Molecular Design and Synthesis of Ivermectin Hybrids Targeting Hepatic and Erythrocytic Stages of *Plasmodium* Parasites

Lovepreet Singh,[a] Diana Fontinha,[b] Denise Francisco,[b] Antonio M. Mendes,[b] Miguel Prudêncio[b] and Kamaljit Singh[a,*]

[a]Department of Chemistry, Guru Nanak Dev University, Amritsar – 143 005, India

[b]Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal

Table of Contents

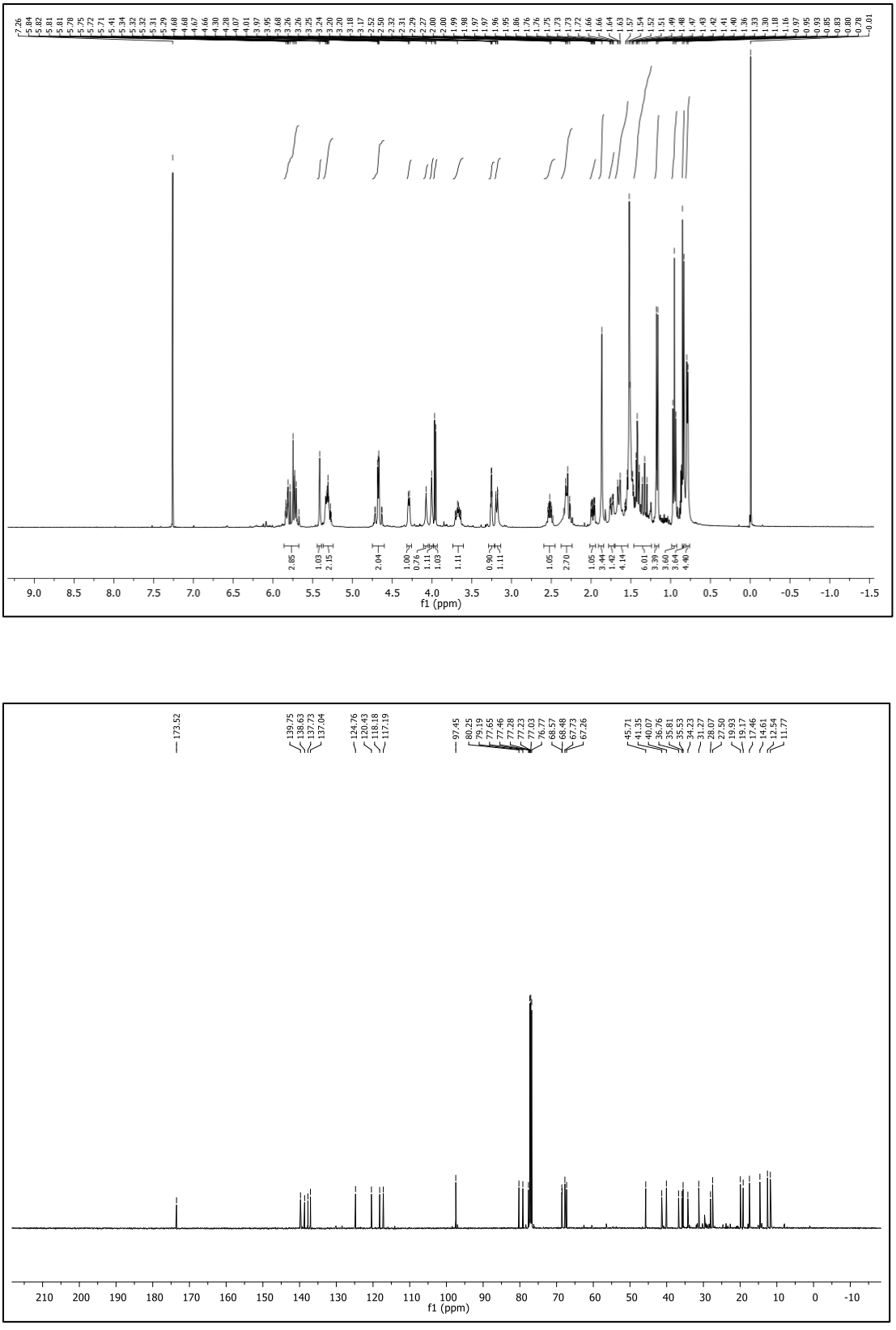


Figure S1.[1]H NMR (400 MHz, $CDCl_3$) and [13]C NMR (125 MHz, $CDCl_3$) of **3**.

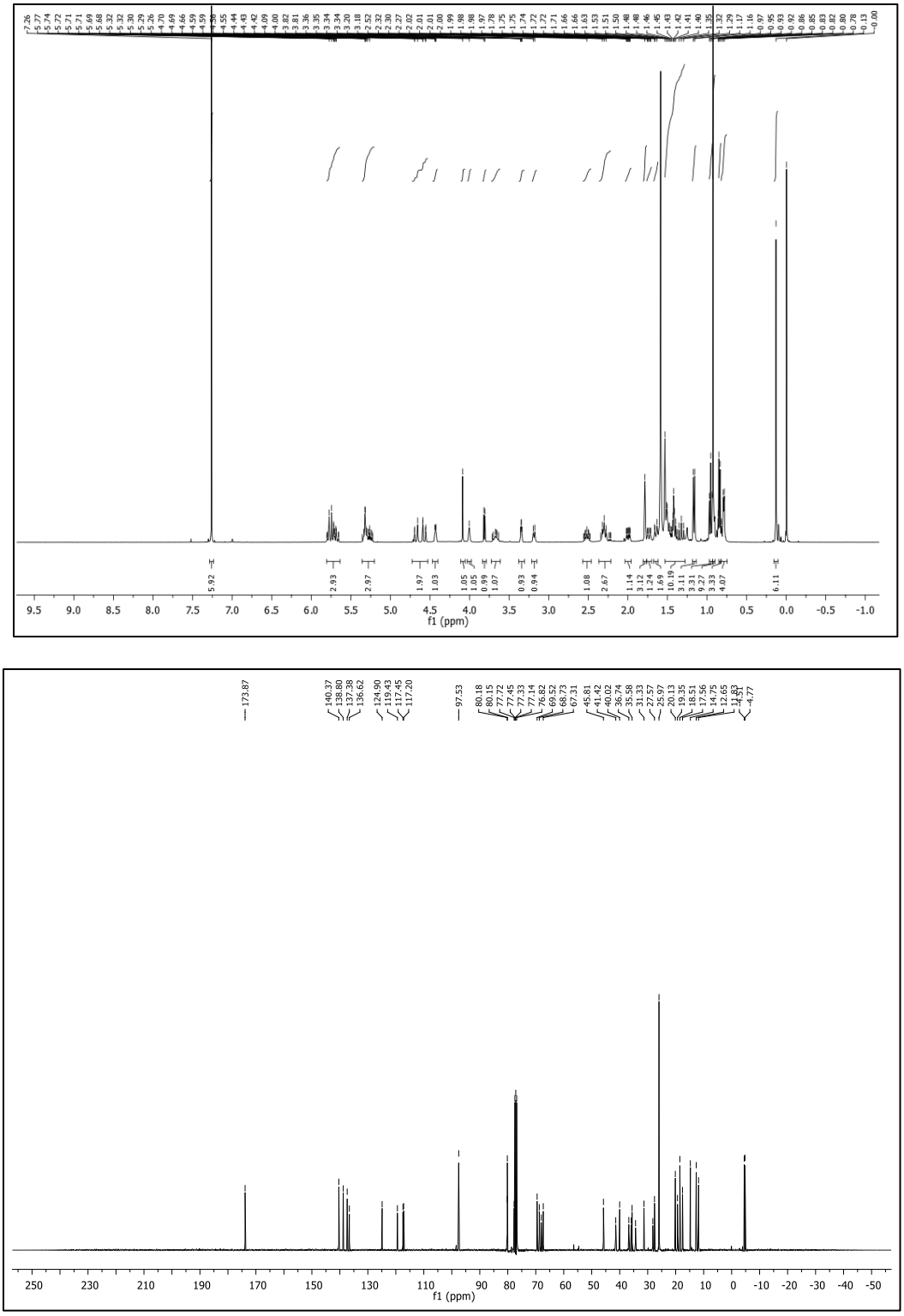


Figure S2.[1]H NMR (400 MHz, $CDCl_3$) and [13]C NMR (100 MHz, $CDCl_3$) of **5**.

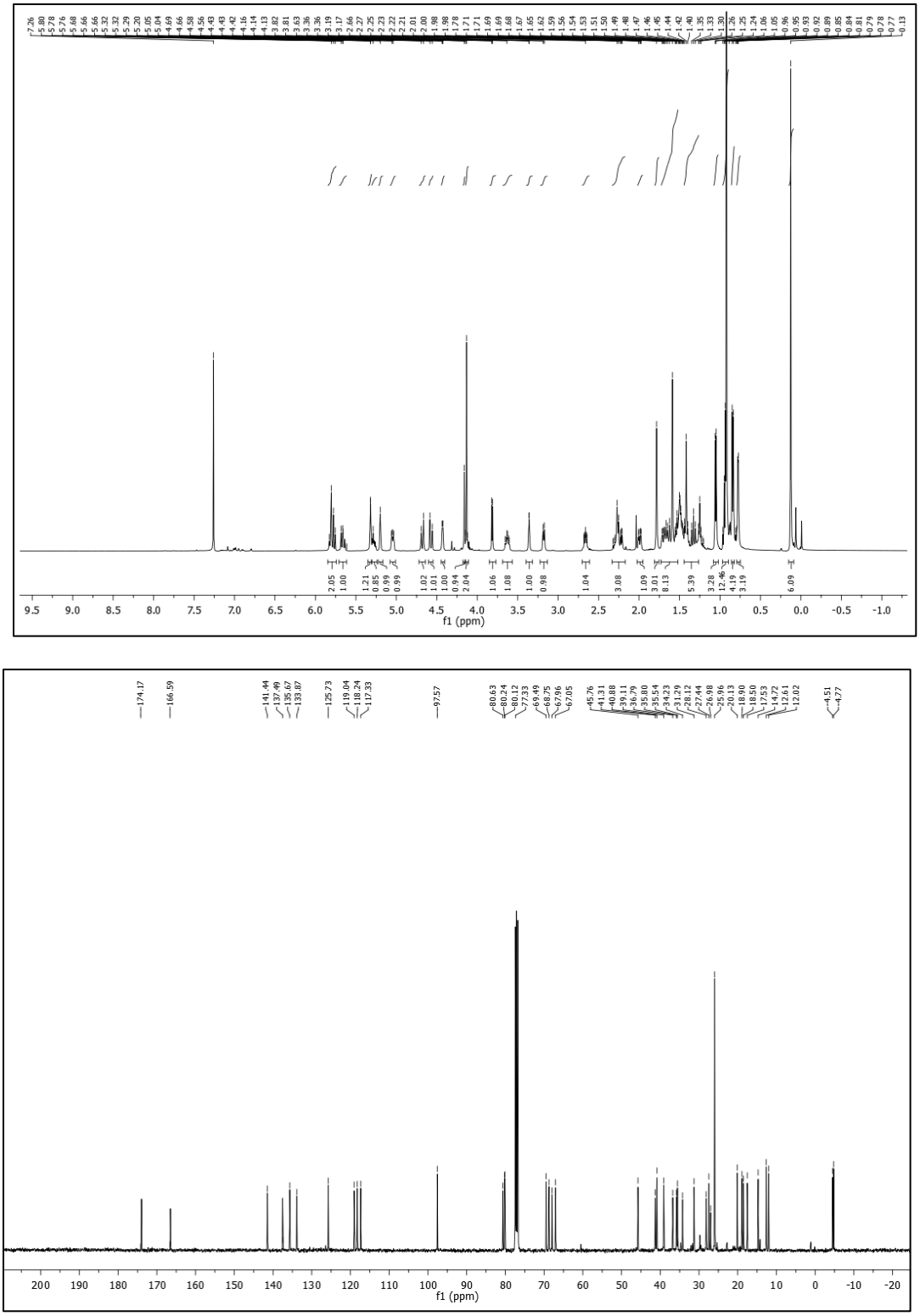


Figure S3.[1]H NMR (400 MHz, $CDCl_3$) and [13]C NMR (100 MHz, $CDCl_3$) of **6**.

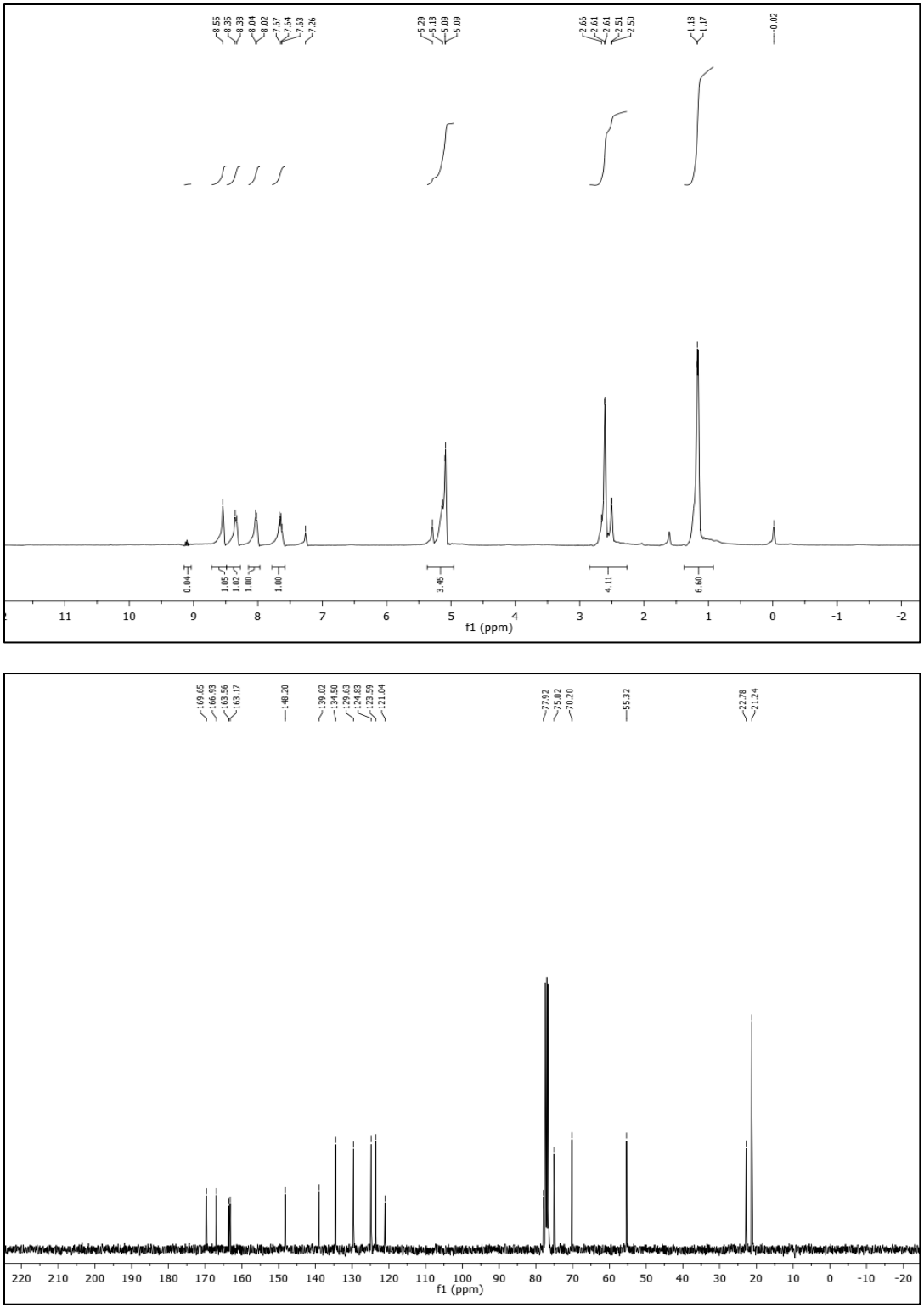


Figure S4.[1]H NMR (300 MHz, $CDCl_3$) and[13]C NMR (75 MHz, $CDCl_3$) of **10a**.

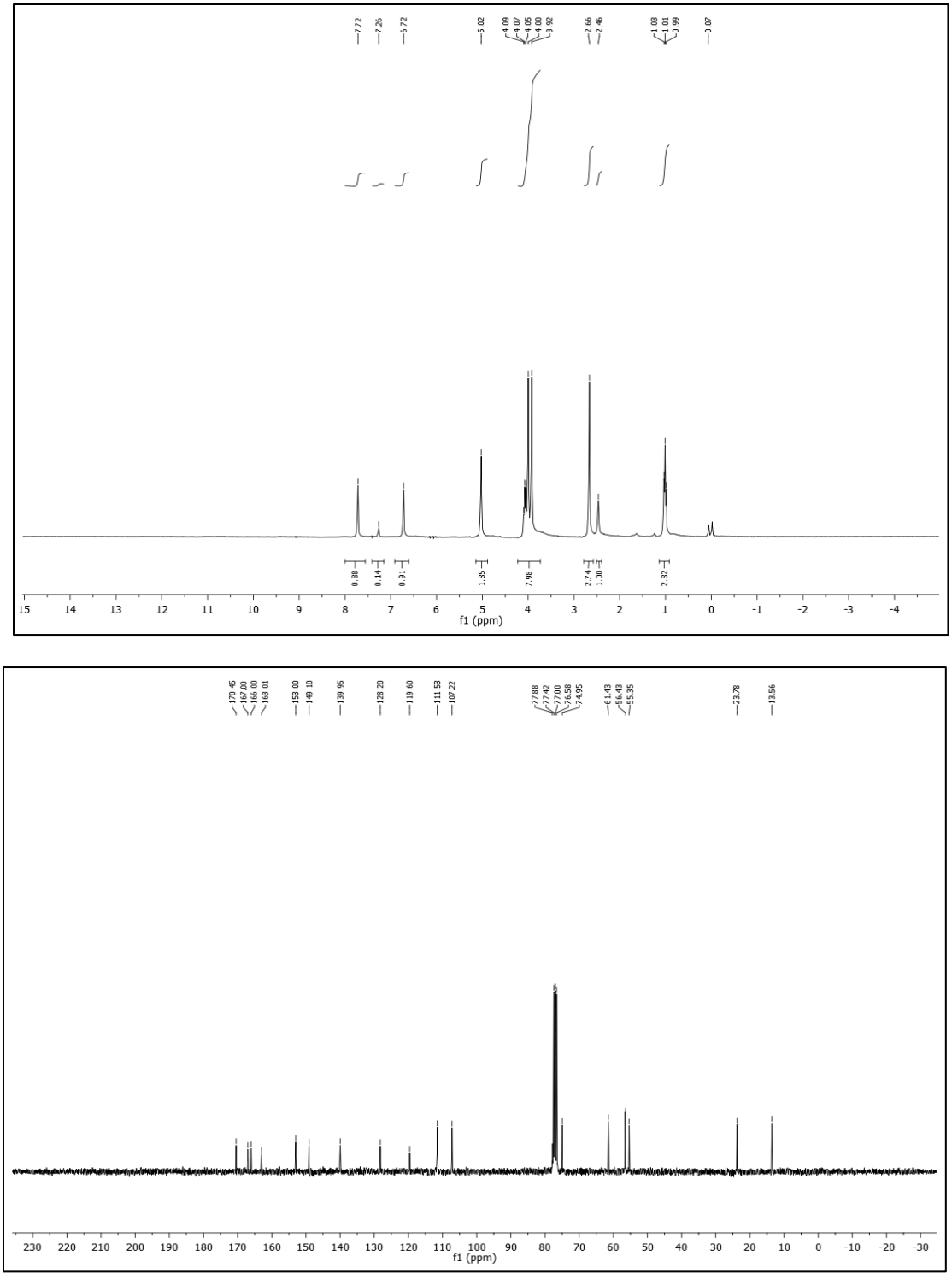


Figure S5.[1]H NMR (300 MHz, $CDCl_3$) and [13]C NMR (75 MHz)**10b**.

