported.[29]

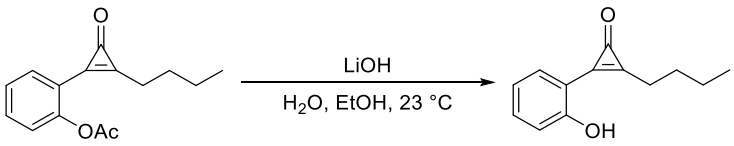


S2a **S3a**

2-(2-Butyl-3-oxocycloprop-1-en-1-yl)phenyl acetate (**S3a**)

General procedure B was used at ambient temperature with the following reagents: **S2a** (0.373 g, 1.73 mmol), NaI (0.570 g, 3.81 mmol, 2.20 equiv.), $TMSCF_3$, (0.52 mL, 3.5 mmol, 2.0 equiv.), and anhydrous THF (8.0 mL, 0.22 M); silica (10 g). The crude residue was purified with flash chromatography (eluting with 3% acetone in CH_2Cl_2) to give compound **S3a** as an orange oil (0.26 g, 62%).

^{1}H NMR (400 MHz, $CDCl_3$) 7.63 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.48 (app td, *J* = 8.1, 1.5 Hz, 1H), 7.28 (app td, *J* = 7.6, 0.8 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 2.71 (t, *J* = 7.3 Hz, 2H), 2.41 (s, 3H), 1.75–1.61 (m, 2H), 1.47–1.33 (m, 2H), 0.88 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.4, 156.0, 155.9, 151.1, 150.0, 133.8, 131.0, 126.4, 123.1, 117.7, 28.4, 26.3, 22.2, 21.0, 13.4. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{15}H_{16}O_3Na$, 267.0997; found, 267.0993.

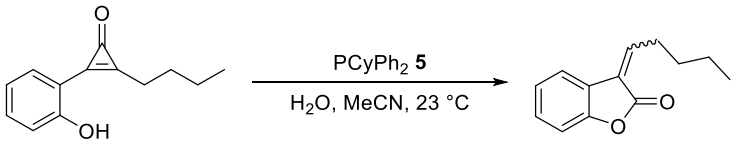


S3a **1a**

2-Butyl-3-(2-hydroxyphenyl)cycloprop-2-en-1-one (**1a**)

General procedure D was used with the following reagents: **S3a** (0.261 g, 1.07 mmol, 1.00 equiv.), LiOH (0.5 M, 2.36 mL, 1.18 mmol, 1.10 equiv.), and EtOH (12 mL, 0.089 M). Following extraction, the mixture was concentrated *in vacuo* to yield **1a** as an off-white solid (0.180 g, 84.0%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.46 (s, 1H), 7.59 (d, *J* = 7.1 Hz, 1H), 7.39 (t, *J* = 7.4 Hz, 1H), 7.16 (d, *J* = 8.2 Hz, 1H), 6.90 (t, *J* = 7.4 Hz, 1H), 2.85 (t, *J* = 7.3 Hz, 2H), 1.83–1.73 (m, 2H), 1.53–1.40 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 159.7, 159.0, 151.3, 150.78, 135.4, 132.2, 119.7, 117.2, 111.2, 28.8, 26.5, 22.6, 13.7. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{13}H_{14}O_2Na$, 225.0892; found, 225.0902.

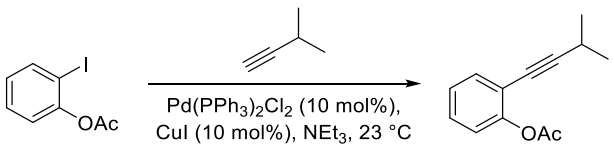


1a **3a**

3-Pentylidenebenzofuran-2(3H)-one (3a)

Compound **1a** (0.033 g, 0.17 mmol, 1.0 equiv.) was dissolved in a mixture of $H_2O/C_6H_6/CH_3CN$ mixture (0.15 mL H_2O, 0.6 mL C_6H_6, 2.0 mL CH_3CN, 0.062 M CpO) in a scintillation vial with a stir bar. Cyclohexyldiphenylphosphine (**5**, $PCyPh_2$, 0.044 g, 0.17 mmol, 1.0 equiv.) was added as a solid to the mixture, and the reaction was stirred at room temperature. The mixture was concentrated *in vacuo*. To ease purification, the remaining $PCyPh_2$ was oxidized using a dilute hydrogen peroxide solution (H_2O_2, 3% in H_2O). The crude residue was purified by flash chromatography (eluting with 5% EtOAc in hexanes) to yield compounds **3a** as a 10:1 mixture of stereoisomers (0.013 g, 38%) in the form of a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ (major stereoisomer) 7.59 (d, *J* = 7.5 Hz, 1H), 7.34 (app td, *J* = 7.9, 1.3 Hz, 1H), 7.20–7.11 (m, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 1.70–1.56 (m, 2H), 1.54–1.42 (m, 2H), 1.01–0.94 (m, 4H). (minor stereoisomer) 7.50–7.44 (m, 1H), 7.42 (dd, *J* = 7.9, 1.3 Hz, 1H), 7.20–7.11 (m, 3H), 7.01 (t, *J* = 8.0 Hz, 1H), 2.97 (app dd, *J* = 15.2, 7.8 Hz, 1H), 1.70–1.56 (m, 2H), 1.54–1.42 (m, 2H), 1.01–0.94 (m, 4H). ^{13}C NMR (151 MHz, $CDCl_3$) δ (major stereoisomer) 168.2, 154.0, 146.9, 130.0, 124.1, 123.6, 123.4, 123.0, 111.2, 30.56, 29.6, 22.7, 14.0. (minor stereoisomer) 166.8, 152.8, 147.0, 129.7, 124.4, 123.9, 123.8, 122.8, 111.0, 31.1, 28.2, 22.6, 14.0. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{13}H_{14}O_3Na$, 225.0802; found, 225.0902.

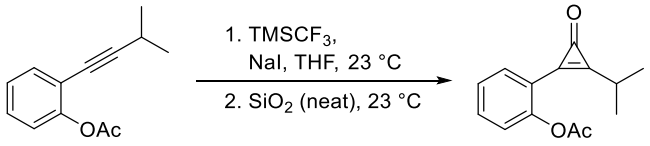


S1 **S2b**

2-(3-Methylbut-1-yn-1-yl)phenyl acetate (**S2b**)

General procedure A was used with the following amounts of reagents: 2-iodophenol acetate (**S1**, 0.300 g, 1.15 mmol, 1.00 equiv.), $Pd(PPh_3)_2Cl_2$ (0.080 g, 0.11 mmol, 10 mol%), CuI, (0.021 g, 0.11 mmol, 10 mol%), 3-methyl-1-butyne (0.34 mL, 3.3 mmol, 3.0 eq), and anhydrous Et_3N (3.0 mL, 0.038 M). The crude residue was purified with flash chromatography (eluting with 1.5% EtOAc in hexanes) to give compound **S2b** as an orange oil (0.24 g, 95%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.29 (app td, *J* = 7.9, 1.4 Hz, 1H), 7.17 (app t, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 8.1 Hz, 1H), 2.80 (hept, *J* = 6.9 Hz, 1H), 2.34 (s, 3H), 1.27 (d, *J* = 6.9 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.9, 151.7, 133.1, 128.8, 125.9, 122.1, 118.2, 101.0, 74.8, 23.1, 21.4, 21.0. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{13}H_{14}O_2Na$, 225.0892; found, 225.0889.

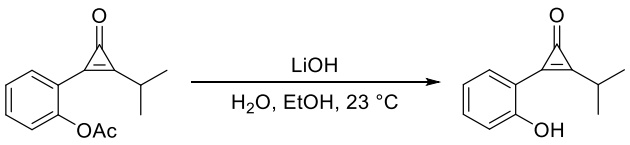


S2b **S3b**

2-(2-Isopropyl-3-oxocycloprop-1-en-1-yl)phenyl acetate (**S3b**)

General procedure B was used at ambient temperature with the following reagents: **S2b** (0.243 g, 1.20 mmol, 1.00 equiv.), NaI (0.375 g, 2.60 mmol, 2.20 equiv.), $TMSCF_3$, (0.36 mL, 2.4 mmol, 2.0 equiv.), and anhydrous THF (5.0 mL, 0.24 M); silica (5 g). The crude residue was purified with flash chromatography (eluting with 10% acetone in CH_2Cl_2) to give compound **S3b** as a yellow oil (0.16 g, 57%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.62 – 7.55 (m, 1H), 7.38 (td, *J* = 7.6, 1.1 Hz, 1H), 7.24 (dd, *J* = 8.2, 1.0 Hz, 1H), 3.17 (hept, *J* = 7.0 Hz, 1H), 2.52 (s, 3H), 1.42 (d, *J* = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.9, 160.3, 156.2, 151.9, 149.2, 134.1, 131.3, 126.7, 123.6, 117.9, 27.9, 21.4, 20.4. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{14}H_{14}O_3Na$, 253.0841; found, 253.0843.

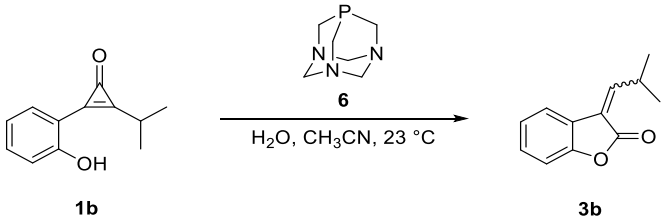


S3b **1b**

2-(2-Hydroxyphenyl)-3-isopropylcycloprop-2-en-1-one (**1b**)

General procedure D was used with the following reagents: **S3b** (0.101 g, 0.440 mmol, 1.00 equiv.), LiOH (0.50 M, 1.2 mL, 0.63 mmol, 1.1 equiv.), and EtOH (6 mL). The mixture was concentrated *in vacuo* to yield compound **1b** as a white solid (0.010 g, 80%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.44 (ddd, *J* = 8.8, 7.4, 1.7 Hz, 1H), 7.16 (dd, *J* = 8.3, 0.6 Hz, 1H), 6.98–6.91 (m, 1H), 3.19 (hept, *J* = 7.0 Hz, 1H), 1.45 (d, *J* = 7.0 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 160.6, 158.5, 154.4, 150.0, 135.6, 131.3, 120.0, 117.5, 110.8, 27.6, 20.4.
HRMS–GC-MS (*m/z*): $[M]^+$ calcd for $C_{12}H_{12}O_2$, 188.0837; found, 188.0844.

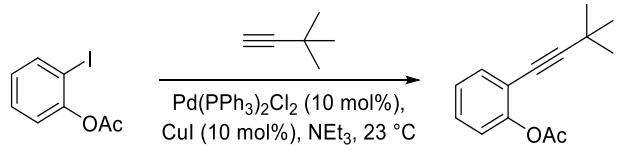


1b **3b**

3-(2-Methylpropylidene)benzofuran-2(3H)-one (3b)

Compound **1b** (0.015 g, 0.080 mmol, 1.0 equiv.) was dissolved in a mixture of H_2O/CH_3CN mixture (4 mL H_2O, 4.0 mL CH_3CN, 0.01 M CpO) in a scintillation vial with a stir bar. 1,3,5-Triaza-7-phosphaadamantane (**6**, PTA, 0.025 g, 0.16 mmol, 2.0 equiv.) was added as a solid to the mixture, and the reaction was stirred at room temperature. The mixture was concentrated *in vacuo*. The crude residue was purified by flash chromatography (eluting with 5% EtOAc in pentanes) to yield compound **3b** as a 10:1 mixture of stereoisomers (0.0024 g, 16%) as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ (major stereoisomer) δ 7.58 (d, *J* = 7.6 Hz, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.17 (t, *J* = 7.6 Hz, 1H), 7.15–7.12 (m, 1H), 6.97 (d, *J* = 10.0 Hz, 1H), 3.25 (dhept, *J* = 10.3, 6.6 Hz, 1H), 1.24 (d, *J* = 6.7 Hz, 6H). (minor stereoisomer) 7.44–7.41 (m, 1H), 7.31 (t, *J* = 7.9 Hz, 1H), 7.15–7.12 (m, 1H), 7.08 (d, *J* = 7.9 Hz, 1H), 6.80 (d, *J* = 10.2 Hz, 1H), 4.00–3.90 (m, 1H), 1.17 (d, *J* = 6.6 Hz, 6H).^{13}C NMR (151 MHz, $CDCl_3$) δ (major stereoisomer) 168.6, 154.0, 152.8, 130.0, 124.1, 123.6, 122.6, 121.6, 111.3, 29.1, 21.7. (minor stereoisomer) 166.6, 152.9, 152.9, 129.7, 123.9, 123.8, 120.9, 119.8, 111.0, 27.2, 22.3. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{12}H_{12}O_2Na$, 211.0837; found, 211.0830.

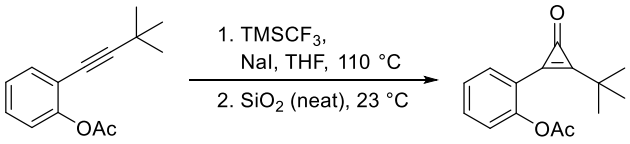


S1 **S2c**

2-(3,3-Dimethylbut-1-yn-1-yl)phenyl acetate (**S2c**)

General procedure A was used with the following amounts of reagents: **S1** (0.359 g, 1.37 mmol, 1.0 equiv.), $Pd(PPh_3)_2Cl_2$ (0.080 g, 0.11 mmol, 8 mol%), CuI, (0.021 g, 0.11 mmol, 8 mol%), 3,3-dimethyl-1-butyne (0.39 mL, 1.4 mmol, 3.0 equiv.), and anhydrous Et_3N (25 mL, 0.55 M). The crude residue was purified with flash chromatography (eluting with 10% EtOAc in hexanes) to give compound **S2c** as an orange oil (0.26 g, 89%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.29 (app td, *J* = 7.8, 1.6 Hz, 1H), 7.17 (app td, *J* = 7.6, 1.1 Hz, 1H), 7.06 (dd, *J* = 8.1, 0.9 Hz, 1H), 2.34 (s, 3H), 1.32 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.8, 151.6, 133.1, 128.7, 125.9, 122.1, 118.2, 103.8, 74.1, 31.1, 28.3, 21.0.
HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{14}H_{16}O_2Na$, 239.1048; found, 239.1047.

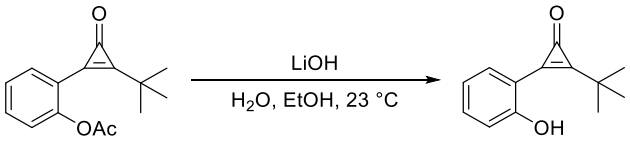


S2c **S3c**

2-(2-(*tert*-Butyl)-3-oxocycloprop-1-en-1-yl)phenyl acetate (**S3c**)

General procedure B was used with heating at 110 °C with the following reagents: **S2c** (0.108 g, 0.500 mmol), NaI (0.165 g, 1.10 mmol, 2.2 equiv.), $TMSCF_3$, (0.15 mL, 1.0 mmol, 2.0 equiv.), and anhydrous THF (8.0 mL, 0.063 M alkyne); silica (5 g). The crude residue was purified with flash chromatography (eluting with 10% acetone in CH_2Cl_2) to give compound **S3c** as an orange oil (0.078 g, 57%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75–7.69 (m, 1H), 7.58 (ddd, *J* = 8.2, 7.5, 1.7 Hz, 1H), 7.38 (app td, *J* = 7.6, 1.2 Hz, 1H), 7.25–7.18 (m, 1H), 2.53 (s, 3H), 1.45 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.1, 162.8, 156.4, 152.3, 148.3, 134.0, 131.1, 126.7, 123.7, 118.0, 34.3, 28.4, 21.5. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{15}H_{16}O_3Na$, 267.0997; found, 267.1002.

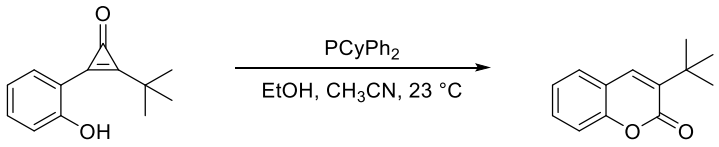


S3c **1c**

2-(*tert*-Butyl)-3-(2-hydroxyphenyl)cycloprop-2-en-1-one (**1c**)

General procedure D was used with the following reagents: **S3c** (0.078 g, 0.32 mmol, 1.0 equiv.), LiOH in water (0.5 M, 0.70 mL, 0.35 mmol, 1.1 equiv.), and EtOH (8.0 mL, 0.040 M CpO). The mixture was concentrated *in vacuo* to yield **1c** as an off-white solid (0.056 g, 87%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.56 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.47–7.38 (m, 1H), 7.17 (d, *J* = 8.3 Hz, 1H), 6.94 (app t, *J* = 7.5 Hz, 1H), 1.48 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.0, 158.5, 156.6, 149.2, 135.6, 131.2, 119.8, 117.6, 110.6, 77.4, 77.2, 77.0, 33.9, 28.4. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{13}H_{14}O_2Na$, 225.0892; found, 225.0890.

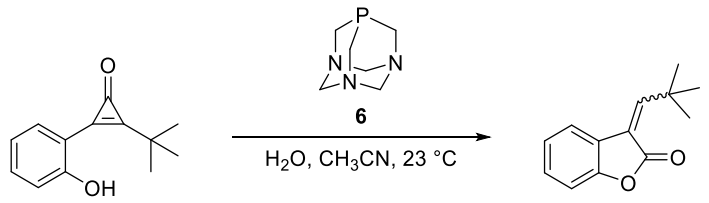


1c **2c**

3-(*tert*-Butyl)-2H-chromen-2-one (**3c**)

Compound **1c** (0.012 g, 0.059 mmol, 1.0 equiv.) was dissolved in a mixture of organic solvents (0.10 mL EtOH, 2.5 mL CH_3CN, 0.022 M) in a scintillation vial with a stir bar. Cyclohexyldiphenylphosphine (**5**, $PCyPh_2$, 0.016 g, 0.059 mmol, 1.0 equiv.) was added as a solid to the mixture, and the reaction was stirred at room temperature. The mixture was concentrated *in vacuo*. To ease chromatographic separation, remaining phosphine **5** was oxidized with a dilute hydrogen peroxide solution (0.1% H_2O_2, H_2O, CH_3CN). The mixture was then concentrated *in vacuo*. The crude residue was purified by flash chromatography

(eluting with CH_2Cl_2) to yield compound **2c** as a white solid (0.064 g, 48%). Spectra matched those previously reported.[8]

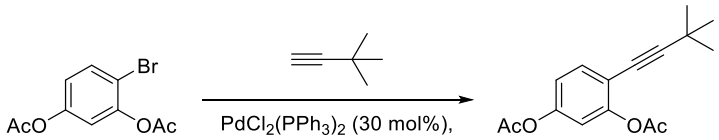


1c **3c**

3-(2,2-Dimethylpropylidene)benzofuran-2(3H)-one (**3c**)

Compound **1c** (0.020 g, 0.099 mmol, 1.0 equiv.) was dissolved in a mixture of H_2O and CH_3CN (4 mL H_2O, 4.0 mL CH_3CN, 0.01 M CpO) in a scintillation vial with a stir bar. 1,3,5-Triaza-7-phosphaadamantane (**6**, PTA, 0.031 g, 0.20 mmol, 2.0 equiv.) was added as a solid to the mixture, and the reaction was stirred at room temperature. The reaction was then concentrated *in vacuo*. The crude residue was purified with flash chromatography (eluting with CH_2Cl_2). During purification and characterization, the mixture of stereoisomers converted to a single product **3c** (0.0040 g, 20%) as a beige solid.

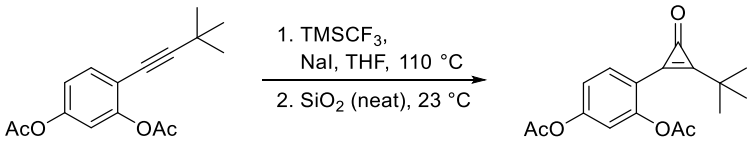
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.35 (td, *J* = 7.8, 1.3 Hz, 1H), 7.27 (s, 1H), 7.19 (td, *J* = 7.7, 1.1 Hz, 1H), 7.14 (ddd, *J* = 8.0, 1.1, 0.5 Hz, 1H), 1.41 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.5, 157.5, 154.6, 130.1, 126.4, 123.9, 122.2, 121.3, 111.4, 33.5, 29.1. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{13}H_{16}O_2Na$, 227.1048; found, 227.1066.



S4 **S5**

4-(3,3-Dimethylbut-1-yn-1-yl)-1,3-phenylene diacetate (**S4**)

4-Bromo-1,3-phenylene diacetate (**S4**) was prepared as previously described.[23] To an oven-dried round-sealed tube with a stir bar was added **S4** (0.400 g, 1.48 mmol, 1.00 equiv.), $Pd(PPh_3)Cl_2$ (0.312 g, 0.440 mmol, 30.0 mol%), CuI (0.020 g, 0.15 mmol, 10 mol%), and anhydrous NEt_3 (14 mL, 0.11 M aryl iodide). 3,3-Dimethyl-1-butyne (0.92 mL, 7.4 mmol, 5.0 equiv.) was added dropwise to the reaction over a period of 30 min. The tube was then sealed, and the mixture was stirred at 60 °C overnight. The resultant slurry was then washed with saturated NH_4Cl (1 x 40 mL). and extracted into EtOAc (3 x 20 mL) The organic layers were combined, dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (eluting with 5% EtOAc in hexanes) to afford an amber oil. This oil comprised an inseparable mixture of the desired aryl alkyne product **S5** and remaining aryl bromide starting material (90% purity, 9 **S5** : 1 aryl bromide **S4**). This mixture was used directly in the next step without further purification.

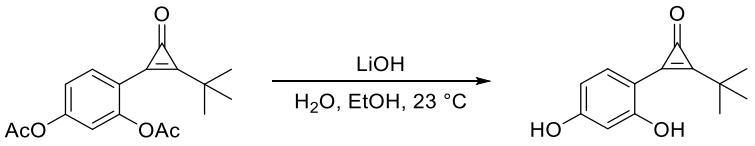


S5 **S6**

4-(2-(*tert*-Butyl)-3-oxocycloprop-1-en-1-yl)-1,3-phenylene diacetate (**S6**)

General procedure B was used with heating at 110 °C with the following reagents: **S5** (0.100g, 0.360 mmol, 1 equiv., 90% purity), NaI (0.120 g, 0.800 mmol, 2.2 equiv.), $TMSCF_3$, (0.11 mL, 0.72 mmol, 2.0 equiv.), and anhydrous THF (4.0 mL, 0.090 M alkyne); silica (3 g). The crude residue was purified with flash chromatography (eluting with 3% acetone in CH_2Cl_2) to give compound **S6** as a yellow oil (0.029 g, 27% over two steps from **S4**).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.5 Hz, 1H), 7.17 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.08 (d, *J* = 2.2 Hz, 1H), 2.52 (s, 3H), 2.32 (s, 3H), 1.44 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.6, 168.4, 162.2, 156.0, 154.5, 153.1, 147.5, 131.7, 120.0, 117.3, 115.5, 34.2, 28.4, 21.4, 21.3. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{17}H_{18}O_5Na$, 325.1052; found, 325.1053.

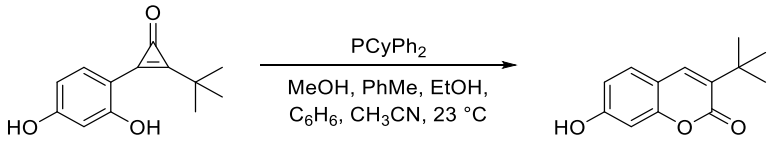


S6 **7**

2-(*tert*-Butyl)-3-(2,4-dihydroxyphenyl)cycloprop-2-en-1-one (**7**)

General procedure D was used with the following reagents: **S6** (0.029 g, 0.096 mmol, 1.0 equiv.), LiOH in water (0.25 M, 1.5 mL, 0.38 mmol, 4.0 equiv.), and EtOH (3.6 mL, 0.027 M CpO). The mixture was quenched with saturated $NaHCO_3$ (5 mL) and concentrated *in vacuo* to remove EtOH. The mixture was extracted with EtOAc (3 x 5 mL). The organic layers were combined, dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (eluting with 30% acetone in CH_2Cl_2) to afford compound **7** as a yellow solid (0.0096 g, 46%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.4 Hz, 1H), 6.44–6.36 (m, 2H), 1.41 (s, 9H). ^{13}C NMR (151 MHz, CD_3OD) δ 165.8, 161.8, 159.9, 153.4, 148.6, 136.8, 109.3, 104.1, 103.3, 34.8, 28.7. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{13}H_{14}O_3Na$, 241.0841; found, 241.0832.

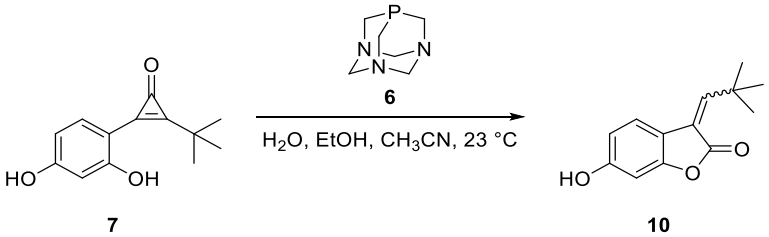


7 **9**

3-(*tert*-Butyl)-7-hydroxy-2H-chromen-2-one (**9**)

Compound **7** (0.0097 g, 0.044 mmol, 1.0 equiv.) was dissolved in a mixture of organic solvents (0.11 mL MeOH, 0.22 mL PhMe, 0.26 mL C_6H_6, 0.44 mL EtOH, 0.79 mL CH_3CN, 0.023 M) in a scintillation vial with a stir bar. Cyclohexyldiphenylphosphine (**5**, $PCyPh_2$, 0.012 g, 0.044 mmol, 1.0 equiv.) was added as a solid to the mixture, and the reaction was covered in aluminum foil and stirred at room temperature. The mixture was concentrated *in vacuo*. The crude residue was purified by flash chromatography (eluting with 20% EtOAc in hexanes) to yield compound **9** as a white solid (0.064 g, 66%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.55 (s, 1H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.07 (d, *J* = 2.3 Hz, 1H), 6.87 (dd, *J* = 8.5, 2.3 Hz, 1H), 1.40 (s, 9H). δ 7.50 (s, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 6.84 (d, *J* = 2.5 Hz, 1H), 6.77 (dd, *J* = 8.4, 2.4 Hz, 1H), 1.39 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 160.6, 158.3, 154.8, 137.0, 133.7, 129.05, 113.5, 112.8, 102.6, 77.3, 77.2, 77.0, 34.9, 28.8. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{13}H_{14}O_3Na$, 241.0841; found, 241.0840.

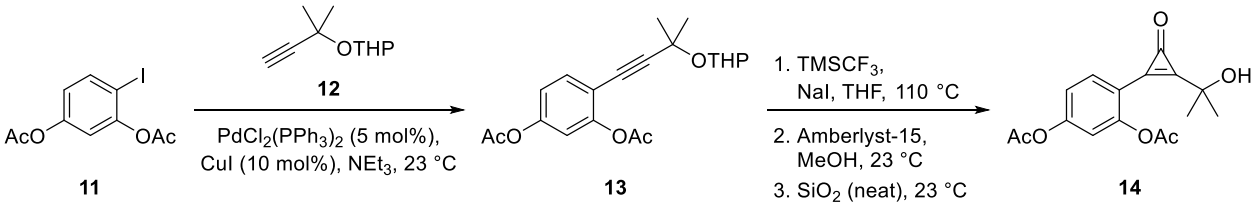


7 **10**

3-(2,2-Dimethylpropylidene)-6-hydroxybenzofuran-2(3H)-one (**10**)

Compound **7** (0.043 g, 0.20 mmol, 1.0 equiv.) was dissolved in a mixture of EtOH/H_2O/CH_3CN mixture (0.50 mL EtOH, 0.40 mL H_2O, 3.6 mL CH_3CN, 0.044 M) in a scintillation vial with a stir bar. PTA (**6**, 0.062 g, 0.40 mmol, 2.0 equiv.) was added as a solid to the mixture, and the reaction was stirred at room temperature. The mixture was concentrated *in vacuo*. The crude residue was purified by flash chromatography (eluting with 20% EtOAc in hexanes) to yield compound **10** as a 1.1:1 mixture of stereoisomers (0.033 g, 77%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ (major stereoisomer) 7.25–7.20 (m, 1H), 6.90 (s, 1H), 6.72–6.66 (m, 2H), 6.65–6.58 (m, 2H), 1.39 (s, 9H). (minor stereoisomer) 7.57 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.09 (s, 1H), 6.72–6.66 (m, 2H), 6.65–6.60 (m, 2H), 1.37 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (major stereoisomer) 170.7, 157.9, 155.8, 154.4, 127.4, 121.8, 117.8, 111.2, 98.9, 34.0, 29.3. (minor stereoisomer) 165.9, 157.5, 154.1, 154.0, 122.1, 120.4, 113.9, 111.4, 99.4, 33.3, 29.1. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{13}H_{14}O_3Na$, 241.0841; found, 241.0846.



