

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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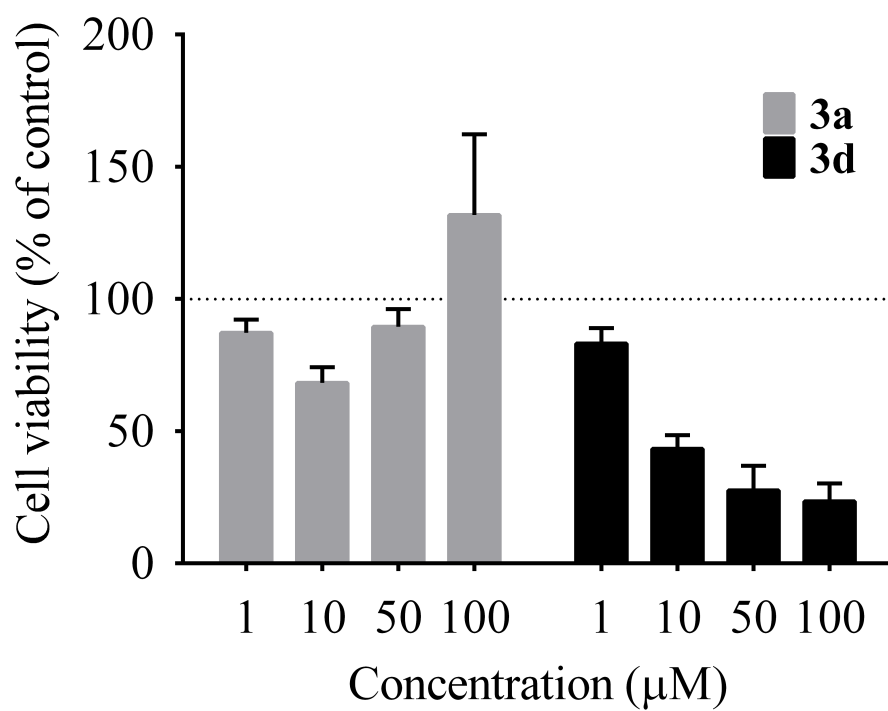


Figure S1. Cytotoxicity of compound **3a** and **3d** in unstimulated HEK293 cells with low background of Wnt pathway activity. Cells were incubated with the compounds for 72 hours. Data are reported as mean \pm SD from quadruplicates.

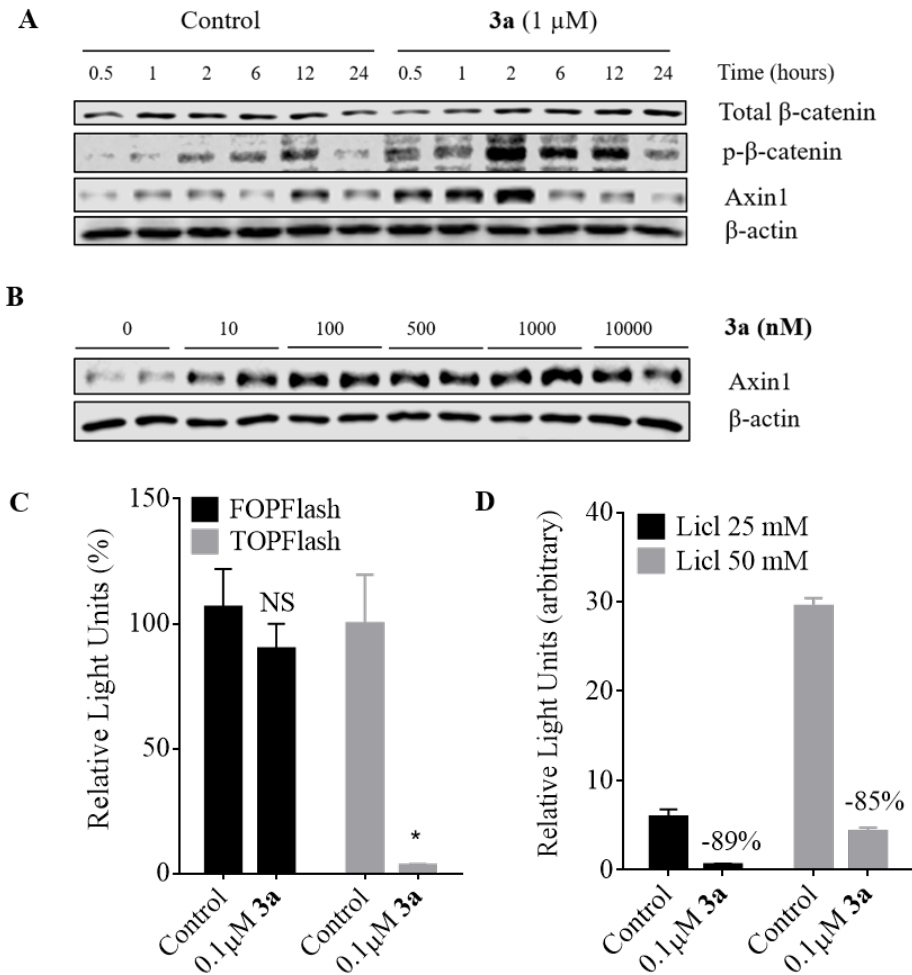


Figure S2. Time and concentration dependent regulation by compound **3a** on the Wnt/ β -catenin pathway. (A) Time dependent effect of compound **3a** on cytoplasmic levels of Wnt/ β -catenin pathway proteins in the presence of Wnt3a. (B) Concentration dependent effect of compound **3a** in the Wnt/ 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 \pm standard deviation, $n=3$. * $p < 0.05$. NS, not significant.

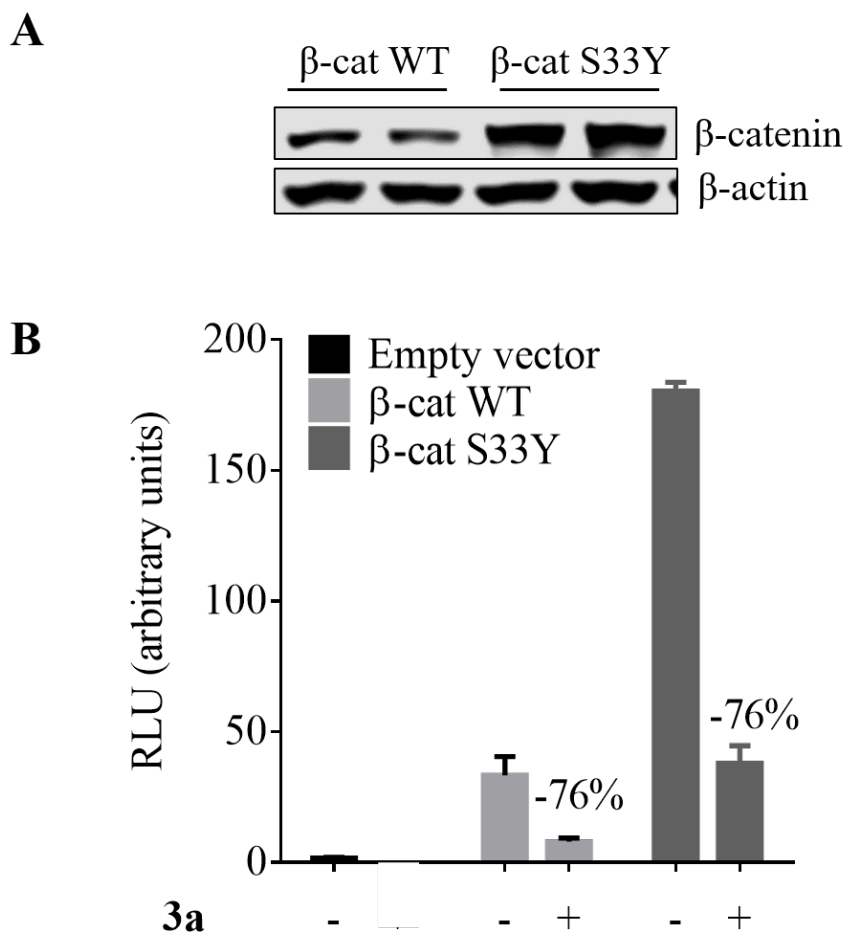


Figure S3. Inhibition of Wnt signaling activity by compound **3a** in HEK293 cells overexpressing β -catenin. (A) Western blot analysis of β -catenin protein expression in cells transiently overexpressing wild type and S33Y-mutant (*GSK3 β* phosphorylation site) β -catenin. The cells were transfected 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 \pm SD, n=3.

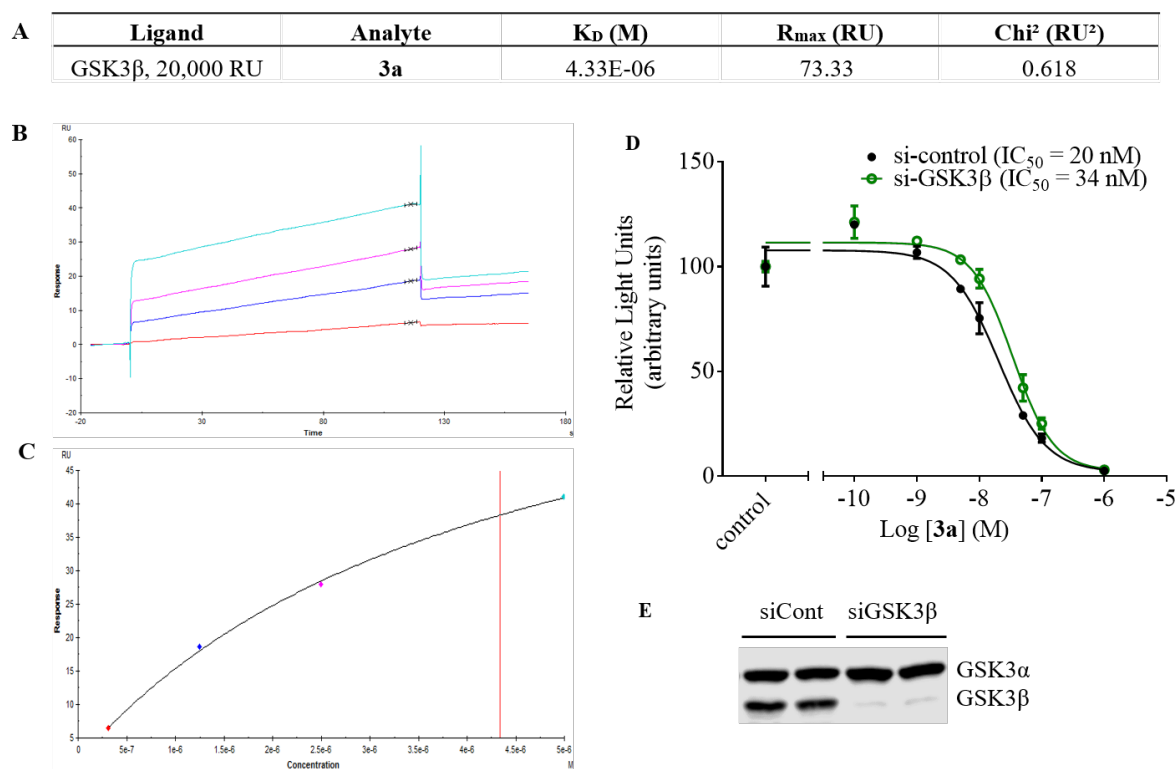


Figure S4. Direct but weak binding of compound **3a** to GSK3 β . (A) SPR analysis of **3a** binding to recombinant GSK3 β protein. (B) & (C) Concentration dependent binding of **3a** to GSK3 β . The concentrations used were 0.313 (red), 1.25 (navy blue), 2.50 (pink) and 5.00 μ M (cyan). (D) Inhibition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

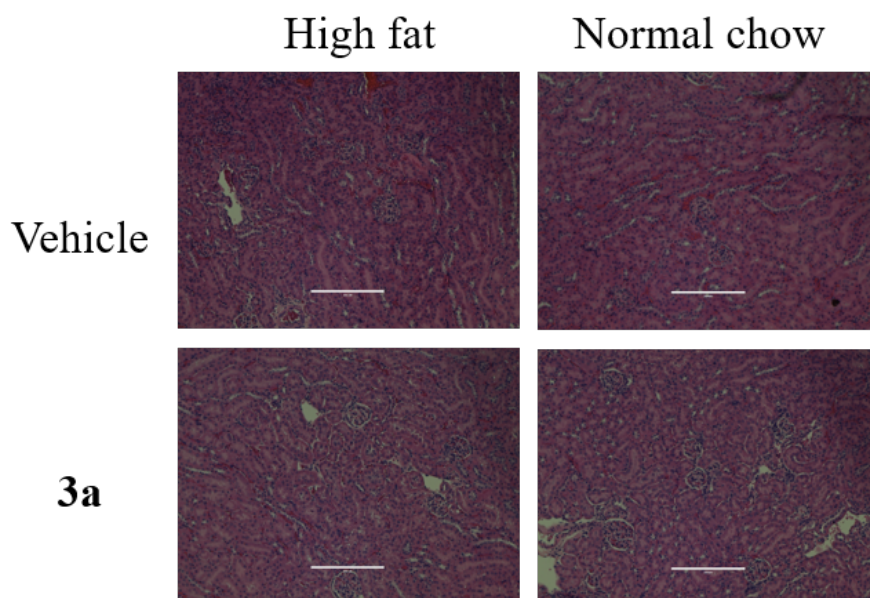
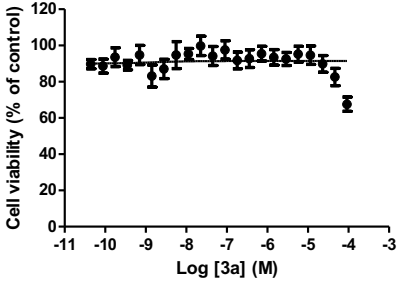
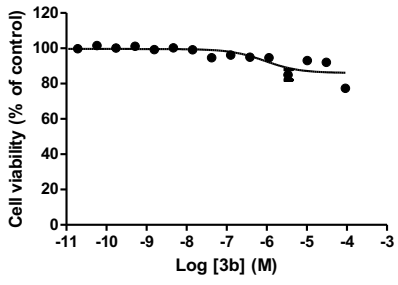
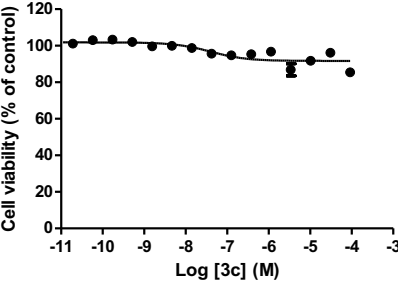
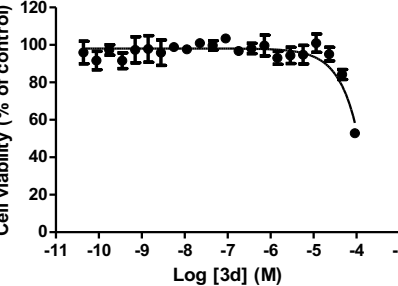


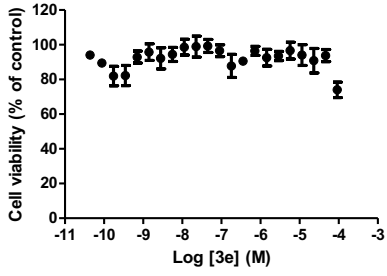
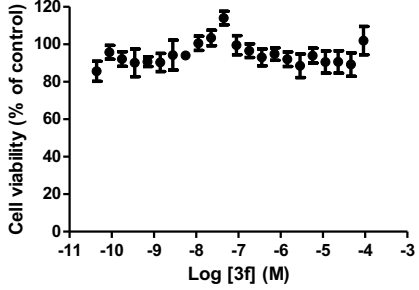
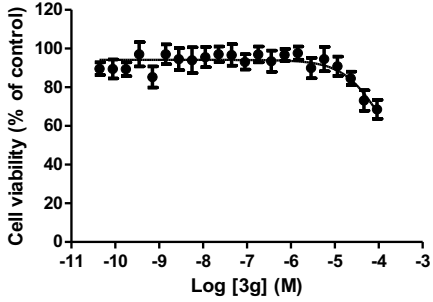
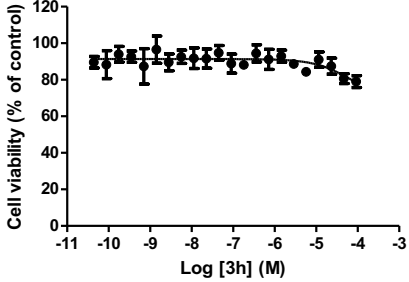
Figure S5. Compound **3a** treatment has no noticeable toxic effects on mouse kidney histology. The mice fed on either a high fat diet or a normal chow diet received *i.p.* injection of 40 mg/kg **3a** or vehicle (corn oil) every two days for 11 weeks. The isolated kidney tissues were staining by Haematoxylin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

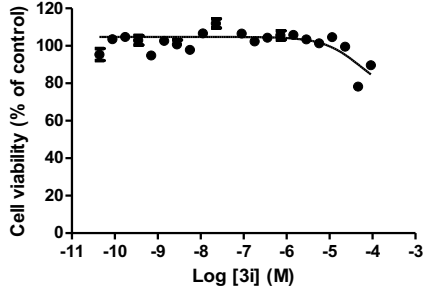
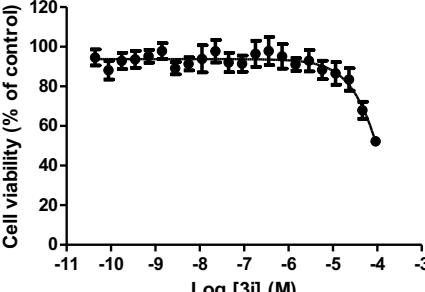
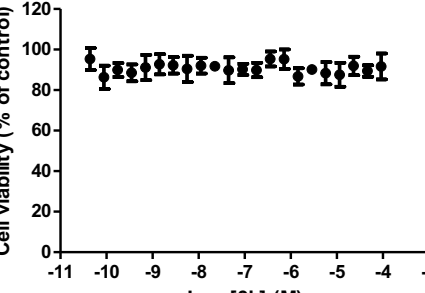
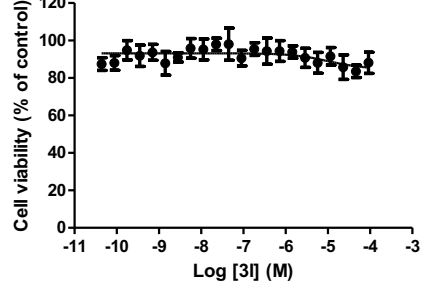
Table S1. Human primers used in the experiments

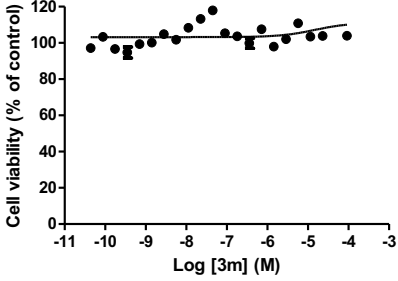
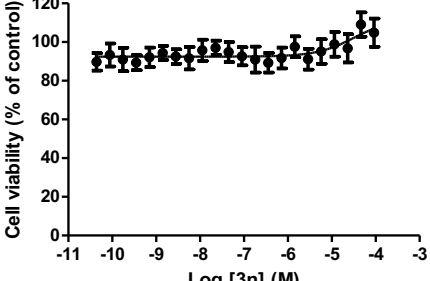
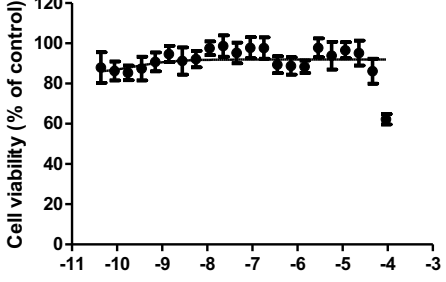
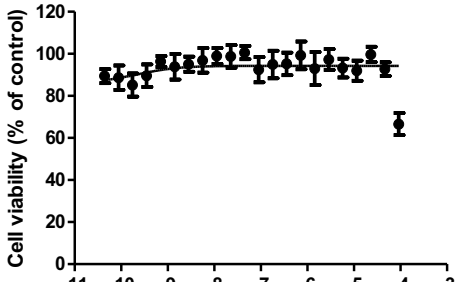
Gene	Forward	Reverse
AXIN2	GAGTGGACTTGTGCCGACTTCA	GGTGGCTGGTGCAAAGACATAG
CYCD1	CAATGACCCCGCACGATTTC	CATGGAGGGCGGATTGGAA
PEPCK	GTCAGCCTGATCACATCCACA	CCGTCTTGCTTTCGATCCTG
G6PASE	CTACTACAGCAACACTTCCGTG	GGTCGGCTTTATCTTTCCCTGA
ACAT2	GCGGACCATCATAGGTTCCCTT	ACTGGCTTGTCTAACAGGATTCT
ACOT4	CCCAGGTAAAAGGCCCAGG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

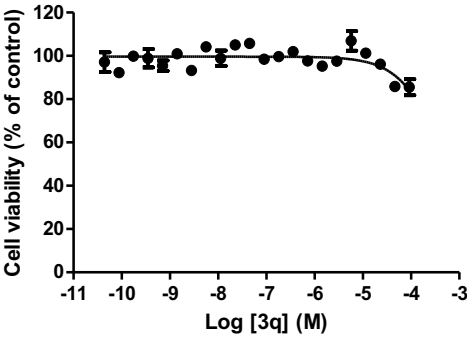
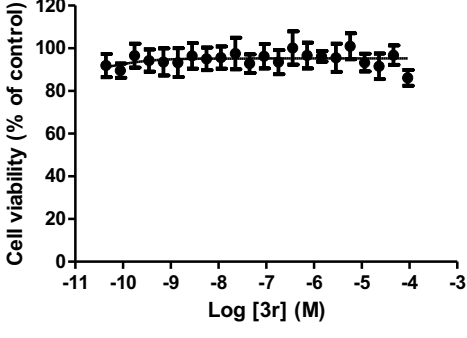
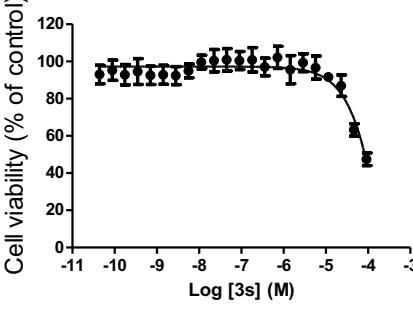
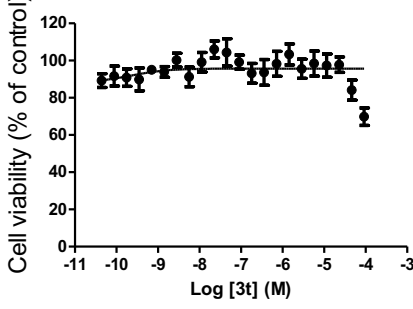
Table S2. The effect of compounds **3a-3u** on HEK293 cell viability during the incubation (24 hours) of the reporter assay for measuring Wnt signaling activity.

