Electronic Supplementary Information

Hydroxylation of Anilides by Engineered Cytochrome $P450_{BM3}$

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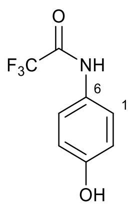
General Experimental Details

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a Bruker AVIII600 (600/151 MHz), Bruker AVII500 (500/126 MHz), Bruker AVIII400 (400/101 MHz), or Bruker AV400 (400/101 MHz) spectrometers. Proton and carbon chemical shifts (δ) are quoted in ppm downfield of tetramethylsilane with residual protiated solvent as the internal standard. Peak assignments were made on the basis of chemical shift, coupling constants, COSY, NOESY, DEPT, HSQC, and HMBC data, and by comparison with spectra of related compounds. Coupling constants (*J*) are quoted as observed and rounded to the nearest 0.5 Hz. Atom numbering, shown below, is given for the purpose of NMR peak assignments and does not necessarily correspond to the IUPAC nomenclature.



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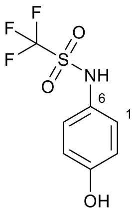

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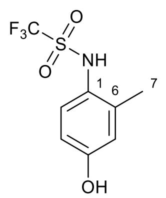

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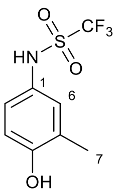

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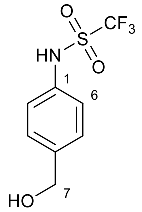

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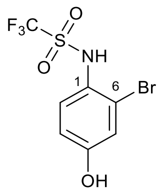

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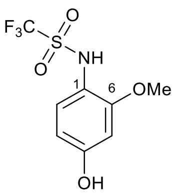

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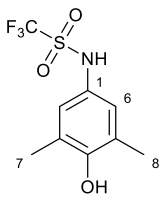

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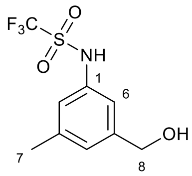

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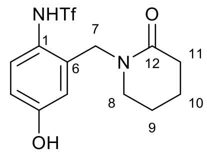

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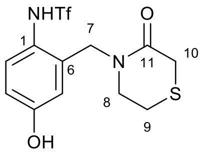

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Low-resolution mass spectra were recorded on a Micromass LCT Premier spectrometer (ES) with values reported in Daltons. High-resolution mass spectra were recorded by the Mass Spectrometry service of the Department of Chemistry, University of Oxford, using a Bruker microTOF Spectrometer. High-resolution values are calculated to 4 decimal places from the chemical formula with all found values being within a tolerance of 5 ppm. Ultra performance liquid chromatography mass spectrometry analysis was carried out on a Waters Acquity (SB-C18, 50 × 4.6 mm, 1.8 µm column, UV, mass spectrometry, corona detection). Compounds were separated using a linear gradient system comprising water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) at a constant flow rate of 2.0 mL min^{-1}. Gas chromatographic analysis was performed on a Thermo Finnigan TRACE gas chromatograph equipped with a flame ionisation detector (FID) and an AS3000 autosampler on a DB-1 capillary GC column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The injector temperature was 280 °C and the FID was at 250 °C.

Infrared spectra were recorded on a Bruker Tensor 27 Fourier transform spectrometer as a thin film on a diamond ATR module. Absorption maxima (ν_{max}) are quoted in wavenumbers (cm^{-1}) and identified as strong (s), medium (m), or weak (w) as a measure of peak intensity. Melting points were recorded on a Griffin melting point apparatus.

Thin-layer chromatography was performed on Merck DC-Alufolien 60 F254 0.2 mm pre-coated plates with reaction progress visualised using basic potassium permanganate dip, acidic anisaldehyde dip, or ultraviolet light. Retention factors (R_f) are quoted with the solvent system in parentheses. Column chromatography was performed on Merck Geduran® 60 SiO_2 (40–63 µm) and the solvent system used is recorded in parentheses. Solvents were either used as commercially supplied or purified by standard techniques. Tetrahydrofuran was freshly distilled over Na and benzophenone prior to use. Petrol refers to the fraction of light petroleum ether boiling at 30–40 °C. Ether refers to diethyl ether. Brine refers to a saturated aqueous solution of sodium chloride. All reactions were carried out under an atmosphere of dry argon in oven-dried flasks unless otherwise stated.

Enzymes and Molecular Biology

The P450$_{BM3}$ enzymes were produced in *Escherichia coli* BL21 (DE3) and purified by anion exchange chromatography.[1] Alternatively, the enzymes were purified by addition of $(NH_4)_2SO_4$ to the cell-free extract to 40% saturation and centrifugation at 10,000 g for 10 min at 4 °C; the residue was discarded and $(NH_4)_2SO_4$ was added to a concentration of 60% saturation. The solution was submitted to centrifugation at 10,000 g for 10 min at 4 °C, the supernatant was discarded and the residue was resuspended in phosphate buffer. After filtration through a 0.22 μm syringe filter the enzymes were stored as stock solutions in 1.0 mL aliquots at –20 °C and thawed at RT before use.

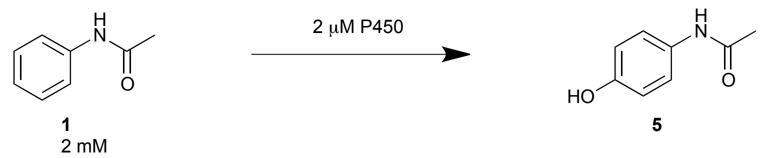
General Procedure for Screening of Compounds

Compounds were screened using an NADPH regeneration system in 100 mM phosphate buffer (pH 7.5), at a total volume of 0.5 mL, contained in 14 mL glass vials or 24 well plates. The substrate was added as a 100 mM stock solution in ethanol, methanol, isopropanol, or dimethylsulfoxide (final concentration 2.0 mM) and the mutant P450$_{BM3}$ enzyme was added as a 10 μM stock solution in phosphate buffer (pH 7.5, final concentration 2.0 μM). The NADPH regeneration system consisted of glucose dehydrogenase, added as a 2 U/μL stock solution in 100 mM phosphate buffer (pH 7.5, final concentration 40 U/mL), glucose, added as a 1.0 M stock solution in 100 mM phosphate buffer (pH 7.5, final concentration 100 mM), and $NADP^+$, added as a 4.0 mM stock solution in 100 mM phosphate buffer (pH 7.5, final concentration 80 μM). The reactions were shaken at RT for 16 h, extracted with 300 μL of ethyl acetate and, following centrifugation at 14,300 g for 2 min, the organic phase was analysed by gas chromatography or liquid-chromatography mass spectrometry.

Table of Mutants

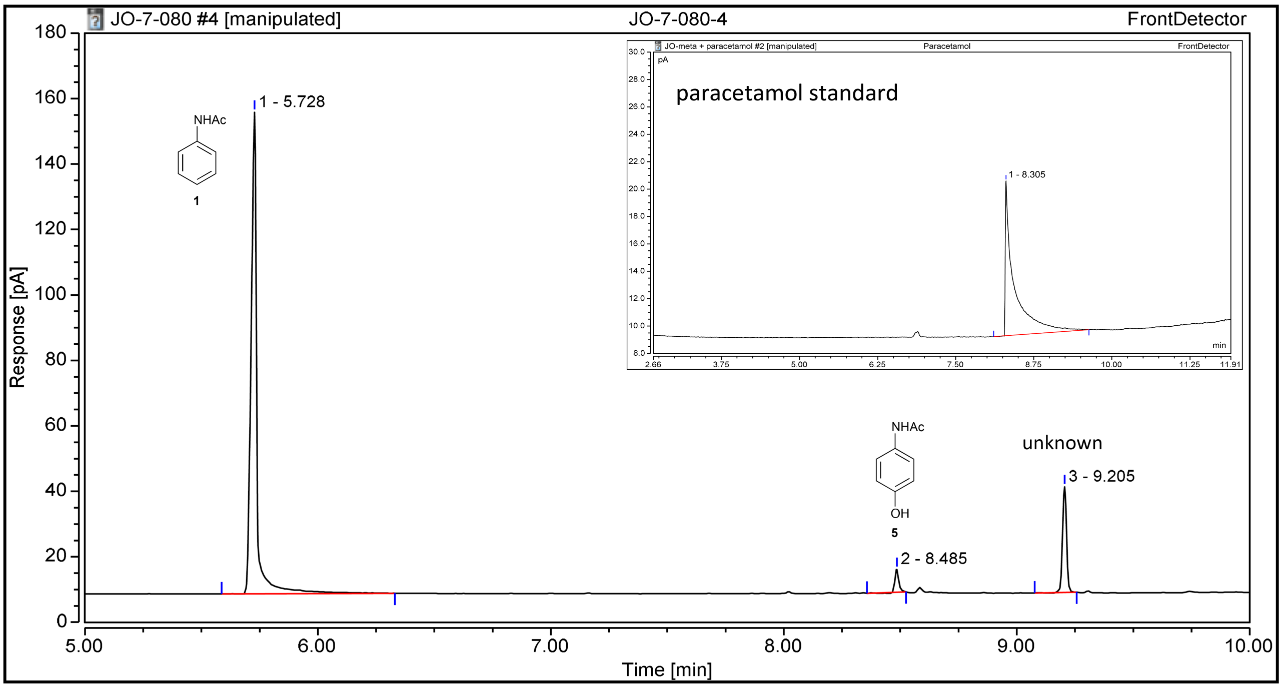
Mutant	Mutations
Library A	
RLYF/KSK19/AI	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328I
KSK19/AM	F87A/A82M/H171L/Q307H/N319Y
KSK19/AI/Y51L	F87A/ H171L/I263A/Q307H/N319Y/A328I
KSK19/AI/AI	F87A/H171L/A184I/Q307H/N319Y/A328I
RP/FV/EV/L437VL	R47L/Y51F/F87V/E267V/I401P/L437VL
KSK19/I263A	F87A/H171L/I263A/Q307H/N319Y
RP	R47L/Y51F/I401P
RP/EV	R47L/Y51F/E267V/I401P
RT2/F81W	R47L/Y51F/F81W/A191T/N239H/I259V/A276T/L353I
RP/FV/EV	R47L/Y51F/F87V/E267V/I401P
KSK19/AI/F87V	F87V/H171L/Q307H/N319Y/A328I
RP/FV/EV/L437LV	R47L/Y51F/F87V/E267V/I401P/L437LV
KSK19/AI/V78I	V78I/F87A/H171L/Q307H/N319Y/A328I
RT2/AP/I401M	R47L/Y51F/A191T/N239H/I259V/A276T/A330P/L353I/I401M
RT2/AP/AM	R47L/Y51F/A82M/A191T/N239H/I259V/A276T/A330P/L353I
RT2/IP	R47L/Y51F/A191T/N239H/I259V/A276T/L353I/I401P
RT2/AP	R47L/Y51F/A191T/N239H/I259V/A276T/A330P/L353I
RT2/AP/A184I	R47L/Y51F/A184I/A191T/N239H/I259V/A276T/A330P/L353I
RT2/AP/V78I	R47L/Y51F/V78I/A191T/N239H/I259V/A276T/A330P/L353I
RT2	R47L/Y51F/A191T/N239H/I259V/A276T/L353I
RP/IA/EV	R47L/Y51F/I263A/E267V/I401P
WT	–
RP/AP	R47L/Y51F/A330P/I401P
RP/FV	R47L/Y51F/F87V/I401P
Library B	
A330P	A330P
RP/FW	R47L/Y51F/I401P/ F81W
RT2/AP/AI/SA	R47L/Y51F/S72A/A184I/A191T/N239H/I259V/A276T/A330P/L353I
RP/HL/IG	R47L/Y51F/H171L/I263G/I401P
RT2/AP/PV	R47L/Y51F/A191T/N239H/I259V/A276T/P329V/A330P/L353I
GVQ	A74G/F87V/L188Q
RT2/AW	R47L/Y51F/A191T/N239H/I259V/A276T/A330W/L353I
F87A	F87A
RLYF/KSK19	R47L/Y51F/F87A/H171L/Q307H/N319Y
RP/AM/IA	R47L/Y51F/A82M/I263A/I401P
RT2/VF	R47L/Y51F/V78F/A191T/N239H/I259V/A276T/L353I
RLYF	R47L/Y51F
KSK19/AM/EF	A82M/F87A/H171L/E267F/Q307H/N319Y
RP/HL	R47L/Y51F/H171L/I401P
RP/EG	R47L/Y51F/E267G/I401P
KSK48	R179H/L233V/S270I/Q307H/N319Y
KSK19/QP/FV	F87V/H171L/Q307H/N319Y/Q403P
RT2/AP/VI/AI	R47L/Y51F/V78I/A184I/A191T/N239H/I259V/A276T/A330P/L353I
RP/FV/EV/LQ	R47L/Y51F/F87V/L188Q/E267V/I401P
RP/FV/EV/VF	R47L/Y51F/V78F/F87V/E267V/I401P
RT2/FW/AI	R47L/Y51F/F81W/A191T/N239H/I259V/A276T/A328I/L353I
RP/EM	R47L/Y51F/E267M/I401P
KSK19/AI/IA	F87A/ H171L/I263A/Q307H/N319Y/A328I
RT2/SW/AW	R47L/Y51F/S72W/A191T/N239H/I259V/A276T/A330W/L353I

Table S1: Substrate conversion for the initial screen of the oxidation of phenylacetamide **1**



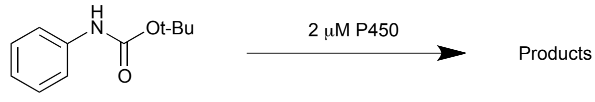
No.	Enzyme	Conversion	4-OH Product (5)	Other Products	TTN[a]
1	KSK19/AI/AI	17%	18%	82%	170
2	RP/IA/EV	~2%	100%	0%	~20
3	RT2/AP/A184I	~2%	0%	100%	~20
4	RT2	~1%	0%	100%	~10
5	RT2/IP	~1%	100%	0%	~10
6	RP	~1%	0%	100%	~10

[a] TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme



GC trace for the screening run with KSK19/AI/AI showing production of paracetamol (**5**, R_t = 8.49 min) and an unknown metabolite (R_t = 9.21 min).

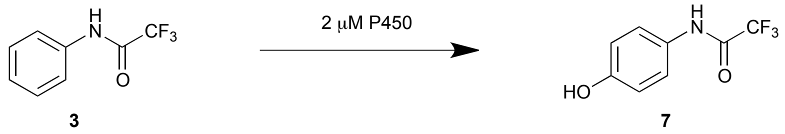
Table S2: Substrate conversion for the initial screen of the oxidation of *t*-butyl phenylcarbamate **2**



No.	Enzyme	Conversion	R_t 1.99[a]	R_t 7.07	R_t 8.65	Other Products	TTN[b]
1	RT2/AP/AM	93%	42%	0%	0%	58%	930
2	RP/FV/EV/L437VL	84%	98%	0%	2%	0%	840
3	RP/FV/EV/L437LV	83%	52%	18%	24%	6%	830
4	RP/AP	77%	94%	0%	4%	2%	770
5	RT2/AP/A184I	72%	96%	0%	3%	1%	720
6	RP/EV	67%	73%	6%	2%	19%	670
7	RP/FV/EV	65%	78%	9%	0%	13%	650
8	RP	61%	97%	0%	0%	3%	610
9	RT2/AP/V78I	46%	4%	32%	0%	64%	460
10	KSK19/AI/Y51L	41%	0%	83%	0%	17%	410
11	RT2/AP/I401M	40%	83%	0%	3%	14%	400
12	RT2	38%	13%	42%	0%	45%	380
13	RP/FV	37%	68%	15%	11%	6%	370
14	RT2/IP	32%	91%	0%	9%	0%	320
15	RT2/F81W	31%	92%	0%	0%	8%	310
16	KSK19/AM	24%	100%	0%	0%	0%	240
17	RT2/AP	16%	81%	12%	0%	7%	160
18	KSK19/AI/AI	9%	33%	55%	0%	12%	90
19	KSK19/I263A	8%	5-%	0%	25%	25%	80
20	WT	5%	0%	0%	0%	100%	50
21	RLYF/KSK19/AI	2%	100%	0%	0%	0%	20
22	RP/IA/EV	2%	100%	0%	0%	0%	20

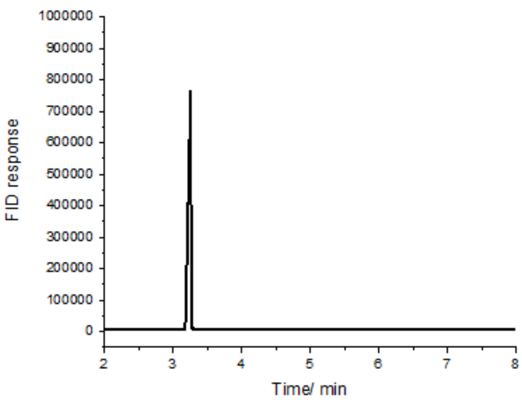
[a] Mass spectrometric (ESI^+) analysis of this product showed *m/z* = 441 (M_2Na^+, 28%) and 232 (MNa^+, 100%), corresponding to M = $C_{11}H_{15}NO_3$ and indicating monohydroxylation of substrate **2** ($C_{11}H_{15}NO_2$); [b] TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

Table S3: Substrate conversion for the initial screen of the oxidation of 2,2,2-trifluoro-*N*-phenylacetamide **3**

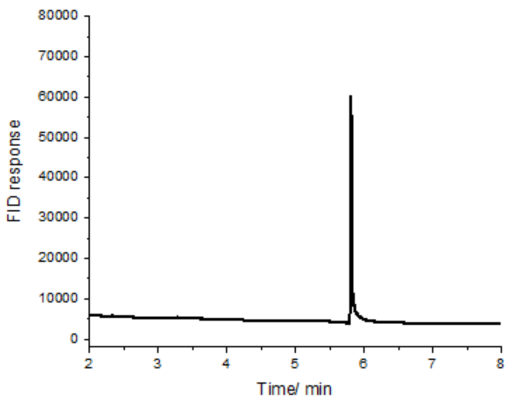


No.	Enzyme	Conversion	4-OH Product (7)	Other Products	TTN[a]
1	KSK19/AI/AI	100%	100%	0%	1000
2	RP/FV	98%	85%	15%	980
3	RP/FV/EV/L437LV	96%	100%	0%	960
4	RLYF/KSK19/AI	90%	100%	0%	900
5	RT2/AP/A184I	84%	88%	12%	840
6	KSK19/AM	81%	100%	0%	810
7	RP/FV/EV	74%	100%	0%	740
8	KSK19/AI/Y51L	67%	100%	0%	670
9	KSK19/AI/F87V	67%	100%	0%	670
10	RT2/AP/AM	49%	47%	53%	490
11	RP/FV/EV/L437VL	45%	100%	0%	450
12	RT2/IP	43%	72%	28%	430
13	RP/AP	43%	72%	28%	430
14	RP/AP/AM	42%	26%	74%	420
15	KSK19/AI/V78I	40%	80%	20%	400
16	RT2	37%	47%	53%	370
17	KSK19/I263A	28%	71%	29%	280
18	RP	23%	100%	0%	230
19	RT2/AP/V78I	23%	65%	35%	230
20	RP/EV	8%	0%	100%	8

[a] TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme. When the conversion reaches >95% the intrinsic, maximum turnover number TON, of the enzyme for the reactions exceeds 1,000.

