Chlorinated Coumarins from the Polypore Mushroom *Fomitopsis officinalis* and Their Activity against *Mycobacterium tuberculosis*

Chang Hwa Hwang,[†,‡] Birgit U. Jaki,[†,‡] Larry L. Klein,[‡] David C. Lankin,[†] James B. McAlpine,[†] José G. Napolitano,[†] Nicole A. Fryling,[§] Scott G. Franzblau,[‡] Sang Hyun Cho,[‡] Paul E. Stamets,[§] Yuehong Wang,[‡] and Guido F. Pauli[†,‡,*]

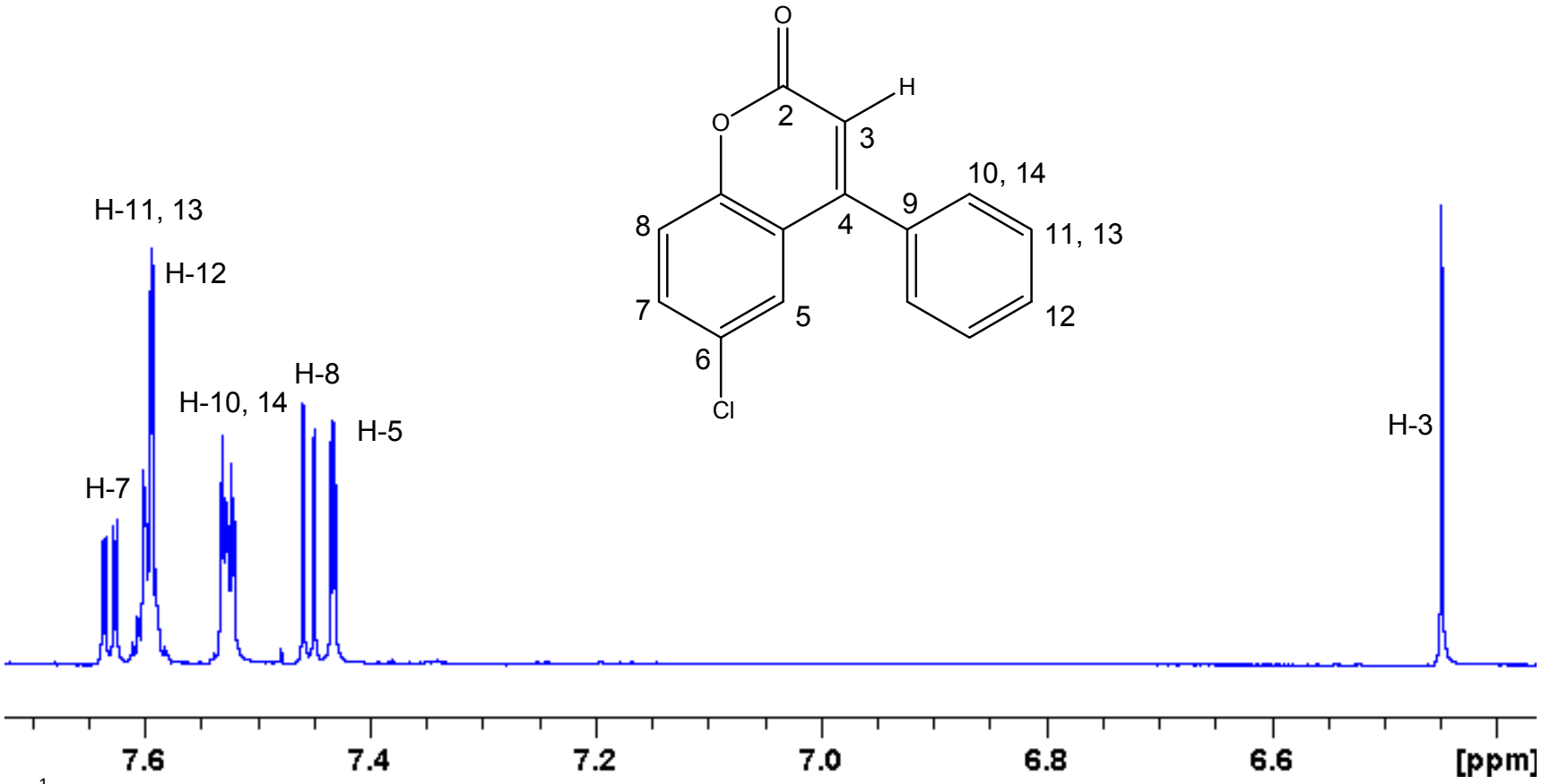
[†]Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, United States

[‡]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, United States

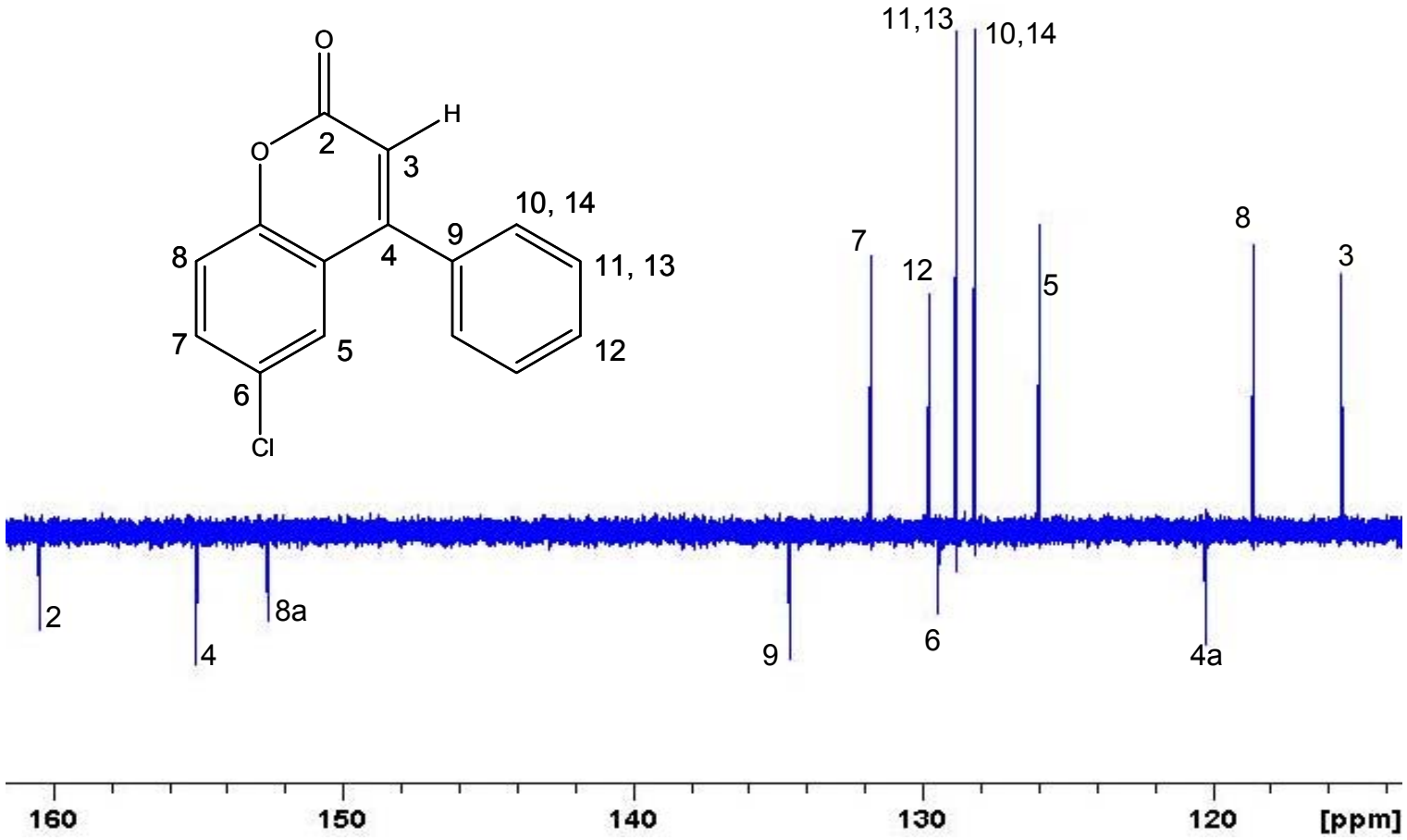
[§]Fungi Perfecti, P.O. Box 7634, Olympia, Washington 98507, United States