11 **13** **14**

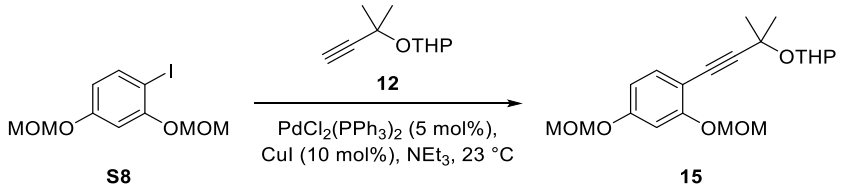
4-(2-(2-Hydroxypropan-2-yl)-3-oxocycloprop-1-en-1-yl)-1,3-phenylene diacetate (**14**)

4-Iodo-1,3-phenylene diacetate (**11**) and 2-((2-Methylbut-3-yn-2-yl)oxy)tetrahydro-2*H*-pyran (**12**) was prepared as previously described.[19,28] General procedure A was used with the following amounts of reagents: **11** (0.256 g, 0.790 mmol, 1.00 equiv.), $Pd(PPh_3)_2Cl_2$ (0.028 g, 0.040 mmol, 5.0 mol%), CuI, (0.016 g, 0.079 mmol, 10 mol%), **12** (0.239 g, 1.42 mmol, 2.00 equiv.), and anhydrous Et_3N (7.0 mL, 0.11 M aryl iodide). The crude residue was purified with flash chromatography (eluting with 20% EtOAc in hexanes) to give compound **13** as an orange oil. This oil comprised the desired product **13** and the aryl

iodide starting material **11** (10 alkyne product **13** : 1 aryl iodide **11**, 0.22 g, 78%). The material was used without further purification.

To prepare CpO **14**, general procedure C was used with heating at 110 °C with the following amounts of reagents: **13** (0.18 g, 0.51 mmol, 1.0 equiv.), NaI (0.168 g, 1.12 mmol, 2.20 equiv.), $TMSCF_3$ (0.15 mL, 1.02 mmol, 2.0 equiv.), and anhydrous THF (6.0 mL, 0.085 M alkyne); Amberlyst-15 (70 mg) and anhydrous MeOH (8.0 mL, 0.064 M CpO); silica (3 g). The crude residue was purified with flash chromatography (eluting with EtOAc) to give compound **14** as a yellow oil (0.0042 g, 3%). An insufficient amount of material was isolated for ^{13}C NMR.

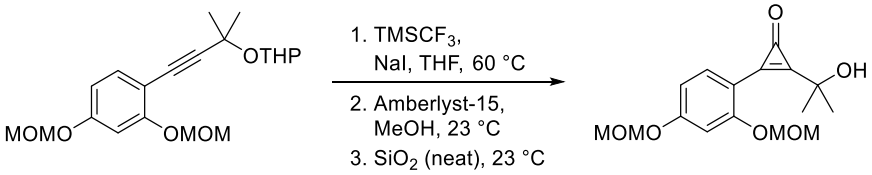
^1H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.5 Hz, 1H), 7.14 (ddd, *J* = 8.5, 2.2, 1.0 Hz, 1H), 7.06 (dd, *J* = 2.2, 0.6 Hz, 1H), 2.50 (s, 3H), 2.32 (s, 3H), 1.64 (s, 6H). HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{16}H_{16}O_6Na$, 327.0845; found, 327.0859.



S8 **15**

2-((4-(2,4-Bis(methoxymethoxy)phenyl)-2-methylbut-3-yn-2-yl)oxy)tetrahydro-2H-pyran (**15**)

1-Iodo-2,4-bis(methoxymethoxy)benzene (**S8**) was prepared as previously described.[24] General procedure A was used with the following amounts of reagents: **S8** (0.199 g, 0.614 mmol, 1.00 equiv.), $Pd(PPh_3)_2Cl_2$ (0.022 g, 0.031 mmol, 5.0 mol%), CuI, (0.012 g, 0.061 mmol, 10.0 mol%), 3,3-dimethyl-1-butyne (0.17 mL, 0.74 mmol, 1.2 equiv.), and anhydrous Et_3N (6.1 mL, 0.10 M alkyne). The crude residue was purified with flash chromatography (eluting with 10% EtOAc in hexanes) to give compound **15** as an orange oil (0.18 g, 81%). This oil comprised the desired product **15** and the aryl iodide **S8** (9 alkyne **15** : 1 aryl iodide **S8**). The material was used without further purification.



15 **16**

2-(2,4-Bis(methoxymethoxy)phenyl)-3-(2-hydroxypropan-2-yl)cycloprop-2-en-1-one (**16**)

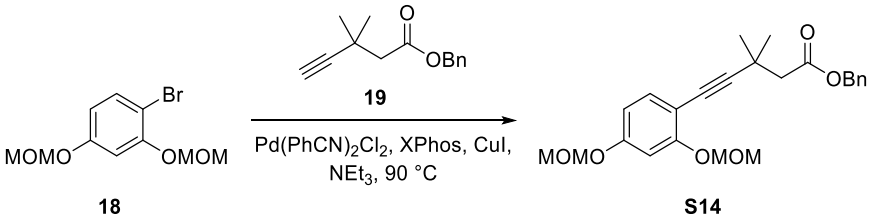
To prepare CpO **16**, general procedure C was used with heating at 60 °C with the following amounts of reagents: **15** (0.401 g, 1.10 mmol, 1.00 equiv.), NaI (0.363 g, 2.42 mmol, 2.20 equiv.), $TMSCF_3$ (0.33 mL, 2.2 mmol, 2.0 equiv.), and anhydrous THF (11.0 mL, 0.1 M alkyne); Amberlyst-15 (70 mg) and anhydrous MeOH (8.0 mL, 0.064 M CpO); silica (4 g). The crude residue was purified with flash chromatography (eluting with EtOAc) to give compound **16** as a yellow oil (0.12 g, 34% over two steps).

^1H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.6 Hz, 1H), 6.89 (d, *J* = 2.3 Hz, 1H), 6.79 (dd, *J* = 8.6, 2.3 Hz, 1H), 5.35 (s, 2H), 5.22 (s, 2H), 4.30 (s, 1H), 3.54 (s, 3H), 3.48 (s, 3H), 1.62 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.8, 157.1, 155.2, 153.3, 146.9, 136.7, 109.9, 107.2, 103.1, 95.19, 94.4, 70.2, 57.1, 56.6, 28.8. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{16}H_{20}O_6Na$, 309.1338; found, 309.1325.

Benzyl 3,3-dimethylpent-4-ynoate (**19**)

To an oven-dried round-bottom flask with a stir bar was added **S10** (0.279 g, 2.21 mmol, 1.00 equiv.) and anhydrous DMF (6.0 mL, 0.37 M alkyne). Ground and dried K_2CO_3 (0.468 g, 3.39 mmol, 1.53 equiv.) and BnBr (0.31 mL, 2.6 mmol, 1.2 equiv.) were added. The mixture was stirred at ambient temperature overnight. Water (20 mL) was then added and the mixture was extracted into EtOAc (3 x 20 mL). The combined organic layers were washed with brine (1 x 40 mL), dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (eluting with 5% Et_2O in hexanes) to afford **19** as an off-clear oil (0.32 g, 71%).

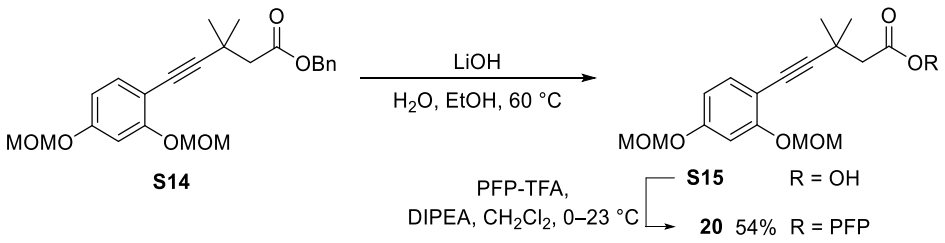
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.31 (m, 5H), 5.15 (s, 2H), 2.53 (s, 2H), 2.14 (s, 1H), 1.37 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.7, 136.1, 128.7, 128.5, 128.3, 90.2, 68.6, 66.4, 47.0, 29.9, 29.3. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{14}H_{16}O_2Na$, 239.1048; found, 239.1050.



Benzyl 5-(2,4-bis(methoxymethoxy)phenyl)-3,3-dimethylpent-4-ynoate (**S14**)

Compound **18** was prepared as previously described.[19] To an oven-dried Schlenk tube with a stir bar was added $Pd(PhCN)_2Cl_2$ (0.372 g, 0.970 mmol, 50.0 mol%), XPhos (0.920 g, 1.93 mmol, 100 mol%), and CuI (0.184 g, 0.97 mmol, 100 mol%), and **18** (0.532 g, 1.93 mmol, 1.00 equiv.) in a solution of anhydrous NEt_3 (15.4 mL, 0.125 M aryl bromide.). Alkyne **19** (0.624 g, 2.89 mmol, 1.50 equiv.) was added in a solution of anhydrous NEt_3 (4.6 mL, 0.20 M alkyne). The vessel was sealed and heated at 90 °C overnight. After cooling to ambient temperature, the resultant slurry was diluted with saturated NH_4Cl (20 mL) and extracted into EtOAc (3 x 20 mL). The organic layers were combined and dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by flash chromatography (eluting with 15% EtOAc in hexanes) to afford an orange oil (0.79 g, 89%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38–7.29 (m, 1H), 7.21 (d, J = 8.5 Hz, 1H), 6.74 (d, J = 1.7 Hz, 1H), 6.63 (dd, J = 8.5, 1.8 Hz, 1H), 5.18 (s, 2H), 5.16–5.14 (m, 4H), 3.50 (s, 3H), 3.48 (s, 3H), 2.62 (s, 2H), 1.45 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.9, 158.8, 158.0, 136.1, 134.3, 128.6, 128.4, 128.2, 109.6, 108.3, 105.0, 98.4, 95.5, 94.6, 66.3, 56.4, 56.3, 47.5, 30.9, 29.5. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{24}H_{28}O_6Na$, 435.1783; found, 435.1794.

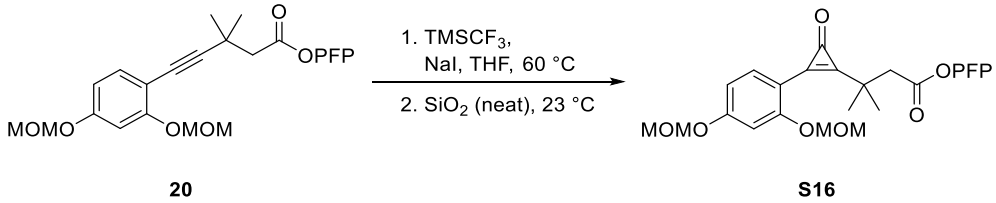


Perfluorophenyl 5-(2,4-bis(methoxymethoxy)phenyl)-3,3-dimethylpent-4-ynoate (**20**)

To an oven-dried round-bottom flask with a stir bar was added **S14** (0.713 g, 1.72 mmol, 1.00 equiv.), LiOH in water (0.40 M, 6.5 mL, 2.6 mmol, 1.5 equiv.), and EtOH (17 mL, 0.10 M aryl alkyne). The mixture was heated overnight at 60 °C. After cooling to ambient temperatures, the ethanol was removed by concentration *in vacuo*. The crude reaction mixture was then acidified to pH 1 using 2 N HCl and extracted into EtOAc (3 x 20 mL). The organic layers were combined and dried with Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (eluting with 0.1% AcOH in 30% EtOAc in hexanes) to give the corresponding carboxylic acid (**S15**) as a yellow solid. This material was used directly in the next step without further purification.

To an oven-dried round-bottom flask with a stir bar was added **S15** (0.493 g, 1.53 mmol, 1.0 equiv.), DIPEA (0.53 mL, 3.1 mmol, 1.2 equiv.), and anhydrous CH_2Cl_2 (5.0 mL, 0.30 M aryl alkyne). The solution was stirred under an atmosphere of N_2 and cooled in an ice bath. Pentafluorophenol trifluoroacetate (0.40 mL, 2.3 mmol, 1.5 equiv.) was added dropwise. The reaction was warmed to room temperature and stirred overnight. Excess solvent was removed *in vacuo*, and the crude reaction mixture was purified by flash column chromatography (eluting with 20% Et_2O in hexanes) to give **20** as a yellow oil (0.45 g, 54% over 2 steps).

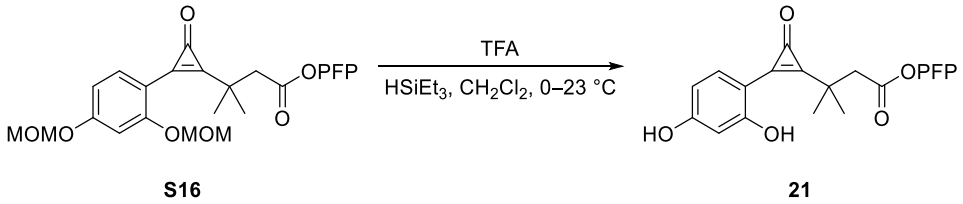
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (d, *J* = 8.3 Hz, 1H), 6.75 (d, *J* = 2.4 Hz, 1H), 6.65 (dd, *J* = 8.6, 2.2 Hz, 1H), 5.19 (s, 2H), 5.15 (s, 2H), 3.51 (s, 3H), 3.47 (s, 3H), 2.91, (s, 2H), 1.54 (s, 6H). ^{19}F NMR (376 MHz, $CDCl_3$) δ -152.29 – -152.64 (m), -158.61 (t, *J* = 21.7 Hz), -162.93 (app tq, *J* = 8.9, 4.3 Hz). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.6, 158.7, 158.1, 142.2–142.0 (m), 140.8–140.1, 139.3–138.2 (m), 137.6–136.8 (m), 134.2, 125.1 (app td, *J* = 14.5, 4.9 Hz), 109.6, 107.9, 104.8, 97.1, 95.3, 94.5, 77.3, 56.2, 56.1, 46.3, 30.9, 29.2. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{23}H_{21}O_6F_5Na$, 511.1156; found, 511.1150.



Perfluorophenyl 3-(2-(2,4-bis(methoxymethoxy)phenyl)-3-oxocycloprop-1-en-1-yl)-3-methylbutanoate (**S16**)

General procedure B was used at 60 °C with the following reagents: **20** (0.25 g, 0.52 mmol), NaI (0.172 g, 1.13 mmol, 2.20 equiv.), $TMSCF_3$ (0.15 mL, 1.0 mmol, 2.0 equiv.), and anhydrous THF (5.3 mL, 0.099 M); silica (5 g). The crude residue was purified with flash chromatography (eluting with EtOAc) to give compound **S16** as a yellow oil (0.161 g, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.6 Hz, 1H), 6.91 (d, *J* = 2.2 Hz, 1H), 6.79 (dd, *J* = 8.6, 2.2 Hz, 1H), 5.35 (s, 2H), 5.22 (s, 2H), 3.53 (s, 3H), 3.49 (s, 3H), 3.18 (s, 2H), 1.61 (s, 6H). ^{19}F NMR (376 MHz, $CDCl_3$) δ -152.4 – -152.5 (m, 2F), -157.8 (t, *J* = 21.7 Hz, 1F), -162.2 – -162.4 (m, 2F). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.6, 162.8, 157.9, 157.0, 153.3, 149.0, 140.3 (m), 137.1 (m), 135.9, 135.5 (m), 124.8 (m), 109.5, 107.6, 103.1, 94.7, 94.4, 56.8, 56.6, 43.9, 35.9, 26.2. HRMS–ESI+ (*m/z*): $[M+Na]^+$ calcd for $C_{24}H_{21}O_7F_5Na$, 539.1105; found, 539.1103.



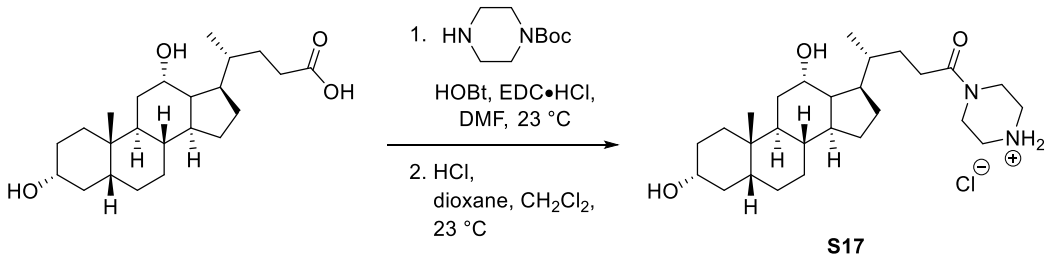
S16 **21**

Perfluorophenyl 3-(2-(2,4-dihydroxyphenyl)-3-oxocycloprop-1-en-1-yl)-3-methylbutanoate (**21**)

This compound was prepared following the general procedure of Luo *et al.* with some modifications.[30] To an oven-dried round bottom flask with a stir bar was added **S16** (0.020 g, 0.039 mmol, 1.0 equiv.) in anhydrous CH_2Cl_2 (1.2 mL). Trifluoroacetic acid (TFA, 1.2 mL, 0.016 M CpO) and triethylsilane ($HSiEt_3$, 0.080 mL, 0.050 mmol, 13 equiv.) were added dropwise. The crude reaction mixture was concentrated *in vacuo*. The reaction was diluted with cold CH_2Cl_2 (3 x 3 mL) and filtered. The filtrate was discarded, and the solid was rinsed with CH_3CN (3 x 3 mL). The filtrate was collected and concentrated i*n vacuo* to give **21** as an orange solid. Compound **21** was used directly without further purification (0.016 g, 87%).

Reagent **21** underwent degradation to the free carboxylic acid in organic solution over time, preventing characterization by ^{13}C NMR.

^{1}H NMR (600 MHz, CD_3CN) δ 7.50 (d, *J* = 8.6 Hz, 1H), 6.49 – 6.45 (m, 2H), 3.22 (s, 2H), 1.53 (s, 6H). ^{19}F NMR (376 MHz, CD_3CN) -154.6 – -154.7 (m, 2F), -160.5 – 160.6 (m, 1F), -164.7 – -164.9 (m, 2F). HRMS–ESI+ (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{14}O_5F_5$, 429.0761; found, 429.0772.



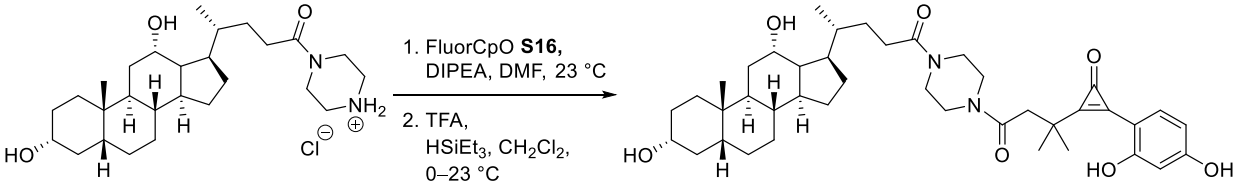
S17

Deoxycholate-piperazine conjugate (S17)

To a flame-dried round bottom flask with a stir bar was added sodium deoxycholate (0.300 g, 0.720 mmol, 1.00 equiv.) in anhydrous DMF (10 mL, 0.072 M). HOBt (0.108 g, 0.800 mmol, 1.10 equiv.), EDC•HCl (207 mg, 1.08 mmol, 1.50 equiv.) were added and the solution was stirred at room temperature for 30 min. 1-Boc-piperazine (0.135 mg, 0.720 mmol, 1.00 equiv.) was added dropwise in anhydrous DMF (5.0 mL, 0.048 M sodium deoxycholate) and stirred at room temperature overnight. The reaction was diluted with EtOAc (50 mL) and washed with H_2O (3 x 50 mL). The organic layer was dried with anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting solid was redissolved in anhydrous CH_2Cl_2 (5 mL)

followed by addition of 4 N HCl in dioxane (10 mL) and stirred at room temperature for 2 h. Solvent was removed *in vacuo* to afford **S17** as a white solid (235 mg, 71%).

^{1}H NMR (600 MHz, CD_3OD) δ 3.97–3.95 (m, 1H), 3.80–3.73 (m, 4H), 3.58–3.49 (m, 1H), 3.21–3.17 (m, 2H), 3.15–3.11 (m, 2H), 2.52–2.47 (m, 1H), 2.40–2.30 (m, 1H), 1.96–1.69 (m, 7H), 1.68–1.24 (m, 14H), 1.04 (d, *J* = 6.6 Hz, 3H), 0.99 (td, *J* = 14.1, 3.2 Hz, 1H), 0.93 (s, 3H), 0.72 (s, 3H).^{13}C NMR (151 MHz, CD_3OD) δ 173.4, 72.7, 71.1, 46.6, 46.2, 43.6, 43.4, 43.0, 42.2, 38.8, 36.1, 35.8, 35.5, 35.0, 33.9, 33.4, 31.0, 29.7, 29.3, 28.5, 27.3, 27.0, 26.1, 23.5, 22.3, 16.3, 11.8. HRMS–ESI+ (*m/z*): $[M+H]^+$ calcd for $C_{28}H_{49}N_2O_3Na$, 461.3743; found, 461.3721.



Deoxycholate-FluorCpO probe (DCA-FluorCpO)

To a flame-dried round bottomed flask with a stir bar was added **S17** (0.057 g, 0.12 mmol, 1.2 equiv.) dissolved in anhydrous DMF (2.0 mL, 0.060 M). DIPEA (0.07 mL, 0.4 mmol, 4 equiv.) was added, then a solution of **S16** (0.051 g, 0.090 mmol, 1.0 equiv.) in anhydrous DMF (2.0 mL, 0.030 M) was added dropwise. The reaction was stirred at room temperature overnight. The following day, the solvent was removed under reduced pressure, and the crude residue was purified by flash chromatography (eluting with 10% MeOH in CH_2Cl_2). The purified residue was dissolved in anhydrous CH_2Cl_2 (3.0 mL) and cooled to 0 °C. Trifluoroacetic acid (TFA, 3.0 mL) and triethylsilane ($HSiEt_3$, 0.05 mL, 0.32 mmol, 3.2 equiv.) were then added dropwise, and the solution was allowed to warm to room temperature over 1.5 h with stirring. The solvent was removed *in vacuo*, and the crude residue was triturated with cold n-pentane (3 x 5 mL) and washed with CH_2Cl_2 (6 x 10 mL). The filtrate was discarded, and the remaining solid was dissolved in MeOH and concentrated to give **DCA-FluorCpO** as a white solid (27 mg, 38% over two steps). A portion of the material was analyzed by HPLC (see Figure S11).

^{1}H NMR (600 MHz, CD_3OD) δ 7.55 (d, *J* = 8.1 Hz, 1H), 6.41 (d, *J* = 10.0 Hz, 1 H), 6.38 (s, 1H), 3.98 (s, 1H), 3.65–3.46 (m, 9 H), 3.01 (s, 1H), 2.99 (s, 1H), 2.47 (m, 1H), 2.33 (m, 1H), 1.99–1.56 (m, 10H), 1.54–1.06 (m, 14H), 1.06–0.91 (m, 7H), 0.72 (m, 3H). HRMS–ESI+ (*m/z*): $[M+H]^+$ calcd for $C_{42}H_{61}N_2O_7$, 705.4479; found, 705.4459.

