Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Development of thiazole appended novel hydrazones as a new class of *α*-amylase inhibitors with anticancer assets: An *in silico* and *in vitro* approach

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Table S 1:2D NMR correlation of ***5aa***

Proton	Chemical shift	Carbon	Chemical shift	Cosy	HMBC	HSQC	Noesy
H17	8.59	C17	149.26	(8.59, 7.38), (8.59, 8.09, (8.59, 7.84)	(8.59, 124.80), (8.59, 137.23)	(8.59, 149.26)	
H16	7.38	C16	124.80	(7.38, 8.59), (7.38, 8.09)	(7.38, 149.26)	(7.38, 124.80)	
H15	7.84	C15	137.23	(7.84, 8.09), (7.84,8.59)	(7.84, 154.10)	(7.84, 137.23)	
H14	8.09	C14	120.95	(8.09, 8.59), (8.09, 7.38), (8.09, 7.84)	(8.09, 124.80)	(8.09,120.95)	
		C13	154.10				
		C11	144.65				
		C10	154.10				
		C4	125.35				
H3a	7.35	C3	129.35		(7.35, 129.35), (7.35, 140.04)		
H2	7.4	C2	119.43	(7.35, 7.75)	(7.4, 119.43)		
		C1	140.04				
H6	7.75	C6	119.43	(7.75, 7.31), (7.75, 7.35)	(7.75, 119.43),	(7.75, 119.43)	(7.75, 10.57)
H5	7.31	C5	129	(7.31, 7.75)		(7.31, 129)	
H12	2.37	C12				(2.37,149.26) (2.37, 154.0)	(2.37, 10.41)
NH	10.57						(10.57, 2.37)
NH	10.44						(10.44, 2.37)

