Supporting information

Triazole-Based Inhibitors of the Wnt/*β*-Catenin Signaling Pathway Improves Glucose and Lipid Metabolism in Diet-Induced Obese Mice

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Contents:

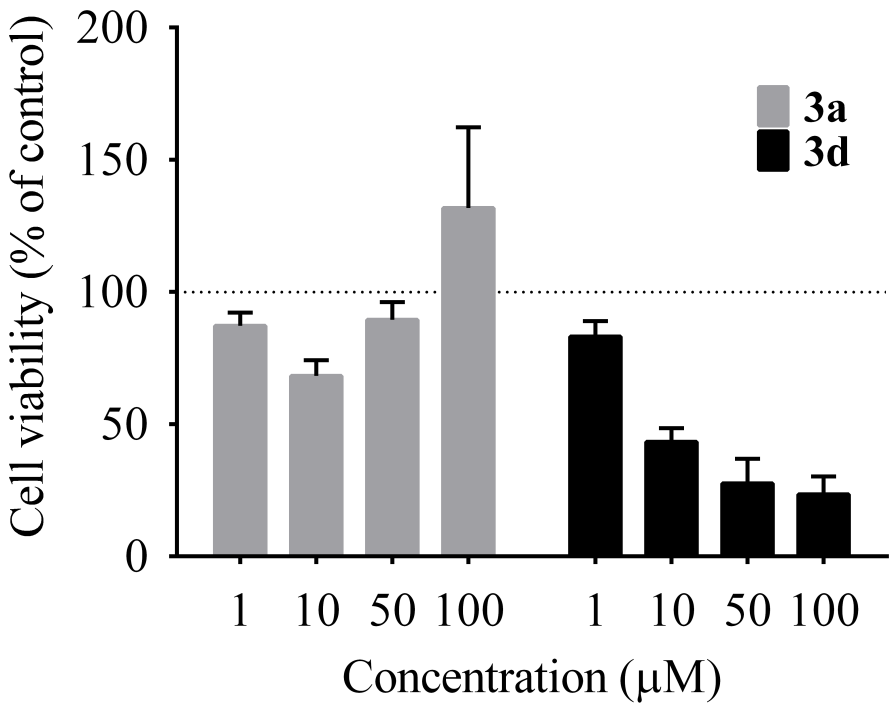


Figure S1. Cytotoxicity of compound **3a** and **3d** in unstimulated HEK293 cells with low background of Wnt pathway activity. Cells were incubated with the compounds for 72 hours. Data are reported as mean ± SD from quadruplicates.

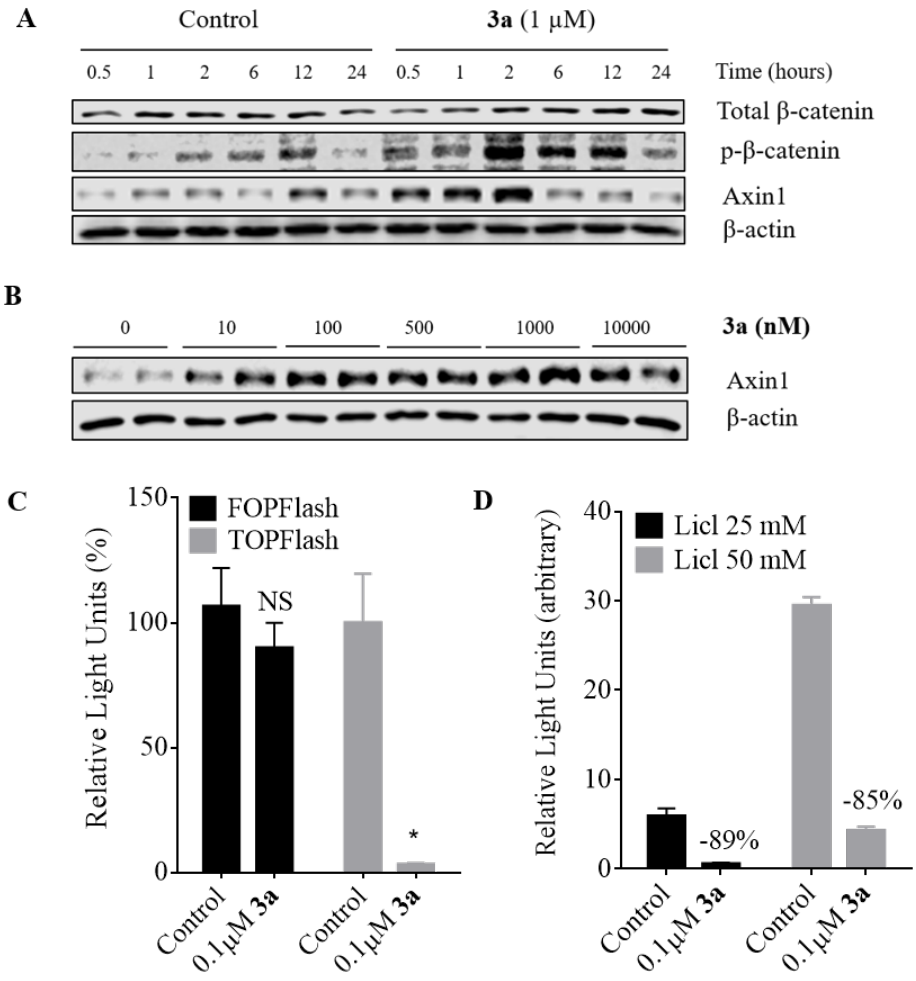


Figure S2. Time and concentration dependent regulation by compound **3a** on the Wnt/*β*-catenin pathway. (A) Time dependent effect of compound **3a** on cytoplasmic levels of Wnt/β-catenin pathway proteins in the presence of Wnt3a. (B) Concentration dependent effect of compound **3a** in the Wnt/*β*-catenin pathway in HEK293 cells treated for 2 h. (C) Reporter assay of compound **3a** in cells overexpressing TCF/LEF response elements (TOPFlash) or scrambled non-function TCF/LEF response element (FOPFlash) treated with Wnt3a-conditioned medium. (D) Varying concentrations of LiCl effect on the efficacy of compound **3a**. Data are presented as mean ± standard deviation, n =3. *$p < 0.05$. NS, not significant.

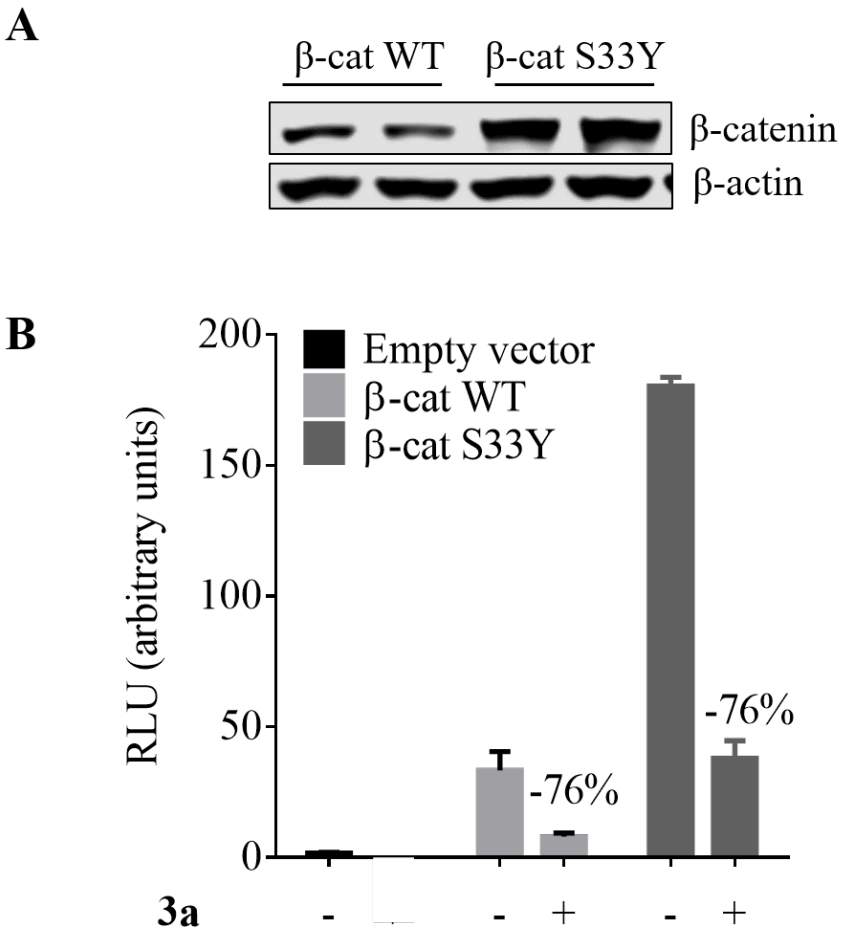


Figure S3. Inhibition of Wnt signaling activity by compound **3a** in HEK293 cells overexpressing β-catenin. (A) Western blot analysis of β-catenin protein expression in cells transiently overexpressing wild type and S33Y-mutant (GSK3*β* phosphorylation site) β-catenin. The cells were transfected with 500 ng of the plasmids and harvested 48 hours later. (B) TCF/LEF gene reporter assay of the transfected cells treated with compound **3a**. Cells were co-transfected with both the indicated β-catenin plasmids and the TOPflash reporter constructs After 48 hours, cells were treated with 100 nM of compound **3a** for 24 hours. Data are presented as mean ± SD, n =3.

A

Ligand	Analyte	K_D (M)	R_{max} (RU)	Chi² (RU²)
GSK3β, 20,000 RU	**3a**	4.33E-06	73.33	0.618

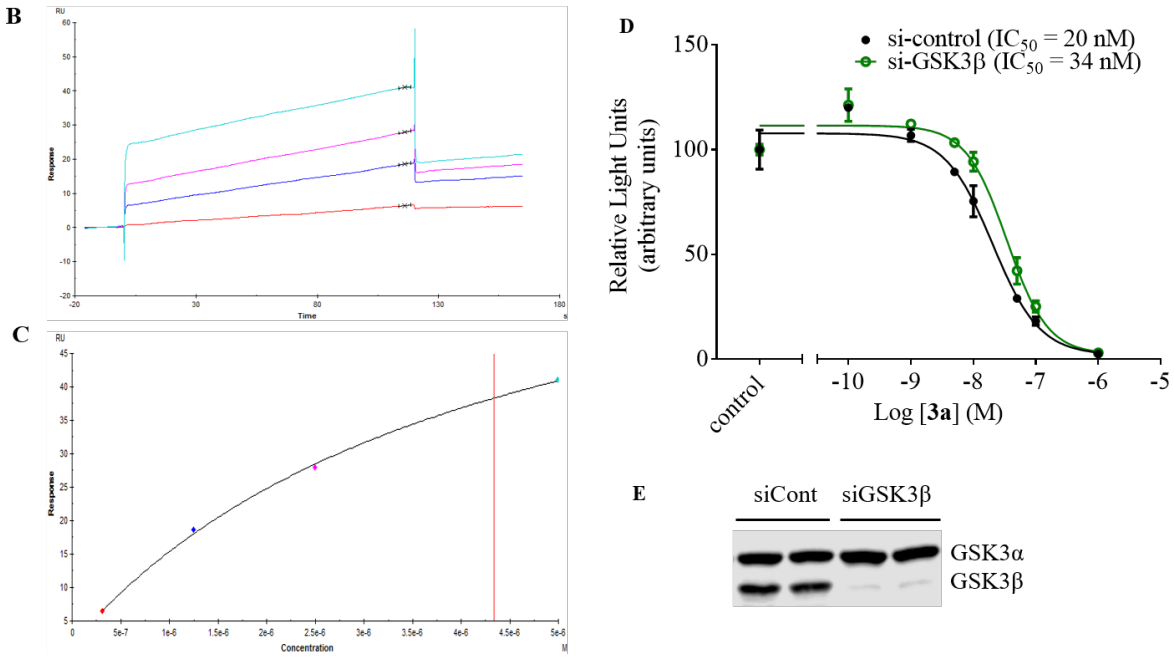


Figure S4. Direct but weak binding of compound **3a** to GSK3β. (A) SPR analysis of **3a** binding to recombinant GSK3*β* protein. (B) & (C) Concentration dependent binding of **3a** to GSK3*β*. The concentrations used were 0.313 (red), 1.25 (navy blue), 2.50 (pink) and 5.00 µM (cyan). (D) Inhibition of Wnt activity by compound **3a** in the presence and absence of GSK3β knockdown, as determined by the luciferase reporter assay. (E) Knockdown of GSK3β in HEK293 cells by a small interference RNA.

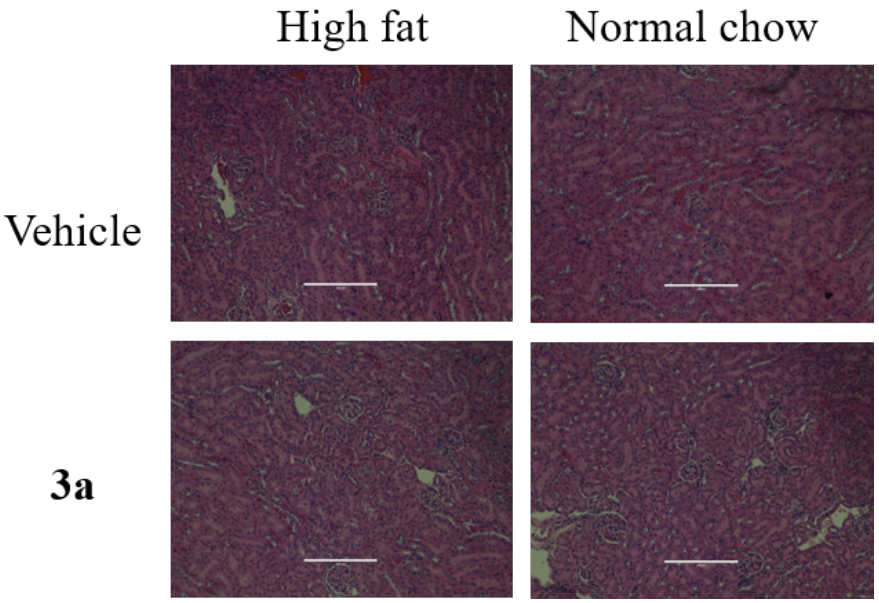
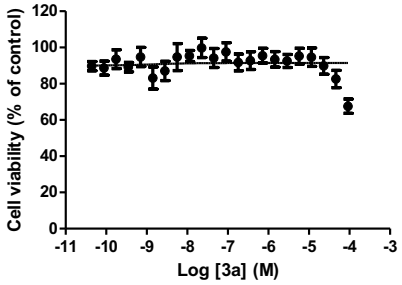
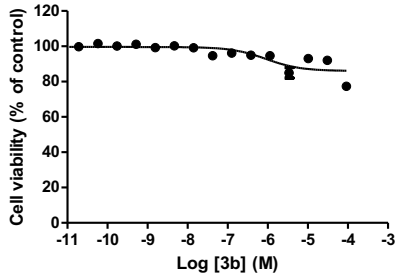
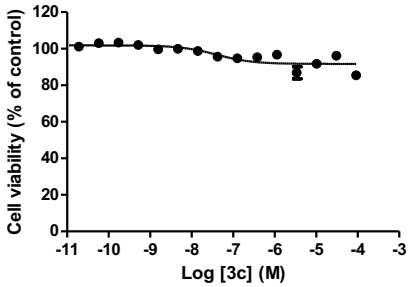
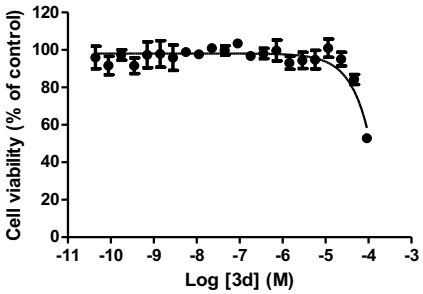


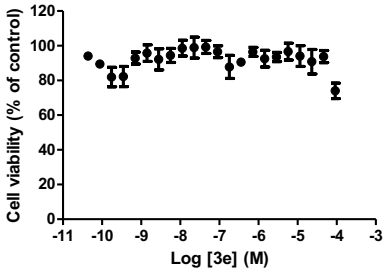
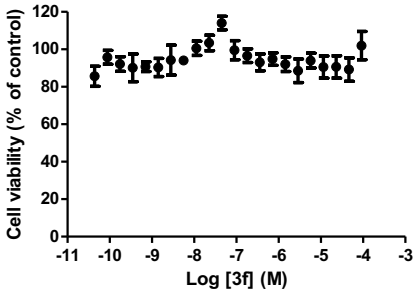
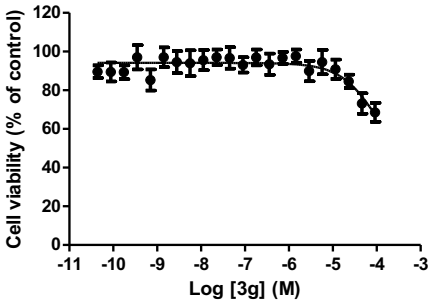
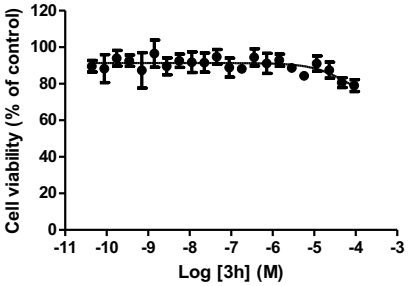
Figure S5. Compound **3a** treatment has no noticeable toxic effects on mouse kidney histology. The mice fed on either a high fat diet or a normal chow diet received *i.p.* injection of 40 mg/kg **3a** or vehicle (corn oil) every two days for 11 weeks. The isolated kidney tissues were staining by Haematoxylin and Eosin (H&E). The images were taken under an amplification of 20 X.

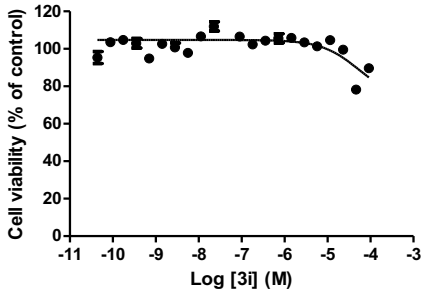
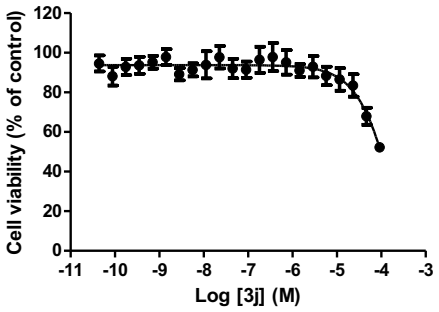
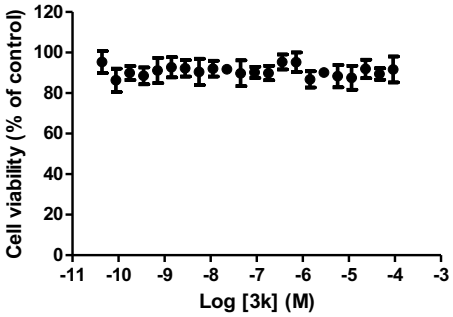
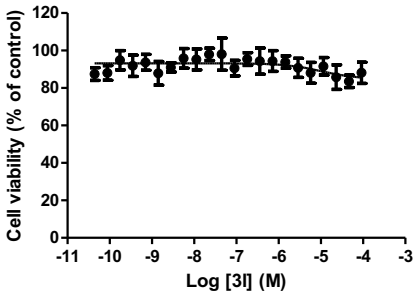
Table S1. Human primers used in the experiments

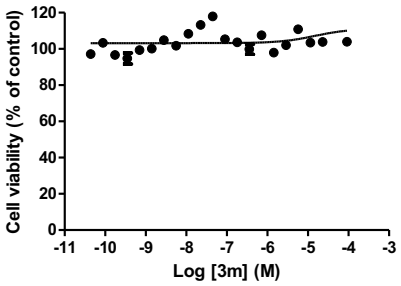
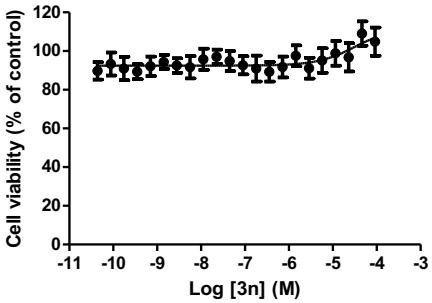
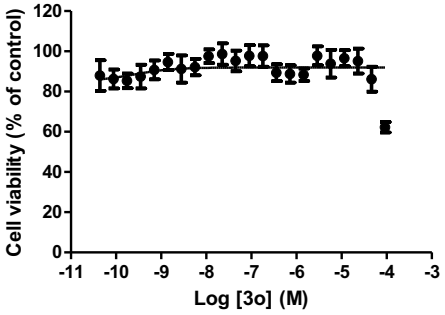
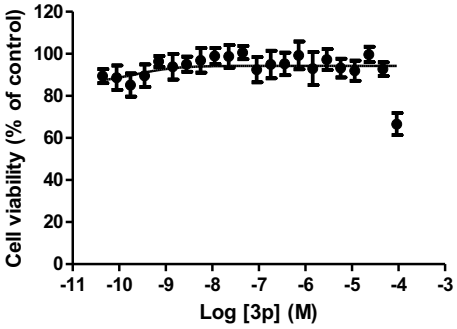
Gene	Forward	Reverse
AXIN2	GAGTGGACTTGTGCCGACTTCA	GGTGGCTGGTGCAAAGACATAG
CYCD1	CAATGACCCCGCACGATTTC	CATGGAGGGCGGATTGGAA
PEPCK	GTCAGCCTGATCACATCCACA	CCGTCTTGCTTTCGATCCTG
G6PASE	CTACTACAGCAACACTTCCGTG	GGTCGGCTTTATCTTTCCCTGA
ACAT2	GCGGACCATCATAGGTTCCTT	ACTGGCTTGTCTAACAGGATTCT
ACOT4	CCCAGGTAAAAGGCCCAGG	GTGTTCCCACTGATCCCAGAT
SCD1	GCCCCTCTACTTGGAAGACGA	AAGTGATCCCATACAGGGCTC
ACAA1	GCGGTTCTCAAGGACGTGAAT	GTCTCCGGGATGTCACTCAGA
FASN	CCGAGACACTCGTGGGCTA	CTTCAGCAGGACATTGATGCC

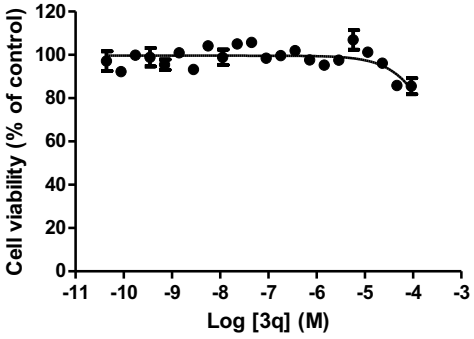
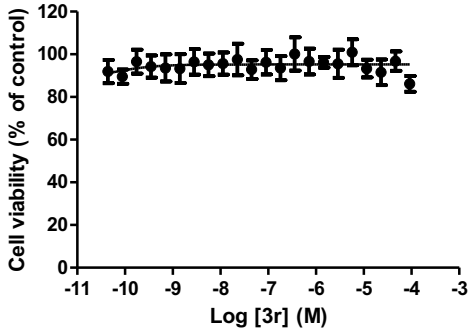
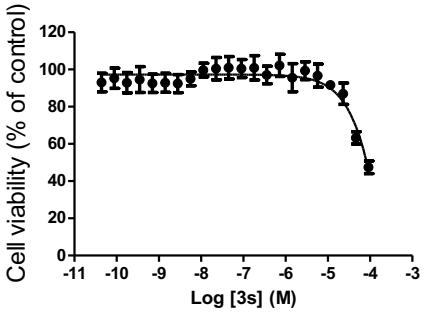
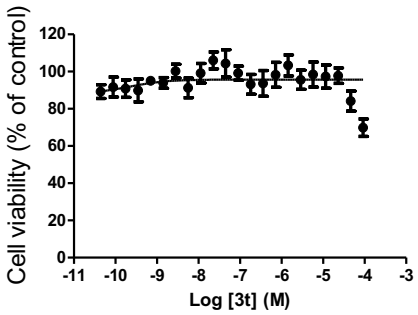
Table S2. The effect of compounds **3a-3u** on HEK293 cell viability during the incubation (24 hours) of the reporter assay for measuring Wnt signaling activity.

