Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Azastilbenes: A cut off to p38 MAPK inhibitors

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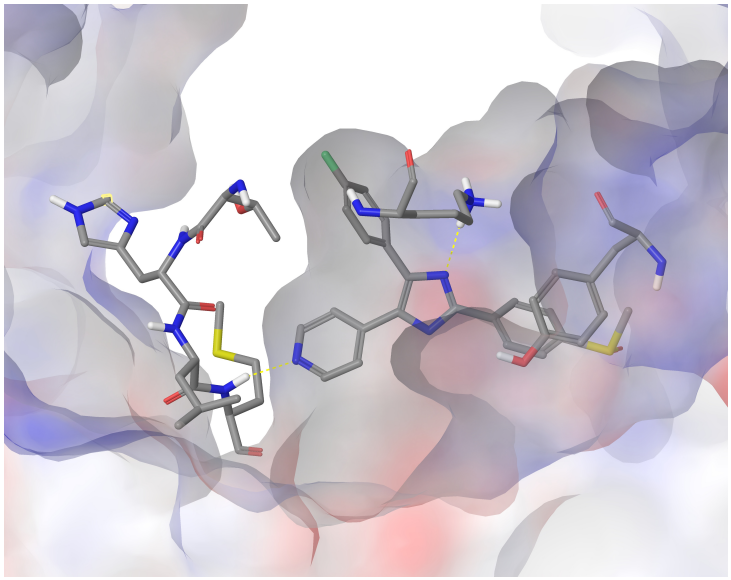
Supporting Information

Contents

General and Materials.

All reagents and chemicals were purchased from Sigma-Aldrich and used without further purification. ^{1}H NMR (399.97 MHz) and ^{13}C NMR (100.58 MHz) spectra were recorded on a Varian Unity 400 spectrometer, using solvent residual peaks (1H: $CDCl_3$: ∂ 7.26 ppm, DMSO-d_6: ∂ 2.50 ppm; 13C: $CDCl_3$: ∂ 77.0 ppm, DMSO-d_6: ∂ 39.52 ppm) as an indirect reference to TMS. Chemical shifts and literature NMR shifts were used as references in identification and characterization of the synthesized compounds. Flash column chromatography was performed using Merck silica gel (0.04-0.06 mm). Thin Layer Chromatography (TLC) was performed on ALUGRAM® SIL G/UV254 plates (0.2 mm), using UV-light (254 nm) for visualization. IR spectra were recorded on a Perkin-Elmer Spectrum One (ATR Technique). HPLC analysis was performed using a Young Lin (YL-9100) system equipped with a Kromasil 5-CellCoat column and using hexane and isopropanol as an eluent (detection: 220 nm and 254 nm). The protein preparation was prepared according to the standard procedure in the Schrödinger package. Docking of compounds was done in Glide (Schrödinger) with extraprecision (XP) settings and standard parameters for ligand docking.

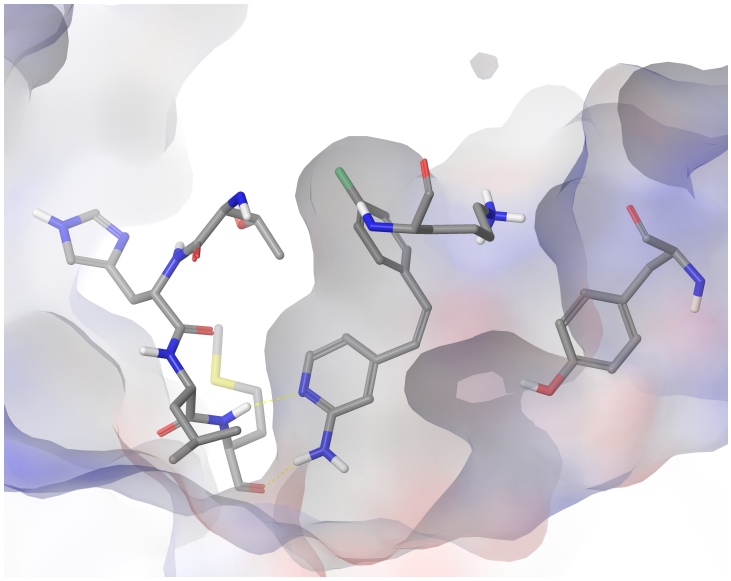
Docking of compounds 6a-b, 7, 9, 14a-b, 15a-b, 16a-c



SB203580

Docking score (XP):

-7.38



Compound 7

Docking score (XP):

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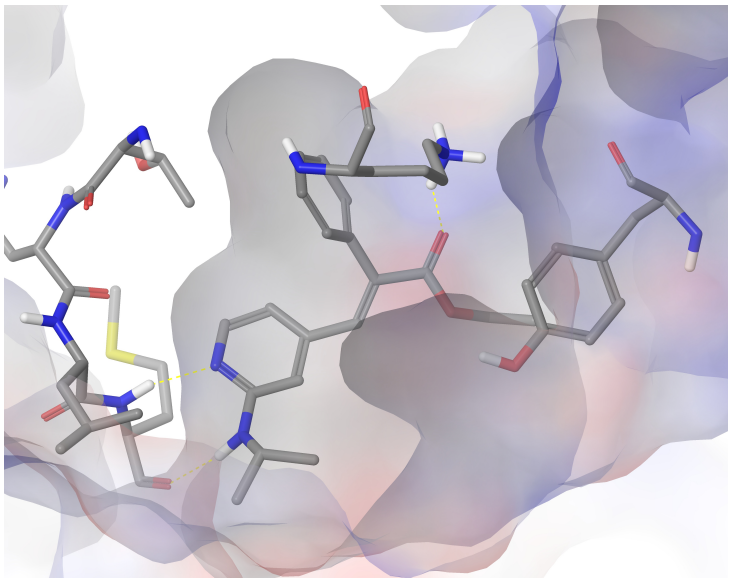

Compound 16c

Docking score (XP):

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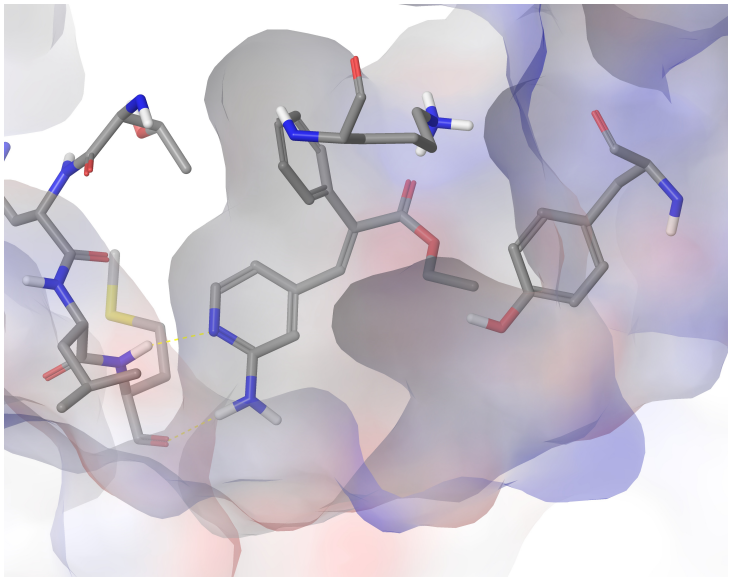
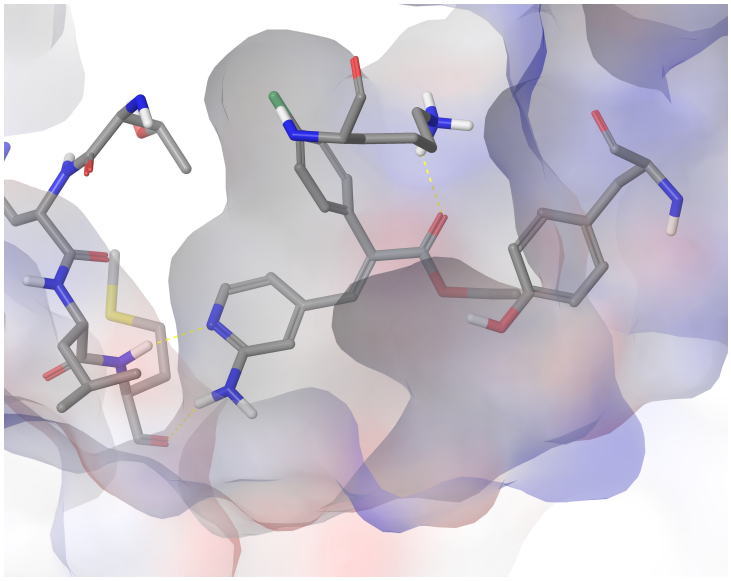


Compound 16a

Docking score (XP):

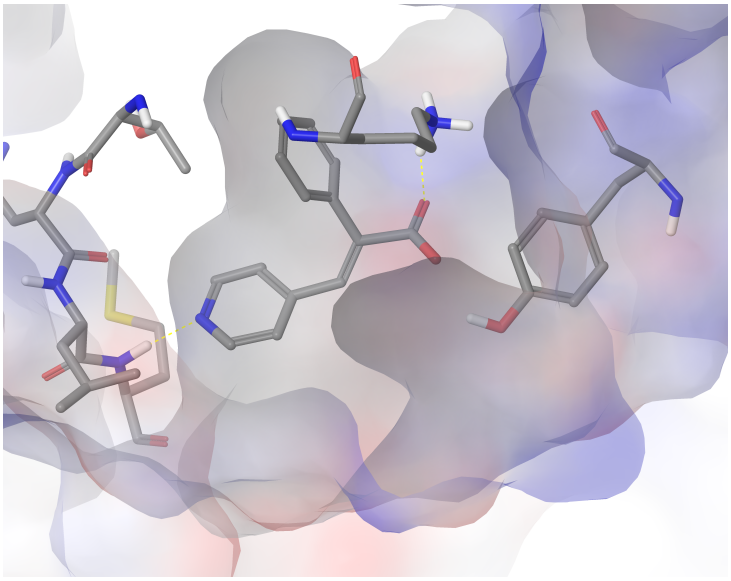
-7.11



Compound 16b

Docking score (XP):

-7.06



Compound 15a

Docking score (XP):

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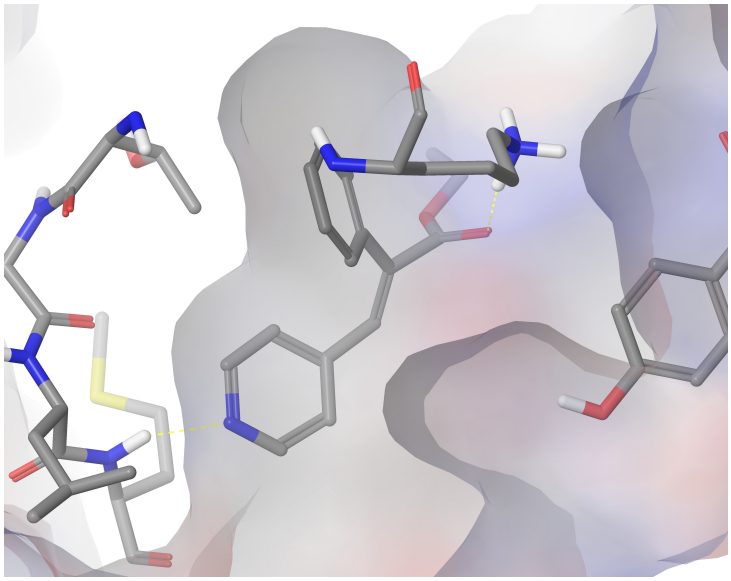
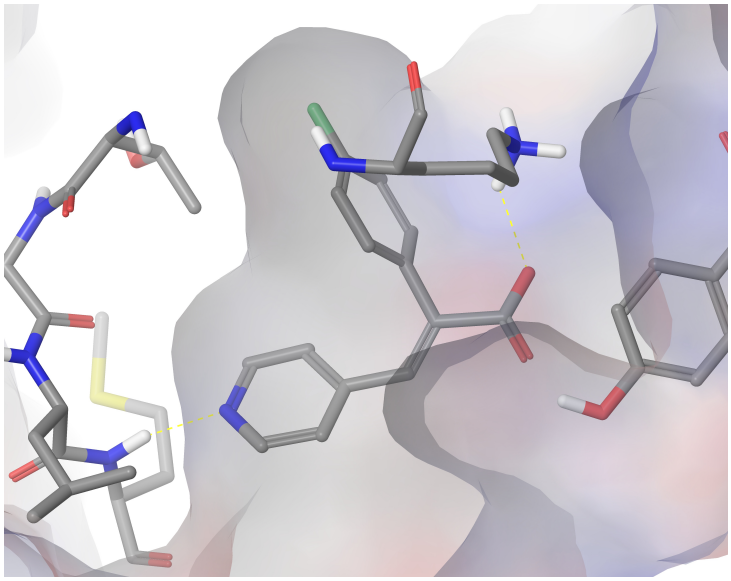


Compound 14a

Docking score (XP):

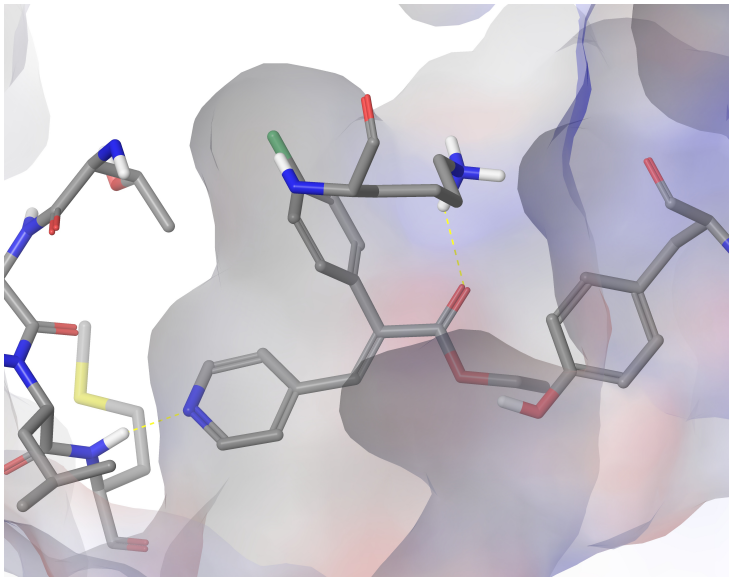
-6.62



Compound 15b

Docking score (XP):

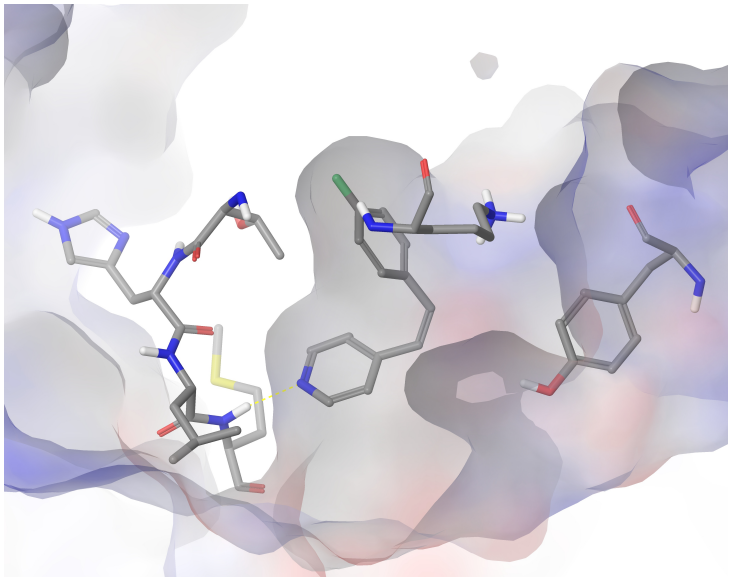
-6.35



Compound 14b

Docking score (XP):

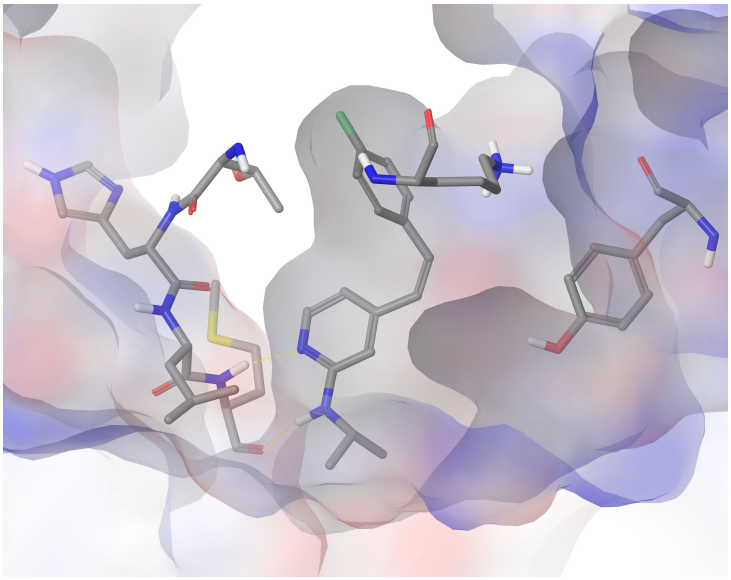
-6.25



Compound 6b

Docking score (XP):

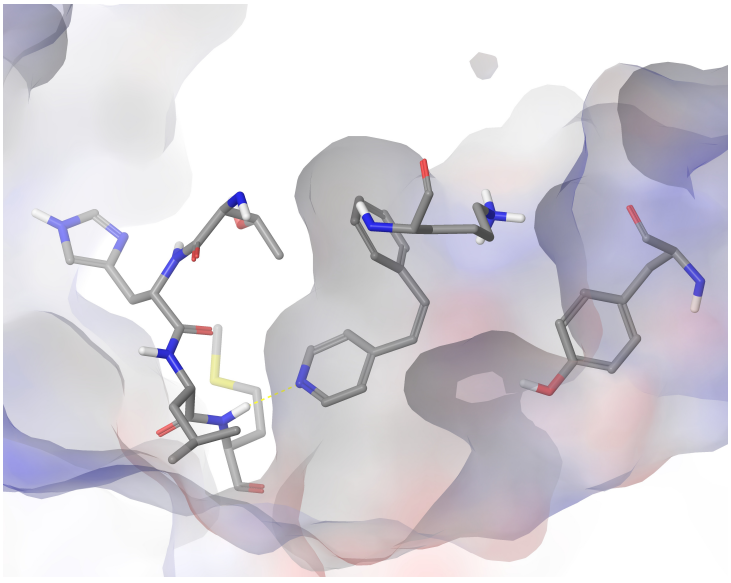
-6.04



Compound 9

Docking score (XP):

-5.94



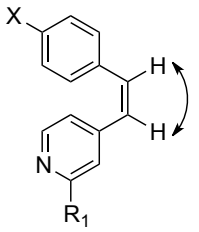
Compound 6a

Docking score (XP):

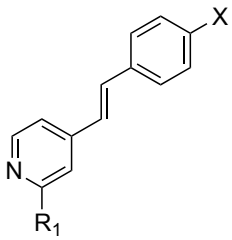
-5.87

Confirmation of E/Z stereochemistry for compounds 6a-b, 7, 9

The stereochemistry was determined by comparison of coupling constants and chemical shifts (see Table below). Vinylic protons with a *trans*-configuration is known to have a larger coupling constant (around 16 Hz) compared the vinylic protons with a *cis*-configuration (around 10-12 Hz).[1] All the synthesized compounds with *cis*-configuration **6a-b, 7, 9** have coupling constants lower than the compounds from the *trans*-configuration (formed as a by-product in the synthesis).



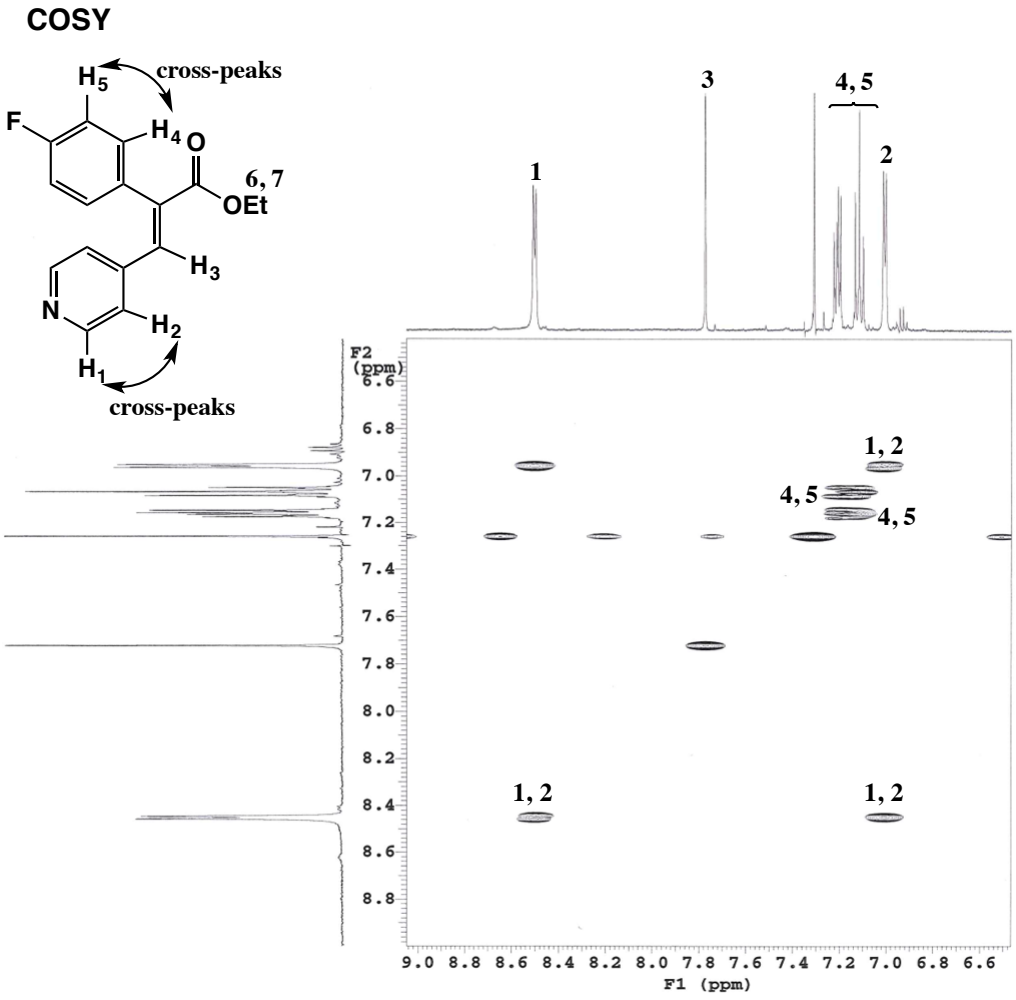
	Coupling constant / Hz	Chemical shift (ppm)
6a	12.2	6.82, 6.53
6b	12.4	6.67, 6.43
7	12.5	6.62
8	12.2	6.71, 6.49



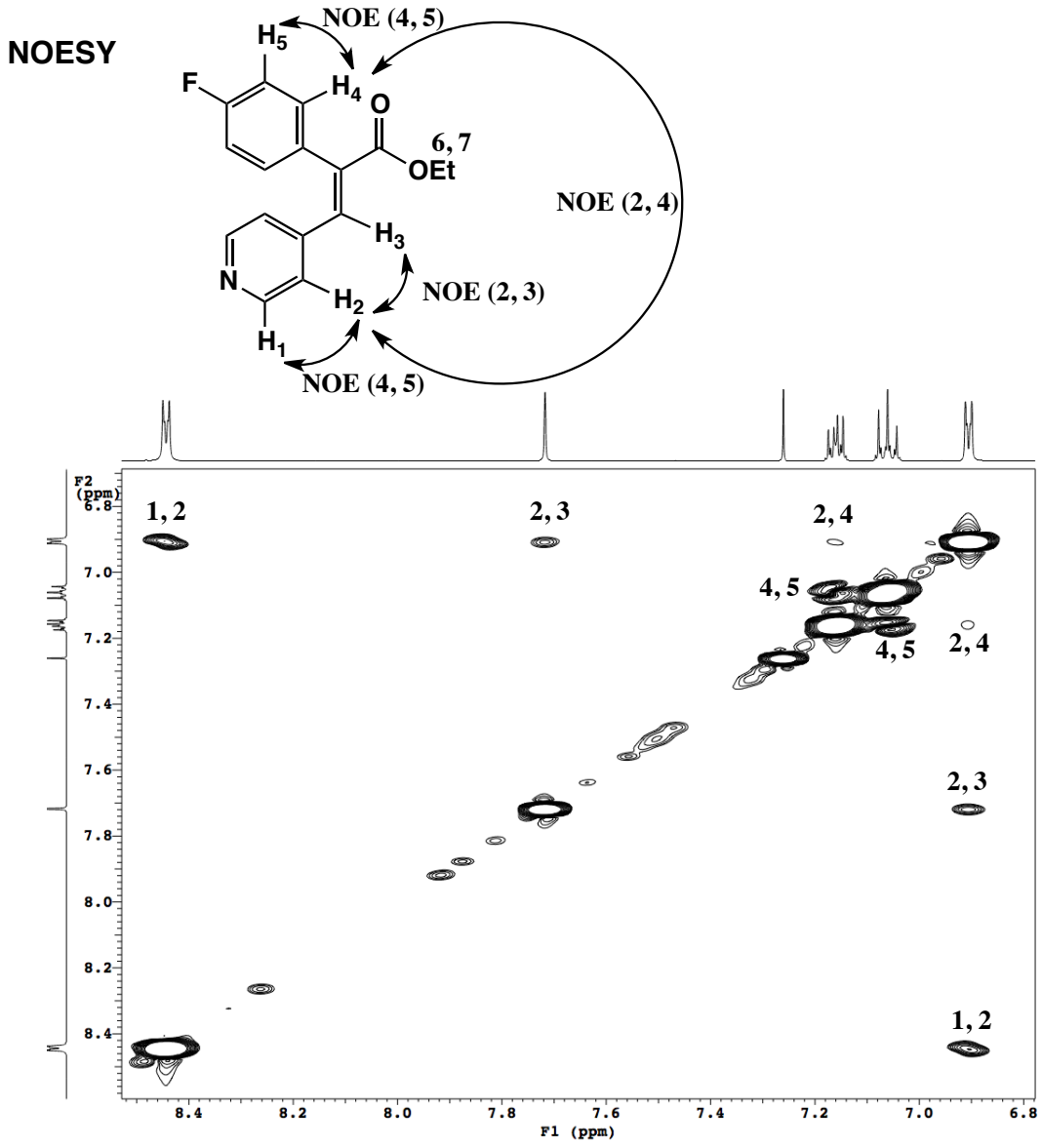
	Coupling constant / Hz	Chemical shift (ppm)
(*E*)-6a	16.0	7.02
(*E*)-6b	16.4	7.25, 6.92
(*E*)-7	16.4	7.04
(*E*)-8	16.4	7.30, 6.96

Confirmation of E/Z stereochemistry for compounds 14a-d, 15a-b, 16a-c

^{1}H NMR signals were assigned with a relayh-COSY experiment (^{1}H NMR spectrum available later in the supporting information) of compound **14b**. Cross-peaks were observed between the aromatic protons H1 and H2 on the pyridine ring and between H4 and H5 on the *p*-fluorophenyl ring. Protons H6 and H7 (-CH_2 and -CH_3) were assigned by chemical shift (^{1}H NMR spectrum available later in the supporting information).