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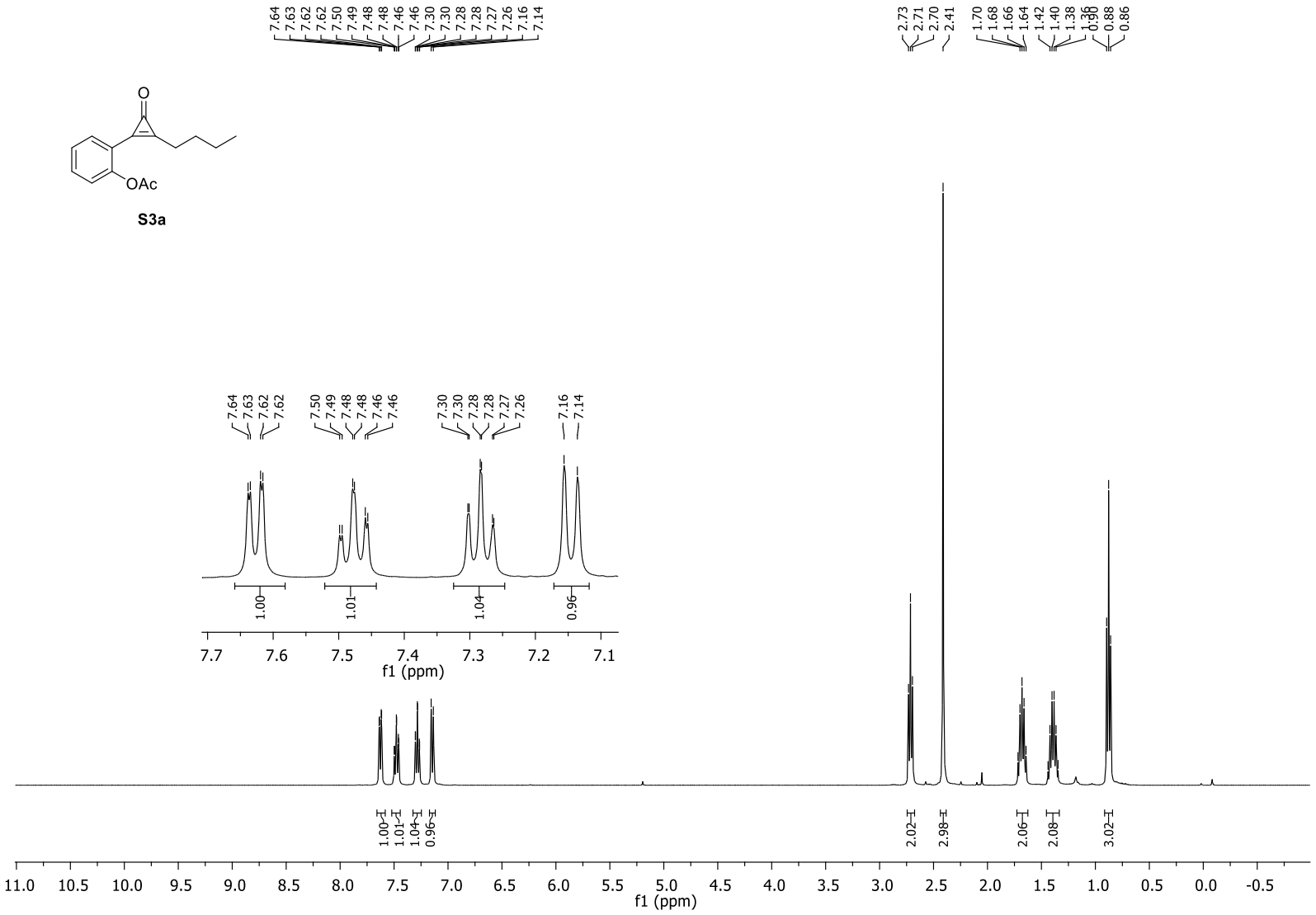
NMR spectra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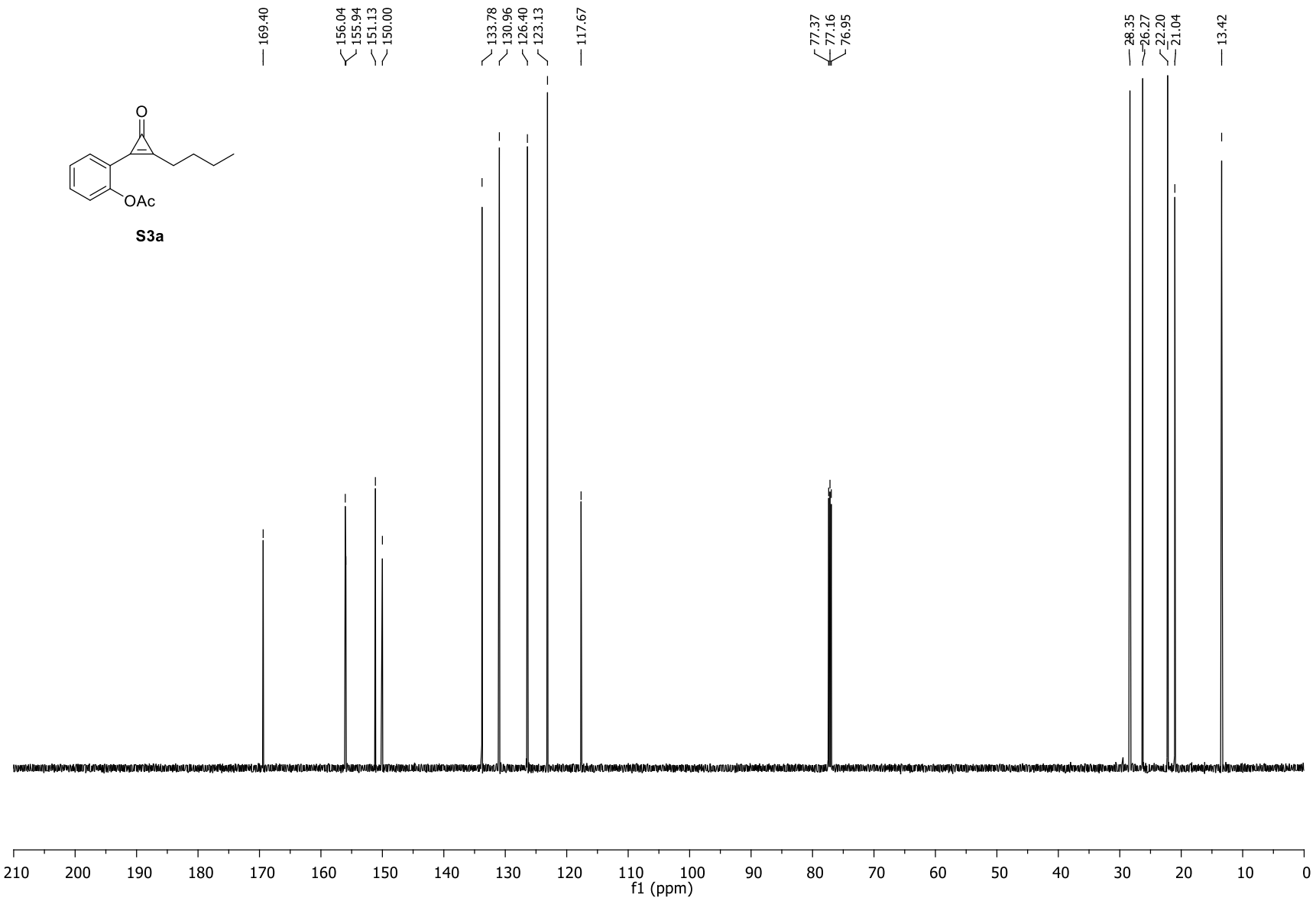
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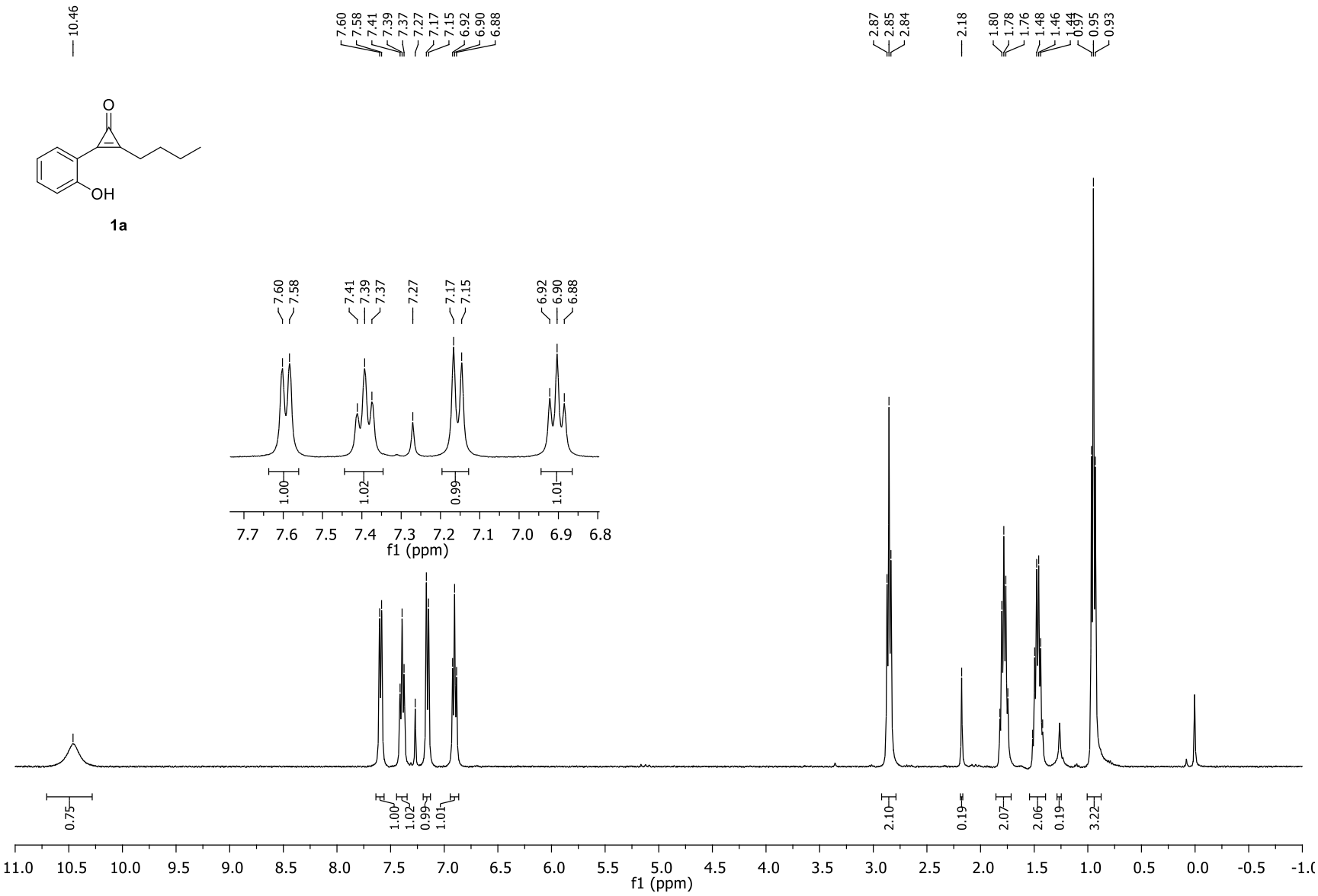
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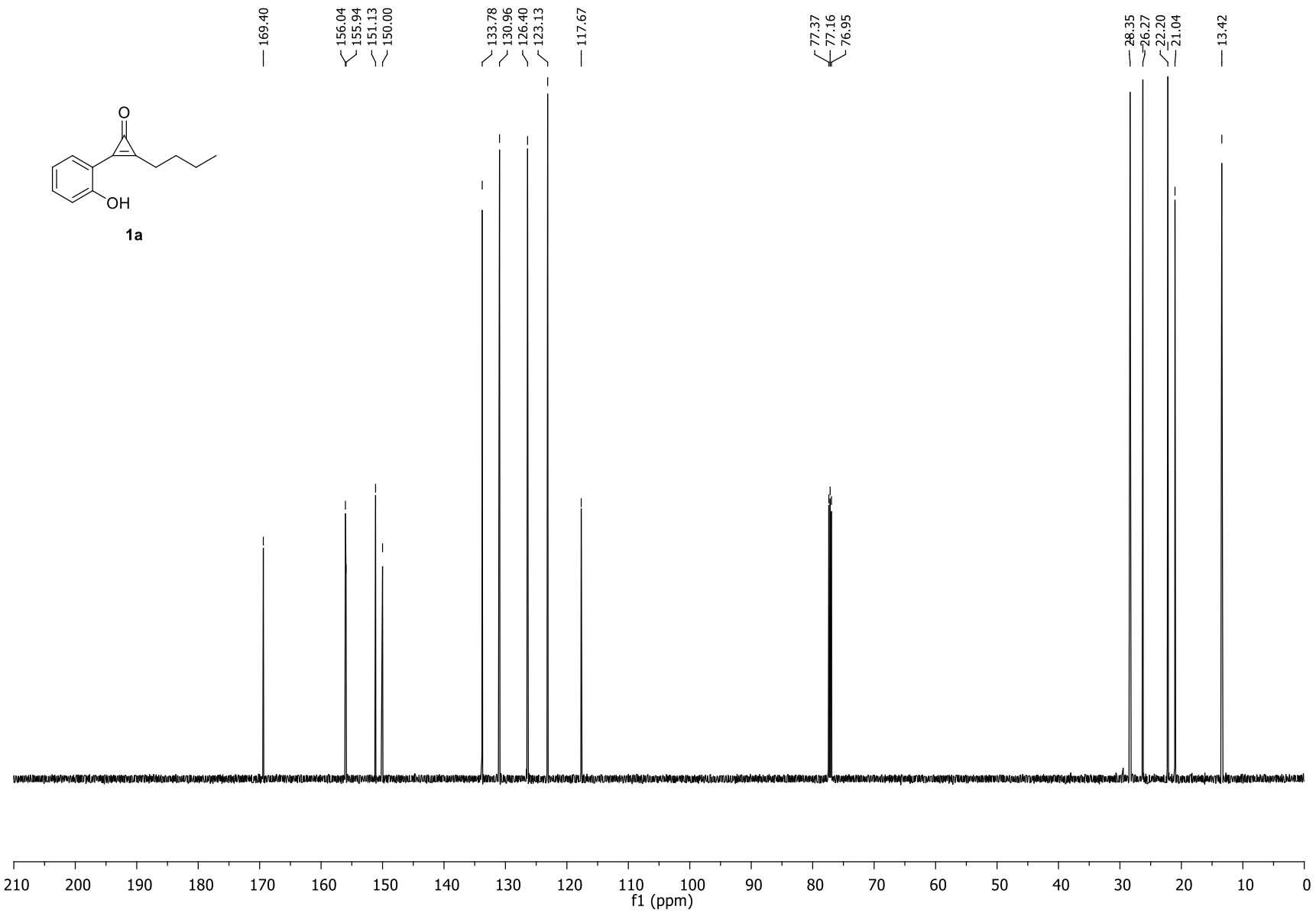
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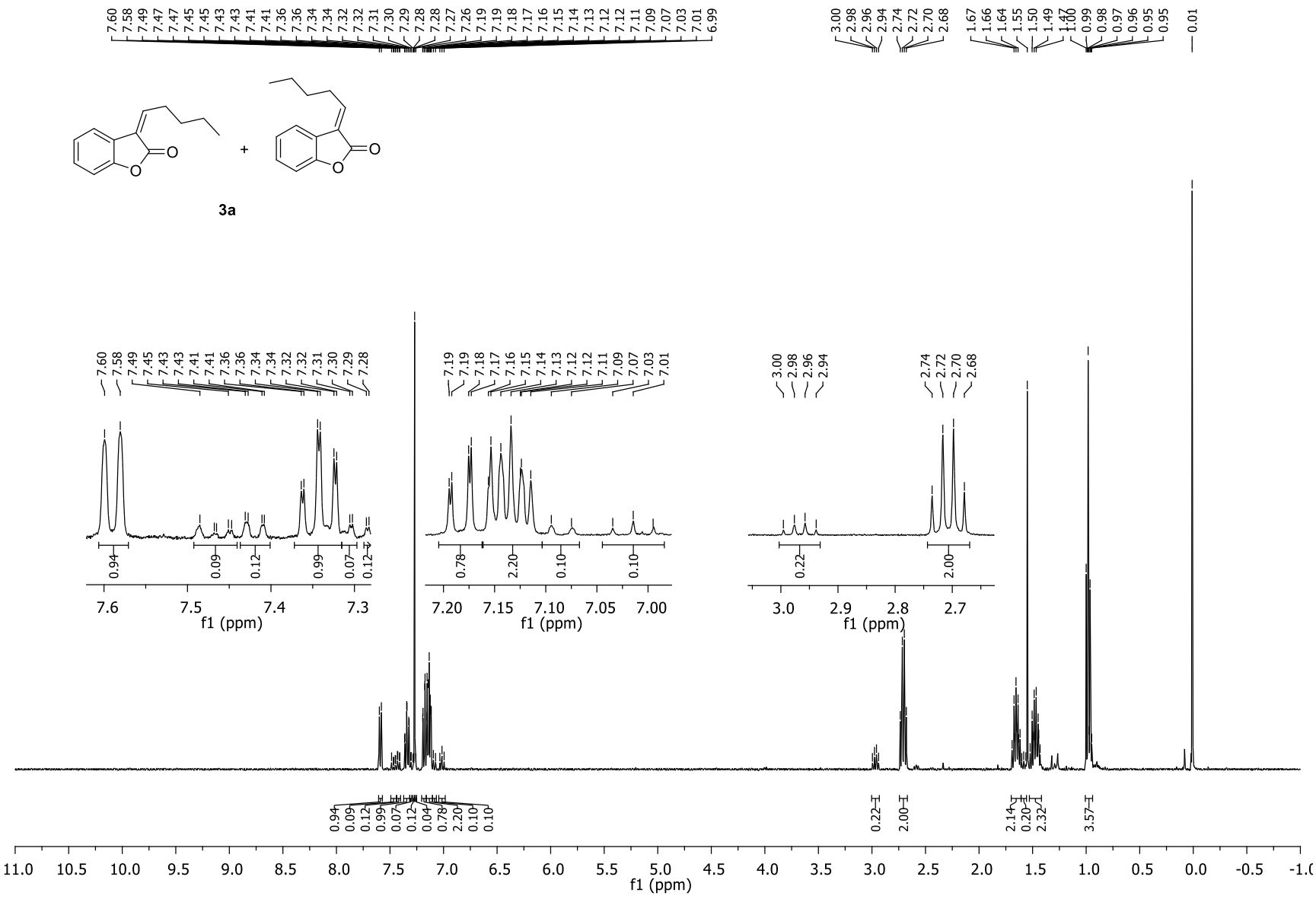
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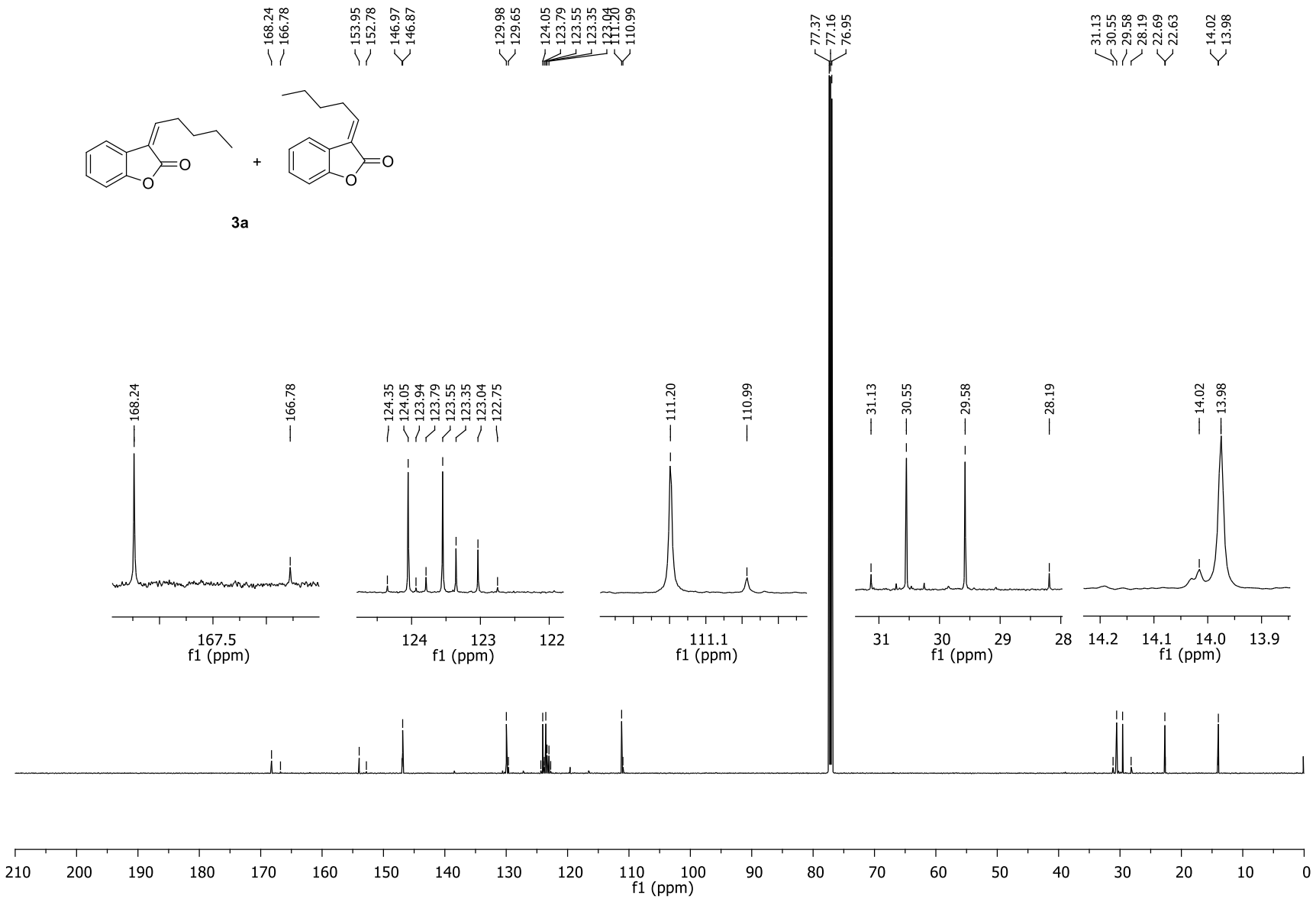
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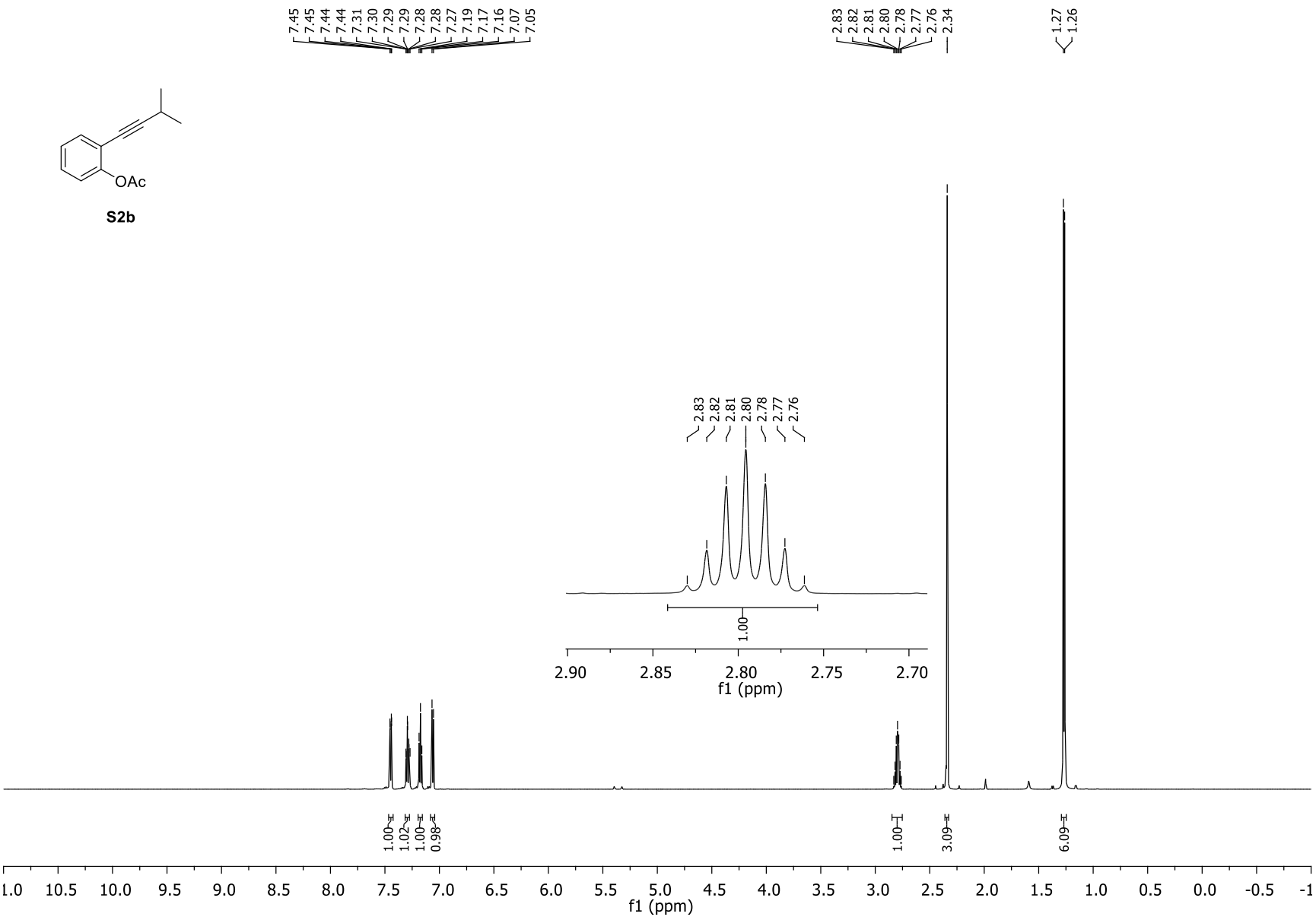
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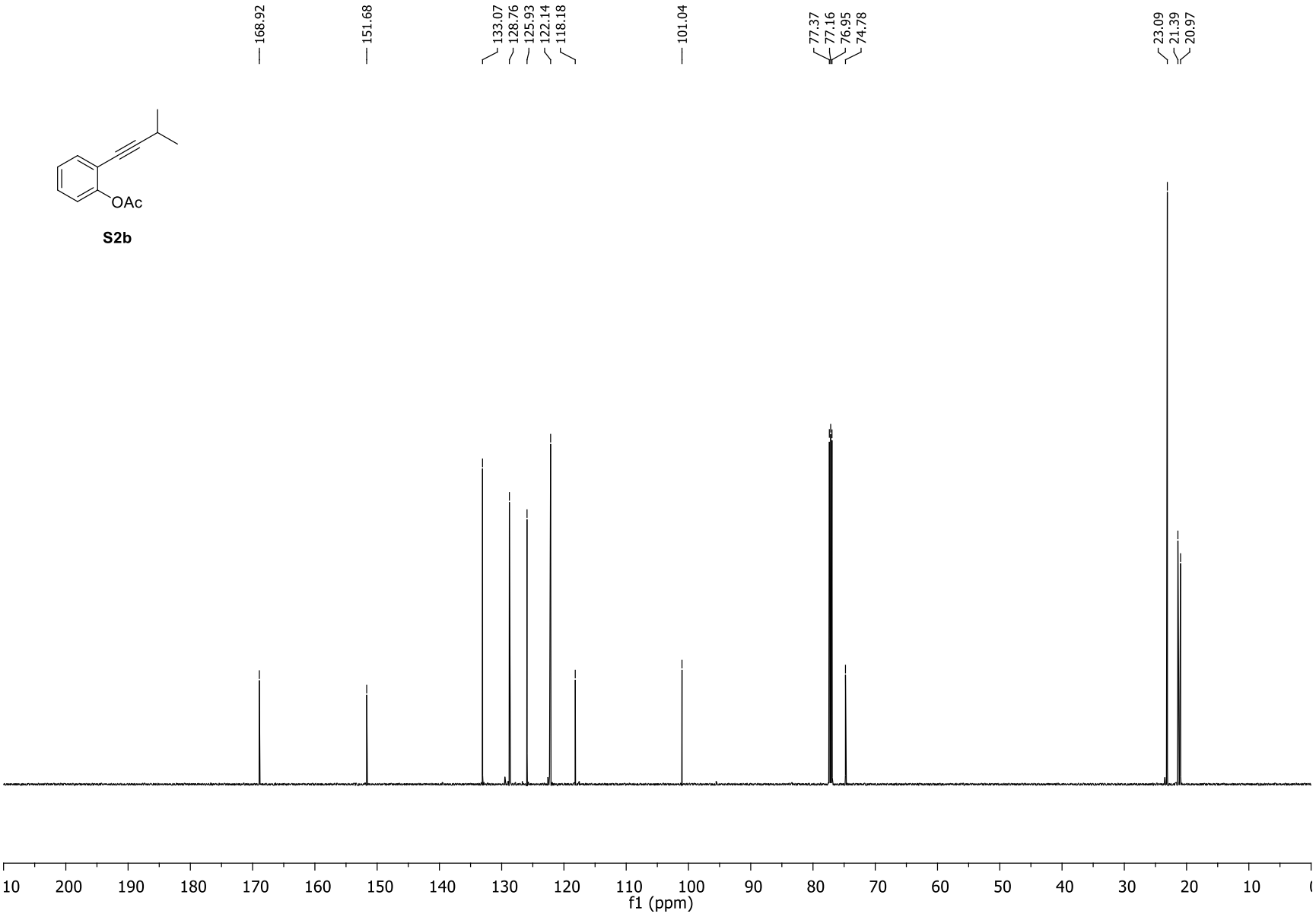
2.70
f1 (ppm)
1.00
1.02
1.00
0.98
1.00
3.09
6.09
f1 (ppm)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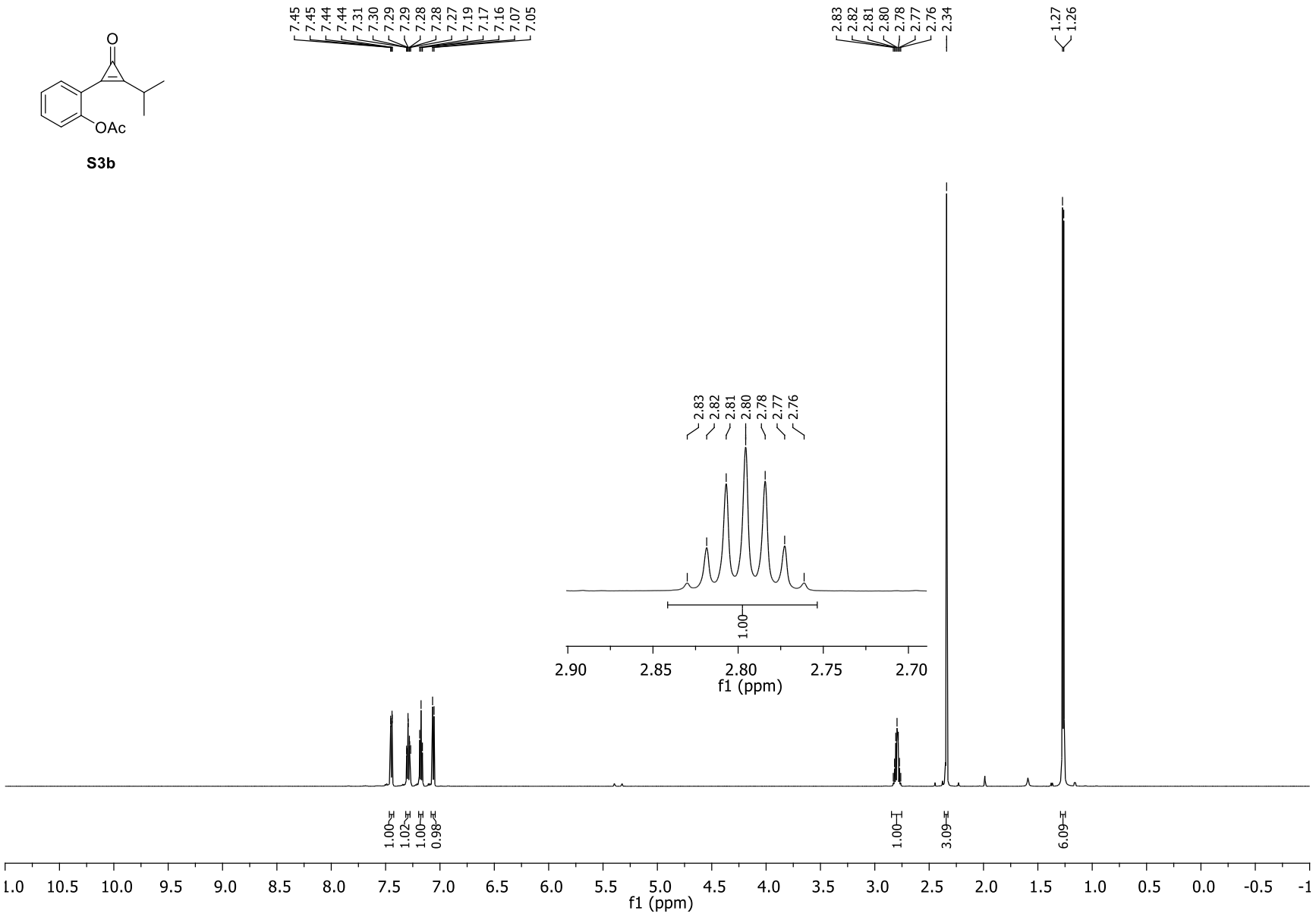
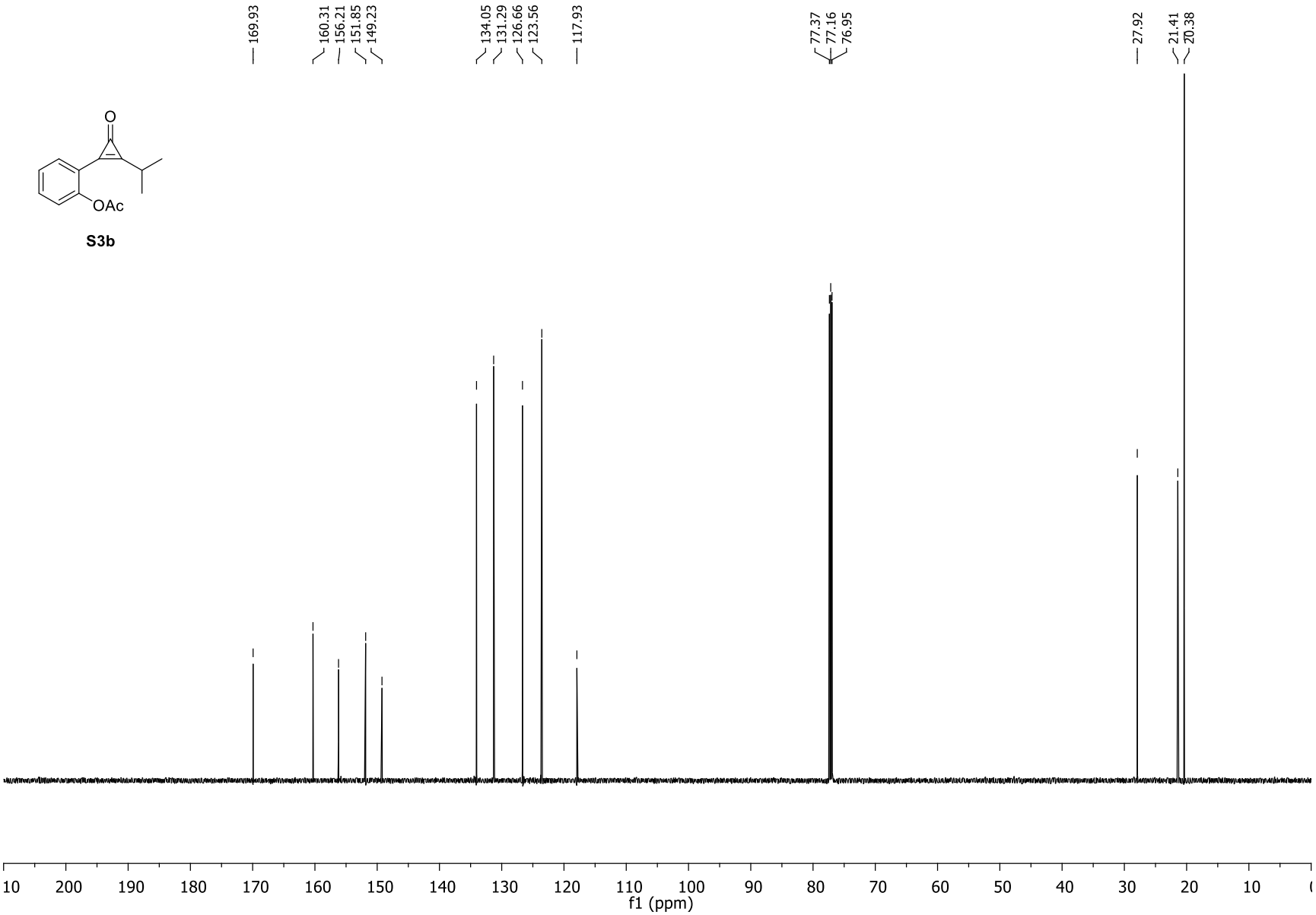
151.68
133.07
128.76
125.93
122.14
118.18
101.04
77.37
77.16
76.95
74.78
23.09
21.39
20.97
OAc
S2b
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


