Supporting Information

Installation of Minimal Tetrazines Through Silver-mediated Liebeskind-Srogl Coupling with Arylboronic Acids

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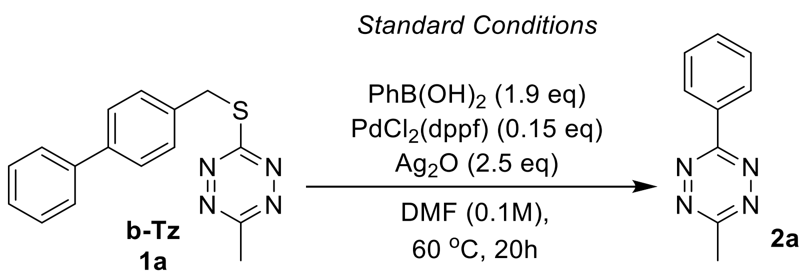
General Considerations

All cross-coupling reactions were conducted in flame dried schlenk flasks or 4 mL sealed vials. All other reactions were conducted in flame dried round-bottom flasks. Additionally, all reactions were run under N_2 unless otherwise noted. Silica gel chromatography was performed on Silicycle Siliaflash P60 silica gel (40-63 µm, 60Å) or on Yamazen reverse-phase prepacked Universal Column C18-silica gel (40-60 µm, 120Å). Automated column chromatography was performed on a Teledyne Isco Combiflash Rf. Commercially available chemicals were purchased from Sigma-Aldrich, Combi-Blocks, Acros Organics, Alfa Aesar, Oakwood Chemical, TCI Chemicals, and Frontier Scientific. Solvents were purchased from Thermo Fisher Chemical, Acros Organics, Decon Laboratories Inc., Mediatech, Inc., and Sigma-Aldrich. Human brain vascular pericytes and pericyte growth supplement were purchased from ScienCell Research Laboratories. Anhydrous dichloromethane was freshly prepared by an alumina column solvent purification system. Anhydrous tetrahydrofuran was freshly distilled from sodium/benzophenone. Deuterated solvents were purchased from Cambridge Isotope. A Bruker AV400 was used to record NMR spectra (^{1}H: 400 MHz, ^{13}C: 101 MHz, ^{19}F: 376 MHz). Chemical shifts are reported in ppm and all spectra are referenced to their residual non-deuterated solvent peaks as follows: $CDCl_3$ (^{1}H: 7.26 ppm, ^{13}C: 77.16 ppm), Methanol-*d*4 (^{1}H: 3.31 ppm, ^{13}C: 49.00 ppm), DMSO-*d*6 (^{1}H: 2.50 ppm, ^{13}C: 39.52 ppm). Coupling constants (*J*) are reported to the nearest 0.1 Hz. Multiplicities are reported as follow: singlet (s), doublet (d), triplet (t), quartet (q), pentet (pent), sextet (sext), heptet (hept), multiplet (m), 'broad' (br), and 'apparent' (app). ^{13}C NMR resonances are proton decoupled and an APT pulse sequence was used to determine type of carbon as follows: quaternary and methylene (C or CH_2) carbons appear 'up' and methine and methyl (CH or CH_3) carbons appear 'down'. Gas chromatography was carried out on a Shimadzu GC-2010 Plus with a Shimadzu AOC-20i auto-injector. Low resolution mass spectra were taken on a Water SQ detector 2 which was attached to a Waters Acquity H-Class UPLC. High resolution mass spectra were obtained using a Waters GCT Premier. Infrared spectra were taken on a Nicolet Magna IR 560 spectrometer. Stopped-flow kinetics were obtained using an Applied Photophysics Ltd. SX 18MV-R stopped-flow spectrophotometer. Differential Scanning Calorimetry was assayed with a Mettler-Toledo Differential Scanning Calorimeter 3+. Optical rotations were measures on a Jasco P-2000 polarimeter equipped with a tungsten-halogen lamp and a 589 nm filter. For fluorescence imagining, gels were scanned with a Typhoon FLA 9500 Biomolecular Imager (GE Healthcare) with the TAMRA channel with 532 nm excitation and a 575 nm long pass emission filter. To measure total protein loading, the gels were scanned with an Odyssey Imager (Li-COR) at the 700 nm channel.

Optimization Studies – Silver(I) Conditions

Table S-1 shows optimization conditions for the palladium catalyzed, silver(I) mediated cross-coupling of tetrazine **1a** and phenylboronic acid. Only the Pd/ligand combinations and silver(I) sources having the best results are shown. All other ligands and silver additives that were screened are listed on Table S-2. Yields were determined by GC using dodecane as an internal standard.



Standard Conditions	Yield %	Conversion %
–	**96**	**100**
Alteration From Standard	**Yield %**	**Conversion %**
No $PdCl_2$(dppf)	0	39
CyJohnPhos (0.4eq) / Pd_2dba_3 (0.15eq)	34	64
N-Xantphos (0.4eq) / Pd_2dba_3 (0.15eq)	26	46
Pd CyjohnPhos G4 (0.15eq)	37	38
No Ag_2O	0	43
Ag_2CO_3	88	93
CuTC	2	92
CuMeSal	4	77
Cu_2O	3	63
+ Cs_2CO_3	0	100
PhB-Pinacol Ester	4	68
$PhBF_3K$	5	74
DMSO	92	100
THF	14	44
ACN	65	77
Acetone	34	56
Toluene	2	55
Dichloroethane	7	9

Table S-1: Summarized optimization of select palladium/ligand sources, Cu/Ag (I) sources, basic conditions, boron nucleophiles, and solvents

Buchwald Ligands	Bidentate Ligands	Pd Precatalysts	Silver (I) Sources
CyJohnPhos*	XantPhos	$PdCl_2$(dppf)*	Ag_2O*
PhDavePhos	N-XantPhos*	Pd CyJohnPhos G4*	Ag_2CO_3*
DavePhos	Dppe	$Pd(PPh_3)_4$	Ag(OAc)
RuPhos	Dppp	$PdCl_2(PPh_3)_2$	Ag(trifluoroacetate)
Aphos	Dppb	PEPPSI Ipr	Ag(OTf)
Sphos	Dpppe	PEPPSI SIPr	Ag_3PO_4
MePhos	Dppf	SingaCycle A1	$AgPF_6$
Xphos	BINAP		$AgBF_4$
TrixiePhos	Tol-BINAP		Ag-Salicylate
BrettPhoss	DPEPhos		Ag-Lactate
Me4tBuXPhos	(+)-DIOP		Ag-2,4-Pentanedionate
CAS 857356-94-6	CAS 65038-36-0		Ag-Cyclohexanebutyrate
CAS 1000171-05-0	CAS 121954-50-5		

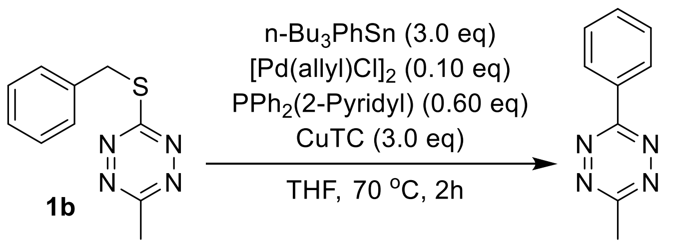
*best of the group, see Table S-1

Table S-2: Listing of ligands, precatalysts, and silver (I) sources that were screened

Optimization Studies – Copper(I) Conditions (Arylstannane nucleophile)

Table S-3 shows optimization conditions for the palladium catalyzed, copper(I) mediated cross-coupling of tetrazine **1b** and tributylphenylstannane. Figure S-4 shows screening of tetrazines **1a**, **1b**, and **1g**. Figure S-5 shows ligand screening using Pd_2dba_3 as a catalyst. Yields were determined by GC using dodecane as an internal standard ([a]isolated yield).

Standard Conditions



Standard Conditions	Yield %
–	58(56[a])
Alteration From Standard	**Yield %**
BrettPhos Pd G3 (20 mol%)	43
$Pd(PhCN)_2Cl_2$ (20 mol%)	35
$PdCl_2$ (20 mol%)	Trace
$Pd(MeCN)_2Cl_2$ (20 mol%)	43
$Pd(OAc)_2$ (20 mol%)	45
Pd_2dba_3 (10 mol%)	49
Pd_2dba_3 (10 mol%), $P(2\text{-furyl})_3$ (40 mol%), DMF	22
Pd_2dba_3 (10 mol%), $AsPh_3$ (60 mol%), DMF	42
Pd_2dba_3 (10 mol%), PPh_3 (60 mol%), DMF	11
Pd_2dba_3 (10 mol%), $AsPh_3$ (60 mol%), DMF, no CuTC	trace
Pd_2dba_3 (10 mol%), $AsPh_3$ (60 mol%), DMF, CuI	trace
Pd_2dba_3 (10 mol%) , $AsPh_3$ (60 mol%), DMF, CuMeSal	12
Pd_2dba_3 (10 mol%) , $AsPh_3$ (60 mol%), DMF, $Cu(I)Ph_2PO_2$	10

Table S-3: Optimization of copper(I) cross-coupling with tributylphenylstannane

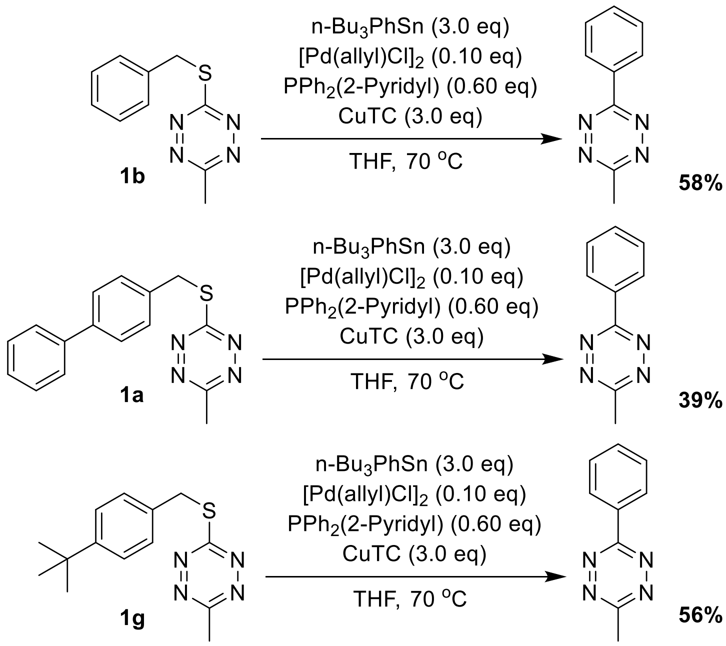


Figure S-4: Tetrazine screening for copper(I) cross-coupling with tributylphenylstannane

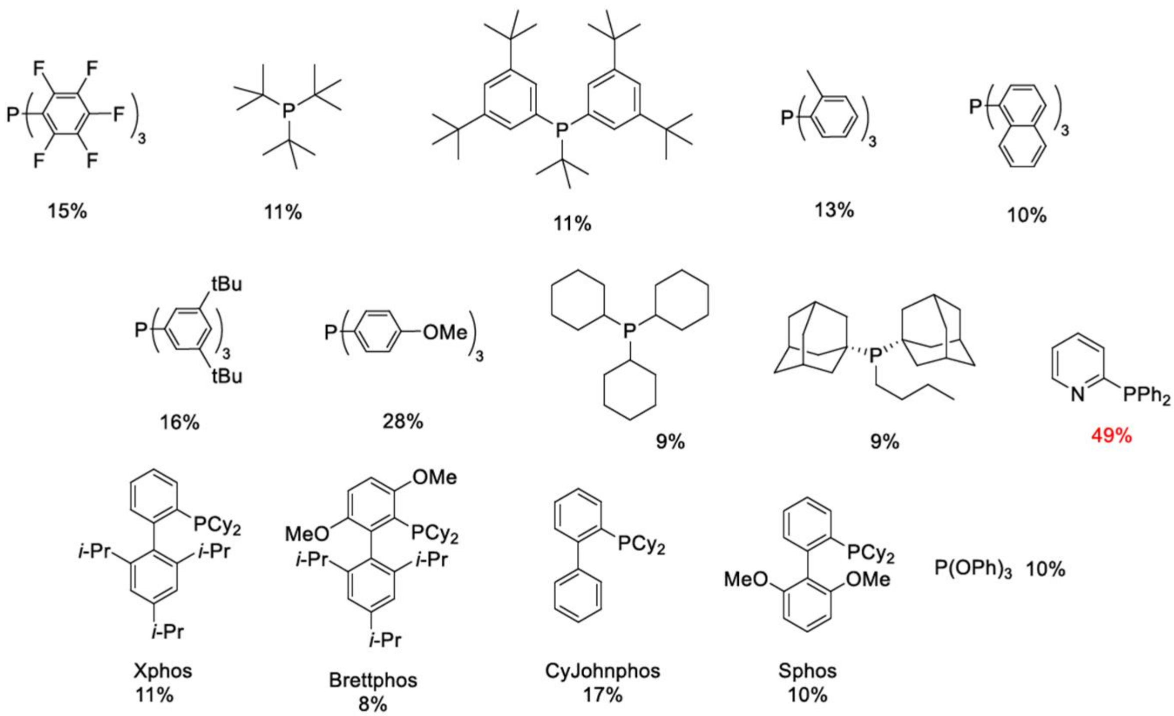
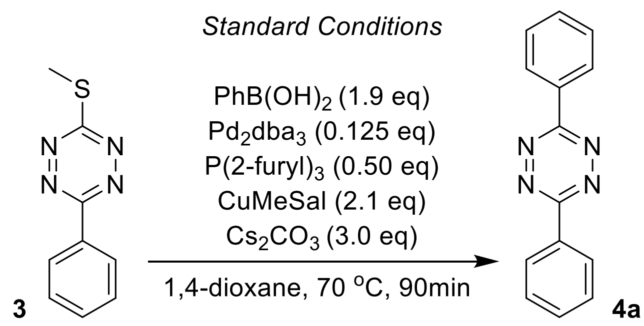


Figure S-5: Ligand screening for copper(I) cross-coupling with tributylphenylstannane and Pd_2dba_3 (10 mol%)

Optimization Studies – Copper(I) Conditions (Arylboronic acid nucleophile)

Table S-6 shows optimization conditions for the palladium catalyzed, copper(I) mediated cross-coupling of tetrazine **3** and phenylboronic acid. These conditions required a base. All other ligands that were screened are listed on Table S-7. Optimized conditions were then used on *S*-benzylic tetrazines **1a** and **1b** as shown on figure S-8. Yields were determined by GC using dodecane as an internal standard.



Standard Conditions	Yield %
–	**80**
Alteration From Standard	**Yield %**
$Pd(OAc)_2$ (20 mol%)	59
$[Pd(allyl)Cl]_2$ (10 mol%)	64
CuMeSal, Toluene	52
CuTC, Toluene	26
THF	68
DMF	Trace
ACN	11
Et_3N	32
CsF	58
LiOtBu	48
K_2CO_3	61
K_3PO_4	22
KHMDS	26

Table S-6: Optimization of copper(I) cross-coupling with phenylboronic acid

Phosphine Ligands	Buchwald Ligands
PPh_3	CyJohnPhos
$AsPh_3$	sPhos
PCy_3	DavePhos
$P(n\text{-}Bu)_3$	JohnPhos
$P(t\text{-}Bu)_3$	XPhos
PPh_2(2-pyridyl)	BrettPhos
Tris(4-methoxyphenyl)phosphine	RuPhos
Tri(*o*-tolyl)phosphine	PhDavePhos
	Me_4-t-Bu-XPhos
	CAS 1000171-05-0

Table S-7: Listing of ligands that were screened

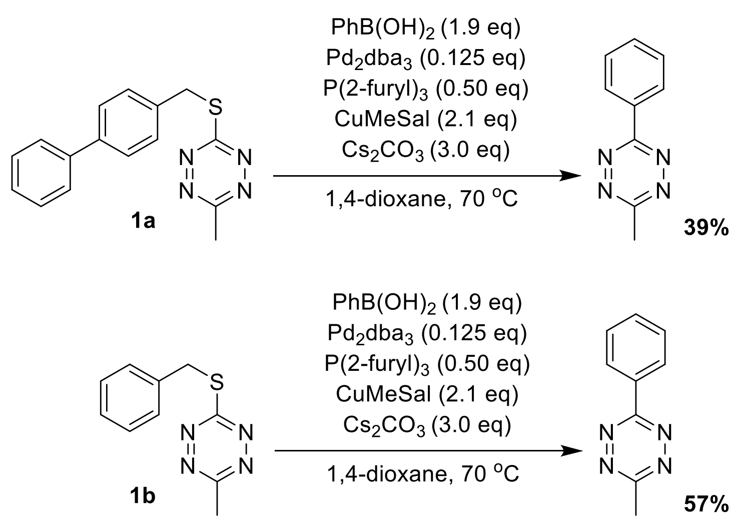


Figure S-8: *S*-benzylic tetrazine screening for copper(I) cross-coupling with phenylboronic acid

Differential Scanning Calorimetry (DSC)

DSC data (Figures S-9 through S-18) was obtained on a Mettler-Toledo Differential Scanning Calorimeter 3+. Samples were loaded into a goldplated high-pressure pan, held at 30°C for 10 minutes, then a gradient of 30°C to 400/500°C at 5°C/min.

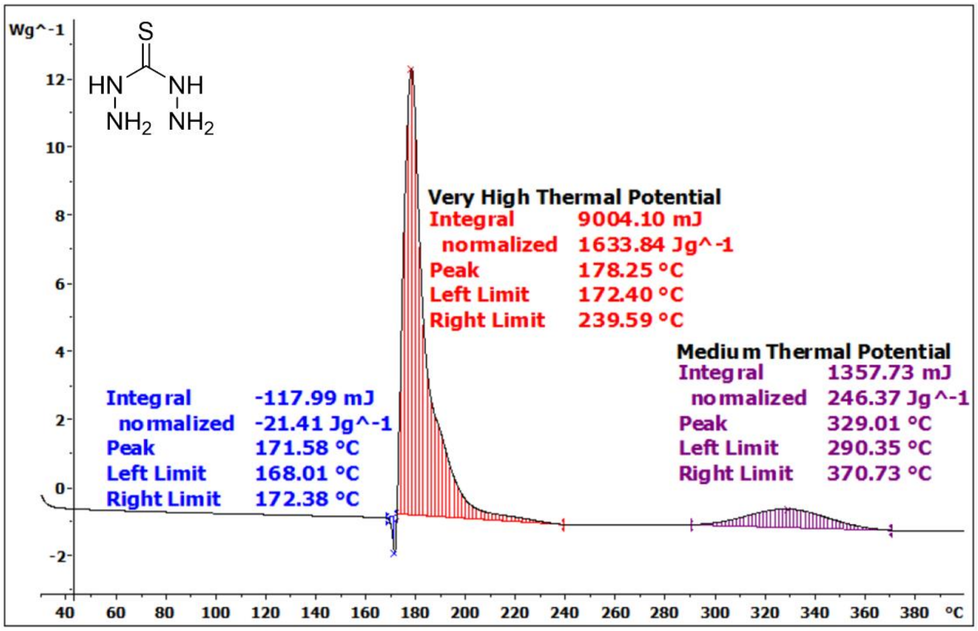


Figure S-9: Thiocarbohydrazide (5.511 mg). As a precaution, all experiments involving thiocarbohydrazide were carried out below 72 °C (100 °C below the DSC transition onset)

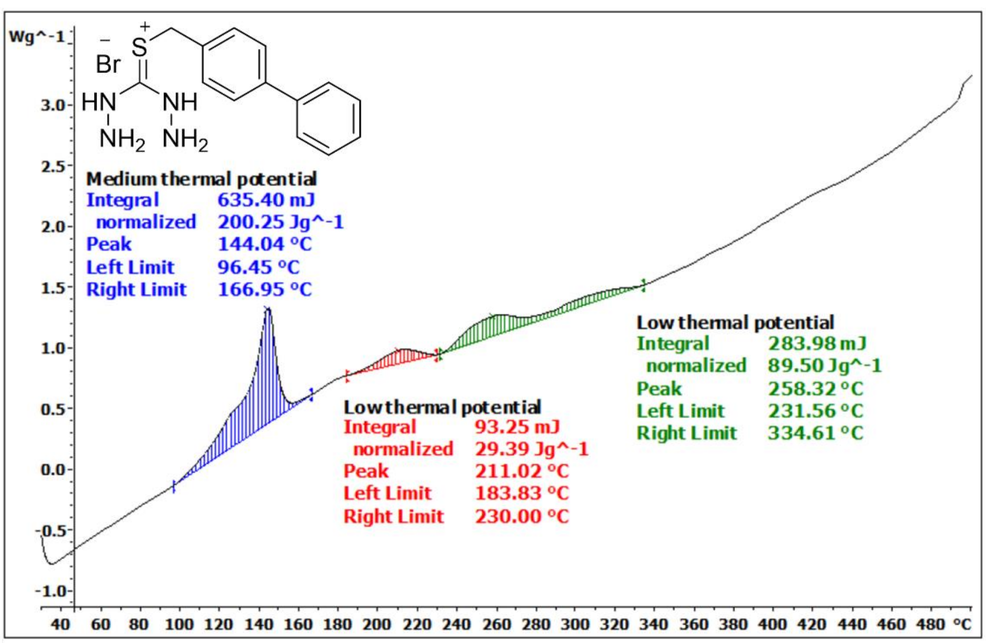


Figure S-10: ([1,1'-biphenyl]-4-ylmethyl)thiocarbohydrazide bromide (3.173 mg)

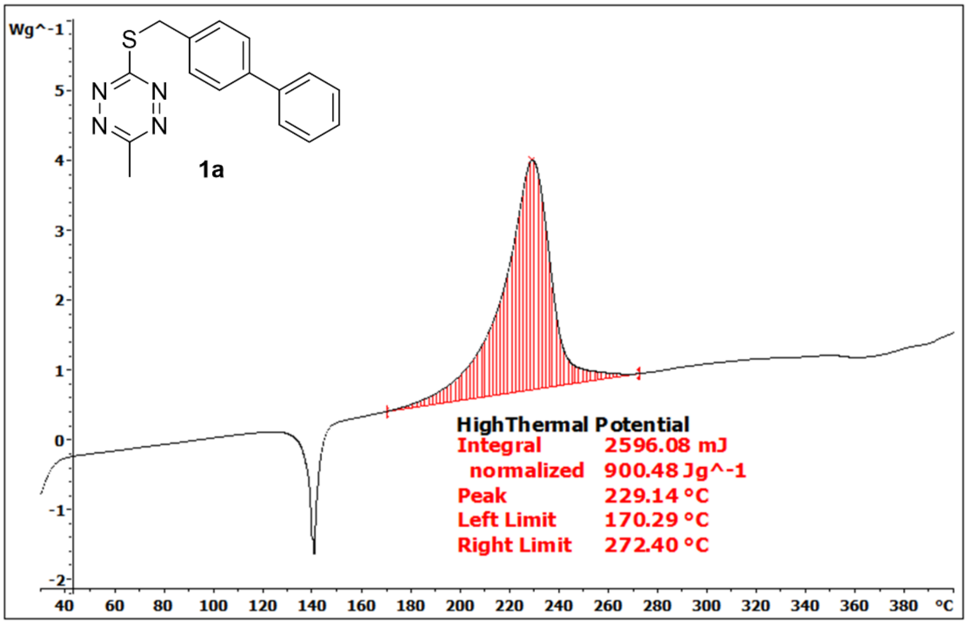


Figure S-11: Compound **1a** (2.883 mg)

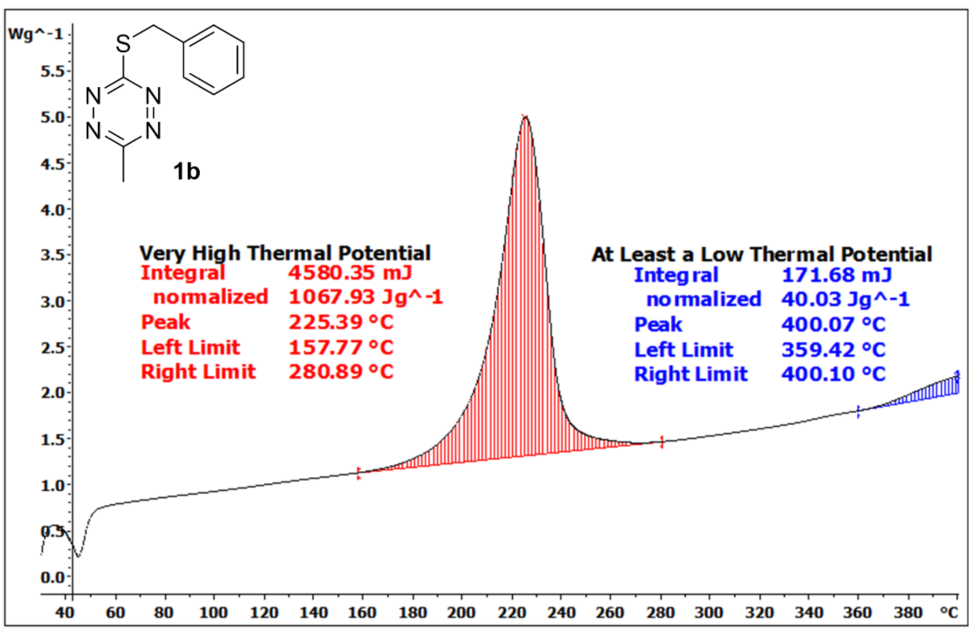


Figure S-12: Compound **1b** (4.289 mg)

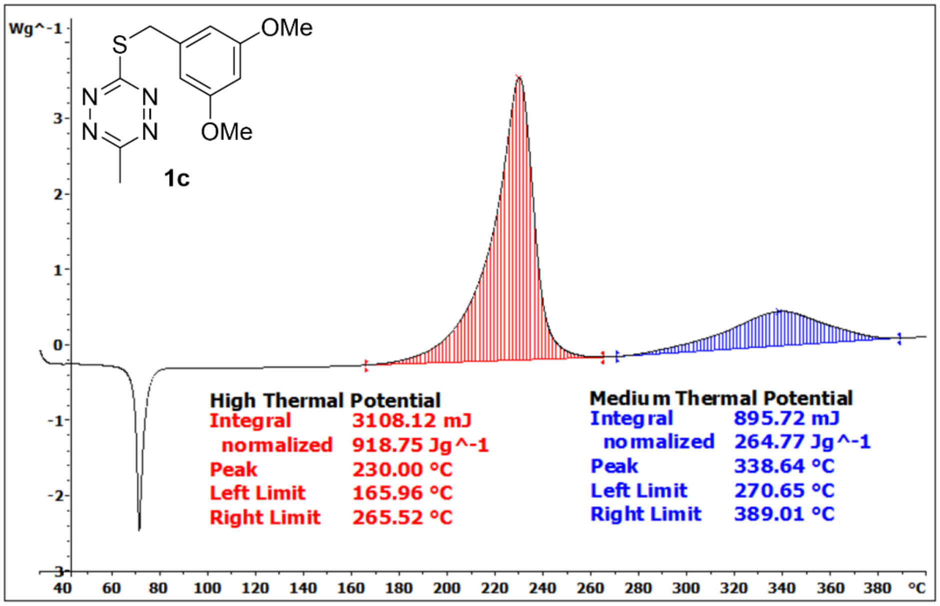


Figure S-13: Compound **1c** (3.383 mg)

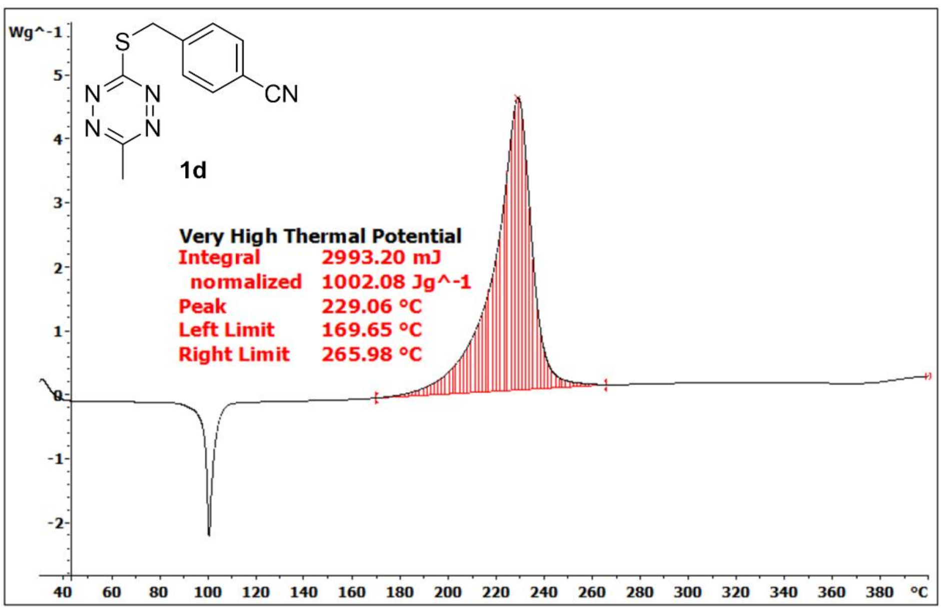


Figure S-14: Compound **1d** (2.987 mg)

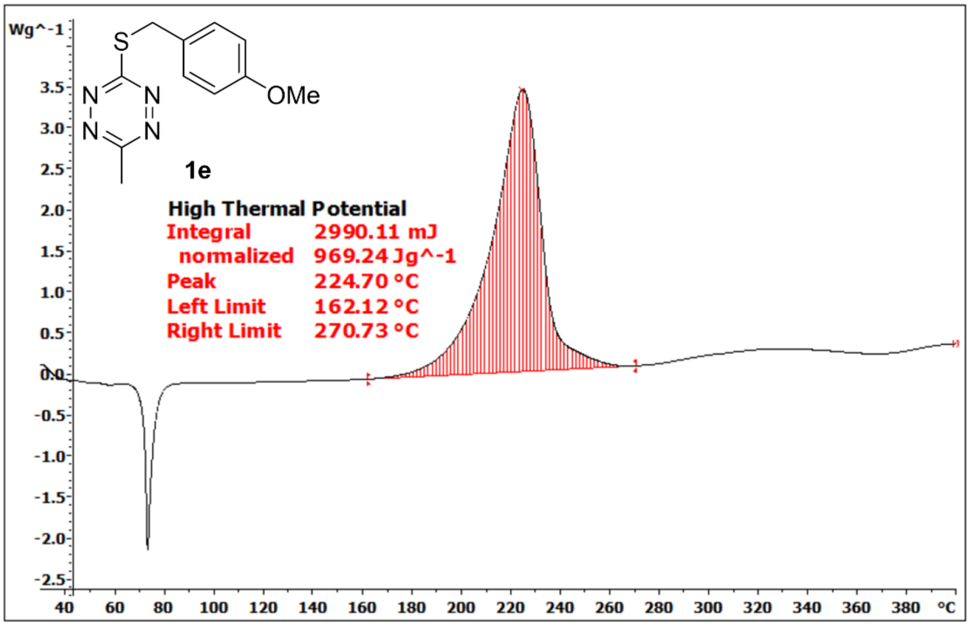


Figure S-15: Compound **1e** (3.085 mg)

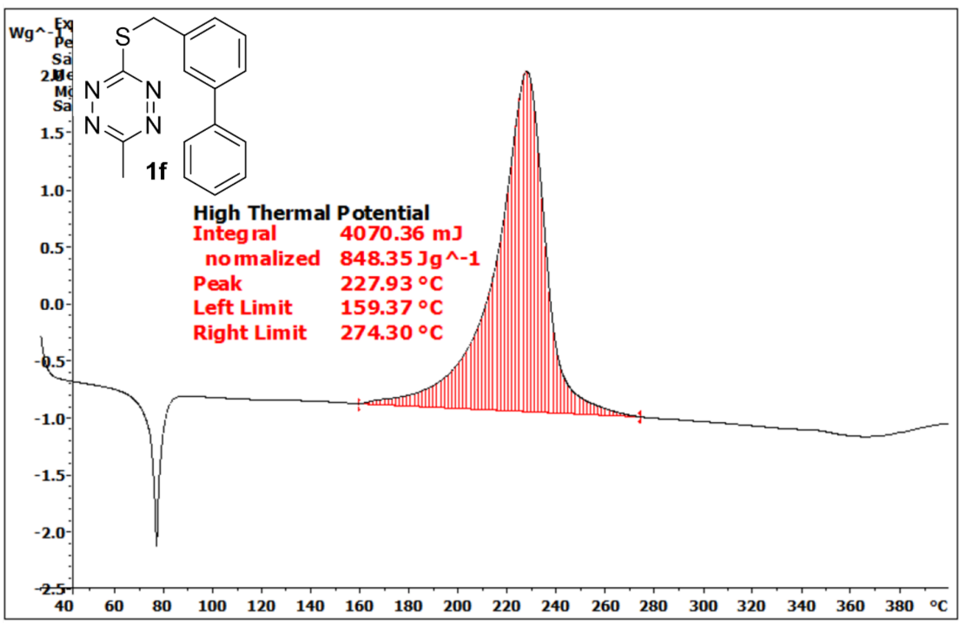


Figure S-16: Compound **1f** (4.798 mg)

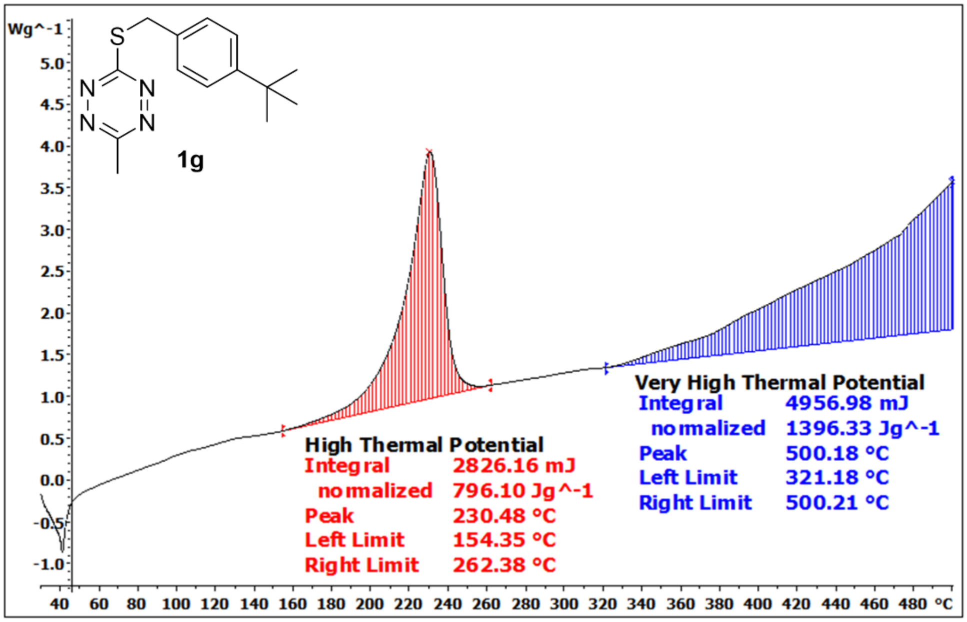


Figure S-17: Compound **1g** (3.550 mg)

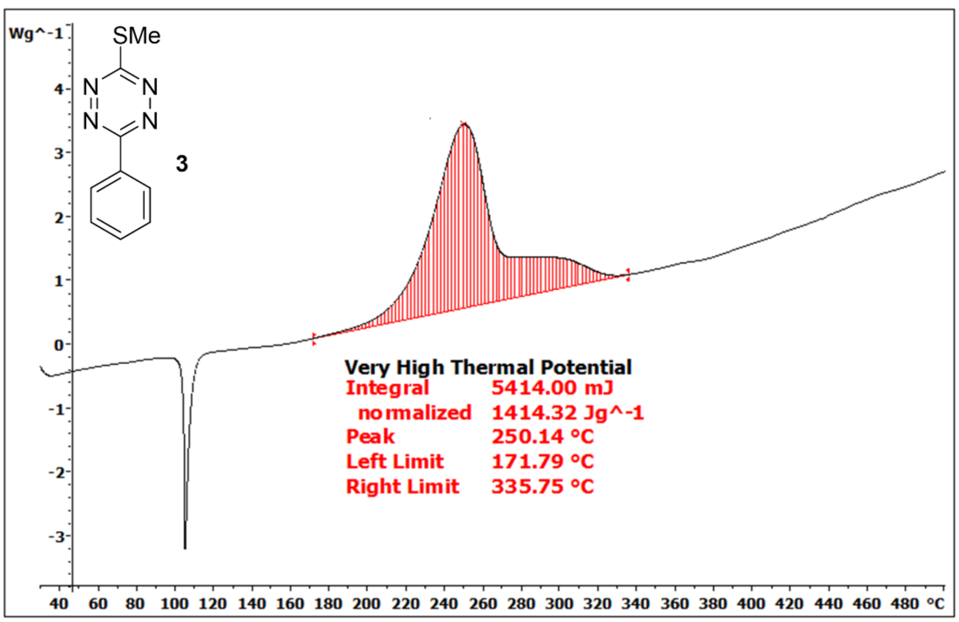
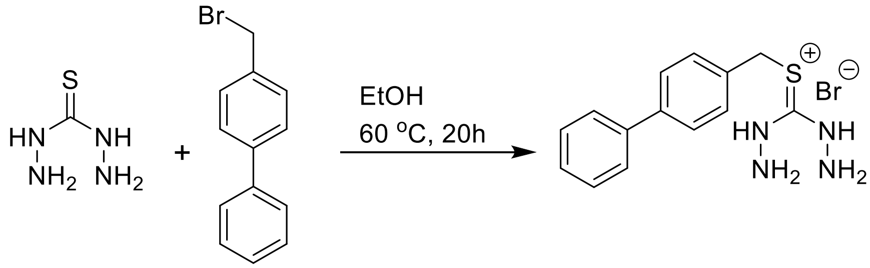


Figure S-18: Compound **3** (3.828 mg)

Synthesis

([1,1’-biphenyl]-4-ylmethyl)thiocarbohydrazide bromide



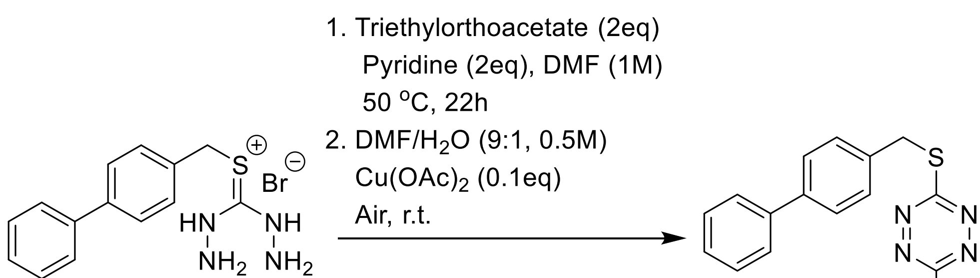
Thiocarbohydrazide (21.20 g, 200 mmol, 1.0 eq) and 4-bromomethyl biphenyl (49.40 g, 200 mmol, 1.0 eq) were suspended in ethanol (300 mL, 0.66 M) in a round bottom flask. The flask was flushed with nitrogen and heated to 60°C for 20h. A thick white slurry forms during the reaction. The reaction was brought to room temperature and the slurry was broken up with 300 mL diethyl ether. The white solids were isolated by vacuum filtration, washed 5x250 mL diethyl ether, and then dried under rotary evaporation to give ([1,1'-biphenyl]-4-ylmethyl)-thiocarbohydrazide bromide as a white powder (65.55 g, 93%).

^{1}H NMR (400 MHz, Methanol-d$_4$) δ 7.66 – 7.60 (m, 4H), 7.49 (app d, *J* = 8.4 Hz, 2H), 7.46 – 7.43 (app t, *J* = 7.9 Hz, 2H), 7.35 (app tt, *J* = 7.6, 2.0 Hz, 1H), 4.28 (s, 2H)

FTIR (KBr, thin film) 3440, 2067, 1637, 533 cm^{-1}

HRMS (ESI+) [M-Br]$^+$ Calculated for $C_{14}H_{17}N_4S^+$ 273.1168; found 273.1173

3-([1,1'-biphenyl]-4-ylmethylthio)-6-methyl-1,2,4,5-tetrazine (b-Tz, 1a)



([1,1'-biphenyl]-4-ylmethyl)-thiocarbohydrazide bromide (65.55 g, 186 mmol, 1.0 eq), Pyridine (33 mL, 371 mmol, 2.0 eq), and DMF (186 mL, 1.0M) were stirred under nitrogen at 50°C. Triethylorthoacetate (70 mL, 371 mmol, 2.0 eq) was added dropwise over 1h and the reaction was stirred an additional 22h. The reaction was then cooled to r.t. and opened to air. DMF (149 mL) and H_2O (37 mL) (9:1 org/aq, 0.5M) were added followed by Cu(II)$(OAc)_2$ (3.38 g, 18.6 mmol, 0.1 eq). Air was then bubbled into the reaction and the solution was stirred vigorously for 24h, after which complete oxidation of tetrazine was observed (monitored by TLC, iodine on silica visualization). Bubbling of air was stopped and 250 mL additional H_2O was added to precipitate tetrazine. The heterogeneous mixture was then filtered and the solids were washed 5x100 mL H_2O. Any remaining solvent was removed by rotary evaporation. The solids were then dissolved in a minimal amount of hot DCM and loaded on to a plug of silica gel (2 in. diameter, 4 in. deep). Tetrazine was eluted with 3:2 DCM/hexanes and then concentrated by rotary evaporation resulting in 3-([1,1'-biphenyl]-4-ylmethylthio)-6-methyl-1,2,4,5-tetrazine (b-Tz) as a bright coral red crystalline solid (27.45 g, 93 mmol, 50%)

^1H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.53 (m, 6H), 7.45 – 7.41 (app t, *J* = 7.4 Hz, 2H), 7.35 (app tt, *J* = 7.2, 2.0 Hz, 1H), 4.57 (s, 2H), 2.98 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.7 (C), 165.5 (C), 140.9 (C), 140.6 (C), 134.8 (C), 129.8 (CH), 128.9 (CH), 127.6 (CH), 127.6 (CH), 127.2 (CH), 34.5 (CH_2), 20.9 (CH_3)

FTIR (KBr, thin film) 3452, 1635, 1370, 740, 694 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{16}H_{15}N_4S^+$ 295.1017; found 295.1015

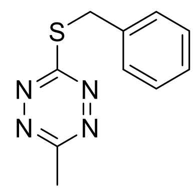
General procedure for the synthesis of tetrazines 1b-1g

*Note: the following procedures **1b-1g** are unoptimized. For an optimized procedure please refer to tetrazine **1a.***

Thiocarbohydrazide (*see below*, 1.0 eq.) and a benzylic bromide reactant (*see below*, 1.0 eq.) were suspended in ethanol (0.2 M) in a round bottom flask. The flask was flushed with nitrogen and heated to 70°C for 2h. The reaction was then brought to room temperature and the solids were isolated by vacuum filtration, washed with diethyl ether, and then dried under rotary evaporation.

The resulting solids (1.0 eq) were dissolved in DMF (0.2 M) under nitrogen and triethylorthoacetate (1.3 eq.) was added. The reaction was stirred at 70°C for 2h and then cooled to 0°C. (Diacetoxyiodo)benzene (1.0 eq.) was added portionwise over 5 min and then the reaction was brought to r.t. and stirred 1h. The reaction was diluted in DCM and washed 4x H_2O, 1x brine, and then dried on $MgSO_4$. The tetrazine products were purified by silica gel chromatography as described below.

3-(benzylthio)-6-methyl-1,2,4,5-tetrazine (1b)



Prepared using Thiocarbohydrazide (4.06 g, 38.3 mmol) and benzyl bromide (4.6 mL, 38.3 mmol). Benzylthiocarbohydrazide bromide was precipitated from the reaction solution with 150 mL hexanes. Silica gel chromatography (25% DCM/hexanes) yielded 3-(benzylthio)-6-methyl-1,2,4,5-tetrazine as a red oil (789 mg, 3.6 mmol, 9%)

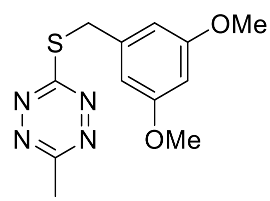
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.46 (m, 2H), 7.36 – 7.27 (m, 3H), 4.54 (s, 2H), 2.97 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.7 (C), 165.4 (C), 135.8 (C), 129.4 (CH), 128.8 (CH), 128.0 (CH), 34.8 (CH_2), 20.8 (CH_3)

FTIR (KBr, thin film) 3446, 3086, 3063, 3030, 2932, 2296, 1495, 1453, 1383, 1316, 1162, 1069, 883, 702 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{10}H_{11}N_4S^+$ 219.0704; found 219.0709

3-((3,5-dimethoxybenzyl)thio)-6-methyl-1,2,4,5-tetrazine (1c)



Prepared using Thiocarbohydrazide (1.15 g, 10.8 mmol) and 3,5-Dimethoxybenzyl bromide (2.49 g, 10.8 mmol). Silica gel chromatography (0-20% ethyl acetate/petroleum ether) yielded 3-((3,5-dimethoxybenzyl)thio)-6-methyl-1,2,4,5-tetrazine as a pink crystalline solid (305 mg, 1.1 mmol, 10%)

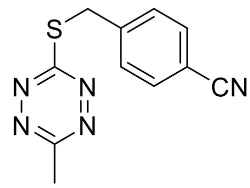
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.61 (d, *J* = 2.0 Hz, 2H), 6.34 (t, *J* = 2.0 Hz, 1H), 4.48 (s, 2H), 3.78 (s, 6H), 2.97 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.8 (C), 165.4 (C), 161.1 (C), 137.9 (C), 107.3 (CH), 100.0 (CH), 55.5 (CH_3), 35.0 (CH_2), 20.9 (CH_3)

FTIR (KBr, thin film) 3459, 3003, 2962, 2838, 2105, 1610, 1316, 1206, 1158, 1066 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{12}H_{15}O_2N_4S^+$ 279.0916; found 279.0922

4-(((6-methyl-1,2,4,5-tetrazin-3-yl)thio)methyl)benzonitrile (1d)



Prepared using Thiocarbohydrazide (530 mg, 5.0 mmol) and 4-(Bromomethyl)benzonitrile (980 mg, 5.0 mmol). Silica gel chromatography (25% ethyl acetate/petroleum ether) yielded 4-(((6-methyl-1,2,4,5-tetrazin-3-yl)thio)methyl)benzonitrile as a pink crystalline solid (350 mg, 1.4 mmol, 29%)

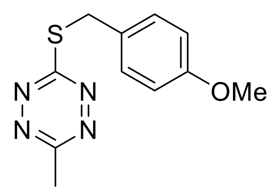
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 – 7.59 (m, 4H), 4.55 (s, 2H), 2.97 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.9 (C), 165.8 (C), 141.7 (C), 132.6 (CH), 130.1 (CH), 118.6 (C), 111.8 (C), 34.1 (CH_2), 20.9 (CH_3)

FTIR (KBr, thin film) 3428, 3086, 3051, 2996, 2229, 1636, 1606, 1384, 1316, 1160, 883cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{11}H_{10}N_5S^+$ 244.0657; found 244.0661

3-((4-methoxybenzyl)thio)-6-methyl-1,2,4,5-tetrazine (1e)



Prepared using Thiocarbohydrazide (1.95 g, 18.4 mmol) and 4-Methoxybenzyl bromide (3.70 g, 18.4 mmol). Silica gel chromatography (0-20% ethyl acetate/petroleum ether) yielded 3-((4-methoxybenzyl)thio)-6-methyl-1,2,4,5-tetrazine as a pink crystalline solid (144 mg, 0.6 mmol, 3%)

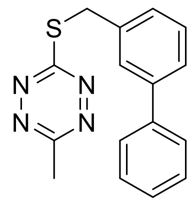
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.37 (app d, *J* = 8,9 Hz, 2H), 6.88 – 6.84 (app d, *J* = 8,9 Hz, 2H), 4.50 (s, 2H), 3.79 (s, 3H), 2.97 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.8 (C), 165.3 (C), 159.3 (C), 130.6 (CH), 127.6 (C), 114.2 (CH), 55.4 (CH_3), 34.4 (CH_2), 20.8 (CH_3)

FTIR (KBr, thin film) 3427, 3034, 3001, 2956, 2934, 2908, 2835, 1610, 1512, 1383, 1316, 1244, 1164, 1032, 884, 833 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{11}H_{13}ON_4S^+$ 249.0810; found 249.0814

3-(([1,1'-biphenyl]-3-ylmethyl)thio)-6-methyl-1,2,4,5-tetrazine (1f)



Prepared using Thiocarbohydrazide (1.07 g, 10.1 mmol) and 3-Phenylbenzyl bromide (2.49 g, 10.1 mmol). Silica gel chromatography (25-50% DCM/hexanes) yielded 3-(([1,1'-biphenyl]-3-ylmethyl)thio)-6-methyl-1,2,4,5-tetrazine as a pink crystalline solid (708 mg, 2.4 mmol, 24%)

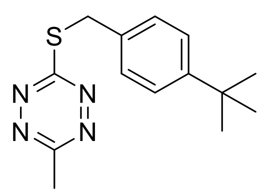
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 7.69 (app t, *J* = 1.9 Hz, 1H), 7.60 – 7.57 (m, 2H), 7.52 (app dt, 7.6, 1.6 Hz, 1H), 7.48 – 7.39 (m, 4H), 7.38 – 7.34 (m, 1H), 4.61 (s, 2H), 2.97 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.7 (C), 165.5 (C), 141.9 (C), 140.7 (C), 136.3 (C), 129.3 (CH), 129.0 (CH), 128.3 (CH), 128.2 (CH), 127.7 (CH), 127.3 (CH), 126.8 (CH), 34.9 (CH_2), 20.9 (CH_3)

FTIR (KBr, thin film) 3456, 3060, 3033, 2932, 1636, 1599, 1479, 1383, 1316, 1160, 1068, 884, 761, 699 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{16}H_{15}N_4S^+$ 295.1017; found 295.1024

3-((4-(*tert*-butyl)benzyl)thio)-6-methyl-1,2,4,5-tetrazine (1g)



Prepared using Thiocarbohydrazide (1.01 g, 9.5 mmol) and 4-*tert*-Butylbenzyl bromide (1.7 mL, 9.5 mmol). ([1-*tert*-butyl-4-ylmethyl)thiocarbohydrazide bromide was precipitated from the reaction solution with 50 mL hexanes. Silica gel chromatography (40% DCM/hexanes) yielded 3-((4-(*tert*-butyl)benzyl)thio)-6-methyl-1,2,4,5-tetrazine as a red oil (727 mg, 2.7 mmol, 28%)

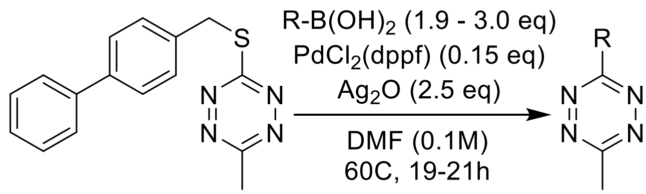
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (app d, *J* = 8.5 Hz, 1H), 7.36 (app d, *J* = 8.5 Hz, 1H), 4.52 (s, 2H), 2.97 (s, 3H), 1.30 (s, 9H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 174.9 (C), 165.4 (C), 151.0 (C), 132.6 (C), 129.1 (CH), 125.8 (CH), 34.5 (CH_2), 31.4 (CH_3), 20.9 (CH_3)

FTIR (KBr, thin film) 3428, 3055, 3027, 2963, 2906, 2868, 1383, 1316, 1162, 1068, 885cm^{-1}

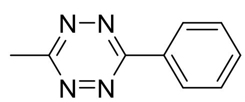
HRMS (ESI+) $[M+H]^+$ Calculated for $C_{14}H_{19}N_4S^+$ 275.1330; found 275.1336

General Procedure for Methyl Tetrazine Cross-coupling



b-Tz **1a** (110 mg, 375 µmol, 1 eq.) boronic acid (713 µmol, 1.9 eq. *or* 1125 µmol, 3.0 eq., *see below*), [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (41 mg, 56 µmol, 0.15 eq.) and silver(I) oxide (218 mg, 938 µmol, 2.5 eq.) were added to a vacuum dried schlenk flask equipped with a stir bar. The solids were dissolved/suspended as a heterogeneous slurry with N,N-Dimethylformamide (3.75 mL, 0.1M) and the flask was flushed with nitrogen and sealed. The reaction was stirred at 60°C for 19-21h, then brought to room temperature and the solvent was removed by rotary evaporation. The crude solids were chromatographed directly on silica gel. Elution systems are described below. Each reaction was run in duplicate to obtain an average yield.

3-methyl-6-phenyl-1,2,4,5-tetrazine (2a)



Prepared using phenylboronic acid (87 mg, 713 µmol, 1.9 eq). Silica gel chromatography (75% DCM/hexanes) yielded an average of 90% as a purple crystalline solid (run 1: 58 mg, 91%; run 2: 57 mg, 88%).

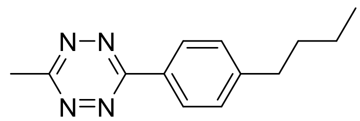
^1H NMR (400 MHz, $CDCl_3$) δ 8.61 – 8.58 (m, 2H), 7.65 – 7.57 (m, 3H), 3.10 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4 (C), 164.2 (C), 132.7 (CH), 131.9 (C), 129.4 (CH), 128.0 (CH), 21.3 (CH_3)

FTIR (KBr, thin film) 3446, 3072, 2918, 2849, 1660, 1402, 1362, 890, 759, 692, 564 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_9H_9N_4^+$ 172.0827; found 173.0827

3-(4-butylphenyl)-6-methyl-1,2,4,5-tetrazine (2b)



Prepared using 4-Butylphenylboronic acid (127 mg, 713 μmol, 1.9 eq). Silica gel chromatography (60% DCM/hexanes) yielded an average of 92% as a purple crystalline solid (run 1: 79 mg, 93%; run 2: 78 mg, 91%).

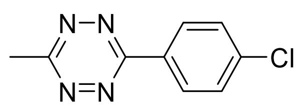
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (app d, *J* = 8.3 Hz 2H), 7.40 (app d, *J* = 8.3 Hz, 2H), 3.08 (s, 3H), 2.72 (app t, *J* = 8.0 Hz, 2H), 1.67 (app pent, *J* = 7.6 Hz, 2H), 1.39 (app sext, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.2 Hz, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1 (C), 164.3 (C), 148.3 (C), 129.5 (CH), 129.3 (C), 128.0 (CH), 35.9 (CH_2), 33.5 (CH_2), 22.5 (CH_2), 21.3 (CH_3), 14.1 (CH_3)

FTIR (KBr, thin film) 3455, 2950, 2927, 2862, 1962, 1606, 1403, 1361, 1088, 889, 570 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{13}H_{17}N_4^+$ 229.1453; found 229.1456

3-(4-chlorophenyl)-6-methyl-1,2,4,5-tetrazine (2c)



Prepared using 4-Chlorophenylboronic acid (111 mg, 713 μmol, 1.9 eq). Silica gel chromatography (60% DCM/hexanes) yielded an average of 91% as a magenta crystalline solid (run 1: 70 mg, 91%; run 2: 70 mg, 91%).

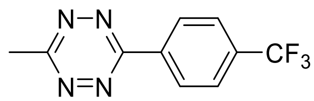
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (app d, *J* = 8.7 Hz, 2H), 7.57 (app d, *J* = 8.7 Hz, 2H), 3.11 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.6 (C), 163.6 (C), 139.3 (C), 130.4 (C), 129.8 (CH), 129.3 (CH), 21.4 (CH_3)

FTIR (KBr, thin film) 3460, 3090, 2075, 1949, 1636, 1396, 1108, 1098, 889, 854, 800, 563 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_9H_8ClN_4^+$ 207.0437; found 207.0439

3-methyl-6-(4-(trifluoromethyl)phenyl)-1,2,4,5-tetrazine (2d)



Prepared using 4-(Trifluoromethyl)phenylboronic acid (136 mg, 713 µmol, 1.9 eq). Silica gel plug (50% DCM/hexanes) then reverse phase C_{18} silica gel chromatography (50-80% MeOH/H_2O) yielded an average of 61% as a magenta crystalline solid (run 1: 56 mg, 62%; run 2: 54 mg, 60%).

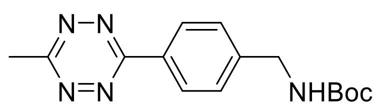
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (app d, J = 8.4 Hz, 2H), 7.86 (app d, J = 8.4 Hz, 2H), 3.14 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.0 (C), 163.4 (C), 135.2 (C, q, $J_{C\text{-}F}$ = 1.0 Hz), 134.2 (C, q, $J_{C\text{-}F}$ = 32.9 Hz), 128.4 (CH), 126.3 (CH, q, $J_{C\text{-}F}$ = 3.7 Hz), 123.8 (CF_3, q, $J_{C\text{-}F}$ = 273.6 Hz), 21.4 (CH_3)

FTIR (KBr, thin film) 3444, 2066, 1636, 1404, 1331, 1162, 1122, 550 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{10}H_8F_3N_4^+$ 241.0701; found 241.0698

***tert*-butyl 4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzylcarbamate (2e)**



Prepared using 4-(N-Boc-aminomethyl)phenylboronic acid (179 mg, 713 µmol, 1.9 eq). Silica gel plug (0.75% acetone/DCM) then reverse phase C_{18} silica gel chromatography (50-80% MeOH/H_2O) yielded an average of 94% as a pink powdery solid (run 1: 110 mg, 97%; run 2: 103 mg, 91%).

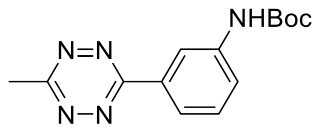
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (app d, J = 8.0 Hz, 2H), 7.50 (app d, J = 8.0 Hz, 2H), 5.00 (NH, br s, 1H), 4.44 (d, J = 6.0 Hz, 2H), 3.09 (s, 3H), 1.48 (s, 9H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3 (C), 164.0 (C), 156.1 (C), 144.1 (C), 130.9 (C), 128.3 (CH), 128.2 (CH), 80.0 (C), 44.5 (CH_2), 28.5 (CH_3), 21.3 (CH_3)

FTIR (KBr, thin film) 3349, 3004, 2977, 2931, 2248, 1696, 1612, 1521, 1405, 1365, 1272, 1250, 1167, 1089, 891, 796, 732, 562 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{15}H_{20}O_2N_5^+$ 302.1617; found 302.1616

tert-butyl (3-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)carbamate (2f)



Prepared using 3-(N-Boc-amino)phenylboronic acid (169 mg, 713 μmol, 1.9 eq). Silica gel chromatography (0.5% acetone, 0.75% ethanol, 98.75% chloroform) yielded an average of 92% as a pink powdery solid (run 1: 98 mg, 91%; run 2: 100 mg, 93%).

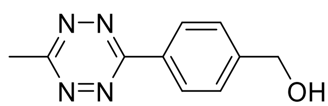
^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.71 (NH, s, 1H), 8.71 (app s, 1H), 8.07 (app d, *J* = 7.6 Hz, 1H), 7.69 (app d, *J* = 7.2 Hz, 1H), 7.53 (app t, *J* = 8.0 Hz, 1H), 3.99 (s, 3H), 1.50 (s, 9H)

^{13}C NMR (101 MHz, DMSO-d$_6$) δ 167.2 (C), 163.3 (C), 153.8 (C), 140.6 (C), 132.4 (C), 129.8 (CH), 121.8 (CH), 121.1 (CH), 116.6 (CH), 79.5 (C), 28.1 (CH$_3$), 20.9 (CH$_3$)

FTIR (KBr, thin film) 3299, 3066, 2979, 2931, 1718, 1541, 1392, 1367, 1235, 1156, 688cm^{-1}

HRMS (ESI+) [M+H]$^+$ Calculated for $C_{14}H_{18}O_2N_5^+$ 288.1460; found 288.1459

(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)methanol (2g)



Prepared using 4-(Hydroxymethyl)phenylboronic acid (171 mg, 1125 μmol, 3.0 eq). Silica gel chromatography (3% acetone/DCM) yielded an average of 68% as a magenta crystalline solid (run 1: 49 mg, 65%; run 2: 53 mg, 70%).

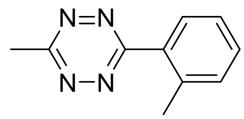
^{1}H NMR (400 MHz, CDCl$_3$) δ 8.59 (app d, *J* = 8.5 Hz, 2H), 7.59 (app d, *J* = 8.5 Hz, 2H), 4.84 (s, 2H), 3.10 (s, 3H), 1.85 (br s, 1H)

^{13}C NMR (101 MHz, CDCl$_3$) δ 167.4 (C), 164.1 (C), 145.7 (C), 131.1 (C), 128.3 (CH), 127.5 (CH), 64.9 (CH$_2$), 21.3 (CH$_3$)

FTIR (KBr, thin film) 3446, 2069, 1636, 1403, 1362, 1038, 562 cm^{-1}

HRMS (ESI+) [M+H]$^+$ Calculated for $C_{10}H_{11}ON_4^+$ 203.0933; found 203.0934

3-methyl-6-(*o*-tolyl)-1,2,4,5-tetrazine (2h)



Prepared using *o*-Tolylboronic acid (97 mg, 713 μmol, 1.9 eq). Silica gel chromatography (90% DCM/hexanes) yielded an average of 50% as a magenta crystalline solid (run 1: 36 mg, 51%; run 2: 35 mg, 49%).

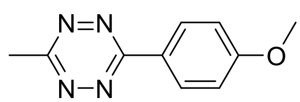
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (app d, *J* = 7.6 Hz, 1H), 7.49 (app td, *J* = 7.6, 1.6 Hz, 1H), 7.44 – 7.39 (m, 2H), 3.12 (s, 3H), 2.63 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3, 166.4, 138.6, 132.0, 131.8, 131.5, 131.0, 126.6, 21.5, 21.4

FTIR (KBr, thin film) 3436, 3079, 2966, 2929, 1602, 1450, 1436, 1398, 1364, 1086, 1041, 1020, 886, 757, 722, 573, 466 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{10}H_{11}N_4^+$ 187.0984; found 187.0986

3-(4-methoxyphenyl)-6-methyl-1,2,4,5-tetrazine (2i)



Prepared using 4-Methoxyphenylboronic acid (108 mg, 713 μmol, 1.9 eq). Silica gel chromatography (90% DCM/hexanes) yielded an average of 86% as a dark pink crystalline solid (run 1: 62 mg, 81%; run 2: 68 mg, 90%).

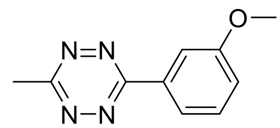
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (app d, *J* = 9.0 Hz, 2H), 7.08 (app d, *J* = 9.0 Hz, 2H), 3.92 (s, 3H), 3.06 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7 (C), 163.9 (C), 163.4 (C), 129.9 (CH), 124.3 (C), 114.8 (CH), 55.7 (CH_3), 21.2 (CH_3)

FTIR (KBr, thin film) 3446, 2042, 1636, 1609, 1405, 1248, 1022, 890, 843, 801, 683, 562 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{10}H_{11}ON_4^+$ 203.0933; found 203.0934

3-(3-methoxyphenyl)-6-methyl-1,2,4,5-tetrazine (2j)



Prepared using 3-Methoxyphenylboronic acid (108 mg, 713 μmol, 1.9 eq). Silica gel chromatography (90% DCM/hexanes) yielded an average of 88% as a dark pink crystalline solid (run 1: 69 mg, 91%; run 2: 65 mg, 85%).

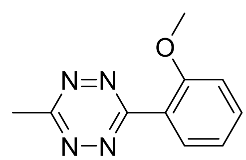
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (app dt, *J* = 8.0, 1.3 Hz, 1H), 8.12 (dd, *J* = 2.8, 1.6 Hz, 1H), 7.50 (app t, *J* = 8.0 Hz, 1H), 7.17 (ddd, *J* = 8.4, 2.8, 1.2 Hz, 1H), 3.93 (s, 3H), 3.10 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5 (C), 164.1 (C), 160.4 (C), 133.1 (C), 130.5 (CH), 120.6 (CH), 119.4 (CH), 112.2 (CH), 55.7 (CH_3), 21.3 (CH_3)

FTIR (KBr, thin film) 3446, 2958, 2041, 1636, 1597, 1220, 1022, 900, 871, 793, 691, 626, 492 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{10}H_{11}ON_4^+$ 203.0933; found 203.0934

3-(2-methoxyphenyl)-6-methyl-1,2,4,5-tetrazine (2k)



Prepared using 2-Methoxyphenylboronic acid (108 mg, 713 μmol, 1.9 eq). Silica gel chromatography (1% acetone/DCM) yielded an average of 19% as a dark pink oil (run 1: 13 mg, 18%; run 2: 14 mg, 19%).

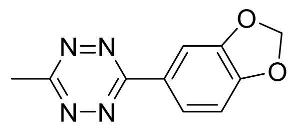
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 7.6, 2.0 Hz, 1H), 7.56 (ddd, *J* = 8.8, 7.2, 1.6 Hz, 1H), 7.16 (app td, *J* = 7.6, 1.2 Hz, 1H), 7.11 (app d, *J* = 8.4 Hz, 1H), 3.90 (s, 3H), 3.11 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.3 (C), 166.0 (C), 158.3 (C), 133.2 (CH), 131.9 (CH), 122.2 (C), 121.2 (CH), 112.2 (CH), 56.2 (CH_3), 21.4 (CH_3)

FTIR (KBr, thin film) 3488, 3078, 3007, 2932, 2838, 1602, 1498, 1467, 1436, 1399, 1363, 1289, 1264, 1239, 1021, 885, 756 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{10}H_{11}ON_4^+$ 203.0933; found 203.0932

3-(benzo[d][1,3]dioxol-5-yl)-6-methyl-1,2,4,5-tetrazine (2l)



Prepared using 3,4-(Methylenedioxy)phenylboronic acid (118 mg, 713 μmol, 1.9 eq). Silica gel chromatography (90% DCM/hexanes) yielded an average of 79% as a salmon colored crystalline solid (run 1: 66 mg, 81%; run 2: 63 mg, 77%).

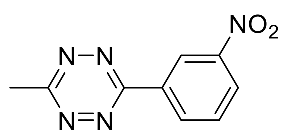
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (dd, *J* = 8.4, 1.6 Hz, 1H), 8.03 (d, *J* = 1.6 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.10 (s, 2H), 3.06 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9 (C), 163.8 (C), 151.7 (C), 148.8 (C), 125.9 (C), 123.6 (CH), 109.2 (CH), 107.8 (CH), 102.0 (CH_2), 21.2 (CH_3)

FTIR (KBr, thin film) 3456, 2361, 2337, 1659, 1395, 1247, 901, 798, 668, 629, 502 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{10}H_9O_2N_4^+$ 217.0726; found 217.0728

3-methyl-6-(3-nitrophenyl)-1,2,4,5-tetrazine (2m)



Prepared using 3-Nitrophenylboronic acid (119 mg, 713 μmol, 1.9 eq). Silica gel chromatography (0.5% acetone/DCM) yielded an average of 48% as a pink crystalline solid (run 1: 39 mg, 48%; run 2: 39 mg, 48%).

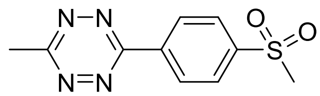
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (app t, *J* = 2.0 Hz, 1H), 8.96 – 8.93 (m, 1H), 8.49 (ddd, *J* = 8.0, 2.4, 1.2 Hz, 1H), 7.82 (app t, *J* = 8.0 Hz, 1H), 3.16 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.3 (C), 162.8 (C), 149.2 (C), 133.8 (C), 133.5 (CH), 130.6 (CH), 127.1 (CH), 123.0 (CH), 21.5 (CH_3)

FTIR (KBr, thin film) 3090, 1621, 1588, 1525, 1482, 1402, 1349, 1107, 1082, 870, 826, 805, 757, 738, 683, 574 cm^{-1}

HRMS (LIFDI) m/z Calculated for $C_9H_7O_2N_5$ 217.0600; found 217.0593

3-methyl-6-(4-(methylsulfonyl)phenyl)-1,2,4,5-tetrazine (2n)



Prepared using 4-(Methanesulfonyl)phenylboronic acid (143 mg, 713 μmol, 1.9 eq). Silica gel chromatography (1% Acetone/DCM) yielded an average of 69% as a magenta crystalline solid (run 1: 60 mg, 64%; run 2: 69 mg, 73%).

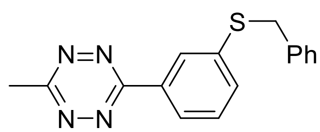
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 – 8.80 (m, 2H), 8.19 – 8.16 (m, 2H). 3.16 (s, 3H), 3.14 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2 (C), 163.1 (C), 144.0 (C), 136.9 (C), 128.9 (CH), 128.4 (CH), 44.5 (CH_3), 21.5 (CH_3)

FTIR (KBr, thin film) 3098, 3008, 2918, 1402, 1365, 1303, 1149, 1085, 968, 891, 778, 572, 545 cm^{-1}

HRMS (LIFDI) m/z Calculated for $C_{10}H_{10}O_2N_4S$ 250.0524; found 250.0529

3-(3-(benzylthio)phenyl)-6-methyl-1,2,4,5-tetrazine (2o)



Prepared using (3-(Benzylthio)phenyl)boronic acid (275 mg, 1125 μmol, 3.0 eq). Due to difficult separation of the resulting product **2o** and unreacted b-Tz, additional reactants were added as follows to consume the remaining b-Tz: 4-Methoxyphenylboronic acid (57 mg, 375 μmol, 1.0 eq), [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (27 mg, 38 μmol, 0.1 eq.) and silver(I) oxide (87 mg, 375 μmol, 1.0 eq.) stirred at 60°C for 6h. Silica gel chromatography (75% DCM/hexanes) yielded an average of 76% as a dark red crystalline solid (run 1: 84 mg, 77%; run 2: 83 mg, 75%).

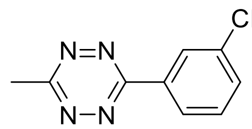
^{1}H NMR (400 MHz, Acetone-d_6) δ 8.49 – 8.48 (m, 1H), 8.34 – 8.32 (m, 1H), 7.63 (ddd, J = 8.0, 2.0, 1.2 Hz, 1H), 7.56 (app td, J = 8.0, 0.8 Hz, 1H), 7.45 – 7.42 (m, 2H), 7.33 – 7.28 (m, 2H) 7.25 – 7.21 (m, 1H), 4.36 (s, 2H), 3.04 (s, 3H)

^{13}C NMR (101 MHz, Acetone-d_6) δ 168.53 (C), 164.30 (C), 139.29 (C), 138.17 (C), 133.97 (C), 133.10 (CH), 130.65 (CH), 129.81 (CH). 129.29 (CH), 128.06 (CH), 128.05 (CH), 125.83 (CH), 38.21 (CH_2), 21.21 (CH_3)

FTIR (KBr, thin film) 3446, 3077, 3028, 2919, 2850, 2361, 2337, 1455, 1394, 1359, 1302, 1076, 1067, 1031, 898, 875, 780, 714, 698, 687, 599, 470 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{16}H_{15}N_4S^+$ 295.1017; found 295.1008

3-(6-methyl-1,2,4,5-tetrazin-3-yl)benzonitrile (2p)



Prepared using 3-Cyanophenylboronic acid (105 mg, 713 µmol, 1.9 eq). Silica gel chromatography (100% DCM) yielded an average of 47% as a bright pink powdery solid (run 1: 34 mg, 46%; run 2: 35 mg, 47%).

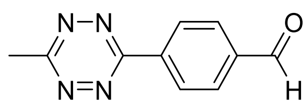
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (app t, *J* = 2.0 Hz, 1H), 8.85 (app dt, *J* = 8.0, 1.6 Hz, 1H), 7.92 (app dt, *J* = 7.6, 1.4 Hz, 1H), 7.74 (app t, *J* = 8.0 Hz, 1H), 3.15 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2 (C), 162.8 (C), 135.7 (CH), 133.3 (C), 131.9 (CH), 131.5 (CH), 130.3 (CH), 118.1 (C), 113.9 (C), 21.4 (CH_3)

FTIR (KBr, thin film) 3445, 2228, 2066, 1636, 1400, 887, 797, 687, 521 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{10}H_8N_5^+$ 198.0780; found 198.0781

4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzaldehyde (2q)



Prepared using 4-formylphenylboronic acid (169 mg, 1125 µmol, 3.0 eq). Silica gel chromatography (0.5% acetone, 0.75% ethanol, 98.75% chloroform) yielded an average of 69% as a magenta crystalline solid (run 1: 51 mg, 68%; run 2: 52 mg, 69%).

