

Asymmetric Chemoenzymatic Synthesis of Podophyllotoxin and Related Aryltetralin Lignans

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We report a concise chemoenzymatic synthesis of podophyllotoxin. Highlights of the synthesis include the use of enzymatic oxidative coupling to generate the tetracyclic core of the natural product, and late stage chemical oxidation to install the requisite secondary alcohol.

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Asymmetric Chemoenzymatic Synthesis of Podophyllotoxin and Related Aryltetralin Lignans

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ABSTRACT: Podophyllotoxin is one of the most potent microtubule depolymerizing agents and has served as an important lead compound in antineoplastic drug discovery. Here, we report a short chemoenzymatic total synthesis of podophyllotoxin and related aryltetralin lignans. Vital to this approach is the use of an enzymatic oxidative C–C coupling reaction to construct the tetracyclic core of the natural product in a diastereoselective fashion. This strategy allows gram scale access to deoxypodophyllotoxin and is readily adaptable to the preparation of related aryltetralin lignans.

The aryltetralin lignans are an important family of secondary metabolites possessing a wealth of antiviral, antibacterial, and antineoplastic activities.¹ The most prominent member of the family, podophyllotoxin (**1**, Figure 1A), is a potent microtubule depolymerizer that binds to the colchicine site of the tubulin subunit.² The use of **1** as a lead compound in anticancer drug development has led to the identification of several synthetic derivatives, including etoposide (**2**) and teniposide, which are currently used to treat several types of cancer.³ In contrast to **1**, the antineoplastic properties of these synthetic derivatives arise from inhibition of topoisomerase II during the late S and early G2 stage of the cell cycle. Several other aryltetralin lignans with alternative substitution patterns on the aryl rings have also been identified in nature (e.g., **3–6**).⁴ Interestingly, investigations into the bioactivities of these lignans have suggested that subtle changes in the substitution patterns of the aromatic rings could significantly affect the microtubule depolymerizing activity and therapeutic window of the scaffold.^{4b,d,e} The medicinal importance of this natural product family has spurred numerous chemical approaches,^{5,6} including a recent Pd-catalyzed C–H arylation strategy to **1** by Maimone.^{6c} Despite this progress, an efficient enantioselective approach to aryltetralin lignans has proven elusive thus far. Herein, we describe an asymmetric, chemoenzymatic approach to **1** that features enantioselective construction of the key dibenzylbutyrolactone precursor and harnesses the biocatalytic utility of a non-heme dioxygenase from podophyllotoxin biosynthesis to forge the key C ring of the natural product. We showcase the scalability and practicality of this approach by conducting the biocatalytic transformation on gram scale in excellent yield. Finally, we demonstrate the general utility of this synthetic strategy in the concise synthesis of related aryltetralin lignin natural products. During the preparation of this manuscript, Kroutil, Fuchs and coworkers reported a complementary chemoenzymatic synthesis of deoxypodophyllotoxin, *epi*-podophyllotoxin and podophyllotoxin via biocatalytic kinetic resolution of racemic dibenzylbutyrolactone precursors.⁷ In contrast, our approach establishes the absolute stereochemistry of the dibenzylbutyrolactone precursor at an early stage

and does not require a kinetic resolution process to provide the enantioenriched tetracyclic product in the key enzymatic cyclization step.

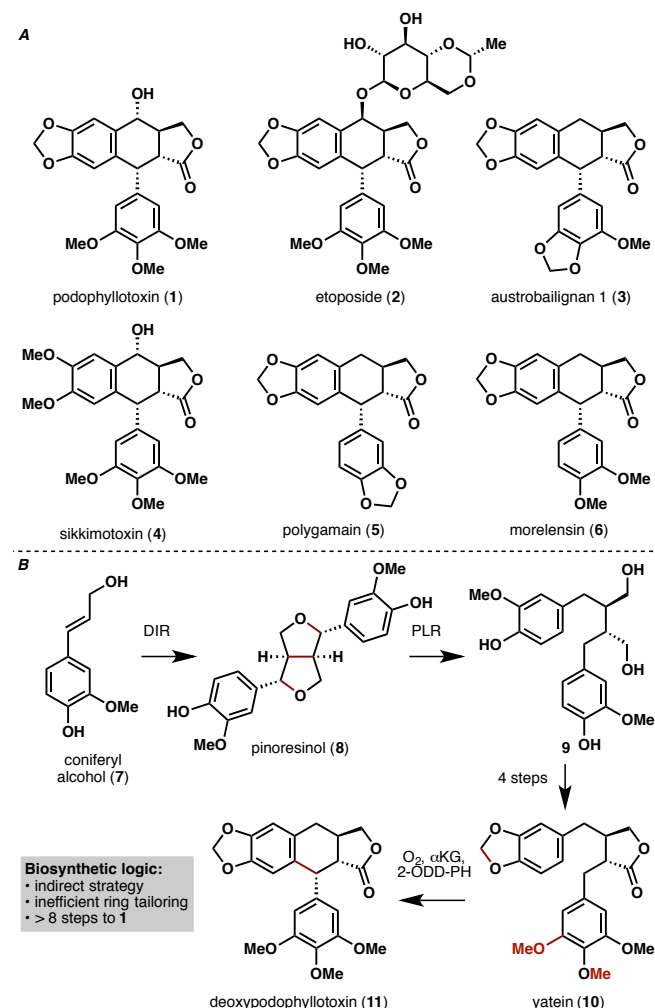


Figure 1. A. Chemical structures of podophyllotoxin (**1**), etoposide (**2**), and related aryltetralin lignans. B. Biosynthetic pathway for the production of deoxypodophyllotoxin (**11**) from coniferyl alcohol (**7**) in *Podophyllum hexandrum*.

Biosynthetically, podophyllotoxin arises from oxidative dimerization of coniferyl alcohol (**7**, Figure 1B).⁸ A proton-coupled electron transfer event in the presence of dirigent protein (DIR) converts **7** to enantioenriched pinoresinol (**8**), which is subsequently reduced by pinocresol lariciresinol reductase (PLR) to the corresponding diol (**9**). Oxidation to the butyrolactone and several ring tailoring events by two P450s and a SAM-dependent methyltransferase affords yatein (**10**). A non-heme

dioxygenase, referred to as 2-ODD-PH, effects an oxidative C–C coupling to convert **10** to deoxypodophyllotoxin (**11**). While the enzymes that are responsible for the conversion of **11** to **1** have not been identified to date, the accumulation of *epi*-podophyllotoxin in tobacco leaves has been observed to be dependent on CYP82D61 expression, implicating the potential role of this enzyme in late-stage tailoring. More recently, *in vitro* studies by Chang, Chien and coworkers provided biochemical confirmation for the role of 2-ODD-PH in the oxidative benzylic coupling step and shed some light on the mechanistic nature of this transformation.⁹

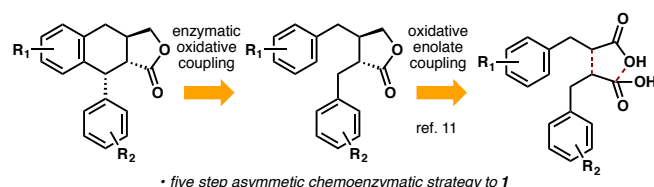
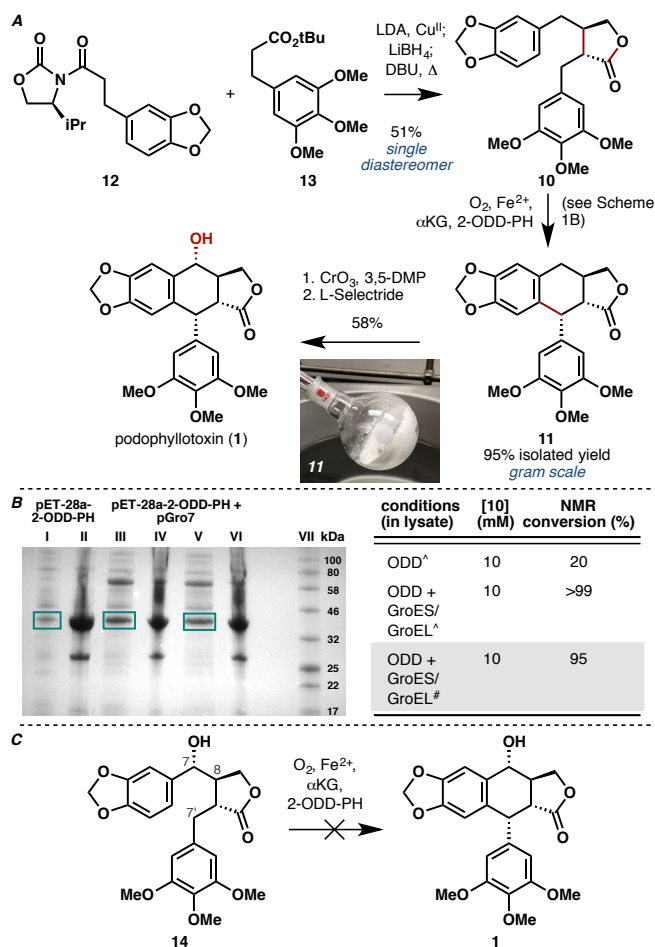


Figure 2. Retrosynthetic analysis of aryltetralin lignans employing chemoenzymatic disconnections.

From synthetic economy perspective,¹⁰ the biosynthetic logic involved in podophyllotoxin production suffers from several non-strategic bond-forming steps. Several steps are expended on the oxidation state adjustments of the butyrolactone moiety as well as on the alteration of the substitution pattern on the aryl rings. We hypothesized that a chemoenzymatic approach that involves rapid asymmetric construction of **10** and harnesses the synthetic utility of 2-ODD-PH in the key C ring formation would enable a more efficient approach towards **11** (Figure 2). A late-stage chemical oxidation on **11** would introduce the requisite secondary alcohol and complete the synthesis of **1**. To this end, we conducted an asymmetric synthesis of **10** through the use of oxidative enolate coupling methodology developed by Baran and coworkers (Scheme 1A).¹¹ Coupling of oxazolidinone **12** and ester **13** in the presence of LDA and Cu^{II} salt effected the formation of the dicarbonyl product as an inconsequential mixture of diastereomers. Subjection of this intermediate to chemoselective reduction with LiBH₄ and thermal equilibration with DBU afforded **10** as a single diastereomer in moderate overall yield and set the stage for the key enzymatic coupling step.

For practical reasons, we elected to conduct the enzymatic transformation using clarified lysate of *E. coli* expressing 2-ODD-PH. When expressed as N-His₆-tagged protein, 2-ODD-PH was observed to exist predominantly in insoluble form (Scheme 1B). Despite the suboptimal enzyme expression, clarified lysate of *E. coli* expressing N-His₆-tagged 2-ODD-PH could be used to catalyze the conversion of **10** to **11** in moderate conversion (ca. 20% conversion on 10 mg scale). To ensure adequate material throughput in our synthesis, we embarked on further enzyme expression optimization. Co-expression of chaperones GroEL and GroES¹² was found to boost the soluble expression level of N-His₆-tagged 2-ODD-PH and in turn enabled full conversion of **10** to **11** using clarified lysate. Gratifyingly, the biotransformation could be conducted on gram scale without any appreciable loss in conversion to afford 95% isolated yield of **11**. Under these conditions, 400 mL of clarified lysate could be produced consistently from 500 mL of expression

culture (pre-lysis OD₆₀₀ = 30) and ca. 1.6 g of **10** could be oxidized in a single pass, translating to a titer yield of more than 3 g/L. With **11** in hand, an efficient strategy to introduce the secondary alcohol at C7 was sought. Prior synthesis of related aryltetralin lignans suggest that a two-step process with NBS and PCC could be employed to introduce ketone functionality at C7.^{6g,13} We found that the use of CrO₃¹⁴ as oxidant allowed for direct access to the C7 ketone product, which in turn could be reduced with L-selectride^{6g} to complete our chemoenzymatic synthesis of podophyllotoxin. Taken together, this route proceeded in five steps in asymmetric fashion with 28% overall yield. It is worth noting that an alternative route to **1** starting from alcohol **14** was also explored as it would hypothetically enable a three-step asymmetric synthesis of **1** from commercial materials (Scheme 1C).¹⁵ However, the use of 2-ODD-PH on **14** failed to elicit the desired C–C coupling to forge the C ring of **1**. This observation is in agreement with the results obtained by Kroutil and Fuchs, who showed that hydroxylation at C7' is favored when **14** is used as substrate.



Scheme 1. **A.** Chemoenzymatic total synthesis of podophyllotoxin (**1**) starting from **12** and **13**. **B.** Improvement in soluble expression of 2-ODD-PH in the presence of chaperones GroES/GroEL and corresponding improvement in the conversion of **10** to **11** using clarified lysate. (I) Induction at OD₆₀₀ = 0.8 with 25 μM IPTG; (II) Insoluble fraction; (III) Induction at OD₆₀₀ = 0.8 with 25 μM IPTG; (IV) Insoluble fraction; (V) Induction at OD₆₀₀ = 1.4 with 25 μM IPTG; (VI) Insoluble fraction; (VII) Protein ladder. ^aReaction conducted at 10 mg scale. [#]Reaction conducted at 1.5 g scale. **C.** Attempted conversion of

advanced intermediate **14** to **1** using 2-ODD-PH as oxidation biocatalyst. See Supporting Information for full experimental details.

To further explore the synthetic utility of this approach, we chose to target the chemoenzymatic preparation of several related aryltetralin lignans (Figure 3). For optimal divergency, dibenzylbutyrolactone precursors **S3**, **S5**, and **S6** were prepared via divergent alkylation¹⁶ of enantiopure butyrolactone **S2**. Despite bearing high structural similarity to yatein, **S3** was unexpectedly converted by 2-ODD-PH to a product (**15**) that is regioisomeric to deoxypodophyllotoxin and deoxysikkimotxin. While **S5** and **S6** could be converted to polygamain (**5**) and morelensin (**6**) by 2-ODD-PH, the reactions had to be performed at lower substrate:enzyme ratio to provide synthetically useful yields of the desired products. Additionally, switching the identity of the southern fragment from 1,2-dimethoxybenzene to 1,2-methylenedioxybenzene led to several fold reduction in reaction conversion. These results indicate that the active site of 2-ODD-PH is highly sensitive to changes in the substitution patterns of the aryl rings. Kroutil and Fuchs showed that the trimethoxybenzene moiety in the southern fragment of the scaffold is essential for successful C–C coupling when substrates containing C7-OH were used. In contrast, our results suggest that substrates containing less electron-rich aromatic rings could still be employed in the reaction when C7-OH is absent. Taken together, these observations point to subtle changes in substrate positioning in the active site when C7-OH is present, which in turn affects the outcome of the oxidative process. Future structural studies on 2-ODD-PH are expected to provide some insights on the key interactions that govern the substrate specificity of the enzyme and inform subsequent engineering work to expand its substrate scope.

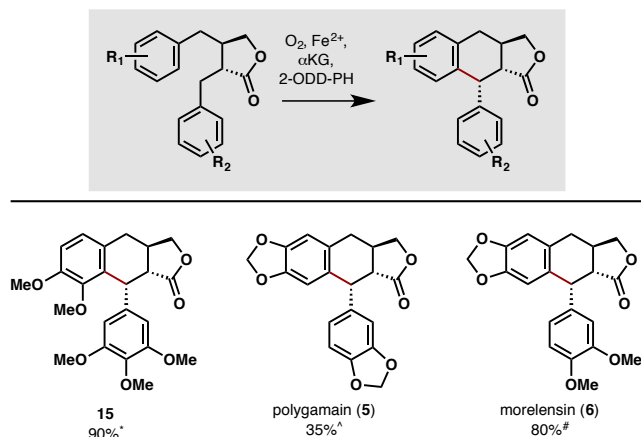


Figure 3. Chemoenzymatic synthesis of **15**, polygamain (**5**), and morelensin (**6**) via late-stage C–C coupling with 2-ODD-PH. *Reaction conducted at 5 mM substrate concentration with pre-lysis OD₆₀₀ = 30. ^Reaction conducted at 1.5 mM substrate concentration with pre-lysis OD₆₀₀ = 30. #Reaction conducted at 4 mM substrate concentration with pre-lysis OD₆₀₀ = 30.

