

Synthesis and Structure-Activity Relationship Study of Biliatresone, a Plant Isoflavonoid That Causes Biliary Atresia

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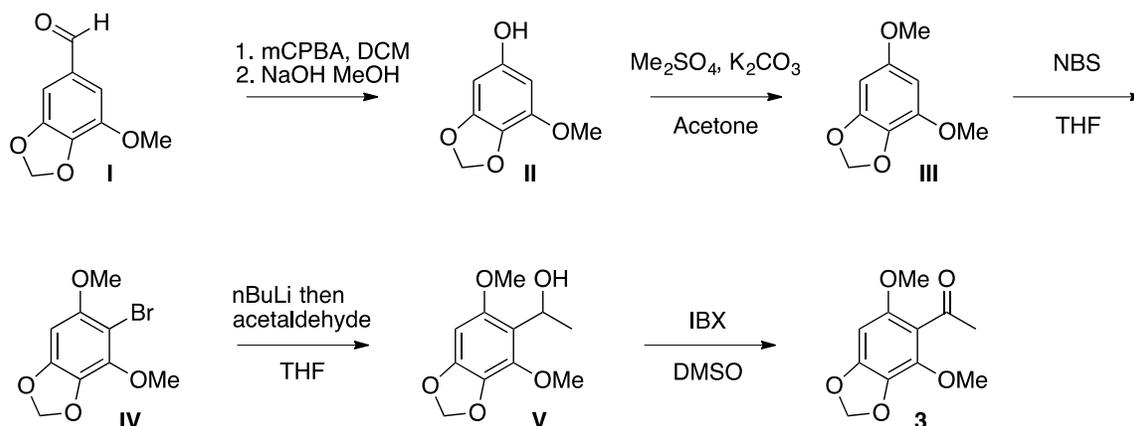
SUPPORTING INFORMATION

Experimental Procedures and Spectral Data for New Compounds and Biological

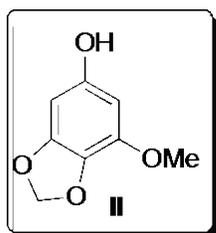
Protocols (64 pages)

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Synthesis of 1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-one (3):



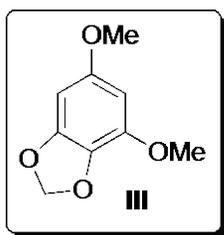
7-methoxy-1,3-benzodioxol-5-ol (II).



To 5-methoxypiperonal (Alfa Aesar; 5.17 g, 28.69 mmol) dissolved in DCM (143 mL) was added NaHCO_3 (4.82 g, 57.39 mmol) followed by m-CPBA (9.90 g, 57.39 mmol) at 0° C. The suspension was then allowed to warm to 25° C and stirred for an additional 1.5 hours. The suspension was then concentrated and then dissolved in MeOH (143 mL). The reaction was then added to a saturated solution of Na_2CO_3 (72 mL) and stirred until the reaction was complete by TLC. The resulting solids were dissolved with water and the MeOH was concentrated in under reduced pressure. The resulting aqueous solution was extracted with DCM (x 3). The combined organic layers were dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The resulting pink solid was then used without further purification (4.82 g, 82%). The analytical data was in accordance to the literature.¹

$^1\text{H NMR}$ (500 MHz, Chloroform-*d*) δ 6.12 – 6.06 (m, 1H), 6.03 (d, $J = 2.3$ Hz, 1H), 5.90 (s, 2H), 3.87 (s, 3H).

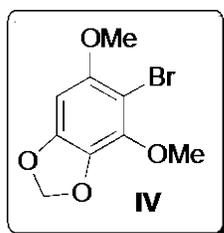
4,6-dimethoxy-1,3-benzodioxole (III).



To 7-methoxy-1,3-benzodioxol-5-ol (II) (1 g, 5.94 mmol) dissolved in acetone (45 mL) was added K_2CO_3 (2.0 g, 14.57 mmol). The resulting suspension was then refluxed and dimethyl sulfate (.620 mL, 6.54 mmol) was added slowly via syringe. After the addition was complete the suspension was stirred an additional 5 hours at reflux. The reaction was then cooled to 25° C, filtered through a pad of celite and then concentrated. The crude material was then added 5% $NaHCO_3$ (50 mL) and warmed to 50° C for 0.5 hours. The reaction was cooled and the resulting biphasic reaction was then extracted with DCM (x 3). The combined organic phases were dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The resulting white solid was then purified by flash chromatography on silica gel (5% EtOAc/hexanes) to afford the product as white solid (0.92 g, 84%). The analytical data was in accordance to the literature.²

1H NMR (500 MHz, Chloroform-*d*) δ 6.18 (d, $J = 2.3$ Hz, 1H), 6.09 (d, $J = 2.3$ Hz, 1H), 5.91 (s, 2H), 3.88 (s, 3H), 3.74 (s, 3H).

5-bromo-4,6-dimethoxy-1,3-benzodioxole (IV).



To 4,6-dimethoxy-1,3-benzodioxole (III) (0.5 g, 2.74 mmol) dissolved in THF (6.3 mL) at 0° C was added NBS (0.493 g, 2.77 mmol). The reaction was then stirred at 0° C for 1 hour and then quenched with water. The resulting solution was extracted with EtOAc (x 3). The combined organic layers were then washed with brine, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The solid was dissolved in

DCM was passed through a pad of silica. The resulting white solid (0.690 g, 96%) was used without further purification.

Physical properties: crystalline solid (mp = 43-50 °C).

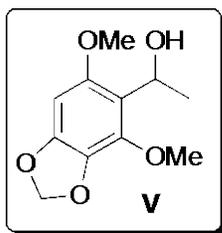
¹H NMR (500 MHz, Chloroform-*d*) δ 6.32 (s, 1H), 5.92 (s, 2H), 4.05 (s, 3H), 3.82 (s, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 152.19, 148.81, 141.24, 131.35, 101.48, 96.00, 89.94, 77.42, 77.16, 76.91, 60.16, 57.22.

FT-IR (cm⁻¹, neat, ATR) 1629, 1603, 1197, 1100, 992.

HRMS (ESI) m/z calc. for C₉H₉BrO₄ (M + H)⁺ 260.9762; Found 260.9763.

1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-ol (V).



To 5-bromo-4,6-dimethoxy-1,3-benzodioxole (IV) (0.560 g, 2.14 mmol) dissolved in THF (7.13 mL) at -78° C was added BuLi (1.29 mL, 2.78 mmol), which then was stirred an additional 15 minutes at -78° C. The reaction was then warmed to 0° C for 45 minutes and then cooled back to -78° C at which time acetaldehyde (0.361 mL, 6.43 mmol) was then slowly added. The reaction was then stirred for 15 minutes at -78° C and then warmed to 0° C for 1 hour. The reaction was then further warmed to 25° C for 30 minutes and then was quenched with water and extracted with EtOAc (x 3). The combined organic layers were then washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure to provide a crude oil. The resulting oil was then purified by flash chromatography on silica gel (40% EtOAc/hexanes) to afford the product as a clear oil (0.375 g, 77%).

Physical properties: clear oil.

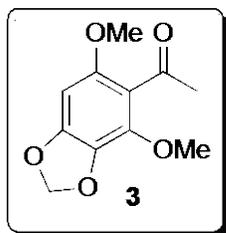
¹H NMR (500 MHz, Chloroform-*d*) δ 6.25 (s, 1H), 5.84 (d, 2H), 5.22 – 5.13 (m, 1H), 3.99 (s, 3H), 3.75 (s, 3H), 3.63 (d, *J* = 11.4 Hz, 1H), 1.46 (d, *J* = 6.7 Hz, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 152.33, 148.19, 141.21, 130.78, 117.37, 100.89, 89.50, 77.41, 77.16, 76.90, 64.32, 59.83, 56.43, 24.25.

FT-IR (cm⁻¹, neat, ATR) 2925, 1618, 1499, 1167, 991.

HRMS (ESI) *m/z* calc. for C₁₁H₁₄O₅ (M + Na)⁺ 249.0739; Found 249.0748.

1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-one (3)



A round bottom flask charged with 1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-ol (V) (0.693 g, 3.09 mmol) dissolved in DMSO. The reaction was then added IBX (1.73 g, 6.18 mmol) at 25° C and was stirred 18 hrs. The reaction was quenched with water. The resulting solid was filtered through a pad of Celite and rinsed with Ethyl acetate. The biphasic solution was then extracted with EtOAc (x 3). The combined organic layers were then washed with water (x 5) and brine (x 1). The organic layers were then dried over Na₂SO₄, filtered and concentrated under reduced pressure to provide a crude oil. The resulting oil was then purified by flash chromatography on silica gel (20% EtOAc/hexanes) to afford the product as a clear oil (0.635 g, 91%).

Physical properties: clear oil.

¹H NMR (500 MHz, Chloroform-*d*) δ 6.24 (s, 1H), 5.90 (s, 2H), 3.98 (s, 3H), 3.74 (s, 3H), 2.45 (s, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 201.23, 152.46, 150.47, 140.62, 130.30, 116.99, 101.27, 89.14, 77.41, 77.16, 76.90, 60.07, 56.62, 32.58.

FT-IR (cm^{-1} , neat, ATR) 1700, 1617, 1420, 1101, 948.

HRMS (ESI) m/z calc. for $\text{C}_{11}\text{H}_{12}\text{O}_5$ ($\text{M} + \text{Na}$) $^+$ 247.0582; Found 247.0579.

HTE Experimental set up

Reaction experimental design was aided by the use of Accelrys Library Studio. 20 μmol scale reactions for the limiting reagent were carried out in 1 mL glass vials (Freeslate, Cat. No. S11500, 8 x 30 mm, 1mL, flat bottom) equipped with magnetic tumble stir bars (V&P Scientific, Cat. No. 7111D-1 and 716-1 for microvials) in 24-well reaction plates (Analytical-Sales, Cat. No. 24249). Liquid handling was done using single and multi-channel Eppendorf pipettors (10, 100, 200, and 1000 μL). On completion of solution dosing the plates were covered by a perfluoroalkoxy alkane (PFA) mat (Analytical-Sales, Cat. No. 96967 and 24261), followed by two silicon rubber mats (Analytical-Sales, Cat. No. 96965 and 24262), and an aluminum cover which was tightly and evenly sealed by 9 screws. Reactions were monitored using a Waters Acquity UPLC-MS. Column: Acquity UPLC HSS C18 1.7 μm 2.1 x 50 mm (Part # 186002350), pH 3.5 Stock Solution: 12.6 g ammonium formate + 7.9 mL formic acid to 1 L water; Mobile Phase A: 1% pH 3.5 stock solution in H_2O , Mobile Phase B: 1% pH 3.5 stock solution in 100 mL H_2O + 900 mL ACN; Strong Wash: 1700 mL ACN, 200 mL H_2O , 100 mL *i*PrOH; Weak Wash: 200 mL ACN + 1800 mL H_2O . The instrument was equipped with an SQD detector with electrospray ionization (ESCI) source in positive and negative mode. High throughput data analysis was carried out with Virscidian Analytical StudioTM software.

General High Throughput Screening Protocol

Stock solutions of the relevant catalyst or ligand were prepared in DCE or THF. Using a 200 μL pipettor, the catalyst or ligand solutions were dispensed into 1 mL glass vials arrayed in a 24 well microtitre plate according to the required Pd/L loading per reaction screen. The solvent was removed *in vacuo*. The vials were then charged with tumble stir

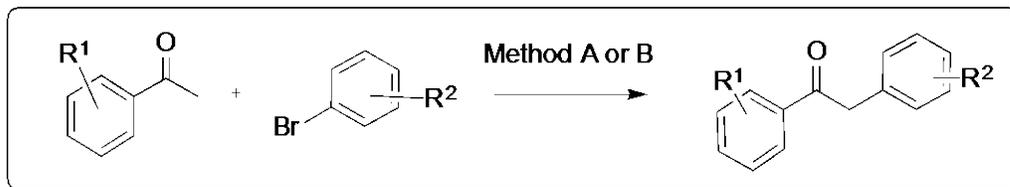
bars. Combined stock solutions of aryl bromide (20 μmol), amine (30 μmol), and base (60 μmol) (100 μL) in either 1,4-dioxane or toluene, was added to each 1 mL vial. The 24 well reaction plate was covered with the PFA film, sealed and stirred at 100 $^{\circ}\text{C}$ for 16h. The reactions were cooled to ambient temperature and quenched with a stock solution of internal standard solution, (biphenyl in ACN (0.002M, 500 μL)) and mixed thoroughly. Aliquots of the quenched reactions were taken (20 μL), further diluted with 700 μL of ACN, and subjected to UPLCMS analysis.

Screen data

Ratios of Product and starting material to Biphenyl internal standard. (Not sorted)

Vial	Catalyst	Ligand	Base	Solvent	Prod/IS	SM-Ketone/IS
A01	tBuXPhos G3	-	NaOtBu	1,4-dioxane	4.8	0.16
A02	XPhos G2	-	NaOtBu	1,4-dioxane	4.6	0.00
A03	tBu3P G2	-	NaOtBu	1,4-dioxane	1.0	0.00
A04	PdCl ₂ (DPPF)	-	NaOtBu	1,4-dioxane	3.1	0.00
A05	SIPr Peppi	-	NaOtBu	1,4-dioxane	2.4	0.00
A06	Pd2dba3	DPEPhos	NaOtBu	1,4-dioxane	4.4	0.00
B01	tBuXPhos G3	-	Cs ₂ CO ₃	toluene	0.0	12.01
B02	XPhos G2	-	Cs ₂ CO ₃	toluene	6.3	10.01
B03	tBu3P G2	-	Cs ₂ CO ₃	toluene	0.0	14.85
B04	PdCl ₂ (DPPF)	-	Cs ₂ CO ₃	toluene	0.2	10.55
B05	SIPr Peppi	-	Cs ₂ CO ₃	toluene	2.0	9.18
B06	Pd2dba3	DPEPhos	Cs ₂ CO ₃	toluene	1.2	12.15
C01	tBuXPhos G3	-	NaOtBu	toluene	0.0	5.21
C02	XPhos G2	-	NaOtBu	toluene	0.6	4.43
C03	tBu3P G2	-	NaOtBu	toluene	0.2	3.95
C04	PdCl ₂ (DPPF)	-	NaOtBu	toluene	0.0	2.37
C05	SIPr Peppi	-	NaOtBu	toluene	0.1	0.51
C06	Pd2dba3	DPEPhos	NaOtBu	toluene	0.7	2.30
D01	tBuXPhos G3	-	Cs ₂ CO ₃	1,4-dioxane	0.0	12.05
D02	XPhos G2	-	Cs ₂ CO ₃	1,4-dioxane	6.1	9.21
D03	tBu3P G2	-	Cs ₂ CO ₃	1,4-dioxane	0.0	11.31
D04	PdCl ₂ (DPPF)	-	Cs ₂ CO ₃	1,4-dioxane	0.0	8.37
D05	SIPr Peppi	-	Cs ₂ CO ₃	1,4-dioxane	2.7	11.24
D06	Pd2dba3	DPEPhos	Cs ₂ CO ₃	1,4-dioxane	0.8	16.99

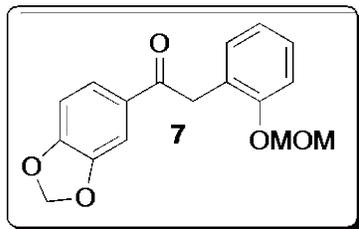
General procedure for the synthesis of ketone intermediates:



Method A: Aryl ketone (1 eq), aryl bromide (1.5 eq) DPE-Phos (3.6 mol %), Pd(dba)₃ (1.5 mol %), NaOtBu (2 eq) were added to a sealed tube fitted with a magnetic stir bar. The flask was evacuated and purged with argon five times and then was added to degassed THF (1 M). The reaction was warmed to 70 °C for 3 hours. The reaction was then cooled to 25° C, filtered through a pad of Celite and rinsed with EtOAc. Water was then added and the biphasic solution was then separated. The aqueous was extracted with EtOAc (x 3). The combined organic layers were then washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was then purified by flash chromatography on silica gel (15% EtOAc/hexanes to 20% EtOAc/hexanes) to afford the product.

Method B: Aryl ketone (1 eq), aryl bromide (1.1 eq) Xphos (G2) (10 mol %), NaOtBu (2 eq) were added to a sealed tube fitted with a magnetic stir bar. The flask was evacuated and purged with argon five times and then was added degassed dioxane (0.3 M). The reaction was warmed to 100 °C for 4 hours. The reaction was then cooled to 25° C and then filtered through a pad of Celite and rinsed with EtOAc. Water was then added and the biphasic solution was then separated. The aqueous was extracted with EtOAc (x 3). The combined organic layers were then washed with brine, dried over Na₂SO₄, filtered and concentrated under reduced pressure.

1-(benzo[1,3]dioxol-5-yl)-2-(2-(methoxymethoxy)phenyl)ethan-1-one (7).



3,4-Methylenedioxyacetophenone (Alfa Aesar; 339 mg, 2.07 mmol) was reacted with 1-bromo-2-(methoxymethoxy)benzene (Combi-Blocks; 300 mg, 1.38 mmol) according to the general procedure (Method A) and

was purified by flash chromatography on silica gel (15% EtOAc/hexanes to 25% EtOAc/hexanes) to afford the product as a clear oil (310 mg, 75%).

Physical properties: clear oil.

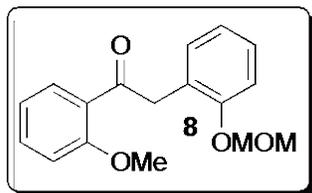
¹H NMR (500 MHz, Chloroform-*d*) δ 7.67 (dd, $J = 8.2, 1.8$ Hz, 1H), 7.51 (d, $J = 1.8$ Hz, 1H), 7.25 – 7.16 (m, 2H), 7.11 (d, $J = 8.2$ Hz, 1H), 6.97 (t, $J = 7.5$ Hz, 1H), 6.84 (d, $J = 8.2$ Hz, 1H), 6.03 (s, 2H), 5.16 (s, 2H), 4.22 (s, 2H), 3.42 (s, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 195.87, 154.98, 151.70, 148.18, 131.85, 131.09, 128.42, 124.69, 124.62, 121.90, 114.15, 108.34, 107.91, 101.87, 94.54, 77.42, 77.16, 76.90, 56.12, 39.98.

FT-IR (cm⁻¹, neat, ATR) 1677, 1602, 1439, 1235, 922.

HRMS (ESI) m/z calc. for C₁₇H₁₆O₅ (M + H)⁺ 301.1076; Found 301.1066.

2-(2-(methoxymethoxy)phenyl)-1-(2-methoxyphenyl)ethan-1-one (8).



2-Methoxyacetophenone (Alfa Aesar; 311 mg, 2.07 mmol) was reacted with 1-bromo-2-(methoxymethoxy)benzene (Combi-Blocks; 300 mg, 1.38 mmol) according to the general procedure (Method A) and was purified by flash chromatography on silica gel (5% EtOAc/hexanes to 10% EtOAc/hexanes) to afford the product as a clear oil (162 mg, 40%).

Physical properties: clear oil.

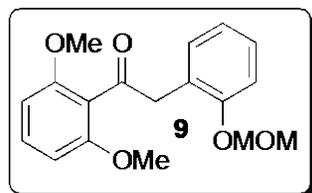
$^1\text{H NMR}$ (500 MHz, Chloroform-*d*) δ 7.68 (dd, $J = 7.6, 1.8$ Hz, 1H), 7.44 (ddd, $J = 8.4, 7.3, 1.9$ Hz, 1H), 7.24 – 7.15 (m, 2H), 7.08 (dd, $J = 8.2, 1.4$ Hz, 1H), 7.02 – 6.93 (m, 3H), 5.10 (s, 2H), 4.29 (s, 2H), 3.92 (s, 3H), 3.40 (s, 3H).

$^{13}\text{C NMR}$ (126 MHz, CDCl_3) δ 200.07, 158.46, 155.47, 133.28, 131.52, 130.47, 128.30, 121.74, 120.72, 114.05, 111.54, 94.54, 77.41, 77.16, 76.91, 56.08, 55.61, 45.78.

FT-IR (cm^{-1} , neat, ATR) 1677, 1596, 1484, 1465, 990.

HRMS (ESI) m/z calc. for $\text{C}_{17}\text{H}_{18}\text{O}_4$ ($\text{M} + \text{Na}$) $^+$ 309.1099; Found 309.1103.

1-(2,6-dimethoxyphenyl)-2-(2-(methoxymethoxy)phenyl)ethan-1-one (9).



2',6'-Dimethoxyacetophenone (Alfa Aesar; 200 mg, 1.10 mmol) was reacted with 1-bromo-2-(methoxymethoxy)benzene (Combi-Blocks; 481 mg, 2.21 mmol) according to the general procedure (Method A) and was purified by flash chromatography on silica gel (10% EtOAc/hexanes to 20% EtOAc/hexanes) to afford the product as a clear oil (53 mg, 15%).

Physical properties: clear oil.

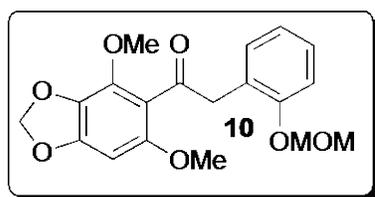
¹H NMR (500 MHz, Chloroform-*d*) δ 7.28 – 7.22 (m, 1H), 7.22 – 7.17 (m, 2H), 7.09 (d, $J = 7.7$ Hz, 1H), 6.95 (td, $J = 7.5, 1.2$ Hz, 1H), 6.53 (d, $J = 8.4$ Hz, 2H), 5.12 (s, 2H), 4.13 (s, 2H), 3.76 (s, 6H), 3.47 (s, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 201.71, 156.95, 155.81, 131.74, 130.64, 128.29, 124.07, 121.64, 120.35, 114.30, 104.06, 94.83, 77.42, 77.16, 76.91, 56.00, 55.92, 46.16.

FT-IR (cm⁻¹, neat, ATR) 1703, 1591, 1492, 1286, 988.

HRMS (ESI) m/z calc. for C₁₈H₂₀O₅ (M + H)⁺ 317.1389; Found 317.1390.

1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)-2-(2-(methoxymethoxy)phenyl)ethan-1-one



(**10**) 1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-one

(**3**) (50 mg, 0.213 mmol) was reacted with 1-bromo-2-(methoxymethoxy)benzene (Combi-Blocks; 53 mg, 0.245

mmol) according to the general procedure (Method B) and was purified by flash chromatography on silica gel (15% EtOAc/hexanes to 20% EtOAc/hexanes) to afford the product as a clear oil (80.3 mg, 95%).

Physical properties: clear oil.

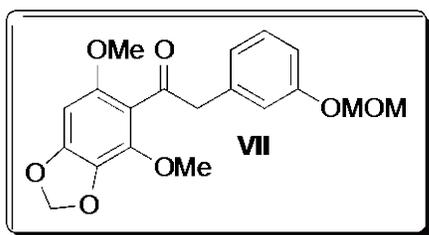
¹H NMR (500 MHz, Chloroform-*d*) δ 7.22 – 7.15 (m, 2H), 7.08 (d, $J = 8.2$ Hz, 1H), 6.94 (t, $J = 7.5$ Hz, 1H), 6.21 (s, 1H), 5.88 (s, 2H), 5.12 (s, 2H), 4.08 (s, 2H), 3.89 (s, 3H), 3.69 (s, 3H), 3.45 (s, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 200.38, 155.75, 152.42, 150.36, 140.69, 131.66, 130.37, 128.31, 124.17, 121.69, 116.70, 114.31, 101.26, 94.79, 89.19, 77.41, 77.16, 76.91, 60.04, 56.65, 56.00, 46.36.

FT-IR (cm^{-1} , neat, ATR) 1703, 1616, 1493, 1230, 991.

HRMS (ESI) m/z calc. for $\text{C}_{19}\text{H}_{20}\text{O}_7$ ($\text{M} + \text{H}$)⁺ 361.1287; Found 361.1306.

1-(4,6-dimethoxybenzo[d][1,3]dioxol-5-yl)-2-(3-(methoxymethoxy)phenyl)ethan-1-one (VII).



1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-one (3) (64.7 mg, 0.288 mmol) was reacted with 1-iodo-3-(methoxymethoxy)benzene (Enamine Building Blocks; 83.8 mg, 0.317 mmol) according to the

general procedure (Method B) and was purified by flash chromatography on silica gel (15% EtOAc/hexanes) to afford the product as a clear oil (58.4 mg, 58%).

Physical properties: clear oil.

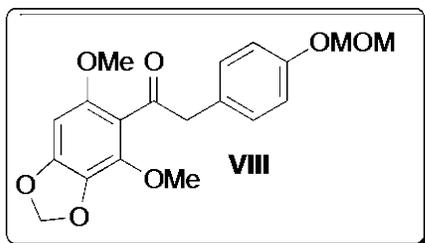
^1H NMR (500 MHz, Chloroform-*d*) δ 7.19 (dd, $J = 8.9, 7.5$ Hz, 1H), 6.91 – 6.88 (m, 2H), 6.86 (d, $J = 7.7$ Hz, 1H), 6.20 (s, 1H), 5.88 (s, 2H), 5.14 (s, 2H), 3.99 (s, 2H), 3.88 (s, 3H), 3.70 (s, 3H), 3.46 (s, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 200.76, 157.34, 152.44, 150.63, 140.63, 136.19, 130.27, 129.30, 123.68, 117.94, 116.31, 114.73, 101.31, 94.61, 89.10, 77.41, 77.16, 76.91, 60.03, 56.64, 56.07, 51.68.

FT-IR (cm^{-1} , neat, ATR) 1698, 1615, 1474, 1248, 951.

HRMS (ESI) m/z calc. for $\text{C}_{19}\text{H}_{20}\text{O}_7$ ($\text{M} + \text{H}$)⁺ 361.1287; Found 361.1298.

1-(4,6-dimethoxybenzo[1,3]dioxol-5-yl)-2-(4-(methoxymethoxy)phenyl)ethan-1-one(VIII).