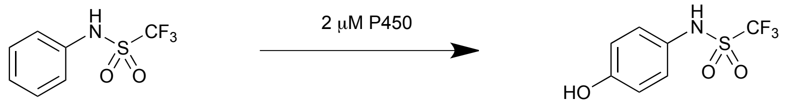


Starting material



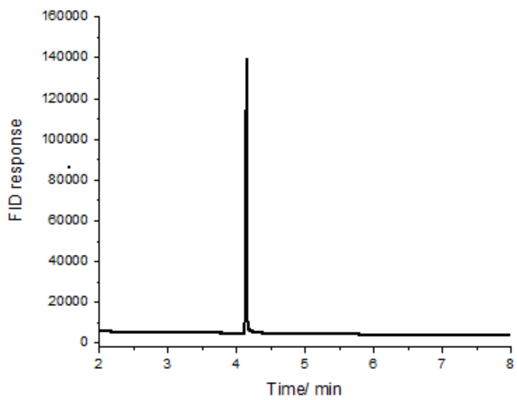
KSK19/AI/AI

Table S4: Substrate conversion for the initial screen of the oxidation of *N*-phenyl-1,1,1-trifluoromethanesulfonamide **4**



No.	Enzyme	Conversion	4-OH Product (8)	Other Products	TTN[a]
1	RP/FV/EV/L437LV	100%	100%	0%	1000
2	RP/IA/EV	100%	100%	0%	1000
3	RP/EV	97%	100%	0%	970
4	KSK19/AI/AI	78%	96%	4%	780
5	RP/FV/EV/L437VL	77%	100%	0%	770
6	RT2/AP/A184I	69%	100%	0%	690
7	KSK19/AM	66%	59%	41%	660
8	KSK19/AI/F87V	56%	80%	20%	560
9	RLYF/KSK19/AI	48%	75%	25%	480
10	RT2/IP	46%	100%	0%	460
11	RP	43%	100%	0%	430
12	RP/AP	23%	100%	0%	230
13	KSK19/AI/Y51L	16%	100%	0%	160

[a] TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme. When the conversion reaches >95% the intrinsic, maximum turnover number TON, of the enzyme for the reactions exceeds 1,000.



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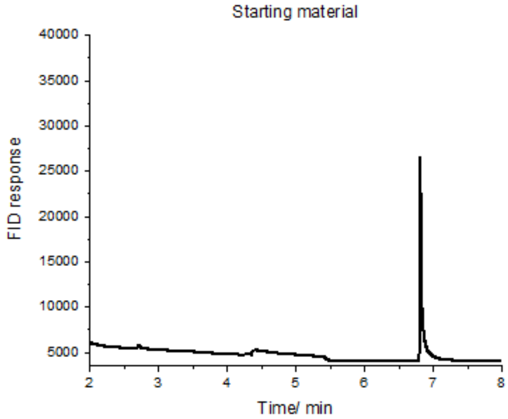
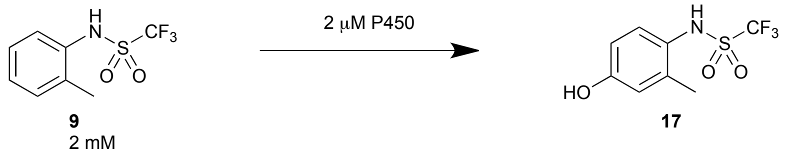


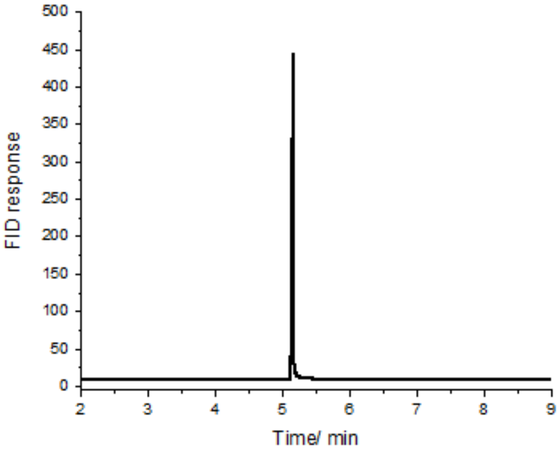
RP/FV/EV/L437LV

Table S5: Substrate conversion for the initial screen of the oxidation of *N*-(*o*-tolyl)-1,1,1-trifluoromethanesulfonamide **9**



No.	Enzyme	Conversion	4-OH Product (17)	Other Products	TTN[a]
1	RP/IA/EV	100%	100%	0%	1000
2	RP/FV/EV	99%	22%	78%	990
3	RP/FV/EV/LQ	99%	22%	78%	990
4	RP/HL/IG	96%	100%	0%	960
5	RP/EV	94%	100%	0%	940
6	KSK19/AM	83%	0%	100%	830
7	RP/FV/EV/L437LV	72%	100%	0%	720
8	RP/EM	72%	100%	0%	720
9	RP/FV	60%	100%	0%	600
10	RP/FV/EV/VF	57%	95%	5%	570
11	RT2/IP	49%	100%	0%	490
12	GVQ	48%	100%	0%	480
13	RT2	41%	100%	0%	410
14	KSK19/AI/AI	37%	100%	0%	370
15	RP/HL	36%	100%	0%	360
16	KSK19/AI/F87V	34%	100%	0%	340
17	KSK19/AM/EF	34%	100%	0%	340
18	KSK19/QP/FV	33%	100%	0%	330
19	RP/FV/EV/L437VL	27%	100%	0%	270
20	RT2/F81W	27%	100%	0%	270
21	RT2/AP/A184I	24%	100%	0%	240
22	RP	20%	100%	0%	200
23	RP/FW	18%	100%	0%	180
24	RT2/AP/PV	14%	100%	0%	140
25	RT2/AP/VI/AI	14%	100%	0%	140
26	RP/AP	10%	100%	0%	100
27	RP/AM/IA	10%	100%	0%	100
28	RT2/AP	7%	100%	0%	70
29	RLYF/KSK19	5%	100%	0%	50

[a] TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme. When the conversion reaches >95% the intrinsic, maximum turnover number TON, of the enzyme for the reactions exceeds 1,000.



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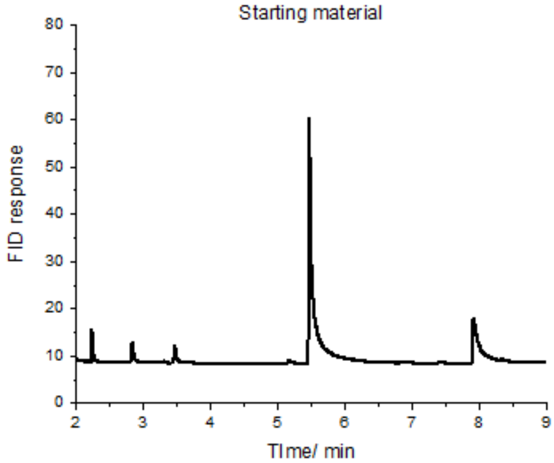
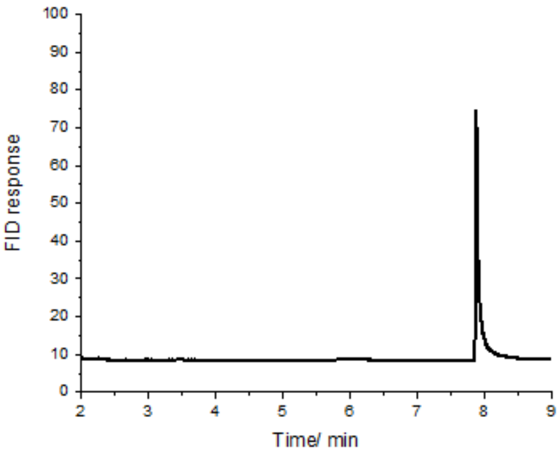
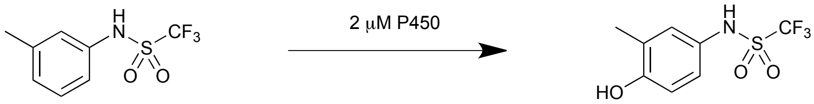


RP/FV/EV



RP/IA/EV

Table S6: Substrate conversion for the initial screen of the oxidation of *N*-(*m*-tolyl)-1,1,1-trifluoromethanesulfonamide **10**

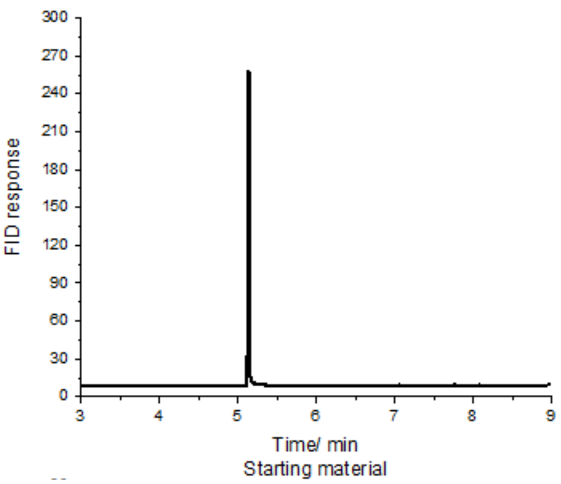


10
2 mM

18

No.	Enzyme	Conversion	4-OH Product (18)	Other Products	TTN[a]
1	RP/FV/EV/L437LV	100%	64%	36%	1000
2	RP/EV	99%	86%	14%	990
3	RT2/F81W	97%	0%	100%	970
4	RP/IA/EV	97%	67%	33%	970
5	RP/FV/EV	93%	78%	22%	930
6	KSK19/AI/AI	91%	83%	17%	910
7	RT2/IP	86%	84%	16%	860
8	KSK19/AM	64%	39%	61%	640
9	RP	62%	58%	42%	620
10	RT2/AP/AM	61%	33%	67%	610
11	RT2/AP/A184I	58%	90%	10%	580
12	RT2/AP	37%	60%	40%	370
13	RP/AP	34%	100%	0%	340
14	KSK19/AI/F87V	24%	100%	0%	240
15	RP/FV/EV/L437VL	11%	100%	0%	110
16	RP/FV	11%	100%	0%	110
17	KSK19/AI/Y51L	10%	100%	0%	100
18	RLYF/KSK19/AI	3%	100%	0%	30
19	RT2/AP/I401M	3%	100%	0%	30

[a] TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme. When the conversion reaches >95% the intrinsic, maximum turnover number TON, of the enzyme for the reactions exceeds 1,000.



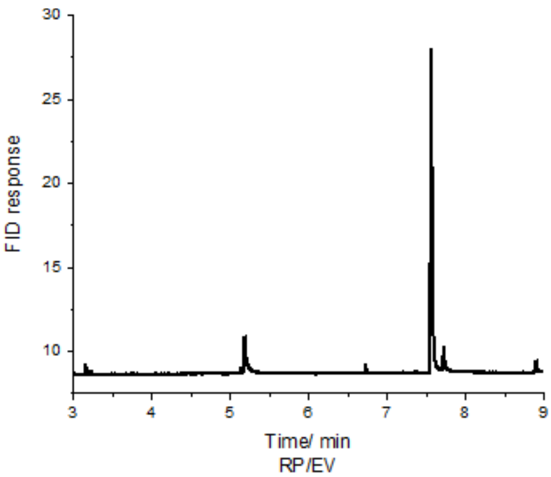
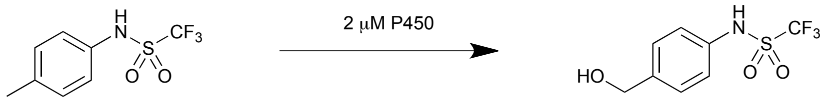
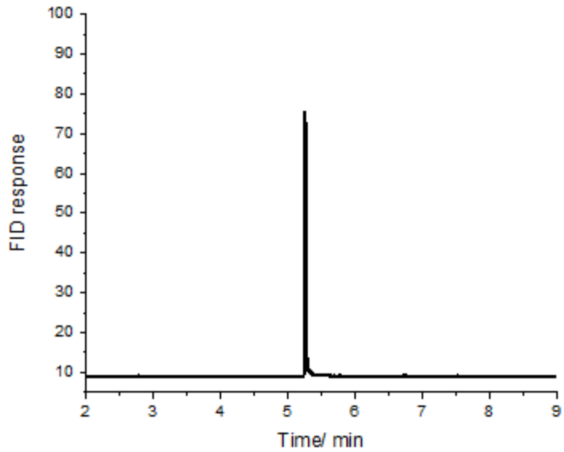


Table S7: Substrate conversion for the initial screen of the oxidation of *N*-(*p*-tolyl)-1,1,1-trifluoromethanesulfonamide **11**

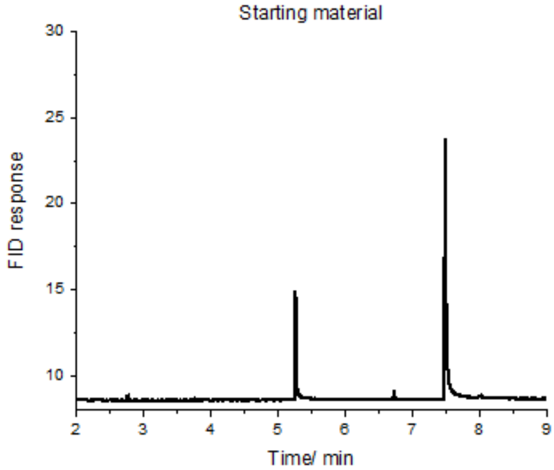


No.	Enzyme	Conversion	Benzylic Alcohol (19)	Other Products	TTN[a]
1	RP/IA/EV	79%	98%	2%	790
2	RP/EV	63%	59%	41%	630
3	RP/FV/EV/L437VL	52%	0%	100%	520
4	RP/FV/EV/L437LV	30%	100%	0%	300
5	WT	28%	100%	0%	280
6	KSK19/AI/AI	19%	0%	100%	190
7	RP/FV/EV	18%	100%	0%	180
8	KSK19/AI/F87V	6%	100%	0%	60

[a] TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

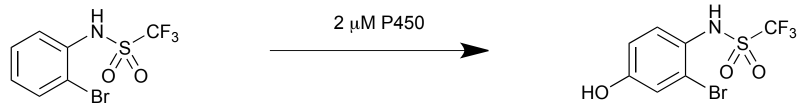


Starting material



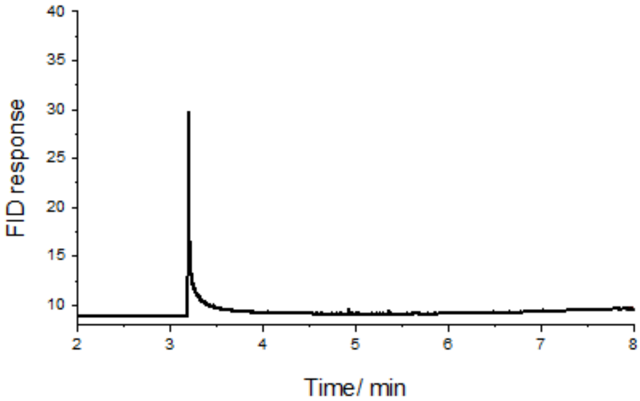
RP/IA/EV

Table S8: Substrate conversion for the initial screen of the oxidation of *N*-(2-bromophenyl)-1,1,1-trifluoromethanesulfonamide **13**