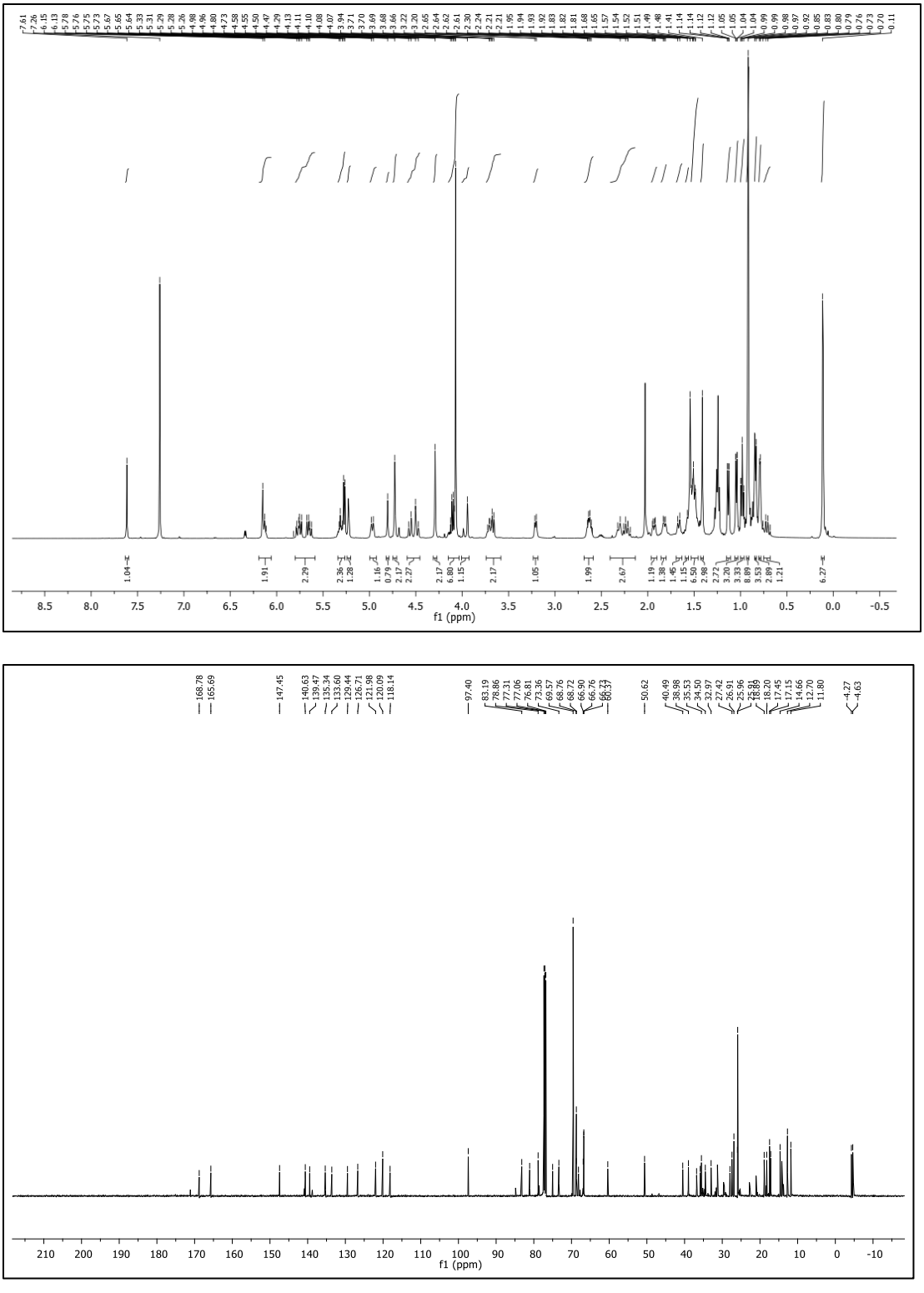


Figure S6.[1]H NMR (500 MHz, $CDCl_3$) and [13]C NMR (125 MHz, $CDCl_3$) of **11**.

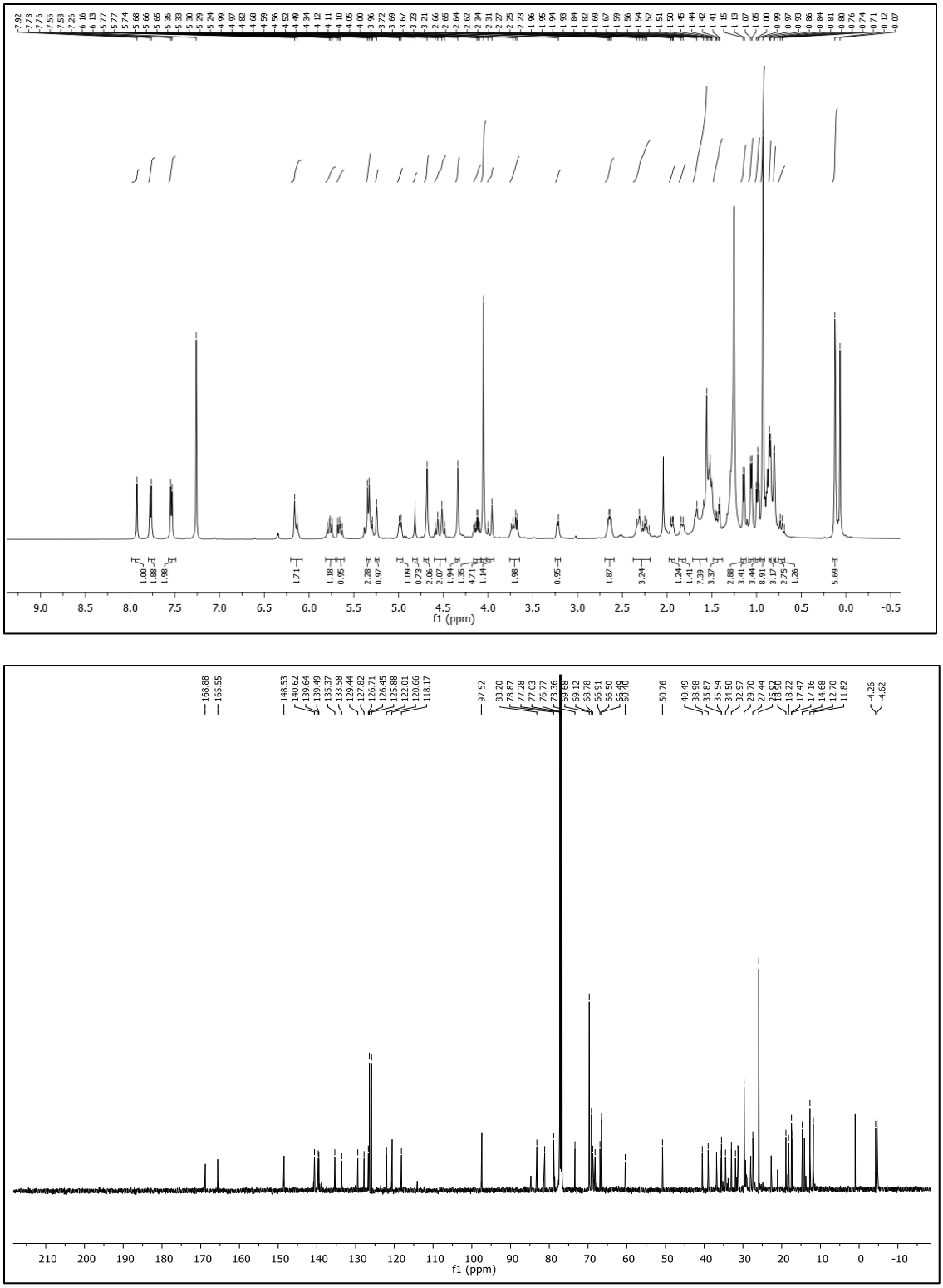


Figure S7.[1]H NMR (500 MHz, $CDCl_3$) and [13]C NMR (125 MHz, $CDCl_3$) of **12**

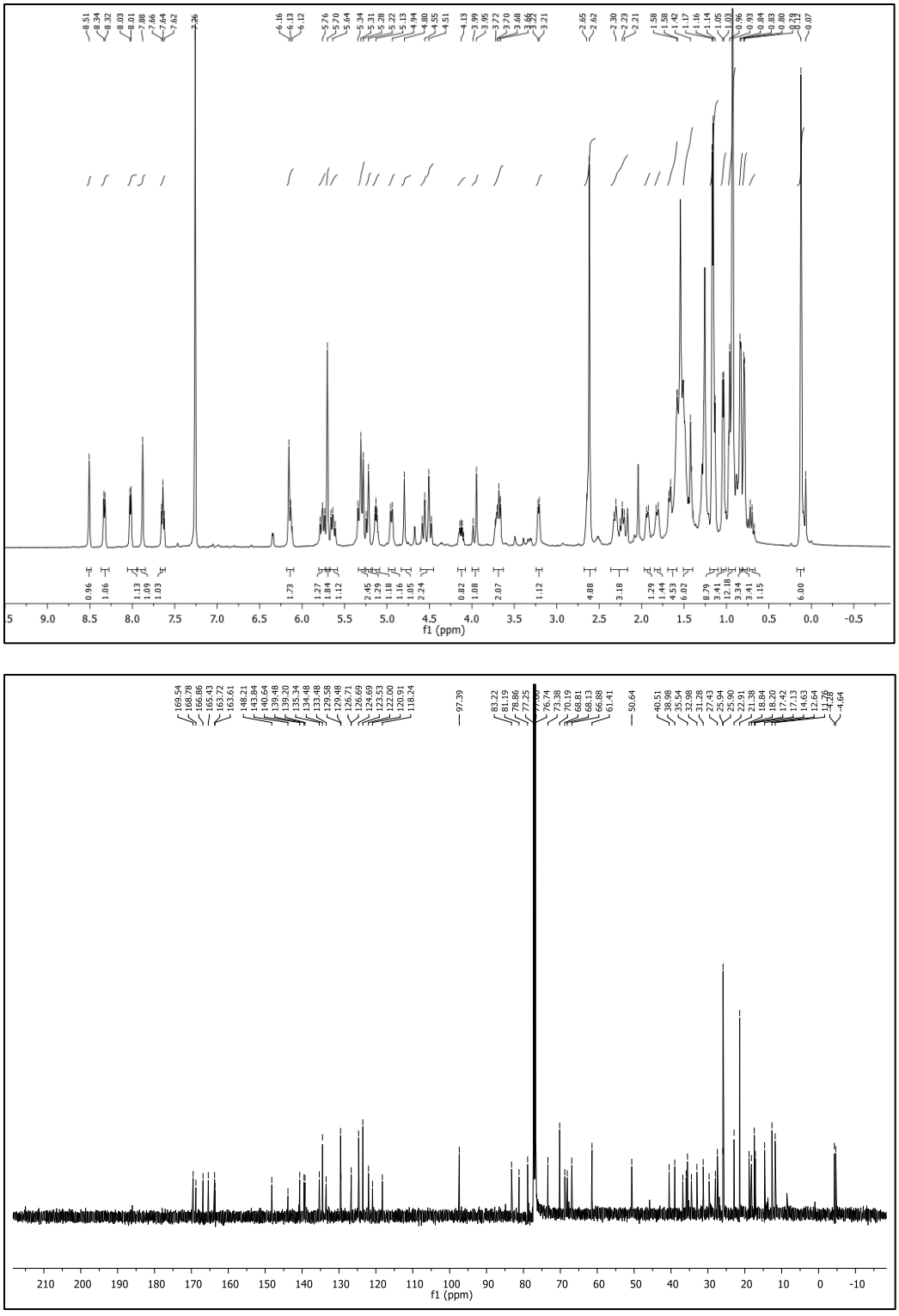


Figure S8.[1]H NMR (500 MHz, $CDCl_3$) and [13]C NMR (125 MHz, $CDCl_3$) of**13**.

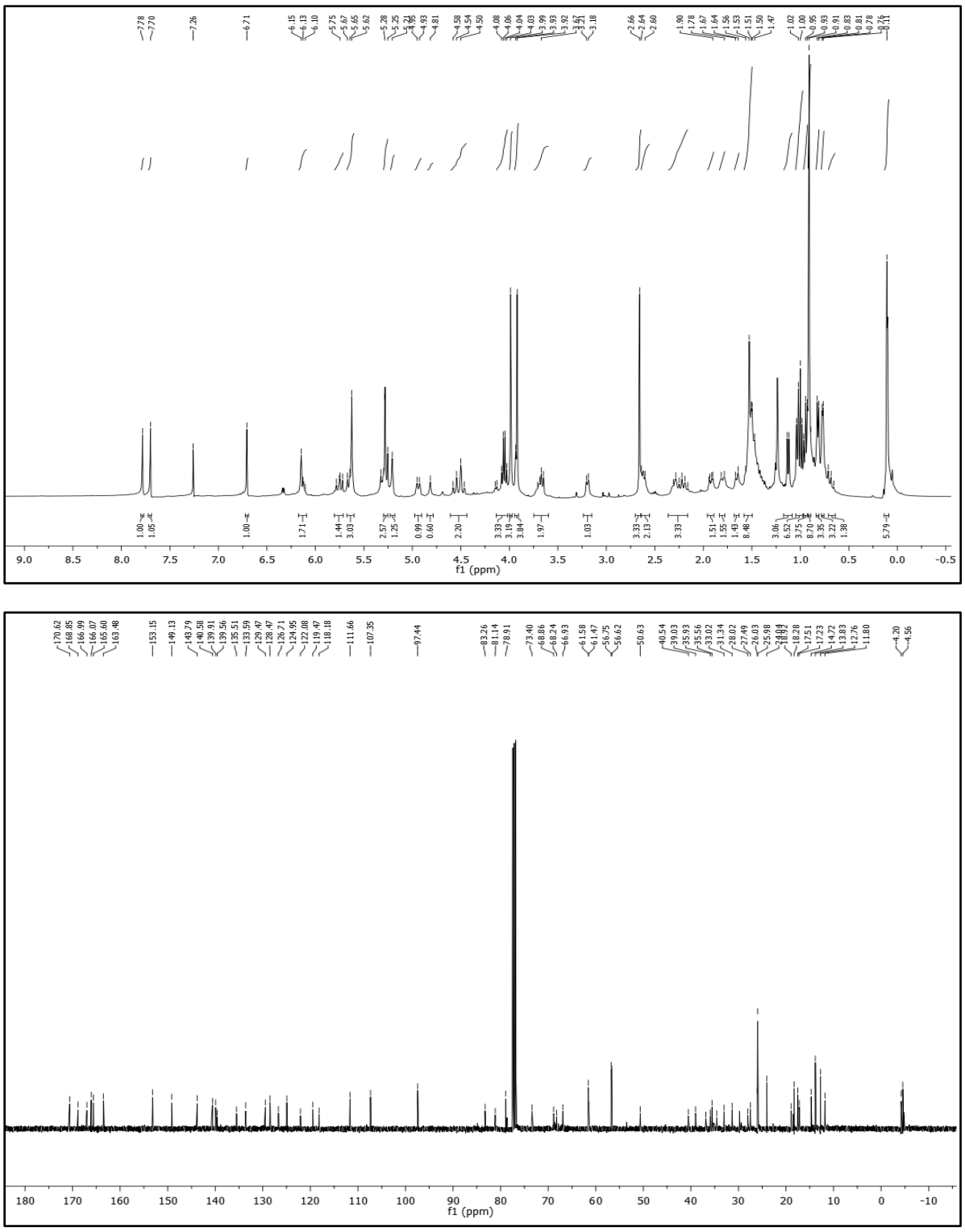


Figure S9.[1]H NMR (400 MHz, $CDCl_3$) and [13]C NMR (100 MHz, $CDCl_3$) of **14.**

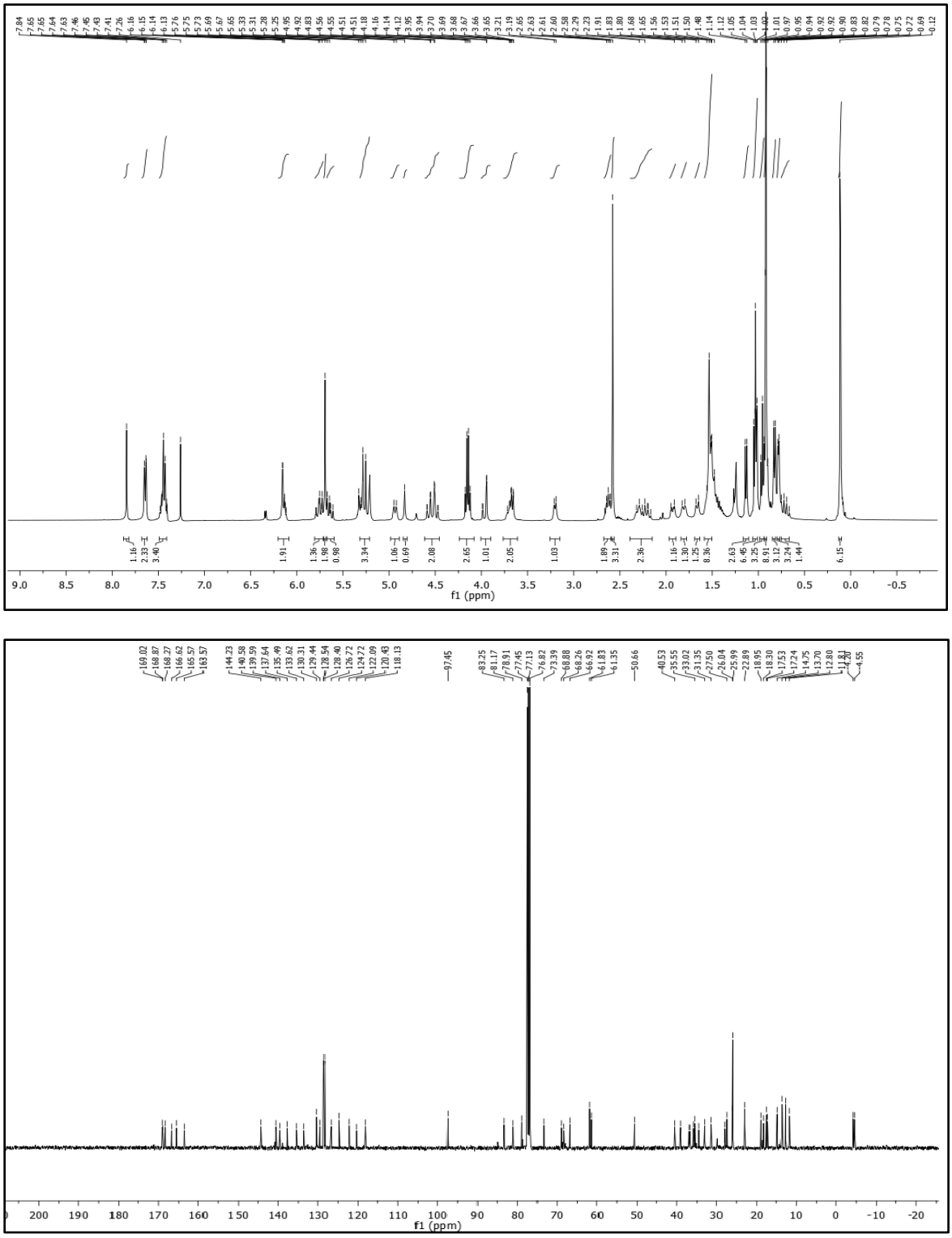


Figure S10.^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) of **15.**

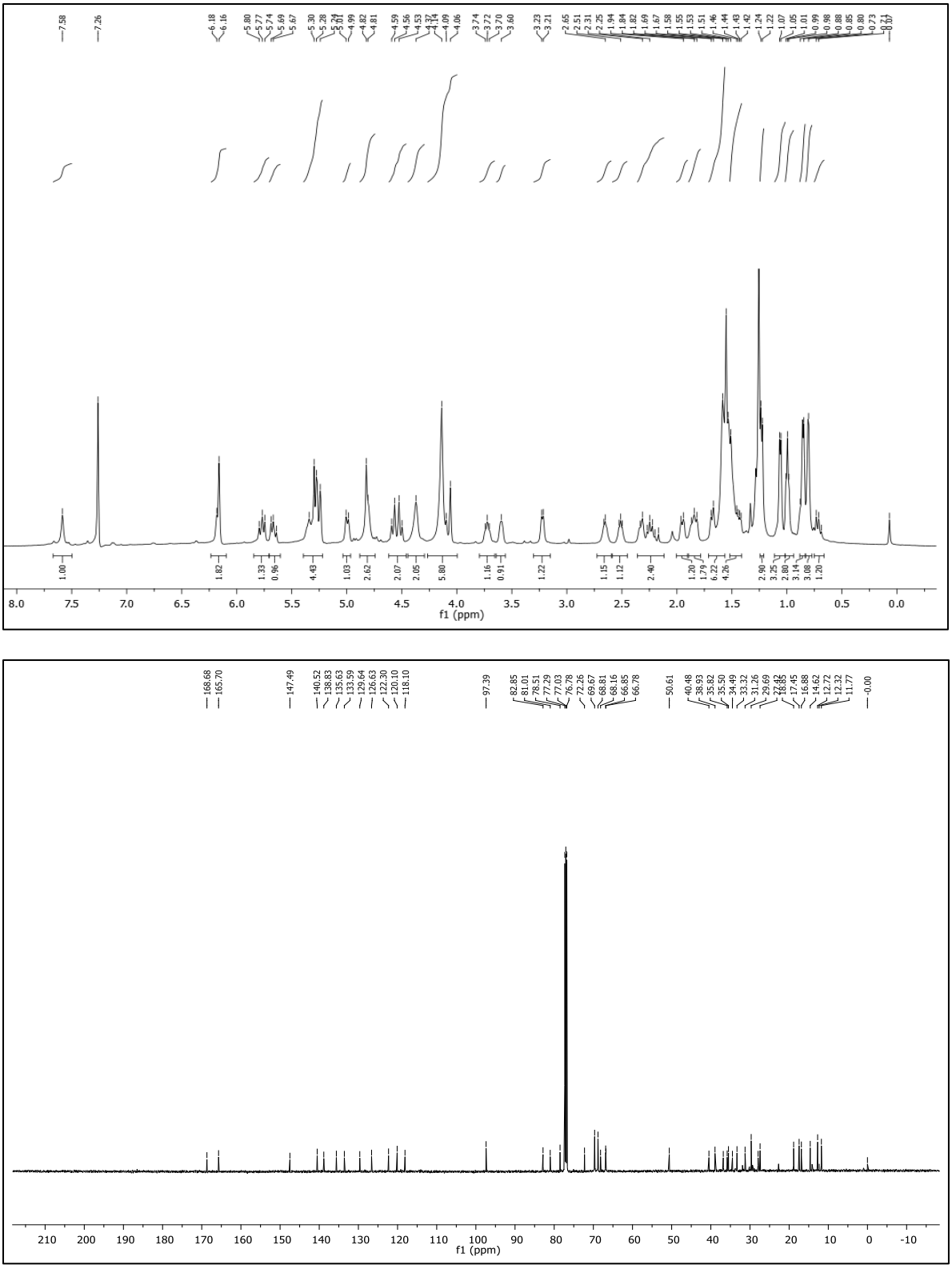


Figure S11.[1]H NMR (500 MHz, $CDCl_3$) and [13]C NMR (125 MHz, $CDCl_3$) of **16**.