In summary, we have developed a concise chemoenzymatic synthesis of podophyllotoxin and related aryltetralin lignans through the use of three oxidative transformations. A key oxidative enolate coupling between oxazolidinone **12** and ester **13** efficiently furnished yatein (**10**) while establishing the absolute stereoconfiguration at C8 of the butyrolactone motif. In the sub-

sequent key step, biocatalytic C–C coupling catalyzed by 2-ODD-PH constructed the tetracyclic core of podophyllotoxin with complete diastereoselectivity. Finally, a late-stage chemoselective oxidation enabled rapid introduction of the final secondary alcohol at C7. The modularity and the scalability of the route, coupled with the complete avoidance of precious metal catalysts, should render it attractive for both scale-up production of **1** and future medicinal chemistry exploration. Similar to our previous work in the use of nonheme dioxygenases in the synthesis of complex alkaloids and morphed peptides,^{12b,17} this work provides further evidence for the biocatalytic utility of non-heme dioxygenases in streamlining the synthesis of medically relevant natural product scaffolds.

Experimental Section

See Supporting Information for Experimental Details.

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Asymmetric Chemoenzymatic Synthesis of Podophyllotoxin and Related Aryltetralin Lignans*Jian Li,⁺ Xiao Zhang,⁺ Hans Renata***Contribution from the Department of Chemistry, The Scripps Research Institute, 130 Scripps Way, Jupiter, FL 33458***SUPPLEMENTARY MATERIAL****Table of Contents**

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General materials and methods

Unless otherwise noted, all chemicals and reagents for chemical reactions were purchased at the highest commercial quality and used without further purification. Reactions were monitored by thin layer chromatography (TLC) and liquid chromatography/mass spectrometry (LC/MS). TLC was performed with 0.25 mm E. Merck silica plates (60F-254) using short-wave UV light as the visualizing agent, and ninhydrin, KMnO_4 , ammonium molybdate, or phosphomolybdic acid and heat as developing agents. LC/MS was performed with Agilent 1260 Infinity System equipped with Poroshell 120 EC-C18 column (3.0 x 50 mm, 2.7 micron). NMR spectra were recorded on a Bruker AVANCE AV400 (400 MHz and 100 MHz) or Bruker AVANCE AV600 (600 MHz and 151 MHz). Signal positions were recorded in ppm with the abbreviations s, d, t, q, br, m and app denoting singlet, doublet, triplet, quartet, broad, multiplet and apparent respectively. All NMR chemical shifts were referenced to residual solvent peaks or to $\text{Si}(\text{CH}_3)_4$ as an internal standard. Spectra recorded in CDCl_3 were referenced to residual CHCl_3 at 7.26 ppm for ^1H or 77.16 ppm for ^{13}C . All coupling constants J are quoted in Hz. Optical rotations were measured on Autopol IV polarimeter (Rudolph Research Analytical). Sonication was performed using a Qsonica Q500 sonicator. Biochemicals and media components were purchased from standard commercial sources.

Protein and DNA sequences

Protein sequence of 2-ODD-PH (Uniprot accession code: A0A0N9HQ36)

MGSTAPLRLPVIDLSMKNLKPGETTSWNSVRTQVREALEEYGCFEAVIDAVSPELQKAVCNKGHE
LLNLPLETKMLNGNKPEYDGFTSIPNLNEGIMGVGRITDLEKVERFTNLMWPEGNKDFCETVYSY
GKRMAEVDHILKMMVFESFGMEKHFDSESTNYLLHFMRYQQPGKDGRSPALSLHKDKSILTI
VNQNDVKGLEFETKDGWILPTADNHIVLLGDCFMAWSNGRLHSPLHRVTLVANQARLSTSSFS
FPKDI IETPAELVDEEHPLLFNPF EITELLAYCF TKEGAKAVCDLKQYKAYTGA*

DNA sequence of 2-ODD-PH

ATGGGCAGCACCGCGCCGCTGCGTTTCCCGGTTATCGATTTGAGCATGAAGAACCTGAAACCTG
GAACCACATCATGGAACAGCGTACGCACTCAGGTGCGTGAAGCTTTGGAAGAATATGGTTGCTT
TGAAGCCGTTATTGATGCGGTTTCCCCAGAGTTGCAAAAAGCTGTCTGCAACAAGGGGCACGAA
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GGTAAGCGCATGGCAGAGGTTGACCATATTCTGAAGATGATGGTATTCGAATCTTTTGGTATGG
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ACCCGGCAAAGACGGTCGCTCTCCCGCATTTGAGTTTACACAAAGATAAATCTATCCTTACGATC
GTCAACCAAAACGATGTAAAAGGACTGGAGTTTCGAAACAAAGGATGGCGAATGGATTTTGCCAA
CAGCCGATAACCACATCGTATTACTTGGAGATTGCTTCATGGCTTGGAGTAACGGTCGCCTGCA
CTCTCCACTGCACCGTGTGACATTAGTGGCAAATCAGGCACGTTTGAGTACCTCCTCATTTAGC
TTCCCAAAAGATATTATTGAGACACCGGCGGAATTGGTGGACGAGGAACATCCGCTTTTATTCA
ATCCCTTCGAGATCACCGAGCTTTTAGCCTACTGTTTTACAAAAGAAGGAGCCAAGGCGGTTTG
CGATTTAAAGCAGTATAAAGCGTACACTGGAGCATGA

Construction of expression vector for 2-ODD-PH and preparation of E. coli BL21(DE3) strain containing pET-28a(+)-2-ODD-PH

The expression vector for 2-ODD-PH was constructed via gene synthesis by Twist Bioscience. Briefly, the aforementioned DNA sequence was inserted between the NdeI and XhoI restriction sites of pET-28a(+) vector. The resulting expression vectors were used directly to transform electrocompetent *E. coli* strain BL21(DE3). *E. coli* strains were stored as glycerol stocks at –80 °C.

Preparation of E. coli BL21(DE3) strain containing pET-28a(+)-2-ODD-PH and pGro7 plasmids

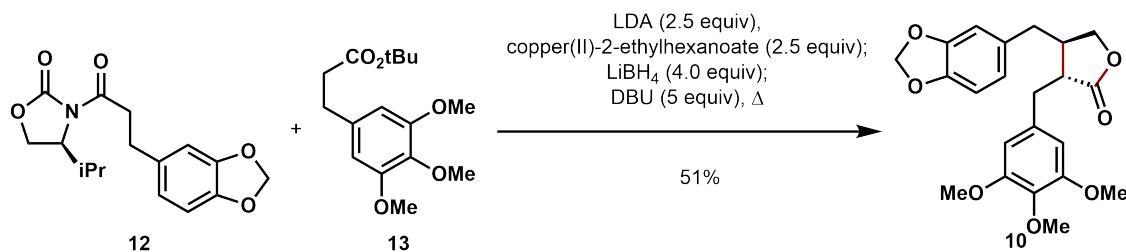
Electrocompetent BL21(DE3) *E. coli* was transformed with isolated pET-28a(+)-2-ODD-PH and

pGro7 plasmids by electroporation and plated on LB/agarose plate containing 25 µg/mL chloroamphenicol and 50 µg/mL kanamycin.

Preparation of clarified lysate of *E. coli* expressing 2-ODD-PH and GroES/GroEL for large-scale biotransformation

An overnight culture of recombinant *E. coli* BL21(DE3) cells was used to inoculate 2 x 500 mL TB media (in 2 x 2 L non-beveled Erlenmeyer flasks) containing 50 µg/mL kanamycin and 25 µg/mL chloramphenicol. The cultures were shaken at 250 rpm at 37 °C for roughly 2.5 hours or until an optical density (OD₆₀₀) of 0.8–1.0 was reached. Cultures were cooled on ice (20 min) and then induced by adding IPTG and arabinose to final concentrations of 0.025 mM and 1 mg/mL, respectively. The cultures were allowed to continue for another 20 hours at 22 °C and shaking at 250 rpm. Cells were harvested by centrifugation (4 °C, 15 min, 4,000xg), and the cell pellet was resuspended to OD₆₀₀ = 30 in 50 mM KPi (pH = 8.0). This procedure reliably yielded *ca.* 2 x 400 mL of cell suspension. Cells were disrupted by sonication (3 min, cycle = 1 s on/4 s off, 50% amplitude) and pelleted by centrifugation at 4,000 rpm for 15 min at 4 °C.

Synthetic procedures



This compound was prepared according to Baran's procedure with minor modifications. To a solution of LDA (1.25 M in THF/hexane 1:1, 20 mL, 2.5 equiv) was added a solution of **12** (3.05 g, 10 mmol, 1.0 equiv) in THF (5 mL) at -78 °C. The reaction mixture was stirred at -78 °C for

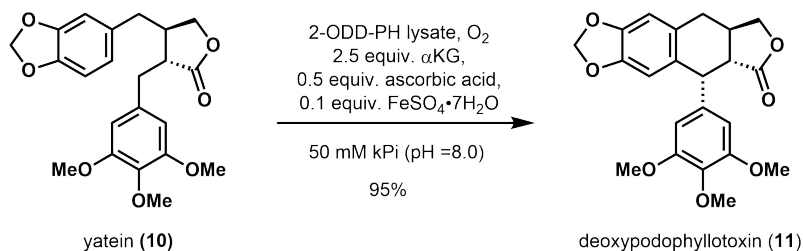
20 minutes before a solution of **13** (2.06 g, 10 mmol, 1.0 equiv) in THF (5 mL) was added. After stirring for 20 minutes, copper (II)-2-ethylhexanoate (8.75 g, 25 mmol, 2.5 equiv) was added in one batch. The reaction mixture was slowly warmed up to 0 °C in 30 min before it was quenched with 2 M NH₄OH (150 mL) and extracted with ethyl acetate (100 mL x 3). The combined organic extracts were washed with brine (200 mL), dried over anhydrous MgSO₄, filtered, and concentrated. The residue was then dissolved into THF (100 mL) and LiBH₄ (2.0 M in THF, 20 mL, 40.0 mmol, 4.0 equiv) was added to the solution at 0 °C. The reaction mixture was stirred at 0 °C for 40 minutes before it was quenched with sat. aq. NH₄Cl (50 mL) and extracted with ethyl acetate (20 mL × 3). The combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The crude oil was dissolved in toluene (100 mL). 1,8-diazabicyclo[5.4.0]undec-7-ene (7.53 mL, 50 mmol, 5 equiv) was added and the reaction was heated to 110 °C for 24 h. Upon cooling to ambient temperature, the reaction was quenched with 5% aqueous citric acid (150 mL). The aqueous layer was extracted with ethyl acetate (100 mL). The organic portions were combined, washed with brine (100 mL), dried over anhydrous MgSO₄, filtered, and concentrated. The resulting oil was purified by flash chromatography (silica gel, hexanes:ethyl acetate 4:1) to yield yatein **10** (2.04 g, 51% yield) as a white foam.

¹H NMR (400 MHz, CDCl₃): δ = 6.69 (dd, *J* = 7.4, 0.8 Hz, 1H), 6.47 (d, *J* = 1.9 Hz, 1H), 6.45 (s, 1H), 6.35 (s, 2H), 5.95–5.90 (m, 2H), 4.17 (dd, *J* = 9.2, 7.2 Hz, 1H), 3.89–3.81 (m, 2 H), 3.82 (s, 7 H), 3.82 (s, 3 H), 2.97–2.81 (m, 2 H), 2.67–2.33 (m, 2 H).

¹³C NMR (101 MHz, CDCl₃): δ = 178.6, 153.4, 148.0, 146.5, 133.5, 131.6, 121.7, 108.9, 108.4, 106.3, 101.2, 71.3, 61.0, 56.2, 46.6, 41.1, 38.5, 35.3.

HRMS (*m/z*): calcd for C₂₂H₂₅O₇⁺ ([M+H]) 401.1595, found 401.1602

$$[\alpha]_D^{24} = -27.4 \text{ (c = 0.6, CHCl}_3\text{)}$$



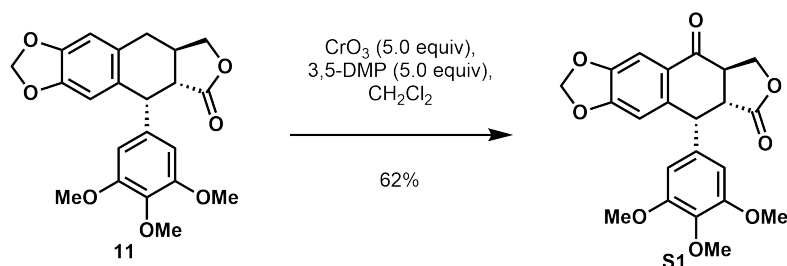
A 2 L Erlenmeyer flask was charged with a solution of yatein (**10**) (1.55 g, 3.875 mmol., 10.0 mM final concentration) in 20 mL DMSO, L-ascorbic acid (341 mg, 1.938 mmol, 0.5 equiv.), and α -ketoglutaric acid (disodium salt dihydrate, 2.19 g, 9.375 mmol, 2.5 equiv.). 388 mL 2-ODD-PH lysate ($OD_{600} = 30$) was added to the flask, followed by FeSO₄·7H₂O (108 mg, 0.388 mmol, 0.1 equiv.). The reaction was shaken for 20 h at 23 °C, 200 rpm under air. The pH of the reaction was adjusted to 5 by 1 N HCl and the mixture was diluted with ethyl acetate (400 mL). The resulting emulsion was filtered through Celite to give a separable two-phase system. The organic layer was washed with water (100 mL), followed by brine (100 mL), dried over anhydrous Na₂SO₄, and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (silica gel, hexanes:ethyl acetate 2:1) to provide **11** (1.46 g, 3.67 mmol, 95%) as a white solid.

¹H NMR (400 MHz, CDCl₃): δ = 6.66 (s, 1H), 6.51 (s, 1H), 6.34 (s, 2H), 5.93 (dd, J = 8.9, 1.4 Hz, 2H), 4.63–4.57 (m, 1H), 4.49–4.39 (m, 1H), 3.94–3.88 (m, 1H), 3.80 (s, 3H), 3.74 (s, 6H), 3.14–2.99 (m, 1H), 2.80–2.66 (m, 3H).

¹³C NMR (101 MHz, CDCl₃): δ = 175.1, 152.6, 147.1, 146.9, 137.1, 136.4, 130.7, 128.4, 110.6, 108.6, 108.4, 101.3, 72.2, 60.9, 56.3, 47.6, 43.8, 33.2, 32.9.

HRMS (m/z): calcd for C₂₂H₂₃O₇⁺ ([M+H]) 399.1438, found 399.1459

$$[\alpha]_{\text{D}}^{24} = -102 \text{ (c = 0.2, CHCl}_3\text{)}$$



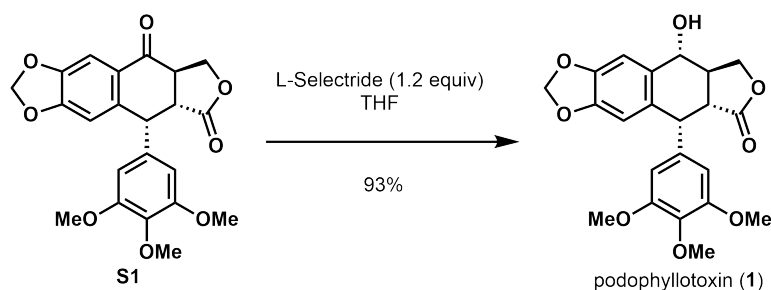
To a solution of CrO_3 (283 mg, 2.84 mmol, 5.0 equiv) in CH_2Cl_2 (1.5 mL) was added 3,5-dimethylpyrazole (273 mg, 2.84 mmol, 5.0 equiv) at 0 °C. The reaction mixture was stirred at 0 °C for 20 minutes before a solution of **11** (226 mg, 0.568 mmol, 1.0 equiv) in CH_2Cl_2 (0.5 mL) was added. The reaction mixture was stirred at 0 °C for 2 h before it was quenched with sat. aq. NaHSO_3 (50 mL) and extracted with ethyl acetate (3×20 mL). The organic portions were combined, washed with brine (100 mL), dried over anhydrous MgSO_4 , filtered, and concentrated. The resulting oil was purified by flash chromatography (silica gel, hexanes:ethyl acetate 4:1) to yield podophyllotoxone **S1** (145 mg, 62% yield) as a white solid.

^1H NMR (400 MHz, CDCl_3): δ = 7.55 (s, 1H), 6.70 (s, 1H), 6.38 (s, 2H), 6.09 (dd, J = 7.6, 1.2 Hz, 3H), 4.84 (d, J = 4.3 Hz, 1H), 4.56 (dd, J = 9.3, 7.6 Hz, 1H), 4.39–4.30 (m, 1H), 3.82 (s, 3H), 3.75 (s, 6H), 3.52 (ddd, J = 15.6, 10.5, 7.6 Hz, 1H), 3.28 (dd, J = 15.5, 4.3 Hz, 1H).