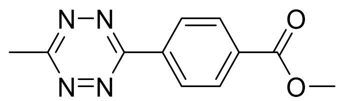
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.16 (s, 1H), 8.79 (app d, *J* = 8.4 Hz, 2H), 8.11 (app d, *J* = 8.4 Hz, 2H), 3.15 (s, 3H). Minor peaks attributable to an impurity were detected at 10.09, 8.00 and 7.81 ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ191.8 (CH), 167.9 (C), 163.6 (C), 139.0 (C), 137.1 (C), 130.5 (CH), 128.6 (CH), 21.5 (CH_3)

FTIR (KBr, thin film) 3446, 2855, 2073, 1684, 1636, 1396, 887, 853, 801, 566 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{10}H_9ON_4^+$ 201.0776; found 201.0778

methyl 4-(6-methyl-1,2,4,5-tetrazin-3-yl)benzoate (2r)



Prepared using 4-Methoxycarbonylphenylboronic acid (203 mg, 1125 μmol, 3.0 eq). Silica gel chromatography (15% EtOAc/hexanes) yielded an average of 66% as a bright pink crystalline solid (run 1: 61 mg, 70%; run 2: 53 mg, 62%).

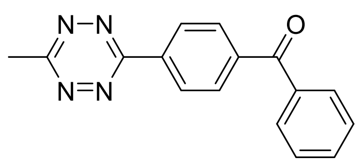
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (app d, *J* = 8.7 Hz, 2H), 8.25 (app d, *J* = 8.7 Hz, 2H), 3.98 (s, 3H), 3.13 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8 (C), 166.5 (C), 163.7 (C), 135.8 (C), 133.7 (C), 130.5 (CH), 128.0 (CH), 52.7 (CH_3), 21.4 (CH_3)

FTIR (KBr, thin film) 3457, 2996, 2951, 2917, 2849, 1722, 1653, 1406, 1276, 1258, 1109, 768, 696, 563 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{11}H_{11}O_2N_4^+$ 231.0882; found 231.0884

(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)(phenyl)methanone (2s)



Prepared using (4-benzoylphenyl)boronic acid (254 mg, 1125 μmol, 3.0 eq). Silica gel chromatography (1% EtOAc/DCM) yielded an average of 66% as a bright pink crystalline solid (run 1: 68 mg, 66%; run 2: 68 mg, 66%).

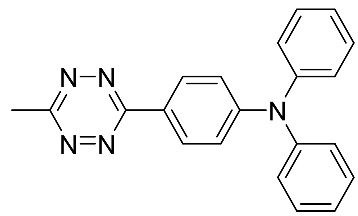
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (app d, *J* = 8.5 Hz, 2H), 8.00 (app d, *J* = 8.5 Hz, 2H), 7.88 – 7.85 (m, 2H), 7.64 (app tt, *J* = 7.6, 1.2 Hz, 1H), 7.55 – 7.51 (m, 2H), 3.15 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 196.2 (C), 167.8 (C), 163.7 (C), 141.0 (C), 137.1 (C), 135.2 (C), 133.1 (CH), 130.8 (CH), 130.3 (CH), 128.6 (CH), 127.9 (CH), 21.4 (CH_3)

FTIR (KBr, thin film) 3447, 2070, 1654, 1403, 894, 867, 789, 742, 700, 585, 551 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{16}H_{13}ON_4^+$ 277.1089; found 277.1093

4-(6-methyl-1,2,4,5-tetrazin-3-yl)-*N,N*-diphenylaniline (2t)



Prepared using 4-(diphenylamino)phenyl boronic acid (325 mg, 1125 μmol, 3.0 eq). Silica gel chromatography (70% DCM/hexanes) yielded an average of 46% as a reddish orange powdery solid (run 1: 61 mg, 48%; run 2: 56 mg, 44%).

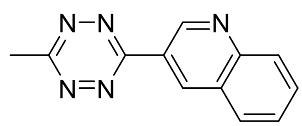
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (app d, *J* = 9.0 Hz, 2H), 7.36 – 7.31 (m, 4H), 7.22 – 7.18 (m, 4H), 7.16 – 7.12 (m, 4H), 3.04 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4 (C), 163.9 (C), 152.0 (C), 146.7 (C), 129.8 (CH), 129.1 (CH), 126.0 (CH), 124.7 (CH), 123.8 (C), 121.1 (CH), 21.2 (CH_3)

FTIR (KBr, thin film) 3462, 2065, 1636, 1591, 1488, 1403, 1364, 1331, 1283, 1177, 1086, 891, 801, 756, 697, 622, 563, 515 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{21}H_{18}N_5^+$ 340.1562; found 340.1566

3-(6-methyl-1,2,4,5-tetrazin-3-yl)quinoline (2u)



Prepared using 3-Quinolineboronic acid (123 mg, 713 μmol, 1.9 eq). Silica gel chromatography (2% acetone/DCM) yielded an average of 63% as a magenta hairy solid (run 1: 54 mg, 64%; run 2: 51 mg, 61%).

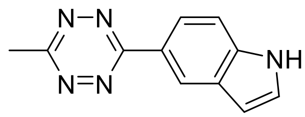
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.04 (d, *J* = 2.0 Hz, 1H), 9.42 (d, *J* = 2.4, 1H), 8.23 (d, *J* = 8.4 Hz, 1H), 8.04 (d, *J* = 8.4, 1H), 7.88 (app t, *J* = 7.8 Hz, 1H), 7.68 (app t, *J* = 7.6 Hz, 1H), 3.17 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.9 (C), 163.4 (C), 149.9 (C), 148.7 (CH), 136.4 (CH), 132.0 (CH), 129.7 (CH), 129.3 (CH), 127.9 (CH), 127.5 (C), 124.8 (C), 21.5 (CH_3)

FTIR (KBr, thin film) 3454, 3058, 2922, 2362, 2337, 1615, 1598, 1497, 1401, 1320, 808, 787, 758, 650, 562, 475 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{12}H_{10}N_5^+$ 224.0936; found 224.0938

5-(6-methyl-1,2,4,5-tetrazin-3-yl)-1H-indole (2v)



Prepared using 5-Indoleboronic acid (115 mg, 713 µmol, 1.9 eq). Silica gel chromatography (2% acetone/DCM) yielded an average of 61% as an orange hairy solid (run 1: 45 mg, 57%; run 2: 51 mg, 65%).

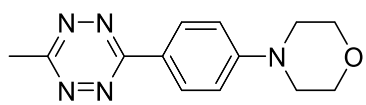
^{1}H NMR (400 MHz, DMSO-d$_6$) δ 11.57 (NH, br s, 1H), 8.59–8.58 (m, 1H), 8.13 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.61 (app t, *J* = 2.8 Hz, 1H), 6.58 – 6.56 (m, 1H), 2.96 (s, 3H)

^{13}C NMR (101 MHz, DMSO-d$_6$) δ 166.4 (C), 164.3 (C), 136.1 (C), 131.0 (C), 129.2 (CH), 124.2 (C), 121.0 (CH), 117.9 (CH), 111.3 (CH), 101.8 (CH), 20.7 (CH$_3$)

FTIR (KBr, thin film) 3448, 2071, 1636, 1401, 1360, 512 cm^{-1}

HRMS (ESI+) [M+H]$^+$ Calculated for $C_{11}H_{10}N_5^+$ 212.0936; found 212.0938

4-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)morpholine (2w)



Prepared using 4-Morpholinophenylboronic Acid (233 mg, 1125 µmol, 3.0 eq). Silica gel chromatography (3% acetone/DCM) yielded an average of 58% as a dark red crystalline solid (run 1: 58 mg, 60%; run 2: 53 mg, 55%).

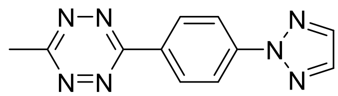
^{1}H NMR (400 MHz, CDCl$_3$) δ 8.49 (d, *J* = 9.1 Hz, 2H), 7.04 (d, *J* = 9.1 Hz, 2H), 3.90 (app t, *J* = 5.0 Hz, 4H) 3.35 (app t, *J* = 5.0 Hz, 4H), 3.04 (s, 3H)

^{13}C NMR (101 MHz, CDCl$_3$) δ 166.3 (C), 163.9 (C), 154.1 (C), 129.5 (CH), 122.1 (C), 114.7 (CH), 66.7 (CH$_2$), 47.9 (CH$_2$), 21.2 (CH$_3$)

FTIR (KBr, thin film) 3428, 2988, 2953, 2926, 2868, 2853, 2361, 1923, 1611, 1415, 1267, 1118, 890, 799, 562 cm^{-1}

HRMS (ESI+) [M+H]$^+$ Calculated for $C_{13}H_{16}ON_5^+$ 258.1355; found 258.1359

3-(4-(2H-1,2,3-triazol-2-yl)phenyl)-6-methyl-1,2,4,5-tetrazine (2x)



Prepared using 4-(triazol-2-yl)phenylboronic acid (213 mg, 1125 μmol, 3.0 eq). Silica gel chromatography (1% EtOAc/DCM) yielded an average of 94% as a dark pink powdery solid (run 1: 84 mg, 94%; run 2: 83 mg, 93%).

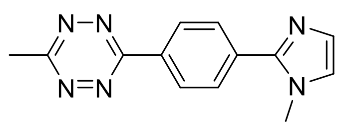
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (app d, *J* = 8.9 Hz, 2H), 8.33 (app d, *J* = 8.9 Hz, 2H), 7.89 (s, 2H), 3.12 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5 (C), 163.6 (C), 142.8 (C), 136.5 (CH), 130.8 (C), 129.3 (CH), 119.5 (CH), 21.4 (CH_3)

FTIR (KBr, thin film) 3435, 2921, 2361, 2356, 2044, 1653, 1635, 1604, 1405, 890, 856, 824, 802, 668, 566 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{11}H_{10}N_7^+$ 240.0998; found 240.0999

3-methyl-6-(4-(1-methyl-1H-imidazol-2-yl)phenyl)-1,2,4,5-tetrazine (2y)



Prepared using 4-(1-methyl-1H-imidazol-2-yl)phenylboronic acid (*see page S37 for synthesis,* 227 mg, 1125 μmol, 3.0 eq). Silica gel plug (2% EtOH/DCM) then reverse phase C_{18} silica gel chromatography (20-60% MeOH/H_2O) yielded an average of 39% as a bright pink crystalline solid (run 1: 36 mg, 38%; run 2: 42 mg, 40%).

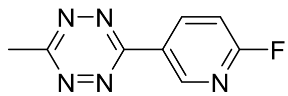
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (app d, *J* = 8.6 Hz, 2H), 7.91 (app d, *J* = 8.6 Hz, 2H), 7.20 (d, *J* = 1.2 Hz, 1H), 7.05 (d, *J* = 1.2 Hz, 1H), 3.86 (s, 3H), 3.12 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5 (C), 163.9 (C), 146.7 (C), 134.4 (C), 131.8 (C), 129.3 (CH), 129.0 (CH), 128.2 (CH), 123.5 (CH), 35.0 (CH_3), 21.4 (CH_3)

FTIR (KBr, thin film) 3445, 2989, 2949, 2066, 1636, 1477, 1407, 1277, 897, 860, 801, 710, 576 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{13}H_{13}N_6^+$ 253.1202; found 253.1201

3-(6-fluoropyridin-3-yl)-6-methyl-1,2,4,5-tetrazine (2z)



Prepared using 6-Fluoro-3-pyridinylboronic acid (101 mg, 713 μmol, 1.9 eq). Silica gel chromatography (1% acetone/DCM) yielded an average of 46% as a bright pink crystalline solid (run 1: 35 mg, 49%; run 2: 31 mg, 43%).

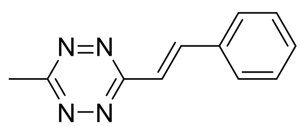
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (d, *J* = 2.4 Hz, 1H), 8.97 (ddd, *J* = 8.8, 7.6, 2.4 Hz, 1H), 7.21 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.14 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2 (C), 166.1 (CF, d, $J_{C\text{-}F}$ = 246.6 Hz), 162.4 (C), 148.5 (CH, d, $J_{C\text{-}F}$ = 16.4 Hz), 140.6 (CH, d, $J_{C\text{-}F}$ = 8.9 Hz), 126.3 (C, d, $J_{C\text{-}F}$ = 4.6 Hz), 110.5 (CH, d, $J_{C\text{-}F}$ = 37.8 Hz), 21.5 (CH_3)

FTIR (KBr, thin film) 3438, 2073, 1635, 1589, 1493, 1412, 1368, 1259, 1126, 1016, 887, 848, 506, 750, 692, 635, 575 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_8H_7FN_5^+$ 192.0685; found 192.0686

(*E*)-3-methyl-6-styryl-1,2,4,5-tetrazine (2aa)



Prepared using *trans*-2-Phenylvinylboronic acid (106 mg, 713 μmol, 1.9 eq). Silica gel chromatography (90% DCM/hexanes) yielded an average of 39% as a salmon colored crystalline solid (run 1: 27 mg, 37%; run 2: 30 mg, 41%).

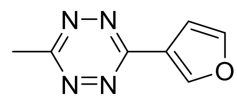
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 16.4 Hz, 1H), 7.70 – 7.67 (m, 2H), 7.49 – 7.40 (m, 4H), 3.06 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.5 (C), 164.9 (C), 141.1 (CH), 135.2 (C), 130.4 (CH), 129.2 (CH), 128.2 (CH), 120.7 (CH), 21.4 (CH_3)

FTIR (KBr, thin film) 3447, 3052, 3027, 2918, 1631, 1449, 1402, 1362, 1004, 748, 687, 470 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{11}H_{11}N_4^+$ 199.0984; found 199.0985

3-(furan-3-yl)-6-methyl-1,2,4,5-tetrazine (2ab)



Prepared using 3-Furanylboronic acid (140 mg, 1125 µmol, 3.0 eq). Silica gel chromatography (70% DCM/hexanes) yielded an average of 53% as a hot pink crystalline solid (run 1: 33 mg, 55%; run 2: 31 mg, 51%).

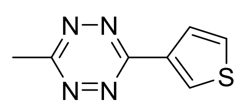
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.61 (app t, *J* = 1.6 Hz, 1H), 7.22 (d, *J* = 1.6 Hz 1H), 3.05 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.1 (C), 161.8 (C), 145.8 (CH), 145.0 (CH), 121.1 (C), 108.8 (CH), 21.4 (CH_3)

FTIR (KBr, thin film) 3176, 3153, 3127, 2920, 2850, 1589, 1517, 1423, 1381, 1360, 1157, 1085, 1000, 870, 801, 753, 648, 601, 515 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_7H_7ON_4^+$ 163.0620; found 163.0620

3-methyl-6-(thiophen-3-yl)-1,2,4,5-tetrazine (2ac)



Prepared using 3-Thienylboronic acid (144 mg, 1125 µmol, 1.9 eq). Silica gel chromatography (60% DCM/hexanes) yielded an average of 52% as a bright pink crystalline solid (run 1: 36 mg, 55%; run 2: 32 mg, 49%).

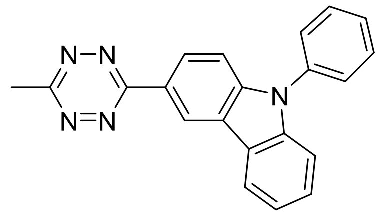
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 3.6 Hz, 1H), 8.04 (d, *J* = 4.8 Hz, 1H), 7.51 (dd, *J* = 5.2, 3.2 Hz, 1H), 3.06 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.9 (C), 161.9 (C), 134.9 (C), 130.0 (CH), 127.6 (CH), 126.6 (CH), 21.4 (CH_3)

FTIR (KBr, thin film) 3118, 3093, 2921, 2851, 1535, 1433, 1339, 892, 789, 662, 507 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_7H_7SN_4^+$ 179.0391; found 179.0391

3-(6-methyl-1,2,4,5-tetrazin-3-yl)-9-phenyl-9H-carbazole (2ad)



Prepared using N-phenyl-9H-carbazol-3-boronic acid (323 mg, 1125 µmol, 3.0 eq). Silica gel chromatography (80% DCM/hexanes) yielded an average of 39% as a salmon colored powdery solid (run 1: 51 mg, 40%; run 2: 47 mg, 37%).

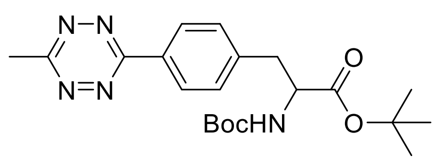
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.43 (d, *J* = 1.6 Hz, 1H), 8.65 (dd, *J* = 8.8, 2.0 Hz, 1H), 8.26 (app d, *J* = 7.6 Hz, 1H), 7.68 – 7.35 (m, 9H), 3.09 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7 (C), 164.8 (C), 143.6 (C), 141.8 (C), 137.1 (C), 130.2 (CH), 128.2 (CH), 127.3 (CH), 126.9 (CH), 125.9 (CH), 124.2 (C), 123.5 (C), 123.5 (C), 121.1 (CH), 120.0 (CH), 120.9 (CH), 110.6 (CH), 110.4 (CH), 21.3 (CH_3)

FTIR (KBr, thin film) 3448, 2072, 1626, 1597, 1502, 1397, 1365, 745, 698, 625 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{21}H_{16}N_5^+$ 338.1406; found 338.1409

***tert*-butyl 2-((*tert*-butoxycarbonyl)amino)-3-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)propanoate (2ae)**



b-Tz **1a** (44 mg, 150 µmol, 1.0 eq.), (4-(3-(*tert*-butoxy)-2-((*tert*-butoxycarbonyl)amino)-3-oxopropyl)phenyl)boronic acid[1] (104 mg, 285 µmol, 1.9 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (17 mg, 23 µmol, 0.15 eq.) and silver(I) oxide (87 mg, 375 µmol, 2.5 eq.) were added to a vacuum dried 4 mL glass vial equipped with a stir bar. The solids were dissolved/suspended as a heterogeneous slurry with N,N-Dimethylformamide (1.5 mL, 0.1M) and the vial was flushed with nitrogen and sealed. The reaction was stirred at 60°C for 20h, then brought to room temperature and the solvent was removed by rotary evaporation. The crude solids were chromatographed directly on silica gel (90-100% DCM/hexanes, then 0-2% acetone/DCM) yielding an average of 96% as a magenta wax (run 1: 61 mg, 98%; run 2: 58 mg, 94%).

^{1}H NMR (400 MHz, Acetone-d_6) δ 8.48 (d, *J* = 8.0 Hz, 2H), 7.56 (d, *J* = 8.0 Hz, 2H), 6.18 (d, *J* = 8.4 Hz, NH), 4.37 (app td, *J* = 8.4, 5.6 Hz, 1H), 3.25 (dd, *J* = 13.6, 5.6 Hz, 1H), 3.12 (dd, *J* = 13.6, 8.4 Hz, 1H), 3.03 (s, 3H), 1.43 (s, 9H), 1.37 (s, 9H)

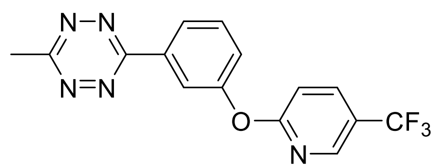
^{13}C NMR (101 MHz, Acetone-d_6) δ 171.65 (C), 168.25 (C), 164.66 (C), 156.15 (C), 143.50 (C), 131.60 (C), 131.21 (CH), 128.29 (CH), 81.79 (C), 79.26 (C), 56.39 (CH), 38.34 (CH_2), 28.49 (CH_3), 28.10 (CH_3), 21.17 (CH_3)

$[\alpha]^{24}_{D}$ = +49.0° (c = 0.11, CH_2Cl_2)

FTIR (KBr, thin film) 3368, 2978, 2932, 1715, 1611, 1498, 1456, 1405, 1366, 1250, 1154, 1089, 1056, 1018, 890, 846, 799, 568 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{21}H_{30}O_4N_5^+$ 416.2298; found 416.2287

3-methyl-6-(3-((5-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)-1,2,4,5-tetrazine (2af)



Prepared using 3-([5-(trifluoromethyl)pyridin-2-yl]oxy)phenylboronic acid (202 mg, 713 µmol, 1.9 eq). Silica gel chromatography (95% DCM/hexanes) yielded an average of 84% as a magenta oil (run 1: 105 mg, 84%; run 2: 103 mg, 83%).

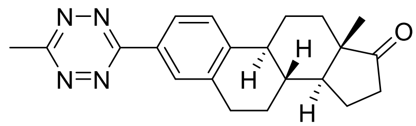
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (app dt, J = 8.0, 1.6 Hz, 1H), 8.44 – 8.43 (m, 1H), 8.40 (app t, J = 2.0 Hz, 1H), 7.96 (dd, J = 8.4, 2.4 Hz, 1H), 7.67 (app t, J = 8.0 Hz, 1H), 7.43 (ddd, J = 8.4, 2.4, 1.2 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 3.11 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7 (C), 165.5 (C, q, $J_{C\text{-}F}$ = 1.1 Hz), 163.6 (C), 154.1 (C), 145.5 (CH, q, $J_{C\text{-}F}$ = 4.3 Hz), 137.1 (CH, q, $J_{C\text{-}F}$ = 3.2 Hz), 133.7 (C), 130.8 (CH), 125.9 (CH), 125.0 (CH), 123.7 (CF_3, q, $J_{C\text{-}F}$ = 272.6 Hz), 122.1 (C, q, $J_{C\text{-}F}$ = 33.4 Hz), 121.1 (CH), 111.9 (CH), 21.4 (CH_3)

FTIR (KBr, thin film) 3449, 3078, 2925, 2853, 2088, 1613, 1588, 1488, 1398, 1363, 1327, 1284, 1282, 1161, 1125, 1077, 1012, 919, 891, 838, 794, 689, 679 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{15}H_{11}OF_3N_5^+$ 334.0916; found 334.0918

(8R,9S,13S,14S)-13-methyl-3-(6-methyl-1,2,4,5-tetrazin-3-yl)-7,8,9,11,12,13,15,16-octahydro-6H-cyclopenta[a]phenanthren-17(14H)-one (2ag)



b-Tz **1a** (59 mg, 200 μmol, 1.0 eq.), estrone-boronic acid[2] (113 mg, 380 μmol, 1.9 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (22 mg, 30 μmol, 0.15 eq.) and silver(I) oxide (116 mg, 500 μmol, 2.5 eq.) were added to a vacuum dried 4 mL glass vial equipped with a stir bar. The solids were dissolved/suspended as a heterogeneous slurry with N,N-Dimethylformamide (2.0 mL, 0.1M) and the vial was flushed with nitrogen and sealed. The reaction was stirred at 60°C for 20h, then brought to room temperature and the solvent was removed by rotary evaporation. The crude solids were chromatographed directly on silica gel (0.5% acetone, 0.75% EtOH, 98.75% chloroform) and then on reverse phase C_{18} silica (50-90% MeOH/H_2O) yielding **2ag** as a magenta crystalline solid (42 mg, 61%).

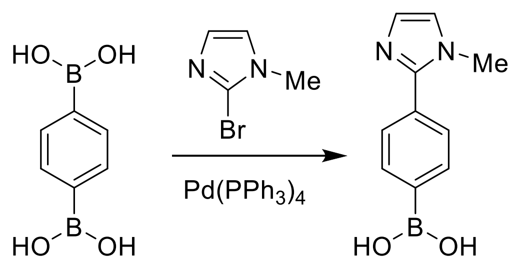
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 – 8.33 (m, 2H), 7.52 (d, *J* = 8.0 Hz, 1H), 3.11 – 3.03 (m, 5H), 2.57 – 2.48 (m, 2H), 2.44 – 2.37 (m, 1H), 2.22 – 1.99 (m, 4H), 1.73 – 1.47 (m, 6H), 0.94 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 220.8 (C), 167.2 (C), 164.2 (C), 145.0 (C), 137.8 (C), 129.3 (C), 128.5 (CH), 126.5 (CH), 125.4 (CH), 50.6 (CH), 48.0 (C), 44.8 (CH), 38.0 (CH), 36.0 (CH_2), 31.7 (CH_2), 29.5 (CH_2), 26.4 (CH_2), 25.7 (CH_2), 21.7 (CH_2), 21.3 (CH_3), 14.0 (CH_3)

FTIR (KBr, thin film) 3442, 2935, 2877, 2857, 2077, 1733, 1635, 1397, 1356, 890, 805, 735, 712, 632, 590 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{21}H_{25}ON_4^+$ 349.2028; found 349.2027

(4-(1-methyl-1H-imidazol-2-yl)phenyl)boronic acid



To a stirred mixture of 1,4-phenylenediboronic acid (69.5 g, 0.42 mol, 1.1 eq.), 2-bromo-1-methyl-1H-imidazole (61.4 g, 0.39 mol, 1.0 eq.) and $Pd(PPh_3)_4$ (4.64 g, 4.1 mmol, 1.1 mol%) in 800 mL of toluene and 700 mL of methanol was added aq. Na_2CO_3 (10% solution in water, 48.6 g, 0.46 mol, 1.2 eq.) at rt in one portion under nitrogen atmosphere. After the addition, the mixture was heated to reflux for 8 h. TLC (DCM/MeOH = 5:1) indicated the complete consumption of the starting material. The mixture was evaporated under reduced pressure; the residue was taken up with 1 L of MeOH, the mixture was filtered to remove inorganic salt, and the filtrate was concentrated. The crude product was re-crystallized from methanol, and then further purification by prep-HPLC in basic condition to afford (4-(1-methyl-1H-imidazol-2-yl)phenyl)boronic acid (2.6 g) as an off-purple solid and by prep-HPLC in acid condition to afford (4-(1-methyl-1H-imidazol-2-yl)phenyl)boronic acid (20.6 g) as a white solid, the total yield was 29%.

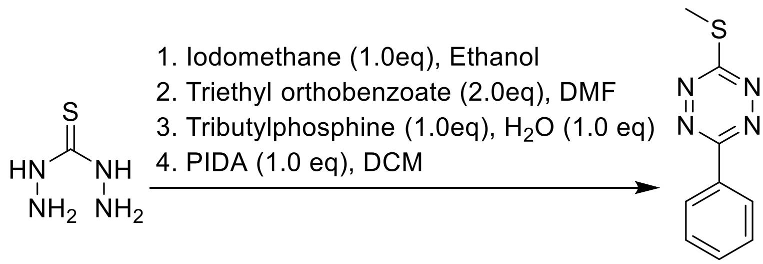
^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.40 (br s, 2H), 8.03 (app d, *J* = 8.4 Hz, 2H), 7.87 (d, *J* = 2.0 Hz, 1H, 7.81 – 7.79 (m, 3H), 3.89 (s, 3H)

^{13}C NMR (101 MHz, DMSO-d$_6$) δ 143.9, 138.2 134.6, 128.4, 124.9, 123.8, 119.2, 35.7

FTIR (KBr, thin film) 3218, 1618, 1597, 1503, 1399, 1332, 1267, 1122, 1010, 709, 683, 641 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{10}H_{12}BO_2N_2^+$ 203.0986; found 203.0980

3-(methylthio)-6-phenyl-1,2,4,5-tetrazine (3)



Step 1: Thiocarbohydrazide (5.30 g, 50.0 mmol, 1.00 eq.) was dissolved in ethanol (75 mL, 0.66 M) in a round bottom flask. The flask was flushed with nitrogen and heated to 60°C. Iodomethane (3.11 mL, 50.0 mmol, 1.0 eq.) was added and the reaction was stirred for 22h. The flask was then cooled to r.t. and 100 mL hexanes was slowly added to precipitate a white solid. The heterogeneous solution was then filtered, and the solids were washed 3x75 mL hexanes and subsequently dried under vacuum to afford methylthiocarbohydrazide iodide as a white solid (9.89 g, 80%)

Step 2: The methylthiocarbohydrazide iodide powder (9.89 g, 39.9 mmol, 1.00 eq.) and pyridine (7.1 mL, 79.8 mmol, 2.0 eq.) were dissolved in DMF (40 mL, 1.0M) and stirred under nitrogen at 50°C. Triethyl orthobenzoate (18.0 mL, 79.8 mmol, 2.0 eq.) was added dropwise over 1 h. The reaction was stirred for 24 h at 50°C to give a mixture of 1,4-dihydrotetrazine and tetrazine products.

Step 3: The tetrazine was reduced *in situ* with tributylphosphine (10.0 mL, 39.9 mmol, 1.00 eq.) and deionized water (717 µL, 1.0 eq.) stirred for 30 minutes. The reaction was then diluted in in 500 mL DCM and washed 1x100 mL aq. sat. $NaHCO_3$, 5x100 mL water and 1x100 mL brine. The crude 1,4-dihydrotetrazine was then preabsorbed on to silica gel, dried by rotary evaporation, and chromatographed (80% DCM/hexanes to elute triethyl orthobenzoate, then 3% MeOH/DCM to elute 1,4-dihydrotetrazine).

Step 4: The 1,4-dihydrotetrazine was dissolved in DCM (200 mL) and cooled to 0°C. (Diacetoxyiodo)benzene (12.84 g, 39.9 mmol, 1.0 eq.) was added slowly and then the reaction was stirred at room temperature for 2h. The solvent was removed by rotary evaporation and the crude mixture was chromatographed directly (35% DCM/hexanes) yielding 3-(methylthio)-6-phenyl-1,2,4,5-tetrazine as a red crystalline solid (4.65 g, 57% steps 2-4 ----- 46% over 4 steps).

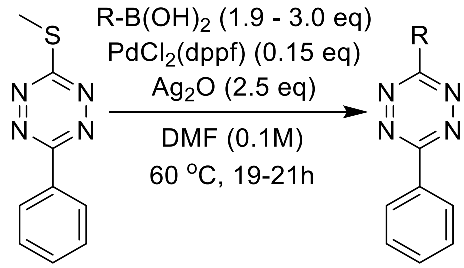
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 – 8.52 (m, 2H), 7.64 – 7.56 (m, 3H), 2.80 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 175.4 (C), 162.4 (C), 132.5 (CH), 131.7 (C), 129.4 (CH), 127.6 (CH), 13.6 (CH_3)

FTIR (KBr, thin film) 3449, 2937, 2067, 1636, 1355, 1196, 897, 760, 694, 561 cm^{-1}

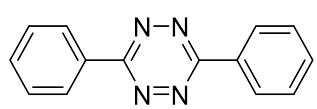
HRMS (ESI+) $[M+H]^+$ Calculated for $C_9H_9N_4S^+$ 205.0548; found 205.0552

General Procedure for Phenyl Tetrazine Cross-coupling



Tetrazine thioether **3** (77 mg, 375 μmol, 1 eq.), boronic acid (713 μmol, 1.9 eq. *or* 1125 μmol, 3.0 eq., *see below*), [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (41 mg, 56 μmol, 0.15 eq.) and silver(I) oxide (218 mg, 938 μmol, 2.5 eq.) were added to a vacuum dried schlenk flask equipped with a stir bar. The solids were dissolved/suspended as a heterogeneous slurry with N,N-Dimethylformamide (3.75 mL, 0.1M) and the flask was flushed with nitrogen and sealed. The reaction was stirred at 60°C for 19-21h, then brought to room temperature and the solvent was removed by rotary evaporation. The crude solids were chromatographed directly on silica gel. Elution systems are described below. Each reaction was run in duplicate to obtain an average yield.

3,6-diphenyl-1,2,4,5-tetrazine (4a)



Prepared using phenylboronic acid (87 mg, 713 μmol, 1.9 eq). Silica gel chromatography (40% DCM/hexanes) yielded an average of 99% as a magenta crystalline solid (run 1: 87 mg, 99%; run 2: 86 mg, 98%).

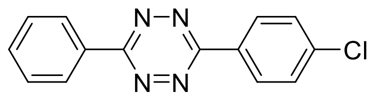
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 – 8.65 (m, 4H), 7.68 – 7.60 (m, 6H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 164.1 (C), 132.9 (CH), 131.9 (C), 129.5 (CH), 128.1 (CH)

FTIR (KBr, thin film) 3440, 1635, 1456, 1393, 919, 774, 767, 689, 589 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{14}H_{11}N_4^+$ 235.0984; found 235.0984

3-(4-chlorophenyl)-6-phenyl-1,2,4,5-tetrazine (4b)



Prepared using 4-Chlorophenylboronic acid (111 mg, 713 μmol, 1.9 eq). Silica gel chromatography (40% DCM/hexanes) yielded an average of 91% as a magenta hairy solid (run 1: 90 mg, 89%; run 2: 93 mg, 92%).

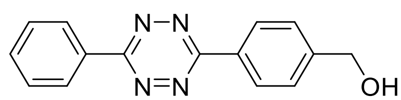
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 – 8.64 (m, 2H), 8.63 – 8.60 (m, 2H), 7.69 – 7.59 (m, 5H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 164.5 (C), 163.5 (C), 139.4 (C), 133.0 (CH), 131.7 (C), 130.4 (C), 129.8 (CH), 129.5 (CH), 129.3 (CH), 128.2 (CH)

FTIR (KBr, thin film) 3444, 2087, 1635, 1395, 915, 813, 756, 687, 586 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{14}H_{10}ClN_4^+$ 269.0594; found 269.0598

(4-(6-phenyl-1,2,4,5-tetrazin-3-yl)phenyl)methanol (4c)



Prepared using 4-(Hydroxymethyl)phenylboronic acid (108 mg, 713 μmol, 1.9 eq). Silica gel chromatography (2% acetone, 0.75% EtOH, 97.25% chloroform) yielded an average of 89% as a magenta hairy solid (run 1: 84 mg, 85%; run 2: 91 mg, 92%).

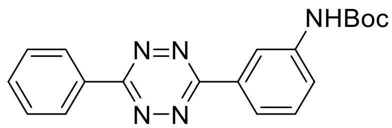
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 – 8.50 (m, 4H), 7.75 – 7.67 (m, 3H), 7.63 (app d, *J* = 8.4 Hz, 2H), 5.45 (OH, t, *J* = 5.6 Hz, 1H), 4.67 (d, *J* = 5.6 Hz, 2H)

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.3 (C), 163.3 (C), 147.7 (C), 132.6 (CH), 132.0 (C), 130.2 (C), 129.5 (CH), 127.5 (CH), 127.5 (CH), 127.2 (CH), 62.5 (CH_2)

FTIR (KBr, thin film) 3439, 2072, 1636, 1394, 588 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{15}H_{13}ON_4^+$ 265.1089; found 265.1091

tert-butyl (3-(6-phenyl-1,2,4,5-tetrazin-3-yl)phenyl)carbamate (4d)



Prepared using 3-(N-Boc-amino)phenylboronic acid (169 mg, 713 µmol, 1.9 eq). Silica gel chromatography (0.5% EtOAc, 0..75% EtOH, 98.75% chloroform) yielded an average of 92% as a pink powdery solid (run 1: 121 mg, 92%; run 2: 119 mg, 91%)

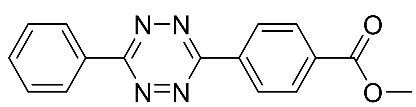
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (NH, s, 1H), 8.80 (app s, 1H), 8.56 – 8.53 (m, 2H), 8.15 (app dt, J = 8.0, 1.6 Hz, 1H), 7.75 – 7.68 (m, 4H), 7.57 (app t, J = 8.0 Hz, 1H), 1.52 (s, 9H)

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.4 (C), 163.3 (C), 152.8 (C), 140.7 (C), 132.7 (CH), 132.3 (C), 131.9 (C), 129.9 (CH), 129.5 (CH), 127.6 (CH), 122.0 (CH), 121.2 (CH), 116.8 (CH), 79.5 (C), 28.2 (CH_3)

FTIR (KBr, thin film) 3445, 3343, 2987, 1697, 1639, 1593, 1532, 1388, 763, 686, 619, 551 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{19}H_{20}O_2N_5^+$ 350.1617; found 350.1610

methyl 4-(6-phenyl-1,2,4,5-tetrazin-3-yl)benzoate (4e)



Prepared using 4-Methoxycarbonylphenylboronic acid (203 mg, 1125 µmol, 3.0 eq). Silica gel chromatography (85% DCM/hexanes) yielded an average of 76% as a purple crystalline solid (run 1: 86 mg, 78%; run 2: 81 mg, 74%).

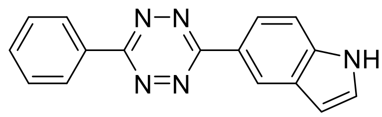
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 – 8.73 (m, 2H), 8.69 – 8.66 (m, 2H), 8.30 – 8.27 (m, 2H), 7.70 – 7.61 (m, 3H), 3.99 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.5 (C), 164.2 (C), 163.6 (C), 135.8 (C), 133.7 (C), 133.2 (CH), 131.6 (C), 130.6 (CH), 129.5 (CH), 128.3 (CH), 128.0 (CH), 52.7 (CH_3)

FTIR (KBr, thin film) 3444, 2077, 1704, 1636, 1396, 1280, 774, 688, 686, 591 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{16}H_{13}O_2N_4^+$ 293.1039; found 293.1038

5-(6-phenyl-1,2,4,5-tetrazin-3-yl)-1H-indole (4f)



Prepared using 5-Indoleboronic acid (123 mg, 713 μmol, 1.9 eq). Silica gel chromatography (1% acetone/DCM) yielded an average of 49% as an orange powdery solid (run 1: 46 mg, 45%; run 2: 54 mg, 52%).

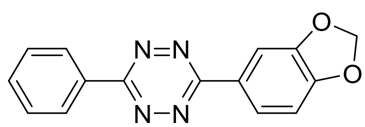
^{1}H NMR (400 MHz, DMSO-d_6) δ 11.65 (NH, br s, 1H), 8.66 (s, 1H), 8.54 – 8.52 (m, 2H), 8.22 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.71 – 7.68 (m, 3H), 7.64 (t, *J* = 2.8 Hz, 1H), 6.60 – 6.59 (m, 1H)

^{13}C NMR (101 MHz, DMSO-d_6) δ 164.4 (C), 162.9 (C), 136.1 (C), 132.3 (CH), 132.1 (C), 131.3 (C), 129.5 (CH), 127.3 (2 x CH), 124.1 (C), 121.1 (CH), 118.1 (CH), 111.6 (CH), 101.9 (CH)

FTIR (KBr, thin film) 3439, 2072, 1636, 1393, 568 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{16}H_{12}N_5^+$ 274.1093; found 274.1093

3-(benzo[d][1,3]dioxol-5-yl)-6-phenyl-1,2,4,5-tetrazine (4g)



Prepared using 3,4-(Methylenedioxy)phenylboronic acid (118 mg, 713 μmol, 1.9 eq). Silica gel chromatography (50% DCM/hexanes) yielded an average of 88% as a dark salmon colored hairy solid (run 1: 90 mg, 86%; run 2: 94 mg, 90%).

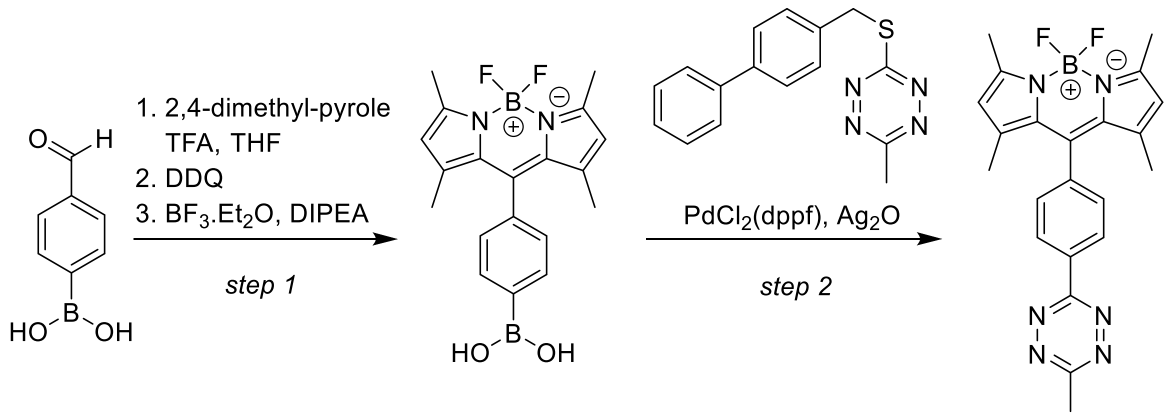
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 – 8.61 (m, 2H), 8.29 (dd, *J* = 8.4, 1.6 Hz, 1H), 8.10 (d, *J* = 1.6 Hz, 1H), 7.66 – 7.59 (m, 3H), 7.03 (d, *J* = 8.0 Hz, 1H), 6.12 (s, 2H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 163.7 (C), 163.6 (C), 151.8 (C), 148.9 (C), 132.6 (CH), 132.0 (C), 129.4 (CH), 127.9 (CH), 125.9 (C), 123.8 (CH), 109.3 (CH), 107.8 (CH), 102.1 (CH_2)

FTIR (KBr, thin film) 3446, 2074, 1636, 1388, 1110, 922, 877, 818, 688, 633, 561 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{15}H_{11}O_2N_4^+$ 279.0882; found 279.0885

3-BODIPY-6-methyltetrazine (6) synthesis (2 steps)



10-(4-boronophenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (5)

4-Formylphenylboronic acid (1.95 g, 13.0 mmol, 1.00 eq.) and 2,4-dimethyl-pyrole (2.81 mL, 27.3 mmol, 2.10 eq.) were dissolved in THF (120 mL) and stirred under nitrogen at room temperature. Trifluoroacetic acid (0.40 mL, 5.20 mmol, 0.40 eq.) was added dropwise and the reaction was stirred until the aldehyde was fully consumed by TLC (1 h). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (2.95 g, 13.0 mmol, 1.00 eq.) was added to the reaction and stirred for 16 h. Boron trifluoride diethyl etherate (16.0 mL, 130 mmol, 10.0 eq.) and N,N-Diisopropylethylamine (15.8 mL, 91.0 mmol, 7 eq.) were then added and the reaction was stirred a further 6 h. The organics were washed with 3x250 mL water and 2x25 mL brine. The organic phase was then dried on $MgSO_4$, filtered, and concentrated by rotary evaporation. The crude material was then dissolved in a minimal amount of hot 10% MeOH/DCM (~30 mL) after which hexanes (~500 mL) were slowly added to precipitate red/black impurities. The mixture was filtered, and the filtrate was concentrated by rotary evaporation. Silica gel chromatography (80% DCM, 19% EtOAc, 1% MeOH) yielded **5** as a dark orange crystalline solid (574 mg, 12%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (app d, *J* = 7.6 Hz, 2H), 7.49 (app d, *J* = 8.0 Hz, 2H), 6.01 (s, 2H), 2.58 (s, 6H), 1.40 (s, 6H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 155.9 (C), 143.1 (C), 141.2 (C), 139.9 (C), 136.5 (CH), 131.2 (C), 130.6 (C), 128.0 (CH), 121.5 (CH), 14.8 (CH_3), 14.6 (CH_3)

FTIR (KBr, thin film) 3738, 3564, 2957, 2925, 1543, 1508, 1469, 1398, 1306, 1194, 1157, 979, 836, 719, 478 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{19}H_{21}B_2F_2O_2N_2^+$ 369.1757; found 369.1754

5,5-difluoro-1,3,7,9-tetramethyl-10-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (6)

b-Tz **1a** (run 1: 110.3 mg, 375 µmol, 1.0 eq.; run 2: 75.7 mg, 257 µmol, 1.0 eq.), **5** (run 1: 262 mg, 713 µmol, 1.9 eq.; run 2: 180 mg, 489 µmol, 1.9 eq.), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (run 1: 41 mg, 56 µmol, 0.15 eq.; run 2: 28 mg, 39 µmol, 0.15 eq.) and silver(I) oxide (run 1: 218 mg, 938 µmol, 2.5 eq.; run 2: 149 mg, 643 µmol, 2.5 eq.) were added to a vacuum dried 4 mL glass vial equipped with a stir bar. The solids were dissolved/suspended as a heterogeneous slurry with N,N-Dimethylformamide (0.1M) and the vial was flushed with nitrogen and sealed. The reaction was stirred at 60°C for 20h, then brought to room temperature and the solvent was removed by rotary evaporation. The crude solids were chromatographed directly on silica gel (90% DCM/hexanes) yielding an average of 78% as a red/orange iridescent solid (run 1: 123 mg, 79%; run 2: 81 mg, 76%).

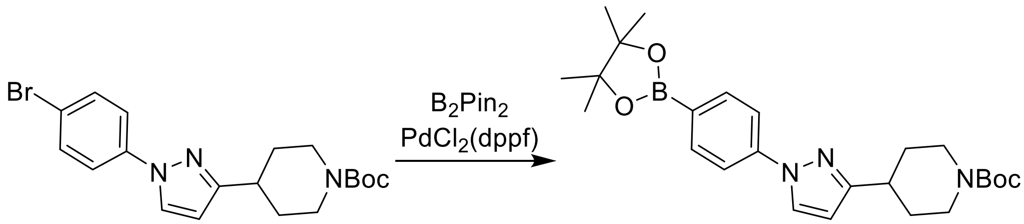
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (app d, *J* = 8.0 Hz, 2H), 7.56 (app d, *J* = 8.0 Hz, 2H), 6.01 (s, 2H), 3.14 (s, 3H), 2.58 (s, 6H), 1.45 (s, 6H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.7 (C), 163.8 (C), 156.2 (C), 143.0 (C), 140.3 (C), 139.6 (C), 132.7 (C), 131.1 (C), 129.4 (CH), 128.7 (CH), 121.7 (CH), 21.4 (CH_3), 14.8 (4 x CH_3)

FTIR (KBr, thin film) 3431, 2963, 2926, 2855, 1546, 1512, 1403, 1308, 1194, 1157, 1084, 982, 711, 477 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{22}H_{22}BF_2N_6^+$ 419.1967; found 419.1962

***tert*-butyl 4-(1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazol-3-yl)piperidine-1-carboxylate**



To a sealed tube was added Pd(dppf)Cl_2 (486 mg, 0.665 mmol, 0.3 eq.), Bis(pinacolato)diboron (1120 mg, 4.43 mmol, 2.0 eq.), potassium acetate (652 mg, 6.65 mmol, 3.0 eq.)), and *tert*-butyl 4-(1-(4-bromophenyl)-1H-pyrazol-3-yl)piperidine-1-carboxylate[3] (900 mg, 2.22mmol, 1.0 eq.)) and degassed 1,4-dioxane (10 mL) under N_2 atmosphere at 25 °C. The reaction mixture was then stirred at 100 °C for 18 h. The mixture was cooled to r.t. and diluted with EtOAc (150 mL). Then the mixture was washed with 1x100 mL water and 1x100 mL brine. The organic layer was concentrated under reduced pressure and the residue was purified by flash column chromatograph (40 g silica gel column, petroleum ether/ EtOAc with EtOAc from 0-30%) to afford the crude product which was triturated with n-hexane (0°C, 30 mL) to afford the *tert*-butyl 4-(1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazol-3-yl)piperidine-1-carboxylate as pale yellow solid (530 mg, 53%).

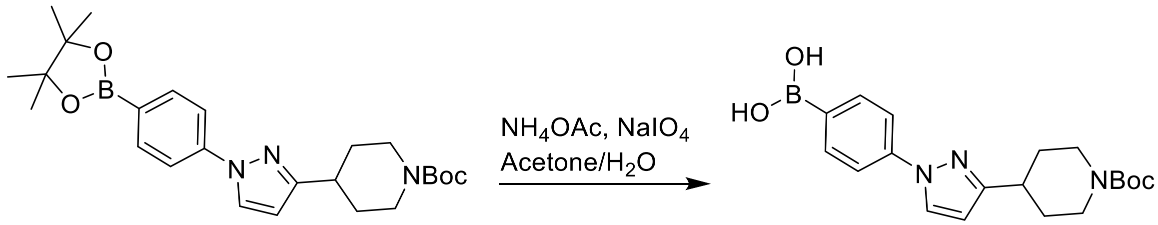
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.85 (m, 3H), 7.66 (app d, *J* = 8.0 Hz, 2H), 6.28 (d, *J* = 2.0 Hz, 1H), 4.18 (app d, *J* = 12.8 Hz, 2H), 2.96 – 2.85 (m, 3H), 1.99 (app d, *J* = 13.2 Hz, 2H), 1.73 – 1.63 (m, 2H), 1.48 (s, 9H), 1.36 (s, 12H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 158.5, 155.0, 142.3, 136.2, 127.5, 117.8, 105.2, 84.1, 79.5, 44.0, 36.0, 32.1, 28.6, 25.0 --- Carbon directly attached to boron not observed

FTIR (KBr, thin film) 2976, 2932, 1685, 1607, 1356, 1232, 1165, 1143, 1092, 946, 858, 733, 653 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{25}H_{37}BO_4N_3^+$ 454.2872; found 454.2857

(4-(3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)-1H-pyrazol-1-yl)phenyl)boronic acid (7)



tert-butyl 4-(1-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-1H-pyrazol-3-yl)piperidine-1-carboxylate (297 mg, 0.66 mmol, 1.00 eq.), ammonium acetate (303 mg, 3.93 mmol, 6.00 eq.) and sodium periodate (842 mg, 3.93 mmol, 6.00 eq.) was stirred in a mixture of 5:2 acetone/H_2O (33 mL) at room temperature for 66 h. The acetone was removed by rotary evaporation and the aqueous phase was extracted with 3x25 mL DCM and washed with 1x25 mL aq. sat. $NaHCO_3$ and 1x25 mL brine. The organics were dried on $MgSO_4$ and concentrated. Silica gel chromatography (3% MeOH/DCM) yielded (4-(3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)-1H-pyrazol-1-yl)phenyl)boronic acid as a flakey white solid (214 mg, 88%).

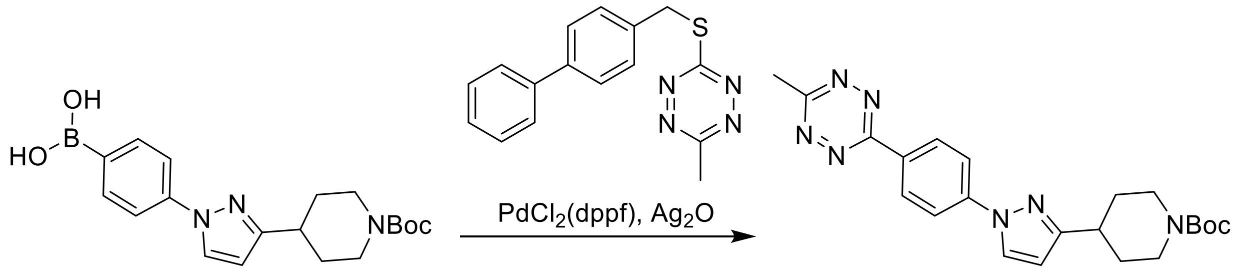
^{1}H NMR (400 MHz, Methanol-d$_4$) δ 8.09 (d, *J* = 2.8 Hz, 1H), 7.82 – 7.61 (m, 4H), 6.34 (d, *J* = 2.8 Hz, 1H), 4.14 – 4.09 (m, 2H), 3.28 – 3.26 (m, 2H), 2.89 (tt, *J* = 11.6, 4.0 Hz, 1H), 1.94 (app dd, *J* = 14.0, 3.6 Hz, 2H), 1.67 – 1.56 (m, 2H), 1.44 (s, 9H) --- $B(OH)_2$ not observed due to solvent exchange

^{13}C NMR (101 MHz, DMSO-d$_6$ + D_2O) δ 157.9 (C), 154.3 (C), 141.2 (C), 135.7 (CH), 131.3 (weak, CB), 128.5 (CH), 117.0 (CH), 105.8 (CH), 79.0 (C), 44.2 (br, CH_2), 43.2 (br, CH_2), 35.2 (CH), 31.7 (br, 2 x CH_2), 28.4 (CH_3)

FTIR (KBr, thin film) 3426, 2979, 2940, 2858, 2079, 1658, 1606, 1367, 1165, 737, 645, 544 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{19}H_{27}BO_4N_3^+$ 372.2095; found 372.2088

tert-butyl 4-(1-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1H-pyrazol-3-yl)piperidine-1-carboxylate (8)



b-Tz **1a** (29 mg, 100 μmol, 1.0 eq.) (4-(3-(1-(*tert*-butoxycarbonyl)piperidin-4-yl)-1H-pyrazol-1-yl)phenyl)boronic acid **7** (71 mg, 190 μmol, 1.9 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (11 mg, 15.0 μmol, 0.15 eq.) and silver(I) oxide (58 mg, 250 μmol, 2.5 eq.) were added to a vacuum dried 4 mL glass vial equipped with a stir bar. The solids were dissolved/suspended as a heterogeneous slurry with N,N-Dimethylformamide (1.0 mL, 0.1M) and the vial was flushed with nitrogen and sealed. The reaction was stirred at 60°C for 20h, then brought to room temperature and the solvent was removed by rotary evaporation. The crude solids were chromatographed directly on silica gel (5% acetone/DCM) yielding *tert*-butyl 4-(1-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1H-pyrazol-3-yl)piperidine-1-carboxylate as a bright pink powder (32 mg, 77%).

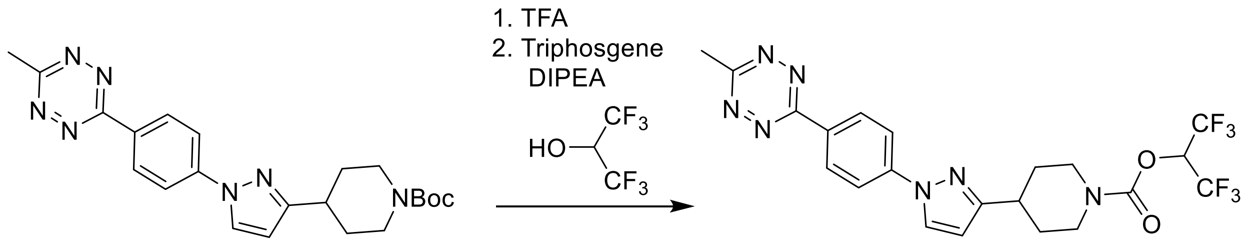
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 – 8.67 (app d, *J* = 9.0 Hz, 2H), 7.79 (d, *J* = 2.4 Hz, 1H), 7.92-7.89 (app d, *J* = 9.0 Hz, 2H), 6.35 (d, *J* = 2.8 Hz, 1H), 4.24-4.16 (m, 2H), 3.10 (s, 3H), 2.99 – 2.87 (m, 3H), 2.05-1.97 (m, 2H), 1.76 – 1.64 (m, 2H), 1.48 (s, 9H)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3 (C), 163.6 (C), 159.2 (C), 155.0 (C), 143.3 (C), 129.4 (CH), 129.1 (C), 127.5 (CH), 119.0 (CH), 106.1 (CH), 79.6 (C), 44.0 (CH_2), 36.0 (CH), 31.9 (CH_2), 28.6 (CH_3), 21.3 (CH_3)

FTIR (KBr, thin film) 3470, 1326, 3109, 3005, 2977, 2937, 2850, 2246, 1676, 1605, 1533, 1408, 1366, 1181, 768, 722, 568 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{22}H_{28}O_2N_7^+$ 422.2304; found 422.2305

1,1,1,3,3,3-hexafluoropropan-2-yl 4-(1-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1H-pyrazol-3-yl)piperidine-1-carboxylate (9)



Step 1: *tert*-butyl 4-(1-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1H-pyrazol-3-yl)piperidine-1-carboxylate **8** (30 mg, 71.6 µmol, 1.0 eq.) and trifluoroacetic acid (82 µL, 1.07 mmol, 15.0 eq.) were stirred in dichloromethane (1.4 mL) for 2h at room temperature. The solvent and *tert*-butanol biproduct was then removed by rotary evaporation leaving the Boc-deprotected reagent.

Step 2: Separately, triphosgene (21 mg, 71.6 µmol, 1.0 eq.) and dichloromethane (630 µL) were stirred at 0°C under a nitrogen line equipped with an in-line column filled with powdered potassium hydroxide. A solution of 1,1,1,3',3',3'-hexafluoro-propanol (23 µL, 215 µmol, 3.0 eq.) and N,N-Diisopropylethylamine (149 µL, 859 µmol, 12.0 eq.) in dichloromethane (315 µL) was then dropwise added to the triphosgene solution. The reaction was stirred for 30min at 0°C, then 2h at room temperature. The solution turns a golden yellow color. The Boc-deprotected reagent was redissolved in dichloromethane (490 µL) and added to the triphosgene reaction which was then stirred for 16h. The reaction was diluted in 30 mL dichloromethane and washed with 2x10 mL H_2O and 1x10 mL brine. The remaining solution was dried on $MgSO_4$, filtered, and the solvent was removed by rotary evaporation. The crude solids were chromatographed on silica gel (2% acetone/DCM) yielding 1,1,1,3,3,3-hexafluoropropan-2-yl 4-(1-(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)-1H-pyrazol-3-yl)piperidine-1-carboxylate as a light pink powder. (29 mg, 78%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (app d, J = 8.8 Hz, 2H), 8.01 (d, J = 2.4 Hz, 1H), 7.92 (app d, J = 8.8 Hz, 2H), 6.38 (d, J = 2.4 Hz, 1H), 5.82 (hept, J = 6.2 Hz, 1H), 4.30 – 4.22 (m, 2H), 3.22 – 3.01 (m, 6H), 2.16 – 2.11 (m, 2H), 1.89 – 1.75 (m, 2H)

^{19}F NMR (376 MHz, $CDCl_3$) δ -73.62 (d, $J_{F\text{-}H}$ = 6.0 Hz)

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3 (C), 163.6 (C), 158.1 (C), 151.6 (C), 143.2 (C), 129.4 (CH), 129.3 (C), 120.9 (CF_3, q, $J_{C\text{-}F}$ = 283.7 Hz), 119.0 (CH), 106.0 (CH), 68.2 (CH, hept, $J_{C\text{-}F}$ = 34.5 Hz), 45.1 (CH_2), 44.5 (CH_2), 35.5 (CH), 31.7 (CH_2), 31.4 (CH_2), 21.3 (CH_3)

FTIR (KBr, thin film) 3435, 2968, 2926, 2854, 1726, 1657, 1606, 1534, 1438, 1409, 1388, 1280, 1250, 1190, 1106, 892, 801, 755, 688, 564 cm^{-1}

HRMS (ESI+) $[M+H]^+$ Calculated for $C_{21}H_{20}F_6O_2N_7^+$ 516.1583; found 516.1601

Effect of Unprotected Heteroatoms on Cross-coupling Yields

Using standard cross-coupling conditions for the synthesis of **2i**, the following additives were included to test the effect of unprotected heteroatoms on overall yield: ethylene glycol (63 µL, 3.0 eq.), ethanolamine (68 µL, 3.0 eq.), or mercaptoethanol (79 µL, 3.0 eq.). Alcohol has little effect on overall yield. Decomposition of b-Tz was observed with ethanolamine. Conversely, mercaptoethanol does not decompose b-Tz, but likely poisons the Pd-catalyst and/or Ag-mediator.

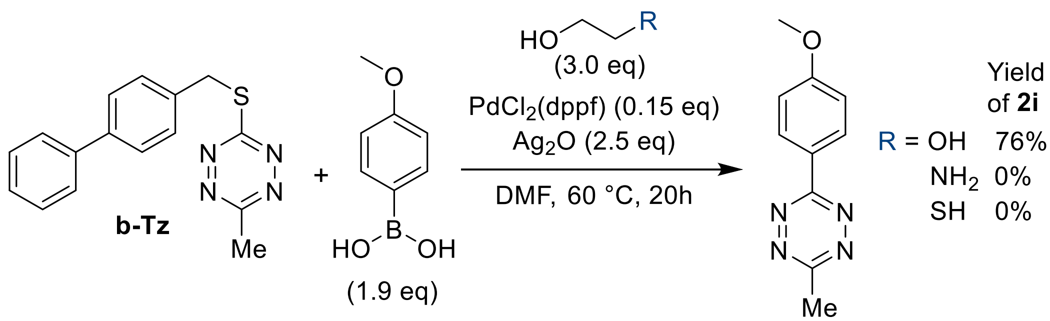
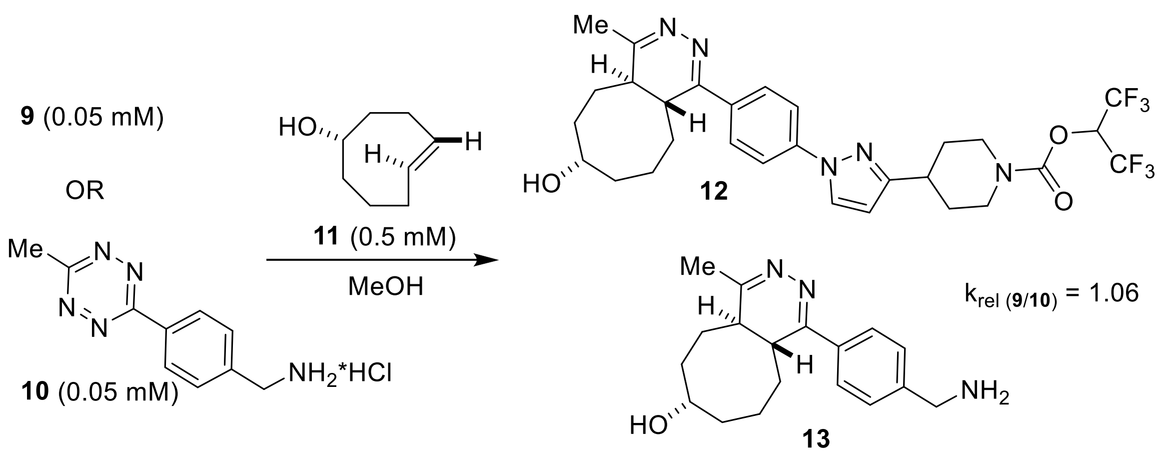


Figure S-19: Effect of ethylene glycol, ethanolamine, or mercaptoethanol on cross-coupling

Stopped-flow Kinetics of Compound 9 Versus 10



0.1 mM solutions (10 mL) in methanol were prepared from stock solutions of **9** (16.3 mg in 632 µL DMF, 50 mM) and **10** (10.0 mg in 422 µL MeOH, 100 mM). A 1.0 mM solution (25 mL) in methanol of axial 5-hydroxy-*trans*-cyclooctene **11** was also prepared from a stock solution (29.0 mg in 460 µL MeOH, 500 mM). The reaction between tetrazine and a *trans*-cyclooctene was measured under pseudo-first order conditions using a SX 18MV-R stopped-flow spectrophotometer (Applied Photophysics Ltd.). The 0.1 mM solution of tetrazines **9** or **10** and the 1.0 mM solution of *trans*-cyclooctene **11** were injected as equal volumes via syringe into the stopped-flow instrument which was held at 25°C, resulting in a final concentration of the tetrazine of 0.05 mM and a final concentration of the trans-cyclooctene of 0.5 mM. The reaction was monitored by the absorbance decay of tetrazine measured at 290 nm. Data points were collected every 0.1 second for 400 seconds and reaction was repeated in triplicate. Prism software was used to obtain the observed rate of each reaction, k_{obs}, which was determined by nonlinear regression analysis resulting in average rate constants of 0.01033 s^{-1} for **9** and 0.00975 s^{-1} for **10**. The relative rate, k_{rel}, of **9** versus **10** was thus determined to be 1.06.

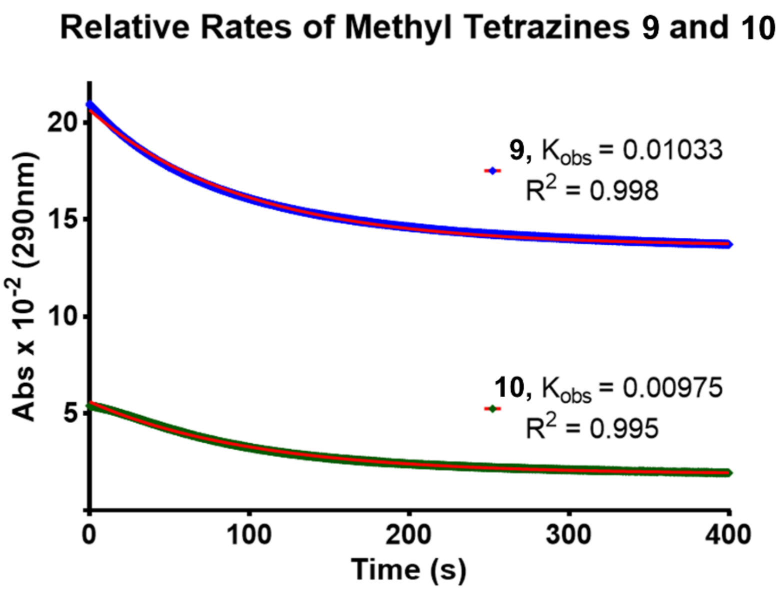


Figure S-20: Psuedo-first order stopped-flow kinetics of the tetrazine ligation between axial 5-hydroxy-*trans*-cyclooctene **11** and tetrazines **9** or **10**. Three trials were run for each tetrazine and the average was computed with Prism software (blue and green curves). The best fit curve was also computed (red curve)

MAGL Protein Assay Using Compound 9

Materials. Tetrazine amine was purchased from Click Chemistry Tools. TCO-TAMRA were synthesized according to literature protocol[4]. Human brain vascular pericytes and pericyte growth supplement were purchased from ScienCell Research Laboratories. Phosphate-based saline (PBS) was purchased from Mediatech, Inc.. Media and other supplements for cell culture were purchased from Thermo Fisher Scientific unless otherwise noted. For cell treatments, all reagents were prepared as 1000x stock solutions in DMSO and stored at -80°C.

Cell culture and probe treatment. Human brain vascular pericytes were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) GlutaMAX media supplemented with 5% heat-inactivated fetal bovine serum (HI FBS), 1x pericyte growth supplement (PGS), and 1x penicillin-streptomycin at 37 °C with 5% CO_2 in a humidified environment. Probe treatment was performed in duplicates. Cells were plated in 6-well plates and cultured overnight in growth media. Live cells were then treated with 300 nM KML29 as a competitor compound (or DMSO as a control) at 37 °C for 1 h. Cells were subsequently treated with probe **9** (0.3 nM – 3.2 μM) at 37 °C for 1 h (cells pre-treated with KML29 were treated with 32 nM of probe **9**), after which the cells were washed with fresh growth media. The media were then placed with fresh media containing 2 μM of TCO-TAMRA, and the cells were incubated at 37 °C for 30 min. To quench the reaction, the media were replaced with PBS containing 100 μM tetrazine amine, and the cells were washed with cold PBS and harvested with a scrapper. The suspensions were centrifuged at 10,000xg for 1 min at 37 °C, and the cell pellets were lysed in PBS containing 0.25% sodium dodecyl sulfate (SDS) with sonication. The protein concentration was measured with a bicinchoninic acid (BCA) assay kit (Thermo Scientific) and normalized.

In-gel fluorescence and data analysis. The proteomes were analyzed with 1.0 mm thick 4-12% bis-tris 15-well protein gels in 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer. The gels were scanned with a Typhoon FLA 9500 Biomolecular Imager (GE Healthcare) with the TAMRA channel with 532 nm excitation and a 575 nm long pass emission filter. To measure the total protein loading, the gels were treated with ClearPage Instant Blue (CBS Scientific) overnight, and after brief destaining with water, scanned with an Odyssey Imager (Li-COR) at the 700 nm channel. The in-gel fluorescence images were processed with ImageJ software (v1.47, NIH), and the intensities were quantified with Image Studio (v5.2, Li-COR) with background subtraction. The coomassie images were processed and quantified with the Image Studio software with background subtraction. For analysis of the cellular potency, the in-gel fluorescence intensities of the two MAGL bands were averaged, normalized with the total coomassie intensity of the corresponding sample, and fitted with a dose-response equation with Prism v7.02 (GraphPad).

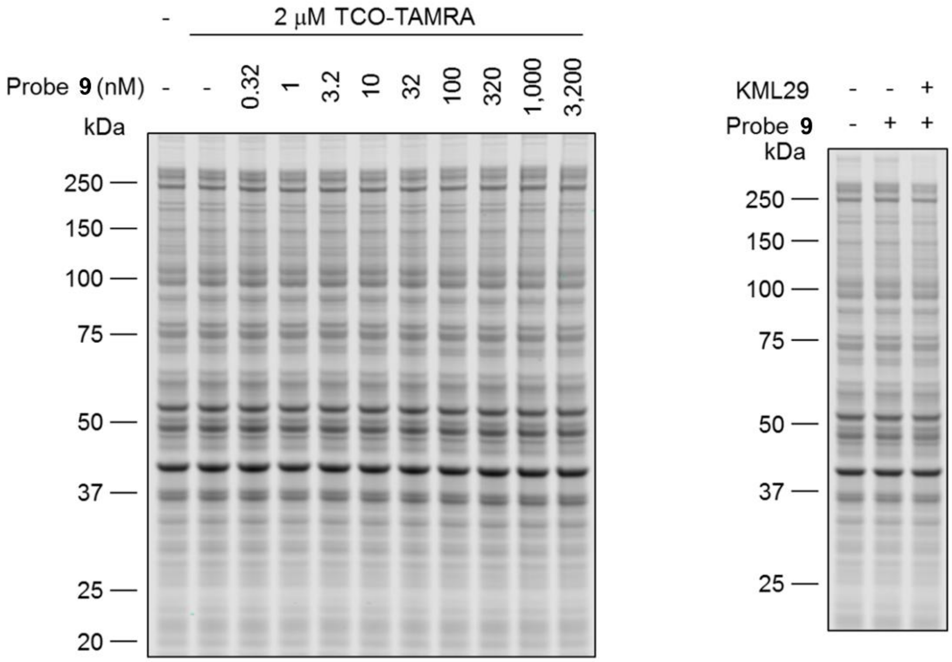


Figure S-21: Coomassie staining of total proteins for gels shown in Figure 7C.

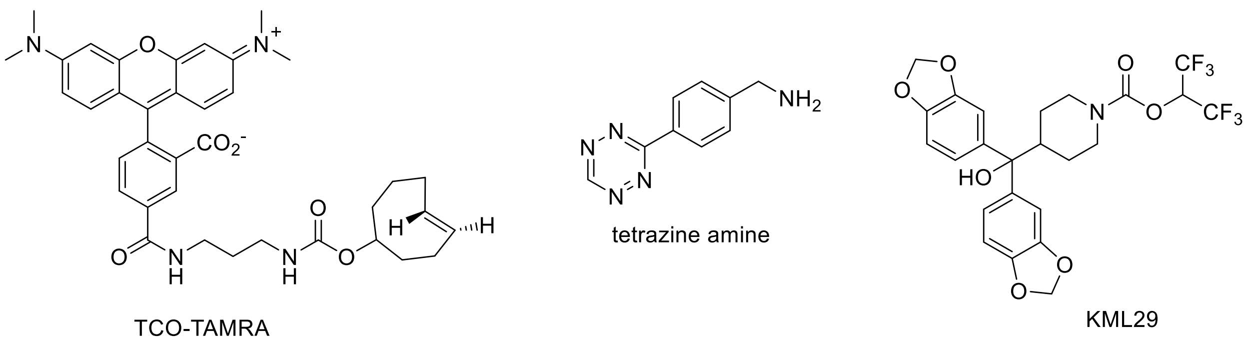


Figure S-22: Structures of the reagents for live cell experiments.