■ Supporting Information

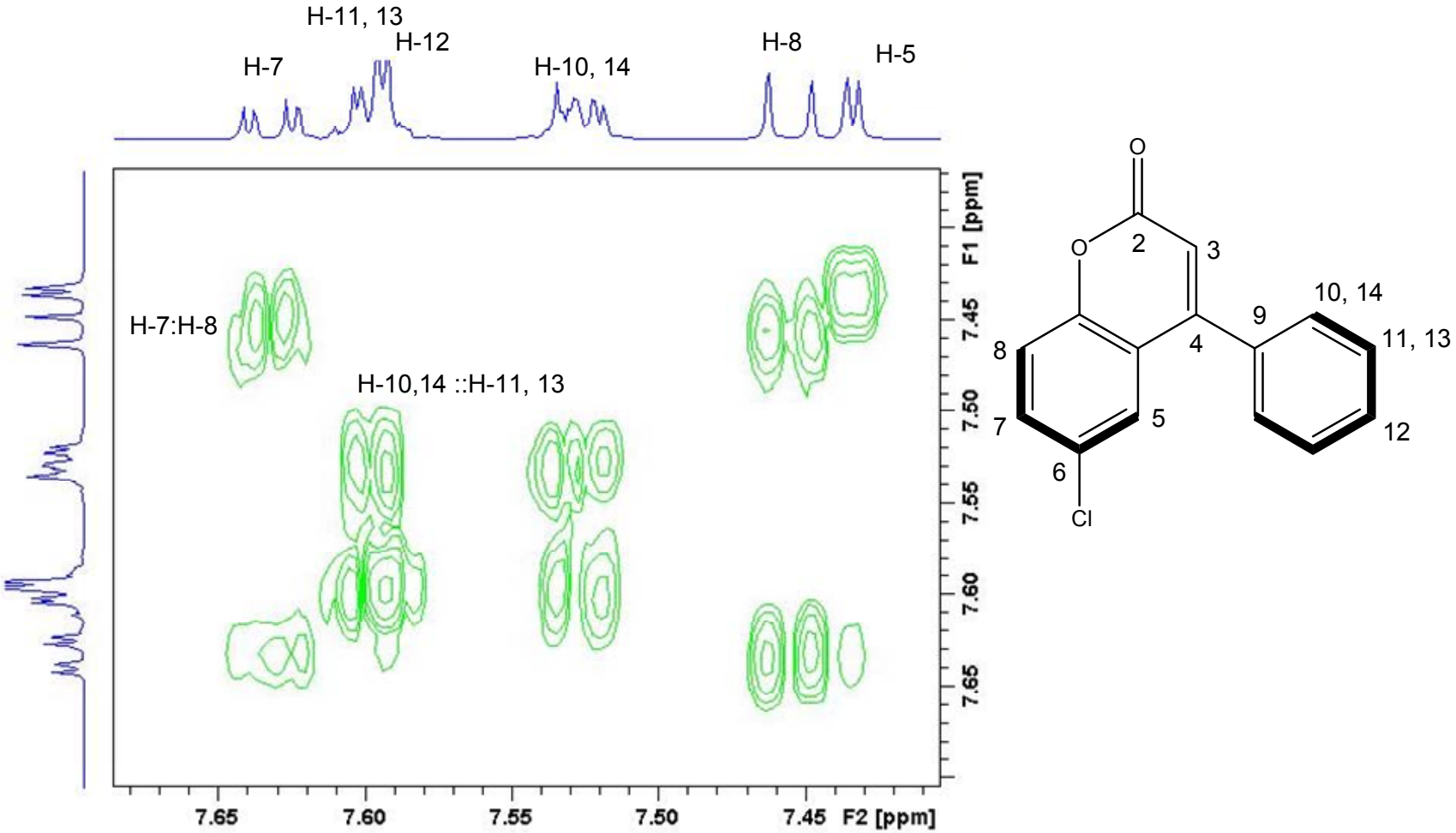
TABLE OF CONTENTS



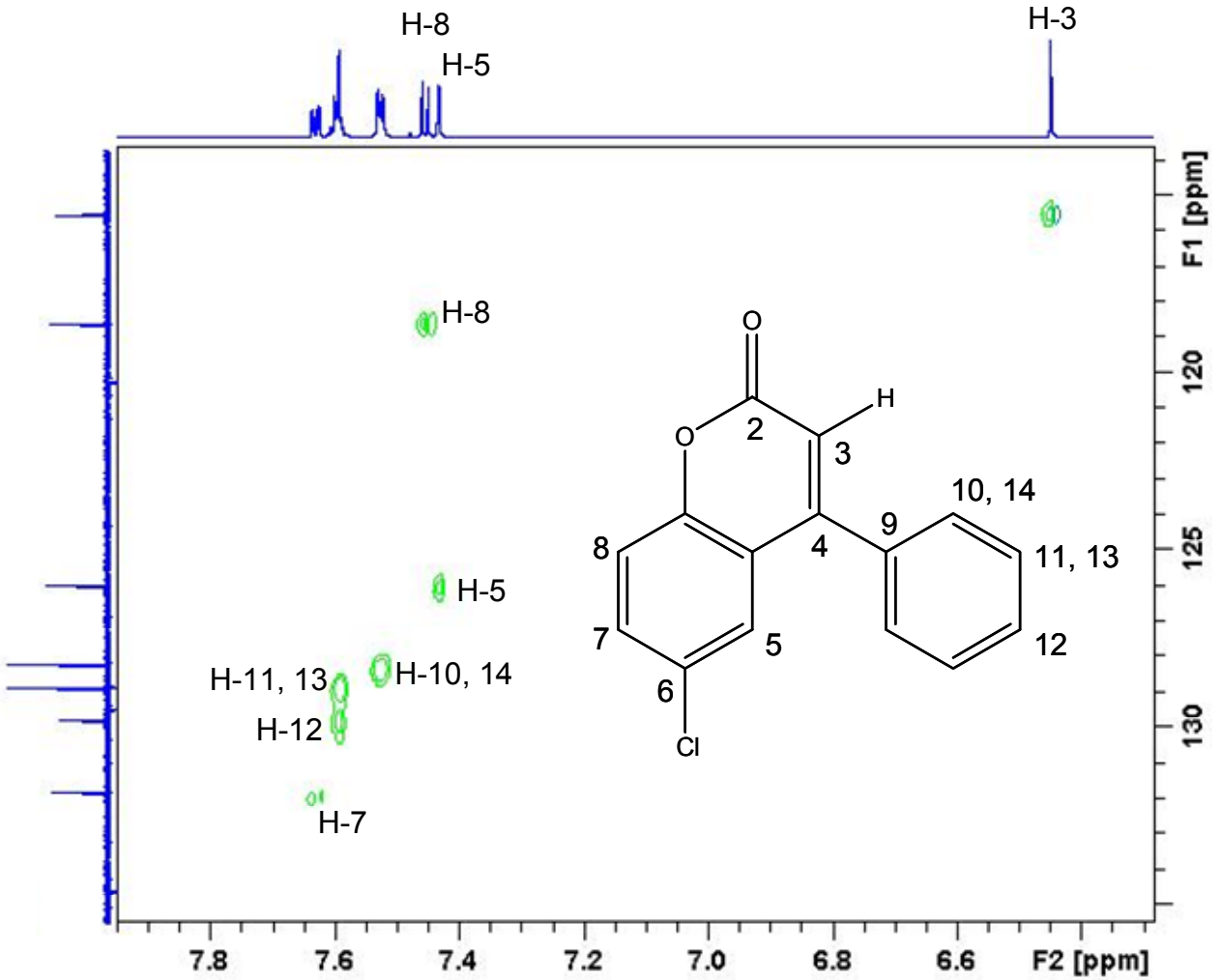
S1. ^{1}H NMR spectrum of isolated **1** (900 MHz, 3 mm tube, 0.5 mg in 200 µL MeOH-d_4)



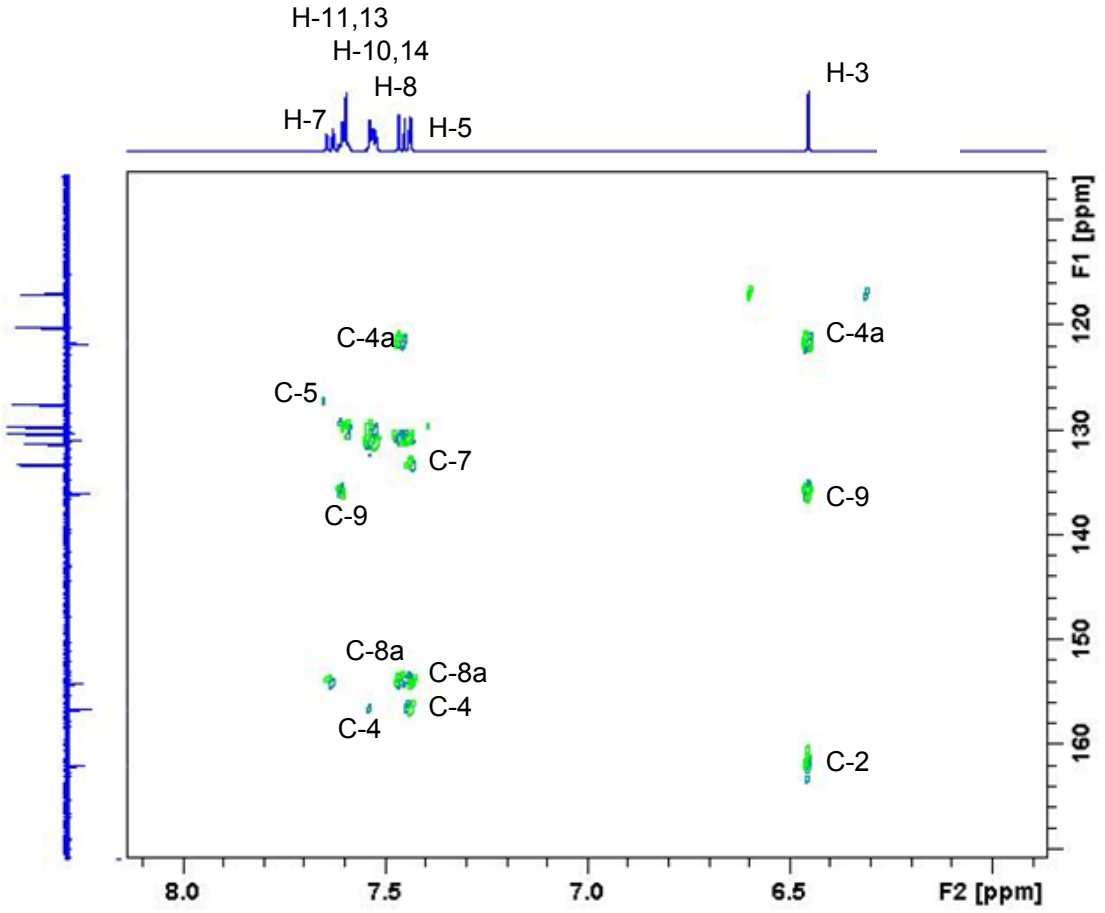
S2. ^{13}C DEPTQ NMR spectrum of isolated **1** (225 MHz, 3 mm tube, 0.5 mg in 200 µL MeOH-d_4)

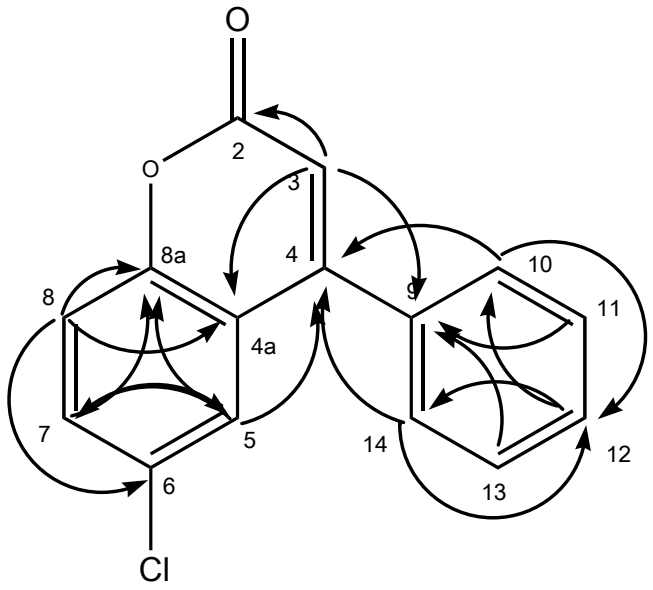


S3. ^{1}H-^{1}H COSY NMR spectrum of isolated **1** (900 MHz, 3 mm tube, 0.5 mg in 200 µL MeOH-d_4)

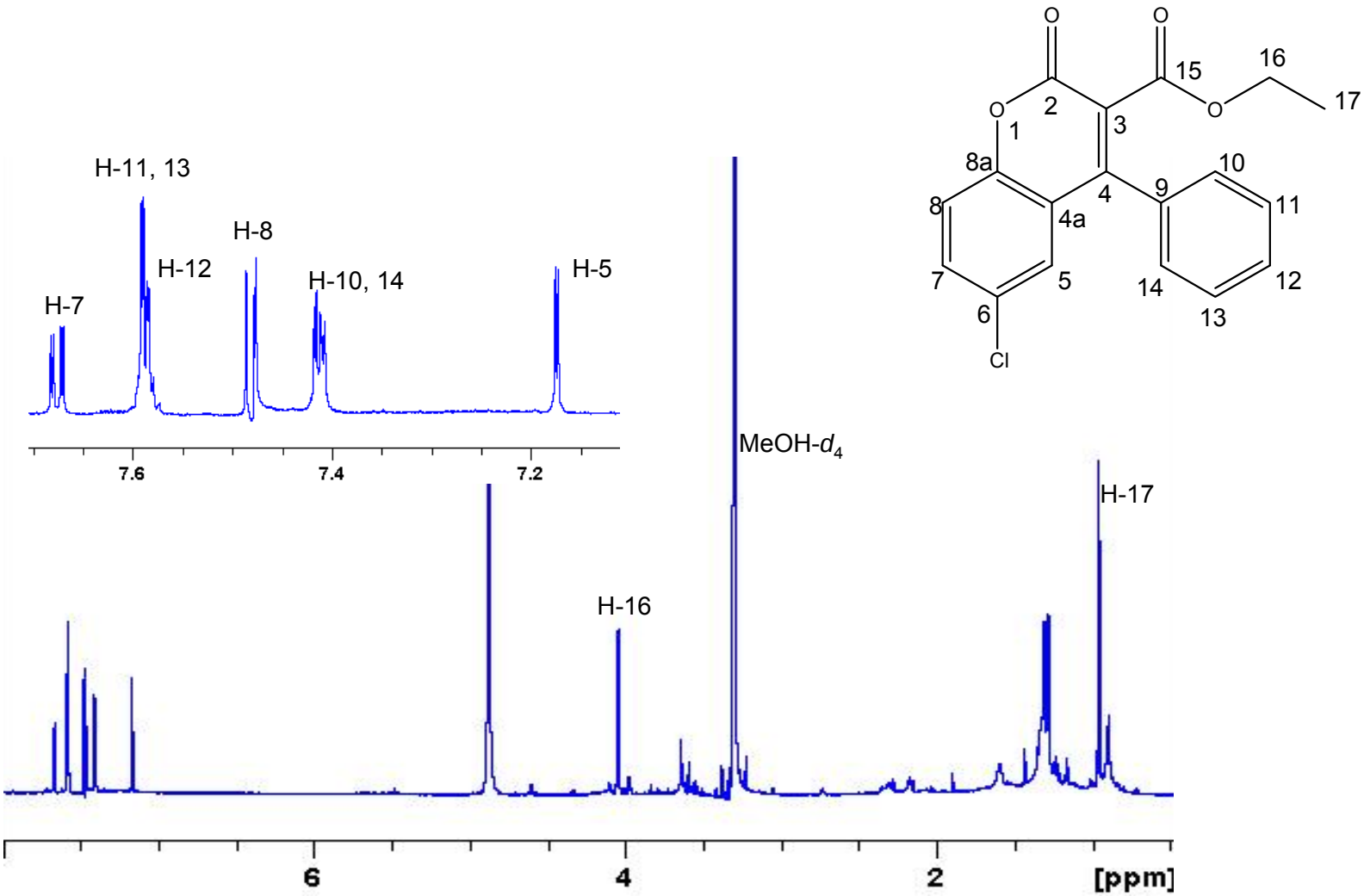


S4. HSQC NMR spectrum of isolated **1** (600 MHz, 3 mm tube, 0.5 mg in 200 µL MeOH-d_4)