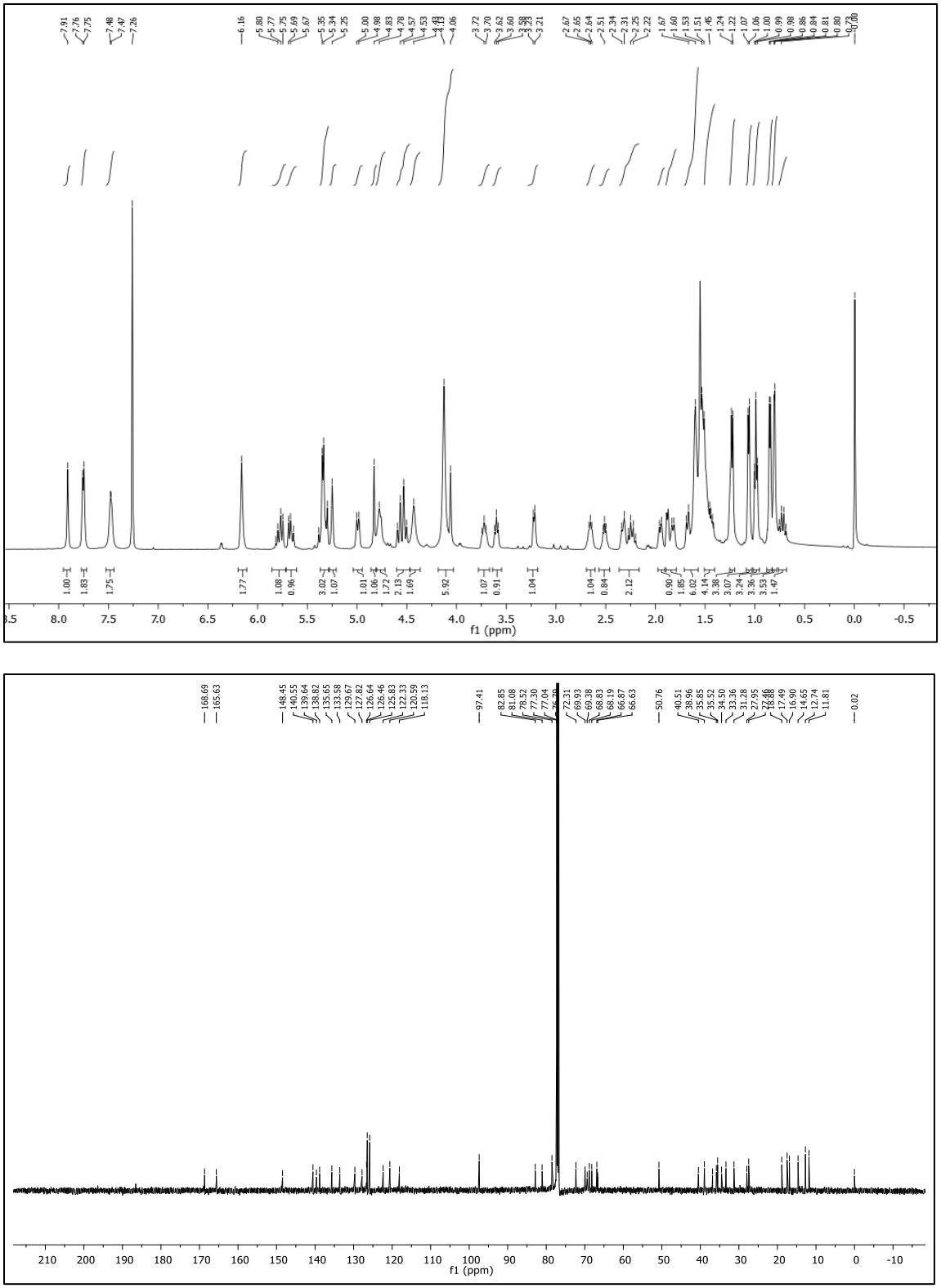


Figure S12.^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) of **17**.

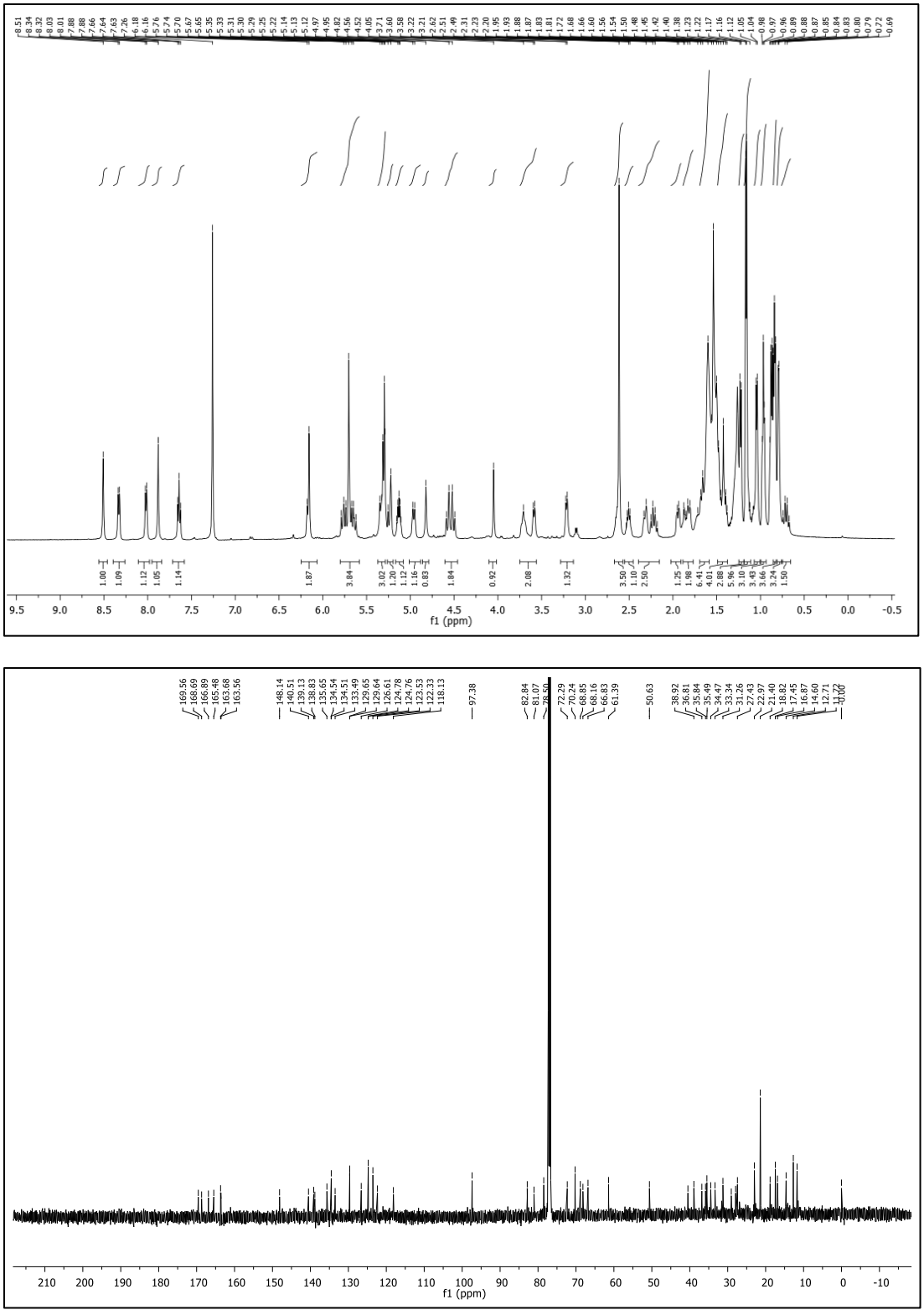
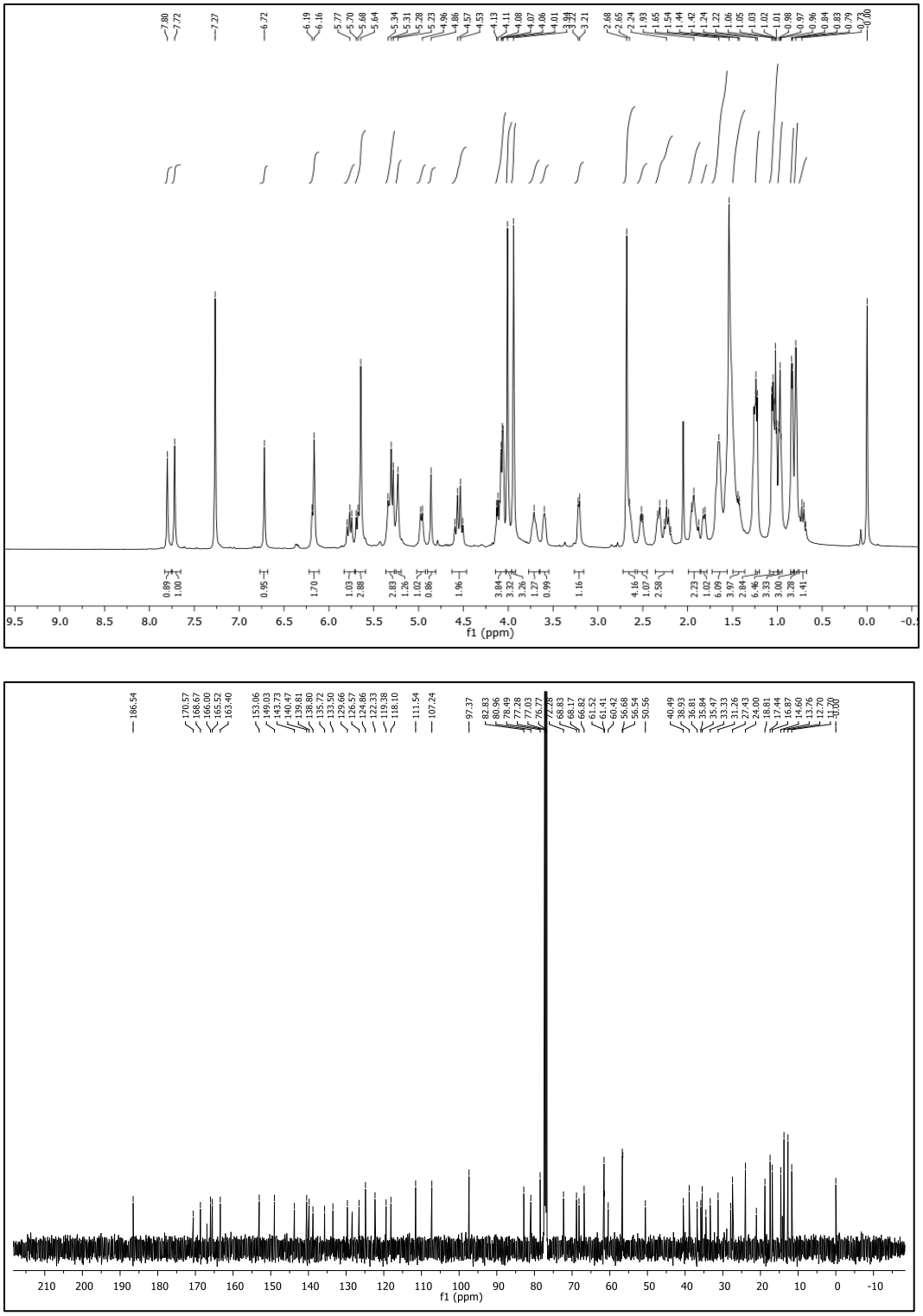


Figure S13.^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$)of **18**.



\Figure S14.[1]H NMR (500 MHz, $CDCl_3$) and [13]C NMR (125 MHz, $CDCl_3$)of **19**.

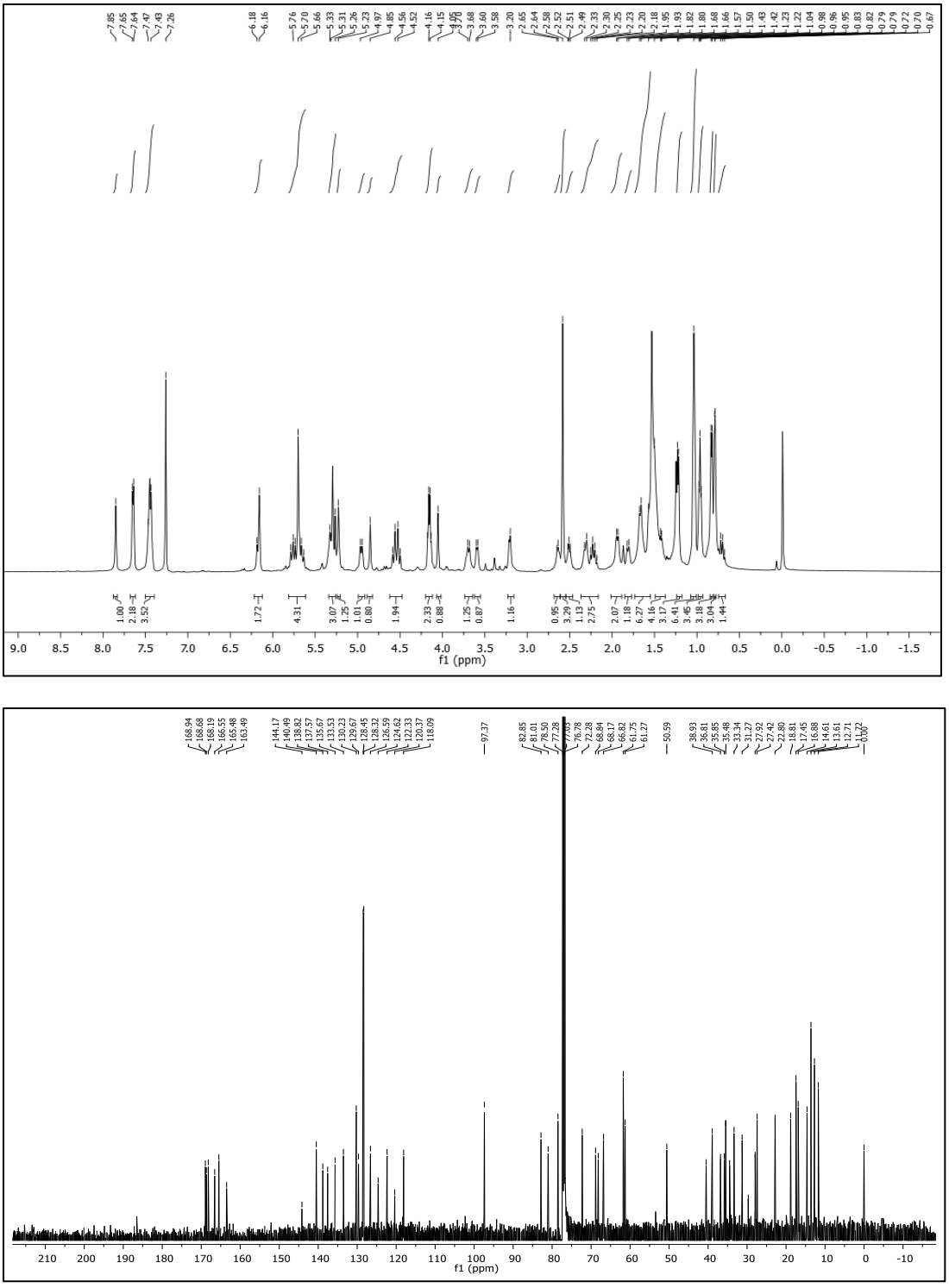


Figure S15.[1]H NMR (500 MHz, $CDCl_3$) and [13]C NMR (125 MHz, $CDCl_3$)of **20**.

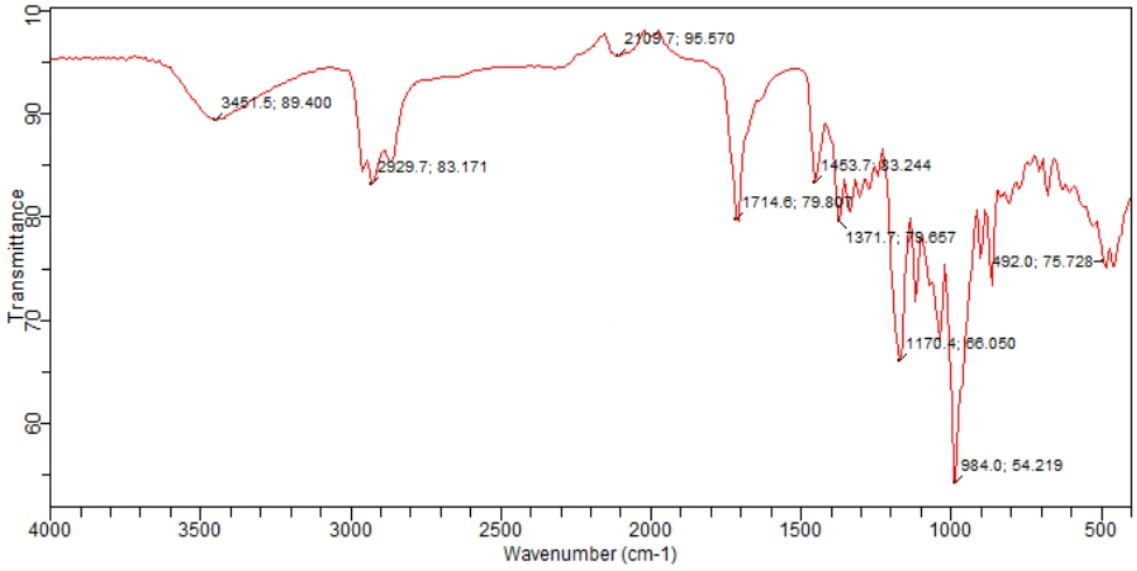


Figure S16: IR spectrum of **3**.

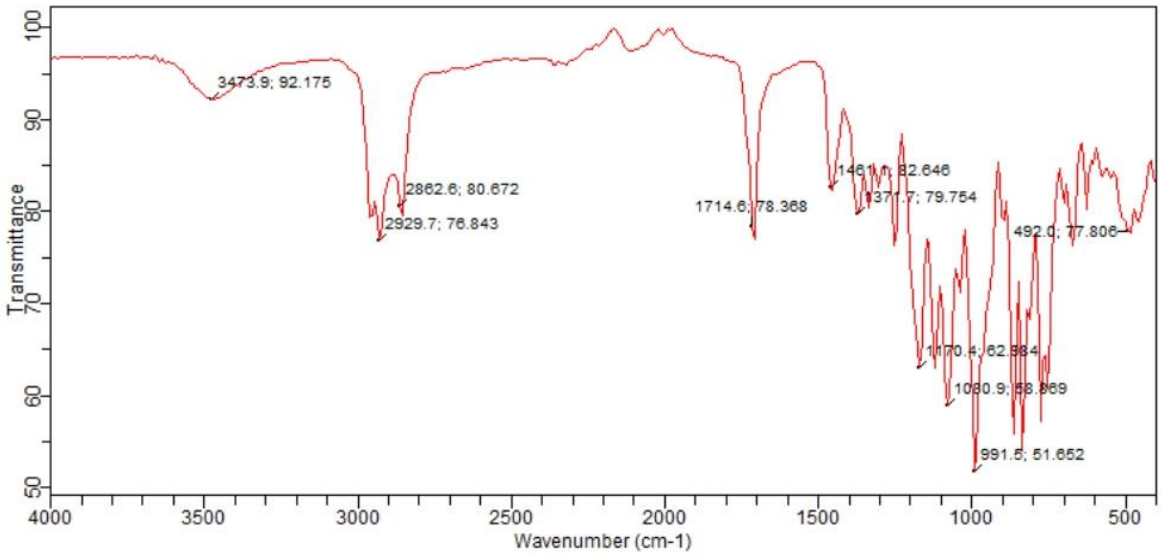


Figure S17: IR spectrum of **5**.