1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-one (3) (55.8 mg, 0.248 mmol) was reacted with 1-Bromo-4-(methoxymethoxy)benzene (Oakwood Chemical; 59 mg, 0.273 mmol) according to the

general procedure (Method B) and was purified by flash chromatography on silica gel (15% EtOAc/hexanes) to afford the product as a clear oil (58.4 mg, 41%).

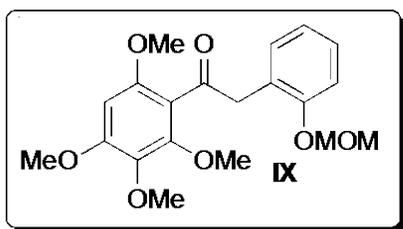
Physical properties: clear oil.

¹H NMR (500 MHz, Chloroform-*d*) δ 7.12 (d, *J* = 8.6 Hz, 2H), 6.96 (d, *J* = 8.6 Hz, 2H), 6.21 (s, 1H), 5.89 (s, 2H), 5.15 (s, 2H), 3.96 (s, 2H), 3.88 (s, 3H), 3.69 (s, 3H), 3.46 (s, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 200.65, 156.26, 152.89, 150.72, 140.95, 132.47, 130.64, 117.01, 116.12, 101.32, 94.60, 89.27, 77.41, 77.16, 76.91, 63.64, 59.97, 56.63, 56.04.

FT-IR (cm⁻¹, neat, ATR) 1698, 1615, 1474, 1230, 996.

HRMS (ESI) *m/z* calc. for C₁₉H₂₀O₇ (M + H)⁺ 361.1287; Found 361.129.



2-(2-(methoxymethoxy)phenyl)-1-(2,3,4,6-tetramethoxyphenyl)ethan-1-one (IX).

2,4,5,6-Tetramethoxyacetophenone (Ark Pharma 100 mg, 0.416 mmol) was reacted with 1-bromo-2-(methoxymethoxy)benzene (Combi-Blocks; 99.3 mg, 0.457 mmol) according to the general procedure (Method B) and was purified by flash chromatography on silica gel

(10% EtOAc/hexanes to 30% EtOAc/hexanes) to afford the product as a clear oil (119 mg, 76%).

Physical properties: clear oil.

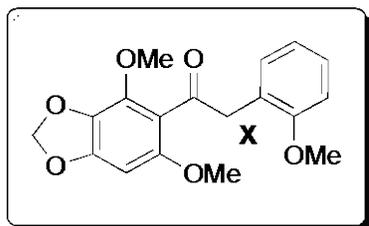
¹H NMR (500 MHz, Chloroform-*d*) δ 7.22 – 7.15 (m, 2H), 7.08 (d, *J* = 7.6 Hz, 1H), 6.94 (td, *J* = 7.4, 1.2 Hz, 1H), 6.23 (s, 1H), 5.13 (s, 2H), 4.12 (s, 2H), 3.87 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.75 (s, 3H), 3.45 (s, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 200.35, 155.74, 154.82, 152.71, 151.30, 136.20, 131.64, 128.33, 124.17, 121.70, 118.31, 114.27, 94.74, 92.46, 77.42, 77.16, 76.91, 61.94, 61.12, 56.26, 56.22, 55.98, 46.42.

FT-IR (cm⁻¹, neat, ATR) 1703, 1596, 1491, 1235, 996.

HRMS (ESI) *m/z* calc. for C₂₀H₂₄O₇ (M + H)⁺ 377.1600; Found 377.1601.

1-(4,6-dimethoxybenzo[1,3]dioxol-5-yl)-2-(2-methoxyphenyl)ethan-1-one (X).



1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-one (3)

(224.2 mg, 0.054 mmol) was reacted with 1-bromo-2-(methoxymethoxy)benzene (Alfa Aesar; 217.06 mg, 0.057 mmol) according to the general procedure (Method

B) and was purified by flash chromatography on silica gel (10% EtOAc/hexanes to 30% EtOAc/hexanes) to afford the product as a clear oil (50.5 mg, 63%).

Physical properties: clear oil.

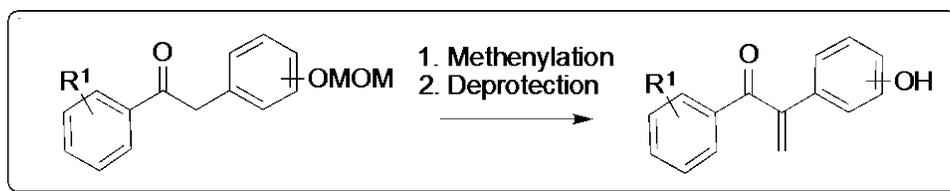
¹H NMR (500 MHz, Chloroform-*d*) δ 7.23 – 7.15 (m, 2H), 6.89 (td, *J* = 7.4, 1.1 Hz, 1H), 6.83 (d, *J* = 8.1 Hz, 1H), 6.20 (s, 1H), 5.88 (s, 2H), 4.05 (s, 2H), 3.90 (s, 3H), 3.76 (s, 3H), 3.69 (s, 3H).

^{13}C NMR (126 MHz, CDCl_3) δ 200.84, 157.85, 152.52, 150.31, 140.78, 131.58, 130.34, 128.25, 123.63, 120.43, 116.78, 110.54, 101.23, 89.11, 77.41, 77.16, 76.90, 60.07, 56.67, 55.55, 46.16.

FT-IR (cm^{-1} , neat, ATR) 1698, 1616, 1494, 1230, 948.

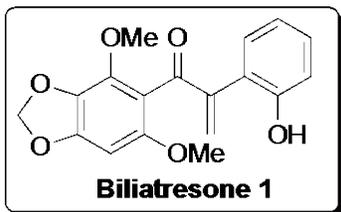
HRMS (ESI) m/z calc. for $\text{C}_{18}\text{H}_{18}\text{O}_6$ ($\text{M} + \text{H}$) $^+$ 331.1182; Found 331.1185.

General procedure for the synthesis of enone analogs:



To the aryl ketone (1 eq) dissolved in DMF (0.05 M) was added paraformaldehyde (1.6 eq) followed by K_2CO_3 (2.2 eq). The reaction was stirred for 16 hours at room temperature and then was quenched by the addition of saturated brine solution. The resulting solution was then extracted with Et_2O (x 3). The combined organic phases were washed with H_2O (x 5) followed by brine. The organic layer was then washed with saturated brine solution, dried over MgSO_4 , filtered and concentrated under reduced pressure. The resulting crude products were then ran through a pad of silica gel using 10% EtOAc/DCM and then concentrated under reduced pressure. The resulting clear oil was then dissolved in THF: 6 M HCl (3:1, 0.01 M) and stirred for 3 hours. The reaction was then slowly quenched with saturated NaHCO_3 and then was extracted with EtOAc (x 3). The combined organic layers was then washed with saturated brine solution, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was then purified by preparative thin layer chromatography (acetone/hexanes).

Biliatresone (1)



1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)-2-(2-(methoxymethoxy)phenyl)ethan-1-one (**10**) (321.6 mg, 0.892 mmol) was reacted according to the general procedure and was purified by thin layer chromatography (25%

acetone/hexanes) to afford the product as a pale yellow oil. The analytical data was in accordance to the literature.⁴

Physical properties: Pale yellow oil; Yield = 138.9 mg (47% yield over 2 steps).

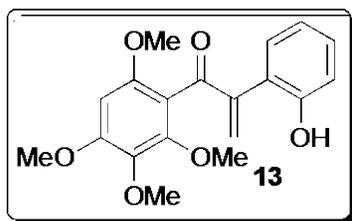
¹H NMR (500 MHz, Acetone-*d*₆) δ 7.98 (s, 1H), 7.21 – 7.12 (m, 2H), 6.90 – 6.81 (m, 2H), 6.48 (s, 1H), 6.06 (d, *J* = 20.1 Hz, 2H), 6.00 (s, 2H), 3.92 (s, 3H), 3.73 (s, 3H).

¹³C NMR (126 MHz, Acetone) δ 206.28, 195.64, 155.39, 153.95, 151.74, 149.38, 141.90, 131.83, 131.33, 130.14, 130.03, 125.77, 120.18, 116.87, 115.68, 102.41, 90.32, 60.28, 57.08, 30.30, 30.15, 29.99, 29.84, 29.69, 29.53, 29.38.

FT-IR (cm⁻¹, neat, ATR) 1698, 1616, 1494, 1230, 948.

HRMS (ESI) *m/z* calc. for C₁₈H₁₆O₆ (M+Na)⁺ 351.0846; Found 351.0845.

2-(2-hydroxyphenyl)-1-(2,3,4,6-tetramethoxyphenyl)prop-2-en-1-one (**13**).



2-(2-(methoxymethoxy)phenyl)-1-(2,3,4,6-tetramethoxyphenyl)ethan-1-one (**IX**) (119.1 mg, 0.316 mmol) was reacted according to the general procedure and was purified by thin layer chromatography (20%

acetone/hexanes) to afford the product as a pale yellow oil.

Physical properties: Pale yellow oil; Yield = 81.5 mg (85% over 2 steps).

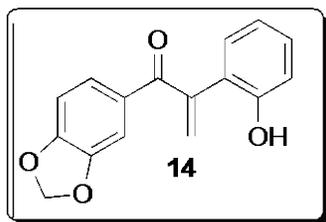
¹H NMR (500 MHz, Chloroform-*d*) δ 7.10 – 7.05 (m, 1H), 6.97 (dd, *J* = 7.6, 1.8 Hz, 1H), 6.78 (d, *J* = 8.2 Hz, 1H), 6.72 (t, *J* = 7.4 Hz, 1H), 6.11 (s, 1H), 5.88 (s, 1H), 5.80 (s, 1H), 3.71 (d, *J* = 4.4 Hz, 6H), 3.61 (d, *J* = 13.7 Hz, 6H) (HO-not reported).

¹³C NMR (126 MHz, CDCl₃) δ 197.33, 155.85, 153.91, 153.45, 151.86, 149.62, 136.22, 131.16, 130.74, 130.30, 124.93, 120.38, 117.22, 115.82, 92.61, 77.42, 77.16, 76.91, 61.98, 61.21, 56.46, 56.35.

FT-IR (cm⁻¹, neat, ATR) 3400, 1666, 1616, 1596, 1485, 989.

HRMS (ESI) *m/z* calc. for C₁₉H₂₀O₆ (M + H)⁺ 345.1338; Found 345.1343.

1-(benzo[1,3]dioxol-5-yl)-2-(2-hydroxyphenyl)prop-2-en-1-one (14).



1-(benzo[1,3]dioxol-5-yl)-2-(2-(methoxymethoxy)phenyl)ethan-1-one (**7**) (44.5 mg, 0.145 mmol) was reacted according to the general procedure and was purified by thin layer chromatography (30%

acetone/hexanes) to afford the product as a pale yellow oil.

Physical properties: Pale yellow oil; Yield 11.2 mg (28% over 2 steps).

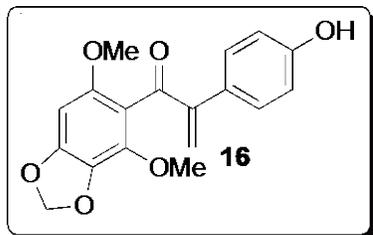
¹H NMR (500 MHz, Chloroform-*d*) δ 9.21 (s, 1H), 7.63 (d, *J* = 8.2 Hz, 1H), 7.49 (s, 1H), 7.29 – 7.17 (m, 2H), 6.99 – 6.83 (m, 3H), 6.08 (s, 2H), 5.93 (s, 1H), 5.73 (s, 1H).

¹³C NMR (126 MHz, CDCl₃) δ 199.02, 154.72, 153.16, 148.42, 146.95, 130.64, 130.45, 130.20, 128.20, 127.29, 125.46, 120.43, 117.87, 109.92, 108.06, 102.33, 77.41, 77.16, 76.91.

FT-IR (cm⁻¹, neat, ATR) 3300, 1650, 1598, 1485, 995.

HRMS (ESI) *m/z* calc. for C₁₆H₁₂O₄ (M + H)⁺ 269.0814; Found 269.0821.

1-(4,6-dimethoxybenzo[1,3]dioxol-5-yl)-2-(4-hydroxyphenyl)prop-2-en-1-one (16).



1-(4,6-dimethoxybenzo[1,3]dioxol-5-yl)-2-(4-(methoxymethoxy)phenyl)ethan-1-one (**VIII**) (34.6 mg, 0.096 mmol) was reacted according to the general procedure and was purified by thin layer chromatography

(30% acetone/hexanes) to afford the product as a white foam.

Physical properties: White foam; Yield = 27.4 mg (86% over 2 steps).

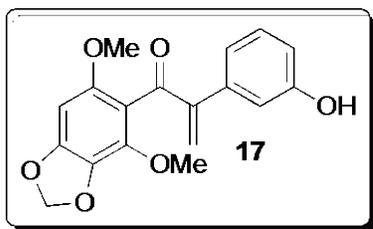
¹H NMR (500 MHz, Chloroform-*d*) δ 7.30 (d, $J = 8.5$ Hz, 2H), 6.79 (d, $J = 8.7$ Hz, 2H), 6.26 (s, 1H), 6.00 (s, 1H), 5.92 (s, 2H), 5.82 (s, 1H), 5.35 (s, 1H), 3.92 (s, 3H), 3.69 (s, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 196.13, 155.90, 153.13, 150.75, 150.14, 141.27, 130.42, 130.02, 129.54, 126.19, 115.30, 115.17, 101.39, 89.43, 77.42, 77.16, 76.90, 60.06, 56.85.

FT-IR (cm⁻¹, neat, ATR) 3400, 1653, 1611, 1513, 1422.

HRMS (ESI) m/z calc. for C₁₈H₁₆O₆ (M+Na)⁺ 351.0866; Found 351.0845.

1-(4,6-dimethoxybenzo[1,3]dioxol-5-yl)-2-(3-hydroxyphenyl)prop-2-en-1-one (17).



1-(4,6-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-2-(3-(methoxymethoxy)phenyl)ethan-1-one (**VII**) (58.4 mg, 0.162 mmol) was reacted according to the general procedure and was purified by thin layer chromatography

(25% acetone/hexanes) to afford the product as a pale yellow oil.

Physical properties: Pale yellow oil; Yield = 18.4 mg (32% over 2 steps).

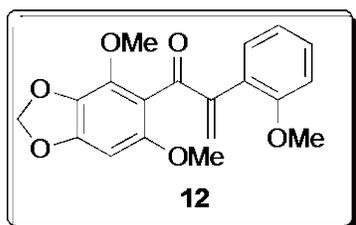
¹H NMR (500 MHz, Chloroform-*d*) δ 7.20 (t, *J* = 7.9 Hz, 1H), 6.99 – 6.93 (m, 2H), 6.80 (d, *J* = 8.1 Hz, 1H), 6.25 (s, 1H), 6.05 (s, 1H), 5.92 (s, 2H), 5.88 (s, 1H), 3.92 (s, 3H), 3.68 (s, 3H) (HO-not reported).

¹³C NMR (126 MHz, CDCl₃) δ 195.67, 155.61, 153.20, 150.84, 150.36, 141.29, 138.49, 130.40, 129.38, 127.59, 120.90, 115.79, 115.32, 115.04, 101.40, 89.40, 77.42, 77.16, 76.90, 60.05, 56.82.

FT-IR (cm⁻¹, neat, ATR) 3500, 1651, 1616, 1579, 1498, 946.

HRMS (ESI) *m/z* calc. for C₁₈H₁₆O₆ (M + H)⁺ 329.1025; Found 329.1036.

1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)-2-(2-methoxyphenyl)prop-2-en-1-one (12).



2-(2-(methoxymethoxy)phenyl)-1-(2,3,4,6-tetramethoxyphenyl)ethan-1-one (**IX**) (50.0 mg, 0.151 mmol) was dissolved in DMF (3.0 mL) and was added paraformaldehyde (7.2 mg, 0.243 mmol) and K₂CO₃ (46.

02 mg, 0.332 mmol). The reaction was stirred for 16 hours at room temperature and then was quenched by the addition of a saturated brine solution. The resulting solution was

then extracted with Et₂O (x 3). The combined organics were washed with H₂O (x 5) followed by brine. The organic layer was then washed with saturated brine solution, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting crude oil was then purified by preparative thin layer chromatography (30% Ethyl acetate/Hexanes) to afford a clear oil (10.9 mg, 21%).

Physical properties: Clear Oil.

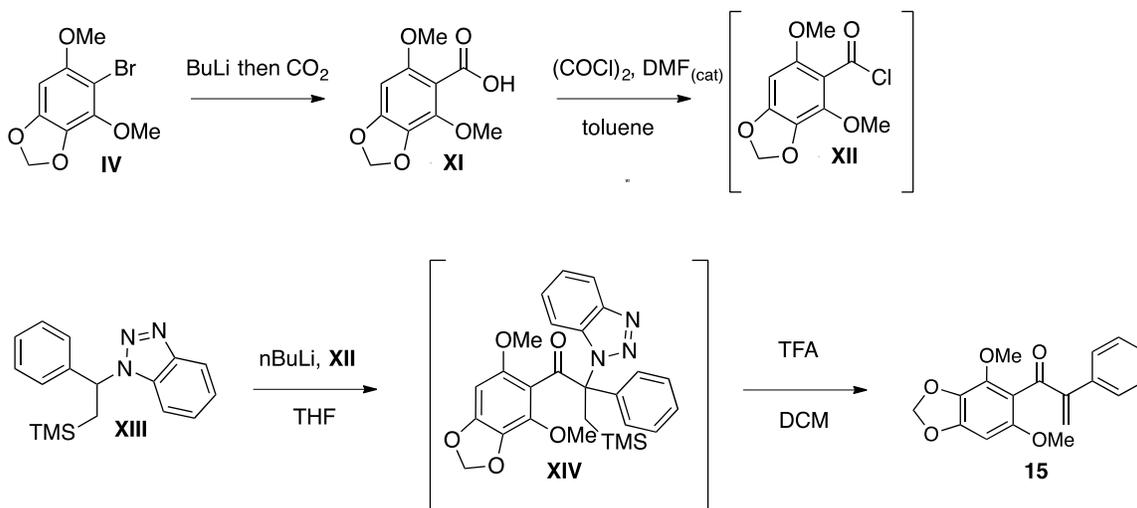
¹H NMR (500 MHz, Chloroform-*d*) δ 7.30 – 7.27 (m, 1H), 7.22 (dd, *J* = 7.5, 1.8 Hz, 1H), 6.94 (td, *J* = 7.5, 1.1 Hz, 1H), 6.86 (d, *J* = 8.4 Hz, 1H), 6.20 (s, 1H), 6.11 (s, 1H), 5.97 (s, 1H), 5.90 (s, 2H), 3.94 (s, 3H), 3.75 (s, 3H), 3.68 (s, 3H).

¹³C NMR (126 MHz, CDCl₃) δ 193.68, 157.34, 153.36, 150.46, 148.43, 141.61, 131.12, 130.59, 129.43, 128.77, 127.27, 120.42, 115.25, 111.08, 101.27, 89.35, 77.41, 77.16, 76.91, 60.22, 56.85, 55.72.

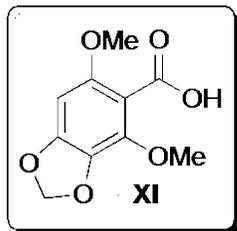
FT-IR (cm⁻¹, neat, ATR) 1671, 1615, 1490, 1473, 1446, 944.

HRMS (ESI) *m/z* calc. for C₁₉H₁₈O₆ (M + H)⁺ 343.1182; Found 343.1184.

Synthesis of deoxybiliatresone (15):



4,6-dimethoxybenzo-1, 3-dioxole-5-carboxylic acid (XI).



To 5-bromo-4,6-dimethoxy-1, 3-benzodioxole (IV) (0.640 g, 2.4 mmol) dissolved in THF (25.0 ml) at -78°C was added BuLi (2.1 mL, 3.4 mmol) and then was stirred an additional 15 minutes at -78°C . The reaction was then warmed to 0°C for 25 minutes and then cooled back to -78°C . Then CO_2 was bubbled through for 15 minutes and a solid precipitated. The reaction was then warmed to 25°C over 35 minutes and then was quenched with 1 M NaOH. The reaction was then extracted with EtOAc (x 3). The aqueous layer was then acidified with 1 M HCl and extracted with EtOAc (x 3). The final three organic extracts were combined, washed with brine, dried over Na_2SO_4 , filtered and concentrated under reduced pressure to provide the product as a white film (0.504 g, 90%). The resulting film was used without further purification in the next step.

Physical properties: Thin Film.

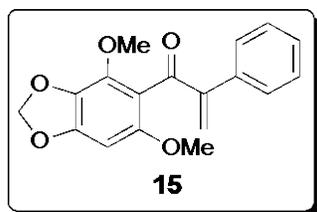
$^1\text{H NMR}$ (500 MHz, Chloroform-*d*) δ 6.29 (s, 1H), 5.95 (s, 2H), 4.05 (s, 3H), 3.83 (s, 3H)(CO_2OH - not reported).

$^{13}\text{C NMR}$ (126 MHz, CDCl_3) δ 168.34, 154.48, 151.73, 142.23, 130.95, 107.38, 101.69, 89.55, 77.41, 77.16, 76.91, 60.58, 57.16.

FT-IR (cm^{-1} , neat, ATR) 2950, 1683, 1622, 1485, 1453, 963.

HRMS (ESI) m/z calc. for $\text{C}_{10}\text{H}_{10}\text{O}_6$ ($\text{M} + \text{H}$) $^+$ 227.0569; Found 227.0556.

Deoxybiliatresone (15)



To 4,6-dimethoxybenzo-1, 3-dioxole-5-carboxylic acid (XI) (0.052 g, 0.229 mmol) in toluene (2.2 mL) at 25°C was added

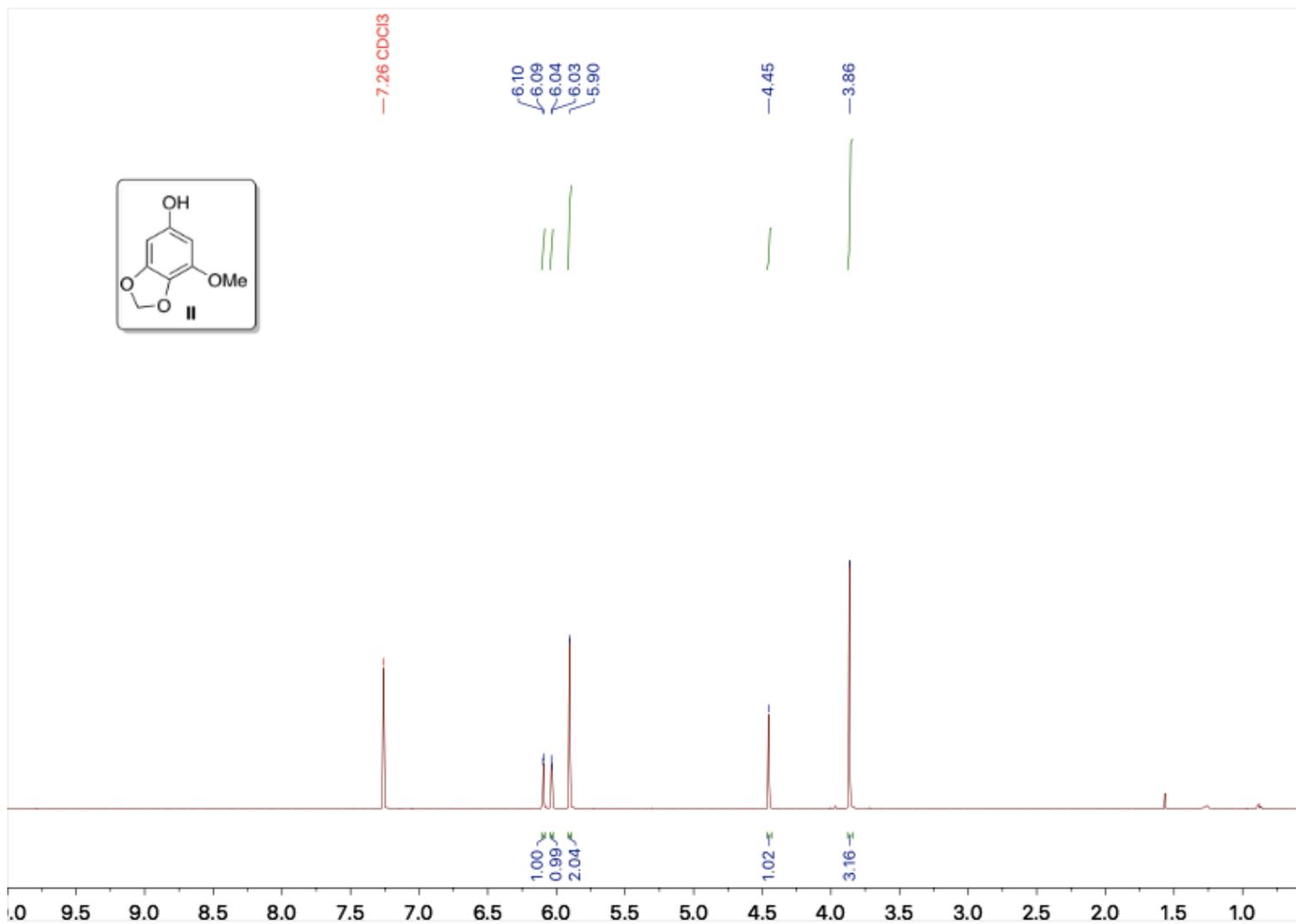
oxalyl chloride (0.197 mL) followed by one drop of DMF. The bubbling reaction was stirred an additional 10 minutes and then was concentrated under reduced pressure. The yellow solid was dissolved in toluene and concentrated three additional times. The resulting acid chloride (XII) was then taken on the next step without further purification. A solution of 1-[1-phenyl-2-(trimethylsilyl)ethyl]-1*H*-1,2,3-benzotriazole (XIII)⁵ dissolved in THF (2.8 mL) was added BuLi (0.114 mL, 0.229 mmol) at -78° C. The solution was then stirred for 15 mins at -78° C and then acid chloride (XII) dissolved in THF (0.8 mL) was added. The reaction was then slowly warmed to 25° C for 18 hours. The reaction was quenched with saturated NH₄Cl solution and extracted with ether (x 3). The organic layers were combined, washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure to provide the product as a crude oil (XIV). The resulting yellow oil (XIV) (0.028 g, 0.05 mmol) was then dissolved in DCM (0.22 mL) at 0° C. Then trifluoroacetic acid (8.5 μL, 1.11 mmol) was added. The reaction was warmed to 25° C and stirred for an additional 8 hours. Then the reaction quenched with 5% NaOH solution and was extracted with DCM (x 3). The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure to provide the product as a crude oil. The crude oil was purified by preparative thin layer chromatography (20% Ethyl acetate/Hexanes) to afford a yellow oil (8 mg, 11% yield).