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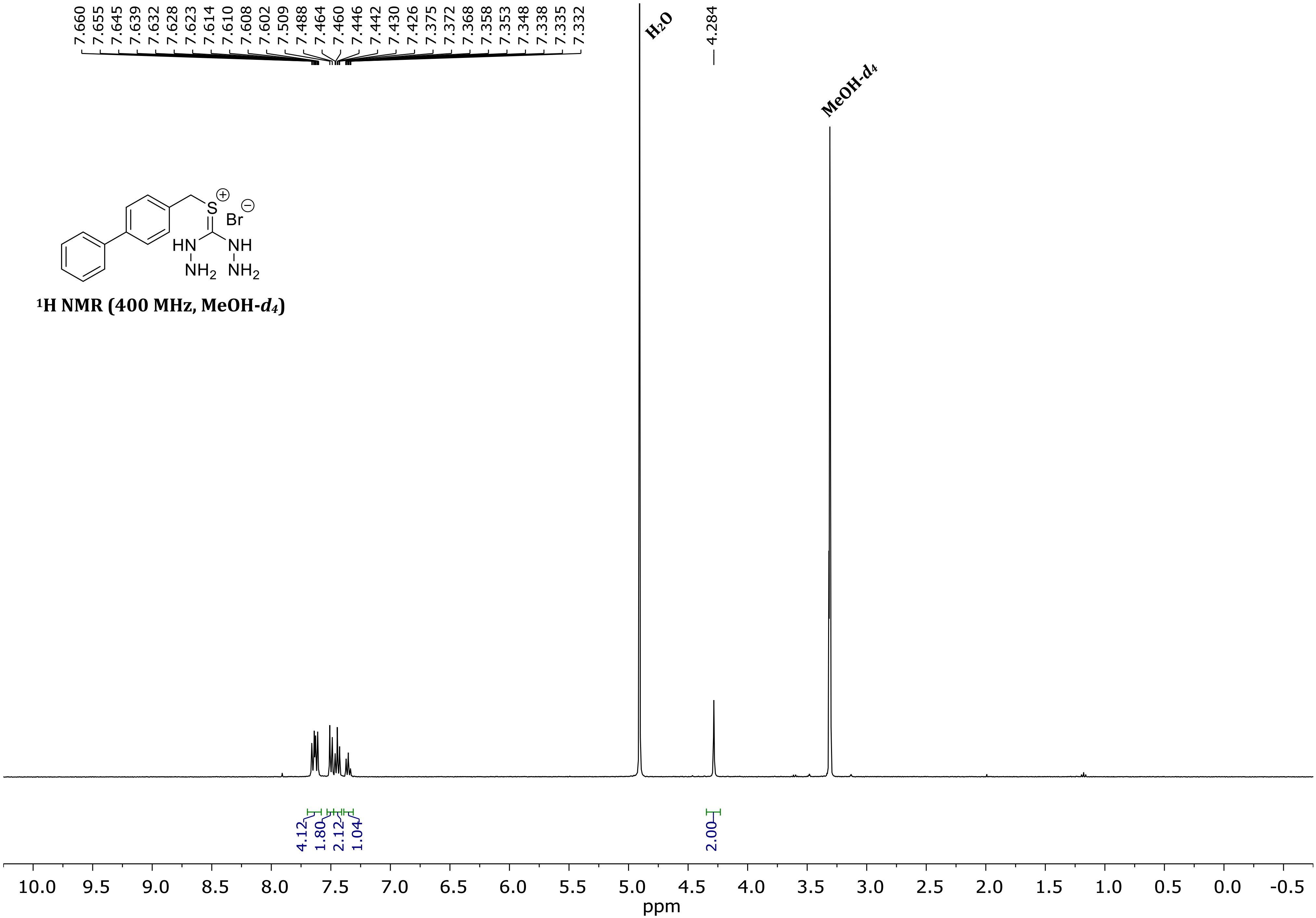
[4] Murrey, H. E.; Judkins, J. C.; Am Ende, C. W.; Ballard, T. E.; Fang, Y.; Riccardi, K.; Di, L.; Guilmette, E. R.; Schwartz, J. W.; Fox, J. M.; Johnson, D. S., Systematic Evaluation of Bioorthogonal Reactions in Live Cells with Clickable HaloTag Ligands: Implications for Intracellular Imaging. *J. Am. Chem. Soc.* **2015,** *137*, 11461-11475.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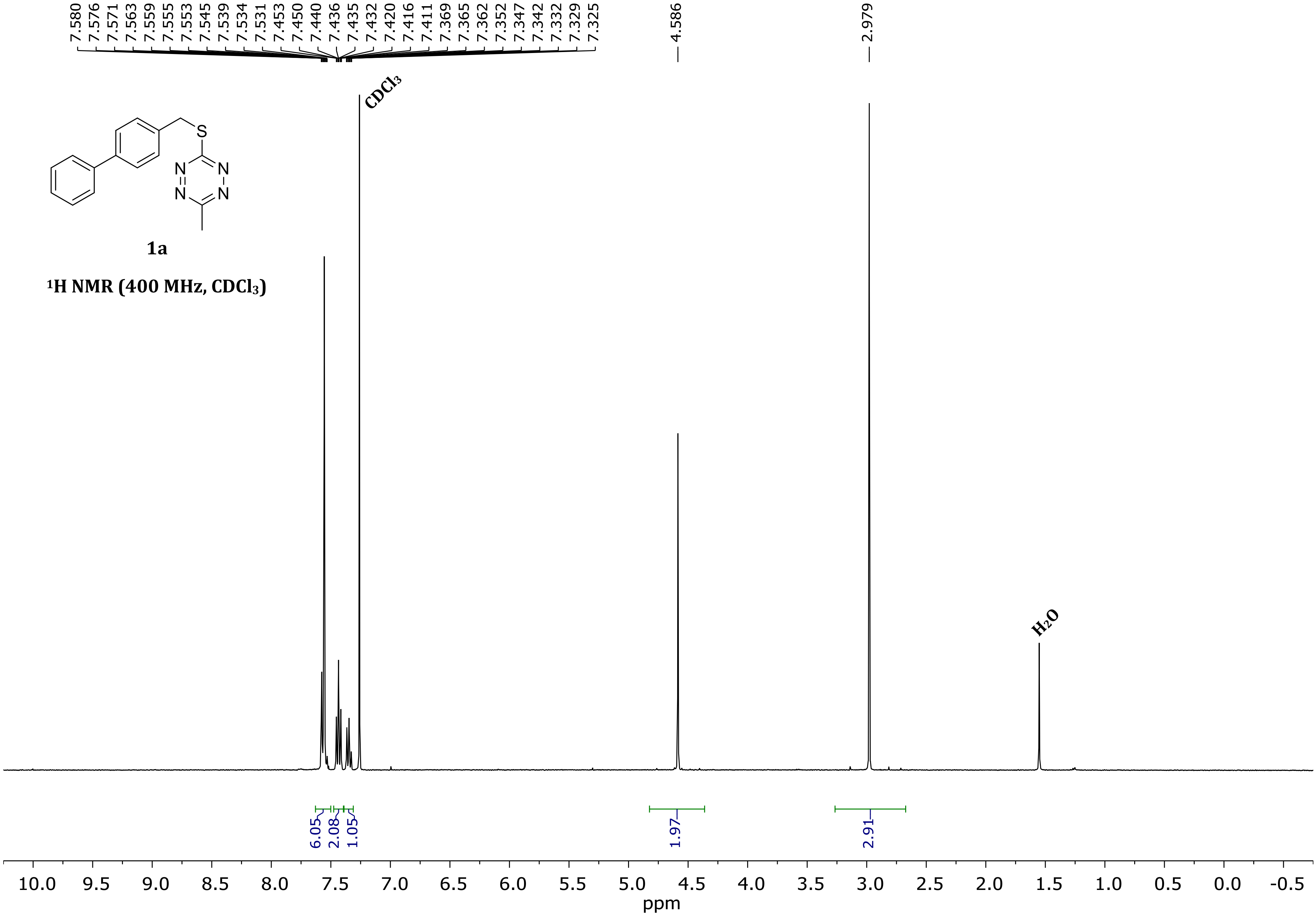
H2O
4.284
MeOH-d4
Br
HN
NH
NH2
NH2
S
1H NMR (400 MHz, MeOH-d4)
4.12
1.80
2.12
1.04
2.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm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7.347
7.342
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7.325
4.586
2.979
CDCl3
S
N
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1H NMR (400 MHz, CDCl3)
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6.05
2.08
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1.97
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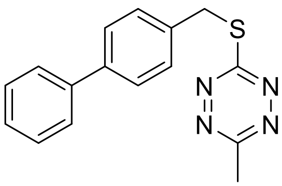
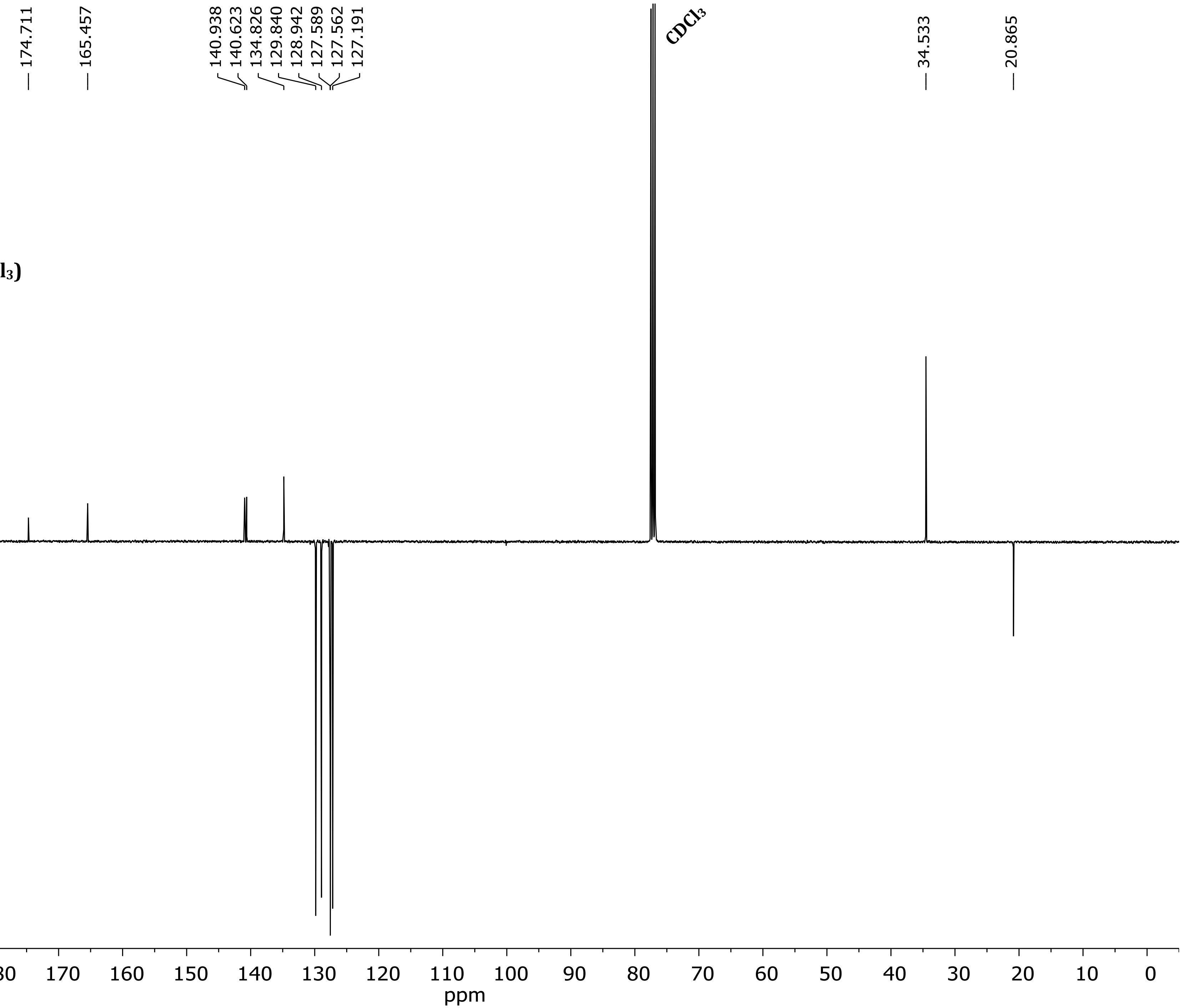
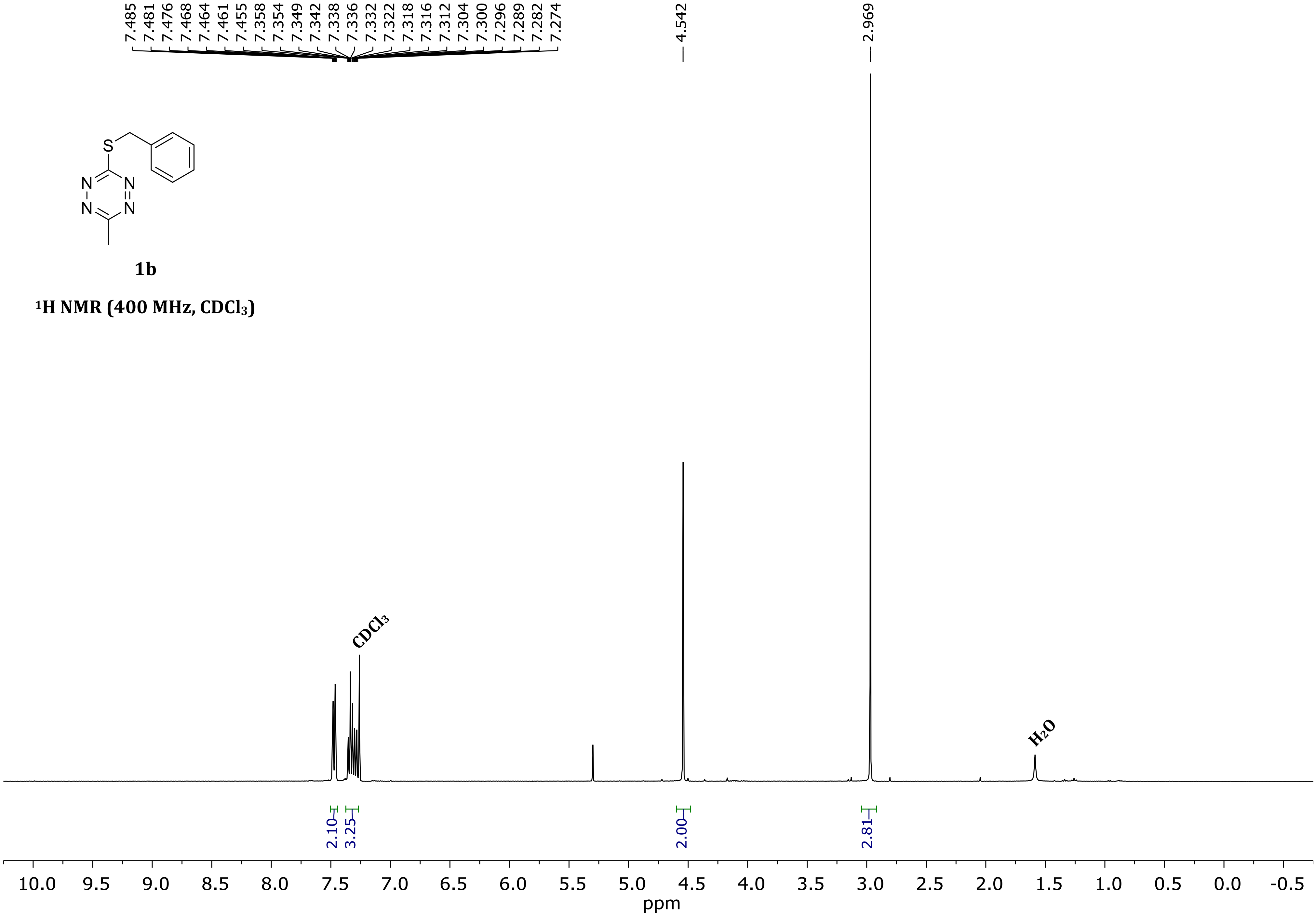
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ppm



1a

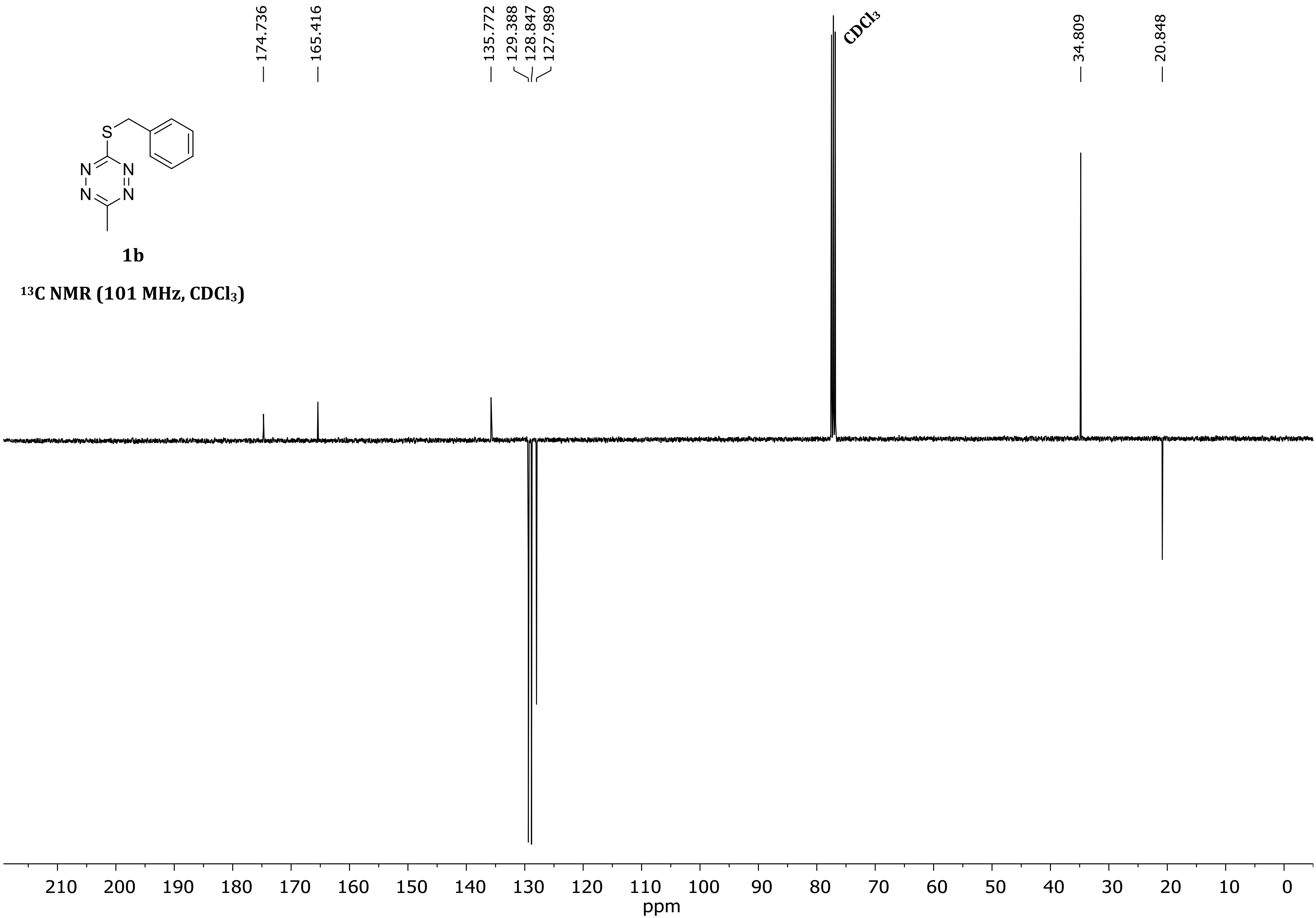
^{13}C NMR (101 MHz, $CDCl_3$)





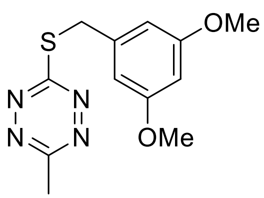
1b

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



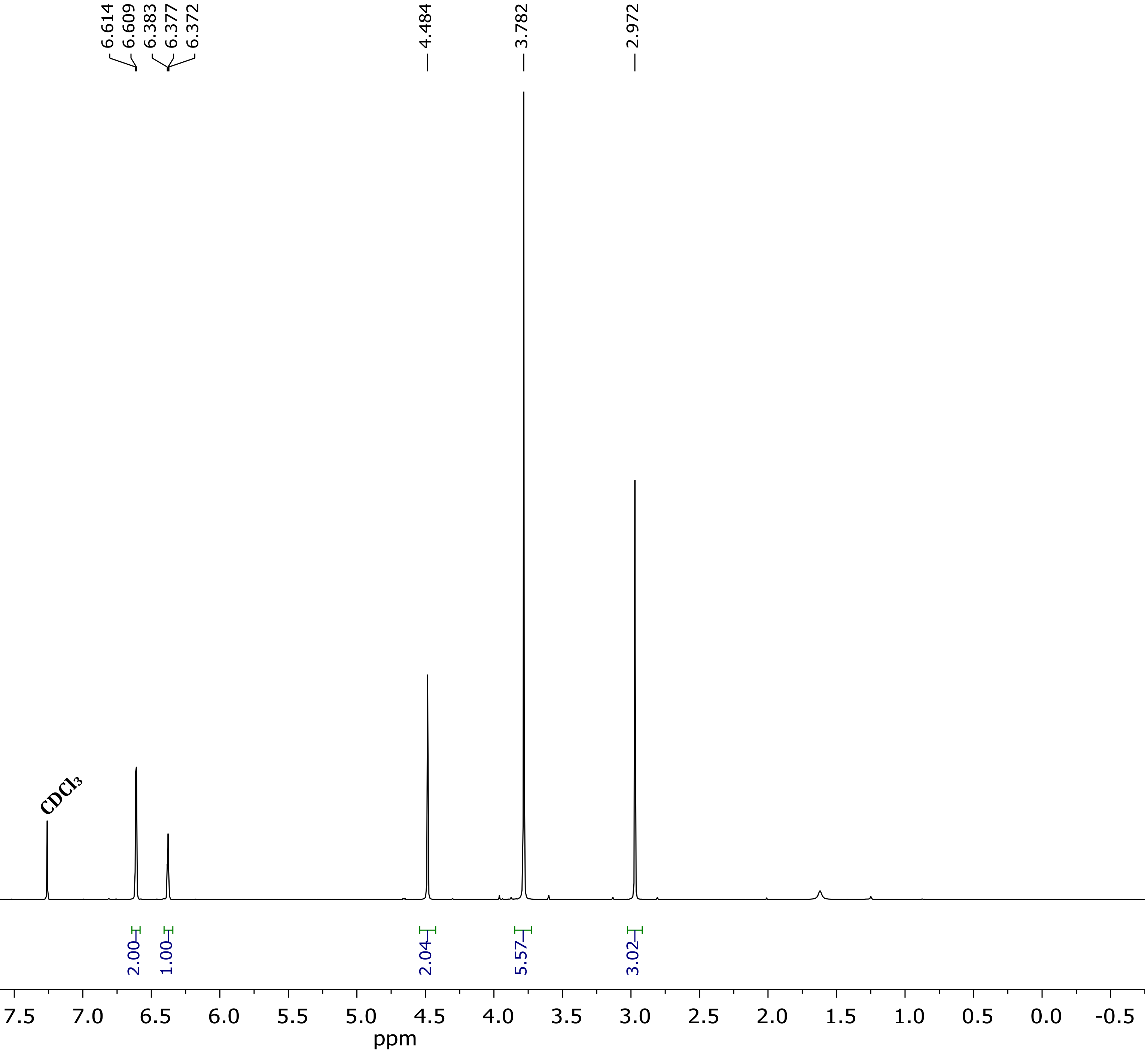
1b

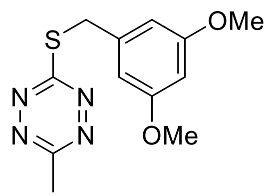
^{13}C NMR (101 MHz, CDCl$_3$)



1c

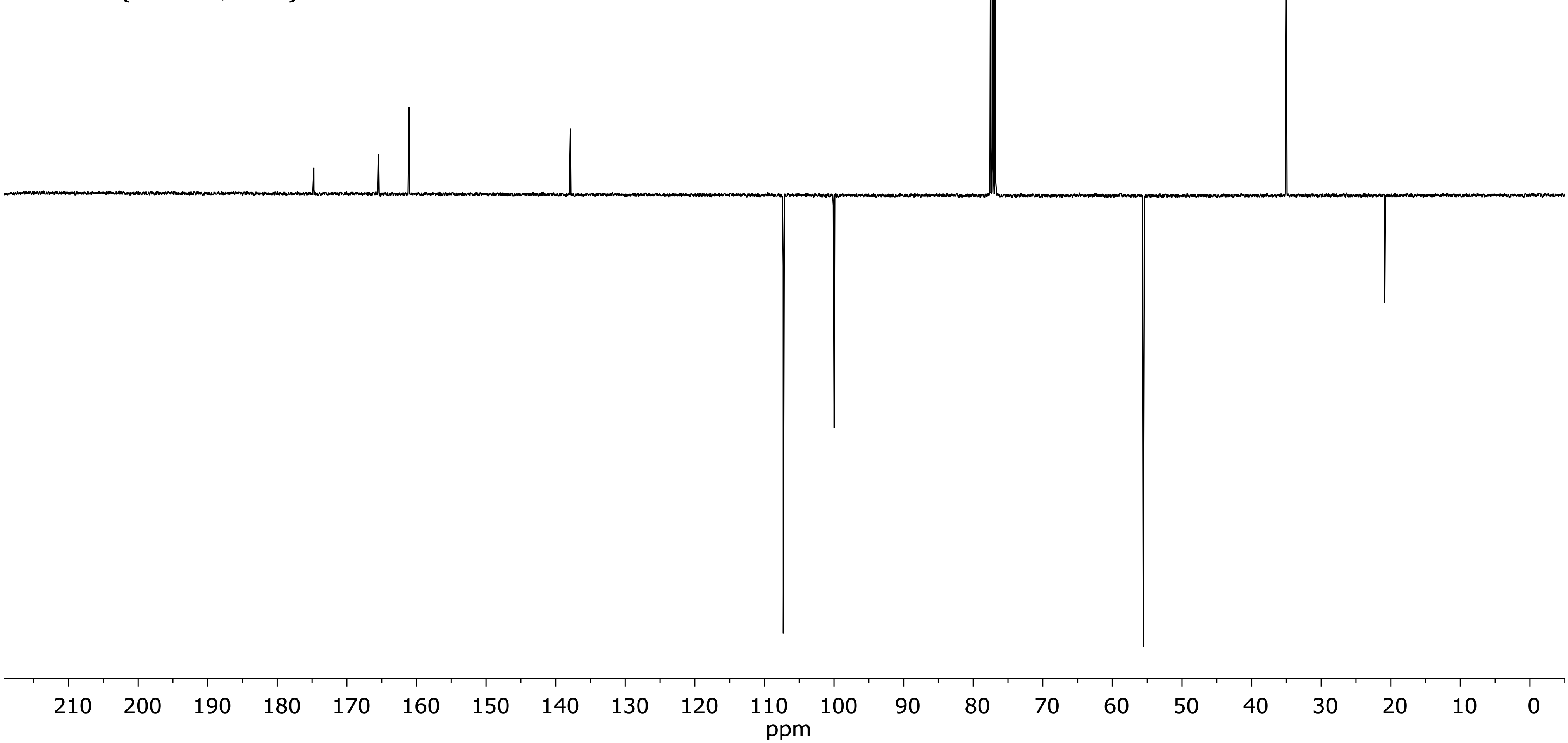
^{1}H NMR (400 MHz, CDCl$_3$)

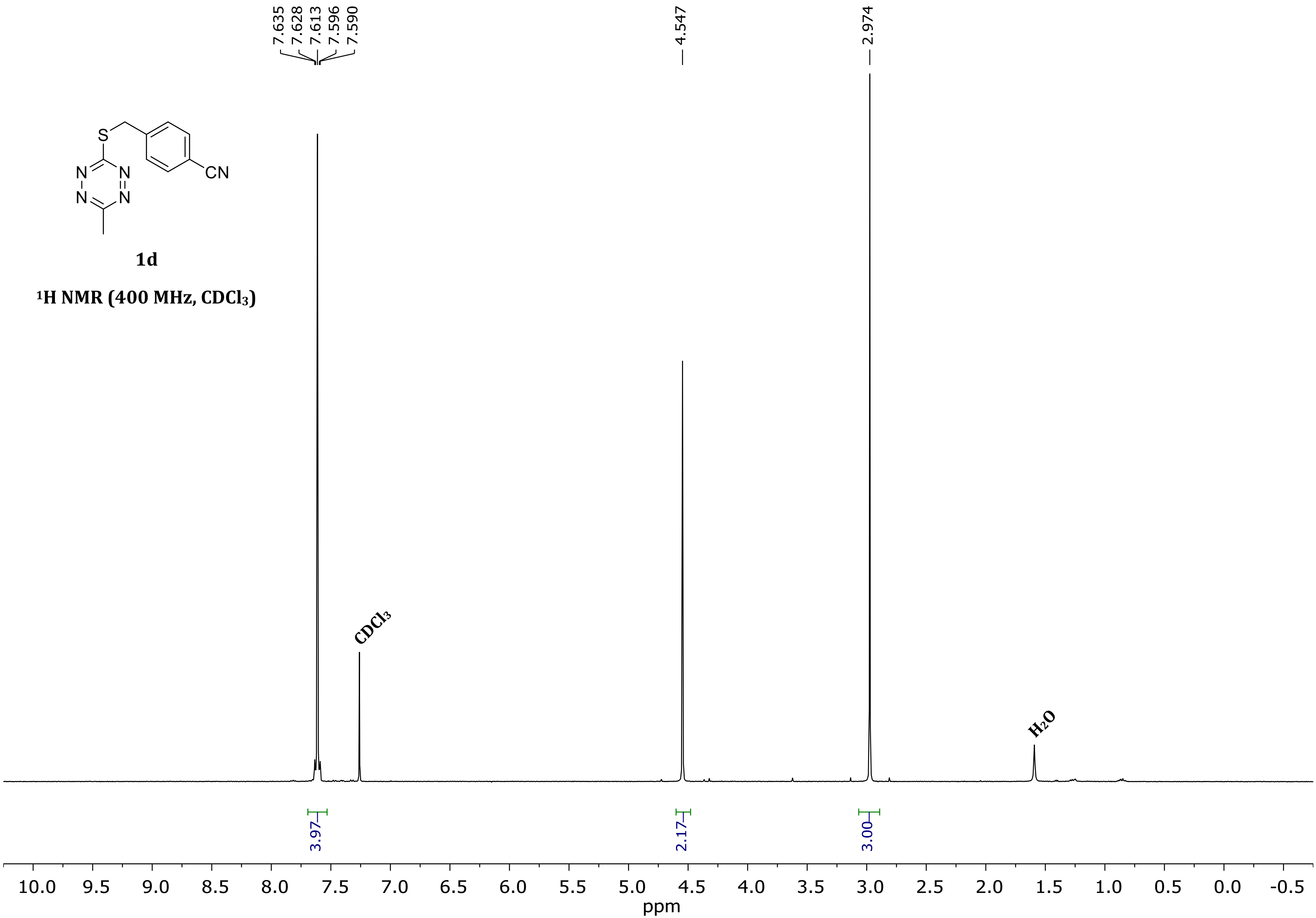


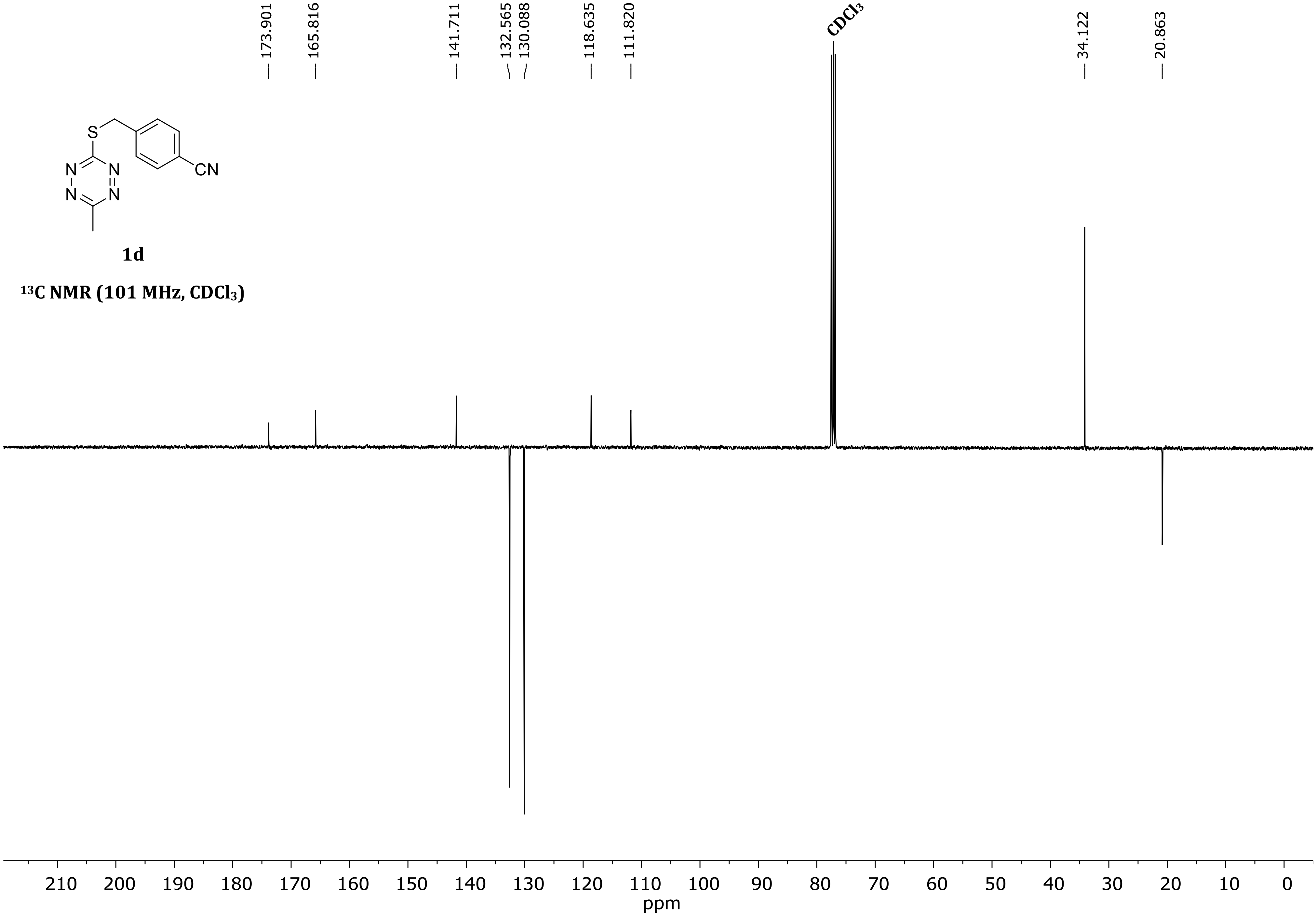


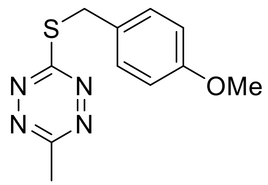
1c

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



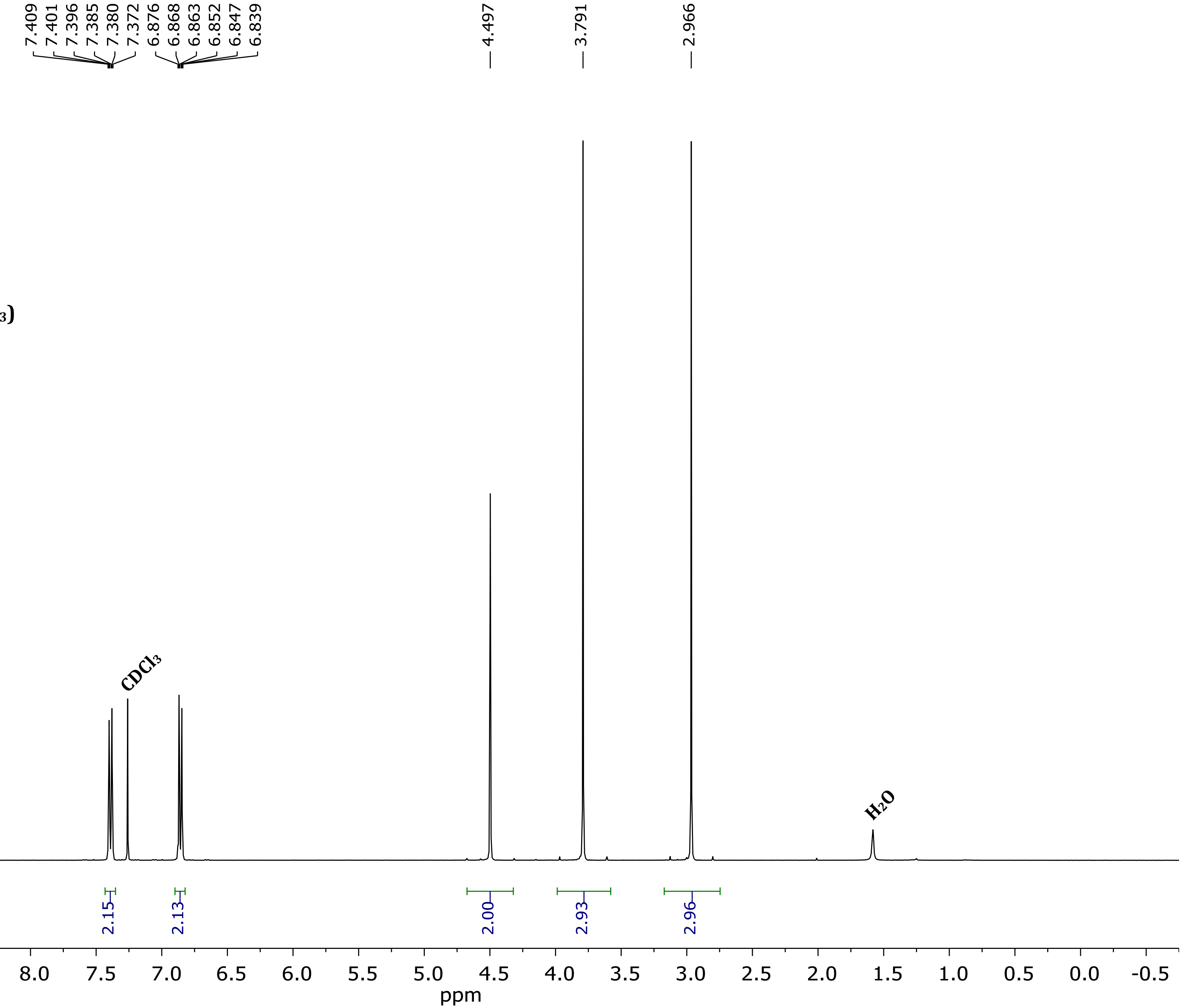

7.635
7.628
7.613
7.596
7.590
4.547
2.974
S
N
N
N
N
CN
1d
¹H NMR (400 MHz, CDCl₃)
CDCl₃
H₂O
3.97
2.17
3.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm

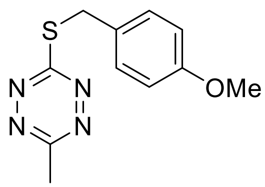

173.901
165.816
141.711
132.565
130.088
118.635
111.820
CDCl3
34.122
20.863
S
N
N
N
N
CN
1d
13C NMR (101 MHz, CDCl3)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm



1e

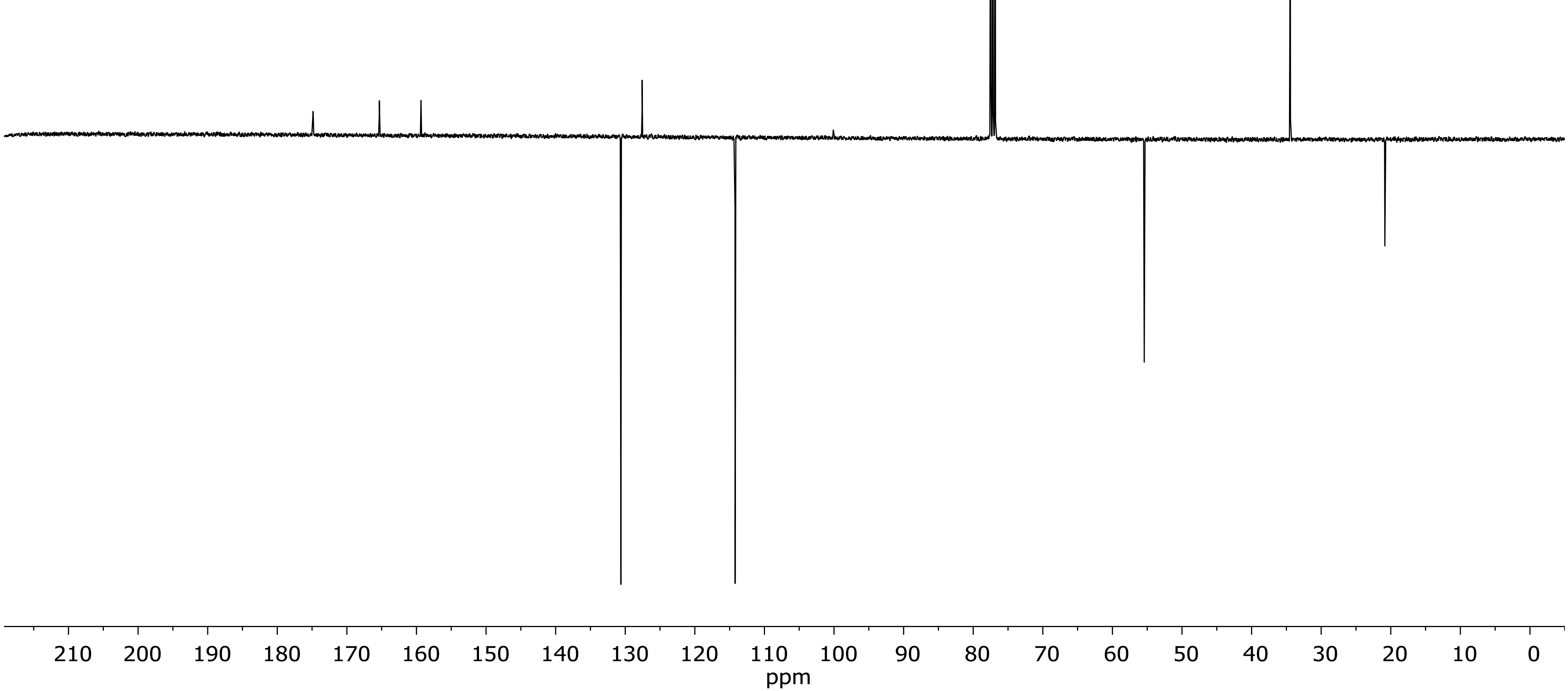
^{1}H NMR (400 MHz, $CDCl_3$)

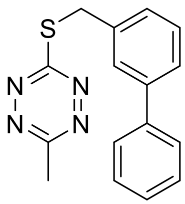




1e

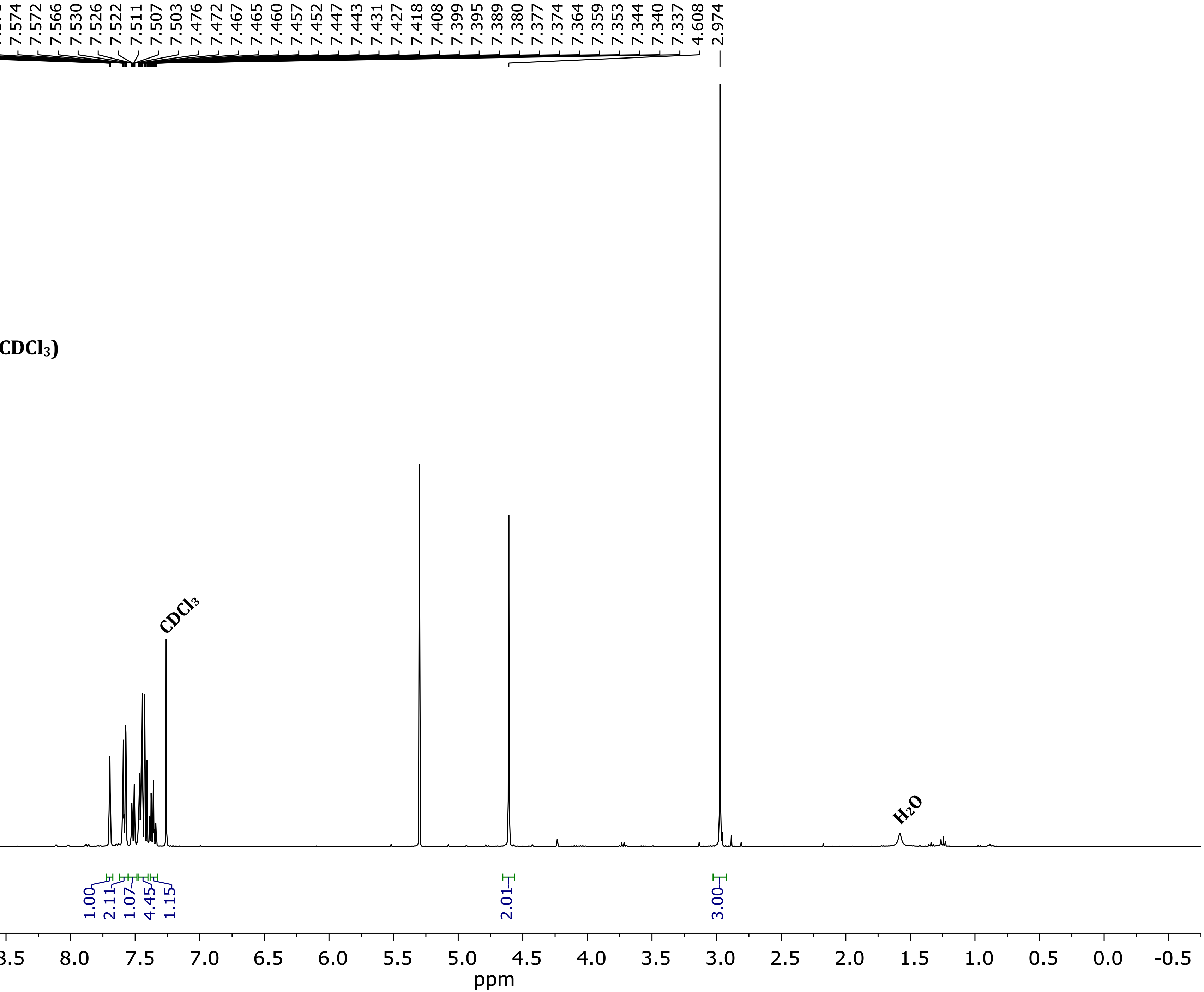
^{13}C NMR (101 MHz, CDCl$_3$)

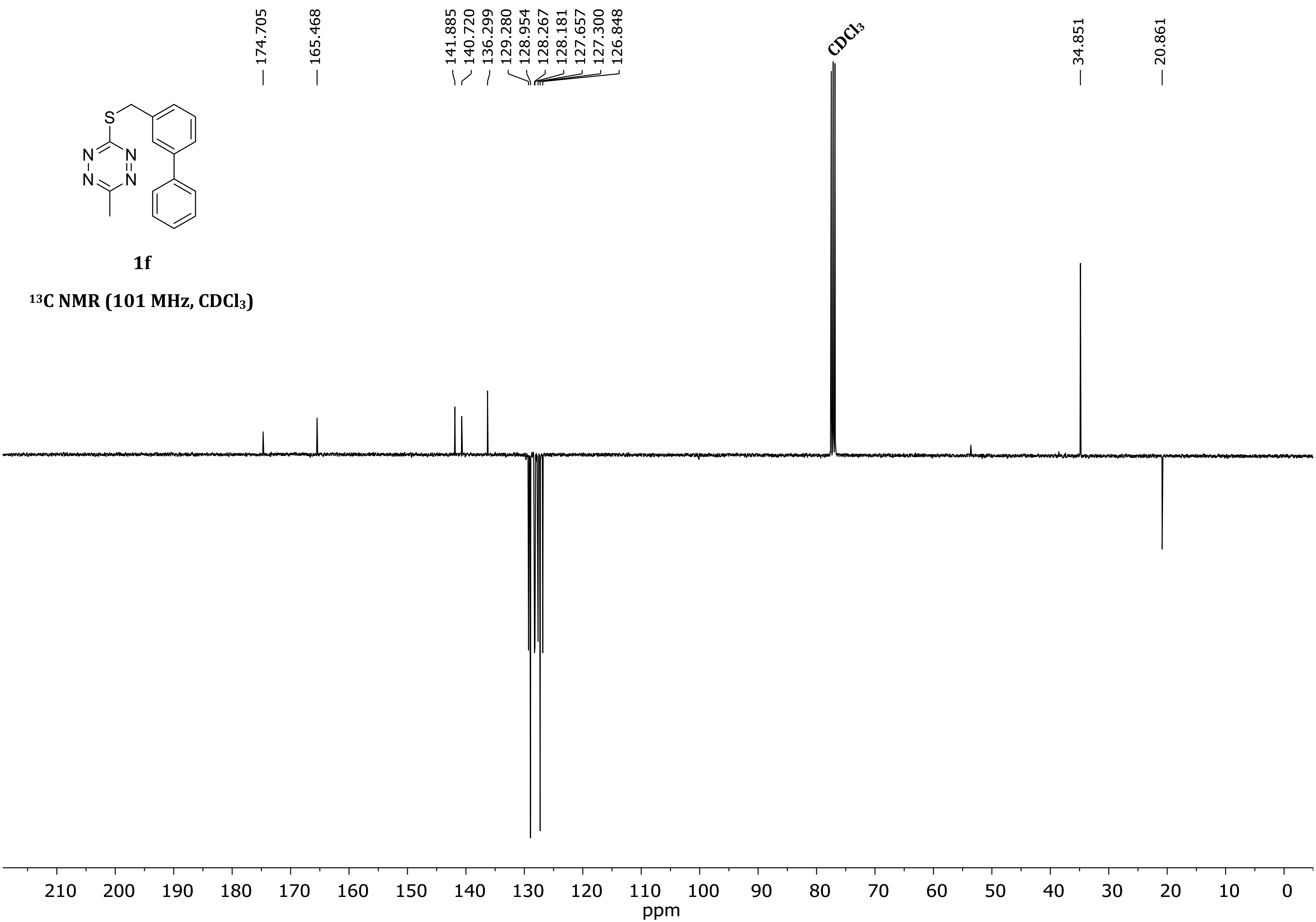




1f

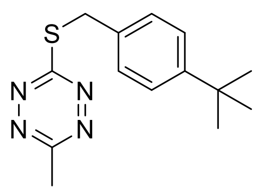
^{1}H NMR (400 MHz, $CDCl_3$)





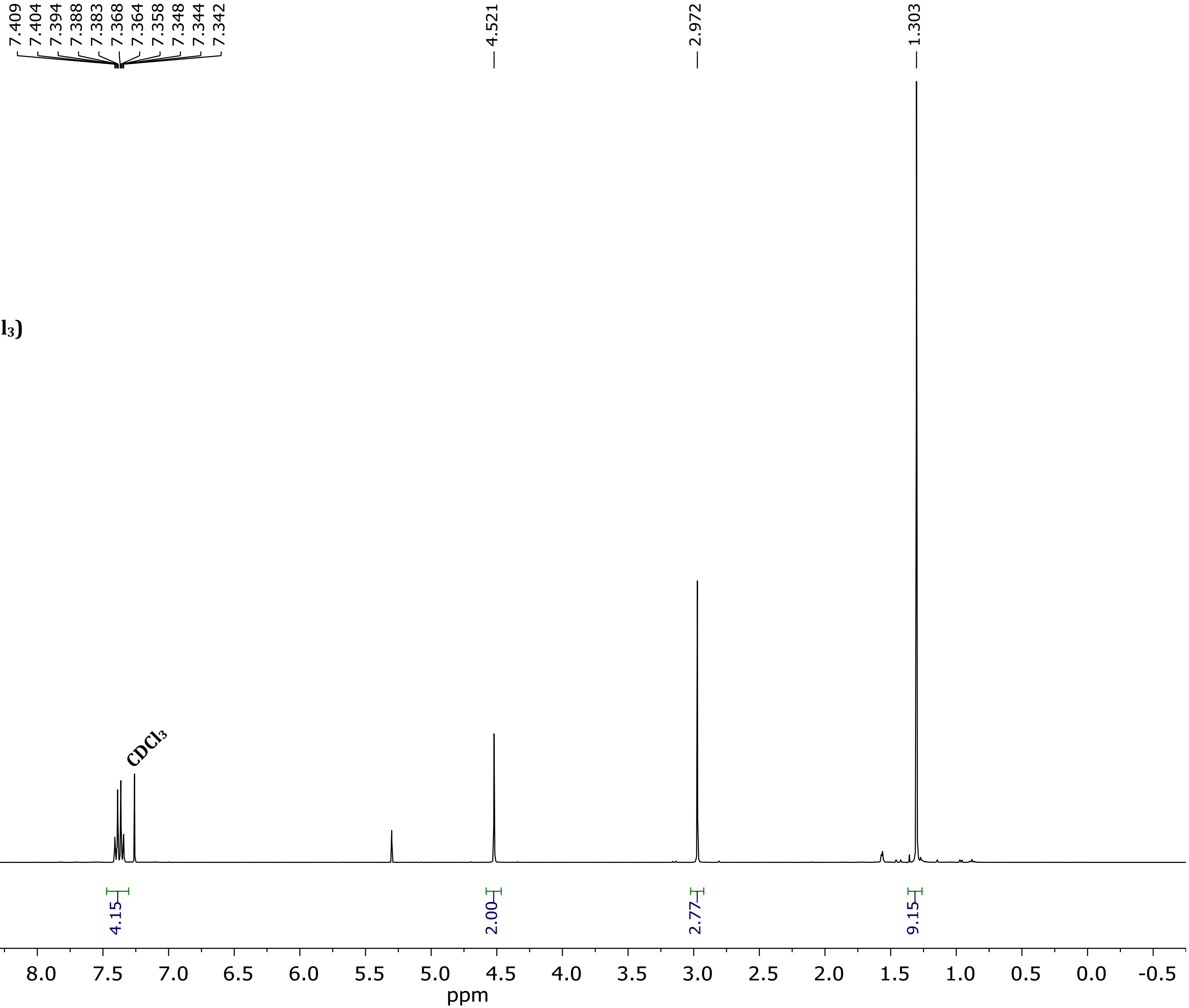
1f

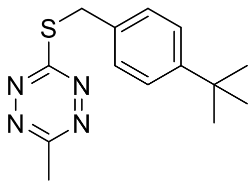
^{13}C NMR (101 MHz, CDCl$_3$)



1g

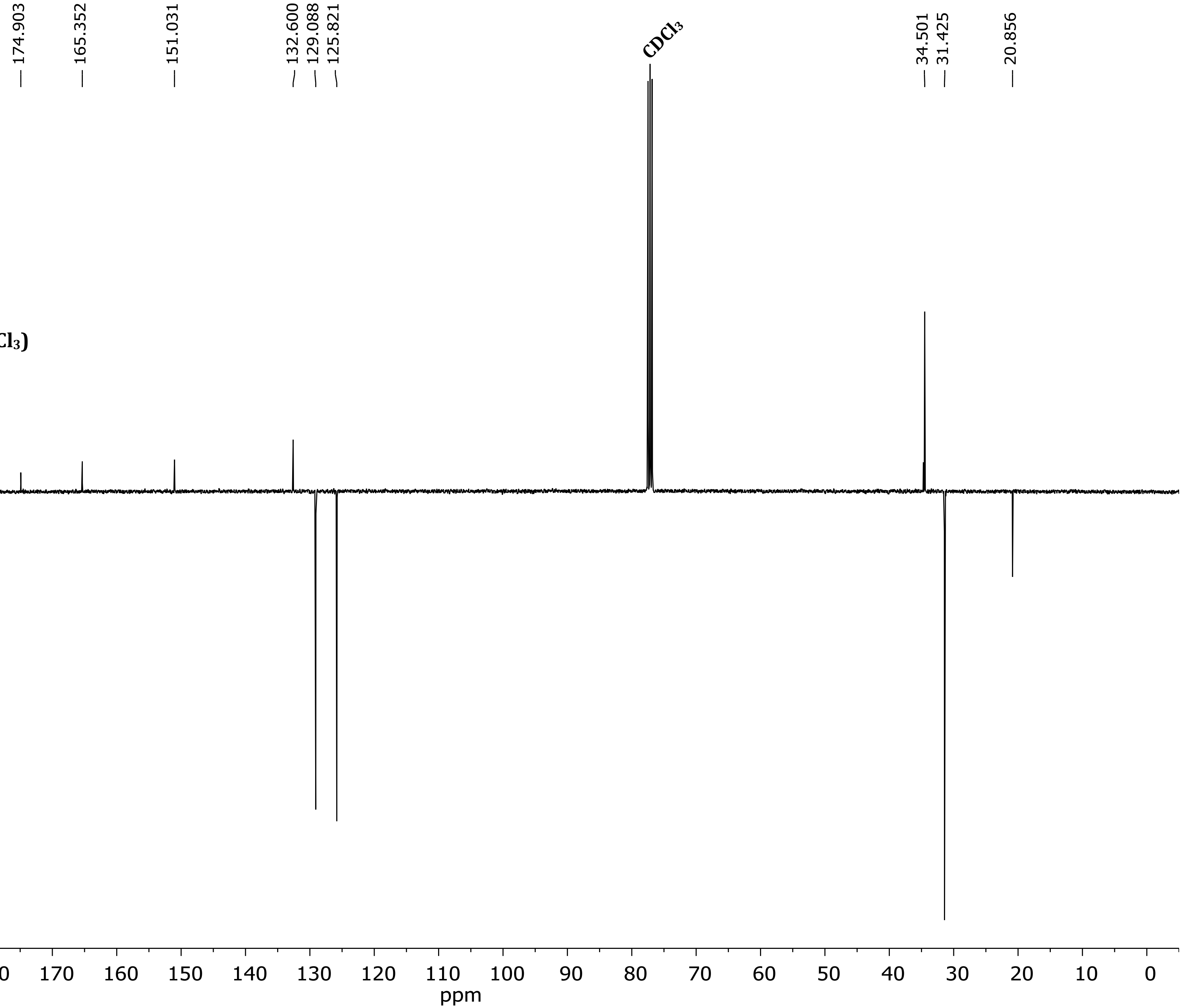
¹H NMR (400 MHz, CDCl$_3$)

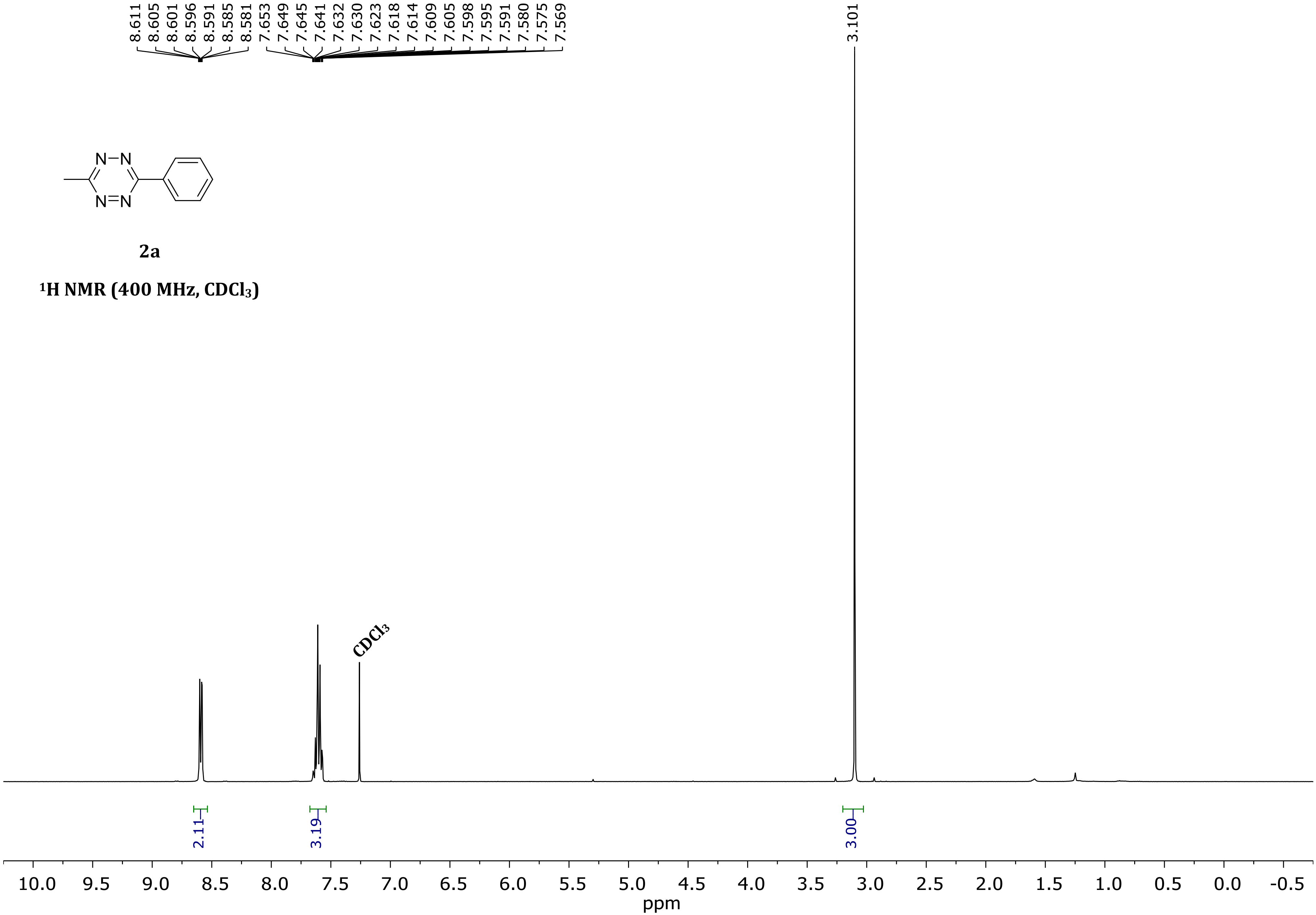




1g

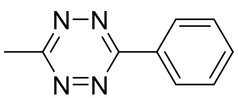
^{13}C NMR (101 MHz, $CDCl_3$)





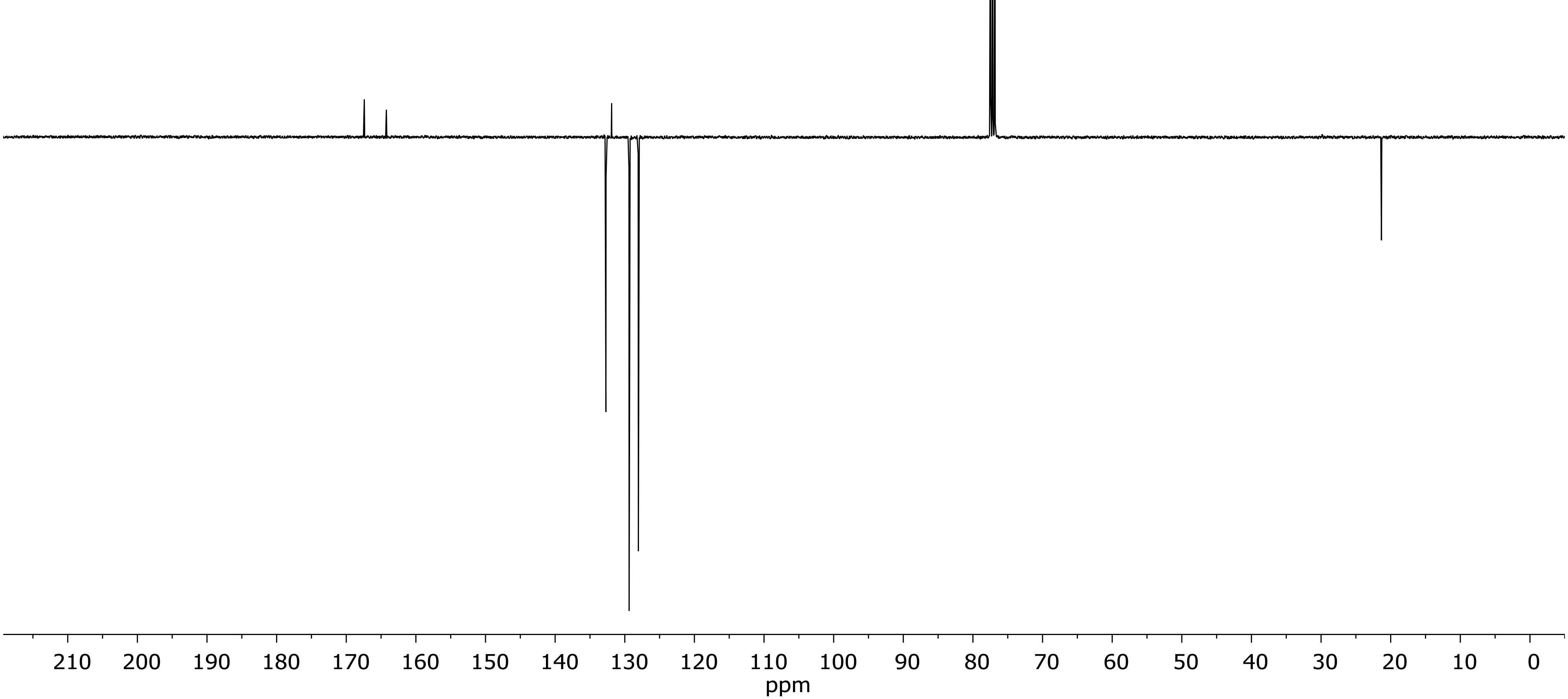
2a

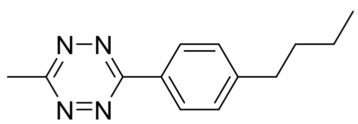
^{1}H NMR (400 MHz, $CDCl_3$)



2a

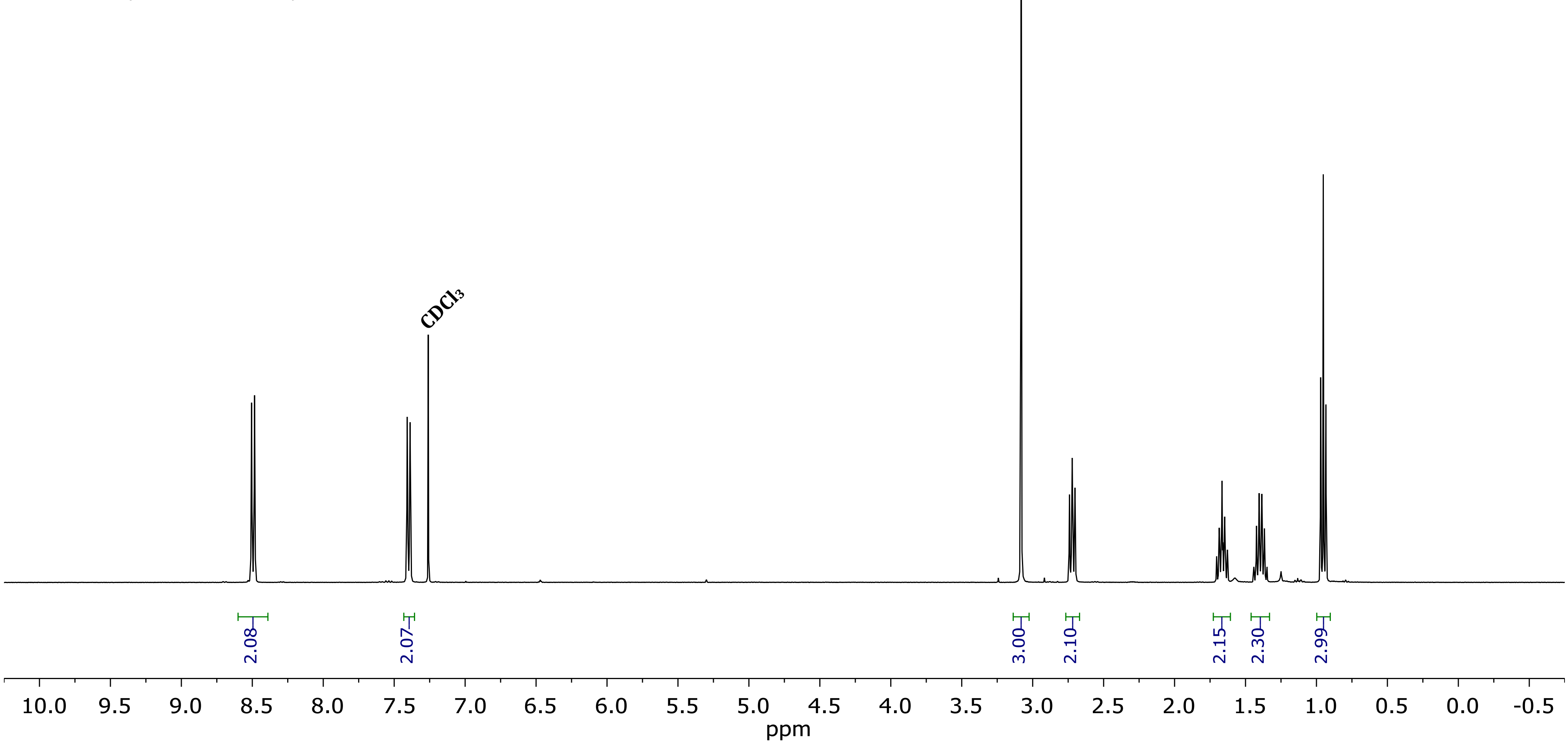
^{13}C NMR (101 MHz, $CDCl_3$)

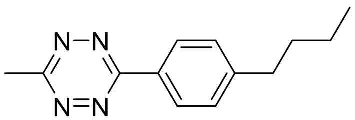




2b

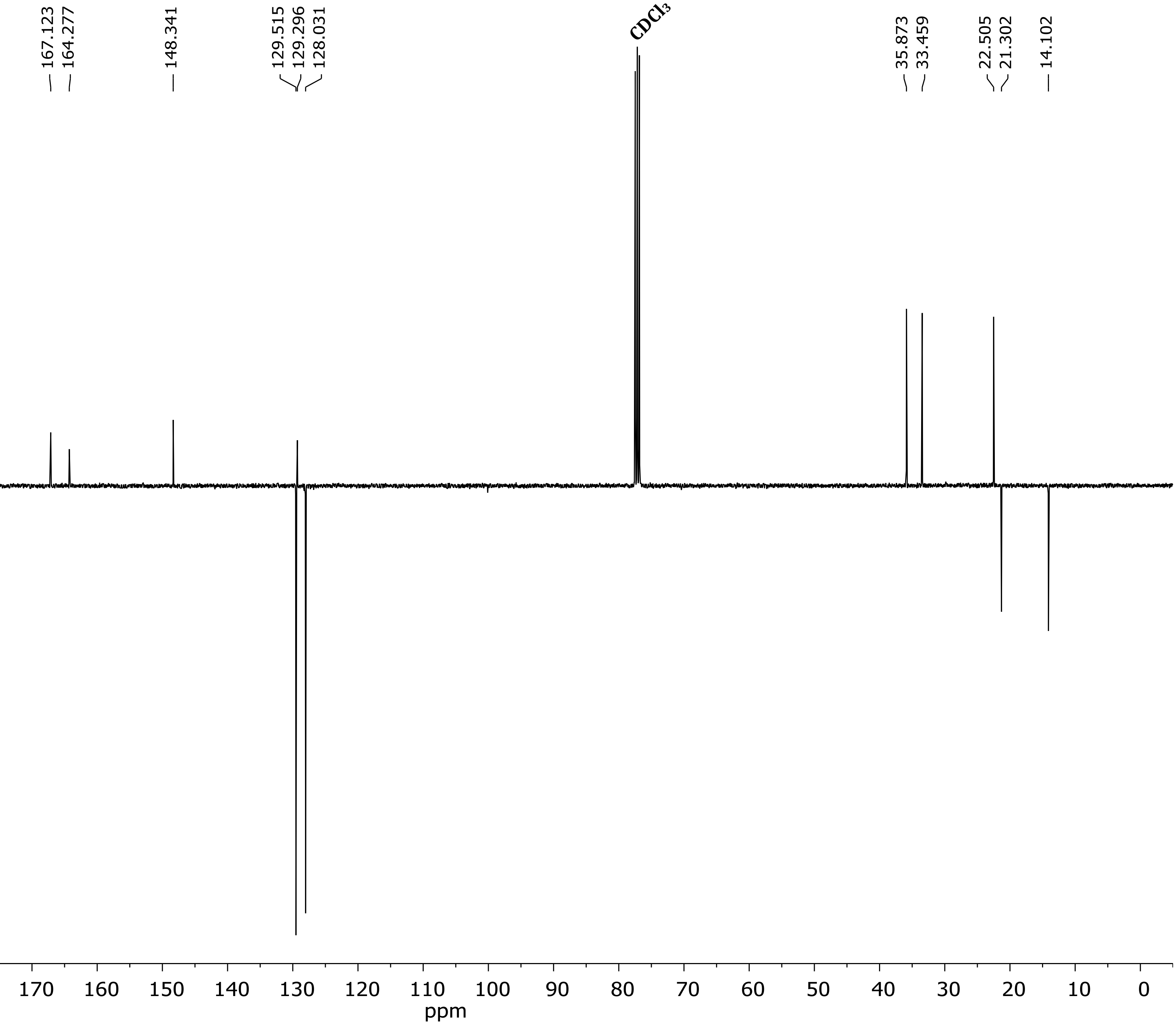
[1]H NMR (400 MHz, $CDCl_3$)