Supplementary information

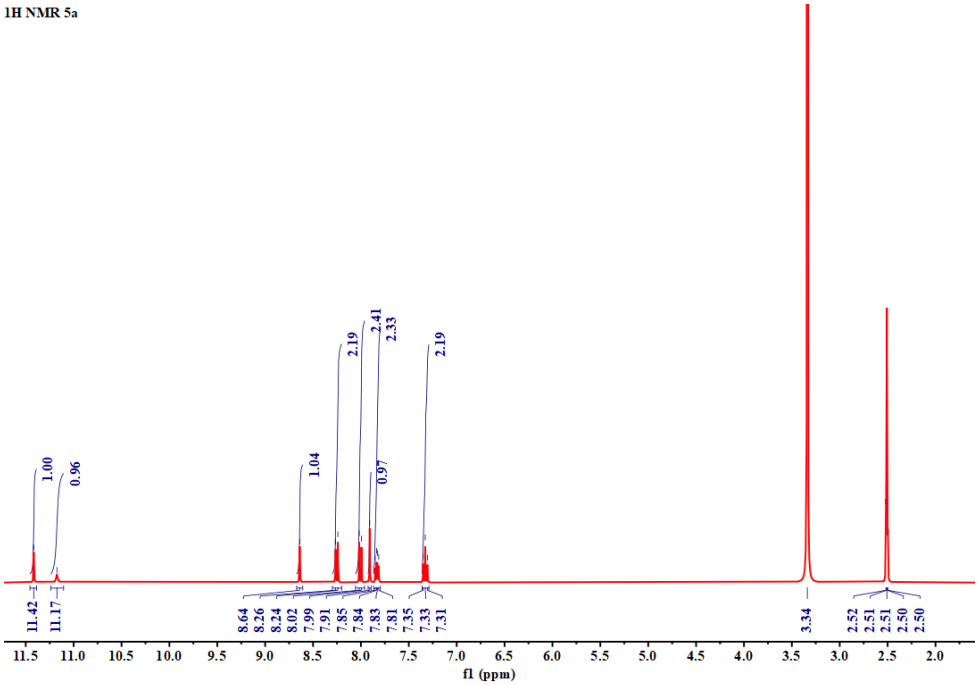


*Figure S1: [1]H NMR spectra of **5a***

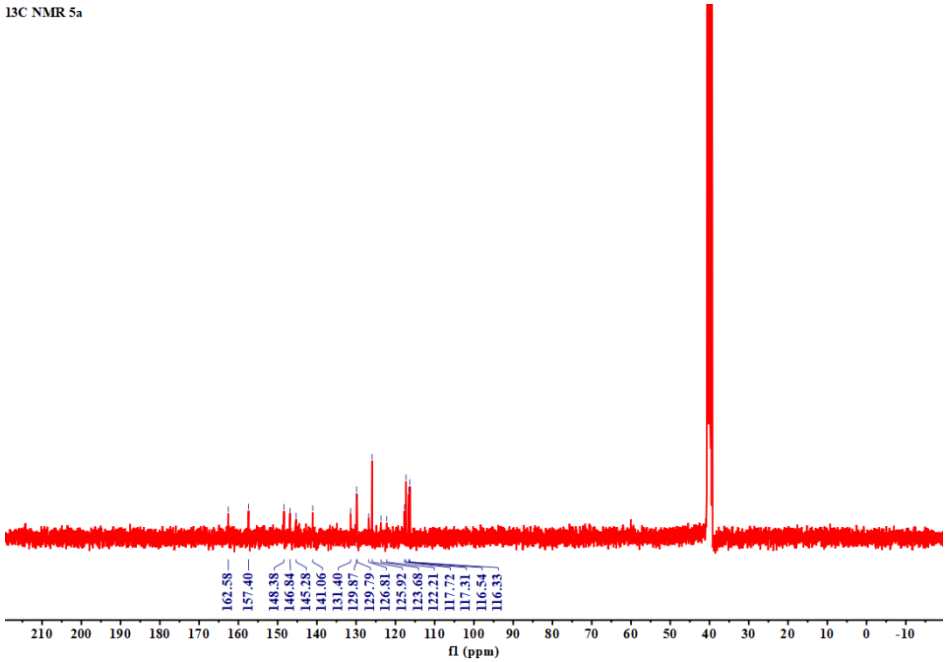


*Figure S2: [13]C NMR spectra of **5a***

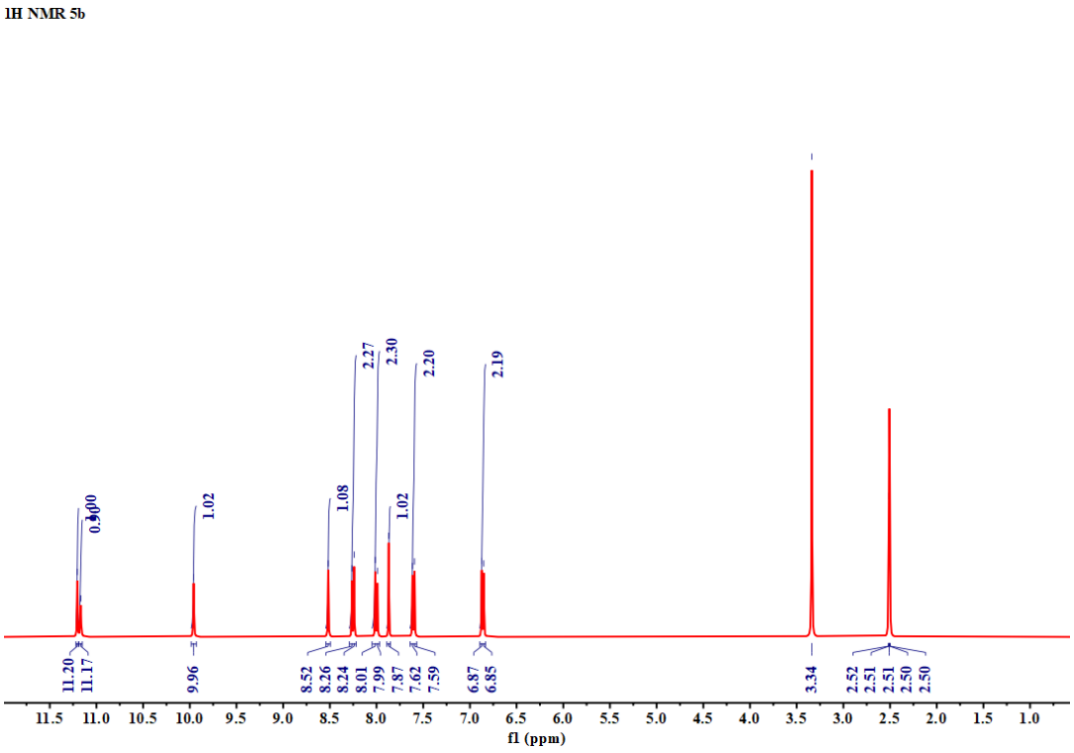


*Figure S3: [1]H NMR spectra of **5b***

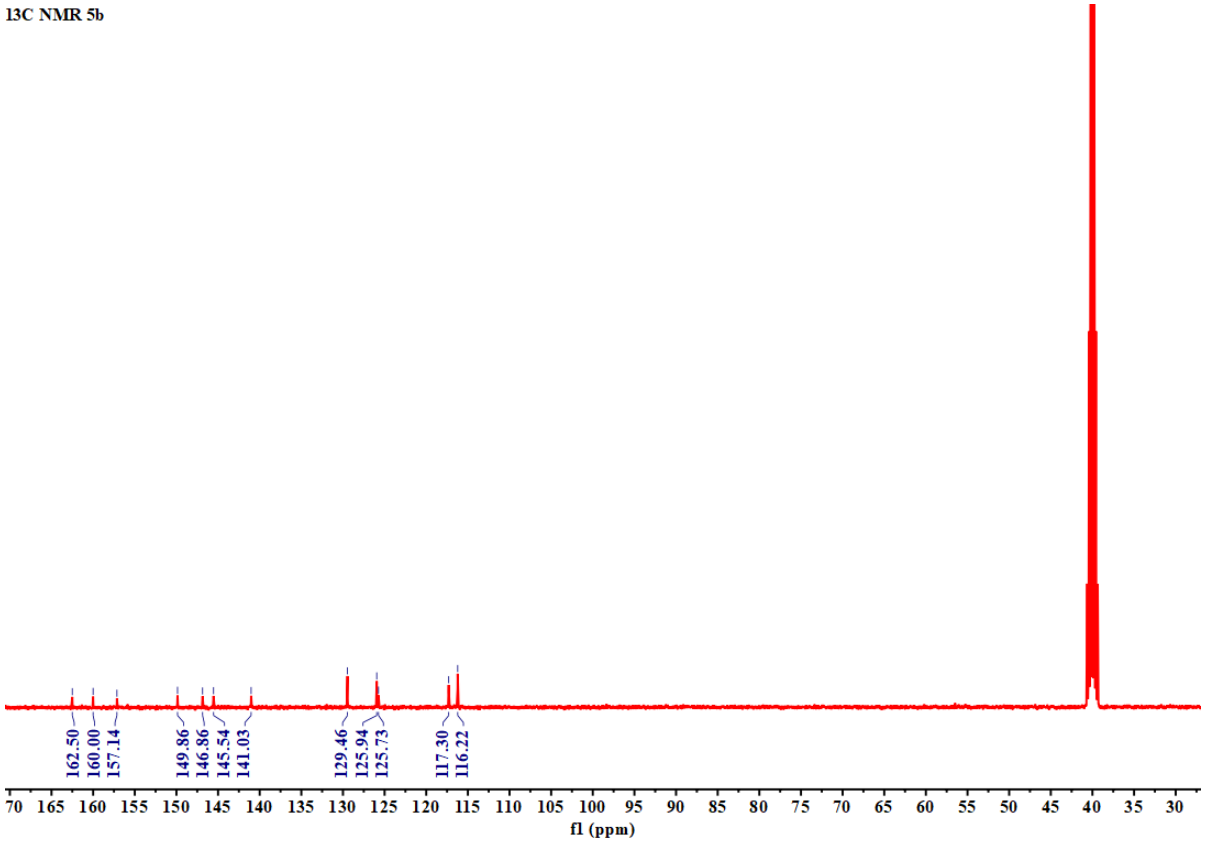


*Figure S4: [13]C NMR spectra of **5b***

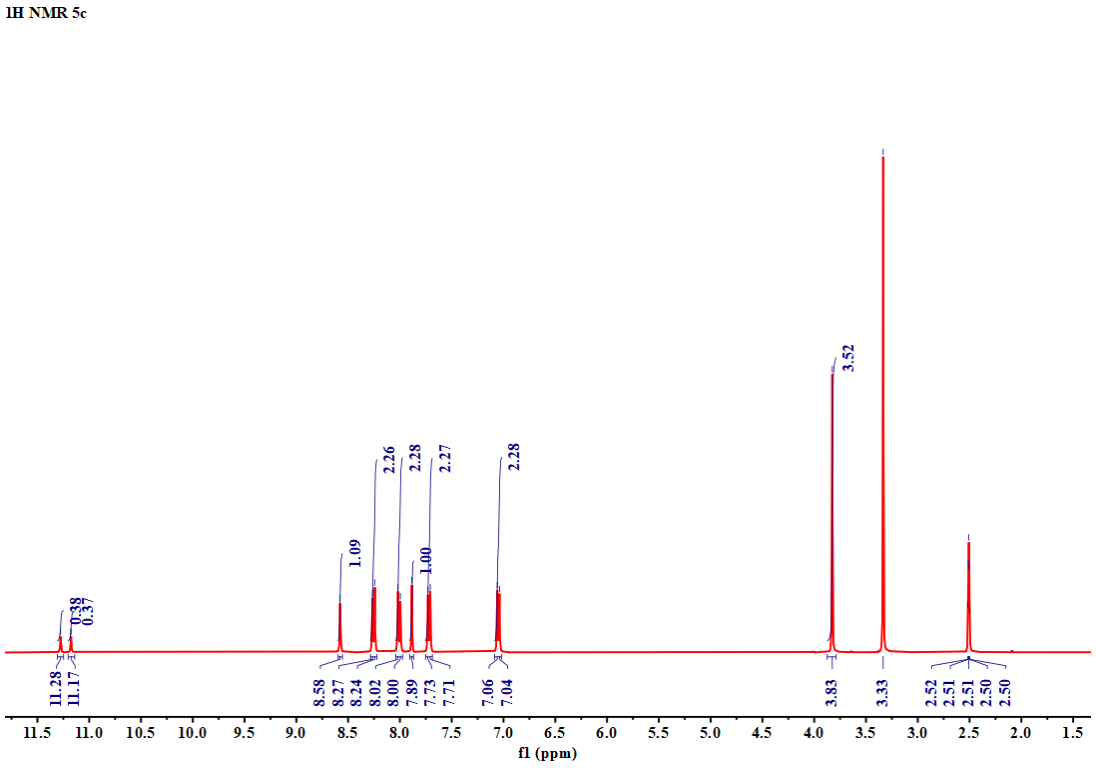


*Figure S5: [1]H NMR spectra of **5c***

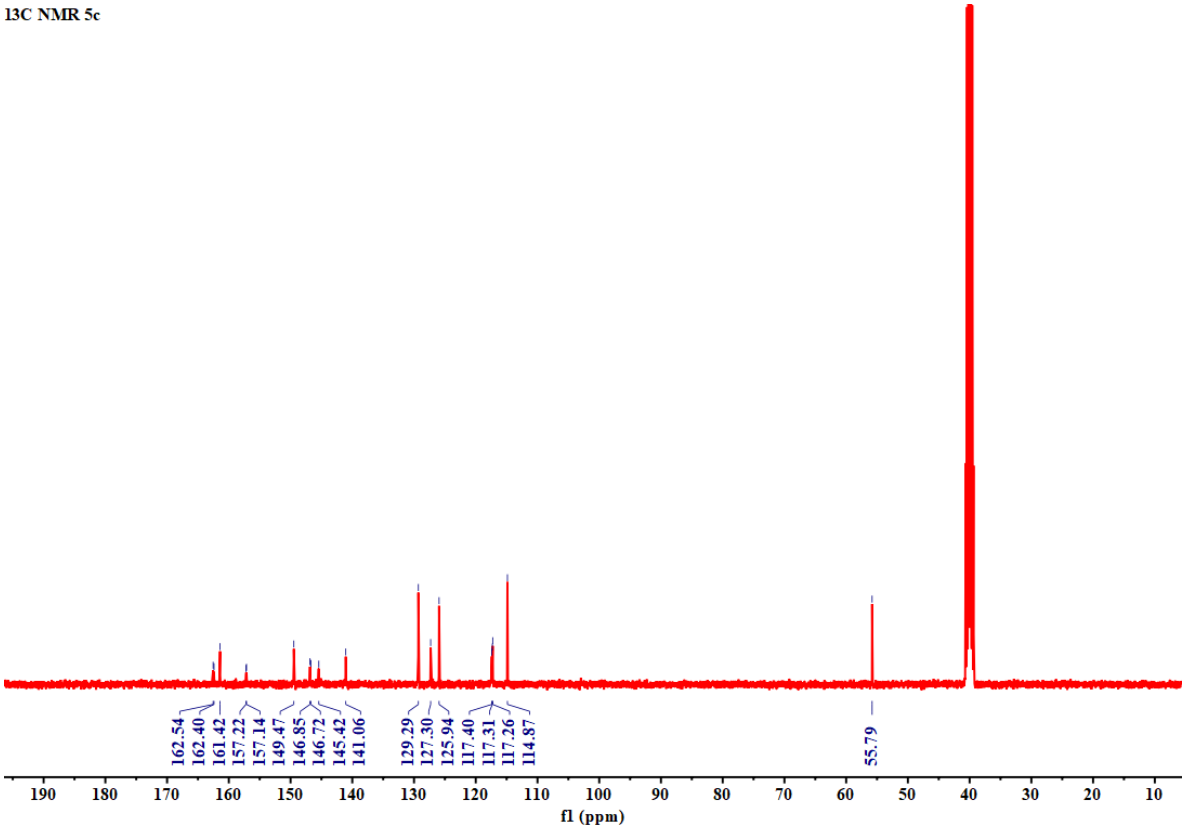


*Figure S6: [13]C NMR spectra of **5c***

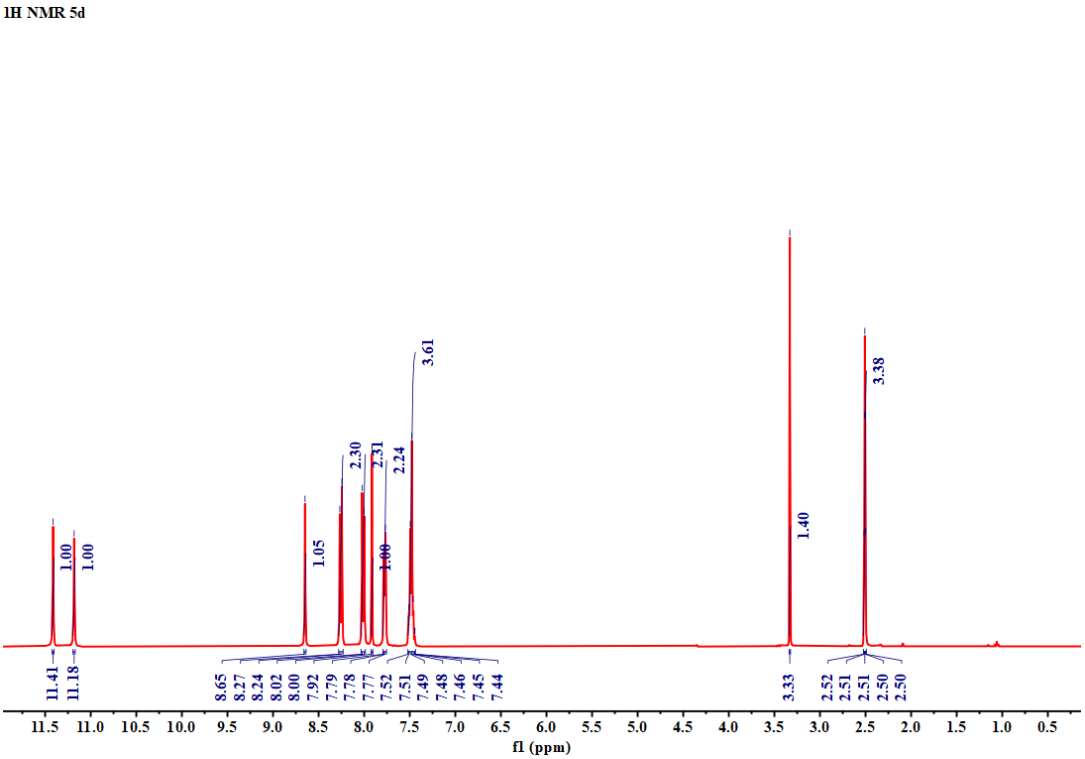


*Figure S7: [1]H NMR spectra of **5d***

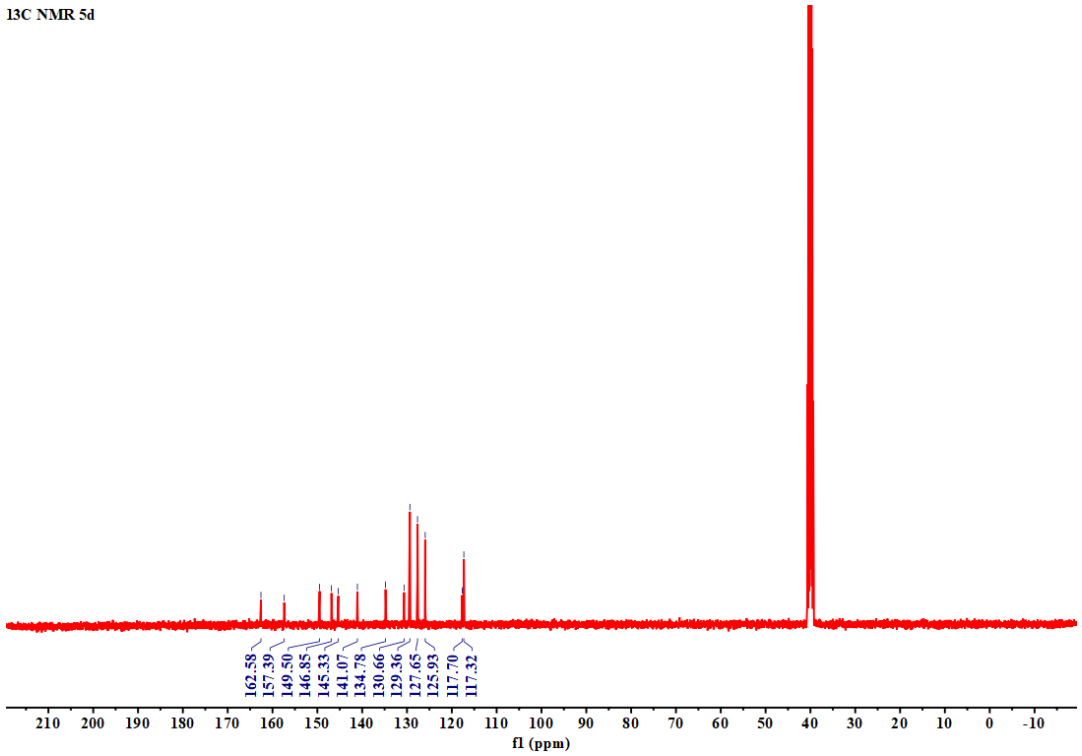


*Figure S8: [13]C NMR spectra of **5d***

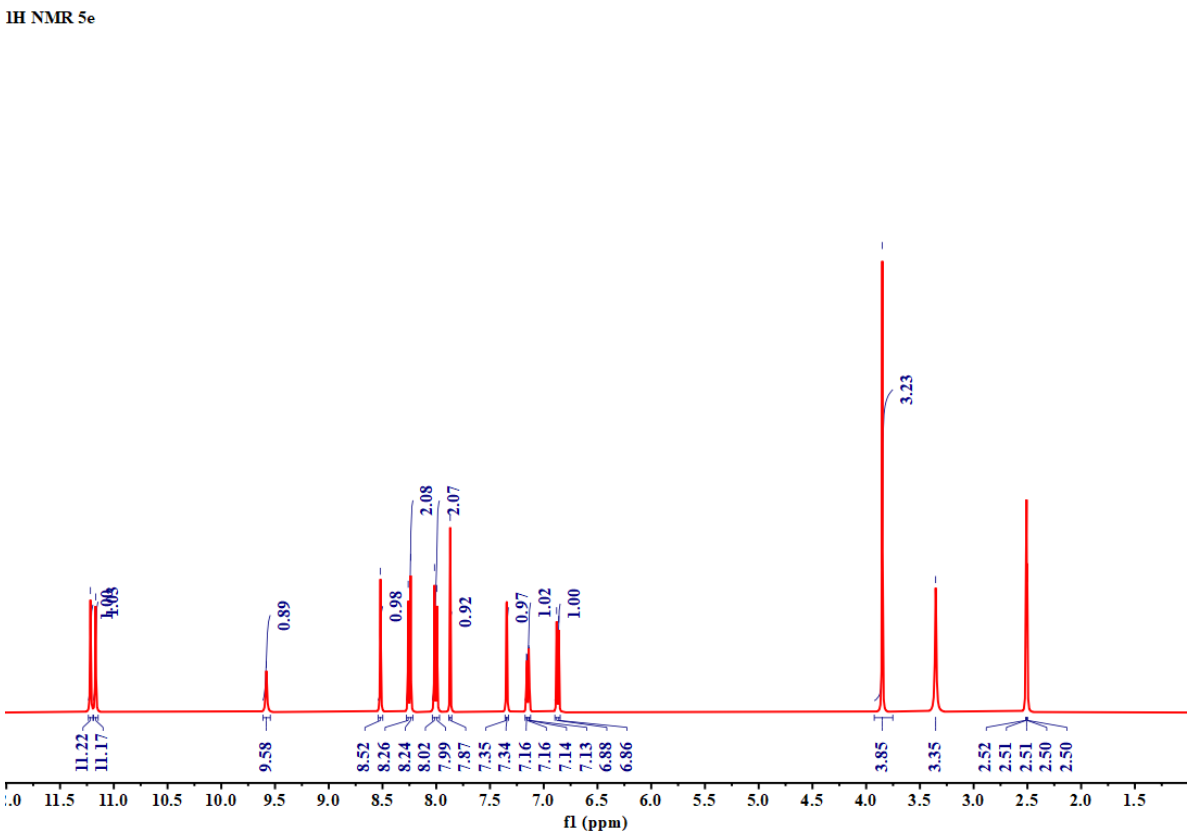


*Figure S9: [1]H NMR spectra of **5e***

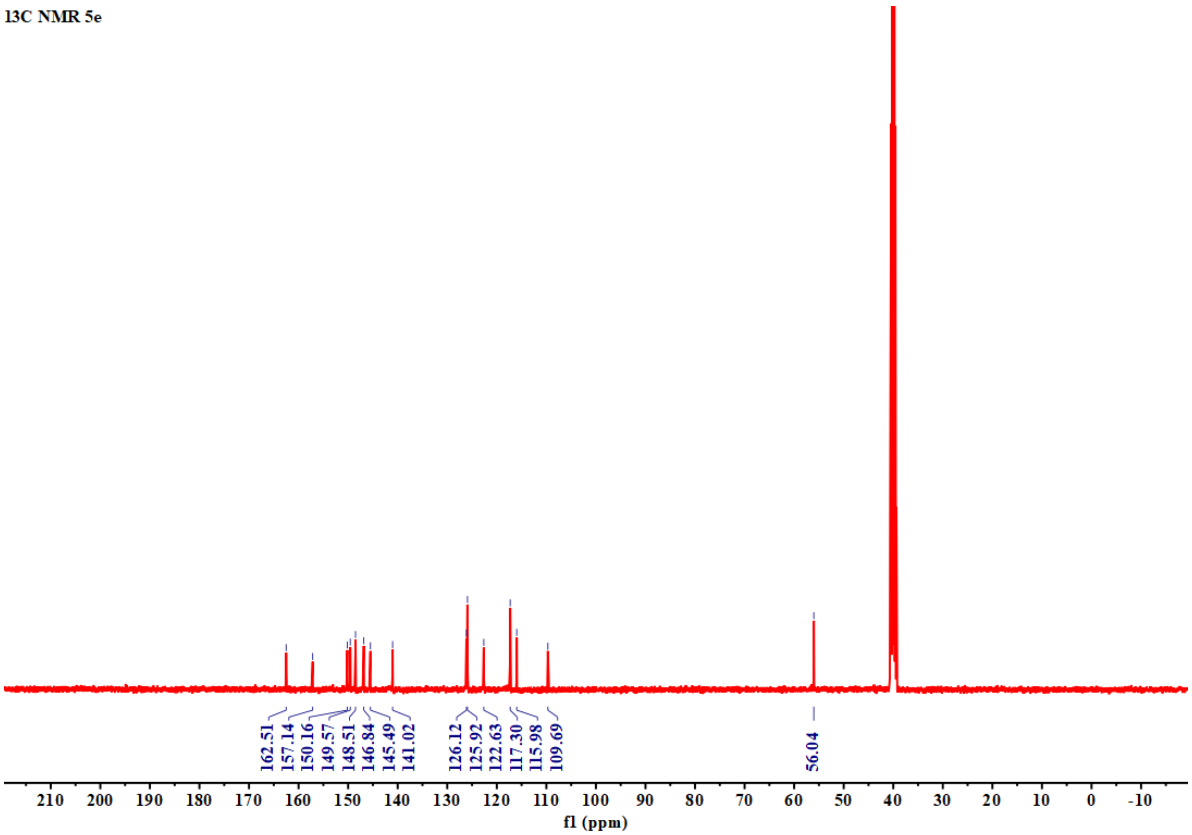


*Figure S10: [13]C NMR spectra of **5e***

1H NMR 5g

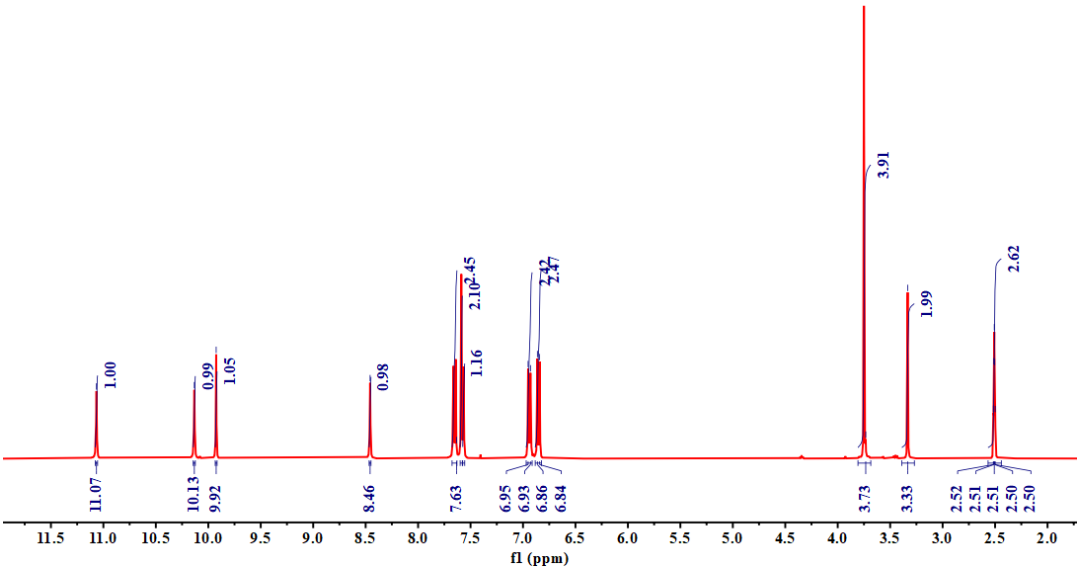


*Figure S11: ^{1}H NMR spectra of **5g***

13C NMR 5g

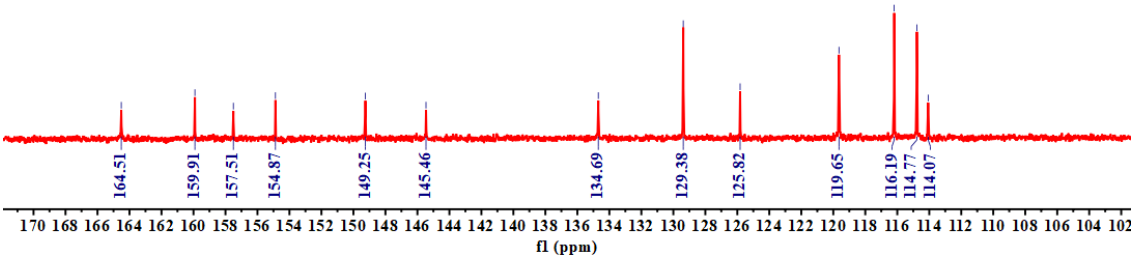


*Figure S12: ^{13}C NMR spectra of **5g***

1H NMR 5i

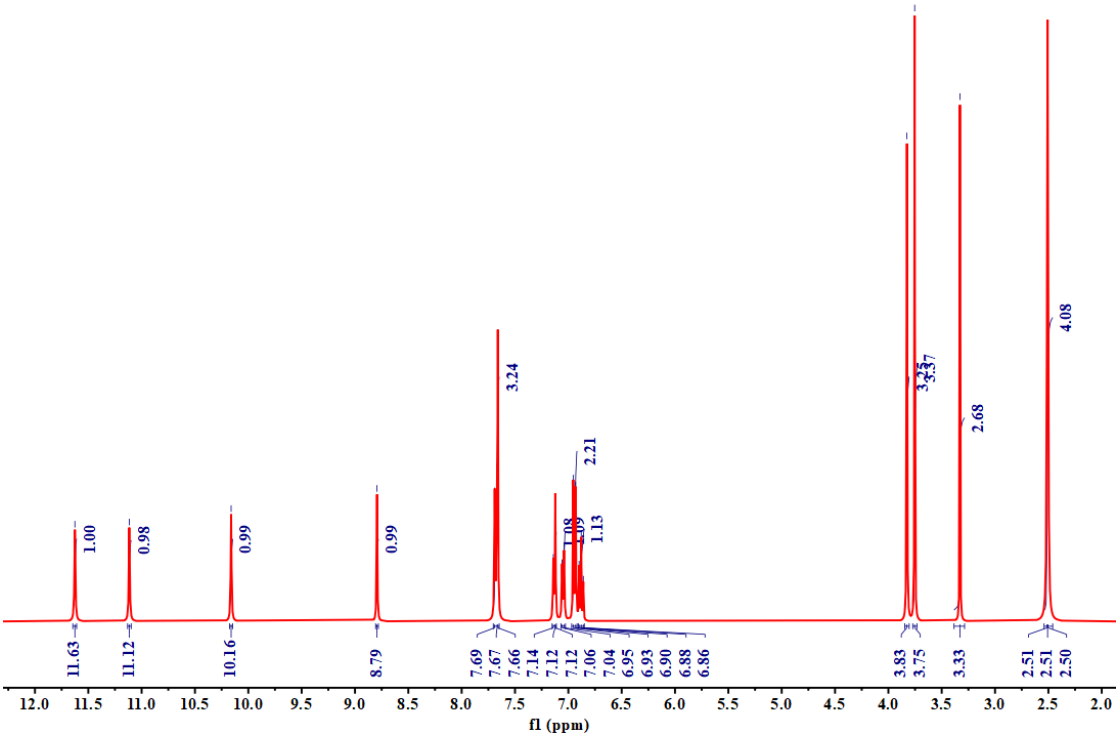


*Figure S13: [1]H NMR spectra of **5i***

13C NMR 5i

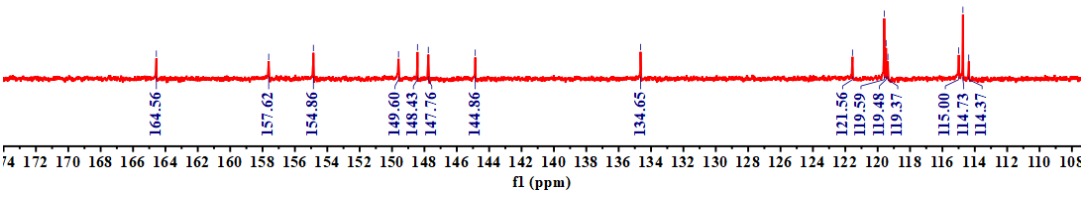


*Figure S14: [13]C NMR spectra of **5i***

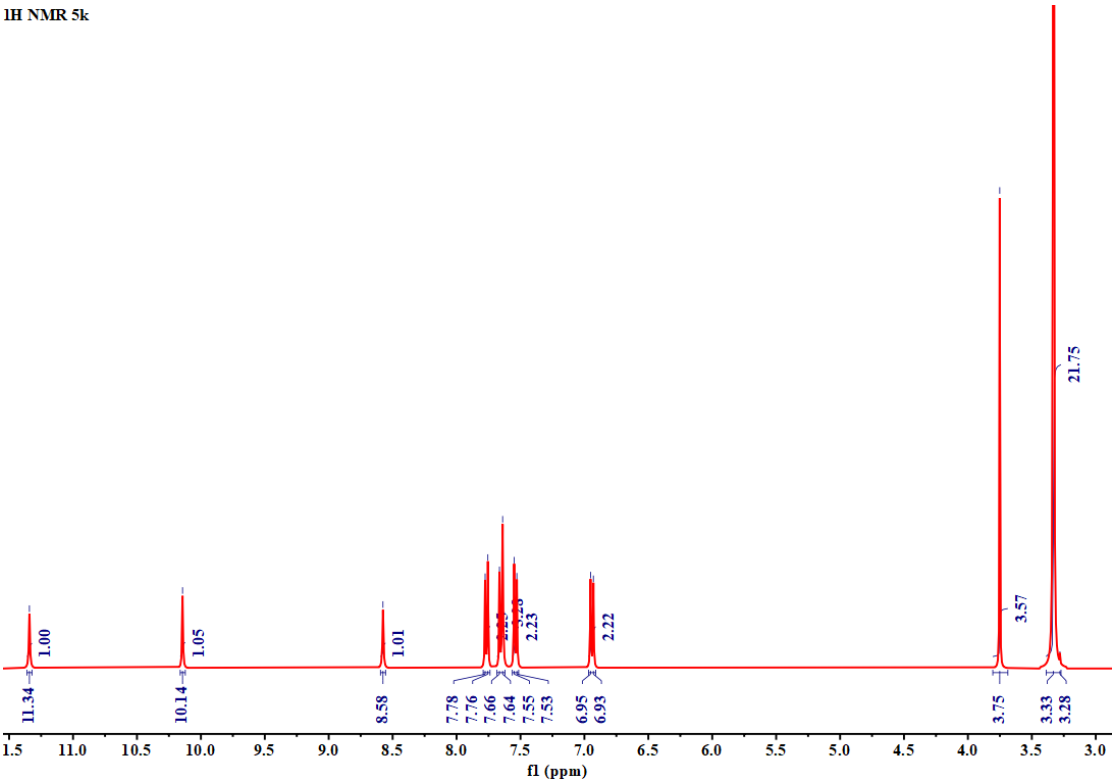


*Figure S15: [1]H NMR spectra of **5k***

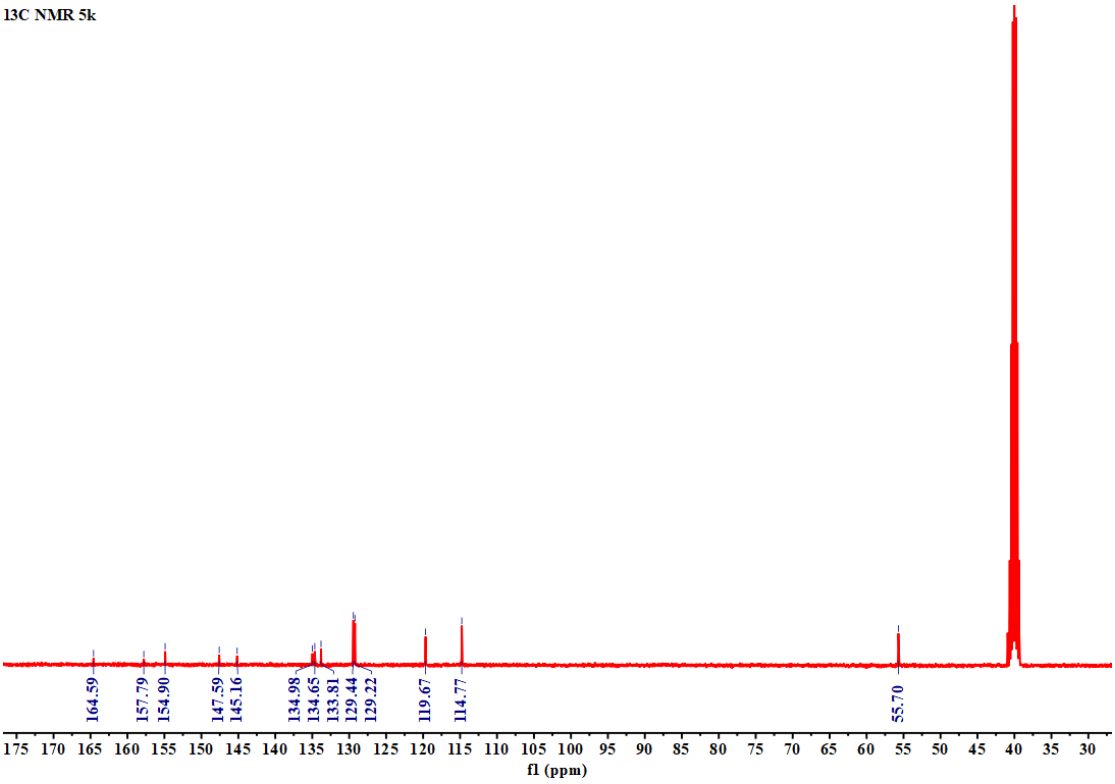


*Figure S16: [13]C NMR spectra of **5k***

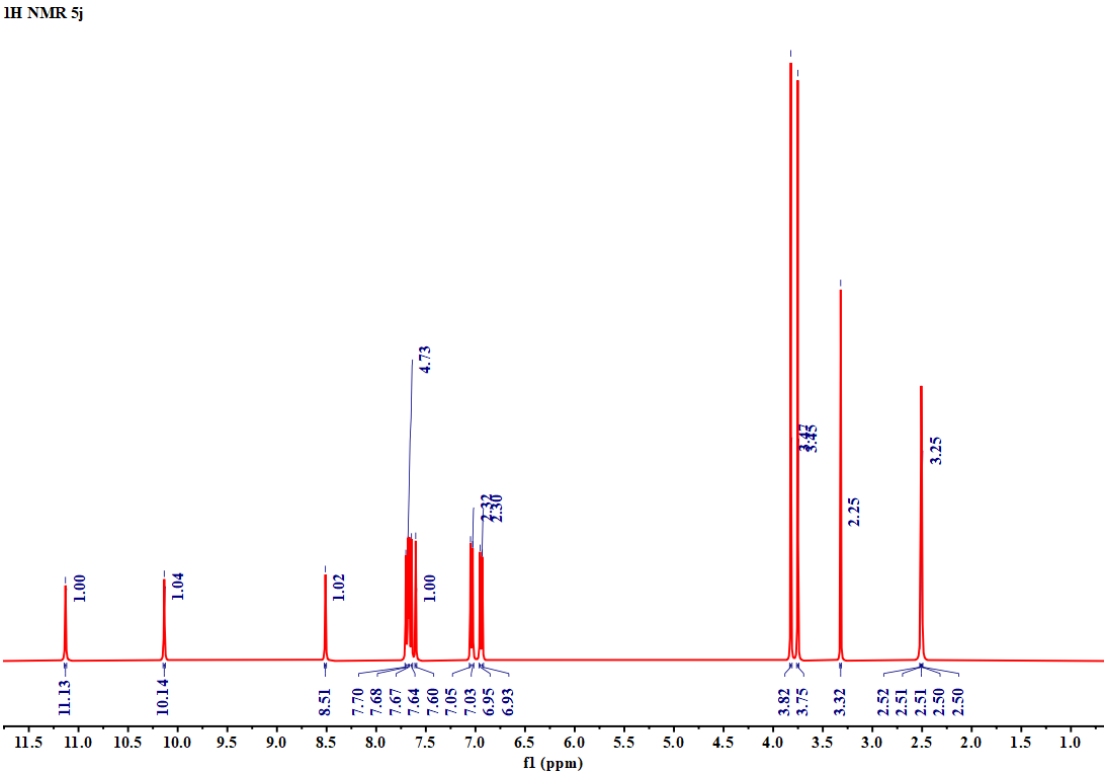


*Figure S17: [1]H NMR spectra of **5j***

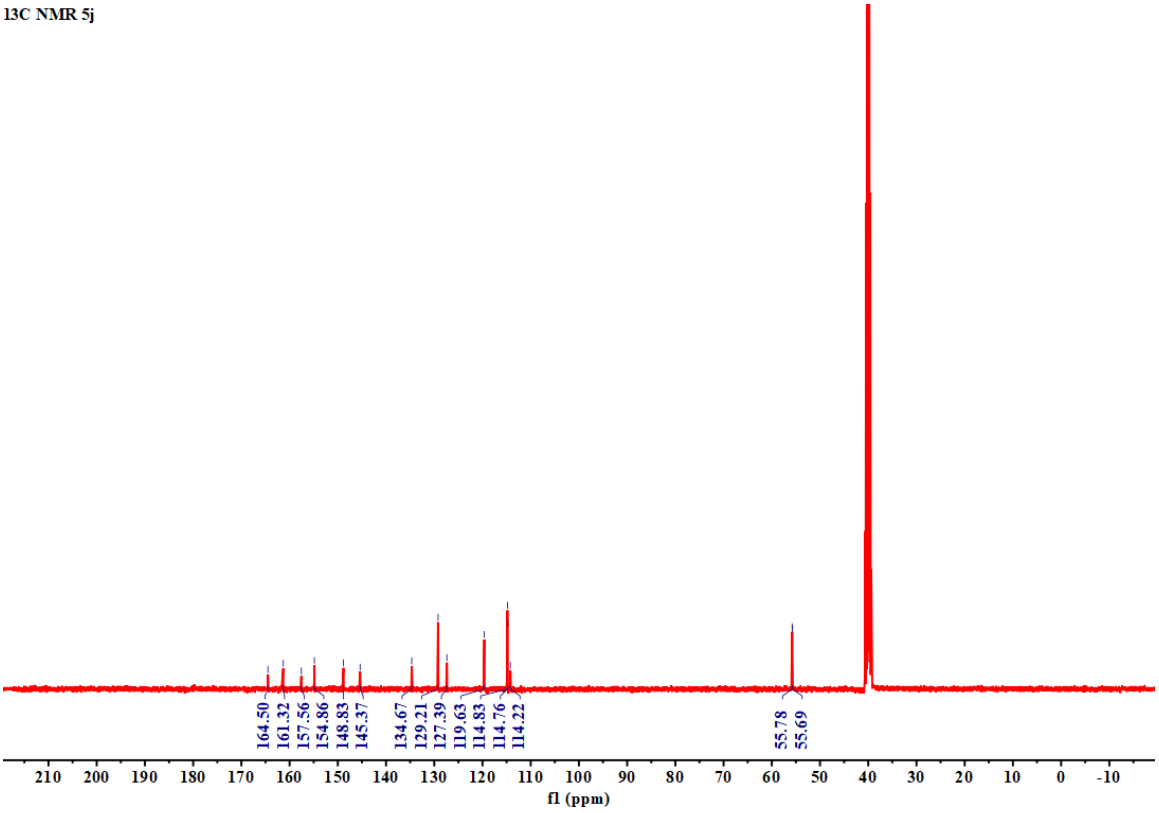


*Figure S18: [13]C NMR spectra of **5j***

1H NMR 5l

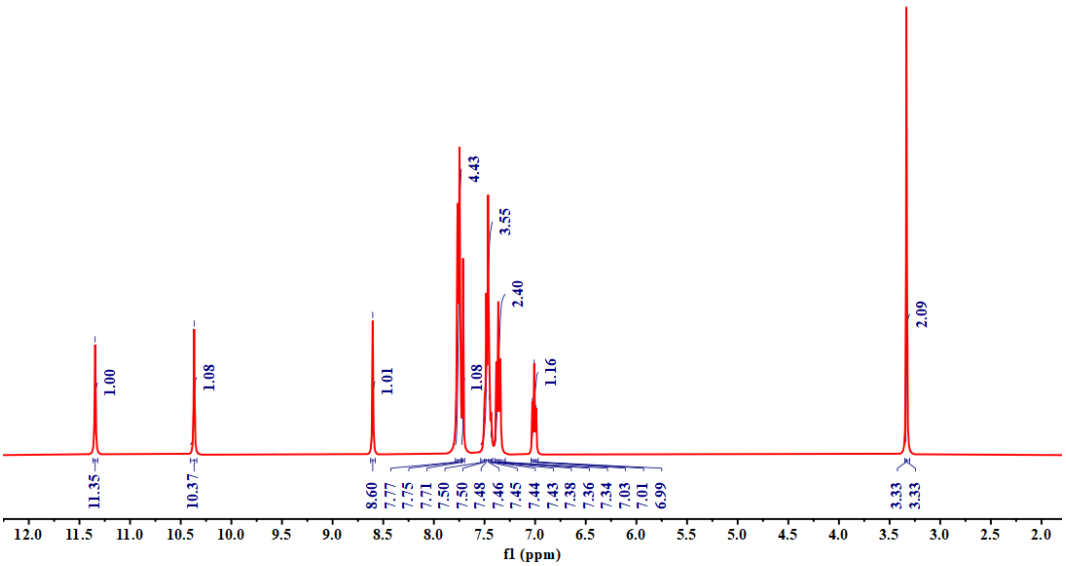


*Figure S19: ^{1}H NMR spectra of **5l***

13C NMR 5l

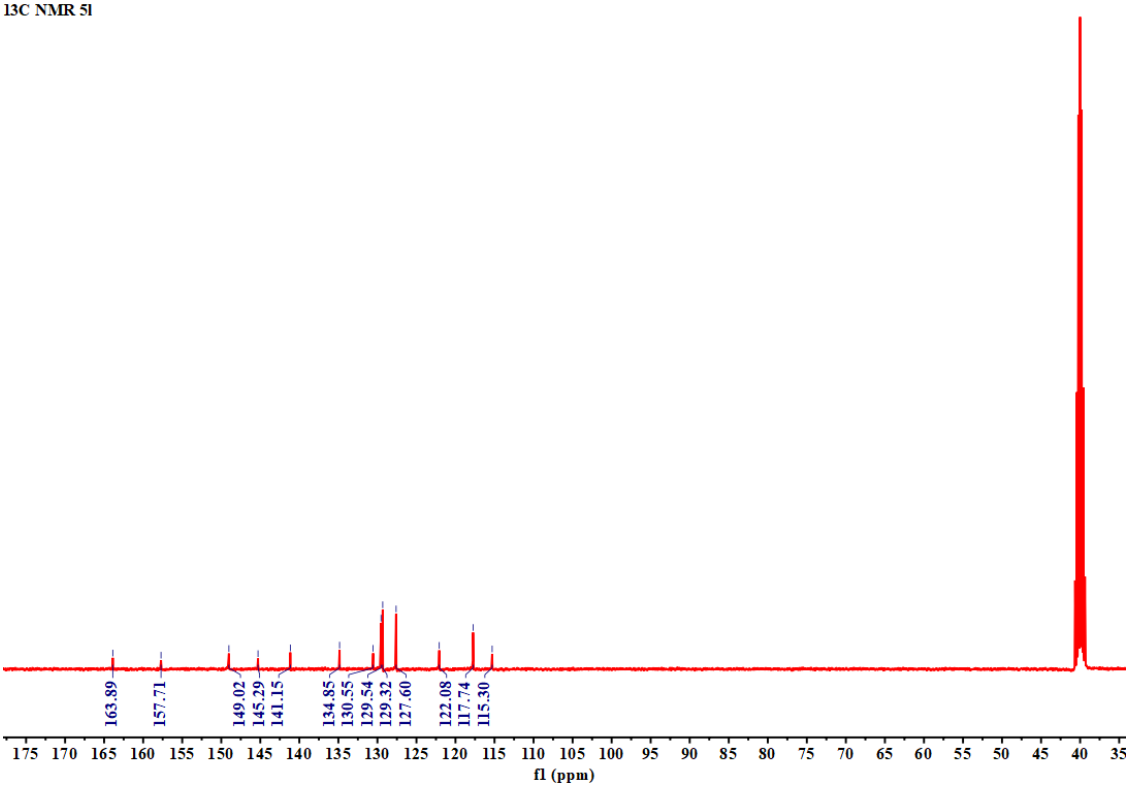


*Figure S20: ^{13}C NMR spectra of **5l***

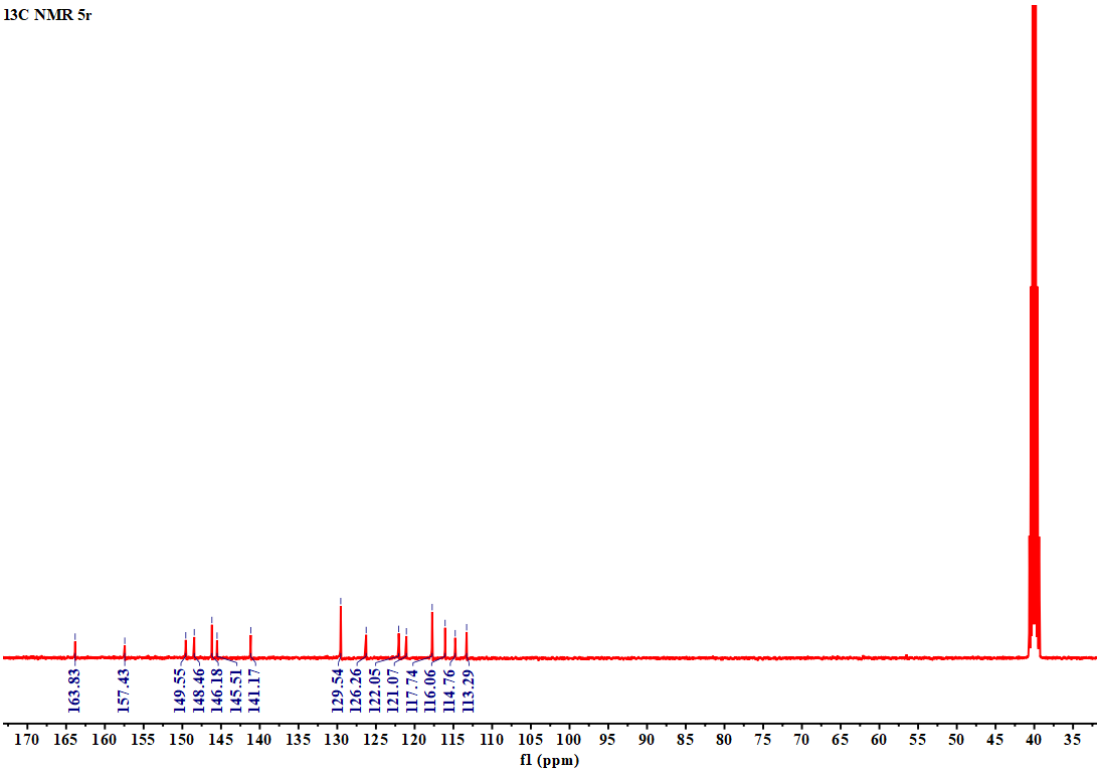