Cmpd	Growth curve	IC_{50} of growth inhibition (μM)
3a		> 92
3b		> 92
3c		> 92
3d		> 92

3e		> 92
3f		> 92
3g		> 92
3h		> 92

3i		> 92
3j		> 92
3k		> 92
3l		> 92

3m		> 92
3n		> 92
3o		> 92
3p		> 92

3q		> 92
3r		> 92
3s		> 92
3t		> 92

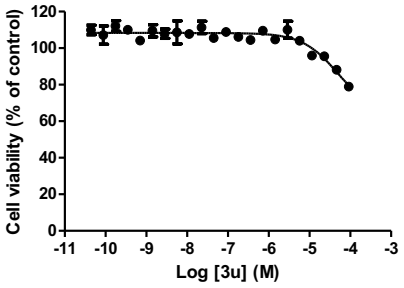
3u	 Cell viability (% of control) 120 100 80 60 40 20 0 -11 -10 -9 -8 -7 -6 -5 -4 -3 Log [3u] (M)	> 92

Table S3. Pharmacokinetic and physicochemical parameters of compound **3a** in mice.

Parameter	Units	Route	
		Oral (N=5)	Intravenous (N=4)
Dose	mg/kg	10	10
$T_{1/2}$	h	3.3 ± 0.83	2.8 ± 0.51
C_{max}	µg/mL	2.2 ± 0.49	
T_{max}	h	0.25	
$AUC_{0\text{-}inf}$	h*µg/mL	4.5 ± 0.76	21 ± 2.1
Bioavailability	%	21	
LogP[a]		3.30	
PSA[a]	A^2	72.7	
pKa[a]		5.93	

[a] The values were calculated using the ACD/Labs version 12.02 software. Pharmacokinetic parameters were calculated using non-compartmental analysis function with a logarithmic trapezoidal method in Phoenix WinNonlin 8.1