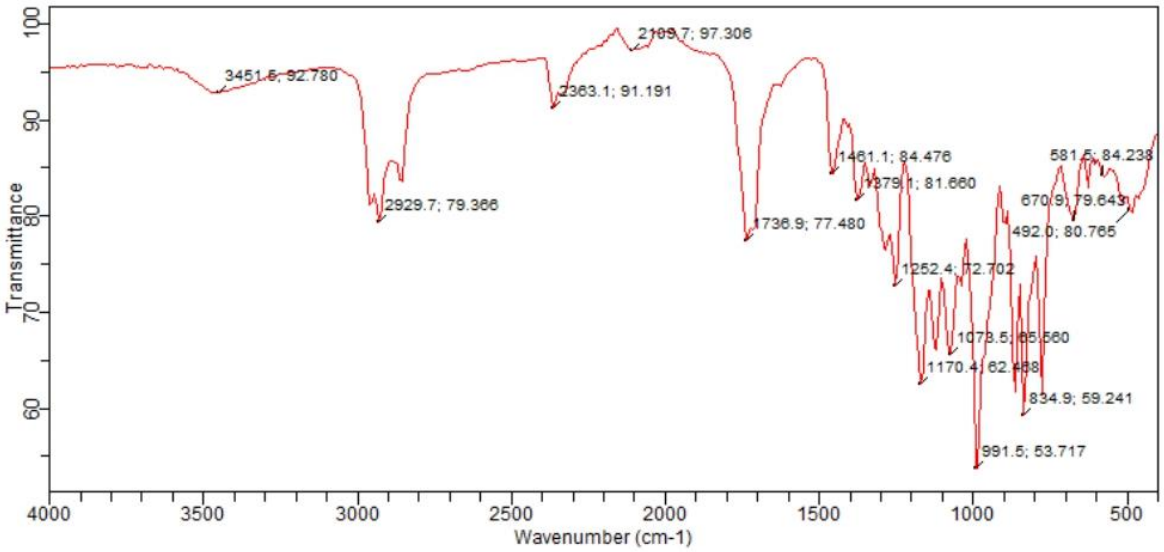


Figure S18: IRspectrum of **6**.

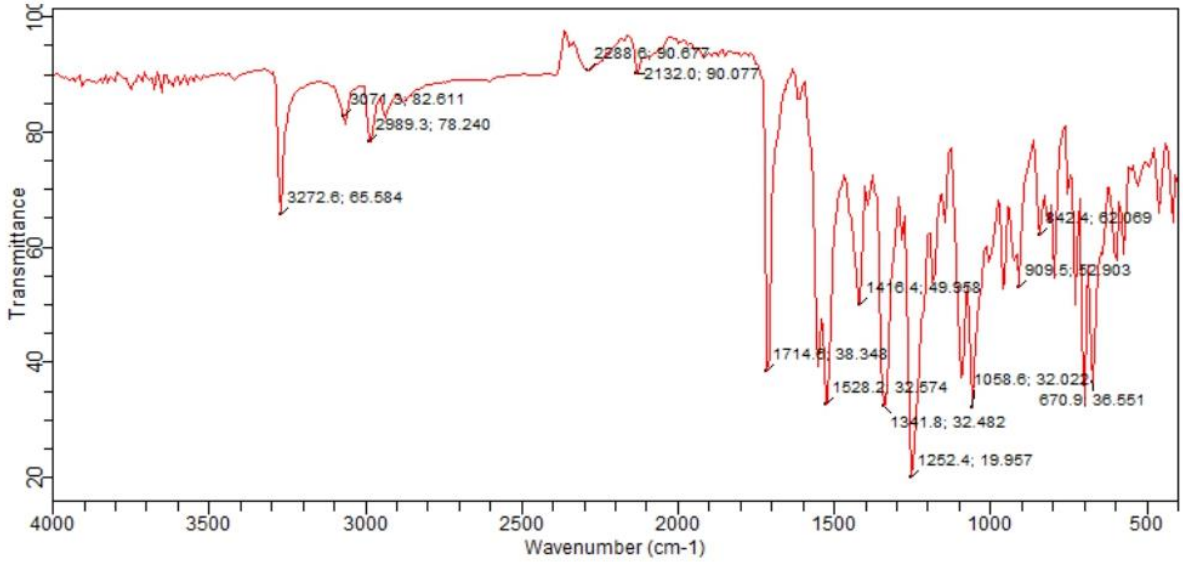


Figure S19: IR spectrum of **10a**.

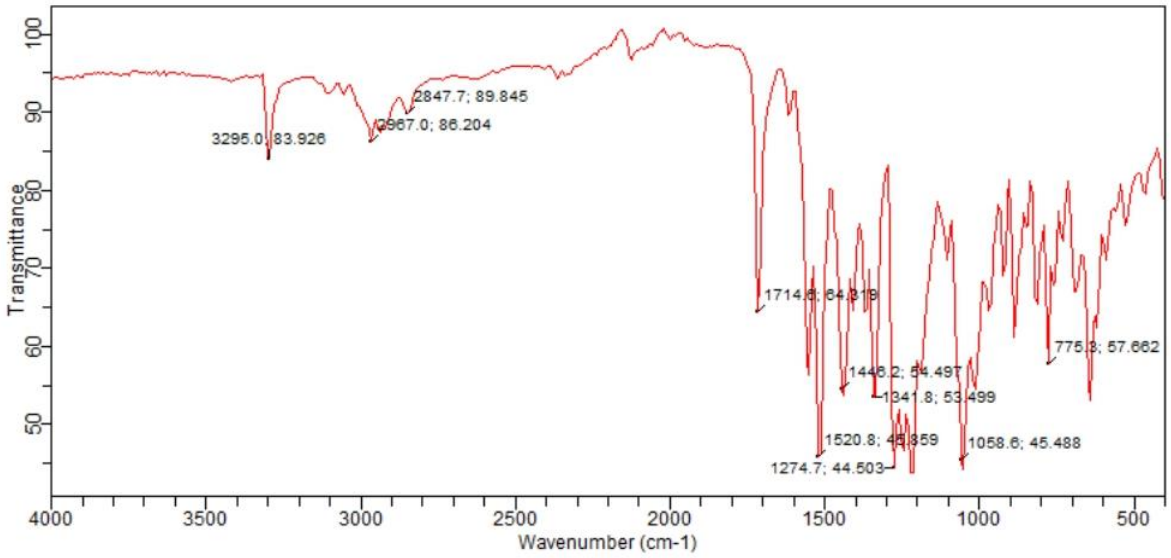


Figure S20: IR spectrum of **10b**.

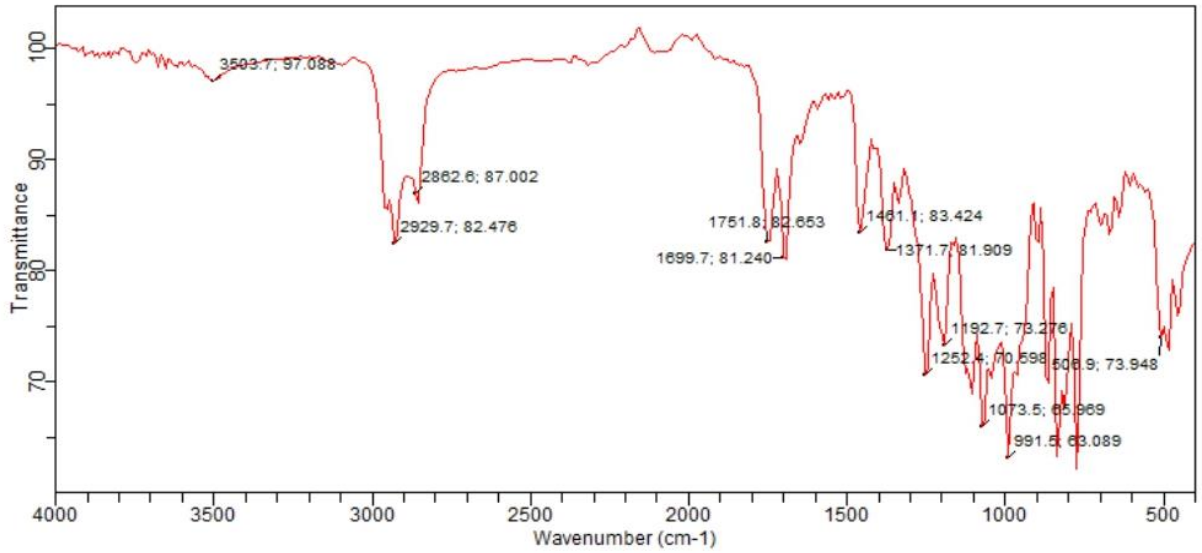


Figure S21: IR spectrum of **11**.

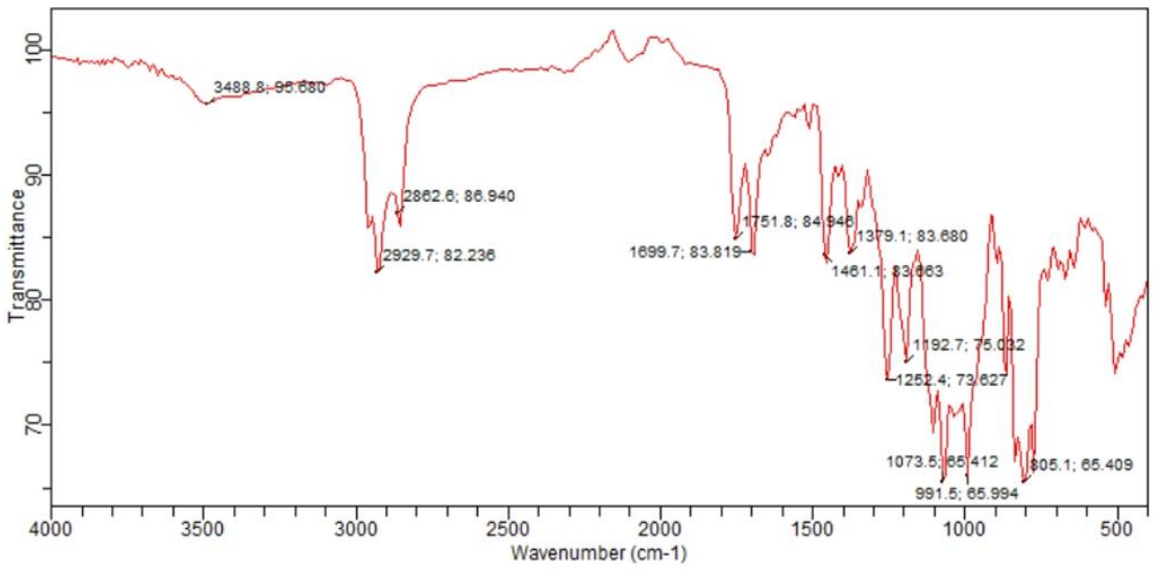


Figure S22: IR spectrum of **12**.

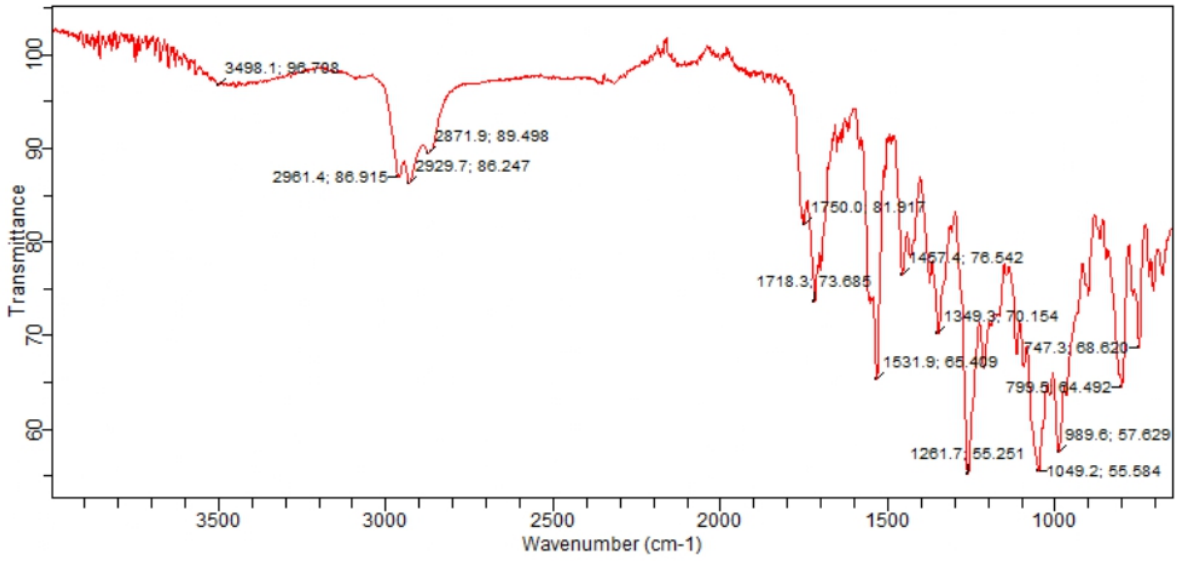


Figure S23: IR spectrum of **13**.

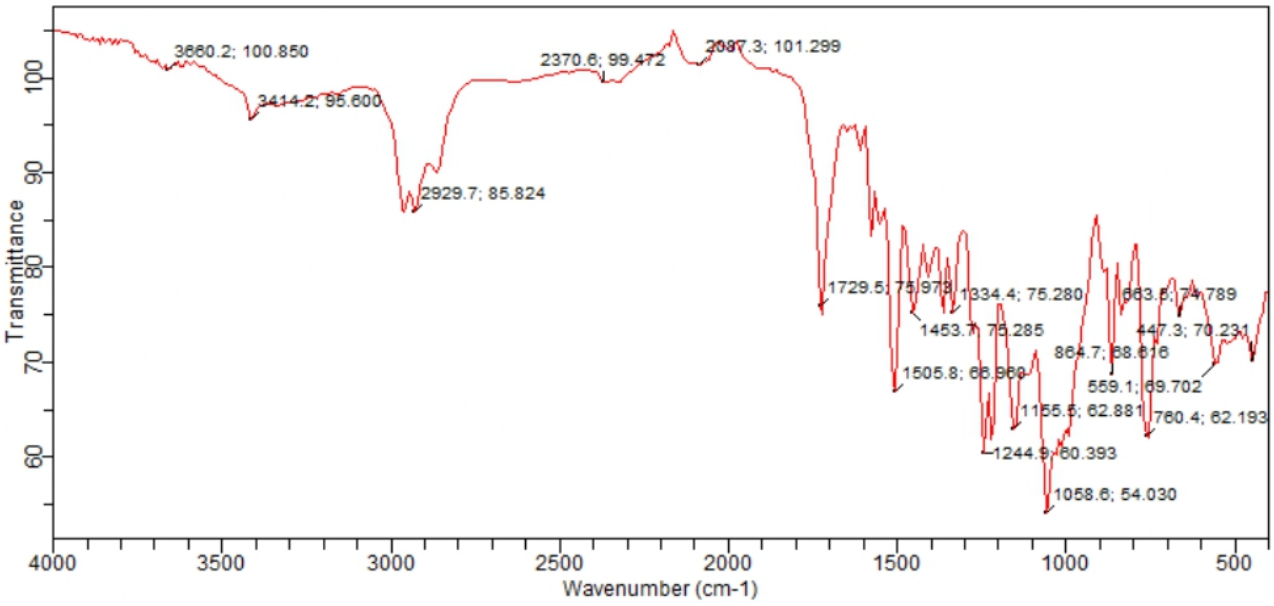


Figure S24: IR spectrum of **14**.

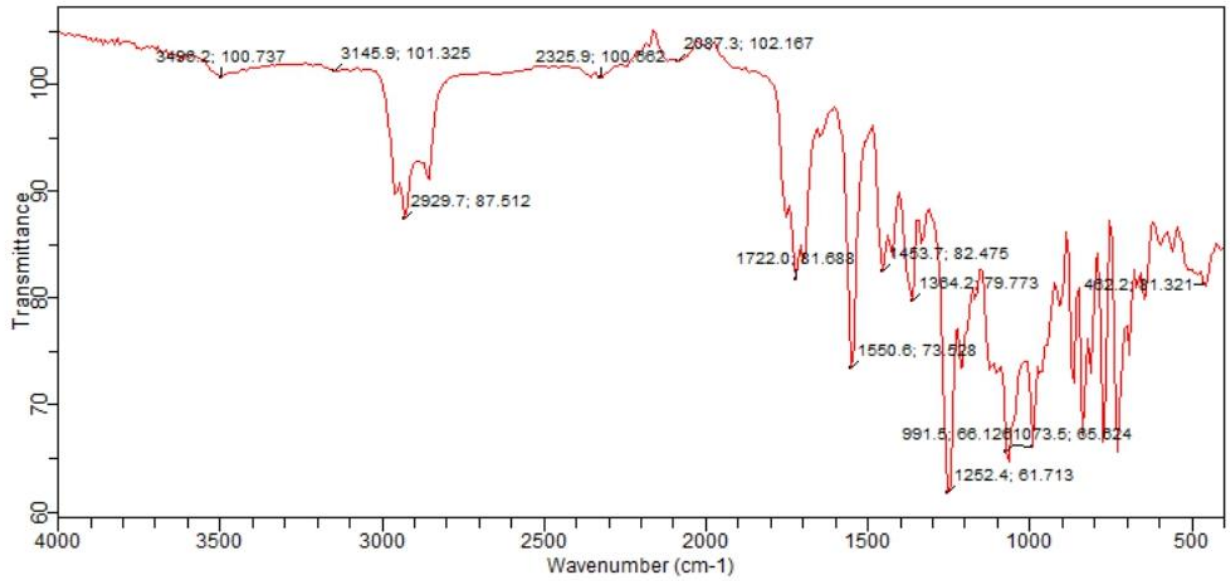


Figure S25: IR spectrum of **15**.

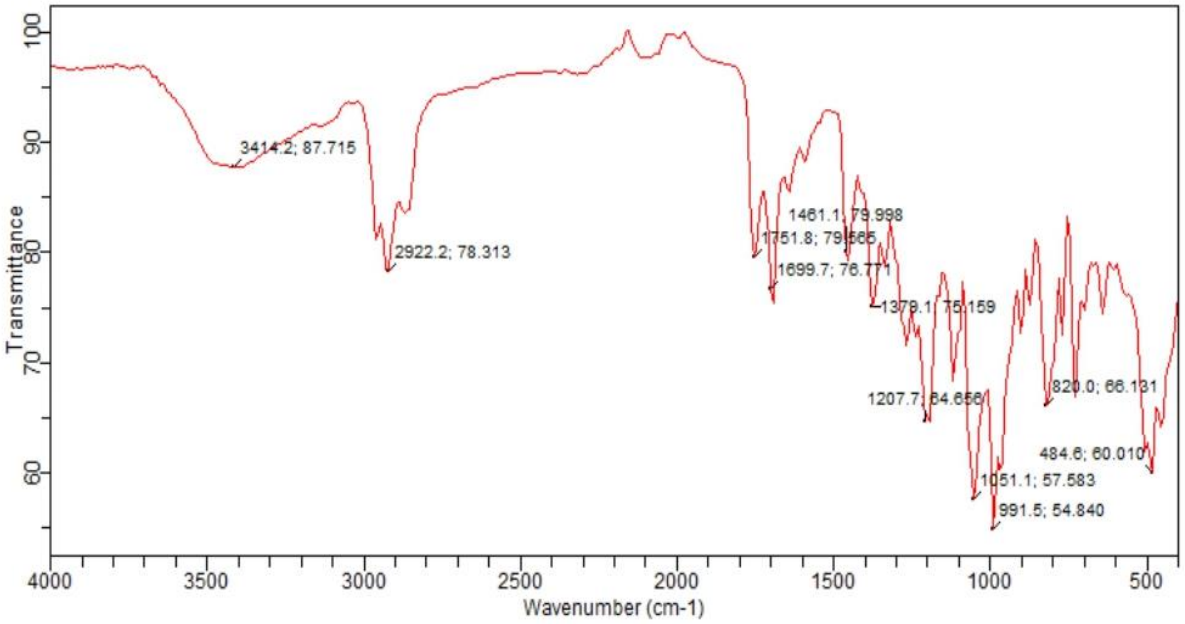


Figure S26: IR spectrum of **16**.