*Figure S21: ^{13}C NMR spectra of **5r***

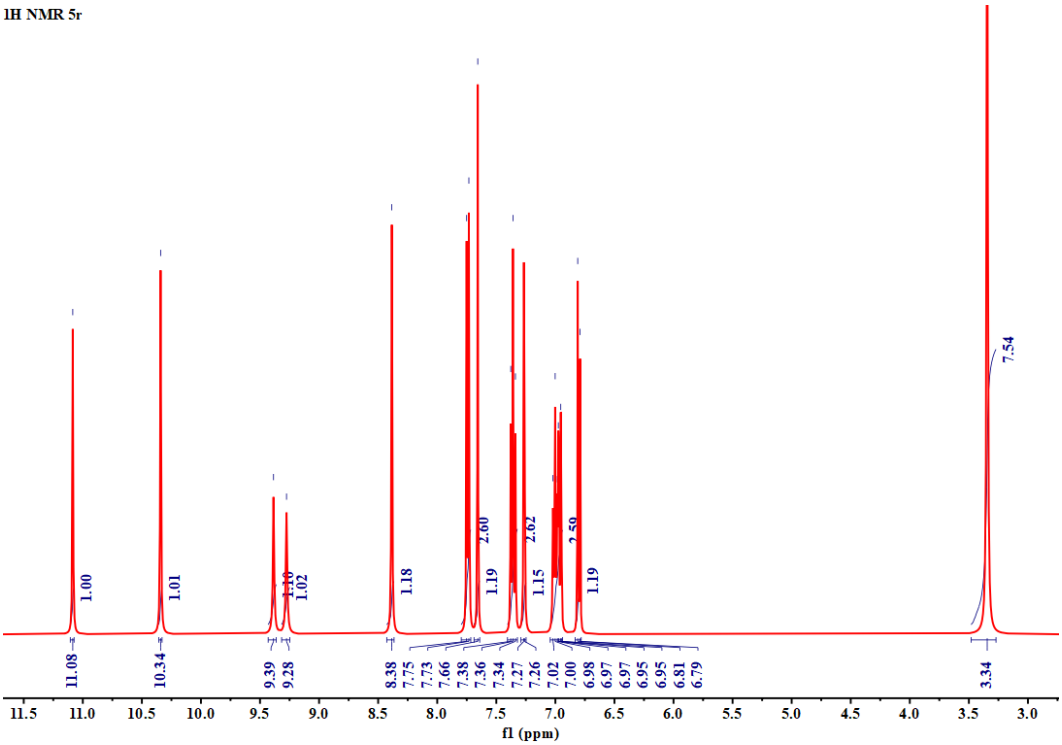


*Figure S22:^{1}H NMR spectra of **5r***

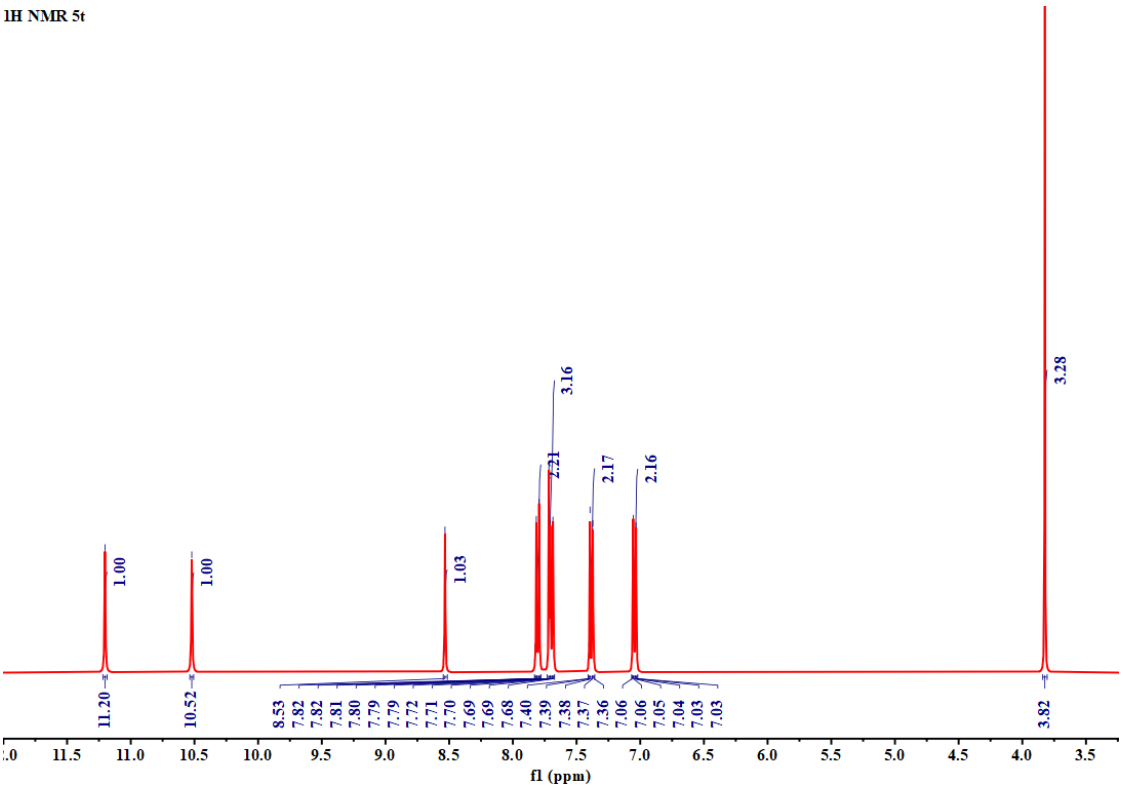


*Figure S23: [1]H spectra of **5t***

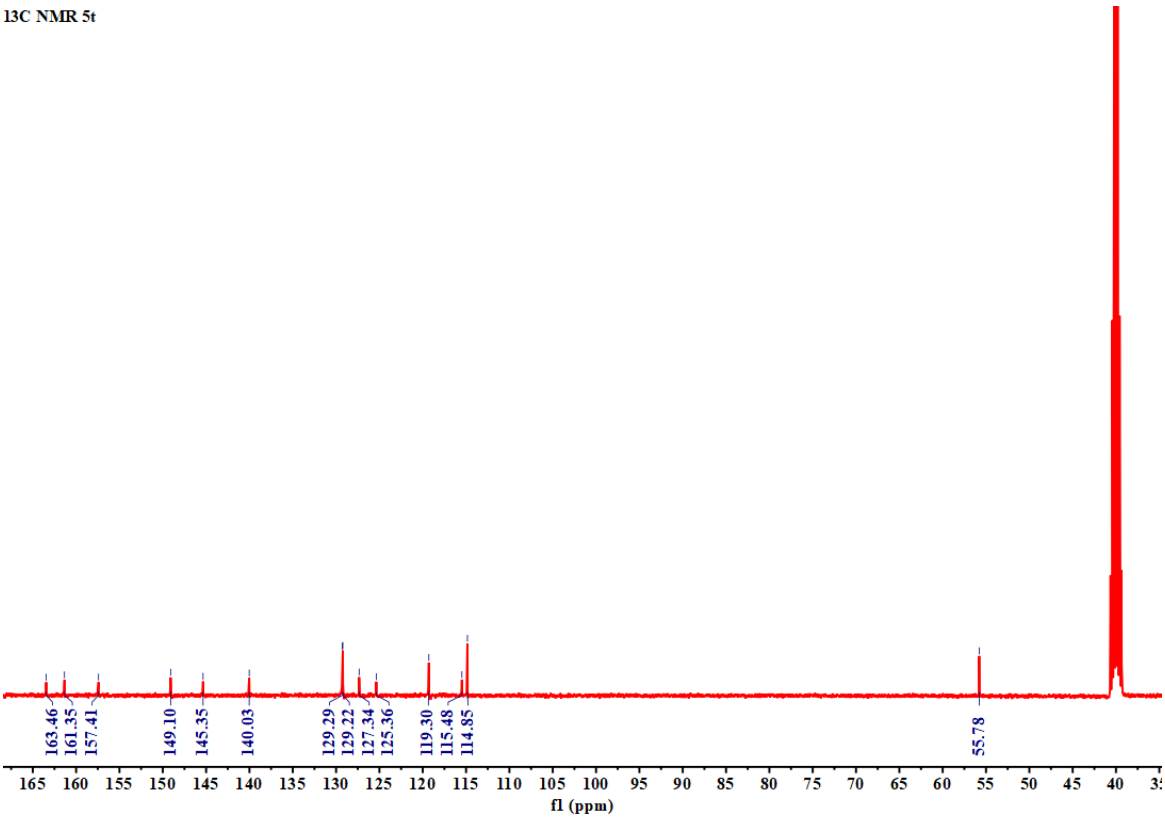


*Figure S24: [13]C NMR spectra of **5t***

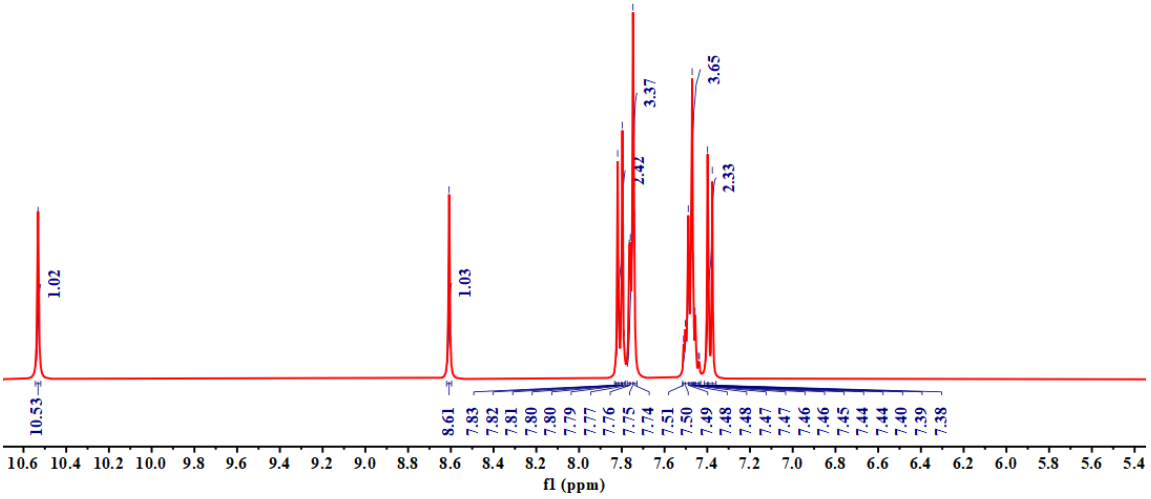


*Figure S25: [1]H NMR spectra of **5u***

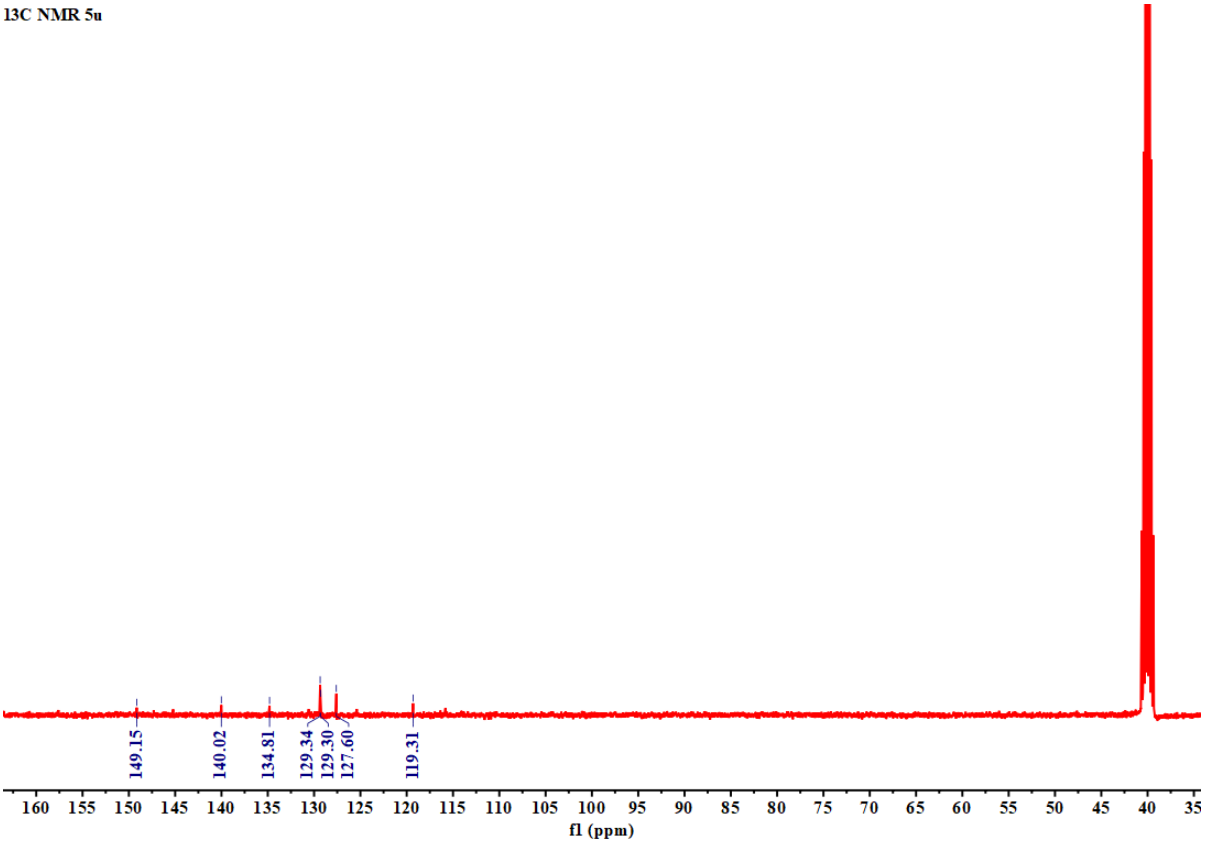


*Figure S26: [13]C NMR spectra of **5u***

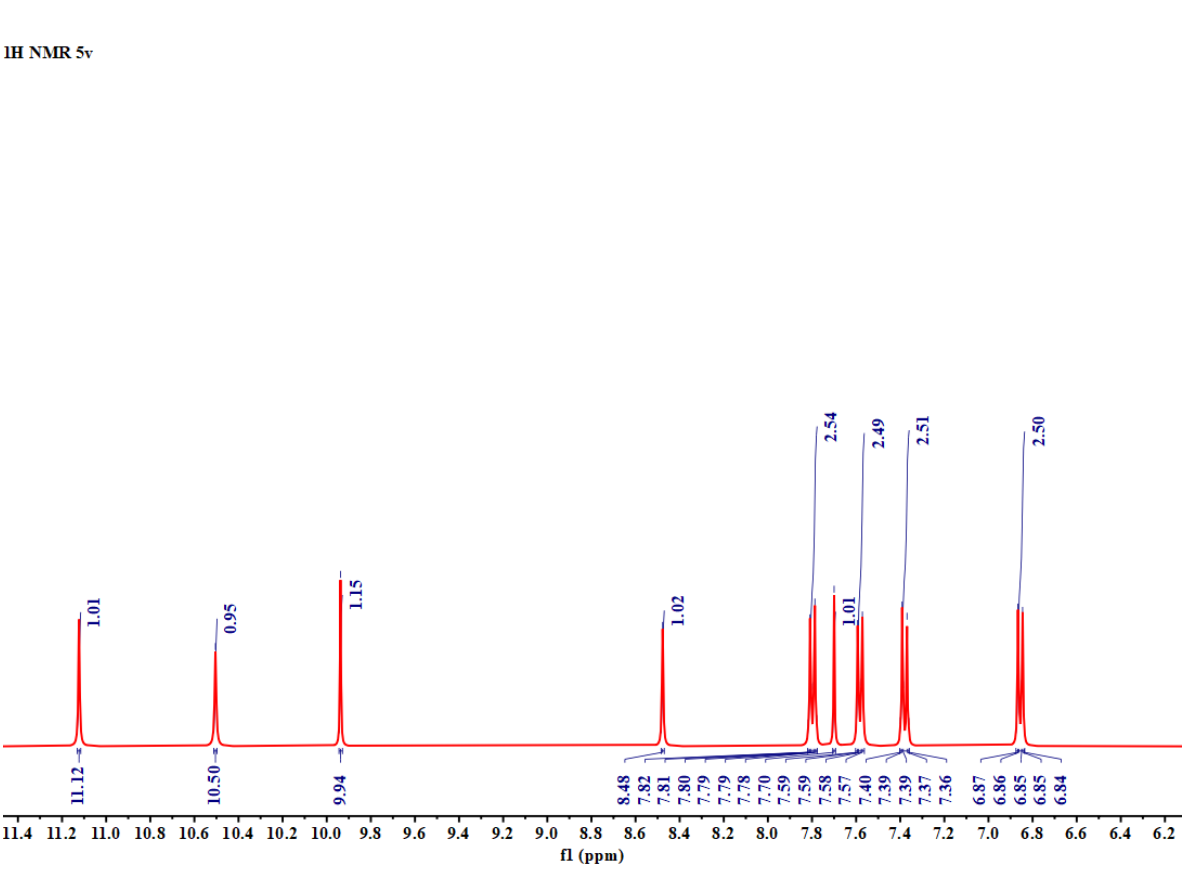


*Figure S27: [1]H NMR spectra of **5v***

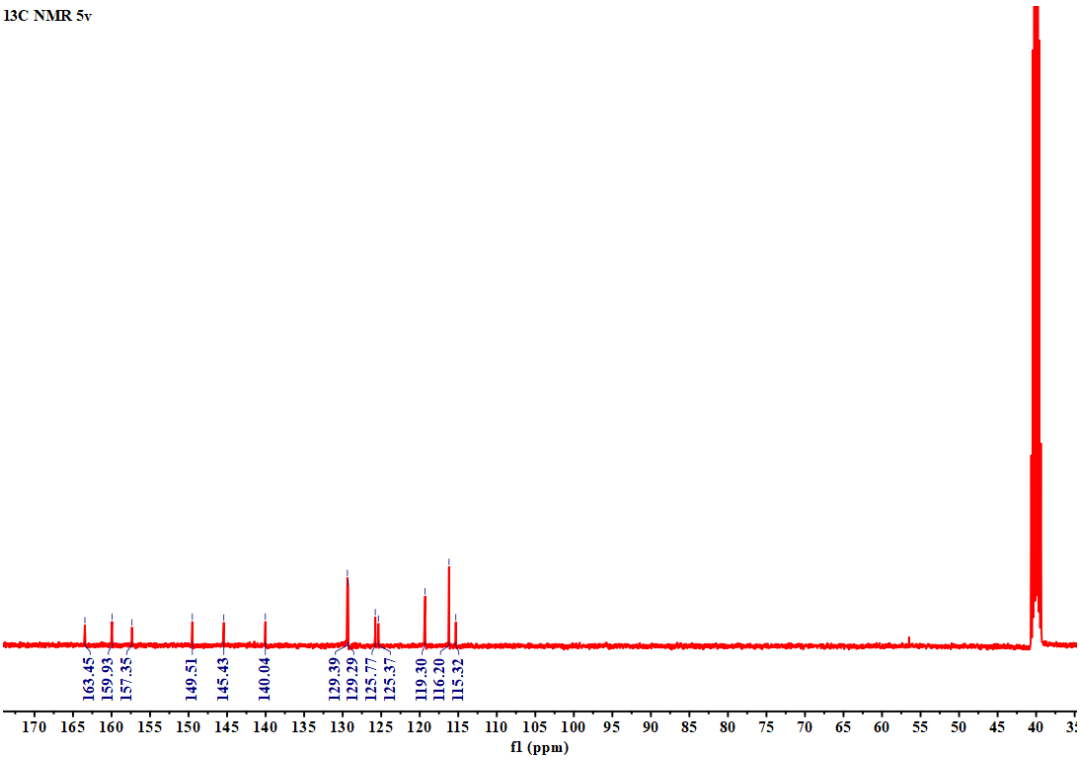


*Figure S28: [13]C NMR spectra of **5v***

1H NMR 5w

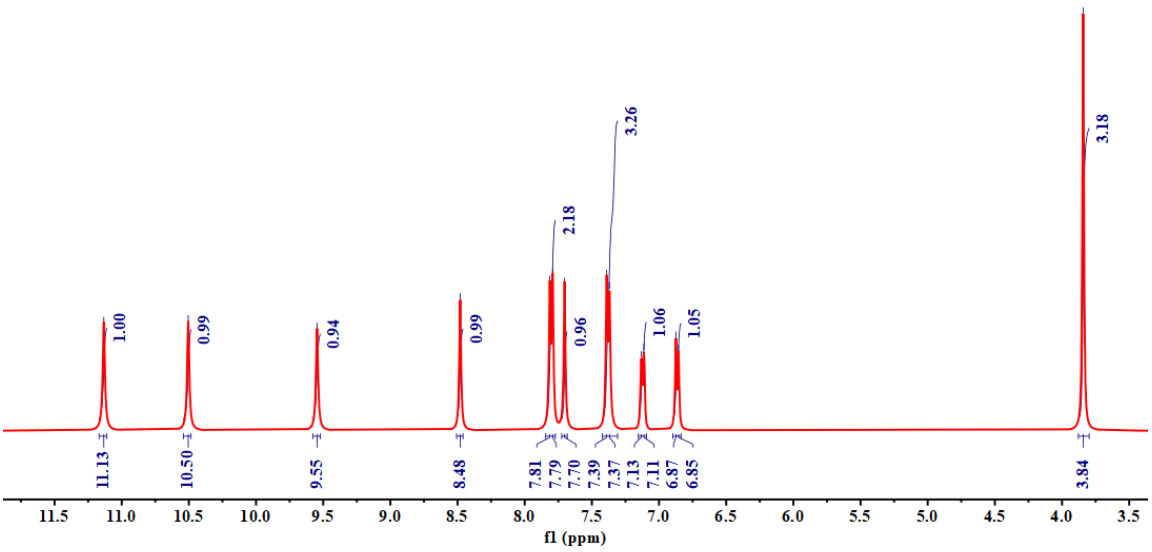


*Figure S29: [1]H NMR spectra of **5w***

13C NMR 5w

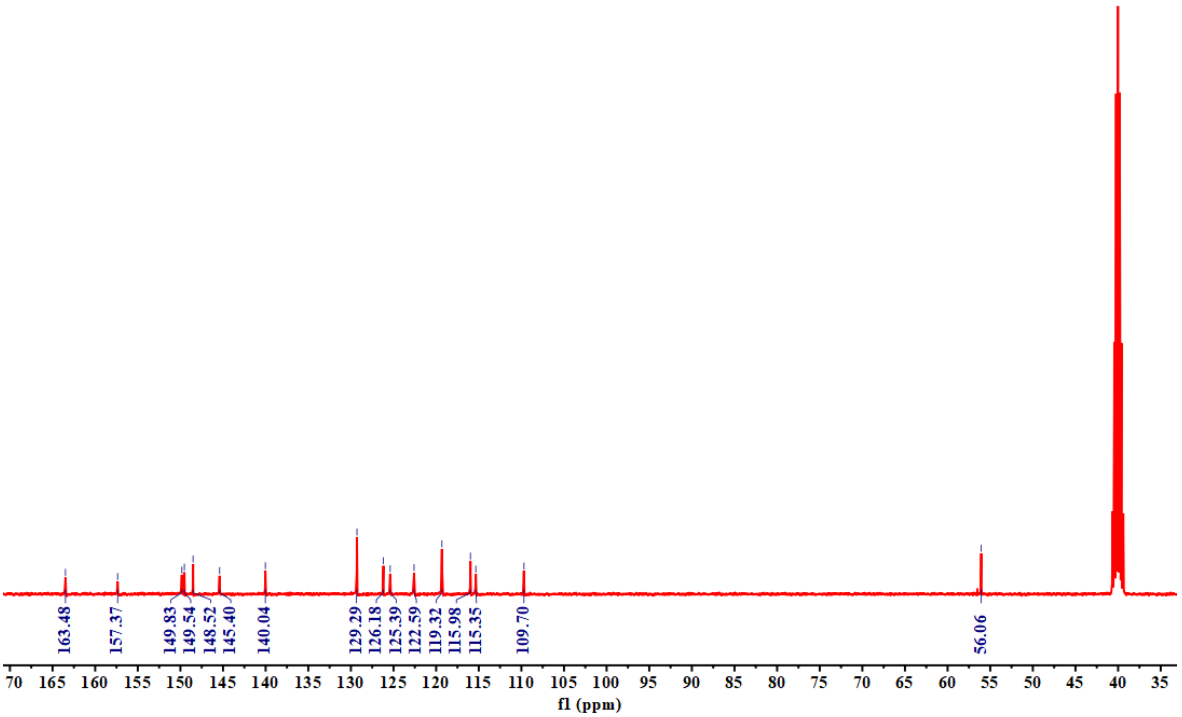


*Figure S30:[13]C NMR spectra of **5w***

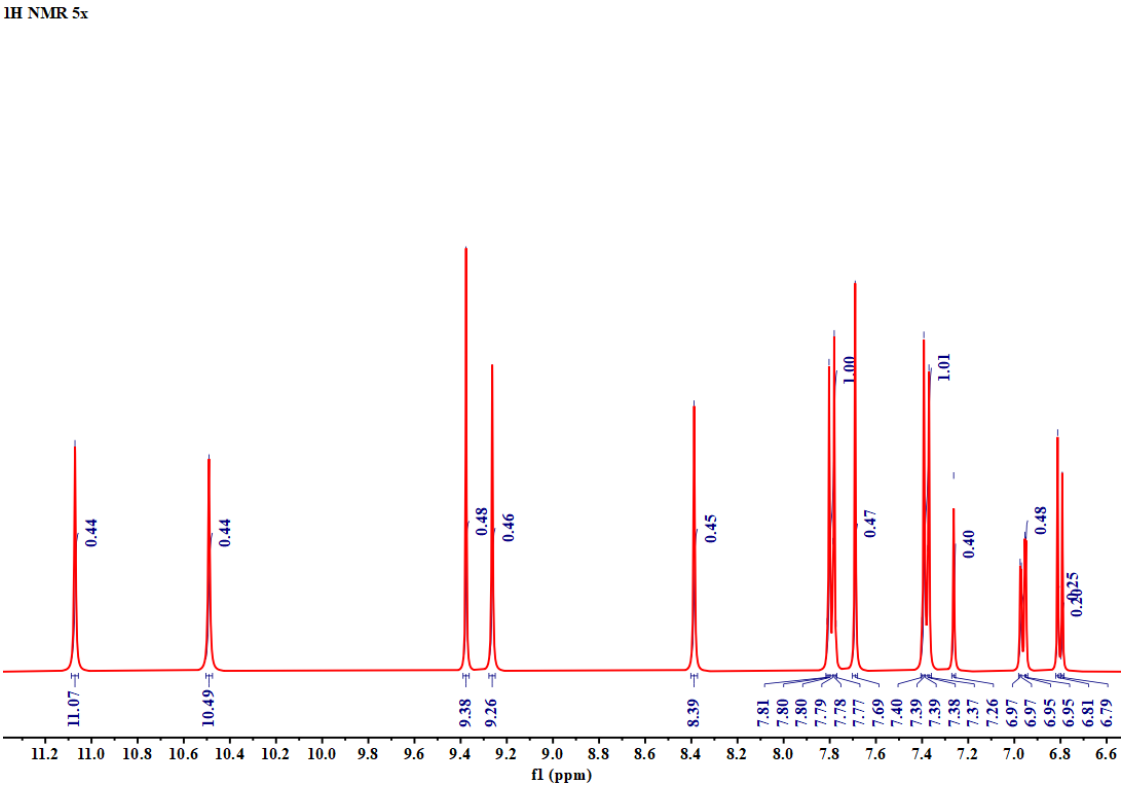


*Figure S31: [1]H NMR spectra of **5x***

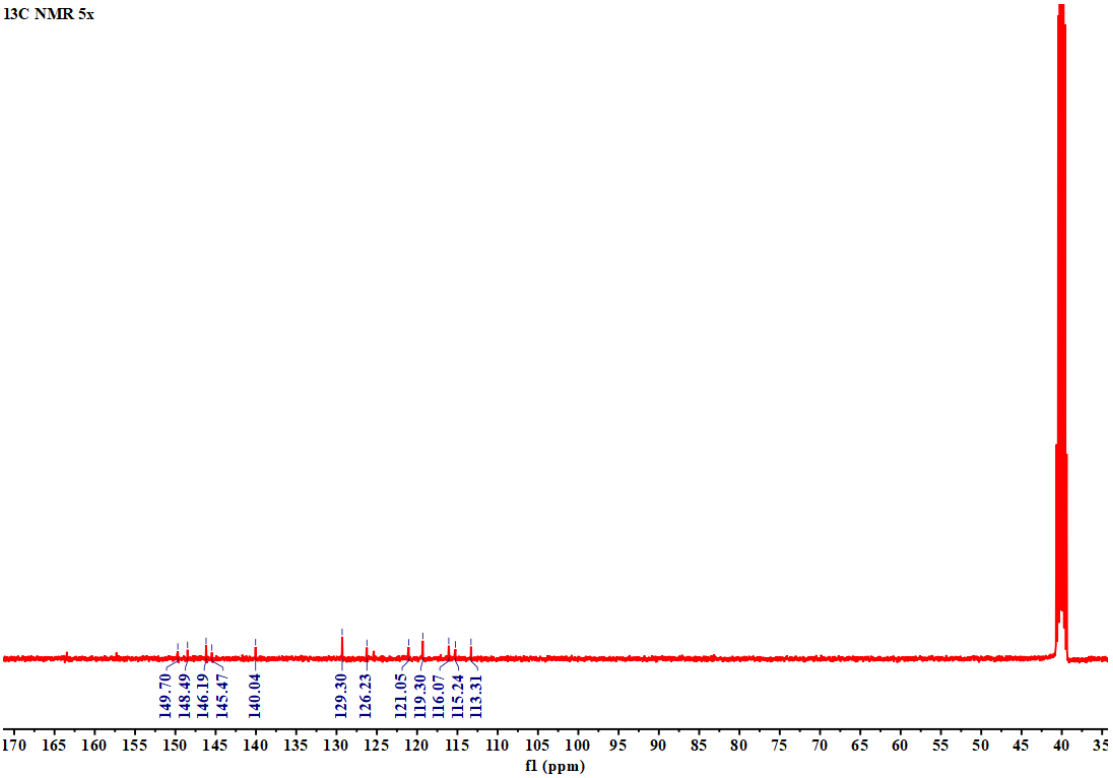


*Figure S32: [13]C NMR Spectra of **5x***

1H NMR 5f

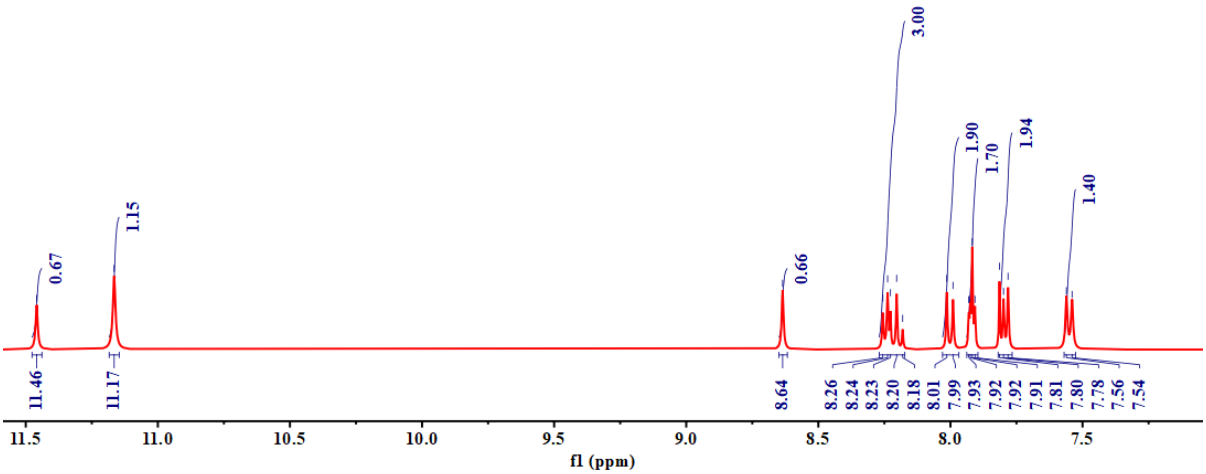


*Figure S33: ^{1}H NMR spectra of **5f***

13C NMR 5f

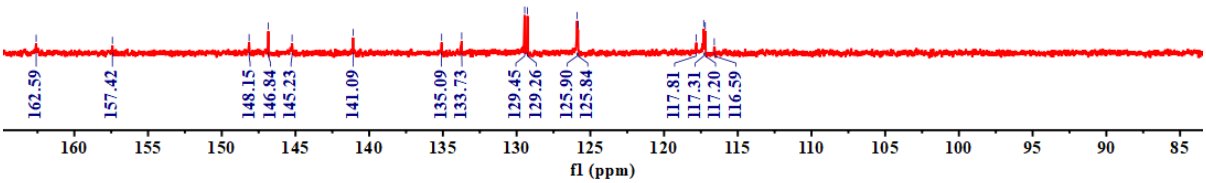


*Figure S34: ^{13}C NMR spectra of **5f***

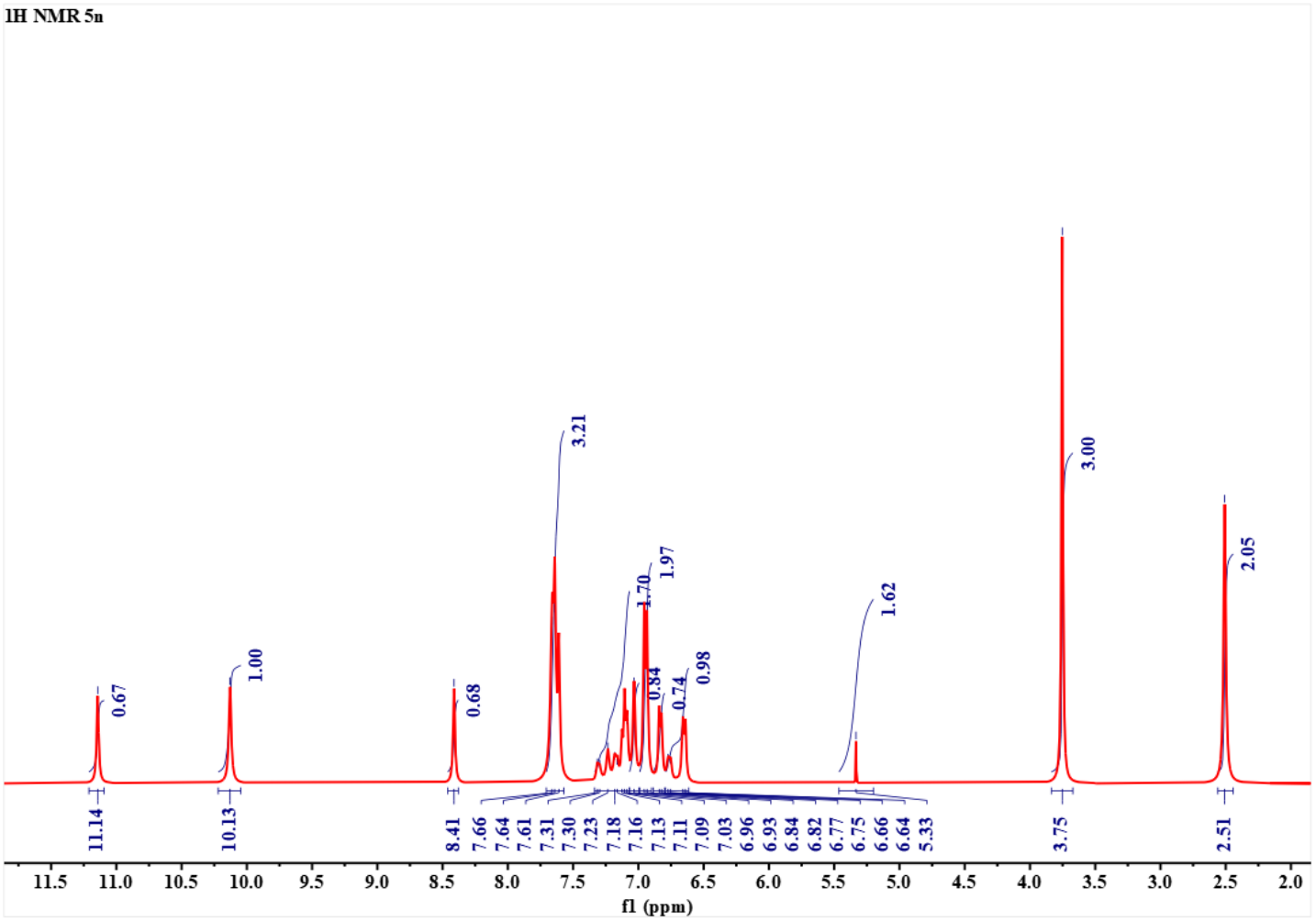


Figure S35: ^{1}H NMR spectra of ***5n***

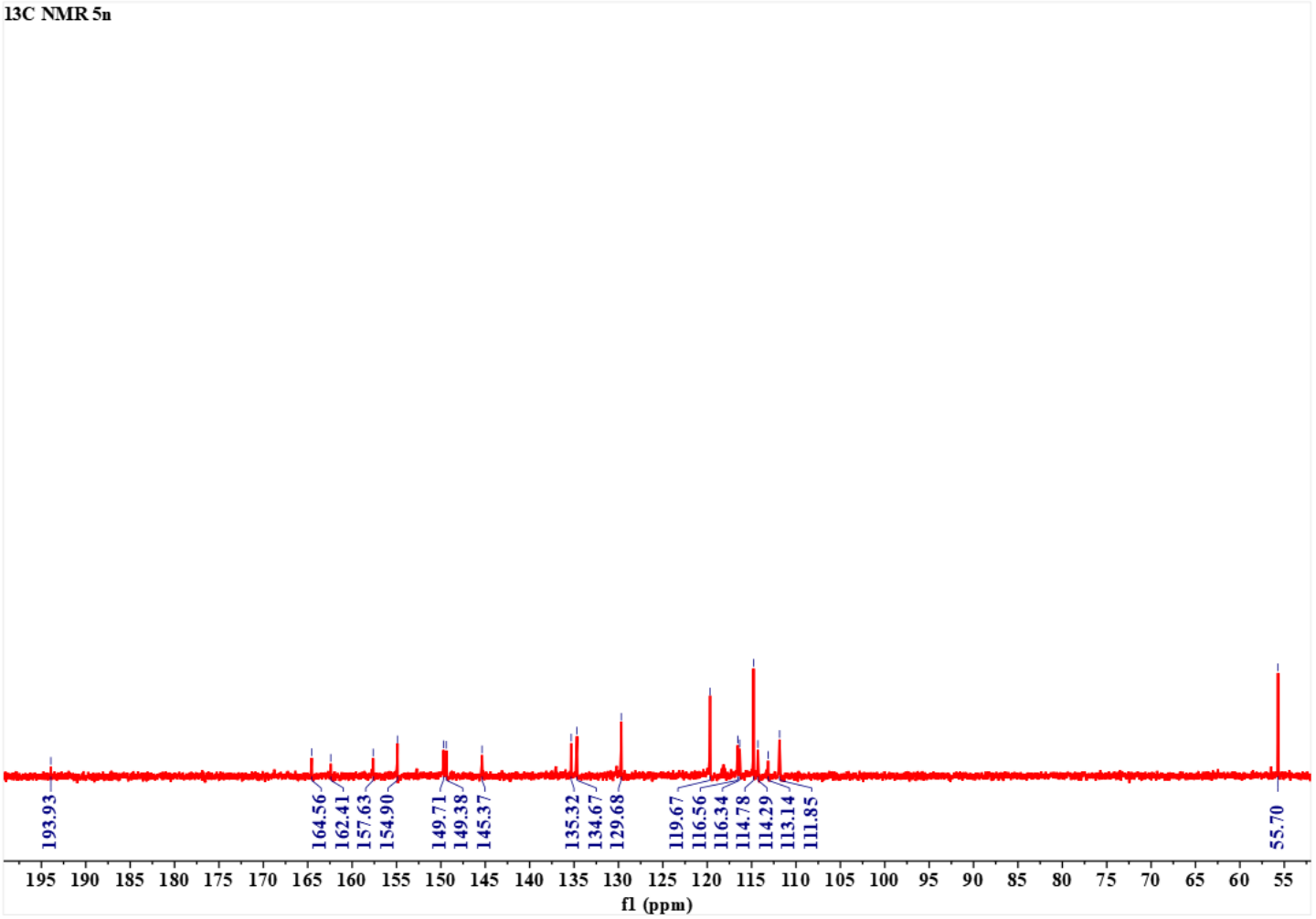


*Figure S36: ^{13}C NMR spectra of **5n***

1H NMR 5o

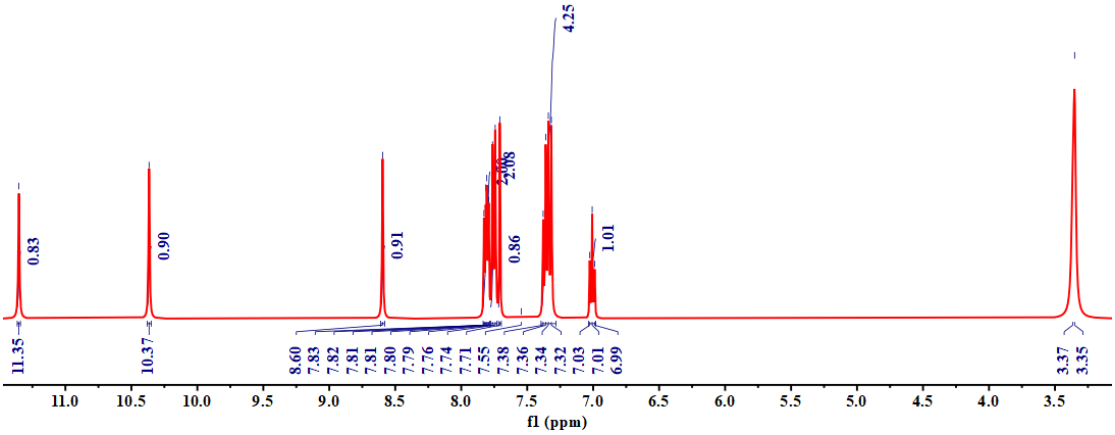


*Figure S37: [1]H NMR spectra of **5o***

13C NMR 5o

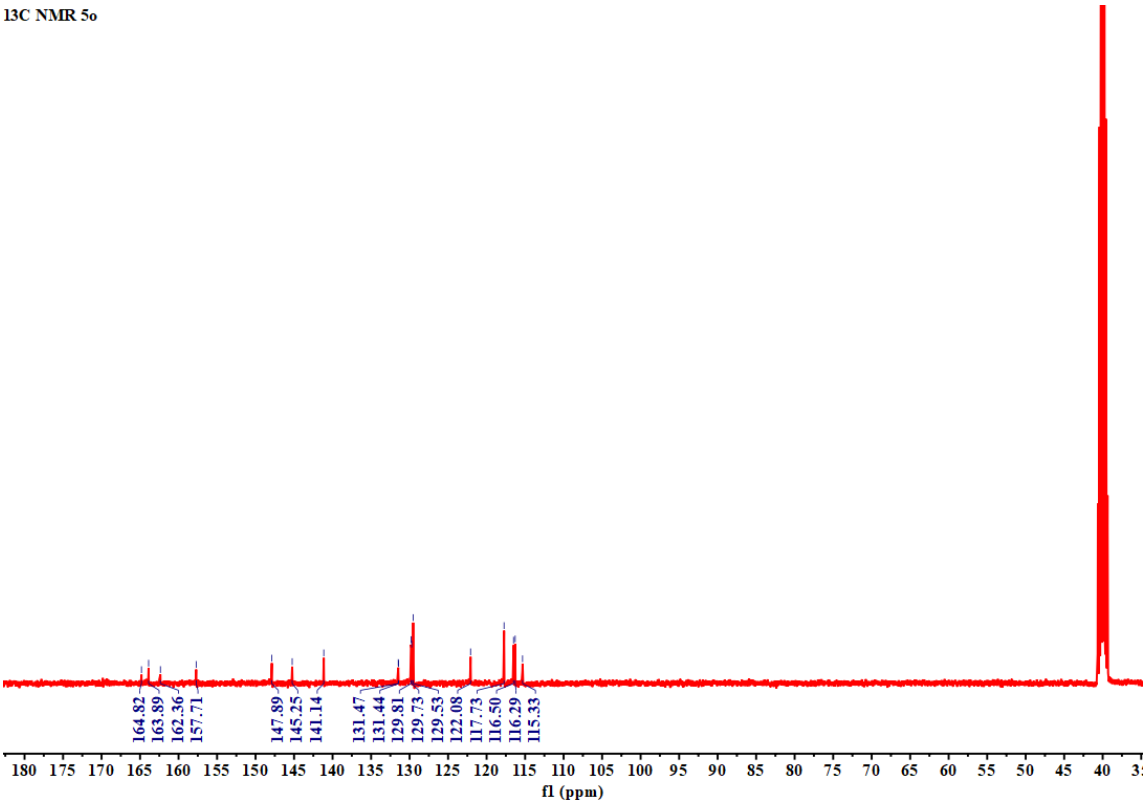


*Figure S38: [13]C NMR spectra of **5o***

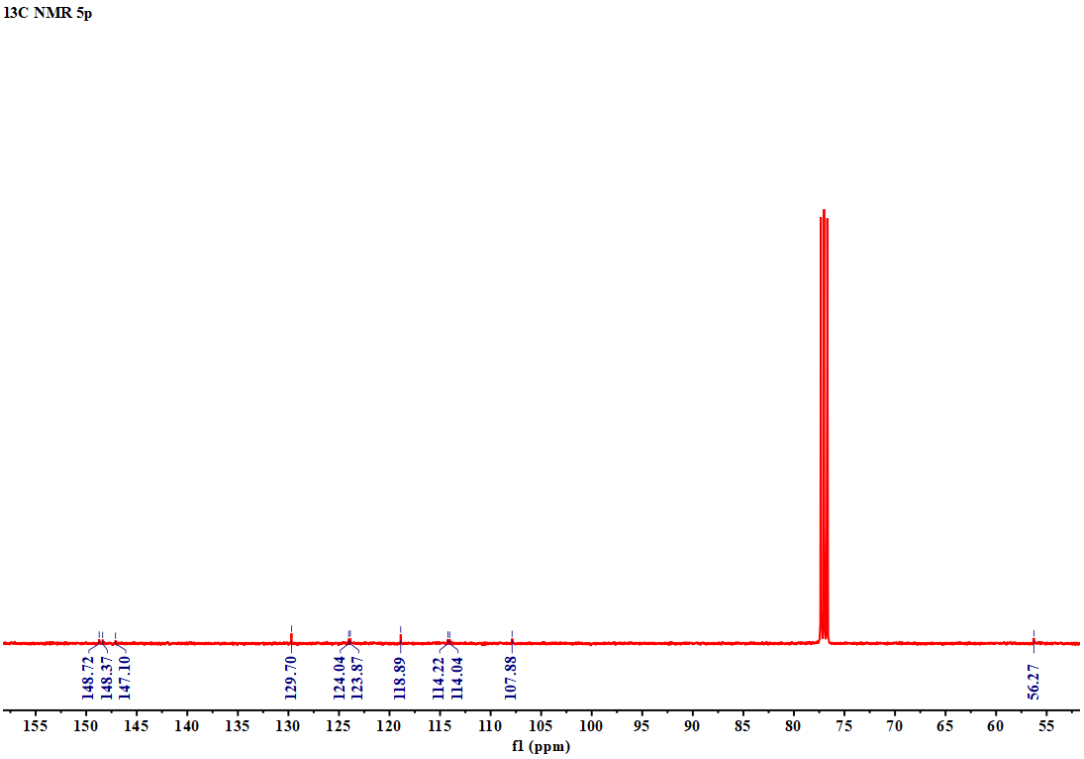


*Figure S39:[1]H NMR spectra of **5p***

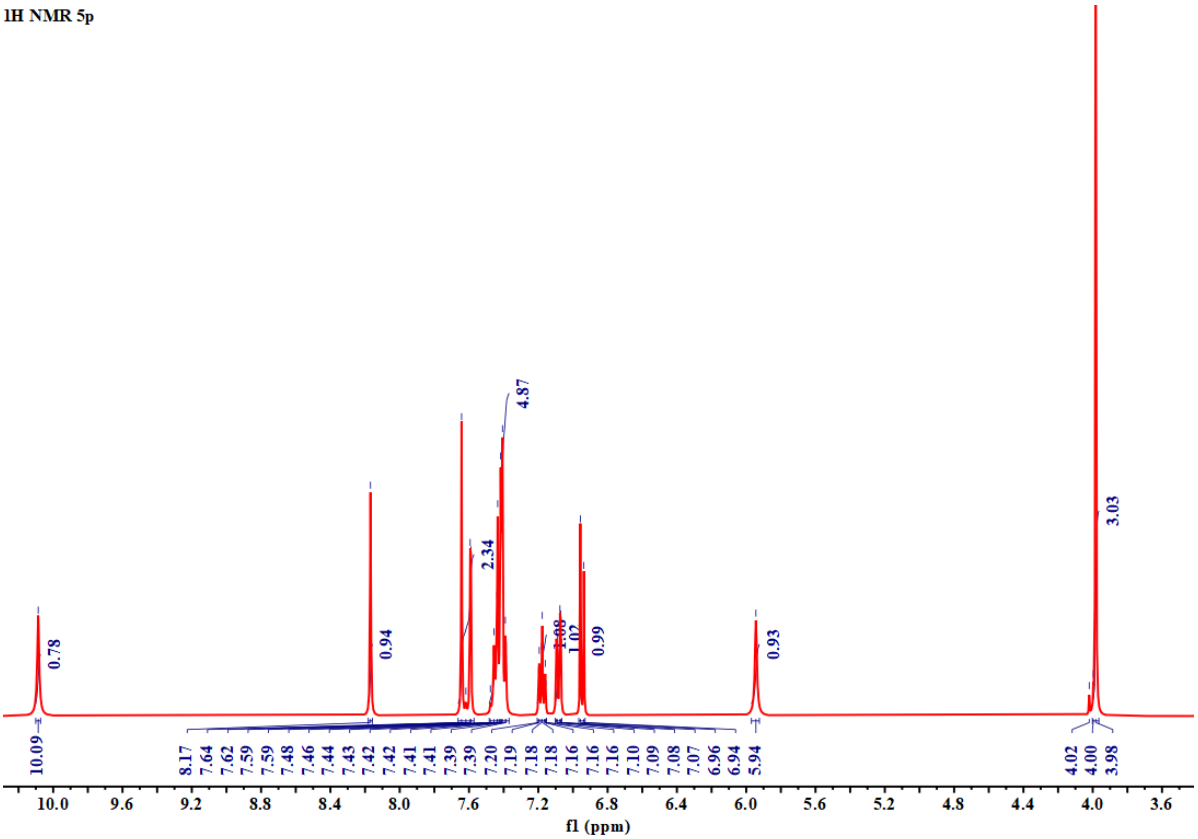


*Figure S40: [13]C NMR spectra of **5p***

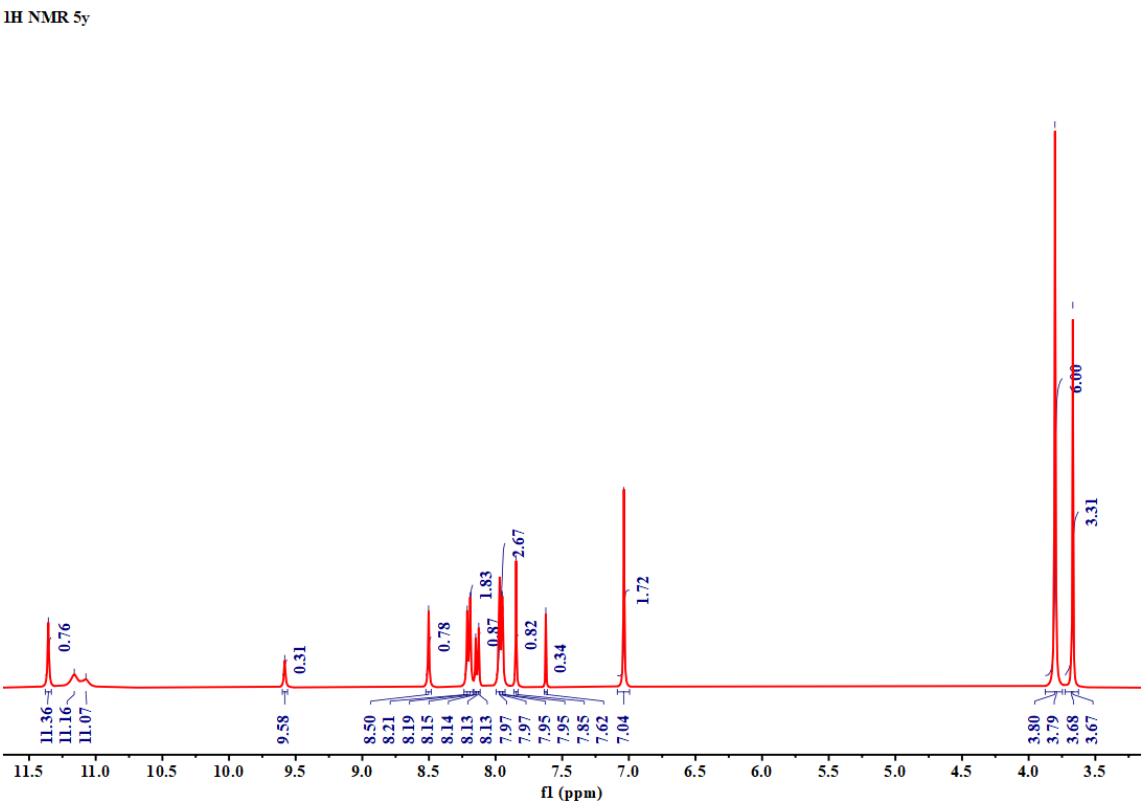


*Figure S41: [1]H NMR spectra of **5y***

