Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Identification, Cloning, Expression and Functional Interrogation of the Biosynthetic Pathway of the Polychlorinated Triphenyls Ambigol A–C from *Fischerella ambigua* 108b

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Supporting Information

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1. General Methods

1.1 Biochemical Methods

Cultivation: *E. coli* cells were grown in liquid culture in LB medium (Carl Roth, Germany) at 37 °C under 200 rpm shaking. *S. elongatus* liquid cultures were grown in BG11 medium (Sigma-Aldrich/Merck, Germany) at 30 °C with 150 rpm shaking and constant 300 LUX illumination from a LED lamp (Fluval A3972 AURA Fresh and Plant Nano LED, 7500 K, Canada). Cells on plates were kept at room temperature with constant 250 LUX illumination growing on BG11 containing 1.5% agarose (Sigma-Aldrich/Merck). Chemically competent *E. coli* DH5α cells were used as a host strain for all cloning steps. Enzymes used for PCR and cloning were purchased from NEB (Germany). Antibiotics were used at the following concentrations: ampicillin 100 mg/L (Carl Roth, Germany), kanamycin 50 mg/L for *E. coli* cells and 5 mg/L for *S. elongatus* cells (Carl Roth), spectinomycin 2 mg/L (Merck/Sigma-Aldrich, Germany) and streptomycin 2 mg/L (Merck/Sigma-Aldrich).

DNA Isolation, Sequencing and Bioinformatic Analysis of the *ab* BGC: To isolate genomic DNA for subsequent sequencing, first an axenic culture of the *Fischerella ambigua* had to be generated, since a *Pseudomonas* strain was associated with it. Therefore, a spheric cyanobacterial colony was dispersed in 500 µL water and the resulting cell suspension was then transferred into 5 mL LB medium supplemented with ampicillin (final concentration 500 µg/mL). The culture was incubated overnight at 37 °C while shaking at 180 rpm. Cyanobacterial cells were recovered by centrifugation, resuspended in 100 mL BG11 medium and grown at 25 °C with shaking at 120 rpm under constant illumination. Cells were harvested after 2–3 weeks of growth, washed three times with sterile water and resuspended in 20 mL SET buffer (75 mM NaCl, 25 mM EDTA, 10 mM Tris-HCl). Using a Potter homogenisator, filaments were broken up and cells separated. SDS 0.5%, proteinase K 500 µg/mL and lysozyme 2.5 mg/mL were added and the suspension was incubated at 55 °C for 2 h. Subsequently, an extraction with one volume of phenol:chloroform:isoamyl alcohol (25:24:1) was performed for 30 min. This step was repeated until no precipitated proteins were visible between the aqueous and the organic phase. The resulting supernatant was purified using genomic tips, which were supplied with the Blood & Cell Culture DNA Mini Kit (Qiagen, Germany) based on the manufacturer's instruction manual. The resulting DNA was submitted to 454 sequencing applying the Roche GS FLX Titanium sequencer (GATC Biotech, Germany). Open reading frames (ORFs) were predicted with the Geneious 8.0.4 software (Biomatters Limited, New Zealand) and bioinformatically analysed using the NCBI/blastx alignment tool (U.S. National Library of Medicine, USA).[1]

Direct Pathway Cloning of the *ab* BGC: The 14.3 kb *ab1-10* BGC was cloned in one piece into pET-28b-SUMO using Gibson Assembly mediated DiPaC.[2] The linearized *ab* PCR amplicon was generated using PCR with Q5 polymerase with 15 ng gDNA per 25 µL reaction setup and integrated into the *Bam*HI (NEB) linearised and dephosphorylated vector backbone. The *gfp* reporter gene was amplified by PCR with Q5 polymerase using 100 ng plasmid DNA per 25 µL reaction setup and integrated into the *Not*I linearized and dephosphorylated pET-28b-SUMO::*ab* plasmid. This expression constructed was transformed into pET-28b-ptetO::*ab-gfp* by exchanging the promoter region with Gibson assembly. Positive clones were selected by Taq screening PCR and confirmed using restriction digest. Mutation free amplification and assembly was verified by Sanger sequencing (GATC Biotech). For expression of the *ab* BGC in the cyanobacterial host *S. elongatus* PCC 7942, the cluster was split in

two parts of roughly equal size with respect to ORF boundaries and integrated into the two neutral sites NSI and NSII of the bacterial chromosome. Therefore, the 6.4 kb *ab1-5* fragment was amplified from plasmid DNA and equipped with homology sequence overhangs by Q5 polymerase PCR and integrated into the *Eco*RI linearised and dephosphorylated pAM5051 vector by Gibson Assembly mediated DiPaC. Positive *E. coli* DH5α clones were selected by Taq screening PCR and confirmed using restriction digest Sanger sequencing of the integration sites. The 7.8 kb *ab6-10* fragment was integrated into pCV0094 in a similar manner. *S. elongatus* cells were naturally transformed with 0.5 µg pCV0094::*ab*5-7 as described previously.[3] After selection of positive clones on $BG11_{Kan}$ plates (BG11 Broth (Sigma-Aldrich/Merck), Trace Metal Mix A5 with Co (Sigma-Aldrich/Merck), 1.5% (w/v) agarose (Sigma-Aldrich/Merck), 1 mM $Na_2S_2O_3$ (Grüssing, Germany) and verifying complete integration of *ab6-10* into NSII on all genome copies by Q5 PCR on liquid cultures[3], the natural transformation and selection procedure was repeated with pAM5051::*ab1-5* resulting in double mutant *S. elongatus* cells harboring *ab1-5* in the chromosomal integration site NSI and *ab6-10* in NSII. Double mutants were grown in BG11 liquid medium or on BG11 agar supplemented with Kan, Spec and Strep.

Heterologous Expression of the *ab* BGC in *E. coli* BAP1 and *S. elongatus* PCC 7942: pET-28b-SUMO::*ab* and pET-28b-SUMO::*ab-gfp* were transformed into chemically competent *E. coli* BAP1 cells. Main cultures were inoculated 1:50 into LB_{Kan} medium (containing 10 g/L NaCl, Carl Roth) from overnight precultures grown in LB_{Kan} and grown at 37 °C 200 rpm until an OD_{600} value of around 0.6. At this point, 20 mg/L 5-aminolevulinic acid (Carl Roth) were added to support formation of the heme cofactor and its incorporation into the target CYP P450 enzymes. Protein expression was induced at OD_{600} around 0.8-1.0 by the addition of 1 mM IPTG (Acros Organics, USA) in case of the pET28b-SUMO based plasmids or 0.5 mg/L tetracycline (Fluka, Switzerland) for pET-28b-ptetO::*ab* plasmid and cells were incubated at 16 °C 200 rpm for 20 h to 5 days with or without the daily addition of 100 mg/L 4-HBA (**2**) (Carl Roth). After harvesting the cells, the pellets were once washed with 0.9% NaCl. Culture supernatant and cell pellets were either directly extracted or stored at -20 °C.
S. elongatus cells harbouring *ab1-5* in NSII and *ab6-10* in NSI were grown in BG11 medium supplemented with Kan, Spec, Strep and 1 g/L NaCl until an OD_{750} of 1.0-1.3. Protein expression was induced with 2 mM theophylline (200 mM stock in DMSO, Acros Organics) and 100 mg/L **2** was added daily for 5 days. After harvesting the cells, the pellets were once washed with 0.9% NaCl. Culture supernatant and cell pellets were either directly extracted or stored at -20 °C.
Culture supernatants were acidified with HCl to a pH of 3 and extracted twice with 1 vol EtOAc. Organic extracts were combined and evaporated *in vacuo*. The residues were resuspended in MeOH or ACN, filtered through a 0.45 µm PTFE syringe filter (Fisher Scientific, Germany) and analysed by HPLC-MS. Cell pellets were resuspended in 2:1 DCM:MeOH and sonicated for 30 min in a Bransonic ultrasonic bath 3510 E-MTH (Branson, USA). After centrifugation at 8.000 g for 10 min at 4 °C, the supernatant was evaporated *in vacuo* and the residues treated as described above.

Cloning and Heterologous Expression of Ab2 and Ab3 in *E. coli*: Codon optimised gene sequences of *ab2* and *ab3* (*ab2*e and *ab3*e) for heterologous overexpression in *E. coli* were ordered at BaseClear B.V. (The Netherlands). *Ab2* was integrated into the pMal expression plasmid by Gibson assembly, thereby deleting the ATG start codon and directly attaching the sequence to the *N*-terminal MBP-tag. *Ab3* was integrated into pMal by restriction cloning using *Bam*HI and *Xho*I and ligation, thus preserving the 102 bp linker sequence between the MBP-tag and the MCS containing a TEV cleavage site.

pMal-SD::*ab2*e and pMal-SD::*ab3*e were transformed into chemically competent *E. coli* BL21 cells. Expression cultures were inoculated 1:50 into TB_{Amp} medium (Carl Roth) from overnight precultures grown in LB_{Amp} medium. Protein expression was otherwise performed as described for the pET-28b-SUMO::*ab-gfp* construct in *E. coli* BAP1 cells but no **2** was added and cells were always harvested after 20 h.

Purification of Ab2 and Ab3 from *E. coli* Heterologous Expression: Cells were resuspended in buffer A (50 mM Tris (Carl Roth) [pH 7.3], 5% (v/v) glycerol (Fisher Scientifc) and sonicated nine times for 10 s with 80% amplitude with a Bandelin SONOPULS Homogenisator HD 2070 MS 72 (Bandelin electronics, Germany). Cell debris was removed by centrifugation at 18.000 g at 4 °C for 30 min. Protein purification was carried out at 4 °C with an Äkta Pure 25 M system (GE Healthcare Life Science, Germany) and a MBPtrap HP 5 ml (GE Healthcare) using the following protocol: loading at 4 mL/min, washing with 100 mL buffer A at 4 mL/min, elution with 3.5 mL buffer B (buffer A containing 10 mM maltose, Carl Roth) at 2 mL/min. Protein concentration was determined using an Implen Nanophotometer P330 5382 (Implen, Germany) and the following parameters: Ab2: 97537.67 Da, ε = 126420; Ab3: 101088.93 Da, ε = 124930.

Ab2 and Ab3 *in vitro* Enzymatic Assays: Purified enzymes were used for enzymatic conversion of 2,4-DCP (**6**) without further concentration. Assays consisted of 20-60 µM Ab2 and/or Ab3, 3 mM 2,4-DCP (**6**) (300 mM stock in DMSO, Sigma-Aldrich), 4 mM NADPH (100 mM stock in H_2O, Carl Roth), 20 µM spinach ferredoxin (PetF) and 10 µM ferredoxin reductase (Fpr). Assays were conducted in 250 µL scale in 1.5 mL microreaction tubes under air permeable conditions and shaking with 400 rpm at 30 °C for 15 h. After acidifying the mixture with HCl to a pH of 3, organic compounds were extracted with 1 vol EtOAc. After evaporation, the remaining solid was solubilised in MeOH or ACN, filtered through a 0.45 µm PTFE syringe filter and analysed by HPLC-MS.

Cloning of Ab2 and Ab3 in *S. elongatus* PCC 7942: For chromosomal integration and expression of *ab2* and *ab3* in *S. elongatus*, the native gene sequences were integrated into the *Eco*RI linearized pCV0094 plasmid using Gibson assembly. After verifying the successful cloning by Taq screening PCR, analytical restriction digest and Sanger sequencing, *ab2, ab3* or both genes joined together with a 4 bp linker sequence were integrated into the NSII site of the cyanobacterial chromosome by natural transformation. Successful and complete integration into all genome copies was verified by Q5 screening PCR (see Supporting Information Figure S4).[3]

Ab2 and Ab3 *in vivo* 2,4-DCP Feeding Assays in *E. coli* and *S. elongatus*: To check for Ab2 and Ab3 induced 2,4-DCP (**6**) conversion *in vivo*, *E. coli* BL21 cells carrying pMal-SD::*ab2* or pMal::*ab3* were grown and induced alone or together as described above for heterologous protein production, but were additionally fed with 50 µM 2,4-DCP (**6**) (300 mM stock in DMSO) and cultivated for 1-3 days. *S. elongatus* cells carrying either *ab2*, *ab3* or both genes were grown to an OD_{750} value of 0.3-0.4 before induction with 2 mM theophylline (200 mM stock in DMSO) and feeding of 50 µM 2,4-DCP (**6**). Cells were harvested after 48 h. Culture supernatant and cell pellets of both *E. coli* and *S. elongatus* cells were extracted as described above. As negative controls, *E. coli* BL21 and *S. elongatus* wild type cells were cultivated and extracted under the same conditions.

HPLC-MS Analysis of *in vitro* and *in vivo* Extracts: Samples were analyzed using an UltiMate 3000 LC System system coupled to a LCQ Fleet Ion Trap Mass Spectrometer (Thermo Scientific). Interpretation of the recorded data was performed using the Thermo Xcalibur Qual Browser 2.2 SP1.48 software. The chromatographic HPLC separation was carried out on a Hypersil Gold AQ C18 (150 x 2.1 mm, 3.0 µm particle size) HPLC column (Thermo Fisher) with the following gradient with water (A) and acetonitrile (B) as the eluents, both buffered with 0.1% formic acid, was applied: 5% B (0 min) → 5% B (4.0 min) → 95% B (24.0 min) → 100% B (24.5 min) → 5% B (25.0 min) → 5% B (26.0 min). The flow rate was kept constant at 0.7 mL/min. Alternatively, a Eurosphere II 100-3 C18 A (150 x 4.6 mm, 3 µm particle size) HPLC column (Knauer, Germany) was used with the following gradient with water (A) and acetonitrile (B) as the eluents, both buffered with 0.1% formic acid, was applied: 5% B (0 min) → 5% B (5.0 min) → 30% B (10.0 min) → 30% B (40.0 min) → 40% B (45.0 min) → 100% B (45.5 min) → 100% B (50.5 min) → 5% B (51.0 min) → 5% B (52.0 min). The flow rate was kept constant at 1.0 mL/min.

Preparative HPLC-based Purification of Ab2 *in vitro* and *in vivo* Assay Products: For structural identification of the products formed by Ab2, large scale *in vitro* and *in vivo* assays were purified by preparative HPLC after extraction using a Eurospher II 100-5 C18 A (250 x 16 mm, 5 µm particle size) HPLC column with precolumn (30 x 16 mm) (Knauer) on a Jasco HPLC system consisting of an UV-1575 Intelligent UV/VIS Detector, two PU-2068 Intelligent Preparative Pumps, a MIKA 1000 Dynamic Mixing Chamber (1000 µl, Portmann Instruments AG, Switzerland), a LC-NetII/ADC and a Rheodyne injection valve. The following gradient with water (A) and acetonitrile (B) as eluents, both buffered with 0.05% TFA, was applied: 5% B (0 min) → 5% B (2 min) → 100% B (45 min) → 100% B (54 min) → 5% B (55 min) → 5% B (60 min). The flow rate was kept constant at 10 mL/min.

1.2 Chemical Methods

Reagents: Solvents for HPLC and MS analysis such as acetonitrile and methanol were purchased from Fisher Scientific and VWR in a purity of over 99% (HPLC-grade). Water was purified using a TKA GenPure water treatment system and deionized. Dry solvents such as diethyl ether, dichloromethane, 1,4-dioxane, tetrahydrofuran and toluene for procedures under inert atmosphere were prepared by distillation and drying over molecular sieve (3 Å or 4 Å). Commercial materials and other solvents were purchased at the highest commercial quality from the providers Acros Organics, Alfa Aesar, Carbolution, Carl Roth, Merck, Sigma Aldrich, VWR, TCI Chemicals and Thermo Fisher Scientific.

NMR: ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectra (NMR) were recorded on *Bruker* AV-HD400 and AV-HD300 spectrometers at 298 K. The chemical shifts are given in δ-values (ppm) and are calibrated on the residual peak of the deuterated solvent ($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.0 ppm; DMSO-d_6: δ_H = 2.50 ppm, δ_C = 39.5 ppm). The coupling constants *J* are given in Hertz [Hz]. Following abbreviations were used for the allocation of signal multiplicities: bs - broad signal, s - singlet, d - doublet, dd - doublet of doublets, t - triplet.

MS: Elektrospray-Ionisation Mass spectra (ESI-MS) were recorded on an *Advion* expression^{L} CMS system using a single-quadrupole mass analyzer, a *Peak Scientific* N118LA nitrogen generator, an *Edwards* RV12 high vacuum pump and a *Jasco* PU-1580 Intelligent HPLC Pump, or a LCQ Fleet ion trap system (*Thermo Scientific*), which was combined with an UltiMate 300 HPLC system. For High

resolution mass spectrometry (HRMS) a Thermo LTQ FT Ultra mass spectrometer was used, and analyses of the recorded spectra were again performed using *Thermo* Xcalibur Qual Browser 2.2 SP1.48 Software.

Chromatography: Thin-layer chromatography (TLC) was performed on precoated plates of silica gel F_{254} (Merck) with UV detection at 254 and 365 nm. Column chromatography was performed on silica gel 60 Geduran® Si 60 (40-60 µm) (Merck). High Performance Liquid Chromatograms (HPLC) were recorded on a computer-controlled Jasco system including a UV-1575 Intelligent UV/VIS Detector, DG-2080-53 3-Line Degaser, two PU-1580 Intelligent HPLC Pumps, AS-1550 Intelligent Sampler, HG-1580-32 Dynamic Mixer. A Eurospher II 100-3 C18 A (150 × 4.6 mm) column with integrated precolumn manufactured by Knauer was used. The eluent system consisted of A = H_2O + 0.05% TFA, B = MeCN + 0.05% TFA. The analytical method used the following elution gradient: 0-2 min (5% B), 2-25 min (linear increase to 95% B), 25-28 min (95% B), 28-31 min (5% B) with a flow of 1.0 mL/min. For medium pressure liquid chromatography (MPLC) the Reveleris® X2 MPLC system (Grace) was used together with Reverleris® Reverse Phase (RP) C18 columns (Grace) using UV-detection at 220 nm, 254 nm, and 280 nm. Air- and moisture-sensitive reactions were performed under argon atmosphere (4.6 = 99.996%) using a Schlenk line. Before application, the flasks were repeatedly evacuated (external heating) and refilled with argon.

2. Cloning of the Ambigol BGC and the Two Cyctochrome P450 Monooxygenases *ab2* and *ab3*

2.1 List of Strains, Primers and Plasmids

Table 1 List of strains.

Name	Description	Reference /Source
E. coli DH5α	F^- ϕ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *rec*A1 *end*A1 *hsd*R17(r_K^-, m_K^+) *pho*A *sup*E44 λ^- *thi*-1 *gyr*A96 *rel*A1, host strain for cloning	NEB
E. coli BL21	F^- *omp*T *hsd*S_B (r_B^-, m_B^-) *gal dcm* (DE3), heterologous expression strain for *ab2*/*ab3* expression and *in vivo* feeding assays	NEB
E. coli BAP1	F^- *omp*T *hsd*S_B (r_B^-, m_B^-) *gal dcm* (DE3) Δ*prp*RBCD::T7prom-*sfp*,T7prom-*prpE,* heterologous expression strain for *ab* BGC expression	[4]
S. elongatus PCC 7942	Host strain for *ab* BGC recombinant expression	[5]

Table 2 List of primers.

Name	Sequence (5' → 3')	Description
GA_ab:T7_for	CTCACAGAGAACAGATTGGTGGATCCatgtctaatttaccaaaatctac	amplification of the ambigol BGC with homology sequences (capitals) to the pET-28b-SUMO plasmid
GA_ab:T7_rev	TGGCGGCCGTTACTAGTGtcaagcagtaattaaactcg	
GA_ab2e:pMal-SD_for	CCCTGAAAGACGCGCAGACTctgtaccaggaggttgcatc	amplification of the codon optimized *ab2* gene with homology sequences (capitals) to the pMal plasmid without spacer sequence between MBP-tag and MCS
GA_ab2e:pMal-SD_rev	CAAGCTTGAATTCCTCGAGGGTttagaagctcggtttgacgac	
GA_ab2::pCV0094_for	GCTAAGGAGGCAACAAGatgttgtatcaagaagtagcc	amplification of the *ab*2 gene with homology sequences (capitals) to the pCV0094 plasmid including a *C*-terminal *Pme*I restricition site (underlined)
GA_ab2::pCV0094_rev	CGAGGTCGAGACGGCTCATGgtttaaactaaaagctaggttttacaacaac	
ab3e_BamHI_for	ggacggatccatggtctctcagc	amplification of the codon optimized *ab3* gene *BamH*I with restriction site (underlined)
ab3e_XhoI_rev	atatctcgagttaaaagcgcggtttcg	amplification of the codon optimized *ab3* gene *Xho*I with restriction site (underlined)
GA_ab3::pCV0094_for	GCTAAGGAGGCAACAAGatggtttcacaacaatcagc	amplification of the *ab3* gene with homology sequences (capitals) to the pCV0094 plasmid including a *C*-terminal *Pme*I restricition site (underlined)
GA_ab3::pCV0094_rev	CGAGGTCGAGACGGCTCATGgtttaaactagaagcgaggttttgctataactttatag	
GA_gfp::T7-ab_for	CAGATATCCATCACACTGGCatggttagcaaaggtgaag	amplification of the *gfp* gene with homology sequences (capitals) to the pET-28b-SUMO::*ab* plasmid
GA_gfp::T7-ab_rev	CAGTGGTGGTGGTGGTGCTCGAGTGCttagctgcctttatacagttc	
Ab1_for	atgtctaatttaccaaaatctacaaaggtattagttg	amplification of the pET-28b-SUMO::*ab_gfp* plasmid without the T7 promoter, lac operator, 6xHis, thrombin site and SUMO-tag
pET28b_rev	gagatctcgatcctctacgc	
GA_ptetO::ab-GFP_for	GCGTAGAGGATCGAGATCTCAATTC	amplification of the lox71 site and tetracycline inducible promoter from pET28b-ptetO plasmid with homology sequences to the pET28b-SUMO::*ab_gfp* plasmid
GA_ptetO::ab-GFP_for	GATTTTGGTAAATTAGACATggtcgatcctcttctctatc	
GA_pMal-SD_for	accctcgaggaattcaagc	amplification of the pMal plasmid without the 105 bp spacer sequence between MBP-tag and MCS
GA_pMal-SD_rev	agtctgcgcgtctttcag	
T7	taatacgactcactataggg	sequencing and screening primers binding plasmid backbones and facing the MCS
SUMO_for	ttggacatggaggataacg	
pET-RP	ctagttattgctcagcgg	
pRSET-RPnew	gggttatgctagttattgc	
PaadA_for	tgccttcatccgtttccac	
aphI_rev	attccgtcagccagtttag	
S7942NSI-LA_for	cagatcaatgcccgtggtttg	screening primers binding the homologous recombination site NSI in the chromosome of *S. elongatus* PCC7942
S7942NSI-RA_rev	gaaaagctcaagcggaaggg	

S7942NSII-LA_for	ctccagtaaagtcttcgcccgtaac	screening primers binding the homologous recombination site NSII in the chromosome of *S. elongatus* PCC7942
S7942NSII-RA_rev	ttggtgctgttcagtctggatgc	
ab1_screen_rev	ggcgcacttgataactg	sequencing and screening primers binding inside the *ab* BGC
ab1_seq_for	cctttgctttcaactggag	
ab2_seq_for	ccacagtactagaagctg	
ab2_seq_rev	agaacaccccaccgtaac	
ab3_seq_for	caagctcaacaatggtgc	
ab3_seq_rev	tgaagtgacattgaggtgg	
ab3_seq_rev_2	caaactgcgtcaacgagc	
ab4_seq_for	gagaaggaaccagaaagc	
ab5_screen&seq_for	acttgtctcattggctcac	
ab6_screen_rev	agaacaactccatttaggcg	
ab6_seq_rev	agctttgacgagttcacc	
ab7_seq_for	aaggattattgacagcacg	
ab7_seq_rev	accaccaccacgtaaaatg	
ab8_seq_for	gagttattgcccgatattgg	
ab9_seq_for	tacctacagaccgtgcc	
ab10_seq_rev	cctatcaagaacgacaactgag	
ab10_seq-rev_2	acgatcaagttccaccatc	
ab10_screen_for	agaagacacaatgccagag	

Table 3 List of plasmids.

Name	**Description**	**Reference /Source**
pET-28b-SUMO	IPTG inducible expression plasmid (T7 promoter), *N*- and *C*-terminal 6xHis-tag, thrombin site, SUMO-tag, ColE1, Kan^R	[6]
pET-28b-ptetO	Tetracycline inducible expression plasmid (ptetO promoter), *N*- and *C*-terminal 6xHis-tag, thrombin site, ColE1, Kan^R	[7]
pMal	IPTG inducible expression plasmid (T7 promoter), 6xHis-tag, MBP-tag, TEV site, ColE1, Amp^R	[8]
pAM5051	Theophylline inducible expression plasmid (PconII promoter), S7942NSI recombination site for the *Synechococcus elongatus* PCC7942 chromosome, ColE1, $Spec/Strep^R$	[9]
pCV0094	Theophylline inducible expression plasmid (PconII promoter), S7942NSII recombination site for the *Synechococcus elongatus* PCC7942 chromosome, ColE1, Tet^R, Kan^R	[5]
pET-28b-SUMO::*ab*	IPTG inducible expression plasmid (T7 promoter), *N*- and *C*-terminal 6xHis-tag, thrombin site, SUMO-tag, ColE1, Kan^R harboring the *ab* BGC	This study
pET-28b-SUMO::*ab-gfp*	IPTG inducible expression plasmid (T7 promoter), *N*- and *C*-terminal 6xHis-tag, thrombin site, SUMO-tag, ColE1, Kan^R harboring the *ab* BGC and the *gfp* gene	This study
pET-28b-ptetO::*ab-gfp*	Tetracycline inducible expression plasmid (ptetO promoter), *N*- and *C*-terminal 6xHis-tag, thrombin site, ColE1, Kan^R harboring the *ab* BGC and the *gfp* gene	This study

pAM5051::*ab1-5*	Theophylline inducible expression plasmid (PconII promoter), S7942NSI recombination site for the *Synechococcus elongatus* PCC7942 chromosome, ColE1, Spec/Strep^R harboring the genes *ab1-5* including the respective intergenic regions in the *ab* BGC	This study
pCV0094::*ab6-10*	Theophylline inducible expression plasmid (PconII promoter), S7942NSII recombination site for the *Synechococcus elongatus* PCC7942 chromosome, ColE1, Tet^R, Kan^R harboring the genes *ab6-10* including the respective intergenic regions in the *ab* BGC	This study
pMal-SD::*ab2e*	IPTG inducible expression plasmid (T7 promoter), 6xHis-tag, MBP-tag, ColE1, Amp^R harboring the codon optimized *ab2* gene directly attached to the MBP-tag	This study
pMal::*ab3e*	IPTG inducible expression plasmid (T7 promoter), 6xHis-tag, MBP-tag, TEV site, ColE1, Amp^R harboring the codon optimized *ab3* gene	This study
pCV0094::*ab2*	Theophylline inducible expression plasmid (PconII promoter), S7942NSII recombination site for the *Synechococcus elongatus* PCC7942 chromosome, ColE1, Tet^R, Kan^R harboring the *ab2* gene	This study
pCV0094::*ab3*	Theophylline inducible expression plasmid (PconII promoter), S7942NSII recombination site for the *Synechococcus elongatus* PCC7942 chromosome, ColE1, Tet^R, Kan^R harboring the *ab3* gene	This study
pCV0094::*ab2-3*	Theophylline inducible expression plasmid (PconII promoter), S7942NSII recombination site for the *Synechococcus elongatus* PCC7942 chromosome, ColE1, Tet^R, Kan^R harboring the ab2 and the *ab3* gene joint by a 4 bp linker sequence	This study

2.2 Cloning Methods

Q5 Polymerase PCR Setup: A standard 25 µL PCR reaction batch for long-amplicon cycling reactions consisted of: 1x Q5 reaction buffer, 200-400 µM deoxynucleotide triphosphates, 500 nM of forward and reverse primer, 15 ng gDNA or 100 ng plasmid DNA template and 0.01-0.02 U/µL Q5 High-Fidelity DNA polymerase (NEB). Cycling was conducted using a T100 Thermal Cycler (Biorad) as follows: 1.) Initial denaturation, 98 °C for 1 min; 2.) Denaturation, 98 °C for 10 sec; 3.) Annealing, t_a °C for 20 sec; 4.) Extension, 72 °C for 30-45 sec/kb; steps 2.) to 4.) were repeated in total for 30 cycles; 5.) Final extension, 72 °C for 60 sec/kb, and 6.) End phase, 16 °C. Primer annealing temperatures were determined using the NEB Tm Calculator online tool (http://tmcalculator.neb.com).

Taq Polymerase PCR Setup: Colony screening PCRs were performed using *Taq* DNA polymerase (NEB). Clones were picked and resuspended in 50 µL of pure water and examined in a 25 µL PCR batch composed as follows: *Taq* buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3 at 25 °C), 4% DMSO, 100 µM deoxynucleotide triphosphates, 200 nM of forward and reverse primer, 5 µL DNA template (bacterial suspension in water) and *Taq* DNA polymerase (0.025 U/µL, NEB. Cycling was conducted using a T100 Thermal Cycler (Biorad) as follows: 1.) Initial denaturation, 95 °C for 5 min; 2.) Denaturation, 95 °C for 45 sec; 3.) Annealing, t_a °C for 30 sec; 4.) Extension, 72 °C for 60 sec/kb; steps 2.) to 4.) were repeated in total 34 times; 5.) Final extension, 72 °C for 120 sec/kb, and 6.) End phase, 16 °C. Primer annealing temperatures were determined using the NEB Tm Calculator online tool.

2.3 Plasmid Maps

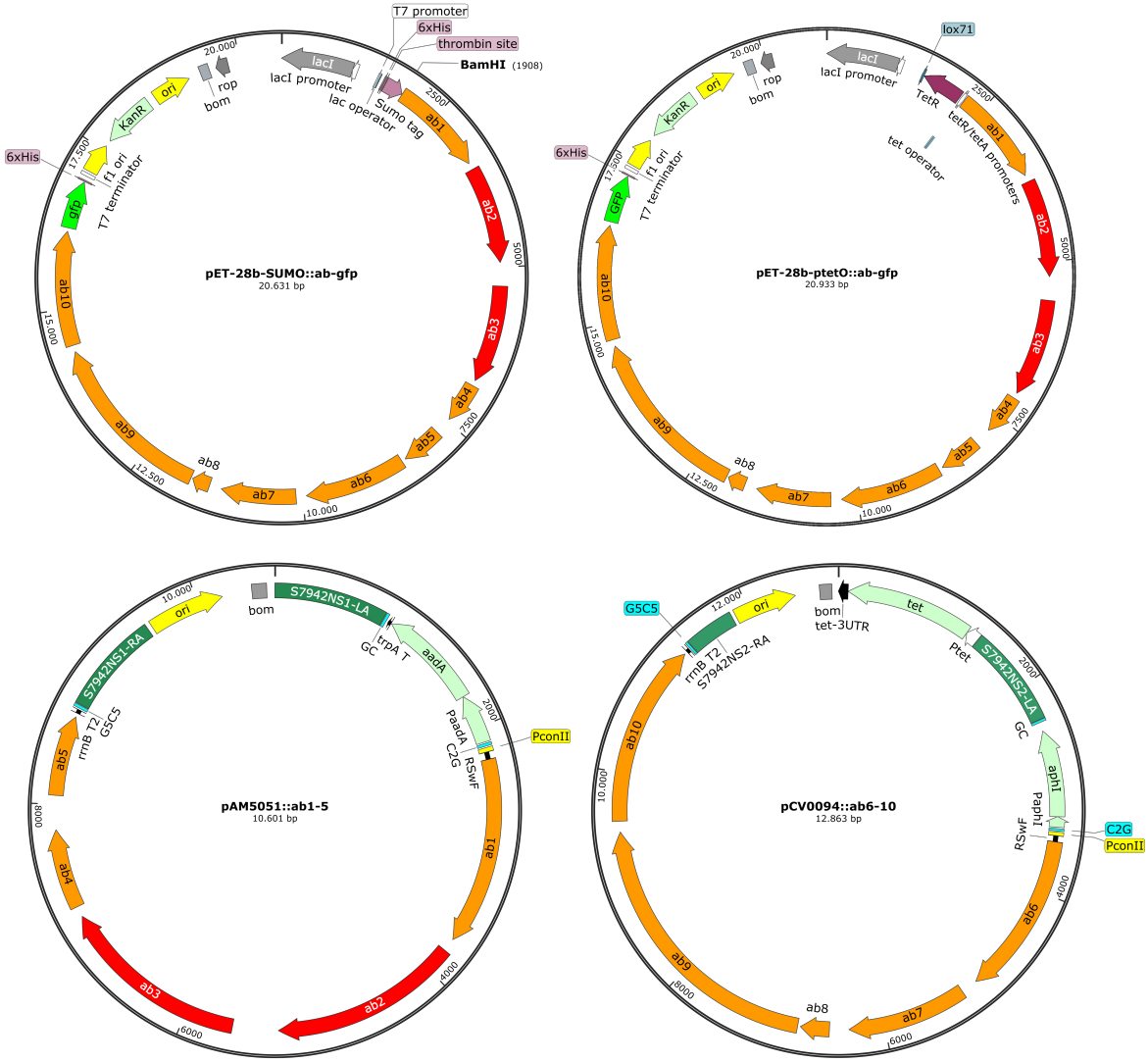

Fig. S1 Plasmids used for heterologous expression of the *ab* BGC in *E. coli* and *S. elongatus*.

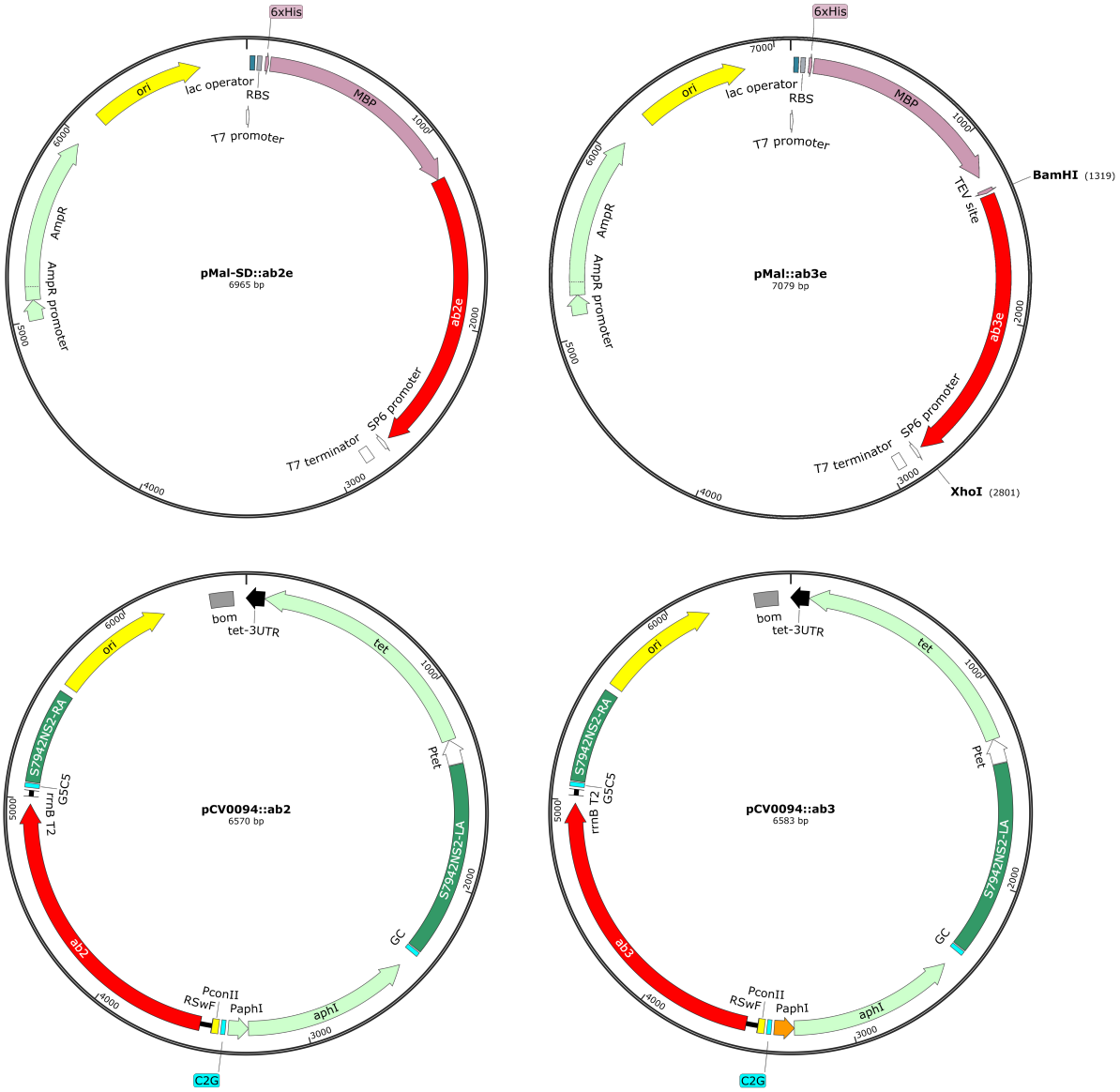

Fig. S2 Plasmids used for heterologous expression of *ab2* and *ab3* in *E. coli* and *S. elongatus*.

2.4 Agarose Gels of Q5 Screening PCRs of Transformed *S. elongatus* Cells

Integration of *ab1-5* into NSI was confirmed by PCR using primer pair PaadA_for/ab1_screen_rev expecting 563 bp to verify upstream integration, ab5_screen&seq_for_1/S7942NSI-RA_rev expecting 581 bp to verify downstream integration and S7942NSI-LA_for/S7942NSI-RA_rev expecting 8140 bp for complete integration of *ab1-5* and 1713 bp for the empty NSI site. Integration of *ab6-10* into NSII was confirmed by PCR using primer pair aphI_rev/ab6_screen_rev_1 expecting 701 bp to verify upstream integration, ab10_screen_for/S7942NSII-RA_rev expecting 447 bp to verify downstream integration and S7942NSII-LA_for/S7942NSII-RA_rev expecting 9417 bp for complete integration of *ab6-10* and 1660 bp for the empty NSII site. Sequenced plasmid DNA of pAM5051::*ab1-5* and pCV0094::*ab6-10*

was used as positive control, water and the empty plasmids pAM5051 and pCV0094 were used as negative controls for the PCR.

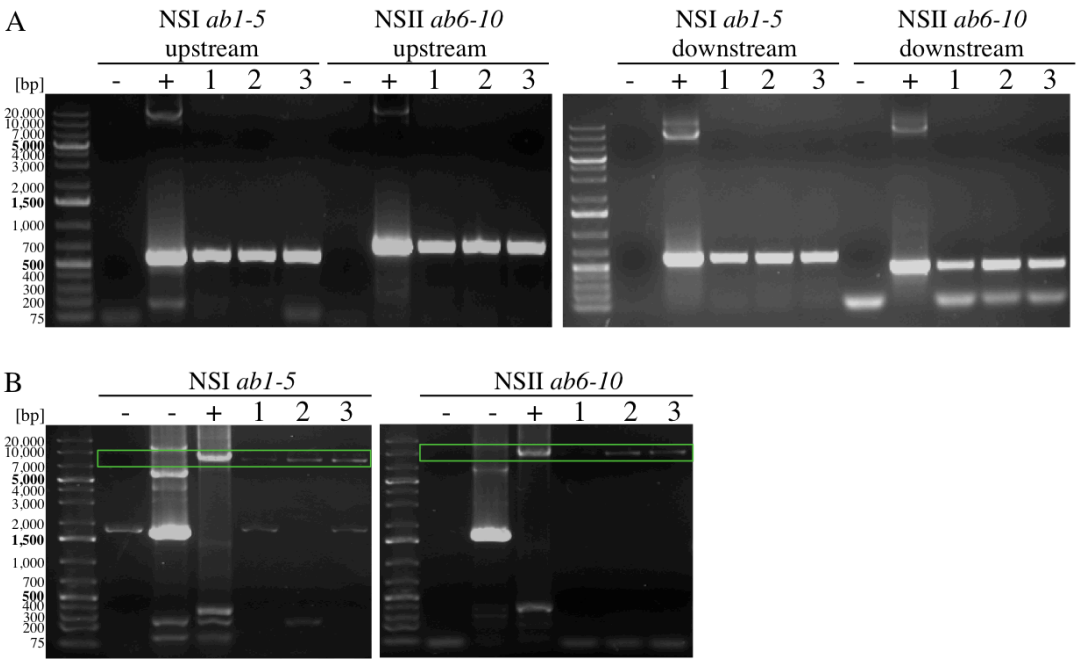

Fig. S3 Agarose gels of Q5 screening PCRs on three *S. elongatus* clones harbouring *ab1-5* in NSI and *ab6-10* in NSII. Verification of A) upstream (left) and downstream (right) integration and of B) complete integration of the respective *ab* cluster parts.

Integration of the cytochrome P450 genes *ab2* and/or *ab3* into NSII was confirmed by PCR using primer pair S7942NSII-LA_for/S7942NSII-RA_rev expecting 3124 bp for *ab2*, 3137 bp for *ab3*, 4605 bp for both *ab2* and *ab3* in succession. Water was used as negative control and sequenced pCV0094::*ab2* plasmid DNA as positive control for the PCR.

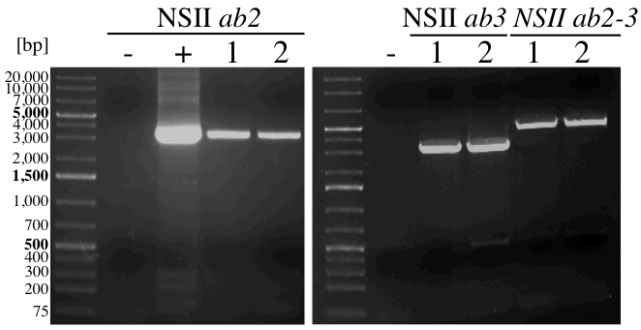

Fig. S4 Agarose gels of Q5 screening PCRs on two *S. elongatus* clones harboring either *ab2*, *ab3* or *ab2-3* in NSII to verify complete integration.

2.5 DNA Sequences

Sequence of the ambigol BGC of *F. ambigua* (Näg.) Gomont 108b, ORFs indicated by color.

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Ab2 codon optimised sequence for expression in *E. coli*.

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Ab3 codon optimised sequence for expression in *E. coli*.

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TTTTACCCACCTGATGCACAAATGCAAATTCGAGAAAGTACCGGGCGAAAAACTGAGCCTGGACCCGCAGCCGG
CGATCATCCTGCCGCCGCAGAATTACAAAGTTATCGCGAAACCGCGCTTTTAA

3. Heterologous Expression of the *ab* BGC in *E. coli* BAP1

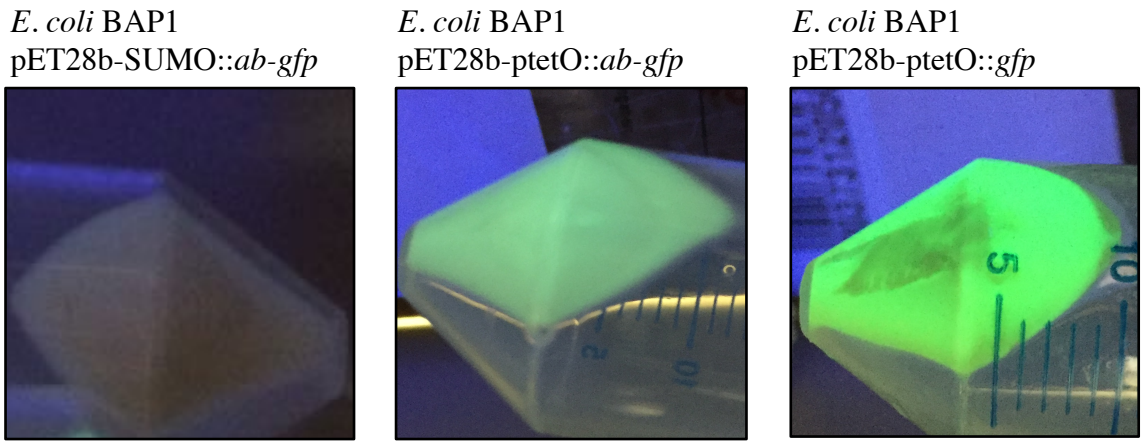


Fig. S5 Cell pellets harvested 24 h after induction of expression. No fluorescence is detected for *E. coli* BAP1 pET28b-SUMO::*ab-gfp*, therefore indicating incomplete gene transcription. In contrast, *E. coli* BAP1 pET28b-ptetO::*ab-gfp* and *E. coli* BAP1 pET28b-ptetO::*gfp* (positive control) show bright green fluorescence indicating full gene transcription.

4. SDS PAGE Gels of Purified Ab2, Ab3, Fpr and PetF used for *in vitro* Assays

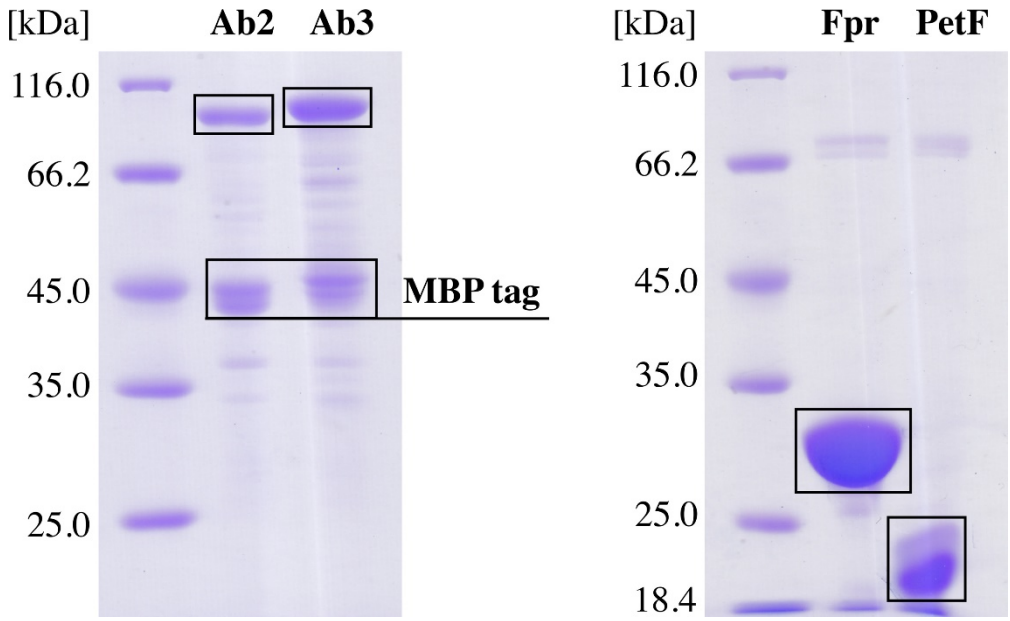
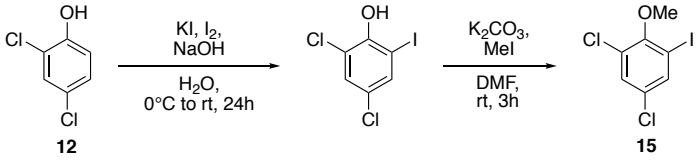


Figure S6 SDS-PAGE analysis of the purified enzymes Ab2 (97.5 kDa), Ab3 (101.1 kDa), Fpr (30.2 kDa) and PetF (12.9 kDa, occurs as dimer) used for *in vitro* assays.

5. Experimental Procedures

5.1 Synthesis of Methoxy Protected Aryl Iodides (15–17)

1,5-Dichloro-3-iodo-2-methoxybenzene (15)



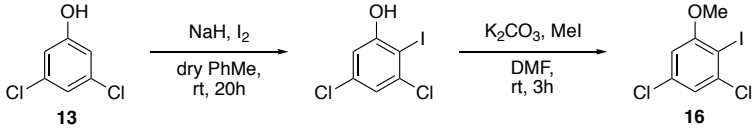
2,4-Dichloro-6-iodophenol was prepared according to a published procedure. Analytical data were in agreement with literature values.[10]

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.61 (d, *J* = 2.4 Hz, 1 H), 7.34 (d, *J* = 2.4 Hz, 1 H), 5.90 (bs, 1 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 150.1, 137.1, 129.4, 126.5, 119.3, 83.4. **MS** (ESI–): *m/z* = 308.9 $[M+Na-2H]^-$, 324.9 $[M+K-2H]^-$.