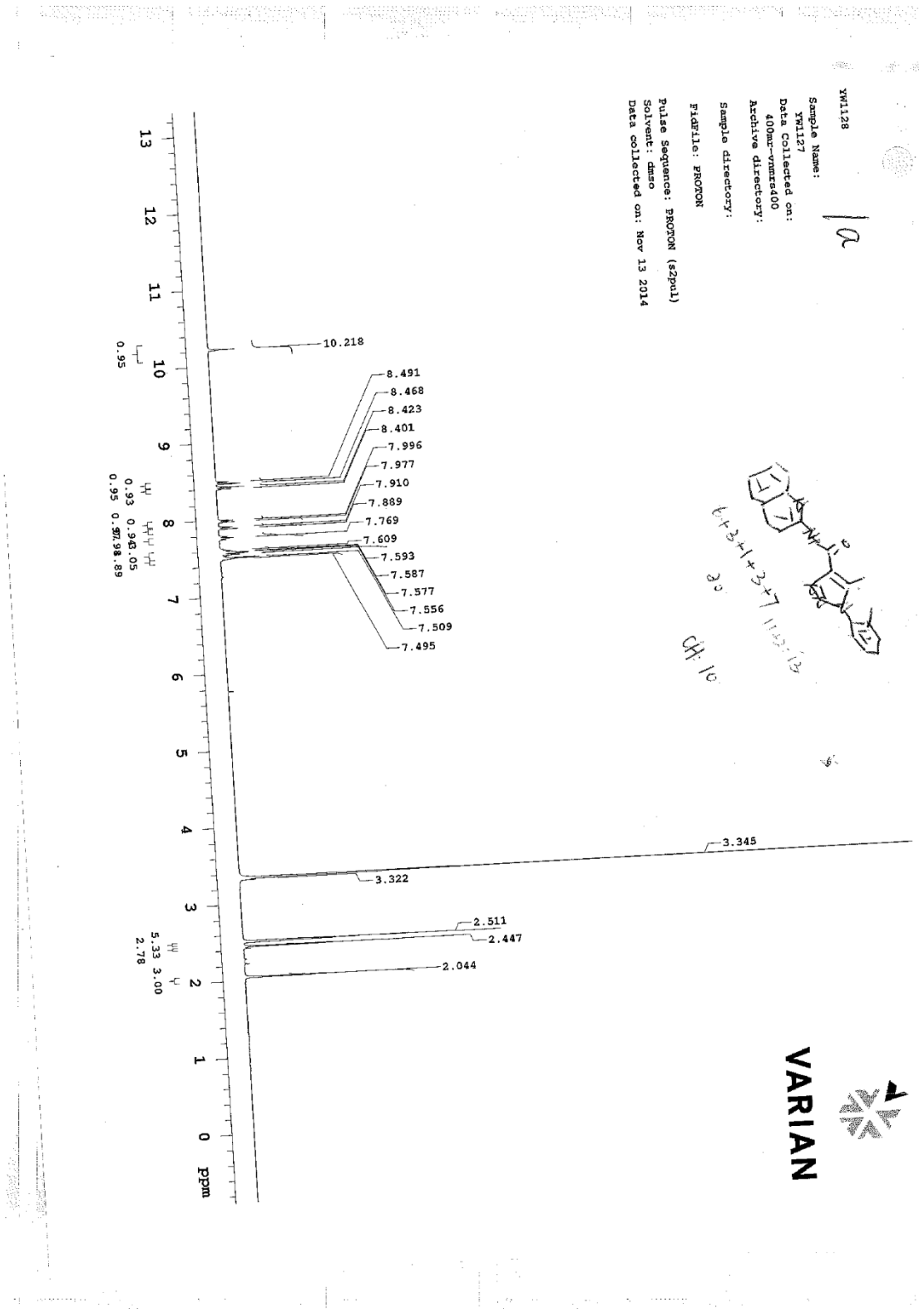


Figure S6. ^{1}H NMR spectrum of compound **3a**

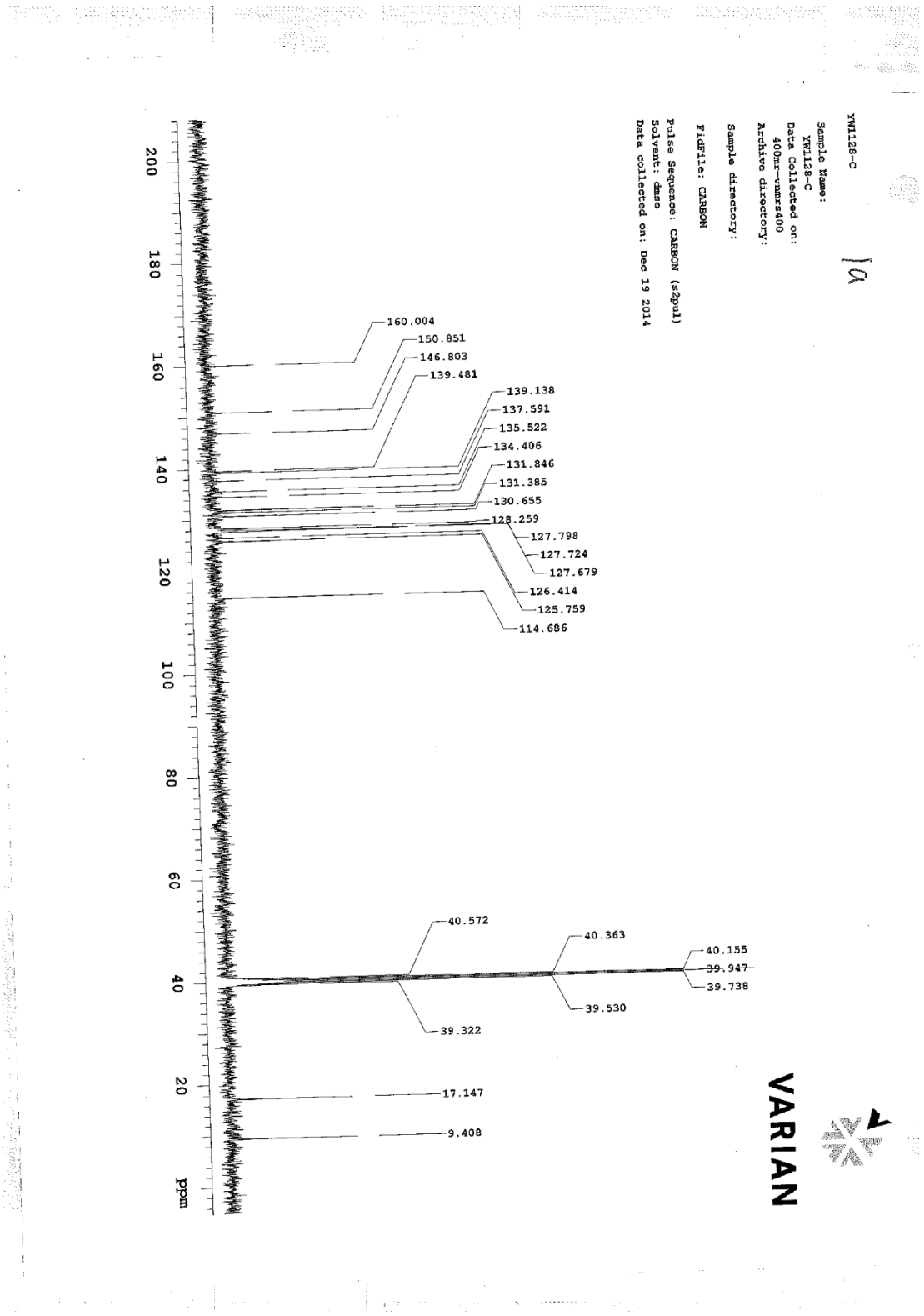


Figure S7. ^{13}C NMR spectrum of compound **3a**

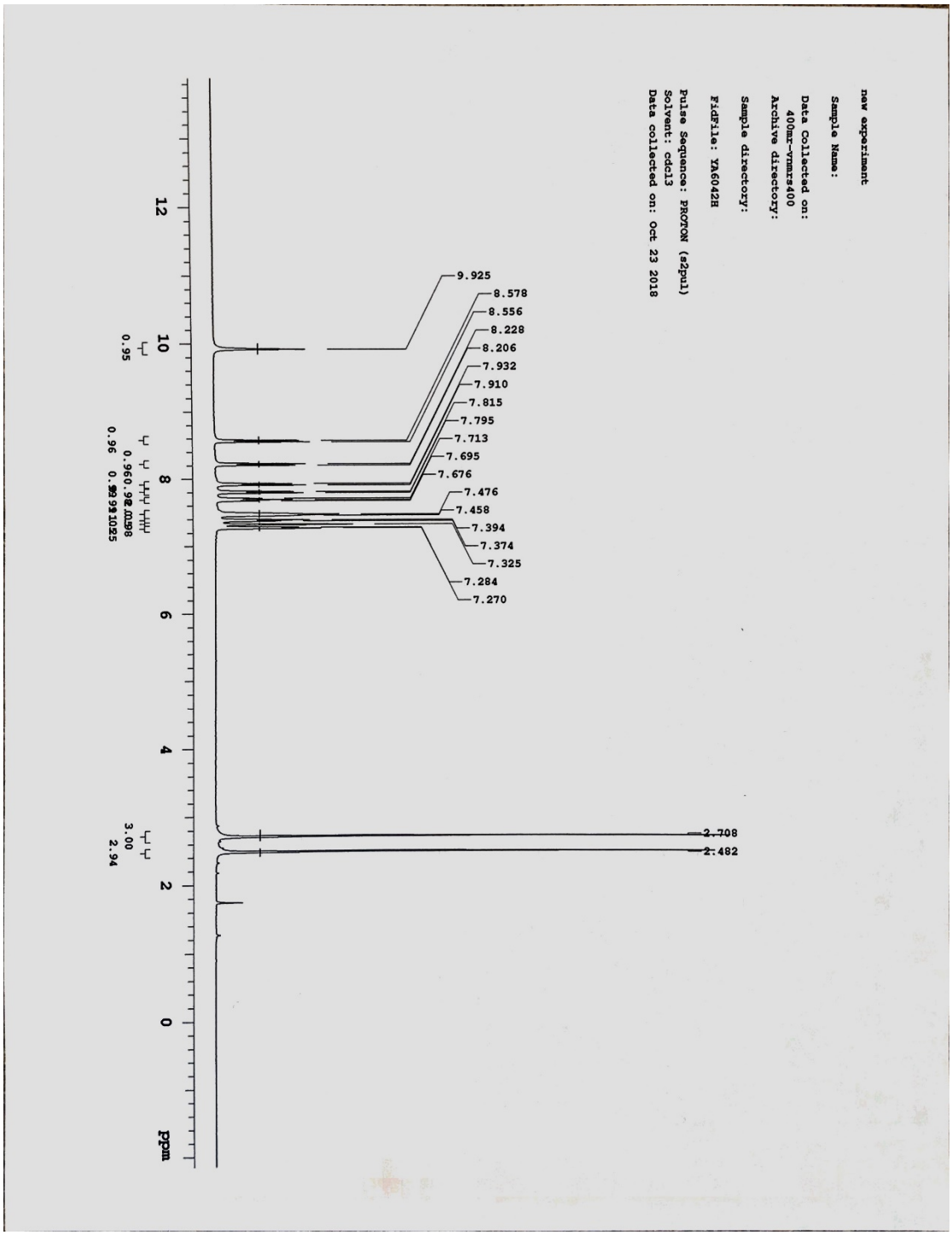


Figure S8. ^{1}H NMR spectrum of compound **3b**

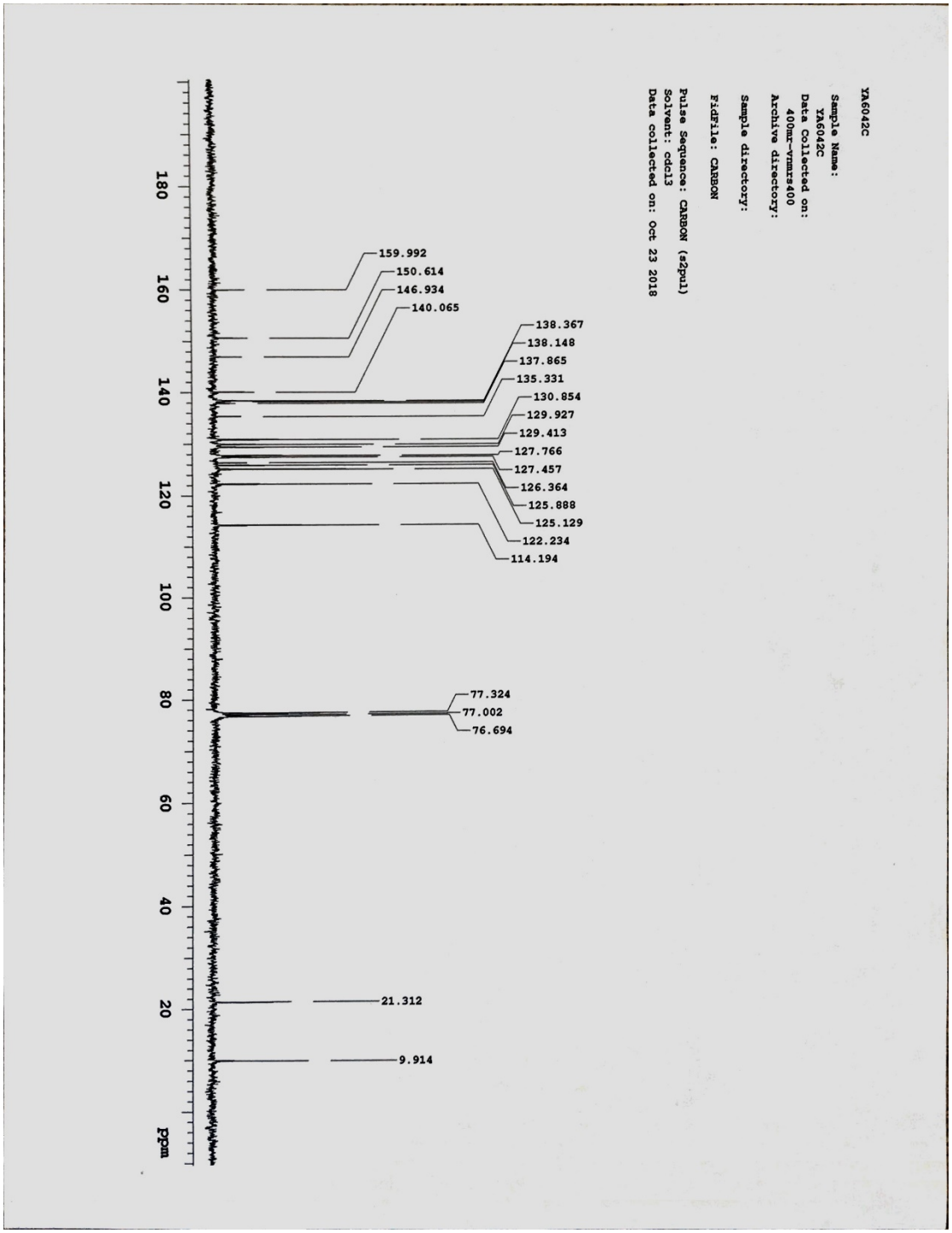


Figure S9. ^{13}C NMR spectrum of compound **3b**

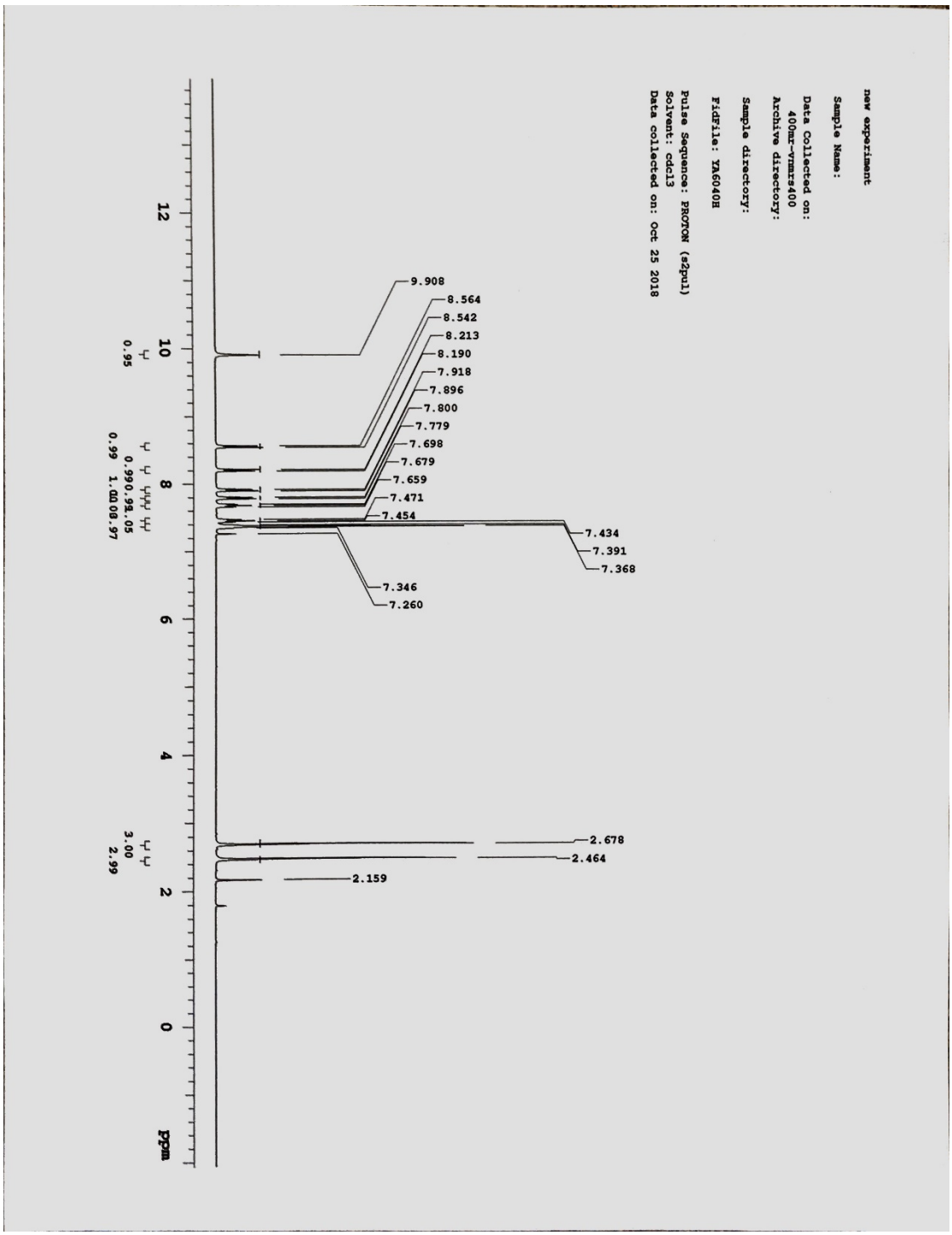


Figure S10. ^{1}H NMR spectrum of compound **3c**

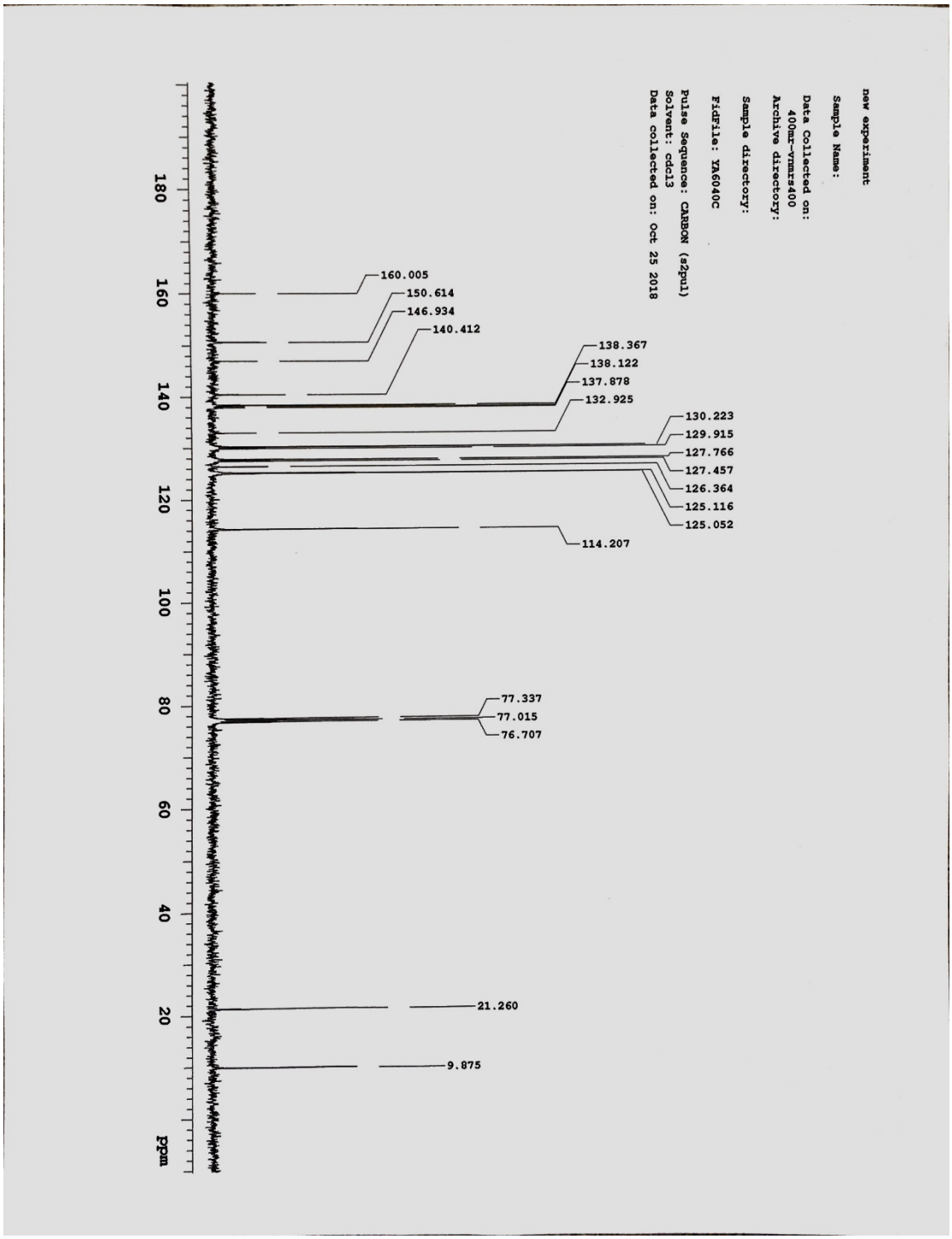


Figure S11. ^{13}C NMR spectrum of compound **3c**

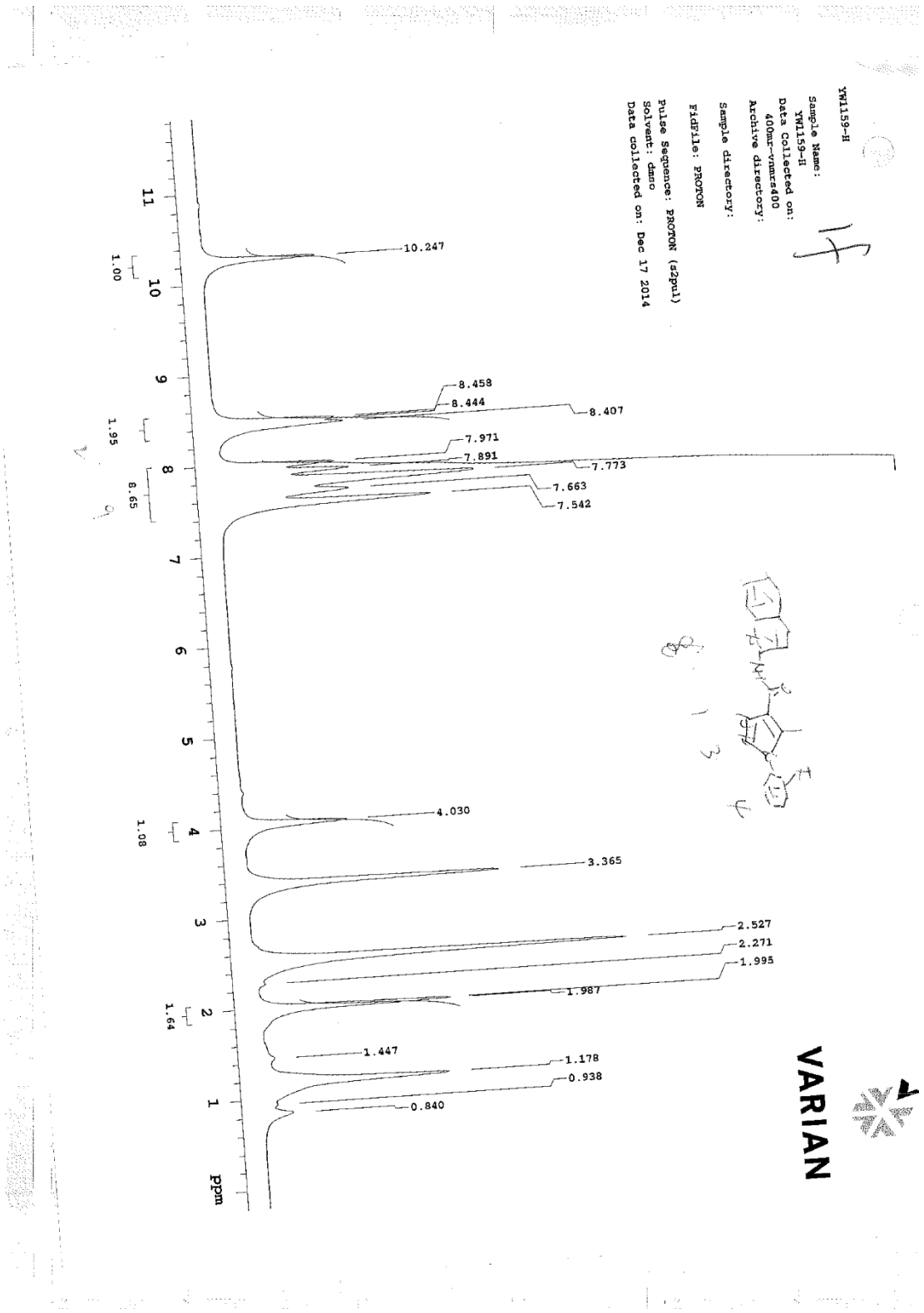


Figure S12. ^{1}H NMR spectrum of compound **3d**