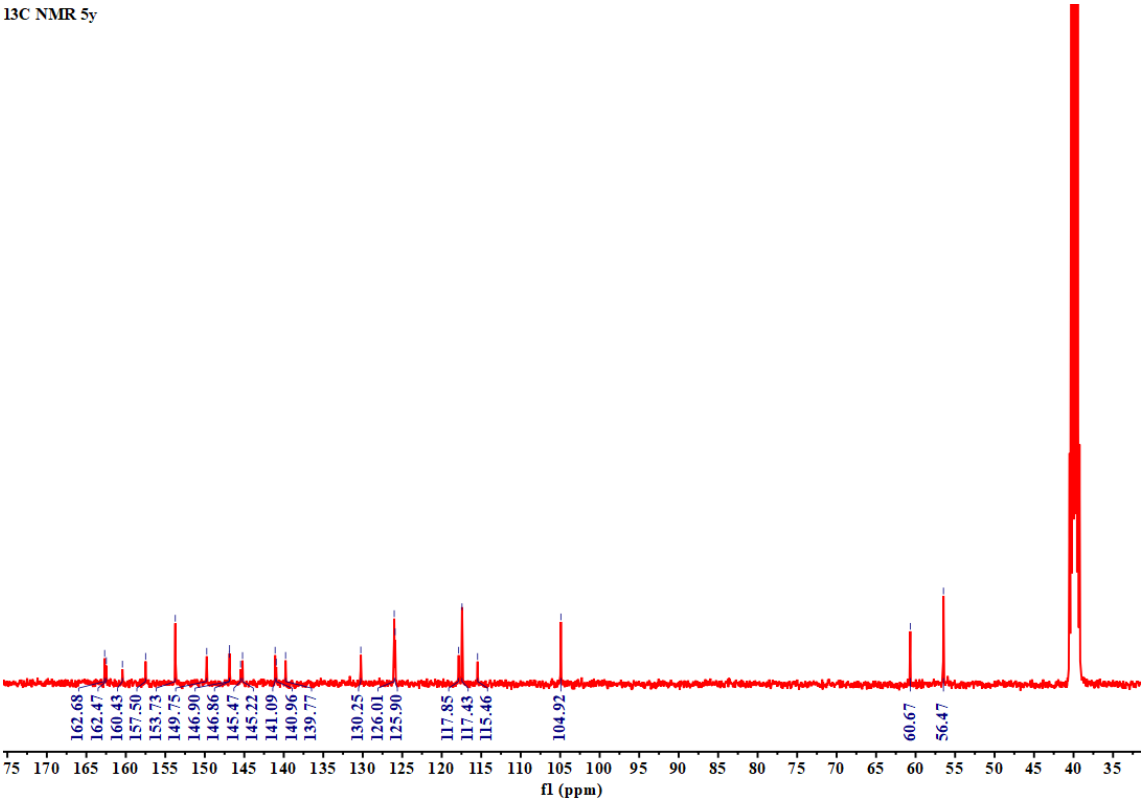


*Figure S42: [13]C NMR spectra of **5y***

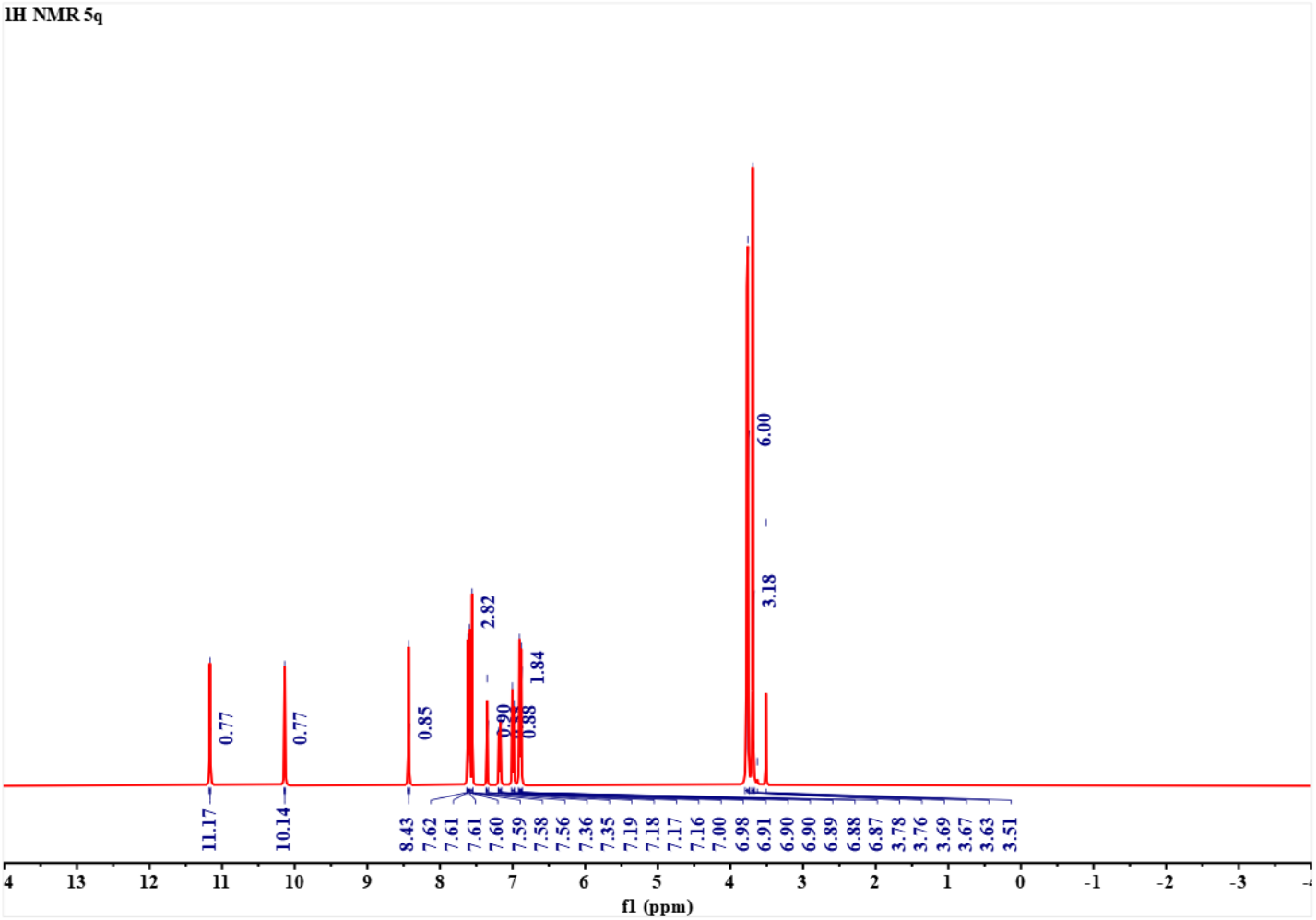


*Figure S43: ^{1}H NMR spectra of **5q***

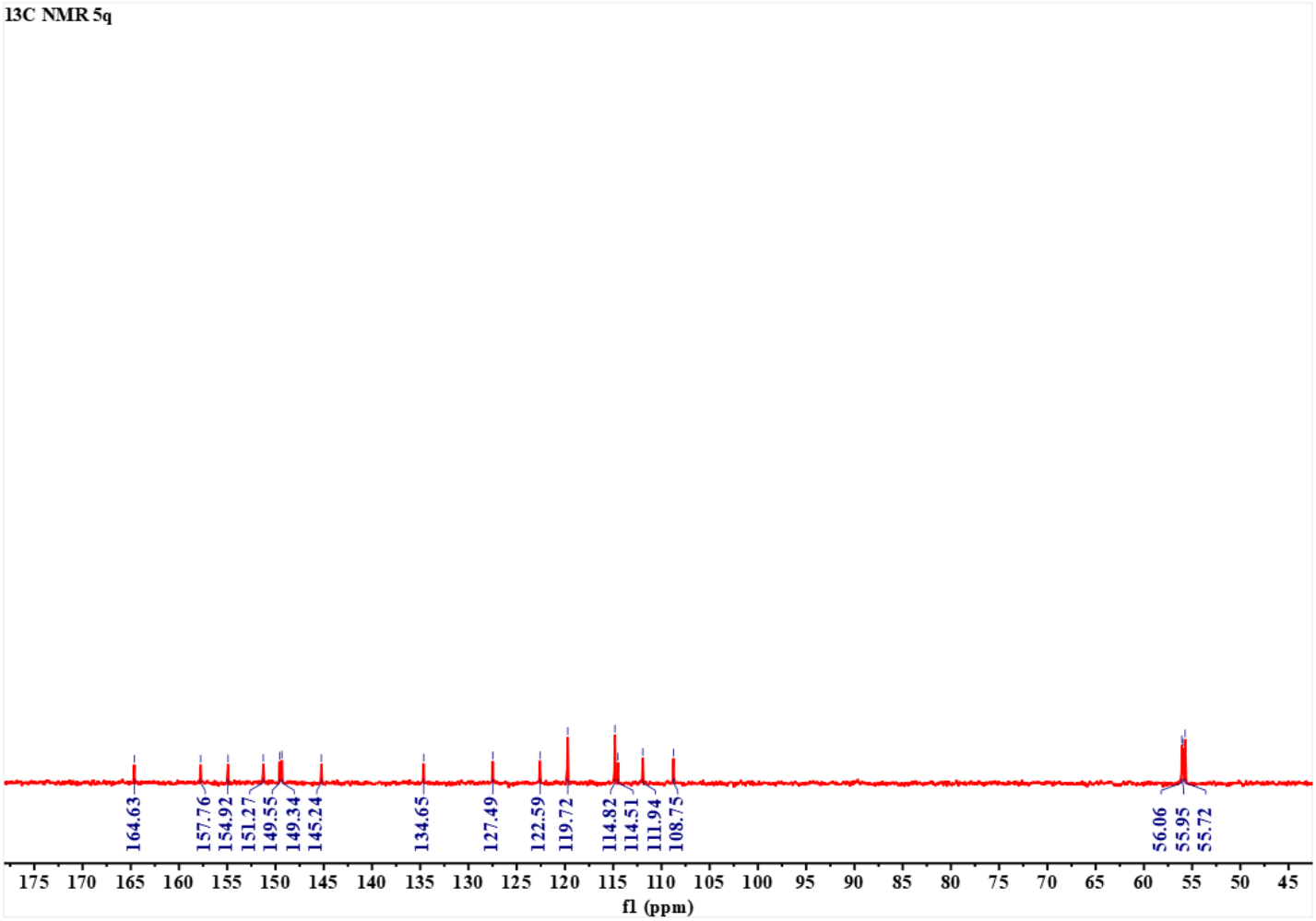


*Figure S44: ^{13}C NMR spectra of **5q***

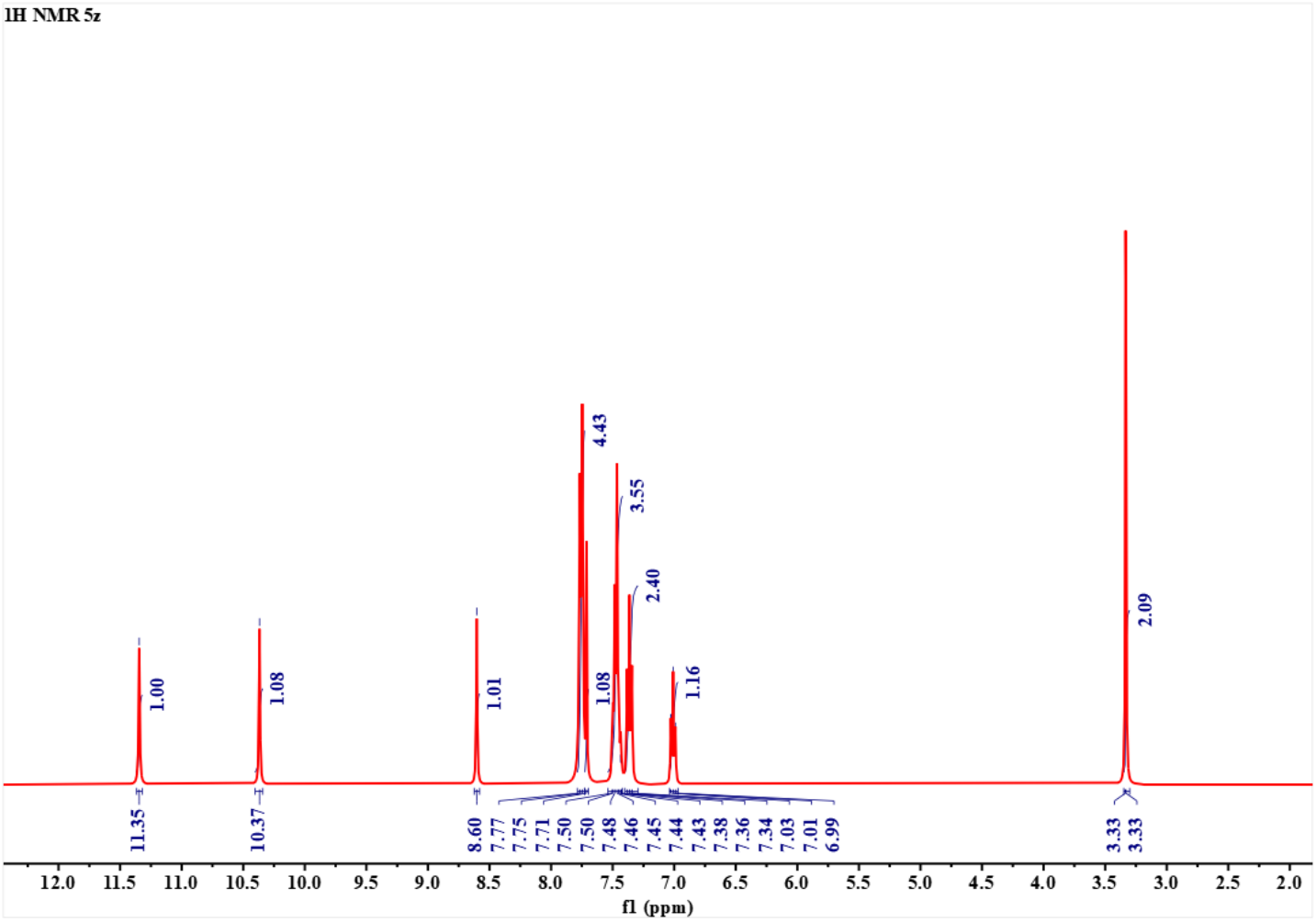


*Figure S45: ^{1}H NMR spectra of **5z***

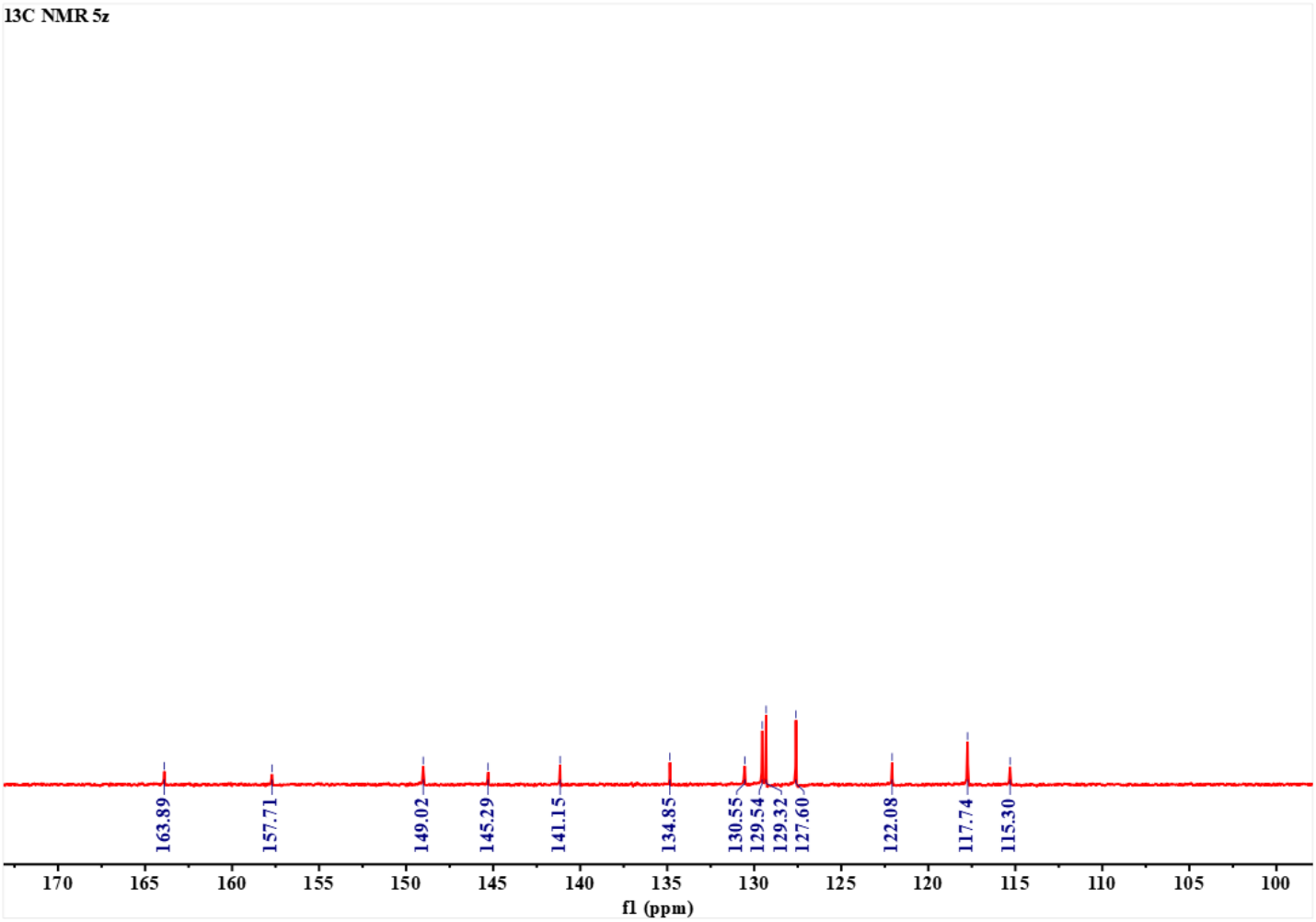


*Figure S46: ^{13}C NMR spectra of **5z***

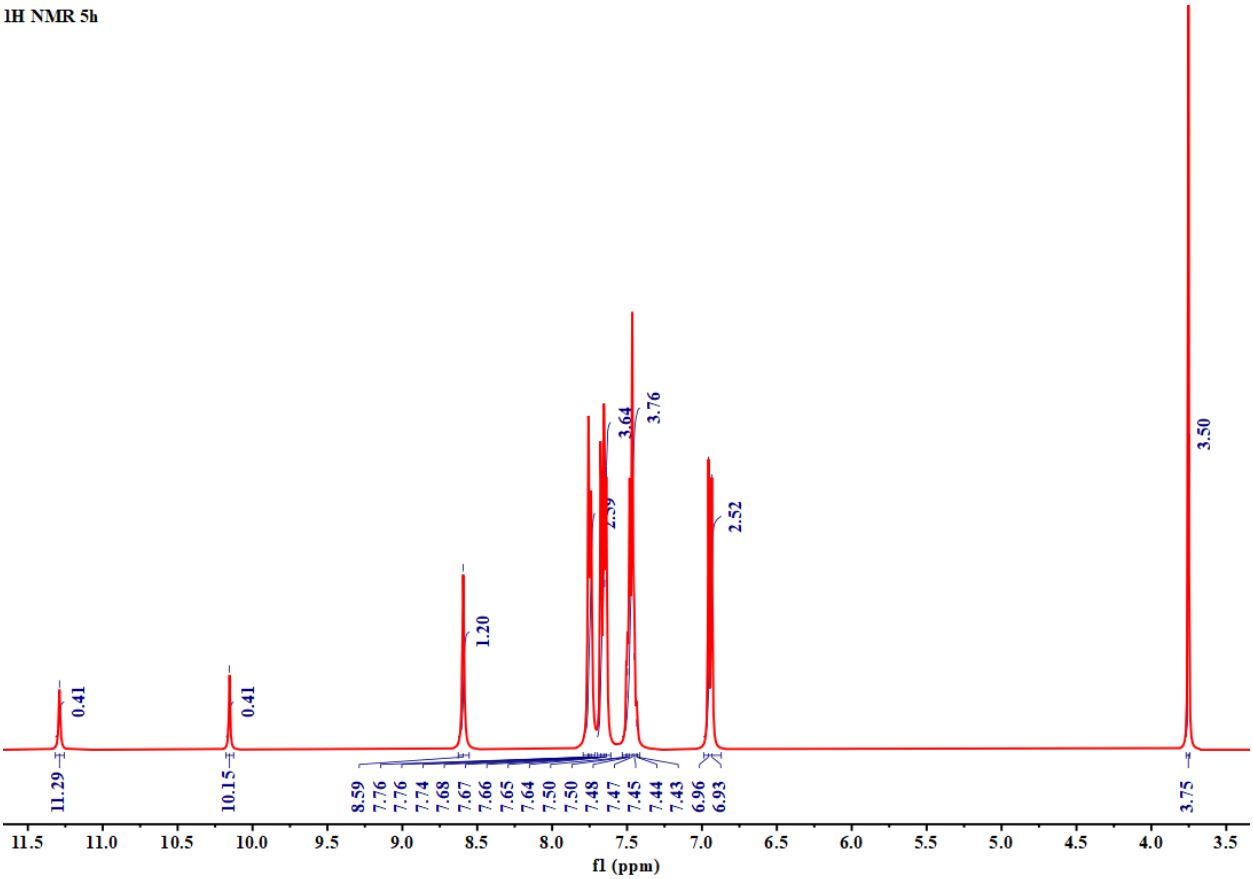


*Figure S47: ^{1}H NMR spectra of **5h***

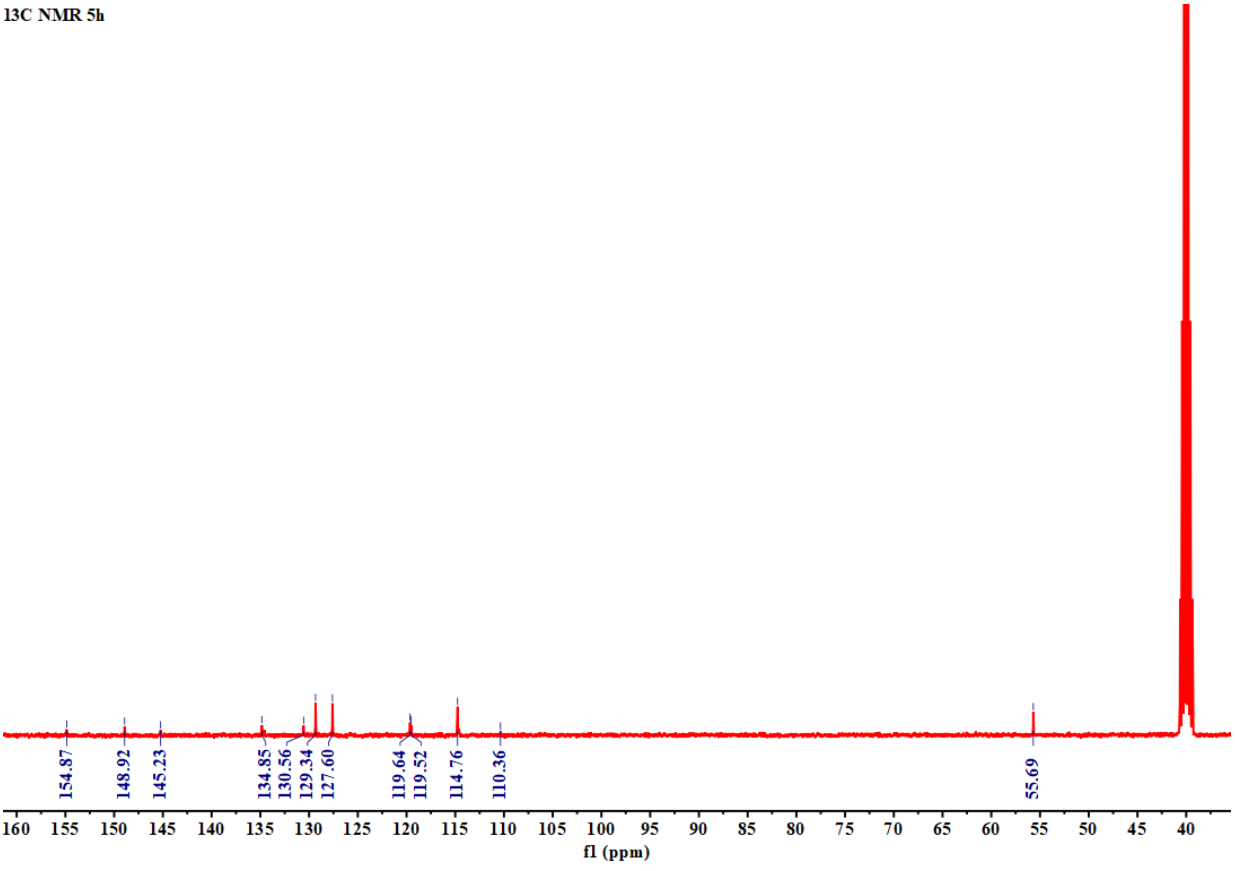


*Figure S48: ^{13}C NMR spectra of **5h***

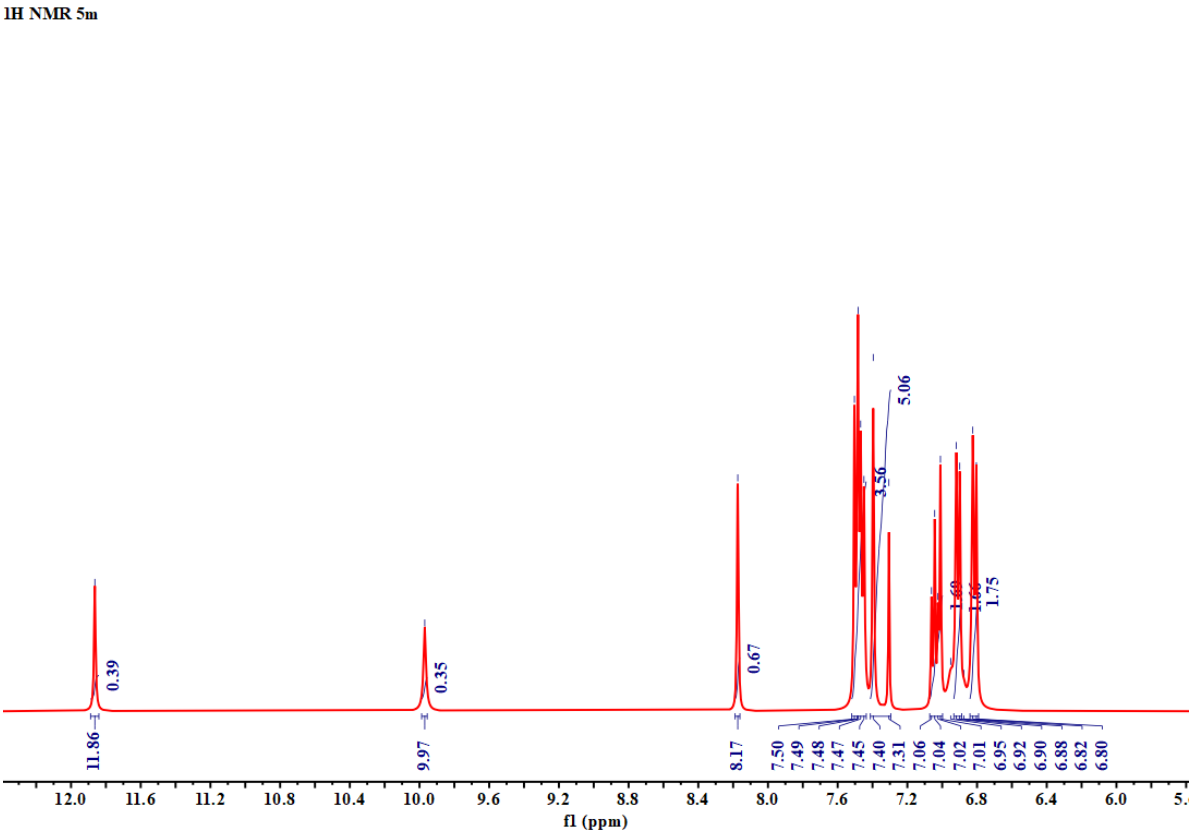


*Figure S49: ^{1}H NMR spectra of **5m***

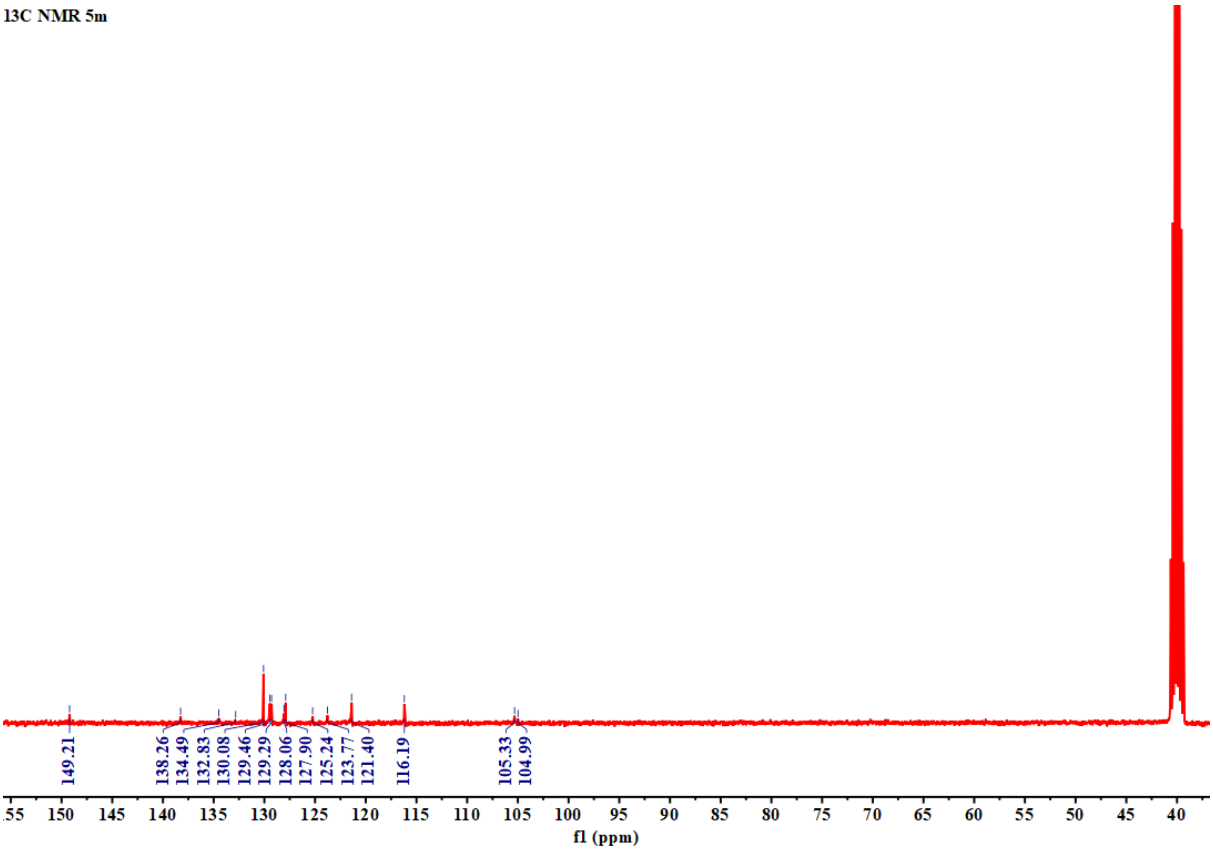


*Figure S50: ^{13}C NMR spectra of **5m***

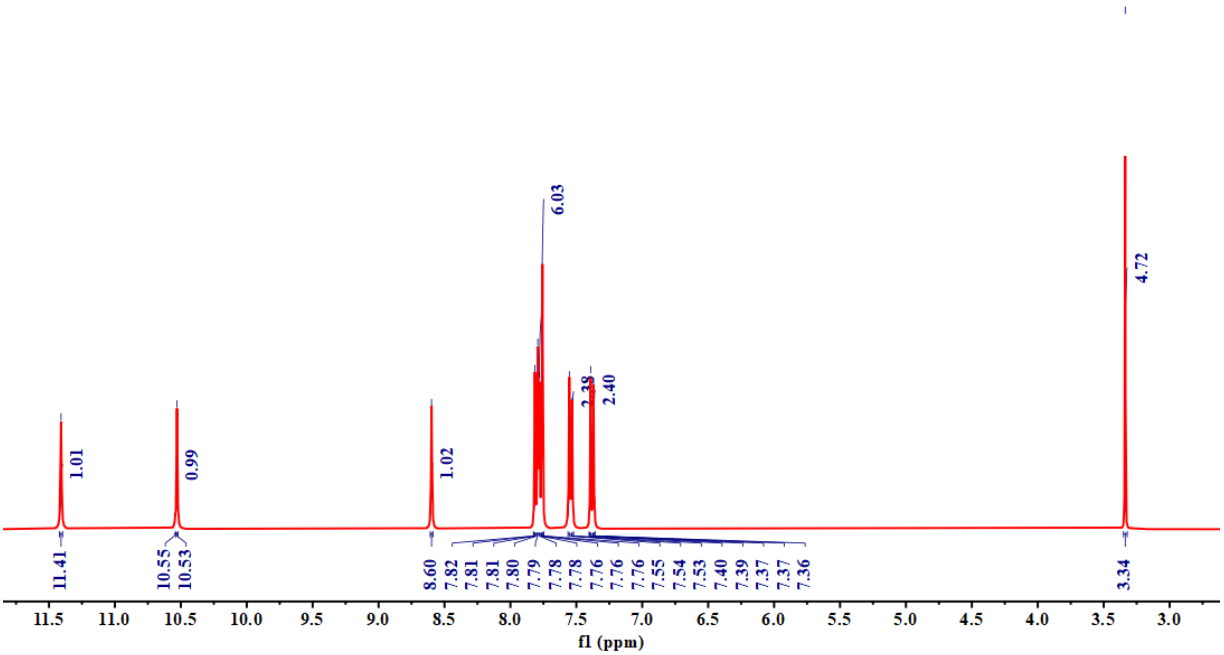


*Figure S51: [1]H NMR spectra of **5s***

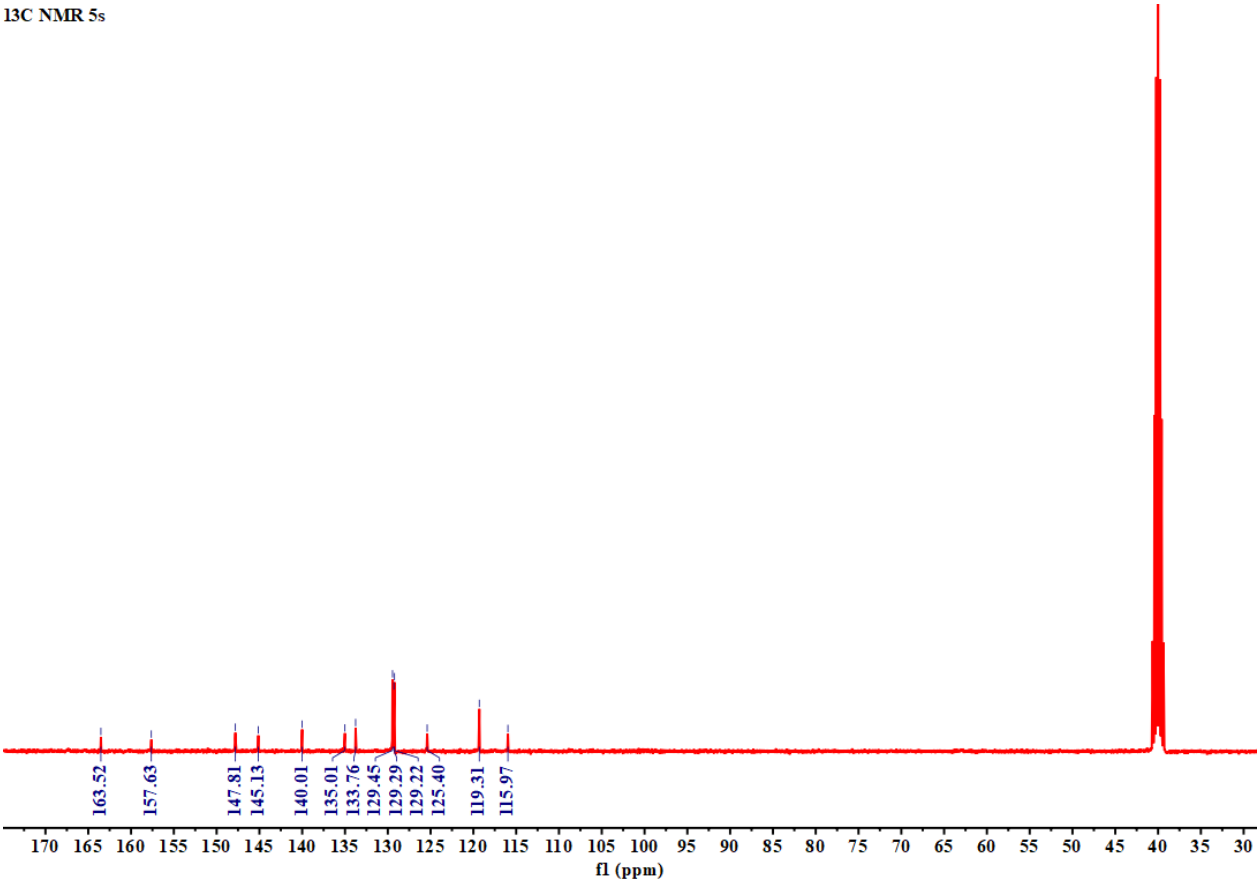


*Figure S52: [13]C NMR spectra of **5s***

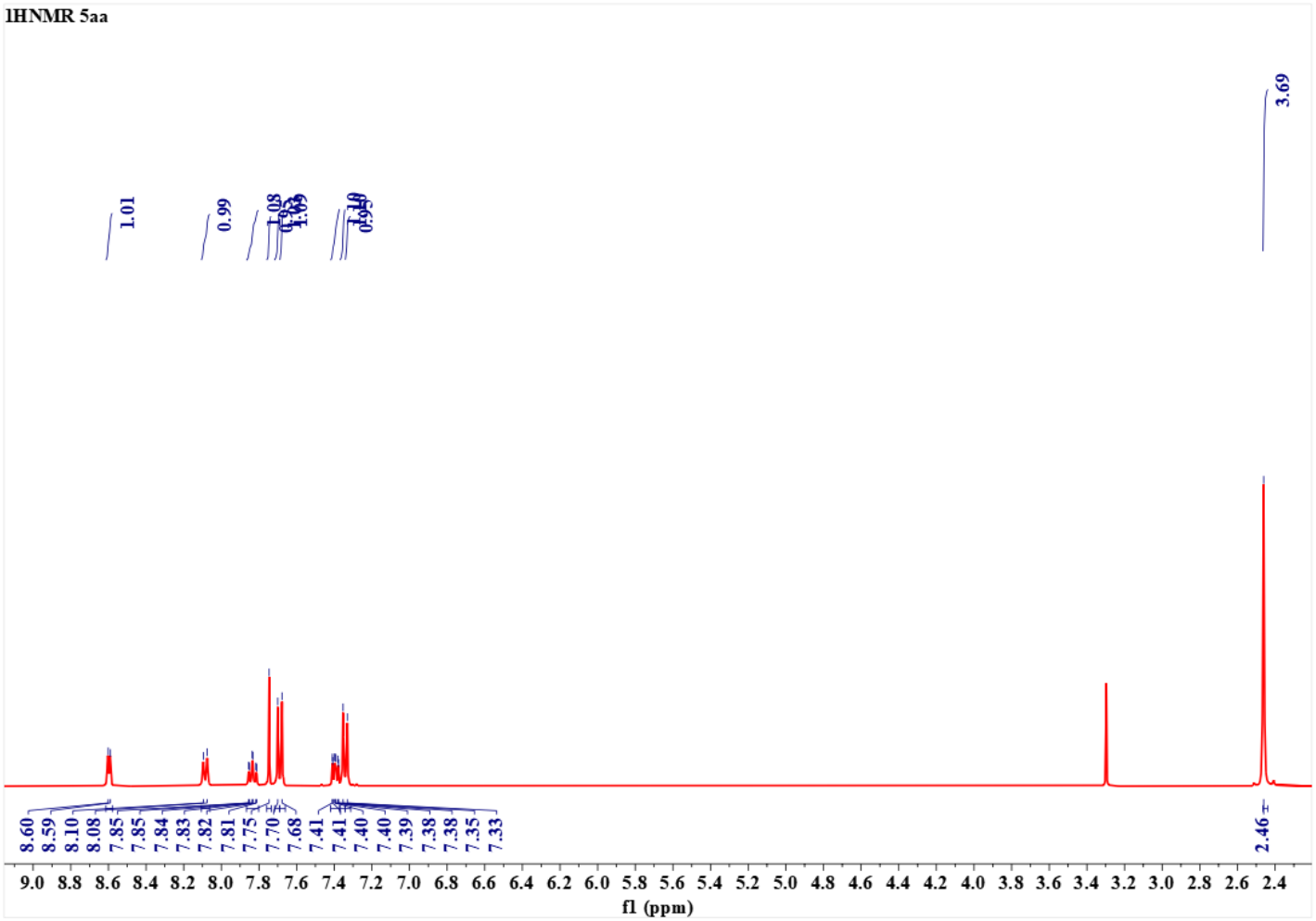


*Figure S53: ^{1}H NMR spectra of **5aa***

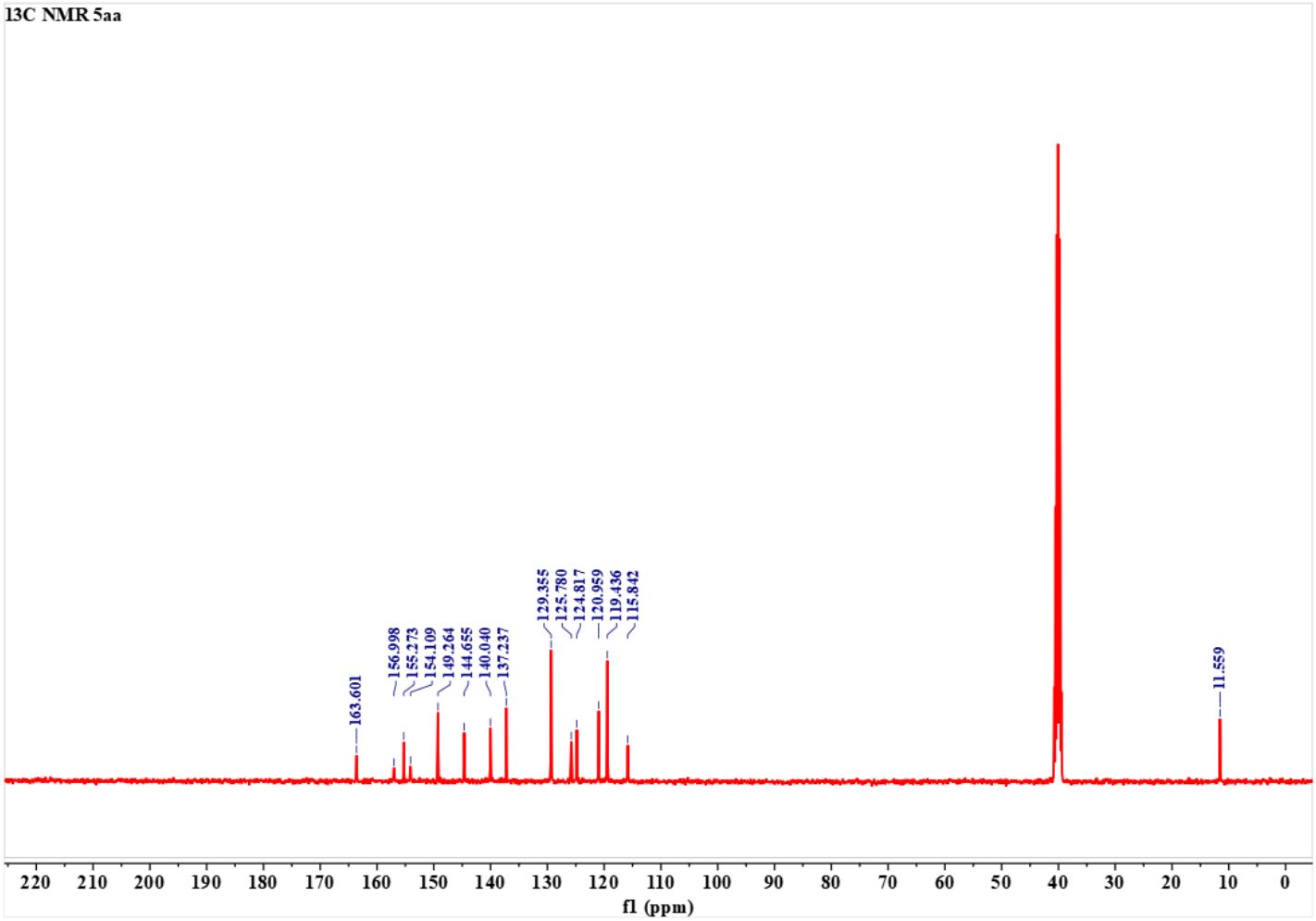


*Figure S54: ^{13}C NMR spectra of **5aa***

2D NMR

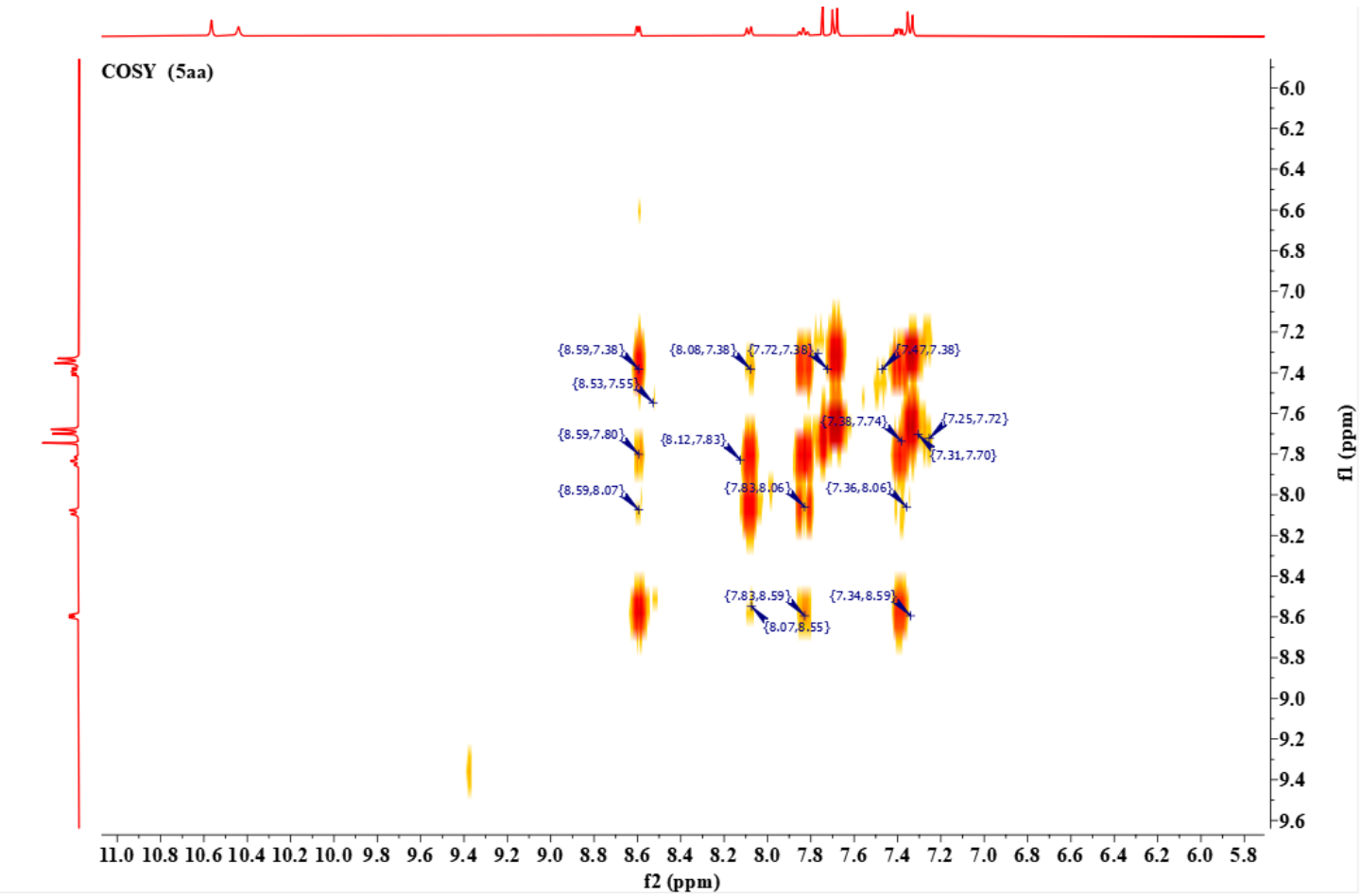


Figure S55: COSY correlation of **5aa**

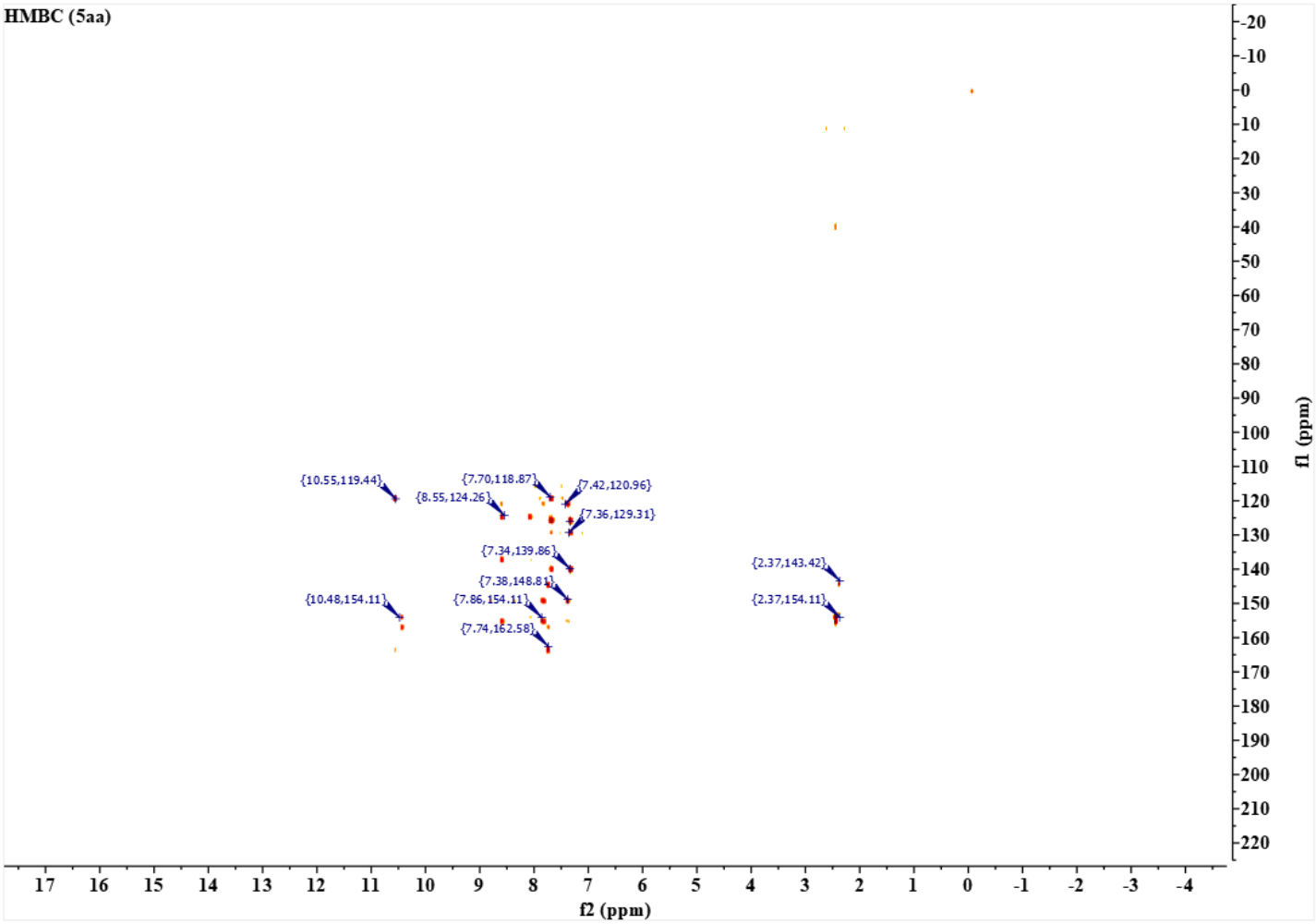


Figure S56: HMBC correlation of **5aa**

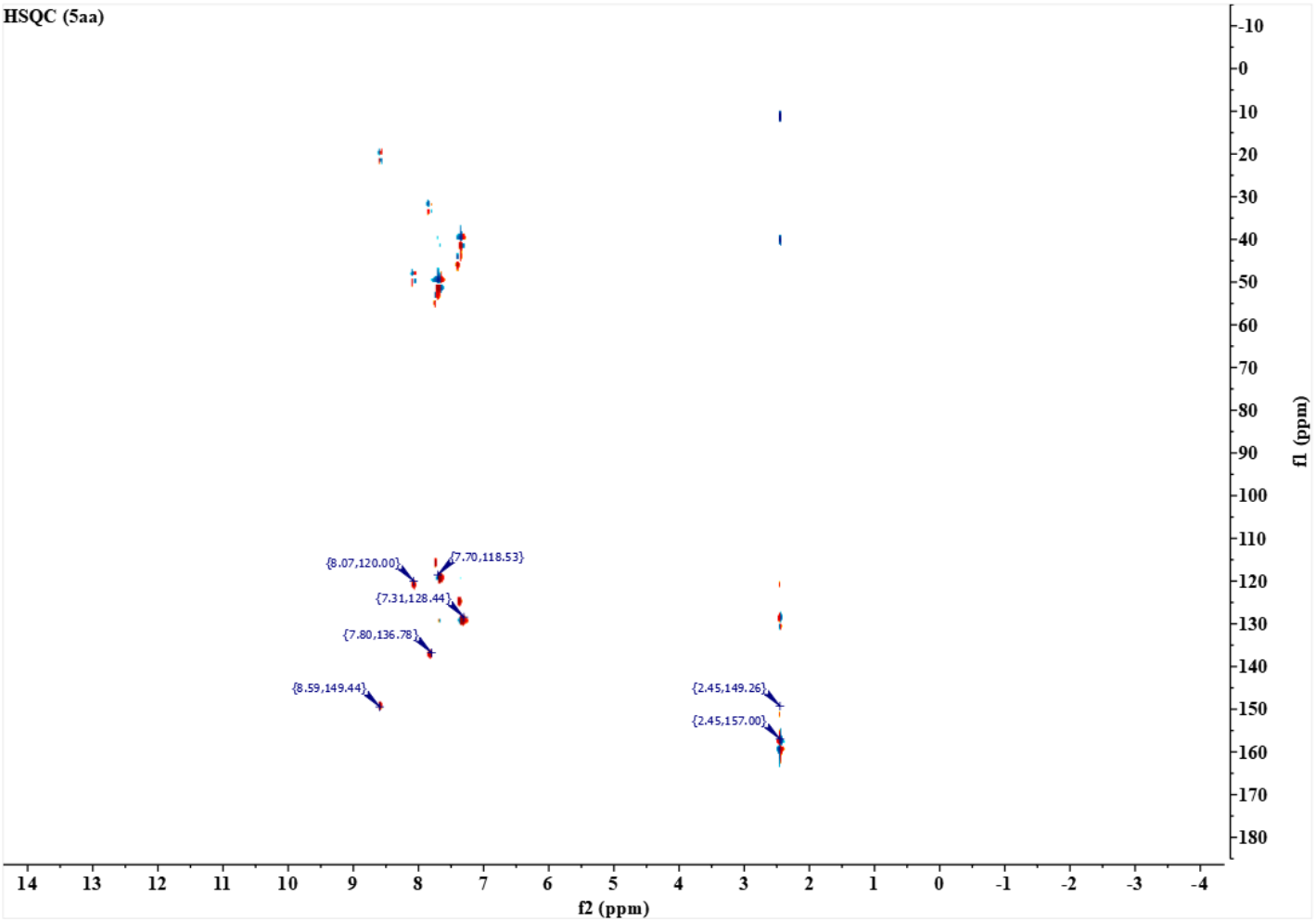


Figure S57: HSQC correlation of **5aa**

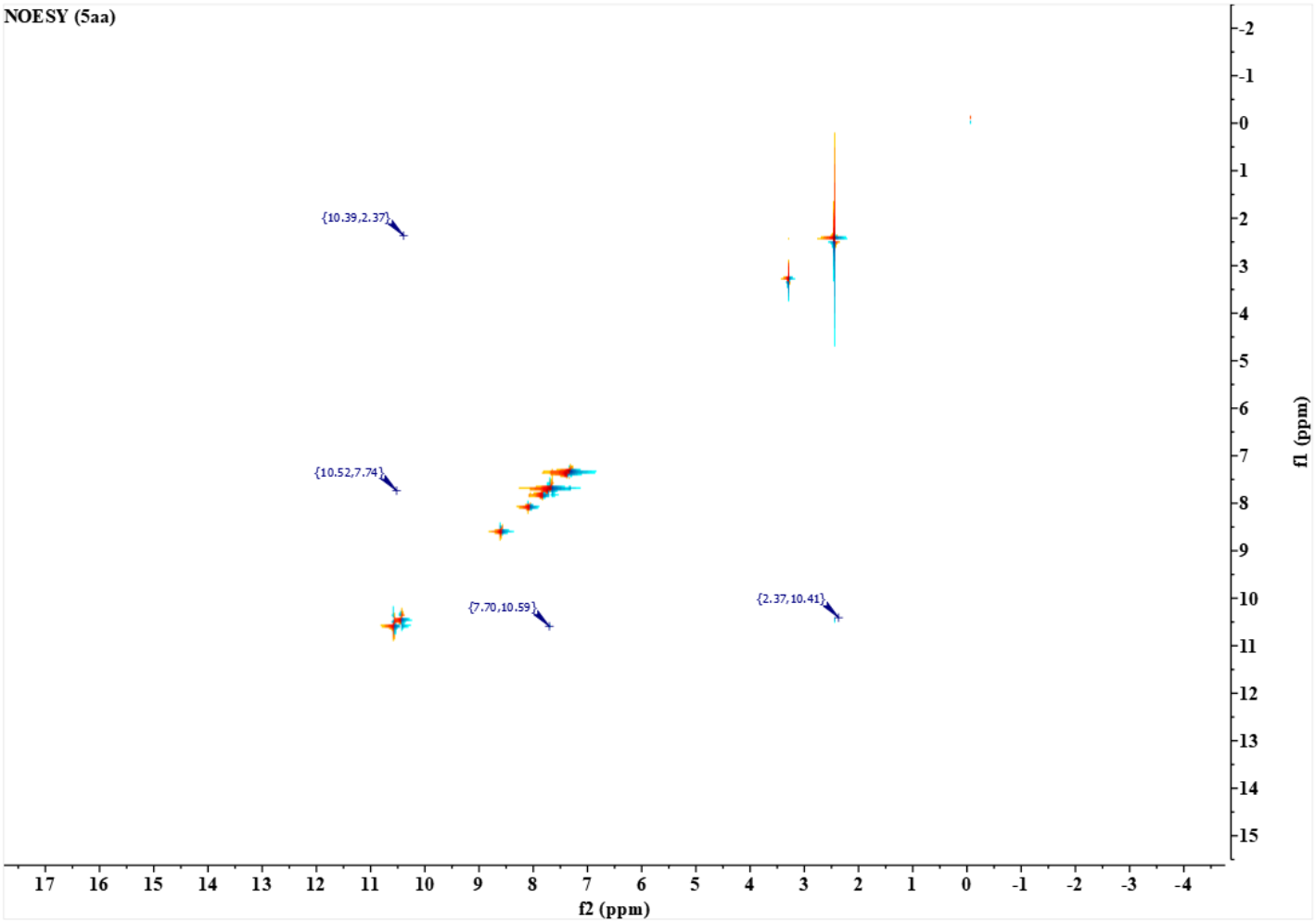


Figure S58: NOESY correlation of **5aa**

IR Data

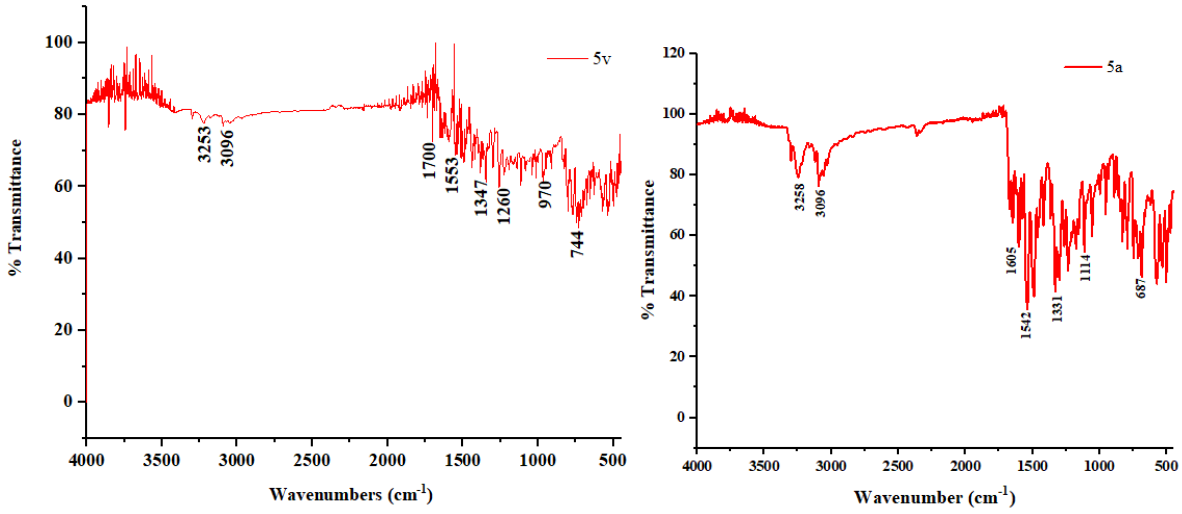


Figure S59: IR spectra of **5v** and **5a**

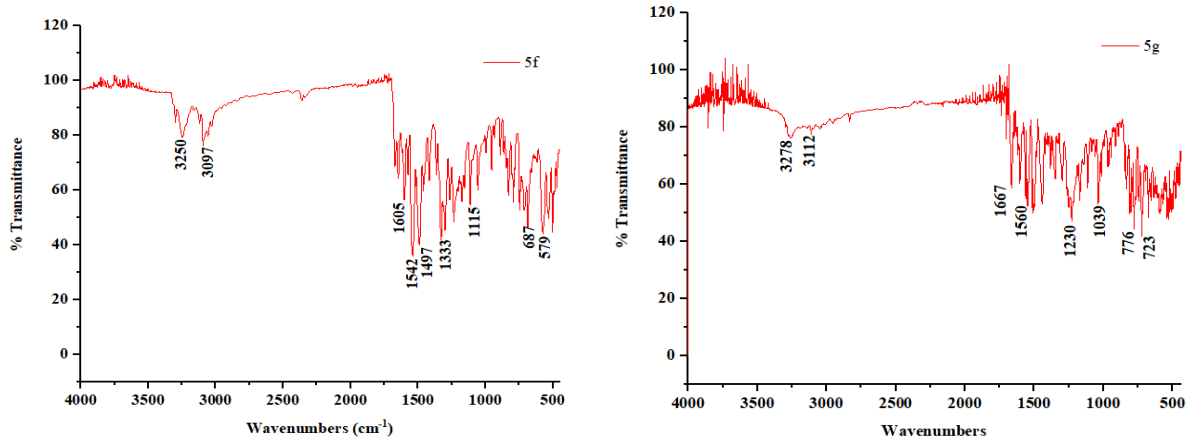


Figure S60: IR spectra of **5f** and **5g**

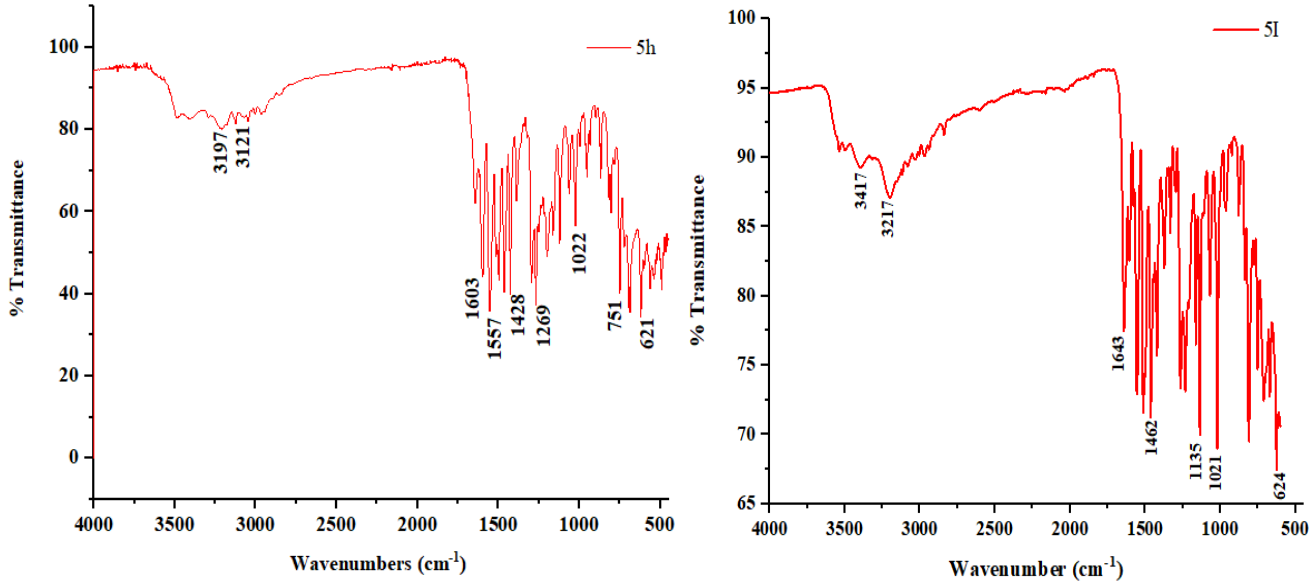


Figure S61: IR spectra of **5h** and **5l**

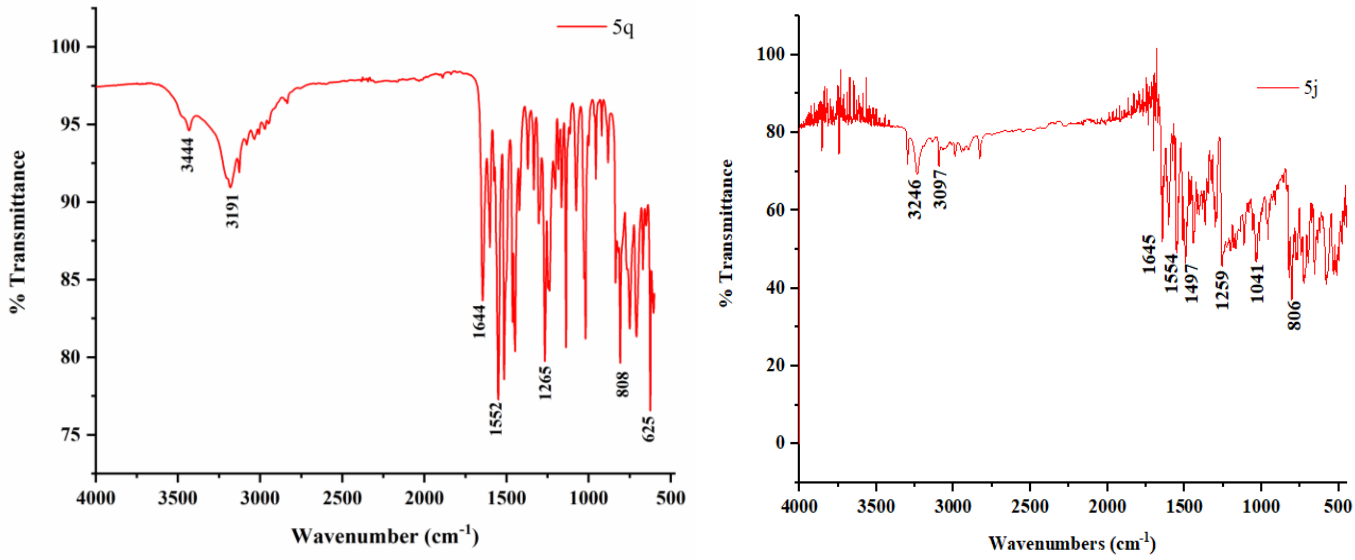


Figure S62: IR spectra of **5q** and **5j**