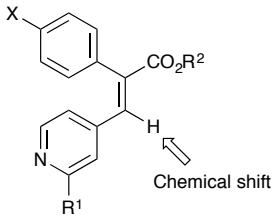


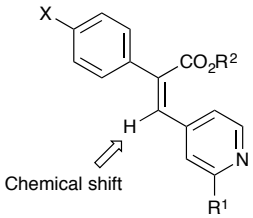
The stereochemistry of compound **14b** was assign with a NOESY experiment. In the NOESY spectrum, NOE were observed between H1-H2, H4-H5, and H2-H3 (negative compared to the positive diagonal). More importantly, NOE was also observed between H2-H4 (negative compared to the positive diagonal) in the two different aromatic rings, which suggest that the two aromatic rings are placed on the same side of the double bond.



The main difference in the ^{1}H NMR of the (*Z*)- and *(E)*-compounds is in the absorption of the olefinic proton. The stereochemistry was determined by comparison of chemical shifts of the vinylic proton with a (*E*)-configuration and a (*Z*)-configuration. Previously, it has been observed that in the (*Z*)-isomer, the vinylic proton resonates at 6.9 ppm, while in the (*E*)-isomer, the absorption of this proton moves downfield for about 1 ppm to occur at 7.9 ppm due to the deshielding effect of the nearby carbonyl and the aromatic ring in similar compounds.[1,2] In compounds **14a-14d**, one clearly can see the difference in chemical shift between the (*E*)- and the (*Z*)-isomer and the same trend is observed for compounds **15a-b, 16a-c** (see Table below).

Table. Chemical shift of the vinylic proton for (*Z*)- and (*E*)-compounds.





X	R^1	R^2	Chemical shift (ppm)	Chemical shift (ppm)
H	H	Et	7.67 (**14a**)	6.92
F	H	Et	7.69 (**14b**)	6.87
H	NHBoc	Et	7.93 (**14c**)	6.93-6.96
F	NHBoc	Et	7.87 (**14d**)	6.87
H	H	H	7.70 (**15a**)	-
F	H	H	7.73 (**15b**)	-
H	NH_2	Et	7.63 (**16a**)	-
F	NH_2	Et	7.62 (**16b**)	-
F	*i*-Pr-NH	Et	7.64 (**16c**)	-

Biological evaluation

Biochemical inhibition. The IC_{50}-values were determined using Millipore's IC50-profiler services (www.millipore.com/life_sciences/flx4/ld_kinases). The technical details of the assay protocal is available at: http://www.millipore.com/techpublications/tech1/pf3036.

General Assay Protocols: SAPK2a (h) is incubated with 25 mM Tris pH 7.5, 0.02 mM EGTA, 0.33 mg/mL myelin basic protein, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 µL of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

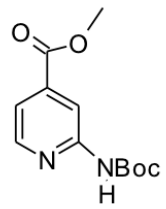
Compound Preparation and Assay Controls: All compounds are prepared to 50x final assay concentration in 100% DMSO. This working stock of the compound is added to the assay well as the first component in the reaction, followed by the remaining components as detailed in the general assay protocols below. In the standard KinaseProfiler service, there is no pre-incubation step between the compound and the kinase prior to initiation of the reaction. Our positive control wells contain all components of the reaction, except the compound of interest; however, DMSO (at a final concentration of 2%) is included in these wells to control for solvent effects. Our blank wells contain all components of the reaction, with a reference inhibitor replacing the compound of interest. This abolishes kinase activity and establishes the baseline (0% kinase activity remaining).

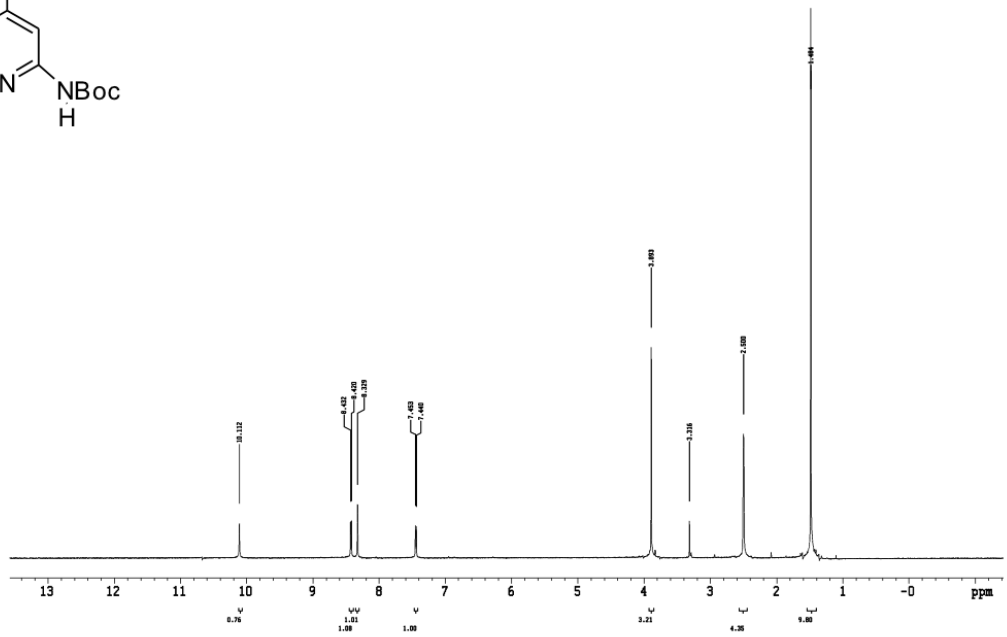
References

1. Tian, J.; Zhang, Z; Yang, X.; Fun, H.; Xu, J.; J.Org. Chem 2001, 66,8230-8235.
2. Boros, L.; Felföldi, K.; Pálinkó, I. *Molecules* **2004**, *9*, 256-263.

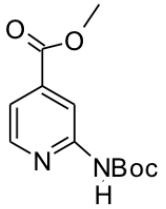
Compound **2**

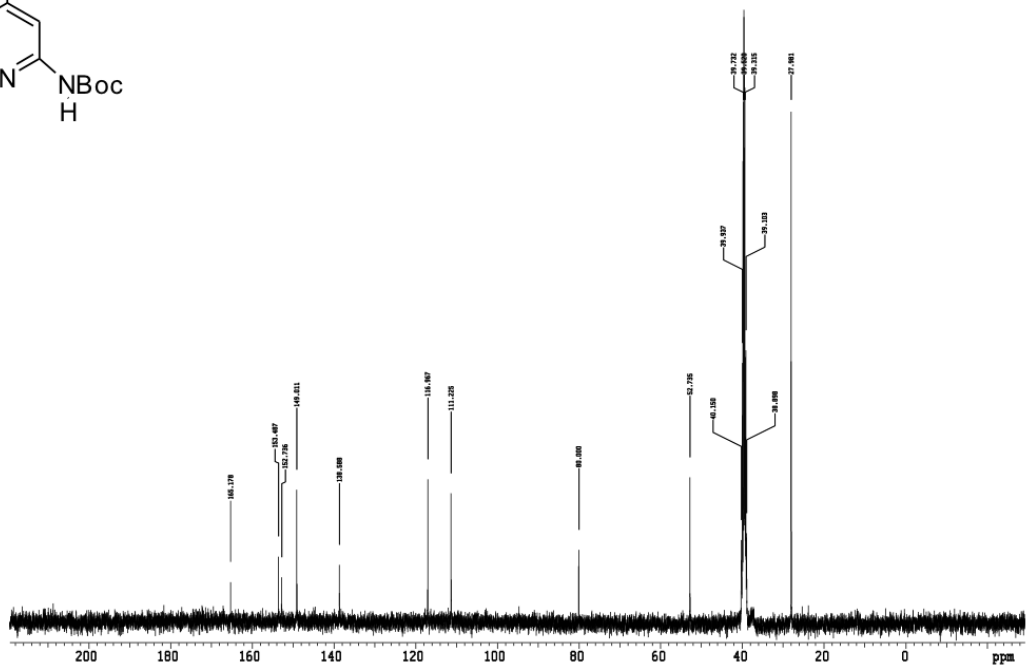
^{1}H NMR





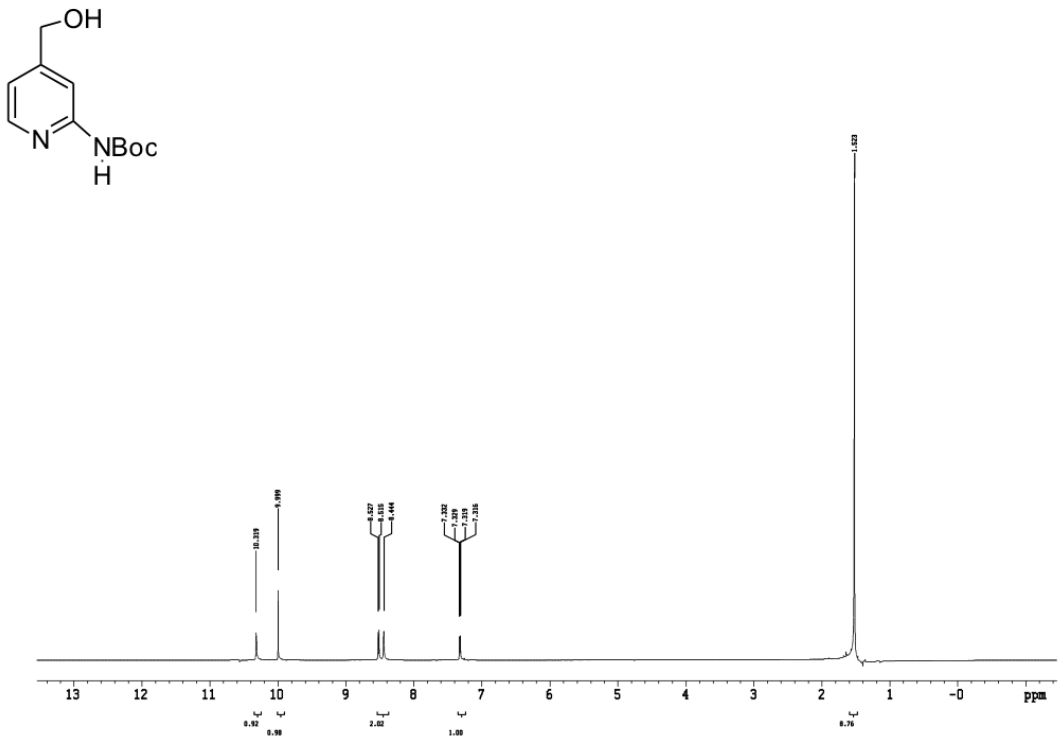
^{13}C NMR



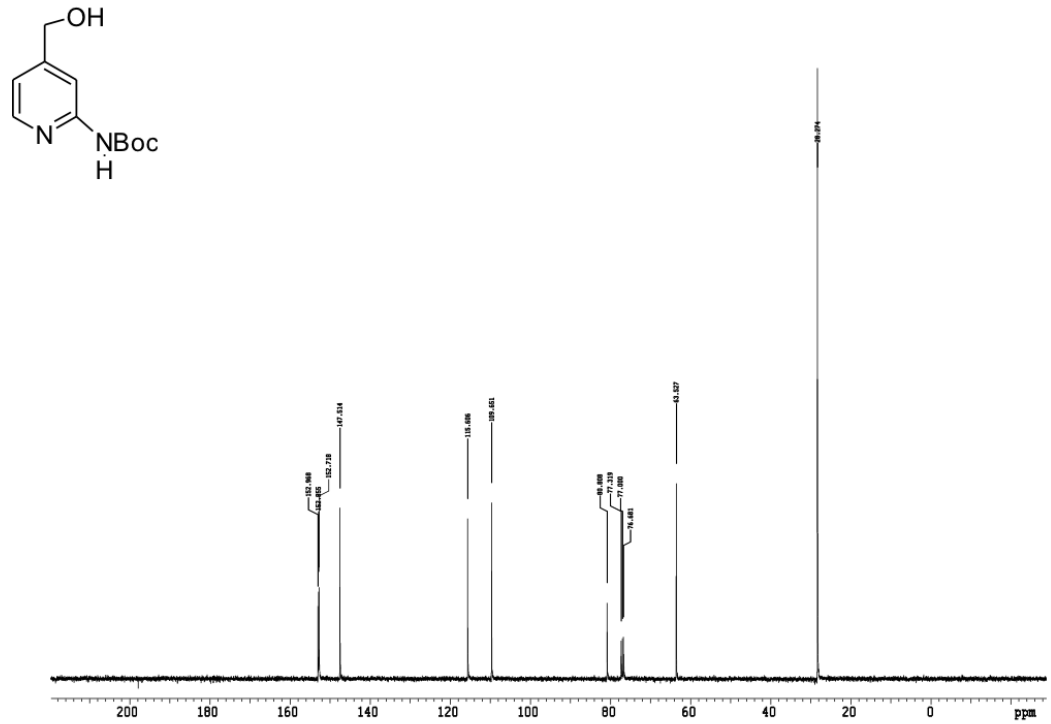


Compound **3**

^{1}H NMR

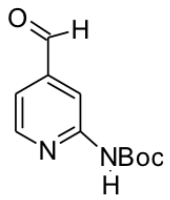


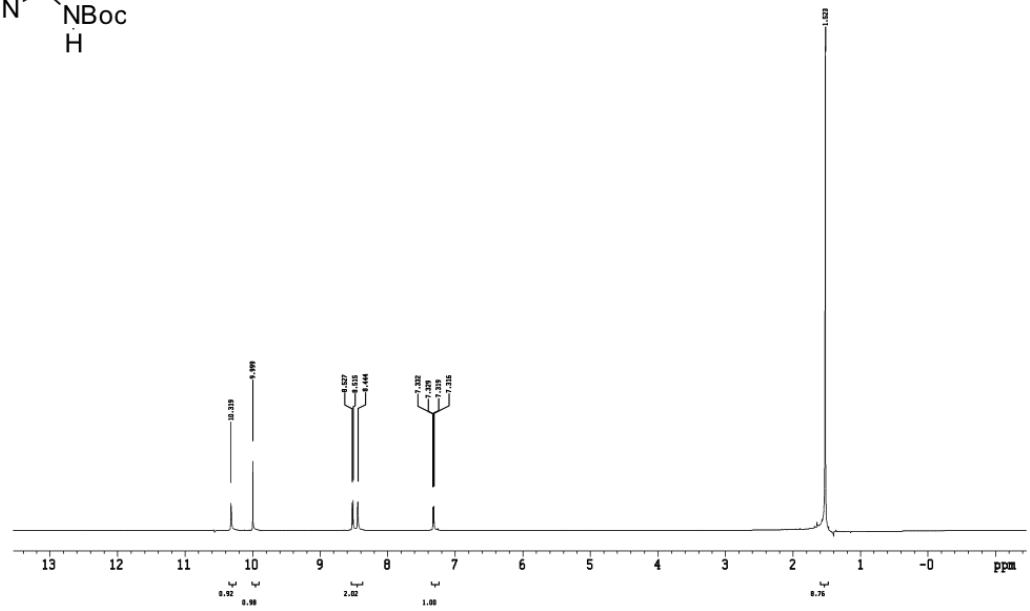
^{13}C NMR



Compound **4b**

^{1}H NMR





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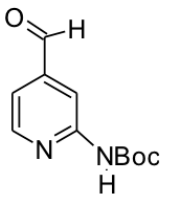
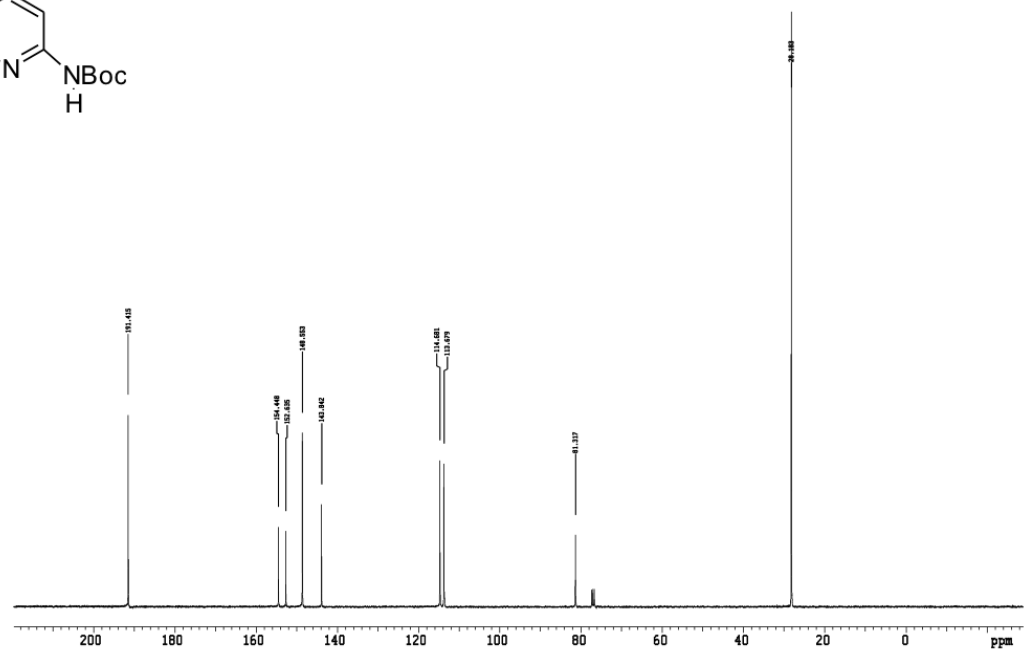
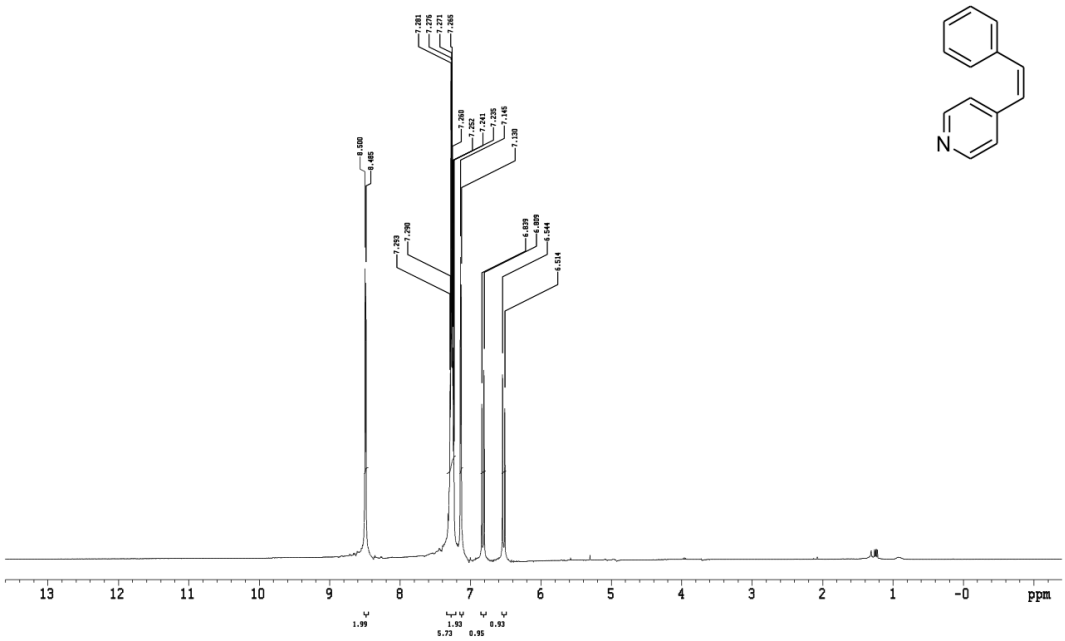




