Supporting Information

Enantioselective Synthesis of Dilignol Model Compounds and Their Stereodiscrimination Study with A Dye-Decolorizing Peroxidase

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1. General information

All chemicals were purchased at the highest purity grade. All solvents were anhydrous. All reactions were performed under argon atmosphere unless otherwise specified. Thin layer chromatography (TLC) was performed using 60 mesh silica gel plates and visualization was performed using short wavelength UV light (254 nm) and basic $KMnO_4$ staining. HPLC was performed with a Waters Breeze 2 system consisting of a 1525 pump and a 2998 photodiode array detector. NMR spectra were recorded on a Varian 400 MHz spectrometer. Chemical shifts of proton (^{1}H NMR) and carbon (^{13}C NMR) were reported in ppm relative to the residual solvent peaks. High-resolution mass spectrometry (HRMS) was recorded on a Waters G2-XS QTof mass spectrometer. Surface plasmon resonance (SPR) was performed using a Biacore T200 instrument (GE Healthcare).

2. General procedures for preparing stereospecific dilignols

2-(2-methoxyphenoxy)acetic acid ***2***.[1] To a solution of guaiacol (51.6 g, 1 equiv.) and ethyl bromoacetate (83.5 g, 1.2 equiv.) in acetone (206 mL) in a 500 mL three-neck flask was added K_2CO_3 (115 g, 2 equiv.) at r.t. The resulting suspension was refluxed overnight and then cooled to r.t. The solid was filtered off and washed with acetone. The solvent in the filtrate was removed under a reduced pressure. The residue was dissolved in MeOH (206 mL) and subjected to hydrolysis using 33 wt% NaOH (150 g). After the hydrolysis was complete as determined by TLC, the MeOH was removed under a reduced pressure. The residue was acidified by adding 6M HCl (aq.) until pH reached 2. The product would precipitate out in few hours to give **2** (62.4 g, 82%) as a white crystal. m.p.: 119−120°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.35 (br, 1H), 7.03−6.99 (m, 1H), 6.92−6.88 (m, 3H),

4.67 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.6, 149.5, 147.0, 123.0, 121.1, 115.0, 112.1, 66.8, 55.8.

*General Procedures for preparation of compound **4**.* To a solution of compounds **2** (1.3 equiv.), **3** (1 equiv.), and Et_3N (4 equiv.) in toluene was added pivaloyl chloride (1.5 equiv.) at 80 °C. The mixture was refluxed overnight and then quenched with water. The organic layer was separated, washed with water twice, and concentrated to dryness. The residue was purified by crystallization (for **4a**, **4b**, and **4g**) using Et_2O/toluene (10/1) or silica gel chromatography (for the rest) eluting with hexane/EtOAc (3/2) to yield **4**.

*(S)-3-(2-(2-methoxyphenoxy)acetyl)-4-phenyloxazolidin-2-one **4a**.* Product **4a** was obtained as a white crystal (5.40 g, 82%) from compound **3c** (3.26 g, 20 mmol). m.p.: 92–93°C; $[\alpha]^{25}_D$ = +101° (c 1.33, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36–7.28 (m, 5H), 6.94–6.74 (m, 4H), 5.41 (dd, *J* = 8.7, 3.6 Hz, 1H), 5.33 (d, *J* = 17.8 Hz, 1H), 5.21 (d, *J* = 17.8 Hz, 1H), 4.73 (t, *J* = 8.9 Hz, 1H), 4.33 (dd, *J* = 9.3, 3.6 Hz, 1H), 3.81 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.8, 153.8, 149.6, 147.2, 138.4, 129.2, 128.9, 126.2, 122.4, 120.6, 114.5, 112.1, 71.2, 68.6, 57.3, 55.9; HRMS: calc. for $C_{18}H_{17}O_5NNa^+$ $[M+Na]^+$: 350.0999, found: 350.1010.

*(R)-3-(2-(2-methoxyphenoxy)acetyl)-4-phenyloxazolidin-2-one **4b**.* NMR data are the same as compound **4a**.

*(S)-4-benzyl-3-(2-(2-methoxyphenoxy)acetyl)oxazolidin-2-one **4c**.* Product **4c** was obtained as a viscous oil (12.72 g, 93%) from compound **3e** (7.09 g, 40 mmol). $[\alpha]^{25}_D$= +112° (c 3.54, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33–7.24 (m, 3H), 7.20–7.17 (m, 2H), 7.00–6.86 (m, 4H), 5.29 (s, 2H), 4.72–4.66 (m, 1H), 4.30–4.21 (m, 2H), 3.88 (s, 3H), 3.31 (dd, *J* = 13.5, 3.3 Hz, 1H,), 2.83 (dd, *J* = 13.5, 9.3, 1H,); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 168.1, 153.3, 149.4, 147.0, 134.7, 129.1, 128.7, 127.1, 122.2, 120.4, 114.4, 112.0, 68.3, 67.2, 55.6, 54.5, 37.2; HRMS: calc. for $C_{19}H_{19}O_5NNa^+$ $[M+Na]^+$: 364.1155, found: 364.1179.

*(R)-4-benzyl-3-(2-(2-methoxyphenoxy)acetyl)oxazolidin-2-one **4d**.* NMR data are the same as compound **4c**.

*(S)-4-isopropyl-3-(2-(2-methoxyphenoxy)acetyl)oxazolidin-2-one **4e**.*[2] Product **4e** was obtained as a viscous oil (2.68 g, 46%) from compound **3a** (2.58 g, 20 mmol). $[\alpha]^{25}_D$= +77.3° (c 1.71, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.99–6.84 (m, 4H), 5.29 (s, 2H), 4.48–4.44 (m, 1H), 4.37 (t, *J* = 8.8 Hz, 1H), 4.28 (dd, *J* = 9.1, 3.2 Hz, 1H), 3.88 (s, 3H), 2.48–2.40 (m, 1H), 0.90 (dd, *J* = 8.4, 7.0 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.7, 153.7, 149.1, 146.8, 121.8, 120.1, 113.9, 111.7, 67.8, 64.1, 57.6, 55.3, 27.7, 17.1, 14.0.

*(S)-3-(2-(2-methoxyphenoxy)acetyl)-5,5-dimethyl-4-phenyloxazolidin-2-one **4f**.* Product **4f** was obtained as a white crystal (0.96 g, 90%) from compound **3b** (0.57 g, 3 mmol). m.p.: 94−95°C; $[\alpha]^{25}_D$= +80.4° (c 1.10, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.32–7.26 (m, 3H), 7.11–7.08 (m, 2H), 6.88–6.74 (m, 4H), 5.30 (q, *J* = 17.8 Hz, 2H), 5.05 (s, 1H), 3.76 (s, 3H), 1.55 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.6, 152.7, 149.1, 146.8, 135.4, 128.3, 128.1, 125.9, 121.8, 120.1, 114.0, 111.7, 83.7, 68.1, 65.9, 55.3, 28.1, 23.1; HRMS: calc. for $C_{20}H_{21}O_5NNa^+$ $[M+Na]^+$: 378.1312, found: 378.1321.

*(S)-1-(4-benzyl-2-thioxothiazolidin-3-yl)-2-(2-methoxyphenoxy)ethan-1-one **4g**.* Product **4g** was obtained as a yellow crystal (0.67 g, 60%) from compound **3e** (0.63 g, 3 mmol). m.p.: 133–134°C; $[\alpha]^{25}_D$= +138° (c 1.10, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36–7.26 (m, 5H), 7.01–6.82 (m, 4H), 5.69 (d, *J* = 17.6 Hz, 1H), 5.51 (d, *J* = 17.5 Hz, 1H), 5.41 (ddd, *J* = 10.8, 7.2, 3.7 Hz, 1H), 3.91 (s, 3H), 3.51 (dd, *J* = 11.6, 7.3 Hz, 1H), 3.26 (dd, *J* = 13.1, 3.8 Hz, 1H), 3.07 (dd, *J* = 13.2, 10.6 Hz, 1H), 2.99 (d, *J* = 11.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 200.9, 169.3, 149.6, 147.2, 136.2, 129.5, 129.0, 127.3, 122.4, 120.8, 114.3, 112.2, 70.8, 68.3, 56.0, 36.7, 33.2; HRMS: calc. for $C_{19}H_{19}O_3S_2NNa^+$ $[M+Na]^+$: 396.0699, found: 396.0706.

*General procedures for preparation of **5**.* To a mixture of compound **4** (1 mmol), vanillin (1.2 equiv.), $MgCl_2$ (0.2 equiv.) and organic base (4 equiv.) in a solvent (5 mL) was added TMSCl (3 equiv.) at 0–

5 °C. The slurry was stirred at 0–5 °C for 24 h and then the water was added. The mixture was stirred for additional 30 min. The organic layer was separated and washed with water 3 times. Fifty μL organic layer was withdrawn and the solvent was evaporated. The residue was dissolved in 200 μL MeOH and analyzed by HPLC for diastereoselectivity. HPLC was performed with an analytical column (Luna C18-2, 5 μm, 4.6 mm × 250 mm) that was eluted at 1.00 mL/min using a linear gradient from 60 to 73% methanol in water over 65 min. A typical HPLC is shown in Figure 2 and results are summarized in Table 1. The remaining organic extractions were combined, dried with anhydrous $MgSO_4$, and concentrated to dryness. The residue was purified by silica gel chromatography eluting with hexane/EtOAc (2/1) to yield **5**.

(S)-3-((2R,3R)-3-(3-methoxy-4-((trimethylsilyl)oxy)phenyl)-2-(2-methoxyphenoxy)-3-((trimethylsilyl)oxy)propanoyl)-4-phenyloxazolidin-2-one (referred as di-TMS-protected aldol product in main text; see its NMR spectra on pages S27-29). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43–7.30 (m, 5H), 7.09 (d, *J* = 2.0 Hz, 1H), 6.99 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.86–6.76 (m, 2H), 6.69 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.55 (td, *J* = 7.7, 1.5 Hz, 1H), 6.33 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.10 (d, *J* = 8.5 Hz, 1H), 5.53 (dd, *J* = 8.4, 2.8 Hz, 1H), 4.89 (d, *J* = 8.5 Hz, 1H), 4.66 (t, *J* = 8.7 Hz, 1H), 4.40 (dd, *J* = 8.9, 2.8 Hz, 1H), 3.80 (s, 3H), 3.38 (s, 3H), 0.22 (s, 9H), -0.02 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.4, 153.7, 150.8, 147.6, 144.5, 139.2, 135.0, 129.2, 128.7, 126.4, 123.4, 120.7, 120.7, 120.4, 118.9, 112.7, 111.7, 81.3, 76.9, 70.2, 57.6, 55.7, 55.6, 0.4, 0.2; HRMS: calc. for $C_{32}H_{41}O_8Si_2NNa^+$ $[M+Na]^+$: 646.2263, found: 646.2320.

(S)-3-((2R,3R)-3-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-3 ((trimethylsilyl)oxy)propanoyl)-4-phenyloxazolidin-2-one **5a**. Product 5a was obtained as a viscous oil (0.38 g, 70%) from compound 4a (0.33 g, 1.00 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.35–7.28 (m, 5H), 7.11 (d, *J* = 1.9 Hz, 1H), 7.04 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.84 (dd, *J* = 17.7, 7.9 Hz, 2H), 6.70 (dd, *J* = 8.3, 1.5 Hz, 1H), 6.60 (dd, *J* = 7.9, 1.5 Hz, 1H), 6.41 (dd, *J* = 7.9, 1.5 Hz, 1H), 6.11 (d, *J* = 8.4 Hz, 1H), 5.59 (s, 1H), 5.52 (dd, *J* = 8.4, 2.8 Hz, 1H), 4.91 (d, *J* = 8.3 Hz, 1H), 4.67 (t, *J* = 8.7 Hz, 1H), 4.39 (dd, *J* = 8.9, 2.9 Hz, 1H), 3.87 (s, 3H), 3.43 (s, 3H), 0.00 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.3, 153.7, 150.5, 147.3, 146.4, 145.6, 139.1, 133.3, 129.2, 128.7, 126.2, 123.1, 121.2, 120.7, 118.1, 113.8, 112.7, 110.3, 80.9, 77.5, 77.1, 76.8, 76.8, 70.2, 57.6, 56.0, 55.7, 0.2; HRMS: calc. for $C_{29}H_{33}O_8SiNNa^+$ $[M+Na]^+$: 574.1868, found: 574.1882.

(R)-3-((2S,3S)-3-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)-3 ((trimethylsilyl)oxy)propanoyl)-4-phenyloxazolidin-2-one **5b**. NMR data are the same as compound **5a**.

*General procedures for preparation of **6**.* The organic layer containing **5** was added 2 drops of TFA and stirred for 30 min. The resulting mixture was quenched with saturated $NaHCO_3$ (aq.) and extracted with CH_2Cl_2. The organic extractions were combined and concentrated to dryness. The residue was purified by silica gel chromatography eluting with hexane/EtOAc (2/1) to yield **6**.

*(S)-3-((2R,3R)-3-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propanoyl)-4-phenyloxazolidin-2-one **6a**.* Product **6a** was obtained as a foamy solid (3.60 g, 75%) from compound **4a** (3.27 g, 10 mmol). $[\alpha]^{25}_D$= +231° (c 1.70, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.31–7.26 (m, 3H), 7.19–7.17 (m, 2H), 7.07–7.02 (m, 2H), 6.94–6.90 (m, 2H), 6.78 (d, *J* = 8.1 Hz, 1H), 6.71–6.64 (m, 2H), 6.27 (dd, *J* = 6.7, 0.8 Hz, 1H), 5.74 (s, 1H), 5.28 (dd, *J* = 8.3, 2.9 Hz, 1H), 5.11 (d, *J* = 6.6 Hz, 1H), 4.40 (t, *J* = 8.5 Hz, 1H), 4.20 (dd, *J* = 8.5, 2.6 Hz, 1H), 3.86 (s, 3H), 3.58 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 169.7, 153.9, 150.2, 146.5, 146.5, 145.6, 138.6, 131.5, 129.0, 128.5, 125.8, 123.4, 120.8, 119.7, 117.7, 114.1, 112.5, 110.0, 79.4, 74.7, 70.5, 57.7, 55.9, 55.6; HRMS: calc. for $C_{26}H_{25}O_8NNa^+$ $[M+Na]^+$: 502.1472, found: 502.1461.

*(R)-3-((2S,3S)-3-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propanoyl)-4-phenyloxazolidin-2-one **6b**.* NMR data are the same as compound **6a**.

*General procedures for preparation of compounds **1a** and **1b**.* To a solution of compound **6** in THF was added 25 wt% aqueous solution of $NaBH_4$ (4 equiv.) at 0–5 °C. The mixture was stirred at 0–5 °C for 1 h and then quenched with 1M HCl (aq). Solvents were removed under a reduced pressure. The crude product was extracted with CH_2Cl_2 (50 mL×2). The organic layers were combined and concentrated to dryness. The residue was purified by silica gel chromatography eluting with

hexane/EtOAc (1/1) to yield **1**. Enantiomeric excess (*ee*) was determined by HPLC using a Chiralpak® AD(-H) column (4.6 mm×250 mm, Daicel Chemical Industries, Exton, PA) eluted at 0.5 mL/min with 15% isopropanol in hexane. HPLC profiles are shown in Figure S1. Optical rotations are summarized in Table S1.

*(1R, 2S)-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol **1a**.* Product **1a** was obtained as a viscous oil (0.95 g, 83%) from compound **6a** (1.71 g, 3.54 mmol). *ee*>99%;^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.08–7.04 (m, 1H), 6.98–6.81 (m, 6H), 5.64 (s, 1H), 4.97 (t, *J* = 3.7 Hz, 1H), 4.16 (td, *J* = 5.5, 3.7 Hz, 1H), 3.93–3.87 (m, 7H), 3.69–3.65 (m, 1H), 3.51 (d, *J* = 3.3 Hz, 1H), 2.78 (t, *J* = 6.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 150.7, 146.8, 146.6, 144.9, 132.0, 123.3, 121.2, 119.5, 119.0, 114.3, 112.0, 109.1, 86.0, 72.6, 60.6, 55.5 (2); HRMS: calc. for $C_{17}H_{20}O_6Na^+$ $[M+Na]^+$: 343.1152, found: 343.1143.

*(1S, 2R)-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol **1b**.* NMR and HRMS data are the same as compound **1a**.

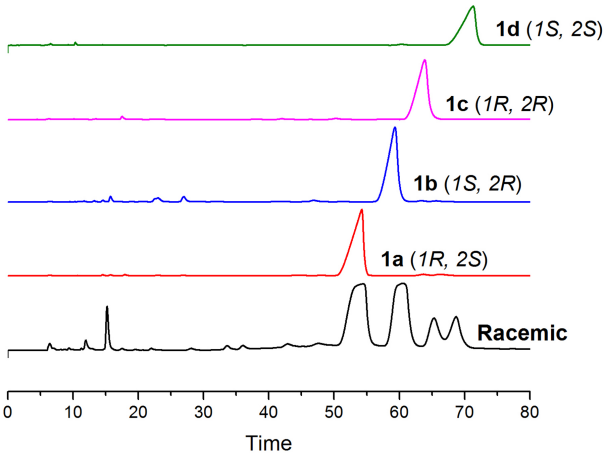


Figure S1. Chiral HPLC profiles monitored at 276 nm for a racemic mixture and four stereoisomers.

Table S1. Optical rotations of stereoisomers **1a-1d** in MeOH

compound	configuration	concentration (g/100 mL)	$[\alpha]_D^{25}$ our study	$[\alpha]_D^{25}$ reported[a]
1a	*1R, 2S*	1.76	−36.9°	−39.2°
1b	*1S, 2R*	1.66	7.3°	8.7°
1c	*1R, 2R*	1.56	−9.8°	−8.5°
1d	*1S, 2S*	1.44	40.4°	43.8°

[a]See reference 3.

*General procedures for preparation of compound **7**.* To a solution of compound **4** (1 mmol) in CH_2Cl_2 (3 mL) was added a solution of *n*Bu_2BOTf (1.2 equiv.) in CH_2Cl_2 (1.0 M) at 0–5 °C. After 15 min, DIPEA (1.5 equiv.) was added to the red solution and the red color faded immediately. After stirring at 0–5 °C for additional 15 min, the reaction mixture was cooled to –78 °C and a solution of protected vanillin (1.2 equiv.) in CH_2Cl_2 (2 mL) was added slowly. After stirring for 1 h, the mixture was warmed to r.t. and stirred for additional 3 h. The reaction was quenched with water. The organic layer was separated and 50 μL was withdrawn. After the solvent was evaporated, the residue was dissolved in 200 μL MeOH and analyzed by HPLC for diastereoselectivity as described above. A typical HPLC is shown in Figure S2 and the results are summarized in Table S2. The remaining organic layer was concentrated to dryness. The residue was purified by silica gel chromatography eluting with hexane/EtOAc (2/1) to yield **7**.

(S)-4-benzyl-3-((2S,3R)-3-(4-(benzyloxy)-3-methoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)-propanoyl)oxazolidin-2-one ***7c***. Product **7c** was obtained as a viscous oil (3.85 g, 56%,) from compound **4c** (3.60 g, 11.46 mmol). $[\alpha]^{25}_{D}$= +128° (c 0.88, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43–7.34 (m, 3H), 7.28–7.22 (m, 4H), 7.09–6.81 (m, 10H), 6.15 (d, *J* = 6.5 Hz, 1H), 5.13–5.06 (m, 4H), 4.13–4.09 (m, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.83 (d, *J* = 1.8 Hz, 1H), 3.33 (t, *J* = 8.3 Hz, 1H), 3.11 (dd, *J* = 13.5, 3.3 Hz, 1H), 2.70 (dd, *J* = 13.5, 9.3 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 169.8, 152.8, 150.0, 149.5, 147.9, 146.9, 136.9, 134.7, 130.9, 129.4, 128.8, 128.5, 127.8, 127.3, 127.2, 123.4, 121.0, 119.2, 117.2, 113.2, 112.5, 110.6, 81.6, 75.9, 70.7, 66.4, 55.9, 55.8, 55.7, 37.6; HRMS: calc. for $C_{34}H_{33}O_8NNa^+$ $[M+Na]^+$: 606.2098, found: 606.2144.

(R)-4-benzyl-3-((2R,3S)-3-(4-(benzyloxy)-3-methoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)-propanoyl)oxazolidin-2-one ***7d***. NMR data are the same as compound **7c**.

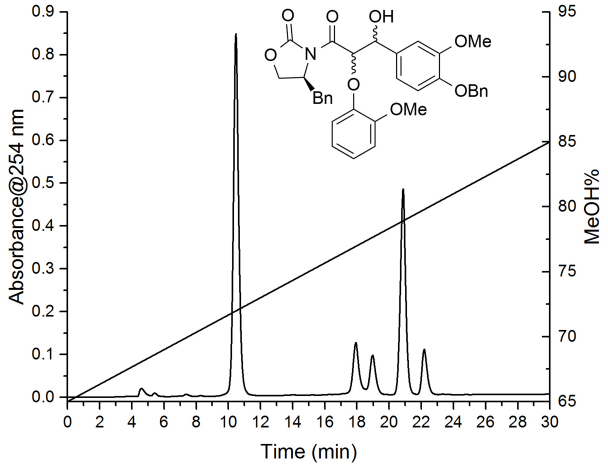
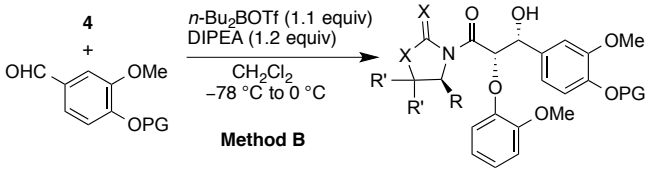


Figure S2. HPLC profile of diastereoselectivity using methods B. The straight line indicates MeOH gradient. The peak at 10.8 min represents unreacted aldehyde.

Table S2. Optimization of aldol reactions using method B



7: stereochemistry of major isomer

entry	compd	PG[a]	Diastereoselectivity[b]
1	**4a**	Bn	2:9:39:50
2	**4c**	Bn	**15:11:62:12**
3	**4e**	Bn	8:9:32:51
4	**4f**	Bn	15:31:36:18
5	**4g**	Bn	trace product
6	**4c**	TMS	no product
7	**4c**	TBDMS	trace product

[a]Protecting group; [b]Diastereoselectivity was determined by HPLC. See Experimental for details.

*General procedures for preparation of compound **8**.* The synthetic procedures were the same as the one for preparing compounds **1a** and **1b** described above, using compound **7** as the starting material. The residue from organic extractions was used directly for next step without purification.

*(1R,2R)-1-(4-(benzyloxy)-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol **8c**.* Product **8c** was obtained as a viscous oil (1.50 g, 83%,) from compound **7c** (2.58 g, 4.42 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.44–7.26 (m, 5H), 7.13–7.01 (m, 3H), 6.94–6.84 (m, 4H), 5.14 (s, 2H), 4.96 (d, *J* = 7.8 Hz, 1H), 4.05–4.02 (m, 1H), 3.88 (d, 6H), 3.76 (s, 1H), 3.63 (d, *J* = 12.6 Hz, 1H), 3.49–3.46 (m, 1H), 2.85–2.80 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 150.8, 149.5, 147.7, 147.5, 136.9, 133.0, 128.3, 127.7, 127.2, 123.6, 121.4, 120.1, 119.3, 113.7, 112.0, 110.5, 88.3, 73.5, 70.8, 60.8, 55.8, 55.6; HRMS: calc. for $C_{24}H_{26}O_6Na^+$ $[M+Na]^+$: 433.1622, found: 433.1624.

*(1S,2S)-1-(4-(benzyloxy)-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol **8d**.* NMR data are the same as compound **8c**.

*General procedures for preparation of compounds **1c** and **1d**.* To a solution of crude **8** in MeOH was added 10% Pd/C powder. The reaction was hydrogenated at atmospheric pressure and vigorously stirred for 12 hours at r.t. The powder was removed by Celite filtration. After the solvent was evaporated, the residue was purified by silica gel chromatography eluting with hexane/EtOAc (1/1) to yield **1**. Chiral HPLC was performed as described for **1a/1b** to determine *ee* and is shown in Figure S1. Their optical rotations are summarized in Table S1.