Physical properties: Clear oil.

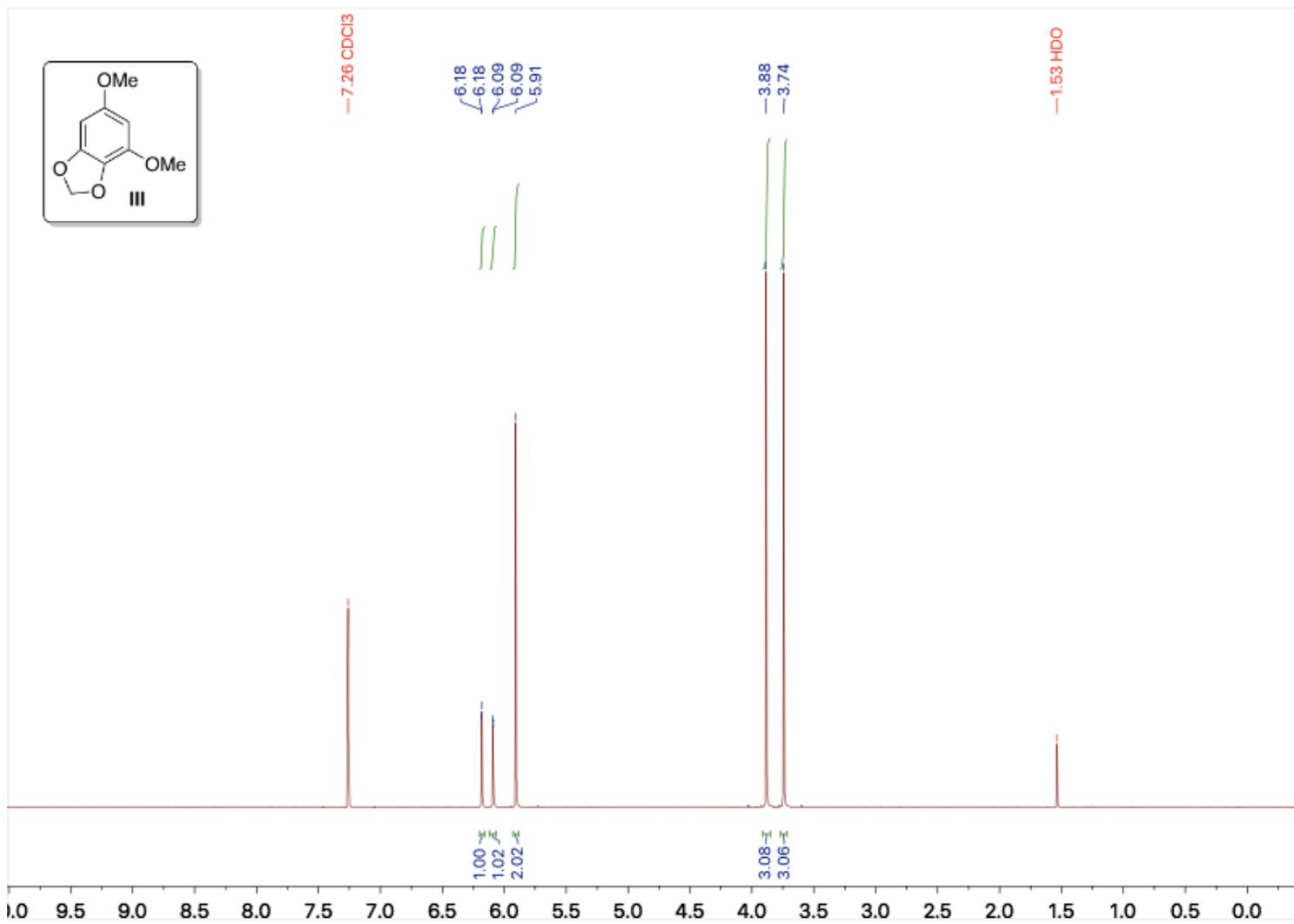
¹H NMR (500 MHz, Chloroform-*d*) δ 7.45 – 7.41 (m, 1H), 7.38 – 7.31 (m, 4H), 6.26 (s, 1H), 6.06 (s, 1H), 5.92 (s, 2H), 5.91 (s, 1H), 3.93 (s, 3H), 3.70 (s, 3H).

FT-IR (cm⁻¹, neat, ATR) 1671, 1618, 1475, 1422, 1229, 926.

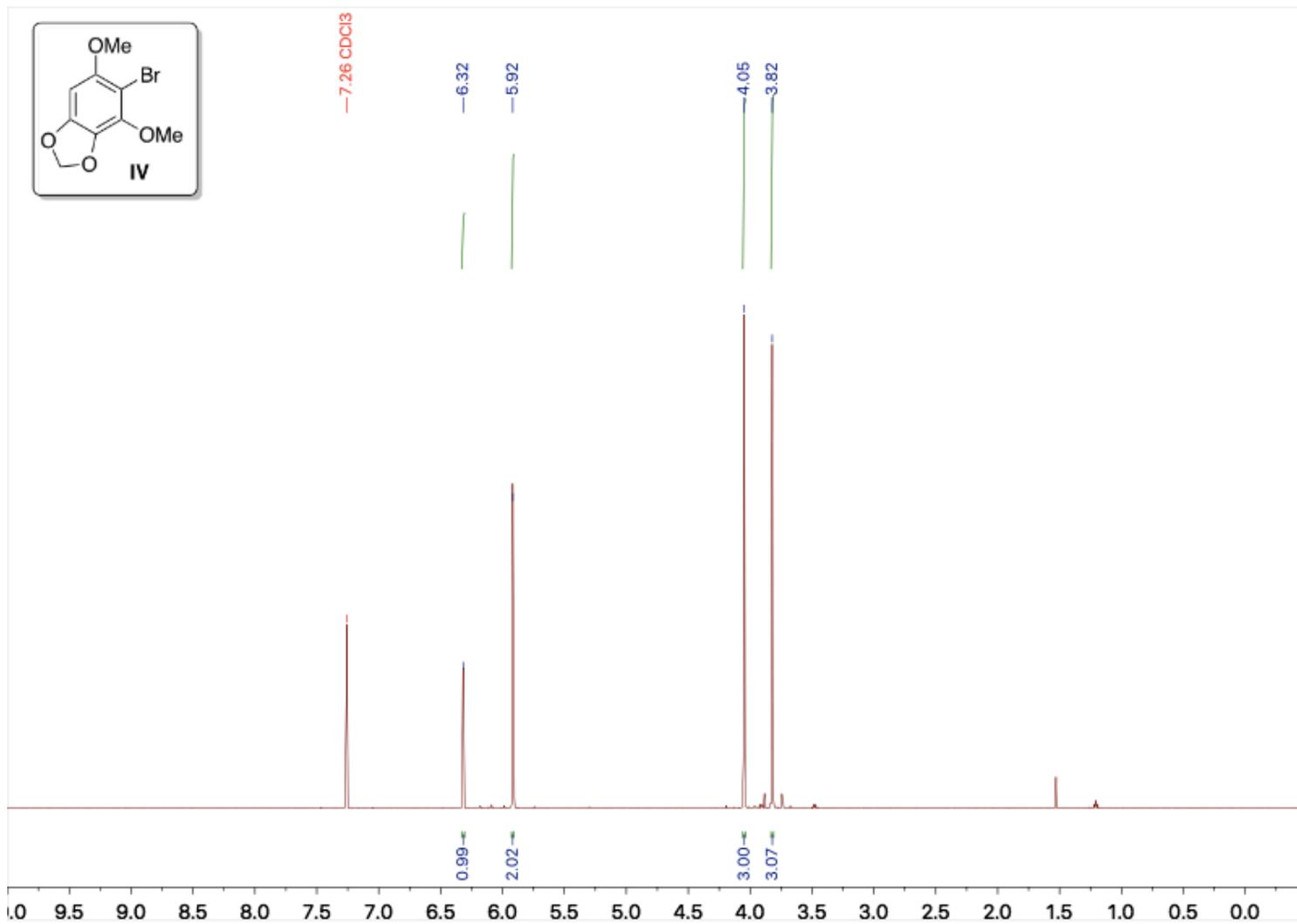
HRMS (ESI) m/z calc. for C₁₈H₁₆O₅ (M + Na)⁺ 335.0895; Found 335.0906.



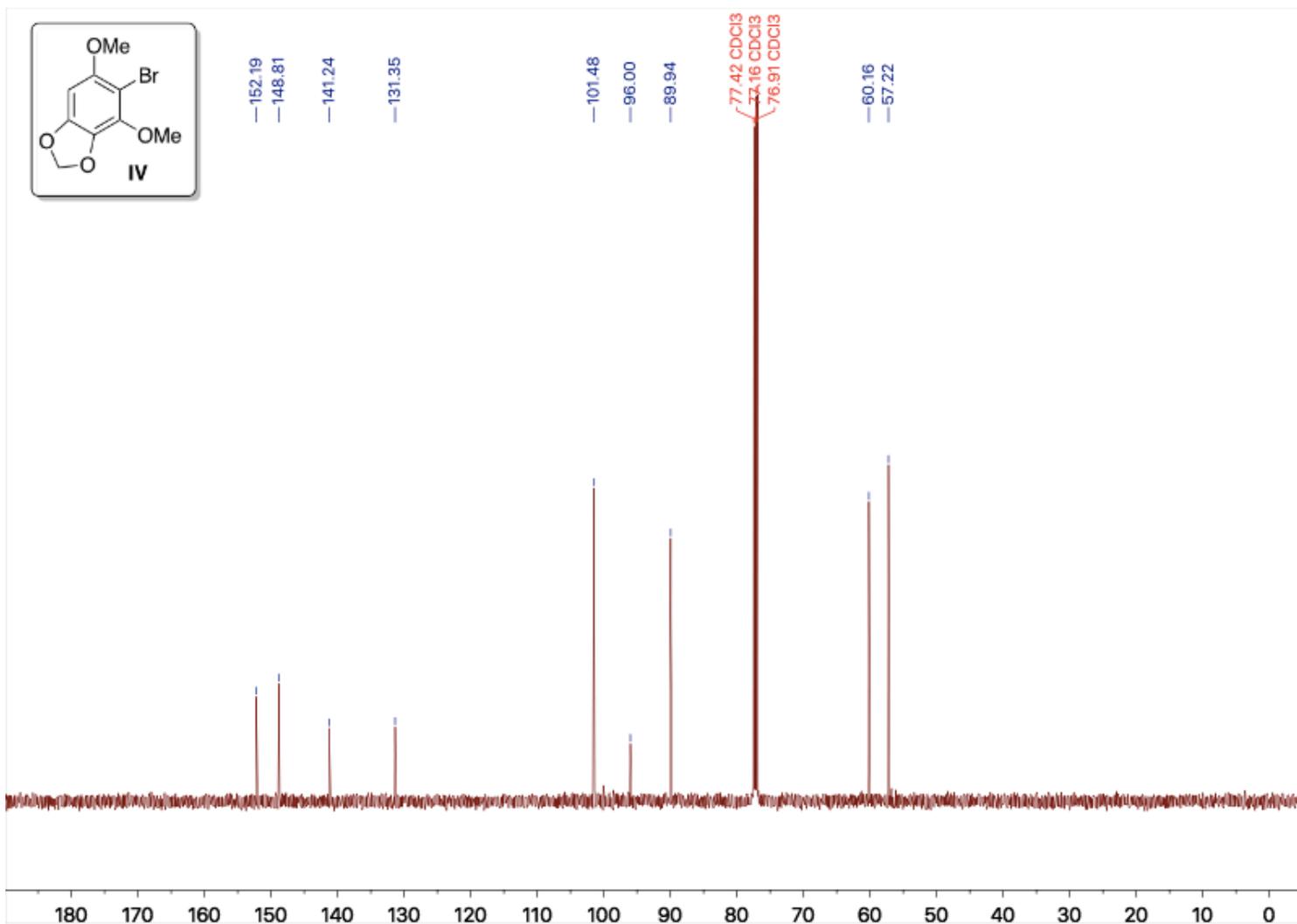
¹H NMR (500 MHz, CDCl₃) Spectrum of 7-methoxy-1,3-benzodioxol-5-ol (II).



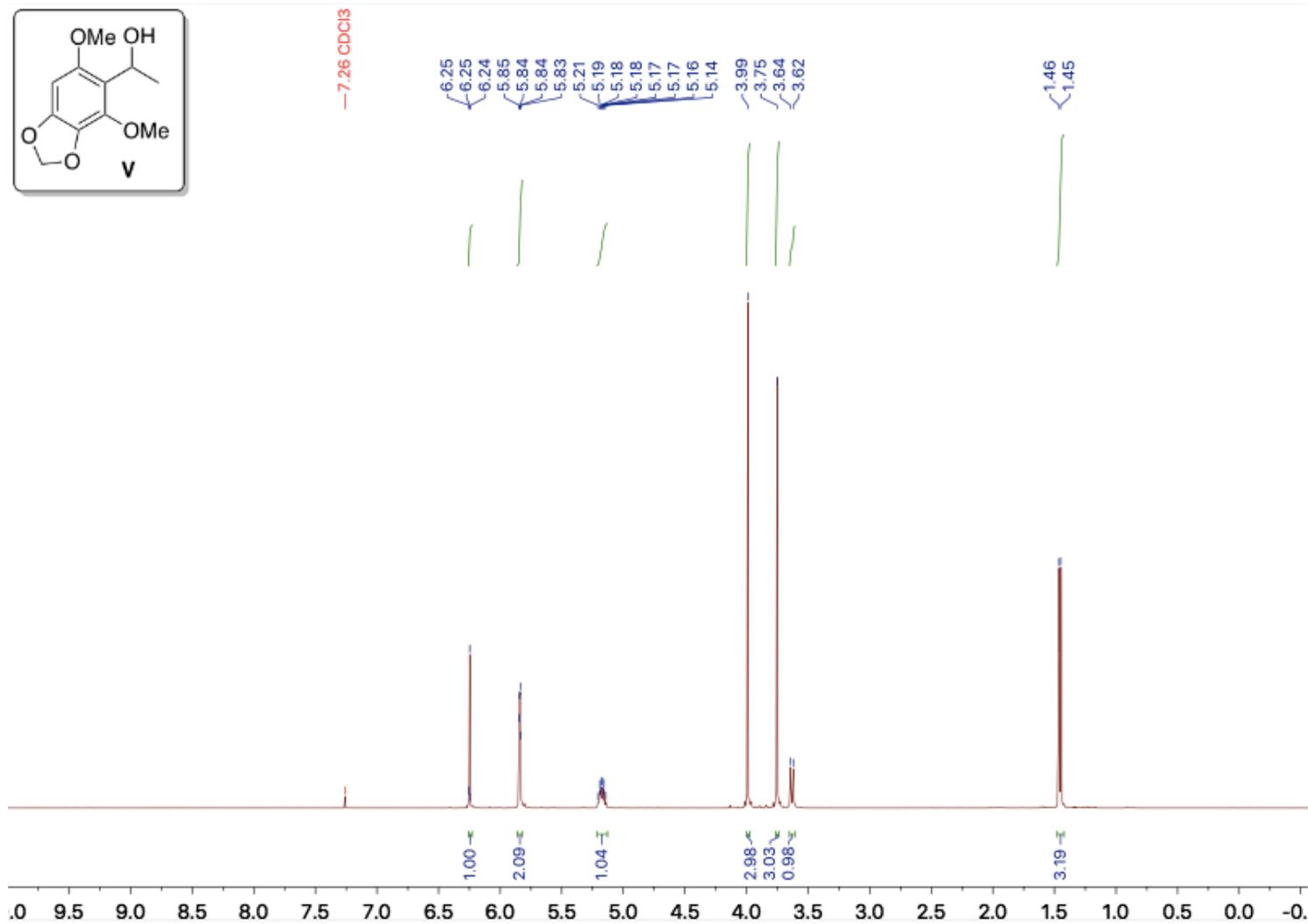
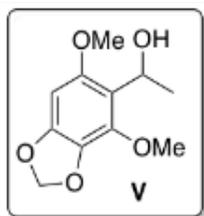
¹H NMR (500 MHz, CDCl₃) Spectrum of 4,6-dimethoxy-1,3-benzodioxole (III).



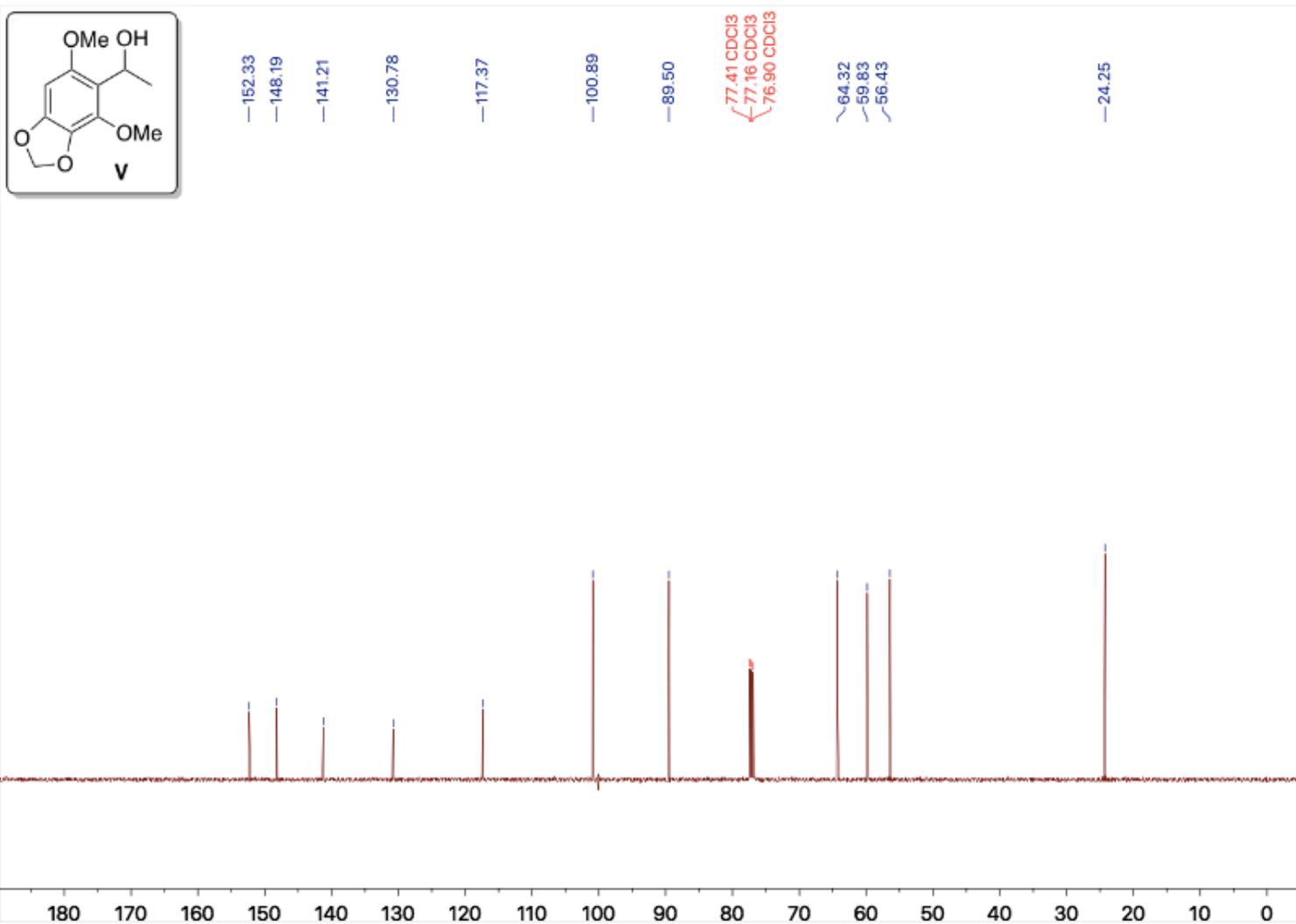
¹H NMR (500 MHz, CDCl₃) Spectrum of 5-bromo-4,6-dimethoxy-1,3-benzodioxole (IV).



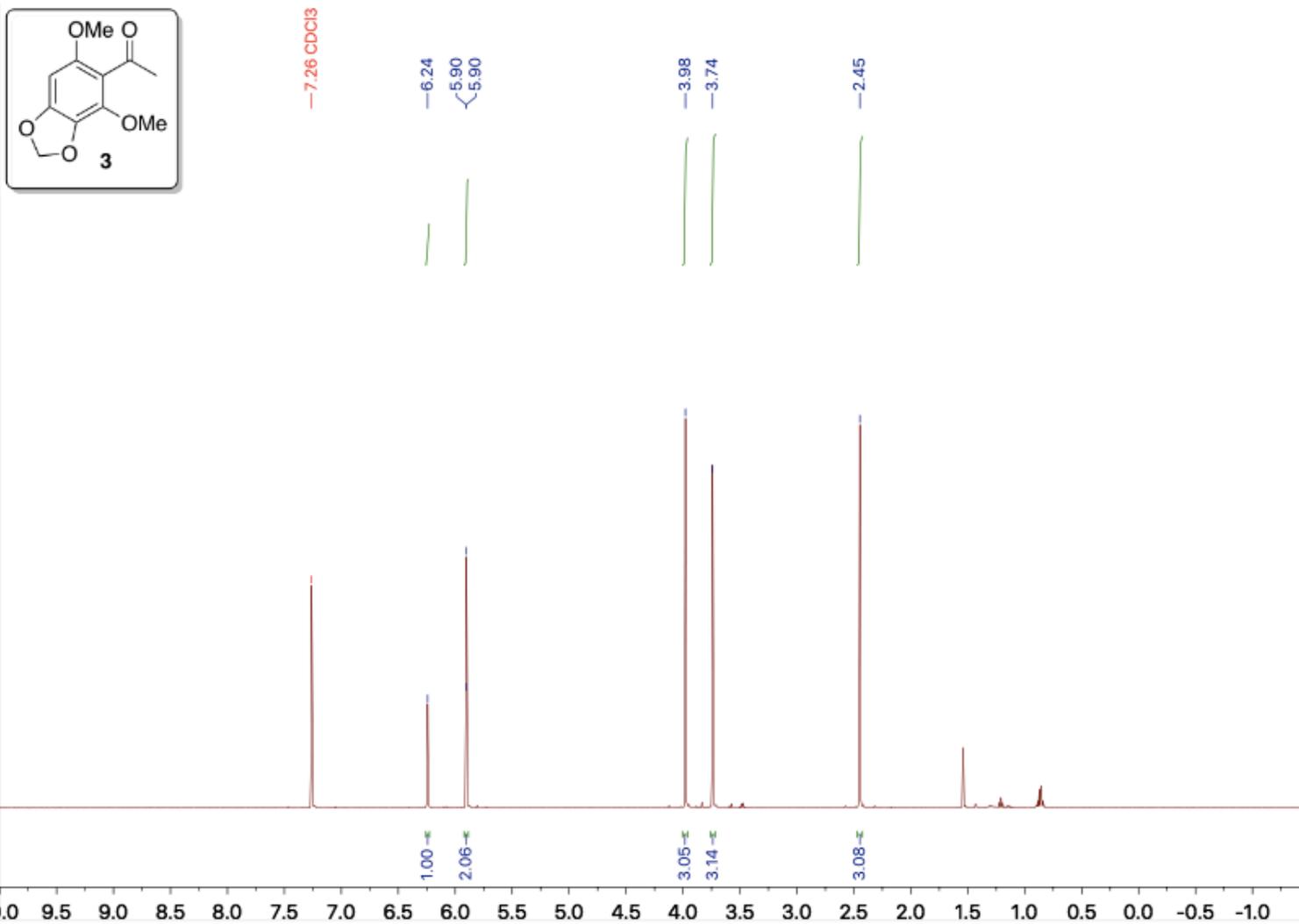
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 5-bromo-4,6-dimethoxy-1,3-benzodioxole (IV).