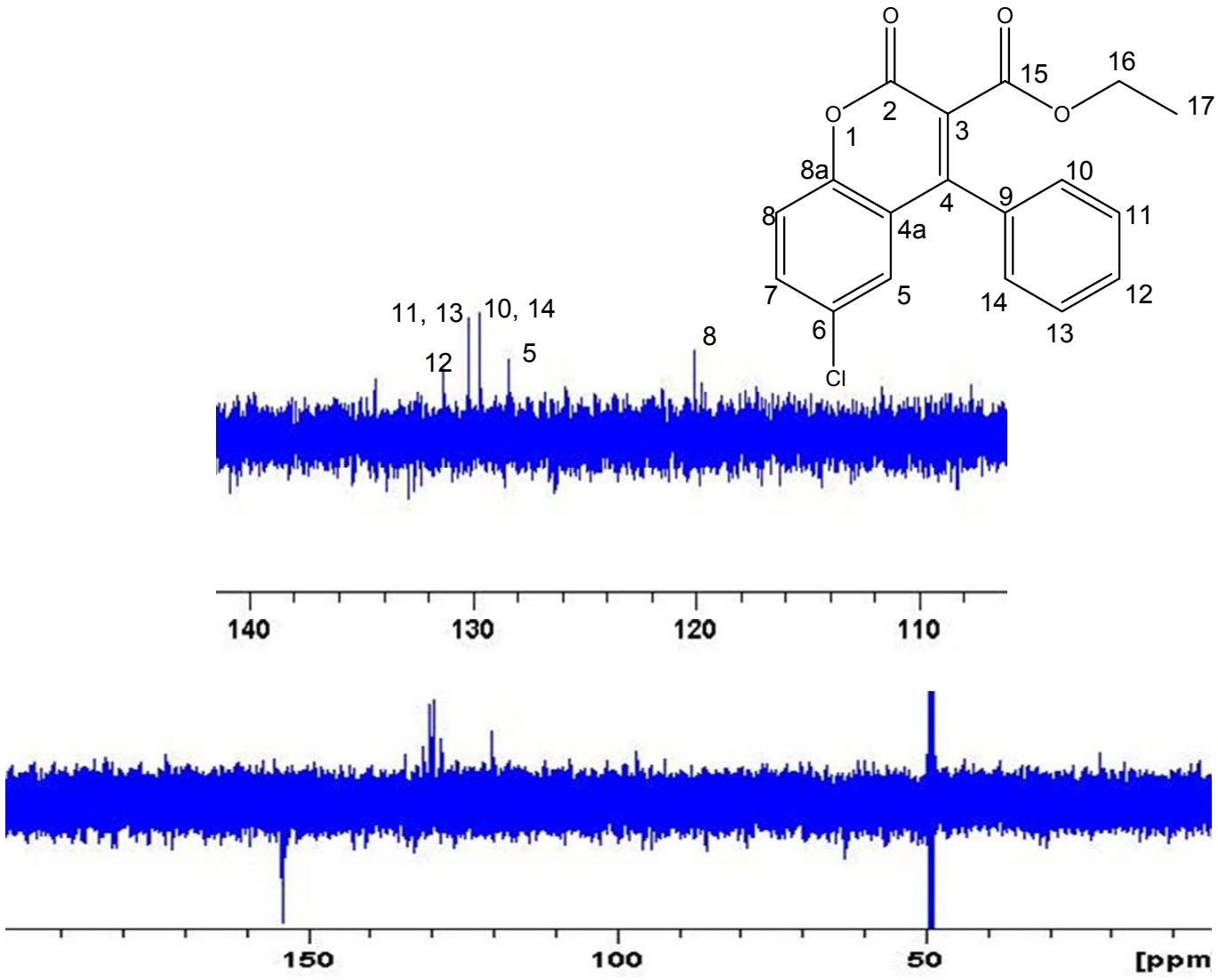




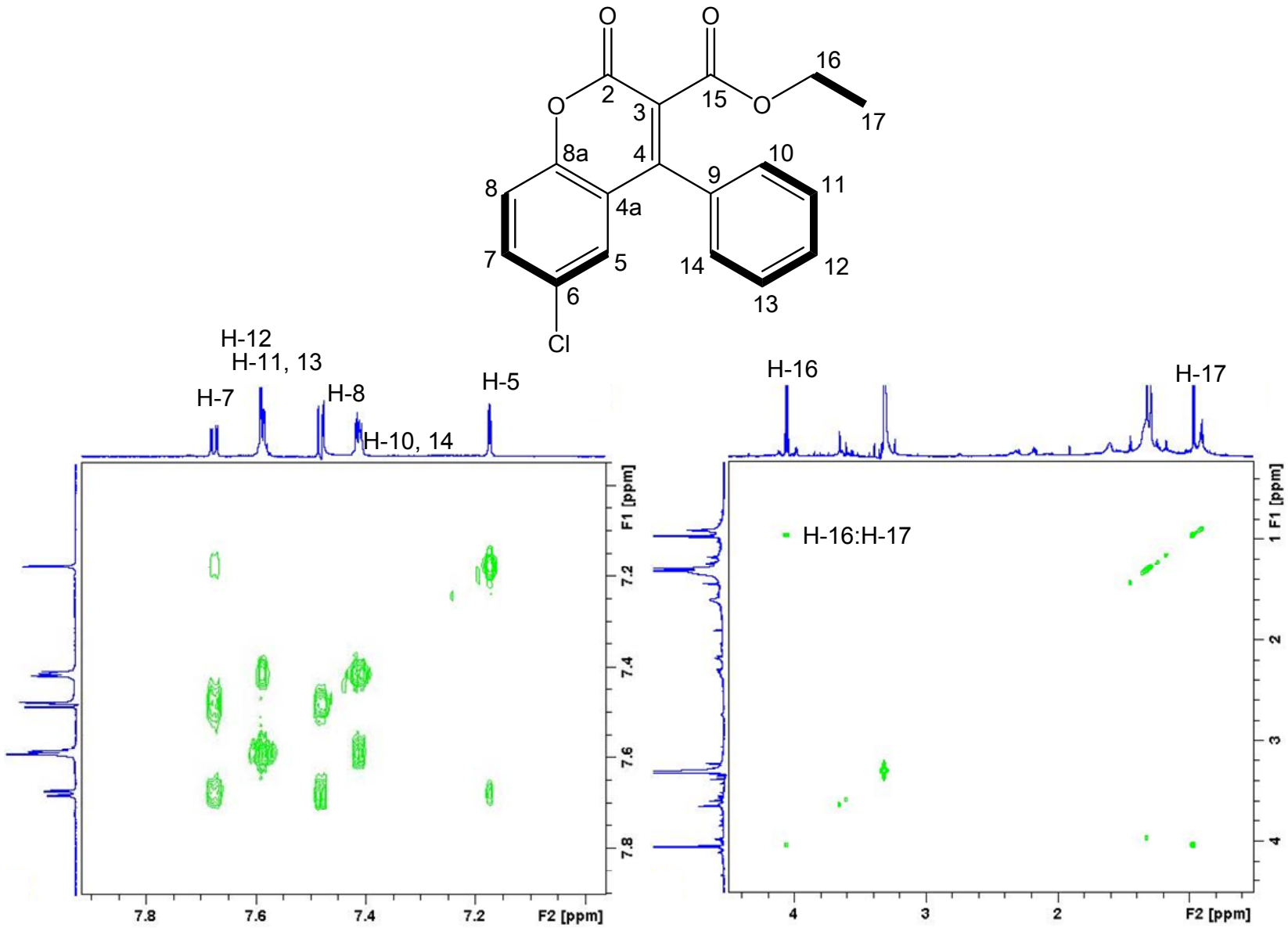
S5. HMBC NMR spectrum of isolated **1** (600 MHz, 3 mm tube, 0.5 mg in 200 µL MeOH-d_4)



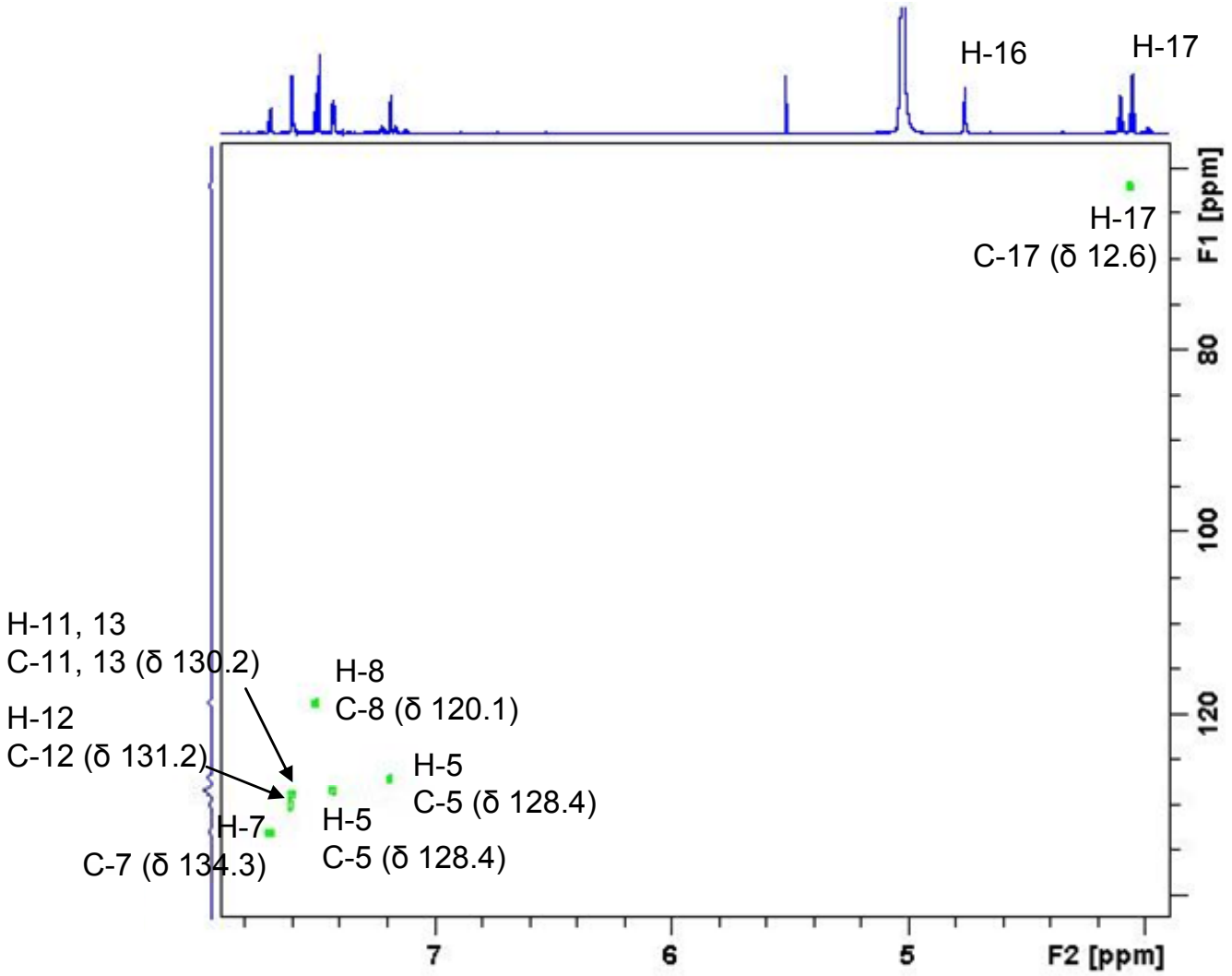
S6. ^{1}H NMR spectrum of isolated **2** (900 MHz, 1.7 mm tube, 0.2 mg in 50 µL MeOH-d_4)