*(1R, 2R)-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol **1c**.* Product **1c** was obtained as a viscous oil (0.65 g, 93%) from compound **8c** (0.90 g, 2.18 mmol). *ee*>99%;^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.14–7.04 (m, 2H), 6.97–6.87 (m, 5H), 5.74 (s, 1H), 4.90 (d, *J* = 8.0 Hz, 1H), 4.04–4.00 (m, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 3.71 (br, 1H), 3.63 (dd, *J* =12.5, 3.3 Hz, 1H), 3.48 (dd, *J* = 12.4, 4.0 Hz, 1H), 2.79 (br, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 151.2, 147.7, 146.8, 145.6, 131.6, 124.1, 121.7, 120.8, 120.2, 114.5, 112.2, 109.6, 89.2, 74.0, 61.1, 56.0, 55.9; HRMS: calc. for $C_{17}H_{20}O_6Na^+$ $[M+Na]^+$: 343.1152, found: 343.1143.

*(1S, 2S)-1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)propane-1,3-diol **1d**.* NMR and HRMS data are the same as compound **1c**.

3. Enzyme purification

His-tagged *Tc*DyP was purified by following the published protocol.[4] Its SDS-PAGE after Ni-NTA purification is shown in Figure S3. The molecular weight of *Tc*DyP was calculated at 43.6 KDa. Its specific activity (SA) with Reactive Blue 19 at r.t. was determined to be 122 μmol $min^{-1}mg^{-1}$.

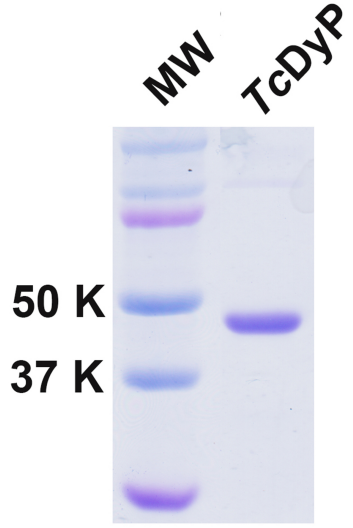


Figure S3. SDS-PAGE of purified *Tc*DyP.

4. Surface plasmon resonance (SPR) experiments

Direct binding of stereospecific model lignin compounds with *Tc*DyP was measured by SPR using a Biacore T200 instrument (GE Healthcare) at 25°C. To begin, a CMD-500M sensorchip (XanTec Bioanalytics GmbH) was used to create *Tc*DyP biosensor by conventional, random amine coupling chemistry. Briefly, an equal volume mixture of 0.1 M N-hydroxysuccinimide (NHS) and 0.4 M ethyl(dimethylaminopropyl) carbodiimide (EDC) was injected for 7 min at 5 µl•min^{-1} over a single flow cell. Then, 50 µg•ml^{-1} DyP protein diluted in 10 mM sodium acetate (pH 6.0) was injected for 15 min and immediately followed by a 7-min injection of 1M ethanolamine (pH 9.0) to quench remaining reactive groups. A final immobilization level of 13,500 response units (RU) was achieved. A reference surface was created by EDC/NHS activation followed by immediate quenching using ethanolamine.

All binding experiments were performed in a running buffer consisting of 20 mM HEPES (pH 7.4), 140 mM NaCl, 0.005% (v/v) Tween 20 using a flow rate of 30 µl•min^{-1}. To evaluate lignin model compounds as prospective ligands for *Tc*DyP, a working stock of each compound was diluted with running buffer to prepare solutions at 500, 250, 125, 62.5, 31.25, and 7.8 µM. Running buffer alone was used as a blank injection. Samples were injected over the biosensor for 30 s, followed by 30 s of dissociation phase. A wash step consisting of 2 M NaCl in *dd*H_2O (30 s contact time) was used to regenerate the surface. Quadruplicate injections were performed for all samples, including the buffer controls.

The sensorgrams obtained from each experimental series were analyzed using Biacore T200 Evaluation software v3.0. Reference corrected curves were generated by subtracting the signal from the reference surface from each experimental surface. The signal just prior to injection stop of these subtracted sensorgrams was treated as the DyP-binding response. Because the on-rates of analytes (dilignols) were fast and the surface density was high, kinetic fitting was not performed. Instead, steady-state affinity analysis was used to characterize the apparent binding affinity (K_D). Thus, the five seconds of each curve immediately prior to injection stop were averaged and plotted against analyte concentrations. Representative sensorgrams and global fittings based on a 1:1 binding model are shown in Figures S4 and S5, respectively. They were used to determine the parameters reported in Table 2. The K_D value is defined as the concentration of ligand, at which a half-maximal RU_{max} is achieved.

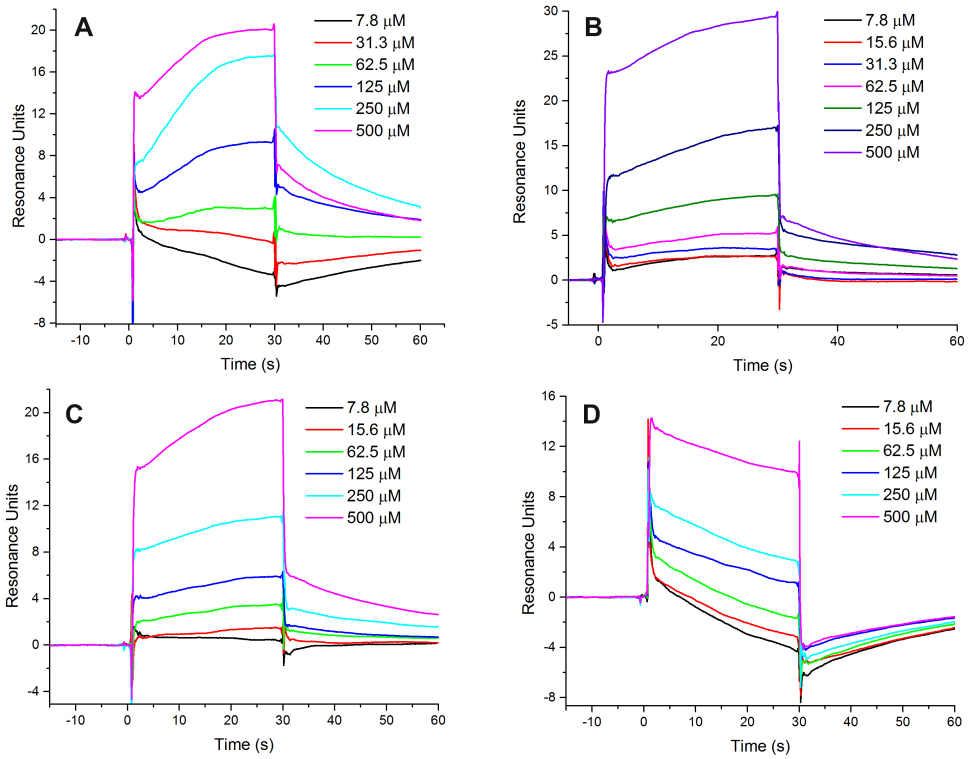


Figure S4. Representative sensorgrams of *Tc*DyP with stereoisomers **1a** (A), **1b** (B), **1c** (C), and **1d** (D) at a series of concentrations.

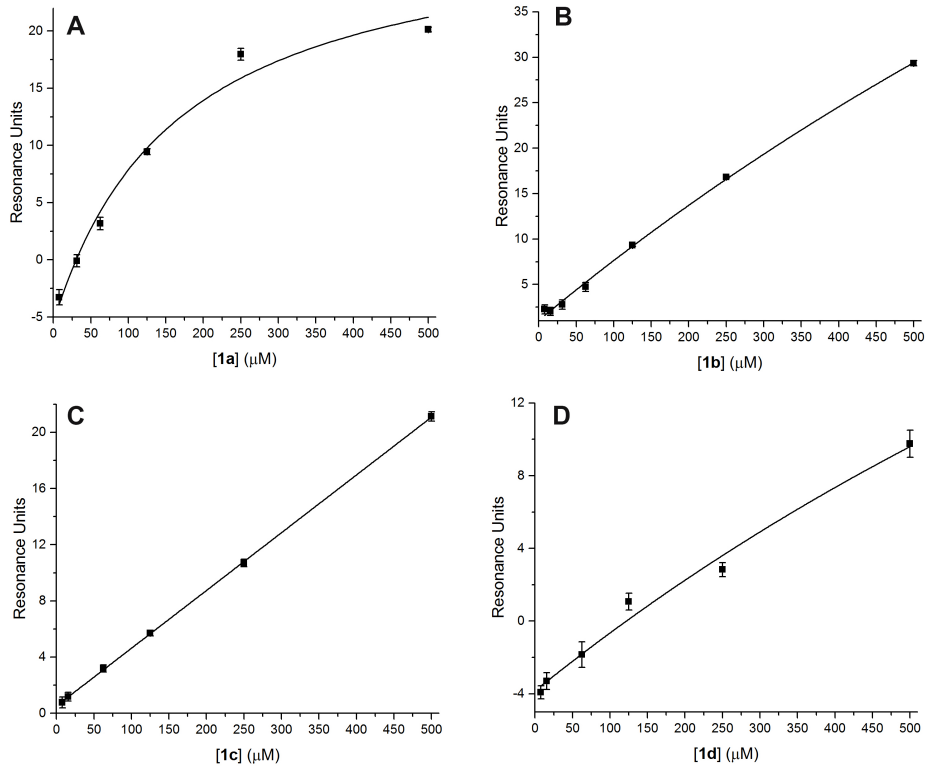


Figure S5. Steady-state affinity analysis of stereoisomers **1a** (A), **1b** (B), **1c** (C), and **1d** (D). The solid lines represent global fitting of the four trials based on a 1:1 binding model.

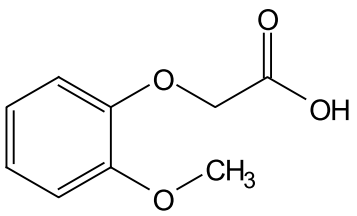
5. Enzyme assays

All enzymatic reactions were carried out at r.t. In a final volume of 1000 μL consisting of 50 mM sodium citrate (pH 3.0), 1.4 mM H_2O_2, 1 mg/mL BSA and 0.7mM model compound **1**, enzyme was added to a final concentration of 0.5 μM to initiate the degradation. At defined time points, 90 μL aliquots were removed from the mixture and the reaction was quenched by heating the sample at 95 °C for 5 min. Each sample was added an internal standard, phenol, to a final concentration of 1.5 mM and then centrifuged to remove protein precipitates. The supernatant was analyzed by HPLC using a column (Luna C18–2, 5 μm, 4.6 mm x 250 mm) eluted at 1.00 ml/min with a linear gradient from 30 to 90% methanol in *dd*H_2O over 30 min. Degradation of the model compounds were monitored and reaction rates were determined by the slope of the initial phase. Enzyme specific activities (SAs) were calculated based on the reaction rate and protein concentration, which are summarized in Table 2.

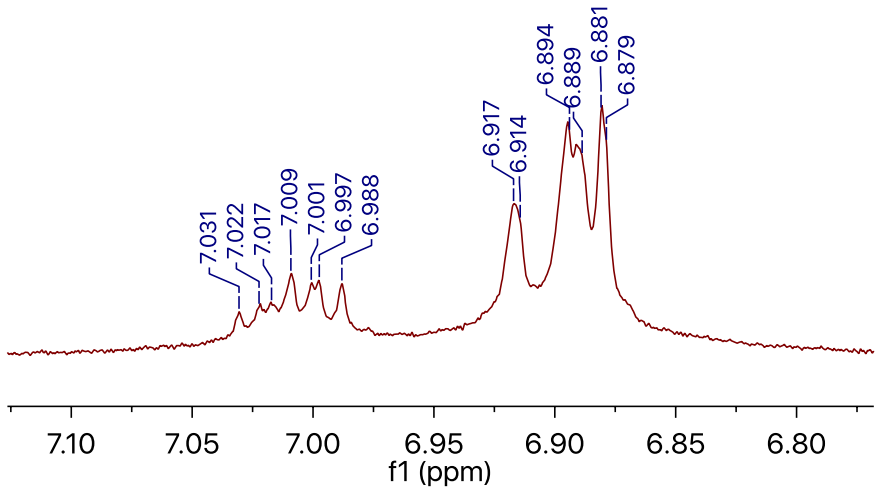
6. References

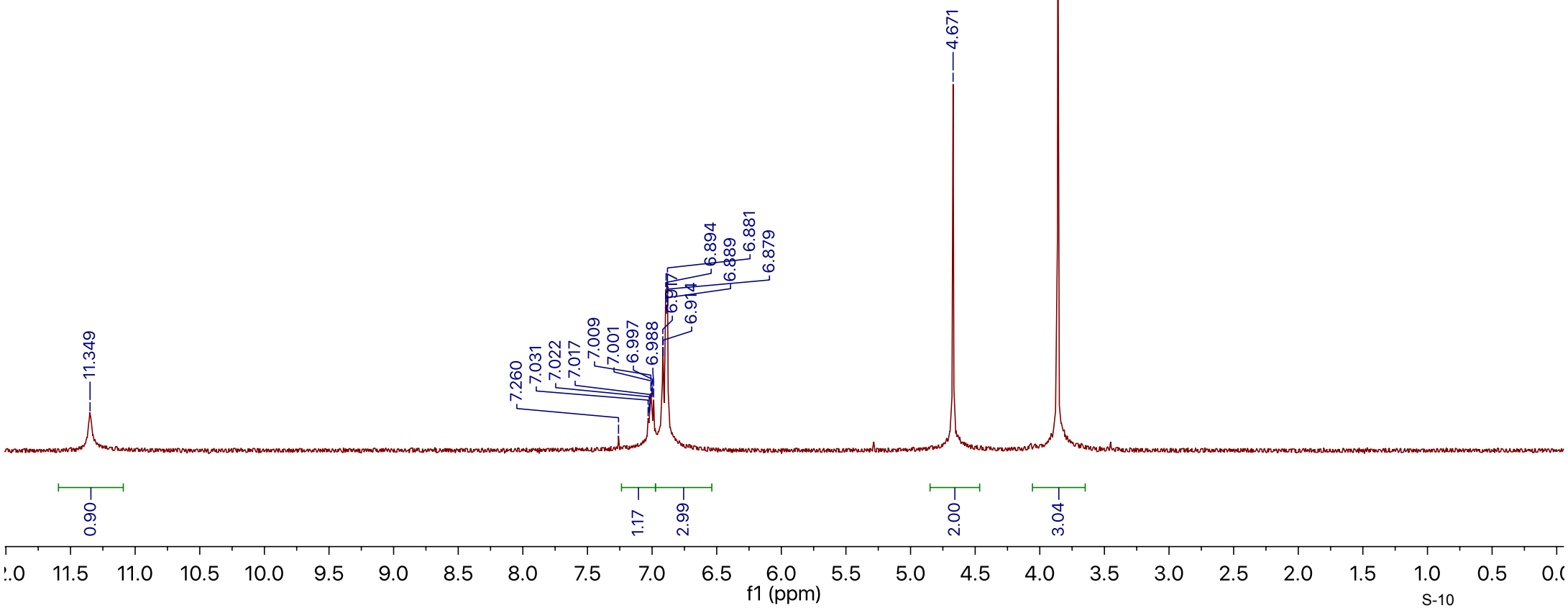
1) Buendia, J.; Mottweiler, J.; Bolm, C., *Chem. Eur. J.* **2011,** *17*, 13877-13882.
2) Njiojob, C. N.; Rhinehart, J. L.; Bozell, J. J.; Long, B. K., *J. Org. Chem.* **2015,** *80*, 1771-1780.
3) Hishiyama, S.; Otsuka, Y.; Nakamura, M.; Ohara, S.; Kajita, S.; Masai, E.; Katayama, Y., *Tetrahedron Lett.* **2012,** *53*, 842-845.
4) Chen, C.; Shrestha, R.; Jia, K.; Gao, P. F.; Geisbrecht, B. V.; Bossmann, S. H.; Shi, J. S.; Li, P., *J. Biol. Chem.* **2015,** *290*, 23447-23463.

^{1}H NMR (400 MHz, $CDCl_3$)

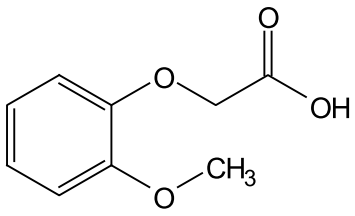


2

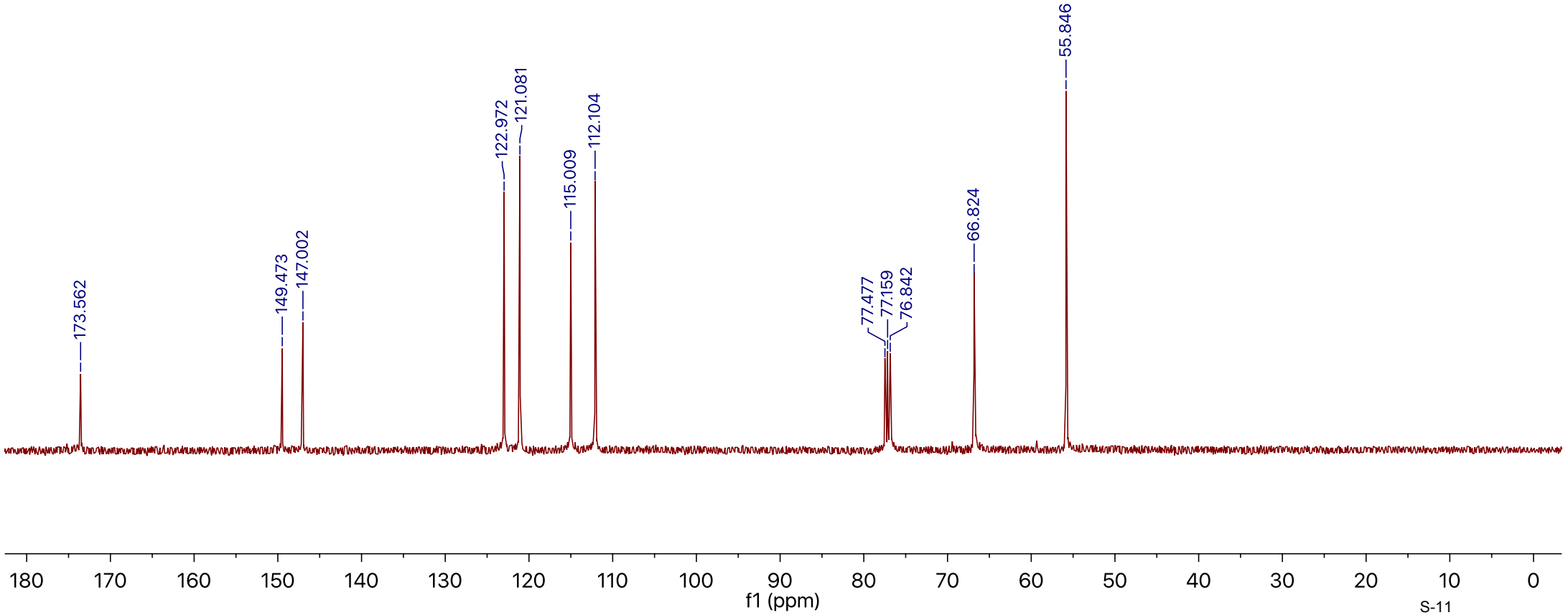




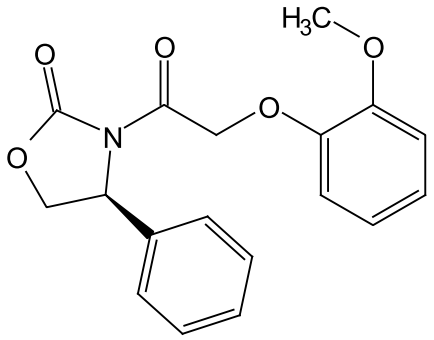
^{13}C NMR (100.6 MHz, $CDCl_3$)



