

# Crystal structures of trimethoprim-resistant DfrA1 rationalize potent inhibition by propargyl-linked antifolates

Michael N. Lombardo, Narendran G-Dayanandan, Dennis L. Wright and Amy C. Anderson

Department of Pharmaceutical Sciences, University of Connecticut, 69 N. Eagleville Rd., Storrs, CT 06269

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## HPLC Purity

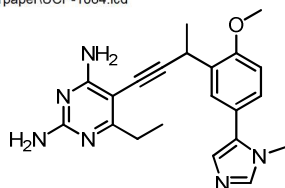
Purity analysis were performed with a reversed phase high performance liquid chromatography performed on a Shimadzu Prominence 20 instrument fitted with a Luna 5 $\mu$  C18(2) 100 Å column (5  $\mu$ M, 4.6 mm x 250 mm, Phenomenex) and using UV diode array detection at 254 nm. Two separate determinations - Method A : isocratic- 40% (v/v) MeCN/ 60% (v/v) 50 mM Potassium Phosphate, monobasic, pH=7.0 and Method B : isocratic- 80% (v/v) MeOH/ 20% (v/v) 50 mM Potassium Phosphate, monobasic, pH=7.0 were made to determine compound purity. Flow rate was 1.5 mL/min for Method A and 1.5 mL/min for Method B

Compounds were diluted in the HPLC grade methanol and filtered prior to analysis. Sample concentrations were 1 mg/ml and injection volume was 5 $\mu$ L.

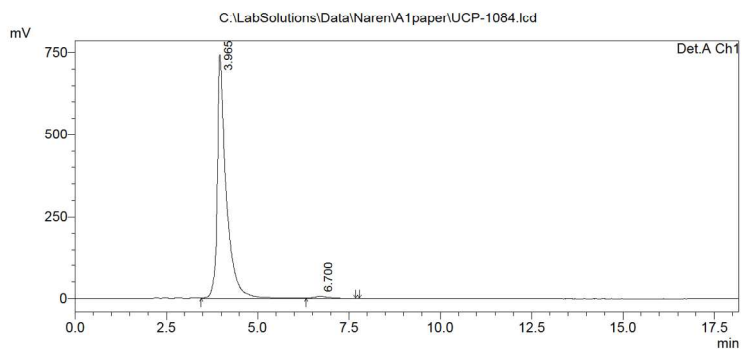
Compound	Method A		Method B	
	t <sub>R</sub> (min)	%	t <sub>R</sub> (min)	%
12	3.96	98.7	5.89	98.9
13	3.32	98.7	5.11	98.6
14	3.21	98.5	4.84	98.6
15	3.31	99.8	5.01	99.6
16	4.94	99.0	7.04	98.7
17	5.02	99.5	7.07	99.2

==== Shimadzu LCsolution Analysis Report ====

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 Sample ID : UCP-1084  
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 Vial # : 2  
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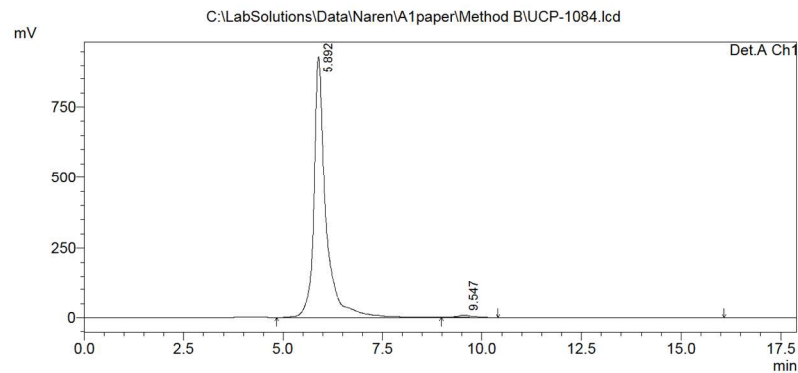
Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.965	12797710	743165	98.832	99.186
2	6.700	151293	6100	1.168	0.814
Total		12949004	749265	100.000	100.000

==== Shimadzu LCsolution Analysis Report ====

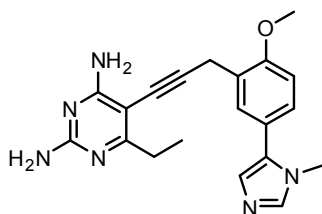
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Detector A Ch1 254nm

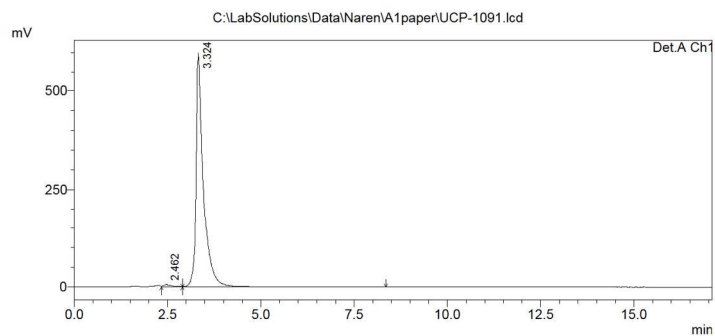
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.892	19771354	929348	98.988	99.195
2	9.547	202041	7544	1.012	0.805
Total		19973395	936891	100.000	100.000



==== Shimadzu LCsolution Analysis Report ====

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 Sample ID : UCP-1091  
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 Vail # : 3  
 Injection Volume : 5 uL  
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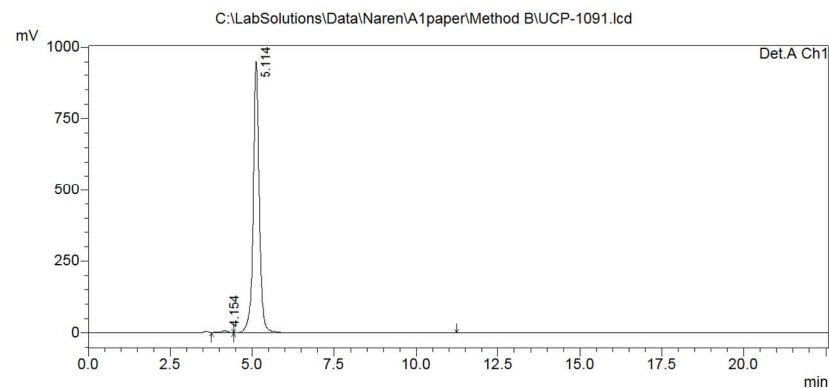
Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.462	107169	6546	1.281	1.089
2	3.324	8257874	594396	98.719	98.911
Total		8365043	600942	100.000	100.000

==== Shimadzu LCsolution Analysis Report ====

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 Sample ID : UCP1091  
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 Vail # : 3  
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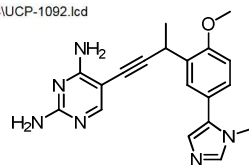


Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.154	165231	8362	1.312	0.872
2	5.114	12429651	950593	98.688	99.128
Total		12594882	958955	100.000	100.000

==== Shimadzu LCsolution Analysis Report ====

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 Sample ID : UCP1092  
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 Vial # : 1  
 Injection Volume : 5 uL  
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 Method File Name : Test.lcm  
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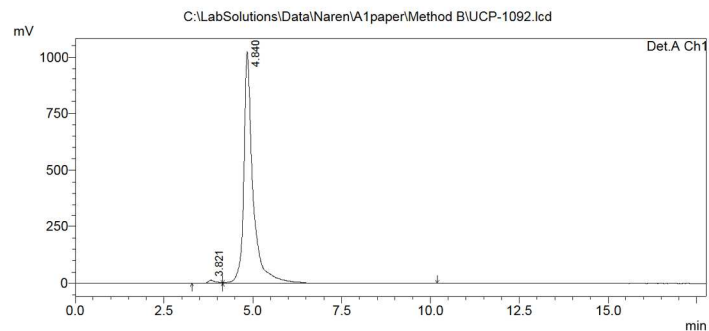


Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.821	246981	14215	1.309	1.371
2	4.840	18615683	1022416	98.691	98.629
Total		18862663	1036632	100.000	100.000