1,5-Dichloro-3-iodo-2-methoxybenzene (**15**) was prepared according to a published procedure. Analytical data were in agreement with literature values.[10]

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.66 (d, *J* = 2.5 Hz, 1 H), 7.37 (d, *J* = 2.4 Hz, 1 H), 3.85 (s, 3 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 154.9, 137.3, 130.7 (two signals), 128.1, 92.7, 60.9. **MS** (ESI–): *m/z* = 322.9 $[M+Na-2H]^-$.

1,5-Dichloro-2-iodo-3-methoxybenzene (16)



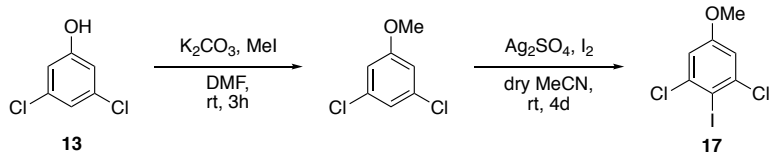
3,5-Dichloro-2-iodophenol was prepared according to a published procedure. Analytical data were in agreement with literature values.[11]

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.08 (d, *J* = 2.3 Hz, 1 H), 6.91 (d, *J* = 2.3 Hz, 1 H), 5.62 (bs, 1 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 157.0, 139.2, 136.1, 121.7, 113.5, 89.1. **MS** (ESI–): *m/z* = 309.0 $[M+Na-2H]^-$, 324.9 $[M+K-2H]^-$.

3,5-Dichloro-2-iodophenol (7.41 g, 25.6 mmol, 1.0 eq.) was dissolved in DMF (26.0 mL, 1.00 mL/mmol) and potassium carbonate (4.01 g, 30.8 mmol, 1.2 eq.) was added. After dropwise addition of methyl iodide (4.37 g, 1.92 mL, 30.8 mmol, 1.2 eq.), the reaction solution was stirred for 3 h at room temperature. After completion, the reaction was diluted with water and Et_2O. The aqueous layer was extracted with Et_2O (2×). The combined organic layers were washed with 1N HCl (6×), brine, dried over Na_2SO_4 and filtered. The solvent was removed to give 1,5-dichloro-2-iodo-3-methoxybenzene (**16**, 7.44 g, 24.6 mmol, 96%) as a white solid. Analytical data were in agreement with literature values.[12]

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.13 (d, *J* = 2.1 Hz, 1 H), 6.68 (d, *J* = 2.1 Hz, 1 H), 3.89 (s, 3 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 160.3, 140.4, 135.7, 121.7, 109.6, 89.2, 57.2. **MS** (ESI+): *m/z* = 303.0 $[M+H]^+$.

1,3-Dichloro-2-iodo-5-methoxybenzene (17)



3,5-Dichlorophenol (**13**, 4.00 g, 24.5 mmol, eq.) was dissolved in DMF (24.5 mL, 1.00 mL/mmol) and potassium carbonate (3.83 g, 29.4 mmol, 1.2 eq.) was added. After dropwise addition of methyl iodide (4.17 g, 1.83 mL, 29.4 mmol, 1.2 eq.), the reaction solution was stirred for 3 h at room temperature. After completion, the reaction was diluted with water and Et_2O. The aqueous layer was extracted with Et_2O (2×). The combined organic layers were washed with 1N HCl (6×), brine, dried over Na_2SO_4 and filtered. The solvent was removed to give 1,3-dichloro-5-methoxybenzene (4.14 g, 23.4 mmol, 95%) as a white solid. Analytical data were in agreement with literature values.[13]

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 6.95 (t, *J* = 1.8 Hz, 1 H), 6.79 (d, *J* = 1.8 Hz, 2 H), 3.79 (s, 3 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 160.8, 135.5, 121.1, 113.3, 55.8. **MS** (ESI+): *m/z* = 176.8 $[M+H]^+$.

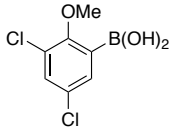
1,3-Dichloro-2-iodo-5-methoxybenzene was prepared according to a published procedure. Analytical data were in agreement with literature values.[14]

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.95 (s, 2 H), 3.79 (s, 3 H). **^{13}C -NMR** (100 MHz, $CDCl_3$): δ = 160.3, 140.8, 114.0, 92.3, 56.0. **MS** (ESI+): *m/z* = 271.0 $[M–MeOH+H]^+$. **MS** (ESI–): *m/z* = 269.0 $[M–MeOH–H]^–$, 322.8 $[M+Na–2H]^–$.

5.2 General Procedure for the Synthesis of Arylboronic Acids (18–20)

The reaction was performed under dry conditions. Methoxy protected aryl iodide (1.0 eq.) was dissolved in dry diethyl ether (5.00 mL/mmol) and cooled down to –15 °C. 2M ^{i}PrMgCl solution in Et_2O (1.5 eq.) was added dropwise to the reaction solution, which was stirred at –15 °C for 1 h. Freshly distilled trimethyl borate (2.0 eq.) was added rapidly. The reaction was stirred at –10 °C to 0 °C for 2 h before being treated with 4M HCl solution (1.00 mL/mmol). After another 30 min at room temperature, the reaction solution was extracted with Et_2O (3x). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and filtered. The solvent was removed, and the obtained residue was washed with hexane to give the corresponding arylboronic acid as a white solid.

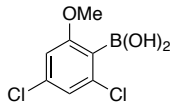
(3,5-Dichloro-2-methoxyphenyl)boronic acid (18)



Synthesised from 1,5-dichloro-3-iodo-2-methoxybenzene (**15**, 5.00 g, 16.5 mmol) in 92% yield (3.36 g, 15.2 mmol) as a white solid.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 8.40 (bs, 2 H), 7.59 (d, *J* = 2.6 Hz, 1 H), 7.35 (d, *J* = 2.6 Hz, 1 H), 3.78 (s, 3 H). **^{13}C-NMR** (75 MHz, DMSO-d_6): δ = 156.9, 132.1, 129.9, 127.6, 126.7, 61.1. C–B signal not observed due to quadrupolar relaxation. **MS** (ESI–): *m/z* = 219.0 $[M–H]^–$. **HRMS** (ESI–) *m/z* calcd for $C_7H_6BCl_2O_3$ $[M–H]^–$: 218.9793, found: 218.9793.

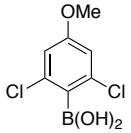
(2,4-Dichloro-6-methoxyphenyl)boronic acid (19)



Synthesised from 1,5-dichloro-2-iodo-3-methoxybenzene (**16**, 1.00 g, 3.30 mmol) in 74% yield (538 mg, 2.44 mmol) as a white solid.

^{1}H-NMR (300 MHz, DMSO- d_6): δ = 8.30 (bs, 2 H), 7.06 (d, *J* = 1.6 Hz, 1 H), 6.98 (d, *J* = 1.6 Hz, 1 H), 3.76 (s, 3 H). **^{13}C-NMR** (75 MHz, DMSO-d_6): δ = 162.2, 135.9, 133.8, 119.9, 109.4, 56.0. C–B signal not observed due to quadrupolar relaxation. **MS** (ESI–): *m/z* = 218.9 $[M–H]^–$. **HRMS** (ESI–) *m/z* calcd for $C_7H_6BCl_2O_3$ $[M–H]^–$: 218.9793, found: 218.9794.

(2,6-Dichloro-4-methoxyphenyl)boronic acid (20)



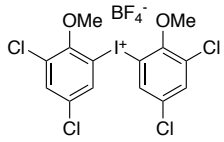
Synthesised from 1,3-dichloro-2-iodo-5-methoxybenzene (**17**, 3.00 g, 9.90 mmol) in 38% yield (843 mg, 3.82 mmol) as a white solid.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 8.20 (bs, 2 H), 6.96 (s, 2 H), 3.78 (s, 3 H). **^{13}C-NMR** (75 MHz, DMSO-d_6): δ = 160.2, 136.4, 112.9, 56.0. C–B signal not observed due to quadrupolar relaxation. **MS** (ESI–): *m/z* = 218.9 $[M–H]^–$. **HRMS** (ESI–) *m/z* calcd for $C_7H_6BCl_2O_3$ $[M–H]^–$: 218.9793, found: 218.9794.

5.3 General Procedure for the Synthesis of Diaryliodonium Tetrafluoroborates (21–23)

The symmetrical iodonium salts were prepared according to a published procedure.[15] *meta*-Chloroperbenzoic acid (77% active oxidant, 1.1 eq.) was added to a Schlenk tube and dissolved in dry DCM (2.00 mL/mmol). The iodated aryl species (1.0 eq.) was added and the reaction mixture was heated up to 80 °C. After 10 min of stirring at 80 °C, the mixture was cooled down to –78 °C. A 0 °C cold solution of $BF_3{\cdot}OEt_2$ (48% BF_3, 2.2 eq.) and arylboronic acid (1.0 eq.) in dry DCM (2.00 mL/mmol) was transferred into the reaction mixture. The obtained yellow solution was stirred for 30 min at –78 °C and warmed to room temperature. The crude reaction solution was purified by flash column chromatography (DCM to DCM/MeOH = 20:1). The product fraction was concentrated and to the obtained residue was added diethyl ether to induce precipitation of the iodonium salt. The solid was washed with diethyl ether and dried under reduced pressure to give the corresponding iodonium salt as a white to beige solid.

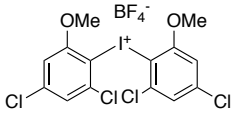
Bis(3,5-dichloro-2-methoxyphenyl)iodonium tetrafluoroborate (21)



Synthesised from 1,5-dichloro-3-iodo-2-methoxybenzene (**15**, 908 mg, 3.00 mmol, 1.0 eq.) and (3,5-dichloro-2-methoxyphenyl)boronic acid (**18**, 663 mg, 3.00 mmol, 1.0 eq.) in 52% yield (876 mg, 1.55 mmol) as a white solid.

^{1}H-NMR (300 MHz, DMSO-d_6): δ = 8.55 (d, *J* = 2.4 Hz, 2 H), 8.05 (d, *J* = 2.4 Hz, 2 H), 4.00 (s, 6 H). **^{13}C-NMR** (75 MHz, DMSO-d_6): δ = 153.3, 135.2, 135.1, 130.4, 127.4, 115.2, 62.5. **MS** (ESI+): *m/z* = 476.8 $[M–BF_4]^+$. **HRMS** (ESI+) *m/z* calcd for $C_{14}H_{10}Cl_4IO_2$ $[M–BF_4]^+$: 476.8474, found: 476.8476.

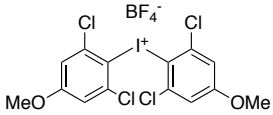
Bis(2,4-dichloro-6-methoxyphenyl)iodonium tetrafluoroborate (22)



Synthesised from 1,5-dichloro-2-iodo-3-methoxybenzene (**16**, 454 mg, 1.50 mmol, 1.0 eq.) and (2,4-dichloro-6-methoxyphenyl)boronic acid (**19**, 332 mg, 1.50 mmol, 1.0 eq.) in 24% yield (200 mg, 0.35 mmol) as a white solid.

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 7.61 (d, *J* = 2.0 Hz, 2 H), 7.37 (d, *J* = 2.0 Hz, 2 H), 3.92 (s, 6 H). **^{13}C-NMR** (100 MHz, DMSO-d_6): δ = 159.3, 140.0, 138.8, 121.9, 112.2, 108.8, 64.9. **MS** (ESI+): *m/z* = 476.7 $[M–BF_4]^+$. **HRMS** (ESI+) *m/z* calcd for $C_{14}H_{10}Cl_4IO_2$ $[M–BF_4]^+$: 476.8474, found: 476.8475.

Bis(2,6-dichloro-4-methoxyphenyl)iodonium tetrafluoroborate (23)



Synthesised from 1,3-dichloro-2-iodo-5-methoxybenzene (**17**, 490 mg, 1.62 mmol, 1.0 eq.) and (2,6-dichloro-4-methoxyphenyl)boronic acid (**20**, 357 mg, 1.62 mmol, 1.0 eq.) in 16% yield (143 mg, 0.25 mmol) as a white solid.

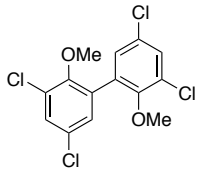
^{1}H-NMR (400 MHz, DMSO-d_6): δ = 7.44 (s, 4 H), 3.88 (s, 6 H). **^{13}C-NMR** (100 MHz, DMSO-d_6): δ = 163.4, 140.0, 115.4, 56.9. **MS** (ESI+): *m/z* = 476.7 $[M–BF_4]^+$. **HRMS** (ESI+) *m/z* calcd for $C_{14}H_{10}Cl_4IO_2$ $[M–BF_4]^+$: 476.8474, found: 476.8474.

5.4 General Procedure for the Suzuki Coupling (24–26)

The reaction was performed under dry conditions. $Pd_2(dba)_3$ (5 mol%) and DavePhos (15 mol%) were added in a Schlenk tube equipped with a stir bar. The Schlenk tube was evacuated and then refilled with argon. Dry 1,4-dioxane (5.00 mL/mmol) was added and three more evacuation-refill cycles (degassing) were performed. The catalyst mixture was heated up to 90 °C using a preheated oil bath. After 20 min, aryl iodide (1.0 eq.), boronic acid (2.0 eq.), potassium carbonate (2.0 eq.) and silver(I)

oxide (0.5 eq.) were added and another three evacuation-refill cycles were performed. The reaction mixture was stirred at 90 °C for 24 h before being diluted with EtOAc at room temperature. The reaction solution was filtered, evaporated and the obtained residue was purified by MPLC to give the corresponding methoxy protected C–C dimers as yellow oils.

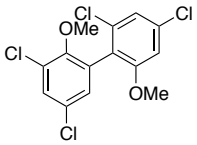
3,3',5,5'-Tetrachloro-2,2'-dimethoxy-1,1'-biphenyl (24)



Synthesised from 1,5-dichloro-3-iodo-2-methoxybenzene (**15**, 300 mg, 0.99 mmol, 1.0 eq.) and (3,5-dichloro-2-methoxyphenyl)boronic acid (**18**, 437 g, 1.98 mmol, 2.0 eq.) in 37% yield (132 mg, 0.37 mmol) as a yellow oil. The substrate 1,5-dichloro-3-iodo-2-methoxybenzene (84.0 mg, 0.28 mmol) was re-isolated in 28% yield, based on which the desired product was obtained in 52% isolated yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.44 (d, *J* = 2.6 Hz, 2 H), 7.19 (d, *J* = 2.6 Hz, 2 H), 3.61 (s, 6 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 152.7, 133.1, 130.4, 129.5, 129.2 (two signals), 61.2. **MS** (ESI–): *m/z* = 385.1 $[M+Cl]^-$.

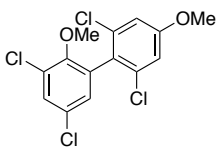
2',3,4',5-Tetrachloro-2,6'-dimethoxy-1,1'-biphenyl (25)



Synthesised from 1,5-dichloro-2-iodo-3-methoxybenzene (**15**, 300 mg, 0.99 mmol, 1.0 eq.) and (3,5-dichloro-2-methoxyphenyl)boronic acid (**19**, 437 mg, 1.98 mmol, 2.0 eq.) in 45% yield (158 mg, 0.45 mmol) as a yellow oil. The substrate 1,5-dichloro-2-iodo-3-methoxybenzene (**10**, 41.0 mg, 0.14 mmol) was re-isolated in 14% yield, based on which the desired product was obtained in 53% isolated yield.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.42 (d, *J* = 2.6 Hz, 1 H), 7.13 (d, *J* = 1.9 Hz, 1 H), 7.01 (d, *J* = 2.6 Hz, 1 H), 6.88 (d, *J* = 1.9 Hz, 1 H) 3.75 (s, 3 H), 3.57 (s, 3 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 158.5, 153.2, 135.4, 135.2, 131.6, 130.2 (two signals), 129.1, 128.9, 123.8, 121.7, 110.4, 61.0, 56.5. **MS** (ESI–): *m/z* = 385.1 $[M+Cl]^-$.

2',3,5,6'-Tetrachloro-2,4'-dimethoxy-1,1'-biphenyl (26)



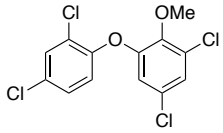
Synthesised from 1,3-dichloro-2-iodo-5-methoxybenzene (**15**, 300 mg, 0.99 mmol, 1.0 eq.) and (3,5-dichloro-2-methoxyphenyl)boronic acid (**20**, 437 mg, 1.98 mmol, 2.0 eq.) in 54% yield (188 mg, 0.53 mmol) as a yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.45 (d, *J* = 2.5 Hz, 1 H), 7.04 (d, *J* = 2.5 Hz, 1 H), 6.98 (s, 2 H), 3.85 (s, 3 H), 3.60 (s, 3 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 160.0, 153.1, 135.6, 133.5, 130.5, 130.2, 129.2, 129.1, 127.0, 114.2, 61.0, 56.0. **MS** (ESI–): *m/z* = 385.0 $[M+Cl]^-$.

5.5 General Procedure for the Biaryl Ether Formation (27–32)

The diphenyl ethers were prepared according to a published procedure.[16] KO^tBu (1.1 eq.) was dissolved in dry THF (4.5 mL/mmol) and cooled down to 0 °C. Dichlorophenol (1.0 eq.) was added and the reaction solution was stirred for 15 min at 0 °C. After addition of the diaryliodonium salt (1.2 eq.), the reaction mixture was stirred at 40 °C for 1 h. H_2O was slowly added at 0 °C and the organic phase was separated. The aqueous layer was extracted with Et_2O (2×). The combined organic layers were washed with brine, dried over Na_2SO_4 and filtered. The solvent was removed and the obtained residue was purified by MPLC to give the corresponding biaryl ether.

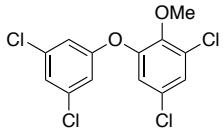
1,5-Dichloro-3-(2,4-dichlorophenoxy)-2-methoxybenzene (27)



Synthesised from 2,4-dichlorophenol (**12**, 35.9 g, 0.22 mmol, 1.0 eq.) and bis(3,5-dichloro-2-methoxyphenyl)iodonium tetrafluoroborate (**21**, 150 mg, 0.27 mmol, 1.2 eq.) in 97% yield (72.0 mg, 0.21 mmol) as a yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.44 (d, *J* = 2.5 Hz, 1 H), 7.10 (d, *J* = 2.3 Hz, 1 H), 7.05 (dd, *J* = 8.8, 2.5 Hz, 1 H), 6.90 (d, *J* = 2.3 Hz, 1 H), 6.41 (d, *J* = 8.8 Hz, 1 H), 3.78 (s, 3 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 153.8, 151.9, 138.7, 131.8, 130.4, 129.6, 127.6 (two signals), 123.5, 122.1, 115.2, 112.3, 56.7. **MS** (ESI+): *m/z* = 337.0 $[M+H]^+$.

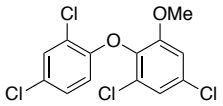
1,5-Dichloro-3-(3,5-dichlorophenoxy)-2-methoxybenzene (28)



Synthesised from 3,5-dichlorophenol (**13**, 96.0 mg, 0.59 mmol, 1.0 eq.) and bis(3,5-dichloro-2-methoxyphenyl)iodonium tetrafluoroborate (**21**, 400 mg, 0.71 mmol, 1.2 eq.) in 82% yield (163 mg, 0.48 mmol) as a yellow oil.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.27 (d, *J* = 2.5 Hz, 1 H), 7.11 (t, *J* = 1.8 Hz, 1 H), 6.94 (d, *J* = 2.5 Hz, 1 H), 6.84 (d, *J* = 1.8 Hz, 2 H), 3.82 (s, 3 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 158.1, 149.2, 147.5, 136.0. 130.2, 129.5, 126.9, 124.0, 121.0, 116.2, 61.4. **MS** (ESI+): *m/z* = 336.9 $[M+H]^+$.

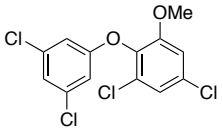
1,5-Dichloro-2-(2,4-dichlorophenoxy)-3-methoxybenzene (29)



Synthesised from 2,4-dichlorophenol (**12**, 96.0 g, 0.59 mmol, 1.0 eq.) and bis(2,4-dichloro-6-methoxy–phenyl)iodonium tetrafluoroborate (**22**, 400 mg, 0.71 mmol, 1.2 eq.) in 48% yield (96.0 mg, 0.28 mmol) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.44 (d, *J* = 2.5 Hz, 1 H), 7.10 (d, *J* = 2.3 Hz, 1 H), 7.05 (dd, *J* = 8.8, 2.5 Hz, 1 H), 6.90 (d, *J* = 2.3 Hz, 1 H), 6.41 (d, *J* = 8.8 Hz, 1 H), 3.78 (s, 3 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 153.8, 151.9, 138.7, 131.8, 130.4, 129.6, 127.6 (two signals), 123.5, 122.1, 115.2, 112.3, 56.7. **MS** (ESI+): *m/z* = 336.9 $[M+H]^+$.

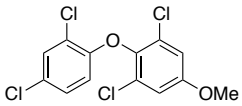
1,5-Dichloro-2-(3,5-dichlorophenoxy)-3-methoxybenzene (30)



Synthesised from 3,5-dichlorophenol (**13**, 96.2 g, 0.59 mmol, 1.0 eq.) and bis(2,4-dichloro-6-methoxy–phenyl)iodonium tetrafluoroborate (**22**, 400 mg, 0.71 mmol, 1.2 eq.) in 29% yield (56.2 mg, 0.17 mmol) as a white solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.10 (d, *J* = 2.3 Hz, 1 H), 7.04 (t, *J* = 1.8 Hz, 1 H), 6.92 (d, *J* = 2.3 Hz, 1 H), 6.72 (d, *J* = 1.8 Hz, 2 H), 3.80 (s, 3 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 158.4, 153.8, 138.0, 135.7, 132.0, 129.7, 123.0, 122.1, 114.2, 112.3, 56.7. **MS** (ESI+): *m/z* = 336.9 $[M+H]^+$.

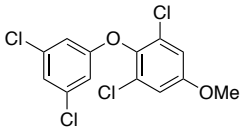
1,3-Dichloro-2-(2,4-dichlorophenoxy)-5-methoxybenzene (31)



Synthesised from 2,4-dichlorophenol (**12**, 48.4 mg, 0.30 mmol, 1.0 eq.) and bis(2,6-dichloro-4-methoxy-phenyl)iodonium tetrafluoroborate (**23**, 185 mg, 0.33 mmol, 1.1 eq.) in 43% yield (43.0 mg, 0.13 mmol) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.46 (d, *J* = 2.5 Hz, 1 H), 7.07 (dd, *J* = 8.8, 2.5 Hz, 1 H), 6.95 (s, 2 H), 6.39 (d, *J* = 8.8 Hz, 1 H), 3.82 (s, 3 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 157.4, 151.6, 140.5, 130.6, 129.9, 127.8, 127.6, 123.5, 122.1, 115.0, 56.2. **MS** (ESI+): *m/z* = 305.1 $[M–MeOH+H]^+$, 336.9 $[M+H]^+$.

1,3-Dichloro-2-(3,5-dichlorophenoxy)-5-methoxybenzene (32)



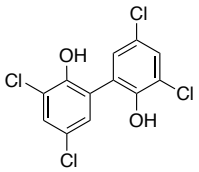
Synthesised from 3,5-dichlorophenol (**13**, 48.4 mg, 0.30 mmol, 1.0 eq.) and bis(2,6-dichloro-4-methoxyphenyl)iodonium tetrafluoroborate (**23**, 185 mg, 0.33 mmol, 1.1 eq.) in 16% yield (16.0 mg, 0.05 mmol) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.05 (t, *J* = 1.7 Hz, 1 H), 6.95 (s, 2 H), 6.72 (d, *J* = 1.7 Hz, 2 H), 3.83 (s, 3 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 158.2, 157.5, 139.9, 135.8, 129.9, 123.1, 115.1, 114.2, 56.2. **MS** (ESI+): *m/z* = 305.1 $[M–MeOH+H]^+$, 337.1 $[M+H]^+$, 375.1 $[M+K]^+$, 381.1 $[M+2Na–H]^+$.

5.6 General Procedure for the Methoxy Deprotection (33–41)

The reaction was performed under dry conditions. The corresponding methoxy protected dimer was dissolved in dry DCM (10.0 mL/mmol) and cooled down to –78 °C. 1M Boron tribromide solution (5.0 eq. for biaryl, 2.5 eq. for biaryl ether) was added dropwise and the reaction solution was stirred for 2 h at –78 °C and for 24 h at room temperature. The reaction was quenched with water at 0 °C and the organic phase was separated. The aqueous layer was extracted with DCM (2×). The combined organic layers were washed with brine, dried over Na_2SO_4 and filtered. The solvent was removed and the obtained residue was purified by MPLC to give the corresponding deprotected phenols.

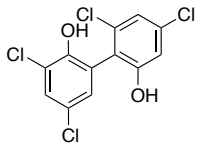
3,3',5,5'-Tetrachloro-2,2'-dihydroxy-1,1'-biphenyl (33)



Synthesised from 3,3',5,5'-tetrachloro-2,2'-dimethoxy-1,1'-biphenyl (**24**, 125 mg, 0.36 mmol) in 29% yield (33.0 mg, 0.10 mmol) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.41 (d, *J* = 2.5 Hz, 2 H), 7.19 (d, *J* = 2.5 Hz, 2 H), 5.84 (bs, 2 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 147.6, 130.1, 129.1, 125.9, 125.6, 121.9. **^{1}H-NMR** (300 MHz, DMSO-d_6): δ = 9.60 (bs, 2 H), 7.53 (d, *J* = 2.6 Hz, 2 H), 7.17 (d, *J* = 2.6 Hz, 2 H). **^{13}C-NMR** (75 MHz, DMSO-d_6): δ = 149.9, 129.5, 128.6, 127.7, 122.7, 122.1. **MS** (ESI–): *m/z* = 320.8 $[M–H]^–$. **HRMS** (ESI–) *m/z* calcd for $C_{12}H_5Cl_4O_2$ $[M–H]^–$: 320.9049, found: 320.9050; calcd for $C_{24}H_{11}Cl_8O_4$ $[2M–H]^–$: 642.8171, found: 642.8174. **HPLC**: t_R = 15.9 min (λ_{max} = 208, 296 nm).

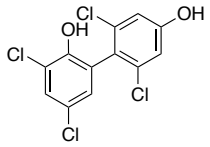
2',3,4',5-Tetrachloro-2,6'-dihydroxy-1,1'-biphenyl (34)



Synthesised from 2',3,4',5-tetrachloro-2,6'-dimethoxy-1,1'-biphenyl (**25**, 153 mg, 0.43 mmol) in 65% yield (91.0 g, 0.28 mmol) as a white solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.47 (d, *J* = 2.5 Hz, 1 H), 7.134 (d, *J* = 2.6 Hz, 1 H), 7.126 (d, *J* = 2.1 Hz, 1 H), 6.96 (d, *J* = 2.0 Hz, 1 H), 5.68 (bs, 1 H), 5.09 (bs, 1 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 154.7, 148.4, 135.8, 135.2, 130.8, 130.0, 126.2, 122.2, 121.9, 121.8, 120.6, 115.5. **MS** (ESI–): *m/z* = 320.9 $[M–H]^–$. **HRMS** (ESI–) *m/z* calcd for $C_{12}H_5Cl_4O_2$ $[M–H]^–$: 320.9049, found: 320.9050; calcd for $C_{24}H_{11}Cl_8O_4$ $[2M-H]^–$: 642.8171, found: 642.8174. **HPLC**: t_R = 14.2 min (λ_{max} = 204, 292 nm).

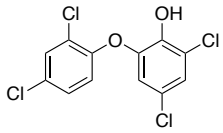
2',3,5,6'-Tetrachloro-2,4'-dihydroxy-1,1'-biphenyl (35)



Synthesised from 2',3,5,6'-tetrachloro-2,4'-dimethoxy-1,1'-biphenyl (**26**, 105 mg, 0.30 mmol) in 93% yield (90.0 mg, 0.28 mmol) as a white solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.41 (d, *J* = 2.5 Hz, 1 H), 7.05 (d, *J* = 2.5 Hz, 1 H), 6.93 (s, 2 H), 5.52 (bs, 2 H). **^{13}C-NMR** (75 MHz, $CDCl_3$): δ = 156.4, 148.1, 135.9, 130.4, 129.0, 126.3, 125.9, 125.3, 121.1, 115.7. **MS** (ESI–): *m/z* = 320.8 $[M–H]^–$. **HRMS** (ESI–) *m/z* calcd for $C_{12}H_5Cl_4O_2$ $[M–H]^–$: 320.9049, found: 320.9050; calcd for $C_{24}H_{11}Cl_8O_4$ $[2M–H]^–$: 642.8171, found: 642.8169. **HPLC**: t_R = 13.8 min (λ_{max} = 204, 288 nm).

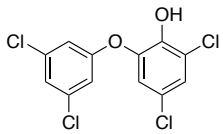
2,4-Dichloro-6-(2,4-dichlorophenoxy)phenol (36)



Synthesised from 1,5-dichloro-3-(2,4-dichlorophenoxy)-2-methoxybenzene (**27**, 149 mg, 0.44 mmol) in 81% yield (116 mg, 0.36 mmol) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.51 (d, *J* = 2.5 Hz, 1 H), 7.28 (dd, *J* = 6.6, 2.1 Hz, 1 H), 7.15 (d, *J* = 2.4 Hz, 2 H), 7.00 (d, *J* = 8.7 Hz, 1 H), 6.63 (d, *J* = 2.4 Hz, 1 H), 5.82 (bs, 1 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 149.9, 144.8, 142.1, 131.0, 128.6, 126.9, 125.1, 124.8, 121.8, 116.3. **^{1}H-NMR** (300 MHz, DMSO-d_6): δ = 10.37 (bs, 1 H), 7.76 (d, *J* = 2.5 Hz, 1 H), 7.40, (dd, *J* = 8.8, 2.6 Hz, 1 H), 7.37 (d, *J* = 2.5 Hz, 1 H), 7.02 (d, *J* = 8.8 Hz, 1 H), 6.91 (d, *J* = 2.5 Hz, 1 H). **^{13}C-NMR** (75 MHz, DMSO-d_6): δ = 150.9, 145.0, 144.1, 130.1, 128.7, 128.3, 125.0, 124.7, 122.7 (two signals), 120.5, 118.1. **MS** (ESI–): *m/z* = 320.8 $[M–H]^–$. **HRMS** (ESI–) *m/z* calcd for $C_{12}H_5Cl_4O_2$ $[M–H]^–$: 320.9049, found: 320.9050; calcd for $C_{24}H_{11}Cl_8O_4$ $[2M–H]^–$: 642.8171, found: 642.8175. **HPLC**: t_R = 21.4 min (λ_{max} = 204, 284 nm).

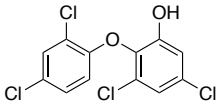
2,4-Dichloro-6-(3,5-dichlorophenoxy)phenol (37)



Synthesised from 1,5-dichloro-3-(3,5-dichlorophenoxy)-2-methoxybenzene (**28**, 138 mg, 0.41 mmol) in 82% yield (108 mg, 0.33 mmol) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.23 (d, *J* = 2.4 Hz, 1 H), 7.15 (t, *J* = 1.8 Hz, 1 H), 6.90 (d, *J* = 2.5 Hz, 1 H) 6.89 (d, *J* = 1.8 Hz, 2 H), 5.64 (bs, 1 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 157.6, 143.3, 143.2, 136.1, 125.9, 125.4, 124.5, 122.2, 199.7, 116.6. **MS** (ESI–): *m/z* = 320.8 $[M–H]^–$. **HRMS** (ESI–) *m/z* calcd for $C_{12}H_5Cl_4O_2$ $[M–H]^–$: 320.9049, found: 320.9050; calcd for $C_{24}H_{11}Cl_8O_4$ $[2M–H]^–$: 642.8171, found: 642.8169. HPLC: t_R = 25.6 min (λ_{max} = 204, 284 nm).

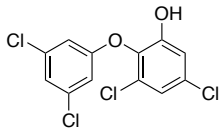
3,5-Dichloro-2-(2,4-dichlorophenoxy)phenol (38)



Synthesised from 1,5-dichloro-2-(2,4-dichlorophenoxy)-3-methoxybenzene (**29**, 83.0 mg, 0.25 mmol) in 93% yield (74.0 mg, 0.23 mmol) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.47 (d, *J* = 2.5 Hz, 1 H), 7.11 (dd, *J* = 8.8, 2.5 Hz, 1 H), 7.06-7.00 (m, 2 H), 6.54 (d, *J* = 8.8 Hz, 1 H), 5.60 (bs, 1 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 151.0, 150.3, 137.1, 132.2, 130.8, 128.9, 128.3, 128.1, 123.8, 122.4, 116.2, 115.8. **MS** (ESI–): *m/z* = 320.8 $[M–H]^–$. **HRMS** (ESI–) *m/z* calcd for $C_{12}H_5Cl_4O_2$ $[M–H]^–$: 320.9049, found: 320.9050; calcd for $C_{24}H_{11}Cl_8O_4$ $[2M–H]^–$: 642.8171, found: 642.8170. **HPLC**: t_R = 23.3 min (λ_{max} = 204, 284 nm).

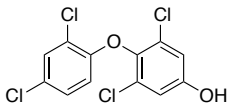
3,5-Dichloro-2-(3,5-dichlorophenoxy)phenol (39)



Synthesised from 1,5-dichloro-2-(3,5-dichlorophenoxy)-3-methoxybenzene (**30**, 31.3 mg, 92.6 µmol) in 69% yield (20.7 mg, 63.9 µmol) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.10 (t, *J* = 1.7 Hz, 1 H), 7.05 (d, *J* = 2.4 Hz, 1 H), 7.02 (d, *J* = 2.4 Hz, 1 H), 6.79 (d, *J* = 1.7 Hz, 2 H), 5.54 (bs, 1 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 157.5, 150.3, 136.3, 136.2, 132.4, 128.6, 124.0, 122.5, 116.3, 114.4. **MS** (ESI–): *m/z* = 320.9 $[M–H]^–$. **HRMS** (ESI–) *m/z* calcd for $C_{12}H_5Cl_4O_2$ $[M–H]^–$: 320.9049, found: 320.9050; calcd for $C_{24}H_{11}Cl_8O_4$ $[2M–H]^–$: 642.8171, found: 642.8168.

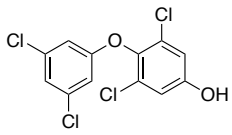
3,5-Dichloro-4-(2,4-dichlorophenoxy)phenol (40)



Synthesised from 1,3-dichloro-2-(2,4-dichlorophenoxy)-5-methoxybenzene (**31**, 43.0 mg, 127 µmol) in 51% yield (21.0 mg, 64.8 µmol) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.46 (d, *J* = 2.5 Hz, 1 H), 7.07 (dd, *J* = 8.8, 2.5 Hz, 1 H), 6.91 (s, 2 H), 6.40 (d, *J* = 8.8 Hz, 1 H), 5.42 (bs, 1 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 153.5, 151.5, 140.7, 130.6, 130.0, 127.8, 127.7, 123.5, 116.5, 115.1. **MS** (ESI–): *m/z* = 320.7 $[M–H]^–$, 642.8 $[2M–H]^–$. **HRMS** (ESI) *m/z* calcd for $C_{12}H_5Cl_4O_2$ $[M–H]^–$: 320.9049, found: 320.9051; calcd for $C_{24}H_{11}Cl_8O_4$ $[2M–H]^–$: 642.8171, found: 642.8165.

3,5-Dichloro-4-(3,5-dichlorophenoxy)phenol (41)



Synthesised from 1,3-dichloro-2-(3,5-dichlorophenoxy)-5-methoxybenzene (**32**, 16.0 mg, 47.0 µmol) in 13% yield (2.00 mg, 6.17 µmol) as a white solid.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.05 (t, *J* = 1.7 Hz, 1 H), 6.92 (s, 2 H), 6.72 (d, *J* = 1.7 Hz, 2 H), 5.15 (bs, 1 H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ = 158.1, 153.6, 140.2, 135.8, 130.0, 123.1, 116.6, 114.2. **MS** (ESI–): *m/z* = 320.9 $[M–H]^-$, 356.8 $[M+Cl]^-$, 642.7 $[2M–H]^-$. **HRMS** (ESI–) *m/z* calcd for $C_{12}H_5Cl_4O_2$ $[M–H]^-$: 320.9049, found: 320.9049; calcd for $C_{24}H_{11}Cl_8O_4$ $[2M–H]^-$: 642.8171, found: 642.8161.

6. LC-MS Data

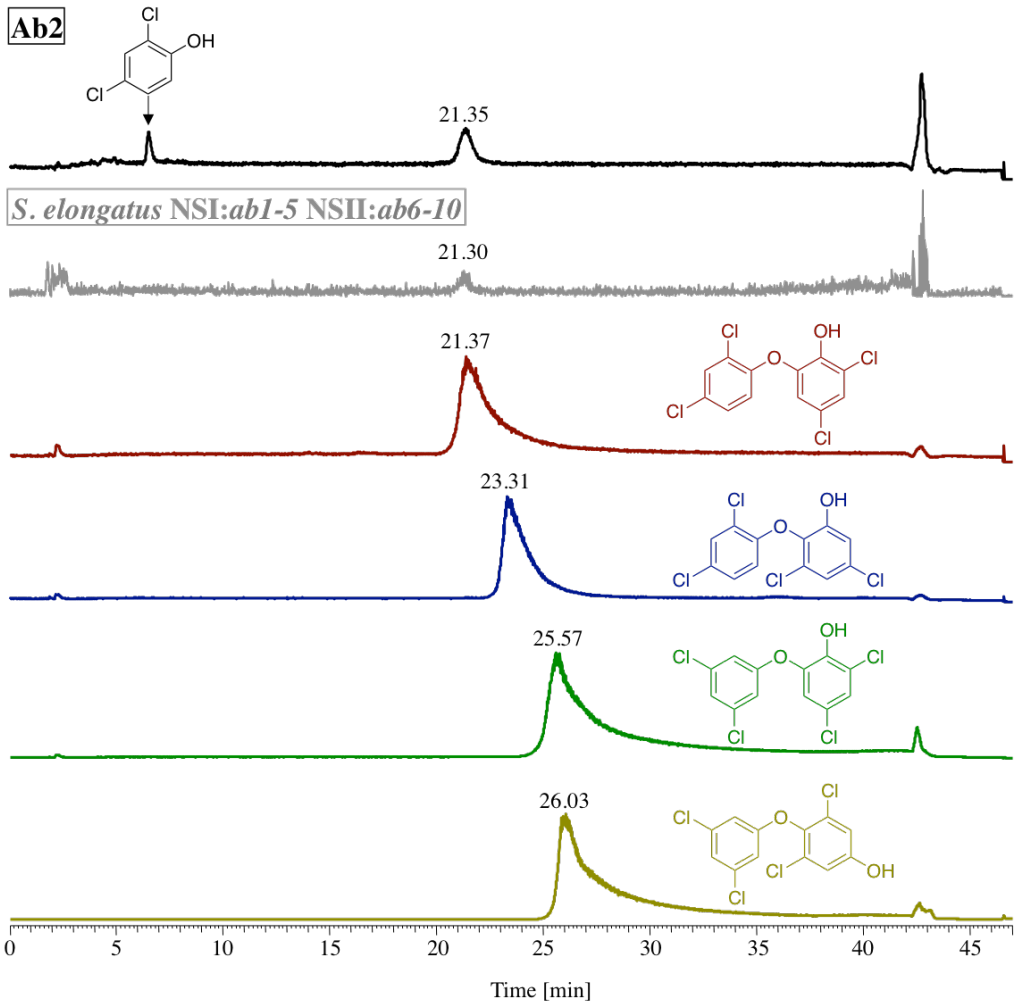


Fig. S7 Comparison of retention times of synthesised dichlorophenol-biaryl-ether-dimers with Ab2 2,4-DCP *in vivo* assay (black) and *S. elongatus* NSI:ab1-5 NSII:*ab6-10* expression (grey).

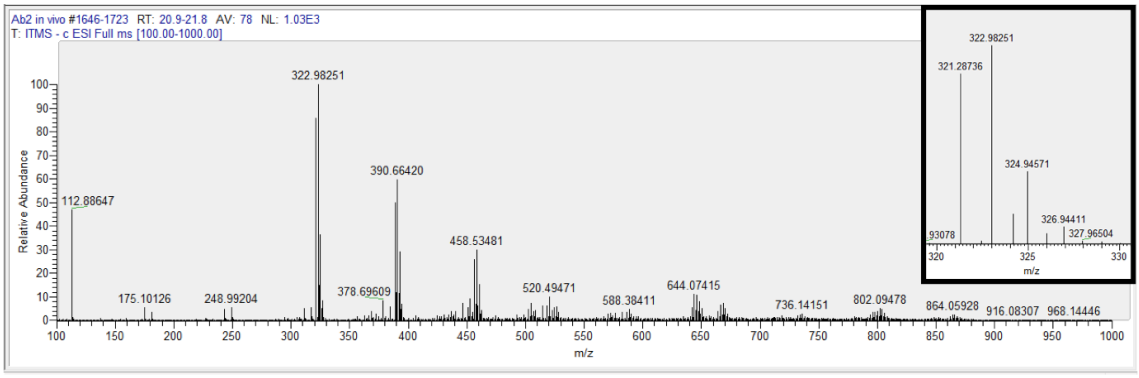


Fig. S8 ESI– spectrum of the 2,4-DCP dimer produced by Ab2, including the characteristic isotopic pattern for a tetrachlorinated substance.

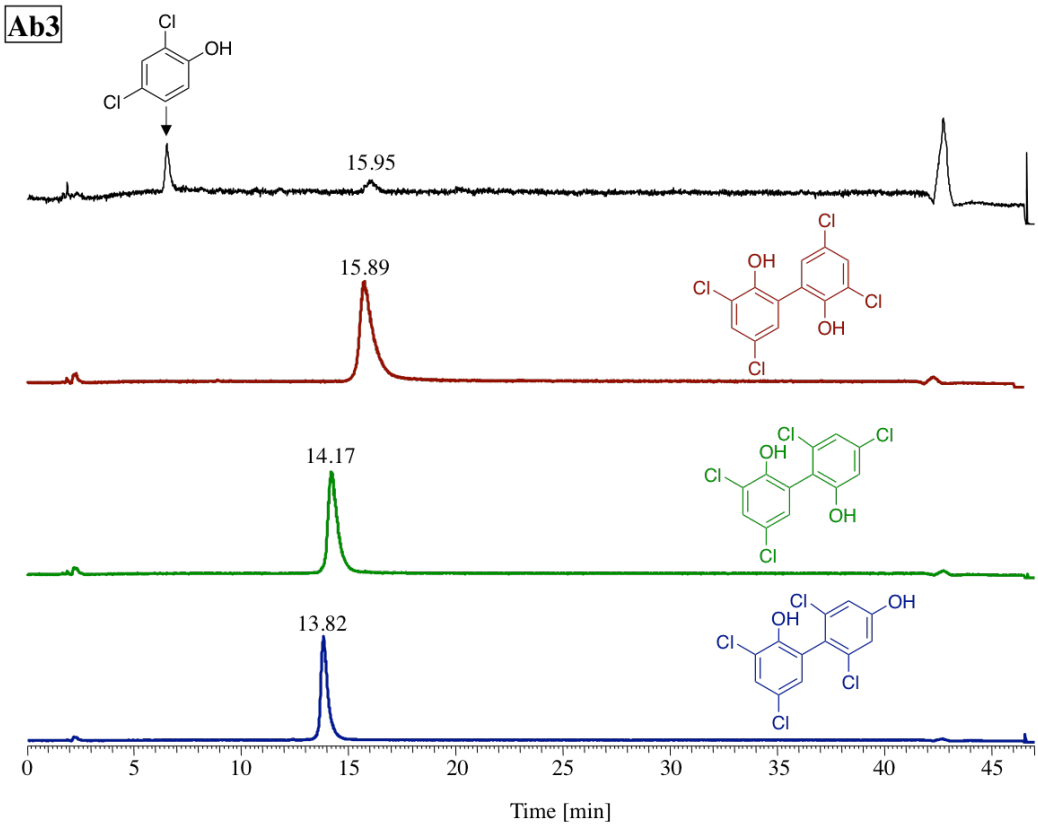


Fig. S9 Comparison of retention times of synthesised dichlorophenol-biaryl-dimers with Ab3 2,4-DCP *in vitro* assay.

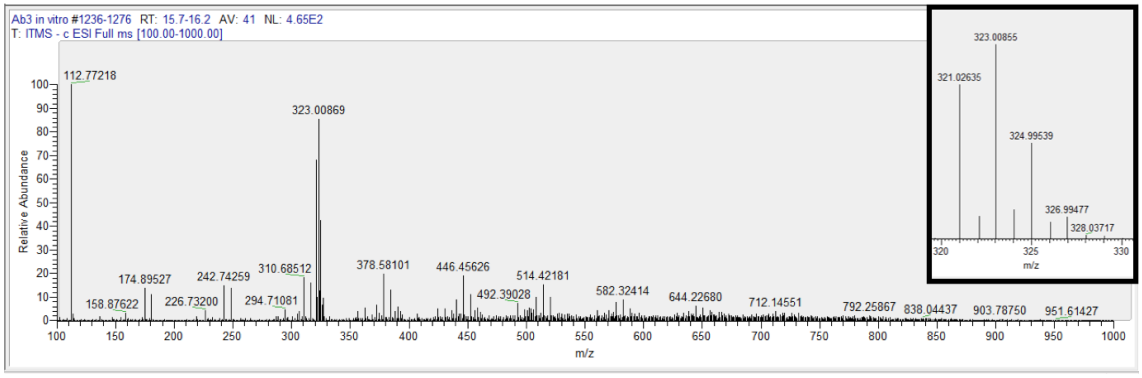


Fig. S10 ESI- spectrum of the 2,4-DCP dimer produced by Ab3, including the characteristic isotopic pattern for a tetrachlorinated substance.

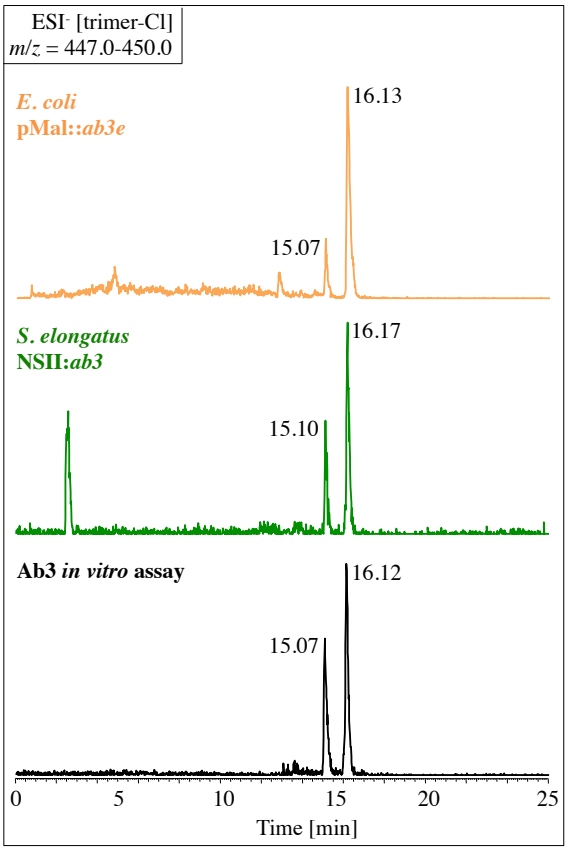


Fig. S11 HPLC-MS analysis (EIC⁻ *m/z* = 447.0–450.0) of *in vivo* and *in vitro* catalytic activity of Ab3 when incubated with 2,4-DCP. The formation of two DCP-trimers lacking one chlorine can be observed.