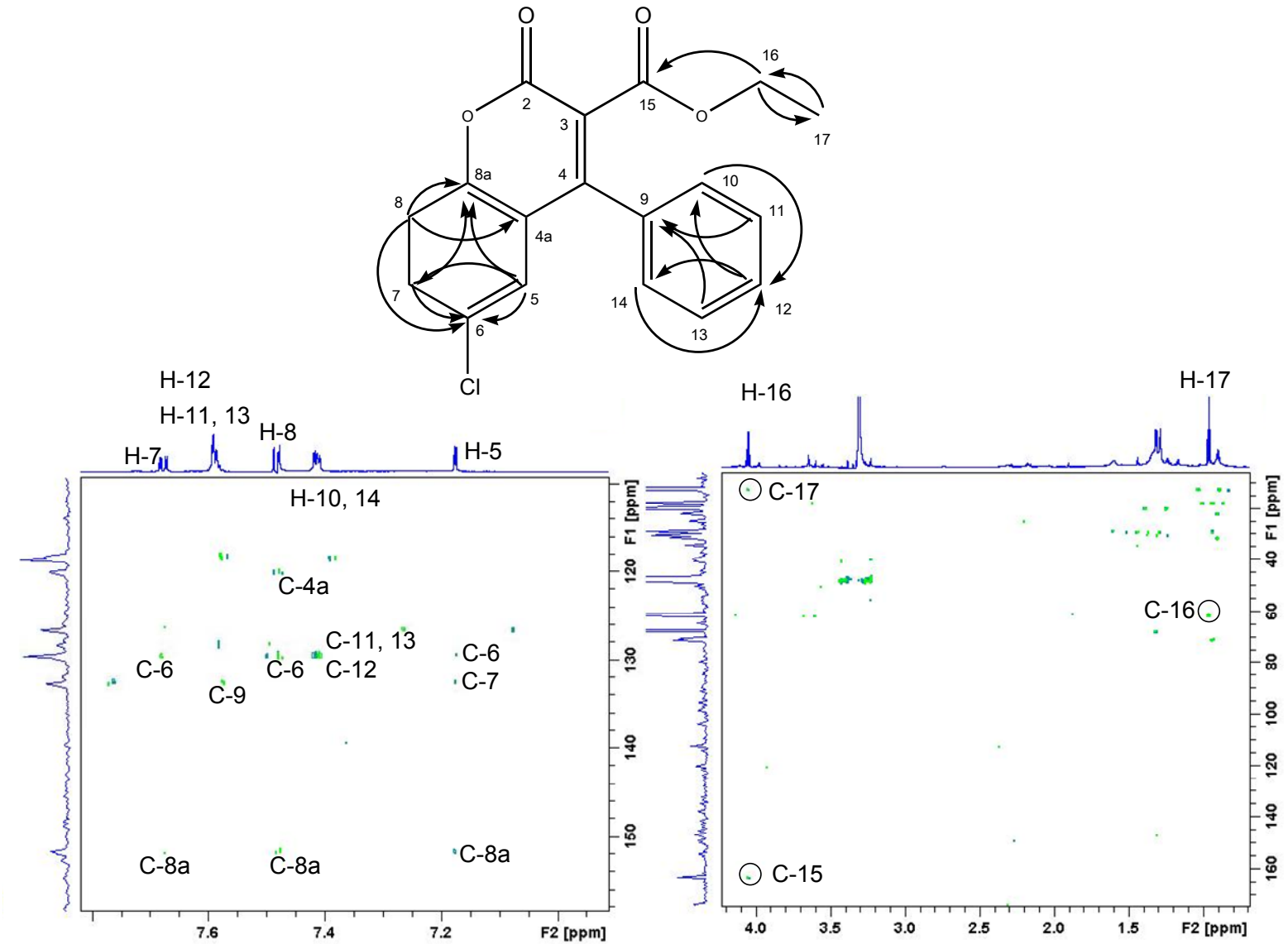
S7. ^{13}C DEPTQ NMR spectrum of isolated **2** (225 MHz, 1.7 mm tube, 0.2 mg in 50 µL MeOH-d_4)



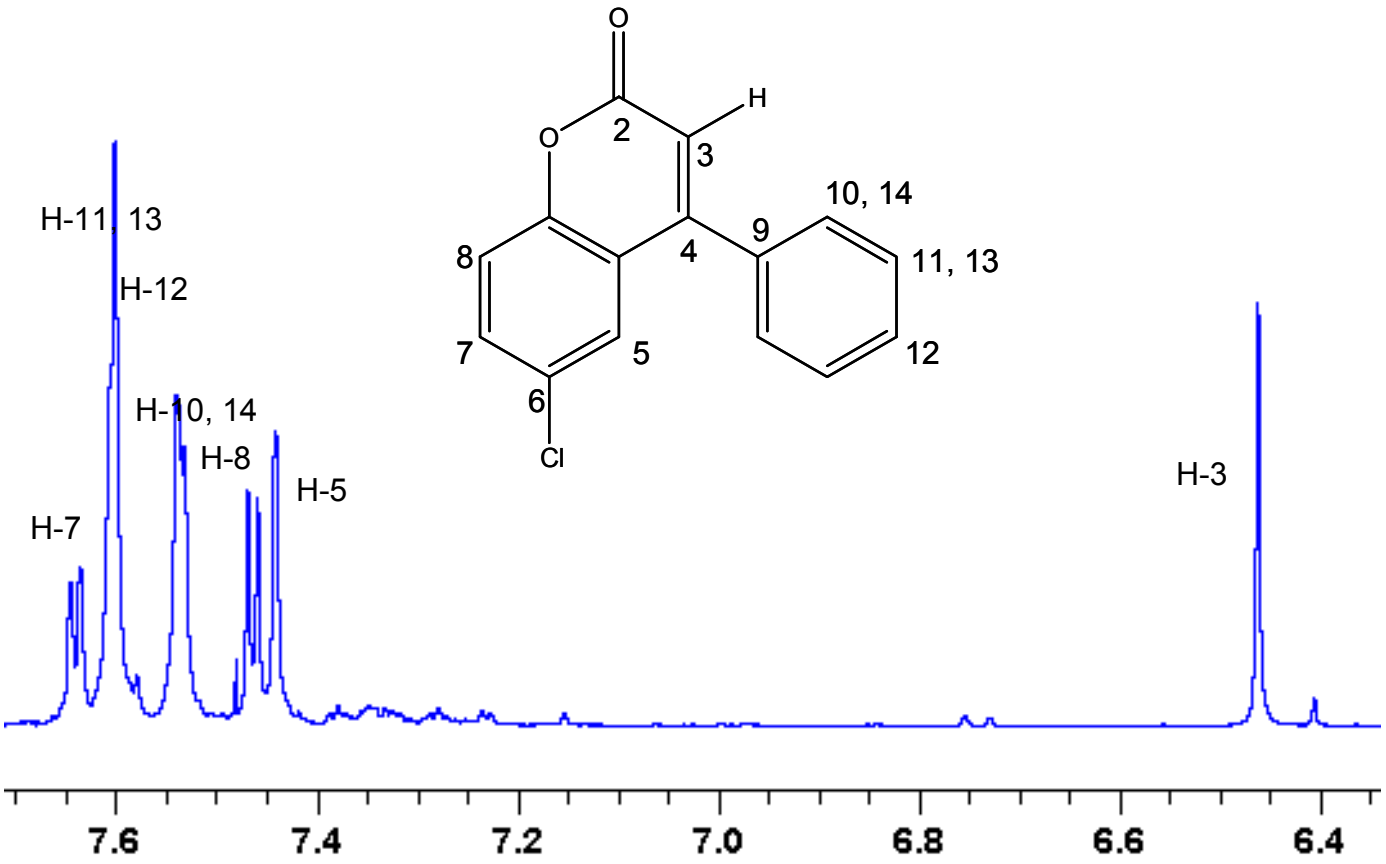
S8. ^{1}H-^{1}H COSY NMR spectrum of isolated **2** (900MHz, 1.7 mm tube, 0.2 mg in 50 µL MeOH-d_4)



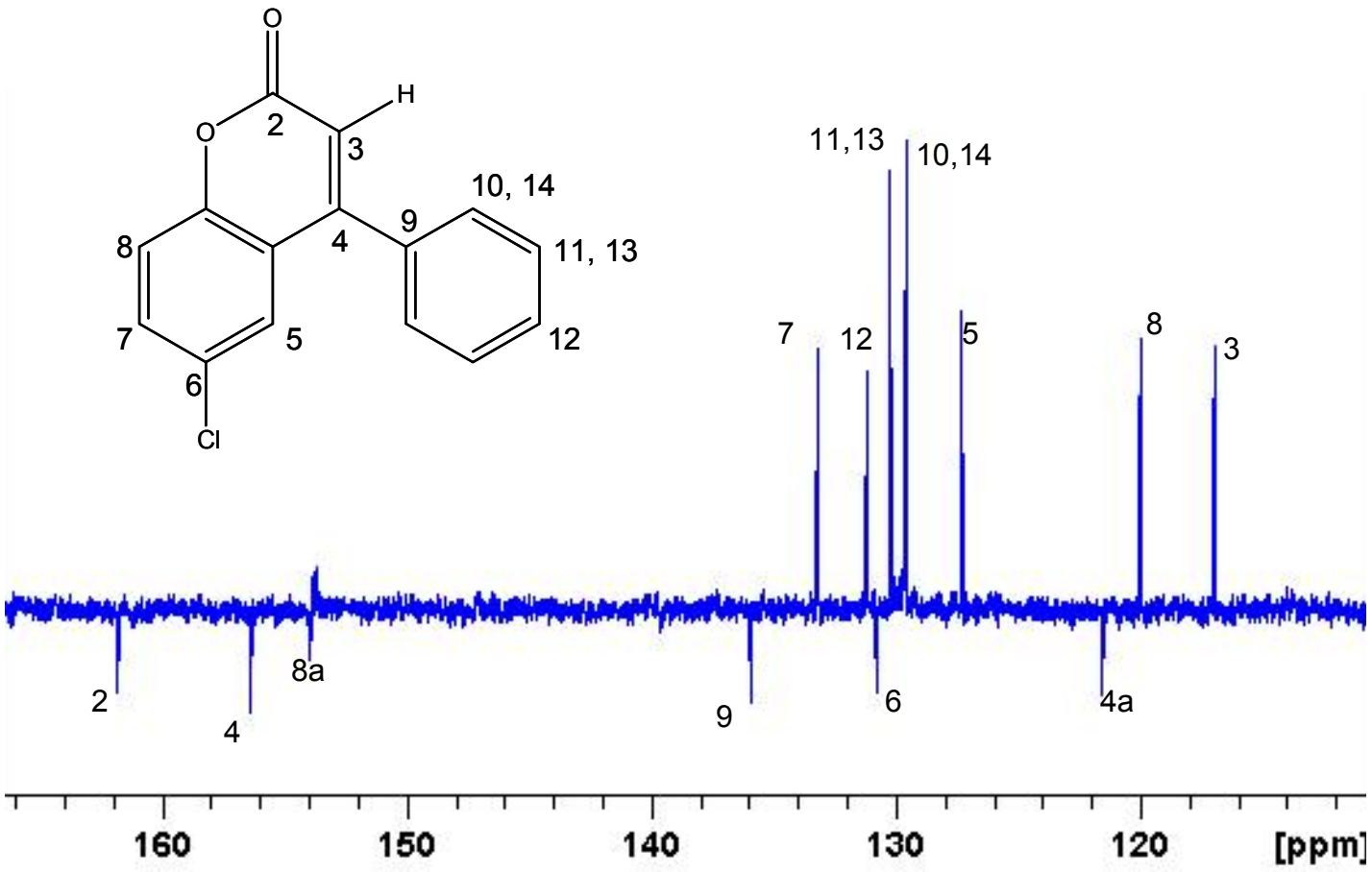
S9. HSQC NMR spectrum of isolated **2** (900 MHz, 3 mm tube, 0.2 mg in 200 µL MeOH-d_4)