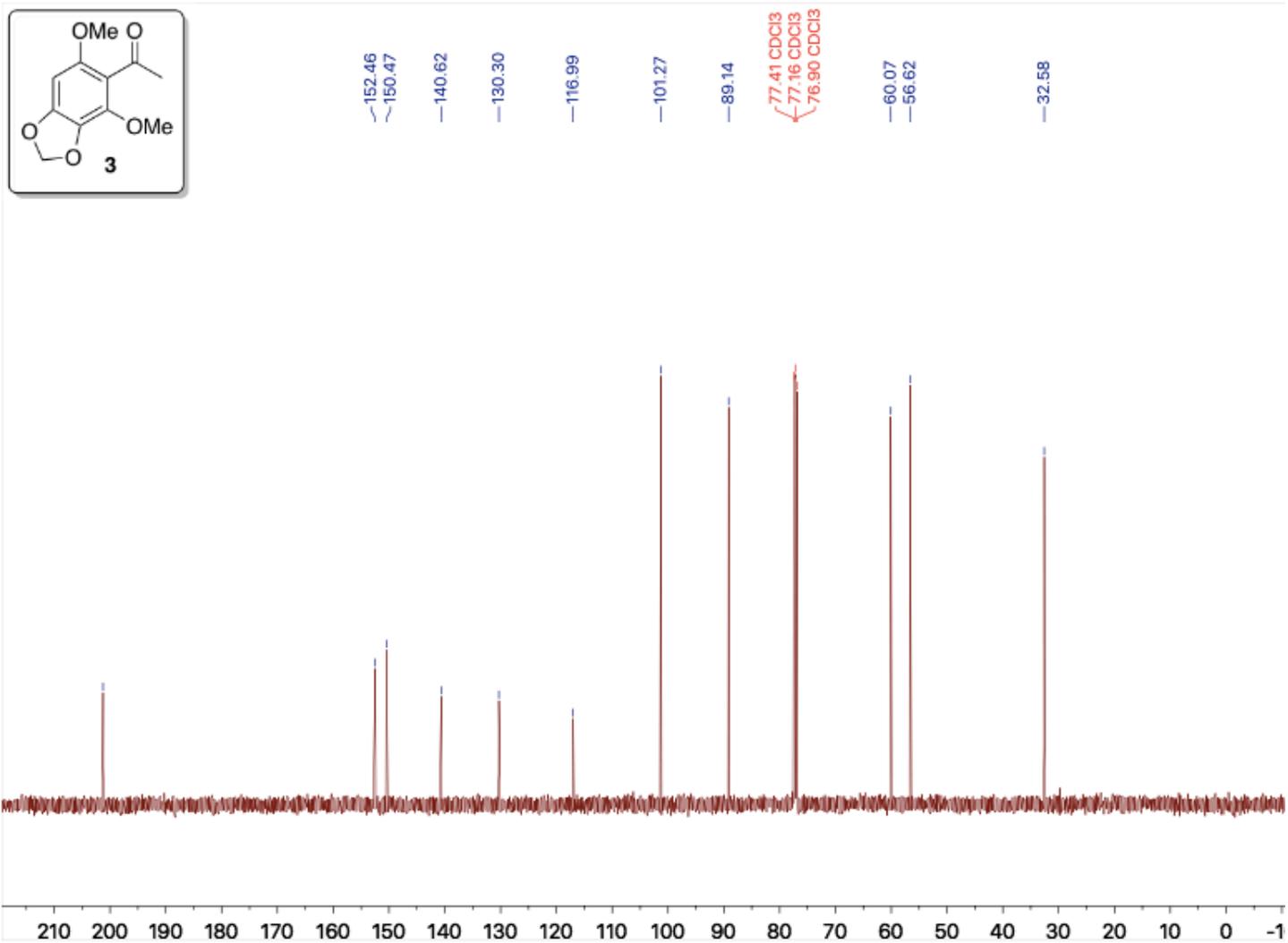
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-ol (V).



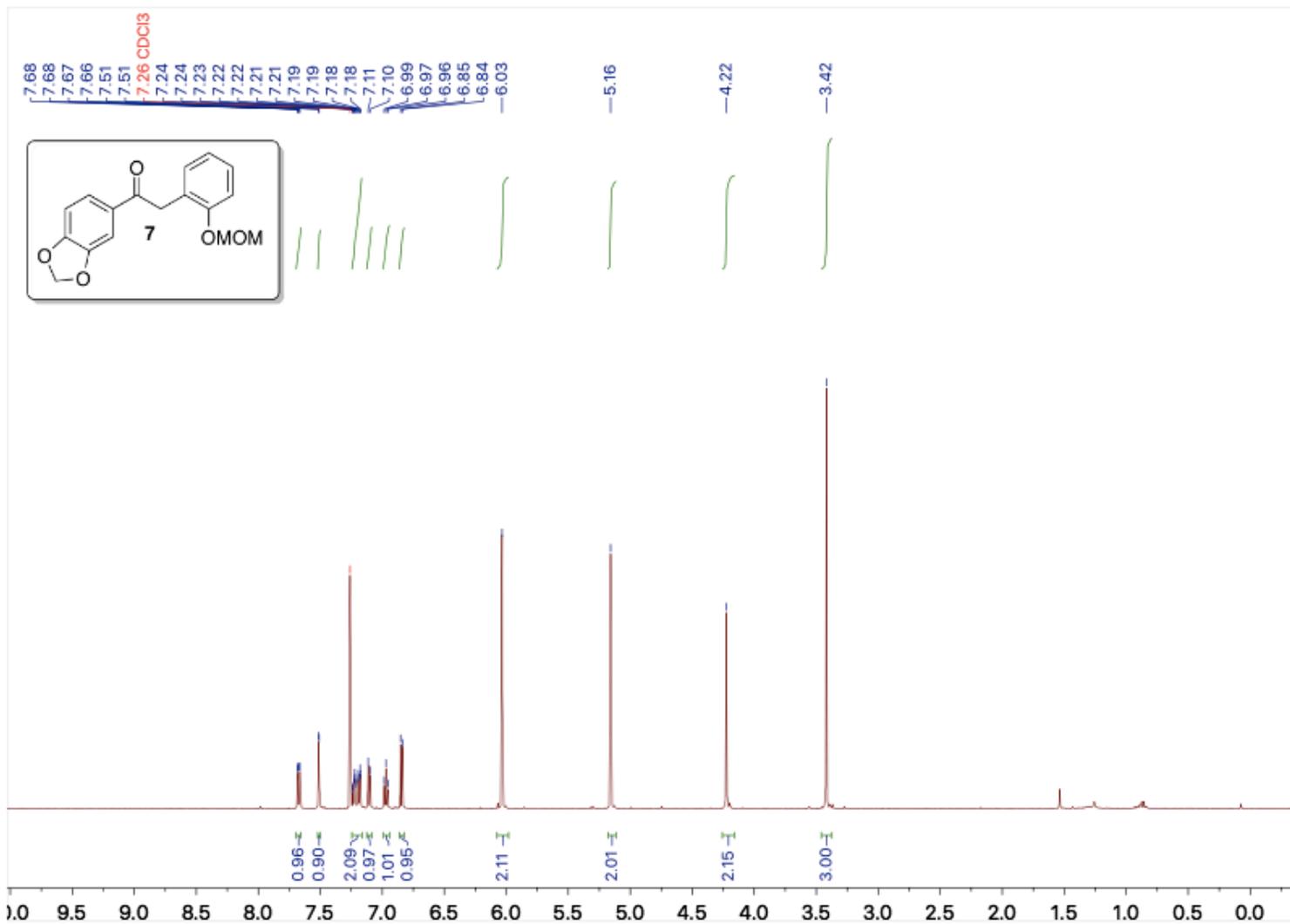
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-ol (V).



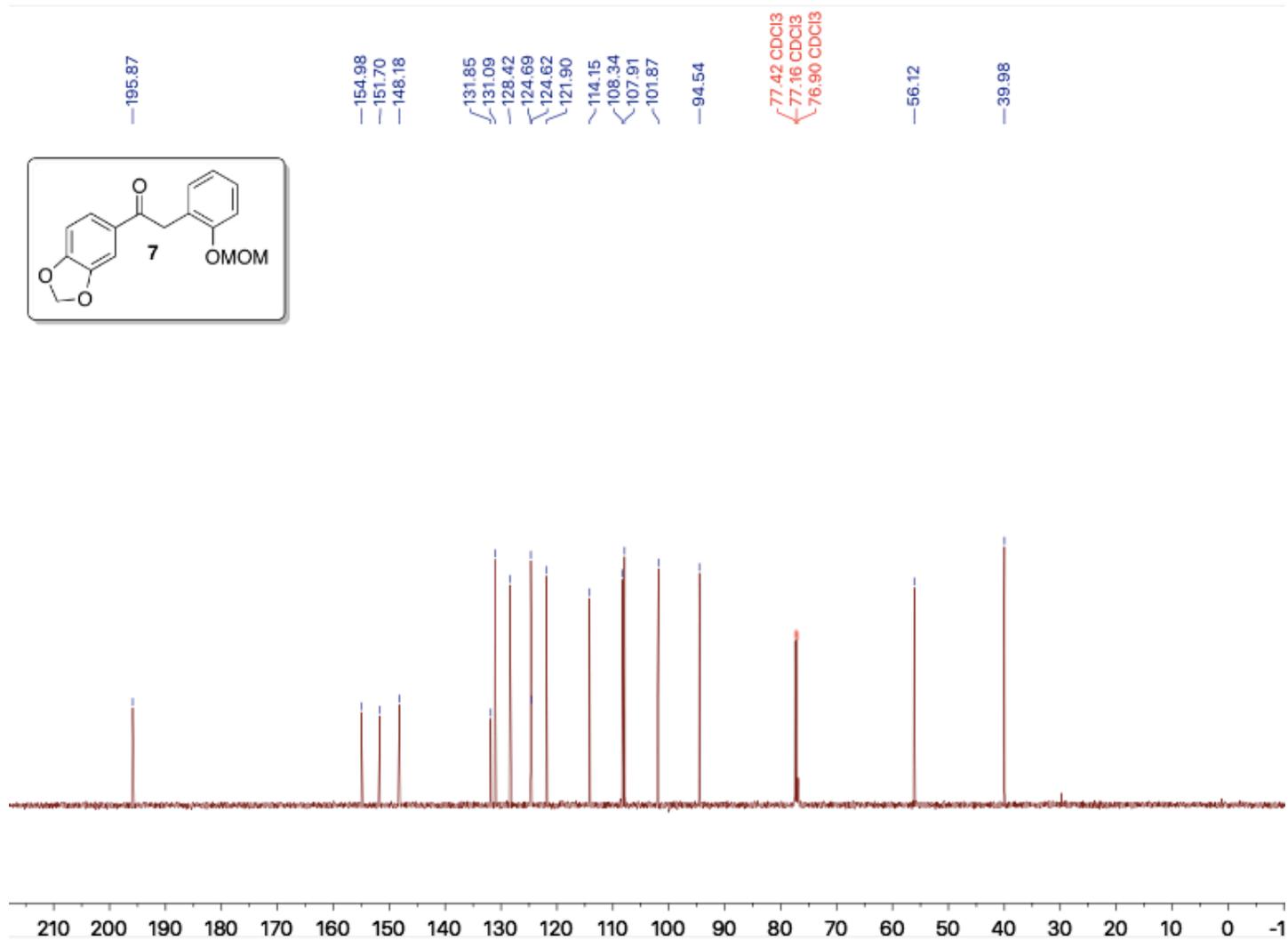
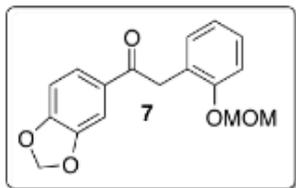
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-one (3).



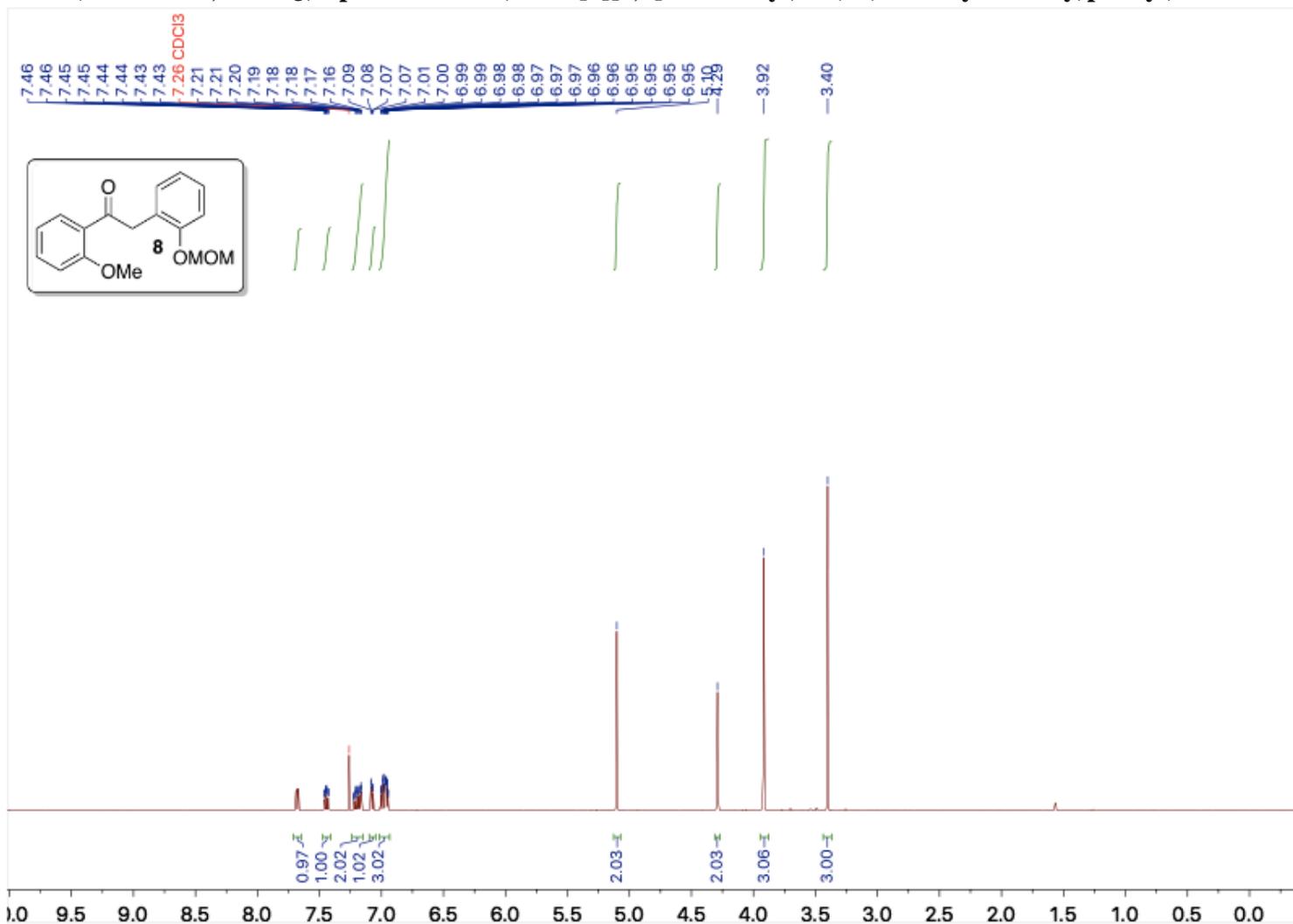
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)ethan-1-one (**3**).



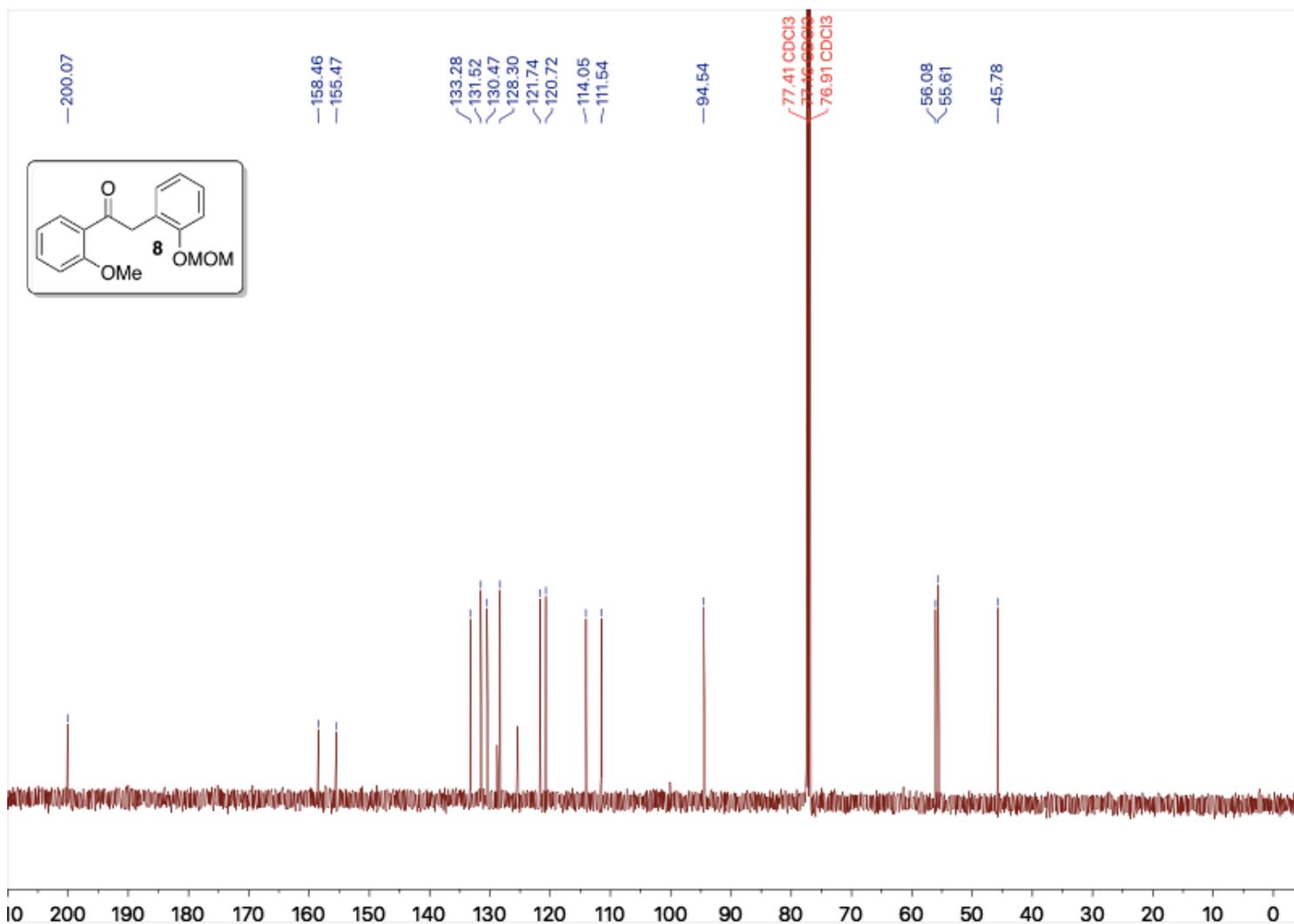
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(benzo[d][1,3]dioxol-5-yl)-2-(2-(methoxymethoxy)phenyl)ethan-1-one (7).



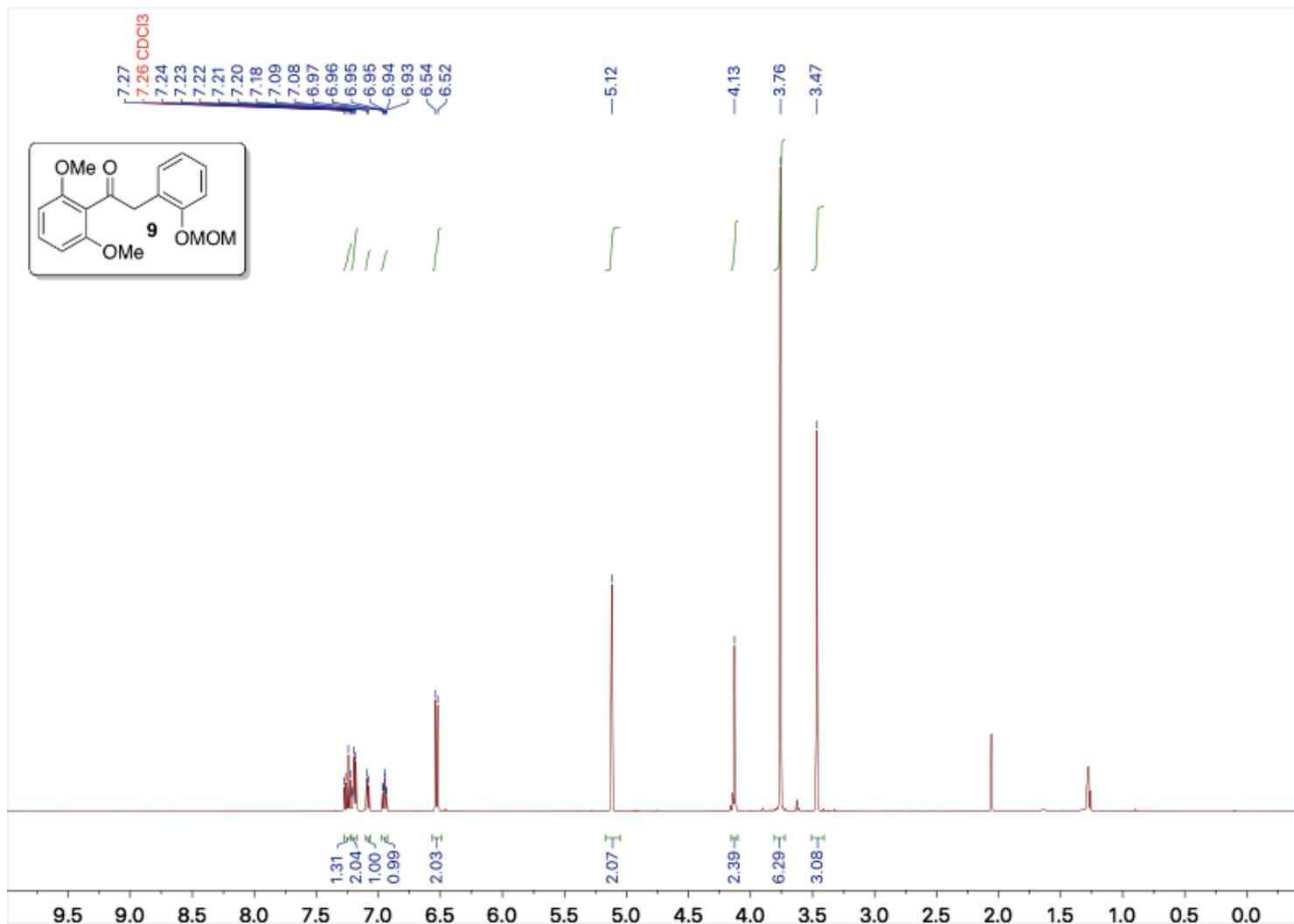
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(benzo[*d*][1,3]dioxol-5-yl)-2-(2-(methoxymethoxy)phenyl)ethan-1-one (7).



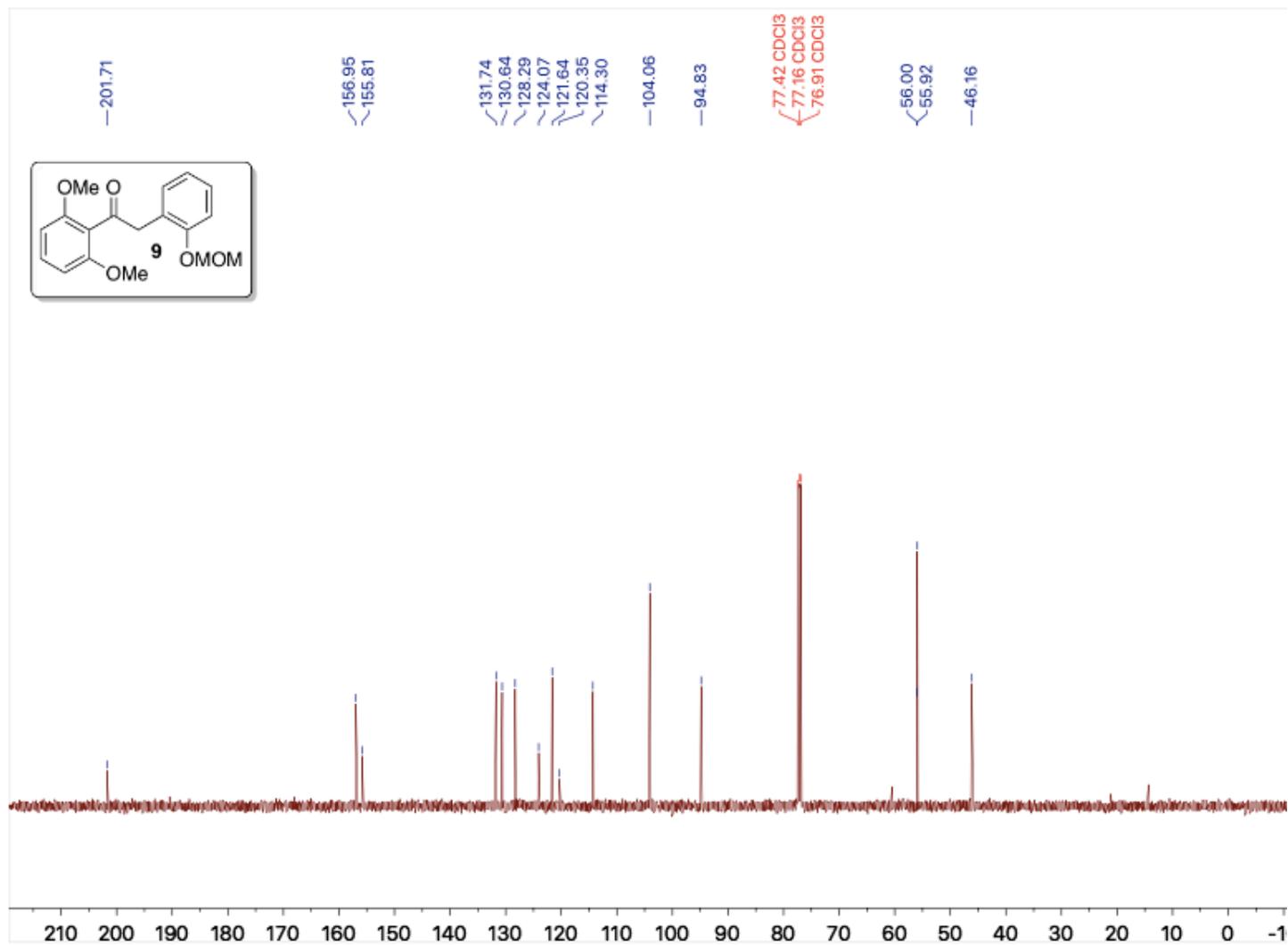
¹H NMR (500 MHz, CDCl₃) Spectrum of 2-(2-(methoxymethoxy)phenyl)-1-(2-methoxyphenyl)ethan-1-one (8).