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	 <p>Cell viability (% of control)</p> <p>Log [3i] (M)</p>	> 92
3j	 <p>Cell viability (% of control)</p> <p>Log [3j] (M)</p>	> 92
3k	 <p>Cell viability (% of control)</p> <p>Log [3k] (M)</p>	> 92
3l	 <p>Cell viability (% of control)</p> <p>Log [3l] (M)</p>	> 92

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

3q	 <p>Cell viability (% of control)</p> <p>Log [3q] (M)</p>	> 92
3r	 <p>Cell viability (% of control)</p> <p>Log [3r] (M)</p>	> 92
3s	 <p>Cell viability (% of control)</p> <p>Log [3s] (M)</p>	> 92
3t	 <p>Cell viability (% of control)</p> <p>Log [3t] (M)</p>	> 92

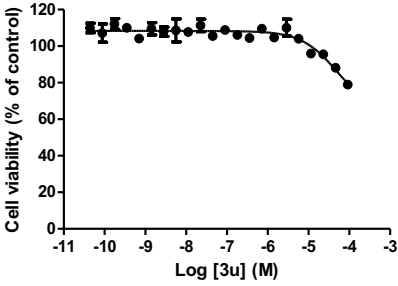
3u	 <p>The graph displays the cell viability of a control group (100%) in response to increasing concentrations of 3u. The x-axis represents the logarithm of the concentration of 3u in Molar (M), ranging from -11 to -3. The y-axis represents the cell viability as a percentage of the control, ranging from 0 to 120. The data points show that cell viability remains near 100% for concentrations up to approximately 10⁻⁶ M, after which it begins to decrease, reaching about 80% viability at 10⁻⁴ M.</p> <table border="1"><thead><tr><th>Log [3u] (M)</th><th>Cell viability (% of control)</th></tr></thead><tbody><tr><td>-10</td><td>105</td></tr><tr><td>-9</td><td>105</td></tr><tr><td>-8</td><td>105</td></tr><tr><td>-7</td><td>105</td></tr><tr><td>-6</td><td>105</td></tr><tr><td>-5</td><td>105</td></tr><tr><td>-4</td><td>80</td></tr></tbody></table>	Log [3u] (M)	Cell viability (% of control)	-10	105	-9	105	-8	105	-7	105	-6	105	-5	105	-4	80	> 92
Log [3u] (M)	Cell viability (% of control)																	
-10	105																	
-9	105																	
-8	105																	
-7	105																	
-6	105																	
-5	105																	
-4	80																	

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-inf}	h*µg/mL	4.5 ± 0.76	21 ± 2.1
Bioavailability	%	21	
LogP ^a		3.30	
PSA ^a	Å 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