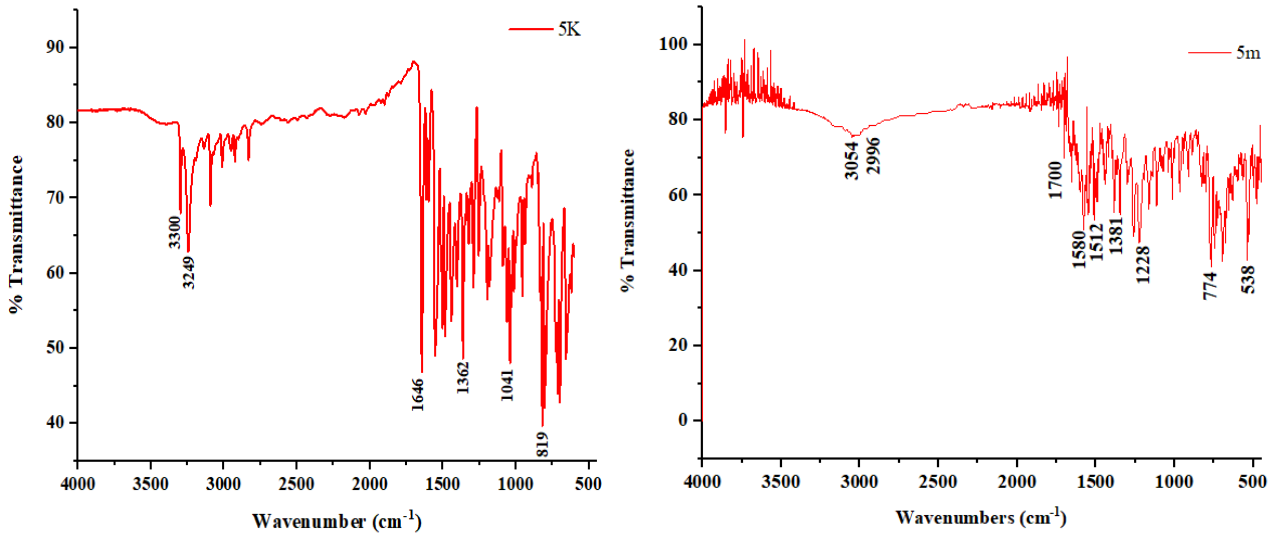


Figure S63: IR spectra of **5k** and **5m**

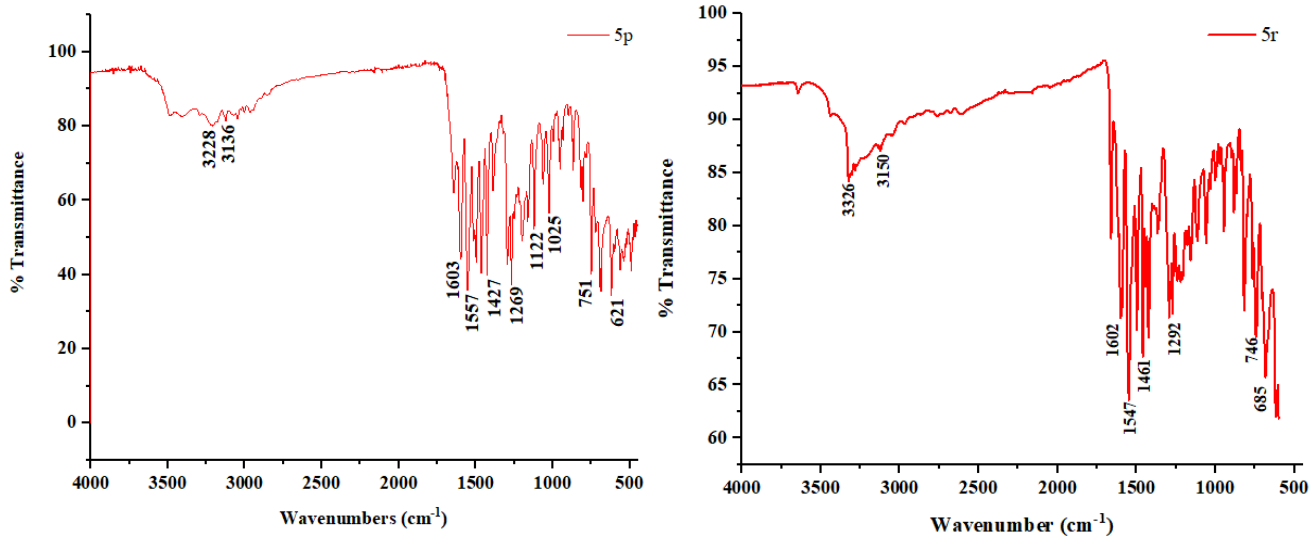


Figure S64: IR spectra of **5p** and **5r**

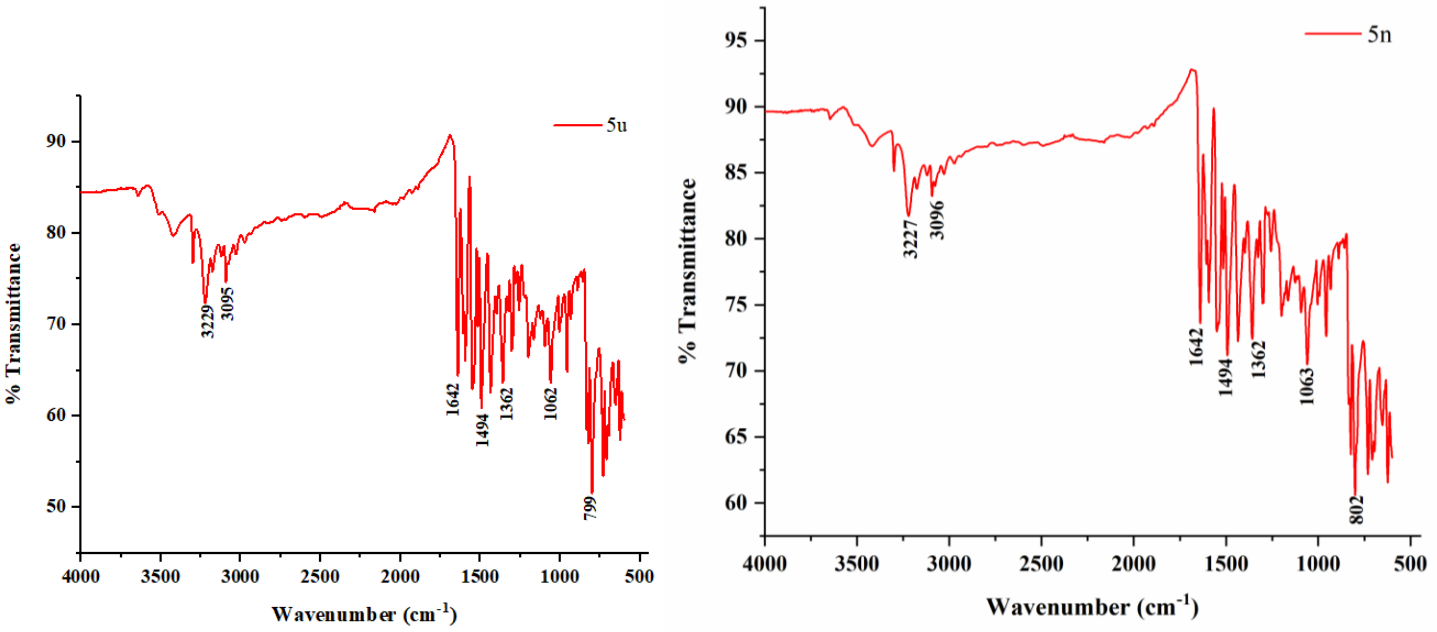


Figure S65: IR spectra of **5u** and **5n**

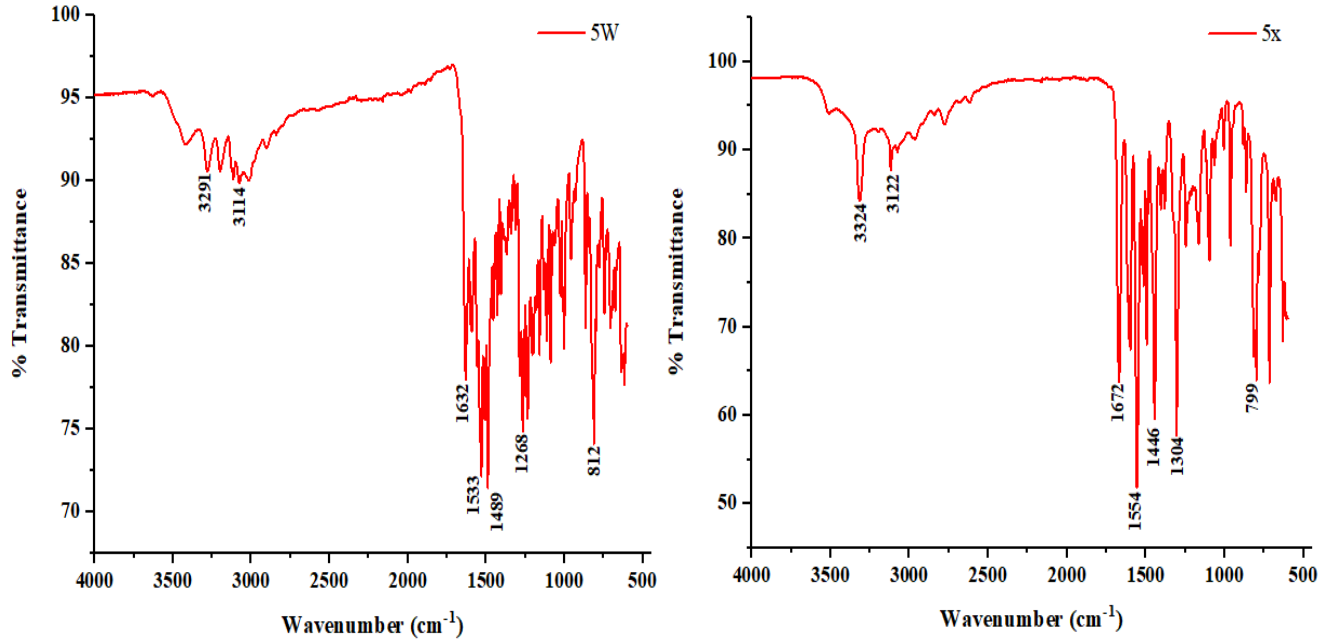


Figure S66: IR spectra of **5w** and **5x**

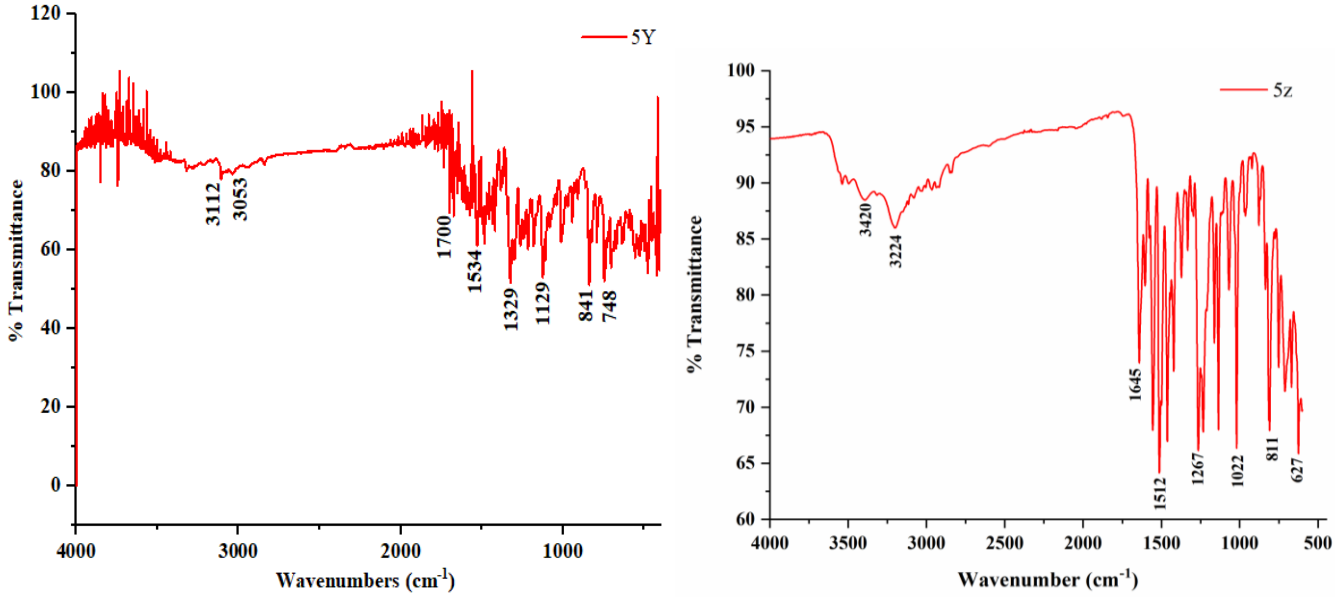


Figure S67: IR spectra of **5y** and **5z**

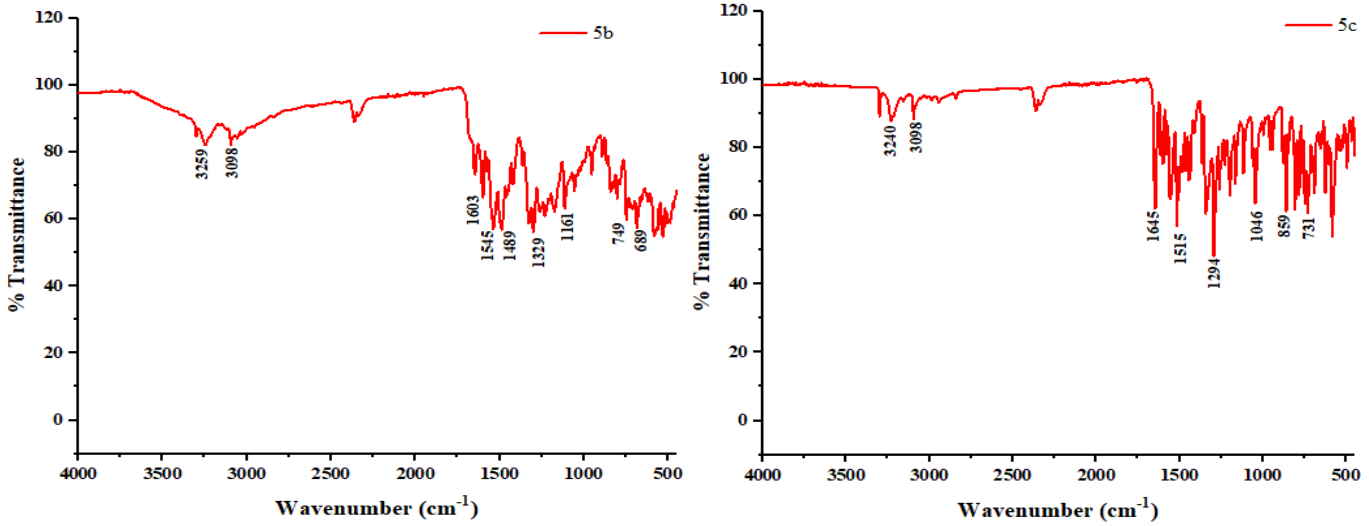


Figure S68: IR spectra of **5b** and **5c**

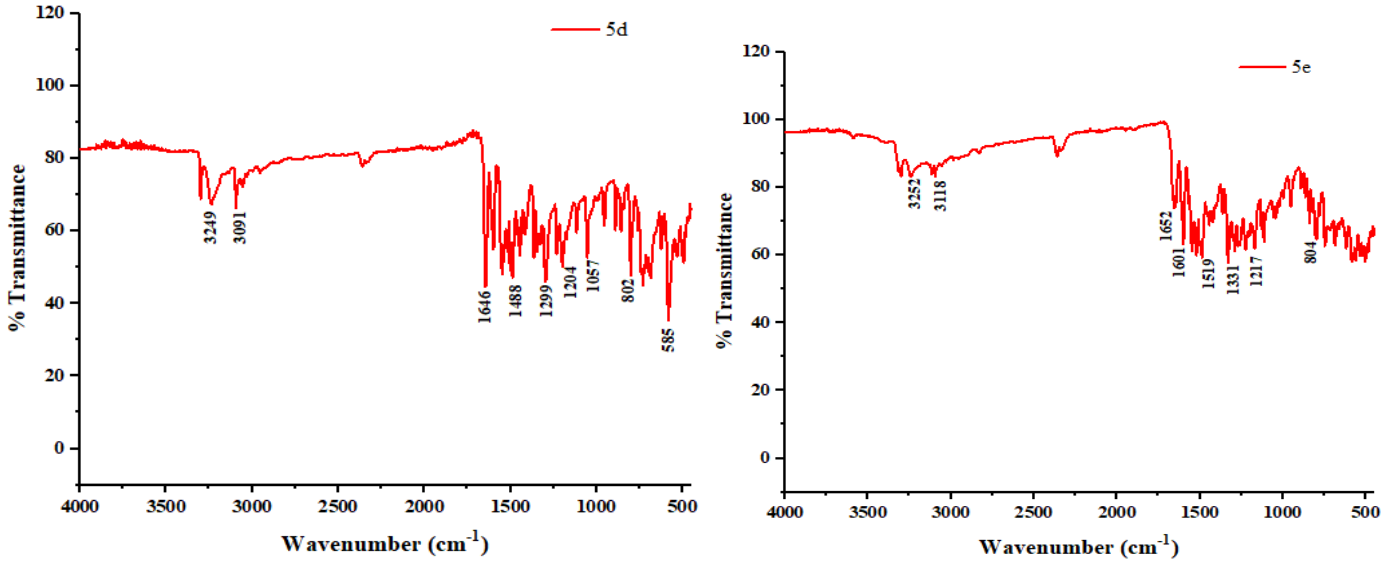


Figure S69: IR spectra of **5d** and **5e**

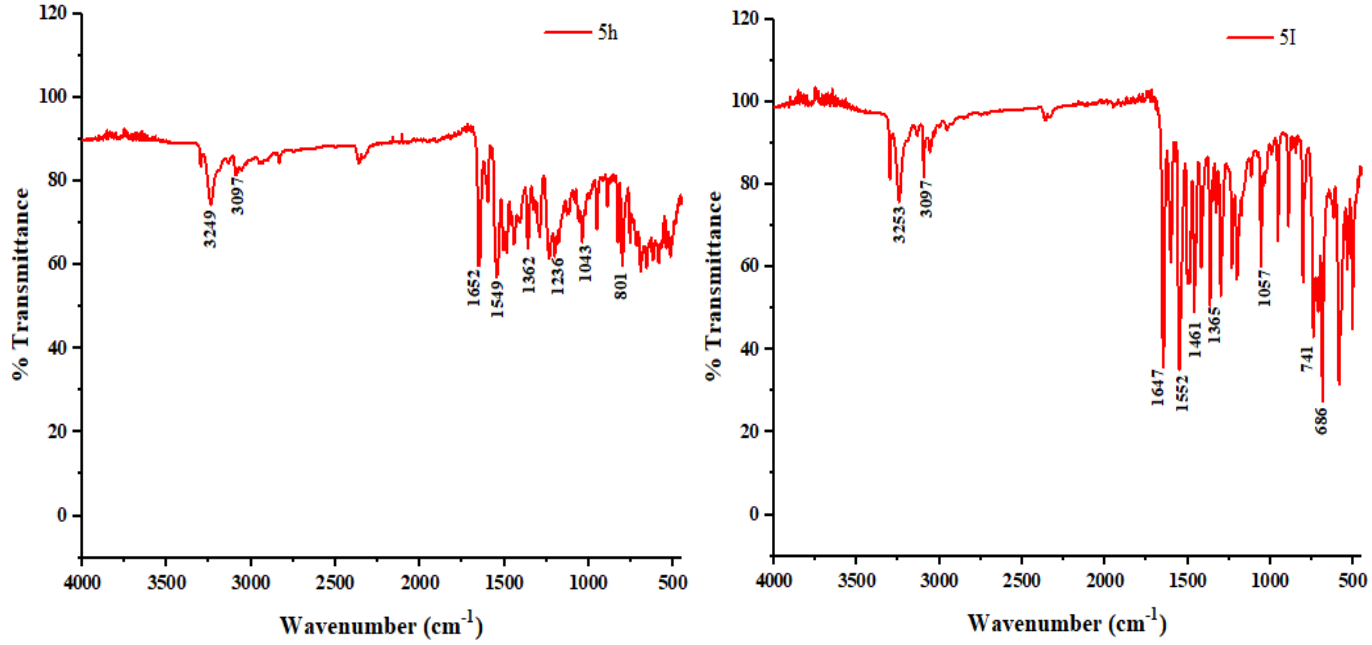


Figure S70: IR spectra of **5h** and **5l**

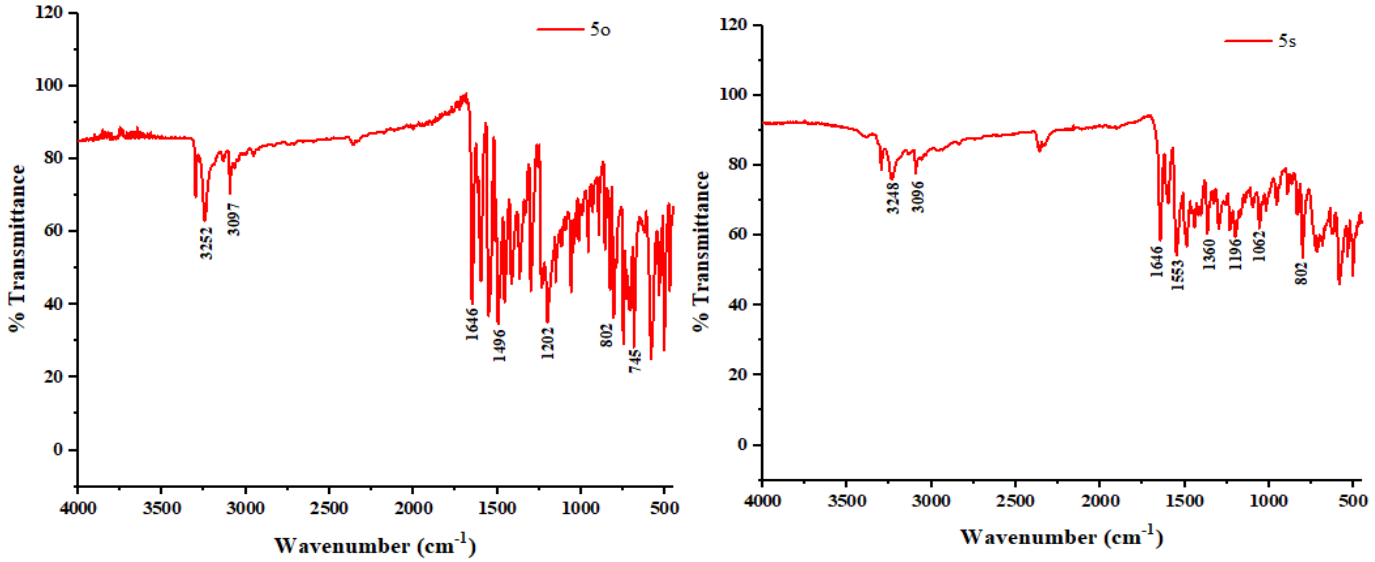


Figure S71: IR spectra of **5o** and **5s**

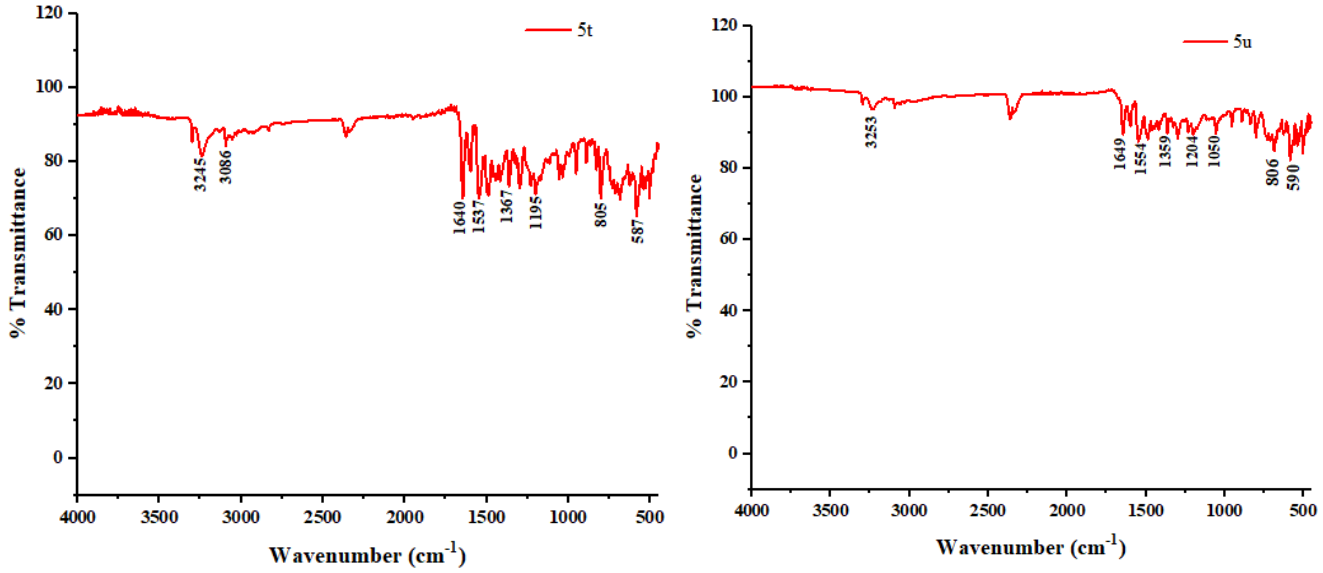


Figure S72: IR spectra of **5t** and **5u**

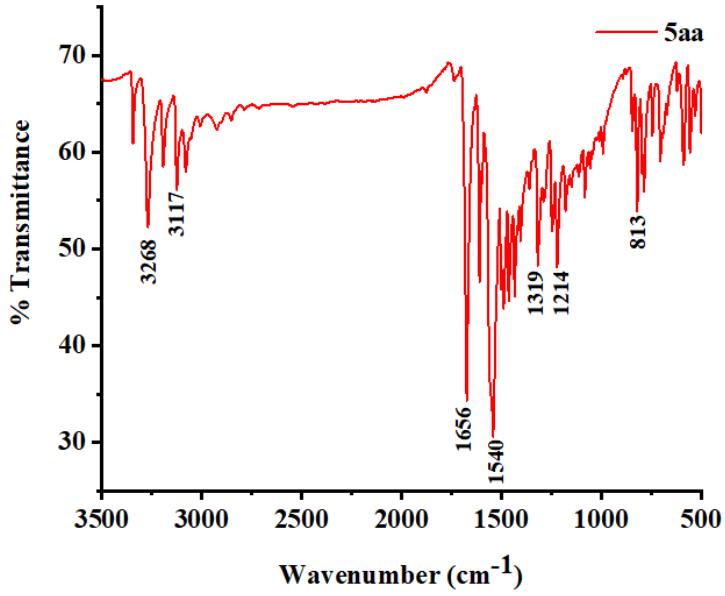


Figure S73: IR spectra of **5aa**

Table S2: **Bond Lengths for 5aa**

Bond Lengths for 5aa

Atom	Atom	Length/Å	Atom	Atom	Length/Å
S	C7	1.746(3)	N1	C17	1.327(4)
S	C8	1.715(3)	C9	C10	1.484(3)
Cl	C4	1.741(3)	C1	C2	1.396(3)
O	C10	1.217(3)	C1	C6	1.390(3)
N3	N2	1.368(3)	C13	C14	1.381(4)
N3	C10	1.348(3)	C8	C9	1.341(3)

N4	C7	1.303(3)	C2	C3	1.365(4)
N4	C9	1.381(3)	C3	C4	1.376(4)
N5	C1	1.402(3)	C6	C5	1.374(4)
N5	C7	1.359(3)	C4	C5	1.375(4)
N2	C11	1.286(3)	C14	C15	1.375(4)
C11	C13	1.479(3)	C15	C16	1.361(5)
C11	C12	1.494(4)	C16	C17	1.364(5)
N1	C13	1.329(3)			

Table S3: Crystal data and structure refinement parameters for **5b**

Identification code	**Data**
Empirical formula	$C_{17}H_{13}ClN_5OS$
Formula weight	370.83
Temperature/K	293(2)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	11.5221(5)
b/Å	15.2245(7)
c/Å	10.1523(5)
α/°	90
β/°	105.553(5)
γ/°	90
Volume/Å^3	1715.69(15)