O
OAc
S3b
7.45
7.45
7.44
7.44
7.31
7.30
7.29
7.29
7.28
7.28
7.27
7.19
7.17
7.16
7.07
7.05
2.83
2.82
2.81
2.80
2.78
2.77
2.76
2.34
1.27
1.26
1.00
2.90
2.85
2.80
2.75
2.70
f1 (ppm)
1.00
1.02
1.00
0.98
1.00
3.09
6.09
f1 (ppm)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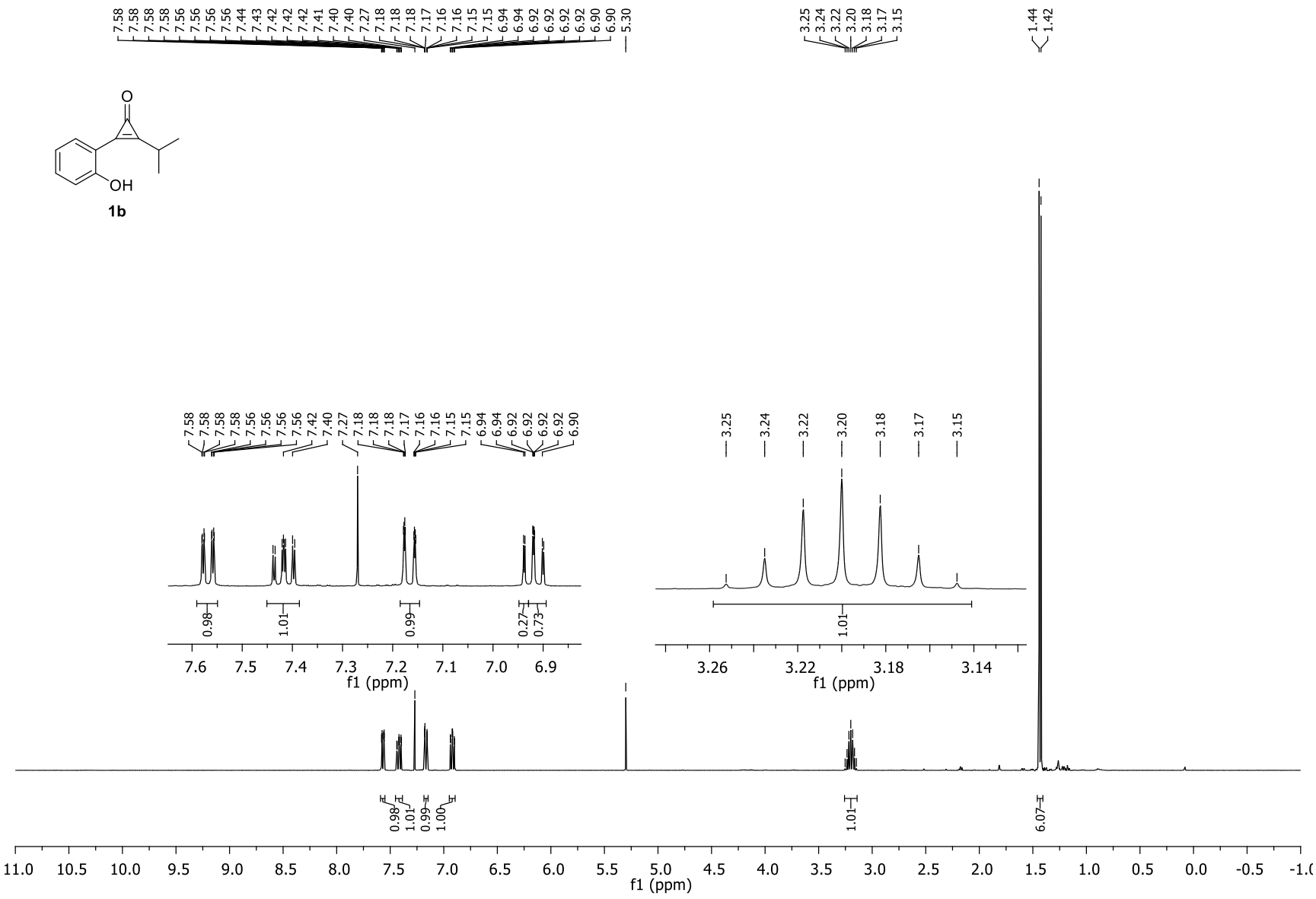
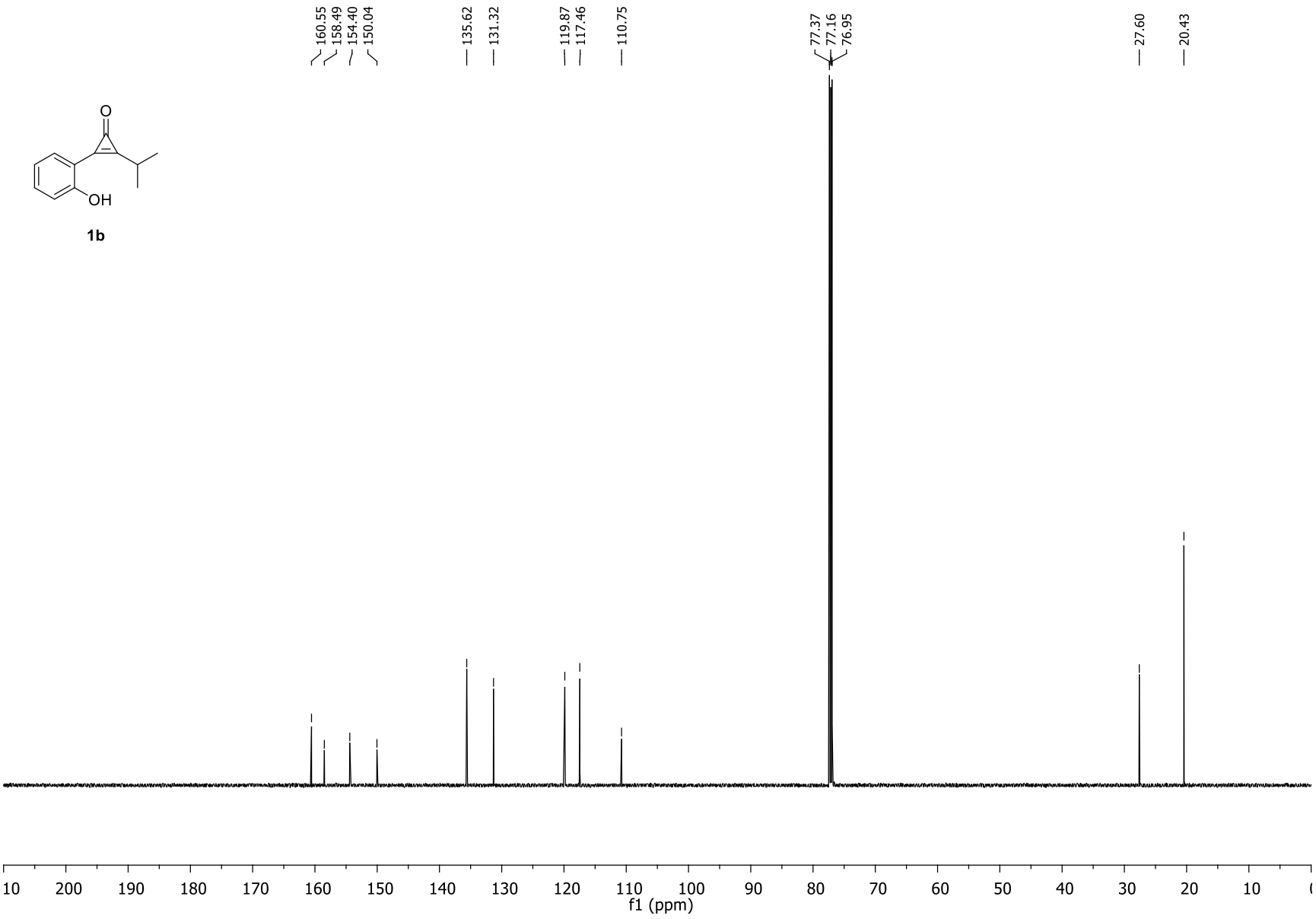
156.21
151.85
149.23
134.05
131.29
126.66
123.56
117.93
77.37
77.16
76.95
27.92
21.41
20.38
O
OAc
S3b
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


7.58 7.58 7.58 7.58 7.56 7.56 7.56 7.44 7.43 7.42 7.42 7.42 7.41 7.40 7.40 7.27 7.18 7.18 7.17 7.16 7.16 7.15 7.15 6.94 6.94 6.92 6.92 6.92 6.92 6.90 6.90 5.30
3.25 3.24 3.22 3.20 3.18 3.17 3.15
1.44 1.42
O
OH
1b
0.98 1.01 0.99 0.27 0.73
1.01
7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9
f1 (ppm)
3.26 3.22 3.18 3.14
f1 (ppm)
0.98 1.01 0.99 1.00
1.01
6.07
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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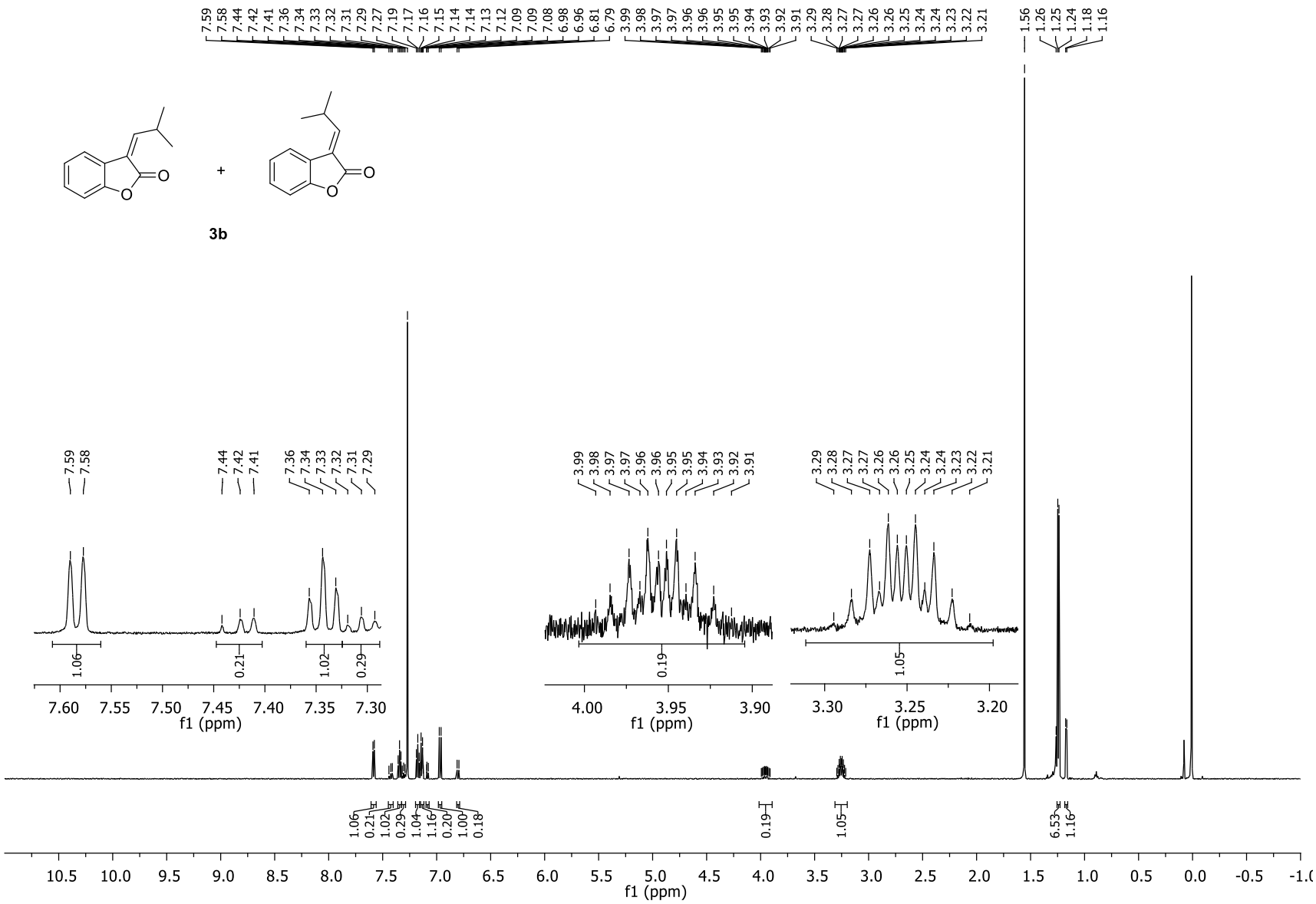
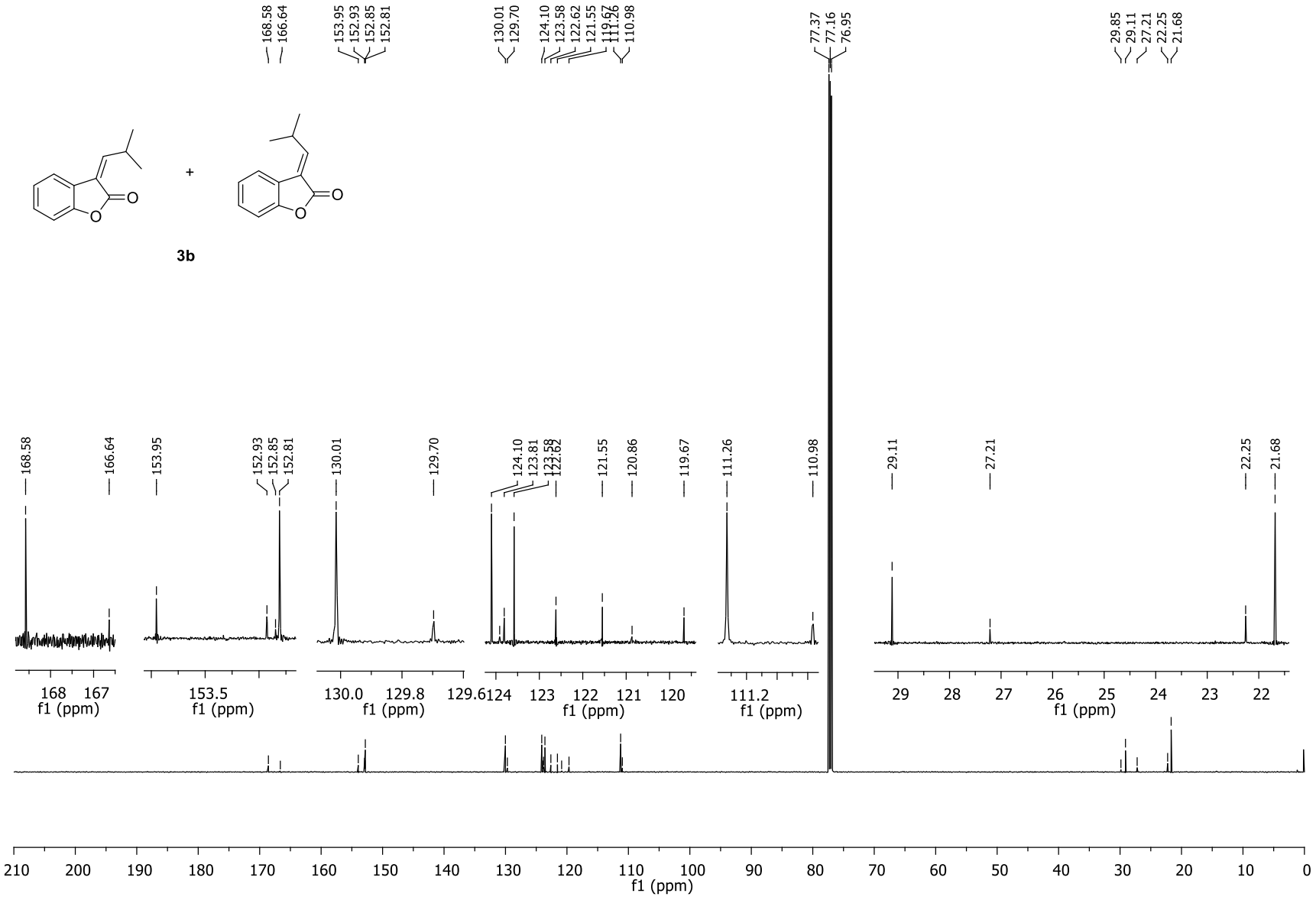
158.49
154.40
150.04
135.62
131.32
119.87
117.46
110.75
77.37
77.16
76.95
27.60
20.43
O
OH
1b
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


3b
+
f1 (ppm)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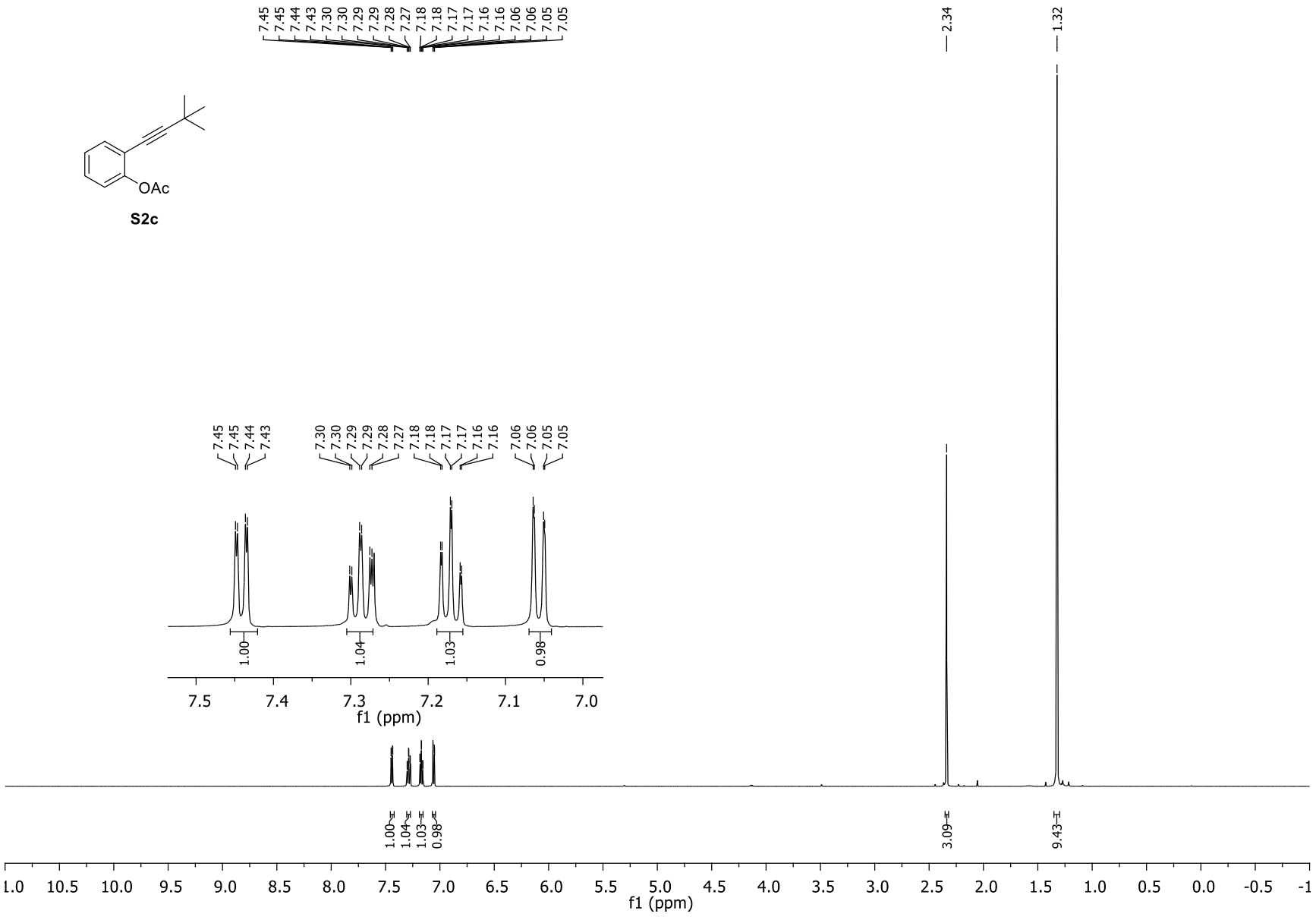
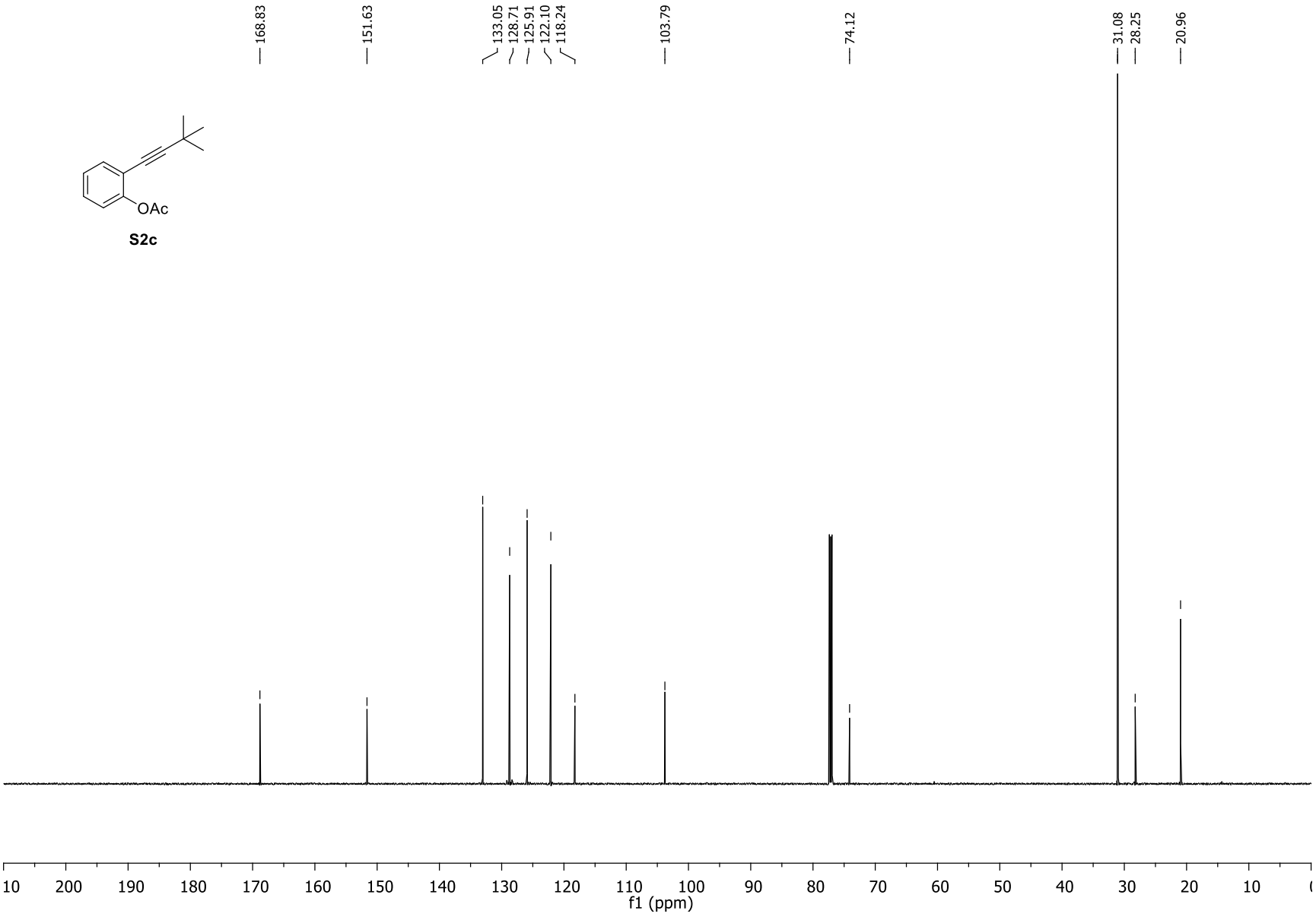
166.64
153.95
152.93
152.85
152.81
130.01
129.70
124.10
123.58
122.62
121.55
110.98
77.37
77.16
76.95
29.85
29.11
27.21
22.25
21.68
3b
f1 (ppm)