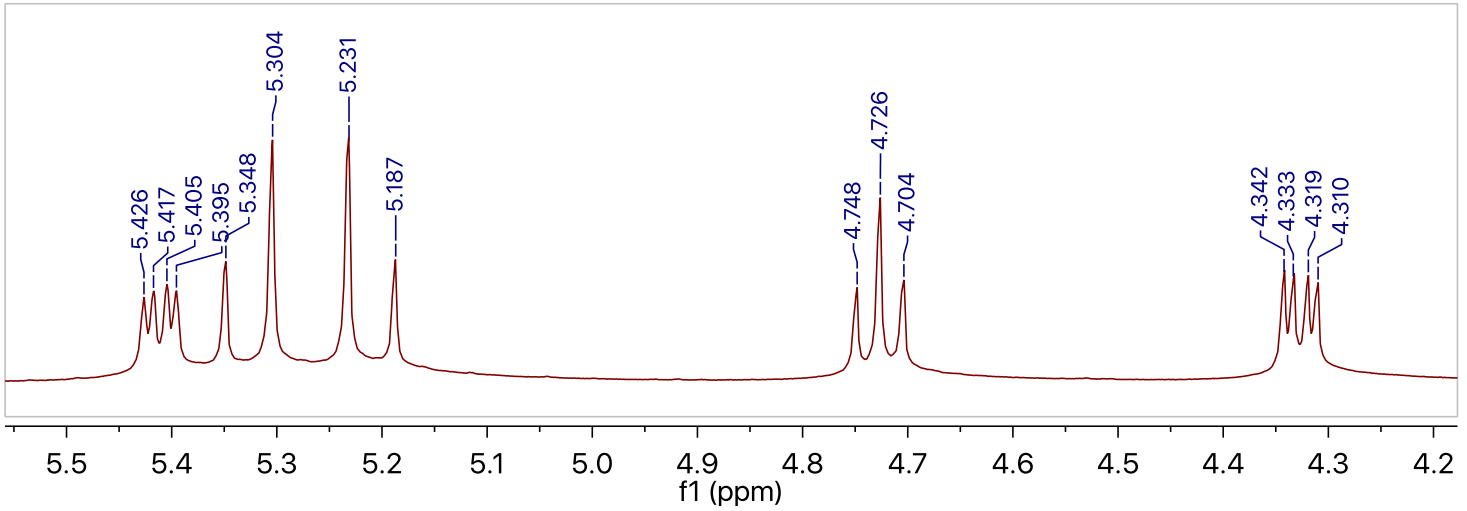
2

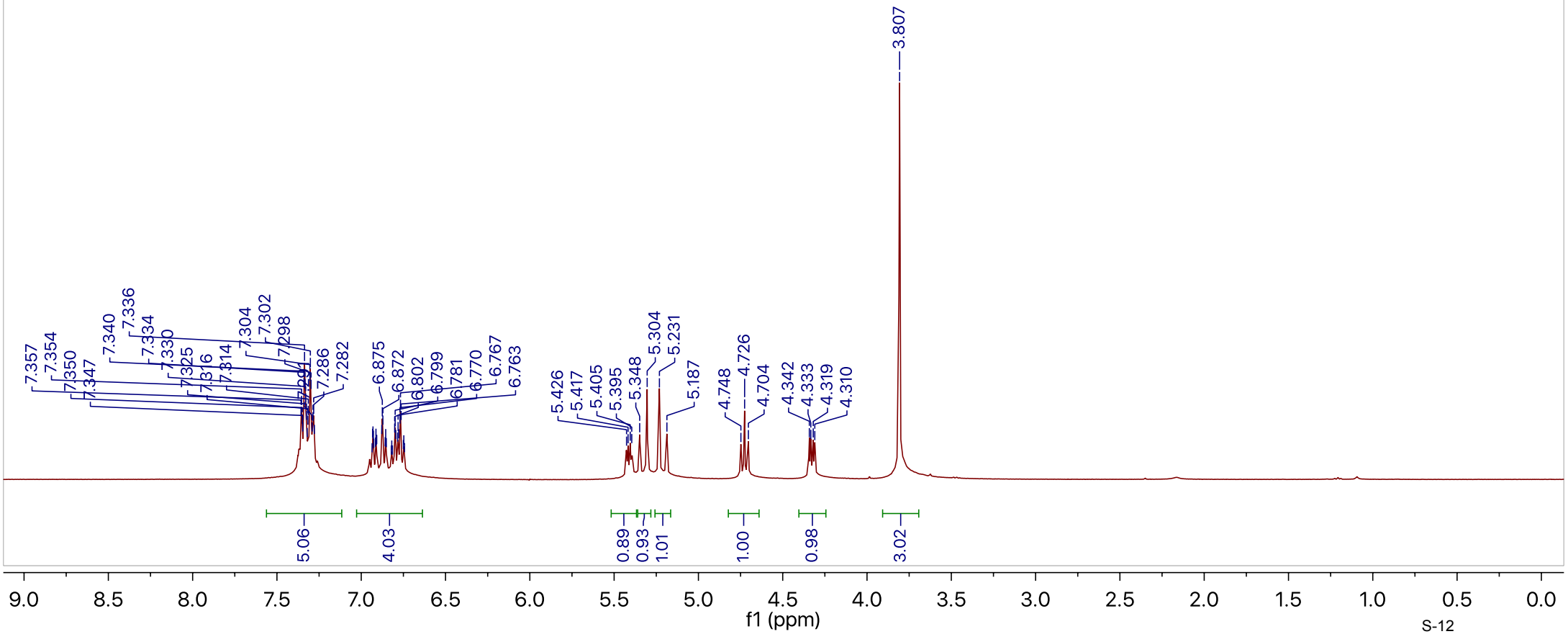


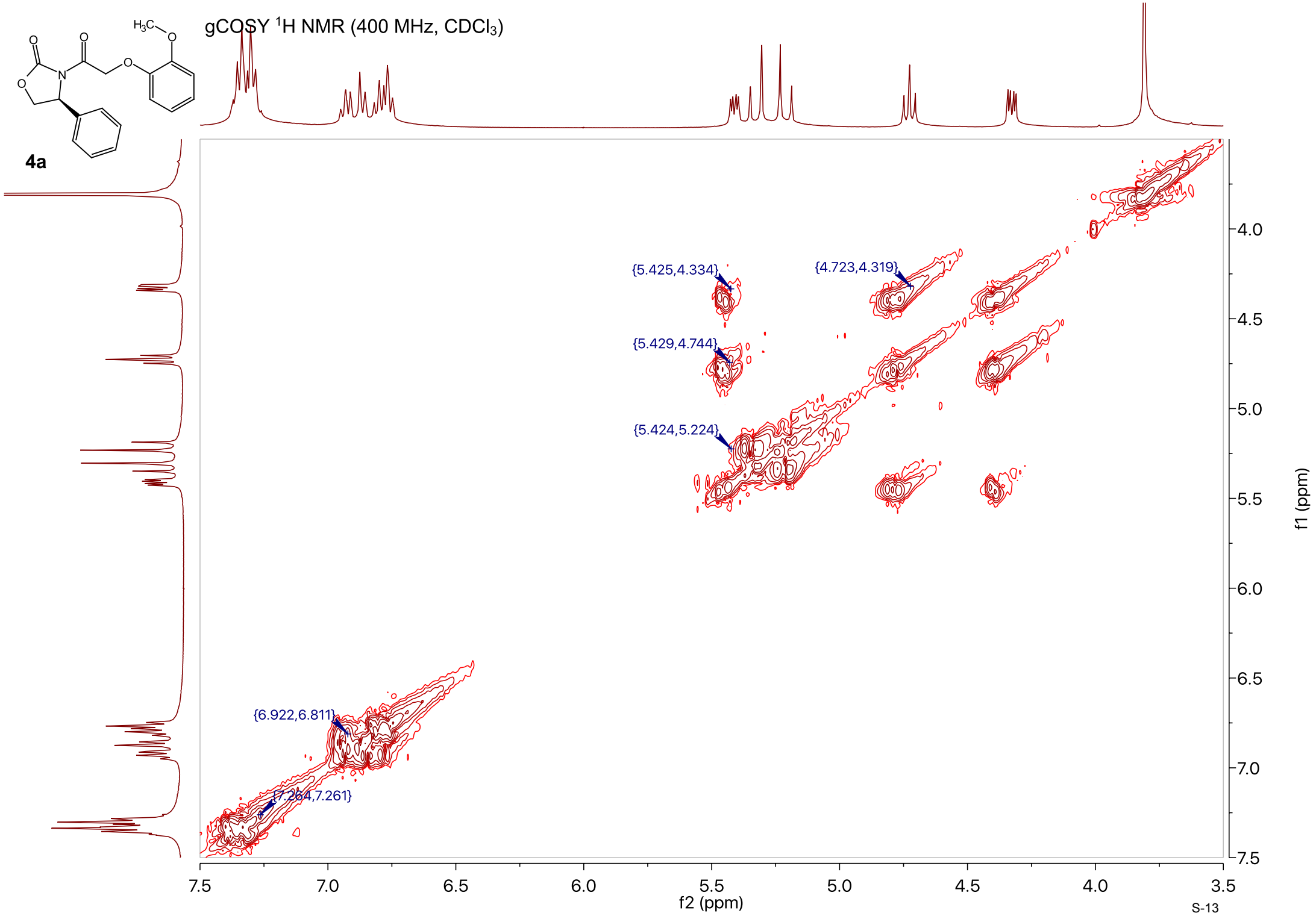
^{1}H NMR (400 MHz, $CDCl_3$)



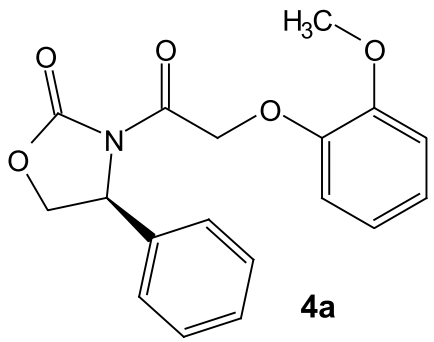
4a



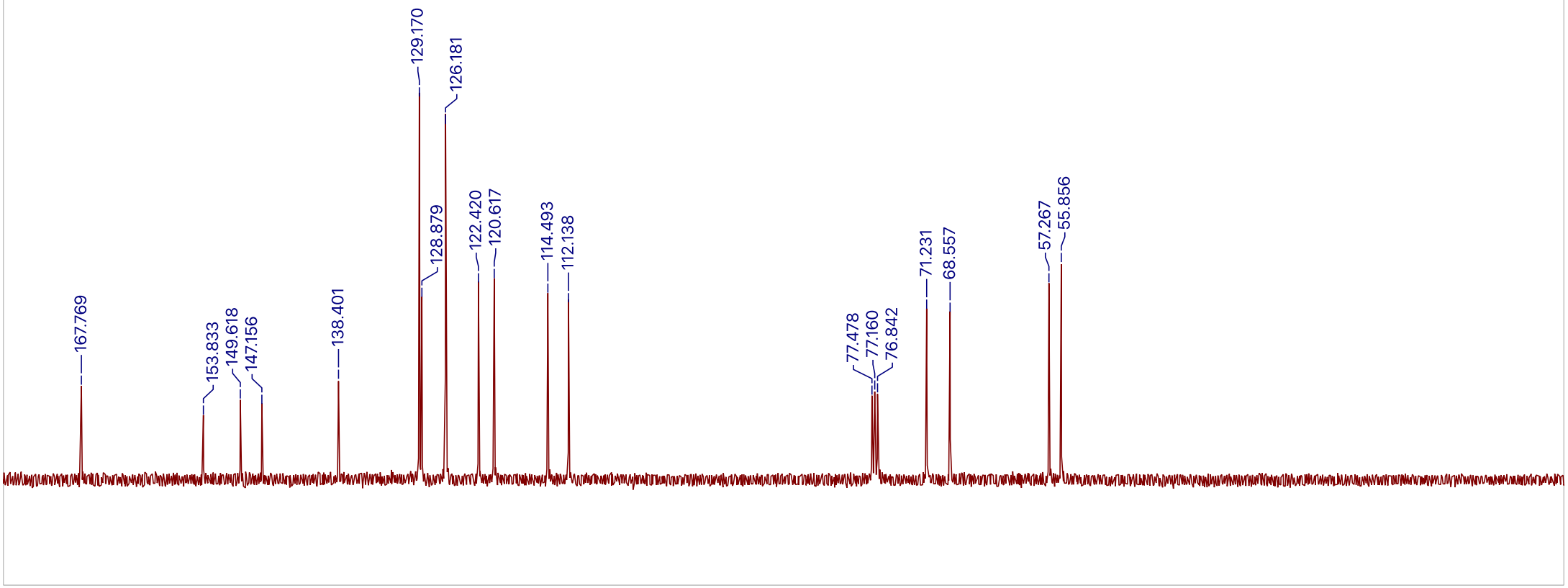


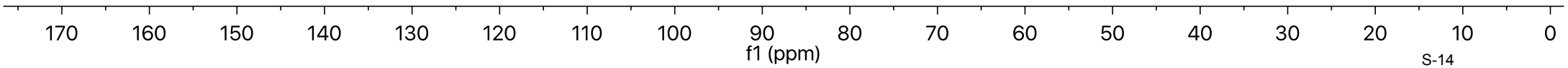

gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)
4a
{5.425,4.334}
{4.723,4.319}
{5.429,4.744}
{5.424,5.224}
{6.922,6.811}
{7.264,7.261}
f2 (ppm)
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5

^{13}C NMR (100.6 MHz, $CDCl_3$)

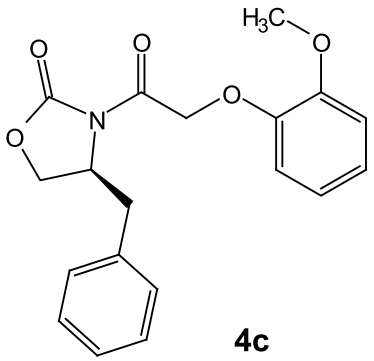


4a

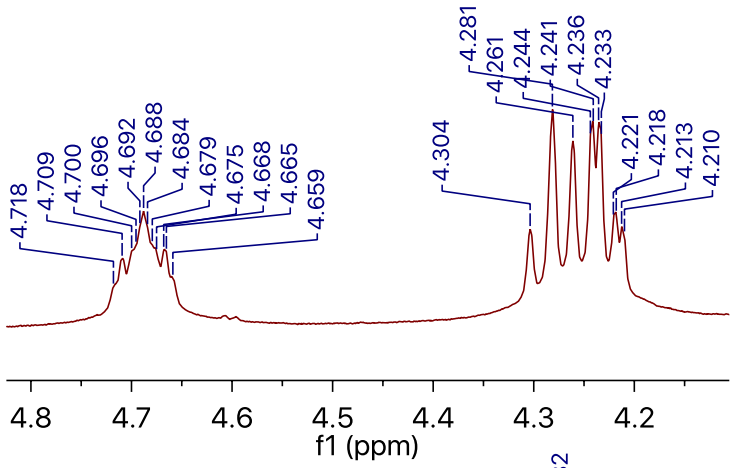


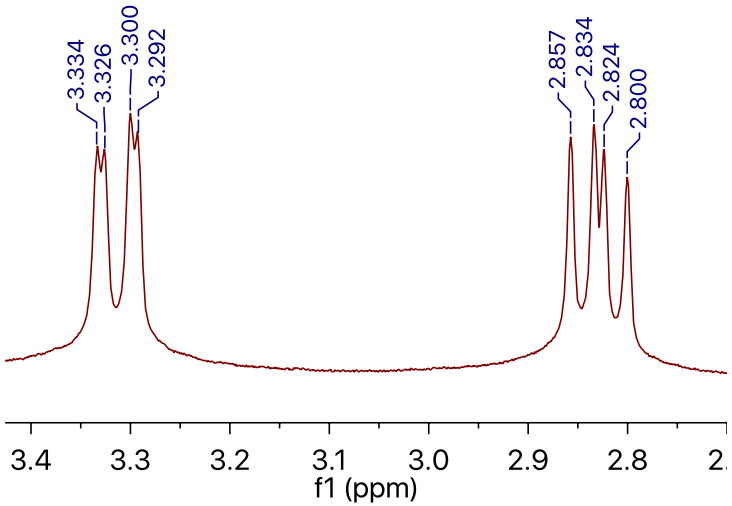


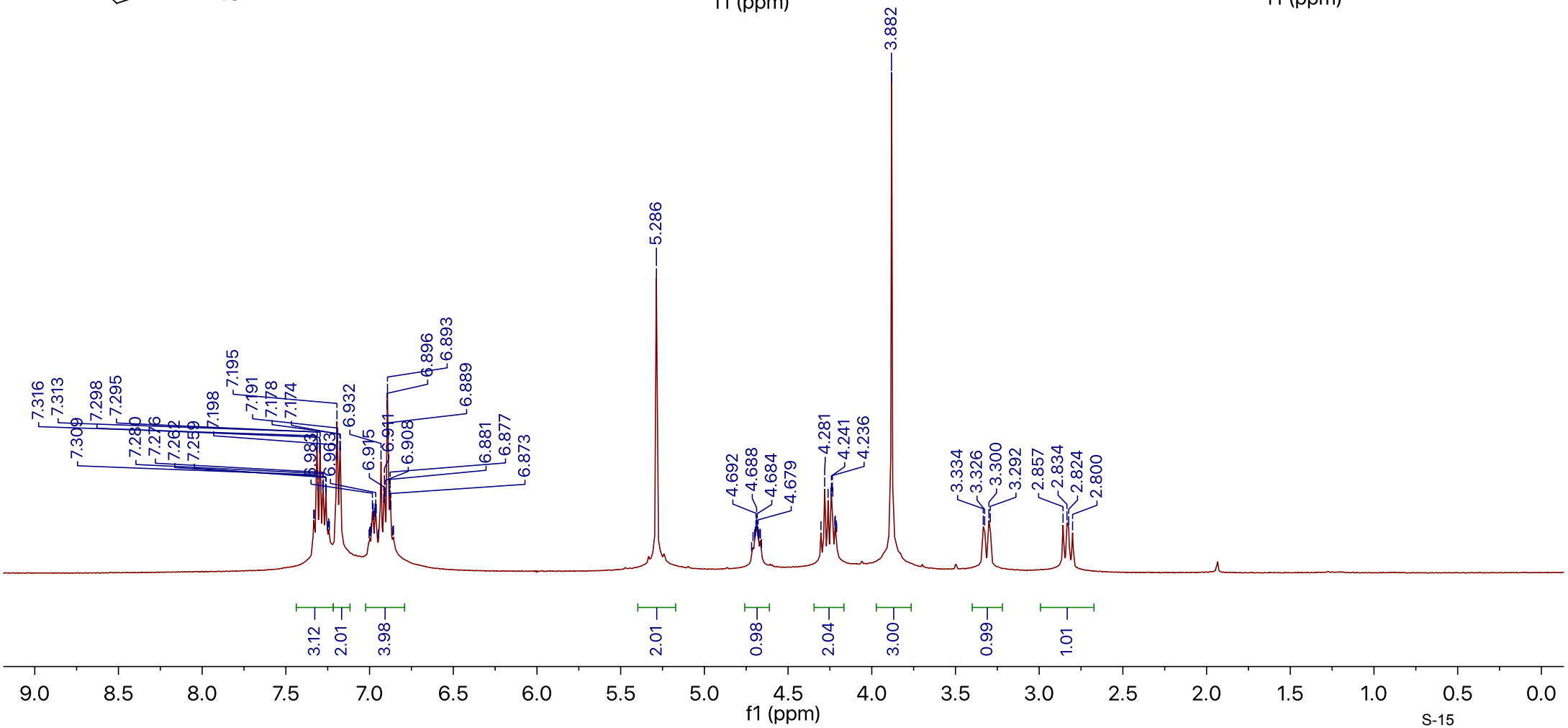
^{1}H NMR (400 MHz, $CDCl_3$)

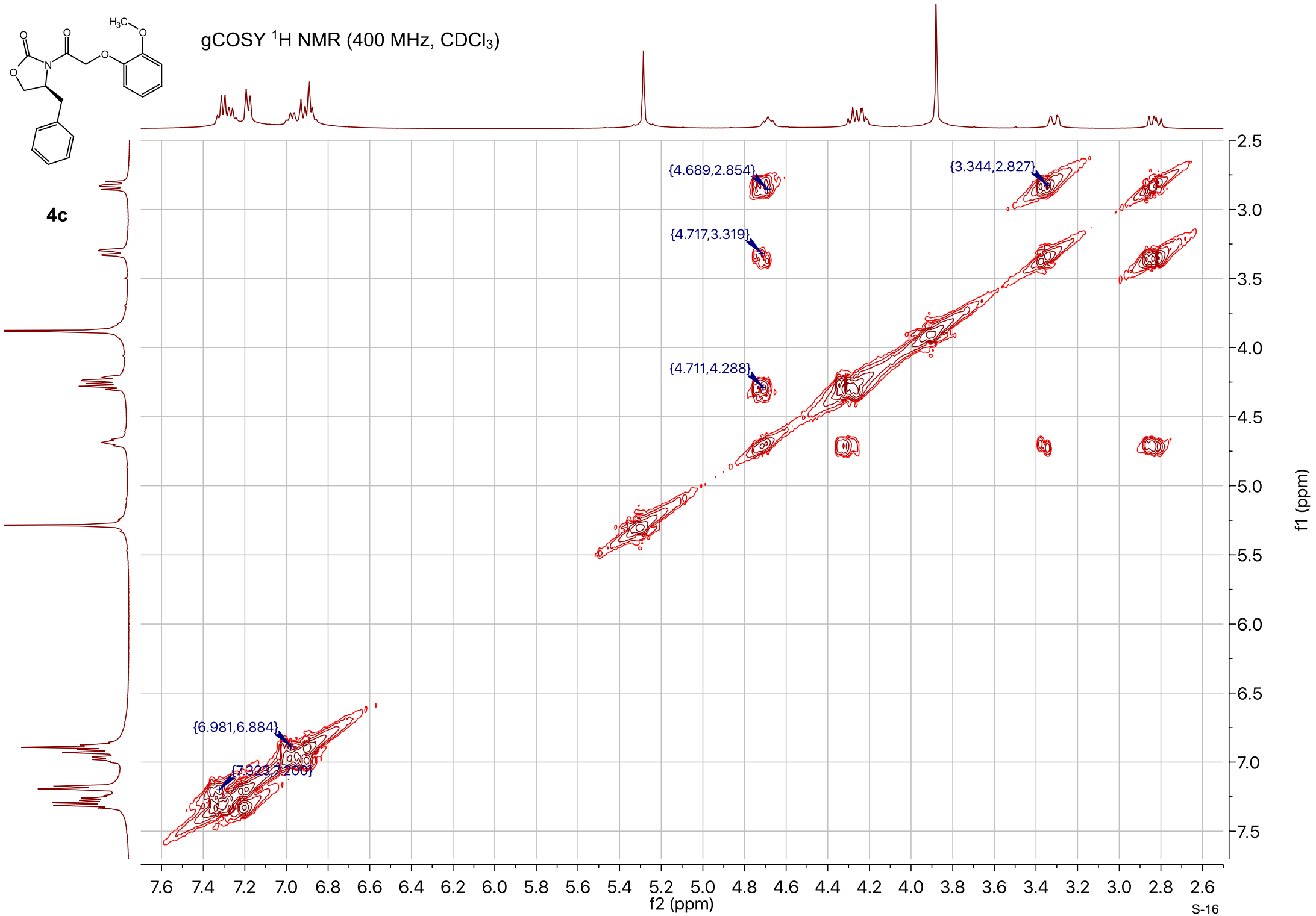


4c

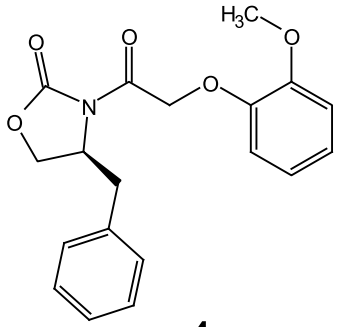




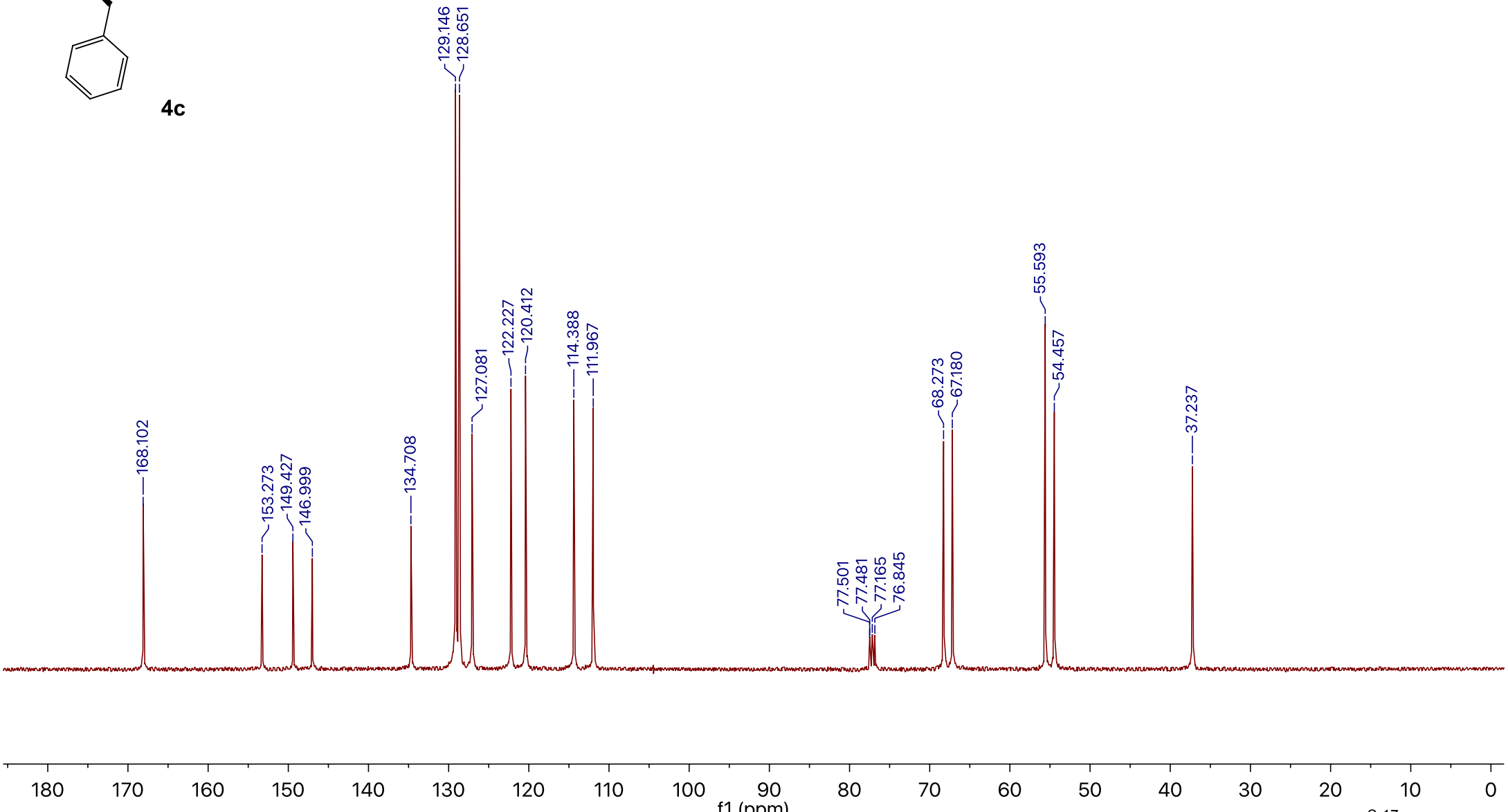



gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)
4c
{4.689,2.854}
{3.344,2.827}
{4.717,3.319}
{4.711,4.288}
{6.981,6.884}
{7.023,7.200}
f2 (ppm)
f1 (ppm)