8/6/2015 11:28:07 1 / 2

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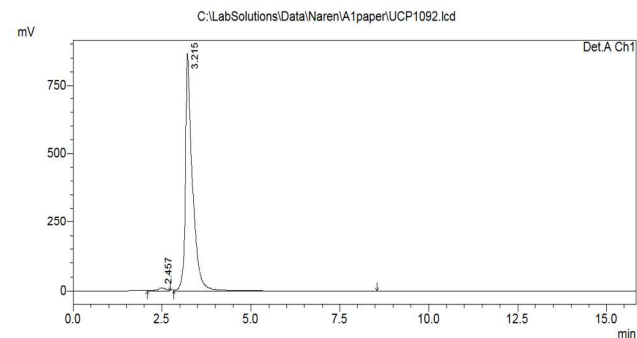
Detector A Ch1 254nm

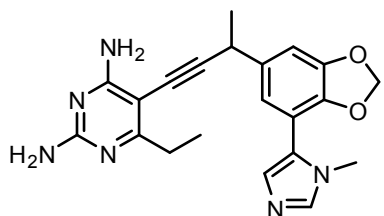
Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.457	180482	11187	1.499	1.279
2	3.215	11861230	863408	98.501	98.721
Total		12041712	874596	100.000	100.000

==== Shimadzu LCsolution Analysis Report ====

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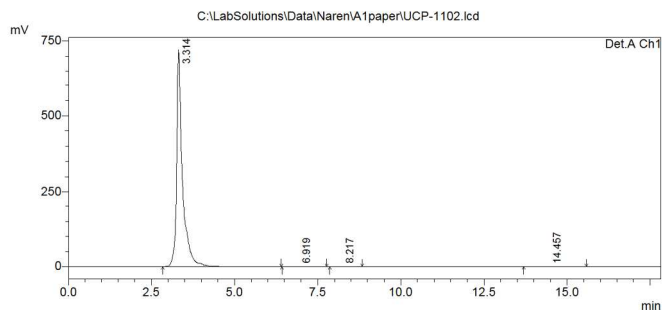




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 Sample ID : UCP-1102  
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 Vial # : 4  
 Injection Volume : 5 uL  
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 Method File Name : Test.lcm  
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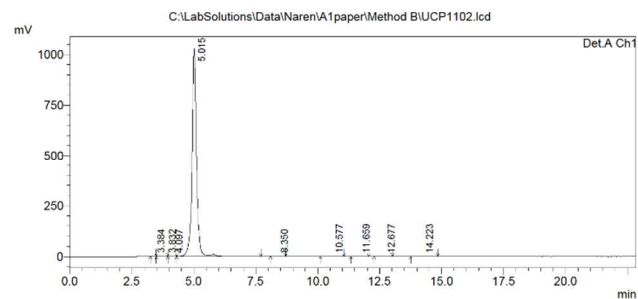
PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.314	8851122	718507	99.808	99.940
2	6.919	6627	182	0.075	0.025
3	8.217	2835	92	0.032	0.013
4	14.457	7547	161	0.085	0.022
Total		8868130	718942	100.000	100.000

==== Shimadzu LCsolution Analysis Report ====

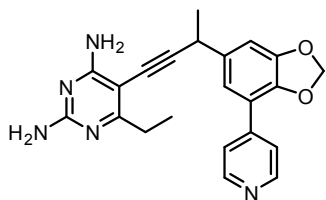
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 Sample ID : UCP1102  
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 Vial # : 4  
 Injection Volume : 5 uL  
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Detector A Ch1 254nm

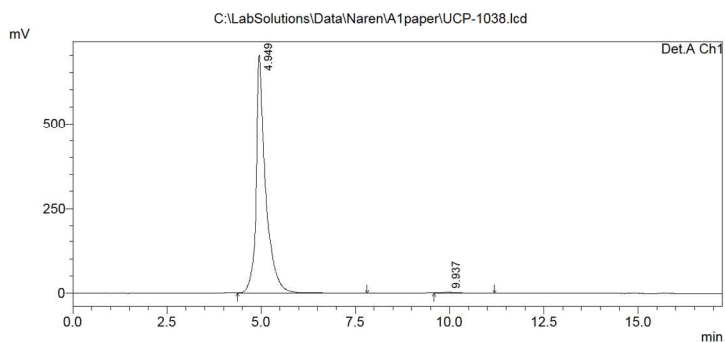
Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.384	927	140	0.007	0.014
2	3.832	10339	686	0.078	0.067
3	4.097	7700	875	0.058	0.085
4	5.015	13162759	1027668	99.628	99.692
5	8.350	8120	508	0.061	0.049
6	10.577	8257	356	0.062	0.035
7	11.659	3449	171	0.026	0.017
8	12.677	2339	116	0.018	0.011
9	14.223	7963	318	0.060	0.031
Total		13211853	1030838	100.000	100.000



==== Shimadzu LCsolution Analysis Report ====

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 Sample ID : UCP-1038  
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 Vail # : 5  
 Injection Volume : 5 uL  
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 Method File Name : Test.lcm  
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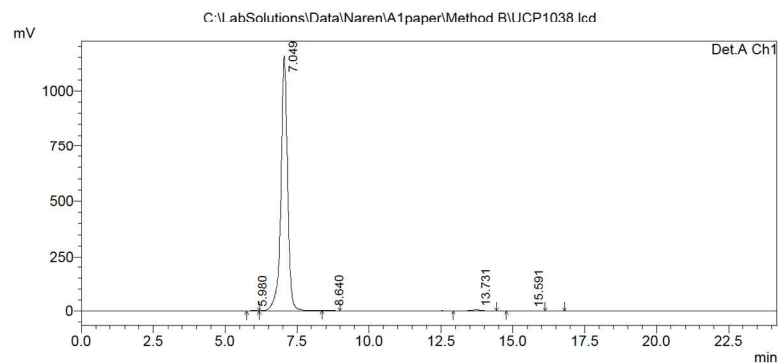
Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.949	12371740	698305	99.067	99.480
2	9.937	116540	3649	0.933	0.520
Total		12488280	701954	100.000	100.000

==== Shimadzu LCsolution Analysis Report ====

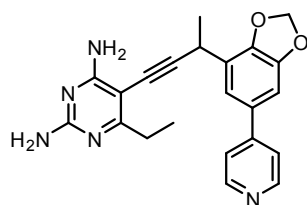
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 Sample ID : UCP1038  
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 Injection Volume : 5 uL  
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Detector A Ch1 254nm

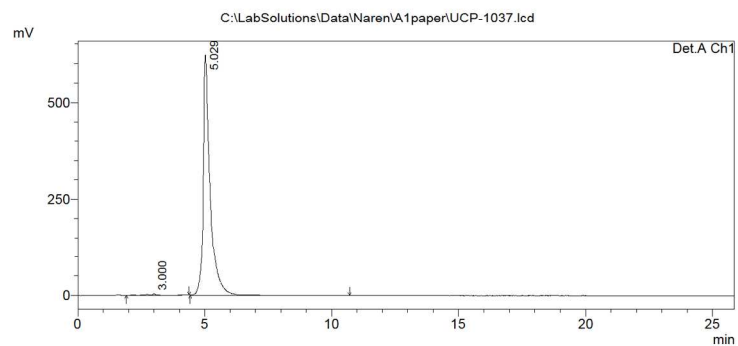
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.980	37681	2823	0.196	0.242
2	7.049	19005034	1157313	98.763	99.049
3	8.640	35931	2319	0.187	0.199
4	13.731	154183	5713	0.801	0.489
5	15.591	10167	260	0.053	0.022
Total		19242996	1168429	100.000	100.000



==== Shimadzu LCsolution Analysis Report ====

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 Sample Name : diox Pyridine w Me  
 Sample ID : UCP-1037  
 Tray# : 1  
 Vial # : 6  
 Injection Volume : 5 uL  
 Data File Name : UCP-1037.lcd  
 Method File Name : Test.lcm  
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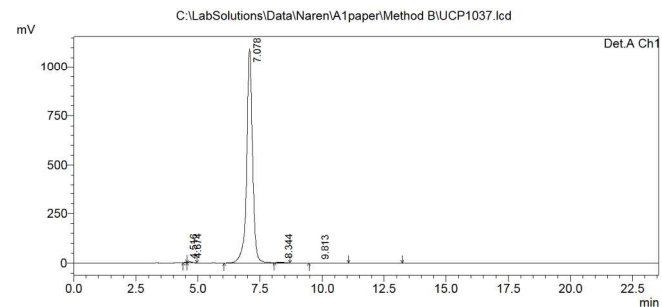
PeakTable