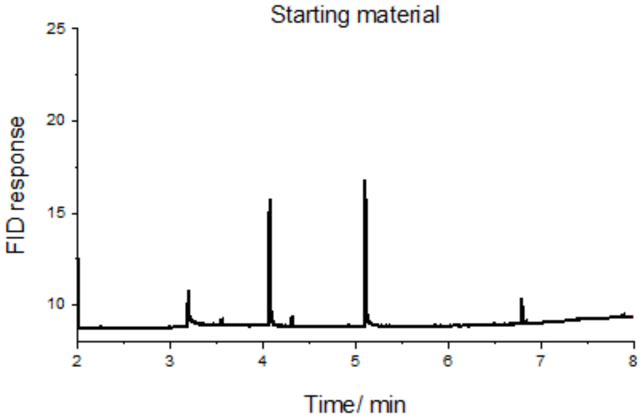


No.	Enzyme	Conversion	4-OH Product (20)	Other Products	TTN[a]
1	RP/IA/EV	100%	44%	56%	1000
2	RP/EV	85%	47%	53%	850
3	RP/FV/EV	81%	23%	77%	810
4	RP/FV/EV/L437LV	78%	55%	45%	780
5	RT2/AP/I401M	60%	0%	100%	600
6	RP/FV	31%	55%	45%	310
7	RT2/AP/AM	30%	0%	100%	300
8	RT2/IP	28%	61%	39%	280
9	RT2/F81W	21%	43%	57%	210
10	KSK19/AI/AI	16%	0%	100%	160
11	RP/FV/EV/L437VL	15%	34%	66%	150
12	RT2	12%	42%	58%	120
13	RP	10%	0%	100%	100
14	RP/AP	10%	0%	100%	100
15	RT2/AP	6%	33%	67%	60
16	KSK19/AM	5%	0%	100%	50
17	KSK19/AI/F87V	5%	0%	100%	50
18	RT2/AP/A184I	5%	20%	80%	50
19	RT2/AP/V78I	5%	0%	100%	50
20	RLYF/KSK19/AI	2%	0%	100%	20
21	WT	2%	0%	100%	20

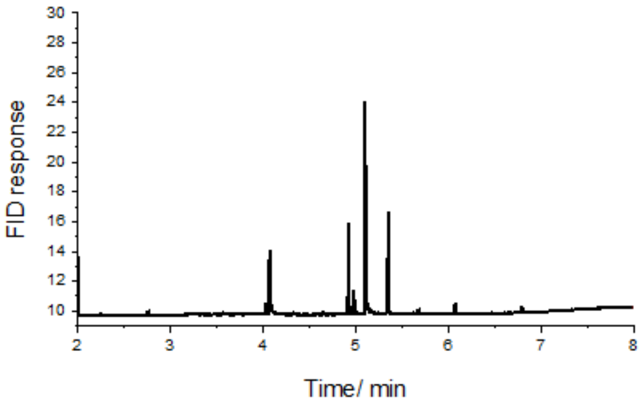
[a] TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme. When the conversion reaches >95% the intrinsic, maximum turnover number TON, of the enzyme for the reactions exceeds 1,000.



Starting material

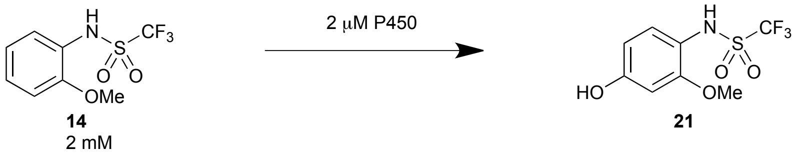


RP/EV



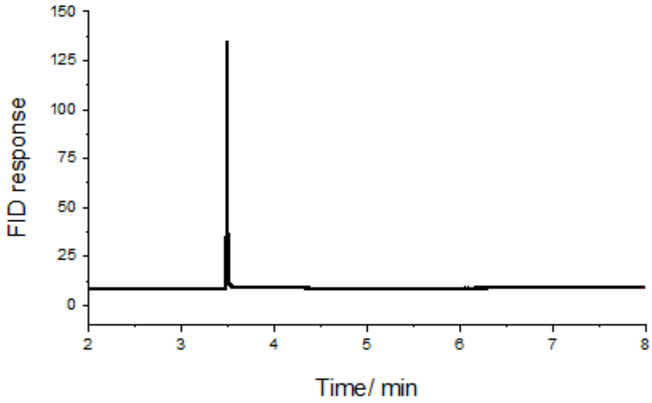
RP/IA/EV

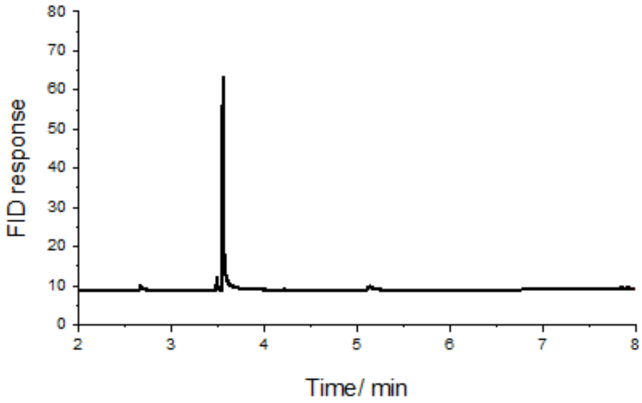
Table S9: Substrate conversion for the initial screen of the oxidation of *N*-(2-methoxyphenyl)-1,1,1-trifluoromethanesulfonamide **14**



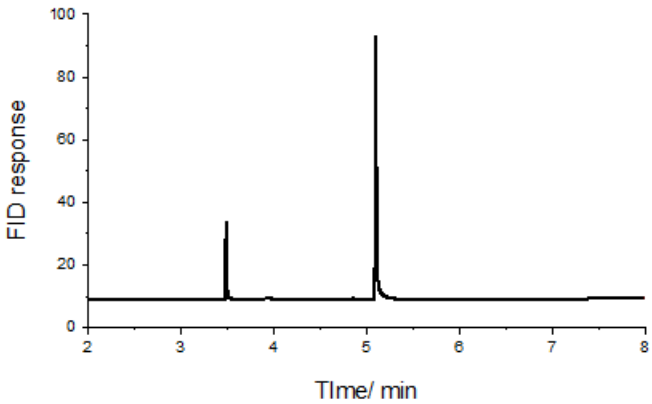
No.	Enzyme	Conversion	4-OH Product (21)	Other Products	TTN[a]
1	RP/FV/EV	97%	6%	94%	970
2	RP/IA/EV	81%	99%	1%	810
3	RP/EV	49%	98%	2%	490
4	KSK19/AM	30%	13%	87%	300
5	RT2/F81W	13%	100%	0%	130
6	RT2/IP	12%	100%	0%	120
7	RT2	9%	100%	0%	90
8	RP/FV/EV/L437VL	7%	100%	0%	70
9	RT2/AP/I401M	3%	100%	0%	30
10	RP/AP	3%	100%	0%	30
11	RLYF/KSK19/AI	2%	100%	0%	20
12	KSK19/AI/Y51L	2%	100%	0%	20

[a] TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme



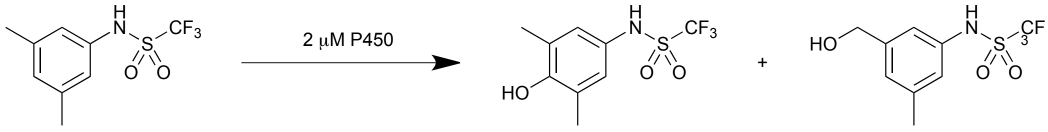


RP/FV/EV



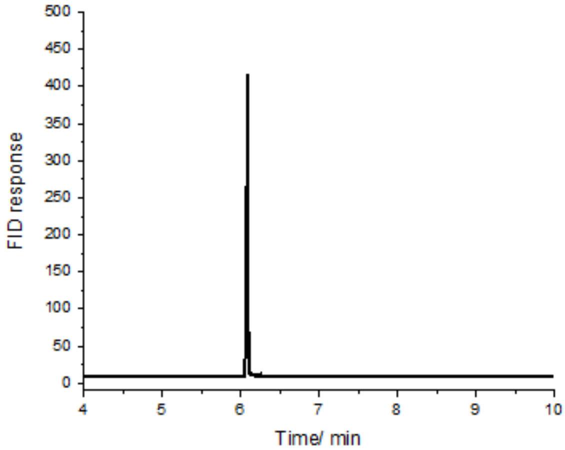
RP/IA/EV

Table S10: Substrate conversion for the initial screen of the oxidation of *N*-(3,5-dimethylphenyl)-1,1,1-trifluoromethanesulfonamide **15**

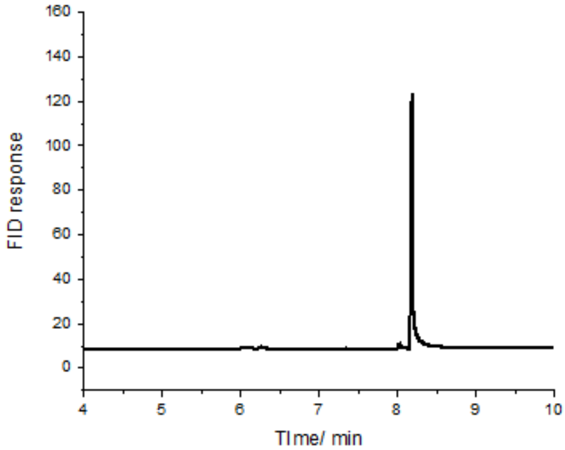


No.	Enzyme	Conversion	4-OH Product (22)	Benzylic Alcohol (23)	Other Products	TTN[a]
1	RP/IA/EV	100%	85%	9%	6%	1000
2	KSK19/AM	99%	13%	18%	69%	990
3	KSK19/AI/F87V	99%	4%	96%	0%	990
4	RP/EV	97%	29%	64%	7%	970
5	KSK19/AI/AI	90%	60%	40%	0%	900
6	KSK19/AI/Y51L	80%	46%	54%	0%	800
7	RT2	80%	4%	89%	7%	800
8	RT2/F81W	68%	37%	63%	0%	680
9	RP/FV/EV	50%	38%	62%	0%	500
10	KSK19/AI/V78I	50%	70%	30%	0%	500
11	RLYF/KSK19/AI	45%	56%	44%	0%	450
12	RP	43%	30%	70%	0%	430
13	RT2/IP	41%	44%	56%	0%	410
14	RT2/AP/A184I	26%	100%	0%	0%	260
15	RP/FV/EV/L437LV	13%	70%	30%	0%	130
16	RT2/AP/AM	10%	70%	30%	0%	100
17	RT2/AP/I401M	7%	100%	0%	0%	70
18	WT	7%	29%	71%	0%	70
19	RP/FV/EV/L437VL	3%	100%	0%	0%	30
20	RT2/AP	3%	100%	0%	0%	30
21	RP/AP	3%	100%	0%	0%	30
22	RP/FV	3%	67%	33%	0%	30

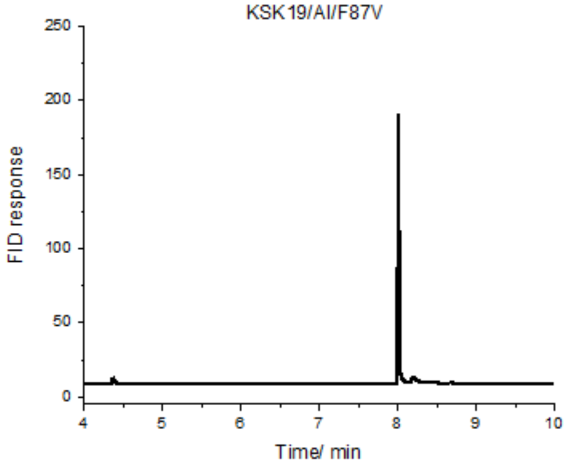
[a] TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme. When the conversion reaches >95% the intrinsic, maximum turnover number TON, of the enzyme for the reactions exceeds 1,000.



Starting material

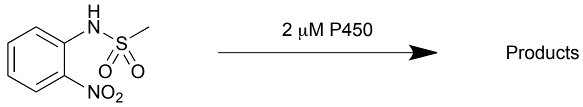


KSK19/AI/F87V



RP/IA/EV

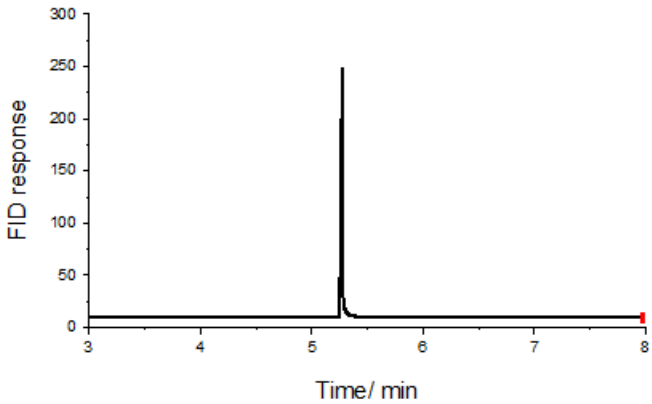
Table S11: Substrate conversion for the initial screen of the oxidation of *N*-(2-nitrophenyl)methanesulfonamide **16**



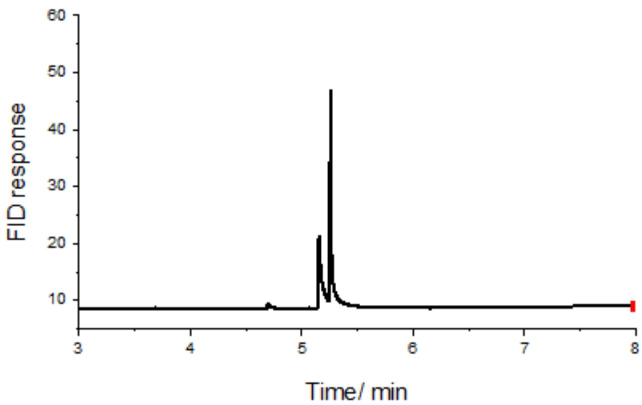
16
2 mM

No.	Enzyme	Conversion	R_t 7.83	R_t 8.61	Other Products	TTN[a]
1	RT2/AP/I401M	35%	6%	94%	0%	350
2	RT2/IP	28%	29%	54%	17%	280
3	RP/FV/EV/L437VL	21%	14%	86%	0%	210
4	RP/FV/EV	13%	0%	0%	100%	130
5	RP/EV	10%	30%	60%	10%	100
6	RT2/AP/V78I	9%	33%	67%	0%	90
7	KSK19/AI/V78I	8%	25%	75%	0%	80
8	KSK19/AM	7%	0%	0%	100%	70
9	KSK19/AI/F87V	1%	100%	0%	0%	10
10	RP/FV/EV/L437LV	1%	100%	0%	0%	10

[a] TTN = Total Turnover Number, reported as mol substrate converted per mol enzyme