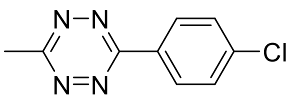




2b

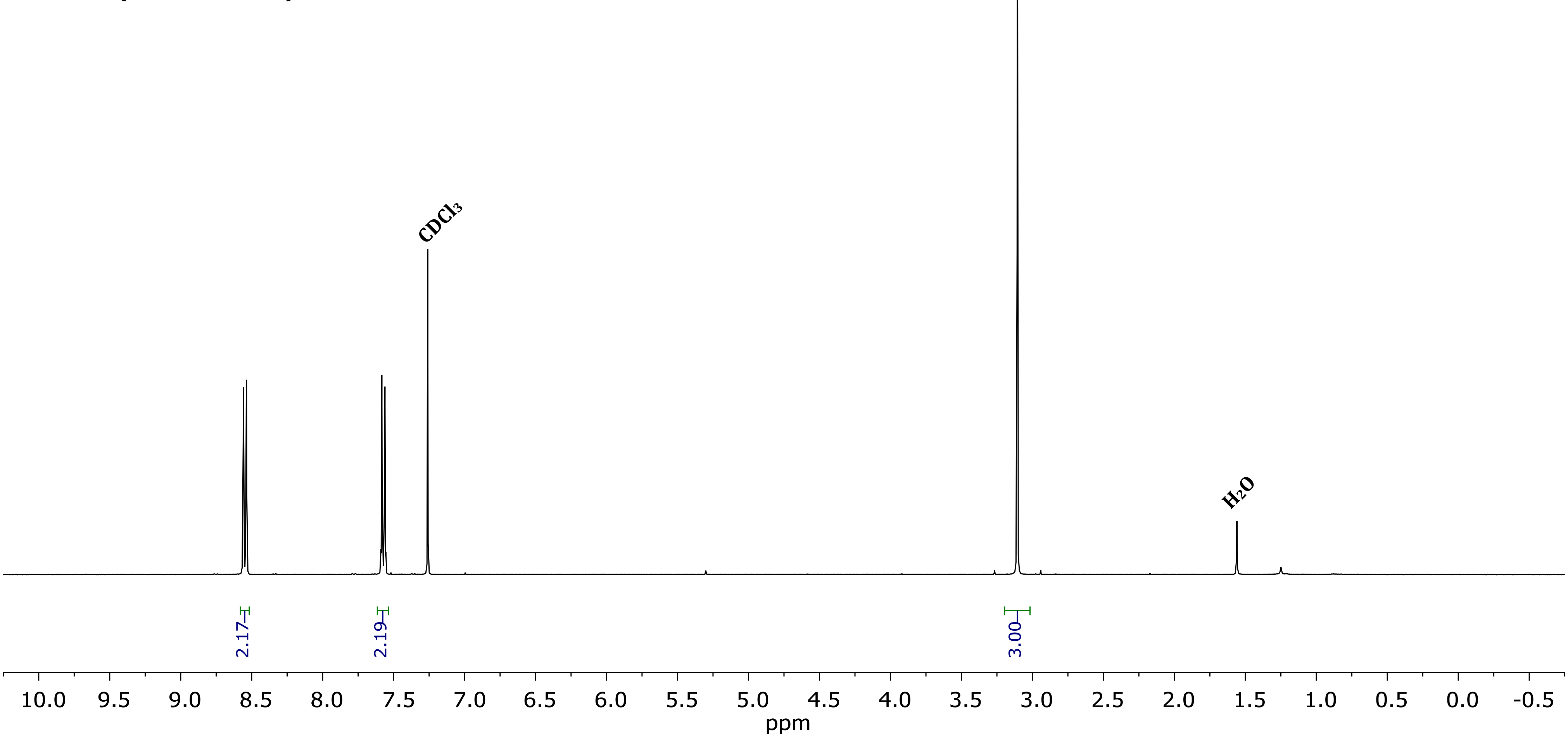
^{13}C NMR (101 MHz, $CDCl_3$)

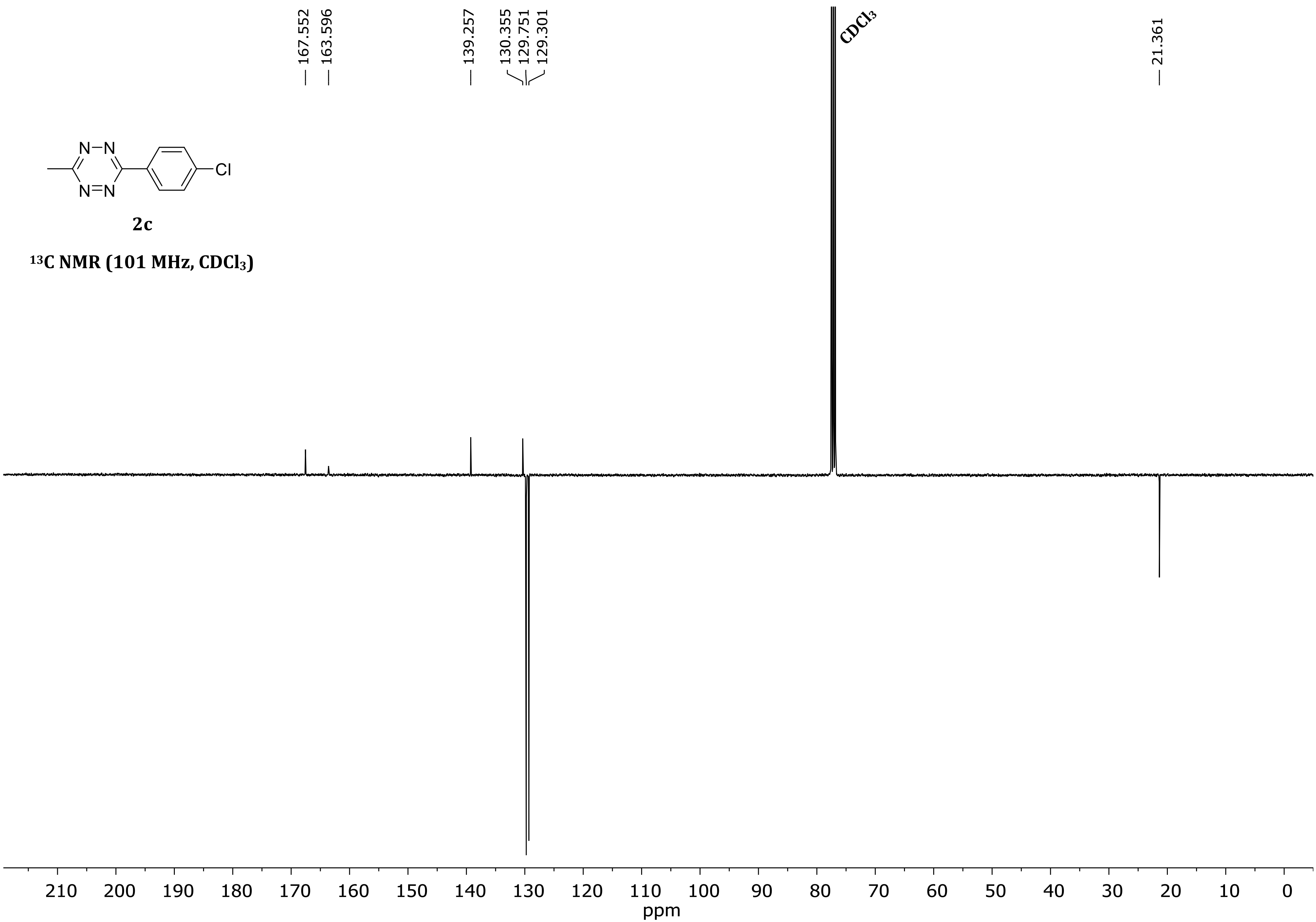




2c

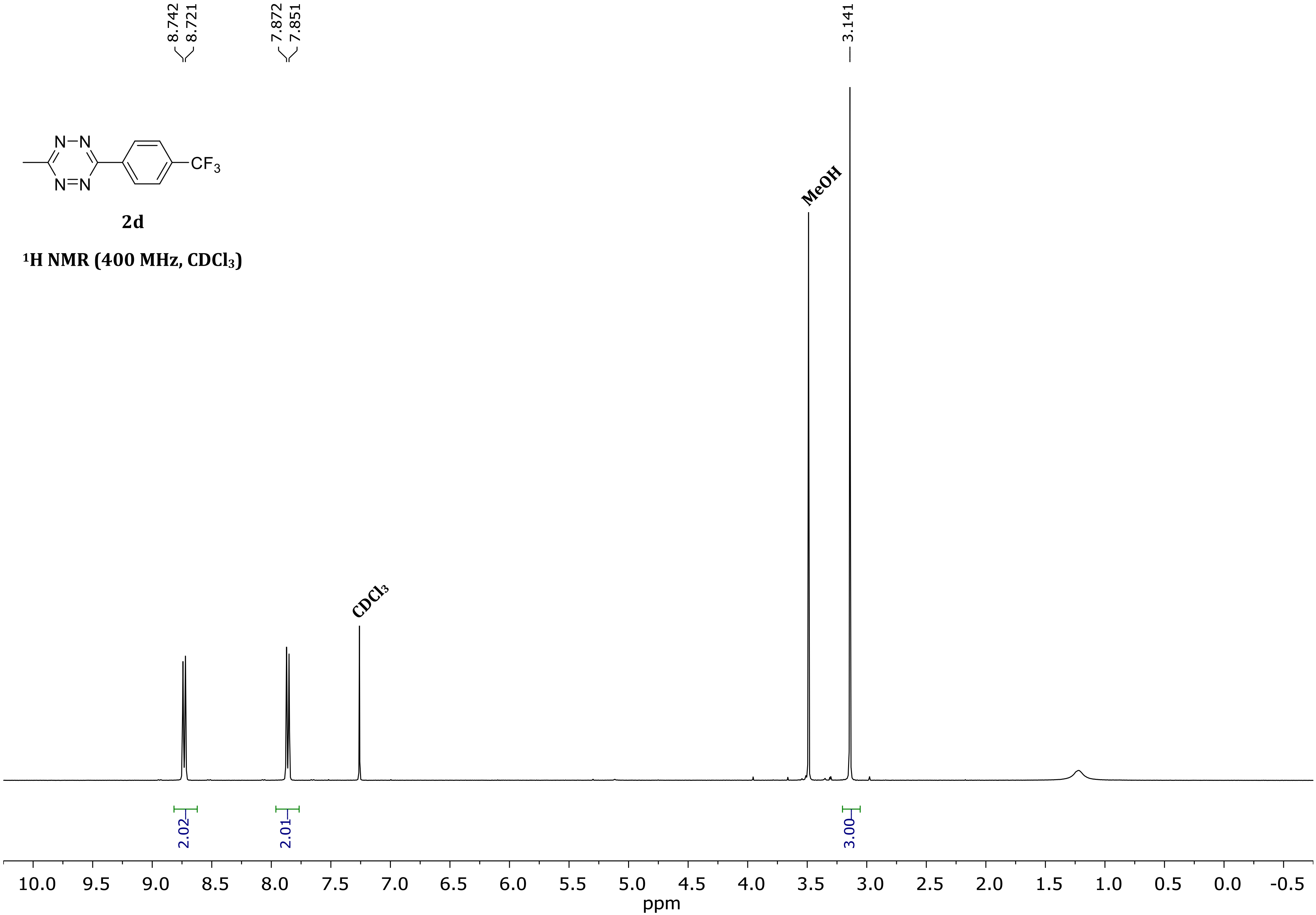
^{1}H NMR (400 MHz, $CDCl_3$)

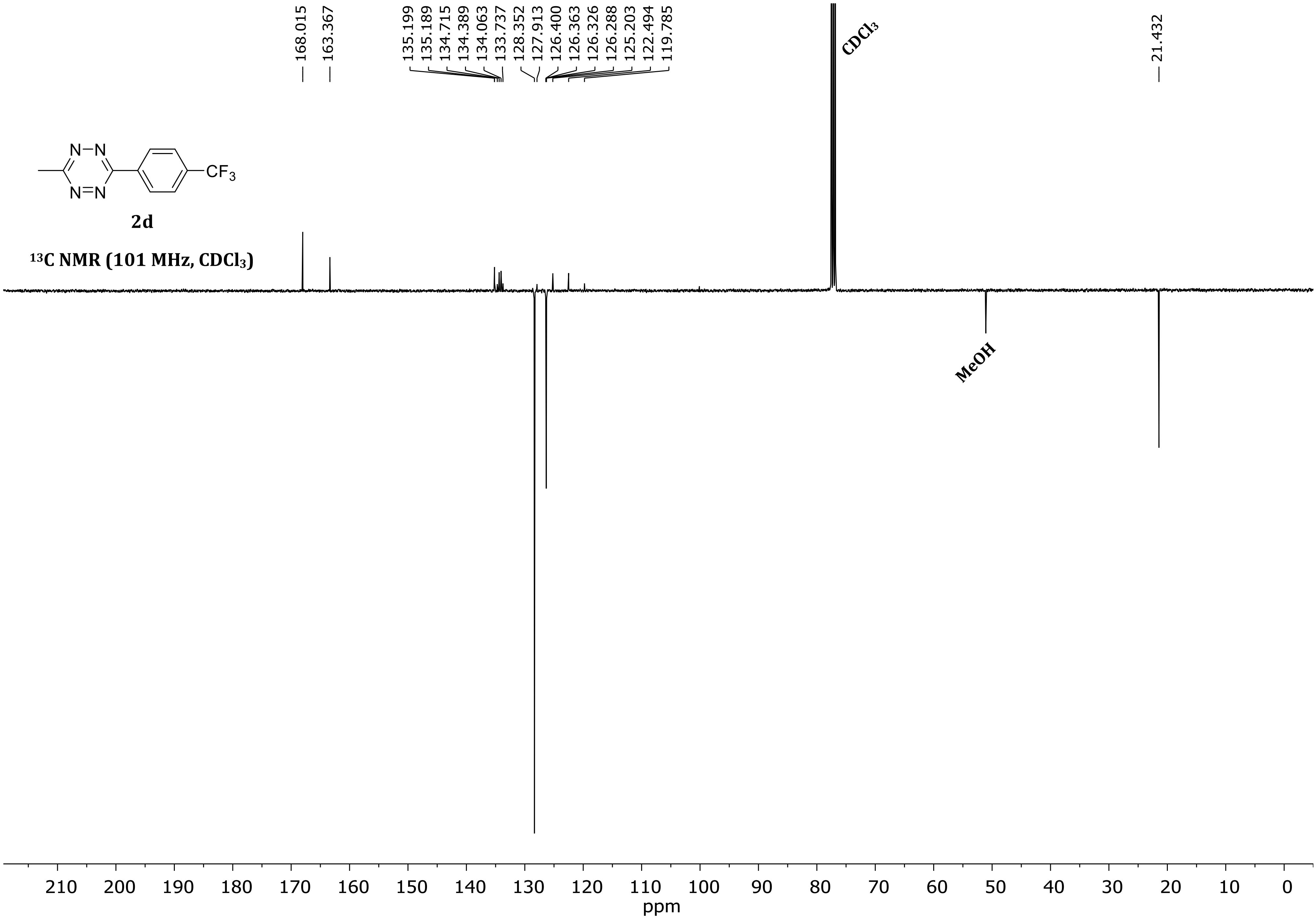


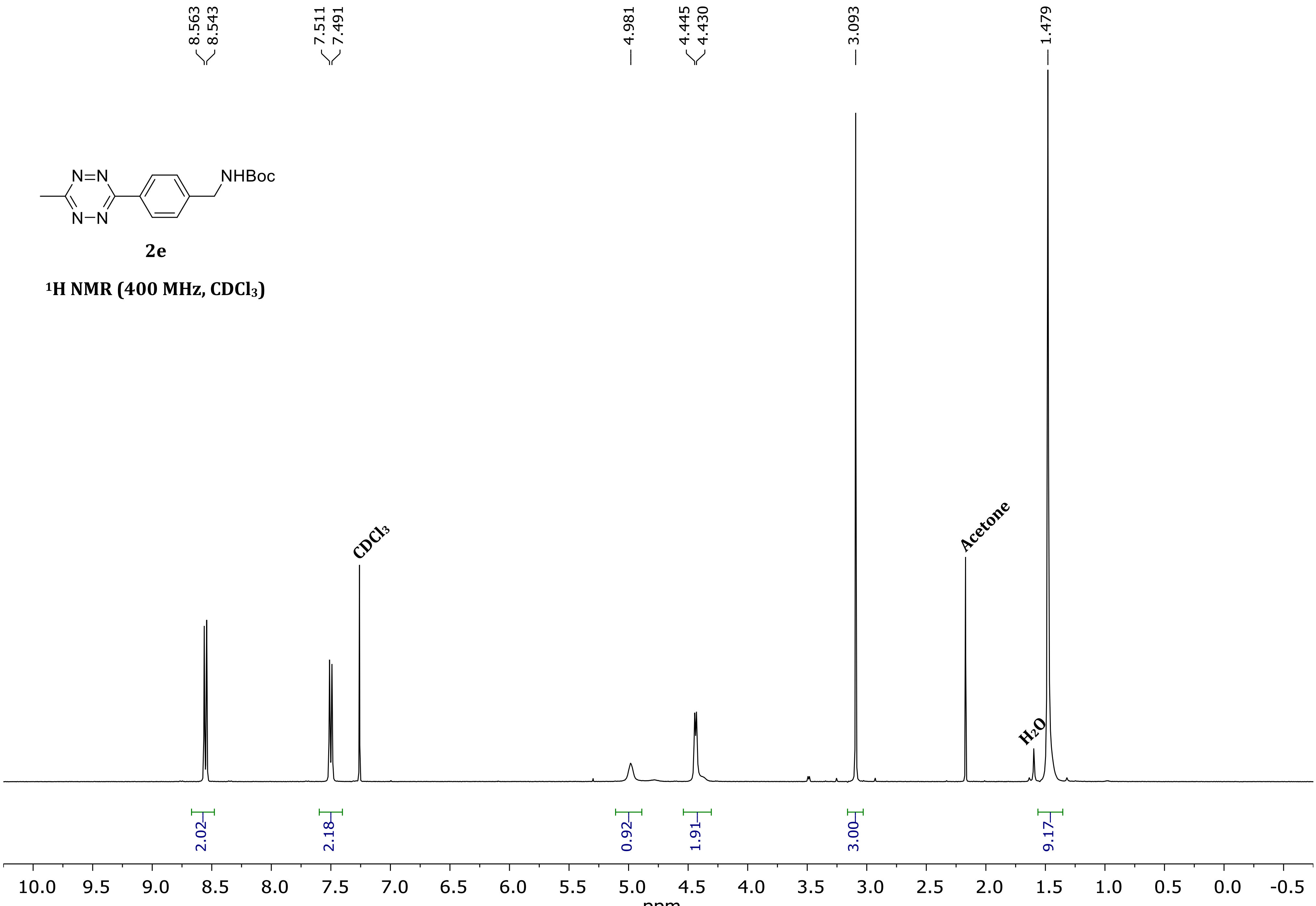


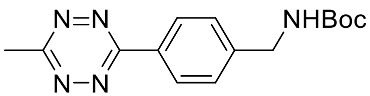
2c

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


8.742
8.721
7.872
7.851
3.141
2d
^{1}H NMR (400 MHz, $CDCl_3$)
MeOH
$CDCl_3$
2.02
2.01
3.00
10.0 9.5 9.0 8.5 8.0 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 0.0 -0.5
ppm

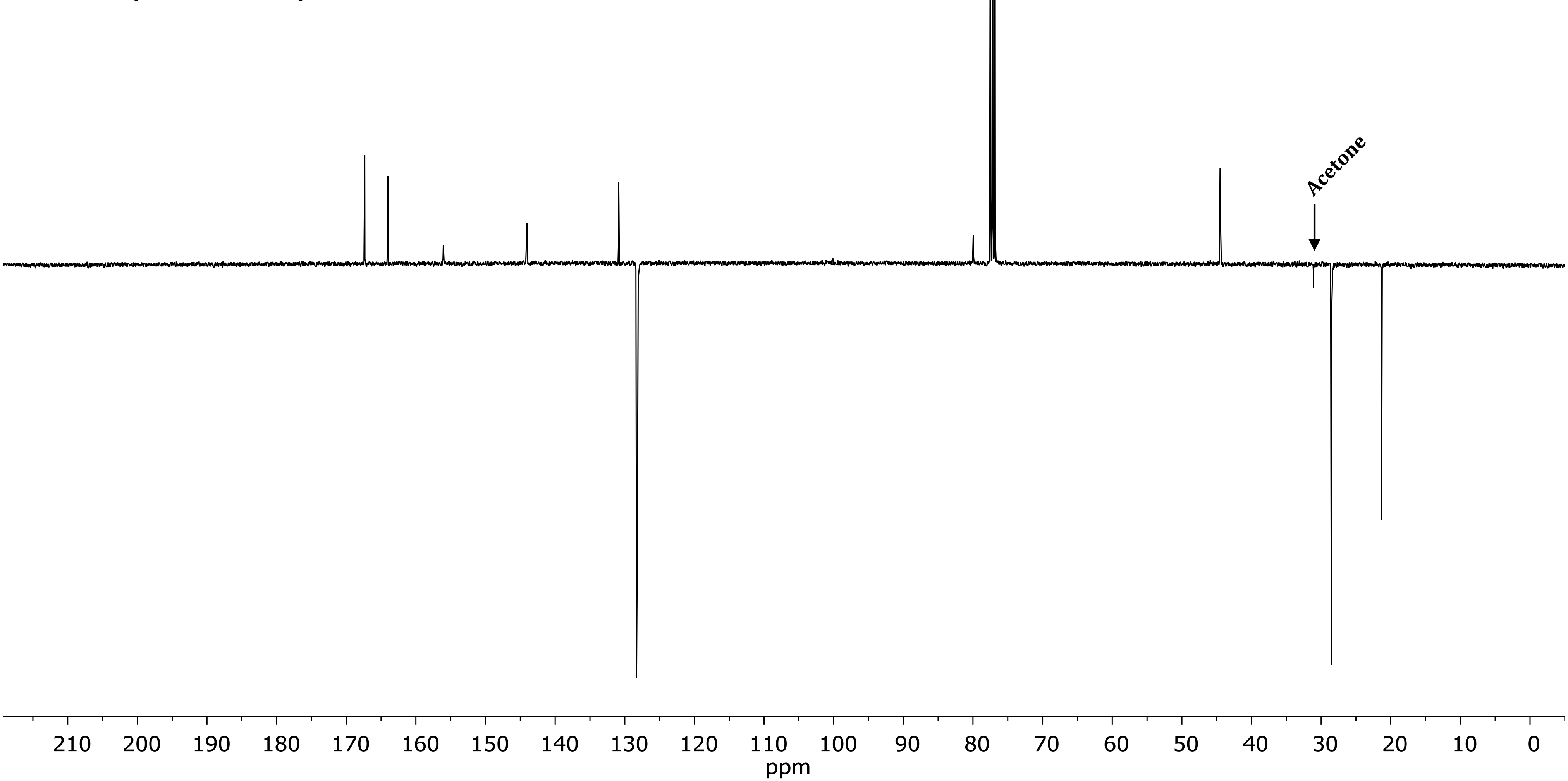

168.015
163.367
135.199
135.189
134.715
134.389
134.063
133.737
128.352
127.913
126.400
126.363
126.326
126.288
125.203
122.494
119.785
CDCl3
21.432
N–N
N=N
CF3
2d
13C NMR (101 MHz, CDCl3)
MeOH
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

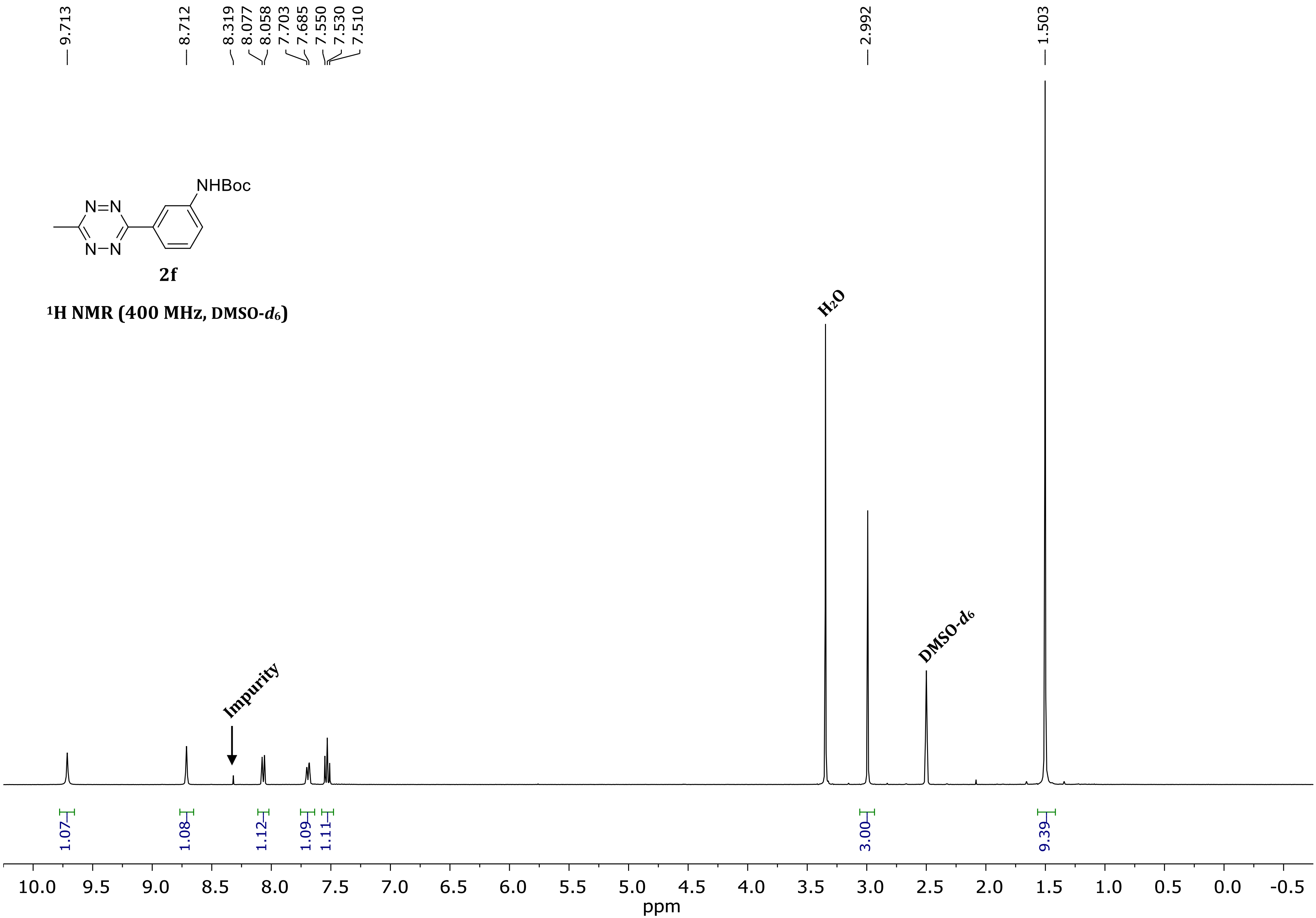

8.563
8.543
7.511
7.491
4.981
4.445
4.430
3.093
1.479
NHBoc
N=N
N−N
2e
^{1}H NMR (400 MHz, $CDCl_3$)
$CDCl_3$
Acetone
H_2O
2.02
2.18
0.92
1.91
3.00
9.17
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ppm



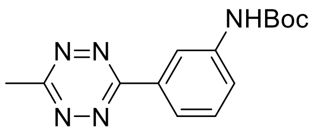
2e

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



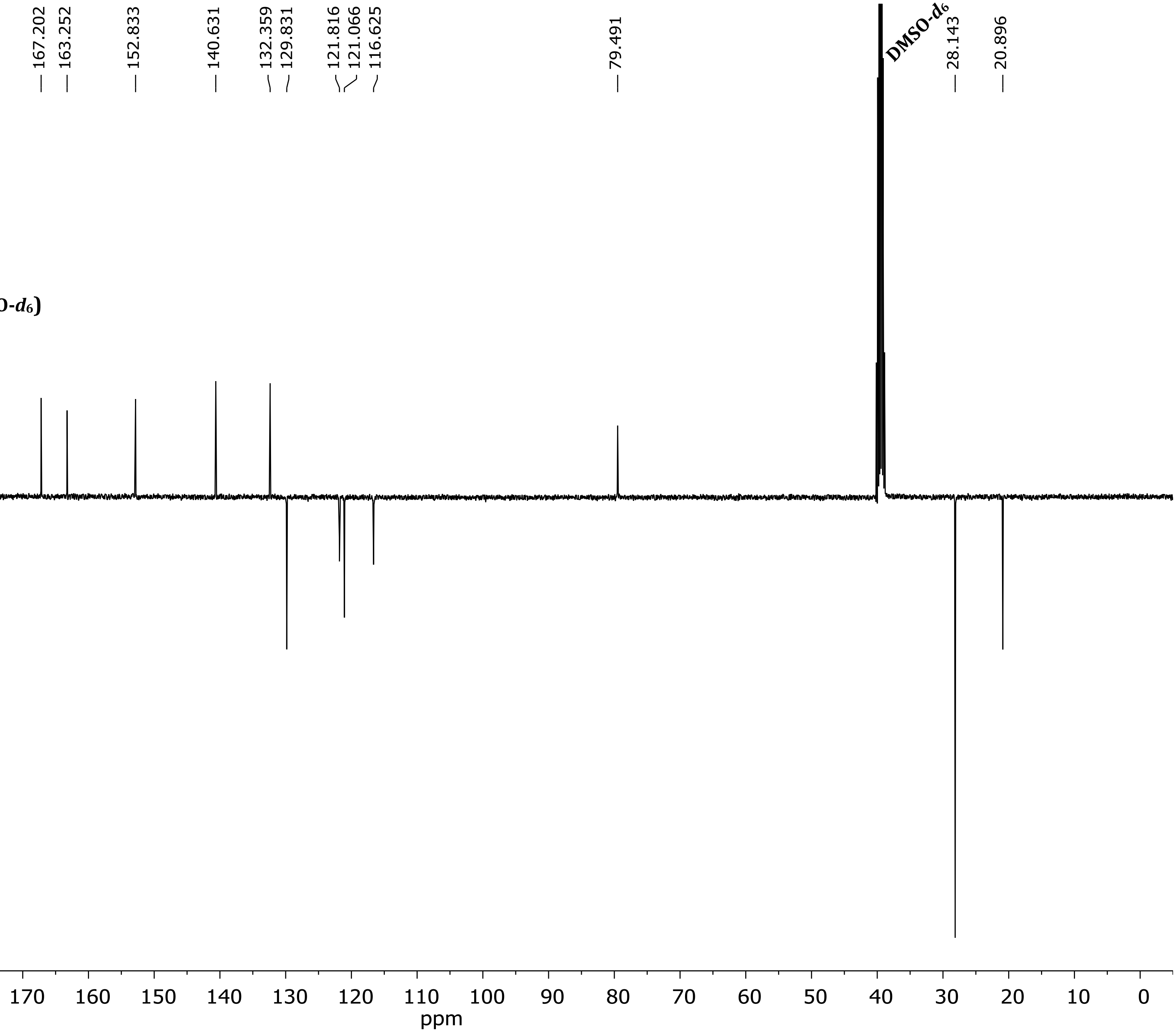


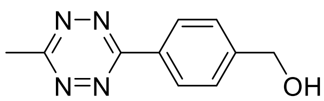
9.713
8.712
8.319
8.077
8.058
7.703
7.685
7.550
7.530
7.510
2.992
1.503
NHBoc
N=N
N–N
2f
¹H NMR (400 MHz, DMSO-d6)
H2O
DMSO-d6
Impurity
1.07
1.08
1.12
1.09
1.11
3.00
9.39
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ppm



2f

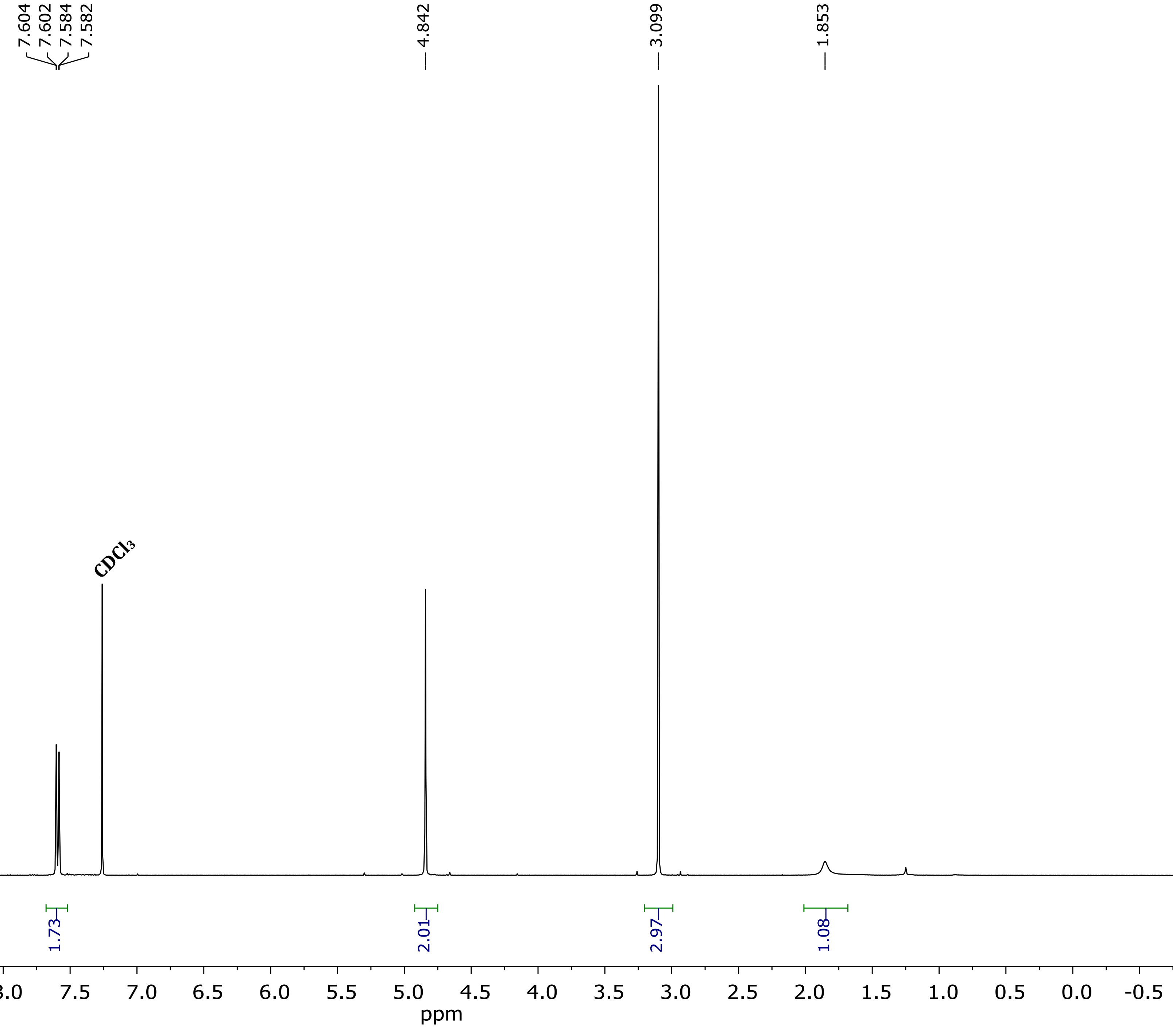
^{13}C NMR (101 MHz, DMSO-d_6)

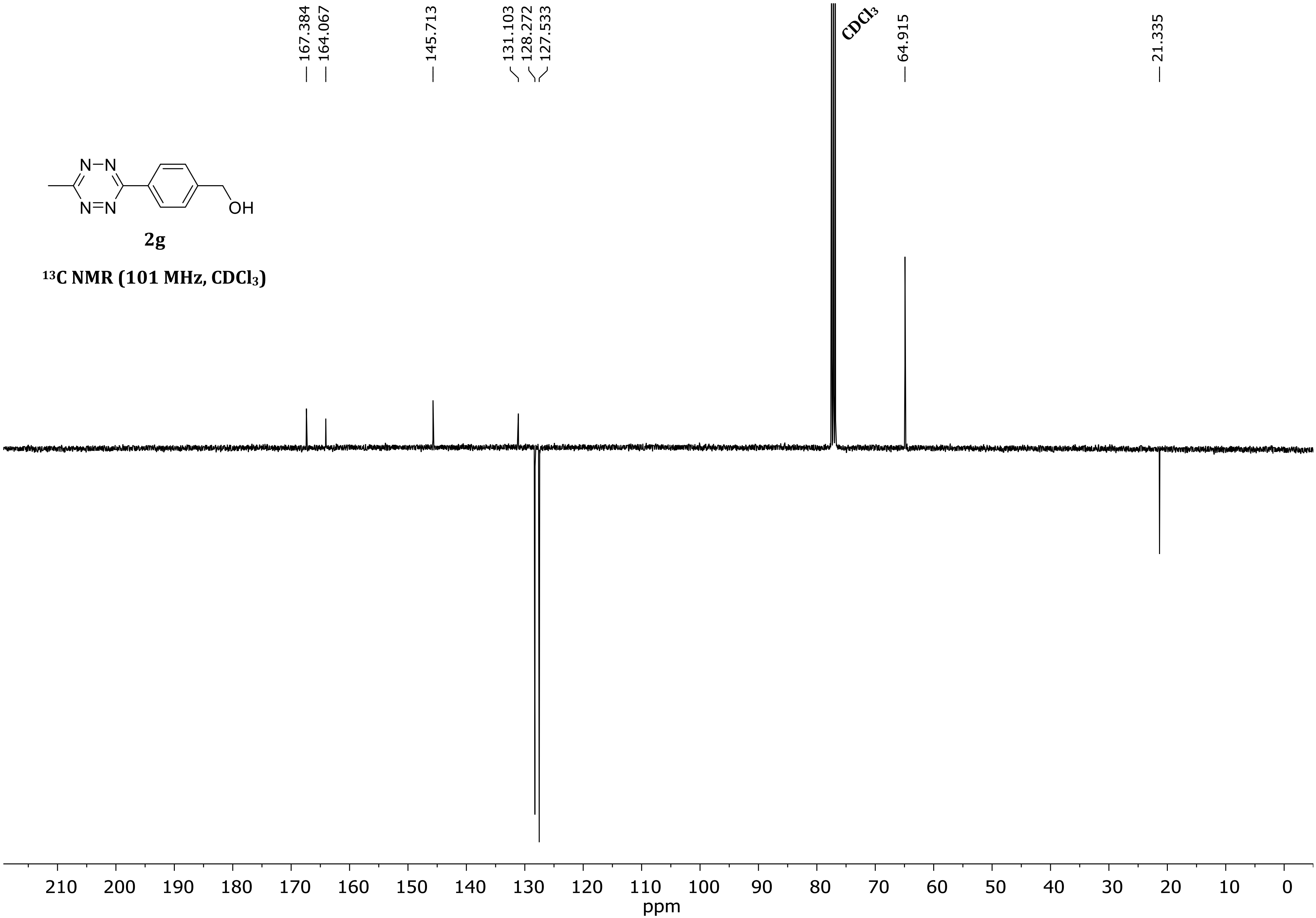


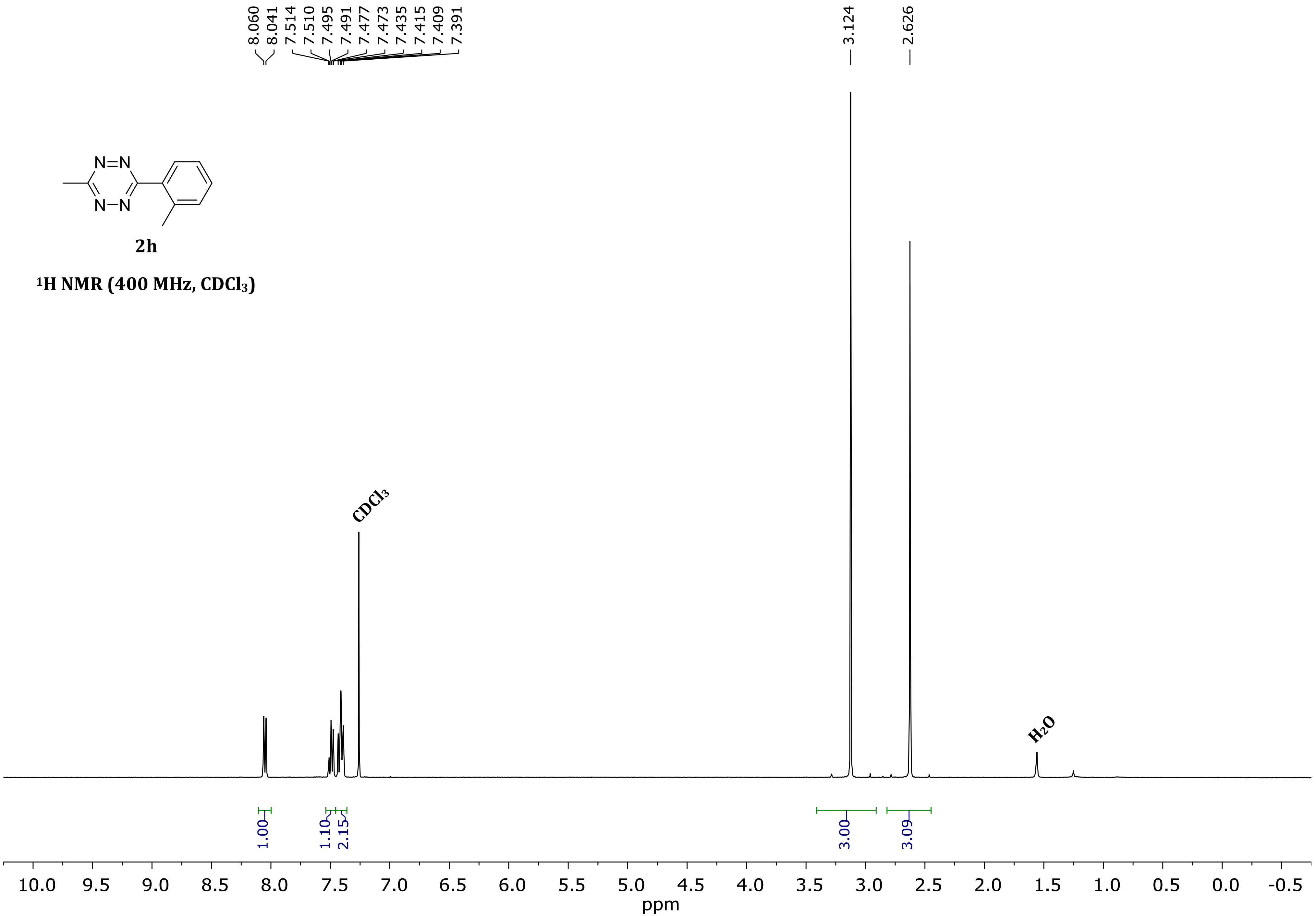


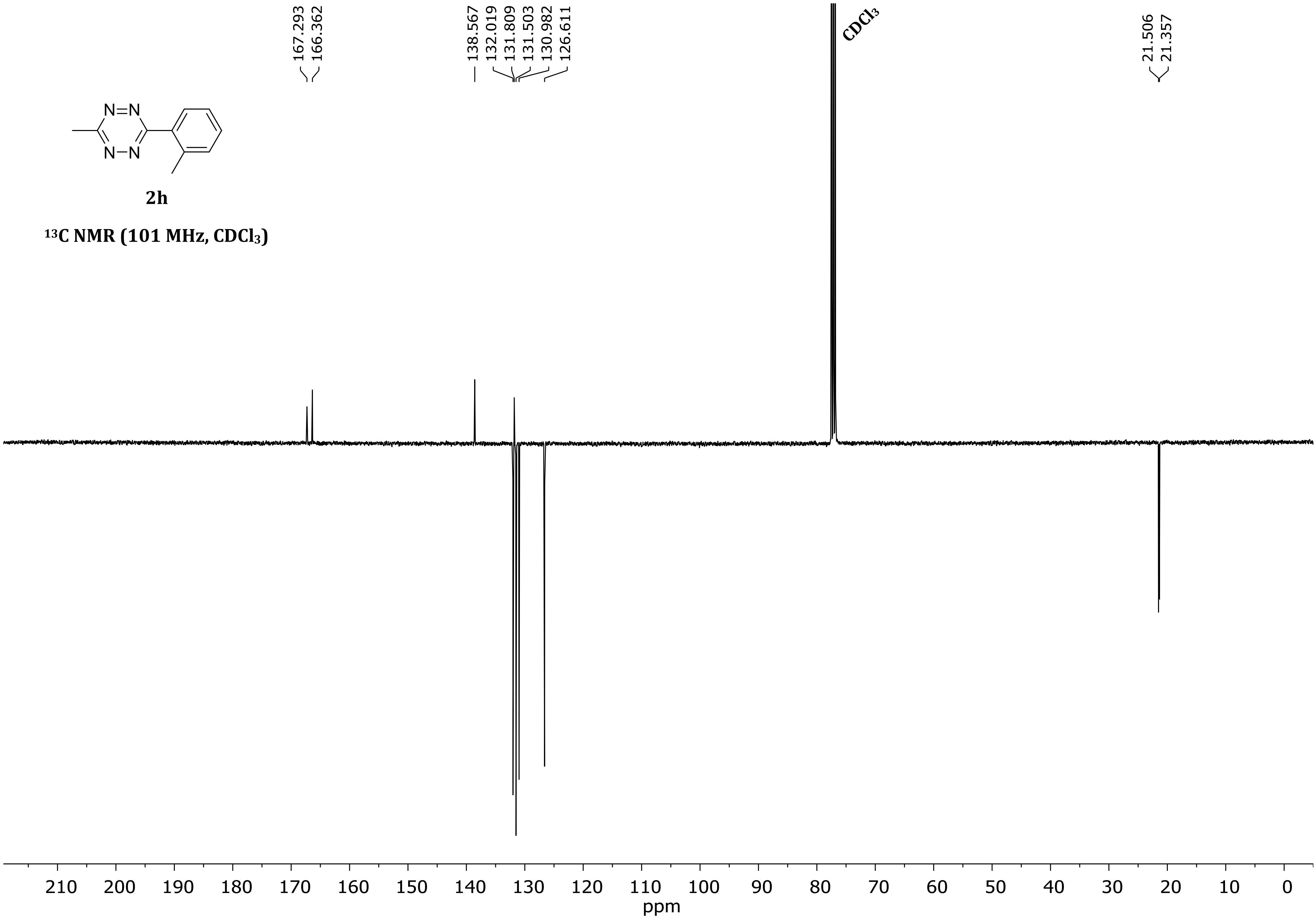
2g

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

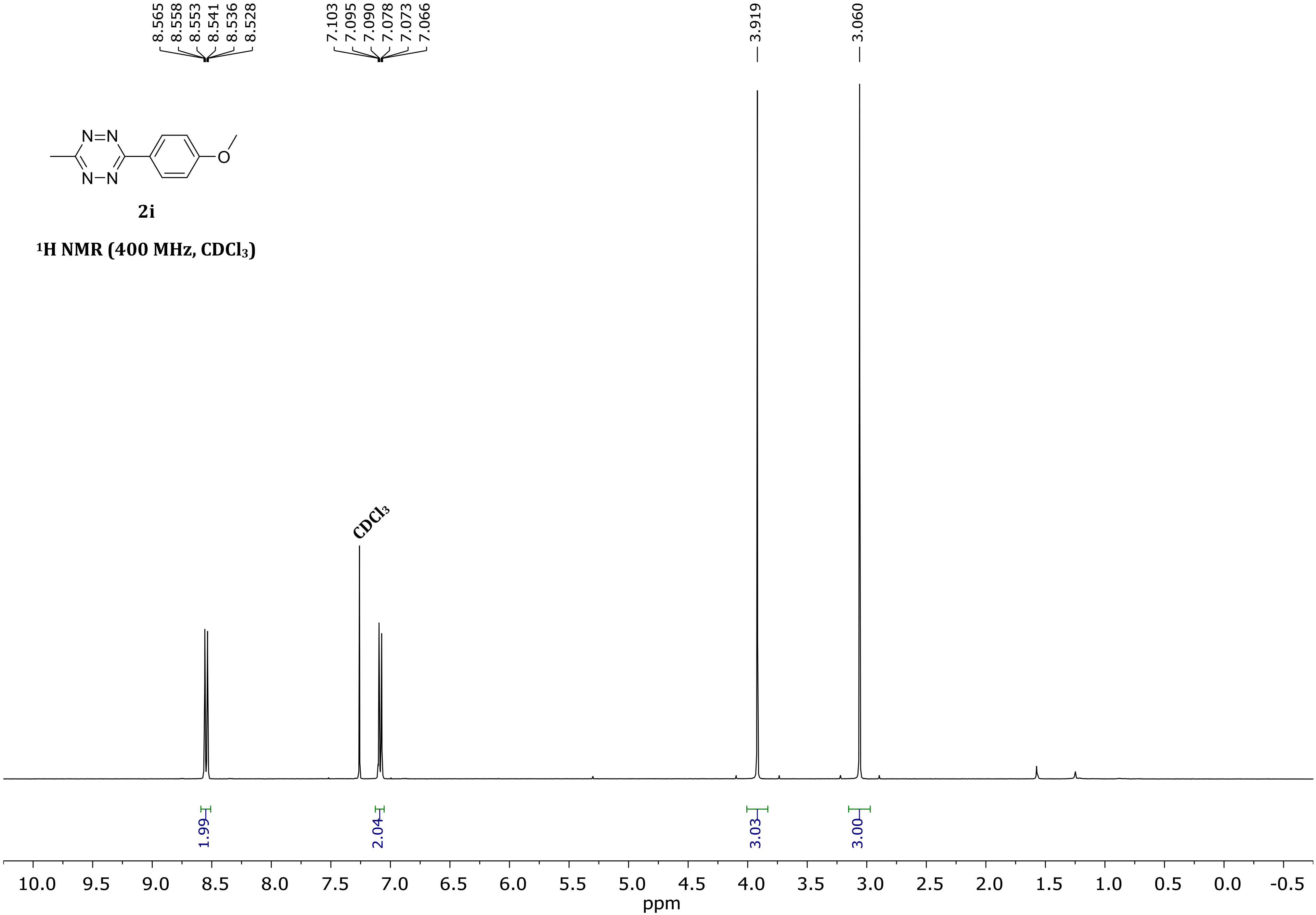



167.384
164.067
145.713
131.103
128.272
127.533
CDCl3
64.915
21.335
N−N
N=N
OH
2g
13C NMR (101 MHz, CDCl3)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm


8.060
8.041
7.514
7.510
7.495
7.491
7.477
7.473
7.435
7.415
7.409
7.391
3.124
2.626
2h
¹H NMR (400 MHz, CDCl₃)
CDCl₃
H₂O
1.00
1.10
2.15
3.00
3.09
10.0 9.5 9.0 8.5 8.0 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 0.0 -0.5
ppm

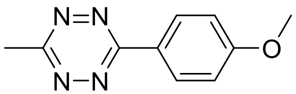


167.293
166.362
138.567
132.019
131.809
131.503
130.982
126.611
CDCl3
21.506
21.357
2h
13C NMR (101 MHz, CDCl3)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm



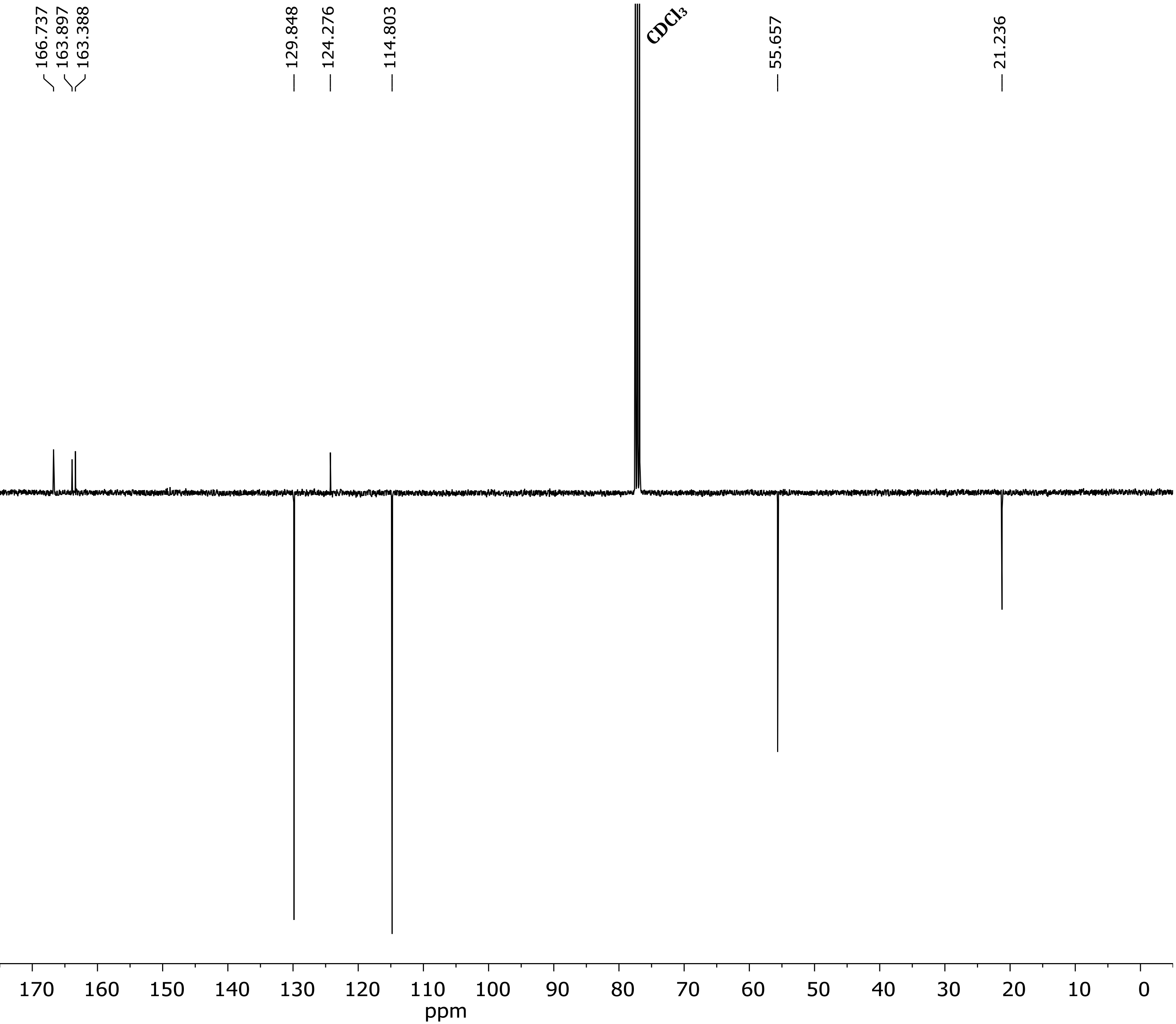
2i

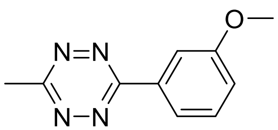
^{1}H NMR (400 MHz, $CDCl_3$)



2i

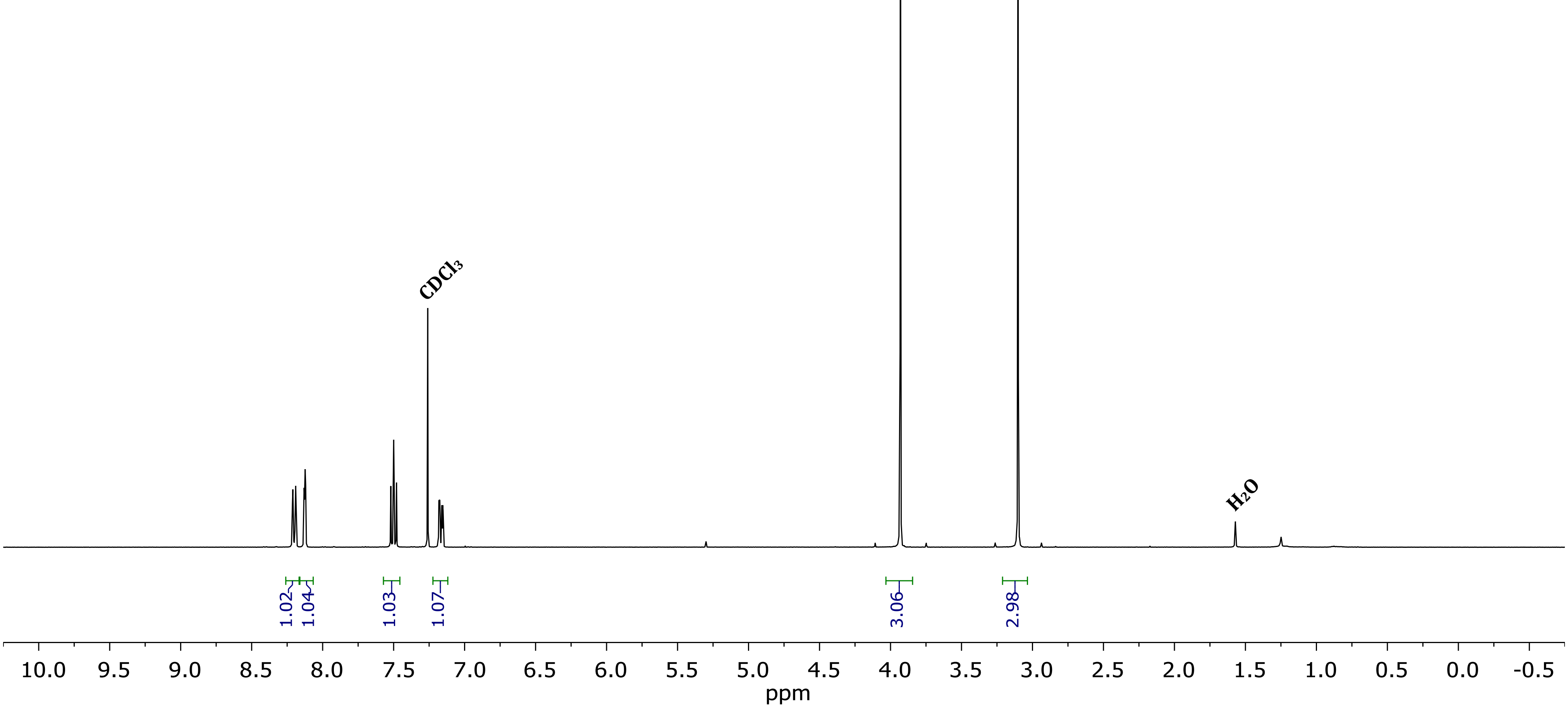
^{13}C NMR (101 MHz, $CDCl_3$)

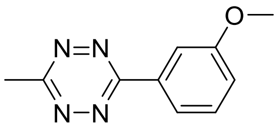




2j

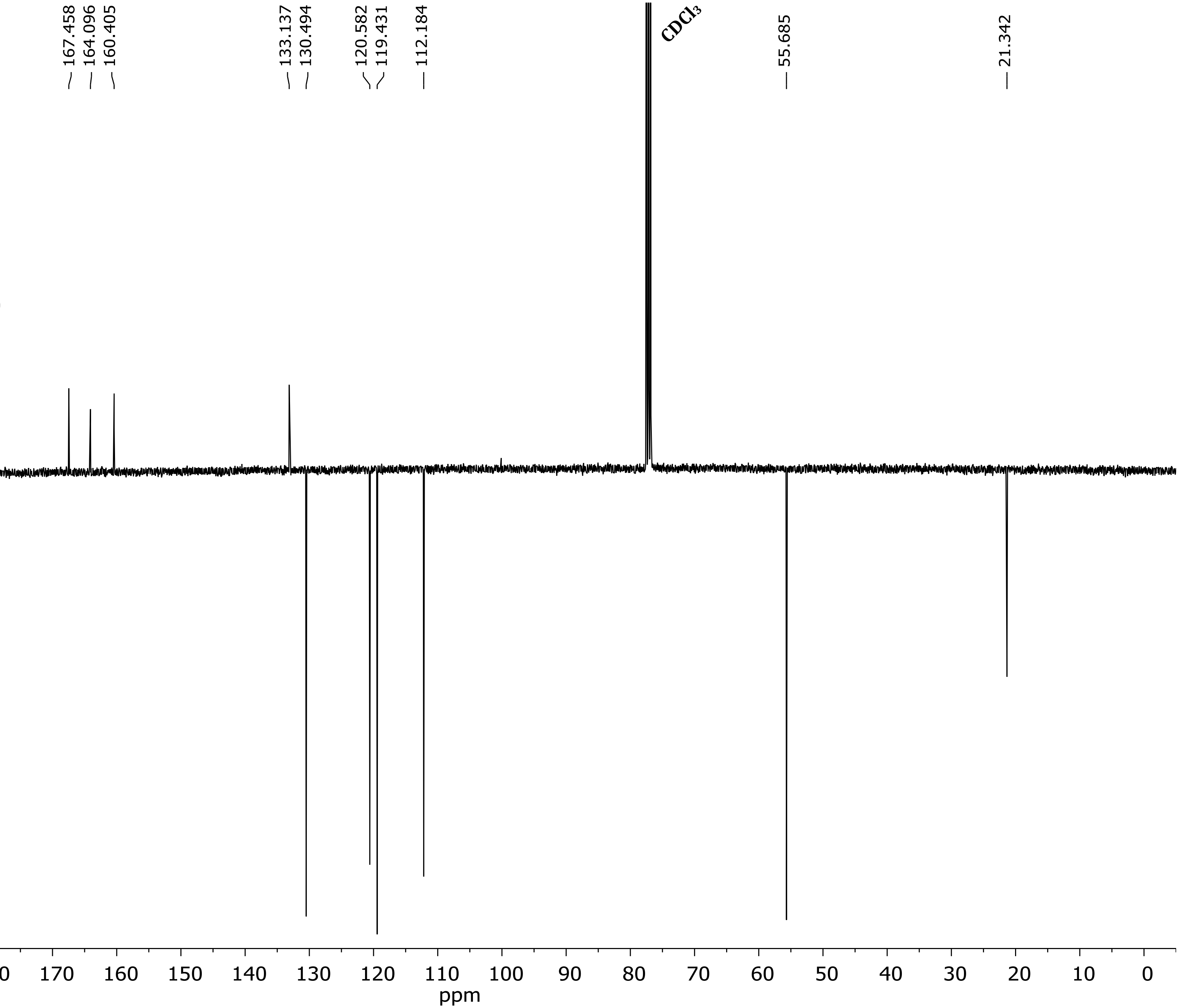
^{1}H NMR (400 MHz, $CDCl_3$)

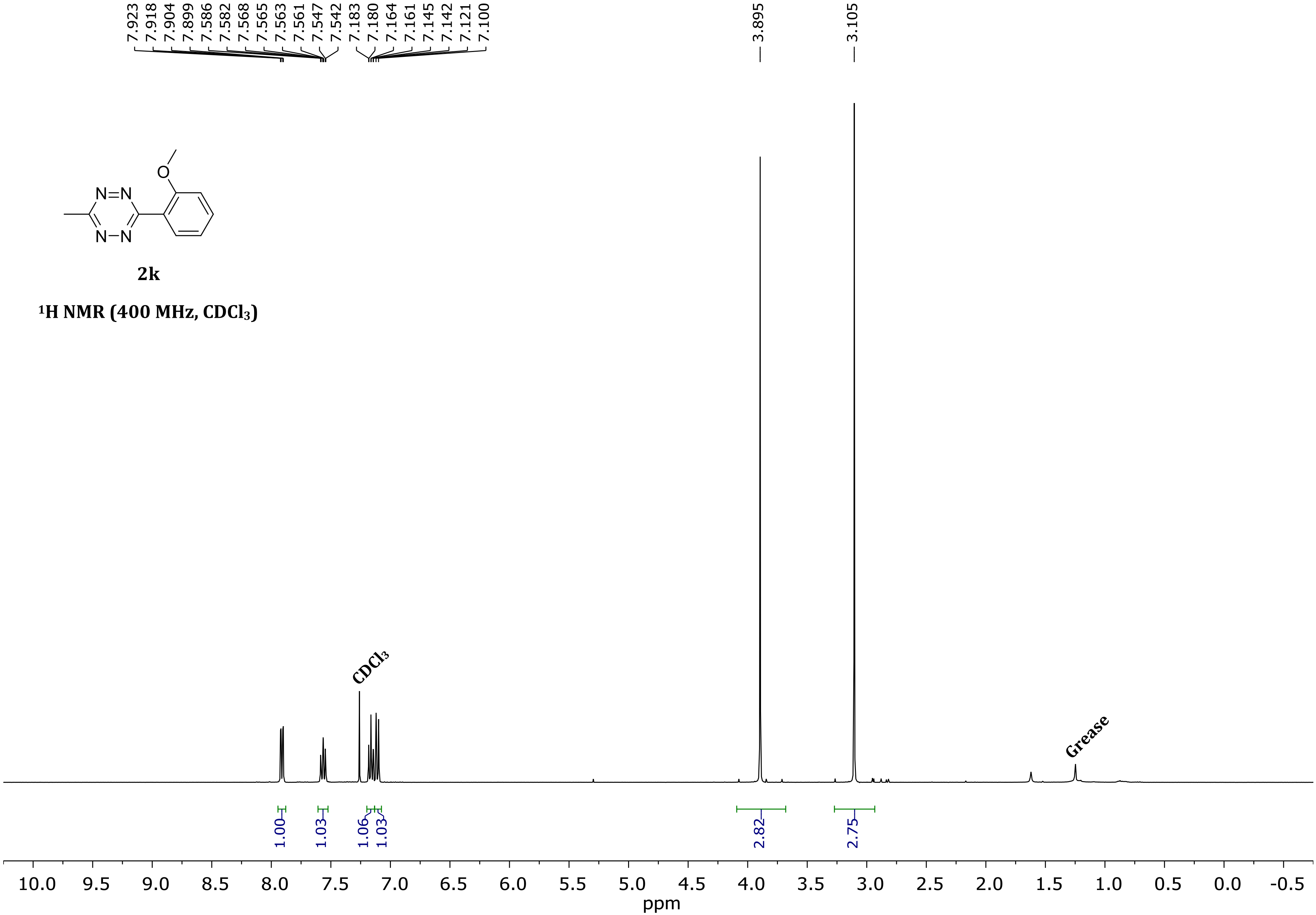


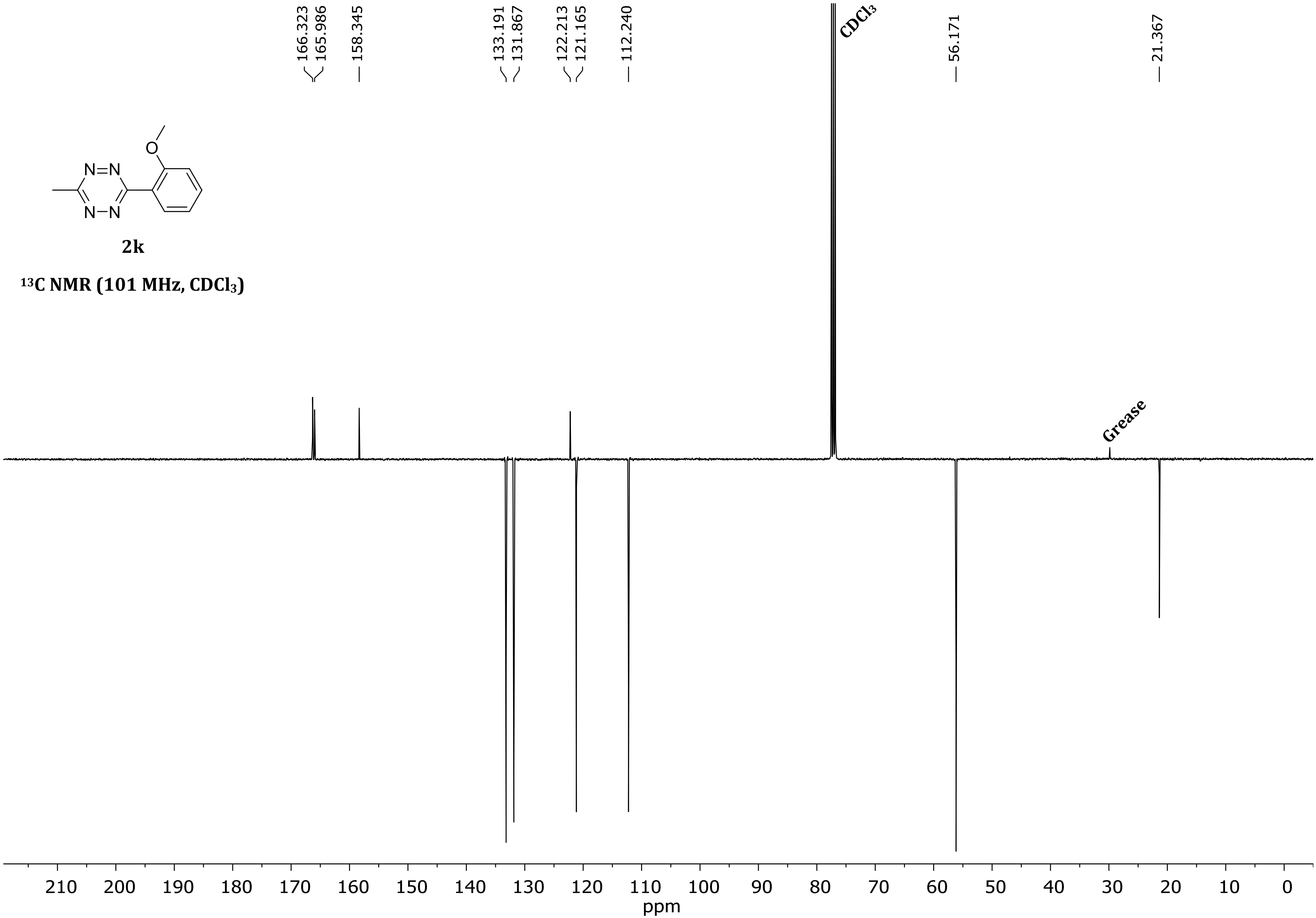


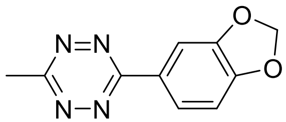
2j

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



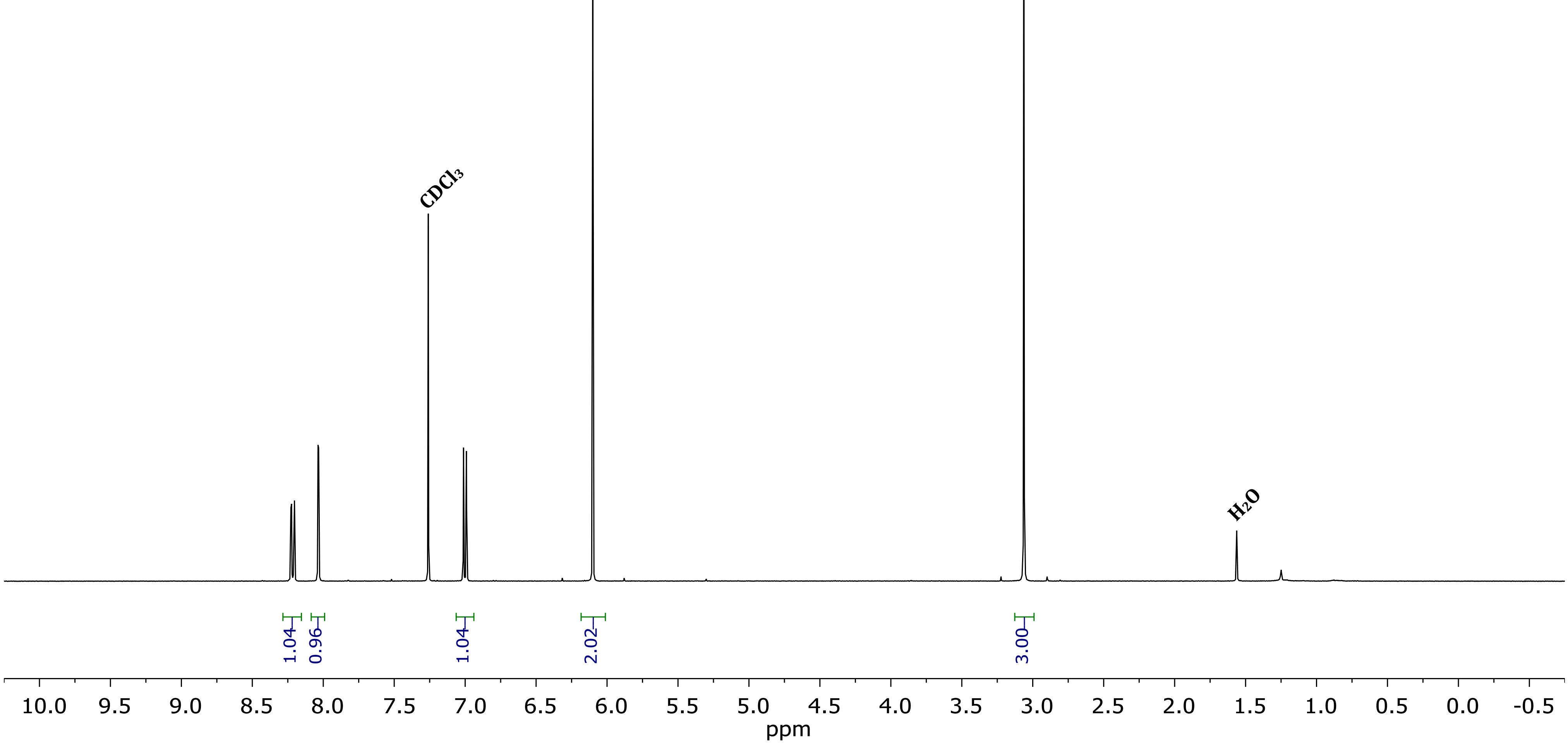

7.923
7.918
7.904
7.899
7.586
7.582
7.568
7.565
7.563
7.561
7.547
7.542
7.183
7.180
7.164
7.161
7.145
7.142
7.121
7.100
3.895
3.105
2k
^{1}H NMR (400 MHz, $CDCl_3$)
$CDCl_3$
Grease
1.00
1.03
1.06
1.03
2.82
2.75
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ppm