^{13}C NMR (101 MHz, CDCl_3): δ = 192.6, 173.2, 153.4, 153.2, 148.3, 141.7, 132.3, 128.3, 109.8, 107.8, 106.3, 102.5, 67.2, 60.9, 56.4, 46.9, 44.8, 43.6.

HRMS (m/z): calcd for $\text{C}_{22}\text{H}_{21}\text{O}_8^+$ ($[\text{M}+\text{H}]$) 413.1231, found 413.1251

$$[\alpha]_{\text{D}}^{24} = -114 \text{ (c = 0.8, CHCl}_3\text{)}$$



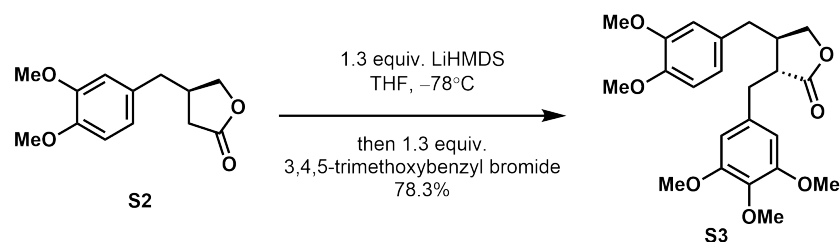
To a solution of **S1** (145 mg, 0.352 mmol, 1.0 equiv) in THF (5 mL) was added L-Selectride (1 M in THF, 0.42 mL, 1.2 equiv.) at $-78\text{ }^{\circ}\text{C}$. The reaction mixture was stirred at $-78\text{ }^{\circ}\text{C}$ for 15 min before it was quenched with sat. aq. NH_4Cl (5 mL) and extracted with ethyl acetate ($3 \times 10\text{ mL}$). The organic phase was washed with brine (100 mL), dried over anhydrous MgSO_4 , filtered, and concentrated. The resulting oil was purified by flash chromatography (silica gel, hexanes:ethyl acetate 4:1 to 1:1) to yield podophyllotoxin **1** (136 mg, 93% yield) as a white solid.

^1H NMR (600 MHz, CDCl_3): δ = 7.11 (s, 1H), 6.51 (s, 1H), 6.37 (s, 2H), 5.99 (d, J = 1.4 Hz, 1H), 5.97 (d, J = 1.4 Hz, 1H), 4.77 (t, J = 8.2 Hz, 1H), 4.63–4.58 (m, 2H), 4.09 (dd, J = 10.2, 8.8 Hz, 1H), 3.81 (s, 3H), 3.76 (s, 6H), 2.84 (dd, J = 14.3, 4.7 Hz, 1H), 2.81–2.72 (m, 1H), 2.06 (br, 1H).

^{13}C NMR (151 MHz, CDCl_3): δ = 174.5, 152.8, 148.0, 147.9, 137.4, 135.5, 133.2, 131.4, 110.0, 108.5, 106.4, 101.6, 73.0, 71.4, 60.9, 56.5, 45.5, 44.2, 41.0.

HRMS (m/z): calcd for $\text{C}_{22}\text{H}_{23}\text{O}_8^+$ ($[\text{M}+\text{H}]$) 415.1387, found 415.1385

$[\alpha]_{\text{D}}^{24}$ = -110 (c = 1.0, CHCl_3)



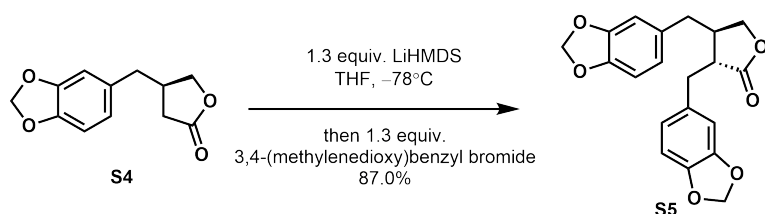
To a stirred solution of **S2**^{S1} (100 mg, 0.423 mmol) in 4 mL dry THF at -78°C was added 1.0 M solution of LHMDS in THF (0.55 mL, 1.3 equiv.). The reaction was allowed to stir for 1 hour before 3,4,5-trimethoxybenzyl bromide (144 mg, 0.55 mmol, 1.3 equiv.) in 2 mL THF was added dropwise. After two hours, TLC analysis showed complete consumption of starting material. The reaction was quenched by addition of 1 mL of saturated aqueous NH_4Cl . The mixture was extracted with 10 mL ethyl acetate and the organic layer was washed with water (10 mL), followed by brine (10 mL) and then dried over anhydrous Na_2SO_4 . Flash chromatography purification (silica gel, hexanes:ethyl acetate 2:1) gave **S3** (138 mg, 0.331 mmol, 78%) as colorless oil.

^1H NMR (600 MHz, CDCl_3): δ = 6.75 (d, J = 8.1 Hz, 1H), 6.55 (dd, J = 8.1, 2.0 Hz, 1H), 6.50 (d, J = 2.0 Hz, 1H), 6.36 (s, 2H), 4.17 (dd, J = 9.1, 7.3 Hz, 1H), 3.92–3.86 (m, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.82 (s, 3H), 3.82 (s, 6H), 2.97–2.87 (m, 2H), 2.67 (dd, J = 13.5, 6.0 Hz, 1H), 2.63–2.47 (m, 2H).

^{13}C NMR (151 MHz, CDCl_3): δ = 178.8, 153.4, 149.2, 148.0, 137.0, 133.6, 130.5, 120.6, 112.0, 111.5, 106.4, 71.4, 61.0, 56.3, 56.0, 56.0, 46.7, 41.2, 38.3, 35.2.

HRMS (m/z): calcd for $\text{C}_{23}\text{H}_{29}\text{O}_7^+$ ($[\text{M}+\text{H}]$) 417.1908, found 417.1908

$[\alpha]_D^{24}$ = -28.0 (c = 0.5, CHCl_3)



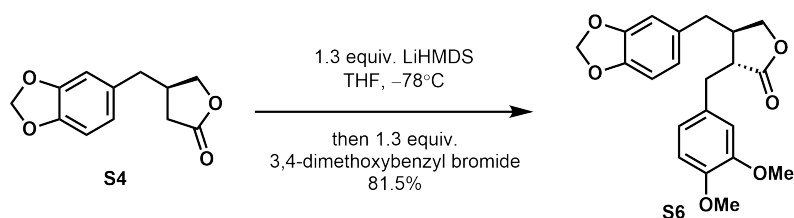
To a stirred solution of **S4**^{S2} (100 mg, 0.454 mmol) in 4 mL dry THF at -78°C was added 1.0 M solution of LHMDS in THF (0.59 mL, 1.3 equiv.). The reaction was allowed to stir for 1 hour before 3,4-(methylenedioxy)benzyl bromide (127 mg, 0.59 mmol, 1.3 equiv.) in 2 mL THF was added dropwise. After two hours, TLC showed complete consumption of starting material. The reaction was quenched by addition of 1 mL of saturated aqueous NH_4Cl . The mixture was extracted with 10 mL ethyl acetate and the organic layer was washed with water (10 mL), followed by brine (10 mL) and then dried over anhydrous Na_2SO_4 . Flash chromatography purification (silica gel, hexanes:ethyl acetate 2:1) gave **S5** (140 mg, 0.395 mmol, 87%) as colorless oil.

^1H NMR (600 MHz, CDCl_3): δ = 6.73 (d, J = 7.9 Hz, 1H), 6.70 (d, J = 8.3 Hz, 1H), 6.63 (d, J = 1.7 Hz, 1H), 6.60 (dd, J = 7.9, 1.8 Hz, 1H), 6.49–6.44 (m, 2H), 5.96–5.90 (m, 4H), 4.12 (dd, J = 9.3, 7.1 Hz, 1H), 3.86 (dd, J = 9.2, 7.2 Hz, 1H), 2.98 (dd, J = 14.1, 5.1 Hz, 1H), 2.84 (dd, J = 14.1, 7.4 Hz, 1H), 2.63–2.56 (m, 1H), 2.53 (ddd, J = 8.2, 7.3, 5.1 Hz, 1H), 2.50–2.41 (m, 2H).

^{13}C NMR (151 MHz, CDCl_3): δ = 178.6, 148.0, 148.0, 146.6, 146.5, 131.7, 131.5, 122.4, 121.7, 109.6, 108.9, 108.5, 108.4, 101.2, 101.1, 71.3, 46.6, 41.4, 38.5, 35.0.

HRMS (m/z): calcd for $\text{C}_{20}\text{H}_{19}\text{O}_6^+$ ($[\text{M}+\text{H}]$) 355.1176, found 355.1192.

$[\alpha]_D^{24}$ = -25.3 (c = 1.0, CHCl_3)



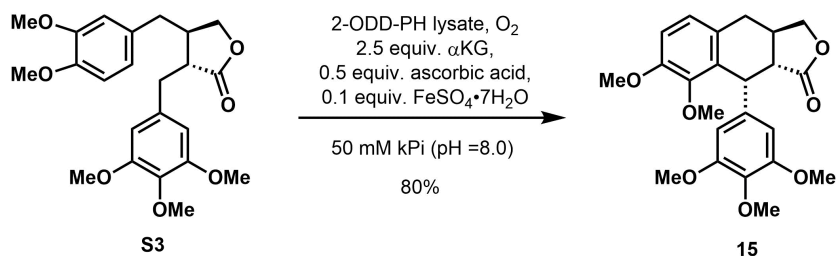
To a stirred solution of **S4** (100 mg, 0.454 mmol) in 4 mL dry THF at -78°C was added 1.0 M solution of LHMDS in THF (0.59 mL, 1.3 equiv.). The reaction was allowed to stir for 1 hour before 3,4-dimethoxybenzyl bromide (154 mg, 0.59 mmol, 1.3 equiv.) in 2 mL THF was added dropwise. After two hours, TLC showed complete consumption of starting material. The reaction was quenched by addition of 1 mL of saturated aqueous NH_4Cl . The mixture was extracted with 10 mL ethyl acetate and the organic layer was washed with water (10 mL), followed by brine (10 mL) and then dried over anhydrous Na_2SO_4 . Flash chromatography purification (silica gel, hexanes:ethyl acetate 2:1) gave **S6** (137 mg, 0.370 mmol, 82%) as colorless oil.

^1H NMR (600 MHz, CDCl_3): δ = 6.78 (d, J = 8.1 Hz, 1H), 6.71–6.64 (m, 3H), 6.45 (dd, J = 7.8, 1.8 Hz, 1H), 6.43 (d, J = 1.7 Hz, 1H), 5.93 (d, J = 1.5 Hz, 1H), 5.92 (d, J = 1.5 Hz, 1H), 4.11 (dd, J = 9.2, 7.1 Hz, 1H), 3.89–3.87 (m, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 2.96 (dd, J = 14.1, 5.1 Hz, 1H), 2.89 (dd, J = 14.1, 7.1 Hz, 1H), 2.63–2.53 (m, 2H), 2.52–2.44 (m, 2H).

^{13}C NMR (151 MHz, CDCl_3): δ = 178.7, 149.2, 148.1, 148.0, 146.5, 131.7, 130.2, 121.7, 121.5, 112.3, 111.2, 108.9, 108.4, 101.2, 71.3, 56.0, 55.9, 46.6, 41.2, 38.4, 34.7.

HRMS (m/z): calcd for $\text{C}_{21}\text{H}_{23}\text{O}_6^+$ ($[\text{M}+\text{H}]$) 371.1489, found 371.1515.

$[\alpha]_{\text{D}}^{24}$ = -22.4 (c = 1.0, CHCl_3)



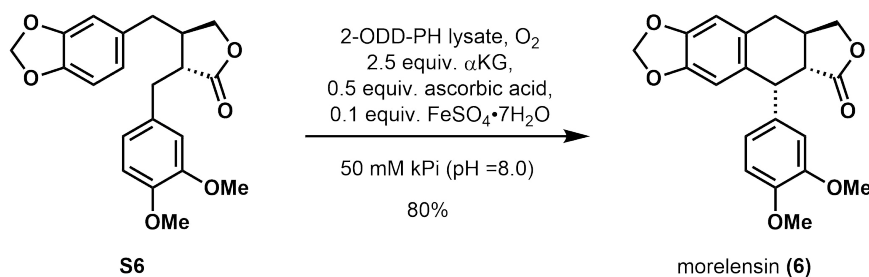
A 100 mL Erlenmeyer flask was charged with a solution of **S3** (41.7 mg, 0.10 mmol, 5.0 mM final concentration) in 1.5 mL DMSO, L-ascorbic acid (8.8 mg, 0.05 mmol, 0.5 equiv.), and α -ketoglutaric acid (disodium salt dihydrate, 56.5 mg, 0.25 mmol, 2.5 equiv.). 20 mL 2-ODD-PH lysate ($OD_{600} = 30$) was added to the flask, followed by $FeSO_4 \cdot 7H_2O$ (2.78 mg, 0.01 mmol, 0.1 equiv.). The reaction was shaken for 20 h at 23 °C, 200 rpm under air. The pH of the reaction was adjusted to 5 by addition of 1 N HCl and the mixture was diluted with ethyl acetate (20 mL). The resulting emulsion was filtered through Celite to give a separable two-phase system. The organic layer was washed with water (10 mL), followed by brine (10 mL), dried over anhydrous Na_2SO_4 , then concentrated *in vacuo*. The crude residue was purified by flash chromatography (silica gel, hexanes:ethyl acetate 2:1) to provide **15** (33.0 mg, 0.0795 mmol, 80%) as colorless oil.

1H NMR (600 MHz, $CDCl_3$): δ = 6.95 (d, J = 8.5 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 6.37 (s, 2H), 5.02 (d, J = 4.8 Hz, 1H), 4.47 (dd, J = 8.5, 6.6 Hz, 1H), 3.91 (dd, J = 10.1, 8.4 Hz, 1H), 3.83 (s, 3H), 3.79 (s, 3H), 3.73 (s, 6H), 3.38 (s, 3H), 3.16 (d, J = 10.5 Hz, 1H), 2.83–2.73 (m, 2H), 2.67–2.57 (m, 1H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 175.2, 152.5, 151.5, 147.3, 137.1, 136.8, 132.7, 127.8, 124.5, 112.2, 108.1, 72.3, 60.9, 60.2, 56.4, 56.0, 47.5, 38.6, 33.0, 32.7.

HRMS (m/z): calcd for $C_{23}H_{27}O_7^+$ ($[M+H]$) 415.1751, found 415.1762.

$[\alpha]_D^{24}$ = -42.7 (c = 0.7, $CHCl_3$)



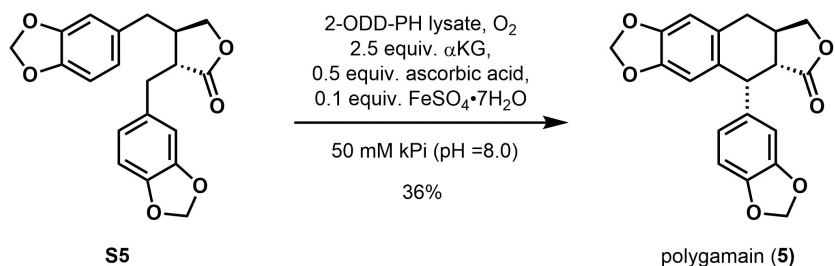
A 100 mL Erlenmeyer flask was charged with a solution of **S6** (37.0 mg, 0.10 mmol, 4.0 mM final concentration) in 1.5 mL DMSO, L-ascorbic acid (8.8 mg, 0.05 mmol, 0.5 equiv.), and α -ketoglutaric acid (disodium salt dihydrate, 56.5 mg, 0.25 mmol, 2.5 equiv.). 25 mL 2-ODD-PH lysate ($OD_{600} = 30$) was added to the flask, followed by $FeSO_4 \cdot 7H_2O$ (2.78 mg, 0.01 mmol, 0.1 equiv.). The reaction was shaken for 20 h at 23 °C, 200 rpm under air. The pH of the reaction was adjusted to 5 by addition of 1 N HCl and the mixture was diluted with ethyl acetate (20 mL). The resulting emulsion was filtered through Celite to give a separable two-phase system. The organic layer was washed with water (10 mL), followed by brine (10 mL), dried over anhydrous Na_2SO_4 , then concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (silica gel, hexanes:ethyl acetate 4:1) to provide **6** (29.5 mg, 0.0801 mmol, 80%) as colorless oil.