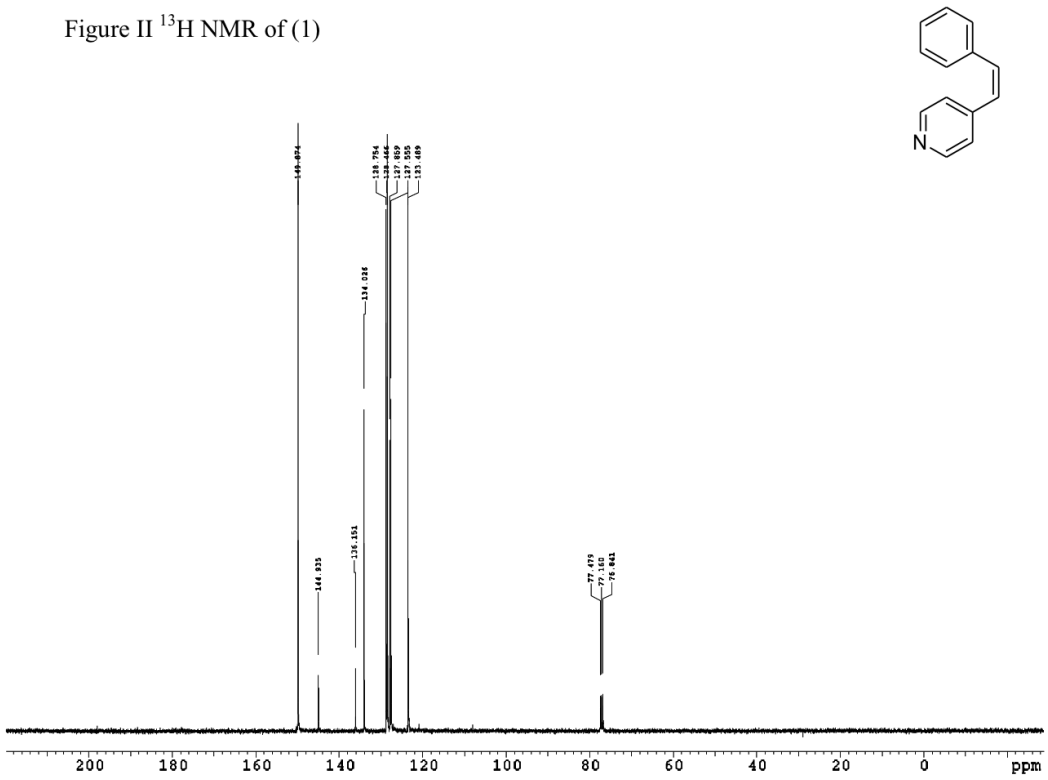
Compound **6a**

^{1}H NMR



^{13}C NMR

Figure II ^{13}H NMR of (1)



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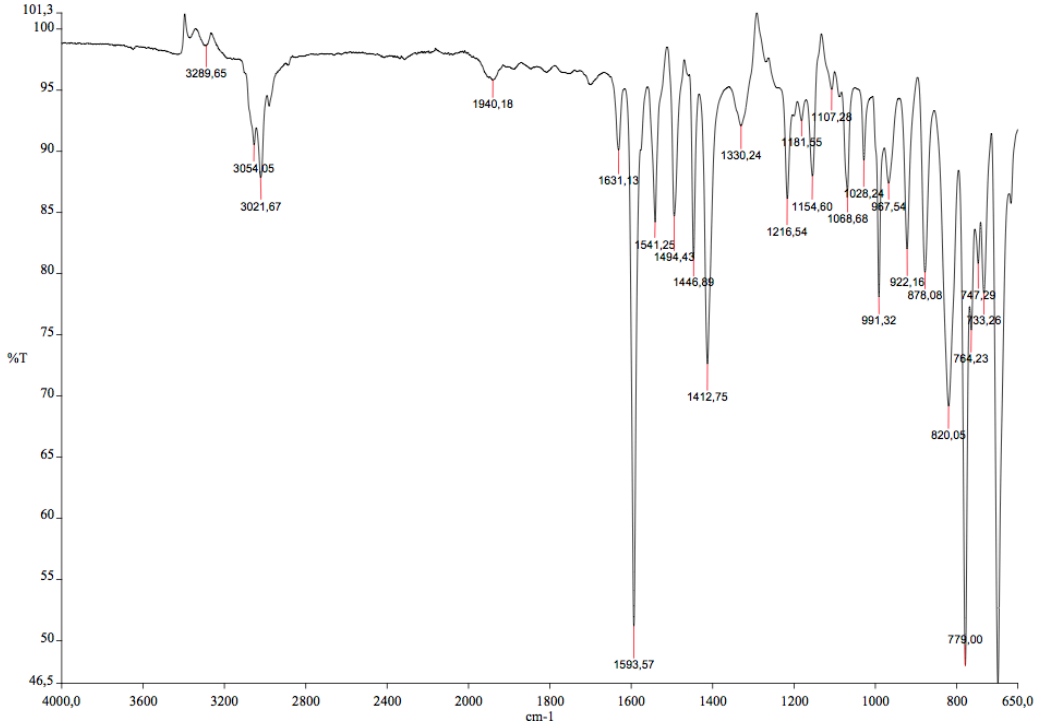
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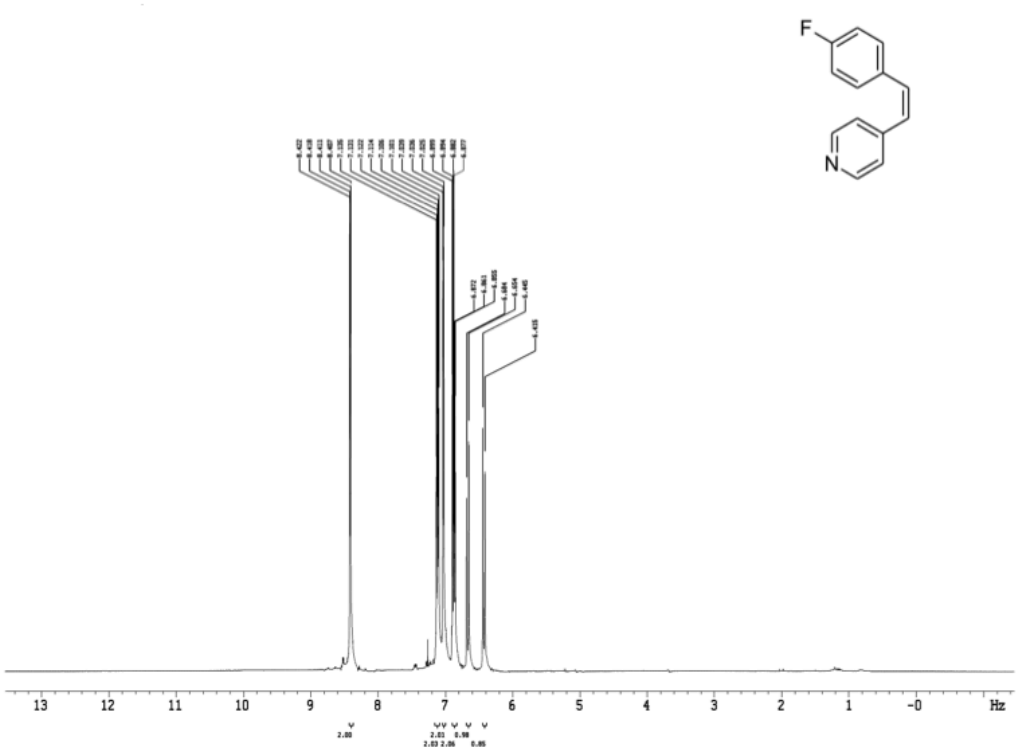
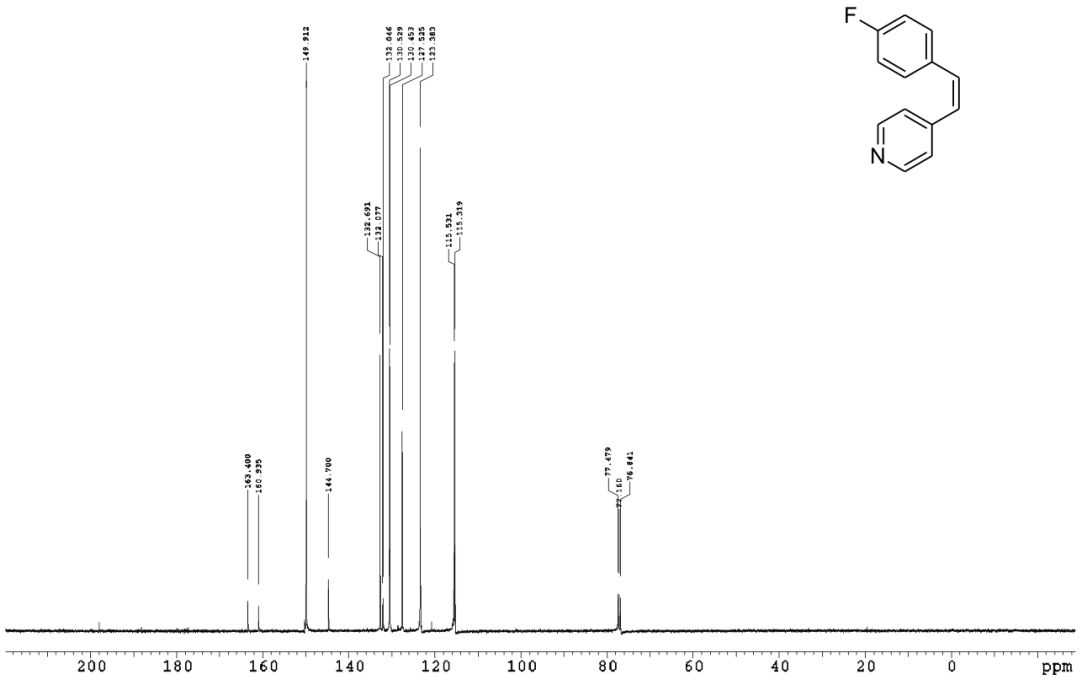
Compound **6b**

^{1}H NMR



^{13}C NMR



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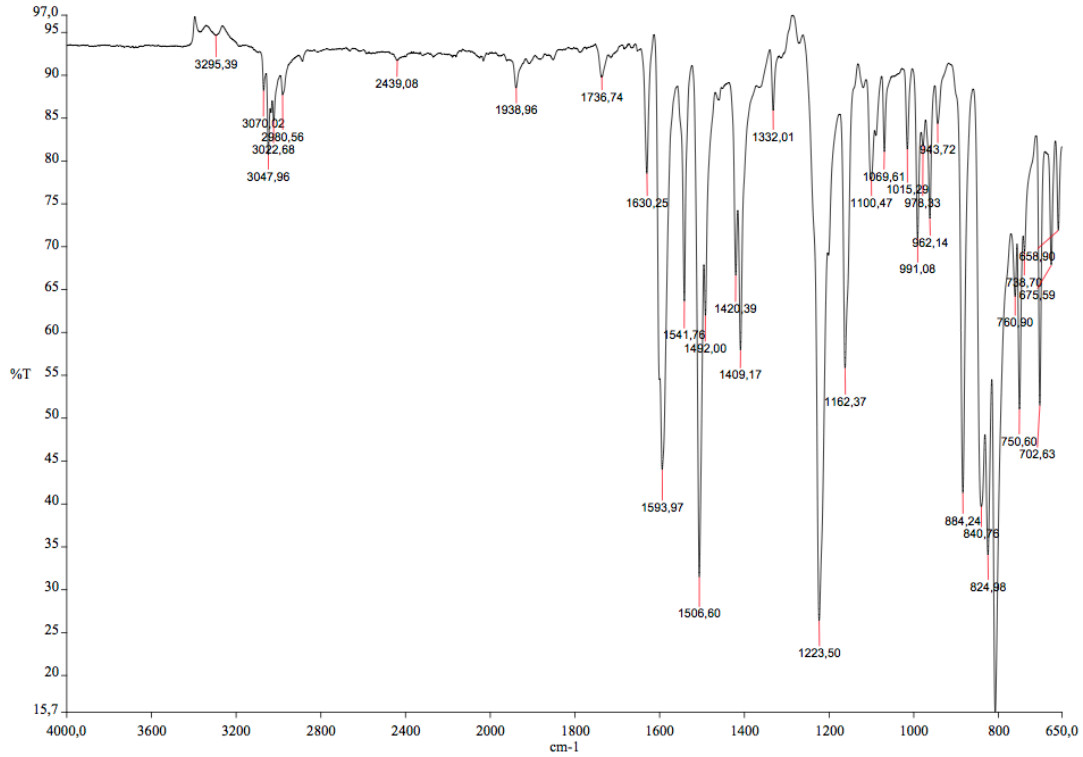
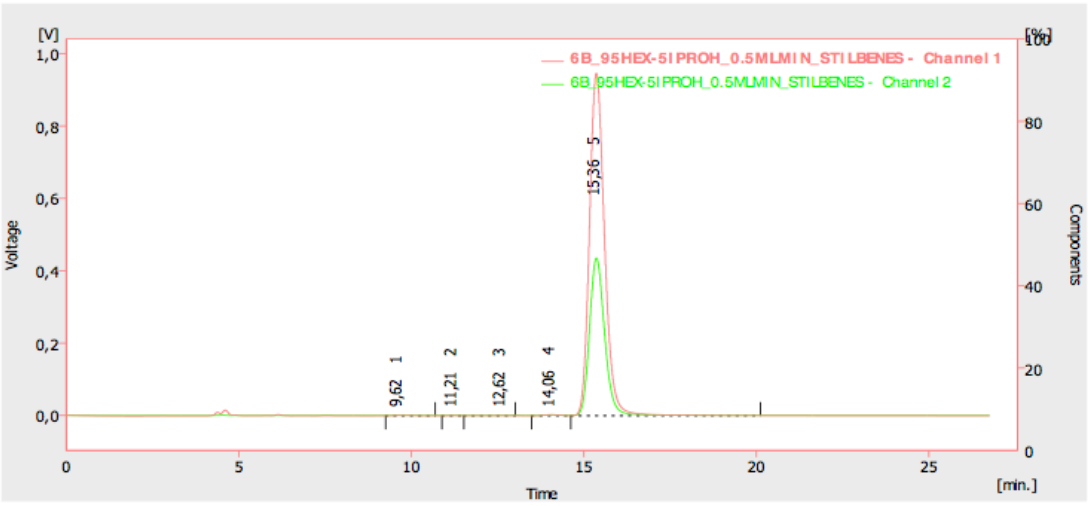


HPLC



Result Table (Uncal - 6B_95HEX-5IPROH_0.5MLMIN_STILBENES - Channel 1)

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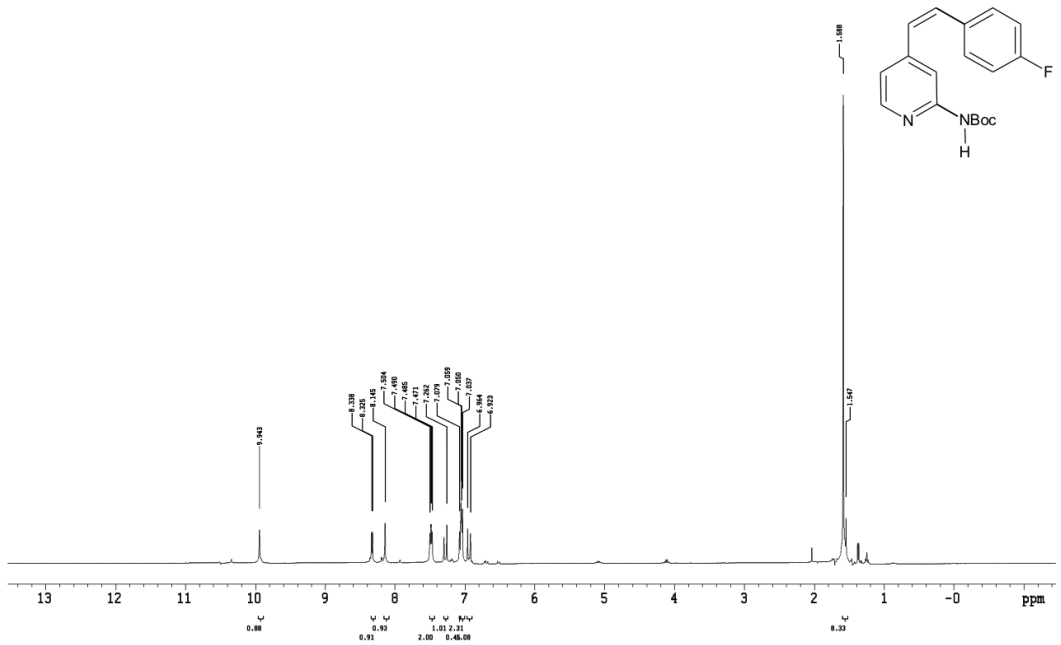
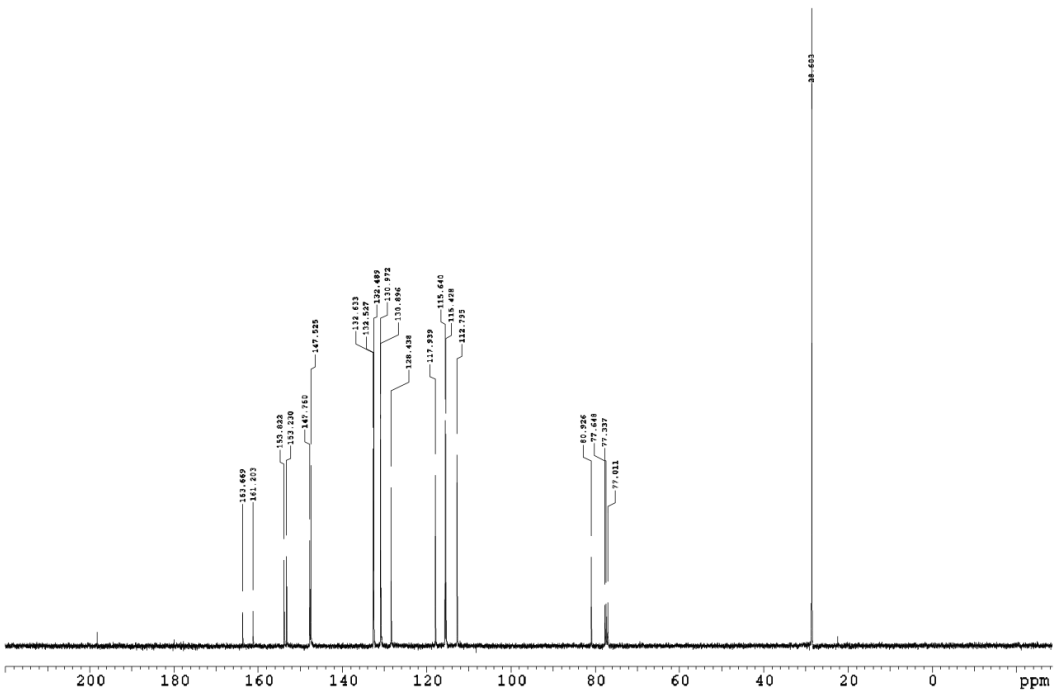
1	9,623	31,855	0,804	0,1	0,1	0,65	
2	11,207	8,355	0,474	0,0	0,0	0,29	
3	12,623	8,204	0,231	0,0	0,0	0,27	
4	14,057	84,476	2,907	0,3	0,3	0,45	
5	15,357	28797,292	946,426	99,5	99,5	0,45	
	Total	28930,181	950,842	100,0	100,0		

Compound **6c**

^{1}H NMR



^{13}C NMR



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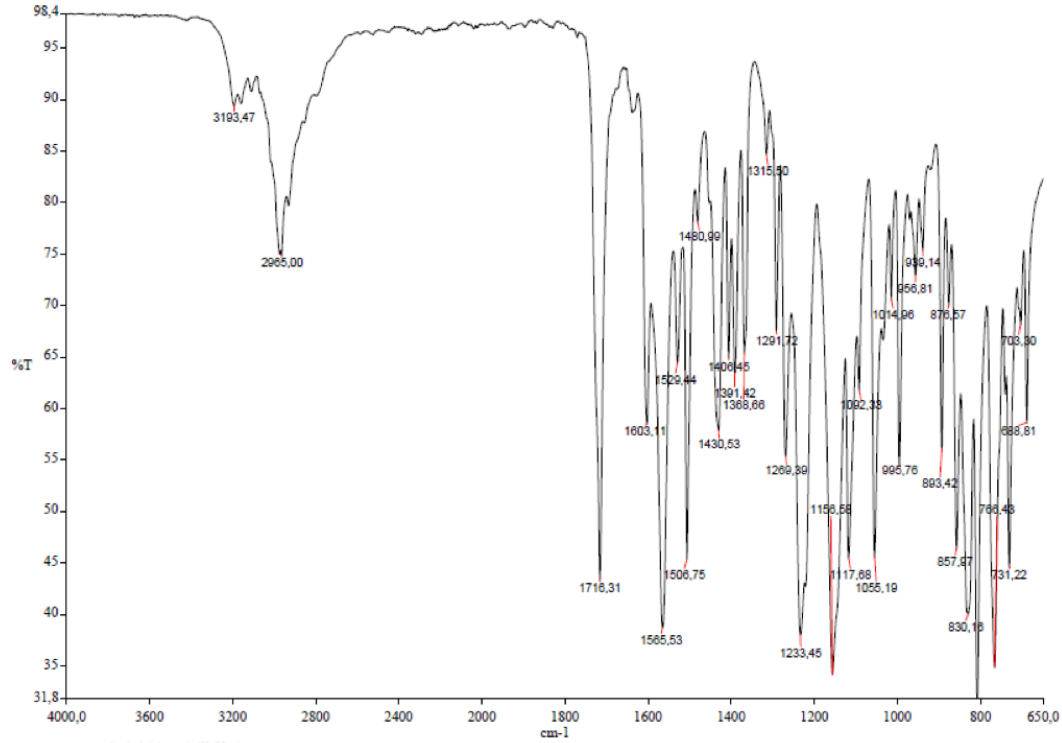

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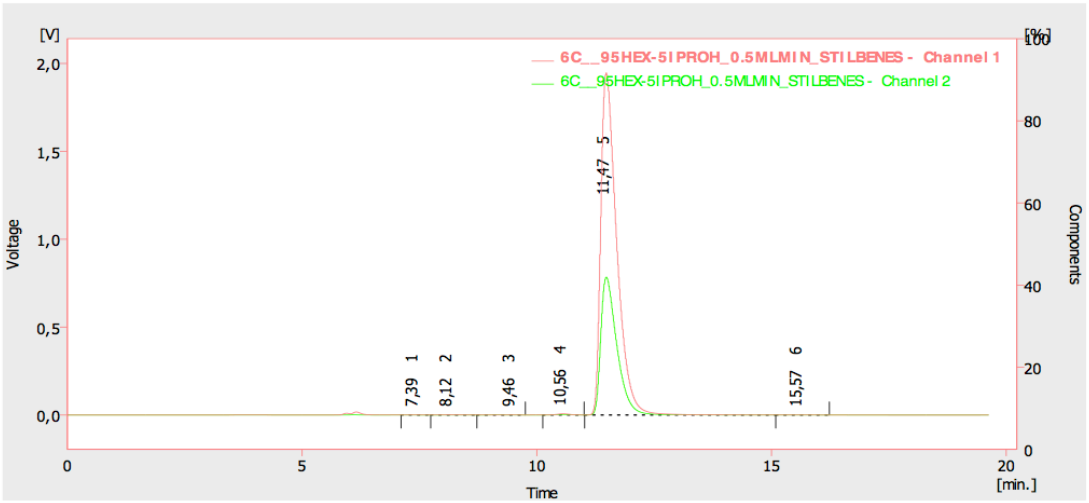

Result Table (Uncal - 6C__95HEX-5IPROH_0.5MLMIN_STILBENES - Channel 1)