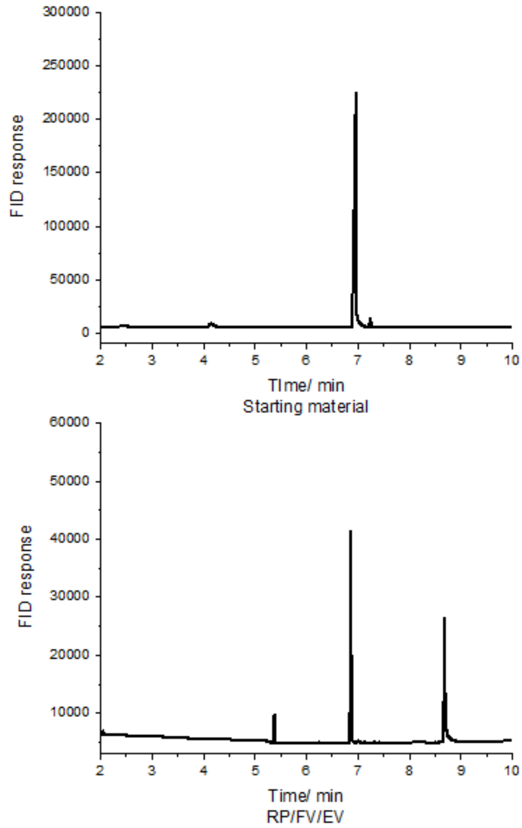


Starting material

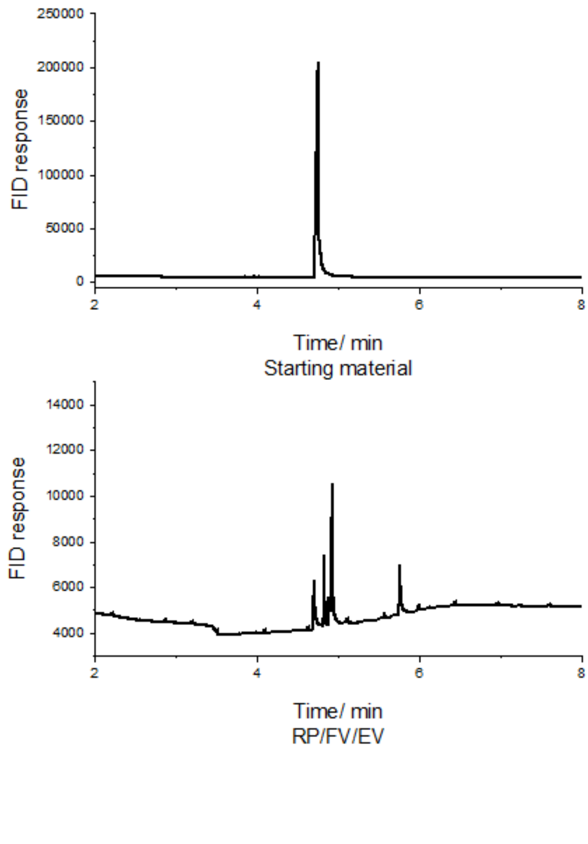


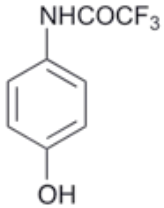
RT2/AP/IM

***N*-4-Hydroxy-2-[(2-oxopiperidin-1-yl)methyl]phenyl-1,1,1-trifluoromethanesulfonamide 26**



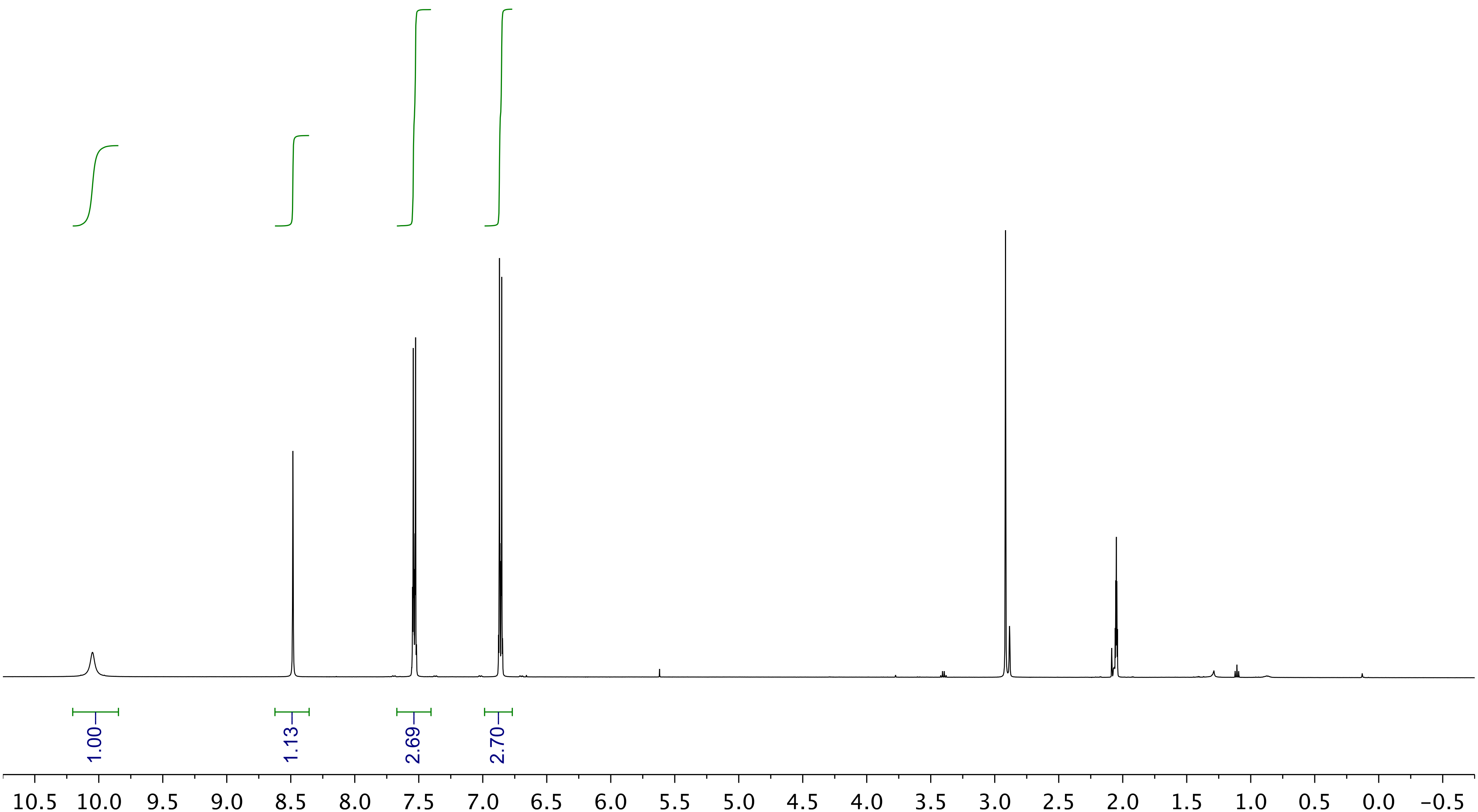
***N*-4-Hydroxy-2-[(3-oxothiomorpholino)methyl]phenyl-1,1,1-trifluoromethanesulfonamide 27**





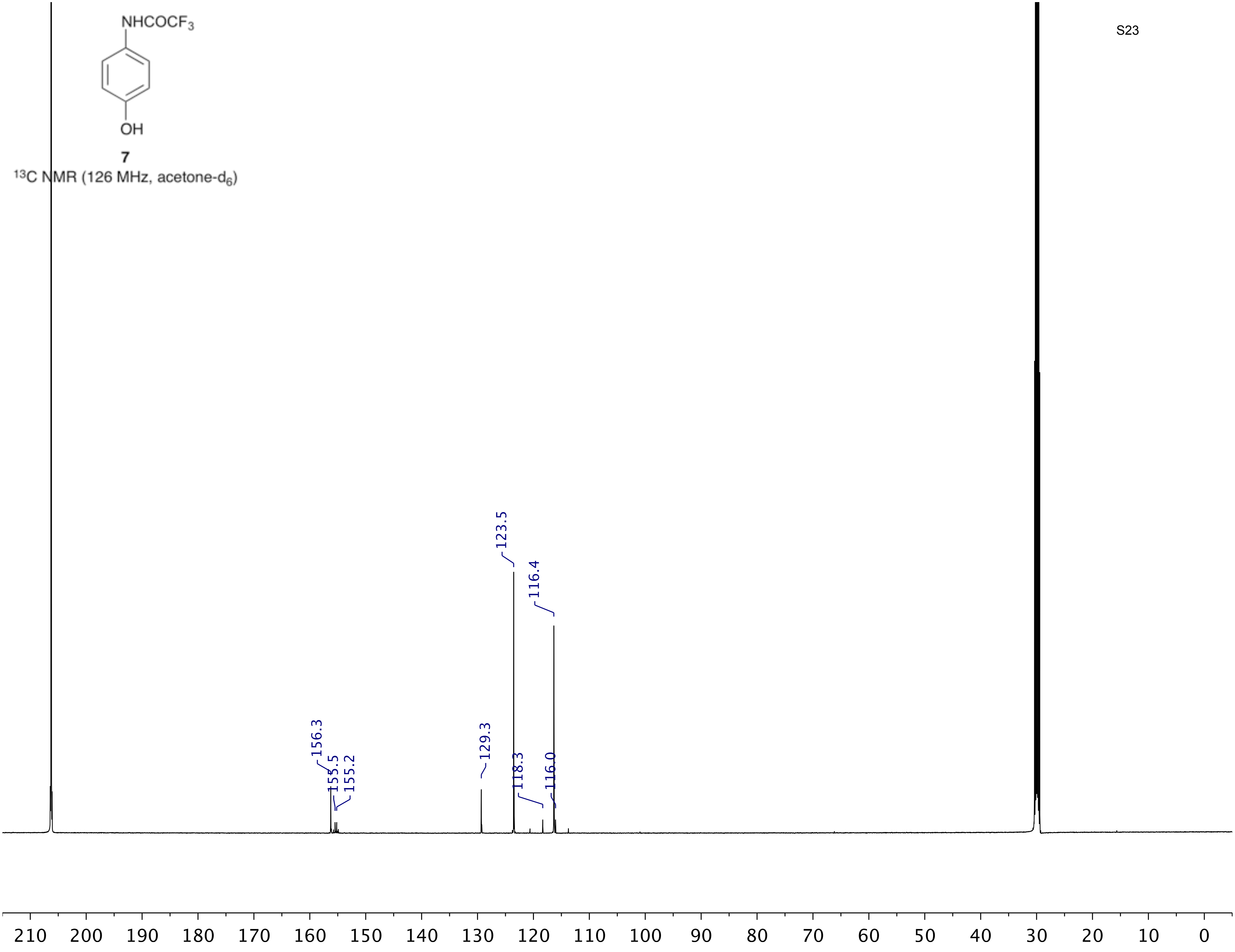
7

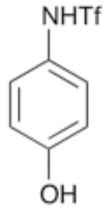
^{1}H NMR (500 MHz, acetone-d_6)



7

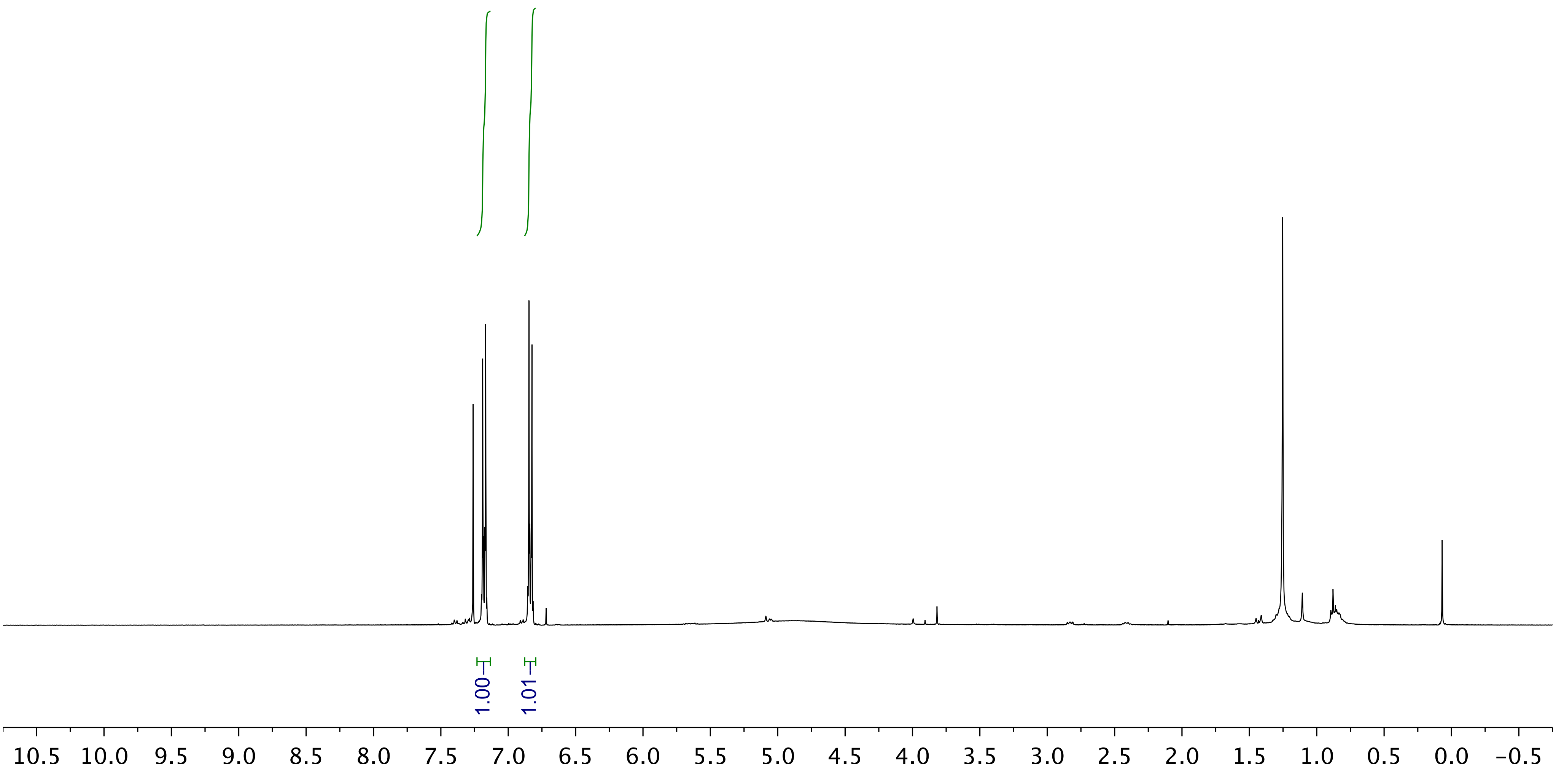
^{13}C NMR (126 MHz, acetone-d_6)

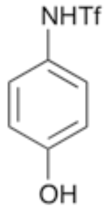




8

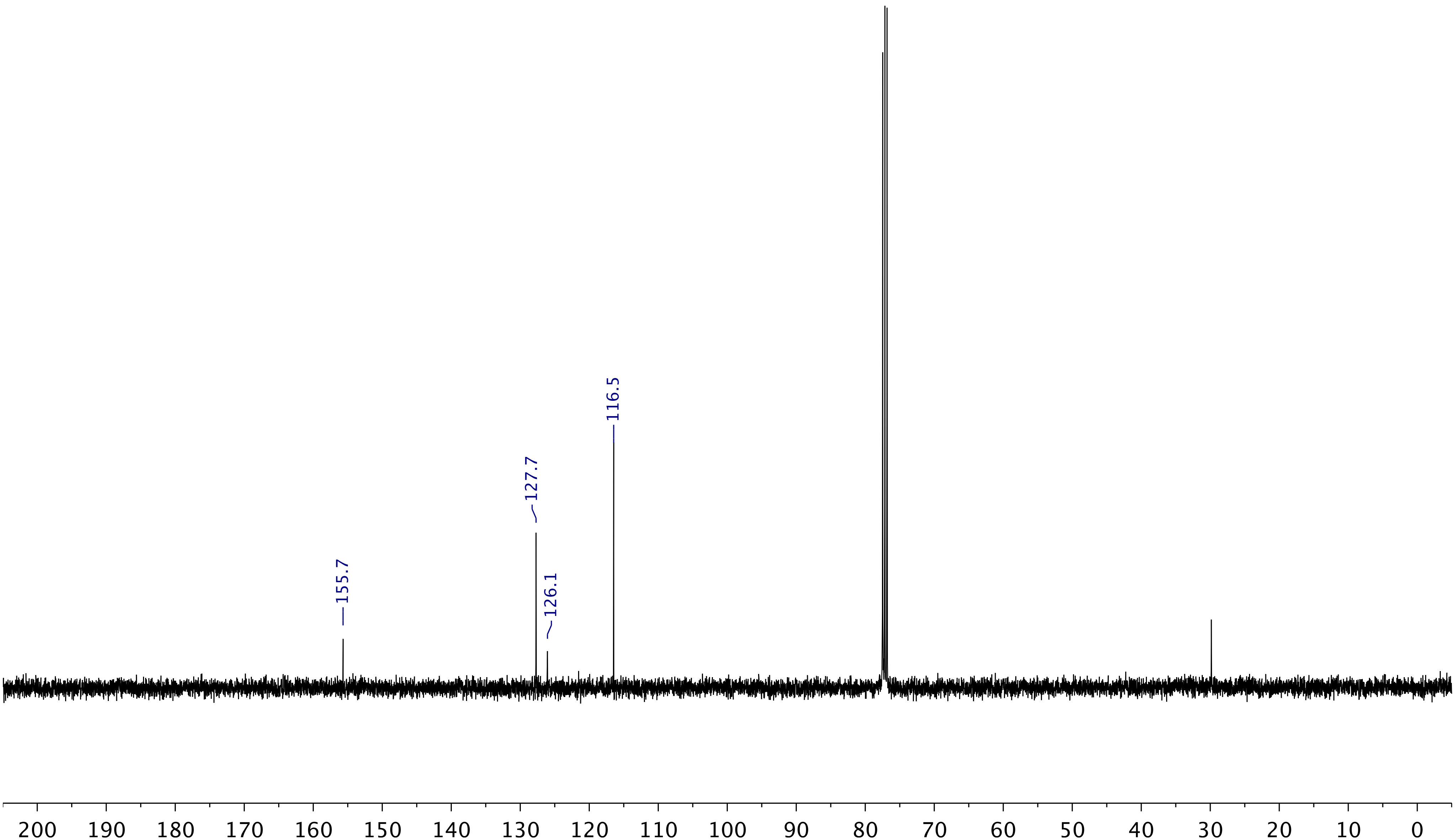
^{1}H NMR (400 MHz, $CDCl_3$)