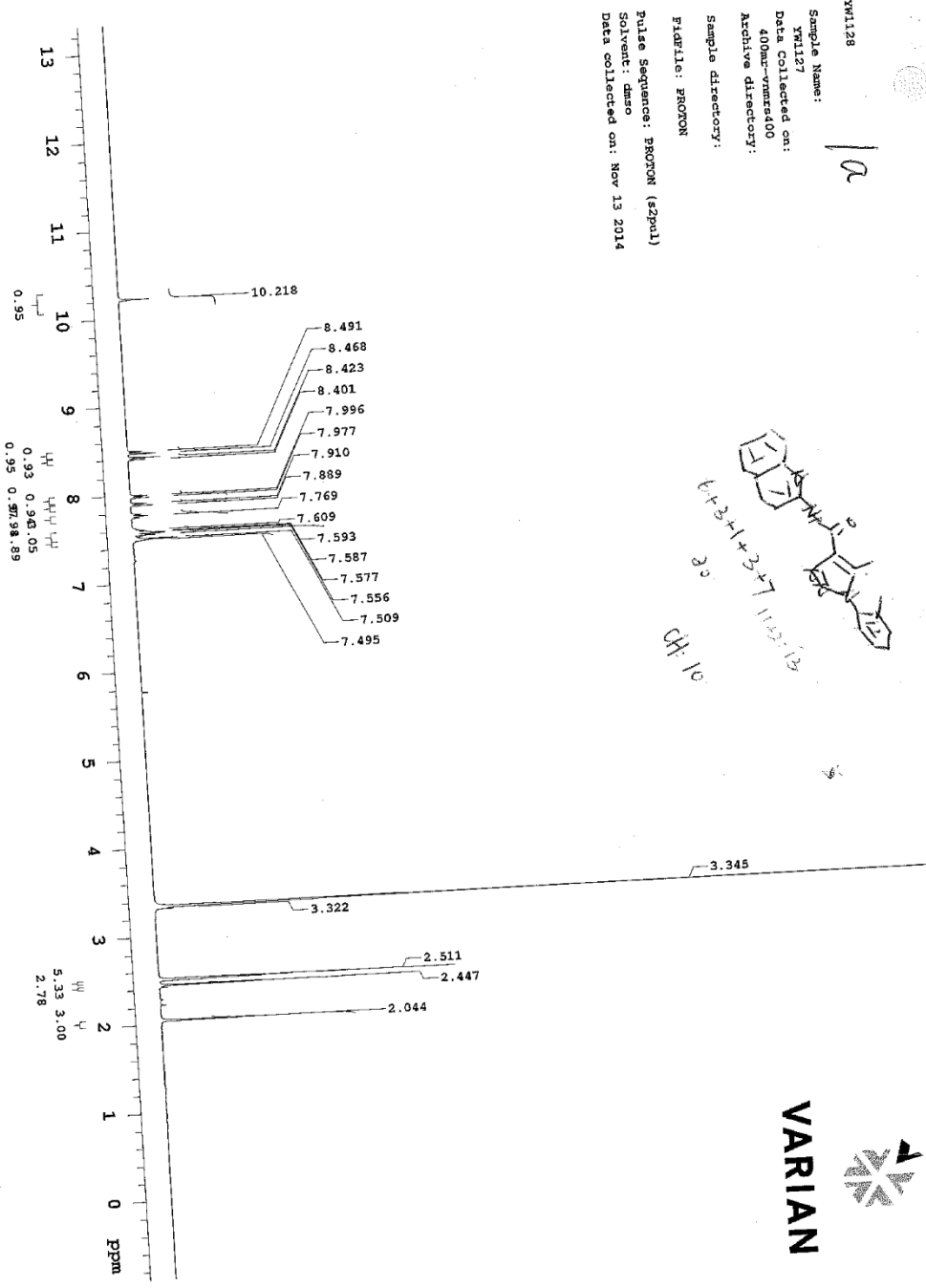


Figure S6. ¹H NMR spectrum of compound 3a

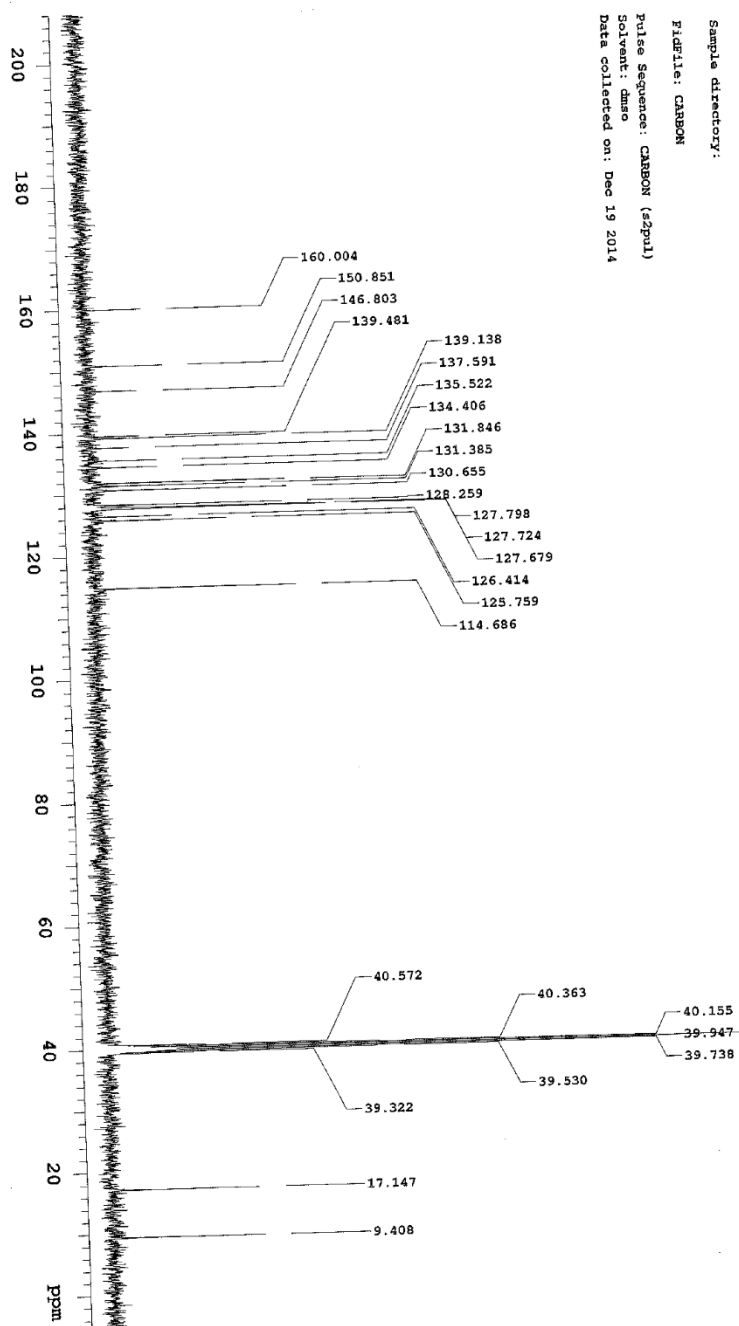


Figure S7. ^{13}C NMR spectrum of compound 3a

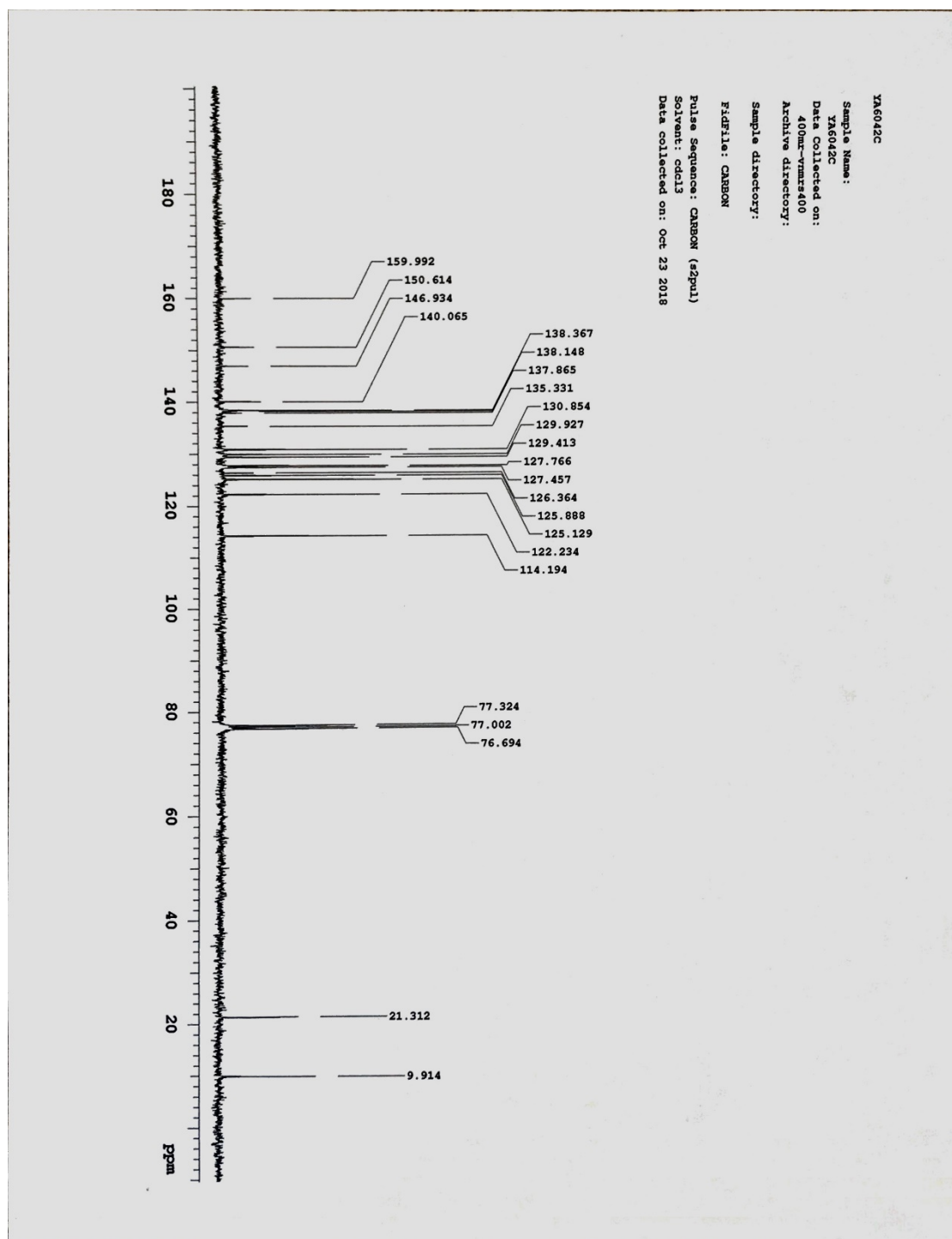


Figure S9. ^{13}C NMR spectrum of compound **3b**

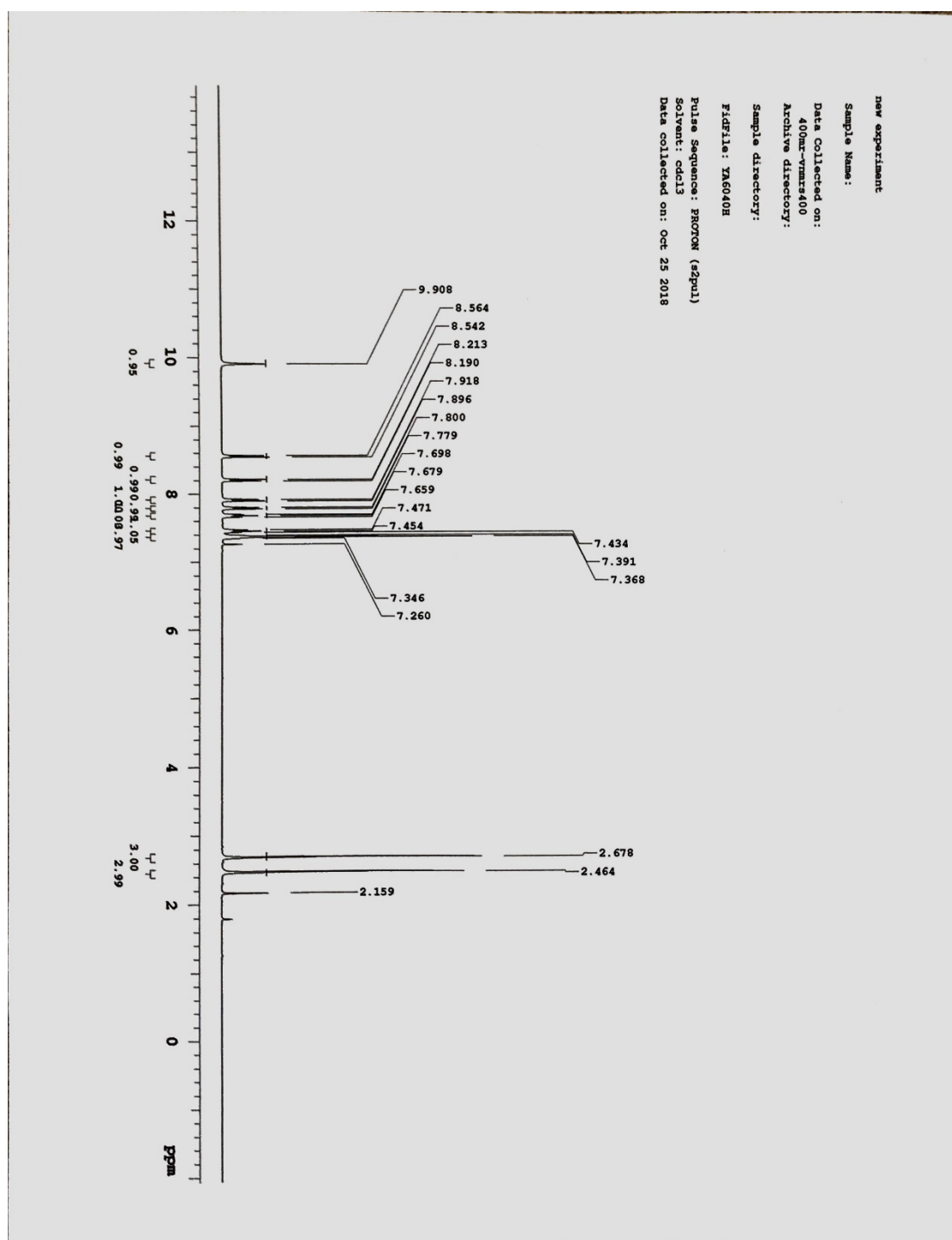


Figure S10. ^1H NMR spectrum of compound **3c**

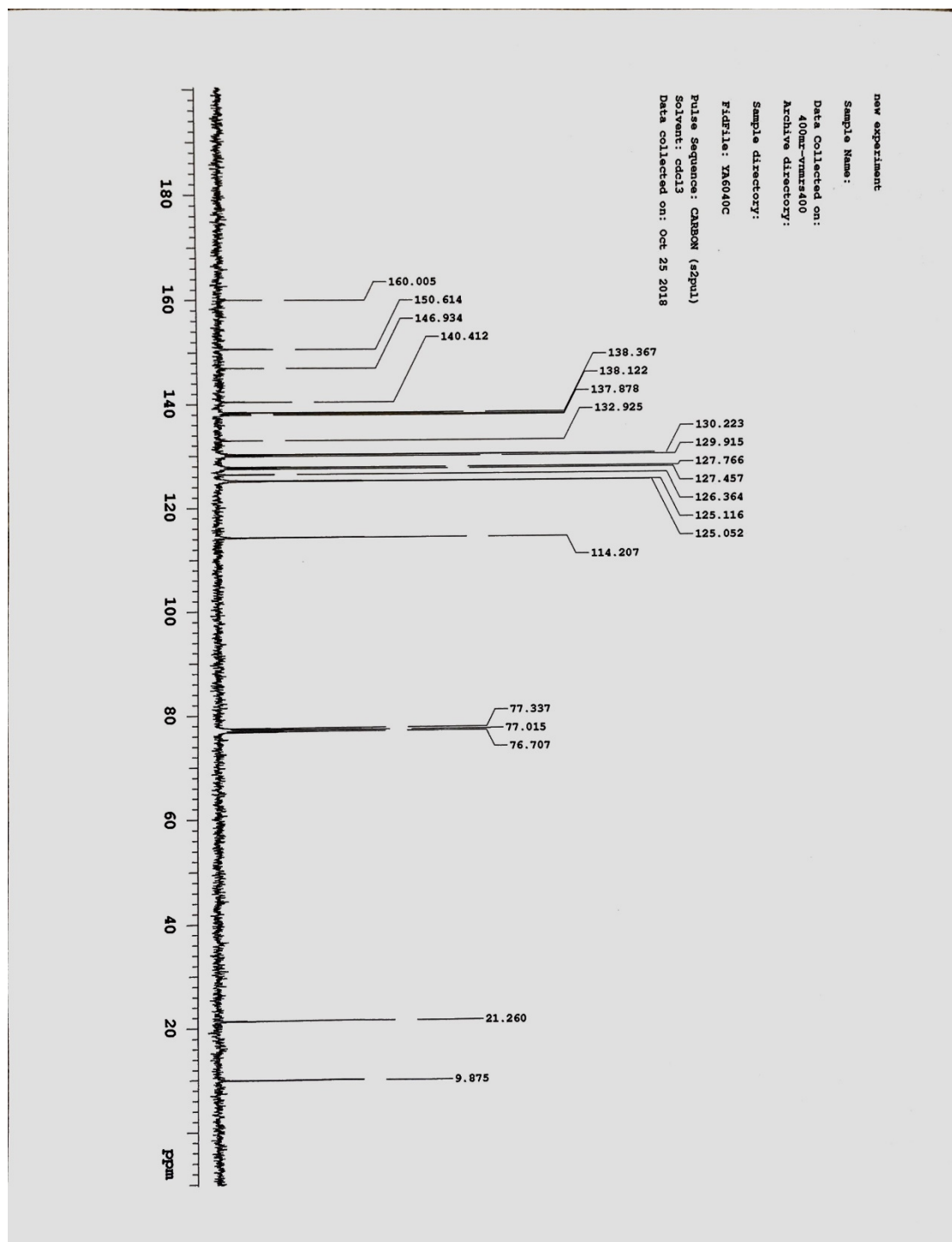


Figure S11. ^{13}C NMR spectrum of compound **3c**

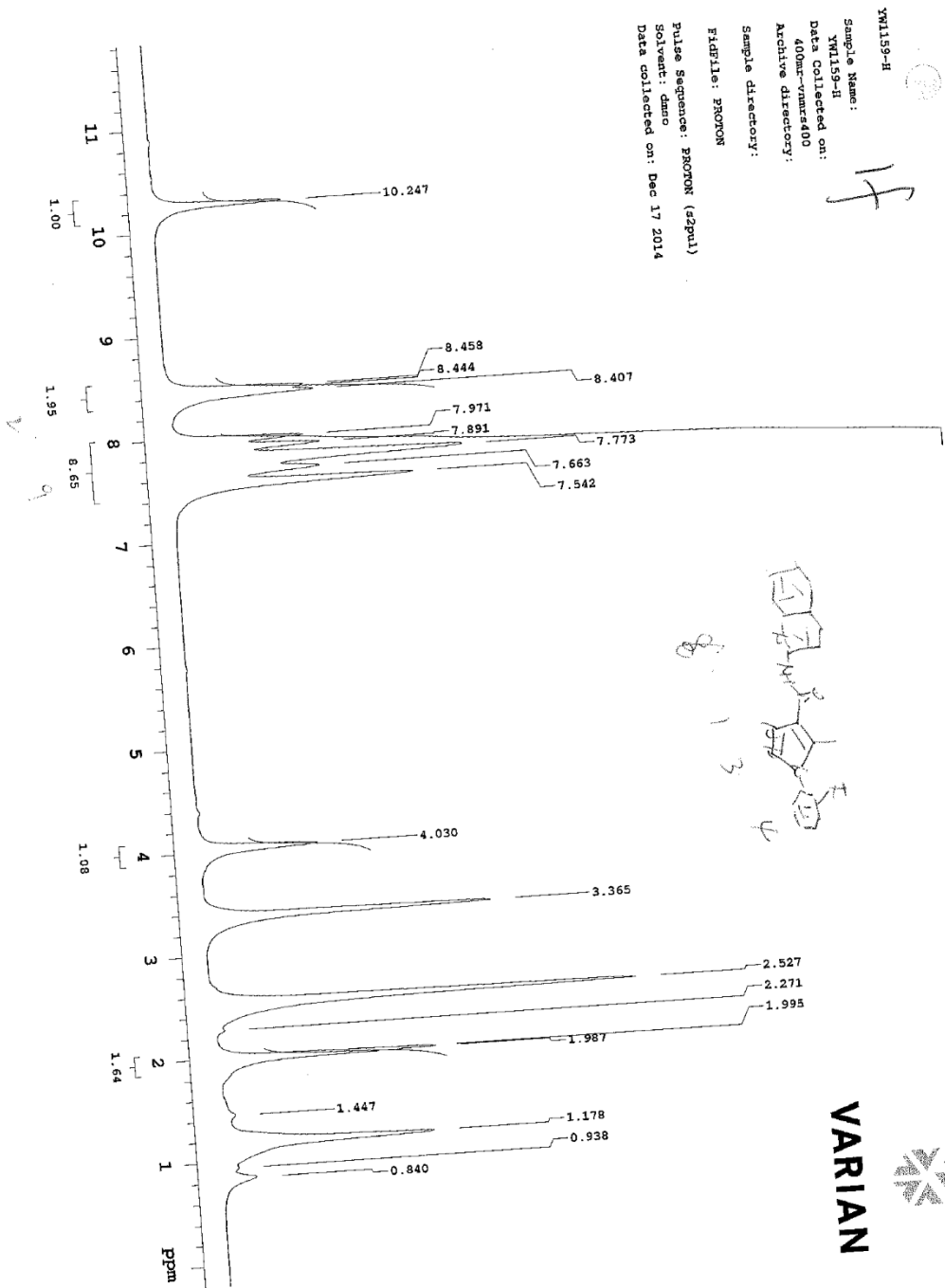


Figure S12. ¹H NMR spectrum of compound 3d

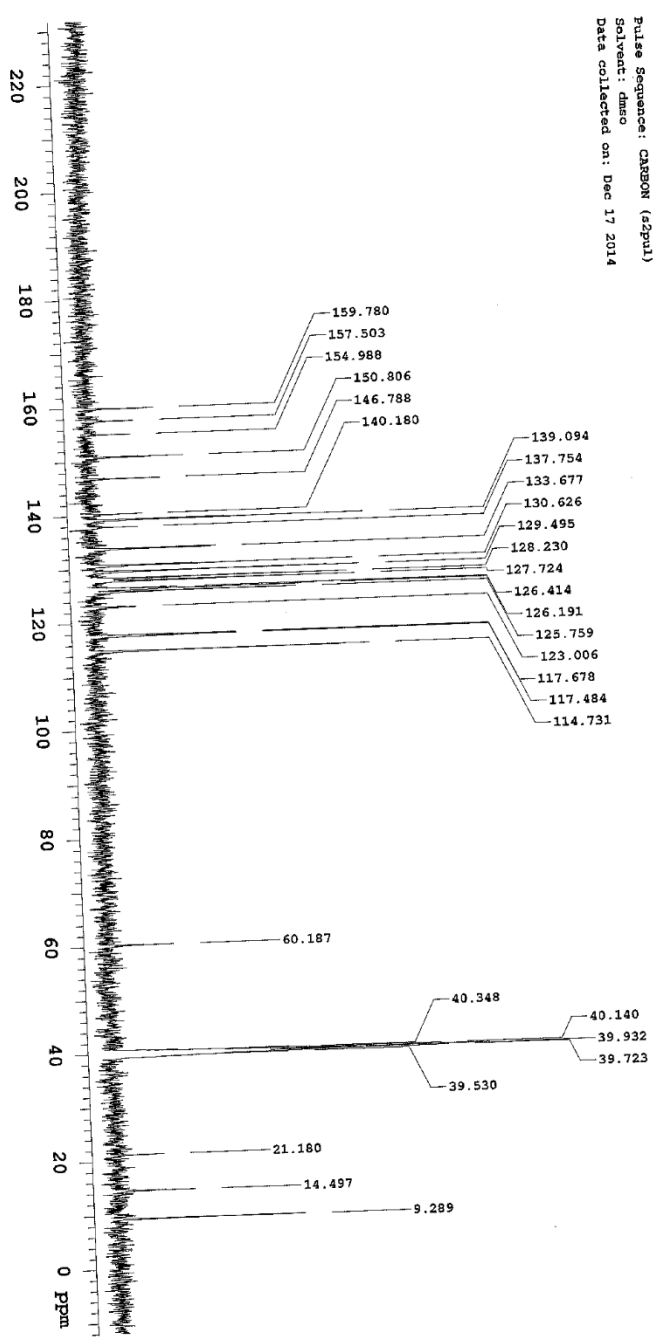


Figure S13. ¹³C NMR spectrum of compound 3d

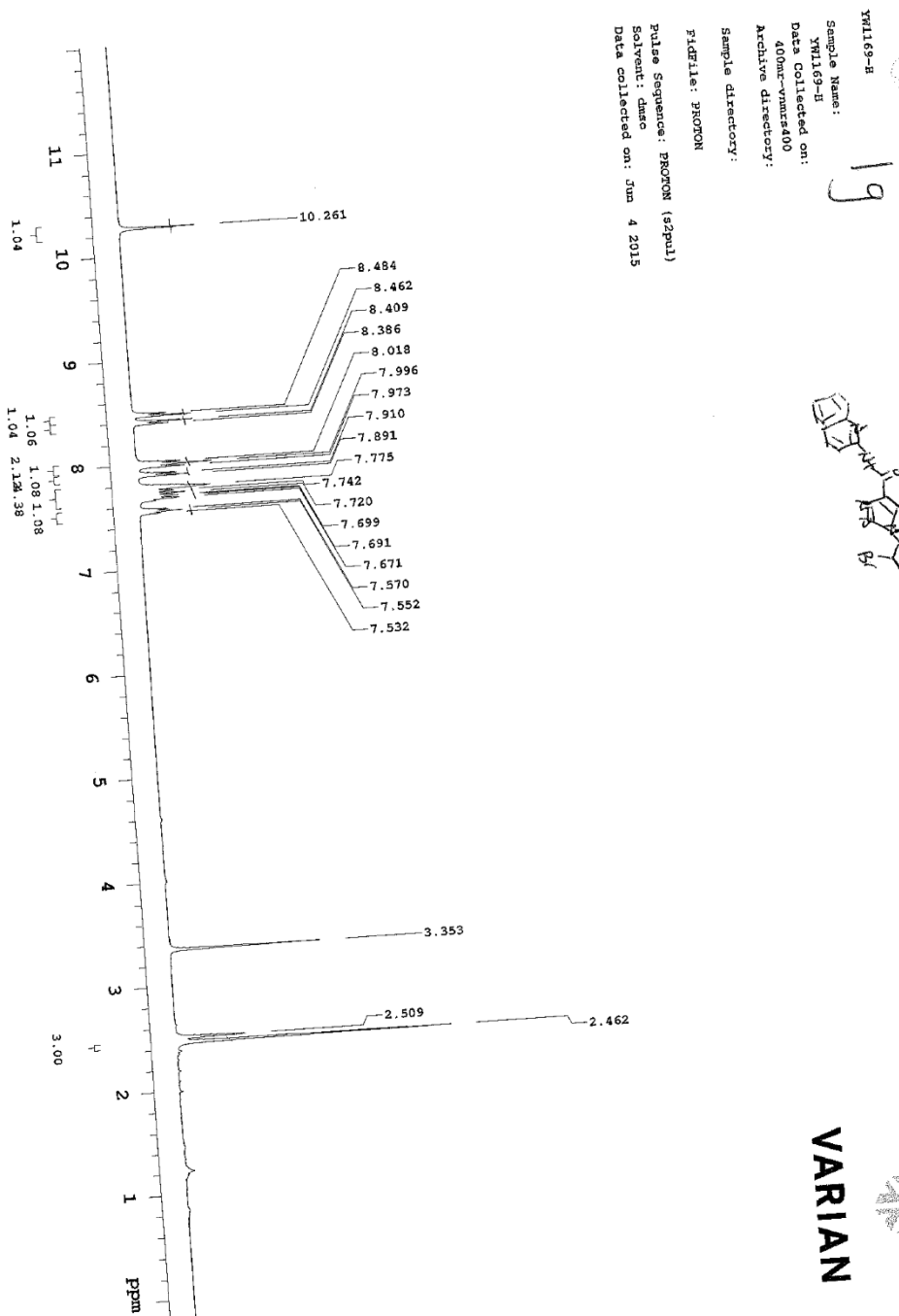


Figure S14. ¹H NMR spectrum of compound 3e

xm1169-c

19

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Data collected on: Jun 4 2015