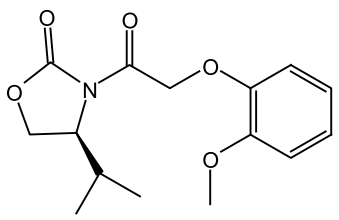
^{13}C NMR (100.6 MHz, $CDCl_3$)



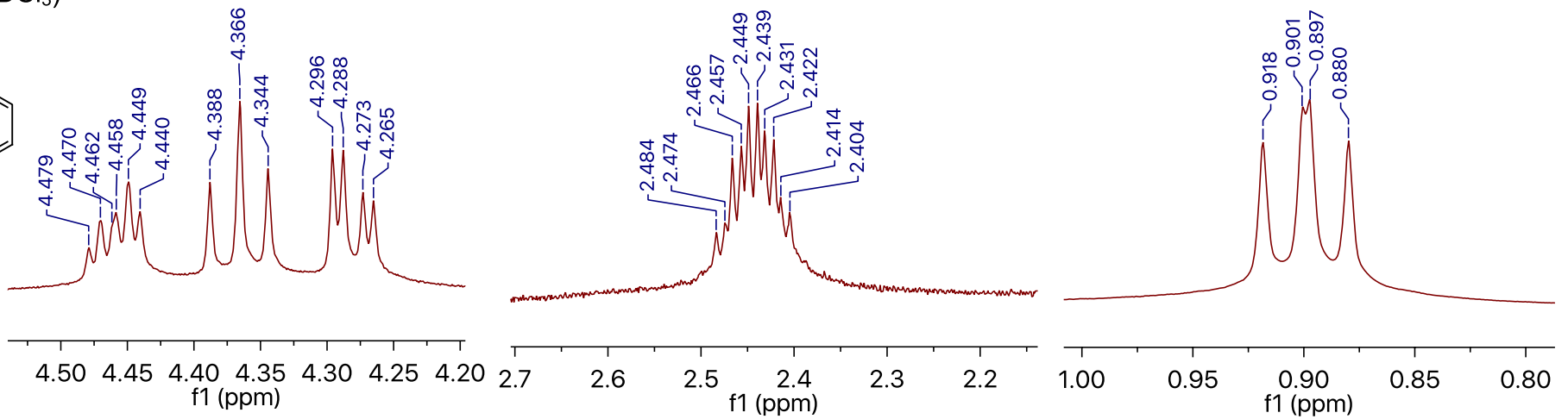
4c

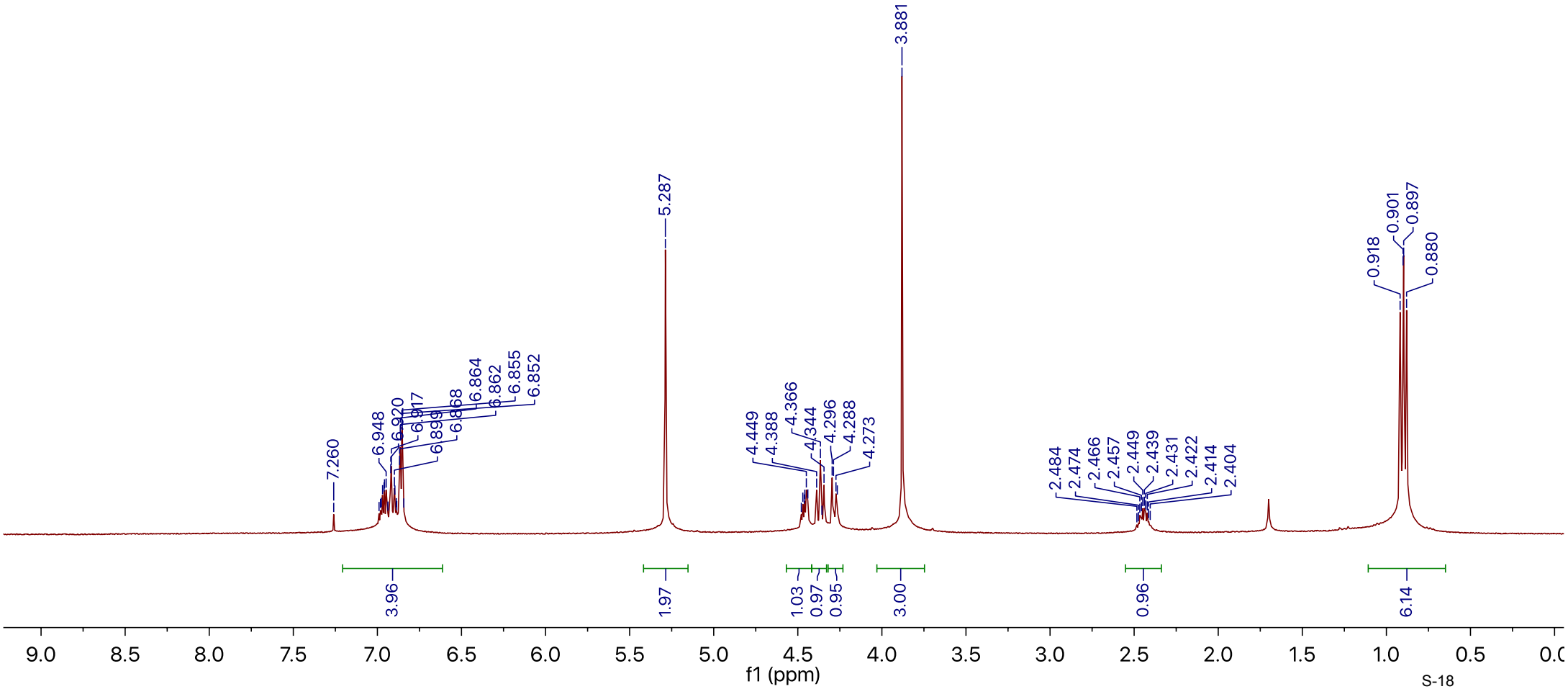


^{1}H NMR (400 MHz, $CDCl_3$)

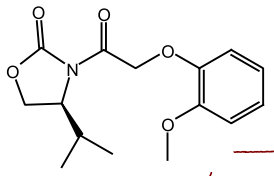


4e





gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)



4e

{0.884,2.443}

{4.363,4.466}

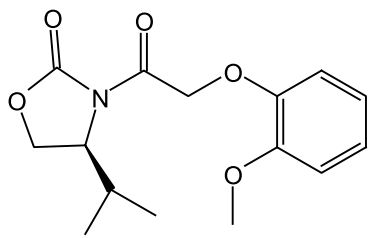
{4.276,4.359}

{2.448,4.453}

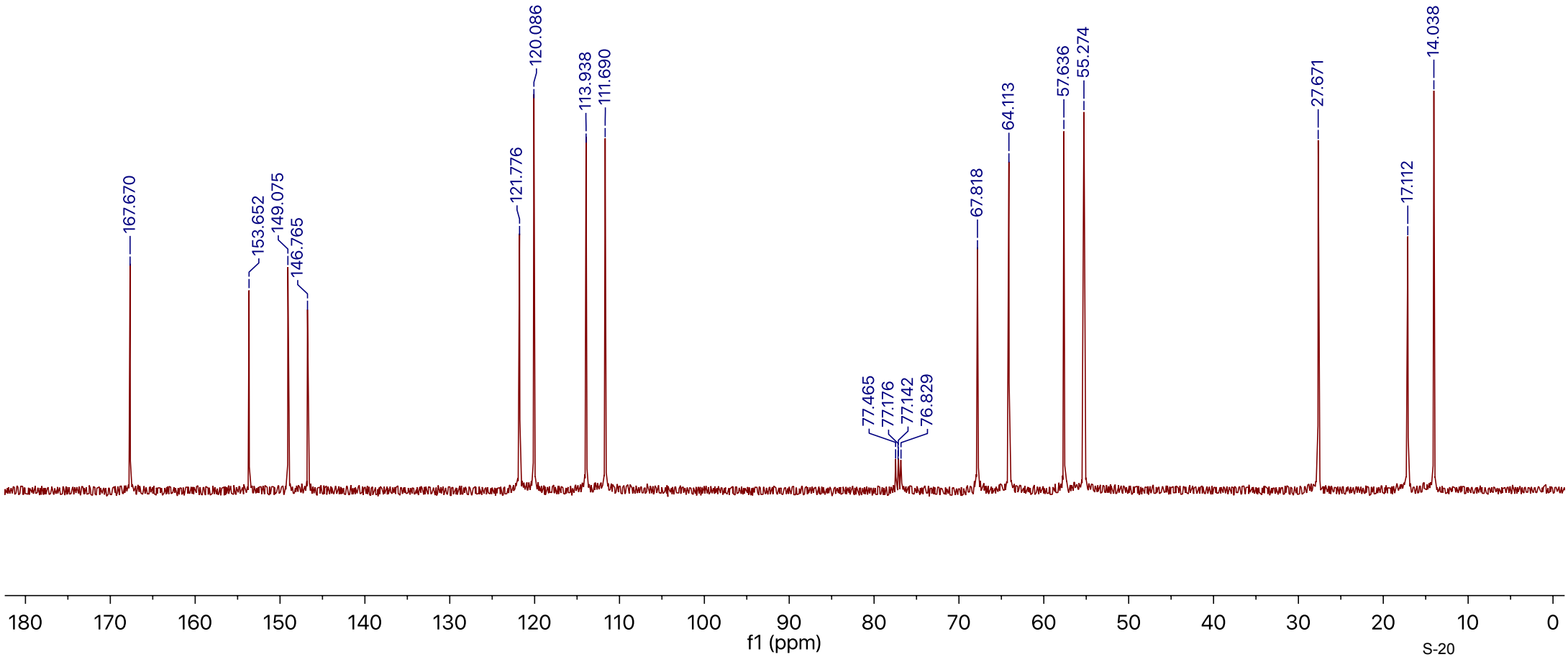
f2 (ppm)

f1 (ppm)

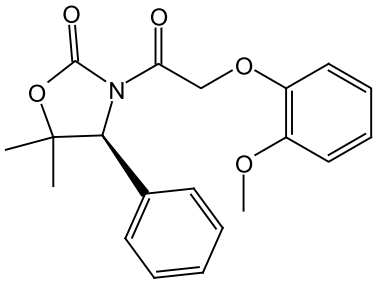
^{13}C NMR (100.6 MHz, $CDCl_3$)



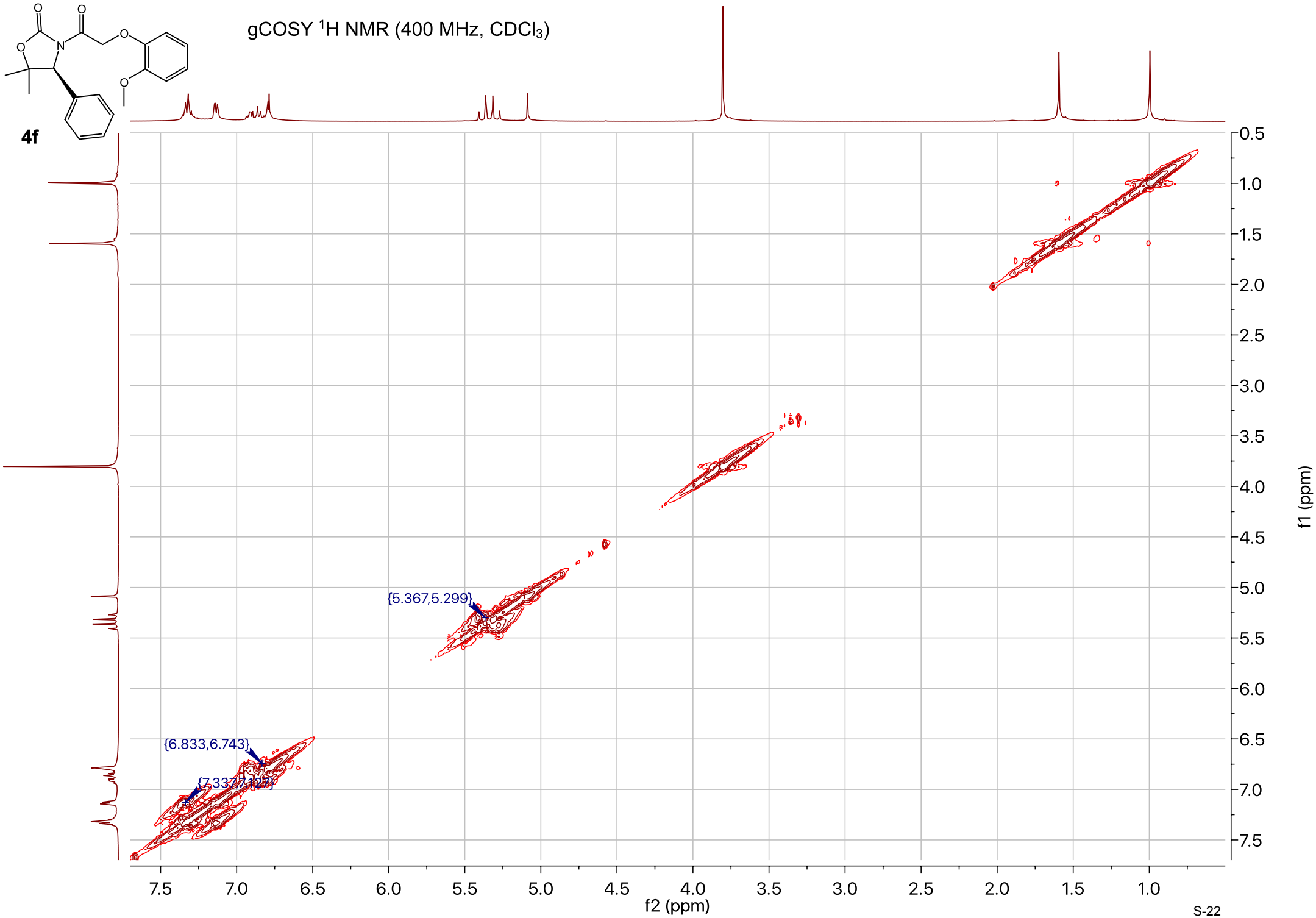
4e



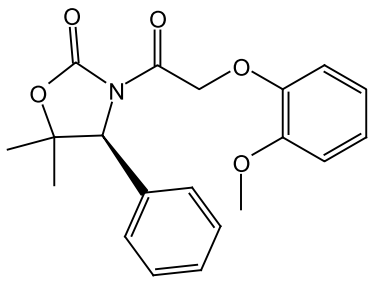
^{1}H NMR (400 MHz, $CDCl_3$)



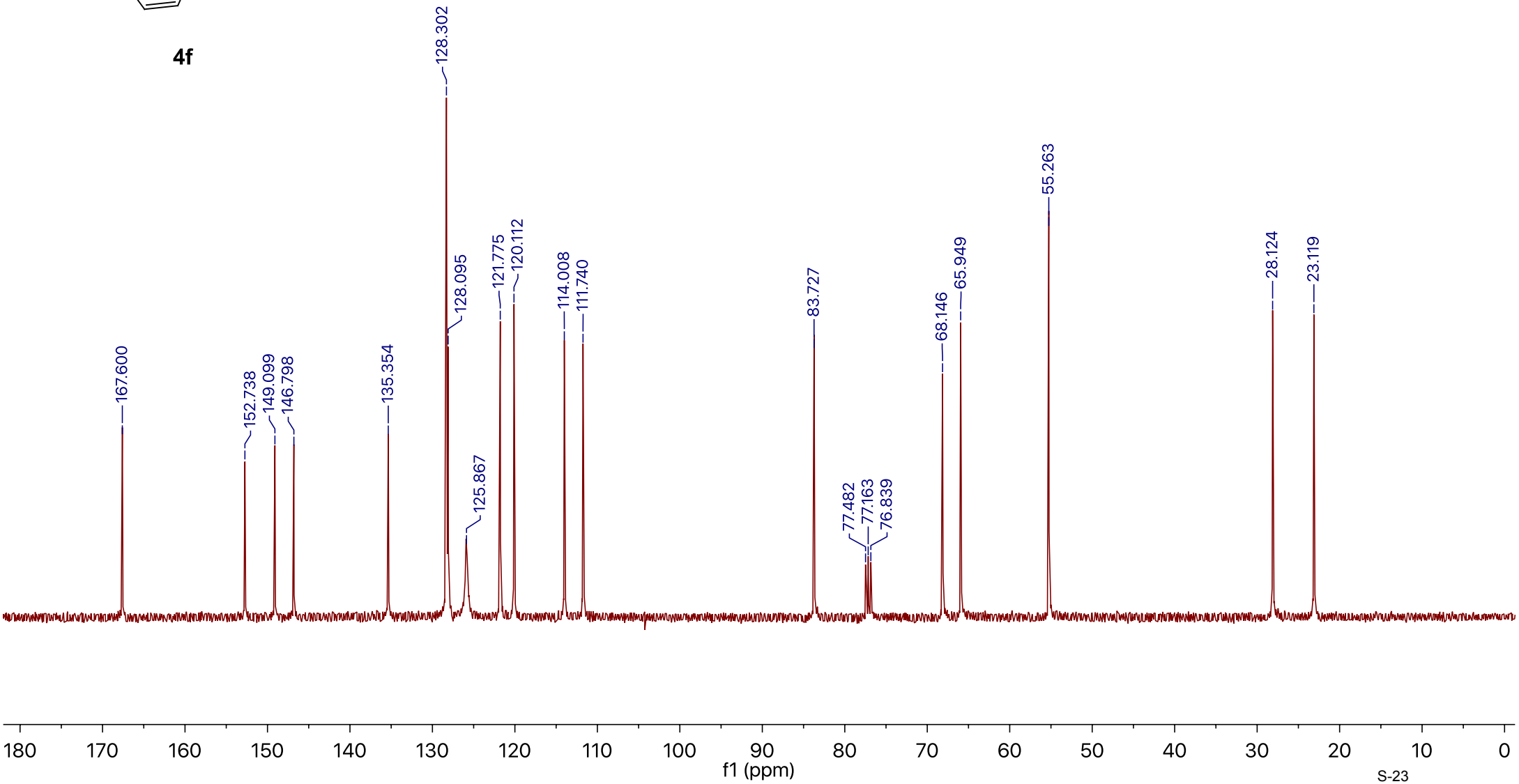
4f


gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)
4f
{5.367,5.299}
{6.833,6.743}
f2 (ppm)
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

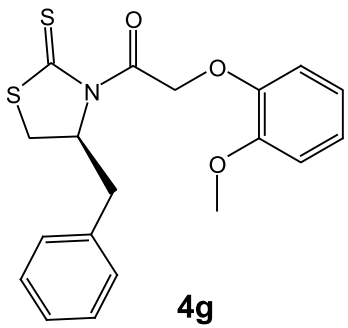
^{13}C NMR (100.6 MHz, $CDCl_3$)



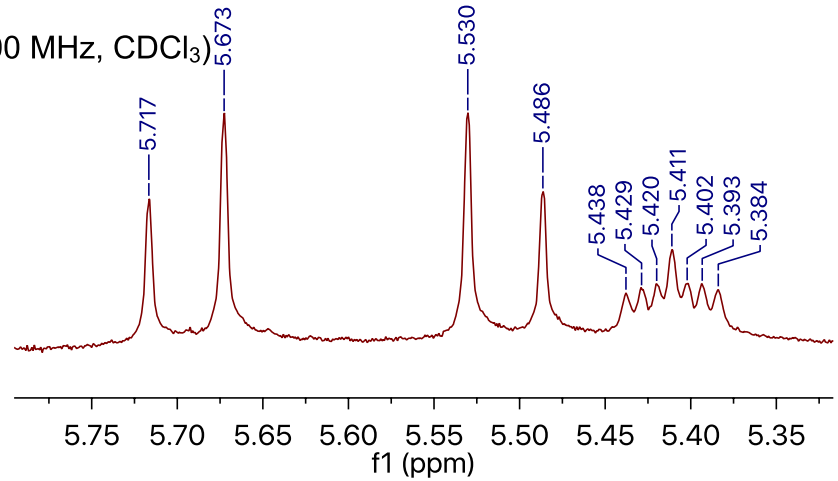
4f

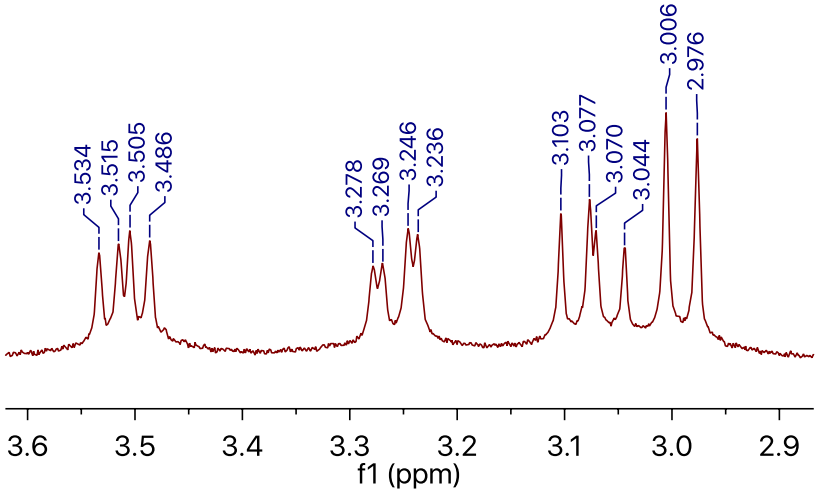


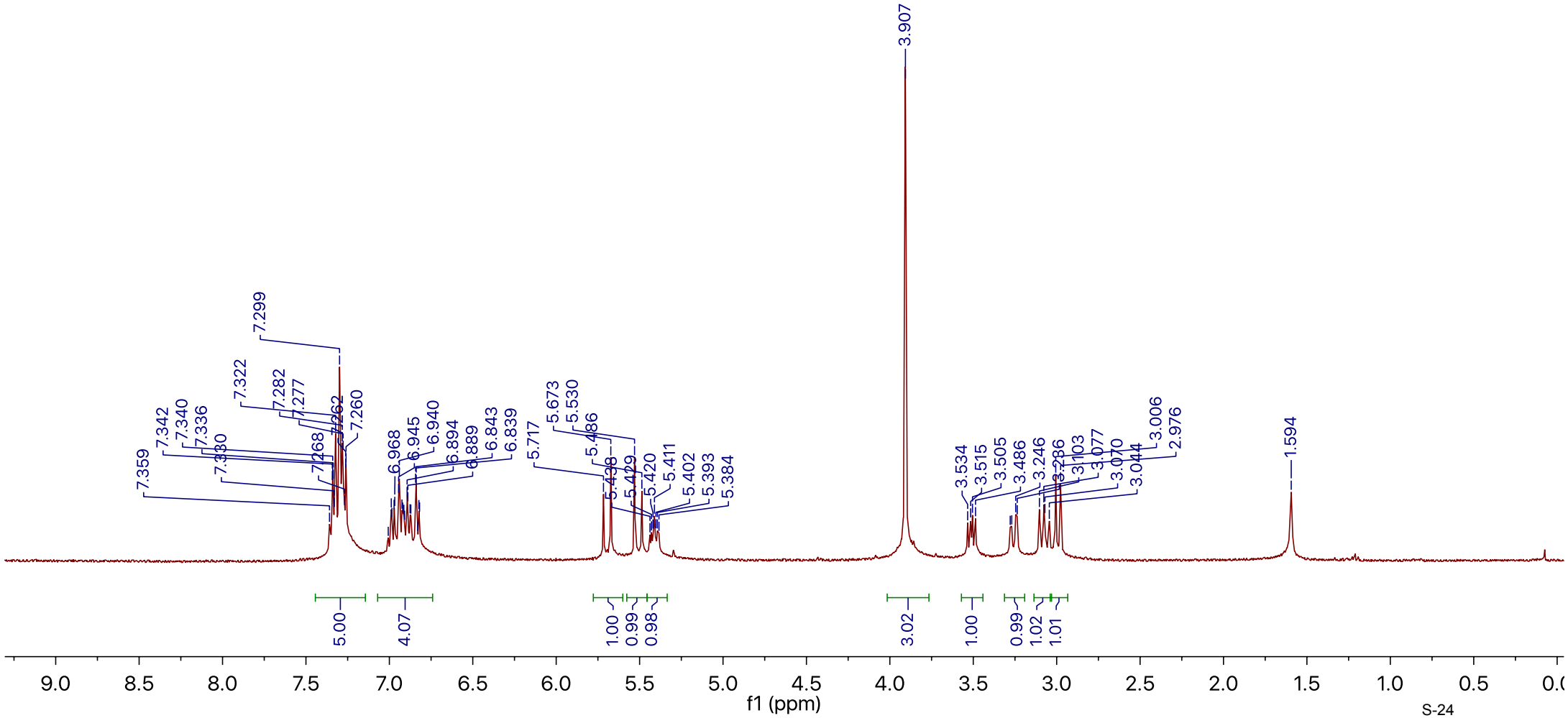
^{1}H NMR (400 MHz, $CDCl_3$)