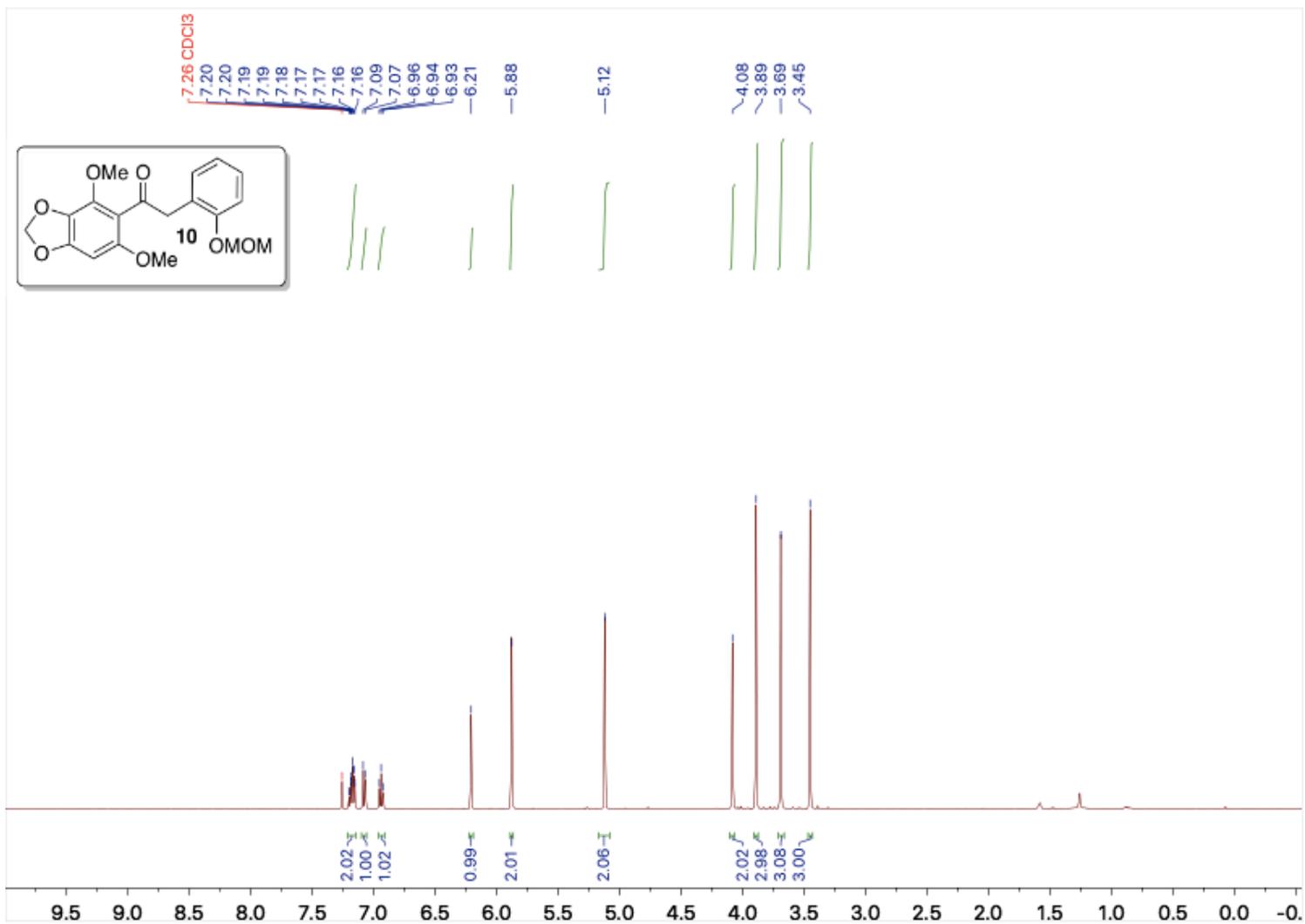
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 2-(2-(methoxymethoxy)phenyl)-1-(2-methoxyphenyl)ethan-1-one (8).



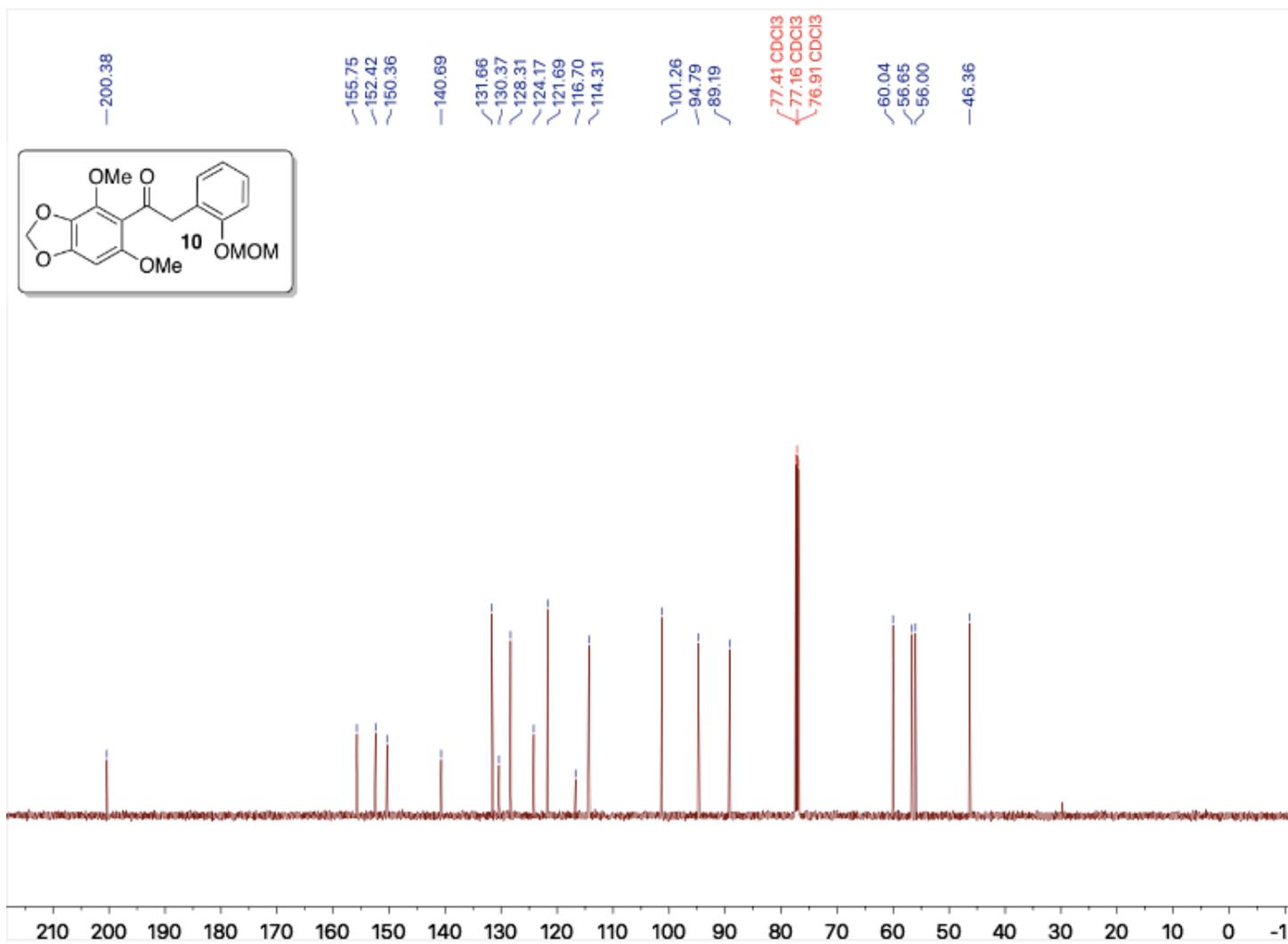
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(2,6-dimethoxyphenyl)-2-(2-(methoxymethoxy)phenyl)ethan-1-one (9).



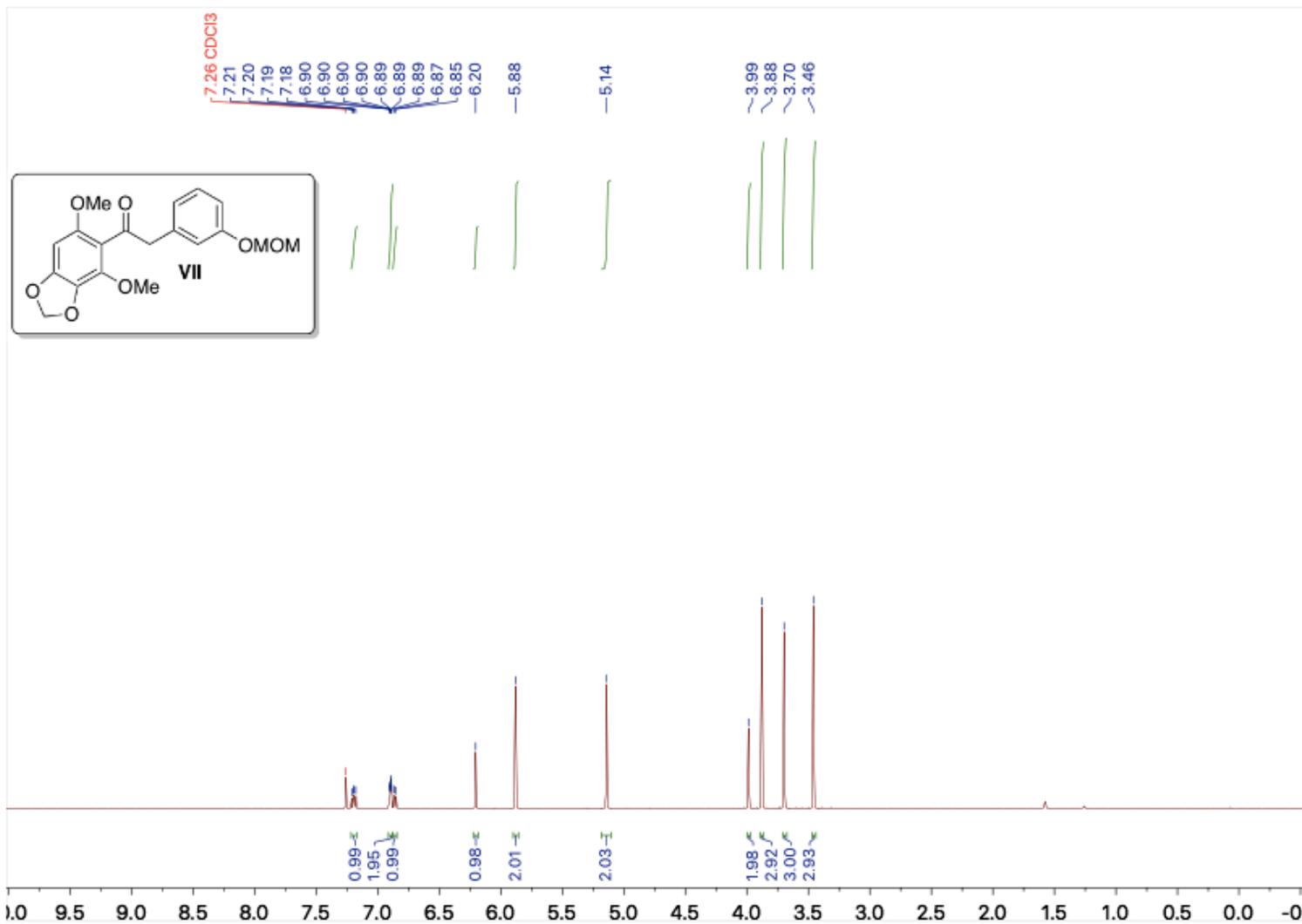
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(2,6-dimethoxyphenyl)-2-(2-(methoxymethoxy)phenyl)ethan-1-one (9).



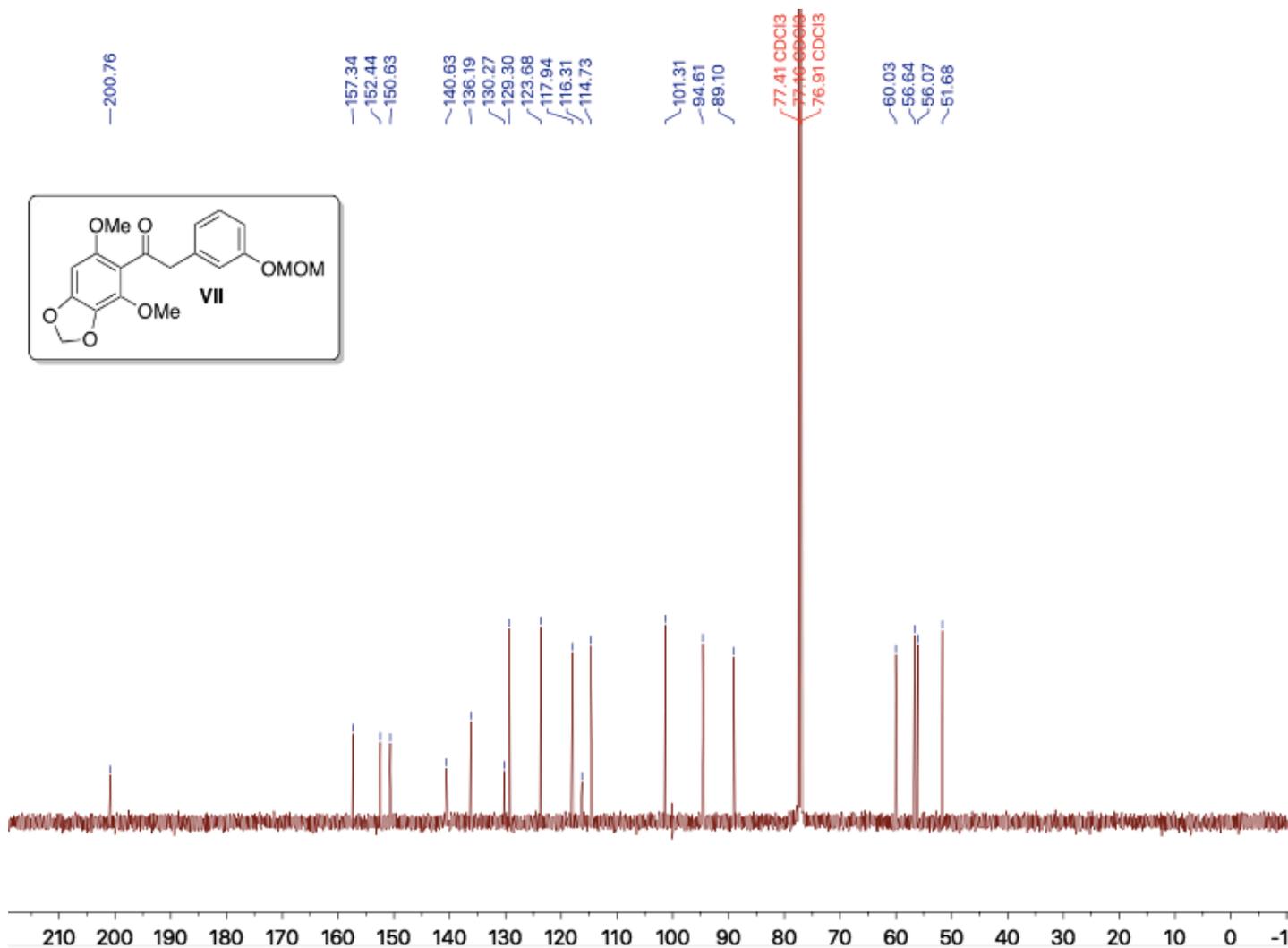
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)-2-(2-(methoxymethoxy)phenyl)ethan-1-one (10).



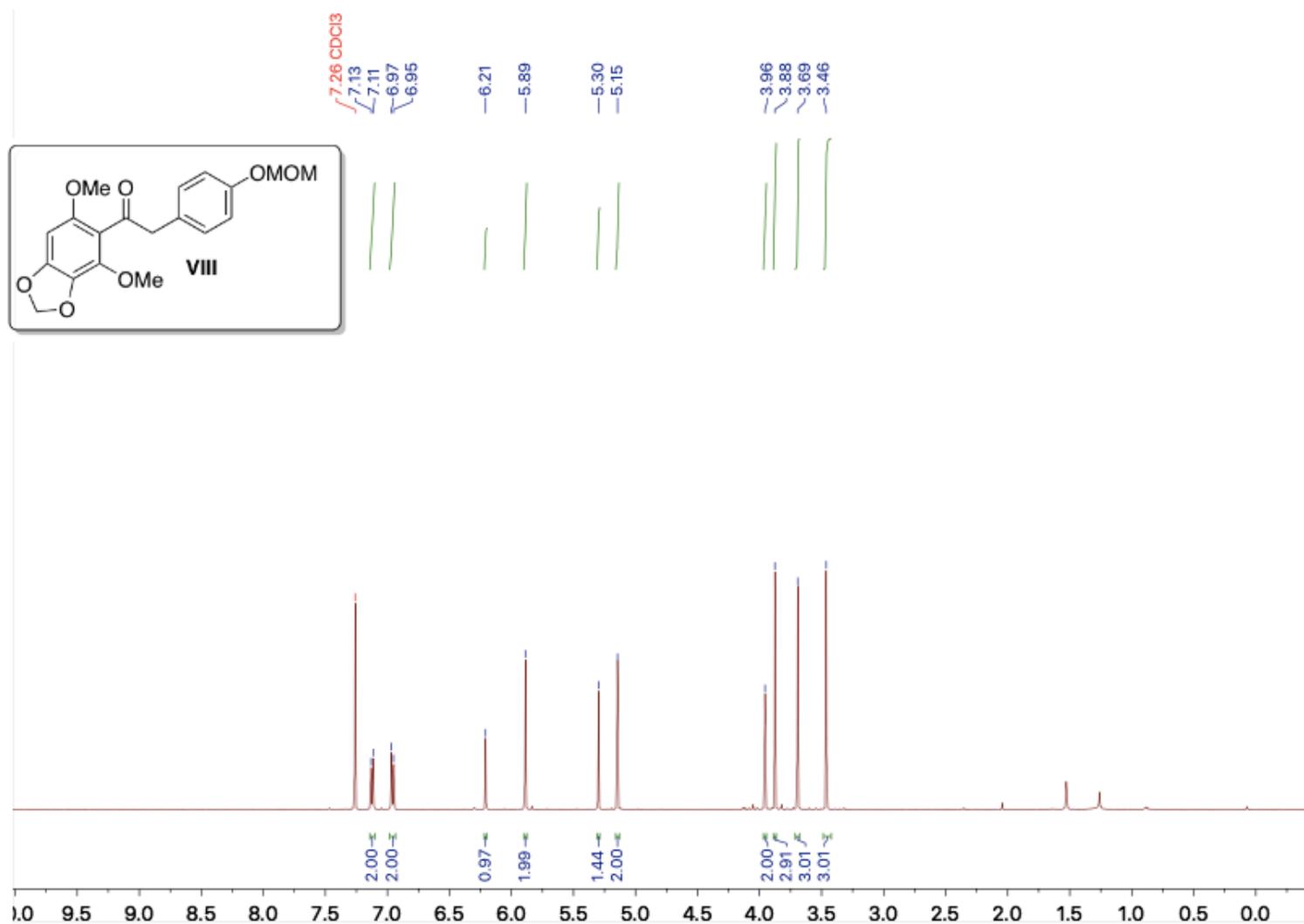
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)-2-(2-(methoxymethoxy)phenyl)ethan-1-one (10).



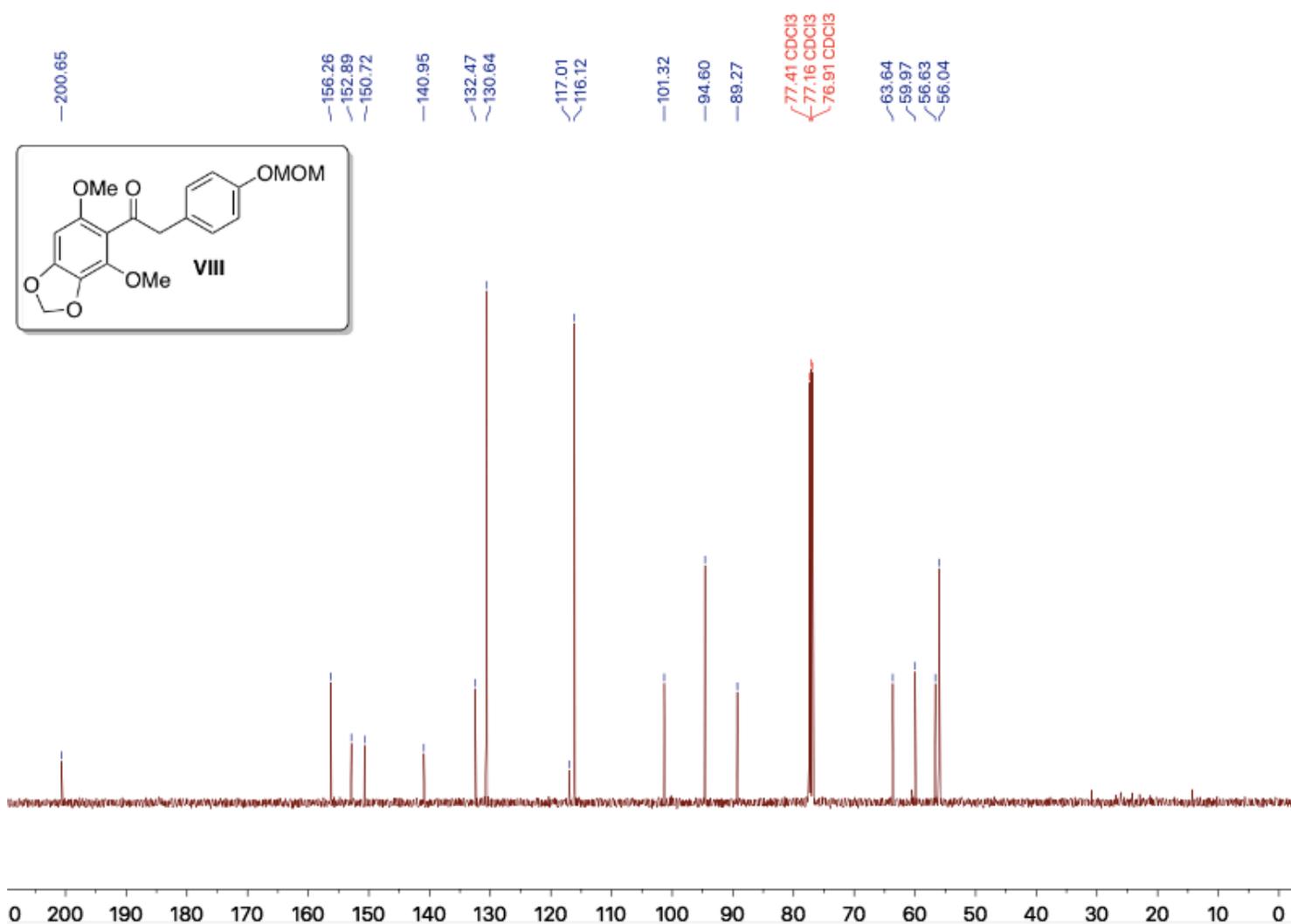
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-2-(3-(methoxymethoxy)phenyl)ethan-1-one (VII).



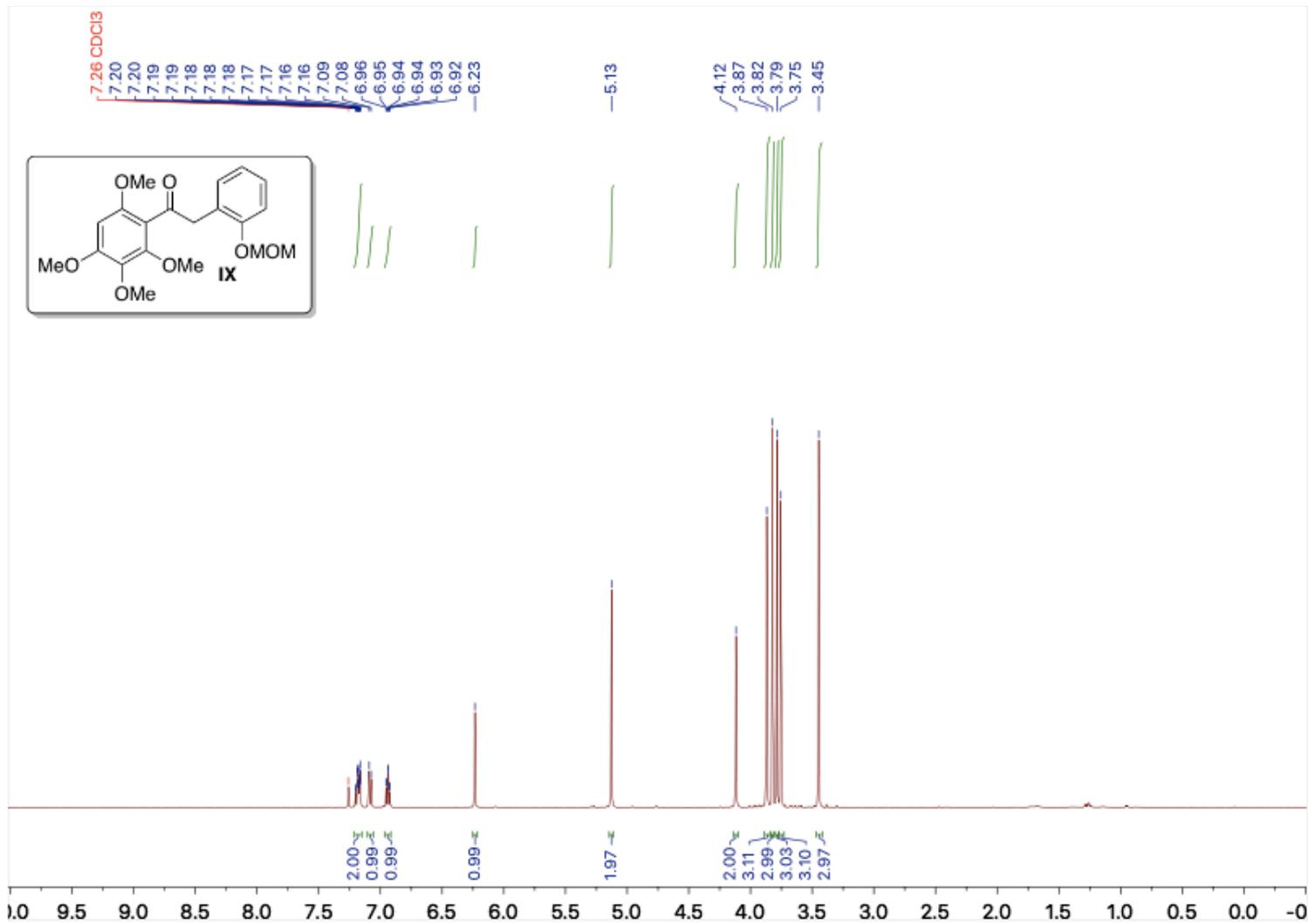
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-2-(3-(methoxymethoxy)phenyl)ethan-1-one (VII).