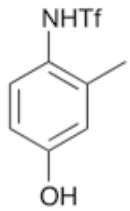




8

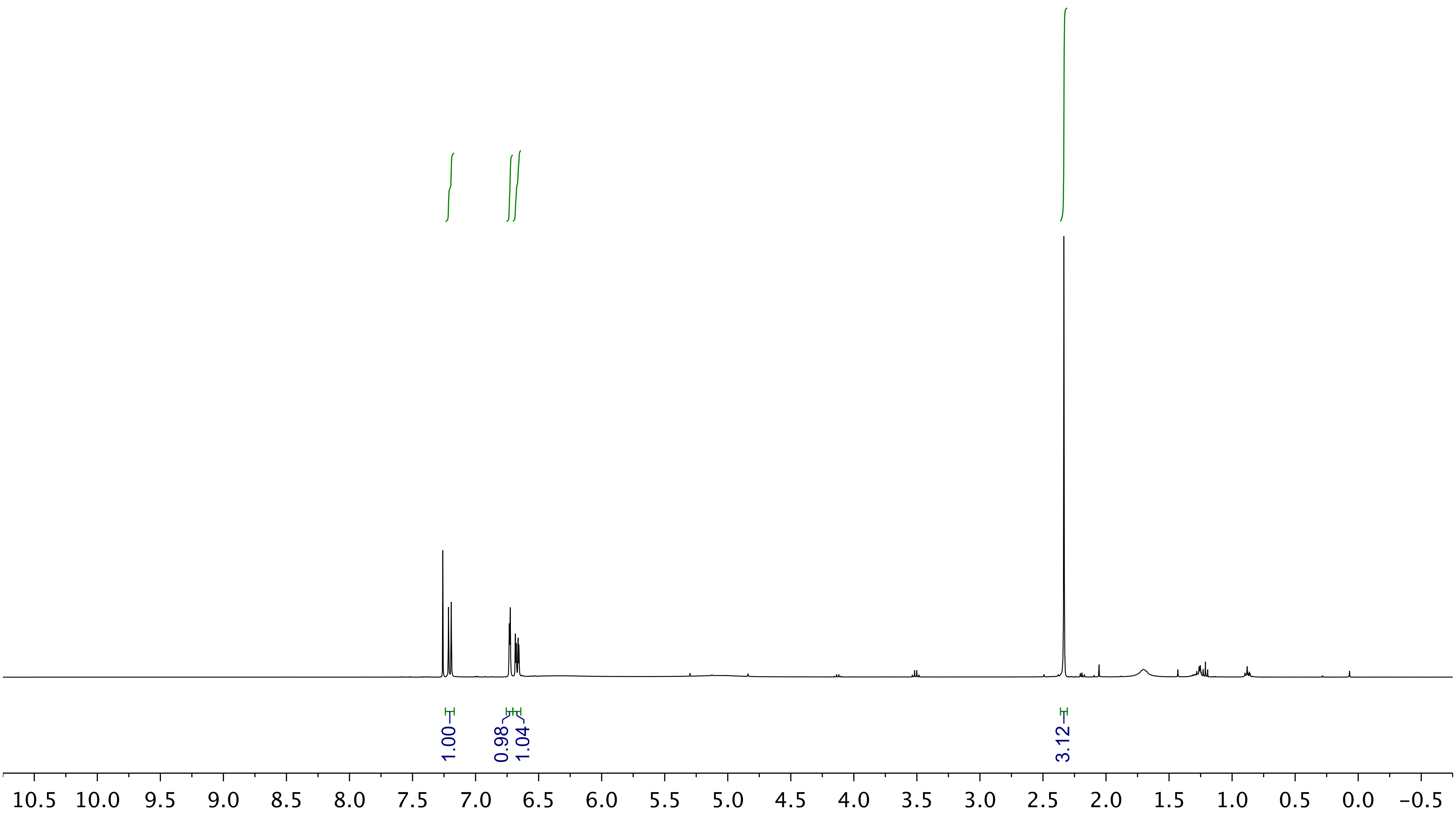
^{13}C NMR (101 MHz, $CDCl_3$)

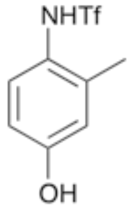




17

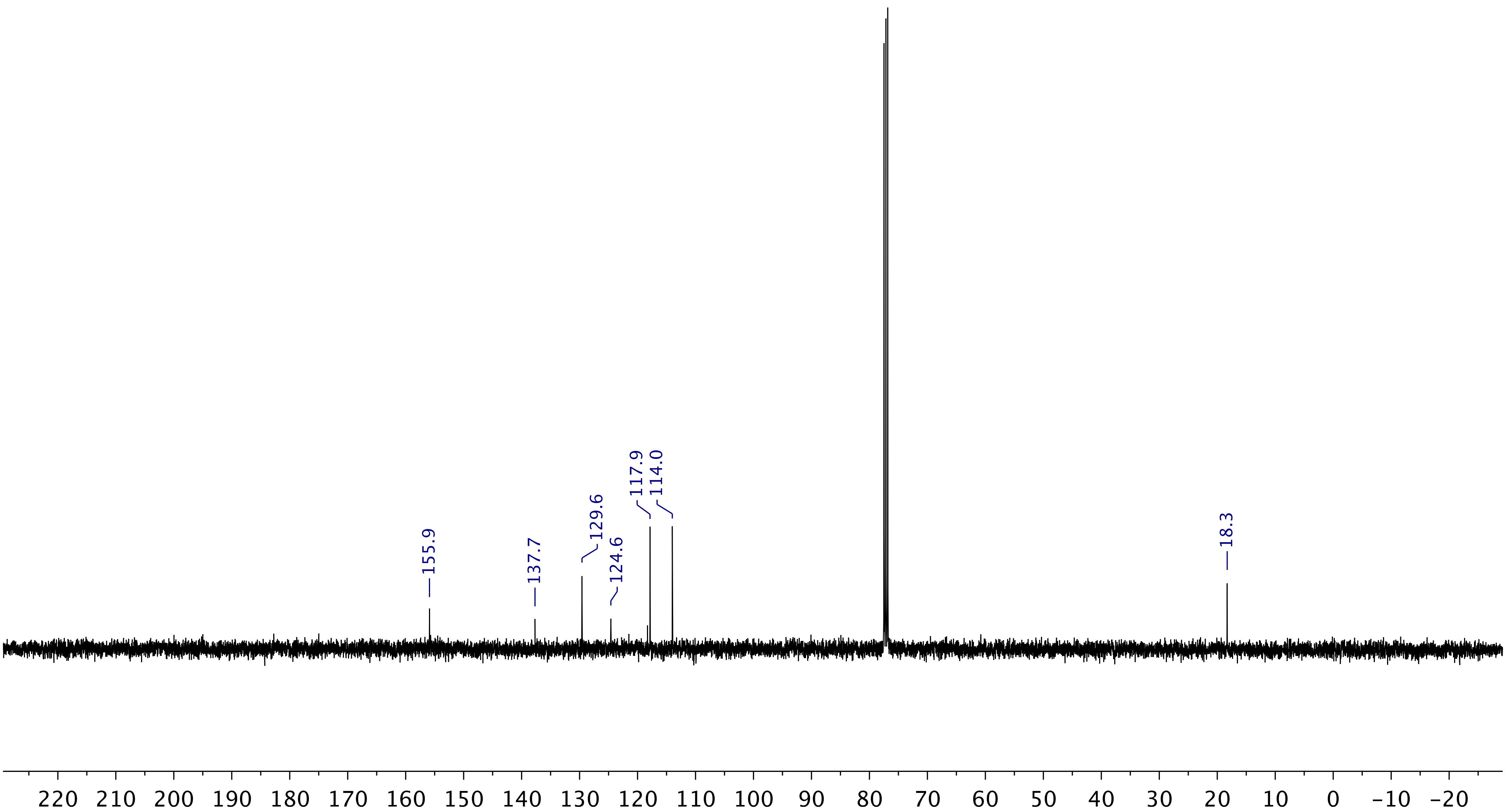
^{1}H NMR (400 MHz, $CDCl_3$)

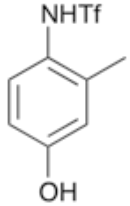




17

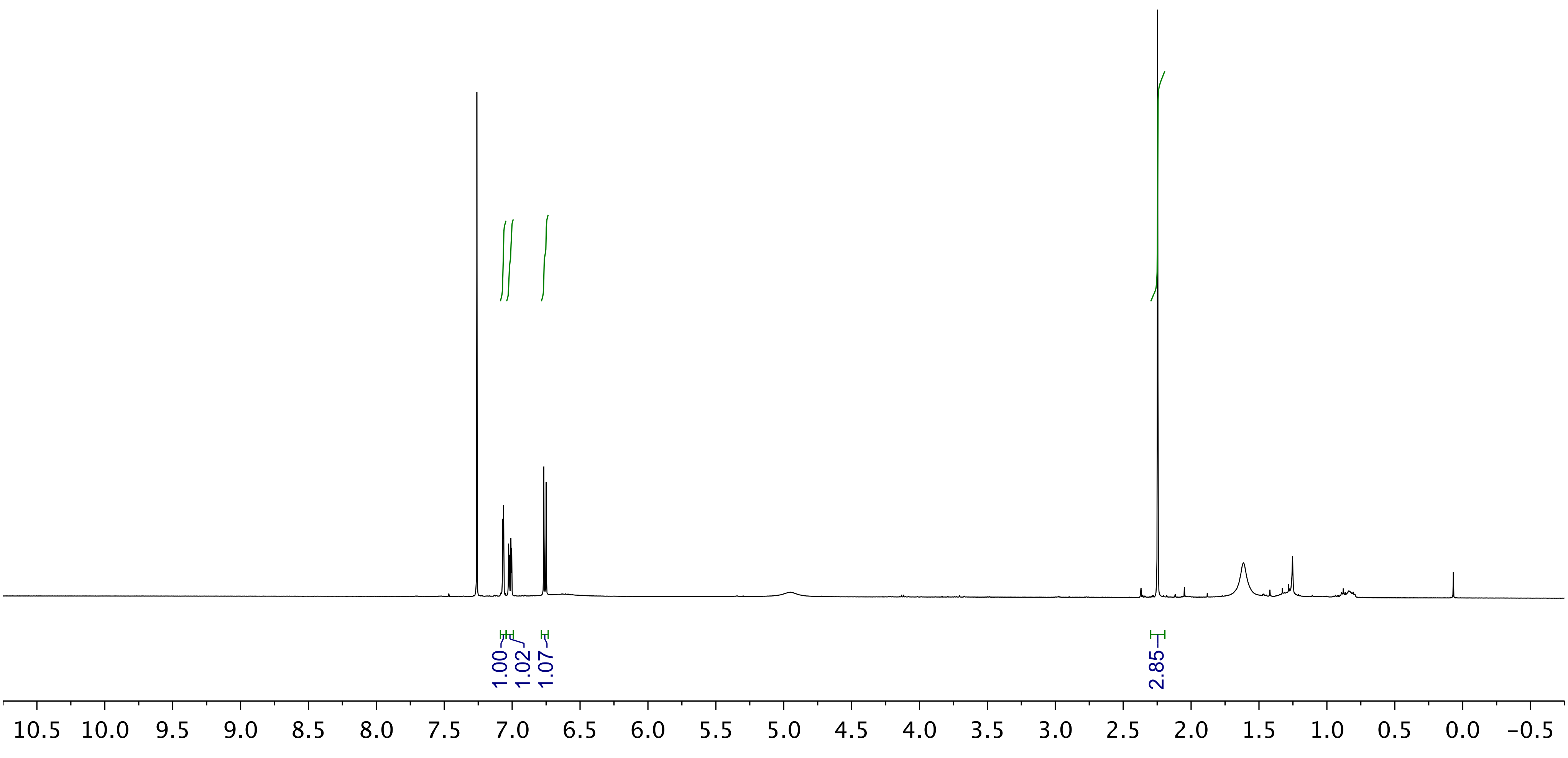
^{13}C NMR (101 MHz, $CDCl_3$)

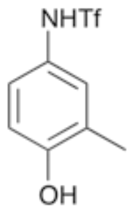




17

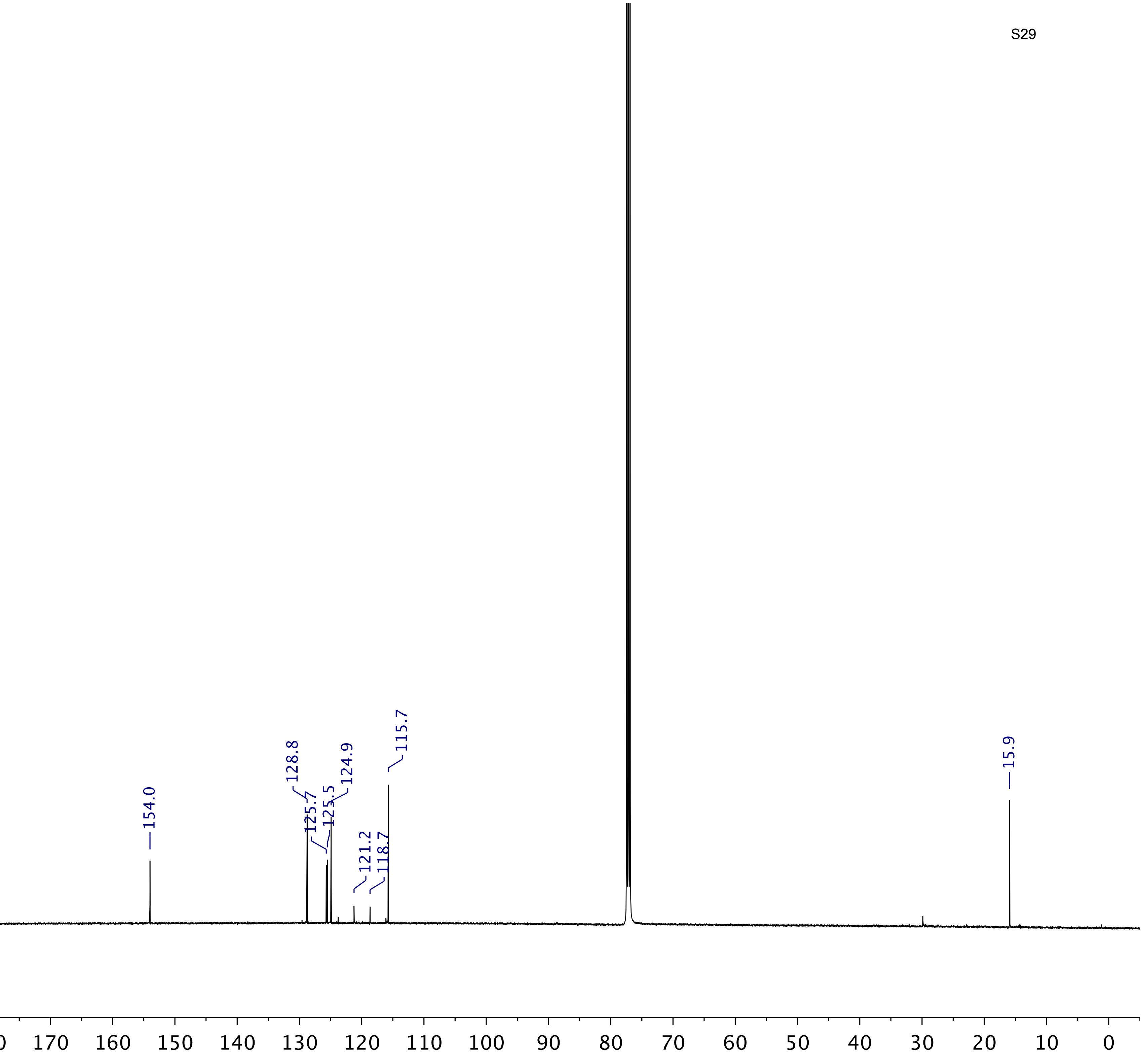
^{13}C NMR (101 MHz, $CDCl_3$)

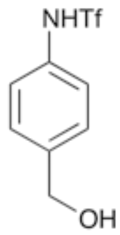




18

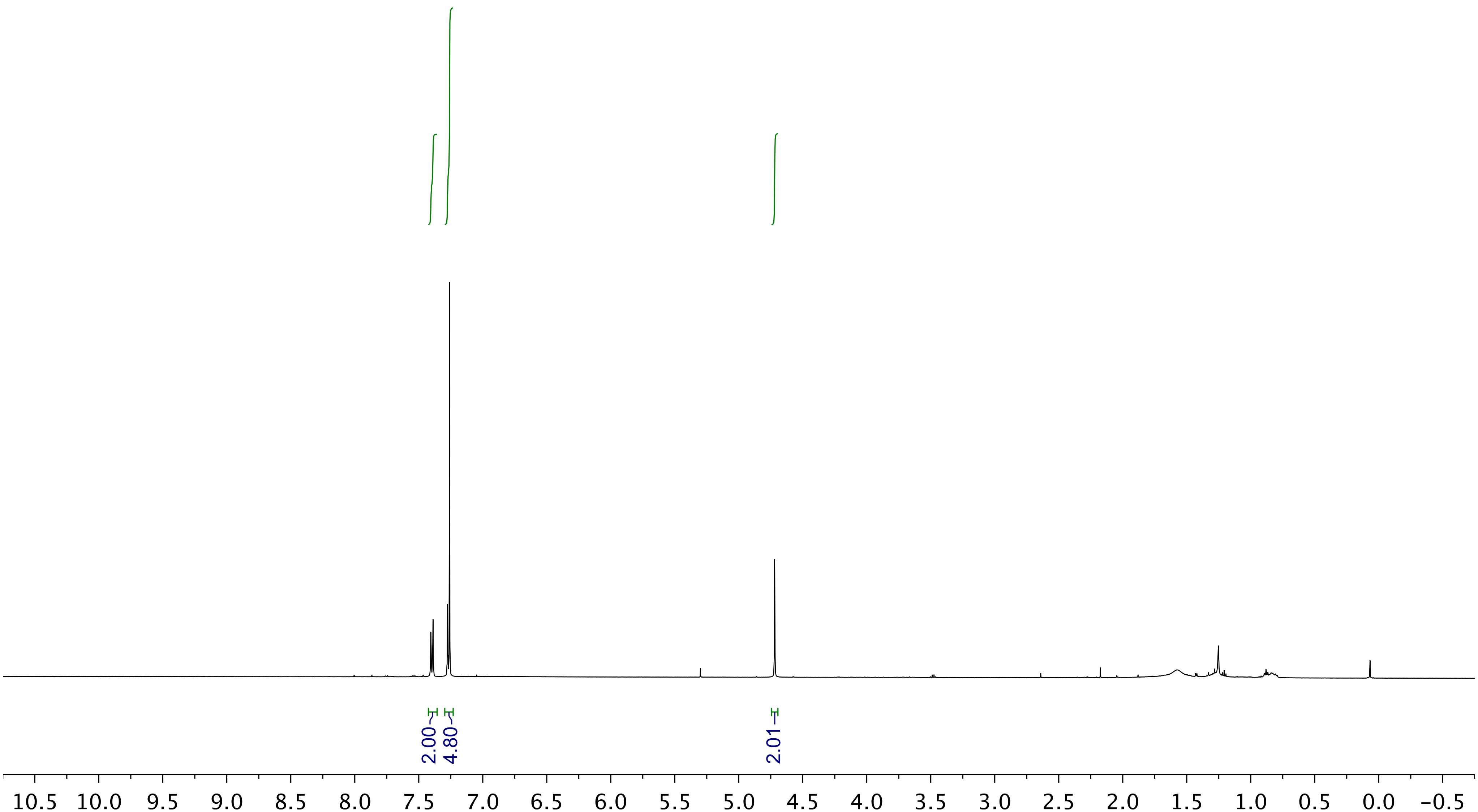
^{13}C NMR (126 MHz, $CDCl_3$)