7. ^{1}H-NMR and ^{13}C-NMR Spectra

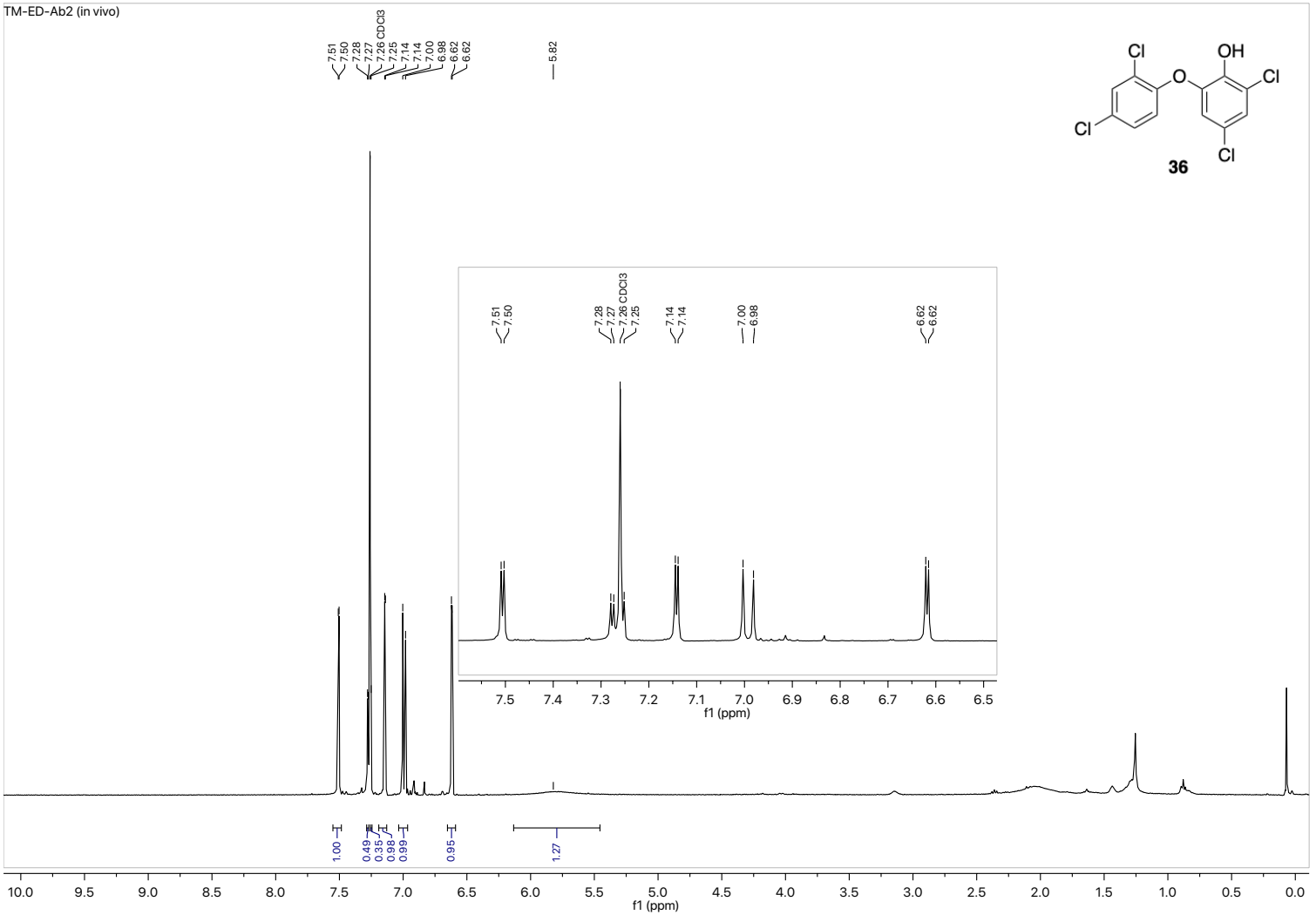


Fig. S12 ^{1}H-NMR spectrum of the major Ab2 2,4-DCP dimeric coupling product extracted and purified from *E. coli* BL21 pMal-SD::*ab2e*.

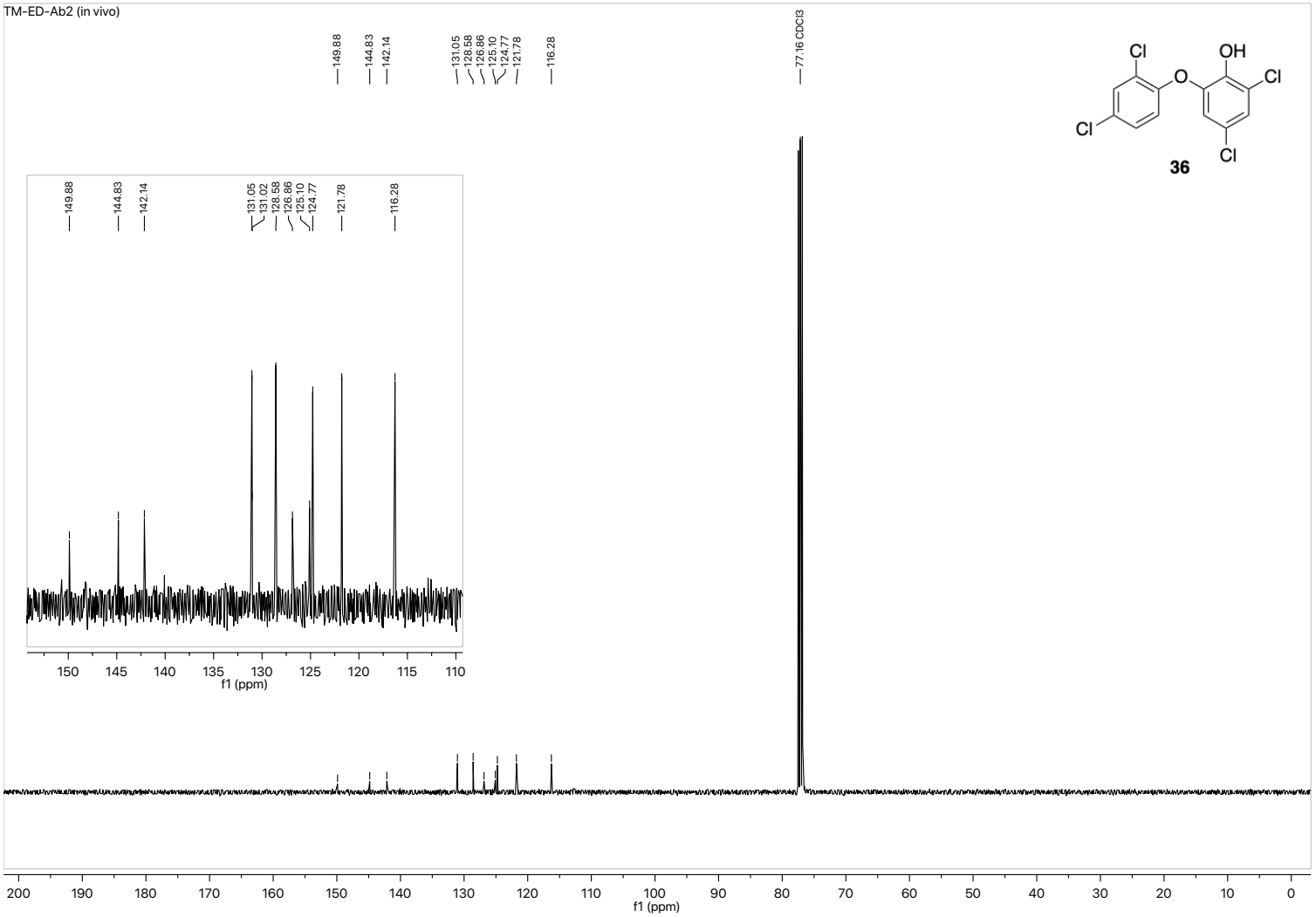


Fig. S13 ^{13}C-NMR spectrum of the major Ab2 2,4-DCP dimeric coupling product extracted and purified from *E. coli* BL21 pMal-SD::*ab2e*.

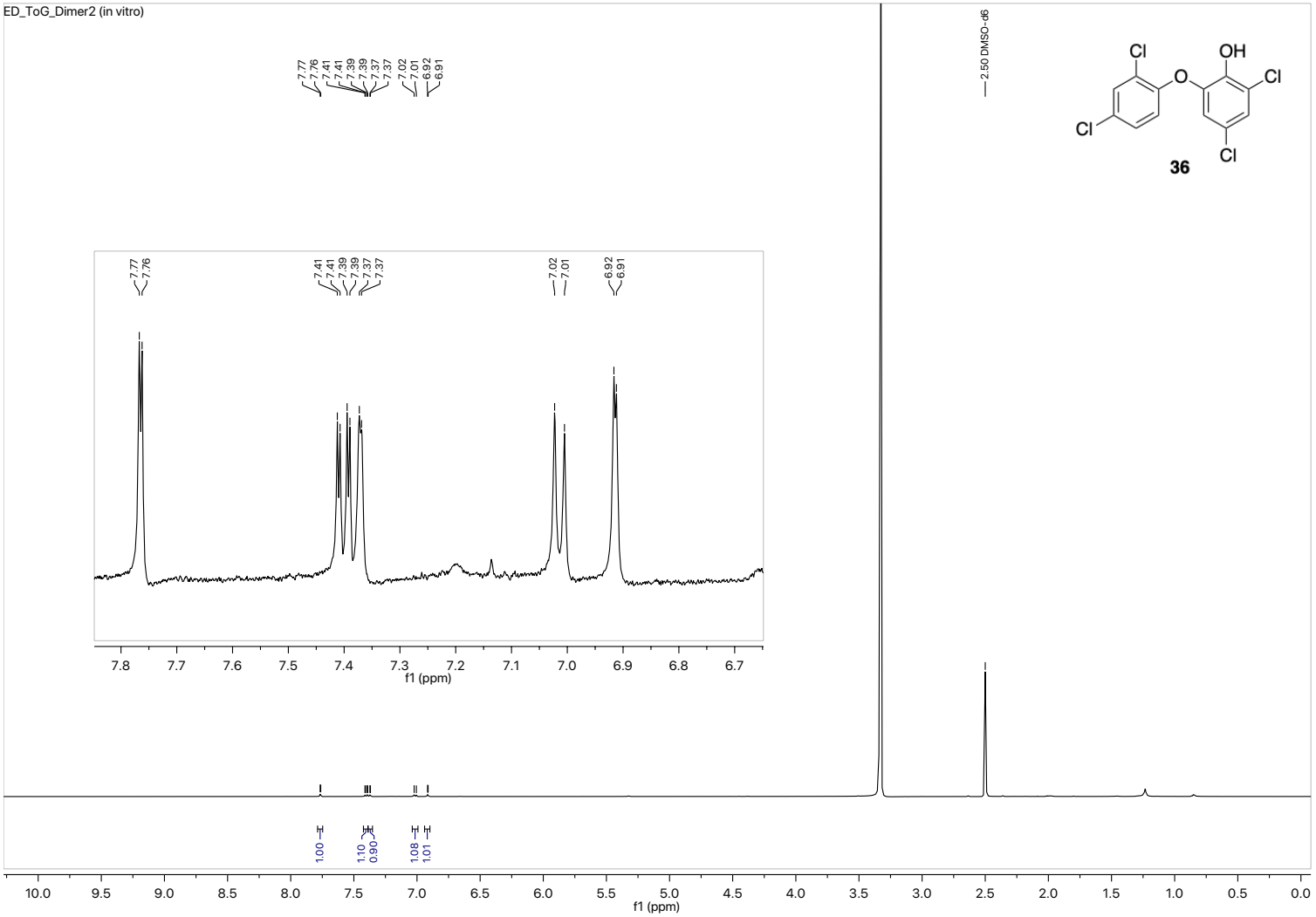


Fig. S14 ^{1}H-NMR spectrum of the major Ab2 2,4-DCP dimeric coupling product isolated from a large scale *in vitro* assay.

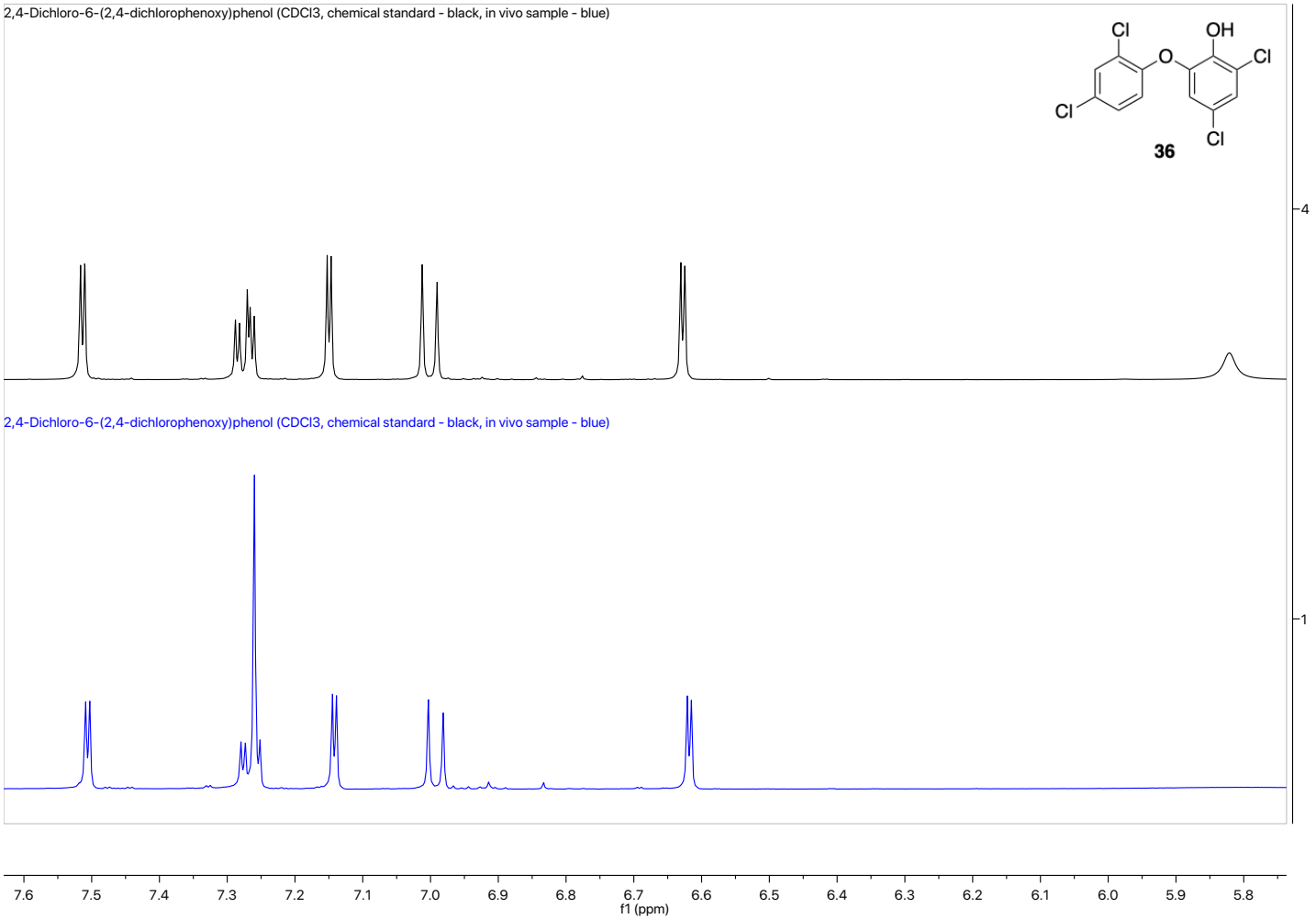


Fig. S15 Comparison of ^{1}H NMR spectra of 2,4-dichloro-6-(2,4-dichlorophenoxy)phenol (**36**) produced by synthesis (black) and *in vivo* (blue). Measured in $CDCl_3$.

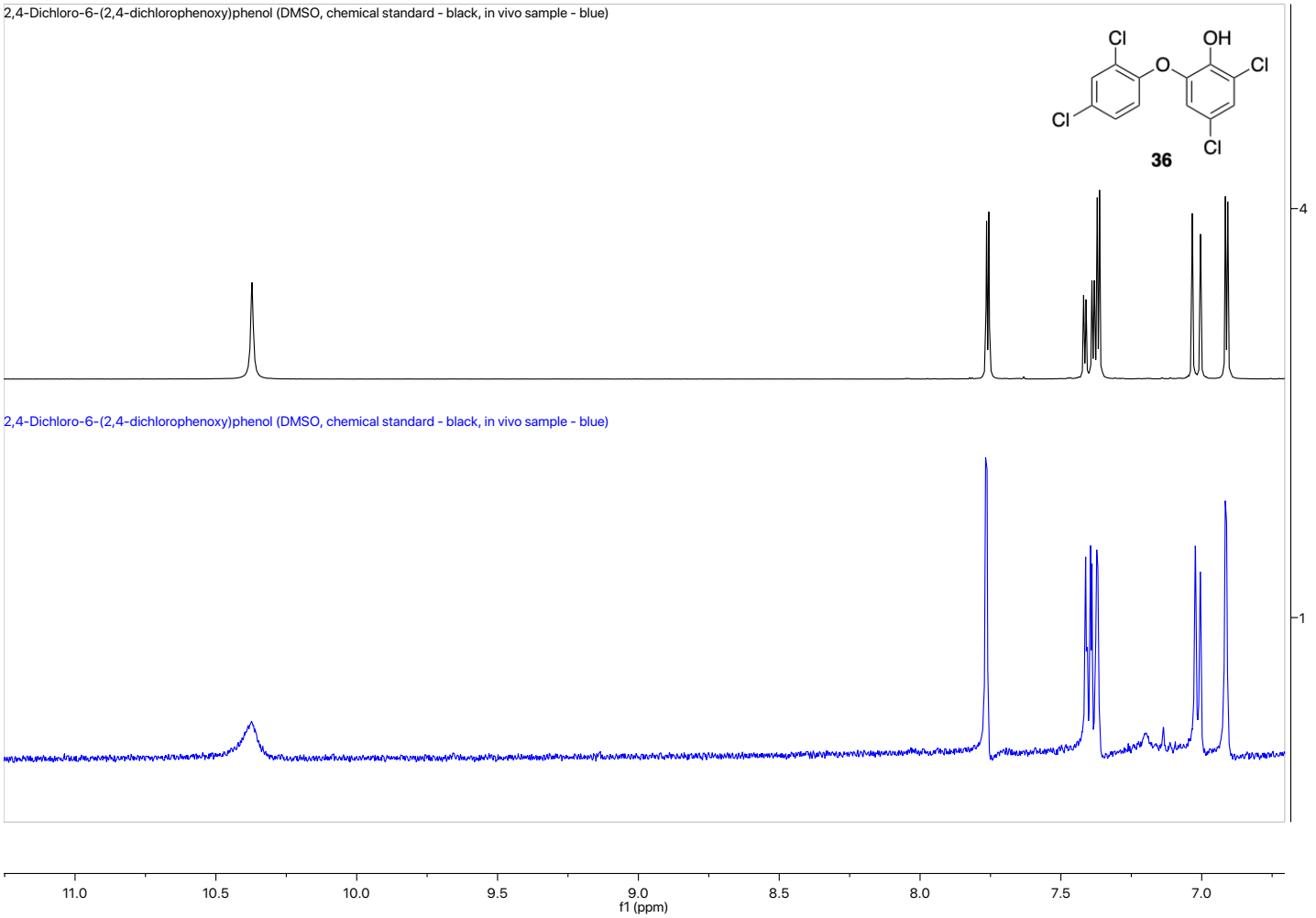


Fig. S16 Comparison of ^{1}H NMR spectra of 2,4-dichloro-6-(2,4-dichlorophenoxy)phenol (**36**) produced by synthesis (black) and *in vivo* (blue). Measured in DMSO-d_6.

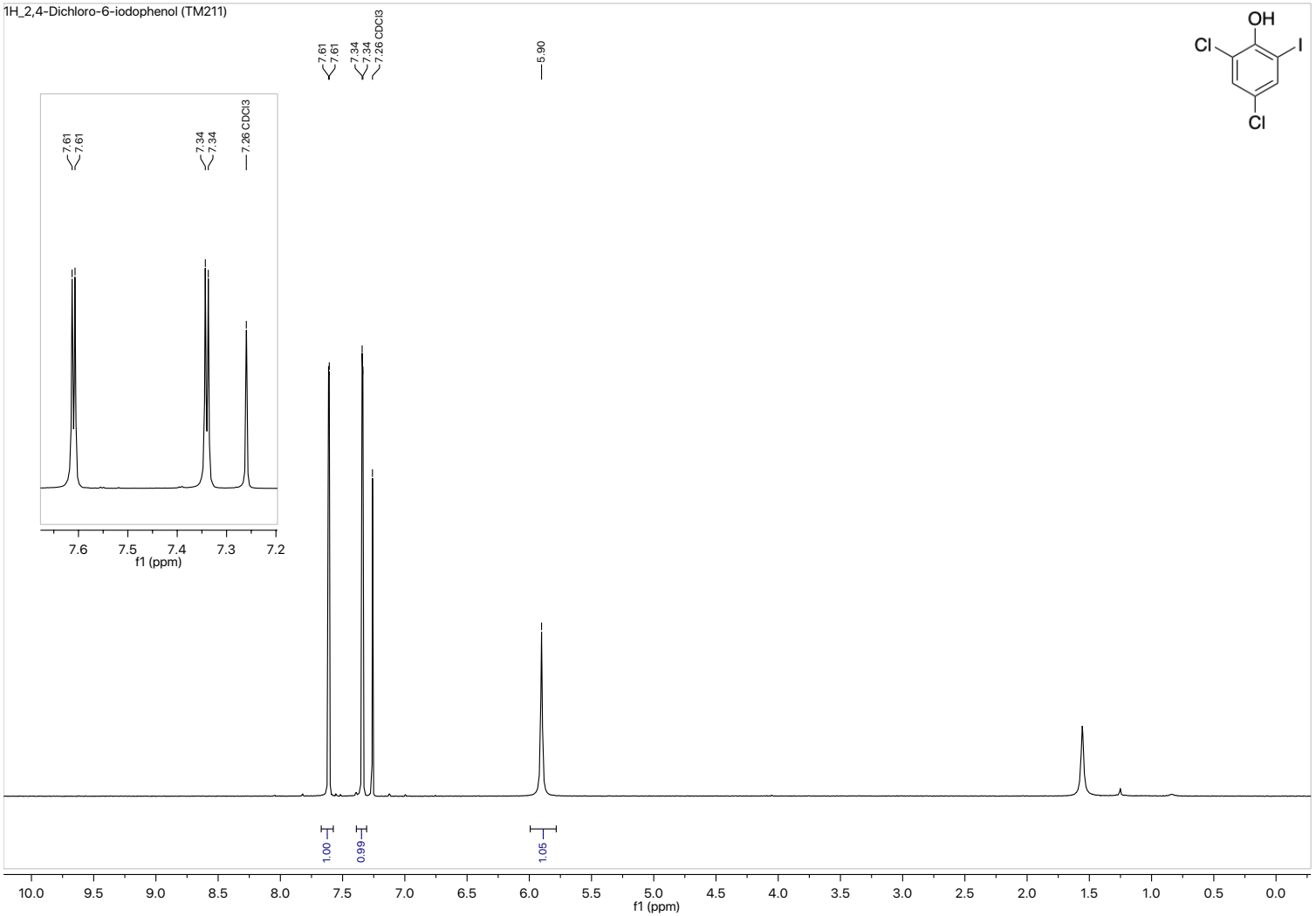


Fig. S17 ^{1}H-NMR spectrum of 2,4-dichloro-6-iodophenol.

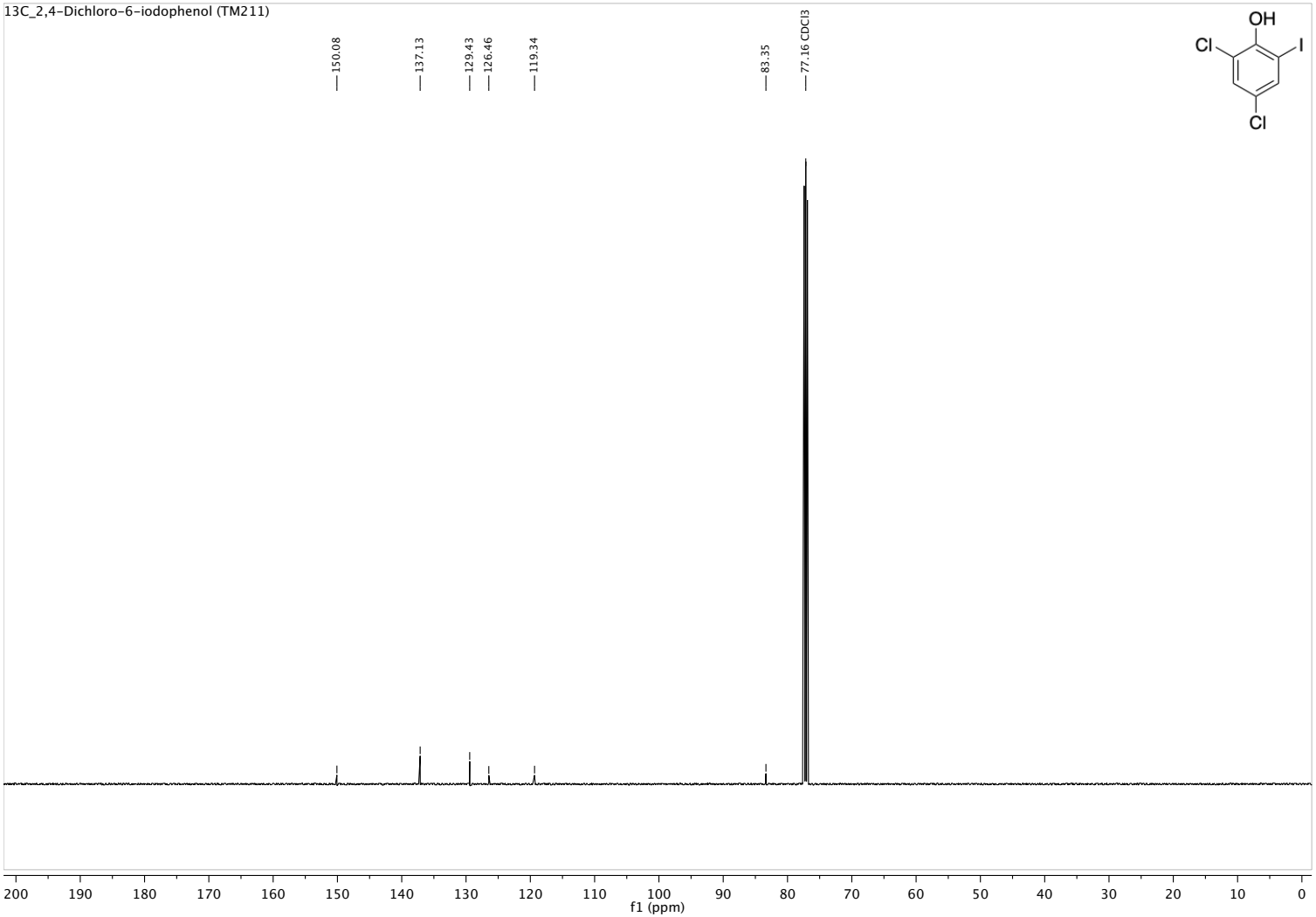


Fig. S18 ^{13}C-NMR spectrum of 2,4-dichloro-6-iodophenol.

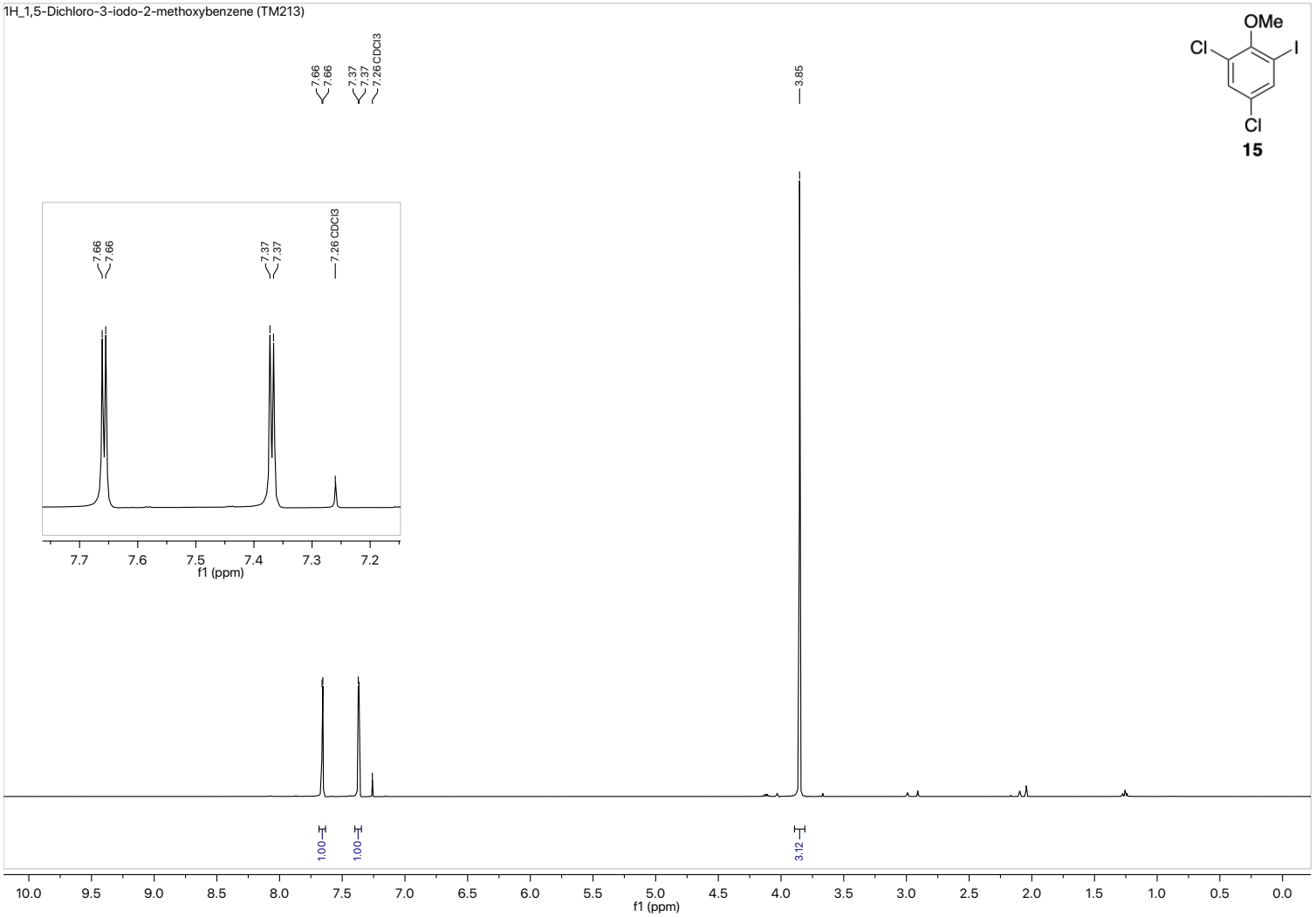


Fig. 19 ^{1}H-NMR spectrum of 1,5-dichloro-3-iodo-2-methoxybenzene (**15**).

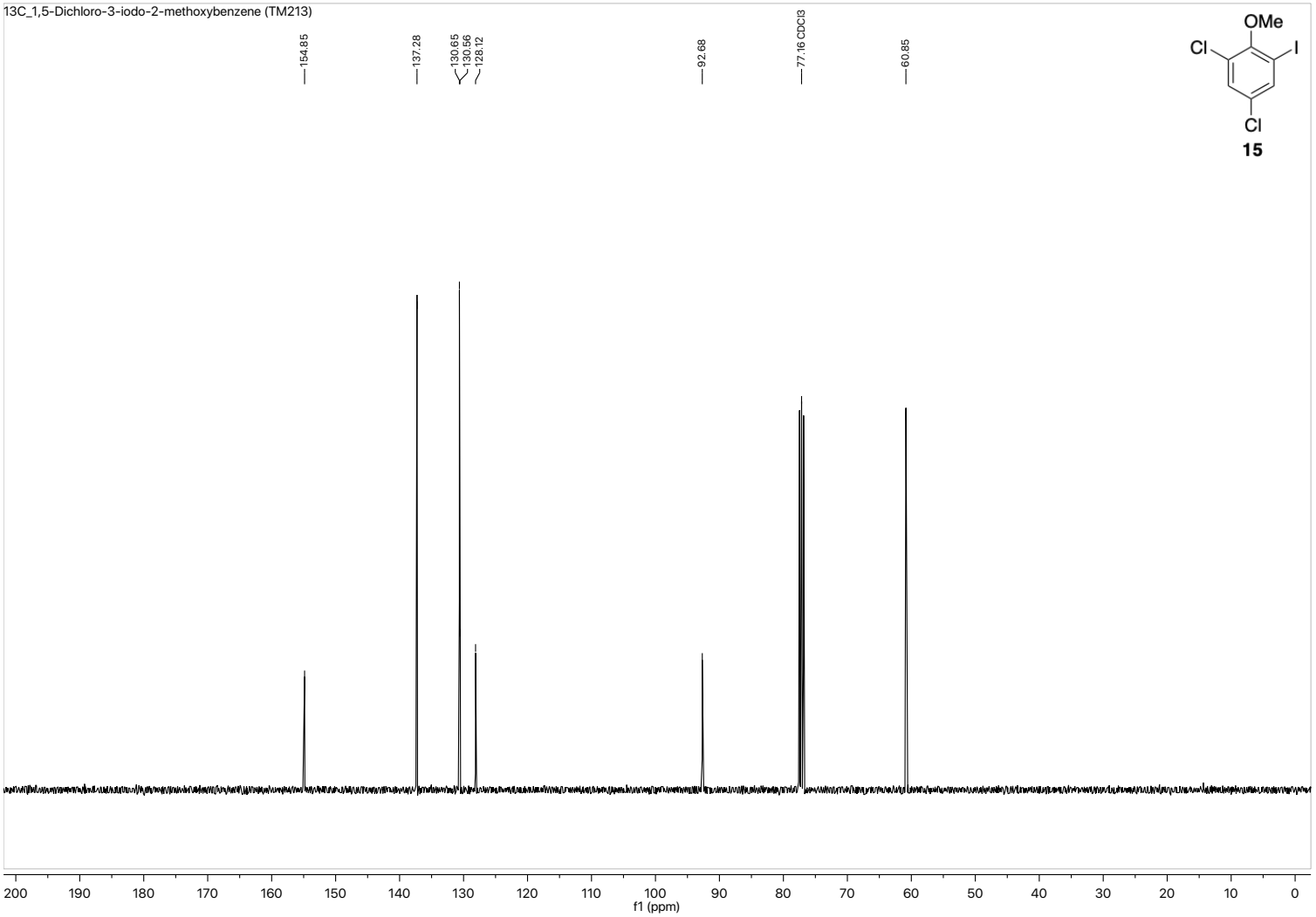


Fig. S20 ^{13}C-NMR spectrum of 1,5-dichloro-3-iodo-2-methoxybenzene (**15**).

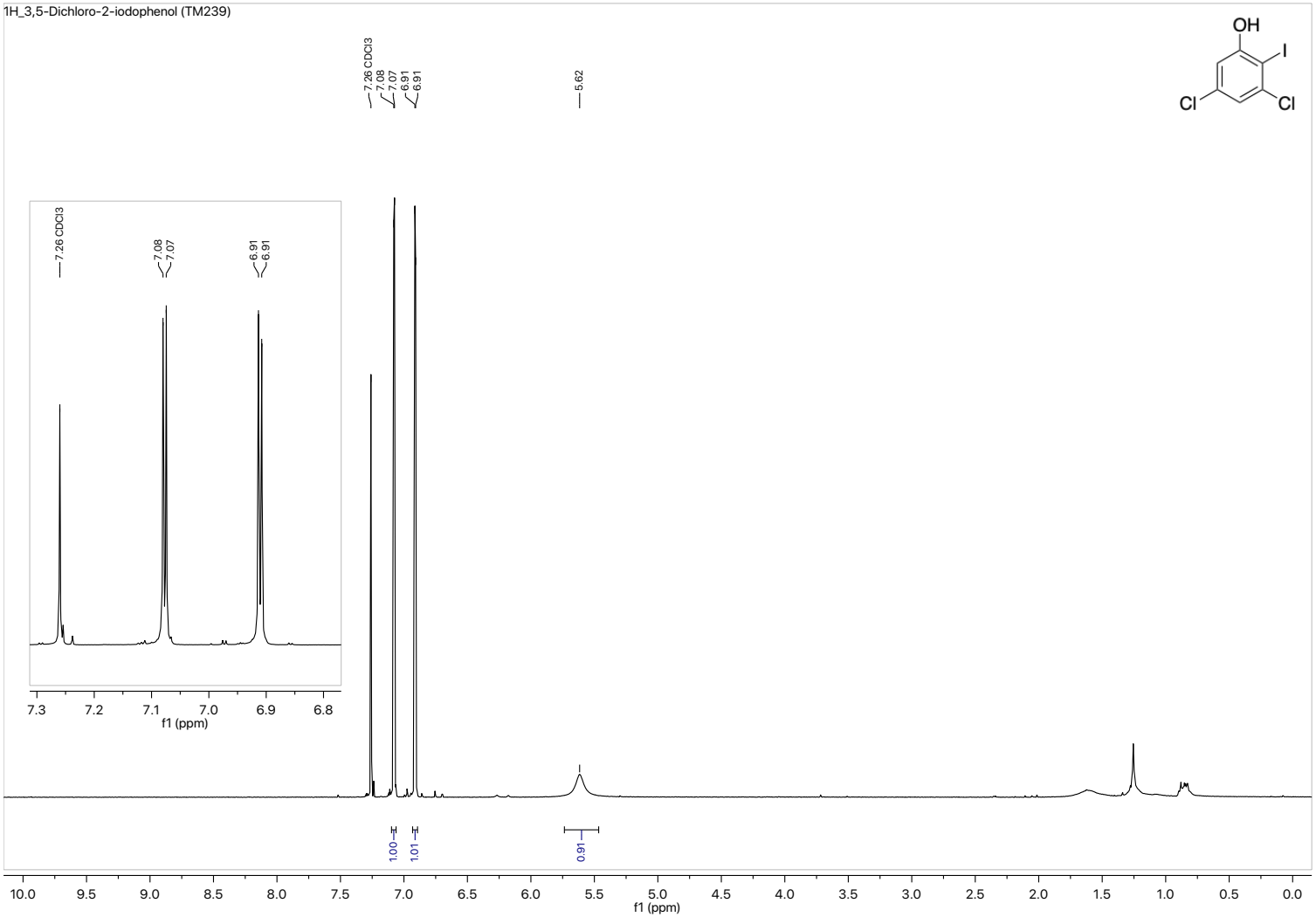


Fig. S21 ^{1}H-NMR spectrum of 3,5-dichloro-2-iodophenol.

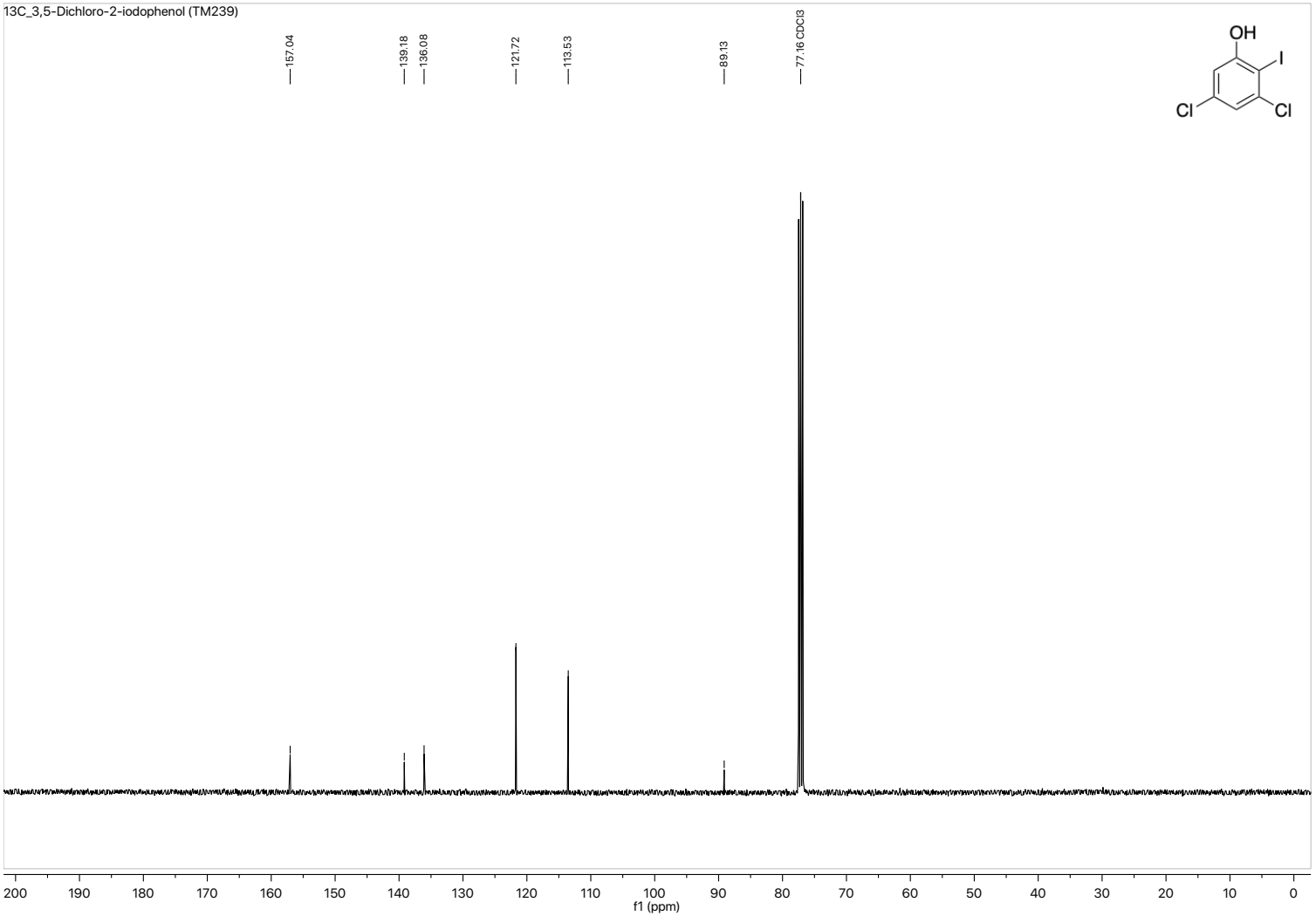


Fig. S22 ^{13}C-NMR spectrum of 3,5-dichloro-2-iodophenol.

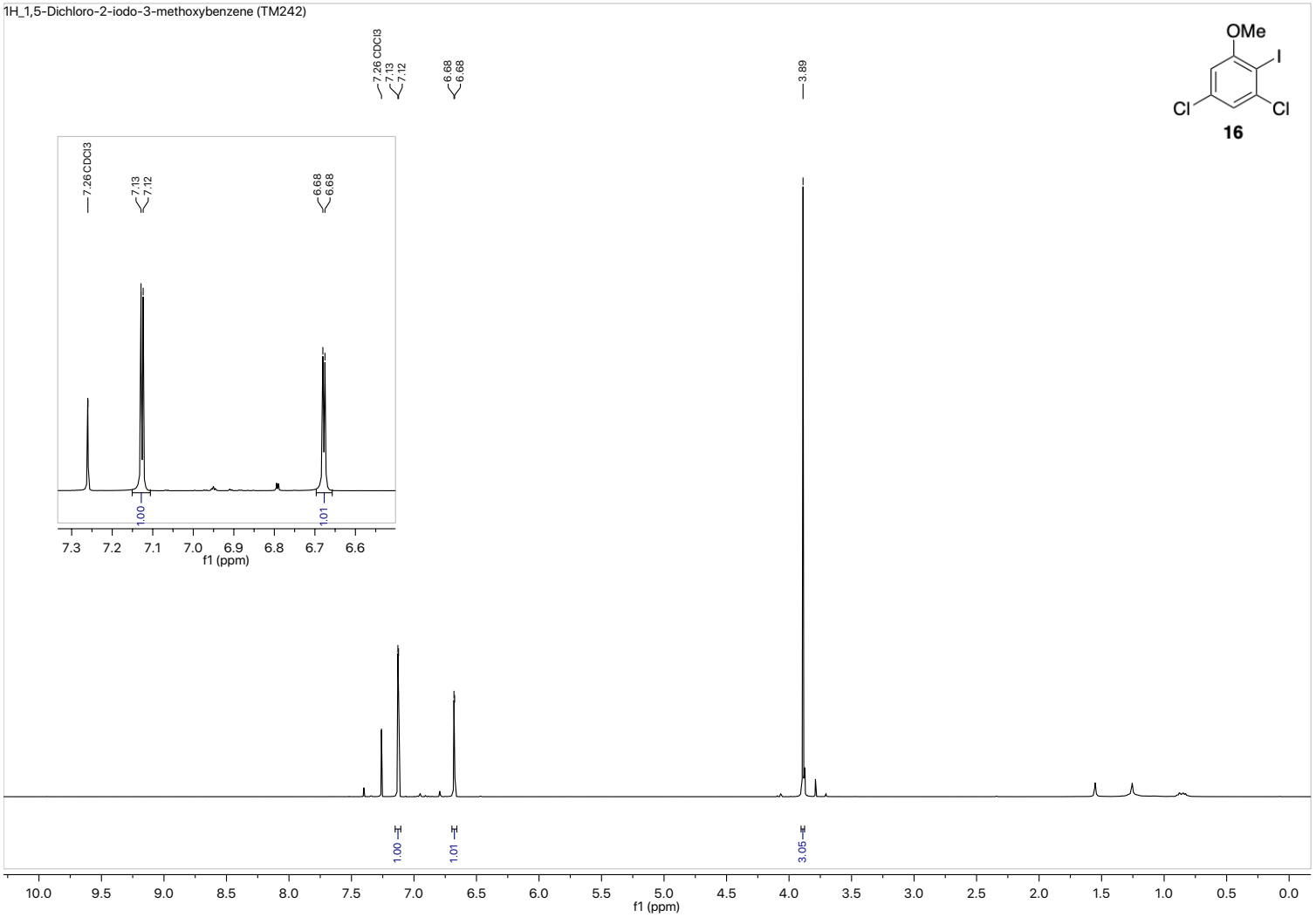


Fig. S23 ^{1}H-NMR spectrum of 1,5-dichloro-2-iodo-3-methoxybenzene (**16**).

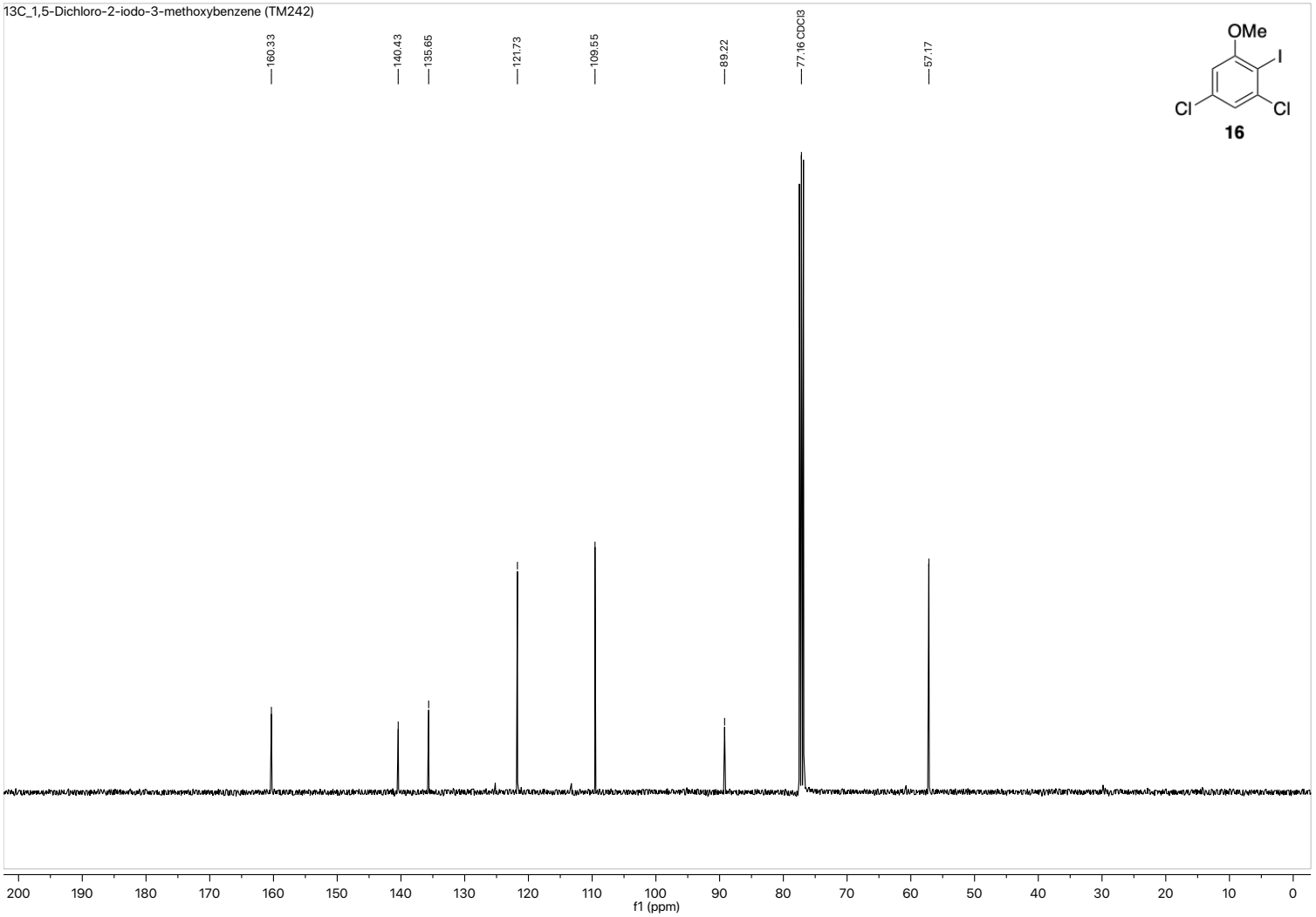


Fig. S24 ^{13}C-NMR spectrum of 1,5-dichloro-2-iodo-3-methoxybenzene (**16**).