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.000	52560	3513	0.441	0.562
2	5.029	11856569	621636	99.559	99.438
Total		11909129	625149	100.000	100.000

==== Shimadzu LCsolution Analysis Report ====

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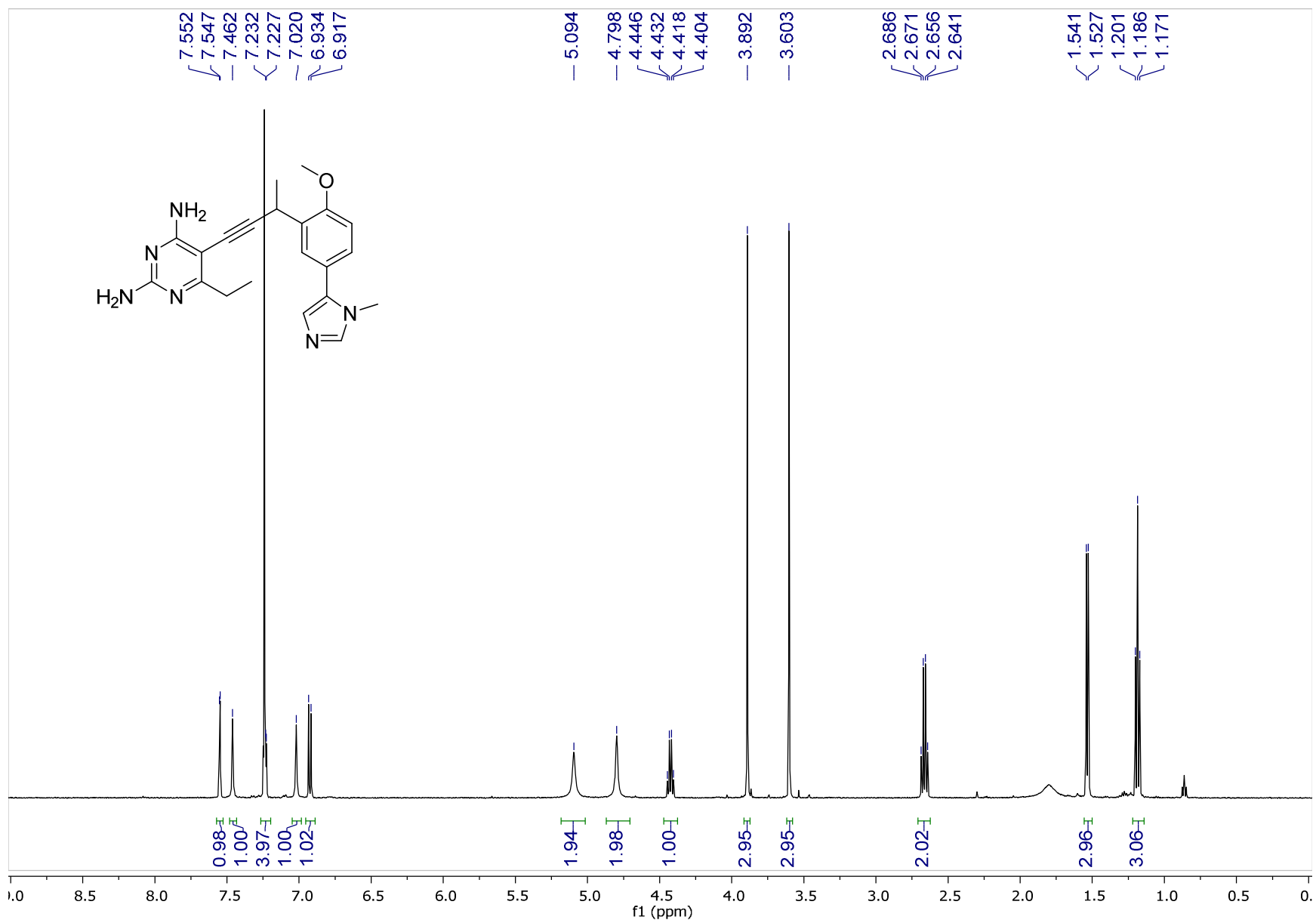
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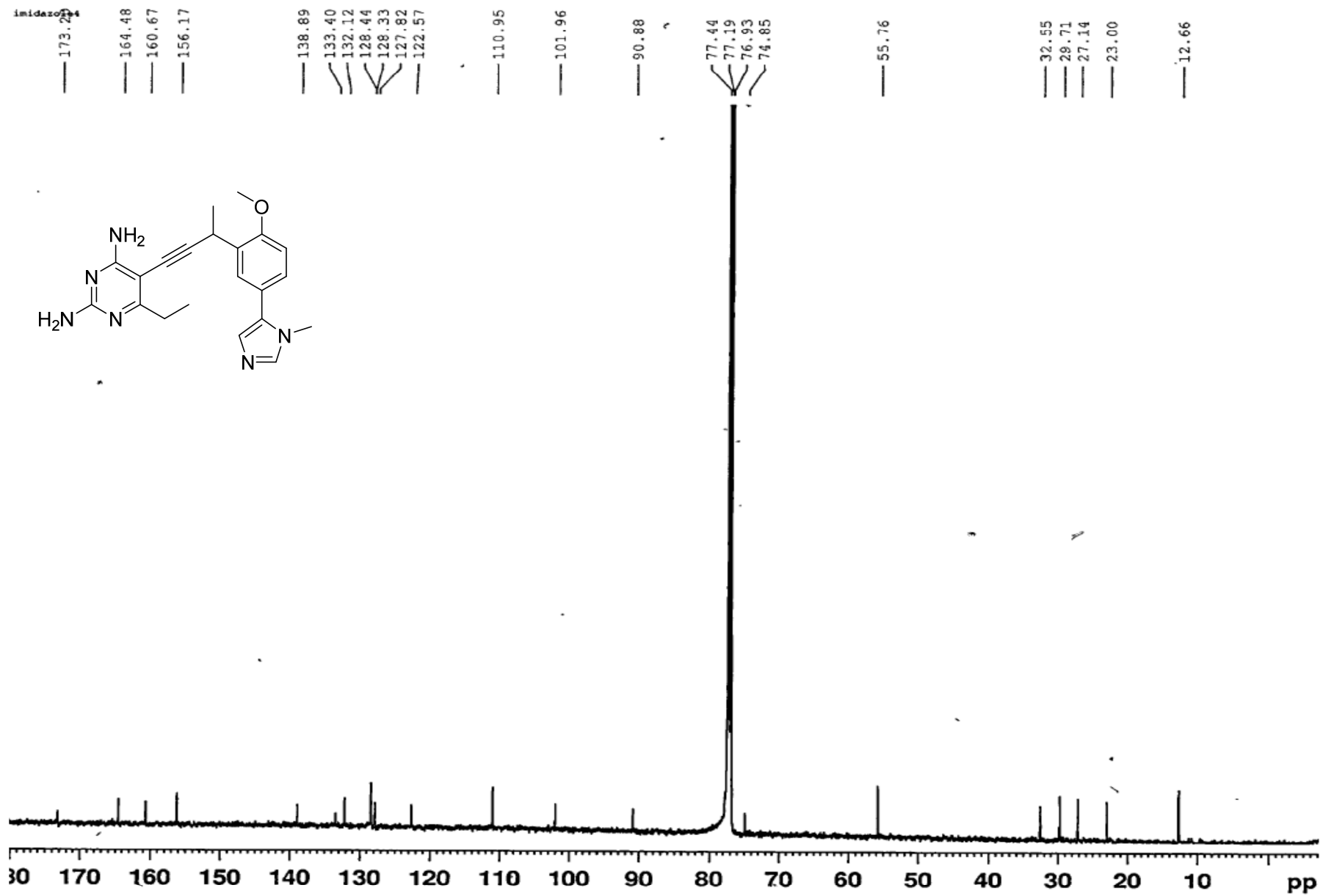