1H NMR (600 MHz, $CDCl_3$): δ = 6.96 (d, J = 2.1 Hz, 1H), 6.68 (d, J = 8.3 Hz, 1H), 6.66 (s, 1H), 6.51 (s, 1H), 6.37 (ddd, J = 8.3, 2.2, 0.6 Hz, 1H), 5.96–5.88 (m, 2H), 4.61 (d, J = 3.6 Hz, 1H), 4.49–4.35 (m, 1H), 3.97–3.87 (m, 1H), 3.85 (s, 3H), 3.81 (s, 3H), 3.10–2.97 (m, 1H), 2.79–2.64 (m, 3H).

^{13}C NMR (151 MHz, $CDCl_3$): δ = 175.2, 148.4, 148.1, 147.1, 146.9, 133.5, 131.2, 128.4, 122.7, 114.6, 110.6, 110.4, 108.6, 101.3, 72.3, 56.1, 55.9, 47.6, 43.3, 33.3, 32.7.

HRMS (m/z): calcd for $C_{21}H_{21}O_6^+$ ($[M+H]$) 369.1333, found 369.1334.

$[\alpha]_D^{24}$ = –97.2 (c = 0.4, $CHCl_3$)



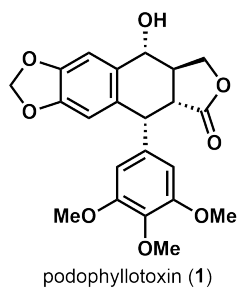
A 250 mL Erlenmeyer flask was charged with a solution of **S5** (35.5 mg, 0.10 mmol., 1.5 mM final concentration) in 3.35 mL DMSO, L-ascorbic acid (8.8 mg, 0.05 mmol, 0.5 equiv.), and α -ketoglutaric acid (disodium salt dihydrate, 56.5 mg, 0.25 mmol, 2.5 equiv.). 67 mL 2-ODD-PH lysate ($OD_{600} = 30$) was added to the flask, followed by $FeSO_4 \cdot 7H_2O$ (2.78 mg, 0.01 mmol, 0.1 equiv.). The reaction was shaken for 20 h at 23 °C, 200 rpm under air. The pH of the reaction was adjusted to 5 by addition of 1 N HCl and the mixture was diluted with ethyl acetate (30 mL). The resulting emulsion was filtered through Celite to give a separable two-phase system. The organic layer was washed with water (10 mL), followed by brine (10 mL), dried over anhydrous Na_2SO_4 , then concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (silica gel, hexanes:ethyl acetate 4:1) to provide **5** (12.5 mg, 0.0355 mmol, 36%) as colorless oil.

1H NMR (600 MHz, $CDCl_3$): δ = 6.68 (dd, J = 7.9, 0.6 Hz, 1H), 6.65 (s, 1H), 6.62–6.59 (m, 2H), 6.47 (s, 1H), 5.92 (s, 2H), 5.90 (d, J = 1.5 Hz, 1H), 5.89 (d, J = 1.5 Hz, 1H), 4.56 (d, J = 4.6 Hz, 1H), 4.47–4.41 (m, 1H), 3.94–3.88 (m, 1H), 3.06 (d, J = 10.8 Hz, 1H), 2.82–2.65 (m, 2H).

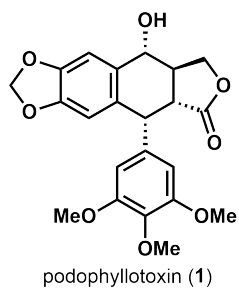
^{13}C NMR (151 MHz, $CDCl_3$): δ = 174.9, 147.4, 147.2, 147.0, 146.6, 134.6, 131.2, 128.3, 124.3, 111.3, 110.5, 108.6, 107.8, 101.3, 101.1, 72.2, 47.5, 43.4, 33.2, 32.7.

HRMS (m/z): calcd for $C_{20}H_{17}O_6^+$ ($[M+H]$) 353.1020, found 353.1033.

$[\alpha]_D^{24}$ = –34.0 (c = 0.1, $CHCl_3$)

Table S1. ^1H NMR comparison table for podophyllotoxin (CDCl_3).

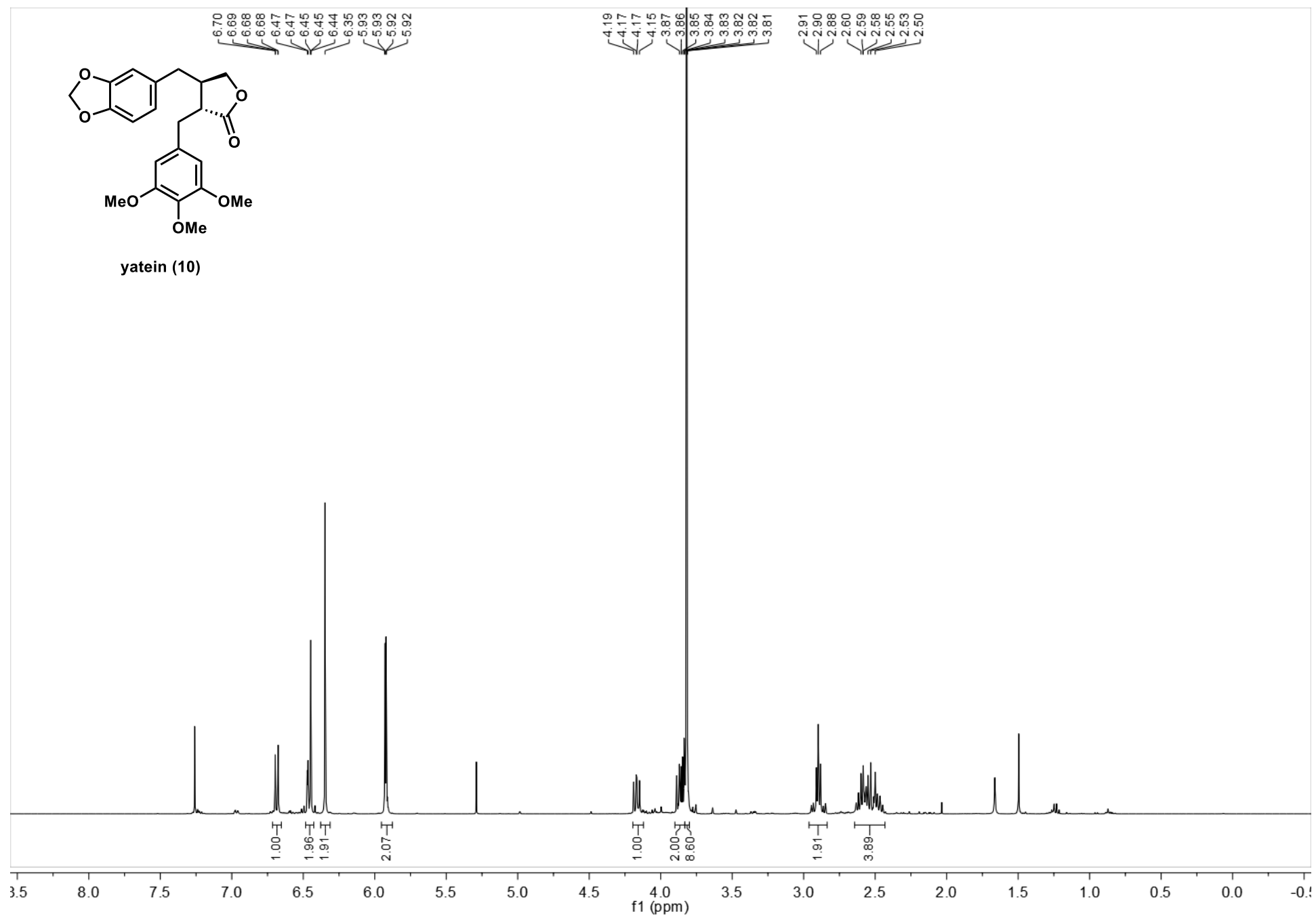
ref. S3 δ ^1H [ppm, mult, J (Hz)] 360 MHz		Synthetic δ ^1H [ppm, mult, J (Hz)] 600 MHz		Err $\Delta\delta$ (ppm)
7.11	1 H, s	7.11	1 H, s	0
6.51	1 H, s	6.51	1 H, s	0
6.37	2 H, s	6.37	2 H, s	0
5.99	1 H, s	5.99	1 H, d, $J = 1.4$ Hz	0
5.97	1 H, s	5.97	1 H, d, $J = 1.4$ Hz	0
4.77	1 H, t, $J = 8.4$	4.77	1H, t, $J = 8.2$ Hz	0
4.60	1 H, t, $J = 8.0$	4.63–4.58	2 H, m	–
4.59	1 H, d, $J = 4.4$			
4.08	1 H, dd, $J = 9.6, 8.8$	4.09	1 H, dd, $J = 10.2, 8.8$ Hz,	–0.01
3.81	3 H, s	3.81	3 H, s	0
3.75	6 H, s	3.75	6 H, s	0
2.84	1 H, dd, $J = 14.0, 4.4$ Hz,	2.84	1 H, dd, $J = 14.3, 4.7$ Hz,	0
2.83–2.74	1 H, m	2.81–2.72	1 H, m	–

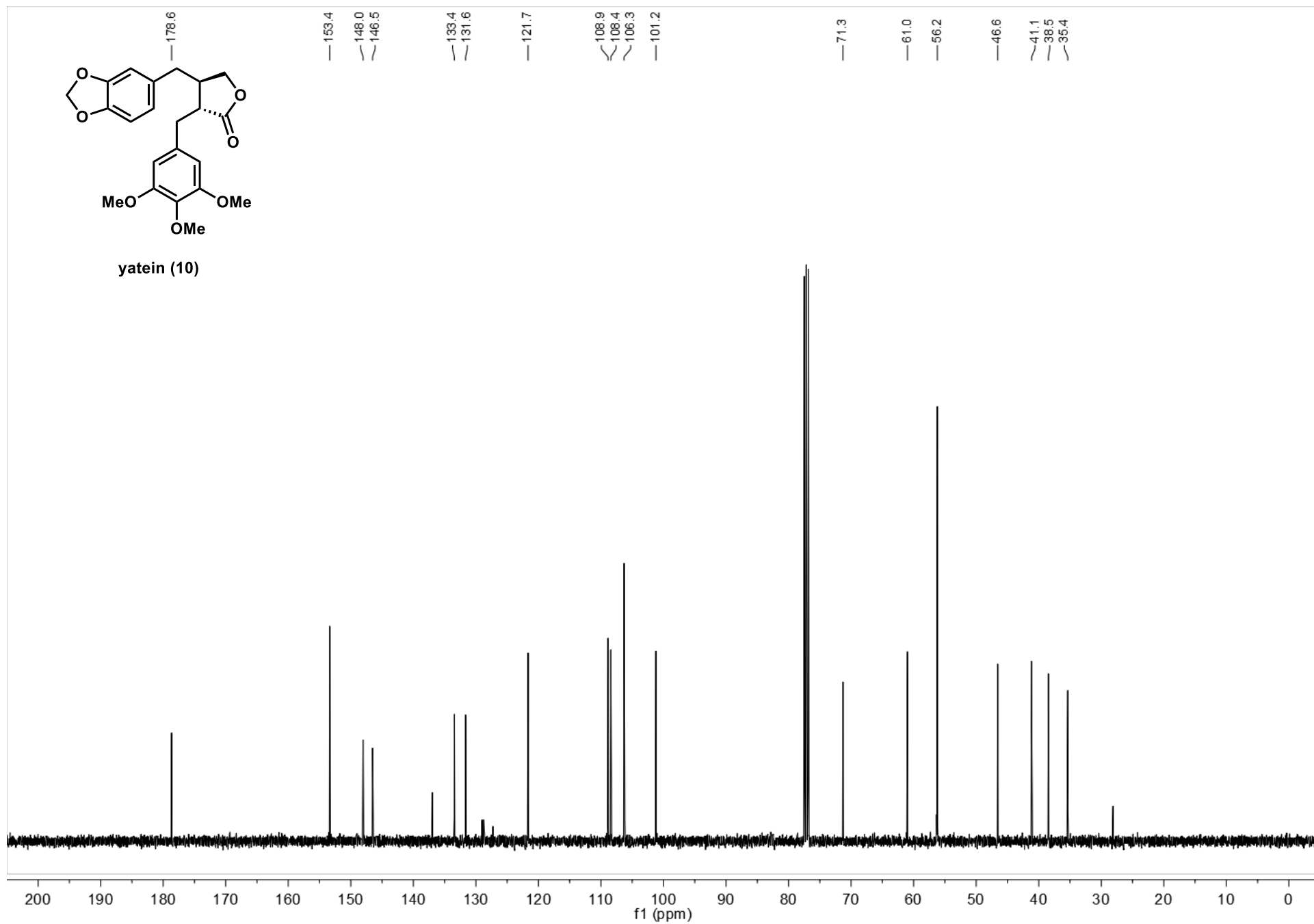
Table S2. ^{13}C NMR comparison table for podophyllotoxin (CDCl_3).

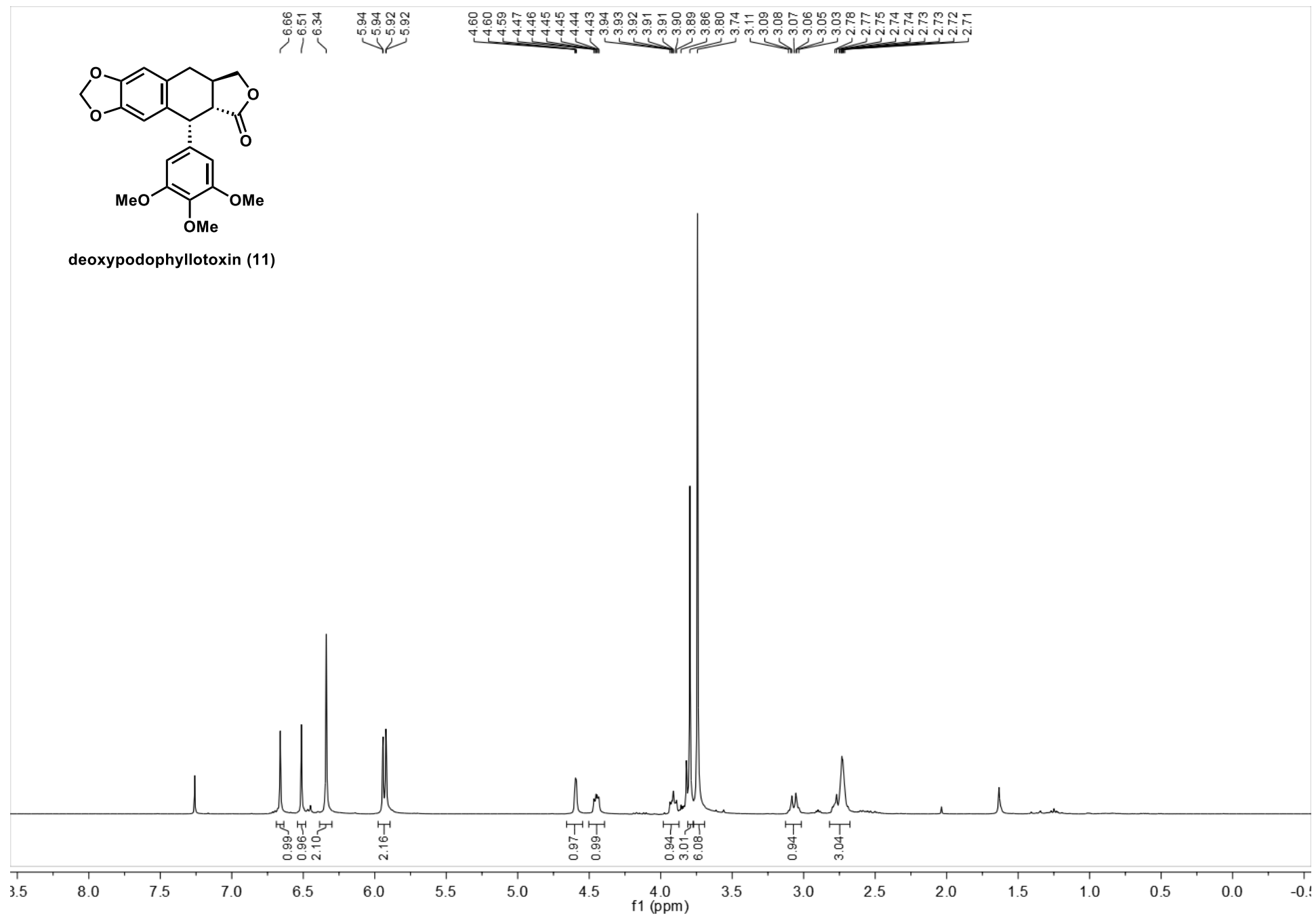
ref. S3 $\delta^{13}\text{C}$ (ppm) 101 MHz	Synthetic $\delta^{13}\text{C}$ (ppm) 151 MHz	Err $\Delta\delta$ (ppm)
174.5	174.5	0
152.6	152.8	-0.2
147.7	148.0	-0.3
147.6	147.9	-0.3
137.2	137.4	-0.2
135.4	135.5	-0.1
133.2	133.2	-0.2
131.1	131.4	-0.3
109.7	110.0	-0.3
108.3	108.5	-0.2
106.3	106.4	-0.1
101.4	101.6	-0.2
72.7	73.0	-0.3
71.3	71.4	-0.1
60.7	60.9	-0.2
56.3	56.5	-0.2
45.3	45.5	-0.2
44.1	44.2	-0.1
40.7	41.0	-0.3