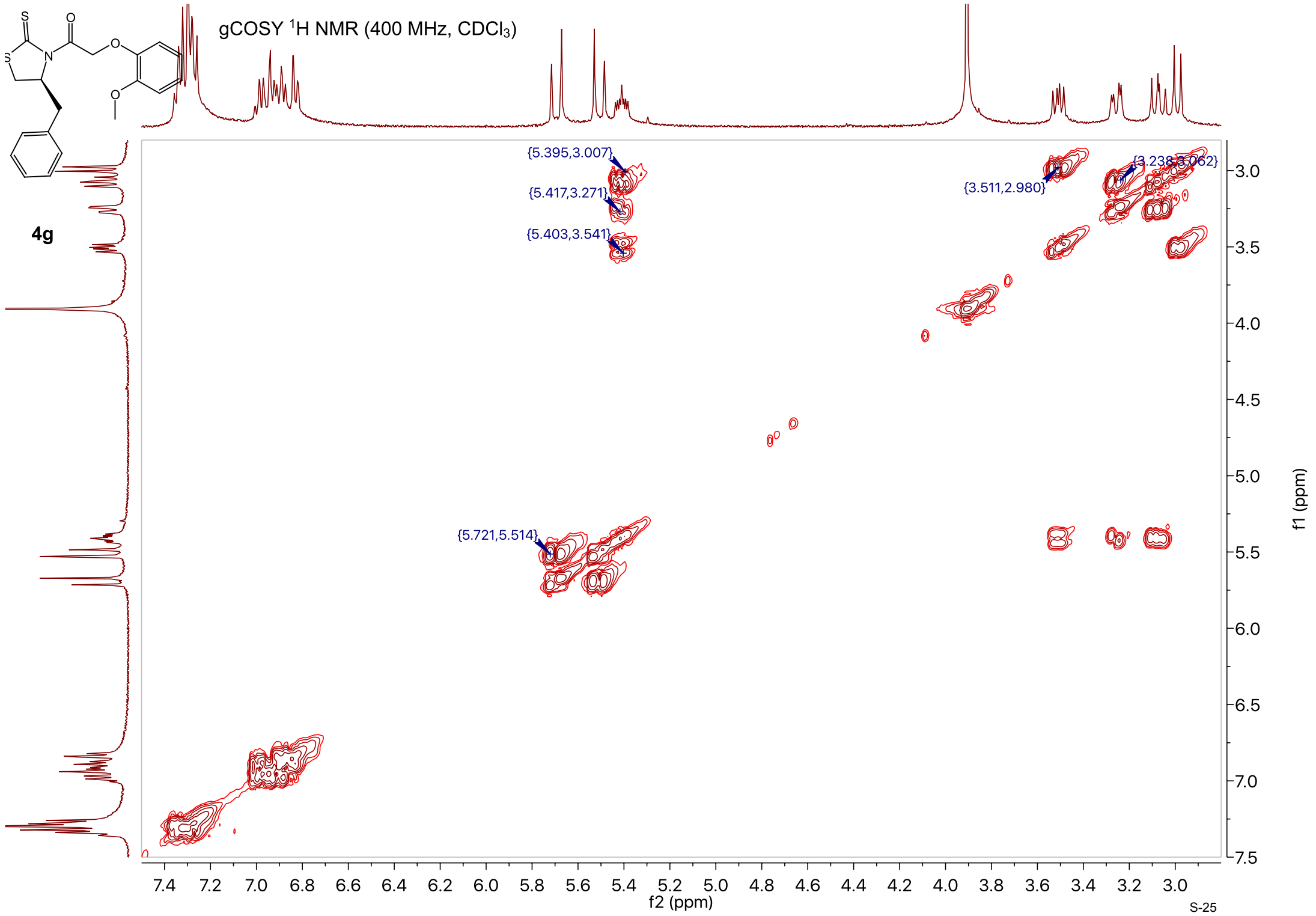


4g

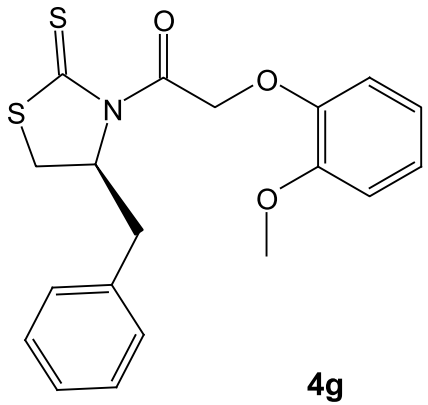




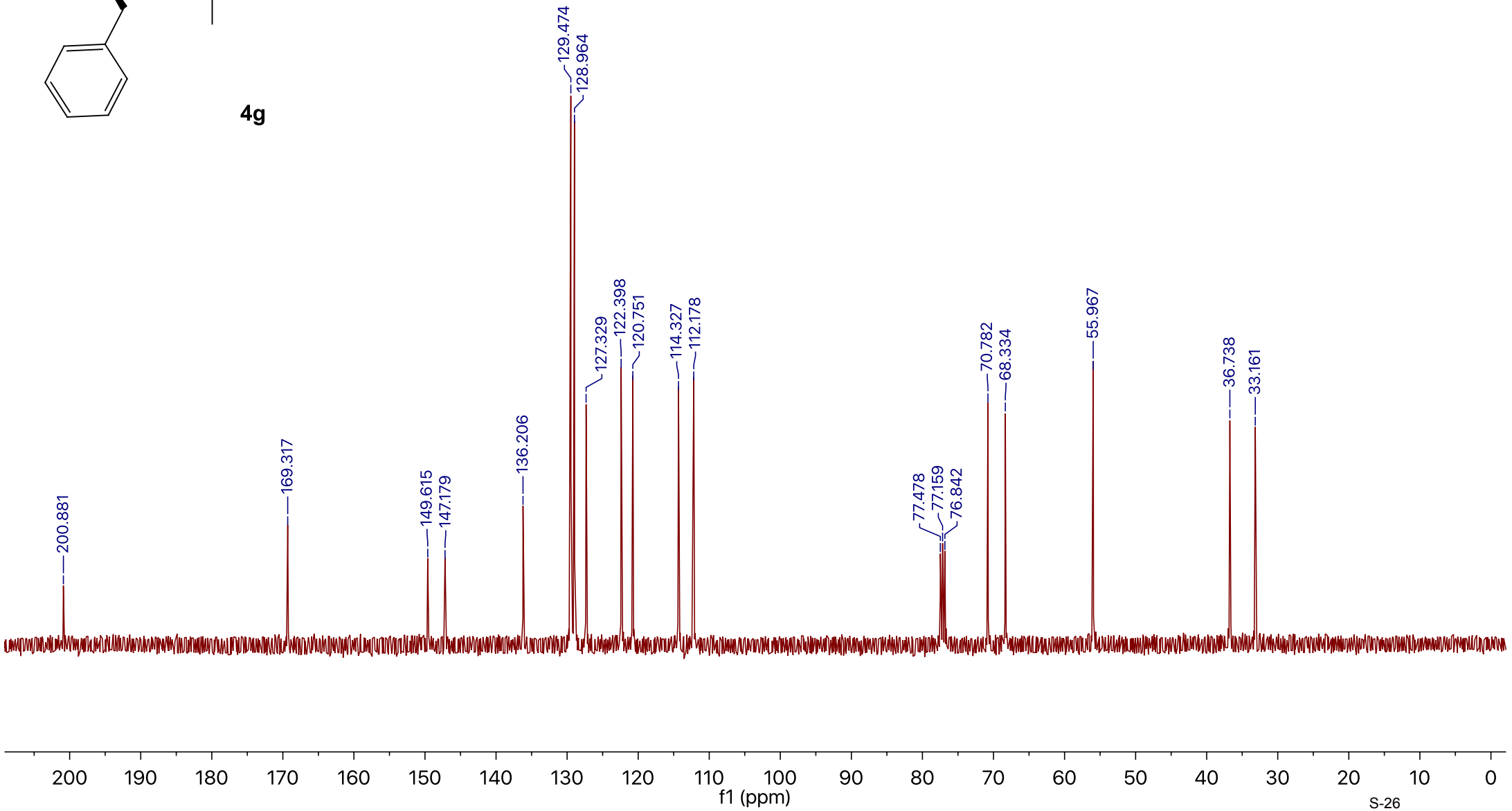



gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)
4g
{5.395,3.007}
{5.417,3.271}
{5.403,3.541}
{5.721,5.514}
{3.511,2.980}
{3.238,3.062}
f2 (ppm)
f1 (ppm)
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0
3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5

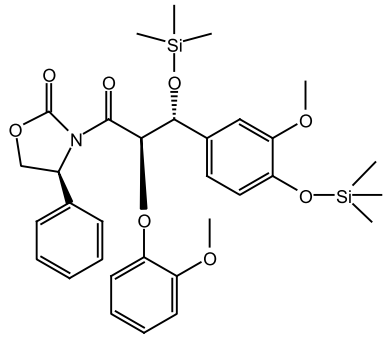
^{13}C NMR (100.6 MHz, $CDCl_3$)

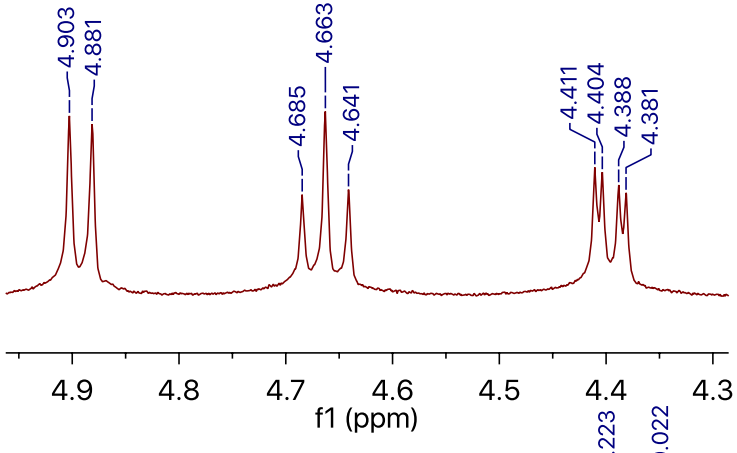


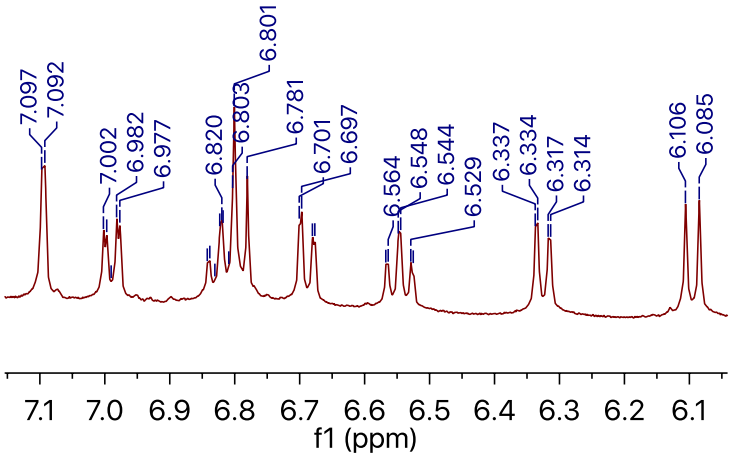
4g

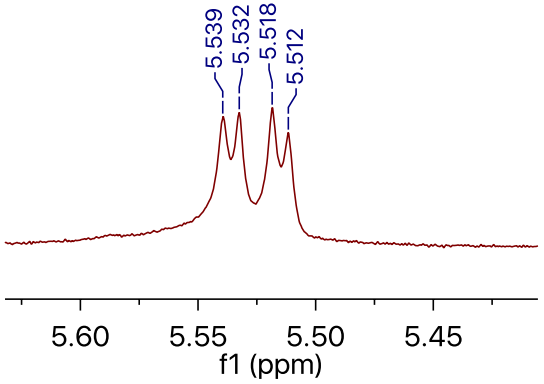


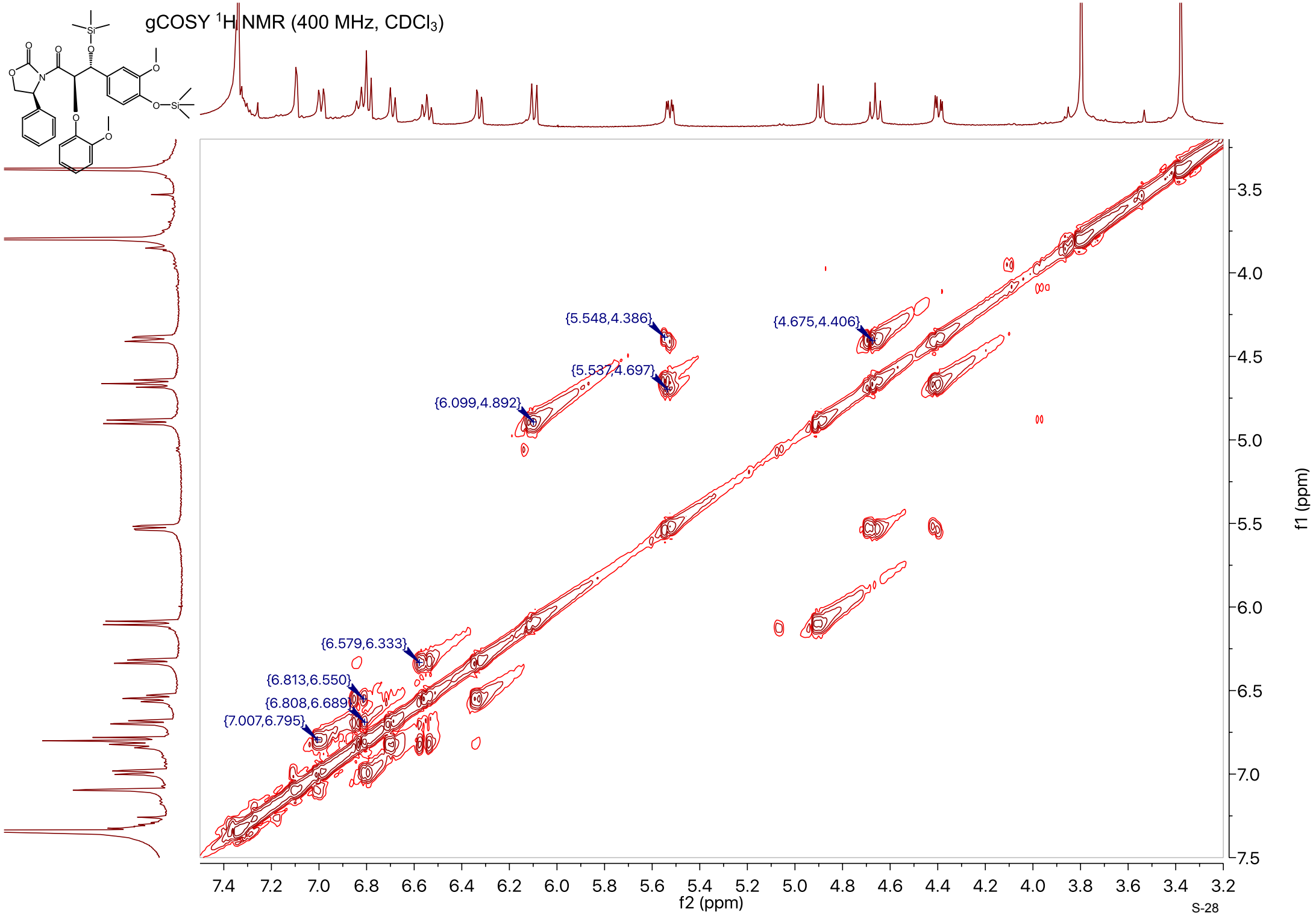
^{1}H NMR (400 MHz, $CDCl_3$)



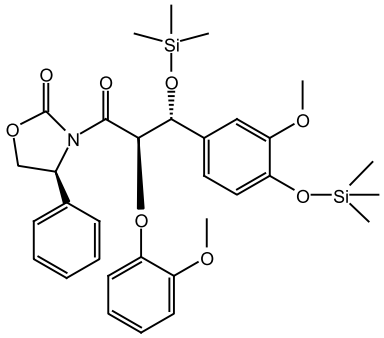


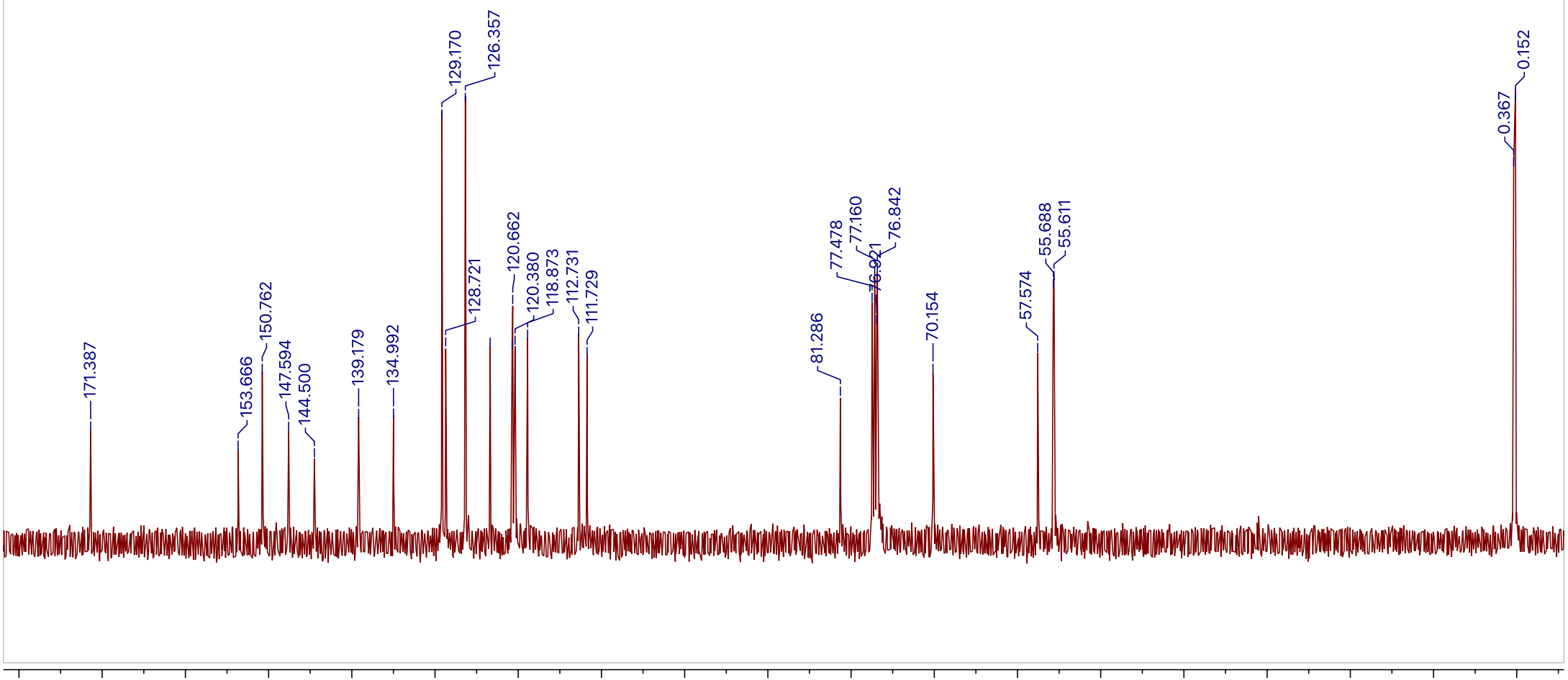


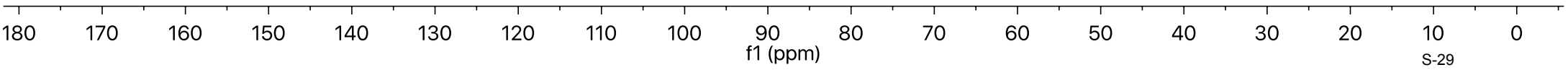



gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)
{5.548,4.386}
{4.675,4.406}
{5.537,4.697}
{6.099,4.892}
{6.579,6.333}
{6.813,6.550}
{6.808,6.689}
{7.007,6.795}
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2
f2 (ppm)
3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5
f1 (ppm)

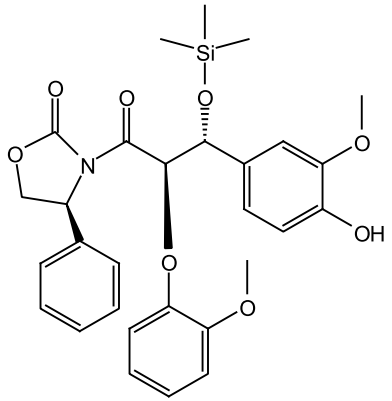
^{13}C NMR (100.6 MHz, $CDCl_3$)