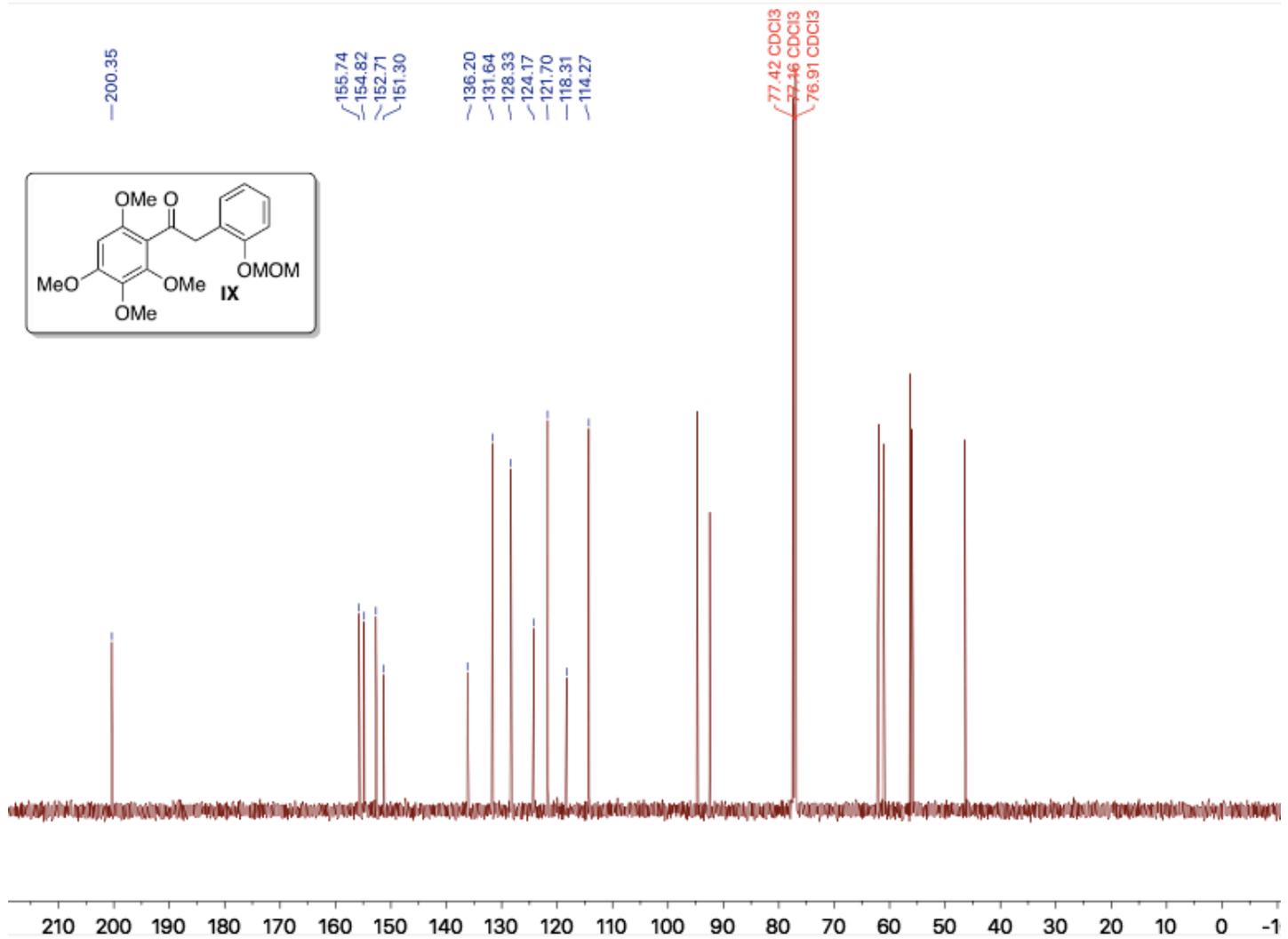
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-2-(4-(methoxymethoxy)phenyl)ethan-1-one (VIII).



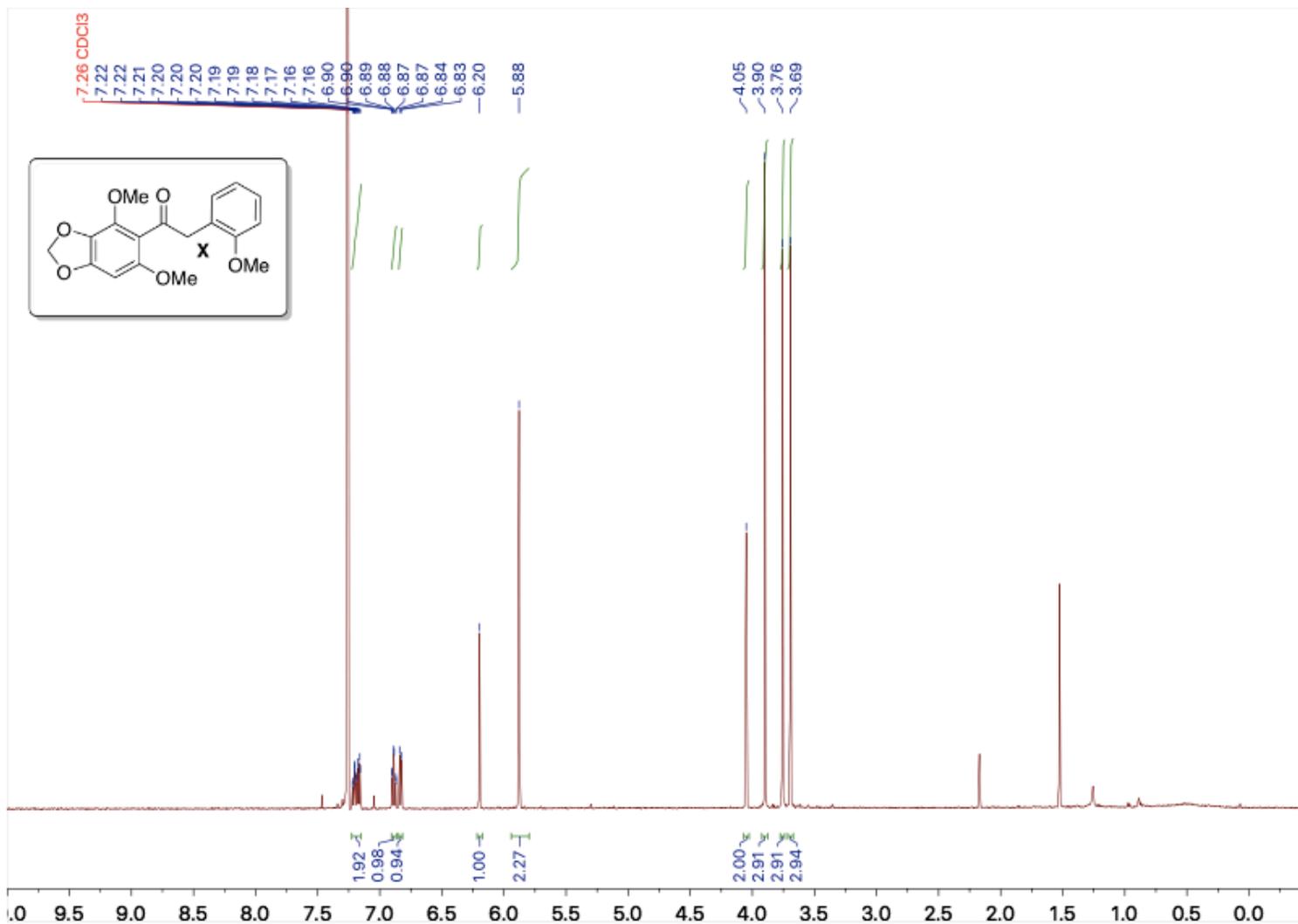
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo[d][1,3]dioxol-5-yl)-2-(4-(methoxymethoxy)phenyl)ethan-1-one (VIII).



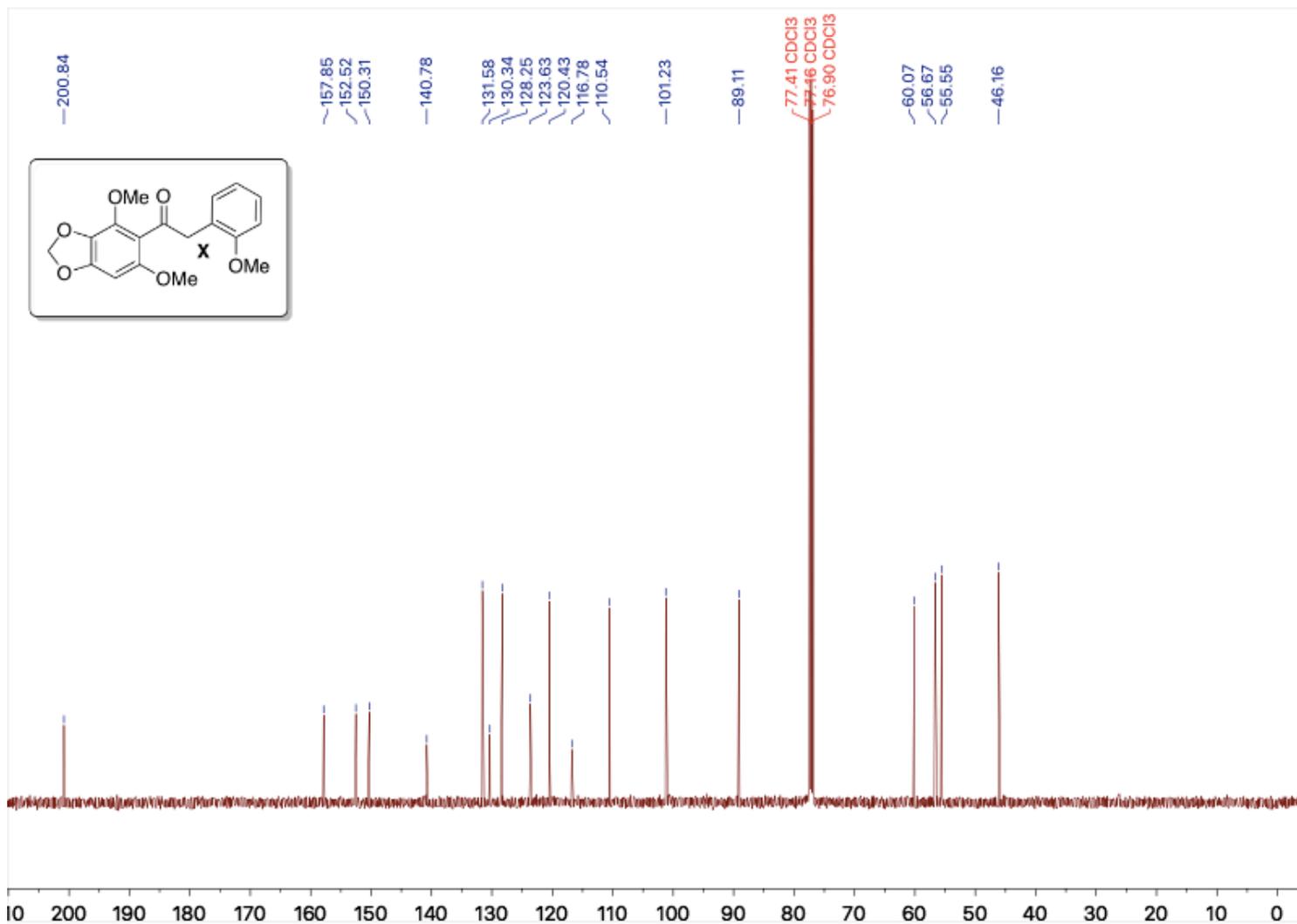
^1H NMR (500 MHz, CDCl_3) Spectrum of 2-(2-(methoxymethoxy)phenyl)-1-(2,3,4,6-tetramethoxyphenyl)ethan-1-one (IX).



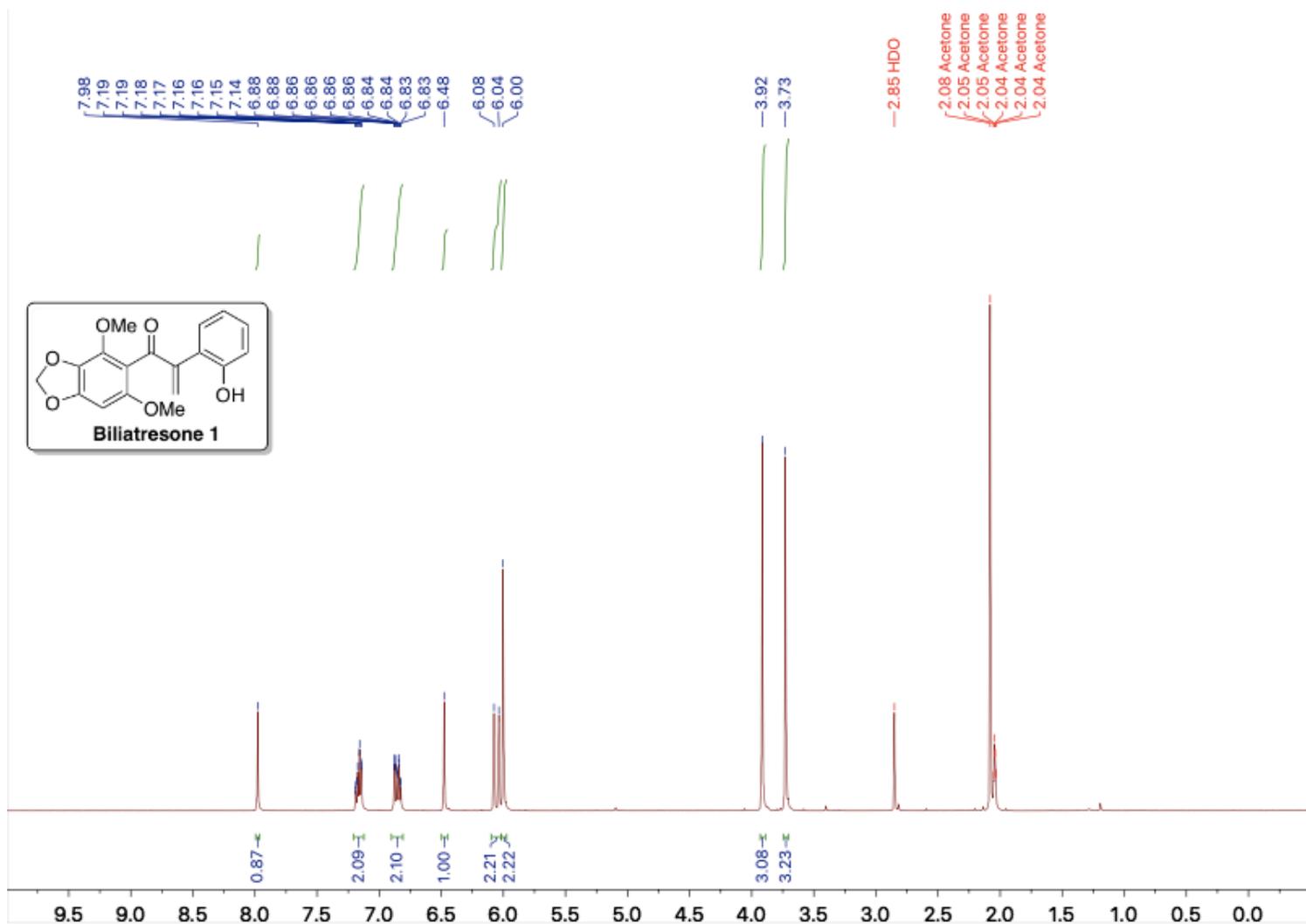
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 2-(2-(methoxymethoxy)phenyl)-1-(2,3,4,6-tetramethoxyphenyl)ethan-1-one (IX).



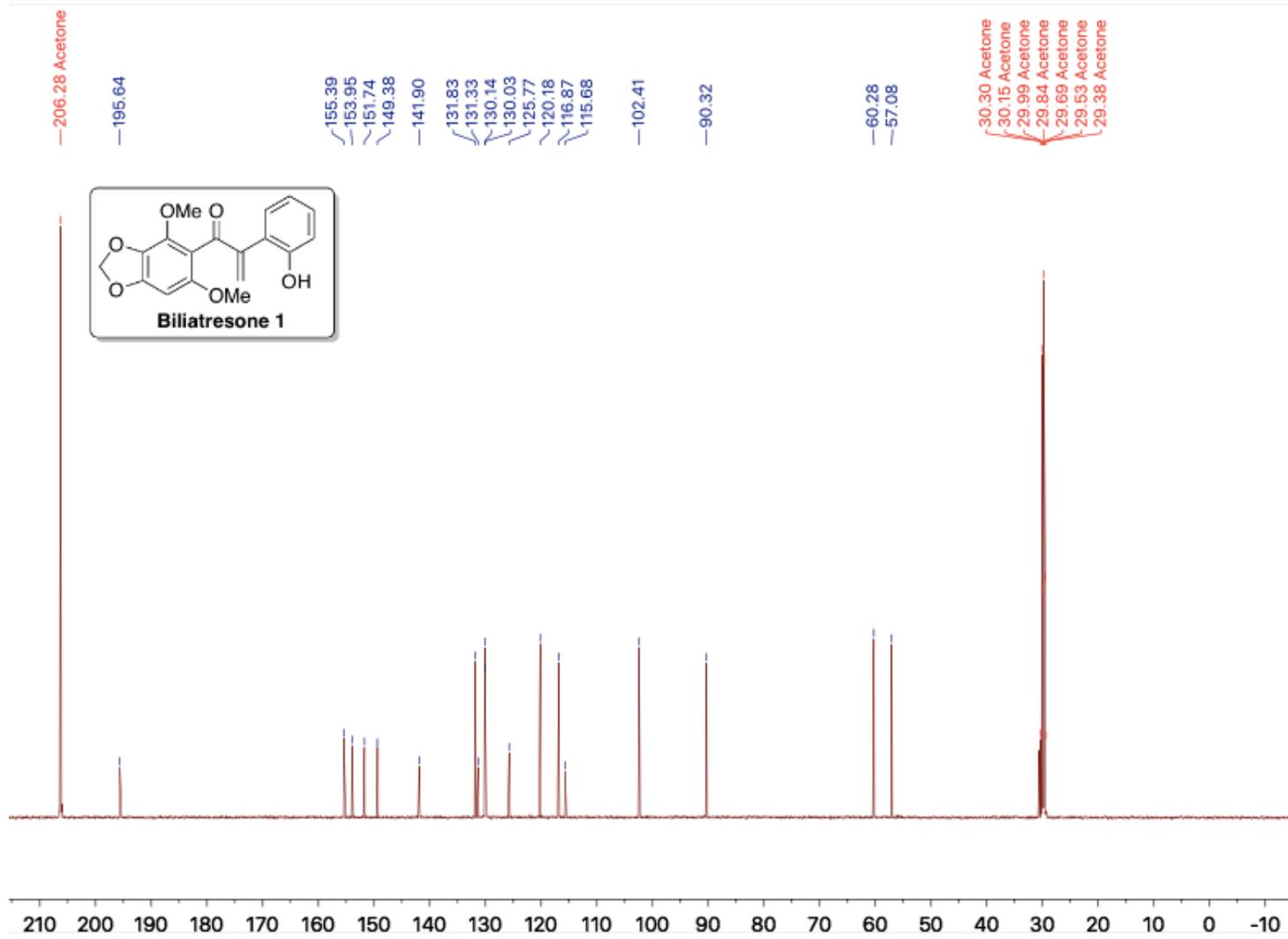
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo[1,3]dioxol-5-yl)-2-(2-methoxyphenyl)ethan-1-one (X).



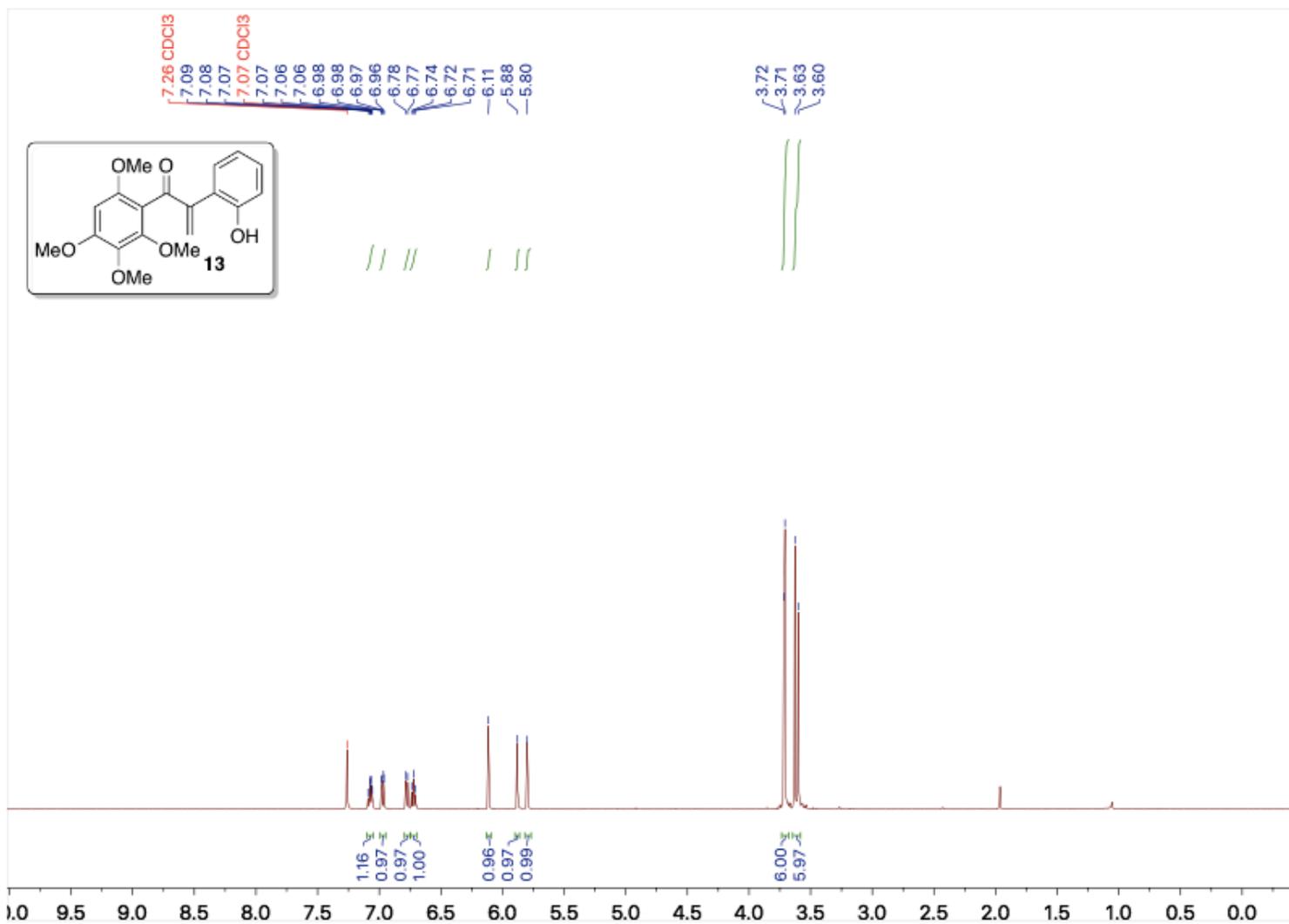
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo[1,3]dioxol-5-yl)-2-(2-methoxyphenyl)ethan-1-one (X).



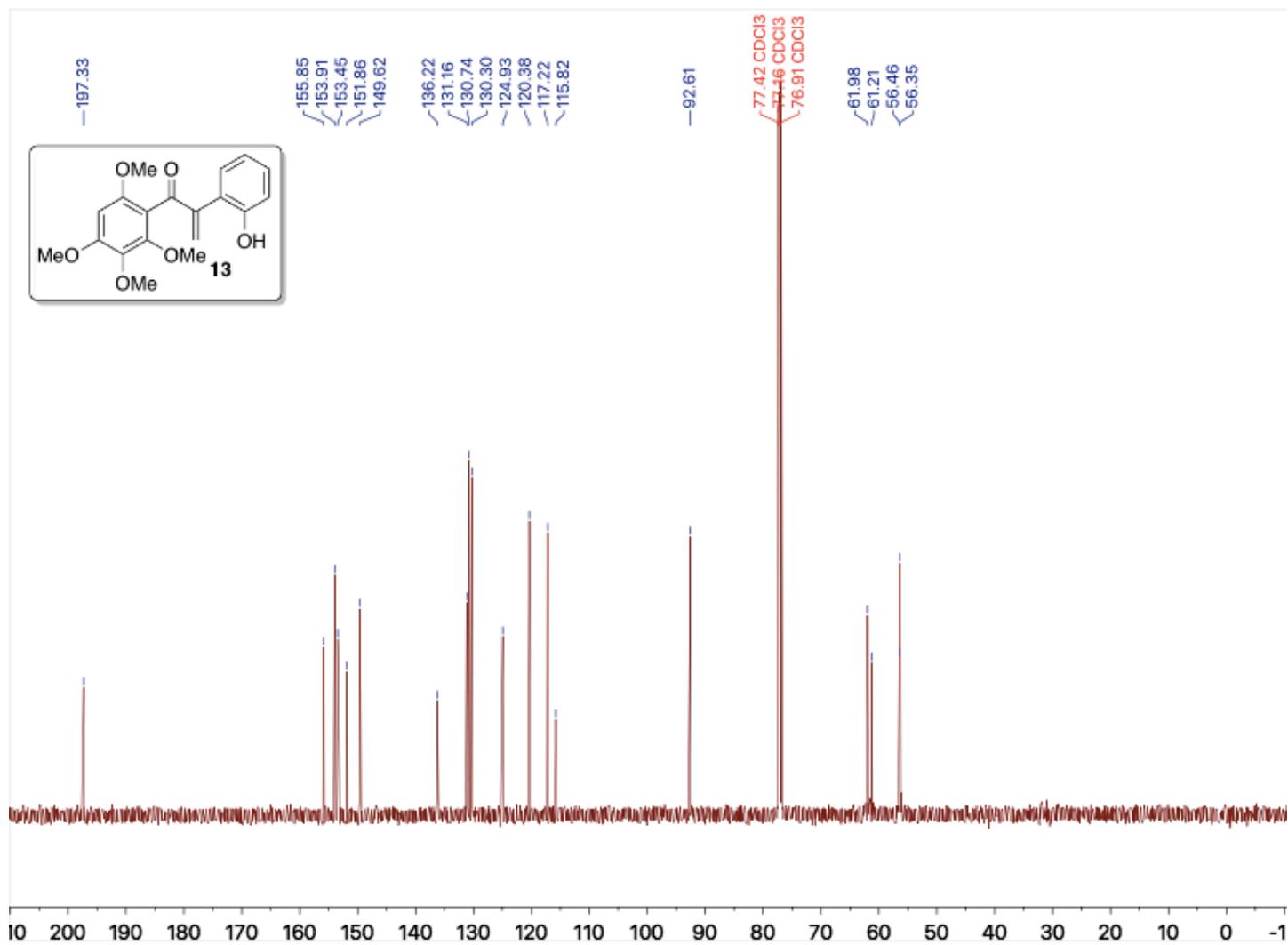
¹H NMR (500 MHz, Acetone-*d*₆) Spectrum of bilitresone (1).