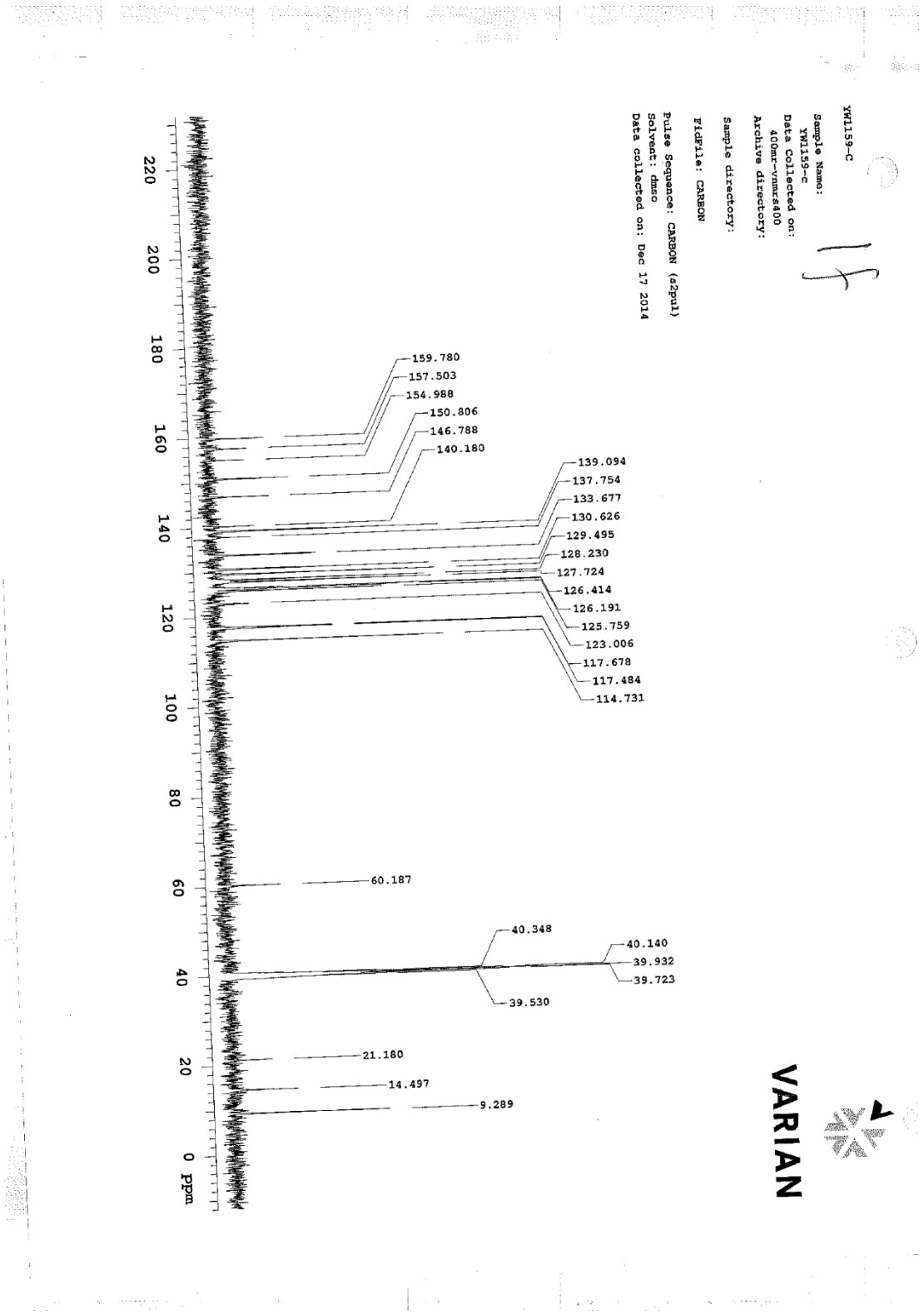


Figure S13. ^{13}C NMR spectrum of compound **3d**

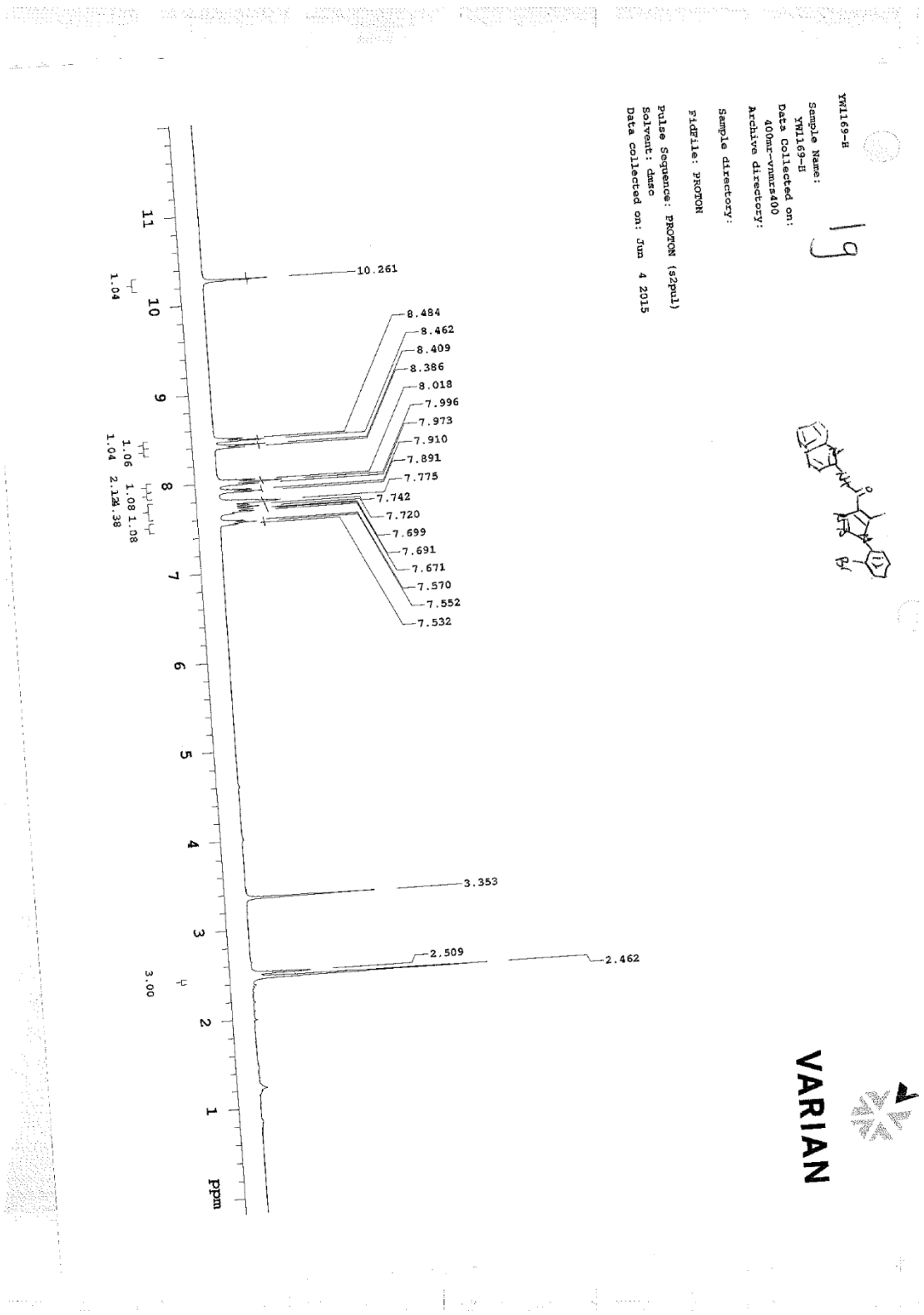


Figure S14. ^{1}H NMR spectrum of compound **3e**

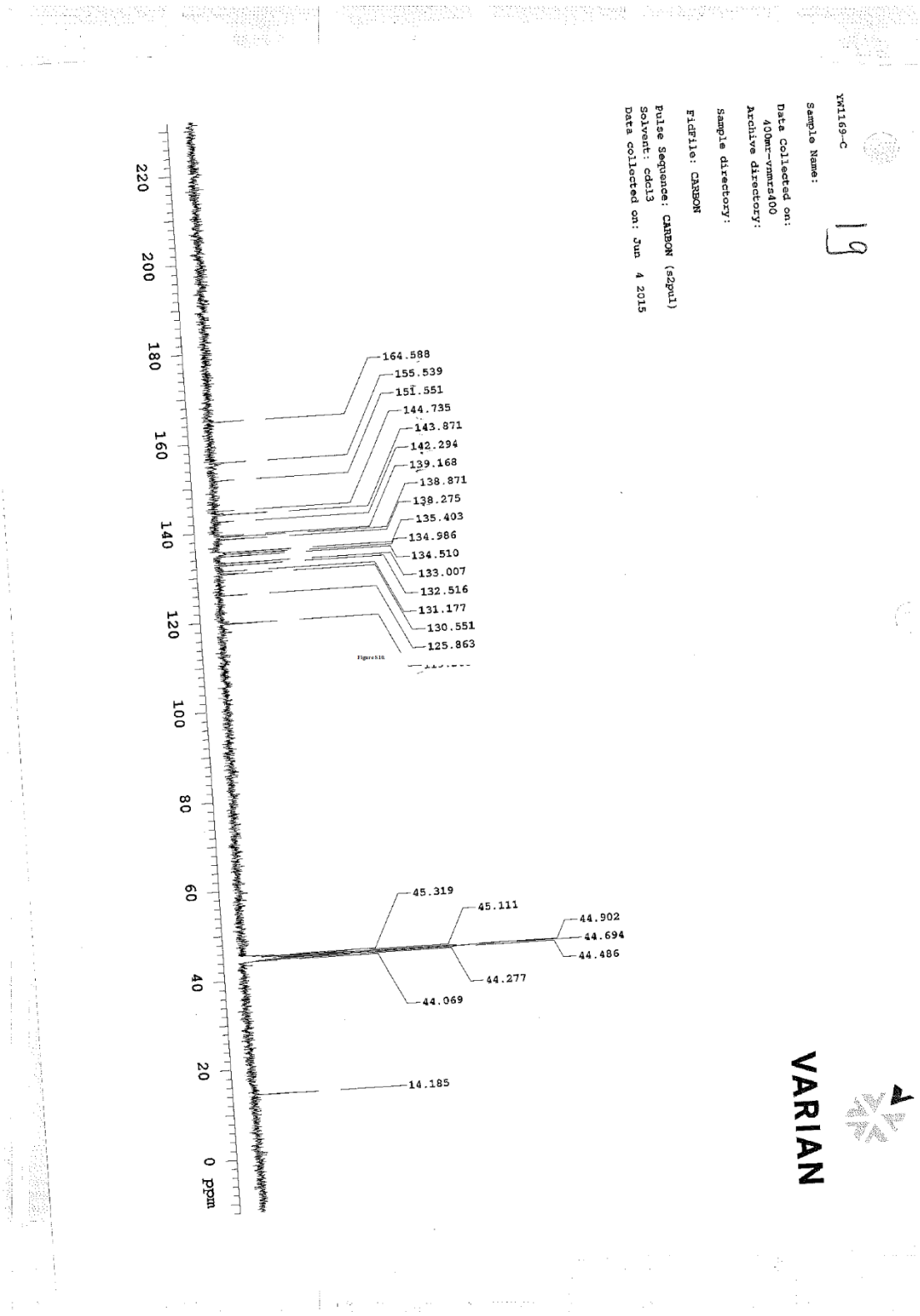


Figure S15. ^{13}C NMR spectrum of compound **3e**

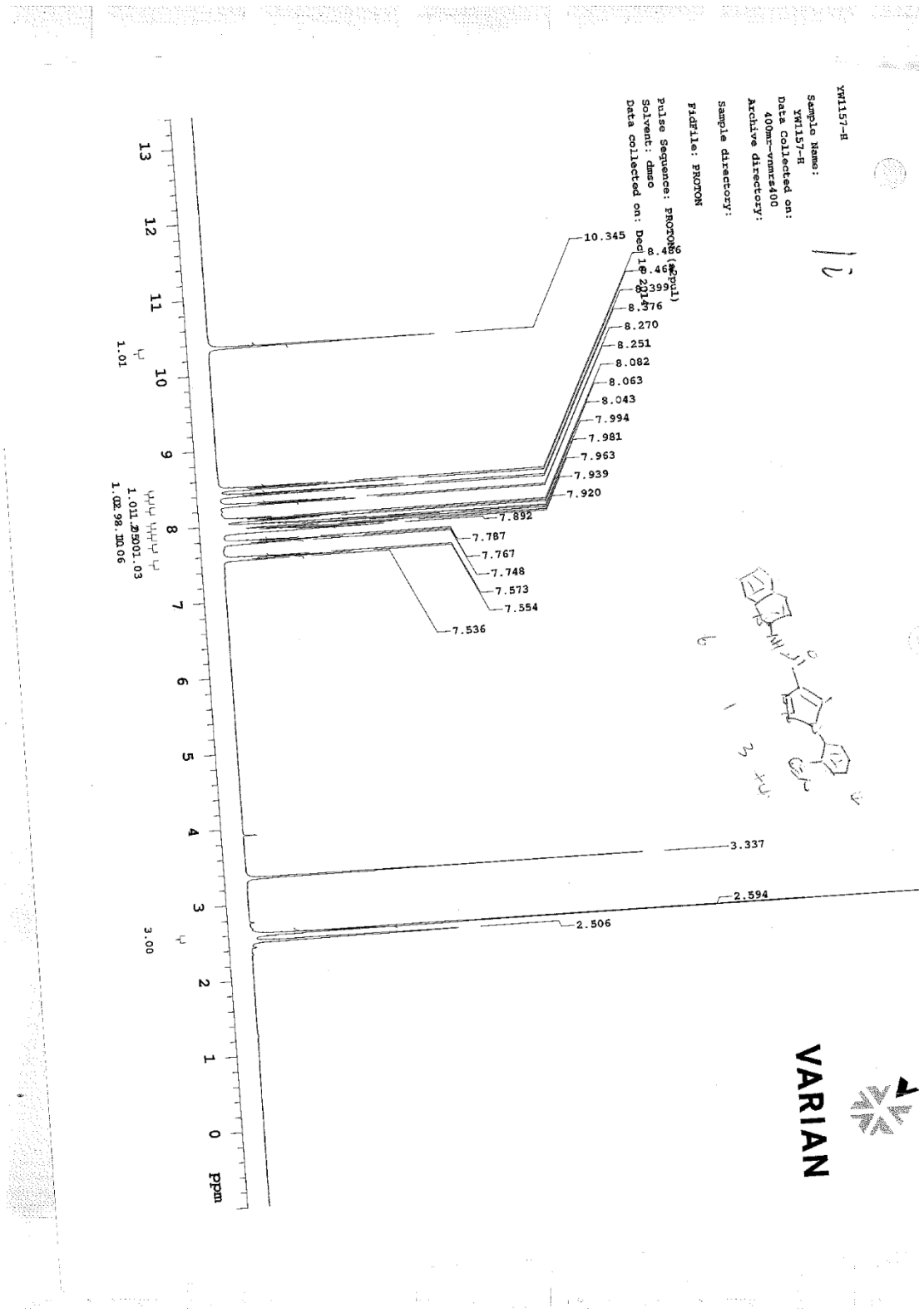


Figure S16. ^{1}H NMR spectrum of compound **3f**

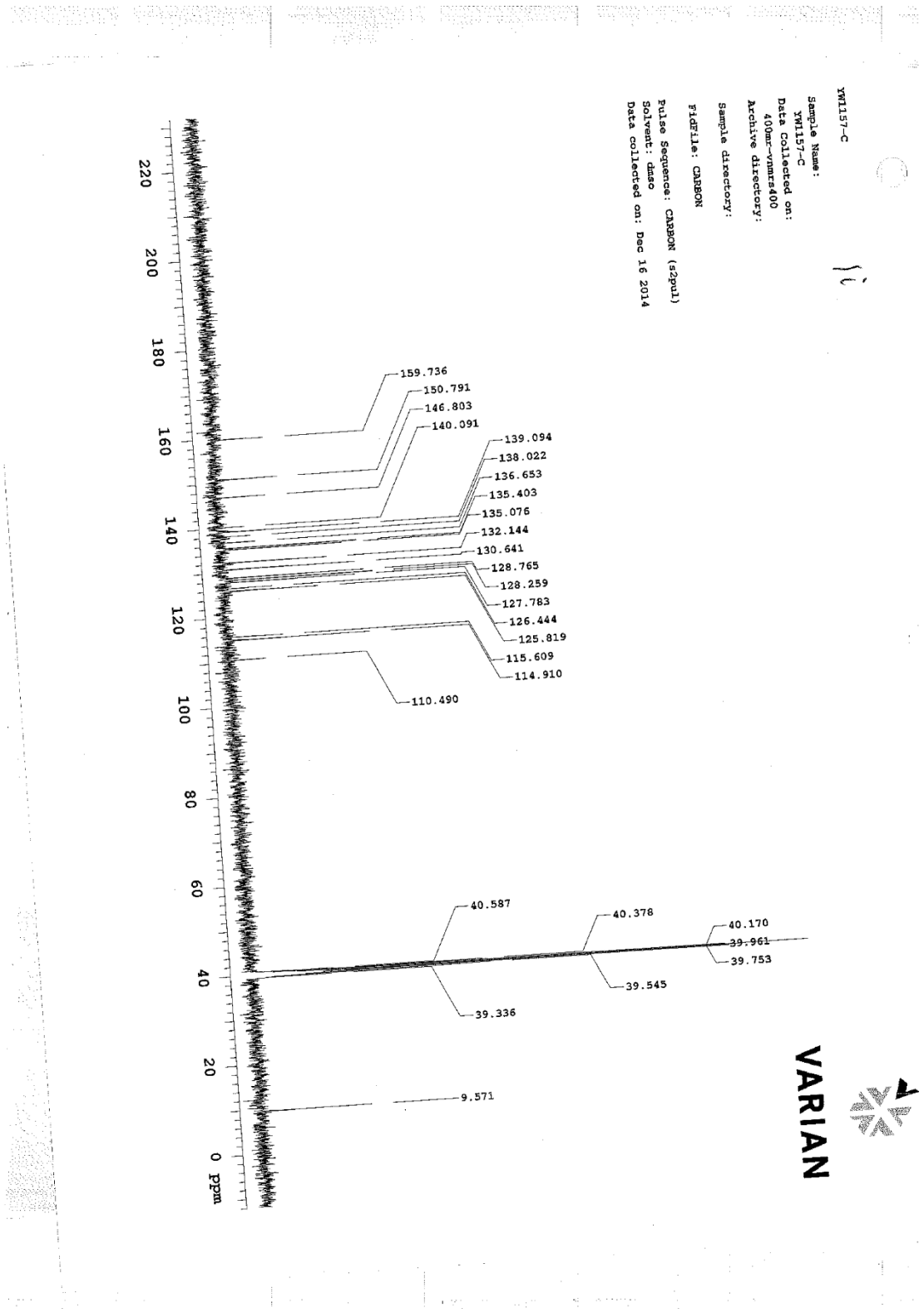


Figure S17. ^{13}C NMR spectrum of compound **3f**

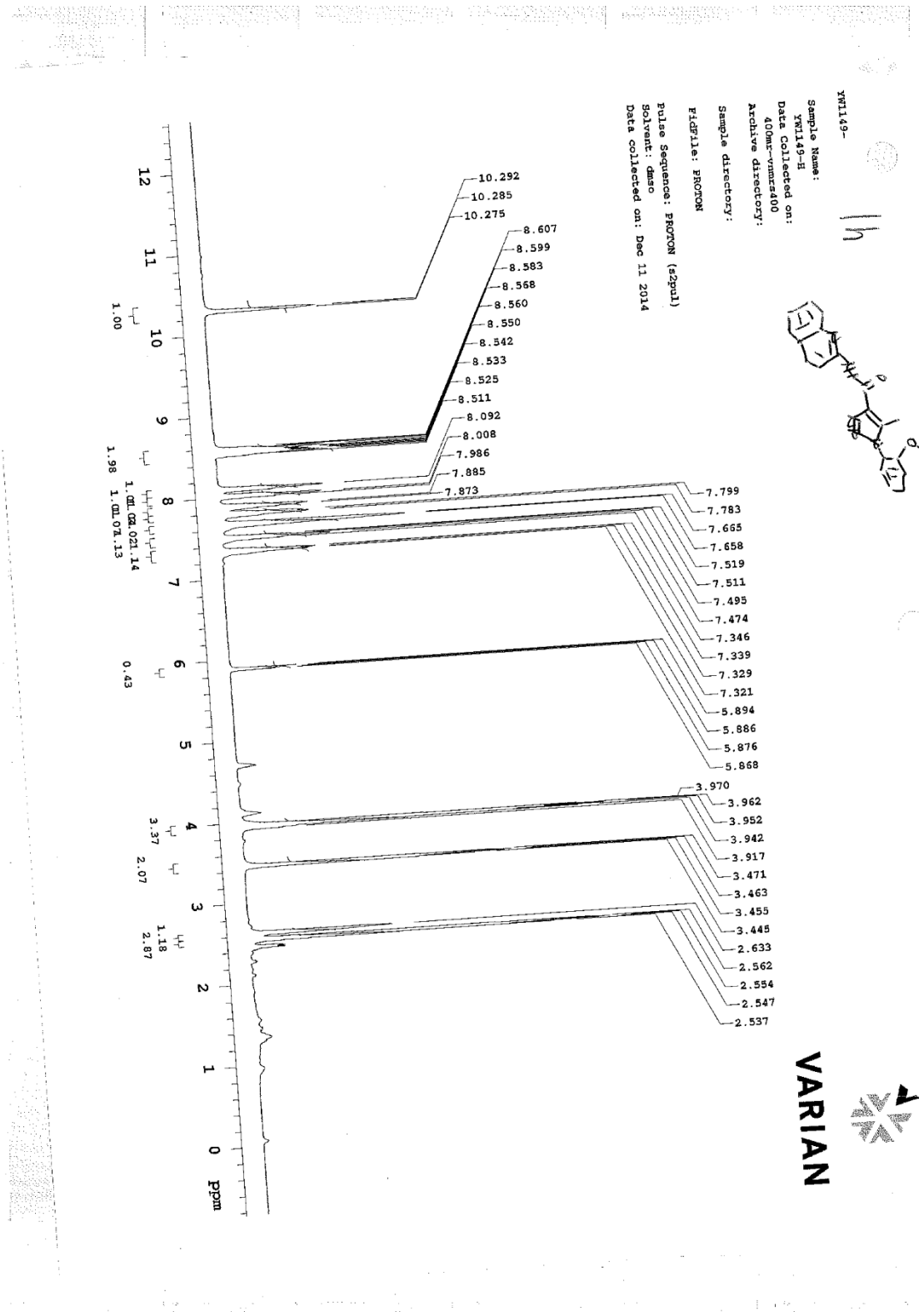


Figure S18. ^{1}H NMR spectrum of compound **3g**

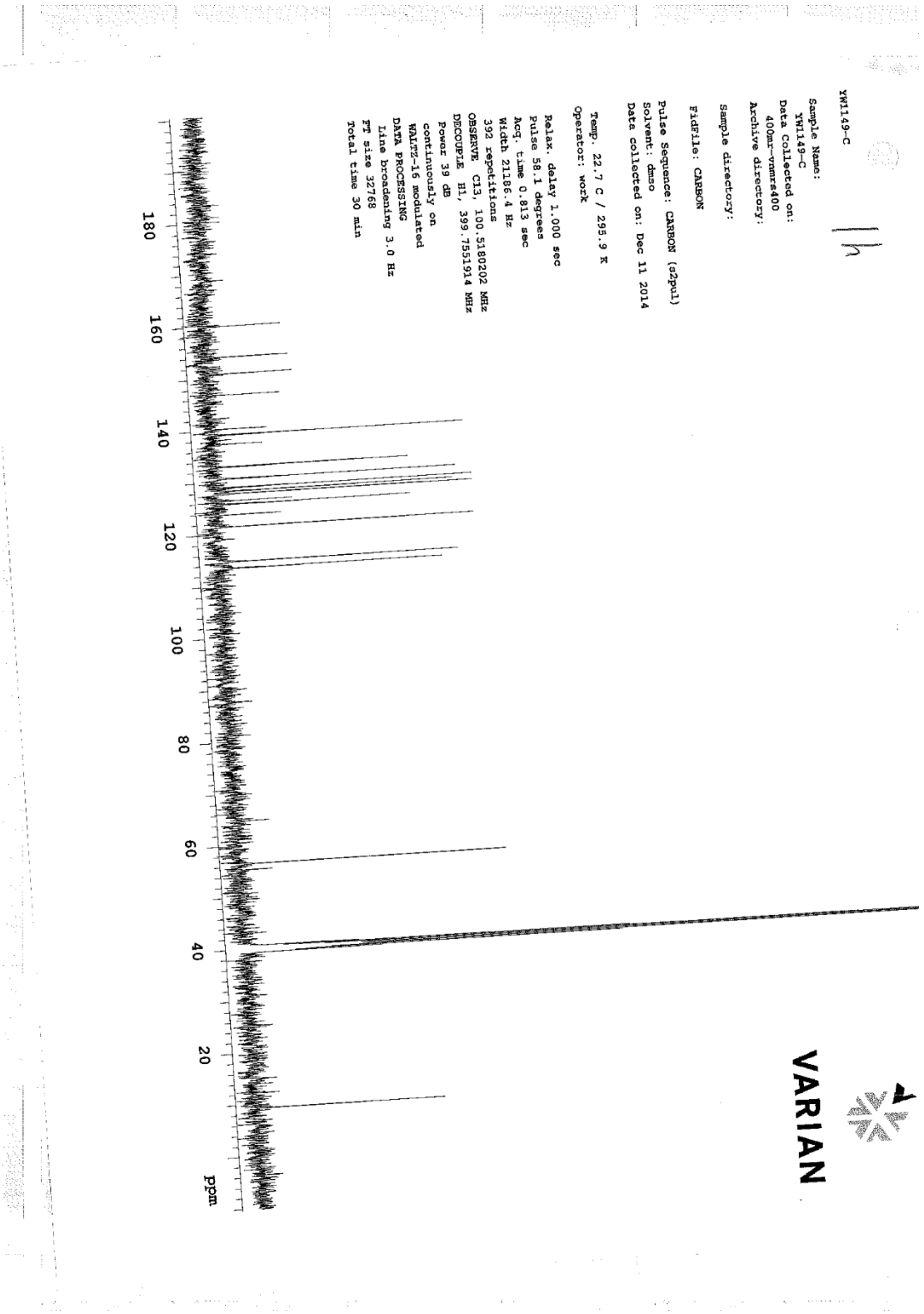


Figure S19. ^{13}C NMR spectrum of compound **3g**