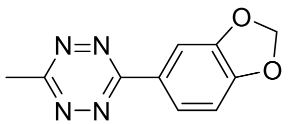

166.323
165.986
158.345
133.191
131.867
122.213
121.165
112.240
CDCl3
56.171
21.367
2k
13C NMR (101 MHz, CDCl3)
Grease
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm



2l

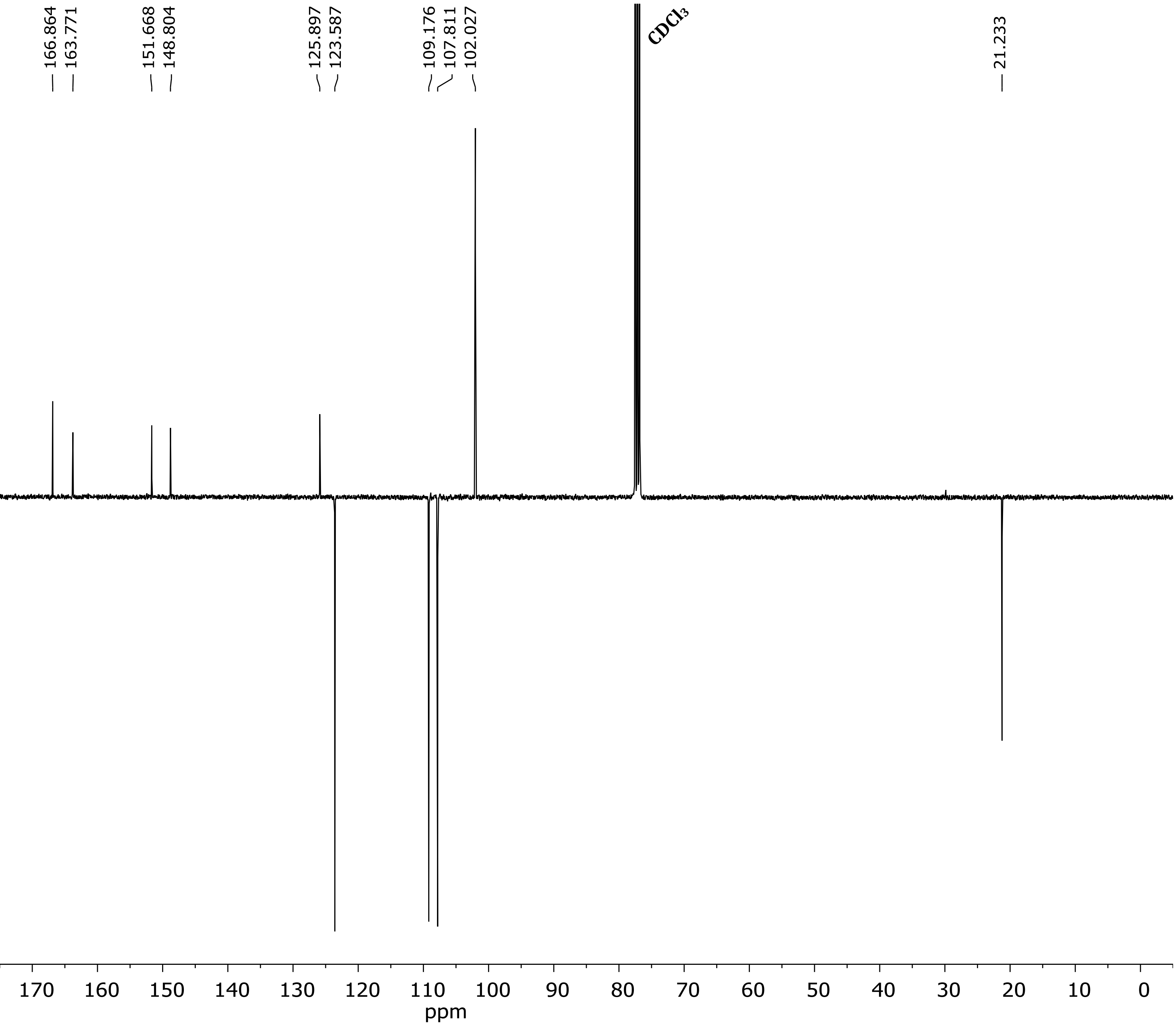
^1H NMR (400 MHz, $CDCl_3$)

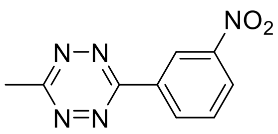




2l

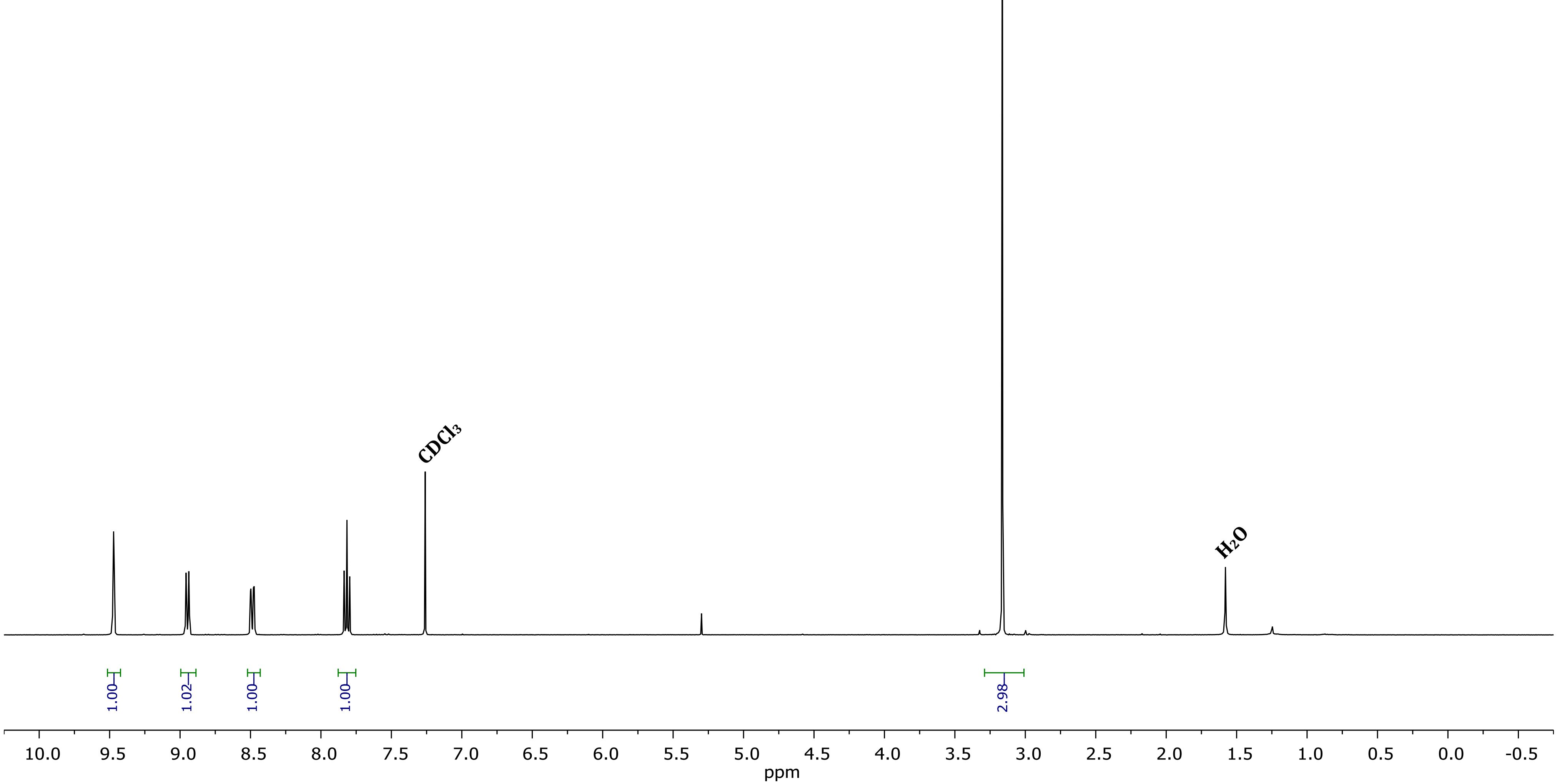
^{13}C NMR (101 MHz, CDCl$_3$)

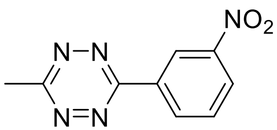




2m

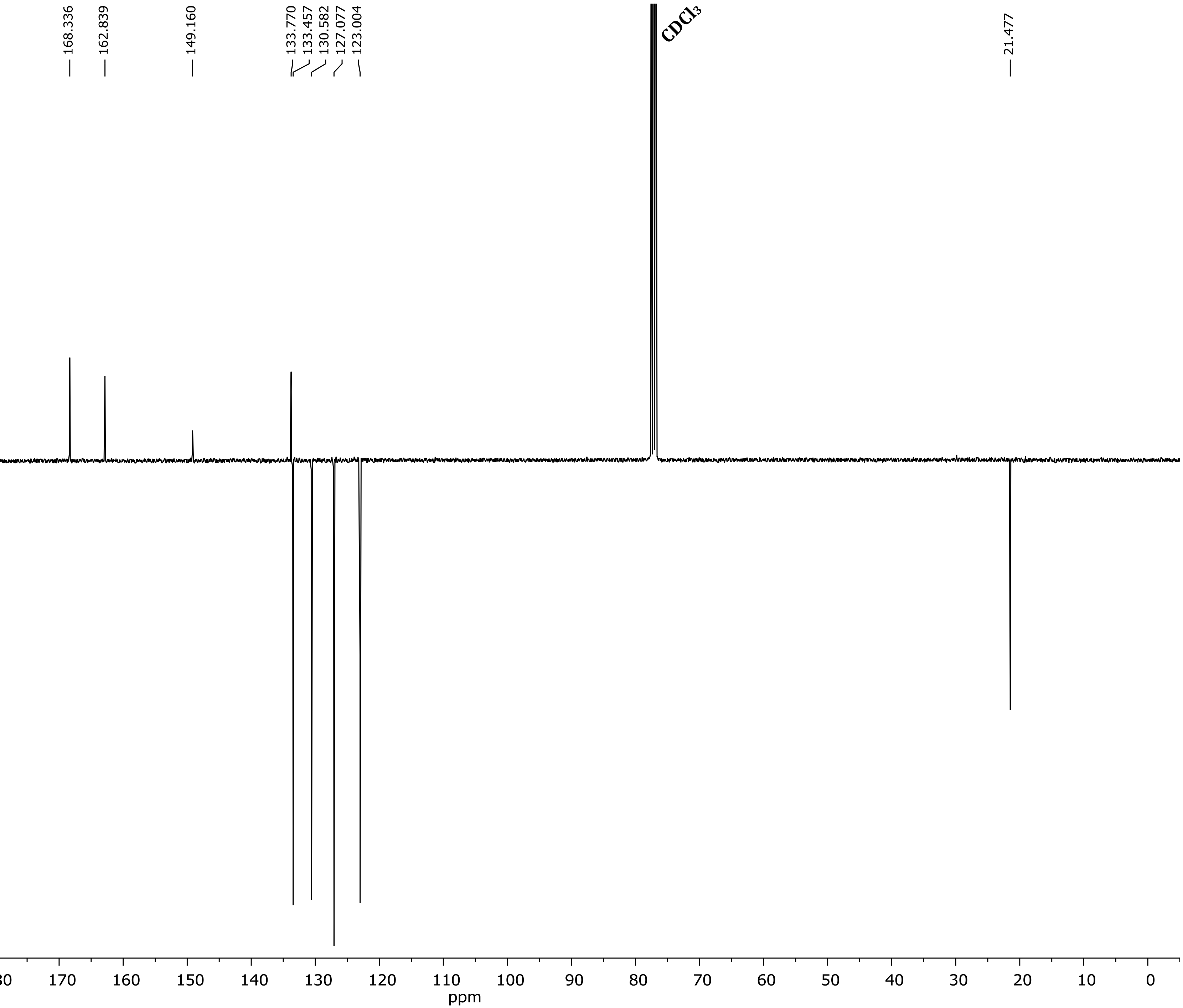
^{1}H NMR (400 MHz, $CDCl_3$)

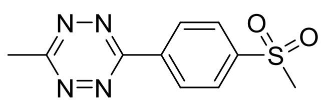




2m

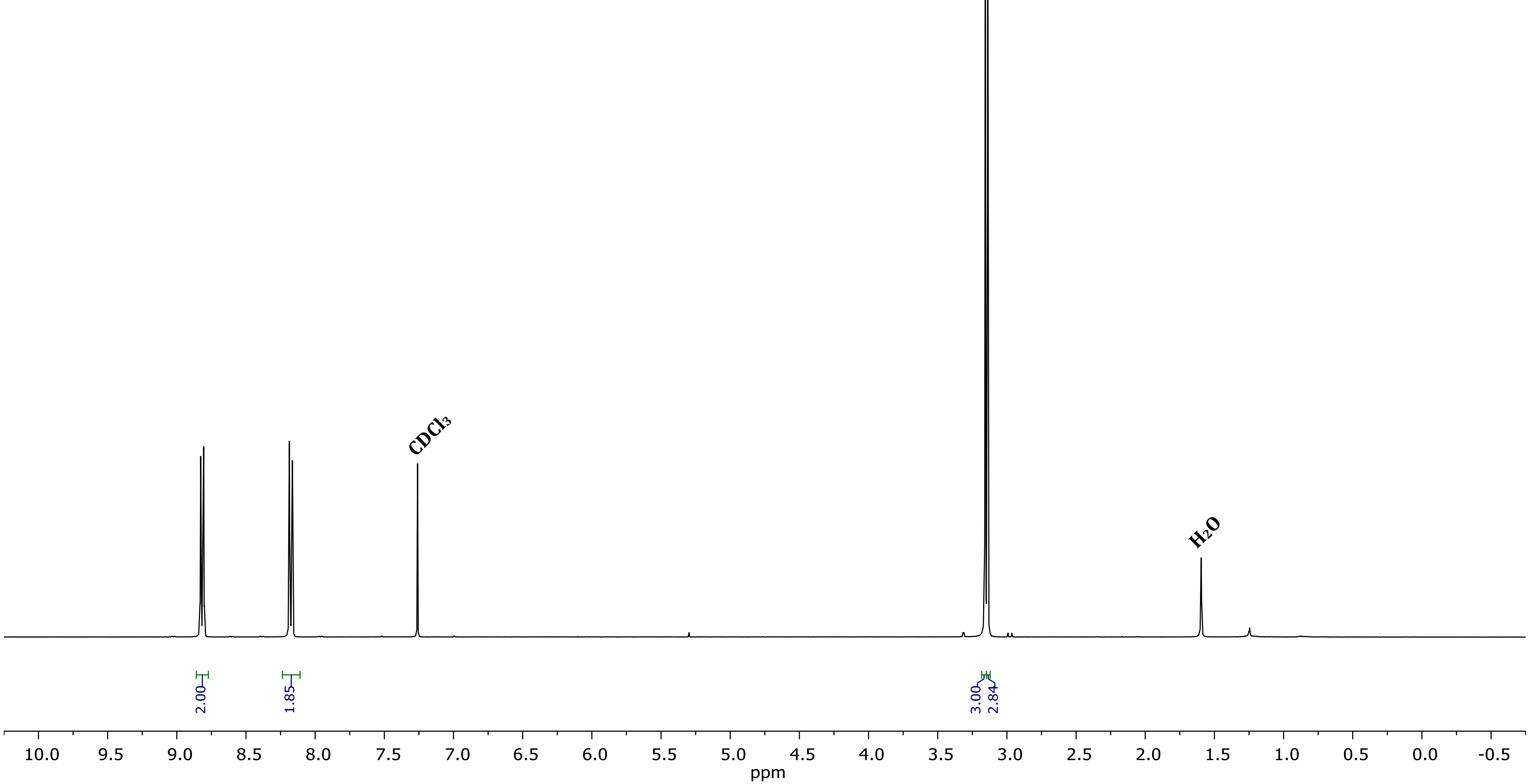
^{13}C NMR (101 MHz, $CDCl_3$)

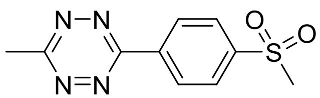




2n

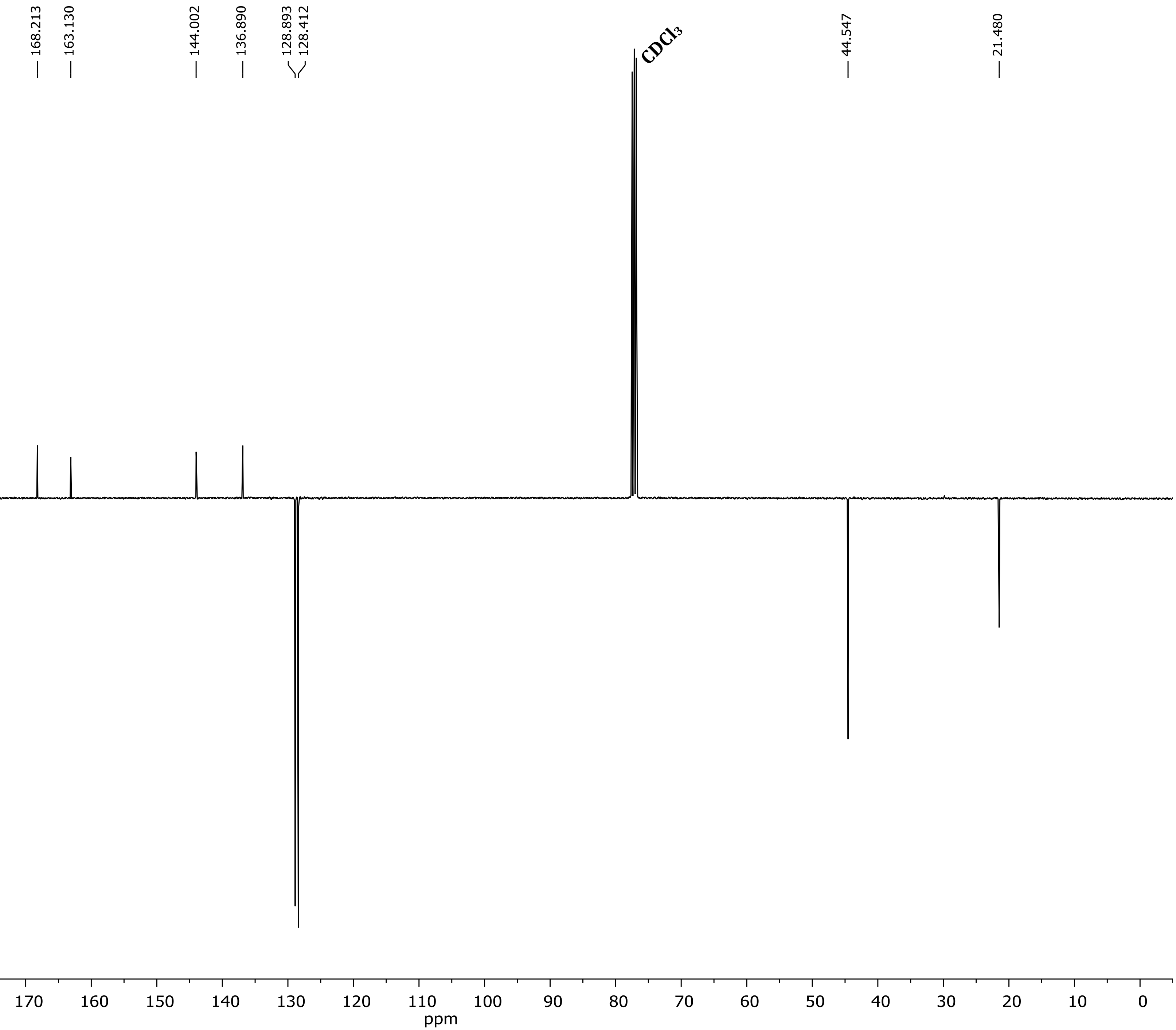
^{1}H NMR (400 MHz, $CDCl_3$)

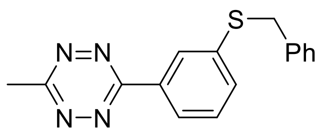




2n

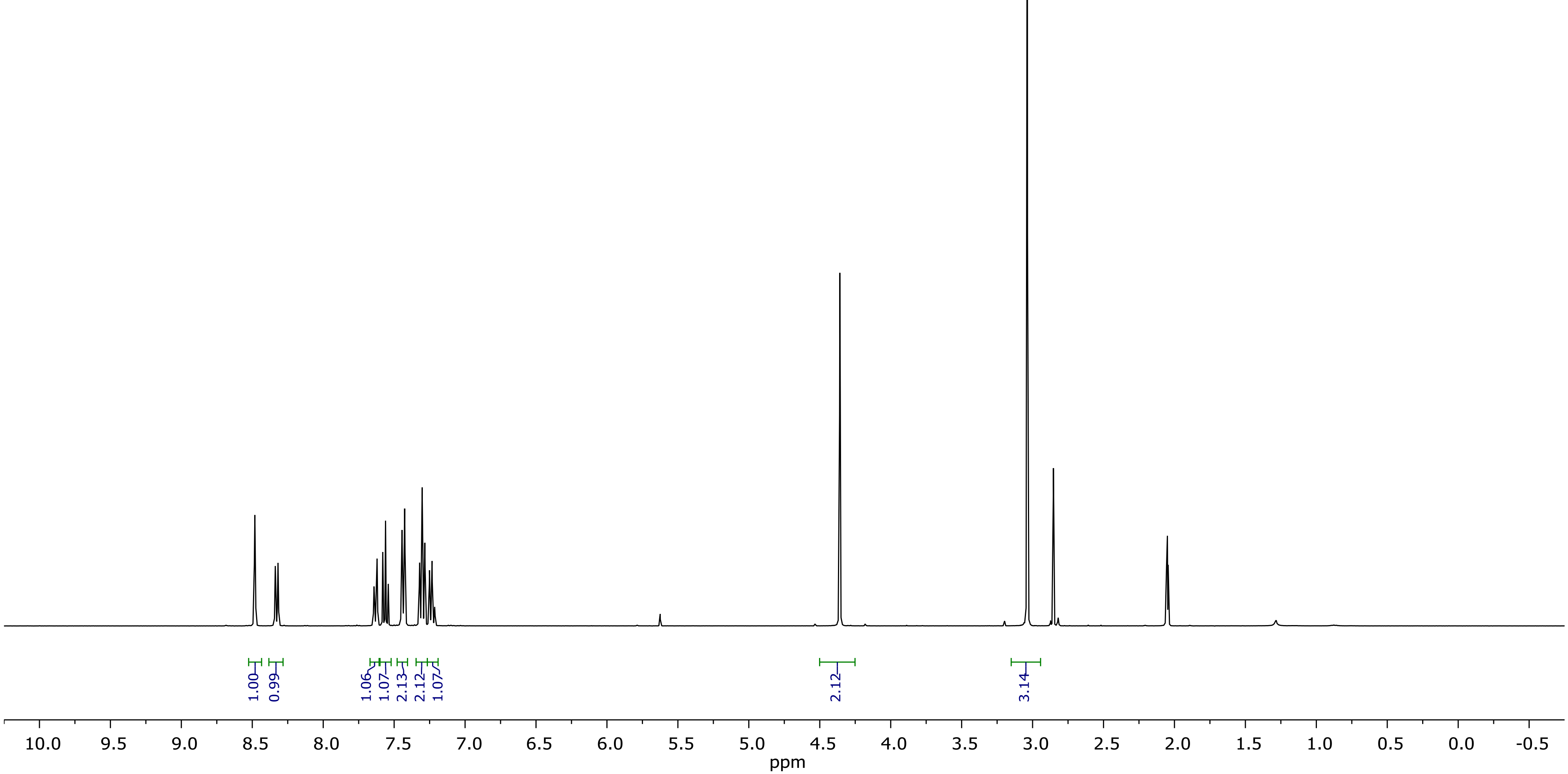
^{13}C NMR (101 MHz, $CDCl_3$)

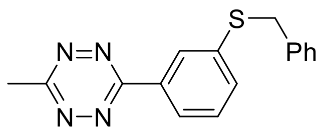




2o

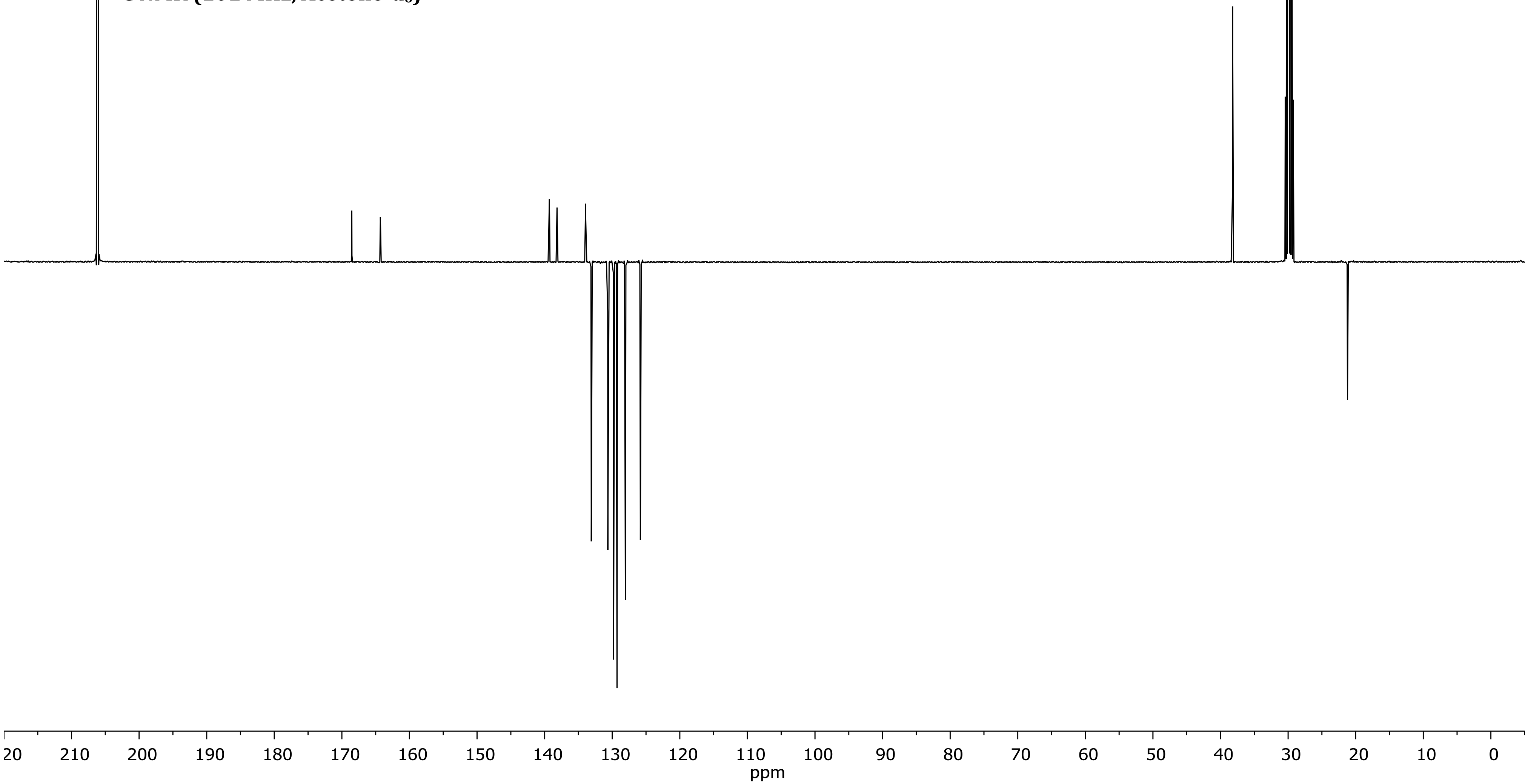
^{1}H NMR (400 MHz, Acetone-d_6)

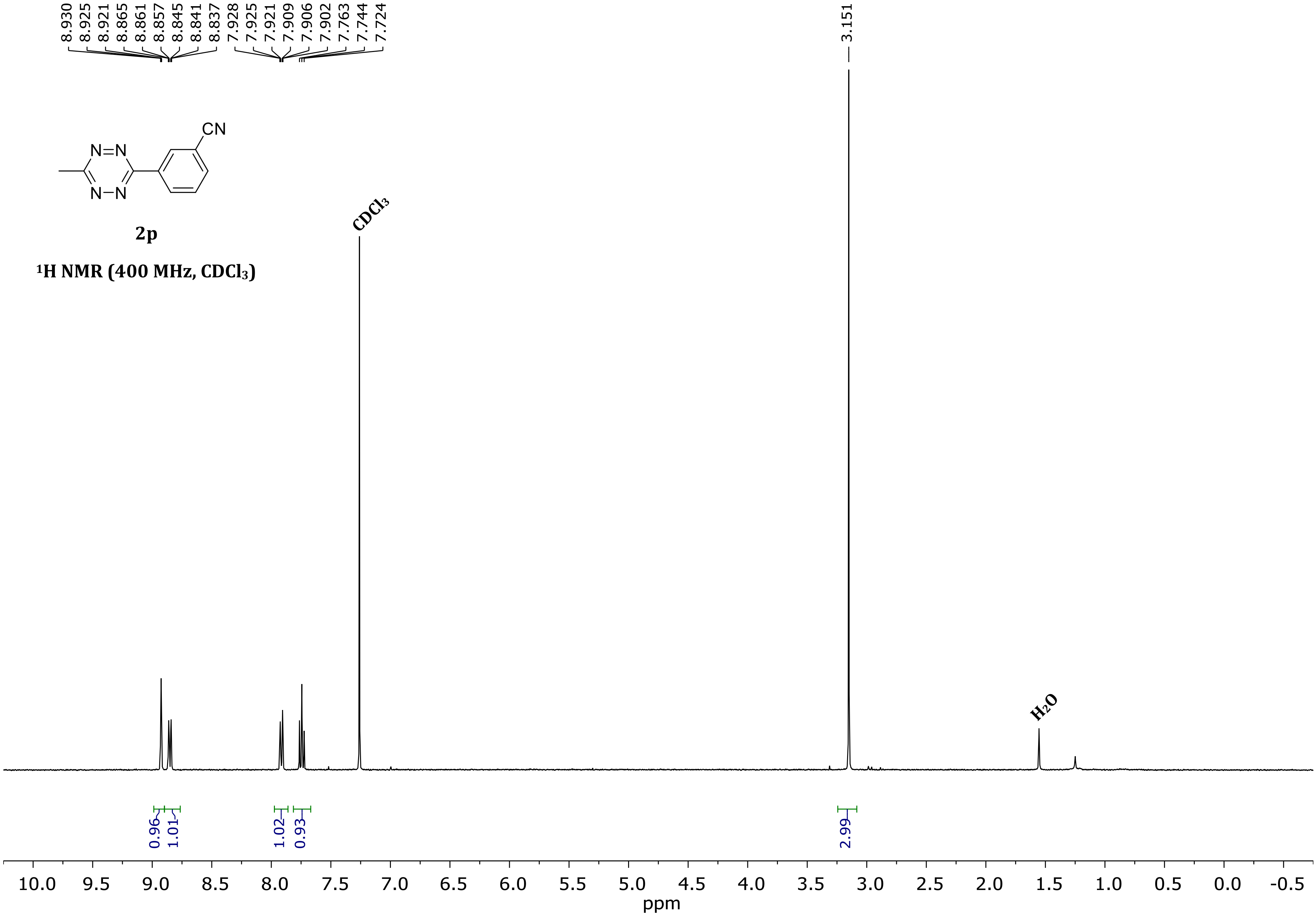




2o

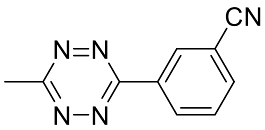
^{13}C NMR (101 MHz, Acetone-d$_6$)





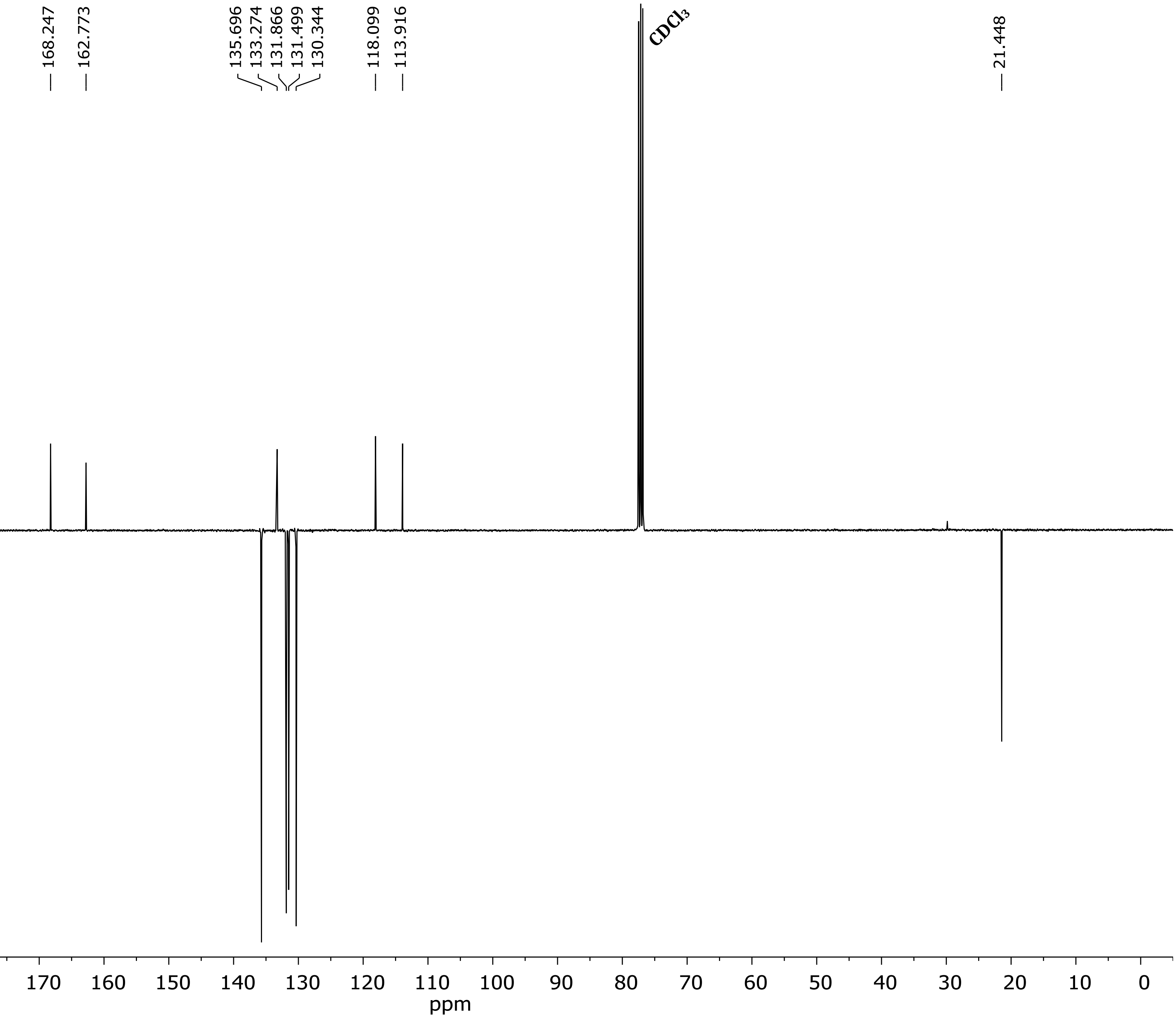
2p

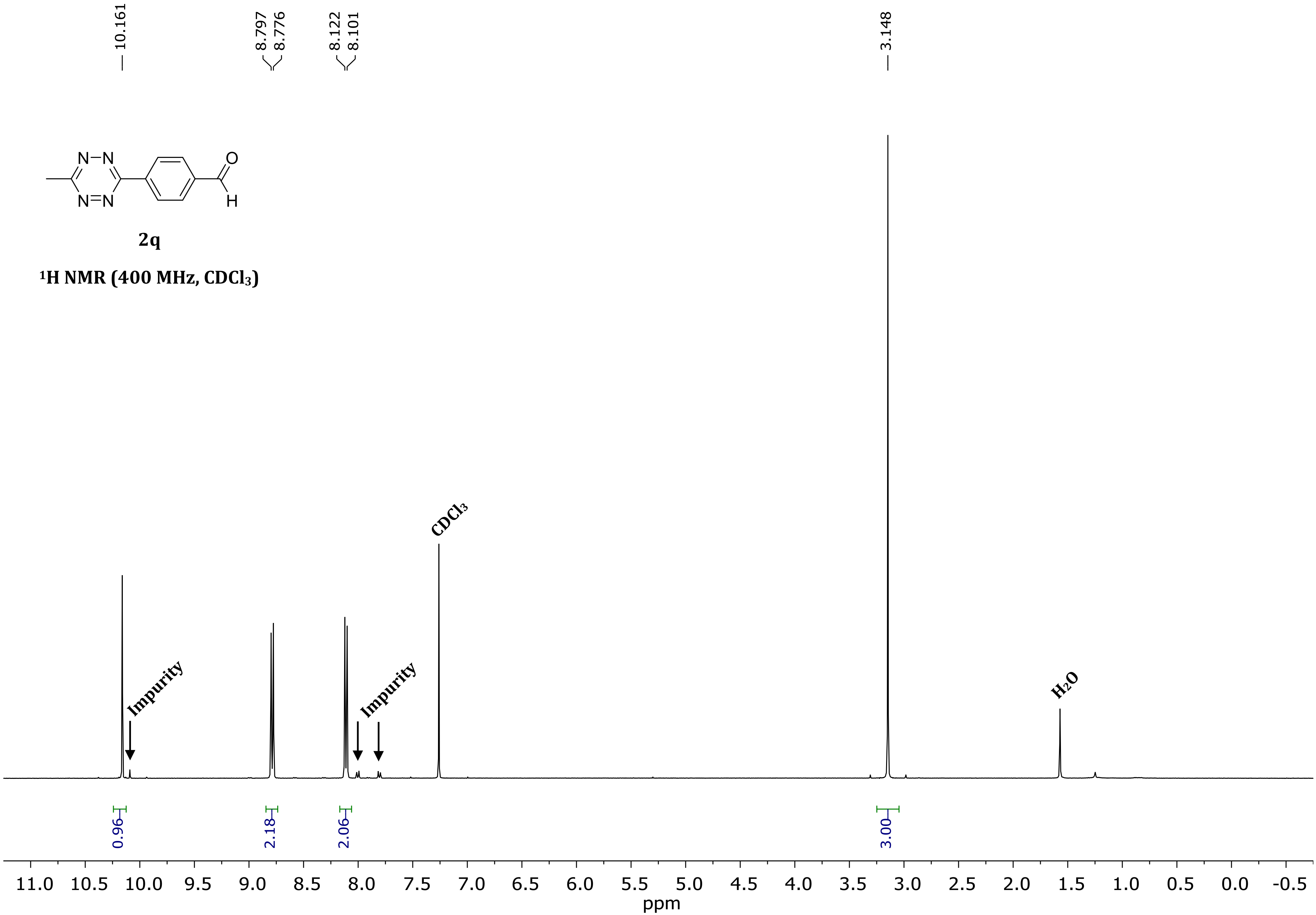
^{1}H NMR (400 MHz, $CDCl_3$)

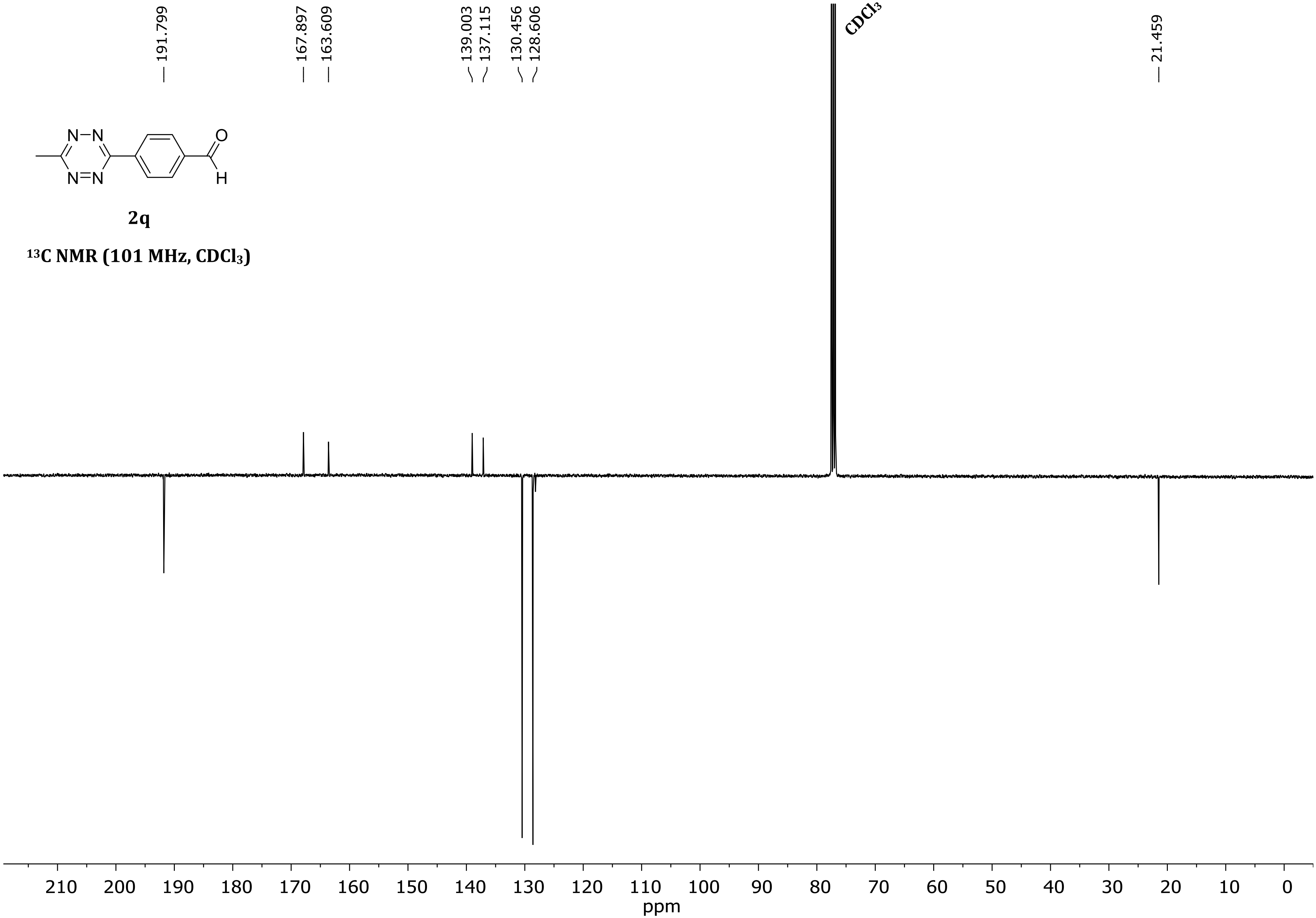


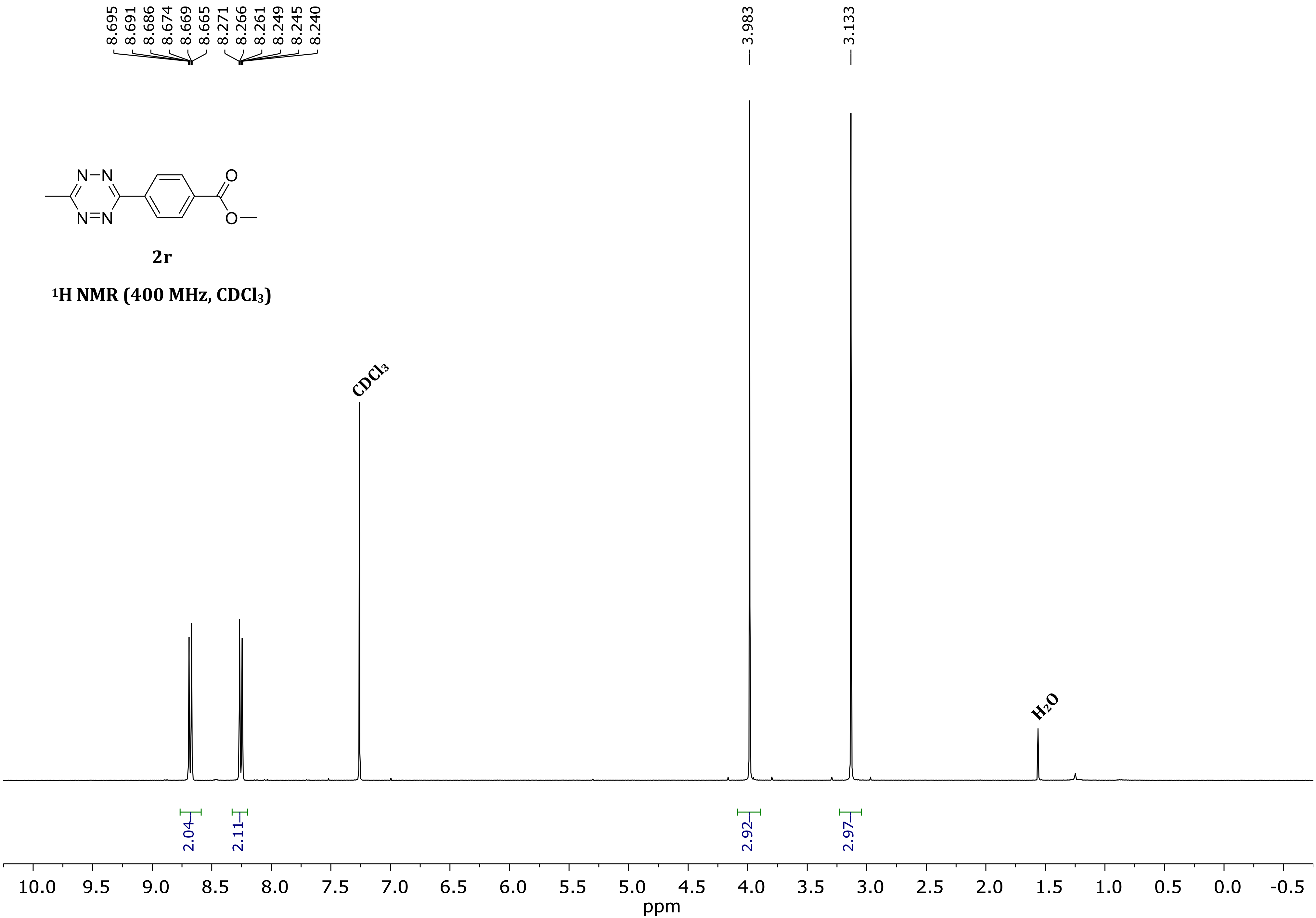
2p

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

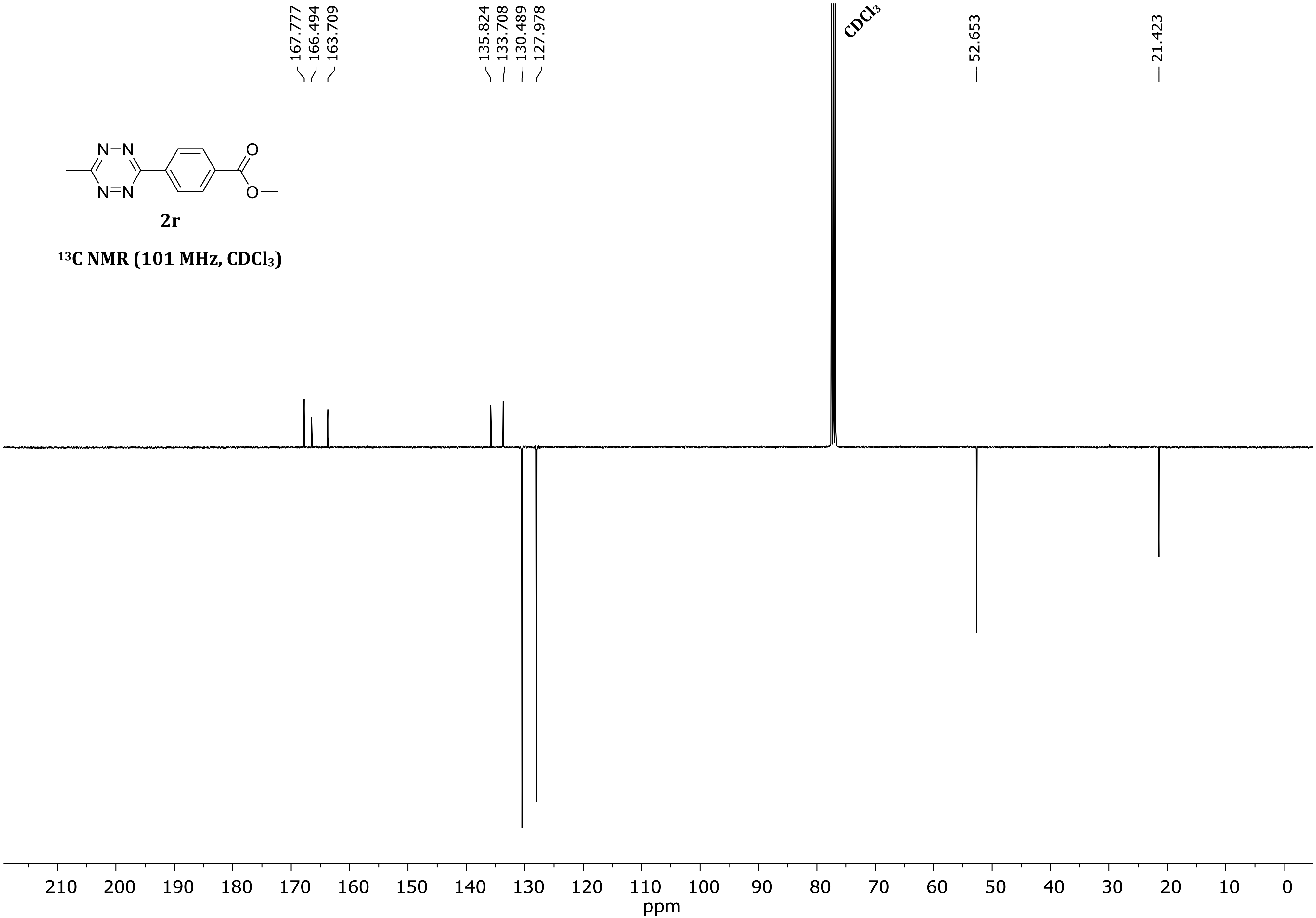



10.161
8.797
8.776
8.122
8.101
3.148
2q
^{1}H NMR (400 MHz, $CDCl_3$)
Impurity
Impurity
$CDCl_3$
H_2O
0.96
2.18
2.06
3.00
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm

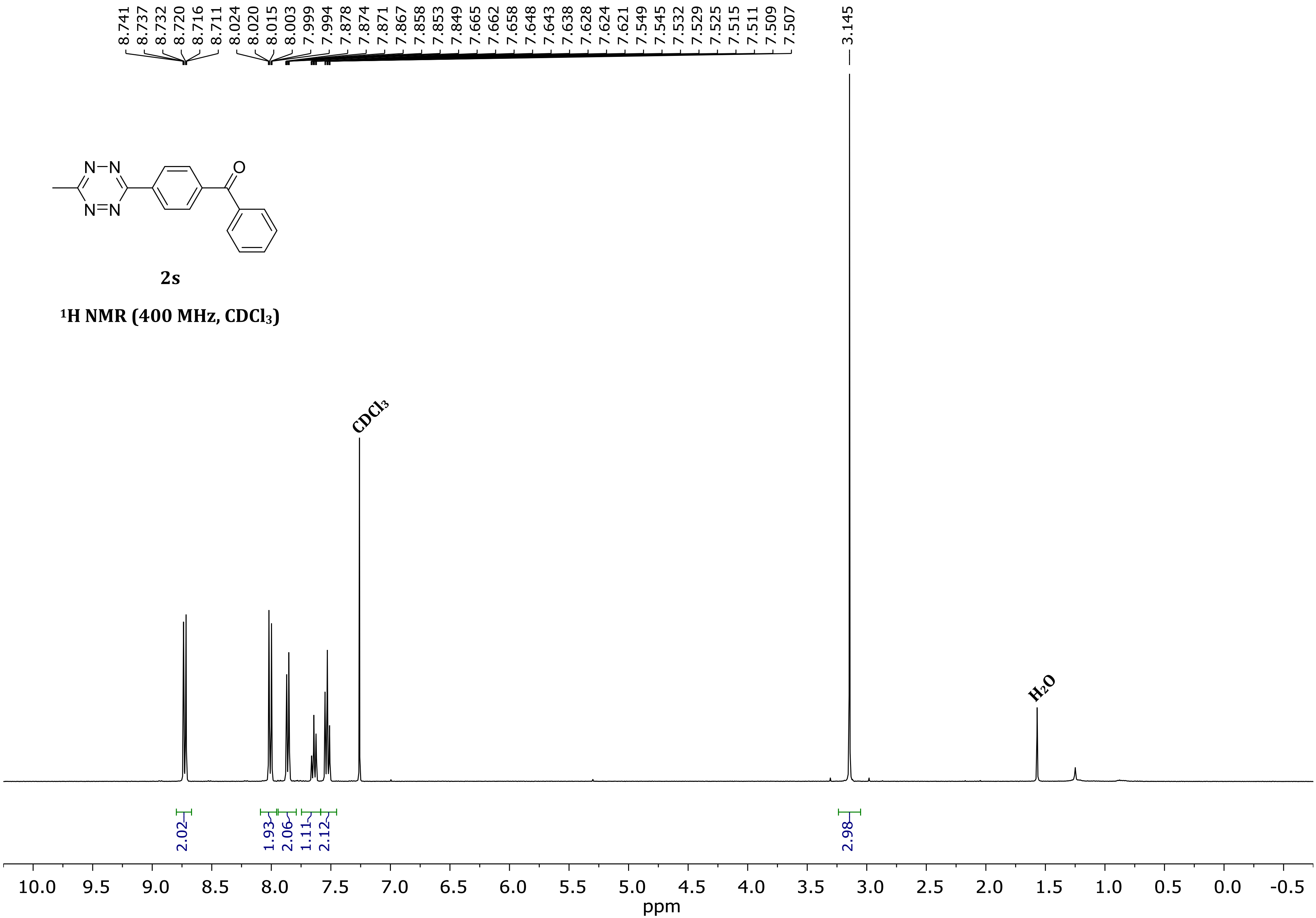

191.799
167.897
163.609
139.003
137.115
130.456
128.606
CDCl3
21.459
2q
13C NMR (101 MHz, CDCl3)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm



8.695
8.691
8.686
8.674
8.669
8.665
8.271
8.266
8.261
8.249
8.245
8.240
3.983
3.133
2r
^{1}H NMR (400 MHz, $CDCl_3$)
$CDCl_3$
H_2O
2.04
2.11
2.92
2.97
10.0 9.5 9.0 8.5 8.0 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 0.0 -0.5
ppm

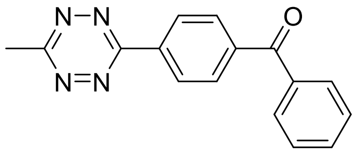


^{13}C NMR (101 MHz, $CDCl_3$)



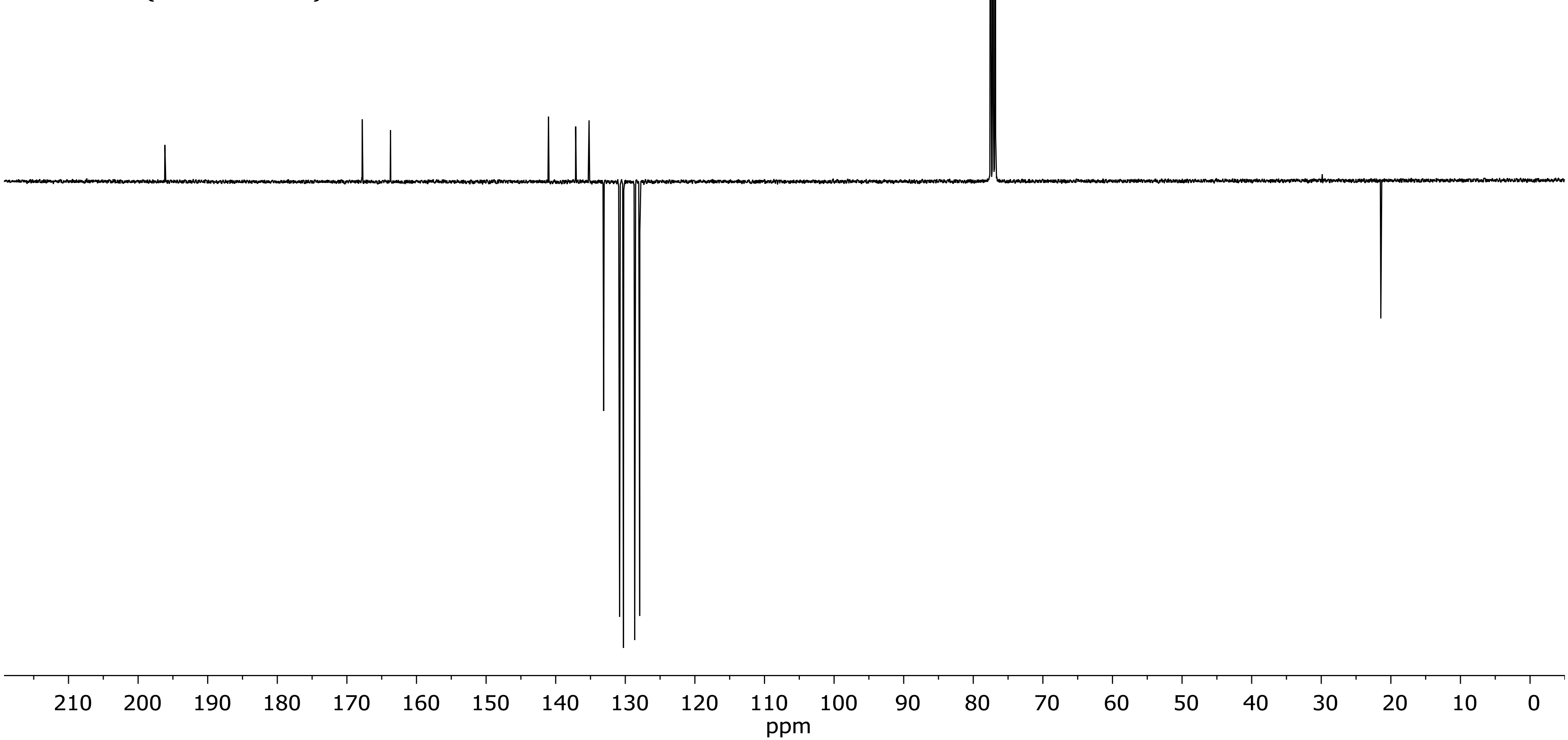
2s

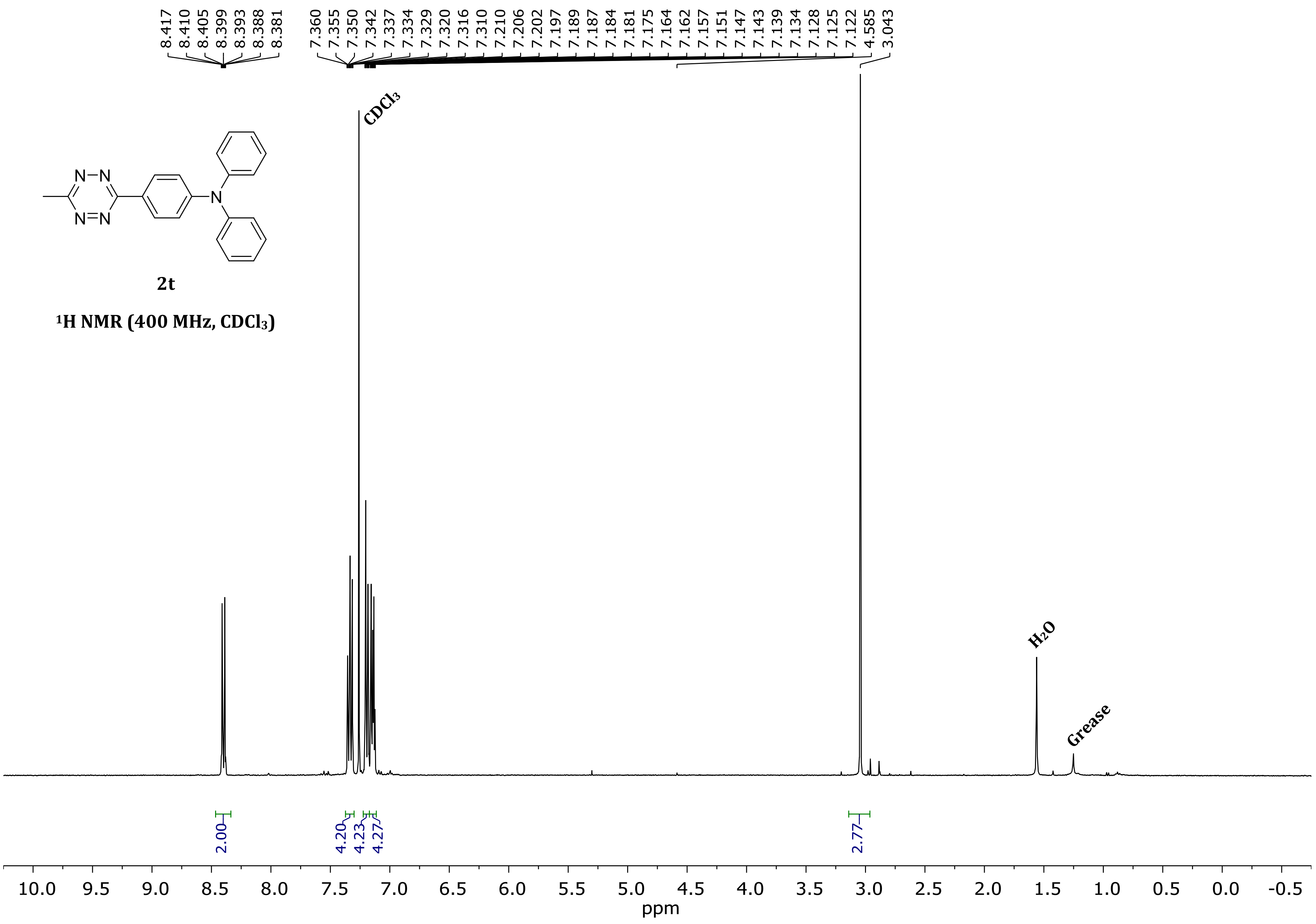
¹H NMR (400 MHz, $CDCl_3$)

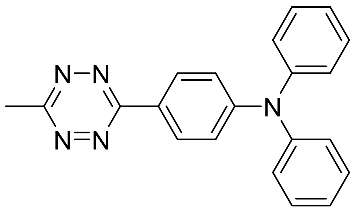


2s

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

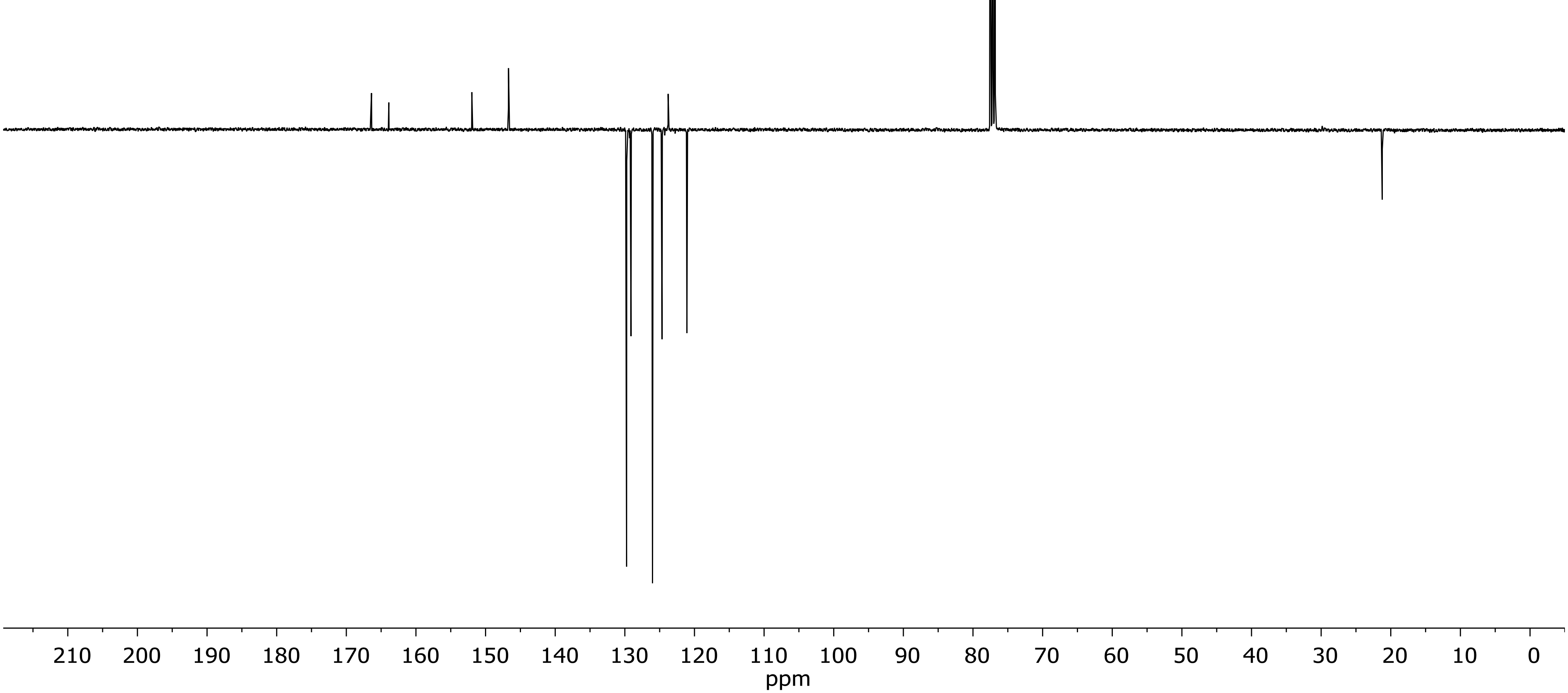


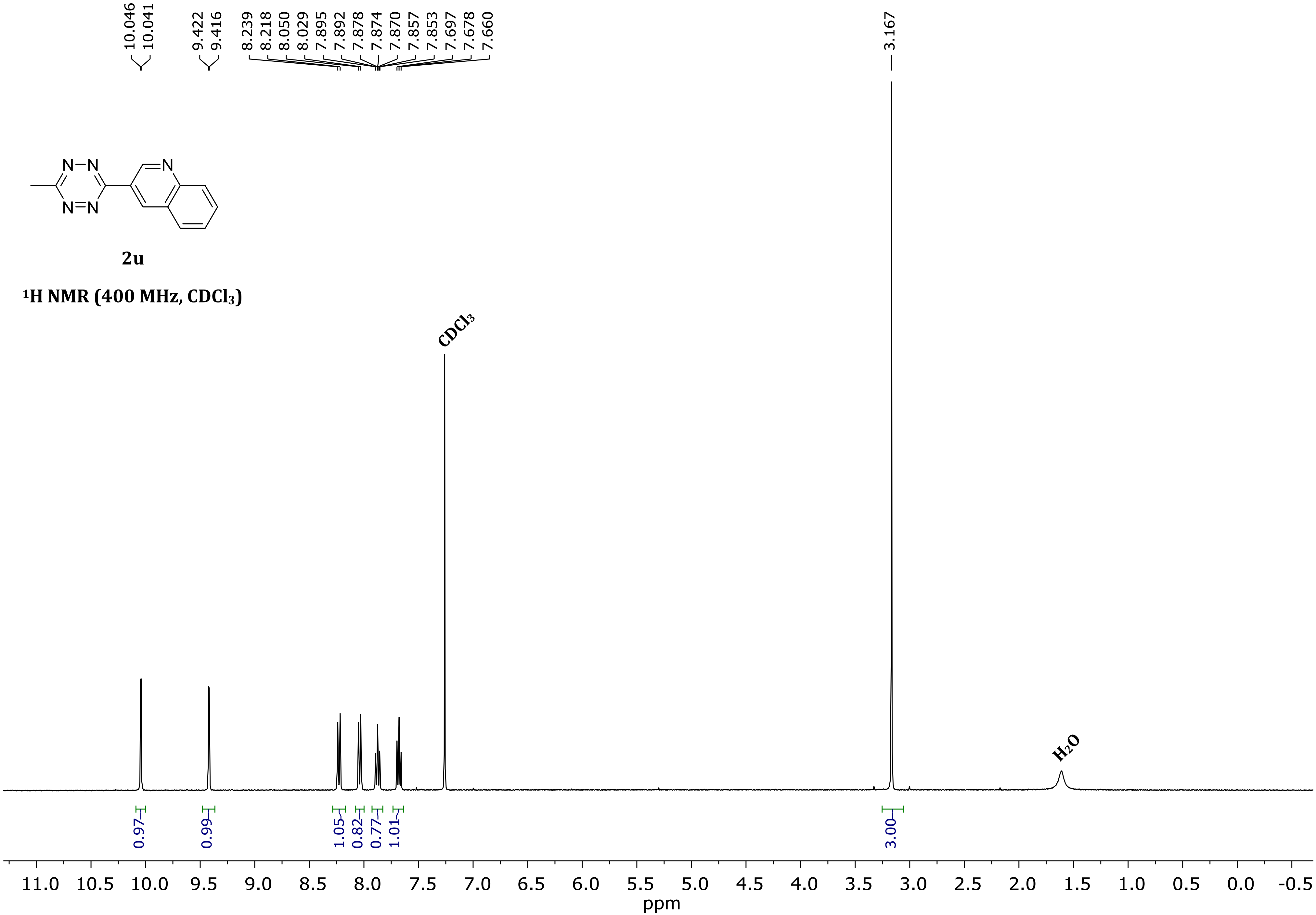

8.417
8.410
8.405
8.399
8.393
8.388
8.381
7.360
7.355
7.350
7.342
7.337
7.334
7.329
7.320
7.316
7.310
7.210
7.206
7.202
7.197
7.189
7.187
7.184
7.181
7.175
7.164
7.162
7.157
7.151
7.147
7.143
7.139
7.134
7.128
7.125
7.122
4.585
3.043
CDCl3
N
N
N
N
N
2t
1H NMR (400 MHz, CDCl3)
H2O
Grease
2.00
4.20
4.23
4.27
2.77
10.0
9.5
9.0
8.5
8.0
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
0.0
-0.5
ppm