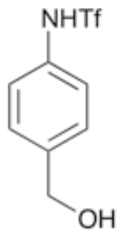




19

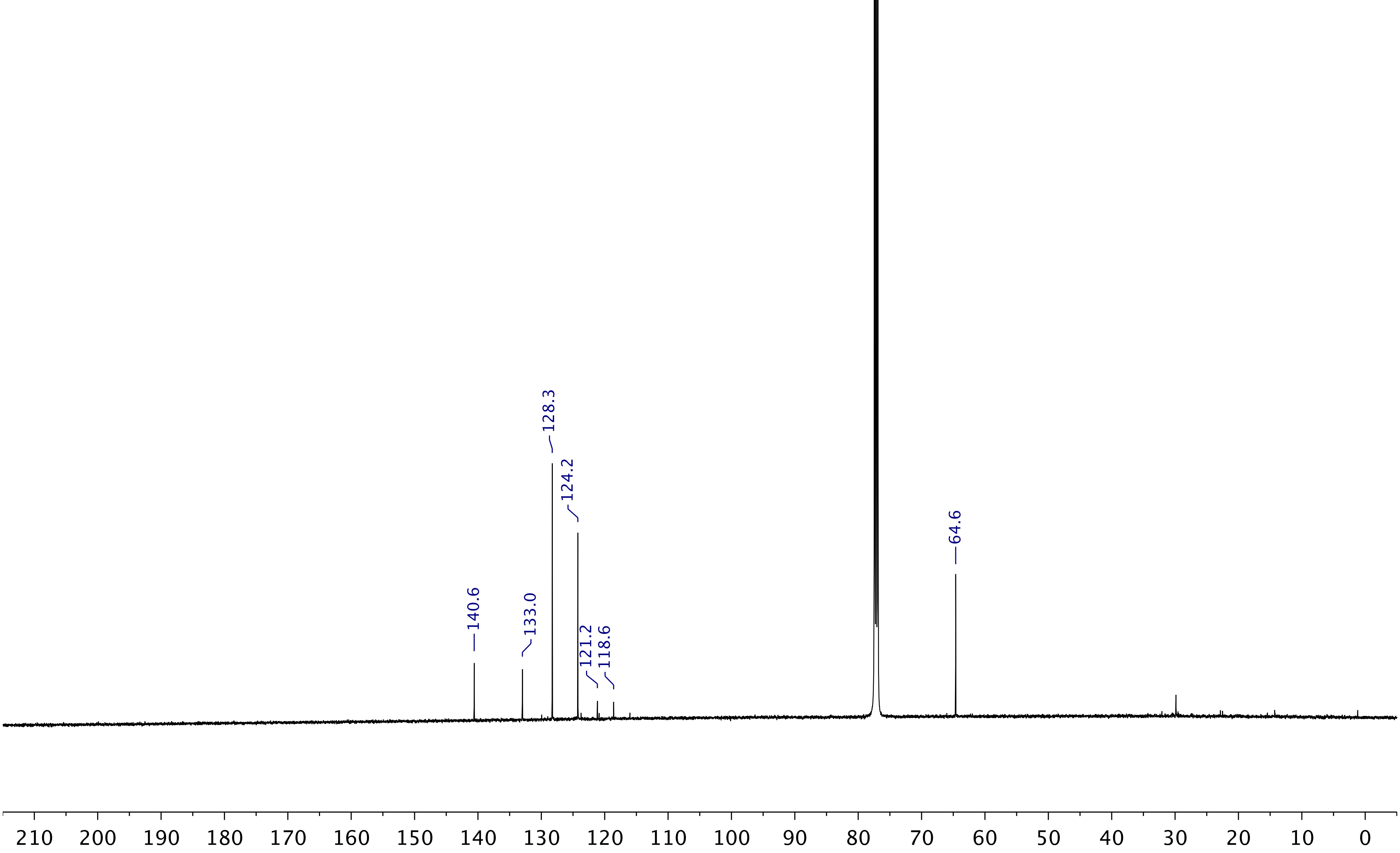
^1H NMR (500 MHz, $CDCl_3$)

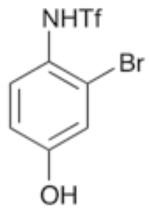




19

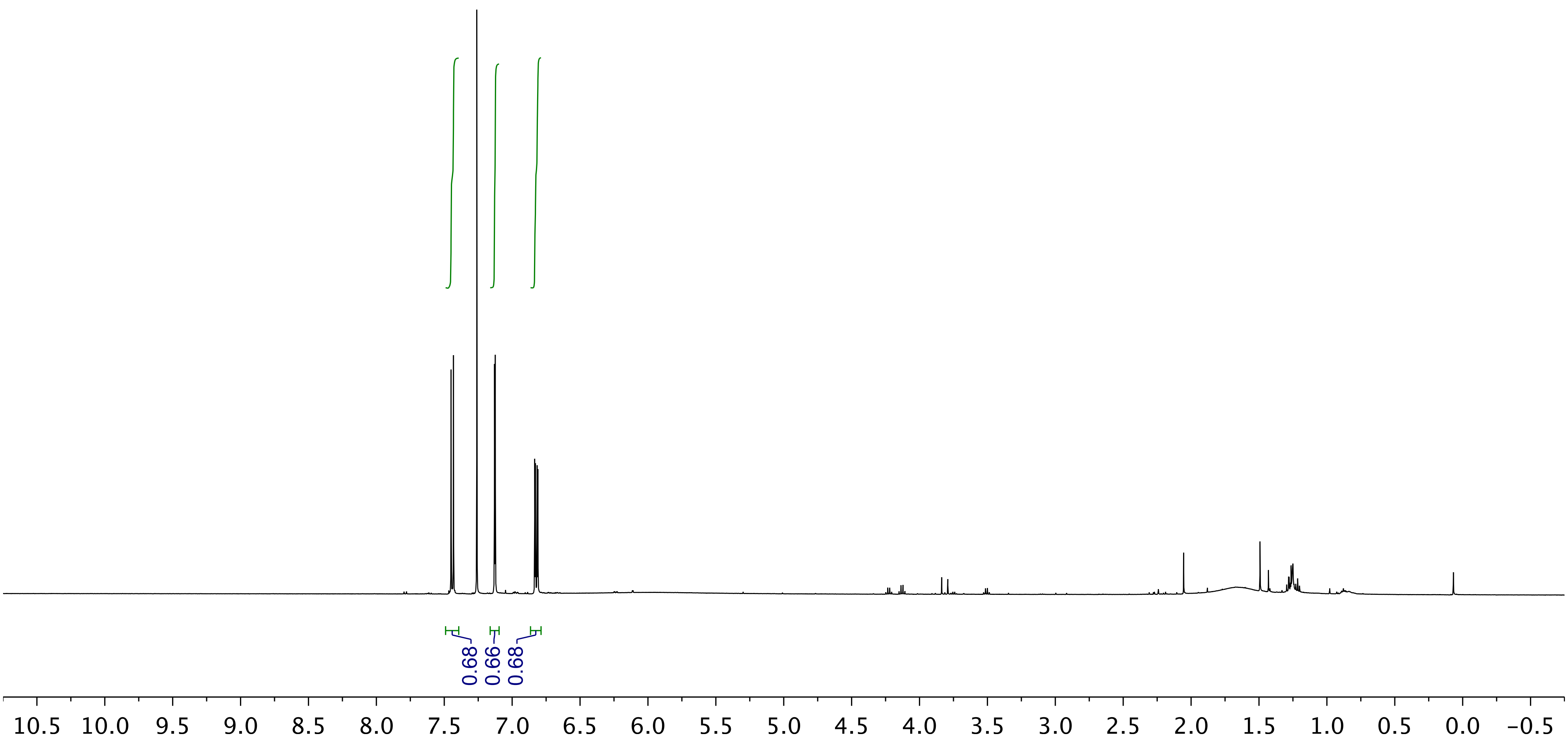
^{13}C NMR (126 MHz, $CDCl_3$)

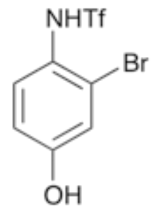




20

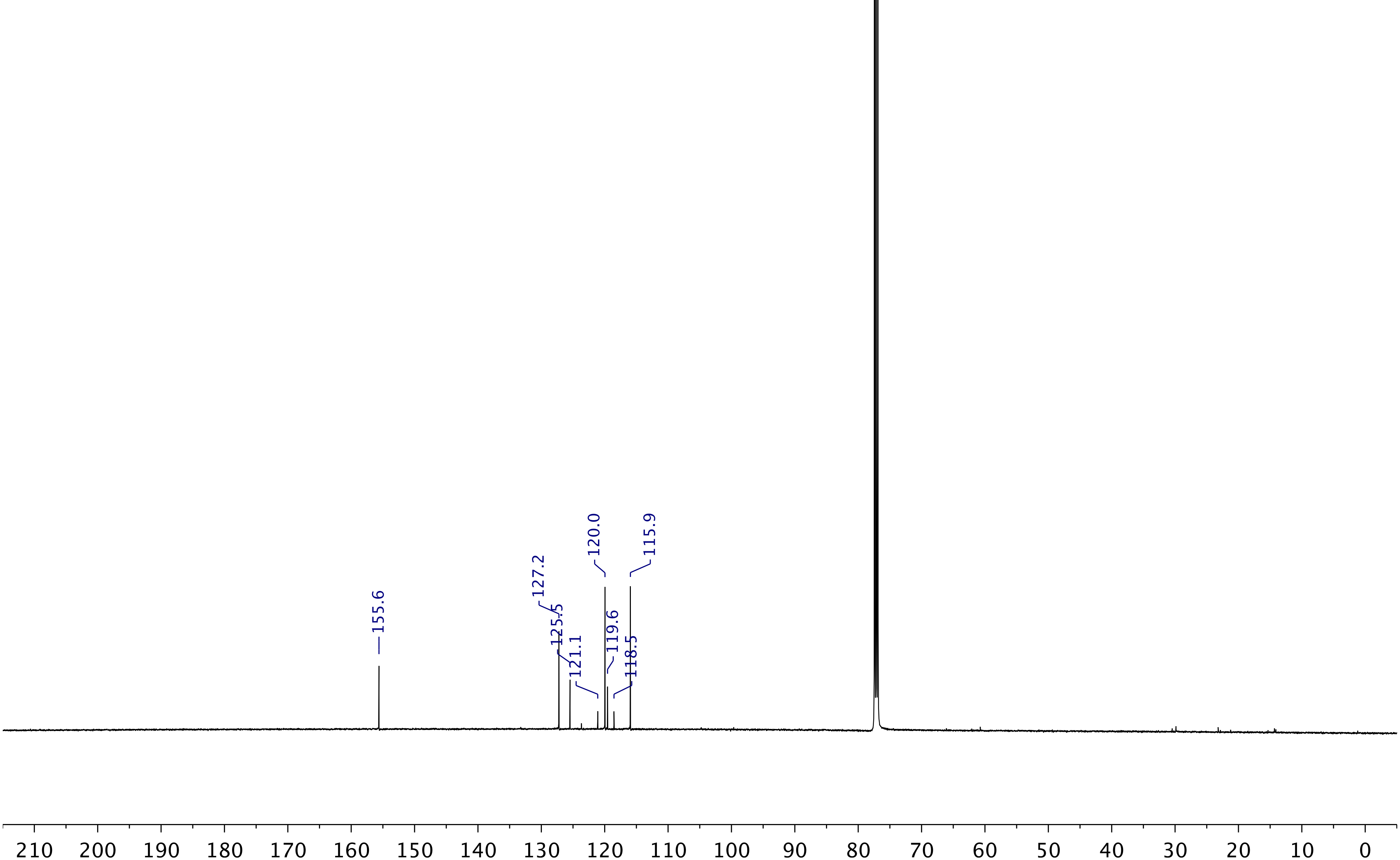
^{1}H NMR (500 MHz, $CDCl_3$)

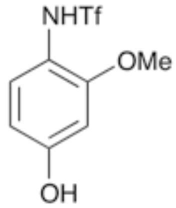




20

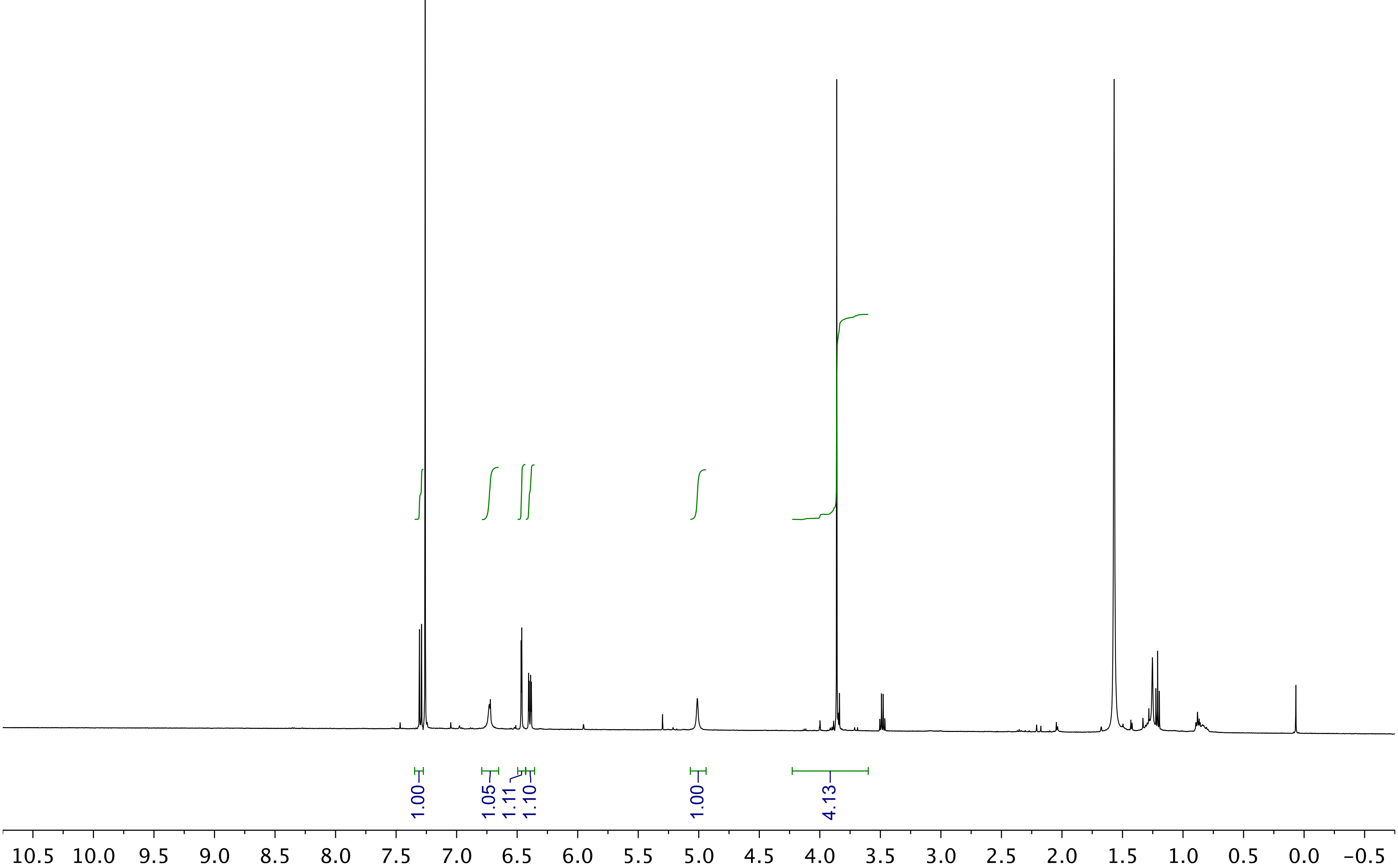
^{13}C NMR (126 MHz, $CDCl_3$)