OAc
S2c
7.45
7.45
7.44
7.43
7.30
7.30
7.29
7.29
7.28
7.27
7.18
7.18
7.17
7.17
7.16
7.16
7.06
7.06
7.05
7.05
2.34
1.32
1.00
1.04
1.03
0.98
3.09
9.43
f1 (ppm)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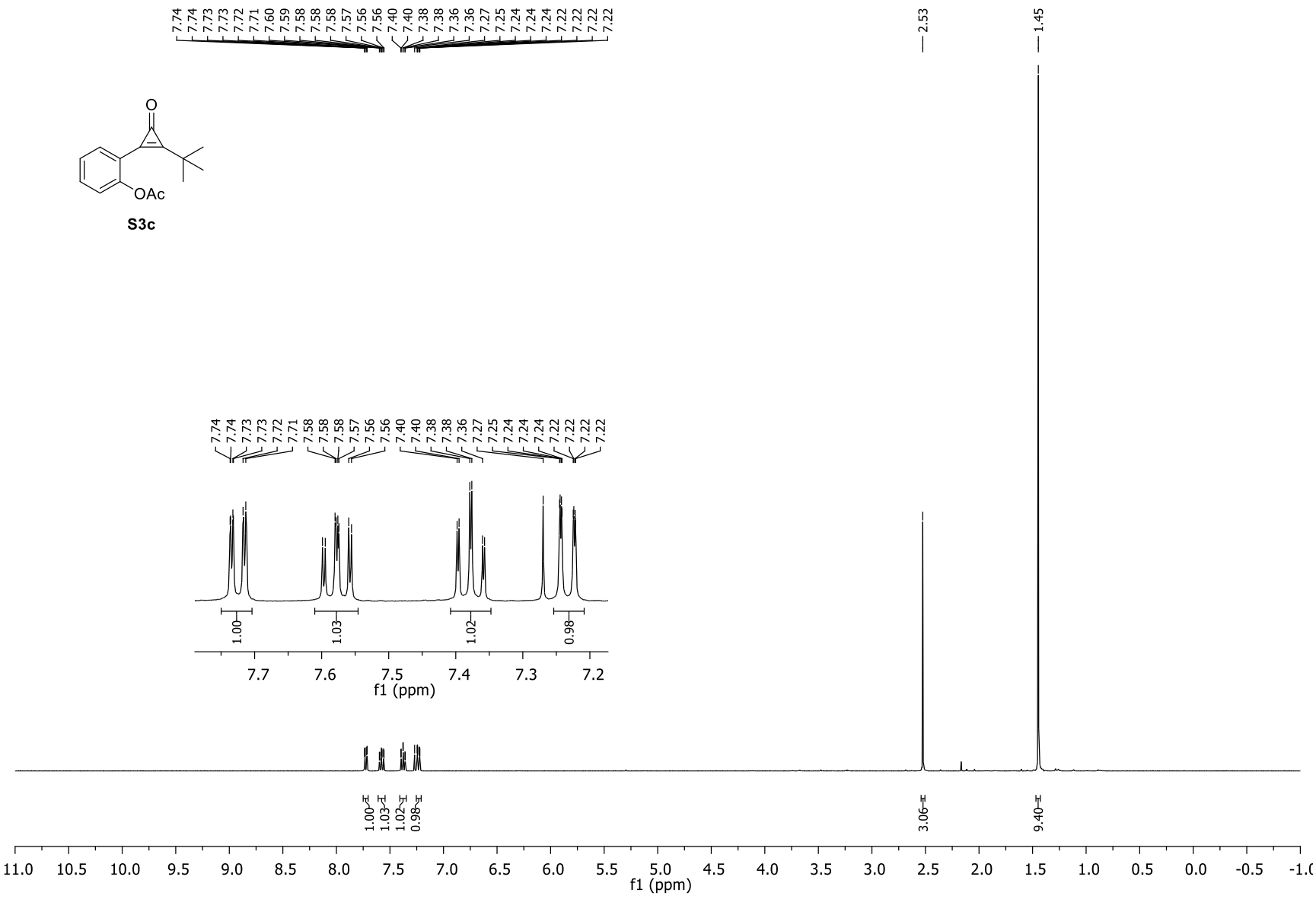
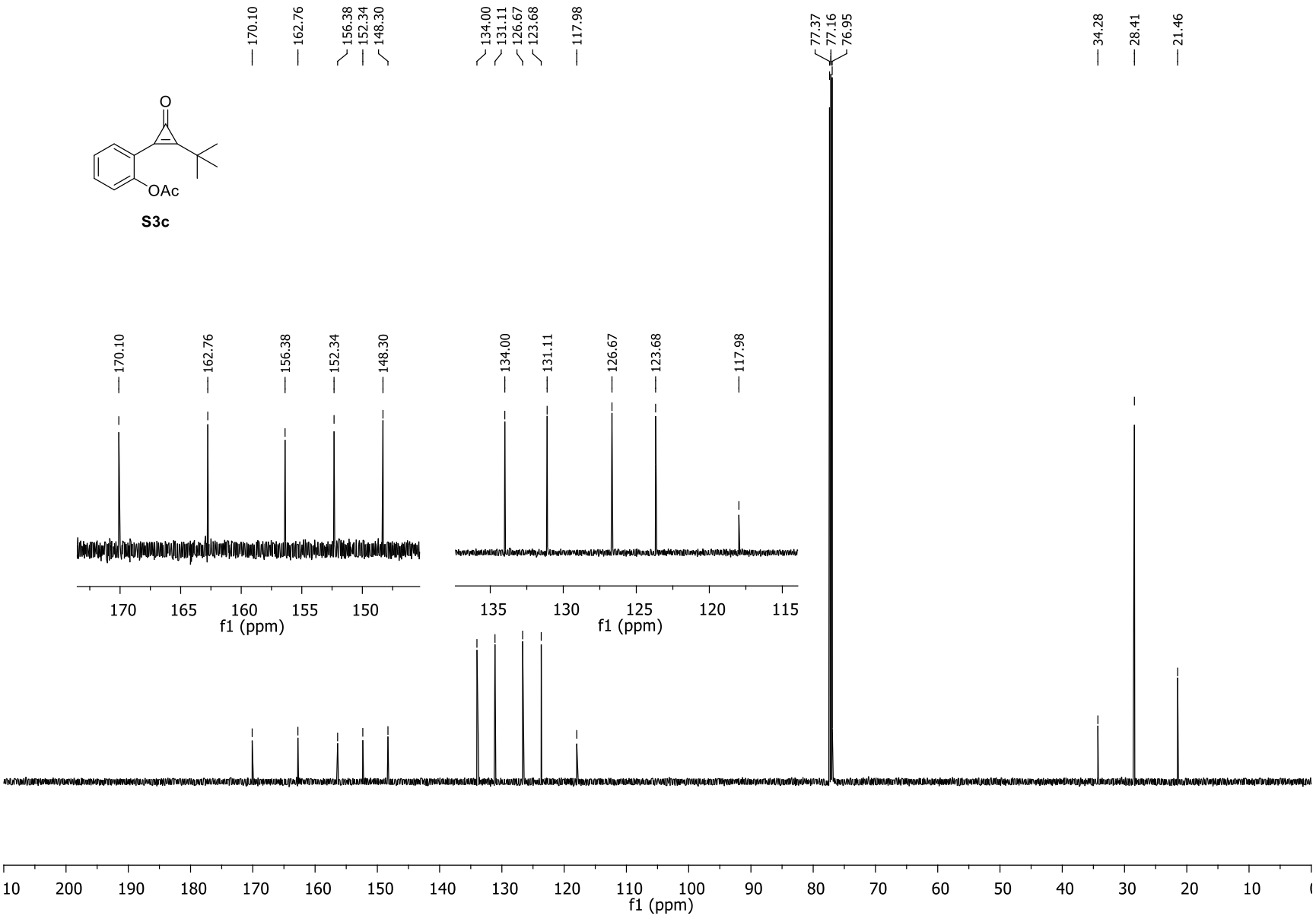
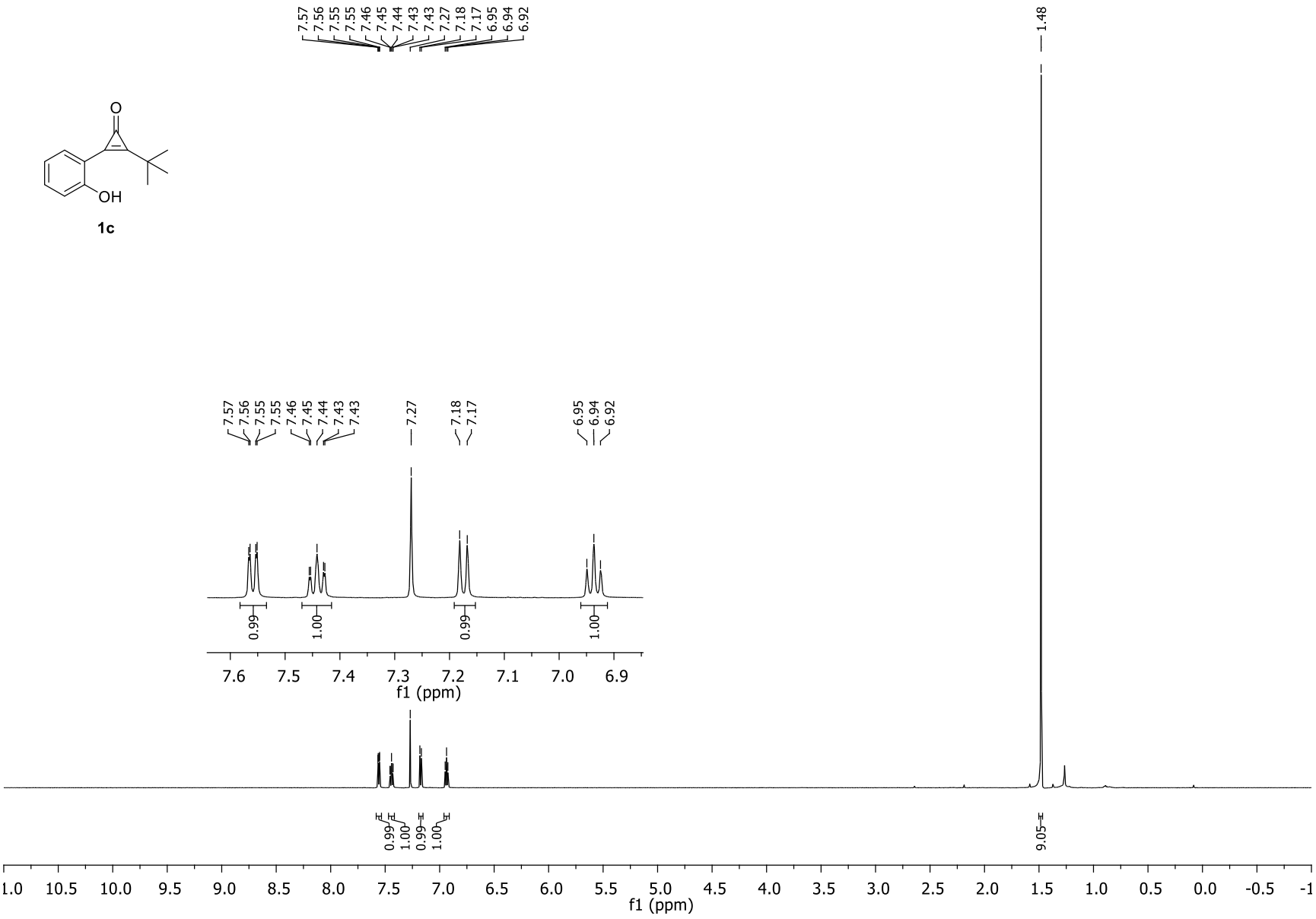
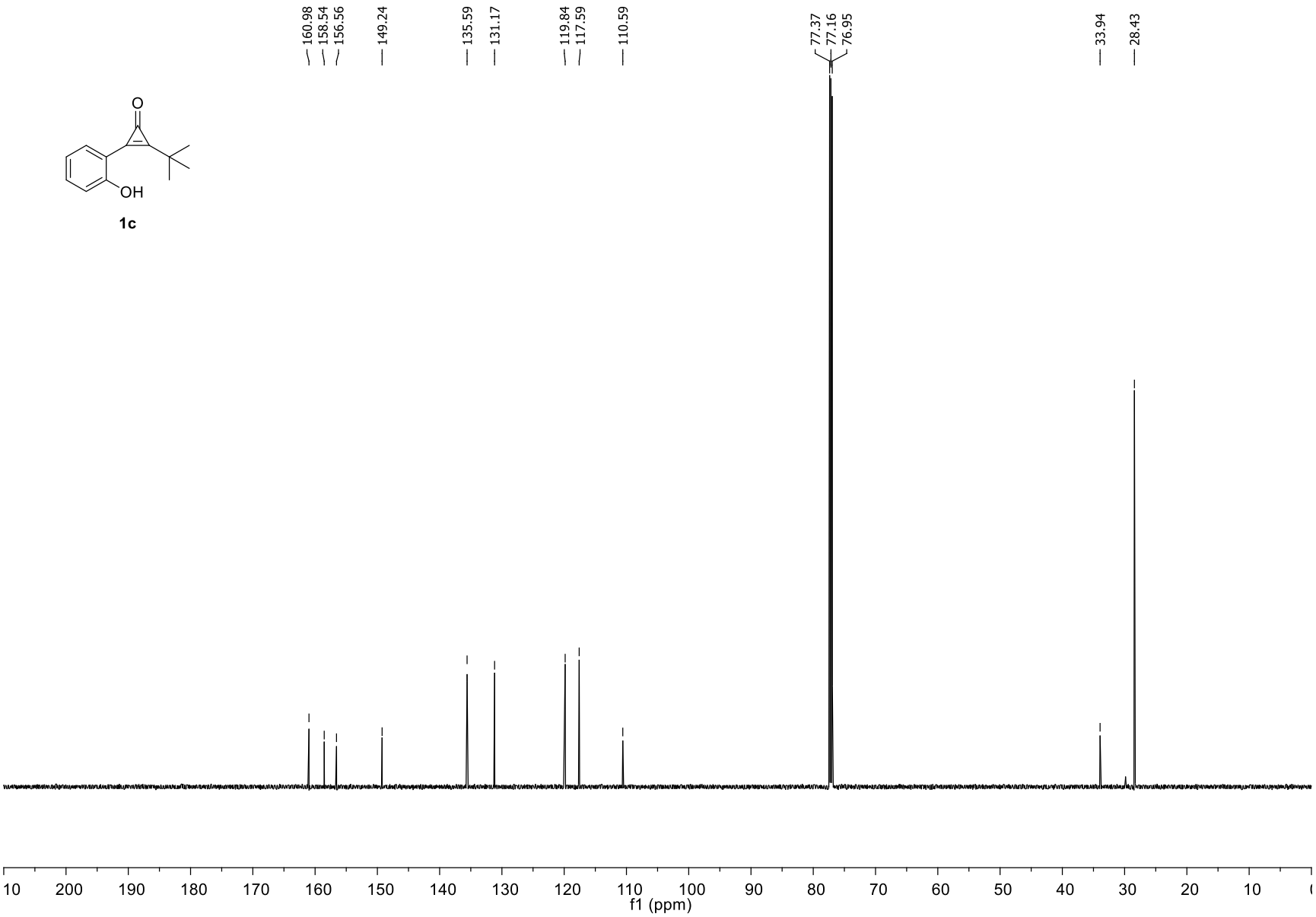
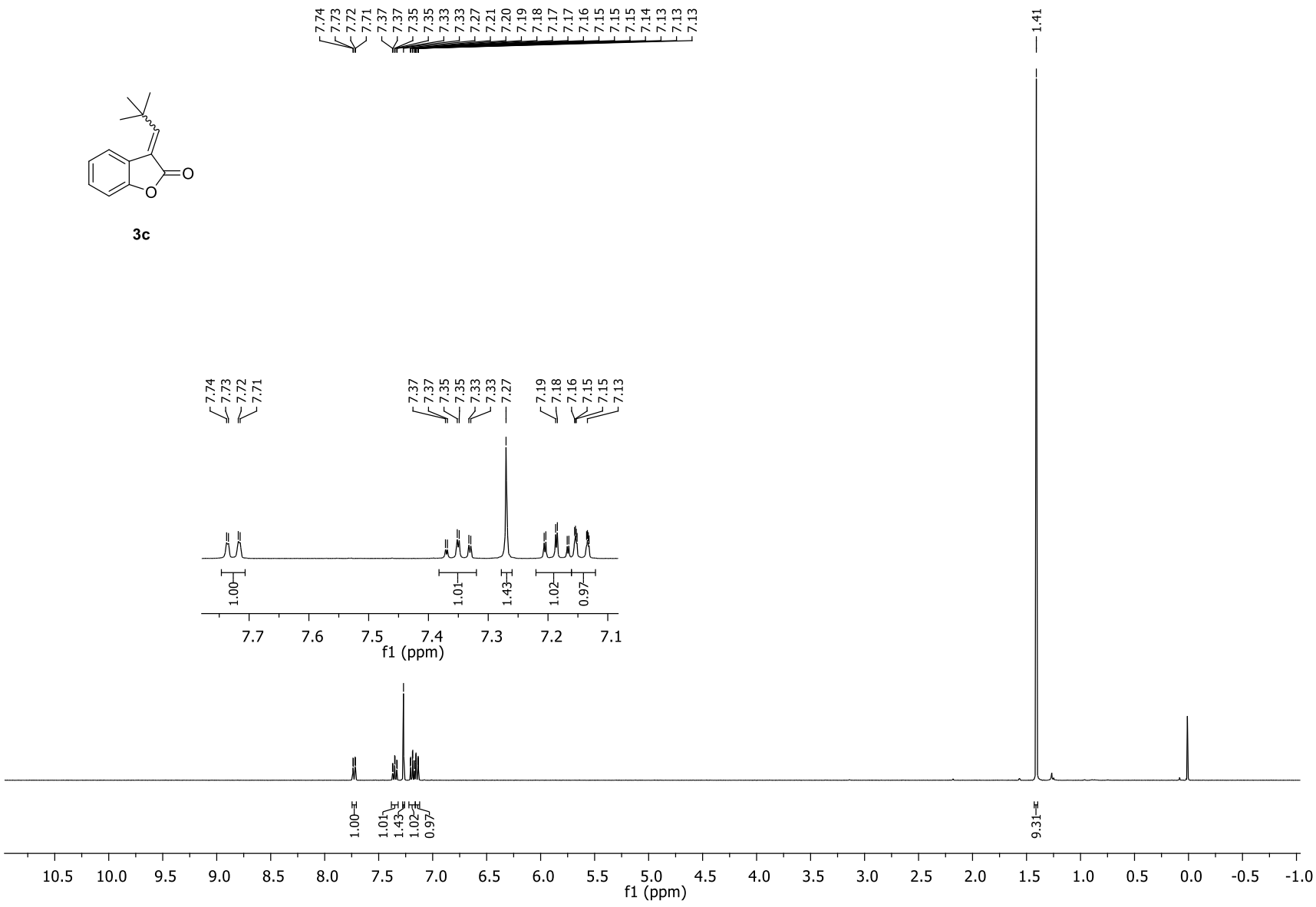
151.63
133.05
128.71
125.91
122.10
118.24
103.79
74.12
31.08
28.25
20.96
OAc
S2c
f1 (ppm)

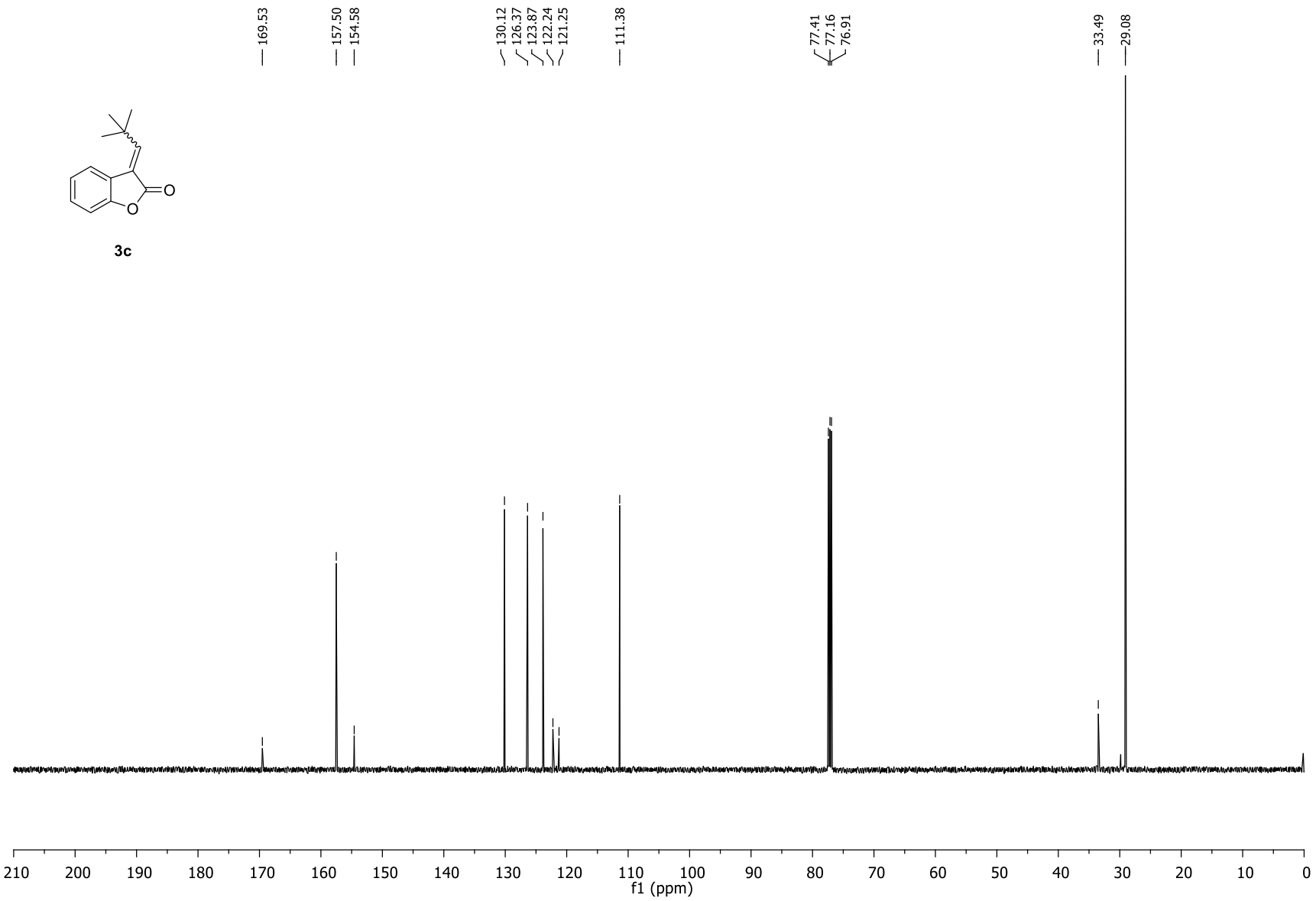

7.74
7.74
7.73
7.73
7.72
7.71
7.60
7.59
7.58
7.58
7.58
7.57
7.56
7.56
7.40
7.40
7.38
7.38
7.36
7.27
7.25
7.24
7.24
7.24
7.22
7.22
7.22
2.53
1.45
O
OAc
S3c
1.00
1.03
1.02
0.98
7.7
7.6
7.5
7.4
7.3
7.2
f1 (ppm)
3.06
9.40
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


170.10
162.76
156.38
152.34
148.30
134.00
131.11
126.67
123.68
117.98
77.37
77.16
76.95
34.28
28.41
21.46
O
OAc
S3c
f1 (ppm)
f1 (ppm)
f1 (ppm)


7.57
7.56
7.55
7.55
7.46
7.45
7.44
7.43
7.43
7.27
7.18
7.17
6.95
6.94
6.92
1.48
O
OH
1c
0.99
1.00
0.99
1.00
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
f1 (ppm)
0.99
1.00
0.99
1.00
9.05
1.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1
f1 (ppm)


160.98
158.54
156.56
149.24
135.59
131.17
119.84
117.59
110.59
77.37
77.16
76.95
33.94
28.43
O
OH
1c
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


7.74
7.73
7.72
7.71
7.37
7.37
7.35
7.35
7.33
7.33
7.27
7.21
7.20
7.19
7.18
7.17
7.17
7.16
7.15
7.15
7.15
7.14
7.13
7.13
7.13
1.41
O
O
3c
1.00
1.01
1.43
1.02
0.97
9.31
f1 (ppm)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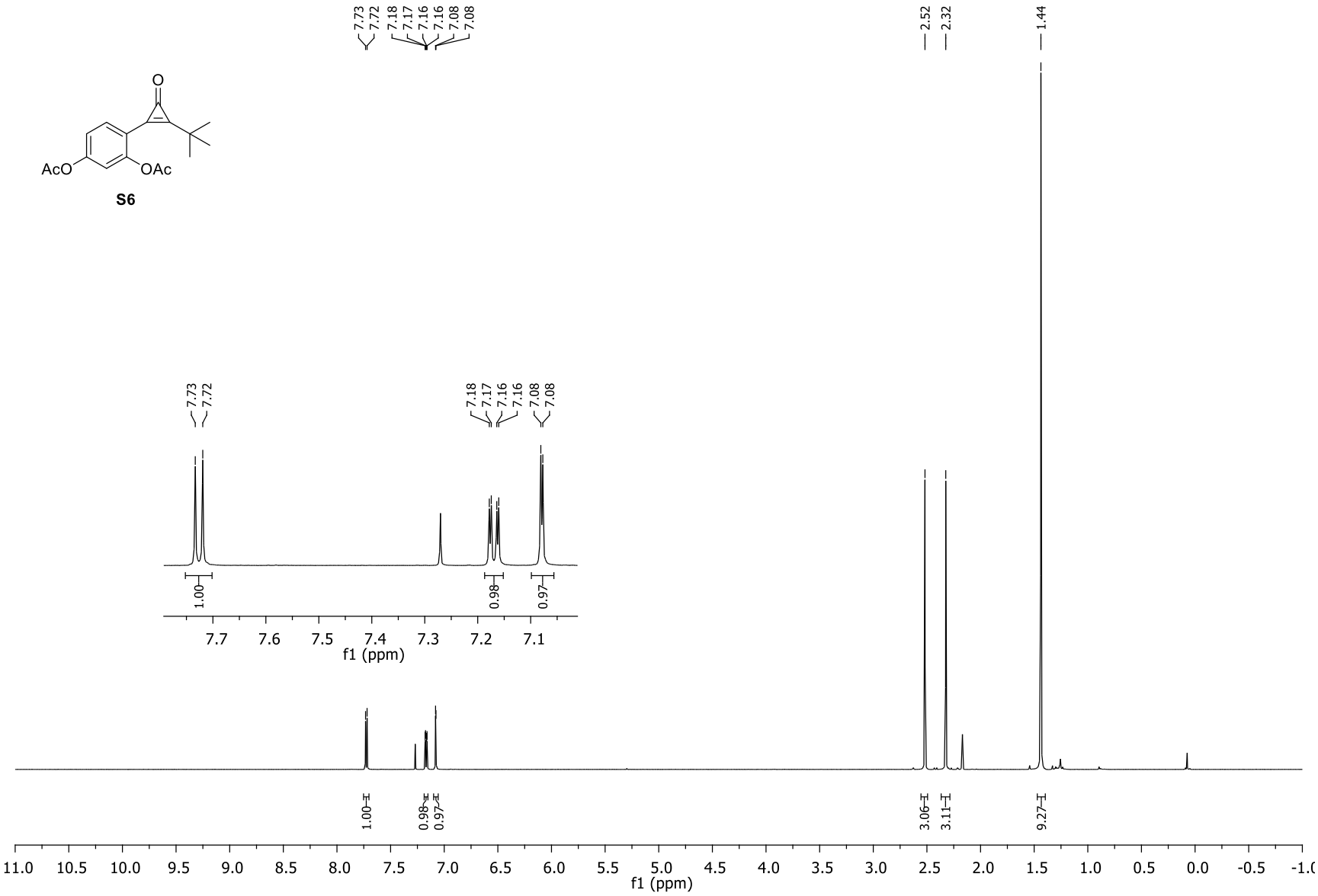
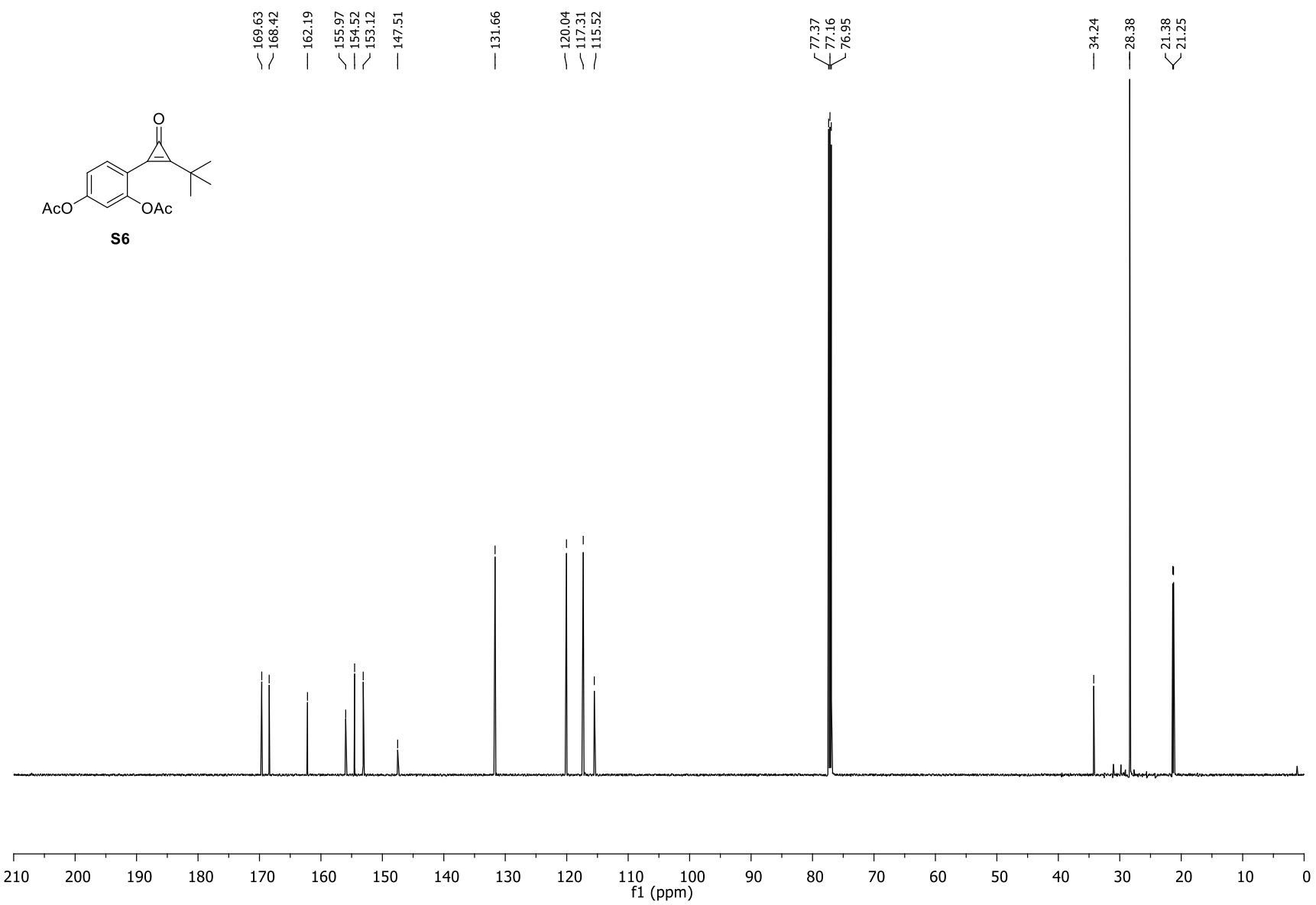
157.50
154.58
130.12
126.37
123.87
122.24
121.25
111.38
77.41
77.16
76.91
33.49
29.08
O
O
3c
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