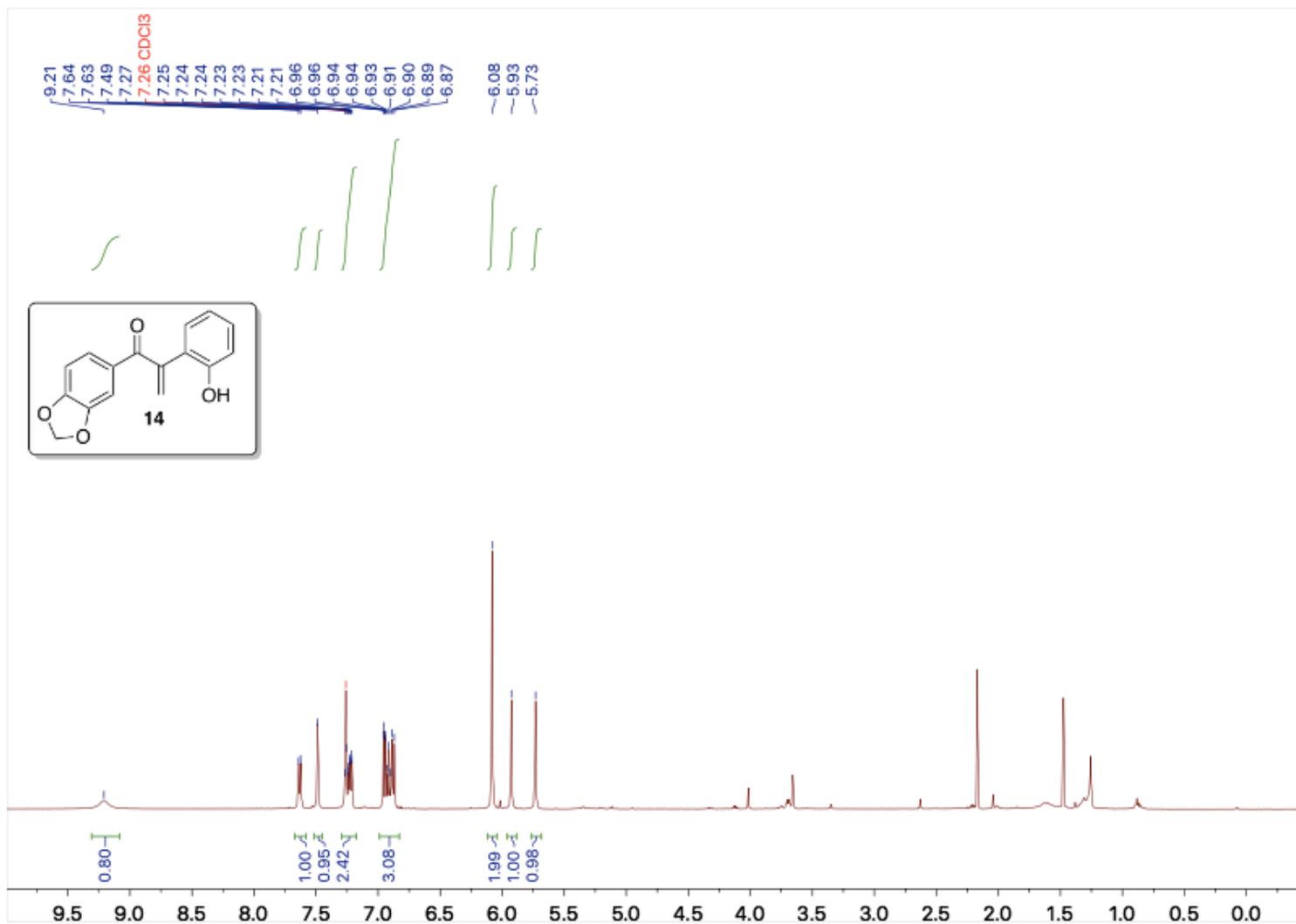
¹³C NMR (125.8 MHz, Acetone-d₆) Spectrum of biliatresone (1).



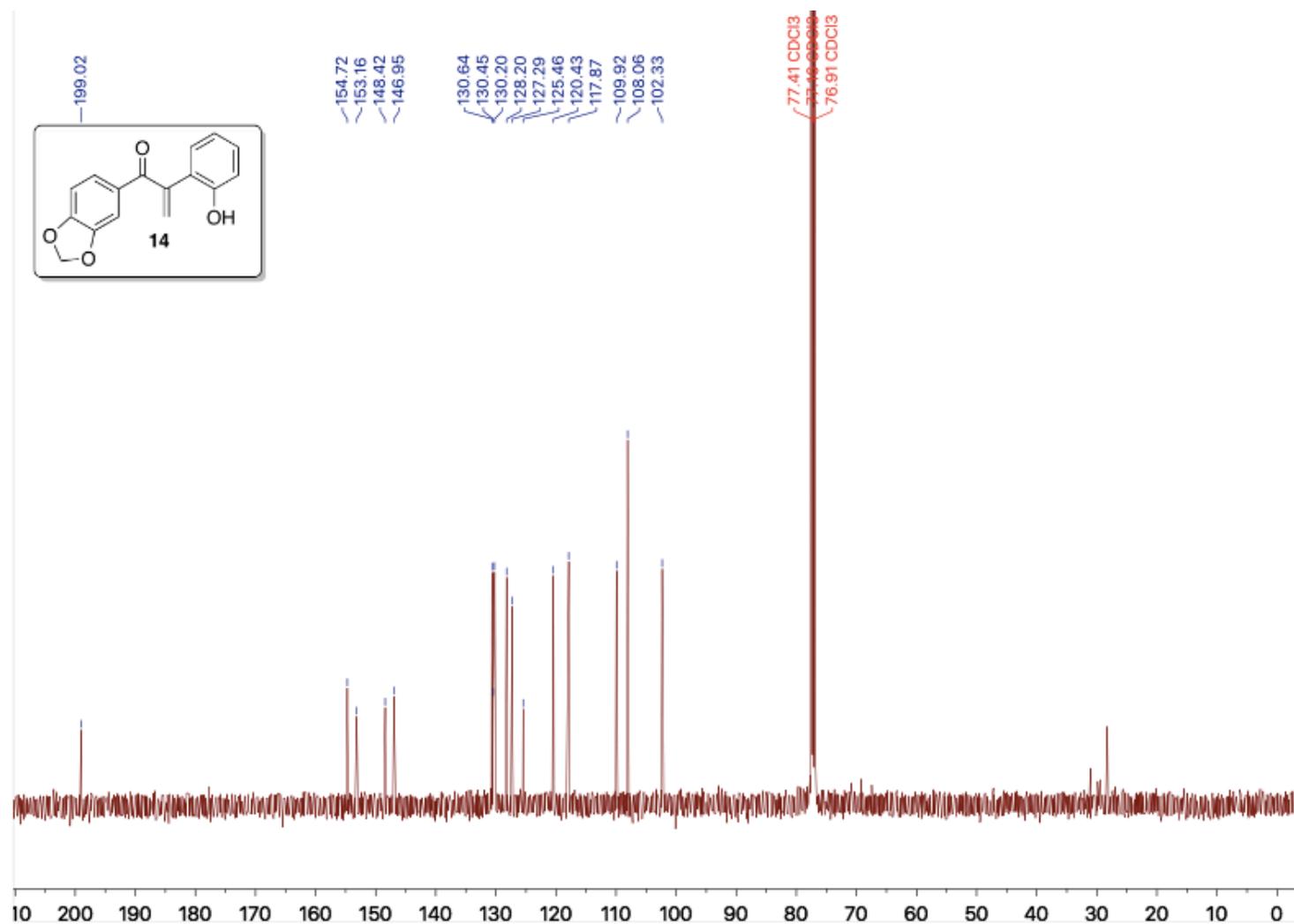
¹H NMR (500 MHz, CDCl₃) Spectrum of 2-(2-hydroxyphenyl)-1-(2,3,4,6-tetramethoxyphenyl)prop-2-en-1-one (13).



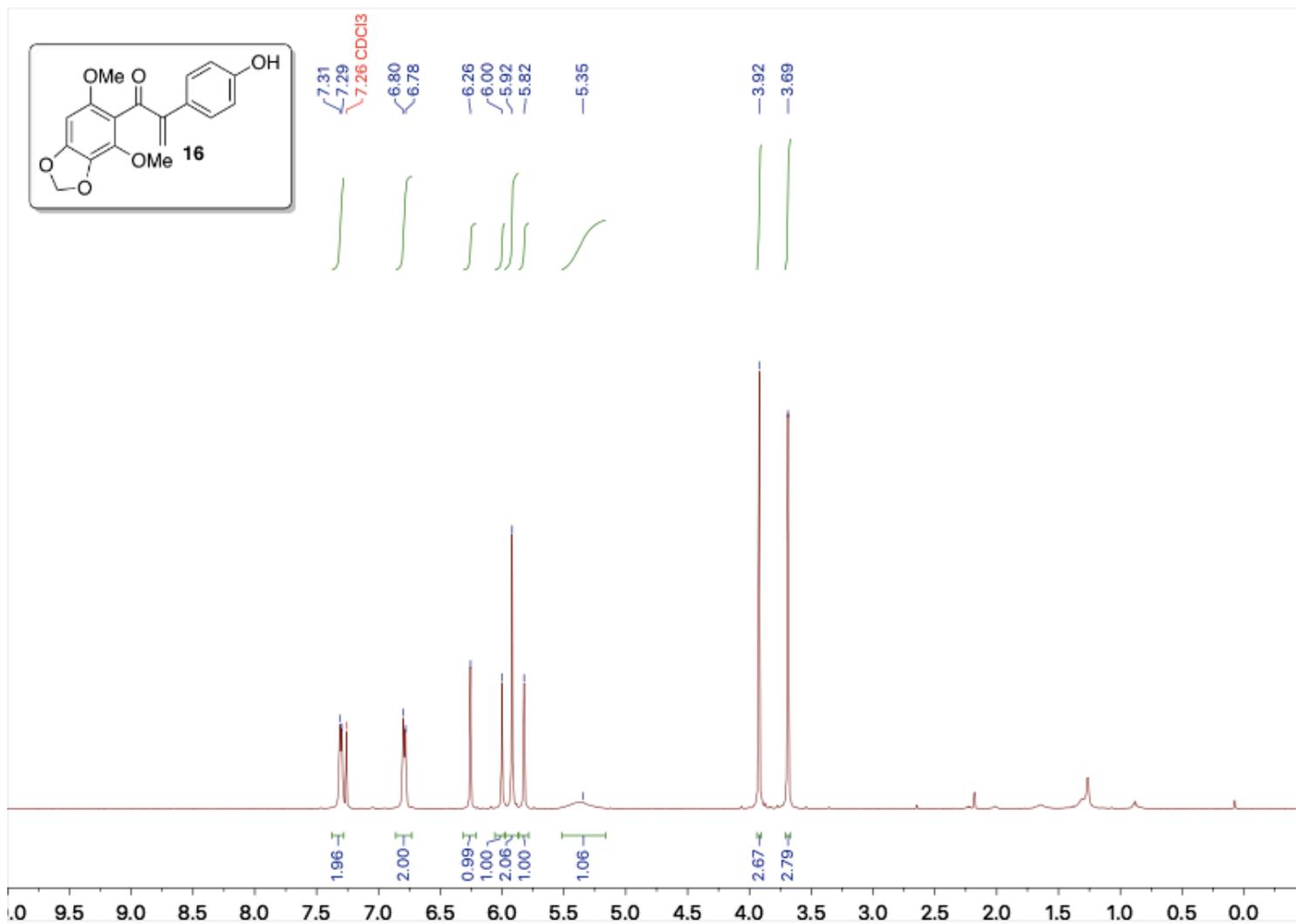
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 2-(2-hydroxyphenyl)-1-(2,3,4,6-tetramethoxyphenyl)prop-2-en-1-one (13).



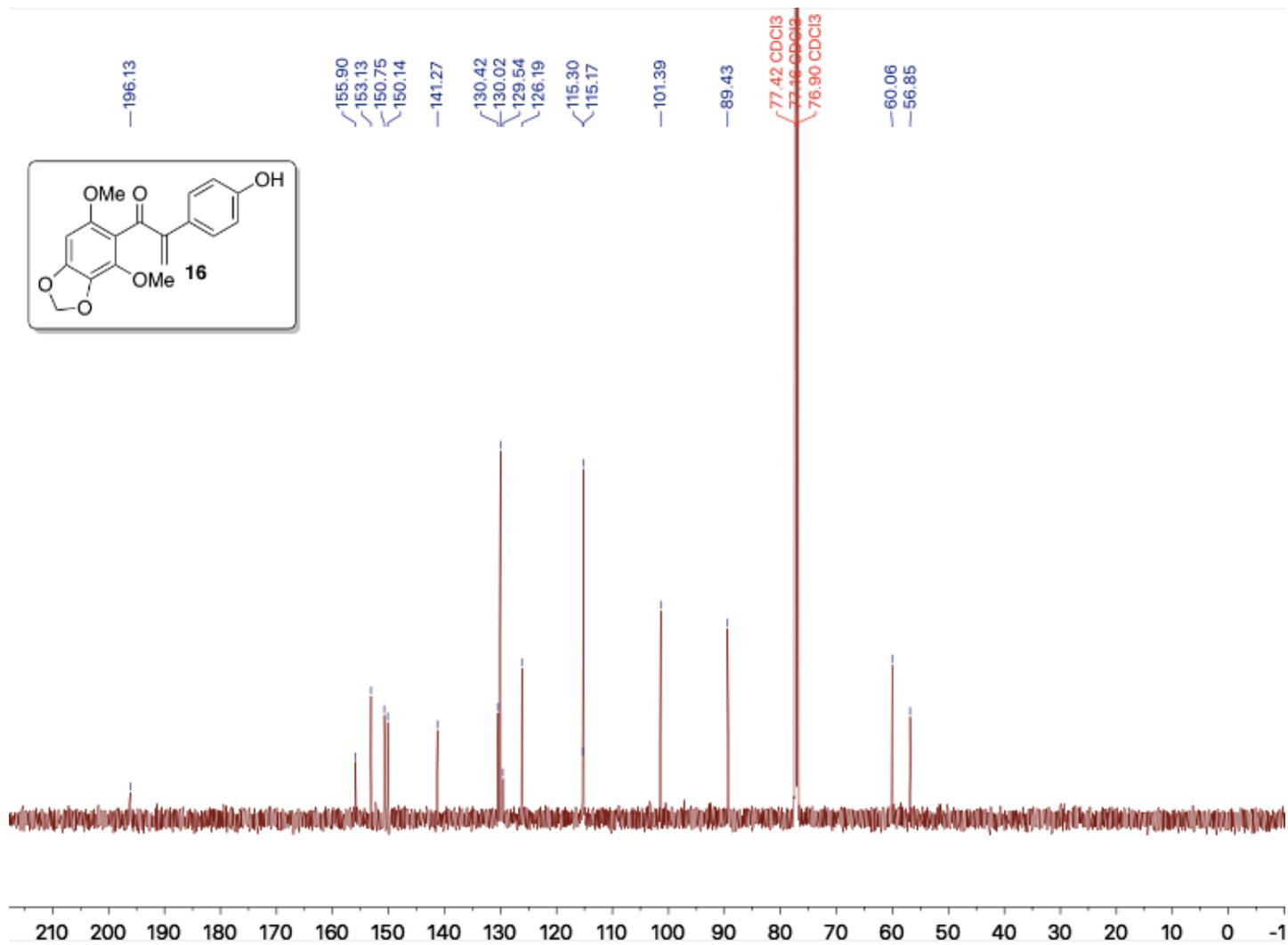
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(benzo[1,3]dioxol-5-yl)-2-(2-hydroxyphenyl)prop-2-en-1-one (14).



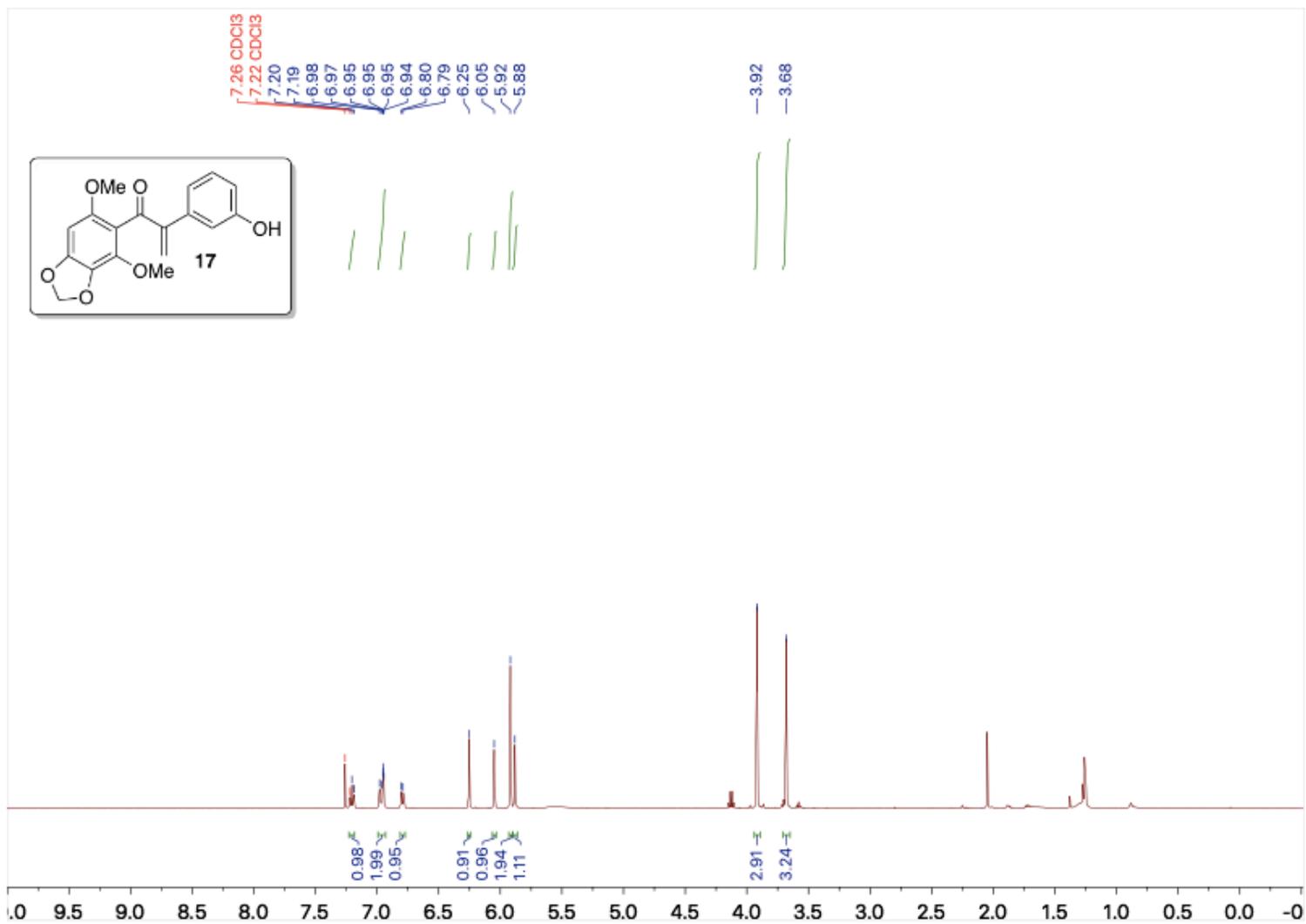
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(benzo[1,3]dioxol-5-yl)-2-(2-hydroxyphenyl)prop-2-en-1-one (14).



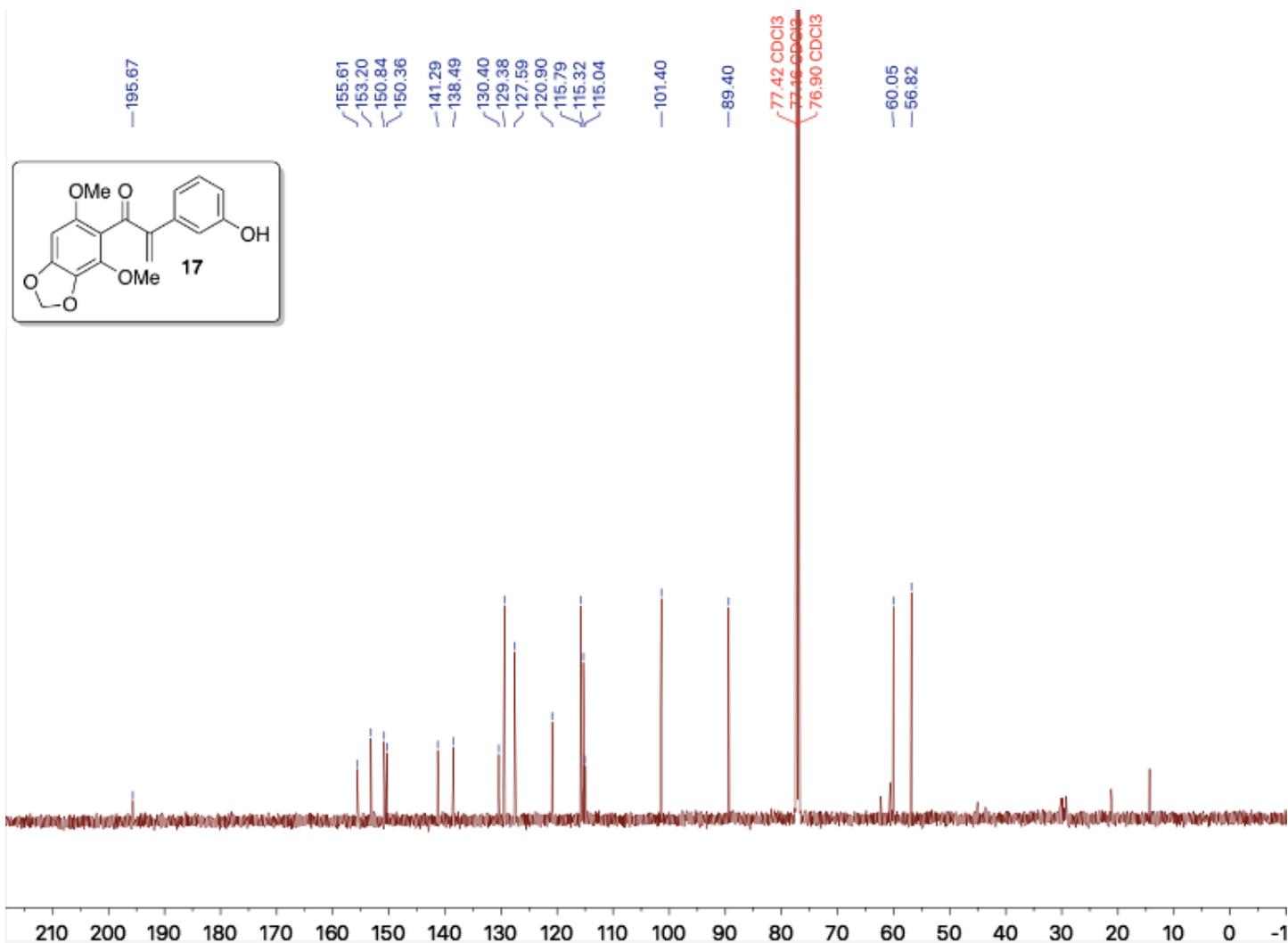
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo[1,3]dioxol-5-yl)-2-(4-hydroxyphenyl)prop-2-en-1-one (16).