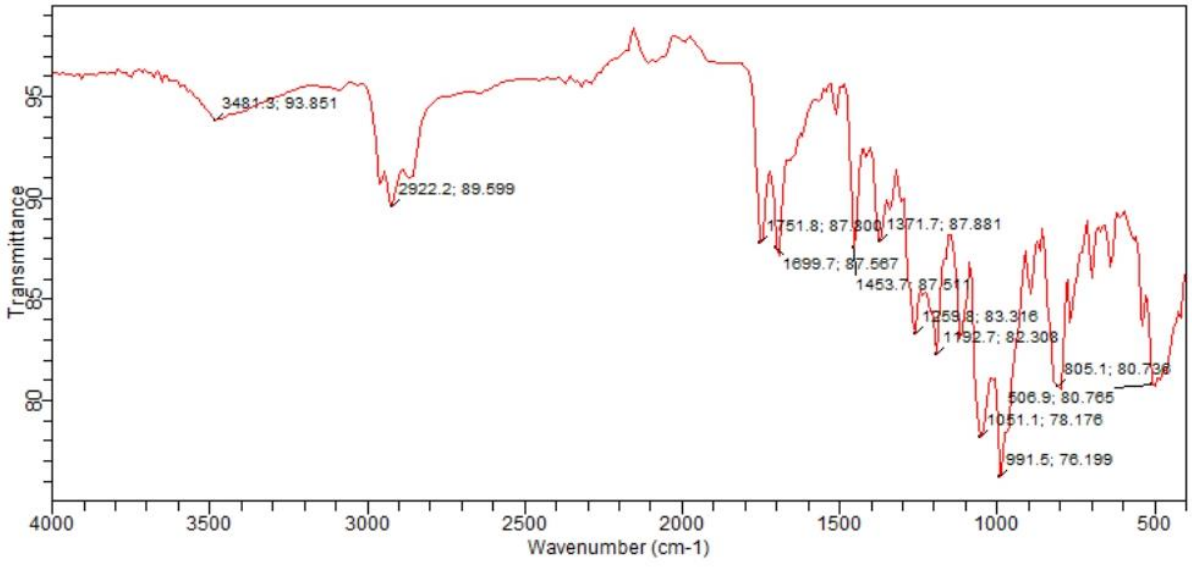


Figure S27: IR spectrum of **17**

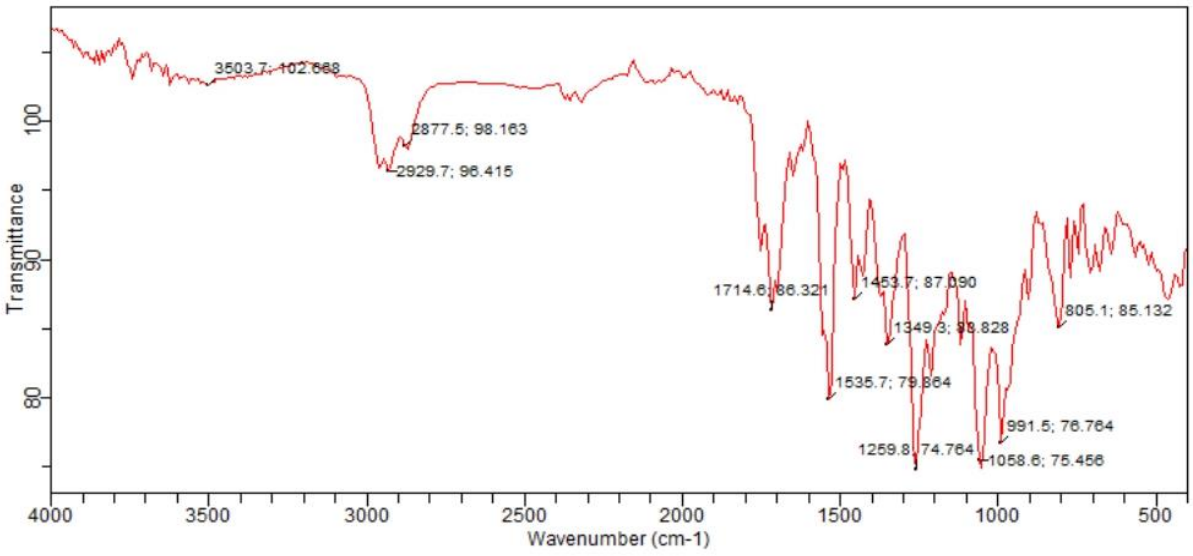


Figure S28: IR spectrum of **18**.

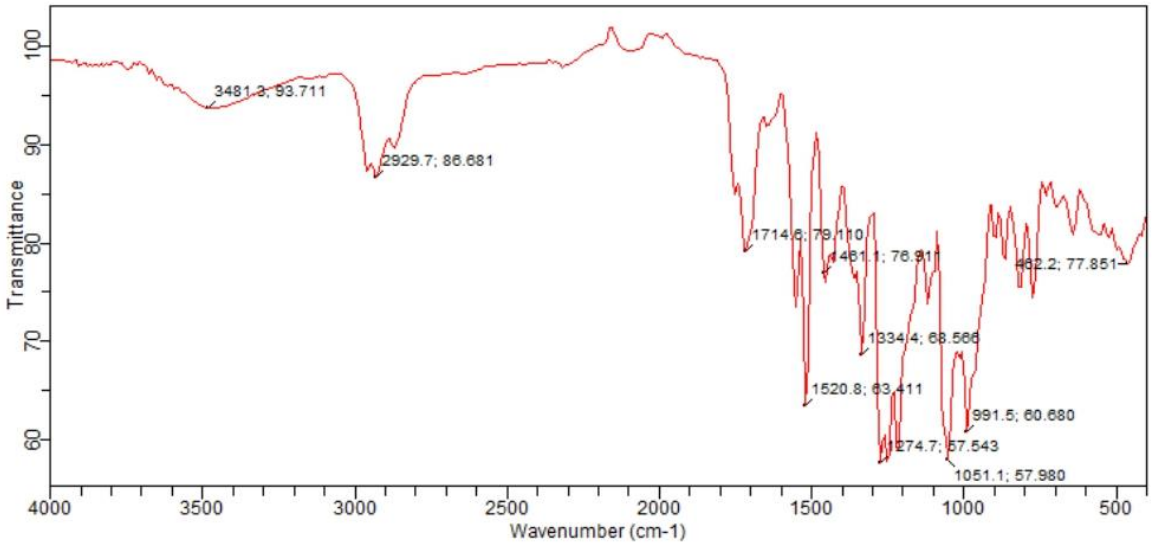


Figure S29: IR spectrum of **19**.

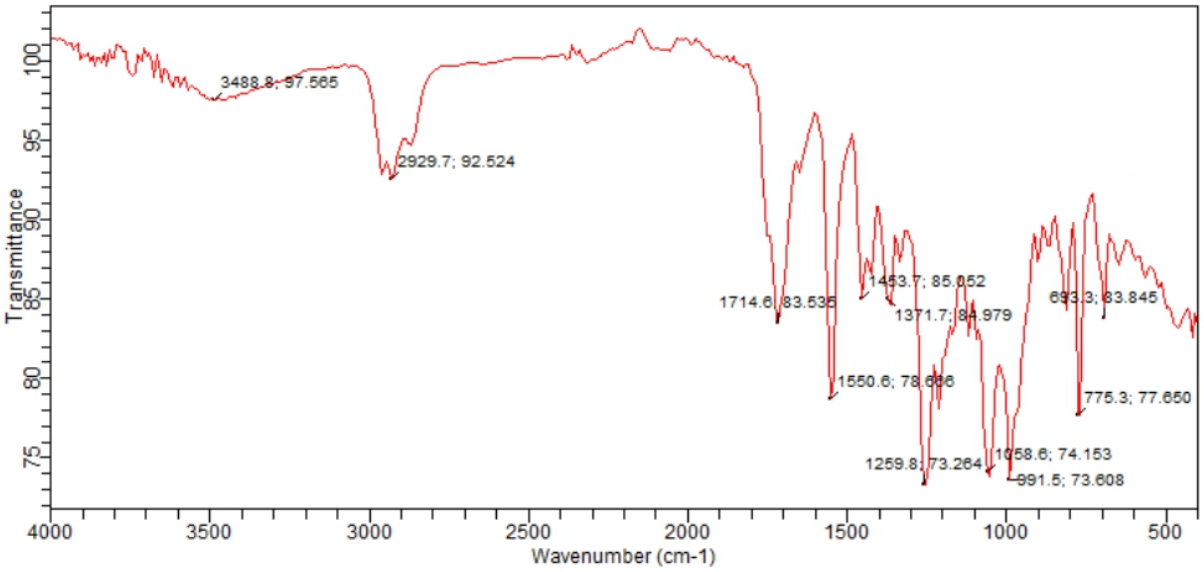


Figure S30: IR spectrum of **20**.

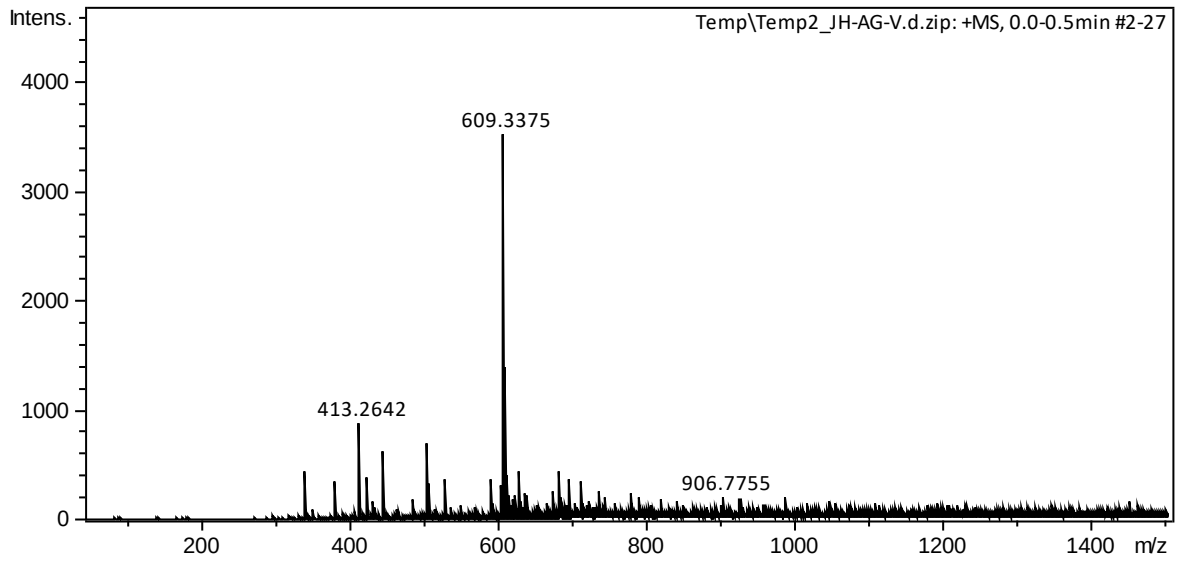


Figure S31: HRMS of **3**.

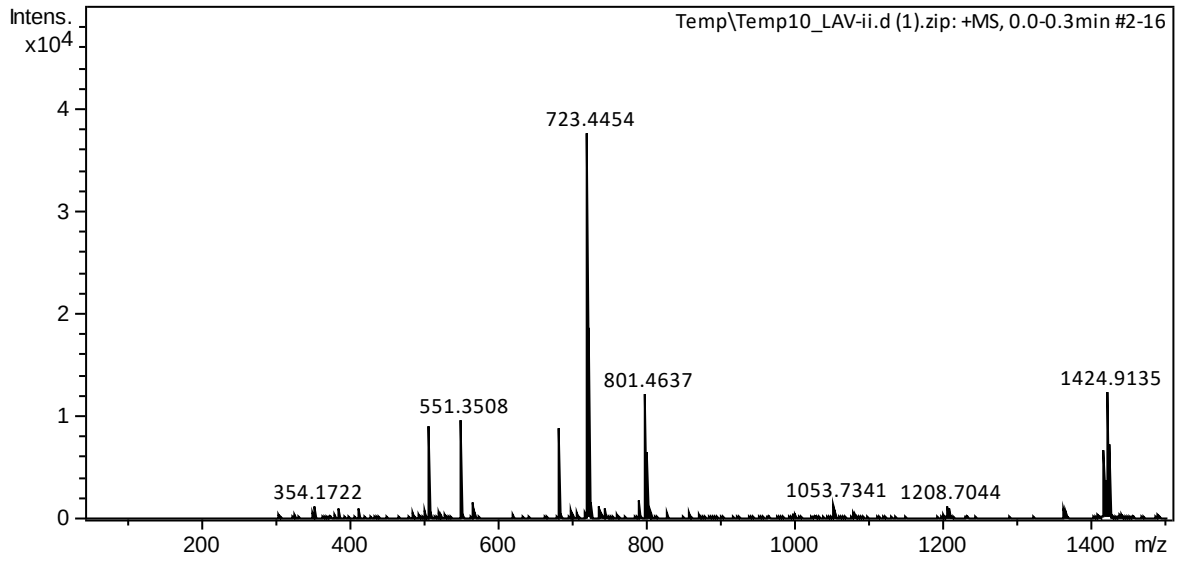


Figure S32: HRMS of **5**.

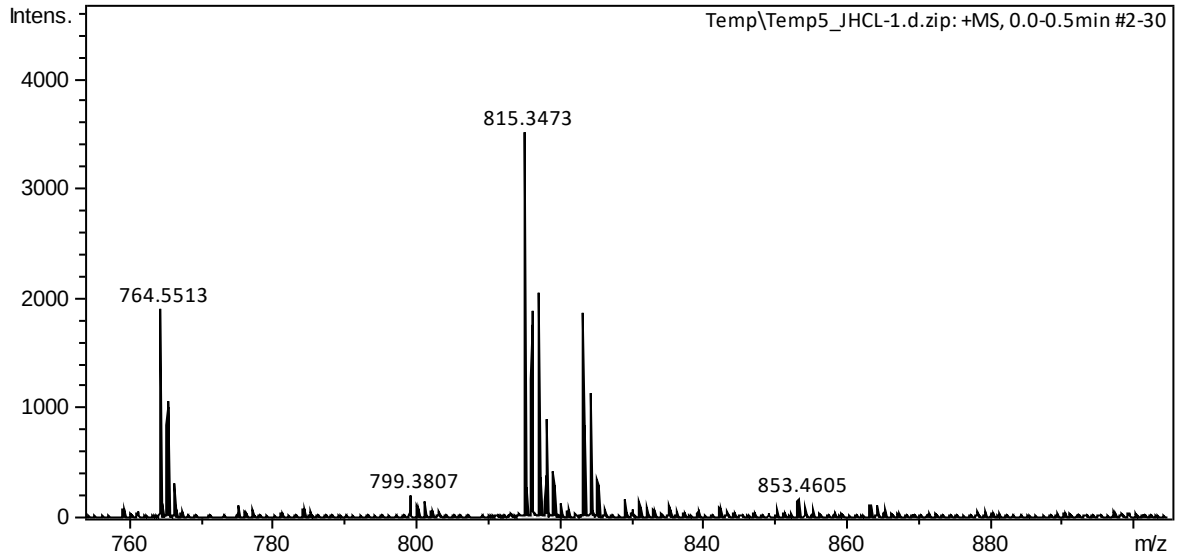


Figure S33: HRMS of **6**.

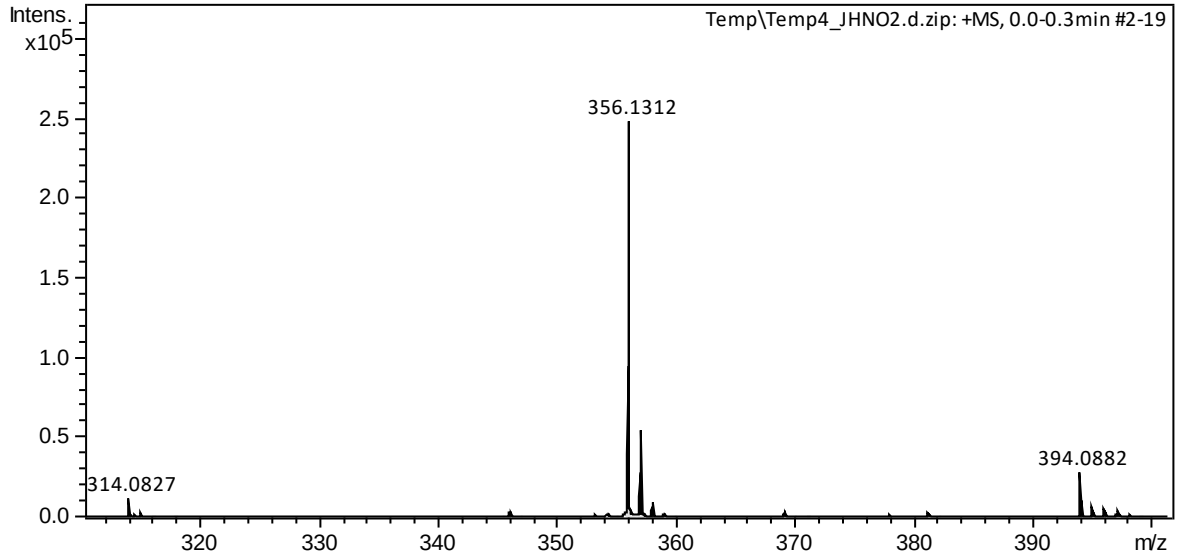


Figure S34: HRMS of **10a**.

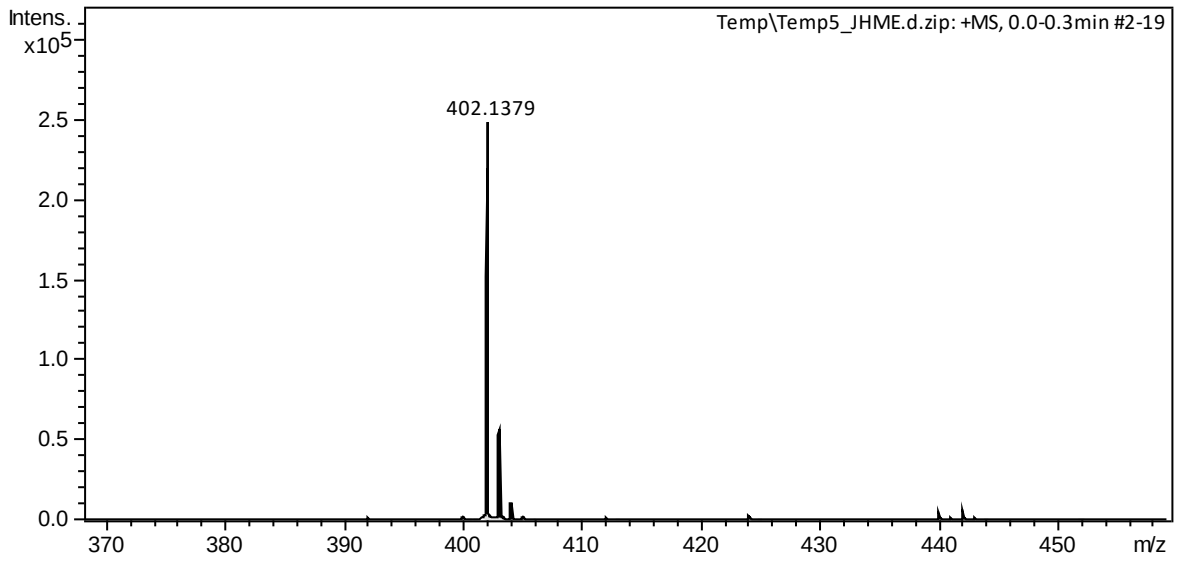


Figure S35: HRMS of **10b**.