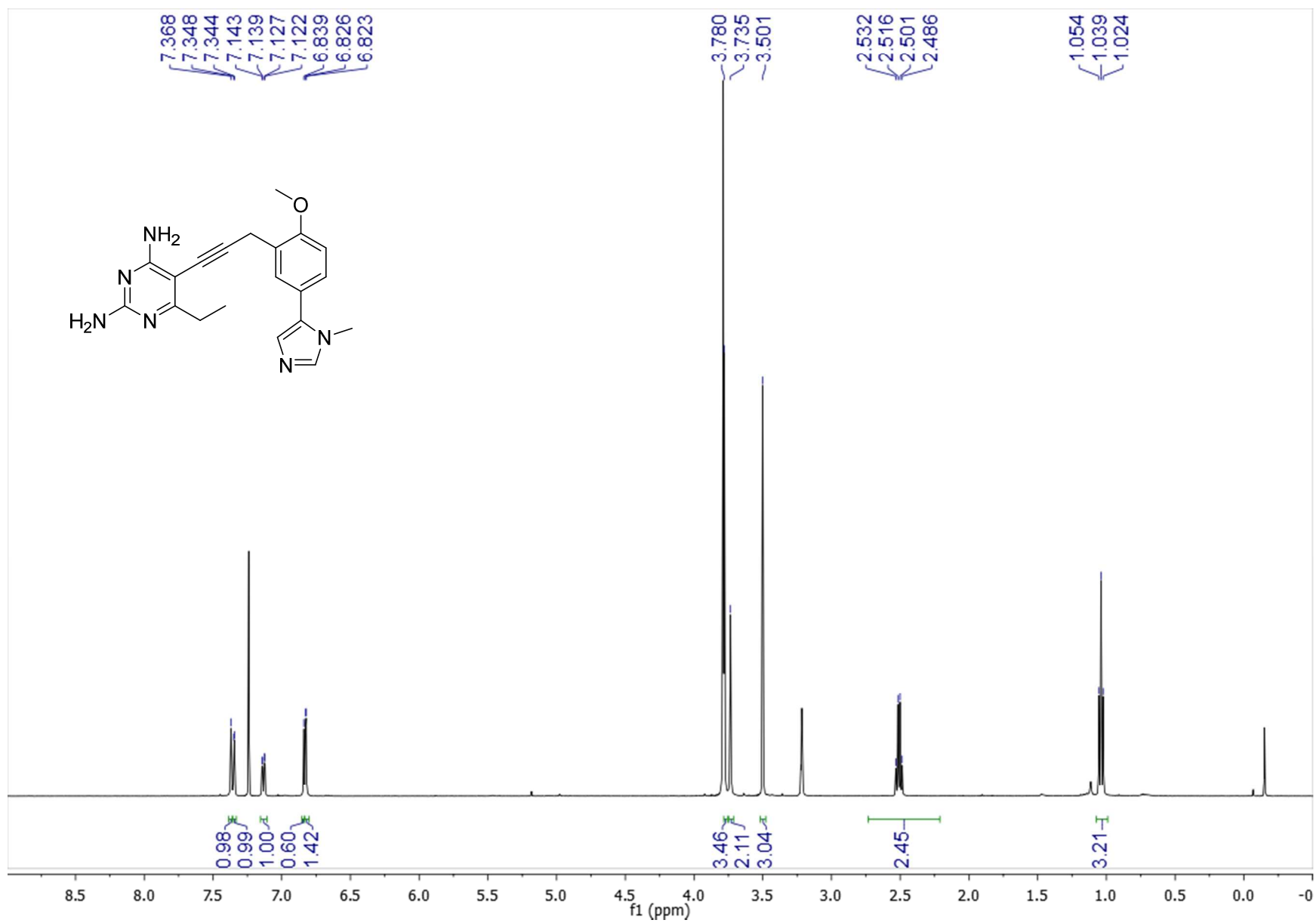
Detector A Ch1 254nm

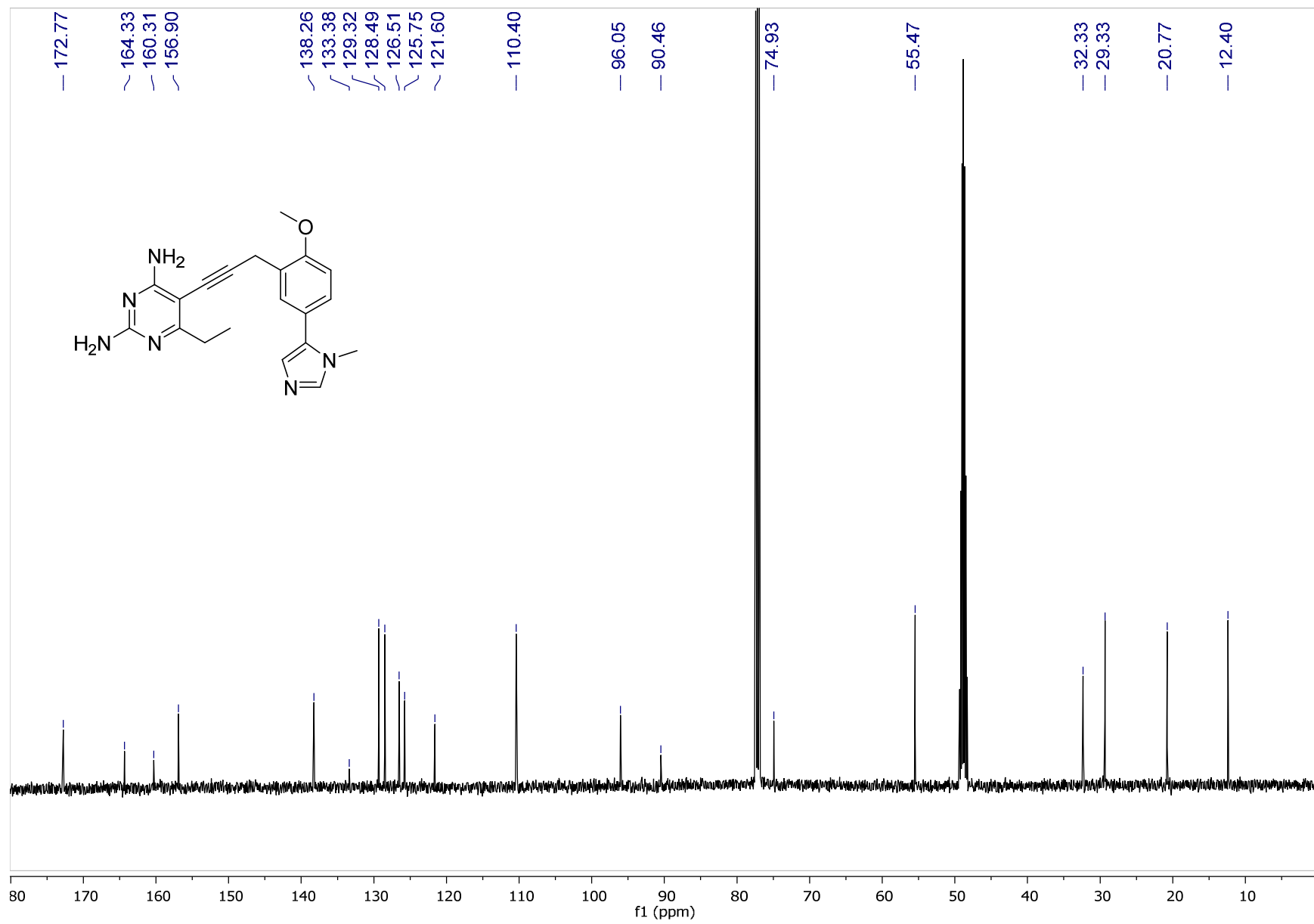
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.516	20225	3088	0.112	0.279
2	4.674	69112	6267	0.381	0.567
3	7.078	17993484	1092406	99.203	98.823
4	8.344	48132	3140	0.265	0.284
5	9.813	7174	516	0.040	0.047
Total		18138127	1105418	100.000	100.000