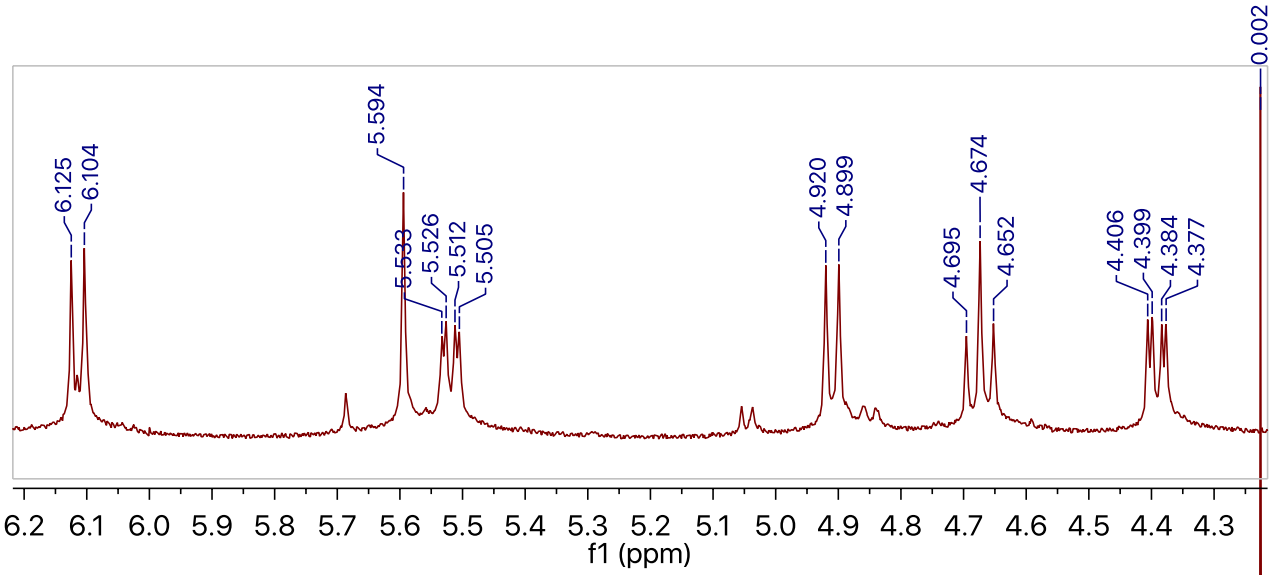


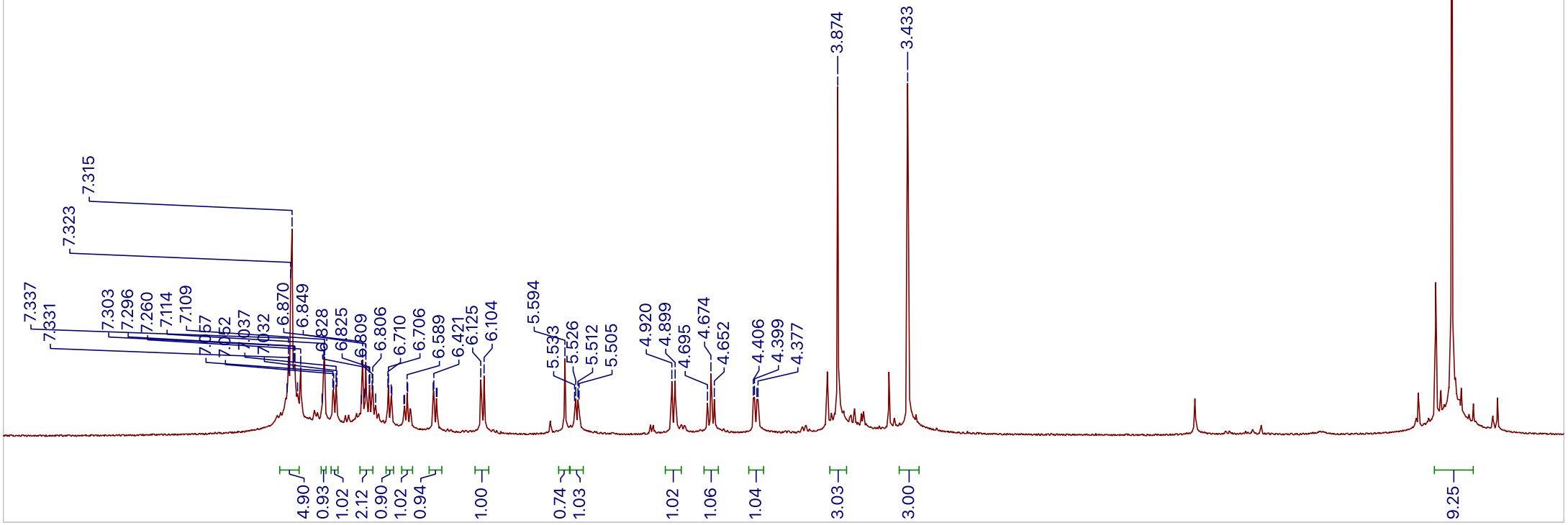


^{1}H NMR (400 MHz, $CDCl_3$)

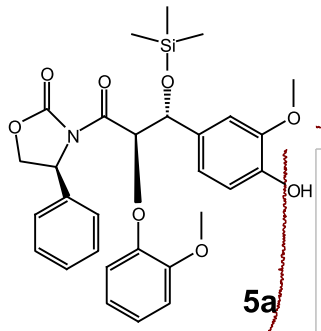


5a





gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)

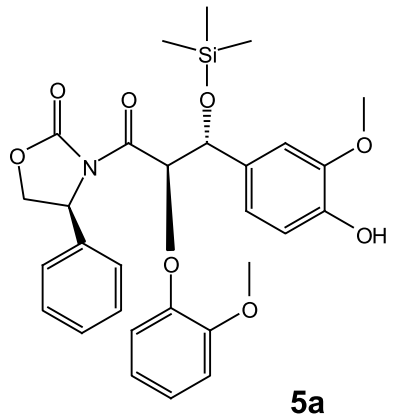


5a

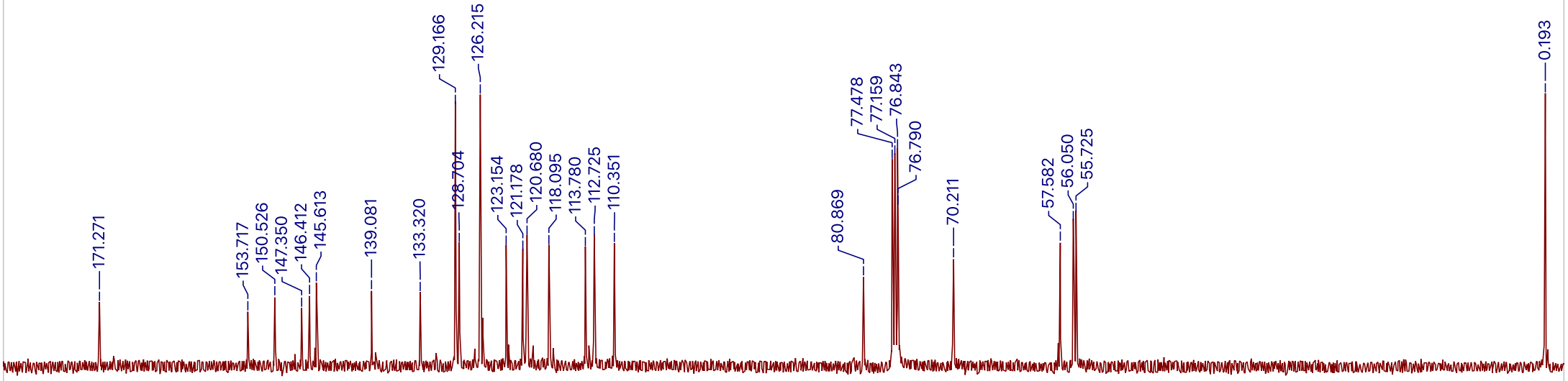
{5.531,4.401}
{5.505,4.693}
{6.114,4.911}
{4.698,4.403}

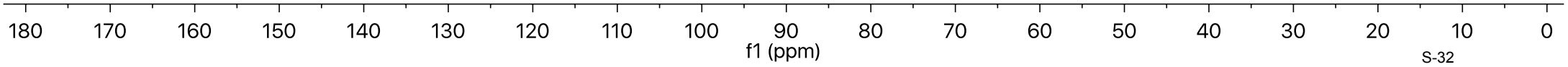
f2 (ppm)
f1 (ppm)

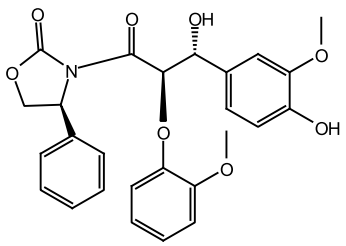
^{13}C NMR (100.6 MHz, $CDCl_3$)



5a

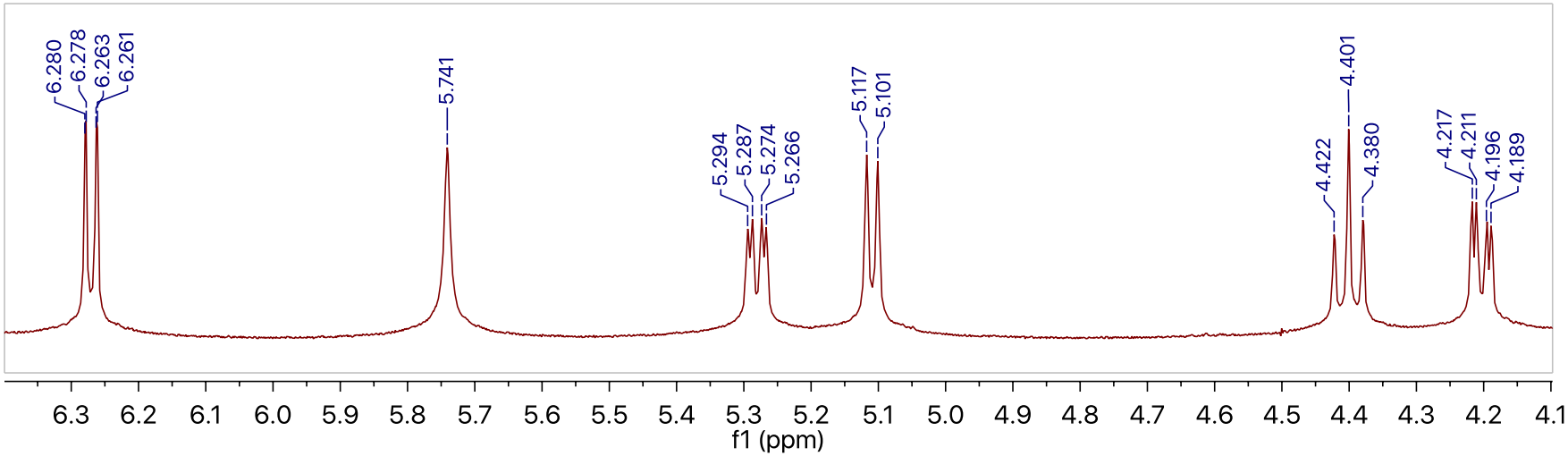


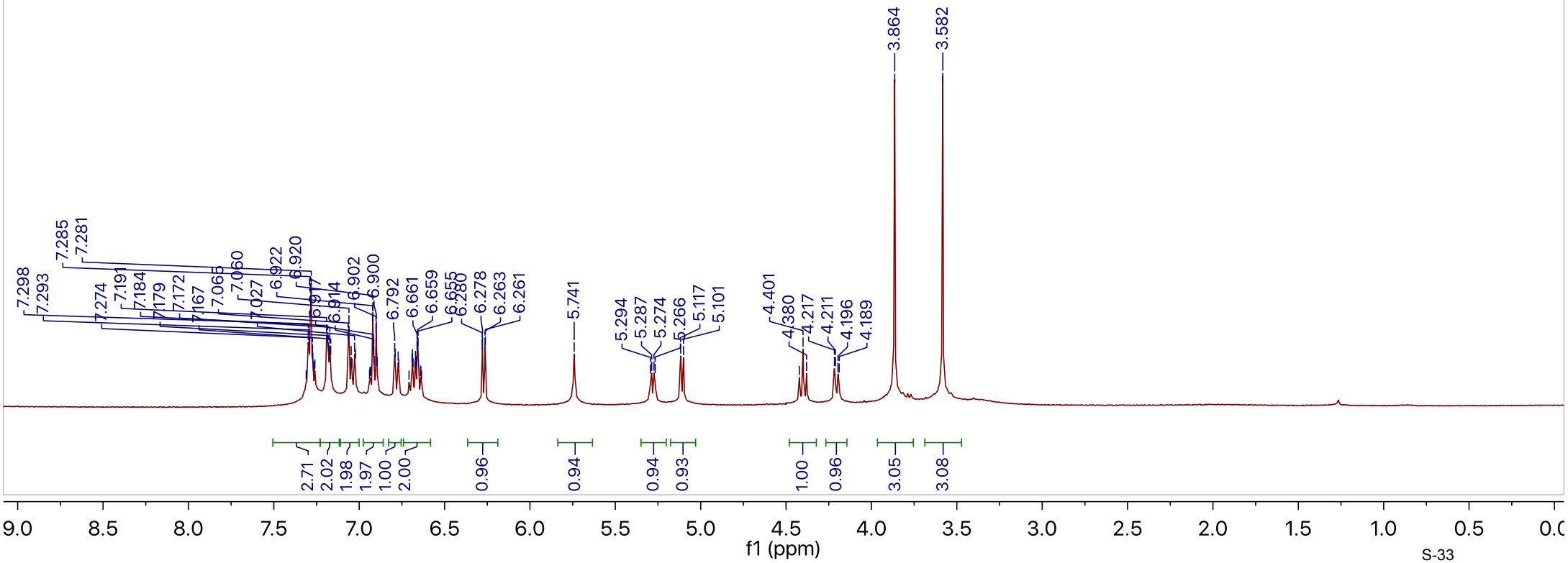


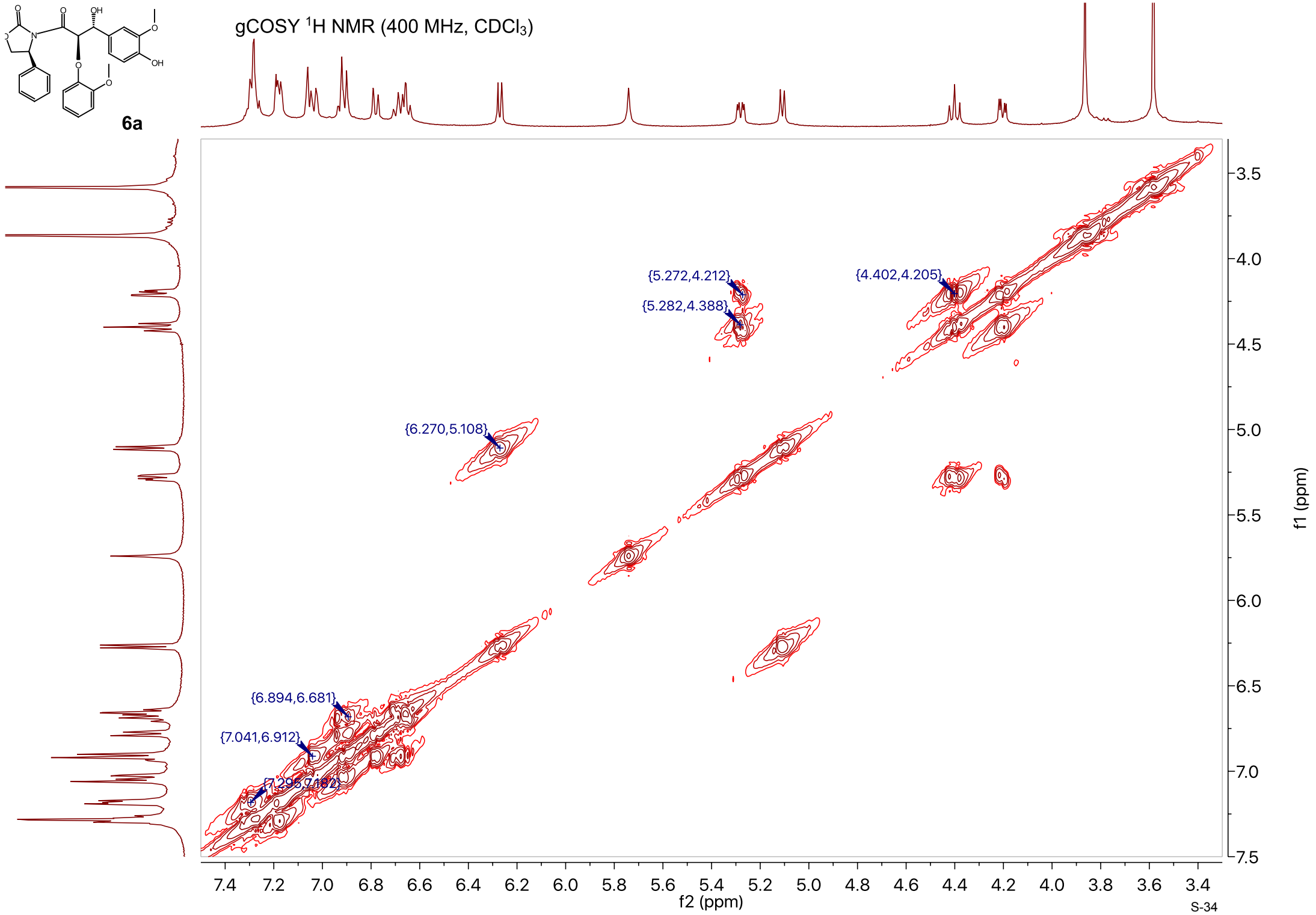


6a

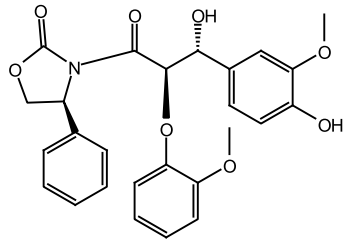
^{1}H NMR (400 MHz, $CDCl_3$)



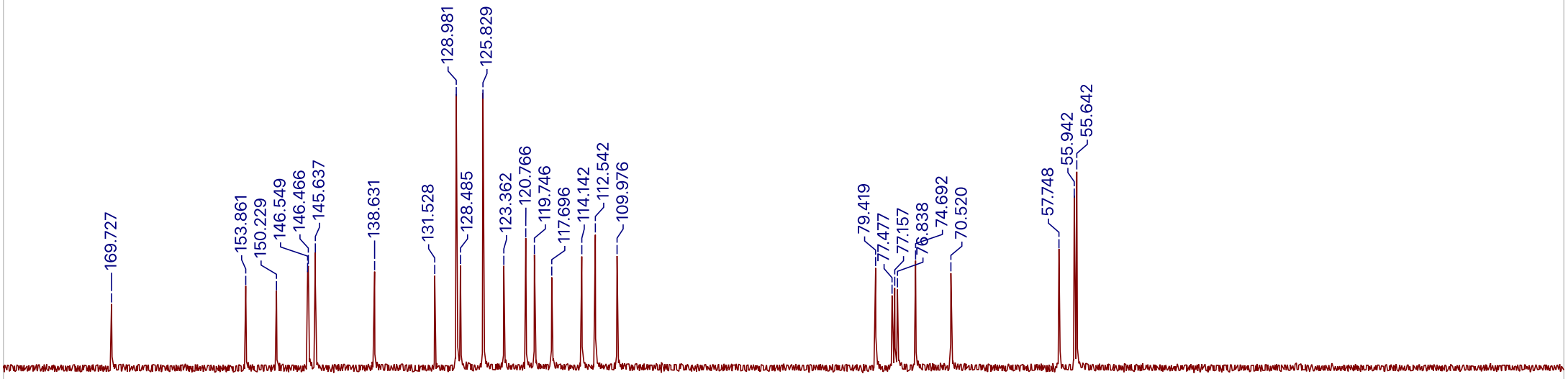


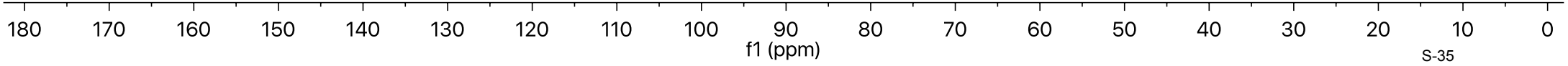

6a
gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)
{5.272,4.212}
{5.282,4.388}
{4.402,4.205}
{6.270,5.108}
{6.894,6.681}
{7.041,6.912}
{7.295,7.182}
f2 (ppm)
f1 (ppm)
S-34

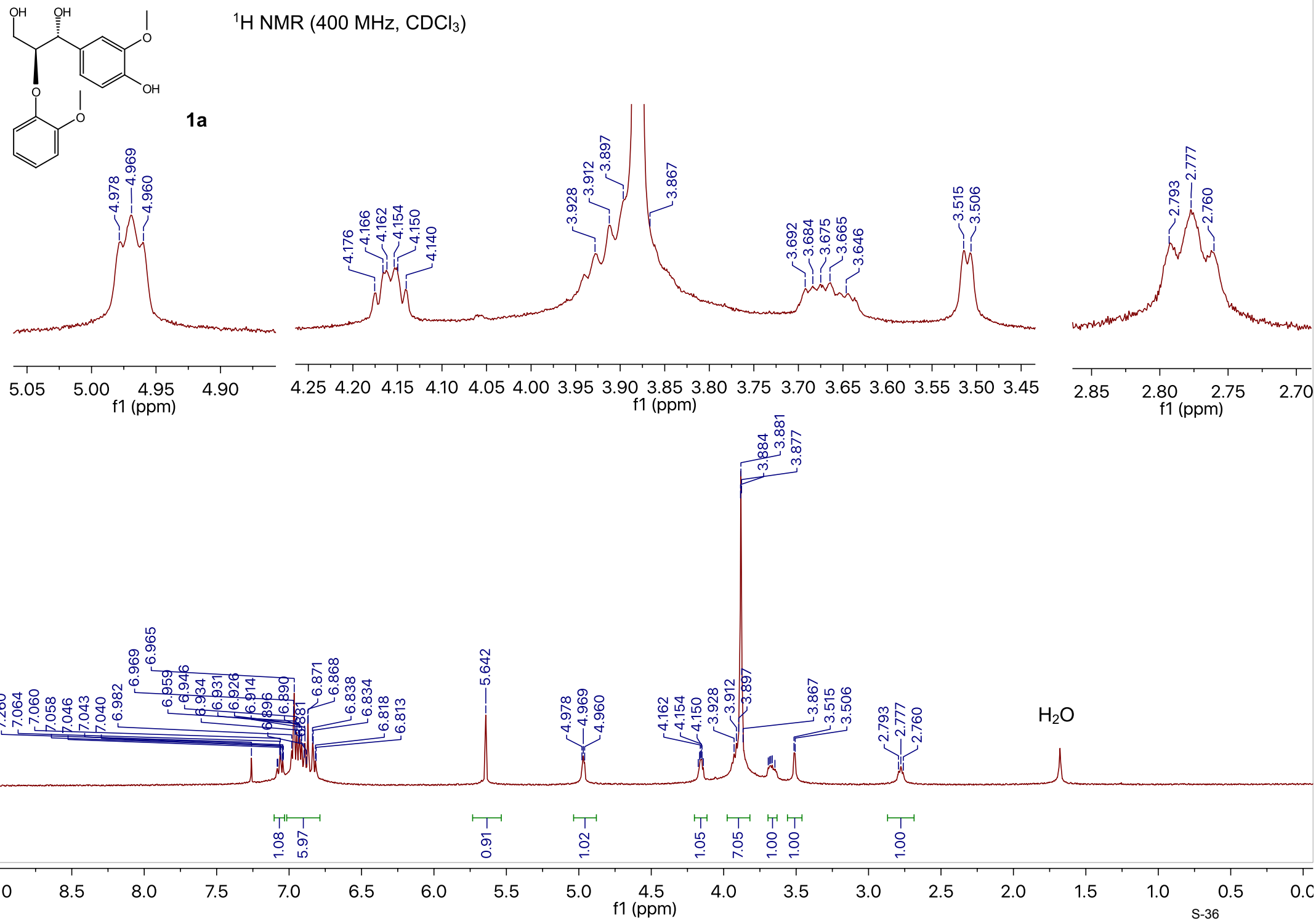
^{13}C NMR (100.6 MHz, $CDCl_3$)