Z	4
$_{\rho calc}$g/cm^3	1.436
μ/mm^{-1}	0.360
F(000)	764.0
Radiation	MoK_α (λ = 0.71073)
2Θ range for data collection/°	6.49 to 54.746
Index ranges	$-14 \leq h \leq 14$, $-18 \leq k \leq 18$, $-12 \leq l \leq 12$
Reflections collected	14897
Independent reflections	3646 [R_{int} = 0.0554, R_{sigma} = 0.0443]
Data/restraints/parameters	3646/0/227
Goodness-of-fit on F^2	1.050
Final R indexes [I > = 2σ (I)]	R_1 = 0.0526, wR_2 = 0.1369
Final R indexes [all data]	R_1 = 0.0902, wR_2 = 0.1613
Largest diff. peak/hole / e Å^{-3}	0.44/-0.26

BSA and alpha amylase Plots:

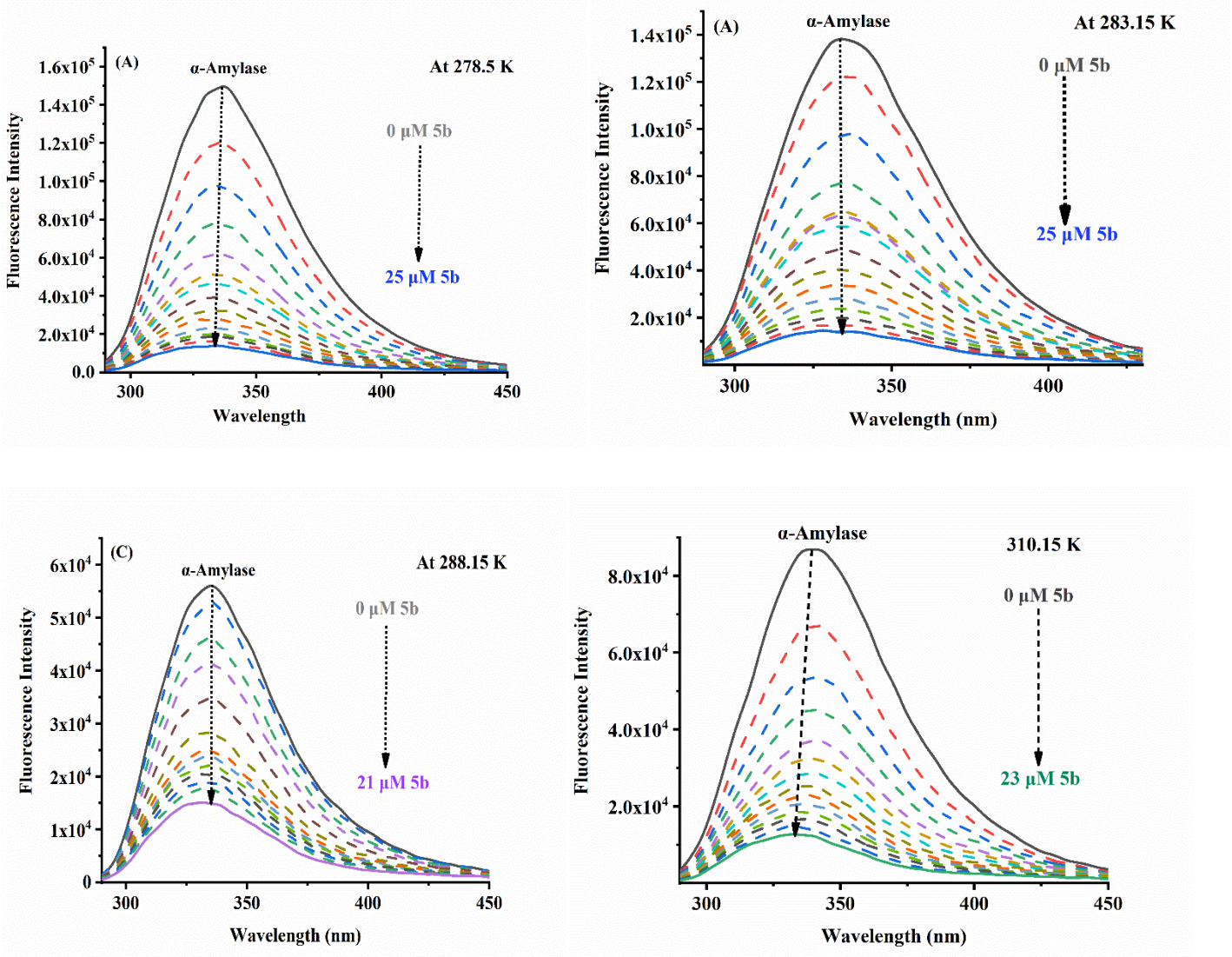
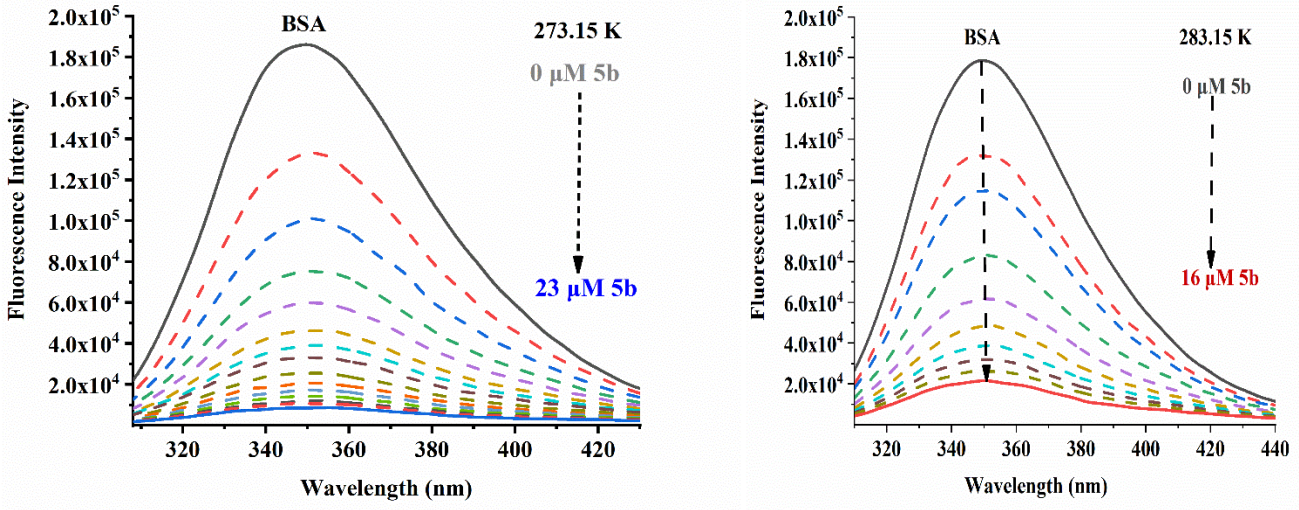


Figure S74: Fluorescence spectra of α-amylase titration with **5b** at different temperature such as 278.15 K, 283.15 K, 288.15 K, 310.15 K.



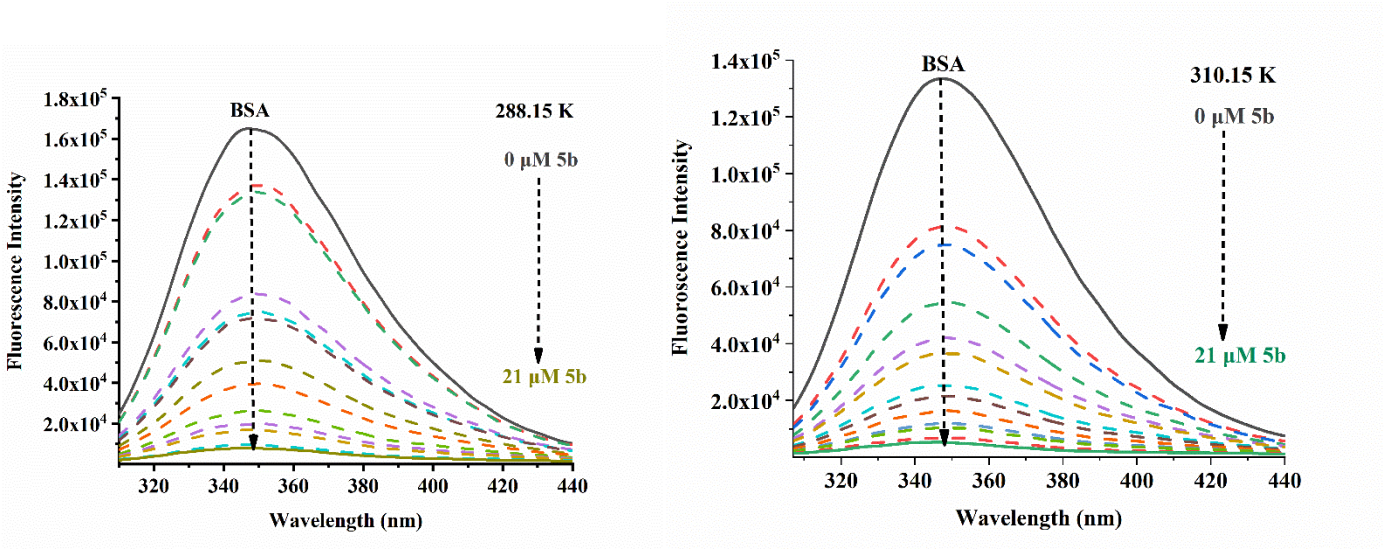


Figure S75: Fluorescence spectra of BSA titration with **5b** at different temperature such as 273.15 K, 283.15 K, 288.15 K, 310.15 K.

Docking images:

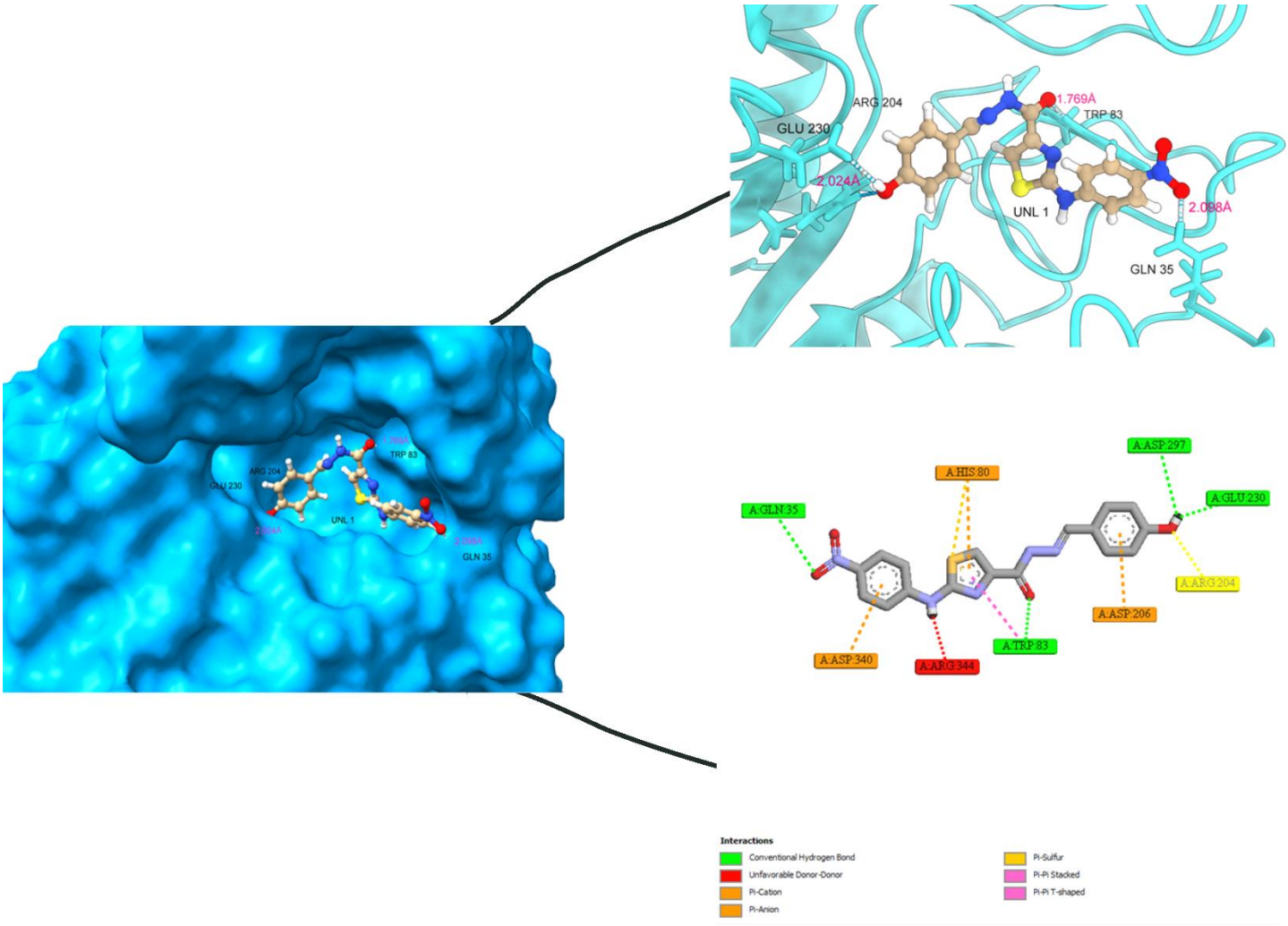


Figure S76: The ligand-protein interaction profile of *α*-amylase and **pose A** of 5b

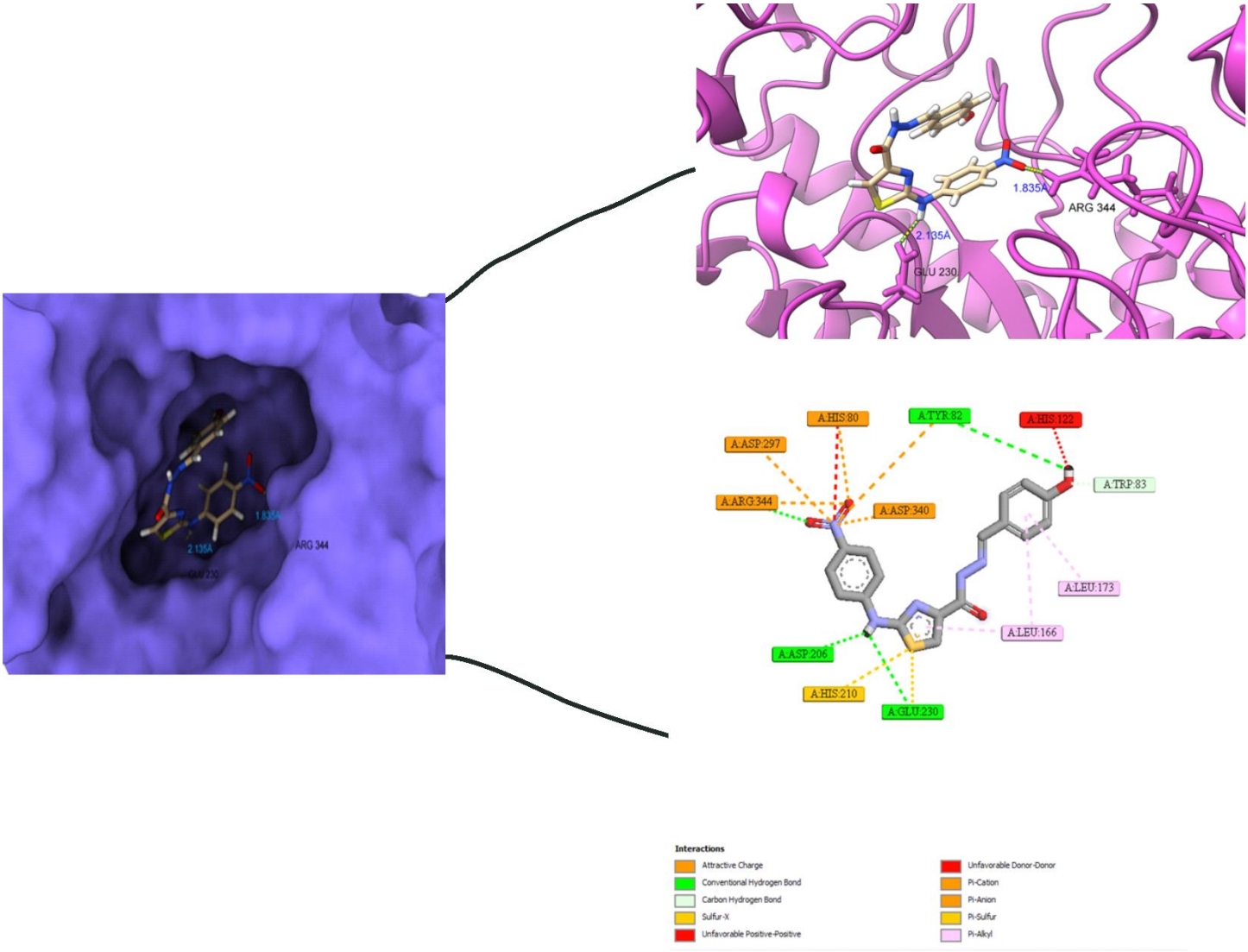


Figure S77: The ligand-protein interaction profile of α-amylase and **pose D** of 5b.

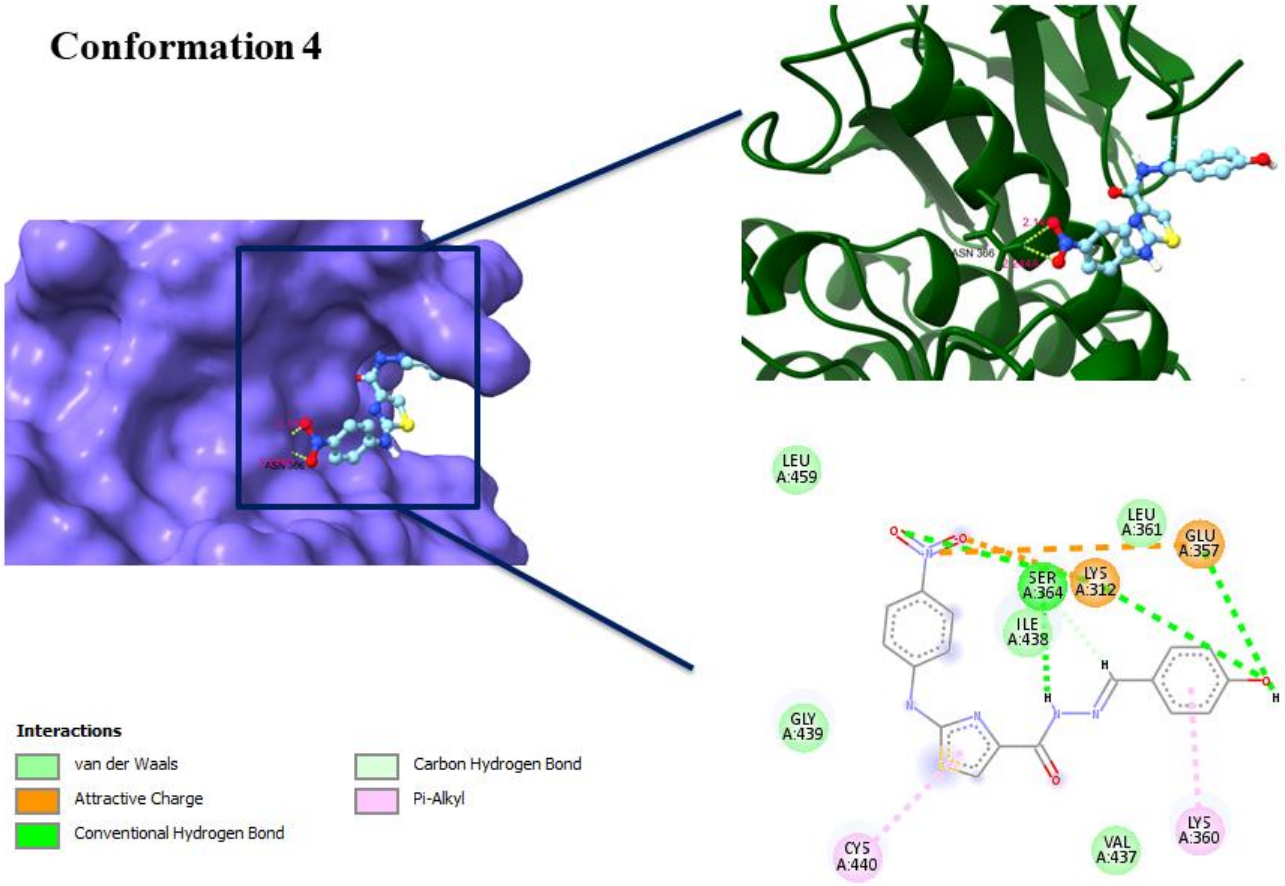


Figure S78: The ligand-protein interaction profile of α-amylase and **pose C** of 5b

Molecular dynamics graph:

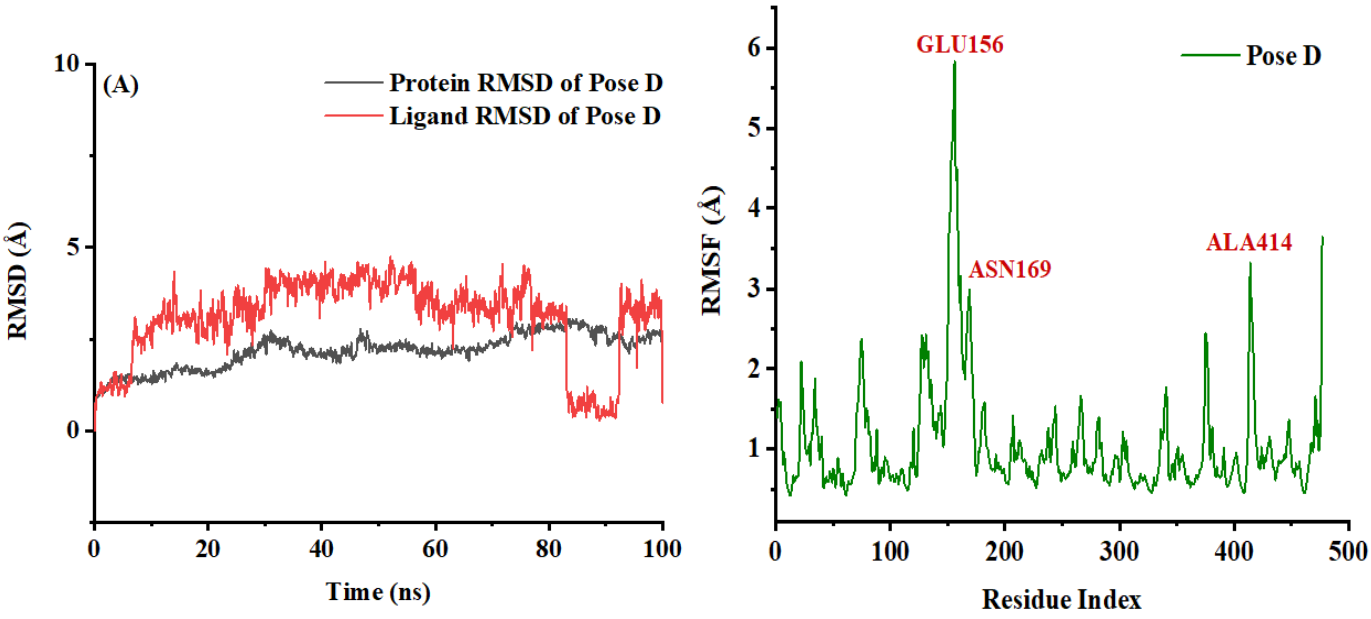


Figure S79: (A) RMSD of protein and ligand in 100 ns molecular dynamics simulation with **pose D** and (B) RMSF of complex in 100 ns molecular dynamics simulation.

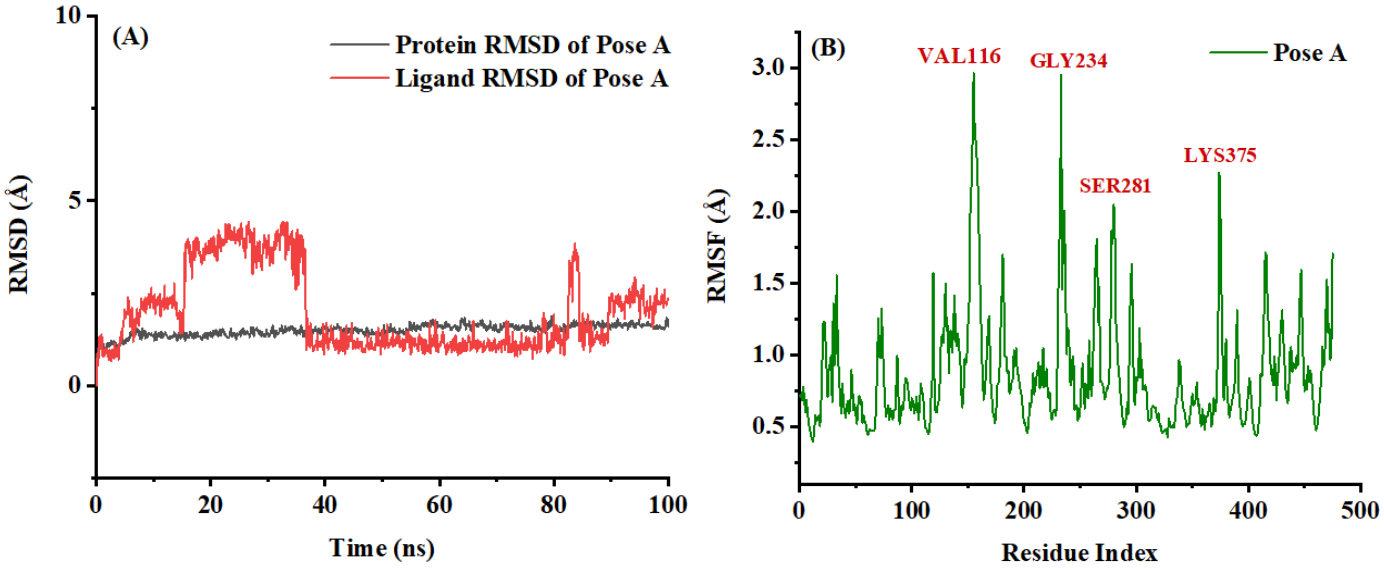


Figure S80: (A) RMSD of protein and ligand in 100 ns molecular dynamics simulation with **pose A** and (B) RMSF of complex in 100 ns molecular dynamics simulation.