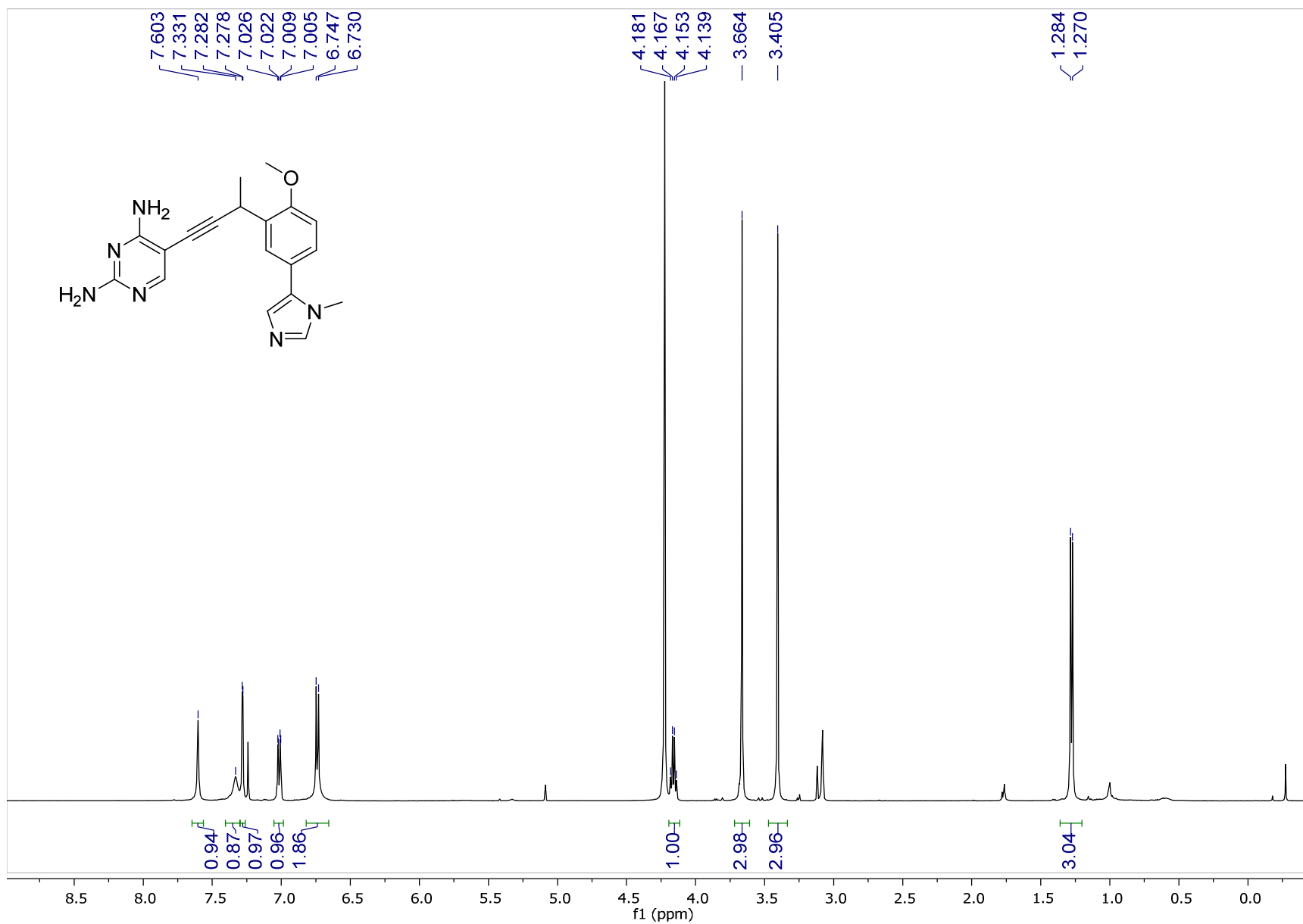


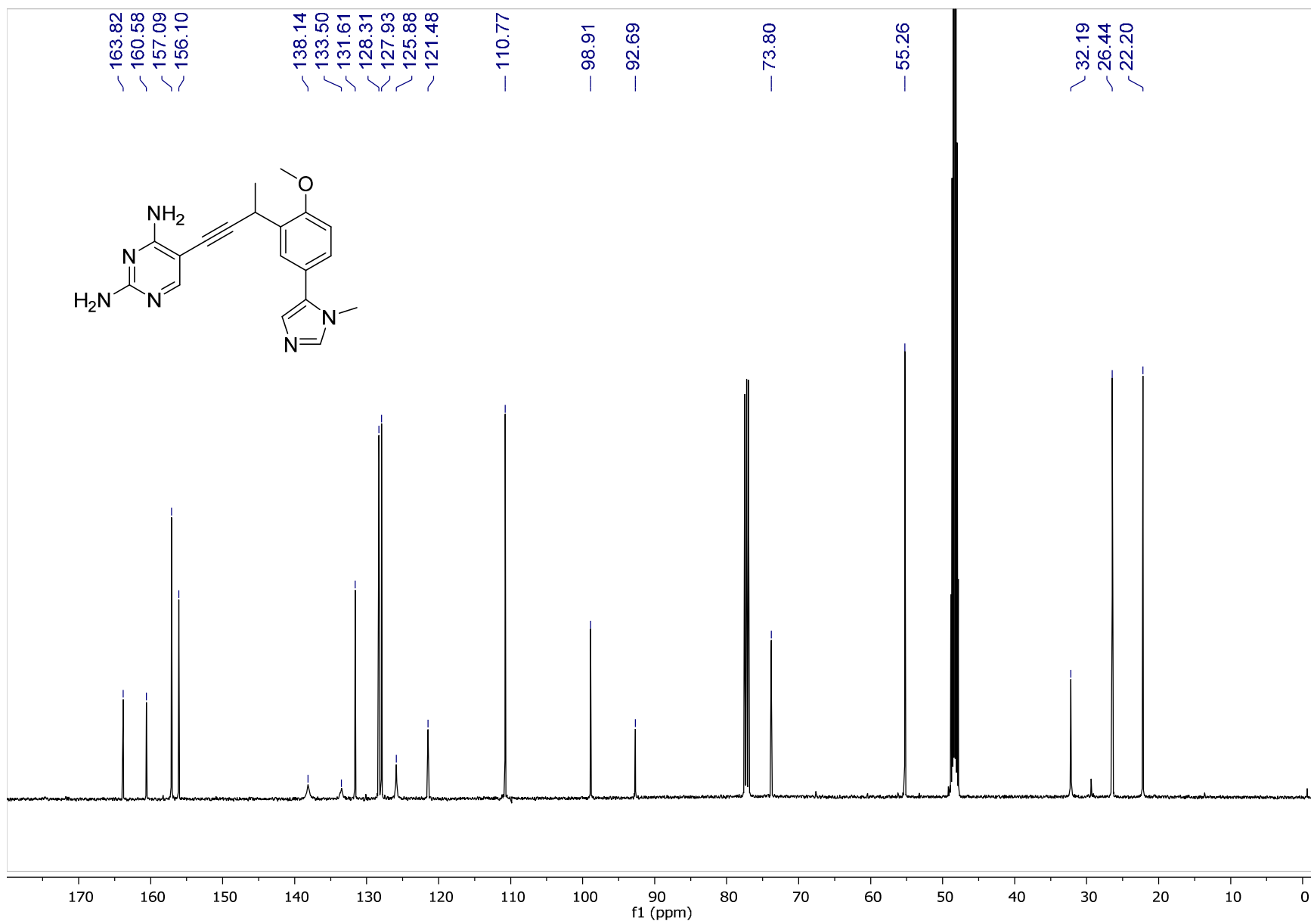


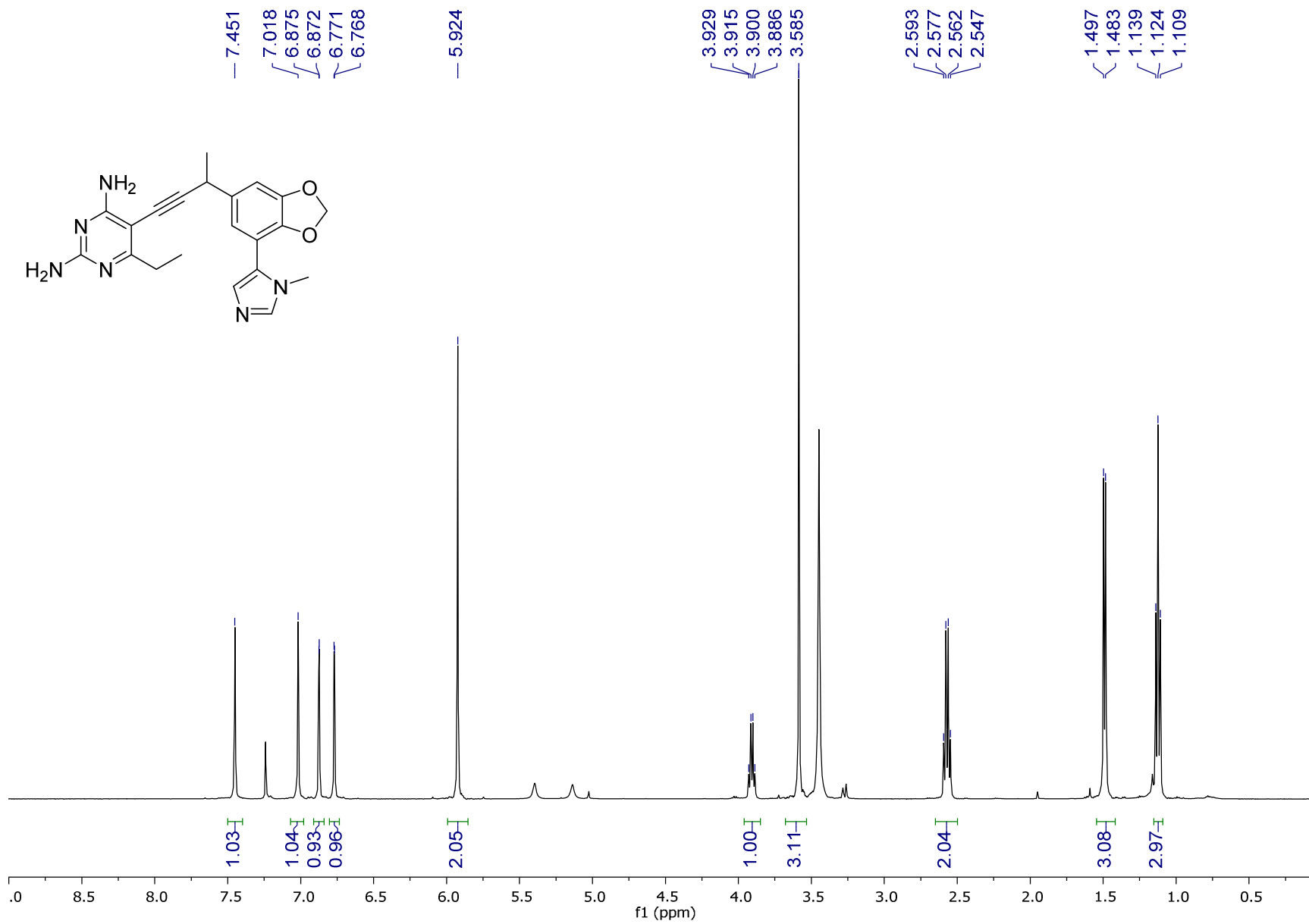