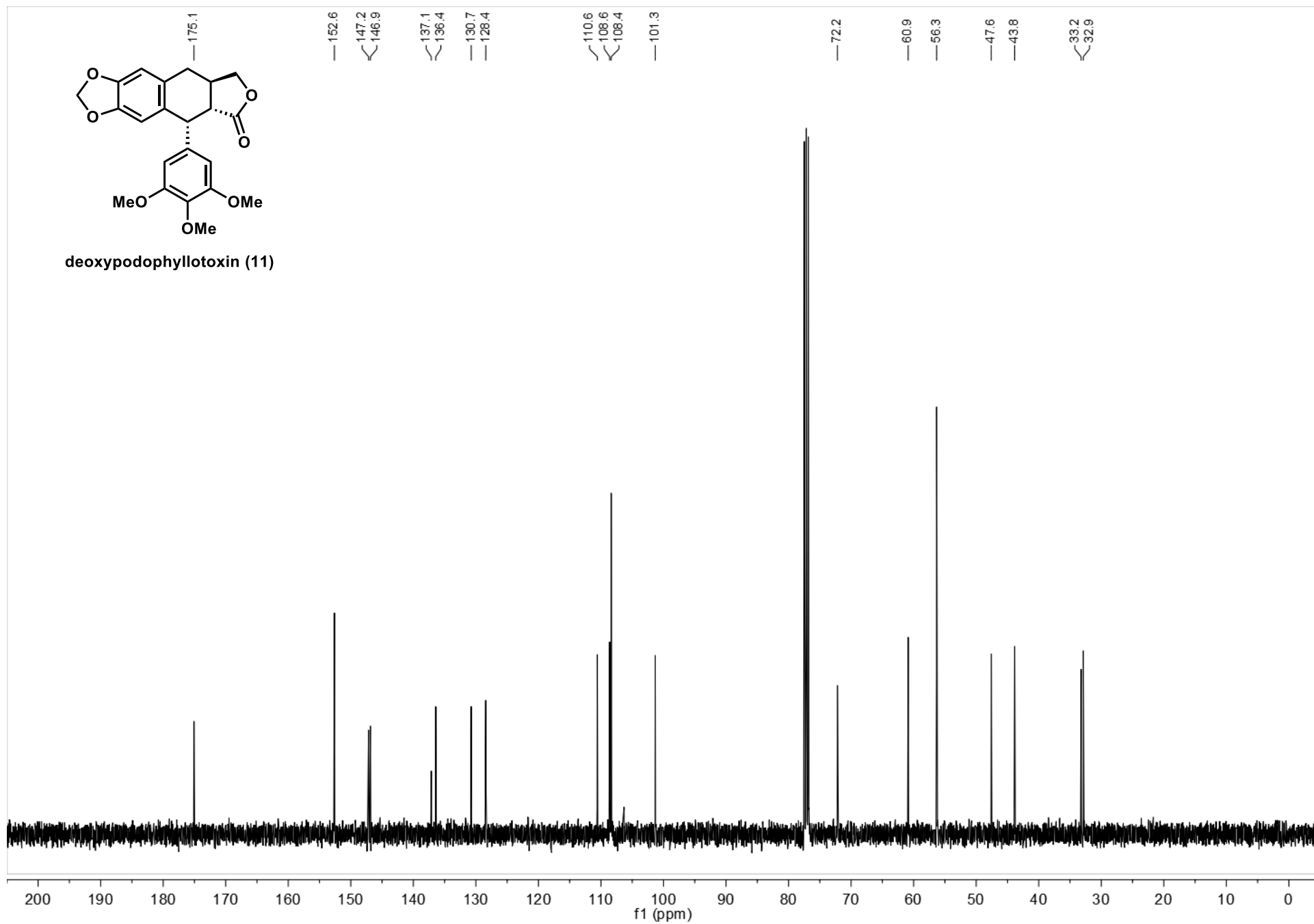
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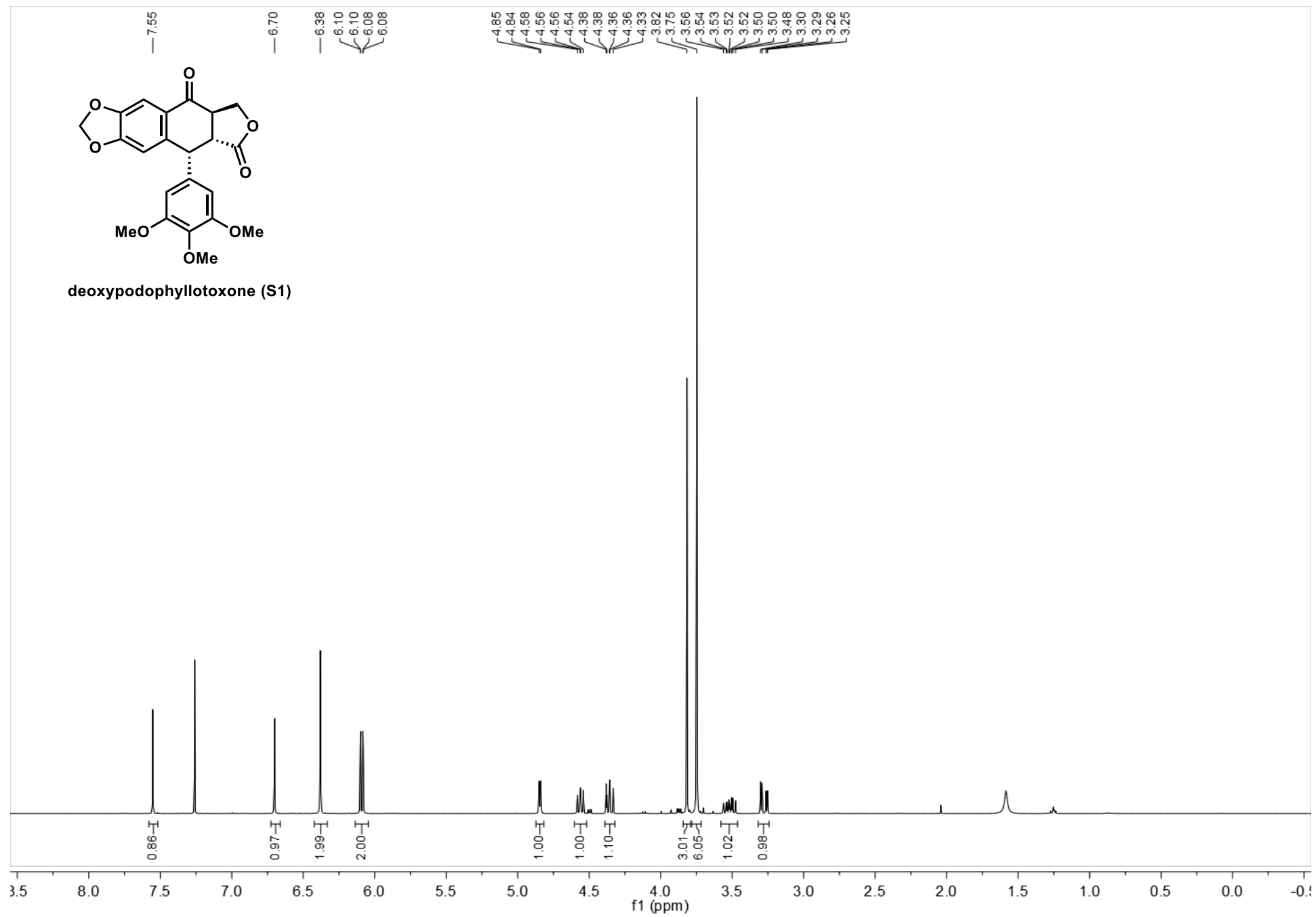
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- (S2) J. M. L. Vanderlei, F. Coelho, W. P. Almeida, *Synth. Commun.* **1998**, 28, 3047–3055.
- (S3) D. Stadler, T. Bach, *Angew. Chem. Int. Ed.* **2008**, 47, 7557–7559.