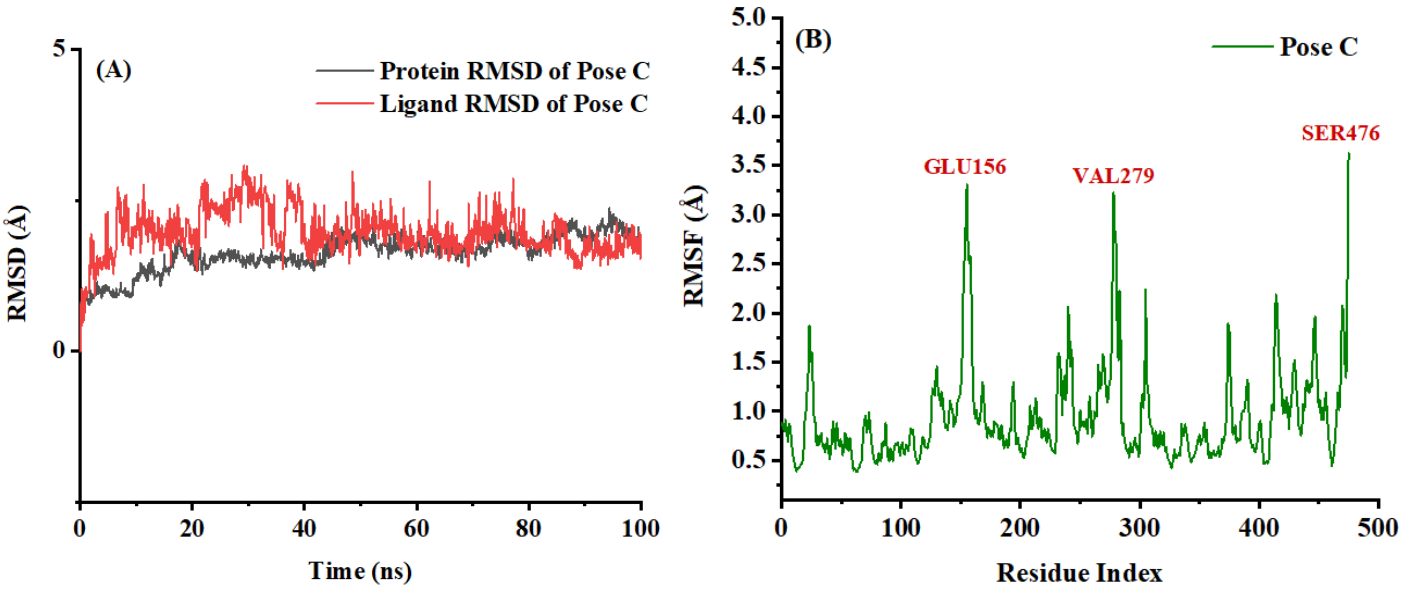


Figure S81: (A) RMSD of protein and ligand in 100 ns molecular dynamics simulation with **pose C** and (B) RMSF of complex in 100 ns molecular dynamics simulation.

Table S4: Interaction detail for pose B against the α-amylase enzyme

Name	Distance	Category	Type	From	From chemistry	To	To chemistry
Interactions of pose B							
CYS475-UNL1	2.13352	H Bond	Conventional H-Bond	CYS475:HN	H-Donor	UNL1:N5	H-Acceptor
GLY56- UNL1	2.97914	H Bond	C-H Bond	GLY56:CA	H-Donor	UNL1:O3	H-

ALA367-UNL1	3.09702	H Bond	C-H Bond	ALA367:CA	H-Donor	UNL1:O1	H-Acceptor
ALA367-UNL1	3.30973	Hydrophobic	π-σ	ALA367:CB	C-H	UNL1	π-Orbitals
LYS473-UNL1	2.93767	Other	π -Lone Pair	LYS473:O	Lone Pair	UNL1	π-Orbitals
UNL1-ALA363	4.77633	Hydrophobic	π -Alkyl	UNL1	π-Orbitals	ALA363	Alkyl
UNL1-ALA367	4.30228	Hydrophobic	π -Alkyl	UNL1	π-Orbitals	ALA367	Alkyl
UNL1-ALA363	4.75605	Hydrophobic	π -Alkyl	UNL1	π-Orbitals	ALA363	Alkyl
UNL1-LYS473	5.48311	Hydrophobic	π -Alkyl	UNL1	π-Orbitals	LYS473	Alkyl

Table S5: Interaction detail for pose D against the α-amylase enzyme

Pose D							
Name	Distance	category	Type	From	From chemistry	To	To chemistry
ARG344-UNL1	1.83522	H Bond	Conventional H Bond	ARG344:HH22	H-Donor	UNL1:O	H-Acceptor
UNL1- ASP206	2.73149	H Bond	Conventional H Bond	UNL1:H	H-Donor	ASP206:OD1	H-Acceptor
UNL1- GLU230	2.1354	H Bond	Conventional H Bond	UNL1:H	H-Donor	GLU230:OE1	H-Acceptor
UNL1-TYR82	2.15874	H Bond	Conventional H Bond	UNL1:H	H-Donor	TYR82:O	H-Acceptor
TRP83-UNL1	3.08257	H Bond	C-H Bond	TRP83:CD1	H-Donor	UNL1:O	H-Acceptor
UNL1- GLU230	2.957	Other	Sulfur-X	UNL1:S	Sulfur	GLU230:OE1	O,N,S
UNL1-HIS210	4.99723	Other	π-Sulfur	UNL1:S	Sulfur	HIS210	π-Orbitals
UNL1-UNL1	4.03737	Hydrophobic	π-π Stacked	UNL1	π-Orbitals	UNL1	π-Orbitals
UNL1-LEU166	5.41429	Hydrophobic	π-Alkyl	UNL1	π-Orbitals	LEU166	Alkyl
UNL1- LEU166	5.03294	Hydrophobic	π-Alkyl	UNL1	π-Orbitals	LEU166	Alkyl
UNL1-LEU173	4.5615	Hydrophobic	π-Alkyl	UNL1	π-Orbitals	LEU173	Alkyl

Table S6: Interaction detail for pose A against the α-amylase enzyme.

Pose A							
Name	Distance	category	Type	From	From chemistry	To	To chemistry
TYR335- UNL1	2.08639	H Bond	Conventional Hydrogen Bond	TYR335:HH	H-Donor	UNL1:O3	H-Acceptor
UNL1-TYR335	1.73456	H Bond	Conventional Hydrogen Bond	UNL1:H2	H-Donor	TYR335:OH	H-Acceptor
UNL1-ALA346	2.61165	H Bond	Conventional Hydrogen Bond	UNL1:H2	H-Donor	ALA346:O	H-Acceptor
UNL1- THR32	2.0567	H Bond	Conventional Hydrogen Bond	UNL1:H3	H-Donor	THR32:OG1	H-Acceptor
THR29-UNL1	2.81299	H Bond	Carbon Hydrogen Bond	THR29:CA	H-Donor	UNL1:O2	H-Acceptor
LEU349-UNL1	3.66429	Hydrophobic	π-σ	LEU349:CD1	C-H	UNL1	π -Orbitals
UNL1-UNL1	4.73062	Hydrophobic	π-π T-shaped	UNL1	π -Orbitals	UNL1	π -Orbitals
UNL1-LEU349	5.41643	Hydrophobic	π-Alkyl	UNL1	π -Orbitals	LEU349	Alkyl

Table S7: Interaction detail for pose C against the α-amylase enzyme.

Pose C							
Name	Distance	category	Type	From	From chemistry	To	To chemistry
UNL1-UNL1	5.37382	Hydrophobic	π-π T-shaped	UNL1	π -Orbitals	UNL1	π -Orbitals
UNL1-VAL171	5.43811	Hydrophobic	π -Alkyl	UNL1	P π-Orbitals	VAL171	Alkyl
UNL1-ASP340	2.16191	H Bond; Electrostatic	Salt Bridge; Attractive Charge	UNL1:H2	H Donor; Positive	ASP340:OD2	H-Acceptor; Negative
UNL1-VAL171	1.80986	H Bond	Conventional H Bond	UNL1:H3	H-Donor	VAL171:O	H-Acceptor
UNL1-ASP297	5.43741	Electrostatic	Attractive Charge	UNL1:N4	Positive	ASP297:OD1	Negative
UNL1-UNL1	5.59105	Other	π -Sulfur	UNL1:S1	Sulfur	UNL1	π -Orbitals
UNL1-TRP83	5.72309	Other	π -Sulfur	UNL1:S1	Sulfur	TRP83	π-Orbitals
ARG344-UNL1	1.87237	H Bond	Conventional H Bond	ARG344:HH22	H-Donor	UNL1:O3	H-Acceptor
HIS210-UNL1	2.35849	H Bond	Conventional H Bond	HIS210:HE2	H-Donor	UNL1:O1	H-Acceptor
HIS80 -UNL1	5.21914	Hydrophobic	π-π T-shaped	HIS80	π-Orbitals	UNL1	π -Orbitals
LEU166CD-UNL1	3.66839	Hydrophobic	π-Sigma	LEU166:CD1	C-H	UNL1	π -Orbitals

MMGBSA

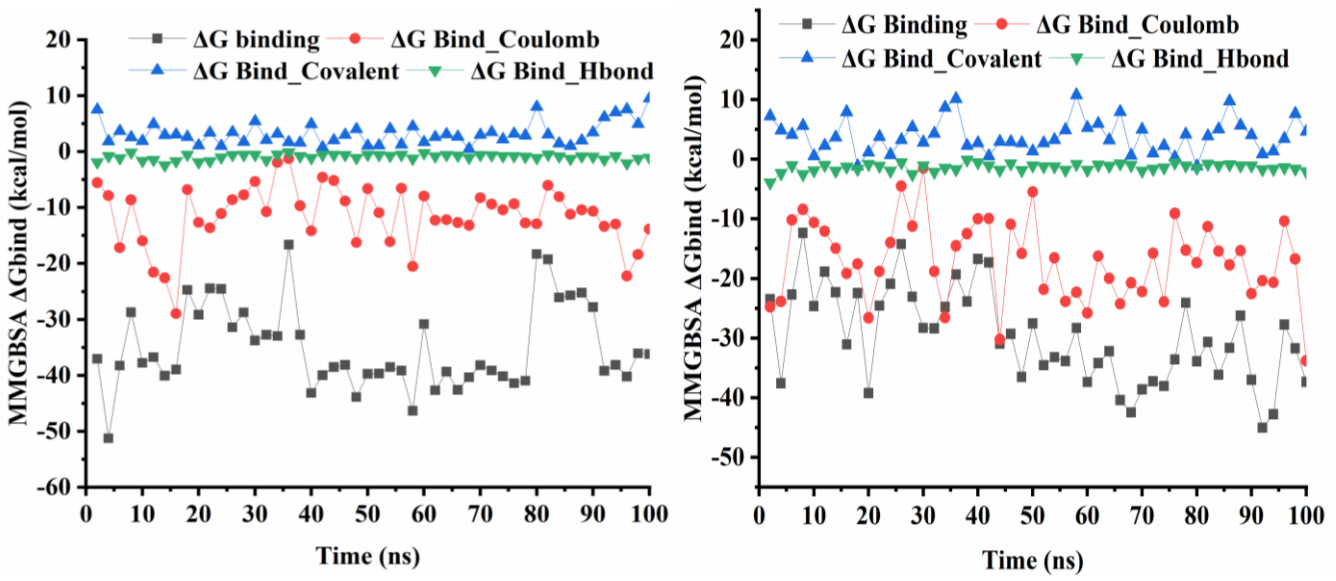


Figure S82: ***Pose A and pose B***

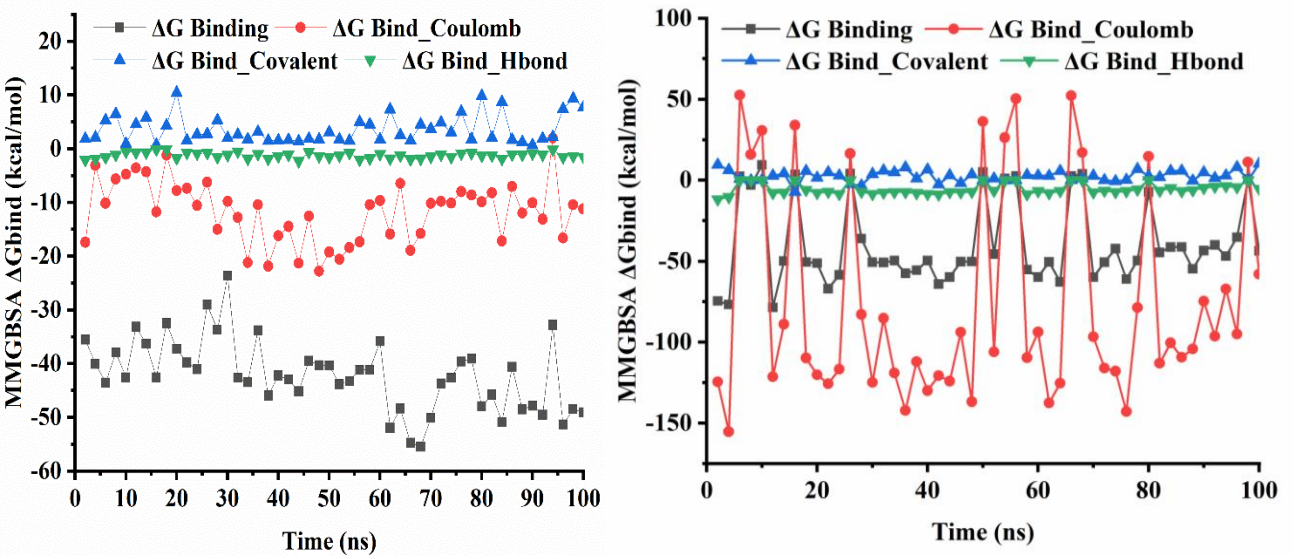


Figure S83: ***Pose D*** and **acarbose**

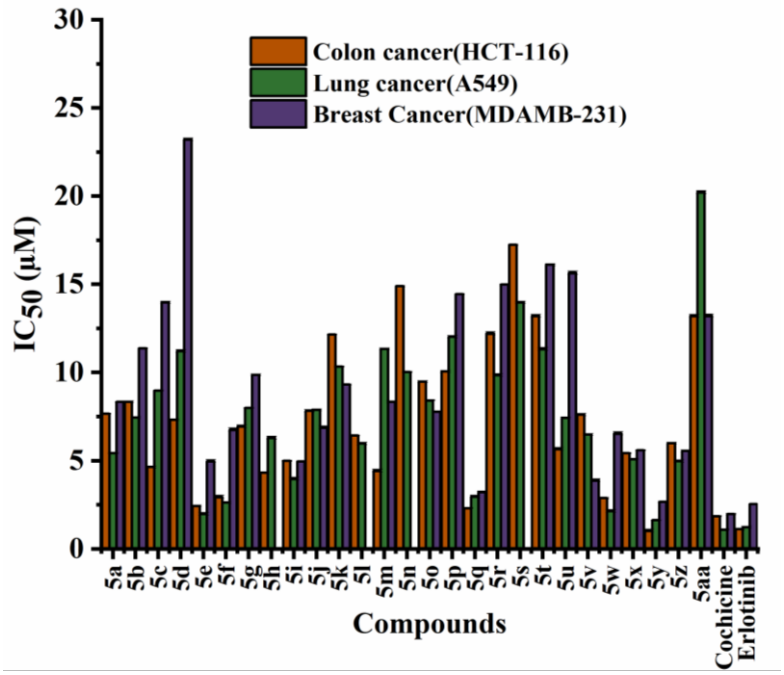


Figure S84: Comparative analysis of IC_{50} values of the synthesized compounds with standard drug cochicine and erlotinib.