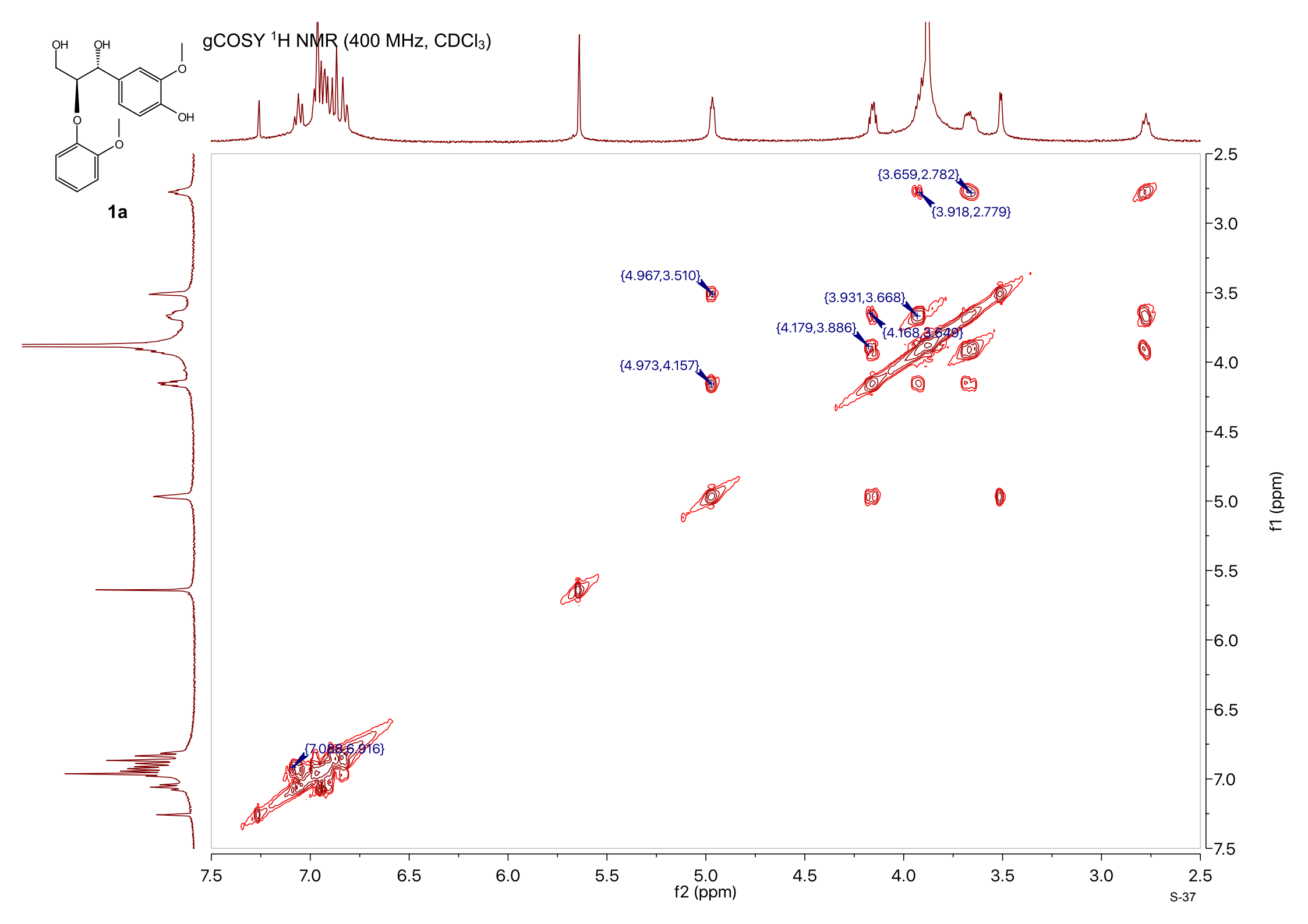


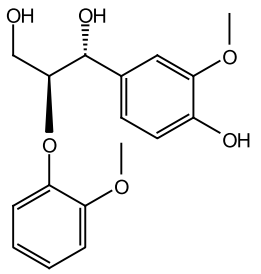
6a





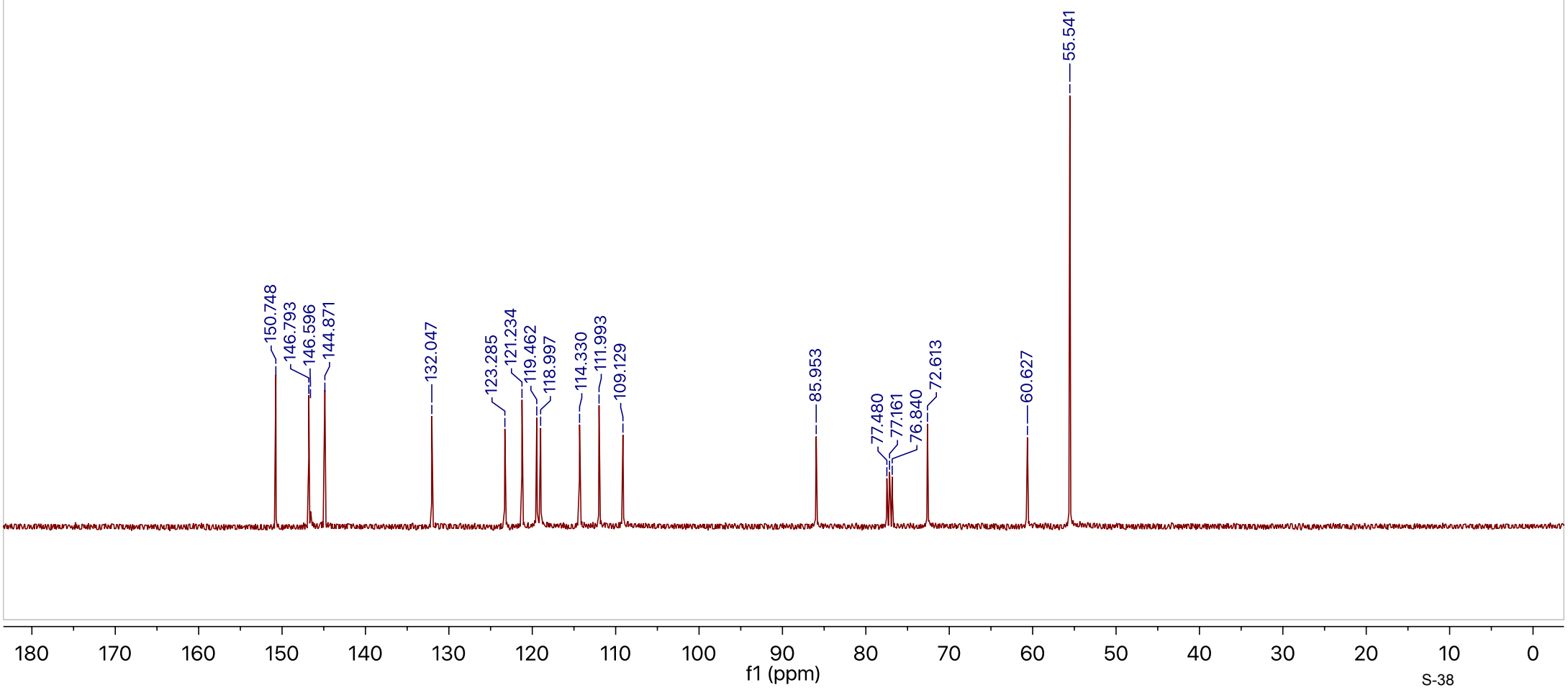

1a
^{1}H NMR (400 MHz, $CDCl_3$)
4.978
4.969
4.960
4.176
4.166
4.162
4.154
4.150
4.140
3.928
3.912
3.897
3.867
3.692
3.684
3.675
3.665
3.646
3.515
3.506
2.793
2.777
2.760
f1 (ppm)
7.260
7.064
7.060
7.058
7.046
7.043
7.040
6.982
6.969
6.965
6.959
6.946
6.934
6.931
6.926
6.914
6.896
6.890
6.881
6.871
6.868
6.838
6.834
6.818
6.813
5.642
3.884
3.881
3.877
H_2O
1.08
5.97
0.91
1.02
1.05
7.05
1.00
1.00
1.00
S-36


gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)
OH
OH
O
OH
O
O
1a
{3.659,2.782}
{3.918,2.779}
{4.967,3.510}
{3.931,3.668}
{4.179,3.886}
{4.168,3.649}
{4.973,4.157}
{7.088,6.916}
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
f1 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f2 (ppm)

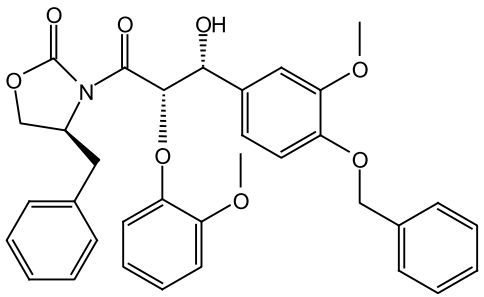


^{13}C NMR (100.6 MHz, $CDCl_3$)

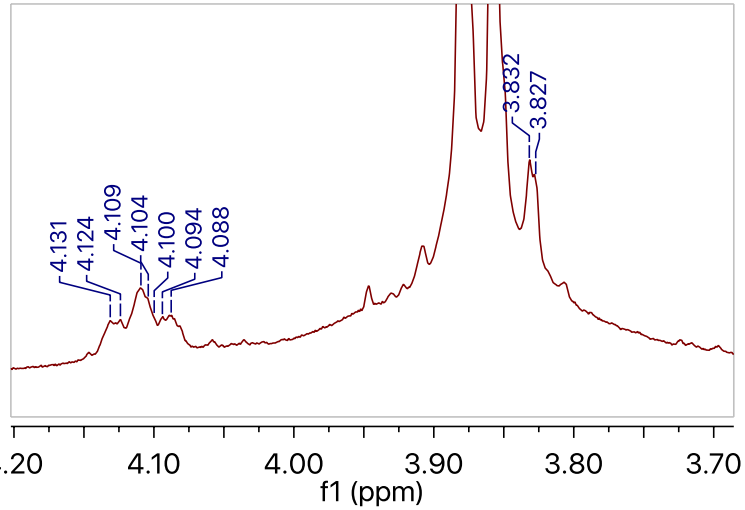
1a

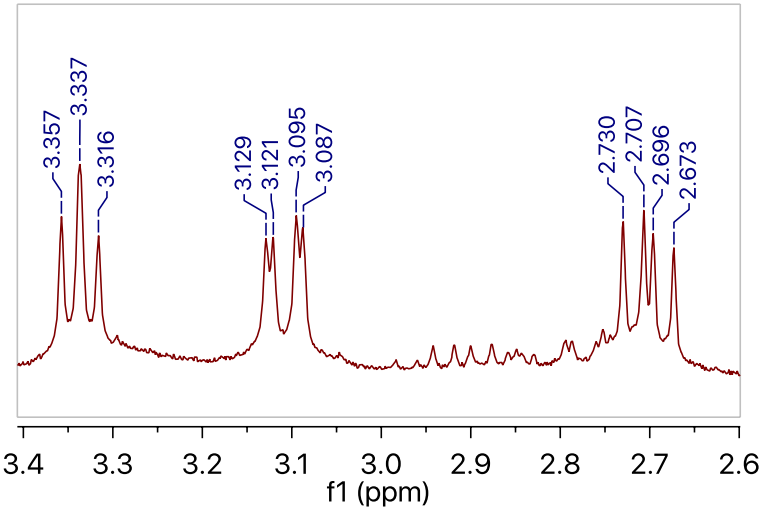


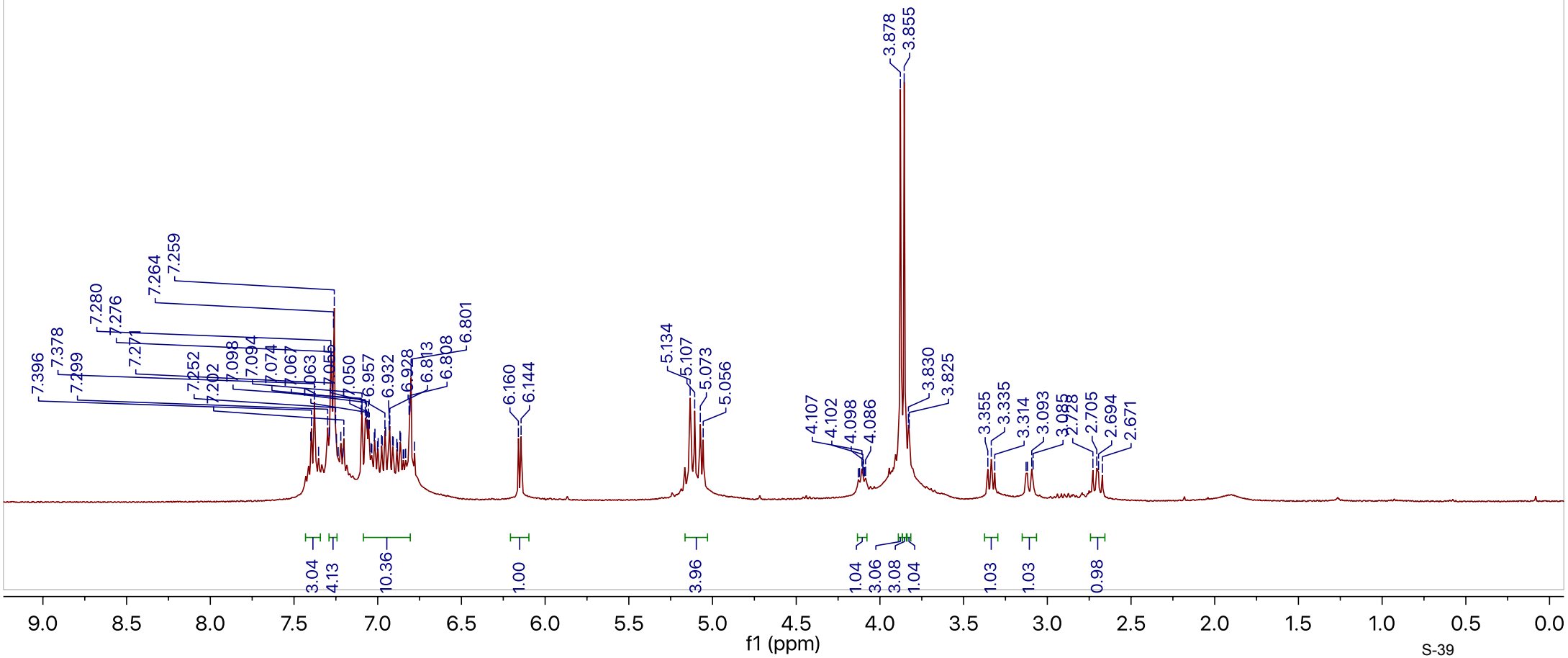
^{1}H NMR (400 MHz, $CDCl_3$)



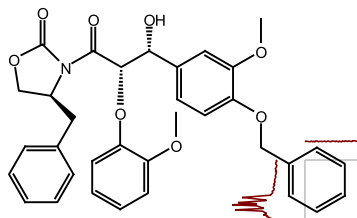
7c





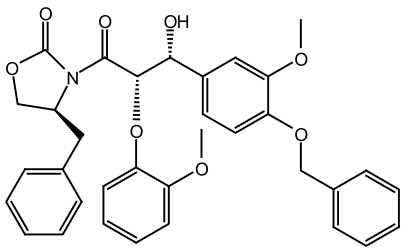


gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)

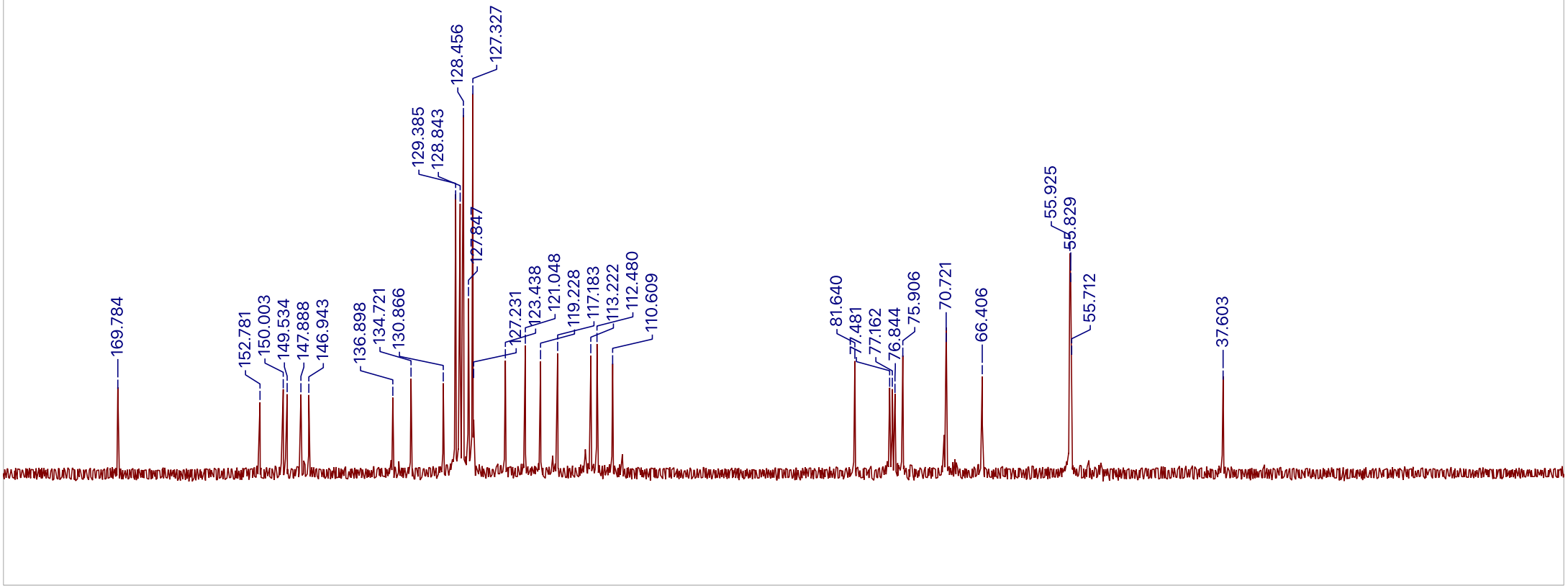


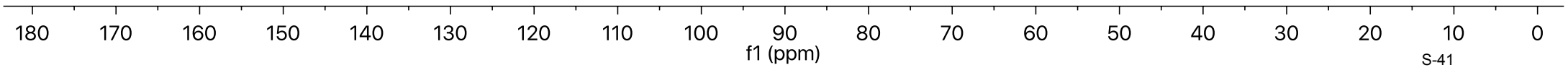
7c

^{13}C NMR (100.6 MHz, $CDCl_3$)

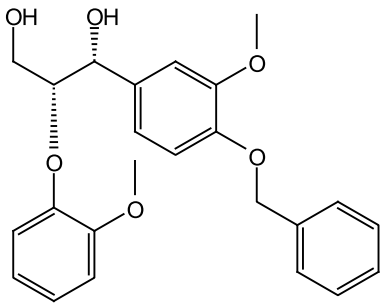


7c

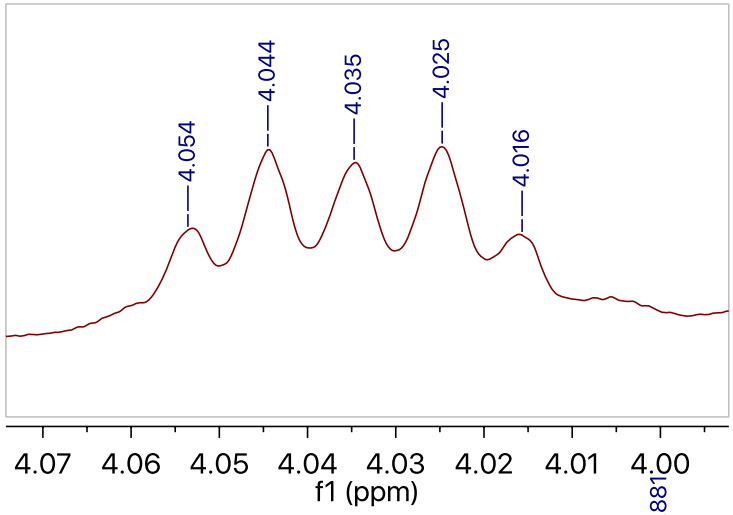


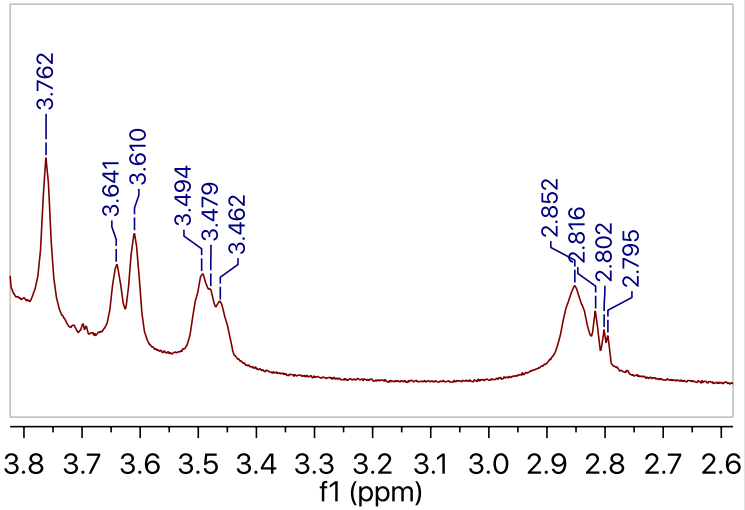


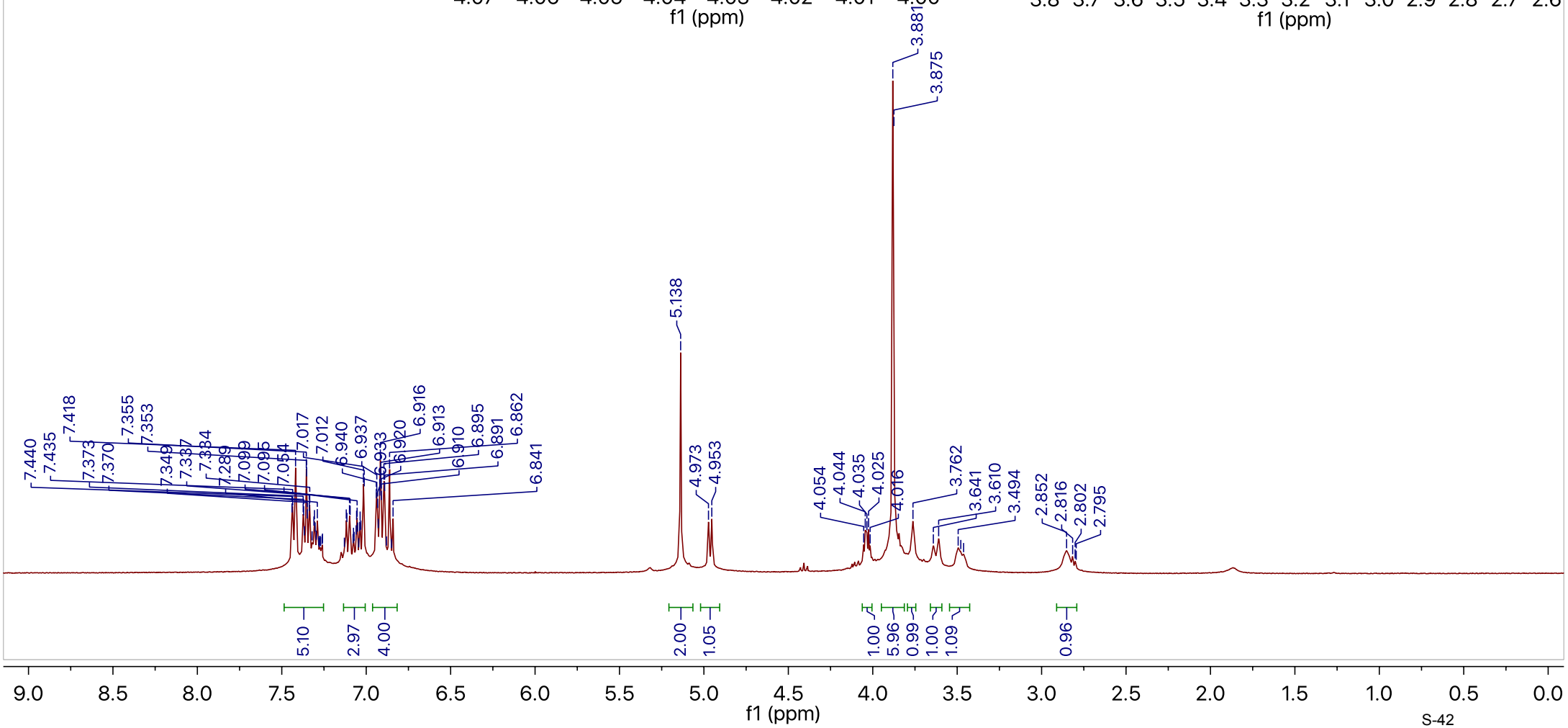
^{1}H NMR (400 MHz, $CDCl_3$)