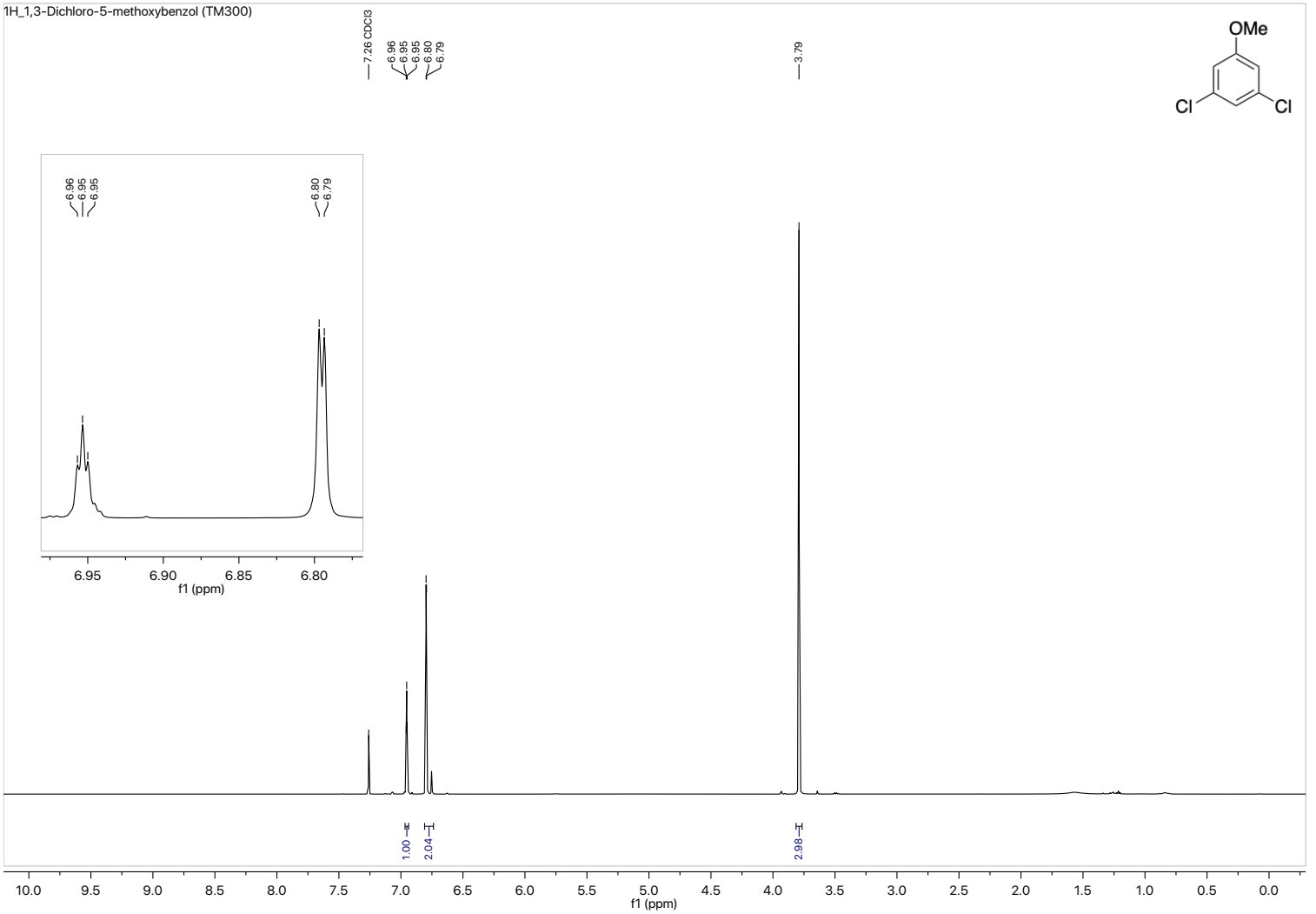


Fig. S25 ^{1}H-NMR spectrum of 1,3-dichloro-5-methoxybenzene.

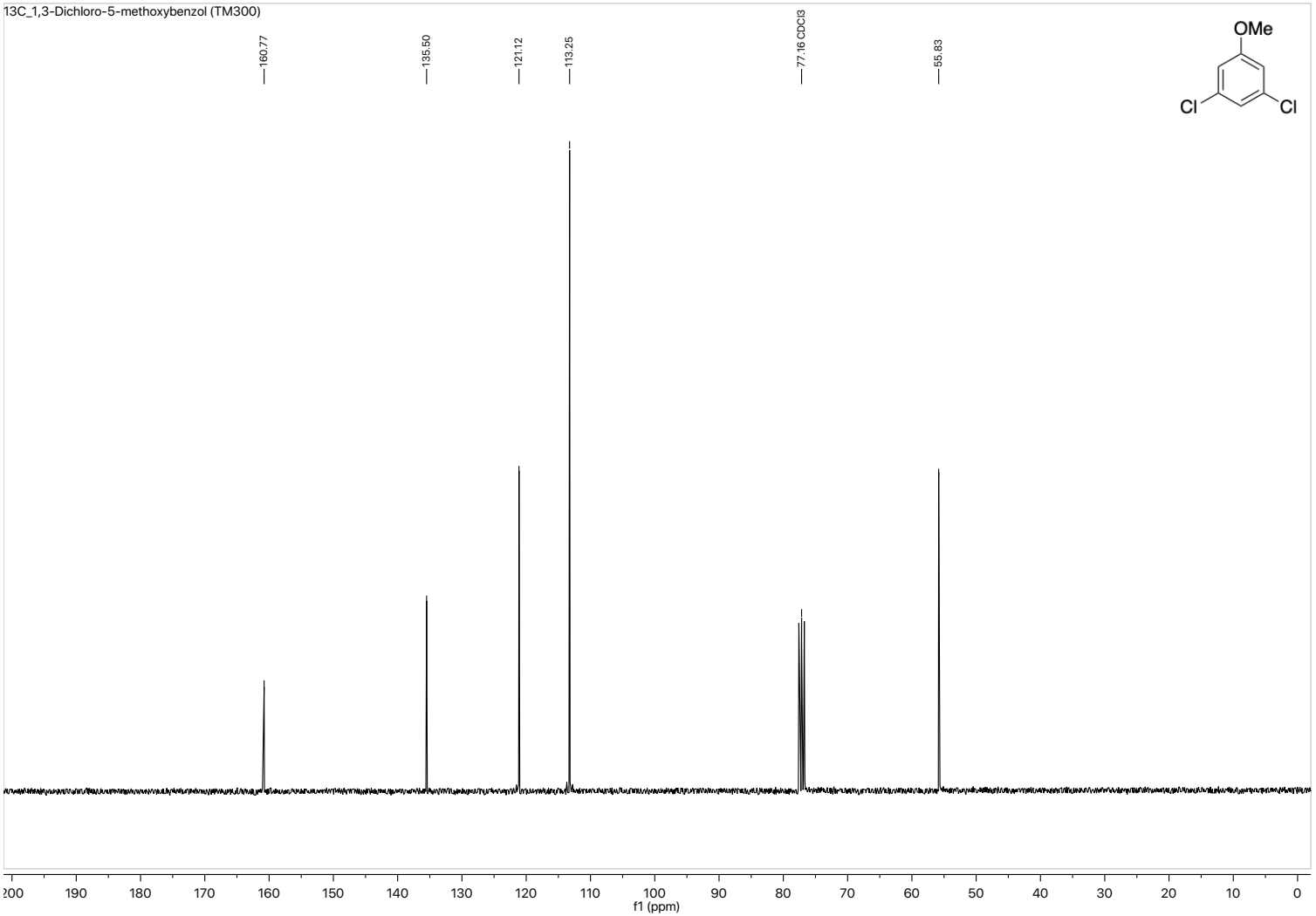


Fig. 26 ^{13}C-NMR spectrum of 1,3-dichloro-5-methoxybenzene.

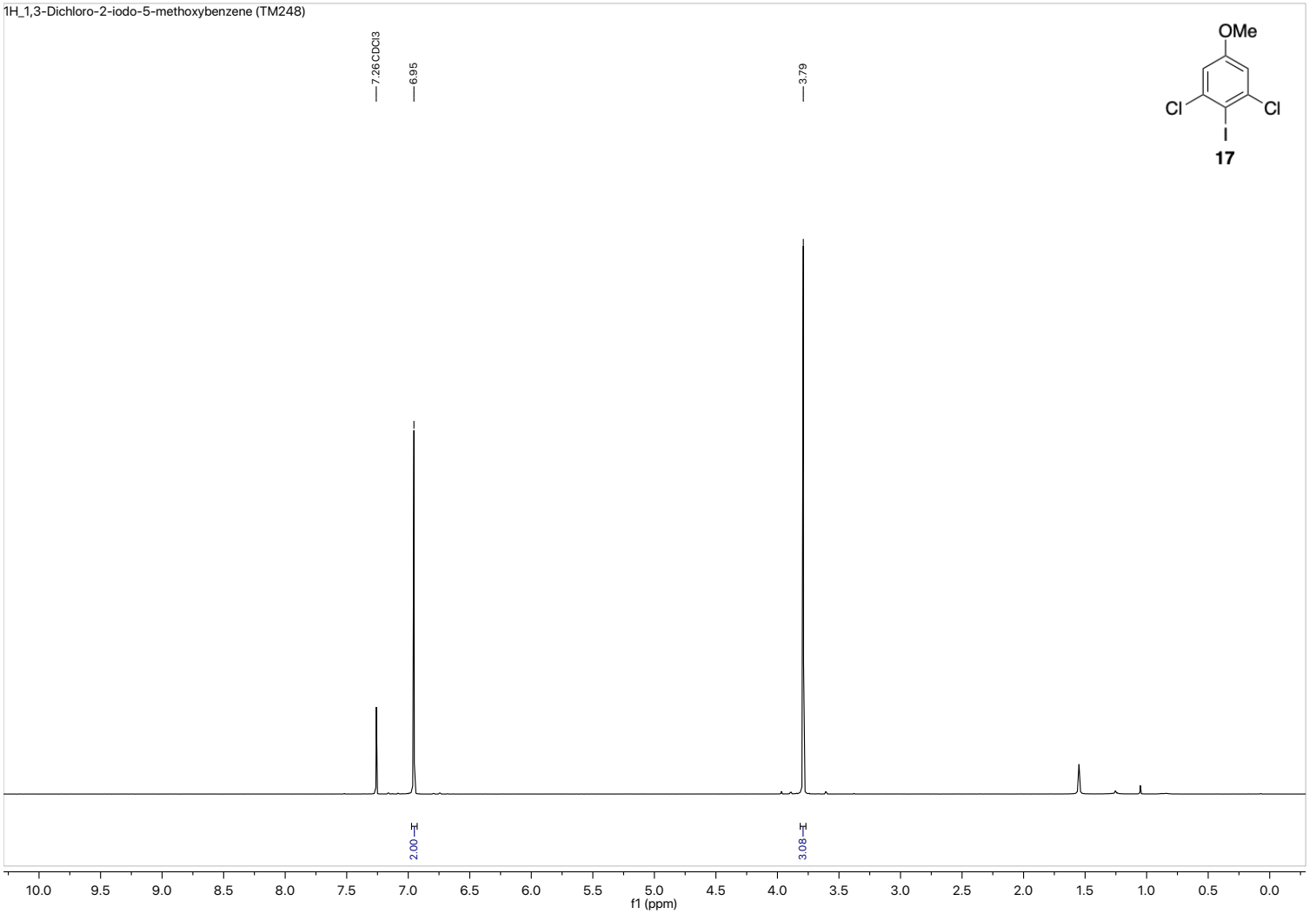


Fig. 27 ^{1}H-NMR spectrum of 1,3-dichloro-2-iodo-5-methoxybenzene (**17**).

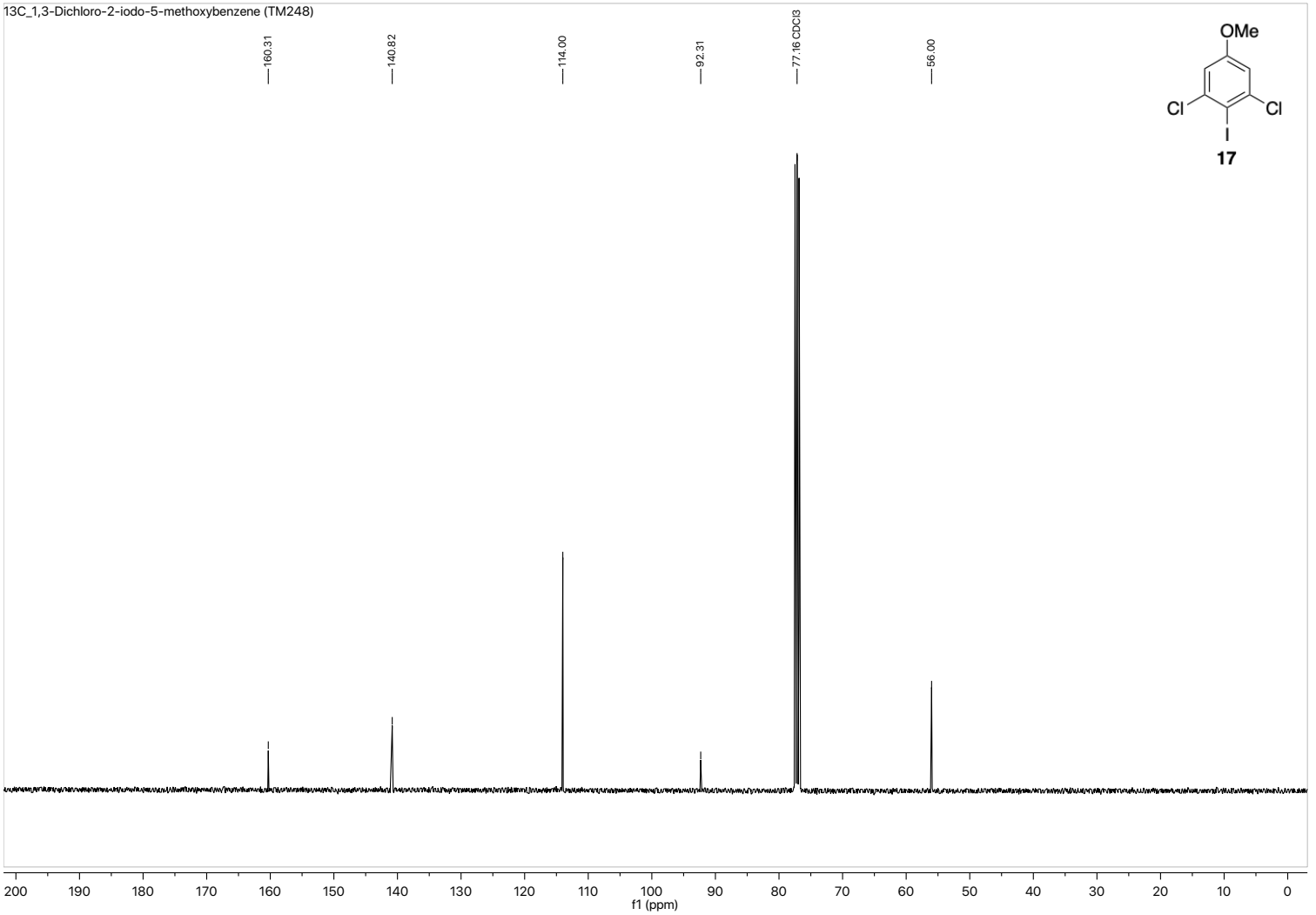


Fig. S28 ^{13}C-NMR spectrum of 1,3-dichloro-2-iodo-5-methoxybenzene (**17**).

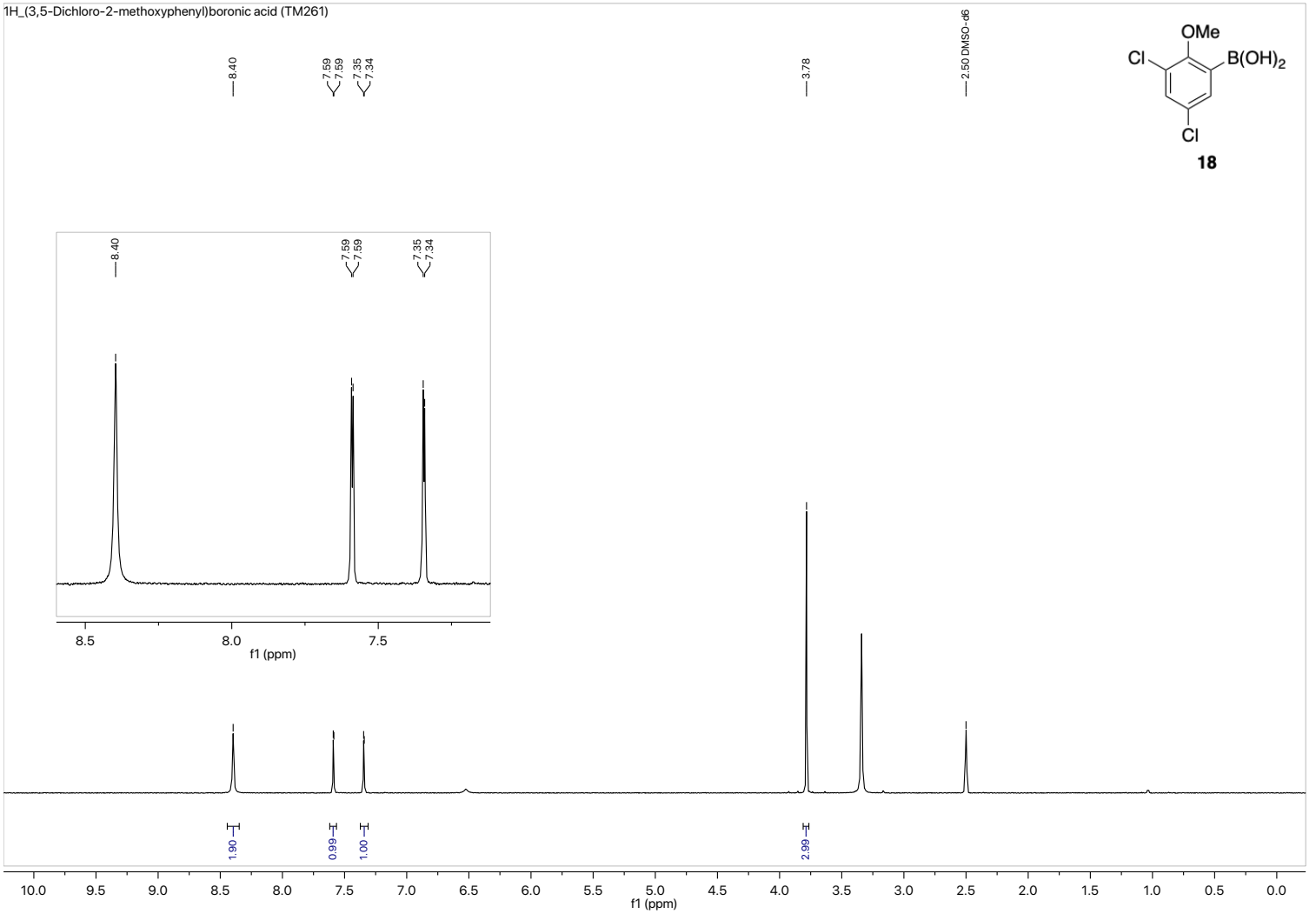


Fig. S29 ^{1}H-NMR spectrum of (3,5-dichloro-2-methoxyphenyl)boronic acid (**18**).

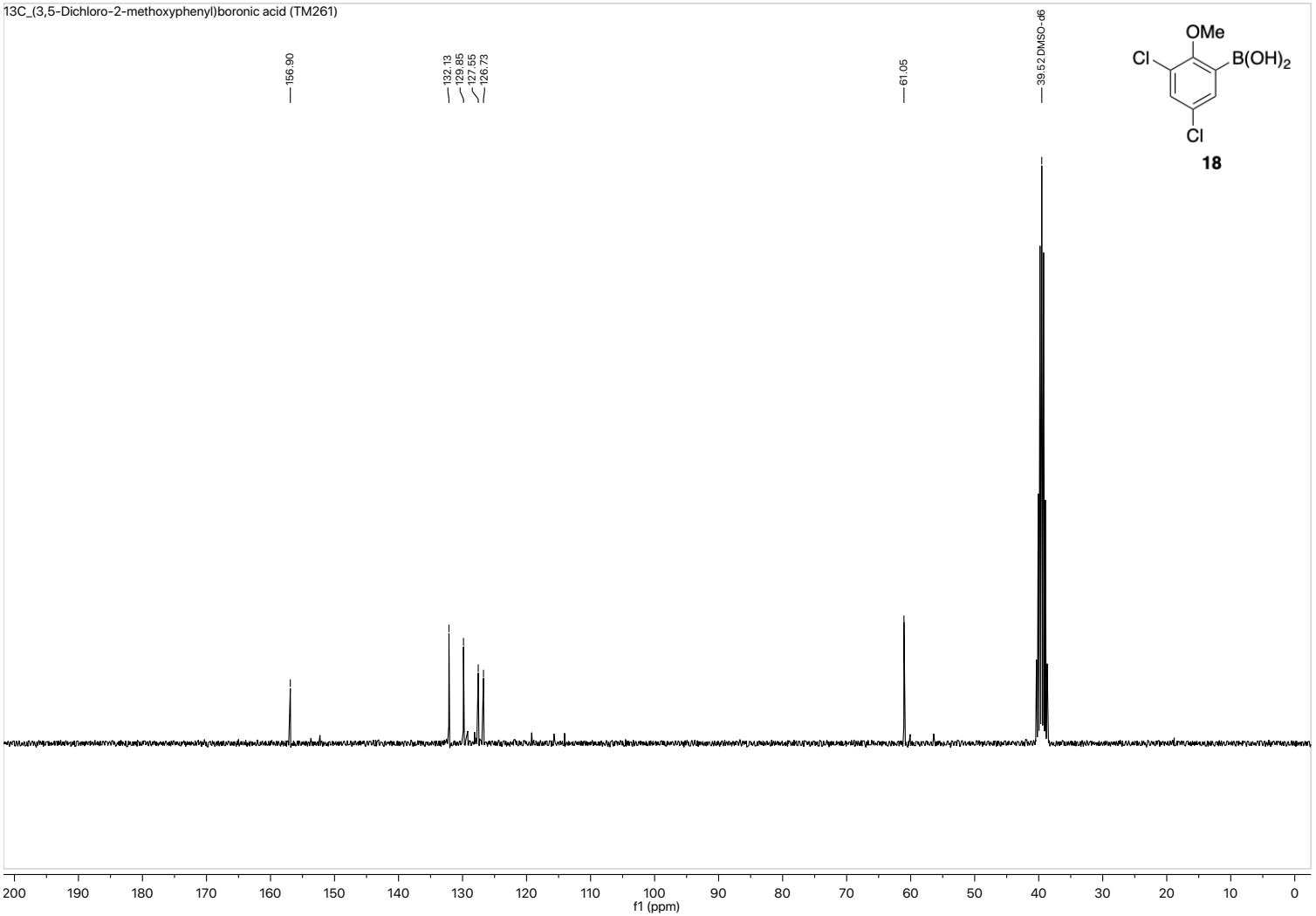


Fig. S30 ^{13}C-NMR spectrum of (3,5-dichloro-2-methoxyphenyl)boronic acid (**18**).

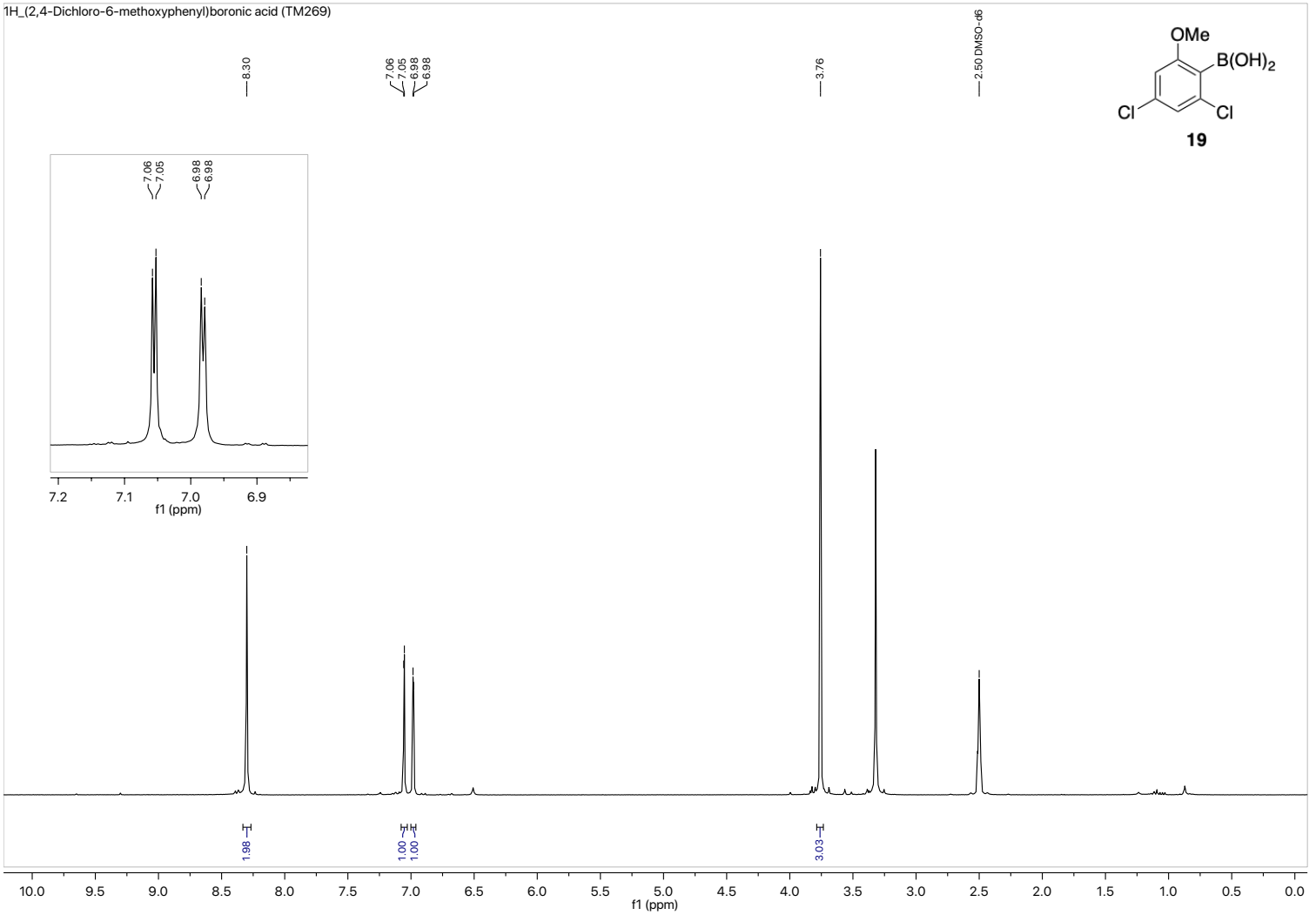


Fig. S31 ^{1}H-NMR spectrum of (2,4-dichloro-6-methoxyphenyl)boronic acid (**19**).

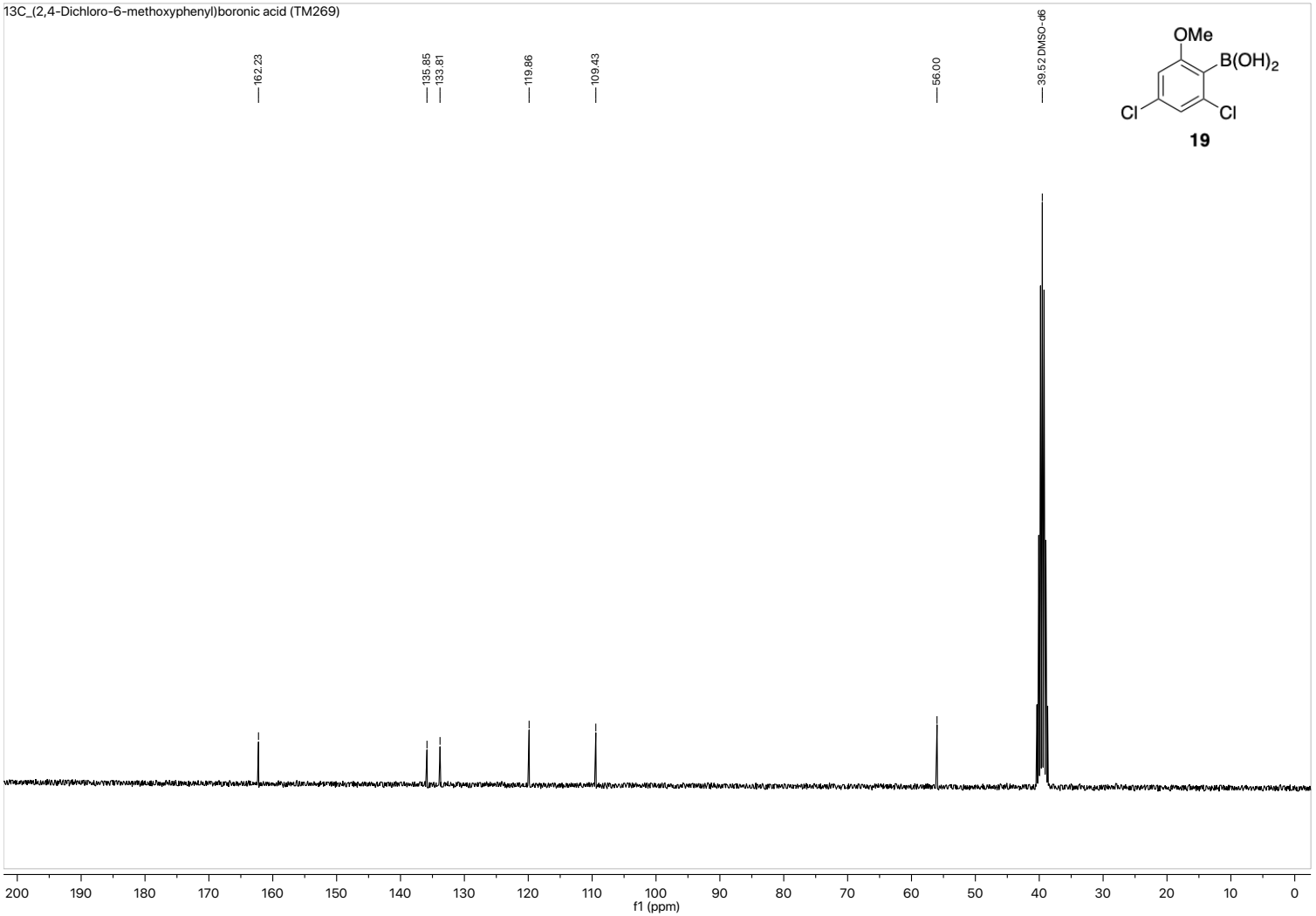


Fig. S32 ^{13}C-NMR spectrum of (2,4-dichloro-6-methoxyphenyl)boronic acid (**19**).

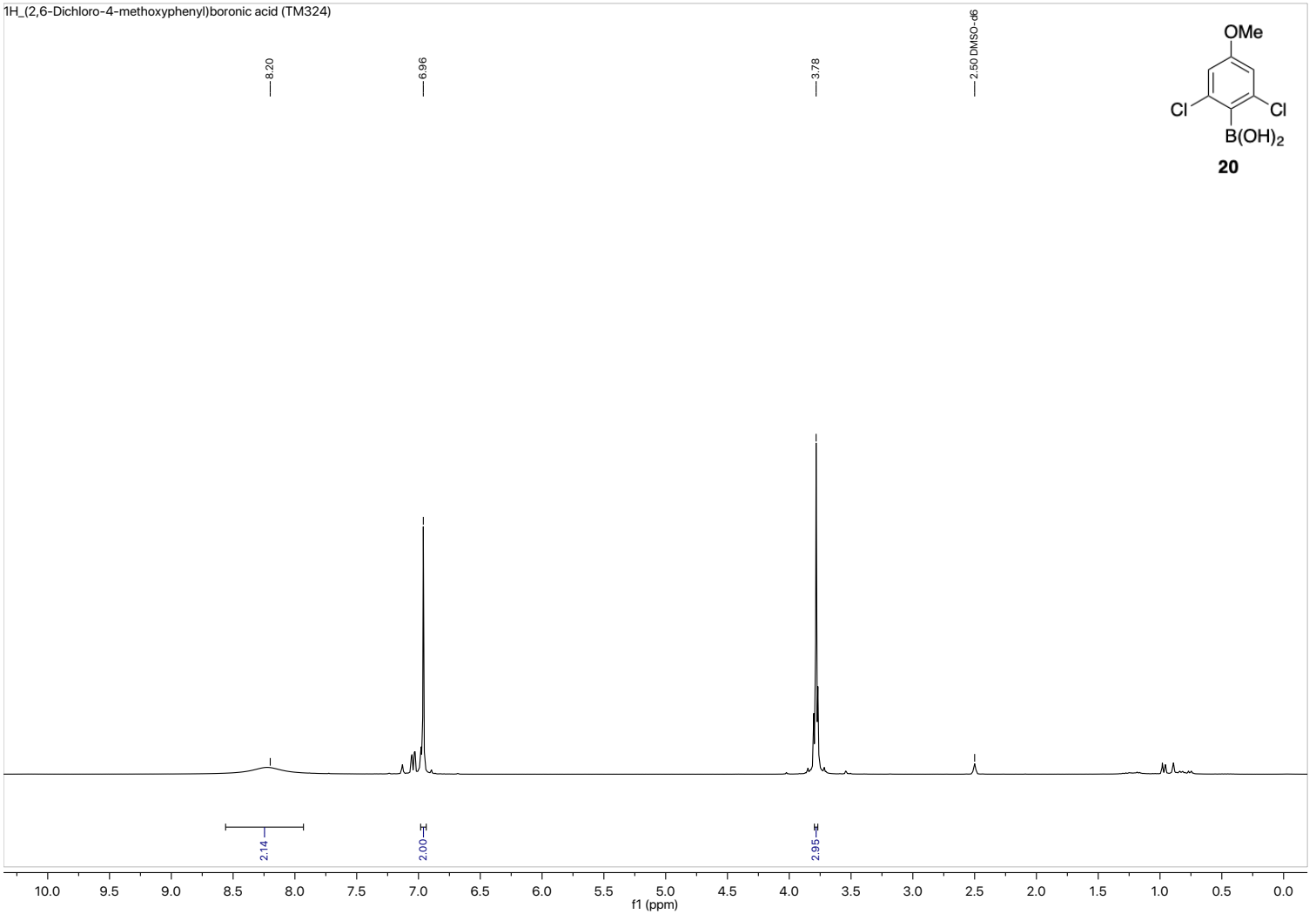


Fig. S33 ^{1}H-NMR spectrum of (2,6-dichloro-4-methoxyphenyl)boronic acid (**20**).

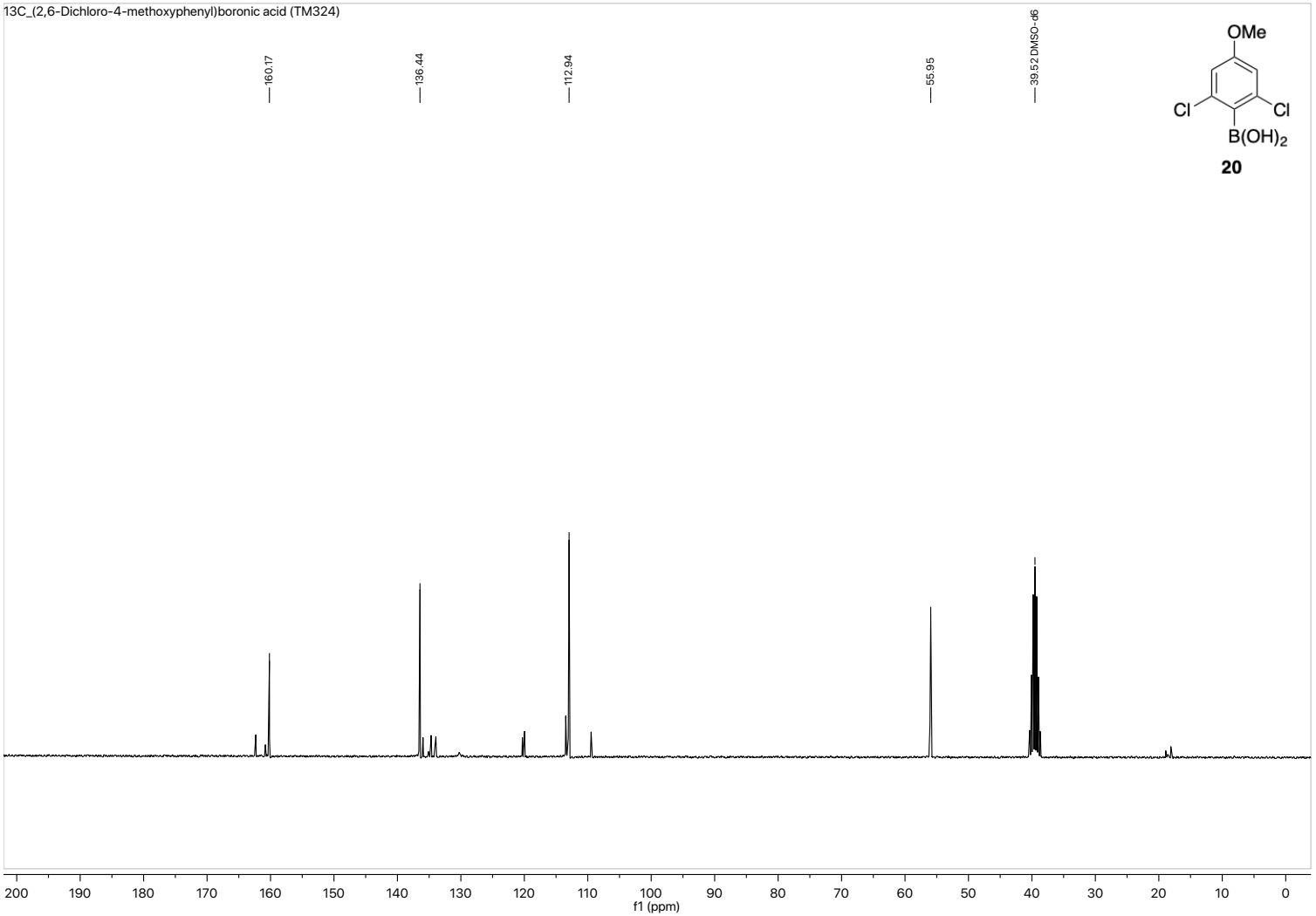


Fig. S34 ^{13}C-NMR spectrum of (2,6-dichloro-4-methoxyphenyl)boronic acid (**20**).

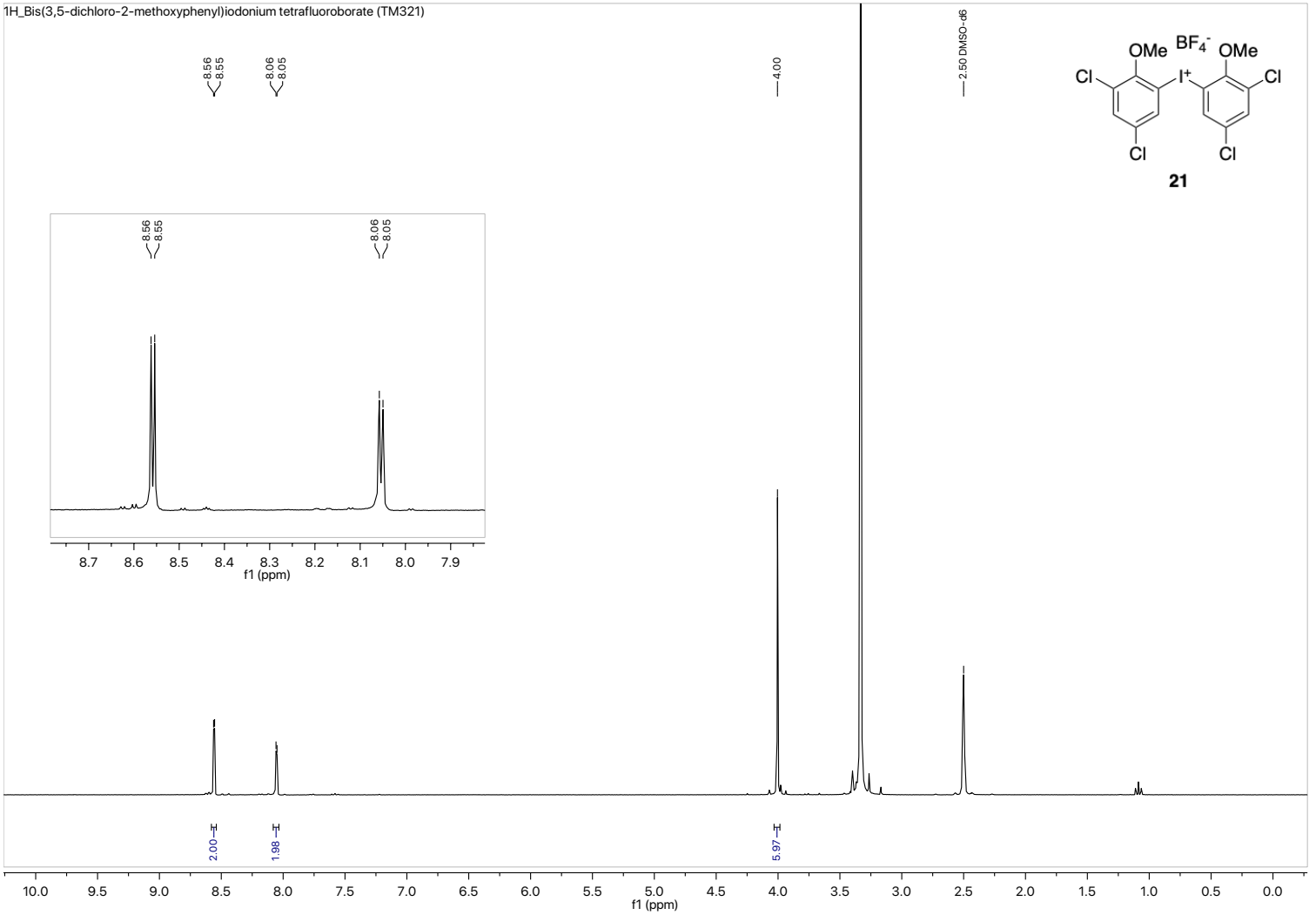


Fig. S35 ^{1}H-NMR spectrum of bis(3,5-dichloro-2-methoxyphenyl)iodonium tetrafluoroborate (**21**).

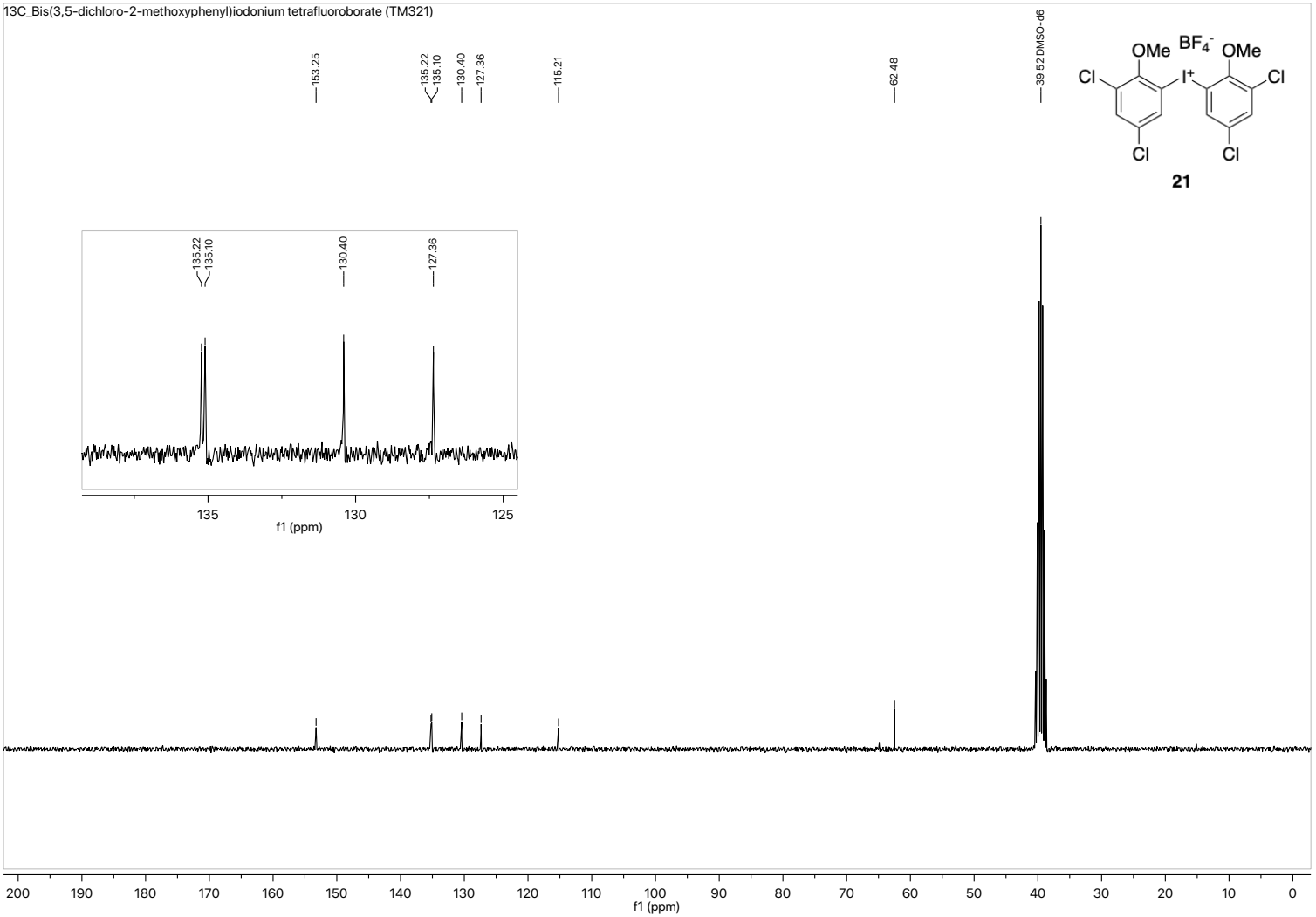


Fig. S36 ^{13}C-NMR spectrum of bis(3,5-dichloro-2-methoxyphenyl)iodonium tetrafluoroborate (**21**).