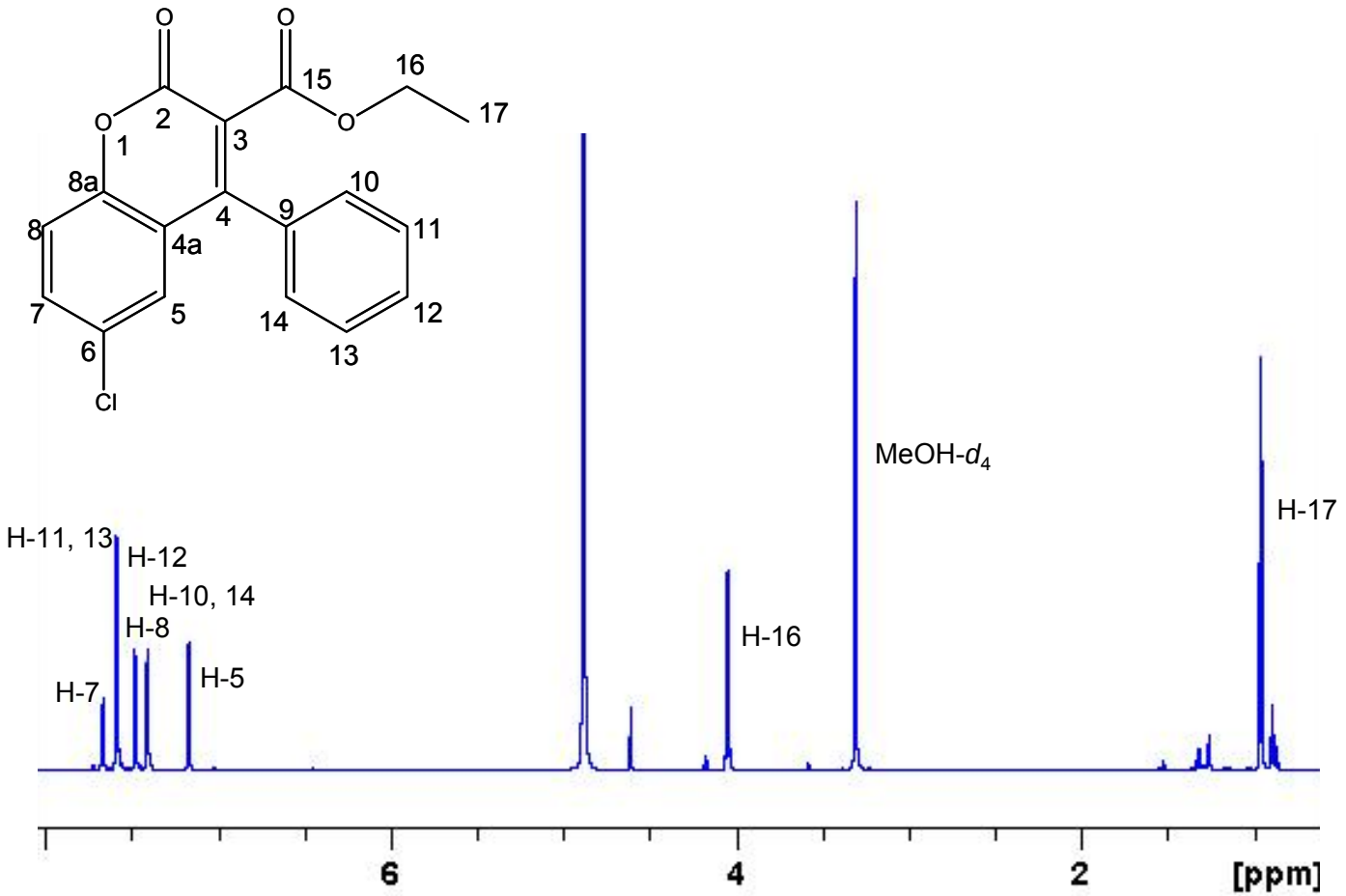
S10. HMBC NMR spectrum of isolated **2** (900MHz, 1.7 mm tube, 0.2 mg in 50 µL MeOH-d_4)



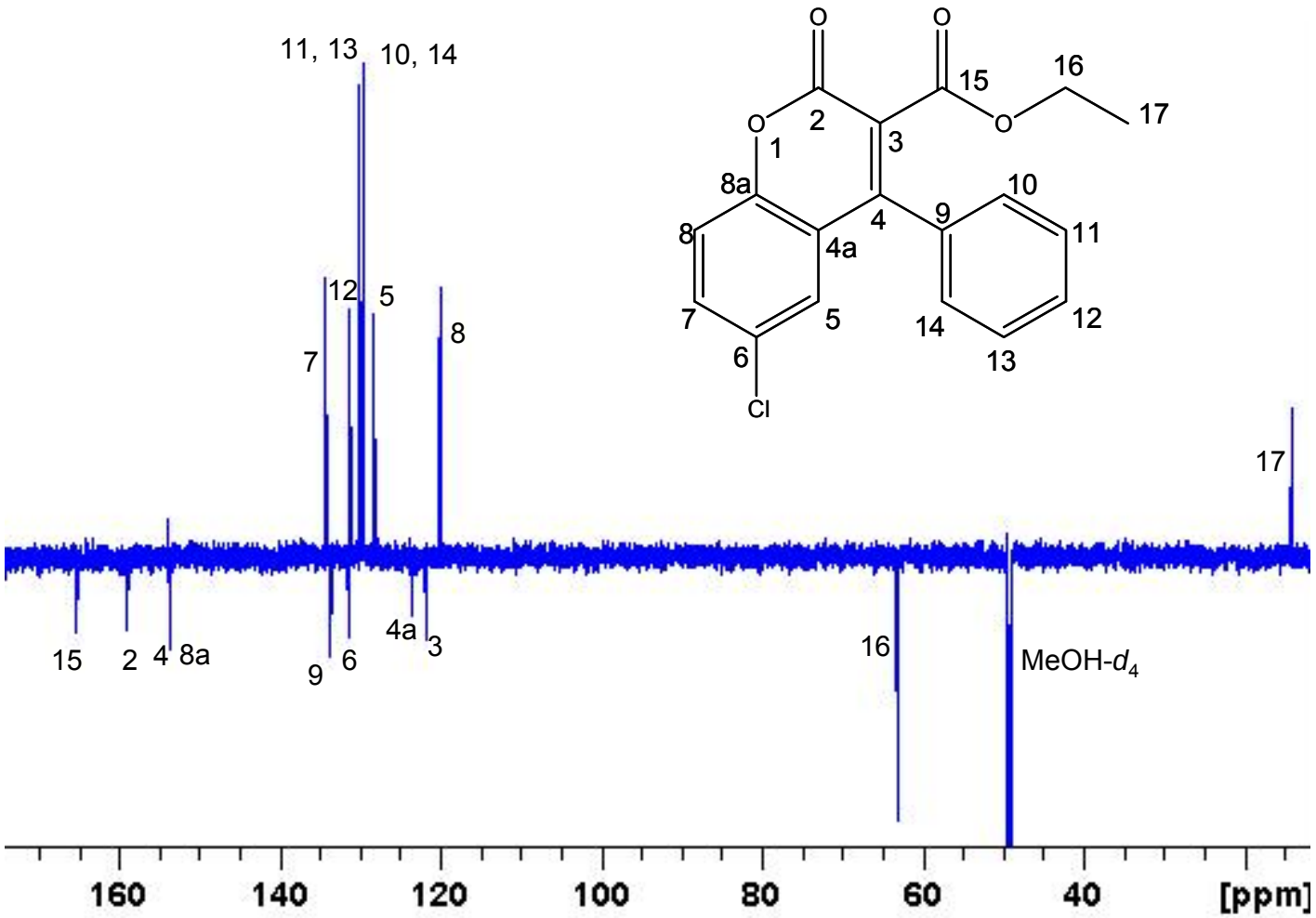
S11. ^{1}H NMR spectrum of synthetic **1** (900 MHz, 3 mm tube, 2 mg in 200 µL MeOH-d_4)



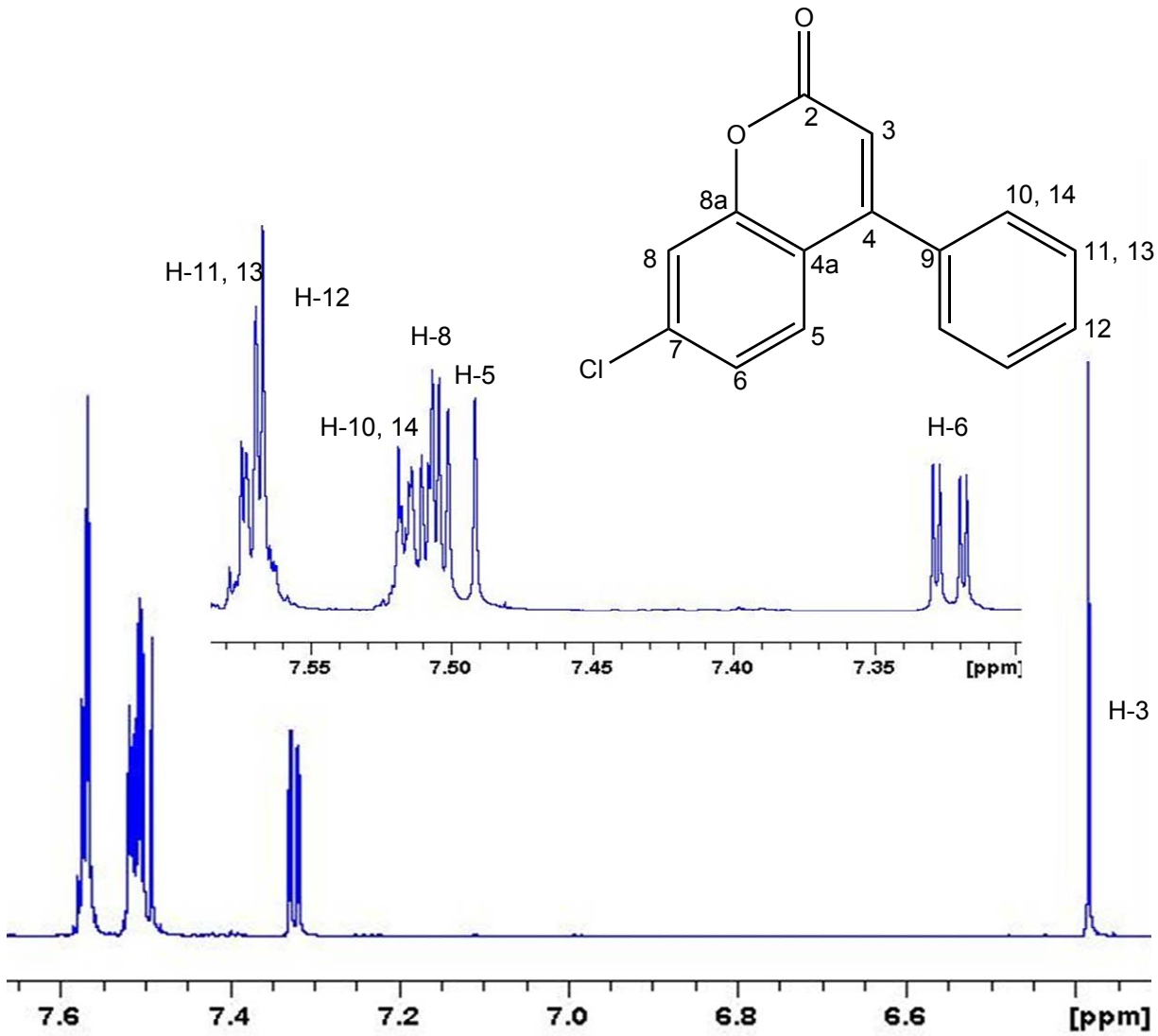
S12. ^{13}C DEPTQ NMR spectrum of synthetic **1** (225 MHz, 3 mm tube, 2 mg in 200 µL MeOH-d_4)



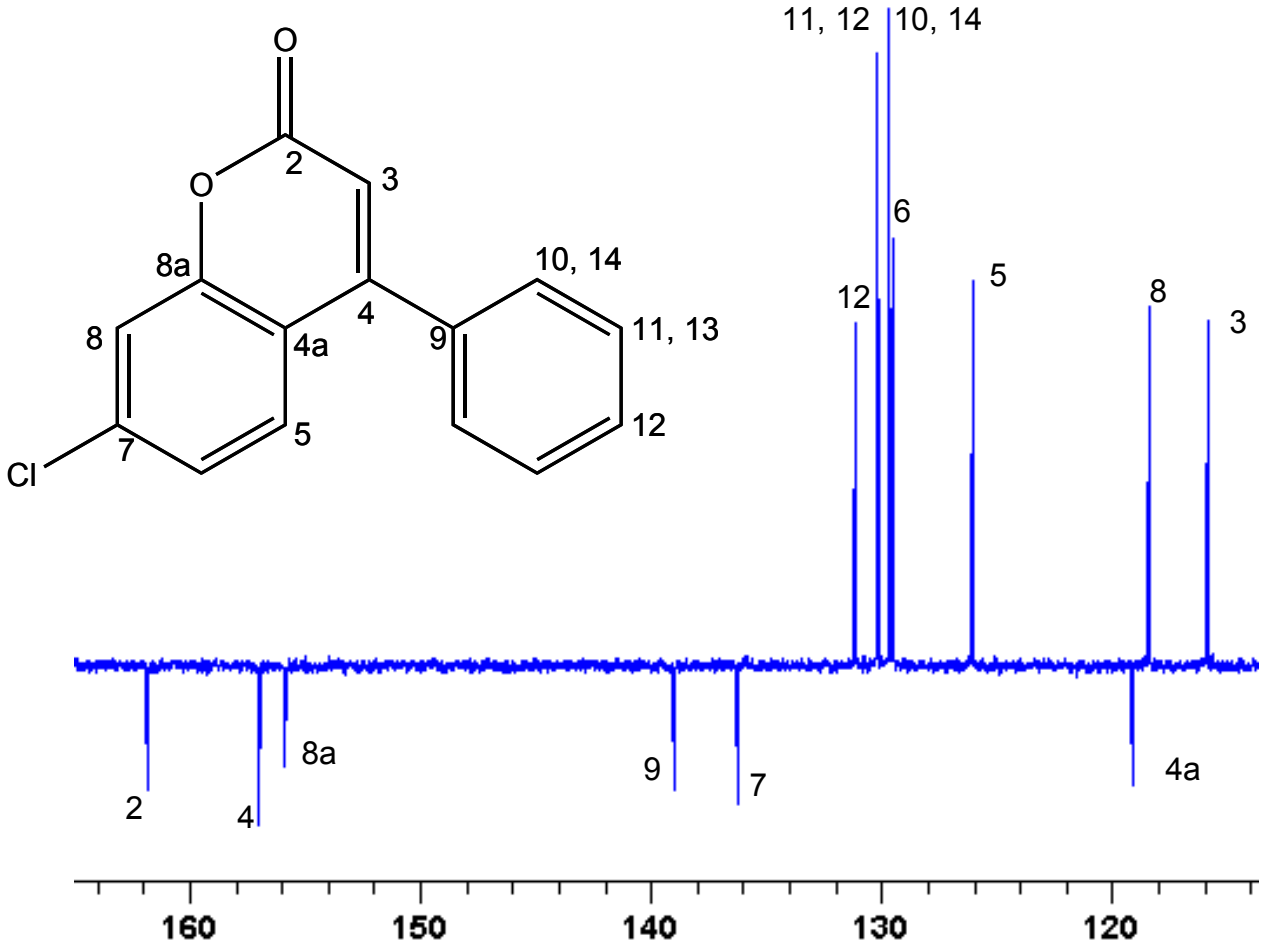
S13. ^{1}H NMR spectrum of synthetic **2** (900 MHz, 3 mm tube, 2 mg in 200 µL MeOH-d_4)