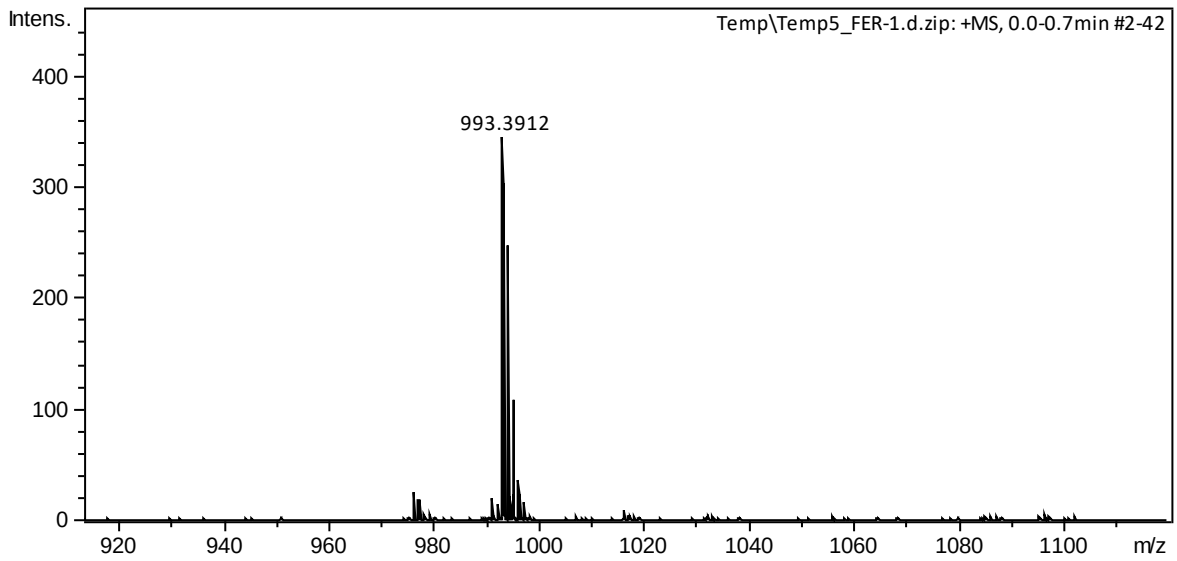


Figure S36: HRMS of **11**

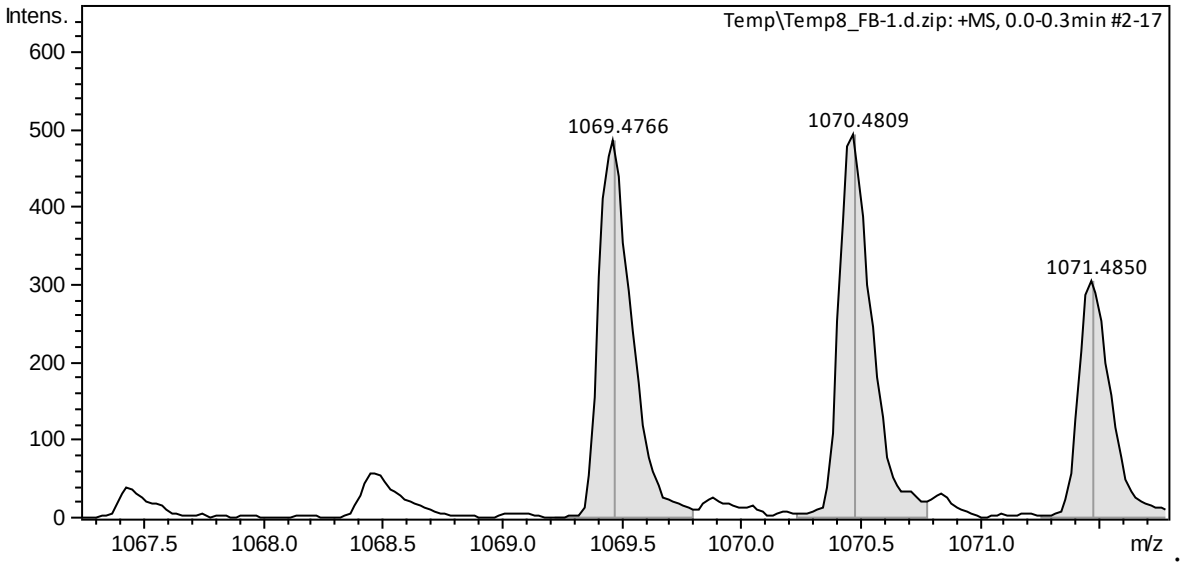


Figure S37: HRMS of **12**

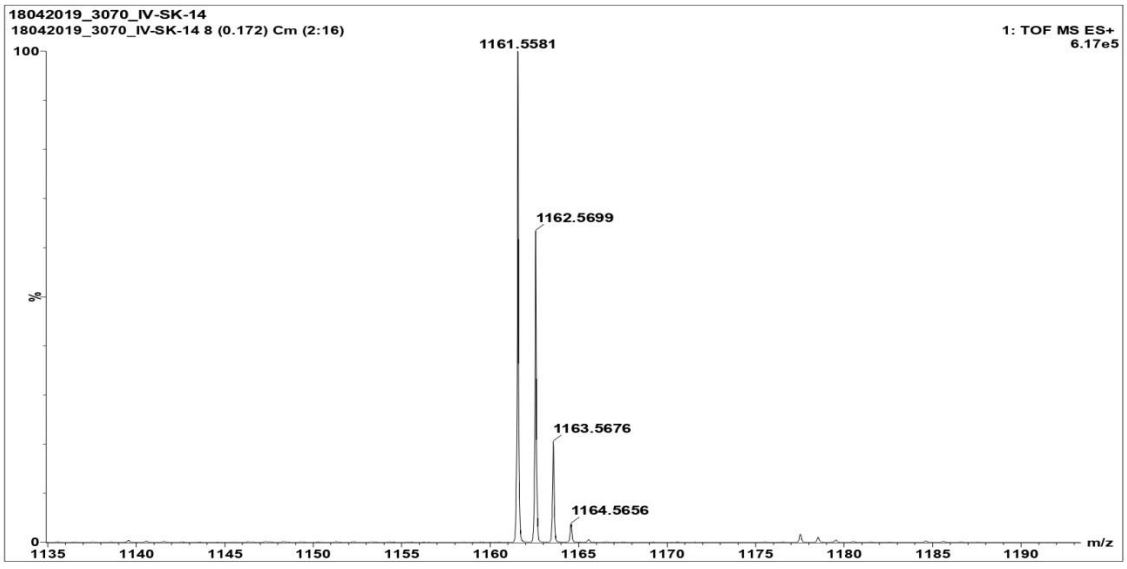


Figure S38: HRMS of **13.**

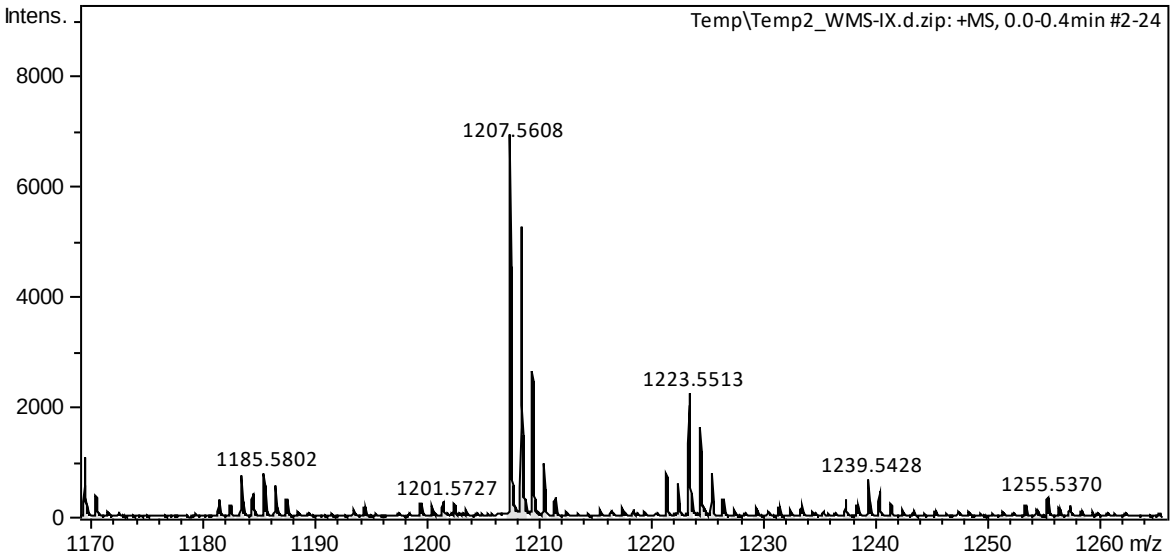
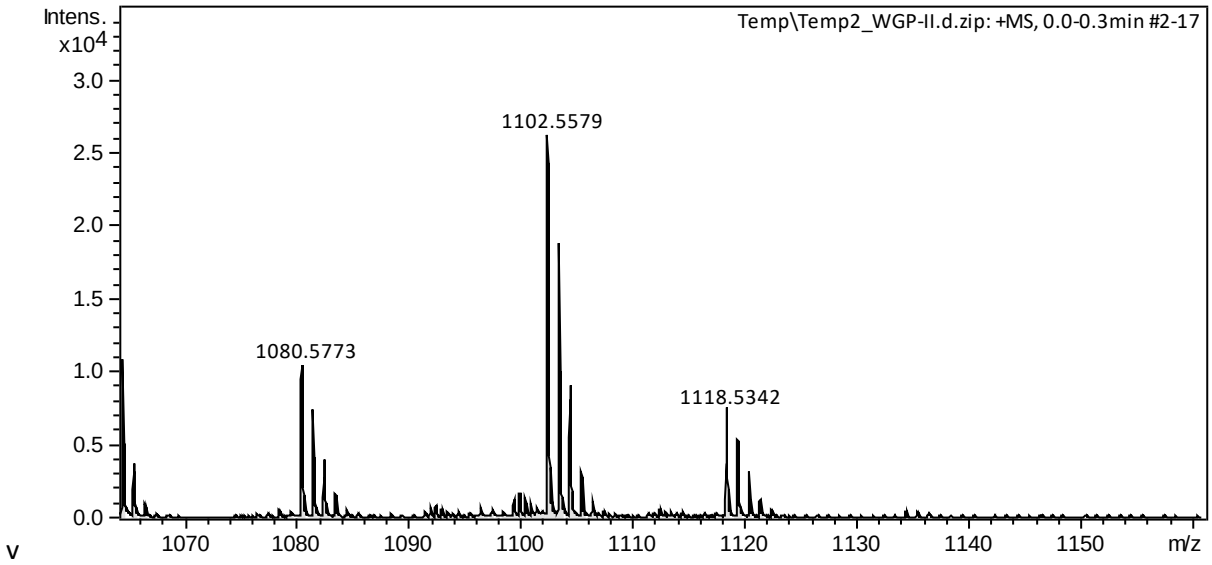


Figure S39: HRMS of **14.**



v

Figure S40: HRMS of **15.**

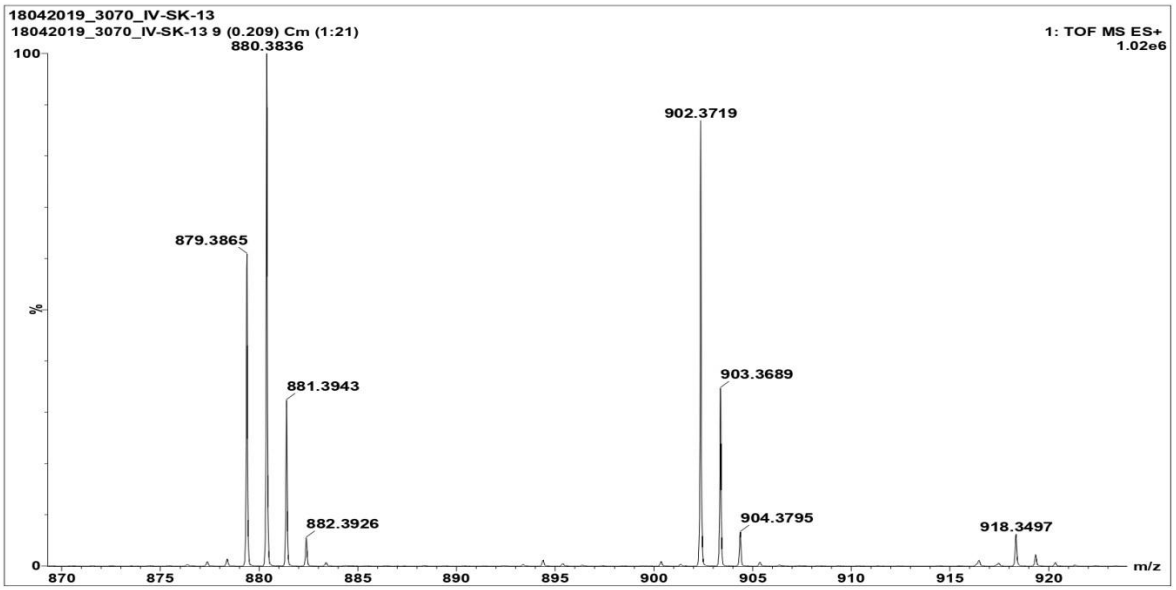


Figure S41: HRMS of **16.**

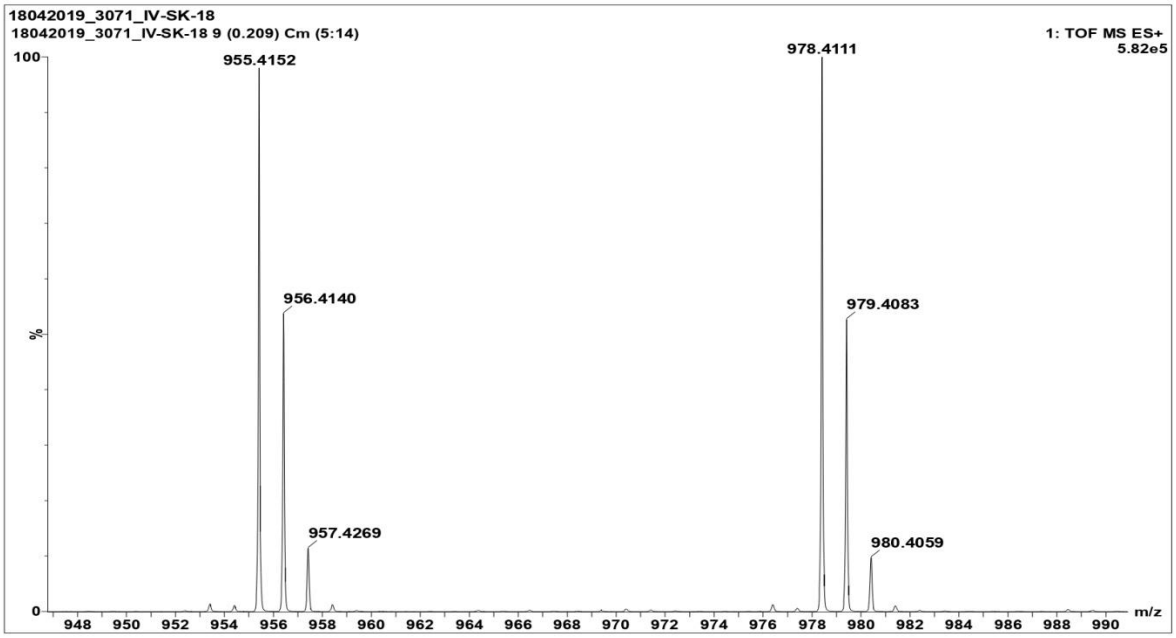


Figure S42: HRMS of **17.**

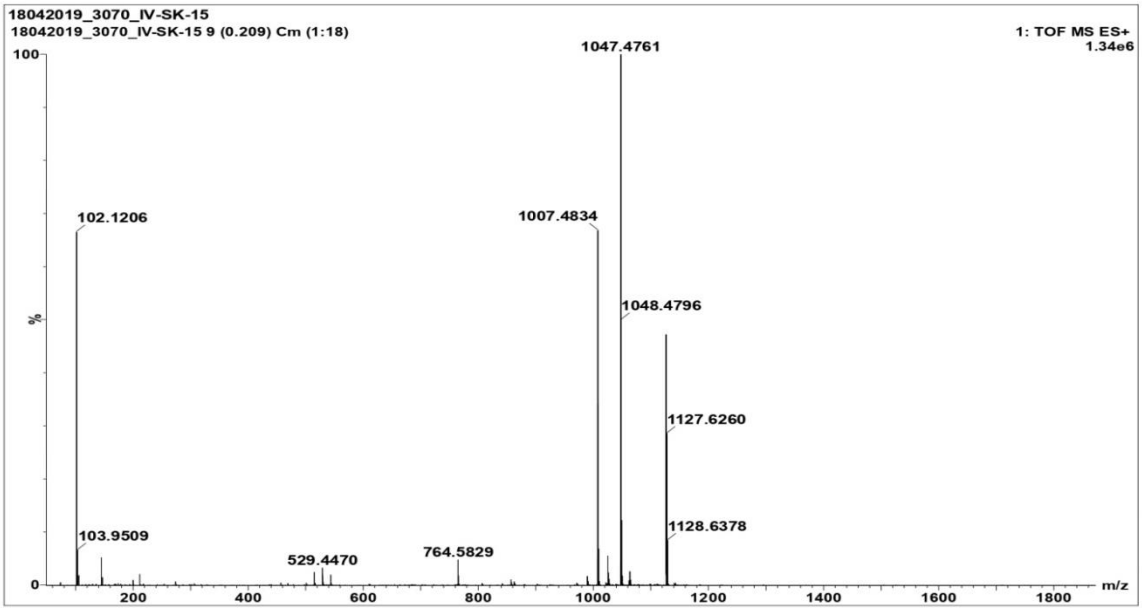


Figure S43: HRMS of **18.**

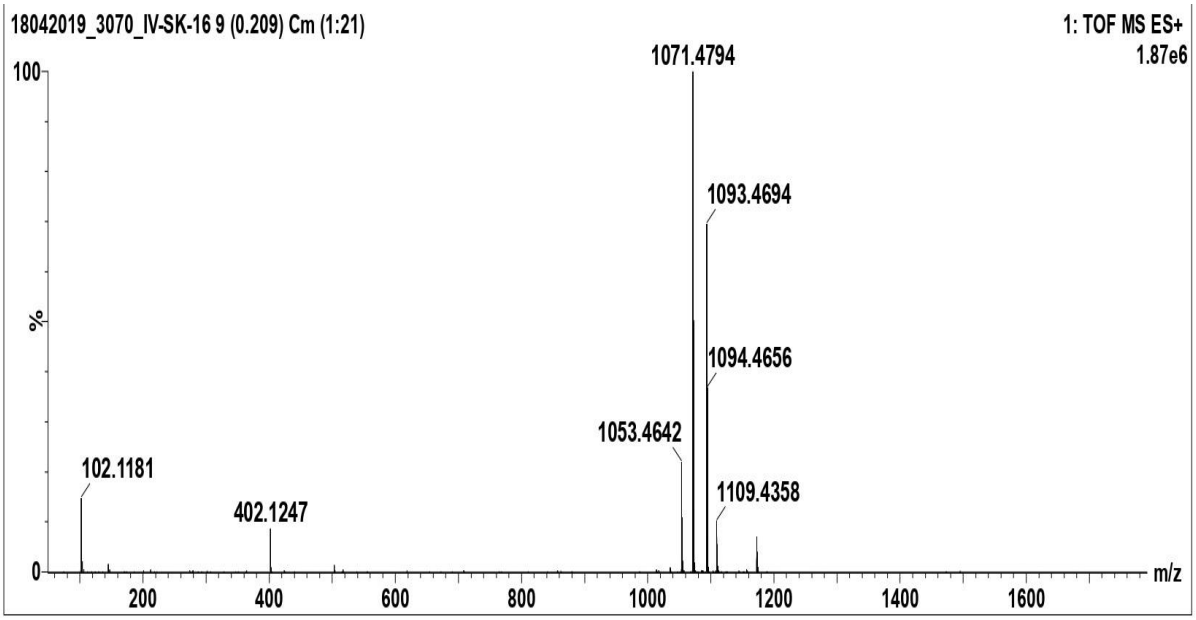


Figure S44: HRMS of **19**.

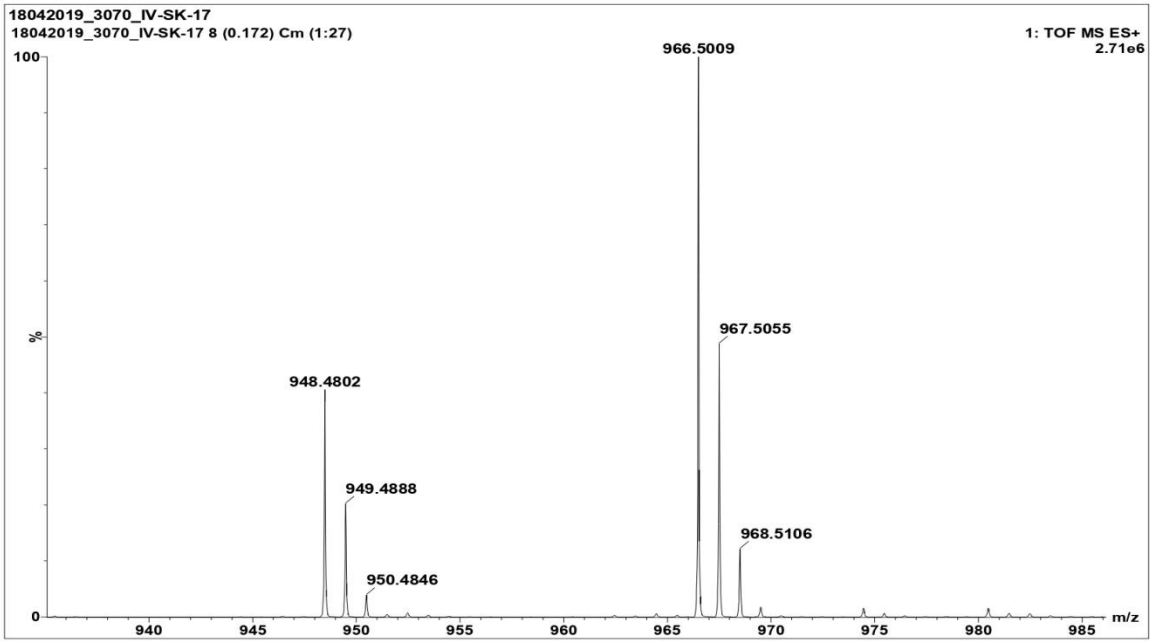


Figure S45: HRMS of **20**.