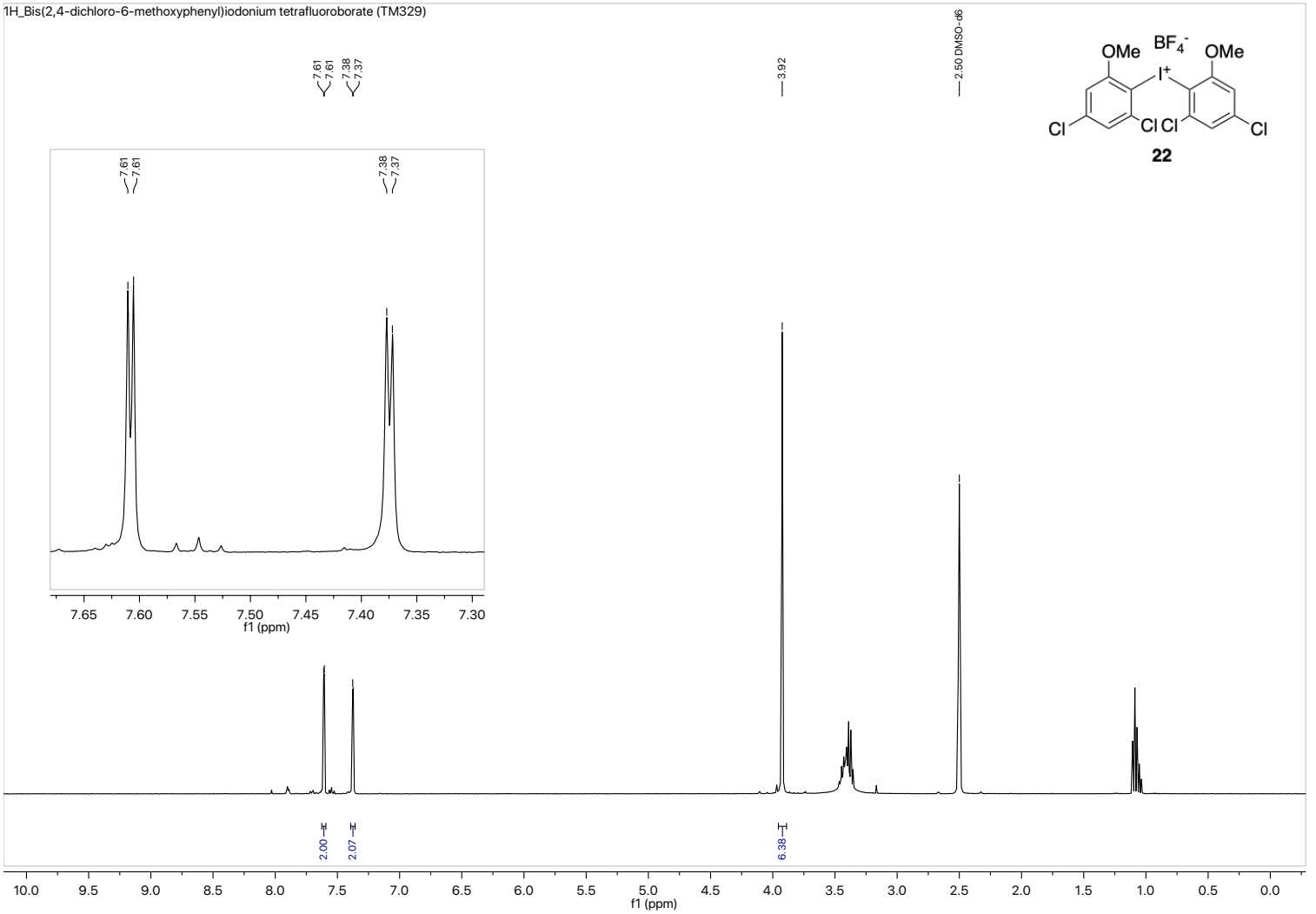


Fig. S37 ^{1}H-NMR spectrum of bis(2,4-dichloro-6-methoxyphenyl)iodonium tetrafluoroborate (**22**).

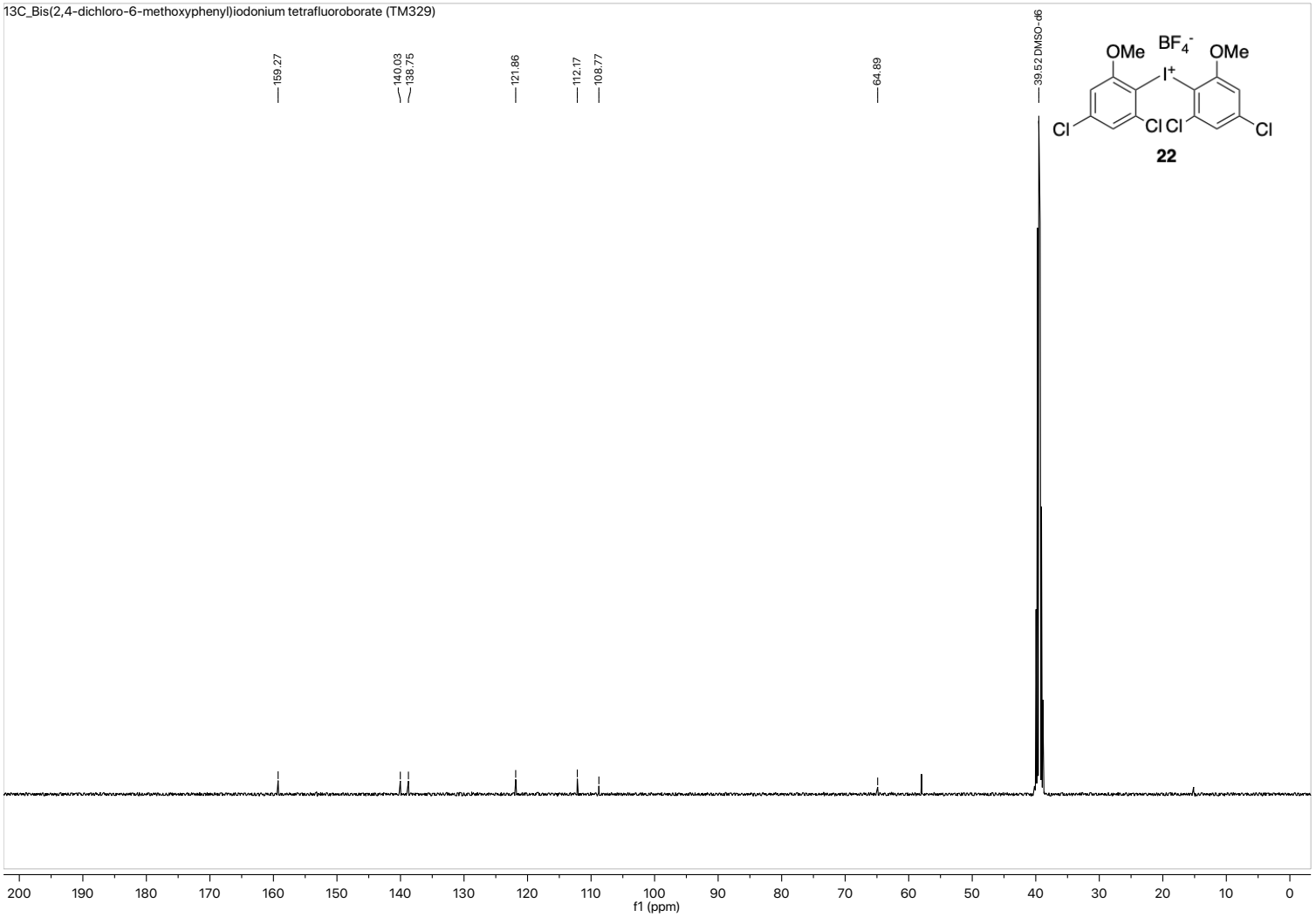


Fig. S38 ^{13}C-NMR spectrum of bis(2,4-dichloro-6-methoxyphenyl)iodonium tetrafluoroborate (**22**).

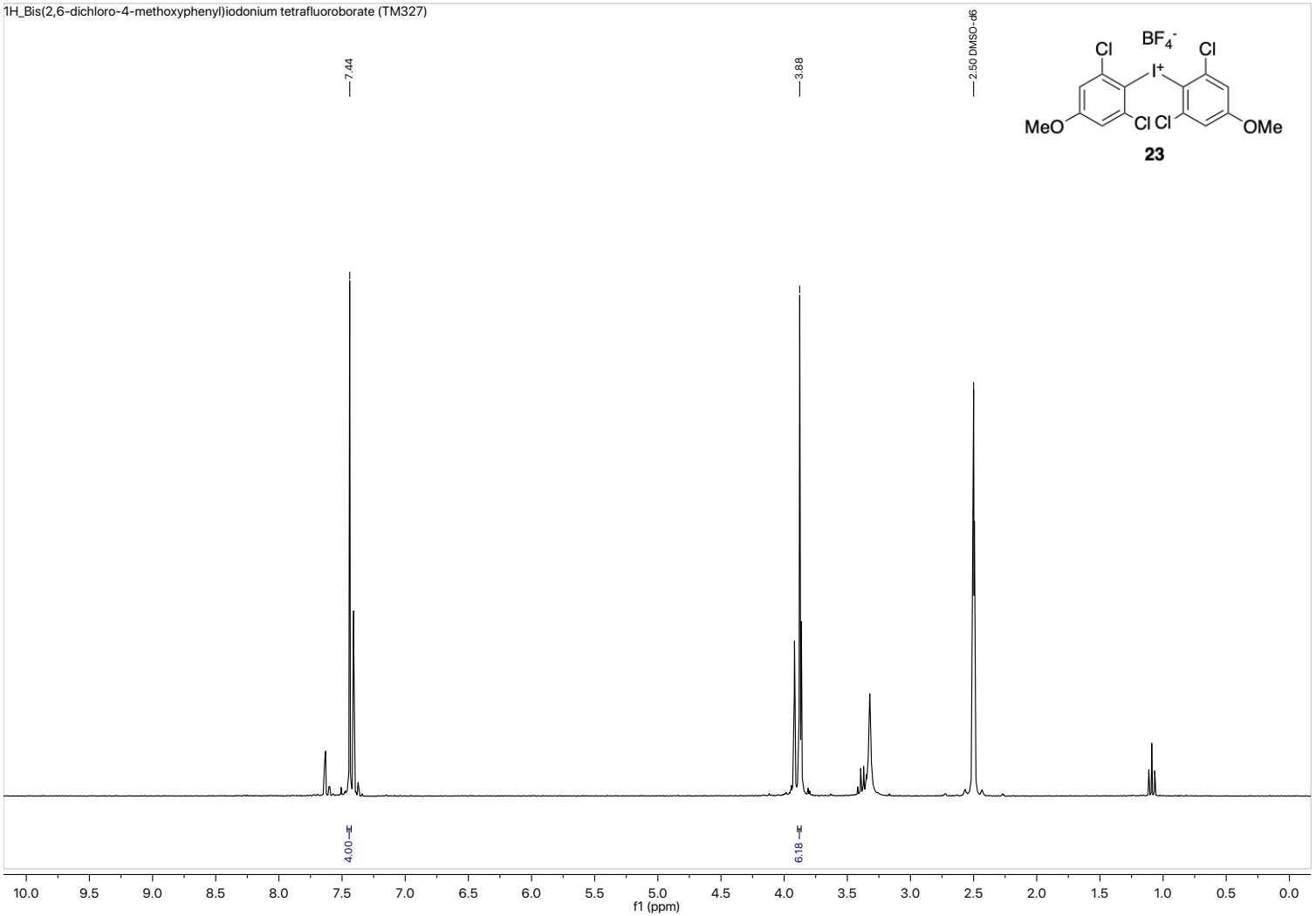


Fig. S39 ^{1}H-NMR spectrum of bis(2,6-dichloro-4-methoxyphenyl)iodonium tetrafluoroborate (**23**).

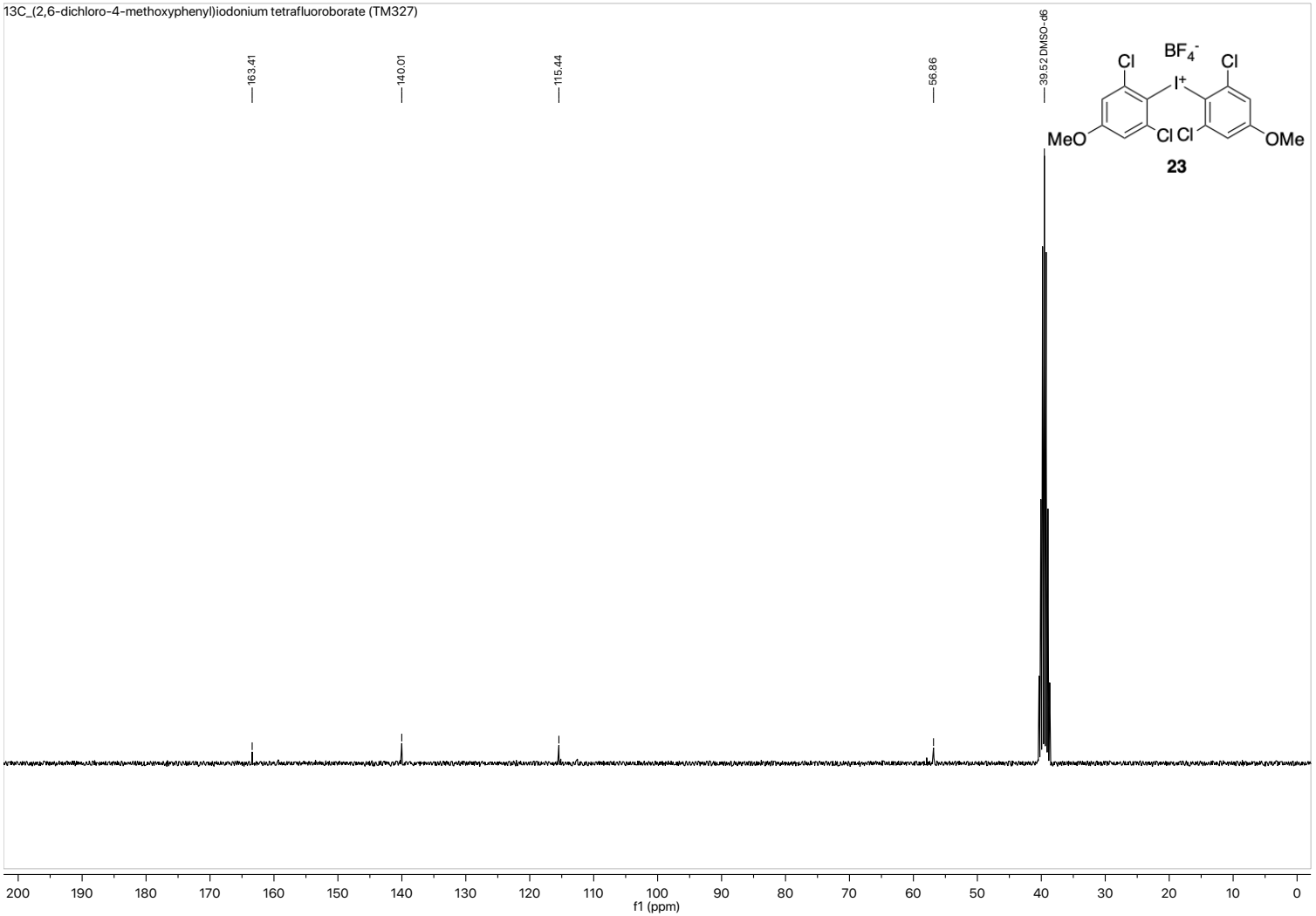


Fig. S40 ^{13}C-NMR spectrum of bis(2,6-dichloro-4-methoxyphenyl)iodonium tetrafluoroborate (**123**).

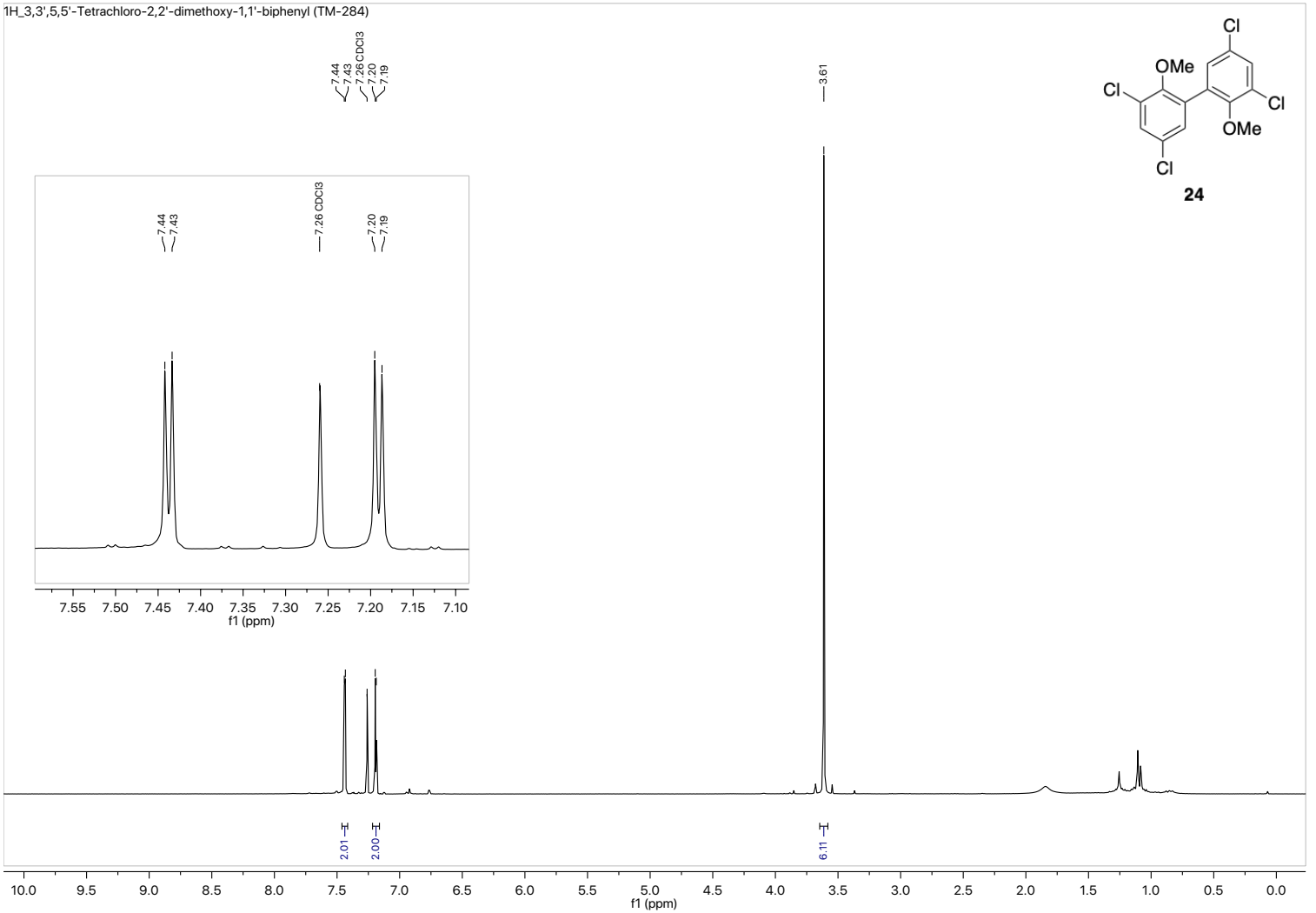


Fig. S41 ^{1}H-NMR spectrum of 3,3',5,5'-tetrachloro-2,2'-dimethoxy-1,1'-biphenyl (**24**).

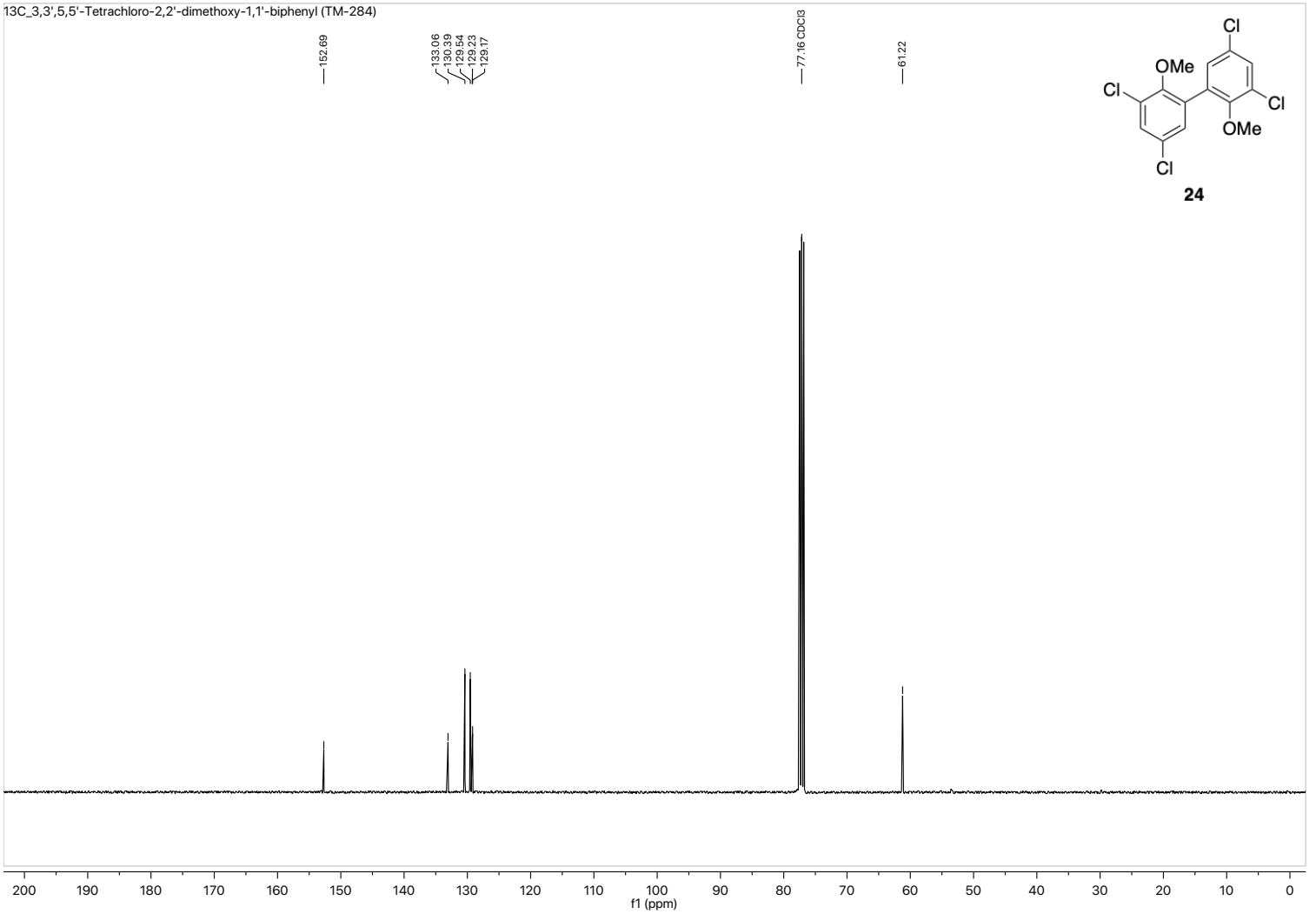


Fig. S42 ^{13}C-NMR spectrum of 3,3',5,5'-tetrachloro-2,2'-dimethoxy-1,1'-biphenyl (**24**).

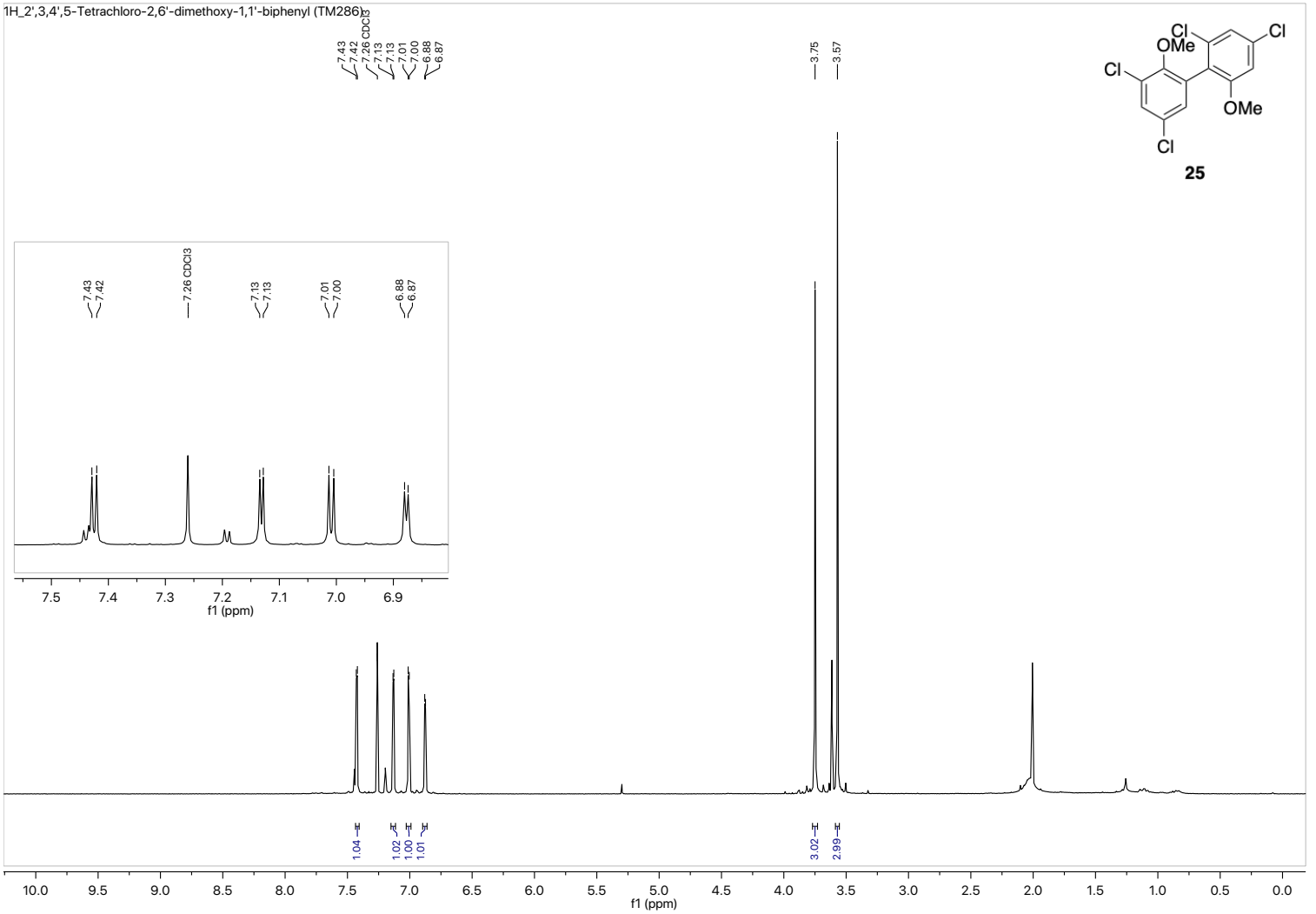


Fig. S43 ^{1}H-NMR spectrum of 2',3,4',5-tetrachloro-2,6'-dimethoxy-1,1'-biphenyl (**25**).

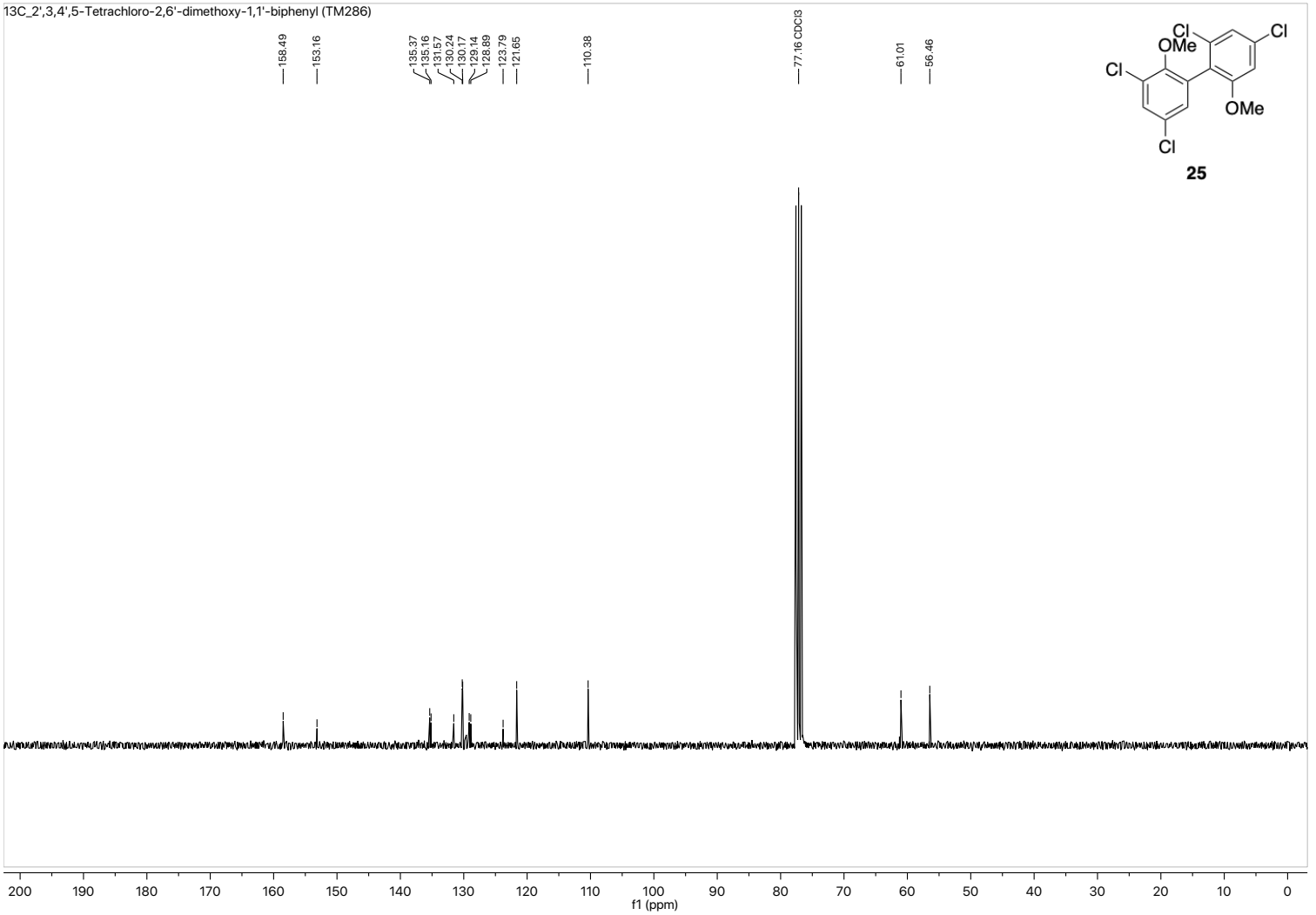


Fig. S44 ^{13}C-NMR spectrum of 2',3,4',5-tetrachloro-2,6'-dimethoxy-1,1'-biphenyl (**25**).

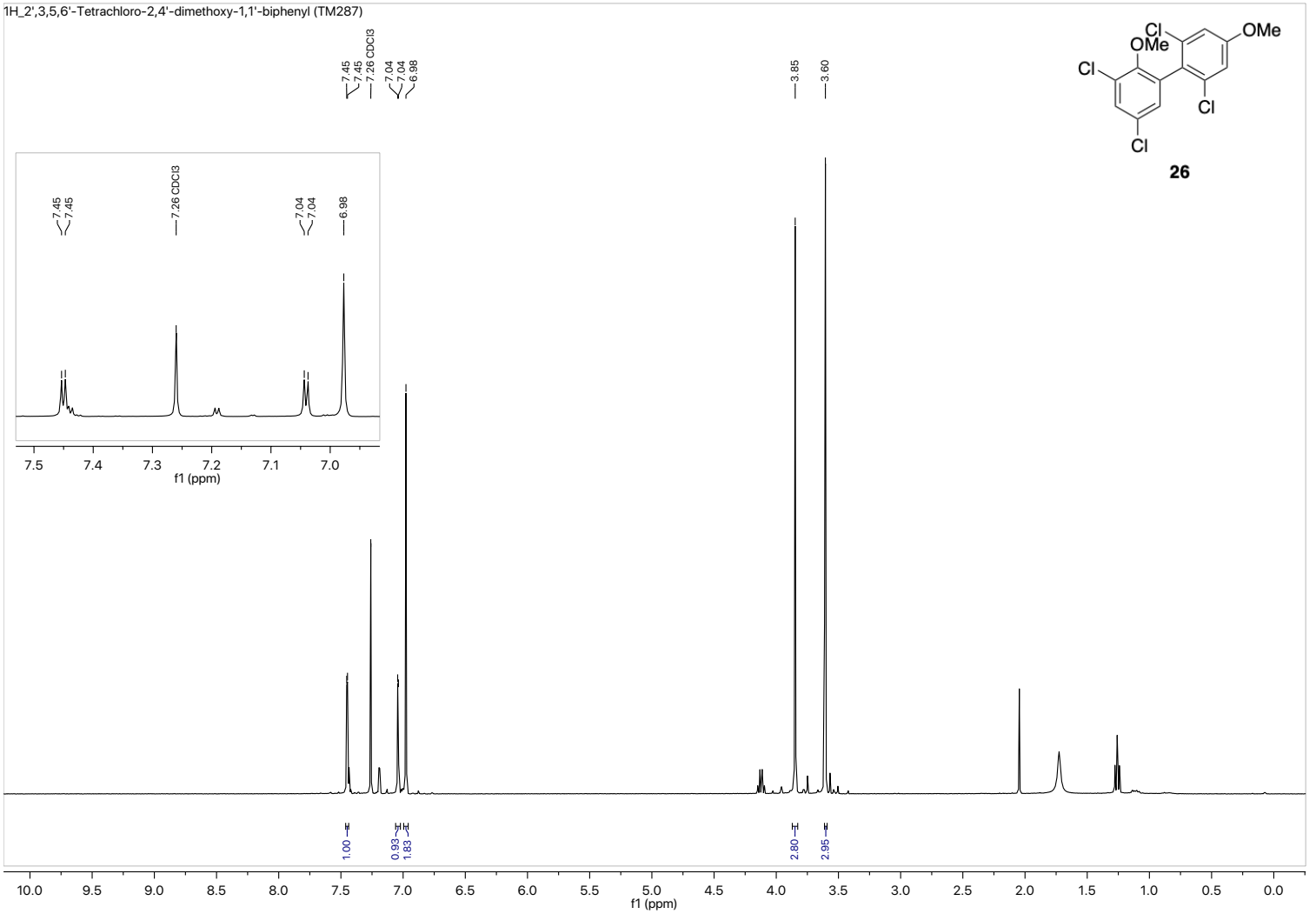


Fig. S45 ^{1}H-NMR spectrum of 2',3,5,6'-tetrachloro-2,4'-dimethoxy-1,1'-biphenyl (**26**).

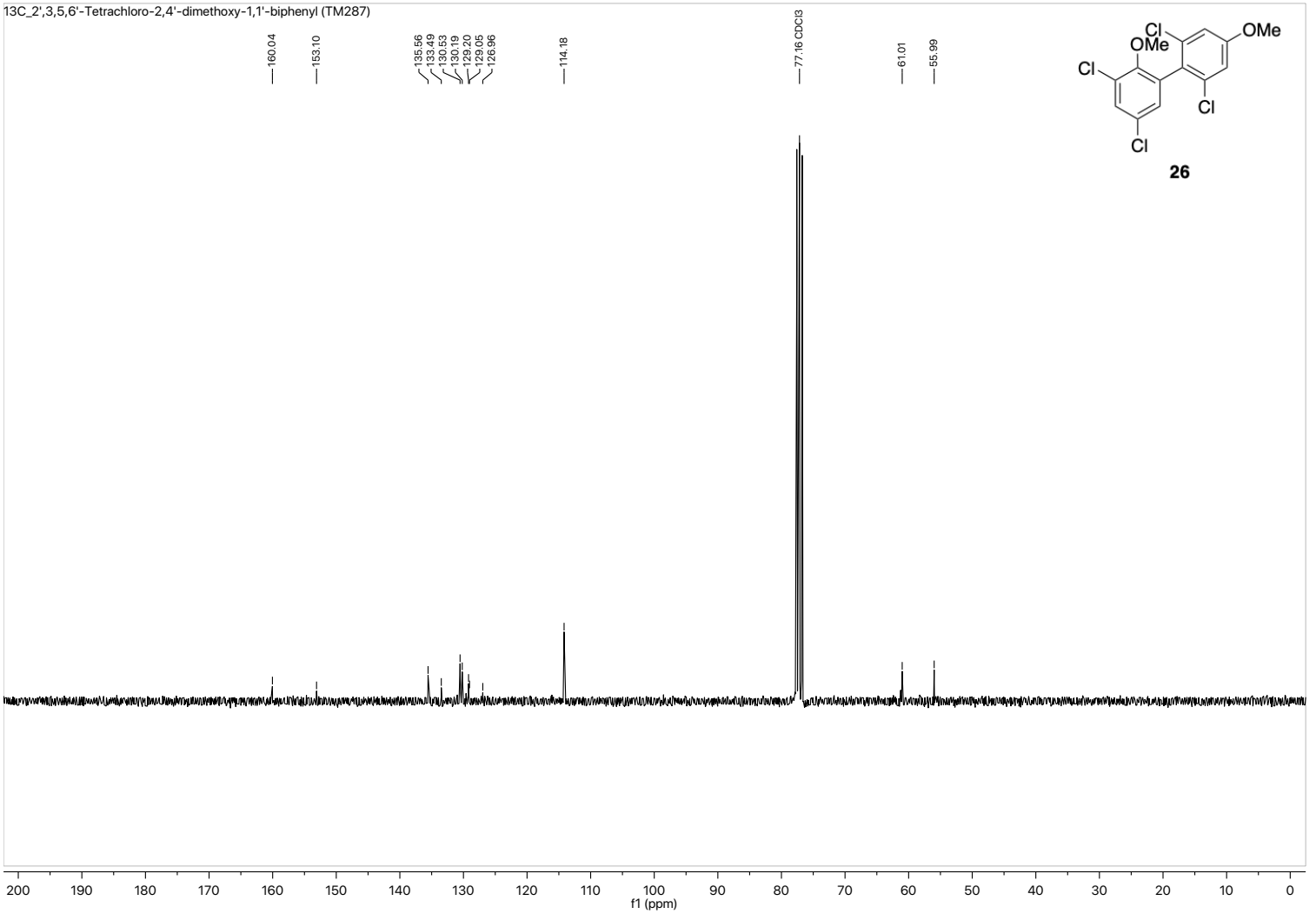


Fig. S46 ^{13}C-NMR spectrum of 2',3,5,6'-tetrachloro-2,4'-dimethoxy-1,1'-biphenyl (**26**).

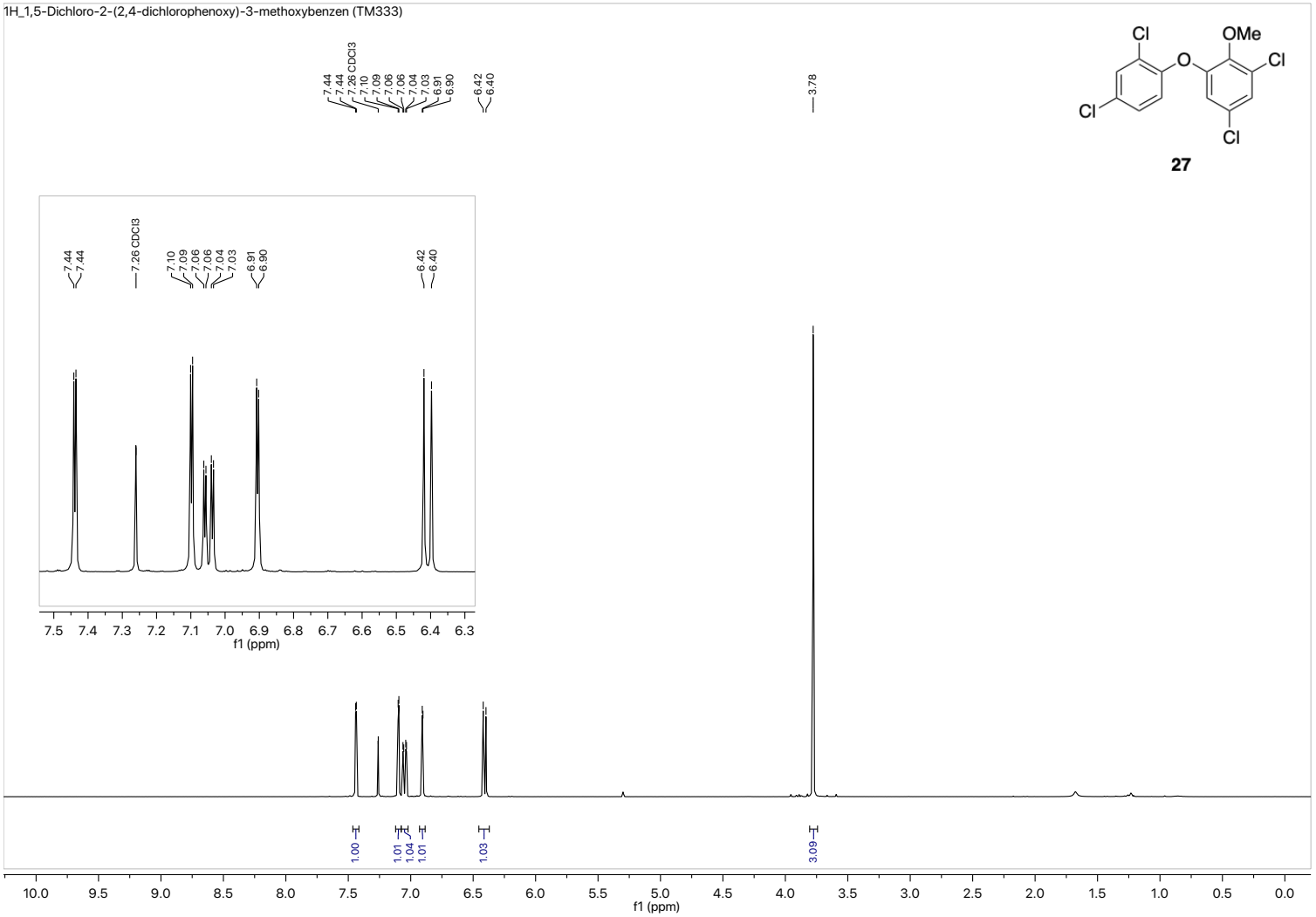


Fig. S47 ^{1}H-NMR spectrum of 1,5-dichloro-3-(2,4-dichlorophenoxy)-2-methoxybenzene (**27**).

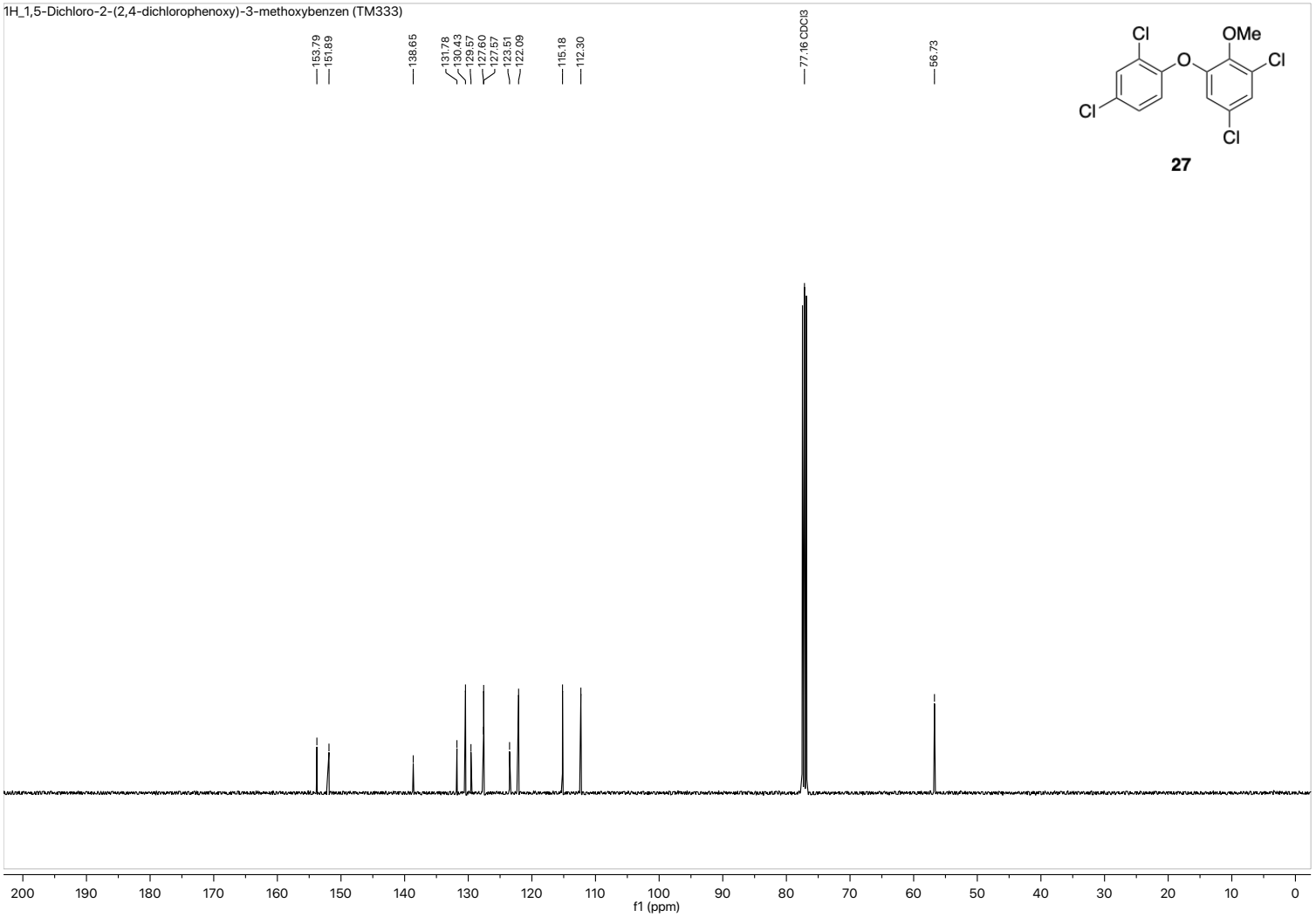


Fig. S48 ^{13}C-NMR spectrum of 1,5-dichloro-3-(2,4-dichlorophenoxy)-2-methoxybenzene (**27**).

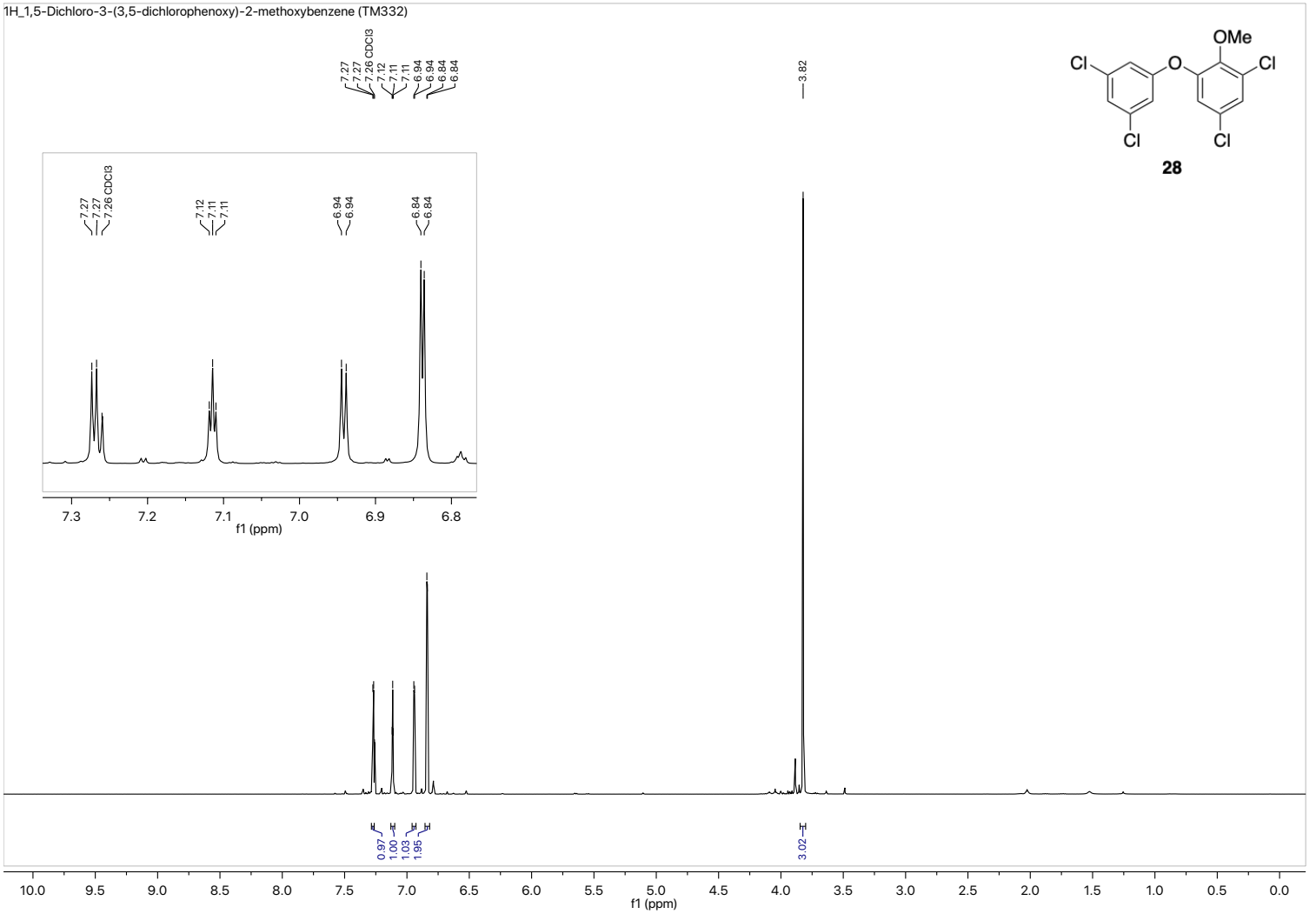


Fig. S49 ^{1}H-NMR spectrum of 1,5-dichloro-3-(3,5-dichlorophenoxy)-2-methoxybenzene (**28**).