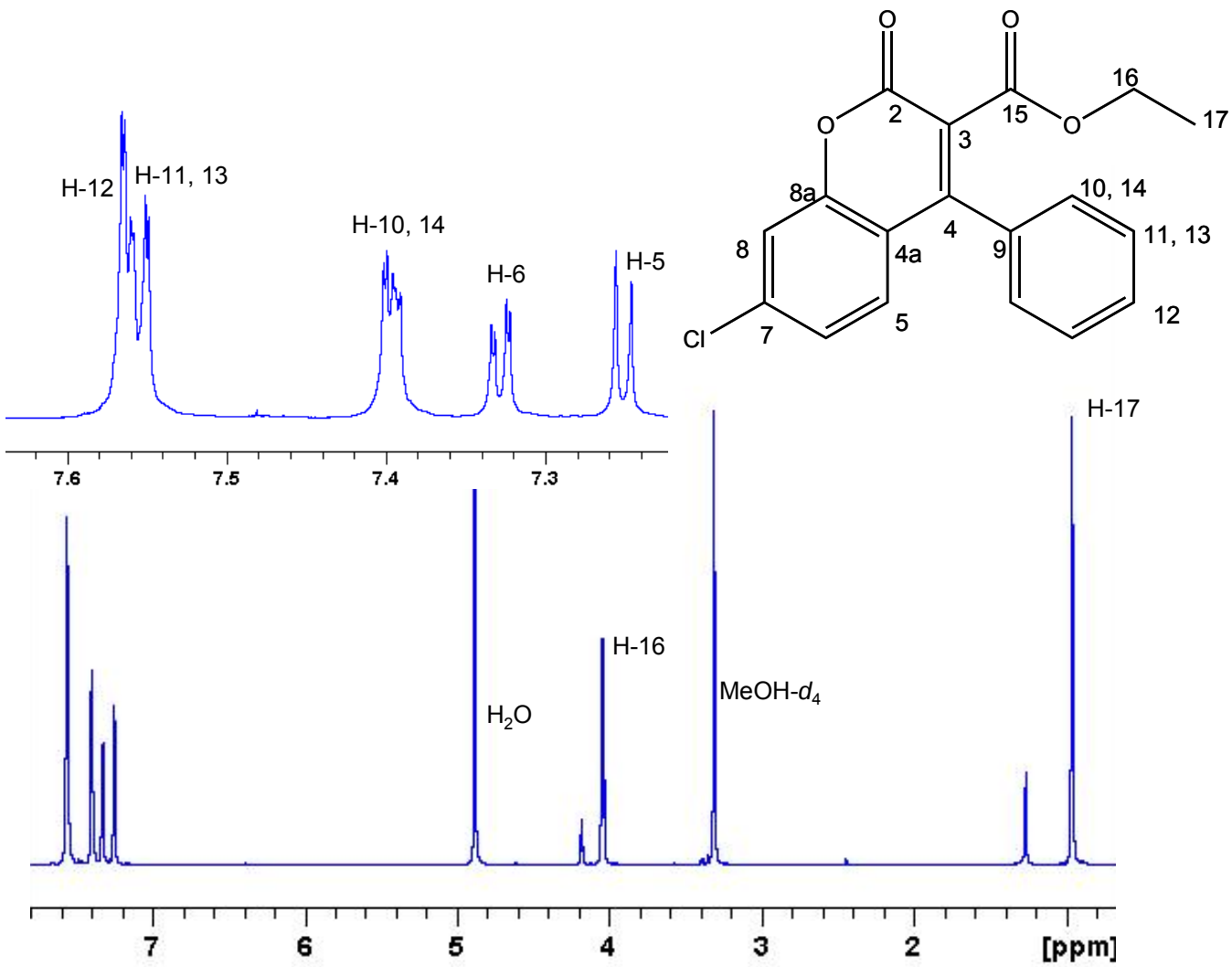
S14. ^{13}C DEPTQ NMR spectrum of synthetic **2** (225 MHz, 3 mm tube, 2 mg in 200 µL MeOH-d_4)



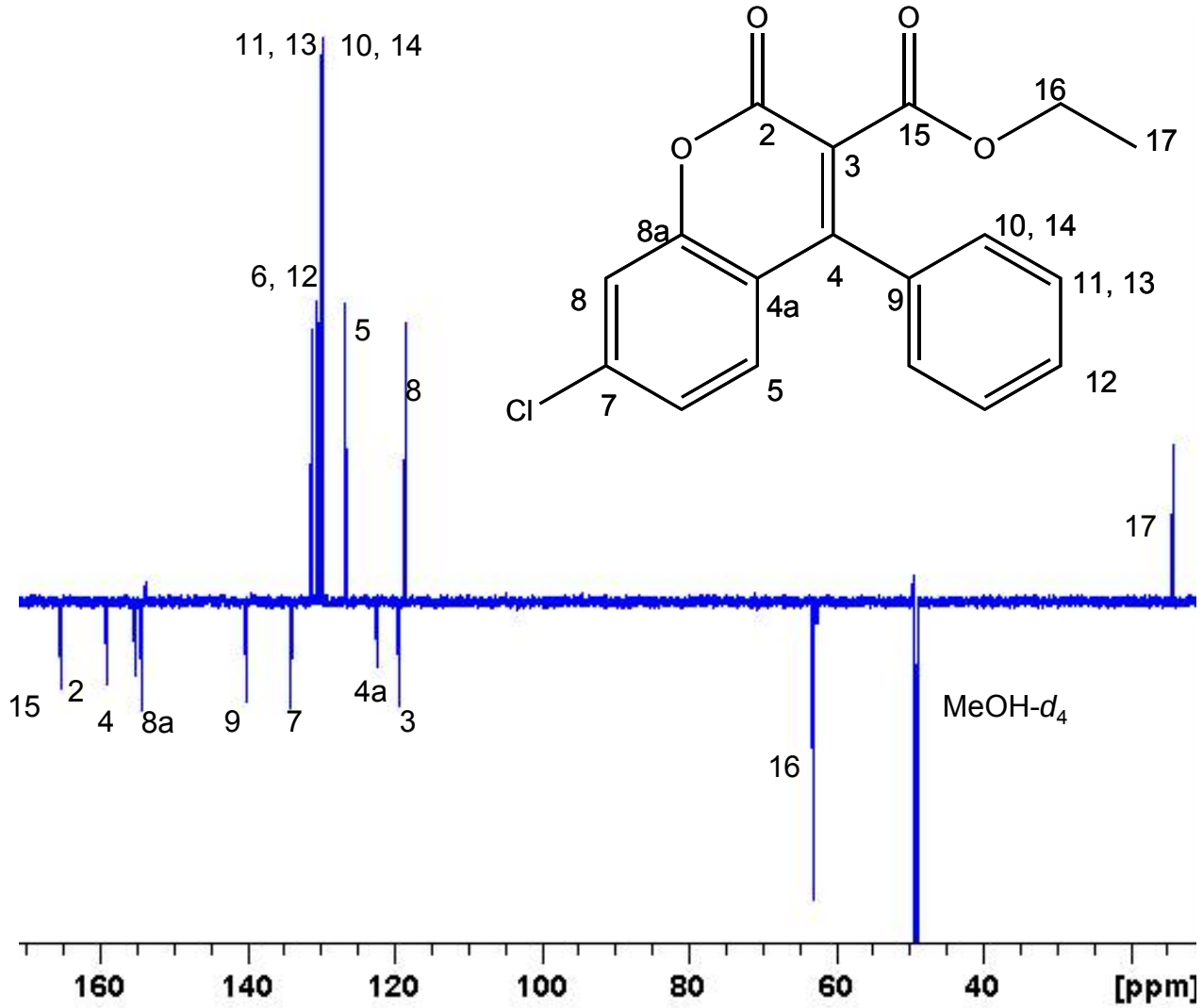
S15. ^{1}H NMR spectrum of synthetic **3** (900 MHz, 3 mm tube, 2 mg in 200 µL MeOH-d_4)



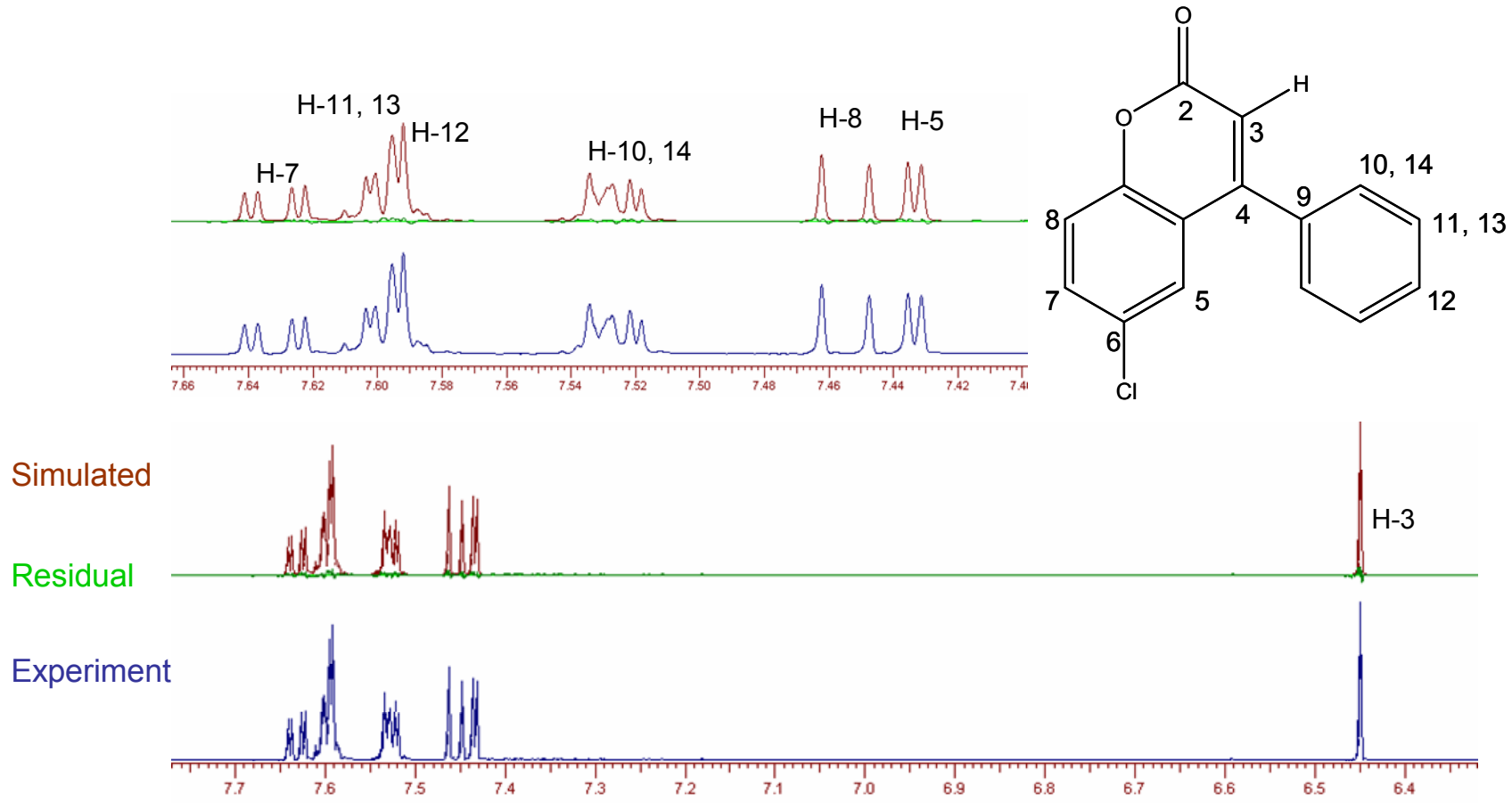
S16. ^{13}C DEPTQ NMR spectrum of synthetic **3** (225 MHz, 3 mm tube, 2 mg in 200 µL MeOH-d_4)