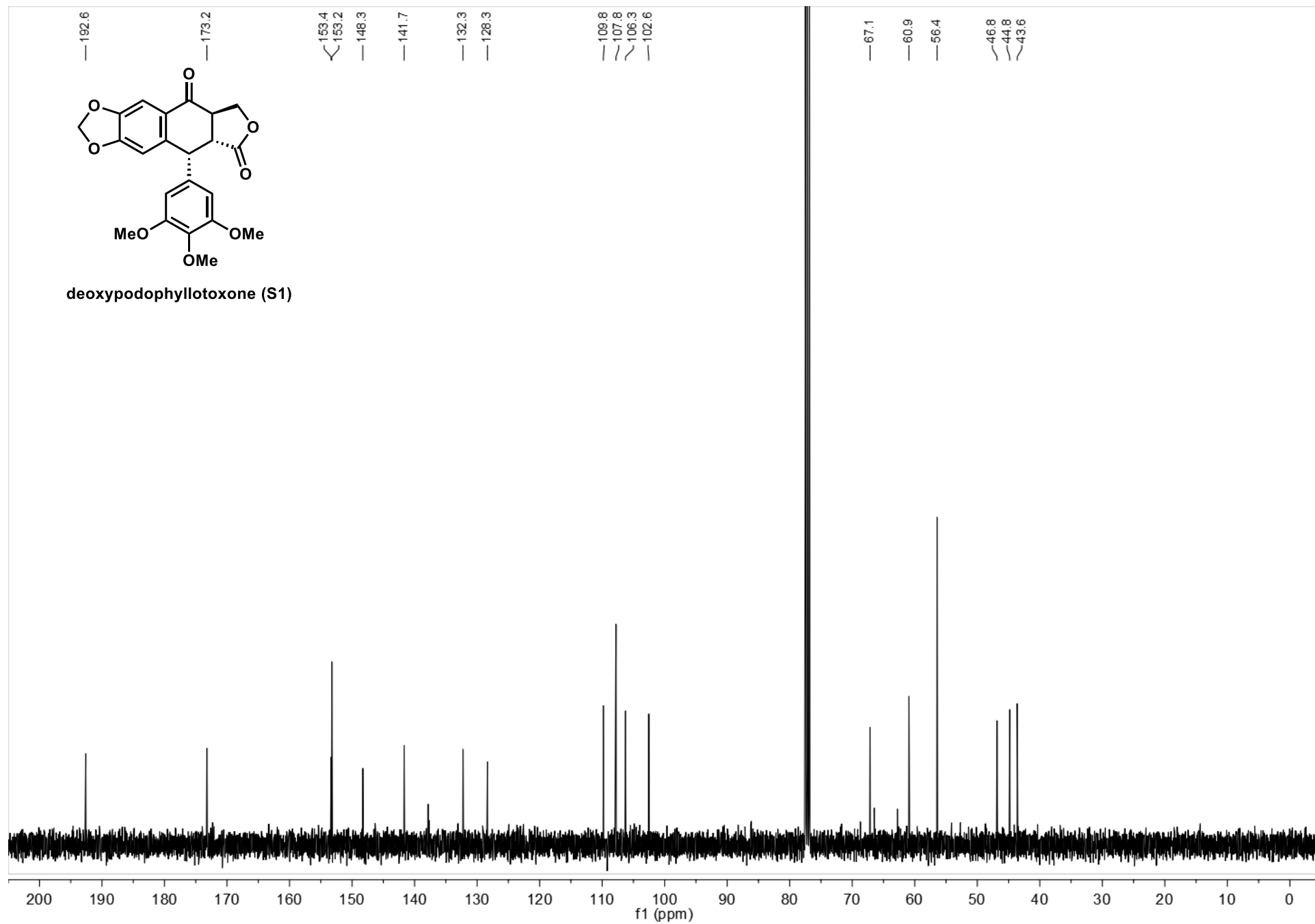


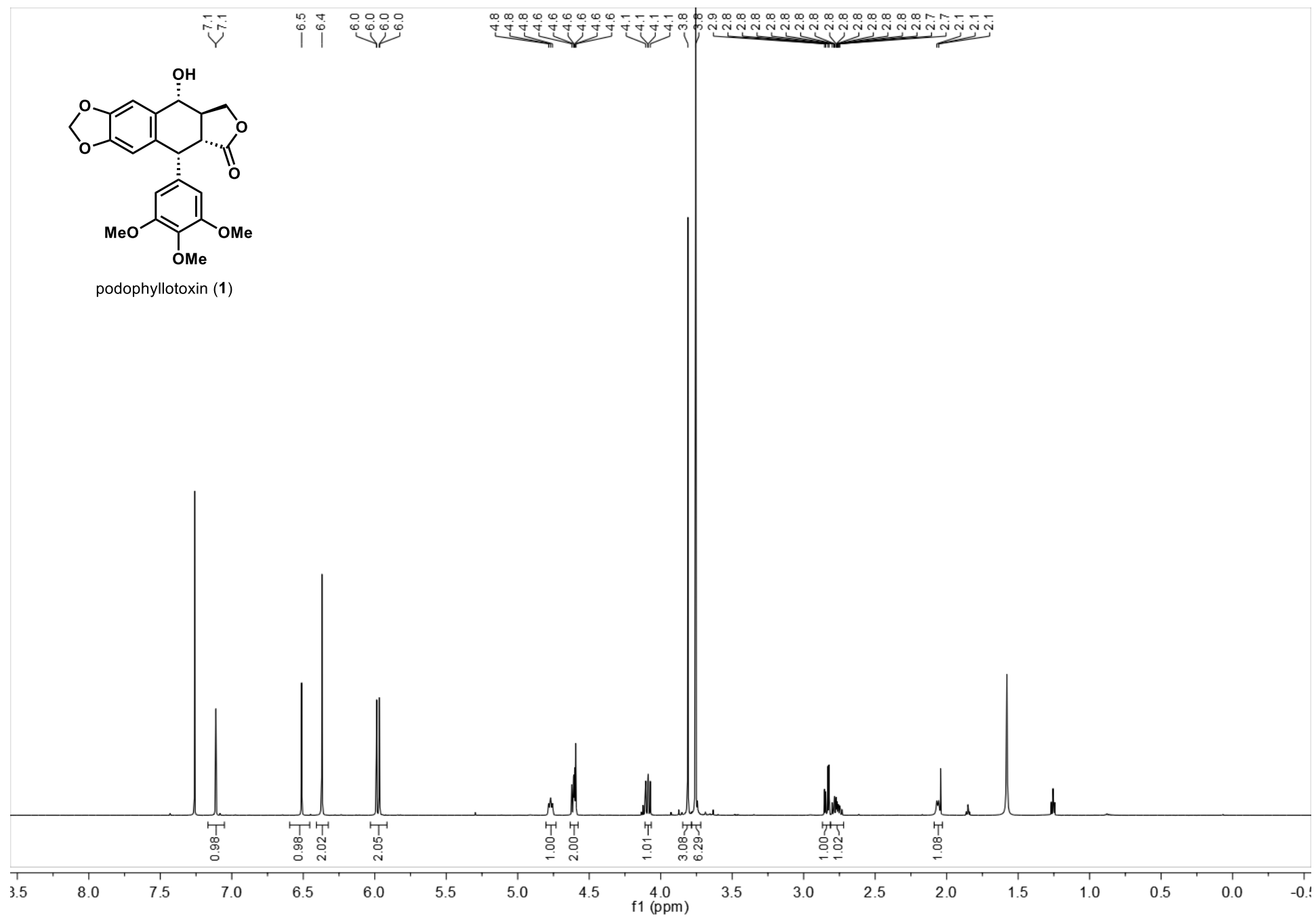


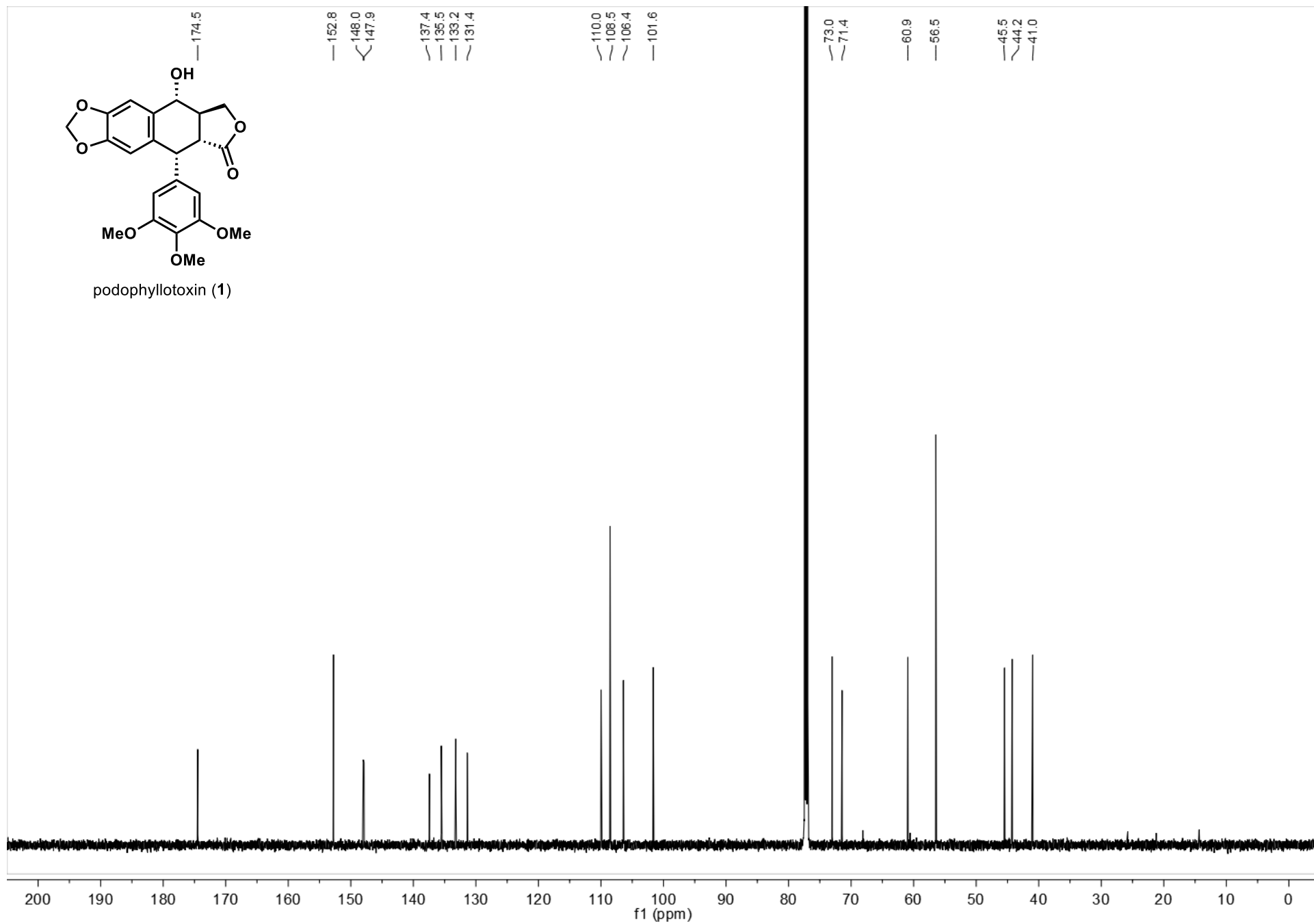


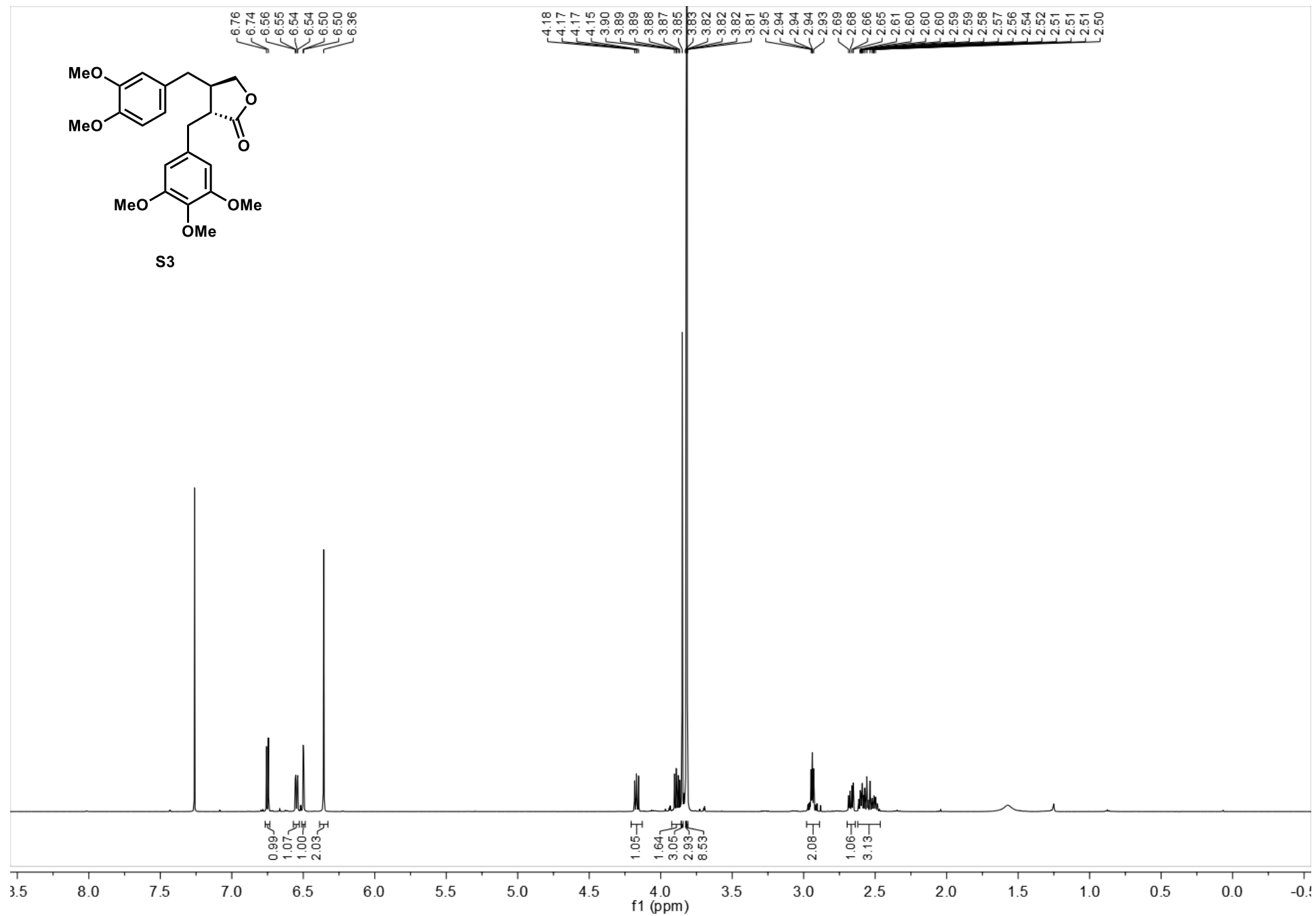


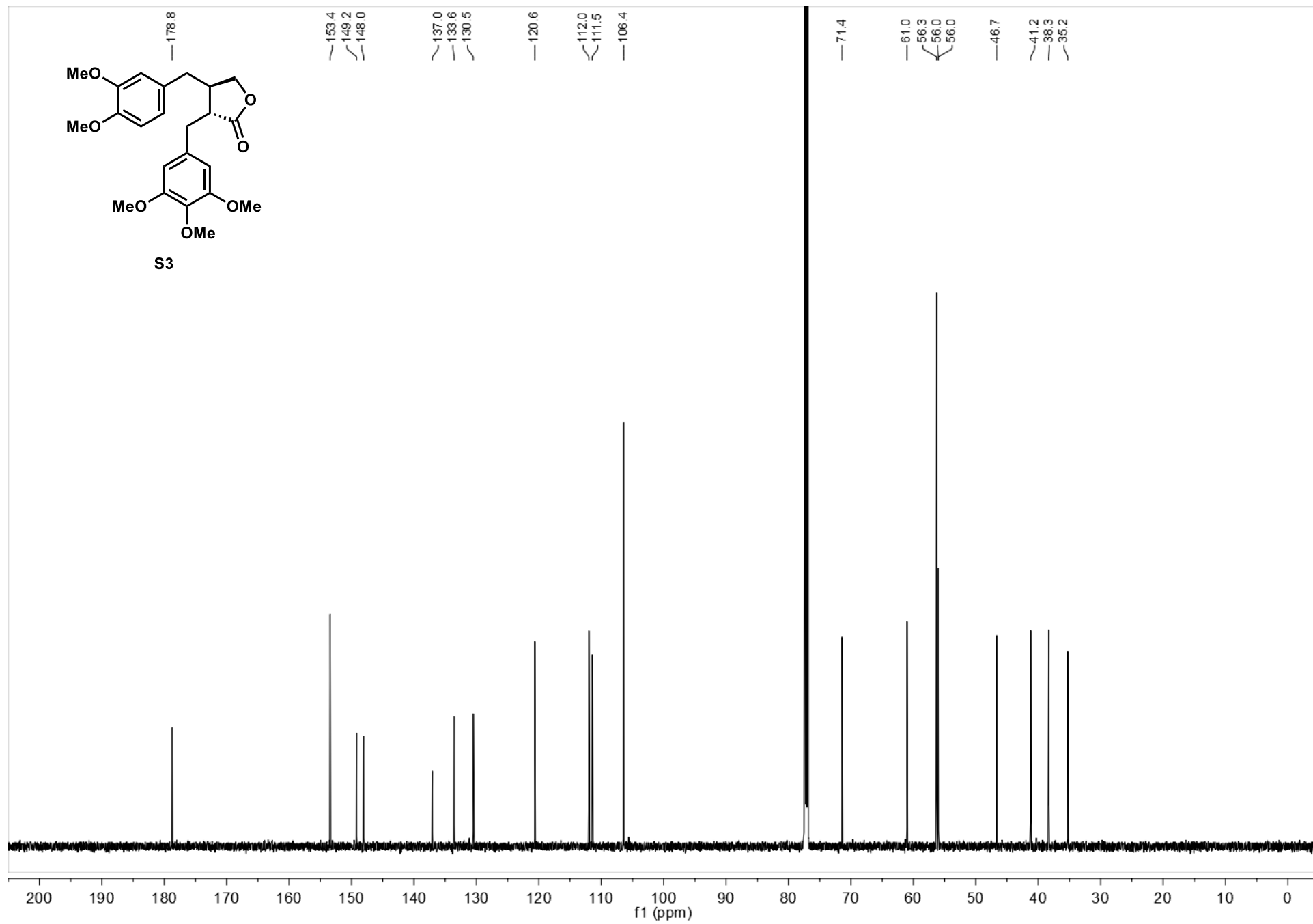