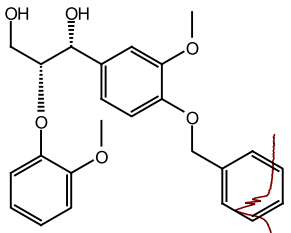
8c



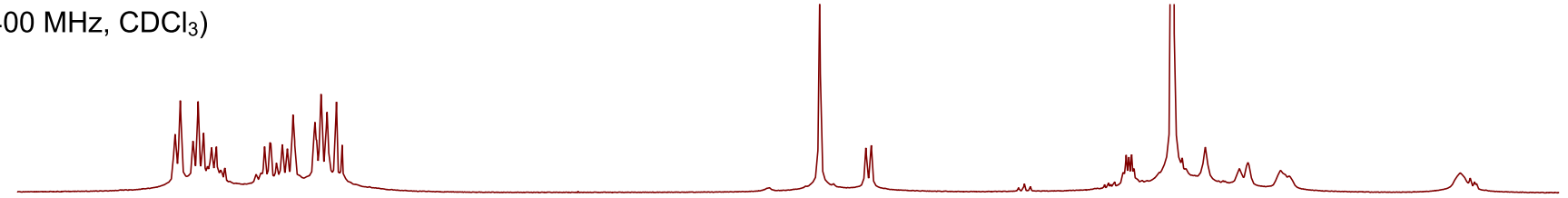


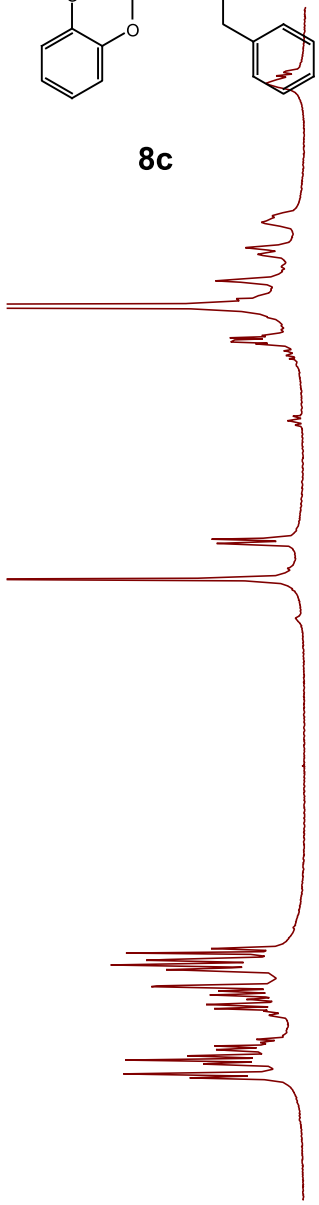


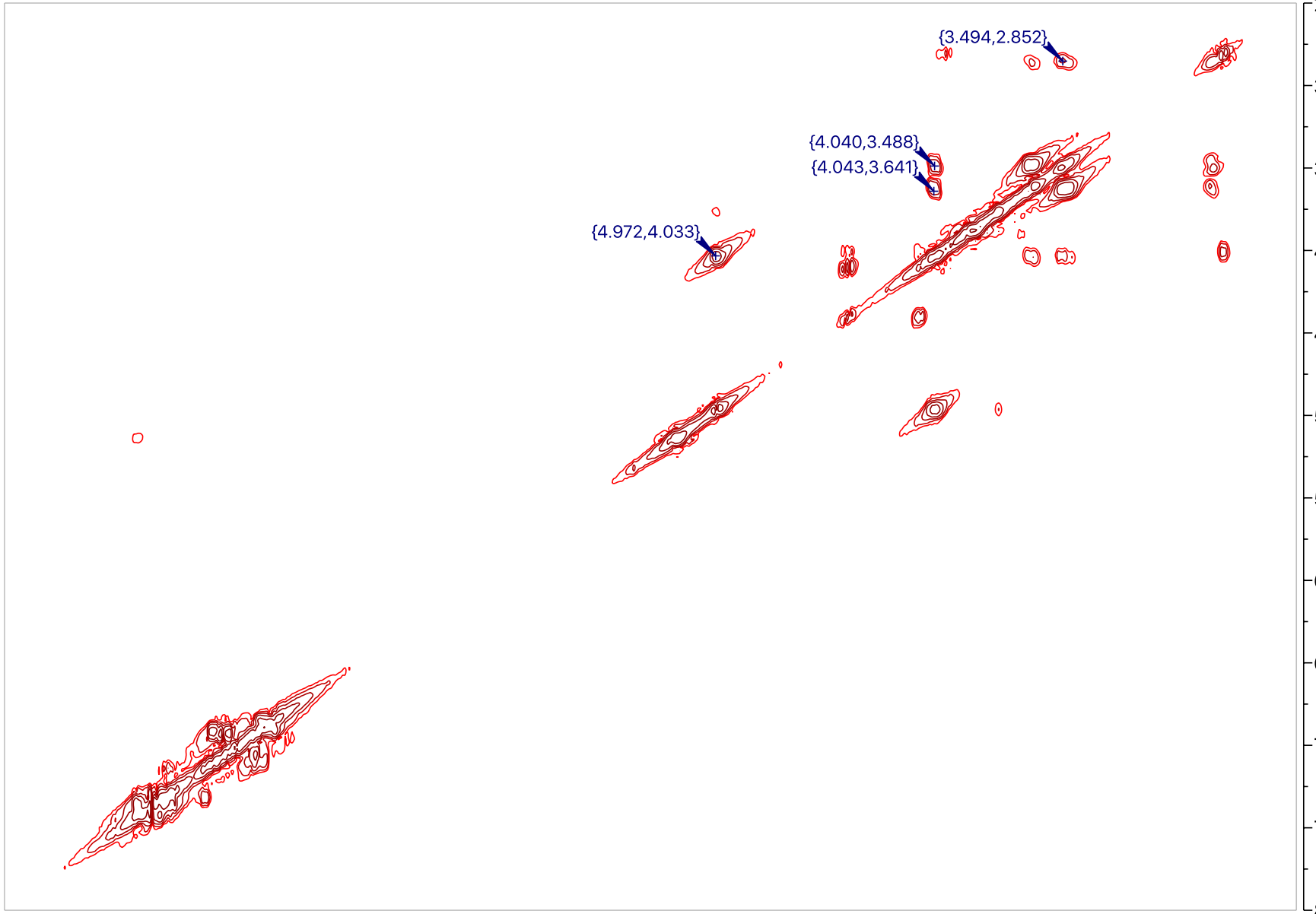
gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)

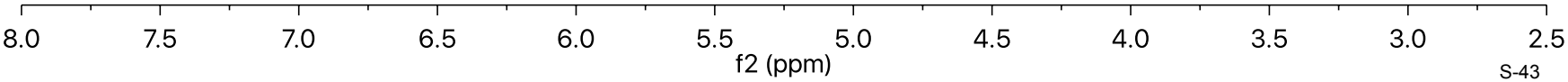


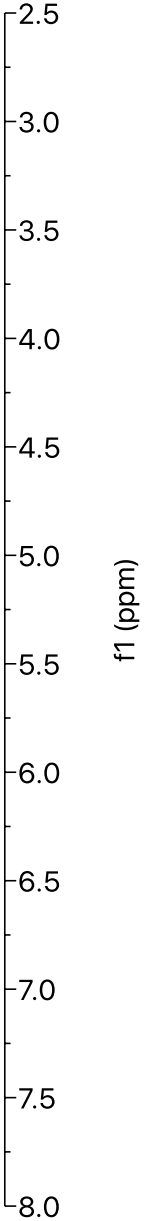
8c



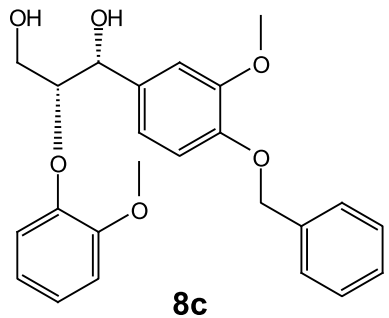




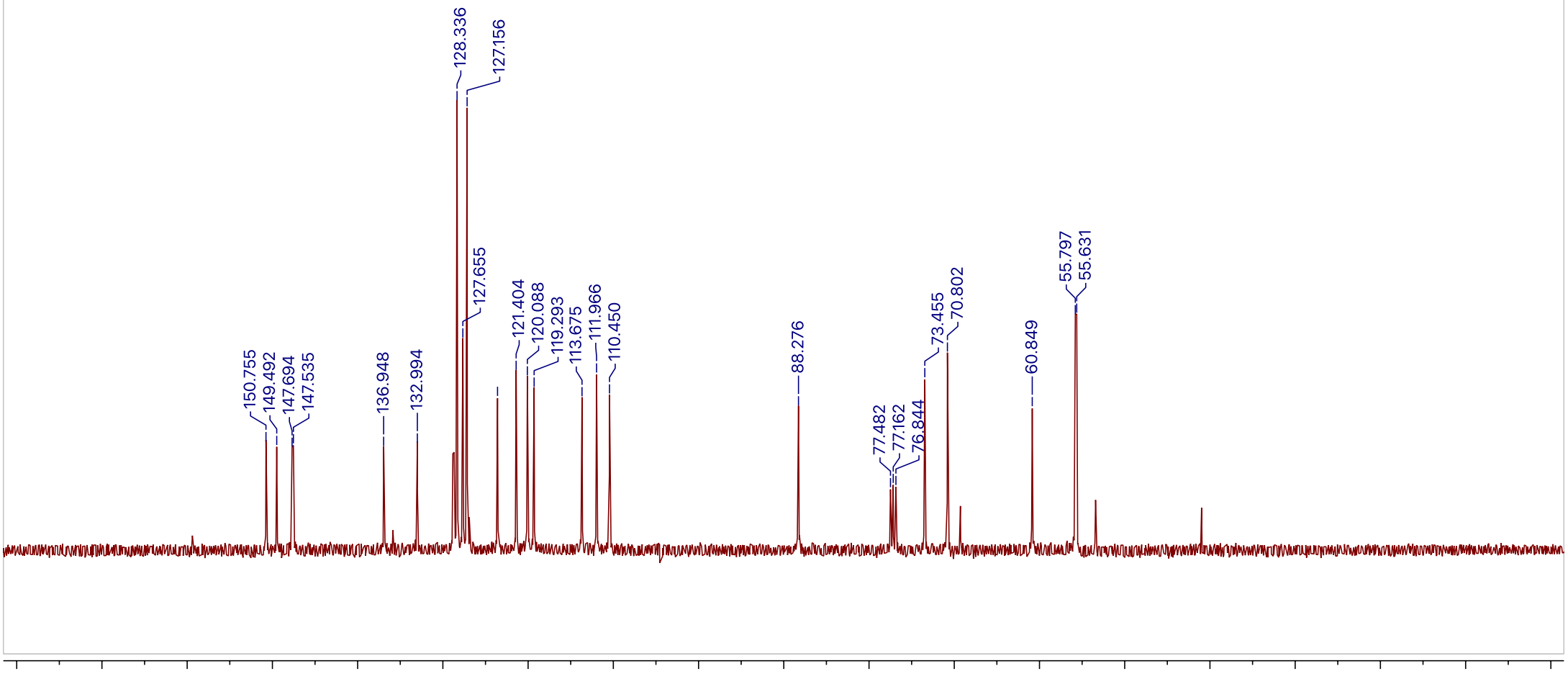


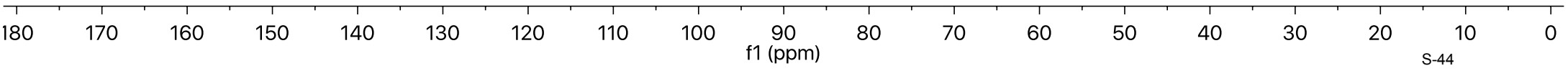


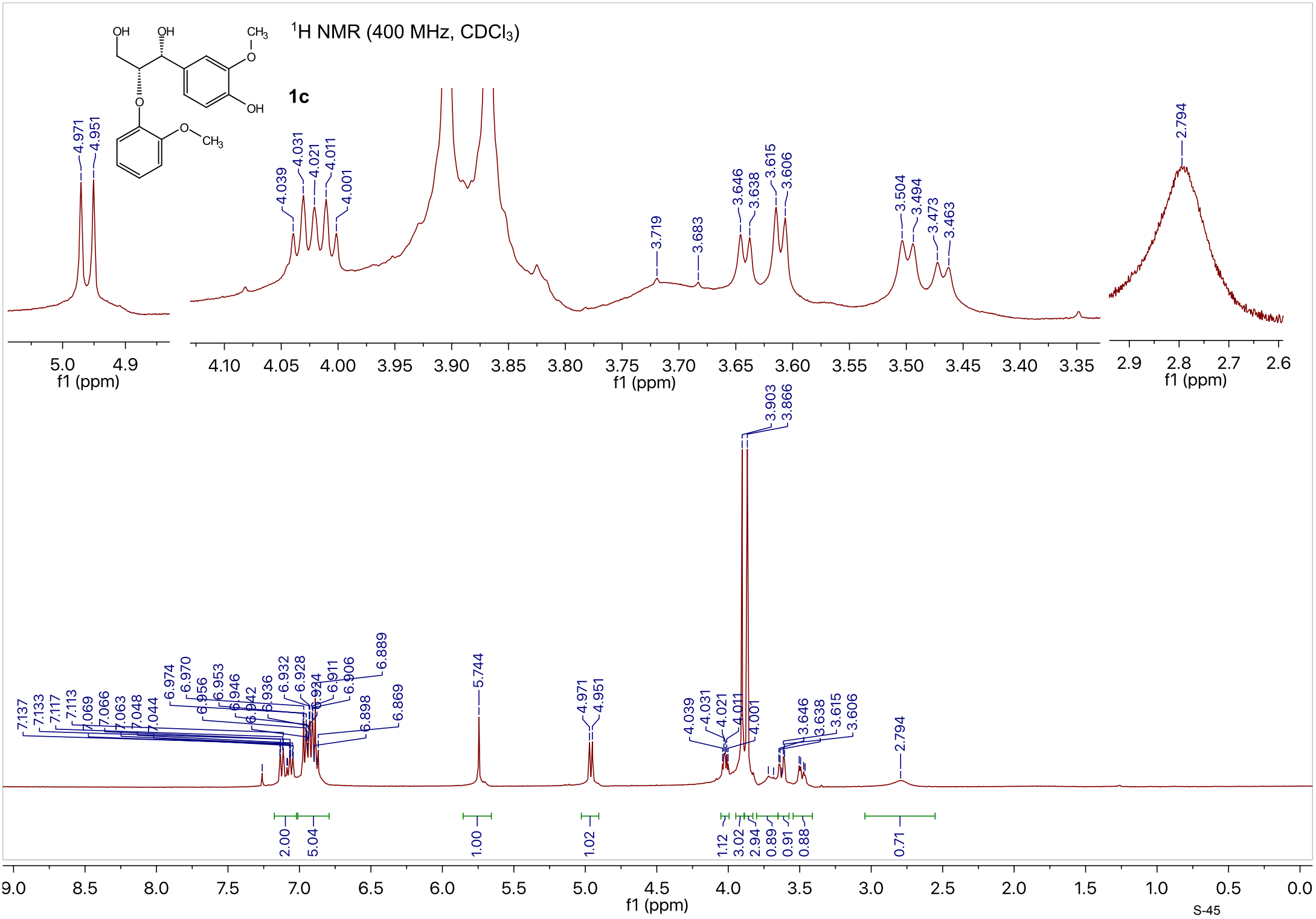
^{13}C NMR (100.6 MHz, $CDCl_3$)



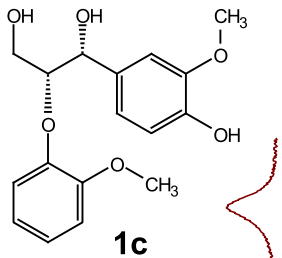
8c



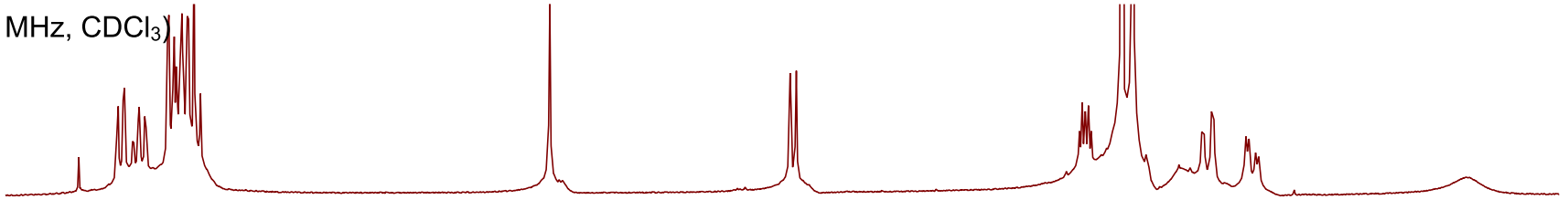


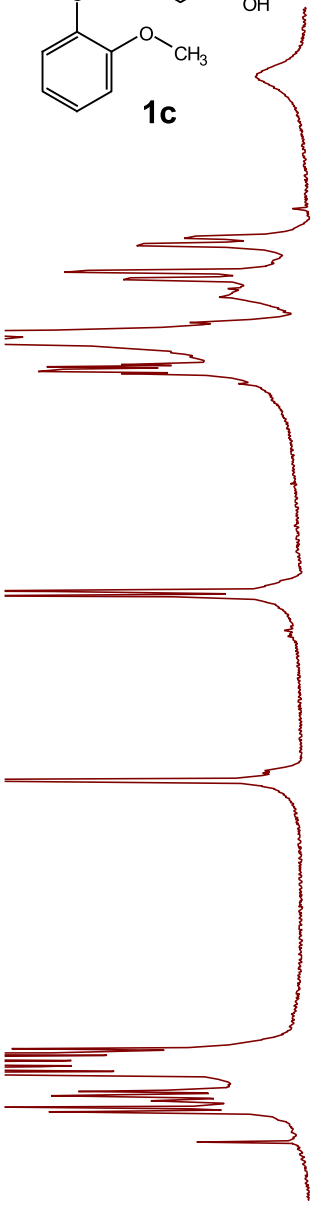

^{1}H NMR (400 MHz, $CDCl_3$)
1c
OH
OH
CH_3
O
O
OH
O
CH_3
4.971
4.951
4.039
4.031
4.021
4.011
4.001
3.719
3.683
3.646
3.638
3.615
3.606
3.504
3.494
3.473
3.463
2.794
f1 (ppm)
7.137
7.133
7.117
7.113
7.069
7.066
7.063
7.048
7.044
6.974
6.970
6.956
6.953
6.946
6.942
6.936
6.932
6.928
6.924
6.911
6.906
6.898
6.889
6.869
5.744
3.903
3.866
2.00
5.04
1.00
1.02
1.12
3.02
2.94
0.89
0.91
0.88
0.71
f1 (ppm)

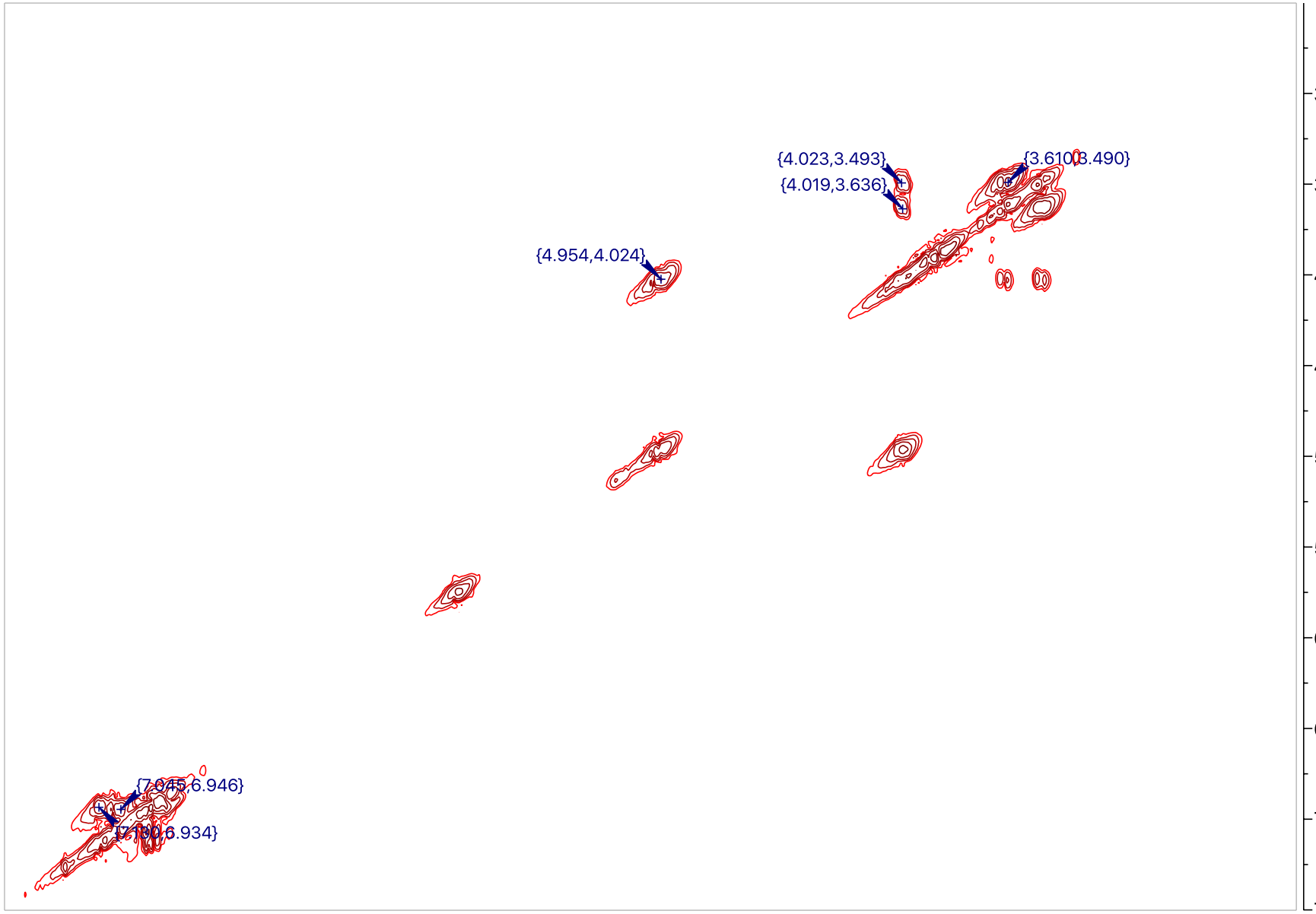
gCOSY ^{1}H NMR (400 MHz, $CDCl_3$)

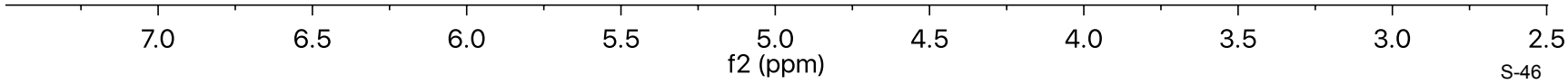


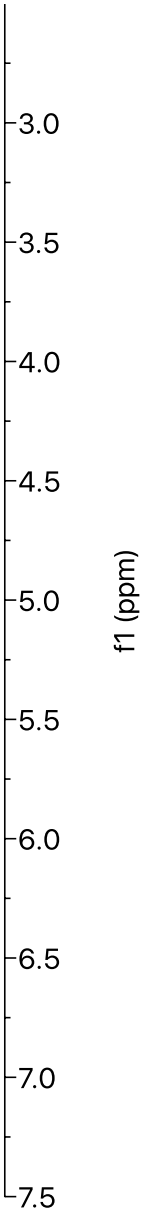
1c



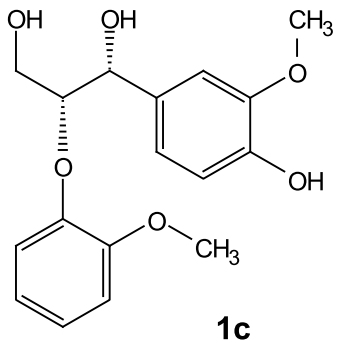








^{13}C NMR (100.6 MHz, $CDCl_3$)



1c