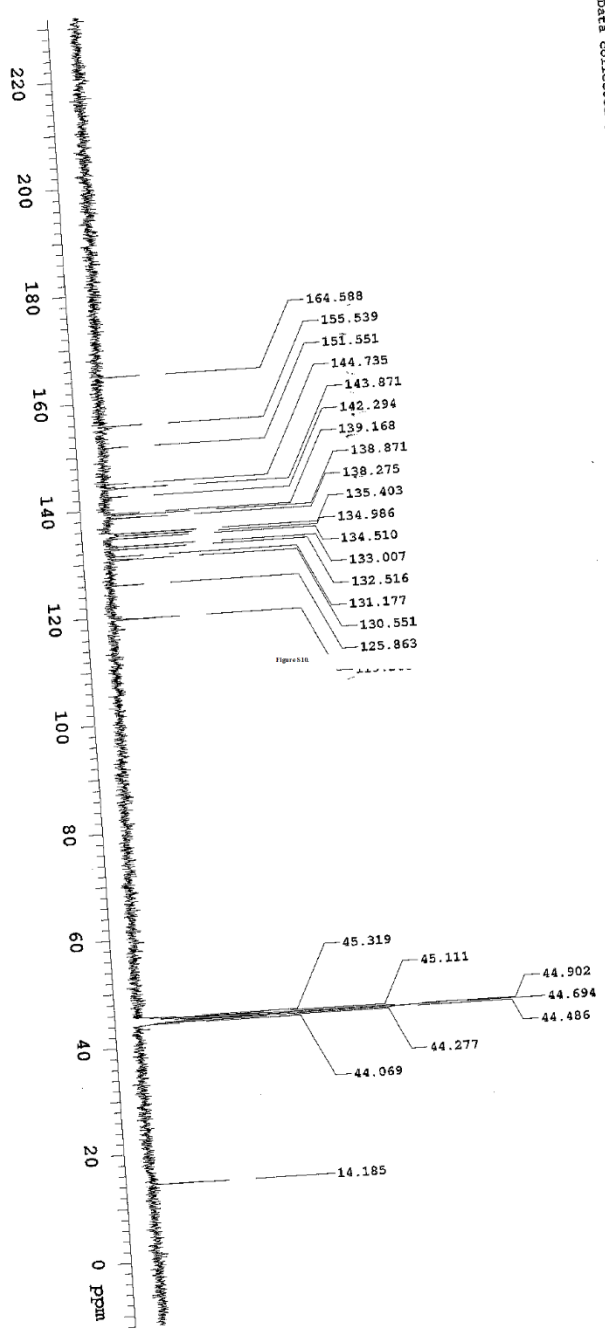


Figure S15. ¹³C NMR spectrum of compound 3e

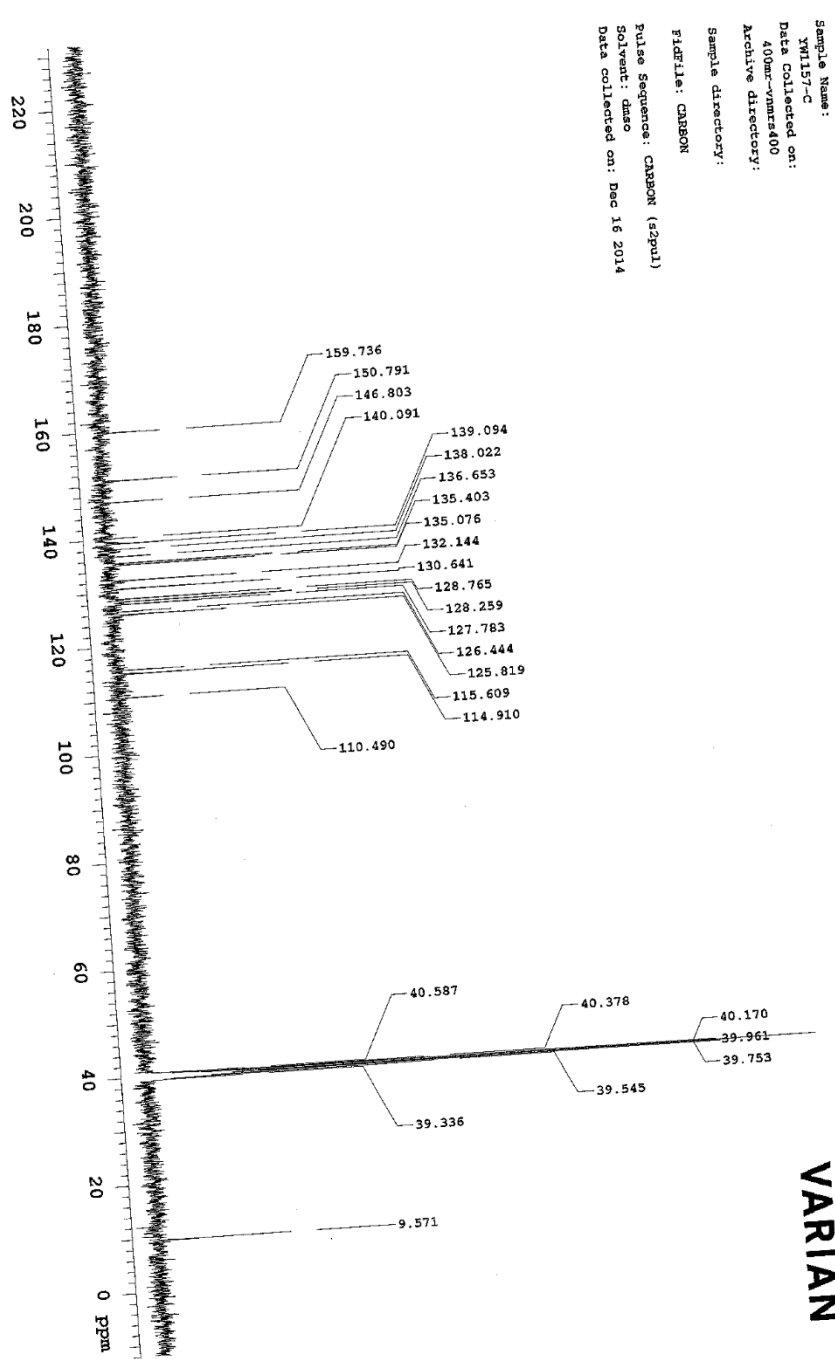


Figure S17. ¹³C NMR spectrum of compound 3f

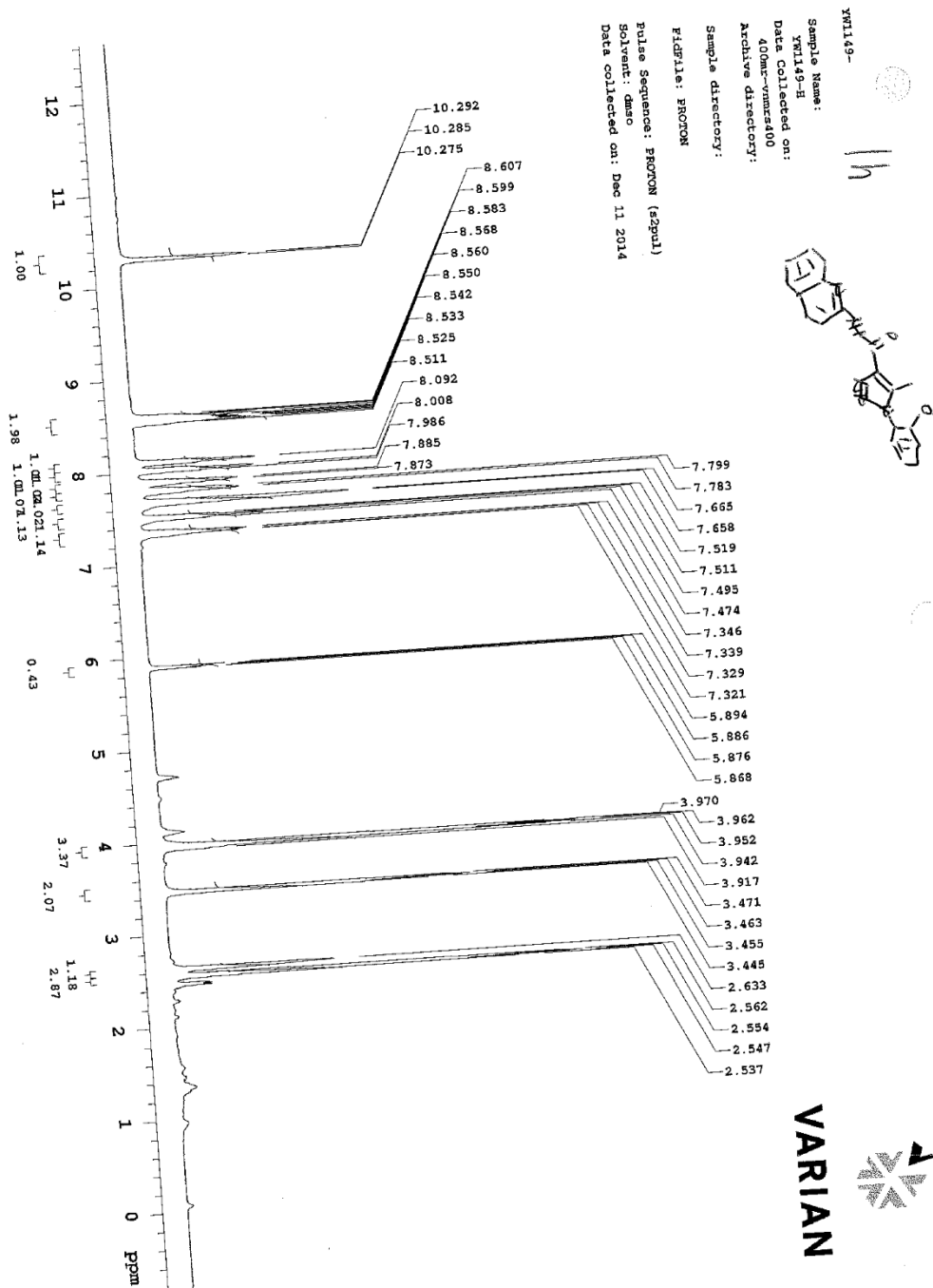


Figure S18. ¹H NMR spectrum of compound 3g

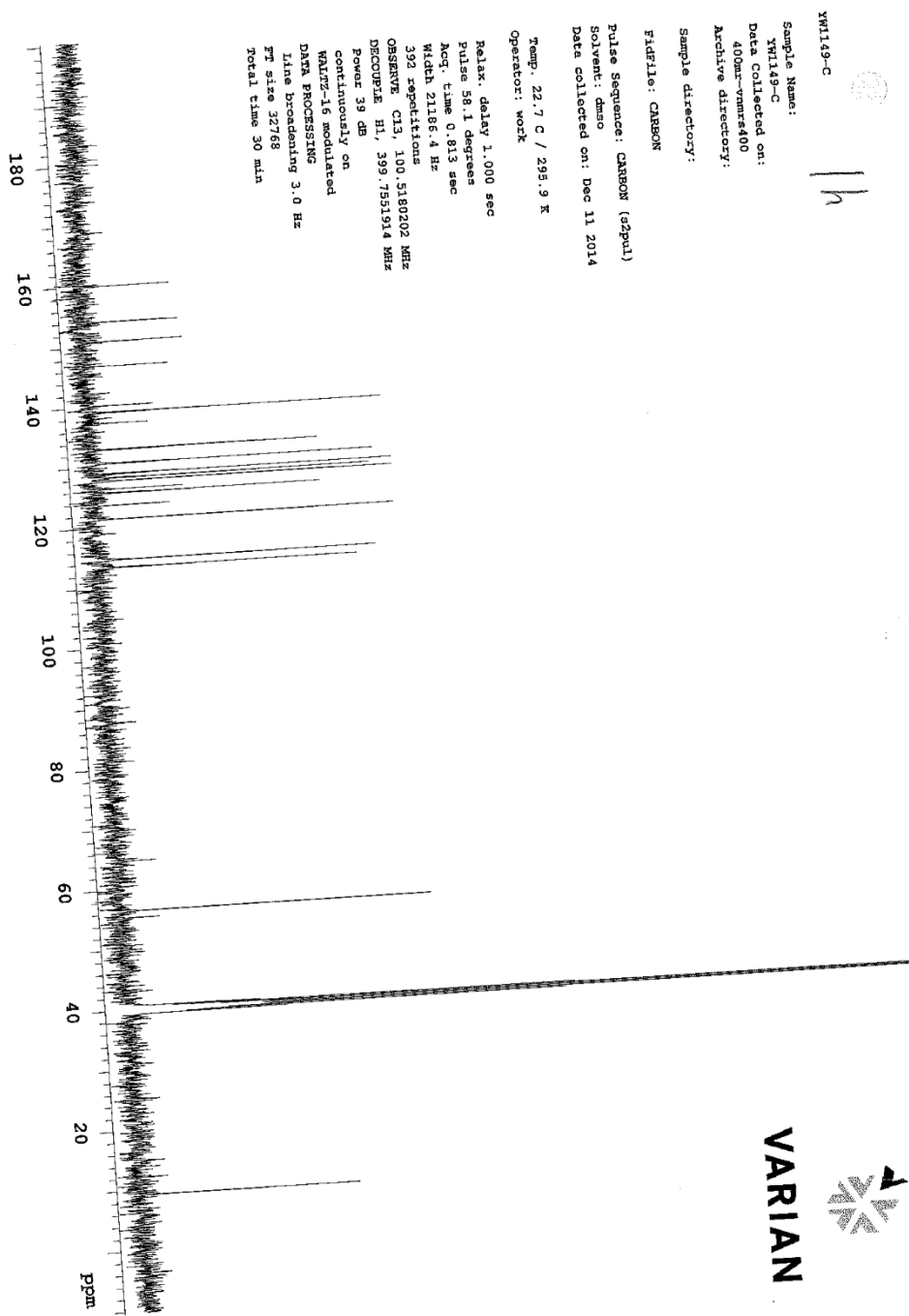
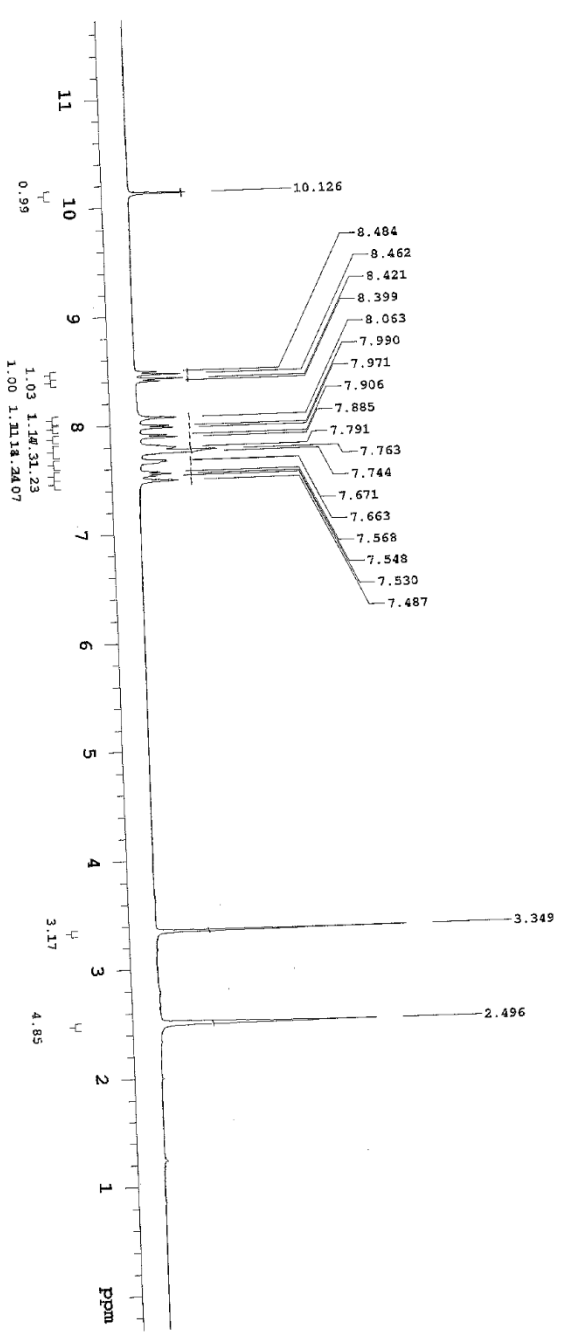


Figure S19. ^{13}C NMR spectrum of compound **3g**



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 Data collected on: Jun 6 2015

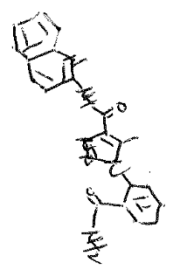


Figure S20. ¹H NMR spectrum of compound 3h

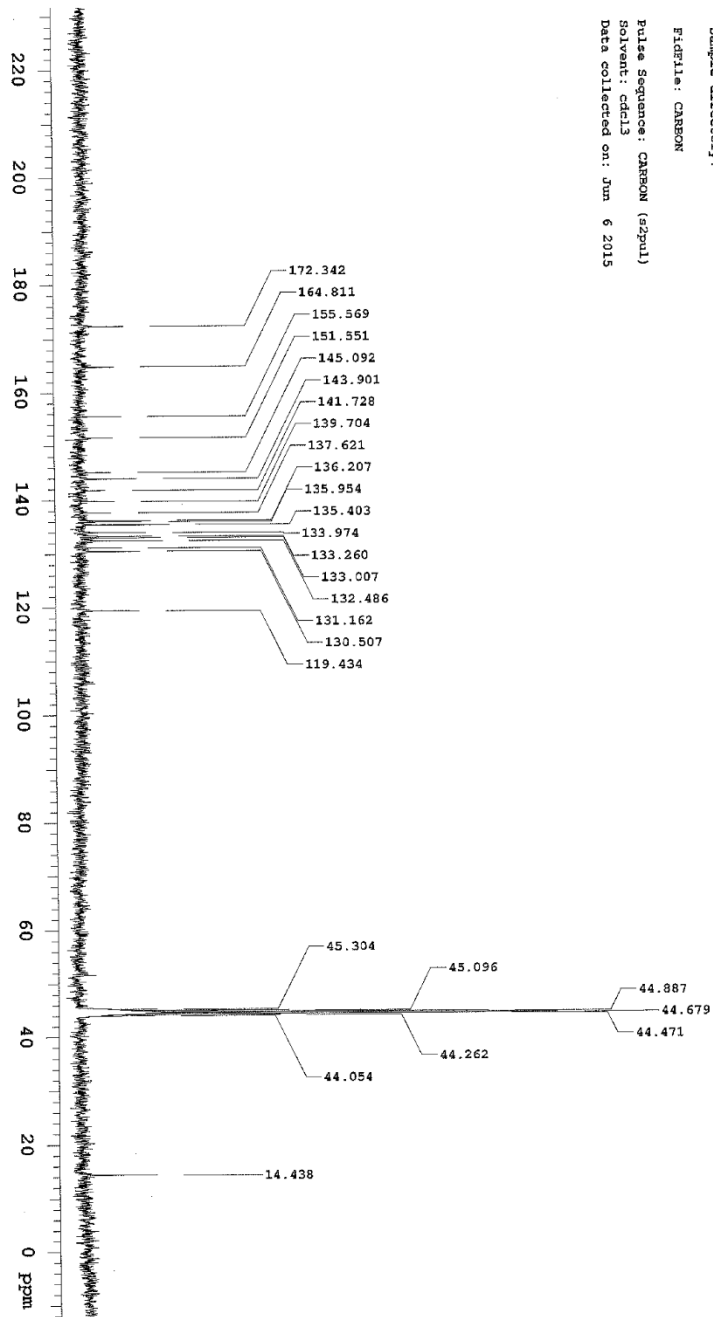
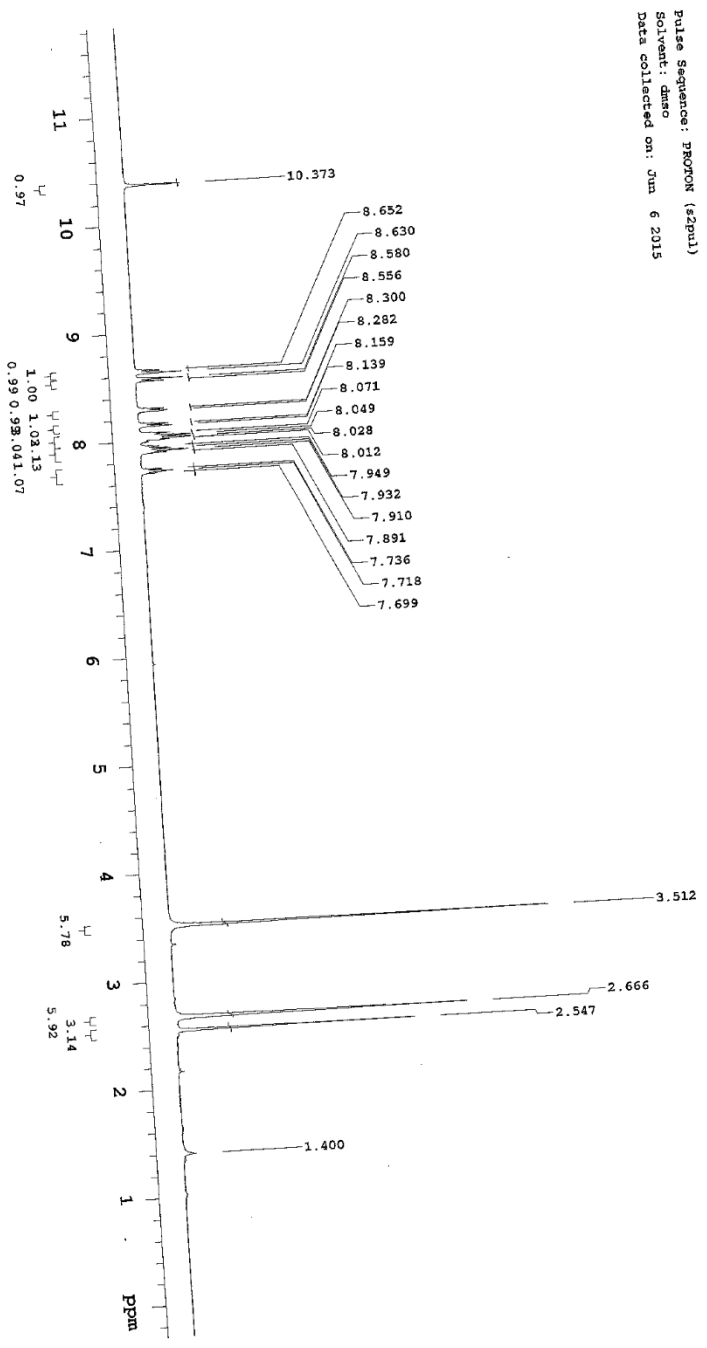


Figure S21. ^{13}C NMR spectrum of compound **3h**



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 Data collected on: Jun 6 2015