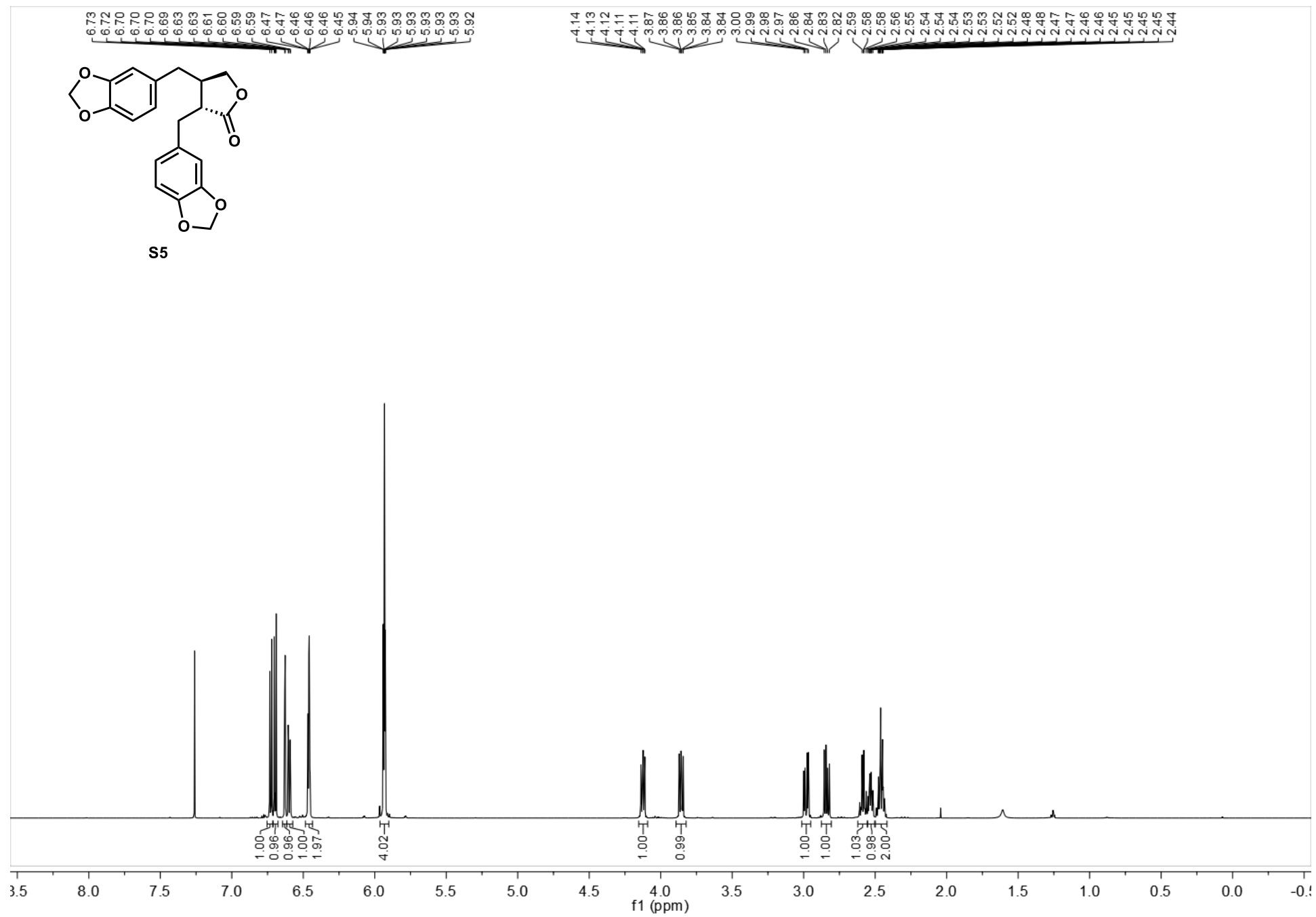


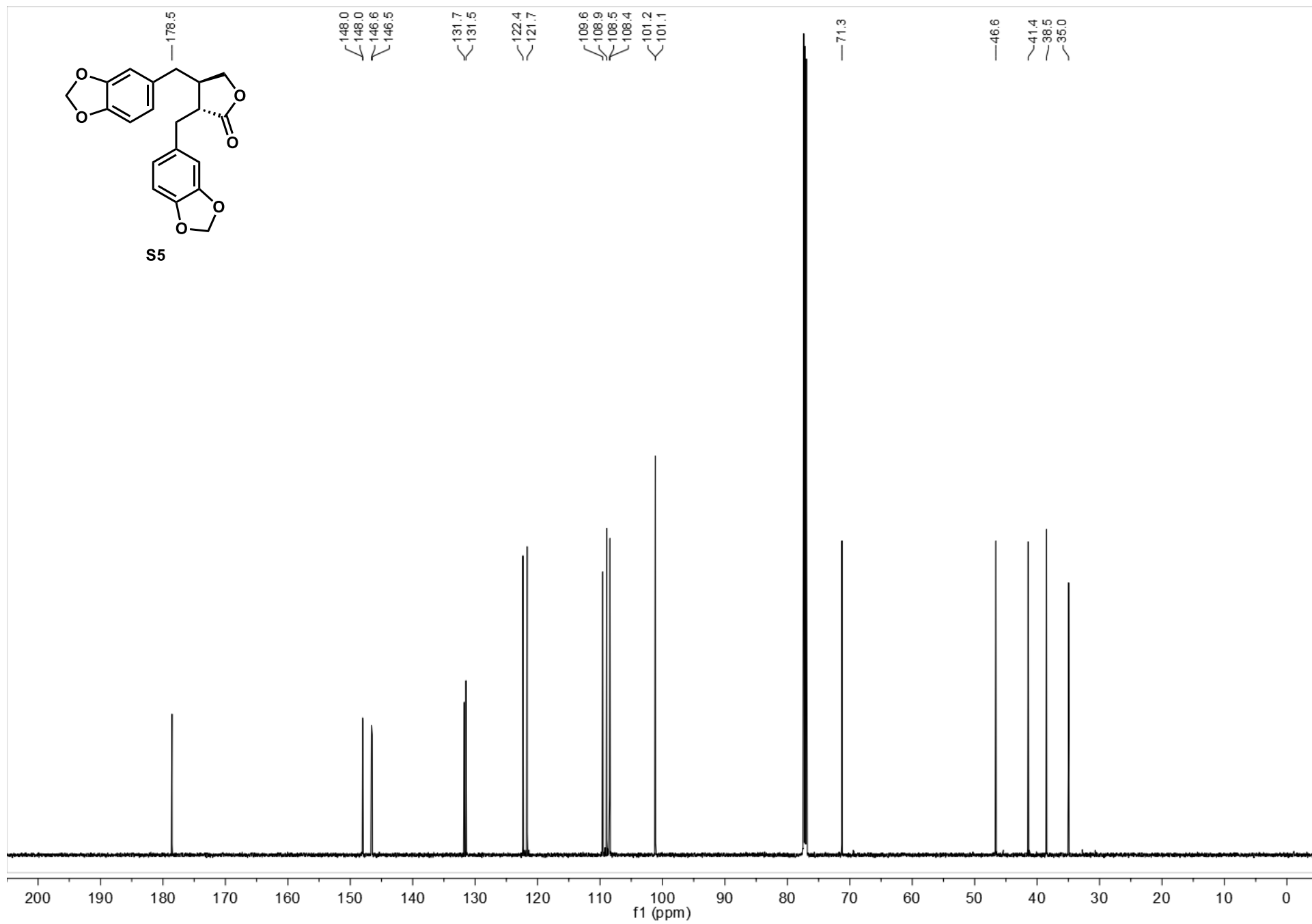


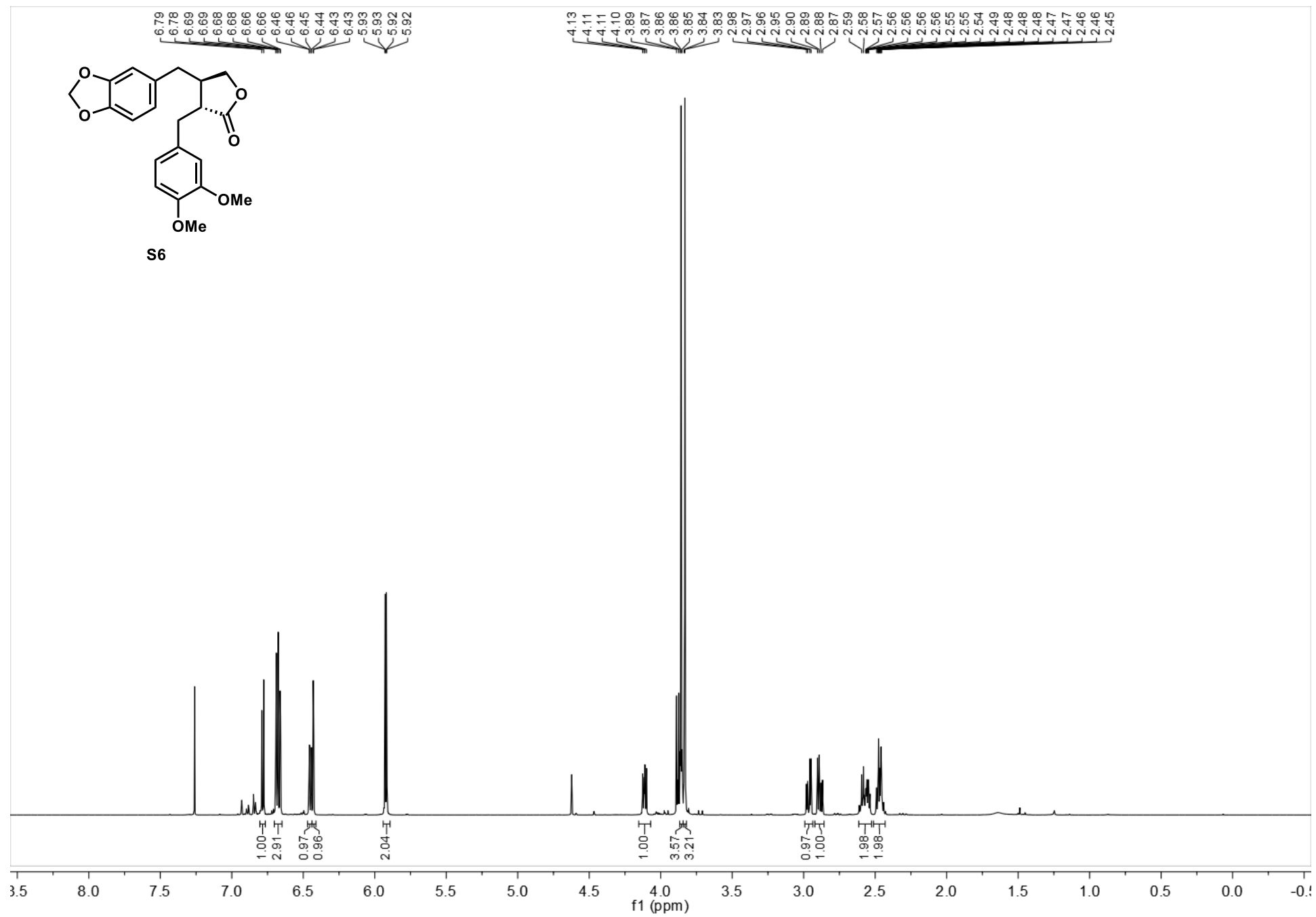


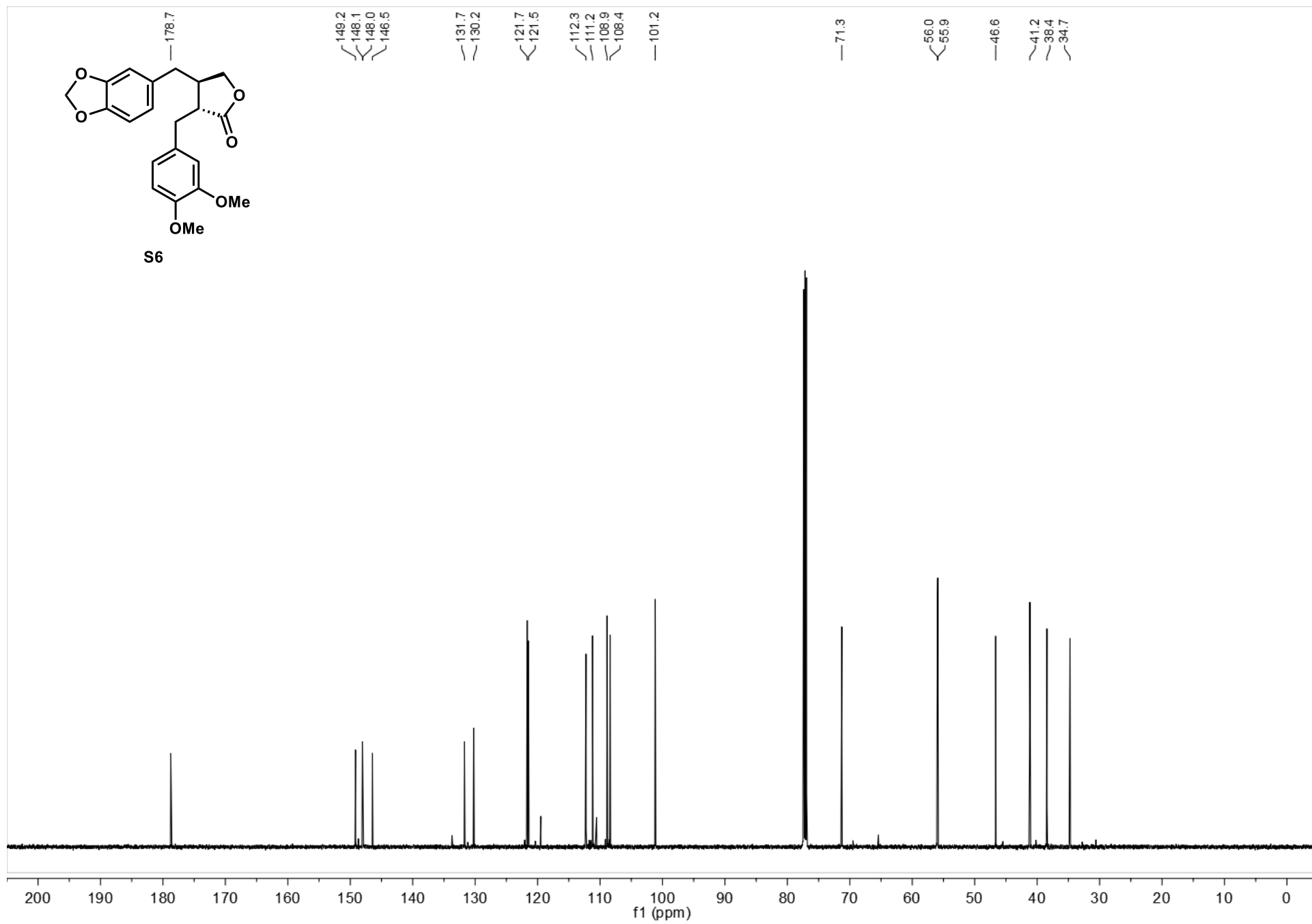


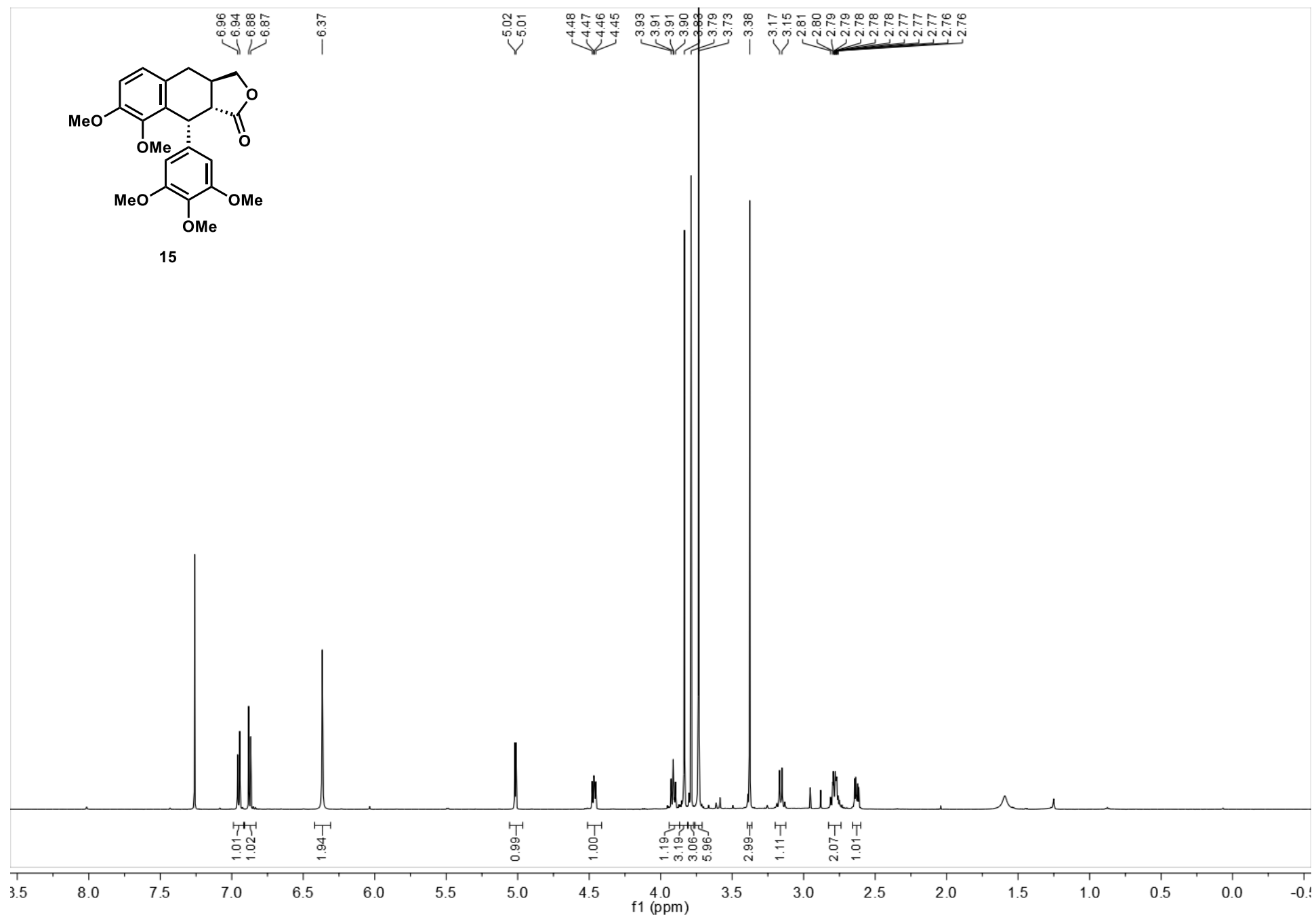


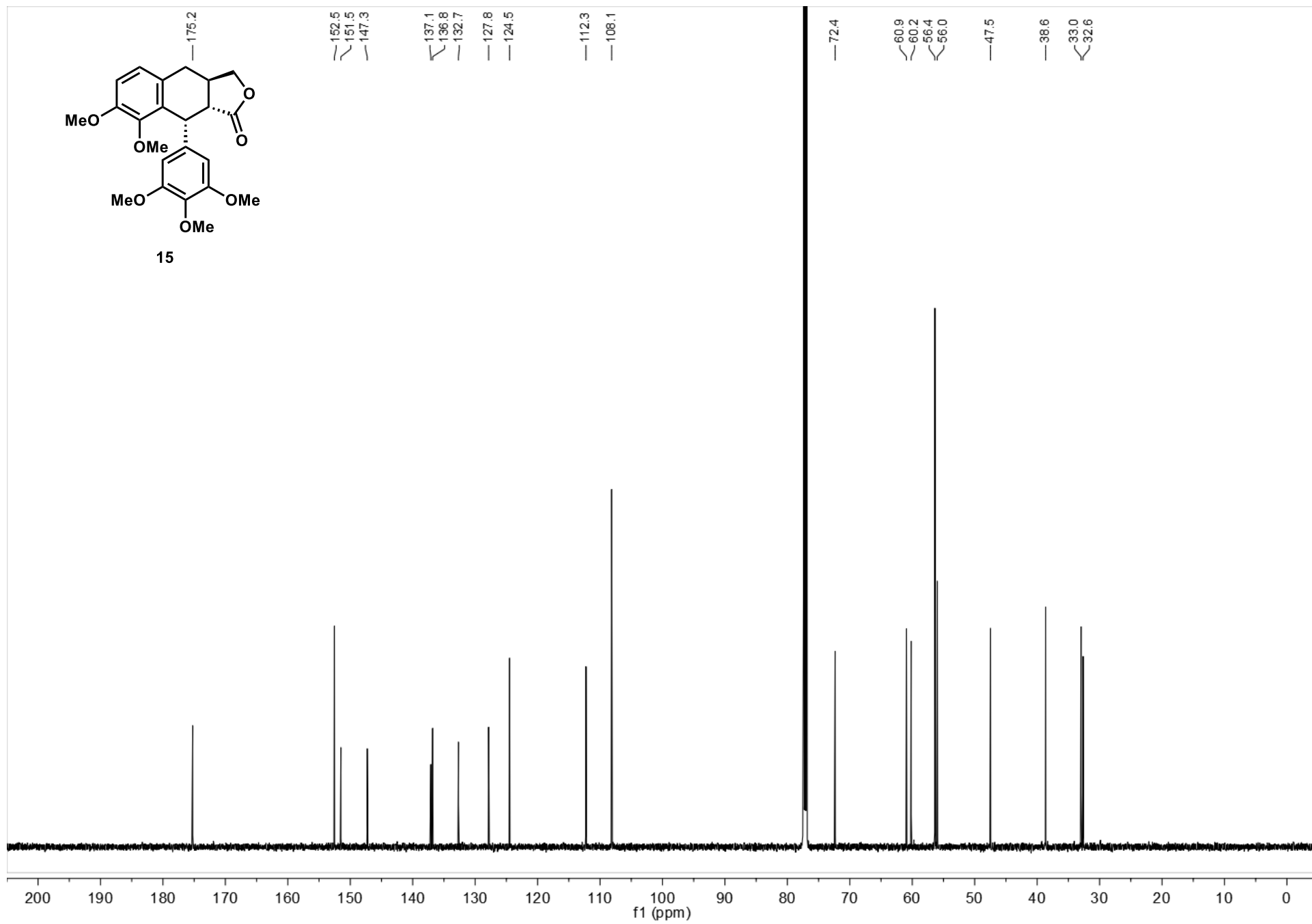