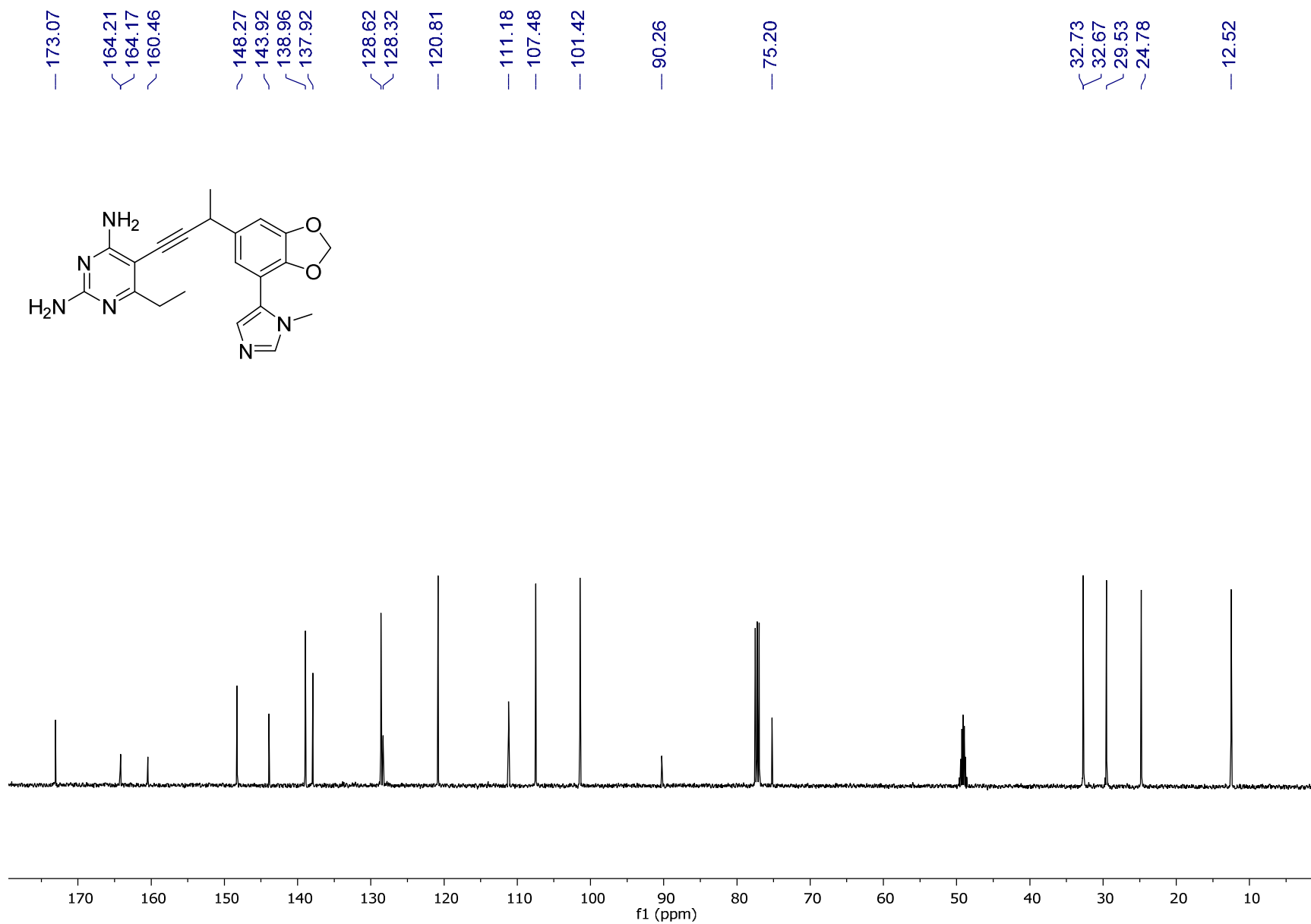




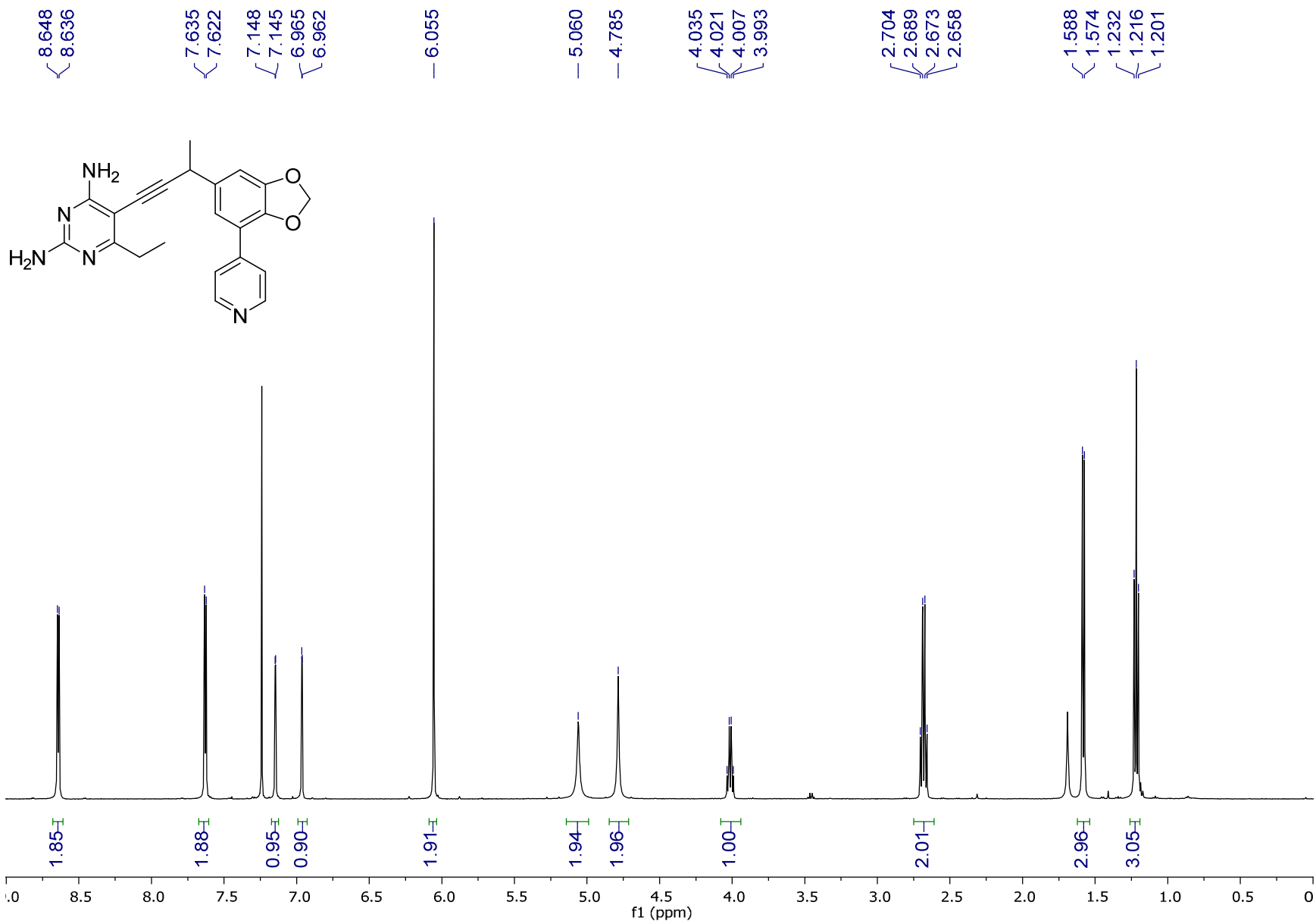


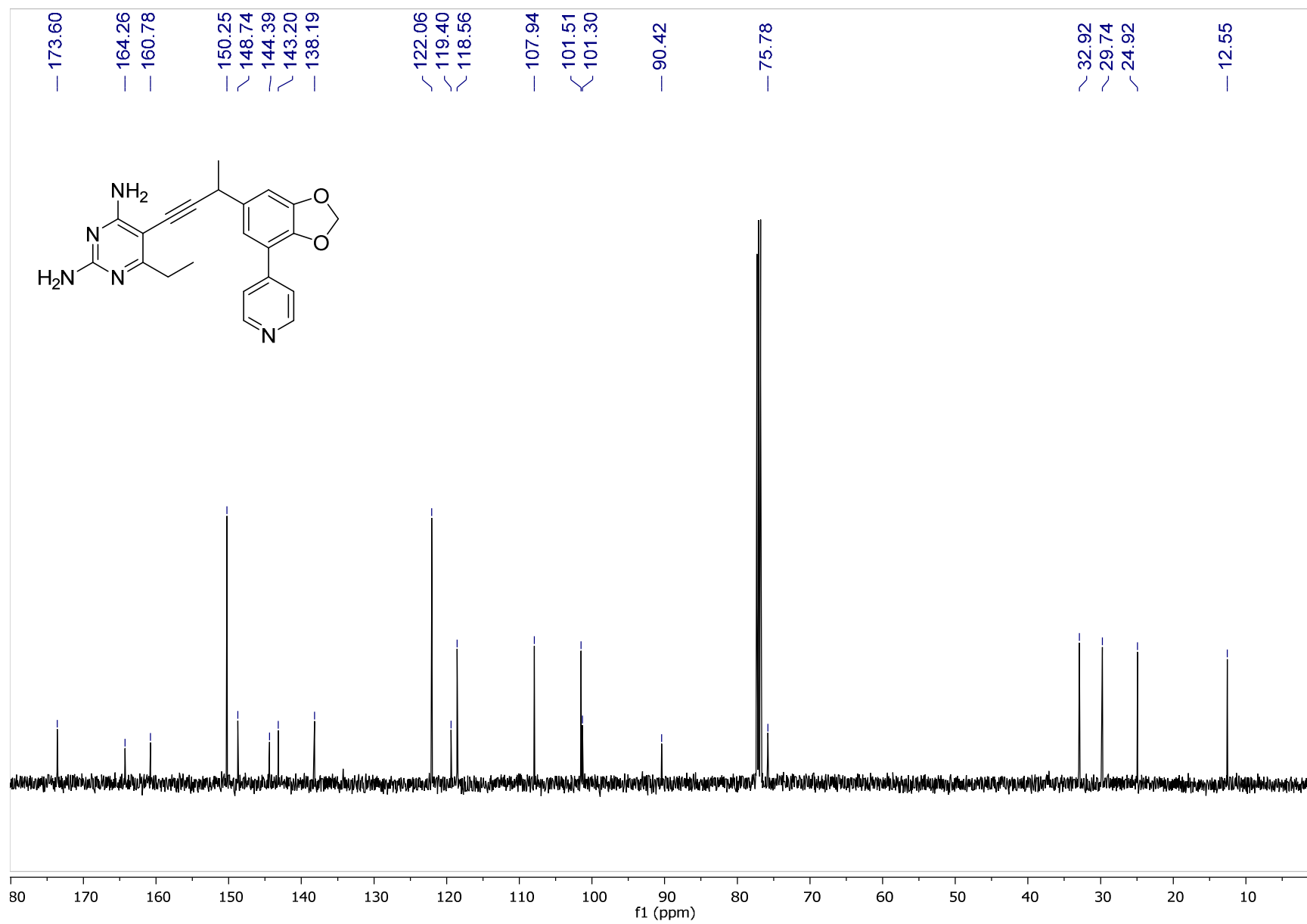