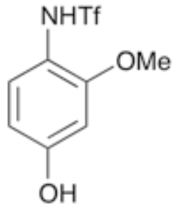




21

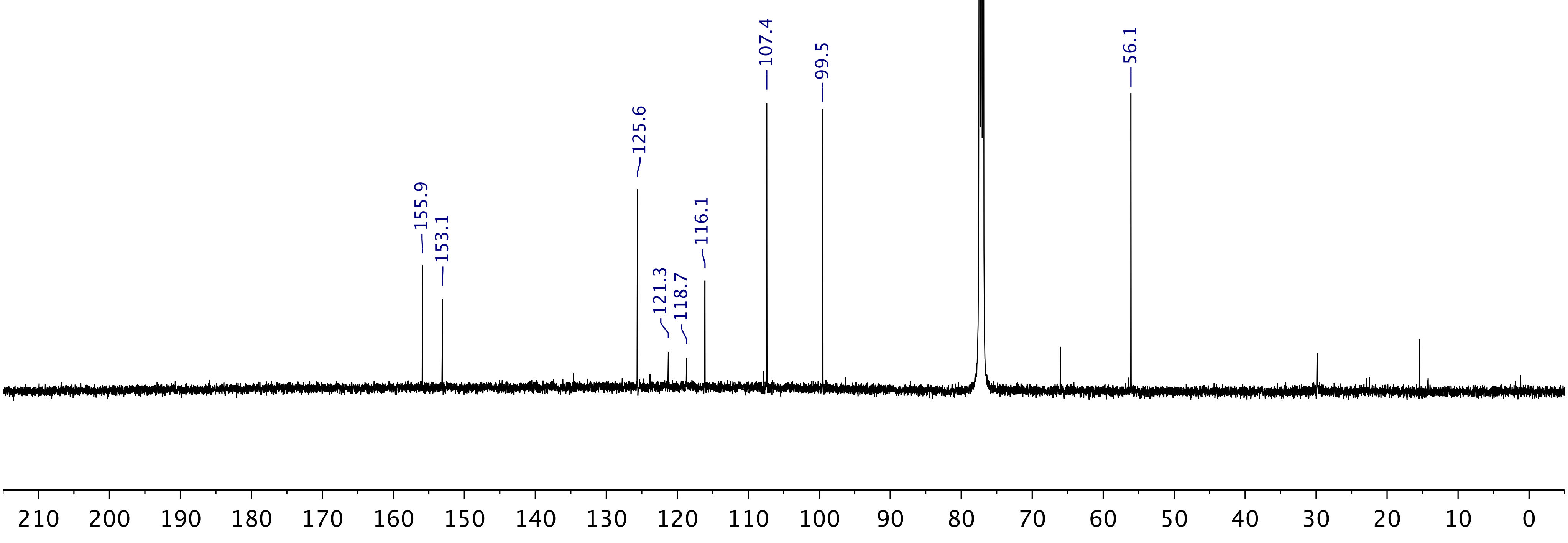
^{1}H NMR (500 MHz, $CDCl_3$)

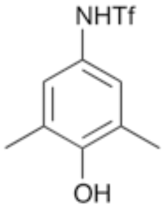




21

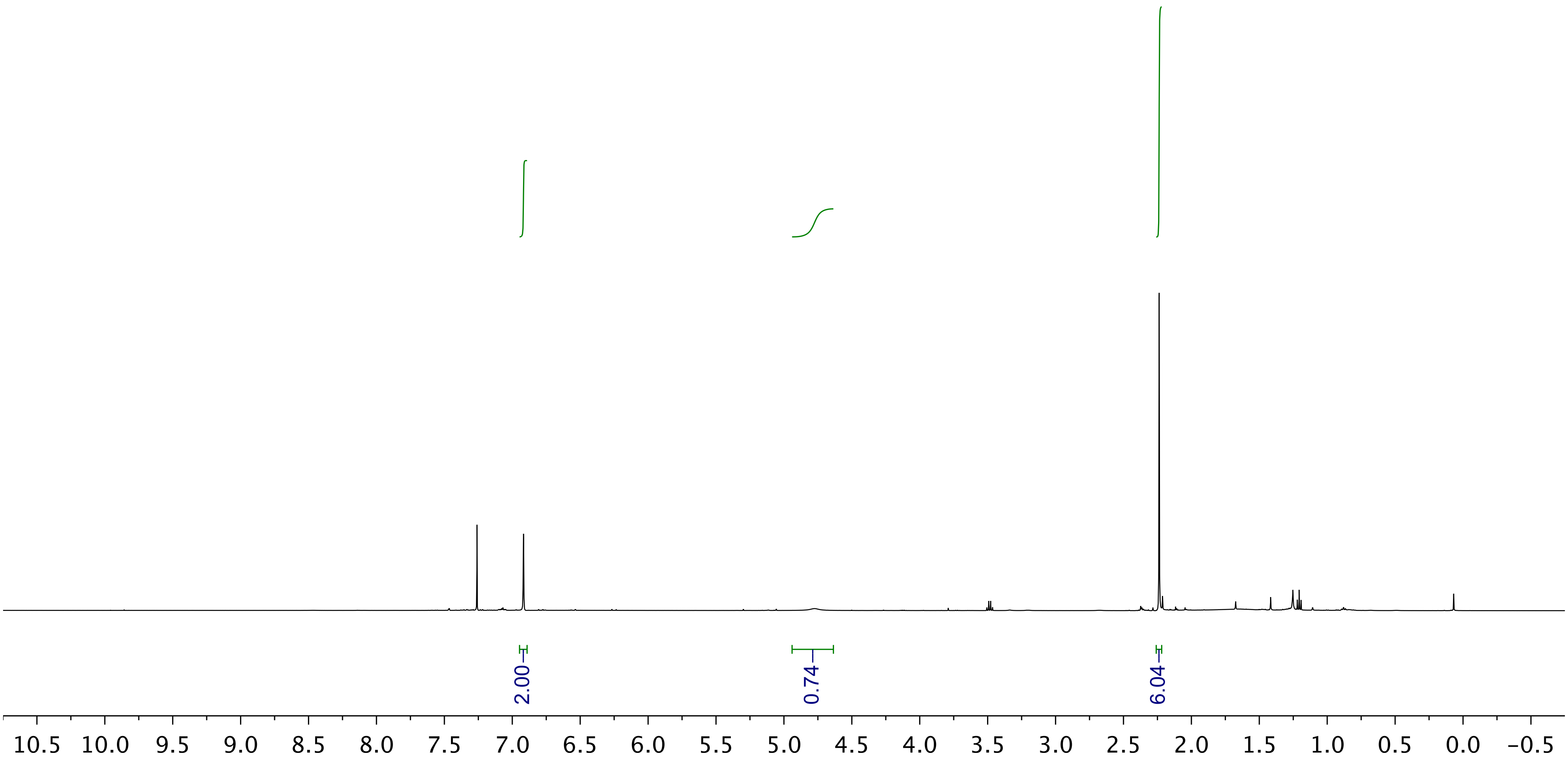
^{13}C NMR (126 MHz, $CDCl_3$)

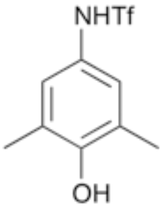




22

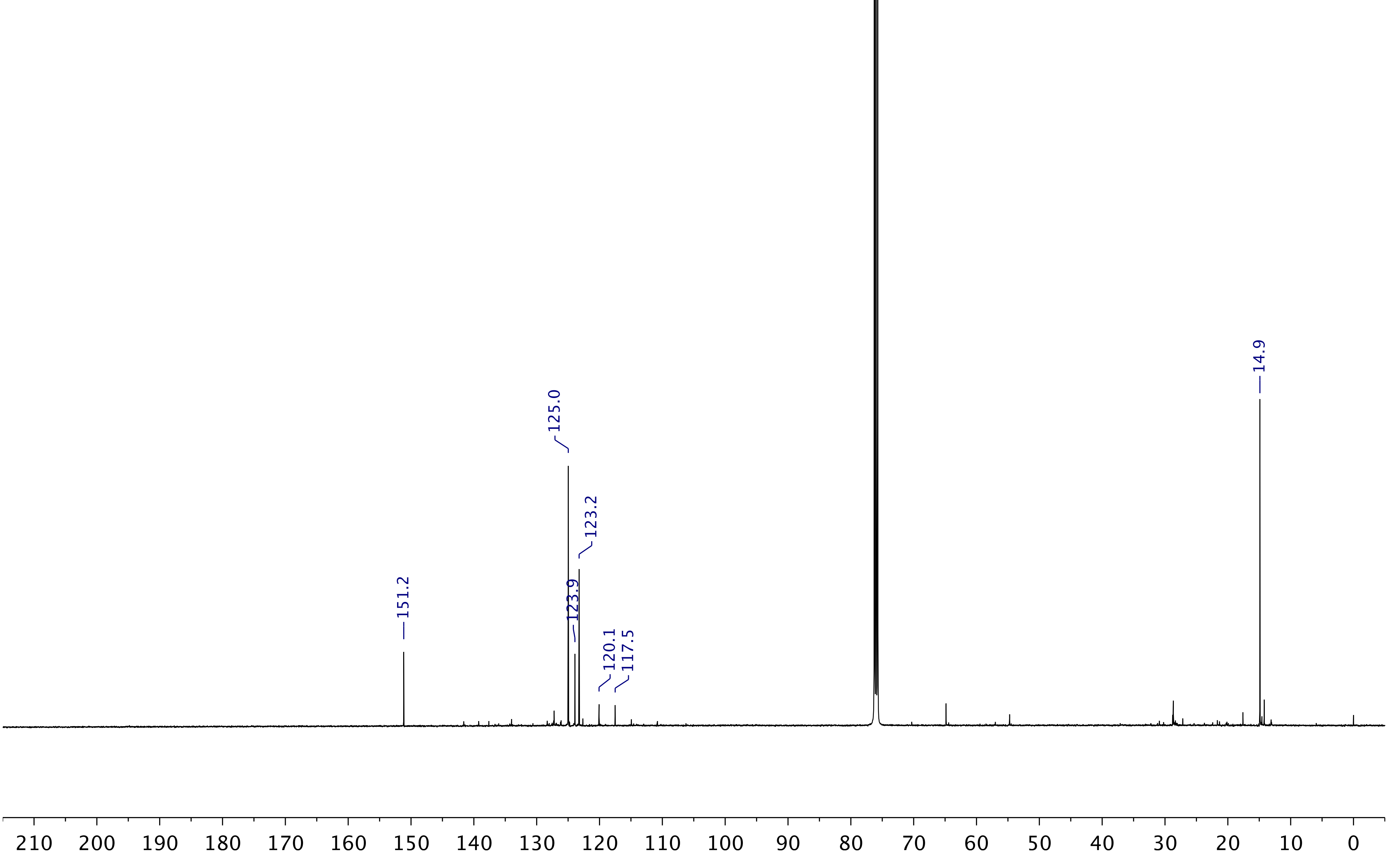
^{1}H NMR (500 MHz, $CDCl_3$)

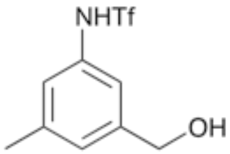




22

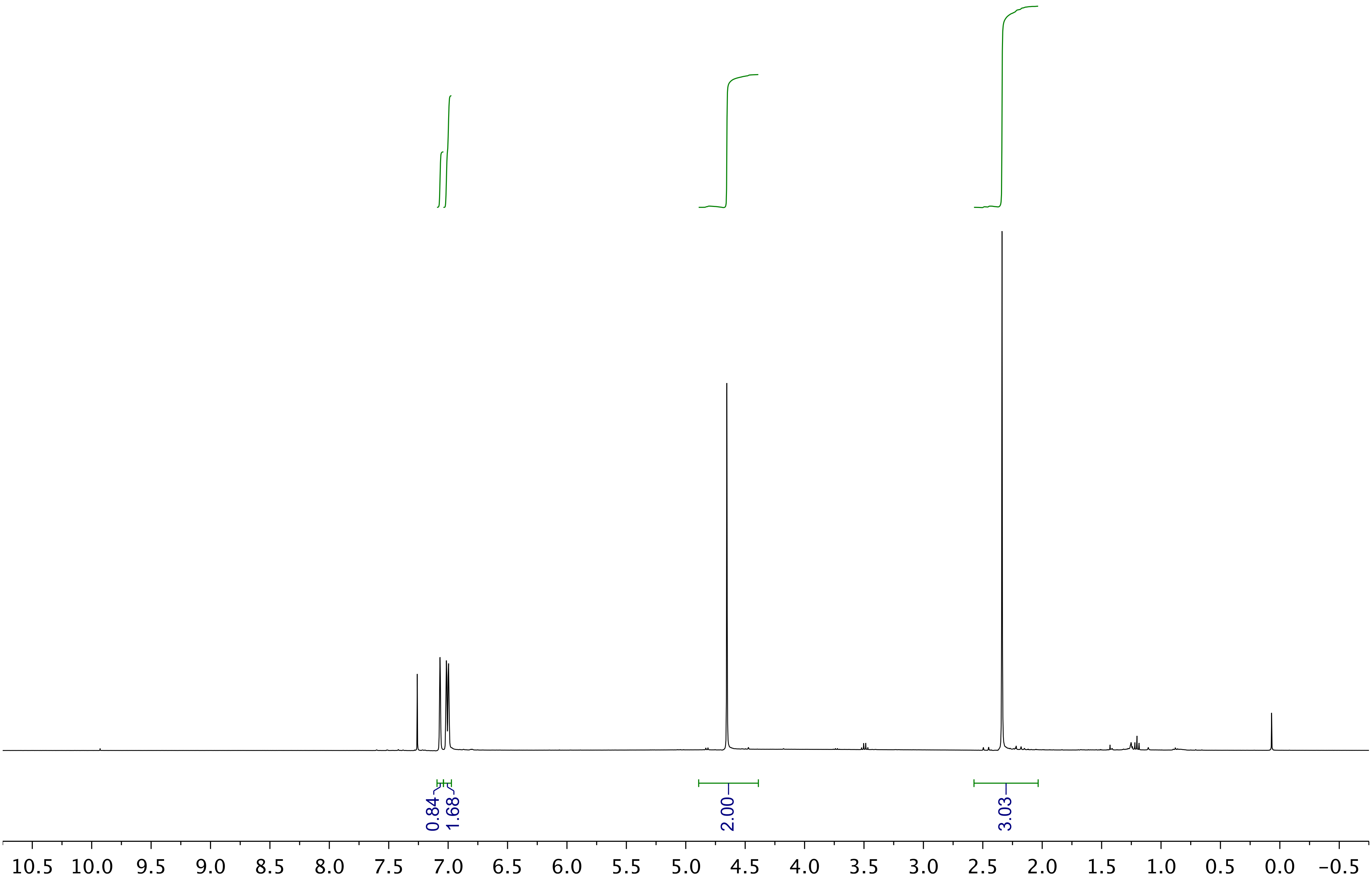
^{13}C NMR (126 MHz, $CDCl_3$)

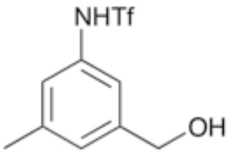




23

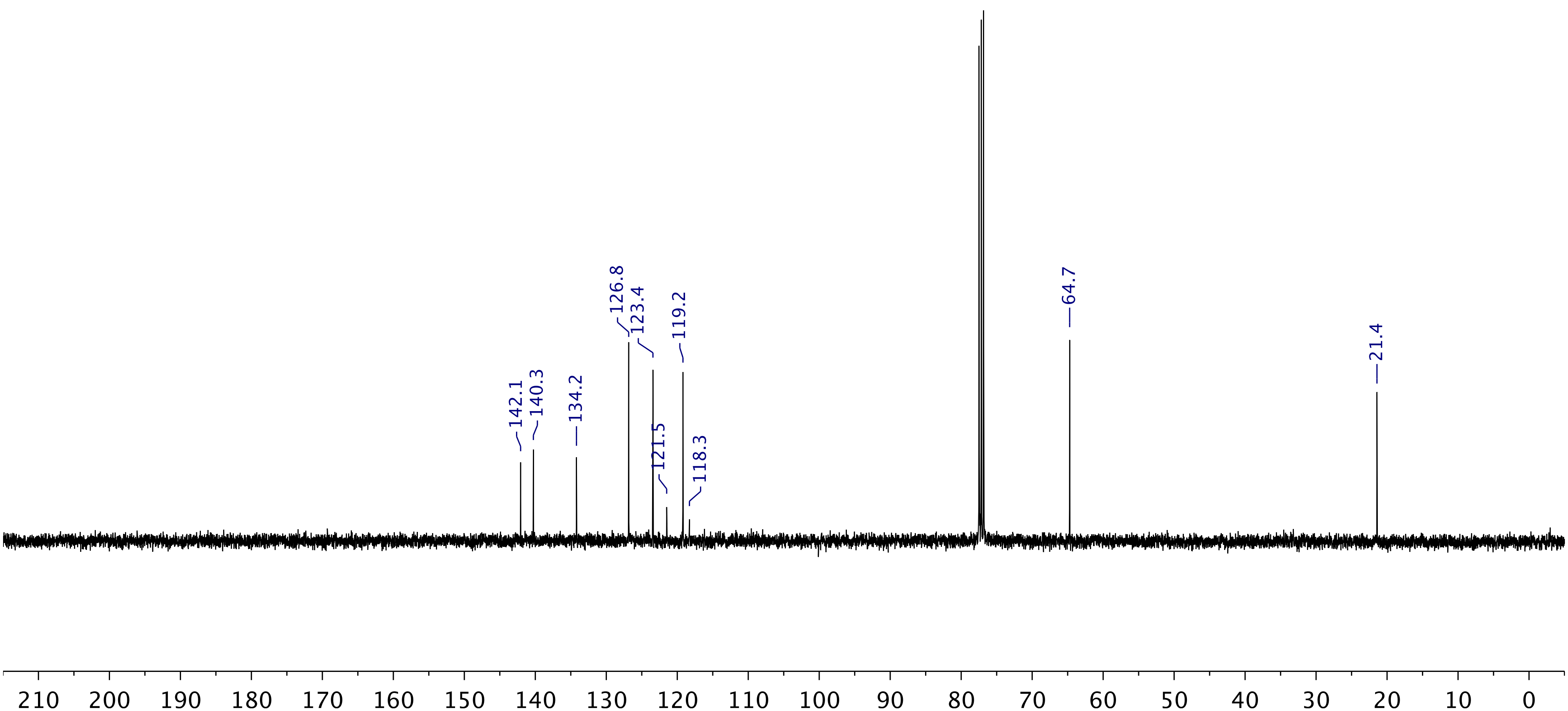
^{1}H NMR (400 MHz, $CDCl_3$)