O
AcO
OAc
S6
7.73
7.72
7.18
7.17
7.16
7.16
7.08
7.08
2.52
2.32
1.44
1.00
0.98
0.97
3.06
3.11
9.27
f1 (ppm)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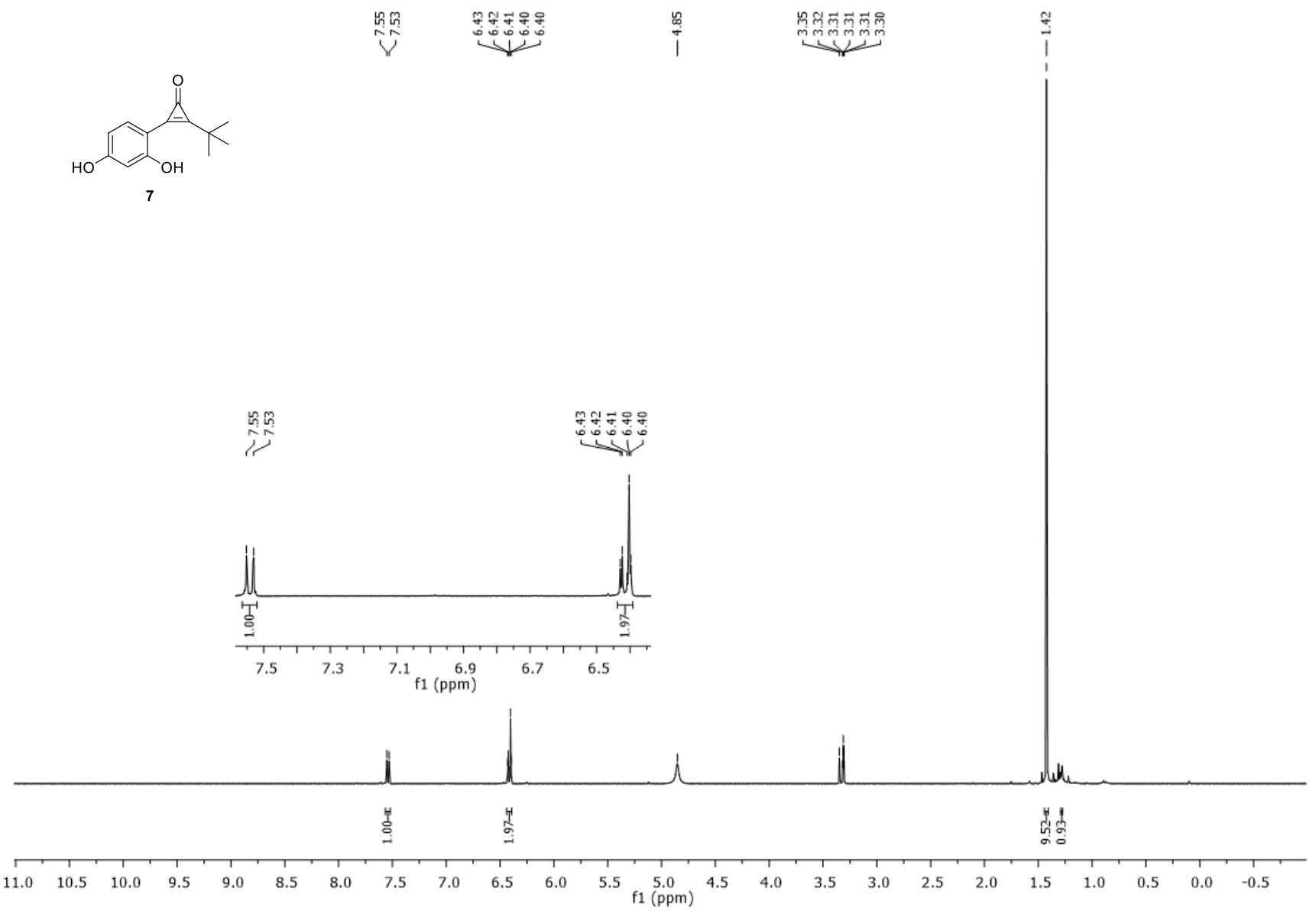
162.19
155.97
154.52
153.12
147.51
131.66
120.04
117.31
115.52
77.37
77.16
76.95
34.24
28.38
21.38
21.25
O
AcO
OAc
S6
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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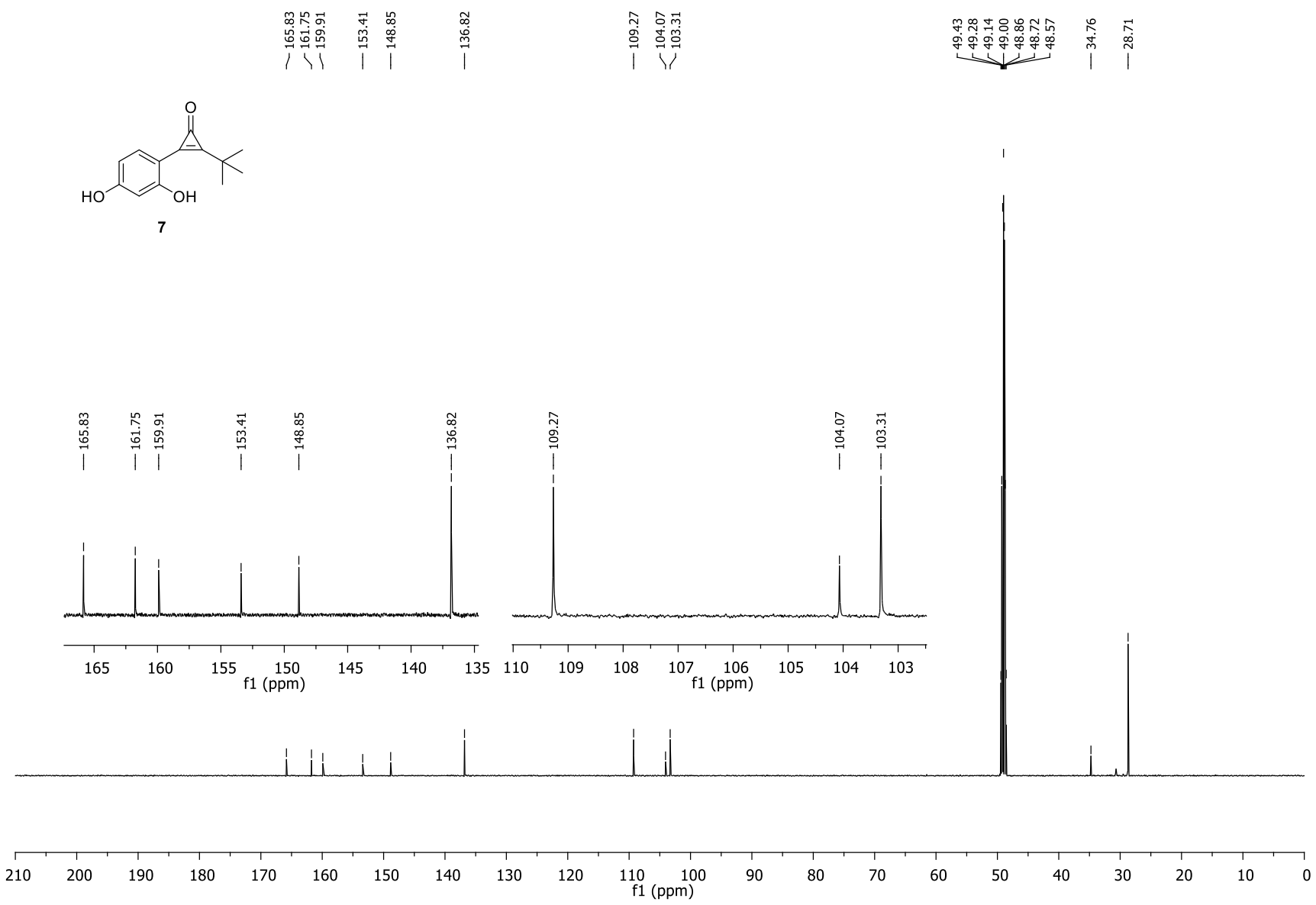
7.53
6.43
6.42
6.41
6.40
6.40
4.85
3.35
3.32
3.31
3.31
3.31
3.30
1.42
HO
OH
O
7
1.00
1.97
f1 (ppm)
1.00
1.97
9.52
0.93
f1 (ppm)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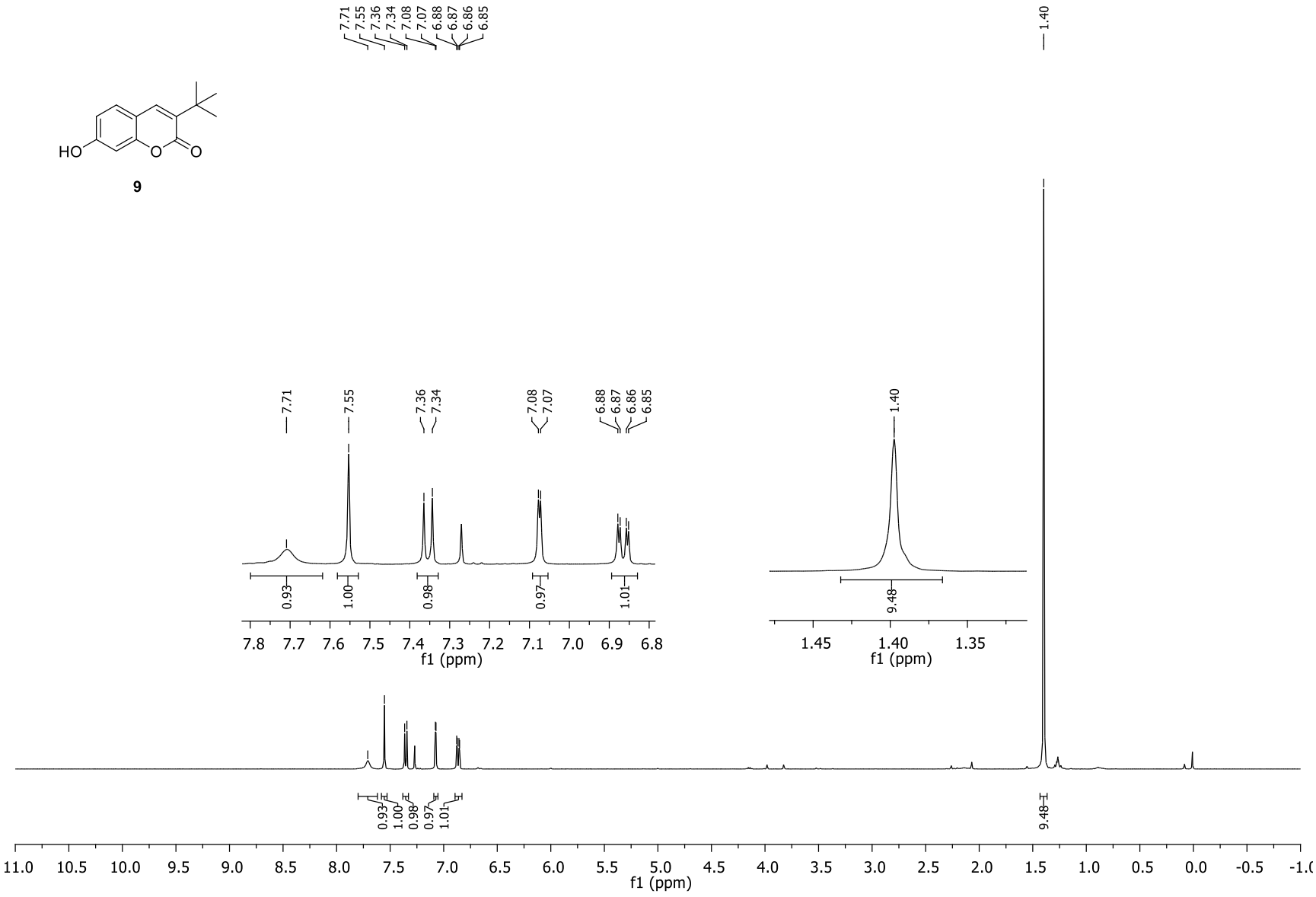
161.75
159.91
153.41
148.85
136.82
109.27
104.07
103.31
49.43
49.28
49.14
49.00
48.86
48.72
48.57
34.76
28.71
O
HO
OH
7
f1 (ppm)
f1 (ppm)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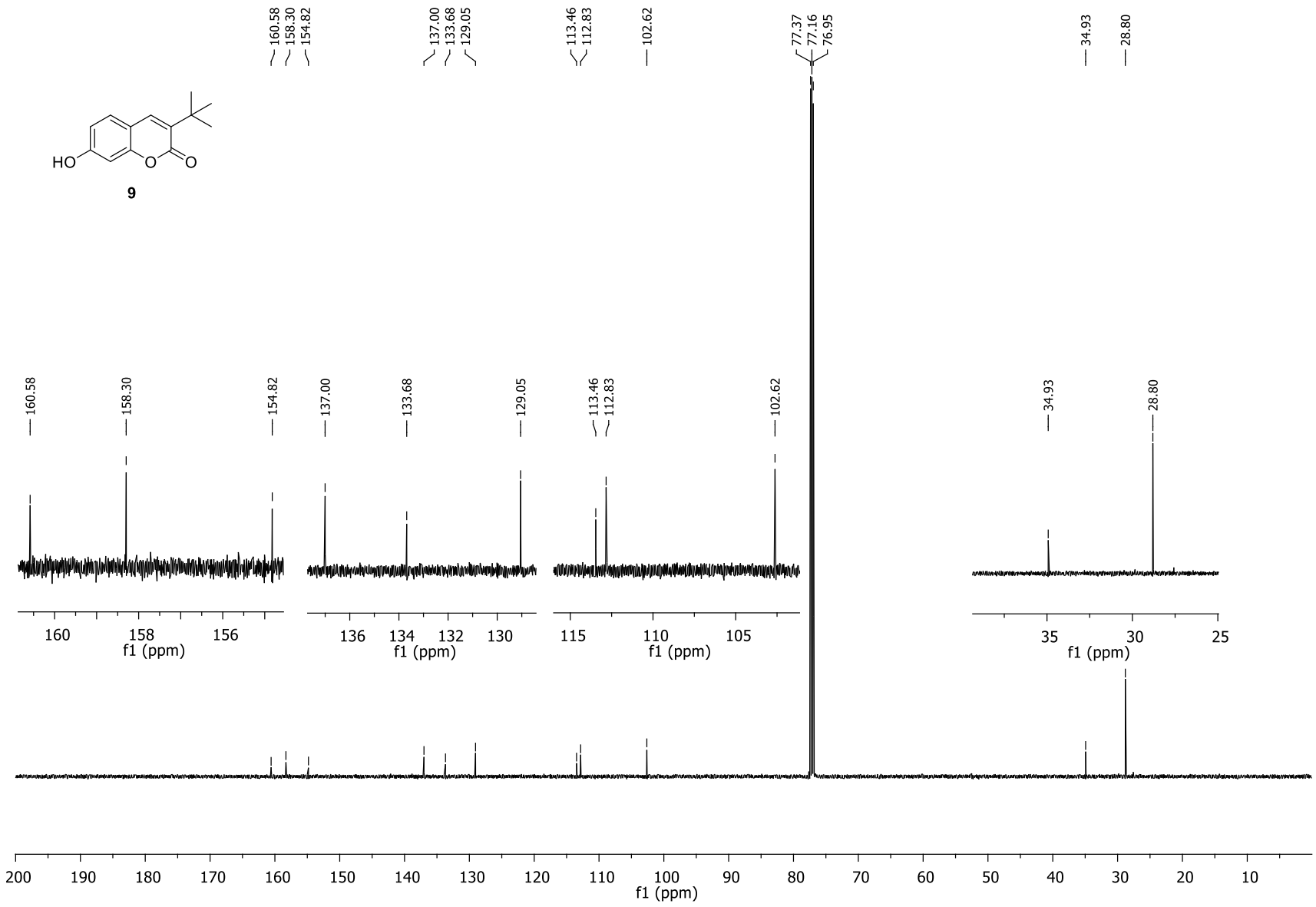
7.55
7.36
7.34
7.08
7.07
6.88
6.87
6.86
6.85
1.40
HO
O
O
9
0.93
1.00
0.98
0.97
1.01
9.48
f1 (ppm)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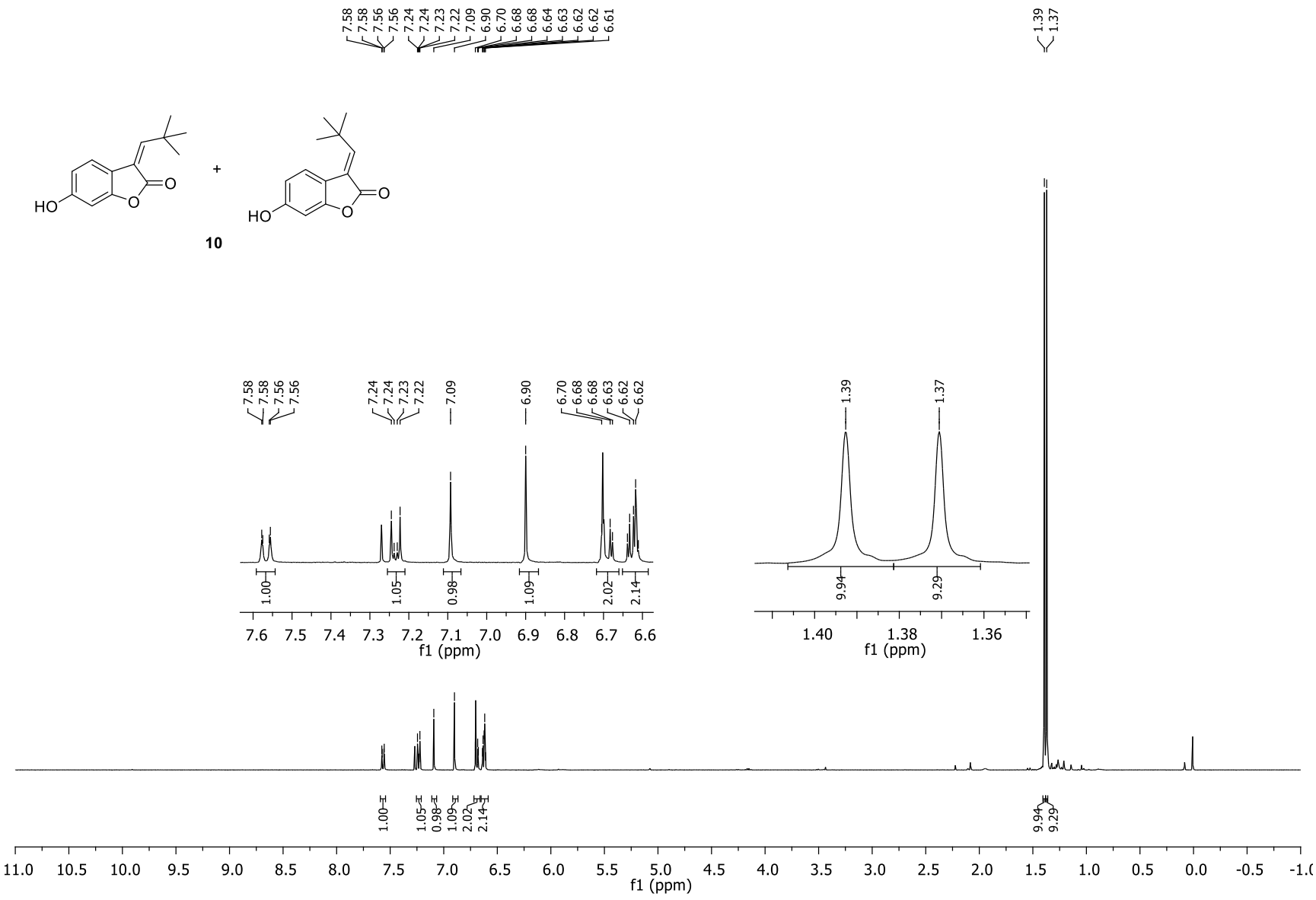
154.82
137.00
133.68
129.05
113.46
112.83
102.62
77.37
77.16
76.95
34.93
28.80
HO
O
O
9
f1 (ppm)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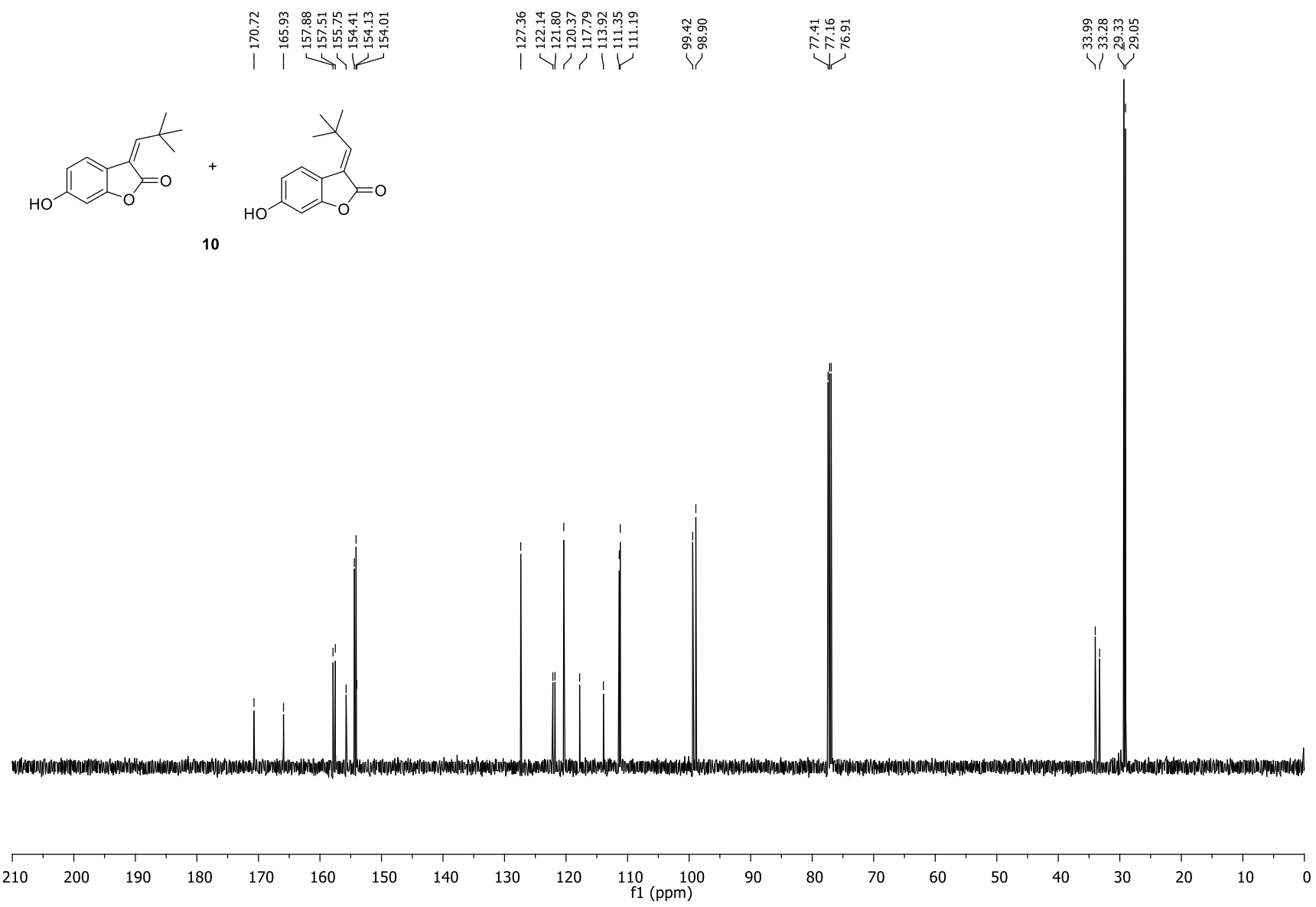
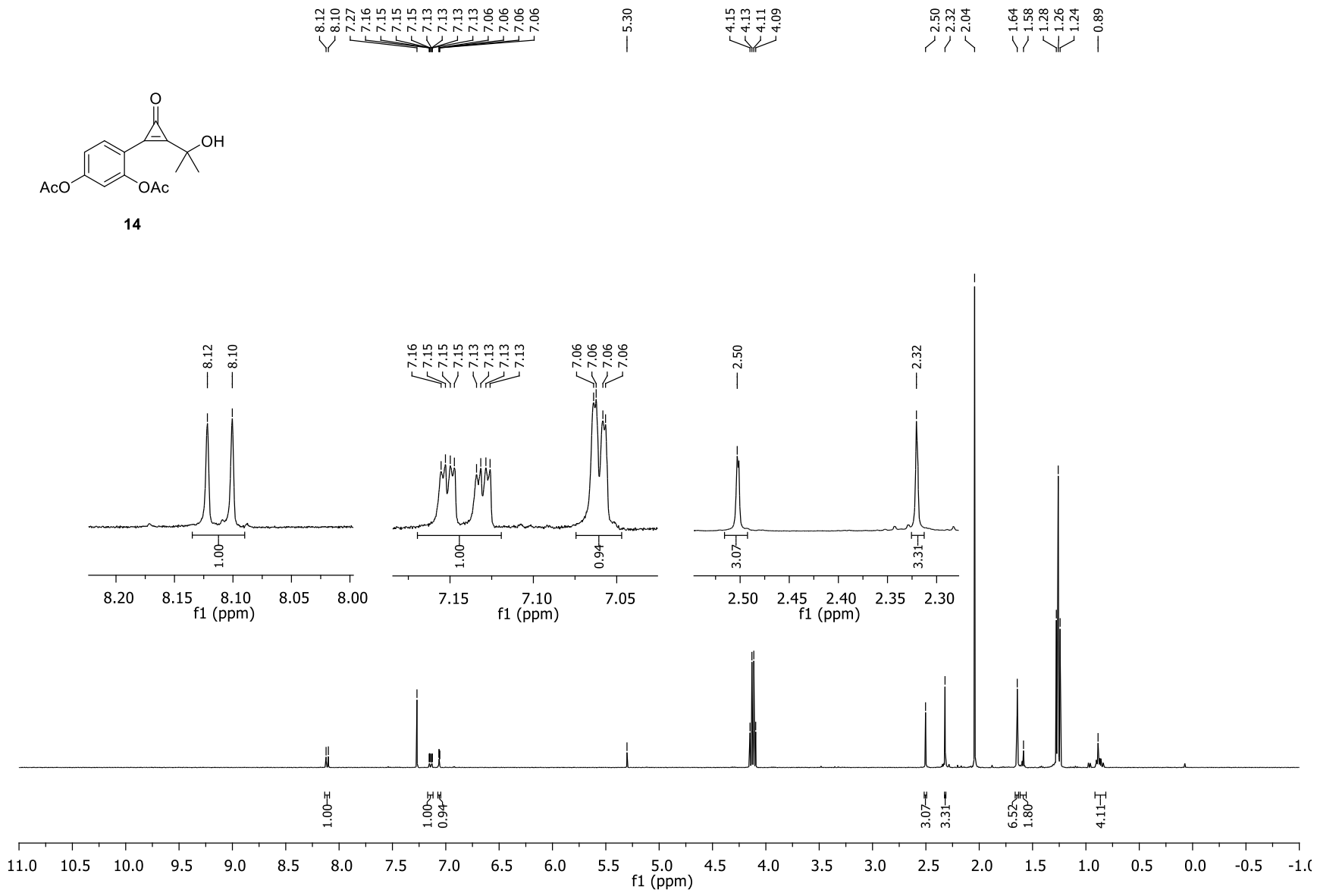
7.58
7.56
7.56
7.24
7.24
7.23
7.22
7.09
6.90
6.70
6.68
6.68
6.64
6.63
6.62
6.62
6.61
1.39
1.37
HO
O
O
+
HO
O
O
10
1.00
1.05
0.98
1.09
2.02
2.14
9.94
9.29
f1 (ppm)