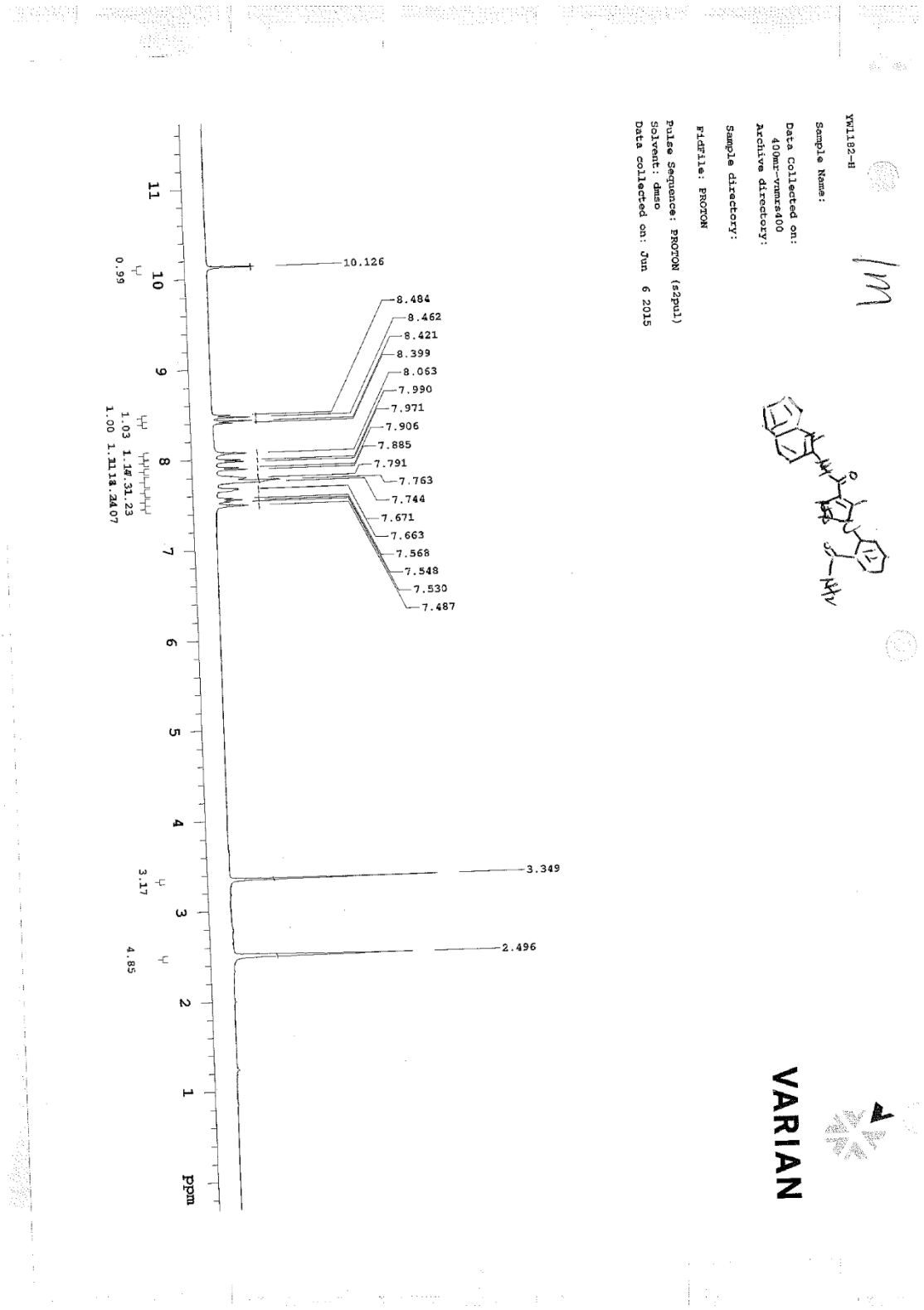


Figure S20. ^{1}H NMR spectrum of compound **3h**

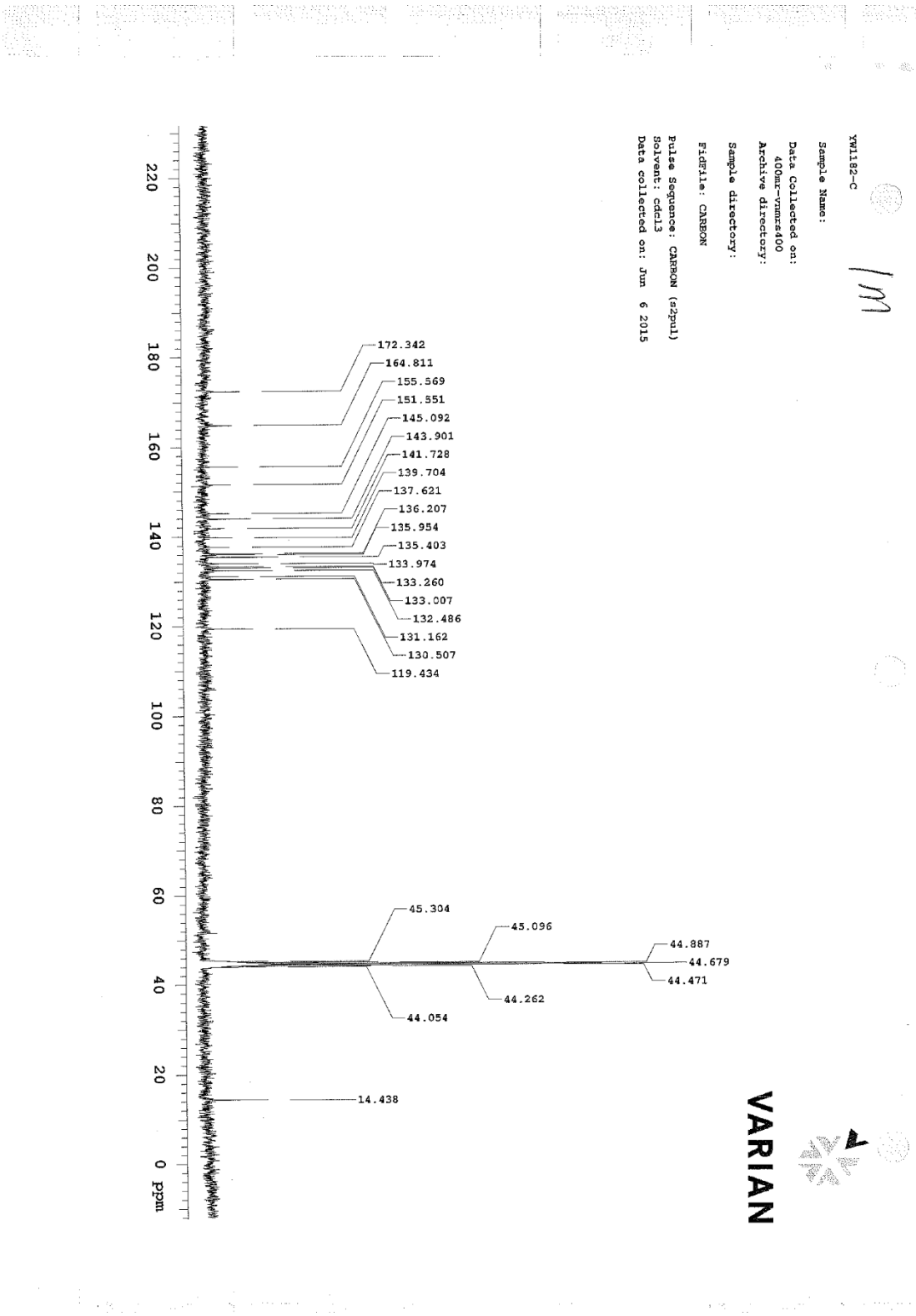


Figure S21. ^{13}C NMR spectrum of compound **3h**

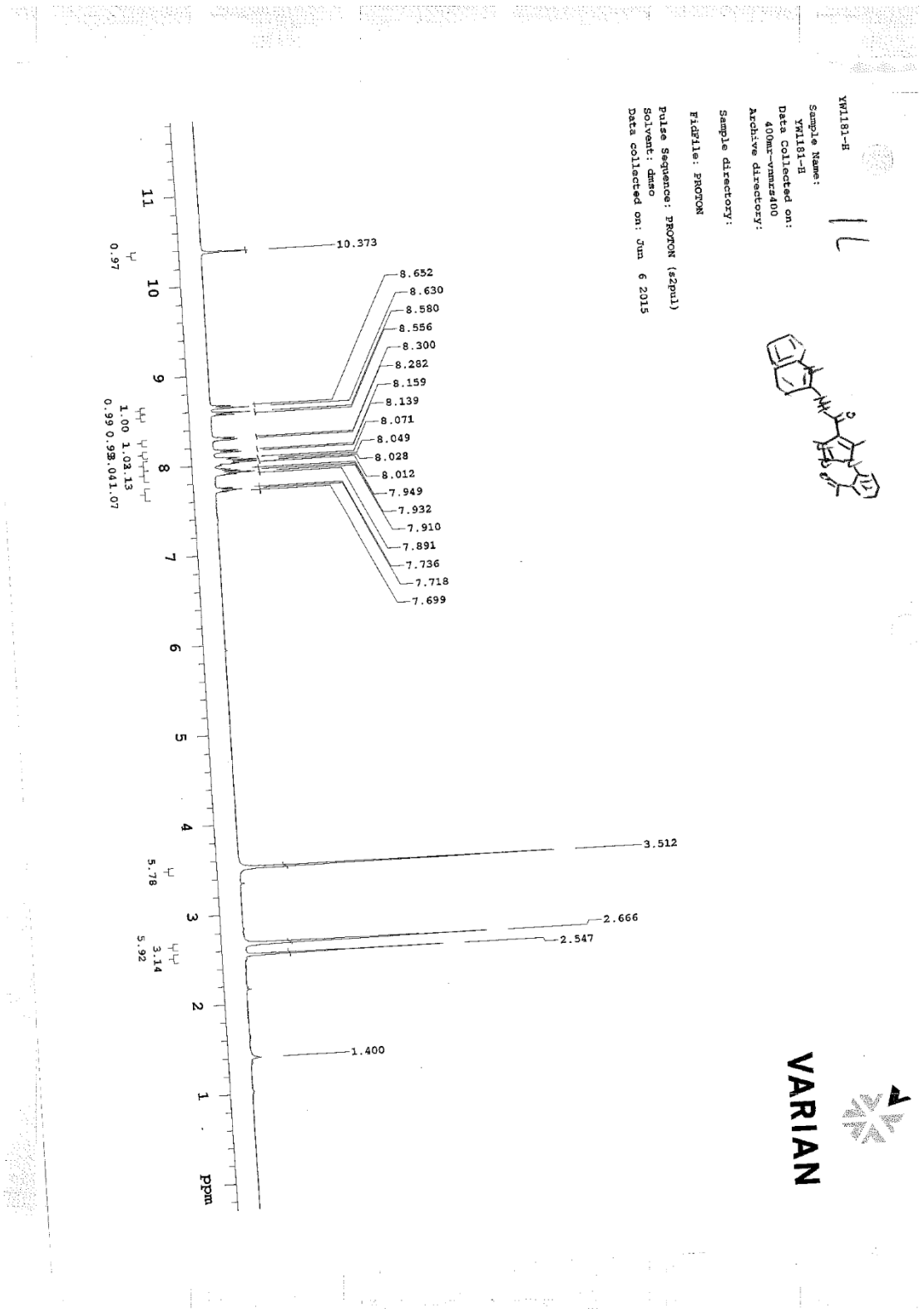


Figure S22. ^{1}H NMR spectrum of compound **3i**

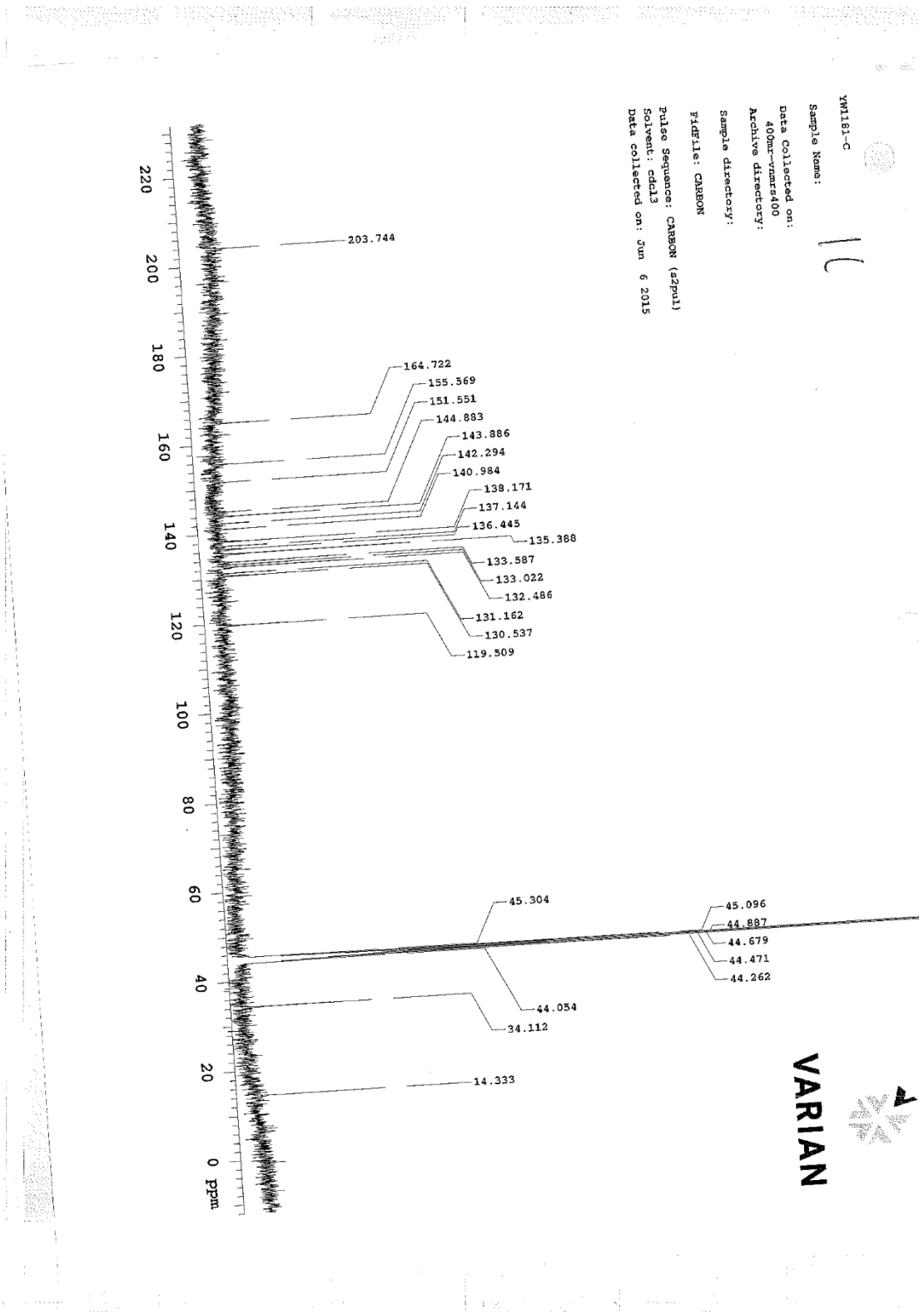


Figure S23. ^{13}C NMR spectrum of compound **3i**

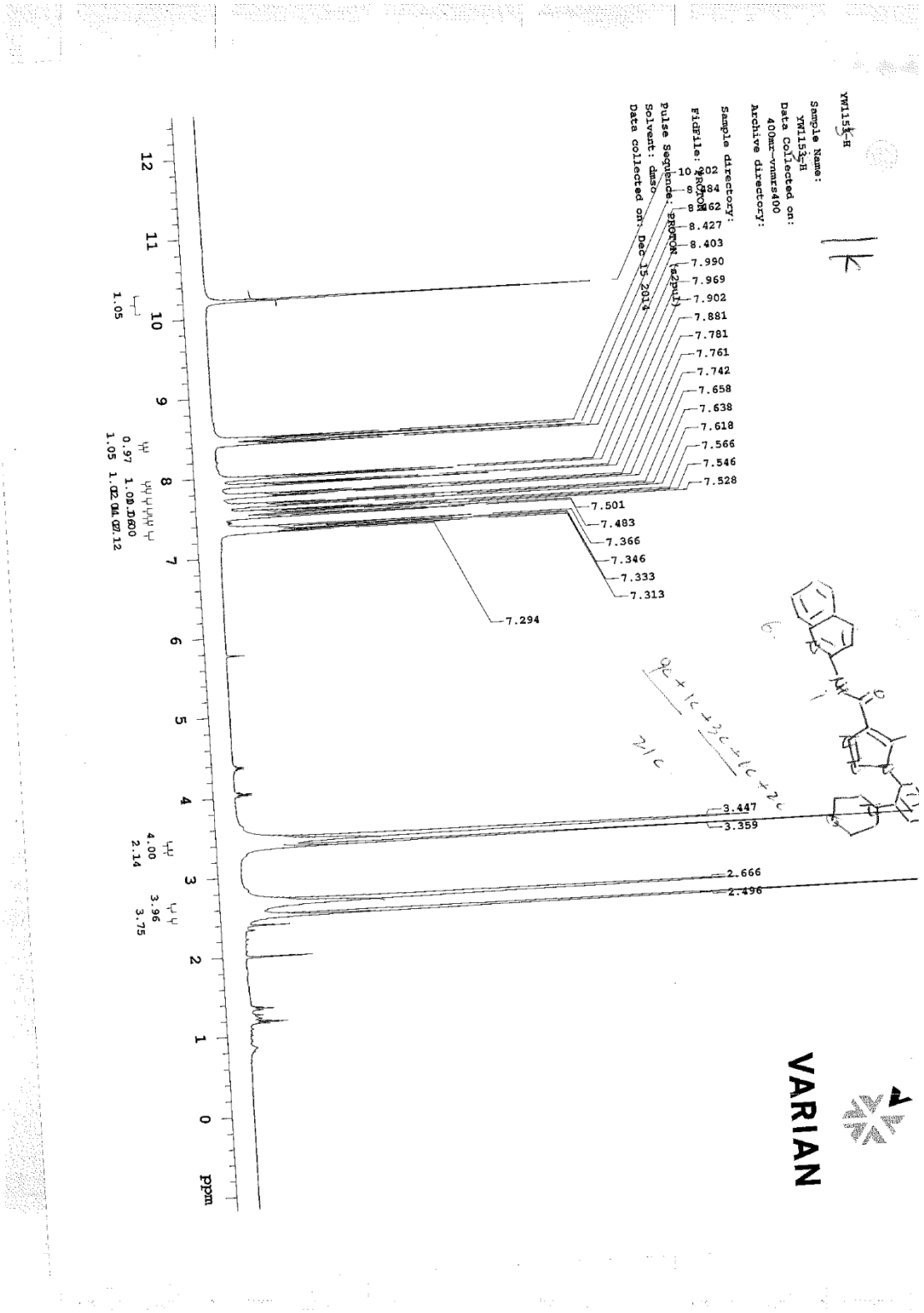


Figure S24. ^{1}H NMR spectrum of compound **3j**

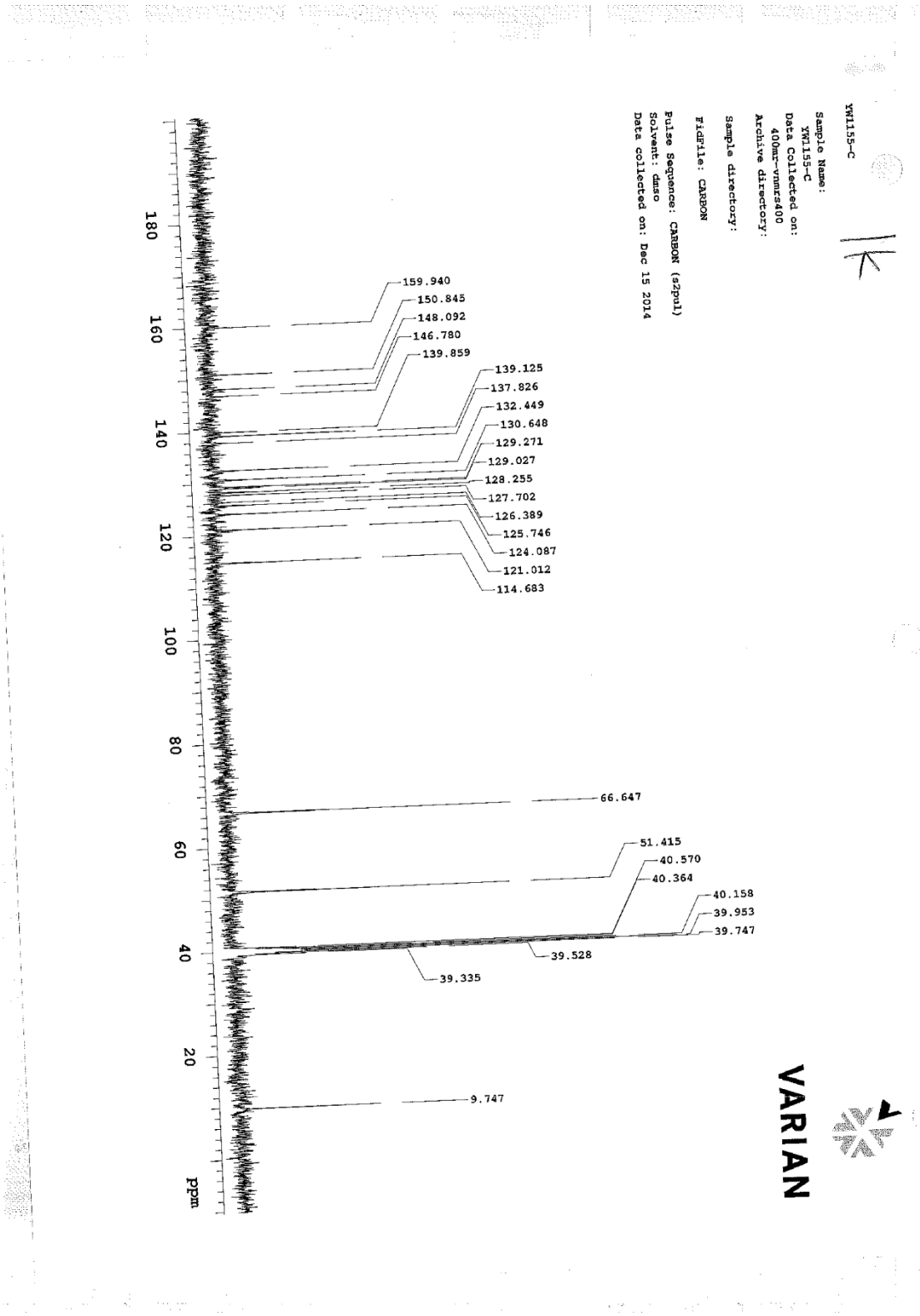


Figure S25. ^{13}C NMR spectrum of compound **3j**

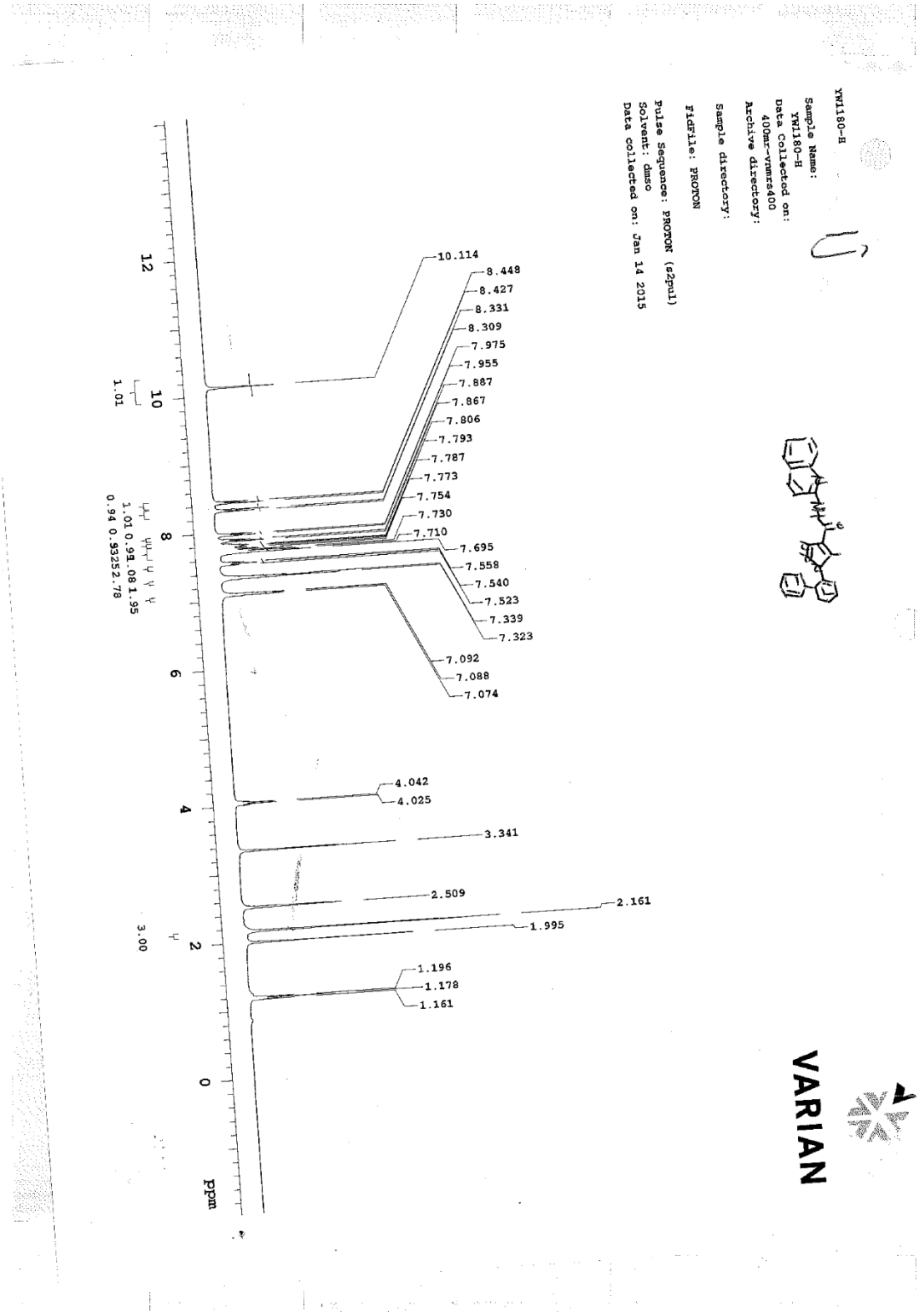


Figure S26. ^{1}H NMR spectrum of compound **3k**