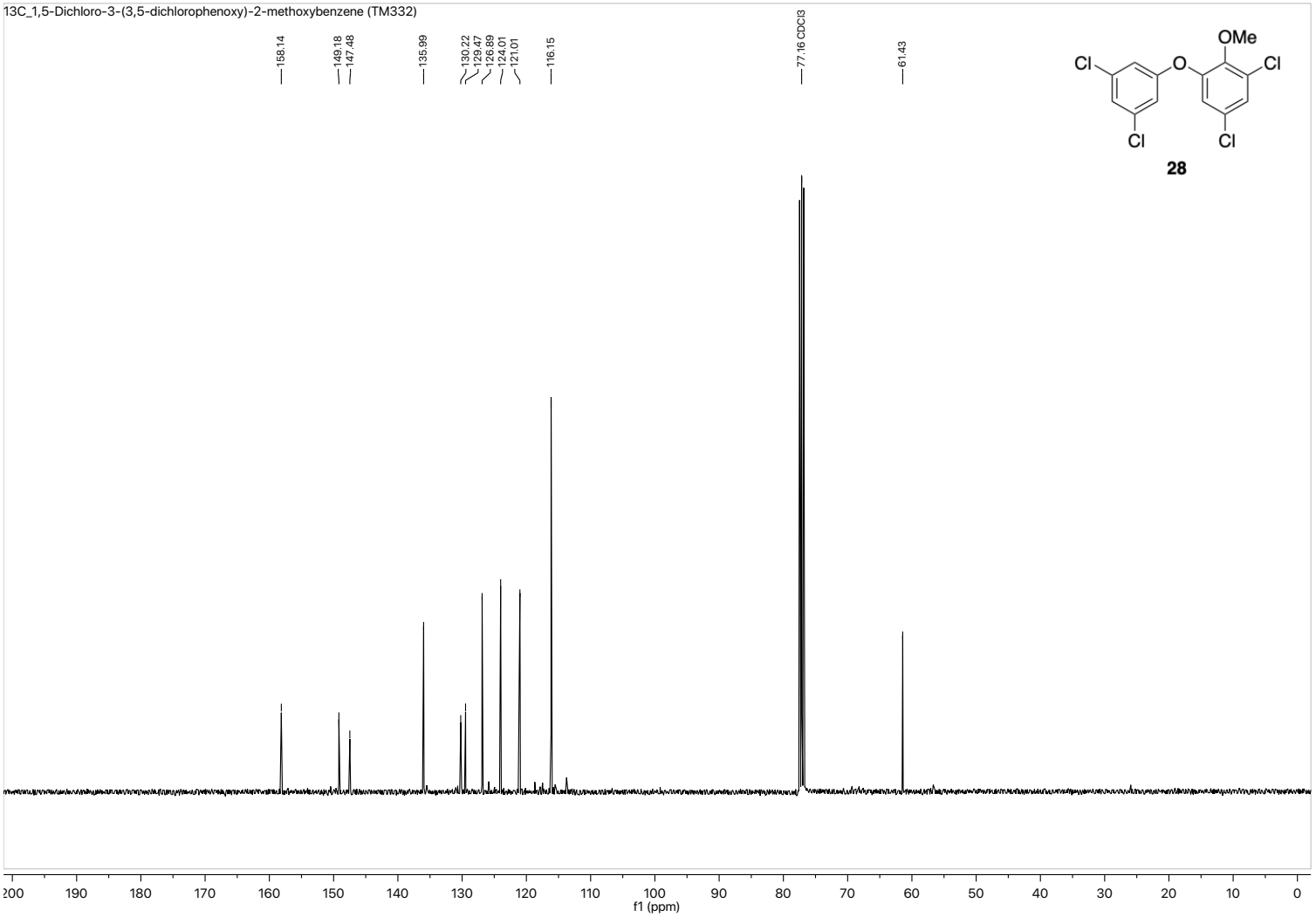


Fig. S50 ^{13}C-NMR spectrum of 1,5-dichloro-3-(3,5-dichlorophenoxy)-2-methoxybenzene (**28**).

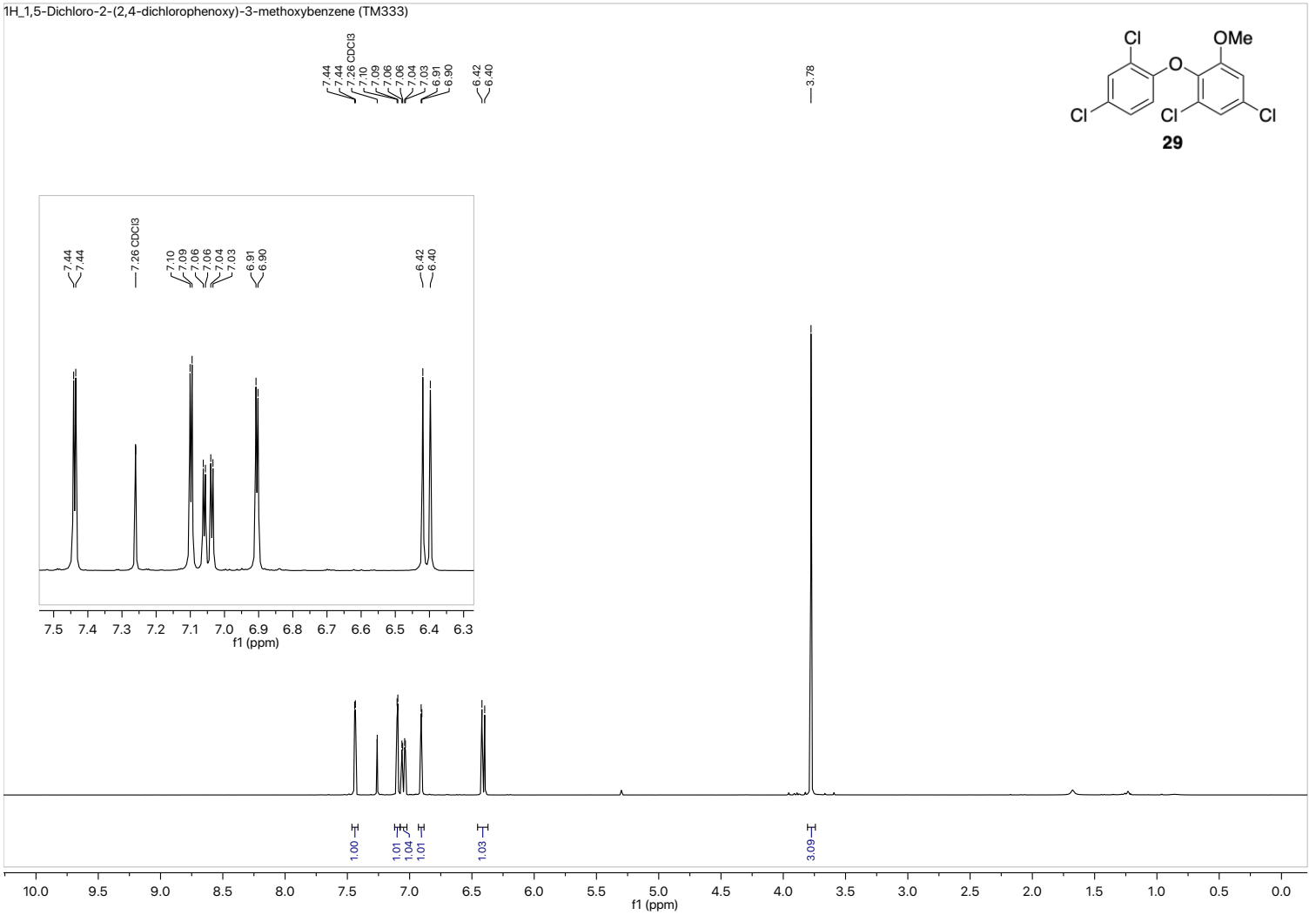


Fig. S51 ^{1}H-NMR spectrum of 1,5-dichloro-2-(2,4-dichlorophenoxy)-3-methoxybenzene (**29**).

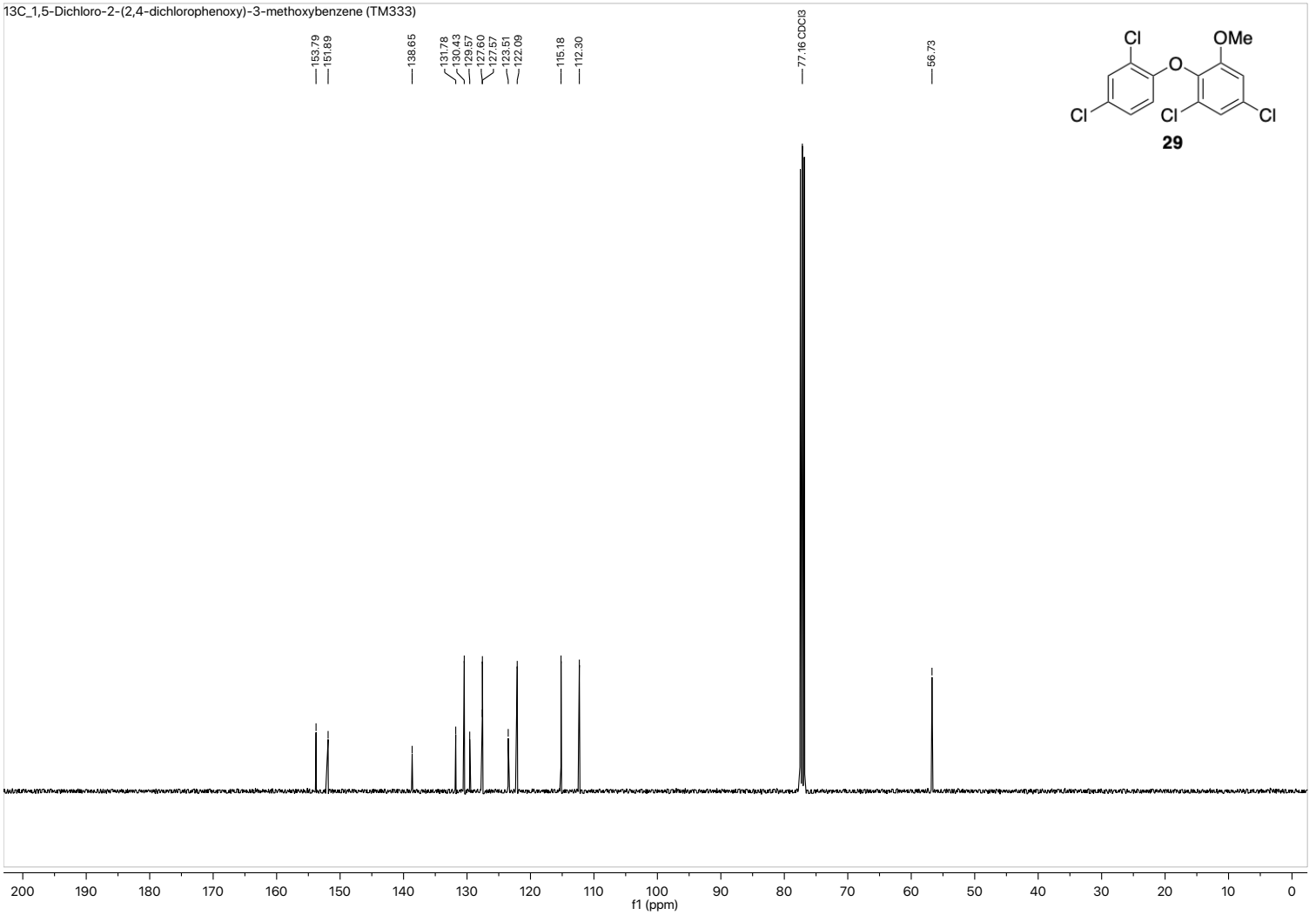


Fig. S52 ^{13}C-NMR spectrum of 1,5-dichloro-2-(2,4-dichlorophenoxy)-3-methoxybenzene (**29**).

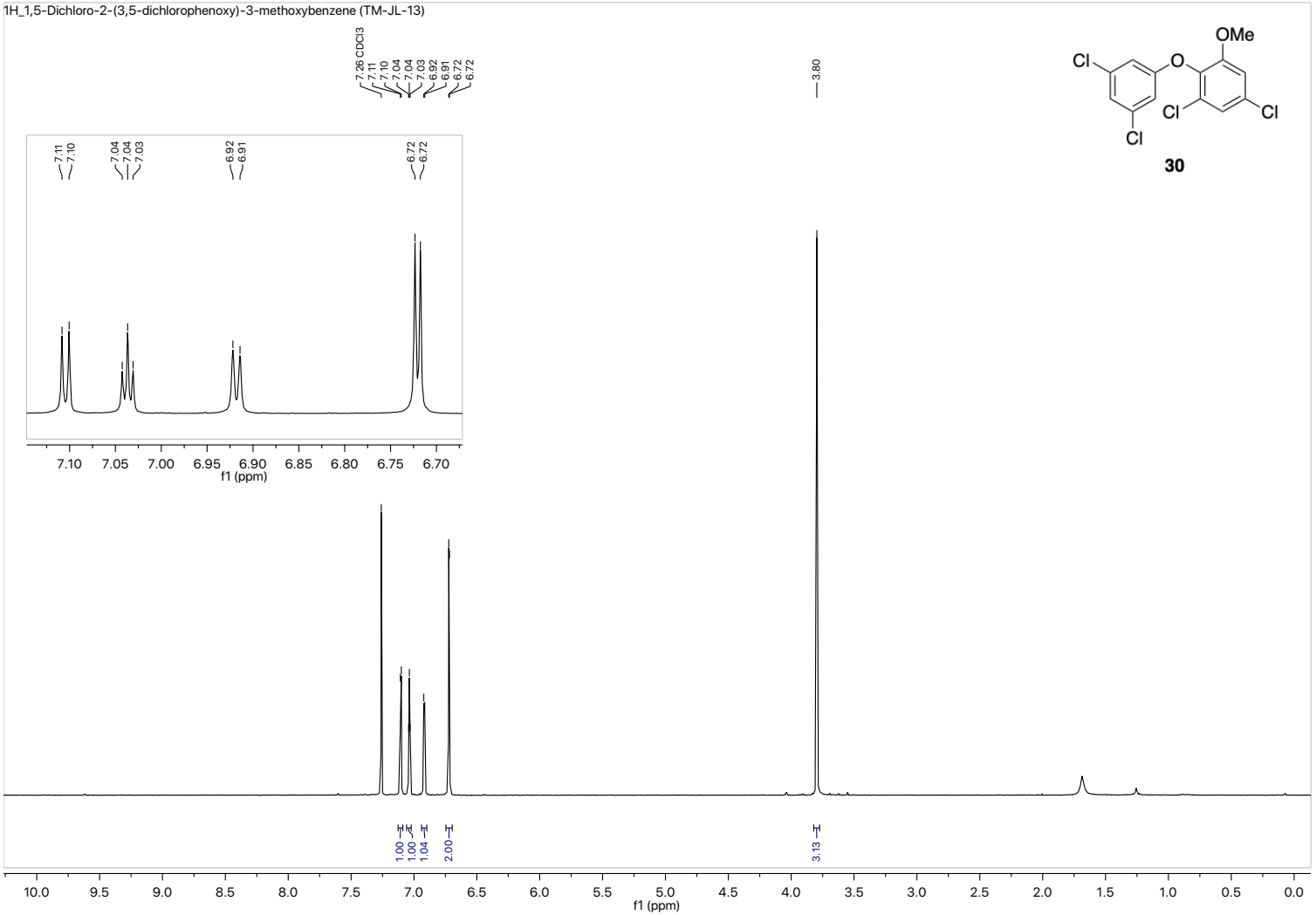


Fig. S53 ^{1}H-NMR spectrum of 1,5-dichloro-2-(3,5-dichlorophenoxy)-3-methoxybenzene (**30**).

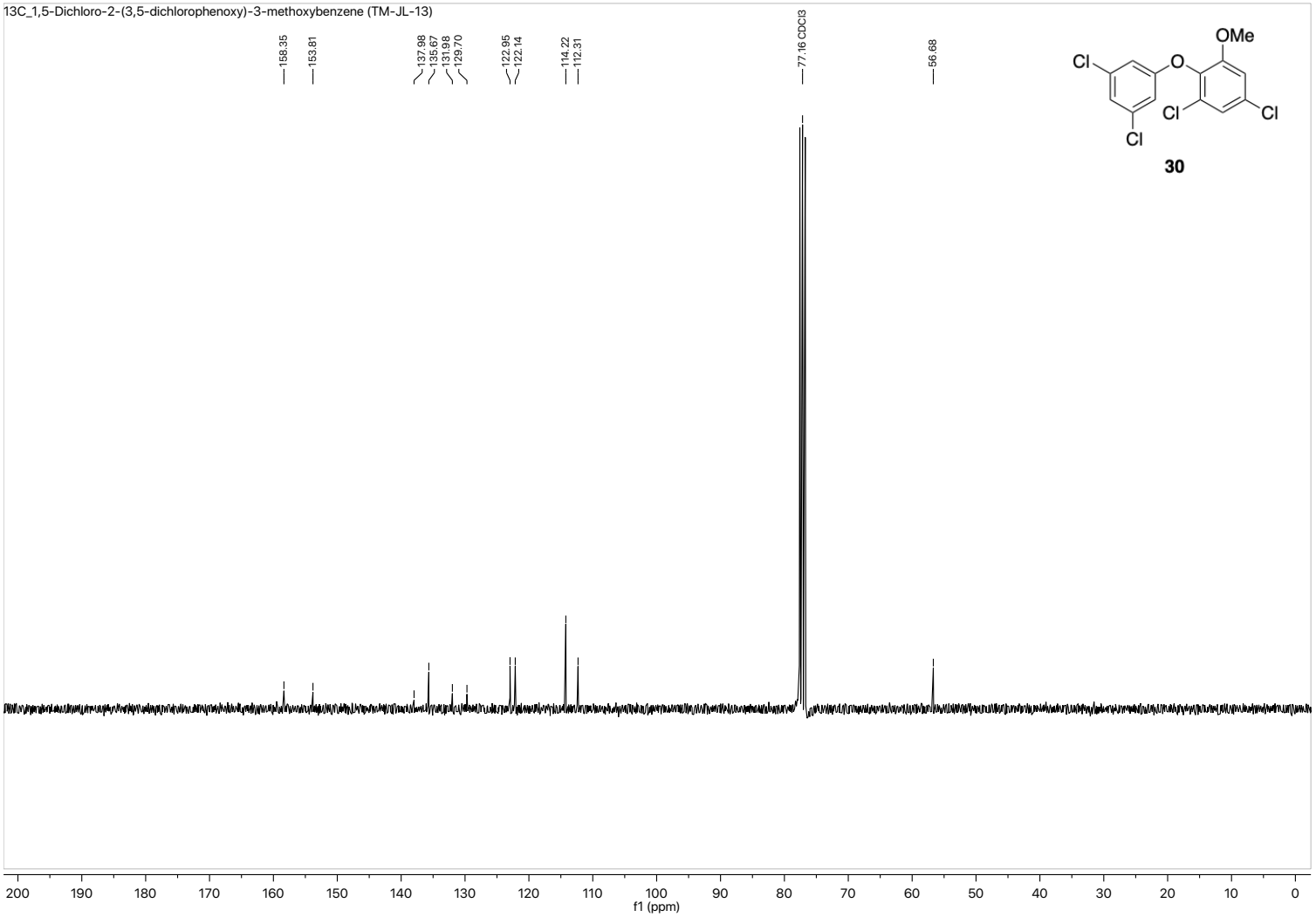


Fig. S54 ^{13}C-NMR spectrum of 1,5-dichloro-2-(3,5-dichlorophenoxy)-3-methoxybenzene (**30**).

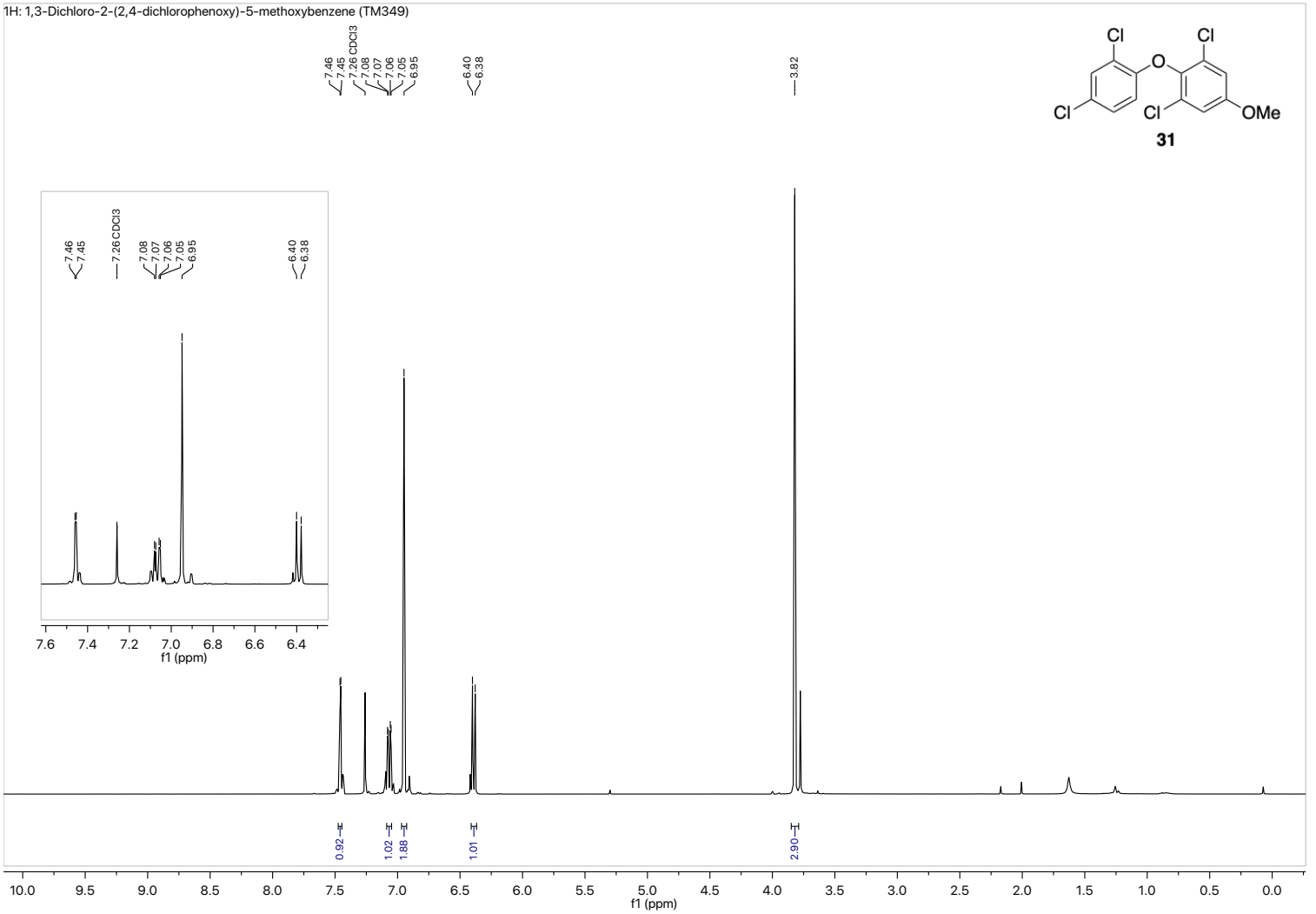


Fig. S55 ^{1}H-NMR spectrum of 1,3-dichloro-2-(2,4-dichlorophenoxy)-5-methoxybenzene (**31**).

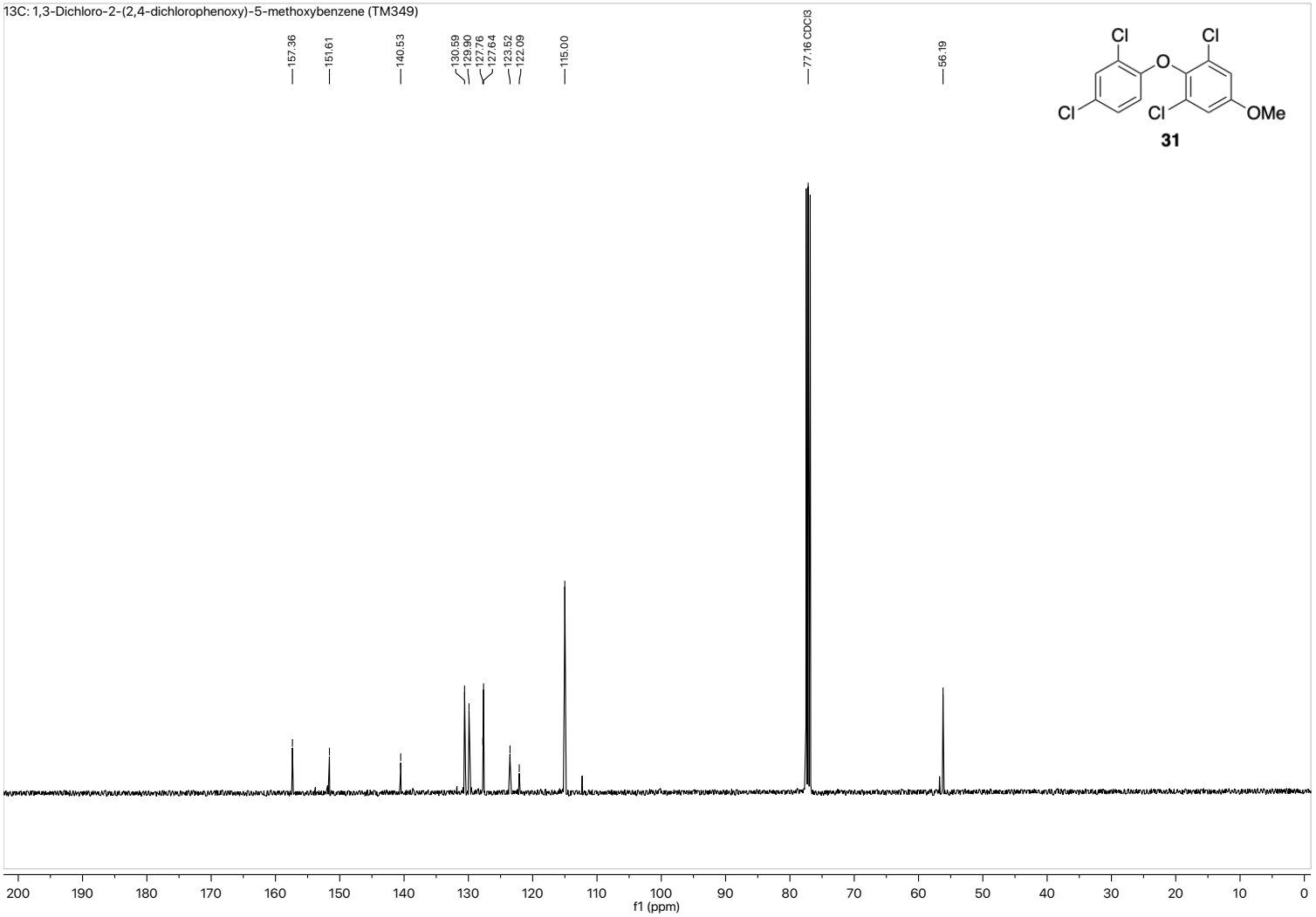


Fig. S56 ^{13}C-NMR spectrum of 1,3-dichloro-2-(2,4-dichlorophenoxy)-5-methoxybenzene (**31**).

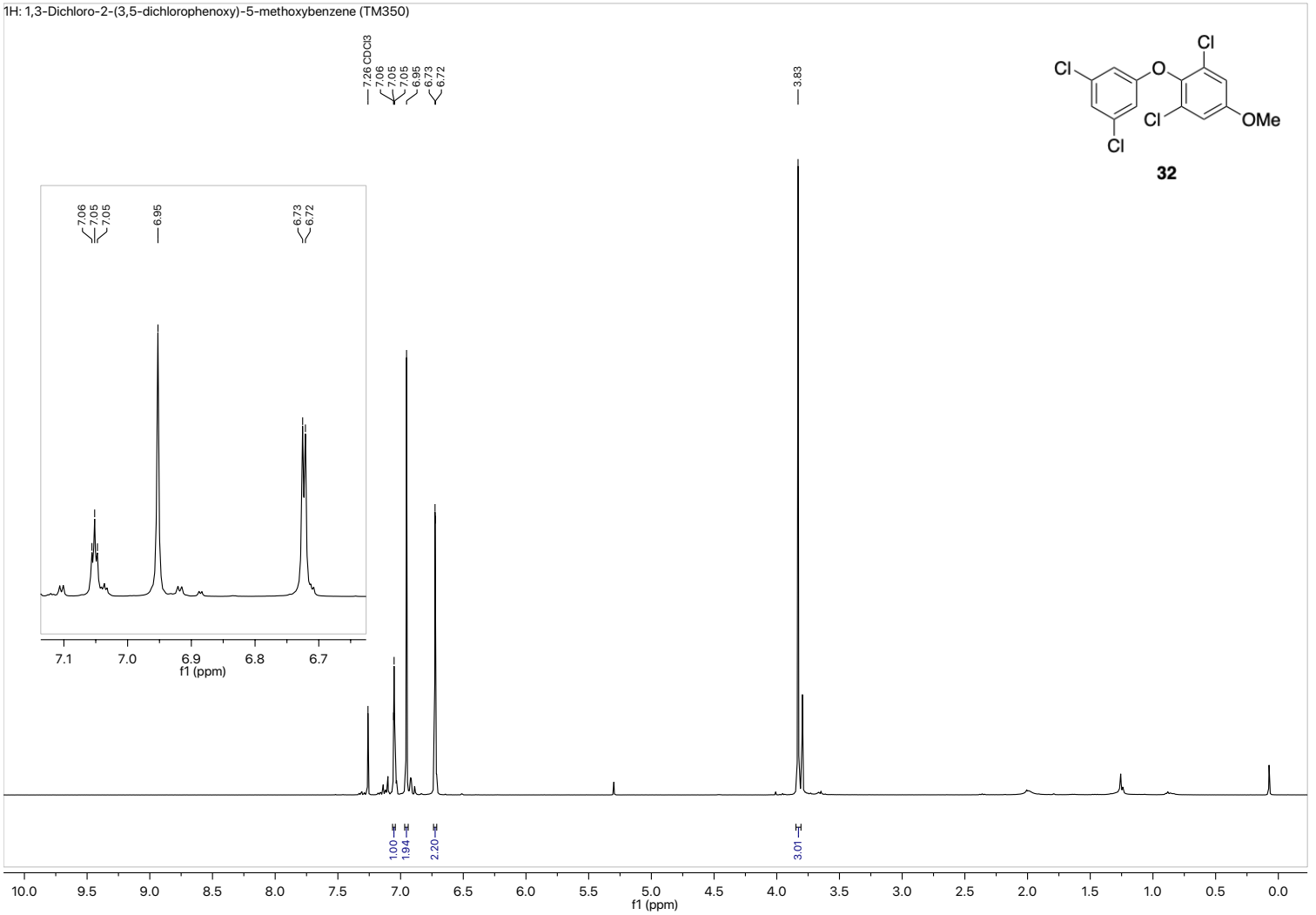


Fig. S57 ^{1}H-NMR spectrum of 1,3-dichloro-2-(3,5-dichlorophenoxy)-5-methoxybenzene (**32**).

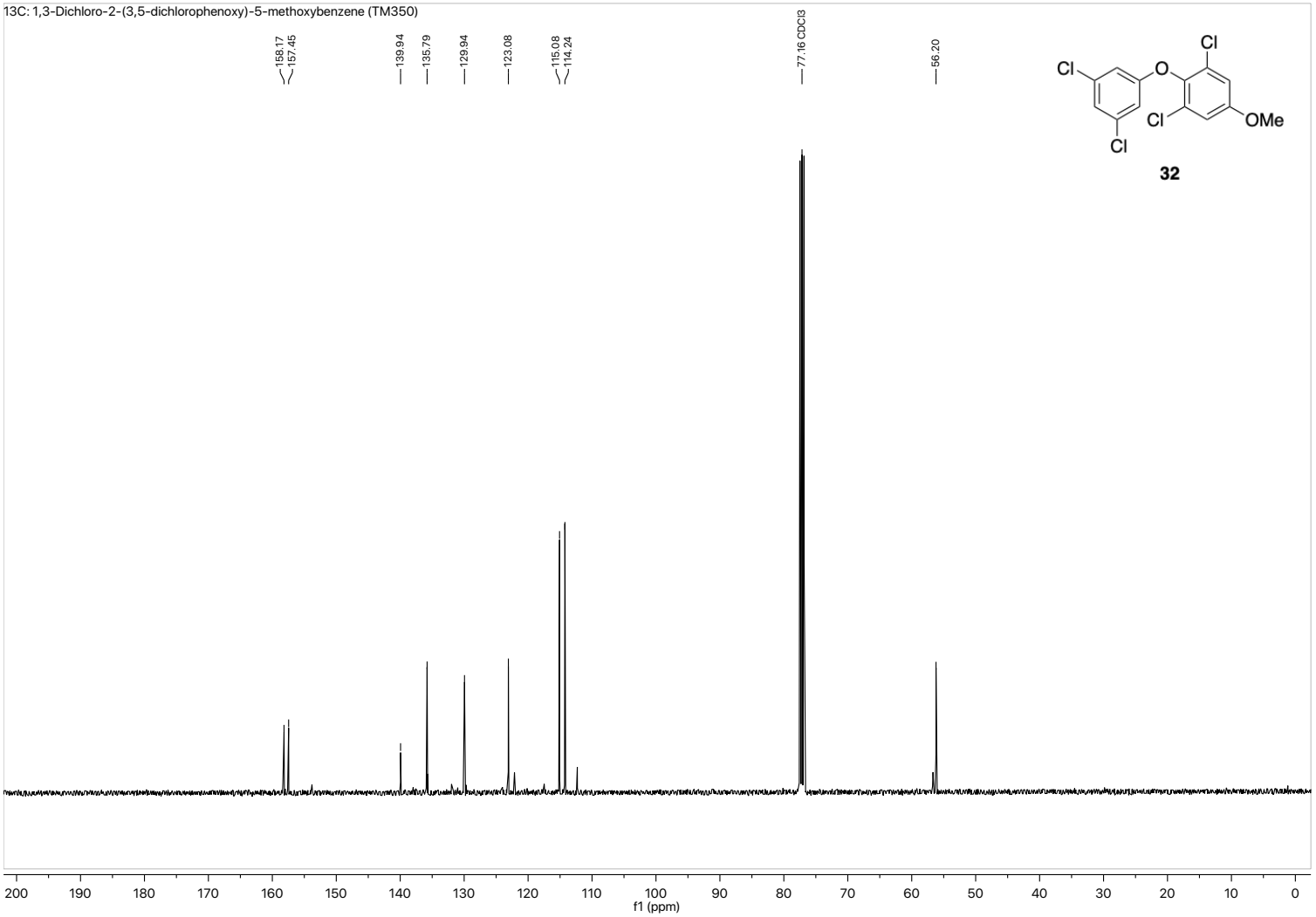


Fig. S58 ^{13}C-NMR spectrum of 1,3-dichloro-2-(3,5-dichlorophenoxy)-5-methoxybenzene (**32**).

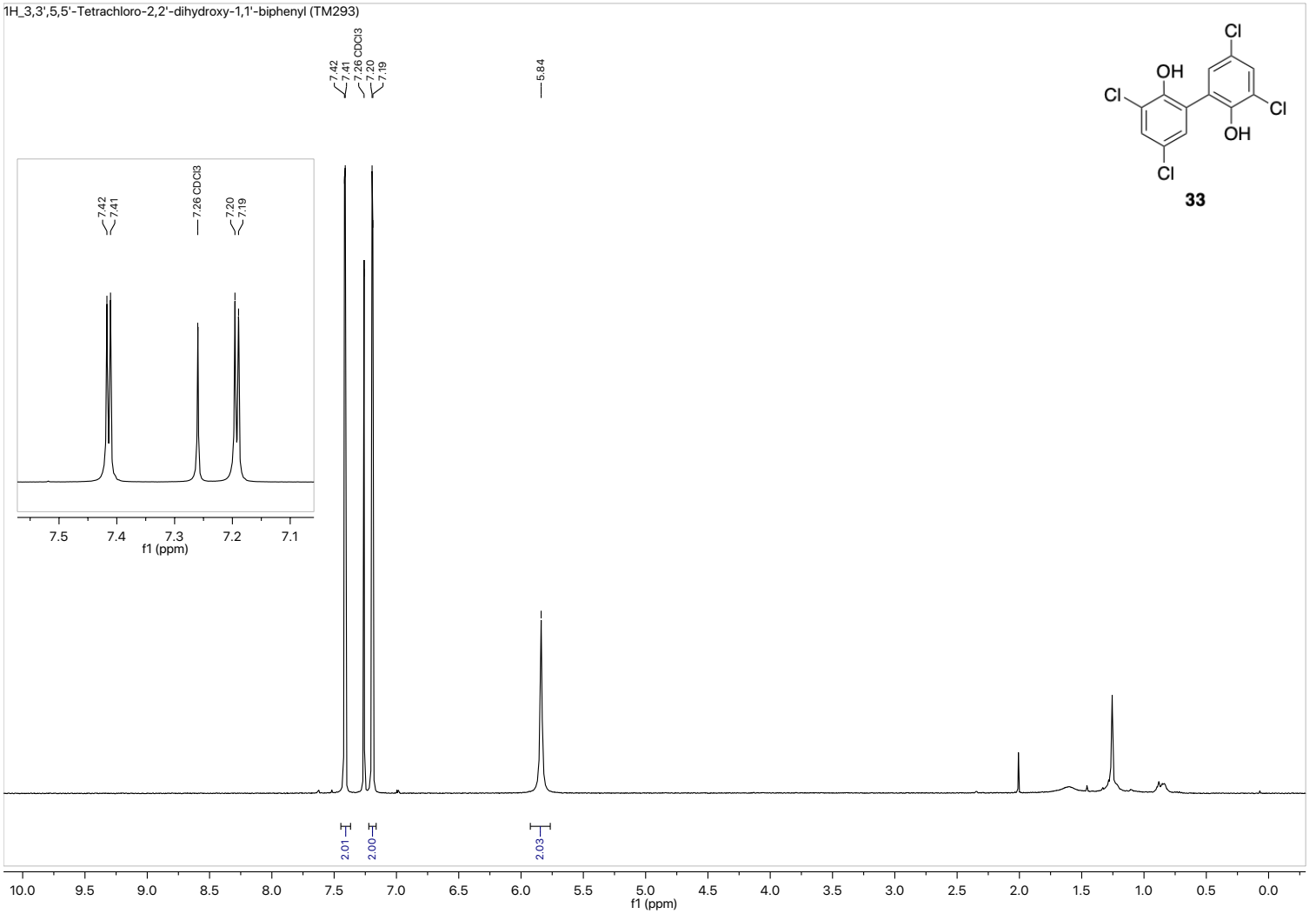


Fig. S59 ^{1}H-NMR spectrum of 3,3',5,5'-tetrachloro-2,2'-dihydroxy-1,1'-biphenyl (**33**). Measured in $CDCl_3$.

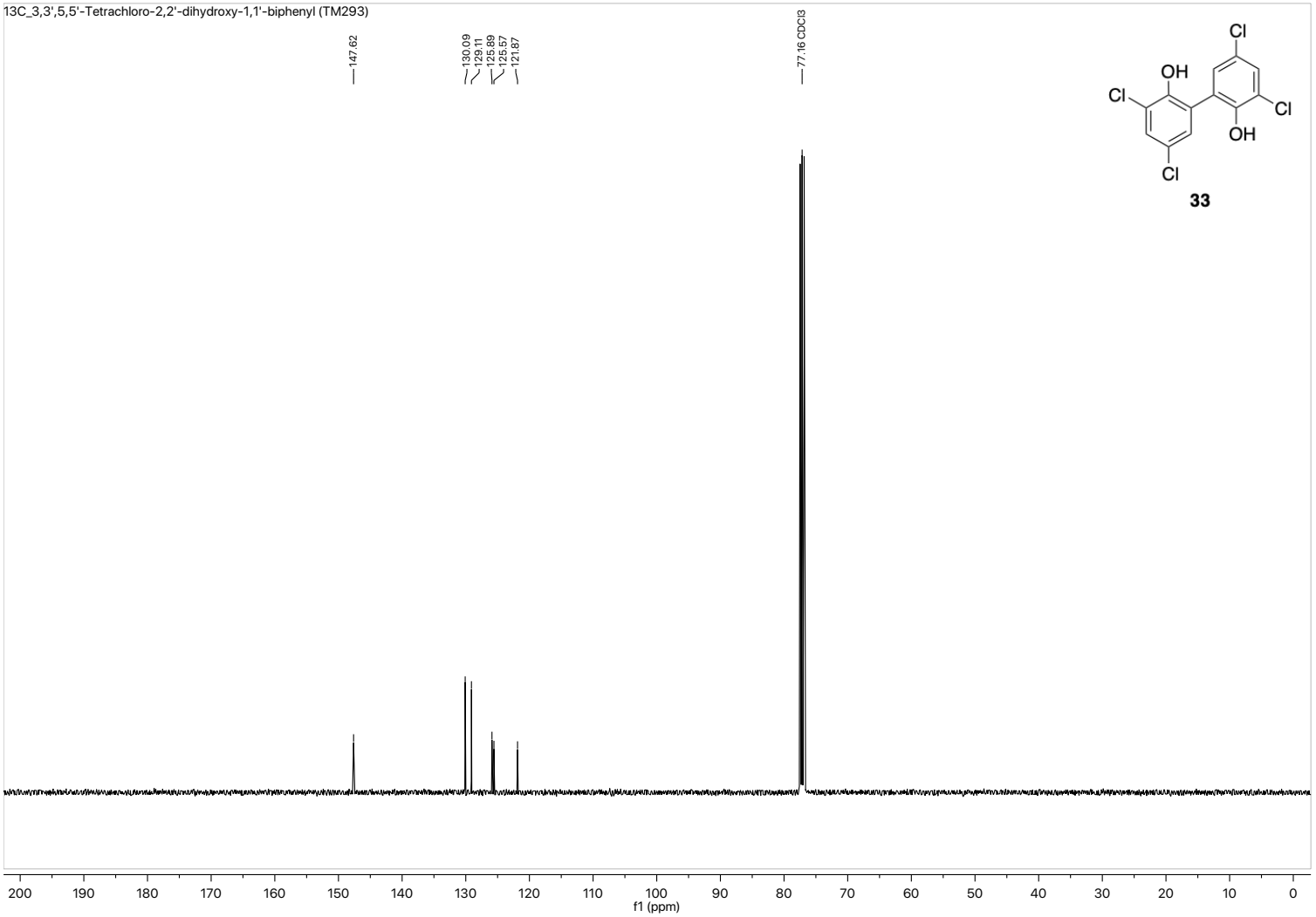


Fig. S60 ^{13}C-NMR spectrum of 3,3',5,5'-tetrachloro-2,2'-dihydroxy-1,1'-biphenyl (**33**). Measured in $CDCl_3$.

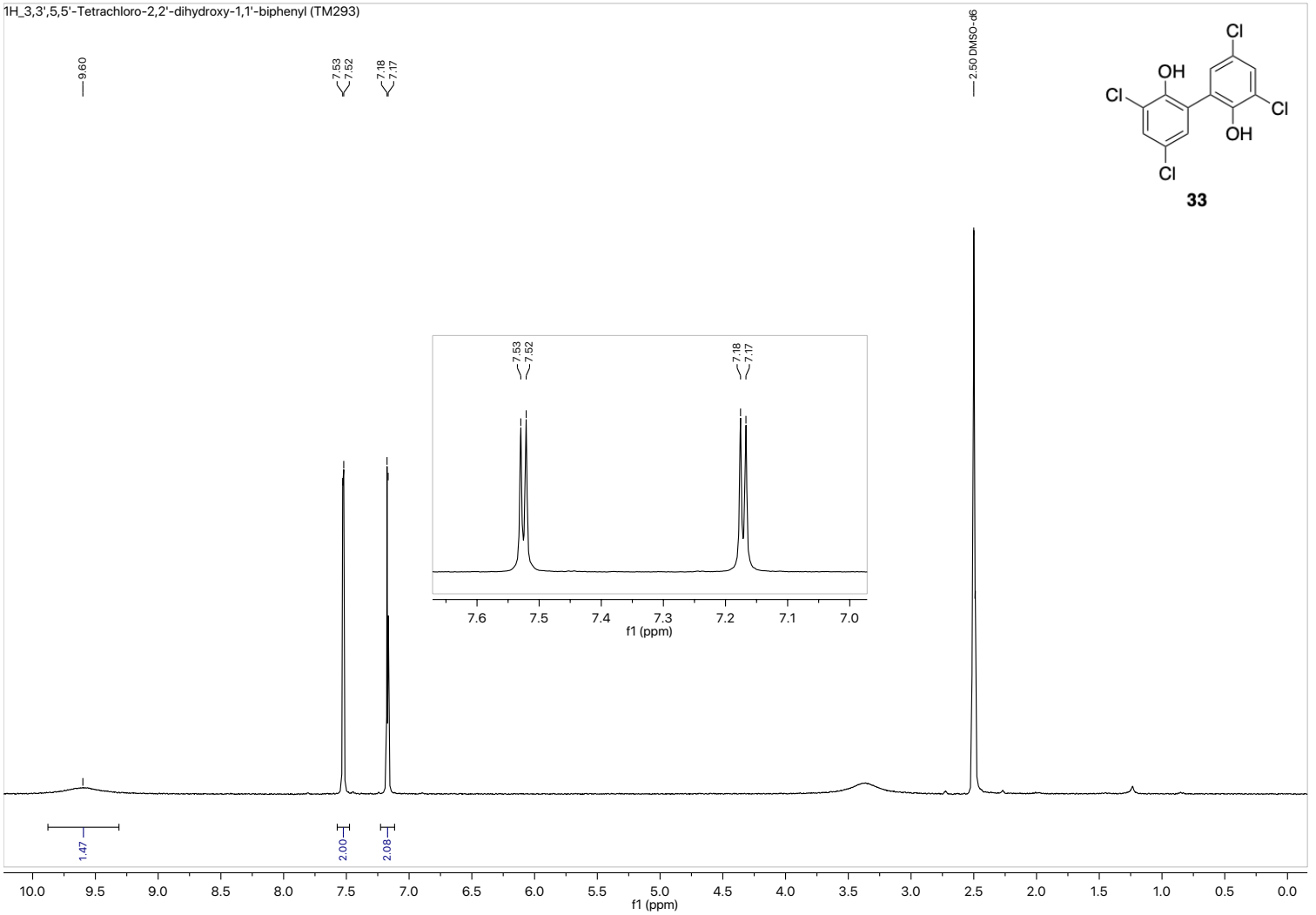


Fig. S61 ^{1}H-NMR spectrum of 3,3',5,5'-tetrachloro-2,2'-dihydroxy-1,1'-biphenyl (**33**). Measured in DMSO-d_6.