2t

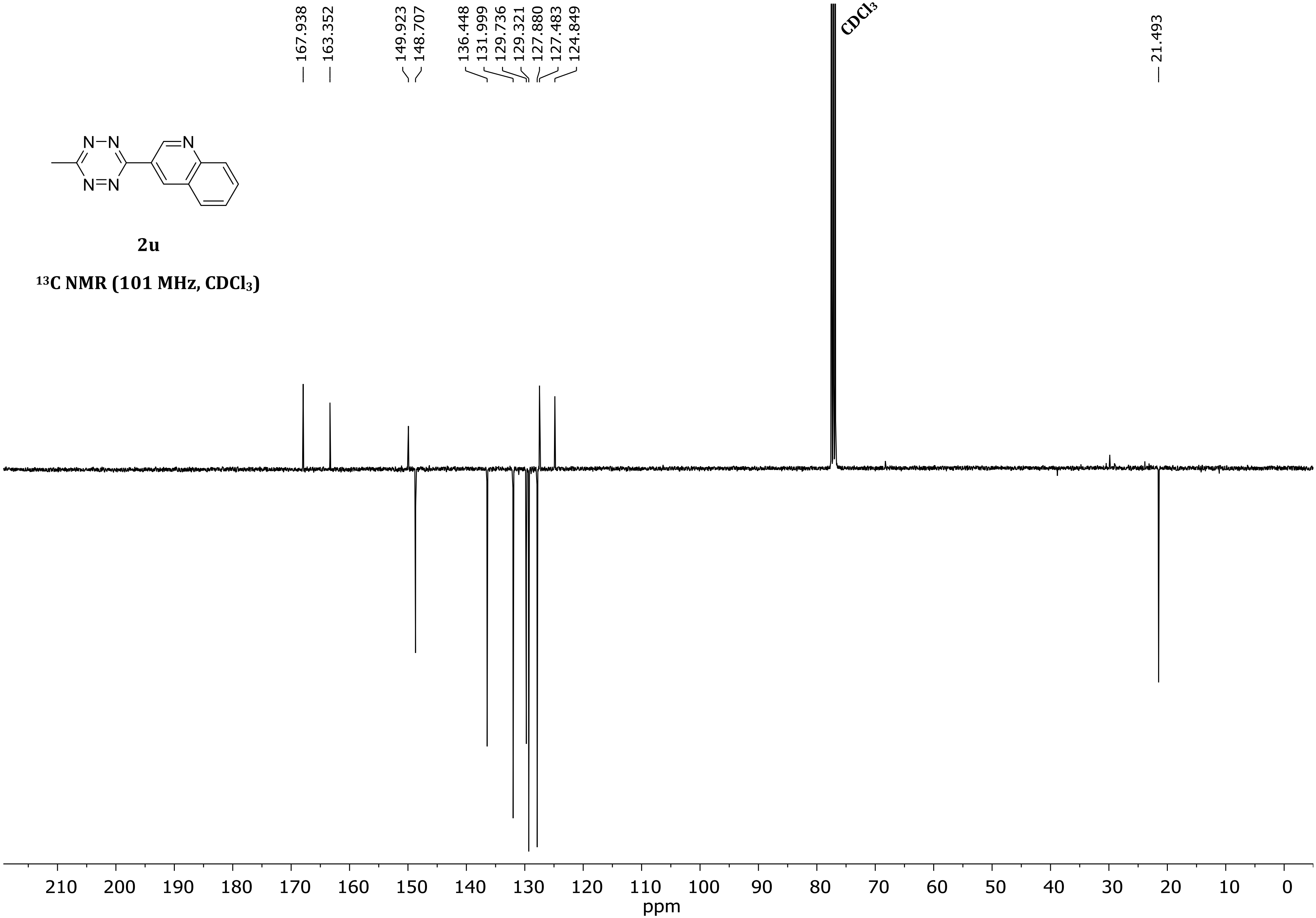
^{13}C NMR (101 MHz, CDCl$_3$)

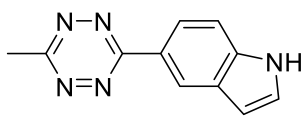



10.046
10.041
9.422
9.416
8.239
8.218
8.050
8.029
7.895
7.892
7.878
7.874
7.870
7.857
7.853
7.697
7.678
7.660
3.167
N–N
N=N
N
2u
¹H NMR (400 MHz, CDCl₃)
CDCl₃
H₂O
0.97
0.99
1.05
0.82
0.77
1.01
3.00
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm

2u

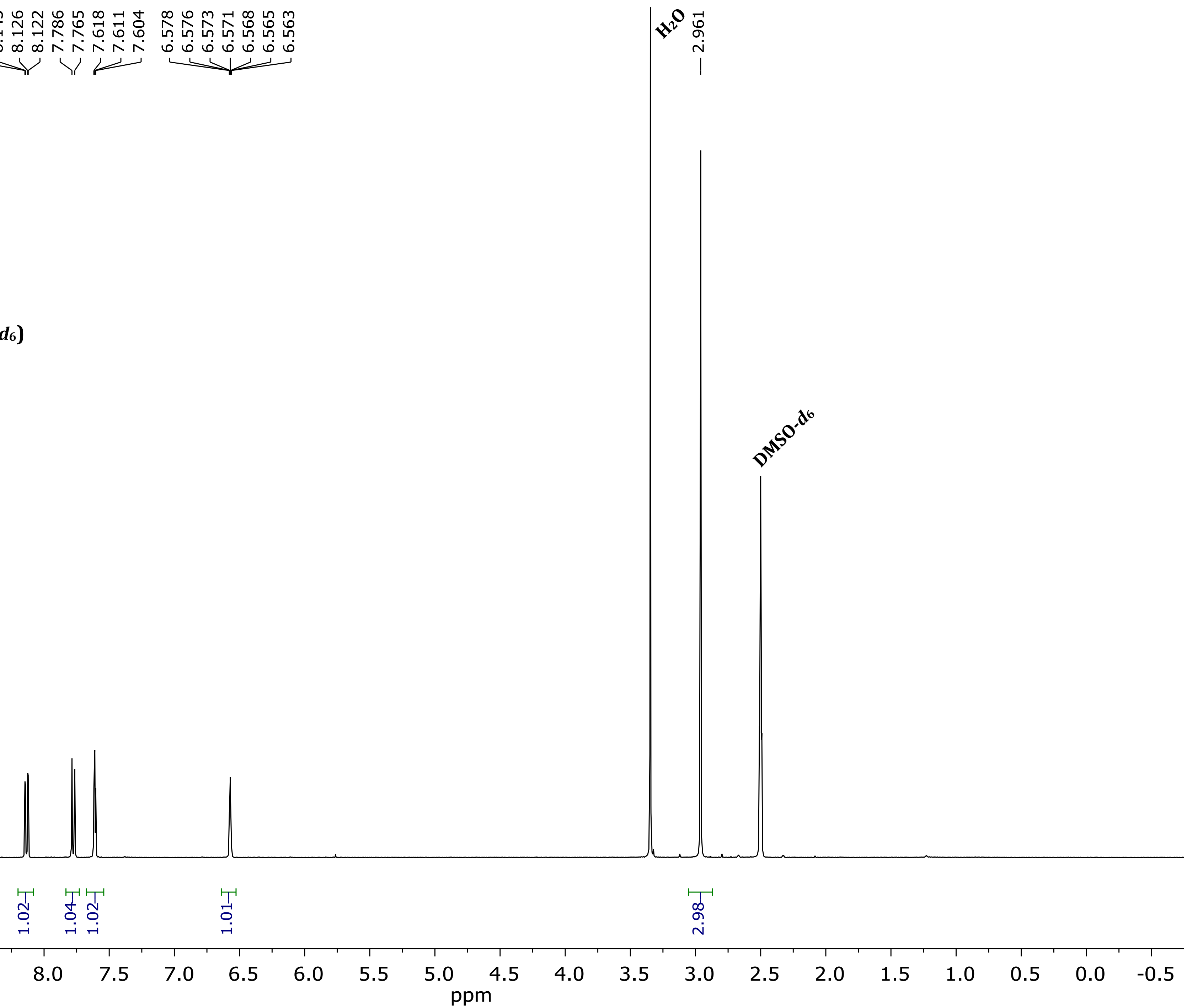
^{13}C NMR (101 MHz, $CDCl_3$)

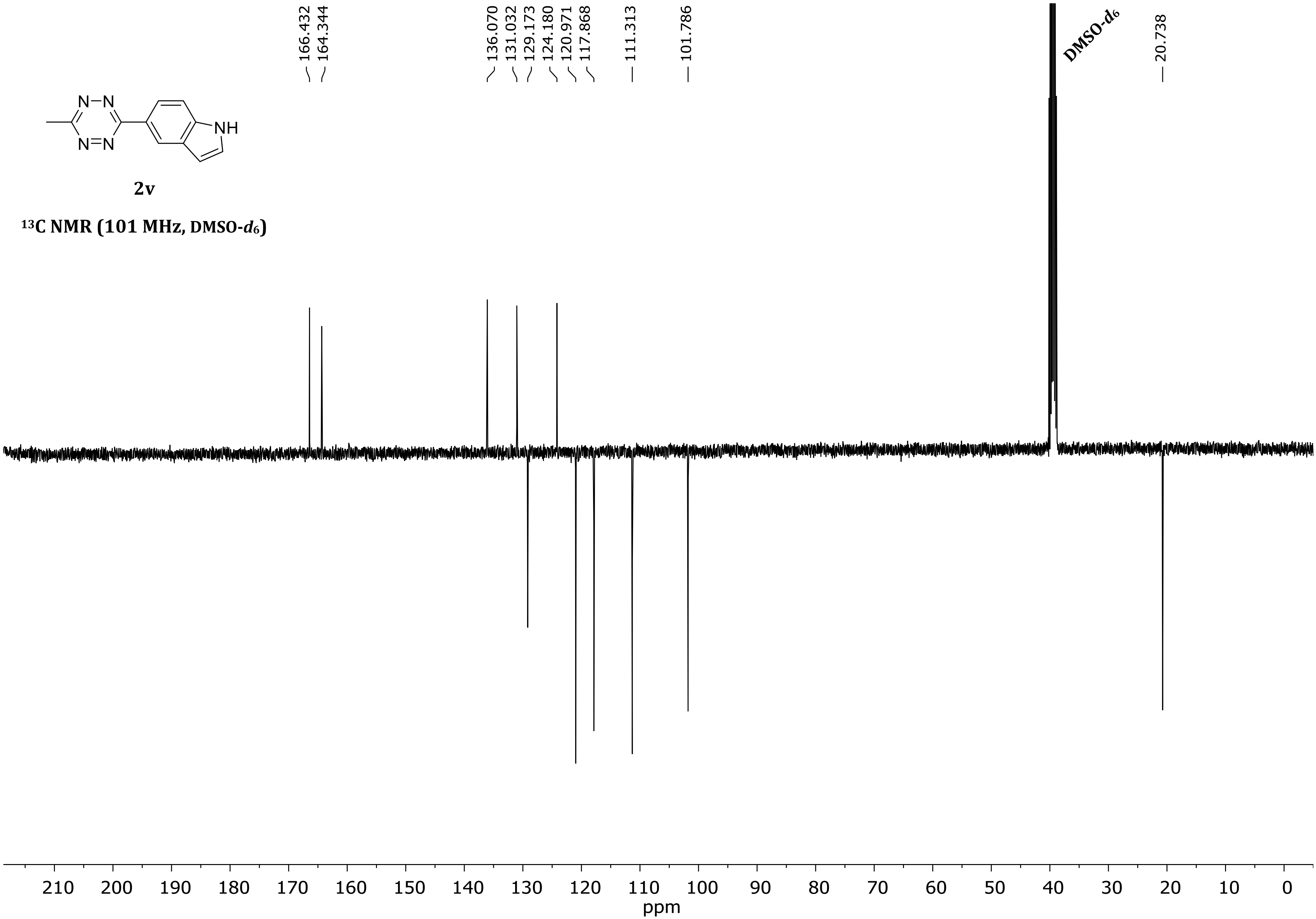




2v

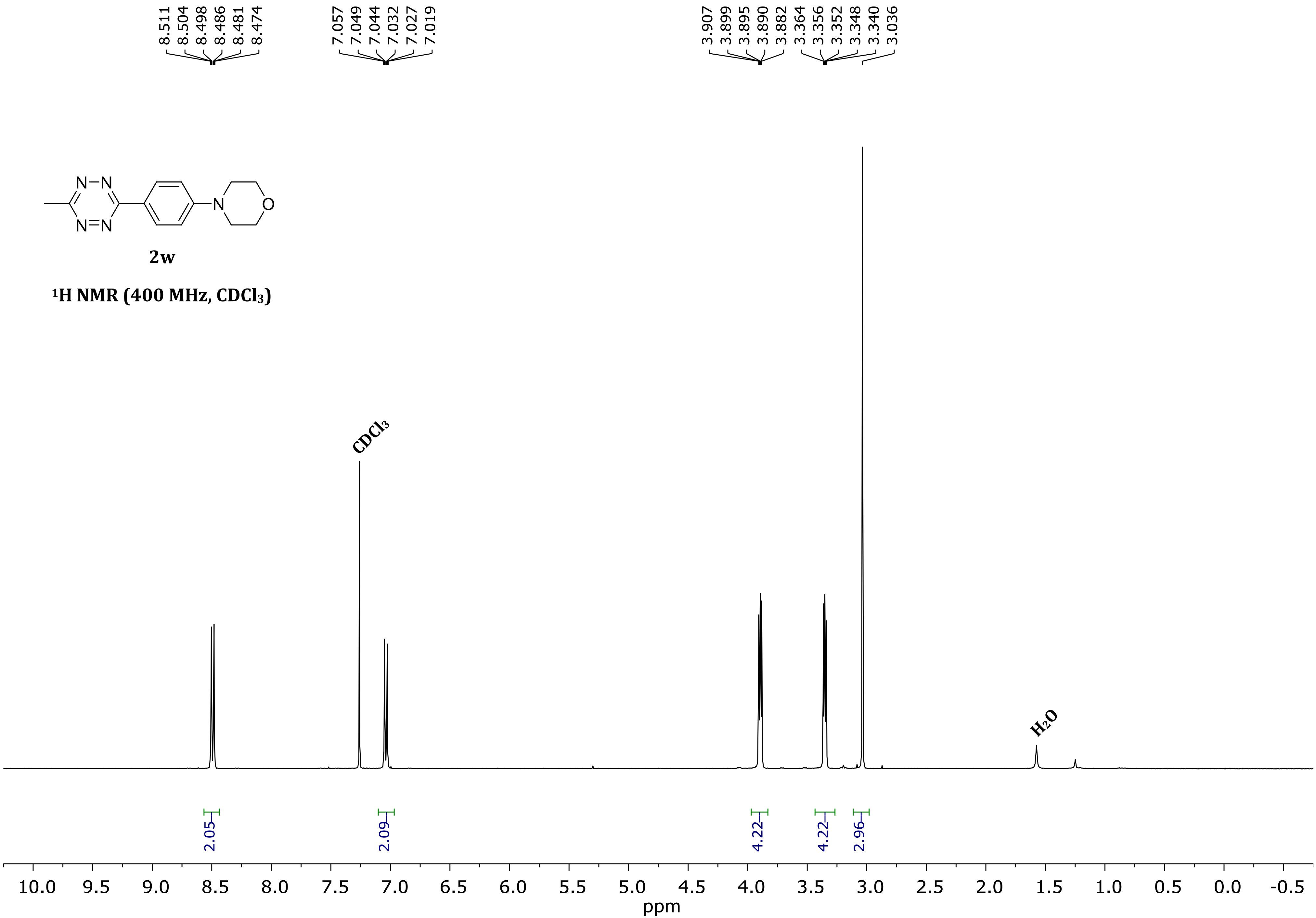
^{1}H NMR (400 MHz, DMSO-d_6)

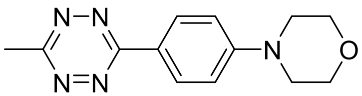




2v

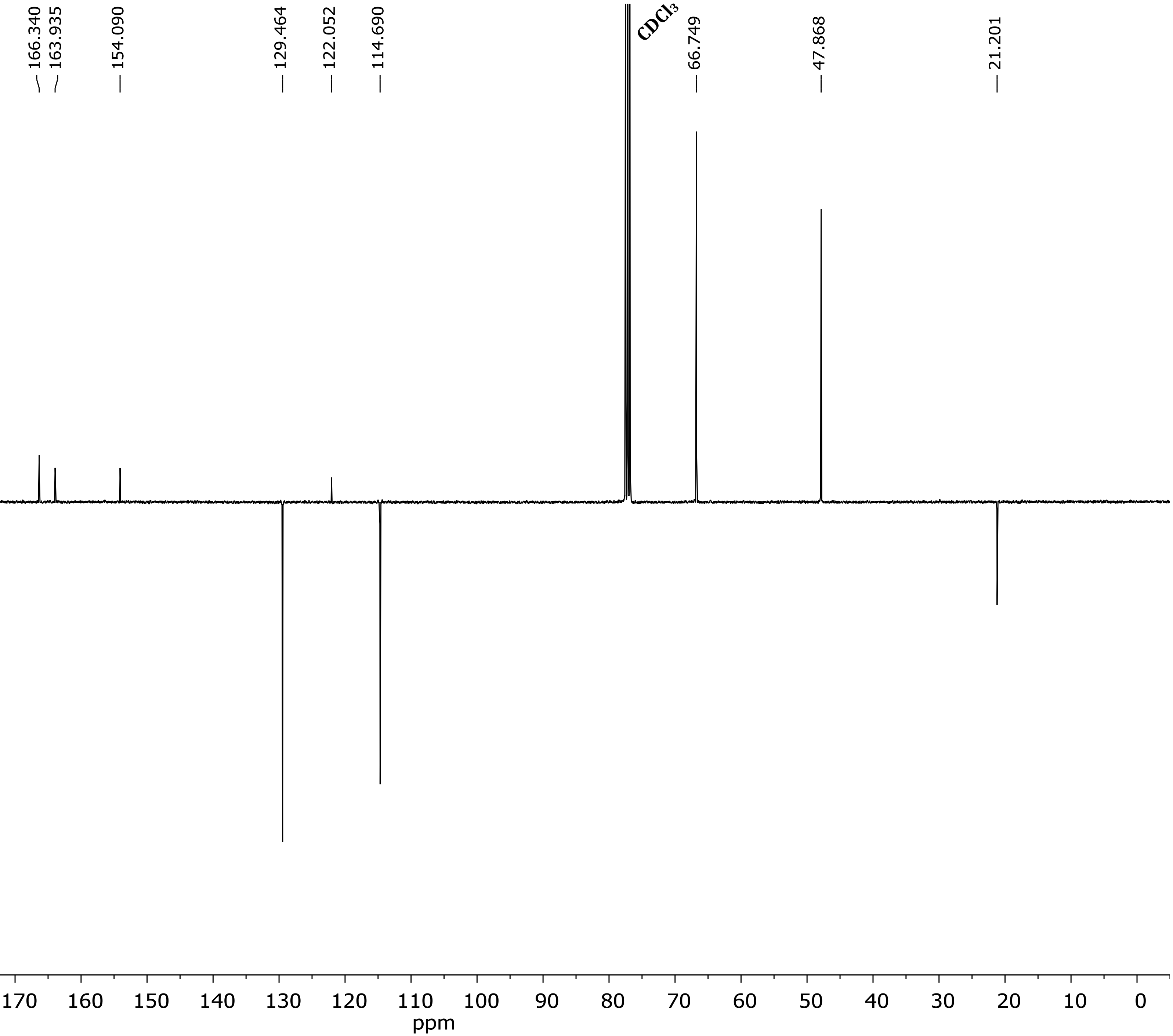
^{13}C NMR (101 MHz, DMSO-d_6)

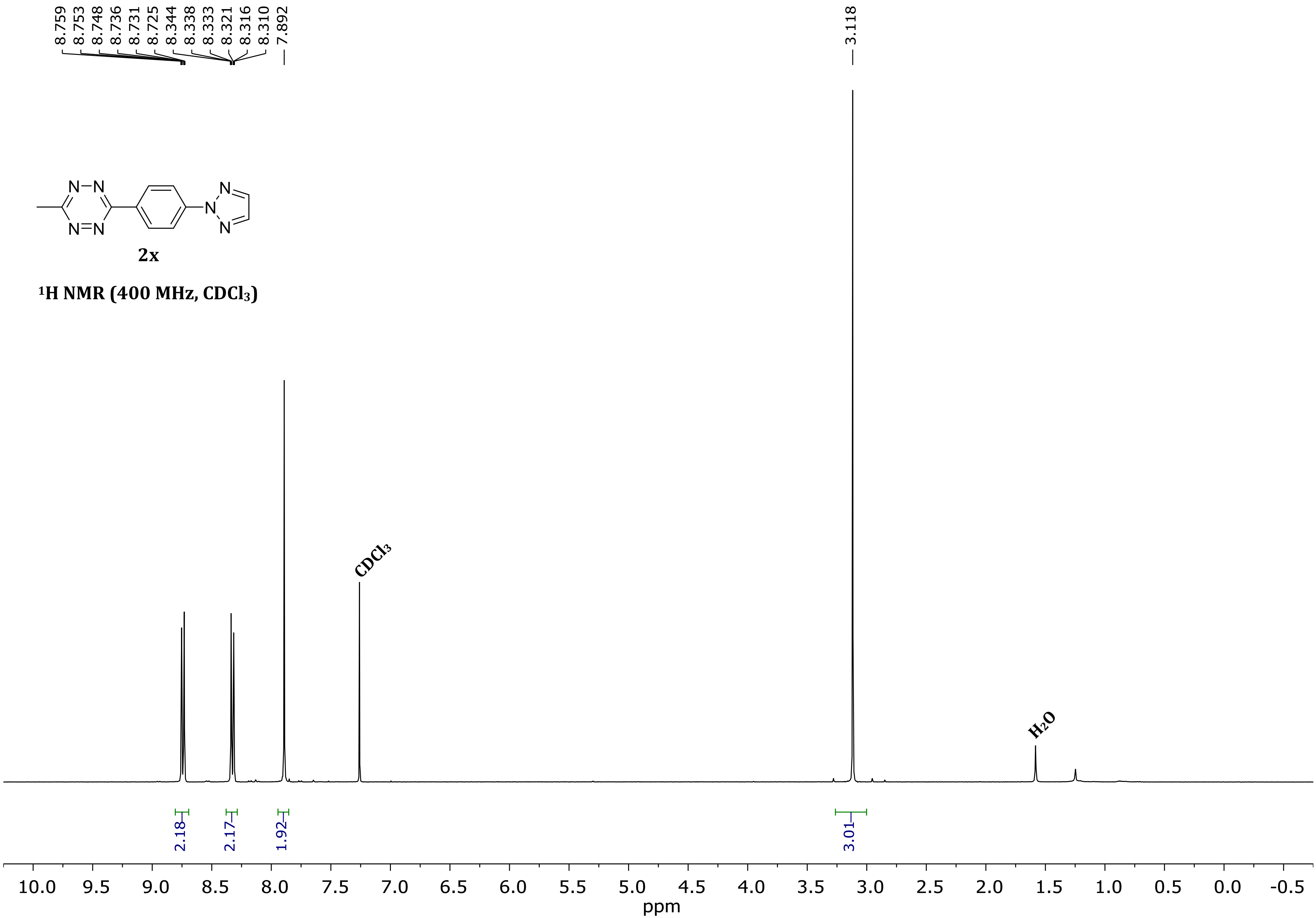

8.511
8.504
8.498
8.486
8.481
8.474
7.057
7.049
7.044
7.032
7.027
7.019
3.907
3.899
3.895
3.890
3.882
3.364
3.356
3.352
3.348
3.340
3.036
2w
¹H NMR (400 MHz, CDCl₃)
CDCl₃
H₂O
2.05
2.09
4.22
4.22
2.96
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm



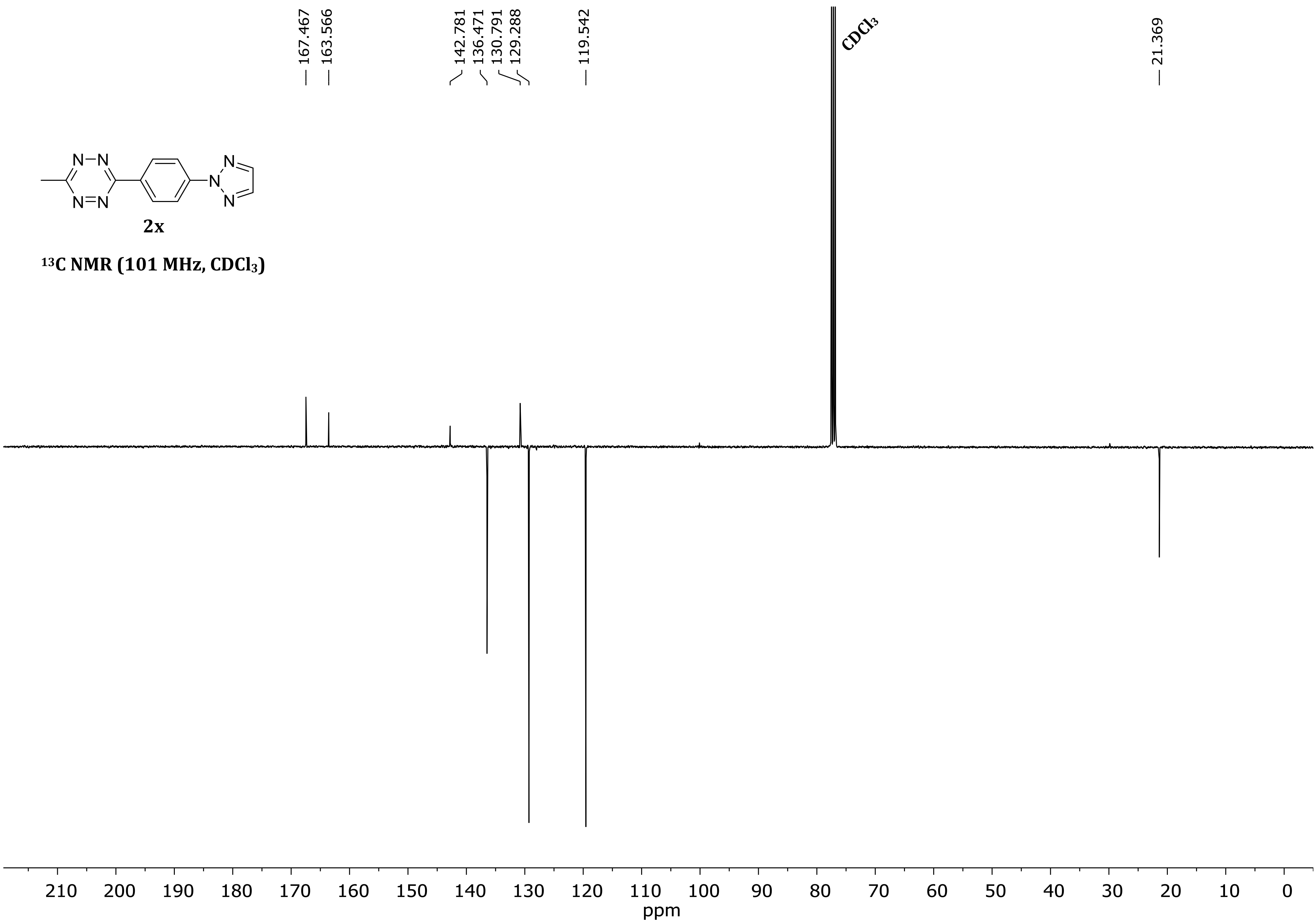
2w

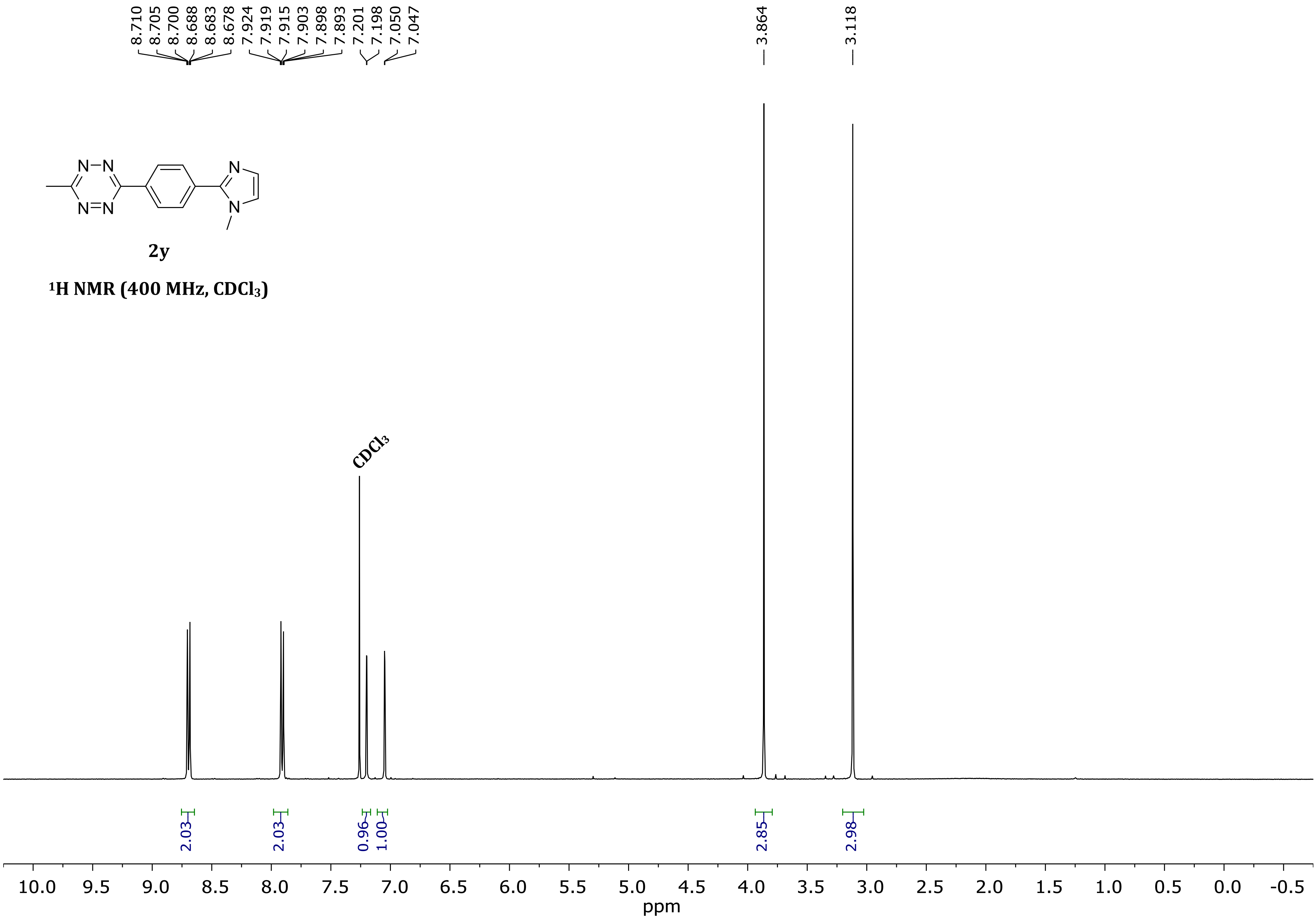
^{13}C NMR (101 MHz, $CDCl_3$)

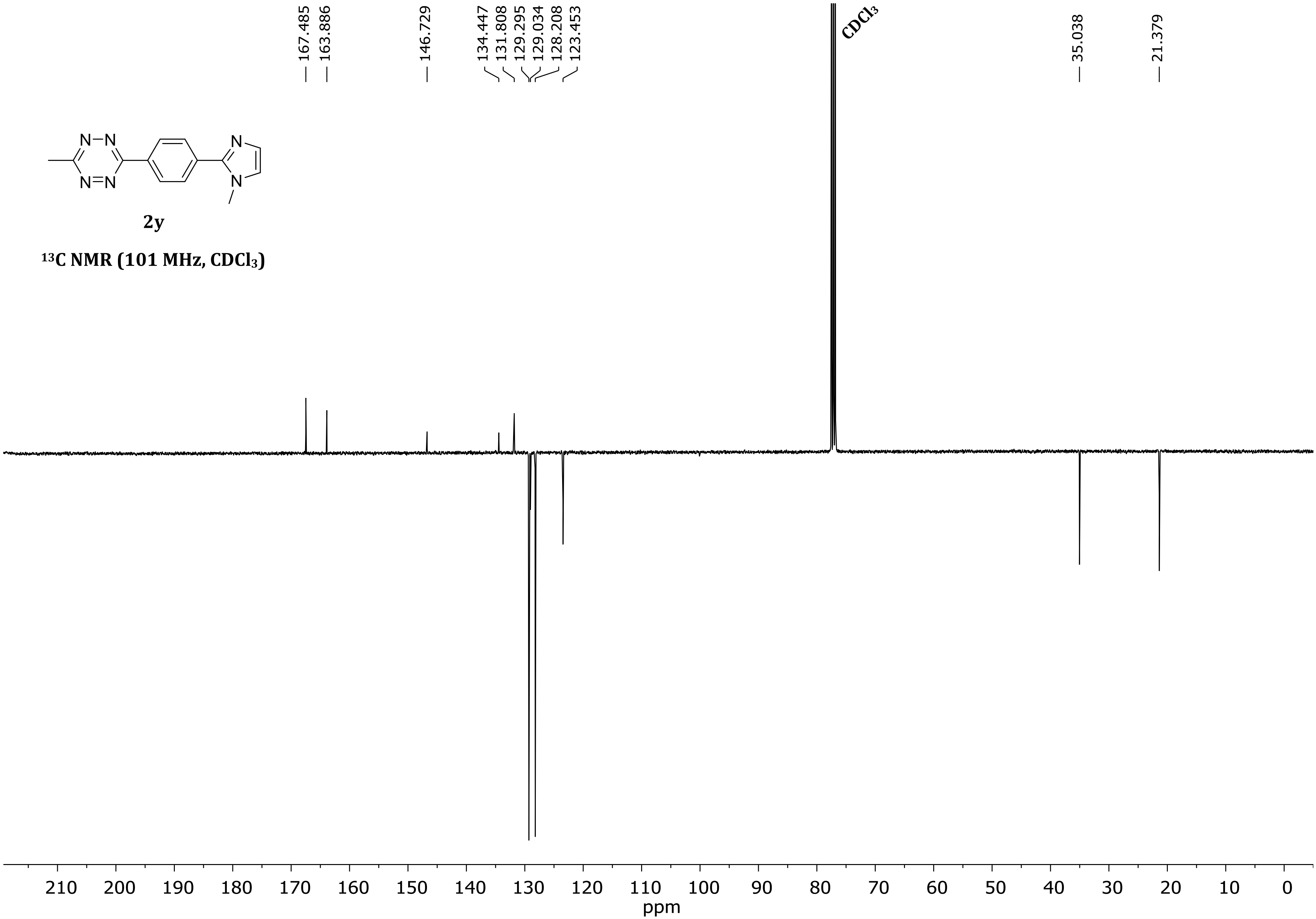




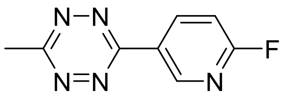
8.759
8.753
8.748
8.736
8.731
8.725
8.344
8.338
8.333
8.321
8.316
8.310
7.892
3.118
2x
1H NMR (400 MHz, CDCl3)
CDCl3
H2O
2.18
2.17
1.92
3.01
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm


167.467
163.566
142.781
136.471
130.791
129.288
119.542
CDCl3
21.369
2x
13C NMR (101 MHz, CDCl3)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm


8.710
8.705
8.700
8.688
8.683
8.678
7.924
7.919
7.915
7.903
7.898
7.893
7.201
7.198
7.050
7.047
3.864
3.118
2y
1H NMR (400 MHz, CDCl3)
CDCl3
2.03
2.03
0.96
1.00
2.85
2.98
10.0
9.5
9.0
8.5
8.0
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
0.0
-0.5
ppm

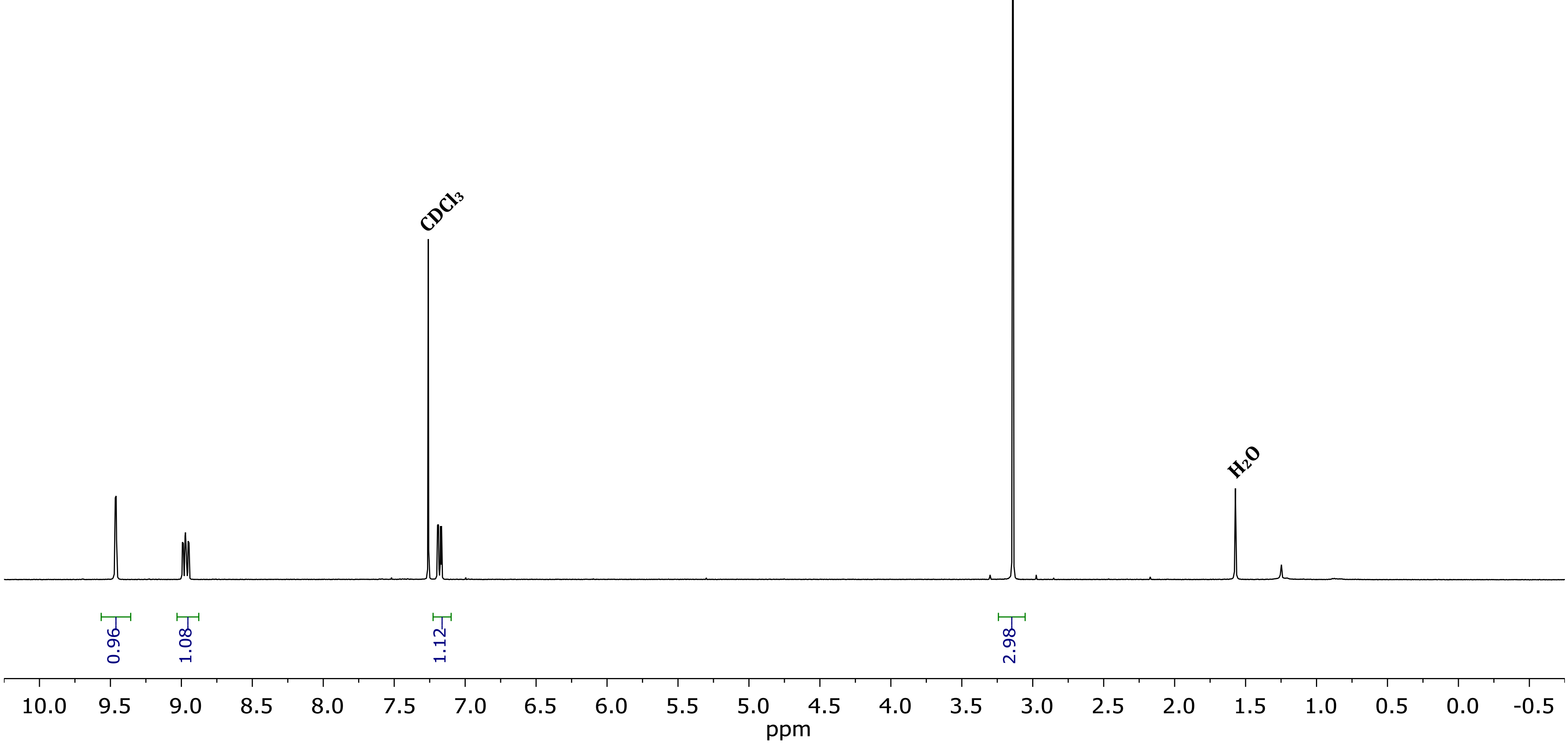


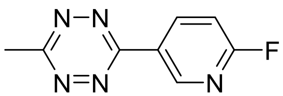
167.485
163.886
146.729
134.447
131.808
129.295
129.034
128.208
123.453
CDCl3
35.038
21.379
2y
13C NMR (101 MHz, CDCl3)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm



2z

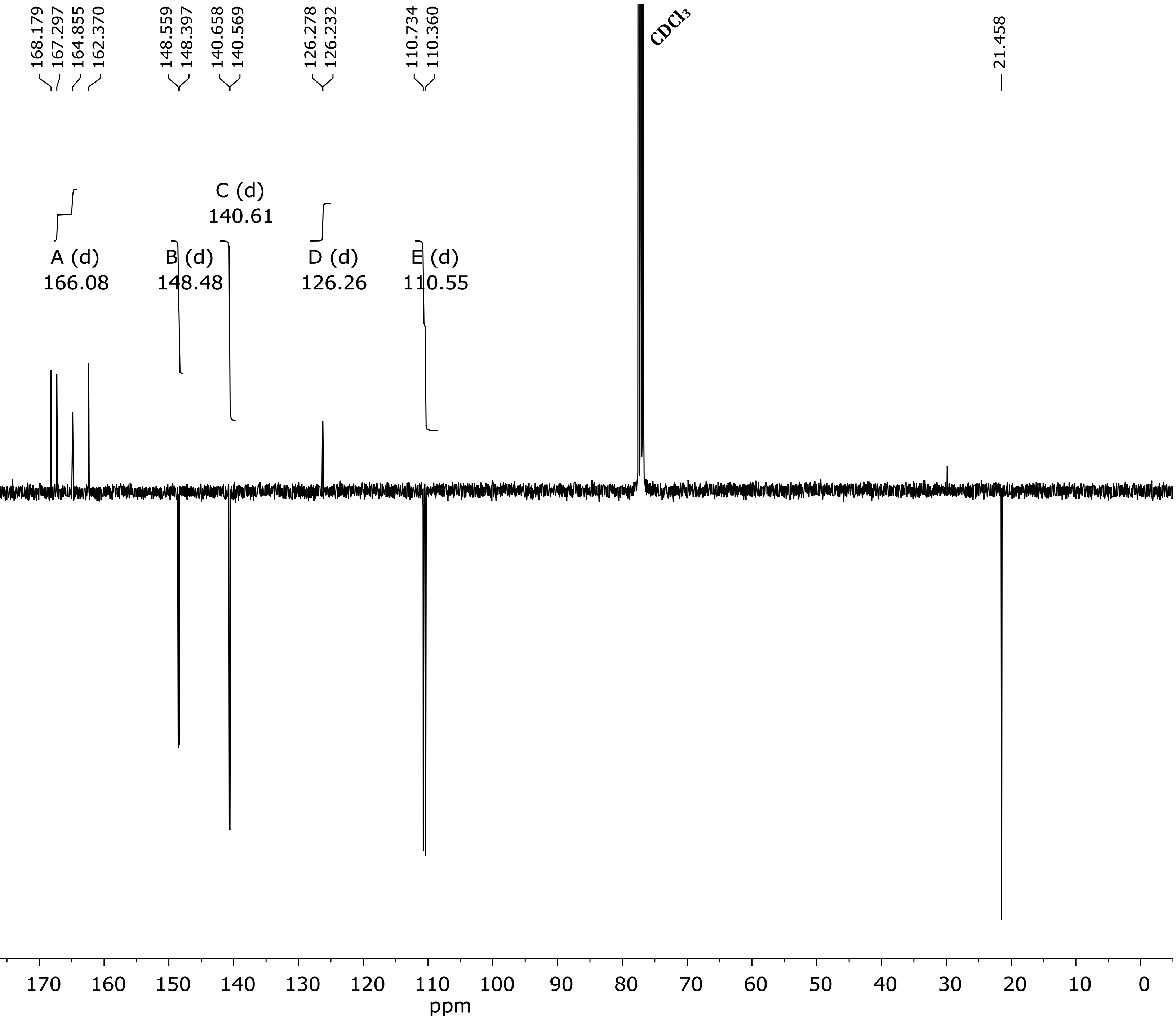
^{1}H NMR (400 MHz, $CDCl_3$)

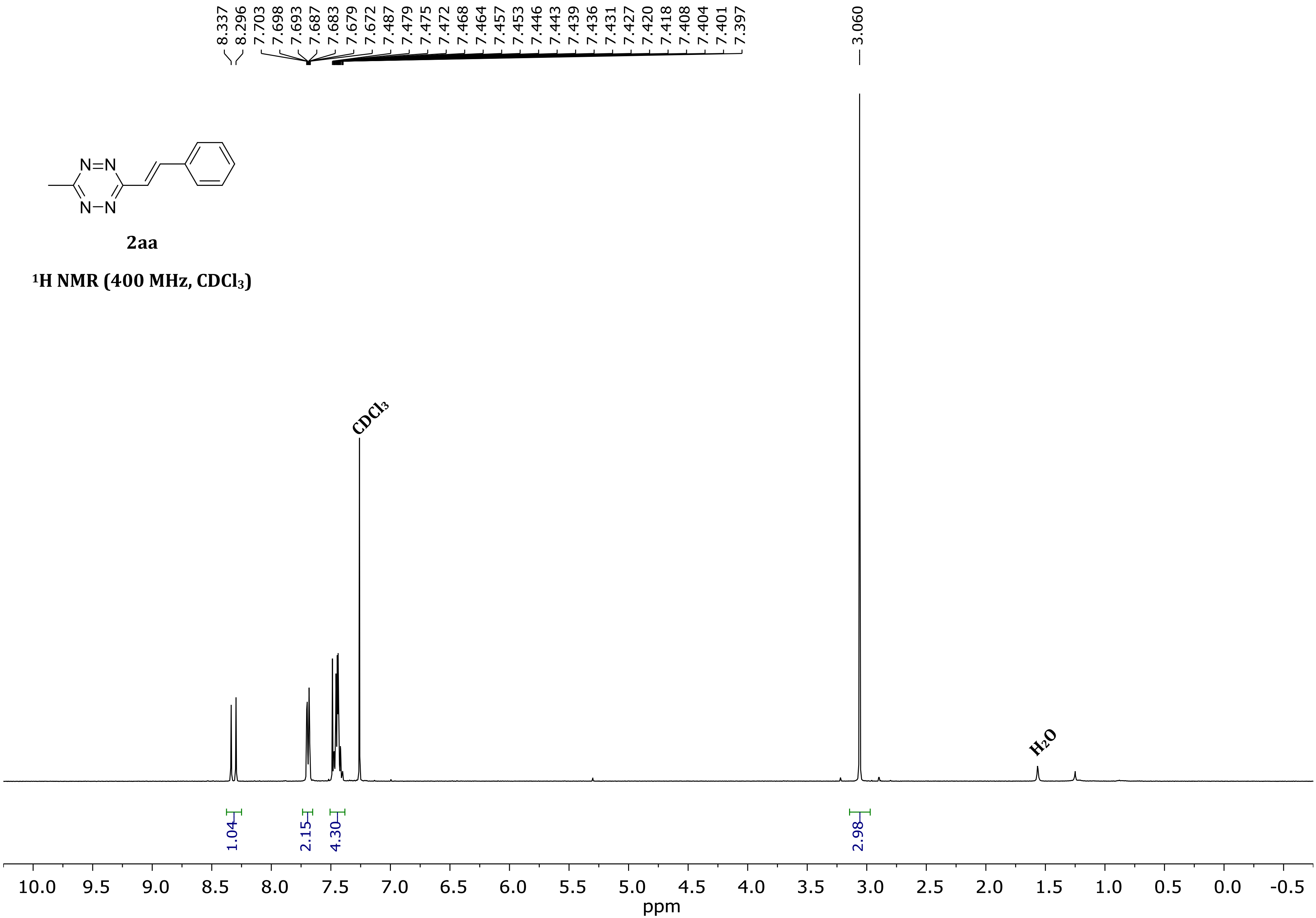


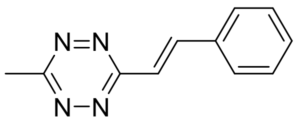


2z

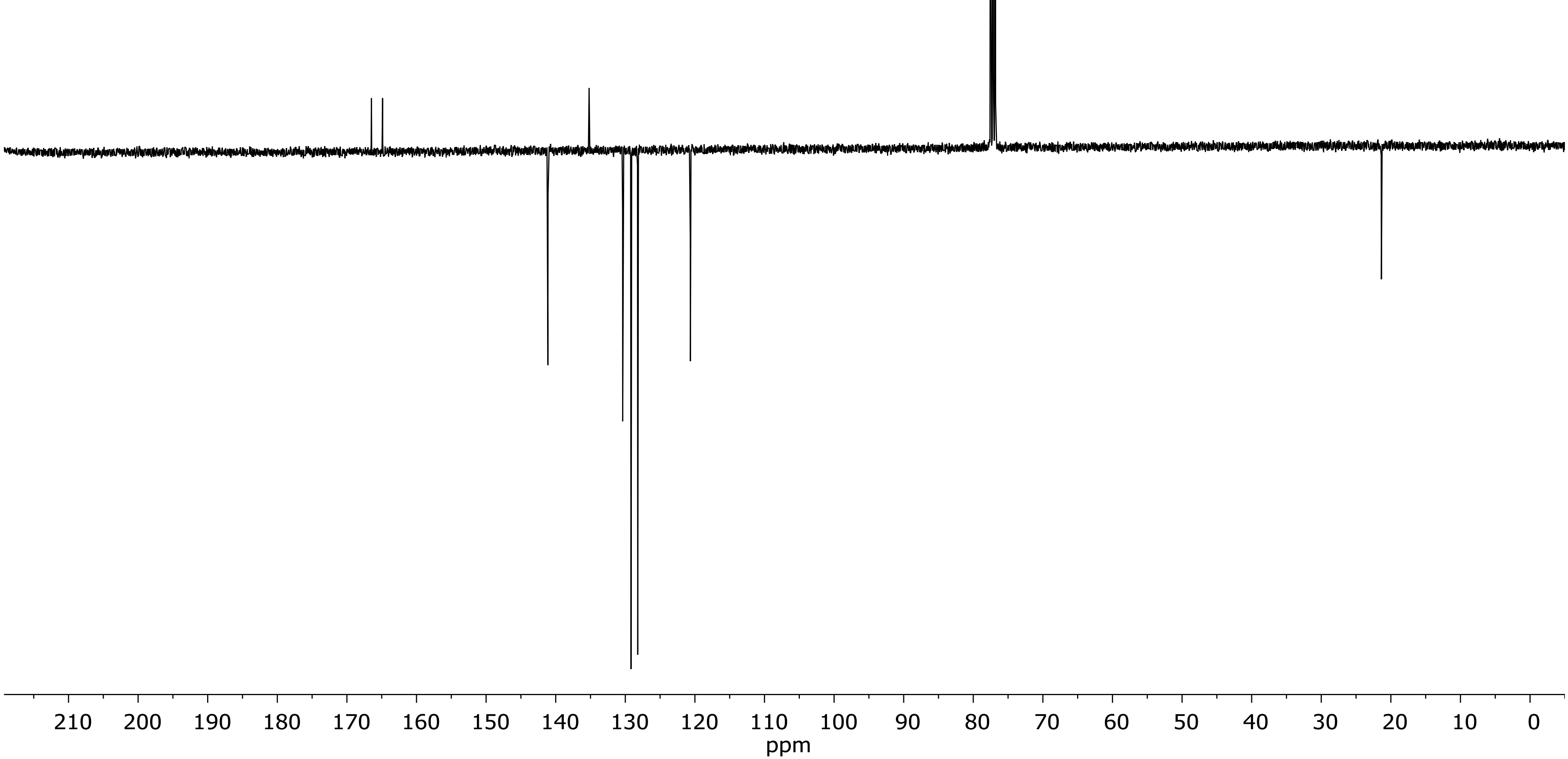
^{13}C NMR (101 MHz, $CDCl_3$)




8.337
8.296
7.703
7.698
7.693
7.687
7.683
7.679
7.672
7.487
7.479
7.475
7.472
7.468
7.464
7.457
7.453
7.446
7.443
7.439
7.436
7.431
7.427
7.420
7.418
7.408
7.404
7.401
7.397
3.060
N=N
N−N
2aa
^{1}H NMR (400 MHz, $CDCl_3$)
$CDCl_3$
H_2O
1.04
2.15
4.30
2.98
10.0 9.5 9.0 8.5 8.0 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 0.0 -0.5
ppm



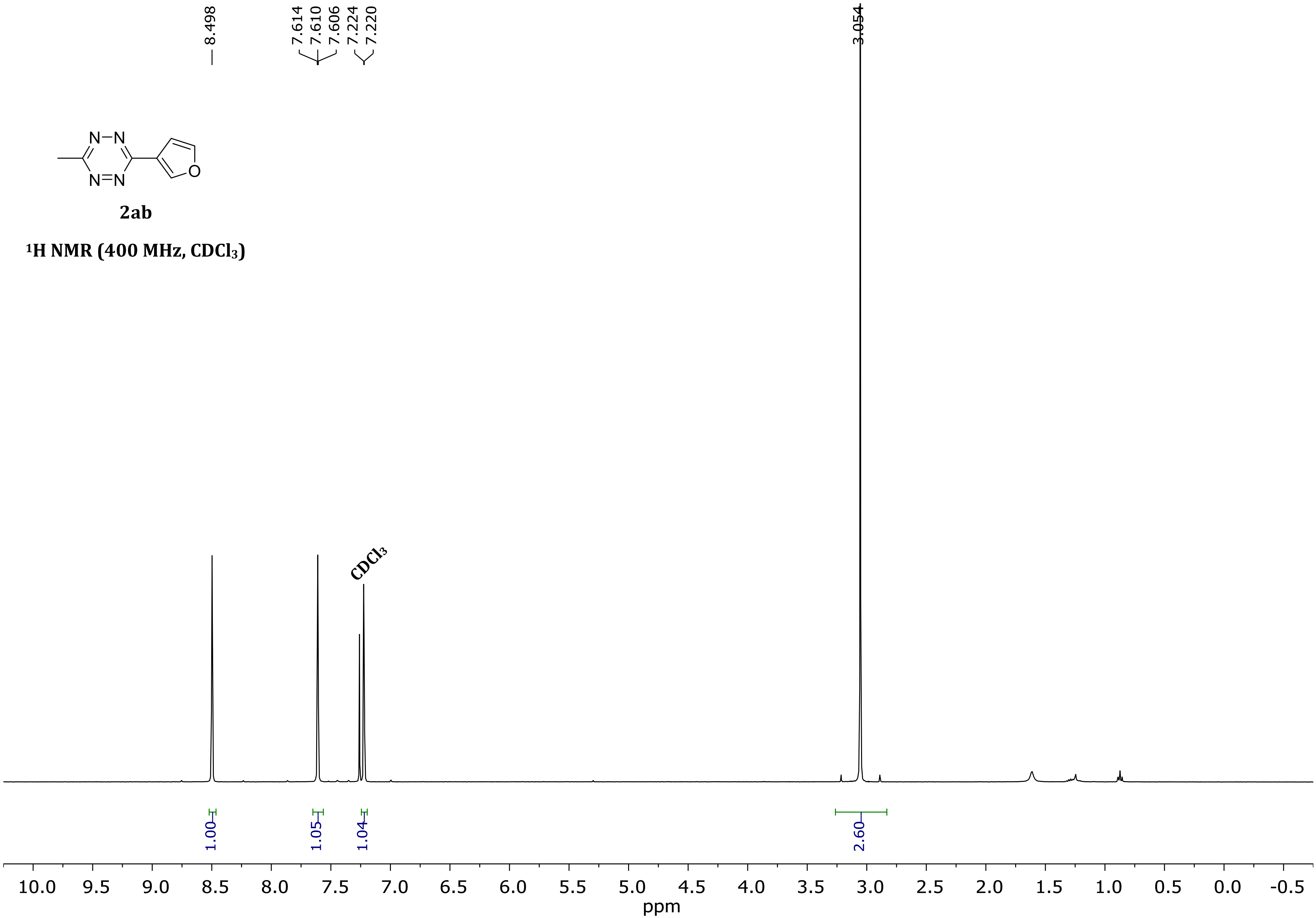
2aa

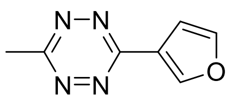


^{13}C NMR (101 MHz, $CDCl_3$)

2ab

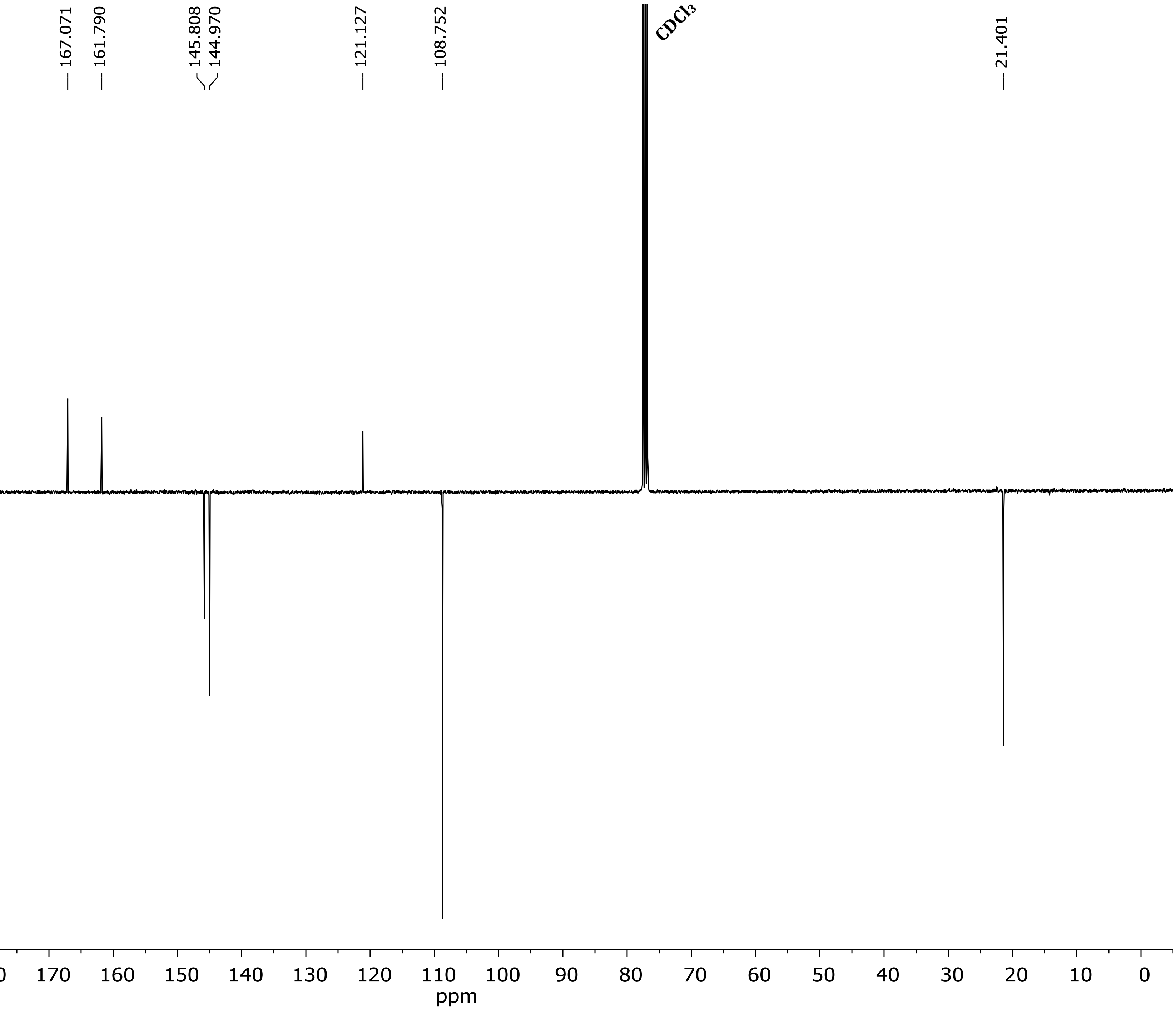
^{1}H NMR (400 MHz, $CDCl_3$)

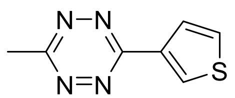




2ab

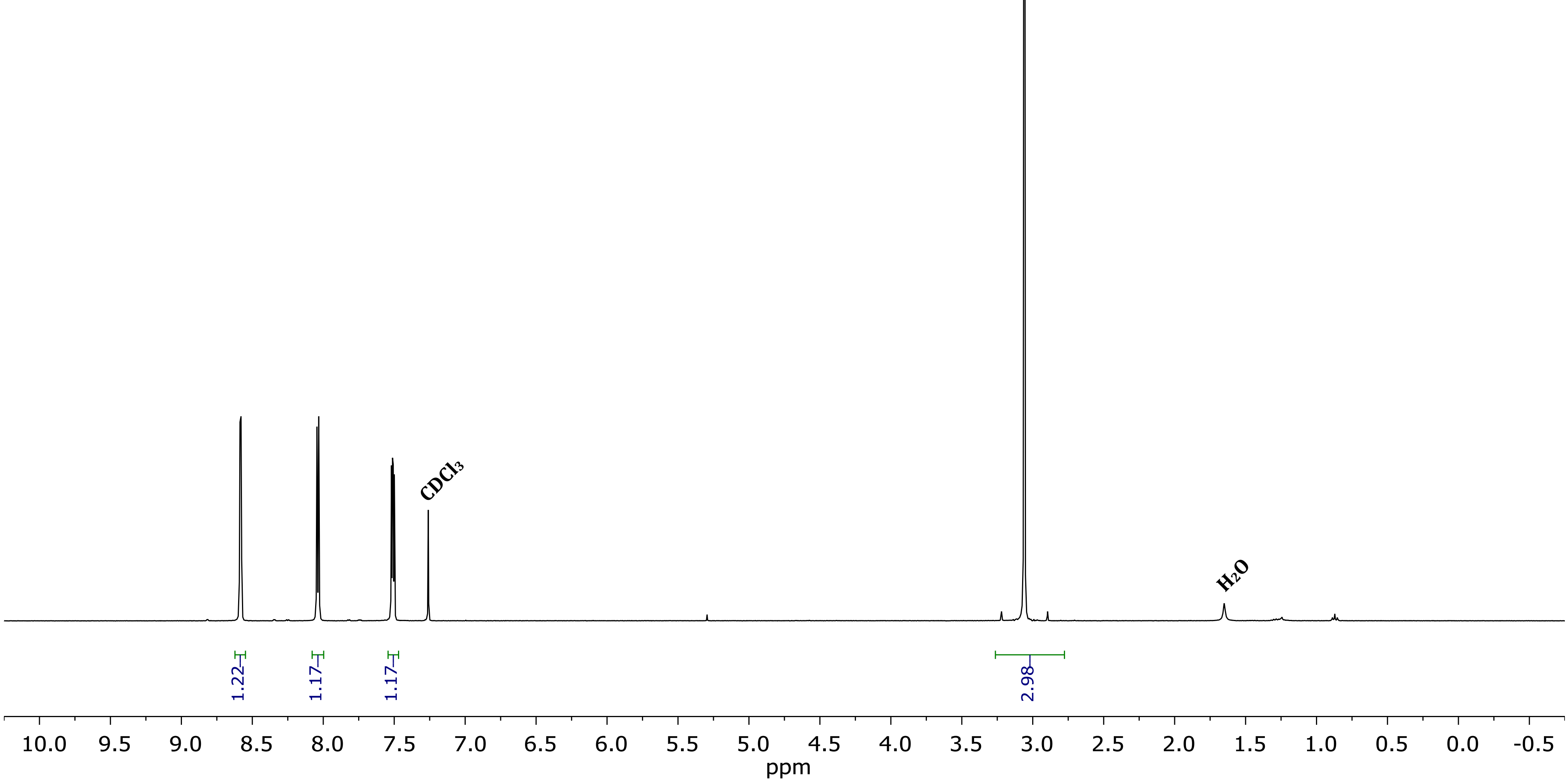
^{13}C NMR (101 MHz, CDCl$_3$)

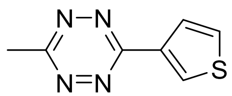




2ac

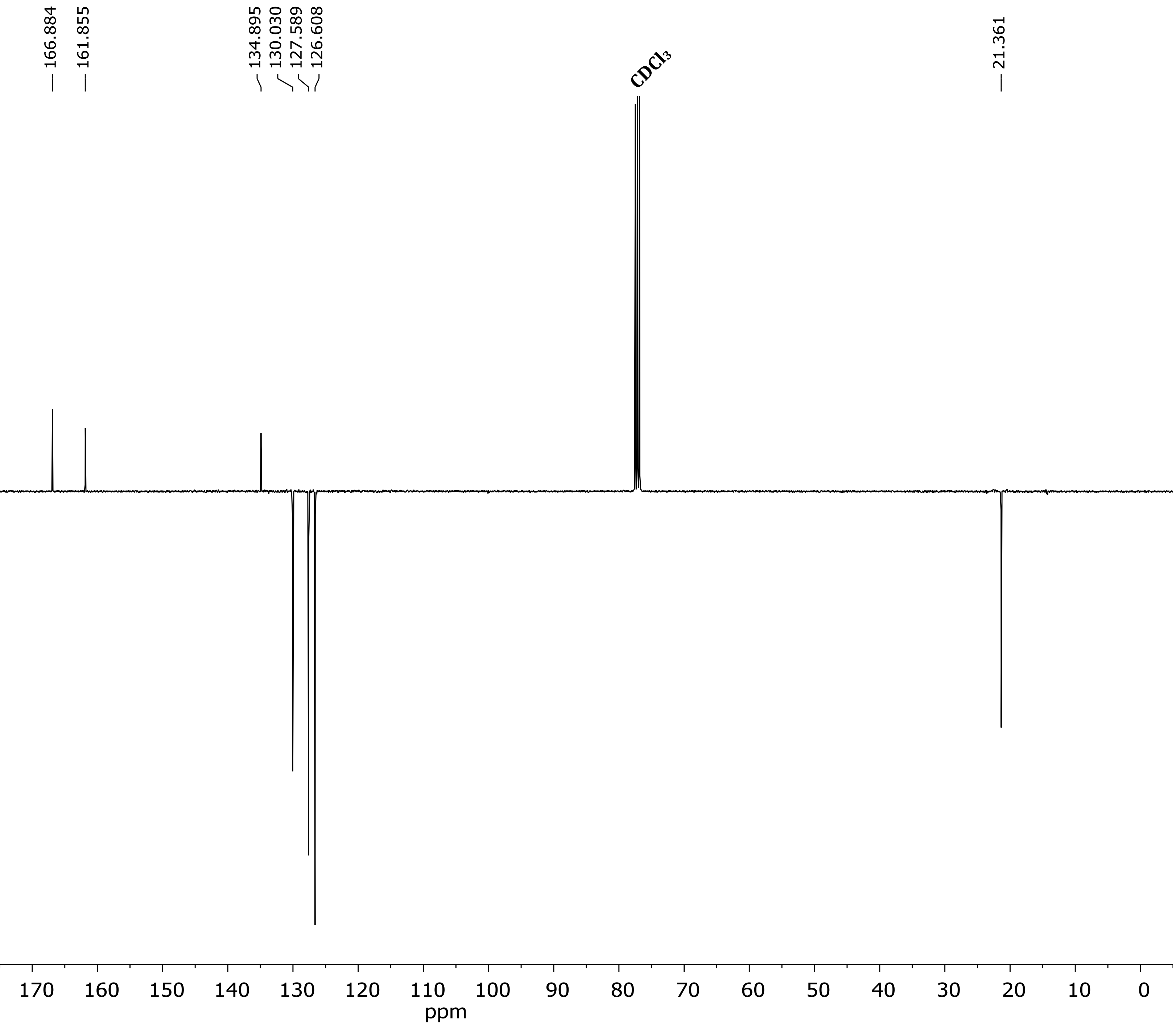
^{1}H NMR (400 MHz, $CDCl_3$)