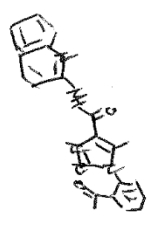


Figure S22. ¹H NMR spectrum of compound 3i

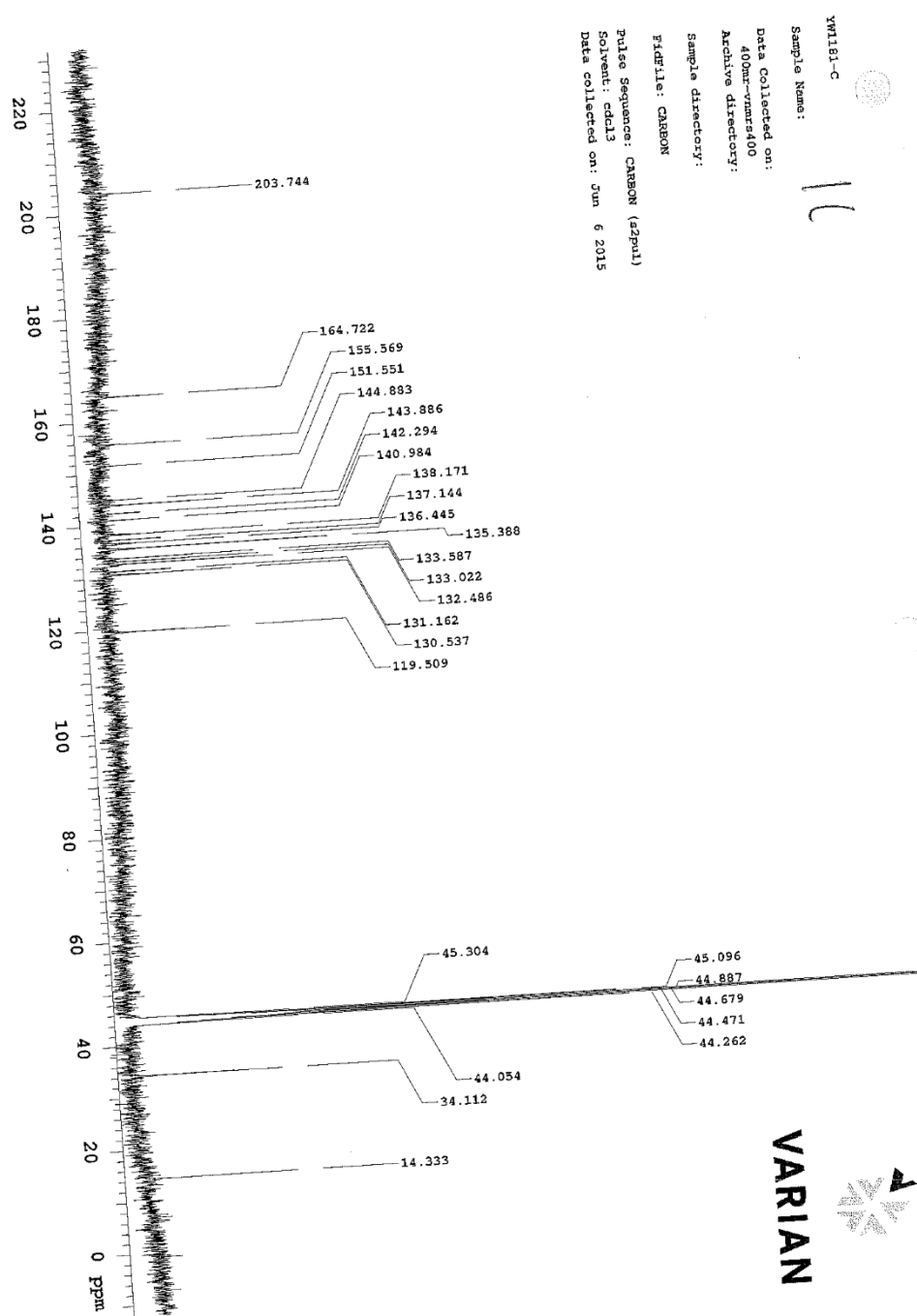


Figure S23. ¹³C NMR spectrum of compound 3i

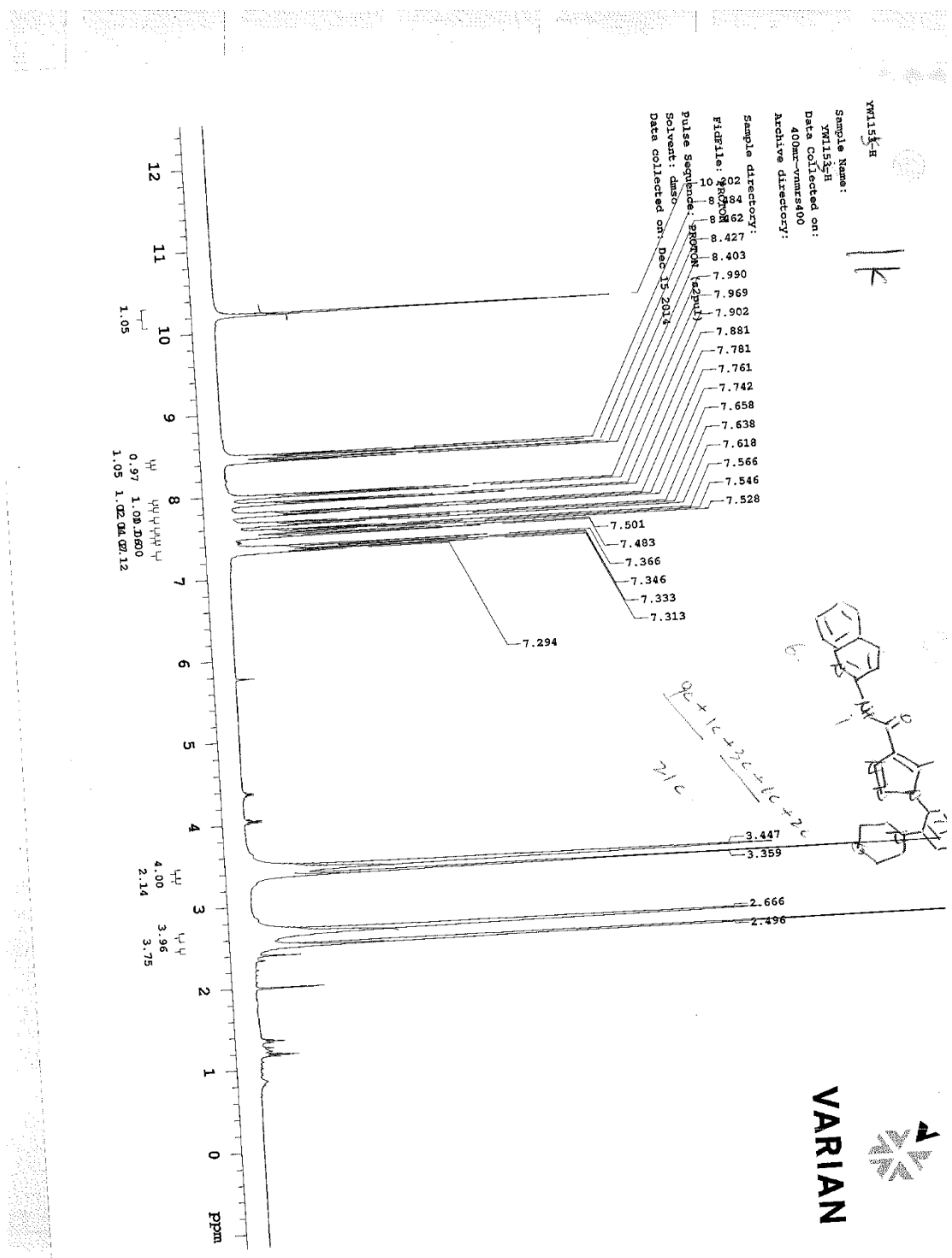


Figure S24. ¹H NMR spectrum of compound 3j

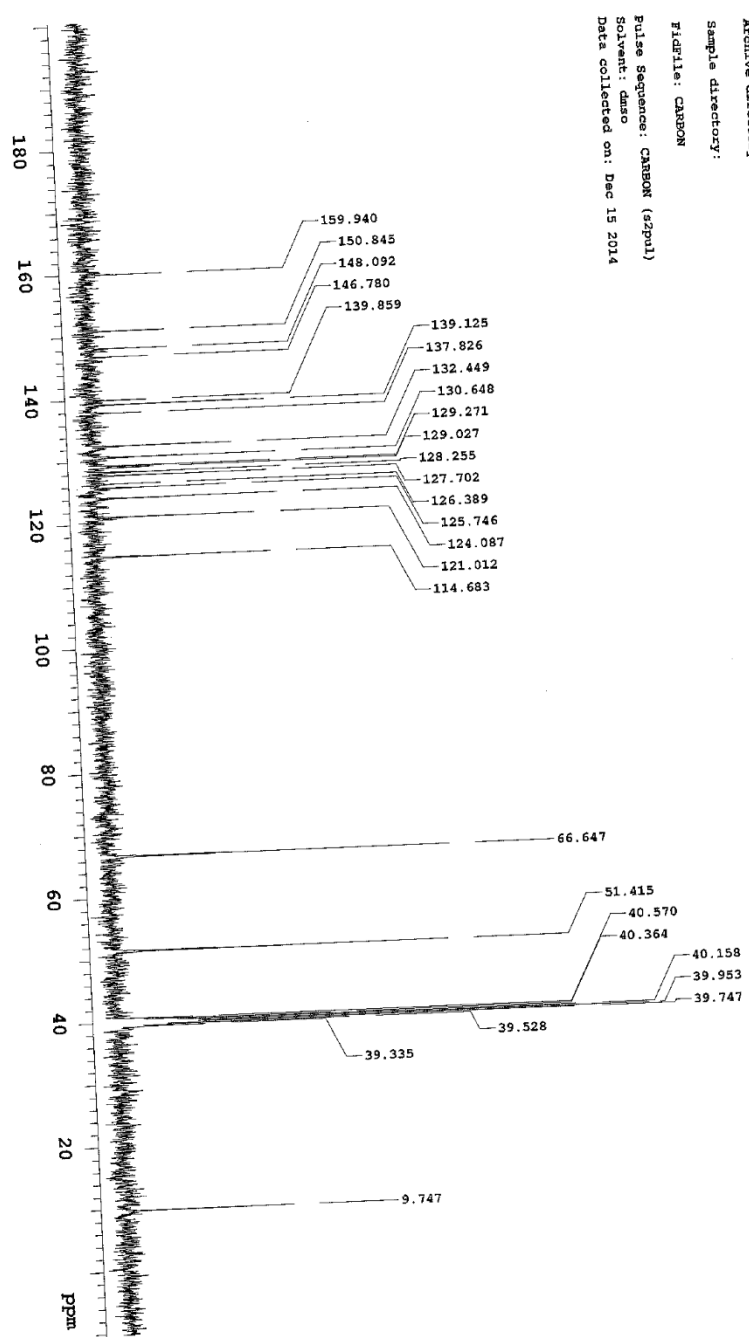


Figure S25. ¹³C NMR spectrum of compound 3j

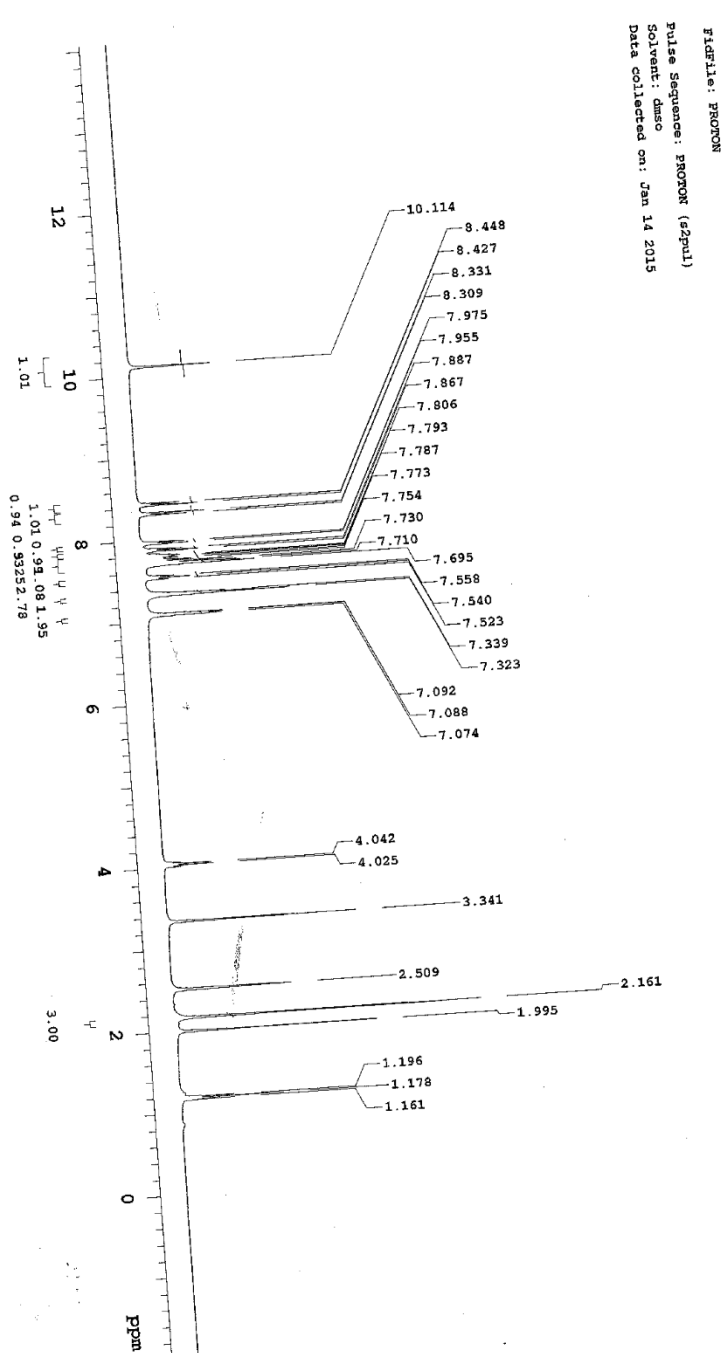


Figure S26. ¹H NMR spectrum of compound 3k

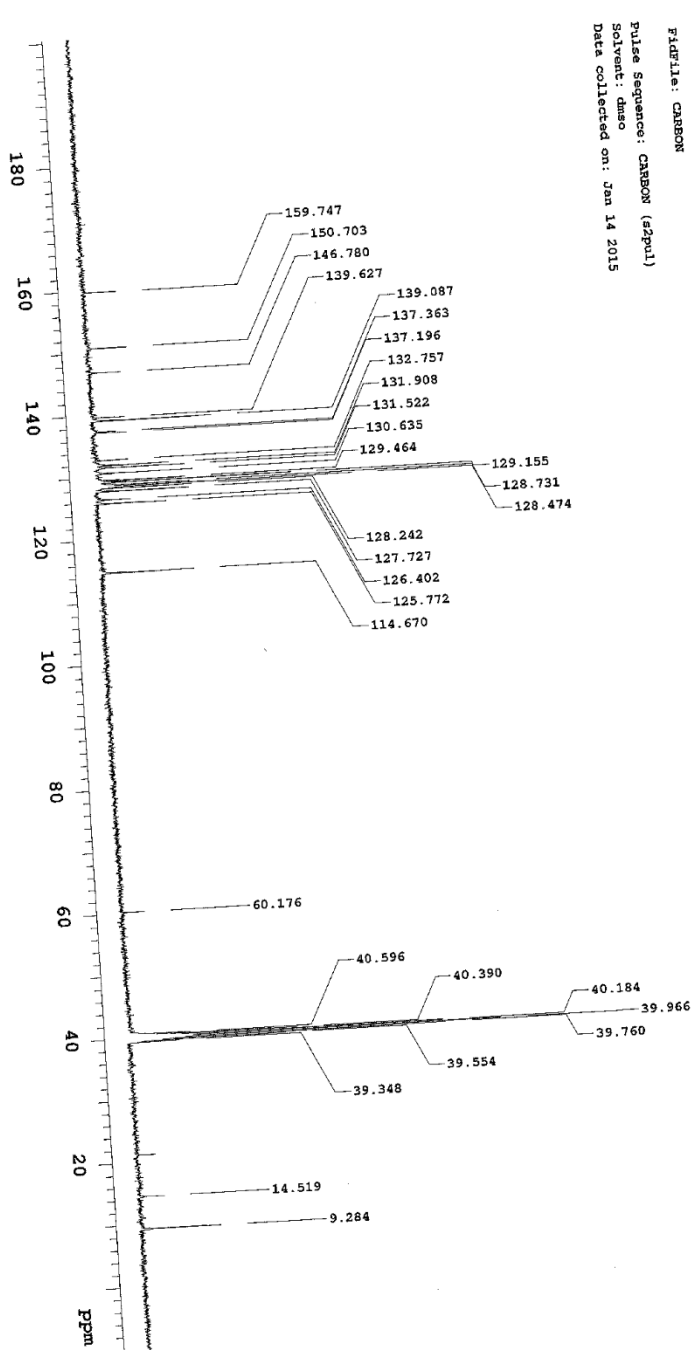


Figure S27. ¹³C NMR spectrum of compound 3k

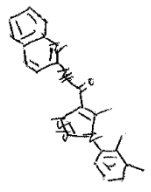
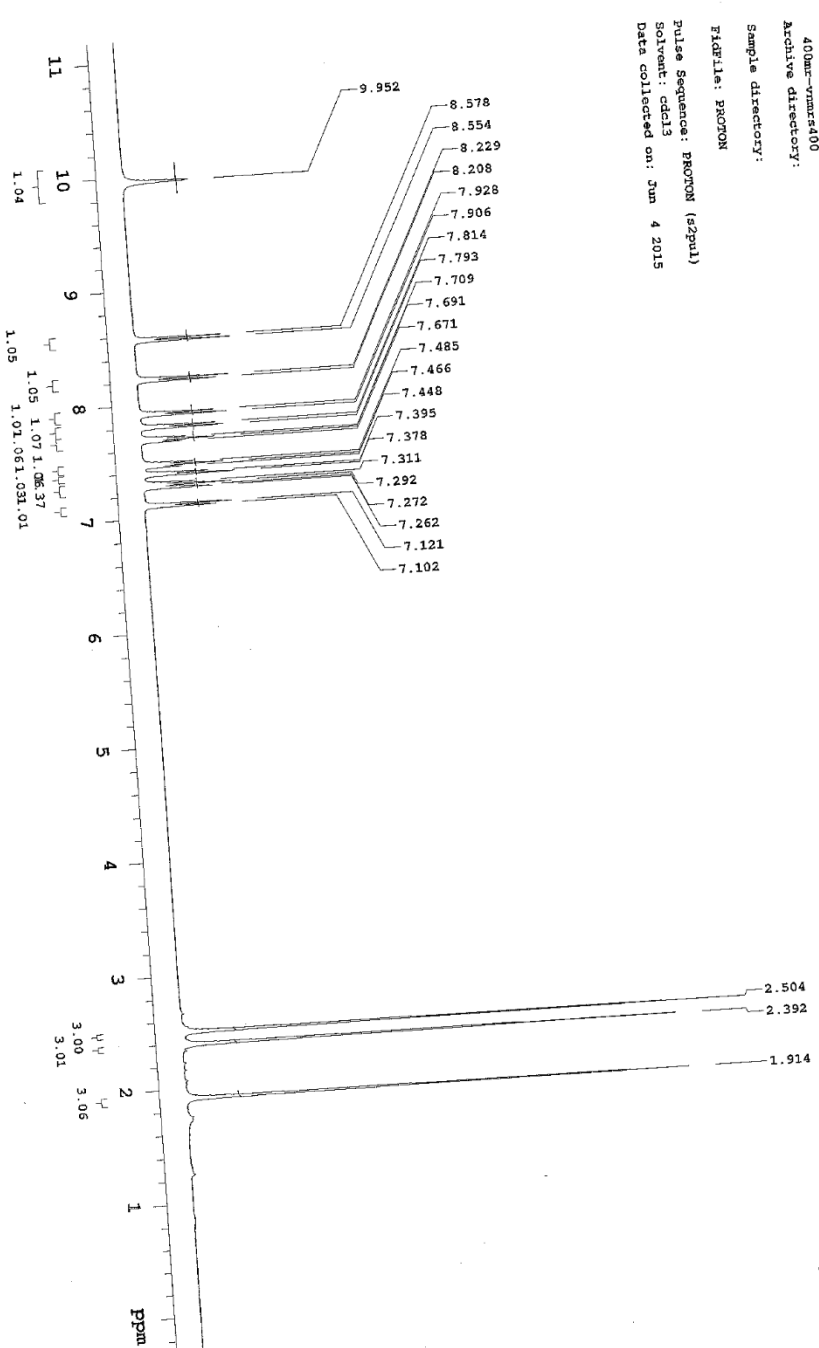


Figure S28. ¹H NMR spectrum of compound 3I

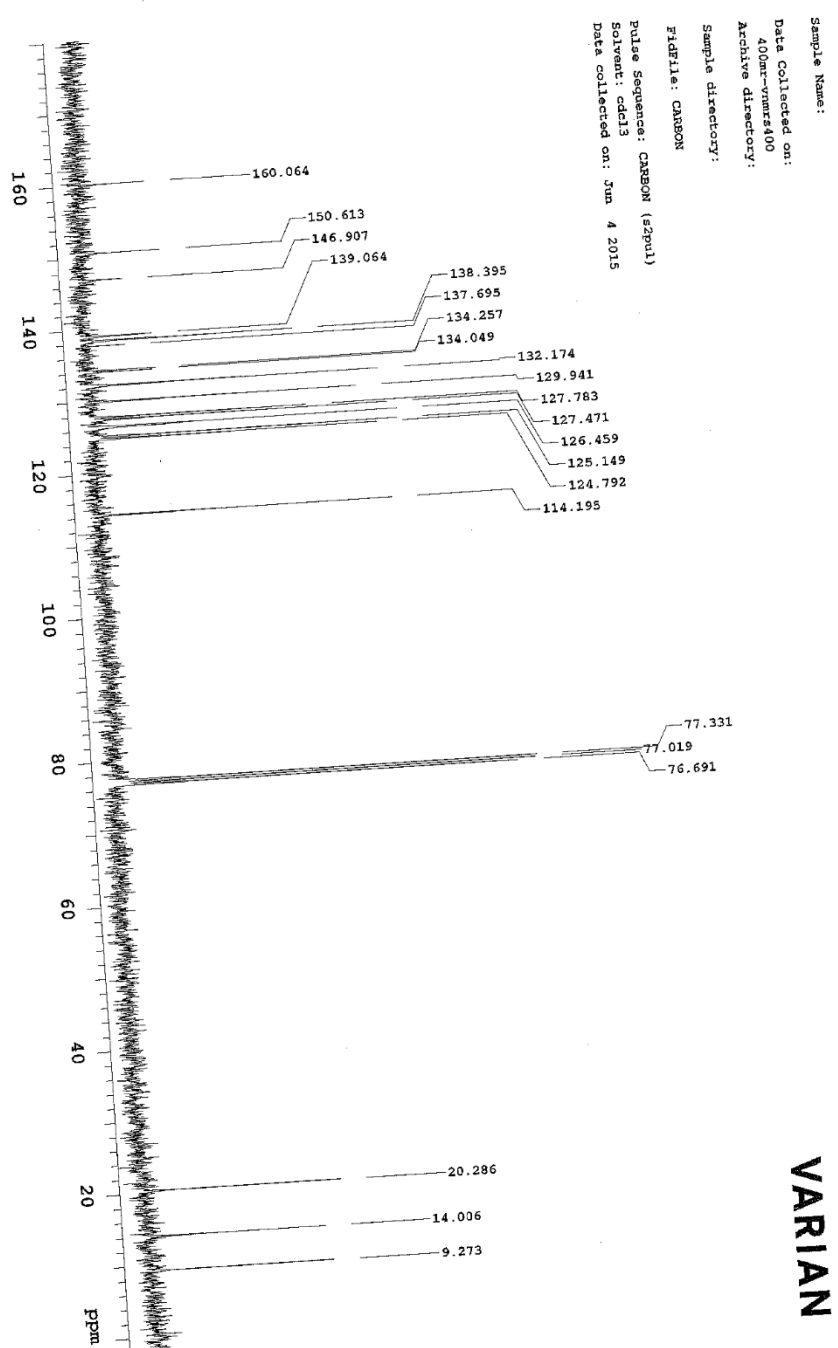
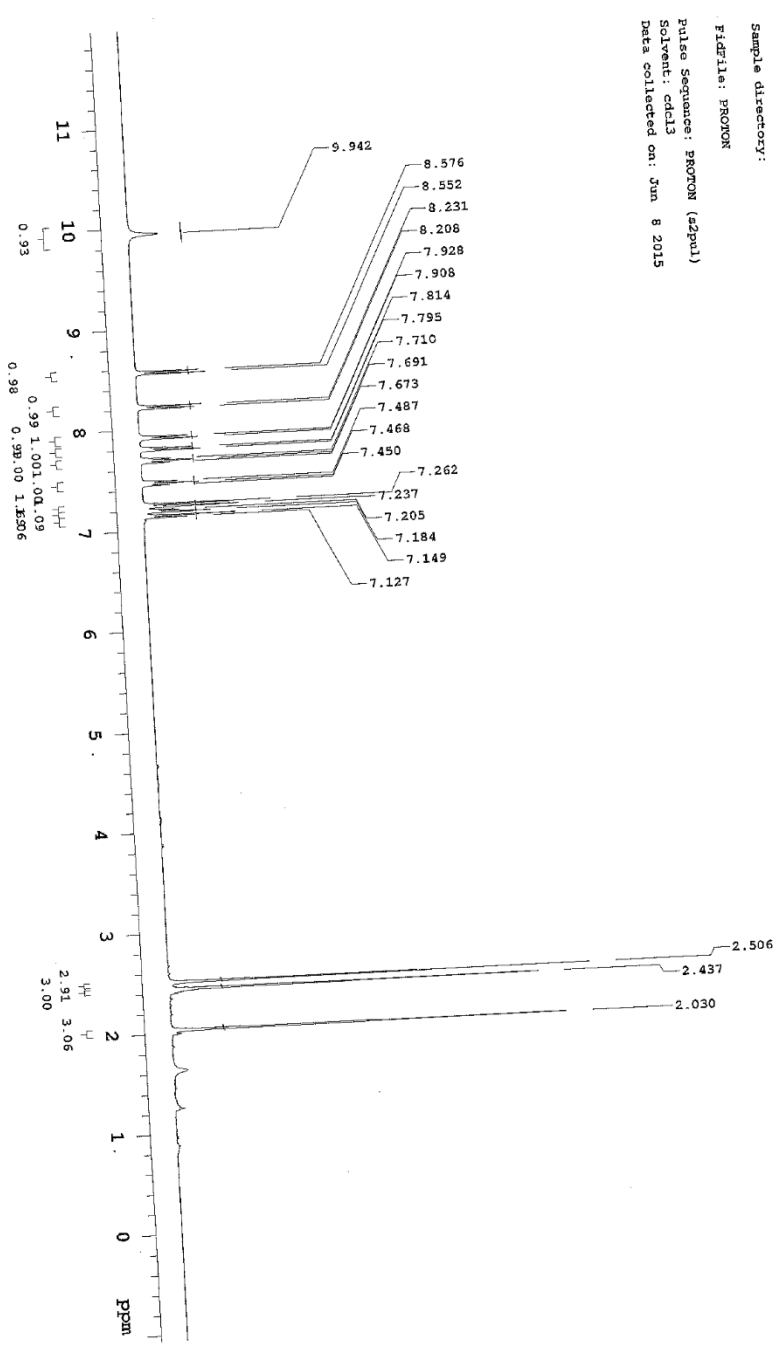