170.72
165.93
157.88
157.51
155.75
154.41
154.13
154.01
127.36
122.14
121.80
120.37
117.79
113.92
111.35
111.19
99.42
98.90
77.41
77.16
76.91
33.99
33.28
29.33
29.05
HO
O
O
+
HO
O
O
10
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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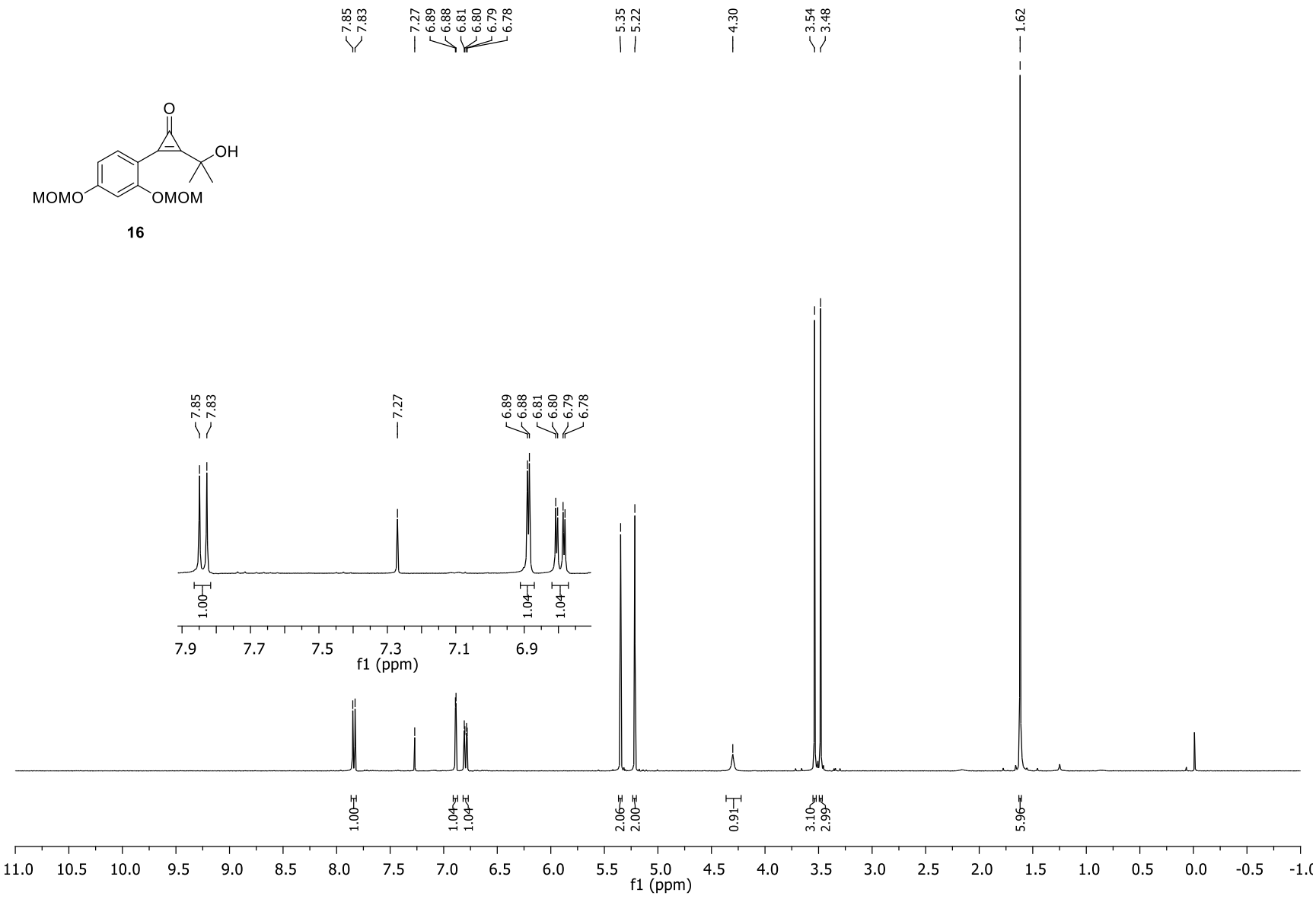
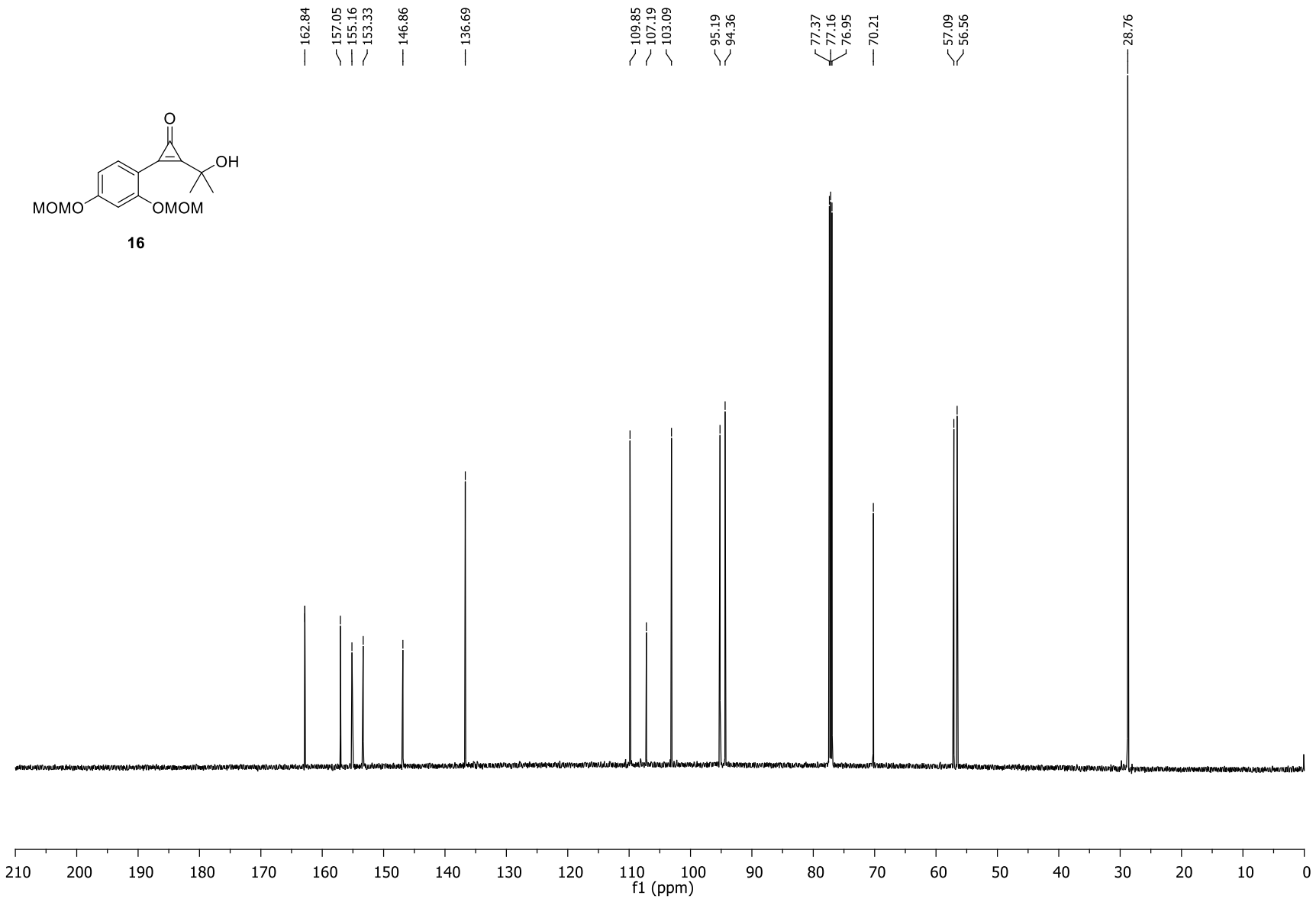
8.10
7.27
7.16
7.15
7.15
7.15
7.13
7.13
7.13
7.06
7.06
7.06
5.30
4.15
4.13
4.11
4.09
2.50
2.32
2.04
1.64
1.58
1.28
1.26
1.24
0.89
O
OH
AcO
OAc
14
f1 (ppm)


O
OH
MOMO
OMOM
16
7.85
7.83
7.27
6.89
6.88
6.81
6.80
6.79
6.78
5.35
5.22
4.30
3.54
3.48
1.62
1.00
1.04
1.04
2.06
2.00
0.91
3.10
2.99
5.96
f1 (ppm)


162.84
157.05
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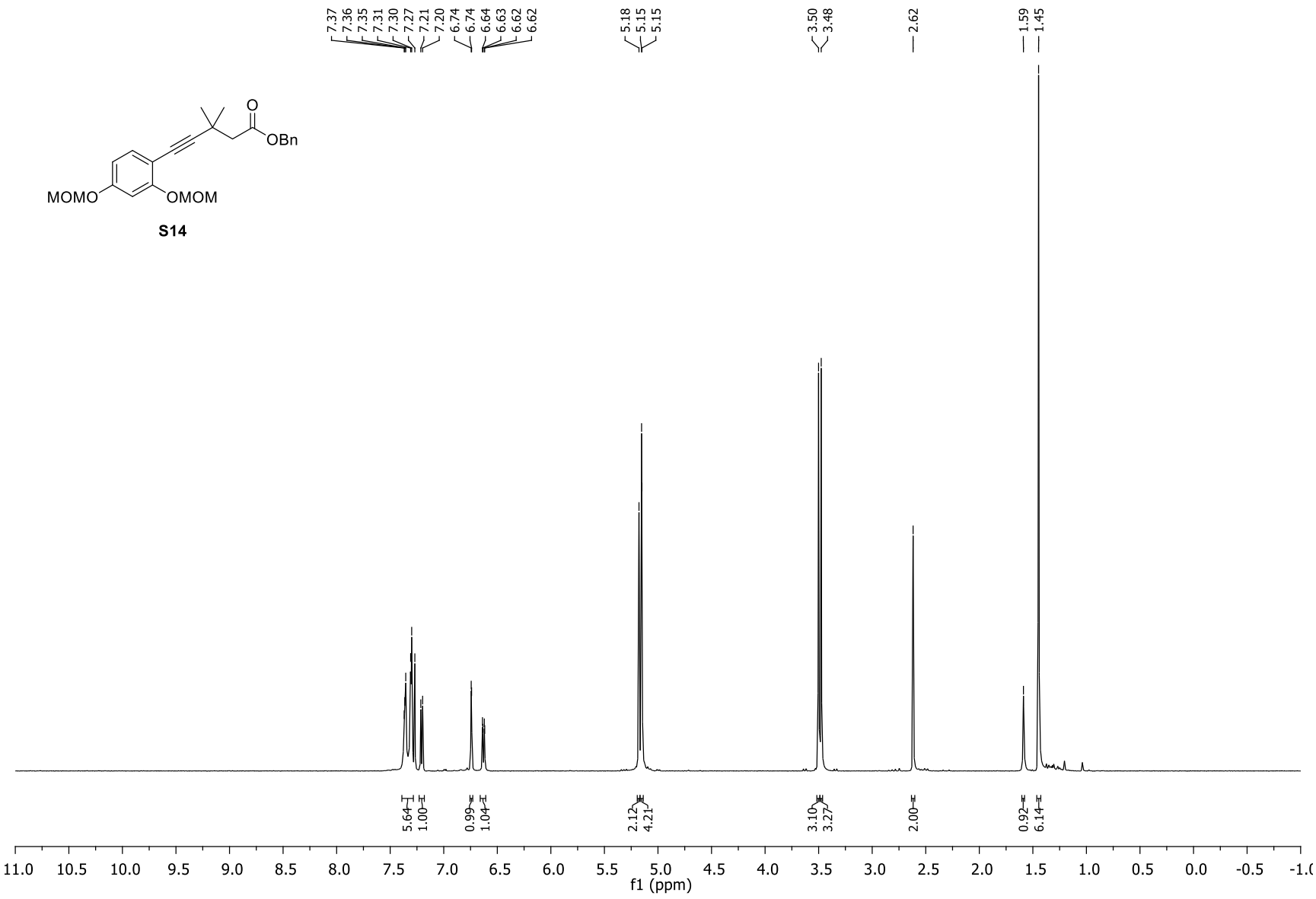
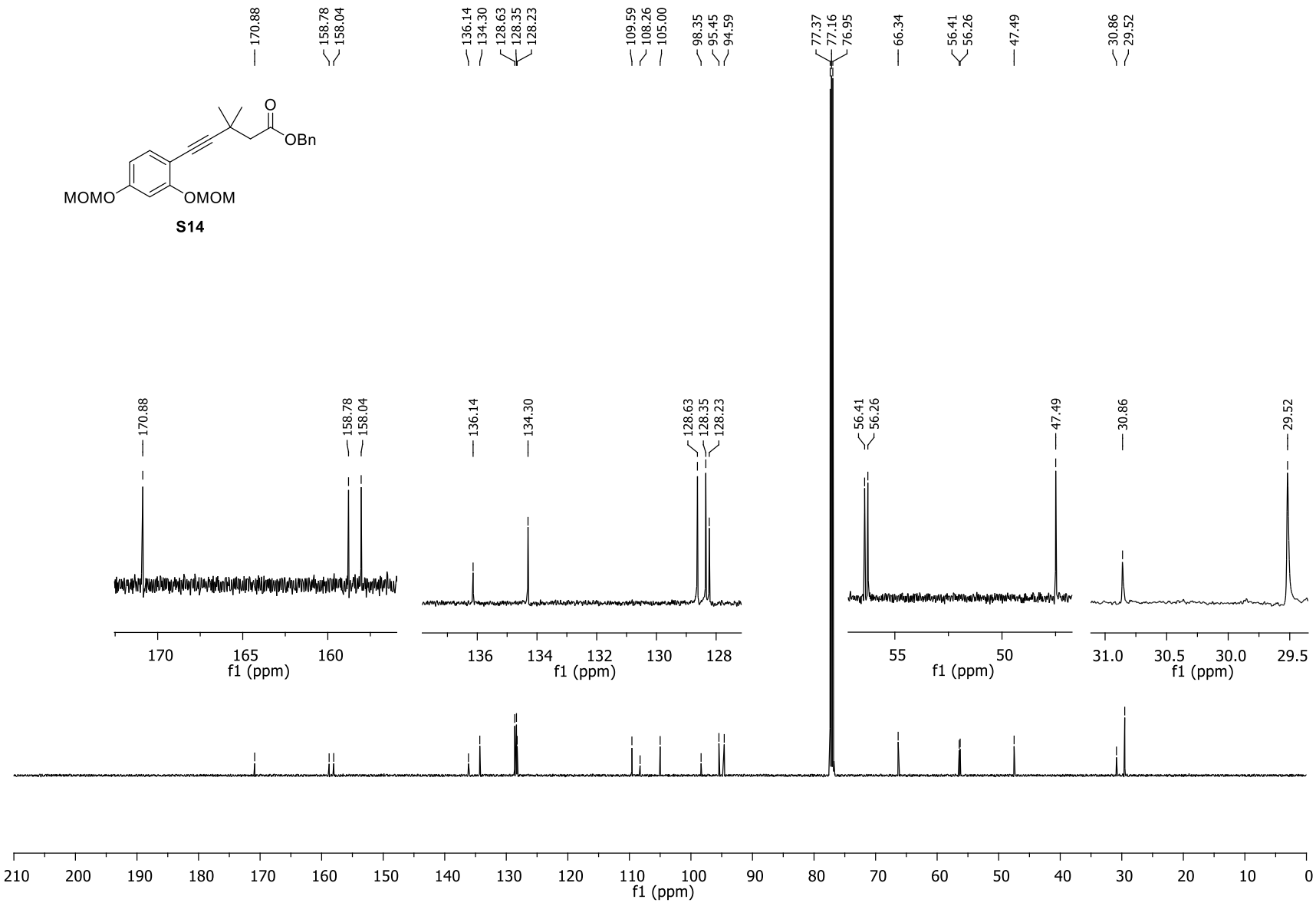
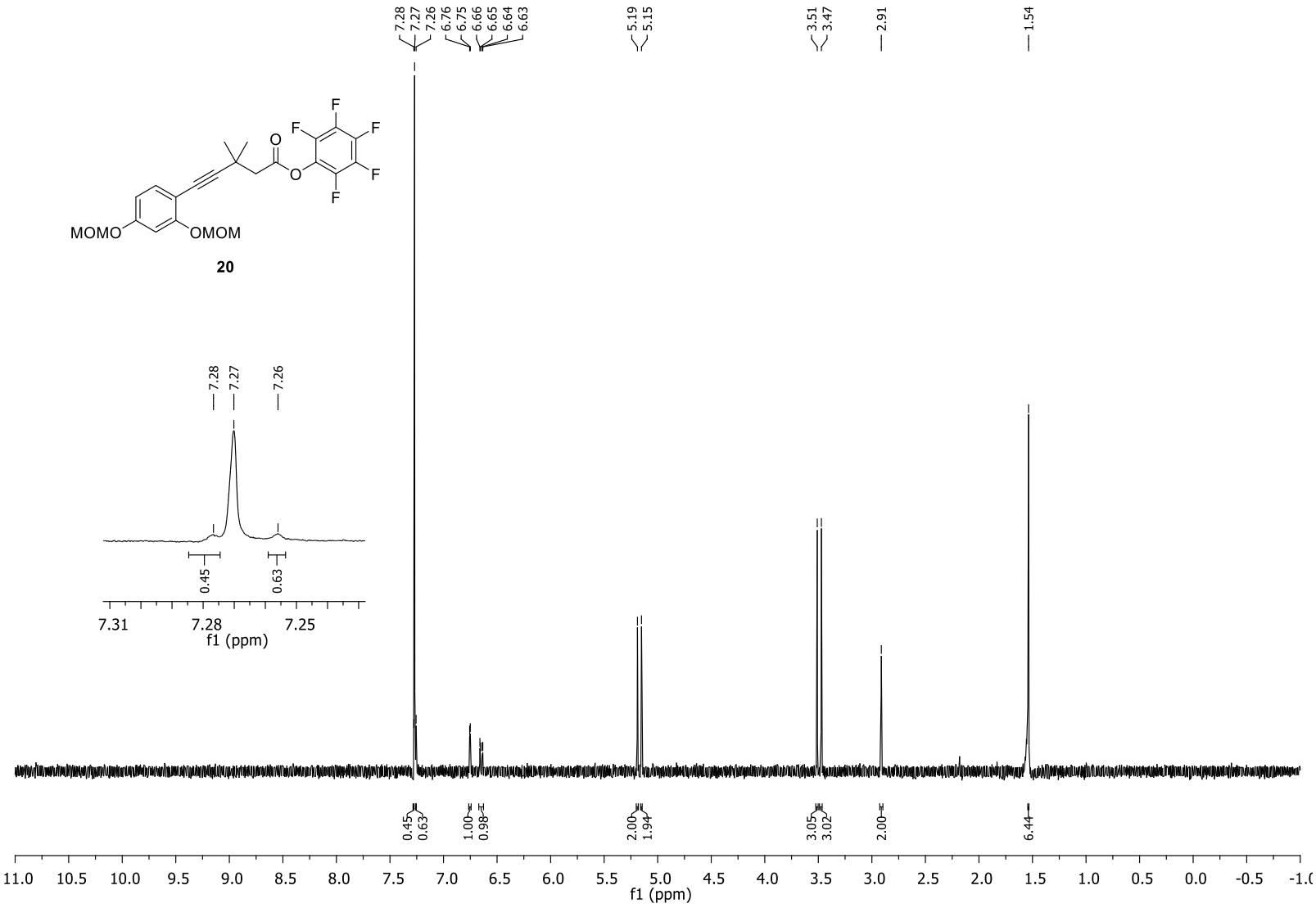
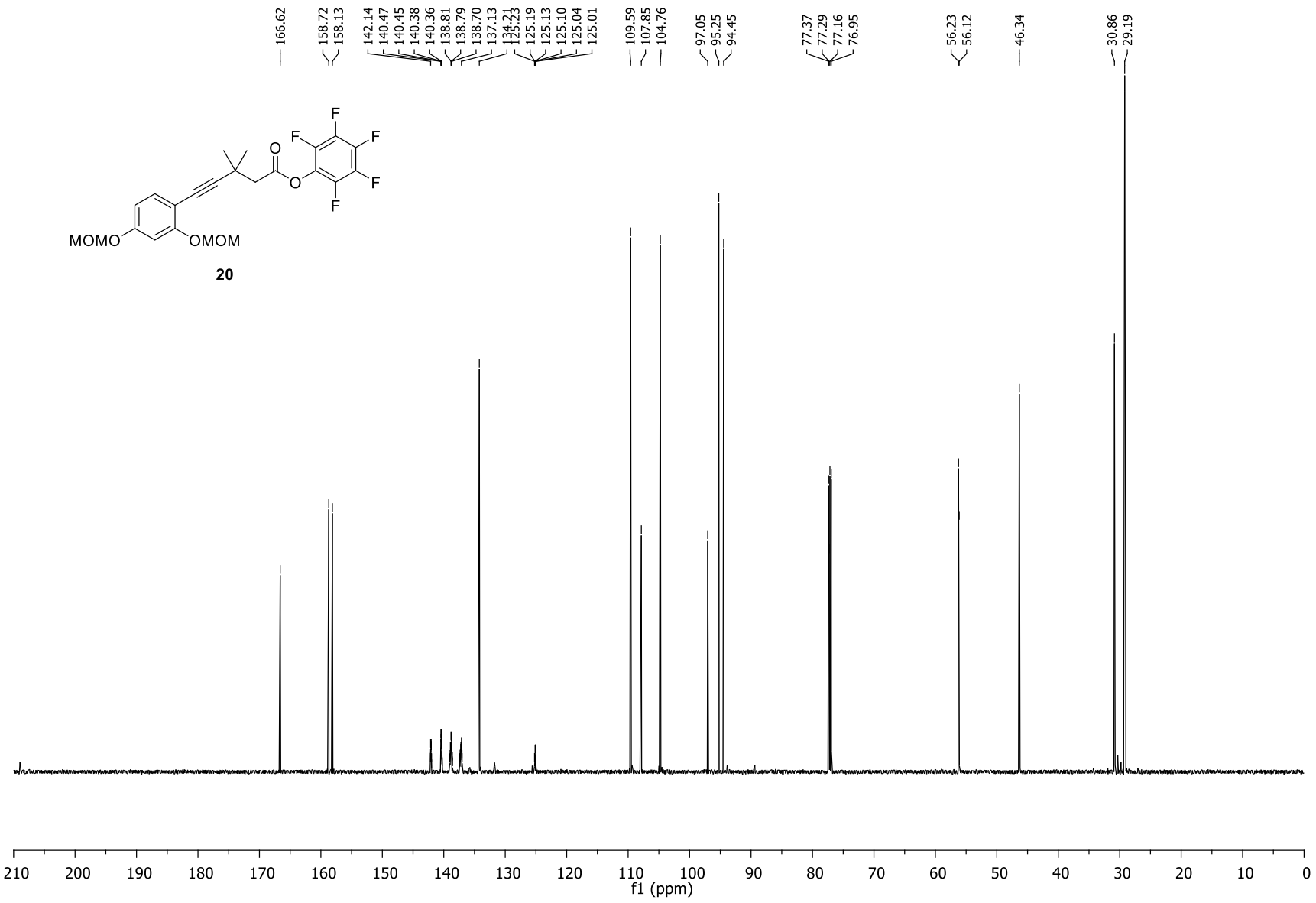
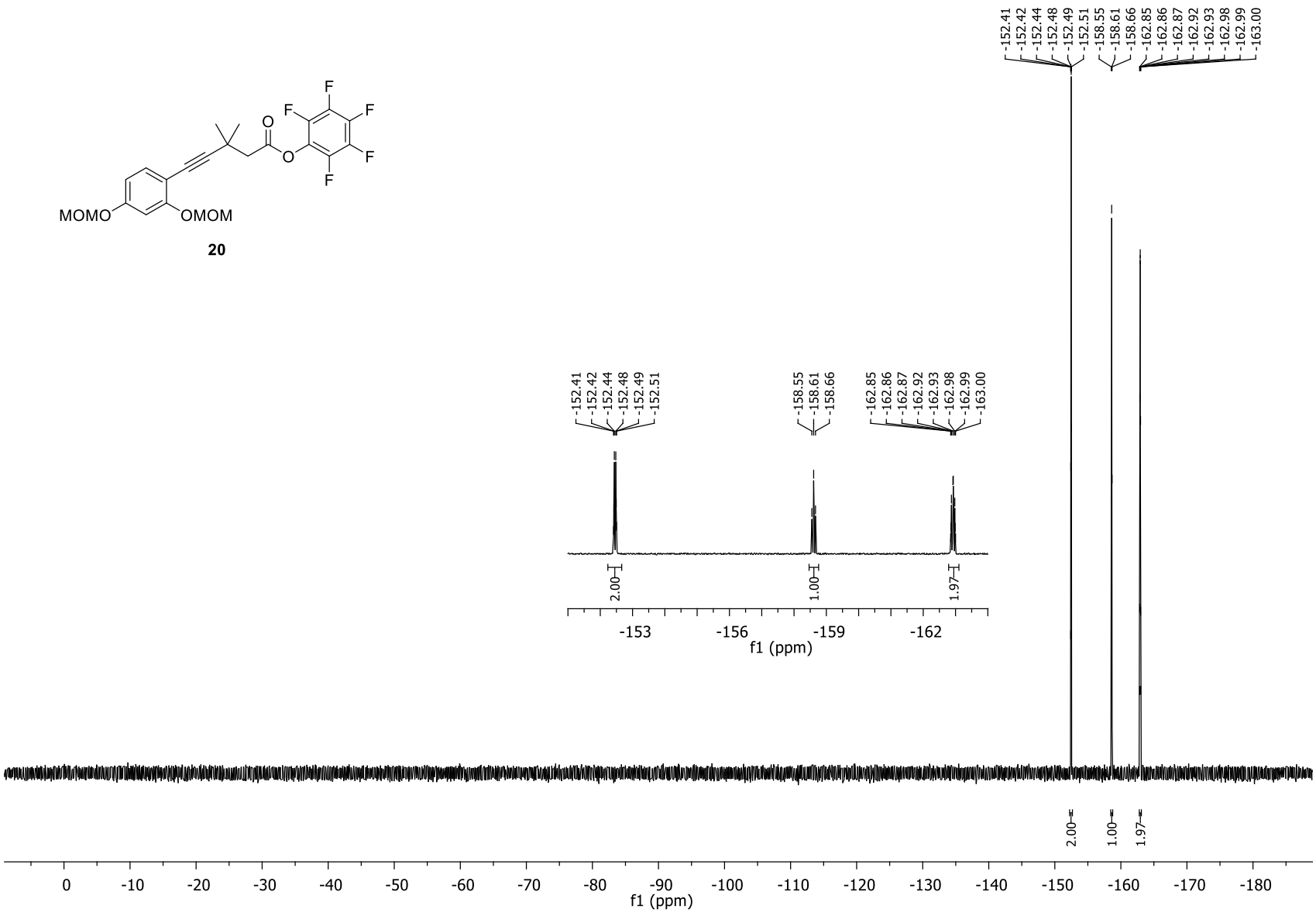
153.33
146.86
136.69
109.85
107.19
103.09
95.19
94.36
77.37
77.16
76.95
70.21
57.09
56.56
28.76
O
OH
MOMO
OMOM
16
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

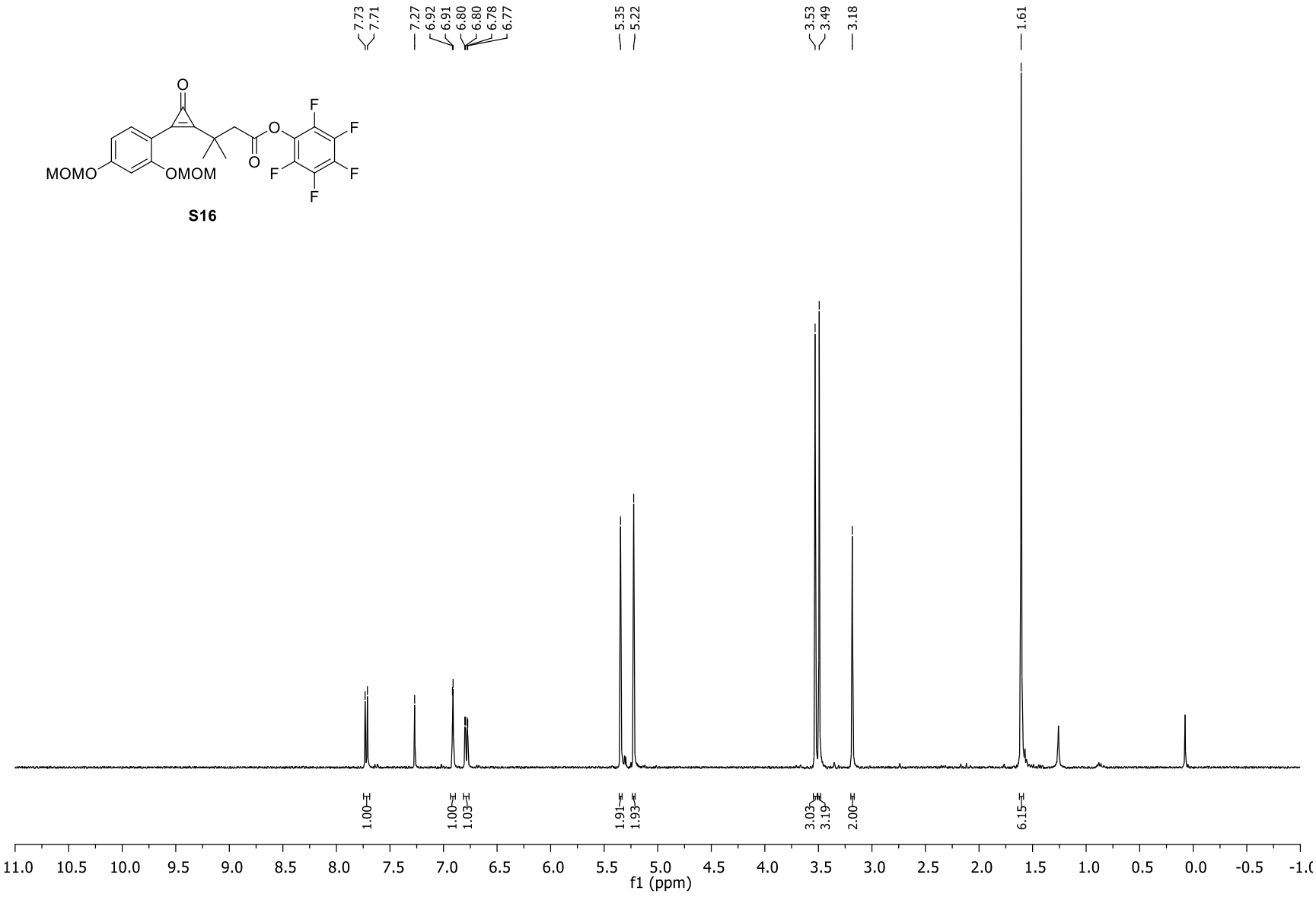

7.37
7.36
7.35
7.31
7.30
7.27
7.21
7.20
6.74
6.64
6.63
6.62
6.62
5.18
5.15
5.15
3.50
3.48
2.62
1.59
1.45
O
OBn
MOMO
OMOM
S14
5.64
1.00
0.99
1.04
2.12
4.21
3.10
3.27
2.00
0.92
6.14
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

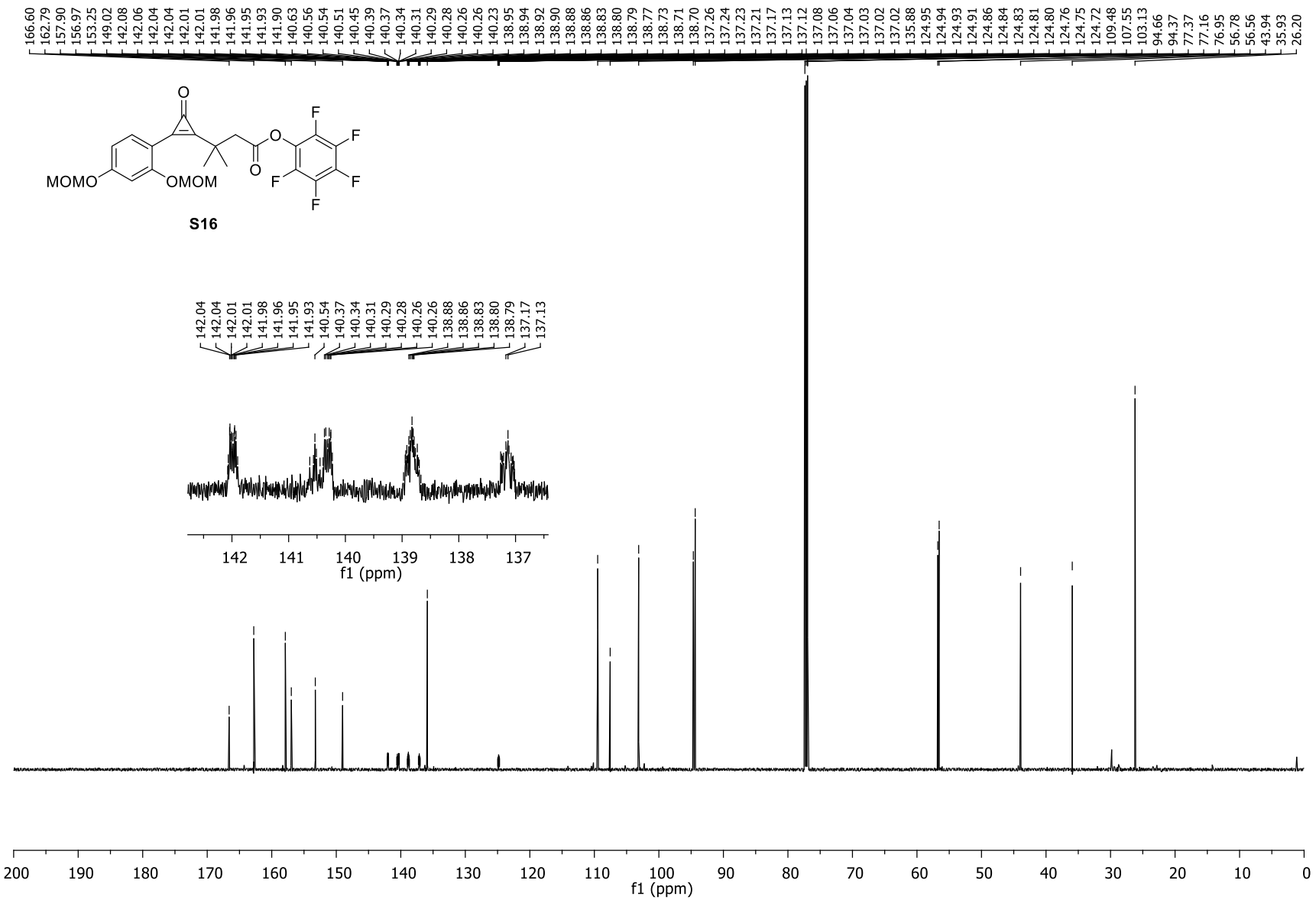

170.88
158.78
158.04
136.14
134.30
128.63
128.35
128.23
109.59
108.26
105.00
98.35
95.45
94.59
77.37
77.16
76.95
66.34
56.41
56.26
47.49
30.86
29.52
O
OBn
MOMO
OMOM
S14
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