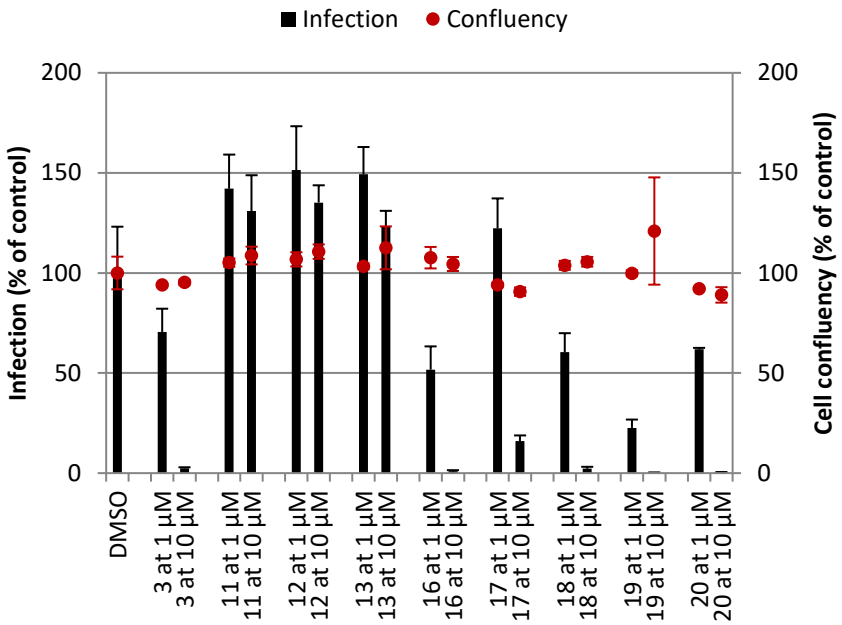


Figure S46: Assessment of *in vitro* activity of LP compounds/ivermectin derivatives against the hepatic stage of *P. berghei* infection. Total parasite load (infection scale, bars) and cell viability (cell confluency scale, dots) are shown. Results were normalized to the negative control, the drug vehicle dimethyl sulphoxide (DMSO), and are represented as mean ± SD, n = 1.

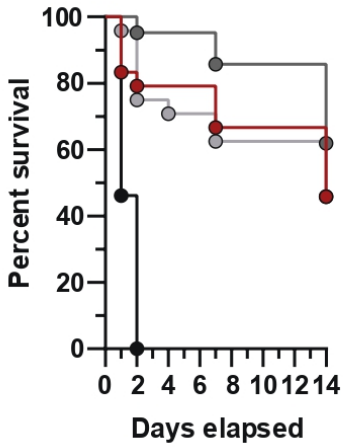
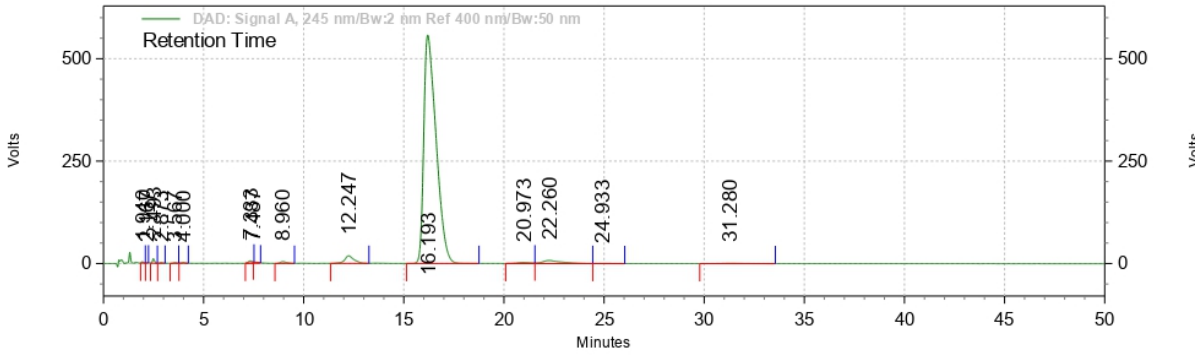


Figure S47: Mouse survival following spz administration and treatment using **19** and **3**. (Red line: DMSO; Light grey: **19**; Dark grey: **3**.)

Area % Report

Data File: C:\EZChrom Elite\Enterprise\Projects\Default\Data\IVM R1f 28-08-2019
Method: C:\EZChrom Elite\Enterprise\Projects\Default\Method\Baldev Singh\IAA Final method\ABA.met
Acquired: 8/28/2019 7:30:18 PM
Printed: 8/29/2019 3:51:14 PM



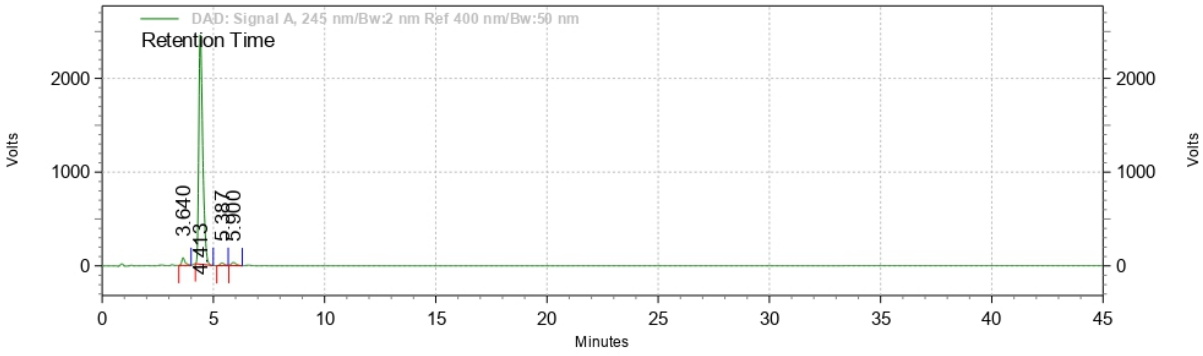
DAD: Signal A,
245 nm/Bw:2 nm
Ref 400
nm/Bw:50 nm
Results

Retention Time	Area	Area %	Height	Height %
1.940	39272	0.07	7368	0.57
2.167	5537	0.01	1341	0.10
2.493	160894	0.30	23220	1.78
2.873	36109	0.07	4144	0.32
3.567	35780	0.07	3640	0.28
4.000	42927	0.08	3813	0.29
7.333	107241	0.20	11779	0.90
7.487	68704	0.13	5975	0.46
8.960	192087	0.36	9036	0.69
12.247	1254617	2.36	38987	2.99
16.193	49806716	93.64	1169744	89.81
20.973	256022	0.48	5689	0.44
22.260	1045411	1.97	15747	1.21
24.933	39467	0.07	734	0.06
31.280	100430	0.19	1300	0.10
Totals	53191214	100.00	1302517	100.00

Figure S48. HPLC chromatograph of **1**.

Area % Report

Data File: C:\EZChrom Elite\Enterprise\Projects\Default\Data\Sample 3 16-10-2019
Method: C:\EZChrom Elite\Enterprise\Projects\Default\Method\untitled.met
Acquired: 10/16/2019 1:41:07 PM
Printed: 10/18/2019 5:52:07 PM



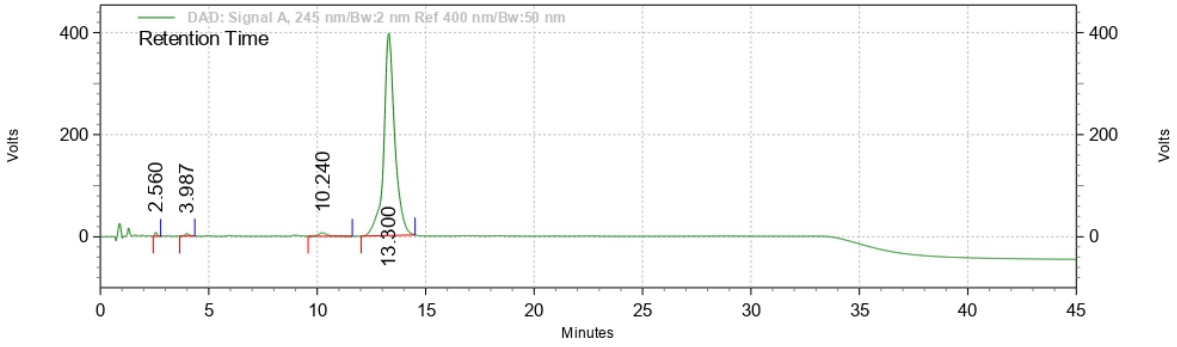
DAD: Signal A,
245 nm/Bw:2 nm
Ref 400
nm/Bw:50 nm
Results

Retention Time	Area	Area %	Height	Height %
3.640	1823847	2.73	173782	3.20
4.413	63404712	94.74	5133048	94.43
5.387	716779	1.07	59849	1.10
5.900	982134	1.47	69321	1.28
Totals	66927472	100.00	5436000	100.00

Figure S49. HPLC chromatograph of **3**.

Area % Report

Data File: C:\EZChrom Elite\Enterprise\Projects\Default\Data\Sample 16 16-10-2019
Method: C:\EZChrom Elite\Enterprise\Projects\Default\Method\untitled.met
Acquired: 10/16/2019 2:28:01 PM
Printed: 10/18/2019 5:55:21 PM



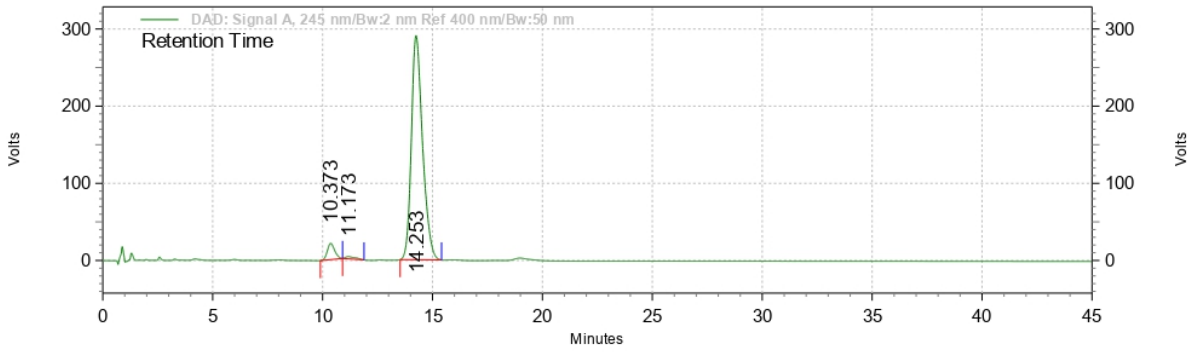
DAD: Signal A,
245 nm/Bw:2 nm
Ref 400
nm/Bw:50 nm
Results

Retention Time	Area	Area %	Height	Height %
2.560	109998	0.37	15500	1.78
3.987	136832	0.46	10690	1.23
10.240	419195	1.40	13940	1.60
13.300	29234535	97.77	831055	95.39
Totals	29900560	100.00	871185	100.00

Figure S50. HPLC chromatograph of **16**.

Area % Report

Data File: C:\EZChrom Elite\Enterprise\Projects\Default\Data\Sample 18 16-10-2019
Method: C:\EZChrom Elite\Enterprise\Projects\Default\Method\untitled.met
Acquired: 10/16/2019 4:01:48 PM
Printed: 10/18/2019 5:44:15 PM



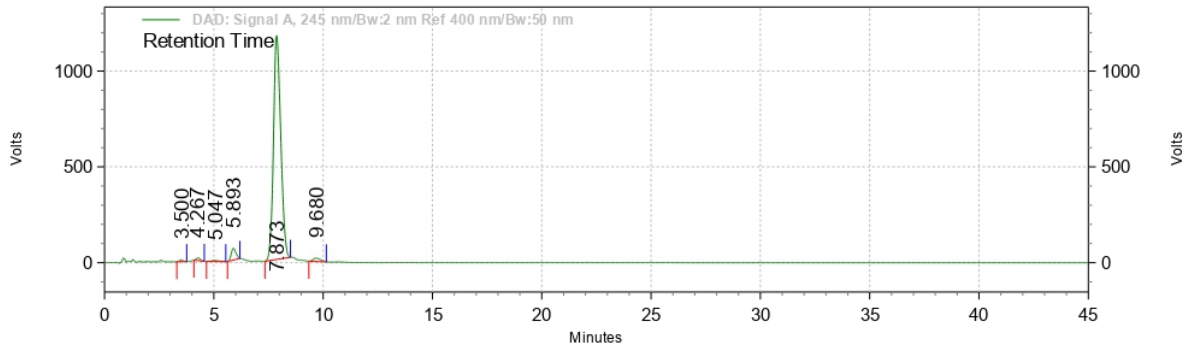
DAD: Signal A,
245 nm/Bw:2 nm
Ref 400
nm/Bw:50 nm
Results

Retention Time	Area	Area %	Height	Height %
10.373	1022971	4.49	44295	6.71
11.173	200795	0.88	6277	0.95
14.253	21567186	94.63	609596	92.34
Totals	22790952	100.00	660168	100.00

Figure S51. HPLC chromatograph of **18**.

Area % Report

Data File: C:\EZChrom Elite\Enterprise\Projects\Default\Data\sample 19 chem 22-10-19.dat
Method: C:\EZChrom Elite\Enterprise\Projects\Default\Method\untitled.met
Acquired: 10/22/2019 4:32:28 PM
Printed: 10/23/2019 12:05:37 PM



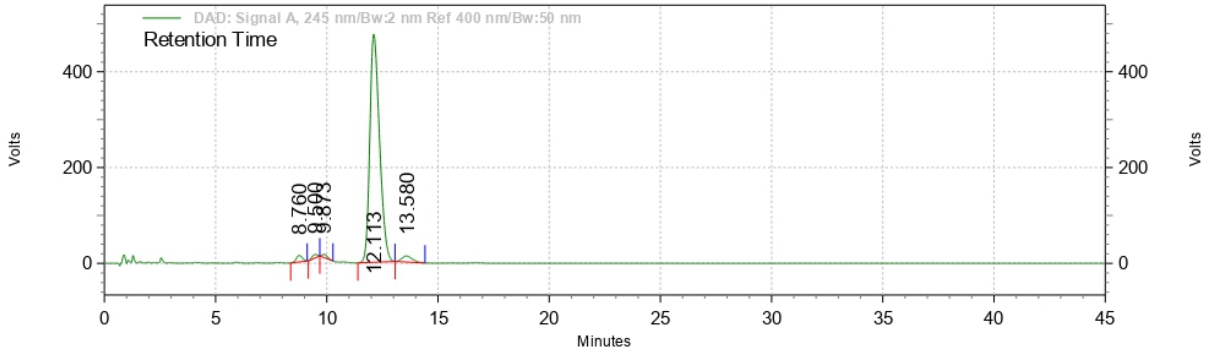
DAD: Signal A,
245 nm/Bw:2 nm
Ref 400
nm/Bw:50 nm
Results

Retention Time	Area	Area %	Height	Height %
3.500	204172	0.34	19488	0.73
4.267	384909	0.64	30151	1.13
5.047	256402	0.43	12753	0.48
5.893	1903977	3.16	128653	4.80
7.873	56590922	94.00	2451386	91.48
9.680	864158	1.44	37263	1.39
Totals	60204540	100.00	2679694	100.00

Figure S52. HPLC chromatograph of **19**.

Area % Report

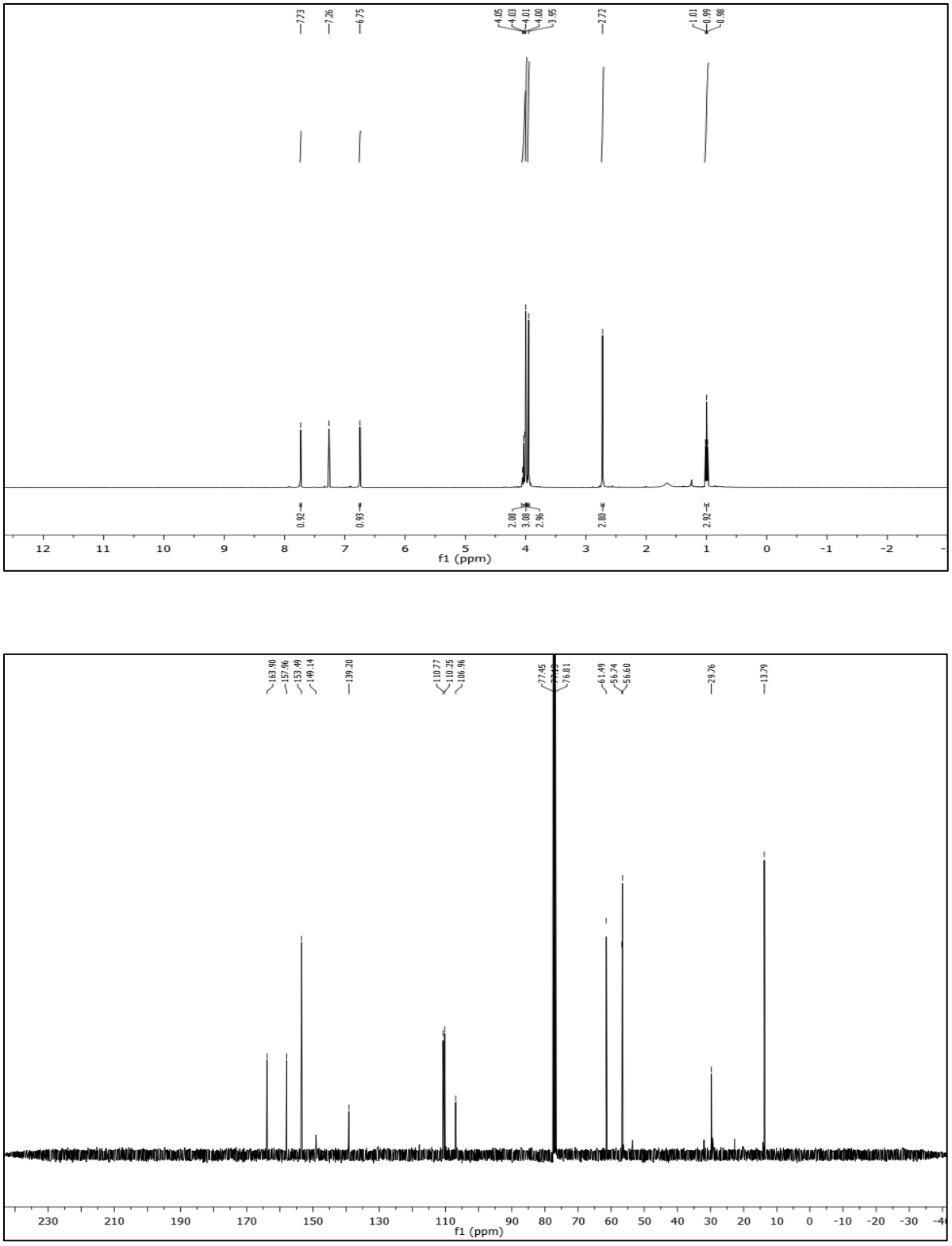
Data File: C:\EZChrom Elite\Enterprise\Projects\Default\Data\sample 20 18-10-19.dat
Method: C:\EZChrom Elite\Enterprise\Projects\Default\Method\untitled.met
Acquired: 10/18/2019 1:08:46 PM
Printed: 10/18/2019 5:48:19 PM



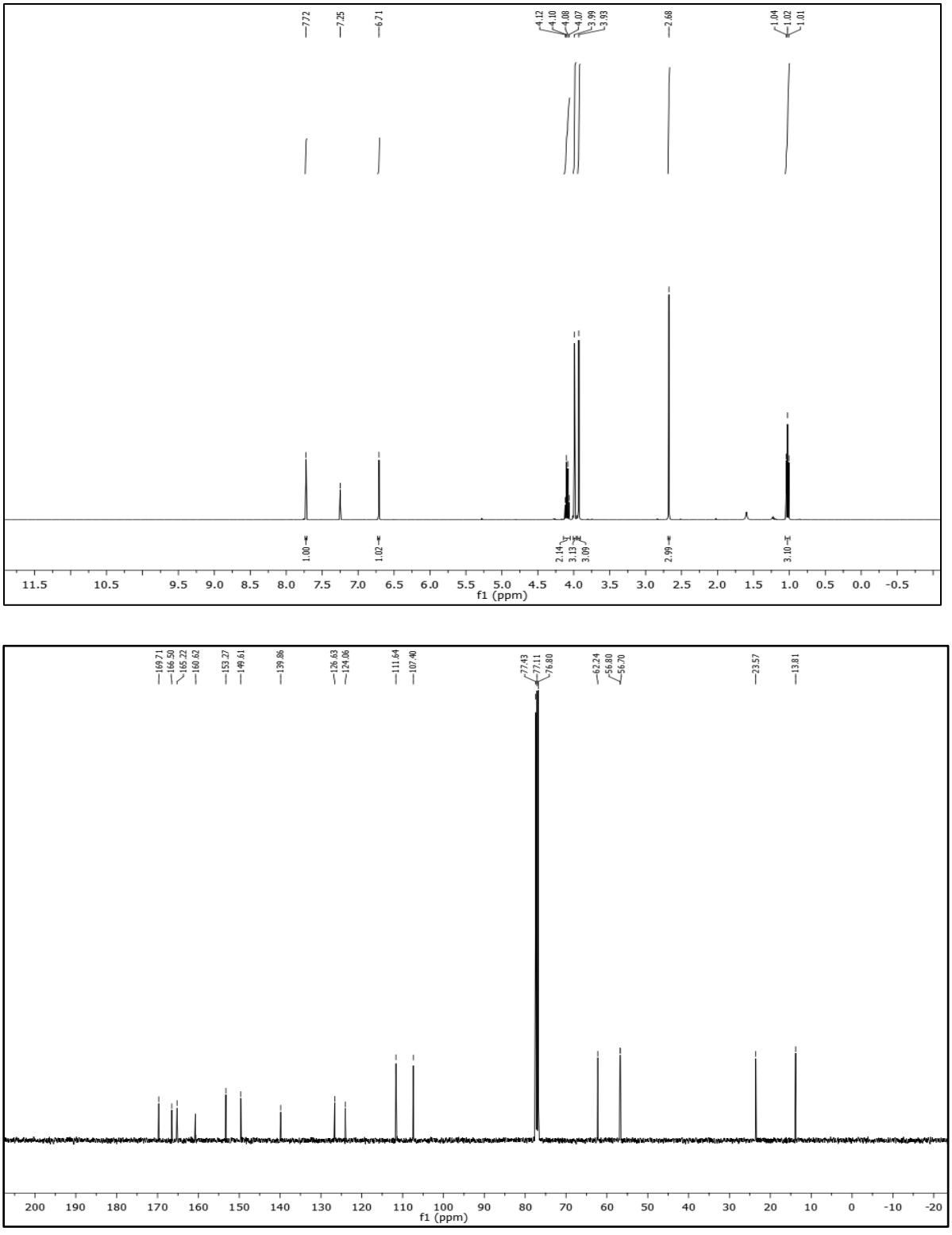
DAD: Signal A,
245 nm/Bw:2 nm
Ref 400
nm/Bw:50 nm
Results