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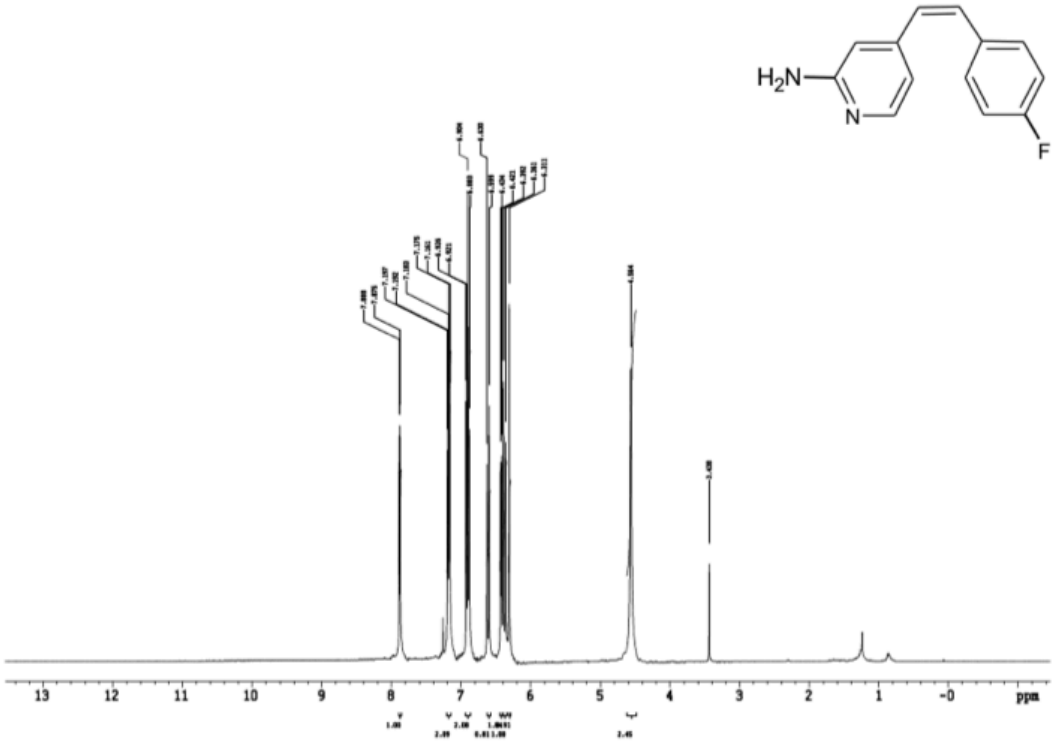
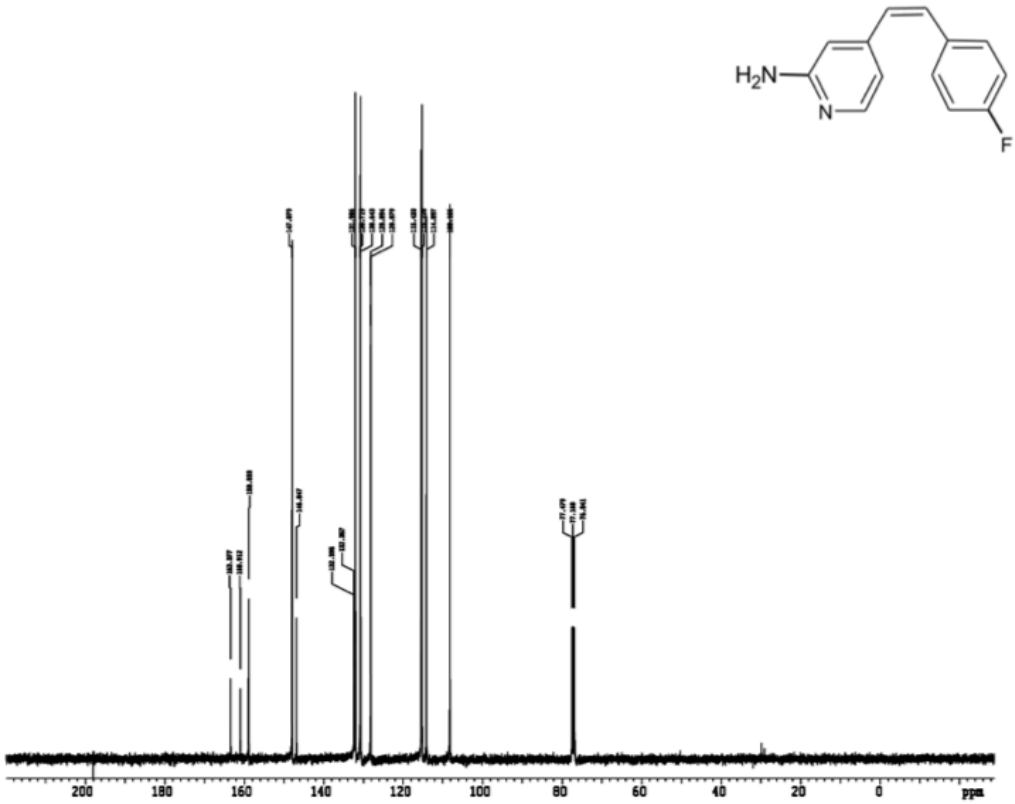
2	8,123	27,041	0,768	0,1	0,0	0,57	
3	9,457	13,390	0,562	0,0	0,0	0,25	
4	10,557	130,334	6,813	0,3	0,3	0,29	
5	11,473	46094,064	1946,198	99,6	99,5	0,35	
6	15,573	14,357	0,452	0,0	0,0	0,47	
	Total	46286,640	1955,200	100,0	100,0		

Compound **7**

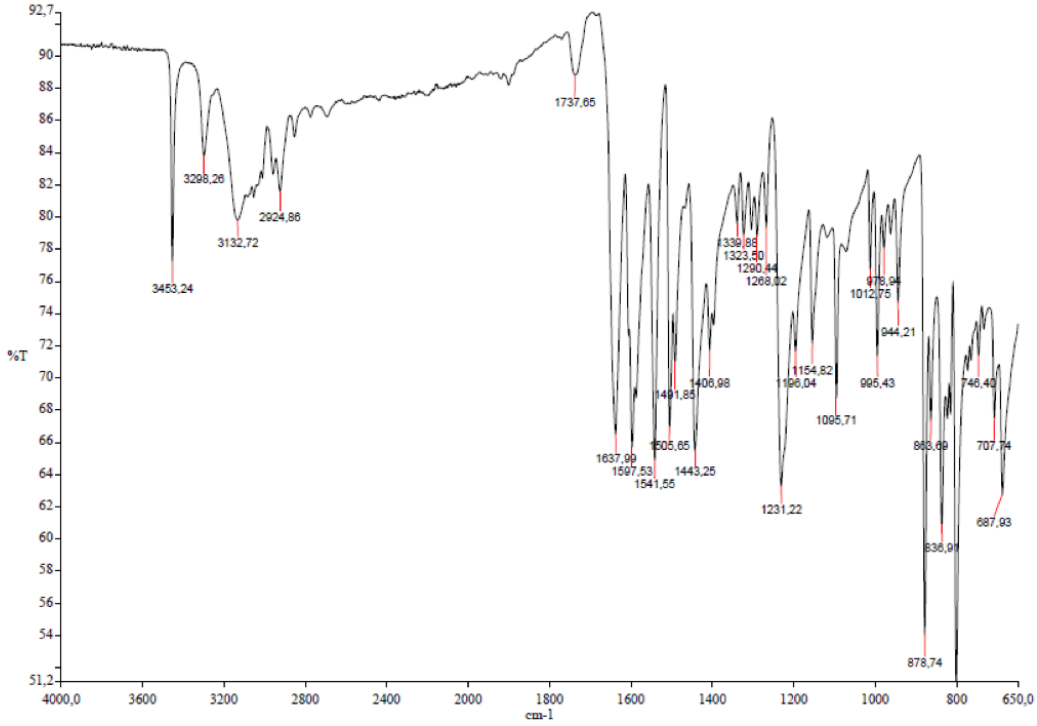
^{1}H NMR



^{13}C NMR



IR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3298,26
3132,72
2924,86
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1597,53
1541,55
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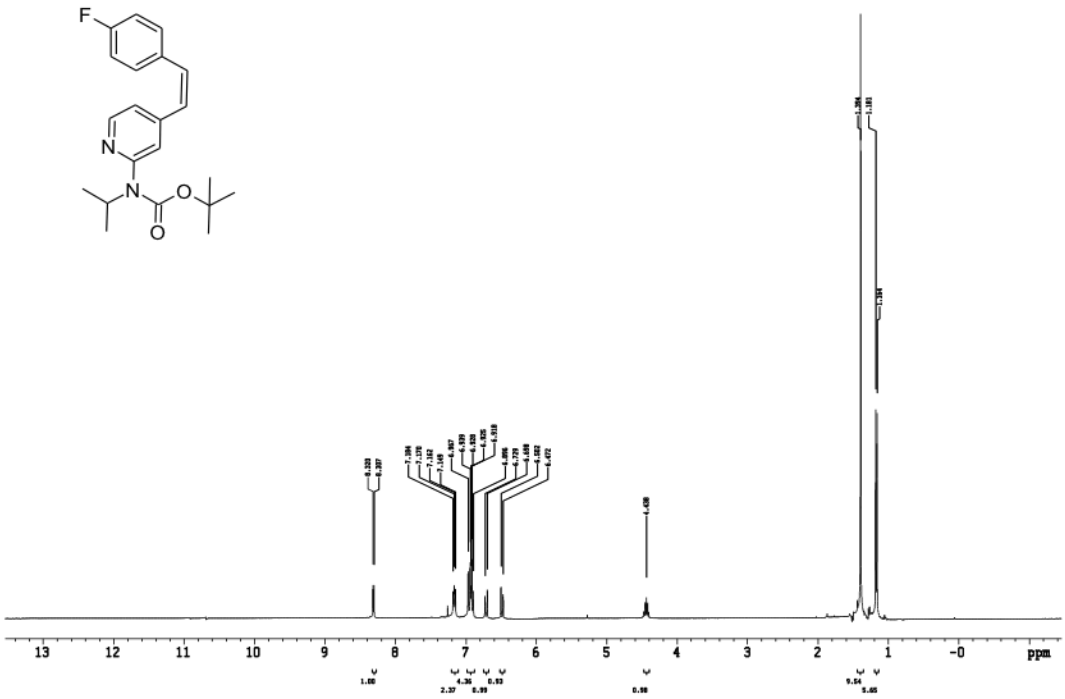
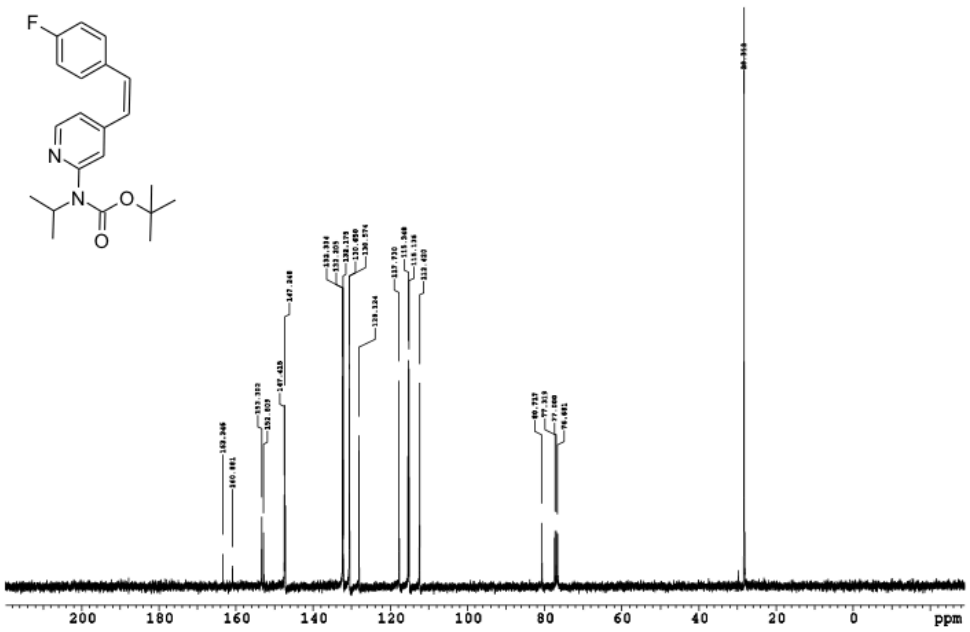
1491,85
1443,25
1406,98
1338,85
1323,50
1290,44
1268,02
1231,22
1196,04
1154,82
1095,71
1012,75
995,43
978,94
944,21
878,74
836,91
746,40
707,74
687,93

Compound **8**

^{1}H NMR



^{13}C NMR



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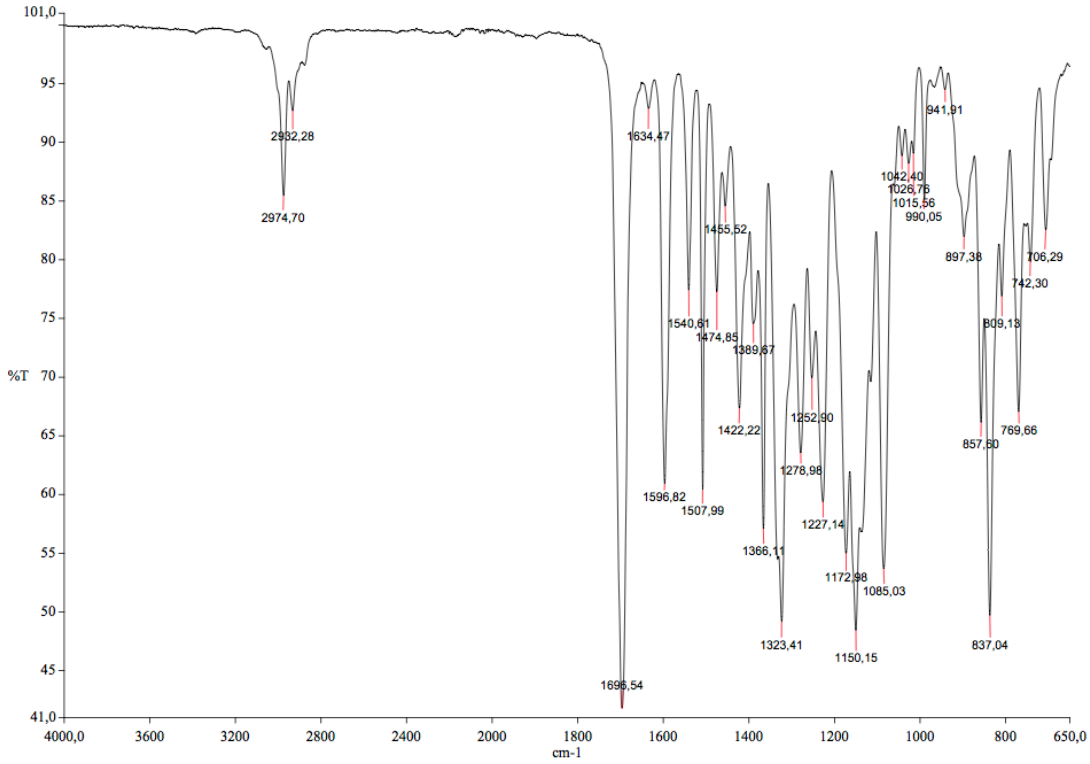
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2400
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650,0
cm-1
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2974,70
1634,47
1455,52
1540,61
1474,85
1389,67
1422,22
1596,82
1507,99
1366,11
1323,41
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1252,90
1278,98
1227,14
1172,98
1150,15
1085,03
1042,40
1026,76
1015,56
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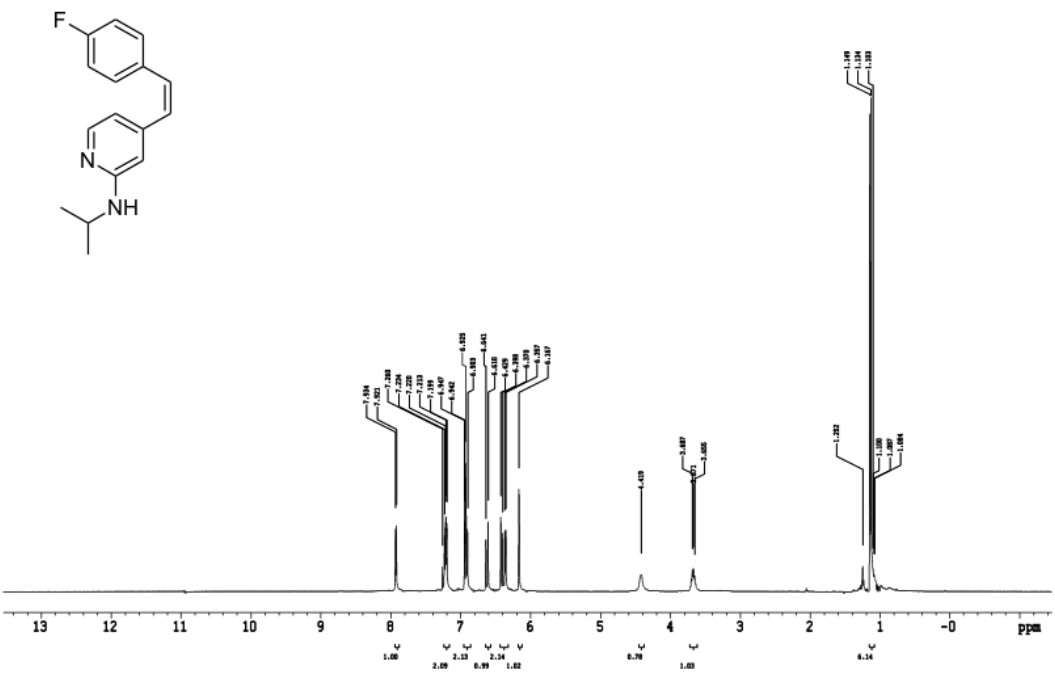
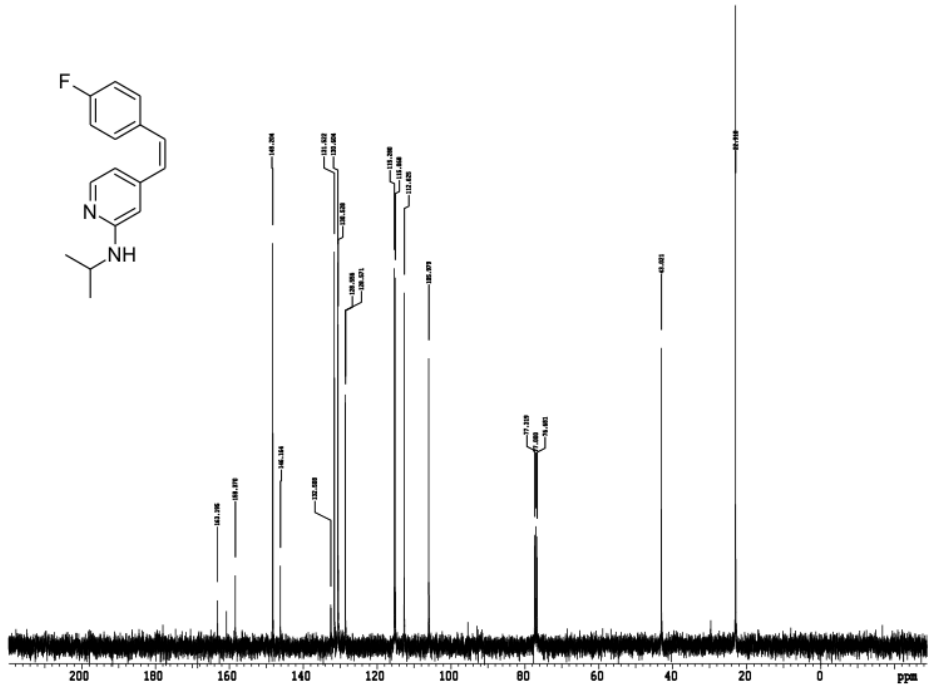
706,29
742,30
809,13
857,80
769,66
837,04

Compound **9**

^{1}H NMR



^{13}C NMR



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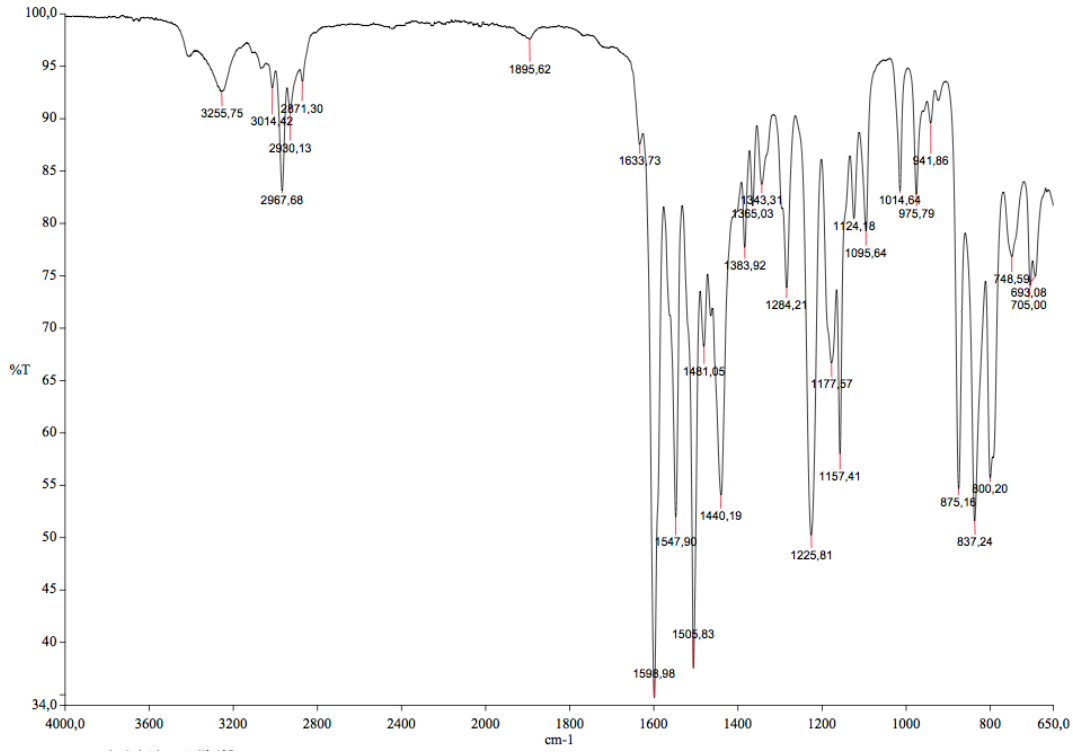
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2967,68
1895,62
1633,73
1598,98
1547,90
1505,83
1481,05
1440,19
1383,92
1365,03
1343,31
1284,21
1225,81
1177,57
1157,41
1124,18
1095,64
1014,64
975,79
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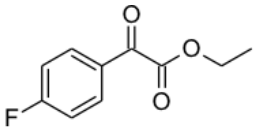
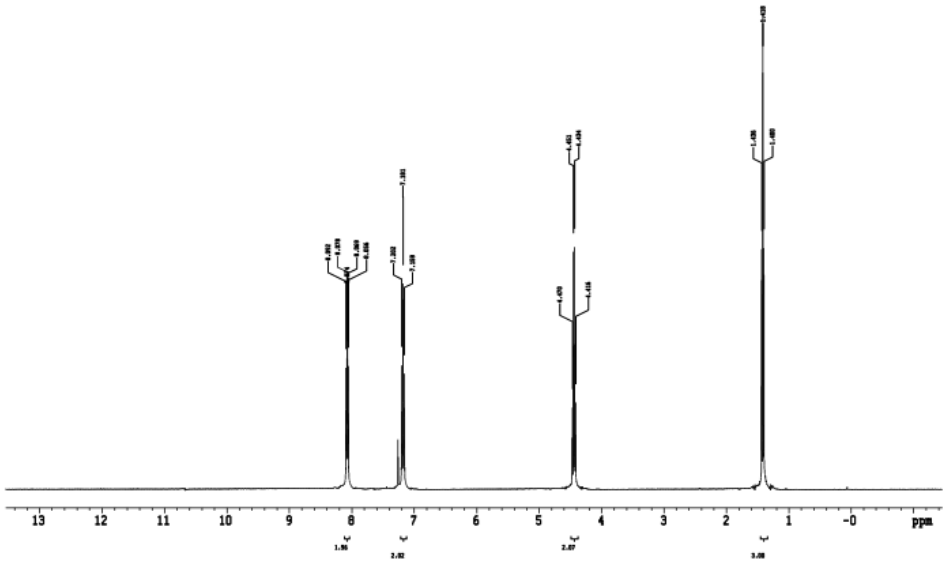
875,16
837,24
800,20
748,59
705,00
693,08