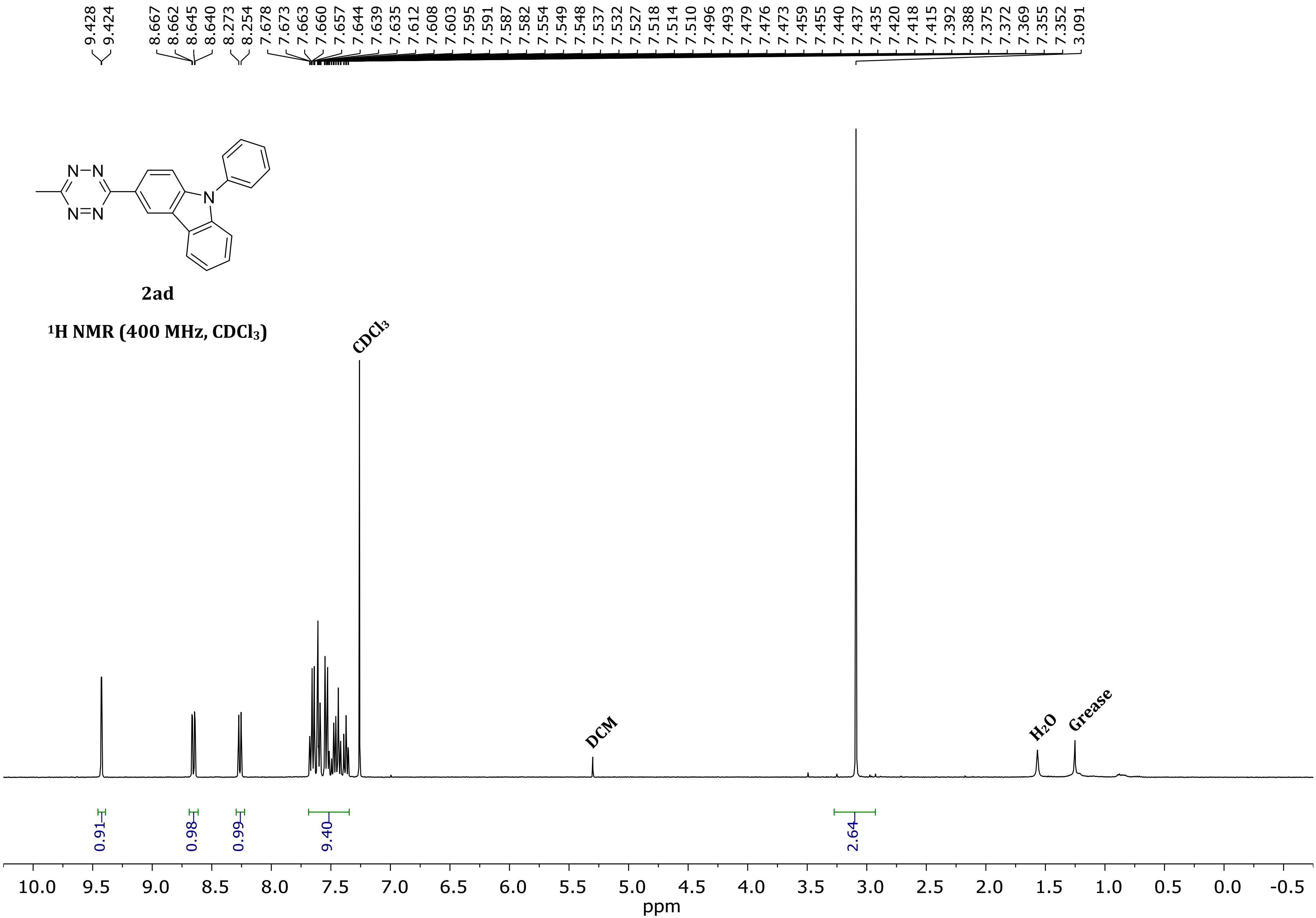




2ac

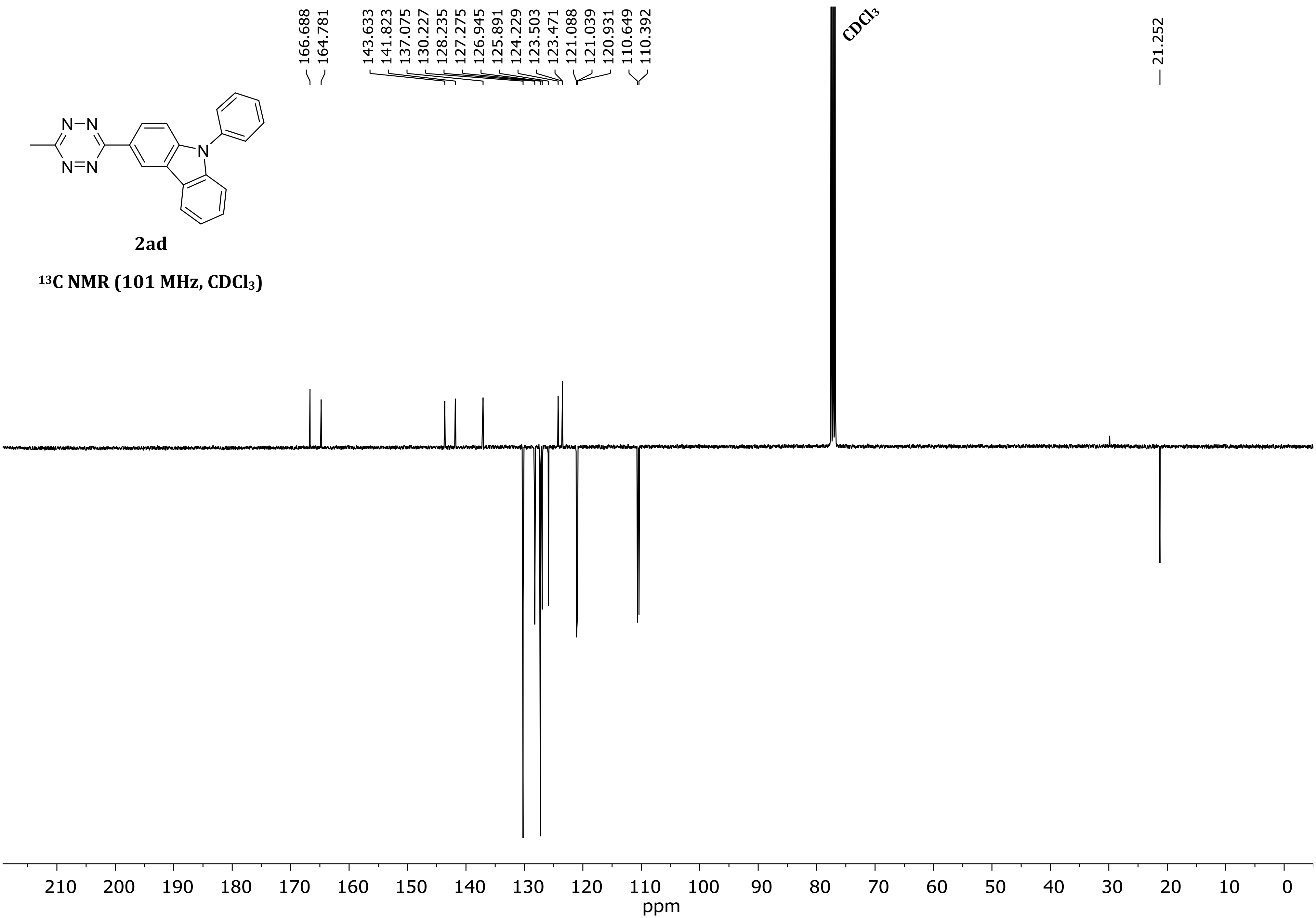
^{13}C NMR (101 MHz, $CDCl_3$)





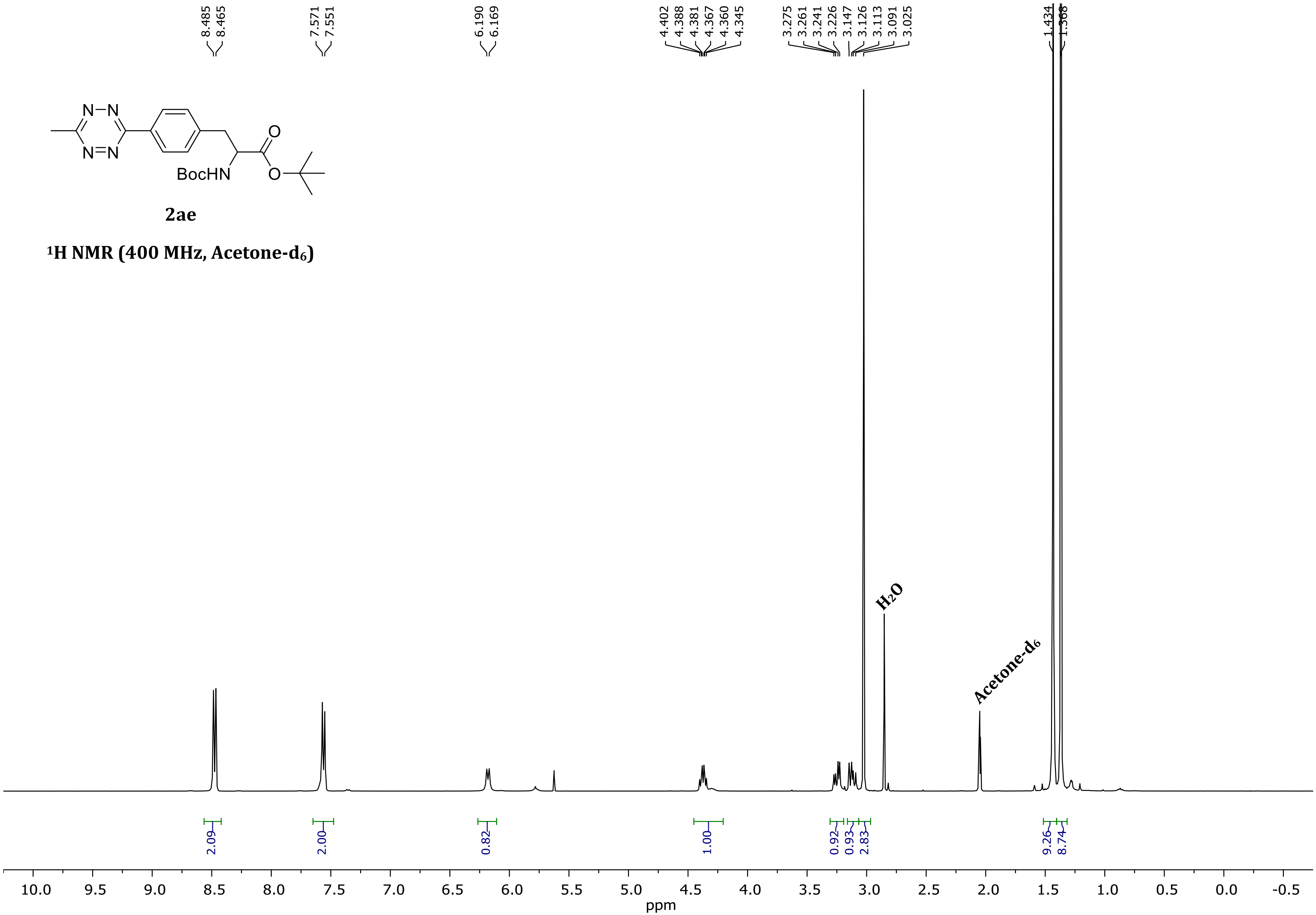
2ad

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



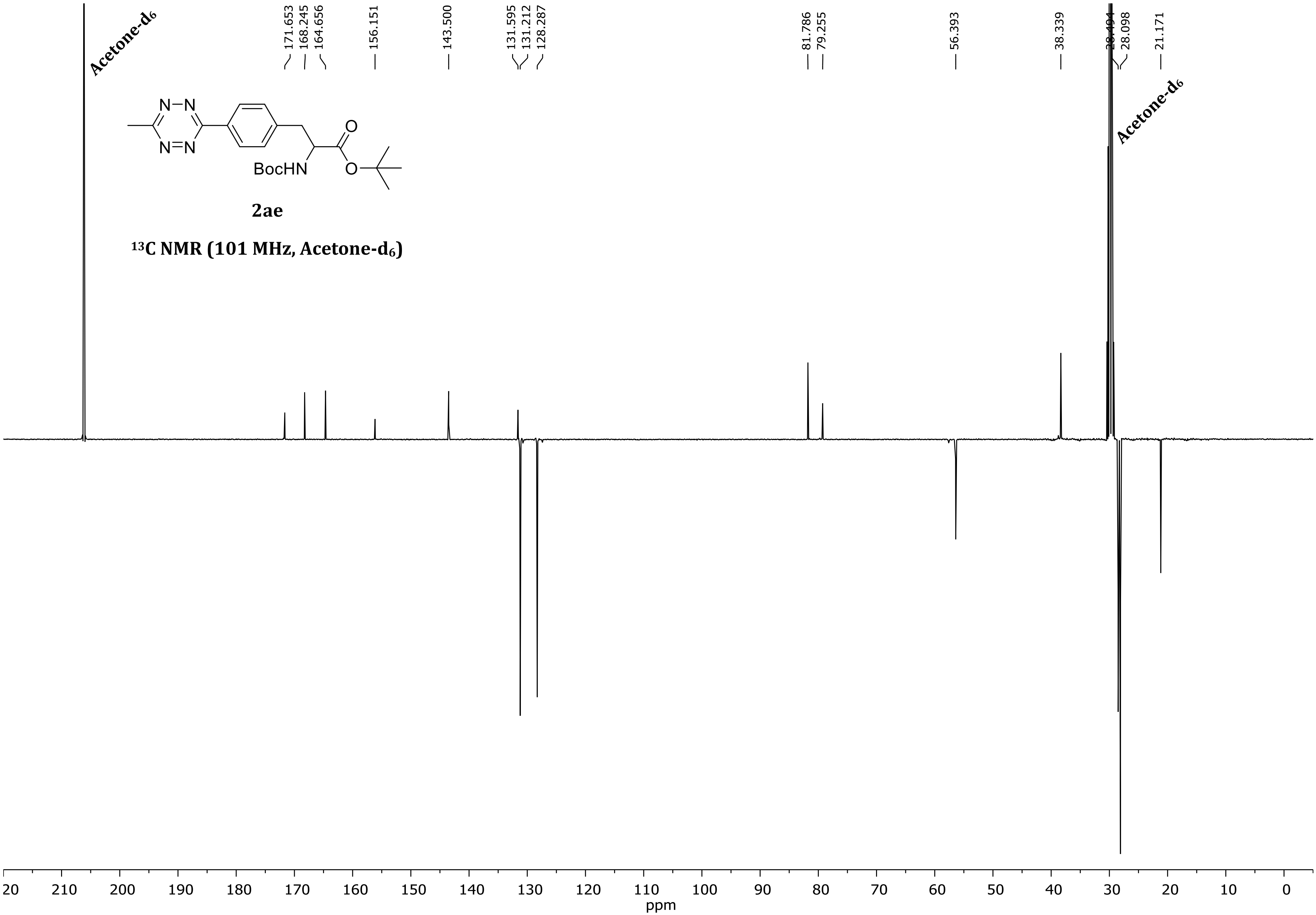
2ad

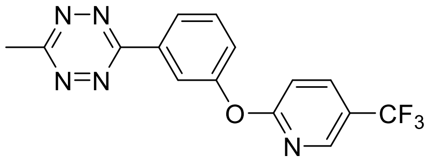
^{13}C NMR (101 MHz, $CDCl_3$)



2ae

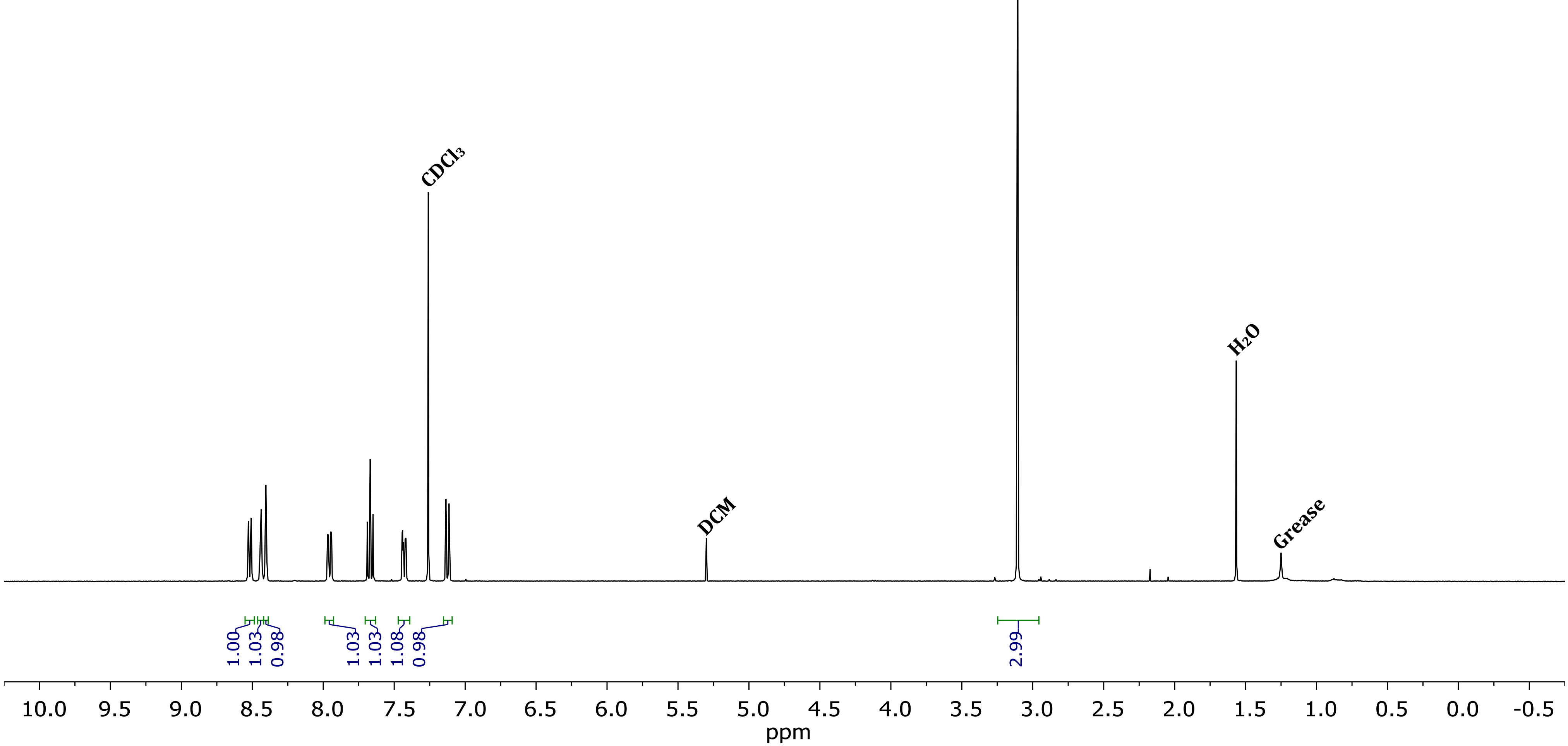
^{1}H NMR (400 MHz, Acetone-d_6)

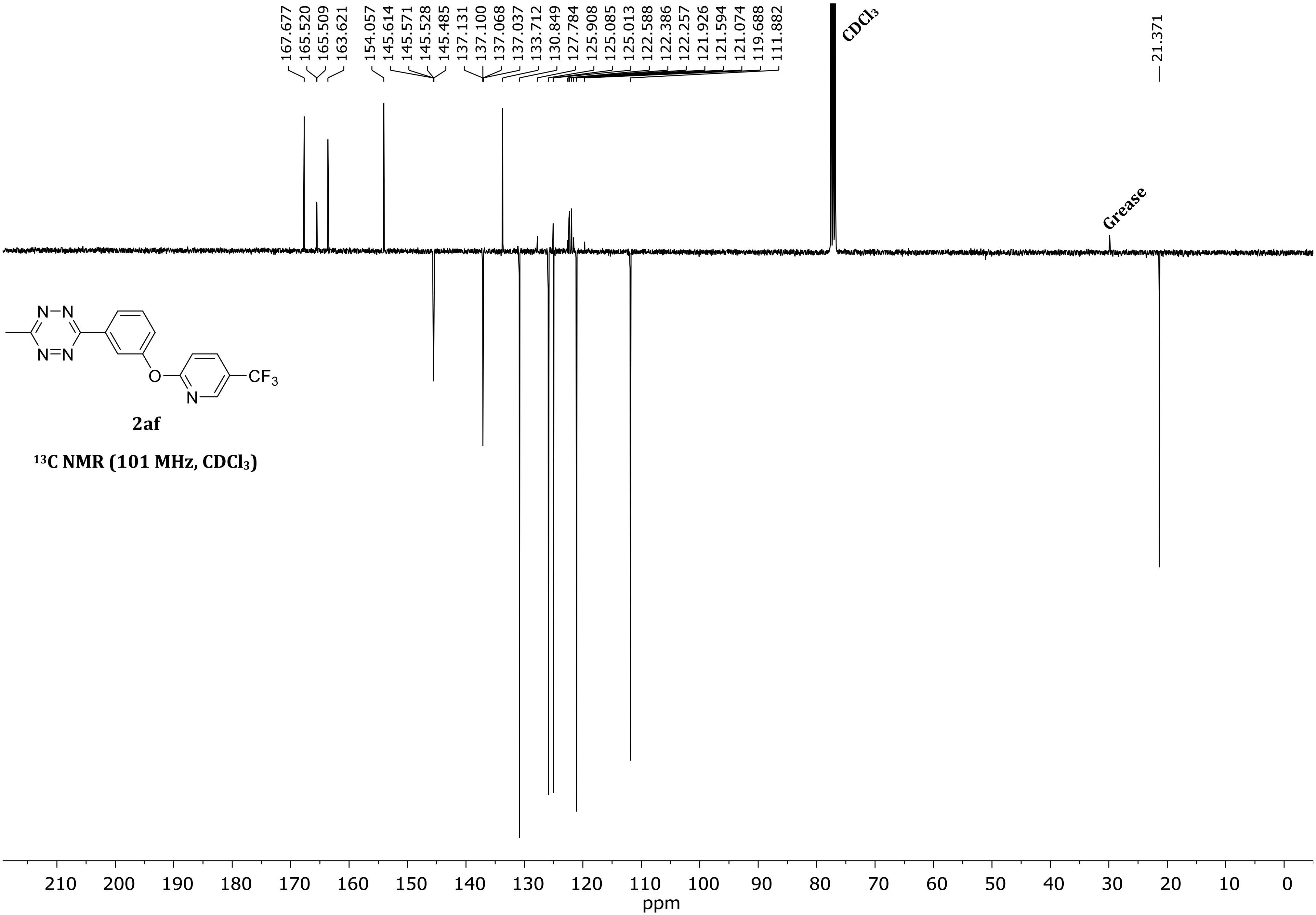

Acetone-d6
171.653
168.245
164.656
156.151
143.500
131.595
131.212
128.287
81.786
79.255
56.393
38.339
28.494
28.098
21.171
Acetone-d6
BocHN
2ae
13C NMR (101 MHz, Acetone-d6)
ppm



2af

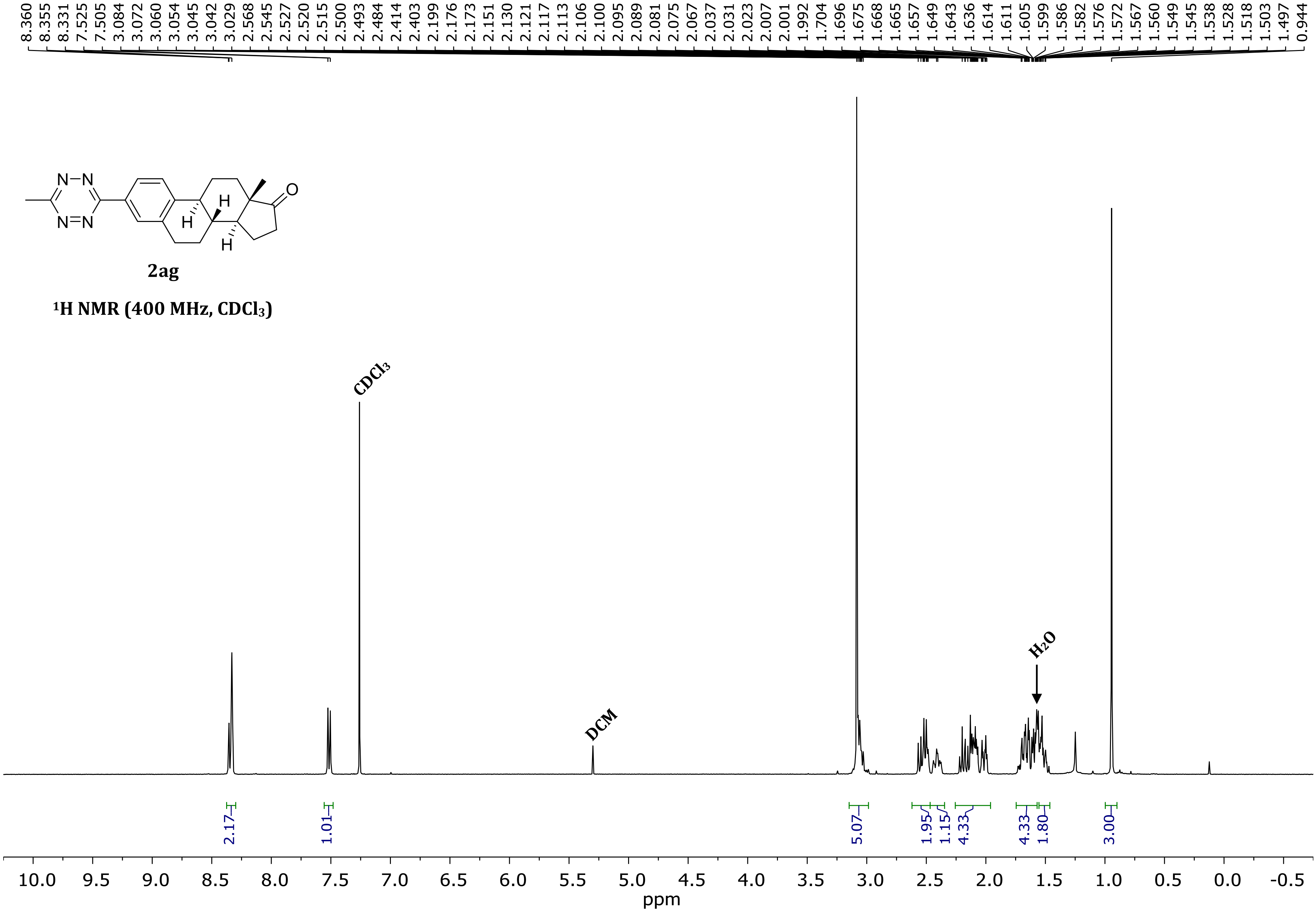
^{1}H NMR (400 MHz, $CDCl_3$)

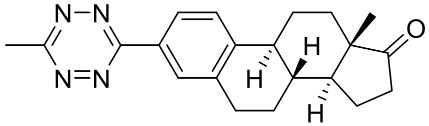




2af

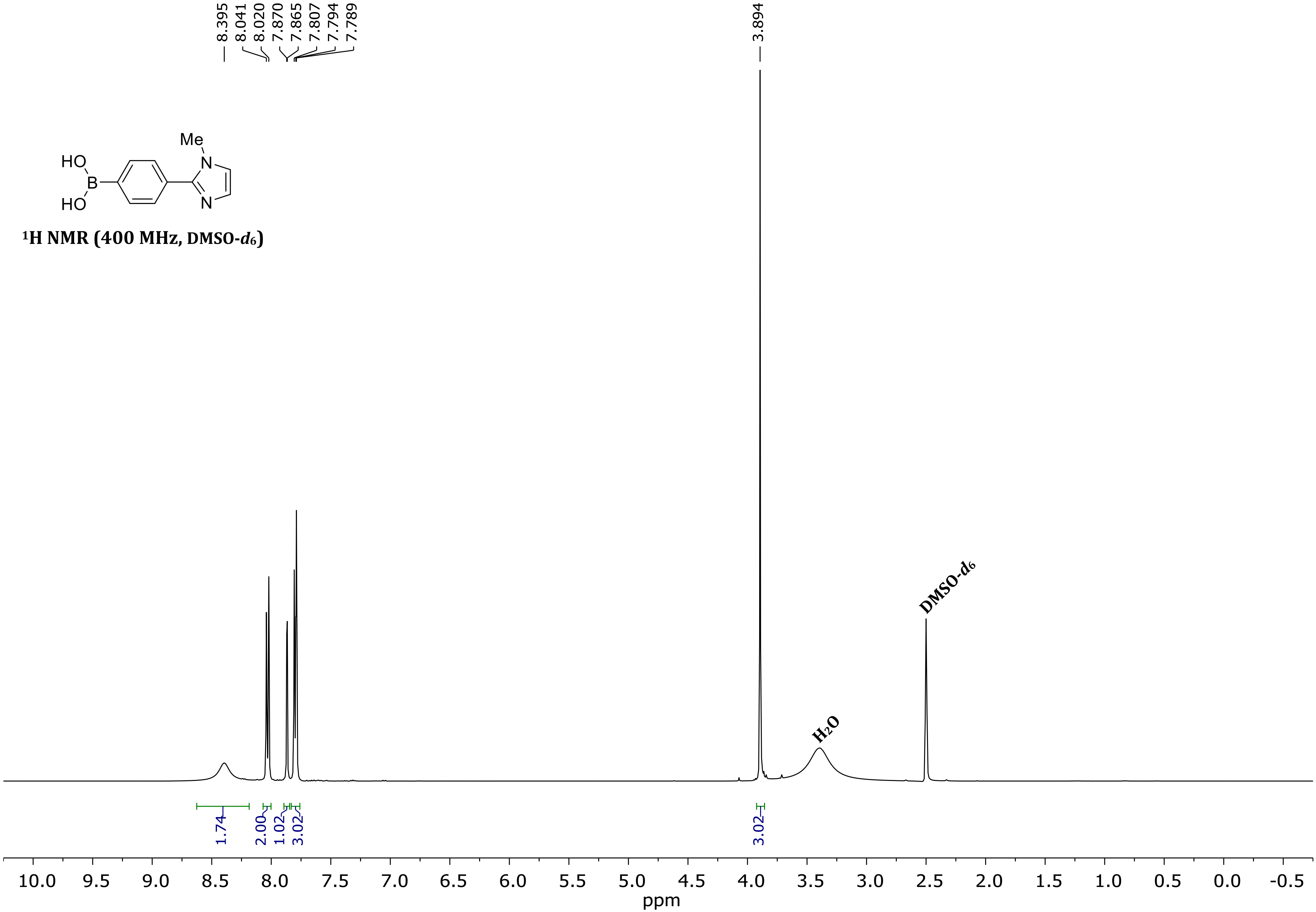
^{13}C NMR (101 MHz, $CDCl_3$)


2ag
¹H NMR (400 MHz, CDCl₃)
CDCl₃
DCM
H₂O
ppm

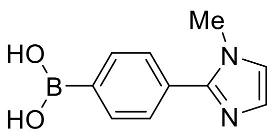


2ag

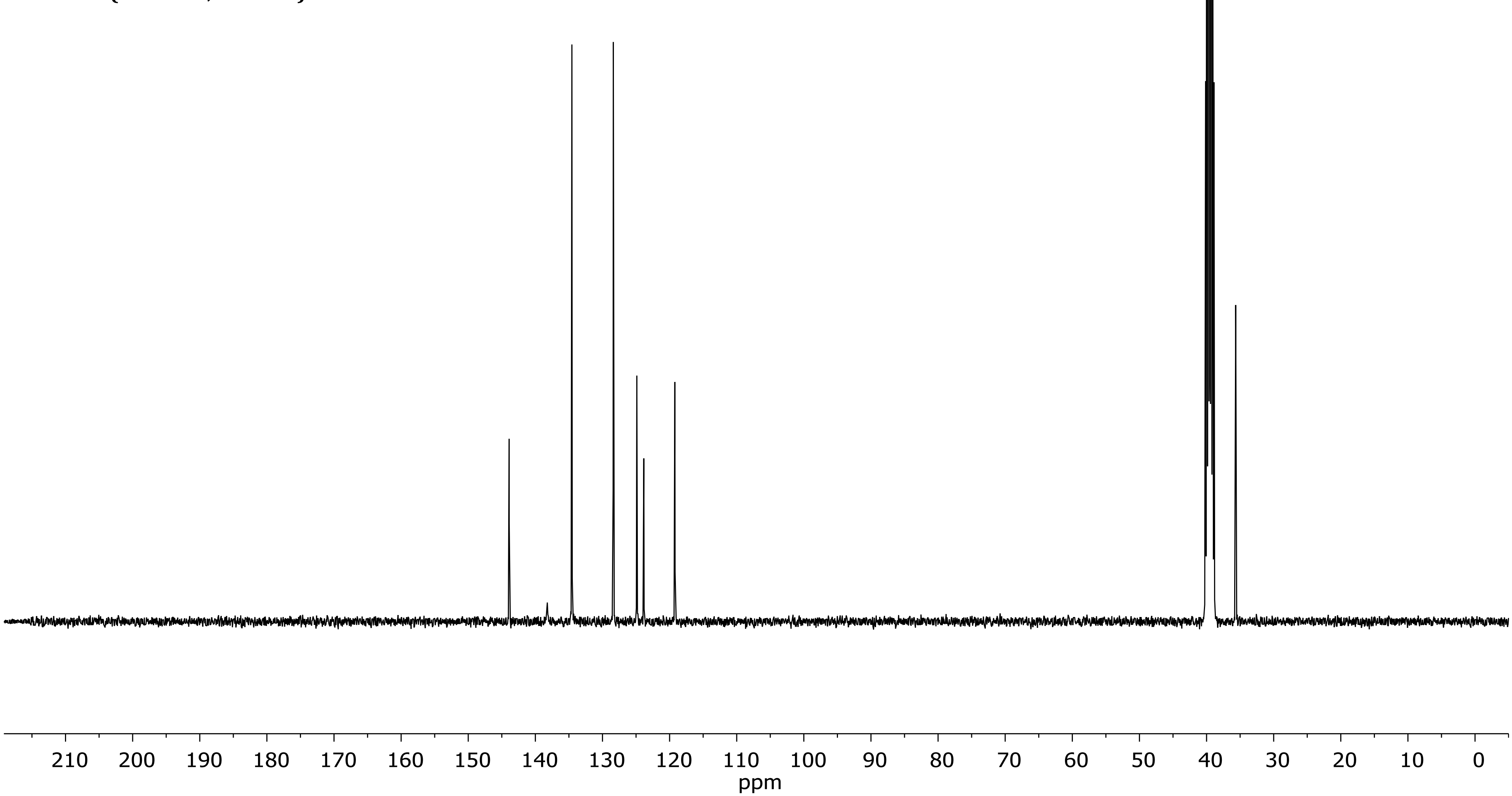
^{13}C NMR (101 MHz, $CDCl_3$)

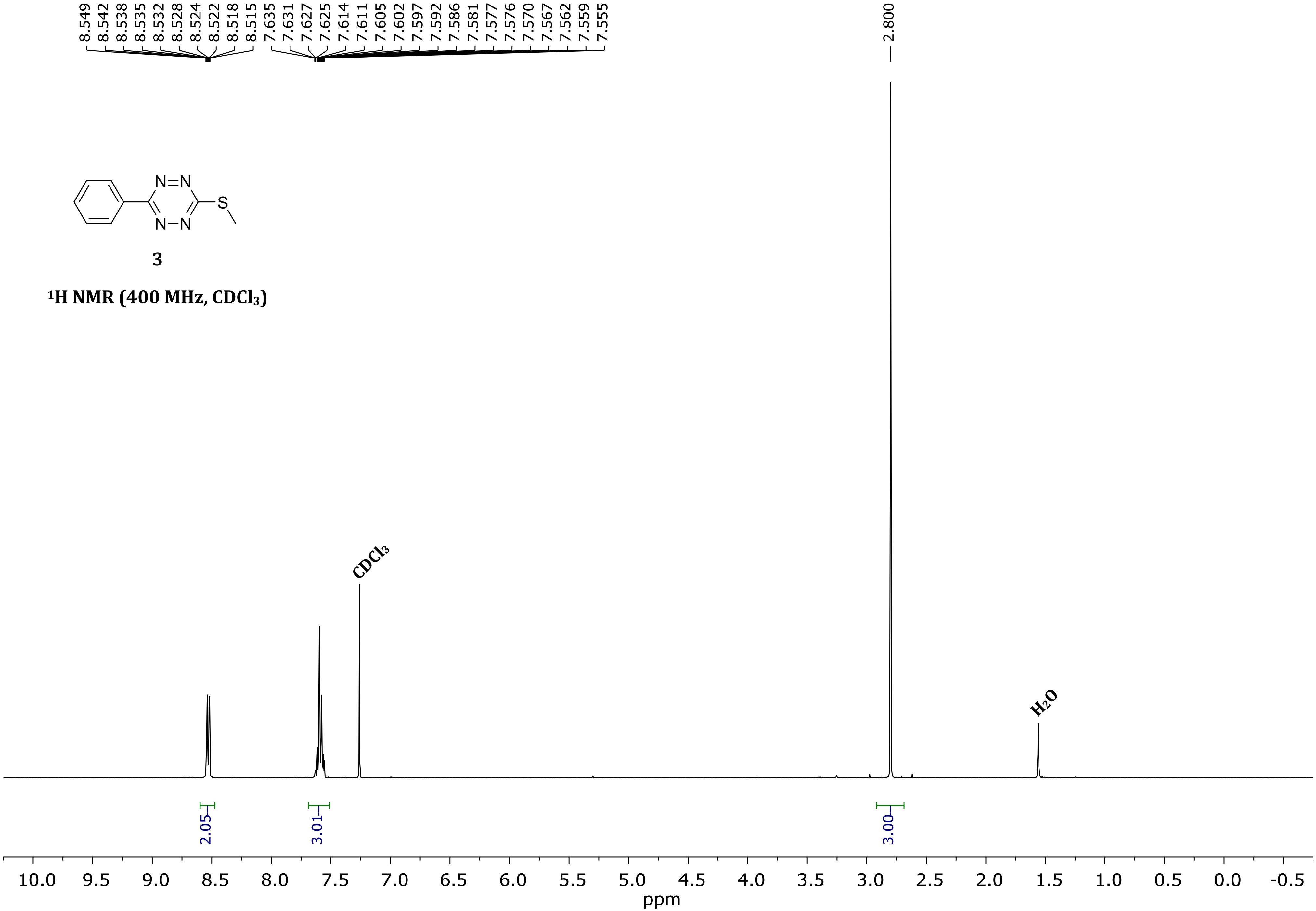


^{1}H NMR (400 MHz, DMSO-d_6)



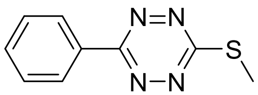
^{13}C NMR (101 MHz, DMSO-d_6)





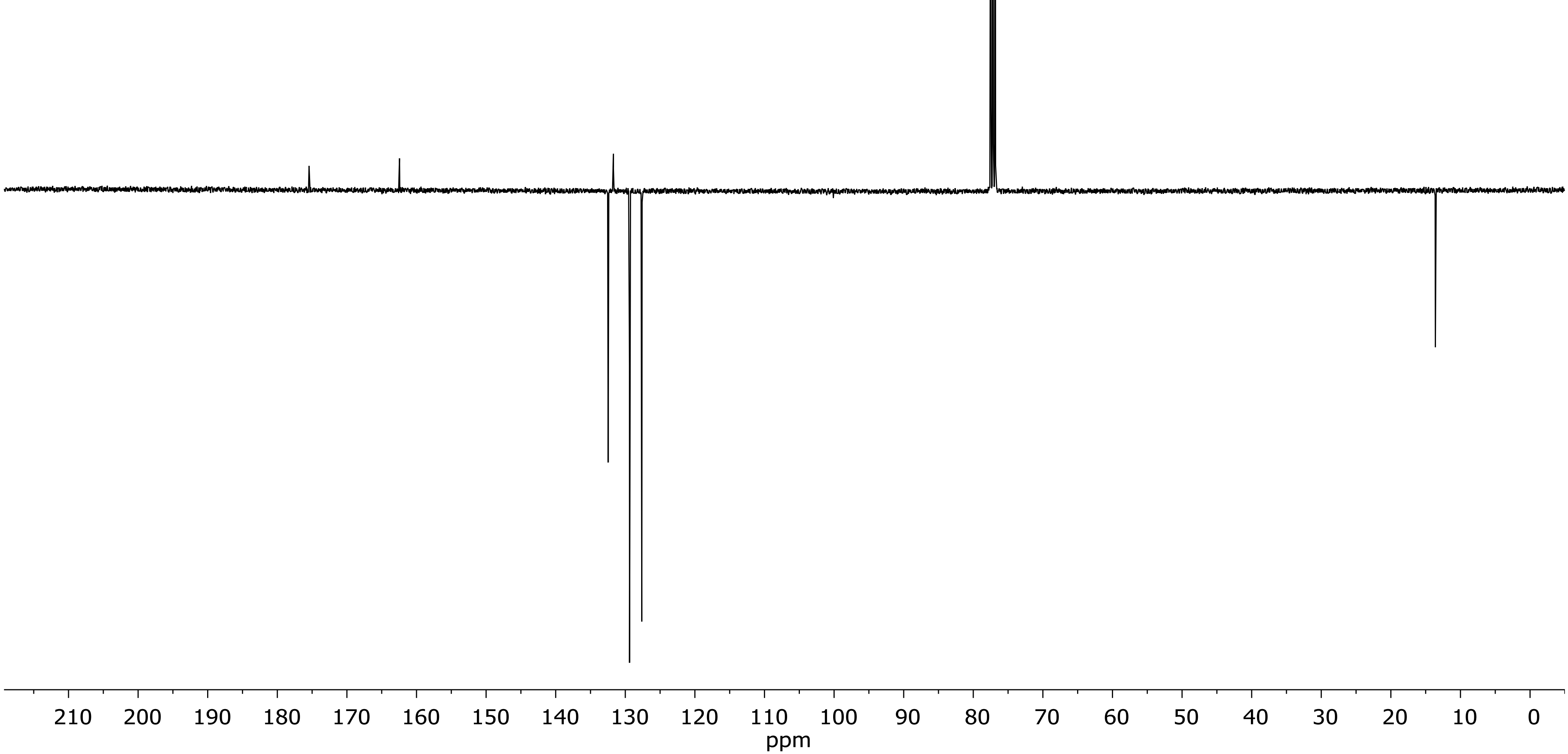
3

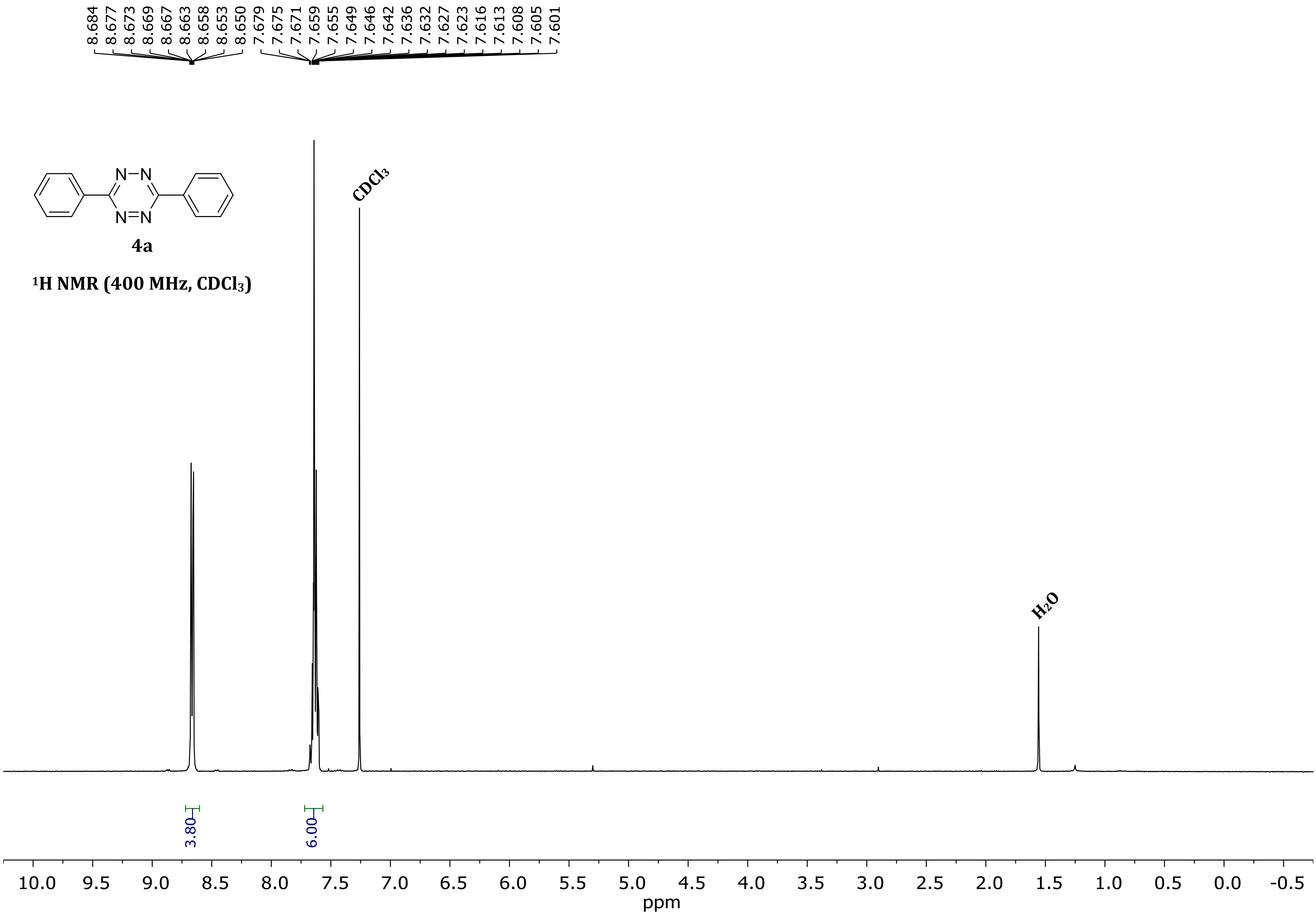
^{1}H NMR (400 MHz, $CDCl_3$)

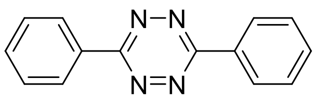


3

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

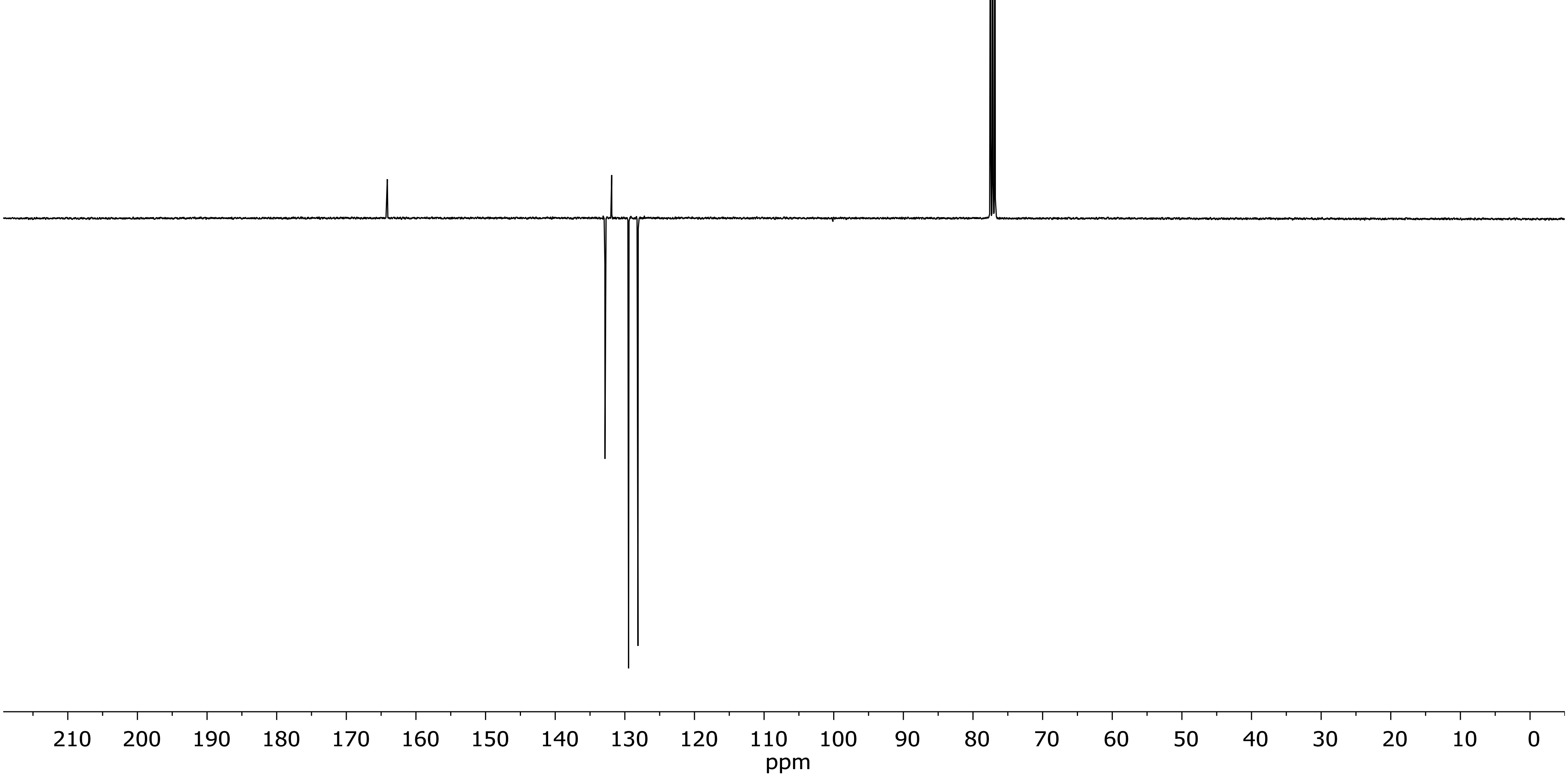


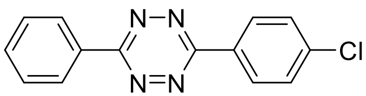

8.684
8.677
8.673
8.669
8.667
8.663
8.658
8.653
8.650
7.679
7.675
7.671
7.659
7.655
7.649
7.646
7.642
7.636
7.632
7.627
7.623
7.616
7.613
7.608
7.605
7.601
4a
1H NMR (400 MHz, CDCl3)
CDCl3
H2O
3.80
6.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm



4a

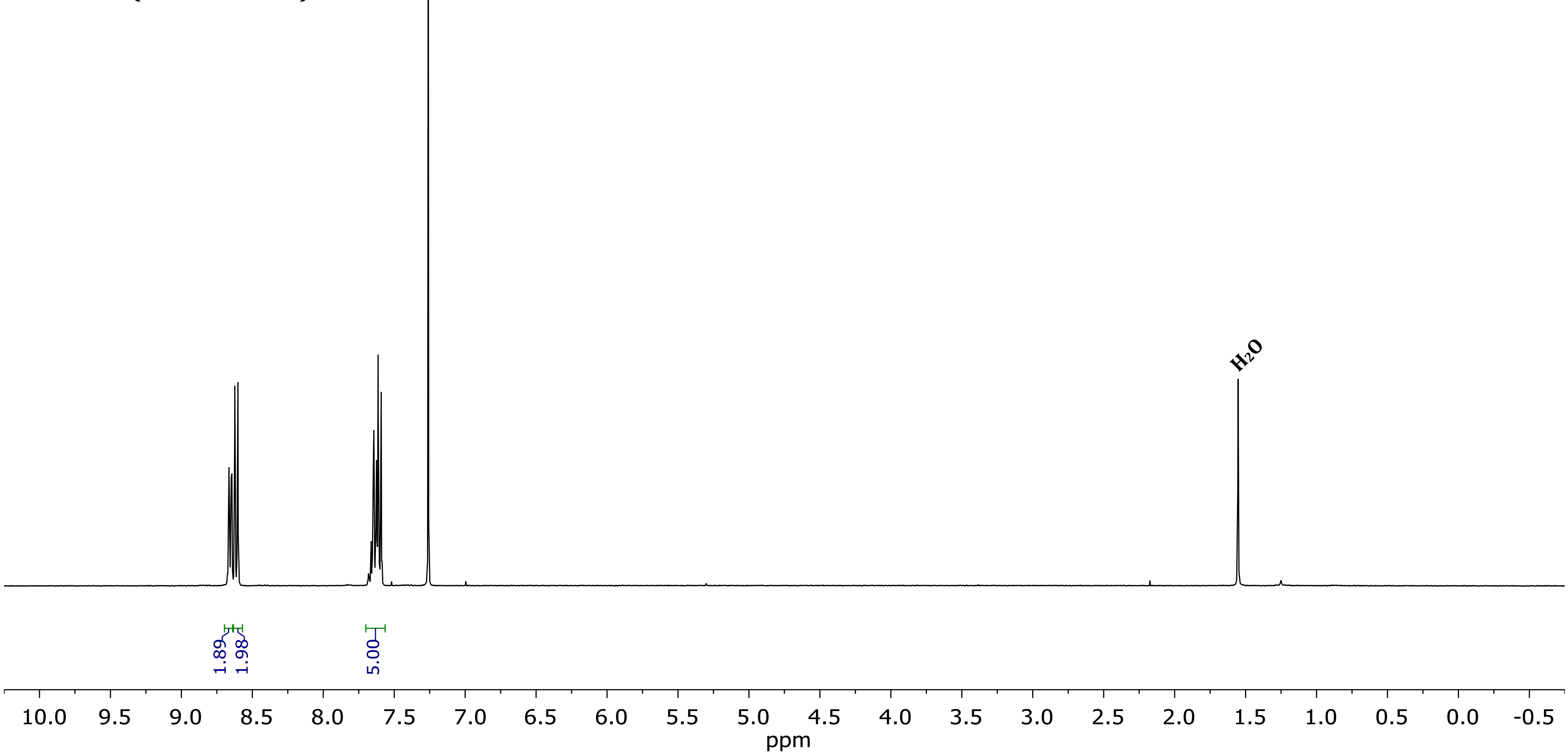
^{13}C NMR (101 MHz, $CDCl_3$)

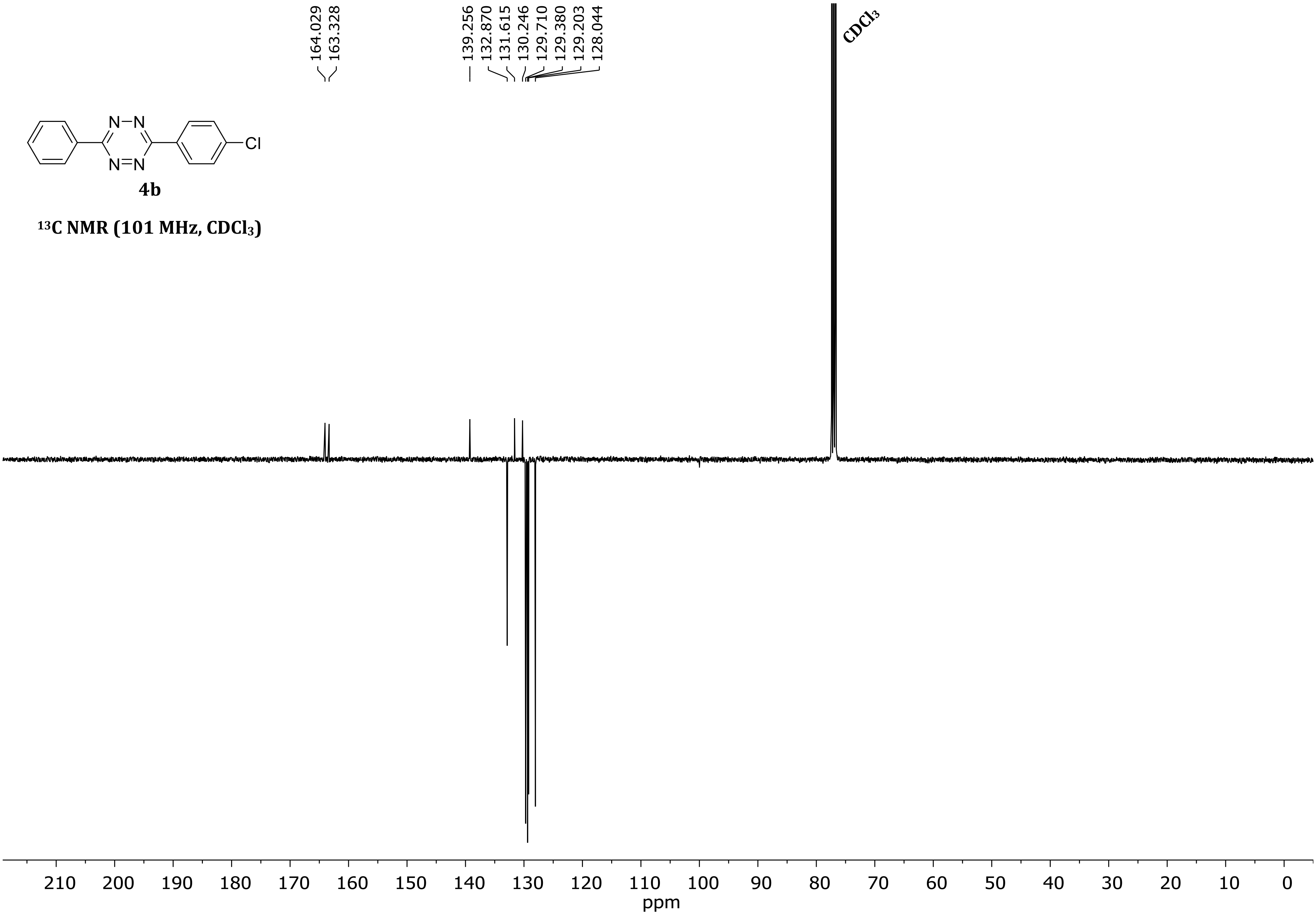




4b

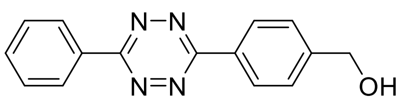
^{1}H NMR (400 MHz, $CDCl_3$)





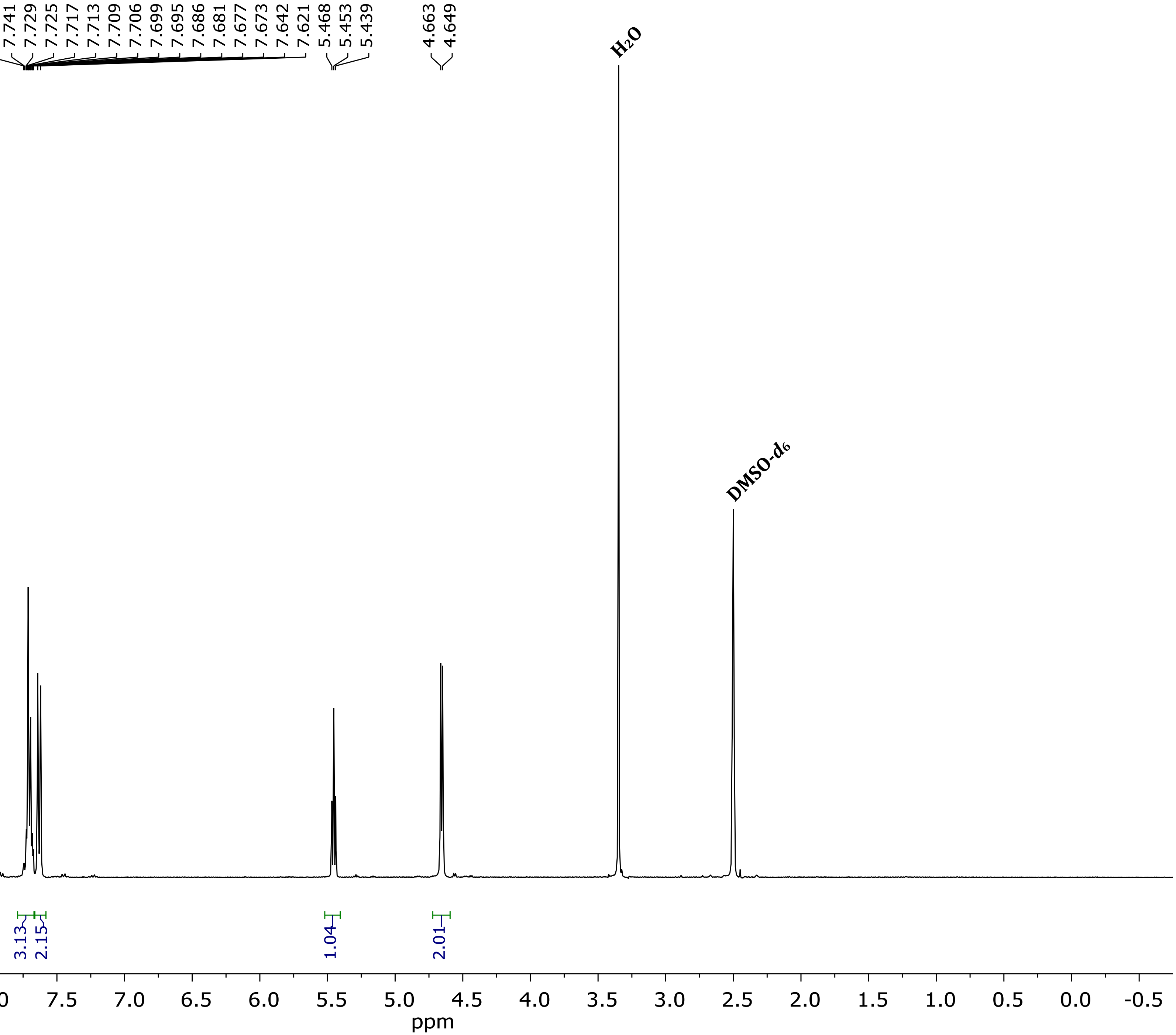
4b

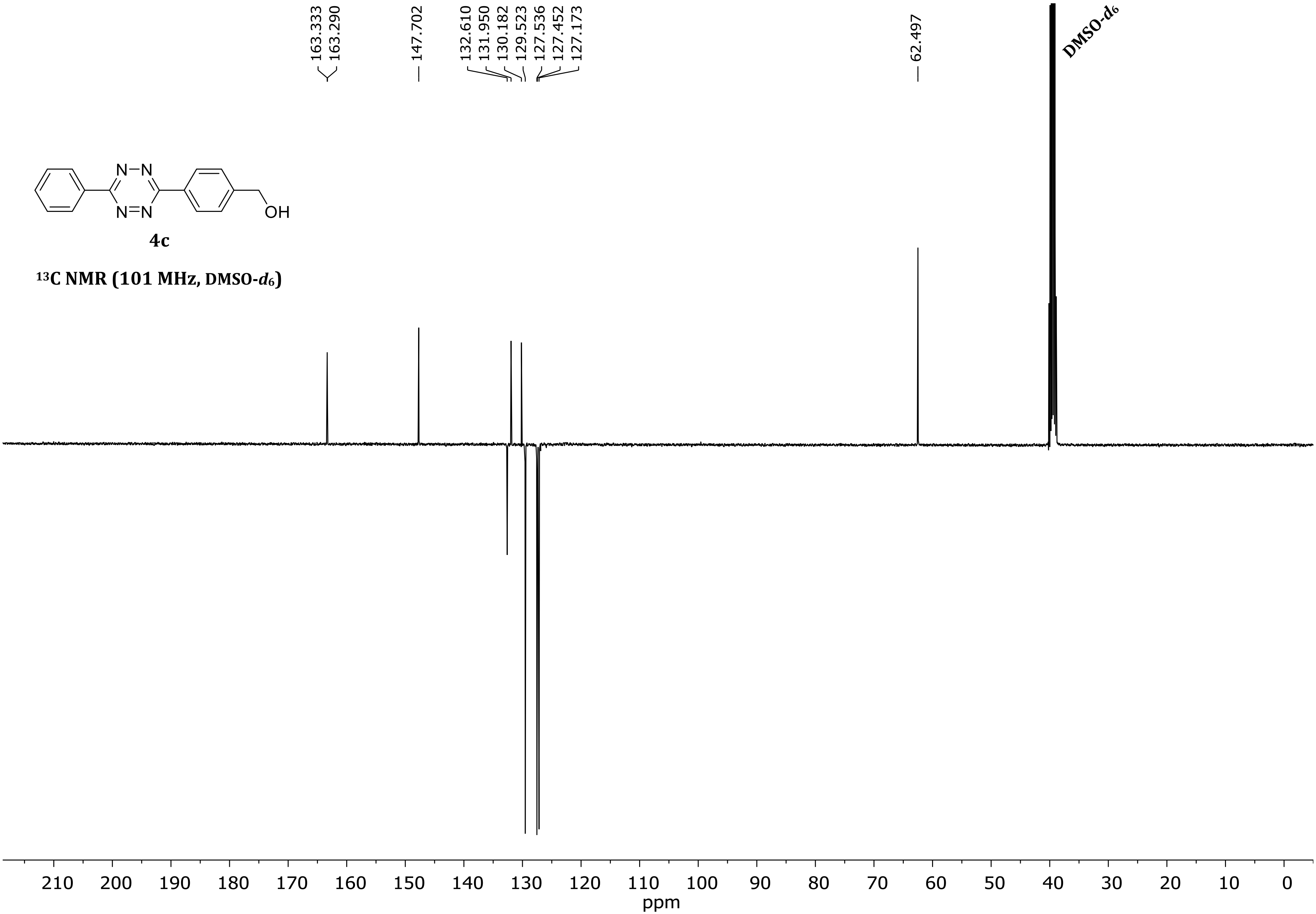
^{13}C NMR (101 MHz, $CDCl_3$)



4c

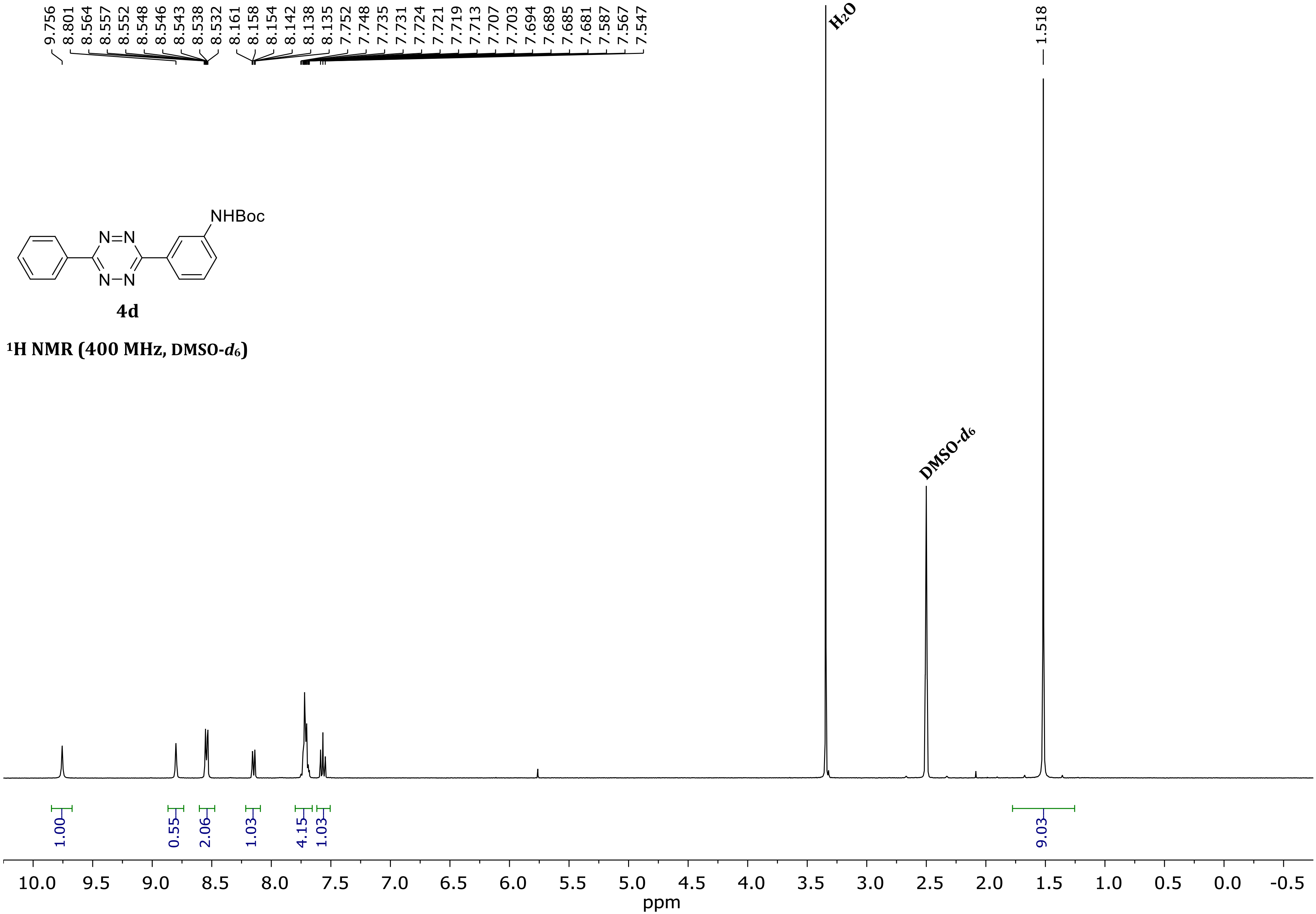
^{1}H NMR (400 MHz, DMSO-d_6)

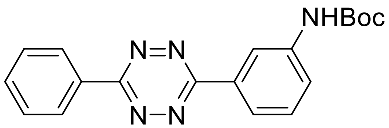




4c

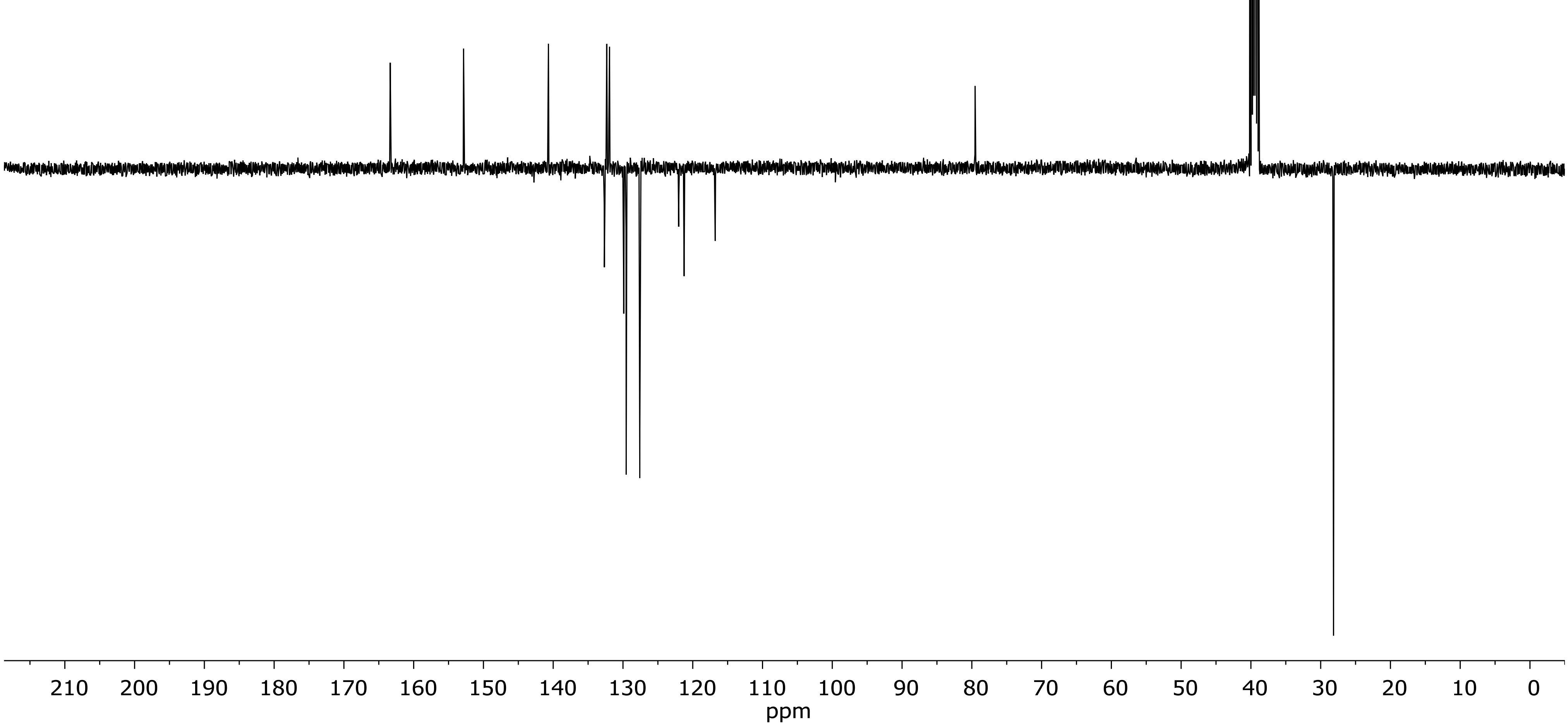
^{13}C NMR (101 MHz, DMSO-d_6)

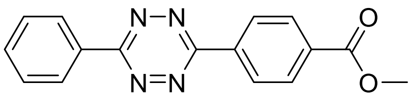

NHBoc
N=N
N–N
4d
1H NMR (400 MHz, DMSO-d6)
9.756
8.801
8.564
8.557
8.552
8.548
8.546
8.543
8.538
8.532
8.161
8.158
8.154
8.142
8.138
8.135
7.752
7.748
7.735
7.731
7.724
7.721
7.719
7.713
7.707
7.703
7.694
7.689
7.685
7.681
7.587
7.567
7.547
1.518
H2O
DMSO-d6
1.00
0.55
2.06
1.03
4.15
1.03
9.03
10.0 9.5 9.0 8.5 8.0 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 0.0 -0.5
ppm