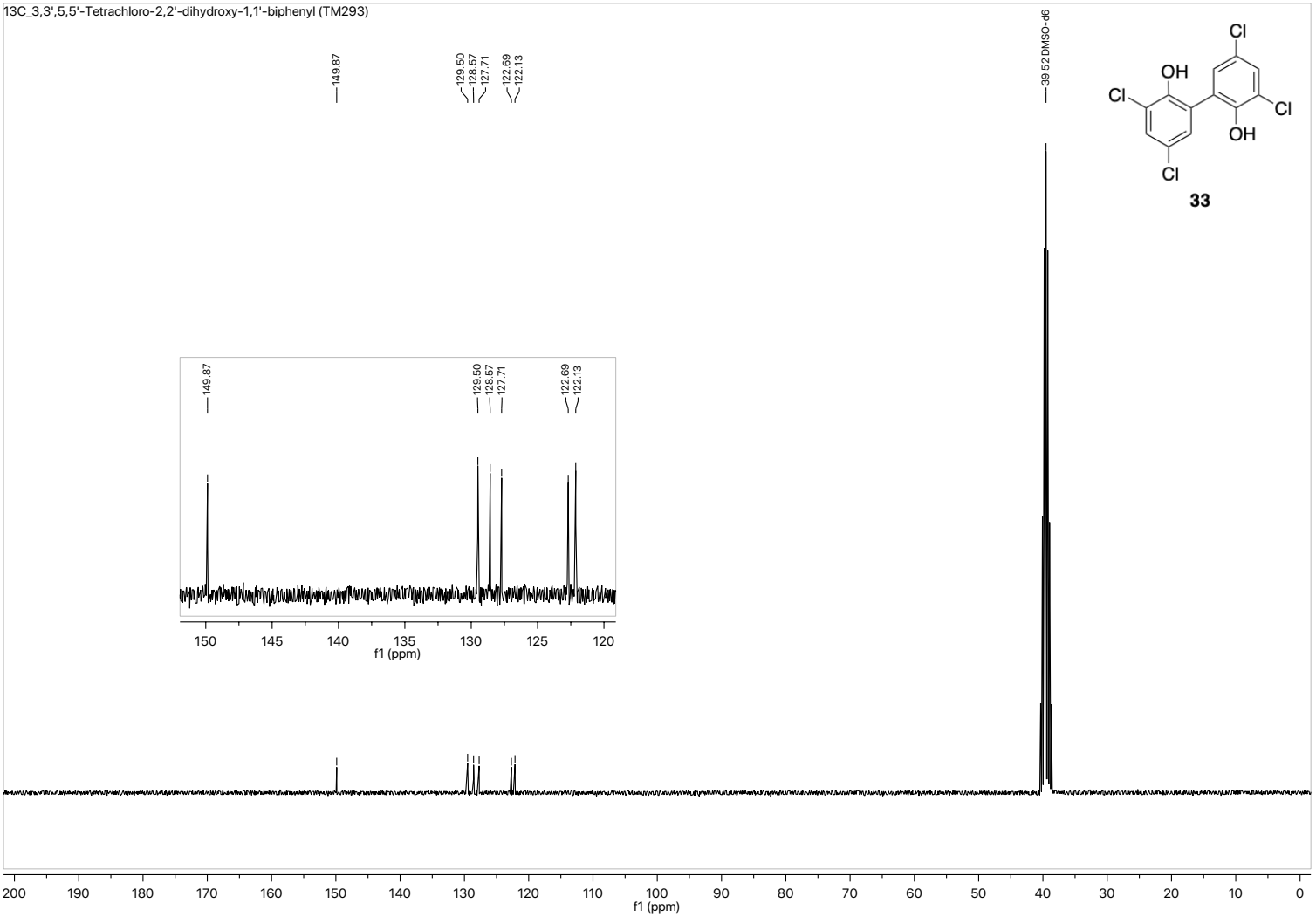


Fig. S62 ^{13}C-NMR spectrum of 3,3',5,5'-tetrachloro-2,2'-dihydroxy-1,1'-biphenyl (**33**). Measured in DMSO-d_6.

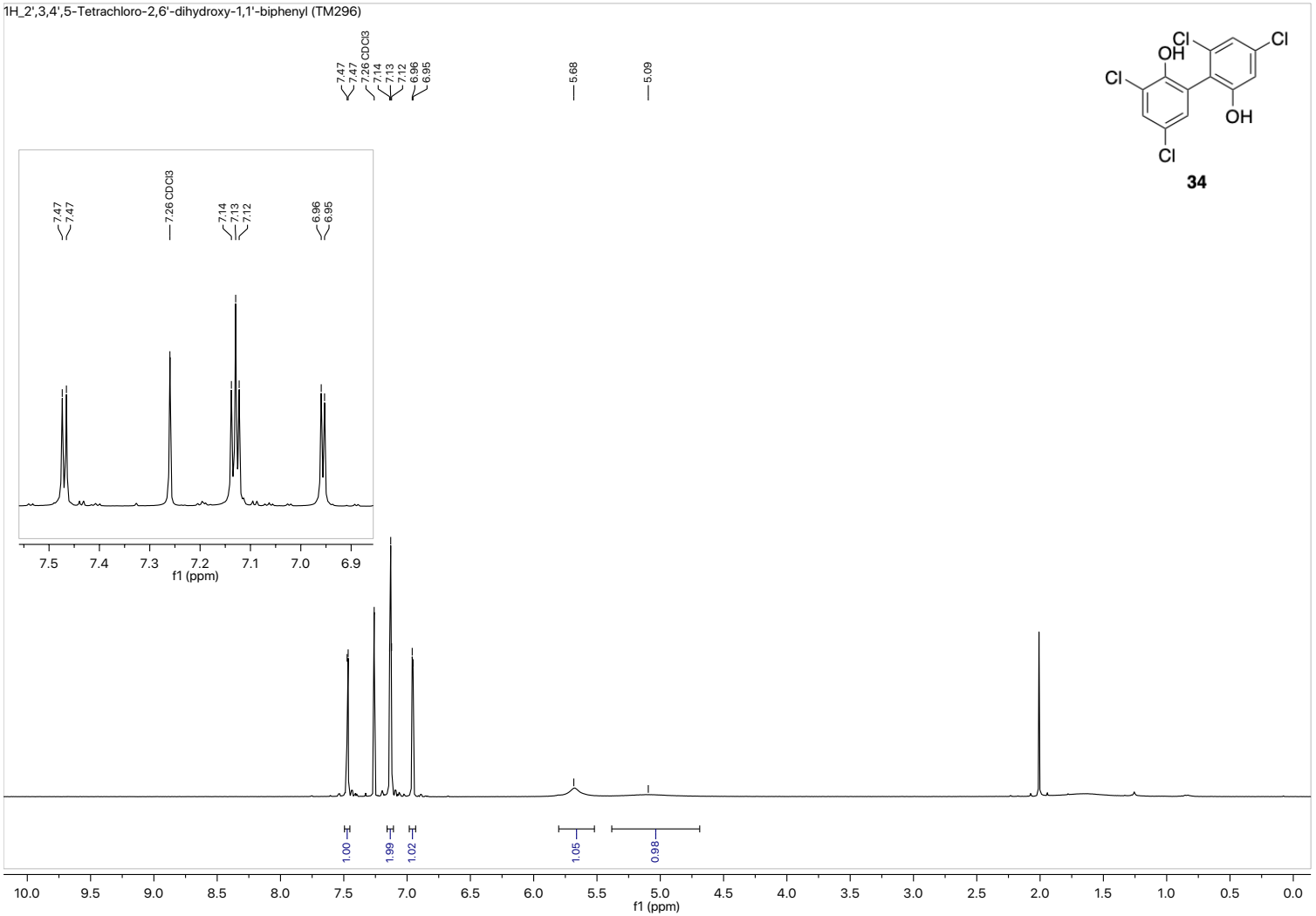


Fig. S63 ^{1}H-NMR spectrum of 2',3,4',5-tetrachloro-2,6'-dihydroxy-1,1'-biphenyl (**34**).

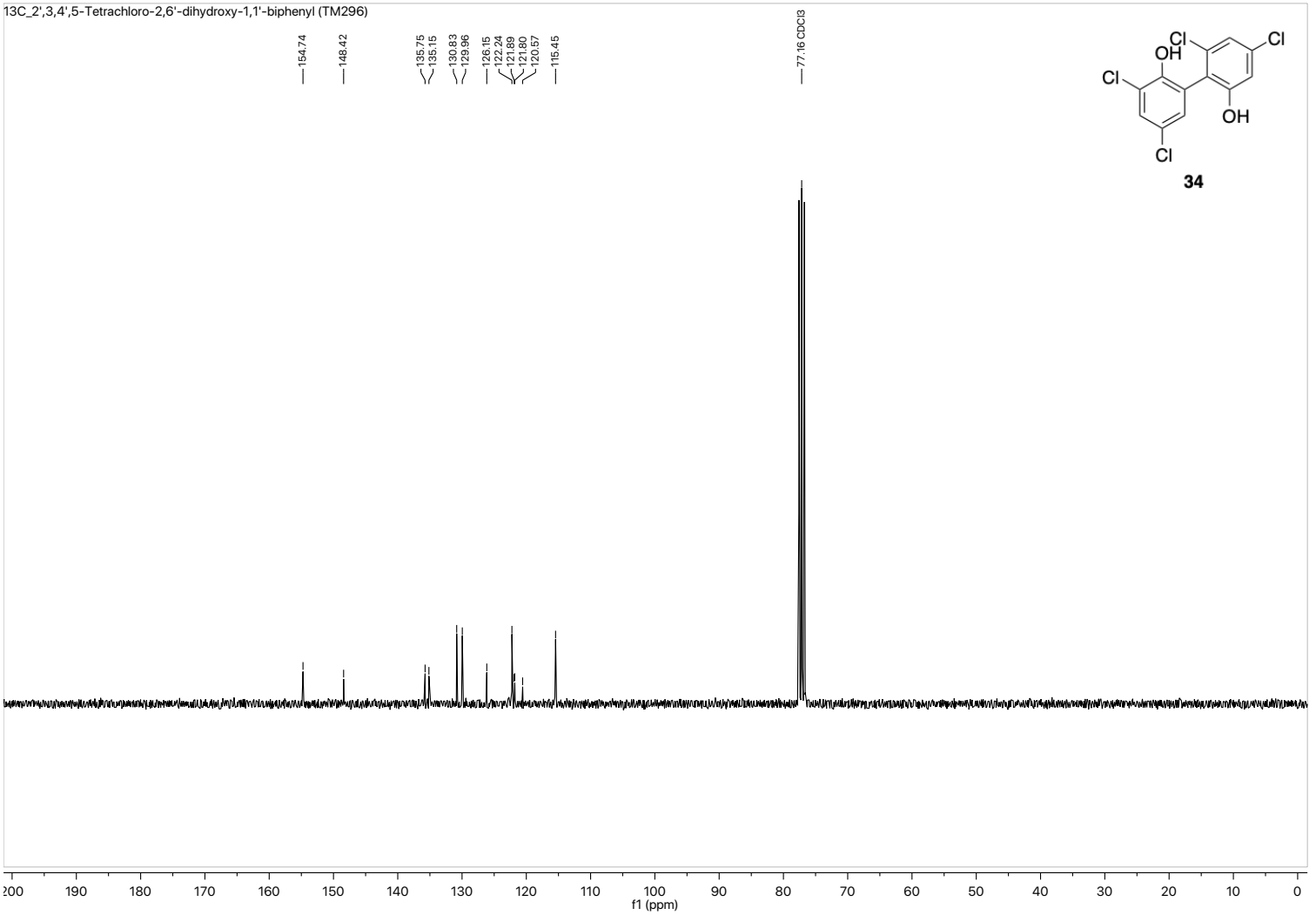


Fig. S64 ^{13}C-NMR spectrum of 2',3,4',5-tetrachloro-2,6'-dihydroxy-1,1'-biphenyl (**34**).

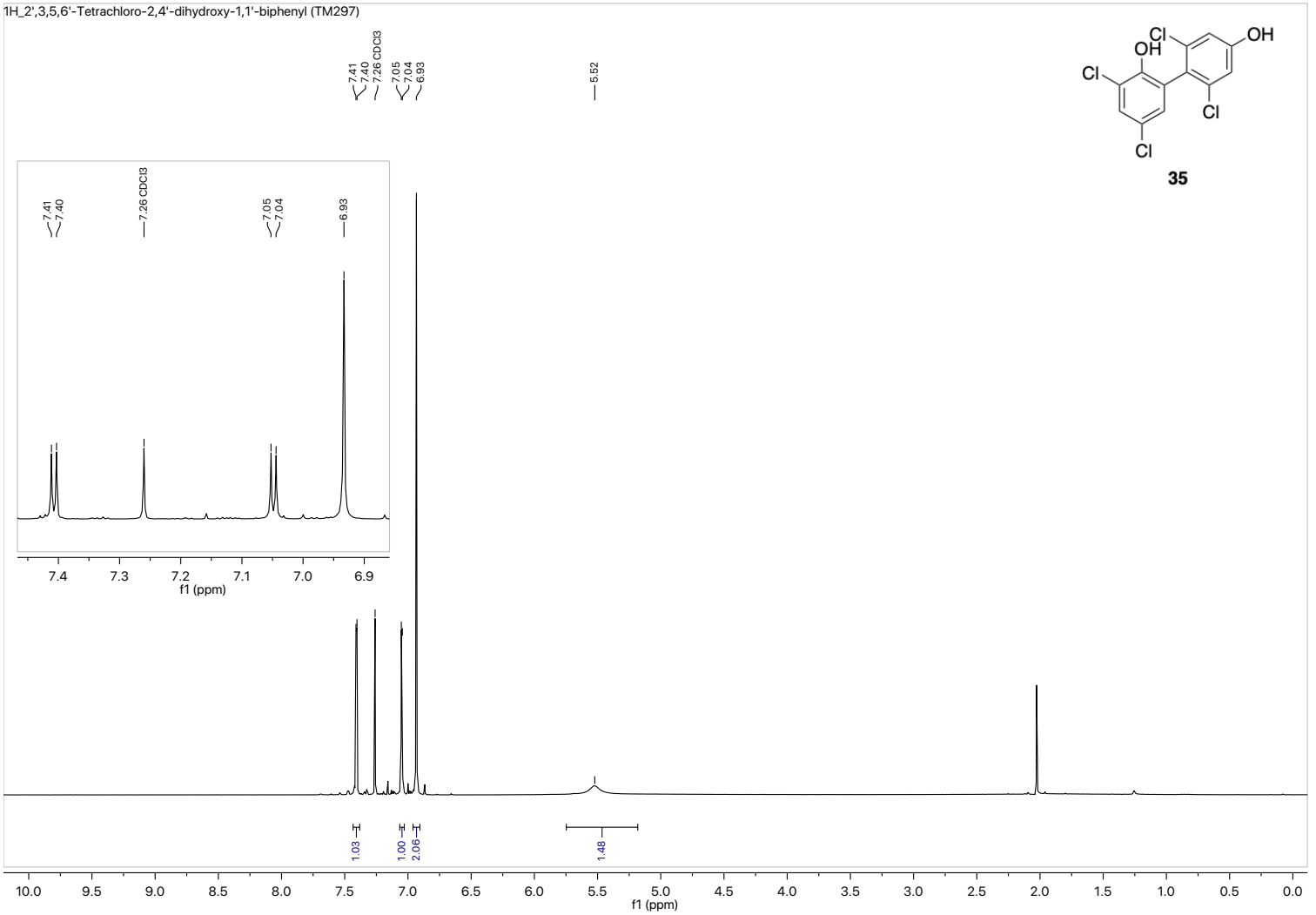


Fig. S65 ^{1}H-NMR spectrum of 2',3,5,6'-tetrachloro-2,4'-dihydroxy-1,1'-biphenyl (**35**).

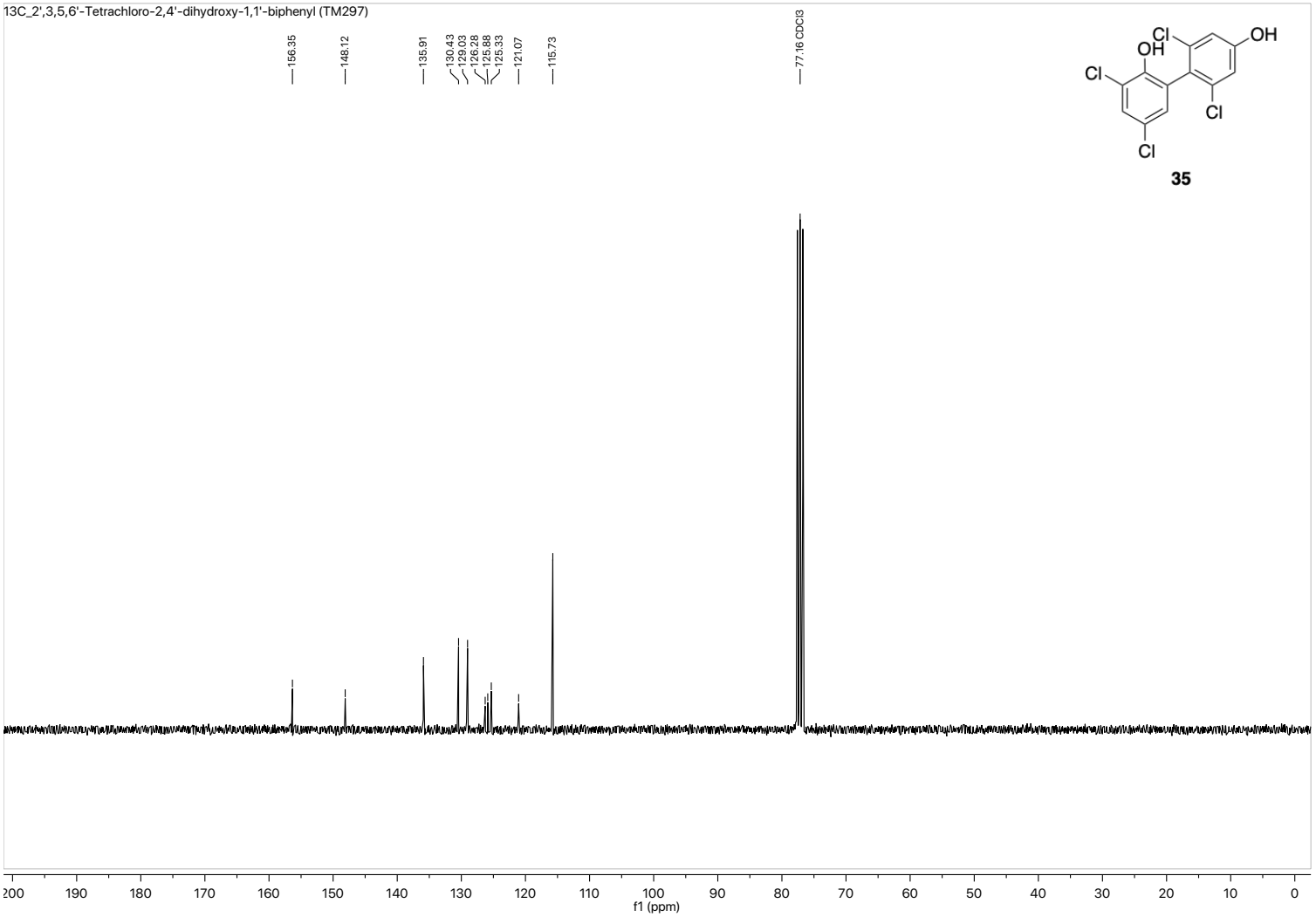


Fig. S66 ^{13}C-NMR spectrum of 2',3,5,6'-tetrachloro-2,4'-dihydroxy-1,1'-biphenyl (**35**).

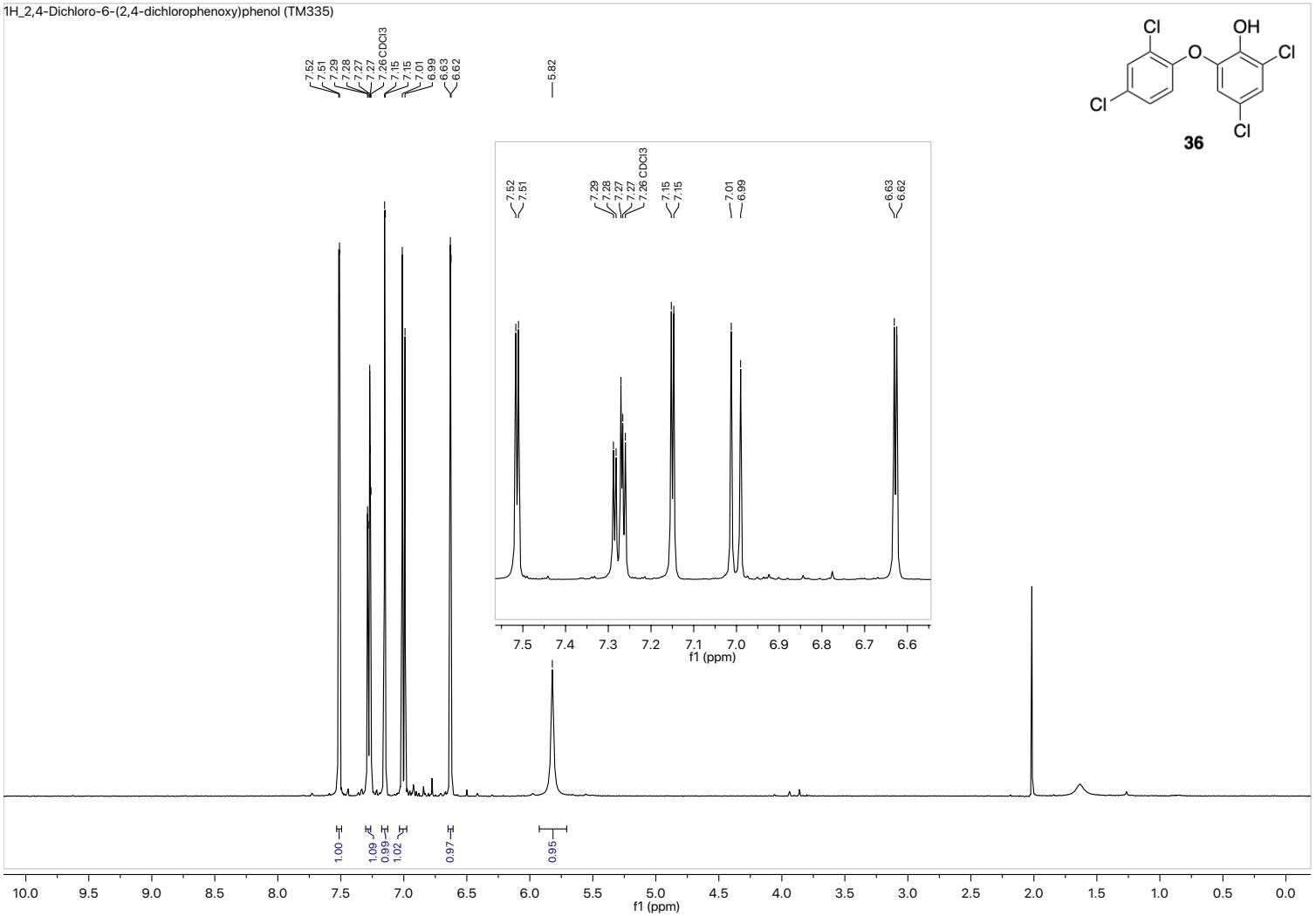


Fig. S67 ^{1}H-NMR spectrum of 2,4-dichloro-6-(2,4-dichlorophenoxy)phenol (**36**). Measured in $CDCl_3$.

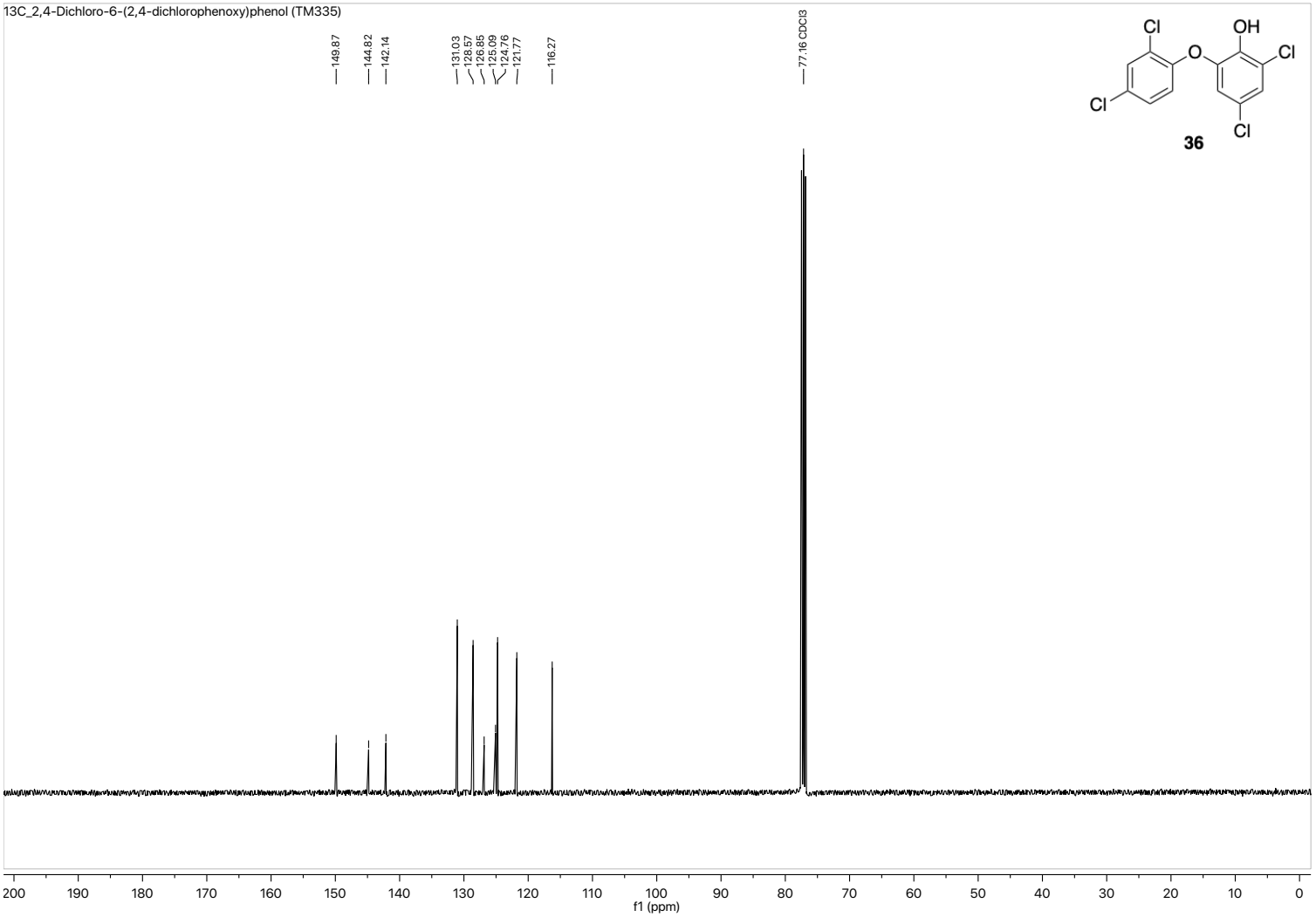


Fig. S68 ^{13}C-NMR spectrum of 2,4-dichloro-6-(2,4-dichlorophenoxy)phenol (**36**). Measured in $CDCl_3$.

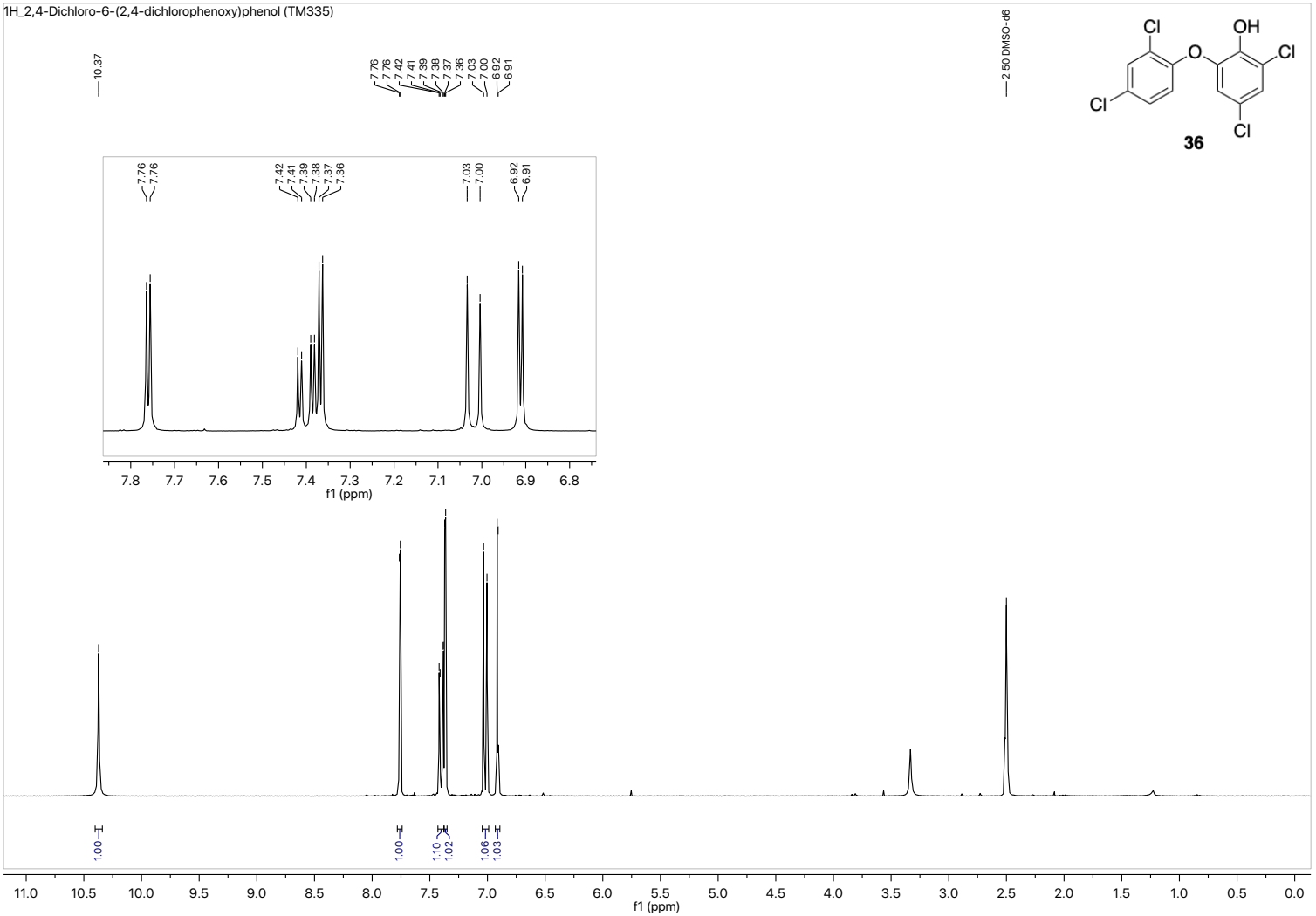


Fig. S69 ^{1}H-NMR spectrum of 2,4-dichloro-6-(2,4-dichlorophenoxy)phenol (**36**). Measured in DMSO-d_6.

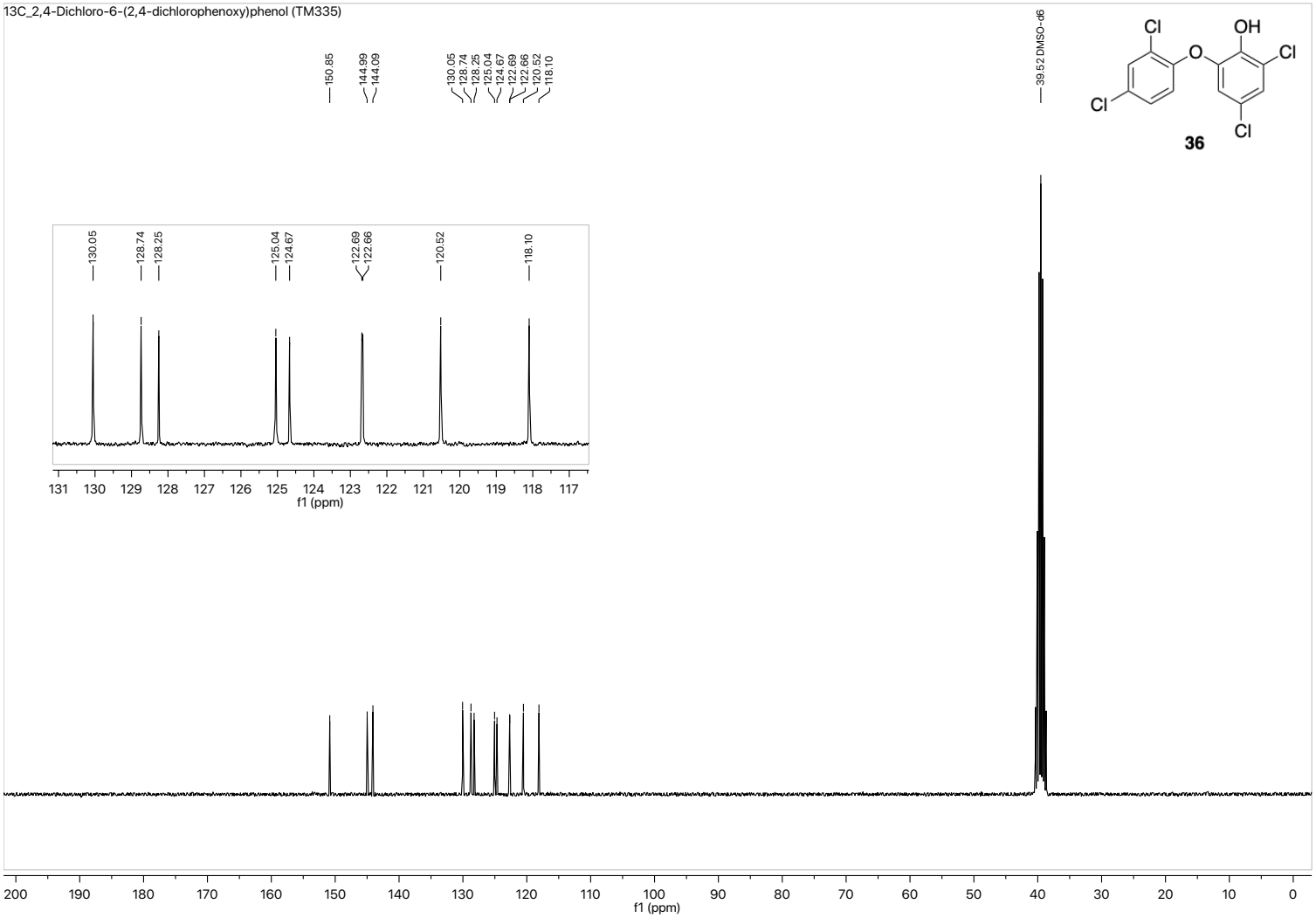


Fig. S70 ^{13}C-NMR spectrum of 2,4-dichloro-6-(2,4-dichlorophenoxy)phenol (**36**). Measured in DMSO-d_6.

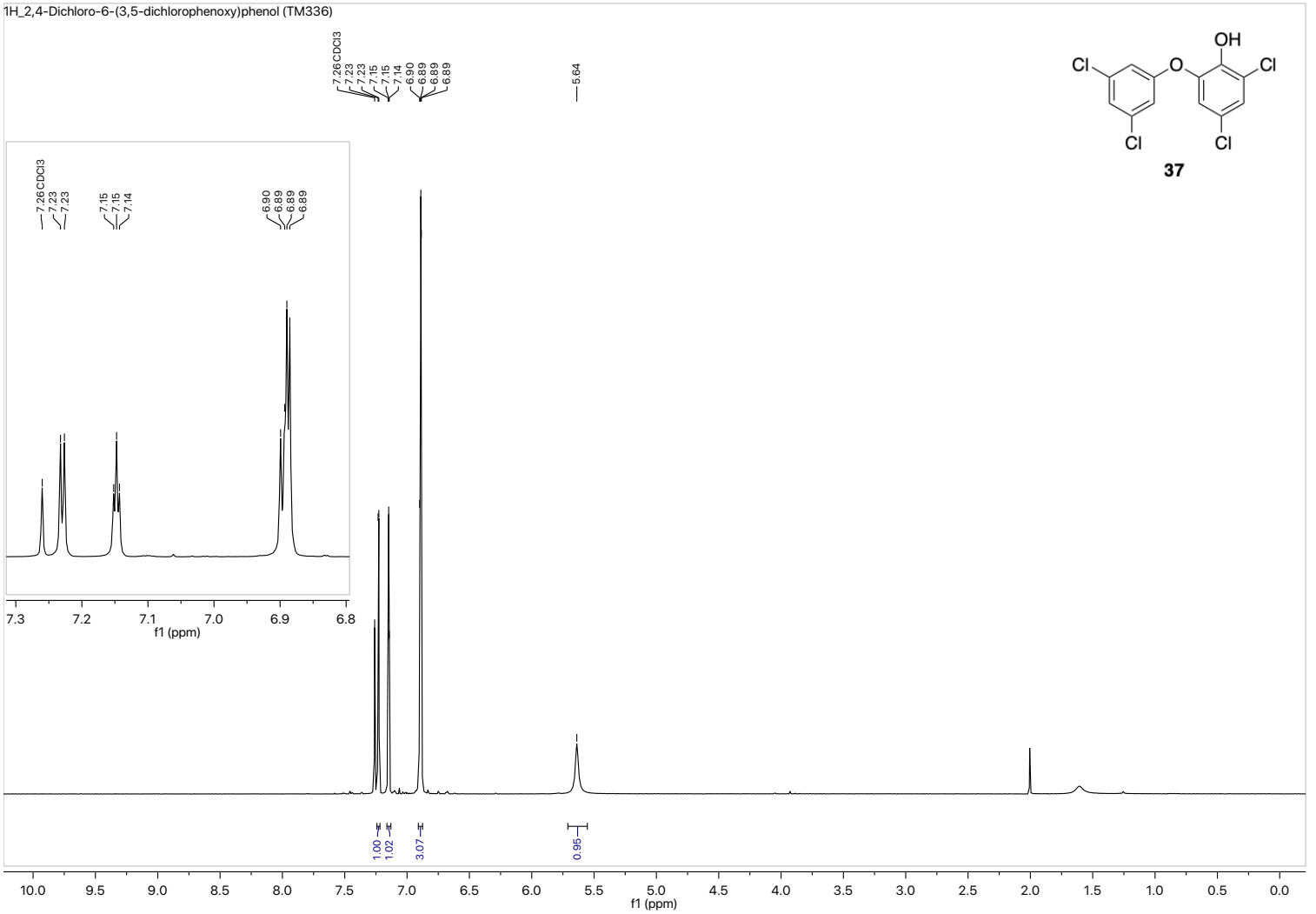


Fig. S71 ^{1}H-NMR spectrum of 2,4-dichloro-6-(3,5-dichlorophenoxy)phenol (**37**).

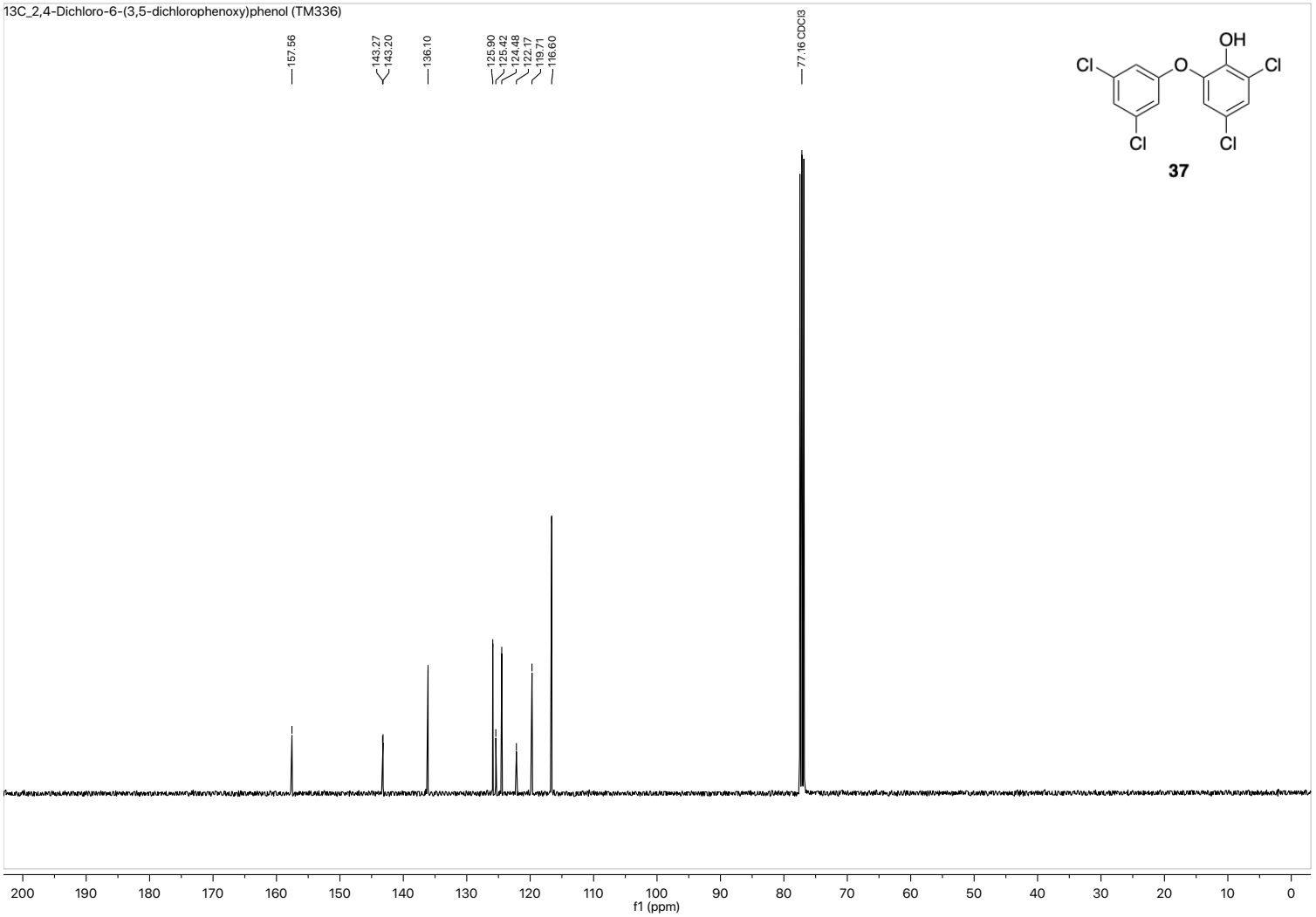


Fig. S72 ^{13}C-NMR spectrum of 2,4-dichloro-6-(3,5-dichlorophenoxy)phenol (**37**).

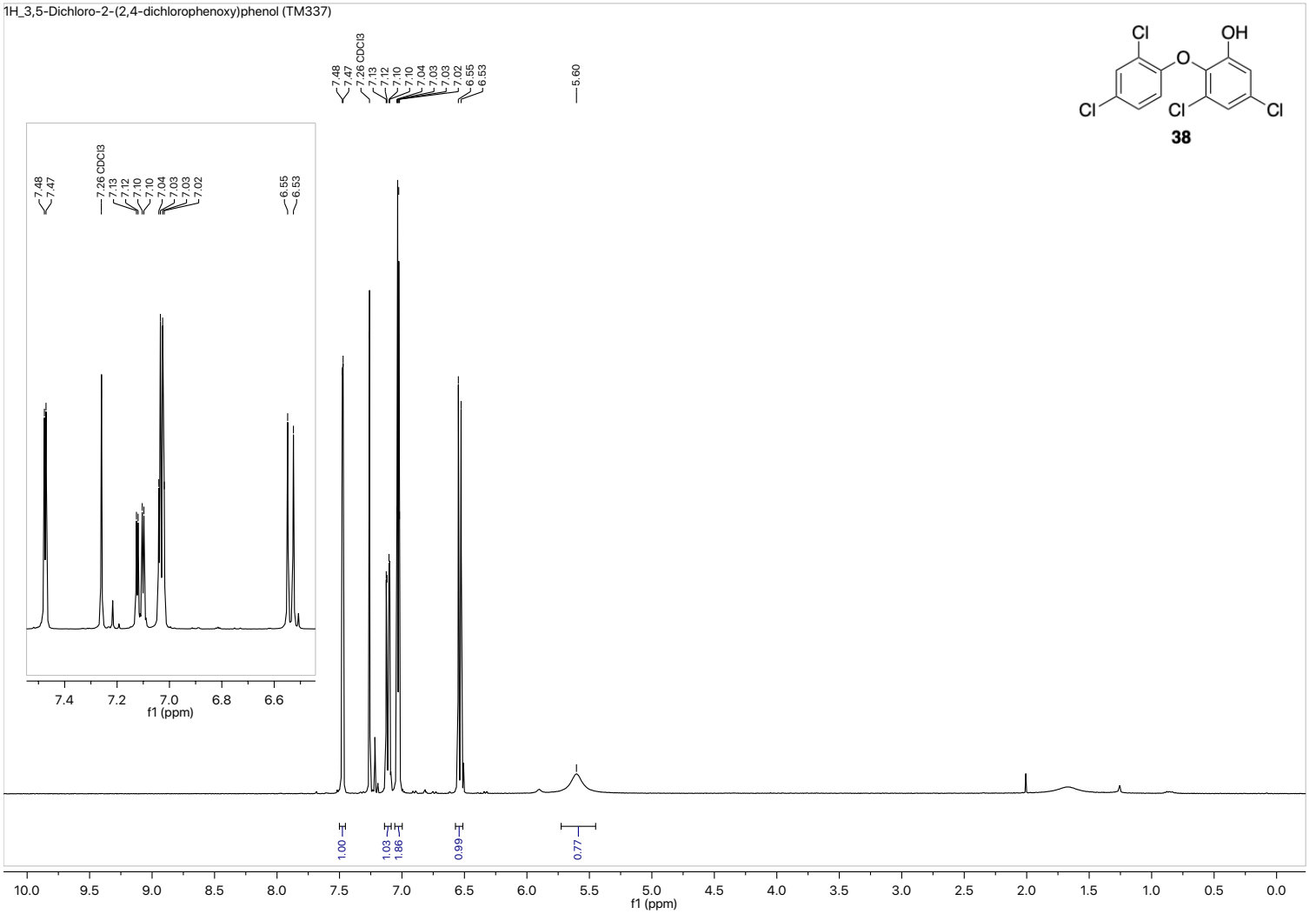


Fig. S73 ^{1}H-NMR spectrum of 3,5-dichloro-2-(2,4-dichlorophenoxy)phenol (**38**).

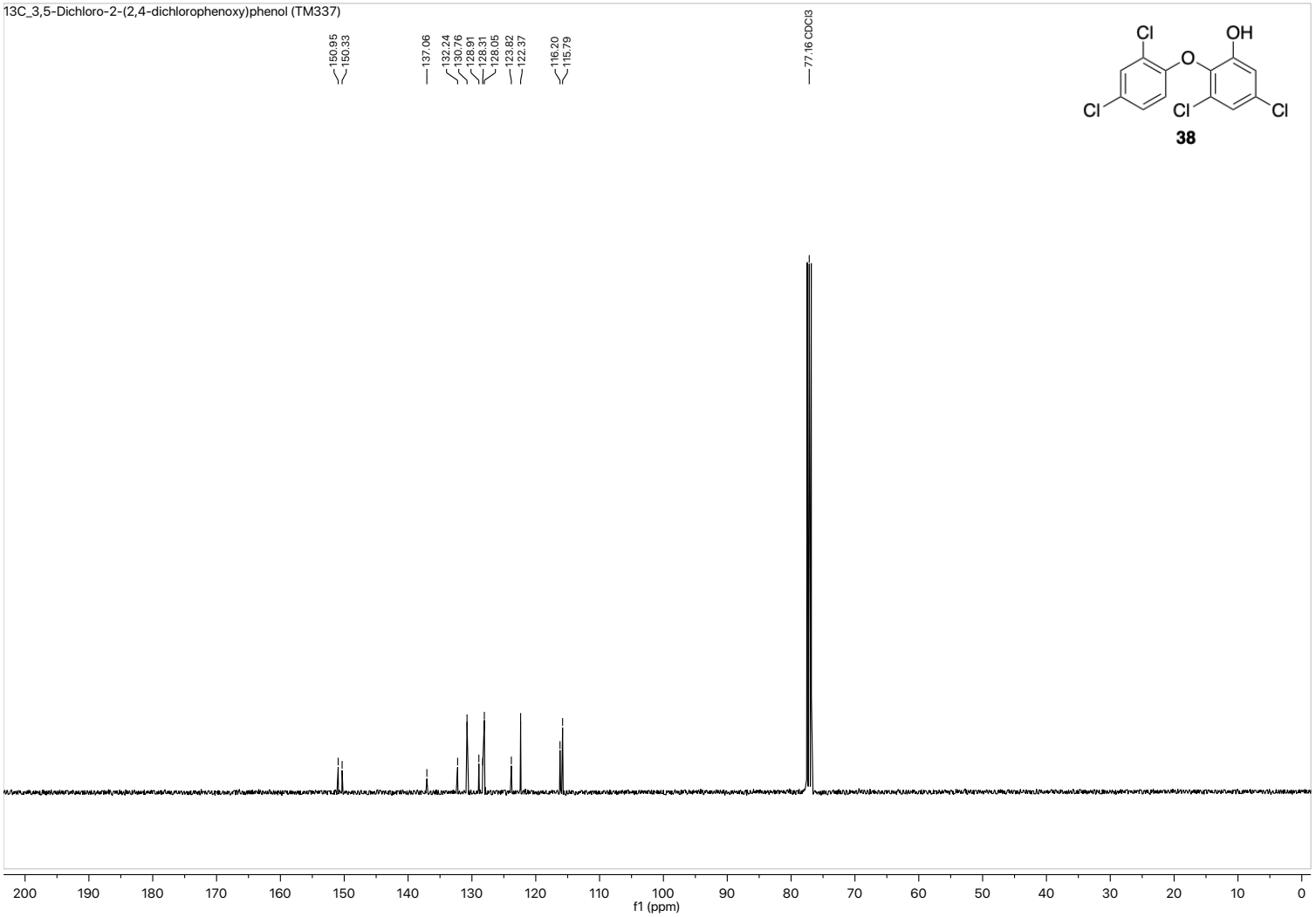


Fig. S74 ^{13}C-NMR spectrum of 3,5-dichloro-2-(2,4-dichlorophenoxy)phenol (**38**).