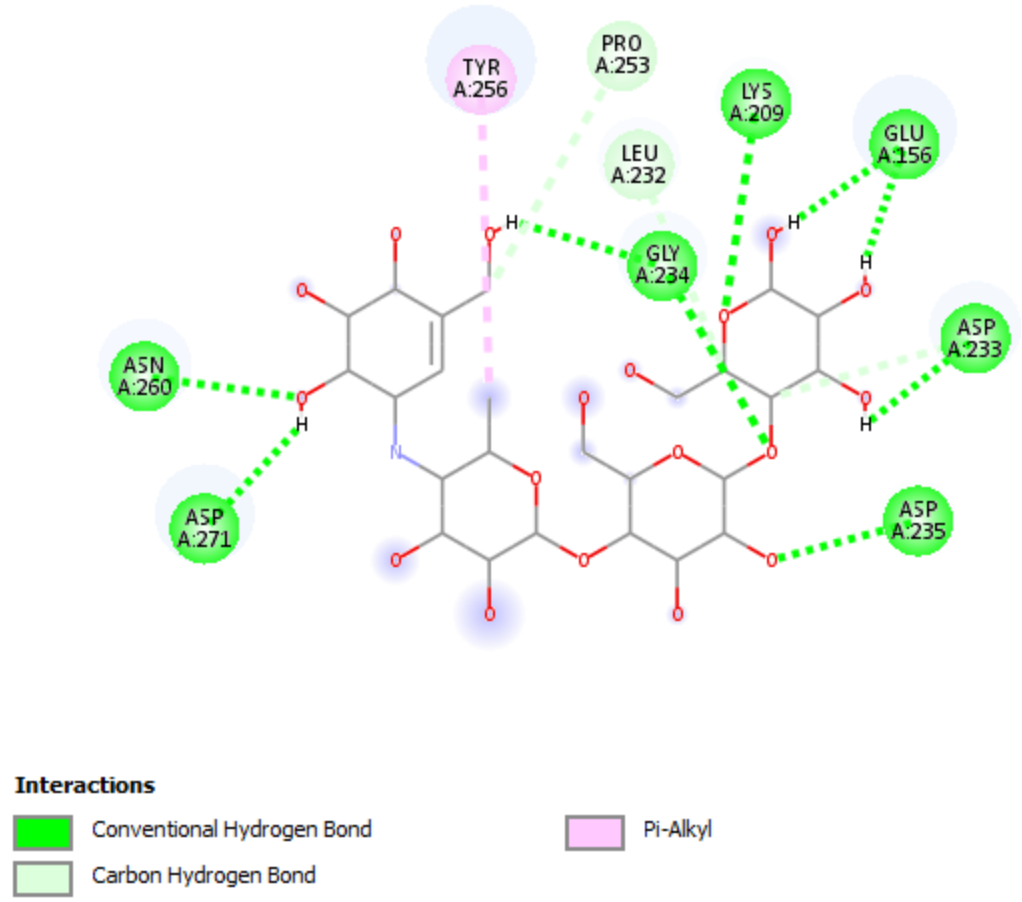


Figure S85: Acarbose 2D interctions

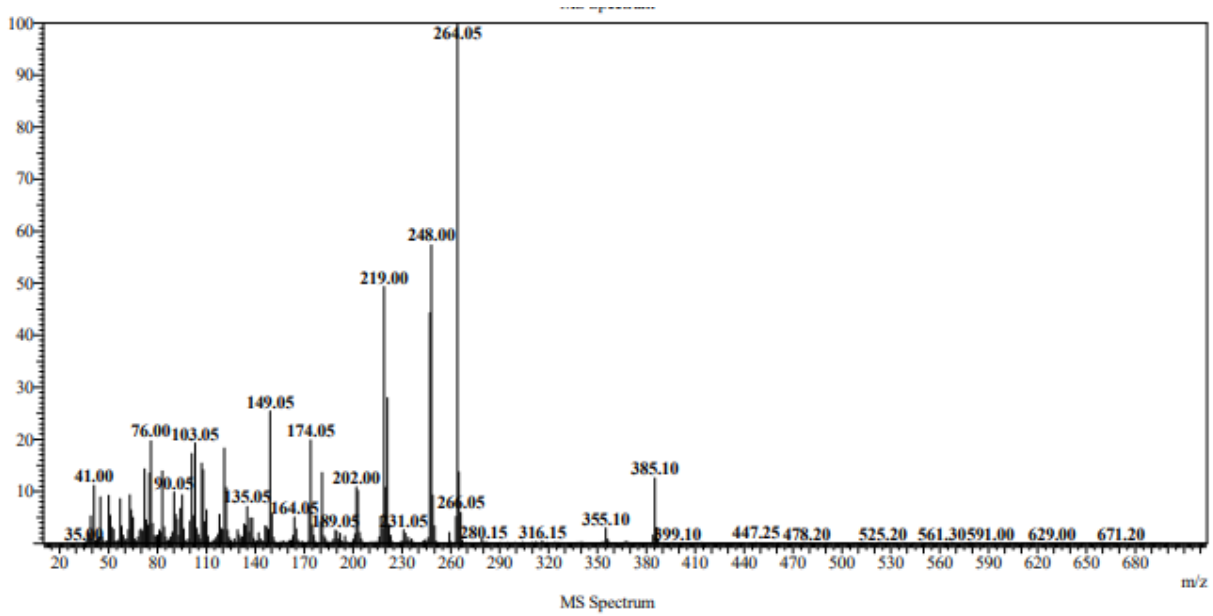


Figure S86: Mass spectrum of ***5a***

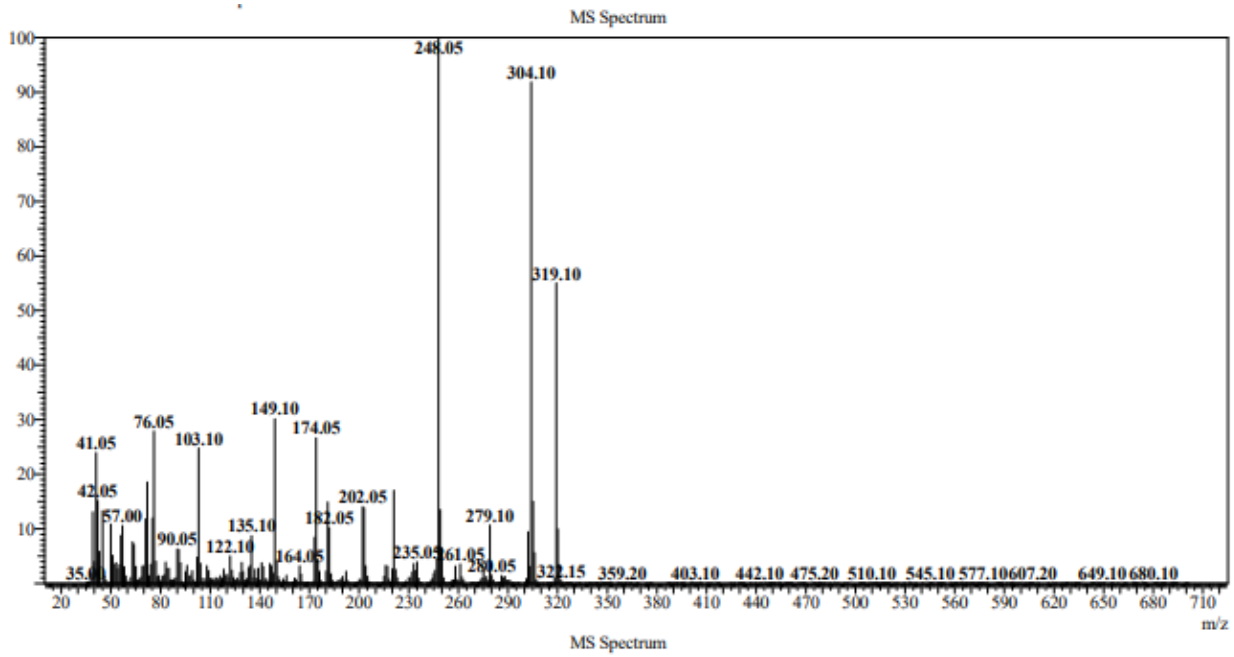


*Figure S87: Mass spectrum of **5b***

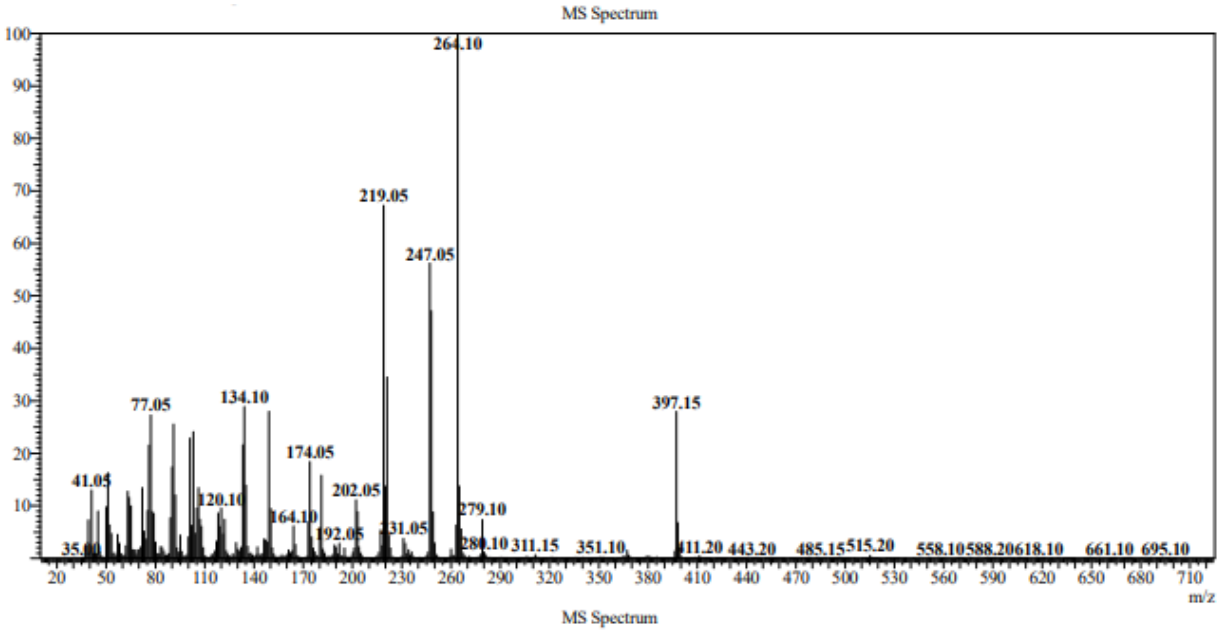


*Figure S88: Mass spectrum of **5c***

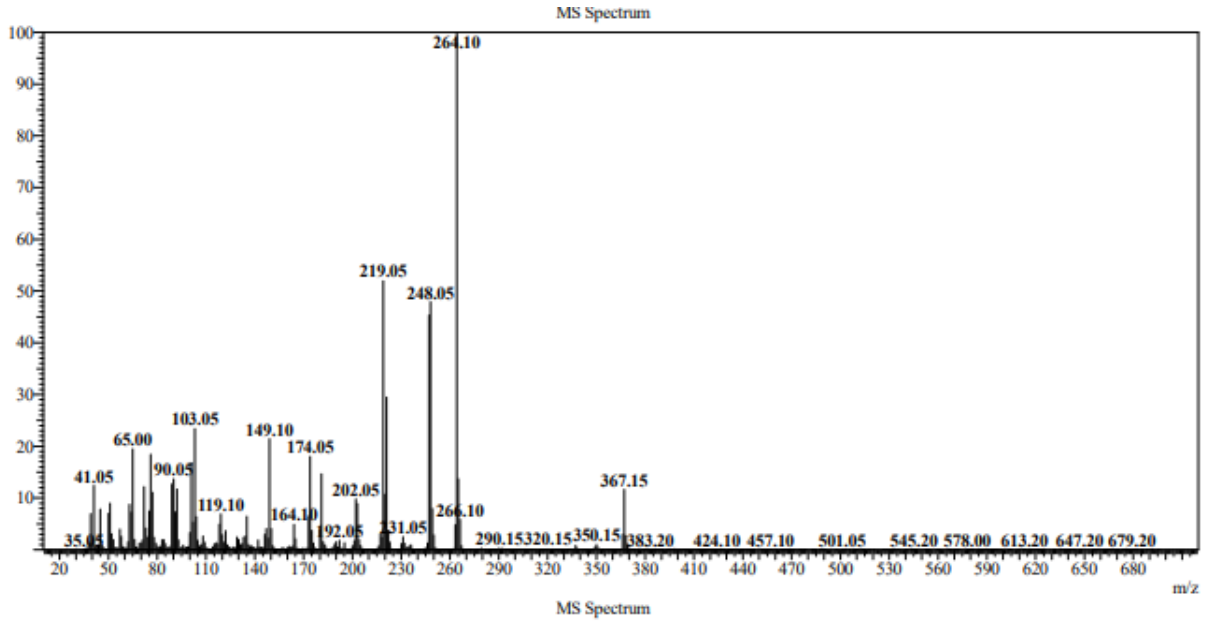


*Figure S89: Mass spectrum of **5d***

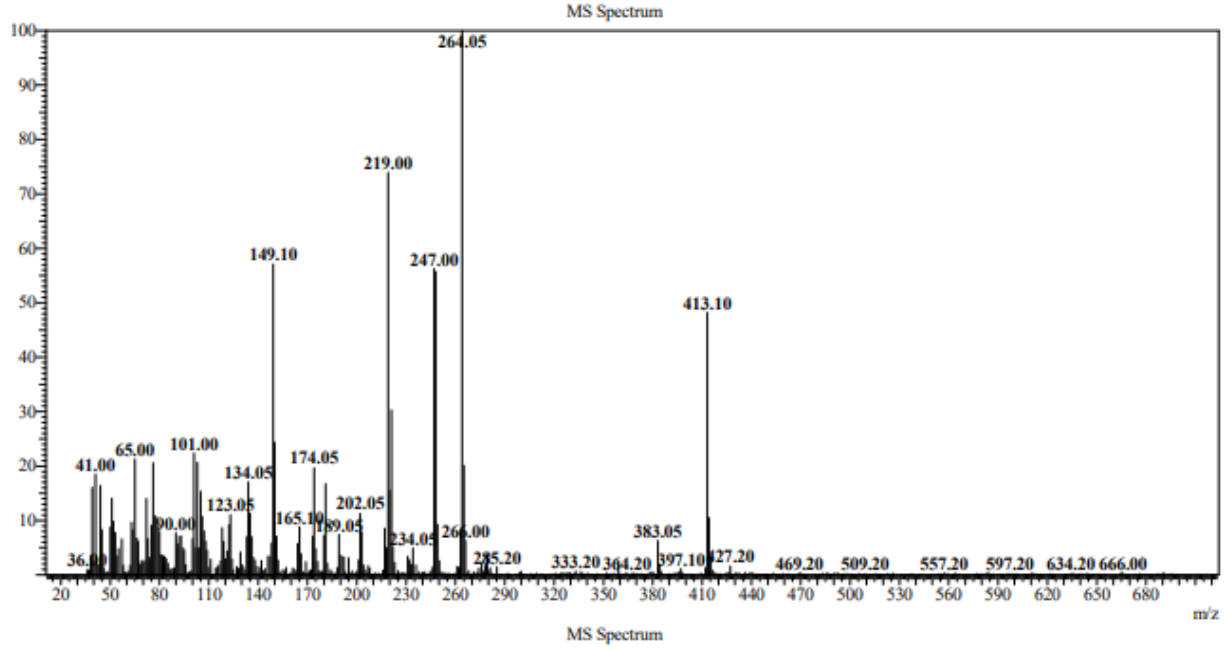


*Figure S90: Mass spectrum of **5e***

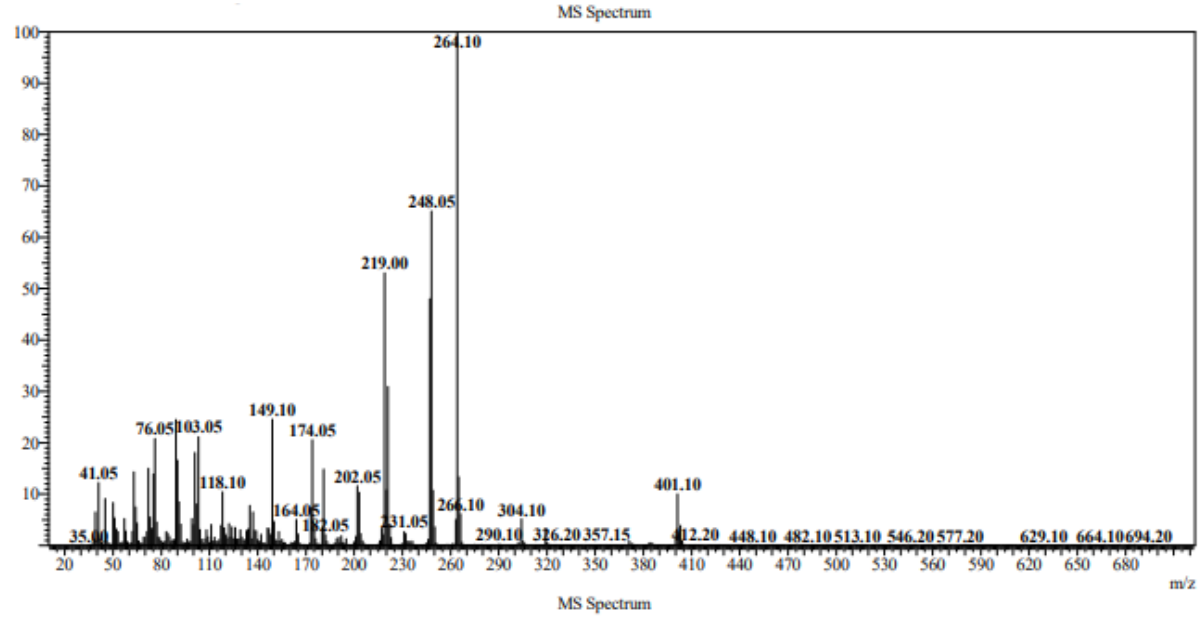


*Figure S91: Mass spectrum of **5f***

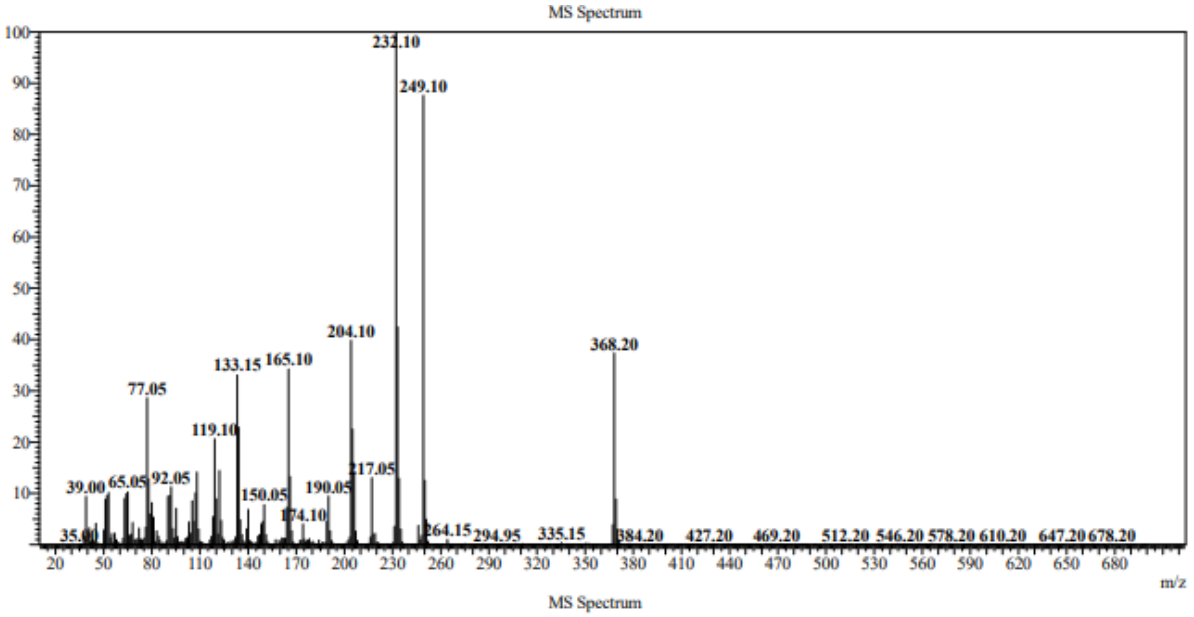


*Figure S92: Mass spectrum of **5g***

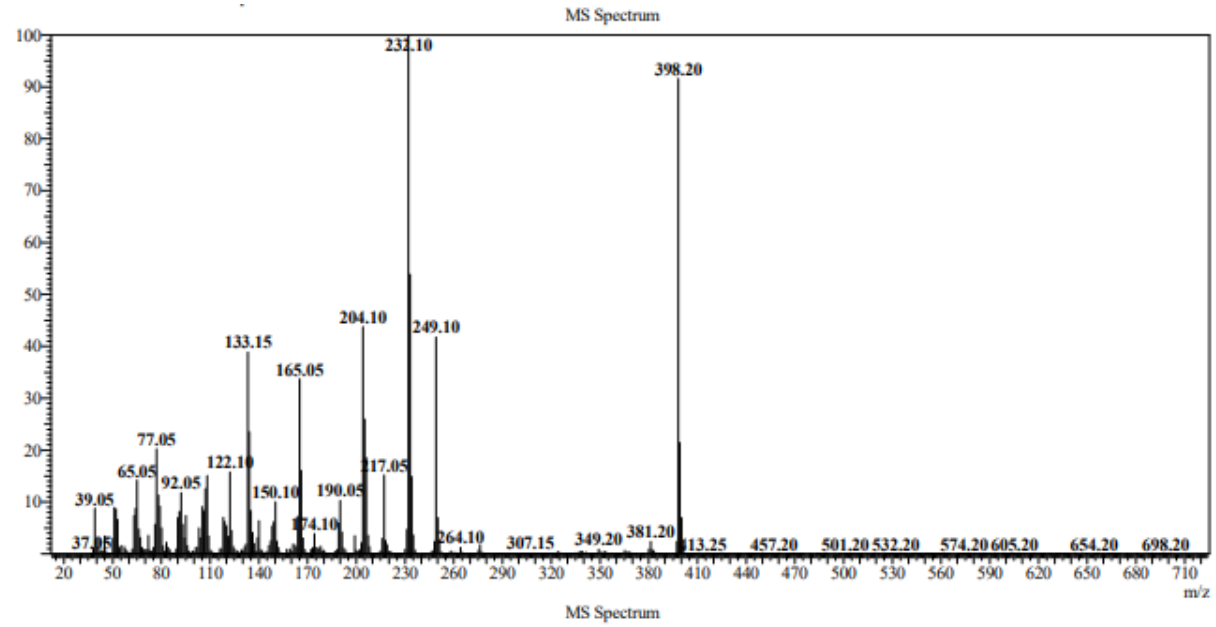


Figure S93: Mass spectrum of ***5z***

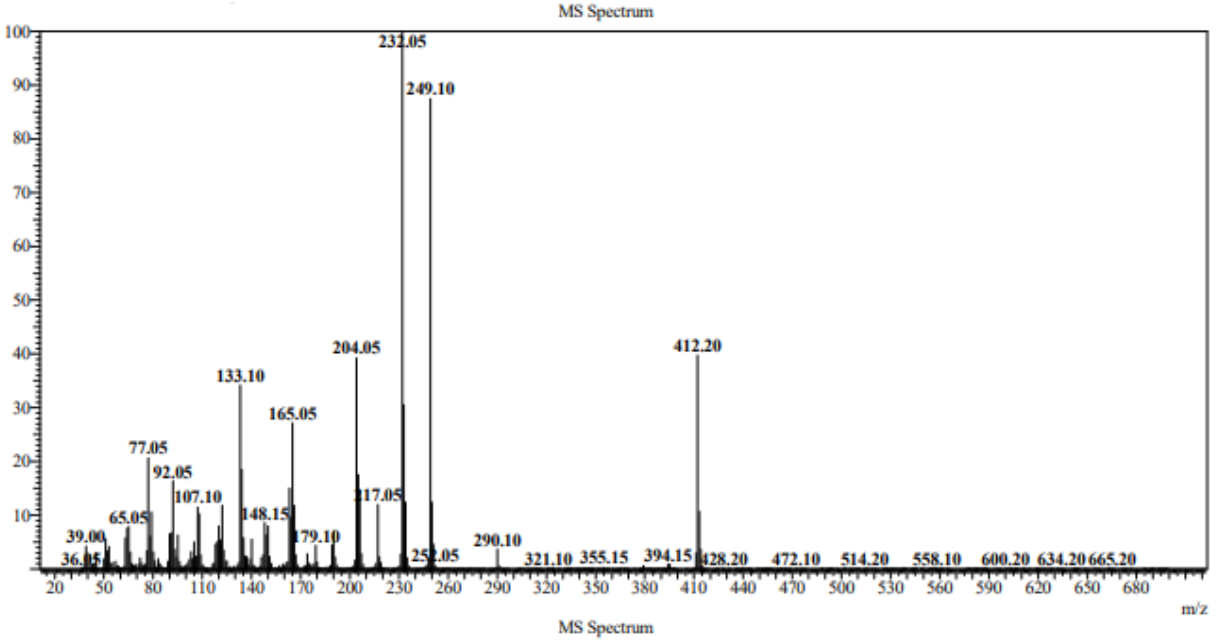


Figure S94: Mass spectrum of ***5q***

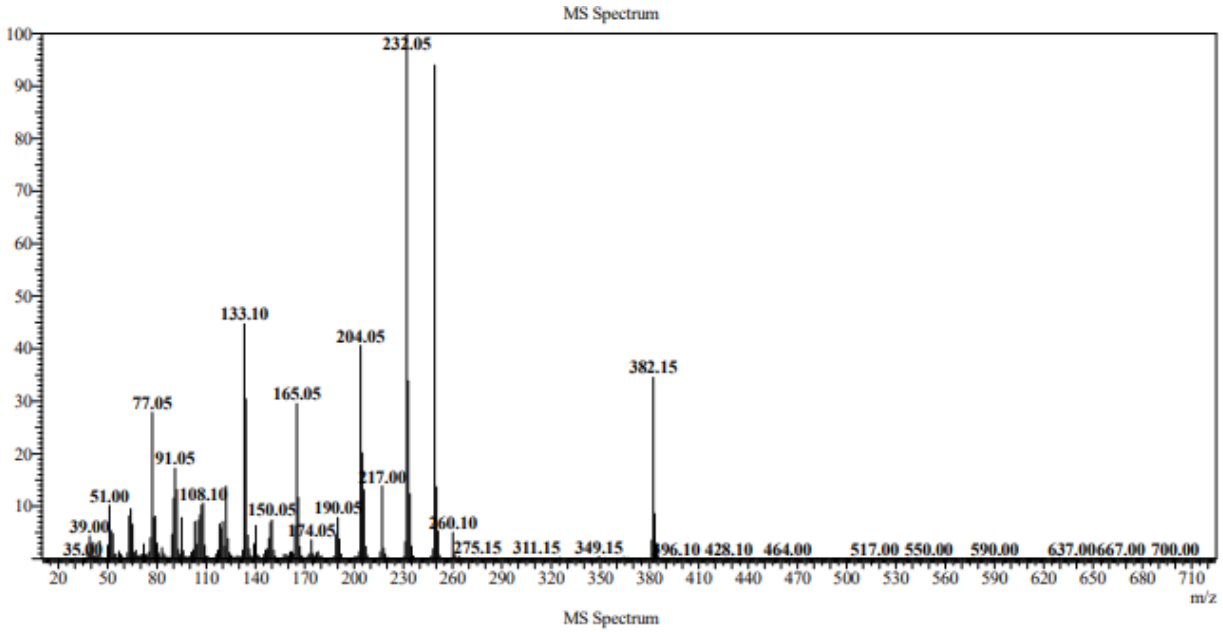


Figure S95: Mass spectrum of ***5J***

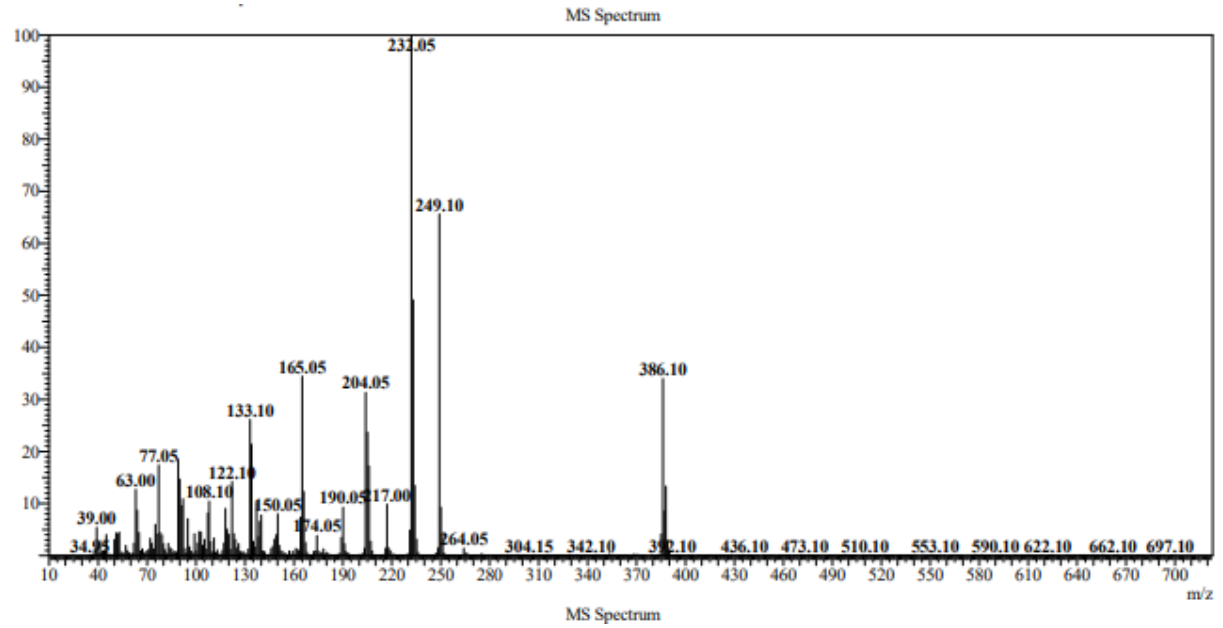


Figure S96: Mass spectrum of ***5k***

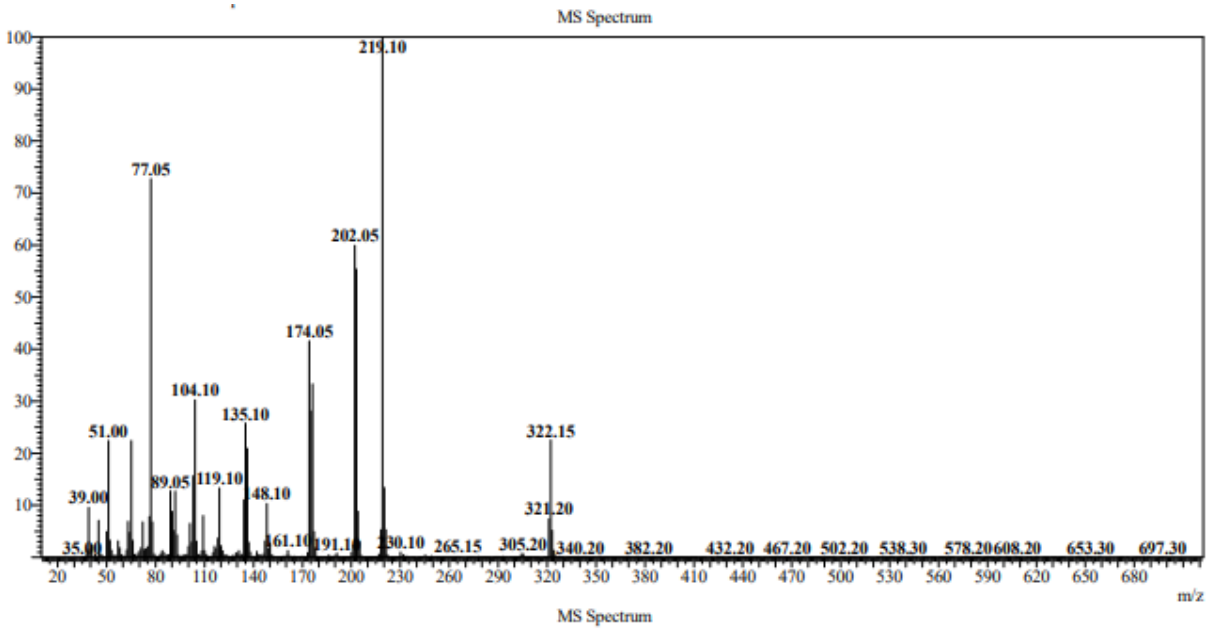


*Figure S97: Mass spectrum of **5l***

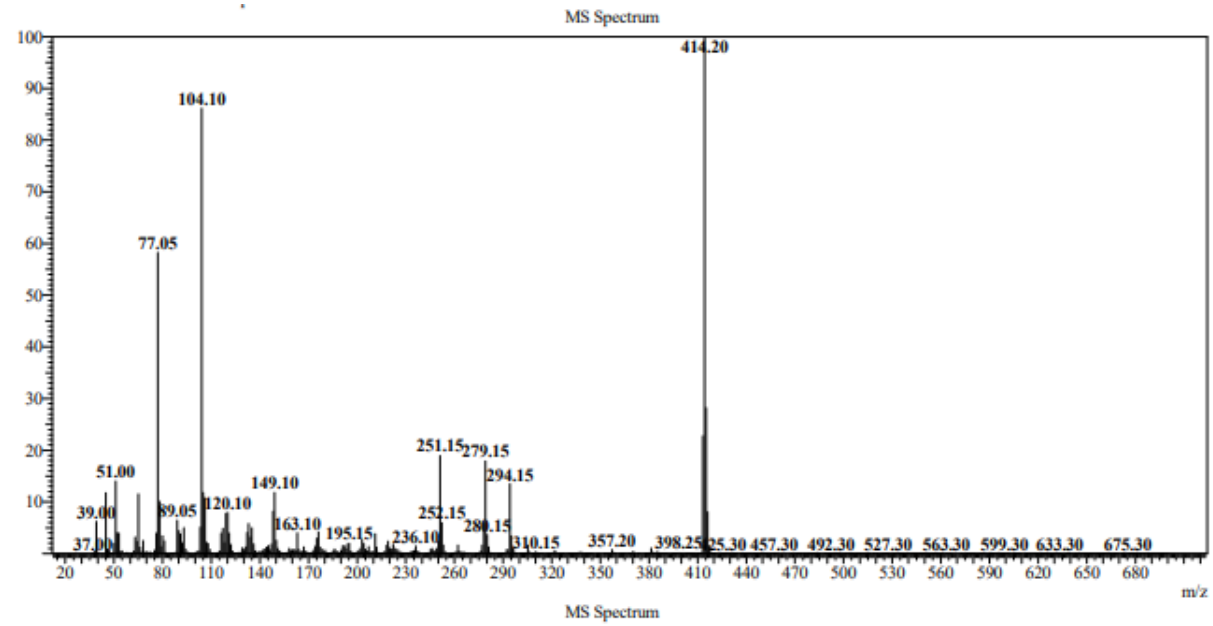


*Figure S98: Mass spectrum of **5m***

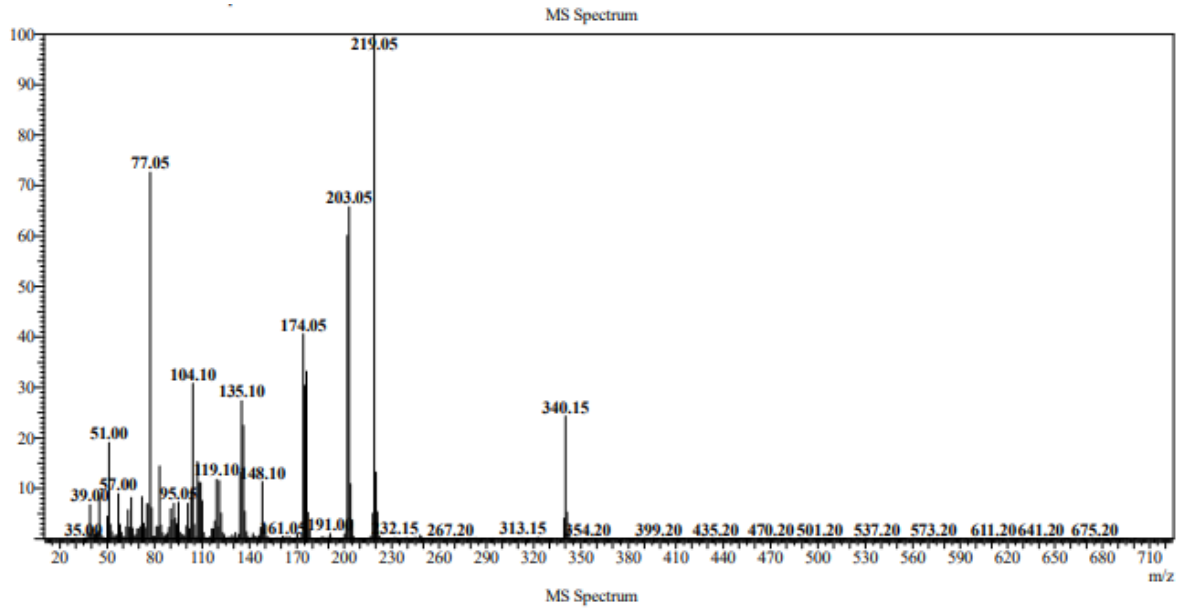


Figure S99: Mass spectrum of ***5o***

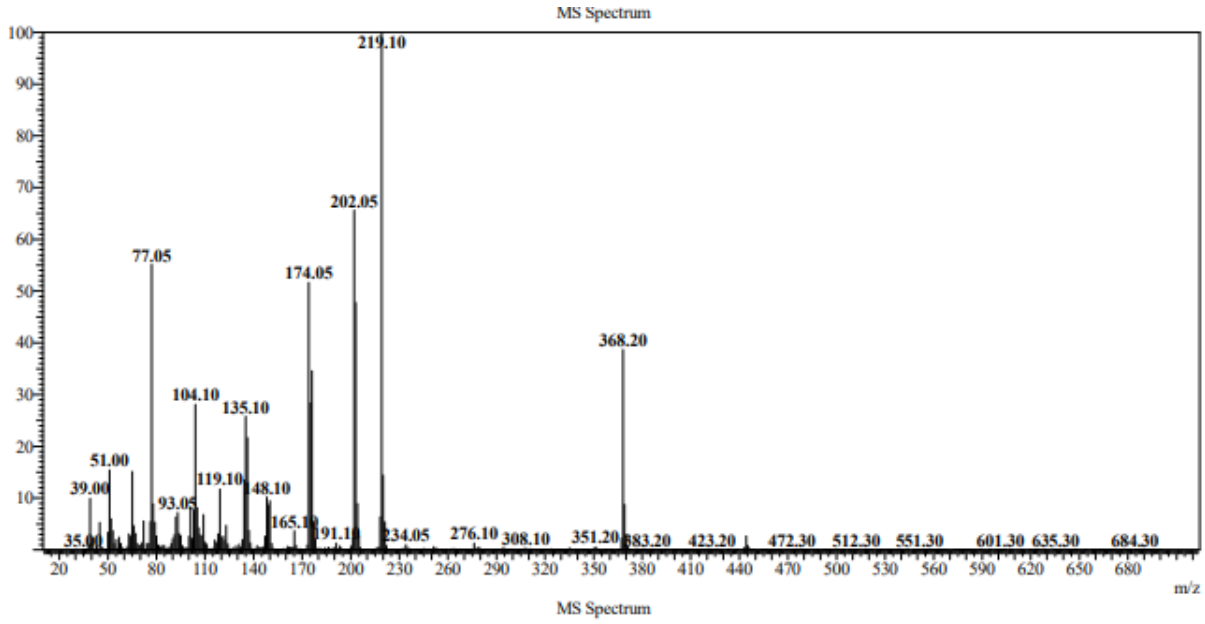


Figure S100: Mass spectrum of ***5p***

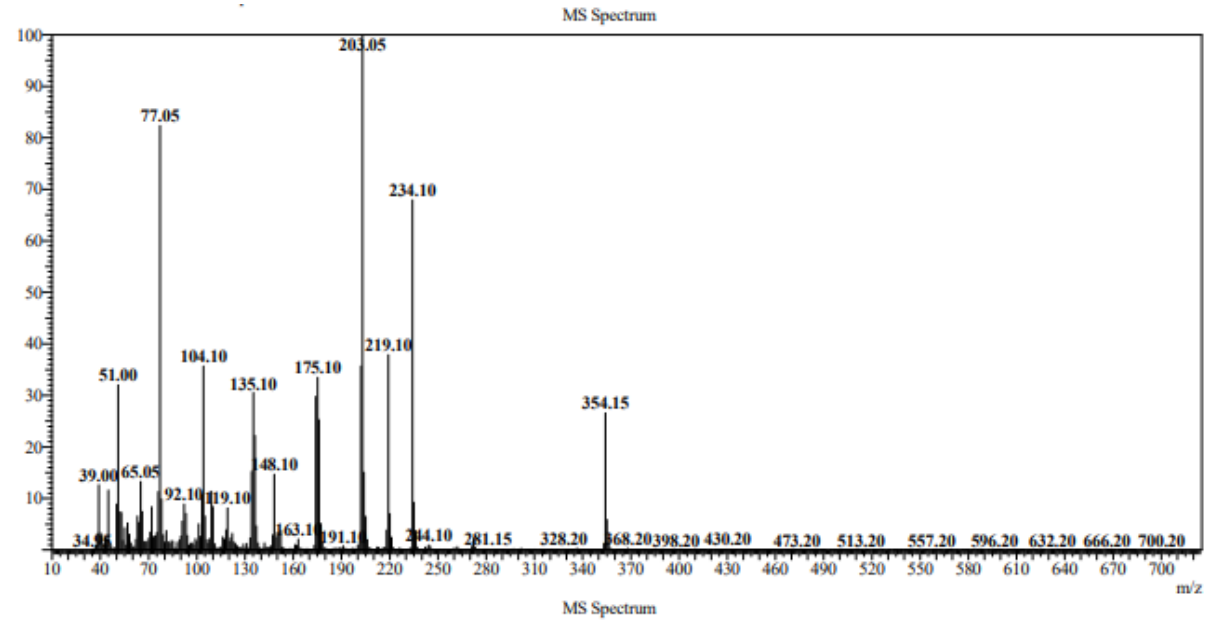


*Figure S101: Mass spectrum of **5r***

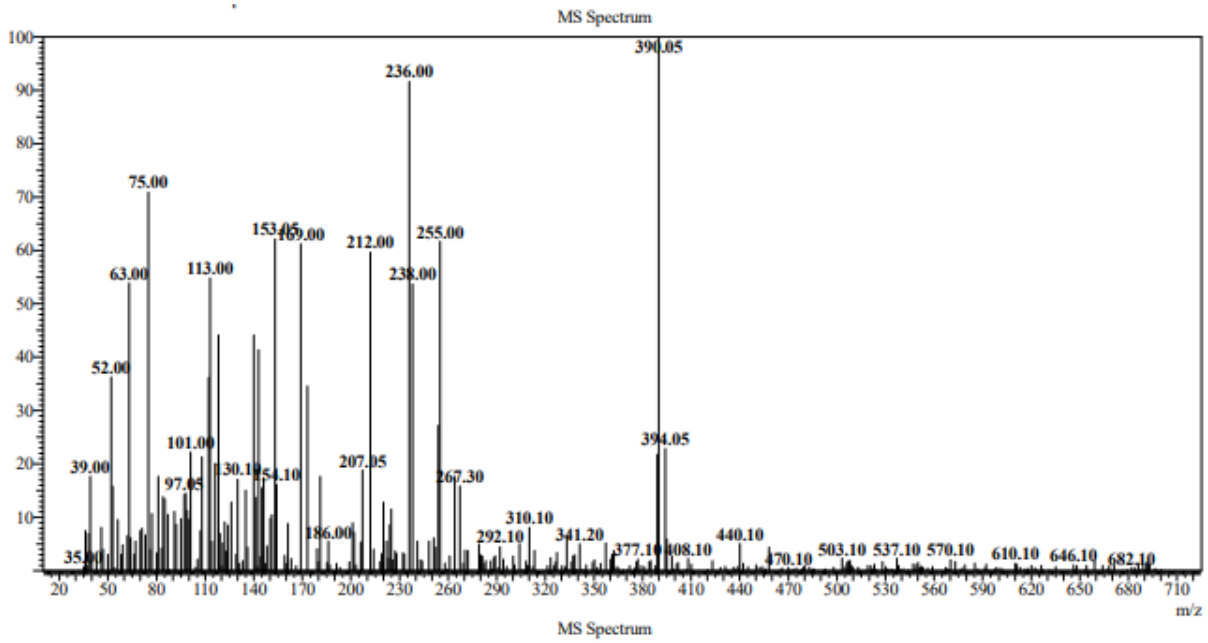


*Figure S102: Mass spectrum of **5s***

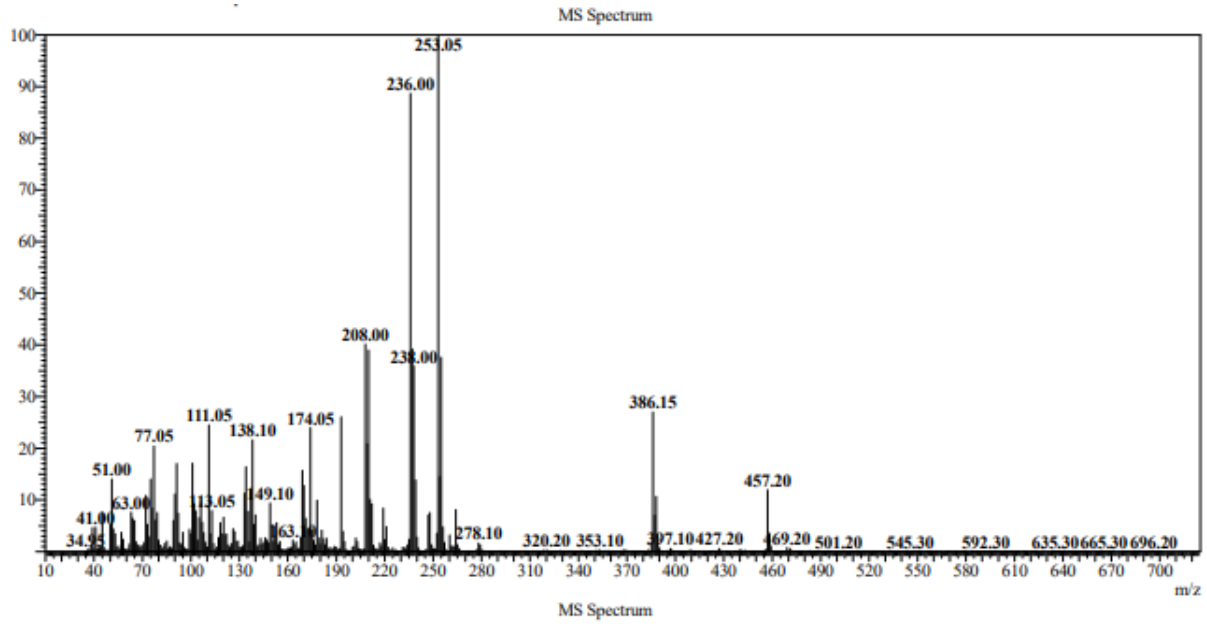


*Figure S103: Mass spectrum of **5t***

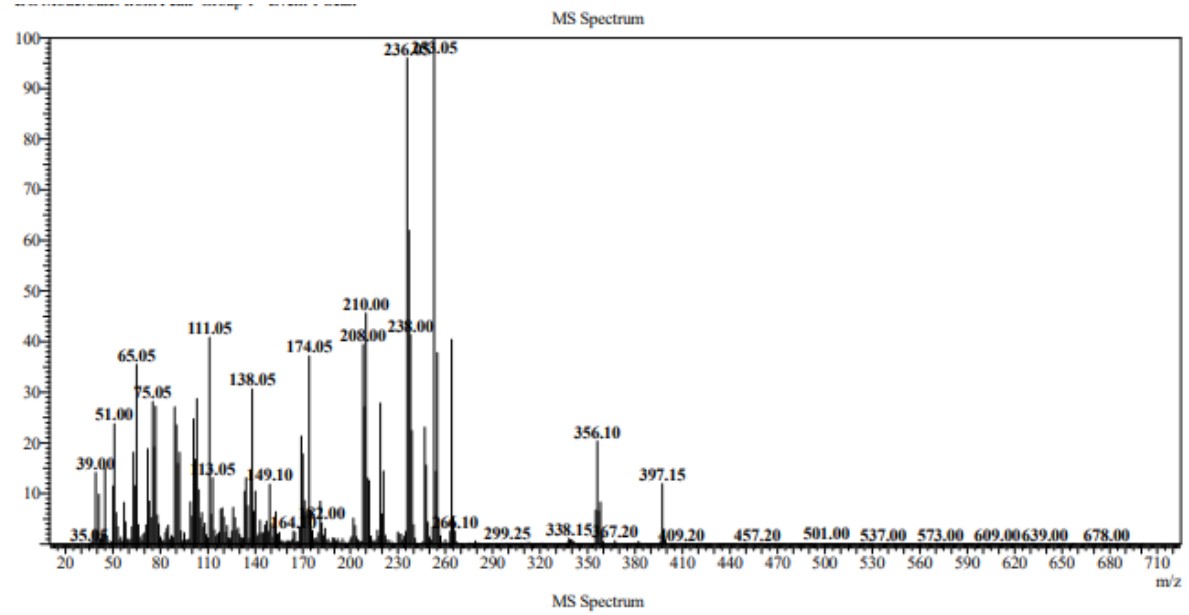


*Figure S104: Mass spectrum of **5u***

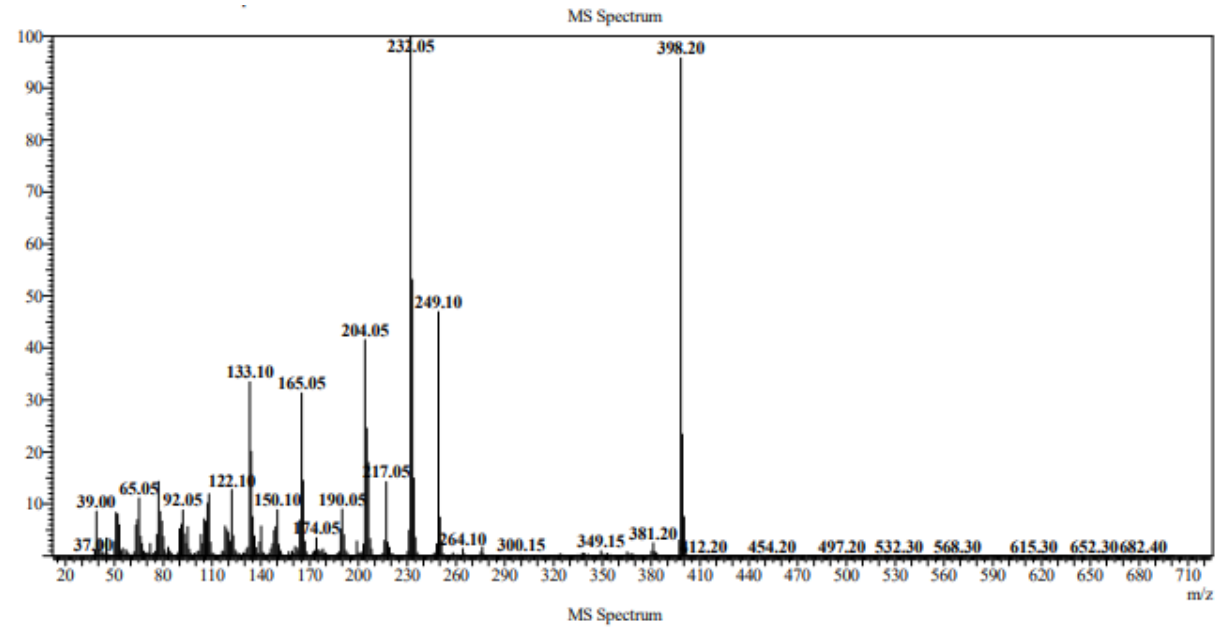


*Figure S105: Mass spectrum of **5n***

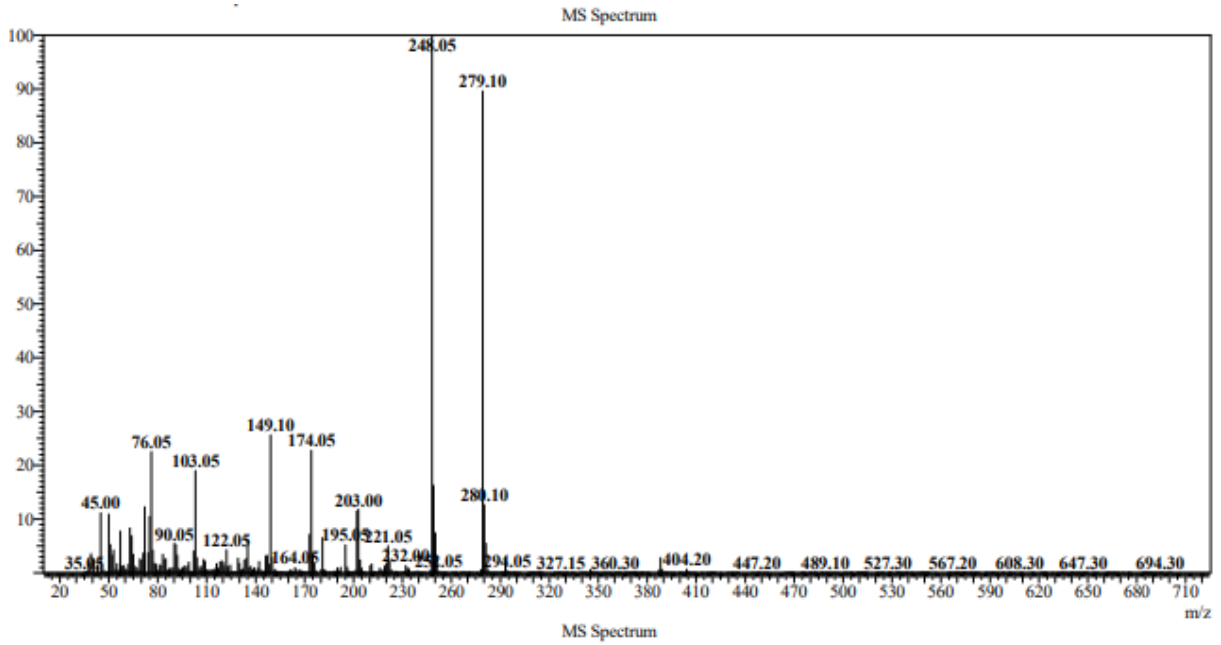


*Figure S106: Mass spectrum of **5y***

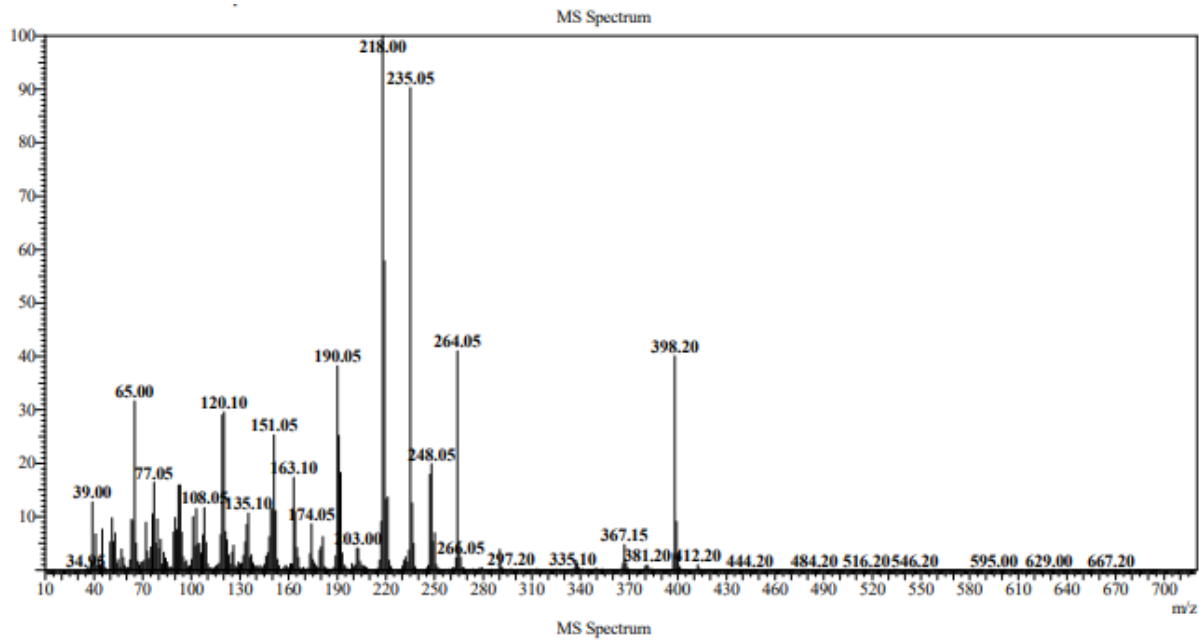


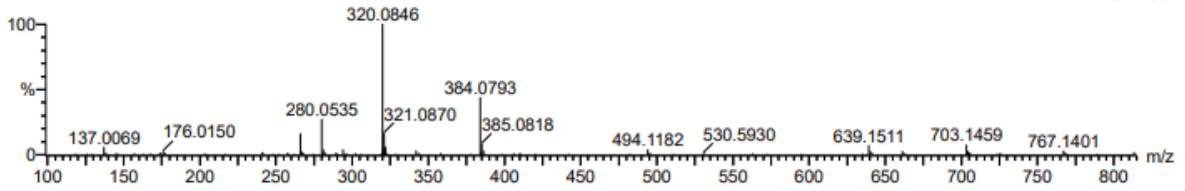
Figure S107: Mass spectrum of ***5i***

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Odd and Even Electron Ions
42 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-22 H: 0-50 N: 0-4 O: 0-2 S: 0-1

200123_5b 20 (0.223) IITRPR XEVO G2-XS QTOF
Test Name : 200123_5b
1: TOF MS ES+
1.04e+007



Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
384.0793	384.0807	-1.4	-3.6	17.5	893.4	n/a	n/a	C22 H14 N3 O2 S

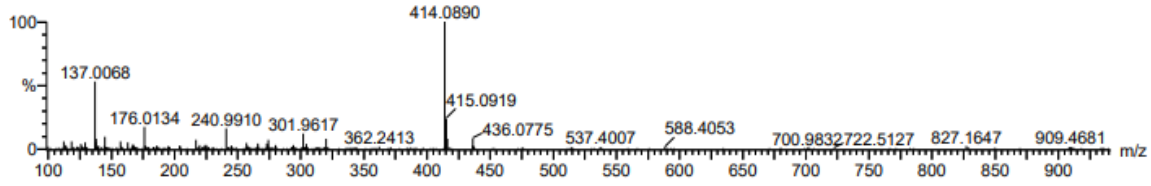
Figure S108: HRMS of ***5b***

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
117 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-22 H: 0-50 N: 0-5 O: 0-5 S: 0-1

200123_5e 52 (0.543) IITRPR XEVO G2-XS QTOF
Test Name : 200123_5e
1: TOF MS ES+
5.69e+005



Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
414.0890	414.0872	1.8	4.3	13.5	696.3	n/a	n/a	C18 H16 N5 O5 S

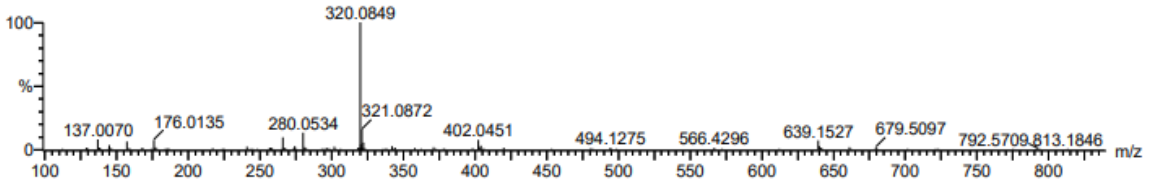
Figure S109: HRMS of ***5e***

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
100 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-17 H: 0-50 N: 0-5 O: 0-3 S: 0-1 Cl: 0-1

200123_5f 22 (0.240) IITRPR XEVO G2-XS QTOF
Test Name : 200123_5f
1: TOF MS ES+
7.54e+006



Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
402.0451	402.0428	2.3	5.7	13.5	743.2	n/a	n/a	C17 H13 N5 O3 S Cl

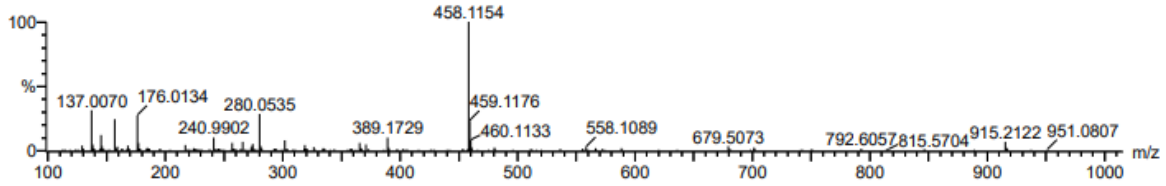
Figure S110: HRMS of ***5f***

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
86 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-20 H: 0-50 N: 0-5 O: 0-6 S: 0-1

200123_5y 24 (0.257) IITRPR XEVO G2-XS QTOF
Test Name : 200123_5y
1: TOF MS ES+
1.94e+006



Minimum:				-1.5				
Maximum:		5.0	10.0	50.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
458.1154	458.1134	2.0	4.4	13.5	732.2	n/a	n/a	C20 H20 N5 O6 S

Figure S111: HRMS of ***5y***