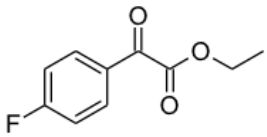
Compound **10**

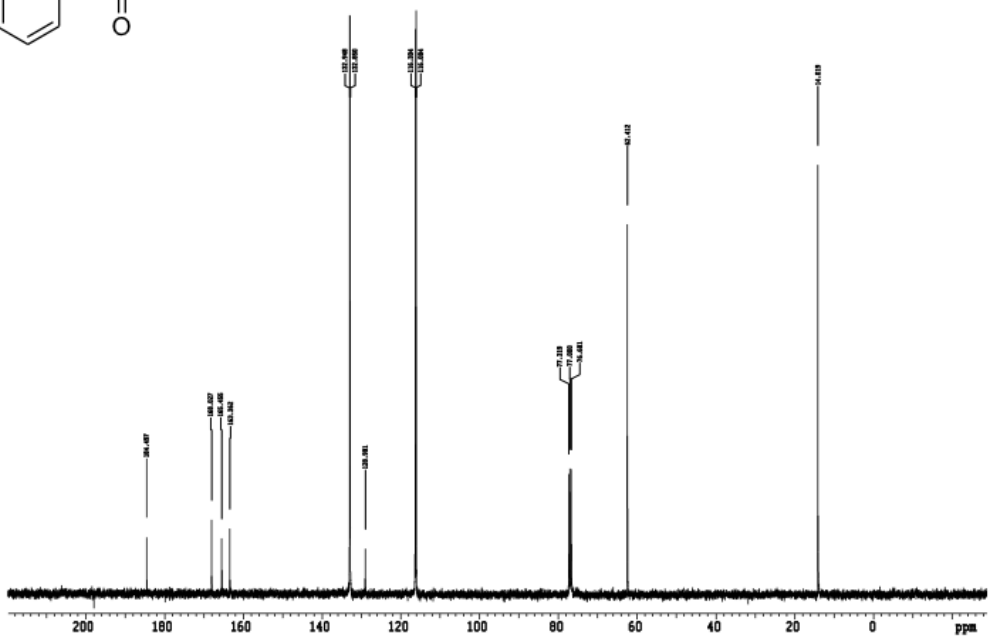
^{1}H NMR





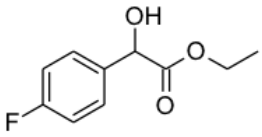
^{13}C NMR

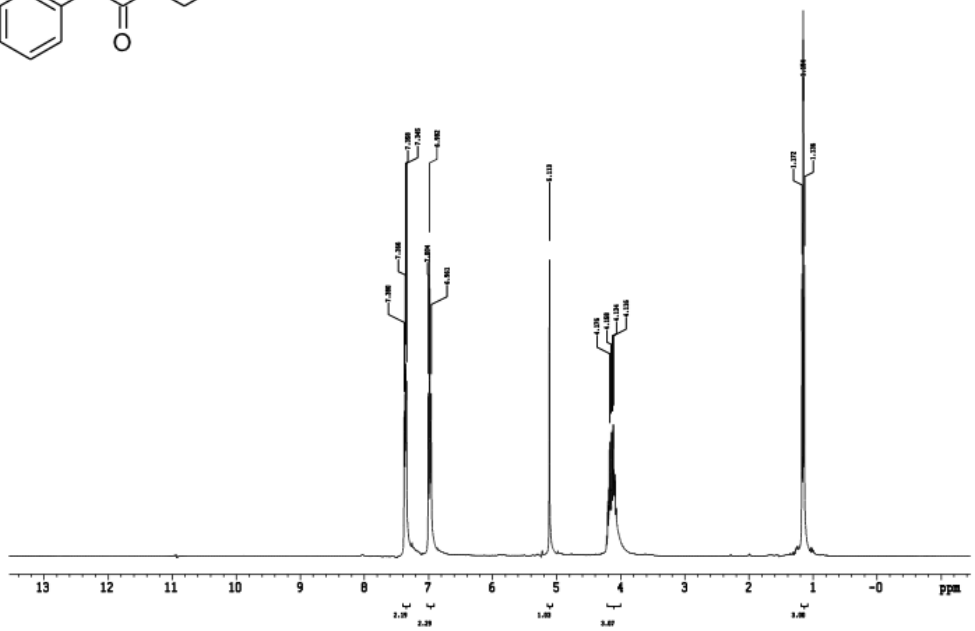




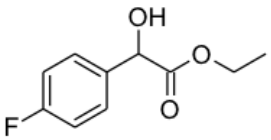
Compound **11**

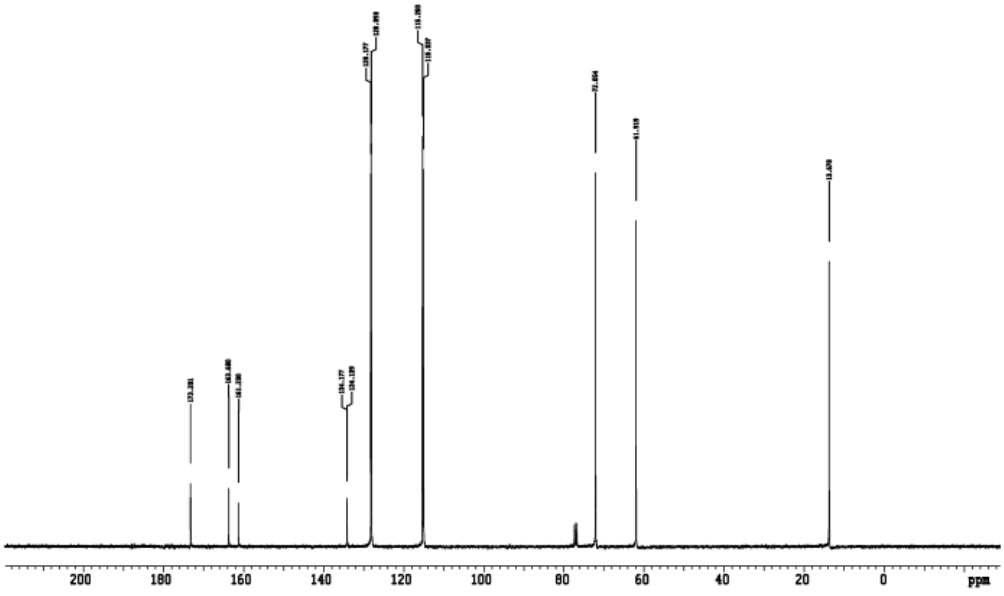
^{1}H NMR





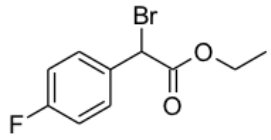
^{13}C NMR

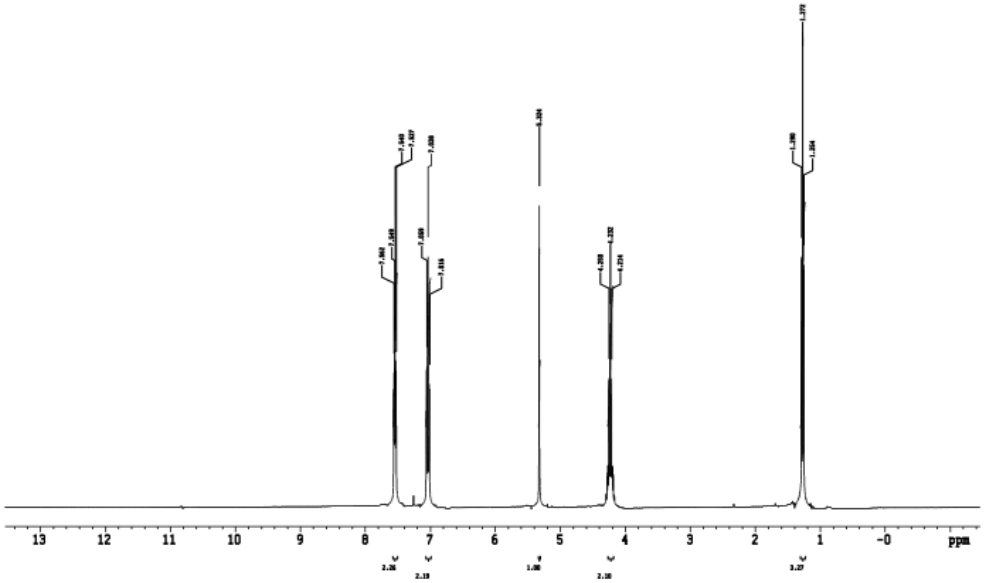




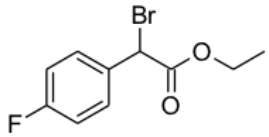
Compound **12b**

^{1}H NMR



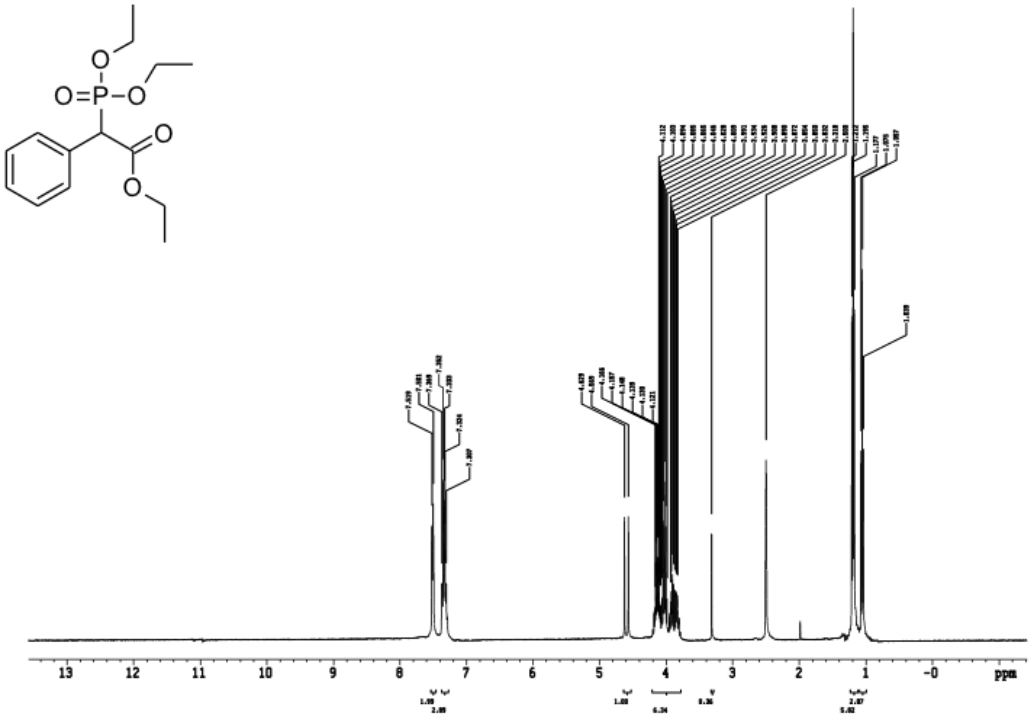


^{13}C NMR

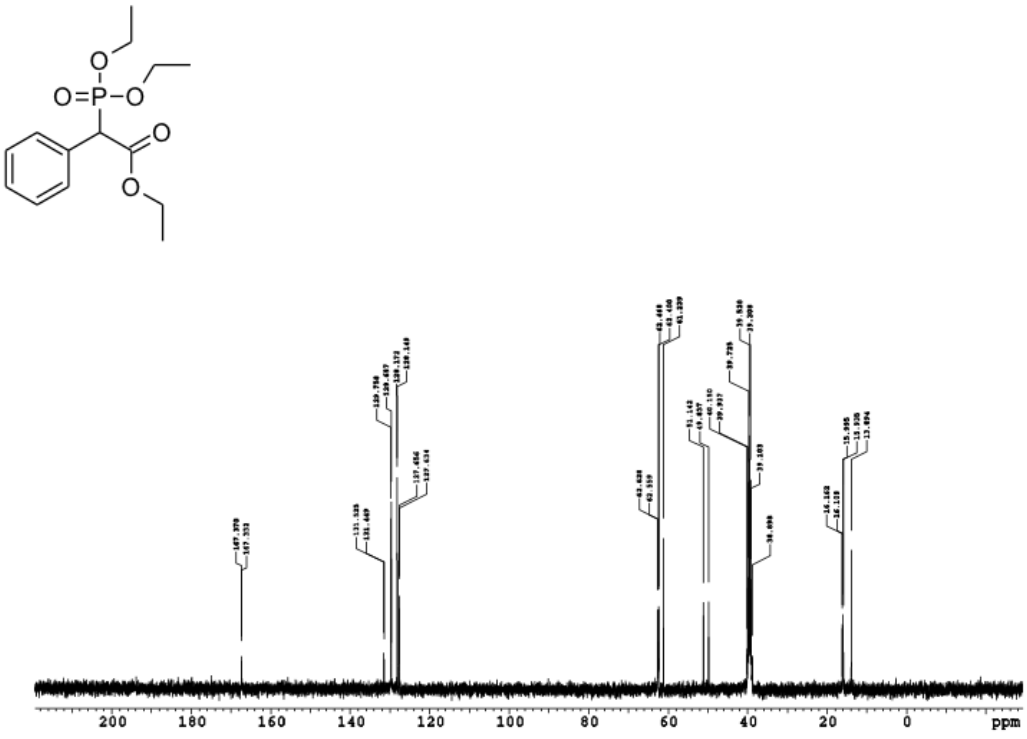


Compound **13a**

^{1}H NMR

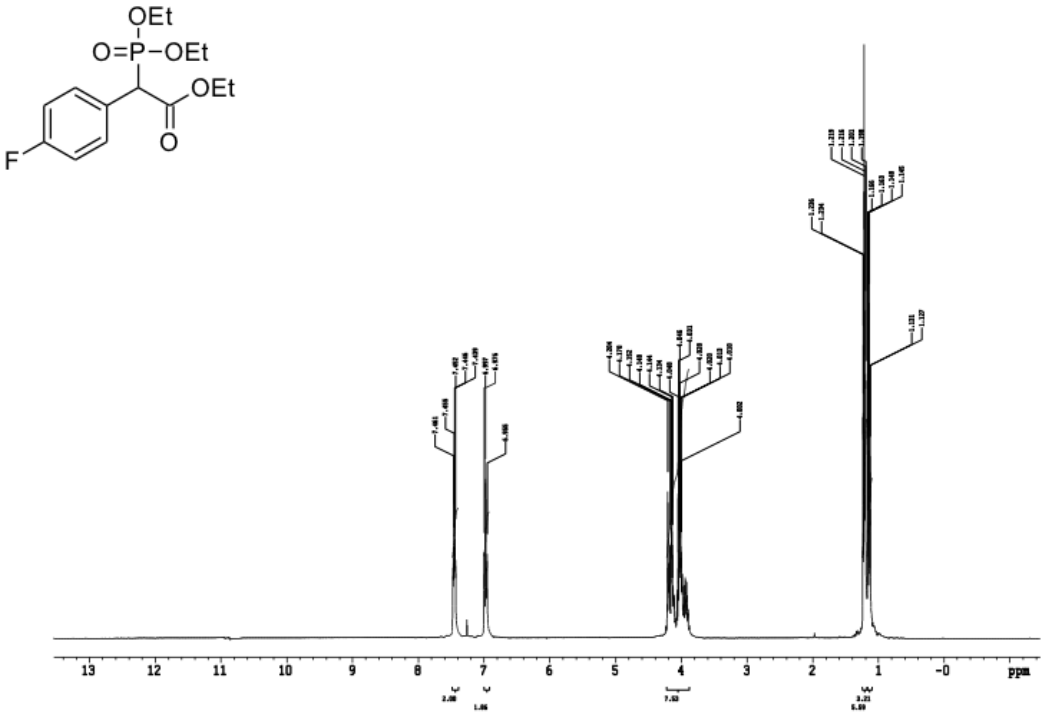


^{13}C NMR

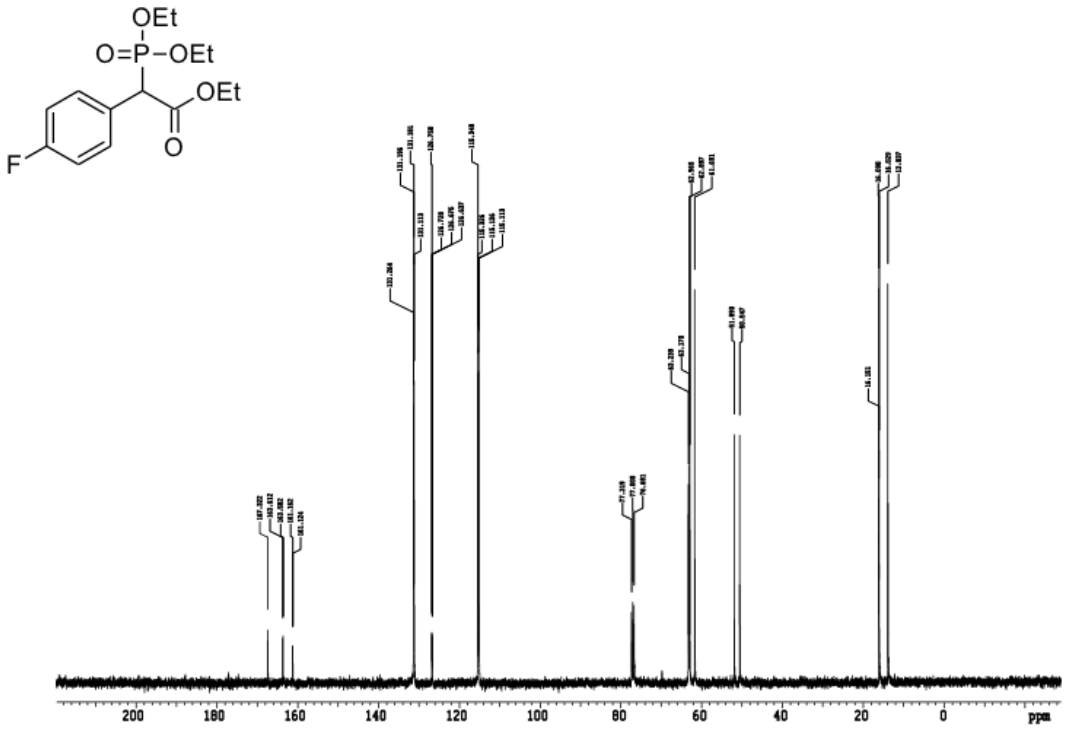


Compound **13b**

^{1}H NMR

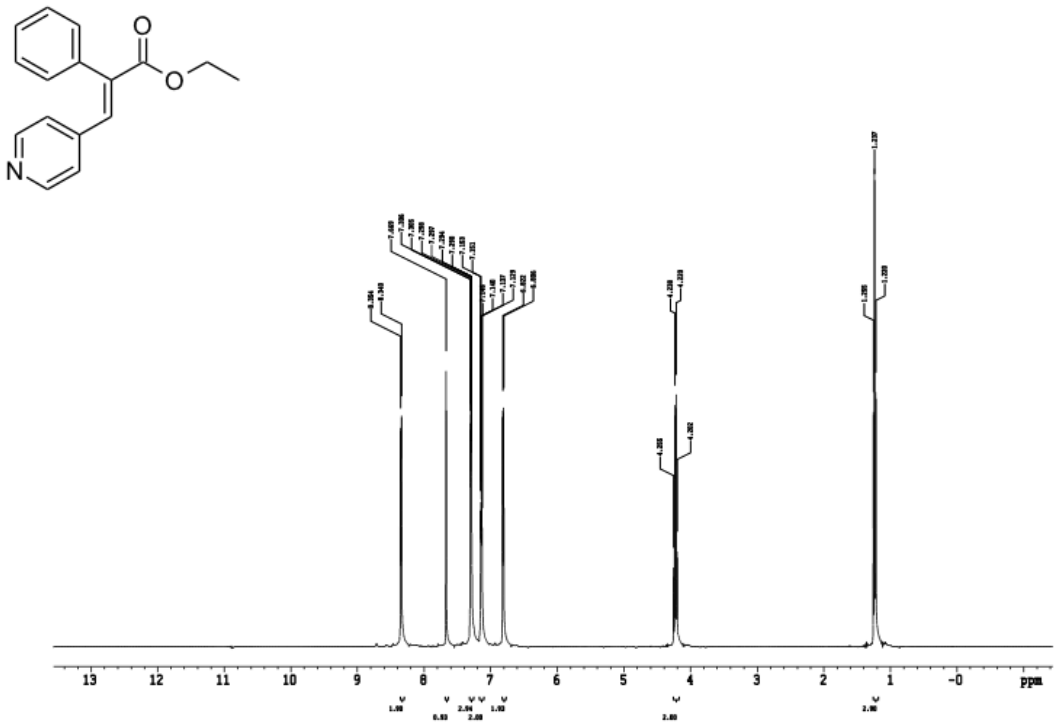


^{13}C NMR

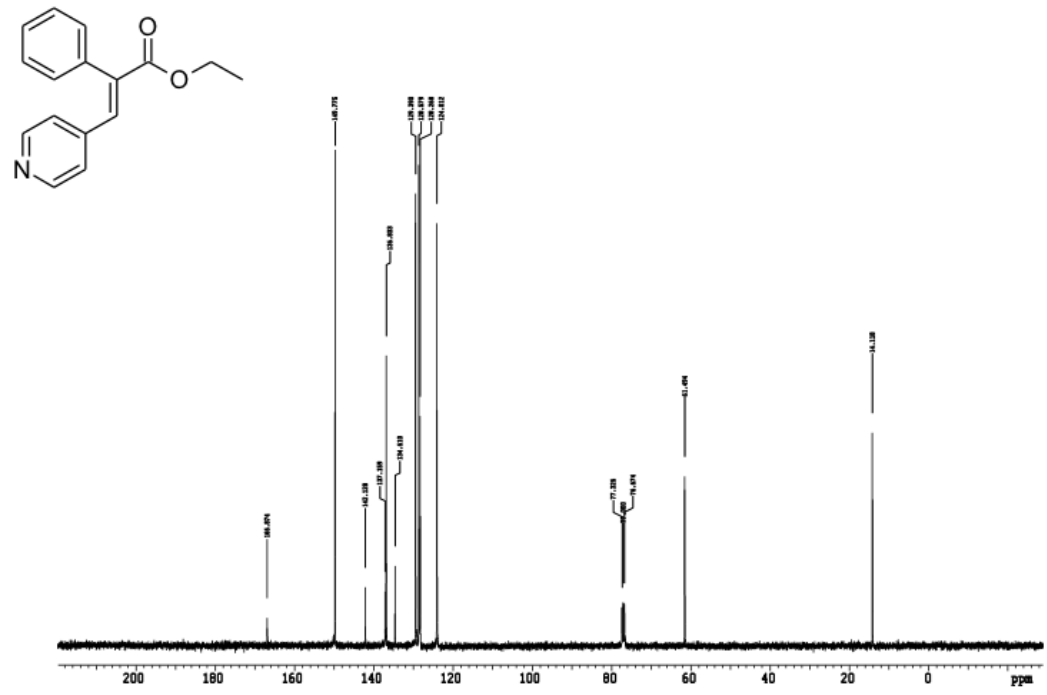


Compound **14a**

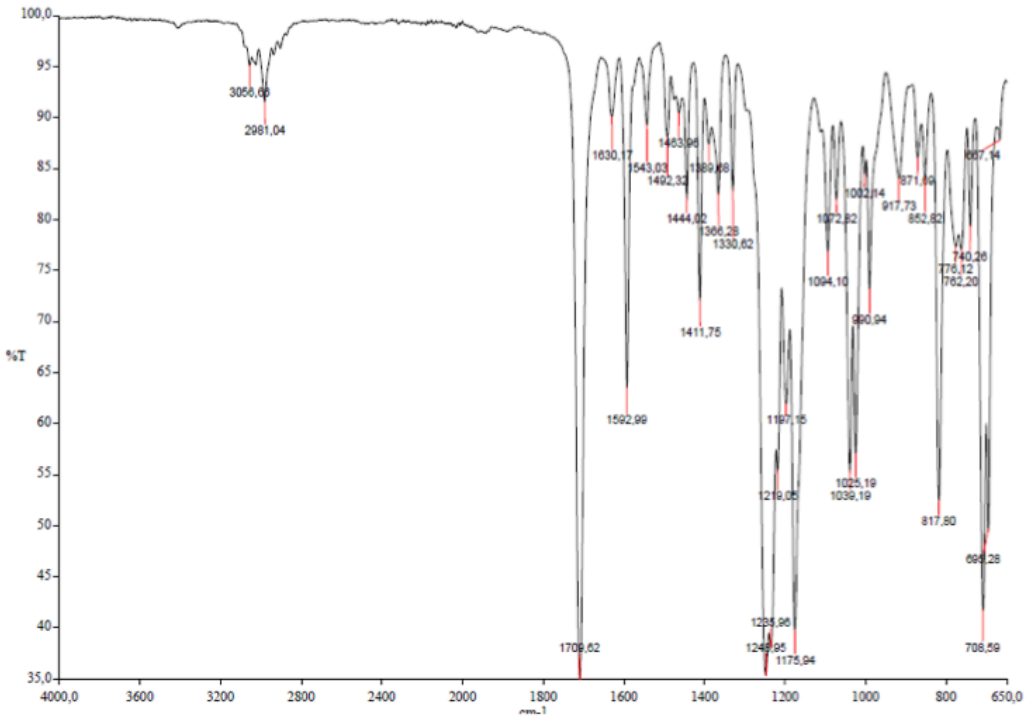
^{1}H NMR



^{13}C NMR

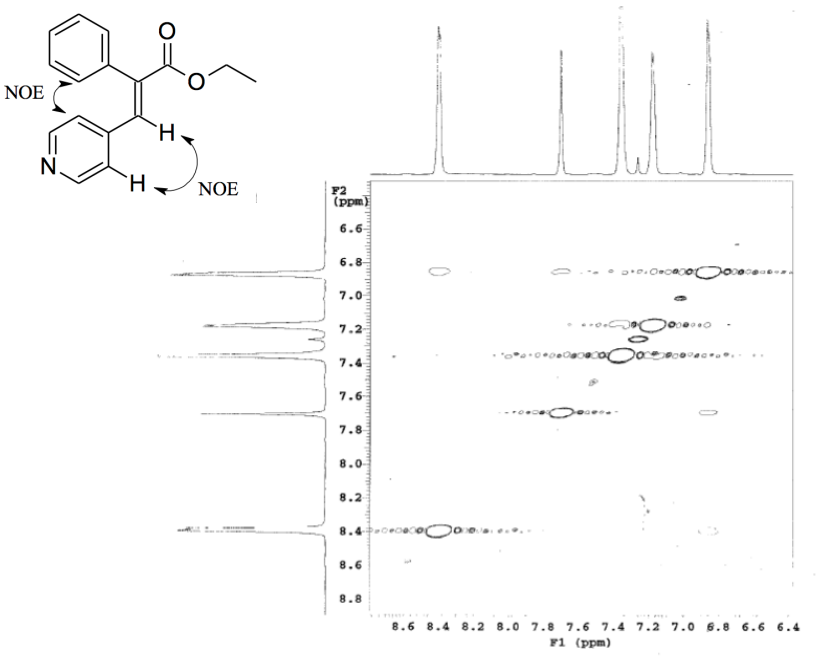


IR



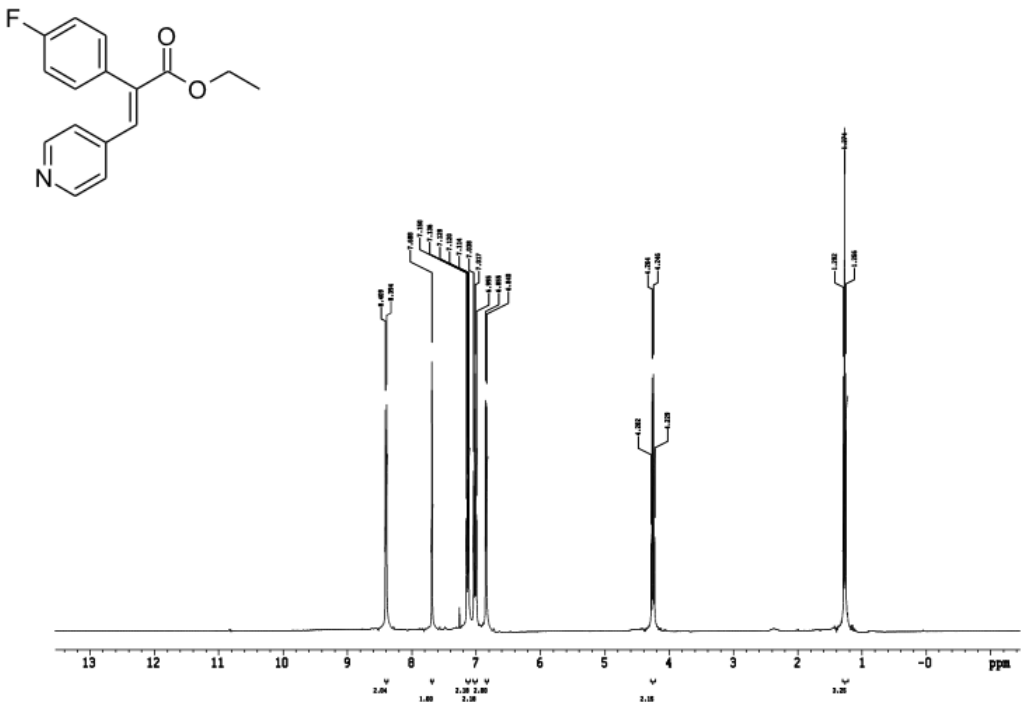
NOESY

^{1}H NMR ($CDCl_3$): δ 8.35 (d, *J* = 5.6 Hz, 2H, NCH), 7.67 (s, 1H, PyrCH), 7.15-7.67 (m, 3H, NCHC*H,* PyrCHCCCHCHC*H*), 7.13-7.15 (m, 2H, PyrCHCCCHC*H*), 6.81 (d, *J* = 6.4 Hz, 2H, PyrCHCCC*H*), 4.23 (q, *J* = 7.2 Hz, 2H, CH_2), 1.24 (t, *J* = 7.2 Hz, 3H, CH_3).