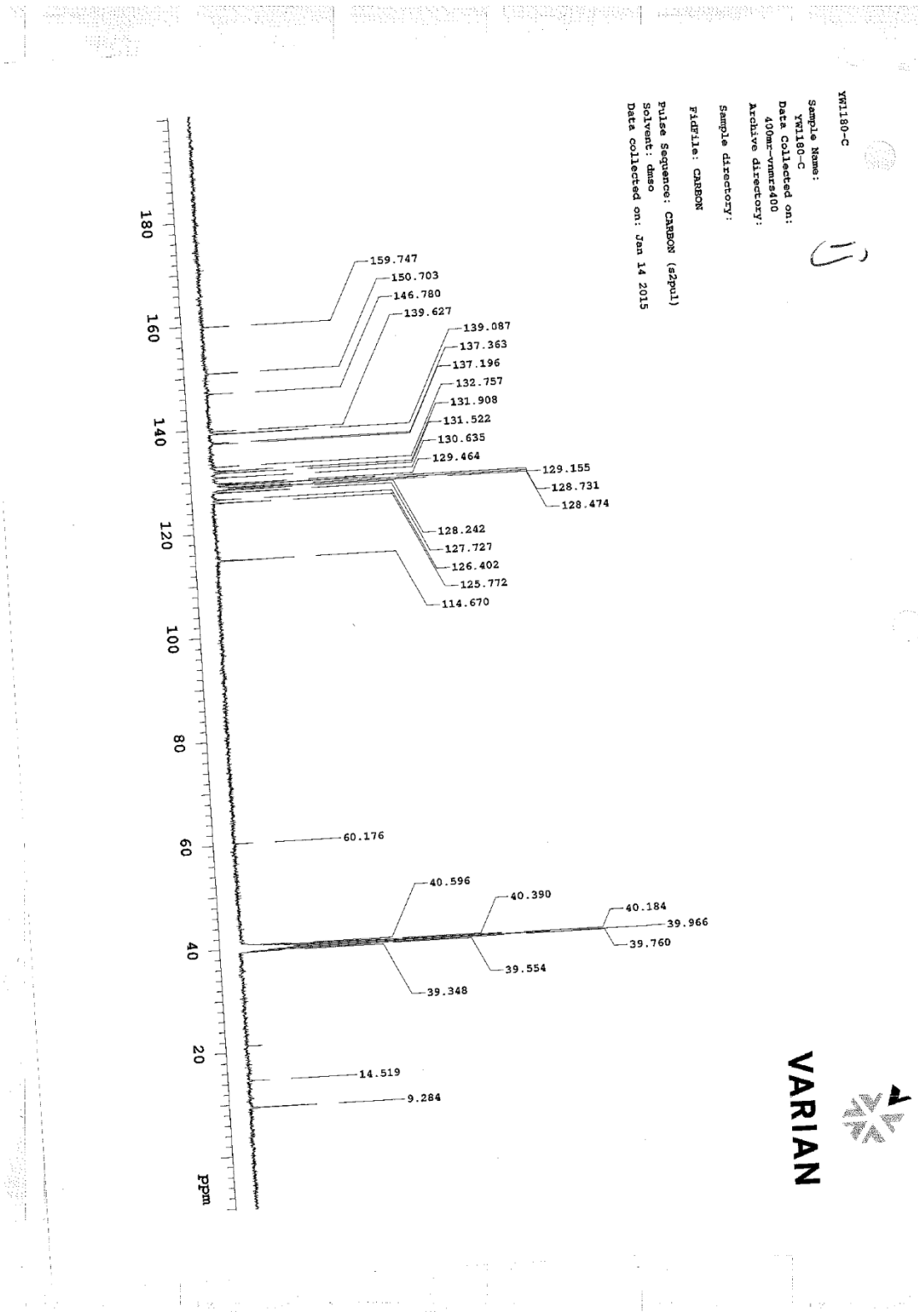


Figure S27. ^{13}C NMR spectrum of compound **3k**

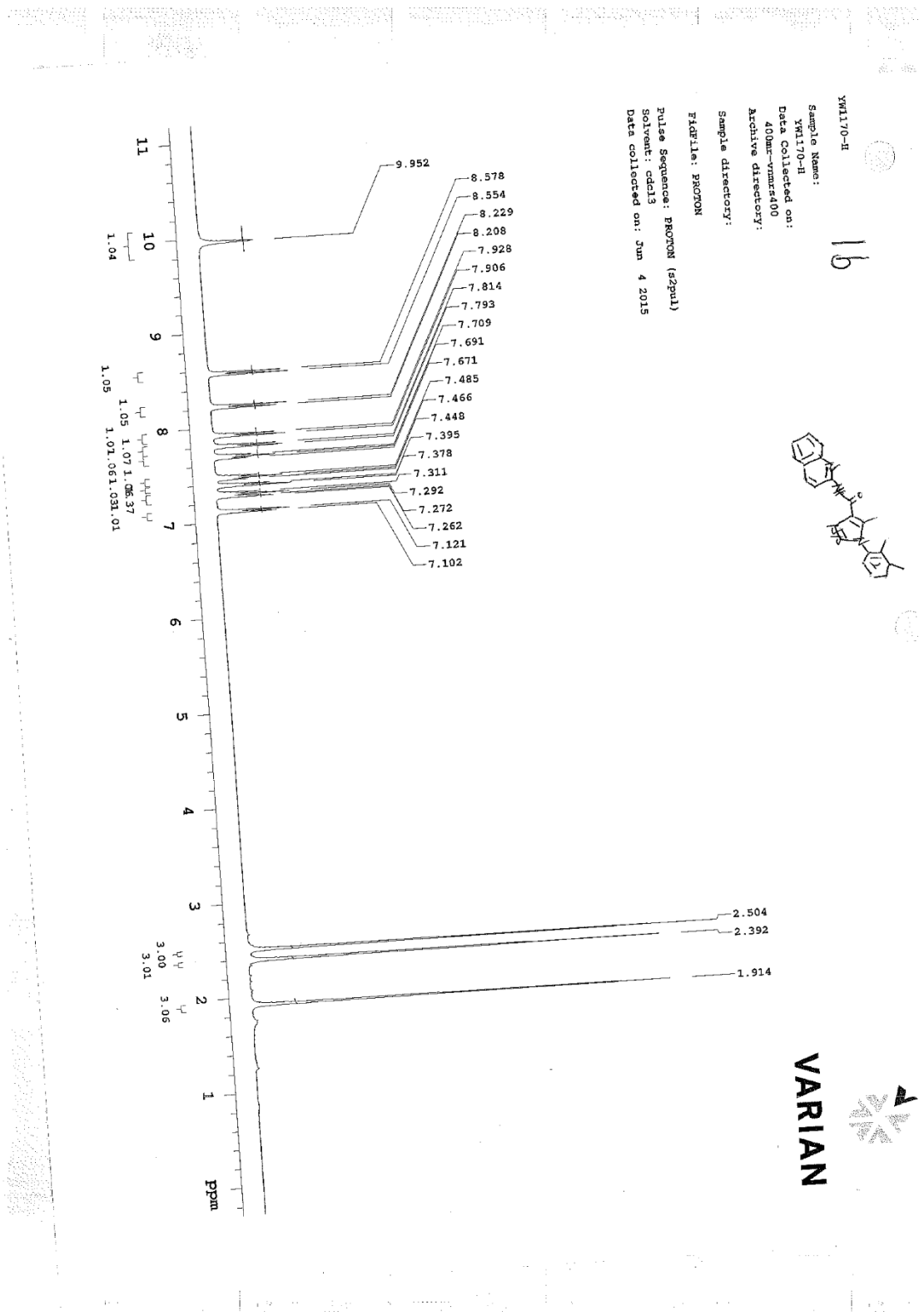


Figure S28. ^{1}H NMR spectrum of compound **3l**

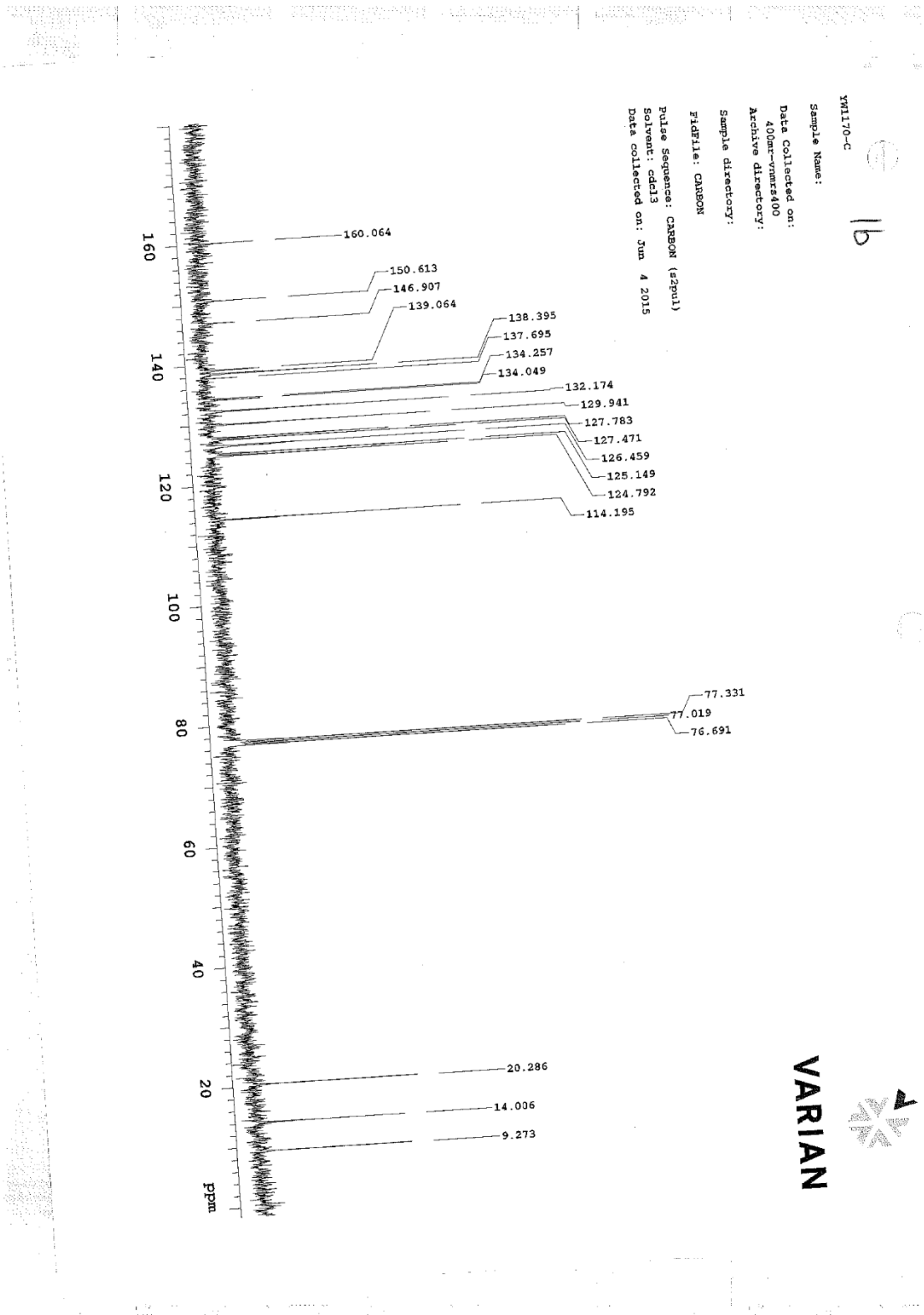


Figure S29. ^{13}C NMR spectrum of compound **3l**

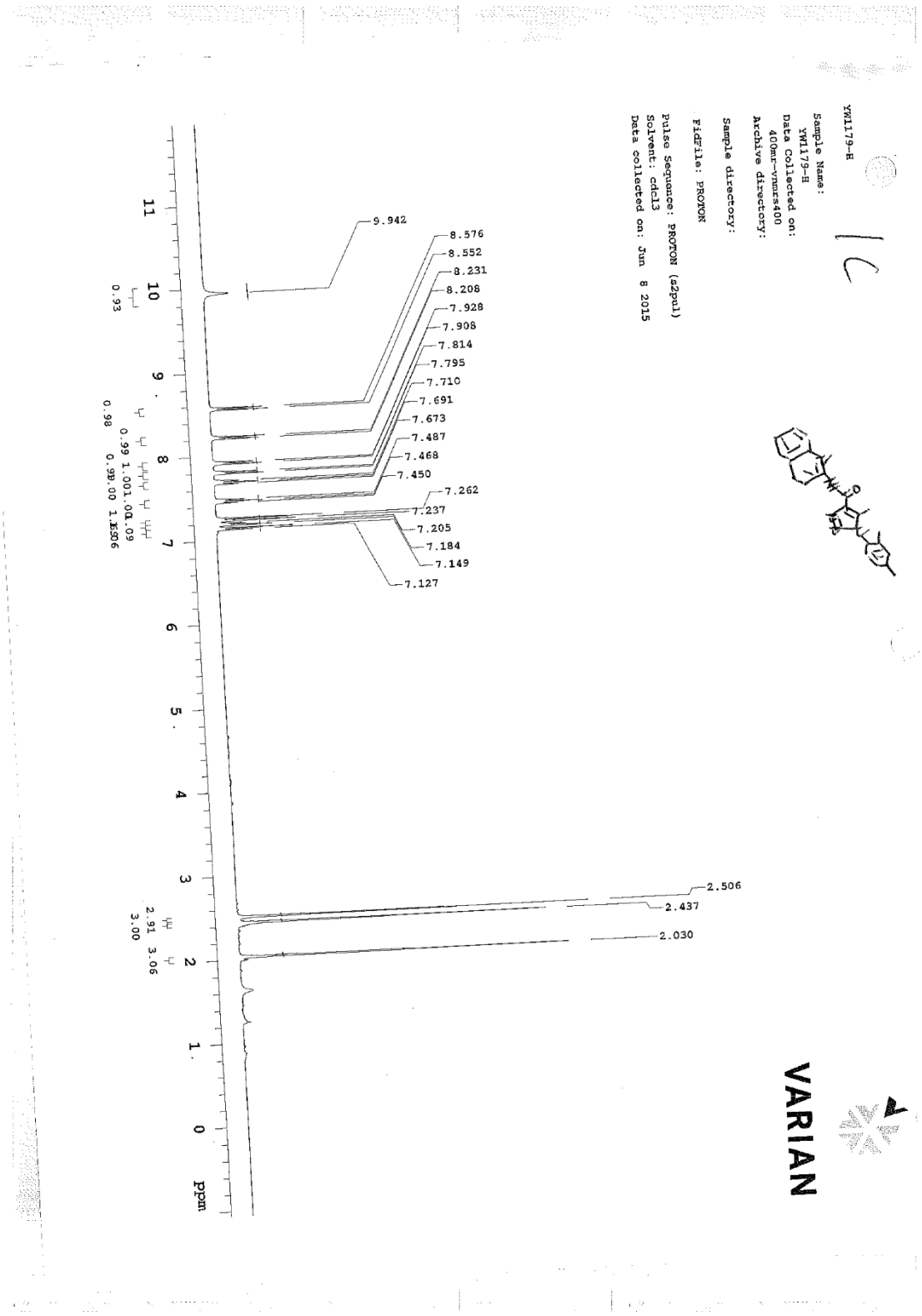


Figure S30. ^{1}H NMR spectrum of compound **3m**

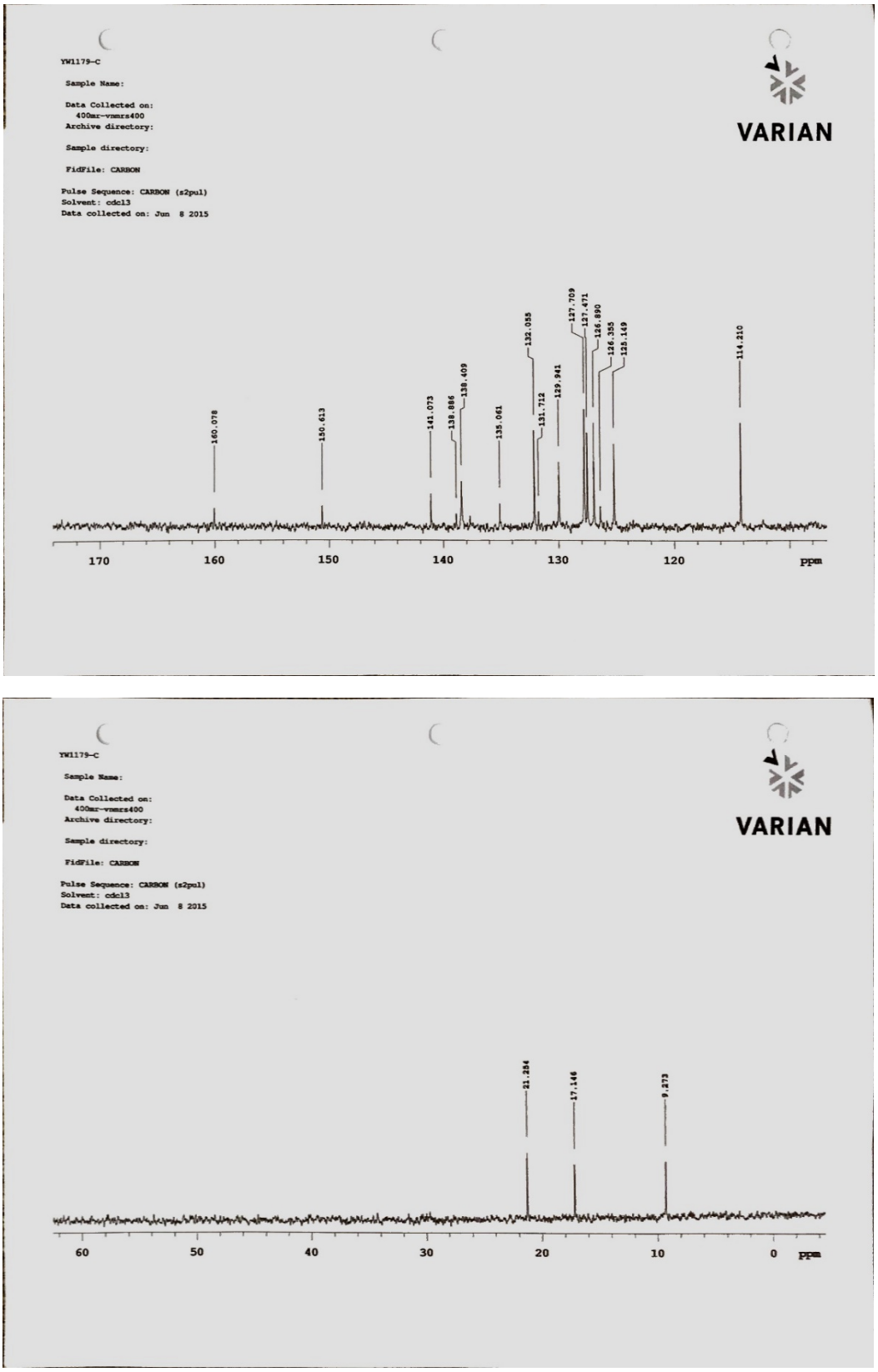


Figure S31. ^{13}C NMR spectrum of compound **3m**

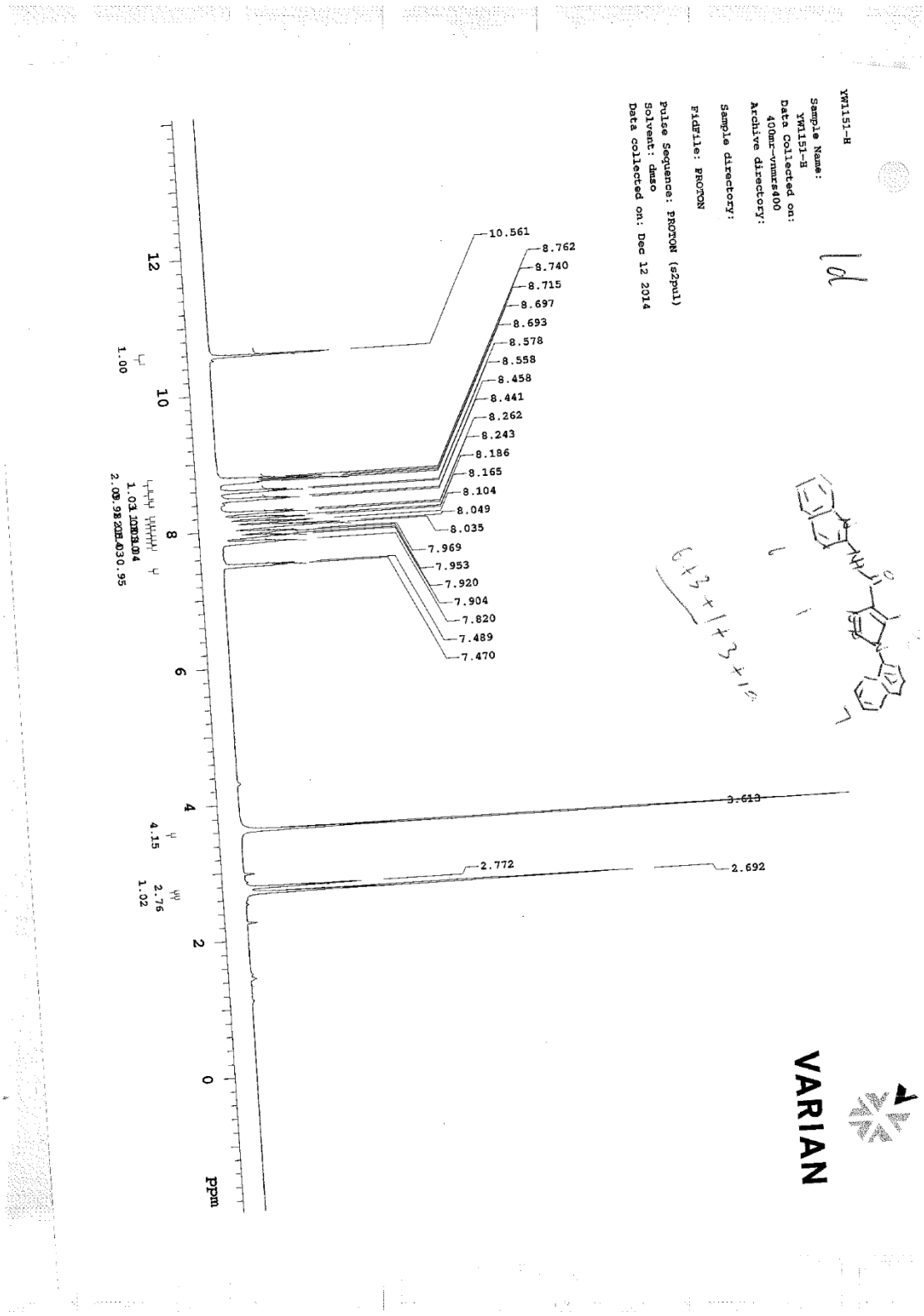


Figure S32. ^{1}H NMR spectrum of compound **3n**

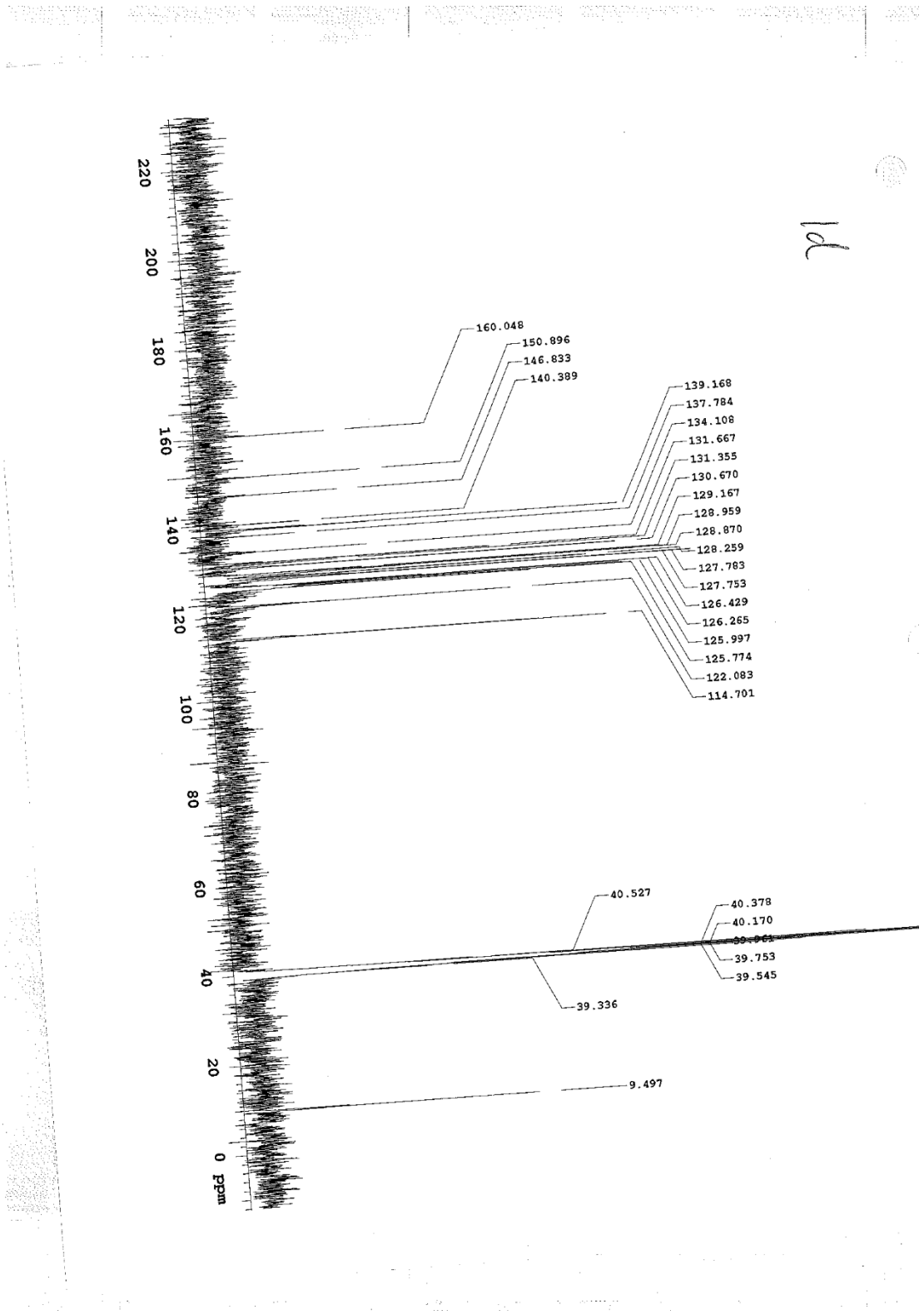


Figure S33. ^{13}C NMR spectrum of compound **3n**