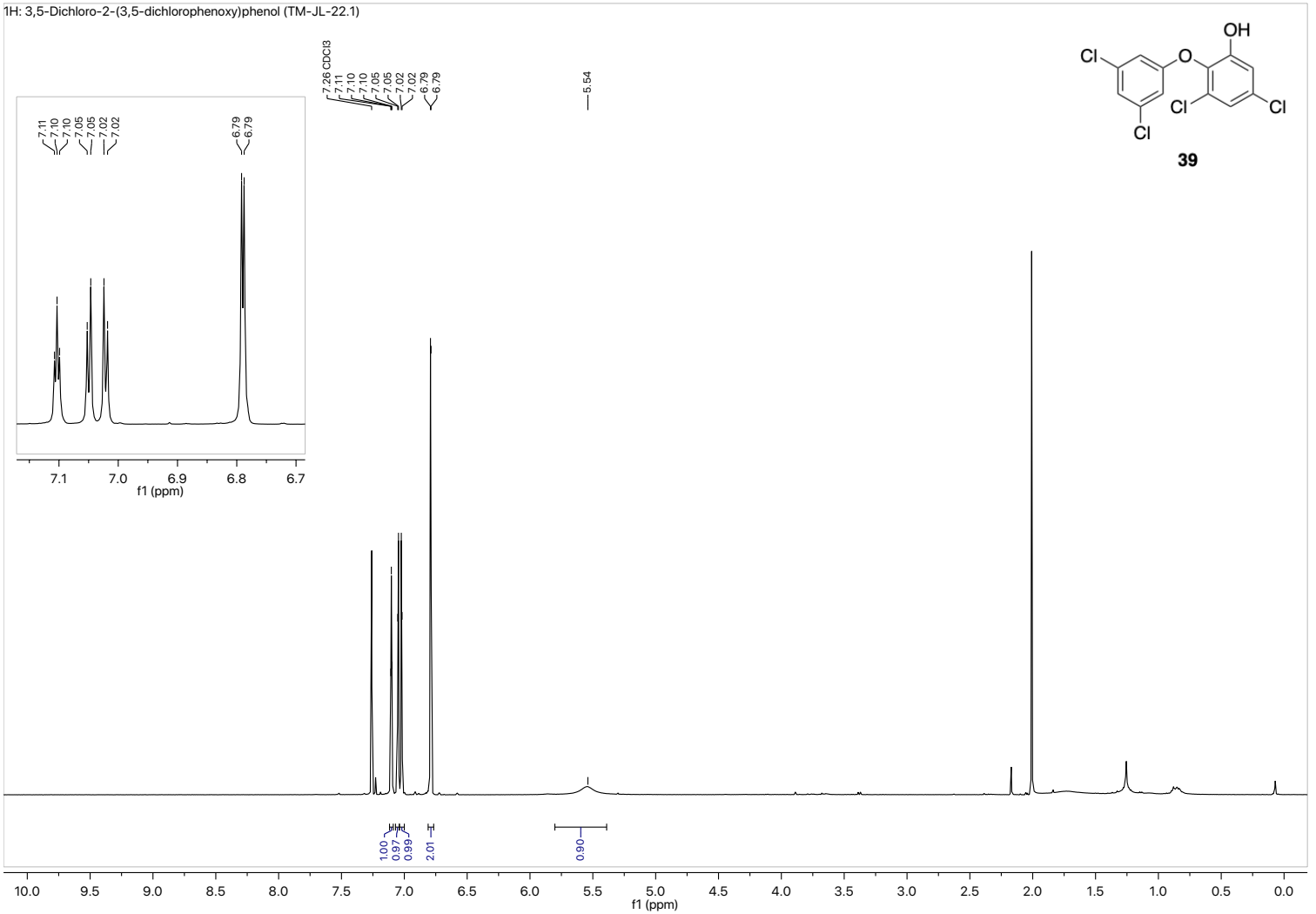


Fig. S75 ^{1}H-NMR spectrum of 3,5-dichloro-2-(3,5-dichlorophenoxy)phenol (**39**).

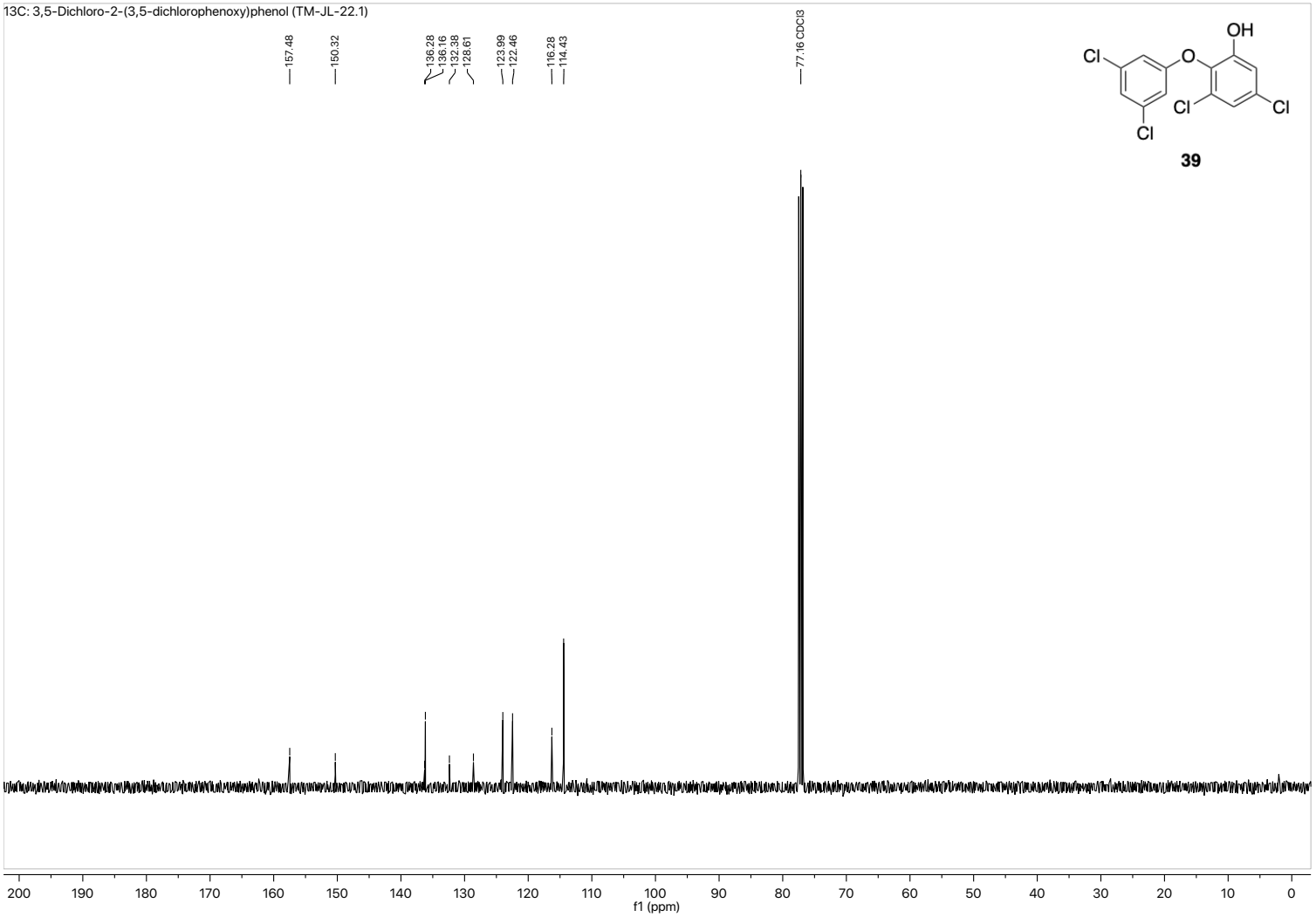


Fig. S76 ^{13}C-NMR spectrum of 3,5-dichloro-2-(3,5-dichlorophenoxy)phenol (**39**).

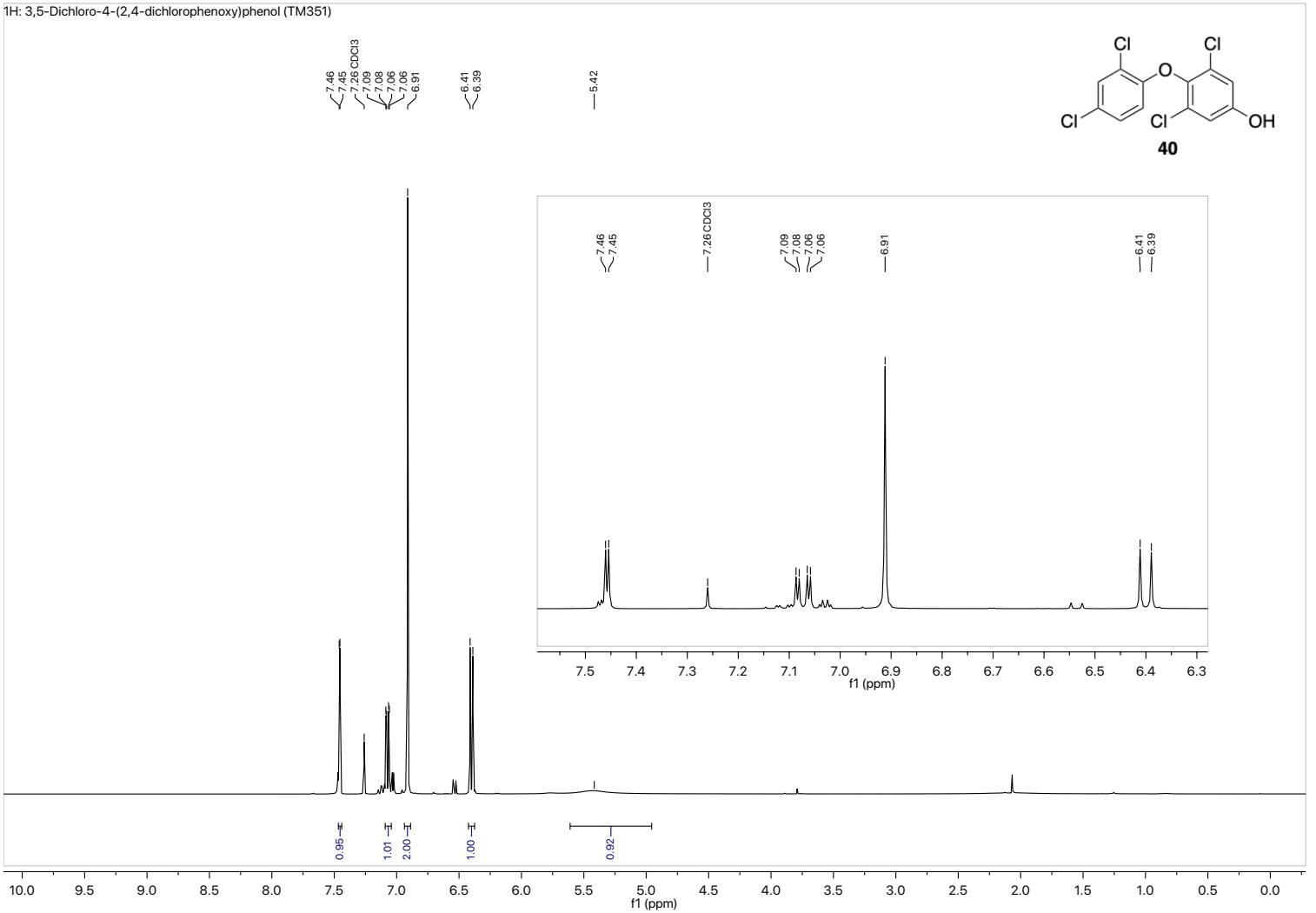


Fig. S77 ^{1}H-NMR spectrum of 3,5-dichloro-4-(2,4-dichlorophenoxy)phenol (**40**).

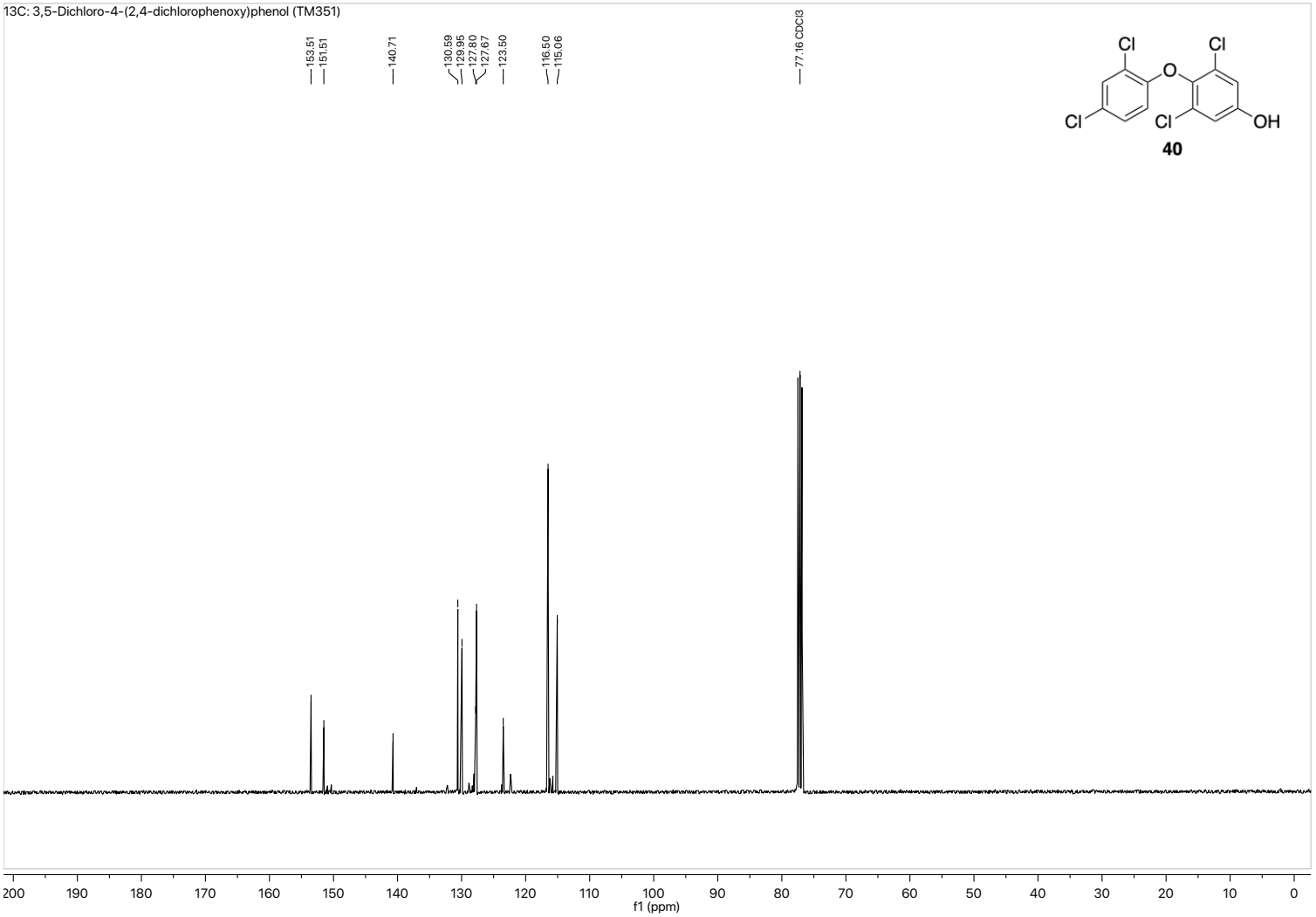


Fig. S78 ^{13}C-NMR spectrum of 3,5-dichloro-4-(2,4-dichlorophenoxy)phenol (**40**).

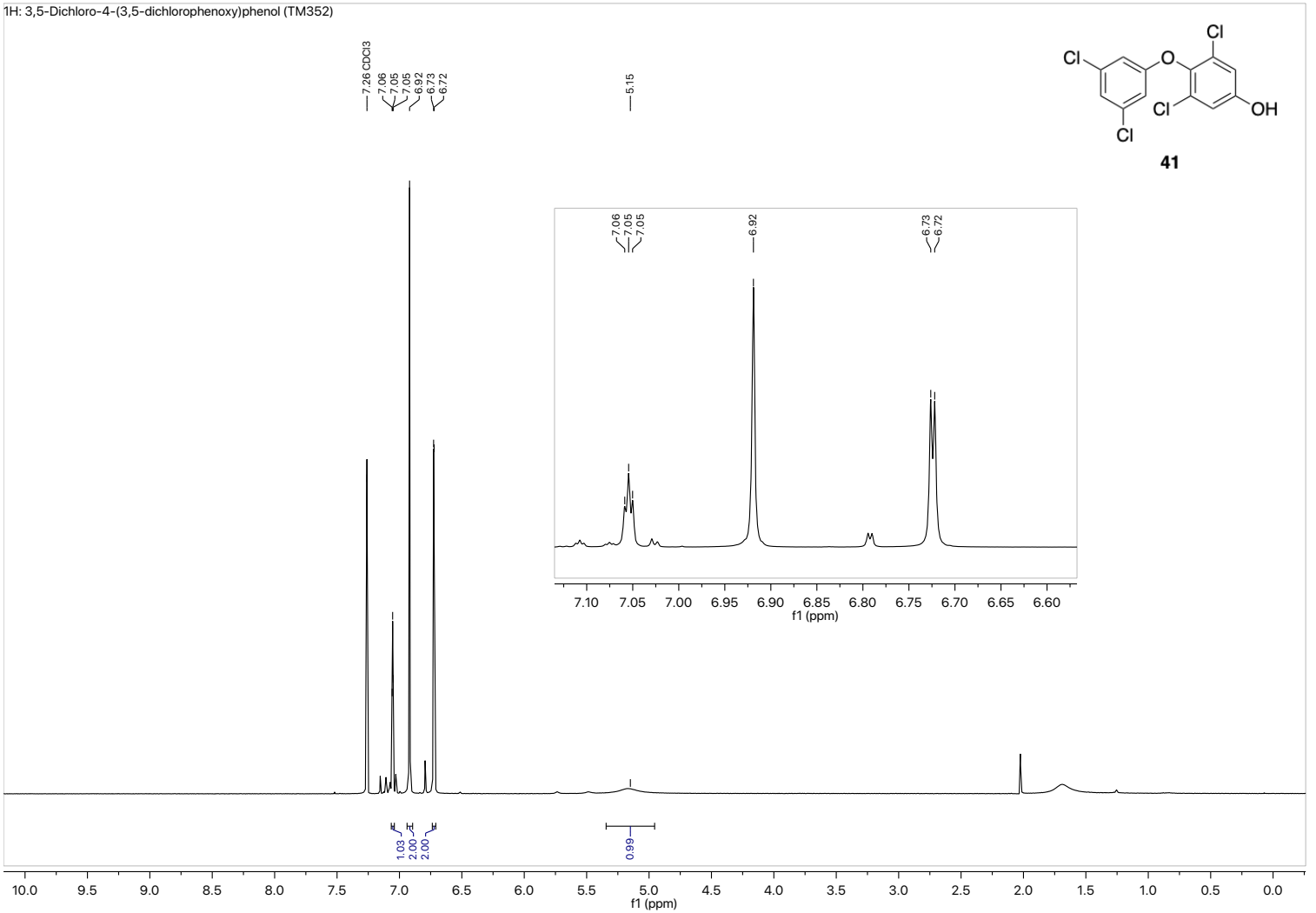


Fig. S79 ^{1}H-NMR spectrum of 3,5-dichloro-4-(3,5-dichlorophenoxy)phenol (**41**).

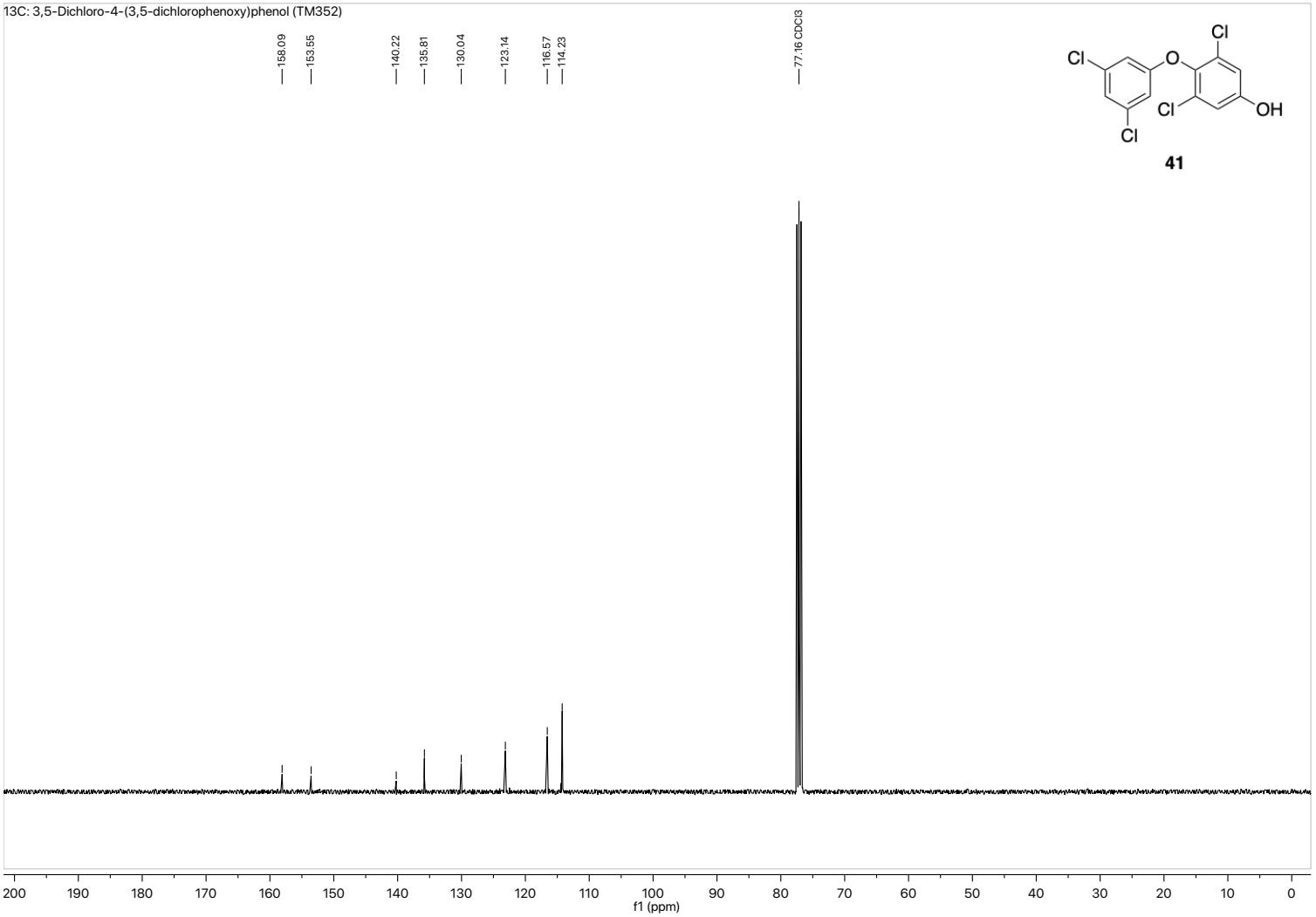


Fig. S80 ^{13}C-NMR spectrum of 3,5-dichloro-4-(3,5-dichlorophenoxy)phenol (**41**).

8. UV-absorbance Measurements

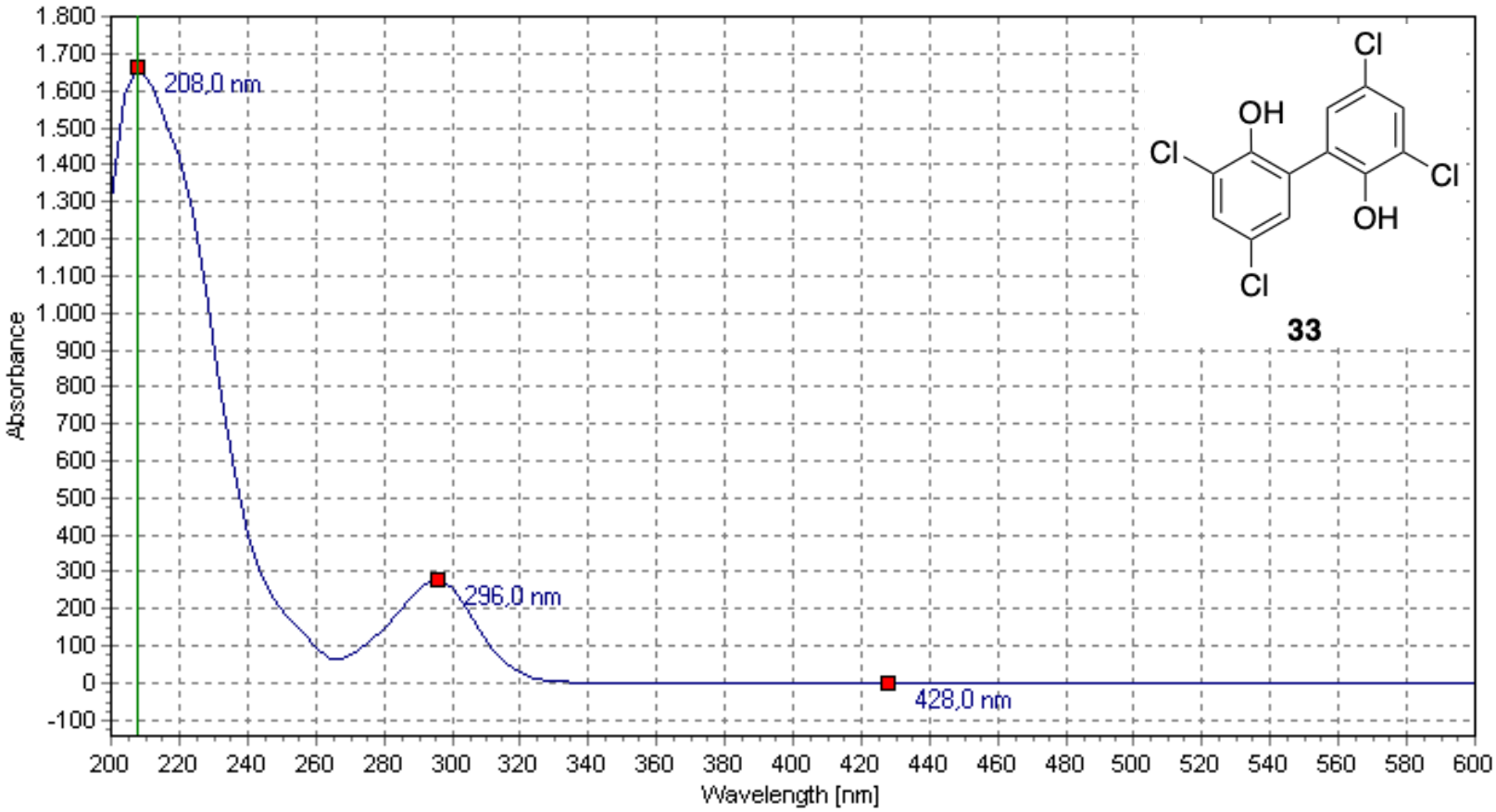


Fig. S81 UV-absorbance measurement of 3,3',5,5'-tetrachloro-2,2'-dihydroxy-1,1'-biphenyl (**33**).

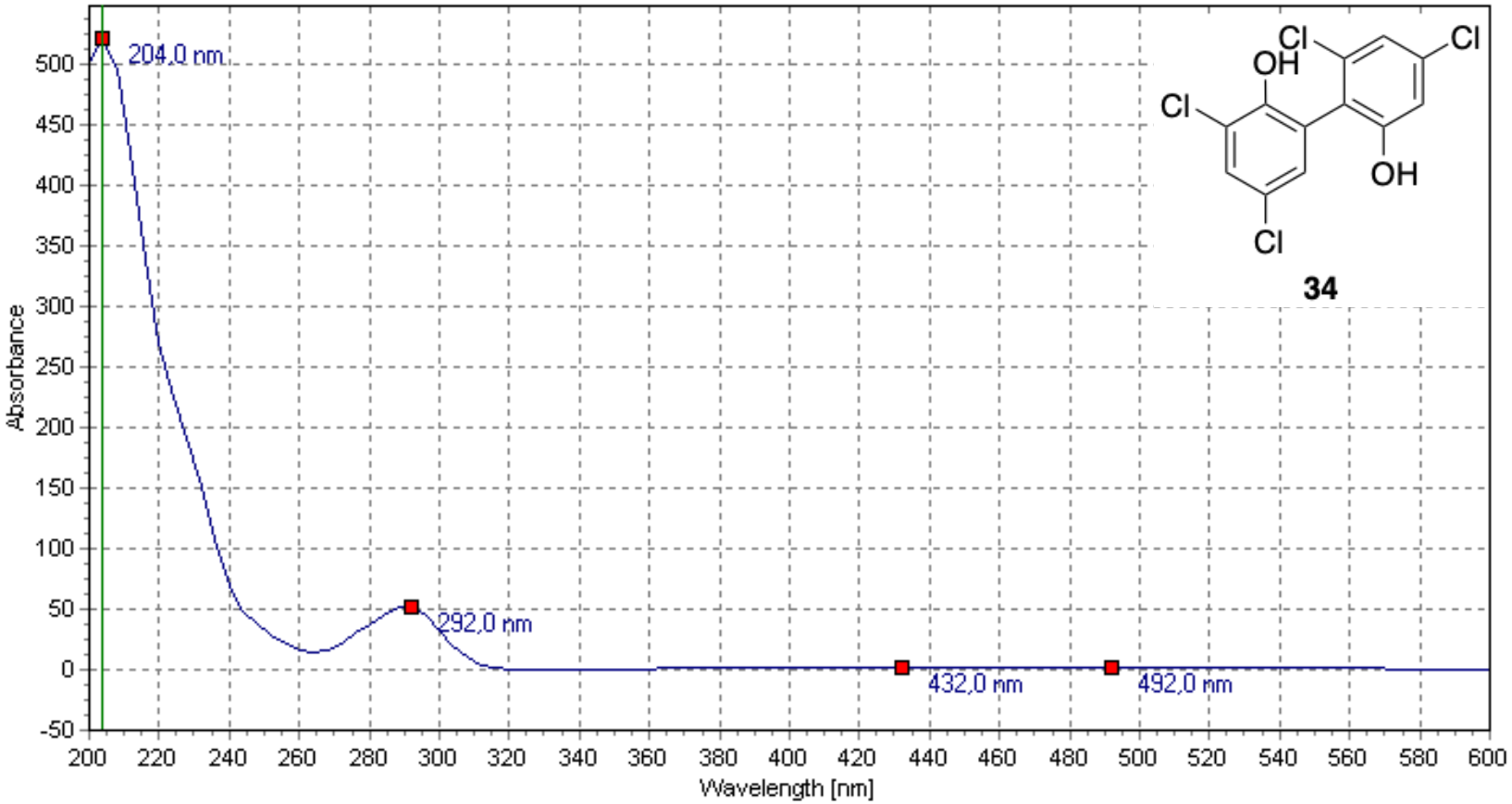


Fig. S82 UV-absorbance measurement of 2',3,4',5-tetrachloro-2,6'-dihydroxy-1,1'-biphenyl (**34**).

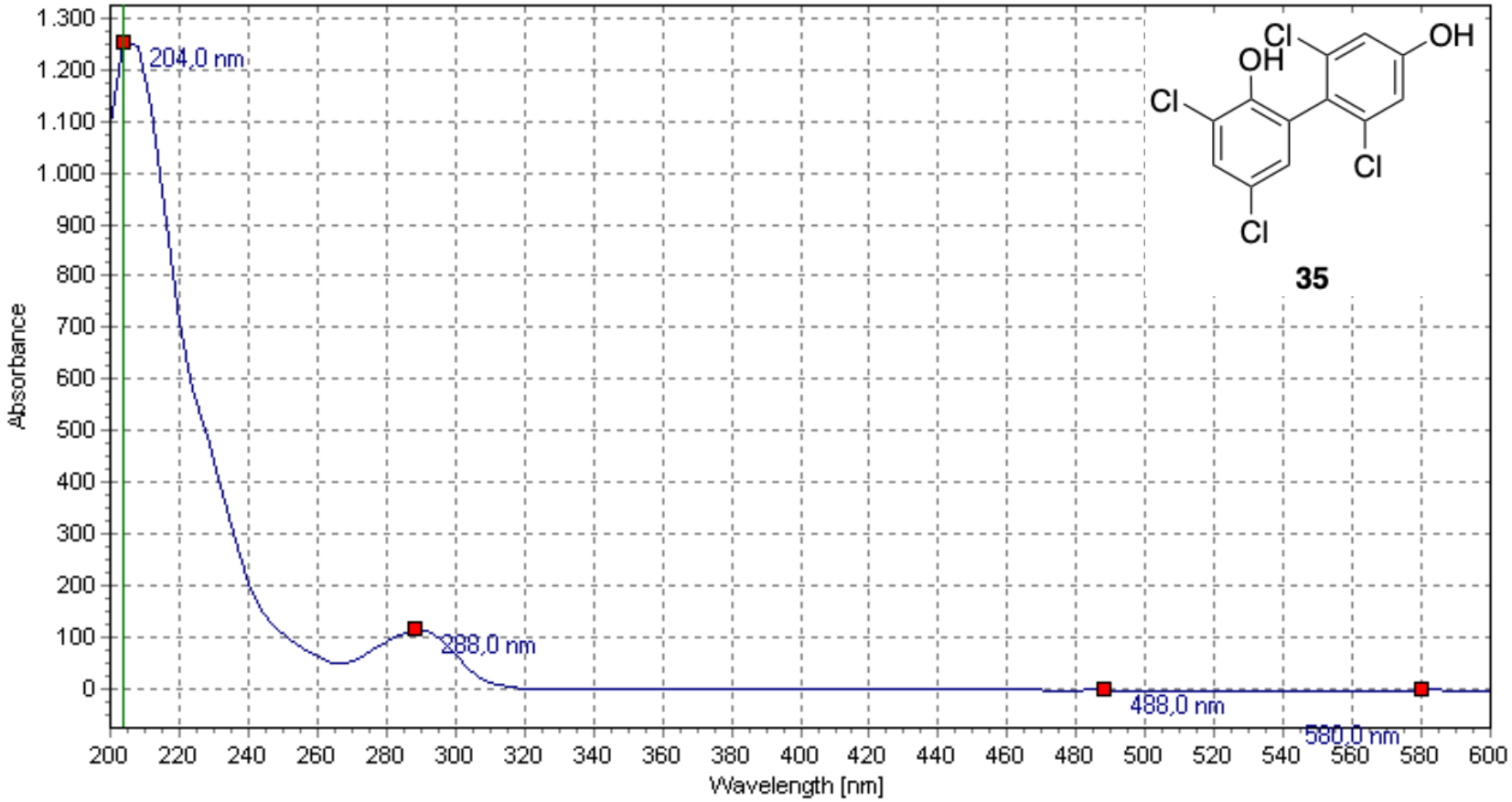


Fig. S83 UV-absorbance measurement of 2',3,5,6'-tetrachloro-2,4'-dihydroxy-1,1'-biphenyl (**35**).

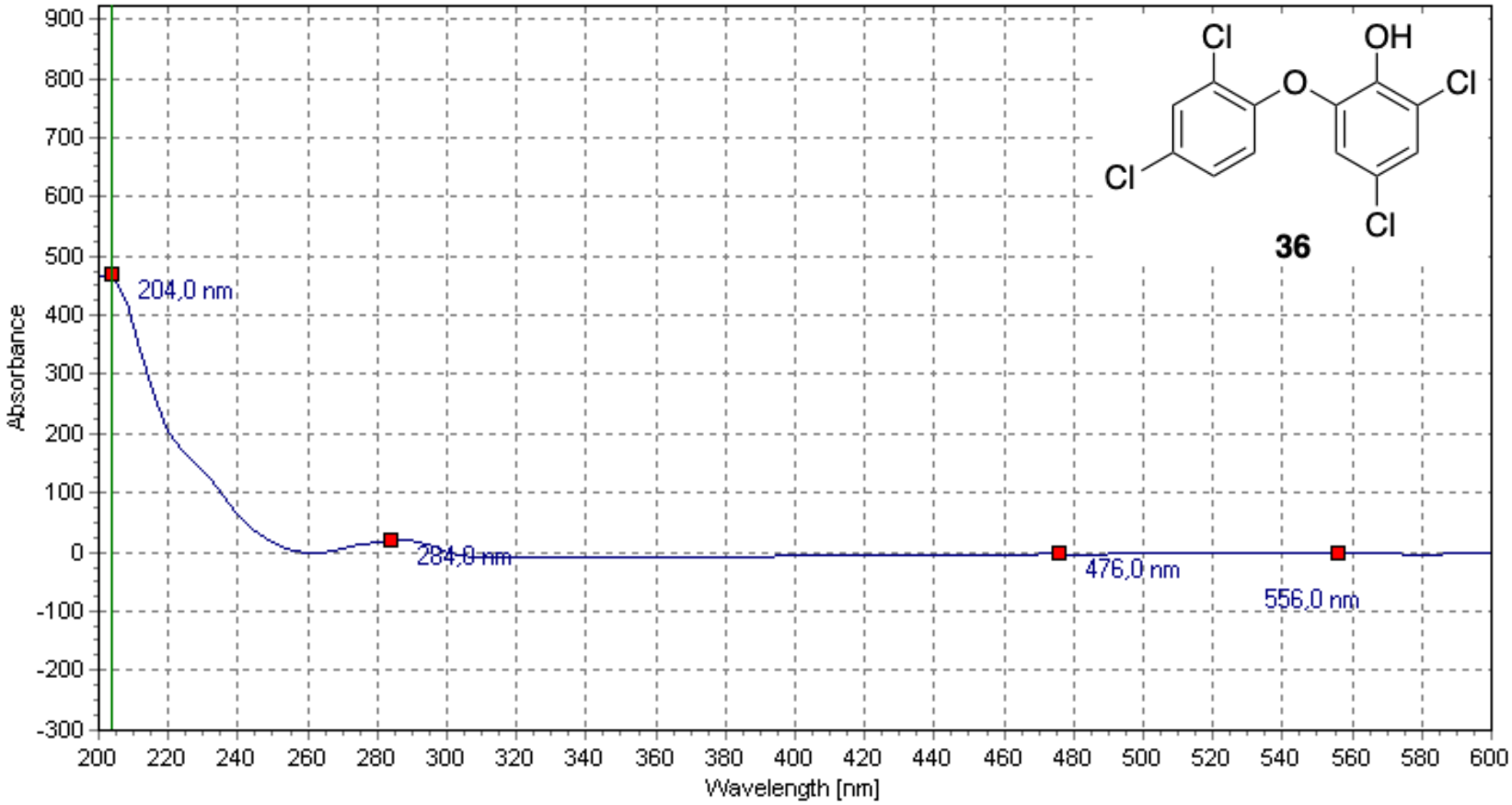


Fig. S84 UV-absorbance measurement of 2,4-dichloro-6-(2,4-dichlorophenoxy)phenol (**36**).

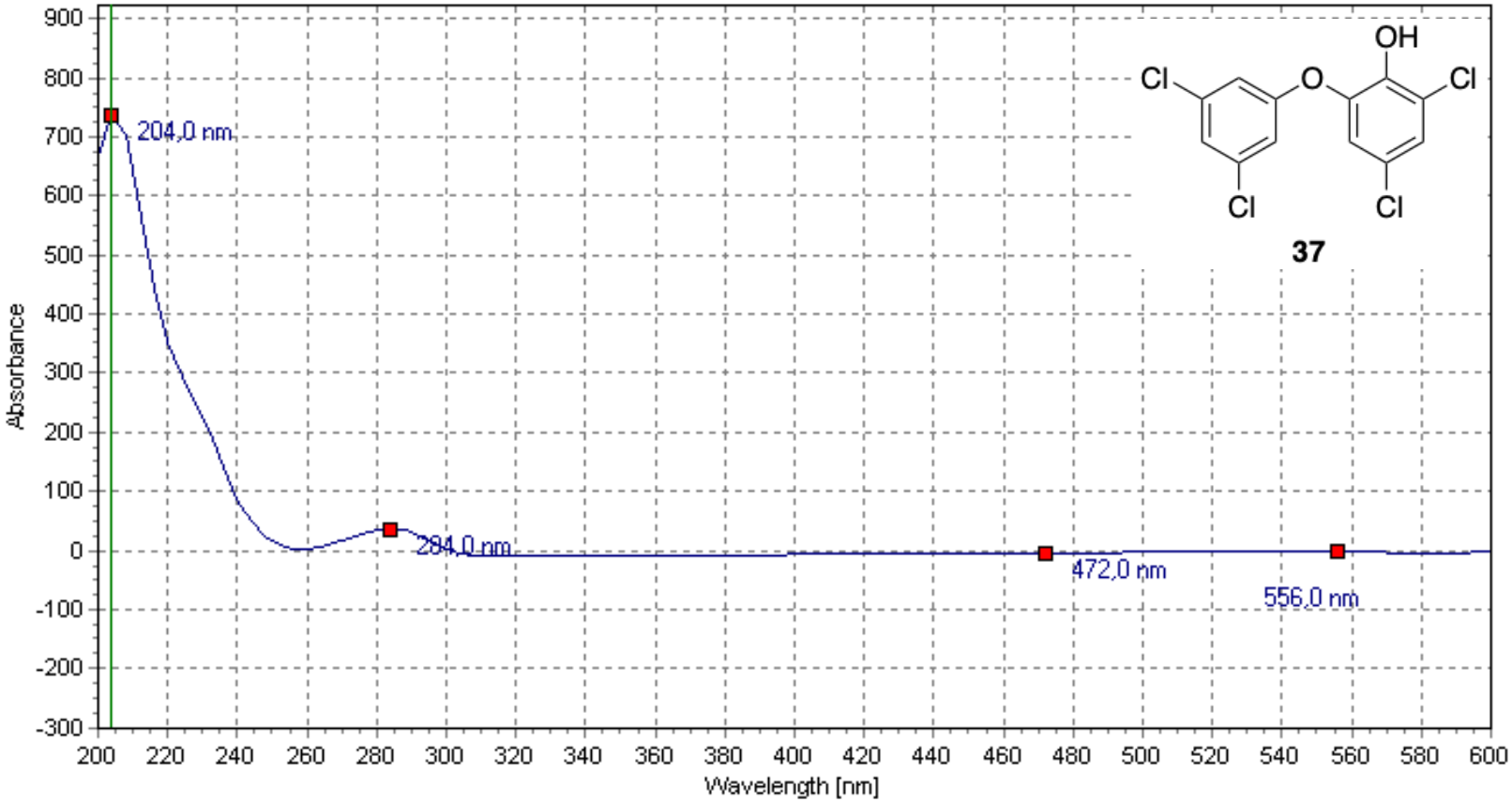


Fig. S85 UV-absorbance measurement of 2,4-dichloro-6-(3,5-dichlorophenoxy)phenol (**37**).

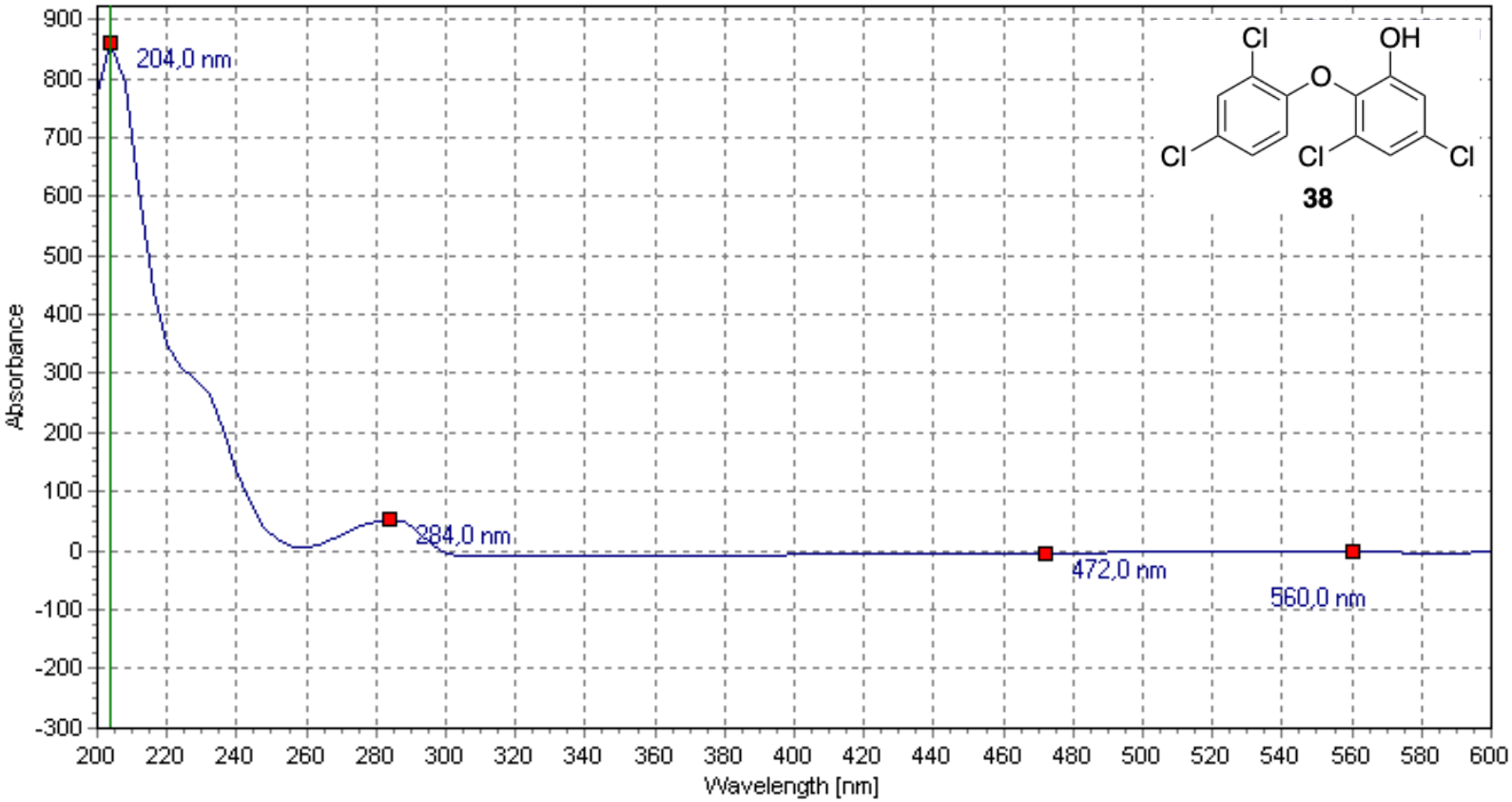


Fig. S86 UV-absorbance measurement of 3,5-dichloro-2-(2,4-dichlorophenoxy)phenol (**38**).

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