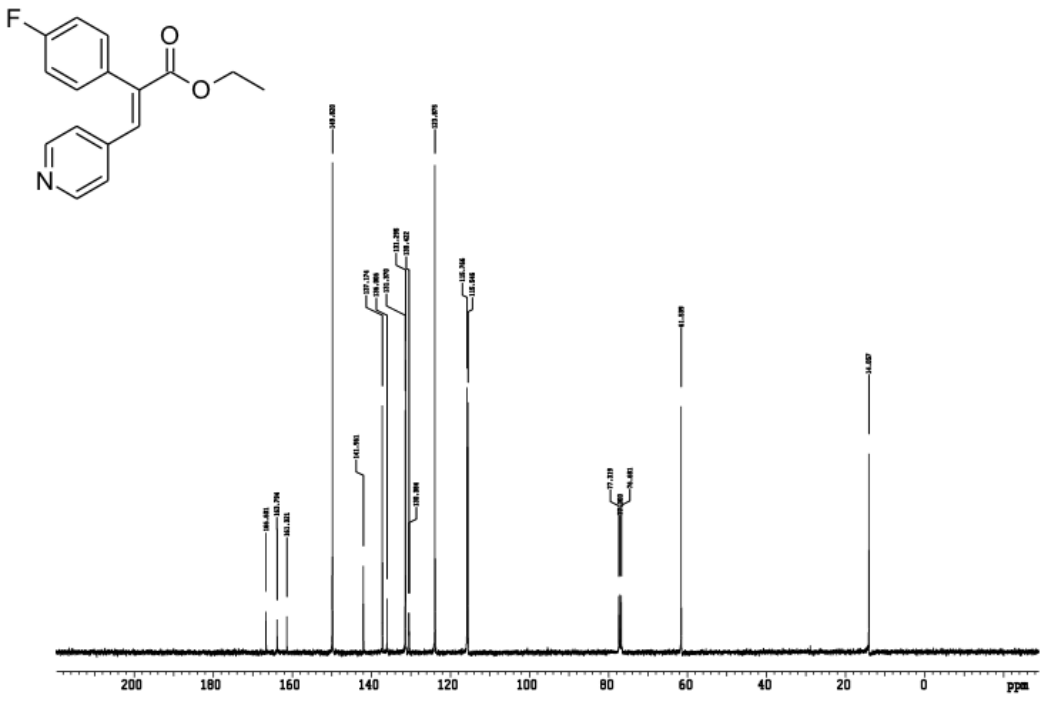


Compound **14b**

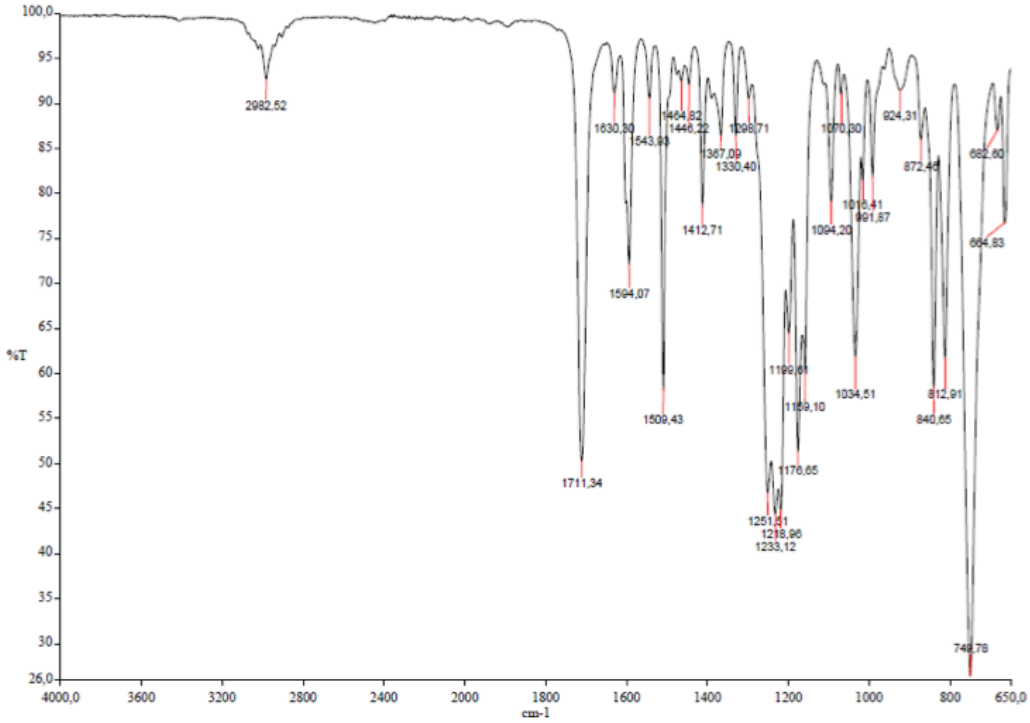
^{1}H NMR



^{13}C NMR



IR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1200
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cm-1
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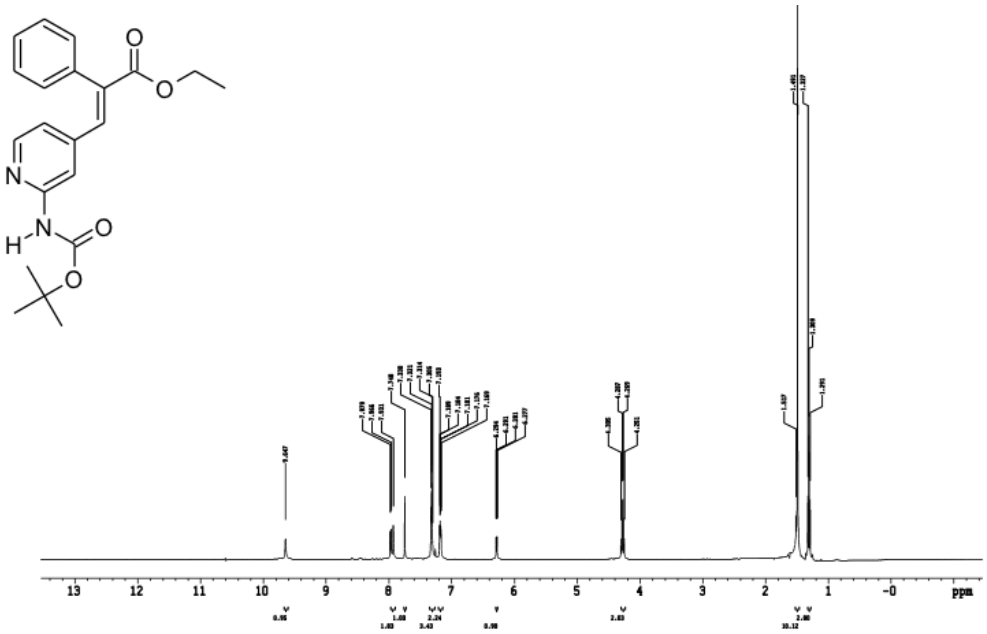
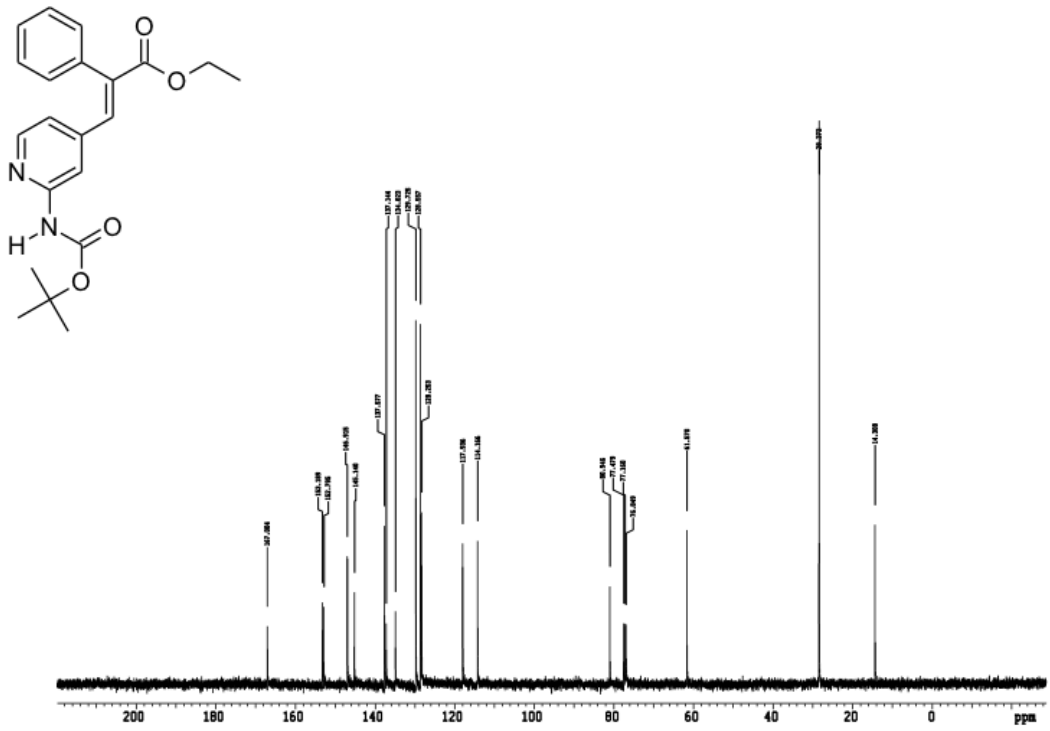
1711,34
1630,30
1594,07
1543,93
1509,43
1464,82
1446,22
1412,71
1367,09
1330,40
1298,71
1251,51
1218,96
1233,12
1199,61
1176,65
1159,10
1094,20
1070,30
1034,51
1016,41
991,87
924,31
872,46
840,65
812,91
749,78
682,60
664,83

Compound **14c**

^{1}H NMR



^{13}C NMR



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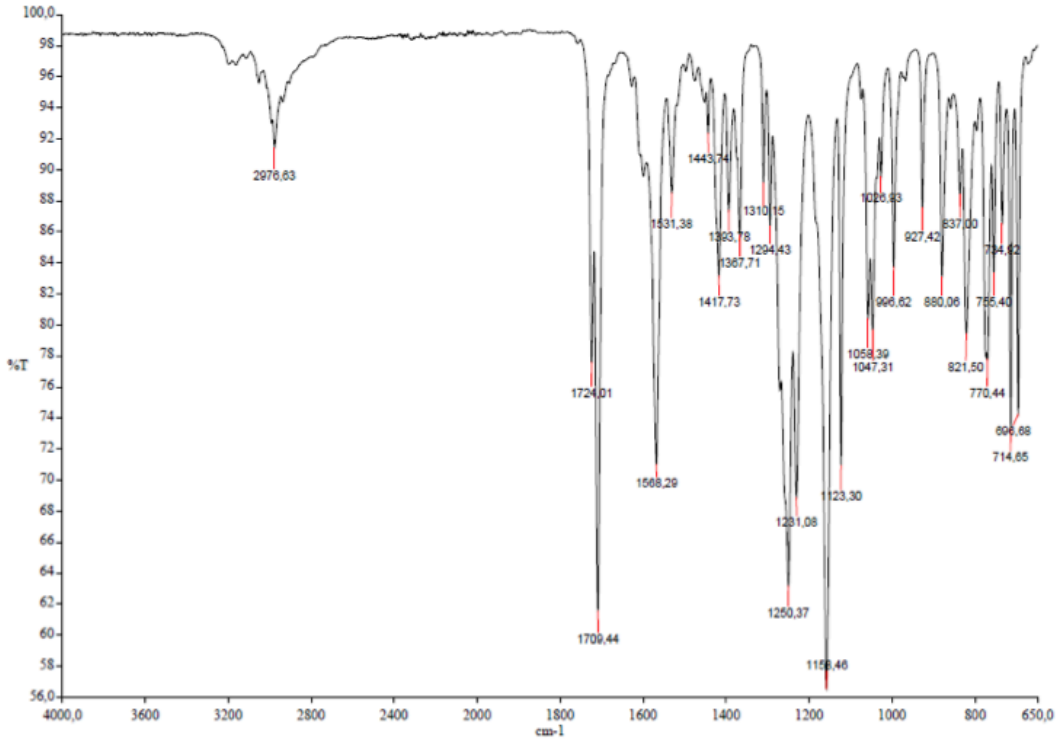
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70
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62
60
58
56,0
%T
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3200
2800
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1600
1400
1200
1000
800
650,0
cm-1
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1724,01
1709,44
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1058,39
1047,31
1026,83
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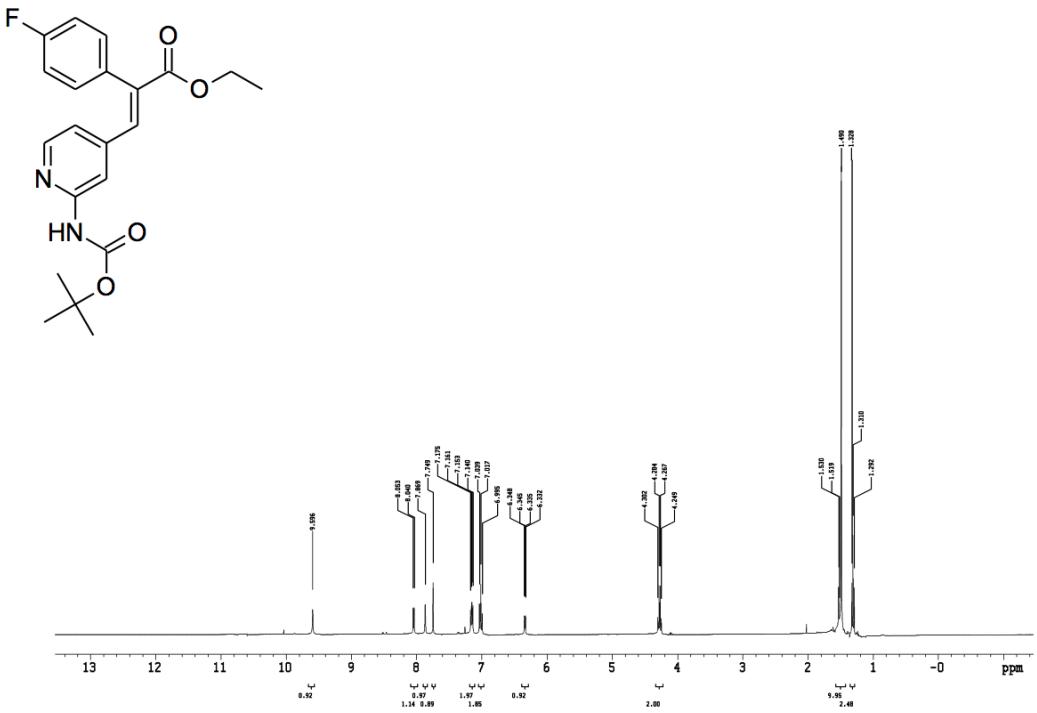
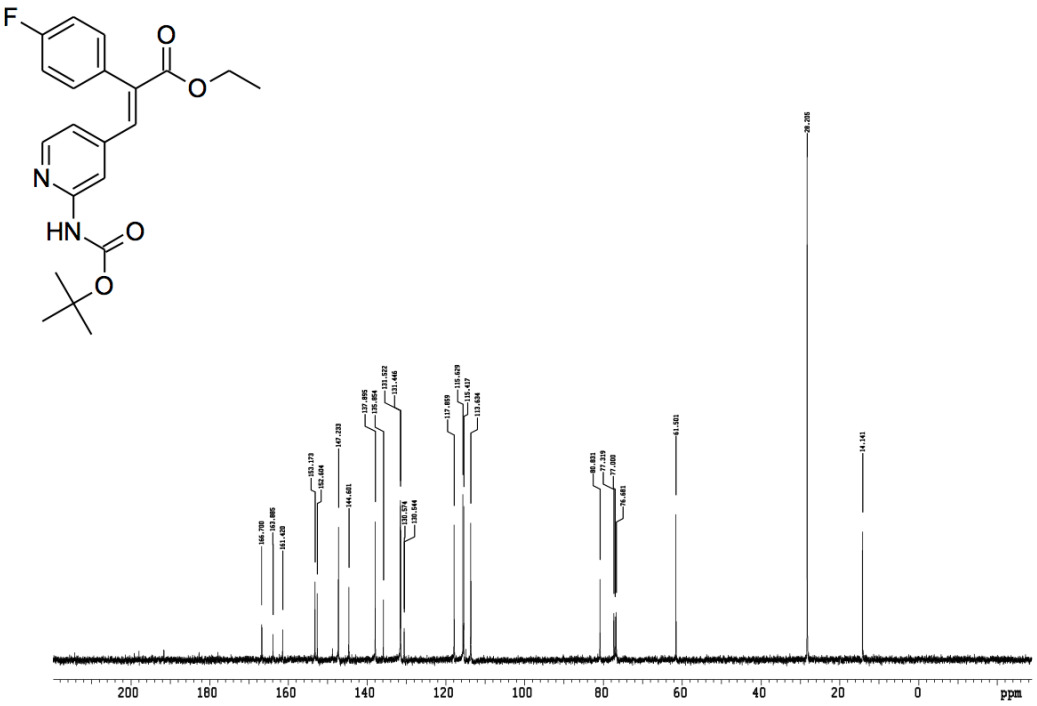
714,65