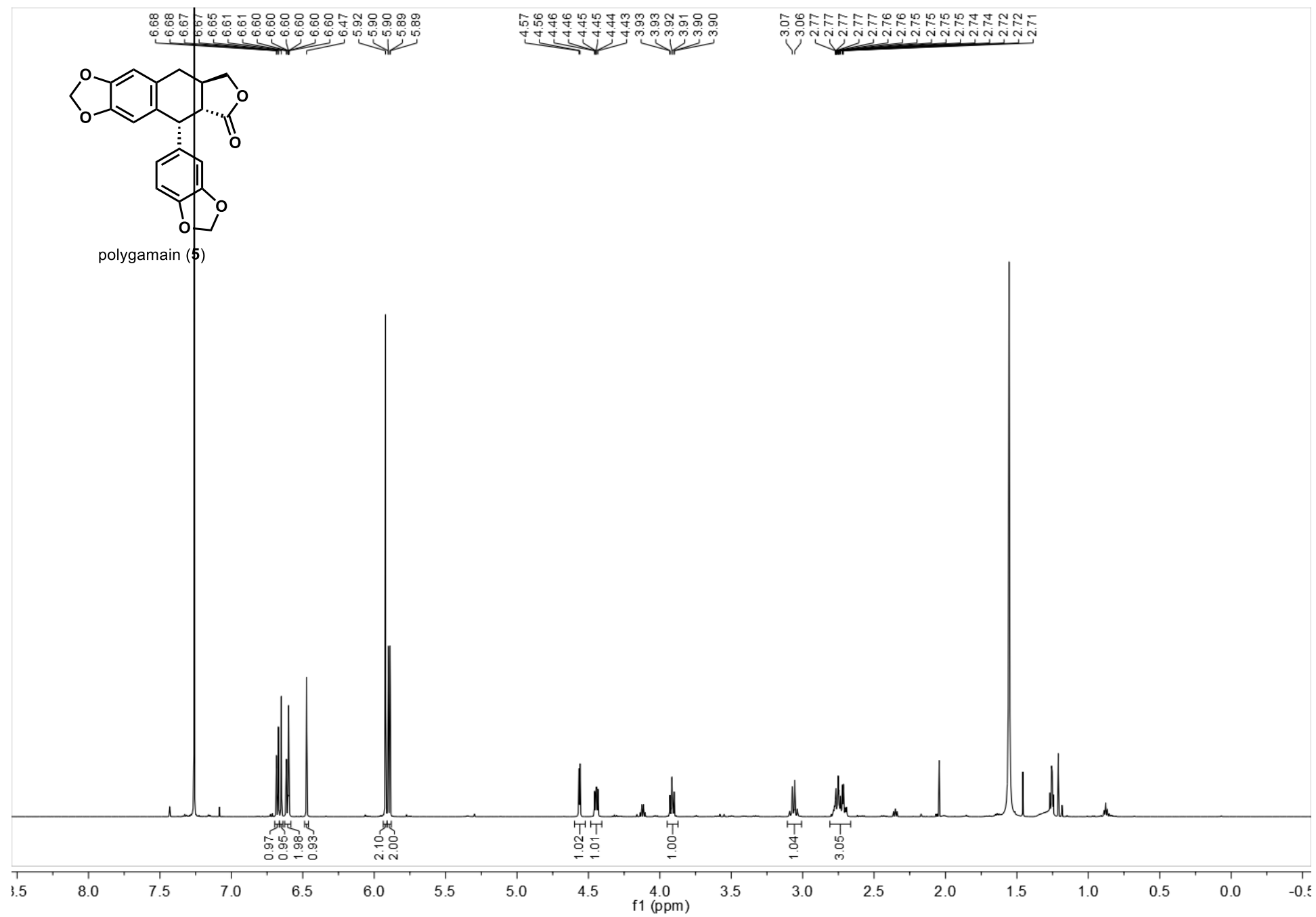


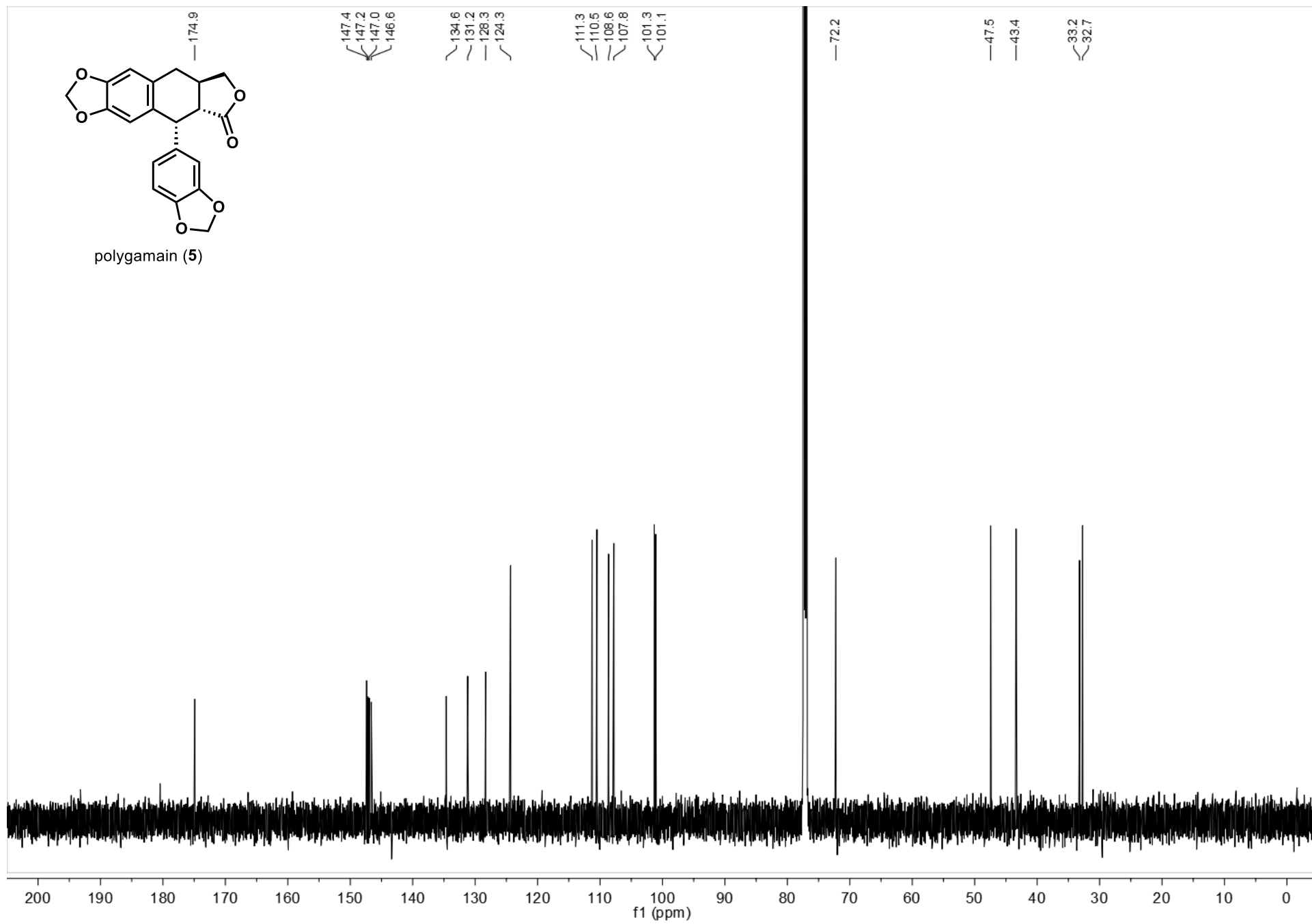


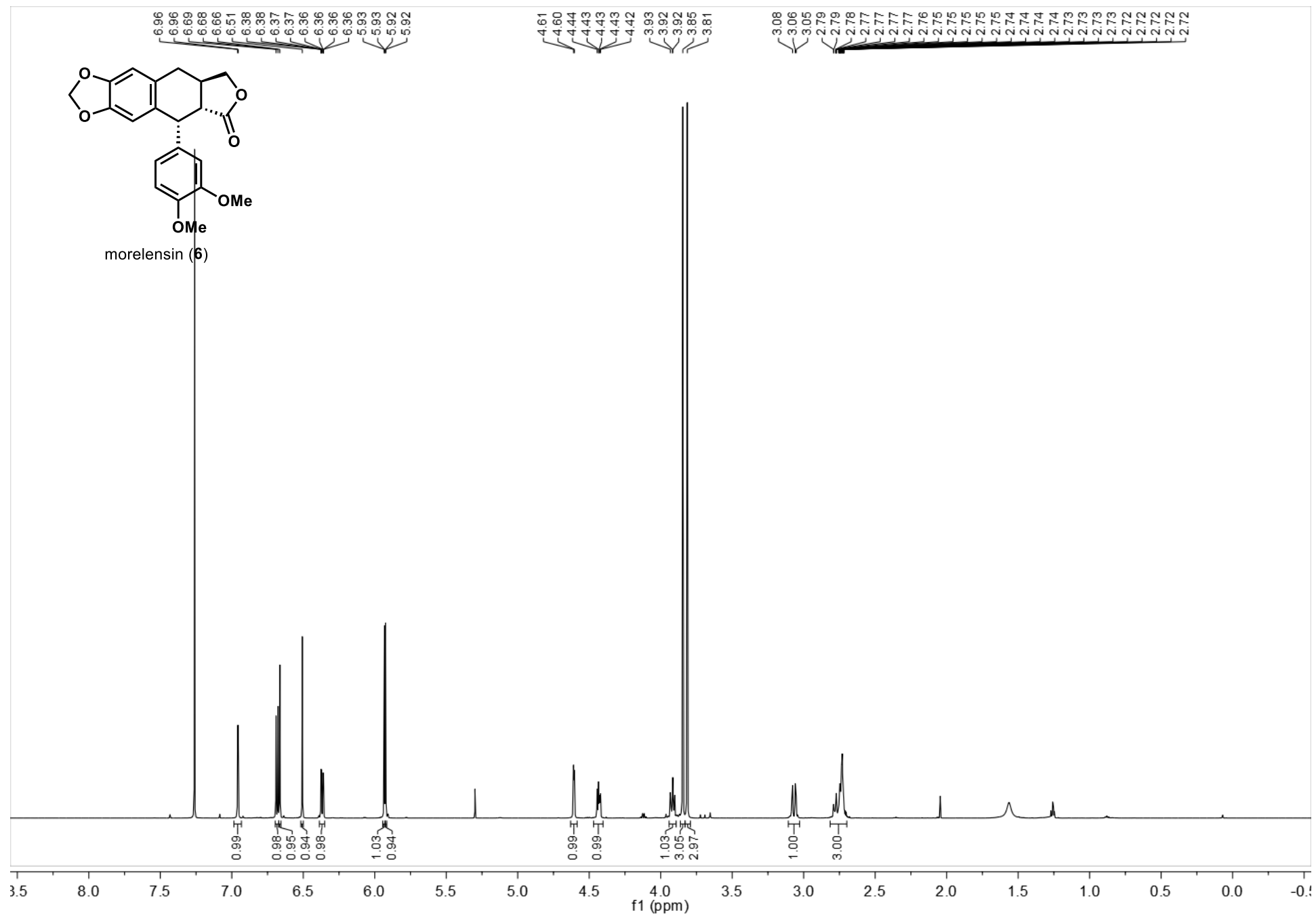


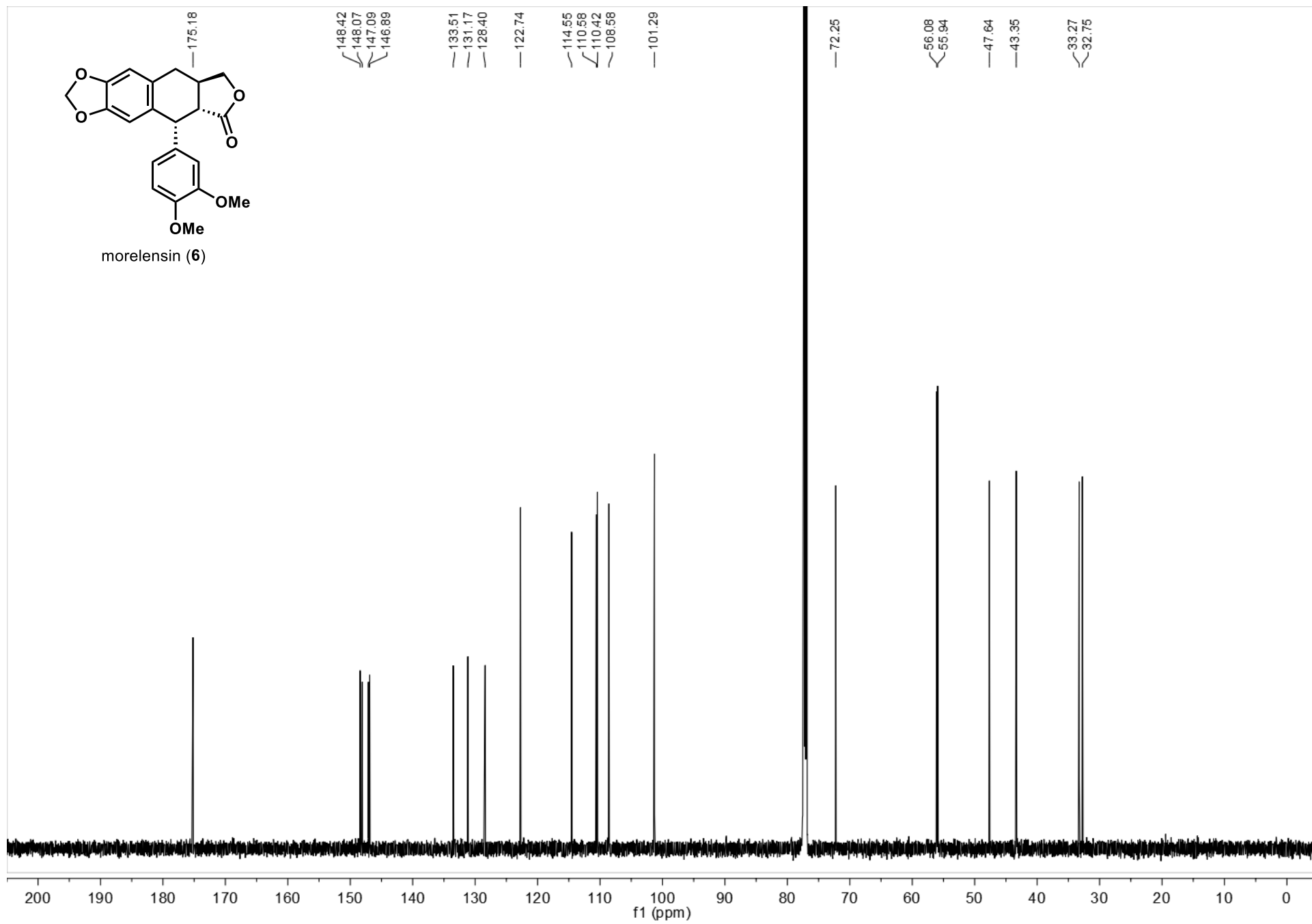












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