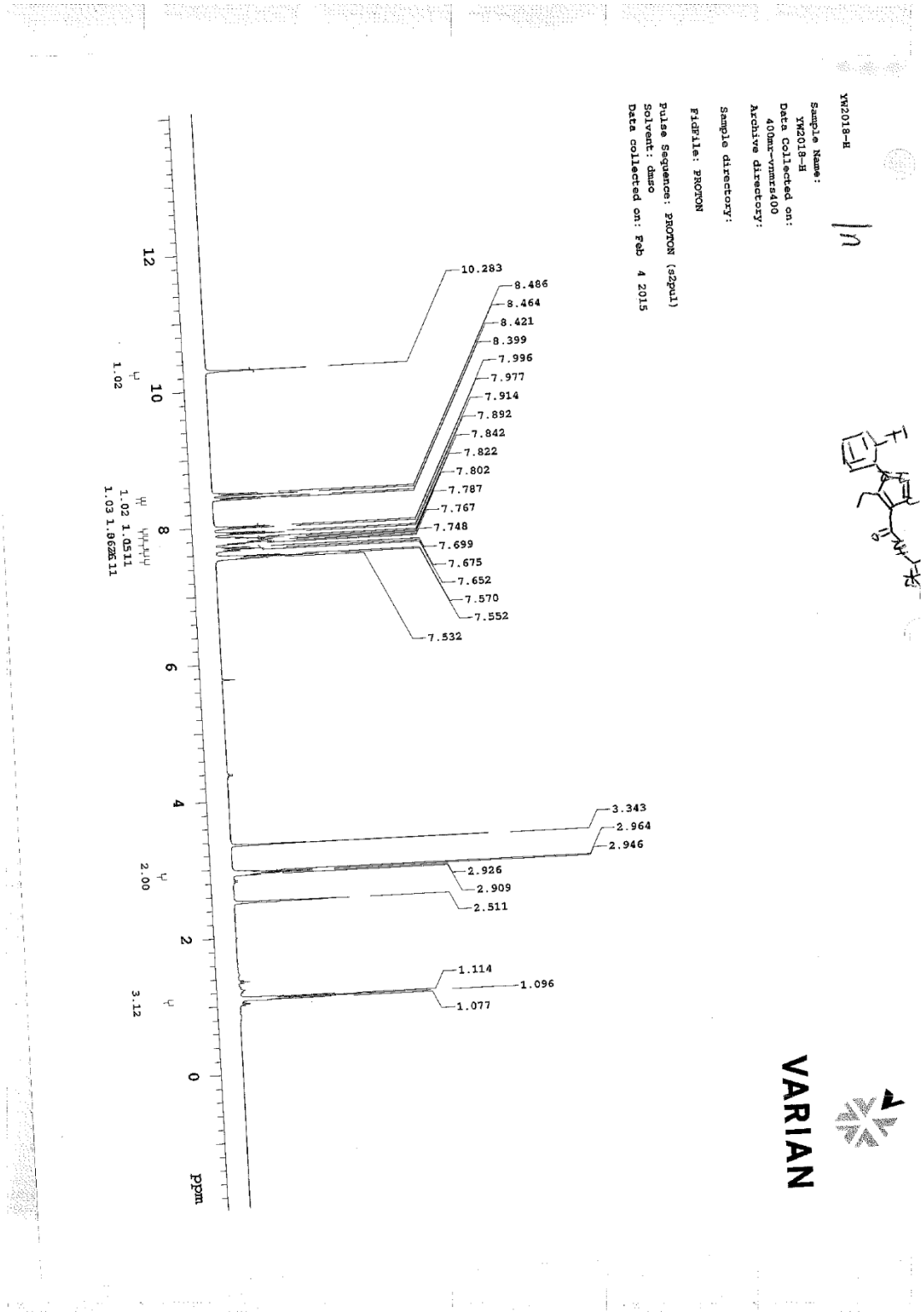


Figure S34. ^{1}H NMR spectrum of compound **3o**

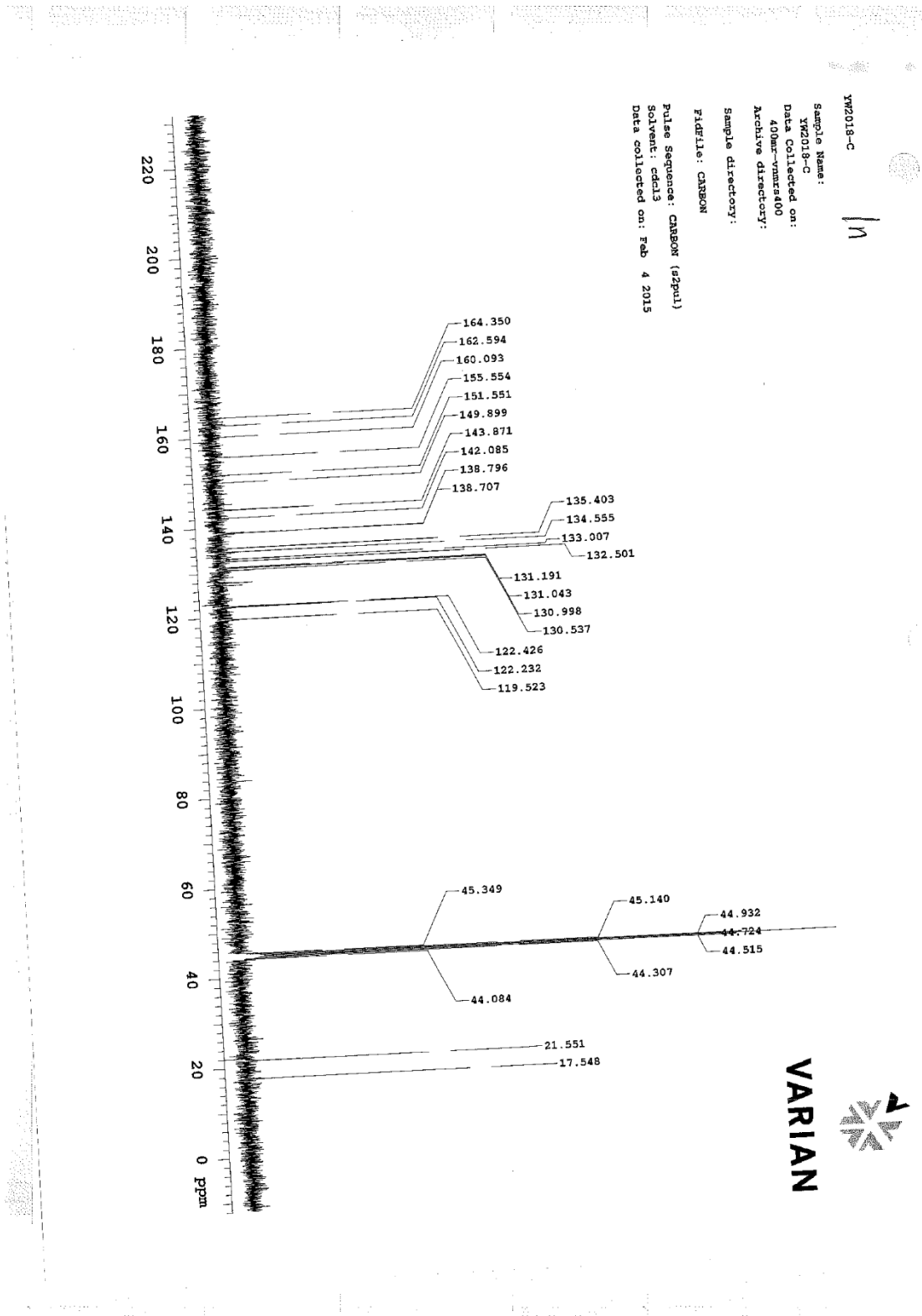


Figure S35. ^{13}C NMR spectrum of compound **3o**

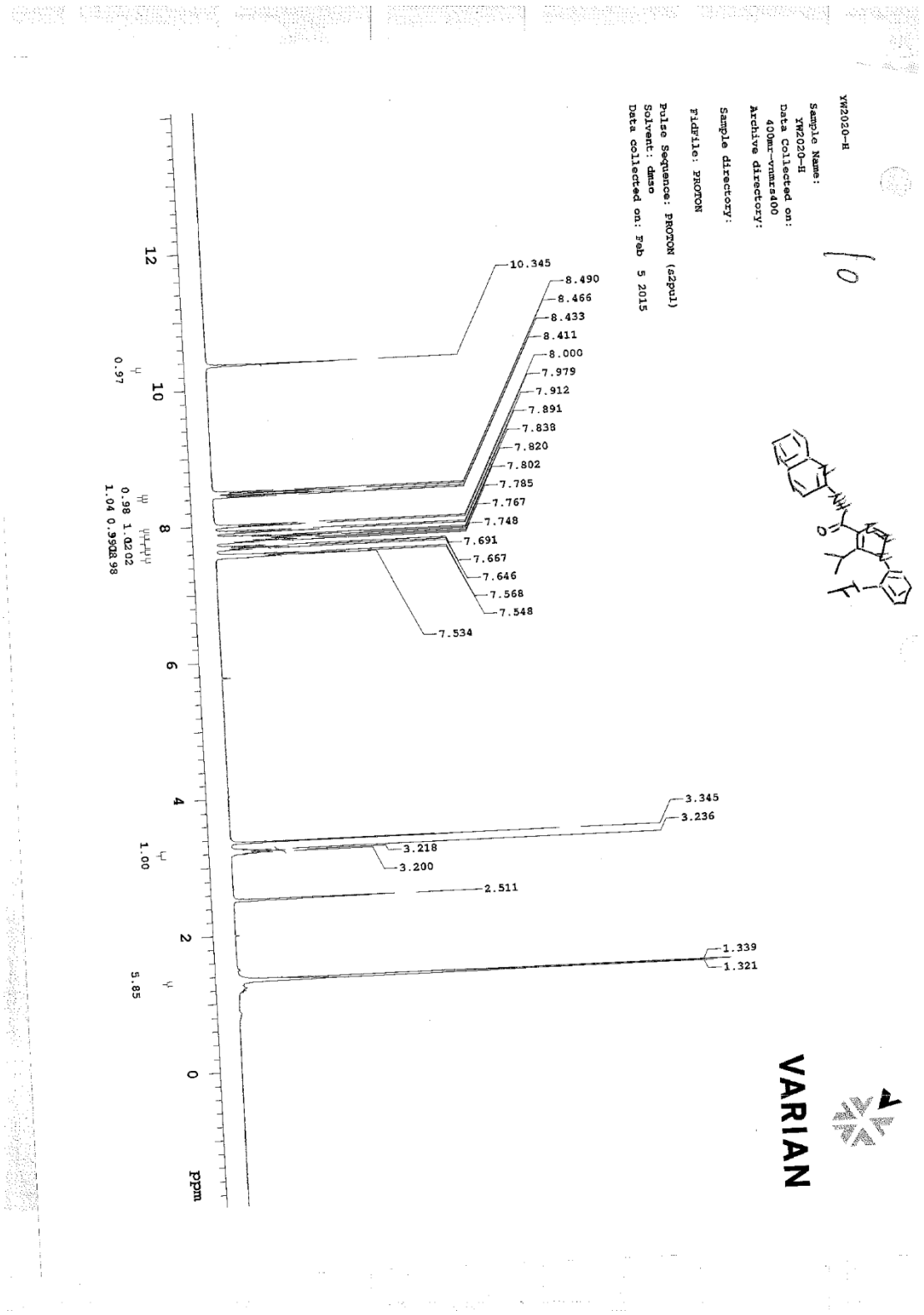


Figure S36. ^{1}H NMR spectrum of compound **3p**

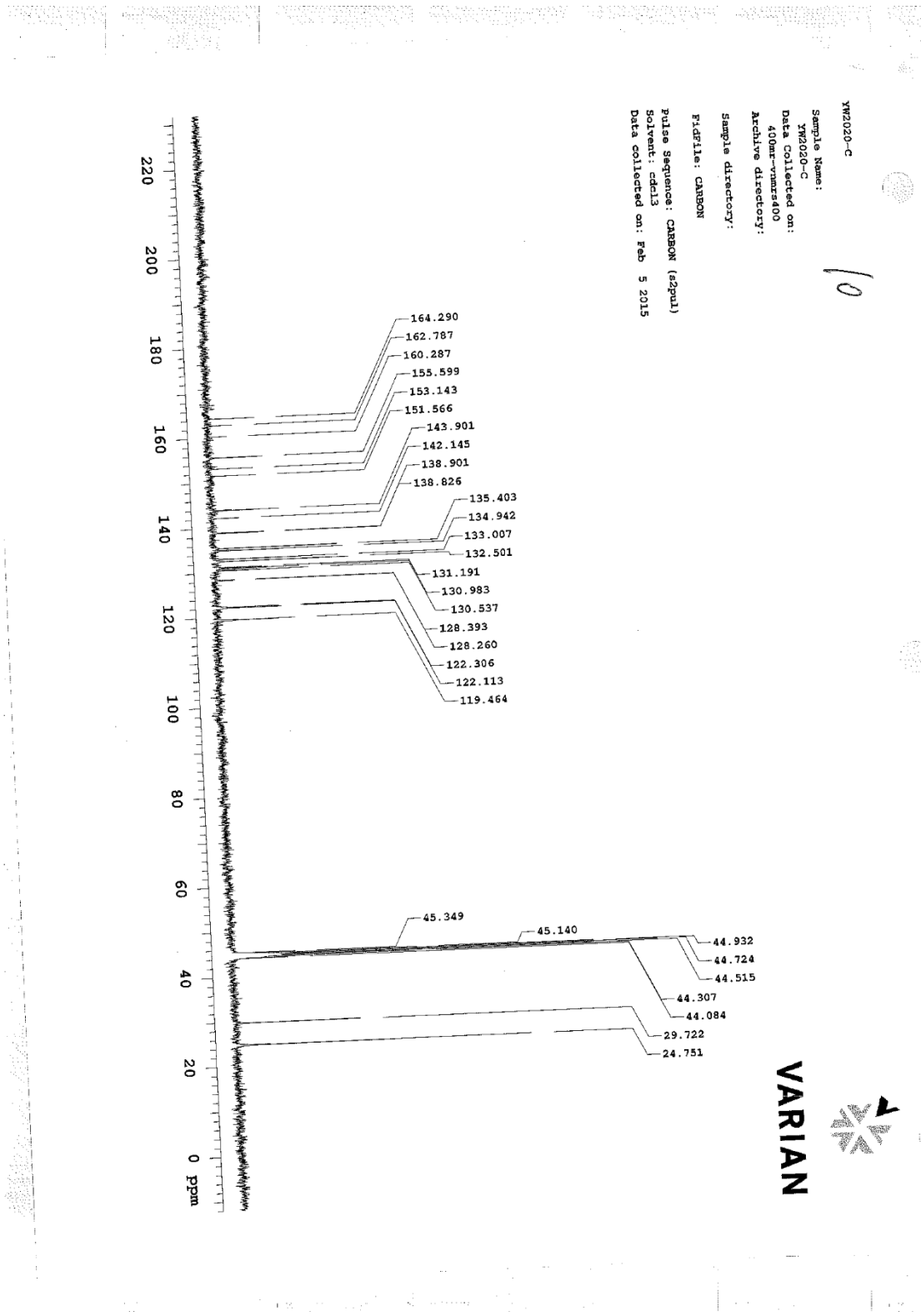


Figure S37. ^{13}C NMR spectrum of compound **3p**

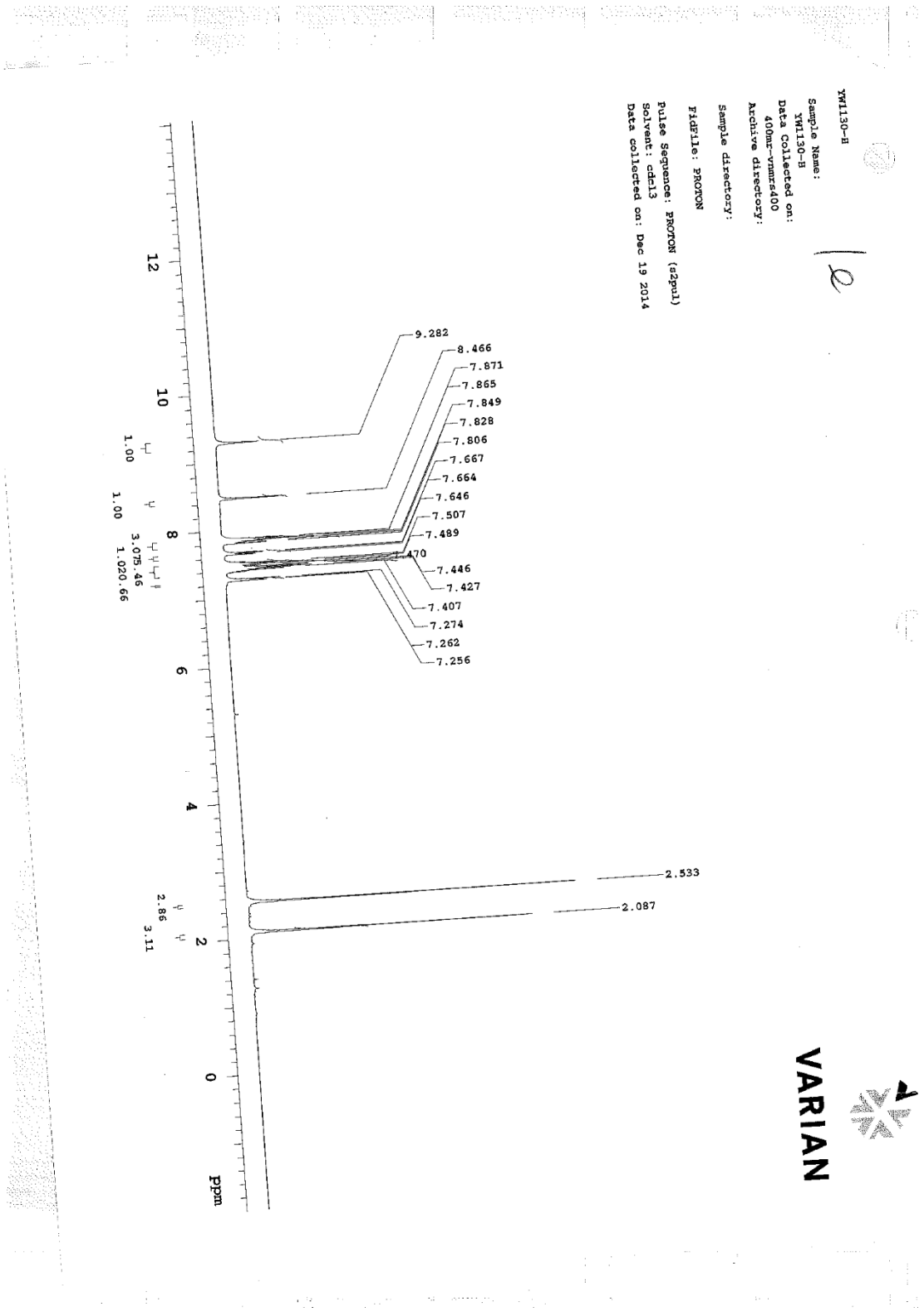


Figure S38. ^{1}H NMR spectrum of compound **3q**

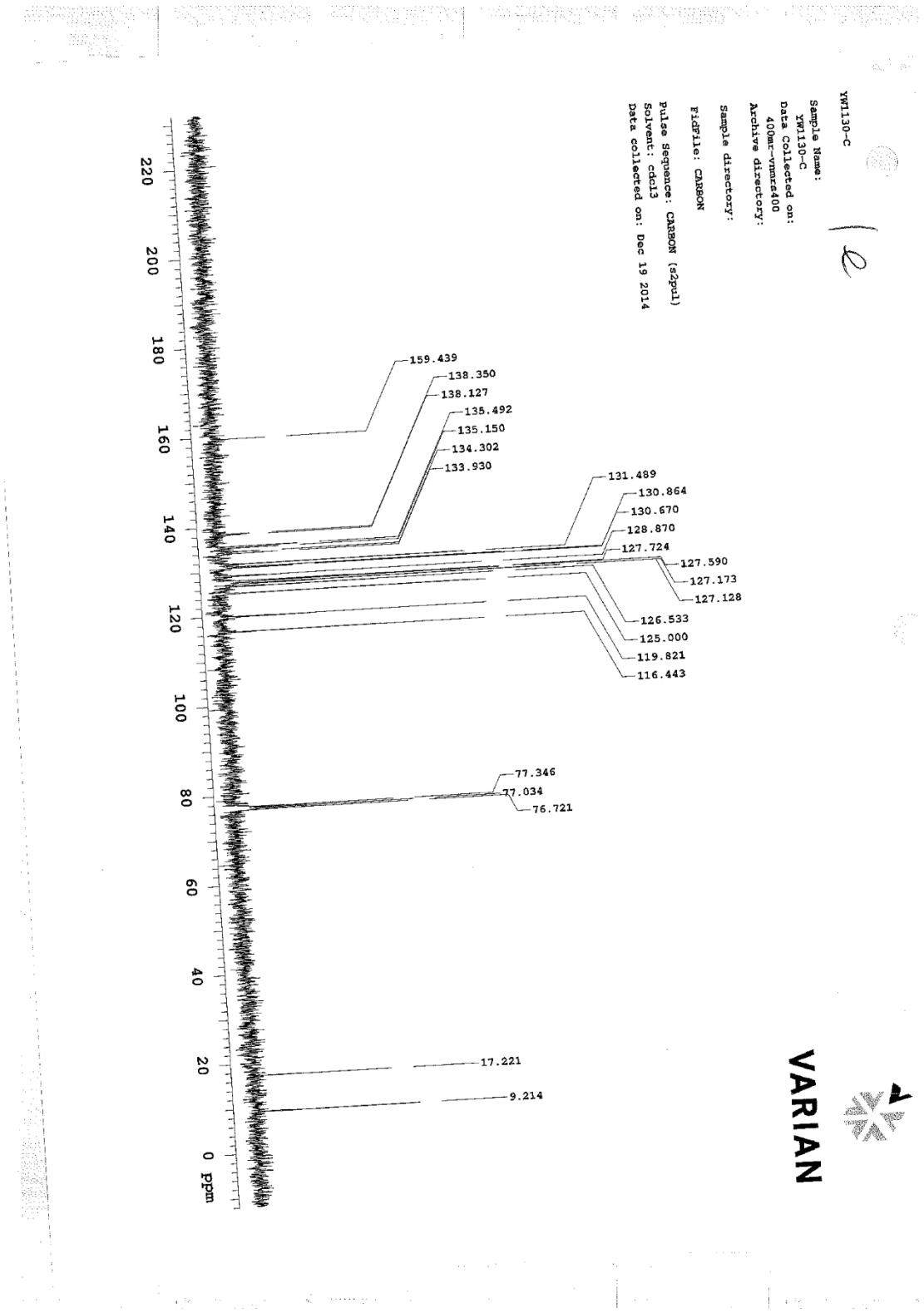


Figure S39. ^{13}C NMR spectrum of compound **3q**

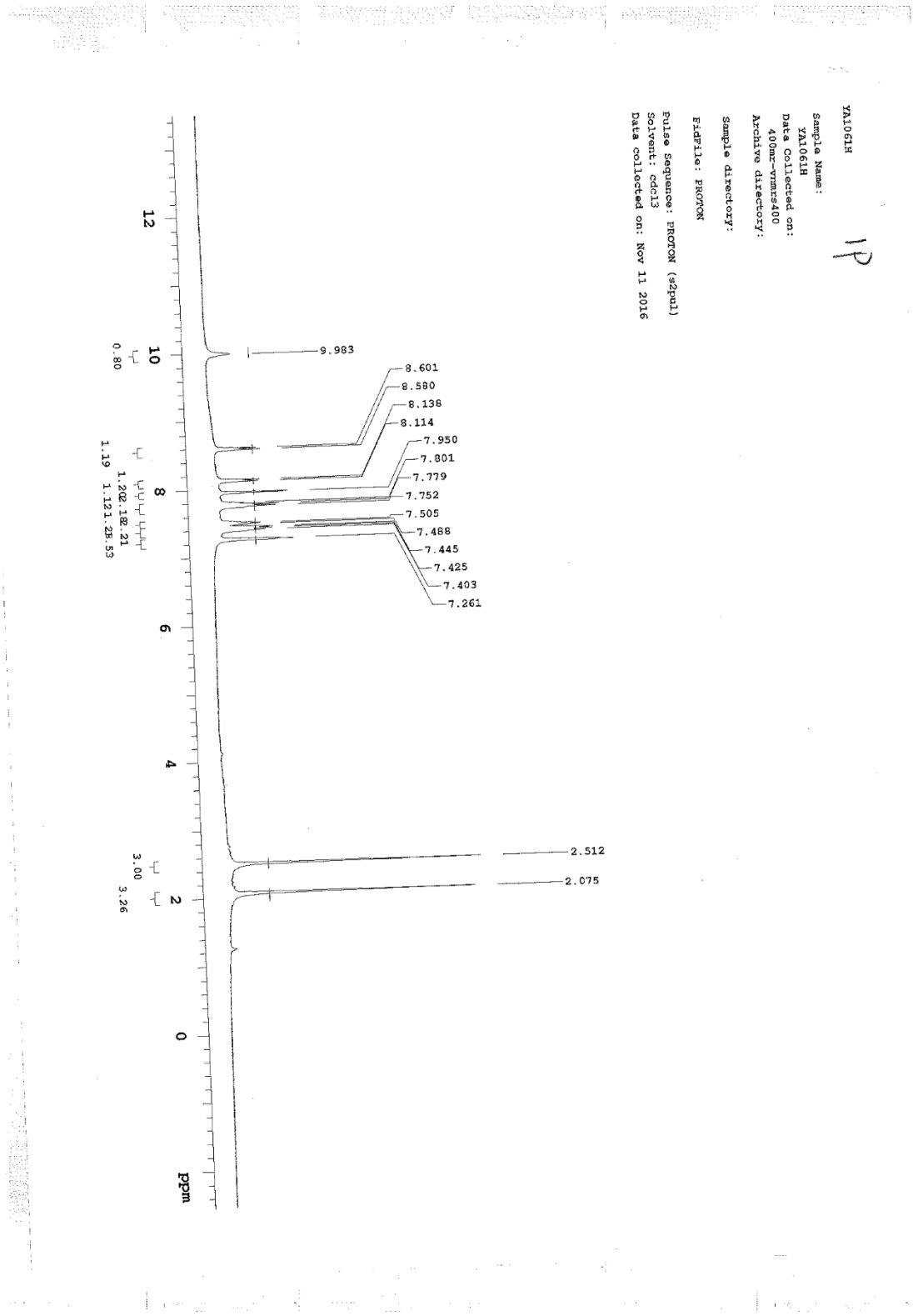


Figure S40. ^{1}H NMR spectrum of compound **3r**