Compound **14d**

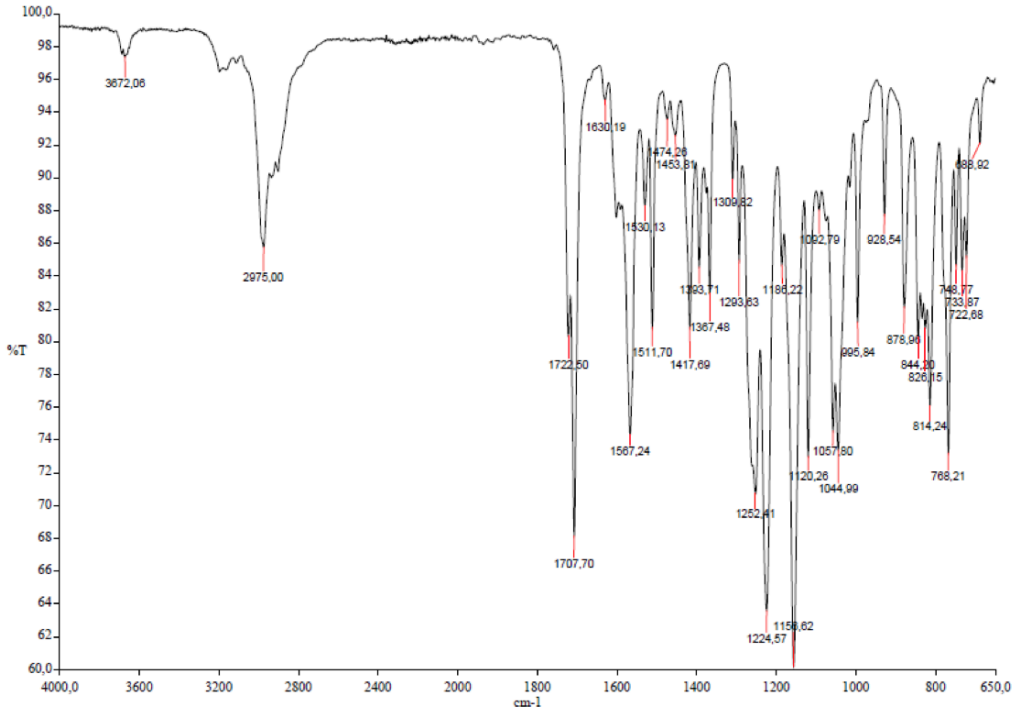
^{1}H NMR



^{13}C NMR



IR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cm-1
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1044,99
995,84
928,54
878,96
844,20
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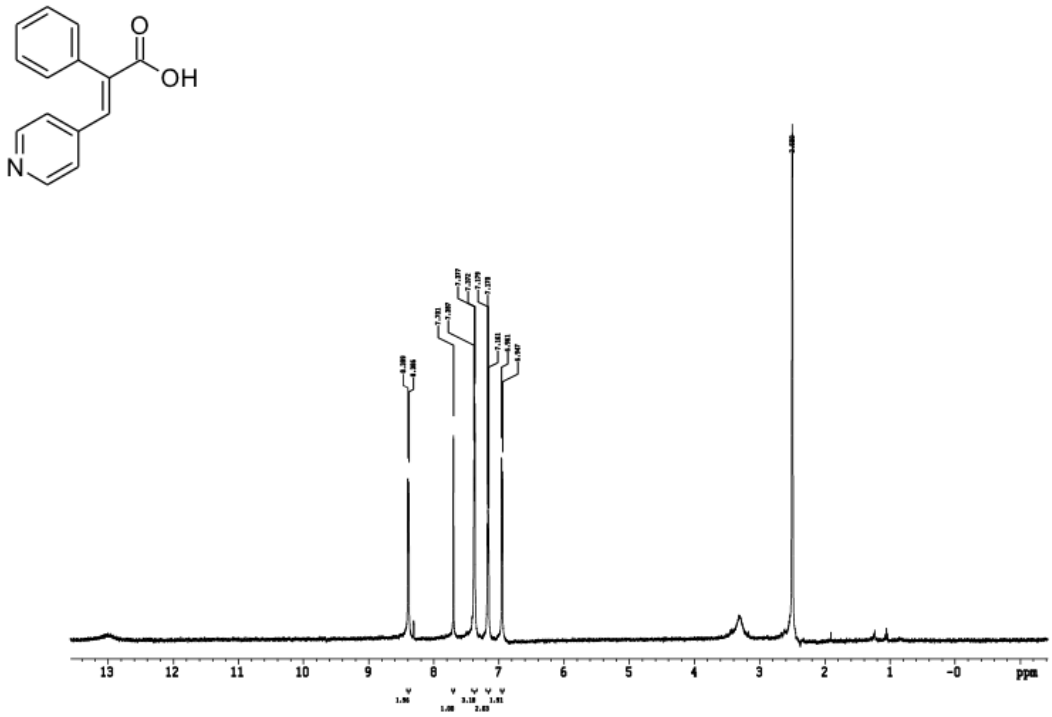
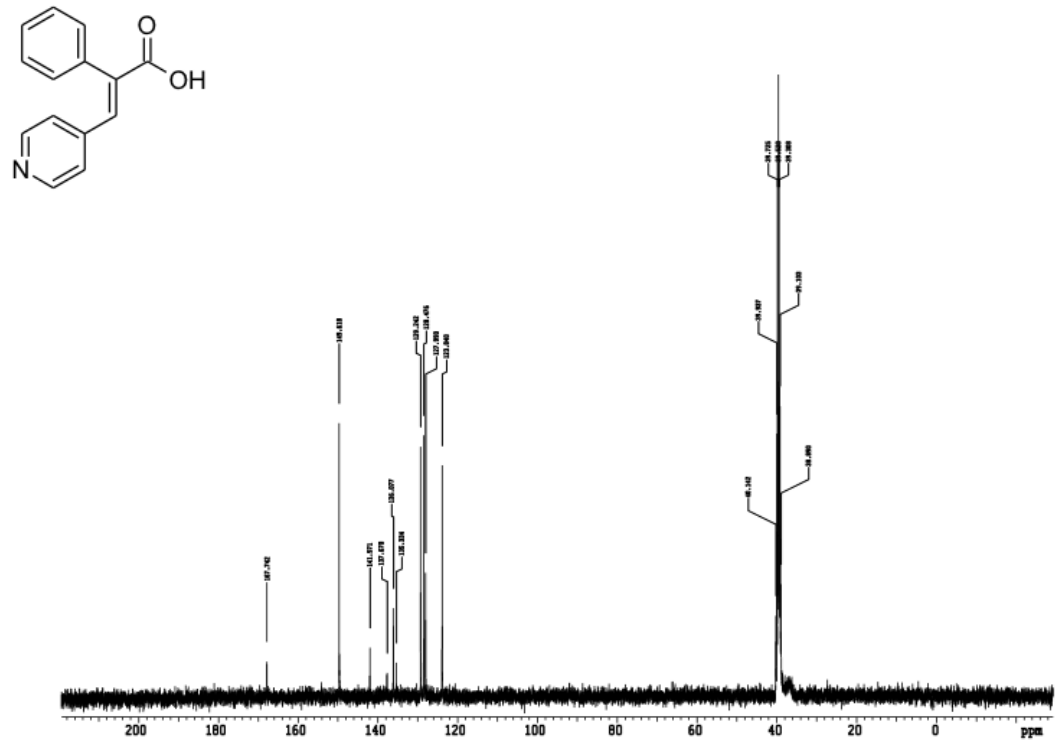
814,24
768,21
748,77
733,87
722,68
688,92

Compound **15a**

^{1}H NMR



^{13}C NMR



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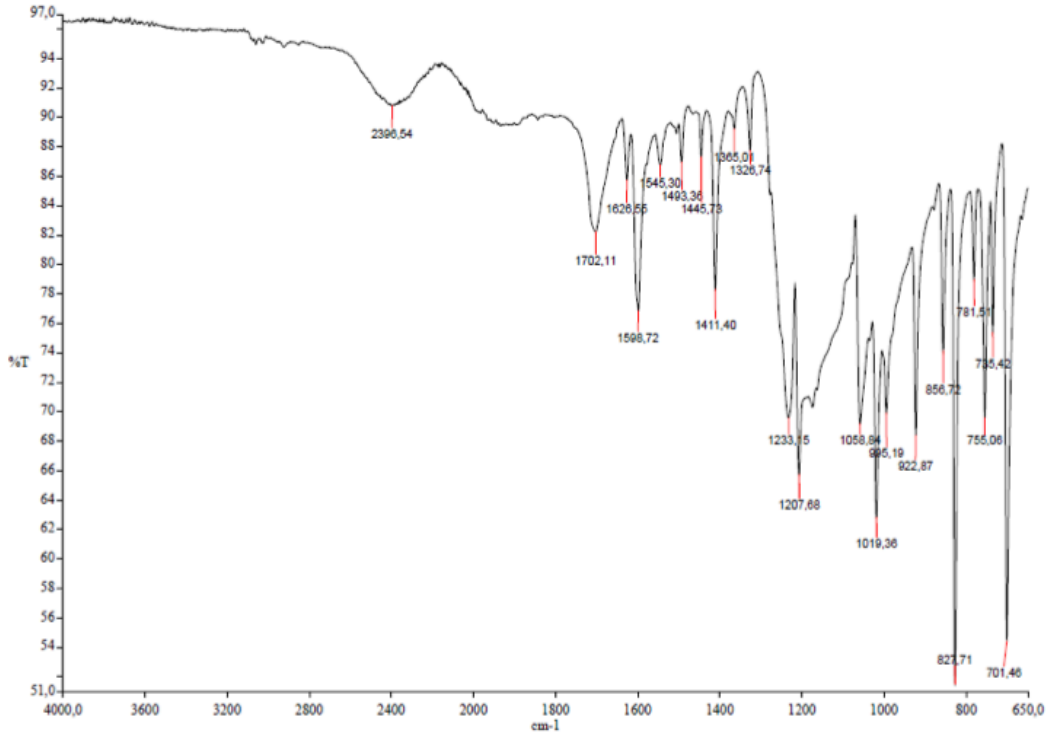
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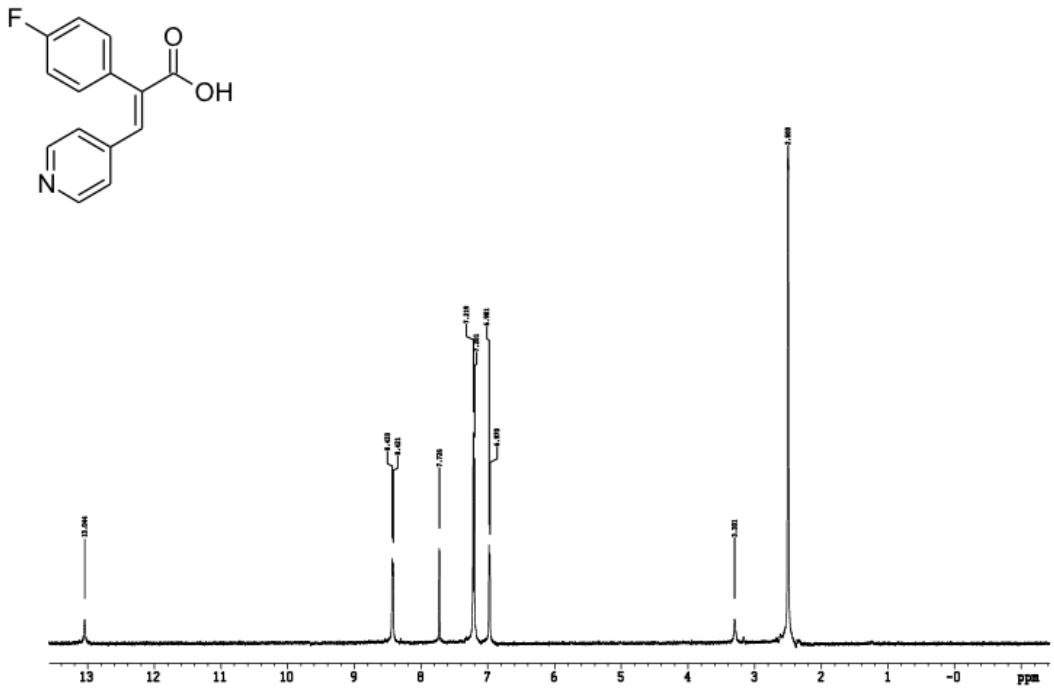
1233,15
1207,68
1058,84
1019,36
995,19
922,87
856,72
823,71
781,51
755,42
755,06
701,46

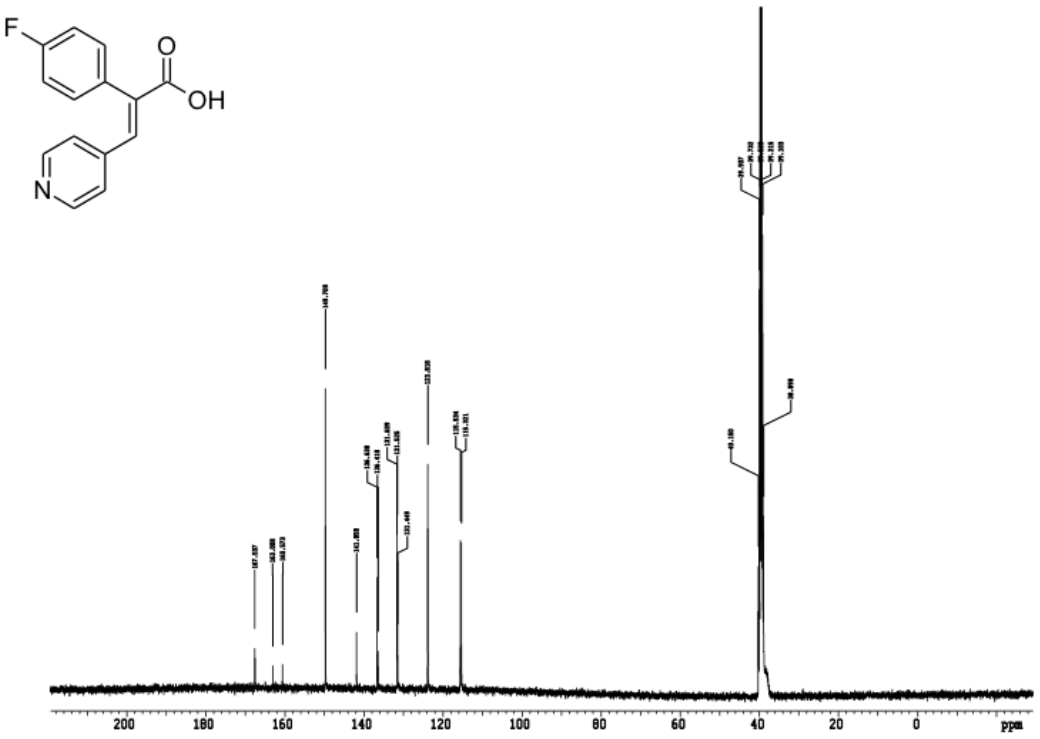
HPLC

Compound **15b**

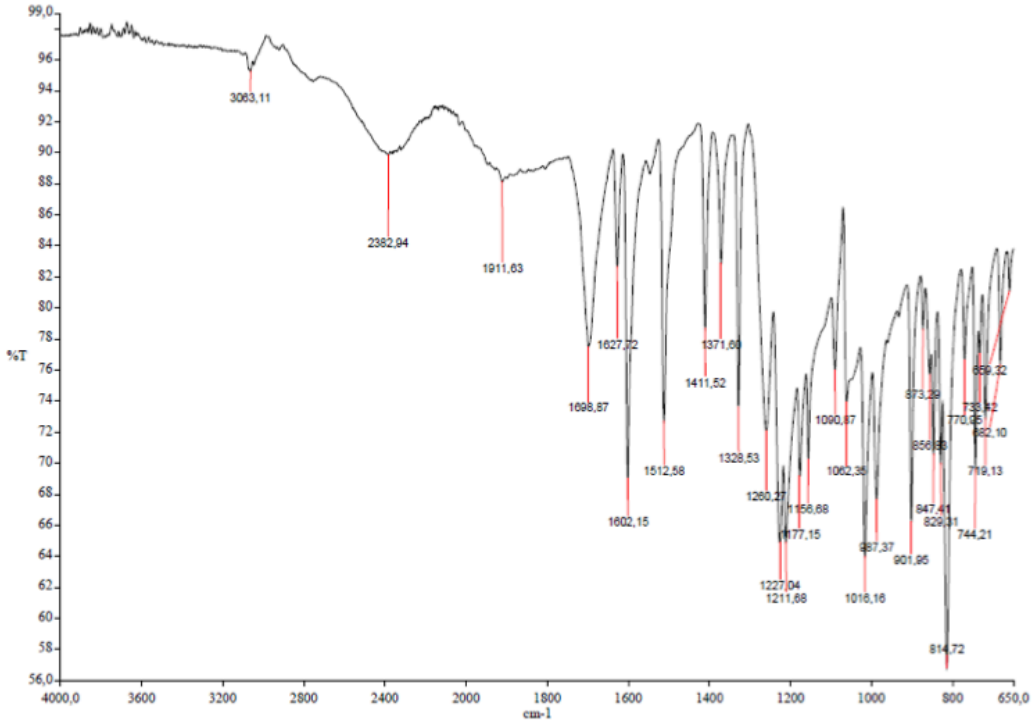
^{1}H NMR



^{13}C NMR



IR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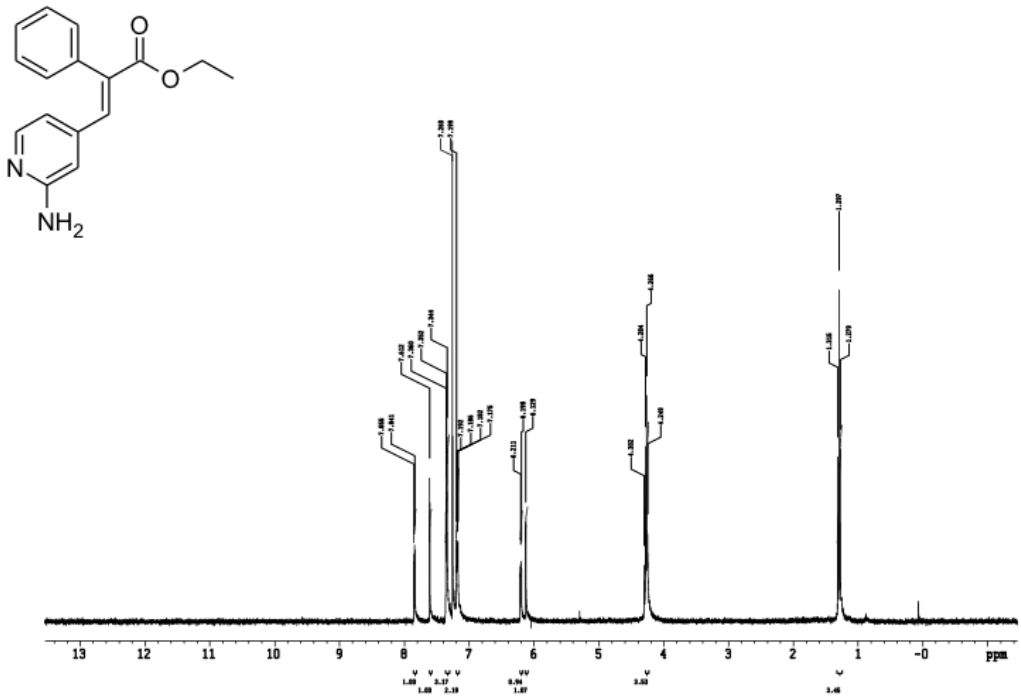
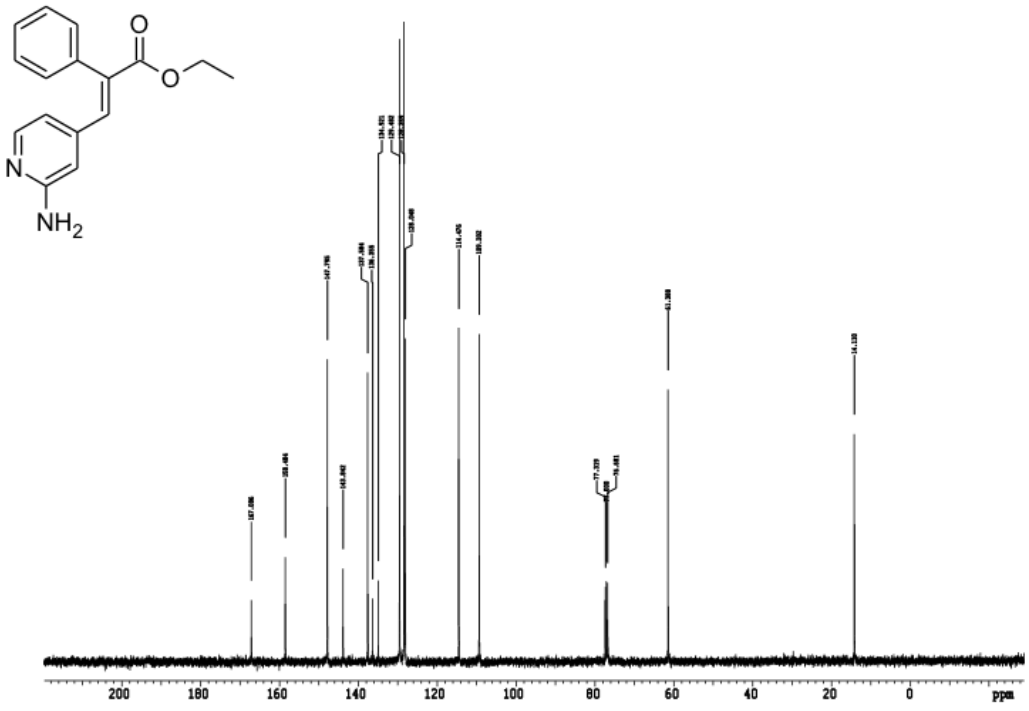
856,68
847,41
829,31
814,72
744,21
719,13
659,32
733,92
770,95
682,10

Compound **16a**

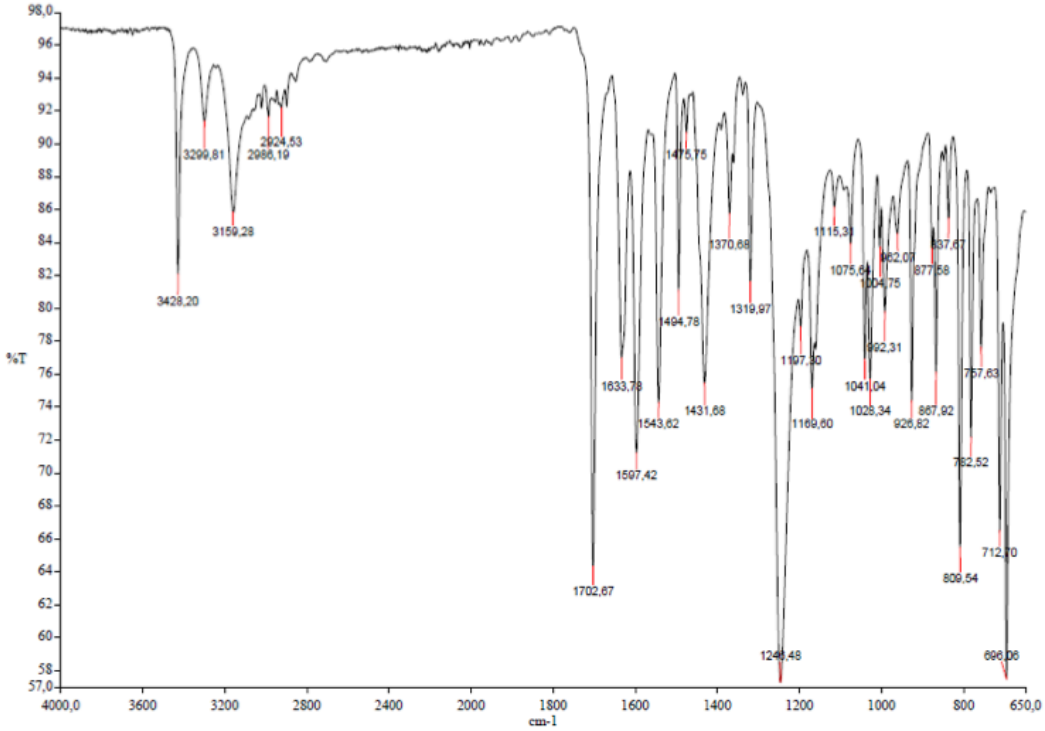
^{1}H NMR



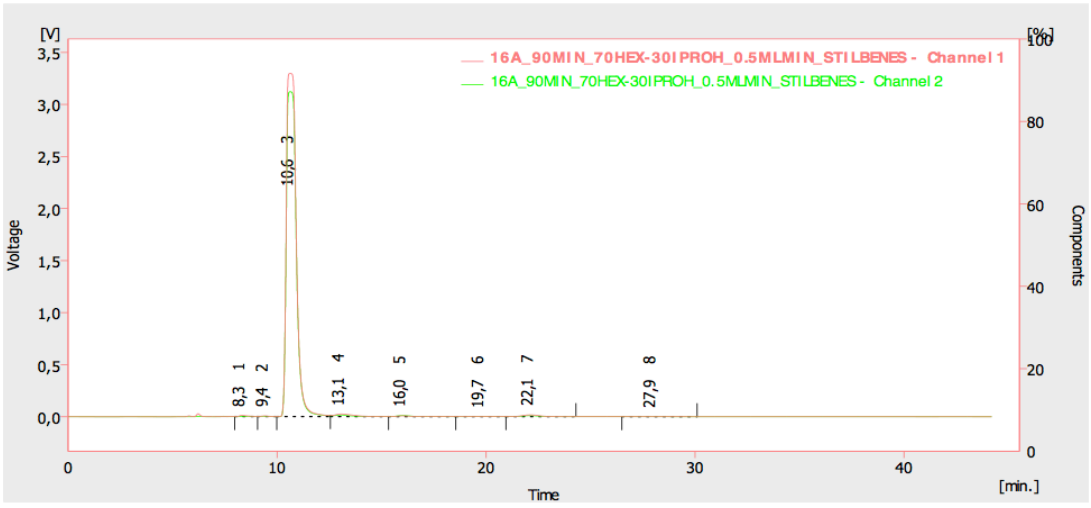
^{13}C NMR



IR



HPLC

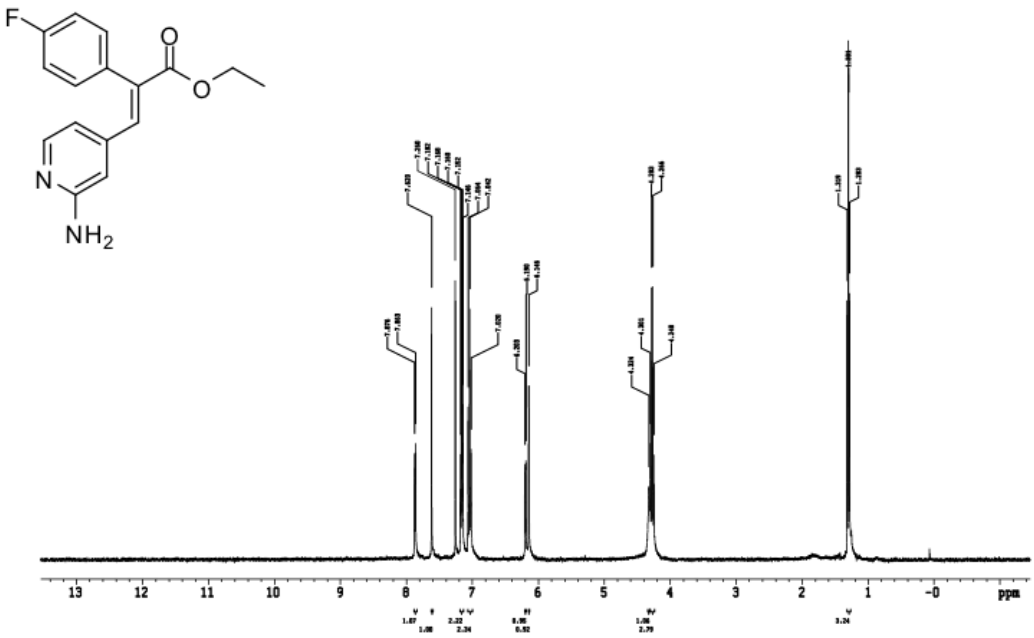


Result Table (Uncal - 16A_90MIN_70HEX-30IPROH_0.5MLMIN_STILBENES - Channel 1)