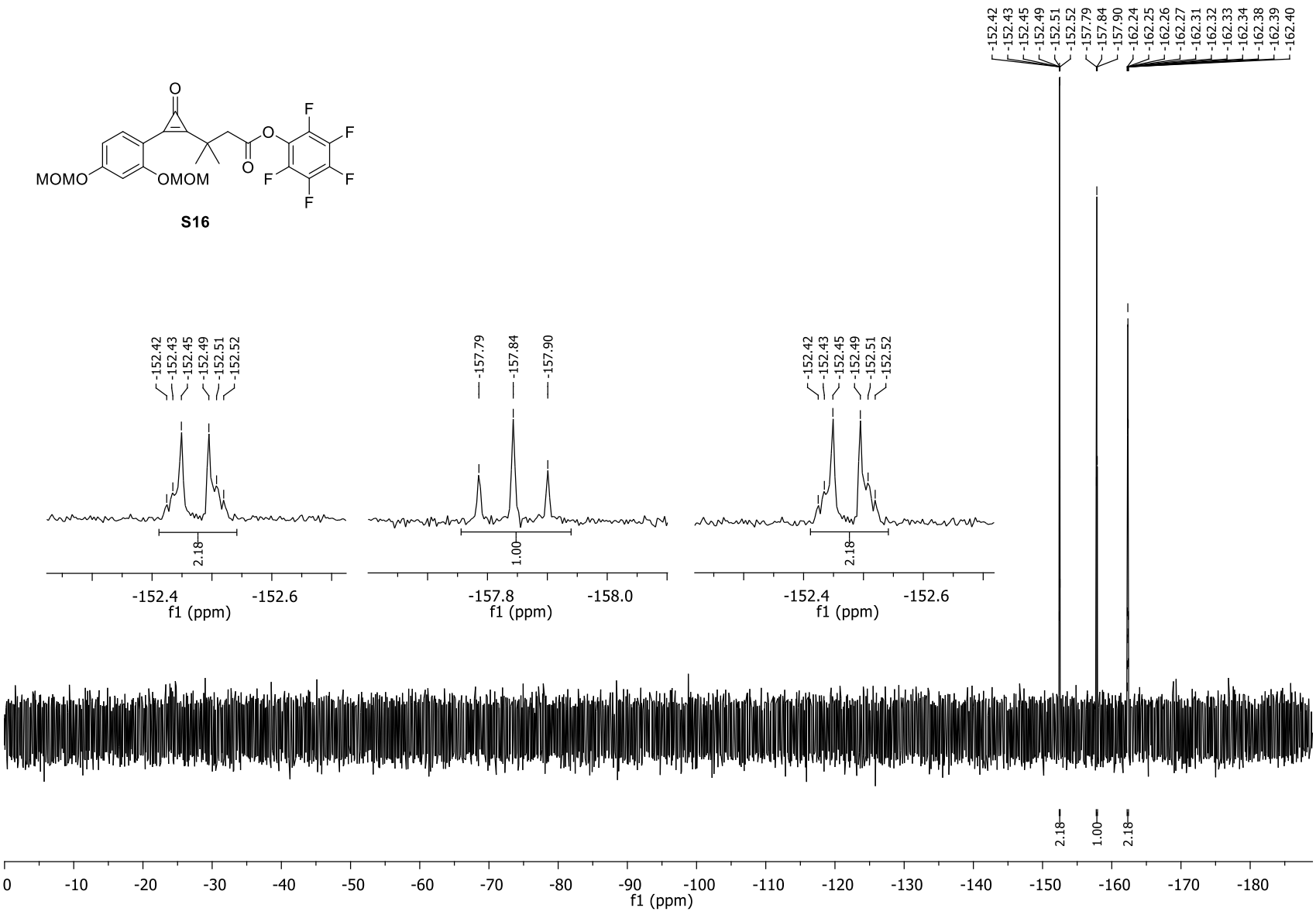

7.28
7.27
7.26
6.76
6.75
6.66
6.65
6.64
6.63
5.19
5.15
3.51
3.47
2.91
1.54
F
F
F
F
F
O
O
MOMO
OMOM
20
0.45
0.63
7.31
7.28
7.25
f1 (ppm)
0.45
0.63
1.00
0.98
2.00
1.94
3.05
3.02
2.00
6.44
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

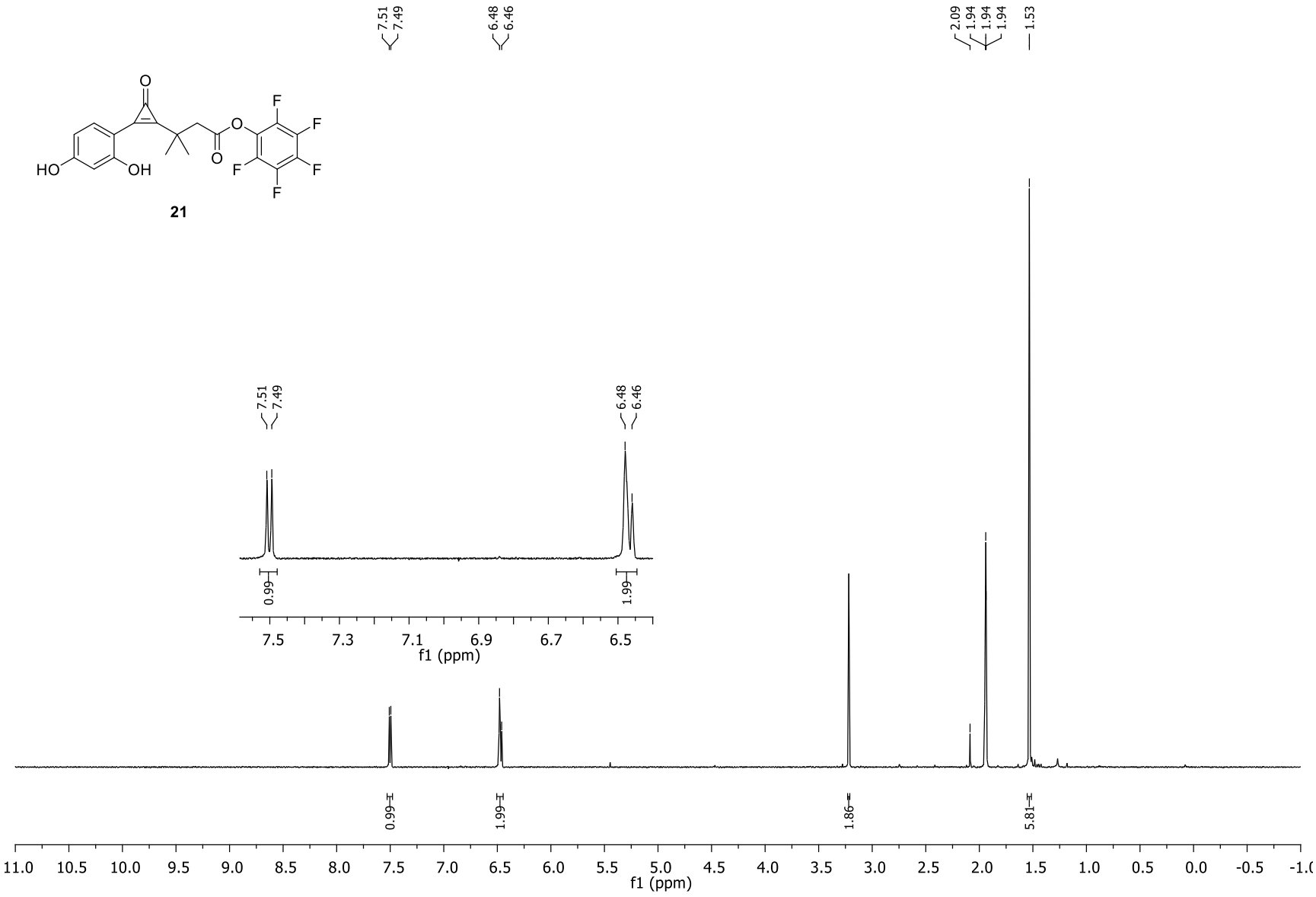

166.62
158.72
158.13
142.14
140.47
140.45
140.38
140.36
138.81
138.79
138.70
137.13
125.19
125.13
125.10
125.04
125.01
109.59
107.85
104.76
97.05
95.25
94.45
77.37
77.29
77.16
76.95
56.23
56.12
46.34
30.86
29.19
F
F
F
F
F
O
O
MOMO
OMOM
20
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

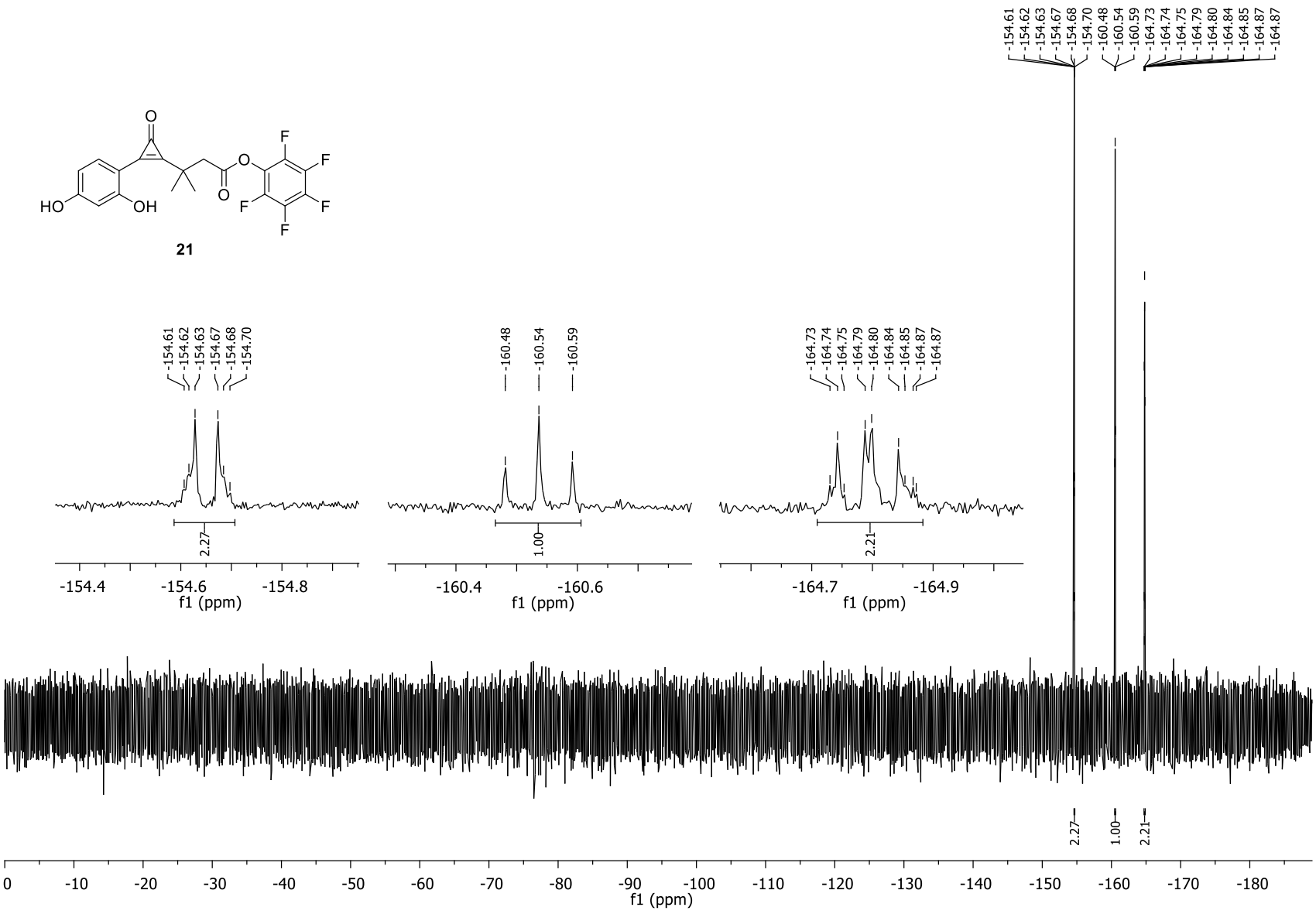

MOMO
OMOM
O
O
F
F
F
F
F
20
-152.41
-152.42
-152.44
-152.48
-152.49
-152.51
-158.55
-158.61
-158.66
-162.85
-162.86
-162.87
-162.92
-162.93
-162.98
-162.99
-163.00
2.00
1.00
1.97
-153
-156
-159
-162
f1 (ppm)
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
f1 (ppm)

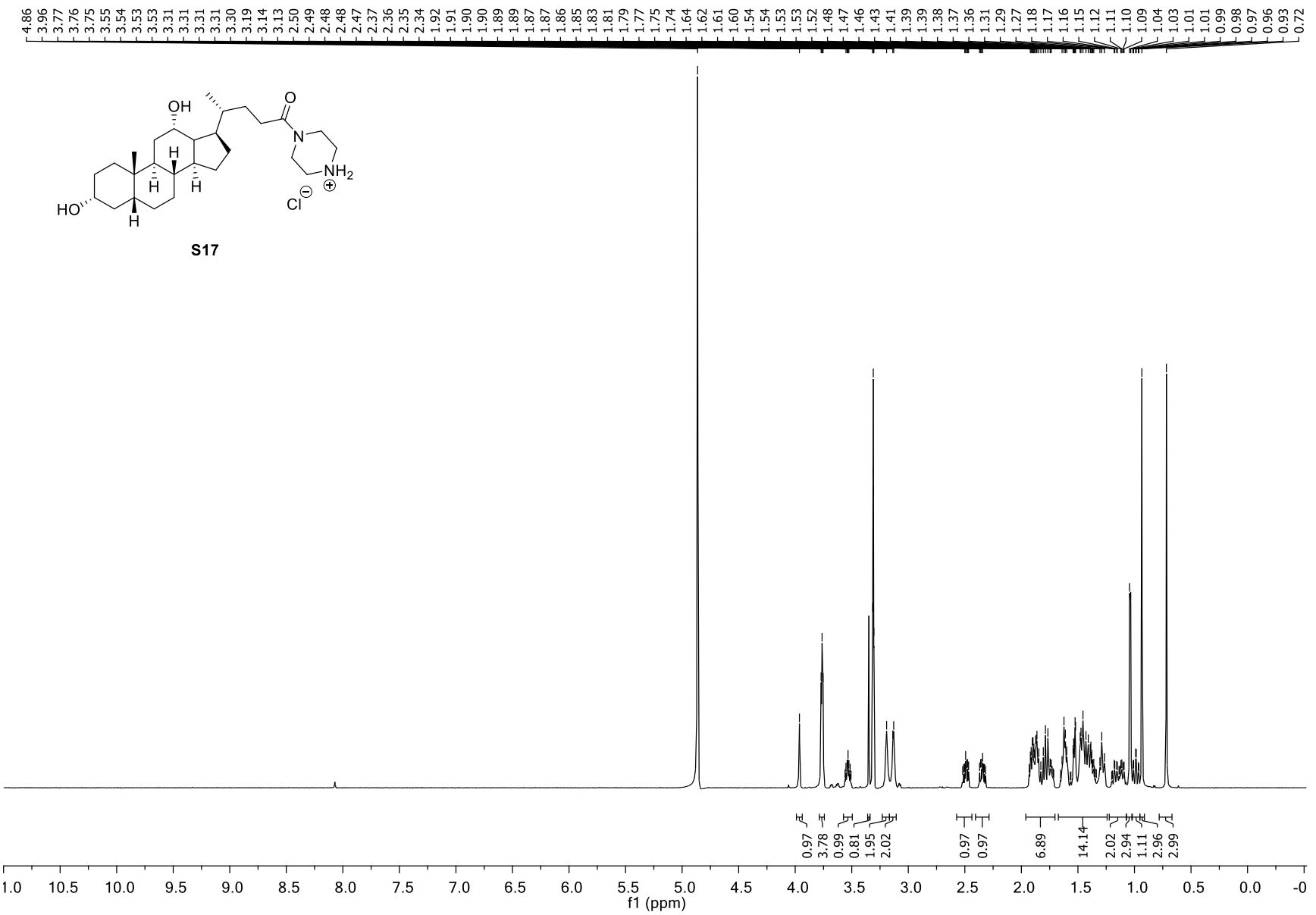

7.73
7.71
7.27
6.92
6.91
6.80
6.78
6.77
5.35
5.22
3.53
3.49
3.18
1.61
O
O
O
O
F
F
F
F
F
MOMO
OMOM
S16
1.00
1.00
1.03
1.91
1.93
3.03
3.19
2.00
6.15
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

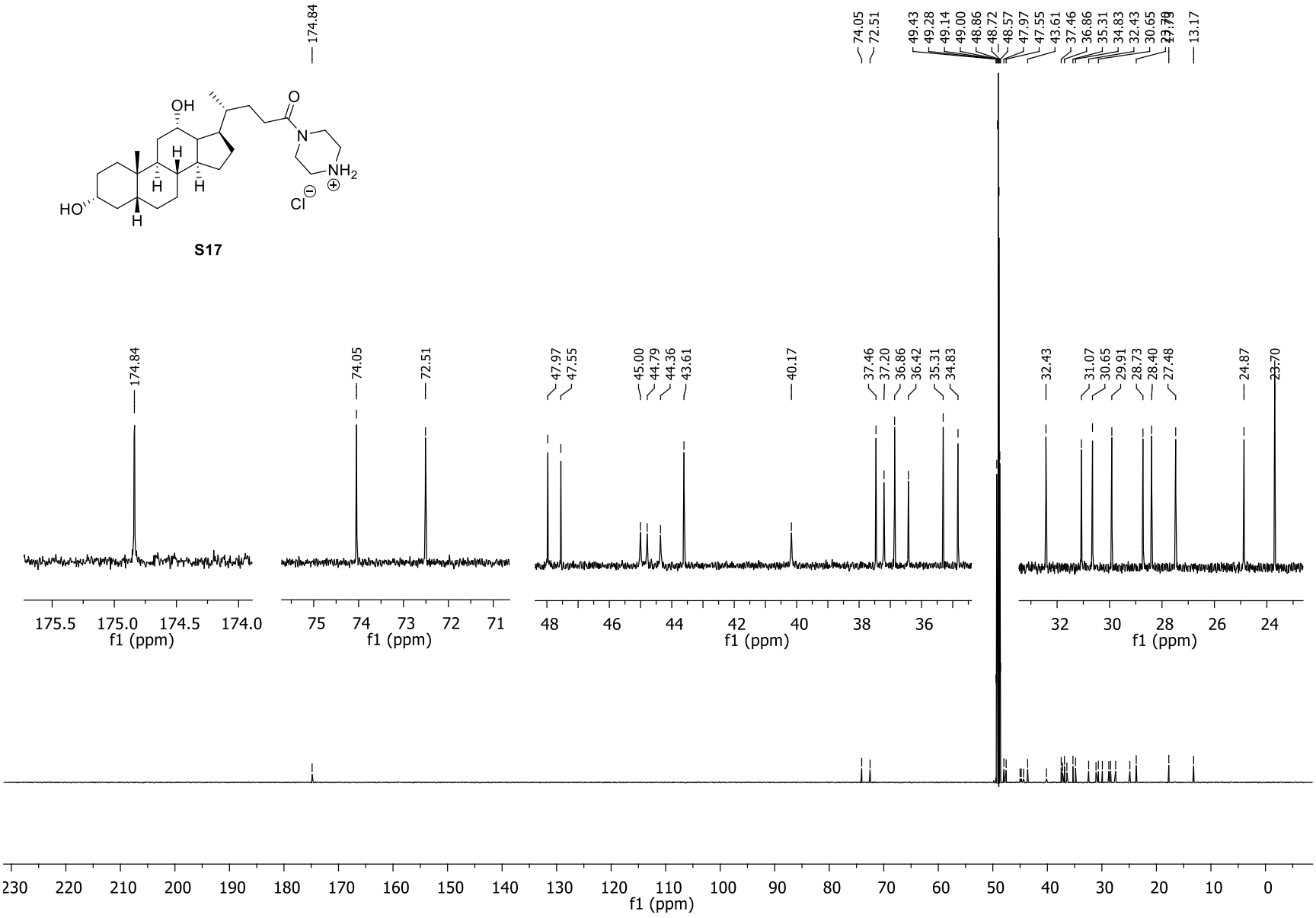

166.60
162.79
157.90
156.97
153.25
149.02
142.08
142.06
142.04
142.04
142.01
142.01
141.98
141.96
141.95
141.93
141.90
140.63
140.56
140.54
140.51
140.45
140.39
140.37
140.34
140.31
140.29
140.28
140.26
140.23
138.95
138.94
138.92
138.90
138.88
138.86
138.83
138.80
138.79
138.77
138.73
138.71
138.70
137.26
137.24
137.23
137.21
137.17
137.13
137.12
137.08
137.06
137.04
137.03
137.02
137.02
135.88
124.95
124.94
124.93
124.91
124.86
124.84
124.83
124.81
124.80
124.76
124.75
124.72
109.48
107.55
103.13
94.66
94.37
77.37
77.16
76.95
56.78
56.56
43.94
35.93
26.20
O
O
O
F
F
F
F
F
MOMO
OMOM
S16
142.04
142.04
142.01
142.01
141.98
141.96
141.95
141.93
140.54
140.37
140.34
140.31
140.29
140.28
140.26
140.26
138.88
138.86
138.83
138.80
138.79
137.17
137.13
142
141
140
139
138
137
f1 (ppm)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

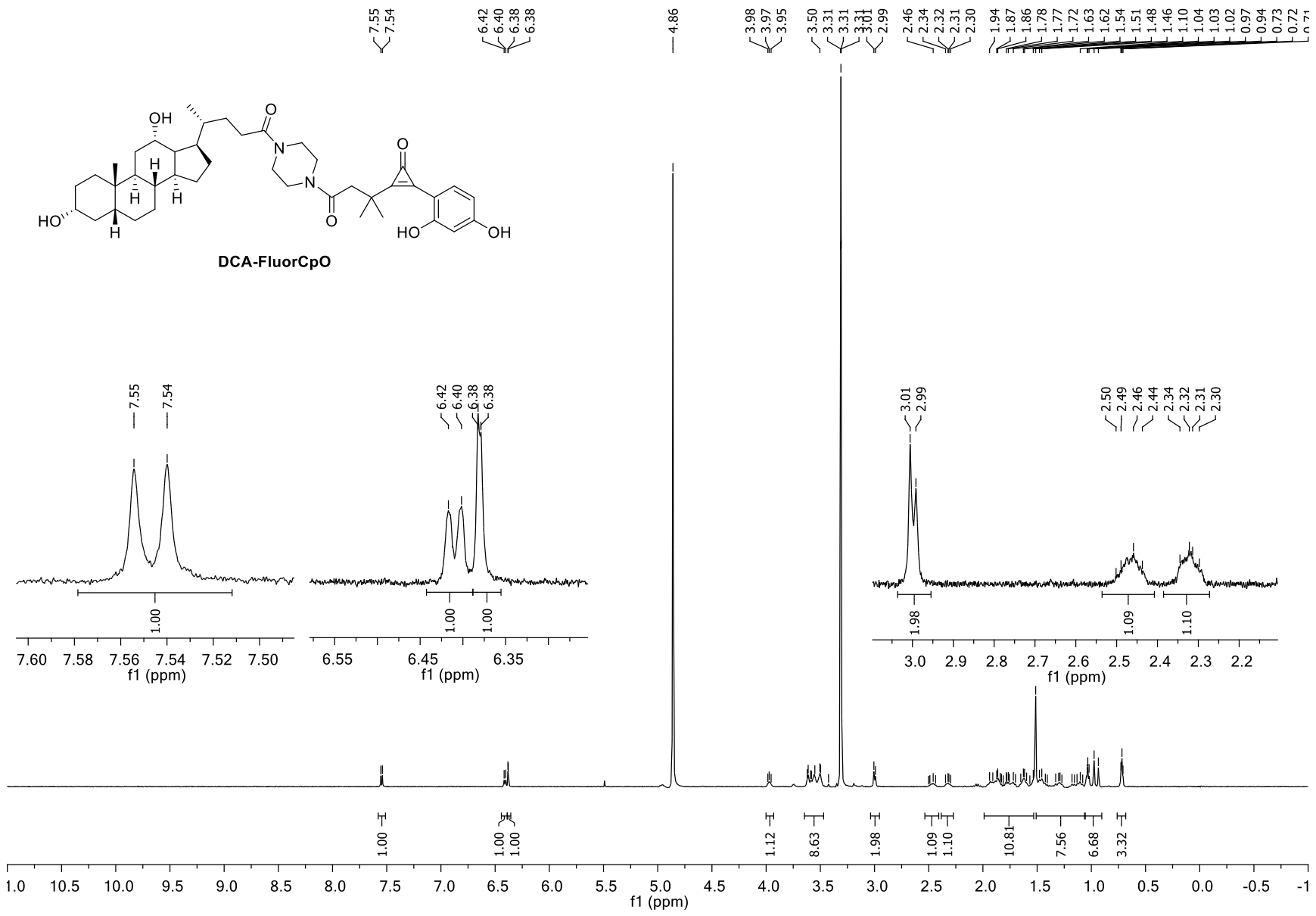

-152.42
-152.43
-152.45
-152.49
-152.51
-152.52
-157.79
-157.84
-157.90
-162.24
-162.25
-162.26
-162.27
-162.31
-162.32
-162.33
-162.34
-162.38
-162.39
-162.40
O
O
O
F
F
F
F
F
MOMO
OMOM
S16
2.18
1.00
2.18
-152.4
-152.6
-157.8
-158.0
f1 (ppm)
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180


7.51
7.49
6.48
6.46
2.09
1.94
1.94
1.94
1.53
O
HO
OH
O
O
F
F
F
F
F
21
0.99
1.99
1.86
5.81
f1 (ppm)


-154.61
-154.62
-154.63
-154.67
-154.68
-154.70
-160.48
-160.54
-160.59
-164.73
-164.74
-164.75
-164.79
-164.80
-164.84
-164.85
-164.87
-164.87
O
O
O
F
F
F
F
F
HO
OH
21
2.27
1.00
2.21
-154.4
-154.6
-154.8
f1 (ppm)
-160.4
-160.6
f1 (ppm)
-164.7
-164.9
f1 (ppm)
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
f1 (ppm)


4.86 3.96 3.77 3.76 3.75 3.55 3.54 3.53 3.53 3.31 3.31 3.31 3.31 3.30 3.19 3.14 3.13 2.50 2.49 2.48 2.48 2.47 2.37 2.36 2.35 2.34 1.92 1.91 1.90 1.90 1.89 1.89 1.87 1.87 1.86 1.85 1.83 1.81 1.79 1.77 1.75 1.74 1.64 1.62 1.61 1.60 1.54 1.54 1.53 1.53 1.52 1.48 1.47 1.46 1.43 1.41 1.39 1.39 1.38 1.37 1.36 1.31 1.29 1.27 1.18 1.17 1.16 1.15 1.12 1.11 1.10 1.09 1.04 1.03 1.01 1.01 0.99 0.98 0.97 0.96 0.93 0.72
OH
O
N
NH2
Cl
H
H
H
HO
H
S17
0.97 3.78 0.99 0.81 1.95 2.02 0.97 0.97 6.89 14.14 2.02 2.94 1.11 2.96 2.99
1.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0
f1 (ppm)


174.84
74.05
72.51
49.43
49.28
49.14
49.00
48.86
48.72
48.57
47.97
47.55
43.61
37.46
36.86
35.31
34.83
32.43
30.65
13.17
OH
O
N
NH2
Cl
HO
H
S17
174.84
74.05
72.51
47.97
47.55
45.00
44.79
44.36
43.61
40.17
37.46
37.20
36.86
36.42
35.31
34.83
32.43
31.07
30.65
29.91
28.73
28.40
27.48
24.87
23.70
f1 (ppm)


DCA-FluorCpO
f1 (ppm)