Figure S29. ¹³C NMR spectrum of compound 31



YMH179-H
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 Data collected on: Jun 8 2015

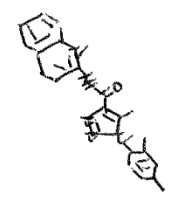


Figure S30. ¹H NMR spectrum of compound 3m

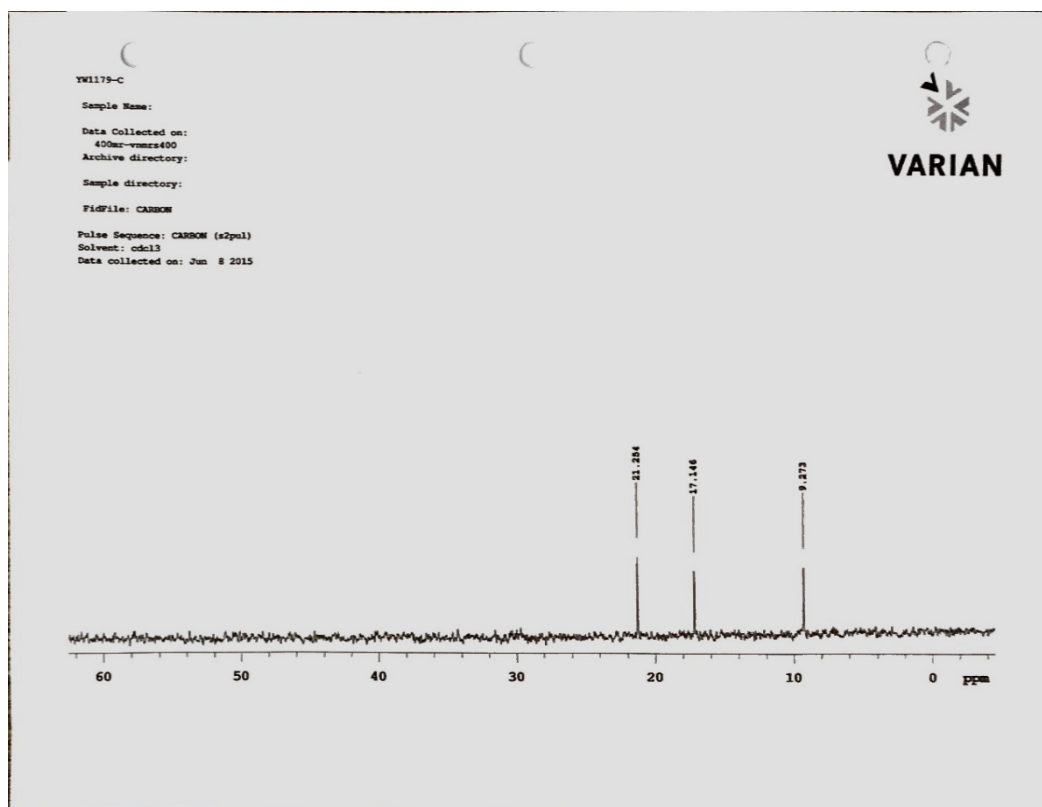
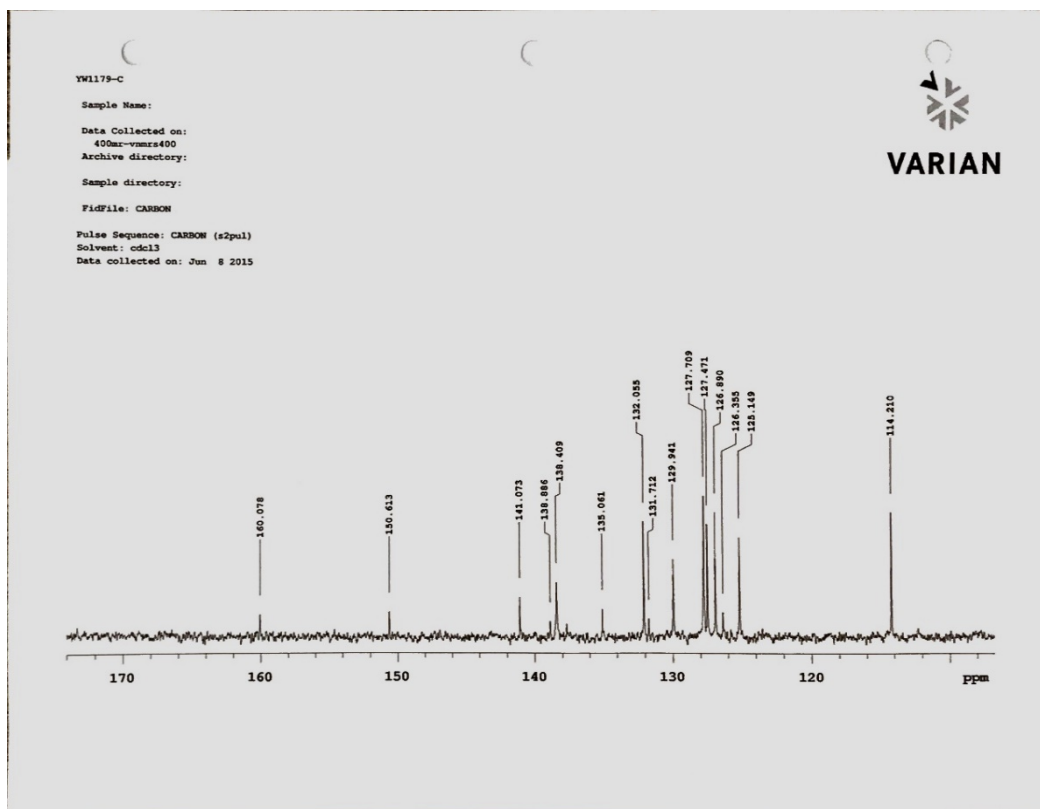


Figure S31. ^{13}C NMR spectrum of compound **3m**

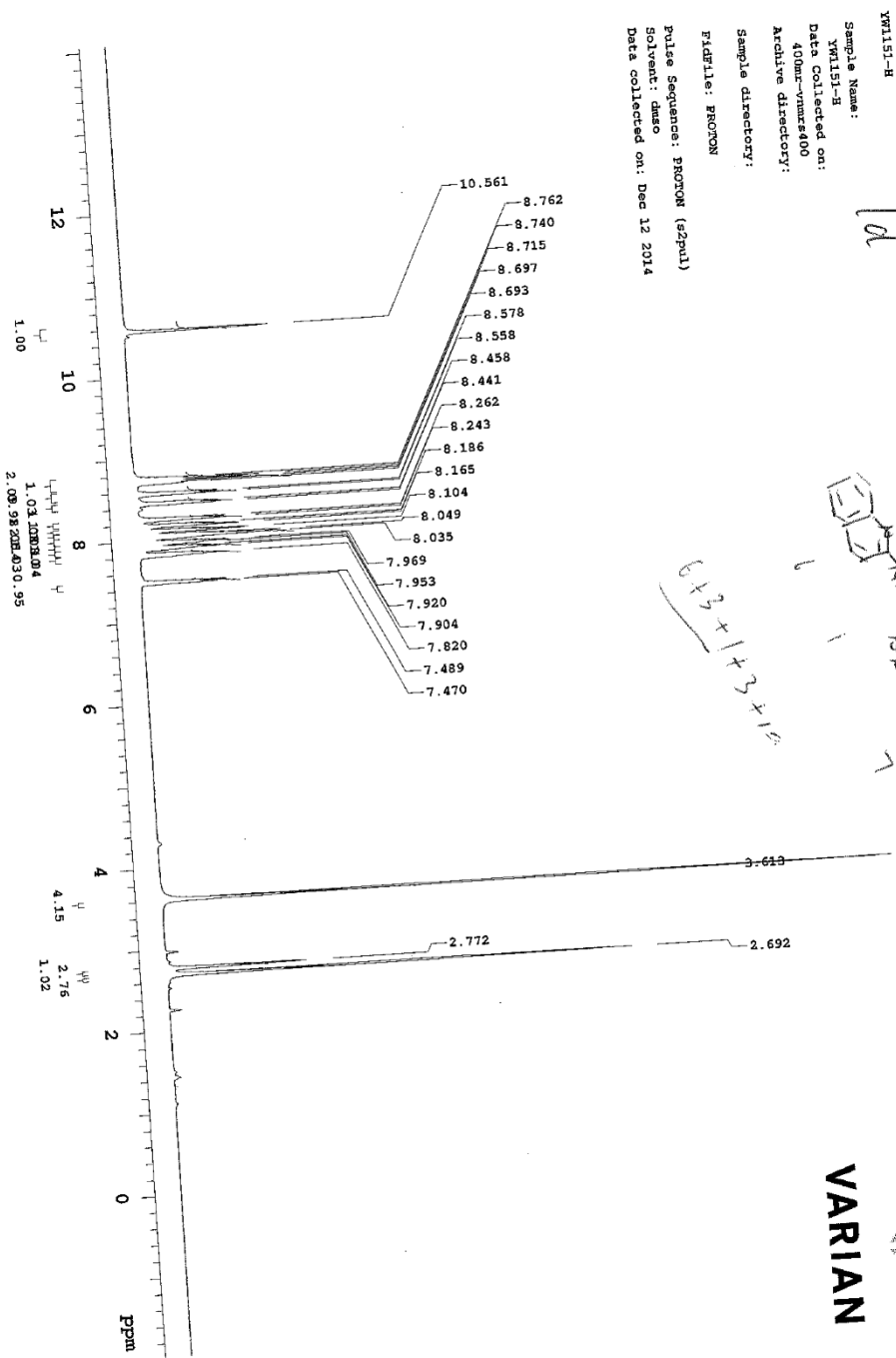


Figure S32. ¹H NMR spectrum of compound 3n

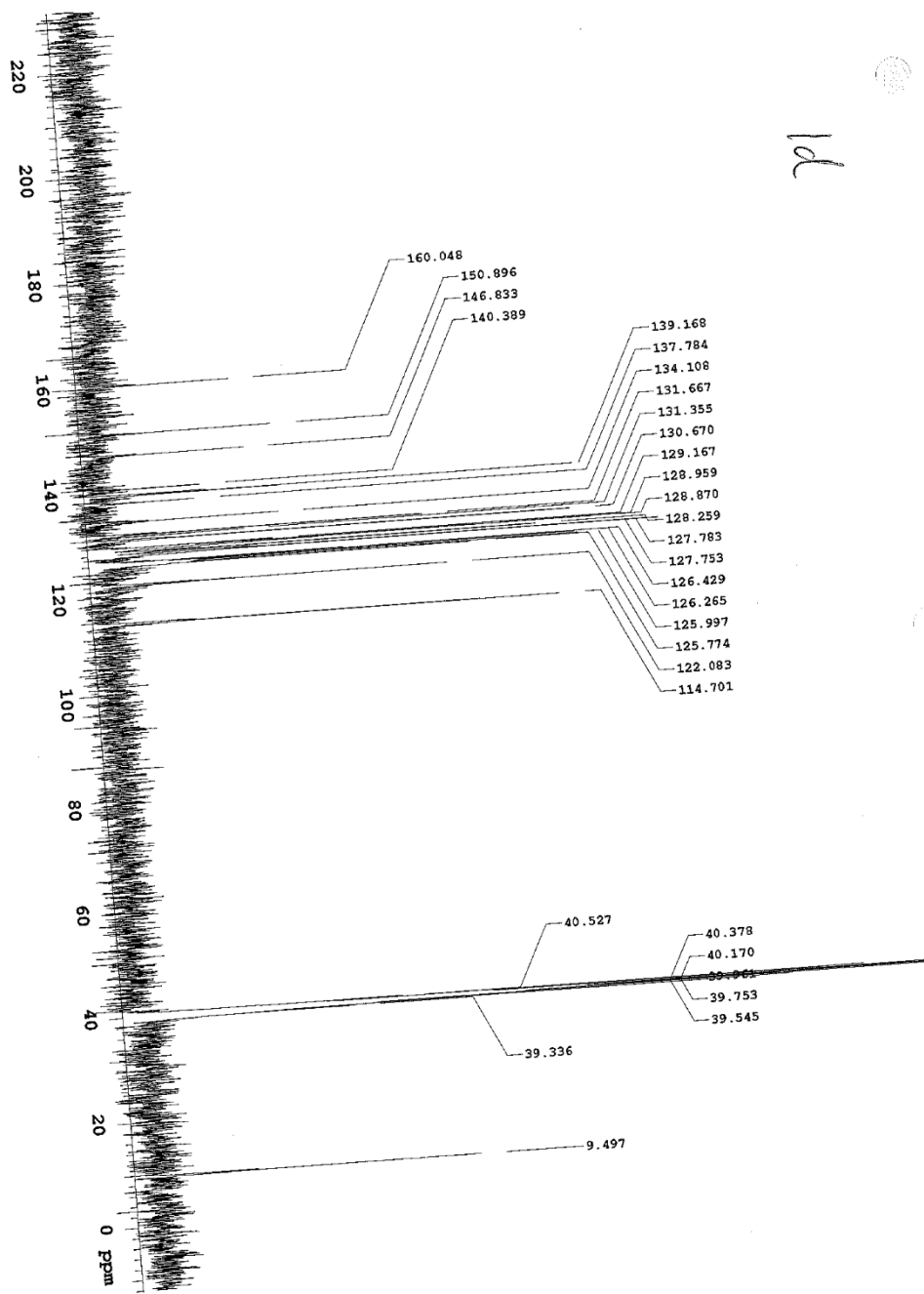
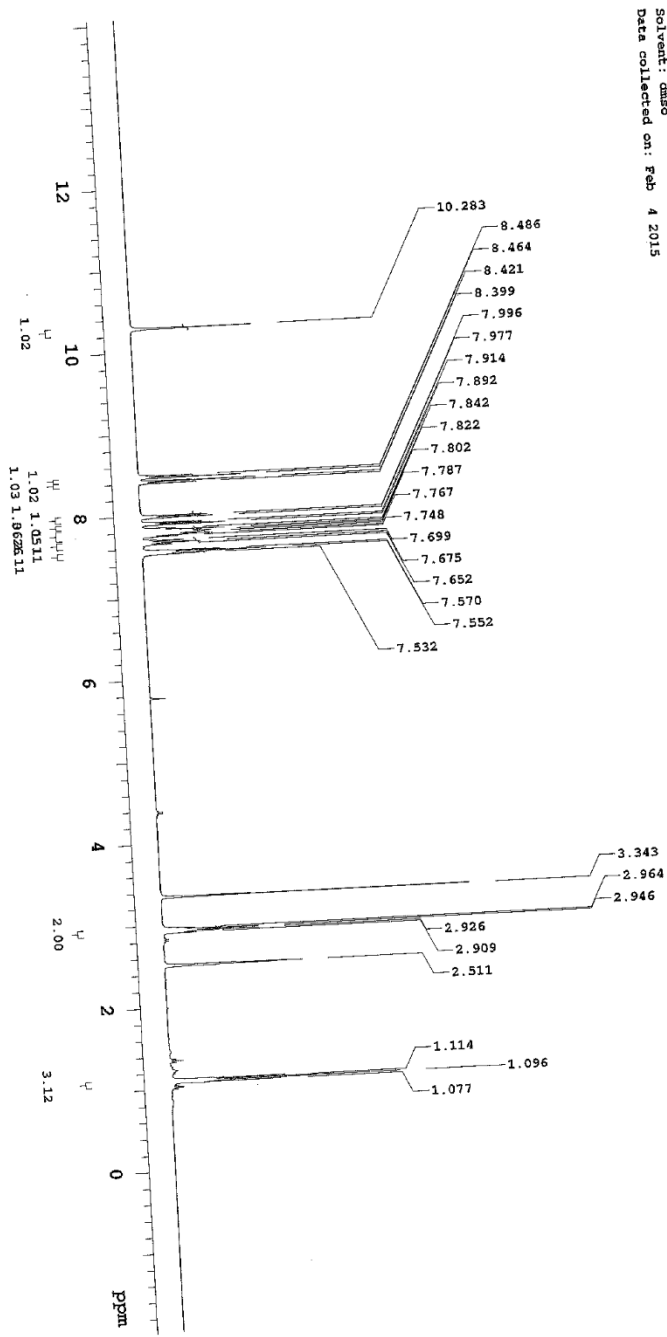


Figure S33. ¹³C NMR spectrum of compound 3n



Sample Name: YW2013-B
 Data Collected on: 4/08/2015
 Archive directory:
 Sample directory:
 FID file: PROTON
 Pulse Sequence: PROTON (zgpg3)
 Solvent: dmso
 Data collected on: Feb 4 2015