Retention Time	Area	Area %	Height	Height %
8.760	545137	1.69	28759	2.66
9.500	258482	0.80	14575	1.35
9.873	249417	0.77	14191	1.31
12.113	30317009	94.06	998675	92.20
13.580	861735	2.67	26975	2.49
Totals	32231780	100.00	1083175	100.00

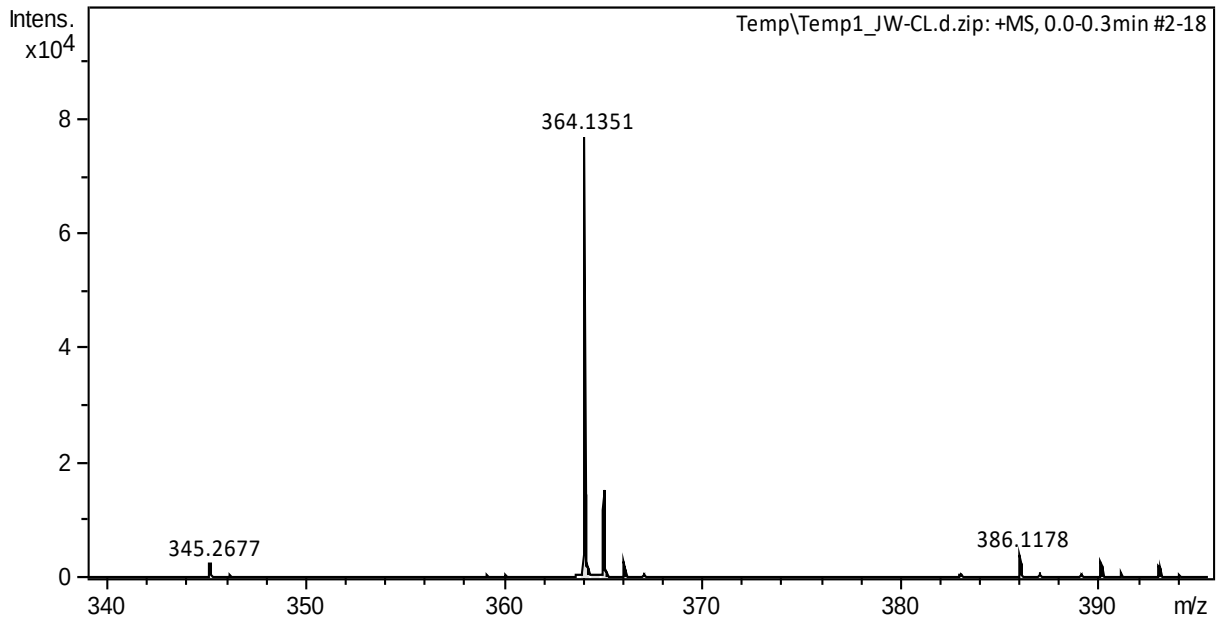
Figure S53. HPLC chromatograph of **20**.



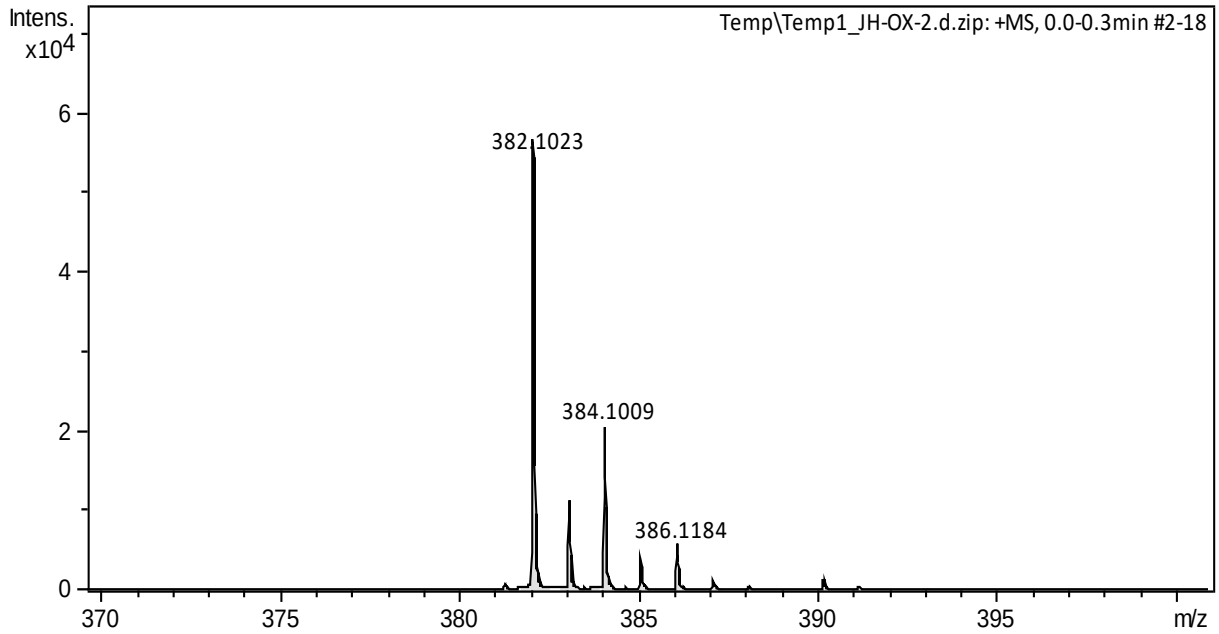
FigureS54.[1]H NMR (400 MHz, $CDCl_3$) and[13]C NMR (100 MHz, $CDCl_3$)of **22**.



FigureS55.[1]H NMR (400 MHz, $CDCl_3$) and[13]C NMR (100 MHz, $CDCl_3$)of **23**.



FigureS56: HRMS of **22.**



FigureS57: HRMS of **23.**

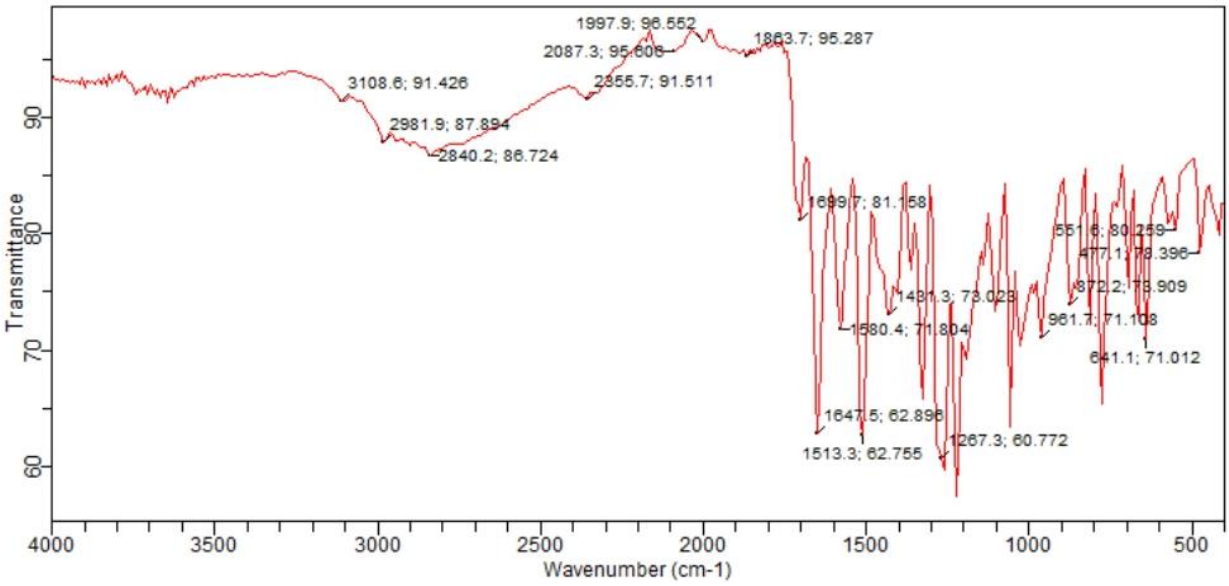


Figure S58: IR spectrum of **22**.

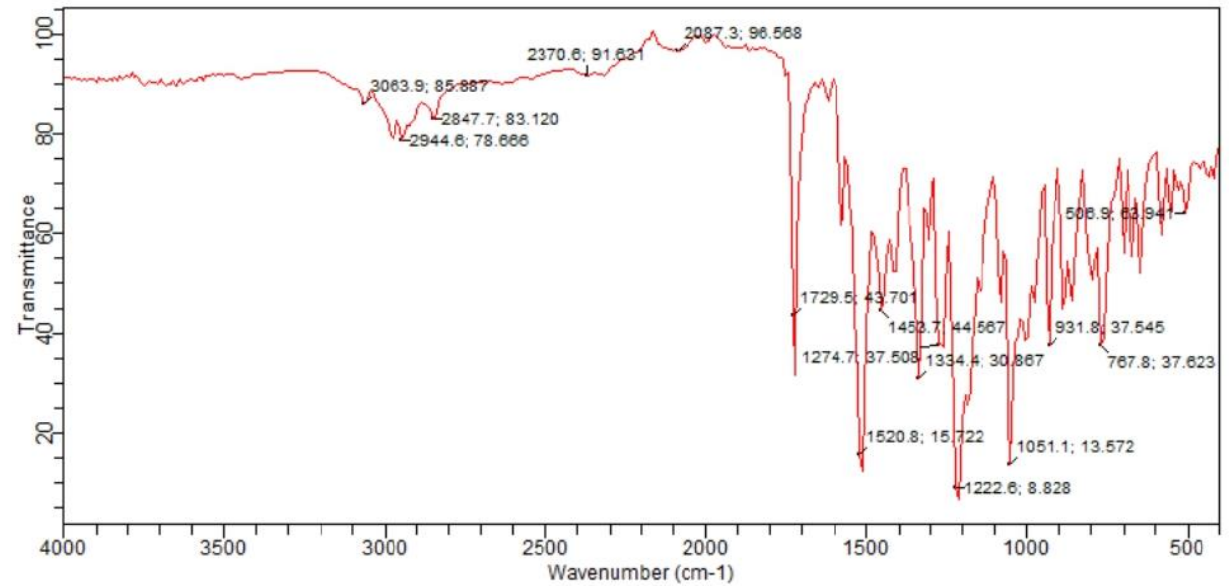


Figure S59: IR spectrum of **23**.

Procedure for Molecular docking studies

The 2D structure of the ligand was drawn using 2D sketcher (Schrödinger_Suites_2019-3, LLC, New York, 2019). The 3D structure of the ligand with the lowest energy was generated using LigPrep module of Schrödinger. The correct ionization states (pH 7.0±2.0) of the ligand was generated and optimized with default settings (Ligprep, Schrödinger_Suites_2019-3). The 3D crystal structure of *C. elegans* Glutamate-gated chloride channel (GluCl) in complex with Fab and Ivermectin (PDB ID:3RHW; resolution 3.26 Å) was retrieved from protein data bank (www.rcsb.org). The proteins were prepared for docking using Protein Preparation Wizard (Maestro 12.1, Schrödinger_Suites_2019-3). Further to refine the structure, OPLS-3E force field parameter was used to alleviate steric clashes. The location of co-crystalized ligand Ivermectin was used to choose the center and size of the receptor grid, which was generated using Glide (Schrödinger_Suites_2019-3, LLC, New York, 2019) with default settings for all parameters. The grid size was chosen sufficiently large to include all active site residues involved in substrate binding. The ligand was docked to the receptor grid file (GluCl) using Glide Standard precision (SP) mode. Default settings were used for the refinement and scoring.

Compound number	Smiles	Antiplasmodial activity	
		IC_{50} (µM) *P. berghei*	IC_{50} (nM) *Pf*NF54
3	O=C([C@@H]1C=C(C)C(O)[C@]2([H])[C@@]1(O)/C(CO2)=C/C=C/[C@H](C)[C@H](O)/C(C)=C/C3)O[C@H]4C[C@]3([H])O[C@]5(O[C@H]([C@H](C)CC)[C@@H](C)CC5)C4	4.557 ± 1.981	
5	O=C([C@@H]1C=C(C)C(O[Si](C)(C)C(C)(C)C)[C@]2([H])[C@@]1(O)/C(CO2)=C/C=C/[C@H](C)[C@H](O)/C(C)=C/C3)O[C@H]4C[C@]3([H])O[C@]5(O[C@H]([C@H](C)CC)[C@@H](C)CC5)C4		
6	O=C([C@@H]1C=C(C)C(O[Si](C)(C)C(C)(C)C)[C@]2([H])[C@@]1(O)/C(CO2)=C/C=C/[C@H](C)[C@H](OC(CCl)=O)/C(C)=C/C3)O[C@H]4C[C@]3([H])O[C@]5(O[C@H]([C@H](C)CC)[C@@H](C)CC5)C4		
7	O=C([C@@H]1C=C(C)C(O[Si](C)(C)C(C)(C)C)[C@]2([H])[C@@]1(O)/C(CO2)=C/C=C/[C@H](C)[C@H](OC(CN=[N+]=[N-])=O)/C(C)=C/C3)O[C@H]4C[C@]3([H])O[C@]5(O[C@H]([C@H](C)CC)[C@@H](C)CC5)C4		
8	[][Fe][].C#Cc1cccc1.c2cccc2		
9	[][Fe][].C#CC(C=C1)=CC=C1c2cccc2.c3cccc3		
10a	O=C(OC(C)C)C1=C(C)N=C(OCC#C)N=C1C2=CC=CC([N+]([O-])=O)=C2		
10b	O=C(OCC)C1=C(C)N=C(OCC#C)N=C1C2=C([N+]([O-])=O)C=C(OC)C(OC)=C2		
10c	O=C(OCC)C1=C(C)N=C(OCC#C)N=C1C2=CC=CC=C2		
11	O=C([C@@H]1C=C(C)C(O[Si](C)(C(C)(C)C)C)[C@]2([H])[C@@]1(O)/C(CO2)=C/C=C/[C@H](C)[C@H](OC(CN3C=C(c4cccc4)N=N3)=O)/C(C)=C/C5)O[C@H]6C[C@]5([H])O[C@]7(O[C@H]([C@H](C)CC)[C@@H](C)CC7)C6.[][Fe][].c8cccc8		
12	O=C([C@@H]1C=C(C)C(O[Si](C)(C(C)(C)C)C)[C@]2([H])[C@@]1(O)/C(CO2)=C/C=C/[C@H](C)[C@H](OC(CN3C=C(C(C=C4)=CC=C4c5cccc5)N=N3)=O)/C(C)=C/C6)O[C@H]7C[C@]6([H])O[C@]8(O[C@H]([C@H](C)CC)[C@@H](C)CC8)C7.[][Fe][].c9cccc9		
13	O=C(OC(C)C)C1=C(C)N=C(OCC(N=N2)=CN2CC(O[C@@H]([C@@H](C)/C=C/C=C(CO3)/[C@]4(O)[C@@]3([H])C(O[Si](C)(C(C)(C)C)C)C(C)=C[C@H]4C(O5)=O)/C(C)=C/C[C@]6([H])C[C@H]5C[C@@]7(O[C@H]([C@H](C)CC)[C@@H](C)CC7)O6)=O)N=C1C8=CC=CC([N+]([O-])=O)=C8		
14	O=C(OCC)C1=C(C)N=C(OCC(N=N2)=CN2CC(O[C@@H]([C@@H](C)/C=C/C=C(CO3)/[C@]4(O)[C@@]3([H])C(O[Si](C)(C(C)(C)C)C)C(C)=C[C@H]4C(O5)=O)/C(C)=C/C[C@]6([H])C[C@H]5C[C@@]7(O[C@H]([C@H](C)CC)[C@@H](C)CC7)O6)=O)N=C1C8=C([N+]([O-])=O)C=C(OC)C(OC)=C8		
15	O=C(OCC)C1=C(C)N=C(OCC(N=N2)=CN2CC(O[C@@H]([C@@H](C)/C=C/C=C(CO3)/[C@]4(O)[C@@]3([H])C(O[Si](C)(C(C)(C)C)C)C(C)=C[C@H]4C(O5)=O)/C(C)=C/C[C@]6([H])C[C@		

	H]5C[C@@]7(O[C@H]([C@H](C)CC)[C@@H](C)CC7)O6)=O)N=C1C8=CC=CC=C8		
16	O=C([C@@H]1C=C(C)C(O)[C@]2([H])[C@@]1(O)/C(CO2)=C/C=C/[C@H](C)[C@H](OC(CN3C=C(c4cccc4)N=N3)=O)/C(C)=C/C5)O[C@H]6C[C@]5([H])O[C@]7(O[C@H]([C@H](C)CC)[C@@H](C)CC7)C6.[][Fe][].c8cccc8	0.990 ± 0.068	256.7 ± 46.6
17	O=C([C@@H]1C=C(C)C(O)[C@]2([H])[C@@]1(O)/C(CO2)=C/C=C/[C@H](C)[C@H](OC(CN3C=C(C(C=C4)=CC=C4c5cccc5)N=N3)=O)/C(C)=C/C6)O[C@H]7C[C@]6([H])O[C@]8(O[C@H]([C@H](C)CC)[C@@H](C)CC8)C7.[][Fe][].c9cccc9		
18	O=C(OC(C)C)C1=C(C)N=C(OCC(N=N2)=CN2CC(O[C@@H]([C@@H](C)/C=C/C=C(CO3)/[C@]4(O)[C@@]3([H])C(O)C(C)=C[C@H]4C(O5)=O)/C(C)=C/C[C@]6([H])C[C@H]5C[C@@]7(O[C@H]([C@H](C)CC)[C@@H](C)CC7)O6)=O)N=C1C8=CC=CC([N+]([O-])=O)=C8	0.911 ± 0.076	161.2 ± 40.49
19	O=C(OCC)C1=C(C)N=C(OCC(N=N2)=CN2CC(O[C@@H]([C@@H](C)/C=C/C=C(CO3)/[C@]4(O)[C@@]3([H])C(O)C(C)=C[C@H]4C(O5)=O)/C(C)=C/C[C@]6([H])C[C@H]5C[C@@]7(O[C@H]([C@H](C)CC)[C@@H](C)CC7)O6)=O)N=C1C8=C([N+]([O-])=O)C=C(OC)C(OC)=C8	0.503 ± 0.002	50.2 ± 24.5
20	O=C(OCC)C1=C(C)N=C(OCC(N=N2)=CN2CC(O[C@@H]([C@@H](C)/C=C/C=C(CO3)/[C@]4(O)[C@@]3([H])C(O)C(C)=C[C@H]4C(O5)=O)/C(C)=C/C[C@]6([H])C[C@H]5C[C@@]7(O[C@H]([C@H](C)CC)[C@@H](C)CC7)O6)=O)N=C1C8=CC=CC=C8	0.990 ± 0.050	110.5 ± 64
21	O=C(OCC)C1=C(C)NC(NC1C2=CC=C(OC)C(OC)=C2)=O		
22	O=C(OCC)C1=C(C)NC(N=C1C2=C([N+]([O-])=O)C=C(OC)C(OC)=C2)=O		
23	O=C(OCC)C1=C(C)N=C(Cl)N=C1C2=C([N+]([O-])=O)C=C(OC)C(OC)=C2		