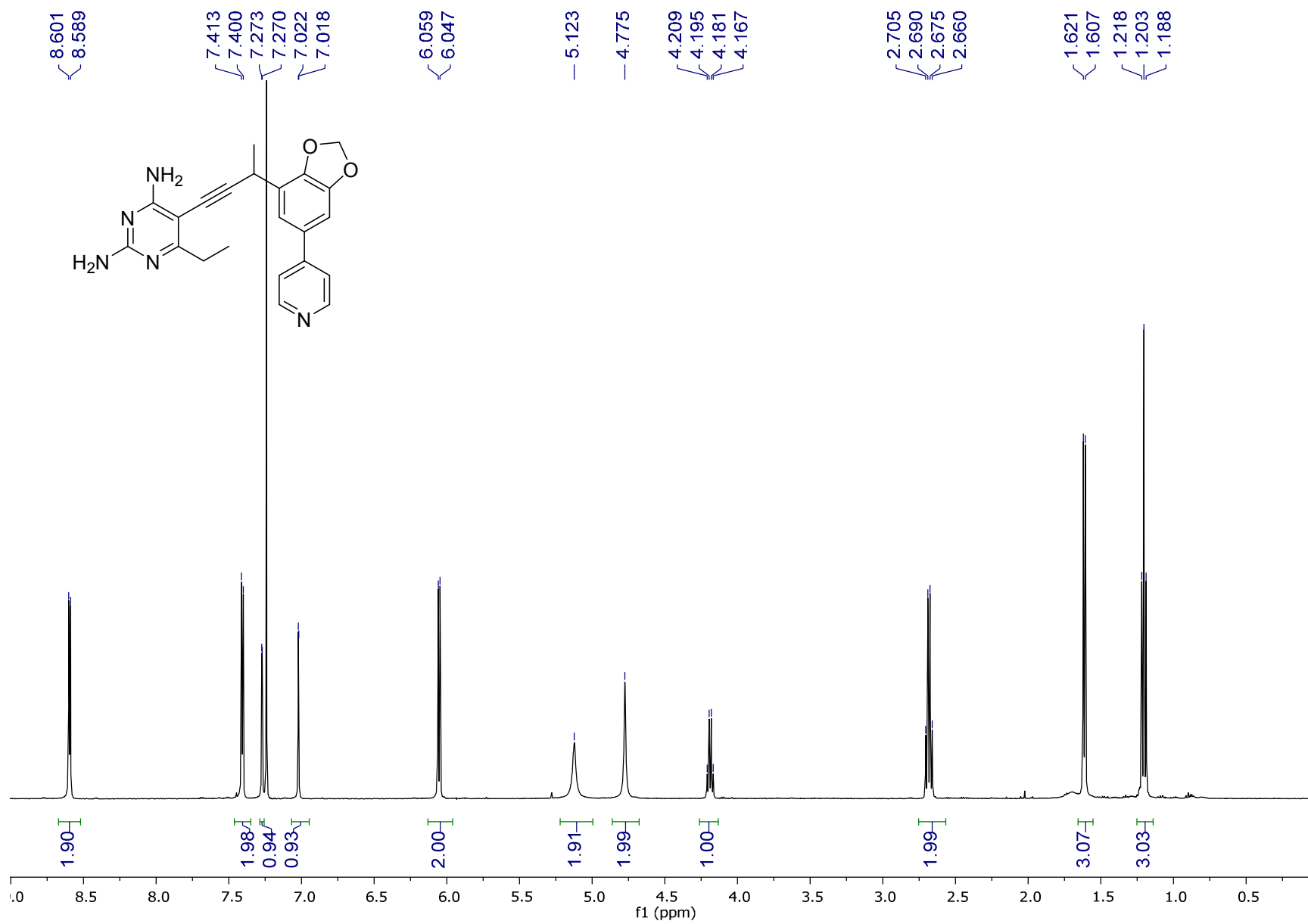


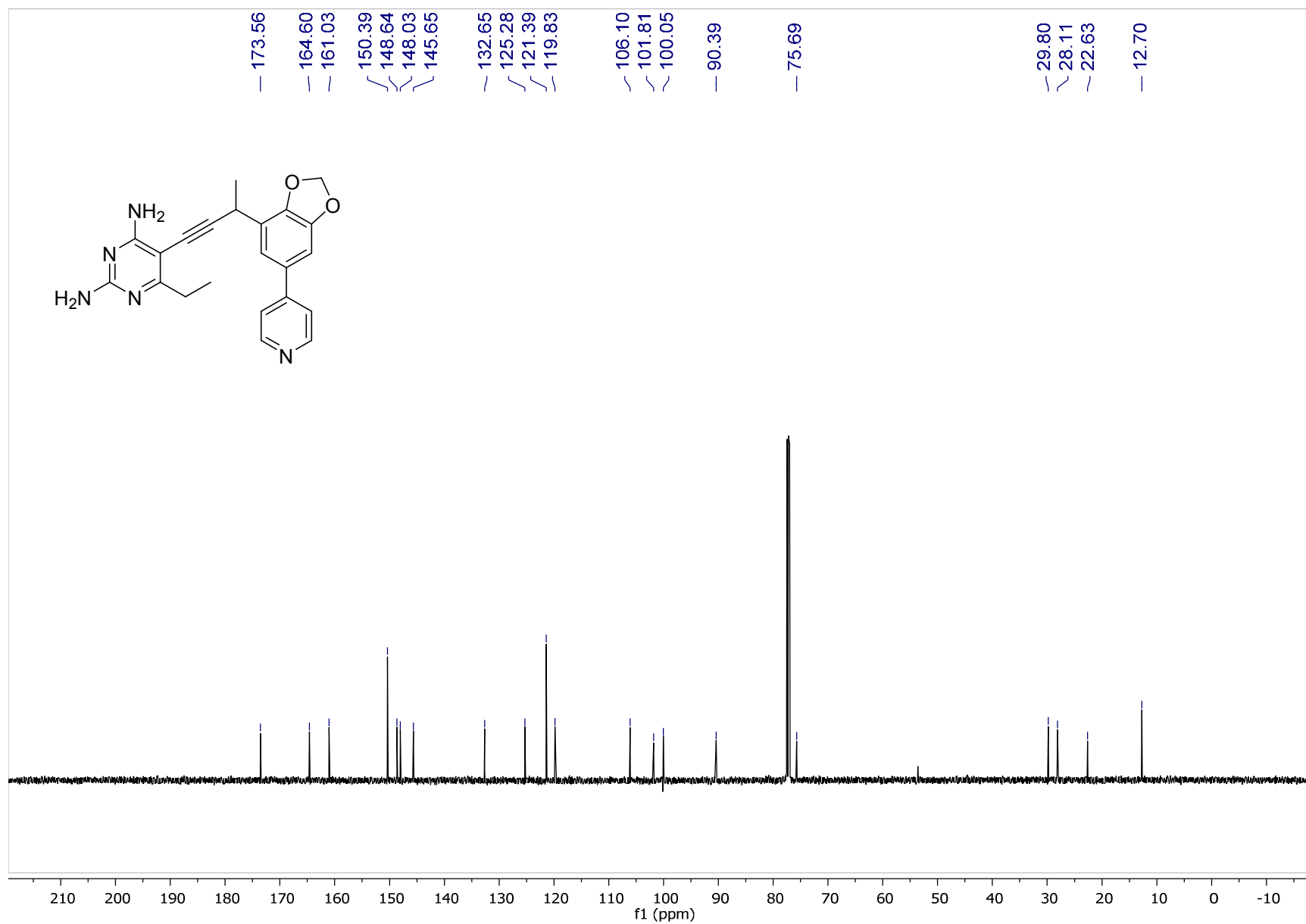












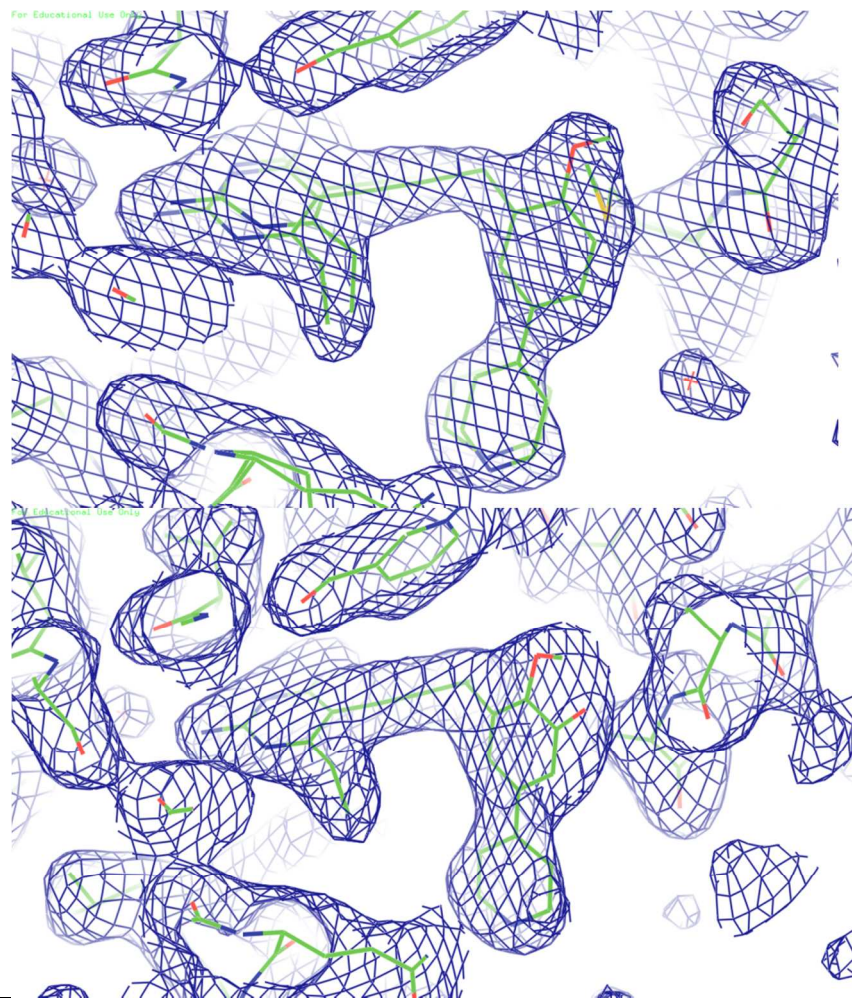


Figure S1. Simulated annealing omit electron density for the ligands: a) compound **3** and b) compound **17**.