4d

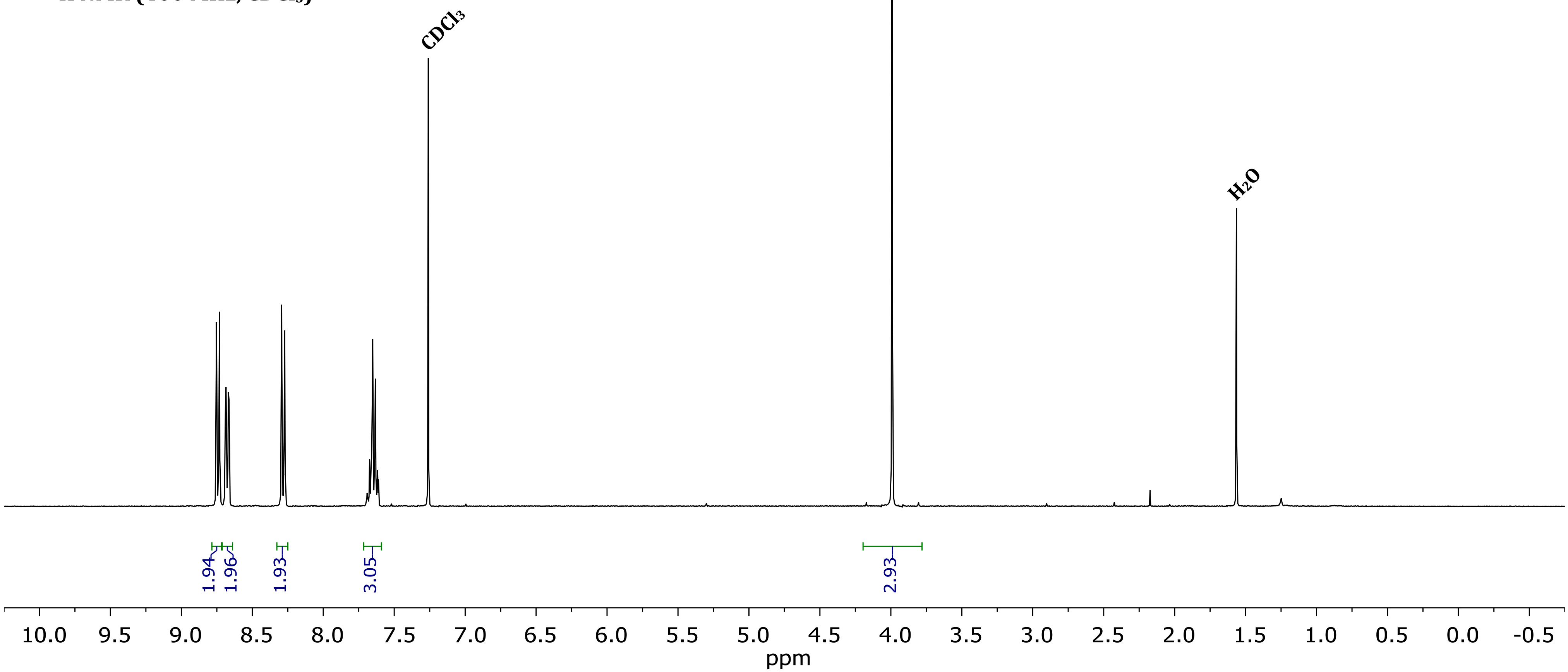
^{13}C NMR (101 MHz, DMSO-d_6)

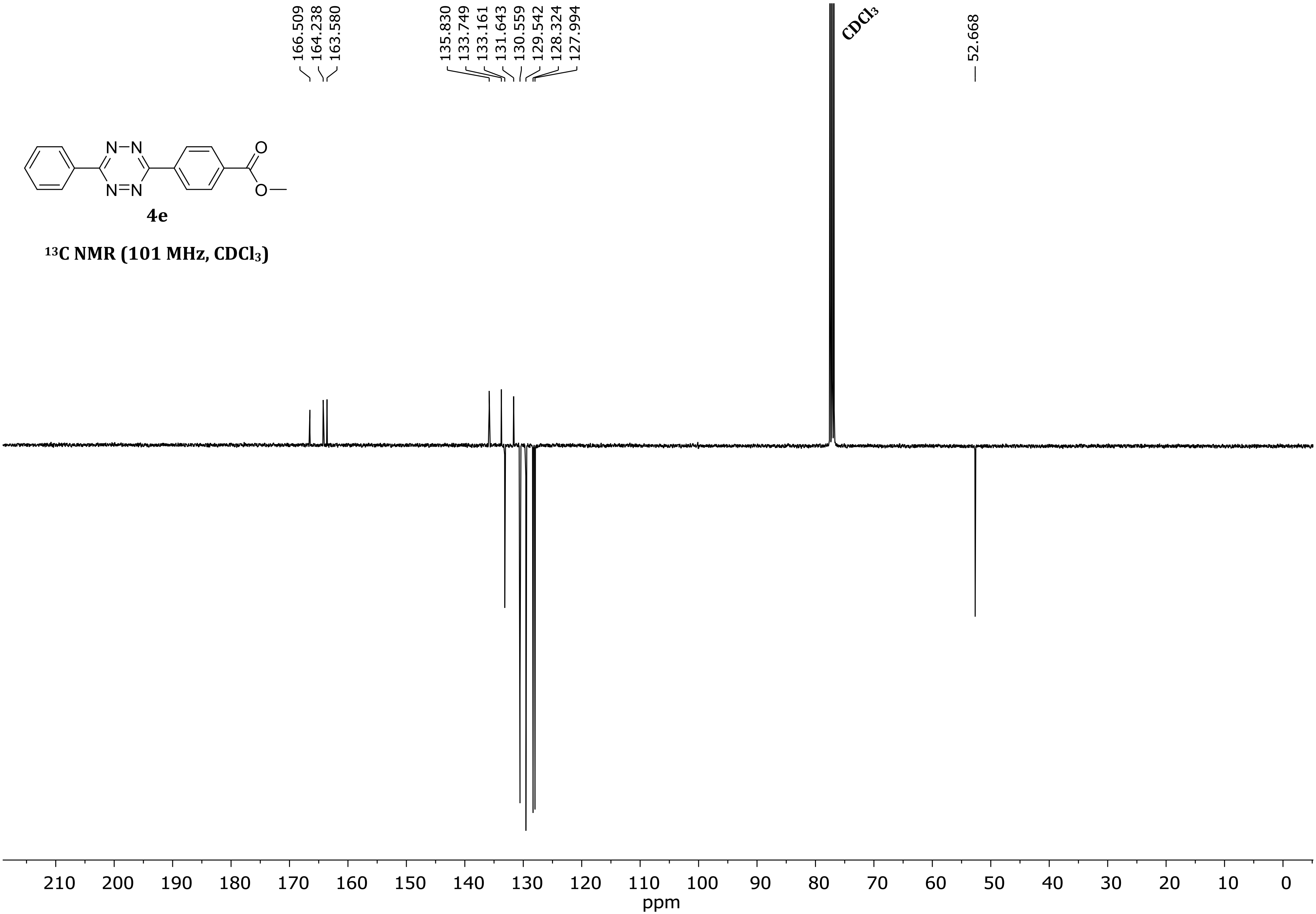




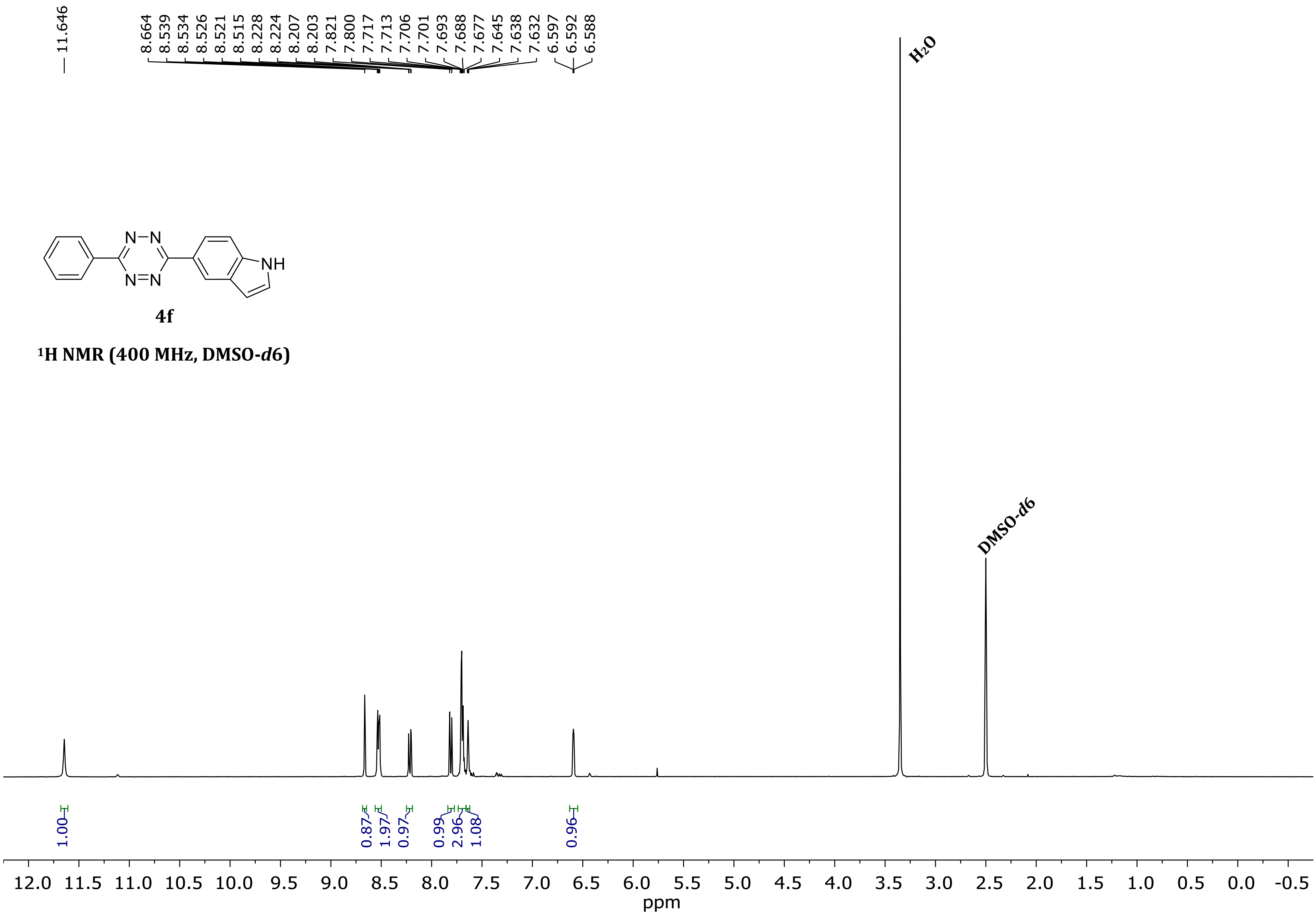
4e

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

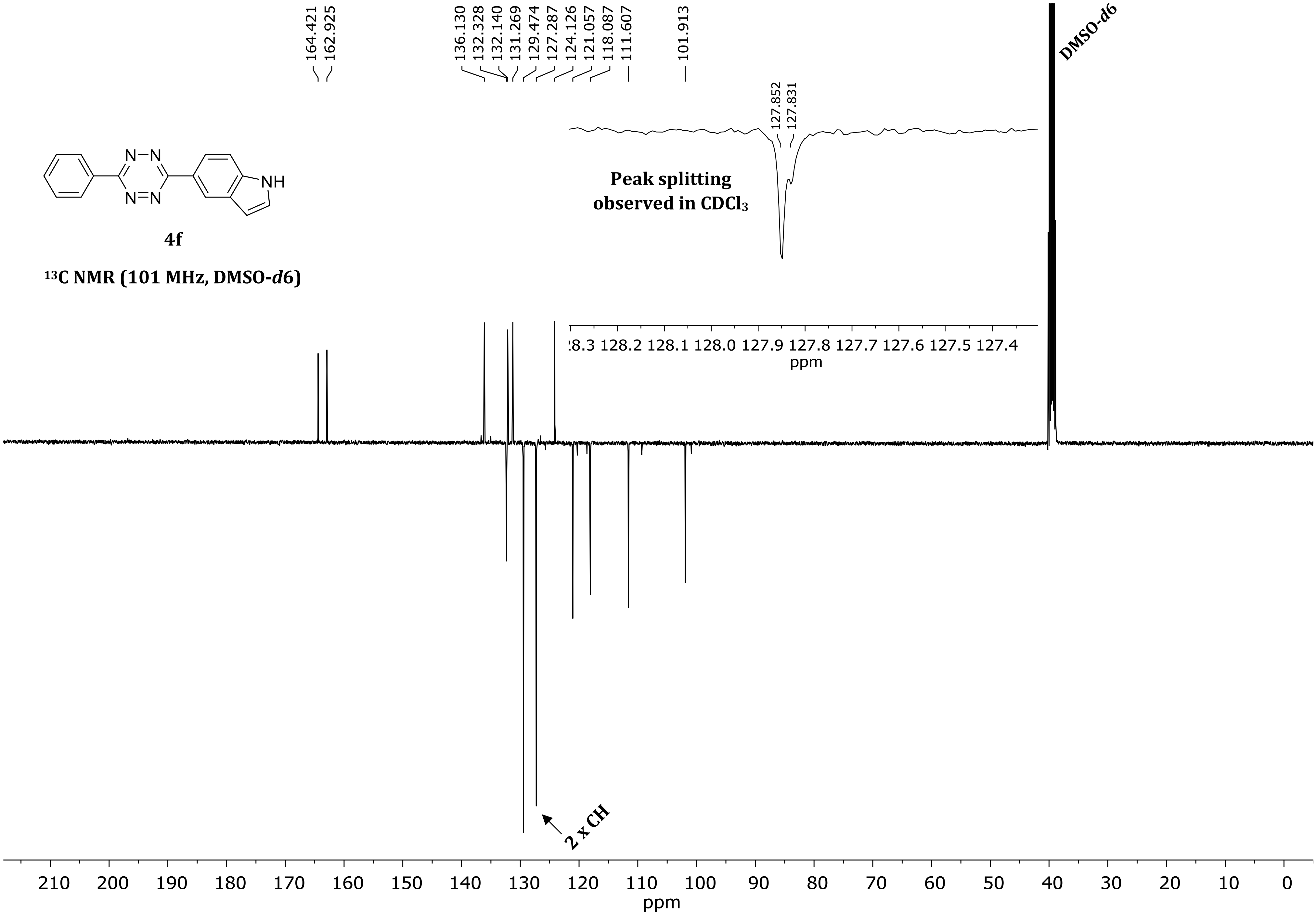


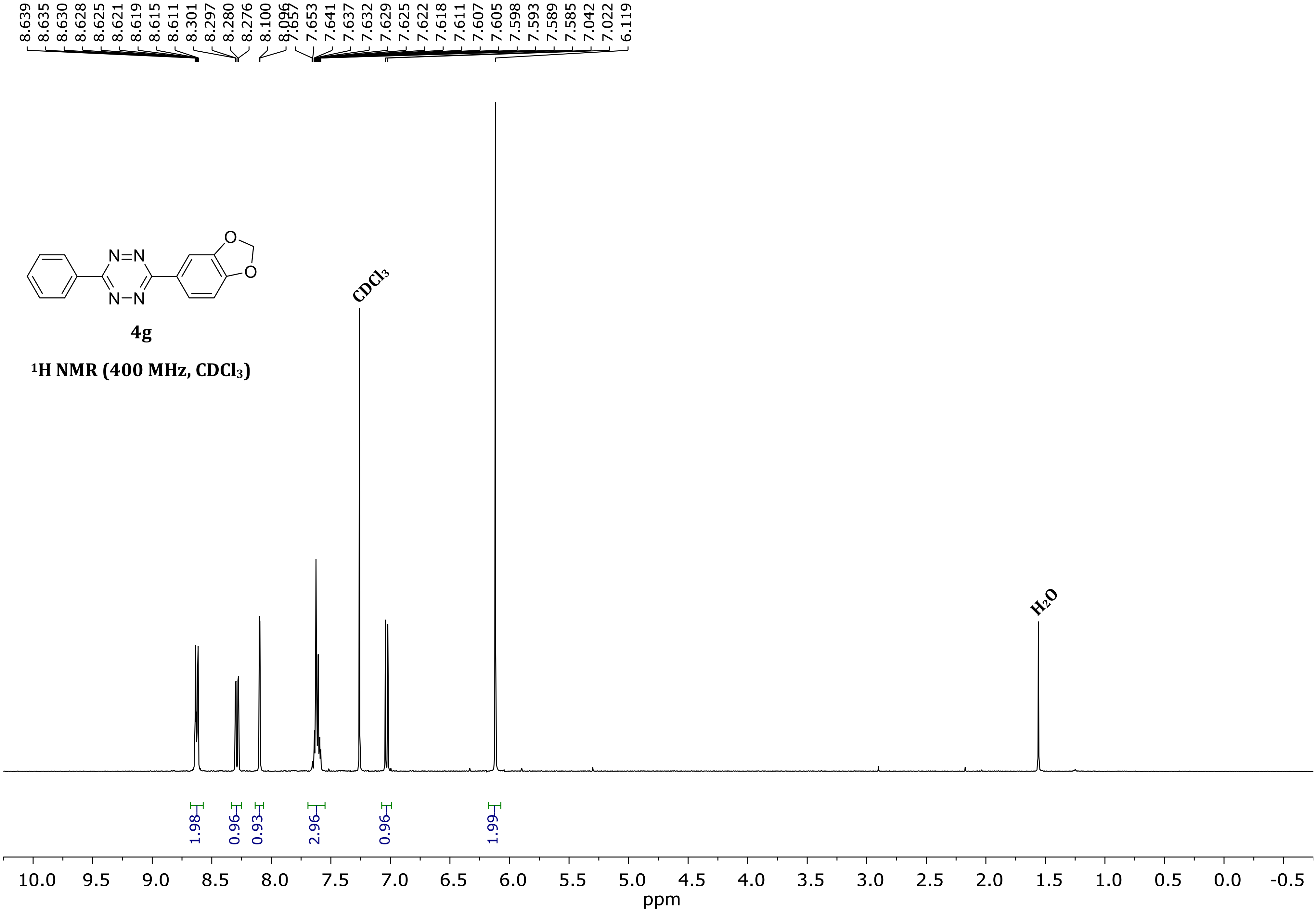


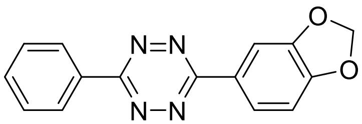
166.509
164.238
163.580
135.830
133.749
133.161
131.643
130.559
129.542
128.324
127.994
CDCl3
52.668
4e
13C NMR (101 MHz, CDCl3)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm



11.646
8.664
8.539
8.534
8.526
8.521
8.515
8.228
8.224
8.207
8.203
7.821
7.800
7.717
7.713
7.706
7.701
7.693
7.688
7.677
7.645
7.638
7.632
6.597
6.592
6.588
N–N
N=N
NH
4f
¹H NMR (400 MHz, DMSO-d6)
H₂O
DMSO-d6
1.00
0.87
1.97
0.97
0.99
2.96
1.08
0.96
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 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 0.0 -0.5
ppm

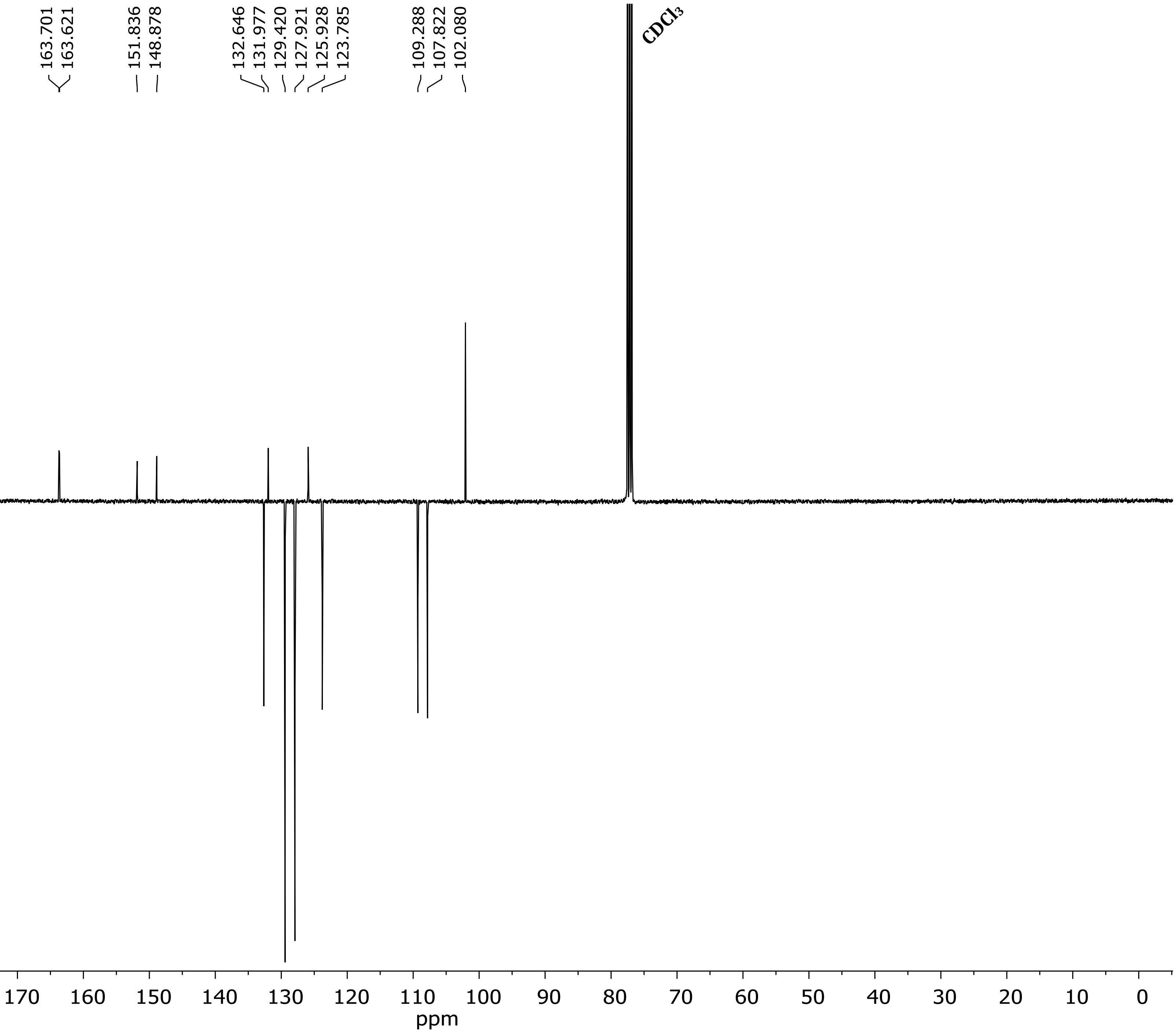

164.421
162.925
136.130
132.328
132.140
131.269
129.474
127.287
124.126
121.057
118.087
111.607
101.913
DMSO-d6
127.852
127.831
NH
N–N
N=N
4f
13C NMR (101 MHz, DMSO-d6)
Peak splitting observed in CDCl3
28.3 128.2 128.1 128.0 127.9 127.8 127.7 127.6 127.5 127.4
ppm
2 x CH
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

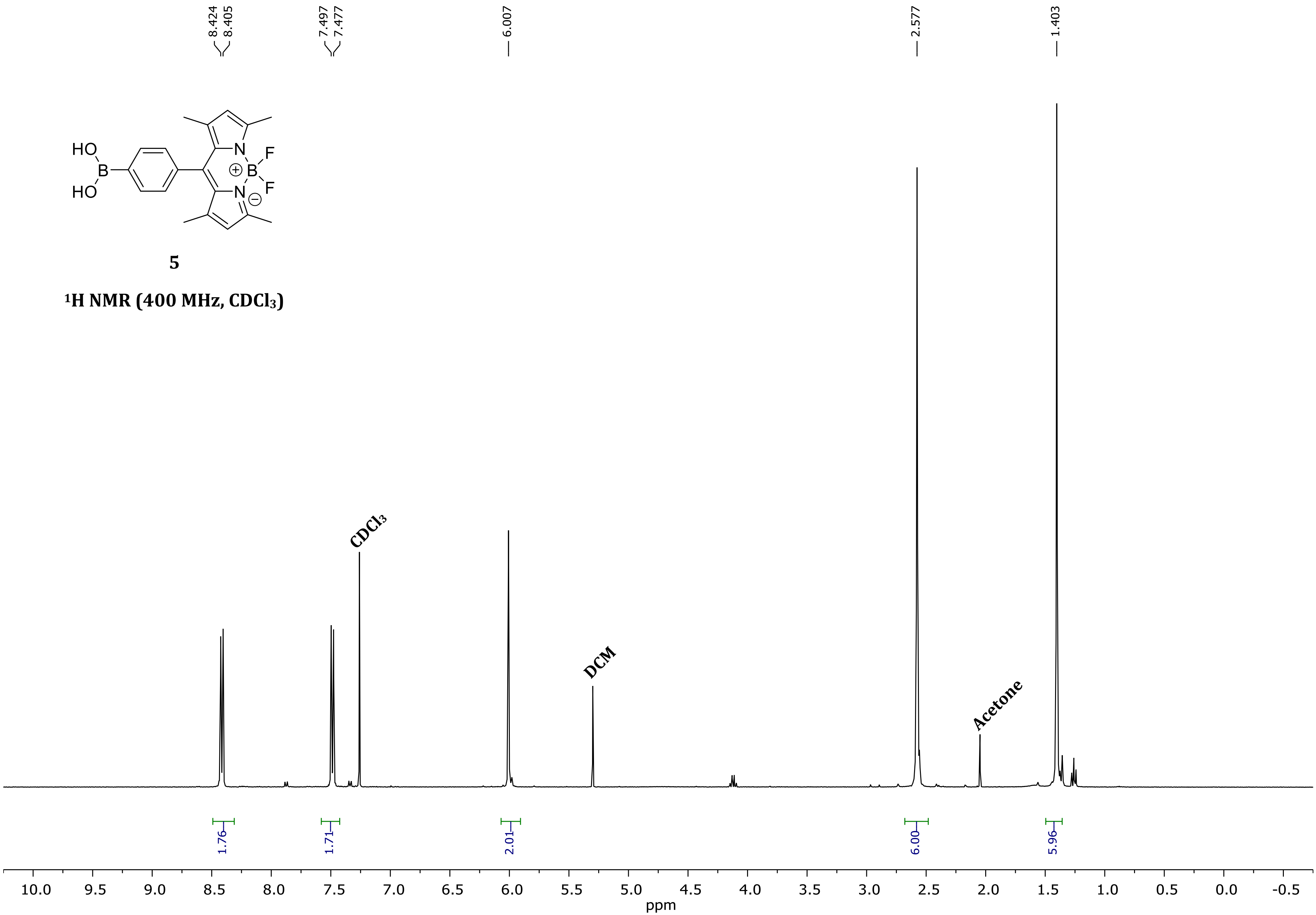

8.639
8.635
8.630
8.628
8.625
8.621
8.619
8.615
8.611
8.301
8.297
8.280
8.276
8.100
8.096
7.657
7.653
7.641
7.637
7.632
7.629
7.625
7.622
7.618
7.611
7.607
7.605
7.598
7.593
7.589
7.585
7.042
7.022
6.119
N=N
N–N
O
O
4g
¹H NMR (400 MHz, CDCl₃)
CDCl₃
H₂O
1.98
0.96
0.93
2.96
0.96
1.99
10.0 9.5 9.0 8.5 8.0 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 0.0 -0.5
ppm



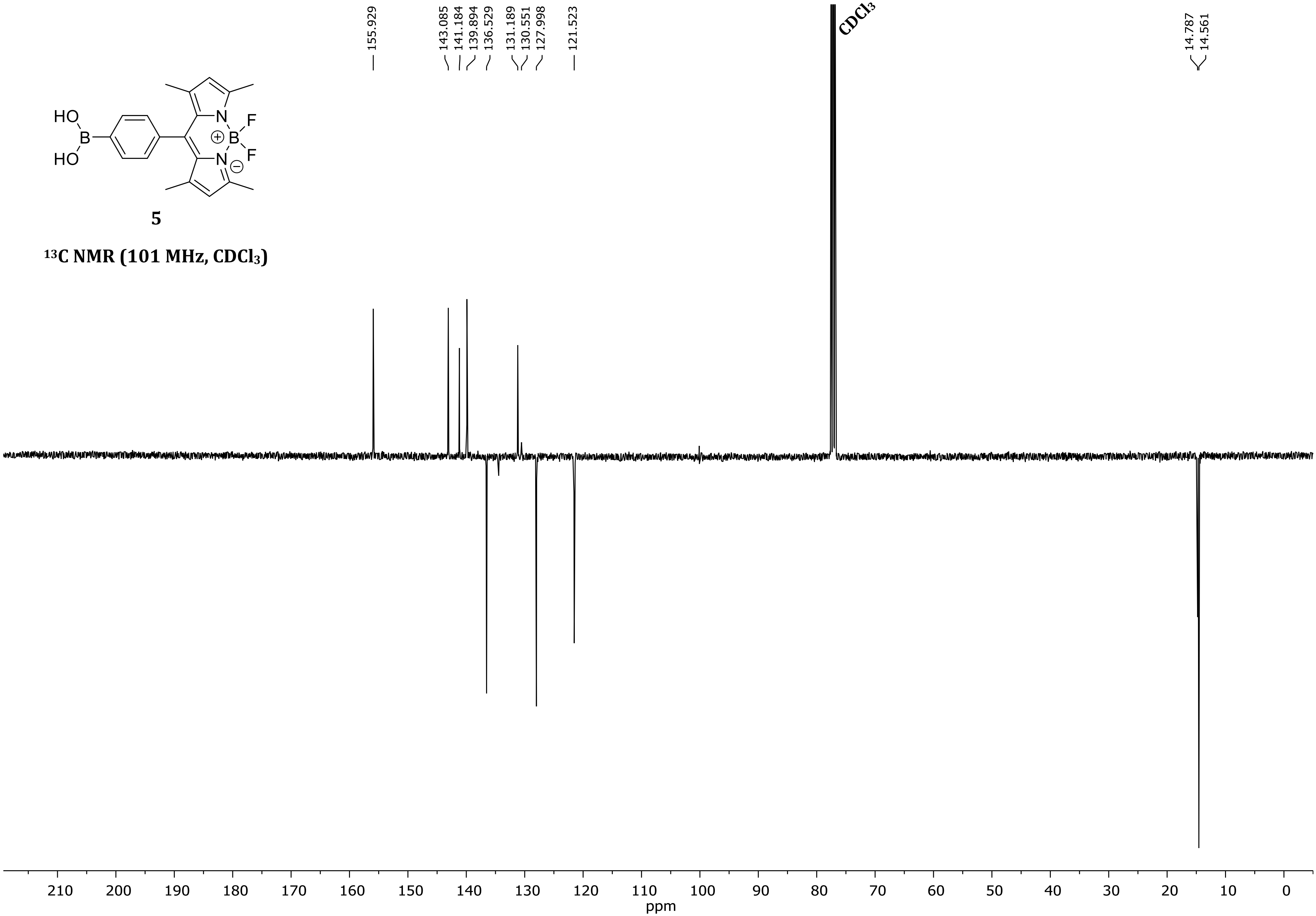
4g

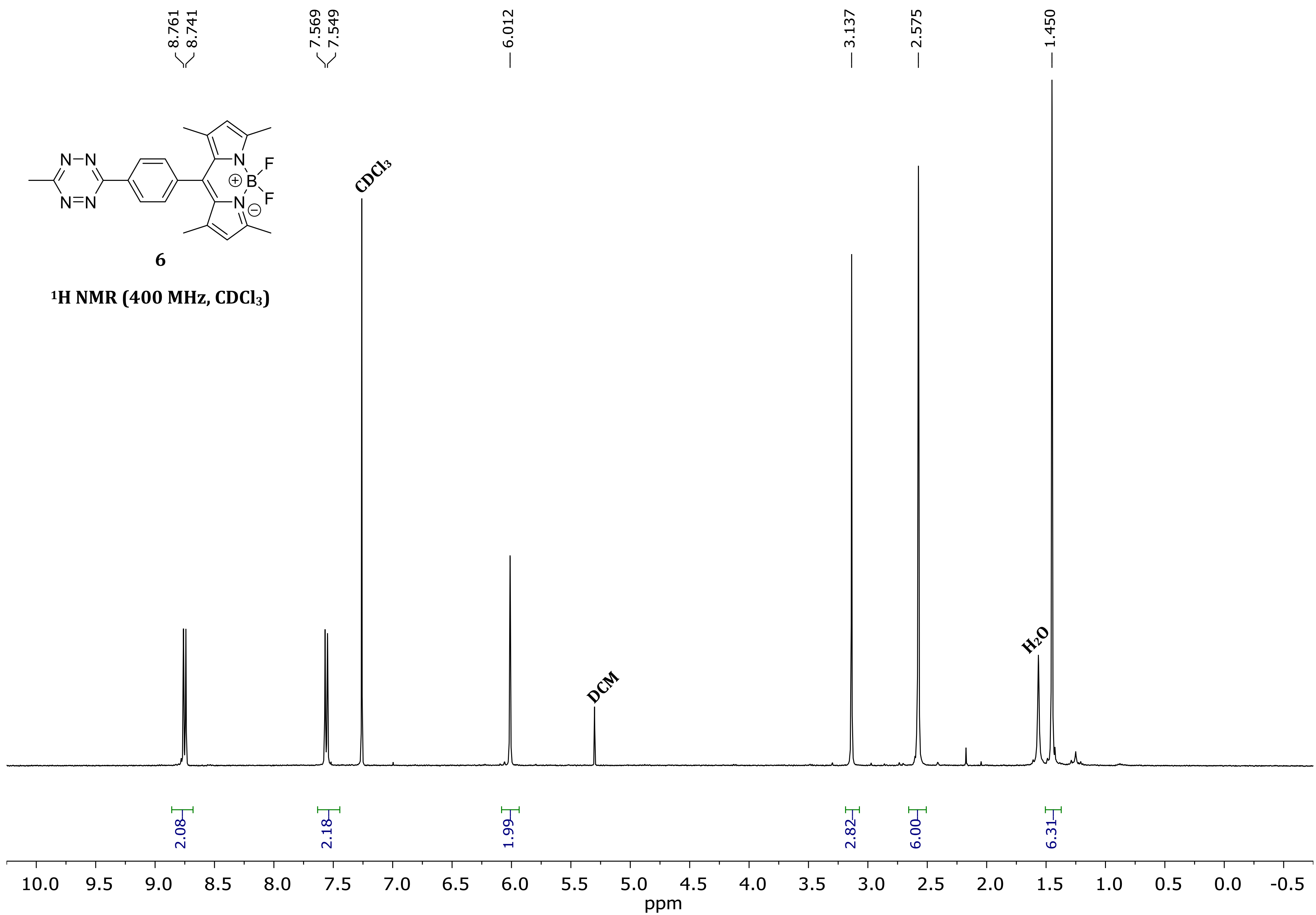
^{13}C NMR (101 MHz, CDCl$_3$)

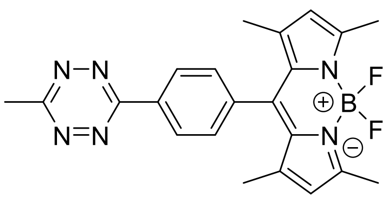




8.424
8.405
7.497
7.477
6.007
2.577
1.403
HO
B
HO
N
F
B
F
N
5
^{1}H NMR (400 MHz, $CDCl_3$)
$CDCl_3$
DCM
Acetone
1.76
1.71
2.01
6.00
5.96
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm

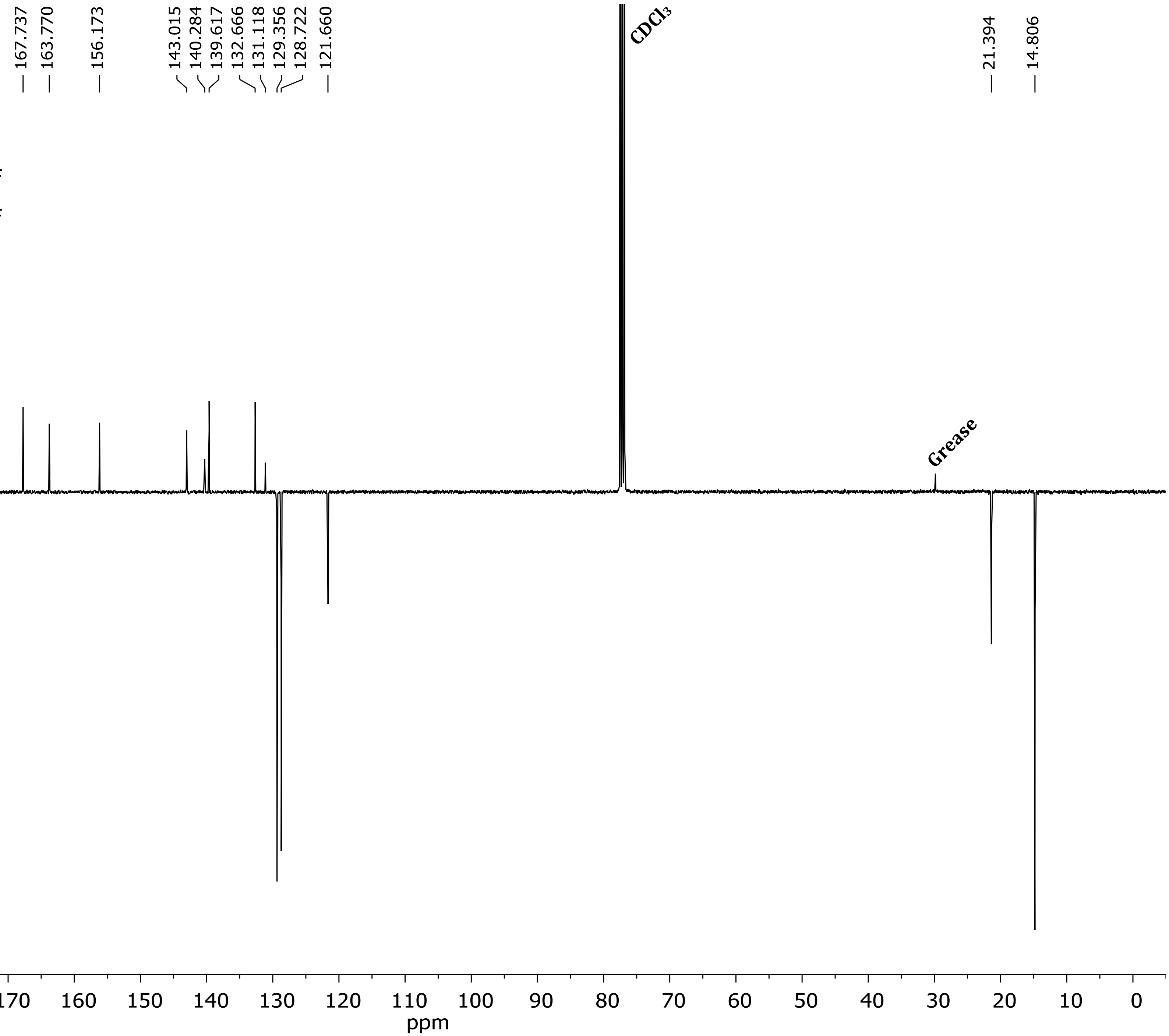

155.929
143.085
141.184
139.894
136.529
131.189
130.551
127.998
121.523
CDCl3
14.787
14.561
HO
B
HO
N
F
B
N
F
5
13C NMR (101 MHz, CDCl3)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

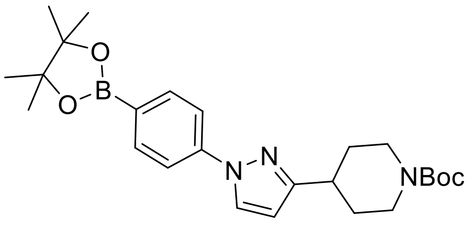

8.761
8.741
7.569
7.549
6.012
3.137
2.575
1.450
CDCl3
DCM
H2O
6
1H NMR (400 MHz, CDCl3)
2.08
2.18
1.99
2.82
6.00
6.31
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm



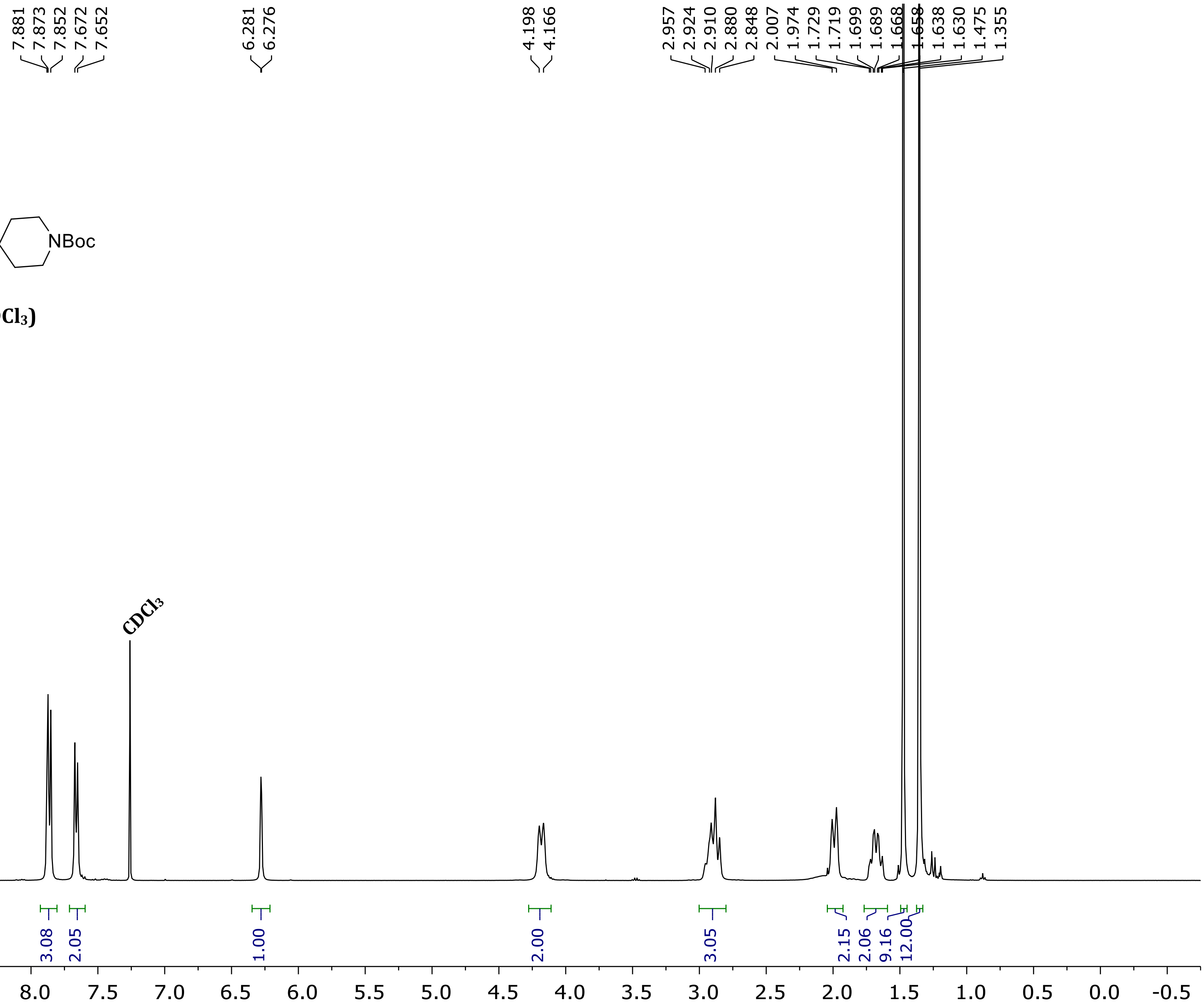
6

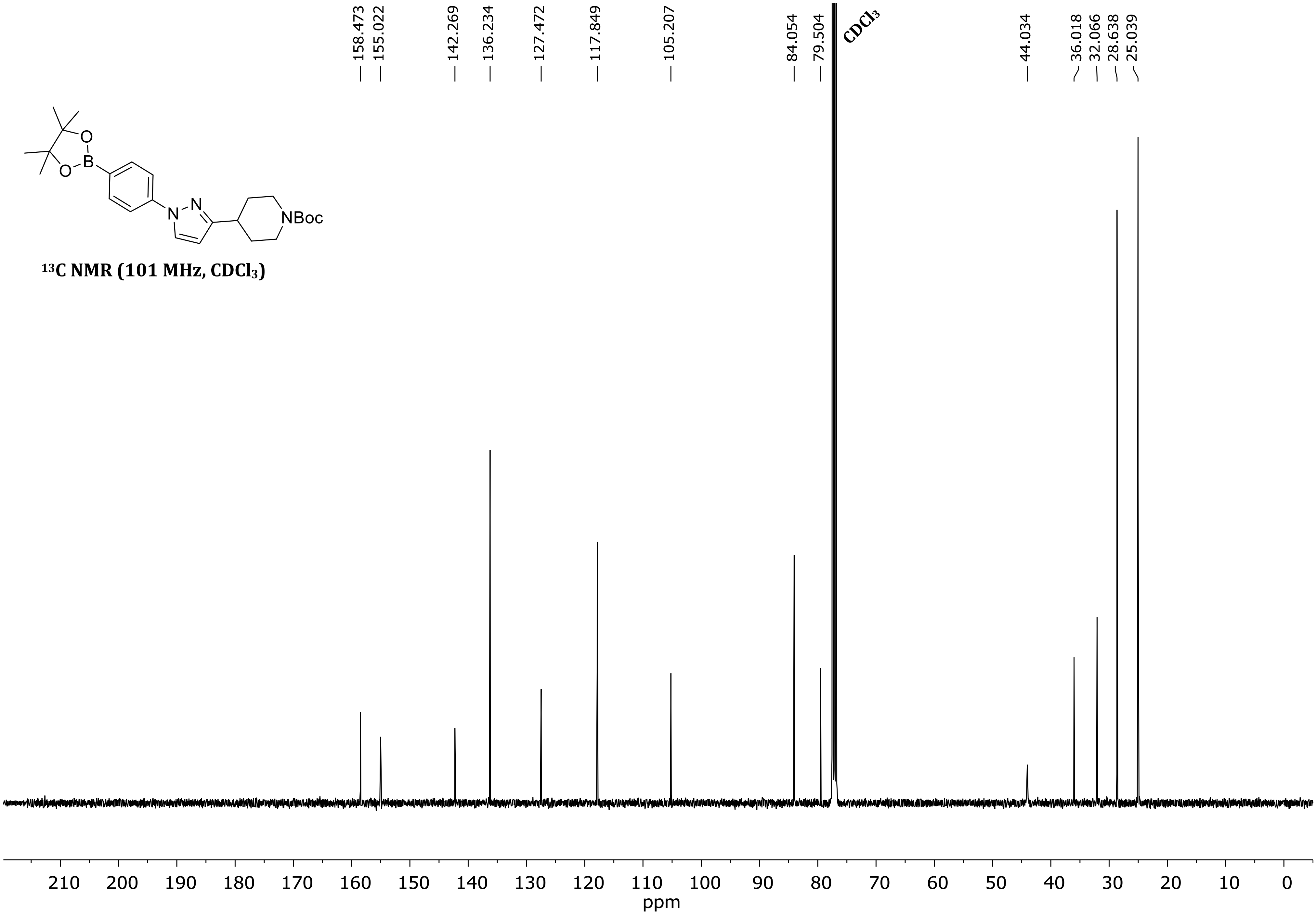
^{13}C NMR (101 MHz, $CDCl_3$)

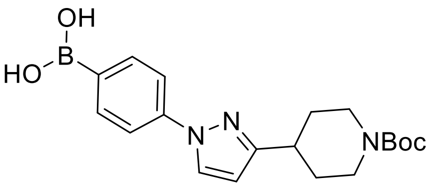




^{1}H NMR (400 MHz, $CDCl_3$)

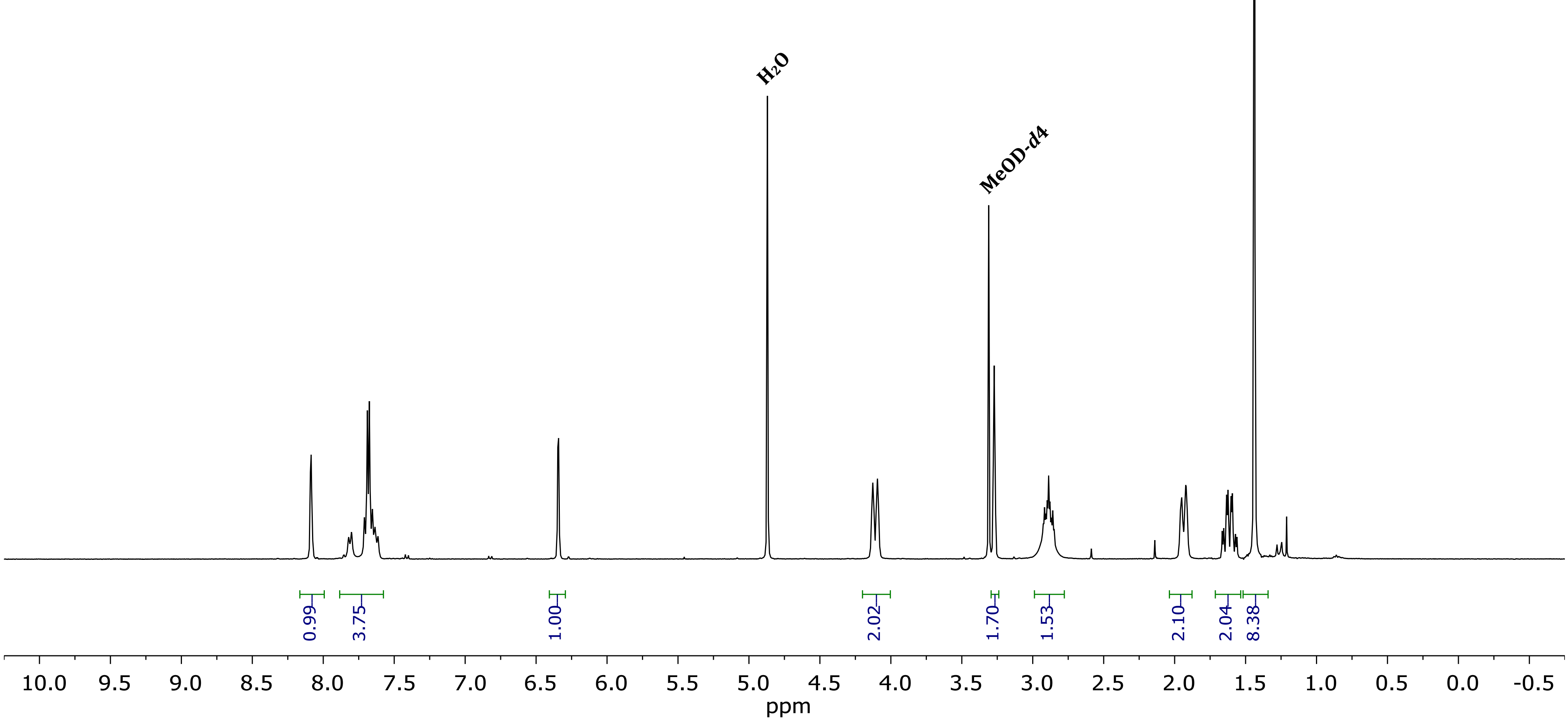


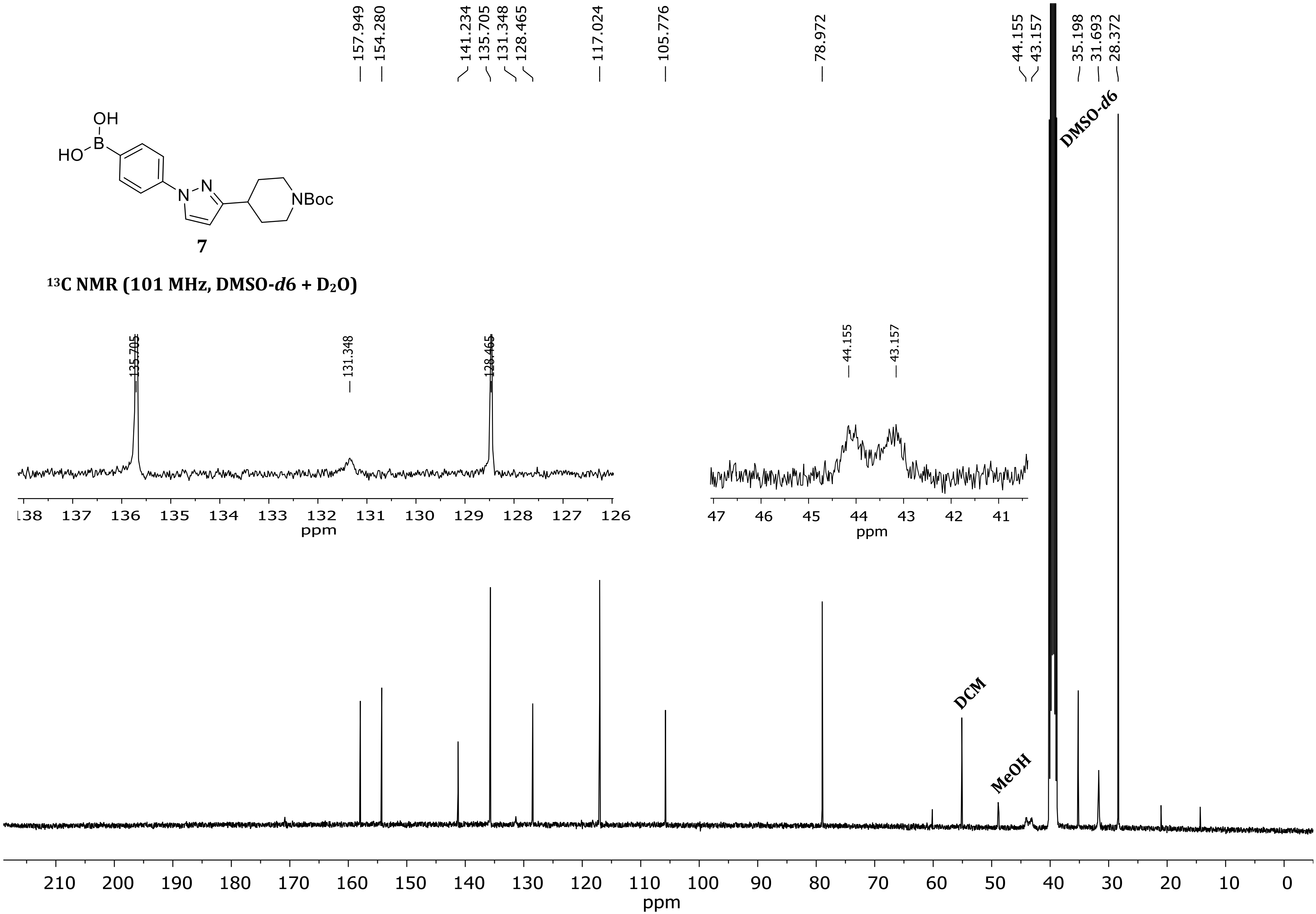

158.473
155.022
142.269
136.234
127.472
117.849
105.207
84.054
79.504
CDCl3
44.034
36.018
32.066
28.638
25.039
O
B
O
N
N
NBoc
13C NMR (101 MHz, CDCl3)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm



7

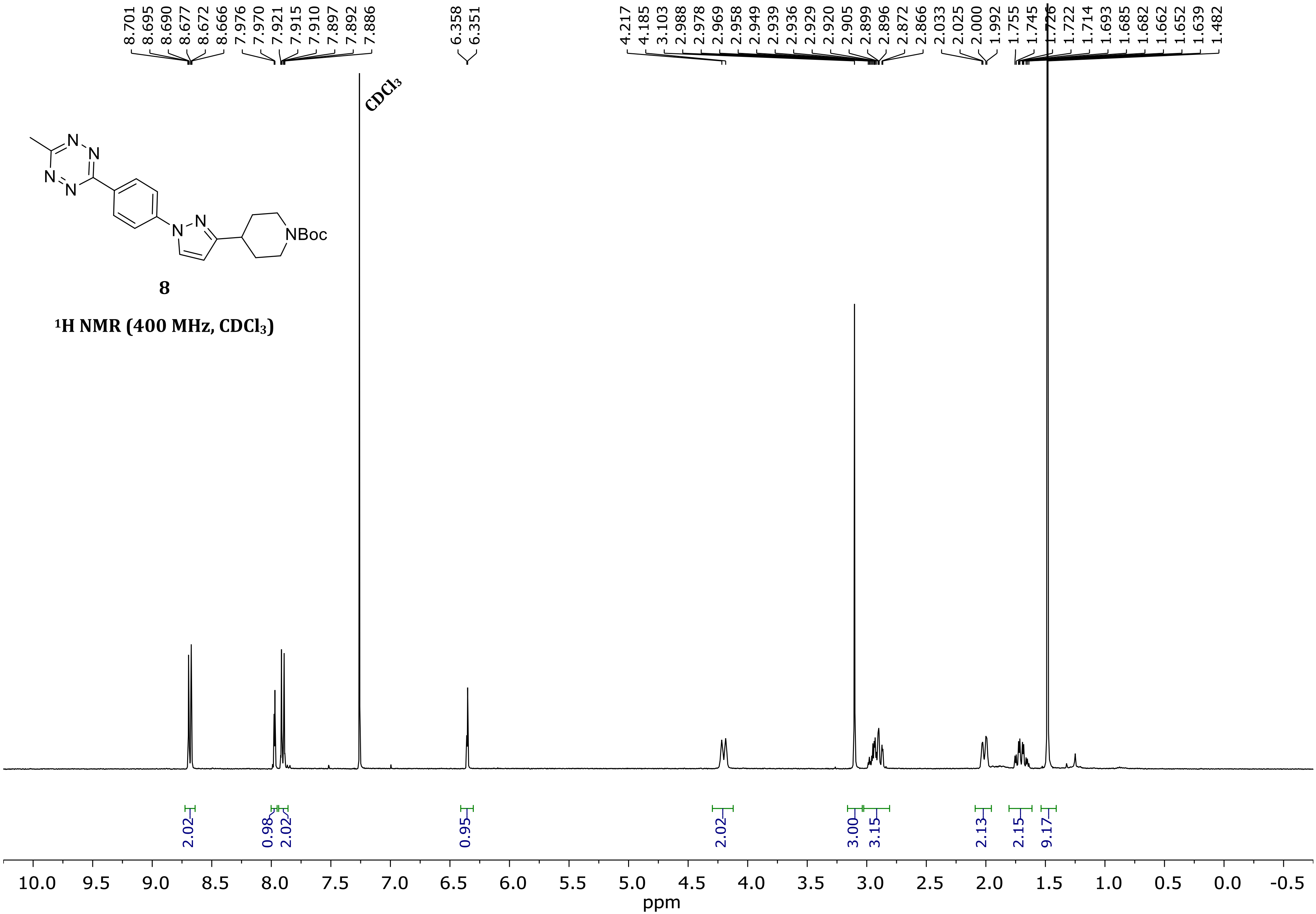
[13]C NMR (101 MHz, MeOD-*d*4)

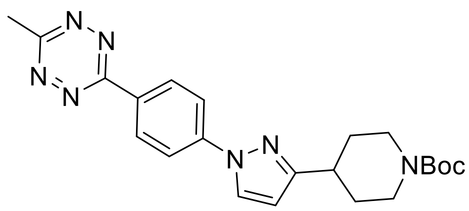




7

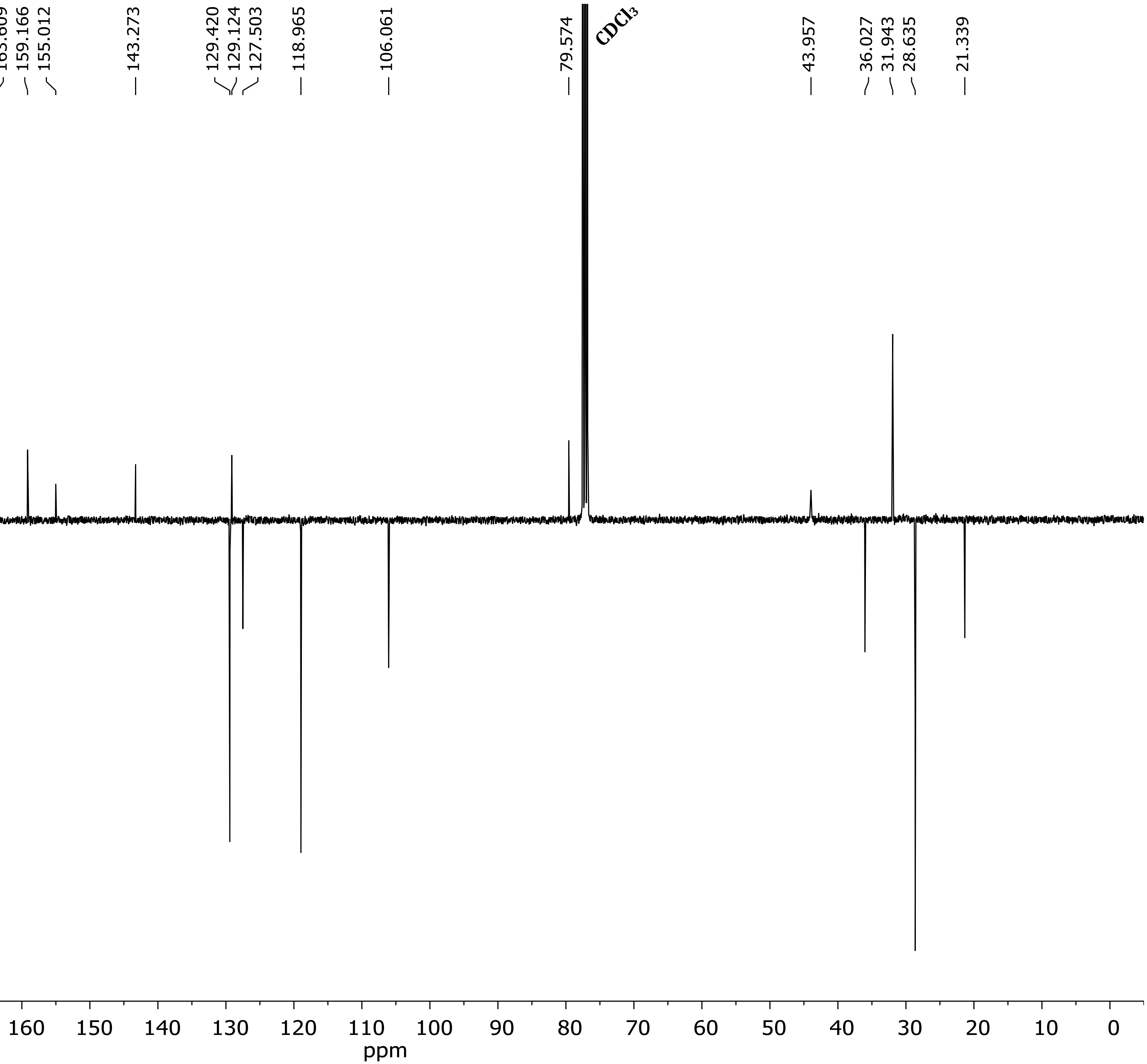
^{13}C NMR (101 MHz, DMSO-*d6* + D_2O)

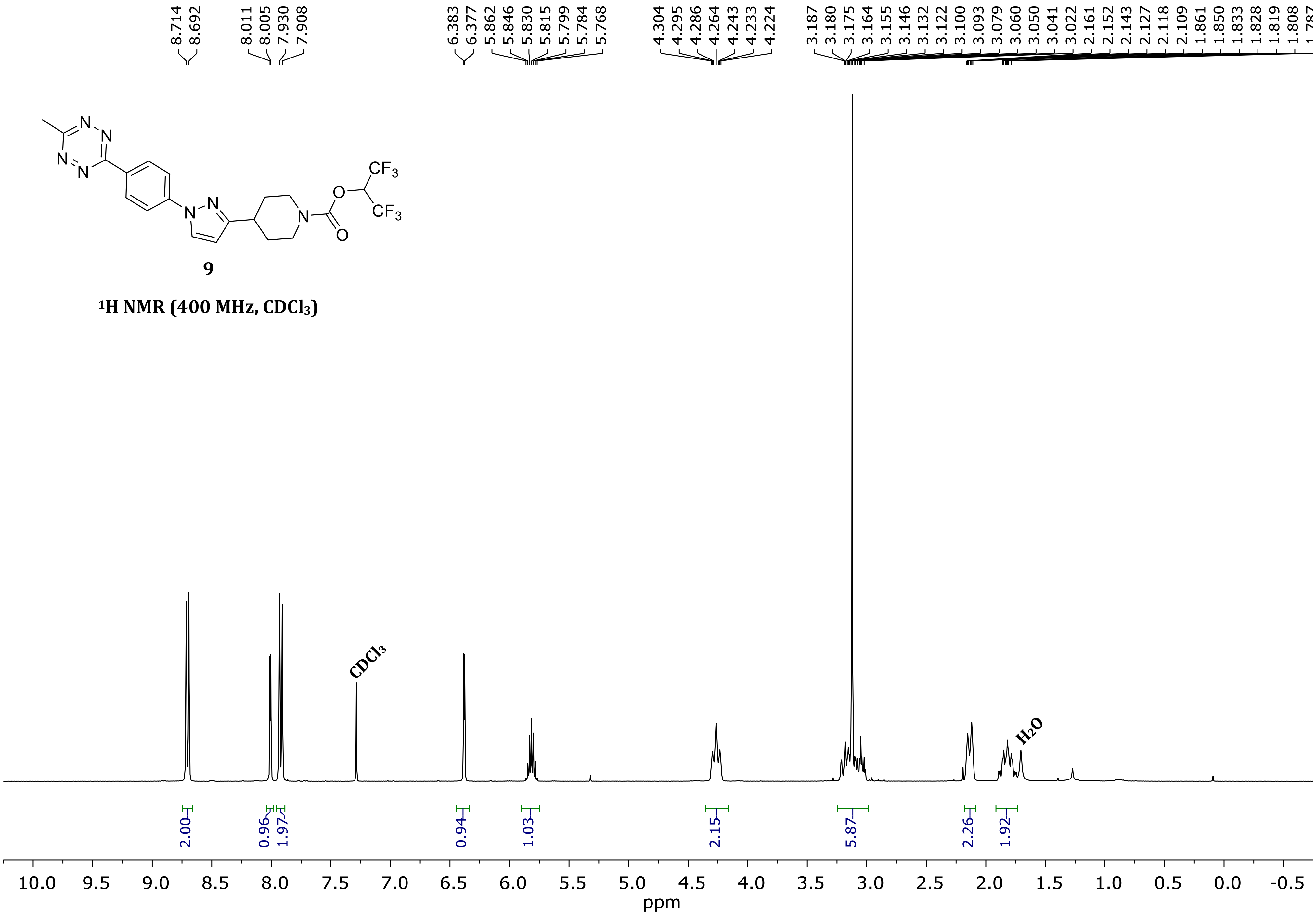

8.701
8.695
8.690
8.677
8.672
8.666
7.976
7.970
7.921
7.915
7.910
7.897
7.892
7.886
6.358
6.351
4.217
4.185
3.103
2.988
2.978
2.969
2.958
2.949
2.939
2.936
2.929
2.920
2.905
2.899
2.896
2.872
2.866
2.033
2.025
2.000
1.992
1.755
1.745
1.726
1.722
1.714
1.693
1.685
1.682
1.662
1.652
1.639
1.482
CDCl3
NBoc
8
1H NMR (400 MHz, CDCl3)
2.02
0.98
2.02
0.95
2.02
3.00
3.15
2.13
2.15
9.17
ppm



8

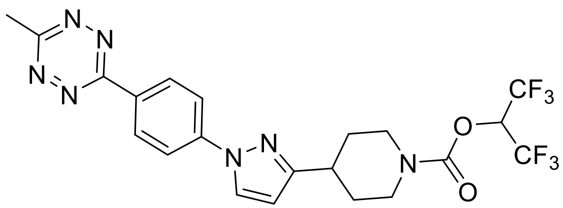
^{13}C NMR (101 MHz, $CDCl_3$)





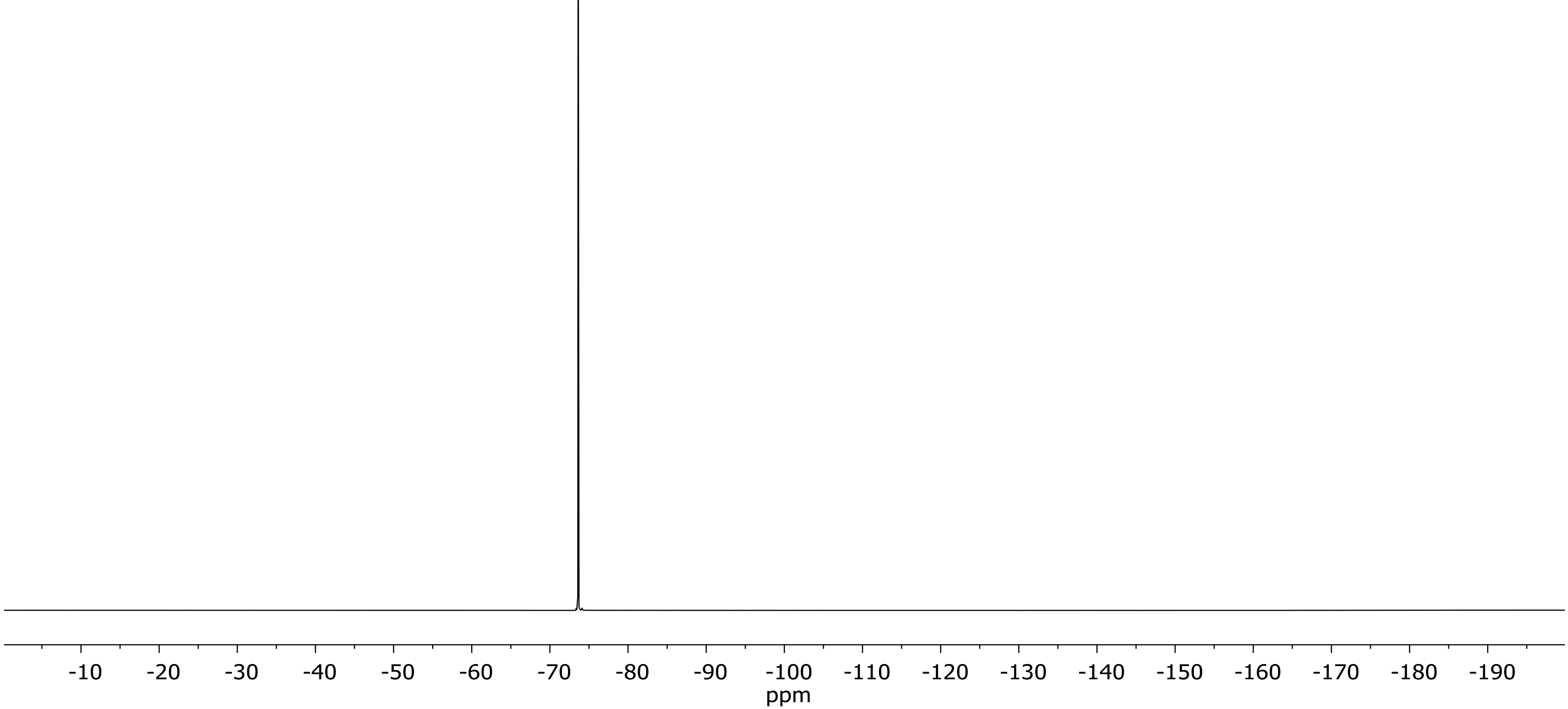
9

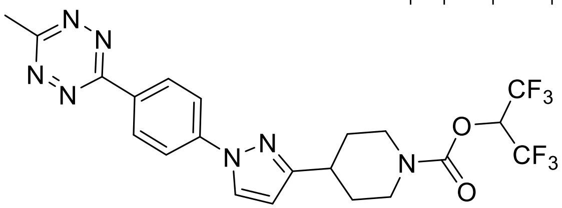
^{1}H NMR (400 MHz, $CDCl_3$)



9

^{19}F NMR (376 MHz, CDCl$_3$)





9

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