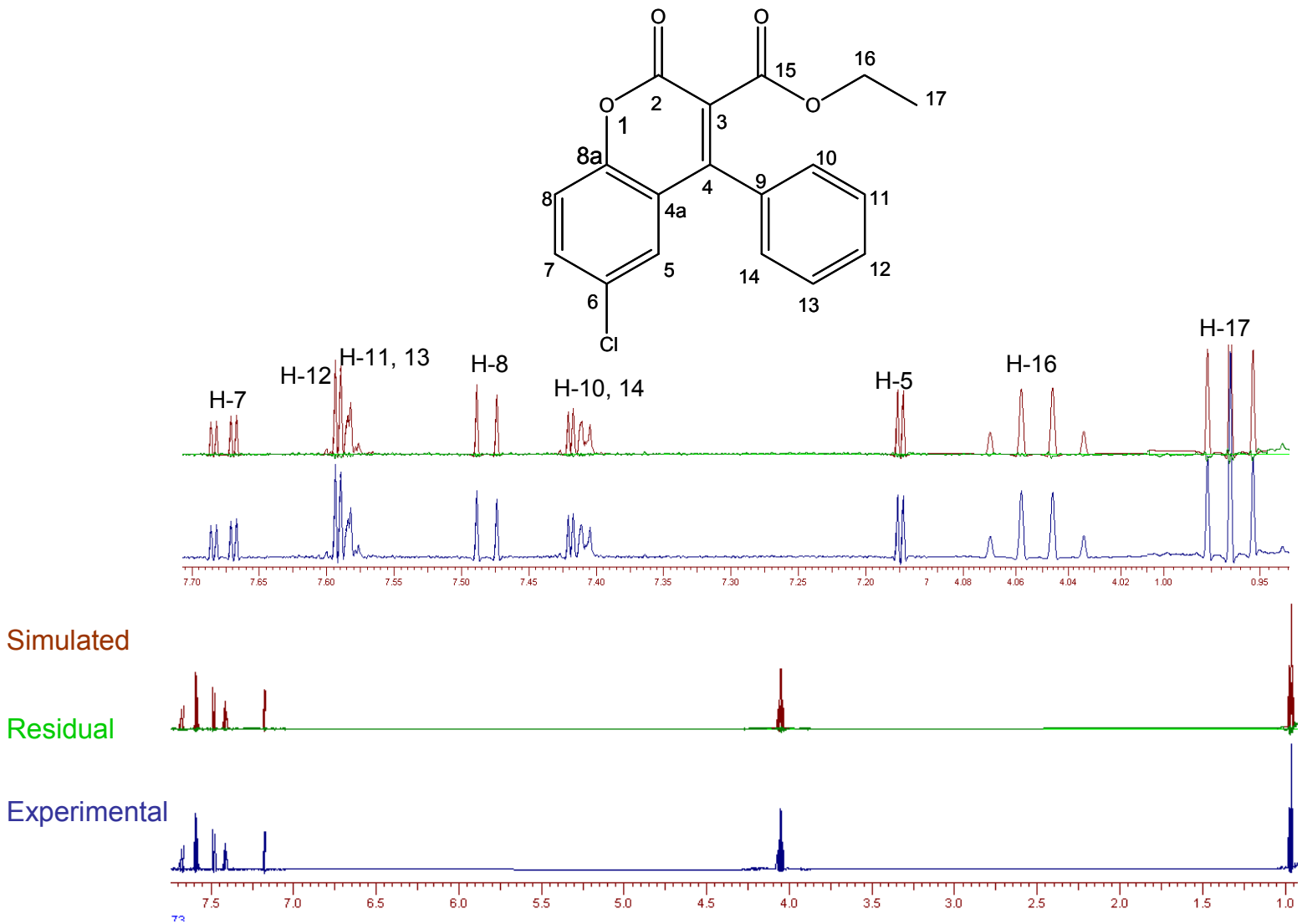
S17. ^{1}H NMR spectrum of synthetic **4** (900 MHz, 3 mm tube, 2 mg in 200 µL MeOH-d_4)



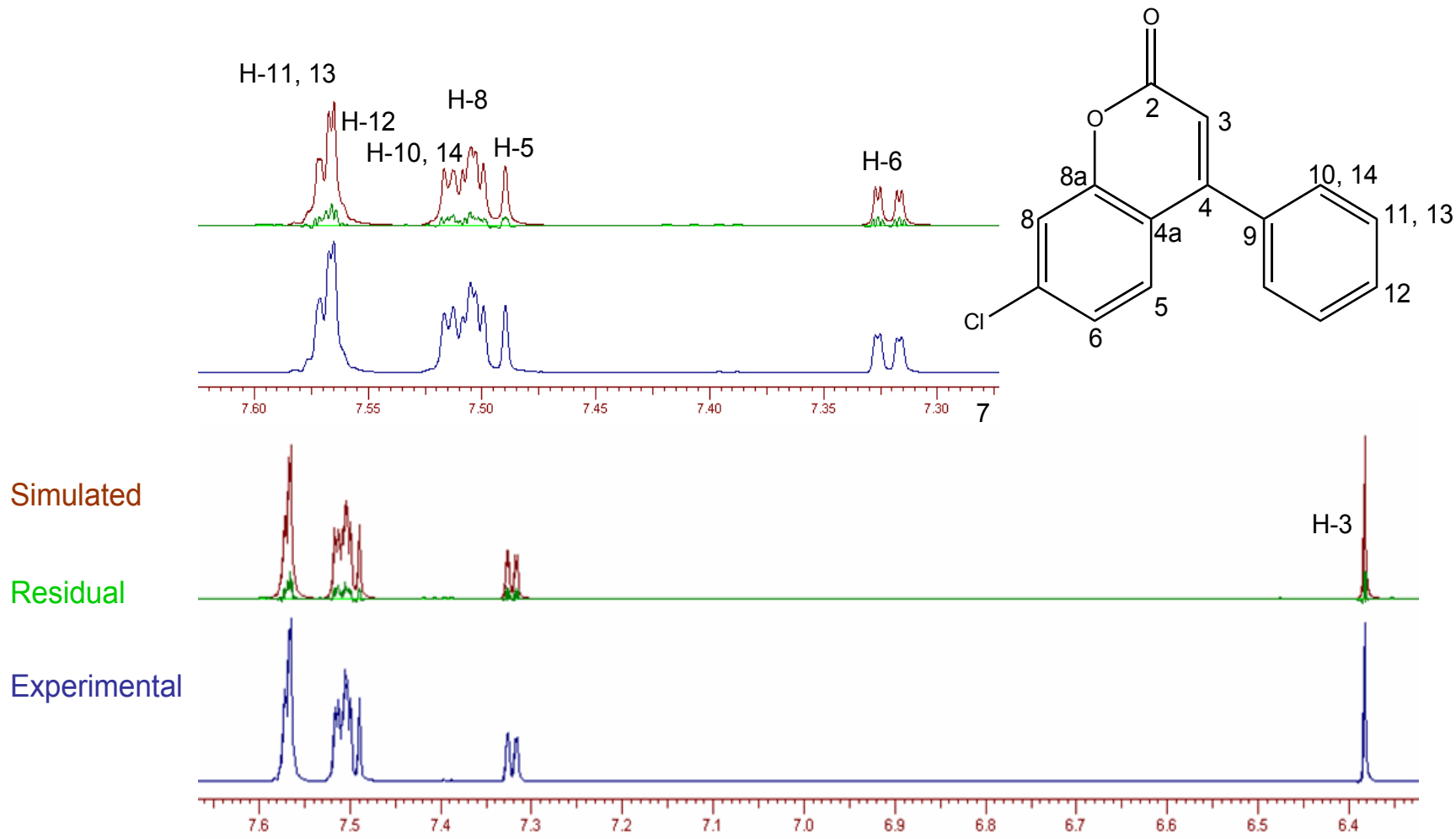
S18. ^{13}C DEPTQ NMR spectrum of synthetic **4** (225 MHz, 3 mm tube, 2 mg in 200 µL MeOH-d_4)



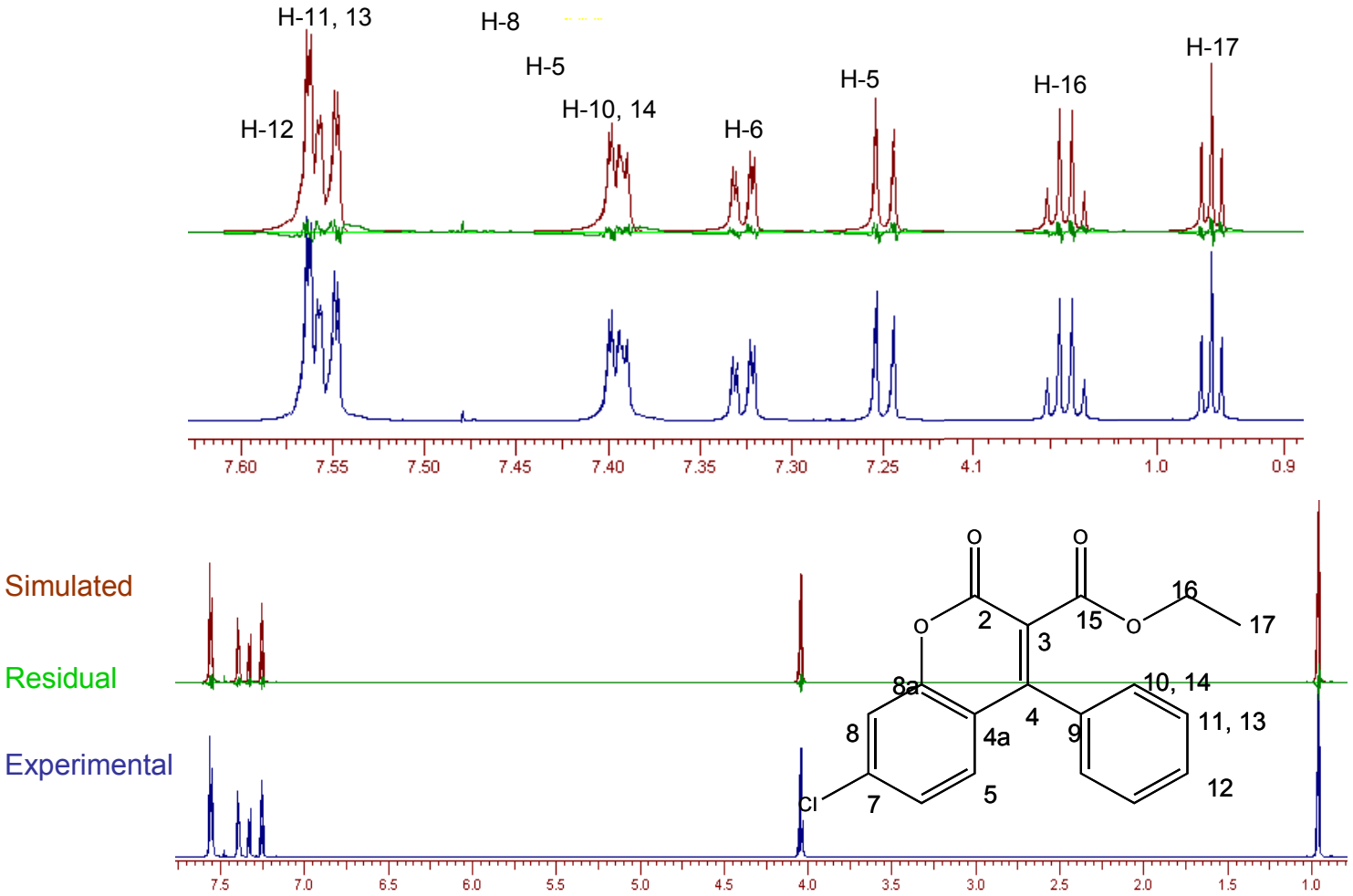
S19. ^{1}H iterative full spin analysis by PERCH iteration of 900 MHz data of the synthetic **1** (simulated spectra in red: experimental spectra in blue: residual in green) Total RMS = 0.05%