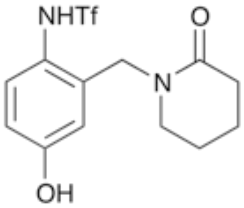




23

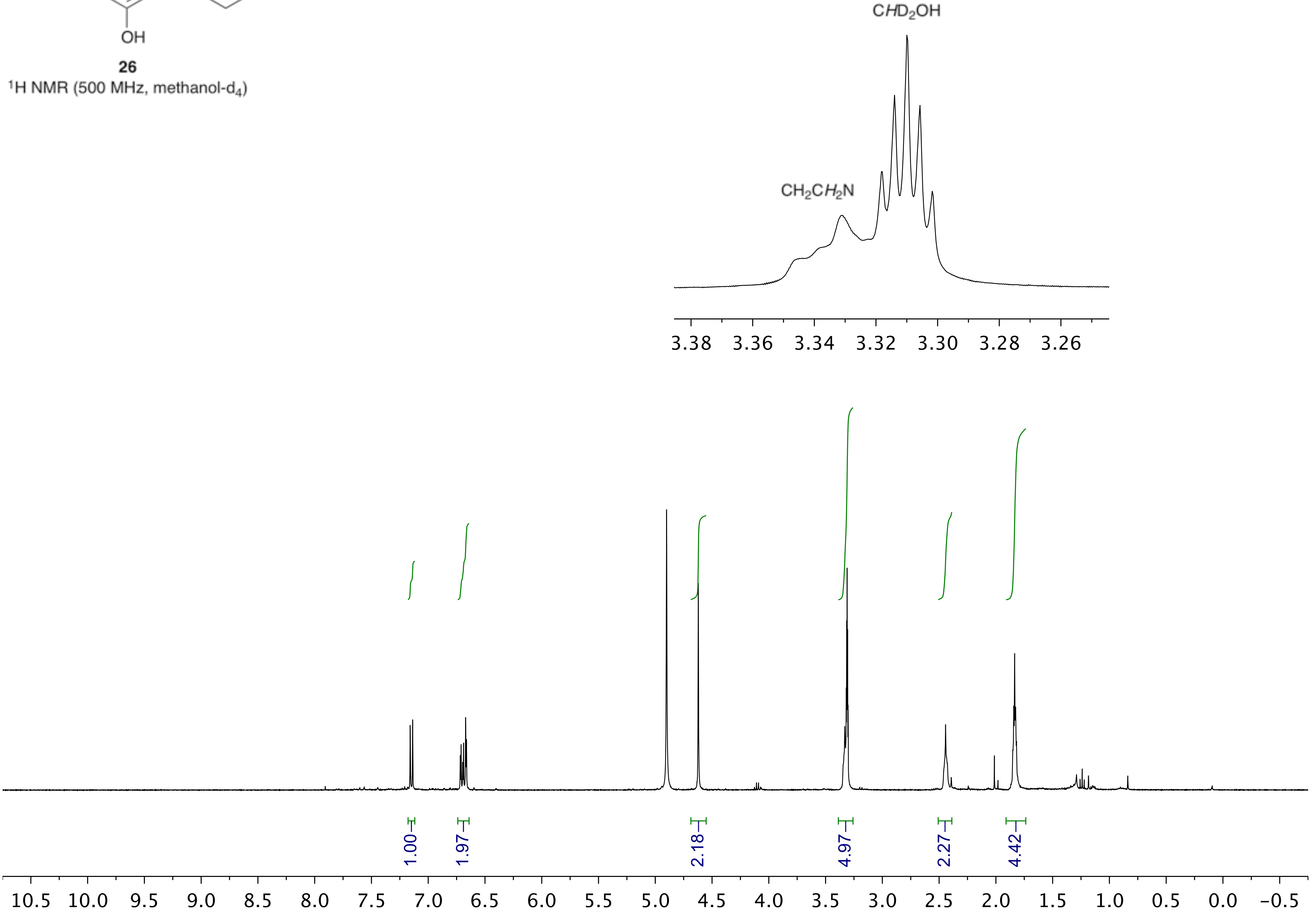
^{13}C NMR (101 MHz, $CDCl_3$)

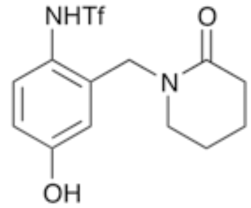




26

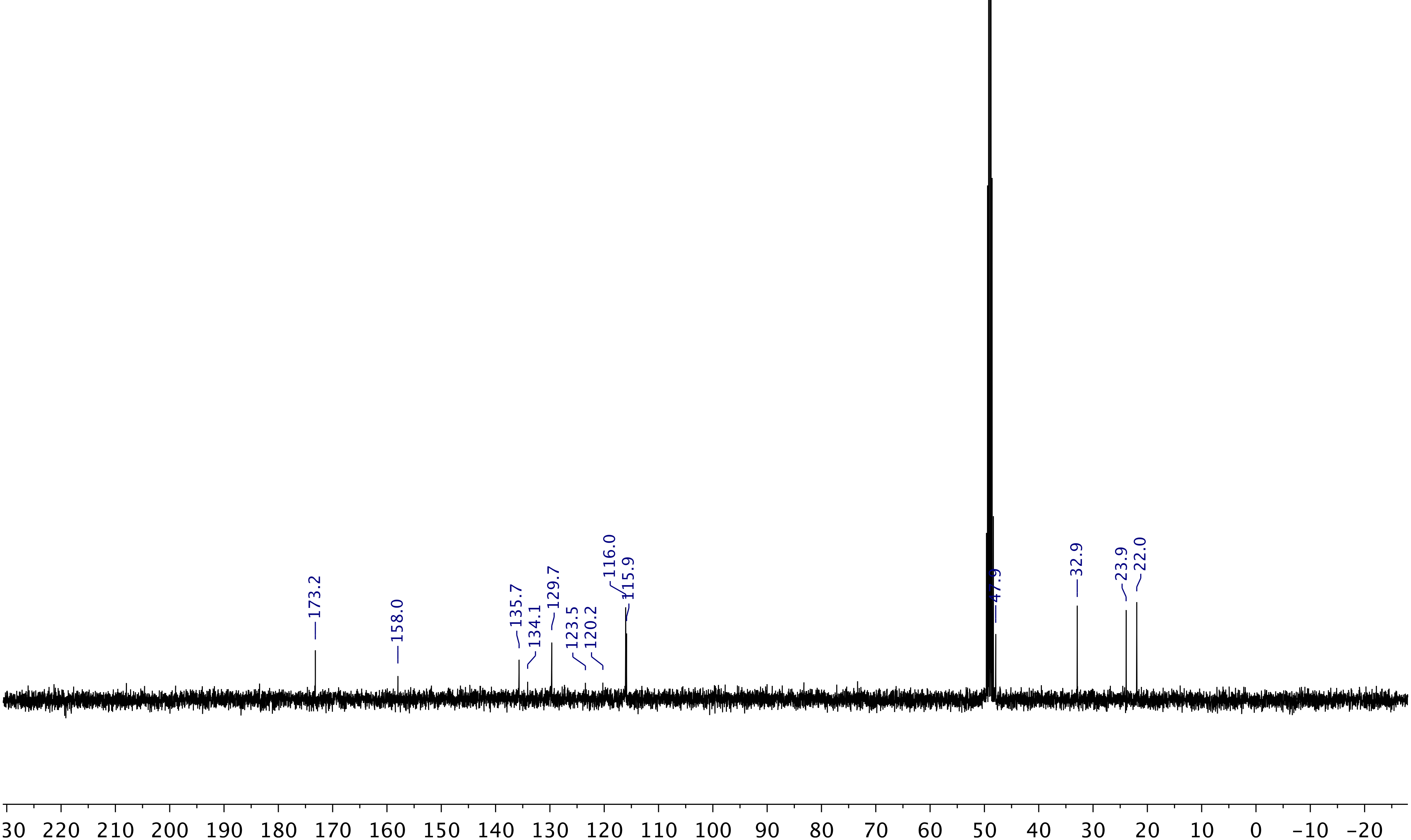
^{1}H NMR (500 MHz, methanol-d$_4$)

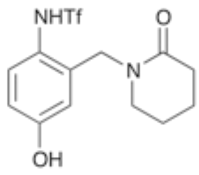




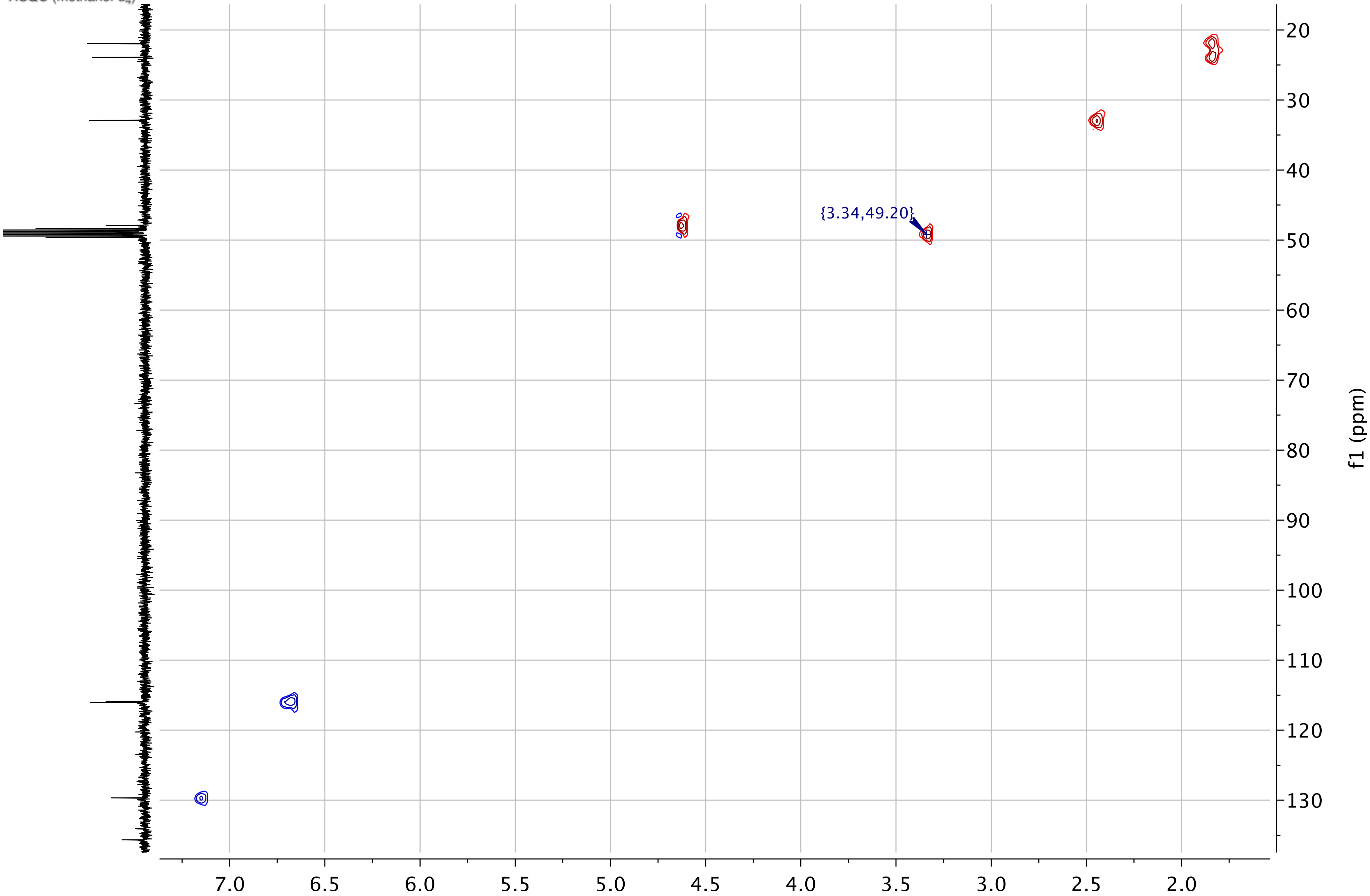
26

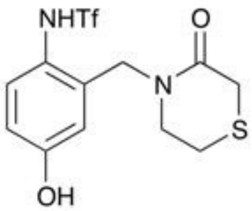
^{13}C NMR (126 MHz, methanol-d_4)





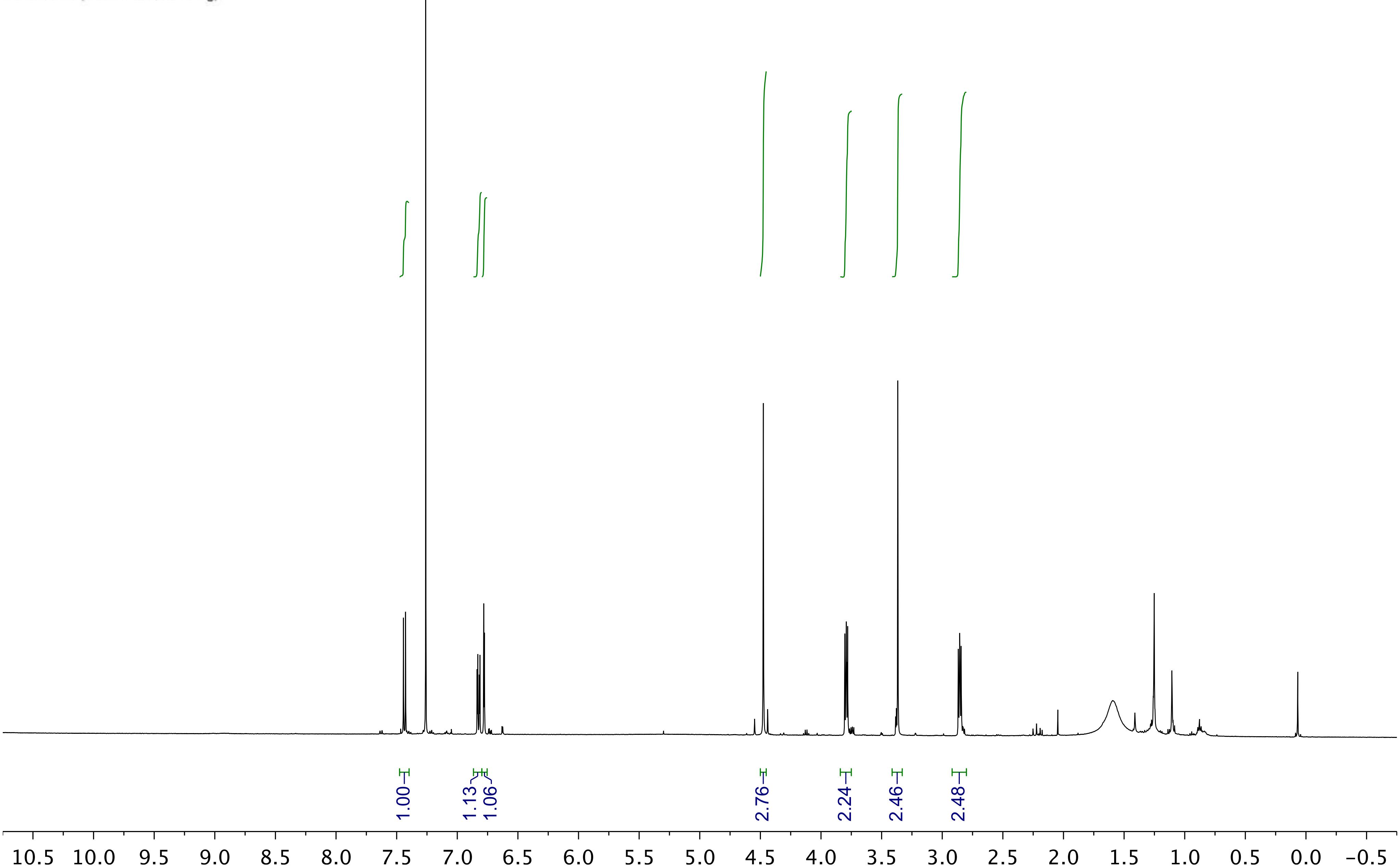
26
HSQC (methanol-d_4)

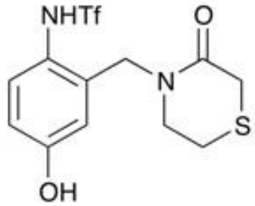




27

^{1}H NMR (500 MHz, $CDCl_3$)





27

^{13}C NMR (126 MHz, $CDCl_3$)