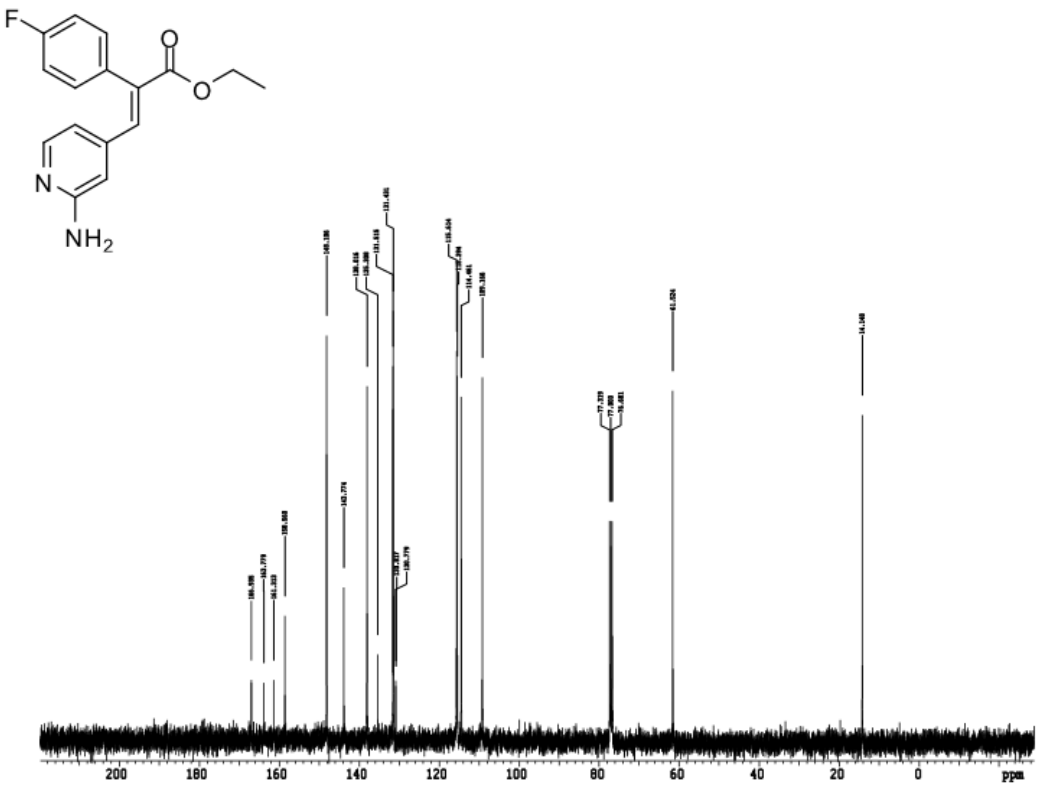
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8,300	342,807	12,164	0,3	0,4	0,42	
2	9,400	167,578	6,593	0,1	0,2	0,39	
3	10,617	109712,482	3297,236	96,7	97,9	0,50	
4	13,050	1638,089	25,173	1,4	0,7	0,99	
5	16,000	422,007	7,466	0,4	0,2	0,67	
6	19,733	100,118	1,172	0,1	0,0	1,35	
7	22,100	977,327	16,961	0,9	0,5	0,87	
8	27,950	60,313	0,556	0,1	0,0	1,75	
	Total	113420,722	3367,322	100,0	100,0		

Compound **16b**

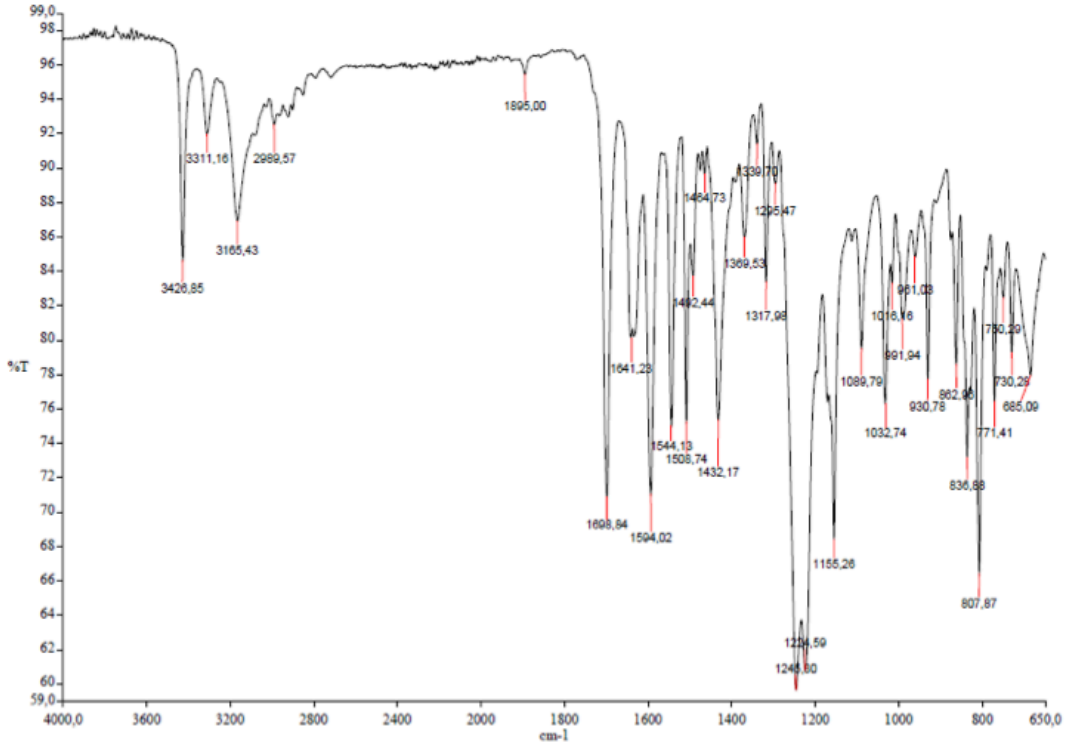
^{1}H NMR



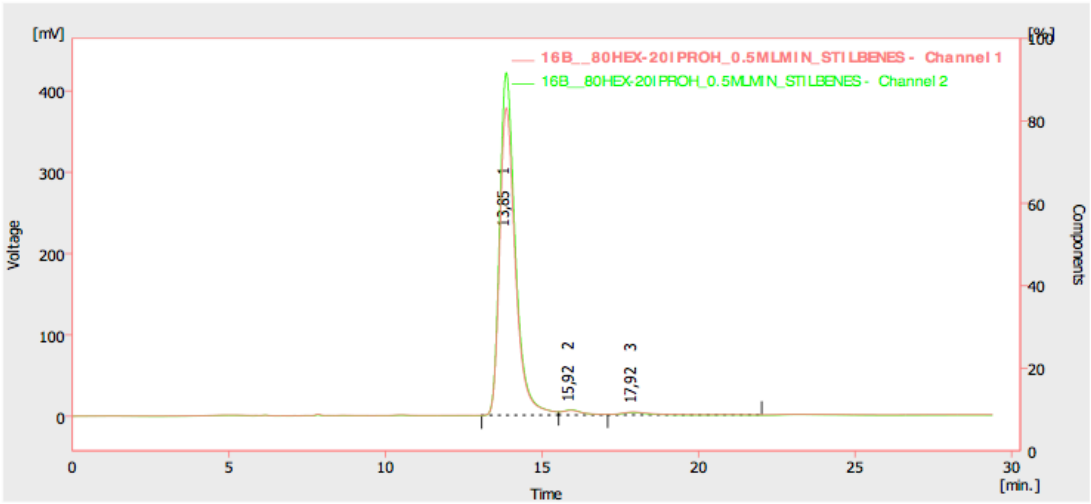
^{13}C NMR



IR



HPLC

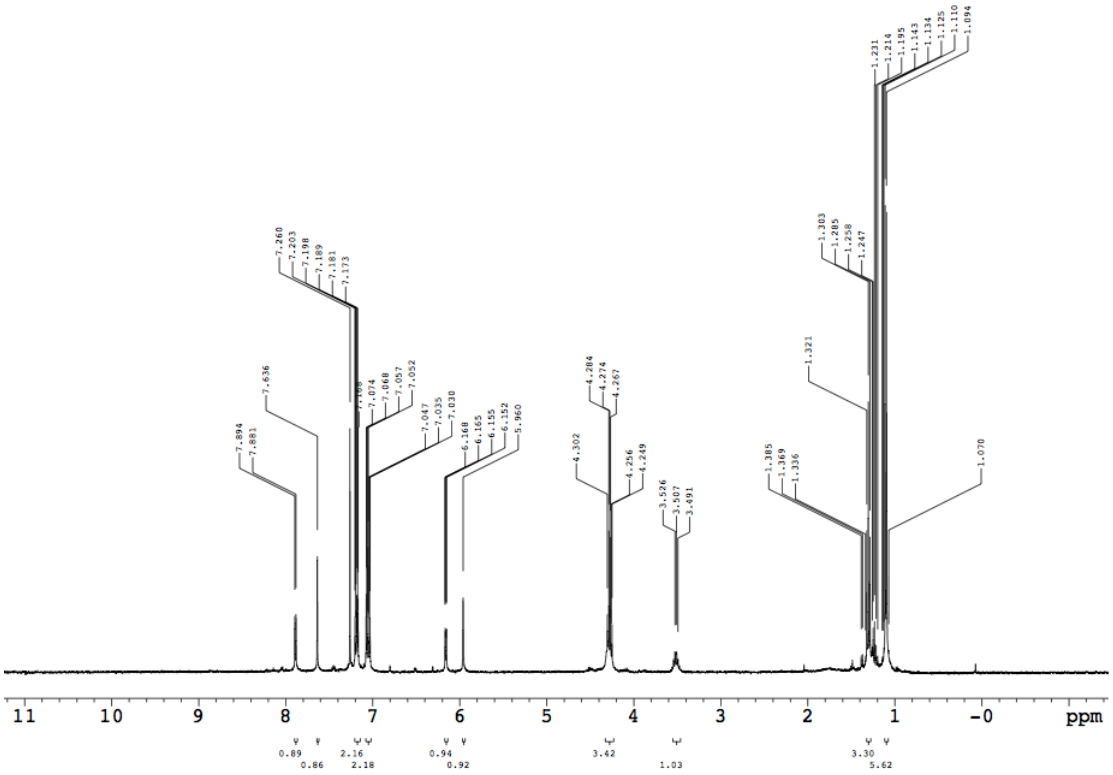


Result Table (Uncal - 16B__80HEX-20IPROH_0.5MLMIN_STILBENES - Channel 1)

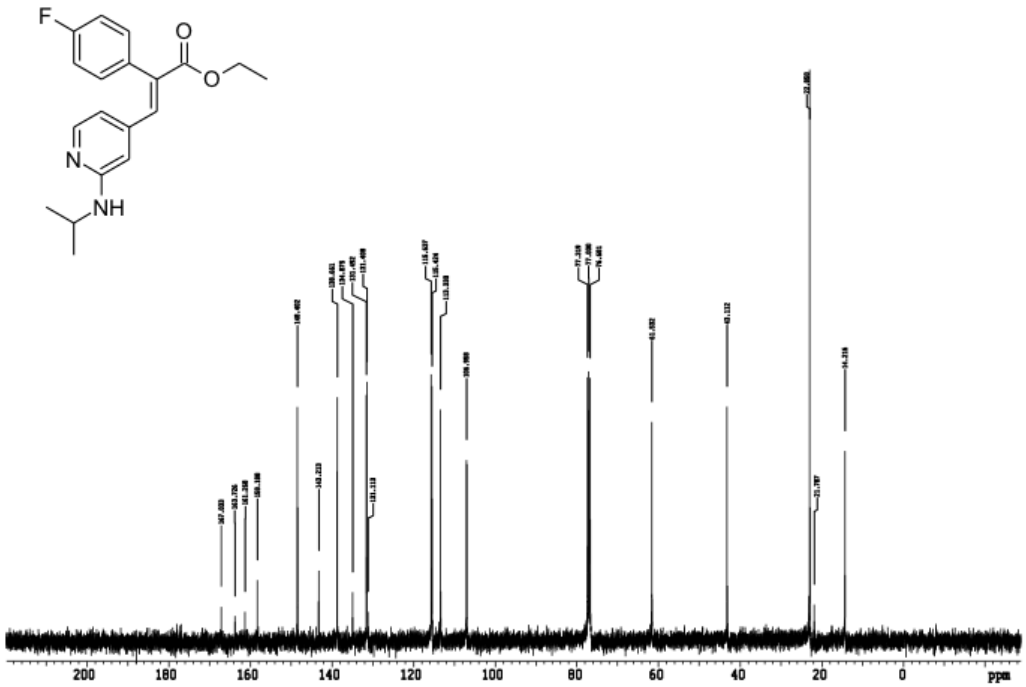
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	13,850	13172,390	377,838	95,8	97,6	0,52	
2	15,917	284,546	5,796	2,1	1,5	0,76	
3	17,917	286,729	3,685	2,1	1,0	0,94	
	Total	13743,666	387,318	100,0	100,0		

Compound **16c**

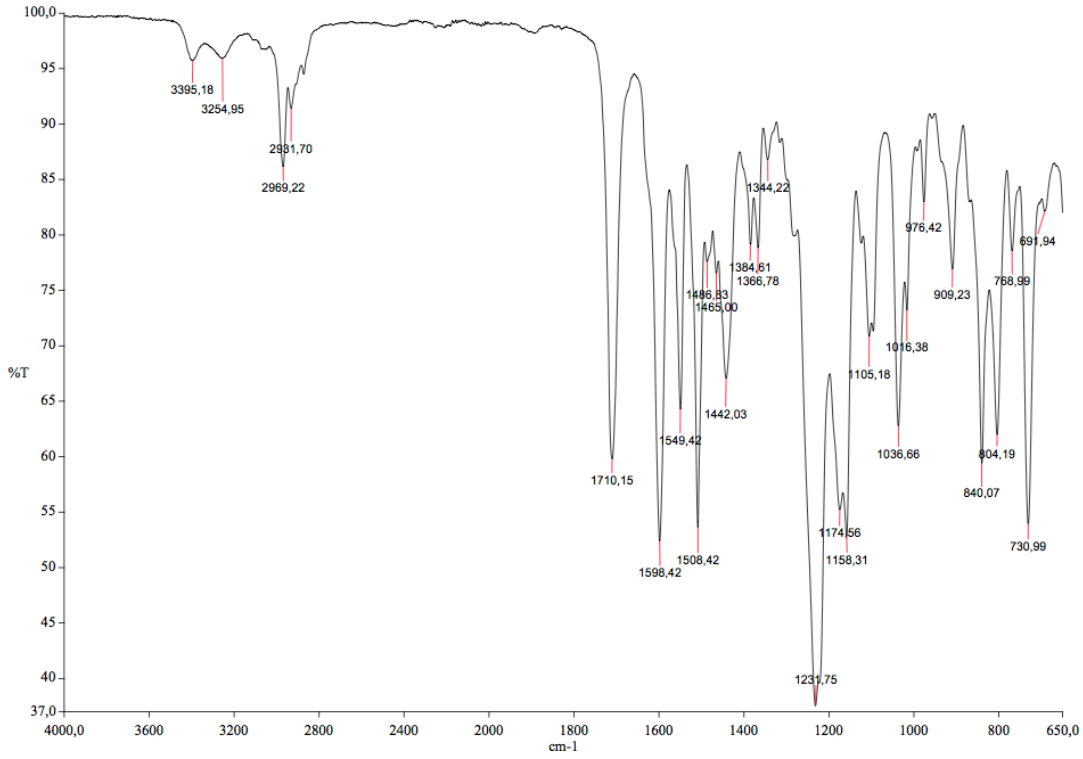
^{1}H NMR



^{13}C NMR

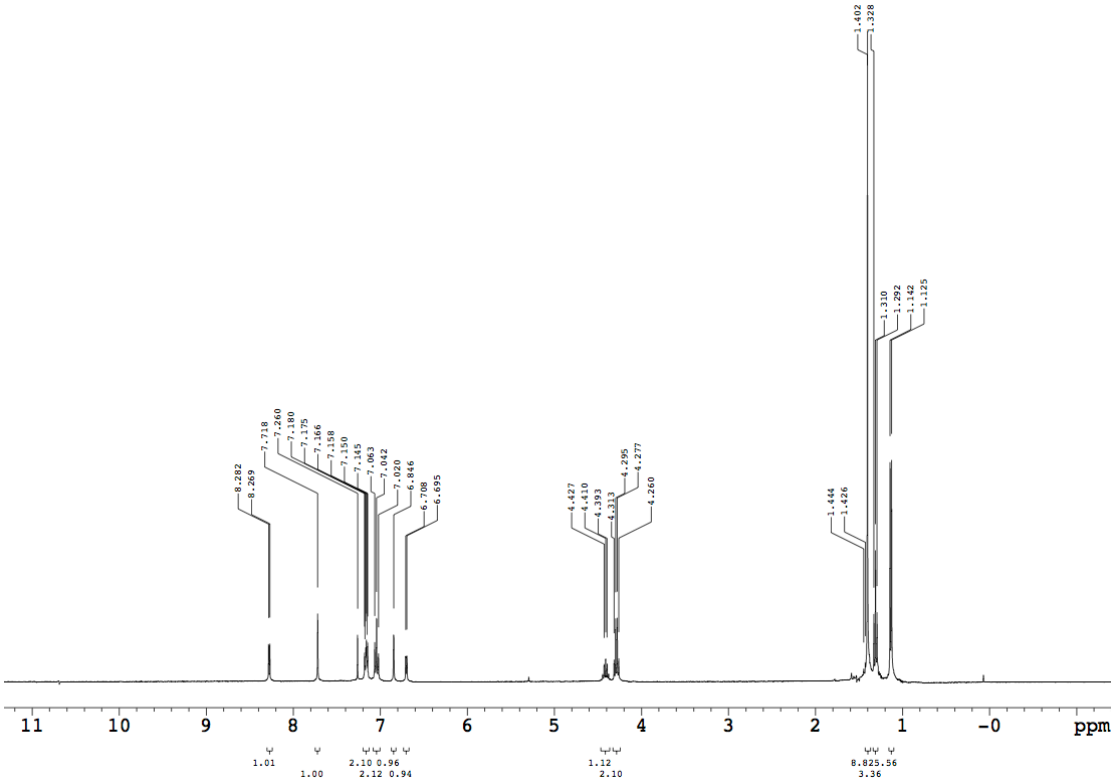


IR

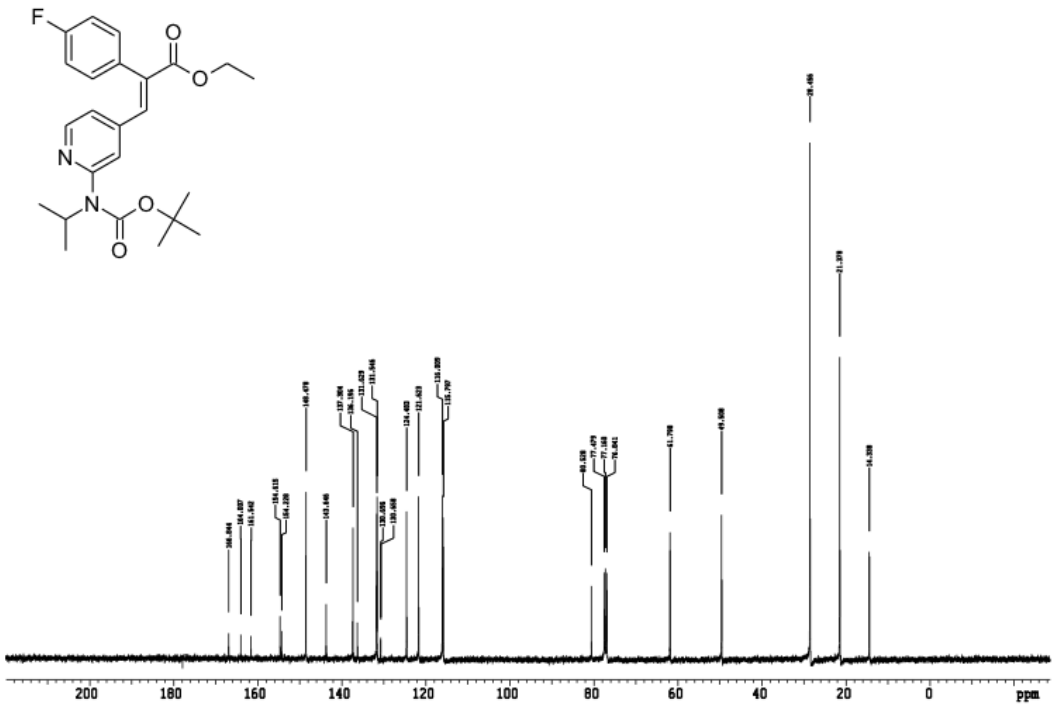

100,0
95
90
85
80
75
70
65
60
55
50
45
40
37,0
%T
4000,0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
650,0
cm-1
3395,18
3254,95
2981,70
2969,22
1710,15
1598,42
1549,42
1508,42
1486,83
1465,00
1442,03
1384,61
1366,78
1344,22
1231,75
1174,56
1158,31
1105,18
1036,66
1016,38
976,42
909,23
840,07
804,19
768,99
730,99
691,94

Compound **17**

^{1}H NMR



^{13}C NMR



IR