Figure S34. ¹H NMR spectrum of compound 30

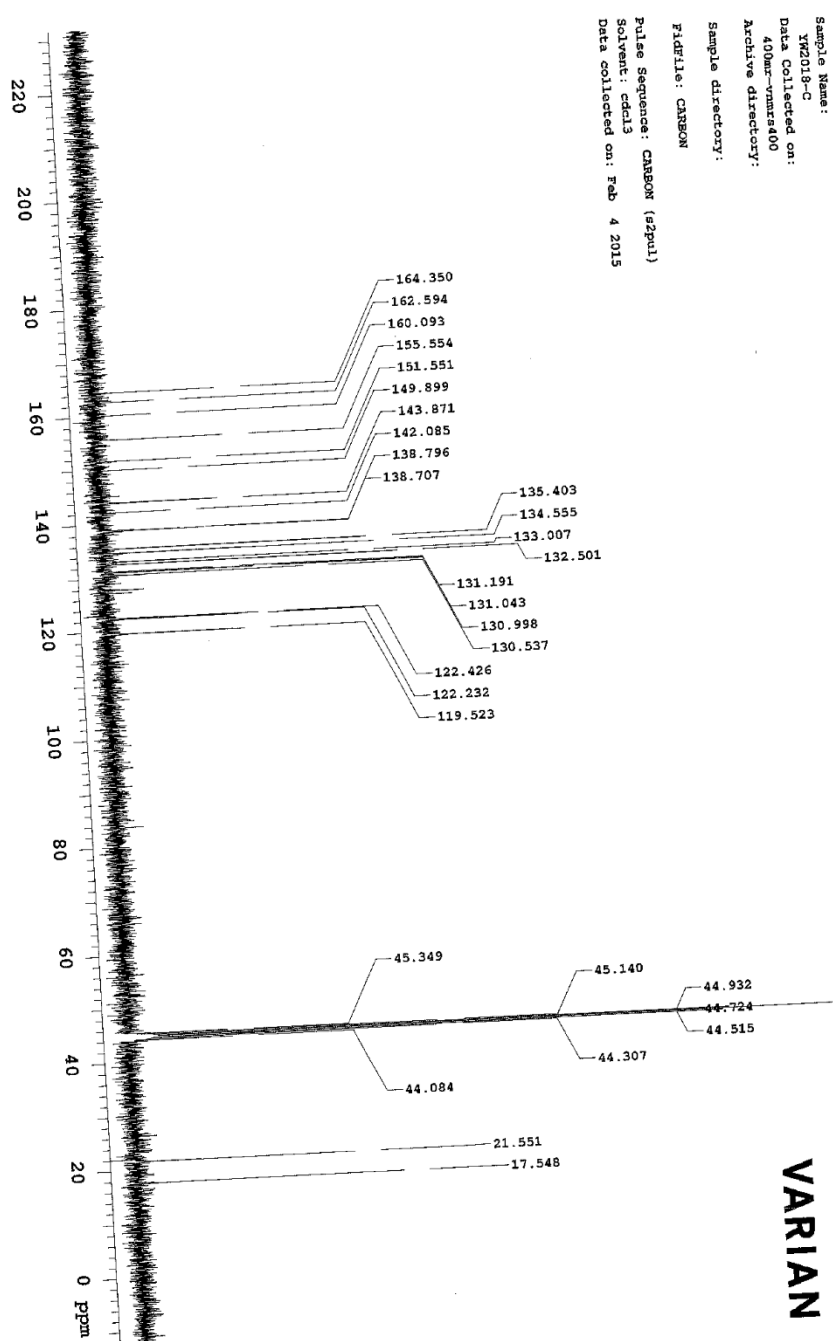


Figure S35. ^{13}C NMR spectrum of compound **30**

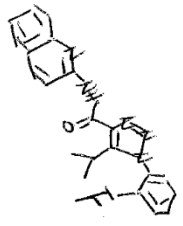
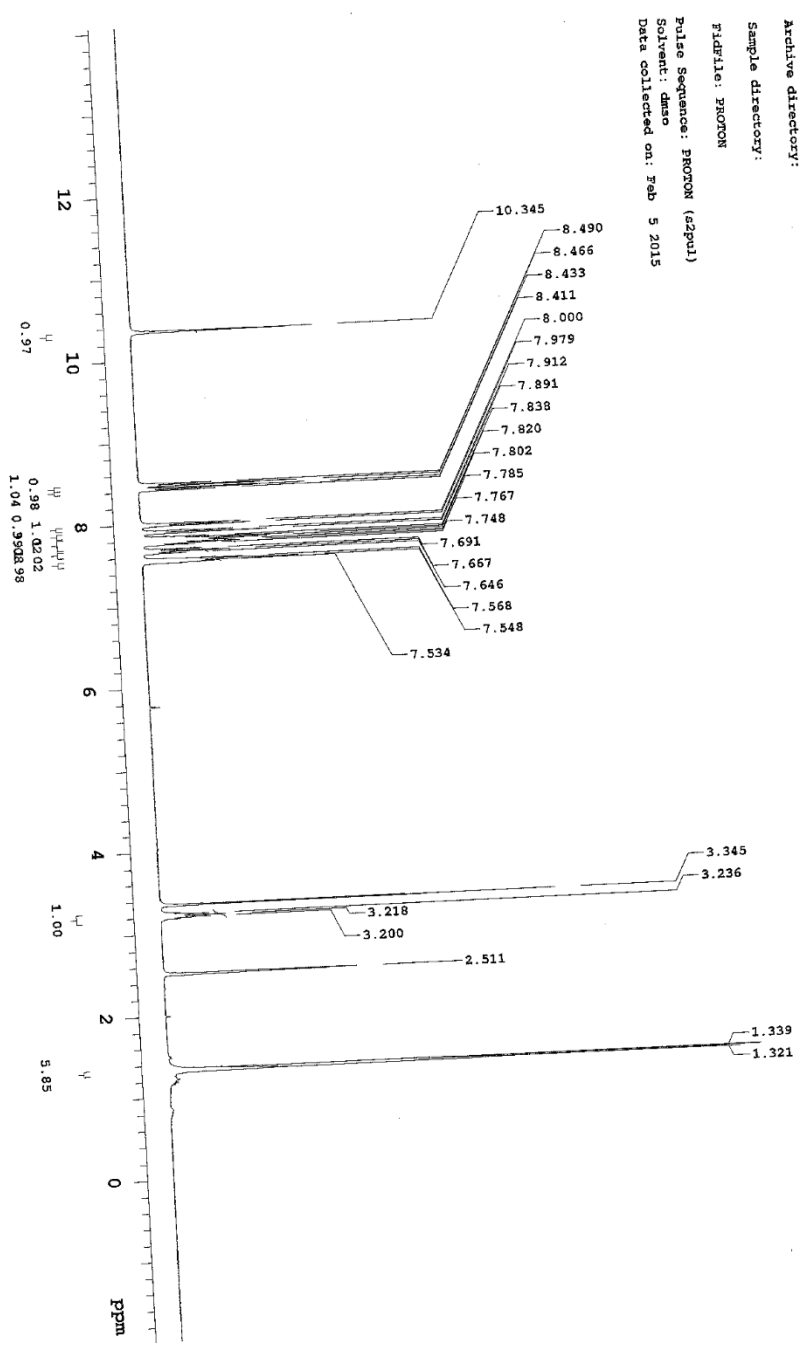


Figure S36. ¹H NMR spectrum of compound 3p

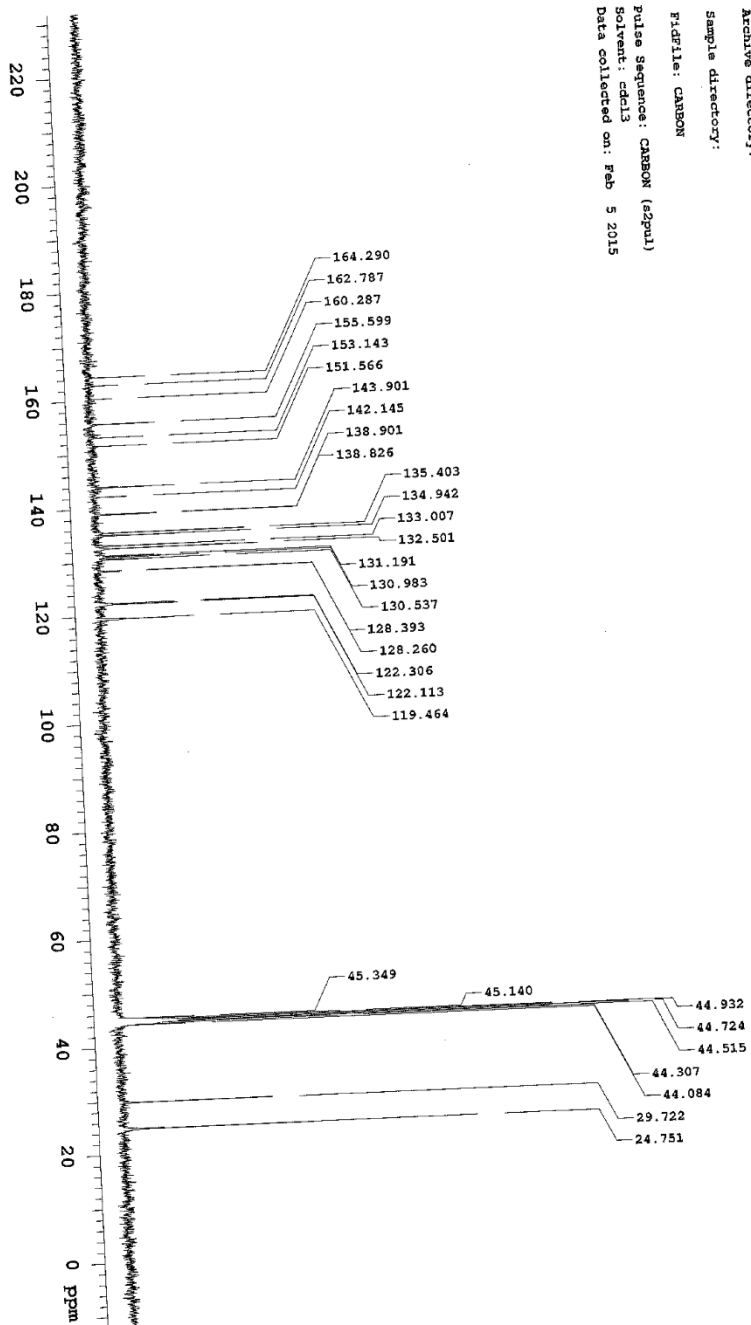


Figure S37. ^{13}C NMR spectrum of compound **3p**

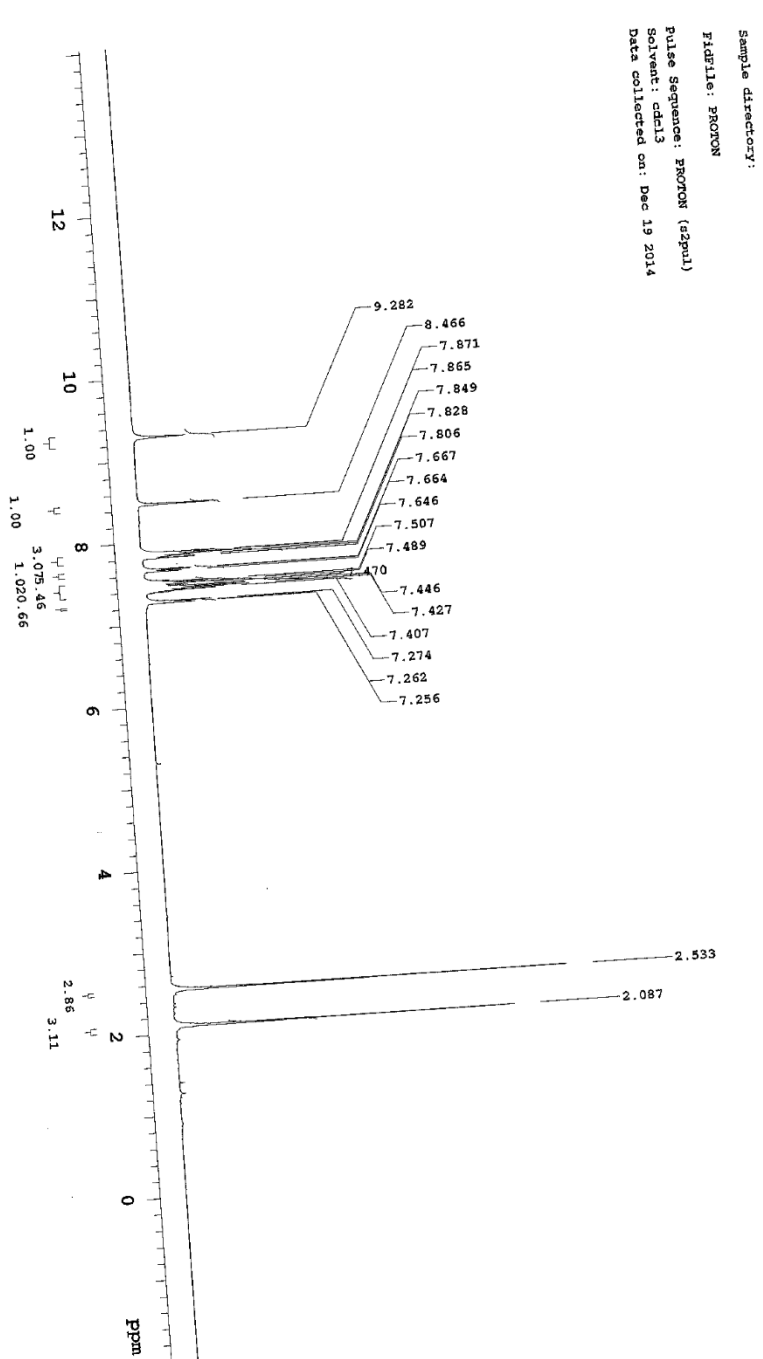
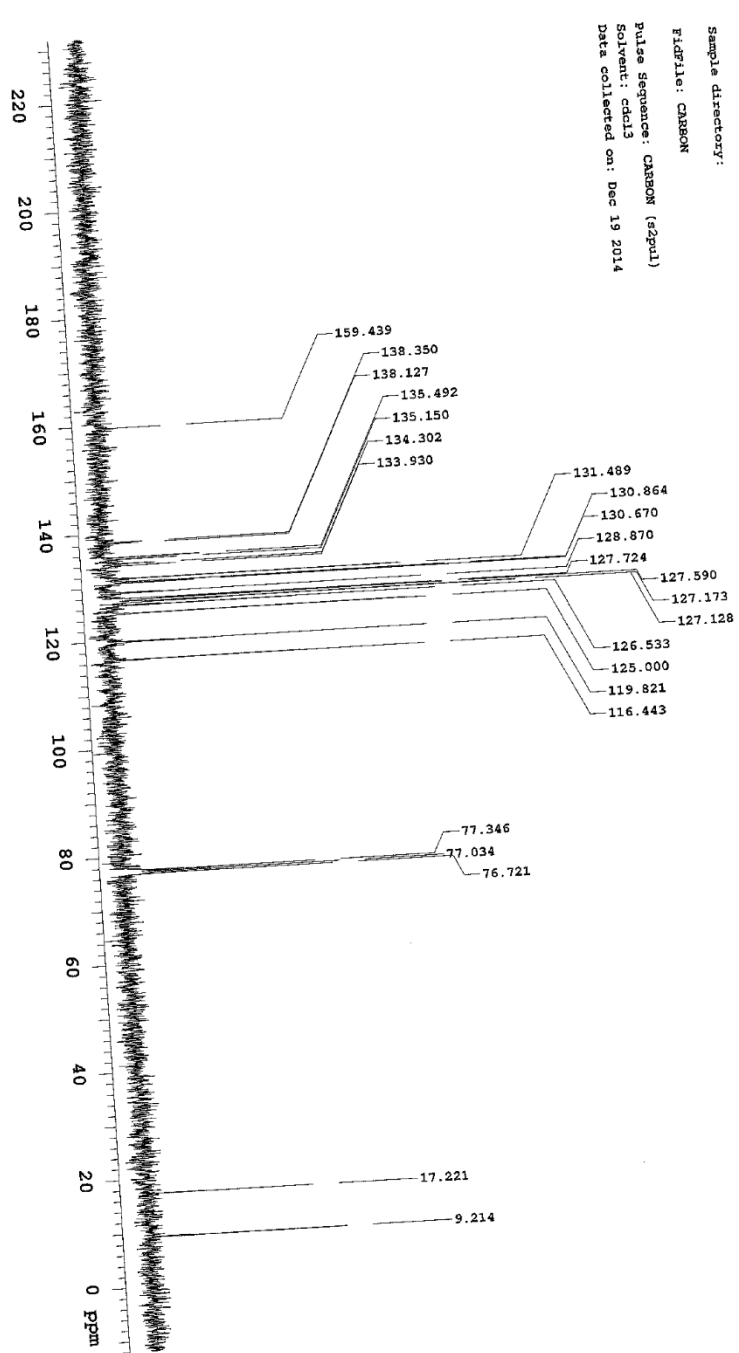