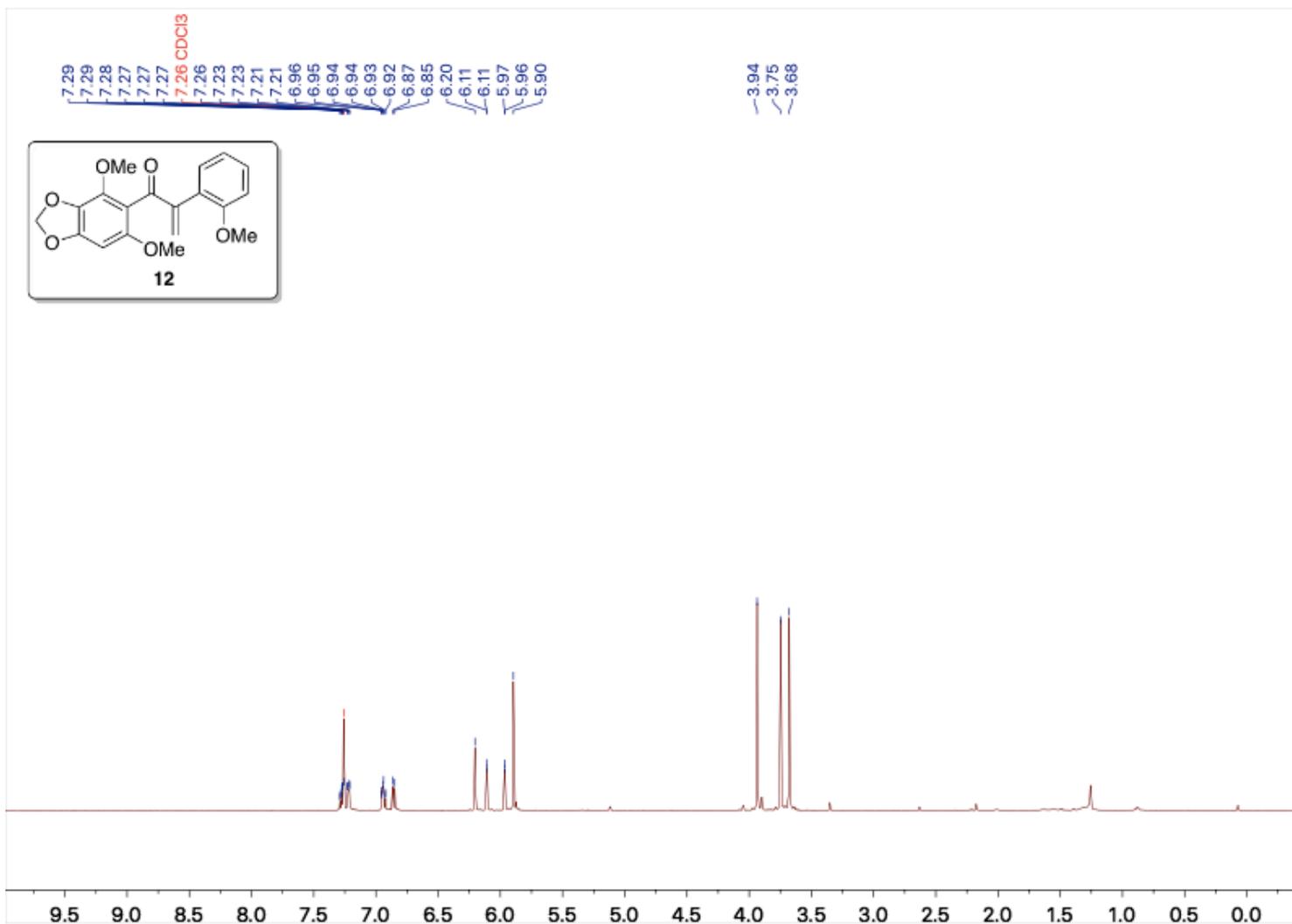
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo[1,3]dioxol-5-yl)-2-(4-hydroxyphenyl)prop-2-en-1-one (16).



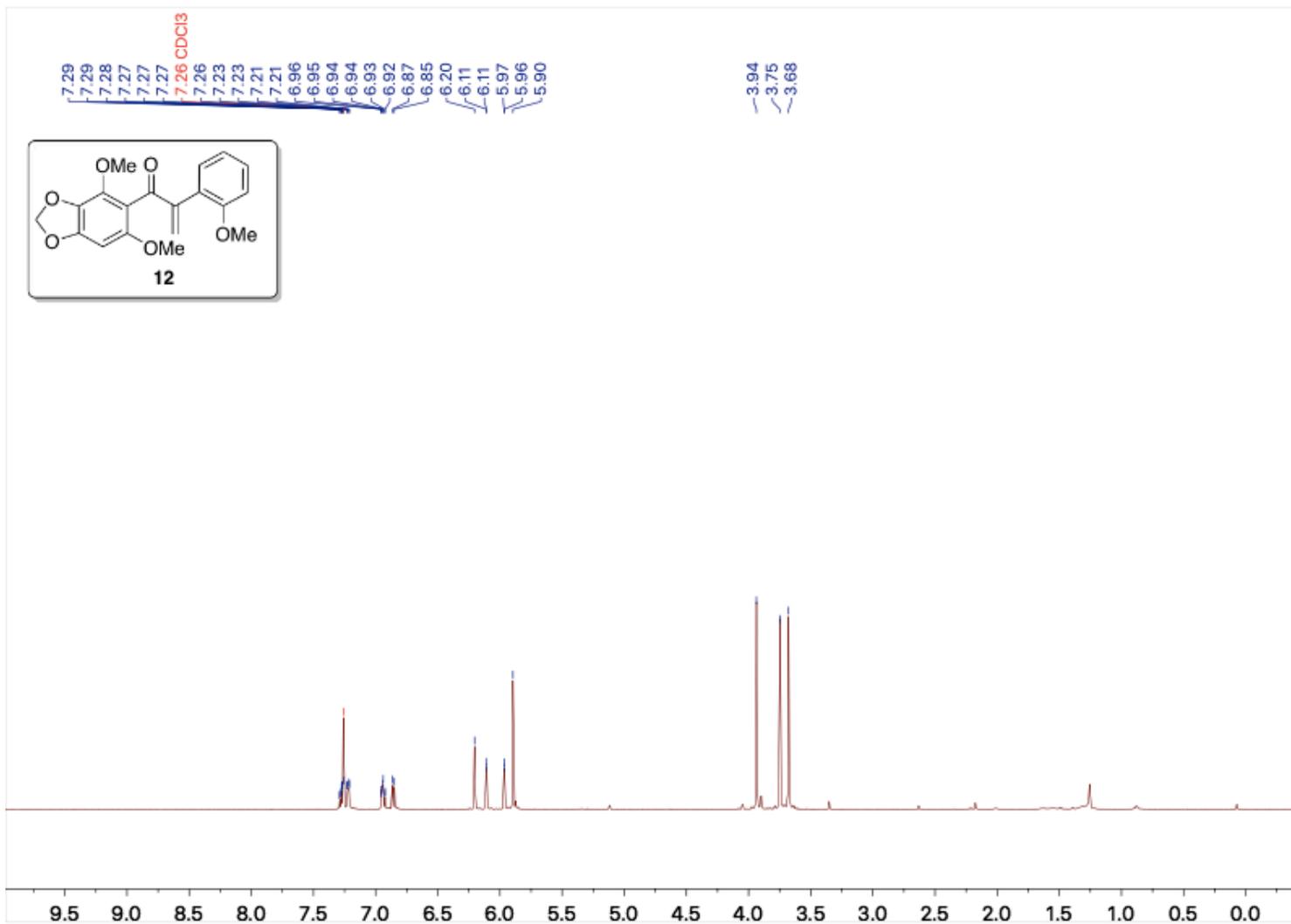
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo[d][1,3]dioxol-5-yl)-2-(3-hydroxyphenyl)prop-2-en-1-one (17).



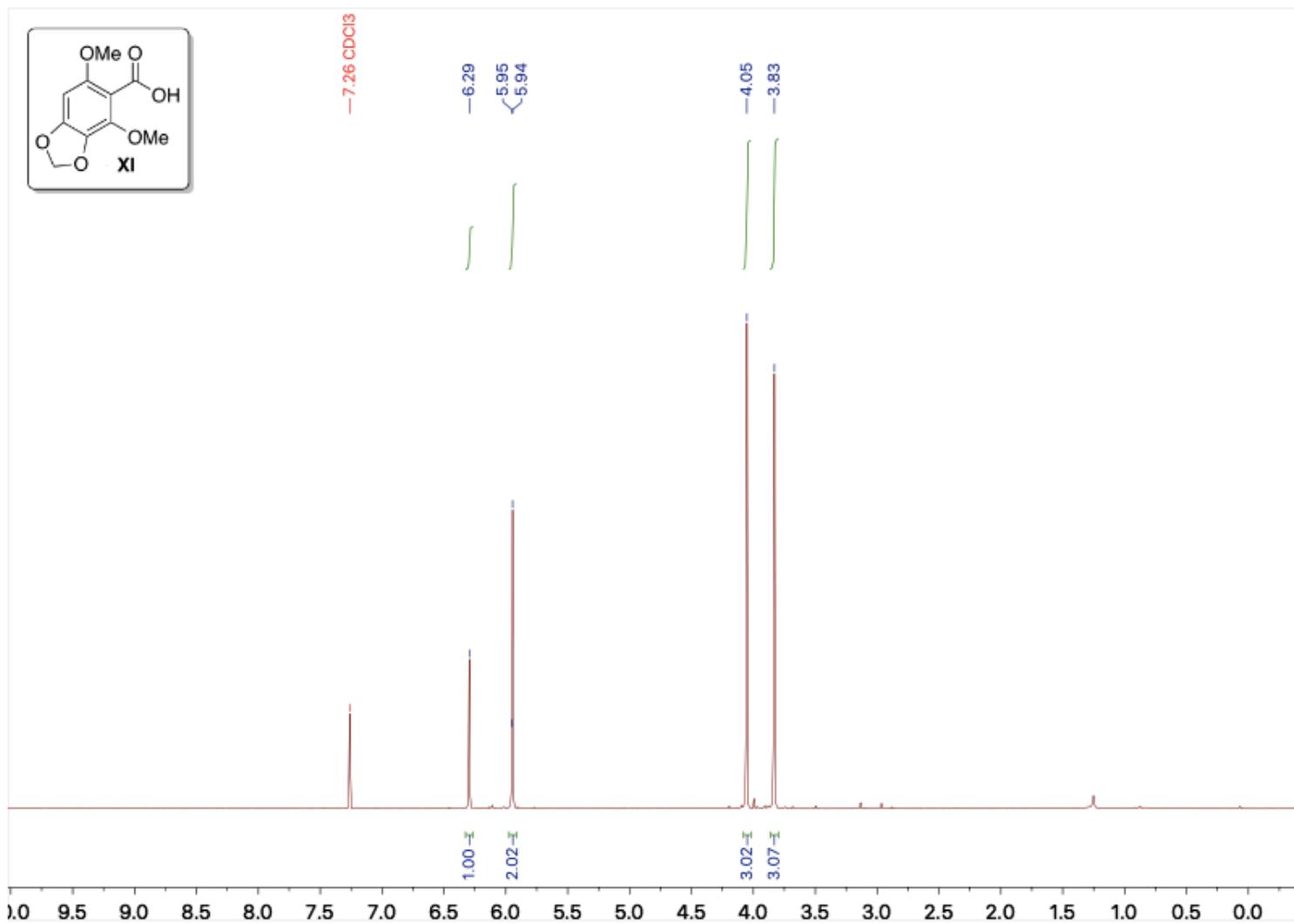
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo[*d*][1,3]dioxol-5-yl)-2-(3-hydroxyphenyl)prop-2-en-1-one (17).



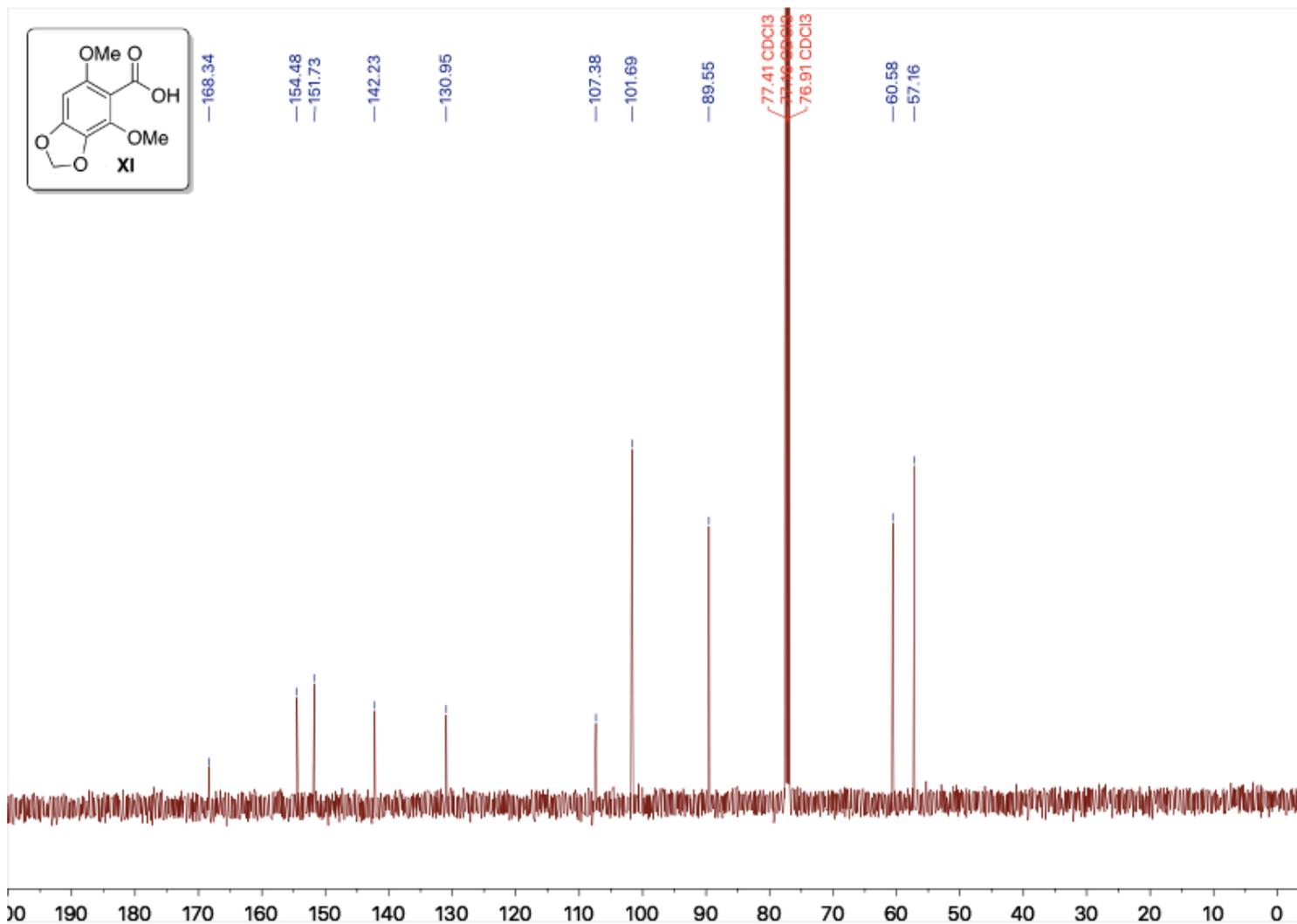
¹H NMR (500 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)-2-(2-methoxyphenyl)prop-2-en-1-one (12).



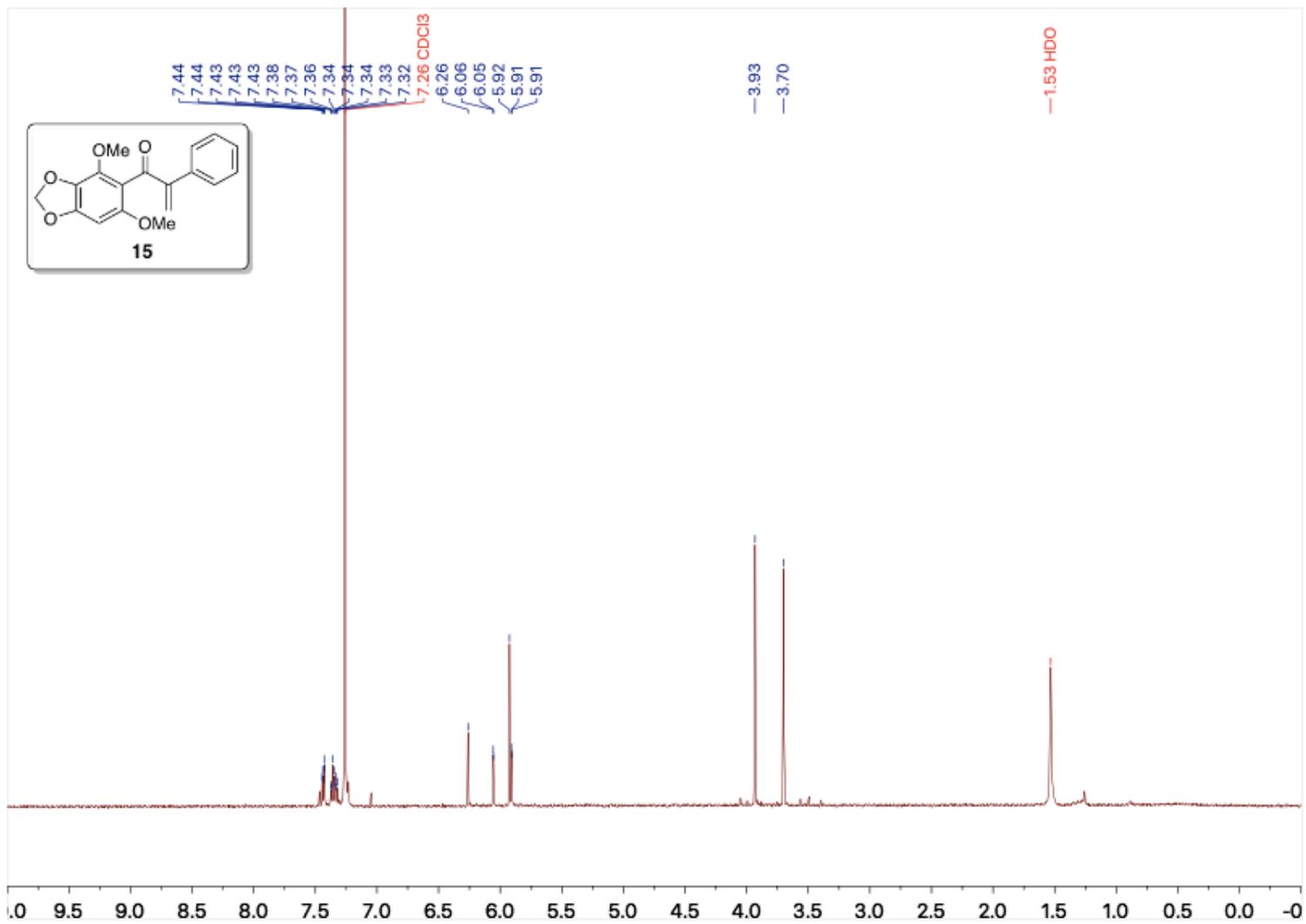
¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 1-(4,6-dimethoxybenzo-1,3-dioxol-5-yl)-2-(2-methoxyphenyl)prop-2-en-1-one (12).



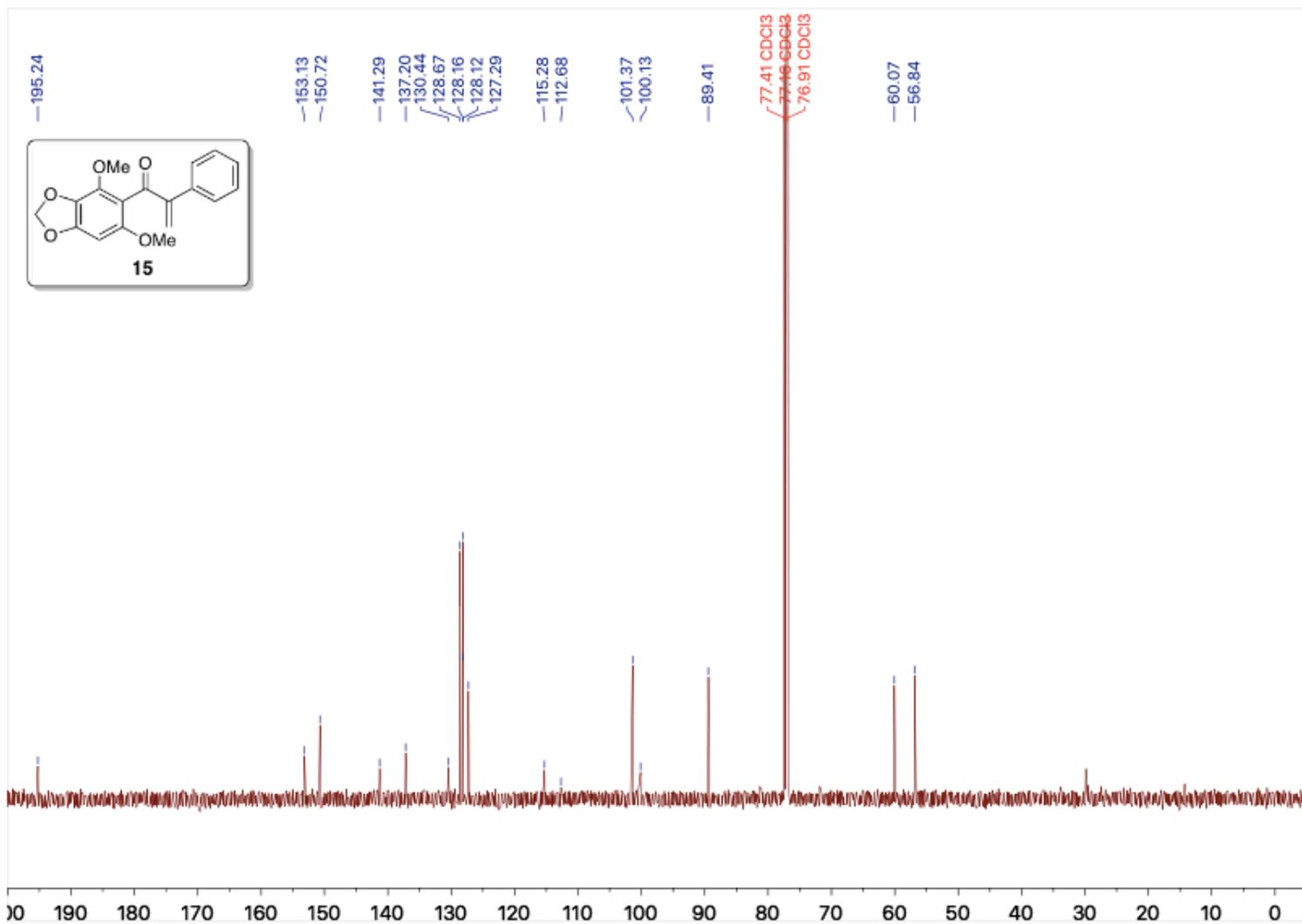
¹H NMR (500 MHz, CDCl₃) Spectrum of 4,6-dimethoxybenzo-1,3-dioxole-5-carboxylic acid (XI).



¹³C NMR (125.8 MHz, CDCl₃) Spectrum of 4,6-dimethoxybenzo-1,3-dioxole-5-carboxylic acid (XI).



¹H NMR (500 MHz, CDCl₃) Spectrum of deoxybilitresone (15).

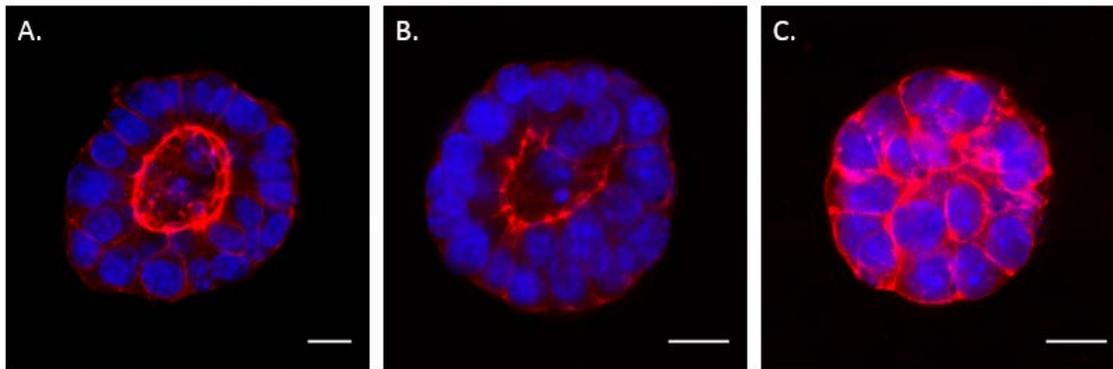


¹³C NMR (125.8 MHz, CDCl₃) Spectrum of deoxybilibatresone (15).

Biological Protocols

a) Cholangiocyte organoid assay

A small cholangiocyte cell line⁵ from mouse intrahepatic bile ducts was cultured in 3D in a mixture of Matrigel and collagen.⁶ In 3D culture, single cholangiocytes divide, polarize, and form a single-cell thick, hollow spheroid over 7-8 days. After 8 days in culture, spheroids were treated with biliaryresone (**1**, 2 μ g/mL), DMSO (control, 2 μ g/mL), and analogs **14-18** in varying concentrations (from 1-3 μ g/mL) for 24h. Spheroids were then fixed with 4% formalin and stained for F-actin (1:1000; phalloidintetramethylrhodamine B isothiocyanate; Santa Cruz Biotechnology, catalog #301530) and DAPI (nuclear stain). Spheroids were imaged using a confocal microscope at 40x magnification (Leica Confocal TCS SP8). For each condition, 100 organoids were counted and lumens were assessed as open, closed (no lumen visible), or partially open (partial lumen closure, monolayer disruption; see Supplemental Figure).



Supplemental Fig. Cholangiocyte organoid assay. (A-C) Cholangiocyte organoids after treatment with vehicle (A) or biliaryresone (B,C) demonstrating an open (A), partially closed (B) or fully closed (C) phenotype.

b) Zebrafish Studies

Zebrafish Lines. The wild-type strains (TL, AB) were used for the present studies. All zebrafish were raised in the University of Pennsylvania School of Medicine Zebrafish Facility in accordance with protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Zebrafish bioassay and microscopy. Lyophilized biliaryresone and analogs resuspended in anhydrous DMSO were added to zebrafish larvae reared in E3 embryo media in 24-well plates a final concentrations of 0.25 and 0.50 μ g/ml, 1% DMSO. All assays were repeated in triplicate with at least 30 treated larvae and an equal number of control larvae. After chemical treatments for 72 hours, larvae were fixed in 4% paraformaldehyde for 2 hours, washed in PBS and immunostained with mouse monoclonal anti-Annexin A4 antibody x (ABCAM) 1:100 dilution overnight at 4°C, followed by incubation in goat anti-mouse IgG-Alexa 488 (Invitrogen) 1:600 dilution for 4 hours at room temperature. Larvae were then washed and processed for confocal microscopy as described.⁷ Biliary morphology was scored in a subset of compound-treated live larvae using Bodipy-C16 as previously described.⁸

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