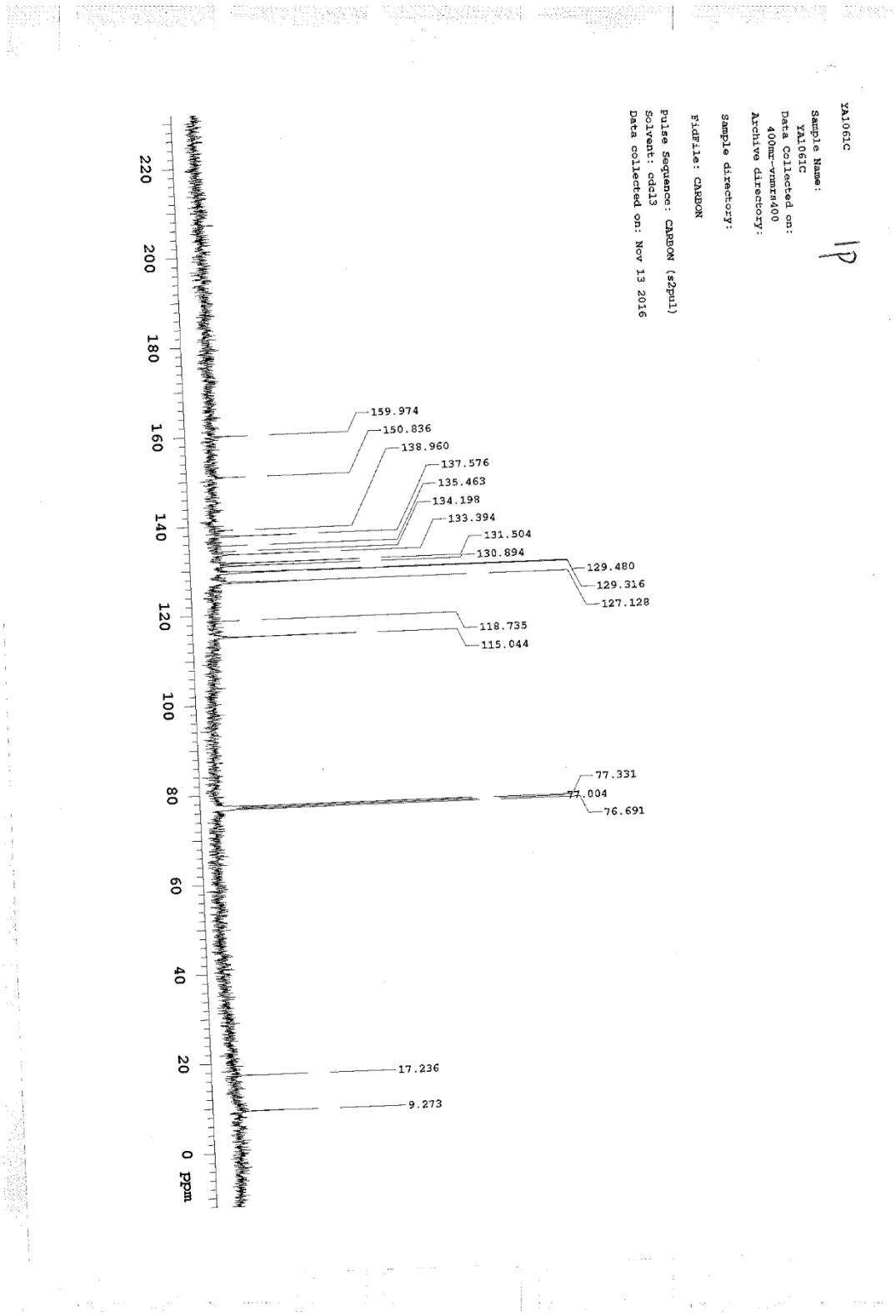


Figure S41. ^{13}C NMR spectrum of compound **3r**

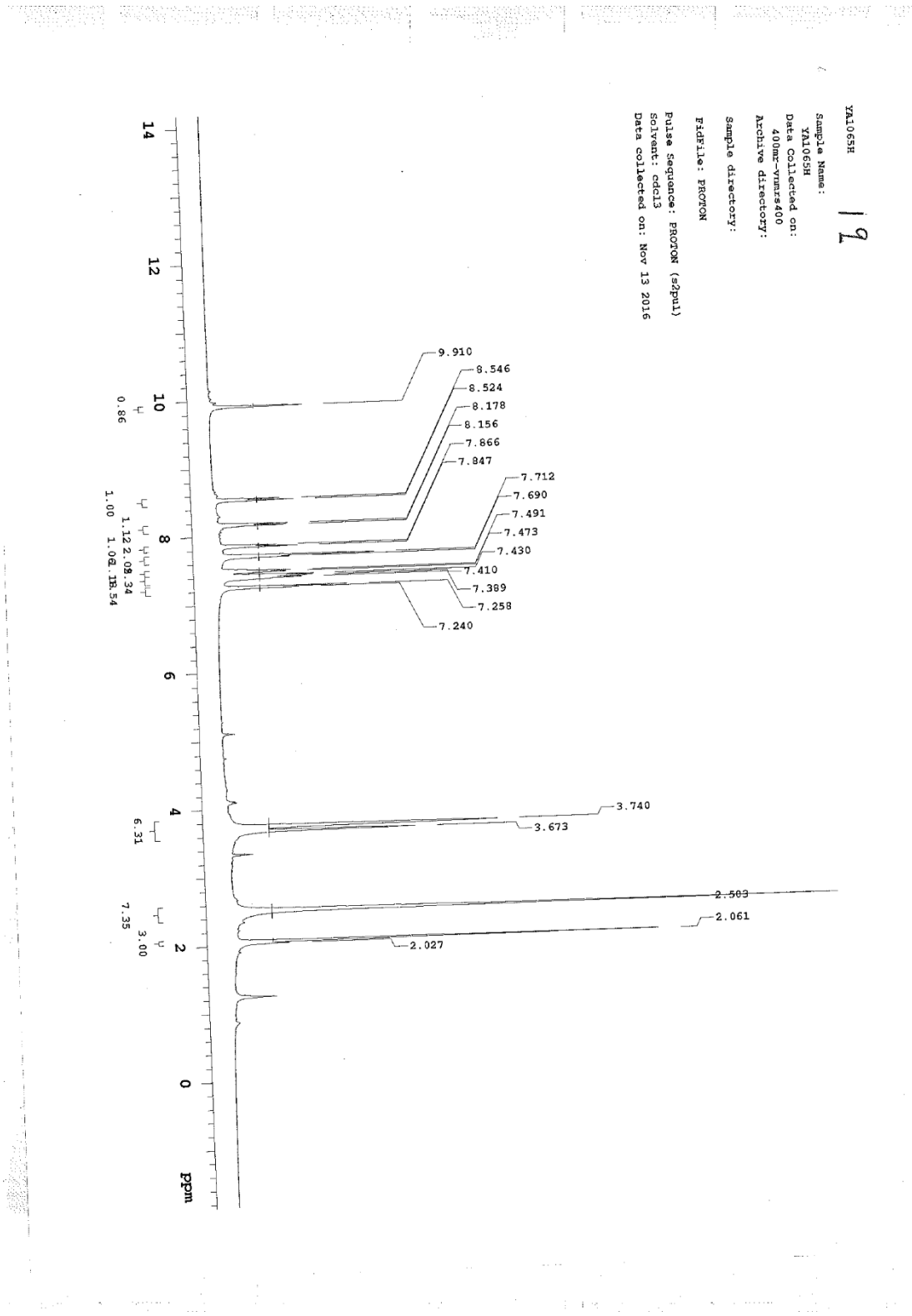


Figure S42. ^{1}H NMR spectrum of compound **3s**

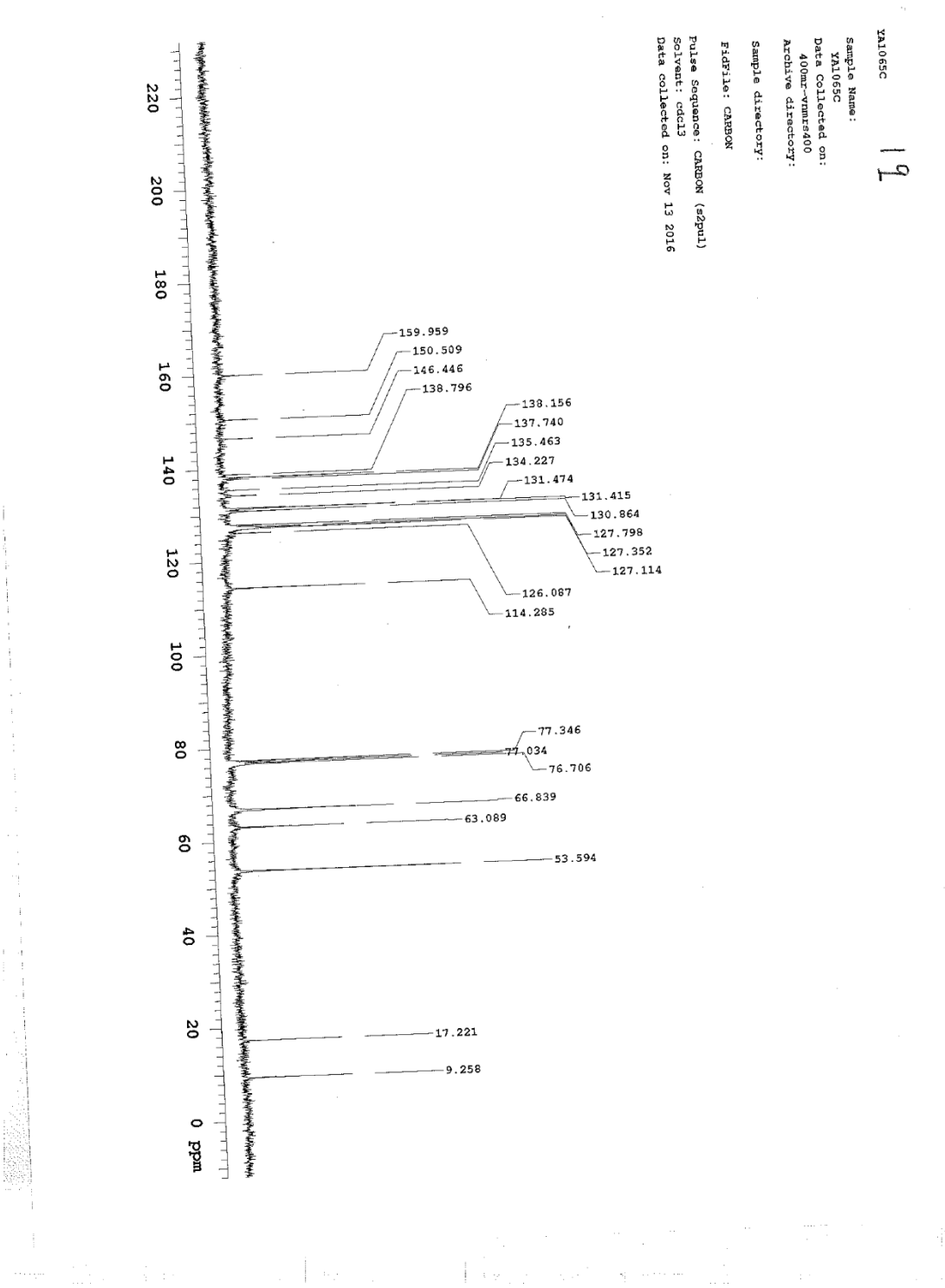


Figure S43. ^{13}C NMR spectrum of compound **3s**

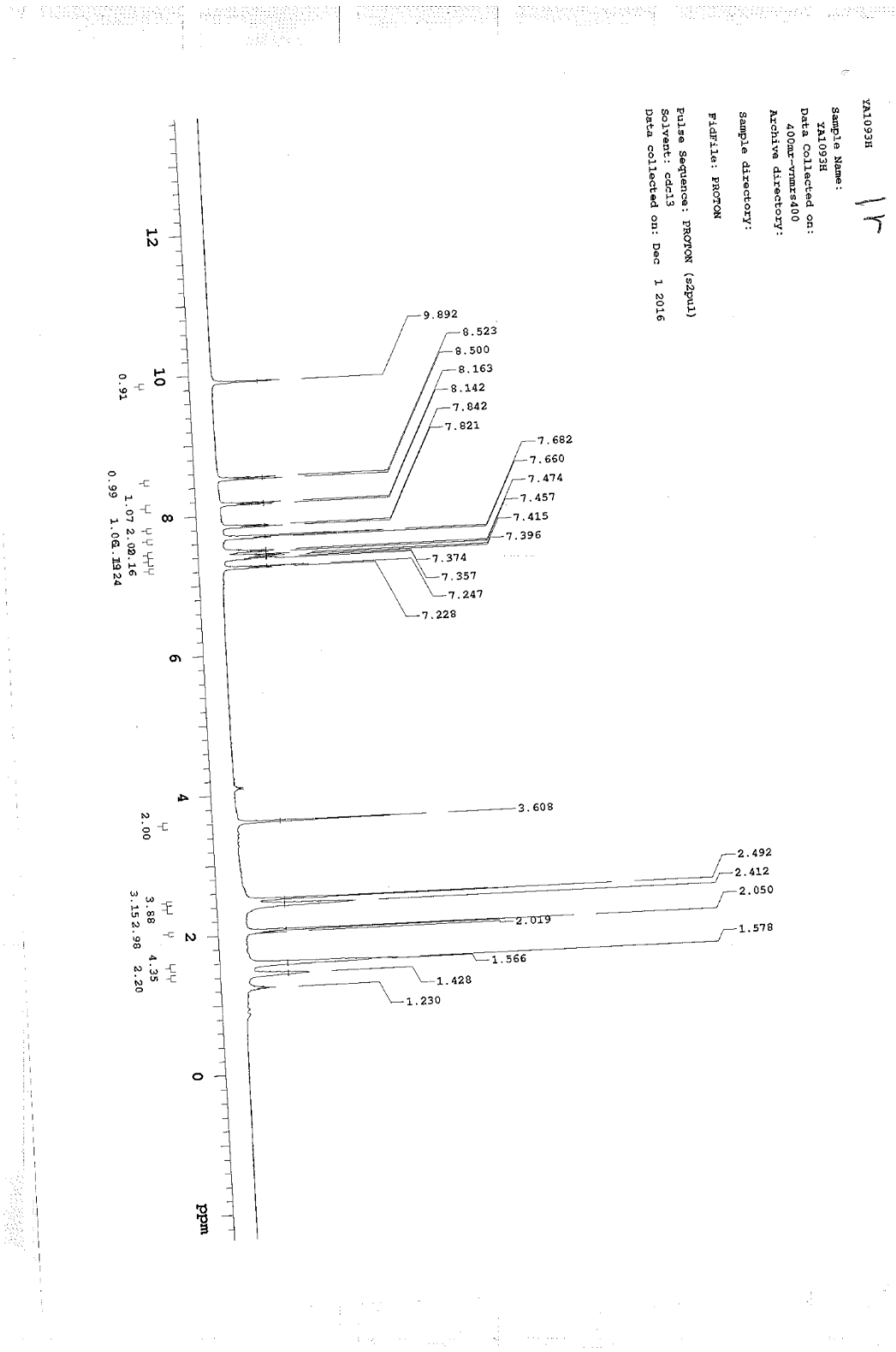


Figure S44. ^{1}H NMR spectrum of compound **3t**

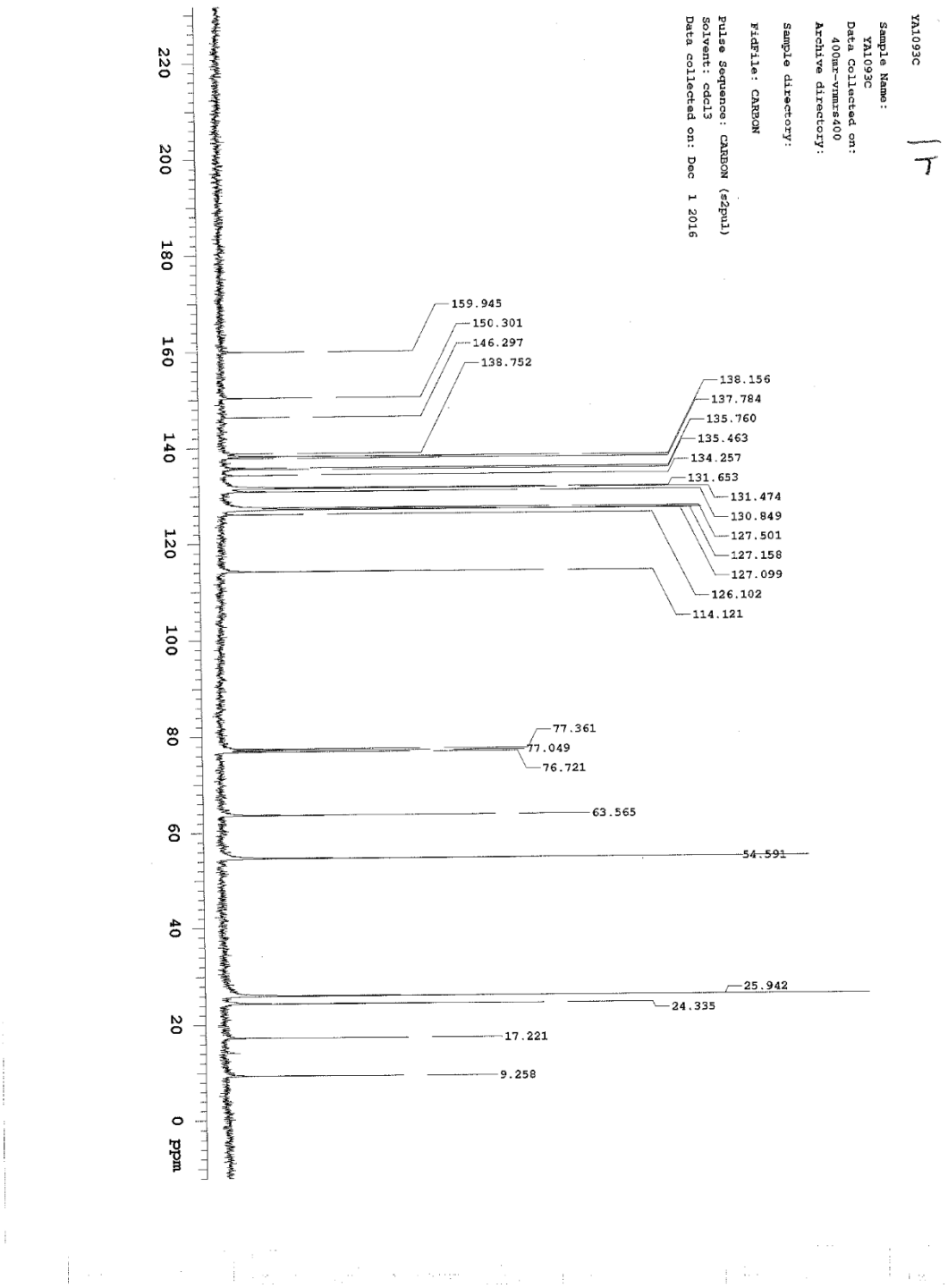


Figure S45. ^{13}C NMR spectrum of compound **3t**

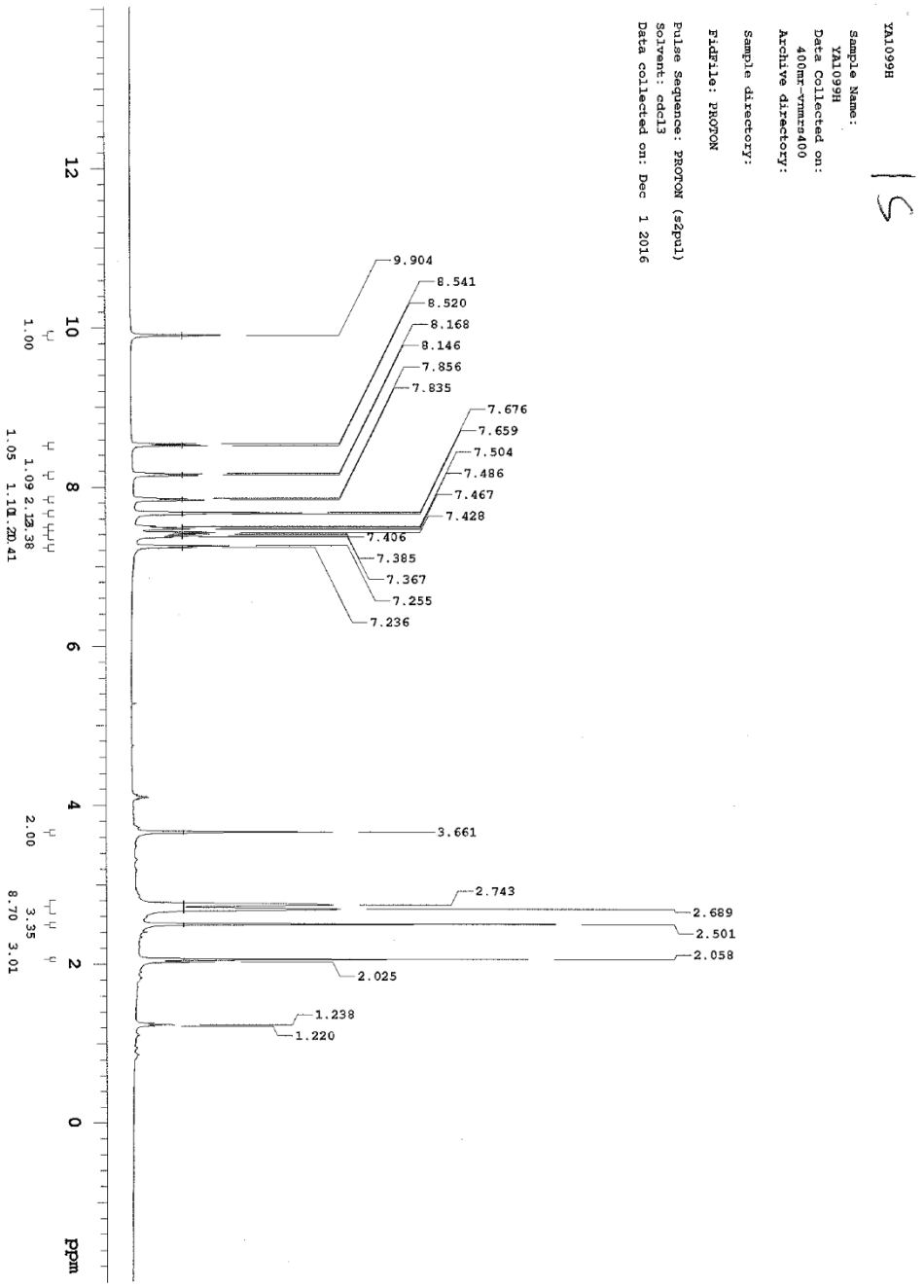


Figure S46. ^{1}H NMR spectrum of compound **3u**

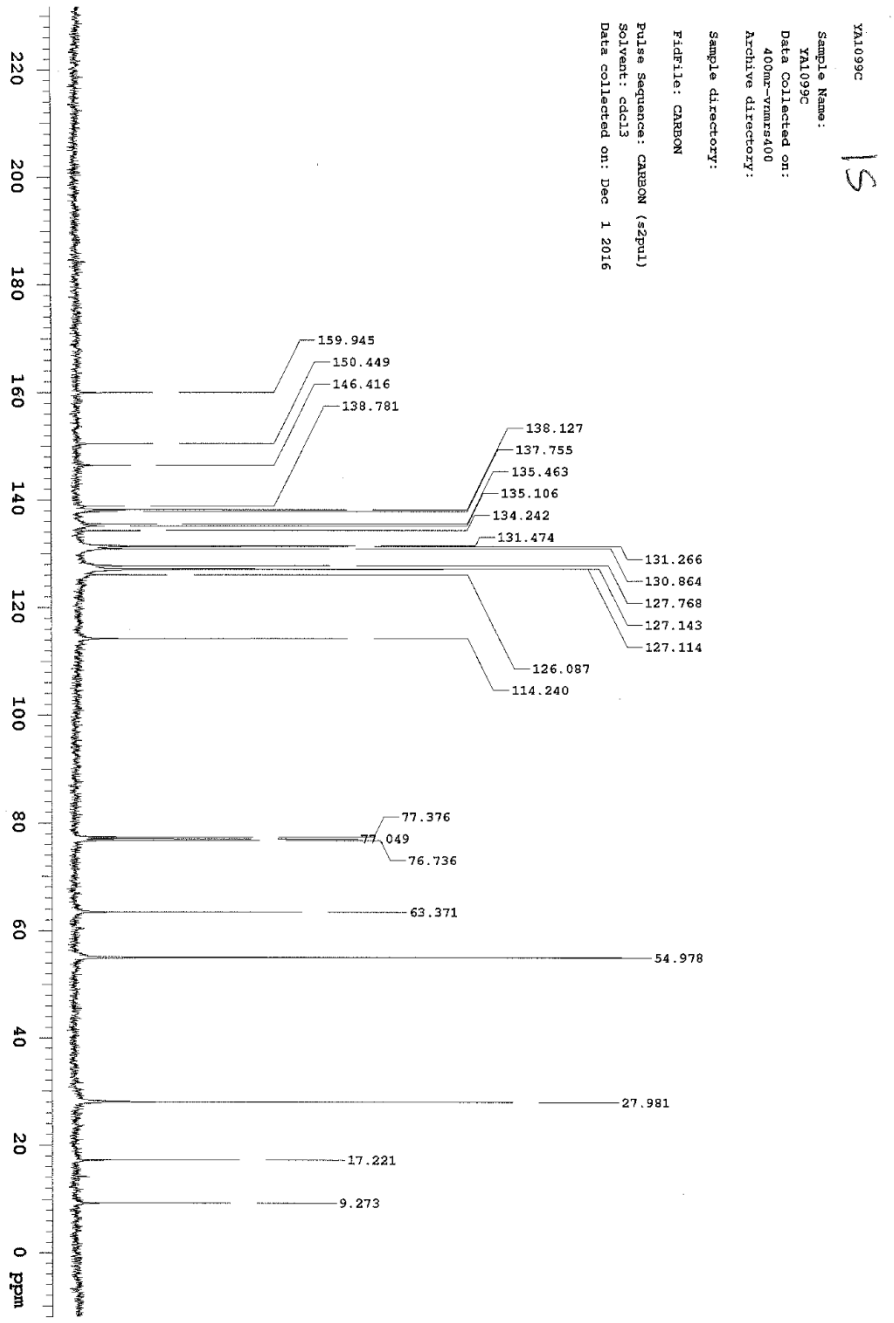


Figure S47. ^{13}C NMR spectrum of compound **3u**