Figure S38. ¹H NMR spectrum of compound 3q



VARIAN

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

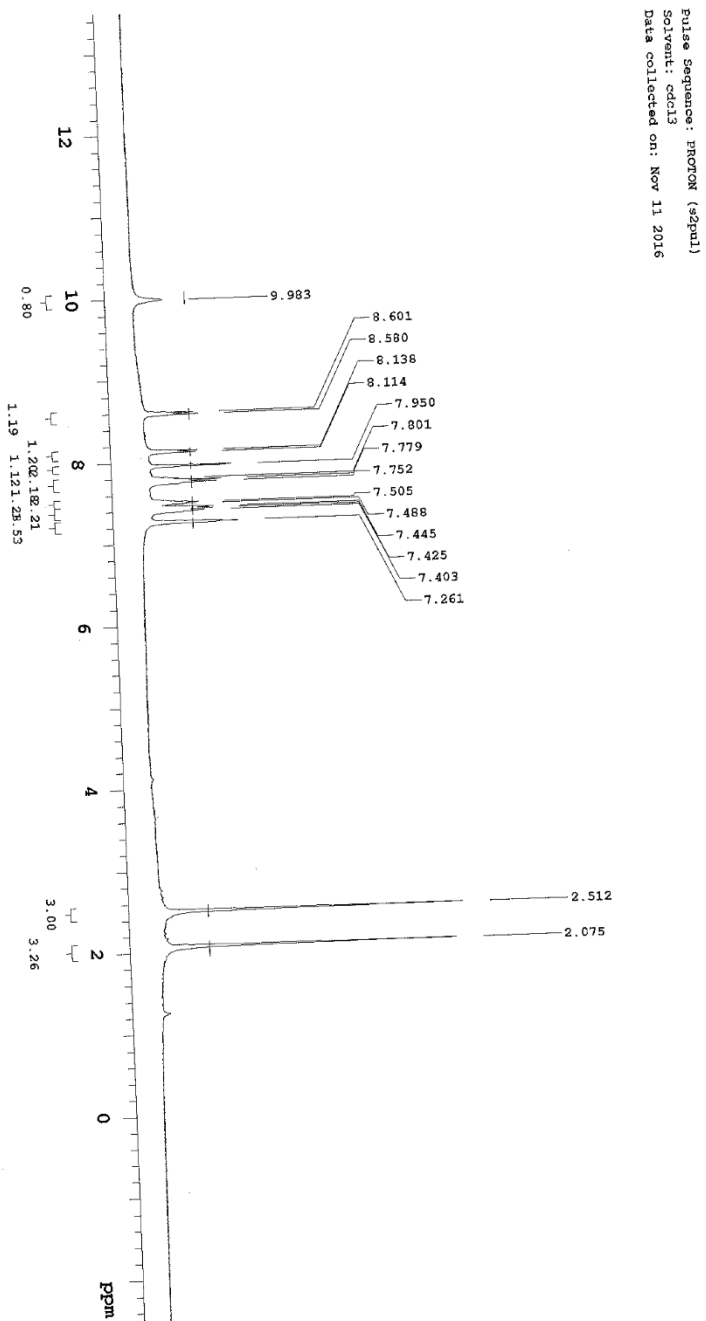


Figure S40. ¹H NMR spectrum of compound 3r

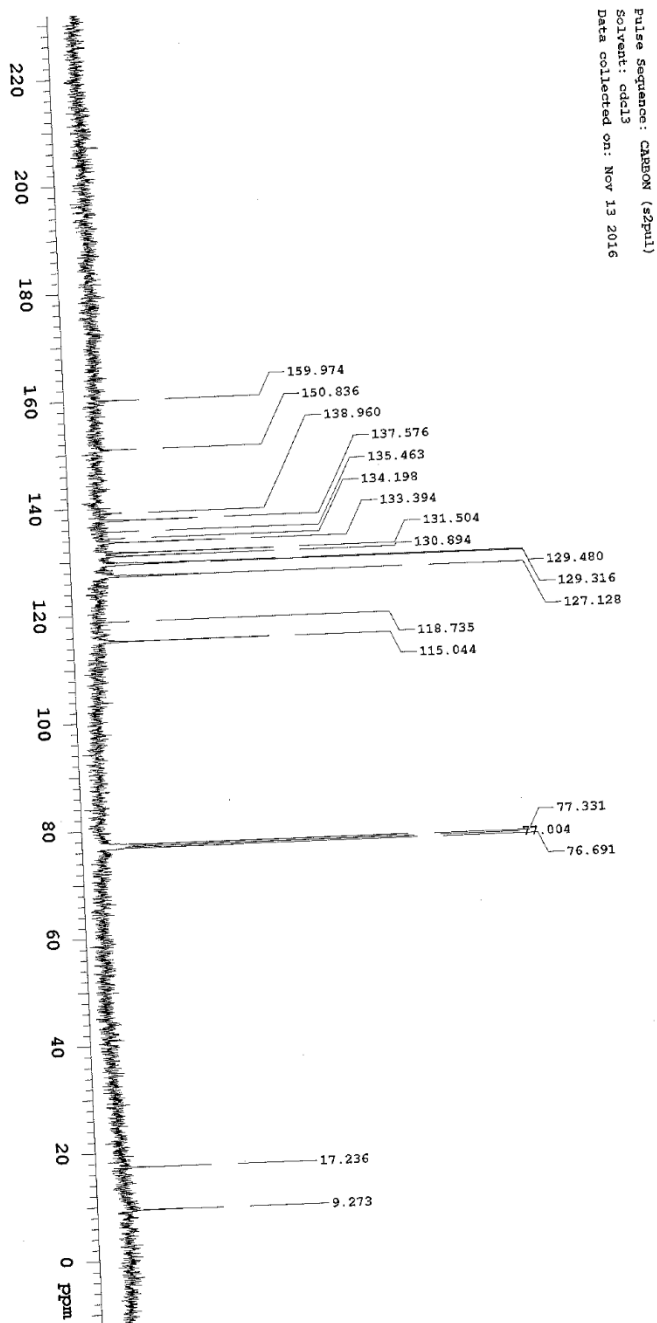


Figure S41. ^{13}C NMR spectrum of compound **3r**

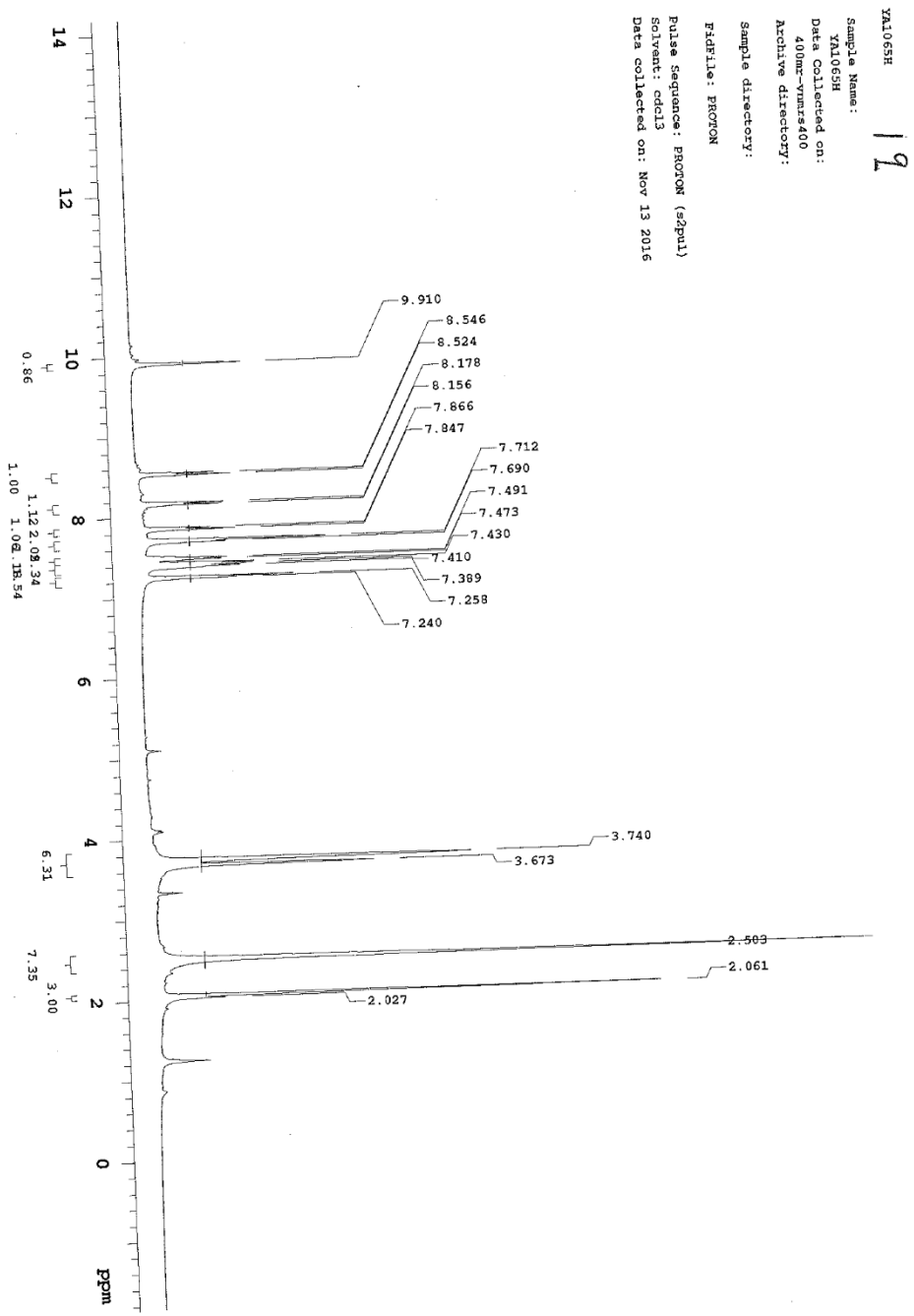


Figure S42. ¹H NMR spectrum of compound 3s

YAI065C

19

Sample Name:
YAI065C
Data collected on:
4/06/2016
Archive directory:
Sample directory:
FIDFile: CARBON
Pulse Sequence: CARBON (a2pu1)
Solvent: cdcl3
Data collected on: Nov 13 2016

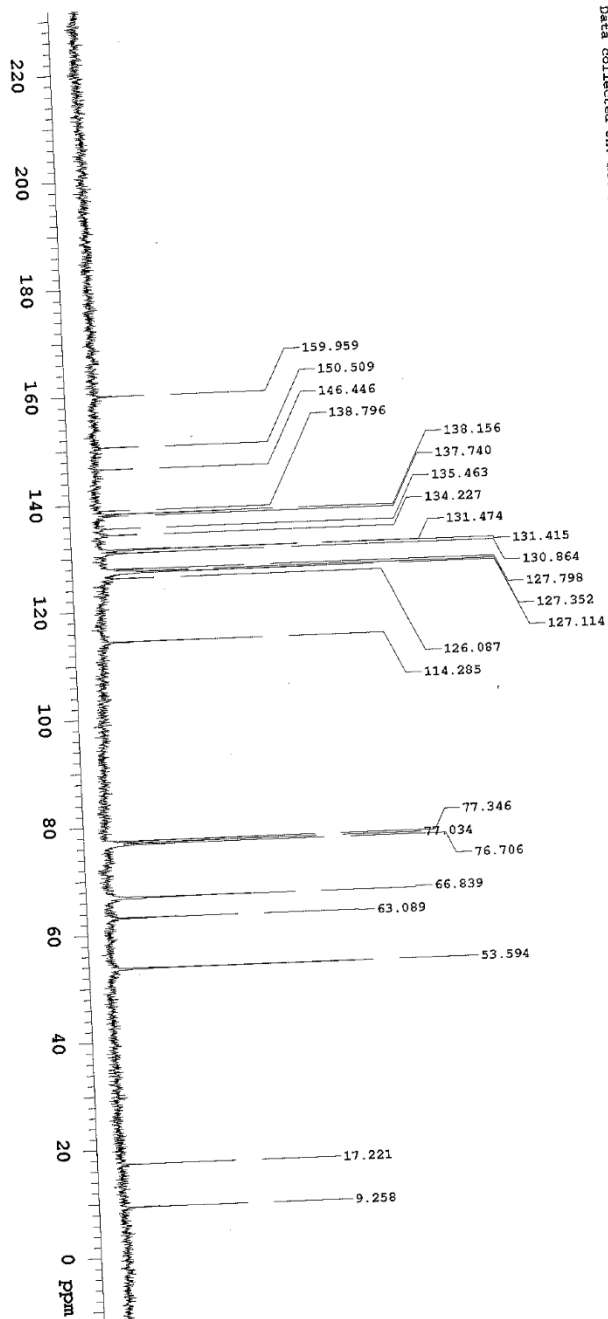
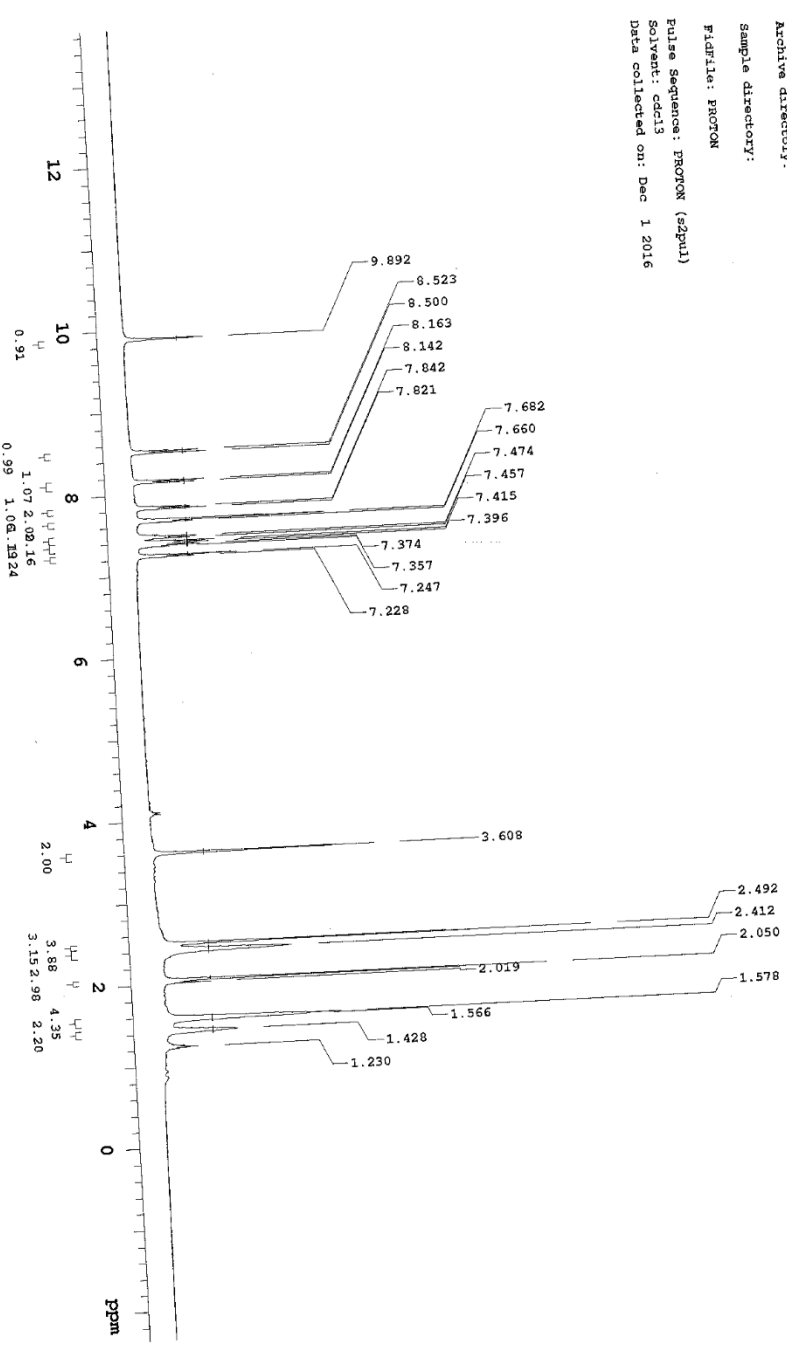


Figure S43. ^{13}C NMR spectrum of compound 3s



YA1093H
 Sample Name: YA1093H
 Data Collected on: 400MHz-mmcs400
 Archive directory:
 Sample directory:
 FIDFile: PROTON
 Pulse Sequence: PROTON (szpml)
 Solvent: cdc13
 Data collected on: Dec 1 2016

Figure S44. ¹H NMR spectrum of compound 3t

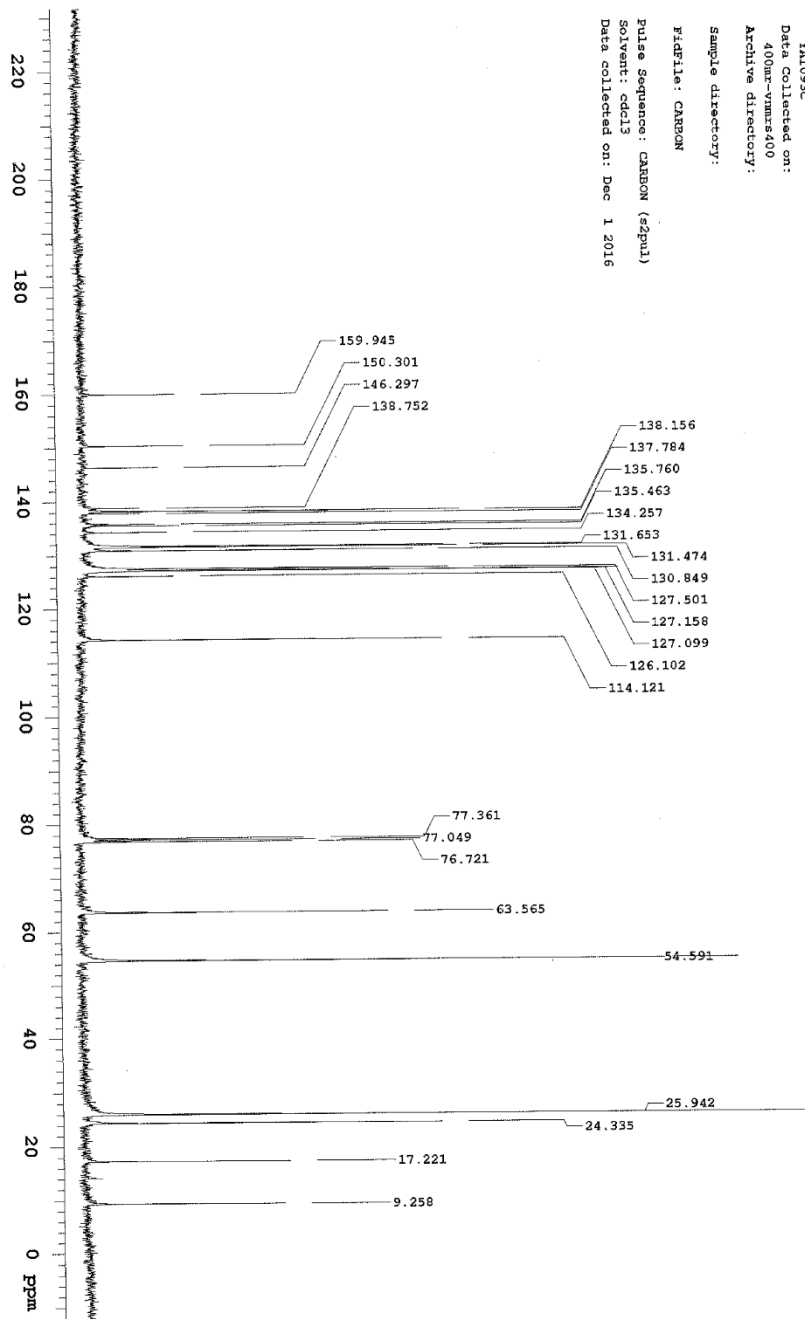


Figure S45. ^{13}C NMR spectrum of compound **3t**

YAL099H
15

Sample Name: YAL099H
Data Collected on: 400MHz-mmz400
Archive directory:
Sample directory:
FIDFile: PROTON
Pulse Sequence: PROTON (zgpg3)
Solvent: cdcl3
Data collected on: Dec 1 2016

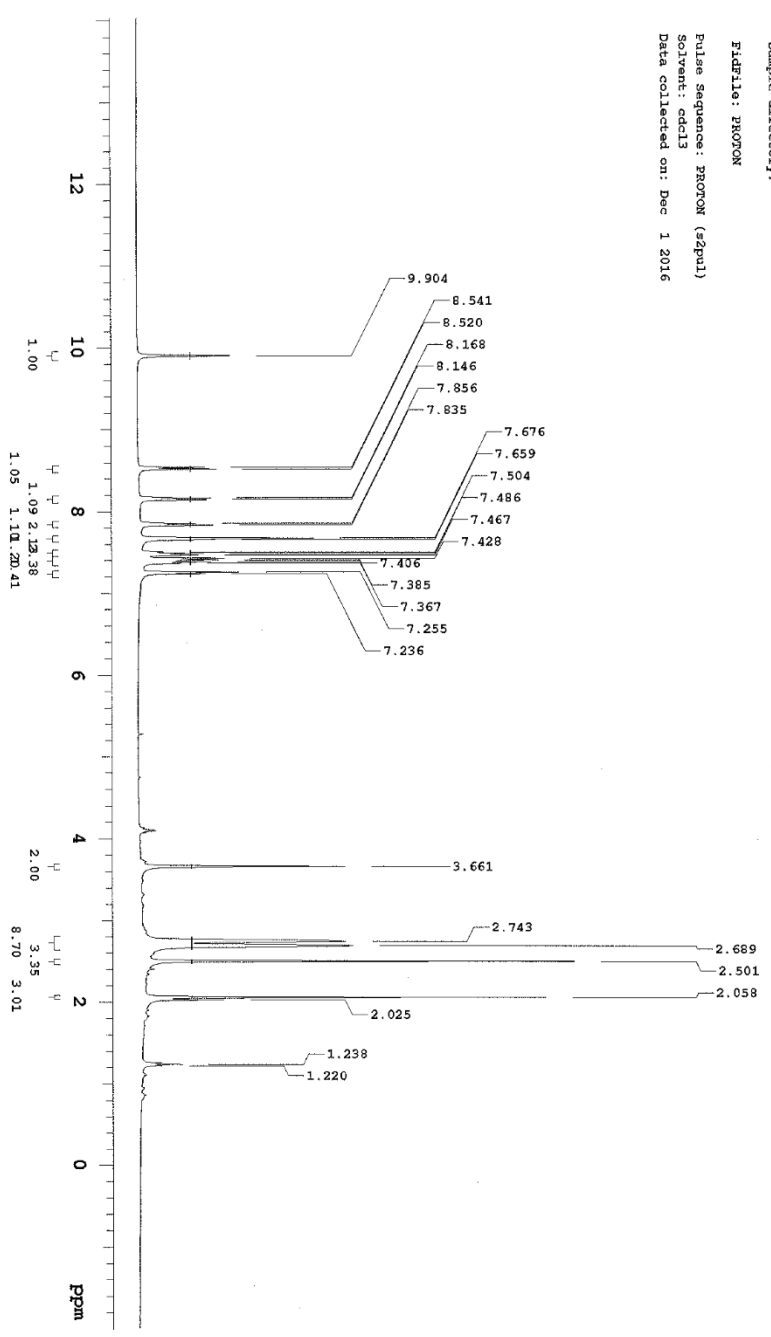


Figure S46. ¹H NMR spectrum of compound 3u

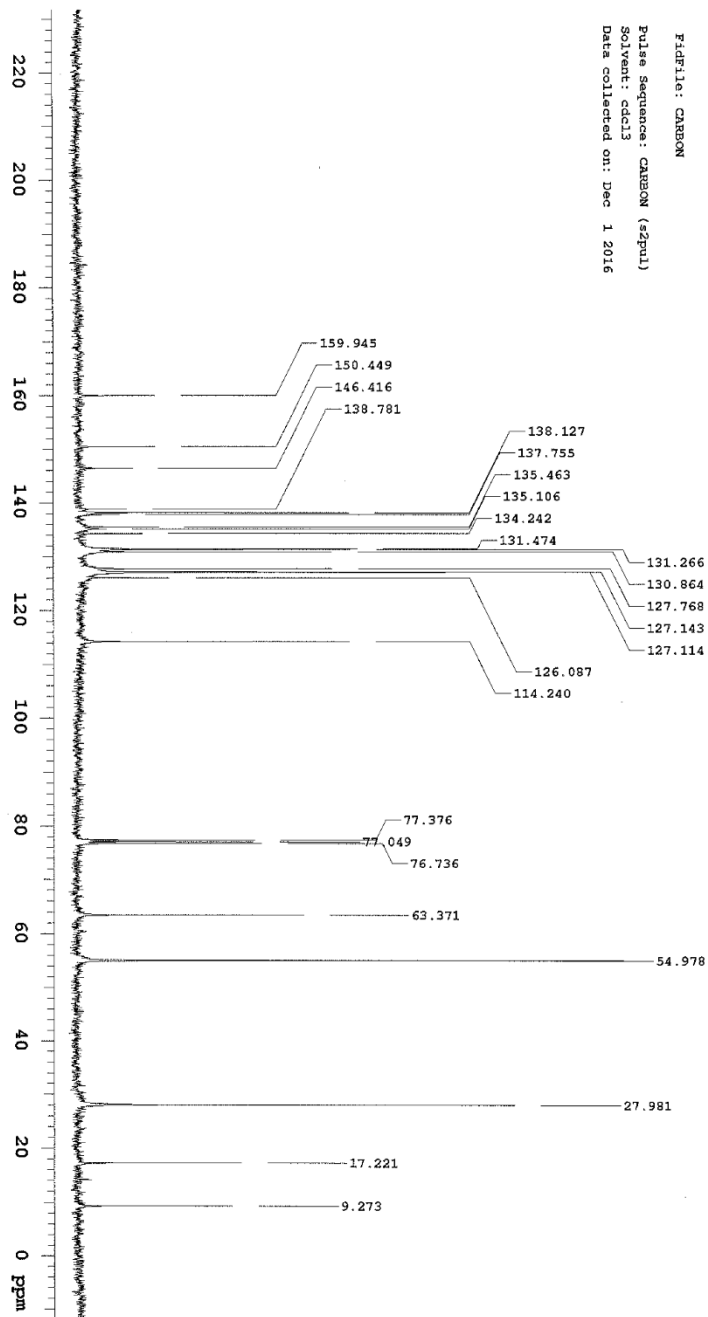


Figure S47. ¹³C NMR spectrum of compound **3u**