S20. ^{1}H iterative full spin analysis by PERCH iteration of 900 MHz data of the synthetic **2** (simulated spectra in red: experimental spectra in blue) Total RMS = 0.19%



S21. ^{1}H iterative full spin analysis by PERCH iteration of 900 MHz data of the synthetic **3** (simulated spectra in red: experimental spectra in blue) Total RMS = 0.04%



S22. ^{1}H iterative full spin analysis by PERCH iteration of 900 MHz data of the synthetic **4** (simulated spectra in red: experimental spectra in blue) Total RMS = 0.05%

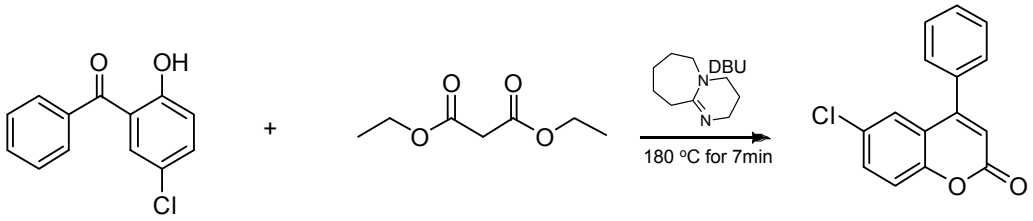
S23. Spectrum of Activity and Non-tuberculosis Mycobacterium Activity

	MIC_{90} (μg/mL)						
compound	*S. aureus*	*A. baumanii*	*P. aeruginosa*	*E. faecalis*	*E. coli*	*C. albicans*	*M. smegmatis*
1	> 100	> 100	> 100	> 100	> 100	> 100	> 100
2	> 100	> 100	> 100	> 100	> 100	> 100	> 100
3	> 100	> 100	> 100	> 100	> 100	> 100	> 100
4	> 100	> 100	> 100	> 100	> 100	> 100	> 100
ampicillin	1.07				8.75		
gentamicin	0.28	0.74	0.68	11.84	1.60		
doxycycline		0.11					
demeclocycline		0.17					
minocycline		0.39					
kanamycin		1.45					
ciprofloxacin			0.15	0.55	0.61		< 0.39
ofloxacin			0.81	1.14	1.14		0.84
rifampin			10.3				45.0
vancomycin					0.15		
moxifloxacin							0.12
amphotericin B						0.89	
ketoconazole						<0.01	

	MIC_{90} (μg/mL)					
compound	*M. chelonae*	*M. abscessus*	*M. marinum*	*M. kansasii*	*M. avium*	*M. bovis*
1	> 100	> 100	> 100	> 100	> 100	> 100
2	> 100	> 100	97.1	> 100	> 100	49.7
3	> 100	> 100	> 100	49.3	> 100	44.7
4	> 100	> 100	> 100	> 100	> 100	47.3
rifampin			0.05	0.16	> 0.29	0.02
moxifloxacin	0.10	5.10	0.19	0.10	2.77	< 0.02

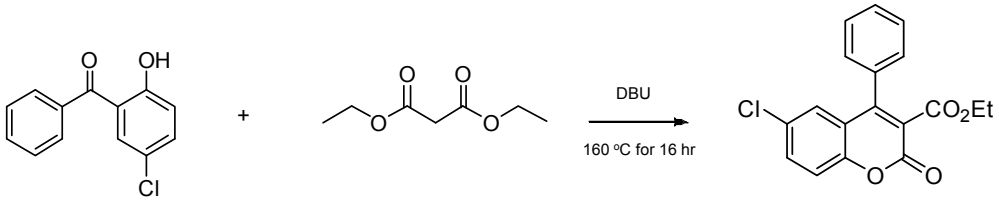
S24. Syntheses of **1** - **4**

6-Chloro-4-phenyl-*2H*-chromen-2-one (1) (ref 20)



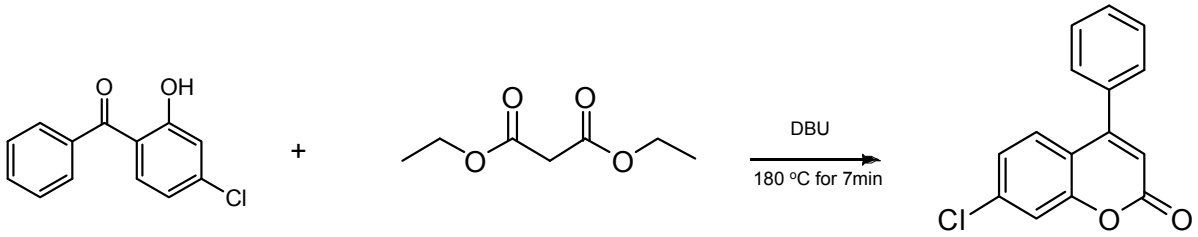
5-Chloro-2-hydroxybenzophenone (0.25 g, 1.075 mmol), DBU (82 mg, 0.537 mmol) and diethyl malonate (0.26 g, 1.6 mmol) were combined in a 10 mL vessel and heated to 180 °C via microwave for 7 min. after which TLC (10% EtOAc–Hex) showed no starting material. The crude residue was collected in dichloromethane (DCM) and washed with sat. ammonium chloride (NH_4Cl). The organic layer was separated and dried over sodium sulfate, and the solvents were evaporated. The crude product was purified via BioTage silica gel Flash cartridge 12 L using 7.5% EtOAc/Hex to give 58.3 mg (24%) white solid from the pure fractions.

Ethyl 6-chloro-2-oxo-4-phenyl-*2H*-chromene-3-carboxylate (2) (ref 21)



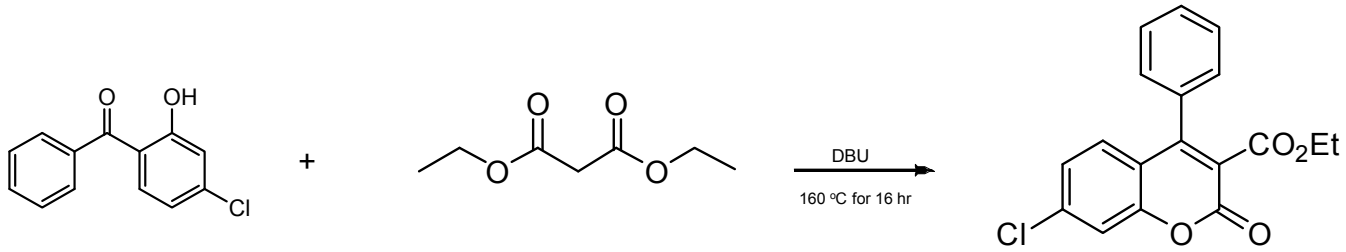
5-Chloro-2-hydroxybenzophenone (0.20 g, 0.86 mmol), DBU (13 mg, 0.09 mmol) and diethyl malonate (0.27 g, 1.72 mmol) were combined in a 10 mL vessel and heated in an oil bath at 160 °C for 16 h. After the starting material was not detectable on TLC (10% EtOAc–Hex), the crude residue was collected in dichloromethane (DCM) and washed with sat. NH_4Cl. The organic layer was separated and dried over sodium sulfate, and the solvents were evaporated. The crude product was purified via BioTage silica gel Flash cartridge 12 L using 10% EtOAc/Hex to give 94.4 mg (35%) as a white solid from the pure fractions.

7-Chloro-4-phenyl-*2H*-chromen-2-one (3) (refs 20, 22)



4-Chloro-2-hydroxybenzophenone (0.2 g, 0.86 mmol), DBU (65 mg, 0.43 mmol), and diethyl malonate (0.21 g, 1.29 mmol) were combined in 10 mL vessel and heated to 180 °C via microwave for 7 min after which TLC (EtOAc–Hex, 1:3) shows no starting material. The crude residue was collected in DCM and washed with sat. NH_4Cl. The organic layer was separated and dried over sodium sulfate, and the solvents were evaporated. The crude product was purified via BioTage silica gel Flash cartridge 12 L using 7.5% EtOAc/Hex to give 54 mg (24%) white solid from the pure fractions.

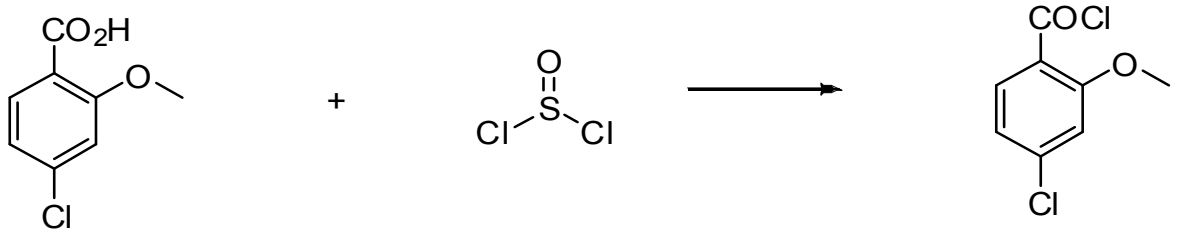
Ethyl 7-chloro-2-oxo-4-phenyl-*2H*-chromene-3-carboxylate (4) (refs 21, 22)



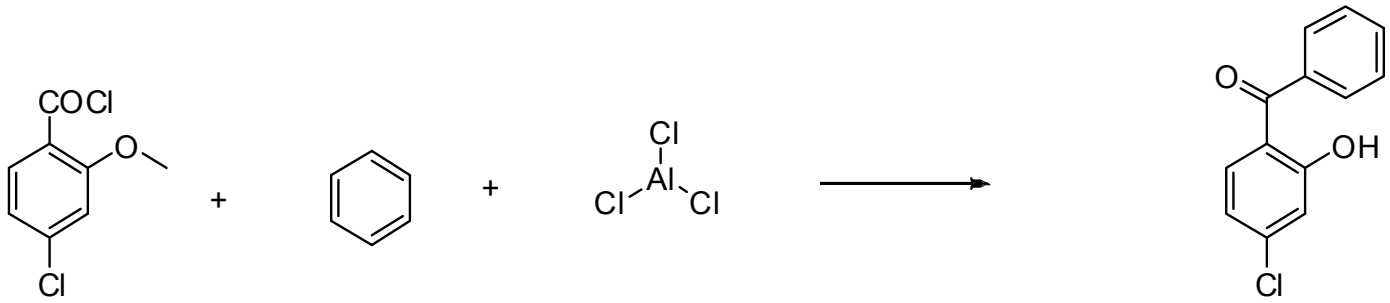
4-Chloro-2-hydroxybenzophenone (0.2 g, 0.86 mmol), DBU (13 mg, 0.086 mmol), and diethyl malonate (0.275 g, 1.72 mmol) were combined in a 10 mL flask and heated in an oil bath at 160 °C for 16 h after which TLC (10% EtOAc–Hex) showed no starting material. The crude residue was collected in DCM and washed with sat. NH_4Cl. The organic layer was separated and dried over sodium sulfate,

and the solvents were evaporated. The crude product was purified via BioTage silica gel Flash cartridge 12 L using 10% EtOAc/Hex to give 132 mg (47%) white solid.

Synthesis of 4-chloro-2-methoxybenzoyl chloride (ref 22)



2-Methoxy-4-chlorobenzoic acid (2 g, 10.72 mmol) was treated with thionyl chloride (2.93 g, 25 mmol) and DMF (1 drop) and heated at reflux for 2 h. The thionyl chloride was evaporated, and the crude residue was used directly for the next reaction.



The acid chloride from above was dissolved in benzene (15 mL). Aluminum chloride (1.57 g, 11.8 mmol) was added portion-wise over 10 min to this solution, and the resulting suspension was refluxed for 1 h (initial vigorous gas evolution upon heating). The mixture was cooled to room temperature, poured into ice (100 g) and con. HCl (20 mL). The organic layer was separated, and the aqueous layer extracted with EtOAc (3x40 mL). The combined organic layer was washed with water (3x20 mL), dried over Na_2SO_4, and the solvents were evaporated to give 2.33 g (crude yield 50%) crystallizing tan solid.